# BAYLIS-HILLMAN ADDUCTS AS SCAFFOLDS FOR THE CONSTRUCTION OF NOVEL COMPOUNDS WITH MEDICINAL POTENTIAL

THESIS

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**DOCTOR OF PHILOSOPHY**

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By

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# DEDICATION

This piece of work is dedicated to the affectionate and evergreen memory of my late mother, **Madam Helen Inyi**, for giving me early in Life, the basic educational foundation I needed to build on.

# ABSTRACT

This project has focused on exploring the application of Baylis-Hillman (BH) {a.k.a.Morita-Baylis-Hillman (MBH)} scaffolds in the construction of various compounds with medicinal potential. A series of 2-nitrobenzaldehydes has been treated under BH conditions, with two different activated alkenes, *viz*., (MVK) and methyl acrylate, using (DABCO) or (3-HQ) as catalyst. While most of the BH reactions were carried out at room temperature, some reactions were conducted using microwave irradiation. The resulting BH adducts have been subjected to dehydration, conjugate addition and allylic substitution to obtain appropriate intermediates, which have been used in turn, to synthesize possible lead compounds, *viz*., cinnamate esters as HIV-1 integrase inhibitors, 3-(aminomethyl)quinolines and quinolones as anti-malarials and cinnamate ester-AZT conjugates as dual-action HIV-1 integrase-reverse transcriptase (IN-RT) inhibitors.

Conjugate addition reactions of methyl acrylate-derived BH β-hydroxy esters with the amines, piperidine, propargylamine and 2-amino-5-(diethylamino)pentane, has afforded a range of products as diastereomeric mixtures in moderate to excellent yields. Catalytic hydrogenation of the aminomethy β-hydroxy esters derivatives, using a palladium-on-carbon (Pd-C) catalyst, has afforded the corresponding, novel 3-aminomethyl-2-quinolone derivatives in moderate yields.

Effective allylic substitution reactions of the MVK-derived BH β-hydroxy ketones (*via* a conjugate addition-elimination pathway) using *in situ*-generated HCl has afforded the corresponding α-chloromethyl derivatives, which have been reacted with various amines, including piperidine, piperazine, propargylamine and 2-amino-5-(diethylamino)pentane, to yield α-aminomethyl derivatives. Catalytic hydrogenation of selected α-aminomethyl derivatives, using a Pd-C catalyst, has afforded the corresponding, novel 3-(aminomethyl)-2-methylquinoline derivatives in low to moderate yields. A bioassay, conducted on a 6-hydroxy-2-methyl-3-[(piperidin-1-yl)methyl]quinoline isolated early in

the study indicated anti-malarial activity and prompted further efforts in the synthesis of analogous compounds.

Reaction of the methyl acrylate-derived BH adducts with $POCl_3$ has provided access to α-(chloromethyl)cinnamate ester derivatives, which have been aminated to afford α-(aminomethyl)cinnamate ester derivatives as potential HIV-1 integrase inhibitors. The α-(propargylaminomethyl)cinnamates were used, in turn, as substrates for the “click chemistry” reaction with 3'-azido-3'-deoxythymidine (AZT– an azide and an established reverse transcriptase HIV-1 inhibitor) to afford cinnamate ester-AZT conjugates as potential dual-action HIV-1 integrase-reverse transcriptase (IN-RT) inhibitors. Computer modelling and docking studies of a cinnamate ester-AZT conjugate into the HIV-1 integrase and reverse transcriptase active-sites revealed potential hydrogen-bonding interactions with amino acid residues within the receptor cavities.

The isolated products have been appropriately characterized using IR, 1- and 2-D NMR and HRMS techniques, while elucidation of the stereochemistry of the double bond in the BH-derived halomethyl derivatives has been assigned on the basis of NOE, computer modelling and X-ray crystallographic data.

# LIST OF ABREVIATIONS.

| | |
|---|---|
| COSY | Correlation spectroscopy ($^1H$-$^1H$). |
| HSQC | Heteronuclear single quantum coherence ($^1H$-$^{13}C$) |
| HMBC | Heteronuclear multiple bond coherence ($^1H$-$^{13}C$). |
| DEPT | Distortionless Enhancement by Polarization Transfer. |
| NOESY | Nuclear Overhauser effect spectroscopy ($^1H$-$^1H$ in space) |
| HOESY | Heteronuclear 2-D NOE. |
| BH | Baylis-Hillman |
| DABCO | 1,4-Diazacyclo[2,2,2]octane |
| 3-HQ | 3-Hydroxyquinuclidine |
| MVK | Methyl vinyl ketone |
| EDC | 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide |
| DCM | dichloromethane |
| THF | tetrahydrofuran |
| DMF | dimethylformamide |
| EtOAc | ethyl acetate |
| TEA | triethylamine |
| DMAP | 4-dimethylaminopyridine |
| MeOH | methanol |
| PCC | pyridinium chlorochromate |

| | |
|---|---|
| HAART | highly active antiretroviral therapy |
| AZT | 3’-azido-3’-deoxythymidine |
| PI | protease inhibitor |
| NRTI | nucleoside reverse transcriptase inhibitor |
| NNRTI | non-nucleoside reverse transcriptase inhibitor |
| INI | integrase inhibitor |
| RTI | reverse transcriptase inhibitor |
| HIV-1 | Human immunodeficiency virus -1 |
| DFT | Density functional theory |
| B3LYP | Becke’s three parameter and the Lee, Yang and Parr Correlation functional |
| DML | designed multiple ligand |

# 1. INTRODUCTION

## 1.1. HISTORY OF HUMAN MALARIA AND ITS TREATMENT.

### 1.1.1. Malaria: an Ancient Disease in a Modern World.

Mankind has been plagued by a number of infectious diseases such as measles and other childhood infection; in some cases vaccines have been found to prevent infection. However, one particular disease, malaria, has resisted all attempts to achieve a permanent cure or to immunize against attack. Malaria is a protozoan parasitic disease of man, caused by *Plasmodium* species and transmitted through mosquito bites. It has one of the highest morbidity and mortality rates of all human infectious diseases with as many as half a billion people at risk of chronic anaemia and the economic consequences.[1-4a,b] Recent statistics show that only tuberculosis has a higher mortality rate than malaria (see Table 1.1).[5a]

**Table 1.1.** Mortality and Burden of Disease Data for Selected "Neglected or Orphan Diseases" (Global Estimates for 2002).[3]

| Disease | Deaths | DALYs (000)a |
|---|---|---|
| **African trypanosomiasis** | 48,000 | 1,525 |
| **Chagas disease** | 15,000 | 667 |
| **Dengue** | 19,000 | 616 |
| **Leishmaniasis** | 51,000 | 2,090 |
| **Malaria** | **1,272,000** | **46,486** |
| **Schistosomiasis** | 15,000 | 1,702 |
| **Tuberculosis** | 1,566,000 | 34,736 |
| **Total** | **2,967,000** | **87,822** |

[a] DALYs: disease-adjusted life years, which is a measure of the economic loss due to the impact of a disease in the productive life of a person.

It should however be noted that many deaths from tuberculosis arise from opportunistic infections associated with HIV/AIDS.[5b] One encouraging finding is that, malaria does not appear to render a patient susceptible to HIV/AIDS nor does the malaria vector (*i.e.* the mosquito) appear to transmit HIV/AIDS. However, an increased rate of placental malaria has been observed in pregnant women who are HIV- positive.[6-7,8] Although HIV/AIDS is a recent disease condition, it shares a similar worldwide distribution pattern with malaria.[6,7]

Malaria occurrence in ancient times has been evidenced by the enlarged spleens found in Egyptian mummies more than 3,000 years old. An ancient papyrus (*ca* 1570 B.C.) records enlargement of the spleen along with various remedies for the associated fevers.[1-3,5a,9] Results from the autopsy of skin and lung samples of mummies from 3200 - 1304 B.C. indicated the presence of the malaria antigen. In his *Book of Epidemics,* the Greek physician, Hippocrates (460 – 370 B.C.) refers to febrile paroxysms which characterize *Plasmodium malariae* and *Plasmodium vivax* malarial infections. He stated that “fever and misery” were associated with harvest time in Greece and “with great epidemiological insight, noted the relationship between enlarged spleens and marshes”.[1] However, Hippocrates never seemed to appreciate that these conditions could have been caused by insects or other microscopic organisms; rather, he attributed the intermittent fevers to disturbances caused by drinking water from stagnant sources. This is also the typical reasoning among traditional healers in malarious region of sub-Saharan Africa, who attribute malarial infections to either long exposure to the sun or to evil spirits. Of course, the early Greek physicians, like the traditional healers today, lacked the scientific knowledge and research facilities required to establish the actual cause of the disease. There is no mention of severe, malignant tertian fevers in Hippocrate’s writings and it has been assumed that *P. falciparum* infections were unusual or non-existent during his time.[1-3,5a,9]

However, it can be inferred from medical literature of the period after 200 B.C., that malarial infection was common in the coastal areas near Osita (the Roman Campagna) since these writings record a precise description of the disease and refer to marshes as its source. Subsequently, the condition became known as the “Roman fever”, and the Italian word, *mal'aria* (meaning "bad air"), began to be used to indicate the source of the disease.[1]

Manson-Bahr noted in his *Malaria Story,*[10] "that ever since the day of Greek and Roman dominance, writers have associated the occurrence of malaria with proximity to marshes; hence the French term, *paludisme*, and the English, miasmatic fever". Based on an earlier article [9] on '*The Nature and Treatment of Miasmatic Fevers*' by Metcalfe in 1862, King concluded that there was a clear relationship between malaria and mosquitoes.[10] The idea that the mosquito, *Aedes oegypti*, could transmit fever, was first proposed by Finlay and confirmed by Reed and his associates, and this undoubtedly influenced thinking about the mode of transmission of malaria.[10] However, in Nigeria, a country in West Africa, malaria is generally called *Iba* (a term used for a feverish condition) and, as mentioned earlier, it is presumed to be caused by long exposure to the scorching sun. Given their limited understanding of the disease, local people treat themselves with various herbal concoctions, which often offer no remedy. Interestingly, natives of the Usumbura Mountains in Tanganyika, East Africa, have the same name, *Mbu,* for both malaria and mosquitoes.[10]

It is estimated that nearly 50% of humanity lives under the menace of this dreaded disease.[11a-13] Global statistics from the World Health Organization indicate that malaria infects close to 500 million people annually, in Africa, South Asia, the Middle East, Oceania and Central and South America,[1-5b,7-13] and that its yearly mortality rate is over one million people, most of whom are children under the age of five years.[9,11a,11b]

There are approximately 100 known species of the genus *Plasmodium*,[13] only four of which cause malaria in humans. The remaining species affect birds, monkeys, livestock, rodents, and reptiles; malaria, therefore, is also an important problem for farm animals, especially chickens.

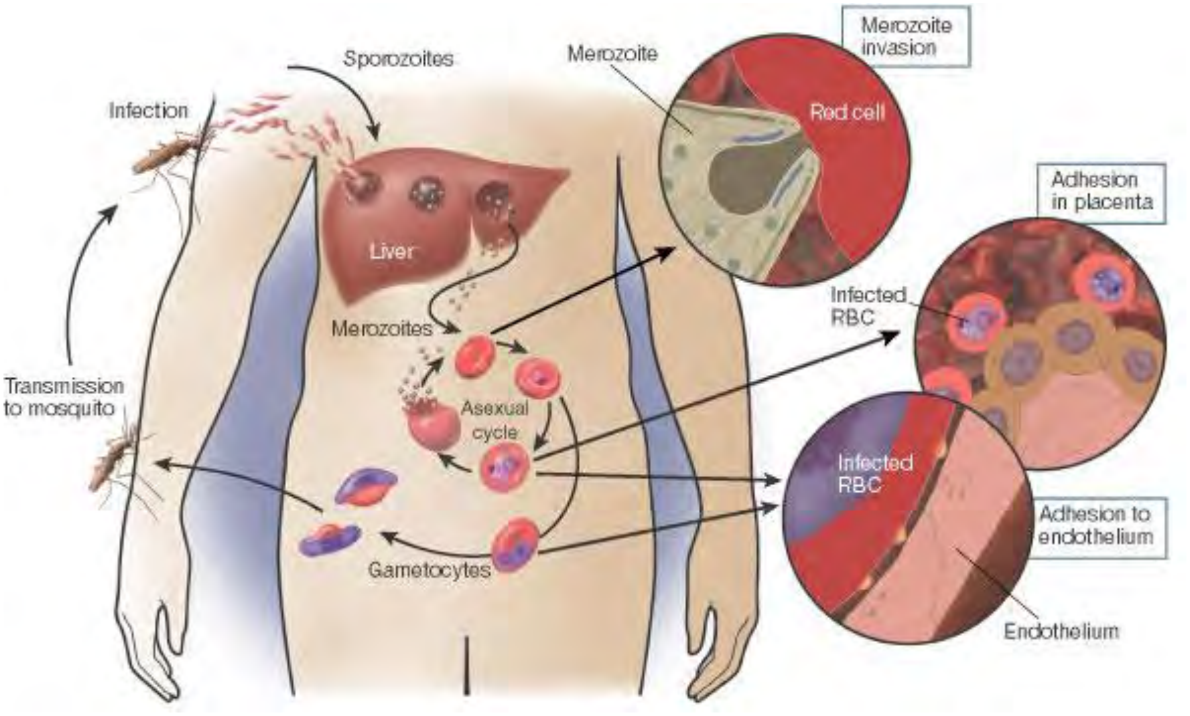


**Figure 1:** Schematic life cycle of the malaria parasite.[5] Reproduced from Google images by permission (malariacycle.ipg).

Of the four plasmodium species known to cause malaria in humans, *Plasmodium falciparum, P. vivax, P. ovale* and *P. malariae*, the most important is *P. falciparum* since it is both the most prevalent and the only one capable of producing fatal complications.[1-5b,6-15] Concomitant infections by more than one of these species occur in endemically affected regions of the world.

Such multiple infections further complicate patient management and the choice of treatment regimens. The parasite requires two hosts, a female *Anopheles* mosquito and a human. The life-cycle of malaria starts with a bite from an infected mosquito on its human prey, thereby withdrawing blood and simultaneously injecting sporozoite-containing saliva into the capillaries of the victim's skin. The sporozoites enter liver cells and multiply to form tens of thousands merozoites each. After several days or a week, the merozoites are released into the blood stream where they enter red blood cells and develop through the trophozoite and schizont stages.[11a,-14]

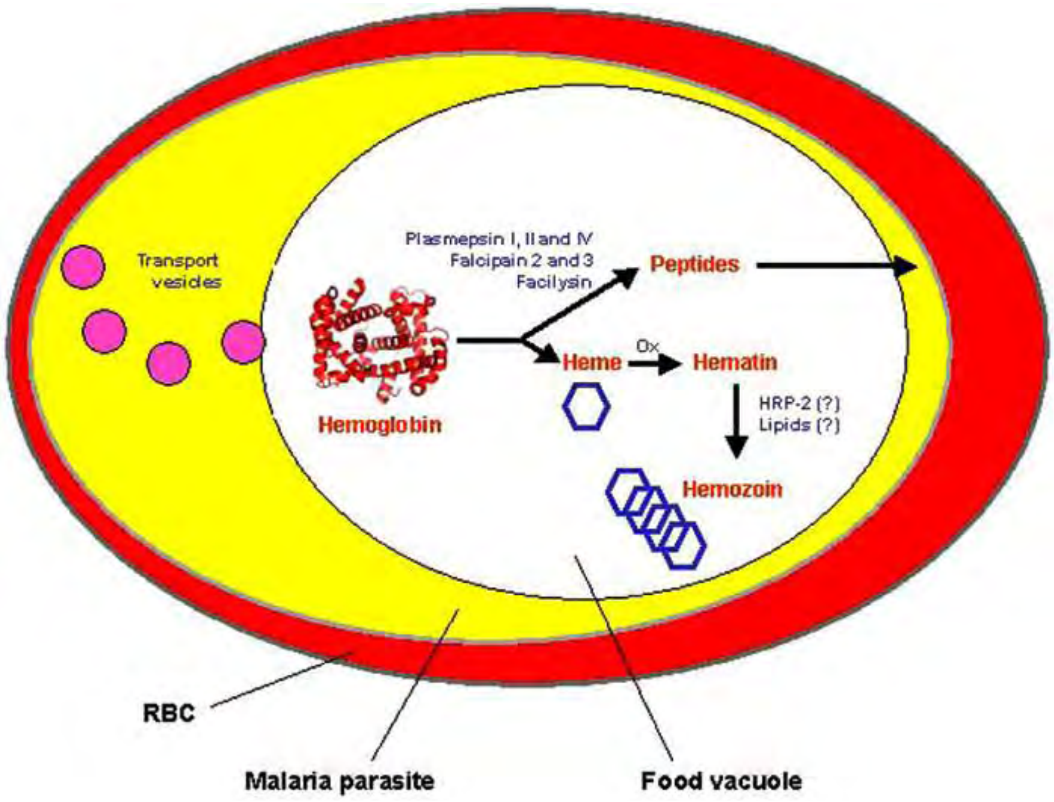


**Figure 2.** Schematic representation of haemoglobin catabolism in *Plasmodium falciparum.*[13] Reproduced by permission**.**

The growth of the malaria parasite is supported by the ingestion of host haemoglobin into an acidic food vacuole where haemoglobin is digested by a series of enzymes, thus serving as the major source of amino acids. A schematic representation of haemoglobin catabolism is shown in Figure 2. Oxidation of released haeme to haematin takes place in the parasite food vacuole.[11a,11b] This haematin is subsequently incorporated into haemozoin (also known as the malaria pigment).[13] It is this intra-erythrocytic stage of the malaria parasite that produces the disease pathology.

Diseases like malaria, which affect the poor (almost 40% of mankind) in poor countries and in developing and developed countries alike, have informed the concept of "neglected" or "orphan diseases". These diseases lead to loss in income and other economic outputs for the countries affected. Significantly, such diseases, which include African trypanosomiasis (sleeping sickness), American trypanosomiasis (Chagas disease), dengue and tuberculosis, often do not offer market interest for the pharmaceutical industry due to the low purchasing power of the affected populations.[3] (See Table 1.1)

Malaria is endemic in all tropical regions of the World (see Figure 3). *P. falciparum* is prevalent in Africa, Southeast Asia, Papua-New Guinea and Haiti, whereas *P. vivax* is commonly found in Central America and parts of South America, North Africa, the Middle East and the Indian sub-continent. Both species are found at comparable levels in other parts of South America, East Asia and Oceania. *P.vivax* strains, with long incubation periods and long intervals between relapses, may be found in China and adjacent countries (Figure 3).[3] Although malaria is accepted as one of the most important tropical and sub-tropical infectious disease, its spread to non-tropical countries is a real possibility.[11]

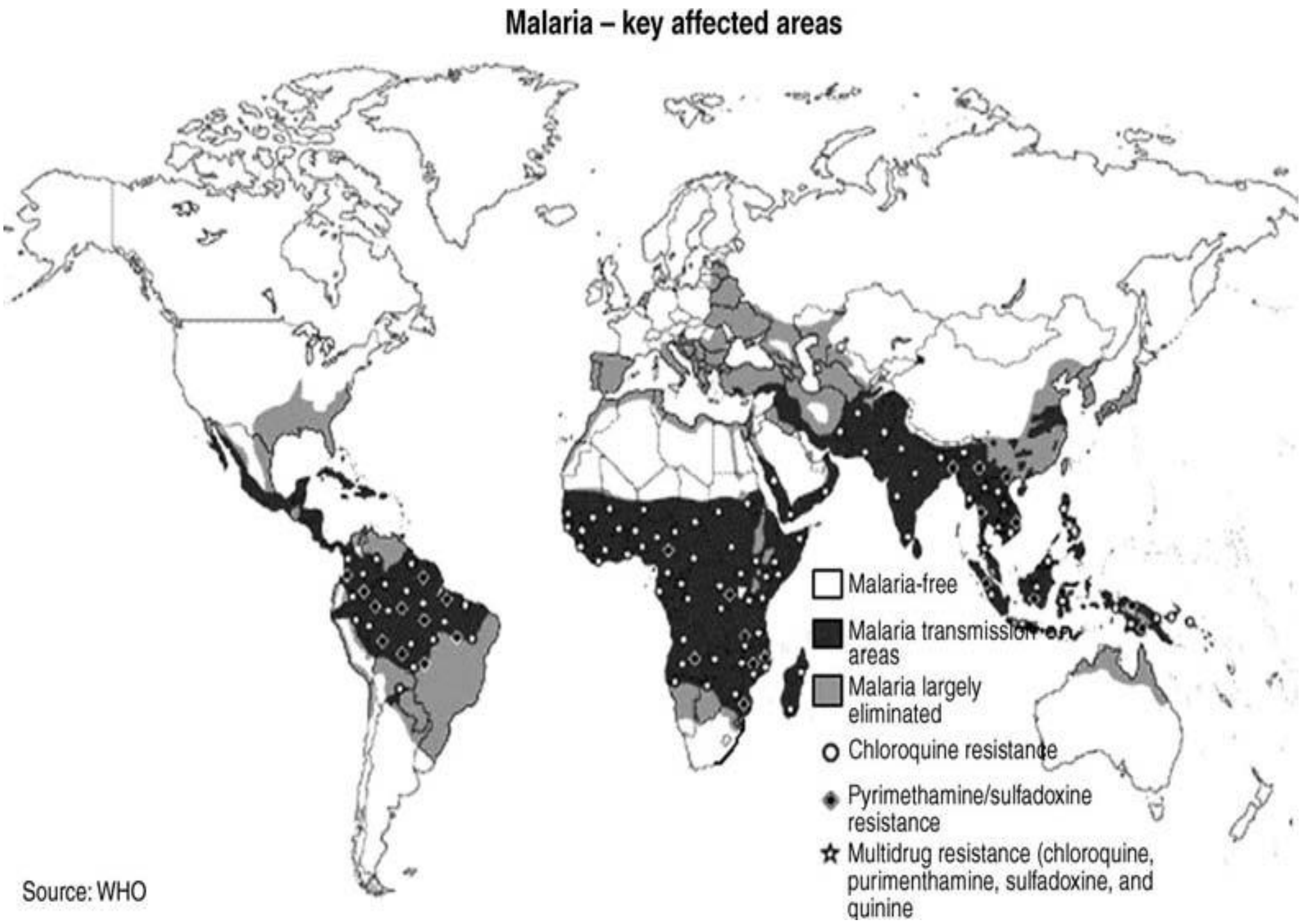


**Figure 3.** Distribution of malaria worldwide (www.childinfo.org/eddb/Malaria/current.htm).[3] Reproduced by permission.

The current increase of the malaria burden is attributable to parasite resistance to existing drugs as well as to resistance to insecticides by the malaria vector, the anopheles mosquito. The above mentioned resistances are as a result of changes in the social and environmental behavior of Communities.[8-13] Such changes include abuse of antimalarial drugs in self-medication, lack of clearing of bushes and improper waste disposal practices that leads to increase in stagnant water and other breeding places for the malarial vector.

### 1.1.2. Overview of Malaria Control Measures and Treatment.

Strategies for controlling malaria include: i) vector control; ii) use of bed nets; iii) insecticides or insecticide-treated bed nets; iv) early diagnosis; v) increasing the availability and effectiveness of therapeutic agents; and vi) development of vaccines.[1-2,9-13, 14-15]

Prophylaxis may be provided by drugs such as pyrimethamine but, once an individual has been infected, therapeutic antimalarial drugs are needed. Chemotherapeutic agents can be classified according to the stages in the plasmodium life-cycle which these agents inhibit. Tissue schizonticides, for example, inhibit the development of the parasites at the liver stage, while blood schizonticides inhibit the development of the intra-erythrocytic stage; gametocides are antimalarial agents that prevent the development of the sexual forms of the malaria parasite in the patient's blood thereby preventing their transmission to mosquitoes during ingestion of blood from their prey.[2,11] There is also the possibility of the parasite being sensitive chemotherapeutic drugs at more than one stage of its development. [12-13,14-15]

Another way of classifying antimalarial agents is based on the stage of the disease in the human host for example, as casual prophylactics, suppressive treatments, clinical cures and radical cures.[2,14-15] Various chemical classes of antimalarial drugs, such as aminoquinolines (e.g. quinine, chloroquine, amodiaquine), 2,4-diaminopyrimidines (pyrimethamine), sulphonamides (e.g. sulfadoxine), hydroxynaptho-quinones (e.g. atovaquone) and antibiotics (e.g. tetracycline, doxycycline, clindamycin) are applied in malaria therapy.[13, 14-15]

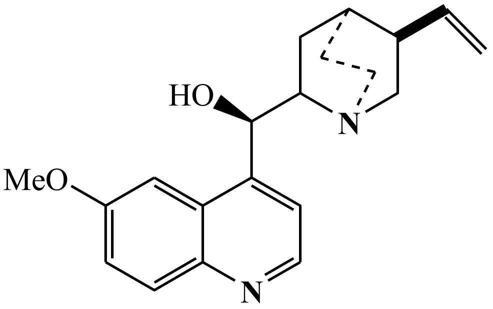
A major challenge confronted in the management of malaria is the development of resistance, by the malaria parasite, to available, established and potent antimalarial drugs, such as chloroquine, artemisinin derivatives, sulfa drugs and atovaquone. Such drug resistance, which involves reduction in the sensitivity of the malaria parasites to normal doses of antimalarial drugs may be caused by various factors.[2,13,14-15] These include: i) *poverty* (malaria infection among the poor, who have limited, if any, access to standard health services); ii) *self-medication*; iii) *poor patient compliance* with treatment regimens; iv) *population movement* carrying infection and thereby spreading the disease; v) *reduced immunity*, and vi) *high levels of transmission intensity*. Other problems associated with the conventional antimalarial drugs include adverse side-effects and the production and sale of sub-standard formulations containing little, if any, of the active drug. Joshi and Viswanathan noted that, in 2001, 38% of the artesunate samples obtained from shops in Burma, Cambodia, Laos, and Thailand contained insufficient or no active antimalarial ingredient.[2,13]

Noteworthy is the application of combination therapies, in which different antimalarial agents, with different modes of action and different plasma half-lives, are used. This approach is particularly efficient in circumventing the parasite's resistance but, unfortunately, it is generally very expensive. One exception is the use of "BlueCQ", which is a cheap antimalarial agent comprising a combination of methylene blue and chloroquine (CQ).[12,13] The availability of efficacious vaccines in the near future seems to be a remote possibility[13] and, consequently, there is an obvious need for low cost, efficient, curative (and possibly preventive) malaria treatments which do not induce resistance.[2,3,11a-13,14-15]

### 1.1.3. Quinoline-based Antimalarials.

Quinoline-containing antimalarials have long been used to combat malaria. The first quinoline antimalarial drugs were alkaloids extracted from the bark of cinchona trees, named after the Countess of Chinchon, who, according to history, had been cured of malaria by a powder made from cinchona bark in 1630.[12] The crushed bark of the "fever tree" was widely distributed in Europe during the 17th century.[12] In time, variations began to appear in the quality of the bark preparations and this resulted in efforts to isolate the active ingredients.[9] Gomes extracted a crude mixture of crystalline alkaloids from cinchona bark in 1810, and Pelletier and Caventou isolated quinine and cinchonine ten years later.[9] Quinine **1** has since been in use as an antimalarial drug.[12.13]

### 1.1.3.1. Quinine.



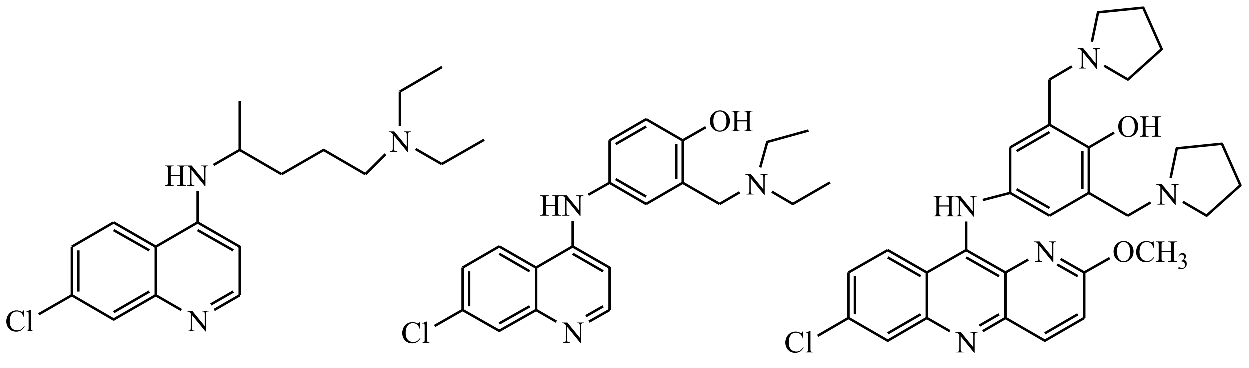
**1** Quinine

Structure of quinine – the first antimalarial drug.

Quinine **1** was listed in the London Pharmacopeia in 1677.[11] It is a 4-quinolinemethanol derivative bearing a substituted quinuclidine ring.[11a,12] A very bitter substance, quinine has been used by millions of malaria sufferers. It has been employed successfully to treat chloroquine-resistant strains of *P. falciparum* and is still considered to be the drug of choice for drug resistant strains.[13] Though the widespread use of quinine is limited by its toxicity, bitter taste and adverse side-effects, such as nausea, tinnitus and deafness, it has served as a starting point for designing new synthetic alternatives with better pharmacological profiles.[11a-14] These include: i) ***4-aminoquinoline derivatives***, such as chloroquine, amodiaquine, and the acridine analogue, pyronaridine; ii) ***aryl-amino alcohols*** related to quinine, such as mefloquine, halofantrine and lumefantrine; and iii) ***8-aminoquinolines*** exemplified by primaquine and tafenoquine.[12-13,14-16] After the Second World War, quinine was largely replaced by chloroquine and pyrimethamine for prophylaxis and routine treatment. This explains why quinine has been spared extensive or prolonged use and, hence, high-level resistance to the drug has been very slow to develop.[11a-13,14-15]

### 1.1.3.2. 4-Aminoquinolines.

Chloroquine (CQ) **2** was discovered as a result of comprehensive antimalarial drug development endeavours in the United States of America during World War II, but the compound was familiar to the Germans as early as 1934 under the trade name, *Resochin.*[12-13,14-15] Synthesized by scientists in the Bayer laboratories in Germany, this drug was patented as an antimalarial compound by the Winthrop Company (United States), under a pre-war disclosure agreement between the two companies. *Resochin* was initially thought to be too toxic for clinical use and, consequently, was not used for a period of ten years. However, during World War II, since the Allied troops, were faced with the problem of malaria infection, with no available solution, re-evaluation of Resochin became necessary. This re-evaluation indicated that Resochin was safe at therapeutic concentrations, and it was renamed chloroquine and taken into clinical trials in1943.[13] Chloroquine has since been used for the treatment of malaria. Its safety (low risk of side-effects) and efficacy, especially when used at prescribed dosage levels, as well as its low cost, brought chloroquine to the forefront in the treatment of malaria, and it was used extensively for almost two decades after its first introduction. Unfortunately, the malaria parasite developed resistance to chloroquine in the 1960s.[12,13] The United States embarked on a major screening programme at the end of World War II, which generated another 4-aminoquinline derivative, amodiaquine (AQ) **3**, as an alternative to chloroquine. Structurally related to CQ, amodiaquine inhibits the development of drug-resistant strains of *P. falciparum* and has been used as a prophylactic agent for over four decades. Although amodiaquine is more efficacious in destroying the malaria parasite than CQ, its therapeutic use has been limited by its hepatoxicity, agranulocytosis and cross-resistance with CQ.[12-13,14-15] Pyronaridine **4**, an acridine derivative that is similar to CQ and AQ, originated from China in 1970 and has demonstrated efficacy against all four *Plasmodium* species affecting humans, including drug-resistant strains.[11-13,14-15]

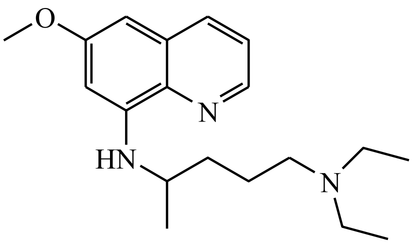


**2** Chloroquine **3** Amodiaquine **4** Pyronaridine

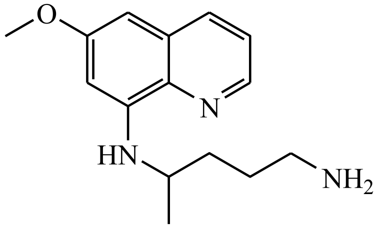
**Figure 4.** Structures of selected 4-aminoquinoline antimalarials.

### 1.1.3.3. 8-Aminoquinolines.

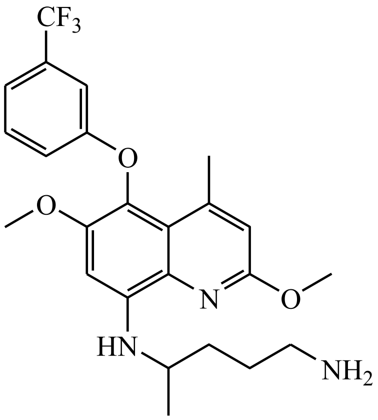
Another class of compounds that has played a role in the development of synthetic antimalarials was the 8-aminoquinolines. The first member in this class, pamaquine **5**, (initially called plasmoquine) was developed in 1925 by German researchers, who observed that methylene blue exhibited some antimalarial activity.[10-13] Pamaquine was given special attention because of its efficacy against liver-stage parasites; however, it was later found to be toxic, and primaquine **6** was synthesized as a less-toxic analogue. Primaquine is capable of targeting pre-erythrocytic stages of *P. falciparum, P. vivax* and *P. ovale*, including hypnozoites, with excellent prophylactic activity and has been shown to inhibit the maturation of fertile gametocytes, but it is not active against blood-stage parasites. Due to toxicological concerns, this compound was subjected to further structural optimization, which led to the development of tafenoquine **7**, which has a longer plasma half-life, lower toxicity and improved activity against both liver- and blood-stage parasites.[10-13]



**5** Pamaquine



**6** Primaquine



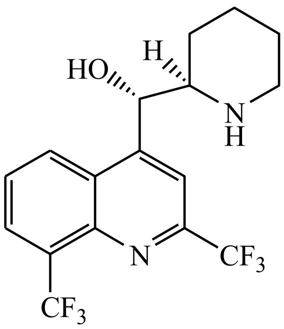
**7** Tafenoquine

**Figure 5.** Structures of selected 8-aminoquinoline antimalarials

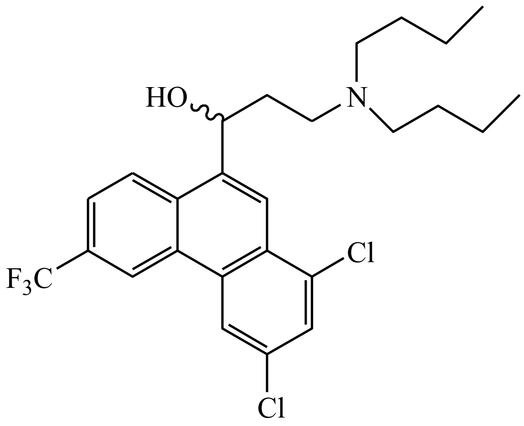
### 1.1.3.4. Quinoline-methanols and aryl alcohols.

In the early 1960's, CQ being very popular and efficacious, was misused unintentionally in population-based dosing programmes aimed at total malaria eradication. In such programmes, tons of CQ were supplied, by the World Health Organization to countries like Brazil,[12-16] for use as a supplement in table salt. This gave rise to the problem of chloroquine-resistant malaria in these areas which has since spread elsewhere. During the Vietnam War, the problem of drug resistance became apparent, and the Walter Reed Army Institute for Research started large-scale screening of potential antimalarial drugs.[12-13] However, only a handful of useful drugs emerged from these studies, the most promising being the 4-quinolinemethanols, which are structural analogues of quinine. Mefloquine **8**, commercially known as 'Lariam', was the first member of this group to be developed.[12-13] Mefloquine proved to be useful prophylatically, but, its long half-life (2-3 weeks) gave rise to resistance and adverse side-effects, such as insomnia, anxiety, vivid dreams and visual disturbances, were reported. As a result, mefloquine was not recommended for people suffering from depression, anxiety or other major psychological disorders.[12-13,14-16]

The 4-quinolinemethanols were another major (although related) class of compounds to emerge from the Walter Reed screening programme. This class comprised of compounds in which the quinoline moiety of the former was replaced by a different aromatic ring system to form the aryl(amino)carbinols. The 9-phenanthrenemethanols, a sub-class of the 4-quinolinemethanol antimalarials, appeared to be promising, and halofantrine **9** was introduced for clinical use.[11a] However, its usefulness has been questioned due to reports of serious cardiotoxicity.[12,15]



**8** Mefloquine



**9** Halofantrine

**Figure 6.** Structures of selected quinolinemethanols and aryl alcohol antimalarial drugs.[14]

It can be seen from the history of the quinoline antimalarial agents, that War has had a major influence in promoting the development of novel drugs. Surprisingly, the massive drug-screening programmes, launched in response to military imperatives, led to the development of only a handful of effective drugs. Unfortunately, these hard-earned and efficacious compounds have, all too often, been wasted by inappropriate use, and some regions of the world are rapidly approaching a situation in which no completely reliable drugs are available for the treatment of malaria.[12] As Western powers have not been involved in any lengthy military exercises in malarial areas since the Vietnam War, the development of novel antimalarial drugs has been neglected. Multinational drug companies have all but abandoned antimalarial drug research,[12,15] and the development of any promising lead compounds has fallen largely to under-resourced organizations, such as the World Health Organization. The outlook, however, is not completely gloomy. In recent years, an understanding of the molecular bases of action of existing quinoline antimalarial drugs, as well as the mechanisms of resistance, has begun to emerge. This raises the possibility that novel drugs might be developed by a process of rational design, which should be much more efficient than the mammoth random screening programmes used in previously.[12,15]

## 1.2 HISTORY OF HIV/AIDS AND ITS TREATMENT.

### 1.2.1. Overview of the HIV/AIDS Pandemic.

An understanding of the HIV/AIDS pandemic and its spread worldwide, must inform the search for global solutions. The Acquired Immunodeficiency Syndrome (AIDS) is a condition in humans in which the immune system begins to fail, leading to life-threatening opportunistic infections. It is caused by two human immune deficiency associated viruses, HIV-1 and HIV-2, belonging to a family of ribonucleic acid (RNA) lentiviruses known as *Retroviridae*, hence the acronym HIV/AIDS.[17-19] These two viruses are responsible for infections which destroy the immune system of the infected host, leaving the body susceptible to further infection from a wide variety of bacteria, fungi and other viruses.[15-17] The severity of HIV/AIDS is defined by the immunity level (CD4 count) and the manifestation of opportunistic infections. Since the knowledge of its existence made news in the early 1980's, AIDS has become a major global health problem with staggering levels of infection and mortality, killing over 25 million people between then and 2006.[18-21]

In 2008, the global estimate of people living with HIV infection stood at 33 million[19] - a decline of 7 million over the preceding two years. [21-23] Over 60% of these people live in Africa[18] and a sizeable percentage of them are children under the age of 15 years. [18-23] World HIV/AIDS distribution patterns in 2008 as published by the United Nations are illustrated in Figure 7.

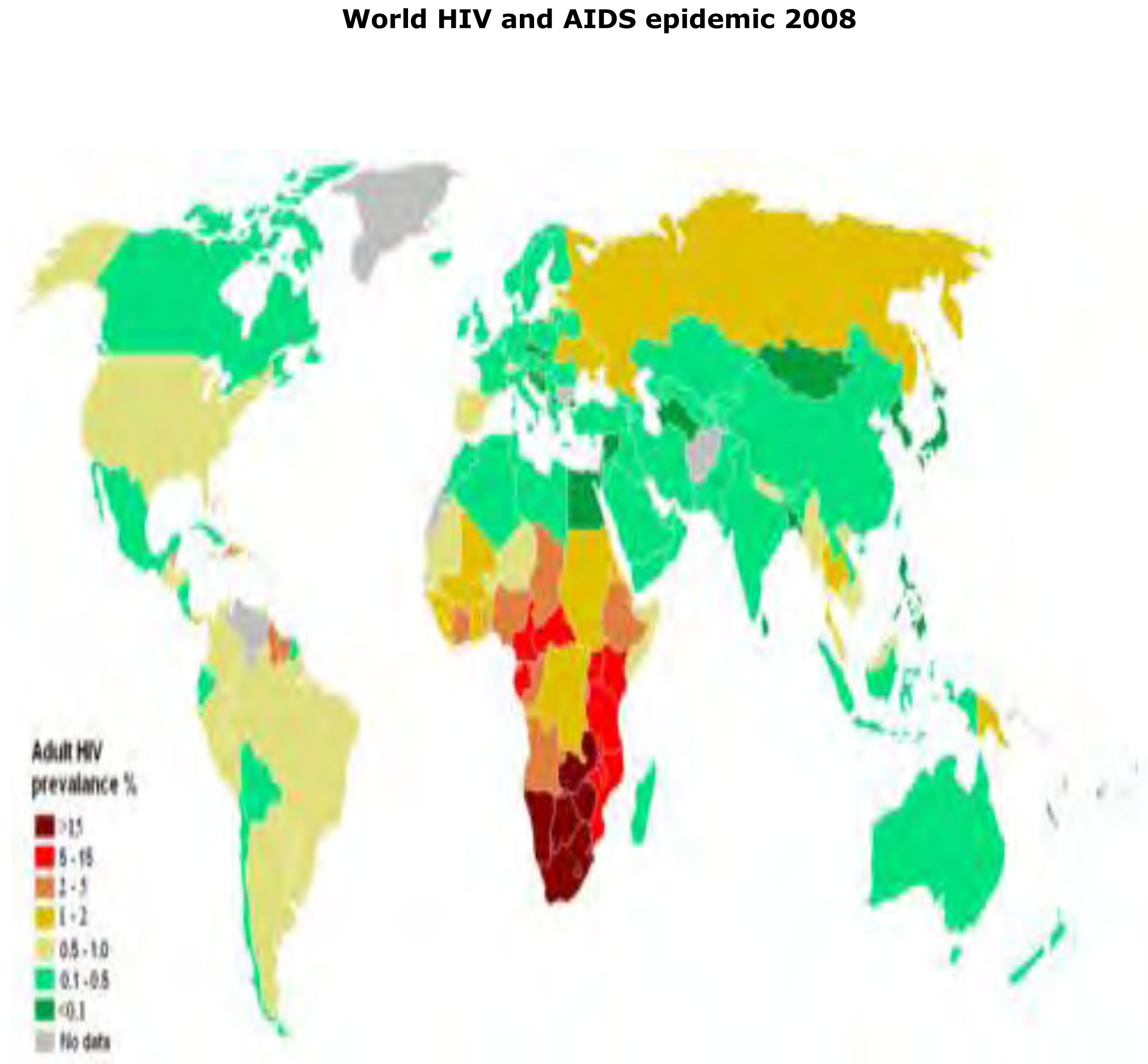
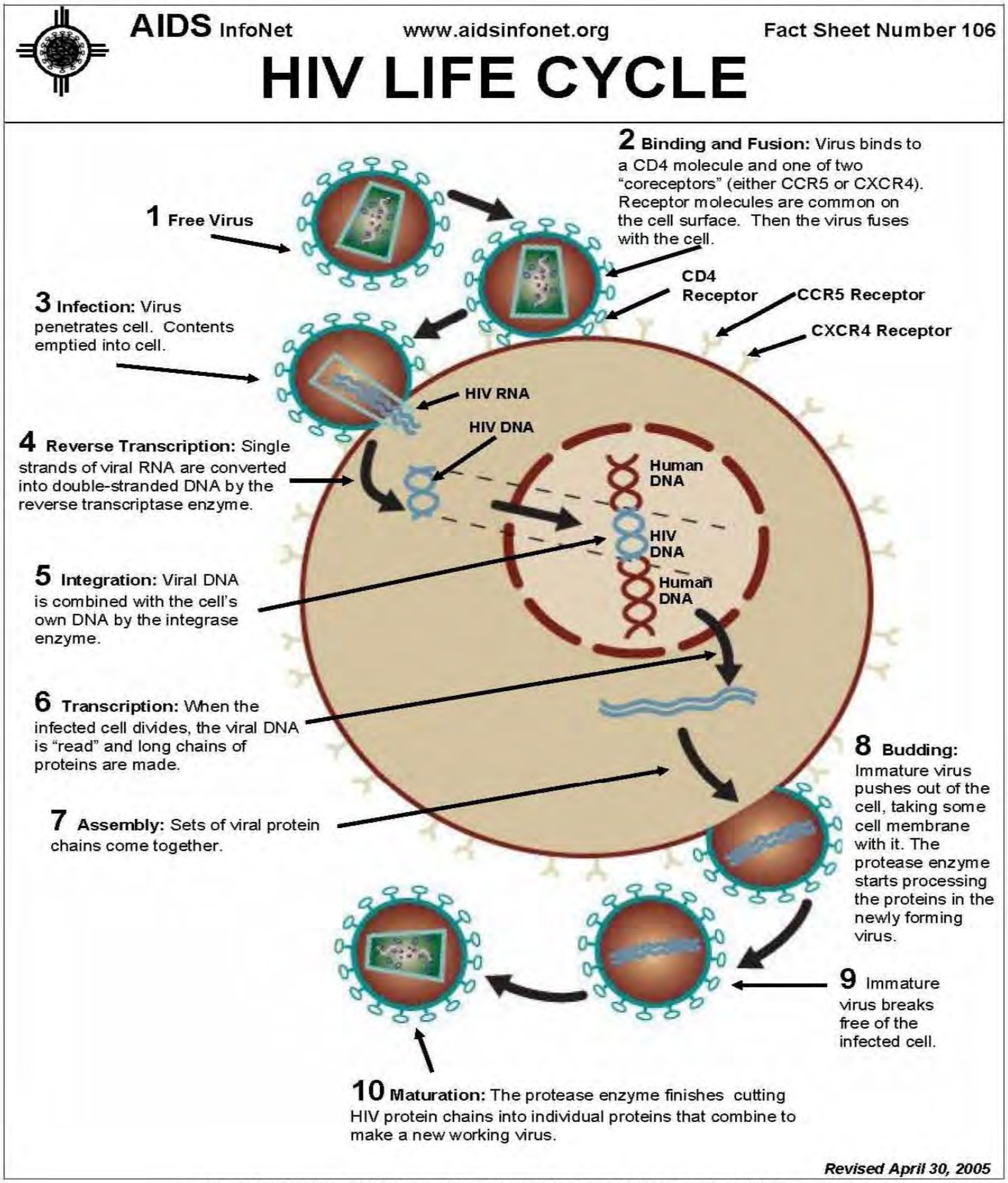


**Figure 7.** Percentage of adults infected by HIV/AIDS by country. Source:UN-UnitedNations(Googleimages).[18]
Reproduced by permission.

High levels of infection continue in spite of great strides in ensuring access to preventive programmes and in developing effective treatments, including the use of highly active antiretroviral therapy (HAART) .[17-23] HAART involves HIV/AIDS treatment regimens which combine therapeutic agents that inhibit the viral enzymes' protease (PR), and reverse transcriptase (RT).[25,38] Enfurvitide, a fusion inhibitor (FI), is sometimes included to supplement HAART programmes.[38] It is apparent that the HIV/AIDS pandemic is yet to be effectively controlled, and this situation calls for more research to find more effective therapeutic agents. [17,22-25, 33-39] It is also important to note, however, that HAART is not a cure for HIV/AIDS, neither is there any vaccine to prevent infection. In attempting to find a cure or vaccine for this pandemic, it is important to understand the HIV life-cycle. Laboratories world-over, through concerted efforts, established the causative organism (HIV) and the mode of transmission (transfer of bodily fluids between humans).[17,18,21] HIV-1, which is endemic in sub-Saharan Africa and responsible for the greatest disease burden, is genetically diverse and due to its complex ability to mutate, presents major drug resistance problems. [17,18]

A schematic representation of the HIV-1 life-cycle is reproduced in Figure 8.[24] Infection of a host by HIV involves the phases shown in the diagram below. In the first stage, the free viral particle (virion) attaches itself to the cell membrane of the host cell, a process in which the coat protein of the virus (gp – 120) attaches itself to CD4 receptors and either CXCR-4 or CCR-5 co-receptors on the human T-cell surface.



