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Macromolecular 4-aminoquinoline compounds as potential antimalarial drugs

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CHAPTER 1: INTRODUCTION

Malaria is a major disease in developing countries causing 1-2 million deaths every year. A large percentage of these deaths occur in Africa; however, malaria is endemic throughout the region of Southeast Asia, the Indian subcontinent, the South Pacific region, and Latin America.[1]

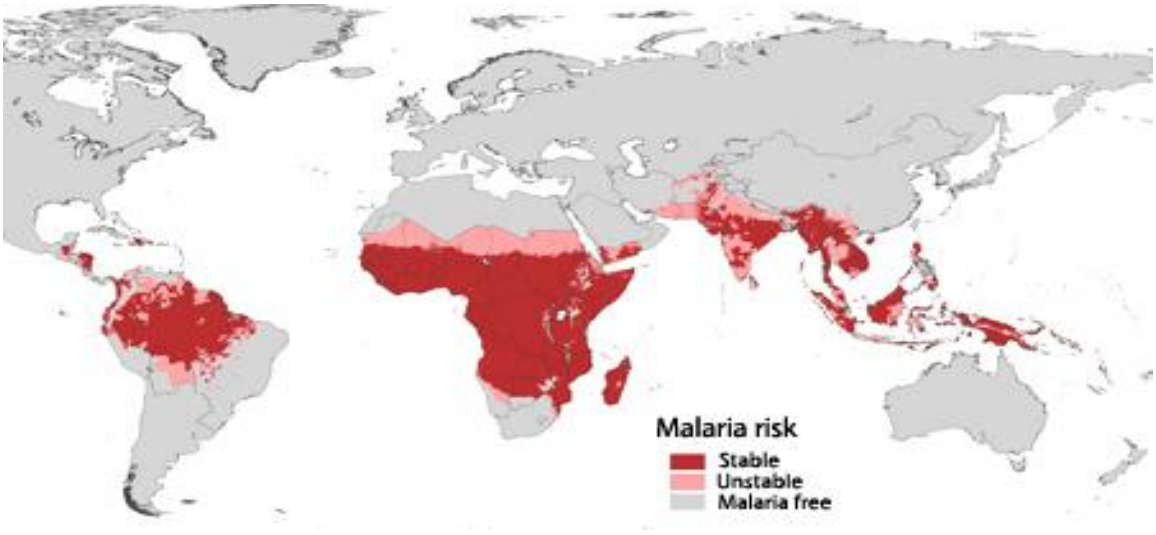

FIGURE 1. World Malaria Atlas 2008[1]

Even in medieval times, it was believed that using large amounts of herbs and spices in cooking, in addition to perfumery material (camphor, sandalwood and incense) or phenols, afforded protection from malaria. Indeed, the constituent of terpenes (like menthol, carvone and thujone) or phenols (like eugenol and myristicin) did work against parasitic infection by either causing paralysis of the worm or disrupting the parasitic life cycle.[2] Malaria is caused by protozoan parasites of the genus *Plasmodium*. Four species of sporozoa are recognised as etiological agents in human malaria: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale* and *Plasmodium malariae*.[2] *Plasmodium falciparum* is responsible for the majority of the deaths due to its resistance to antimalaria drugs.

The parasite is transmitted by the female anopheles mosquito, and transits through the liver and the blood of mammalian host.[3] Malaria is severe and life-threatening because of various factors such as size of the infective dose of sporozoites, nutritional status of the host, level of acquired immunity, host genetic factors such as the presence of sickle cell haemoglobin, parasitic features such as growth rate and drug resistance, and socioeconomic factors such as the basic availability of healthcare and education.[4] The resistance of malarial parasites to drugs has resulted in a resurgence of malaria in many parts of the world, and there is a pressing need for the development of new drugs to combat the resistance problem.

Drug development for antimalaria is challenging for various reasons, such as: (i) it is generally agreed among physicians in malaria endemic countries, that drugs for malaria treatment need to be well tolerated and safe in humans, with no side effect, and this is because of the large number of people who will take antimalarials and the fact that follow-up care is underdeveloped in places where malaria is prevalent; (ii) Antimalarials need to be orally bioavailable for ease of administration in a nonhospital setting; (iii) the concern about compliance and development of resistance; a three-day maximum therapy for cure with once or twice a day dosing is desirable; (iv) drugs need to be used in combination to reduce the development of resistance, which increases the number of new drugs that need to be developed; (v) antimalarials drugs need to have a low cost of goods and be affordable; (vi) a good part of antimalarial drug development occurs at research centres that are not ideally structured for drug discovery (*i.e.* academic and non-profit research institutions).[5]

1.1 POLYMER-DRUG CONJUGATION TECHNIQUE

It is a technique in which selective bioactive agents are reversibly bound to water-soluble polymeric carriers to meet specific biomedical requirements. The technique was pioneered by Ghose[6] and successfully advanced by Ringdorf.[7] It comprises of a linear chain composed of subunits bearing water solubilizing units, other subunits equipped with functional groups for reversible drug anchoring, and finally subunits containing a homing device for directing the molecule to the target tissue. The conjugate represents a

prodrug from which active agents are released into a predestined biological environment by hydrolysis or through enzymatic action. This technique has been accepted as a highly practicably approach towards enhancement of drug efficaciousness.[8-9] This technique is being presently used, *inter alia*, for the treatment of lung cancer and schizophrenia.[10-11]

1.2 AIMS OF RESEARCH

1. Modification of selected agents such as aminoquinolines by amination reactions with different amine groups which are suitable as anchoring moieties for polymer binding. The 4-aminoquinolines will be prepared by a one-step amination reaction between 4,7-dichloroquinoline and selected amines such as tris(2-aminoethyl)amine, 1,3-diaminopropane, *N*,*N*-diethyldiethylenetriamine and diethylenetriamine. The prepared 4-aminoquinolines are to be purified by flash chromatography and characterized by ^{1}H NMR spectroscopy. The 8-aminoquinoline compound used in this project is primaquine which will be liberated as a free base with sodium hydroxide.
2. Preparation of various macromolecular conjugates containing the modified aminoquinoline agents. These carriers are expected to overcome the resistance that is associated with the currently used aminoquinolines.
3. Preparation of various macromolecular conjugates that are biodegradable, non-toxic or have a minimal degree of toxicity, easily conveyed to the target cells or tissues, with different solubilizing groups to ensure rapid dissipation of the conjugate in the central circulation system and reactive functional groups as suitable binding sites for drug attachment. The polymer-drug connective link is to provide delayed and controlled drug release thereby ensuring restriction of drug serum concentration and reduction of organ toxicity.
4. Co-conjugation of an anticancer and an antimalarial agent on a single carrier, whereby the antimalarial is expected to serve as potentiating and resistance reducing agent. Some antimalarial drugs have been found to be anticarcinogenic and the have ability to inhibit tumor development and we aim to take advantage of these finding in this research

5. The antimalarial conjugates will be purified by dialysis against water with 12000 membrane tubing at a pH of 7-8. The carriers will then be characterized by ^{1}H NMR spectroscopy to determine the % incorporation of antimalarial drug before submission for *in vitro* antiplasmodial and toxicity screening tests against a chloroquine-sensitive strain of *plasmodium falciparum.*

1.3 REFERENCES CITED IN CHAPTER ONE

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CHAPTER 2: BACKGROUND AND LITERATURE REVIEW

2.1 MALARIA CONTROL AND PREVENTION

Malaria is a focal disease that differs in its characteristics from country to country and even within the same country. No single strategy is applicable for all situations, and implementing of any of these may be problematic in an area. There are various factors to be taken into account, including (i) the biological, anthropological, cultural, and social characteristics of the population; (ii) the intensity and periodicity of malaria transmission; (iii) the species of malaria parasites and their sensitivity to antimalarial drugs; (iv) the species of the mosquito vector, their behaviour, and their susceptibility to insecticides; (v) the presence of social and ecological change; and (vi) the characteristics of the existing health services.[1]

The world health organisation suggests that there are three essential elements of malaria control. The first is the selective application of vector control either by eliminating, where feasible, or reducing mosquito breeding sites; destroying larval, pupal, and adult mosquito contact. The second elements is early diagnosis and effective and prompt treatment of malarial disease which reduces the source of parasites for infection of mosquitoes as well as reducing morbidity and mortality in all area where people are at risk. The third element is early detection or forecasting of epidemics and rapid application of control measures.[1]

Chemicals continue as the mainstay of mosquito control and broadly falls into five groups, namely, petroleum oils and their derivatives, copper acetoarsenite, natural constituents of the flowers of pyrethrum, pyrethrins, pyrethroids which are synthetic derivatives, organochlorines such as dichlorodiphenyltrichloroethane (DDT) and organophosphates such as malathion and temephos.[2] The last two groups are dangerous in handling, and the problem of resistance have increased with time, and the use of DDT is still frequent where mosquitoes are resistant. Not only can exposure to insecticides lead

to resistant mosquitoes, but it may also result in modification in mosquitoes behaviour whereby mosquitoes and insecticides have fleeting, if any contact.[3,4]

Antimalarial drugs form an important element in control programs in treating cases to remove a source of infection for feeding mosquitoes. Antimalarials are also used prophylactically, but drug resistance has emerged and is spreading.

2.2 THE LIFE CYCLE OF MALARIA PARASITE

The plasmodium parasites have a complex life cycle (figure 2), which begins with a bite by an infected female anopheles mosquito withdrawing blood and at the same time injecting sporozoite-containing saliva into the capillaries of the skin (A). Sporozites from the mosquito's salivary glands travel to the liver and infect the hepatic cells (B). They remain in the liver for 9-16 days, undergoing multiple asexual fission and producing merozoites. After about 5 days, the merozoites are released into the blood stream (C), then enter into the red blood cells, grow and divide to produce more merozoites, which eventually causes the red blood cells to rupture (D). Some of the newly released merozoites go on to infect other red blood cells while other merozoites develop into sex cells known as male and female gametocytes (E). Another mosquito bites the infected human, ingesting the gametocytes (F). In the mosquito's stomach, the gametocytes mature (G). The male and female gametocytes undergo sexual reproduction, uniting to form a zygote. The zygote multiplies to form sporozoites, which travel to the mosquito's salivary glands. If this mosquito bites another human, the cycle continues again (H).[5]

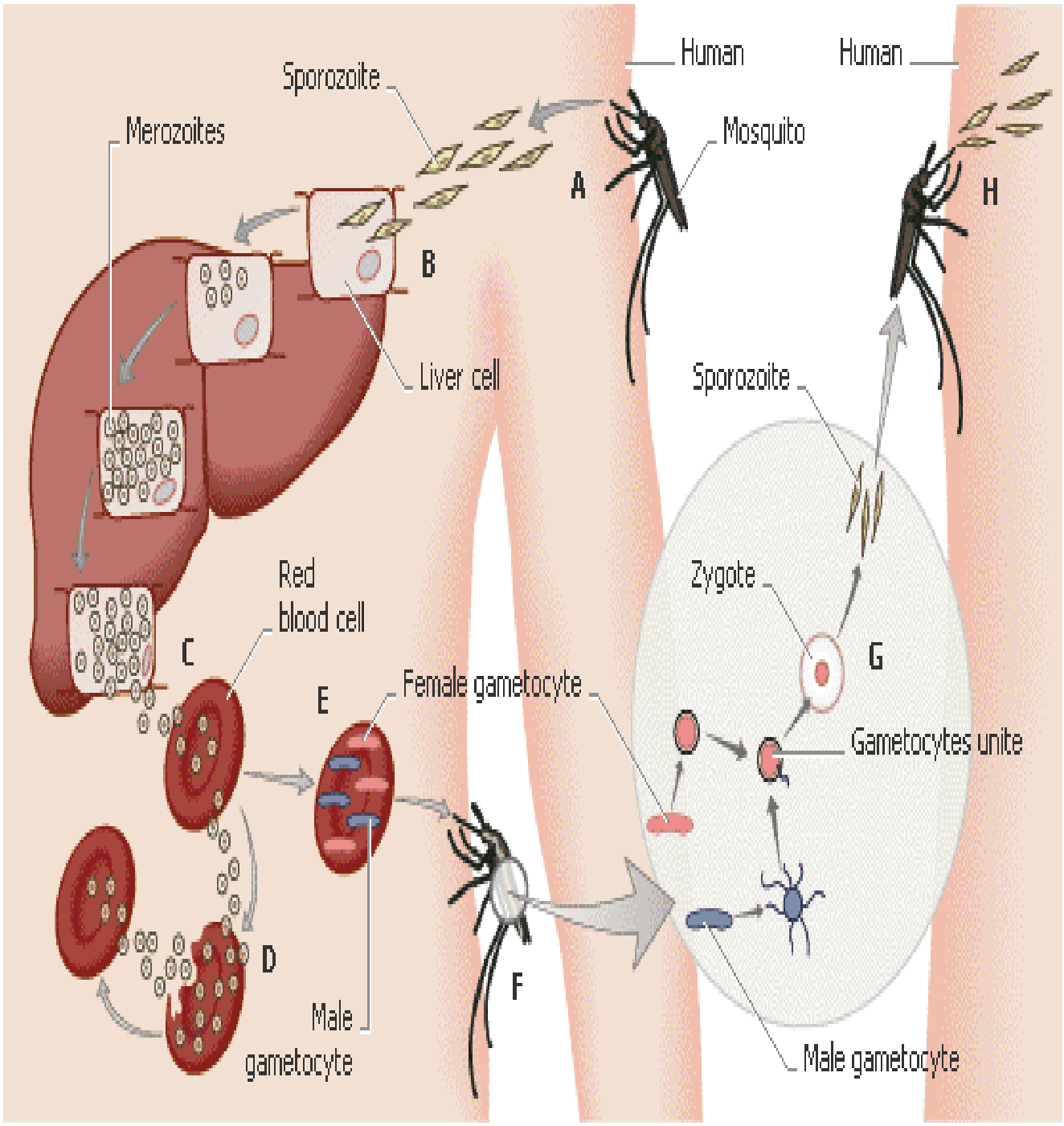


Figure 2. The life cycle of malaria parasite.[5]

2.3 SYMPTOMS OF MALARIA

Symptoms of malaria include fever, shivering, arthralgia i.e. joint pains, vomiting, anemia caused by hemolysis, hemoglobinuria, retinal damage and convulsions.[6] The classic symptom of malaria is coldness, followed by rigor and fever followed by sweating which last 4-7 hours, occurring every two days in *P. vivax* and *P. ovale* infections, while every 3 hours in *P. malariae*.[7] *P. falciparum* can have recurrent fever every 36-48 hours. Malaria has been found to cause cognitive impairments, especially in children. Severe malaria is almost exclusively caused by *P. falciparum* infection and arises 6-14 days after infection.[8] The consequences of severe malaria includes coma and death if untreated, and young children and pregnant women are most vulnerable.

2.4 EVOLUTIONARY PRESSURE OF MALARIA ON HUMAN GENES

Malaria is believed to have the greatest pressure on human genome in recent history, and this is due to high mortality and morbidity caused by malaria, especially the *P. falciparum* species. Malaria is believed to be the evolutionary driving force behind sickle cell disease, thalassemia, glucose 6-phosphate deficiency and other erythrocyte defects.[9]

SICKLE CELL DISEASE

In sickle cell disease there is a mutation of the *HBB* gene which encodes the beta-globin subunit of the haemoglobin. In the merozoites stage, the malaria parasites lives within the red blood cells and its metabolism changes the chemistry within the red blood cells. If the red blood cell contains a mixture of sickle and normal haemoglobin, it becomes deformed and is destroyed. Mutation of the red blood cells produces HbE and HbC haemoglobin types which are common in populations where malaria is endemic, the frequency of sickle-cell genes is around 10%.[9]

THALASSAEMIA

Another set of mutation found in the human genome associated with malaria are those that cause the blood disorder known as thalassaemia. It is an emotological disorder caused by haemoglobin's (red corpuscles protein carrying oxygen to the tissues)

structural anomalites. Recent studies in Sardinia and Papua New Guinea have found that the gene frequency of β-thalassaemias is common where malarial is endemic.[9]

DUFFY ANTIGENS

The duffy antigens are produced by red blood cells during inflammation and they act as chemokine receptors. The *P. vivax* parasite invades the red blood cells causing malaria and red blood cells that lack duffy antigens are resistant to invasion by *P. vivax*. It is possible to express no duffy antigens on red blood cells (Fy-/Fy-) and this genotype is rare in European, Asian and American populations but is found in populations of West and Central Africa. This is thought to be due to high exposure to *P. vivax* in Africa in the last thousand years.[10]

G6PD

Glucose-6-phosphate dehydrogenase (G6PD) is an enzyme that protects from the effects of oxidative stress in the red blood cells. It is present in the cytoplasm of all cells of the body and in red blood cells that lack nuclei mitochondria. *P. falciparum* invades the red blood cells which results in their deficiency leading to G6PD deficiency. A genetic deficiency in this enzyme results in increased protection against severe malaria.[9]

2.5 CURRENTLY USED ANTIMALARIALS

There are several families of drugs used to treat malaria. Chloroquine until recently was very effective and the drug of choice for many years. However, the resistance of *P. falciparum* to chloroquine has spread recently from Asia to Africa making the drug ineffective.[11]

There are several other substances which are used for treatment and, partially, for prevention (prophylaxis), and larger doses are used for the treatment of malaria. A 2006 study by Northwestern University researchers suggested that propranolol may reduce the dosages required for existing drugs to be effective against *P. falciparum* by 5- to 10-fold, suggesting a role in combination therapies.[12]

Below is a list of currently used antimalarial drugs; some of them are used in combination therapy in regions where wide spread drug resistance is evident. [13]

- Quinine
- Chloroquine
- Cotrifazid
- Doxycycline
- Mefloquine
- Primaquine
- Proguanil
- Hydroxychloroquine
- Sulfadoxine-pyrimethamine
- Atovaquone-proguanil
- Artesunate-Sulfadoxine/pyrimethamine
- Artesunate-mefloquine
- Artesunate-amodiaquine
- Artemether-lumefantrine

2.5.1 CHLOROQUINE

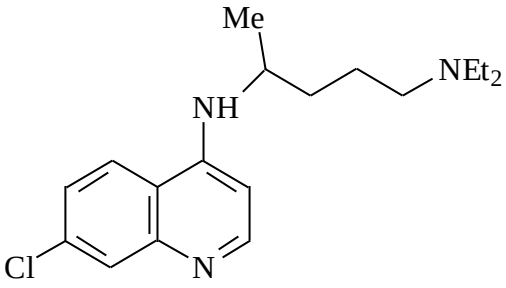


Figure 3: Chloroquine

Chloroquine is a 4-aminoquinoline compound with a complicated and still unclear mechanism of action. It is believed to reach high concentration in the vacuoles of the parasites, which, due to its alkaline nature, raises the internal pH. It controls the conversion of toxic haem to haemozoin by inhibiting the biocrystallization of

haemozoin,[14] thus poisoning the parasite through excess level of toxicity. Other potential mechanisms through which it acts include interfering with the biosynthesis of parasitic nucleic acids, and the formation of chloroquine-haem or chloroquine-DNA complex. Chloroquine also has a significant anti-pyretic and anti-inflammatory effect. The buffering properties of chloroquine-like compounds with lipidic structures have been found to be highly efficient in gene transfer experiments into tested gynecological tumors.[15] Chloroquine have also been found to inhibit the development of drug resistance in cancer cells when used in combination with anticancer drugs.[16] Chloroquine has raised considerable interest because of its anticarcinogenic and antimutagenic properties and its ability to inhibit tumor development and virus replication.[17-26] We aim to take advantage of these findings in this research.

2.5.2 QUININE

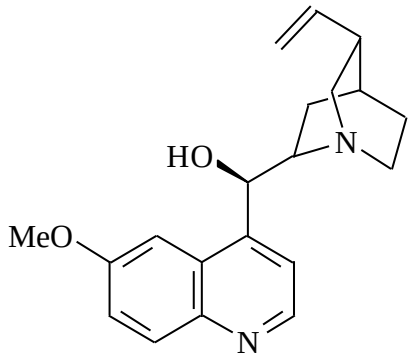


Figure 4: Quinine

The first quinoline antimalarial drugs were alkaloids extracted from the cinchona tree. Quinine is an alkaloid that acts as a blood schizonticide and weak gametocide against *P. vivax* and *P. malariae*. It accumulates in the food vacuoles of plasmodium species such as *P. falciparum*. It acts by inhibiting the heme polymerase enzyme, thus facilitating an aggregation of cytotoxic heme.[27] Quinine is still used in the treatment of acute cases of severe *P. falciparum* infection. It is also used in post-exposure treatment of individuals returning from an area where malaria is endemic. The use of quinine is characterized by a

frequently experienced cinchonism, hearing impediment known as tinnitus, rashes, vertigo, nausea, vomiting and abdominal pains. [2]

2.5.3 MEFLOQUINE

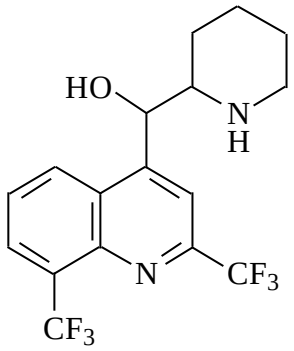


Figure 5: Mefloquine

Mefloquine was developed during the Vietnam War to protect American troops against multi-drug resistant *P. falciparum*. It is believed to act by forming toxic heme complexes that damage the parasitic food vacuoles. It is now used for resistant strains and used in combination with artesunate. Mefloquine frequently produces side effects such as nausea, vomiting, diarrhea, abdominal pains and dizziness. Several associations with neurological events have been made such as anxiety disorders, hallucination, sleep disturbances, convulsion and psychosis.[27]

2.5.4 PRIMAQUINE

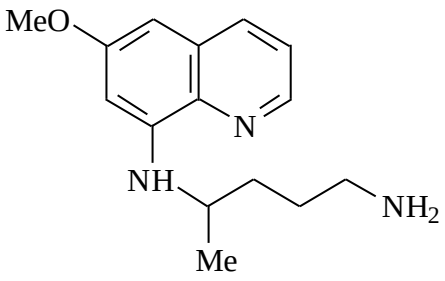


Figure 6: Primaquine

Primaquine is a highly active 8-aminoquinoline that is used to treat all kinds of malaria infections. This treatment is often used in conjugation with another effective blood schizonticidal drug. The mechanism of action is not fully known but it is believed to involve the creation of oxygen-free radicals that interfere with the plasmodial electron transport chain during respiration. There are several side effects that primaquine causes, such as nausea, vomiting, cramps, anemia, abdominal pains and chest weakness.[27]

2.5.5 AMODIAQUINE

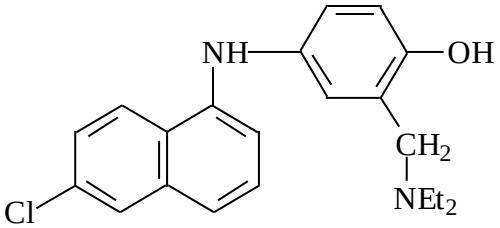


Figure 7: Amodiaquine

Amodiaquine is a 4-aminoquinoline similar in structure to chloroquine and it has a similar mechanism to that of chloroquine. It is frequently used in combination with chloroquine and it is believed to be more effective in clearing out parasites in uncomplicated malaria than chloroquine. It has certain side effects such as itching, nausea, vomiting and abdominal pain. It has been suggested to be effective and less toxic than other combination treatments in HIV positive patients.[27]

2.5.6 PYRIMETHAMINE

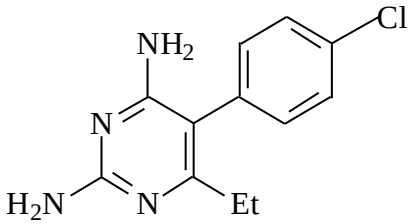


Figure 8: Pyrimethamine

Pyrimethamine is used for protozoal infections and it is commonly used as an antimalarial drug for both prevention and treatment. It is used in combination with sulfadiazine for treatment of *Taxoplasm gondii* infections in HIV–positive patients. Some side effects of pyrimethamine include depletion of folic acid in humans, resulting in hematologic effects associated with folate deficiency including hypersensitivity reactions, megaloblastic anemia and leukopenia.[28]

2.5.7 PROGUANIL

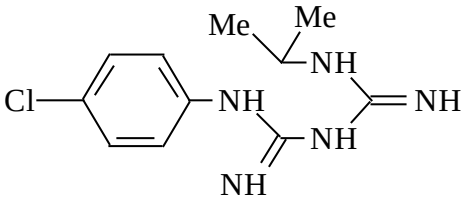


Figure 9: Proguanil

Proguanil is a prophylactic antimalarial drug and it works by stopping the malaria parasite, *Plasmodium falciparum* and *Plasmodium vivax* from reproducing once it is in the red blood cells. It does this by inhibiting the enzyme, dihydrofolate reductase, which is involved in the reproduction of parasite.[29] It is usually taken in combination with another antimalarial drug such as atovaquone for chloroquine-resistant and multi-drug resitant strains of *Plasmodium vivax* and *Plasmodium falciparum*.[30,31] Some of the side effects includes the feeling of sullenness and anxiety to a level that is outside the ordinary.[29]

2.5.8 SULPHADOXINE

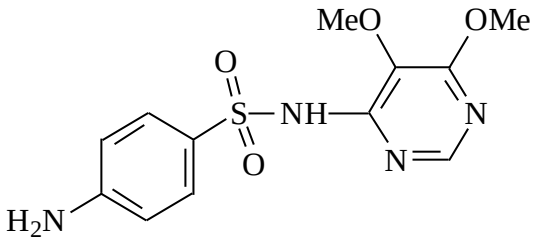


Figure 10: Sulphadoxine

Sulphadoxine is usually administered in combination with pyrimethamine to treat and prevent malaria. Both drugs are antifolates and they inhibit the production of enzymes involved in the synthesis of folic acid within the parasites. [32]

2.5.9. ATOVAQUONE

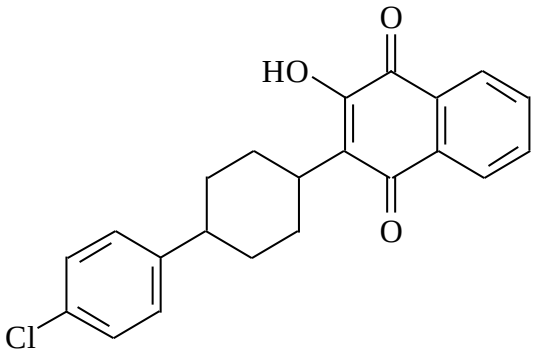


Figure 11: Atovaquone

Atovaquone belongs to the class of naphthoquinones and it is used to treat and prevent pneumocystis pneumomia, taxoplasmosis, and malaria; however, resistance has been observed. It is usually used in combination with proguanil to treat severe malaria. Proguanil acts as mitochondrial sensitizer and synergizes with atovaquone; of concern is that there is a high frequency of cytochrome B mutants, which leads to failure rate if atovaquone is used on its own to treat malaria.[33,34]

2.5.10 HYDROXYCHLOROQUINE

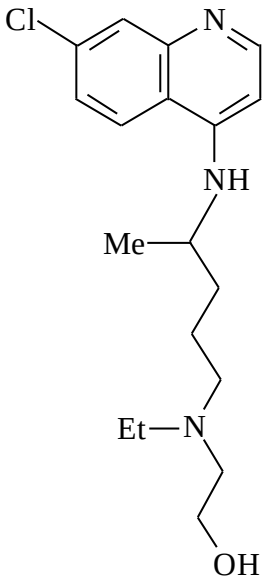


Figure 12: Hydroxychloroquine

The pharamacokinetics of hydrochloroquine is similar to that of chloroquine. It is very water soluble and well absorbed in the gastrointestinal tracts. Severe malnutrition affects absorption, but diarrhea does not. It has a prolonged half-live between 40 and 50 days and a low blood clearance. [35-38]

2.5.11 DOXYCYCLINE

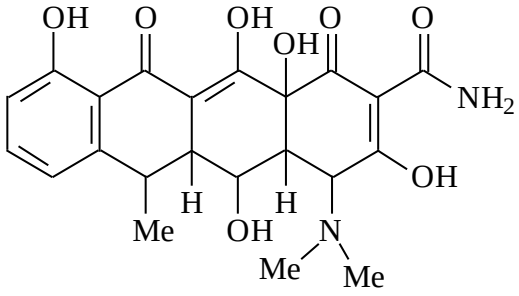


Figure 13: Doxycycline

Doxycycline is a member of the tetracycline antibiotics and it is used for the treatment of a variety of infections. It is used in prophylaxis against malaria, and usually used in

combination with other antimalarial agents such as quinine. One of the side-effects is thin skin and photosensitivity.[39,40]

2.5.12 HALOFANTRIN

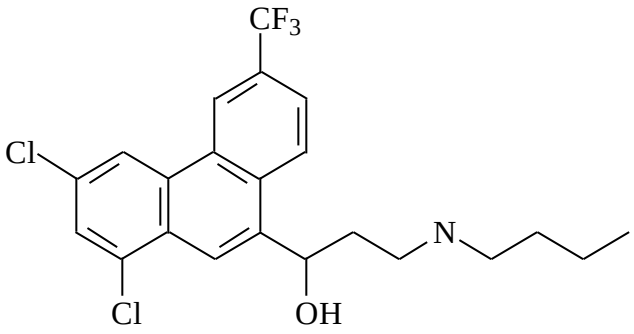


Figure 14: Halofantrin

Halofantrin is a drug used to treat malaria and it contains a substituted phenanthrene. It is used only for treatment of malarial diseases and not for the prevention of malaria because of the risk of toxicity, unreliable absorption and short life span of 1-2 days. Cytotoxic complexes are formed with ferritoporphyrin XI that cause plasmodial membrane damage. Some of the side-effects include rashes, diarrhoea, vomiting and itching.[41]

2.5.13 ARTEMESININ AND DERIVATIVES

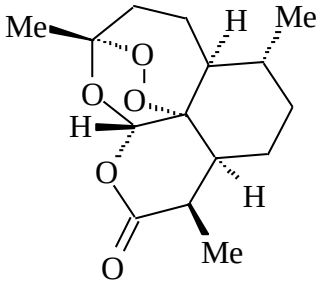


Figure 15: Artemesinin

Artemesinin is a Chinese herb that has been used over 1000 years for the treatment of fever, thus predating the use of quinine in the western world.[42] The active compound was first derived from a plant in 1971 and called artemsinin. It is a sesquiterpene lactone with a chemically rare peroxide bridge linkage. It has been proven to be effective against all

forms of multi-drug resistant *P. falciparum*.[43] It has demonstrated the fastest clearance of all antimalarials currently used and acts primarily on the trophozite phase, thus preventing further progression of the disease. It is also used in combination with other antimalarials and at present is strictly controlled under WHO guidelines .[27] There are few side effects associated with artemesinin such as itching, dark urine, abnormal bleeding, headaches and nausea.

DERIVATIVES OF ARTEMESININ

1. ARTEMETHER

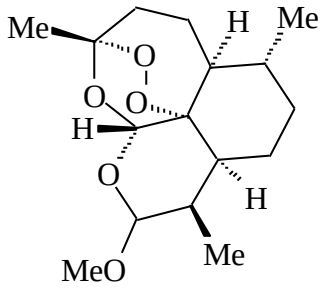


Figure 16: Artemether

Artemether is a methyl derivative of dihydroartemesinin and it has a similar mode of action to that of artemesinin, but it has demonstrated a reduced ability as a hypnozoiticidal compound, instead acting more significantly to decrease gametocyte carriage. It is synthesized by the reduction of dihydroartemesinin.[44]

2. ARTEETHER

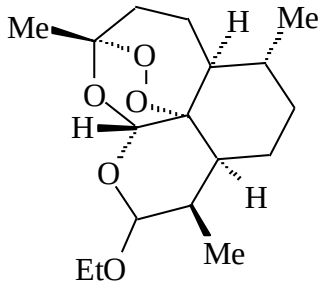


Figure 17: Arteether

Arteether is an ethyl derivative of dihydroartemesinin. It is used in combination therapy for cases of uncomplicated resistant *P. falciparum*. It also prepared by the reduction of dihydroartemesinin.[44]

3. DIHYDROARTEMESININ

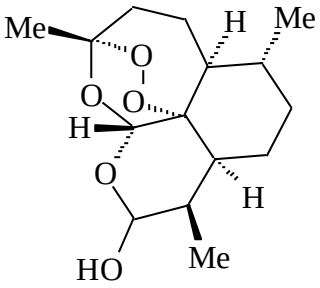


Figure 18: Dihydroartemesinin

Dihydroartemsinin is the active metabolite to which artemesinin is reduced *in vivo*. It is the most effective artemesinin compound and the least stable. It has a strong blood schizonticidal action and reduces gametocyte transmission.[27]

4. ARTESUNATE

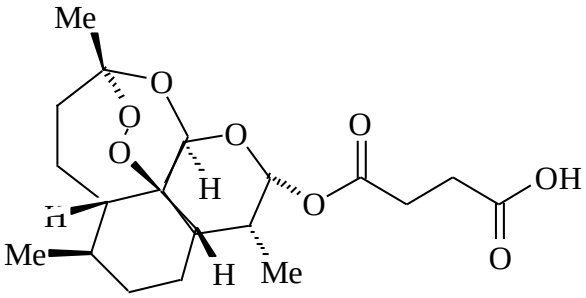


Figure 19: Artesunate

Artesunate is a water-soluble semi-synthetic hemisuccinate derivative of artemesinin. It is the most effective because of its instantaneous bioavailability. It is synthesized by reacting dihydroartemesinin and succinic acid anhydride in an alkaline medium. A combination of mefloquine and artesunate is highly effective in multi-drug resistant malaria.[45]

2.6 RESISTANCE TO ANTIMALARIALS

Antimalarial drug resistance is defined as the "ability of a parasite to survive and multiply despite the absorption of a given drug administered in doses equal or higher than doses usually recommended but with tolerance".[27] Drug resistance occurs selectively in the species *P. falciparum*. The three other species have no documented resistance apart from the regionalized chloroquine resistance observed in *P. vivax*, concentrated in Papua New Guinea and Irian Jaya (Indonesia).[46] The reasons for the resistance and spread of resistance is thought to be due to (i) interaction of drug-use pattern such as misdiagnosis and incorrect doses; (ii) characteristics of the drug itself *i.e*. poor drug quality and poor absorption; (iii) human host factors; (iv) parasite characteristics, which leads to gene mutation that results in the over-expression of the pfmdr1 gene; this causes resistance to many antimalarials; (v) Parasite-vector combination can enhance the transmission of resistant parasites, causing pocket-like areas of resistance, and there has been evidence to suggest this factor and; (vi) environmental factors.[46]

2.7 COMBINATION THERAPY FOR ANTIMALARIAL CHEMOTHERAPHY

Combination therapy involving the use of newer antimalarial agents with older antimalarial agents is still under study in Africa, and other areas with widespread drug resistance. The use of combination antimalarial therapy offers two important potential advantages. First, the combination should improve antimalarial efficacy. Secondly, and most important, the use of combination therapy should slow down the progression of parasite resistance.[47] The combination of drugs is currently divided into two categories:

2.7.1 NON-ARTEMSININ BASED COMBINATIONS

1. Amodiaquine combination with sulfadoxine/pyrimethamine showed excellent antimalarial efficacy in regions of East Africa but with fairly high level of resistance to each individual agent.[48] This combination uniquely provides two available and inexpensive drugs; however, there are serious adverse reactions associated with its use.[27]
2. Quinine and doxycycline combination: despite the increasing level of resistance to quinine, this combination has proven to be particularly efficacious. The problem with this combination is the relatively complicated drug regimen.[27]
3. Chloroquine combination with sulfadoxine/pyrimethamine
4. Mefloquine combination with sulfadoxine/pyrimethamine

2.7.2 ARTEMESININ-BASED COMBINATION

1. Artesunate/mefloquine
2. Artemether/lumefantrine
3. Atovanquone/proquanil

This first three artemesinin combinations are quite expensive and do not contain components with similar pharmacokinetics, and this may have toxicity concerns. These combinations are not ideal for widespread use in many areas, particular in Africa.[49]

2.8 ANTIMALARIAL COMPOUNDS TARGET LOCATION

The most innovative approach to the synthesis of antimalarials requires identification of the target location, design of target molecules and an understanding of the mechanism of the drugs. There are two main classes of antimalarial agents in use, the antifolates and

cinchona alkaloids or quinoline-containing drugs. The antifolates include the diaminopyrimidines such as pyrimethamine, biguanides (e.g. proguanil) and sulfa drugs including sulfonamides and sulfones. The quinoline containing compounds include quinine, quinidine, halofantrine, 4-aminoquinoline and 8-aminoquinoline.[1]

TABLE 1.ANTIMALARIAL COMPOUND'S TARGETS.[47]

Target location	Pathway/mechanism	Target molecule	Existing antimalarial agents
Cytosol	1. Folate metabolism 2.Glycolysis	1. Dihydrofolate reductase 2. Dihydropteroate Synthase 3. Thymidylate synthase 4. Lactate dehydrogenase	Proguanil, sulfadoxine, pyrimethamine, dapsone
Parasite membrane	1.Phospholipids synthesis 2.Membrane transport	Choline transporter Unique channels	
Food Vacuole	1. Heme Polymerization 2. Hemoglobin hydrolysis 3. Free radical generation	Hemozoin Plasmepsins Unknown	Quinolines Artemisinins
Mitochondrion	Electron transport	Cyt. *c* oxidoreductase	Atovaquone, primaquine
Apicoplast	1. Protein synthesis	1. Apicoplast ribosome	Antibiotics
	2. DNA synthesis 3. Transcription	2. DNA gyrase 3. RNA polymerase	Quinolones Rifampin

	4. Type II fatty acid biosynthesis 5. Isoprenoid synthesis 6. Protein farnesylation	4. FabH FabI 5. DOXP reductoisomerase 6. Farnesyl transferase	

2.8.1 CYTOSOLIC TARGETS

The cytosol is the location of numerous metabolic pathways, with hundreds of enzymes that are essential. Folate metabolism is one of the pathways of cytosolic agents which have been proven valuable, and antifolates that treat both bacterial and protozoan infection with minimal toxicity follow this pathway. Glycolysis is another cytosolic pathway, and malaria parasites are dependent on this pathway for energy production. *P. Falciparum lactate dehydrogenase* has been characterized structurally, an it's unique binding sites for NADH offers opportunities for the design of selective inhibitors.[47]

2.8.2 PARASITE MEMBRANE TARGETS

Intraerythrocytic malaria parasites undergo extensive phospholipid synthesis to produce membranes necessary to enclose the parasitophorous vacuole, cytosol and multiple subcellular compartments. The most abundant lipids in plasmodial membrane are phosphatidylcholine. Synthesis of phosphatidylcholine requires host choline, and blockage of choline transport has been identified as a promising therapeutic strategy in antimalarials.[50]

2.8.3 FOOD VACUOLE TARGETS

Malaria parasites contain acidic food vacuoles in which erythrocyte hemoglobin is hydrolyzed. In *P. Falciparum* trophozoites, a single large food vacuole is present and it appears to be the site of action of existing antimalarials. In the food vacuoles, hemoglobin is degraded into heme, which is polymerized into soluble hemozoin pigment and globin, which is hydrolyzed to individual amino acids. 4-Aminoquinolines appear to act by

blocking the formation of hemozoin from heme molecules once they are liberated from hemoglobin.[51]

2.8.4 MITOCHONDRIAL TARGETS

Atovaquone acts against ubiquinol-cytochrome *c* oxidoreductase (complex III), inhibits electron transfer and collapses mitochondrial membrane potential which is required for a number of parasite biochemical processes.[52] The 8-aminoquinolines, such as primaquine, can bring about radical cure of *P. vivax* infections by acting on the gametocyte and hypnozoite stages. Primaquine is thought to be converted in the liver to an active quinone metabolite. Treatment with primaquine causes swelling and thickening of the mitochondria of tissue-stage parasites, suggesting that it exerts its activity by interfering with mitochondrial function.[53-54] The mechanism of action of the 8-aminoquinolines may be related to the action of napthoquinones such as atovaquone, which has been shown to inhibit the cytochrome bc1 complex of the mitochondrial respiratory chain and collapse the mitochondrial membrane potential. Primaquine can cause hemolysis in individuals with glucose 6-phosphate dehydrogenase deficiency[55] as metabolites of primaquine undergo redox cycling in the erythrocyte, leading to an oxidative stress that is poorly tolerated by glucose 6-phosphatedehydrogenase-deficient cells.[56]

2.8.5 APICOPLAST TARGETS

Apicoplast has a separate, prokaryote-like genome, and this explain the antimalarial effects of a number of antibacterial compounds that do not attack the eukaryotes. A number of antibacterial compounds are effective slow-acting antimalarials, and these compounds act by targeting apicoplast and mitochondrial processes that are similar to that of bacteria. Foe example, quinolone antibiotics inhibit DNA gyrase, and rifampin inhibits RNA polymerase.[57]

2.9 RESISTANCE TO 4-AMINOQUINOLINES

The resistance of 4-aminoquinolines (chloroquine) is believed to be due to the development of an efflux mechanism that expels the 4-aminoquinolines from the parasites before attainment of the level required to effectively inhibit the process of haem

polymerization that is necessary to prevent build up of toxic by products formed by haemoglobin digestion.[27] One of the aims of this research project is to overcome this resistance by the conjugation of 4-aminoquinoline to macromolecular carrier. Polymer-drug connective links provide delayed and controlled drug release thereby ensuring restriction of drug serum concentrations well within pharmacologically dictated limits with concomitant reduction of organ toxicity.