**1 Free Virus**

**2 Binding and Fusion:** Virus binds to a CD4 molecule and one of two "coreceptors" (either CCR5 or CXCR4). Receptor molecules are common on the cell surface. Then the virus fuses with the cell.

**3 Infection:** Virus penetrates cell. Contents emptied into cell.

**4 Reverse Transcription:** Single strands of viral RNA are converted into double-stranded DNA by the reverse transcriptase enzyme.

**5 Integration:** Viral DNA is combined with the cell's own DNA by the integrase enzyme.

**6 Transcription:** When the infected cell divides, the viral DNA is "read" and long chains of proteins are made.

**7 Assembly:** Sets of viral protein chains come together.

**8 Budding:** Immature virus pushes out of the cell, taking some cell membrane with it. The protease enzyme starts processing the proteins in the newly forming virus.

**9** Immature virus breaks free of the infected cell.

**10 Maturation:** The protease enzyme finishes cutting HIV protein chains into individual proteins that combine to make a new working virus.

A Project of the New Mexico AIDS Education and Training Center. Partially funded by the National Library of Medicine.
Fact Sheets can be downloaded from the Internet at http://www.aidsinfonet.org

**Figure 8.** The HIV-1 Life-cycle. (Google images).[24] Reproduced by permission.

A fusion process follows, in which the virus mixes its membrane with that of the host cell, and the contents of the viral cell enter the host cell.[17,32-36] As a result, two copies of the single-stranded viral RNA genome and three enzymes crucial to the survival of the virus in the host cell are simultaneously injected into the host cell.[17] These three enzymes are reverse transcriptase (RT), integrase (IN), and protease (PR).

Reverse transcriptase catalyses the formation of double-stranded DNA (dsDNA)– a complementary copy of the viral genome, known as proviral DNA. The integrase enzyme, as its name suggests, catalyses integration of the proviral DNA into the host cell genome; [16,24] viral transcription then follows, whereby numerous copies of viral messenger RNA are produced.[17] Finally, in the maturation process, the protease enzyme, catalyses division of the polyproteins produced during transcription, into the required lengths.[16] This understanding of the various stages in HIV-1 replication in the host cell has permitted targets for drug development to be identified.[17,19,21,25,26] For example, the polymerase activity of the RT enzyme, served as the target for the development, in 1987, of the first HIV-1 drug, zidovudine (AZT), a nucleoside inhibitor (NRTI).[17, 20,25]

Drugs that have thus far been approved by the USA Food and Drug Administration (FDA) for antiretroviral treatment of HIV can be grouped into the following classes: nucleoside reverse transcriptase inhibitors (NRTIs), nucleotide reverse transcriptase inhibitors (NtRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), fusion inhibitors (FIs), co-receptor inhibitors (CRIs), also known as entry Inhibitors (EIs), and maturation inhibitors (MIs).[17,21,25,34] Non-enzymatic viral targets have also been identified and certain natural compounds have been found, which act as HIV enzyme inhibitors. [17,37]

### 1.2.2. Current HIV Treatment and Drug Classes.

The major treatment strategy for HIV/AIDS, is based on inhibition of the activities of various enzymes critical to the replication lifecycle of the virus in the human host as depicted in the Figure 9, which highlights the HIV propagation cycle and the critical targets for therapeutic intervention.[17,34]

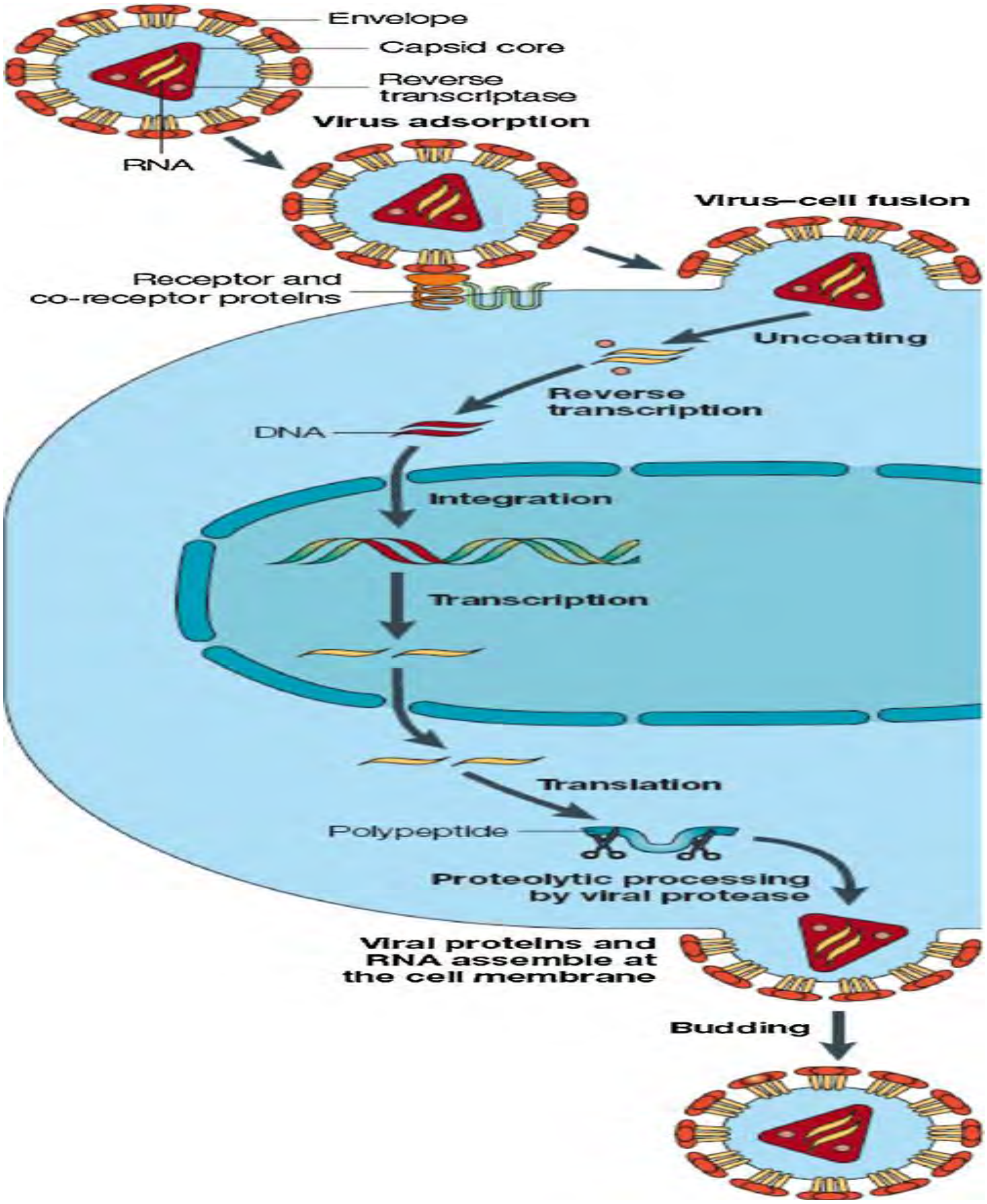
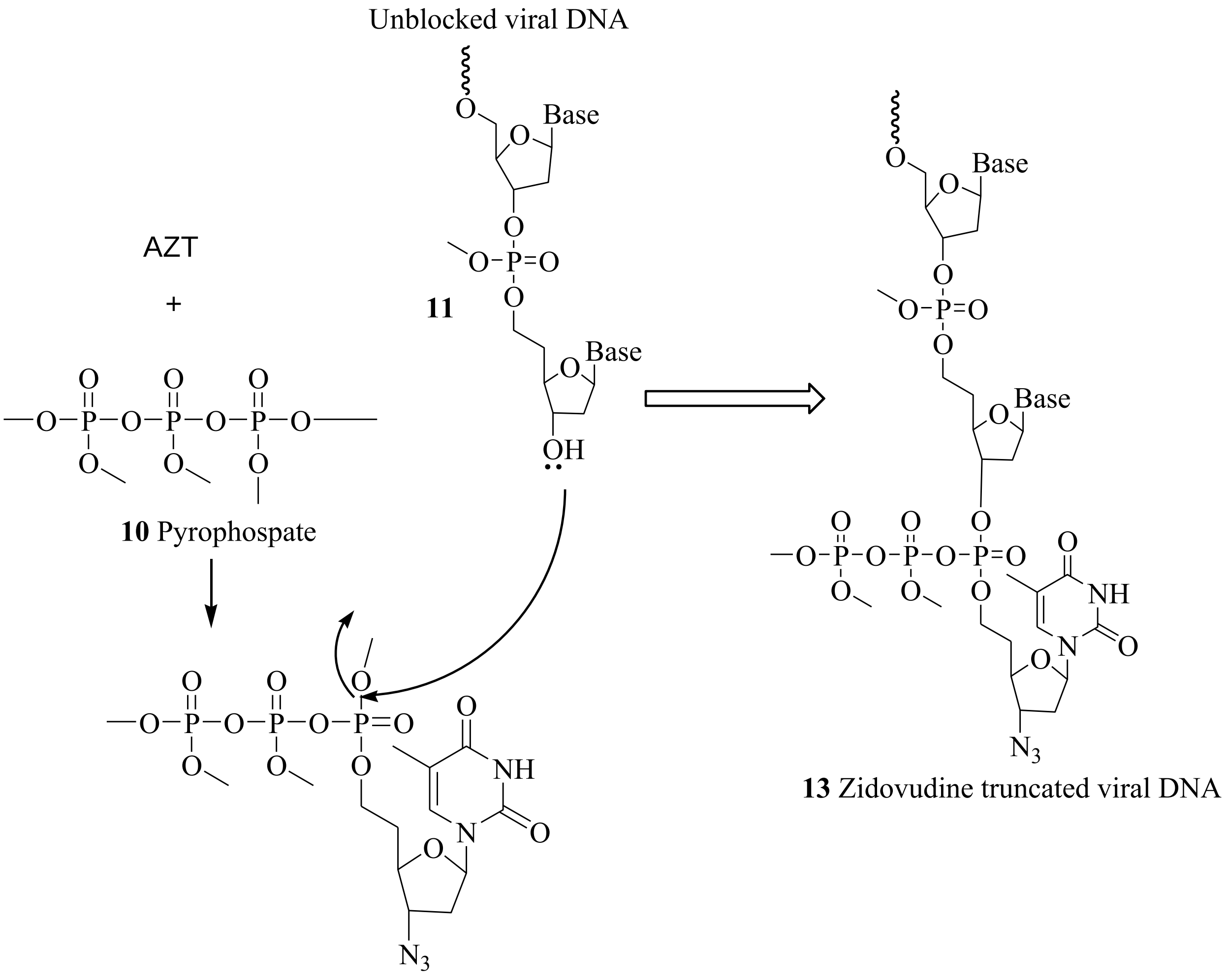


**Figure 9.** HIV replicative cycle, highlighting target areas for therapeutic intervention, *viz*.,(co-receptor interaction; virus-cell fusion; reverse transcription; integration and proteolytic processing. Reproduced by permission. [34,35]

### 1.2.2.1. Nucleoside Reverse Transcriptase inhibitors (NRTIs).

As already indicated, Zidovudine (AZT or Retrovir),[34] was the first drug to be approved for the treatment of HIV/AIDS infections. First synthesized in 1964, its anti-retroviral activity is understood to be due to the 5'-triphosphate (AZT-TP) moiety being accepted, following *in vivo* triphospharylation, as a substrate mimic in the developing complementary proviral DNA chain. However, since AZT-TP lacks a 3'-hydroxy group (replaced by an azido group) the proviral DNA synthesis is truncated, as illustrated in Figure 10. This is believed to be the general mode of inhibition of the other approved NRTI drugs in this class (see Figure 11).



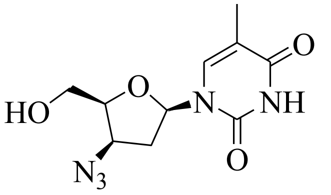
**10** Pyrophospate

**11**

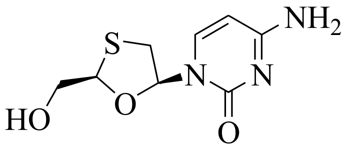
**12** Zidovudine triphospate

**13** Zidovudine truncated viral DNA

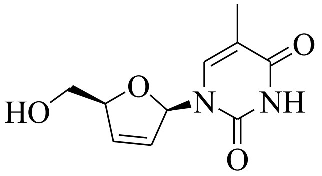
**Figure 10.** Illustration of the AZT inhibition mechanism.[17]



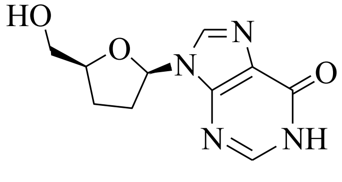
**14** Zidovudine



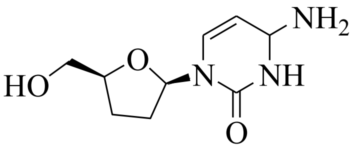
**15** Lamivudine



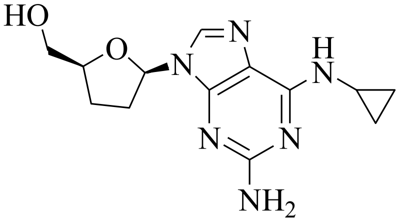
**16** Stavudine



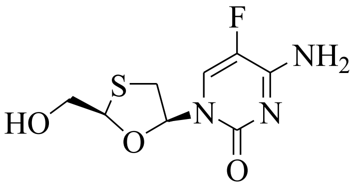
**17** Didanosine



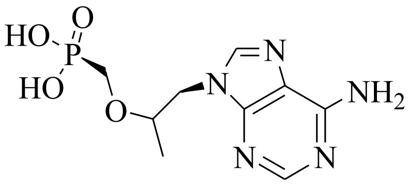
**18** Zalcitabine



**19** Abacavir



**20** Emtricitabine



**21** Tenofovir

**Figure 11.** Structures of nucleoside reverse transcriptase inhibitors **(NRTIs)** currently in clinical use.[17,34-35,37]

A major subgroup in this class are the nucleotide reverse transcriptase inhibitors (NtRTIs), which are monophosphonated analogues, such as Tenofovir.[32] The phosphonate group in such compounds cannot be hydrolysed by *in vivo* esterases.[34] These drugs have been used as monotherapeutic agents, but problems of toxicity, tolerability and the emergence of resistant viral populations soon became evident.[16, 20-33] The RT enzyme has, in fact, two important inhibitor binding sites (see Figure 12) – one for NRTIs and NtRTIs and the other for a further class of RT inhibitors, the non-nucleoside reverse transcriptase inhibitors (NNTIs).[34]

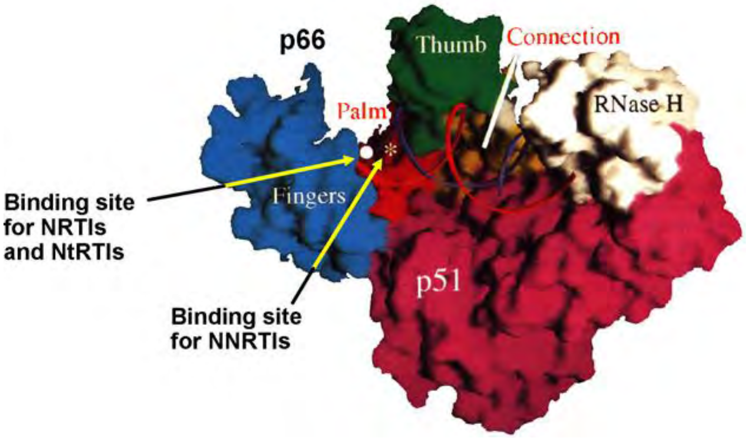


**Figure 12.** HIV-RT enzyme with the binding site for the **NRTIs** and **NtRTIs** as well as the binding site for **NNRTI's**.[34] Reproduced by permission.

### 1.2.2.2. Non-nucleoside Reverse Transcriptase inhibitors (NNRTI's).

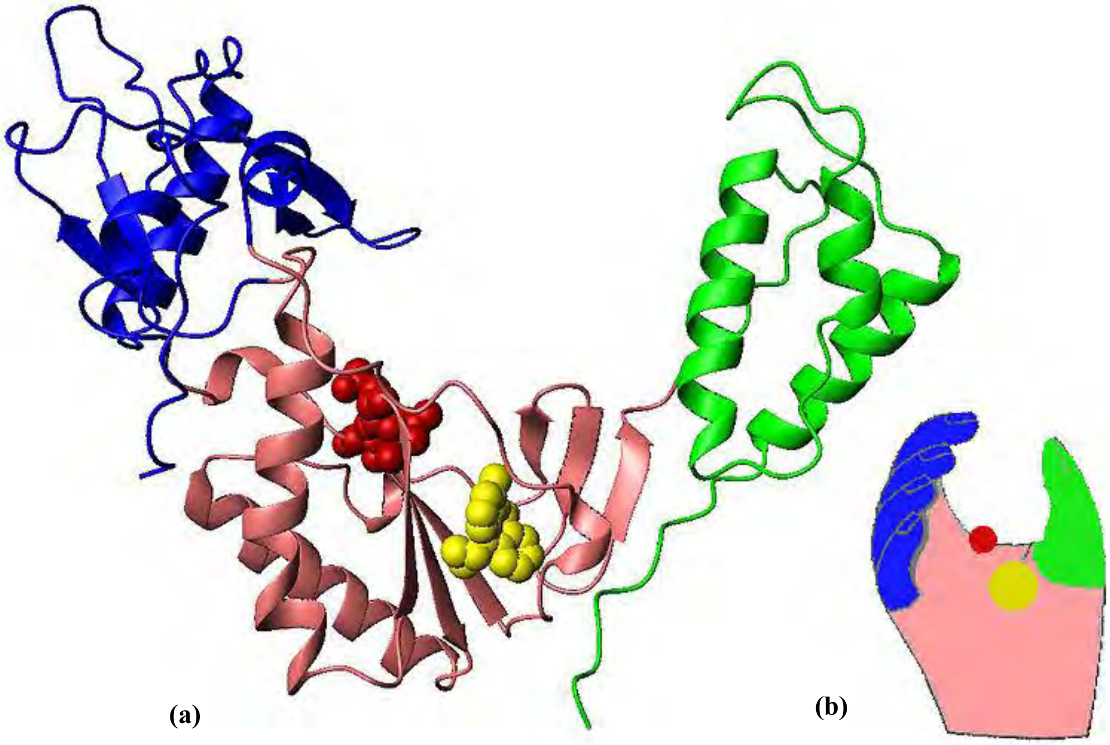
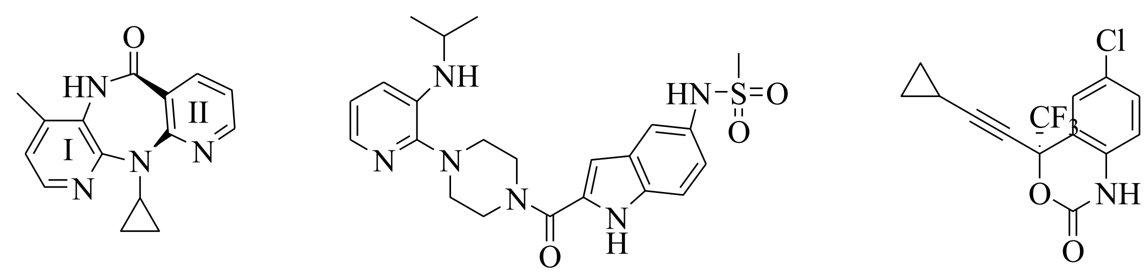


**Figure 13.** Ribbon representation (a) of the active domain of the RT enzyme, illustrating its (b)hand-like structure, showing fingers (blue), palm (pink) and thumb (green). The active site (red atoms), where the length of DNA complex is increased, is in the palm region. An NNRTI drug (yellow) is also shown binding in the allosteric non-nucleoside pocket.Reproduced by permission.[33]

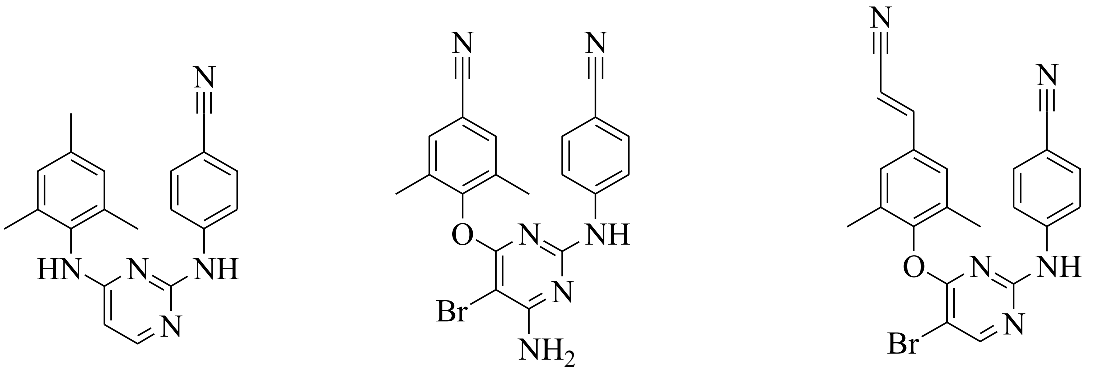
The next major group of drugs to be approved for use as HIV/AIDS drugs were the non-nucleoside reverse transcriptase inhibitors (NNRTIs). A ribbon representation of an enzyme-bound NNRT Inhibitor is shown in Figure 13. While being reverse transcriptase inhibitors, their mode of action is very different from the NRTIs.[17] In fact, it has been found that these compounds bind at an allosteric site rather than the enzyme active-site.[17] Comprehensive research efforts led to the approval of efavirenz (Sustiva, Stocrin) for use in combination therapy; efavirenz was the first HIV/AIDS drug to be administered as a once-a-day dose.[17] Since then, NNRTIs have made an important contribution to modern day treatment regimens.[17,] However, nevirapine is now being used as a substitute for patients who cannot tolerate efavirenz.[17, 27] It is important to note that NNRTIs are HIV-1 specific and have no activity against HIV-2 and other retroviruses.[33] Some examples of NNRTIs are shown in Figure 14.



**22** Nevirapine

**23** Delavirdine

**24** Efavirenz



**25** Dapivirine

(TMC120-R147681)

**26** Etravirine

(TMC125-R165335)

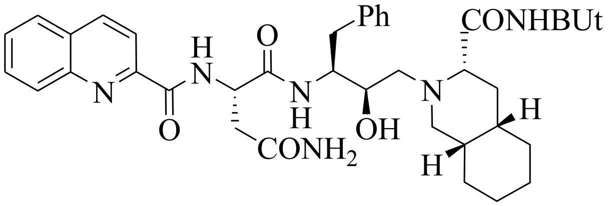
**27** Rilpivirine

(TMC278-R278474)

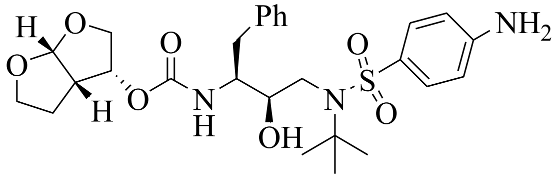
**Figure 14.** Structures of non-nucleoside reverse transcriptase inhibitors (NNRTIs).[27]

### 1.2.2.3. Protease Inhibitors (PIs).

The introduction of HIV protease inhibitors as a new class of inhibitors, in combination with existing drugs, brought about a dramatic change in HIV/AIDS treatment. HIV-1 protease, illustrated in Figure **16** is an enzyme that is involved in the production of mature viral proteins in the host cell. It catalyzes the hydrolysis of peptide bonds in the precursor protein to give a number of structural and functional proteins. Due to the fact that the structures and functions of the HIV protease are significantly different from those of human protease, HIV-1 PR is a good target for antiretroviral therapy. Many of the current HIV-1 PR drugs, for example saquinavir **28**, act by inhibiting the viral protease enzyme. The hydroxyethylene dipeptide scaffold, resembles the normal peptide linkage recognized and cleaved by the HIV-PR enzyme; but the inhibitor is, itself, not cleavable and thereby blocks the proteolytic processing of proviral proteins into daughter viral proteins.[17,27,34,] The last of the ten currently licensed PIs, Darunavir **29**, is illustrated in Figure **15**, bound in the PR active site.[34] However, due to the susceptibility of the HIV-RNA-based genome to random mutations, the protease enzyme is constantly evolving to counter inhibition drugs. Thus, the design of new, efficient and effective drugs to accommodate such evolution remains an important research goal. [17,25-28]



**28** Saquinavir (SQV)



**29** Darunavir TMC-114

**Figure 15.** Structural formulae of saquinavir(SQV) and darunavir (TMC-114).

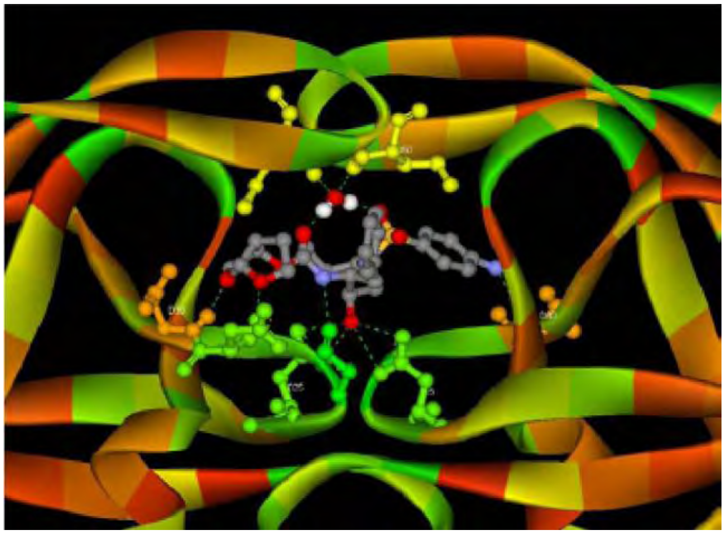


**Figure 16.** HIV- 1 PR ribbon structure with the inhibitor, Darunavir (TMC-114), in the active site.[34] Reproduced by permission.

### 1.2.2.4. Fusion Inhibitors (FIs).

Fusion inhibitors are antiretroviral drugs that interrupt the first step in viral propagation in the host cell. This is the step in which the virion attaches specific proteins on its surface to one or more receptors on the surface of its target. The gp-41 and gp-120 viral proteins are responsible for this attachment. DP-178, now marketed as enfuvirtide (Fuzeon), an N-terminal acetylated, C-terminal amide peptide (Figure **17**), which conforms to residues 643-678 of HIV-1 gp-41, on the extracellular side of the transmembrane region, has shown potent antiretroviral activity at minimal concentrations (1 – 80 ng $L^{-1}$) against HIV-1 but not HIV-2 cells. This compound interrupts both cell-to-cell and cell-free transmission of the HIV-1 cell culture at very low concentration.

$CH_3$CO-Tyr-Thr-Ser-Leu-His-Ser-Leu-IIe-Glu-Glu-Ser-Gln-Asn-Gln-Gln-Glu-Lys-Asn-Glu-Gln-Glu-Leu-Leu-Glu-Leu-Asp-Lys-Trp-Ala-Ser-Leu-Trp-Asn-Trp-Phe-$NH_2$

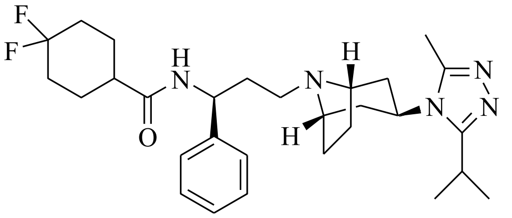
**Figure 17.** The structure of Enfuvirtide (Fuzeon).[34]

This drug has been shown to interrupt the gp-41 refolding necessary for viral fusion, this represents a novel mechanism of action and results in high inhibition efficacy.

Enfuvirtide was thus listed for clinical studies, but when administered intravenously it shows instability to conditions in the intestine. After two weeks of clinical trial, the drug showed a remarkable reduction in the viral load in patients who received twice-daily injection doses of the drug – even in patients whose previous combination therapy had been declining. The drug was eventually given regulatory approval by the FDA in 2003, for the treatment of HIV/AIDS patients who show signs of viral replication while on other antiretrovirals (ARVs). [34]

### 1.2.2.5. Co-receptor Inhibitors (CRIs).

This class of inhibitors is active against the viral co-receptors CCR5 or CXCR4, which are the respective sites, used by M (macrophage-tropic) and T (lymphocyte-tropic) HIV strains to infect target host cells. These strains of viruses are commonly called R5 and X4 strains respectively. Presently, only one drug, a CCR5 antagonist, maraviroc, has been approved in this class.[34] However, maraviroc is a selective inhibitor for R5 strains and there is a need for co-receptor inhibitors that inhibit the X4 strains, if CRIs are to contribute meaningfully in the war against HIV/AIDS.



**30** Maraviroc (Selzentry®; UK-427857)

**Figure 18.** Structural formula of maraviroc (UK-427857; Selzentry®).

HIV/AIDS infection ‘is no more a death sentence’ [23-32] as it was in the early 1980’s because of the availability of a significant number of ARV drugs approved for use in treatment regimens. A critical factor in this success has been the use of triple-action HAART combination therapy. Some of the major difficulties encountered are high cost, adverse side-effects, development of resistance to existing drugs by the virus which is always undergoing mutation. Experience has shown that, in the face of all the above efforts at finding a remedy for HIV/AIDS infection, the virus still remains within what is known as “HIV- sanctuaries” where it “hides” and continues to replicate. Blankson *et al*. gave a classical definition of an HIV-1 sanctuary, (also known as viral reservoirs), “as a cell type or anatomical site associating with a form of the virus, competent of replication, which accumulates and persists with a stable kinetic properties than the main pool of actively replicating virus”.[23] Developing countries, like South Africa (and other countries in sub-Saharan Africa) are experiencing the adverse impact of the HIV/AIDS epidemic and struggling under the heavy burden of the highest global percentage of HIV/AIDS positive population and the continuous risk of new intfections.[22,23] The HIV-sanctuaries are believed to be the underlying factor in persistent infection, even in the presence of HAART. This emphasizes the need for the development of new drugs targeting other processes in the viral life cycle, to enhance the progress already made in the fight against HIV/AIDS.[19-26] The third enzyme, critical in the viral propagation in the host cell, is integrase and this is the focus of new drug development strategies in many laboratories including our own.[23,24-32]

### 1.2.2.6. Integrase Inhibitors (INI’s).

The HIV-1 integrase enzyme facilitates integration of reverse transcribed DNA, into the host cell. It catalyzes polynucleotide transferase activity, which initiates the cleavage of the host DNA genome and the subsequent insertion of a copy of the viral DNA at the site of cleavage.[34]

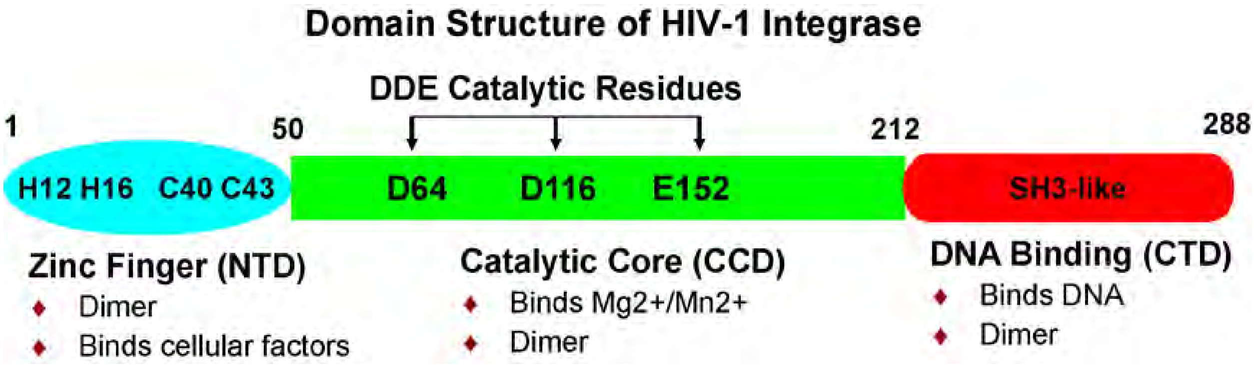
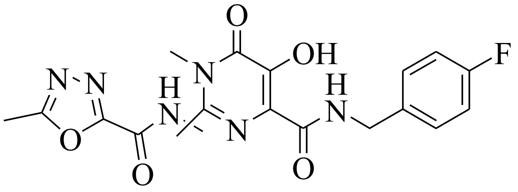


**Figure 19:** Schematic representation of the structural domains of HIV integrase.[35, 39] Reproduced by permission.

This process affords 'proviral DNA' or Cdna in the host cell genome and eventually utilizes the cell instruments in maintaining and replicating itself into daughter viral cells.[17,34,37,38] The integration process, is thus a critical part of the viral propagation cycle.[34] The integrase enzyme has three distinct domains with different functions, but all three are involved in the two integration processes, *viz*., 3'-processing and strand transfer [26, 33, 36] The domains are the amino-terminal domain (NTD), the catalytic core domain (CCD) and the carboxyl-terminal domain (CTD) as shown in Figure 19 below.[35, 37, 38-42] Figure **20** illustrates a ribbon representation of the core domain of HIV-1 integrase complexed with a potential inhibitor.[39]

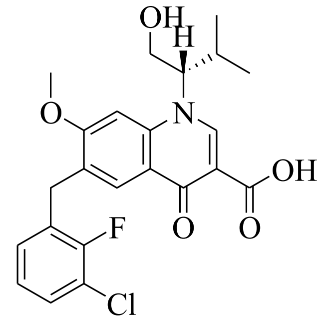
**Figure 20.** Ribbon representation of the core domain of HIV-1 integrase complexed with $Mg^{2+}$ and 1-(5-chloroindol-3-yl)-3-hydroxy-3-(2*H*-tetrazol-5-yl)-propenone(PDB code: 1QS4).[39] Reproduced with permission.

The first integrase (IN) inhibitor to be approved by the FDA for clinical use was raltegravir (Figure 21) from the *N*-methylpyrimidinone family and broadly related to the diketo-acids.[17,32,34,37.] This compound is an integrase strand transfer inhibitor (INSTI) and has proved to be very effective in reducing viral loads in HIV- infected patients.[32] While a number of potential INSTIs such as elvitegravir (GS-9137, JTK-303) Figure 25, are in clinical trials.[37]



MK-0518 Raltegravir Isentress ™

**31**

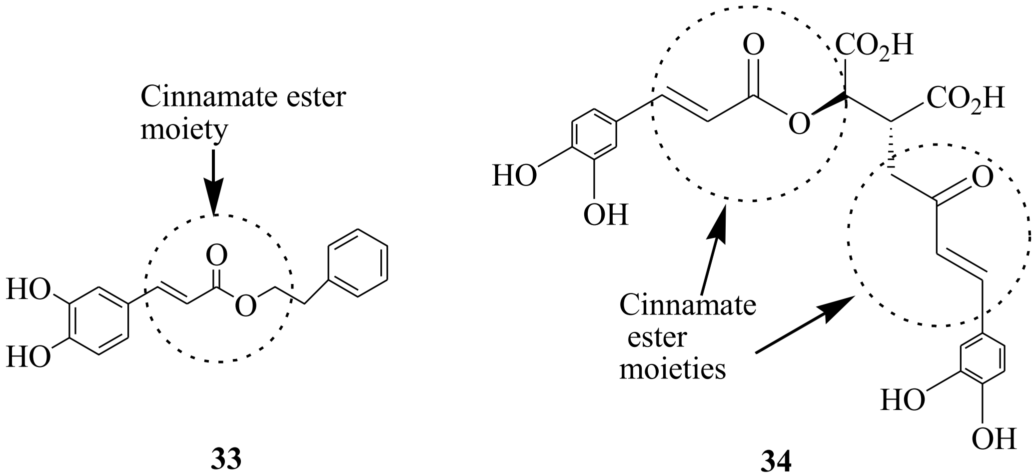


Elvitegravir(GS-9137, JTK-303)

**32**

**Figure 21.** Structural formulae of the HIV IN inhibitor raltegravir (MM-0518) and elvitegravir (GS-9137, JTK-303).[37]

A large number of compounds, both naturally occurring and synthetic, have been reported to show some IN *iv vitro* inhibitory activity, but are often unfit for therapeutic development because of significant cytotoxicity or the absence of substantial antiretroviral activity.[37] The cinnamate ester moiety (Figure 22) has been identified as a significant structural feature in certain compounds which exhibit HIV-1 integrase inhibition, *e.g.* caffeic acid phenyl ester (CAPE) and *L*- chicoric acid (LCA) and their derivatives (Figure 22).[19,35,37,43] Pommier, *et al.* clearly stated in 1993 that CAPE, a non-toxic compound, had shown selective inhibition of the HIV-1 integration step.[42] Twelve years later, in 2005, another report indicated that CAPE and its analogues acted through a specific mechanism, and were considered safe for further research as potential chemotherapeutic agents. [19,46] More recent publications have also reported CAPE and CA derivatives as HIV-1 IN inhibitors.[19] In fact, chicoric acid has exhibited integrase inhibition activity, with an $IC_{50}$ range of 0.1 – 0.5µM for end-processing/strand transfer reactions and 0.1 – 0.2µM for the disintegration step.[19] It has also been shown that chicoric acid inhibits HIV replication by a non-specific gp-120 interaction.



**33**

Caffeic acid phenethyl ester(CAPE)

**34**

L-Chicoric acid (LCA)

**Figure 22.** Structures of caffeic acid phenethyl ester (CAPE) and *L*-chicoric acid (LCA).

Robinson *et al*. showed that L-chicoric acid (CA) shared a similar binding pocket with the diketo acids (DKAs), with even closer contacts with the amino acid residues in the active site.[32] However, the susceptibility of the ester linkage to cleavage by hydrolytic enzymes, limited their oral bioavailability while their conformational flexibility, arising from the number of rotatable bonds is expected to increase the entropic demand for effective binding.[19,35,37,41-42]

In an attempt to overcome these limitations, new classes of potential integrase inhibitors are being designed with the ester moiety being replaced with other functional groups with higher ARV activity, such as the more conformationally restricted amide group.[41] An alternative approach, is to administer these inhibitors containing the ester moiety, intravenously as is the case with enfuvirtide, a fusion inhibitor figure **17**.[22,34] or better still, these ester containing compounds can be utilized in the design of multiple Ligands. This is the approach adopted by our research group.

### 1.2.2.7. Designed Multiple Ligands (DMLs).

Designed Multiple Ligands (DMLs) have been defined by Zhan and Liu as “a new drug discovery term, coined to describe a single therapeutic entity, designed to inhibit multiple targets in the treatment of a particular disease; or as drug compounds that possess activity-related multiple pharmacophores or functions which can interact with multiple activity-sites related to a particular disease, or multiple domains in one single target".[4a,19, 44-45] The intention is to improve patient compliance by using dual-action drugs and thus address the development of resistance against existing drugs which arises from patients' non-compliance, which in turn, is often related to the difficulties of taking multiple drugs continuously. [4a,19, 44-46] Depending on the target sites at which such DMLs are intended to act, three main classes have been proposed, *viz*.,

i) DMLs targeted at adjacent pockets of a single protein;
ii) DMLs targeted at different proteins which recognize similar endogenous ligands; and,
iii) DMLs targeted at different proteins which recognize different non-endogenous ligands.

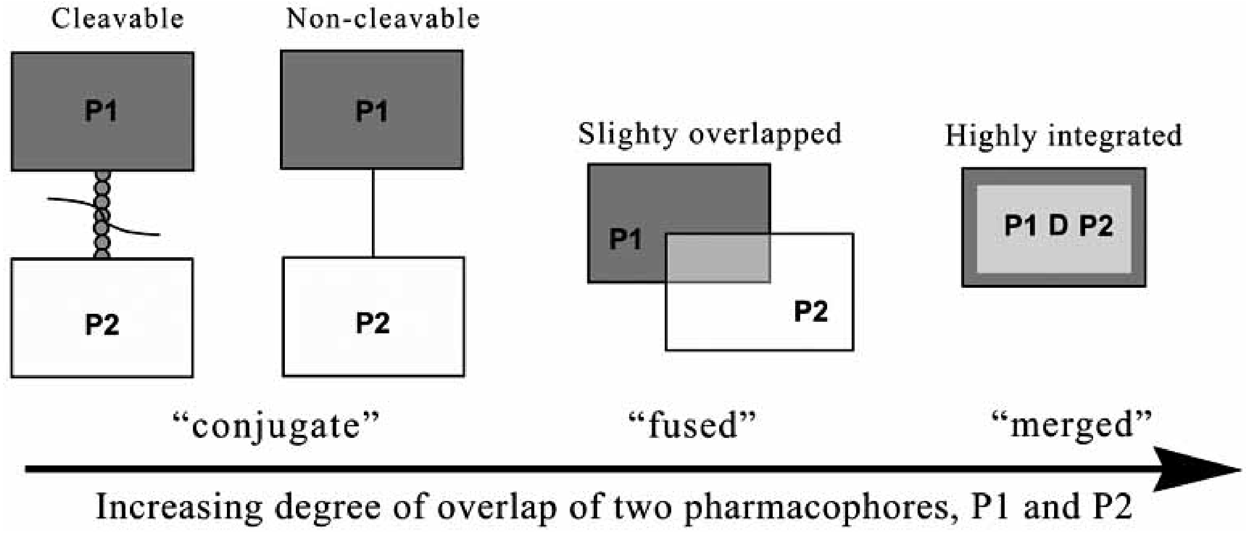


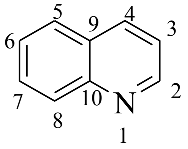
**Figure 23.** Three main clinical scenarios for multiple target therapy.[45]
Reproduced by permission.

Another classification method is based on the mode of linkage used to connect the active ligands, (Figure 23). Thus *Conjugate DMLs* contain ligands joined by a linker, *Fused DMLs* comprise scaffolds in which ligands are coupled directly with no linker, while in *Merged DMLs,* the ligands are integrated into a single scaffold.[44, 45]

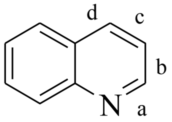
## 1.3. BIOLOGICALLY ACTIVE QUINOLINES AND QUINOLONES.

### 1.3.1. Synthesis of Quinoline and its derivatives.

The quinoline moiety **10**, is found in a number of natural occurring alkaloids (*e.g.* quinine), some synthetic chemotherapeutic agents (such as the antimalarial drug chloroquine), in the cyanine dyes (*e.g.* the pincyanol cation used as photographic sensitizer), and in the analytical reagent, Oxine (8-hydroxyquinoline).[16,17] Its bicyclic structure, first suggested by Korner, [18] was confirmed based on the constitutional, synthetic and chemical properties of quinoline. The structure was also supported by the Friedlander synthesis as well as its degradative oxidation and ring-opening reactions.[18] X-ray crystallographic analysis of its derivatives as well as different spectroscopic techniques have added further supportive confirmation of its structure.[18]

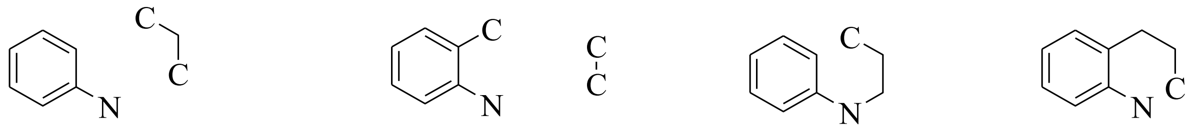


**10a**



**10b**

First isolated in 1834 by Runge, from coal tar, and eight years later from alkaline hydrolysis of the alkaloid, cinchonine, its identity was established forty years later.[19] It derived its name, quinoline, from *quina*, a Spanish version of a local South American name for the bark of quinine-containing *Cinchona* species. The ring is usually numbered as shown in structure **10**. A large number of quinoline and quinolone derivatives are known, and there are various synthetic pathways to access them depending on which of the bonds (a) – (d) in **10b** closes the ring.[16-18] The strategies most commonly used are represented in the synthon types**;** synthons **11** represent the Skraup, Döebner-Miller and Conrad-Limpach syntheses, **12** the Friedlander and Pfitzinger pathways– five well known synthetic routes to quinolines.[16] Synthons **13** and **14** represents newer synthetic methods.