2.10 RESISTANCE TO 8-AMINOQUINOLINES

Primaquine on the other hand is structurally related to the 4-aminoquinolines; it acts in a different manner and on different forms of the parasite. Primaquine is an antimalarial agent and is the essential co-drug with chloroquine in treating all cases of malaria. Primaquine disrupts parasite mitochondrial function that is responsible for supplying it with energy, and also binds to DNA. Primaquine inhibits the gametocyte and the exoerythrocytic forms of the parasite (liver trophozoites),[58] whereas chloroquine interferes with the parasite's ability to metabolize, and plasmodial forms that do not have food vacuole, such as the exoerythrocytes forms, are not affected by chloroquine.[59] This prevents the development of blood forms responsible for relapses with *P. vivax* malaria. Primaquine is not used in the prevention of malaria, only in the treatment. Primaquine has gametocytocidal activity against all plasmodia, including *P. falciparum.*

2.11. LITERATURE REVIEW ON 4-AMINOQUINOLINES

Quinoline-containing drugs, particularly the 4-aminoquinolines, have a long and successful history as antimalarials. One of these, chloroquine, has been in worldwide use; however, resistance to chloroquine has become clinically significant in several areas of the world. Prior work has shown that either shortening or lengthening of the linker of the alkylamine side chain in chloroquine leads to compounds that remain effective against drug-resistant *P. falciparum*.[60-62] Chloroquine is an analogue of quinoline that has a chloro group at the 7-position and an aliphatic amino group at the 4-position. Because of the aromatic ring structure, chloroquine can intercalate DNA and facilitates the unpackaging of DNA from polyplex.[63-65] Chloroquine analogues are prepared by a one-step substitution reaction between 4,7-aminoquinoline and corresponding aliphatic

amines. Chloroquine is a weak base with a pK_a of 8.1 and 10.2, and this is known to buffer the luminal pH of endosomes. The buffering activity of chloroquine could improve the transfection efficiency by facilitating DNA release from the endocytic pathway, and by inhibiting lysosomal enzyme degradation.[66-70]

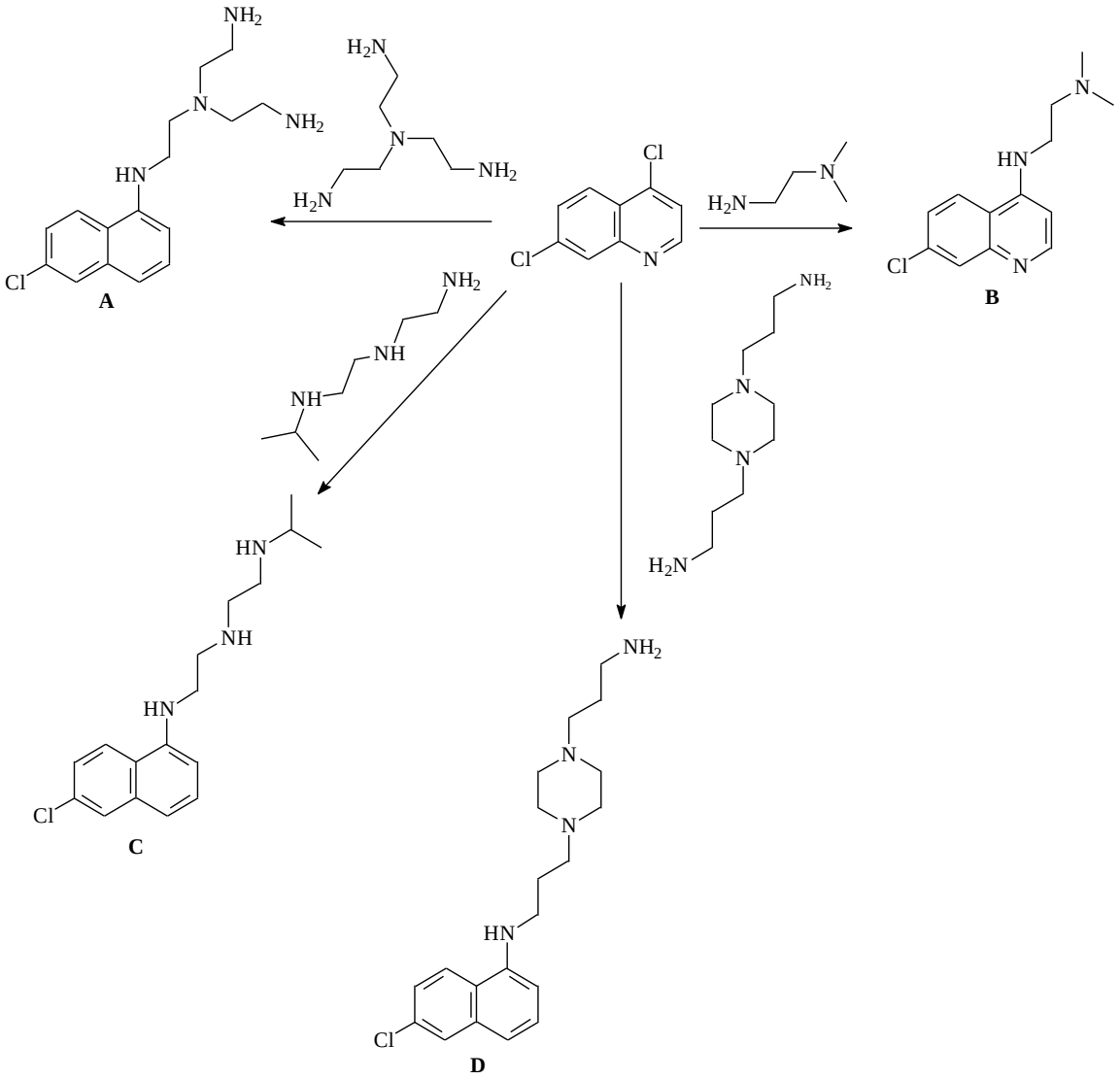
2.11.1 MECHANISM OF ACTION OF CHLOROQUINE

Early studies suggested that interaction of chloroquine with DNA might underlie the antimalarial activity of chloroquine. This idea was derived from the demonstrated interaction of DNA with 4-aminoquinolines, especially chloroquine, and the ability of a number of quinolines to inhibit DNA replication and RNA synthesis.[63-64] Chloroquine is only active against the blood stages of *Plasmodium* parasites and it is not active against the pre-erythrocytes or the hypnozoites stage parasites in the liver, nor against mature gametocytes. It is therefore assumed that chloroquine interferes with the parasite's feeding process, and the food vacuole is believed to be the site of chloroquine action. Thus, it has been proposed that quinoline drugs selectively target the parasite by inhibiting the parasite-specific process of haemoglobin degradation. Chloroquine is a diprotic weak base and in its unprotonated form, it traverses the membrane of the parasitized erythrocytes and moves down the pH gradient to accumulate in the food vacuoles (pH 5.0-5.2). Once protonated the drug becomes membrane impermeable and is trapped in the acidic compartment of the parasite. The level of accumulation of chloroquine in the food vacuole depends on the difference in pH between the external medium and the food vacuole.[71-73]

2.12 SYNTHESIS OF 4-AMINOQUINOLINES

The modification of quinine have led to inexpensive 4-aminoquinoline derivatives which are mostly prepared by a one-step reaction between 4,7-dichloroquinoline and corresponding aliphatic amines. Most of these compounds contain the 7-chloroquinoline nucleus with varying basic amine side chain and research has shown that modification of the basic side chain can produce 4-aminoquinolines that are active against drug-resistant *P. falciparum* strains. Scheme 1 is an example of some 4-aminoquinoline compounds synthesized by M. Davies and co-workers, where 4-aminoquinolines that had more than

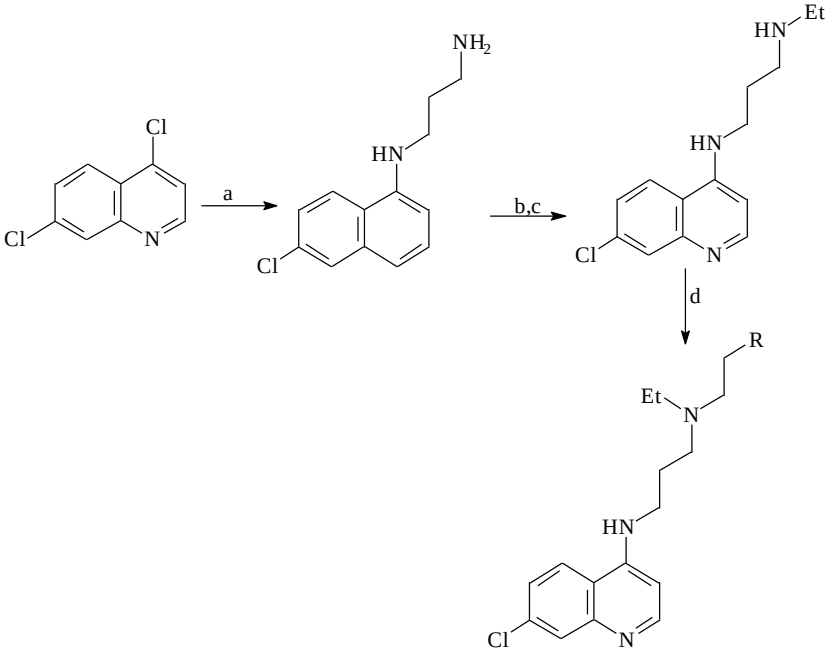
two amino moieties (A, C and D) were found to be more basic than the chloroquine analogue and increasing the number of amine groups resulted in slight increase in the maximal luciferase activity when compared to chloroquine for HepG2 cells. [74]



Scheme 1: Synthesis of 4-aminoquinolines

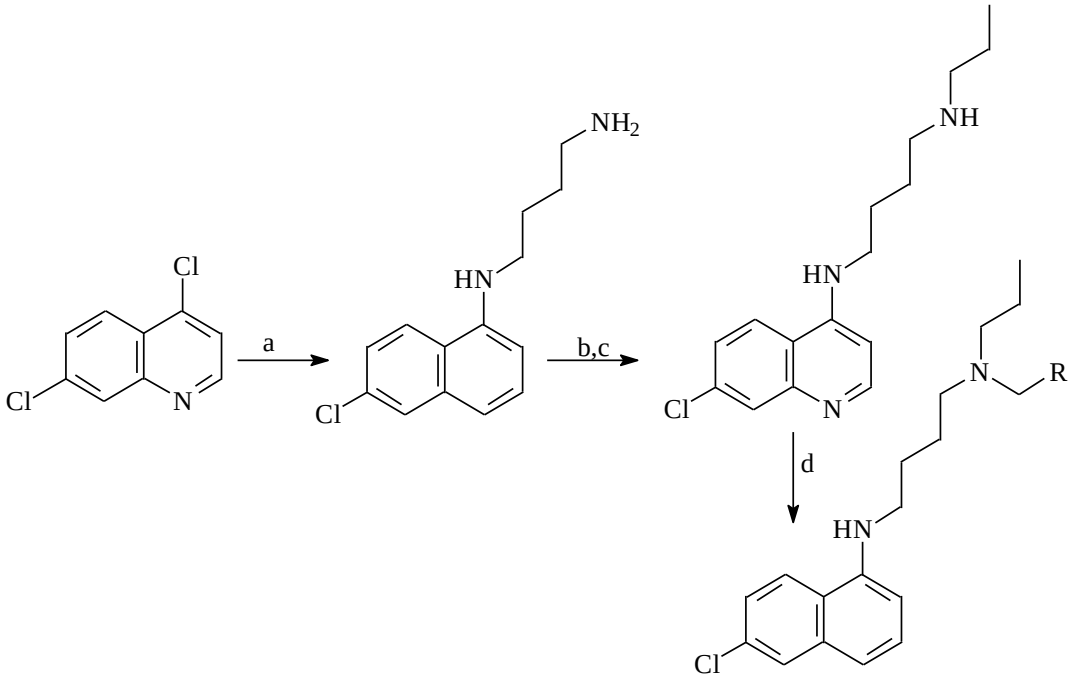
In scheme 2, the 4,7-dichloroquinoline compound was reacted with neat 1,3-diaminopropane and in scheme 3 with 1,4-diaminopropane to afford 4-aminoquinoline

compounds which were then reacted with propionic anhydride followed by reduction using borane-dimethylsulfide in THF to afford the secondary amine intermediates.[60, 75]



(a) 1,3-diaminopropane, reflux 1 h (b) propionic anhydride, $CHCl_3$, pyridine (c) BH_3DMS, THF, reflux 1 h (d) RCHO, $NaBH_3CN$, MeOH

Scheme 2.[75]



(a) 1,4-diaminobutane, reflux, 1h, (b) propionic anhydride, $CHCl_3$, pyridine, (c)BH_3DMS, THF, reflux,

(d) RCHO, $NaBH_3CN$, MeOH,

Scheme 3.[75]

2.13 USE OF ANTIMALARIALS TO REDUCE RESISTANCE OF ANTICANCER DRUGS

Antimalarial drugs have raised considerable interest because of their anticarcinogenic properties and their ability to inhibit tumor development and virus replication.[76] The present invention of antimalarial drugs which are administered in combination with anticancer drug, relates to complex compositions of anticancer drugs which are ministered in combination with antimalarial drugs to enhance the effect of anticancer drugs by inhibiting the development of drug resistance of cancer cells caused by anticancer drugs. Certain anticancer drugs were selected, such as cisplatin and doxorubicin, and the antimalarial drugs selected were chloroquine, primaquine and hydroxychloroquine. To evaluate the effect of hydroxychloroquine co-administered with chemotherapeutic agents, hydroxychloroquine was found to cause the cell viability of malignant glioma to decrease and reverse the chemotherapy resistance significantly when co-administered with the four chemotherapeutic agents (doxorubicin, vincrisitine,

nimustine, cisplatin) as their concentration is increased.[77] The antimalarial drug was found to thus enhance the intracellular concentration of the cytotoxic drug.

Antimalarial drugs are often strong DNA-intercalating agents and are lysosomotropic; both actions in eukaryotic cells modify several cell functions. In cells with high mitotic rate, such as cancer cells, chloroquine and quinacrine are antimutagenic.[78-79] Reasearch has also shown that chloroquine has a radiopotentiating effect in conjunction with mild hyperthermia in the potentiating effect of radiation in Hela cells.[80] There are several mechanisms that are proposed for the physiological action of chloroquine, including retention of drug in the lysosomes,[81] inhibition of proteolytic enzymes,[82] intercalation with DNA and DNA repairs process,[83] and prevention of protein phosphorylation.[84] Another research has shown that chloroquine enhances the anticancer drug cytotoxicity in multiple drug resistant human leukemia cells.[85]

2.14. PHARMACOKINETICS OF ANTIMALARIALS

In general, 4-aminoquinoline derivatives appear to bind to the nucleoprotein and inhibit DNA and RNA polymerase. High drug concentrations are found in the malaria parasite's digestive vacuoles, and this increases the pH of vacuoles and interferes with the ability of the parasite's ability to metabolize and utilize erythrocytes hemoglobin.[59,86] Plasmodial forms that do not have digestive vacuoles and do not utilize hemoglobin, such as exoerythrocytes, are not affected by chloroquine. 8-Aminoquinoline on the other hand follows a different pharmacokinetic pathway by disrupting the parasite mitochondrial function that is responsible for supplying energy.

2.15 LITERATURE REVIEW OF ANTICANCER DRUGS

Another aspect of this research project is the modification of anticancer drugs followed by a co-conjugation of the modified anticancer and the antimalarial drugs on a single polymer carrier where the antimalarial drug is expected to serve as potentiating and resistance reducing agent. Cancer is a class of disease in which the cells undergo uncontrolled growth beyond normal limits, invasion and sometimes metastasis that are

spread to other locations of the body via blood or lymph systems. Cancer affects all ages, but the risk of varieties increases with age, and cancer causes 13% of all deaths.[87] In the United states, every in every three people will contract some form of cancer in his/her lifetime; in South Africa, cancer is the second most common cause of death among the white, coloured and Asian population, and the third cause of death among the black population.[88] Almost all cancers are caused by abnormalities in the genetic material of the transformed cell, and these abnormalities are frequently due to carcinogens, such as tobacco smoke, chemicals, radiation or infective agents.[89]

2.15.1 CLASSIFICATION

Cancer cells are classified according to the tissue from which the cancerous cell originates. Classes: tumors of the stomach, lungs, esophagus, pancreas, colon, spinal column etc. Some of the definitions are below:

1. Tumor: It means abnormal swelling, lump or mass.
2. Neoplasm: It refers to benign or malignant growth
3. Invasive tumor: It refers to invasion of surrounding tissue
4. Non-invasive tumor: This refers to neoplasm which is not invasive but has a potential to progress to cancer if left untreated.[89]

2.15.2 CANCER TREATMENT OPTIONS

There are a number of treatment options available for cancer, and the choice of treatment depends on the location, grade of tumor and the stage of the disease, as well as the general state of the patient.[90]

1. Surgery: This involves the removal of tumors, and this can be performed using conventional instrumention or laser. When cancer has metastasized to other sites in the body prior to surgery, complete surgical excision is usually impossible. Example of surgical operation includes mastectomy for breast cancer, prostatectomy for prostrate cancer.
2. Radiation therapy: This involves the treatment of cancer using ionizing radiation to destroy cancer cells and shrink tumors, thereby making it impossible for these cells to continue to grow. Radiation therapy damages both normal and cancer

cells, but some most of the normal cell affected by the radiation therapy, recover from the effect of radiation and function normally again. It is used to treat almost every type of solid tumors, such as cervix, breast, brain lung, pancreas, skin stomach, uterus, and larynx.

3. Hormone therapy: This is the use of hormones to change the way hormones in the body help cancer cells to grow. Removing or blocking estrogen and testosterone is often an important additional treatment. In certain cancers, administration of hormone agonists, such as progestogens may be therapeutically beneficial.
4. Biological therapy (immunotherapy): This treatment option makes use of the body's immune system either directly or indirectly to fight cancer and lessen the side effects. The contemporary method for generating an immune system against tumor includes intravesical BCG immunotherapy for superficial bladder cancer.
5. Angiogenesis inhibitors: This prevents the excessive growth of blood vessels that the tumor requires to survive.
6. Targeted therapies: This constitutes the use of agents for the deregulated proteins of cancer cells. It also involves the use of small peptides as homing devices which can bind to the cell surface receptors surrounding the tumors. Photodynamic therapy is another type of targeted therapy that often involves the use of photosensitizer, oxygenated tissue and lasers. It is very useful for the removal of traces of malignant tissue after the surgical removal of large tumors.
7. Alternative therapy: this includes acupuncture and homeopathy.
8. Chemotherapy: This is the treatment of cancer with anticancer drugs. Anticancer drugs destroy cancer cells by stopping growth or multiplication. These anticancer drugs can be administered intravenously (into a vein), orally (by mouth), by injection into the muscle depending on the drug and type of cancer.

2.16 TYPES OF CHEMOTHERAPEUTIC DRUGS

Chemotherapeutic drugs are classified based on their chemical structures and the way they act on cancer cells.[91,92]

1. Alkylating agents: Alkylating agents were among the first chemotherapy drugs to be used to treat cancer. They act directly on the DNA causing cross-linking of

DNA strands, abnormal base pairing or DNA strands break, thus preventing cells from dividing. They are effective in the treatment of slow-growing cancers rather than rapidly growing cancers. Examples of alkylating agents are busalfan, thiotepa, Chlorambucil, cyclophosphamide.

2. Antimetabolites: They replace natural substances in DNA molecules, which alters the function of enzymes required for cell metabolism and protein synthesis. They stop nutrients from reaching the cells, so eventually cells starve to death. Examples of antimetabolites are purine antagonists, pyrimidine antagonists and folate antagonists.
3. Plants Alkaloids: This type of chemotherapy drugs is derived from plants and they act on cancer cells and prevent them from dividing themselves. Some drugs of this type act throughout the cell cycle, while others are cycle-specific, that is, more effective during S and M phases of cell. Examples of plant alkaloids are actinomycin, doxorubicin and mitomycin.
4. Antitumor antibiotics: They are not specific to cell cycle and they act on the cells by binding with DNA and thus stopping RNA synthesis, which creates proteins necessary for cell survival. These drugs cause the strands of genetic material that make up DNA to uncoil, which prevents the cell from reproducing. Some commonly used antitumor antibiotics are doxorubicin, mitoxantrone and bleomycin.

2.17. EXAMPLES OF COMMONLY-USED ANTICANCER AGENTS

2.17.1. GEMCITABINE

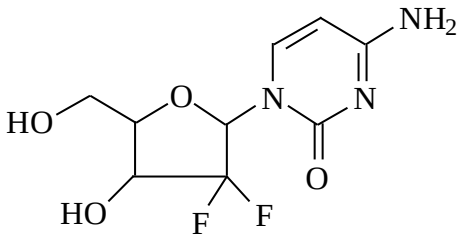


Figure 20: Gemcitabine

Gemcitabine replaces one of the building blocks of nucleic acids, in this case cytidine, during DNA replication. This process arrests tumor growth, as the nucleotides cannot be attached to the faulty nucleoside, resulting in apoptosis. It is used for the treatment of pancreatic, breast, oesophageal, and lung cancer.[93]

2.17.2 FLUOROURACIL

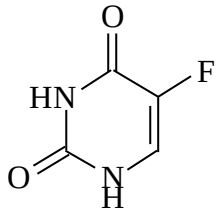


Figure 21: Fluorouracil

Fluorouracil belongs to the family drug called antimetabolites. It is used to treat colorectal cancer and it transformed in the cell into different cytotoxic metabolites which are then incorporated into DNA and RNA, finally inducing cell cycle arrest and apoptosis by inhibiting the cell ability to synthesize DNA. Side effects include dermatitis, diarrhea, cardiac toxicity and mucositis.[94]

2.17.3. CARBOPLATIN

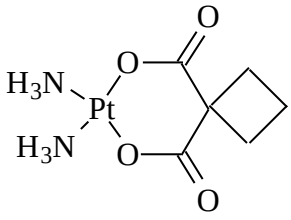


Figure 22: Carboplatin

Carboplatin belongs to the family of drugs called the DNA alkylating agents and it is used, *inter alia,* for the treatment of ovarian, lung, head and neck cancer. It differs from cisplatin in that it has a closed cyclobutane dicarboxylate moiety in contrast to the readily leaving chloro groups found in cisplatin.[94,95]

2.17.4. METHOTREXATE

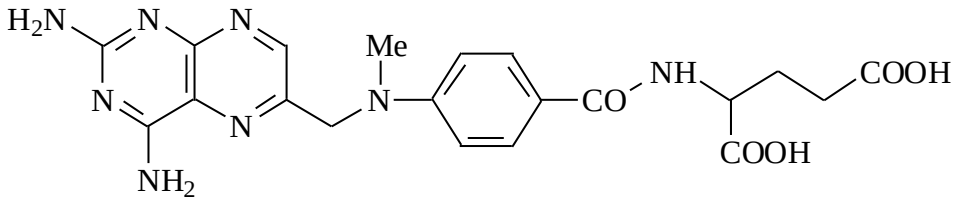


Figure 23: Methotrexate

Methotrexate an alkylating agent and a deoxyfolic acid derivative, has found clinical application for many years as a highly potent anticancer agent. It competitively and reversibly inhibits the dihydrofolate reductase, an enzyme that participates in tetrahydrofolate synthesis. Methotrexate acts specifically during DNA and RNA synthesis. Some of the side effects of methotrexate are toxicity to the rapidly dividing cells of bone marrow and gastrointestinal mucosa,[96-99]reduction of the number of red blood cells, reduction of the production of platelets that help blood to clot, leading to easy bleeding.[100]

2.17.5 FERROCENE

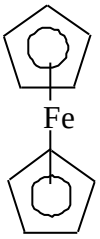


Figure 24: Ferrocene

It is a prototypical metallocene consisting of two cyclopentadienyl rings bound on the opposite side of a central metal atom. Some ferrocenium salts exhibit anticancer activity, and the idea is that it binds with the estrogen sites resulting in cytotoxicity effects.[101]

2.17.6 DOXORUBICIN

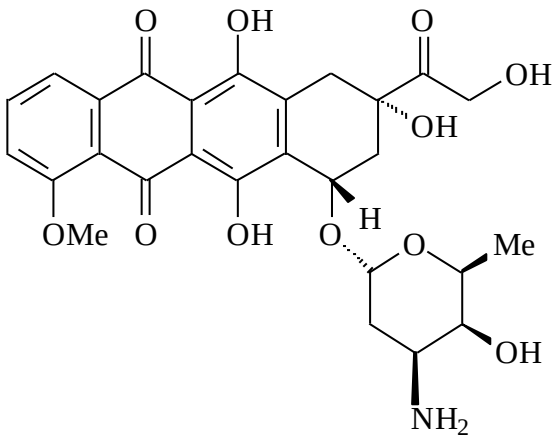


Figure 25: Doxorubicin

It is an antracycline antibiotic which is used in the treatment of various kinds of cancer, such as stomach, lung, breast, bladder, ovaries, thyroid and others.[102] It is thought to react with DNA by intercalation and inhibition of macromolecular biosynthesis.[103] Some of the side effect includes decrease in white blood cells, congestive heart failure, hair loss.

2.18 CAUSES OF RESISTANCE TO ANTICANCER DRUGS

Commonly, patients initially show good response to cancer treatment, only to succumb eventually to the disease. Cancer cells can develop resistance not only to drugs administered, but also to many other drugs similar in structure but acting at different intracellular targets, and this phenomenon is known as multiple drug resistance. Resistance to anticancer drugs can be either intrinsic i.e. present in cancer cell before treatment or acquired after treatment. There are several reasons why anticancer drugs can be prevented from accomplishing their assigned task of killing cancer cells, such as inadequate drug delivery to the cancer cells, or lack of maintenance of adequate drug concentration in the cells; i.e. cancer cells often show alterations in their intracellular compartments resulting in changes in locations and increase in size, and these changes result in capture of anticancer cells, and the drugs do not get to the nucleus but are excreted by the cells. Two gene codes responsible for the formation of proteins that are

involved in the transport of drugs out of the cells (multiple drug resistance codes for P-glycoprotein Pgp, and multiple drug resistance associated protein gene codes for multiple resistance-associated proteins MRP) often perform roles in normal tissue, but become overexpressed in cancer cells. There is also evidence that the simultaneous activity of the two proteins is the decisive factor in the resistance of tumor cells to anticancer drugs.[104] At Technion-Isreal Institute of Technology researchers under Professor Yehuda Assaraf discovered that during chemotherapy, a mutation takes place in the protein ABCG2, and that this mutation gives ABCG2 the ability to transport a wide range of anticancer drugs from the antifolate family out of the malignant cell. As a result, these malignant cells escape the effects of the drugs and become more resistant to anticancer drugs, leading to the rapid spread of the disease. In a second paper by Professor Assaraf, they discovered that cancer cells create "waste baskets" into which anticancer drugs are deposited.[105] Another study by a research group at the University of California, Berkeley, discovered that anticancer drugs are highly toxic when delivered straight, but wrapping them inside a larger molecule can lessen the side effects such as drug resistance and make them more effective.[106]

2.19 PHARMACOLOGICAL DEFICIENCIES IN CURRENTLY USED ANTI-CANCER DRUGS.

The main goal of anti-cancer drugs is to destroy cancer cells but as they kill out fast-growing cancer cells, they also destroy fast growing normal cells. These are some of the pharmacological deficiencies that contribute to the limitations of effectiveness of the currently used anti-cancer drugs: (i) Lack of cell specificity, with ensuing drug distribution into both normal cells and transformed cells, and drug application becomes wasteful; (ii) Inadequate water solubility, hampering swift and efficacious drug distribution in the body's aqueous fluid system, and these results in enhanced exposure to macrophage activity; (iii) Decreased serum half-life as a consequences of catabolism, protein binding by the reticuloendothelial system, or efficacious excretion mechanisms; (iv) Monophasic salt-like or charged structure, inhibiting membrane penetration and cell entry through normal passive diffusion, and as a consequence, only a small fraction of

medicinal agents will successfully enter intracellular space for interaction with nuclear DNA or proteinaceous constituents; (v) Excessive systematic toxicity which grossly diminishes therapeutic drug effectiveness, and often times results in the premature termination of therapy; (vi) Severe nausea and vomiting; (vii) Intensive damage to the lining of the intestine that leads to loss of appetite; (viii) Lack of long term effectiveness because of induced drug resistance with consequent need for premature discontinuation of drug-specific therapy.[107]

2.20 POLYMER-DRUG CONJUGATION CONCEPT

The concept of polymer-anticancer conjugates was first proposed in 1975 by Ringsdorf[108] and the biological rationale for their design was discussed, *inter alia*, by Kopecek and Duncan.[109-110] The macromolecular carrier comprises of a linear polymer chain composed of subunits bearing water-solubilizing groups, functional groups for reversible drug anchoring, and a homing device that directs the conjugate molecule selectively to the target tissue. The conjugate represents a prodrug from which active agents are released into the predestined biological environment. The clinical administration of the polymer bound drug as compared to the free agent, may enhance therapeutic effectiveness in terms of the following:[111]

(a) They facilitation of endocytotic cell entrance, thus circumventing potential problems caused by drug polarity.

(b) Polymer-drug connective linker provides delayed and controlled drug release, thereby ensuring restriction of drug serum concentration.

(c) Polymer conjugates tend to accumulate in solid tumors because of enhanced intratumoral vascular permeability, allowing for substantial leakage of the polymeric molecules into the tumor tissue.

(d) Accelerated and unencumbered distribution in the aqueous central circulation system of the body (the blood), thereby reducing the risks of premature degradation and excretion.

(e) Polymer-bound drugs experience temporary protection from enzymatic attack, serum protein binding and other depletion processes while in transit in the central

circulation. This leads to reduced renal clearance and prolonged serum life time with substantially enhanced drug bioavailability.

The structural features of polymer-drug conjugates required to comply with these attributes include the following:

(i) A highly flexible linear chain comprising structural entities that can produce water solubility.

(ii) A large molecular mass to prevent quick excretion from the body.

(iii) A biodegradable carrier backbone prone to catabolic elimination of the spent polymer main chain after the payload of drug has been delivered to the target tissue.

(iv) Reactive functional groups as suitable binding sites for drug attachment; these sites should be distanced from the main chain by 5-15 constituent atoms to reduce steric bulk effect of polymeric carrier backbone.

(v) One or more biofissionable functions (amide, ester etc.) to be inserted into the carrier-drug connecting link. These must be sufficiently remote from the main chain to permit enzyme approach and cleavage action which results in effective drug release into the target environment.

(vi) The carrier backbone should be non-toxic and possess minimal immunogenicity so as to preclude carrier-induced pathological effects.

(vii) The carrier must contain moieties that enhance drug targeting capability, i.e. a tumor homing device, antispasmodic properties and other desirable physiological effects.

2.21 MACROMOLECULAR CARRIER SYSTEMS

The design of a drug carrier represents the most important task in polymer-drug conjugation, and the therapeutic activity of the conjugate depends on the macromolecular carrier system's physical and chemical properties including solubility. Steric accessibility and reactivity of the anchoring sites contribute to the carrier's functional efficacy. Typical carrier type include:[88]

(i) α,β-DL polyaspartamides

(ii) Polyamidoamines

(iii) 2-Hydroxypropyl-metacrylate polymers (HPMA)

2.21.1 α,β-DL POLYASPARTAMIDES

These are synthesized by high-temperature condensation polymerization of DL-aspartic acid in orthophosphoric acid medium leading to a polysuccinimide intermediate (Scheme 1). The intermediary polysuccinimide is treated sequentially with two amine nucleophiles and occasionally a third amine, whereby the imide ring opening gives rise to polyaspartamides (figure 26).

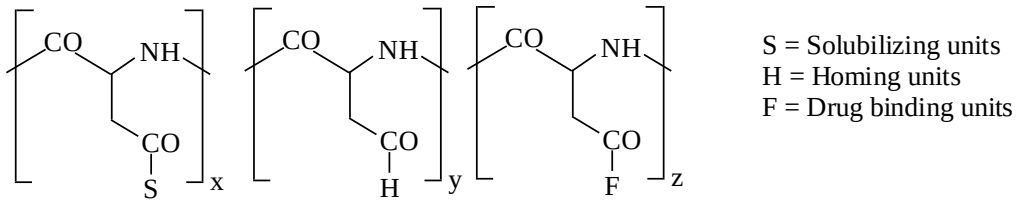
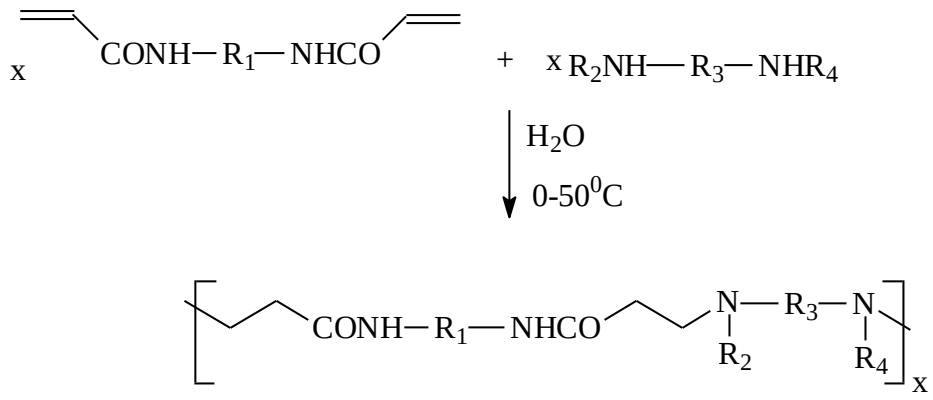


FIGURE 26: Copolyaspartamides equipped with solubilizing, target-directing and drug-binding moities

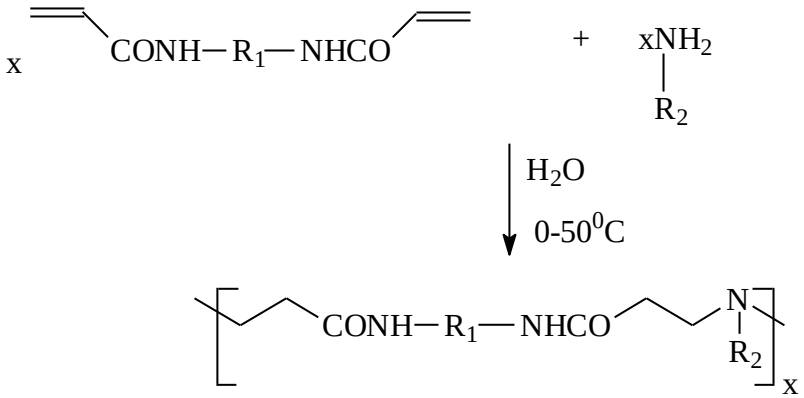
The macromolecular carrier is non-toxic due to the use of aspartic acid as a molecular backbone source and it is hydrolytically biodegradable at the CO-NH links for catabolic fragment elimination through the globular system of the kidneys upon drug release.[88] The extrachain S unit makes the carrier water-soluble, and it typically contains *tert*-amine or hydroxyl group. These functionalities are prone to aquation with the aqueous solvent or protonation with generation of cationic sites. The steric accessibility of the anchoring site F is optimized by insertion of a suitably long aliphatic spacer link between F and the backbone, thus reducing any steric hindrance. The H unit is the homing group which is represented by cationic moieties, tracking the conjugate preferentially to neoplastic tissue as a consequence of electrostatic attraction to the negative surface displayed by many cancer cells.

2.21.2 POLYAMIDOAMINES

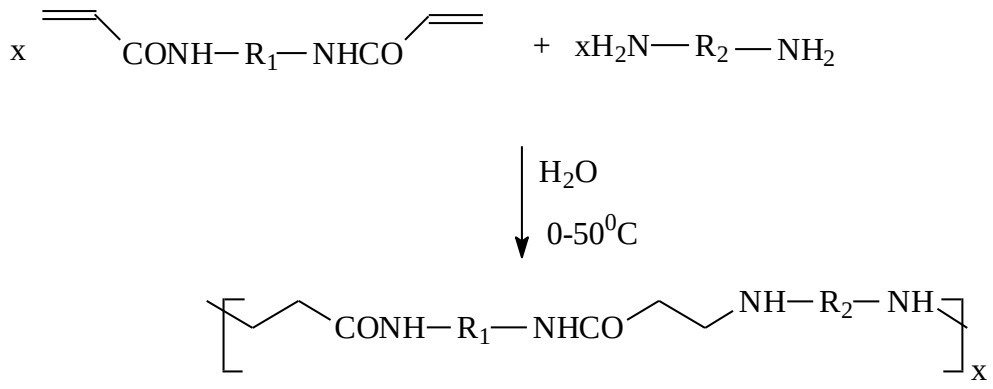
This carrier is prepared by aqueous-phase Micheal addition polymerization of a bis(acrylamido) compound, e.g. methylenebisacrylamide or bisacryloylpiperazine, with comonomers that either contain two secondary amino groups for single substitution on each N group (Scheme 4) or a primary amine (Scheme 5), giving rise to polymer structures comprising of both amide and amine functionalities. Comonomers featuring two primary amino groups are allowed to enter polymerization by single step Michael addition at each $–NH_2$ by modified, carefully controlled conditions to produce polyamidoamines possessing secondary amino groups in the main chain which are for drug binding (Scheme 6).[88]



SCHEME 4



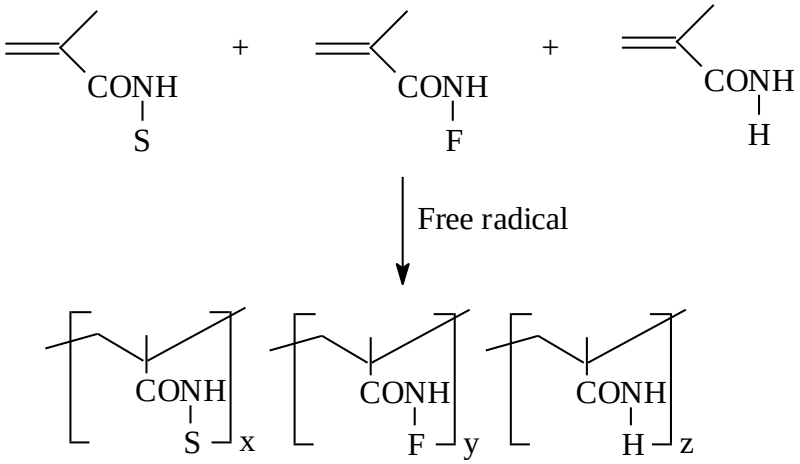
SCHEME 5



SCHEME 6

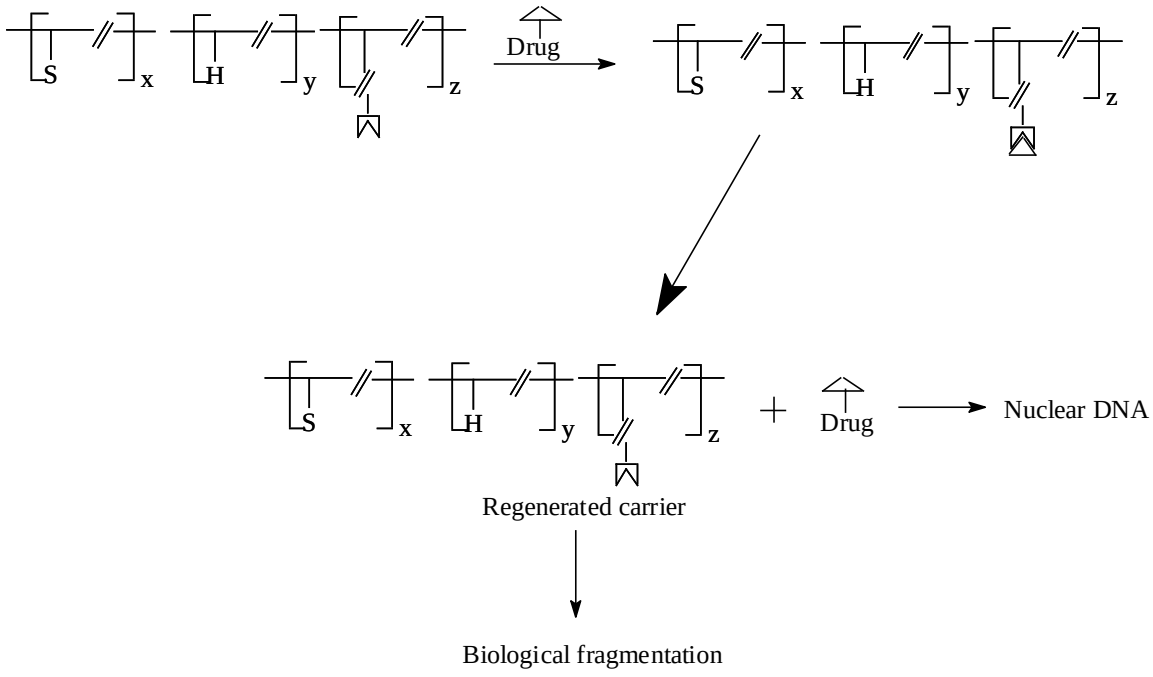
2.21.3. *N*-(2-HYDROXYPROPYL)METHACRYLAMIDES (HPMA)

The carrier is prepared by free-radical copolymerization of two or more methacrylamide monomers bearing water-solubilizing (S), drug anchoring (F) and homing (H) moieties (Scheme 7).



SCHEME 7

2.22 PHARMACOKINETIC PATHWAY OF POLYMER-DRUG CONJUGATES



S - Solubilizing group
H - Homing group
// - bifissionable link
- drug binding functionality
- complementary funtionality on drug
- biocleavable drug binding group

Scheme 8: The pharamacokinetic *in vivo* pathway of polymer-drug conjugate[88]

The polymer acts as a carrier and a transport vehicle that transports the drug, and the homing group takes the polymer-drug conjugate to the target tissue where the drug upon cell entry is released by enzymatic or hydrolytic action. The polymer-drug linker is designed so as to remain intact while the conjugate is in transit and on its way to the target in the ever so slightly basic (pH~7.5) environment. The drug release is mediated by the lysosomal proteolytic enzymes, taking advantage of the acidic (pH~5) intralysosomal

environment. The regenerated carrier, after the release of the drug, then undergoes a biological fragmentation.

2.23 POLYMER CONJUGATION OF ANTICANCER DRUGS

Anticancer drugs are often highly toxic when delivered straight, but incorporating them into larger molecules can lessen the side effects as well as make them more effective. In recent research, the powerful doxorubicin, enveloped in a large polymer, produced a 100 percent cure in mice with induced colon cancer, while all mice treated with the drug proper died.[107] The doxorubicin-polymer combination proved as effective as a liposomal therapy. According to Frechet, branched polymers are better than linear polymers because branched polymers do not readily pass through filtration pores in the kidney.[106] It has been found that it is possible to attach a cocktail of drugs to a single carrier so that the drugs can be delivered together and this is the approach employed in our laboratory.