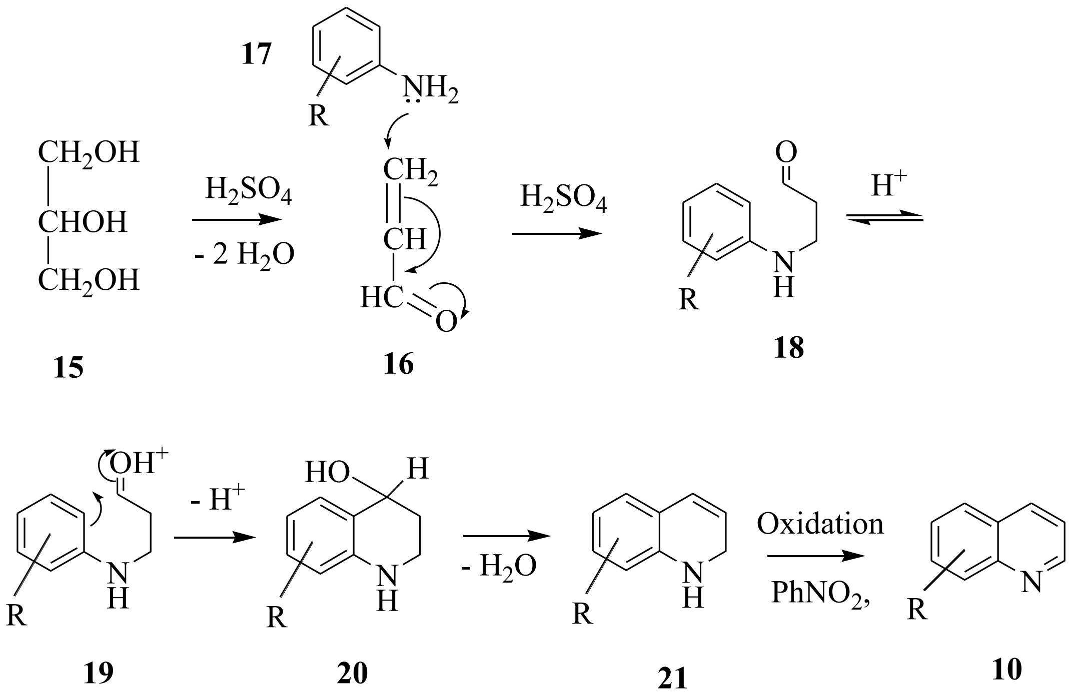
**11** **12** **13** **14**

**Figure 24.** Commonly used disconnection strategies for quinoline construction.[17]

The first reported synthesis of quinoline was by Koenigs, who used two different methods; the first involved passing ethylaniline or alkylanilines vapours over heated litharge, the second involved heating the addition product of aniline and acrolein.[17] These synthetic break-throughs were then followed by those of Skraup, of Döebner and, later, von Miller.[17]

### The Skraup Synthesis of quinoline.

This involved gently heating, a mixture of aniline (or its derivative with a vacant *ortho* position), glycerol, nitrobenzene (as a mild oxidizing agent), iron (II) sulphate and sulphuric acid, resulting in a vigorous exothermic reaction [moderated by the iron (II) sulphate], which is brought to completion by continuous heating.[16] Then residual nitrobenzene is steam-distilled off, and the reaction mixture is basified to afford quinoline, which is also isolated by steam distillation.[16] The reaction is believed to proceed *via* acid-catalyzed elimination of a molecule of water from glycerol **15** to afford acrolein **16** (Scheme 1.1) followed by conjugate addition of acrolein by aniline **17** to give the anilinopropanal intermediate **18**. Acid-catalyzed cyclization resulting from the electrophilic attack by the electron-deficient carbonyl carbon, on the ring position *ortho* to the amino group gave the intermediate **20**. Subsequent dehydration affords the dihydroquinoline **21**, which is further oxidized to the final product quinoline **10.**[16,18]

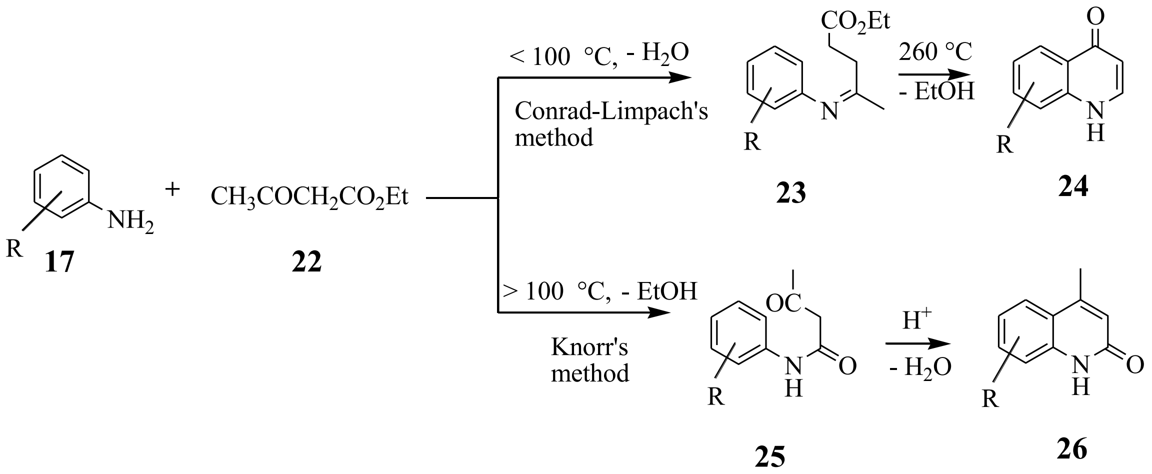


**Scheme 1.1.** Skraup's route to quinoline **10.**

This widely used synthetic protocol, incorporates the substituents on the final product by varying the type and position of ring-substituents on the aniline used in the reaction.[16] The Döebner-von Miller synthetic method to quinoline, is similar to that of Skraup, except that the Döebner's method generates the three-carbon fragment *in situ* by an acid-catalyzed aldol condensation reaction, hence a dehydrogenating agent is not used.[16]

### The Conrad-Limpach and Knorr Synthesis of quinolones.

This involves reacting β-keto-esters (such as ethyl acetoacetate **22**) with anilines either at low temperatures to afford a Schiff base **23**, which is then cyclized to 4-quinolones **24,** or at high temperatures to afford an amide **25**, which on cyclization, affords 2-quinolones **26**.[16-18]



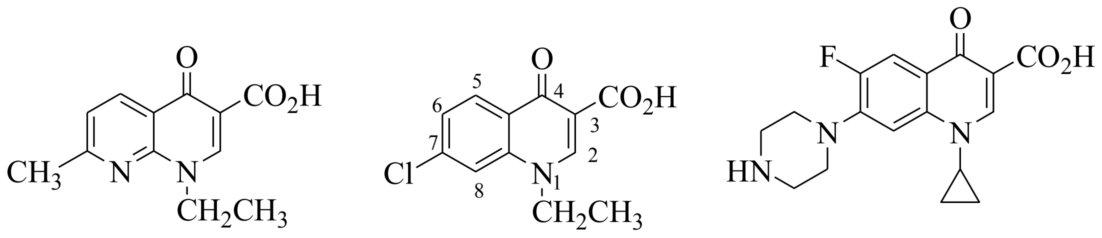
**Scheme 1.2.** The Conrad-Limpach and Knorr routes to quinolones.

There are two types of carbonyl group present in the β-keto ester **22**, at low temperatures (below 100 °C), condensation takes place between the amino group and the more reactive keto carbonyl to give a Schiff base **23**, which cyclizes to afford the 4-quinolone **24** when heated.[16,18] At higher temperatures (above 100 °C), the arylamine **17,** reacts with the β-keto ester **22**, to form the more stable amide **25**. This subsequently cyclizes when heated or in the presence of sulphuric acid, to give the 2-quinolone **26**. The low temperature process is believed to be kinetically controlled and known as (the Conrad method) while the thermodymically-controlled higher temperature reaction, is known as the Knorr method.[18] Again, substituents in the final product is introduced *via* the aniline ring.

Other synthetic routes to quinoline and its derivatives include: i) cyclization of *o*-amino derivatives of cinnamic acid, cited by Claret *et al.* as one of the earliest synthesis of the quinoline ring system;[18] ii) cyclization of cinnamanilides; iii) ring-enlargement methods; and iv) the use of the Vilsmeier-Haack reagent to access quinoline, as reported by Perumal *et al.*[18]

## 1.3.2. Quinolones.

Quinolones are related in structure to quinoline, which is the core of the well known antimalarial, quinine.[46-47a] Over the years, compounds with the 4-quinolone nucleus have also exhibited excellent medicinal properties. For instance, nalidixic acid and its analogues, both natural and synthetic, have been used as antibiotic agents in the treatment of various bacterial infections of different organs in the body, including the respiratory tract (RTIs), urinary tract, (UTIs), gastrointestinal, abdominal, skin and soft tissue infections as well as for bone and sexually transmitted diseases (STDs).[46-47a] Quinolones have also found use as anti-ulcer drugs and in the treatment of allergic asthma and some cancers.[47a] They are also used as an antimalarial drugs.[47b] The 2-(1*H*)-quinolone and 4-(1*H*)-quinolone nuclei, are found in many natural occurring alkaloids.[47a]



Nalidixic acid (NegGram®) 7-chloro-4-Quinolone Ciprofloxacin

**35** **36** **37**

**Figure 25.** Structures of important 4-quinolone derivatives *viz*., (nalidixic acid (NegGram® **35**)(1-ethyl-7-metyl-3-carboxy-4-quinolone), (7-chloro-4-quinolone, **36** (showing the system of numbering quinolone based compounds).and (Ciprofloxacin **37**).

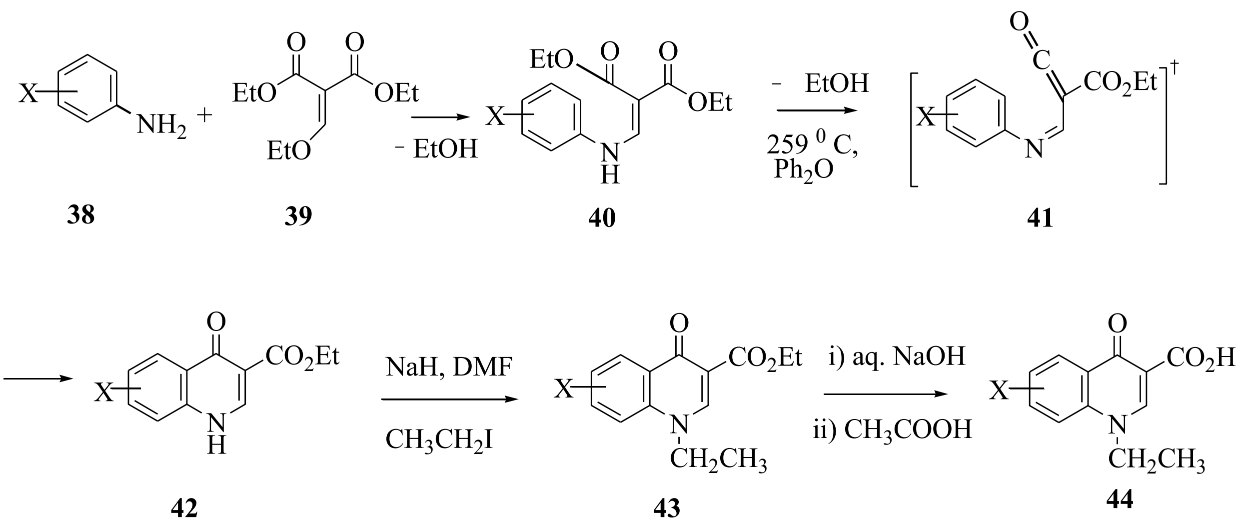
The first quinolone antibiotic to receive FDA approval, nalidixic acid (NegGram), was discovered through serendipity by Lesher and his colleagues in 1962, while conducting research to find a more efficient antimalarial drug than chloroquine **2**. The precursor of nalidixic acid, 7-chloro-4-quinolone **36**, was isolated as a by-product from a recrystalization step in the project, and on further investigation, nalidixic acid was discovered.[26, 46-47a,] Nalidixic acid, having been found to be excreted in urine was soon applied in the treatment of uncomplicated (UTIs), caused by gram-negative bacteria.[46]

Following this initial discovery, these classes of therapeutic agents have undergone various modifications from 2-alkyl-4(1*H*)-quinolones (AQs) to the modern fluoroquinolones with improved coverage of gram-positive bacteria.[26,46-48] Ciprofloxacin **37**, a member of the second generation of these drugs, is one of the most widely prescribed quinolone-based antibiotics. Since its approval by the FDA, in 1986, it has shown excellent antibiotic activity against resistant gram-negative pathogens.[26,46-47a,]

The general mode of antibiotic activity of the quinolones involves inhibiting two type IIA bacterial isomerases, namely DNA gyrase and topoisomerase IV, which result in the immediate death of bacteria.[46-48] This activity, which is accompanied by minimal side-effects, is due to the polar functional groups, present in the quinolone-based antibiotics, which form conjugates with serum proteins, thereby exhibiting the wide-volume distribution necessary for antibacterial activity.[26,46-47a,] It should, be noted however, that quinolones form complexes with cations, such as calcium, magnesium, iron, aluminum and zinc in physiological systems.[49]

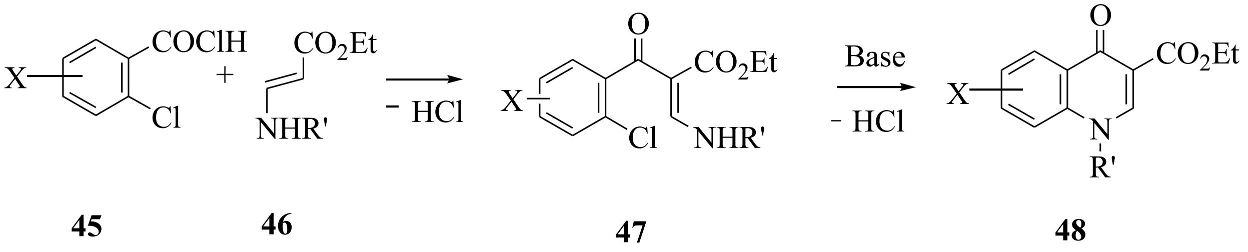
Of importance to the present investigation, Shinkai, and co-workers recently reported access to potential inhibitors of the strand transfer process of the HIV-1 integrase enzyme, by modifying the structure of quinolone antibiotics, suggesting that quinolones may in fact posses antiviral activity. [48]

Quinolone antibiotics, apart from being obtained from animal, plant and microorganism sources, have also been synthesized through the Gould–Jacob's method, shown in Scheme 1.3**.** This involves a reaction between an aniline and diethyl 2-(ethoxymethylene)malonate to yield an enamine, which forms an intermediate ketene when heated. The ketene rearranges to give 4-quinolone-3-carboxylate with different substituents on the aromatic ring.[46] *N*-alkylation then affords the 1-alkyl-4-quinolone derivatives, saponification of which leads to the 1-alkyl-3-carboxy-4-quinolones.[46] However, depending on the position of the ring substituents, regioisomers may be formed during cyclization. Nevertheless, the process affords high yields and can be applied in large-scale production.[46] The problem of regiospecificity has been addressed by adopting an alternative cyclo aracylation route, which involves reacting *ortho*-chlorobenzoic acid as shown in Scheme 1.4.[46]



**Scheme1.3.** Gould-Jacobs synthetic pathway to quinolones.[46]

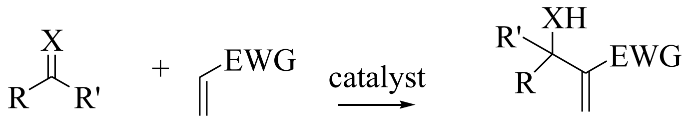
The regiospecificity of the target product is thus established by reacting the *ortho*-chloro and the carboxylic acid groups. Once formed, the aryl acid chloride precursor, is reacted with various enamines making it possible to introduce a variety of substituent other than hydrogen on the nitrogen, [47a] – a vital characteristic for antibacterial activity.[46-47a] This approach permits rapid synthesis of analogues, resulting in the production of libraries of quinolones.[46] From the experience with nalidixic acid, it was noted that an ethyl substituent on the nitrogen, gives optimal antibacterial activity.[46] However, results from four decades of research has indicated that cyclopropyl-substituted quinolones, like ciprofloxacin, exhibits improved antibacterial inhibition over ethyl-substituted quinolones. [26, 46-47a,] The Morita-Baylis-Hillman reaction has also provided access to both quinolone and quinoline derivatives.[53]



**Scheme 1.4.** Cycloaracylation route to quinolones.

## 1.4. THE BAYLIS-HILLMAN REACTION.

The Baylis-Hillman (BH) reaction, also known as the Morita-Baylis-Hillman (MBH) reaction, was first described by Morita *et al.* in 1968, as the reaction of different aldehydes with acrylic compounds, using tricyclohexyphosphine ($PCy_3$) catalysts, to afford vinyl monomers.[49] In 1972, Baylis and Hillman patented[50] the same kind of reaction but used tertiary amines, like 1,4-diazabicyclo[2.2.2]octane (DABCO), as catalysts. This extensively reviewed reaction,[52] is a catalyzed atom–economic carbon-carbon bond-forming process between activated alkenes and carbon electrophiles, affording a variety of highly functionalized addition products, commonly known as Baylis-Hillman adducts.[50] The Baylis-Hillman reaction is also known as an "organocatalyst reaction" because generally, organic compounds are used as catalysts.[50] The reaction typically involves coupling of an aldehyde **49** with an activated alkene **50**, using a tertiary amine as catalyst, to yield densely functionalized adducts **51** as shown in Scheme 1.5.[51] Basavaiah *et al.* have described the reaction as involving three steps, which include aza-Michael, aldol, and elimination processes in one reaction.[52]



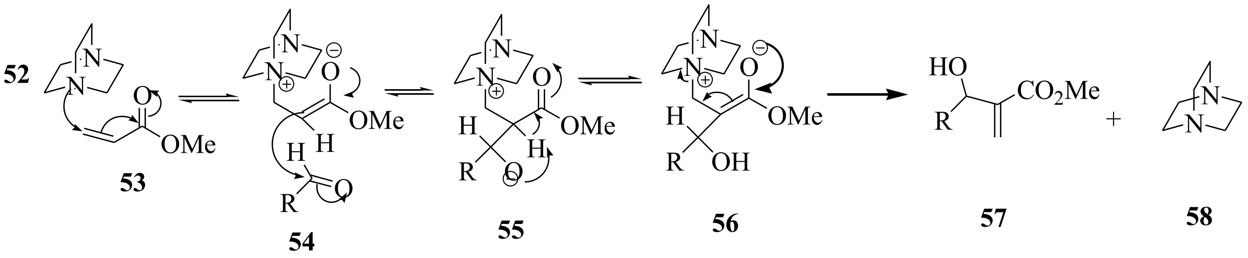
**49** **50** **51**

**R** = Aryl, alkyl, heteroaryl; R' = H, $CO_2R$, Alkyl;
**Catalyst** = tertiary amines, tertiary phosphines or Lewis acids.
X = O, NCOOR, NTs, $NSO_2Ph$, $NPPh_2$ *e.t.c.*,
**EWG** = electron-withdrawing group: COR, CHO, COOR, $PO(OEt)_2$, $SO_3Ph$, $SO_2Ph$, SOPh, $CONR_2$, COSR *e.t.c.*

**Scheme 1.5**. General representation of the Baylis-Hillman reaction.[50,51]

The reaction may thus be viewed as a multiple-component carbon-carbon bond forming reaction with room for a wide range of possible substrates.[50,51] The stereogenic centre created in the adducts, from prochiral electrophiles, provides opportunities as well as challenges for developing asymmetric version of the reaction.[50,51] The highly functionalized adducts coupled with the their proximity, offers the opportunity for variuos synthetic manipulations.[50,51.] There is even the possibility of developing an intramolecular Baylis-Hillman reaction, provided that the functional groups are in suitable positions in the substrate.[50,51]

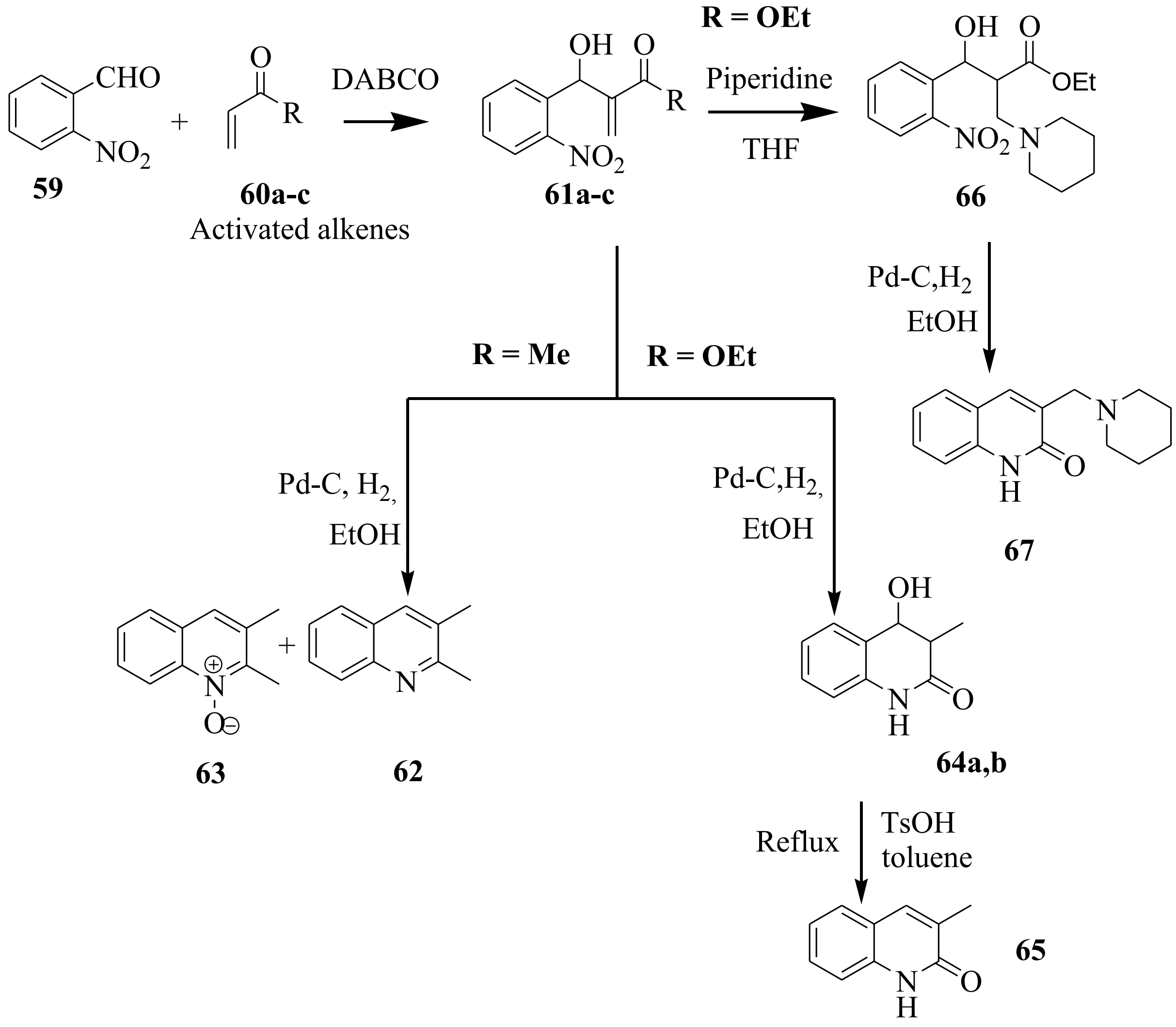
The starting materials are often readily available and releatively cheap and the ease of performance and environmental friendliness (it can be conducted in aqueous mediumor even neat), atom economy, potential chemoselectivity and the opportunity to introduce asymmetry make the Baylis-Hillman reaction a versatile tool for the Synthetic Organic Chemist.[51] The mechanism of the Baylis-Hillman reaction has been the subject of a number of studies and an early kinetic-mechanistic study was reported by our research group, (Scheme 1.6). [52] There is a general agreement about the early phases of the reaction but different explanations have been offered for the final elimination phase.



**Scheme 1.6.** Steps in the Baylis-Hillman reaction mechanism.[50-53]

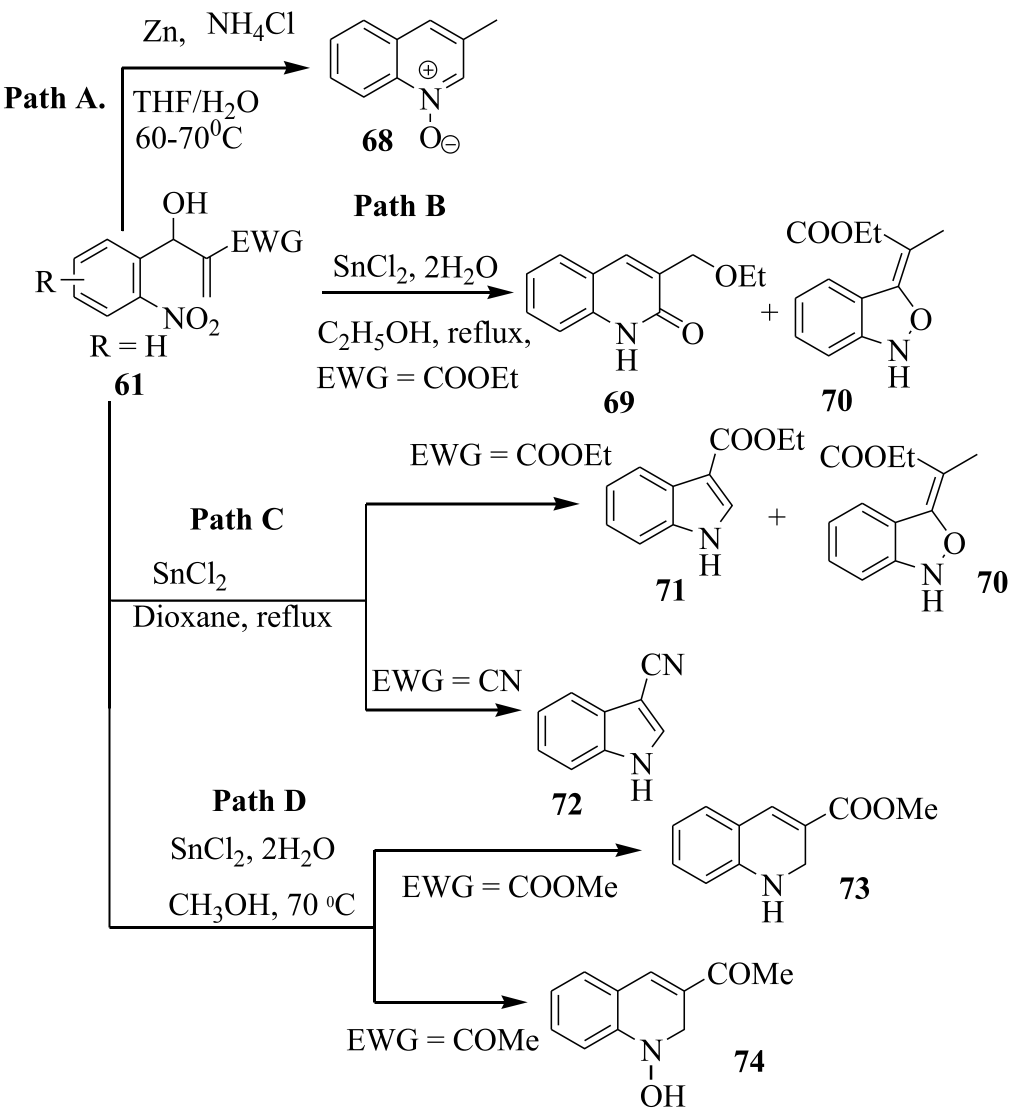
## 1.5. NITROGEN-HETEROCYCLICS *via*. BH METHODOLOGY.

Our research group was the first to report the Morita-Baylis-Hillman route to quinoline and quinolone derivatives from 2-nitrobenzaldehyde precursors in 1998, as illustrated in Scheme 1.7.[53] A later paper, in 2004, provided a fuller account of the application of B-H methodology in the preparation of quinoline and quinolone derivatives.[54] Attention has been given to the use of Baylis-Hillman methodology in the synthesis of indolinzines, chromenes, thiochromenes and coumarins.[54]



**Scheme 1.7.** Baylis-Hillman routes to nitrogen heterocyclics.[55,56]

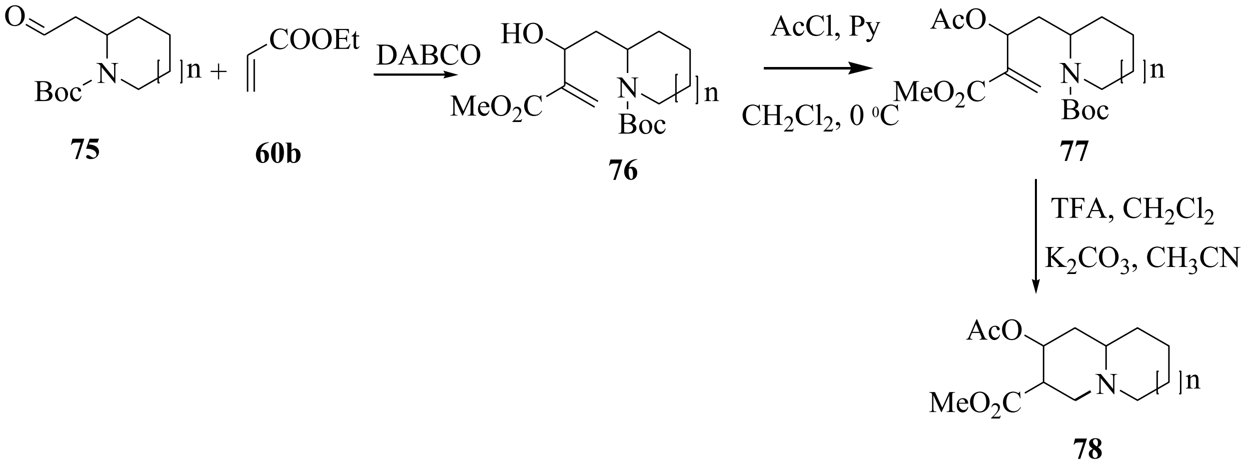
Subsequently, Kim *et al*. reported the use of Morita-Baylis-Hillman adducts **61**, derived from 2-nitrobenzaldehyde and activated alkenes, to access the quinoline-*N*-oxide **68**, and other derivatives.[55,56] using aqueous zinc chloride and ammonium chloride to effectively reduced the nitro group (Path A, Scheme 1.8).[51,55]



**Scheme 1.8.** Various synthetic routes to Nitrogen heterocyclics using Baylis-Hillman adducts.[51,54,69]

Kim *et al*. then found that stannous chloride could be used as an efficient reducing agent permitting the formation of the expected quinolone derivatives **69**, along with benzisoxazoline derivatives **70** (Path B, Scheme 1.8).[51,56] When the reaction was carried out in dioxane, the indole **71** and benzisoxazolines **70** derivatives were isolated ( EWG = COOEt), but for EWG = CN only the indole derivative **72** was isolated (Path C, Scheme 1.8).[51,57] Our group reported the synthesis of the dihydroquinoline, **73** and the *N*-hydroxydihydroquinoline **74** *via* the reductive cyclization of a Baylis-Hillman adduct using $SnCl_2/H_2O$ (Path D, Scheme 6). [51,54,69]

Furthermore, Clive *et al.* have demonstrated the first Morita-Baylis-Hillman reaction between *N*-protected β-amino aldehydes **75** and an acrylate ester **60b**, followed by *O*-acetylation and *N*-deprotection, to afford bicyclic amines **76** with nitrogen at the ring-fusion position (Scheme 7).[51, 58]



**Scheme 1.9.** Baylis-Hillman route to bicyclic amines.[51,58]

## 1.6 PREVIOUS WORK DONE IN THE GROUP AND AIMS OF THE PRESENT STUDY.

In addition to contribution to the system of quinoline derivatives, attention has also been given in our group to the preparation of:– potential antimalarials, such as phosphonated *N*-aryl- and heteroarylcarboxamides as DOXP-reductoisomerase (DXR) inhibitors;[108] novel heterocyclic analogues of the HIV-1 protease inhibitor (PI), ritonavir; [109] and coumarin derivatives, with potential as dual-action HIV-1 protease and reverse transcriptase inhibitors.[78] The constant mutation of disease-causing organisms, results in the development of resistance against current or existing drugs and there is an ever increasing need for the development of new chemotherapeutic agents. The present study involved investigation into the multi-functional and versatile Morita-Baylis-Hillman (MBH) adducts as scaffolds for the preparation of novel lead compounds of medicinal interest; these have included 3-aminomethylquinolines and 2-quinolones as potential antimalarials and cinnamate esters as potential HIV-1 integrase inhibitors and their AZT conjugates as potential HIV-1 integrase-reverse transcriptase inhibitors.

Specific objectives have therefore included the following.

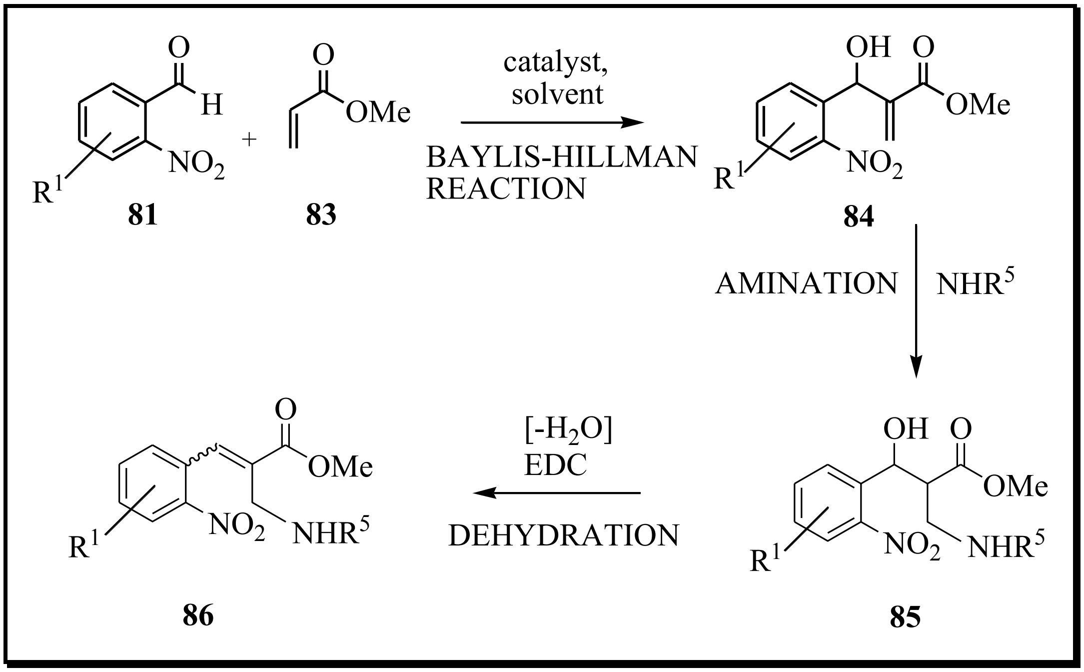
1. The synthesis of 4-chloro- and 5-methoxy-2-nitrobenzaldehydes and their use, in conjunction with other substituted 2-nitrobenzaldehydes as substrates, in the Baylis-Hillman reactions, using MVK, methyl acrylate and ethyl acrylate.
2. The dehydration of the β-hydroxy BH adducts to afford unsaturated BH-derived allyl halides, for use as intermediates for subsequent reaction steps.
3. Conjugate addition of various amines to the BH-derived allyl halides and subsequent reductive cyclization of the conjugate addition products to afford quinolines or quinolones.
4. Application of 'Click Chemistry' in the construction of potential dual-action HIV-1 integrase-reverse transcriptase (IN-RT) inhibitors.
5. Preliminary evaluation of selected products using bioassay and *in silico* enzyme active-site docking data.

# 2. RESULTS AND DISCUSSION.

The discussion follows a number of themes: (i) synthesis of cinnamate esters as potential HIV-1 integrase inhibitors [Section 2.1]; (ii) synthesis of cinnamate-AZT conjugates as potential HIV-1 IN-RT dual-action compounds [Section 2.2); (iii) synthesis, characterization and antiplasmodial activity of 3-(aminomethyl)quinolines and 2-quinolones as potential antimalarials [Section 2.3]; and (iv) molecular modelling and docking studies of selected compounds (Section 2.4].

## 2.1. SYNTHESIS OF CINNAMATE ESTERS.

The synthetic strategy adopted to access the cinnamate esters is illustrated in Scheme 2.1 and involved a number of steps: (i) synthesis of Baylis-Hillman adducts **84**, using methyl acrylate **83** and 2-nitrobenzaldehydes **81**, (ii) conjugate addition of amine nucleophiles to the Baylis-Hillman adducts **84**, and (iii) dehydration of the β-hydroxy aminomethyl derivatives **85** to afford cinnamate esters **86**.

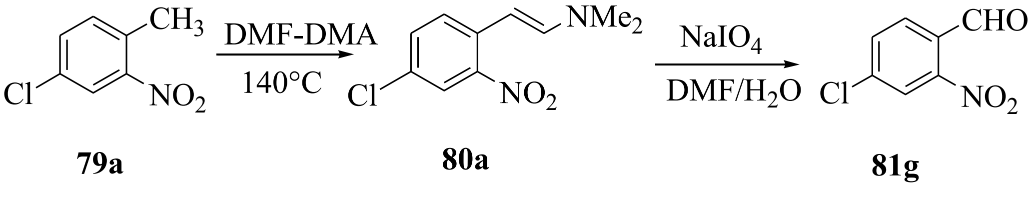


**Scheme 2.1**. *STRATEGIC PLAN I*: Synthesis of cinnamate esters as potential HIV-1 integrase inhibitors.

### 2.1.1. Preparation of 2-nitrobenzaldehyde precursors.

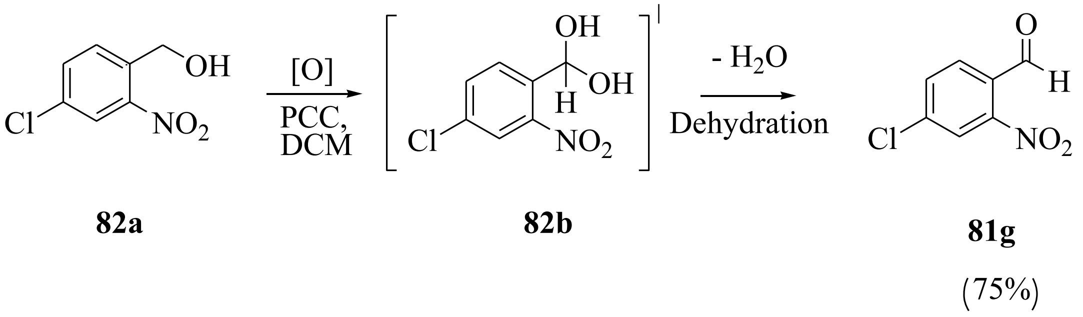
In addition to the objective of synthesizing potential anti-HIV-1 lead compounds, the preparation of novel 3-aminomethylquinolines as potential antimalarials forms part of the present study. Thus, the nitrobenzaldehyde Baylis-Hillman substrates were required as critical intermediates for the construction of *both* cinnamate esters as potential HIV-1 integrase inhibitors *and* novel quinoline derivatives. The targeting of chloroquine and quinine analogues required that 4-chloro-2-nitrobenzaldehyde **81g** and 5-methoxy-2-nitrobenzaldehyde **81h** be used as the respective precursors for the targeted analogues. Due to their cost, these 2-nitrobenzaldehydes were prepared as part of the project. The remaining six 2-nitrobenzaldehydes were purchased from Sigma-Aldrich and used without further purification.

Two different methods were used to access the 4-chloro-2-nitrobenzaldehyde **81g** required for the preparation of the chloroquine analogue. The method reported by Caron *et al.* involves the oxidation of the methyl side chain in 4-chloro-2-nitrotoluene **79a** to afford the aldehyde **81g**, using a mild oxidizing agent, such as the *N*,*N*-dimethylformamide-dimethyl acetal complex (DMF-DMA) at high temperature (Scheme 2.2).[59,60,65]



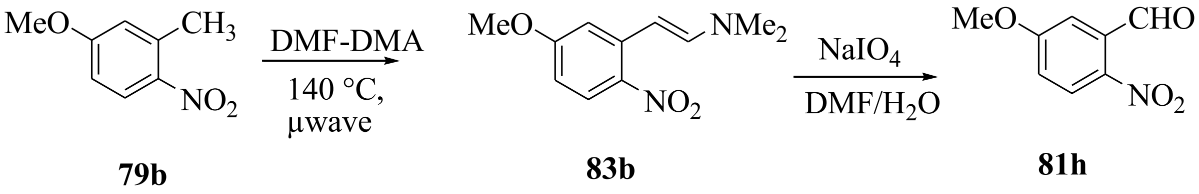
**Scheme 2.2.** Oxidation of 4-chloro-2-nitrotoluene **79a** to 4-chloro-2-nitrobenzaldehyde **81g.**

Due to the poor yield obtained for the desired product **81g** (*ca.* 20%) using this method, a second approach, the method reported by Ahmad *et al.*, [61,62] was adopted to improve the yield. This approach involved oxidation of the hydroxyl group in 4-chloro-2-nitrobenzyl alcohol **82** to the aldehyde **81g**, using pyridinium chlorochromate (PCC) in dichloromethane at room temperature, as outlined in Scheme 2.3.



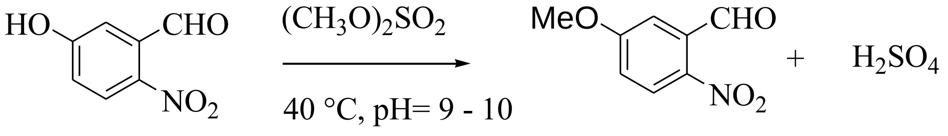
**Scheme 2.3.** Oxidation of 4-chloro-2-nitrobenzyl alcohol **82** to 4-chloro-2-nitrobenzaldehyde **81g**.

Thus, a solution of 4-chloro-2-nitrobenzyl alcohol **82** in dichloromethane was added rapidly to a well-stirred suspension of PCC in dichloromethane in an open glass beaker (Scheme 2.3). Work-up and chromatography of the crude product afforded 4-chloro-2-nitrobenzaldehyde **81g** in much better yield (75%); the aldehyde **81g** gave a satisfactory melting point and was characterized by NMR and IR spectroscopy. [61,62] The $^1$H NMR spectrum of the product **81g** showed the aldehydic proton resonating as a singlet at 10.37 ppm and, as expected, three aromatic proton signals, one as a singlet at 8.09 ppm and two as doublets at 7.94 and 7.76 ppm. The expected carbon signals for the product were observed in the $^{13}$C NMR spectrum (Figure. 2.1) with the carbonyl carbon signal at 186.7 ppm and all six aromatic carbon signals in the region 125 – 160 ppm, three of which were shown by the DEPT 135 NMR spectrum to correspond, as expected; to the three methine carbons.



**Scheme 2.4.** Oxidation route to 5-methoxy-2-nitrobenzaldehyde **81h**.

Access to 5-methoxy-2-nitrobenzaldehyde **81h** was also achieved using two different routes. In the first of these, oxidation of the methyl group in 3-methyl-4-nitroanisole **79b** to the aldehyde was effected, following the method reported by Caron *et al.* (Scheme 2.4).[59,60] In a variation of this approach, microwave irradiation was also employed. The second route involved methylation of the phenolic group in 5-hydroxyl-2-nitrobenzaldehyde **81b** using dimethyl sulphate ($Me_2SO_4$) (Scheme 2.5) - a method reported by Fen-Er Chen *et al.* [63] This involved adding 30% aq. NaOH dropwise to a well stirred aqueous solution of 5-hydroxy-2-nitrobenzaldehyde and $Me_2SO_4$ at 40°C.



**81b** **81h**

**Scheme 2.5.** Methylation route to 5-methoxy-2-nitrobenzaldehyde **81h**.

Work-up and flash chromatography of the crude product afforded in each case, 5-methoxy-2-nitrobenzaldehyde **81h,** which was characterized by NMR and IR analysis, and which gave a satisfactory melting point.[63] Both methods gave yields of 60 – 68%. The $^1$H NMR spectrum of the product **81h** showed the methoxy protons resonating as a singlet at 3.93 ppm the three aromatic proton signals, one as a triplet at 7.14 ppm and the other two as doublets at 7.28 ppm and 8.13 ppm, and the aldehydic proton as a singlet at 10.43 ppm. The expected eight carbon signals for the product **81h** were observed in the $^{13}$C NMR spectrum (Figure.2.2) with the carbonyl carbon resonating at 188.4 ppm, and the methoxy carbon at 56.3 ppm.

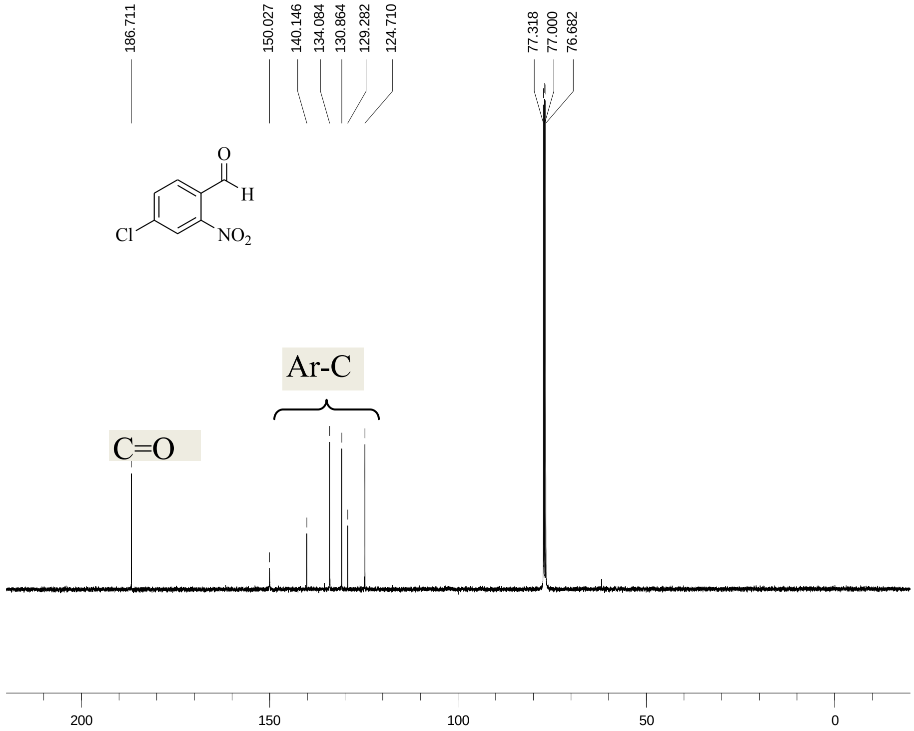


**Figure 2.1.** 100MHz $^{13}$C NMR spectrum of 4-chloro-2-nitrobenzaldehyde **81g** in $CDCl_3$

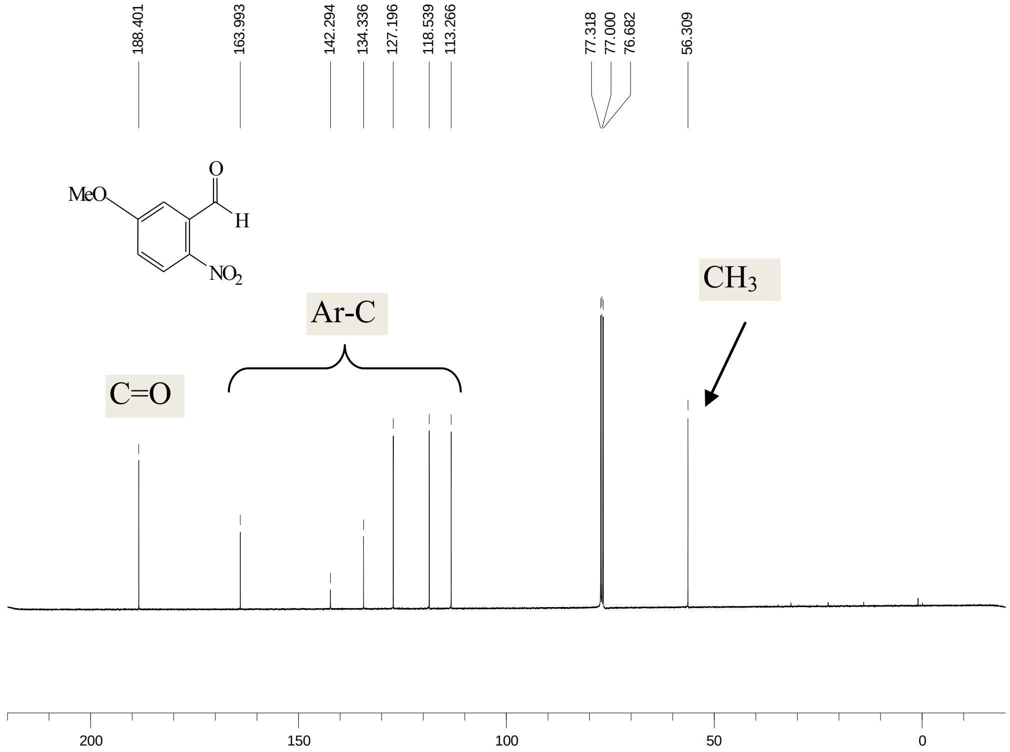
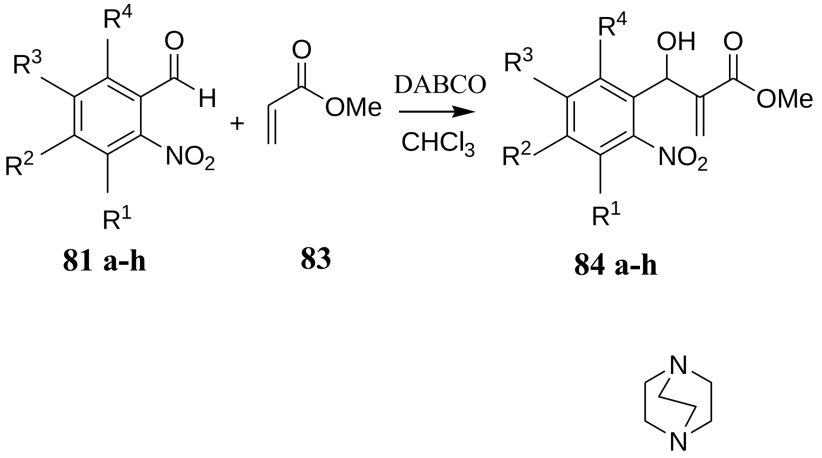


**Figure 2.2.** 100MHz $^{13}$C NMR spectrum of 5-methoxy-2-nitrobenzaldehyde **81h** in $CDCl_3$.

### 2.1.2. Reactions of 2-nitrobenzaldehydes under BH conditions.

Following the strategic plan outlined in Scheme 2.1, the series of 2-nitrobenzaldehydes **81a-h** were reacted with methyl acrylate **83**, using DABCO as catalyst and chloroform as solvent. The reactions were allowed to run at room temperature for periods ranging from two days to six weeks. The progress of the reaction was monitored using TLC analysis and, in a few instances in which there were indications that the reaction was no longer progressing and a significant amount of the starting material remained, additional methyl acrylate and DABCO were added to promote further reaction. The yields correspond those of the isolated products after flash column chromatography.

At the end of each reaction, excess solvent was removed under reduced pressure and the residue purified by flash chromatography, to afford the Baylis-Hillman adducts **84a-h** in up to 91% yield, as shown in Table 2.1.



**81 a-h** **83** **84 a-h**

| | R$^1$ | R$^2$ | R$^3$ | R$^4$ |
|---|---|---|---|---|
| a | H | H | H | H |
| b | H | H | OH | H |
| c | H | O-CH$_2$-O | | H |
| d | H | OMe | OMe | H |
| e | OMe | H | H | H |
| f | H | H | Cl | H |
| g | H | Cl | H | H |
| h | H | H | OMe | H |

1,4-diazabicyclo[2,2,2]octane
**(DABCO)**

**Scheme 2.6.** Synthesis of Baylis-Hillman adducts with methyl acrylate.

The products **84a-h** were all fully characterized by NMR, IR and new compounds, *viz*., **84e,g,h**, by high-resolution mass spectrosmetry (HRMS). The characteristic features in the $^1$H NMR spectra of the BH adducts, which indicate their formation of products, are the three protons signals (typically singlets) between 5.5 and 6.5 ppm, the area generally referred to as the 'BH region'. These signals correspond to the 3-methine proton and the two vinylic protons. In the $^1$H NMR spectrum of the BH adduct **84a** (Figure. 2.3), the characteristic "BH signal" are evident, as a singlet at 5.63 ppm (3-methine proton) and the second as another singlet at 6.12 ppm and the third singlet at 6.23 ppm (vinyl protons) respectively. The signals corresponding to the four aromatic protons, the methoxy protons and the hydroxy proton are also evident. The $^{13}$C NMR spectrum (Figure.2.4) shows the 11 carbon signals expected, with the ester carbonyl carbon signal at 166.8 ppm, while the $^{13}$C signal assignments are supplemented by the DEPT135 data (Figure 2.5).

**Table 2.1.** Isolated yields of methyl acrylate-based Baylis-Hillman products **84a-h.**

| BAYLIS-HILLMAN ADDUCT | $R^1$ | $R^2$ | $R^3$ | $R^4$ | Yield |
|---|---|---|---|---|---|
| **84a** | H | H | H | H | 84% |
| **84b** | H | H | OH | H | 37% |
| **84c** | H | O-CH$_2$-O | | H | 63% |
| **84d** | H | OMe | OMe | H | 66% |
| **84e** | OMe | H | H | H | 91% |
| **84f** | H | H | Cl | H | 55% |
| **84g** | H | Cl | H | H | 78% |
| **84h** | H | H | OMe | H | 80% |

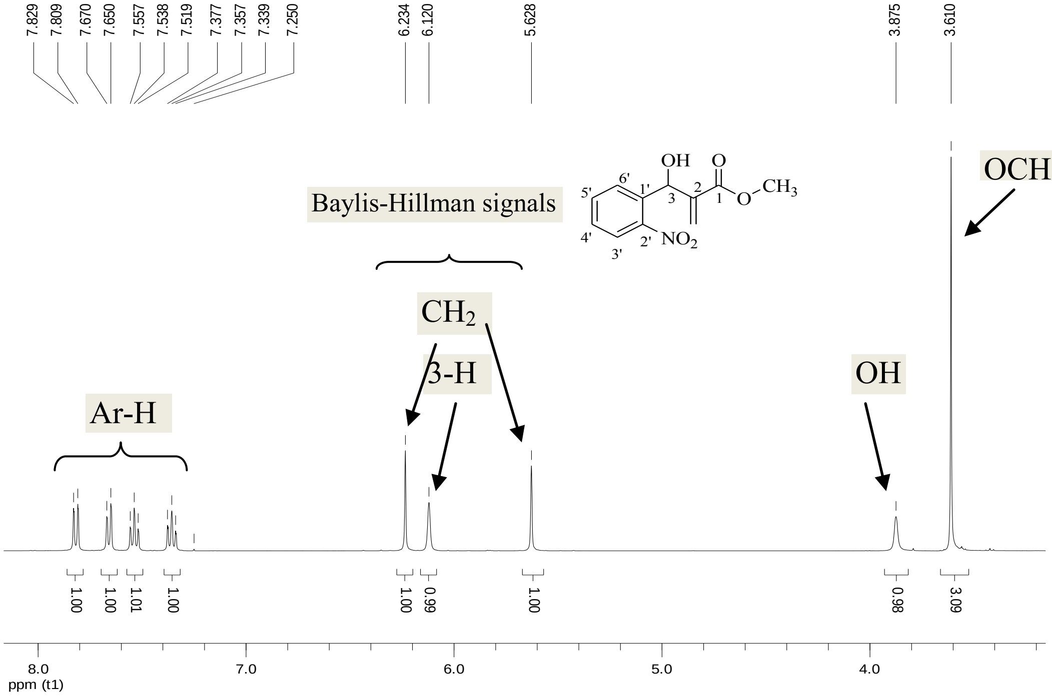


**Figure 2.3.** 400MHz $^1$H NMR spectrum of methyl 3-hydroxy-2-methylene-3-(2-nitrophenyl)propanoate **84a** in CDCl$_3$.

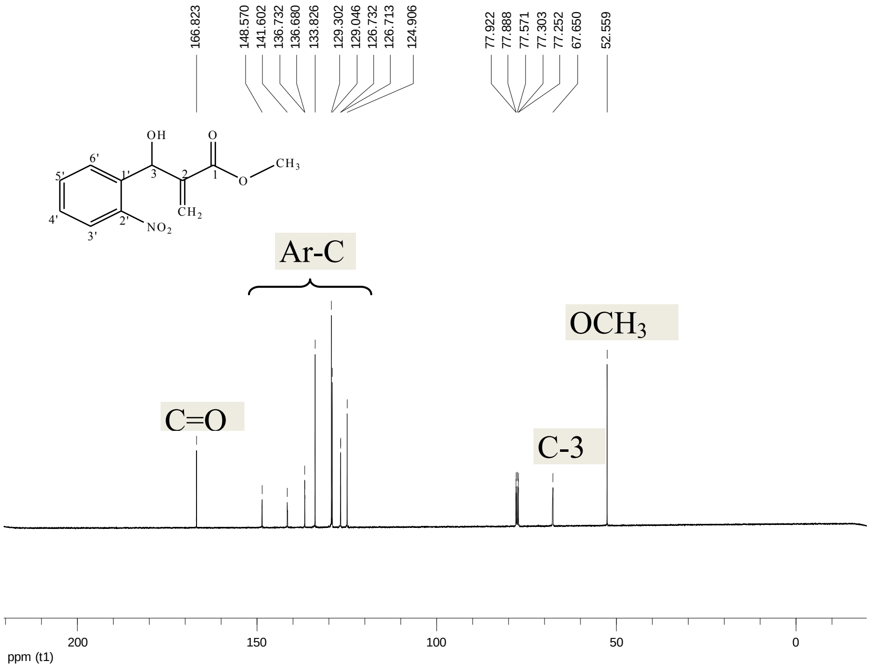


**Figure-2.4.**100 MHz $^{13}$C NMR spectrum of methyl 3-hydroxy-2-methylene-3-(2-nitrophenyl)-propanoate **84a** in CDCl$_3$.

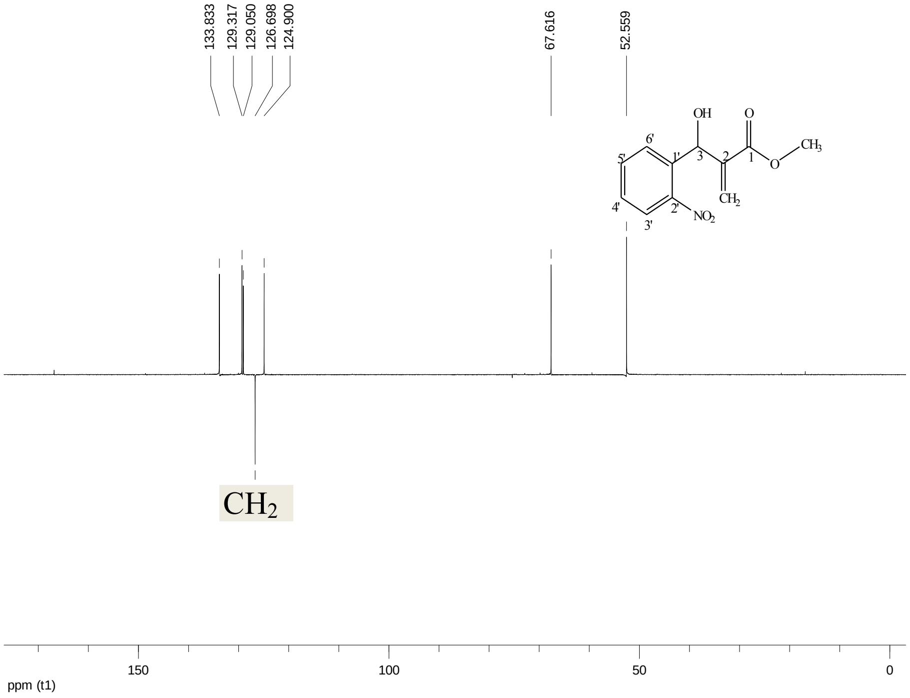
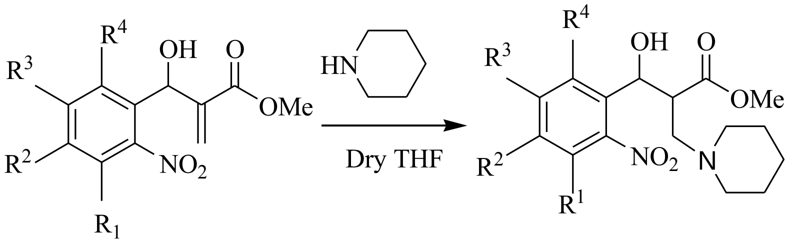


**Figure 2.5.** DEPT135 NMR spectrum of methyl 3-hydroxy-2-methylene-3-(2-nitrophenyl)propanoate **59a** in $CDCl_3$.

### 2.1.3. Conjugate addition reactions of Baylis-Hillman ester adducts.



**84a,e** **85a,e**

| | $R^1$ | $R^2$ | $R^3$ | $R^4$ |
|---|---|---|---|---|
| a | H | H | H | H |
| e | OMe | H | H | H |

**Scheme 2.7.** Conjugate addition reactions of Baylis-Hillman products.

The next step in the reaction sequence involved conjugate addition of secondary amines to the vinylic α,β-unsaturated methyl esters **84.** Piperidine, a secondary amine was the first amine nucleophile initially used for the conjugate addition reaction with the methyl esters **84a,e**, and the reaction was carried out in anhydrous THF (Scheme 2.7). A typical reaction involved treating 1 eq. of the methyl ester with 1 eq. of piperidine. The reaction mixtures were stirred at room temperature, for 2 to 5 days to afford the diastereomeric products, **85a,e** in quantitative yields. The formation of the nucleophilic conjugate addition products as pairs of diastereomers is attributable to the presence of two near stereogenic centres (C2 and C3) in the products **85a,e** (of course, no enantioselectivity is expected and the products are diastereomeric pairs of enantiomers). The $^{1}$H NMR spectrum of the mixture of diastereomers **85a** (Figure. 2.6) shows the 3-methine proton signals between 5.5 and 6.0 ppm. Integration of these signals permitted determination of the diastereomeric ratio in each case. The doublets at 5.53 ppm (*J* = 8.8 Hz) and 5.72 ppm (*J* = 4.0 Hz) correspond to the major and minor diastereomers, respectively, and, from the $^{1}$H NMR integral data in Figure 2.6, the diastereomeric ratio is 1.6:1. The $^{13}$C NMR spectrum (Figure 2.7) also exhibits the doubling of signals. In fact, twice the total number of expected carbon signals is observed, reflecting the presence of two diastereomeric systems. Six pairs of methine carbon signals are also seen in the DEPT 135 NMR spectrum (Figure 2.8), four of which correspond to the four aromatic methine carbons and the remaining pairs to carbons 2 and 3. All efforts at separating the diastereomers proved unsuccessful; hence, the mixture was taken to the next step of the reaction sequence.

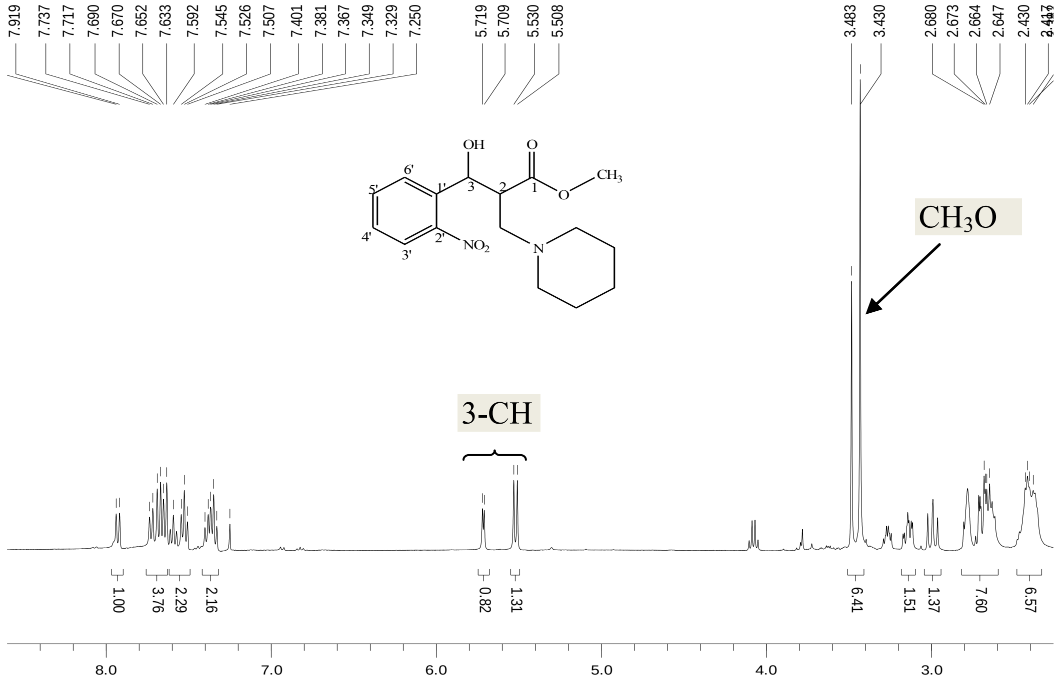


**Figure 2.6.** 400 MHz $^{1}$H NMR spectrum of methyl 3-hydroxy-2-methylene-3-(2-nitrophenyl)-2-[(1-piperidinyl)methyl]propanoate **85a,** as a diastereomeric mixture, in $CDCl_3$.

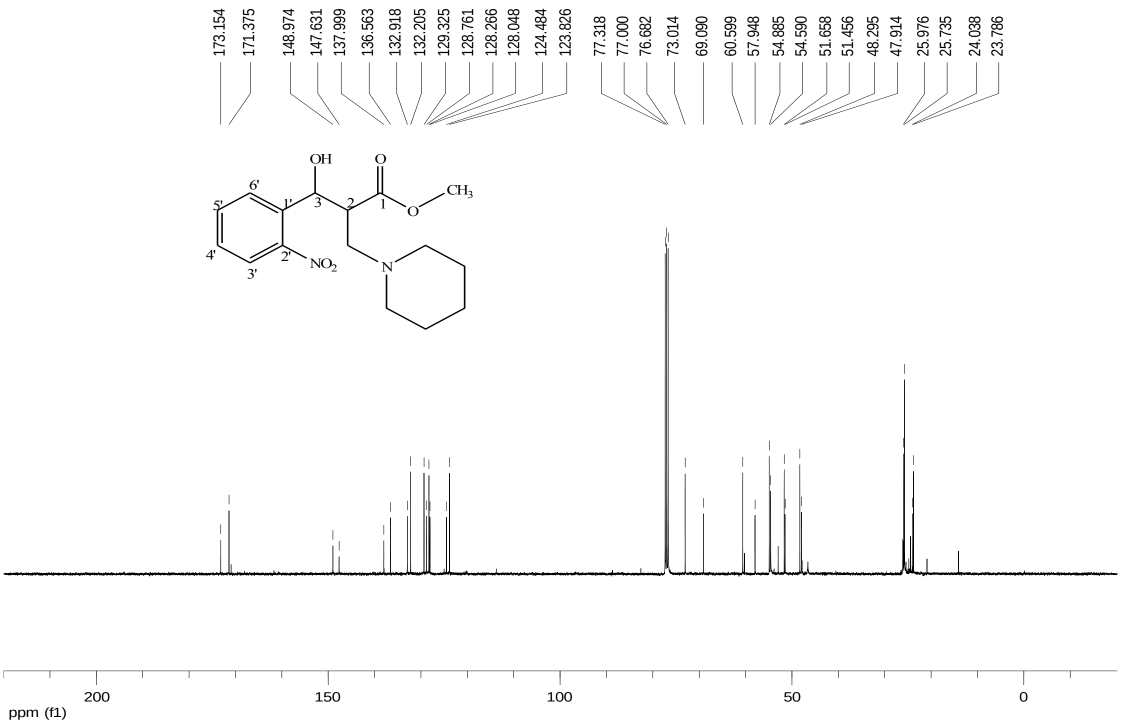


**Figure 2.7.** 100 MHz $^{13}$C NMR spectrum of methyl 3-hydroxy-2-methylene-3-(2-nitrophenyl)-2-[(1-piperidinyl)methyl]propanoate **85a,** as a of diastereomeric mixture, in $CDCl_3$.

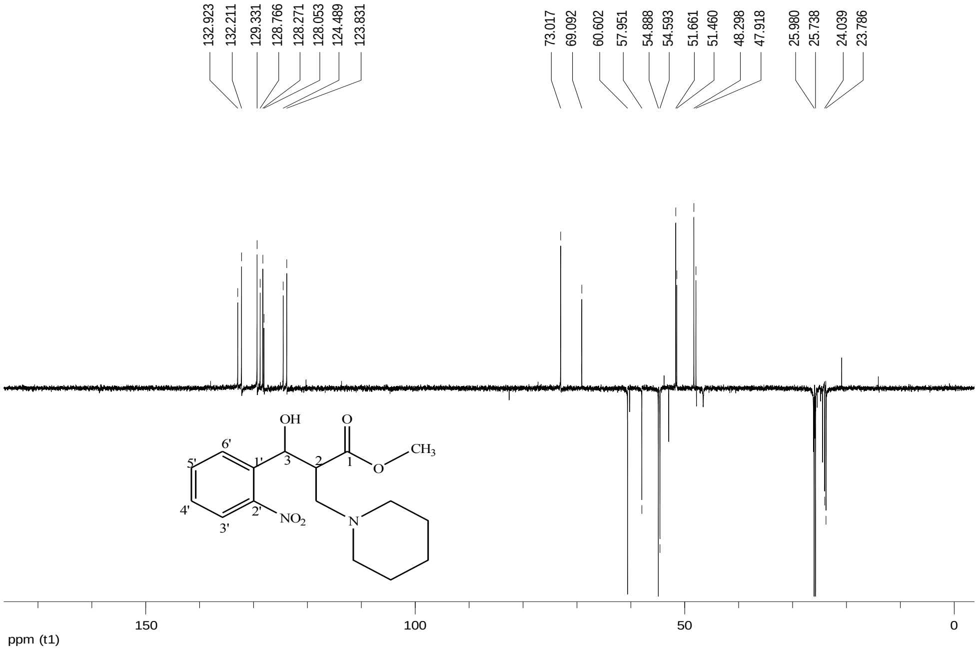
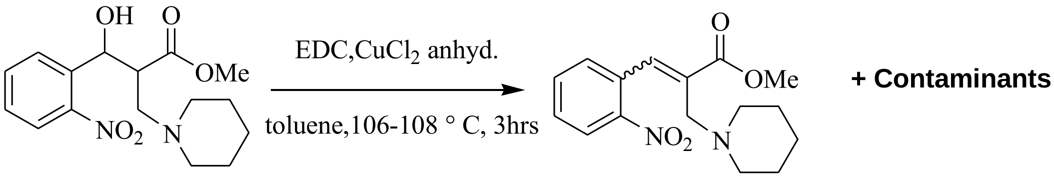


**Figure 2.8.** DEPT135 NMR spectrum of methyl 3-hydroxy-2-methylene-3-(2-nitrophenyl)-2-[(1-piperidinyl)methyl]propanoate **85a**, as a pair of diastereomers, in $CDCl_3$.

### 2.1.4. Dehydration of the aminomethyl β-hydroxy esters.

The final next step in the synthesis of the cinnamate esters was the dehydration of the diastereomeric mixture of the aminomethyl β-hydroxy esters, **85a,e**. The approach initially adopted was that reported by *Sai et al.*[70] involving a stereoselective synthetic route to (*E*)- or (*Z*)- α-substituted cinnamates in which 1-ethyl-3-(3-diethylaminopropyl)carbodiimide (EDC) was used as a dehydrating agent in an $E_2$ elimination reaction of *syn*- or *anti*-α-substituted-β-hydroxyesters.



**85a** **86a**

**Scheme 2.8.** Dehydration of the diastereomeric aminomethyl derivative **85a**, to afford cinnamate esters.

They reported that the s*yn*-β-hydroxyesters undergo stereoselective $E_2$ elimination to afford (*E*)-α-substituted cinnamates in quantitative yields, whereas the reaction with *anti*-β-hydroxyesters was not stereoselective, yielding a (32:68) mixture of (*Z*)- and (*E*)-α-substituted cinnamates. Sai *et al.*[70] observed that refluxing 1 eq. of the β-hydroxyesters with 2 eq. of EDC and 0.1 eq. of $CuCl_2$ in toluene for 2 hours afforded the best yields. Although the β-hydroxyesters **85** used in our study are significantly different from those used by Sai *et al.*, their synthetic protocol was explored for the dehydration of the β-hydroxy ester **85a (**Scheme 2.8**).** This involved refluxing the β-hydroxy ester with EDC in toluene for 2 hours. However, a complex mixture was obtained, which proved impossible to purify. As indicated in the $^1$H NMR spectrum of the dehydration product 8**6a** (Figure.2.9) after chromatography that dehydration of the β-hydroxyester **85a** was confirmed by the disappearance of the pair of 3-methine proton signals that resonate between 5.53 and 5.72 ppm in the $^1$H NMR spectrum of the substrate **85a** (Figure. 2.6). However; it was obvious that extraneous signals from unknown contaminants, made purification of the product(s) impossible.

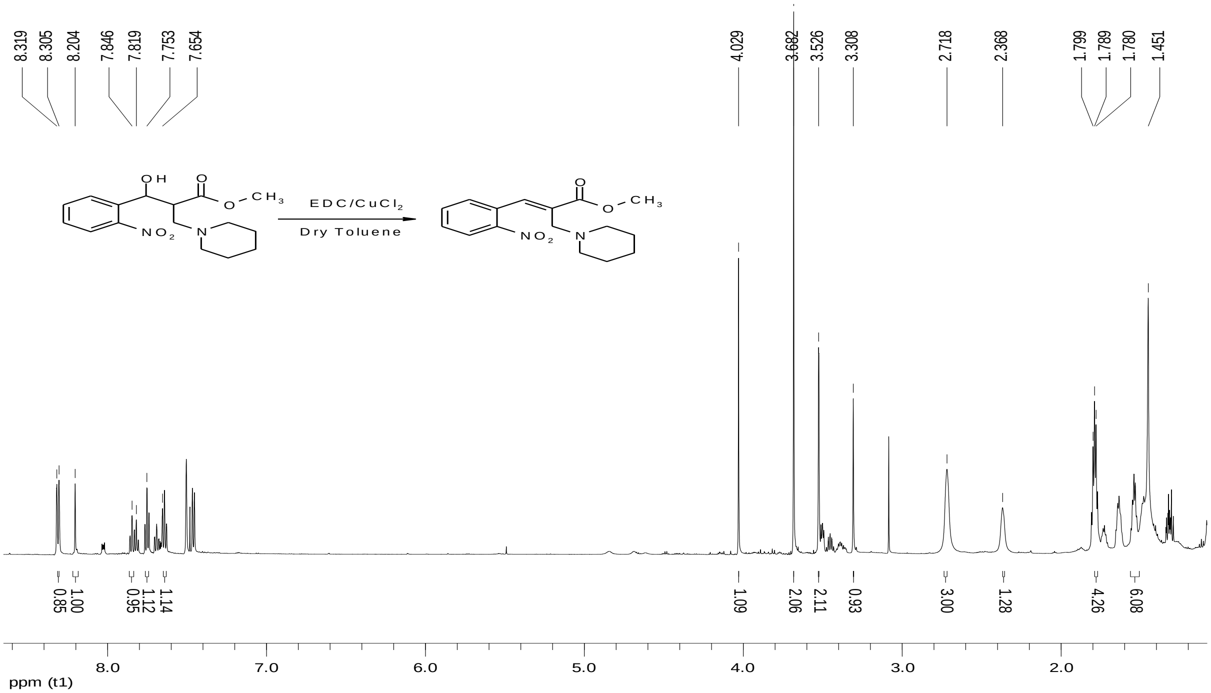
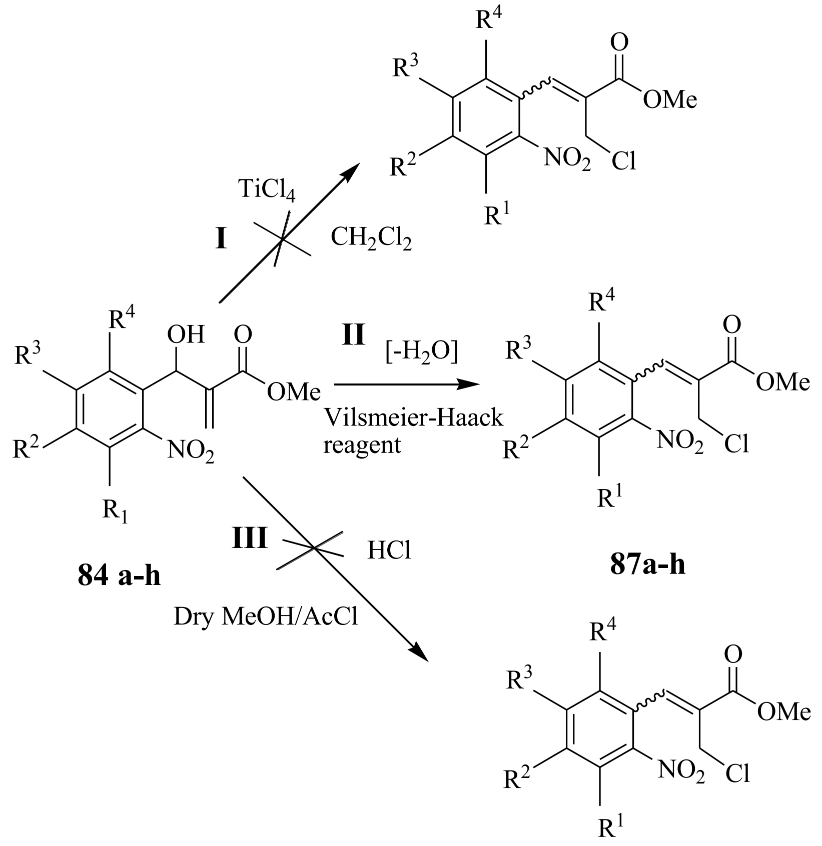


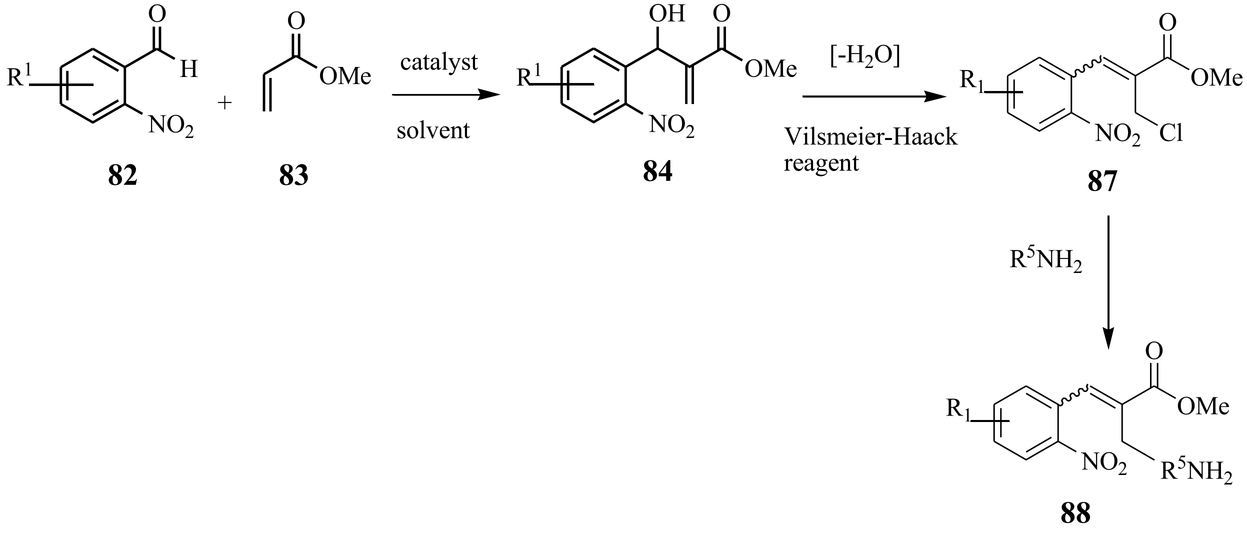
**Figure.2.9.** 400 MHz $^1$H NMR spectrum of the chromatographed material from the attempted dehydration of compound **85a** in $CDCl_3$.

From this result, it became obvious that this dehydration method would not be appropriate for successful access to the desired cinnamate esters and another approach was needed. An attempt was then made at adapting the method reported by Shi *et al*.,[71] in which a combination of Lewis base such as an amine, quaternary ammonium halides and Lewis acid, Titanium (iv) chloride ($TiCl_4$) significantly increased the rate of the Baylis-Hillman reaction of *p*-nitrobenzaldehyde with MVK, and afforded the chlorinated (*Z*)-alkene as the most stable product. It was expected that resulting allyl chlorides **87** (Scheme 2.9) could be reacted with amine nucleophiles to afford the desired α-aminomethylcinnamates **86**. In our case, the BH β-hydroxyester **84a** in dichloromethane (DCM), was stirred with $TiCl_4$ (1.0N in DCM) in ratio 1: 1.4 at room temperature (reaction **I**, Scheme 2.9). There was an immediate vigorous reaction when the $TiCl_4$ was introduced drop-wise, and the clear reaction mixture turned to a colloidal suspension. The $^1H$ NMR spectrum acquired of the crude product obtained after work-up, however, showed that the ester has not undergone any change, in spite of the vigorous reactivity observed.



**Scheme 2.9.** Attempts at modifying our approach to access the cinnamate esters.

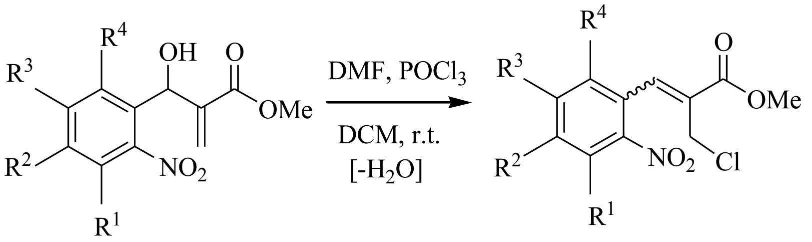
A further attempt to dehydrate the β-hydroxyester **84a** to afford the α-chloromethyl cinnamate ester **87a** was carried out by reacting the β-hydroxy ester with HCl; generated *in situ.* by reacting acetyl chloride (AcCl) cautiously with anhydrous methanol, following a method reported by Yadav *et al.*[72,74] This well-established method has been applied in the dehydration of β-hydroxy ketones in our group,[73,74] and involves adding the BH adduct (1 eq.) to the methanolic HCl solution, generated *in-sit,* by adding AcCl (8 eq.) drop-wise to anhydrous methanol; the reaction of AcCl with methanol is exothermic and cooling on an ice-bath is essential. The flask was fitted with reflux condenser, to avoid loss of material. The reaction mixture was then left to stir overnight at room temperature. Unfortunately, $^1$H NMR analysis of the crude material after work-up showed neither a change in the substrate signals nor the appearance of new signals. The AcCl reaction was then repeated but in this case, refluxing the methanolic solution; however; there was still no change in the $^1$H NMR spectrum of the crude material after work-up. A further search of the literature for a suitable method brought relief, as we found an article by Liu *et al.*,[75] who reported the use of the *in situ.* generated Vilsmeier-Haack reagent (halomethyleneiminium salt) in the stereoselective synthesis of (*Z*)-allyl chlorides from BH adducts in quantitative yields. The reaction between phosphorus oxychloride ($POCl_3$) and DMF at room temperature generated the chloromethyleneiminium salt *in situ,* and a solution of the β-hydroxyester **84a** in DCM was added to the mixture (reaction **II**, Scheme 2.9). Work-up afforded the chloromethyl cinnamate ester **87a** in 61% yield.The success of this dehydration method prompted a change in the approach to the desired cinnamate esters as detailed in Scheme 2.10.



**Scheme 2.10.** MODIFIED APPROACH to the synthesis of cinnamate esters as potential HIV-1 integrase inhibitors.

## 2.1.5. Dehydration of Baylis-Hillman adducts.

Dehydration of the series of β-hydroxy esters **84a-h** was successfully achieved using the Vilsmeier-Haack approach (Scheme 2.11), with the progress of the reactions being monitored to completion by TLC. Work-up and chromatography afforded the allyl chlorides **87a-h** in moderate to excellent yields (Table 2.2).



**82 a-h**

**87a-h**

| | R$^1$ | R$^2$ | R$^3$ | R$^4$ |
|---|---|---|---|---|
| a | H | H | H | H |
| b | H | H | OH | H |
| c | H | O-CH$_2$-O | | H |
| d | H | OMe | OMe | H |
| e | OMe | H | H | H |
| f | H | H | Cl | H |
| g | H | Cl | H | H |
| h | H | H | OMe | H |

**Scheme 2.11.** Dehydration of Baylis-Hillman products to afford the allyl chloromethyl cinnamate esters **87a-h**.

**Table 2.2.** Yields of isolated α-chloromethyl cinnamate esters **87a-h** from the BH adducts **84a-h**.

| Chloromethyl cinnamate esters | R$^1$ | R$^2$ | R$^3$ | R$^4$ | Yield |
|---|---|---|---|---|---|
| **87a** | H | H | H | H | 61% |
| **87b** | H | H | OH | H | 72% |
| **87c** | H | -OCH$_2$O- | | H | 53% |
| **87d** | H | OMe | OMe | H | 96% |
| **87e** | OMe | H | H | H | 61% |
| **87f** | H | H | Cl | H | 82% |
| **87g** | H | Cl | H | H | 84% |
| **87h** | H | H | OMe | H | 81% |

The $^1$H NMR spectrum of the chloromethyl ester **87a** (Figure. 2.10) clearly shows the methyl ester singlet at 3.87 ppm, closely followed by the chloromethylene signal at 4.21 ppm. There are five proton signals in the aromatic region instead of the four in the substrate confirming formation of the cinnamate ester. The $^{13}$C NMR spectrum of compound **87a** (Figure 2.11) reveals the methylene carbon signal at 38.5 ppm, the methyl carbon signal at 52.6 ppm, and the carbonyl carbon signal at 165.6 ppm. These assignments are supported by the DEPT 135 data. The high stereoselectivity of the transformation is reflected in the absence of any additional signals, particularly in the $^{13}$C NMR spectrum (Figure 2.11), attributable to diastereomers. Assignment of *Z*-stereochemistry to the isolated products is based on the absence of any NOE interaction between the vinylic and chloromethylene protons in the NOESY NMR experiment (Figure. 2.12) conducted on the product. The configurational assignment is also consistent with data obtained for the MVK-derived analogues **93a-h** (see Section 2.2).

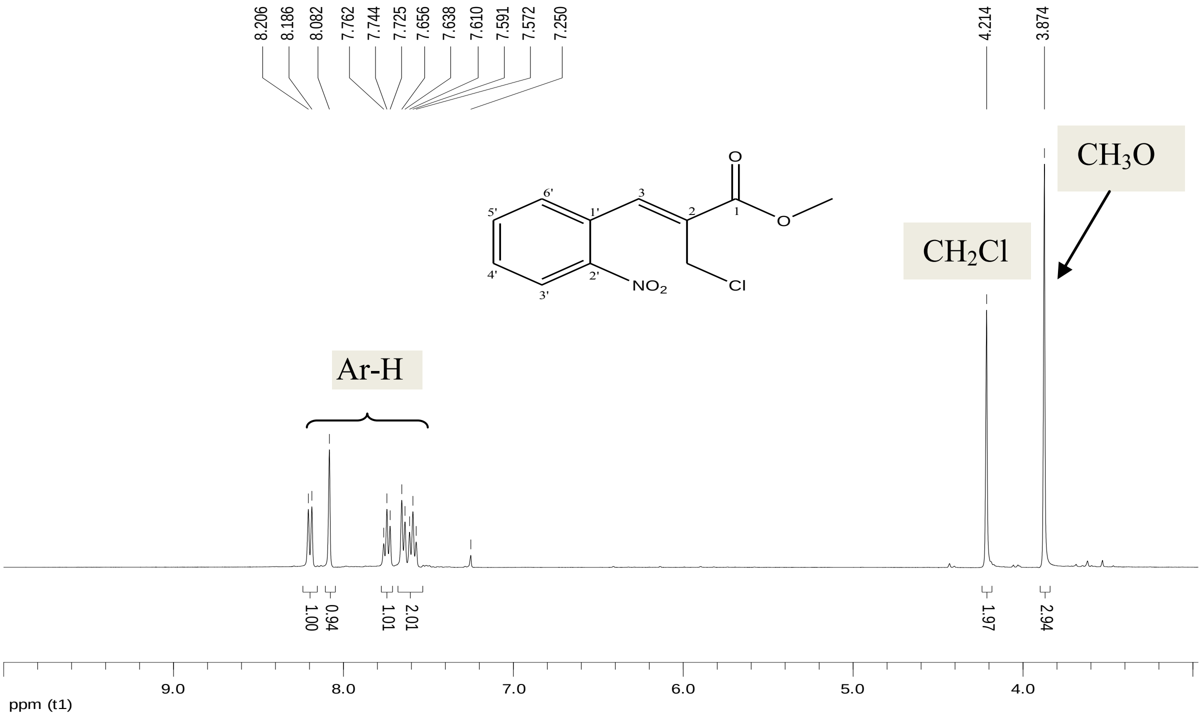


**Figure 2.10.** 400 MHz $^{13}$C NMR spectrum of methyl (*Z*)-2-(chloromethyl)-3-(2-nitrophenyl)propenoate **87a** in CDCl$_3$.

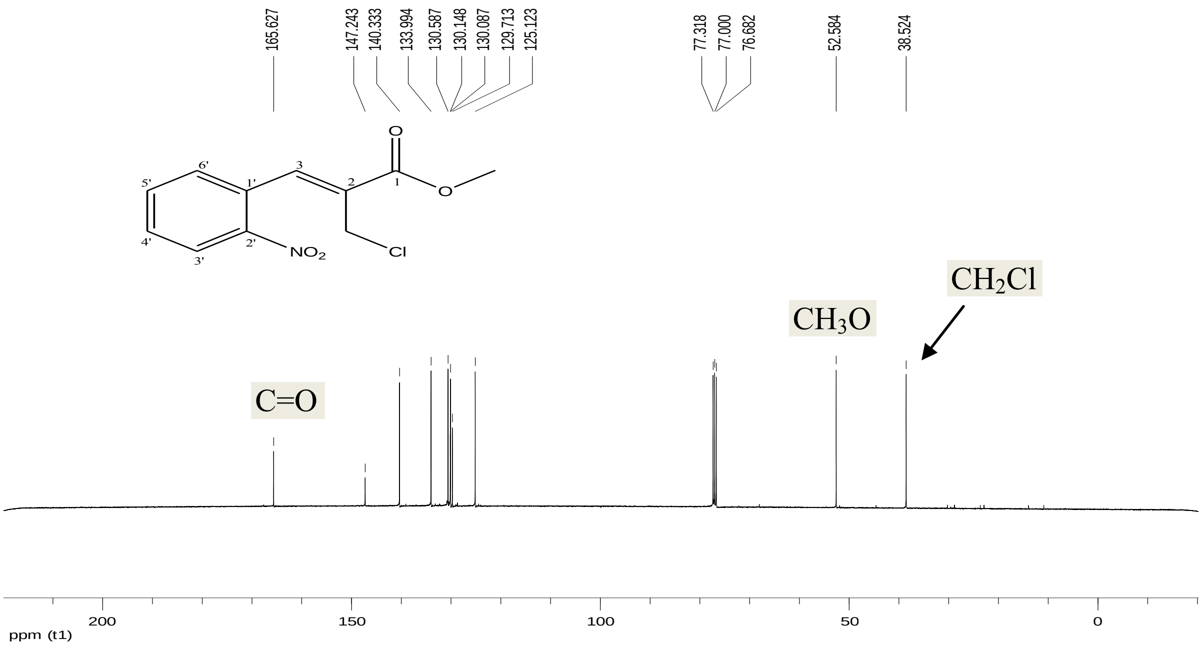


**Figure 2.11.** 100 MHz $^{13}$C NMR spectrum of methyl (*Z*)-2-(chloromethyl)-3-(2-nitrophenyl)propenoate **87a** in $CDCl_3$.

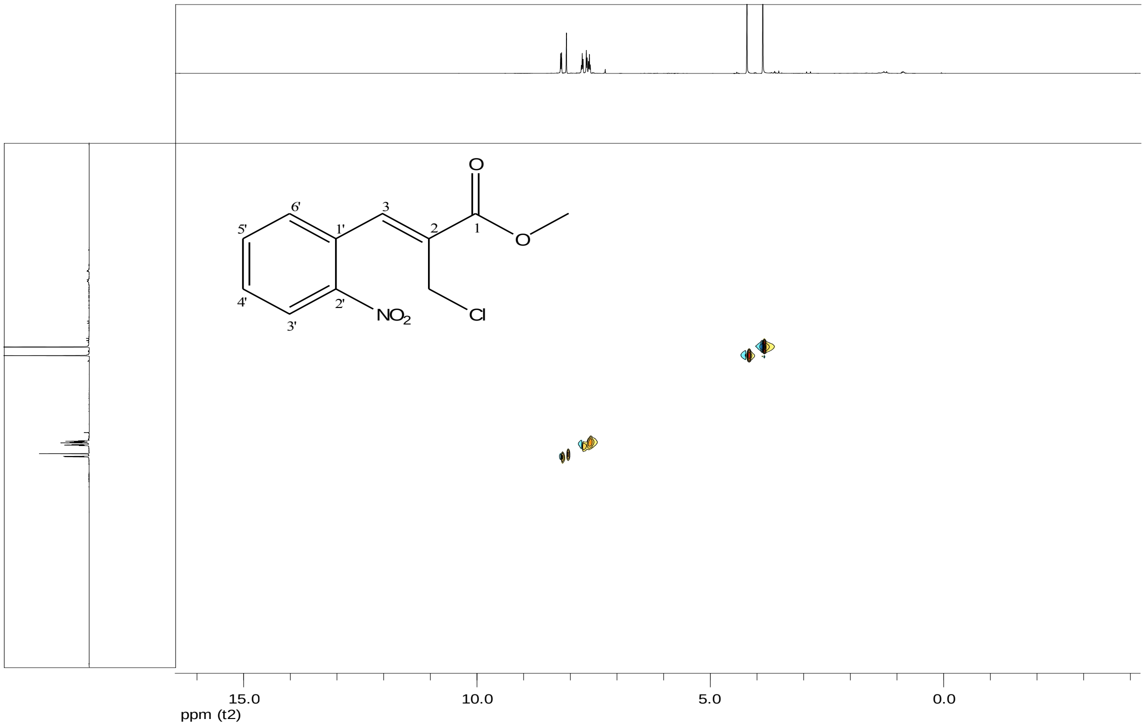
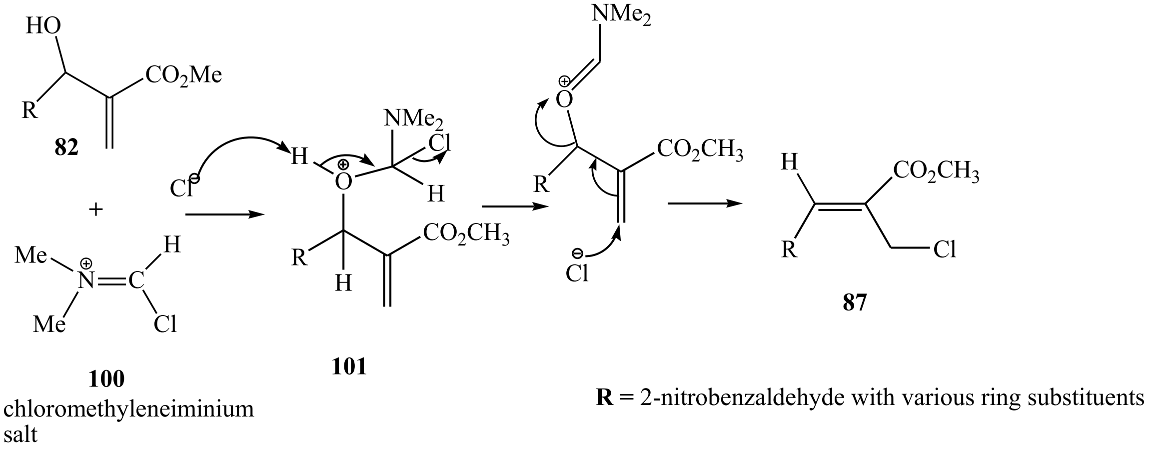


**Figure 2.12.** NOESY NMR spectrum of methyl (*Z*)-2-(chloromethyl)-3-(2-nitrophenyl) propenoate **87a** in $CDCl_3$ showing no NOE correlation.