2.24 NOVEL APPROACH OF POLYMER-DRUG CONJUGATION OF ANTI-CANCER DRUGS

Breast and prostrate cancers are they most common malignancies in Europe, and treatment of these cancers is often dependent on surgery, followed by a combination hormonal therapy and chemotherapy. Here, the use of water-soluble polymers as drug carriers offers the possibility of designing a combination therapy comprising of hormone and chemotherapy along a polymer chain.[112] To prepare the first conjugates to test this concept, P. Vasey and co-workers choose HPMA copolymer as a carrier due to its proven clinical safety, and doxorubicin (DOX) a cytotoxic agent currently a first-line treatment for breast cancer and aminoglutethimide (AGM) a nonsteriodal first generation aromatase inhibitor were used as model drugs (Figure 27 and 28).[113] The conjugates displayed reduced haemolytic activity in comparison with a free drug and also showed evidence of cytotoxicity *in vitro*.

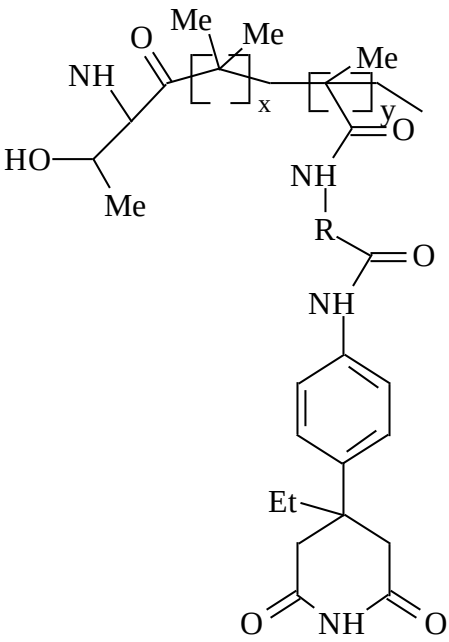


Figure 27: HPMA-R-AGM (R:Gly-Gly peptidyl linkers) (Polymer-AGM conjugates)

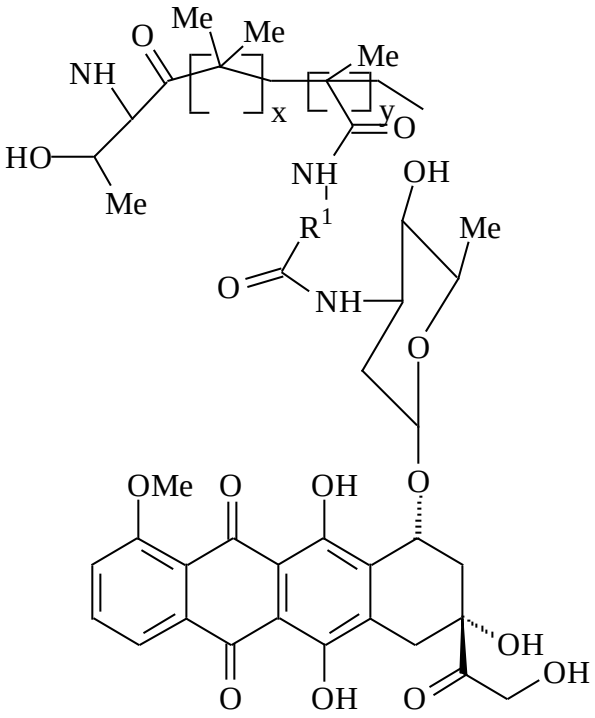
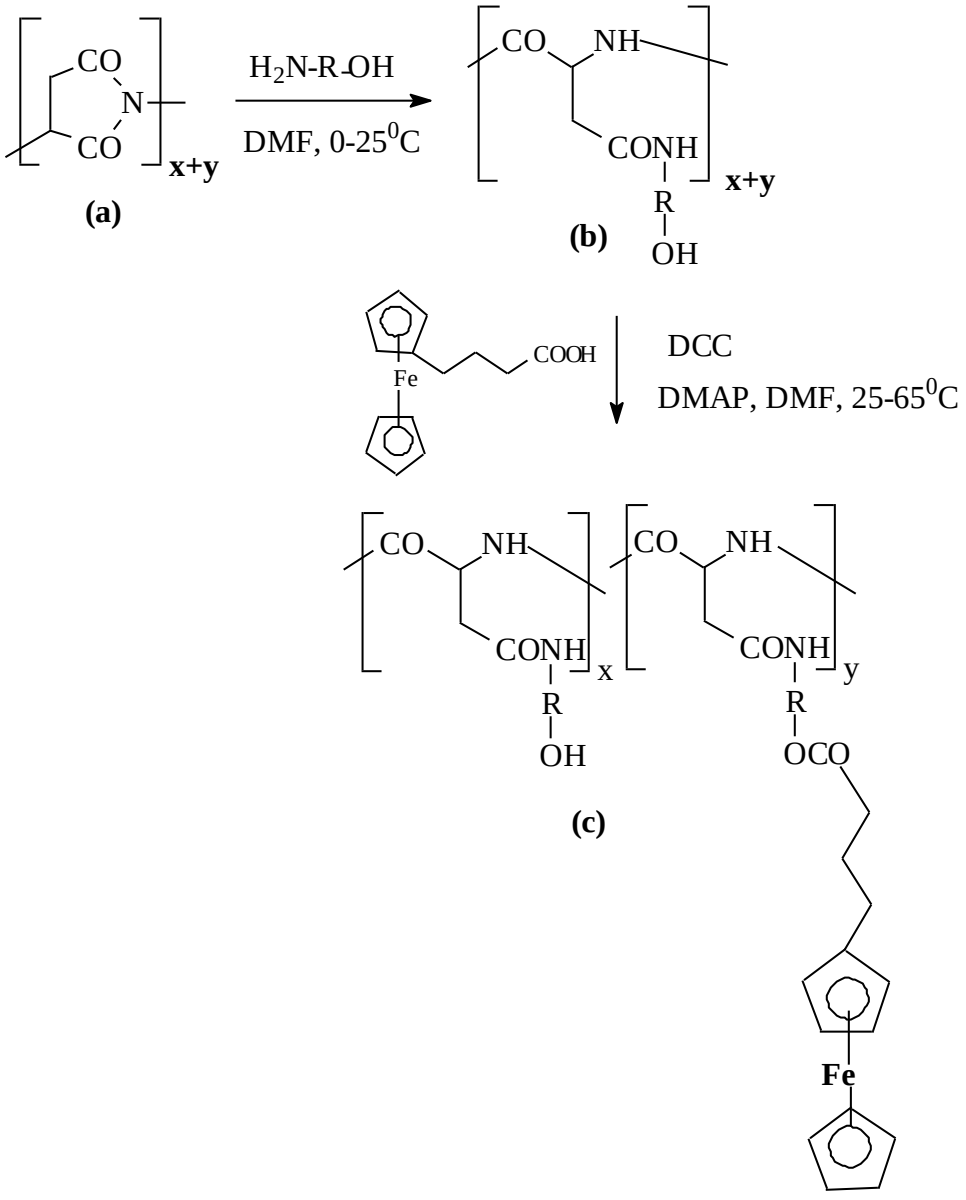
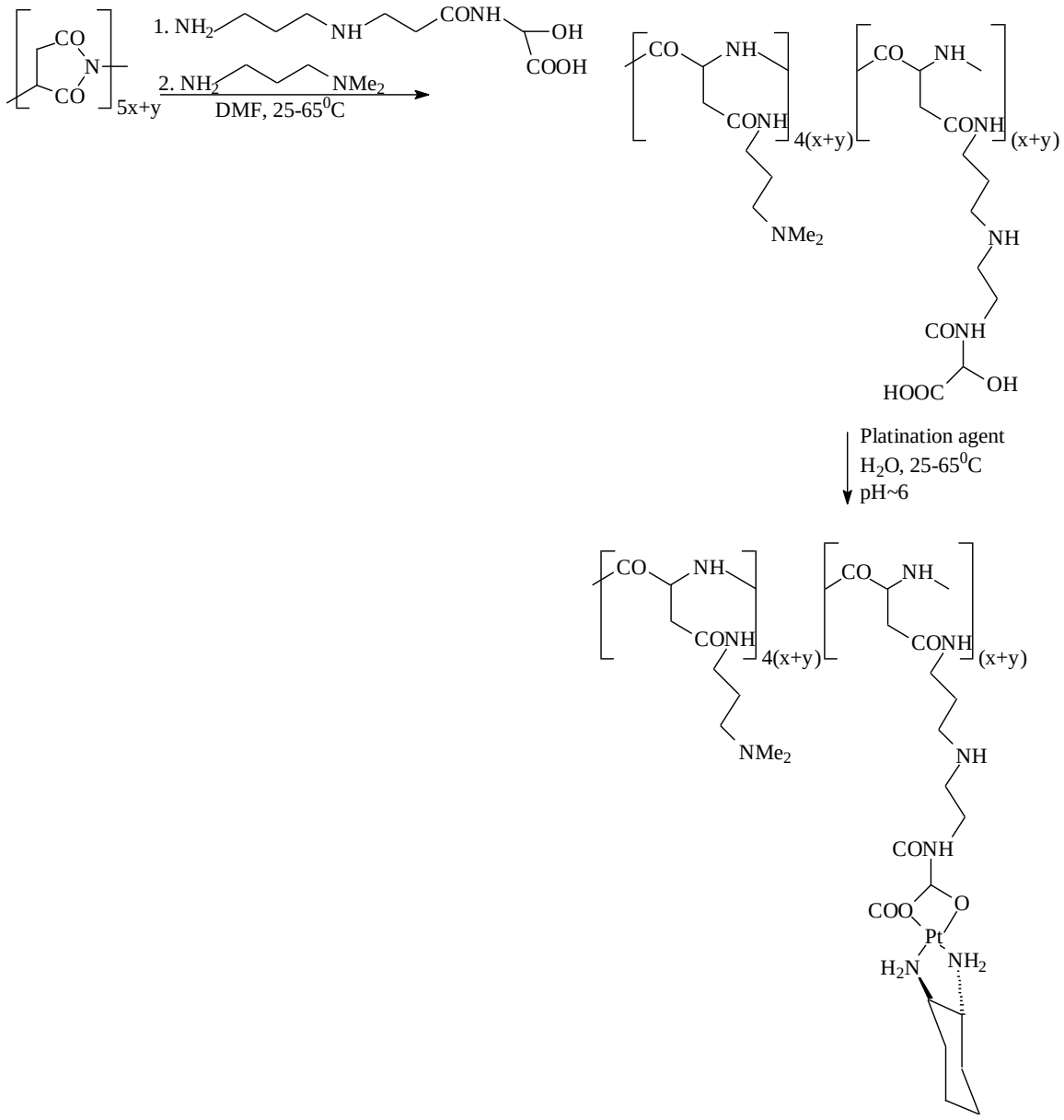


Figure 28: HPMA-R^1 –DOX (R^1 :Gly-Phe-Leu-Gly peptidyl linkers) (Polymer-DOX conjugate)

Another example of polymer-anchored antitumor drugs, a polymer-ferrocene conjugate was synthesized in our laboratory (scheme 9). The conjugate was prepared by ring opening of polysuccinimide intermediate (a) in DMF with amine giving rise to a polyaspartamide (b) which was then coupled with ferrocene in the presence of a coupling agent to afford the conjugate (c). The conjugate (c) was subjected to toxicological test *in vivo*, and it was 20-fold less toxic than the standard non-polymeric ferrocene.



Scheme 9: Ferrocenylation of polyaspartamide



Scheme 10: Synthesis of conjugates of 1,2-carboxylatohydroxylato coordination of platinum.

Conjugate containing platinum drug is another example of polymer-anchored antitumor drug that was synthesized in our laboratory in scheme 10. The synthesis method used in scheme 10 included ring opening reaction performed on poly-D-L-succinimide in addition to low and high temperature solution polycondensation, Michael-type addition and interfacial polymerization. The conjugate was subjected to *in vitro* testing and found to be less toxic than the free platinum drug

2.25 BIO-EVALUATION IN CELL CULTURE TESTS: PRELIMINARY RESULTS

The four conjugates A(80)Pt (figure 29), B(90)Pt (figure 30), C(80)Pt (figure 31, D(90)Pt (figure 32), prepared in the polymer research laboratory, were submitted for biomedical evaluation and toxicological tests at the Department of Pharmacology, University of Pretoria. They were bio-evaluated *in vitro* against the Hela (human adenocarcinoma of the cervix), Colo 320 DM (human colon cancer) and MCF 7 (human breast cancer) cancer cell lines. The Hela line is sensitive to most anti-cancer drugs whereas the Colo 320 DM is considered to be multidrug resistant.

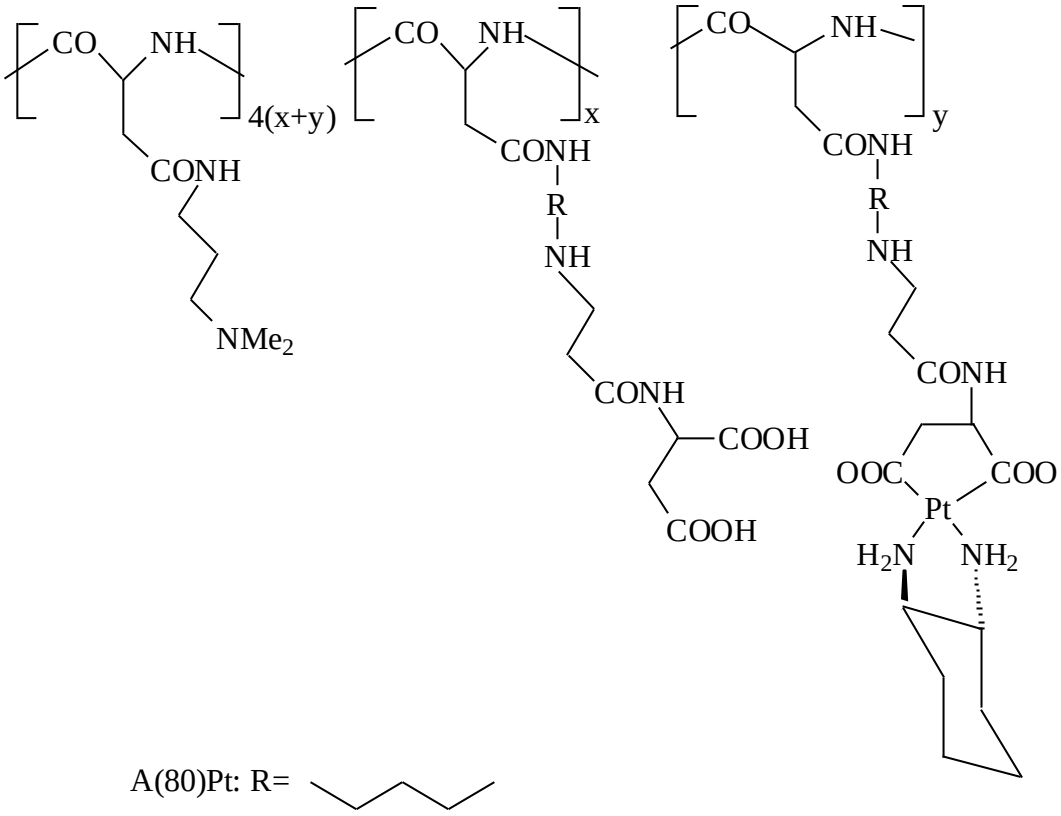


FIGURE 29: Polymer-platinum conjugate (A(80)Pt)

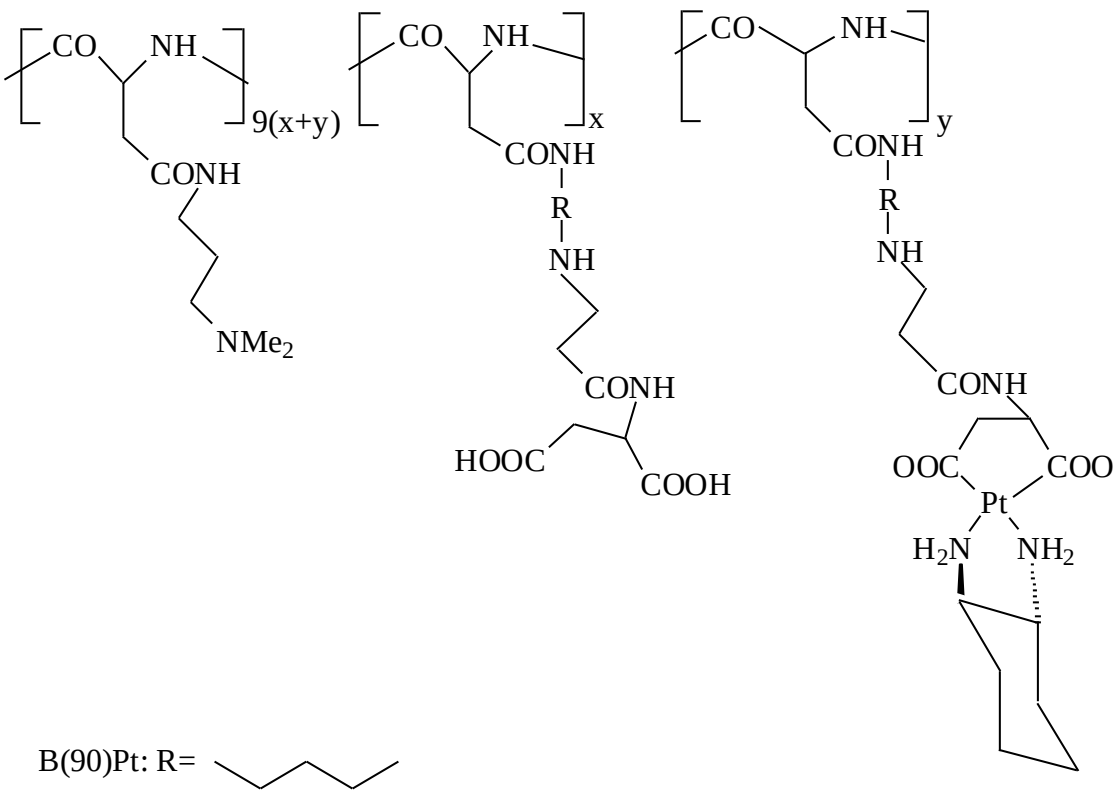
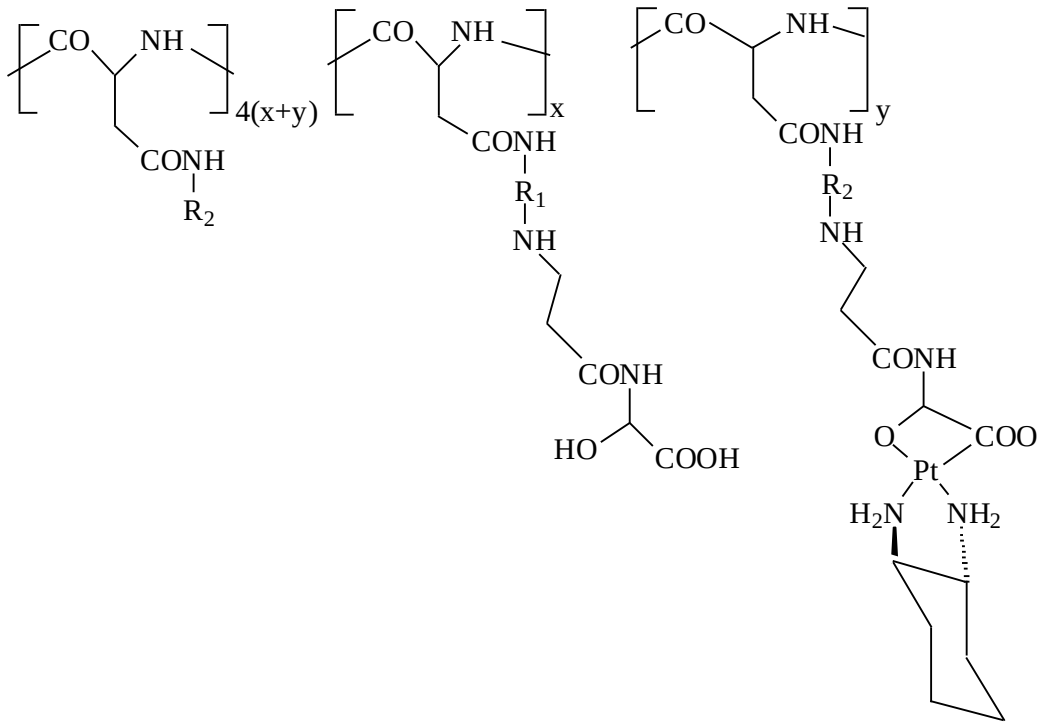
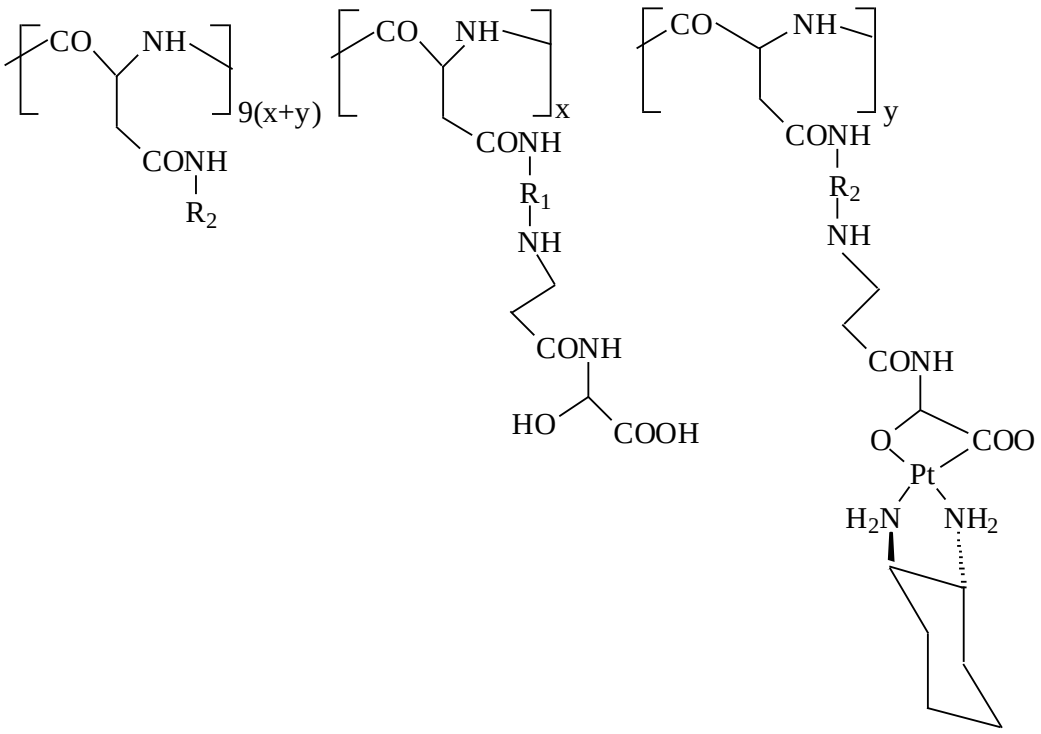


FIGURE 30: Polymer-platinum conjugate (B(90)Pt)



C(80)Pt: R_1= R_2= NMe_2

FIGURE 31: Polymer-platinum conjugate (C(80)Pt)



D(90)Pt: R_1= R_2= NMe_2

FIGURE 32: Polymer-platinum conjugate (D(90)Pt)

Below is the table of the results in terms of IC_{50} , the drug concentration to achieve the 50% cell killing relative to drug-free control. The comparative data on free cisplatin are also included in the table as non-polymeric standard.

TABLE1: ANTIPROLIFERATIVE ACTIVITY OF PLATINUM CONJUGATES (FIGHRE 28, 29, 30 AND 31)

	Designation	%Pt[b]	$IC_{50,}$ (μg Pt/mL)[a]		
			Hela	Colo 320	MCF 7
Conjugate	(i) A(80)Pt (FIGURE 29)	5.74	0.000044	0.02	0.006
	(ii) B(90)Pt (FIGURE 30)		0.0097	2.149	3.959
	(iii) C(80)Pt (FIGURE 31)	6.19	0.000173	0.037	0.007
	(iv)D(90)Pt (FIGURE 32)		0.00022	2.557	8.976
Free Drug	Cisplatin		0.029667	0.172	0.159

[a]IC_{50} defined here as drug concentration, in μg Pt/mL, required to achieve 50% cell killing relative to drug-free control.

[b]Platinum content, by mass found.[114]

A(80)Pt (figure 29) and C(80)Pt (figure 30) were found give the best results in tests against Hela, Colo 320, MCF 7. They were found to be more active when compared to free cisplatin, and it is obvious from the results that polymeric dicarboxylato-chelated platinum and carboxylatohydroxylato-chelated platinum (*i.e.* polymeric anchoring of drugs) are more active than free cisplatin.

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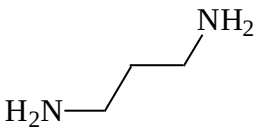
CHAPTER 3: RESULTS AND DISCUSSION

Some antimalarial drugs have been found to be anticarcinogenic and they have the ability to inhibit tumor development. It inhibits the development of drug resistance in cancer cell when used in combination with anticancer drugs. We took advantage of these findings by preparing various macromolecular co-conjugates containing a modified aminoquinoline agent and an anticancer agent on a single polymeric carrier. The aminoquinoline compounds are expected to serve as a potentiating and resistance reducing agent, and they were prepared by a one-step amination reaction between 4,7-dichloroquinoline and selected amines.

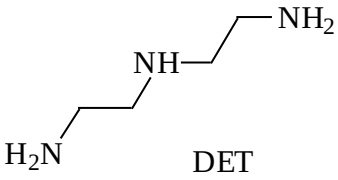
3.1 AMINATION REACTIONS OF 4,7-DICHLOROQUINOLINE

Research has proved that shortening or lengthening the side chain of chloroquine increases the stability and efficacy against the chloroquine resistant strain of *P. Falciparum*.[1] The amination reaction of 4,7-dichloroquinoline was a one-step reaction with selected amine to afford 4-aminoquinolines for polymer anchoring. The amines used were selected because they have a terminal amino group that is useful for polymer anchoring, and the 4-aminoquinolines prepared are close analogs of chloroquine but only differed from chloroquine in the nature of the side chain attached to the 4-aminoquinoline moiety. The selected amines used in this research are shown below (Figure 33):



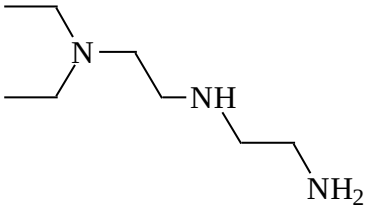
PDA

(1,3-DIAMINOPROPANE)



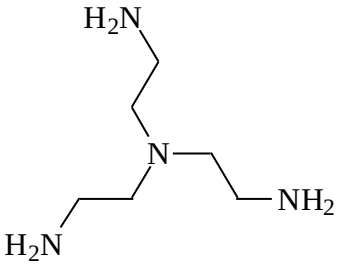
DET

(DIETHYLENETRIAMINE)



NN

(*N*,*N*-DIETHYLDIETHYLENETRIAMINE)



TRIS

(TRIS(2-AMINOETHYL)AMINE)

Figure 33

The number of carbon atoms between the two nitrogen atoms in the diaminoalkane side chain is a major determinant of activity against the drug resistant *P. Falciparum.* The mechanism of action of chloroquine has been proposed in which the drug (i) enters the food vacuole by diffusion of the free base across intervening membranes; (ii) accumulates in the food vacuole, at least in part due to pH trapping of the protonated drug at the low pH of the vacuole; (iii) forms a complex which may further enhance drug accumulation; (iv) Inhibits formation of hemozoin through the formation of the complex; and (v) exerts a toxic effect on the parasite.[2]

3.1.1 *N*-(7-CHLOROQUINOLIN-4-YL)PROPANE-1,3-DIAMINE (PDA.Q)

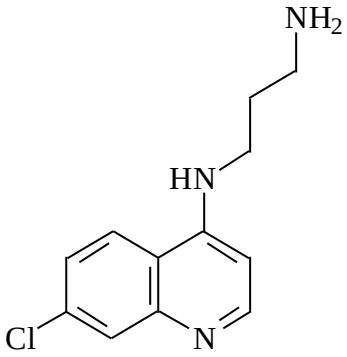


Figure 34: *N*-(7-chloroquinolin-4-yl)propane-1,3-diamine (PDA.Q)

PDA.Q (Figure 34) was prepared using a modification of De's method[3] in which 4,7-dichloroquinoline was allowed to react with neat 1,3-diaminopropane in a molar ratio of 4.5 to 1.0 of 4,7-dichloroquinoline. There was a formation of a coarse precipitate during the washing step of the work up, and analysis revealed that it was made up of the expected compound. PDA.Q isolated was characterized using ^{1}H NMR spectroscopy, and the signal peaks at 3.42 ppm (t) were for 2H, $\mathbf{CH_2}NHAr$, at 2.80 ppm (t) for 2H, $\mathbf{CH_2}NH_2$ and 1.90 ppm (dt) for 2H, $NH_2CH_2\mathbf{CH_2}CH_2NH$. Aromatic signals were found at 8.35 ppm (d), for 1H, 8.07 ppm (d), for 1H, 7.77 ppm (d), for 1H, 7.38 ppm (dd), for 1H and 6.53 ppm (d) for 1H, and confirmed the proposed structure.[3]

3.1.2 N-(7-CHLORO-4-QUINOLYL)-TRIS(2-AMINOETHYL)AMINE (TRIS.Q)

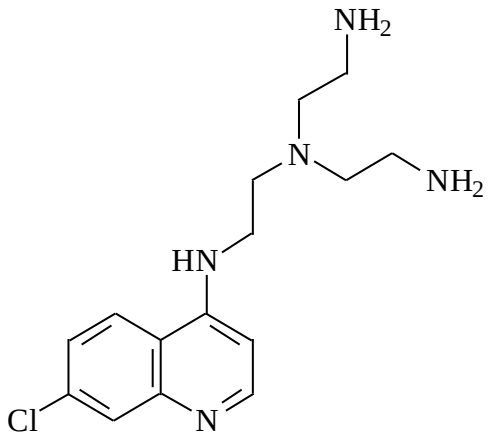


Figure 35: N-(7-chloro-4-quinolyl)-tris(2-aminoethyl)amine **(**TRIS.Q)

TRIS.Q (figure 35) was prepared by a one-step substitution reaction between tris(2-aminoethyl)amine (TRIS) and 4,7-dichloroquinoline using a modified method[4] that involved the use of small amount of dioxane solvent. In initial studies, we encountered a decomposition of the compound, and dioxane was then used to avoid decomposition and thus afford our expected compound. The isolated compound was purified by column chromatography twice using basic alumina, and this could have been responsible for the low yield of 44%. The compound was characterized by ^{1}H NMR spectroscopy, and the signal peaks at 2.74-2.51 ppm (m, 12H, (NH_2**CH_2CH_2**N)$_3$, confirmed the addition of the amine. Aromatic signals were found at 8.23 ppm (d, 1H, Ar-H), 8.01-7.89 ppm (m, 1H, Ar-H), 7.65 ppm (d, 1H, Ar-H), 7.30-7.24 ppm (m, 1H, Ar-H), 6.41 ppm (s, 1H, Ar-H), and confirmed structural assignment of the isolated compound.[4]

3.1.3 *N*-(2-(2-DIETHYLAMINO)ETHYLAMINE)ETHYL)-7-CHLOROQUINOLINE-4-AMINE (NNQ)

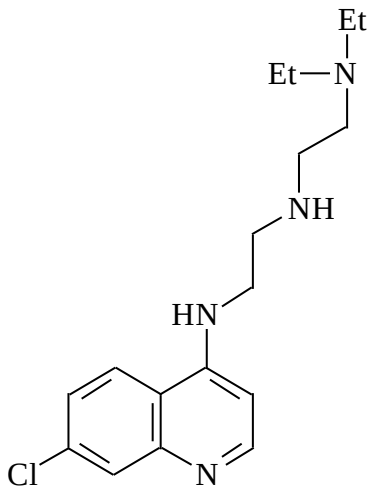


Figure 36: *N*-(2-(2-aminoethylamino)ethyl)-7-chloroquinoline-4-amine (NN.Q)

NN.Q (figure 36) was prepared using a modified method of Keil *et*. *al*[5] by a one-step amination reaction between 4,7-dichloroquinoline and neat *N*,*N*-diethyldiethylenetriamine (NN). The compound was then purified by column chromatography on a basic alumina and characterized by ^{1}H NMR spectroscopy. The signal peaks at 3.65 ppm (bs), 2H for **NH**, 3.35 ppm (dd), 2H for NH**CH_2**CH_2NHAr, 3.02 ppm (t), 2H for **CH_2**NHAr, 2.74 ppm (t), 2H for **CH_2**NH_2, 2.63-2.52 ppm (m), 6H for

$CH_2NCH_2CH_2$ and 1.06-0.90 ppm (m), 6H for $CH_2\mathbf{CH_3}$ was evident. Aromatic signals were seen at 8.47 ppm (d), for 1H, 8.05-7.73 ppm (m), for 2H, 7.72 ppm (dd), for 1H, 6.34 ppm (d), for 1H, and confirmed the proposed structure.[5]

3.1.4 *N*-(2-(2-AMINOETHYLAMINO)ETHYL)-7-CHLOROQUINOLINE-4-AMINE (DET.Q)

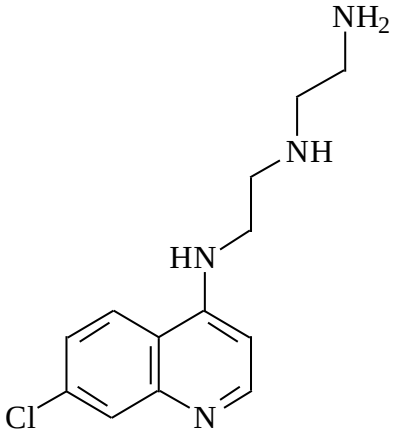


Figure 37: *N*-(2-(2-aminoethylamino)ethyl)-7-chloroquinoline-4-amine (DET.Q)

DET.Q (figure 37) was synthesized by substitution reaction of 4,7-dichlorquinoline with diethylenetriamine (DET) using dioxane solvent to avoid decomposition. The isolated compound was purified by column chromatography on basic alumina and characterized by ^{1}H NMR spectroscopy and signal peaks at 3.70-2.13 (m, 8H) for NH$\mathbf{CH_2CH_2}$NH$\mathbf{CH_2CH_2}$$NH_2$ confirmed the proposed structure.

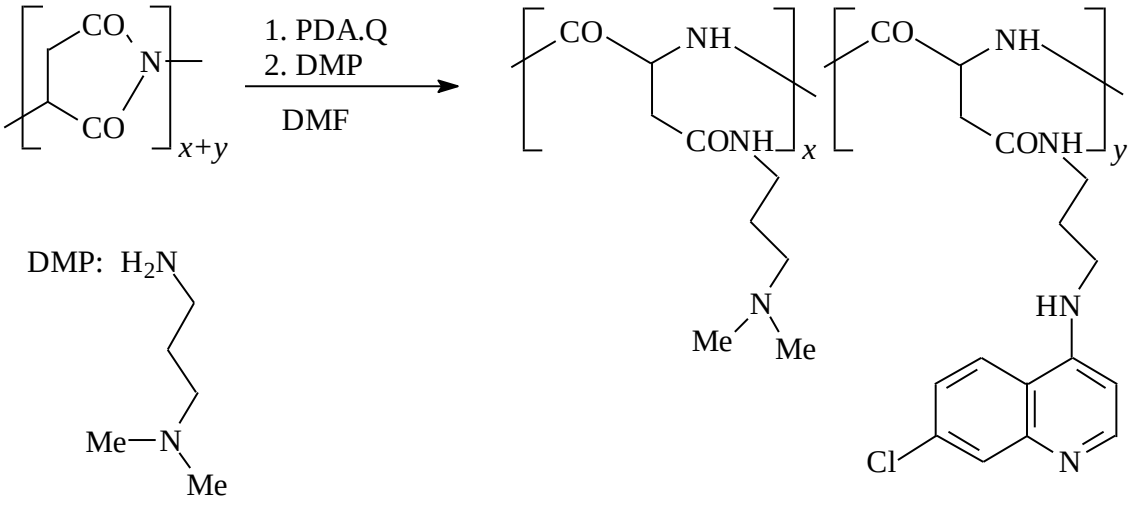
3.2 LIBERATION OF FREE BASE PRIMAQUINE

The primaquine used in his research was liberated as a free base by dissolving 1 mole equivalent of primaquine phosphate salt in water and the addition of 6 mole equivalent of sodium hydroxide. Extraction was then done with dichloromethane, which was dried over anhydrous sodium carbonate and then concentrated under vacuum to afford the free base primaquine as a red viscous liquid. This was then placed under high vacuum to remove any remaining water so as to avoid unwanted hydrolytic ring opening in the substrate polysuccinimide, which can lead to the generation of free carboxylic side groups.

3.3. 4-AMINOQUINOLINE CONJUGATES (PDA.Q)

The macromolecular carrier used in the preparation of 4-aminoquinoline conjugates was α,β-DL-polyaspartamide, a time-proven polymer used extensively for many years in this laboratory. It was prepared from polysuccinimide by an aminolytic ring-opening process in dipolar aprotic medium such as *N,N*-dimethylformamide (DMF). The product is a racemic mixture of α- and β-peptidic repeat units. For convenience, only the α- forms of polyaspartamide will be depicted in further schematic diagrams. The reaction was done under anhydrous conditions to avoid hydrolytic ring opening that can lead to the production of carboxylic side groups and poor % incorporation of the 4-aminoquinoline analogs. The conjugates were all prepared in a ratio of (9:1) (*i.e* ratio of solubilizing unit to drug binding unit (*x/y)*).

3.3.1 PSI. DMP. (90) PDA.Q (10)



SCHEME 11: Synthesis of PSI. DMP (90) PDA.Q (10)

The aminolytic ring opening of the polysuccinimide was done in DMF solvent at room temperature using the 4-aminoquinoline analog (PDA.Q). In the first step, PDA.Q was added before the addition of DMP in a ratio of x/y (9:1). In initial studies, we consistently achieved low incorporation of PDA.Q when DMP was added first before PDA.Q, and this was because DMP is more reactive than PDA.Q. The DMP amine used is a primary as well as a *tert*-amine and it acts as both solubilizing and cell-selecting group. It is prone

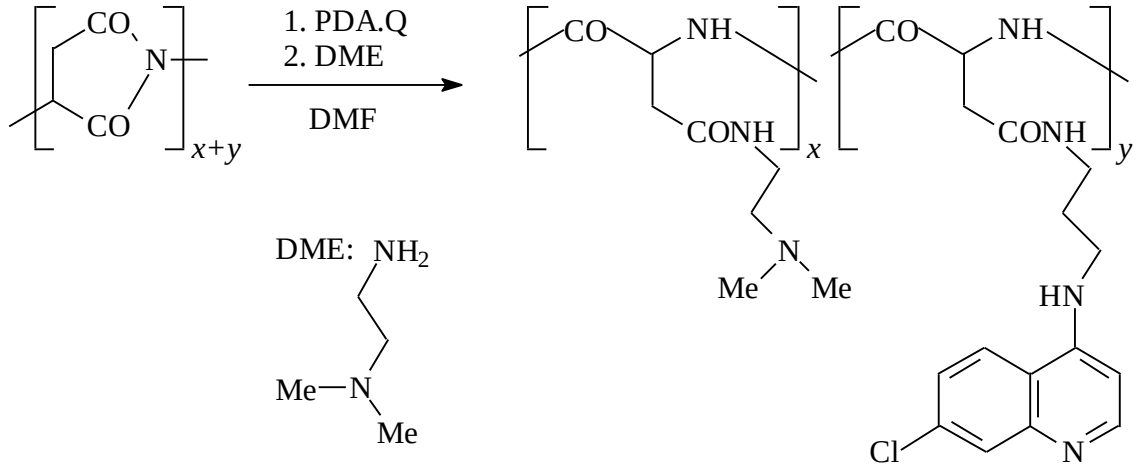
to aquation, *i.e.* hydrogen bond formation with aqueous solvent, thereby making the homopolymer water soluble. This is important as the adsorptive endocytic cell entry of the homopolymer is influenced by its ability to undergo protonation under physiological conditions.

Table 1: ^{1}H NMR result for PSI. DMP (90) PDA.Q (10)

CHEMICAL SHIFT (ppm)	**EXPECTED NUMBER OF PROTONS**	**NUMBER OF PROTONS FOUND**
8.12-6.08	5	4.7
3.10	20	23
2.76	20	23
2.29-2.14	72	75
1.64	20	20

The ^{1}H NMR spectra showed a 100% incorporation of DMP and 94% of PDA.Q (the 4-aminoquinoline analog). The ^{1}H NMR spectra showed the signal peaks for the aromatic protons on PDA.Q at 8.12-6.08 ppm and 4.7 protons were found (table 1). The % incorporation of PDA.Q is the number of found protons divided by the number of expected protons x 100 (4.7/5 x 100 = 94%). The signal for $CONHCH_2\mathbf{CH_2}CH_2NMe_2$ protons were found at 1.64 ppm, therefore, the % incorporation for DMP = 20/20 x 100 = 100%.

3.3.2 PSI. DME (90) PDA.Q



SCHEME 12: Synthesis of PSI. DME (90) PDA.Q

PSI. DME (90) PDA.Q (*x/y*) = 9:1, where *x* is the repeating unit with DME *and y* is the repeating unit with PDA.*Q* was prepared by aminolytic ring opening of polysuccinimide in DMF using DME amine and PDA.Q (4-aminoquinoline analog). In initial studies, the % incorporation of PDA.Q (the 4-aminoquinoline analog), was between 60%-80%. This was because of hydrolytic ring opening that led to the production of carboxylic side groups. The reaction was then performed under anhydrous condition to optimize the % incorporation of PDA.Q. The 4-aminoquinoline was added first, followed by the amine which through its *tert*-amine function acts as a water-solubilizing group. The latter function in DME is closer to the macromolecular backbone, which makes the conjugate less toxic. Research has proved that 4-aminoquinoline analogs with side chain shorter than chloroquine are more effective against the drug resistant *P. Falciparum.*[3,5] The compound was prepared in a ratio of *x*/*y* (9:1).