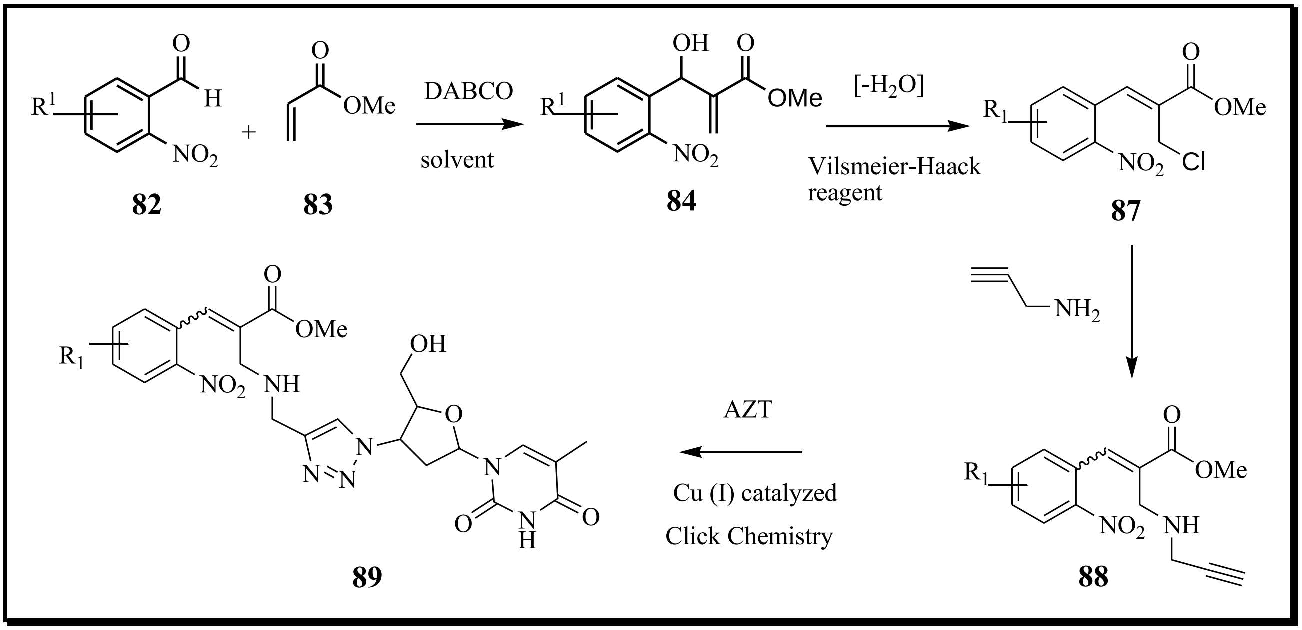


**R** = 2-nitrobenzaldehyde with various ring substituents

**Scheme 2.12.** Proposed mechanism for the dehydration of BH methyl esters.[75]

A mechanism for the dehydration reaction of β-hydroxy esters **82a-h** using the *in situ* generated Vilsmeier-Haack reagent has been proposed by Liu *et al*. and is illustrated in Scheme 2.12.[75]

At this stage in the project, it was decided to explore the use of the chloromethylene products **87a-h** in the construction of potential dual-action HIV-1 IN/RT inhibitors by constructing the cinnamate ester-AZT conjugates **89a-h** following the approach outlined in the Strategic Plan II (Scheme 2.13). This involved reacting the chloromethylene products with propargylamine.

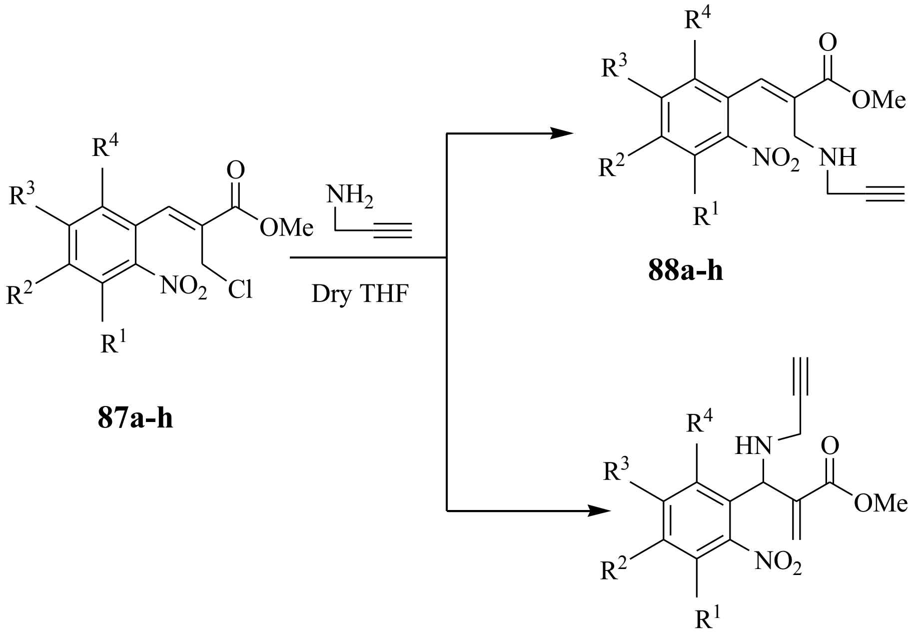


**Scheme 2.13.** ***STRATEGIC PLAN II*****:** Synthesis of cinnamate ester– AZT conjugates as potential HIV-1 dual action IN-RT inhibitors**.**

### 2.1.6. Propargylation of the α-chloromethyl esters 87a-h.

As shown in Scheme 2.14, reaction of the α-chloromethyl esters **87a-h** with propargylamine, a primary amine, in anhydrous THF, affords the 2-(aminomethyl)cinnamates **88a-h**. The method adopted in our research group[73,74] involves reacting 1 eq. of the α-chloromethyl ester **87** with 2 eq. of propargylamine, at room temperature, for a period of 2 – 3 days. The reactions progressed well, affording the desired propargylamine derivative in moderate to excellent yields. However, in some cases, it was observed that, in addition to the expected product, a competition product was isolated, which gave BH type methine and vinylic proton signals in their $^{1}H$ NMR spectra and corresponding signals in the $^{13}C$ NMR spectra. These competition products were identified (and fully characterized) as the isomeric propargylamine derivatives, **90a,b,e-h**. The yields obtained for the expected products **88a-h** and the competition products **90a,b,e-h** are summarisied in Tables 2.3 and 2.4, respectively. Formation of the desired 2-(propargylamino)methyl derivatives **88a-h** may be attributed to direct ($S_N$)

displacement of chloride ion ($\mathrm{Cl}^{\bigcirc}$). In the case of the 3-propargylamino isomers **88a,b,e-h**, however, the reaction could involve either $S_N$' or conjugate addition – elimination pathways. The ratio of the expected product **88** to the competing product **90** was estimated from the $^1$H NMR spectrum of the crude product to be generally (1:1). The $^1$H NMR spectrum of methyl (*E*)-2-[(propynylamino)methyl]-3-(2-nitrophenyl)propenoate **88a** (Figure. 2.14) shows:- a singlet at 2.03 ppm, which accounts for the terminal alkyne proton, overlapping the broad NH proton signal; two methylene proton singlets at 3.33 and 3.48 ppm; a methyl singlet at 3.83 ppm; and signals corresponding to five protons in the aromatic region.



**87a-h**

**88a-h**

**90 a,b,e-h**

| | R$^1$ | R$^2$ | R$^3$ | R$^4$ |
|---|---|---|---|---|
| a | H | H | H | H |
| b | H | H | OH | H |
| c | H | O-CH$_2$-O | | H |
| d | H | OMe | OMe | H |
| e | OMe | H | H | H |
| f | H | H | Cl | H |
| g | H | Cl | H | H |
| h | H | H | OMe | H |

**Scheme 2.14.** Substitution reactions of the α-chloromethyl cinnamate esters with propargylamine.

**Table 2.3.** Yields of 2-(propynylamino)methyl derivatives **88a-h** from the chloromethyl esters **87a-h.**

| **2-[(Propynylamino)methyl derivatives 88a-h** | **R$^1$** | **R$^2$** | **R$^3$** | **R$^4$** | **Yield** |
|---|---|---|---|---|---|
| **88a** | H | H | H | H | 56% |
| **88b** | H | H | OH | H | 42% [a] |
| **88c** | H | O-CH$_2$-O | | H | 78% |
| **88d** | H | OMe | OMe | H | 86% |
| **88e** | OMe | H | H | H | 40% |
| **88f** | H | H | Cl | H | 36% |
| **88g** | H | Cl | H | H | 47% |
| **88h** | H | H | OMe | H | 32% [a] |

[a] Yield estimated from the $^1$H NMR spectrum of the mixture of the two isomers, as it was not possible to isolate the isomers.

**Table 2.4.** Yield of 3-propargylamino isomers **90a,b,e-h.**

| **3-[(Propynylamino)isomer 90a,b,e-h** | **R$^1$** | **R$^2$** | **R$^3$** | **R$^4$** | **Yield** |
|---|---|---|---|---|---|
| **90a** | H | H | H | H | 16% [a] |
| **90b** | H | H | OH | H | 24% [a] |
| **90e** | OMe | H | H | H | 48% |
| **90f** | H | H | Cl | H | 40% |
| **90g** | H | Cl | H | H | 46% |
| **90h** | H | H | OMe | H | 35% [a] |

[a] Yield estimated from the $^1$H NMR spectra of the mixture of the two isomers, as it was not possible to isolate the individual isomers.

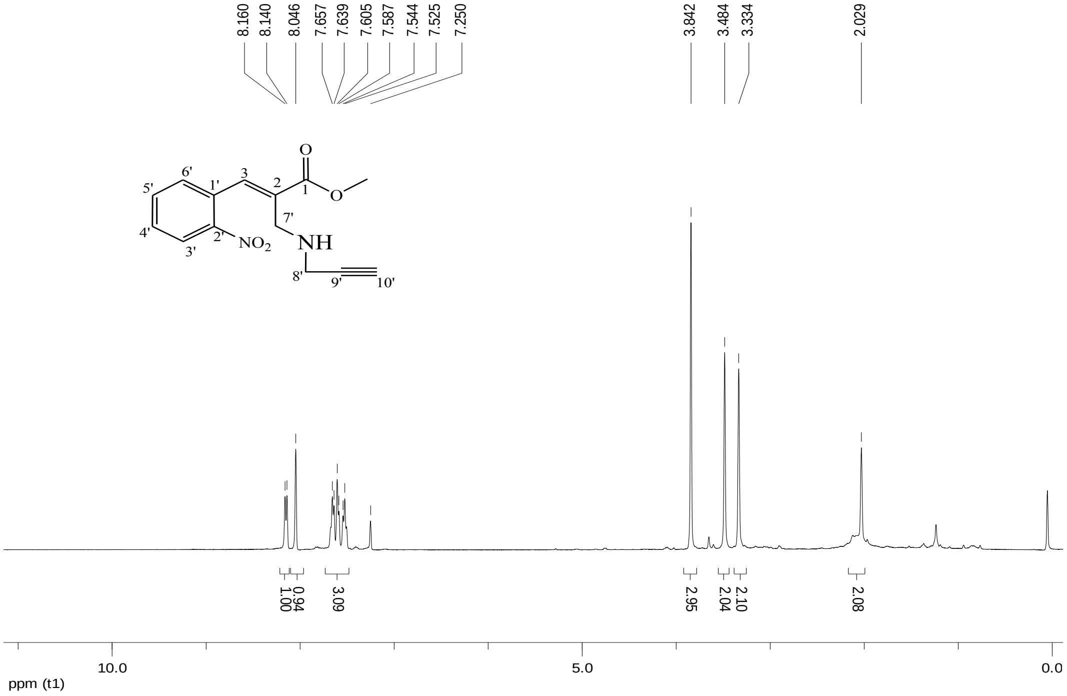


**Figure 2.13.** 400MHz $^{1}$HNMR spectrum of methyl (*E*)-2-[(propynylamino)methyl]-3-(2-nitrophenyl)propenoate **88a** in $CDCl_3$.

The $^{13}$C NMR spectrum of compound **88a** (Figure 2.14) shows all of the 14 carbon signals expected, with the carbonyl carbon resonating at 167.5 ppm. The DEPT 135 NMR spectrum (Figure. 2.15) reveals two methylene carbon signals at 37.5 and 44.9 ppm which account for carbons C-7' and C-8'. An interesting feature in the DEPT 135 NMR spectrum (Figure. 2.15) of compound **88a** is the fact that both the terminal *and* the non-terminal alkyne gave positive signals. The appearance of the signal for the quaternary non-terminal alkyne carbon at 81.4 ppm, in the DEPT135 NMR experiment, is attributable to polarization transfer from the alkyne proton two bonds away, due to the exceptionally large $^{2}J_{CH}$ values observed in these systems. This phenomenon was observed consistently in the DEPT 135 spectra of all the other members of propargylamino cinnamate ester series **88a-h** and their competition products **90a,b,e-h.** The $^{1}J_{CH}$ and $^{2}J_{CH}$ correlation between the terminal alkyne proton of both alkyne carbons is clearly evident in the HSQC spectrum (Figure.2.16).

Although, the Sequence Rule stereochemical assignment has changed from (*Z*) in the precursor to (*E*) in the product, no change is expected in the *actual* configuration about the double bond. This expectation is consistent with the absence of any NOE in the NOESY spectrum of the parent system.

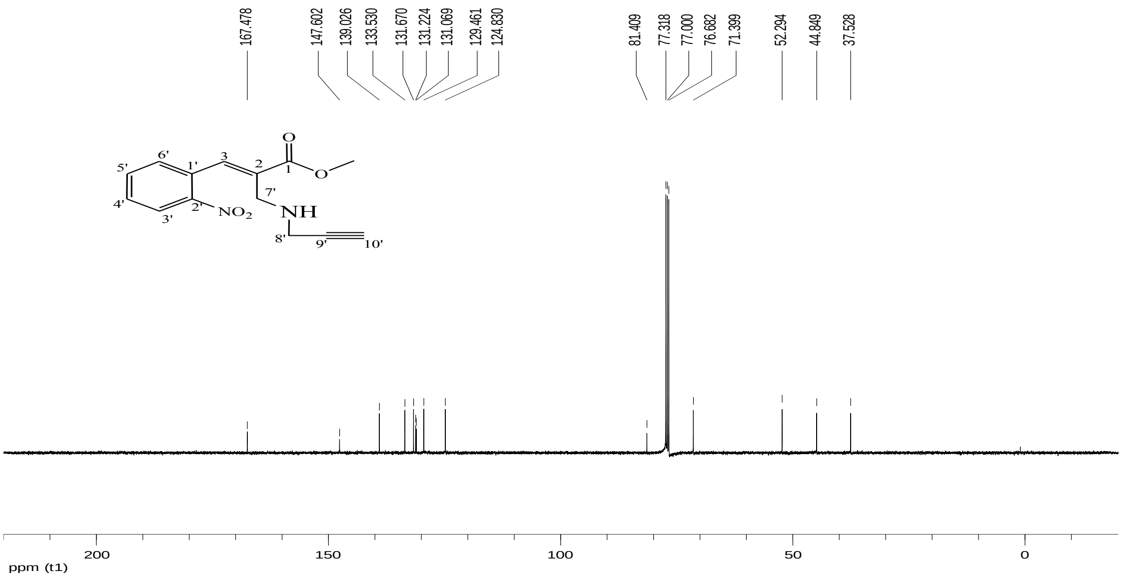


**Figure 2.14.** 100 MHz $^{13}$C NMR spectrum of methyl (*E*)-2-[(propynylamino)methyl]-3-(2-nitrophenyl)propenoate **88a** in CDCl$_3$.

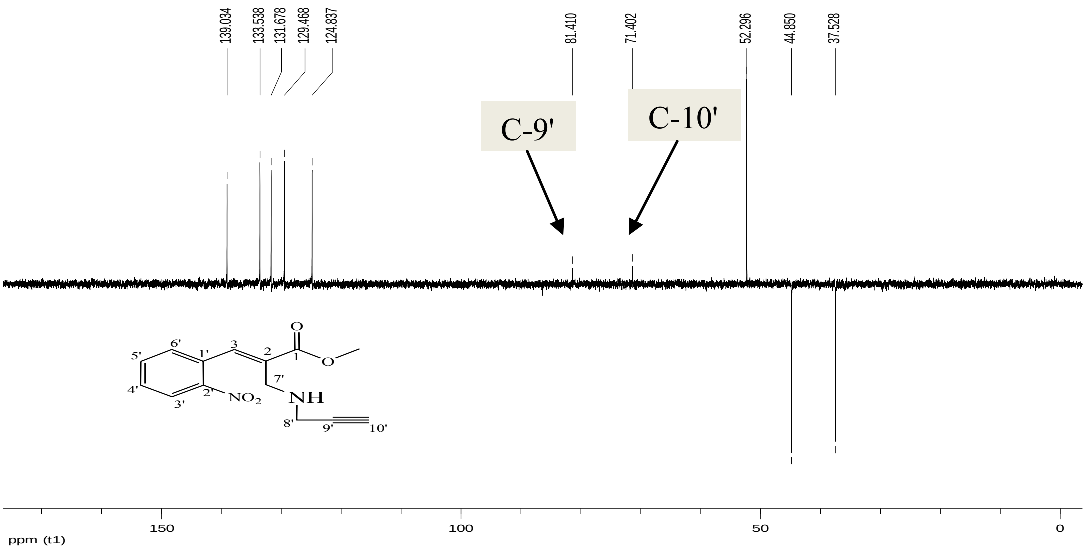


**Figure 2.15.** DEPT 135 NMR spectrum of methyl (*E*)-2-(propynylamino)-3-(2-nitrophenyl)propenoate **88a** in CDCl$_3$.

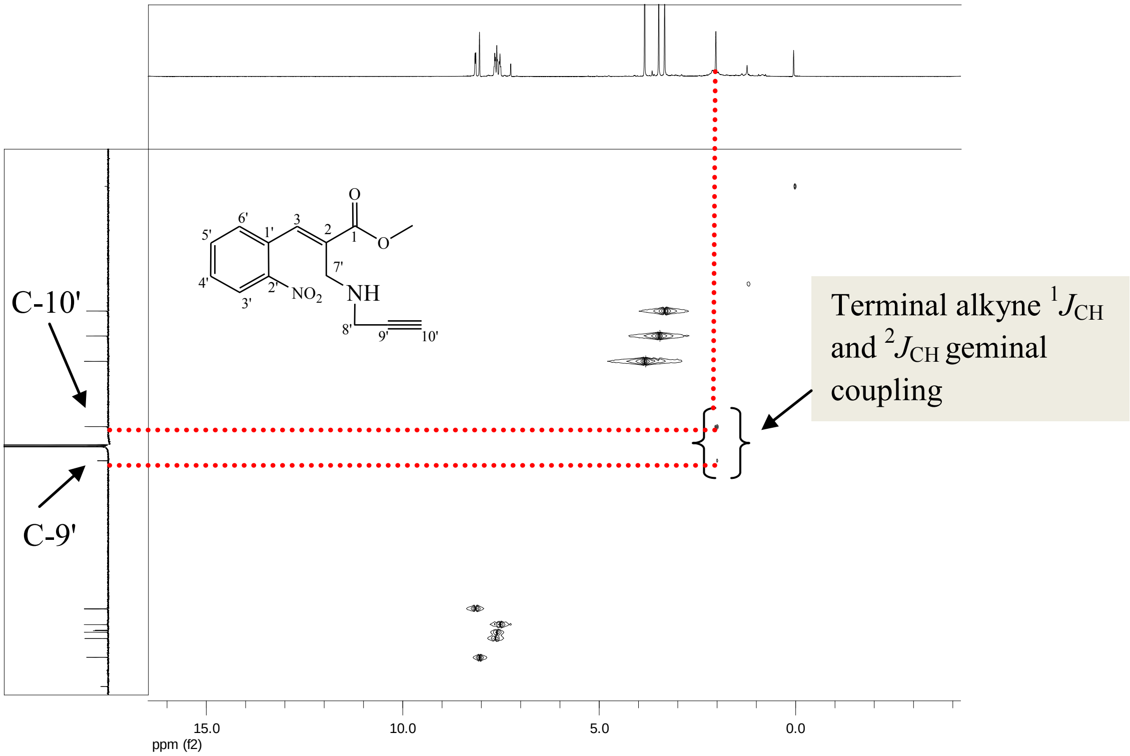


**Figure 2.16.** HSQC spectrum of methyl (*E*)-2-(propynylamino)-3-(2- nitrophenyl)-propenoate **88a** in $CDCl_3$

NMR spectroscopic analysis permitted unambiguous characterization of the isomeric 3-(5-chloro-2-nitrophenyl)-2-methylene-3-(propargylamino)propanoate, **90f**. Thus, the $^{1}H$ NMR spectrum of the 5'-chloro analogue **90f** (Figure.2.17), reveals the broad NH signal at 1.92 ppm, the terminal alkyne proton signal at 2.24 ppm, the aminomethylene singlet at 3.39 ppm, and the methoxy singlet at 3.67 ppm. Of particular significance are the three proton signals, seen in the 'BH region' of the $^{1}H$ NMR spectrum of compound **90f**, due to the 3-methine proton at 5.43 ppm and the vinylic protons (at 5.85 and 6.40 ppm). All 14 carbon signals are accounted for in the $^{13}C$ NMR spectrum (Figure 2.18) with the carbonyl carbonyl resonating at 165.9 ppm and the six aromatic and two vinylic carbon signals evident in the region 125 – 147 ppm, three of which are shown by the DEPT 135 NMR spectrum (Figure 2.19) to correspond, as expected, to the three methine aromatic carbon signals, and the vinylic methylene carbon signal at 127.1 ppm. The DEPT 135 spectrum (Figure 2.19) also illustrates the presence of positive signals for both alkyne carbons at 72.2 and 80.9, while the HSQC spectrum (Figure 2.20) clearly shows the $^1J_{CH}$ and $^2J_{CH}$ interactions between these carbon nuclei and the terminal alkyne proton.

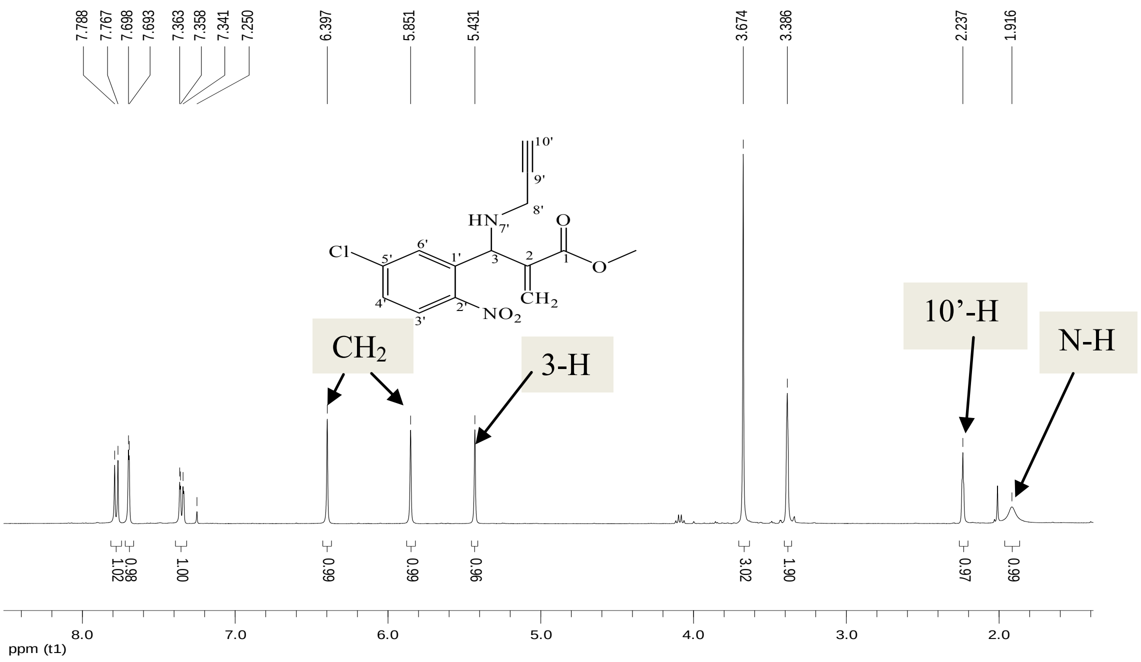


**Fig.2.17.** 400 MHz $^1$H NMR spectrum of methyl 3-(propynylamino)-2-methylene-3-(5-chloro-2-nitrophenyl)propanoate **90f** in $CDCl_3$

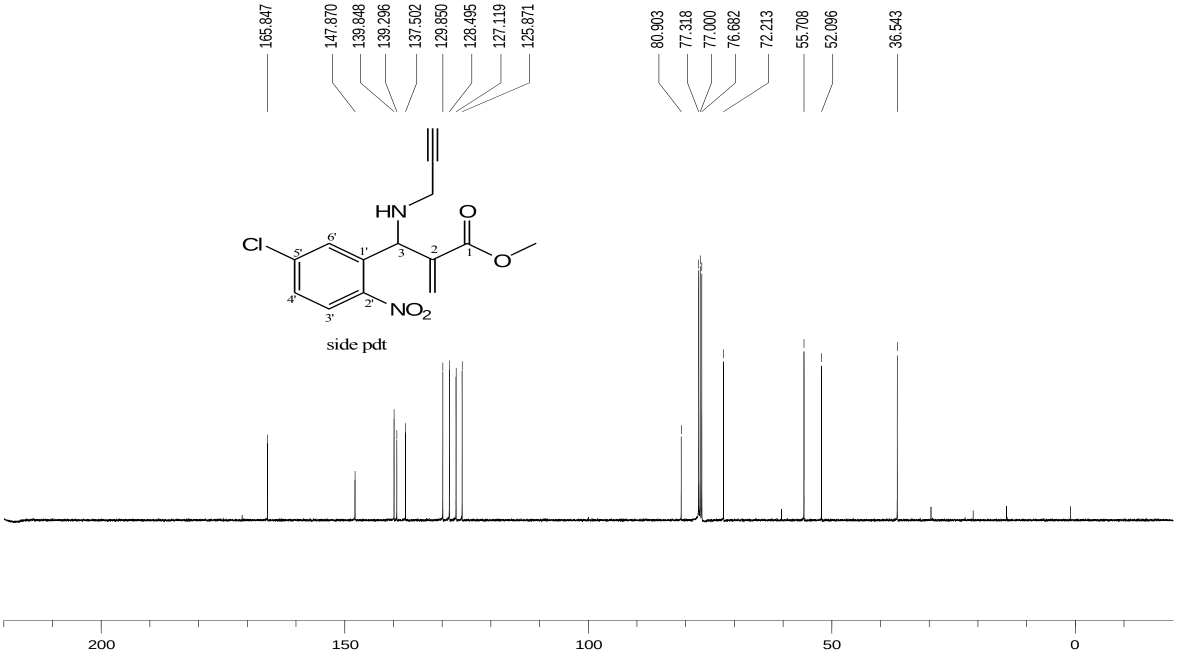


**Figure 2.18.** 100 MHz $^{13}$C NMR spectrum of methyl 3-(propynylamino)-2-methylene-3-(5-chloro-2-nitrophenyl)propanoate **90f** in $CDCl_3$.

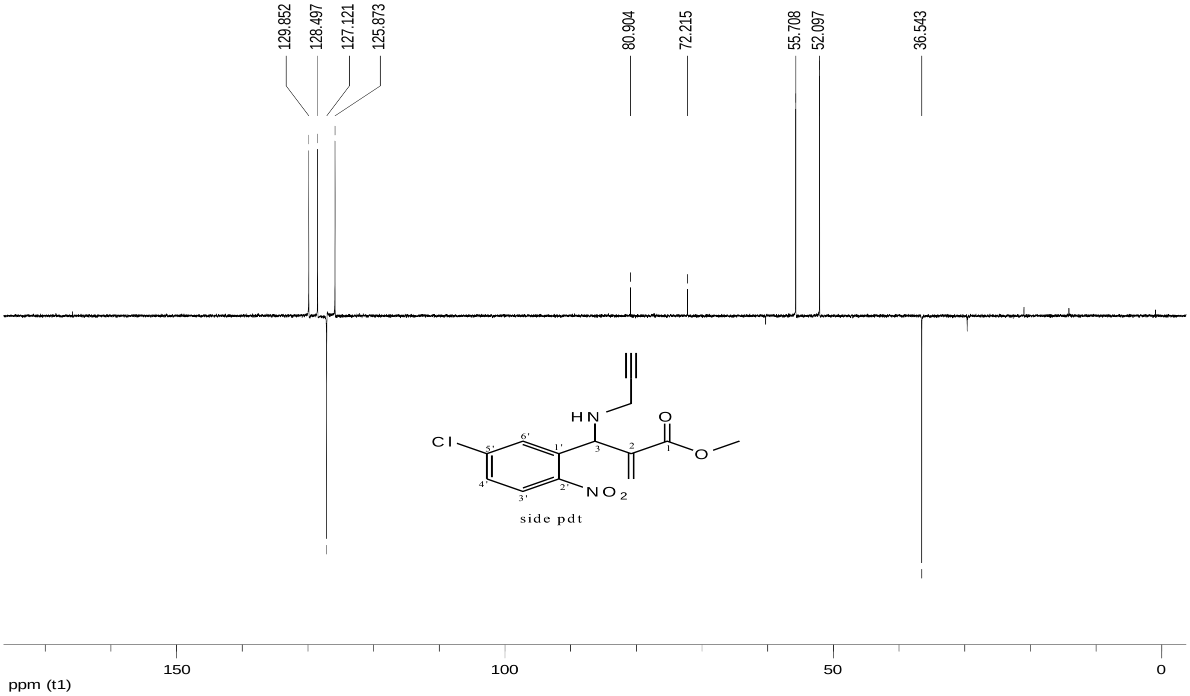


**Figure 2.19.** DEPT 135 NMR spectrum of methyl 3-(5-chloro-2-nitrophenyl)-2-methylene-3-(propynylamino)-3-propanoate **90f** in $CDCl_3$.

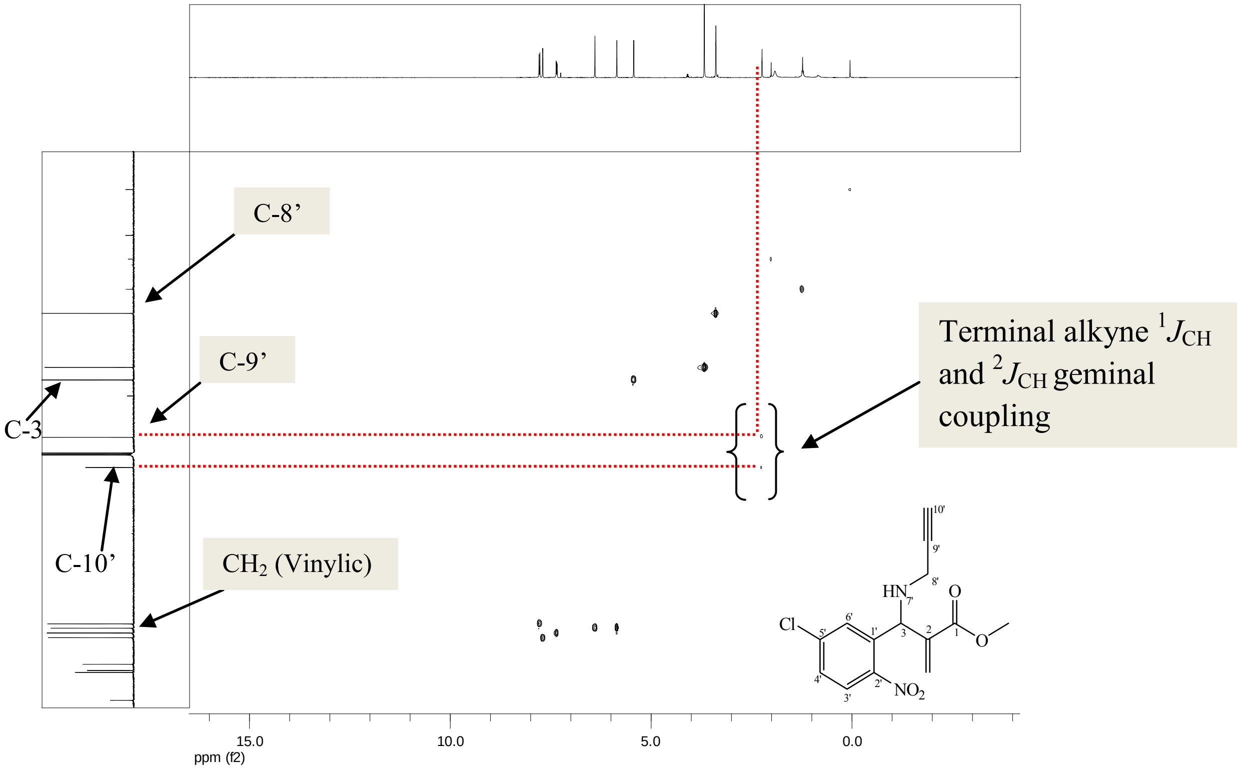
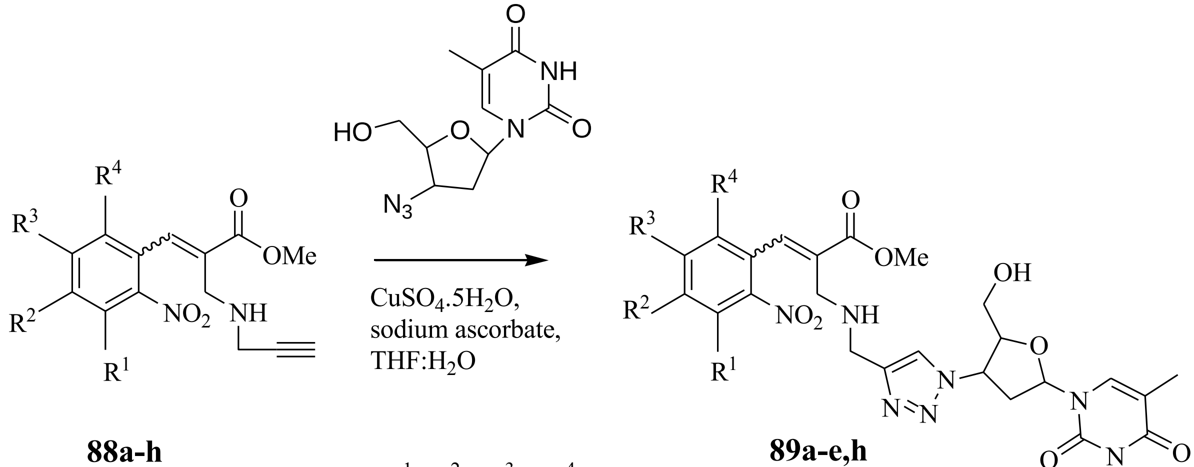


**Figure 2.20.** HSQC spectrum of methyl 3-(5-chloro-2-nitrophenyl)-2-methylene3-(propynylamino)-3-propanoate **90f** in $CDCl_3$.

## 2.2. CINNAMATE ESTER-AZT CONJUGATES.

While the cinnamate esters **88a-h** may be viewed as potential HIV-1 integrase inhibitor, the propargylamine group was introduced with the deliberate intention of using the products **88a-h** in ‘click’ chemistry reactions. This involved reacting the organic azide, 3'-azido-3'-deoxythymidine (AZT), with the terminal alkyne group, in the copper(I) catalyzed 1,3-dipolar Huisgen cycloaddition reaction, [77] to afford cinnamate ester-AZT conjugates with potential as a dual-action HIV-1 IN-RT inhibitors.



**88a-h** **89a-e,h**

| | $R^1$ | $R^2$ | $R^3$ | $R^4$ |
|---|---|---|---|---|
| a | H | H | H | H |
| b | H | H | OH | H |
| c | H | O-$CH_2$-O | | H |
| d | H | OMe | OMe | H |
| e | OMe | H | H | H |
| f | H | H | Cl | H |
| g | H | Cl | H | H |
| h | H | H | OMe | H |

**Scheme 2.15.** Synthesis of cinnamate ester-AZT conjugates as potential dual action IN-RT inhibitors.

The method adopted had been employed previously in our research group in the synthesis of coumarin derivatives as potential dual-action HIV-1 protease and reverse transcriptase inhibitors.[78] The method involved dissolving 3'-azido-3'-deoxythymidine (AZT) in $H_2O$/THF (1:1) and then adding an equimolar amount of the propargyl derivative **88**, sodium ascorbate (0.20 eq.), and copper(II) sulphate pentahydrate ($CuSO_4.5H_2O$) (0.03

eq.). After stirring for 24 h at room temperature, work-up and chromatography afforded the cinnamate ester-AZT conjugate **89**. As indicated in Scheme 2.15, the six propargyl derivatives **88a-e,h** were reacted under these conditions to afford the corresponding products **89a-e,h** in moderate to excellent yields (Table 2.5).

**Table 2.5.** Yields of cinnamate ester-AZT conjugates.

| Cinnamate-AZT conjugates 88a-h | $R^1$ | $R^2$ | $R^3$ | $R^4$ | Yield |
|---|---|---|---|---|---|
| **88a** | H | H | H | H | 44% |
| **88b** | H | H | OH | H | 42% |
| **88c** | H | -OCH$_2$O- | | H | 62% |
| **88d** | H | OMe | OMe | H | 70% |
| **88e** | OMe | H | H | H | 91% |
| **88h** | H | H | OMe | H | 43% |

The $^1$H NMR spectrum of the cinnamate ester-AZT conjugate **89a** (Figure 2.21) reveals a singlet at 1.88 ppm corresponding to the AZT methyl protons, two multiplets at 2.68 and 2.81 ppm corresponding to the diastereotopic 14-methylene protons of the furan ring, and signals for the aromatic protons in the compound appearing between 7.48 and 8.17 ppm. The $^{13}$C spectrum (Figure 2.22) reveals the absence of the substrate alkyne carbon signals and the presence of all the expected 27 carbon signals including the three carbonyl carbons signals between 149.7 and 166.4 ppm.

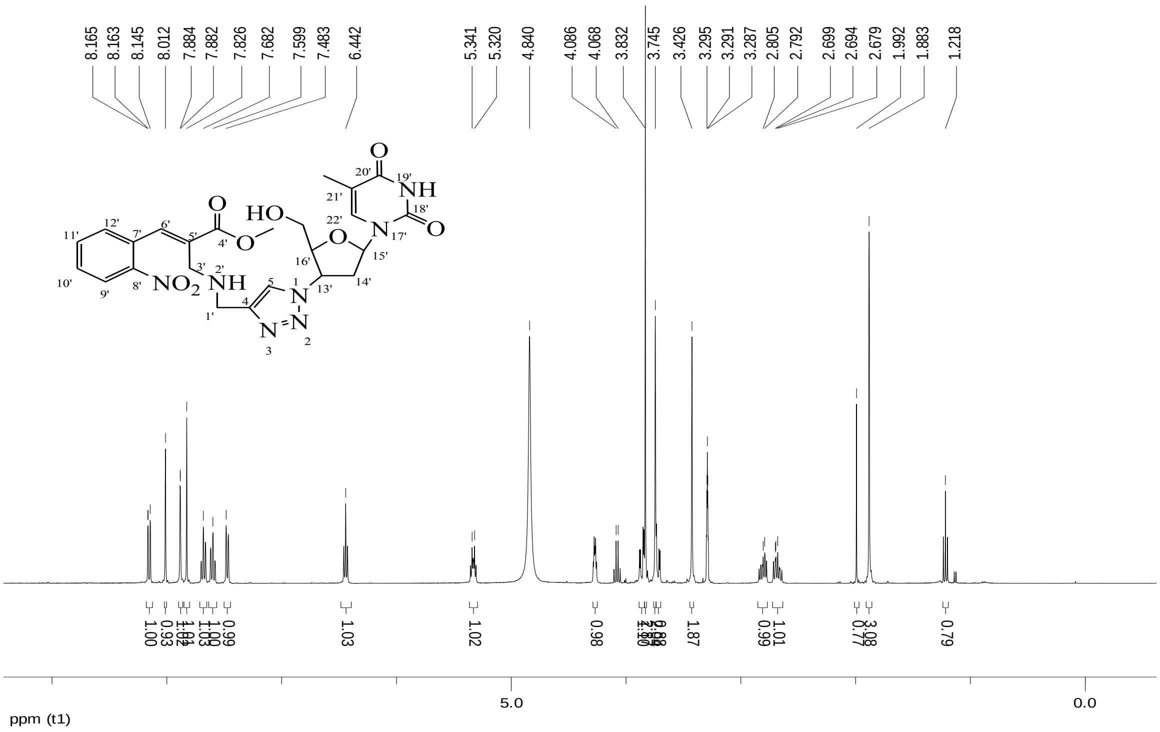


**Figure 2.21.**400MHz $^{1}$H NMR spectrum of the cinnamate ester-AZT conjugate **89a** in methanol-*d*$_4$.

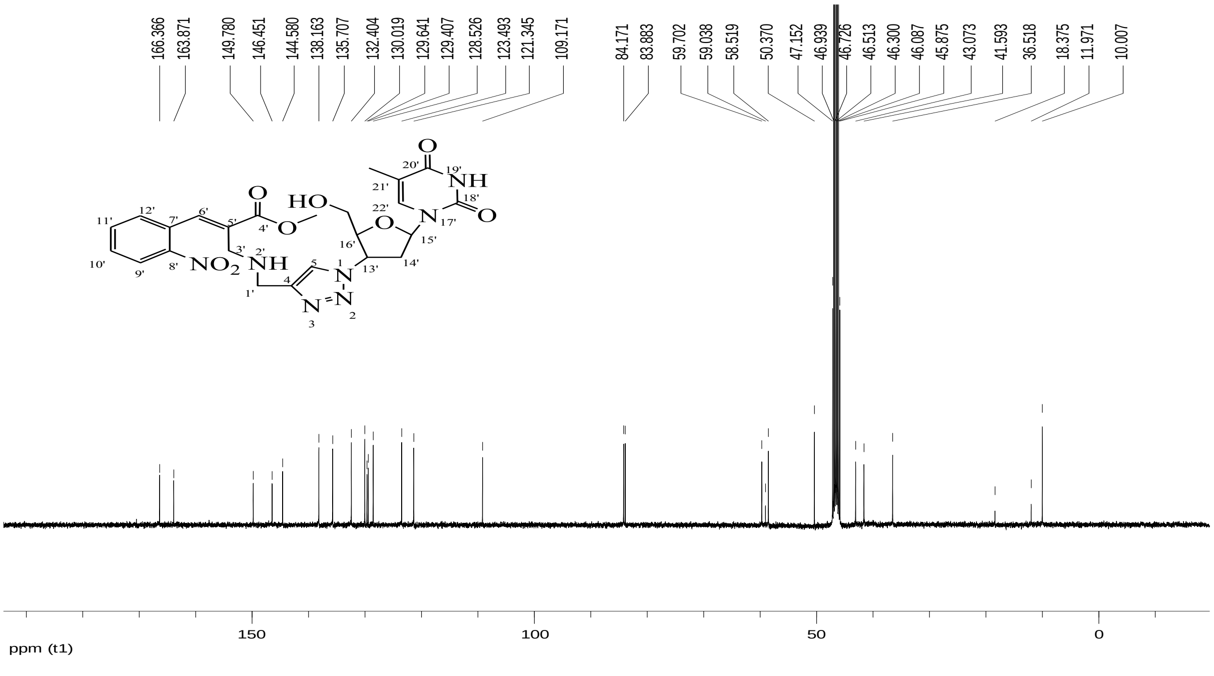


**Figure 2.22.** $^{13}$C NMR spectrum of the cinnamate ester-AZT conjugate **89a** in methanol-*d*$_4$.

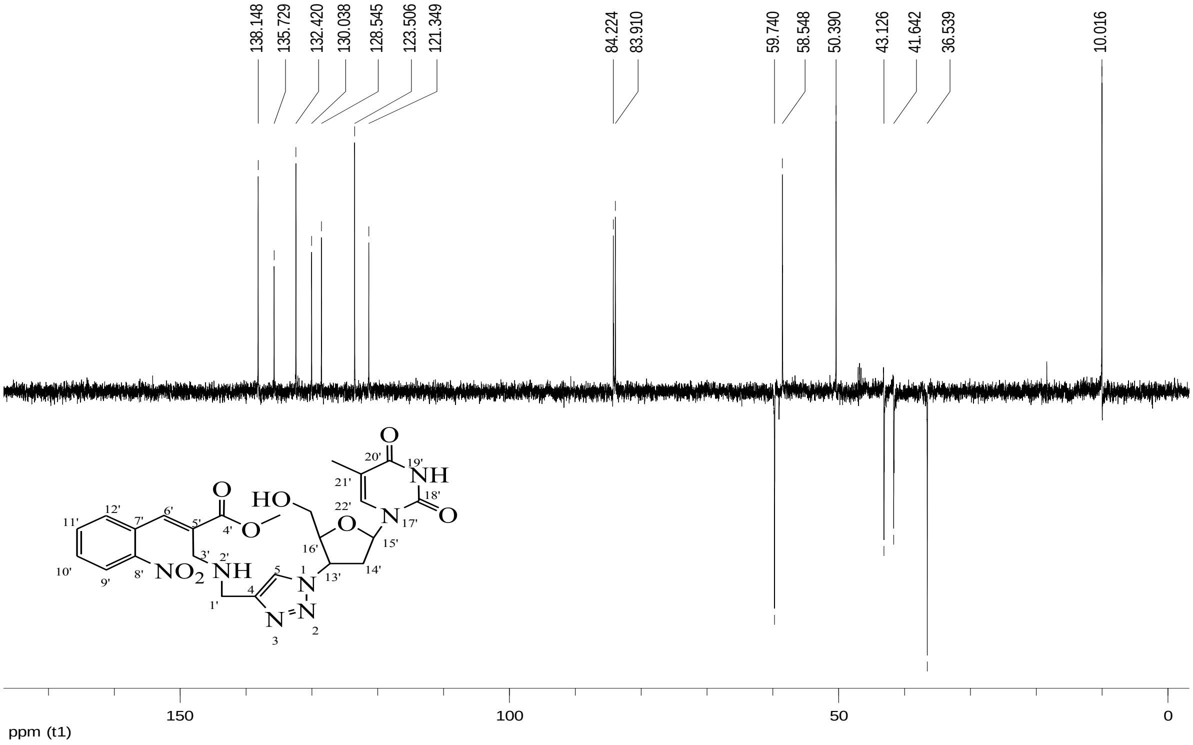


**Figure 2.23.** DEPT 135 NMR spectrum of the cinnamate ester-AZT conjugate **89a** in methanol-$d_4$.

The DEPT 135 NMR spectrum (Figure 2.23) confirms the presence of four methylene carbon signals and seven methine carbon signals in the aromatic region. The COSY spectrum (Figure 2.24) reveals the long range couplings (marked X) between the 22'-methine proton and the AZT- methyl protons (21'-$CH_3$), and between the diastereotropic 14'-methylene protons and the 15'- and 16'-methine protons, (marked Y). The HSQC and HMBC spectra (Figures 2.25 and 2.26) also confirm the assignment of all the signals.

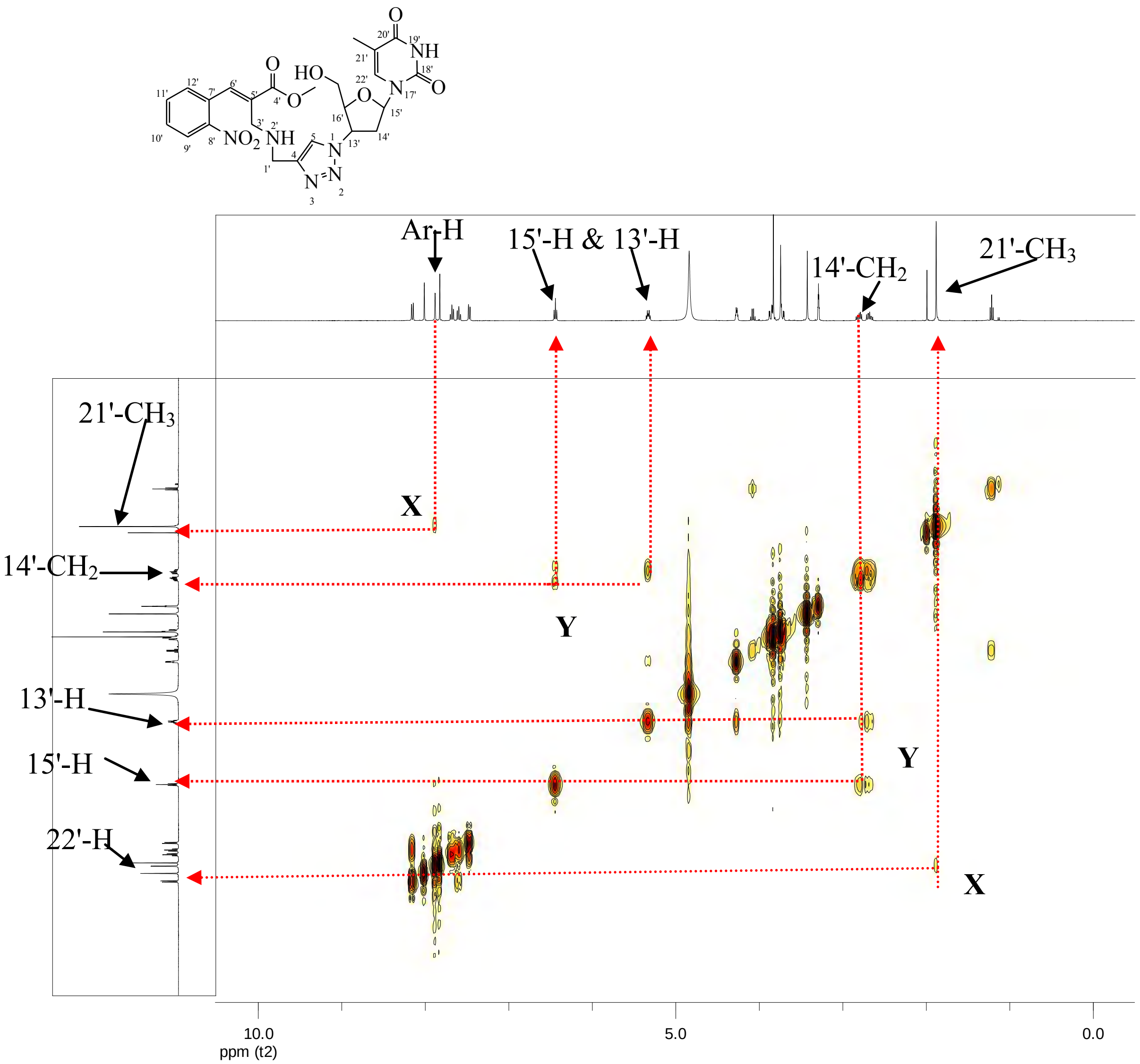


**Figure 2.24.** COSY spectrum of the cinnamate ester-AZT conjugate **89a** in methanol-$d_4$.

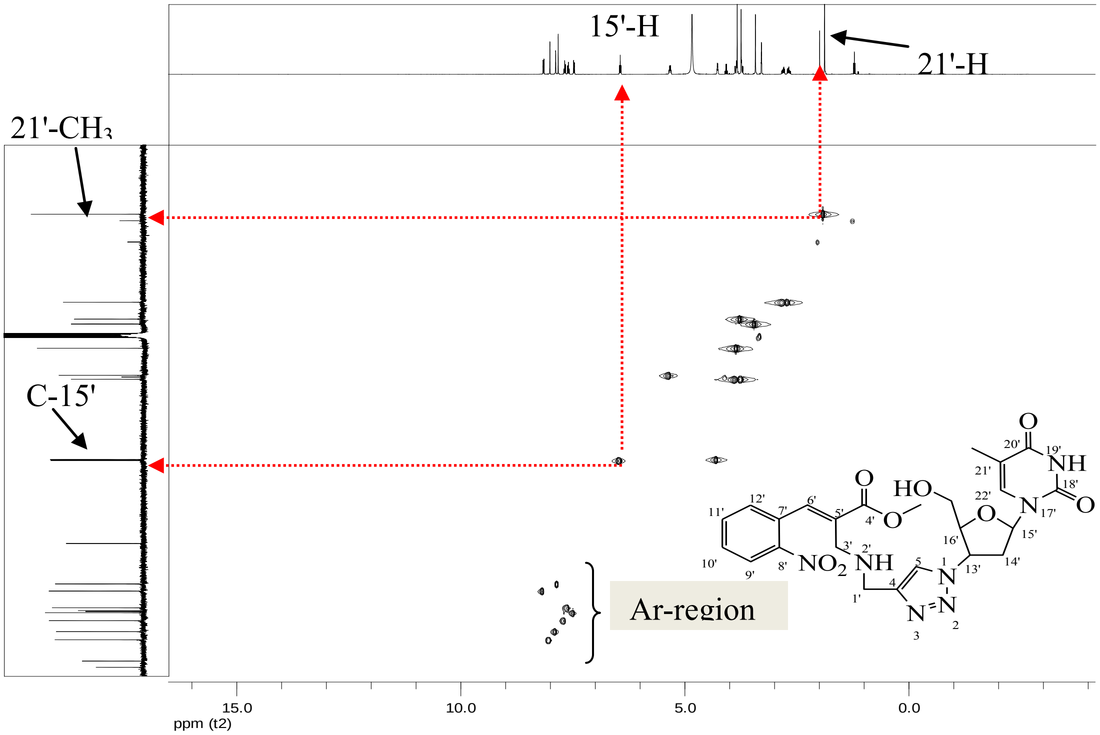


**Figure 2.25.** HSQC spectrum of cinnamate ester-AZT conjugate **89a** in methanol-$d_4$.

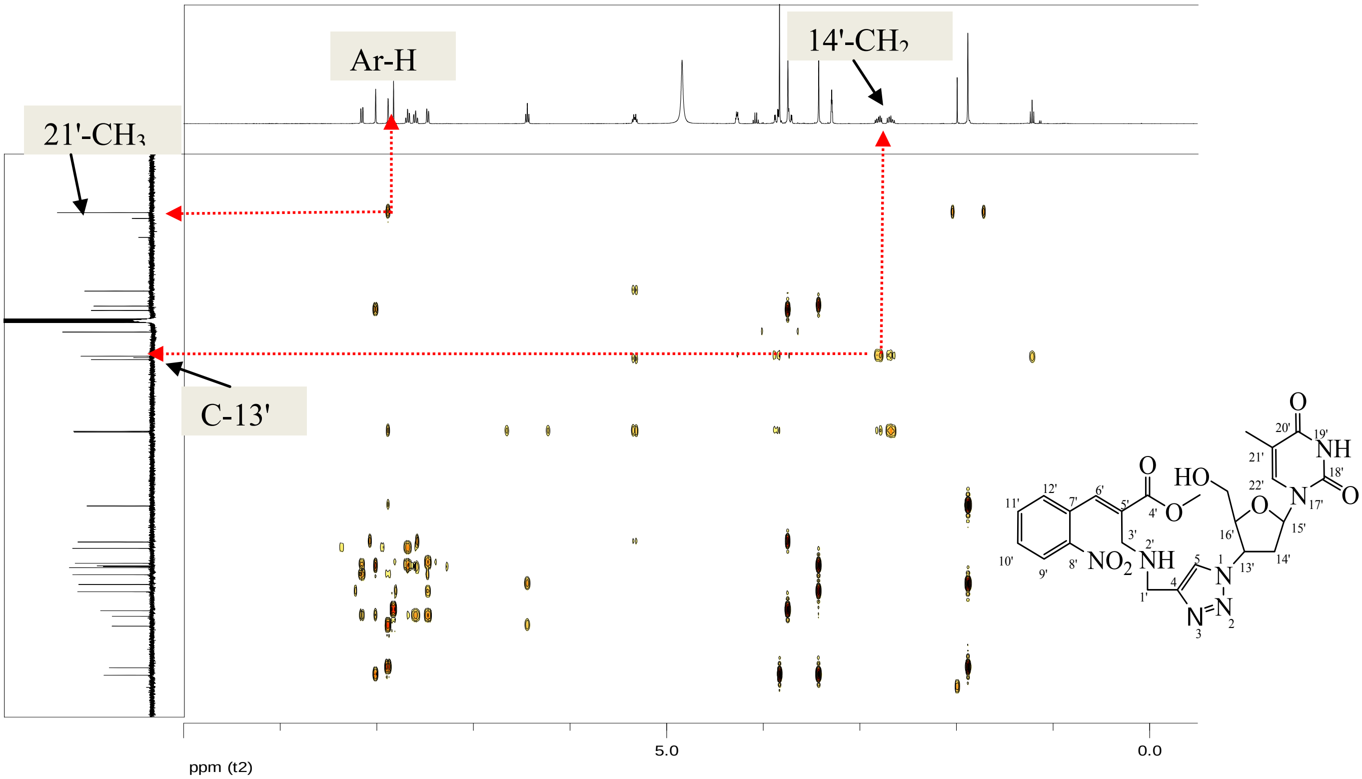
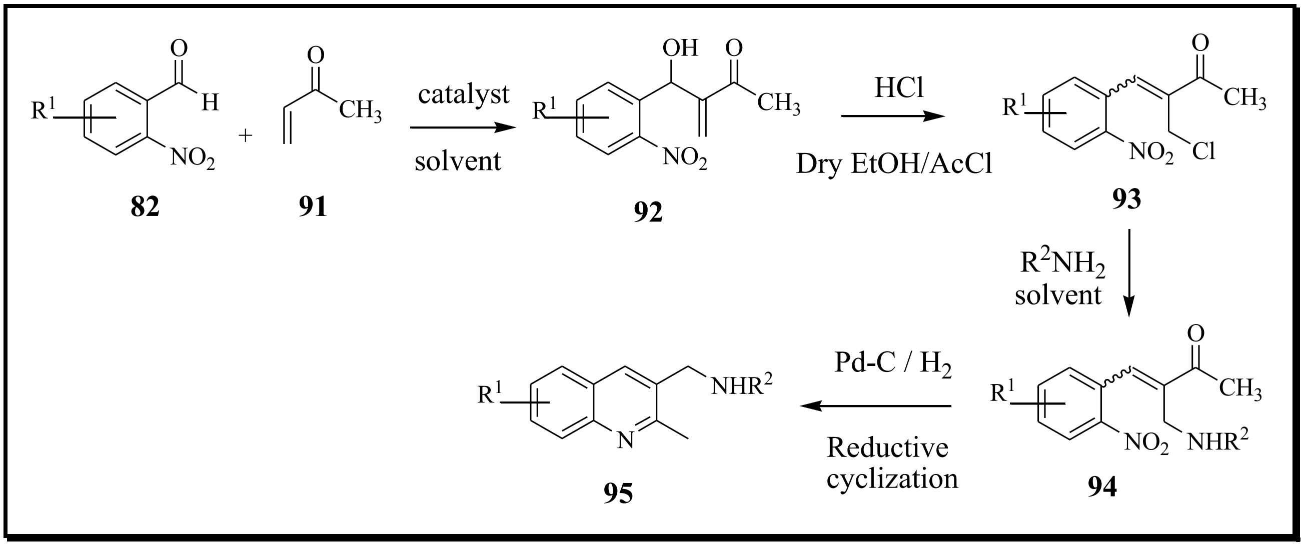


**Figure 2.26.** HMBC spectrum of cinnamate ester-AZT conjugate **89a** in methanol-$d_4$.

## 2.3. SYNTHESIS OF QUINOLINE DERIVATIVES.

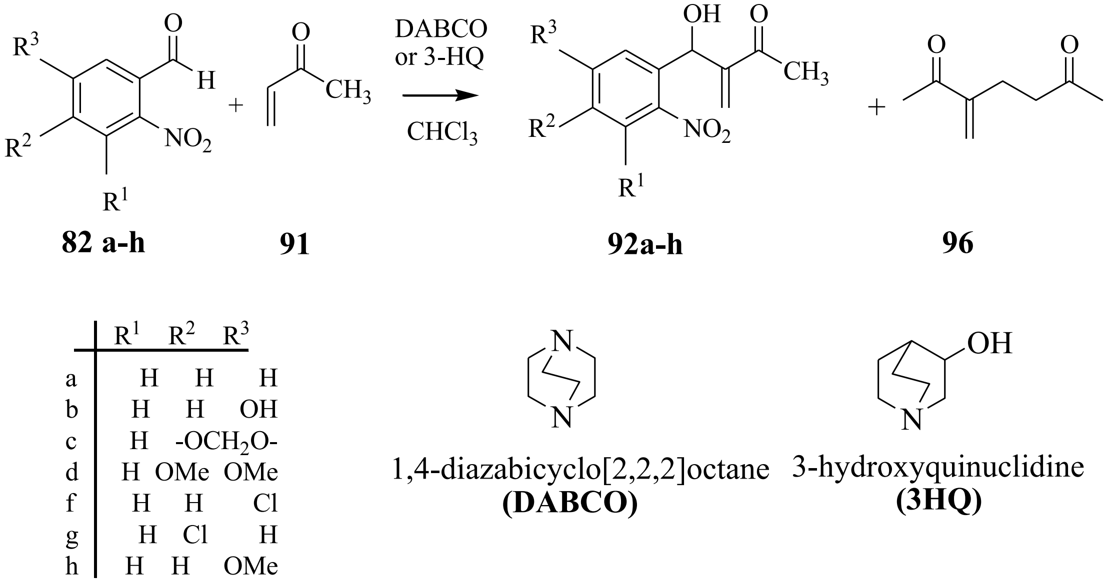
The synthetic strategy adopted to access the 3-(aminomethyl)quinoline is illustrated in Scheme 2.16 and involved a number of steps, namely: (i) synthesis of Baylis-Hillman adducts with MVK; (ii) reaction of Baylis-Hillman adducts with HCl to afford chloromethyl α,β unsaturated ketones; (iii) conjugate addition reactions of the chloromethyl α,β-unsaturated ketones with different amines; and (iv) reductive cyclization to quinoline derivatives



**Scheme 2.16.** ***STRATEGIC PLAN III***: Synthesis of 3-(aminomethyl)quinolines.

### 2.3.1. Reactions of 2-nitrobenzaldehydes with MVK.

As indicated in Scheme 2.17, the series of 2-nitrobenzaldehydes **82a-h** were reacted with methyl vinyl ketone (MVK) **91**, using DABCO or 3-hydroxyquinuclidine (3-HQ) as catalyst and chloroform, or a mixture of chloroform and methanol (1:1), as solvent, at room temperature for periods between two days and six weeks. Two of the reactions, however, were also carried out in the microwave reactor. The progress of the reactions was monitored using TLC analysis. In a few instances, when there were indications that the reaction was no longer progressing additional MVK and catalyst were added to raise the respective molar ratio [from initial: 1(aldehyde):2(MVK):0.05(DABCO) to 1:2:0.1]. At the end of each reaction, the excess solvent was removed under reduced pressure and the residue purified by flash chromatography on silica gel to afford the Baylis-Hillman adducts **92a-h** in yields of 20 - 80% as shown in Table 2.6.



**82 a-h** **91** **92a-h** **96**

| | $R^1$ | $R^2$ | $R^3$ |
|---|---|---|---|
| a | H | H | H |
| b | H | H | OH |
| c | H | $-OCH_2O-$ | |
| d | H | OMe | OMe |
| f | H | H | Cl |
| g | H | Cl | H |
| h | H | H | OMe |

1,4-diazabicyclo[2,2,2]octane
**(DABCO)**

3-hydroxyquinuclidine
**(3HQ)**

**Scheme 2.17.** Synthesis of Baylis-Hillman adducts from 2-nitrobenzaldehydes and MVK.

**Table 2.6.** Isolated yields of MVK-based Baylis-Hillman products **92a-h.**

| Entry | 2-Nitrobenzaldehydes | $R^1$ | $R^2$ | $R^3$ | 3° amine catalyst | Solvent | Yield |
|---|---|---|---|---|---|---|---|
| **1** | **82a** | H | H | H | DABCO | $CHCl_3$ | 18% |
| **2** | | | | | 3HQ | $CHCl_3$ | 28% |
| **3** | | | | | DABCO | Neat [a] | 54% |
| **4** | **82b** | H | H | OH | DABCO | $CHCl_3$/MeOH (1:1) | 39% |
| **5** | | | | | 3HQ | | 52% |
| **6** | **82c** | H | O-$CH_2$-O | | DABCO | $CHCl_3$ | 26% |
| **7** | | | | | 3HQ | $CHCl_3$ | 27% |
| **8** | **82d** | H | OMe | OMe | DABCO | $CHCl_3$ | 16% |
| **9** | | | | | | MeOH[a] | 22% |
| **10** | **82e** | OMe | H | H | DABCO | $CHCl_3$ | 24% |
| **11** | **82f** | H | H | Cl | DABCO | $CHCl_3$ | 80% |
| **12** | **82g** | H | Cl | H | DABCO | $CHCl_3$ | 72% |
| **13** | **82h** | H | H | OMe | DABCO | $CHCl_3$ | 70% |

[a] Under microwave conditions.

The resulting adducts **92a-h** were fully characterized by NMR, IR and HRMS analysis. The characteristic features in the $^1H$ NMR spectrum of such BH adducts are clearly seen in the $^1H$ NMR spectrum of compound **92f** (Figure 2.27), which shows a methyl singlet at 2.36 ppm, a broad hydroxyl proton signal at 3.48 ppm and the three signals characteristic of the BH adduct, as singlets at 5.76 ppm (4-methine proton) and 6.15 and 6.66 ppm (vinylic protons). The signals corresponding to the three aromatic protons appear between 7.40 and 7.95 ppm. The $^{13}C$ NMR spectrum (Figure 2.28) shows the 11 carbon signals expected, with the methyl signal at 25.9 ppm, the 4-methine signal at 67.2 ppm and the carbonyl signal at 199.7 ppm. The presence of the vinylic methylene carbon is confirmed at 126.5 ppm in the DEPT 135 NMR spectrum (Figure 2.29) of the product **92f**, which also confirms the assignment of the signals for the methyl and 4-methine carbons.

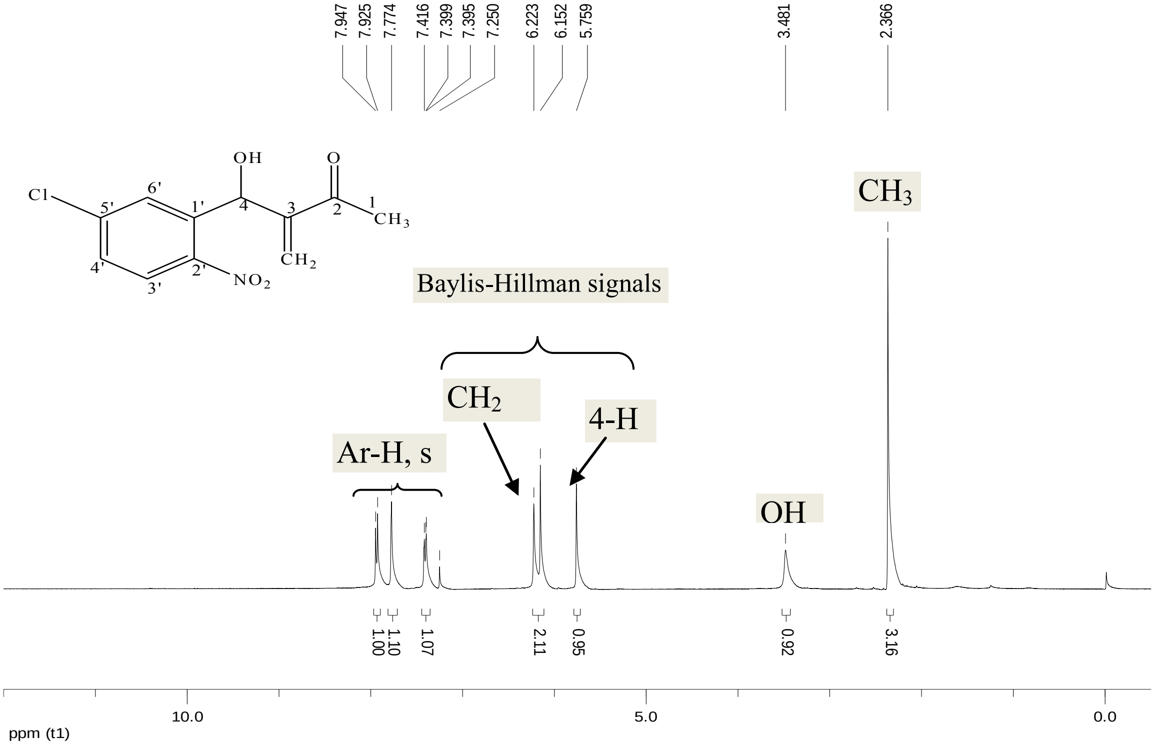


**Figure 2.27.** 400 MHz $^{1}$H NMR spectrum of 4-hydroxy-3-methylene-4-(5-chloro-2-nitrophenyl)butan-2-one **92f** in $CDCl_3$.

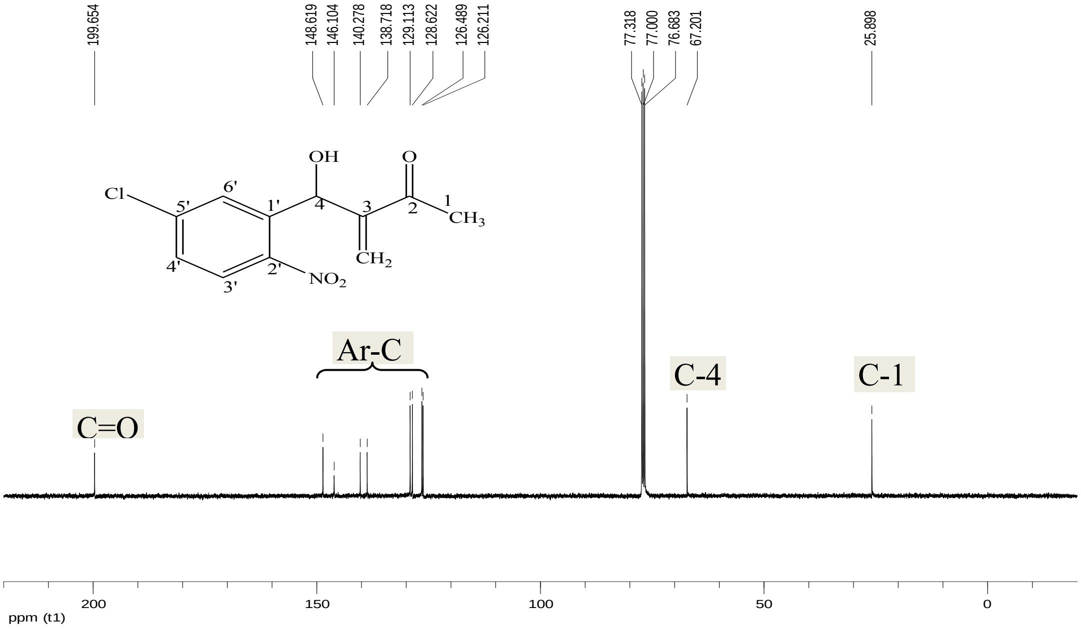


**Figure 2.28.** 100 MHz $^{13}$C NMR spectrums of 4-(5-chloro-2-nitrophenyl)-4-hydroxy--3-methylenebutan-2-one **92f** in $CDCl_3$.

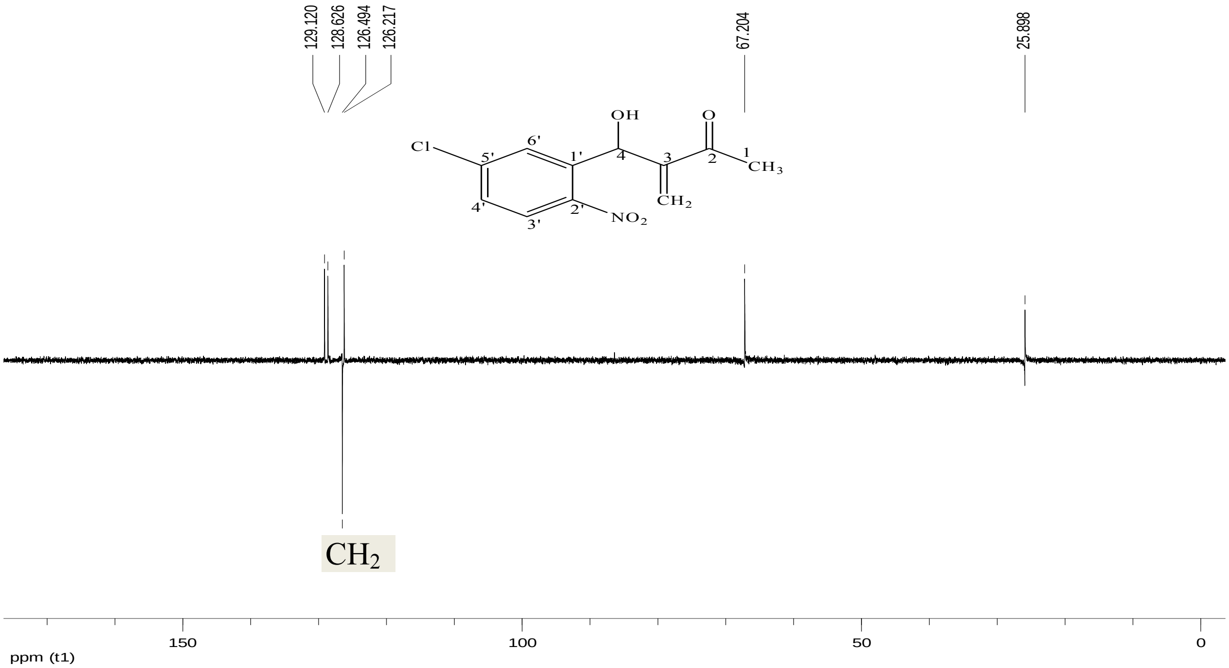
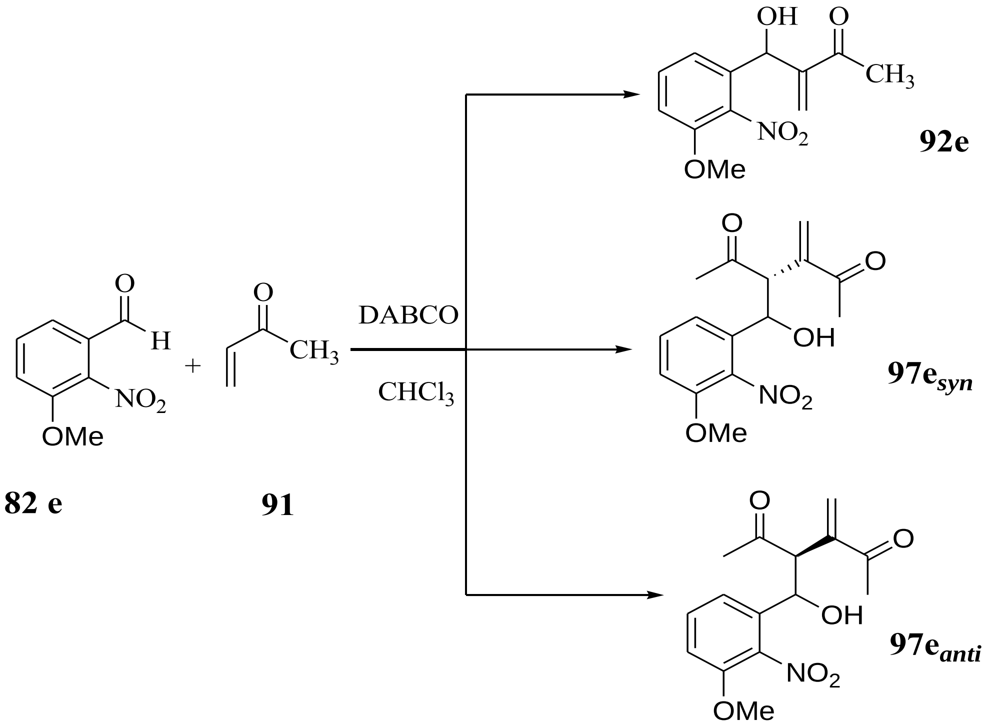


**Figure 2.29.** DEPT-135-NMR-spectrum of 4-(5-chloro-2-nitrophenyl)-4-hydroxy-3-methylene-butan-2-one **92f** in $CDCl_3$.

## 2.3.2. Formation of competition products.

In earlier studies[74,79] it had been observed that Baylis-Hillman reactions using 2-nitrobenzaldehyde and MVK, generate not only the desired β-hydroxy-α-methylene adducts **92** but other products from competing reactions as well. Such competition products include the methyl vinyl ketone (MVK) dimer **96** and the diastereomeric bis-MVK adducts **97e**$_{syn}$ and **97e**$_{anti}$ (Scheme 2.18). These side reactions, may explain the low yields observed for some of three reactions (see Table 2.6 entries 6-10). Shi *et al.* had reported similar results in the reaction between certain aryladehydes with MVK **91** and with ethyl vinyl ketone (EVK).[78] In the present study, the MVK dimer **97** was isolated in three instances and the bis-MVK adducts **97e**$_{syn}$ and **97e**$_{anti}$ from the reaction between 3-methoxy-2-nitrobenzaldehyde **82e** and MVK **91** in the presence of DABCO. [81]



**Scheme 2.18.** Formation of diastereomeric bis-MVK adducts as side-reaction products.[81]

The $^{1}$H NMR spectrum of the MVK dimer **96**, (Figure 2.30) shows two singlets at 1.74 and 1.94 ppm corresponding to the two methyl groups, two sets of multiplets between 2.09 and 2.23 ppm corresponding to the two methylene groups, while the vinylic methylene signals appear at 5.46 and 5.69 ppm. The $^{13}$C NMR spectrum (Figure 2.31) shows the eight carbon signals expected, with the two carbonyl carbon signals at 198.3 and 206.5 ppm. In the DEPT 135 NMR spectrum (Figure 2.32), the signals for the two methylene carbons can be seen at 24.2 and 41.4 ppm, while the two methyl carbon signals appeared at 24.9 and 28.8ppm. The vinylic methylene carbon signal is shown at 125.1 ppm. In the present study, the diastereomeric bis-MVK *syn-* and *anti-* adducts **97e** were also isolated and further purified using HPLC to afford analytically pure samples which were fully characterized. Computational techniques were used to assign the *syn-* and *anti-* diastereomers.[81] These results were critical for the completition of a kinetic-mechanistic study of the transformations detailed in Scheme 2.18, which has now been published.[81]

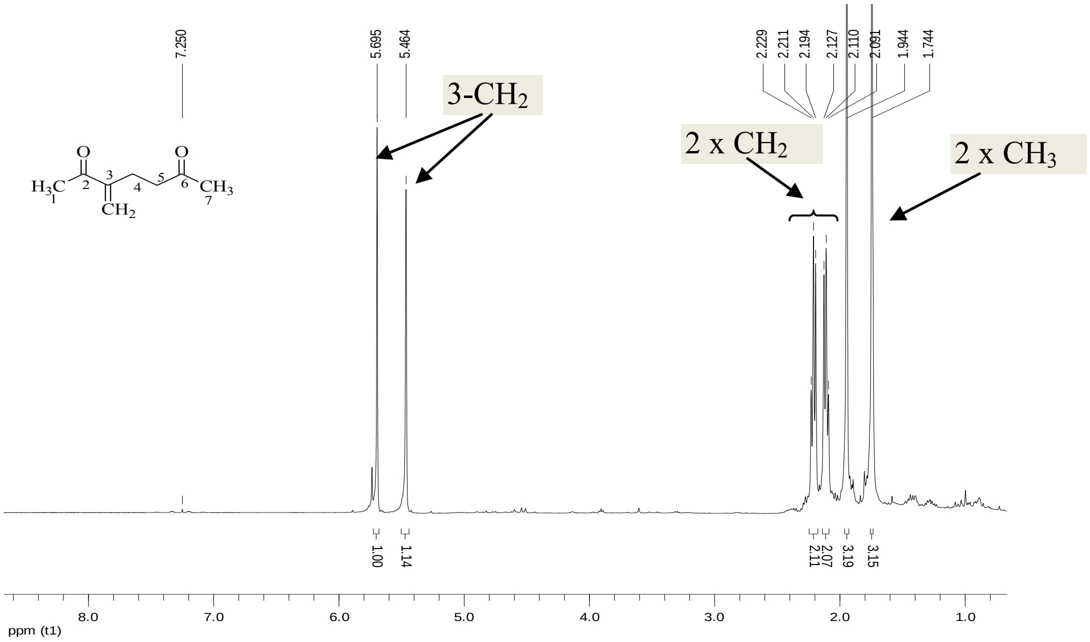


**Figure 2.30.** 400 MHz $^1H$ NMR spectrum of the MVK dimer, 3-methyleneheptan-2,6-dione **96,** in $CDCl_3$.

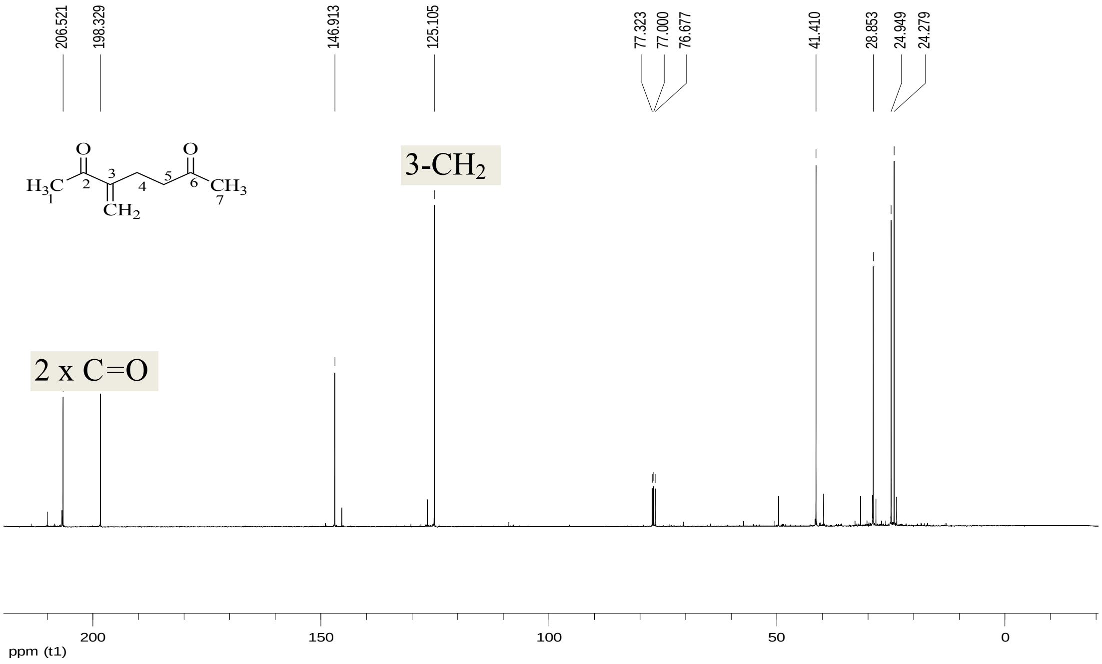


**Figure 2.31.** 100 MHz $^1H$ NMR spectrum of the MVK dimer, 3-methyleneheptan-2,6-dione **96,** in $CDCl_3$.

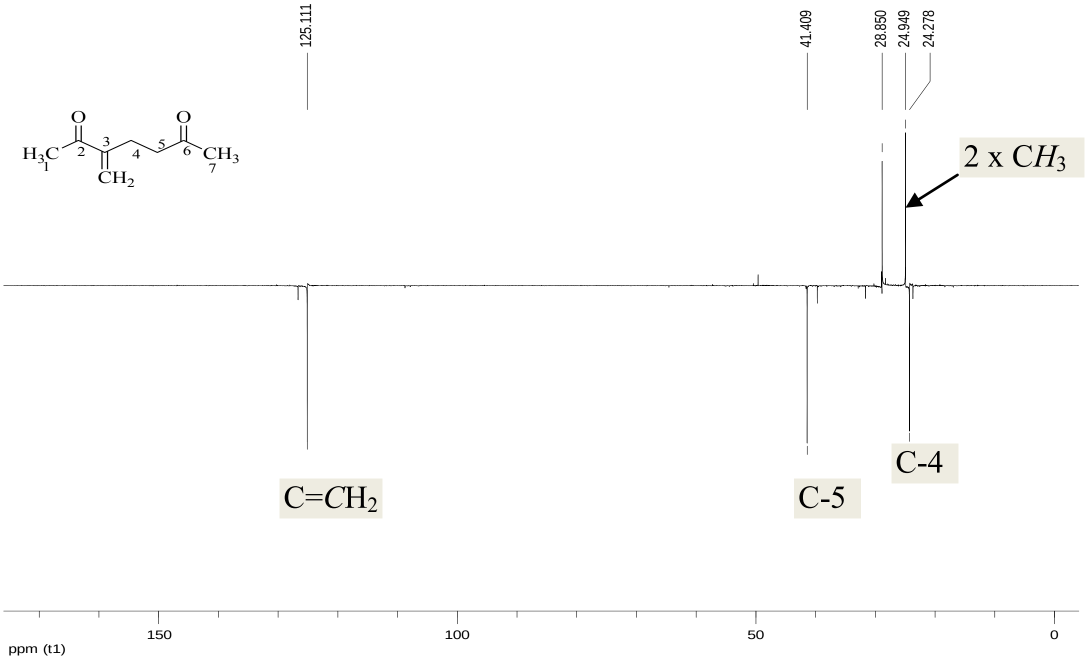


**Figure 2.32.** DEPT 135 NMR spectrum of the MVK dimer, 3-methyleneheptan-2,6-dione **96,** in $CDCl_3$.

Figures 2.33 and 2.34 illustrate the $^1H$ NMR spectra of the diastereomers **97e**$_{syn}$ and **97e**$_{anti}$ and reveal a number of significant differences.[81] In our paper, the stereochemical assignments were based on a relation between the experimental diastereomeric ratio and the relative energies of the modelled structures.[81] The spectrum of the *syn*-isomer features two methyl singlets at 2.07 and 2.24 ppm, corresponding to the methyl groups of the two terminal methyl groups of the diketone. The position of the second methyl singlet is shifted slightly downfield in the spectrum of the *anti*-diastereomer to 2.31 ppm. The methyl signals are closely followed by the 4"-methylene multiplet at 2.61 ppm and another multiplet at 3.21 ppm corresponding to the 3-methine proton in the *syn*-isomer; in the *anti* isomer, the 3-methine multiplet has a similar chemical shift (3.10 ppm) but the 4"-methylene protons resonate as two distinct double doublets at 2.35 and 3.10 ppm. The vinylic methylene proton signals can be seen as two singlets at 5.68 and 5.94 ppm in the *syn*-diastereomer, while the corresponding signals appear further downfield at 5.84 and 6.05 ppm in the spectrum of the *anti*-diastereomer. The $^{13}C$ NMR spectra (Figures 2.35 and 2.36) illustrate the 16 carbon signals expected for each of the diastereomers; again, a number of significant differences can be seen in the positions of various signals. The

DEPT 135 NMR spectra of the two diastereomers (Figures 2.37 and 2.38) facilitated the assignment of signals to the proton-bearing carbons.

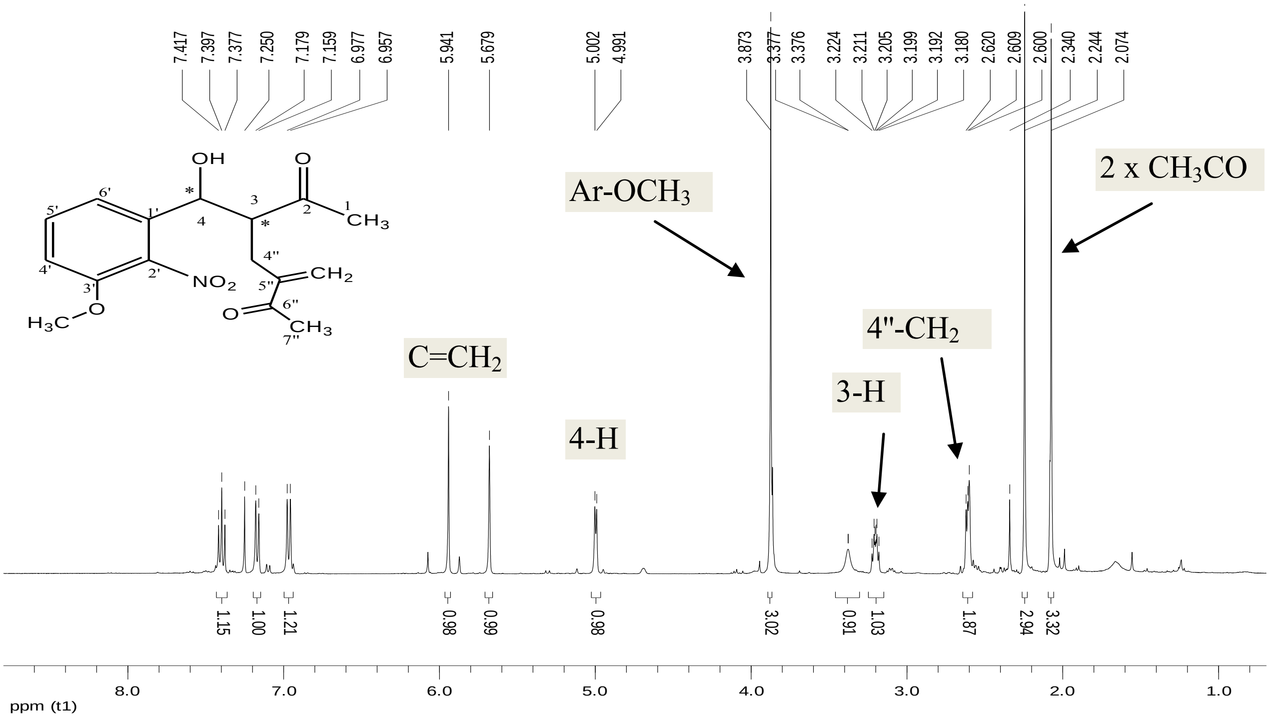


**Figure 2.33.** 400MHz $^1$H NMR spectrum of the bis-MVK adduct, 5-acetyl-6-hydroxy-6-(3-methoxy-2-nitrophenyl)-3-methylene-2-hexanone **97e**$_{syn}$, in CDCl$_3$.

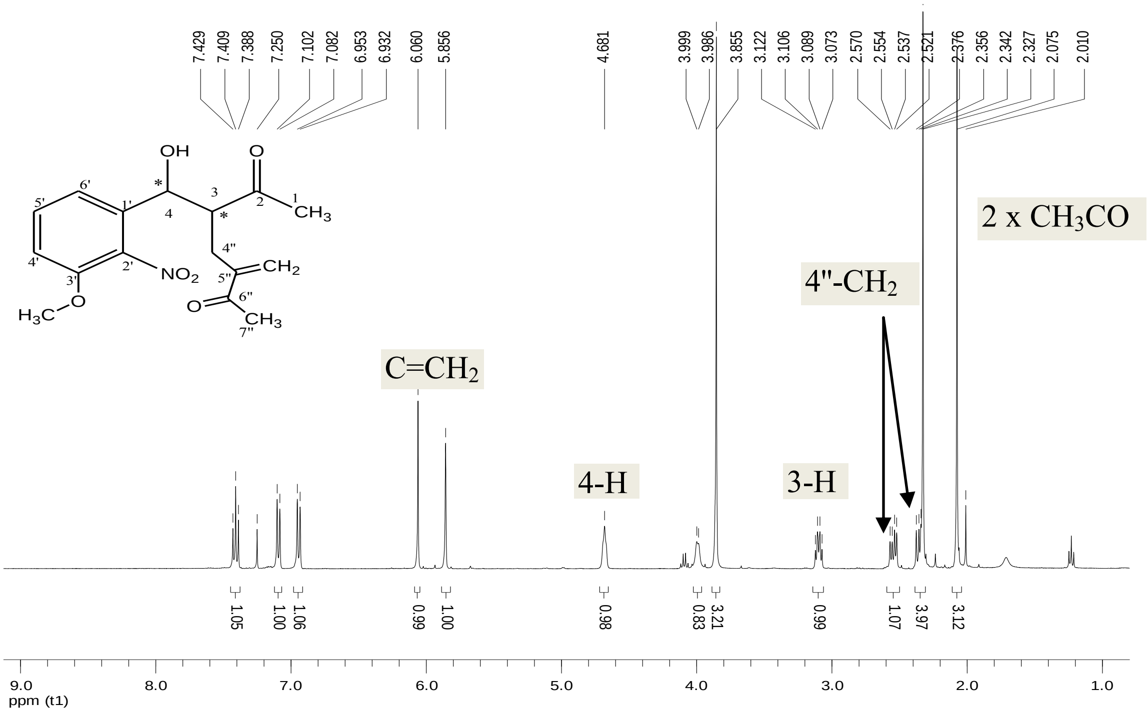


**Figure 2.34.** 400MHz $^{1}$H NMR spectrum of the bis-MVK adduct, 5-acetyl-6-hydroxy-6-(3- methoxy-2- nitrophenyl)-3-methylene-2-hexanone **97e**$_{anti}$, in CDCl$_3$.

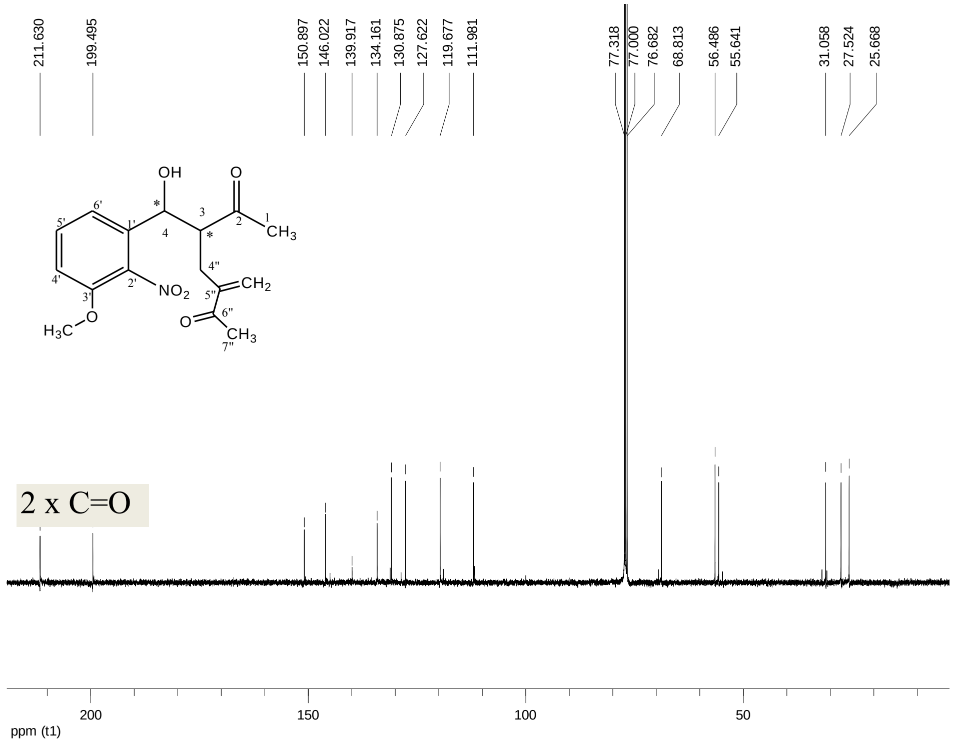


**Figure 2.35.** 100 MHz $^{13}$C NMR spectrum of the bis-MVK adduct, 5-acetyl-6-hydroxy-6-(3-methoxy-2-nitrophenyl)-3-methylene-2-hexanone **97e**$_{syn}$ in CDCl$_3$.