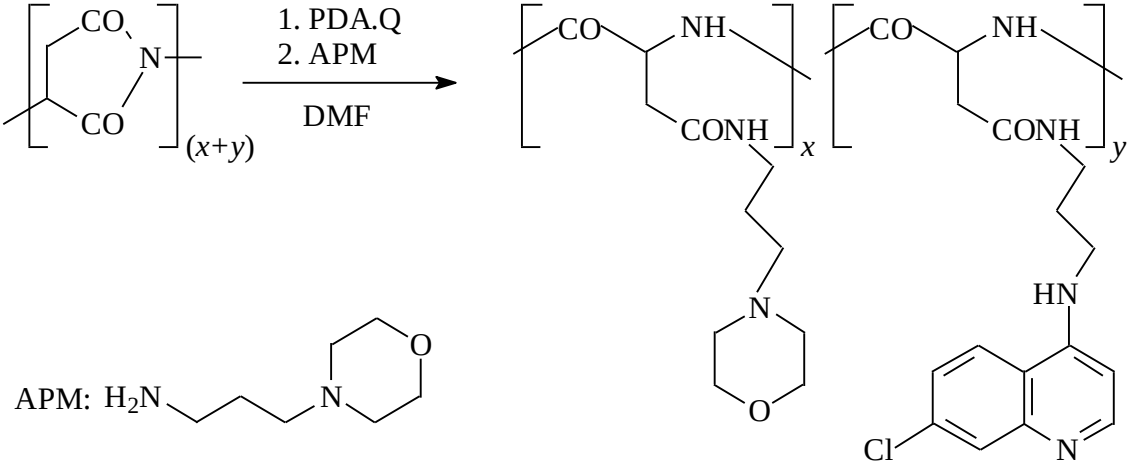
Table 2: ^{1}H NMR result for PSI. DME (90) PDA.Q (10)

CHEMICAL SHIFT (ppm)	EXPECTED NUMBER OF PROTONS	NUMBER OF PROTONS FOUND
8.02-6.04	5	4.8
3.36-3.34	20	24
2.63-2.54	40H	39

2.28	54	51
1.76-1.54	2	2

The ^{1}H NMR spectra proved that there was a 96% incorporation of DME and 96% incorporation of PDA.Q. ^{1}H NMR spectra showed signals for the PDA.Q aromatic protons at 8.02-6.04 ppm for 4.8 protons but the expected number of protons were 5 protons. The % incorporation of PDA.Q, the 4-aminoquinolines compound was found to be 4.8/5 x 100% = 96% (table 2).

3.3.3 PSI. APM (90) PDA.Q (10)



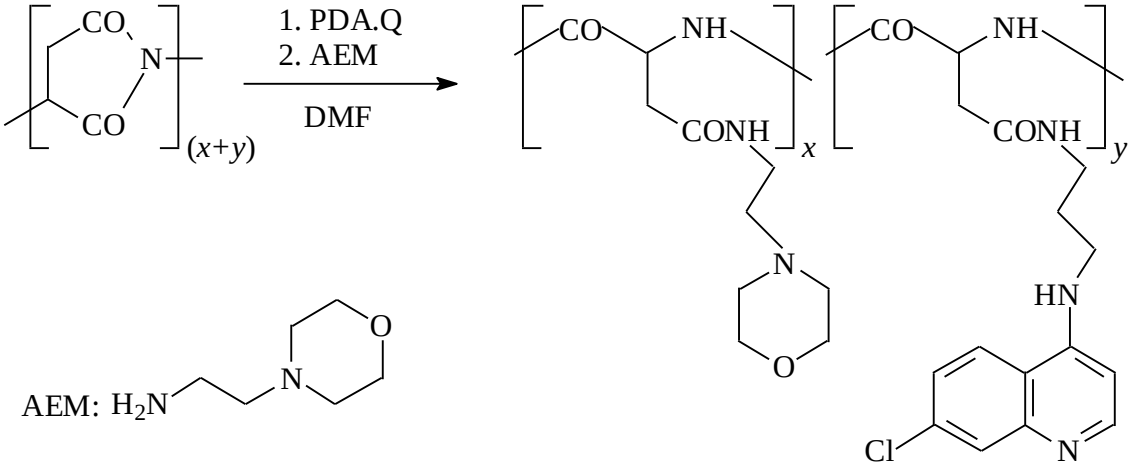
SCHEME 13: Synthesis of PSI. APM (90) PDA.Q (10)

For the synthesis of the third polymer, an aminolytic ring opening of the polysuccinimide, PDA.Q and APM was utilized. The tertiary amine was used as a major reactant (x>y) and it acts as a solubilizing group that makes the macromolecular carrier water soluble. The reaction was performed at room temperature in an (x/y) ratio of (9/1). In both studies, a 100% incorporation of APM was achieved, and the ^{1}H NMR showed a 100% incorporation of PDA.Q and 100% of APM. The ^{1}H NMR signals for the aromatic protons on PDA.Q, the 4-aminoquinoline compound were found at 8.14-6.11 ppm for 5 protons (table 3). The conjugate were prepared in a ratio of x/*y* (9:1), and evaluated as antimalarials.

Table 3: ^{1}H NMR result for PSI. APM (90) PDA.Q (10)

CHEMICAL SHIFT (ppm)	EXPECTED NUMBER OF PROTONS	NUMBER OF PROTONS FOUND
8.14-6.11	5	5
3.72	36	35
3.18-3.17	20	22
2.87-2.60	22	18
2.51-2.49	54	52
1.68-1.67	20	20

3.3.4 PSI. AEM (90) PDA.Q (10)



SCHEME 14: Synthesis of PSI.AEM (90) PDA.Q (10)

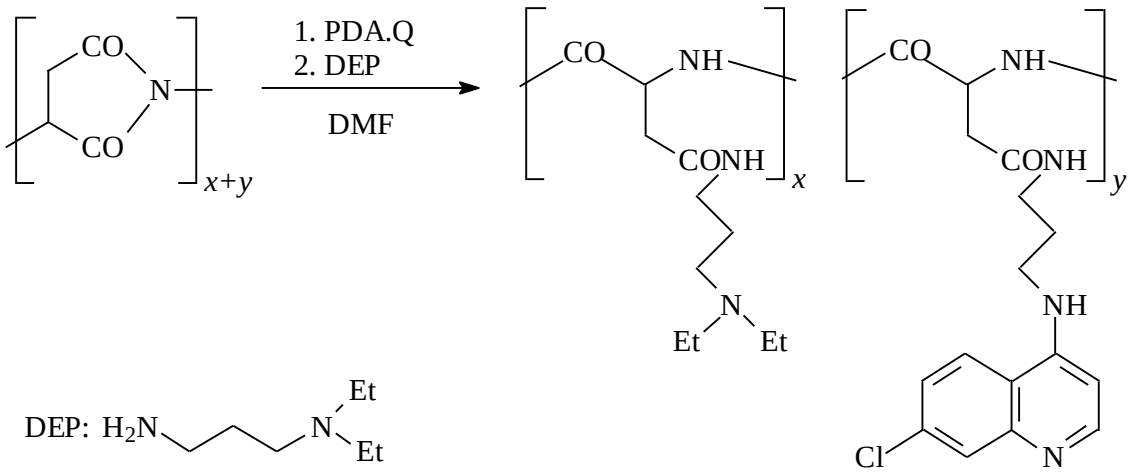
The aminolytic ring opening of the polysuccinimide proceeded by using PDA.Q first, before AEM was added because of the low reactivity of PDA.Q. The reaction was performed at room temperature, and purification was done by dialysis using 12000 molecular mass cut-off membrane for two days at a pH of 7-8. In both studies, the % incorporation of PDA.Q was 96-97%, but in the initial study, 63% incorporation was achieved and reaction was repeated in an anhydrous condition to afford an 83% incorporation of AEM. The ^{1}H NMR spectrum showed that there was 90% incorporation of PDA.Q and 83% incorporation of AEM. The signal peaks for PDA.Q aromatic protons were found at 8.08-6.08 ppm for 4.5 protons instead of 5 protons and the % incorporation

of PDA.Q was found to be 4.5/5 x 100% = 90%. The signal peaks for AEM were found at 3.74 ppm for **CH_2OCH_2** and 30 protons were found instead of 36 protons therefore the % incorporation of AEM was found to be 30/36 x 100% = 83% (table 4).

Table 4: ^{1}H NMR result for PSI.AEM (90) PDA.Q (10)

CHEMICAL SHIFT (ppm)	EXPECTED NUMBER OF PROTONS	NUMBER OF PROTONS FOUND
8.08-6.08	5H	4.5H
3.74	36H	30H
3.36-3.30	20H	19H
2.74	22H	16H
2.50	54H	44H
1.68	2H	2H

3.3.5 PSI.DEP (90) PDA.Q (10)



SCHEME 15: Synthesis of PSI. DEP (90) PDA.Q (10)

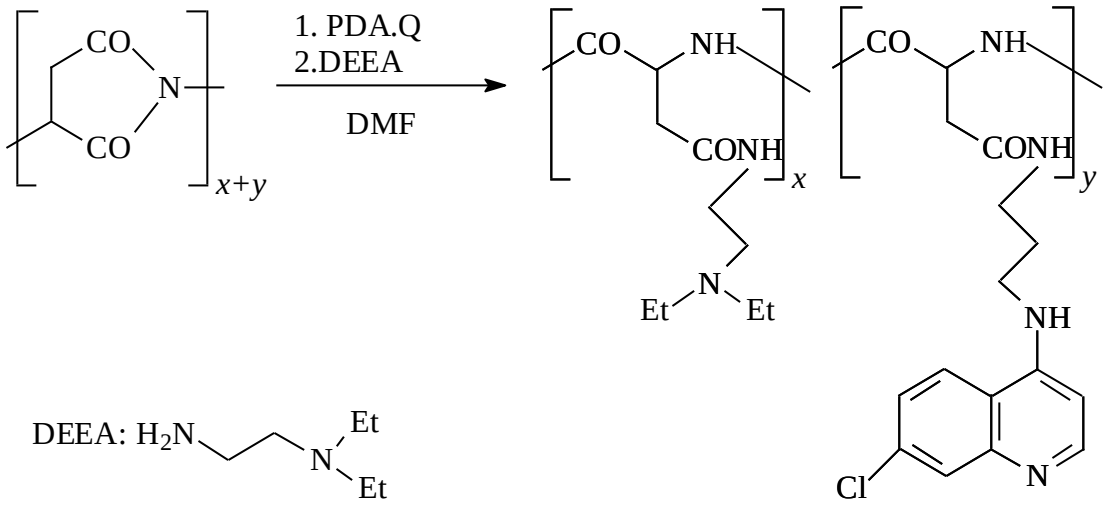
For this polymer PDA.Q was added first, followed by DEP for the aminolytic ring opening. The tert-amine part of DEP used is the water-solubilizing group, and the reaction was performed at room temperature. In both studies, the ^{1}H NMR spectrum showed a 96% incorporation of PDA.Q, and a 100% incorporation of DEP. The conjugates were later evaluated as antimalarials. The signal peaks for PDA.Q, the 4-

aminoquinoline compound were found at 8.25-6.08 ppm for 4.8 protons and the % incorporation of PDA.Q was found to be the number of found protons/number of expected protons = 4.8/5 x 100% = 96%. The signal peak for DEP were found at 0.95 ppm for N(CH_2**CH_3**) and 54 protons were found (table 5).

Table 5: ^{1}H NMR result for PSI.DEP(90) PDA.Q (10)

CHEMICAL SHIFT (ppm)	EXPECTED NUMBER OF PROTONS	FOUND NUMBER OF PROTON
8.25-6.08	5H	4.8H
3.15	20H	18H
2.45-2.43	76H	73H
1.65-1.62	20H	21H
0.95	54H	54H

3.3.6. PSI. DEEA (90) PDA.Q (10)



SCHEME 16: Synthesis of PSI.DEEA (90) PDA.Q (10)

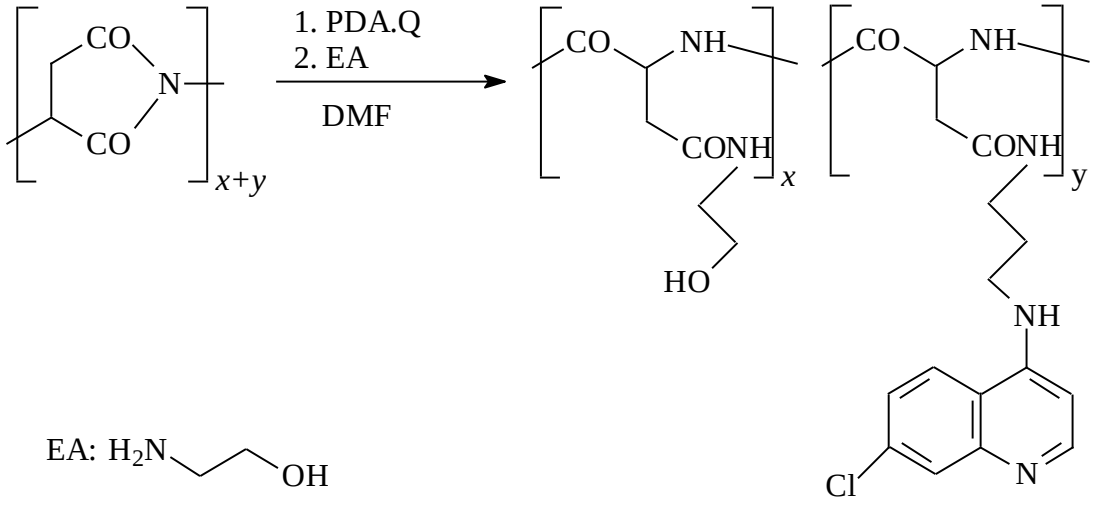
The aminolytic ring opening was performed by the addition of PDA.Q before the addition of DEEA. After the addition of PDA.Q, the reaction was allowed to stir for 20 h at room temperature to afford a high incorporation of PDA.Q. DEEA was added after 20 h of stirring, because DEEA is more reactive than PDA.Q. The ^{1}H NMR spectrum showed a

92% incorporation of PDA.Q, and 92% incorporation of DEEA. The signal peaks for DEEA were found at 0.94 ppm for N(CH_2**CH_3**) 54 protons and at 2.51-1.80 ppm for **CH_2**N(**CH_2**CH_3) 68 protons instead of 78 protons therefore the % incorporation of DEEA was found to be 68 +54/78 +54 x 100% = 92%. The signal peaks for PDA.Q aromatic protons were found at 8.21-6.20 ppm for 4.6 protons instead of 5 proton and the % incorporation of PDA.Q was found to be 4.6/5 x 100% = 92% (table 6).

Table 6: ^{1}H NMR result for PSI.DEEA(90)PDA.Q (10)

CHEMICAL SHIFT (ppm)	EXPECTED NUMBER OF PROTONS	NUMBER OF PROTONS FOUND
8.21-6.20	5H	4.6H
3.28-3.27	20H	21H
2.51-1.80	78H	68H
0.94	54H	54H

3.3.7 PSI.EA (90) PDA.Q (10)



SCHEME 17: Synthesis of PSI.EA (90) PDA.Q (10)

The preparation of PSI.EA (90) PDA.Q (10), was performed at room temperature with the addition of PDA.Q before the solubilizing group (EA). EA, containing a hydroxyl

group, is a water-solubilizing compound that permits the solubility of the polymer in water. The ^{1}H NMR spectrum showed a 70% incorporation of PDA.Q, and a 94% incorporation of EA. The ^{1}H NMR signal peaks for EA protons were found at 3.63 ppm for **CH_2**OH protons and 17 protons were found but 18 protons were expected. The % incorporation of EA was found to be 17/18 x 100 = 94%. The signal peaks for PDA.Q aromatic protons were found at 7.93-6.00 ppm for 3.5 protons instead of 5 protons and % incorporation for PDA.Q, the 4-aminoquinoline compound was found to be 3.5/5 x 100 = 70% (table 7).

Table 7: The ^{1}H NMR result for PSI. EA (90) PDA.Q (10)

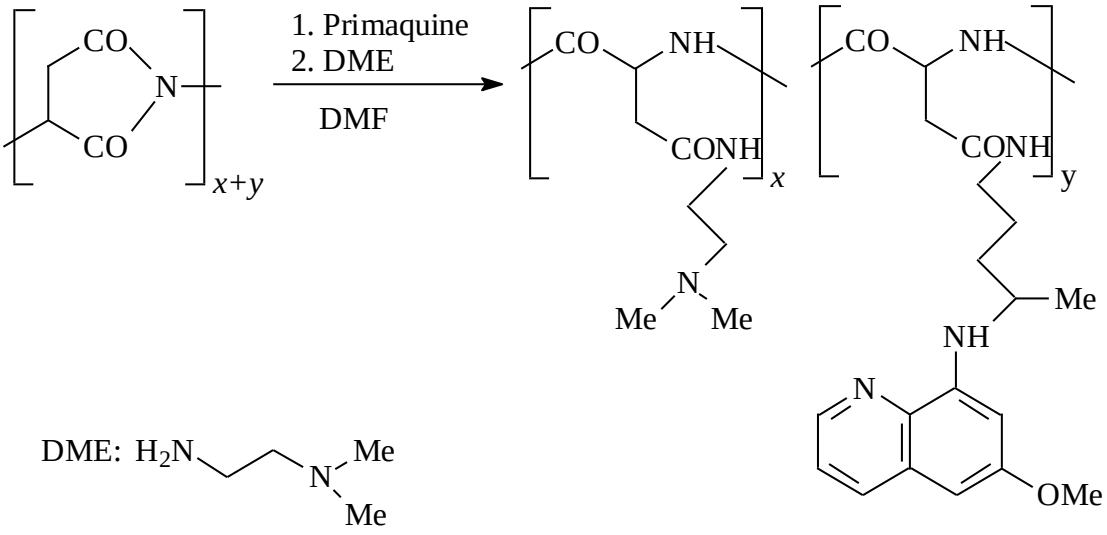
CHEMICAL SHIFT (PPM)	EXPECTED NUMBER OF PROTONS	NUMBER OF PROTONS FOUND
7.93-7.92	1H	0.5H
7.27	2H	1.4H
6.78-6.77	1H	0.6H
6.00	1H	1H
3.63	18H	17H
3.33	20H	18H
2.89-2.71	22H	18H
1.78-1.70	2H	2H

3.4 8-AMINOQUINLINE CONJUGATES

The 8-aminoquinoline conjugate were prepared using primaquine, an 8-aminoquinoline compound, and was liberated as a free base. The macromolecular carrier used was α,β-DL-polyaspartamide, a polymer used extensively for many years in the polymer laboratory, University of Witwatersrand. This polymer has been found to be non-toxic, biodegradable, and it permits co-conjugation of drugs to a single carrier. The conjugates were all prepared under anhydrous conditions to avoid undesired hydrolytic ring opening that leads to generation of free carboxylic side groups, and low incorporation of 8-aminoquinoline analogs. The conjugates were all prepared in a ratio of *x*/*y*, 9:1 (where *x*

and *y* represent the solubilizing unit and drug binding repeating unit respectively), and the solubilizing groups used were thus the major reagent (*x*>y).

3.4.1 PSI.DME (90) PRIMAQUINE (10)



SCHEME 18: Synthesis of PSI. DME (90) PRIMAQUINE (10)

In the synthesis of PSI DME (90) PRIMAQUINE (10) conjugate, DME, a tertiary amine, was used in a ratio of *x*/*y* = 9:1 to the 8-aminoquinoline compound. Primaquine was added first during the aminolytic ring opening to afford high % incorporation, before the addition of the tertiary amine. In various attempts to synthesize the expected compound, a low incorporation of 50-65% of DME was achieved. To optimize the % incorporation of DME, the reaction was then performed under anhydrous conditions to afford an 80-100% incorporation of DME. The signal peaks for the aromatic protons in the primaquine compound were found at 8.54-6.09 ppm for 5 protons and the signal peaks for DME protons ($\mathbf{CH_2CH_2}N(\mathbf{CH_3})_2$) were found at 2.89-2.59 ppm for 91 protons instead of 92 protons. 100% and 92% incorporation of primaquine and DME were found respectively (table 8).

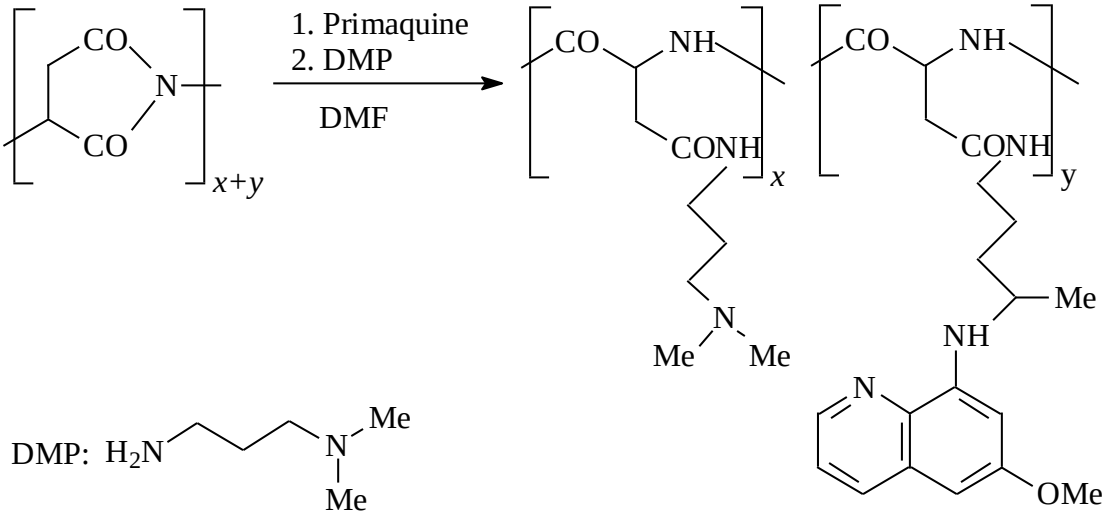
Table 8: ^{1}H NMR result for PSI. DME (90) PRIMAQUINE (10)

CHEMICAL SHIFT (PPM)	EXPECTED NUMBER OF	NUMBER OF PROTONS

	PROTONS	FOUND
8.54-6.09	5H	5H
3.51	20H	21H
2.89-2.59	92H	91H
1.63-1.26	7H	7H

The ^{1}H NMR spectrum showed a 100% incorporation of primaquine and 99% incorporation of DME.

3.4.2 PSI. DMP (90) PRIMAQUINE (10)



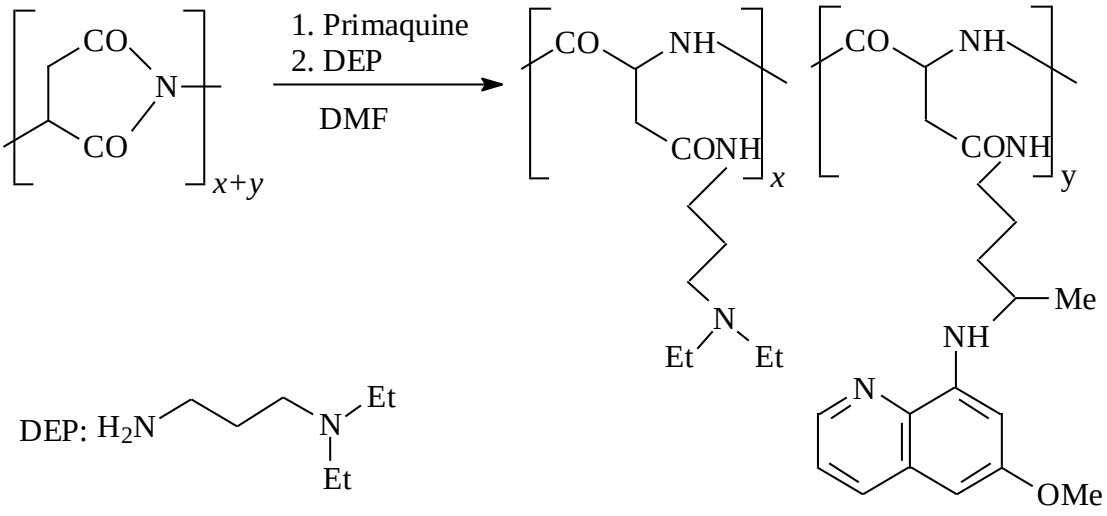
SCHEME 19: Synthesis of PSI. DMP (90) PRIMAQUINE (10)

The above compound was prepared at room temperature, and the solubilizing group, a tertiary amine was the major reagent used in a ratio of 9:1 (*x*/*y*). The ^{1}H NMR spectrum showed a 88% incorporation of DMP, and aromatic signals for the 8-aminoquinoline were found at 8.31 ppm (bs), 1H, 7.25 ppm (bs), 1H, 7.02 ppm (bs),1H and, 6.06 ppm (bs), 2H. The two terminal methyl groups on the DMP signal were found at 2.12 ppm (s), 54H. The macromolecular carrier signals were found at 3.19 ppm (s), 20H for CONH**CH_2** (table 9).

Table 9: ^{1}H NMR result for PSI. DMP (90) Primaquine (10)

CHEMICAL SHIFT (PPM)	EXPECTED NUMBER OF PROTONS	NUMBER OF PROTONS FOUND
8.31-6.02	5H	5H
3.19	20H	24H
2.89	20H	17H
2.12	72H	63H
1.64-0.92	25H	25H

3.4.3 PSI. DEP (90) PRIMAQUINE (10)



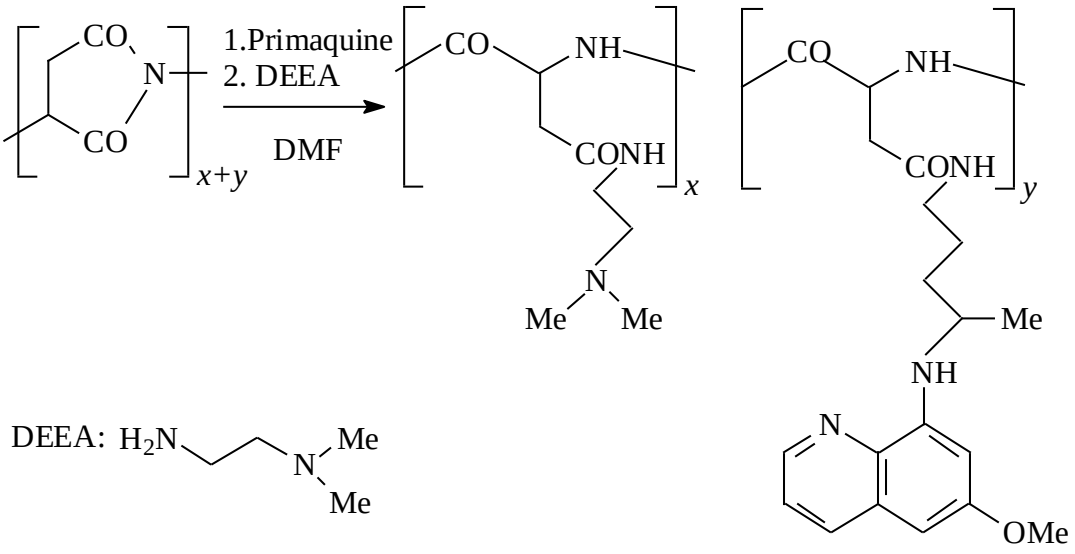
SCHEME 20: Synthesis of PSI. DEP (90) PRIMAQUINE (10)

PSI. DEP (90) Primaquine (10) compound was prepared in a ratio of (9:1) (DEP:Primaquine). ^{1}H NMR spectroscopy showed a 100% incorporation of both DEP and primaquine. DEP the solubilizing group used is a tertiary amine and the signals for the methyl groups in the DEP and primaquine were found at 1.29-1.03 ppm for 54 protons. The signal peaks for the primaquine 5 aromatic protons were found at 8.40-6.18 ppm. The signals for the polymer backbone (*i.e*. polyaspartamide) were found at 3.15-3.12 ppm for -CONH**CH$_2$**- (table 10).

Table 10: ^{1}H NMR spectroscopy result for PSI. DEP (90) PRIMAQUINE (10)

CHEMICAL SHIFT (PPM)	EXPECTED NUMBER OF PROTONS	NUMBER OF PROTONS FOUND
8.40-6.18	5H	5H
3.15-3.12	94H	94H
1.95-1.93	22H	20H
1.29	57H	57H

3.4.4 PSI. DEEA (90) PRIMAQUINE (10)



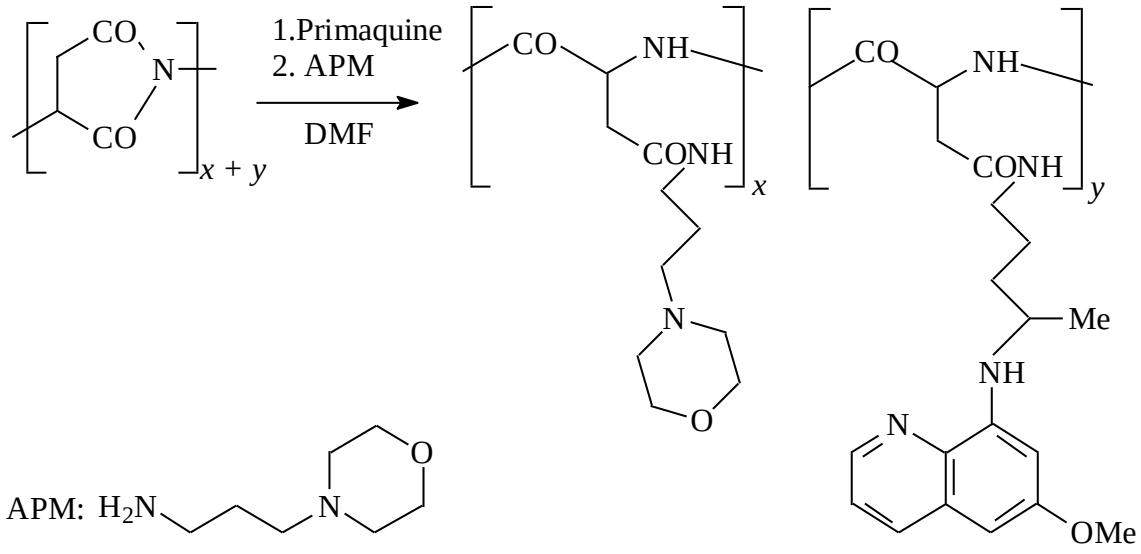
SCHEME 21: Synthesis of PSI. DEEA (90) PRIMAQUINE (10)

In the initial synthesis of PSI. DEEA (90) PRIMAQUINE (10), a 0% incorporation of primaquine was observed and the procedure was reviewed. In the review procedure, aminolytic ring opening was performed with the initial addition of primaquine for 8 h before the addition of DEEA. ^{1}H NMR spectroscopy showed a 22% incorporation of primaquine and a 100% incorporation of DEEA. Initially, the 0% incorporation of primaquine was believed to be a result of generation of carboxylic side group. The signal peaks for the aromatic protons were found at 8.54-6.60 ppm for 1.1 protons instead of 5 protons and the signal peaks for the methyl group on both the DEEA and primaquine compounds were found at 1.28-1.03 ppm for 57 protons (table 11).

Table 11: ^{1}H NMR result for PSI. DEEA (90) PRIMAQUINE (10)

CHEMICAL SHIFT (PPM)	EXPECTED NUMBER OF PROTONS	NUMBER OF PROTONS FOUND
8.54-6.60	5H	1.1H
3.32	20H	21H
2.79-2.75	74H	82H
1.70	4H	2H
1.28-1.03	57H	57H

3.4.5 PSI. APM (90) PRIMAQUINE (10)



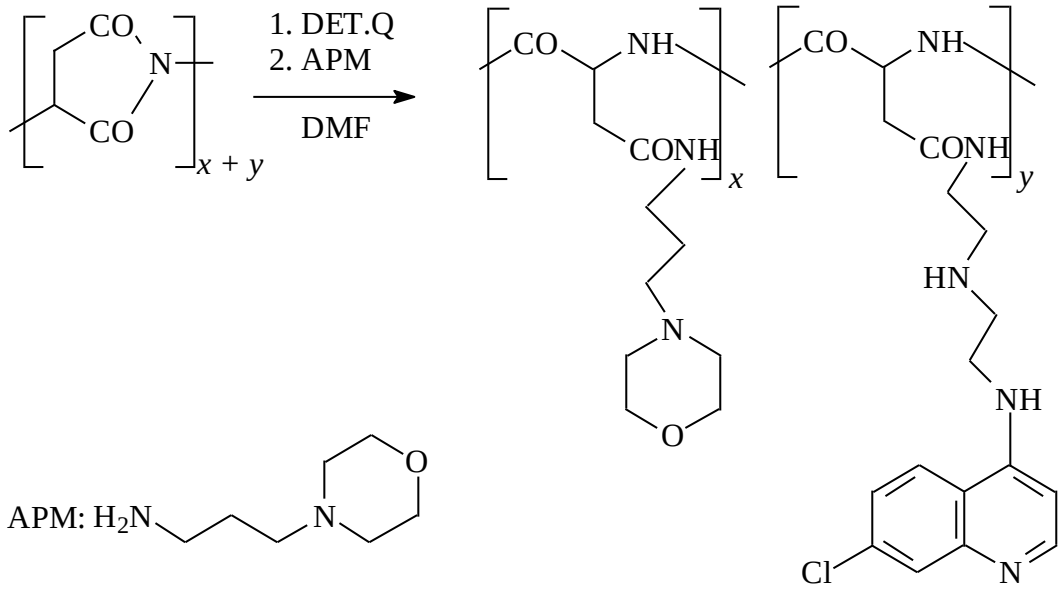
SCHEME 22: PSI.APM (90) PRIMAQUINE (10)

The ^{1}H NMR spectroscopy showed signals for the primaquine compound at 8.40-6.13 ppm for the five aromatic protons and at 1.68-1.67 ppm for $CH_2\mathbf{CH_2CH_2}CH(\mathbf{CH_3})$NH-. The signals for APM were found at 3.72 ppm for CH_2OCH_2, 2.74-2.73 ppm for $-N(\mathbf{CH_2})_3$-. The ^{1}H NMR spectroscopy showed a 92% of APM and 98% of primaquine (table 12).

Table 12: ^{1}H NMR result of PSI. APM (90) PRIMAQUINE (10)

CHEMICAL SHFT (PPM)	EXPECTED NUMBER OF PROTONS	NUMBER OF PROTONS FOUND
8.40-6.13	5H	4.9H
3.72	36H	38H
3.19-3.18	20H	20H
2.74-2.73	74H	68H
1.68-1.67	25H	25H

3.5 PSI. APM (90) DET.Q



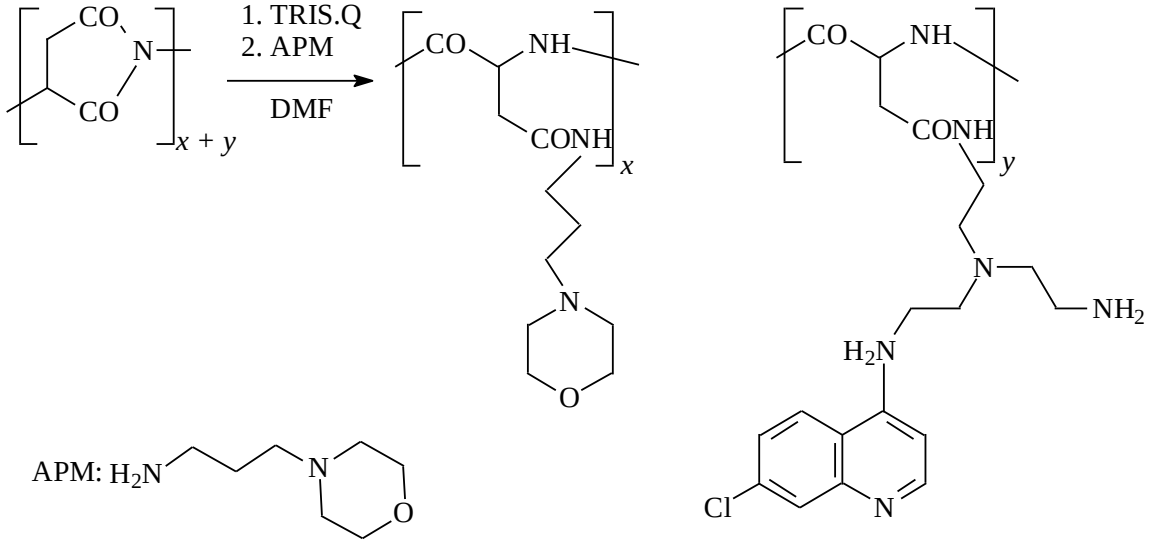
SCHEME 23: Synthesis of PSI. APM (90) DET.Q

^{1}H NMR spectrum showed that there was 90% incorporation of DET.Q and 99% incorporation of APM. The signals for APM, the solubilizing group were found at 3.73ppm for **-CH_2OCH_2-** and at 2.52-2.36ppm for –N(**$CH_2)_3$.** The signal peaks for the aromatic protons on DET.Q were found at 8.22-6.33 pm for 4.5 protons instead of 5 protons. In initial synthesis of the above compound, APM was added first before DET.Q and this resulted in a low incorporation of DET.Q (table 13).

Table 13: H NMR result for PSI. APM (90) DET.Q

CHEMICAL SHIFT (PPM)	EXPECTED NUMBER OF PROTONS	NUMBER OF PROTONS FOUND
8.22-6.33	5H	4.5H
3.73	36H	35H
3.20-3.18	20H	22H
2.78-2.76	26H	26H
2.52-2.36	54H	54H
1.70-1.68	18H	18H

3.6 PSI. APM (90) TRIS.Q (10)



SCHEME 24: Synthesis of PSI. APM (90) TRIS.Q (10)

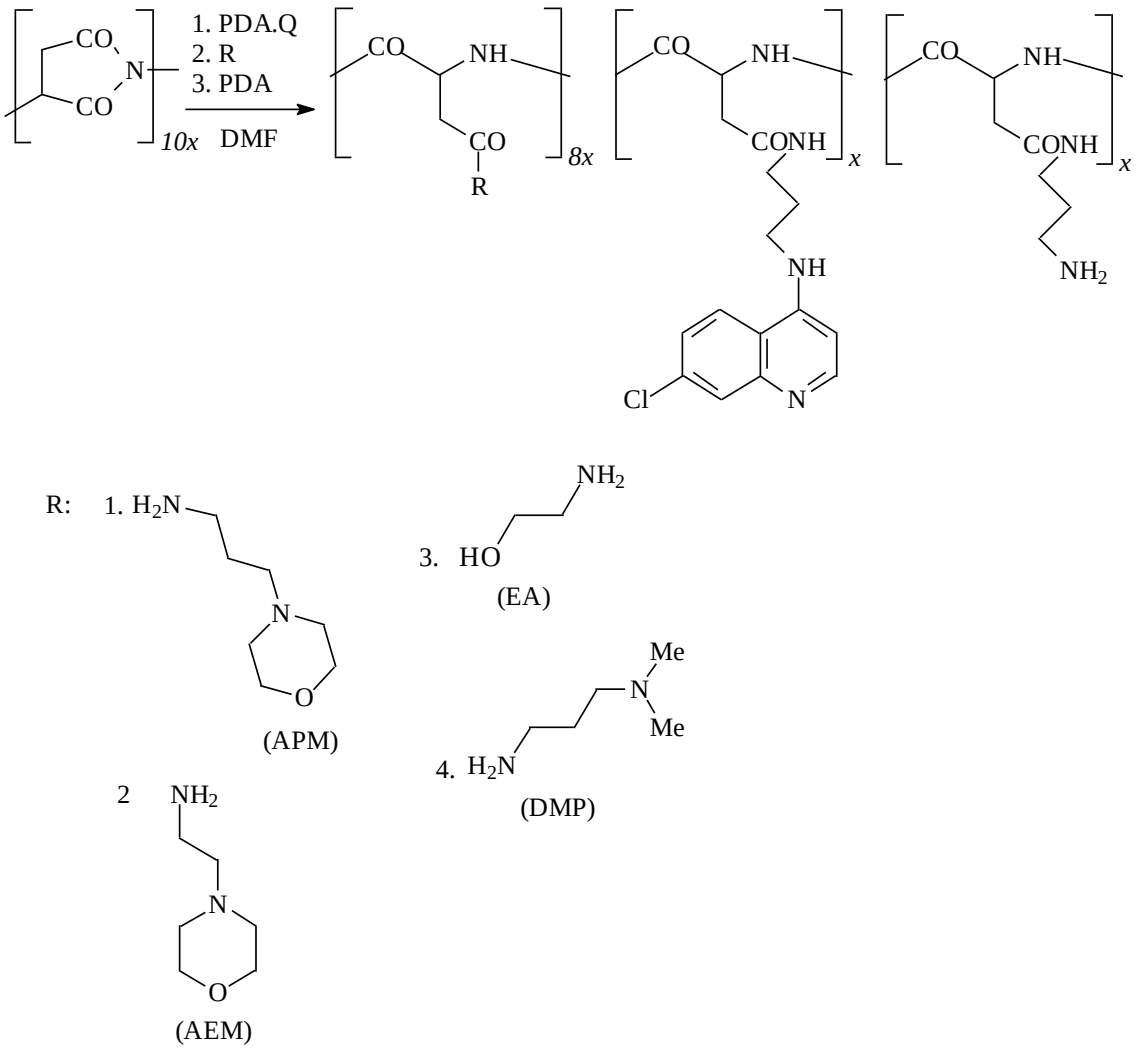
The synthesis of PSI. APM (90) TRIS.Q (10) was performed in an ice-bath to avoid cross-linking. ^{1}H NMR spectrum showed that there was 42% incorporation of TRIS.Q and 94% incorporation of APM. The signal peaks for TRIS.Q, a 4-aminoquinoline were found at 2.79-2.38 ppm for 79 protons of -N(**CH$_2$CH$_2$)$_3$-** instead of 84 protons and at 8.40-6.30 ppm for 2 aromatic protons instead of 5 protons. The signal peaks for APM protons (**CH$_2$OCH$_2$**) were found at 3.75 ppm for 32 protons instead of 36 protons and at 2.79-2.38 ppm for N(**CH$_2$**)$_3$ (table 14).