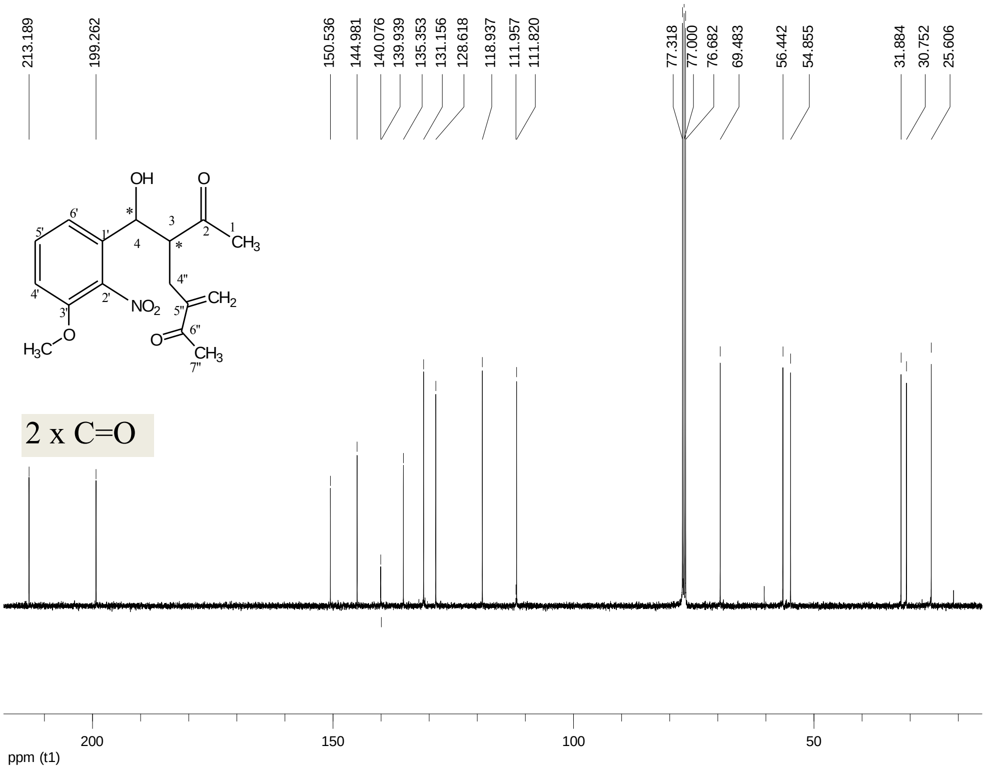


**Figure 2.36.**100MHz $^1$H spectrum of the bis-MVK adduct 5-acetyl-6-hydroxy-6-(3-methoxy- 2-nitrophenyl)-3-methylene-2-hexanone **97e**$_{anti}$, in $CDCl_3$.

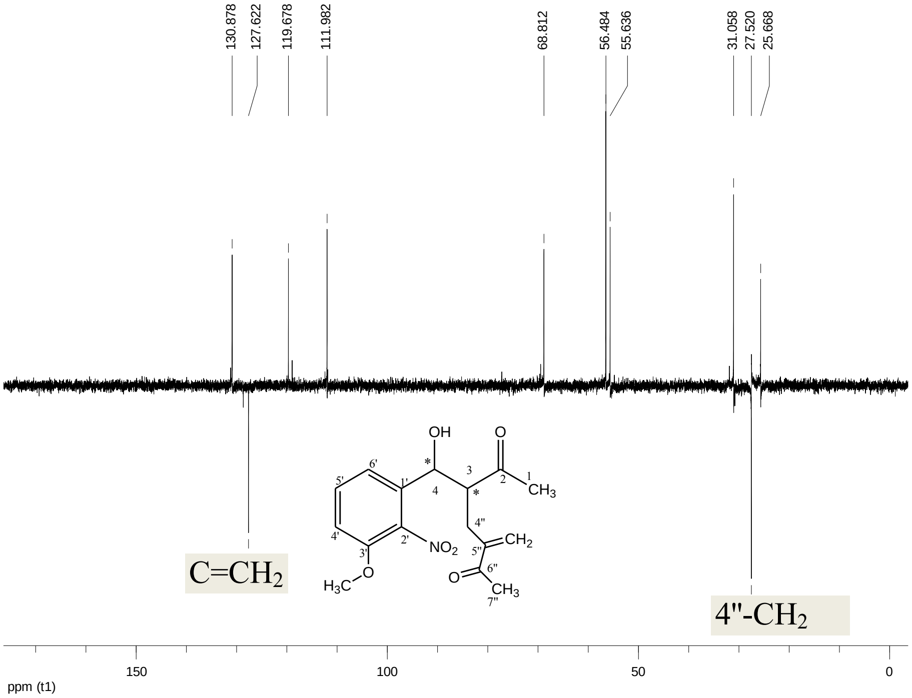


**Figure 2.37.** DEPT 135 NMR spectrum of the bis-MVK adduct 5-acetyl-6-hydroxy-6-(3-methoxy-2-nitrophenyl)-3-methylene-2-hexanone **97e**$_{syn}$, in $CDCl_3$.

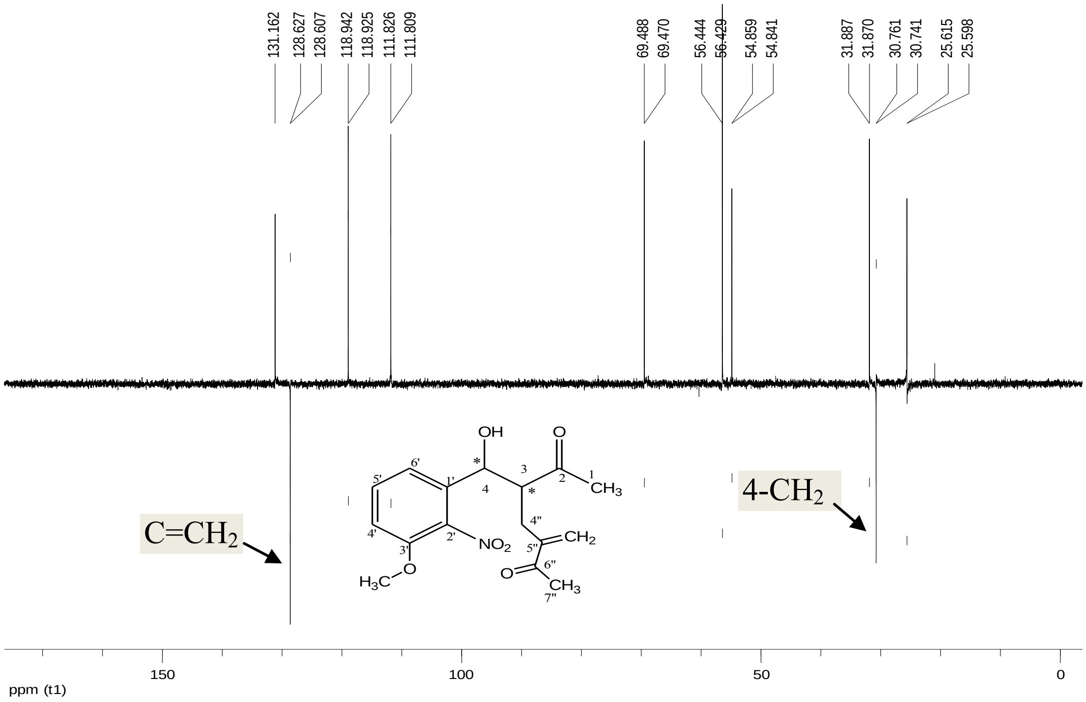
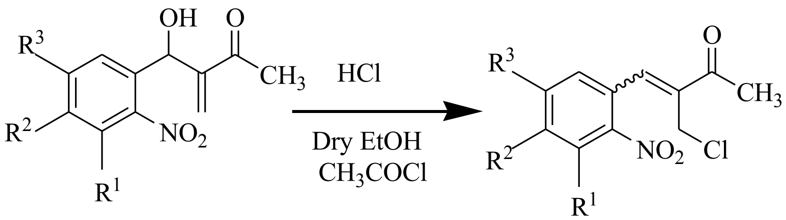


**Figure 2.38.** DEPT 135 NMR spectrum of the bis-MVK adduct, 5-acetyl-6-hydroxy-6-(3-methoxy- 2-nitrophenyl)-3-methylene-2-hexanone **97e**$_{anti}$, in $CDCl_3$.

Since the Baylis-Hillman reaction was the first step in the proposed pathway to the 3-(aminomethyl)quinolines, yield optimization became essential. To this end, the use of two different catalysts was explored (DABCO and 3HQ), along with a slight modification of the solvent system towards more polar solvents in the reactions of compound **82b** and **82d** with MVK **91.** In these two cases, a (1:1) mixture of methanol and chloroform or methanol alone was used as the solvent due to the apparent insolubility of these two substrates in chloroform. Several reports were found,[82-85] including the report by Davies *et al*.,[82] on the effect of solvent on iminium ion-catalysed Baylis-Hillman reactions.[82-85] In our study, the addition of methanol resulted in the dissolution of the reactants, facilitating the reaction, which might otherwise have been slower or even impossible, and affording the products in moderate yield (Table 2.6; (entries 4 and 9). While MeOH-$CHCl_3$ (1:1) was used for **82b**, $CHCl_3$ was used for **82d** in the conventional reaction, but MeOH alone for the microwave-assisted reaction; the yield of **82d** in both cases was low 16 - 22%.

The Baylis-Hillman reaction between the 2-nitrobenzaldehydes **82a-h** and MVK **91** is generally slow and, in an attempt to enhance the reaction rate and perhaps the yield, microwave irradiation was employed in the reactions of two of the substrates, **82a** and **82d.** The typical reaction time, (*ca.* 7 days) under conventional stirring at room temperature, was significantly reduced to 30 minutes and the yields were improved (Table 2.6; entries 3 and 9). When the reaction was carried out under environmentally friendly solvent-free conditions, (Table 2.6; entry 3) a relatively clean product was obtained, resulting in simpler purification step. Replacing DABCO with 3-HQ as catalyst in the reactions of three of the 2-nitrobenzaldehyde **82a-c** with MVK **91** also resulted in a substantial increase in reaction rate resulting in shorter reaction times *ca* 2 days and marginal increase in product yield Table 2.3 (entries 2,5 and 7).

### 2.3.3. Reactions of Baylis-Hillman adducts with HCl.



**92a-h** **93a-h**

| | R$^1$ | R$^2$ | R$^3$ |
|---|---|---|---|
| a | H | H | H |
| b | H | H | OH |
| c | H | -OCH$_2$O- | |
| d | H | OMe | OMe |
| e | OMe | H | H |
| f | H | H | Cl |
| g | H | Cl | H |
| h | H | H | OMe |

**Scheme 2.19.** Reactions of Baylis-Hillman adducts with HCl.

As indicated in the strategic plan (Scheme 2.16), the second step of this study involved hydrohalogenation (hydrochlorination) and dehydration of the BH-derived alcohols **92a-h**. These reactions yielded, exclusively, the rearranged allyl chlorides, with the creation of π-bond facilitating conjugation of the carbonyl group and the aromatic ring. The method reported by Yadav, *et al.* was employed involving treatment of the BH alcohols

**92a-h** with ethanolic HCl generated *in situ.* By adding acetyl chloride cautiously to anhydrous ethanol at 0 °C.[72]

The vinylic group in the substrates **92** is conjugated to the carbonyl group and the terminal vinylic carbon is rendered electron-deficient by the electron-withdrawing effect of the carbonyl group. Acid catalysed conjugate addition of chloride ($Cl^-$) , followed by dehydration, afforded the 3-chloromethyl-4-(2-nitrophenyl)but-3-en-2-ones **93a-h** in essentially quantitative yields (as indicated by NMR analysis) and no further purification was necessary.

The vinylic proton signals characteristic of the substrates **92** (see Figure 2.27) were conspicuously absent in the $^1$H NMR spectrum of the products **93** (see Figure. 2.39); instead, a new proton singlet is evident downfield, towards the aromatic region corresponding to the vinylic proton (4-H). Also, a new signal corresponding to the chloromethylene protons is present at 4.17 ppm, while the methyl signal at 2.31 ppm in the spectrum of the substrate is shifted downfield to 2.51 ppm in the spectrum of the product due, presumably, to deshielding by the extended conjugation involving the aromatic ring and the proximity of the electronegative chloride atom. These changes are confirmations of the transformation that has taken place. The $^{13}$C NMR spectrum of the chloromethyl product **93f** (Figure 2.40) shows the presence of the chloromethylene carbon signal at 37.0 ppm – an assignment confirmed by the DEPT 135 NMR spectrum **93f** (Figure. 2.41). All of the products **93a-h** were fully characterized by IR, NMR and HRMS analysis.

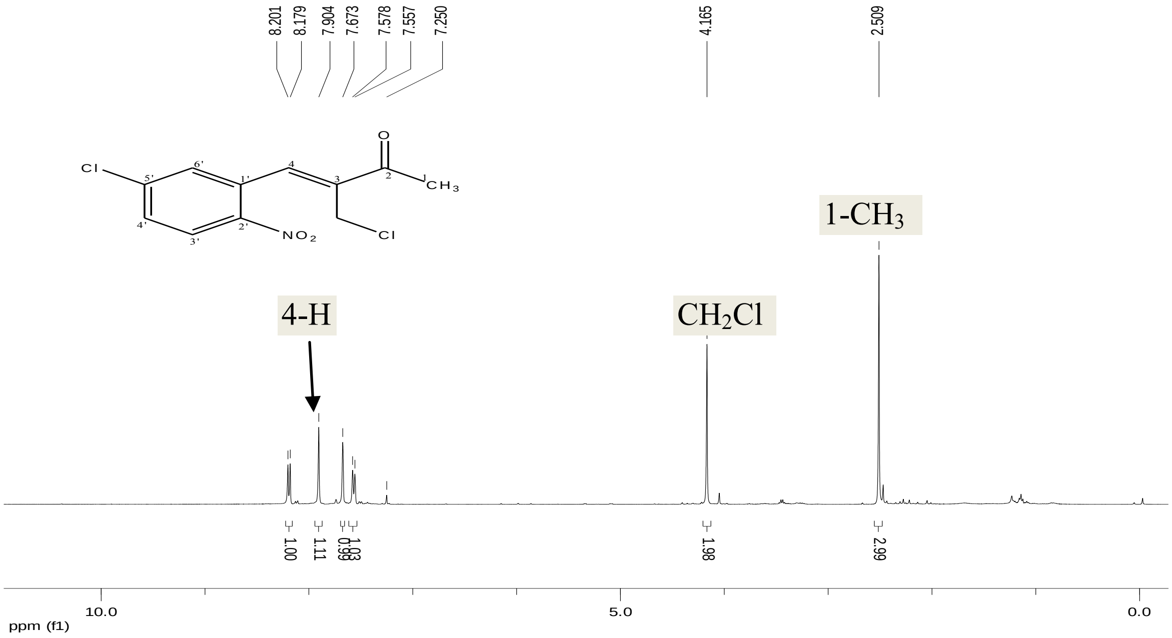


**Figure 2.39.** 400MHz $^1$H NMR spectrum of 3-chloromethyl-4-(5-chloro-2-nitrophenyl)but-3-en-2-one **93f** in $CDCl_3$.

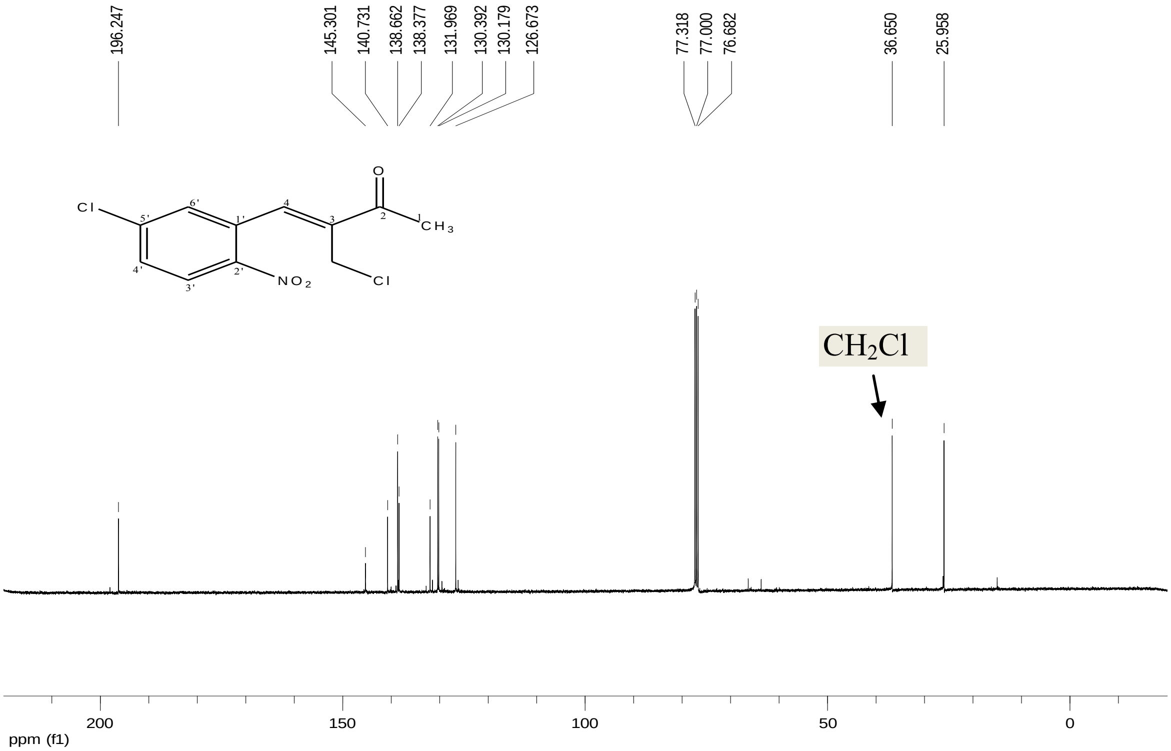


**Figure 2.40.** 100MHz $^{13}$C NMR spectrum of 3-chloromethyl-4-(5-chloro-2-nitrophenyl)but-3-en-2-one **93f** in $CDCl_3$.

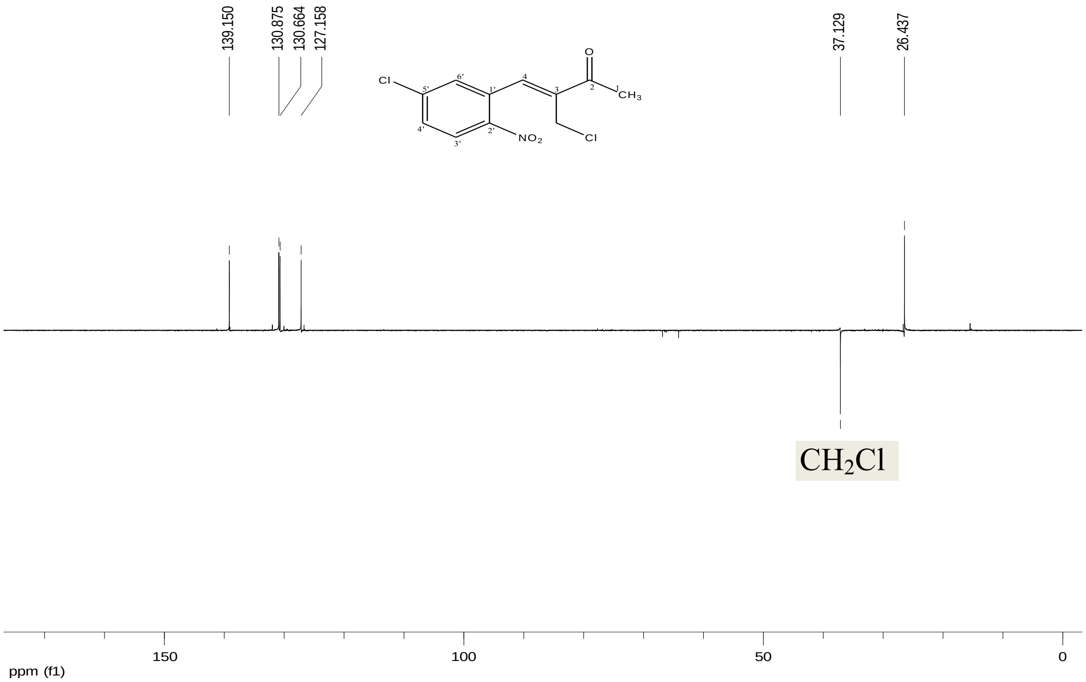


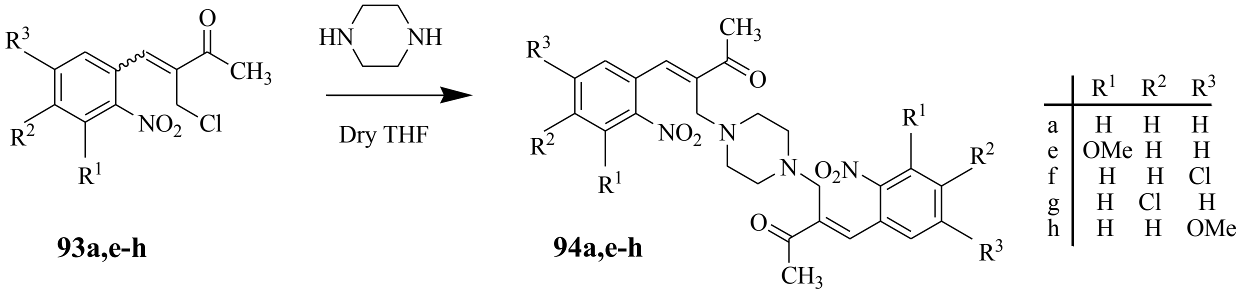
**Figure 2.41.** DEPT 135 NMR spectrum of 3-chloromethyl-4-(5-chloro-2-nitrophenyl)but-3-en-2-one **93f** in $CDCl_3$.

### 2.3.4. Reactions of Baylis-Hillman-derived allyl chlorides.

The chloromethyl derivatives **93a-h**, were treated with various amines including the primary amines, propargylamine and 2-amino-5-diethylaminopentane, and the secondary amines, piperidine and piperazine. Most of the products from these reactions with amines were very difficult to isolate, this problem is reflected in the low yields obtained in some cases, with significant loss of the desired products during the course of purification by chromatography on silica.

#### 2.3.4.1. Reactions with piperazine.

The eight α-chloromethyl α,β-unsaturated ketones **93a-h** were reacted with equimolar quantities of piperazine, using anhydrous THF as solvent. The reactions were terminated after 3-7 days, with the progress of the reaction being monitored by TLC analysis. Excess solvent was removed under reduced pressure to afford the crude products which were flash chromatographed repeatedly on silica gel and concentrated *in vacuo*; trituration of the residue with EtOAc-hexane (1:1) afforded the bis-substituted piperazines **94**.[87] However, only five of the expected products **94a,e-h,** were isolated, and then in very low yields (Table 2.7) due to the multiple purification steps required to obtain the compounds, which were fully characterized.



| | R1 | R2 | R3 |
|---|---|---|---|
| a | H | H | H |
| e | OMe | H | H |
| f | H | H | Cl |
| g | H | Cl | H |
| h | H | H | OMe |

**Scheme 2.20.** Reactions of the chloromethyl α,β-unsaturated ketones with piperazine.

**Table 2.7.** Yields of disubstituted piperazine **94a,e-h.**

| Piperazine products | R$^1$ | R$^2$ | R$^3$ | YIELD |
|---|---|---|---|---|
| **94a** | H | H | H | 19% |
| **94e** | OMe | H | H | 22% |
| **94f** | H | H | Cl | 13% |
| **94g** | H | Cl | H | 9% |
| **94h** | H | H | OMe | >5% |

The $^1$H NMR spectrum of the parent system **94a,** illustrated in Figure 2.42, reveals a broad singlet at 2.01 ppm for the eight piperazine methylene protons. Another singlet at 2.45 ppm corresponds to the two methyl groups and the singlet at 3.04 ppm to the two aminomethyl groups. The integrals for each of the aromatic proton signals are also multiplied by a factor of two to account for the ten protons resonating in the aromatic region. The $^{13}$C NMR spectrum (Figure 2.43) shows the expected 12 carbon signals with the 2- and 2'-aminomethyl signal almost overlapping the piperazine methyl signal, the assignment, being confirmed by the DEPT 135 spectrum (Figure 2.44). The piperazine methylene protons are all equivalent, due to the symmetry of the system.

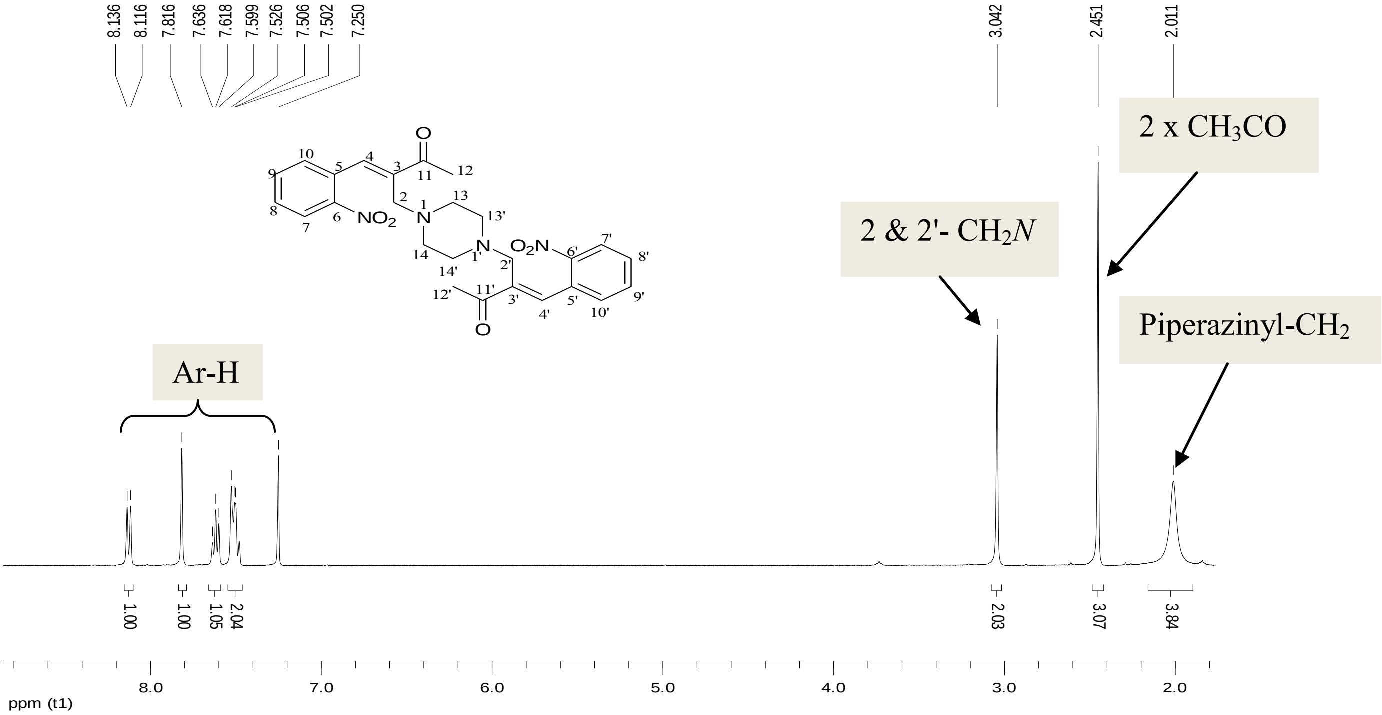


**Figure 2.42.** 400MHz $^1$H NMR spectrum of *N*,*N'*-bis[(*E*)-2-acetyl-3-(2-nitrophenyl)-2-propenyl]-1,4- piperazine **94a** in $CDCl_3$.

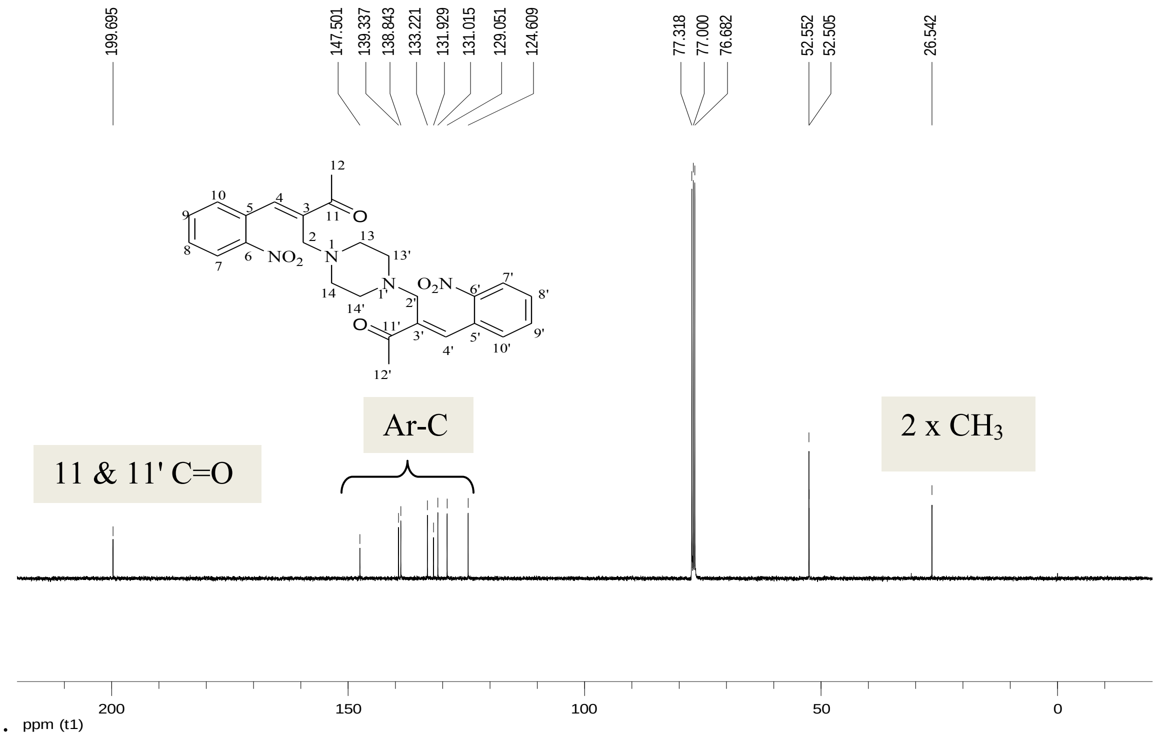


**Figure 2.43.** 100MHz$^{13}$C NMR spectrum of *N*,*N*'-bis[(*E*)-2-acetyl-3-(2-nitrophenyl)-2-propenyl]-1,4-piperazine **94a** in $CDCl_3$.

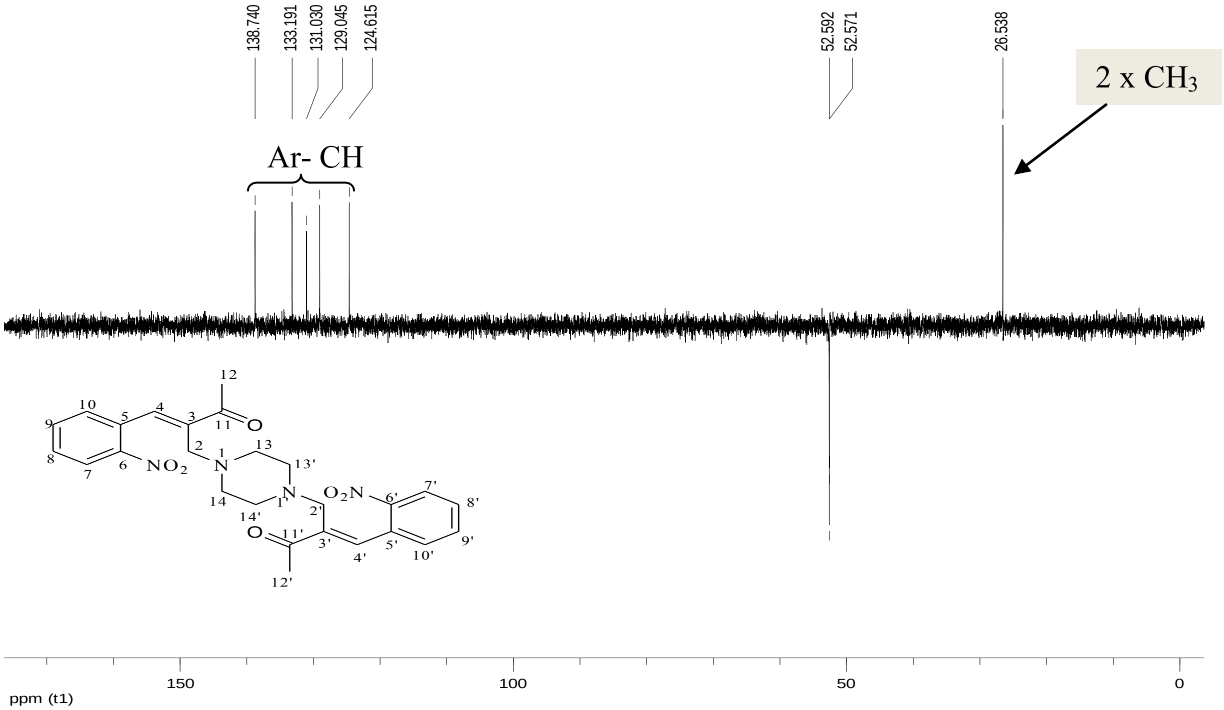


**Figure 2.44.** DEPT 135 spectrum of *N*,*N*'-bis[(*E*)-2-acetyl-3-(2-nitrophenyl)-2-propenyl]-1,4-piperazine **94a** in $CDCl_3$.

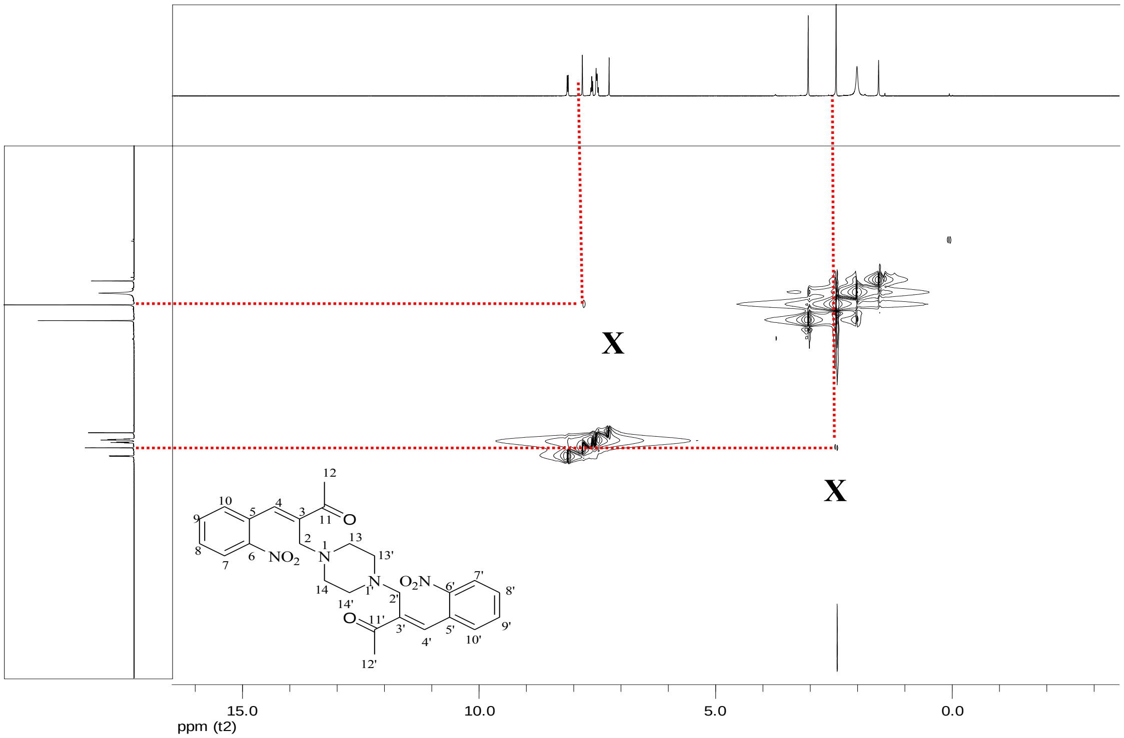


**Figure 2.45.** NOESY spectrum of *N*,*N*'-bis[(*E*)-2-acetyl-3-(2-nitrophenyl)-2- propenyl] -1,4-piperazine **94a** in CDCl$_3$, showing NOE interactions (see Figure 2.46).

In attempting to resolve the configuration about the exocyclic double bond in this series of compounds **94a**,**e-h** and, by extension, that of their α-chloromethyl α,β-unsaturated ketone precursors **93a**,**e-h**, several methods were examined. These included running fully-coupled $^{13}$C NMR experiments on some members of the series, to establish the magnitude of any long-range three-bond ($^3J_{\text{H-C=O}}$) interactions between the carbonyl carbon and the vinylic proton – an approach that has been used to determine configuration in similar system.[110] These experiments gave very complex spectra, which could not be adequately resolved due to further coupling with 2- and 2'-methylene protons. 2D Heteronuclear NOE (HOESY) NMR experiments[88] were also conducted on three members of the series; this again failed to reveal appreciable evidence of long-range interactions. However, the NOESY spectra of all five compounds **94a,e-h** consistently revealed NOE interactions between the methyl and vinylic protons. This is illustrated in the NOESY spectrum of the parent compound **94a** (Figure 2.45), which shows NOE interactions (marked X) between the methyl protons and the vinylic 4- and 4'- protons.

These observations required assignment[89] of an (*E*)-configuration to each of these compounds, **94a,e-h** and, by extension, the (*Z)*-configuration to their α-chloromethyl precursors **93a,e-h**. This assignment was consistent with the lower energy of the arrangements for the (*E*) configuration **94f** [compared to (*Z*)] as determined by Gaussian03 geometry optimization at the B3LYP/6-31G(d) level,[90] (Figure 2.46). Finally, single crystal X-ray analysis of the parent compound **94a (**Figure 2.47) unequivocally confirmed these previous theoretical and spectroscopic data. The synthesis and configurational assignment of these piperazine derivatives and their α-chloromethyl α,β-unsaturated ketone precursor has been published.[87]

The compounds in this series, possess two interesting properties, being diketo aryl (DKA) analogues, such as 5-CITEP[91]as well as having $C_2$-symmetry.[92] Their property as a DKA analogues gives them the potential of being applied as HIV-1 Integrase inhibitors; while their $C_2$ symmetric nature afford them the potential of being $C_2$ symmetric inhibitors of HIV-1 protease.

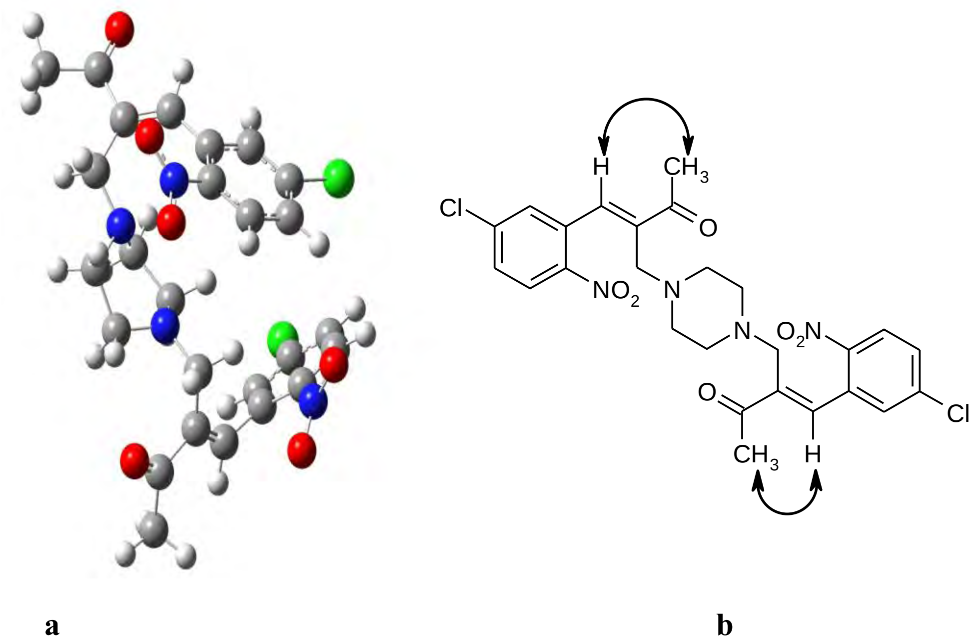


**Figure 2.46.** (**a**) Gaussian03 geometry optimized structure of compound **94f**. (**b**) Line structure illustrating NOE interactions.

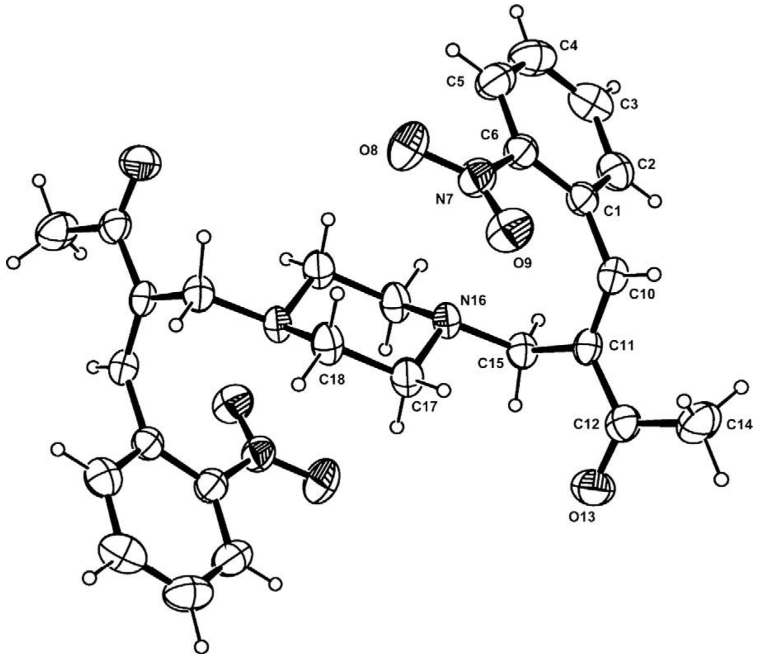
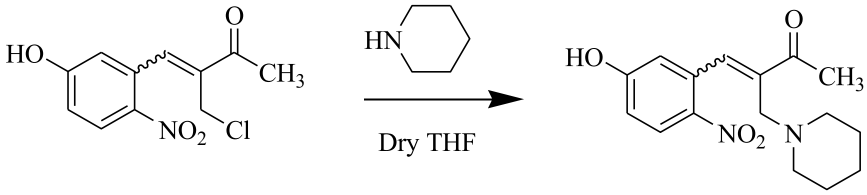


**Figure 2.47.** X-ray structure of N,N-bis[(*E*)-2-acetyl-3-(2-nitrophenyl)-2-propenyl]piperazine **94a**, showing the crystallographic numbering and the double bond configuration. [86]

### 2.3.4.2. Reaction with piperidine.

The chloromethyl α,β-unsaturated ketones **93a-h** were also reacted with piperidine in an attempt to obtain the corresponding aminomethyl derivatives as precursors for the 3-aminomethylquinolines, targeted as potential antimalarials. All eight chloromethyl derivatives **93a-h** were reacted with piperidine in anhydrous THF for 3-7 days. However, as experienced with the piperazine derivatives earlier, isolation of products proved to be very challenging, since the amino products showed a great affinity for silica, particularly when protonated by the released HCl to afford the hydrochloride salts. The crude product mixtures were therefore neutralized as part of the work-up procedure, by washing with saturated aqueous $Na_2CO_3$, followed by brine to remove any residual $Na_2CO_3$. Unfortunately, neutralization of crude material, failed to resolve the separation problem.



**93b**

**95b**

**Scheme 2.21.** Reaction of the chloromethyl α,β-unsaturated ketones **93b** with piperidine.

However, after several attempts, (*E*-3-(5-hydroxy-2-nitrophenyl)-2-(piperidin-1-ylmethyl)-but-3-en-1-one, **95b** was isolated at 23% yield (Scheme 2.21). The $^1$H NMR spectrum of compound **95b** (Figure 2.48) reveals a broad signal at *ca*. 1.27 ppm accounting for the three pairs of piperidnyl methylene protons, the remaining two pairs resonate as another broad signal at 2.08 ppm. The methyl singlet appears at 2.40 ppm; and the aminomethyl singlet at 3.06 ppm. The $^{13}$C spectrum (Figure 2.49) shows two signals for three of the piperidnyl methylene carbons at *ca*. 22.0 ppm, the methyl carbon signal at 26.1 ppm, the signal for the two piperidnyl aminomethylene carbons at 43.5 ppm, and the aminomethylene carbon signal at 52.6 ppm. The eight aromatic carbon signals appear between 116.4 and 163.3 ppm, while the carbonyl signal is revealed at 199.1 ppm. Other aminomethyl derivatives (such as those that would have been **60c**,**d**, and **e**) were equally subjected to the above dehydration protocol. $^1$H NMR analysis of the crude mixture indicated the formation of products, however, separation of crude mixture on silica gel column chromatography, gave fractions, $^1$H NMR analysis of which indicated that none of them corresponds to the expected products.

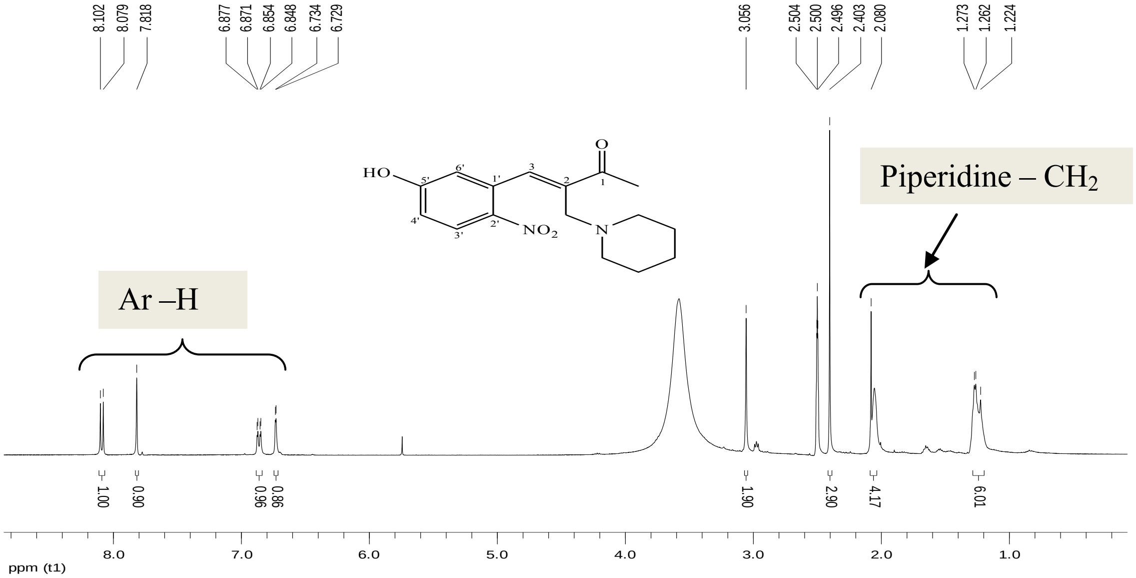


**Figure 2.48.** 400MHz $^1$H NMR spectrum of (*E*)-3-(5-hydroxy-2-nitrophenyl)-2-(piperidin-1-ylmethyl)-but-3-en-1-one, **95b** in DMSO-$d_6$.