Table 14: H NMR result for PSI. APM (90) TRIS.Q (10)

CHEMICAL SHIFT (PPM)	EXPECTED NUMBER OF PROTONS	NUMBER OF PROTONS FOUND
8.40-6.30	5H	2.0H
3.75	36H	32H
3.20	20H	19H
2.79-2.38	84H	79H
1.71-1.70	18H	18H

3.7 SYNTHESIS OF CO-POLYMERS WITH PDA DRUG BINDING SITE

We then decided to take the polymer synthesis one step further by using a different drug binding site, and PDA (1,3-diaminopropane) was selected as a drug binding site. Polysuccinimide substrate was treated with the 4-aminoquinoline compound and two amine nucleophiles. The co-polymers prepared were equipped with solubilizing, target-directing and drug-binding sites. The first step was the ring opening of polysuccinimide with 4-aminoquinoline compound, followed by the addition of the solubilizing group. The resultant solution was added dropwise to a solution of PDA (1,3-diaminopropane) in an ice-bath to avoid crosslinking. Various solubilizing groups were used, and PDA was used as the drug binding site for the coupling of methotrexate, and ferrocene to afford a polymer with two drug agents such as antimalarial and anticancer drugs.



SCHEME 25: Synthesis of co-polymers with PDA.Q and PDA

Table 15: ^{1}H NMR result for PSI. AEM (80) PDA.Q (10) PDA (10)

CHEMICAL SHIFT	EXPECTED NUMBER OF PROTONS	NUMBER OF PROTONS FOUND
8.11-6.10	5H	4.2H
3.74	32H	30H
3.35	20H	19H
2.55-2.50	72H	52H
1.80-1.60	4H	4H

From table 15, the ^{1}H NMR spectroscopy showed that there was 94% incorporation of AEM, 90% of PDA.Q, and 100% of PDA. The signal peaks for the aromatic protons on PDA.Q compound were found at 8.11-6.10 ppm for 4.5 protons instead of 5 protons and the signal peaks for AEM were found at 3.74 ppm for 30 proton of **CH_2OCH_2** instead of 32 protons (table 15).

Table 16: ^{1}H NMR result for PSI. APM(80) PDA.Q (10) PDA (10)

CHEMICAL SHIFT	EXPECTED NUMBER OF PROTONS	NUMBER OF PROTONS FOUND
8.30-6.11	5H	4.6H
3.73	32H	28H
3.21-3.19	20H	22H
2.51-2.49	72H	62H
1.66-1.65	20H	20H

From table 16, the ^{1}H NMR spectroscopy showed that there was an 87% incorporation of APM, 92% of PDA.Q and 87% of PDA. The signals for the aromatic protons of PDA.Q were found at 8.30-6.11 ppm for 4.6 protons and 5 protons were expected, the signal peaks for APM and PDA were found at 2.51-2.49 ppm and 1.66-1.65 ppm respectively (table 16).

Table 17: ^{1}H NMR result for PSI. EA (80) PDA.Q (10) PDA (10)

CHEMICAL SHIFT	EXPECTED NUMBER OF PROTONS	NUMBER OF PROTONS FOUND
8.20-6.00	5H	3.8H
3.63	16H	13H
3.33	20H	17H
2.75-2.71	24H	14H
1.60-1.59	4H	4H

From table 17, the ^{1}H NMR spectroscopy showed that there was 81% incorporation of EA, 75% of PDA.Q, and 58% of PDA. The signal peaks for the aromatic protons of PDA.Q were found at 8.20-6.00 ppm for 3.8 protons instead of 5 protons. The signal peaks for EA protons (**CH_2**OH) were found at 3.63 ppm and 13 protons were found instead of 16 protons (table 17).

Table 18: H NMR result for PSI.DMP (80) PDA.Q (10) PDA (10)

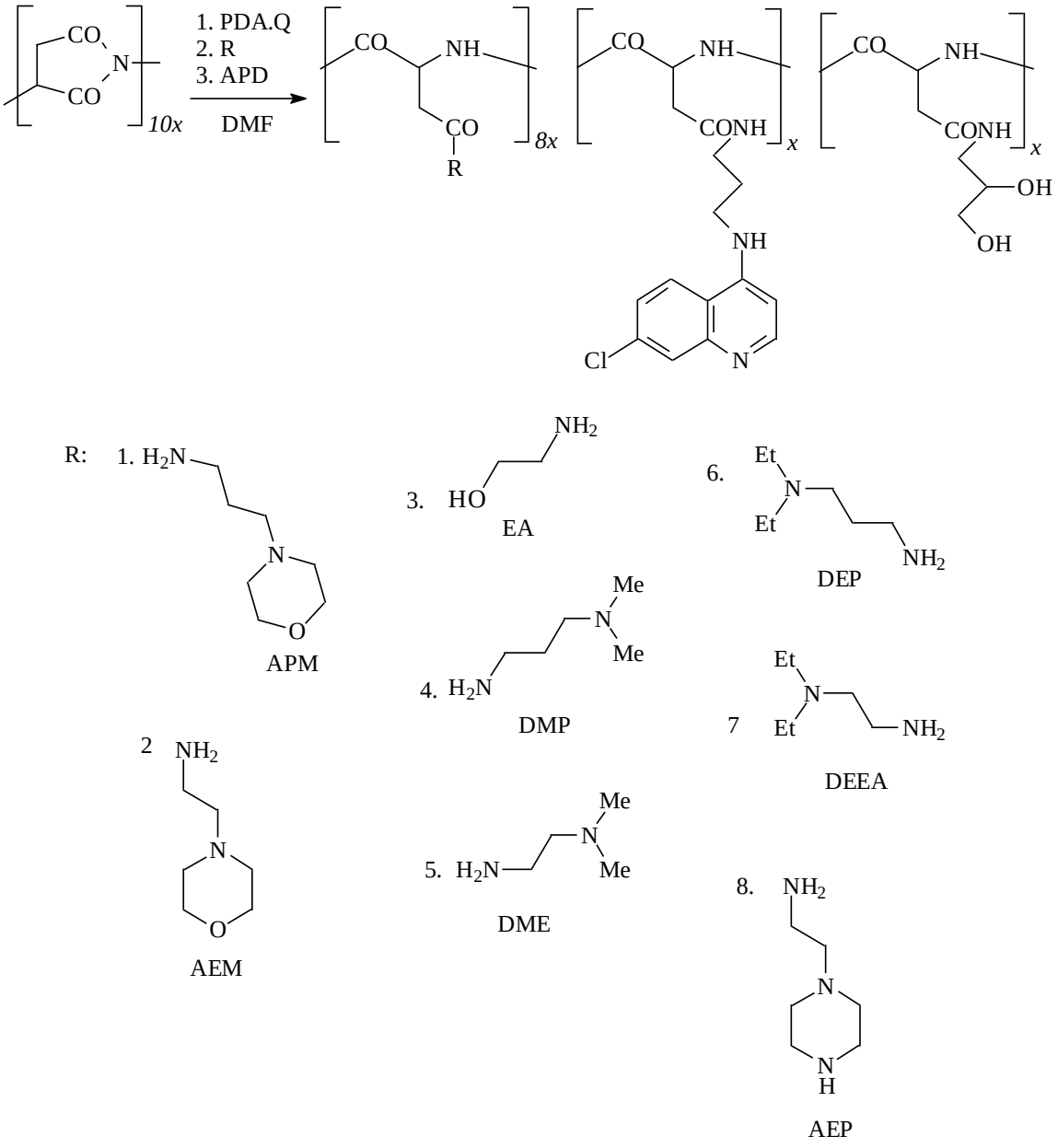
CHEMICAL SHIFT	EXPECTED NUMBER OF PROTONS	NUMBER OF PROTON FOUND
8.10-6.11	5H	4.4H
3.24-3.22	20H	23H
2.75-2.43	88H	84H
1.77-1.76	20H	20H

From table 18, ^{1}H NMR spectroscopy showed that there was 96% incorporation of DMP, 88% of PDA.Q, and 96% of PDA. The signal peaks for PDA.Q aromatic protons were found at 8.10-6.11 ppm for 4.4 protons instead of 5 protons. The signal peaks for PDA and DME protons (-**CH_2**CH_2**CH_2**-) were found at 2.75-2.43 ppm for 84 protons but 88 protons were expected (table 18).

3.8 SYNTHESIS OF CO-POLYMERS WITH APD DRUG BINDING SITE

The co-polymers prepared were equipped with water-solubilizing and homing units, quinoline-binding and dihydroxyl-equipped units in a ratio of (9:1:1). The APD ((±)-3-amino-1,2-propanediol) used is a drug binding site for platinum drug and it keeps the drug temporarily protected from catabolic or enzymatic attack before reaching the target site. The water-solubilizing units used included; APM, AEM, DEP, EA, DEEA, DMP, DME and AEP. Percentage incorporations of the water-solubilizing group ranged from 29-100%, and incorporation of APD were found to be 64-100%. The lowest % incorporation of water-solubilizing group was found to be with AEP, and this was surprising because of the low reactivity of AEP. Finally, the % incorporation of PDA.Q,

the 4-aminoquinoline compound, was found to be from 72 to 100%. The co-polymers were prepared under concentrated conditions.



SCHEME 26: Synthesis of co-polymers with APD group.

Table 19: ^{1}H NMR result of PSI .APM (80) PDA.Q (10) APD (10)

CHEMICAL SHIFT (PPM)	EXPECTED NUMBER OF PROTONS	NUMBER OF PROTONS FOUND
8.20-6.10	5H	4.2H

3.73	36H	36H
3.21-3.19	20H	22H
2.80	20H	20H
2.51-2.49	48H	46H
1.69-1.68	18H	18H

^{1}H NMR spectroscopy showed that there was a 96% incorporation of APM, 83% of PDA.Q, and 100% of APD. The signal peaks for the aromatic protons on PDA.Q compound were found at 8.20-6.10 ppm for 4.2 protons instead of 5 protons. Signal peaks for APD and APM protons were found at 3.73 ppm and 2.51-2.49 ppm respectively (table 19).

Table 20: ^{1}H NMR result of PSI.AEM (80) PDA.Q (10) APD (10)

CHEMICAL SHIFT (PPM)	EXPECTED NUMBER OF PROTONS	NUMBER OF PROTONS FOUND
8.10-6.11	5H	5H
3.74-3.72	36H	30H
3.35-3.33	20H	20H
2.73-2.48	68H	52H
1.63	2H	2H

^{1}H NMR spectroscopy showed that there was an 80% incorporation of AEM, 100% of PDA.Q and 83% of APD. The signal peaks for the PDA.Q aromatic protons were found at 8.10-6.11 ppm for 5 protons and that of APD were found at 3.74-3.72 ppm. The signal peaks for AEM compound were found at 3.74-3.72 ppm and 2.73-2.48 ppm (table 20).

Table 21: ^{1}H NMR result of PSI. DEP (80) PDA.Q (10) APD (10)

CHEMICAL SHIFT (PPM)	EXPECTED NUMBER OF PROTONS	NUMBER OF PROTONS FOUND
8.30-6.20	5H	5H

3.82-3.59	5H	4.8H
3.25-3.23	20H	23H
2.80-2.77	68H	69H
1.79-1.75	18H	18H
1.20	48H	48H

^{1}HNMR spectroscopy showed that there was 100% incorporation of DEP, 100% of PDA.Q and 96% of APD. The signal peaks for the aromatic protons on PDA.Q compound were found at 8.30-6.20 ppm for 5 protons and the signal peaks for APD (**CH_2CH**(OH)**CH_2**OH) were found at 3.82-3.59 ppm for 4.8 protons instead of 5 protons. The signals for the two methyl groups on DEP were found at 1.20 ppm for 48 protons (table 21).

Table 22: ^{1}H NMR result of PSI.EA (80) PDA.Q (10) APD (10)

CHEMCAL SHIFT (PPM)	EXPECTED NUMBER OF PROTONS	NUMBER OF PROTONS FOUND
7.90-5.90	5H	4H
3.79-3.58	20H	15H
3.32	20H	14H
2.72-2.71	20H	10H
1.60	2H	2H

^{1}H NMR spectroscopy showed that there was 72% incorporation of EA, 75% of PDA.Q and 80% of APD. The signal peaks for aromatic protons on PDA.Q were found at 7.90-5.90 ppm for 4 protons instead of 5 protons (table 22).

Table 23: ^{1}H NMR result of PSI. DEEA (80) PDA.Q (10) APD (10)

CHEMICAL SHIFT (PPM)	EXPECTED NUMBER OF PROTONS	NUMBER OF PROTONS FOUND
8.20-6.20	5H	4.6H
3.81	3H	2.8H

3.59-3.37	22H	23H
2.75-2.74	68H	54H
1.62	2H	2H
1.08	48H	39H

^{1}H NMR spectroscopy showed that there was 81% incorporation of DEEA, 92% of PDA.Q and 93% of APD. The peaks for the aromatic protons on PDA.Q were found at 8.20-6.20 ppm for 4.6 protons. The signal peaks for DEEA were found at 1.08 ppm for 39 protons instead of 48 protons (table 23).

Table 24: ^{1}H NMR result of PSI. DME (80) PDA.Q (10) APD (10)

CHEMICAL SHIFT (PPM)	EXPECTED NUMBER OF PROTONS	NUMBER OF PROTONS FOUND
8.00-6.00	5H	4.4H
3.81	3H	3H
3.40-3.36	20H	23H
2.76-2.55	82H	57H
1.85-1.84	2H	2H

^{1}H NMR spectroscopy showed that there was 70% incorporation of DME, 88% of PDA.Q and 100% of APD. Signal peaks for the aromatic protons on PDA.Q compound were found at 8.00-6.00 ppm for 4.4 protons instead of 5 protons and DME signal peaks were found at 2.76-2.55 ppm for 57 protons instead of 82 protons (table 24).

Table 25: ^{1}H NMR result of PSI. DMP (80) PDA.Q (10) APD (10)

CHEMICAL SHIFT (PPM)	EXPECTED NUMBER OF PROTONS	NUMBER OF PROTONS FOUND
8.20-6.20	5H	3.5H
3.83-3.59	20H	23H
3.26	20H	23H
2.75-2.61	84H	87H

1.85-1.84	18H	18H

^{1}H NMR spectroscopy showed that there was 100% incorporation of DMP, 70% of PDA.Q and 100% of APD. The signal peaks for the aromatic protons on PDA.Q were evident at 8.20-6.20 ppm for 3.5 protons instead of 5 protons (table 25).

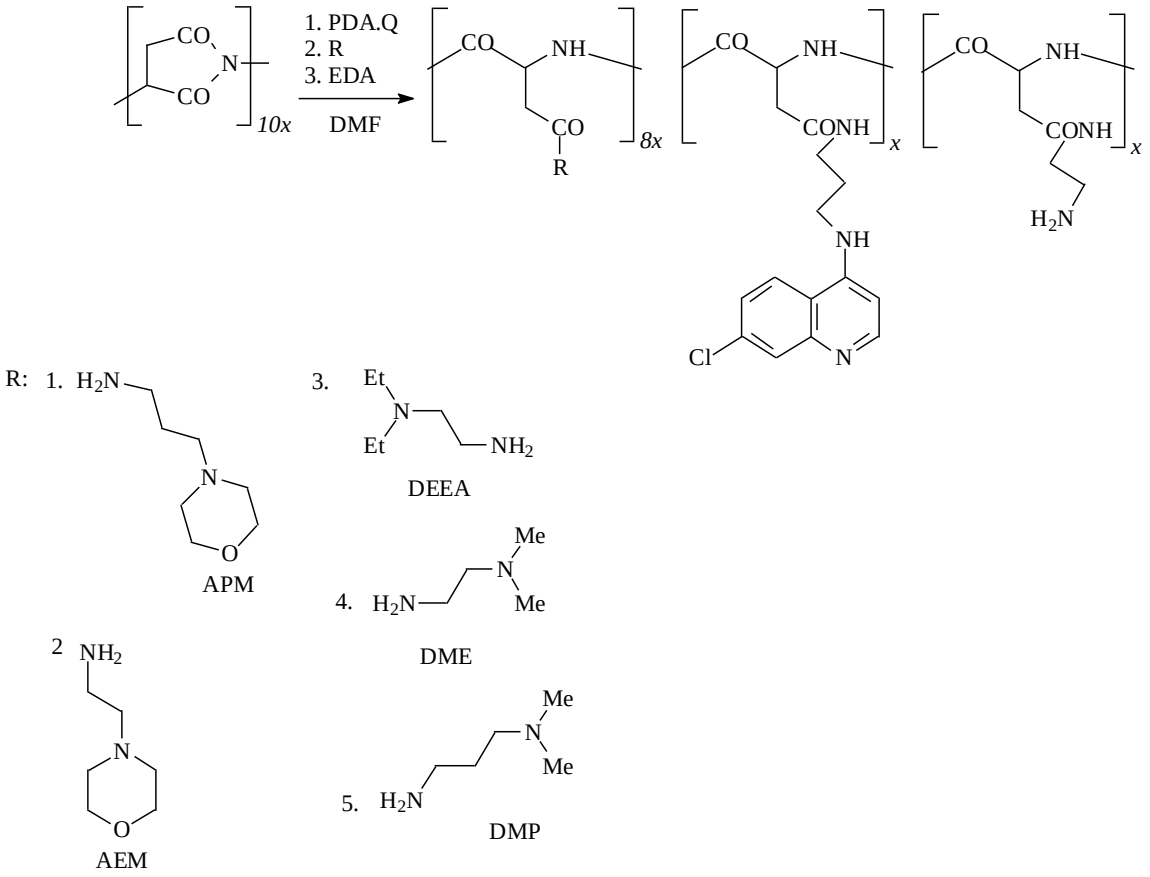
Table 26: ^{1}H NMR result of PSI.AEP (80) PDA.Q (10) APD (10)

CHEMICAL SHIFT (PPM)	EXPECTED NUMBER OF PROTONS	NUMBER OF PROTONS FOUND
8.30-6.20	5H	3.8H
3.80	3H	3.2H
3.60-3.28	22H	27H
2.82-2.64	100H	29H

The ^{1}H NMR spectroscopy showed that there was 29% incorporation of AEP, 76% of PDA.Q and 100% of APD. The signal peaks for PDA.Q aromatic protons were found at 8.30-6.20 ppm for 3.8 protons instead of 5 protons. AEP peaks were visible at 2.82-2.64 for 29 protons instead of 100 protons and APD peak was evident at 3.80 ppm for 3 protons (table 26).

3.9 SYNTHESIS OF CO-POLYMERS WITH AN EDA DRUG BINDING SITE

EDA (ethylenediamine) is a good binding site for methotrexate, ferrocene and other carboxyl-functionalized agents. The solubilizing groups used were APM, AEM, DEEA, DME, DMP, and the % incorporation of the solubilizing groups was between 80 and 100%. The % incorporation of EDA, the drug binding site, was found to be between 83 and 100%, and the % incorporation of the 4-aminoquinoline compound was found to be between 82 and 93%.



SCHEME 27: Synthesis of co-polymers with EDA drug binding site

Table 27: ^{1}H NMR result for PSI. APM (80) PDA.Q (10) EDA (10)

CHEMICAL SHIFT (PPM)	EXPECTED NUMBER OF PROTONS	NUMBER OF PROTONS FOUND
8.30-6.20	5H	4.2H
3.77	32H	32H
3.50	20H	24H
2.75-2.42	72H	71H
1.72	18H	18H

The ^{1}H NMR spectroscopy showed that there was 100% incorporation of APM, 83% of PDA.Q and 99% of EDA. The signal peaks for the aromatic protons on PDA.Q were found at 8.30-6.20 ppm for 4.2 protons instead of 5 protons and APM peaks were visible at 2.75-2.42 ppm for 71 protons instead of 72 protons (table 27).

Table 28: ^{1}H NMR result of PSI. AEM (80) PDA.Q (10) EDA (10)

CHEMICAL SHIFT (PPM)	EXPECTED NUMBER OF PROTONS	NUMBER OF PROTONS FOUND
8.20-6.01	5H	4.5H
3.74-3.73	32H	33H
3.35-3.32	20H	23H
2.71-2.52	72H	67H
1.75	2H	2H

The ^{1}H NMR spectroscopy showed that there was 100% incorporation of AEM, 90% of PDA.Q and 93% of EDA. The signal peaks for the aromatic protons on PDA.Q were evident at 8.20-6.01 ppm for 4.5 protons instead of 5 protons. AEM peaks (**CH_2OCH_2**) were found at 3.74-3.73 ppm for 33 protons. The signal peaks for EDA protons were visible at 2.71-2.52 ppm (table 28).

Table 29: ^{1}H NMR result of PSI. DEEA (80) PDA.Q (10) EDA (10)

CHEMICAL SHIFT (PPM)	EXPECTED NUMBER OF PROTONS	NUMBER OF PROTONS FOUND
8.13-6.16	5H	4.4H
3.40-3.39	20H	21H
2.82-2.79	72H	60H
1.88-1.86	2H	2H
1.11	48H	40H

The ^{1}H NMR spectroscopy showed that there was 83% incorporation of DEEA, 88% of PDA.Q and 83% of EDA. The signal peaks for PDA.Q aromatic protons were found at 8.13-6.16 ppm for 4.4 protons instead of 5 protons. The methyl group protons on DEEA found at 1.11 ppm for 40 protons instead of 48 protons. The CH_2 protons on EDA were visible at 2.82-2.79 ppm (table 29).

Table 30: ^{1}H NMR result of PSI. DME (80) PDA.Q (10) EDA (10)

CHEMICAL SHIFT (PPM)	EXPECTED NUMBER OF PROTONS	NUMBER OF PROTONS FOUND
8.20-6.00	5H	4.7H
3.33-3.25	20H	22H
2.72-2.71	24H	22H
2.48-2.46	16H	16H
2.23-2.19	48H	45H
1.80	2H	2H

The ^{1}H NMR spectroscopy showed that there was 94% incorporation of DME, 93% of PDA.Q and 92% of EDA. The signal peaks for the methyl group on the DME compound were visible at 2.23-2.19 ppm for 45 protons instead of 48 protons. The aromatic peaks were found at 8.20-6.00 ppm for 4.7 protons instead of 5 protons and EDA signal peaks were evident at 2.72-2.71 ppm (table 30).

Table 31: ^{1}H NMR result of PSI. DMP (80) PDA.Q (10) EDA (10)

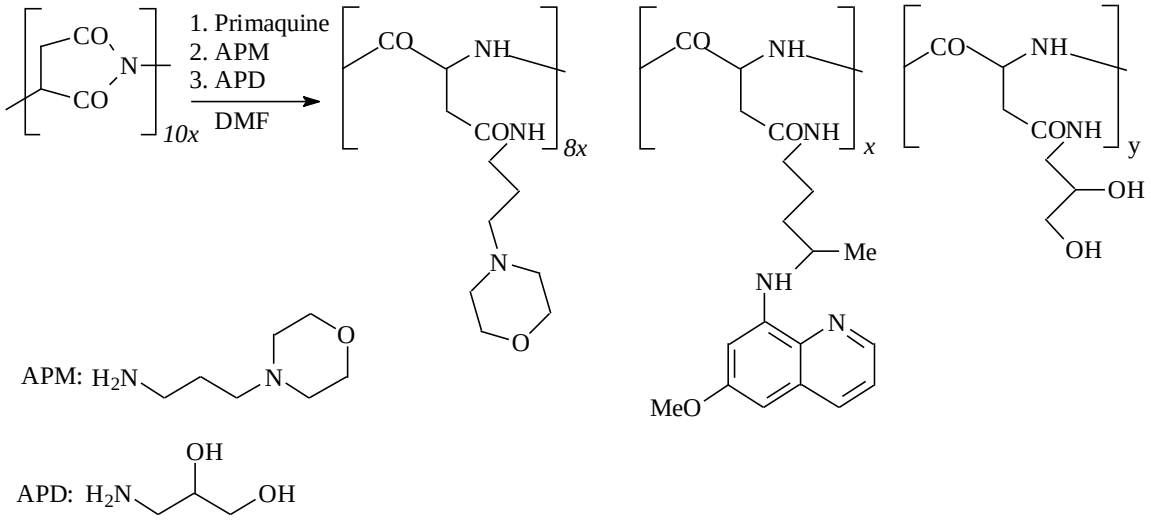
CHEMICAL SHIFT (PPM)	EXPECTED NUMBER OF PROTONS	NUMBER OF PROTONS FOUND
8.20-6.20	5H	4.1H
3.27	20H	20H
2.87-2.67	88H	84H
1.87-1.86	18H	18H

The ^{1}H NMR spectroscopy showed that there was 96% incorporation of DMP, 82% of PDA.Q and 96% of EDA. The signal peaks for -CH_2**C**$\mathbf{H_2}$$CH_2$- protons on DMP were found at 1.87-1.86 ppm and the methyl groups were found at 2.87-2.67 ppm. The aromatic protons were visible at 8.20-6.20 ppm for 4.1 protons instead of 5 protons (table 31). EDA protons were found at 2.87-2.67 ppm.

3.10 CO-POLYMERS WITH PRIMAQUINE

The co-polymers with primaquine, an 8-aminoquinoline, were prepared using APM solubilizing group, and APD drug binding site which is useful for anchoring of platinum

drugs. It was characterized using ^{1}H NMR spectroscopy, and the signal peaks for the aromatic protons on primaquine were found at 8.45-6.20ppm for 4.4 H instead of 5 H, the signal peaks for APM (**CH_2OCH_2**), 40 H were found at 3.73ppm, **CH_2**CH_2**CH_2**N(**CH_2**)$_3$, for 48H at 2.51-2.38ppm. APM is the solubilizing group which also acts as the homing device, and APD is the drug binding site for anticancer drugs such as platinum.



SCHEME 28: Synthesis of PSI. APM (80) Primaquine (10) APD (10)

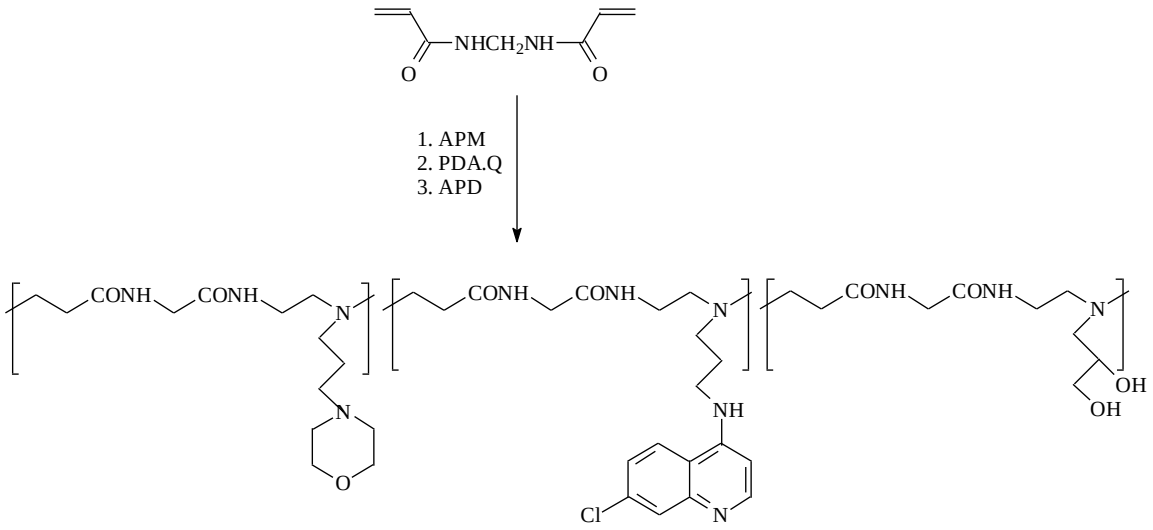
Table 32: ^{1}H NMR result of PSI. APM (80) Primaquine (10) APD (10)

CHEMICAL SHIFT (PPM)	EXPECTED NUMBER OF PROTONS	NUMBER OF PROTONS FOUND
8.45-6.20	5H	4.4H
3.73	34H	40H
3.40-3.19	20H	26H
2.70-2.38	66H	68H
1.68-1.10	23H	23H

The ^{1}H NMR spectroscopy showed that there was 100% incorporation of APM, 88% of Primaquine and 100% of APD (table 32).

3.11 SYNTHESIS OF POLYAMIDOAMINES CONTAINING PDA.Q

Polyamidoamines have proved to be as versatile as the polyaspartamides, allowing for construction of a multitude of unique carrier compositions.[6] They were prepared by Michael addition polymerization reactions involving nucleophilic addition of the amino group across an activated double bond pioneered in Ferruti's laboratory.[7,8] In this research, methylenebisacrylamide was treated in aqueous-phase with APM, APD and PDA.Q, in the ratio of (3:1:1:1), and the major reactant used was methylenebisacrylamide. The yield was low and this is as a result of side reactions such as hydrolysis, which counteract propagation in the aqueous phase. The reaction was performed at room temperature, and APM was used as the solubilizing group while APD was used as the drug binding site for drug anchoring of a platinum-type anticancer drug. The reaction was initially performed using 8-aminoquinoline compound *i.e* primaquine free base, but there was a precipitation of primaquine out of the solution.



SCHEME 29: Synthesis of MBA.APM.PDA.Q.APD (3:1:1:1)

The ^{1}H NMR spectrum showed that signal peaks for APM, 4H, were found at 3.75-3.74 ppm for **$CH_2O$$CH_2$**, APM signal peaks were found at 3.75-3.74 ppm for 5H, **CH_2CH**(OH)**CH_2**OH, and the aromatic protons were at 8.20-6.24 ppm for 5H.

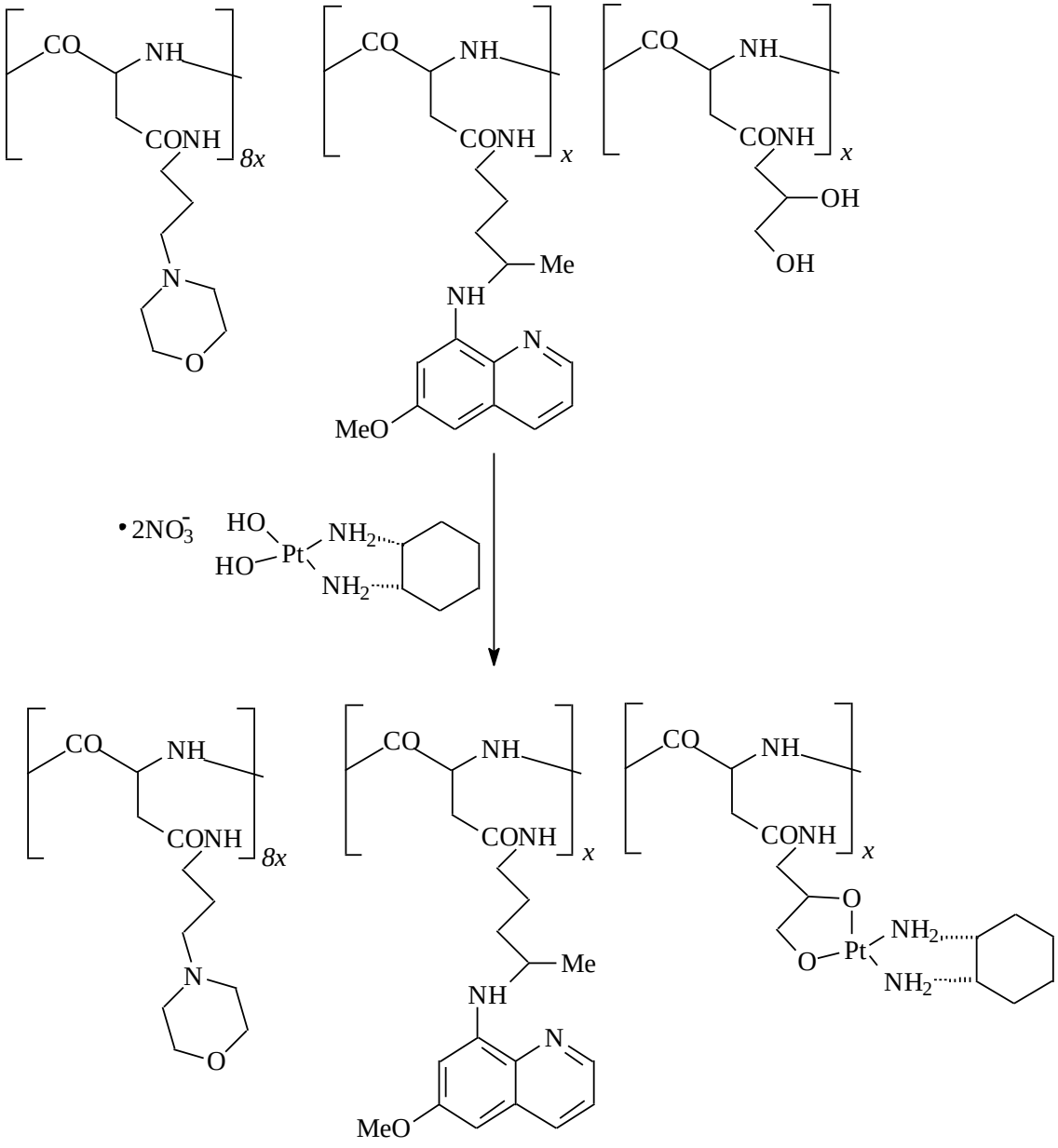
Table 33: ^{1}H NMR result of MBA.APM.PDA.Q. APD (3:1:1:1)

CHEMICAL SHIFT (PPM)	EXPECTED NUMBER OF PROTONS	NUMBER OF PROTONS FOUND
8.20-6.24	5H	4.2H
3.75-3.74	9H	10H
2.79-2.40	42H	44H
1.66-1.64	4H	4H

^{1}H NMR spectroscopy showed a 100% of APM and APD, 84% PDA.Q (table 33).

3.12 POLYMERIC CARRIER WITH TWO DRUG SYSTEMS

3.12.1 PSI. APM (80) PRIMAQUINE (10) APD (10) .Pt

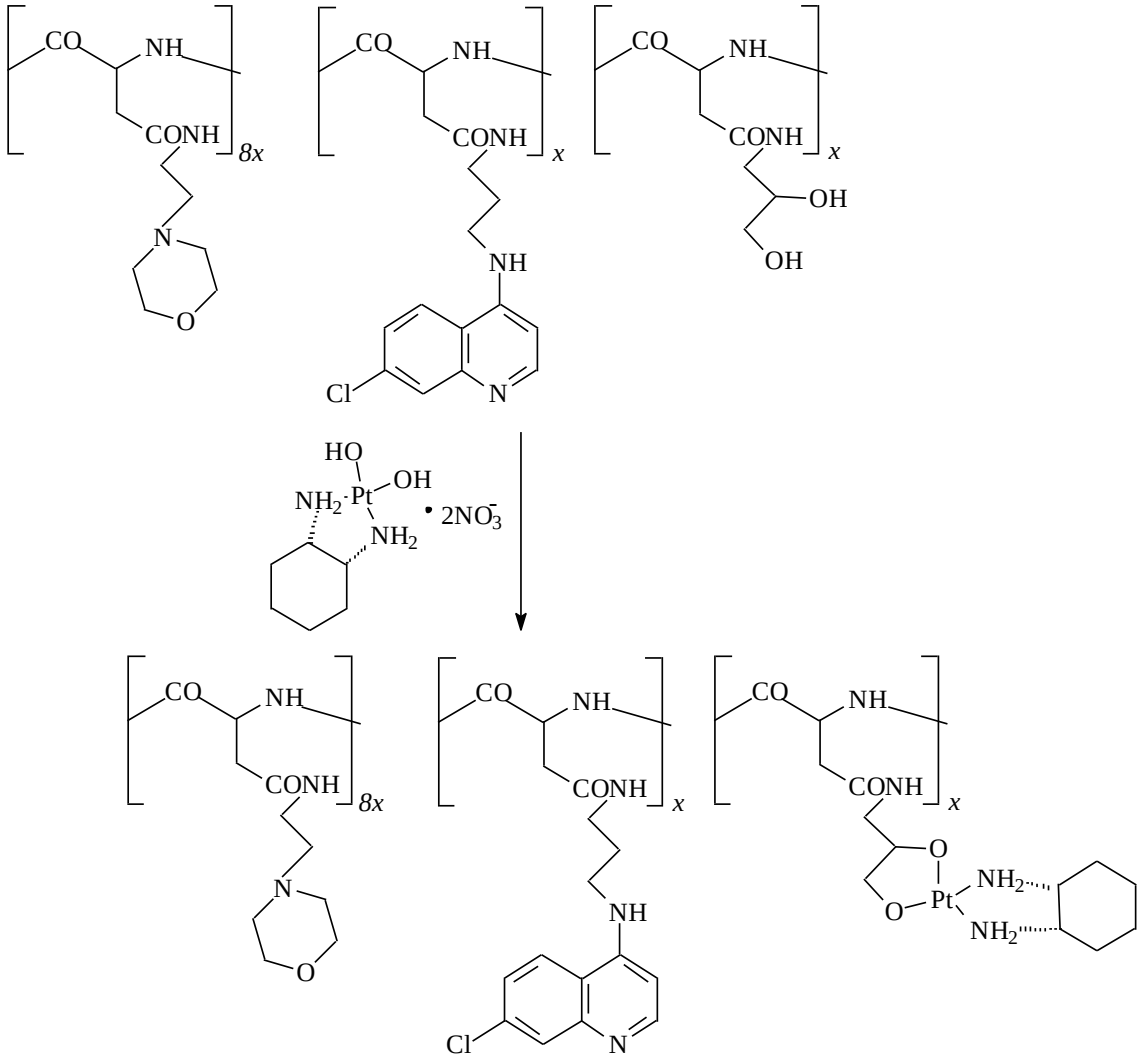


SCHEME 30: Synthesis of PSI. APM (80) Primaquine (10) APD (10).Pt

PSI. APM (80) Primaquine (10) APD (10).Pt conjugate contains two drug systems (i.e primaquine, an antimalarial drug and a cisplatin-type, an anticancer drug). The platinum complex was conjugated through the two oxygen donor ligands provided by the carrier.

These conjugates follow a release mechanism involving platinum-oxygen bond cleavage, thus requiring no special biofissionable links in the carrier for drug liberation. The reaction was performed at a controlled pH of 5.5-6.0, and freeze dried to afford the above conjugates. Further studies will be done to evaluate the effects of antimalarial drug when co-administered with anticancer agent on a single polymeric carrier, and platinum analysis will be performed to determine the % incorporation of platinum.

3.12.2 PSI. AEM (80) PDA.Q (10) APD (10).Pt

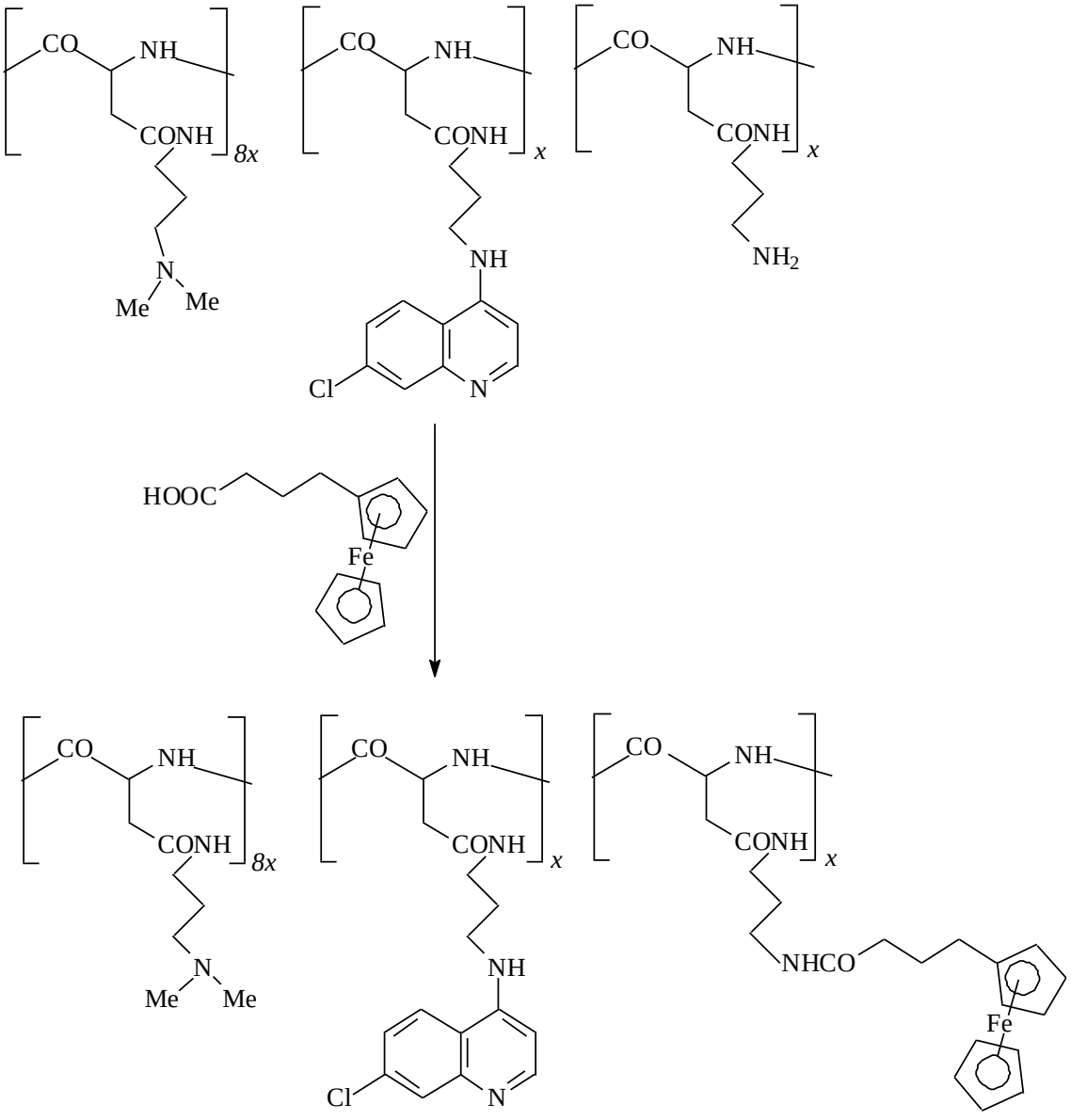


SCHEME 31: Synthesis of PSI. AEM (80) PDA.Q (10) APD (10). Pt

PSI. AEM (80) PDA.Q (10) APD (10).Pt conjugates were prepared at a pH of 5.5-6.0 and a different type of water solubilizing group (AEM), was used. The platinum complex was

conjugated through the two oxygen donor ligands provided by the carrier. The synthesis was a two-step reaction, and the first step was the synthesis of the homopolymer, followed by the platination reaction of the platinum compound to the macromolecular carrier. The conjugates contain a 4-aminoquinoline compound and an anticancer drug Further studies will be performed to determine the % incorporation of platinum, and evaluation of the effect of the 4-aminoquinoline on the activity of the platinum drug.

3.12.3 PSI.DMP(80) PDA.Q (10) PDA (10) .FERROCENE



SCHEME 32: PSI. DMP (80) PDA.Q (10) PDA (10).Ferrocene

The ferrocenylation of PSI.DMP (80) PDA.Q (10) PDA (10) carrier was performed using ferrocenylcarboxylic acid and mediated by the HTBU coupling agent. The drug binding site used was PDA, a diamine and the water-solubilizing group used was DMP, a tertiary amine. ^{1}H NMR spectroscopy showed a 96% incorporation of ferrocene, and the signal peak for ferrocene complex was found at 4.19 ppm. The number of protons expected was 9H, but 8.6 protons were found.

3.13 *IN VITRO* ANTIPLASMODIAL ACTIVITY OF SOME OF THE HOMOPOLYMERS WITH 4- AND 8-AMINOQUINOLINE COMPOUNDS

3.13.1 METHODOLOGY

The samples were screened for *in vitro* antiplasmodial activity at the Division of Pharmacology, University of Cape Town. The test samples were tested in triplicate against chloroquine-sensitive strain of *Plasmodium falciparum* (D10). Continuous *in vitro* cultures of asexual erythrocyte stage of *P. falciparum* were maintained using a modified method of Trager and Jensen.[9] Quantitative assessment of antiplasmodial activity *in vitro* was determined via the parasite lactase dehydrogenase assay using a modified method by Makler *et al*.[10] The test samples were prepared to a 2 mg/mL stock solution in 10% DMSO and sonicated to enhance solubility. All samples were tested as a suspension and chloroquine was used as a reference drug in all experiments. A full dose-response was performed for the selected compounds to determine the concentration sinhibiting 50% of parasite growth (IC_{50}-value). The IC_{50}-values were obtained using a non-linear dose response curve fitting analysis via Graph Pad Prism v.4.0 software.

3.13.2 RESULTS

Table 34: *In vitro* antiplasmodial activity against *P. falciparum* (D10).

SAMPLE	IC_{50}(μg/ml)
PSI.DEP(90)PRIMAQUINE (10)	3.20
PSI.APM (90)DET.Q (10)	94.84

PSI.DME (90)PDA.Q (10)	95.06
PSI.APM (90) PDA.Q (10)	>100
PSI.DEEA (90) PDA.Q (10)	52.24
PSI. EA (90) PDA.Q (10)	>100
PSI.DEP (90) PDA.Q (10)	2.92
PSI.DME (90)PRIMAQUINE (10)	>100
PSI.AEM(90) PRIMAQUINE (10)	>100
PSI. DMP (90) PDA.Q (10)	5.66
CHLOROQUINE	27.86 ng/ml

The result showed that PSI.DEP (90) PRIMAQUINE (10) and PSI. DEP (90) PDA.Q (10) were most active with IC_{50}-values of 3.2 μg/ml and 2.9 μg/ml respectively. PSI. DMP (90) PDA.Q (10) showed moderate activity with an IC_{50}-values of 5.7 μg/ml. All other samples were not active against the chloroquine sensitive strain of *P. falciparum* (D10).

3.14 REFERENCES USED IN CHAPTER THREE

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CHAPTER FOUR: CONCLUSION AND FUTURE WORK

4.1 SUMMARY

The homopolymers and conjugates prepared were all characterised by ^{1}H NMR spectroscopy. They were purified by dialysis using 12000 membrane tubing and this was followed, by freeze drying to afford the isolated compounds. The selected anticancer drugs used in this research were ferrocene and platinum drugs, and the antimalarial drugs used were primaquine free base, DET.Q, PDA.Q, TRIS.Q.

4.1.1 HOMOPOLYMERS WITH PDA.Q

The homopolymers were prepared using various solubilizing groups such as APM, DME, DMP, DEP, AEM, DEEA, and EA. The % incorporation of homopolymer with APM solubilizing group was found to be 100%, DME was 95%, DMP was 100%, DEEA was 98%, DEP was 98%, EA was 95%, APM was 100%, and AEM was 83%. The % incorporation of PDA.Q compound was found to be between 70-100%. They were all prepared in a ratio of (9:1) (x:y), the solubilzing agents were used in greater ratio when compared to that of the 4-aminoquinolines. The homopolymers with DEP and DMP were found to be active against a chloroquine sensitive strain of *Plasmodium falciparum* (D10).

4.1.2 HOMOPOLYMERS WITH 8-AMINOQUINOLINES

The 8-aminoquinoline compound used in this research was primaquine, and it was liberated as a free base before use. The solubilizing groups used were DME, DMP, DEP, APM, DEEA, and they were all used in a ratio of (9:1) (x:y). The % incorporation of DME was found to be100%, DMP was 90%, DEP was 100%, APM was 100% and DEEA was 100%. The % of primaqine was found to be 100% except in the case where DEEA was used as a solubilizing agent. The homopolymers were prepared at room temperature with the addition of primaquine first, before the addition of the solubilizing group. The homopolymer with the DEP solubilizing group was found to be active against chloroquine sensitive strain of *Plasmodium falciparum* (D10).

4.1.3 HOMOPOLYMERS WITH DET.Q AND TRIS.Q

The % incorporation of DET.Q was 90%, and the solubilizing group used was APM with a % incorporation of 100%. The % incorporation of TRIS.Q was found to be 42%, with a % incorporation of 89% for the solubilizing group. Both compounds are water soluble and were characterized by ^{1}H NMR spectroscopy.

4.1.4 CO-POLYMERS WITH PDA.Q AND APD DRUG BINDING SITE

They co-polymers were prepared in a ratio of (8:1:1), and they were equipped with water-solubilizing, homing and drug anchoring units. The solubilizing groups used were AEM, APM, EA, DMP, and the % incorporation was between 80-96%. The % incorporation of PDA.Q was between 58-96%. Some of these co-polymers will be used for future studies to anchor anticancer drugs.

4.1.5 CO-POLYMERS WITH PDA.Q AND EDA DRUG BINDING SITE

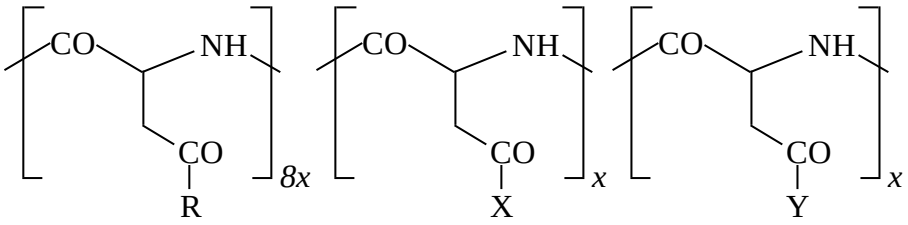
They were prepared in a ratio of (8:1:1) and characterized by ^{1}H NMR spectroscopy. EDA, a diamine, was used to create a drug binding site for anticancer drugs, and the solublizing groups used were APM, AEM, DMP, DME, EA, DEEA, DEP and AEP. The % incorporation of the soubilizing groups used was between 29-100%, and the % incorporation of PDA.Q was between 72-100%. The solubilizing group with the least % incorporation was AEP.

4.1.6 CO-POLYMER WITH PRIMAQUINE

The co-polymer was synthesized in a ratio of (8:1:1), and the % incorporation of APM, the water-solubilizing group, was 97%, and that of the drug binding group was 100%. APD used provides a drug binding site for anticancer drug.

4.2 FUTURE WORK

The future work will involve co-conjugation of an 8-aminoquinoline or 4-aminoquinoline and selected anticancer drugs to a single macromolecular carrier as shown in the diagram below.



R- Solubilizing group

X – 8-aminoquinoline or 4-Aminoquinoline

Y – Selected anticancer drug

The co-conjugates will be characterized by ^{1}H NMR spectroscopy after purification by dialysis against water with 12000 membrane testing. *In vitro* and toxicity tests and studies will be done to determine the effect of the antimalarial drugs on the anticancer drugs because the antimalarial drug is expected to act as a potentiating and resistance reducing agent. The conjugates will also be submitted for *in vitro* and *in vivo* screening tests against human cancer cell lines. The conjugates are expected to be biodegradable, non-toxic and have reactive functional groups suitable for drug binding.

CHAPTER 5: EXPERIMENTAL

5.1 GENERAL PROCEDURES

The 'H NMR spectra were obtained at 300MHz and 400MHz in D_2O solution. Chemical shifts, δ in ppm, were referenced against sodium 3-(trimethylsilyl)-2,2,3,3-d_4-propionate, and to eliminate potential protonation effects, sample solution pH values were adjusted to 10-11 with sodium hydroxide, where applicable. For the ^{1}H NMR assignment, the expected protons counts and assignments are given in parentheses.

Dialysis was performed against distilled water using cellulose membrane spectra (Spectrum Industries, Los Angeles, CA), with a molecular mass cut-off limit of 12000-14000. Freeze-drying of polymer and conjugate solutions was performed in a VIRTIS bench-Top 3 freeze-drier at -30°C and a pressure of 0.1 torr. The freeze-dried polymers were post-dried in a SARTORIUS Thermo Control Infrared drying apparatus and kept in a descicator. Analytical samples were dried using the Abderhalden apparatus, and calcium chloride was used as the drying agent. UV/VIS Spectroscopy was done on a HITACHI 2000 spectrophotometer, at a scan speed of 400nm/min; both analytical methods were performed in the School of Chemistry. Flash chromatography was performed using basic alumina as a solid support. The aprotic solvent, *N*,*N*-dimethylformamide (DMF), was redistilled under nitrogen gas and reduced pressure, and kept over molecular sieves.

5.2 REAGENTS, REACTANTS AND SOLVENTS

The reagents used in this research include: methylenebisacrylamide (MBA), 3-dimethylamino-1-propylamide (DMP), 3-diethylamino-1-propylamide (DEP), 1-(2-aminoethyl)-Piperazine (AEP), 4-(3-aminopropyl)morpholine (APM), diethylenetriamine (DET), ethylenediamine (EDA), 2,2'-(ethylenedioxy)-diethylamine (EDDA), N-N-diethyldiethylenetriamine (NN), tris(2-aminoethyl)amine (Tris), 1,3-diaminopropane (PDA), ethanolamine (EA), 4-(2-aminoethyl)morpholine (AEM), N,N'-dicyclohexylcarbodiimide (DCC), 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium

fluorophosphate (HBTU), 2-dimethylaminoethylamine (DME), 2-diethylaminoethylamine (DEEA), (±)-3-amino-1,2-propanediol (APD), triethylamine (TEA), 2-(2-aminoethoxy)ethanol (AEE), 4,7-dichloroquinoline, sodium hydroxide, primaquine diphosphate salt, sodium sulphate, D,L-aspartic acid.

They solvents used include diethyl ether (Et_2O), methanol (MeOH), dichloromethane (DCM), hexane, ethyl acetate, ethanol, ammonium hydroxide, dioxane, acetone, propyloyl, *N*,*N*-dimethylformamide (DMF), distilled water

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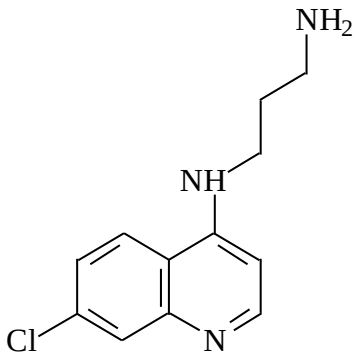
5.3. EXPERIMENTAL PROCEDURES

5.3.1 SYNTHESIS OF 4-AMINOQUINOLINES

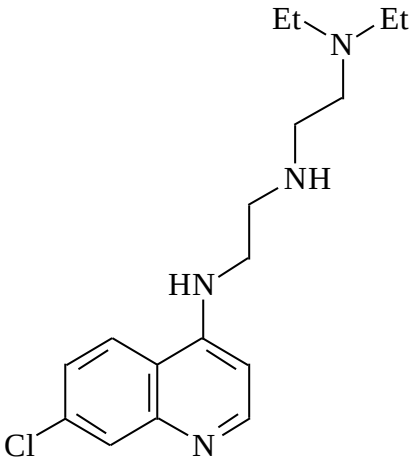
5.3.1.1 *N'*-(7-Chloroquinolin-4-yl-)propane-1,3-diamine (PDA.Q)



A mixture of 4,7-dichloroquinoline (1.00 g, 5.05 mmol) and PDA (1.89 mL, 1.68 mg, 22.7 mmol) was heated to reflux for 8 h with stirring and then allowed to cool, after which the resultant solution was diluted with DCM (20 mL) and the mixture was washed with NaOH solution (1 M, 20 mL) and brine (10 mL). An aqueous layer, organic layer and white coarse particulate precipitate were obtained, and the organic layer was collected and dried over anhydrous sodium sulphate, filtered and then concentrated on a rotary evaporator to afford a white solid.[1-4] (1.02 g, 86% yield). The solids were recrystallized propyloyl to afford three crops with their melting point between 86-89ºC. ^{1}H NMR (CH_3OD) δ/ppm: 8.35 (d, Ar-H), 8.07 (d, Ar-H), 7.77 (d, Ar-H), 7.38 (dd, Ar-

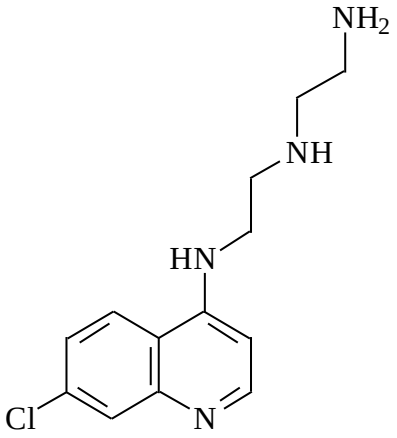
H), 6.53 (d, Ar-H), 3.42 (t, 2H, **CH$_2$**NHAr), 2.80 (t, 2H, **CH$_2$**NH$_2$), 1.90 (dt, 2H, NH$_2$CH$_2$**CH$_2$**CH$_2$NH).

5.3.1.2. *N*-(2-(2-(Diethylamino)ethylamine)ethyl)-7-chloroquinoline-4-amine (NNQ)



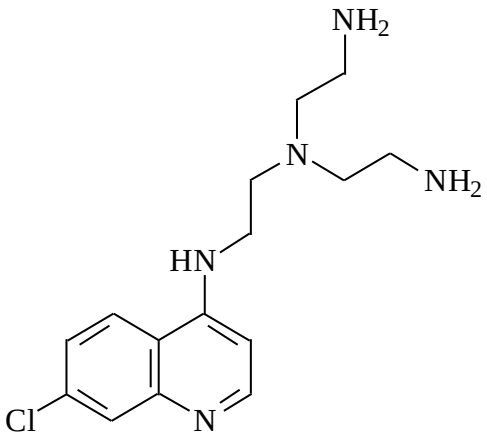
A solution of 4,7-dichloroquinoline (100 mg, 0.51 mmol) and NN (163 mg, 1.02 mmol) in 0.2 mL of dioxane, was heated with reflux at 110°C for (2 days x 8 h). The crude sample was allowed to cool and then dissolved in 30 mL of DCM and washed with 30ml of water to afford an organic and aqueous layer. The organic layer was then collected and dried over sodium sulphate, filtered and concentrated on the rotary evaporator to obtain a brown viscous liquid. Flash chromatography was then performed using basic alumina as the solid support, and eluted with 1:4 (EtOAc:MeOH) to afford a brown viscous liquid (80 mg, 80% yield).[5] ^{1}H NMR ($CDCl_3$) δ/ppm: 8.47 (d, 1H, Ar-H), 8.05-7.73 (m, 2H, Ar-H), 7.72 (dd, 1H, Ar-H), 6.34 (d, 1H, Ar-H), 3.65 (bs, 2H, **NH**), 3.35 (dd, 2H, NH**CH$_2$**CH$_2$NHAr), 3.02 (t, 2H, **CH$_2$**NHAr), 2.74 (t, 2H, **CH$_2$**NH$_2$), 2.63-2.52 (m, 6H, $CH_2NCH_2CH_2$), 1.06-0.90 (m, 6H, CH_2**CH$_3$**).

5.3.1.3. *N*-(2-(2-Aminoethylamino)ethyl)-7-chloroquinoline-4-amine (DET.Q)



A solution of 4,7-dichloroquinoline (2.00 g, 10.1 mmol) and DET (2.08 g, 20.2 mmol) in 0.5 mL of dioxane was heated at reflux at 110°C for (2 days x 8 h). The crude sample was then purified by flash chromatography using basic alumina as solid support and 7:2:1 (MeOH:DCM:NH_4OH) eluent to afford a brown liquid (1.65 g, 50% yield). ^{1}H NMR (CH_3OD) δ/ppm: 8.11 (d, 1H, Ar-H), 7.83 (d, 1H, Ar-H), 7.53 (s, 1H, Ar-H), 7.11 (d, 1H, Ar-H), 6.25 (d, 1H, Ar-H), 3.70-2.13 (m, 8H, NH**CH_2CH_2**NH).

5.3.1.4. *N*-(7-chloro-4-quinolyl)-tris(2-aminoethyl)amine (Tris.Q)



A solution of 4,7-dichloroquinoline (2.00 g, 10.9 mmol) and Tris (2.95 g, 20.2 mmol) in 0.5 mL of dioxane was heated at reflux at 110°C for (2 days + 8 h). The crude sample was then purified by flash chromatography on basic alumina as a solid support and 7:2:1 (MeOH:DCM:NH_4OH) as eluent to afford a brown liquid (44%, 1.37 g).[6] (CH_3OD)

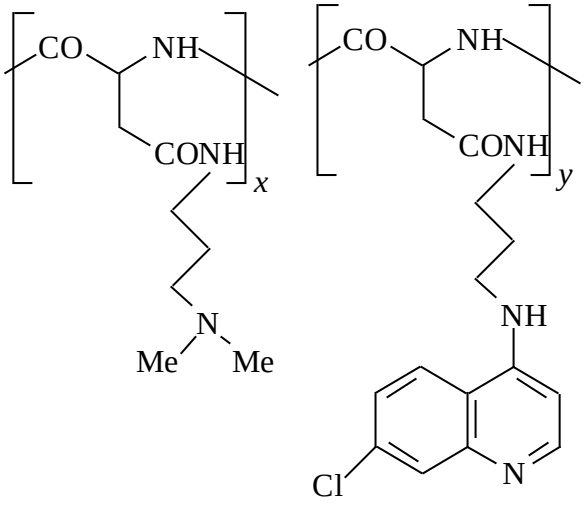
δ/ppm: 8.23(d, 1H, Ar-H), 8.01-7.89 (m, 1H, Ar-H), 7.65 (d, 1H, Ar-H), 7.30-7.24 (m, 1H, Ar-H), 6.41 (s, 1H, Ar-H), 2.74-2.51 (m, 12H, N**CH$_2$CH$_2$**NH$_2$).

5.3.2. PREPARATION OF POLY-αβ-DL-SUCCINIMIDE (PSI)

D,L-Aspartic acid (50g) was mixed with phosphoric acid (25g) in a 2000ml round bottom flask until the mixture was homogenous. The flask was then placed in an oil bath at a temperature of 240 ºC. When polymerization reaction started, it was controlled with nitrogen gas and the temperature was dropped to 190 ºC. The mixture was then left for 2 h until a beige product was obtained. The beige product was then washed with water until the pH was 6, and the product was then filtered off and dried in the oven at 70 ºC overnight. The product was then dissolved in 70 mL of DMF and stirred overnight at room temperature, and the brown solution was then stirred for 1 h in an ice bath, and DCC was then added. The resultant solution was then allowed to stir in an ice bath for 4 h followed by overnight stirring at room temperature. The suspension was then centrifuged off to obtain a clear solution from which the product polymer was precipitated with excess water. The precipitate was filtered off, washed with ethanol solution, and dried in the oven overnight at 70 ºC to afford a brittle white solid, 39.7 g (80%) which was then stored in a dry container.

5.4. SYNTHESIS OF HOMOPOLYMERS WITH PDA.Q

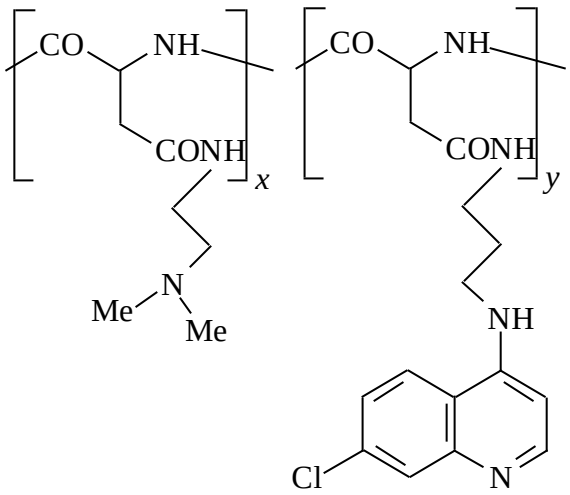
5.4.1. PSI. DMP (90) PDA.Q (10)



PSI (400 mg, 4.25 mmol) was dissolved in 10 mL of DMF, and PDA.Q (169 mg, 0.72 mmol) in 5 mL of DMF was added, followed by addition of TEA (73 mg, 0.72 mmol). The solution was flushed with nitrogen gas, and stirred at room temperature overnight for 20 h. DMP (391 mg, 3.83 mmol) was added, and the resultant solution was flushed with nitrogen gas, and stirred overnight at room temperature. The solution was concentrated on the rotary evaporator at 60°C, to afford a yellow viscous liquid which was allowed to cool before precipitation with 20 mL of diethyl ether:acetone (2:1) to afford a white precipitate. The precipitate was washed twice with boiling toluene, followed by a single washing with boiling acetone. The precipitate was then dissolved in 10 mL of distilled water, the pH was adjusted to 7-8 with hydrochloric acid, and dialysis was performed using 12000 membrane tubing for 2 days against distilled water, followed by freeze-drying to afford the expected homopolymer as a white water soluble solid, 500 mg (57%). The ^{1}H NMR spectrum revealed 94% incorporation of PDA.Q and 100% of DMP.

^{1}H NMR (D_2O) δ/ppm: 8.12-6.01, 4.7H (5H, Ar-H); 3.10, 23H (20H, CONH**CH_2**); 2.76, 23H, (**CH_2**CONH, 20H; **CH_2**NHAr, 2H); 2.29-2.14, 75H (72H, **CH_2**N(**CH_3)$_2$**); 1.64, 20H (20H, CH_2**CH_2**CH_2).

5.4.2 PSI. DME (90) PDA.Q (10)

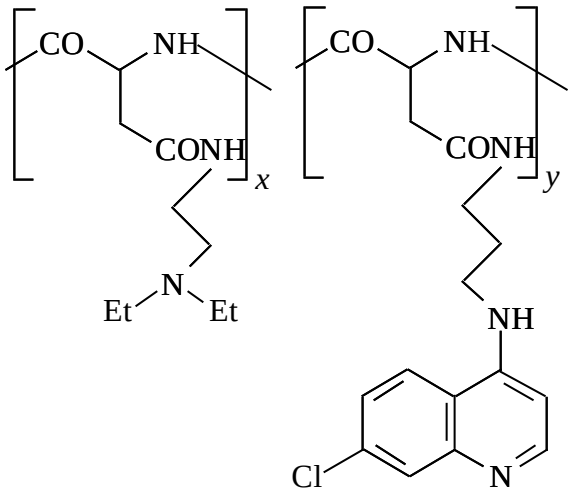


PSI (1.00 g, 10.63 mmol) was dissolved in 9 mL of DMF, and PDA.Q (0.28 g, 1.20 mmol) and TEA (0.12 g, 1.29 mmol) was added, and the solution was flushed with nitrogen gas, and stirred overnight at room temperature. DME (0.84 g, 9.57 mmol) was

added and the resultant solution was flushed with nitrogen gas, and then stirred overnight at room temperature. The solution was concentrated on the rotary evaporator at 60ºC to afford a yellow viscous liquid, which was precipitated after cooling with diethyl ether:acetone (2:1) solution (20 mL). The precipitate was washed twice with boiling toluene, and once with boiling acetone. The precipitate was then dissolved in distilled water (20 mL), and pH adjusted to 7-8 with hydrochloric acid. Dialysis was performed using 12000 membrane tubing against distilled water for two days, followed by freeze-drying to afford a white water soluble solid, 1.04 g (51%). The ^{1}H NMR spectrum showed that there was 96% incorporation of PDA.Q, and 96% of DME.

^{1}H NMR (D_2O) δ/ppm: 8.02-6.04, 4.8H (5H, Ar-H); 3.36-3.34, 24H (20H, CONH**CH_2**); 2.83-2.54, 39H (40H; **CH_2**CH_2CH_2NHAr, (**CH_2CH_2**N$(CH_3)_2$); 2.28, 51H (54H, CH_2N(**$CH_3)_2$**); 1.76, 2H (2H; CH_2**CH_2**CH_2NHAr)

5.4.3 PSI. DEEA (90) PDA.Q (10)

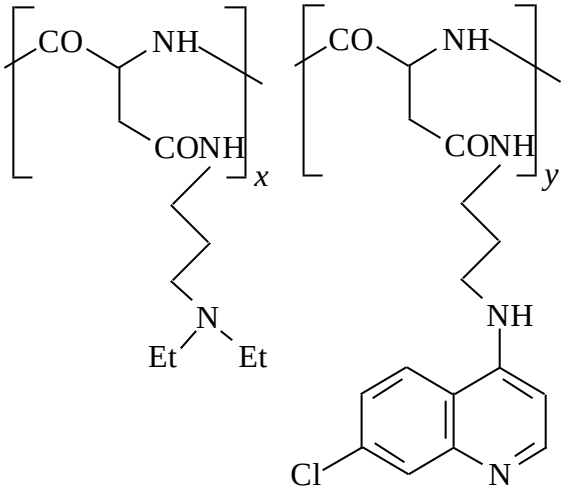


PSI (300 mg, 3.19 mmol) was dissolved in 5 mL of DMF, and PDA.Q (82 mg, 0.35 mmol) was added, followed by TEA (36 mg, 0.35 mmol). The solution was flushed with nitrogen gas, and stirred overnight at room temperature. DEEA (334 mg, 2.87 mmol) was added, flushed with nitrogen gas, and stirred at room temperature for 2 d. The solution was concentrated on the rotary evaporator at 60ºC to afford a viscous liquid, which, was precipitated with diethyl ether:acetone (2:1) solution (15 mL).The precipitate was washed twice with boiling toluene, and once with boiling acetone. The precipitate was dissolved

in distilled water, and the pH was adjusted to 7-8 with hydrochloric acid, followed by dialysis against distilled water for 2 d with 12000 membrane tubing. It was freeze-dried to afford water soluble solid, 510 mg (73%). The ^{1}H NMR spectrum showed a 92% incorporation of PDA.Q, and 92% incorporation of DEEA

^{1}H NMR (D_2O) δ/ppm: 8.21-6.20, 4.6H (5H; Ar-H), 3.28-3.27, 21H (20H; CONH**CH_2**); 2.51-1.80, 68H (78H; **CH_2CH_2**N(**CH_2**$CH_3)_2$, **$CH_2CH_2CH_2$**NHAr); 0.94, 54H (54H; $CH_2CH_2N(CH_2$**$CH_3)_2$**).

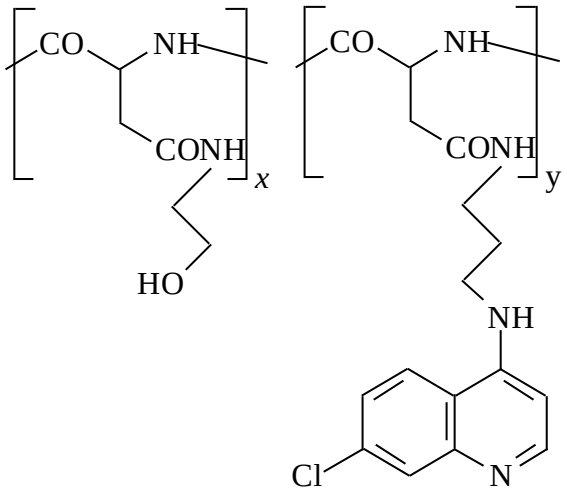
5.4.4. PSI. DEP (90) PDA.Q (10)



PSI (300 mg, 3.19 mmol) was dissolved in 5 mL of DMF, and PDA.Q (82 mg, 0.35 mmol) was added, followed by TEA (36 mg, 0.35 mmol). The solution was flushed with nitrogen gas, and stirred overnight at room temperature. DEP (374 mg, 2.87 mmol) was added, flushed with nitrogen gas, and stirred at room temperature for 2 d. The solution was concentrated on the rotary evaporator at 60ºC to afford a viscous liquid, which, was precipitated with ethylether:hexane (2:1) solution (15 mL). The precipitate was washed twice with boiling toluene, and once with boiling acetone. The precipitate was dissolved in distilled water, and pH was adjusted to 7-8 with hydrochloric acid, followed by dialysis against distilled water for 2 days with 12000 membrane tubing. It was freeze-dried to afford water soluble solid, 500 mg (69%). The ^{1}H NMR spectrum showed that there was a 96% incorporation of PDA.Q, and 100% incorporation of DEP.

^{1}H NMR (D_2O) δ/ppm: 8.25-6.08, 4.8H (5H; Ar-H); 3.15, 18H (20H; CONH**CH_2**); 2.45-2.43, 73H (76H; **CH_2**CH_2**CH_2**N(**CH_2**$CH_3)_2$, and **CH_2**CH_2**CH_2**NHAr); 1.65-1.63, 21H (20H; CH_2**CH_2**$CH_2N(CH_2CH_3)_2$, CH_2**CH_2**CH_2NHAr); 0.95, 54H (54H; $CH_2CH_2N(CH_2$**$CH_3)_2$**).

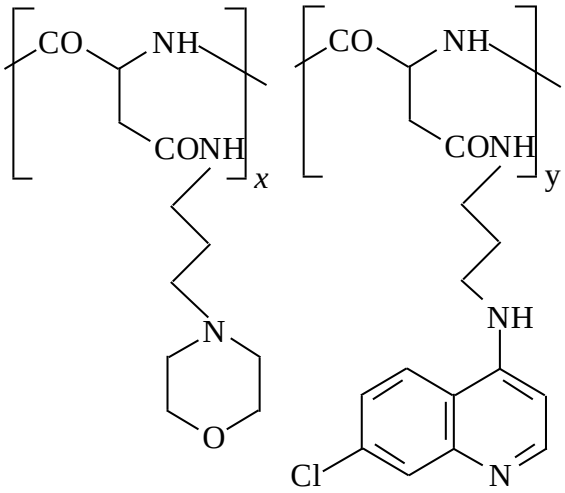
5.4.5 PSI. EA (90). PDA.Q (10)



PSI (300 mg, 3.19 mmol) was dissolved in 5 mL of DMF, and PDA.Q (82 mg, 0.35 mmol) was added, followed by TEA (36 mg, 0.35 mmol), and the solution was then flushed with nitrogen gas, and stirred at room temperature overnight. EA (175 mg, 2.87 mmol) was added, and the resultant solution was flushed with nitrogen gas, and stirred at room temperature for two days. The resultant solution was concentrated on the rotary evaporator at 60º C, and allowed to cool after which precipitation was done using diethyl ether: acetone (2:1) (20 mL). The precipitate was washed twice with boiling toluene, and once with boiling acetone. The precipitate was dissolved in 15 mL of distilled water, and pH adjusted to 7-8 with hydrochloric acid, after which, dialysis was performed using 12000 membrane tubing for two days against distilled water, followed by freeze-drying to afford a water soluble white solid, 400 mg (74%). The 'H NMR spectrum showed a 70% incorporation of PDA.Q, and a 94% incorporation of EA.

'H NMR (D_2O) δ/ppm: 8.00-6.00, 3.5H (5H, Ar-H); 3.63, 17H (18H; $CONHCH_2$ **CH_2**OH); 3.33, 18H (20H; $CONH$**CH_2**); 2.89-2.71, 18H (22H; **CH_2**CH_2**CH_2**$NHAr$, **CH_2**CH_2OH); 1.78-1.70, 2H (2H; CH_2**CH_2**CH_2NHAr).

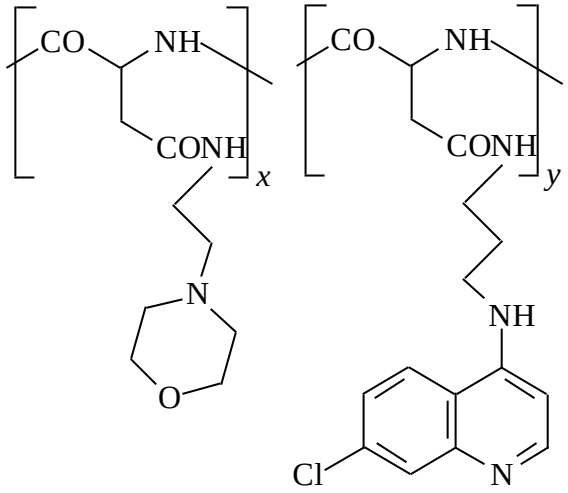
5.4.6 PSI APM (90) PDA.Q (10)



PSI (300 mg, 3.19 mmol) was dissolved in 5 mL of DMF, and PDA.Q (82 mg, 0.35 mmol) was added, followed by TEA (36 mg, 0.35 mmol). The solution was flushed with nitrogen gas, and stirred at room temperature overnight. APM (414 mg, 2.87 mmol) was added, and the resultant solution was flushed with nitrogen gas, and stirred at room temperature for two days. The resultant solution was concentrated on the rotary evaporator at 60ºC, and allowed to cool after which, precipitation was done using diethyl ether: acetone (2:1) (20 mL). The precipitate was washed twice with boiling toluene, and once with boiling acetone. The precipitate was dissolved in 15 mL of distilled water, and the pH was adjusted to 7-8 with hydrochloric acid after which, dialysis was performed using 12000 membrane tubing for two days against distilled water, followed by freeze-drying to afford a water soluble white solid 500 mg (65%). The 'H NMR spectrum showed a 100% incorporation of PDA.Q, and a 100% incorporation of APM.

'H NMR (D_2O) δ/ppm: 8.14-6.11, 5H (5H; Ar-H); 3.72, 35H (36H; **CH_2**O**CH_2**); 3.18-3.17, 22H (20H; $CONH$**CH_2**); 2.87-2.60, 18H (22H; **CH_2**CH_2**CH_2**N); 2.51-2.49, 52H (54H; $N($**CH_2**$)_3$), 1.68-1.67, 20H (20H; CH_2**CH_2**CH_2).

5.4.7 PSI.AEM (90) PDA.Q (10)

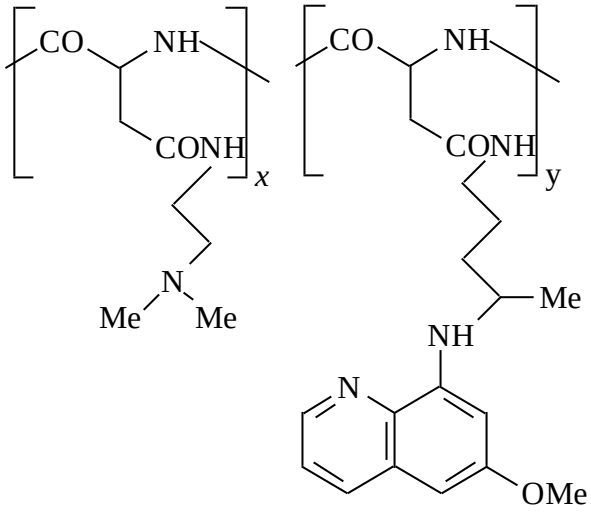


PSI (300 mg, 3.19 mmol) was dissolved in 5 mL of DMF, and PDA.Q (82 mg, 0.35 mmol) was added, followed by TEA (36 mg, 0.35 mmol). The solution was flushed with nitrogen gas, and stirred overnight at room temperature. AEM (374 mg, 2.87 mmol) was added, flushed with nitrogen gas, and stirred at room temperature for 2 d. The resultant solution was then concentrated on the rotary evaporator at 60ºC to afford a viscous liquid, which, was precipitated with diethyl ether:acetone (2:1) solution (15 mL). The precipitate was washed twice with boiling toluene, and once with boiling acetone. The precipitate was dissolved in distilled water, and the pH was adjusted to 7-8 with hydrochloric acid, followed by dialysis against distilled water for 2 d with 12000 membrane tubing. It was freeze-dried to afford water soluble solid, 400 mg (55%). The ^{1}H NMR spectrum showed a 90% incorporation of PDA.Q, and 82% incorporation of AEM.

^{1}H NMR (D_2O) δ/ppm: 8.08-6.08, 4.5H (5H; Ar-H); 3.74, 30H (36H; **CH_2OCH_2**); 3.36-3.30, 19H (20H; CONH**CH_2**); 2.74, 16H (22H; CH_2CH_2**CH_2NHAr** and **CH_2**$CH_2N(CH_2)_2$); 2.50, 44H (54H; CH_2N(**CH_2**$)_3$); 1.68, 2H (2H; CH_2**CH_2**CH_2NHAr).

5.5. SYNTHESIS OF HOMOPOLYMERS WITH PRIMAQUINE

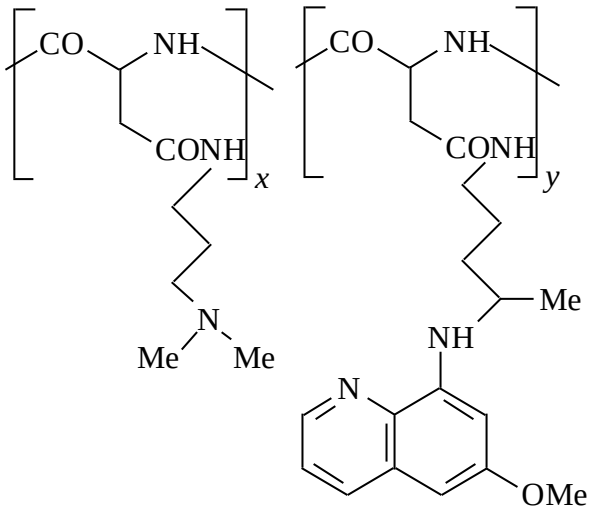
5.5.1 PSI. DME (90) PRIMAQUINE (10)



PSI (500 mg, 5.32 mmol) was dissolved in 7 mL of DMF, and Primaquine free base (152 mg, 0.59 mmol) was added, followed by TEA (65 mg, 0.59 mmol). The solution was then flushed with nitrogen gas, and stirred overnight at room temperature. DME (469 mg, 5.32 mmol) was added, flushed with nitrogen gas, and stirred at room temperature for 2 d. The solution was concentrated on the rotary evaporator to afford a viscous liquid, which was precipitated with diethyl ether:acetone (2:1) solution (15 mL). The precipitate was washed twice with boiling toluene, and once with boiling acetone. The precipitate was then dissolved in distilled water, and the pH was adjusted to 7-8 with hydrochloric acid, followed by dialysis for 2 d with 12000 membrane tubing. It was freeze-dried to afford water soluble solid, 770 mg (74%). The ^{1}H NMR spectrum showed a 100% incorporation of Primaquine, and 99% incorporation of DME.

^{1}H NMR (D_2O) δ/ppm: 8.55-6.09, 5H (5H; Ar-H); 3.51, 21H (20H; CONH**CH_2**); 2.89-2.59, 91H (92H; **CH_2CH_2**N(**CH_3**)$_2$ and **CH_2**CH_2CH_2CH**(**CH_3**)**NHAr); 1.63-1.26, 7H (7H; CH_2**CH_2CH_2**CH**(CH$_3$)** NHAr)

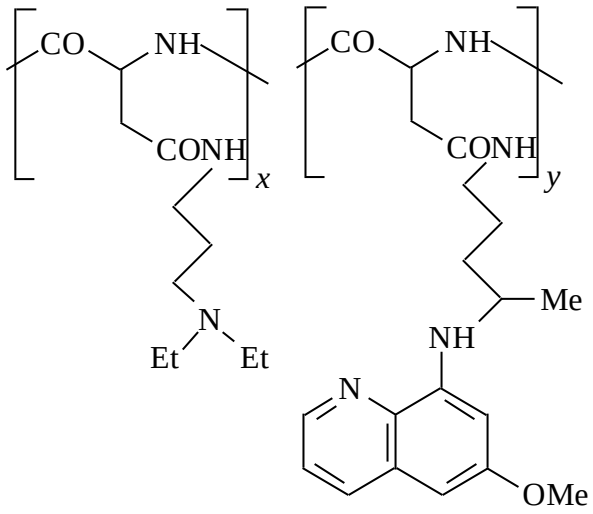
5.5.2 PSI. DMP (90) PRMAQUINE (10)



PSI (200 mg, 2.13 mmol) was dissolved in 3 mL of DMF, and primaquine free base (61 mg, 0.23 mmol) was added, followed by TEA (24 mg, 0.23 mmol). The solution was flushed with nitrogen gas, and stirred overnight at room temperature. DMP (195 mg, 1.91 mmol) was added, flushed with nitrogen gas, and stirred at room temperature for 2 d. The resultant solution was concentrated on the rotary evaporator at 60ºC to afford a viscous liquid, which was precipitated with diethyl ether:acetone (2:1) solution (15 mL). The precipitate was washed twice with boiling toluene, and once with boiling acetone. The precipitate was dissolved in distilled water, and the pH was adjusted to 7-8 with hydrochloric acid, followed by dialysis against distilled water for 2 days with 12000 membrane tubing. It was freeze-dried to afford water soluble solid, 300 mg (67%). The ^{1}H NMR spectrum showed a 100% incorporation of primaquine and 88% of DMP.

^{1}H NMR (D_2O) δ/ppm: 8.31-6.02, 5H (5H, Ar-H); 3.19, 24H (20H; CONH**CH_2**); 2.89, 17H (20H; **CH_2**CH_2CH_2N); 2.12, 63H (72H; CH_2CH_2**CH_2**N(**CH_3**$)_2$); 1.63-0.92, 25H (25H; CH_2**CH_2CH_2**CH**(CH_3)**, CH_2**CH_2**$CH_2N(CH_3)_2$).

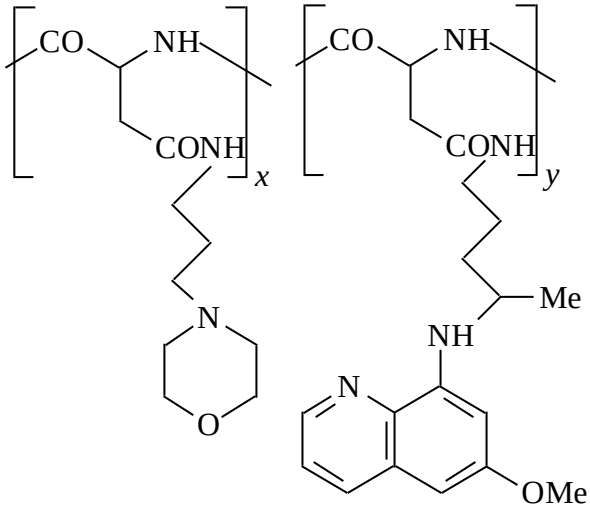
5.5.3 PSI. DEP (90) PRIMAQUINE (10)



PSI (200 mg, 2.13 mmol) was dissolved in 3 mL of DMF, and Primaquine free base (61 mg, 0.23 mmol) was added, followed by TEA (24 mg, 0.23 mmol). The solution was flushed with nitrogen gas, and stirred overnight at room temperature. DEP (249 mg, 1.91 mmol) was added, flushed with nitrogen gas, and stirred at room temperature for 2 d. The solution was concentrated on the rotary evaporator at 60ºC to afford a viscous liquid, which was precipitated with diethyl ether:acetone (2:1) solution (15 mL). The precipitate was washed twice with boiling toluene, and once with boiling acetone. The precipitate was dissolved in distilled water, and the pH was adjusted to 7-8 with hydrochloric acid, followed by dialysis against distilled water for 2 days with 12000 membrane tubing. It was then freeze-dried to afford water soluble solid, 200 mg (41%). ^{1}H NMR spectrum showed that there was a100% incorporation of DEP and Primaquine.

^{1}H NMR (D_2O) δ/ppm: 8.46-6.19, 5H (5H; Ar-H); 3.15-3.12, 94H (94H; CONH$\mathbf{CH_2}$, $\mathbf{CH_2}CH_2CH_2$, $CH_2CH_2\mathbf{CH_2}N(\mathbf{CH_2}CH_3)_2$); 1.95-1.93 20H (22H; $CH_2\mathbf{CH_2}CH_2N(CH_2CH_3)_2$ and $CH_2\mathbf{CH_2}\mathbf{CH_2}CH\mathbf{(}CH_3)NHAr$), 1.29, 57H (57H, $CH_2CH_2CH_2N(CH_2\mathbf{CH_3})_2$ and $CH_2CH_2CH_2CH\mathbf{(CH_3)}NHAr$)

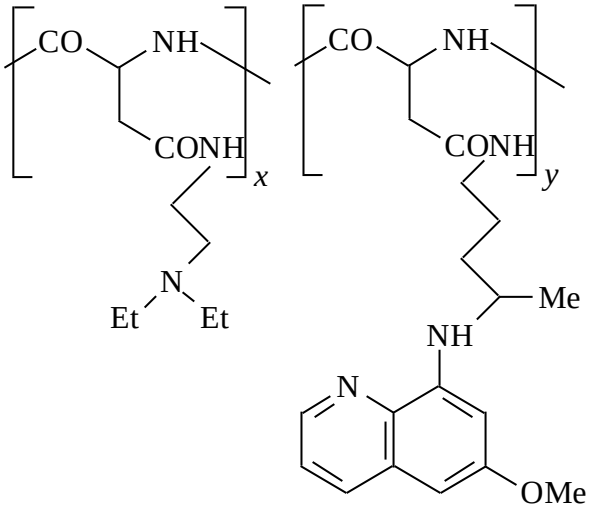
5.5.4. PSI. APM (90) PRIMAQUINE (10)



PSI (200 mg, 2.13 mmol) was dissolved in 4 mL of DMF, and primaquine free base (61 mg, 0.23 mmol) was added, followed by TEA (24 mg, 0.23 mmol). The solution was flushed with nitrogen gas, and stirred overnight at room temperature. APM (276 mg, 1.91 mmol) was added, flushed with nitrogen gas, and stirred at room temperature for 2 d. The resultant solution was concentrated on the rotary evaporator at 60ºC to afford a viscous liquid, which was precipitated with diethyl ether:acetone (2:1) solution (15 mL). The precipitate was washed twice with boiling toluene, and once with boiling acetone. The precipitate was dissolved in distilled water, and the pH was adjusted to 7-8 with hydrochloric acid, followed by dialysis for 2 days with 12000 membrane tubing. It was freeze- dried to afford water soluble solid, 320 mg (62%). ^{1}H NMR spectrum showed that there was 92% incorporation of APM and 98% of primaquine.

^{1}H NMR (D_2O) δ/ppm: 8.40-6.14, 5H (5H; Ar-H); 3.72, 36H (36H; **CH_2OCH_2**); 3.19-3.18, 20H (20H; CONH**CH_2**); 2.74-2.49, 68H (74H; **CH_2**CH_2CH_2, CH_2CH_2**CH_2**N(**CH_2**$)_2$); 1.68-1.67, 25H (25H; CH_2**CH_2**$CH_2N(CH_2)_2$ and $CHCH_2$**CH_2CH_2** CH(**CH_3**) NHAr).

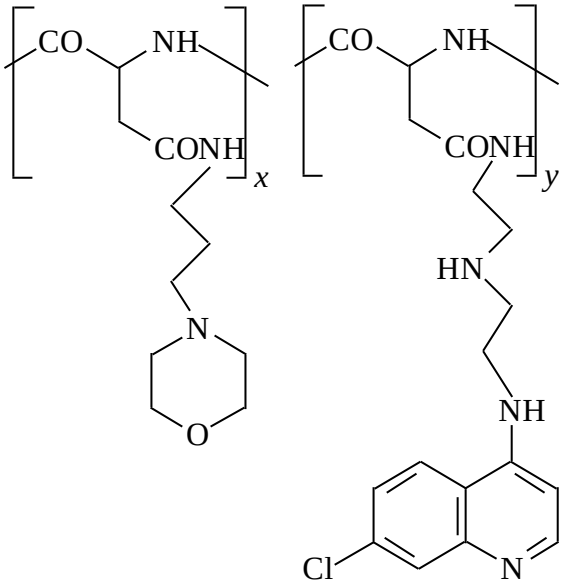
5.5.5 PSI. DEEA (90) PRIMAQUINE (10)



PSI (300 mg, 3.19 mmol) was dissolved in 4 mL of DMF, and Primaquine free base (91 mg, 0.35 mmol) was added, followed by TEA (36 mg, 0.35 mmol). The solution was flushed with nitrogen gas, and stirred overnight at room temperature. DEEA (334 mg, 2.87 mmol) was added, flushed with nitrogen gas, and stirred at room temperature for 2 d. The solution was concentrated on the rotary evaporator at 60ºC to afford a viscous liquid, which was precipitated with diethyl ether:acetone (2:1) solution (15 mL). The precipitate was washed twice with boiling toluene, and once with boiling acetone. The precipitate was then dissolved in distilled water, and the pH was adjusted to 7-8 with hydrochloric acid, followed by dialysis for 2 days with 12000 membrane tubing. It was freeze-dried to afford water soluble solid, 360 mg (51%). ^{1}H NMR spectrum showed that there was 100% incorporation of DEEA, and 22% of Primaquine.

^{1}H NMR (D_2O) δ/ppm: 8.53-6.37, 1.1H (5H; Ar-H); 3.32, 21H (20H; CONH**CH_2**); 2.79-2.75, 82H (74H; **CH_2CH_2**N(**CH_2**CH_3)$_2$ and **CH_2**CONH); 1.70, 2H (4H; CH_2**CH_2CH_2**CH(CH_3)NHAr); 1.28-1.02, 57H (57H; $CH_2CH_2CH_2$CH(**CH_3**)NHAr, CH_2CH_2N(CH_2**CH_3**)$_2$).

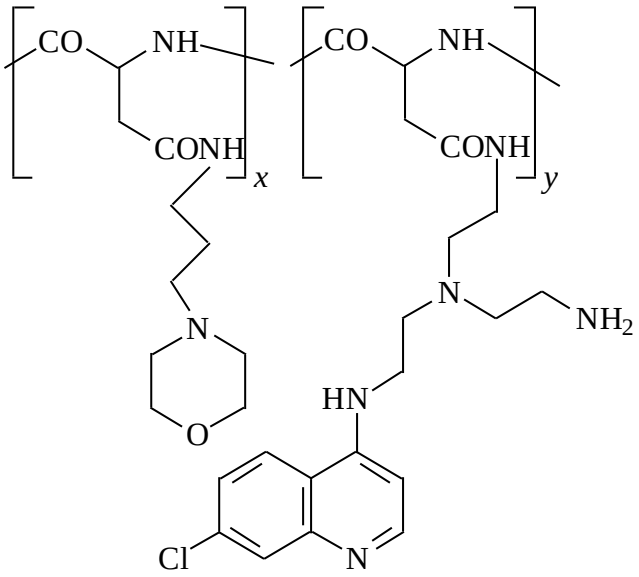
5.6 HOMOPLOYMERS WITH DET.Q (PSI. APM (90) DET.Q (10))



PSI (300 mg, 3.19 mmol) was dissolved in 4 mL of DMF, and DET.Q (100 mg, 0.38 mmol) was added, followed by TEA (36 mg, 0.35 mmol). The solution was flushed with nitrogen gas, and stirred overnight at room temperature. APM (368 mg, 2.55 mmol) was added, flushed with nitrogen gas, and stirred at room temperature for 2 d. The resultant solution was then concentrated on the rotary evaporator at 60ºC to afford a viscous liquid, which was precipitated with diethyl ether:acetone (2:1) solution (15 mL). The precipitate was washed twice with boiling toluene, and once with boiling acetone. The precipitate was dissolved in distilled water, and the pH was adjusted to 7-8 with hydrochloric acid, followed by dialysis for 2 days with 12000 membrane tubing. It was freeze- dried to afford water soluble solid, 520 mg (67%). ^{1}H NMR spectrum showed that there was 90% incorporation of DET.Q, and 99% incorporation of APM.

^{1}H NMR (D_2O) δ/ppm: 8.17-6.33, 4.5H (5H; Ar-H); 3.73, 35H (36H; $\mathbf{CH_2}O\mathbf{CH_2}$); 3.20-3.18, 22H (20H; CONH$\mathbf{CH_2}$); 2.78-2.76 26H (26H; $\mathbf{CH_2CH_2}$NH$\mathbf{CH_2CH_2}$NHAR, $\mathbf{CH_2}CH_2CH_2N(CH_2)_2$), 2.52-2.36, 54H (54H; $CH_2CH_2\mathbf{CH_2}N(\mathbf{CH_2})_2$), 1.70-1.68, 18H (18H; $CH_2\mathbf{CH_2}CH_2N(CH_2)_2$)

5.7 HOMOPOLYMERS WITH TRIS.Q (PSI. APM (90).TRIS.Q (10))

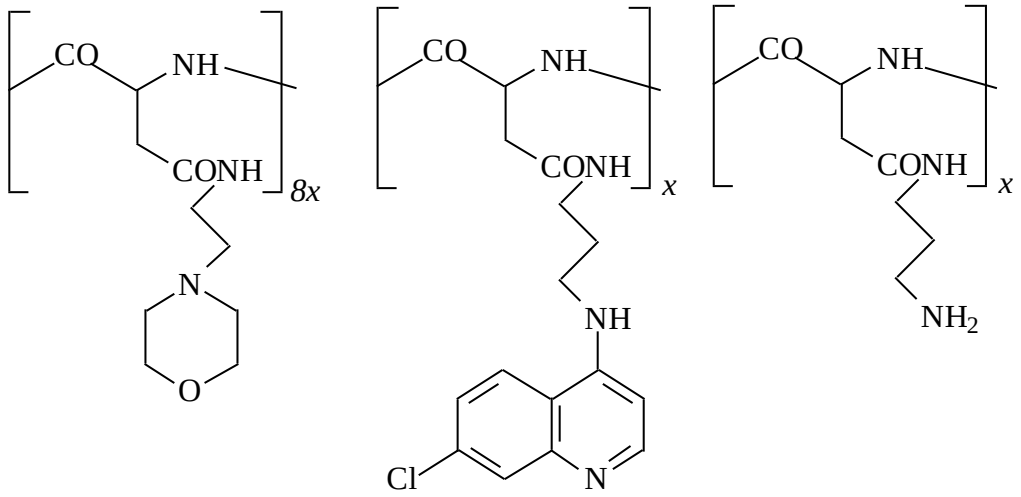


PSI (200 mg, 2.13 mmol) was dissolved in 3 mL of DMF, and APM (276 mg, 1.91 mmol) was added, followed by TEA (23 mg, 0.23 mmol). The solution was flushed with nitrogen gas, and stirred overnight at room temperature. The above solution was added drop-wise to the solution of TRIS.Q (71 mg, 0.23 mmol), in 6 mL of DMF, in an ice-bath. The solution was flushed with nitrogen gas, and stirred at room temperature for 2 d, followed by concentration on the rotary evaporator to afford a viscous liquid, which was precipitated with diethyl ether:acetone (2:1) solution (15 mL). The precipitate was washed twice with boiling toluene, and once with boiling acetone. The precipitate was dissolved in distilled water, and the pH was adjusted to 7-8 with hydrochloric acid, followed by dialysis in distilled water for 2 days with 12000 membrane tubing. It was freeze-dried to afford water soluble solid, 500 mg (94%). ^{1}H NMR spectrum showed that there was a 42% incorporation of TRIS.Q, and 94% incorporation of APM.

^{1}H NMR (D_2O) δ/ppm: 8.40-6.30, 2.1H (5H; Ar-H); 3.20, 19H (20H; CONH**CH_2**); 3.75, 32H (36H; **CH_2OCH_2**); 2.79-2.38, 79H (84H; **CH_2**CONH, CH_2CH_2**CH_2**N(**CH_2**)**$_2$** and **CH_2CH_2**N(**CH_2CH_2**)$_2$); 1.71-1.70, 18H (18H; CH_2**CH_2**CH_2N(CH_2)$_2$).

5.8 SYNTHESIS OF CO-POLYMERS FOR ANTICANCER DRUG ANCHORING

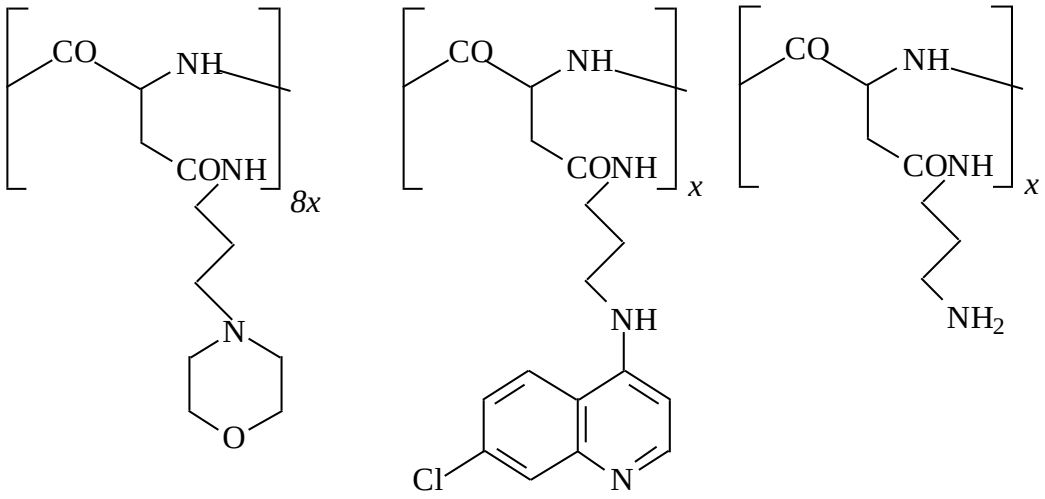
5.8.1 PSI.AEM (80) PDA.Q (10) PDA (10)



PSI (300 mg, 3.19 mmol) was dissolved in 5 mL of DMF, and PDA.Q (82 mg, 0.35 mmol) was added, followed by TEA (36 mg, 0.35 mmol). The solution was the stirred overnight at room temperature after flushing with nitrogen gas. AEM (332 mg, 2.55 mmol) was then added, flushed with nitrogen gas, and stirred overnight at room temperature. The resultant solution was added drop-wise to a solution of PDA (26 mg, 0.35 mmol), in 4 mL of DMF, in an ice-bath. The resultant solution was flushed with nitrogen gas, and stirred overnight in an ice-bath, followed by stirring for one day at room temperature. The solution was concentrated on the rotary evaporator at 60°C, and allowed to cool before precipitation with diethyl ether:acetone (2:1) (10 mL). The precipitate was washed twice with boiling toluene, and once with boiling acetone. The precipitate was dissolved in 10 mL of distilled water, and the pH was then adjusted to 7-8 with hydrochloric acid before dialysis against water with 12000 membrane tubing for two days. It was freeze-dried to afford water soluble solid 490 mg (68%), and the ^{1}H NMR spectrum showed that there was 94% incorporation of AEM, 90% of PDA.Q, and 100% of PDA.

^{1}H NMR (D_2O) δ/ppm: 8.11-6.10, 4.7H (5H; Ar-H); 3.74, 30H (32H, **CH_2OCH_2)**; 3.35, 19H (20H, CONH**CH_2**); 2.55-2.50, 52H (72H, **CH_2CH_2**N(**CH_2**)$_2$, **CH_2**CH_2**CH_2**N); 1.80-1.60, 4H (4H, CH_2**CH_2**CH_2NHAR and CH_2**CH_2**CH_2NH_2).

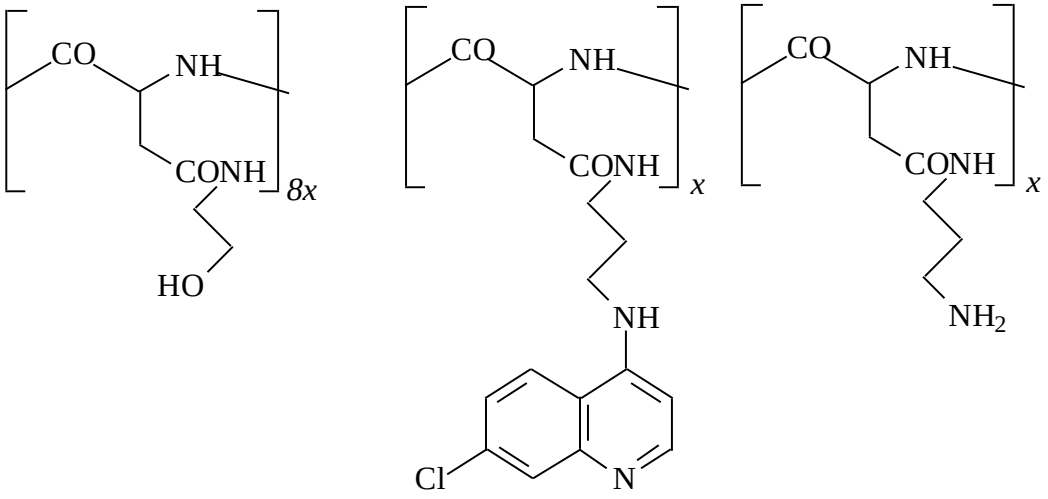
5.8.2 PSI.APM (80) PDA.Q (10) PDA (10)



PSI (300 mg, 3.19 mmol) was dissolved in 5 mL of DMF, and PDA.Q (82 mg, 0.35 mmol) was added, followed by TEA (36 mg, 0.35 mmol). The solution was stirred overnight at room temperature after flushing with nitrogen gas. APM (368 mg, 2.55 mmol) was added, flushed with nitrogen gas, and stirred overnight at room temperature. The above resultant solution was added drop wise to a solution of PDA (26 mg, 0.35 mmol), in 4 mL of DMF, in an ice-bath. The solution was flushed with nitrogen gas, and stirred overnight in an ice-bath, followed by stirring for one day at room temperature. The solution was concentrated on the rotary evaporator at 60ºC, and allowed to cool before precipitation with diethyl ether:acetone (2:1) (10 mL). The precipitate then washed twice with boiling toluene and once with boiling acetone. The precipitate was dissolved in (10 mL) of distilled water, and the pH was adjusted to 7-8 with hydrochloric acid before dialysis against distilled water with 12000 membrane tubing for two days. It was freeze-dried to afford water soluble solid, 640 mg (85%), and the ^{1}H NMR spectroscopy showed that there was an 87% incorporation of APM, 92% of PDA.Q, and 87% of PDA.

^{1}H NMR (D_2O) δ/ppm: 8.30-6.11, 4.6H (5H; Ar-H); 3.73, 28H (32H, **CH_2OCH_2**); 3.21-3.19, 22H (20H, CONH**CH_2**), 2.51-2.49, 62H (72H; **$CH_2CH_2CH_2N(CH_2)_2$**, **$CH_2CH_2CH_2$**NHAR, and **$CH_2CH_2CH_2NH_2$**), 1.66-1.65, 20H (20H; CH_2**CH_2**CH_2NHAR, CH_2**CH_2**CH_2NH_2 and CH_2**CH_2**$CH_2N(CH_2)_2$).

5.8.3 PSI. EA (80) PDA.Q (10) PDA (10)

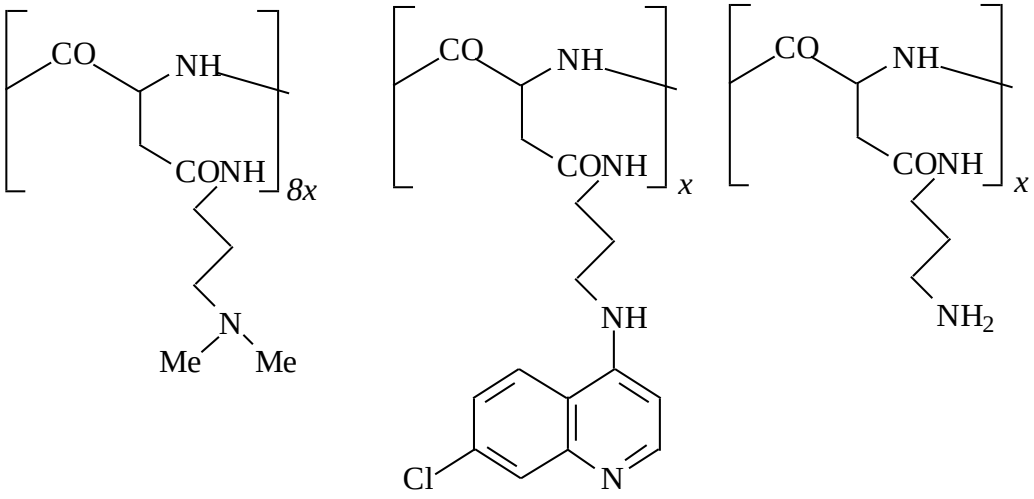


PSI (300 mg, 3.19 mmol) was dissolved in 5 mL of DMF, and PDA.Q (82 mg, 0.35 mmol) was added, followed by TEA (36 mg, 0.35 mmol). The resultant solution was stirred overnight at room temperature after flushing with nitrogen gas. EA (156 mg, 2.55 mmol) was added, flushed with nitrogen gas, and stirred overnight at room temperature. The above resultant solution was added drop-wise to a solution of PDA (26 mg, 0.35 mmol), in 4 mL of DMF, in an ice-bath. The resultant solution was flushed with nitrogen gas, and stirred overnight in an ice-bath, followed by stirring for one day at room temperature. The solution was concentrated on the rotary evaporator at 60ºC, and allowed to cool before precipitation with diethyl ether:acetone (2:1) (10 mL). The precipitate was washed twice with boiling toluene, and once with boiling acetone. The precipitate was dissolved in 10 mL of distilled water, and pH was then adjusted to 7-8 with hydrochloric acid before dialysis against distilled water with 12000 membrane tubing for two days. It was freeze-dried to afford a water soluble solid, 400 mg (73%), and the ^{1}H NMR

spectroscopy showed that there was an 81% incorporation of EA, 75% of PDA.Q, and 58% of PDA.

^{1}H NMR (D_2O) δ/ppm: 8.20-6.00, 3.8H (5H; Ar-H); 3.63, 13H (16H, CH_2**CH_2**OH), 3.33, 17H (20H; CONH**CH_2**), 2.75-2.71, 14H (24H; **CH_2**CH_2OH, **CH_2**CH_2**CH_2**NHAR, and **CH_2**CH_2**CH_2**NH_2), 1.60-1.59, 4H (4H; CH_2**CH_2**CH_2NHAR, CH_2**CH_2**CH_2NH_2).

5.8.4 PSI. DMP (80) PDA.Q (10) PDA (10)

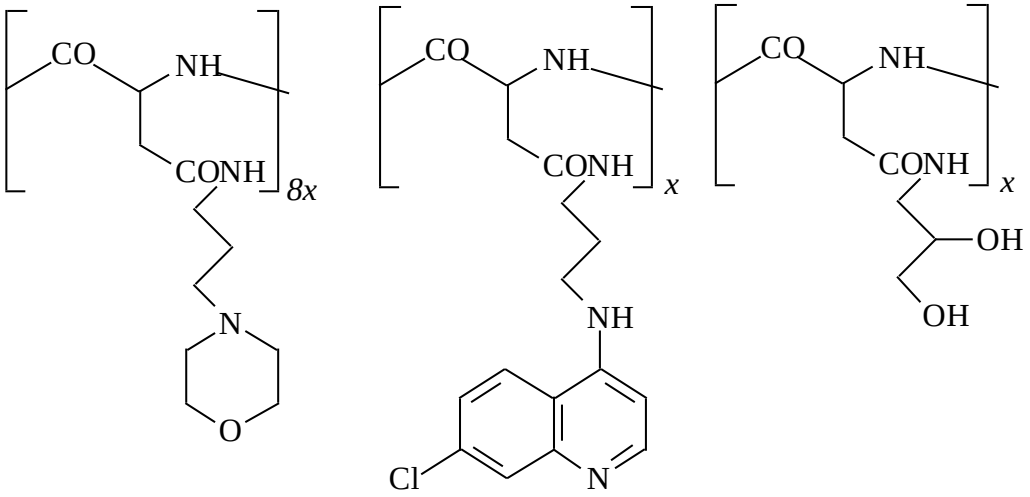


PSI (300 mg, 3.19 mmol) was dissolved in 5 mL of DMF, and PDA.Q (82 mg, 0.35 mmol) was added, followed by TEA (36 mg, 0.35 mmol). The solution was stirred overnight at room temperature after flushing with nitrogen gas. DMP (261 mg, 2.55 mmol) was added, flushed with nitrogen gas, and stirred overnight at room temperature. The solution was then added drop-wise to a solution of PDA (26 mg, 0.35 mmol), in 4 mL of DMF, in an ice-bath. The resultant solution was flushed with nitrogen gas, and stirred overnight in an ice-bath, followed by stirring for one day at room temperature. The solution was then concentrated on the rotary evaporator at 60ºC, and allowed to cool before precipitation with diethyl ether:acetone (2:1) (10 mL). The precipitate was washed twice with boiling toluene and once with boiling acetone. The precipitate was then dissolved in 10 mL of distilled water, and the pH was then adjusted to 7-8 with hydrochloric acid before dialysis with 12000 membrane tubing for two days. It was

freeze-dried to afford water soluble solid, 460 mg (71%), and the ^{1}H NMR spectroscopy showed that there was 96% incorporation of DMP, 88% of PDA.Q, and 96% of PDA.

^{1}H NMR (D_2O) δ/ppm: 8.10-6.11, 4.4H (5H; Ar-H); 3.24-3.22, 23H (20H; CONH**CH_2**); 2.75-2.43, 84H (88H; **CH_2**CH_2**CH_2**N(**CH_3**)$_2$, **CH_2**CH_2**CH_2**NHAR and **CH_2**CH_2**CH_2**NH_2), 1.77-1.76, 20H (20H, CH_2**CH_2**CH_2NHAR and CH_2**CH_2**CH_2NH_2).

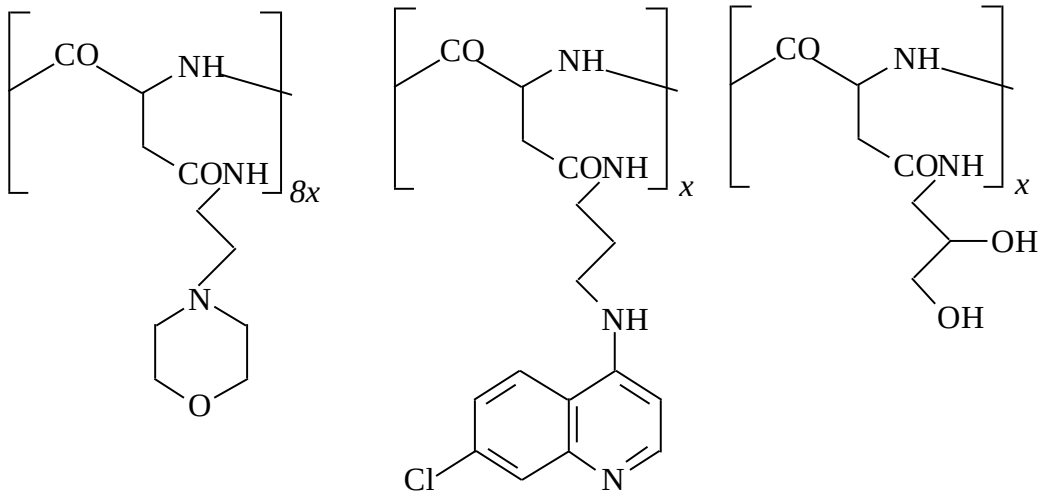
5.8.5 PSI. APM (80) PDA.Q (10) APD (10)



PSI (300 mg, 3.19 mmol) was dissolved in 5 mL of DMF, and PDA.Q (82 mg, 0.35 mmol) was added, followed by TEA (36 mg, 0.35 mmol). The solution was stirred at room temperature after flushing with nitrogen gas for 6 h. APM (368 mg, 2.55 mmol) and APD (32 mg, 0.35 mmol) were added and the resultant solution was flushed with nitrogen gas, and stirred overnight at room temperature. The solution was concentrated on the rotary evaporator at 60ºC, and allowed to cool before precipitation with diethyl ether:acetone (2:1) (10 mL). The precipitate was washed twice with boiling toluene, and once with boiling acetone. The precipitate was dissolved in 10 mL of distilled water, and the pH was adjusted to 7-8 with hydrochloric acid before dialysis against distilled water with 12000 membrane tubing for two days followed. It was freeze-dried to afford water soluble solid, 510 mg (67%), and the ^{1}H NMR spectroscopy showed that there was a 96% incorporation of APM, 83% of PDA.Q, and 100% of APD.

^{1}H NMR (D_2O) δ/ppm: 8.20-6.10, 4.2H (5H; Ar-H); 3.73, 36H (37H; **$CH_2O$$CH_2$**, **$CH_2CH($**OH)**$CH_2$**OH); 3.21-3.19, 22H (20H; CONH**CH_2**); 2.80, 20H (20H; **CH_2**CONH, CH_2CH_2**CH_2**NHAr); 46H (48H; CH_2CH_2**CH_2**N(**CH_2**)$_2$); 1.69-1.68, 18H (18H; CH_2**CH_2**CH_2NHAR and CH_2**CH_2**$CH_2N(CH_2)_2$).

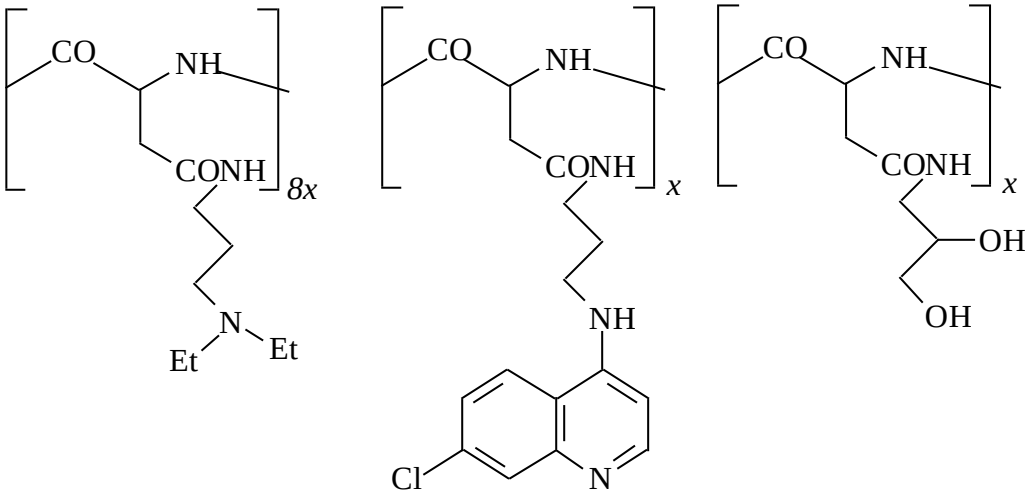
5.8.6 PSI.AEM (80) PDA.Q (10) APD (10)



PSI (300 mg, 3.19 mmol) was dissolved in 5 mL of DMF, and PDA.Q (82 mg, 0.35 mmol) was then added, followed by TEA (36 mg, 0.35 mmol). The solution was stirred at room temperature after flushing with nitrogen gas for 6 h. AEM (332 mg, 2.55 mmol) and APD (32 mg, 0.35 mmol) were added, and the resultant solution was flushed with nitrogen gas, and stirred overnight at room temperature. The solution was concentrated on the rotary evaporator at 60ºC, and allowed to cool before precipitation with diethyl ether:acetone (2:1) (10 mL). The precipitate was washed twice with boiling toluene and once with boiling acetone. The precipitate was dissolved in 10 mL of distilled water, and the pH was adjusted to 7-8 with hydrochloric acid before dialysis against distilled water with 12000 membrane tubing for two days. It was freeze-dried to afford water soluble solid, 330 mg (43%), and the ^{1}H NMR spectroscopy showed that there was a 80% incorporation of AEM, 100% of PDA.Q, and 83% of APD.

^{1}H NMR (D_2O) δ/ppm: 8.10-6.11, 5H (5H, 5Ar-H); 3.74-3.72, 30H (36H; **$CH_2O$$CH_2$**, **$CH_2$**CH(OH)**$CH_2$**(OH)); 3.35-3.33, 20H (20H; CONH**CH_2**); 2.73-2.48, 52H (68H; **CH_2**CH_2**CH_2**NHAR and **CH_2CH_2**N(**CH_2**)$_2$); 1.63, 2H (2H, CH_2**CH_2**CH_2NHAR).

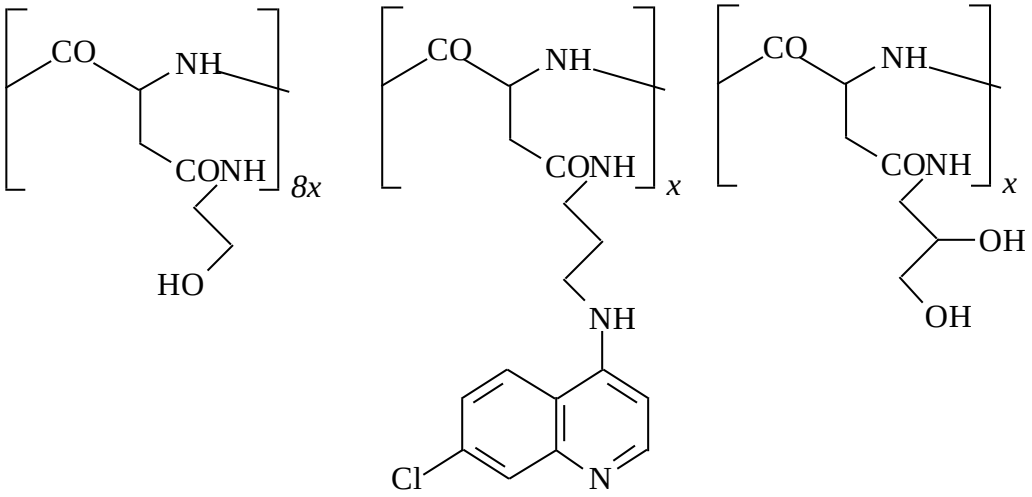
5.8.7 PSI.DEP (80) PDA.Q (10) APD (10)



PSI (300 mg, 3.19 mmol) was dissolved in 5 mL of DMF, and PDA.Q (82 mg, 0.35 mmol) was added, followed by TEA (36 mg, 0.35 mmol). The solution was stirred at room temperature after flushing with nitrogen gas for 6 h. DEP (332 mg, 2.55 mmol) and APD (32 mg, 0.35 mmol) were added, and the solution was flushed with nitrogen gas, and stirred overnight at room temperature. The solution was concentrated on the rotary evaporator at 60ºC, and allowed to cool before precipitation with diethyl ether:acetone (2:1) (10 mL). The precipitate was washed twice with boiling toluene, and once with boiling acetone. The precipitate was dissolved in 10 mL of distilled water, and the pH was adjusted to 7-8 with hydrochloric acid before dialysis with 12000 membrane tubing for two days. It was freeze-dried to afford water soluble solid 610 mg (84%), and the ^{1}H NMR spectroscopy showed that there was a 100% incorporation of DEP, 100% of PDA.Q, and 96% of APD.

^{1}H NMR (D_2O) δ/ppm: 8.30-6.20, 5H (5H; Ar-H); 3.82-3.59, 4.8H (5H; **$CH_2CH($**OH**$)CH_2OH$**); 3.25-3.24, 23H (20H; CONH**CH_2**); 2.80-2.77, 69H (68H; **CH_2**CH_2**CH_2**NHAR and **CH_2**CH_2**CH_2**N(**CH_2**$CH_3)_2$); 1.79-1.75, 18H (18H; CH_2**CH_2**CH_2NHAR and CH_2**CH_2**$CH_2N(CH_2CH_3)_2$); 1.20, 48H (48H; $CH_2CH_2CH_2N(CH_2$**CH_3**$)_2$).

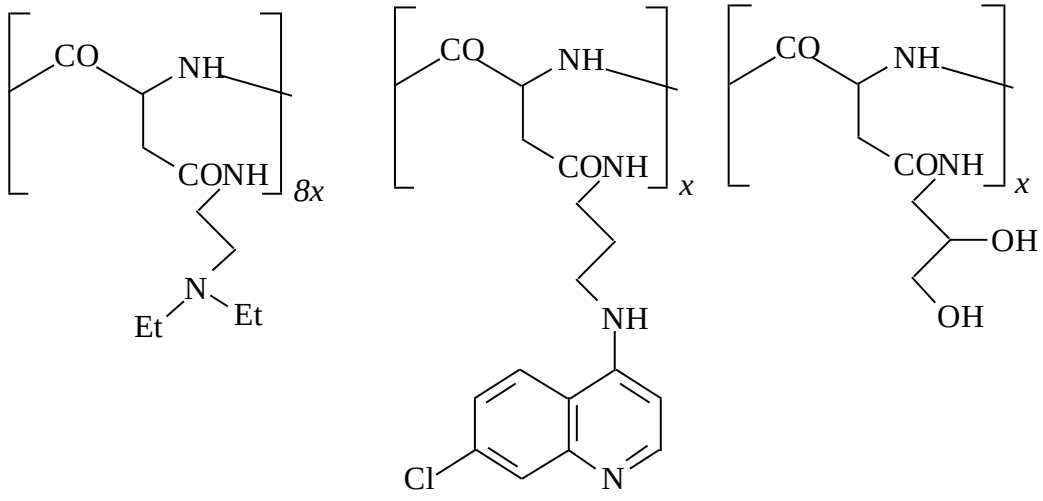
5.8.8 PSI. EA(80) PDA.Q (10) APD (10)



PSI (300 mg, 3.19 mmol) was dissolved in 5 mL of DMF, and PDA.Q (82 mg, 0.35 mmol) was then added, followed by TEA (36 mg, 0.35 mmol). The solution was stirred at room temperature after flushing with nitrogen gas for 6 h. EA (156 mg, 2.55 mmol) and APD (32 mg, 0.35 mmol) were added, and the resultant solution was then flushed with nitrogen gas, and stirred overnight at room temperature. The solution was concentrated on the rotary evaporator at 60ºC, and allowed to cool before precipitation with diethyl ether:acetone (2:1) (10 mL). The precipitate was washed twice with boiling toluene, and once with boiling acetone. The precipitate was dissolved in 10 mL of distilled water, and the pH was adjusted to 7-8 with hydrochloric acid before dialysis against water with 12000 membrane tubing for two days. Freeze-drying was performed to afford water soluble solid 200 mg (36%), and the ^{1}H NMR spectroscopy showed that there was a 72% incorporation of EA, 80% of PDA.Q, and 70% of APD.

^{1}H NMR (D_2O) δ/ppm: 7.90-5.90, 4H (5H; Ar-H); 3.79-3.58, 15H (20H; **CH_2CH(**OH)**CH_2OH**, CH_2**CH_2**OH); 3.32, 14H (20H; CONH**CH_2**); 2.72-2.71, 10H (20H; **CH_2**CH_2**CH_2**NHAR and **CH_2**CH_2OH); 1.60, 2H (2H; CH_2**CH_2**CH_2NHAr).

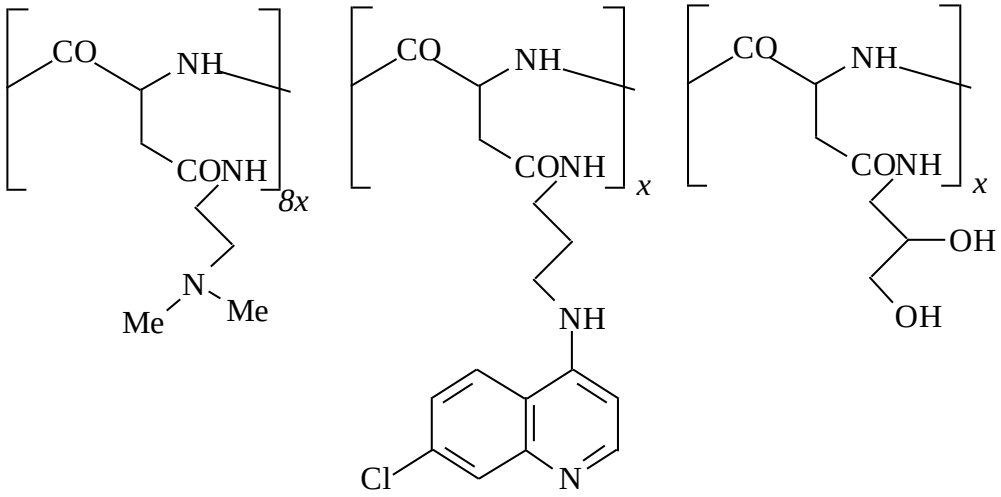
5.8.9. PSI.DEEA (80) PDA.Q (10) APD (10)



PSI (300 mg, 3.19 mmol) was dissolved in 5 mL of DMF, and PDA.Q (82 mg, 0.35 mmol) was added, followed by TEA (36 mg, 0.35 mmol). The solution was stirred at room temperature after flushing with nitrogen gas for 6 h. DEEA (297 mg, 2.55 mmol) and APD (32 mg, 0.35 mmol) were added, and the resultant solution was then flushed with nitrogen gas, and stirred overnight at room temperature. The solution was concentrated on the rotary evaporator at 60ºC, and allowed to cool before precipitation with diethyl ether:acetone (2:1) (10 mL). The precipitate was washed twice with boiling toluene, and once with boiling acetone. The precipitate was dissolved in 10 mL of distilled water, and the pH was adjusted to 7-8 with hydrochloric acid before dialysis against water with 12000 membrane tubing for two days. Freeze-drying was performed water soluble solid, 560 mg (82%), and the ^{1}H NMR spectroscopy showed that there was an 81% incorporation of DEEA, 92% of PDA.Q, and 93% of APD.

^{1}H NMR (D_2O) δ/ppm: 8.20-6.20, 4.6H (5H; Ar-H); 3.81, 2.8H (3H, CH_2**CH(**OH)**CH_2OH**); 3.59-3.37, 23H (22H; CONH**CH_2** and **CH_2**CH(OH)CH_2OH); 2.75-2.74, 54H (68H; **CH_2**CH_2**CH_2**NHAR and **$CH_2$$CH_2$**N(**$CH_2$**$CH_3$)$_2$); 1.62, 2H (2H; CH_2**CH_2**CH_2NHAr); 1.08, 39H (48H; $CH_2CH_2N(CH_2$**CH_3**$)_2$).

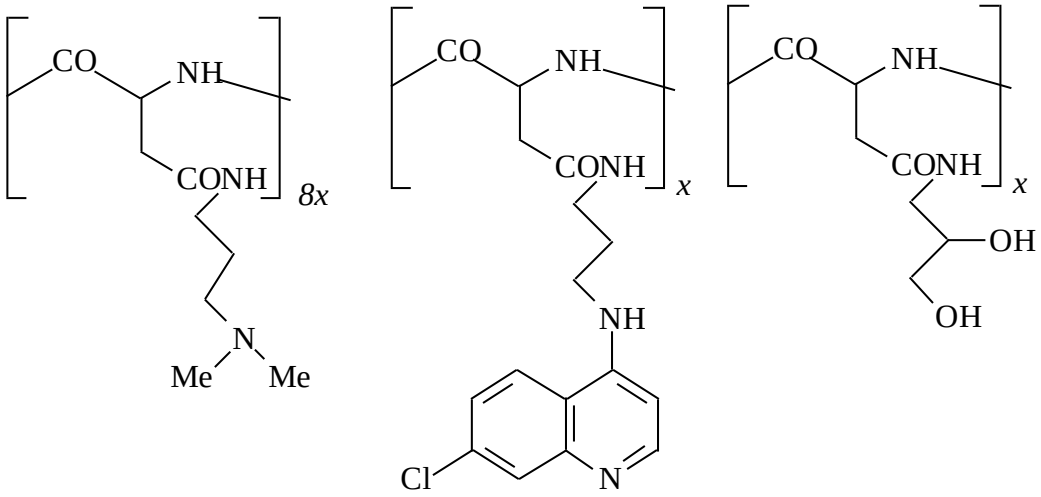
5.8.10 PSI.DME (80) PDA.Q (10) APD (10)



PSI (300 mg, 3.19 mmol) was dissolved in 5 mL of DMF, and PDA.Q (82 mg, 0.35 mmol) was added, followed by TEA (36 mg, 0.35 mmol). The solution was stirred at room temperature after flushing with nitrogen gas for 6 h. DME (225 mg, 2.55 mmol) and APD (32 mg, 0.35 mmol) were added, and the solution was flushed with nitrogen gas, and stirred overnight at room temperature. The solution was concentrated on the rotary evaporator at 60ºC, and allowed to cool before precipitation with diethyl ether:acetone (2:1) (10 mL). The precipitate was washed twice with boiling toluene, and once with boiling acetone. The precipitate was dissolved in 10 mL of distilled water, and the pH was then adjusted to 7-8 with hydrochloric acid before dialysis against water with 12000 membrane tubing for two days. Freeze-drying was performed to afford water soluble solid, 410 mg (90%), and the ^{1}H NMR spectroscopy showed that there was a 70% incorporation of DME, 88% of PDA.Q, and 100% of APD.

^{1}H NMR (D_2O) δ/ppm: 8.00-6.00, 4.4H (5H; Ar-H); 3.81, 3.2H (3H; CH_2**CH(**OH)**CH_2OH**); 3.40-3.36, 23H (23H; CONH**CH_2**, **CH_2**CH(OH)CH_2(OH)); 2.76-2.55, 57H (82H; **CH_2**CH_2**CH_2**NHAR, **CH_2CH_2**$N(CH_3)_2$ and CH_2CH_2N(**CH_3**)$_2$); 1.85-1.84, 2H (2H; CH_2**CH_2**CH_2NHAr).

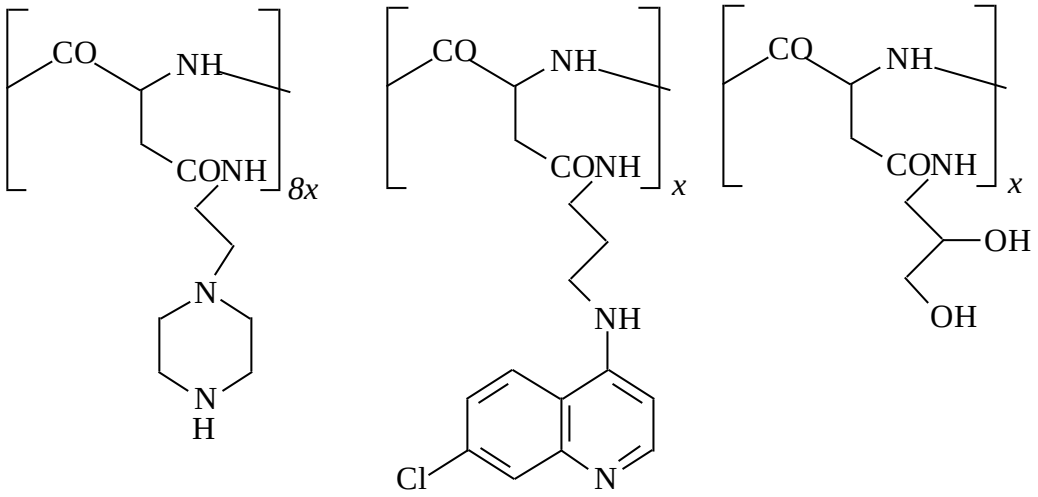
5.8.11 PSI.DMP (80) PDAQ (10) APD (10)



PSI (300 mg, 3.19 mmol) was dissolved in 5 mL of DMF, and PDA.Q (82 mg, 0.35 mmol) was added, followed by TEA (36 mg, 0.35 mmol). The solution was stirred at room temperature after flushing with nitrogen gas for 8 h. DMP (261 mg, 2.55 mmol) and APD (32 mg, 0.35 mmol) were added, and the solution was flushed with nitrogen gas, and stirred overnight at room temperature. The solution was concentrated on the rotary evaporator at 60ºC, and allowed to cool before precipitation with diethyl ether:acetone (2:1) (10 mL). The precipitate was washed twice with boiling toluene, and once with boiling acetone. The precipitate was dissolved in 10 mL of distilled water, and the pH was then adjusted to 7-8 with hydrochloric acid before dialysis against distilled water with 12000 membrane tubing for two days. Freeze-drying was performed to afford water soluble solid, 540 mg (82%), and the ^{1}H NMR spectroscopy showed that there was a 100% incorporation of DMP, 70% of PDA.Q, and 100% of APD.

^{1}H NMR (D_2O) δ/ppm: 8.20-6.20, 3.5H (5H; Ar-H); 3.83-3.59, 5H (5H; **CH_2CH(**OH)**CH_2OH**); 3.26, 23H (20H; CONH**CH_2**); 2.75-2.61, 87H (84H; **CH_2**CH_2**CH_2**NHAR and **CH_2**CH_2**CH_2**N(**CH_3**)$_2$); 1.85-1.84, 18H (18H; CH_2**CH_2**CH_2NHAr and CH_2**CH_2**CH_2N$(CH_3)_2$).

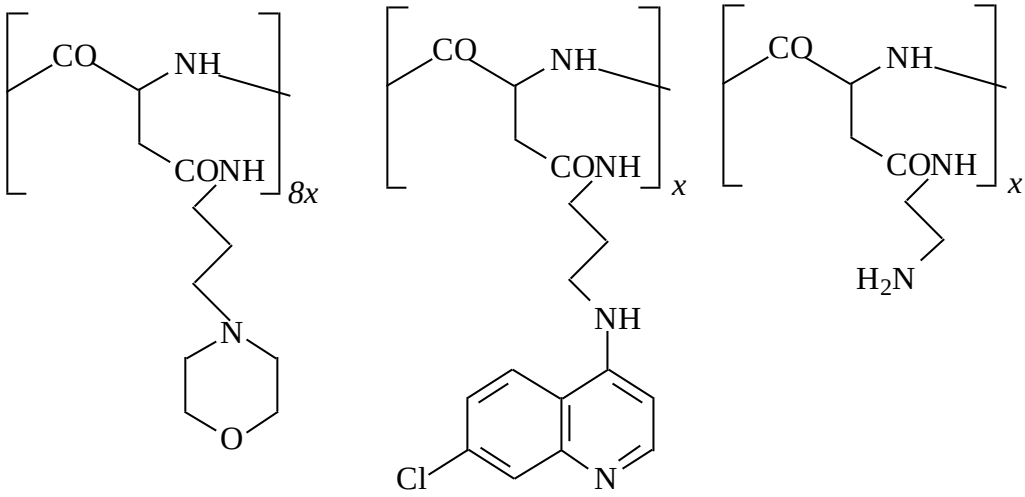
5.8.12 PSI.AEP (80) PDA.Q (10) APD (10)



PSI (300 mg, 3.19 mmol) was dissolved in 5 mL of DMF, and PDA.Q (82 mg, 0.35 mmol) was added, followed by TEA (36 mg, 0.35 mmol). The solution was stirred at room temperature after flushing with nitrogen gas for 8 h. AEP (330 mg, 2.55 mmol) and APD (32 mg, 0.35 mmol) were then added, and the solution was flushed with nitrogen gas, and stirred overnight at room temperature. The solution was concentrated on the rotary evaporator at 60ºC, and allowed to cool before precipitation with diethyl ether:acetone (2:1) (10 mL). The precipitate was washed twice with boiling toluene, and once with boiling acetone. The precipitate was dissolved in 10 mL of distilled water, and the pH was adjusted to 7-8 with hydrochloric acid before dialysis against distilled water with 12000 membrane tubing for two days. Freeze drying was performed to afford water soluble solid, 520 mg (93%), and the ^{1}H NMR spectrum showed that there was a 29% incorporation of AEP, 76% of PDA.Q, and 100% of APD.

^{1}H NMR (D_2O) δ/ppm: 8.30-6.20, 3.8H (5H; Ar-H); 3.80, 3.2H (3H; CH_2**CH(**OH)**CH$_2$**OH); 3.60-3.28, 27H (22H; CONH**CH$_2$**); 2.82-2.64, 100H (29H; **CH$_2$**CH_2**CH$_2$**NHAR and **CH$_2$CH$_2$**N(**CH$_2$**)$_2$**CH$_2$**NH**CH$_2$**); 1.80, 2H (2H; CH_2**CH$_2$**CH_2NHAR).

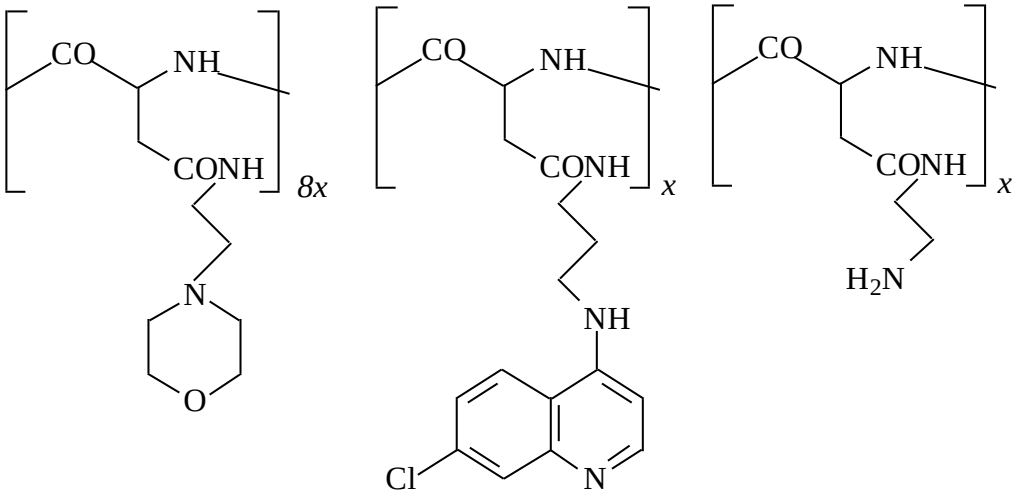
5.8.13 PSI.APM (80) PDA.Q (10) EDA (10)



PSI (300 mg, 3.19 mmol) was dissolved in 5 mL of DMF, and PDA.Q (82 mg, 0.35 mmol) was added, followed by TEA (36 mg, 0.35 mmol). The solution was stirred at room temperature after flushing with nitrogen gas for 8 h. APM (368 mg, 2.55 mmol) was added, and the solution was flushed with nitrogen gas, and stirred overnight at room temperature. The above solution was added drop wise to a solution of EDA (21 mg, 0.35 mmol), in 3 mL of DMF, in an ice bath. The resultant solution was flushed with nitrogen gas and stirred in an ice bath overnight, followed by stirring at room temperature for one day. The solution was concentrated on the rotary evaporator at 60ºC, and allowed to cool before precipitation with diethyl ether:acetone (2:1) (10 mL). The precipitate was washed twice with boiling toluene, and once with boiling acetone. The precipitate was dissolved in 10 mL of distilled water, and the pH was adjusted to 7-8 with hydrochloric acid before dialysis against distilled water with 12000 membrane tubing for two days. Freeze-drying was performed to afford water soluble solid, 510 mg (68%), and the ^{1}H NMR spectroscopy showed that there was a 100% incorporation of APM, 83% of PDA.Q, and 99% of EDA.

^{1}H NMR (D_2O) δ/ppm: 8.30-6.20, 4.2H (5H; Ar-H); 3.71, 32H (32H; **CH_2OCH_2**); 3.50, 24H (20H; CONH**CH_2**); 2.75-2.42, 71H (72H; **$CH_2CH_2NH_2$**, **$CH_2CH_2CH_2$**NHAR and **$CH_2CH_2CH_2N(CH_2)_2$**); 1.72, 18H (18H; CH_2**CH_2**CH_2NHAR and CH_2**CH_2**$CH_2N(CH_2)_2$).

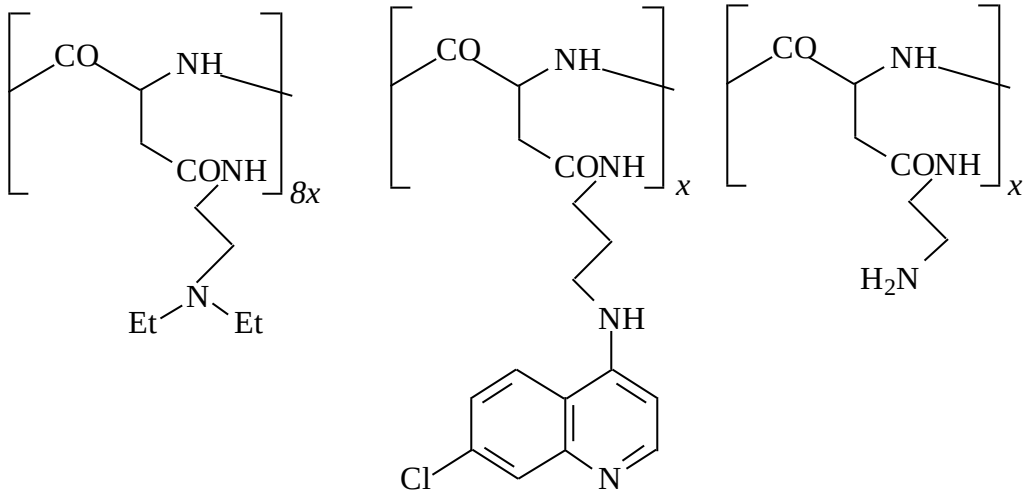
5.8.14 PSI.AEM (80) PDA.Q (10) EDA (10)



PSI (300 mg, 3.19 mmol) was dissolved in 5 mL of DMF, and PDA.Q (82 mg, 0.35 mmol) was added, followed by TEA (36 mg, 0.35 mmol). The solution was stirred at room temperature after flushing with nitrogen gas for 8 h. AEM (332 mg, 2.55 mmol) was added, and the solution was flushed with nitrogen gas, and stirred overnight at room temperature. The above solution was added drop wise to a solution of EDA (21 mg, 0.35 mmol), in 3 mL of DMF, in an ice bath, and the solution was flushed with nitrogen gas, and stirred in an ice bath overnight, followed by stirring at room temperature for one day. The resultant solution was then concentrated on the rotary evaporator at 60ºC, and allowed to cool before precipitation with diethyl ether:acetone (2:1) (10 mL). The precipitate was washed twice with boiling toluene, and once with boiling acetone. The precipitate was then dissolved in 10 mL of distilled water, and the pH was then adjusted to 7-8 with hydrochloric acid before dialysis against distilled water with 12000 membrane tubing for two days. Freeze-drying was performed to afford water soluble solid, 410 mg (58%), and the ^{1}H NMR spectroscopy showed that there was a100% incorporation of AEM, 90% of PDA.Q, and 93% of EDA.

^{1}H NMR (D_2O) δ/ppm: 8.20-6.01, 4.5H (5H; Ar-H); 3.74-3.73, 33H (32H; **CH_2OCH_2**); 3.35-3.34, 23H (20H; CONH**CH_2**); 2.71-2.52, 67H (72H; **$CH_2CH_2NH_2$**, **$CH_2CH_2CH_2$**NHAR and **$CH_2CH_2N(CH_2)_2$**); 1.75, 2H (2H; CH_2**CH_2**CH_2NHAR).

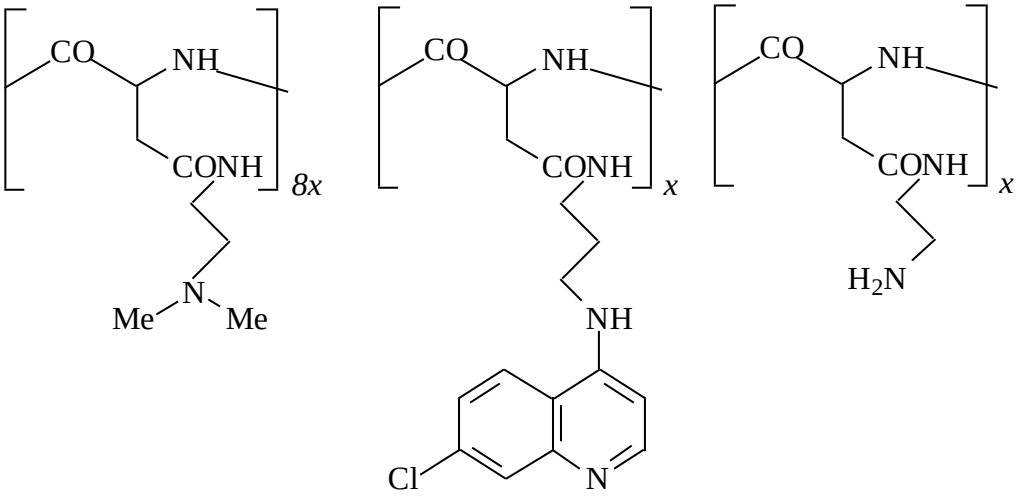
5.8.15 PSI.DEEA (80) PDA.Q (10) EDA (10)



PSI (300 mg, 3.19 mmol) was dissolved in 5 mL of DMF, and PDA.Q (82 mg, 0.35 mmol) was added, followed by TEA (36 mg, 0.35 mmol). The solution was stirred at room temperature after flushing with nitrogen gas for 8 h. DEEA (297 mg, 2.55 mmol) was added, and the solution was then flushed with nitrogen gas, and stirred overnight at room temperature. The above solution was added drop wise to a solution of EDA (21 mg, 0.35 mmol), in 3 mL of DMF, in an ice bath, and the solution was flushed with nitrogen gas, and stirred in an ice bath overnight, followed by stirring at room temperature for one day. The solution was concentrated on the rotary evaporator at 60ºC, and allowed to cool before precipitation with diethyl ether:acetone (2:1) (10 mL). The precipitate was washed twice with boiling toluene, and once with boiling acetone. The precipitate was dissolved in 10 mL of distilled water, and the pH was then adjusted to 7-8 with hydrochloric acid before dialysis for two days. Freeze-drying was performed to afford water soluble solid, 510 mg (75%), and the ^{1}H NMR spectrum showed that there was an 83% incorporation of DEEA, 88% of PDA.Q, and 83% of EDA.

^{1}H NMR (D_2O) δ/ppm: 8.13-6.16, 4.4H (5H; Ar-H); 3.40-3.39, 21H (20H; CONH**CH_2**); 2.82-2.79, 60H (72H; **CH_2CH_2**NH_2, **CH_2**CH_2**CH_2**NHAR and **CH_2CH_2**N(**CH_2**CH_3**)**$_2$); 1.88-1.86, 2H (2H; CH_2**CH_2**CH_2NHAR); 1.11, 40H (48H; $CH_2CH_2N(CH_2$**CH_3**)**$_2$).

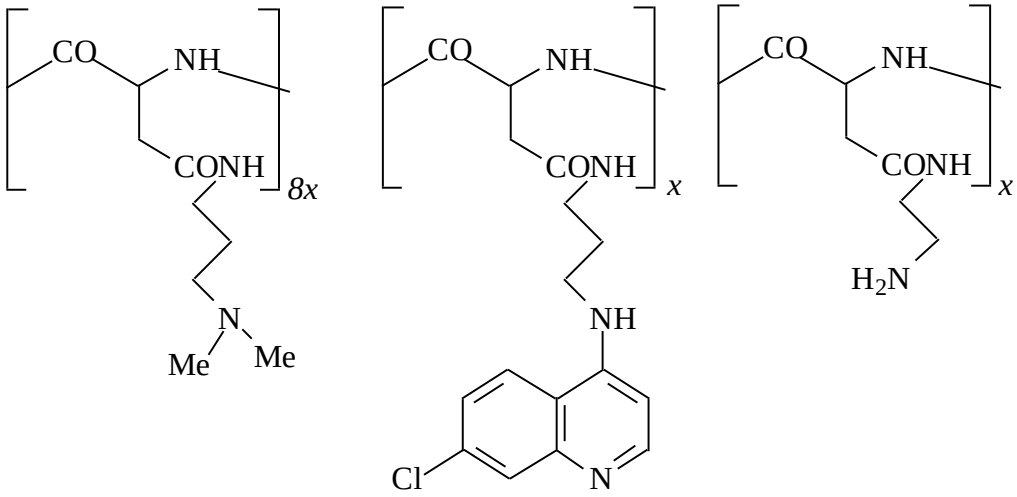
5.8.16 PSI.DME (80) PDA.Q (10) EDA (10)



PSI (300 mg, 3.19 mmol) was dissolved in 5 mL of DMF, and PDA.Q (82 mg, 0.35 mmol) was added, followed by TEA (36 mg, 0.35 mmol). The solution was stirred at room temperature after flushing with nitrogen gas for 8 h. DME (225 mg, 2.55 mmol) was added and the solution was flushed with nitrogen gas, and stirred overnight at room temperature. The above solution was added drop wise to a solution of EDA (21 mg, 0.35 mmol), in 3 mL of DMF, in an ice bath, and the resultant solution was then flushed with nitrogen gas, and stirred in an ice bath overnight, followed by stirring at room temperature for one day. The solution was concentrated on the rotary evaporator at 60°C, and allowed to cool before precipitation with diethyl ether:acetone (2:1) (10 mL). The precipitate was washed twice with boiling toluene, and once with boiling acetone. The precipitate was dissolved in 10 mL of distilled water, and the pH was then adjusted to 7-8 with hydrochloric acid before dialysis with 12000 membrane tubing for two days. Freeze-drying was performed to afford water soluble solid, 510 mg (84%), and the ^{1}H NMR spectroscopy showed that there was a 94% incorporation of DME, 93% of PDA.Q, and 92% of EDA.

^{1}H NMR (D_2O) δ/ppm: 8.20-6.00, 4.7H (5H; Ar-H); 3.33-3.25, 22H (20H; CONH**CH_2**); 2.72-2.71, 22H (24H; **CH_2CH_2**NH_2, **CH_2**CH_2**CH_2**NHAR and **CH_2**$CH_2N(CH_3)_2$); 2.48-2.46, 16H (16H; CH_2**CH_2**$N(CH_3)_2$); 2.23-2.19, 45H (48H; CH_2CH_2N(**CH_3**)$_2$); 1.80, 2H (2H; CH_2**CH_2**CH_2NHAR).

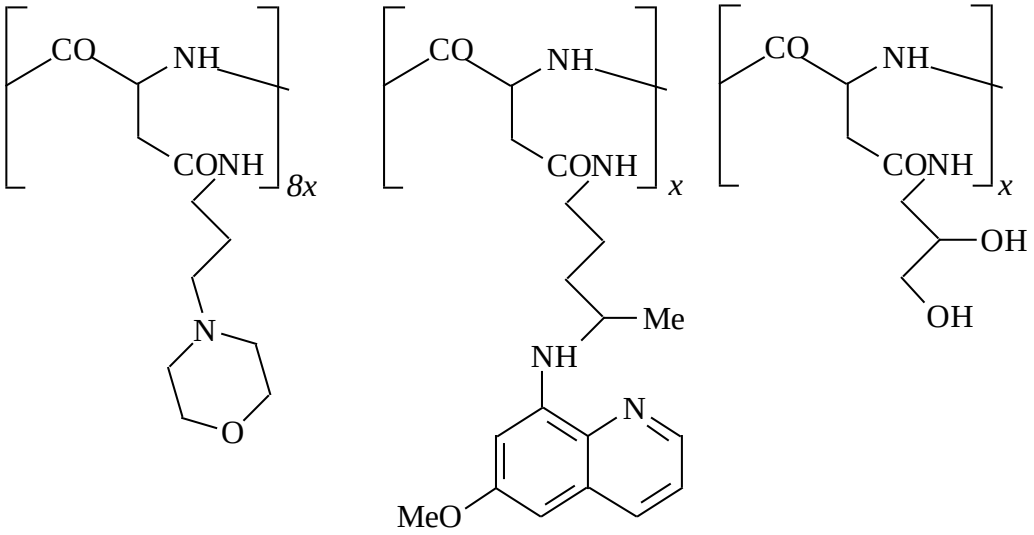
5.8.17 PSI.DMP (80) PDA.Q (10) EDA (10)



PSI (300 mg, 3.19 mmol) was dissolved in 5 mL of DMF, and PDA.Q (82 mg, 0.35 mmol) was added, followed by TEA (36 mg, 0.35 mmol). The solution was stirred at room temperature after flushing with nitrogen gas for 8 h. DMP (261 mg, 2.55 mmol) was added, and the solution was flushed with nitrogen gas, and stirred overnight at room temperature. The solution was added drop wise to a solution of EDA (21 mg, 0.35 mmol), in 3 mL of DMF, in an ice bath, and the solution was flushed with nitrogen gas, and stirred in an ice bath overnight, followed by stirring at room temperature for one day. The solution was concentrated on the rotary evaporator at 60ºC, and allowed to cool before precipitation with diethyl ether:acetone (2:1) (10 mL). The precipitate was washed twice with boiling toluene, and once with boiling acetone. The precipitate was dissolved in 10 mL of distilled water, and the pH was adjusted to 7-8 with hydrochloric acid before dialysis against water with 12000 membrane tubing for two days. Freeze-drying was performed to afford water soluble solid, 510 mg (79%), and the ^{1}H NMR spectrum showed that there was a 96% incorporation of DMP, 82% of PDA.Q, and 96% of EDA.

^{1}H NMR (D_2O) δ/ppm: 8.20-6.20, 4.1H (5H; Ar-H); 3.27, 20H (20H; CONH**CH_2**); 2.87-2.67, 84H (88H; **CH_2CH_2**NH_2, **CH_2**CH_2**CH_2**NHAR and **CH_2**CH_2**CH_2**N(**$CH_3)_2$**); 1.87-1.86, 18H (18H; CH_2**CH_2**CH_2NHAR, CH_2**CH_2**CH_2N(CH_3**)**$_2$).

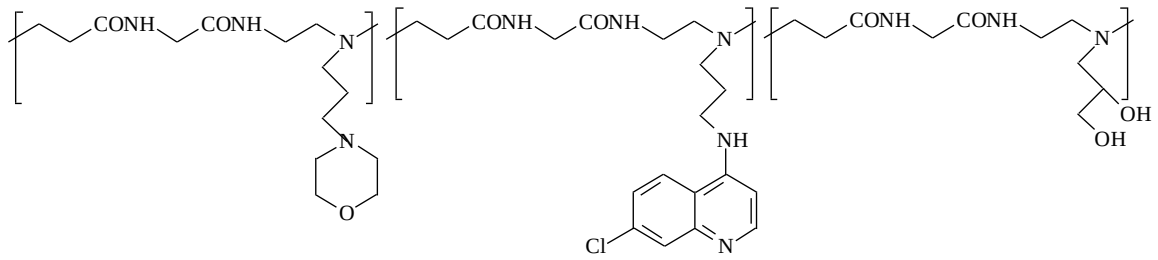
5.8.18 PSI.APM (80) PRIMAQUINE (10) APD (10)



PSI (200 mg, 2.13 mmol) was dissolved in 5 mL of DMF, and primaquine (60 mg, 0.23 mmol) was added, followed by TEA (23 mg, 0.23 mmol) and APD (21 mg, 0.23 mmol). The resultant solution was stirred at room temperature after flushing with nitrogen gas overnight. APM (246 mg, 1.70 mmol) was added, and the solution was flushed with nitrogen gas, and stirred for 2 d at room temperature. The solution was concentrated on the rotary evaporator at 60ºC, and allowed to cool before precipitation with diethyl ether:acetone (2:1) (10 mL). The precipitate was washed twice with boiling toluene, and once with boiling acetone. The precipitate was dissolved in 10 mL of distilled water, and the pH was adjusted to 7-8 with hydrochloric acid before dialysis with 12000 membrane tubing for two days. Freeze-drying was performed to afford water soluble solid, 380 mg (75%), and the ^{1}H NMR spectrum showed that there was a 100% incorporation of APM, 88% of Primaquine, and 100% of APD.

^{1}H NMR (D_2O) δ/ppm: 8.45-6.25, 4.4H (5H; Ar-H); 3.73, 40H (37H; **CH_2OCH_2**, **CH_2CH**(OH)**CH_2**OH); 3.40-3.19, 26H (20H; CONH**CH_2**); 2.70-2.38, 66H (68H; **CH_2**CH_2**CH_2**N(**CH_2**)$_2$, **CH_2**$CH_2CH_2CH(CH_3)$NHAR); 1.68-1.10, 23H (23H, CH_2**CH_2**$CH_2N(CH_2)_2$ and CH_2**$CH_2CH_2CH(CH_3)$**NHAR).

5.8.19 MBA. APM. PDA.Q. APD (3:1:1)

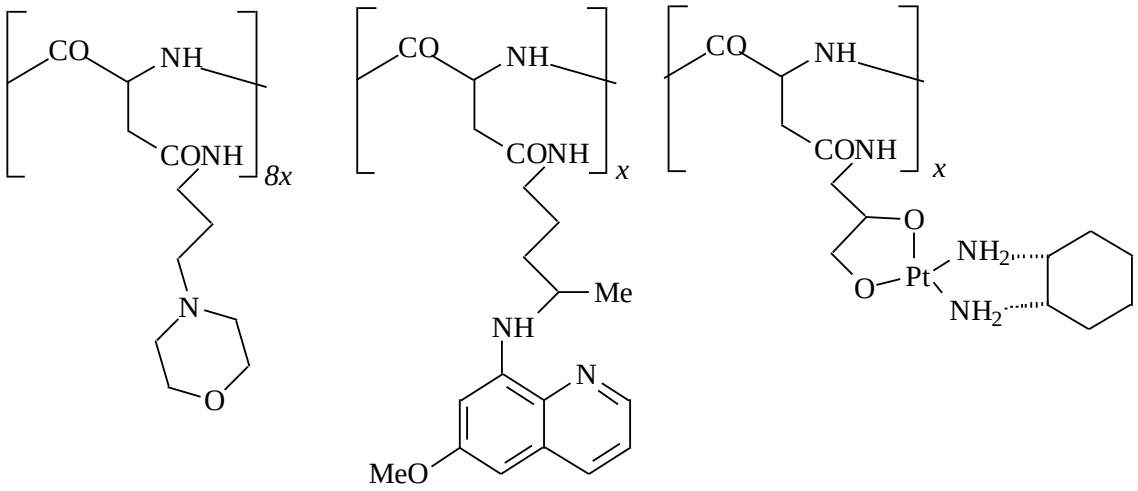


MBA (500 mg, 3.24 mmol) was dissolved in 4 mL of methanol, and PDA.Q (245 mg, 1.08 mmol) and APD (98 mg, 1.08 mmol) were added, followed by TEA (109 mg, 1.08 mmol). The resultant solution was flushed with nitrogen gas, and stirred overnight at room temperature. APM (156 mg, 1.08 mmol) was added, flushed with nitrogen gas, and stirred at room temperature for 4 d. The solution was concentrated on the rotary evaporator at 60ºC to afford a yellow viscous liquid, which, was precipitated with diethyl ether:acetone (2:1), and the precipitate was then washed once with boiling acetone. The precipitate was dissolved in 10 mL of distilled water, and the pH was adjusted to 7-8 with hydrochloric acid, followed by dialysis for 2 d with 12000 membrane tubing. Freeze-drying was performed to afford water soluble solid, 80 mg (16%). ^{1}H NMR spectrum showed that there was a 100% APM, 84% PDA.Q and 100% APD.

^{1}H NMR (D_2O) δ/ppm: 8.20-6.24, 4.2H (5H; Ar-H); 3.75-3.74, 10H (9H; **$CH_2O$$CH_2$**, N**$CH_2$$CH$**(OH)**$CH_2$**OH); 2.79-2.40, 44H (42H; **$CH_2$$CH_2$**CONH**$CH_2$**CONH**$CH_2$$CH_2$**N, N**$CH_2$**$CH_2$**$CH_2$**N(**$CH_2$**)$_2$, N**$CH_2$**$CH_2$**$CH_2$**NHAr); 1.66-1.64, 4H (4H; NCH_2**CH_2**$CH_2N(CH_2)_2$, NCH_2**CH_2**CH_2NHAr).

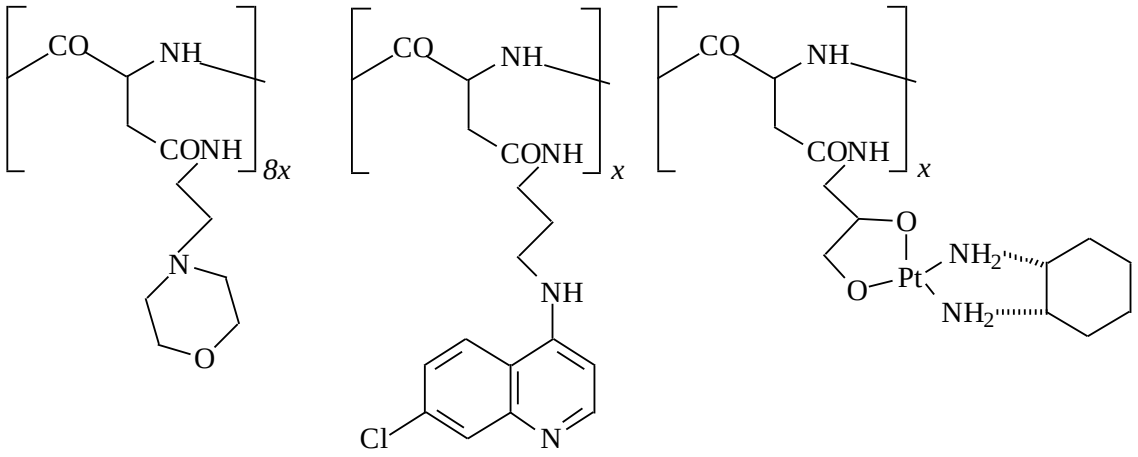
5.9 POLYMERIC TWO DRUGS CO-CONJUGATE

5.9.1 PSI. APM (80) PRIMAQUINE (10) APD (10) Pt



To nitrogen saturated solution of PSI. APM (80) Primaquine (10) APD (10) (200 mg, 0.08 mmol) in 4 mL of distilled water, DACH.Pt (41 mg, 0.08 mmol) in 2 mL of distilled water was added, and the resultant solution was re-saturated with nitrogen gas, and protected from light using aluminium foil. It was stirred at room temperature for 3 d with pH maintained at 5.5-6.0, and at the same pH range for two hours at 50ºC in the incubator. The solution was then filtered, dialyzed for two days in 12000 membrane tubing and freeze-dried to afford a yield of 150 mg of water soluble polymer.

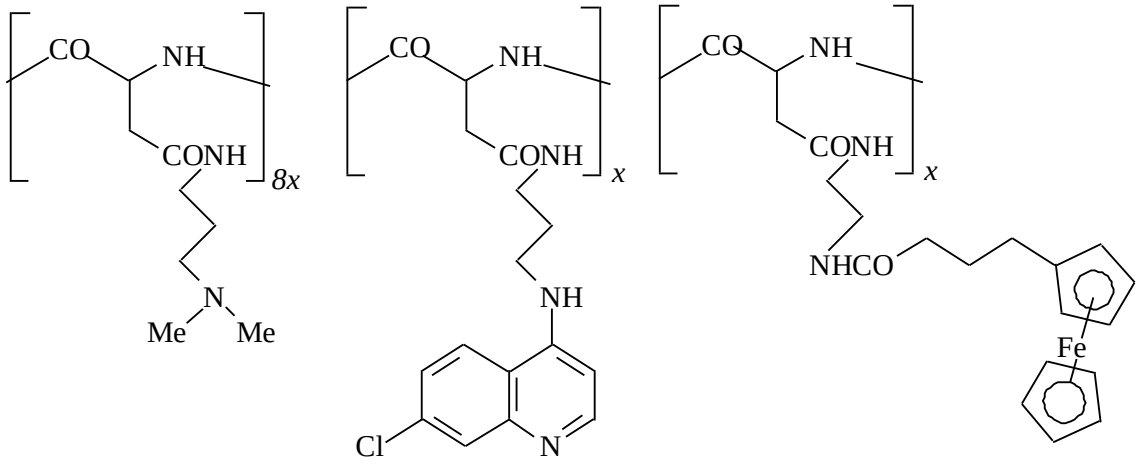
5.9.2 PSI. AEM (80) PDA.Q (10) APD (10) Pt



To nitrogen saturated solution of PSI. AEM (80) PDA.Q (10) APD (10) (150 mg, 0.07 mmol) in 4 mL of distilled water, DACH.Pt (34 mg, 0.07 mmol) in 2 mL of distilled

water was added and the resultant solution was then re-saturated with nitrogen gas, and protected from light using aluminium foil. The resultant solution was stirred at room temperature for three days with pH maintained at 5.5-6.0, and for two hours at 50ºC in incubator. The solution was then filtered, dialyzed for two days in 12000 membrane tubing and then freeze dried to afford a yield of 90 mg water soluble solid.

5.9.3 PSI. DMP (80) PDA.Q (10) PDA (10) .FERROCENE



PSI. DMP (80) PDA.Q (10) PDA (10) (200 mg, 0.09 mmol) was dissolved in 2 mL of methanol, and Ferrocene butanoic acid (32 mg, 0.12 mmol) was added, followed by HBTU (34 mg, 0.11 mmol) in 1 mL of DMF. TEA (12 mg, 0.12 mmol) was added, and the resultant solution was flushed with nitrogen gas, and stirred overnight at room temperature, and it was protected from light with aluminium foil. The resultant solution was precipitated with diethyl ether: acetone (2:1) (10 mL), centrifuged, and the precipitate was dissolved in 10mL of distilled water, the pH was adjusted to 10 using NaOH pellets. The solution was passed through a G25 column using distilled water, and the expected fraction was collected, pH adjusted to 7 with glacial acetic acid, and dialysis was performed for 2 d in 12000 membrane tubing. In the last 6 h of the dialysis, the pH was adjusted to 3 with HCl for 3 min, and to a pH of 6 with ammonia solution, and freeze dried to afford a yield of 80 mg with 96% incorporation of ferrocene.

'H NMR (D_2O), δ/ppm*:* 4.19, 8.6H (Expected: 9H, Fe-H) indicating a 96% incorporation of ferrocene.

5.10 REFERENCES CITED IN CHAPTER FIVE

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APPENDIX 1

(^{1}H NMR SPECTRA)

APPENDIX II

(DOSE-RESPONSE CURVE OF TEST SAMPLES AGAINST CQS CD10 STRAIN OF *P. falciparum*)