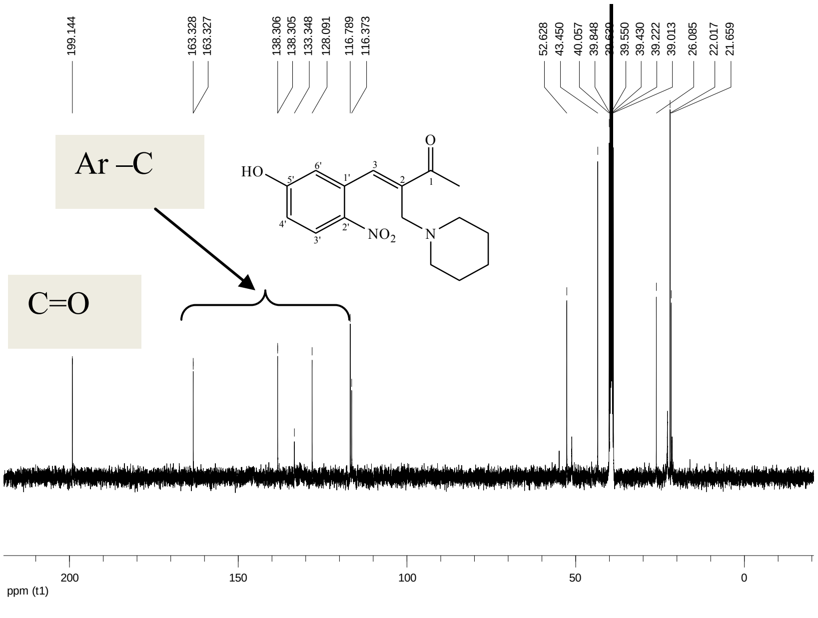
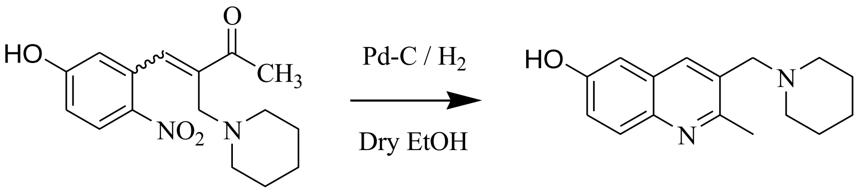


**Figure 2.49.** 100MHz $^{13}$C NMR spectrum of (*E*) -3-(5-hydroxy-2-nitrophenyl)-2-(piperidin-1-ylmethyl)-but-3-en-1-one, **95b**, in DMSO-$d_6$.

### 2.3.4.3 Reductive cyclization of the piperidine derivative 98b.



**98b** **99b**

**Scheme 2.22.** Reductive cyclization to the quinoline derivative **99b**.

As indicated in Scheme 2.16, the concluding step in the synthesis of potential antimalarial compounds requires reductive cyclization. This involves a number of concerted reactions, *viz*., reduction of the *ortho*-nitro group to a nucleophilic *ortho*-amino group, which can then attack the electrophilic carbonyl carbon and thus effect cyclization; susbsequent dehydration then leads to the target 3-aminomethylquinoline derivatives. Compound **98b** was dissolved in ethanol, and then hydrogenated using a 10% palladium on activated carbon (10% Pd-C) catalyst. The reactions were terminated after 3.0 hours, and the crude material was filtered through a bed of Celite to remove excess catalyst. Concentration of the filtrate under reduced pressure, afforded 6-hydroxy-2-methyl-3-[(piperidine-1-yl)methyl]quinoline **99b**, in 55% yield. The $^1$H NMR spectrum of compound **99b** (Figure 2.50) reveals two multiplets at *ca*.1.6 ppm for three pairs of the piperidinyl methylene protons, a singlet at 2.35 ppm for the methyl group attached to the quinoline ring, and a multiplet for the three pairs of *N*-methylene protons at 2.89 ppm. The signals of the four aromatic protons are seen, resonating between 7.01 and 7.79 ppm.

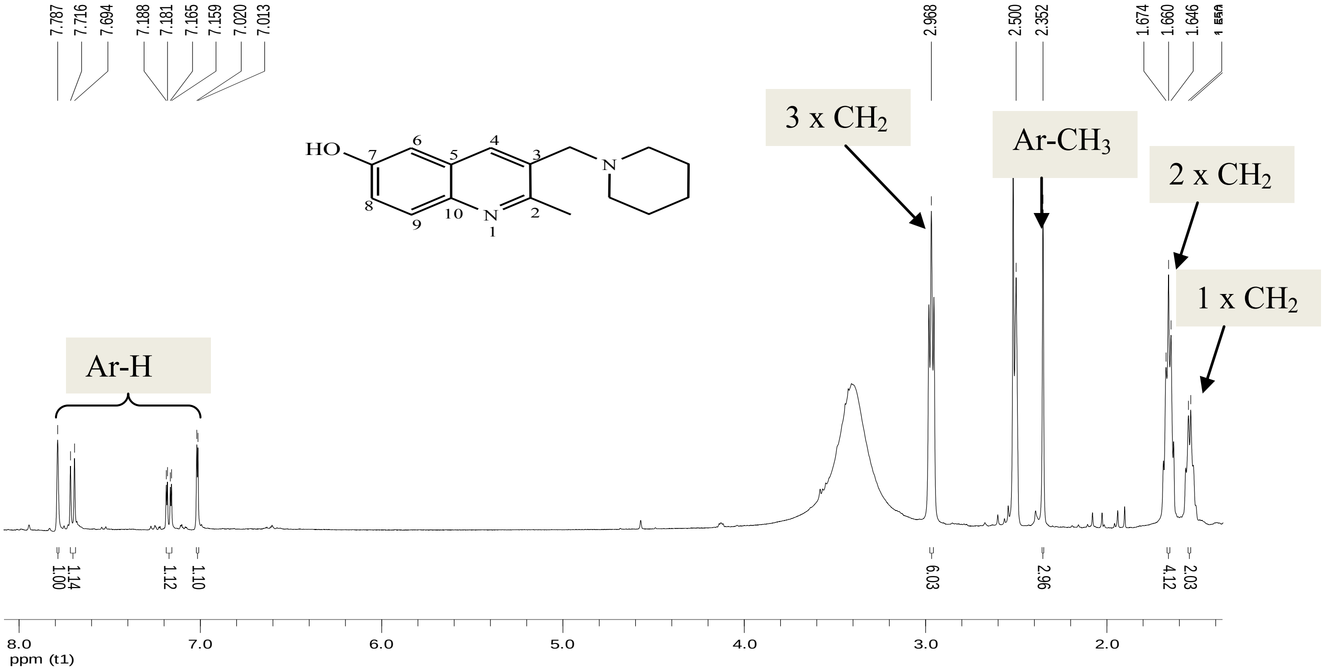


**Figure 2.50.** 400MHz $^1$H NMR spectrum of 6-hydroxy-2-methyl-3-(piperidin-1-ylmethyl)quinoline **99b** in DMSO-$d_6$.

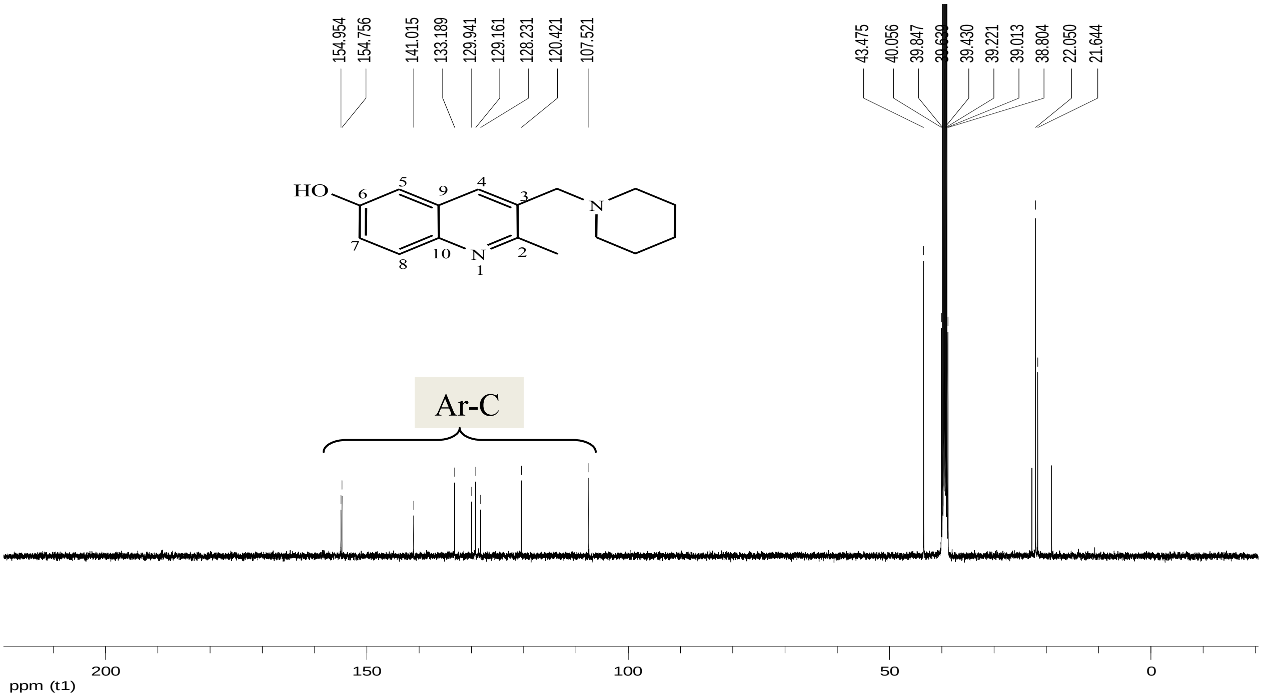


**Figure 2.51.**100 MHz $^{13}$C NMR spectrum of 6-hydroxy-2-methyl-3-[(piperidin-1-yl)methyl]quinoline **99b** in DMSO-$d_6$.

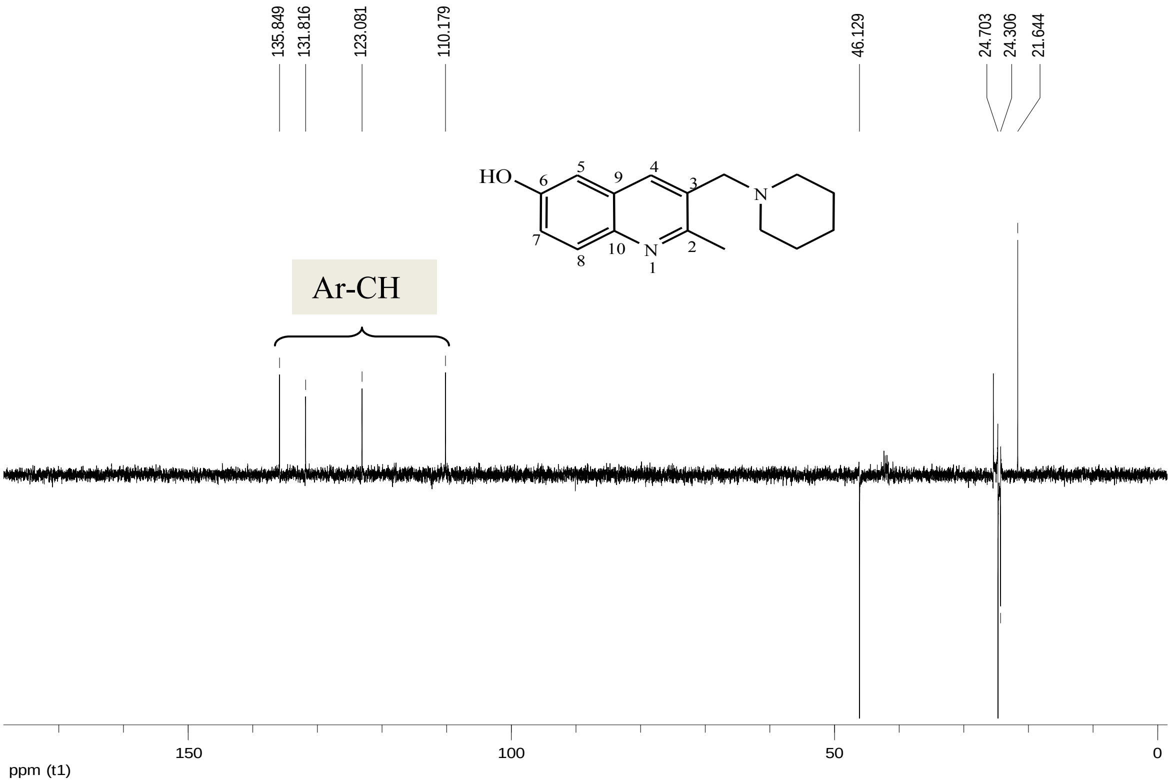


**Figure 2.52**. DEPT 135 spectrum of 6-hydroxy-2-methyl-3-[(piperidin-1-yl)-methyl]quinoline **99b** in DMSO-$d_6$.

One significant feature in the $^{13}$C spectrum of compound **99b** (Figure 2.51) is the absence of the carbonyl signal, which is consistent with reductive cyclization. Five quaternary and four methine (total of nine) carbon signals are revealed in the aromatic region of compound **99b**. The DEPT 135 spectrum (Figure 2.52) confirms the presence of four aromatic methine carbon signals and three methylene carbon signals between 24.3 and 46.1 ppm, accounting for the three aminomethylene groups. A correlation of interest in the COSY spectrum (Figure 2.53), is the long-range methyl proton interaction (marked **X**), with the aromatic proton (4-H), indicating that this methyl group is bonded to the quinoline ring. Furthermore, another interaction is evident in the HMBC spectrum between the methyl protons and a quaternary carbon (marked Y, Figure 2.55) in the aromatic region. As evidenced by the 2-D NMR data illustrated below, (Figure 2.53 - 2.55), the quinoline derivative **99b** was isolated in remarkable purity before chromatography.

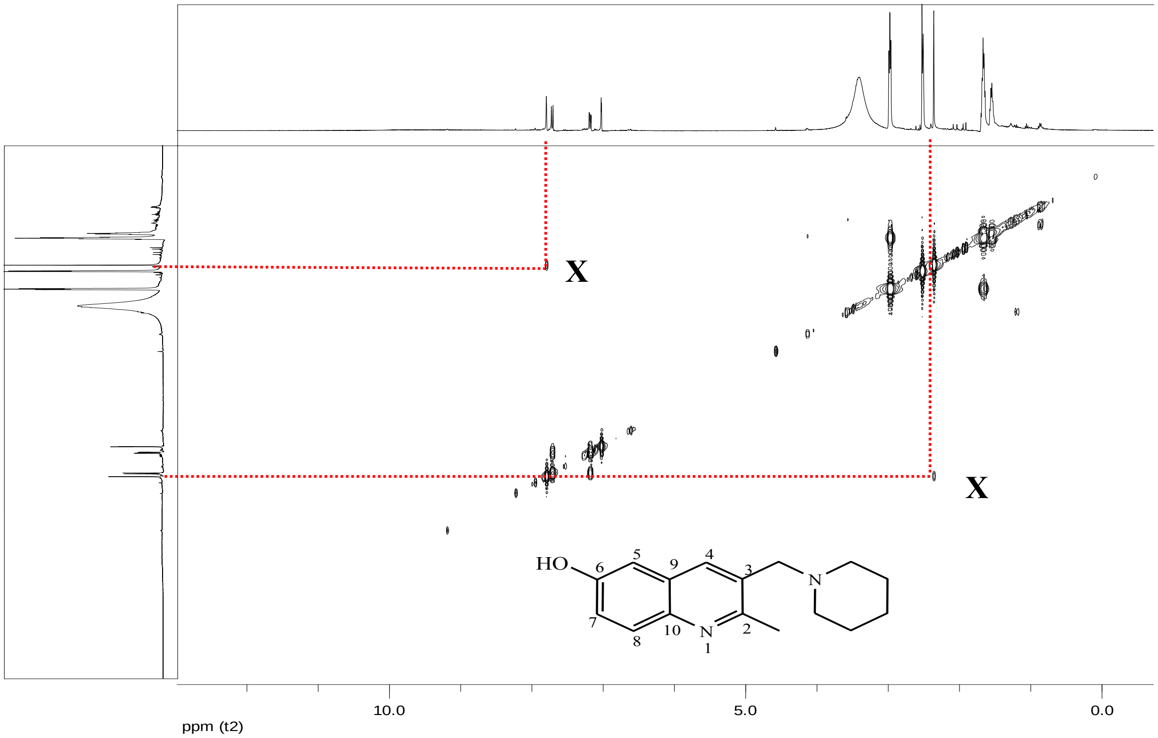


**Figure 2.53**. COSY spectrum of 6-hydroxy-2-methyl-3-(piperidin-1- ylmethyl)quinoline **99b** in DMSO-$d_6$.

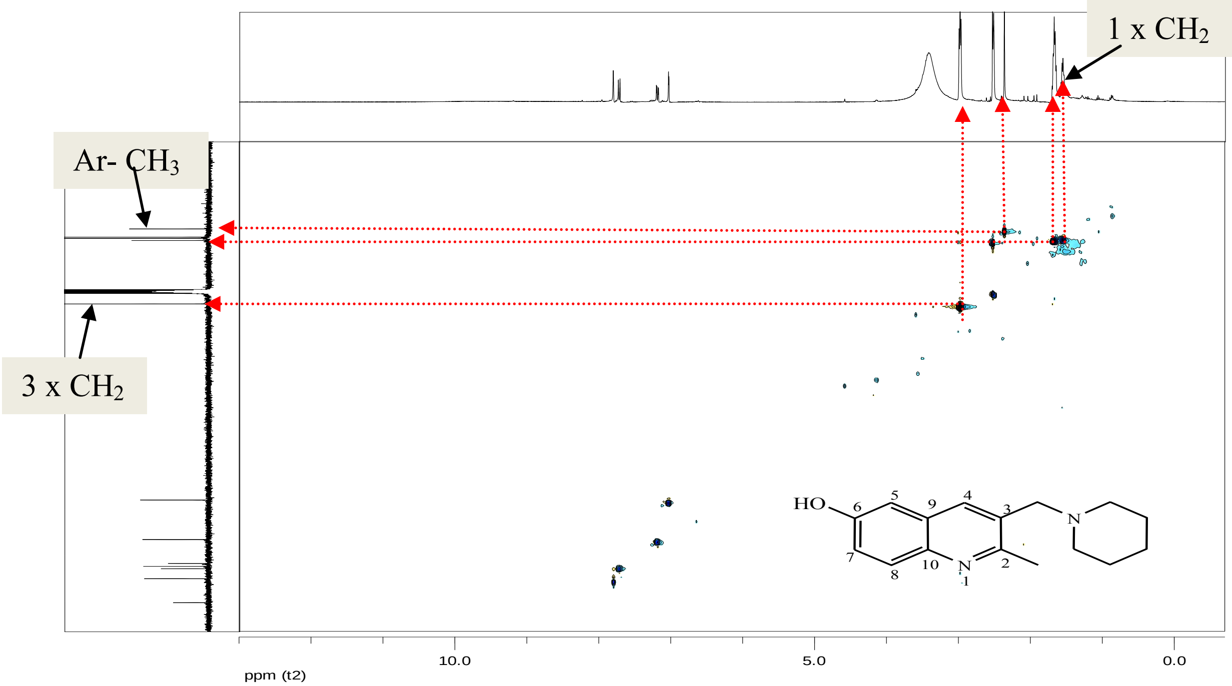


**Figure 2.54.**HSQC spectrum of 6-hydroxy-2-methyl-3-[(piperidin-1-yl)methyl]quinoline **99b** in DMSO-$d_6$.

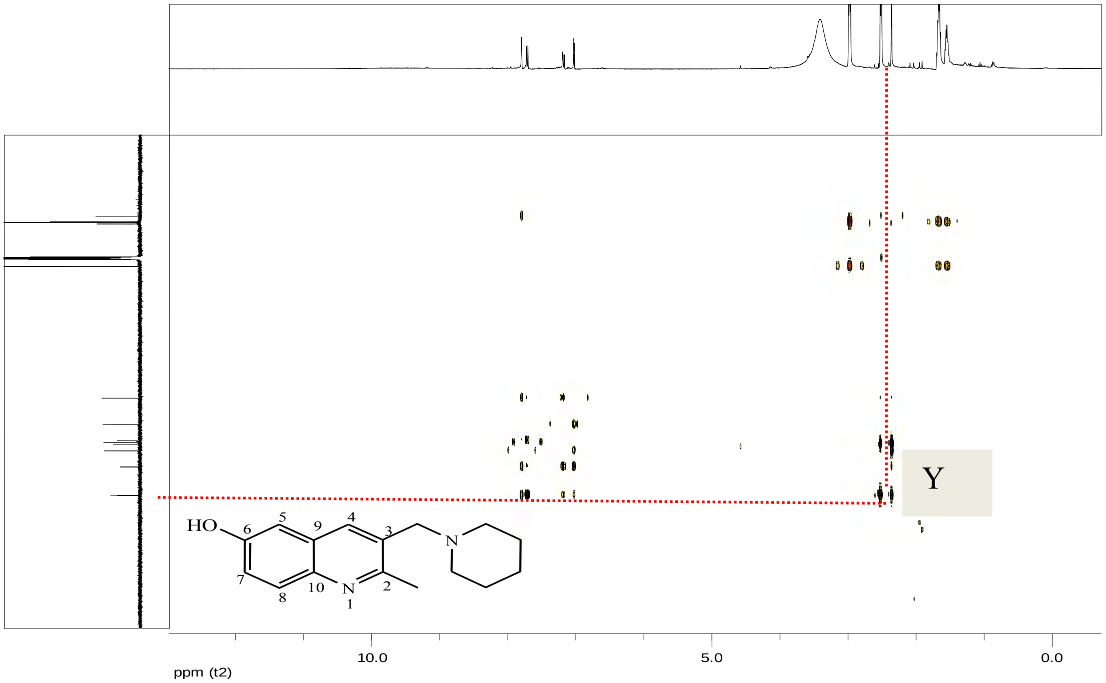
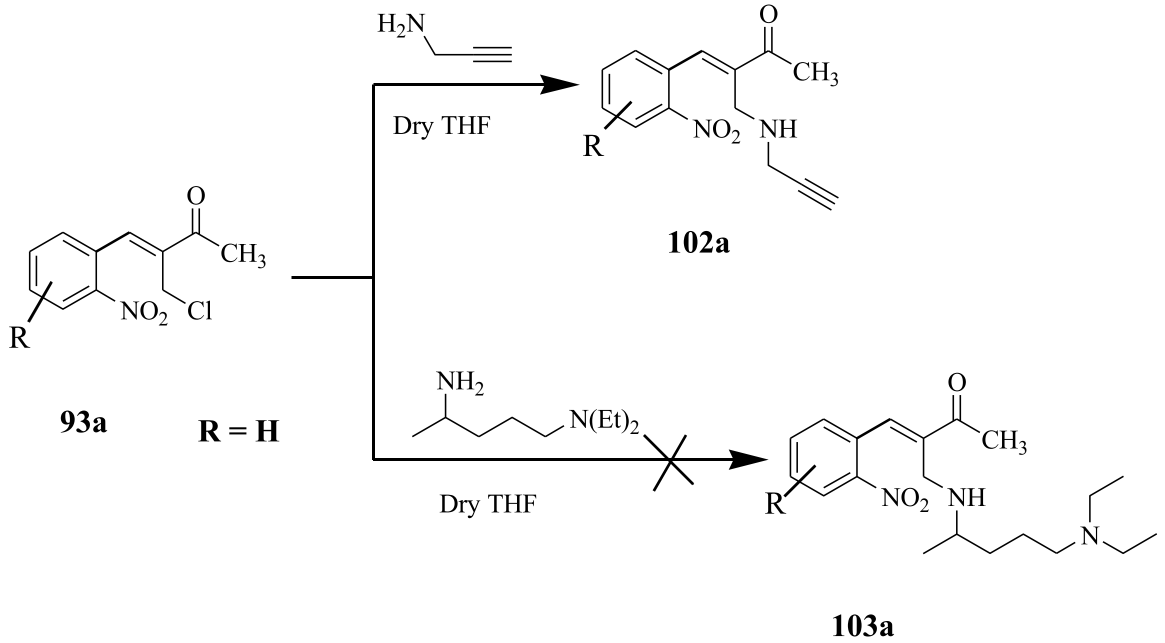


**Figure 2.55**. HMBC spectrum of 6-hydroxy-2-methyl-3-[(piperidin-1-yl)-methyl]quinoline **99b** in DMSO-$d_6$.

### 2.3.4.4. Reaction with primary amines.



**Scheme 2.23.** Reactions of the chloromethyl α,β-unsaturated ketones with primary amines.

As indicated in Scheme 2.23, the chloromethyl derivative **93a,** was treated with two primary amines, propargylamine and 2-amino-5-(diethylamino)pentane (2-A-5-DEAP; (the chloroquine side chain). These reactions were intended to afford two opportunities namely, (i) access to ketone-AZT conjugates and (ii) 3-aminomethylquinoline analogue of chloroquine, the potent antimalarial. The chloromethyl derivative, **93a** was treated with two molar equivalents of each of these primary amines in anhydrous THF for a period of 3-7 days. The reactions were terminated, excess solvent evaporated *in vacuo* and $^1$H and $^{13}$C NMR spectra of the crude material were acquired. Most of these spectra failed to indicate unambiguously the presence of the desired product signals, as only a broad "hump" was seen in the aromatic region, while the upfield signals sugggested a complex mixture. Several attempts to purify these crude mixtures by neutralization with saturated aqueous $Na_2CO_3$, followed by washing with brine, failed to yield any isolable product. While several attempts to react the chloromethyl derivative **93a** with 2-amino-5-

(diethylamino)pentane failed to produce any isolable product, further reactions with propargylamine finally, afforded 4-(2-nitrophenyl)-3-[(2-propenyl)methyl]but-3-en-2-one **102a,** in less than 5% yield. This product was isolated after several purification steps involving normal and reverse phase chromatography, semi-preparative HPLC and trituration. The $^1$H NMR spectrum of 4-(2-nitrophenyl)-3-[(2-propenyl)methyl]but-3-en-2-one **102a** reveals a signal at 1.41 ppm for the terminal alkyne proton, while the DEPT 135 spectrum (Figure 2.56) reveals positive signals for both the terminal and non-terminal alkyne carbons due to $^1J_{CH}$ coupling with the terminal alkyne proton – a phenomenom discussed earlier and clearly illustrated in the HSQC spectrum (Figure 2.57).

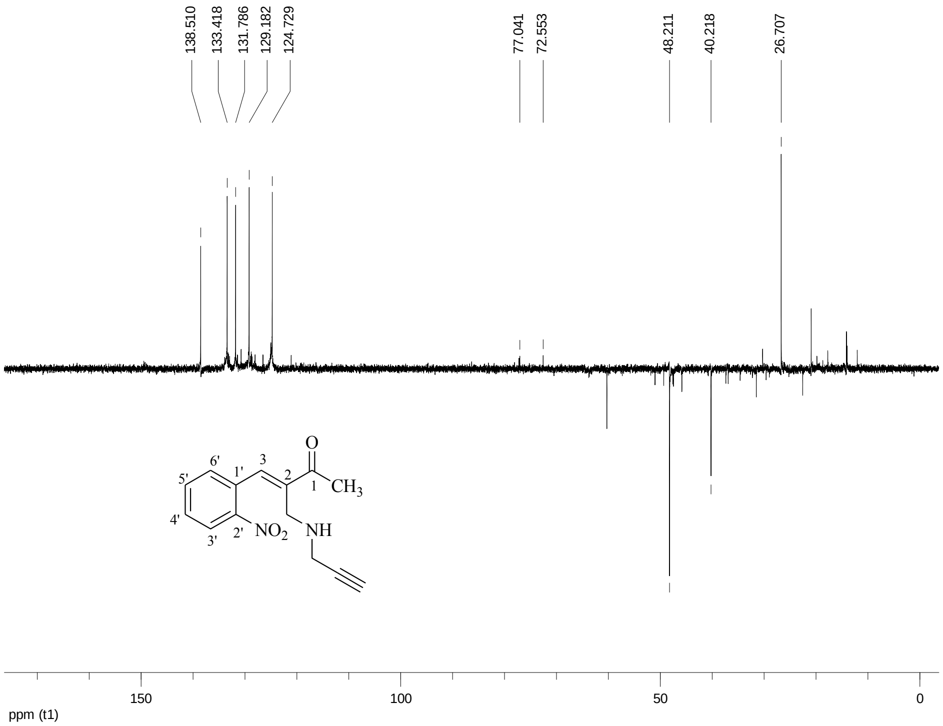


**Figure 2.56**. DEPT 135 spectrum of 4-(2-nitrophenyl)-3-[(2-propynylamino)methyl]but-3-en-2-one **102a** in $CDCl_3$.

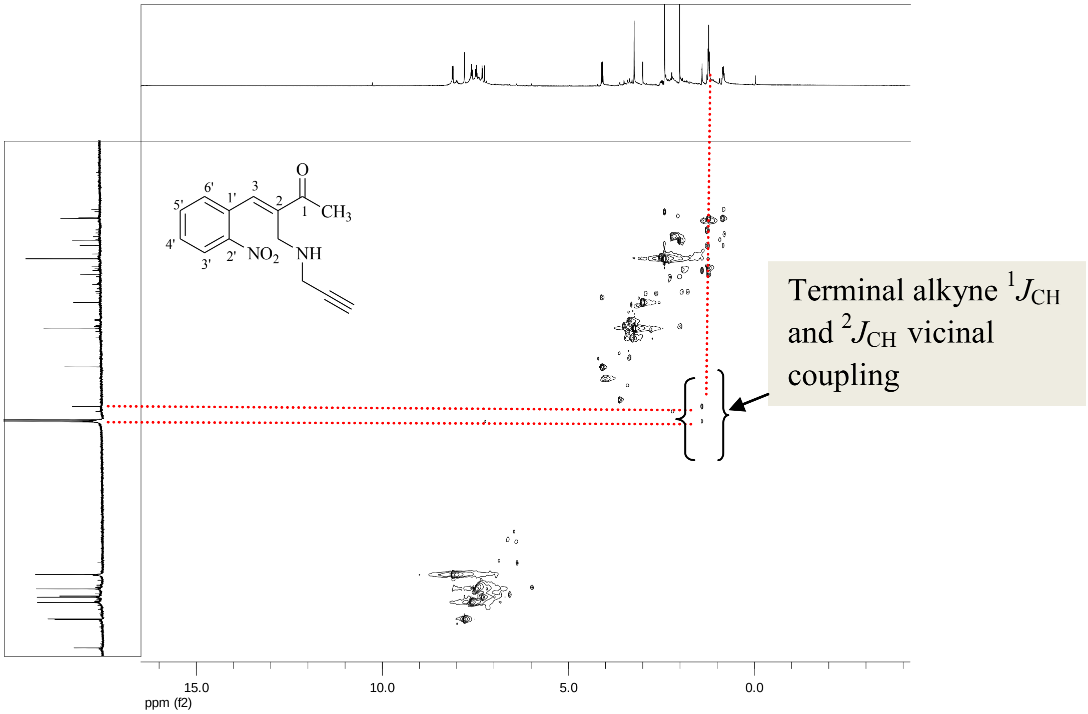


**Figure 2.57**. HSQC spectrum of 4-(2-nitrophenyl)-3-[(2-propynylamino)methyl]but-3-en-2-one **102a,** in $CDCl_3$.

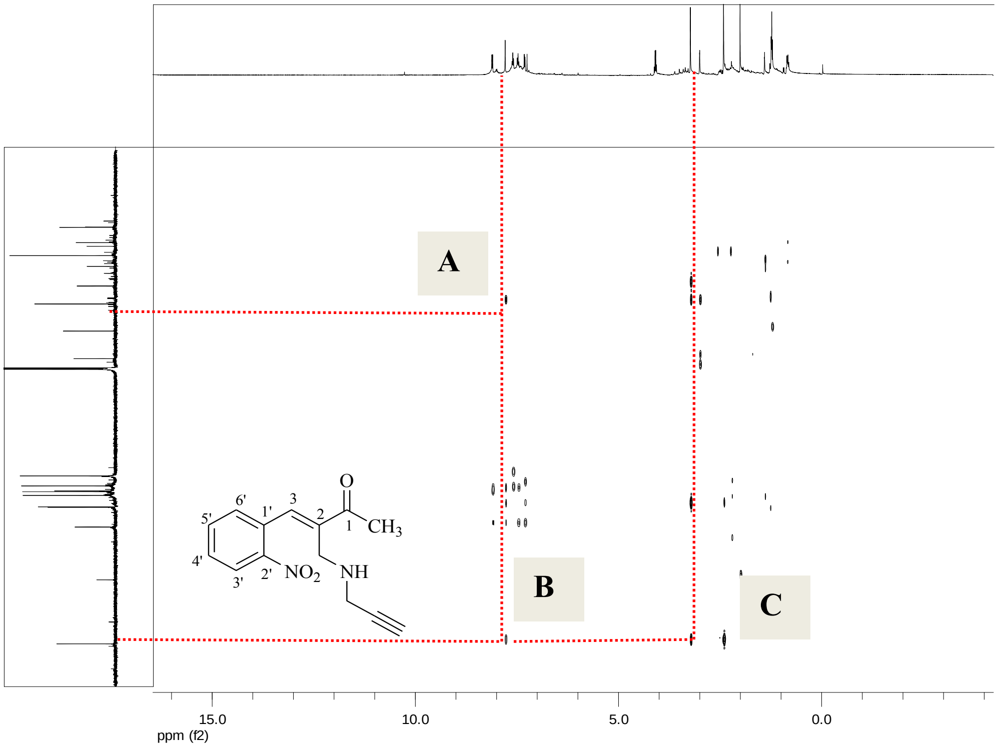
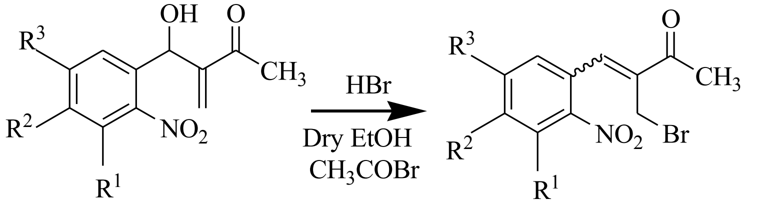


**Figure 2.58**. HMBC spectrum of 4-(2-nitrophenyl)-3-[(2-propynylamino)methyl]but-3-en-2-one **102a,** in $CDCl_3$.

Further confirmation of the structure is provided by the HMBC spectrum (Figure 2.58), showing long-range interaction marked **A**,**B** and **C**. Mark **A** shows the long-range coupling between the vinylic proton (3-H) and the methylene carbon, **B** the coupling between the same vinylic proton and the carbonyl carbon, and **C** long-range coupling between the carbonyl carbon and the methylene protons. Due to very low yield and time constraints, it was not possible to carry out further reaction with this product as earlier planned.

## 2.3.5. Reaction of Baylis-Hillman adducts with HBr.

Re-examining our approach to the 3-aminoquinolines, in view of all the above-mentioned difficulties, and with a firm determination to access our targeted 3-aminomethylquinolines, we reasoned that the bromomethyl derivatives **104** (Scheme 2.24) would be more reactive than the chloromethyl analogues **93**, towards nucleophilic primary and secondary amines, the bromide anion being a better leaving group than chloride in substitution reaction.[93] To this end, acetyl bromide was used to generate the bromomethyl derivatives as indicated in Scheme 2.24.



**92a,f** **104a, f**

| | $R^1$ | $R^2$ | $R^3$ |
|---|---|---|---|
| a | H | H | H |
| f | H | H | Cl |

**Scheme 2.24.** Reaction of Baylis-Hillman adducts with HBr.

Adapting the method reported by Yadav *et al.*, [72] the adducts **92a**,**f** were treated with ethanolic HBr generated *in situ.* by adding acetyl bromide cautiously to anhydrous ethanol at 0 °C Work-up and chromatography afforded the allyl bromides **104a,f** in moderate yields. The $^1H$ NMR spectrum of 3-bromomethyl-4-(2-nitrophenyl)but-3-en-2-one **104a** (Figure 2.59) reveals the methyl singlet at 2.54 ppm and the methylene protons as a singlet at 4.08 ppm. All 11 carbons signals expected for the compound are revealed in the $^{13}$C NMR spectrum (Figure 2.60), with the carbonyl signal at 196.6 ppm and the signals of the eight aromatic carbons resonating between 125.3 and 147.1 ppm. The DEPT 135 spectrum (Figure 2.61) confirms these signal assignments and also reveals the methylene and the methyl carbon signals at 24.4 and 26.5 ppm, respectively.

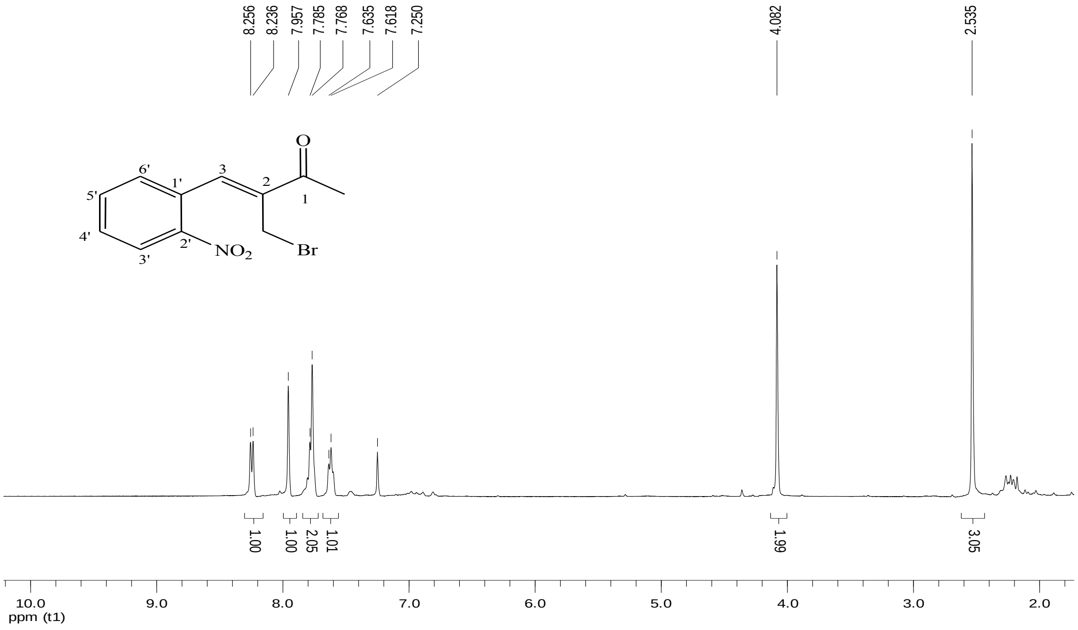


**Figure 2.59.** 400 MHz $^1$H NMR spectrum of 3-bromomethyl-4-(2-nitrophenyl)but-3-en-2-one **104a** in CDCl$_3$.

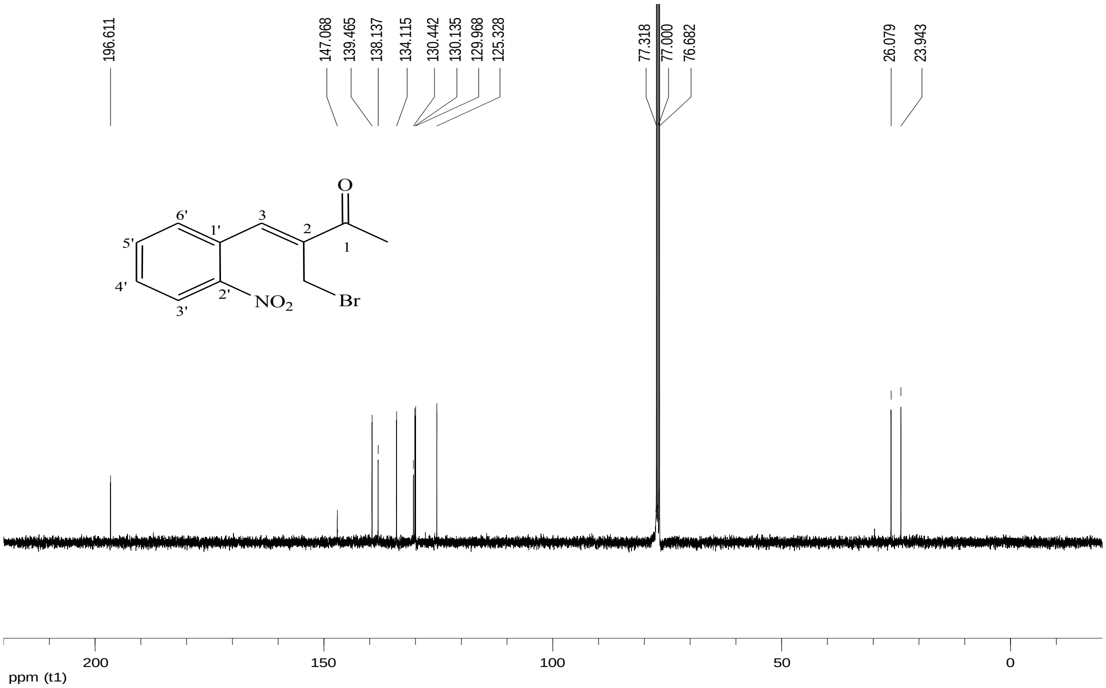


**Figure 2.60.** 100 MHz$^{13}$C NMR spectrum of 3-bromomethyl-4-(2-nitrophenyl)but-3-en-2-one **104a,** in $CDCl_3$.

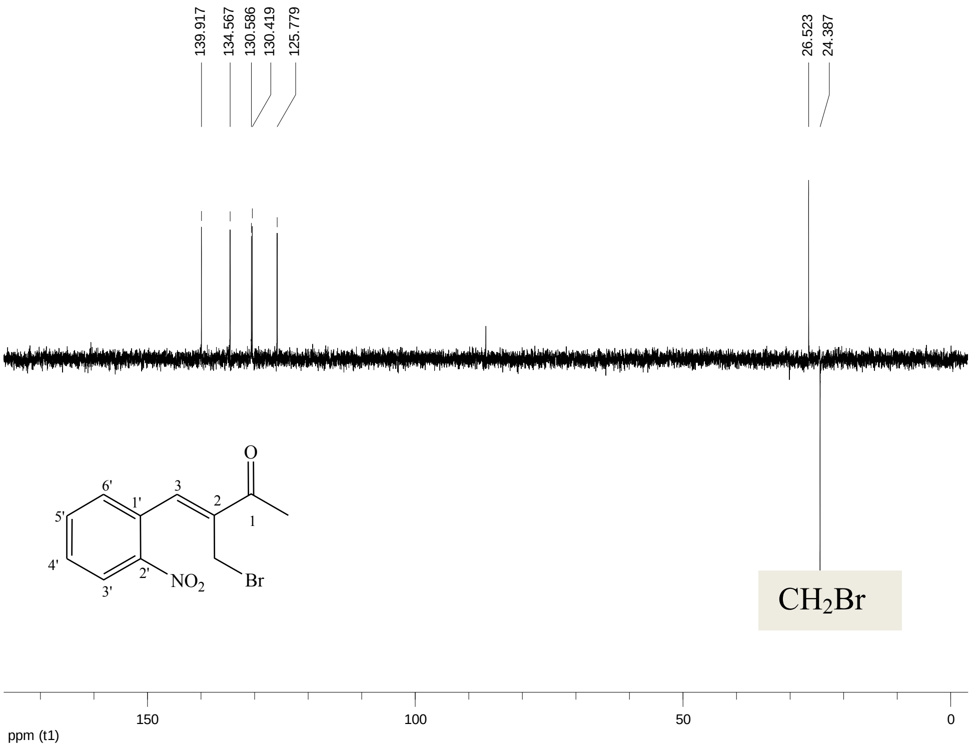
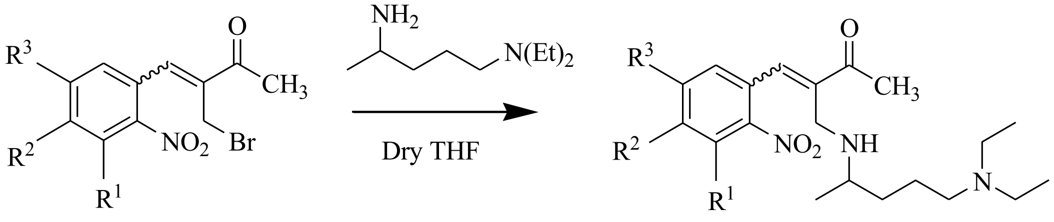


**Figure 2.61.** DEPT 135 spectrum of 3-bromomethyl-4-(2-nitrophenyl)but-3-en-2-one **104a,** in $CDCl_3$.

## 2.3.6. Reactions of the bromomethyl α,β-unsaturated ketones.

Having obtained the derived allyl bromides **104a**,**f**, the next step was to react them with primary amines, particularly 2-amino-5-(diethylamino)pentane. The products **103a**,**f**, from this step would serve as precursors for the 3-(aminomethyl)quinoline bearing the chloroquine side chain as indicated in Scheme 2.25. The allyl bromide **104a** was treated with 2.0 eq. of 2-amino-5-(diethylamino)pentane in anhydrous THF, for 3-7 days. Work-up and chromatography afforded compound **105a**, in 36% yield. Again, the yield is not good, but, at least, some product was obtained. The $^1$H NMR spectrum (Figure 2.62) reveals the five aromatic proton signals between 7.54 and 8.18 ppm, and the allylic aminomethylene signal as two doublets at 3.34 ppm; the signals for the remaining protons tend to overlap further up-field. The $^{13}$C NMR spectrum (Figure 2.63) reveals the carbonyl signal at 200.3 ppm and all of the eight aromatic and vinylic carbon signals between 124.9 ppm and 147.3 ppm. The DEPT 135 spectrum (Figure 2.64) reveals the three methyl signals positive, the aromatic methine signals and expected five signals for the six methylene carbons.



**104 a,f**

**103a,f**

|   | R¹ | R² | R³ |
|---|---|---|---|
| a | H | H | H |
| f | H | H | Cl |

**Scheme 2.25:** Conjugate addition reactions of the bromomethyl α,β-unsaturated ketones with 2- amino-5-(diethylamino)pentane.

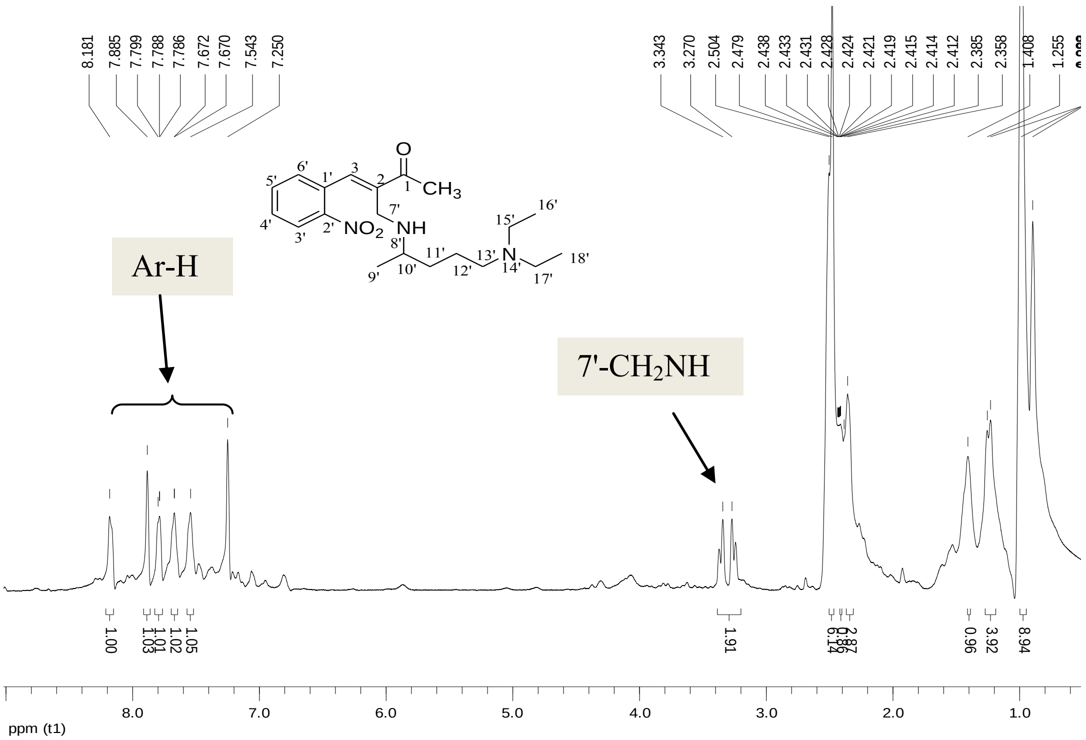


**Figure 2.62.** 400MHz $^1$H NMR spectrum of compound **103a** in CDCl$_3$.

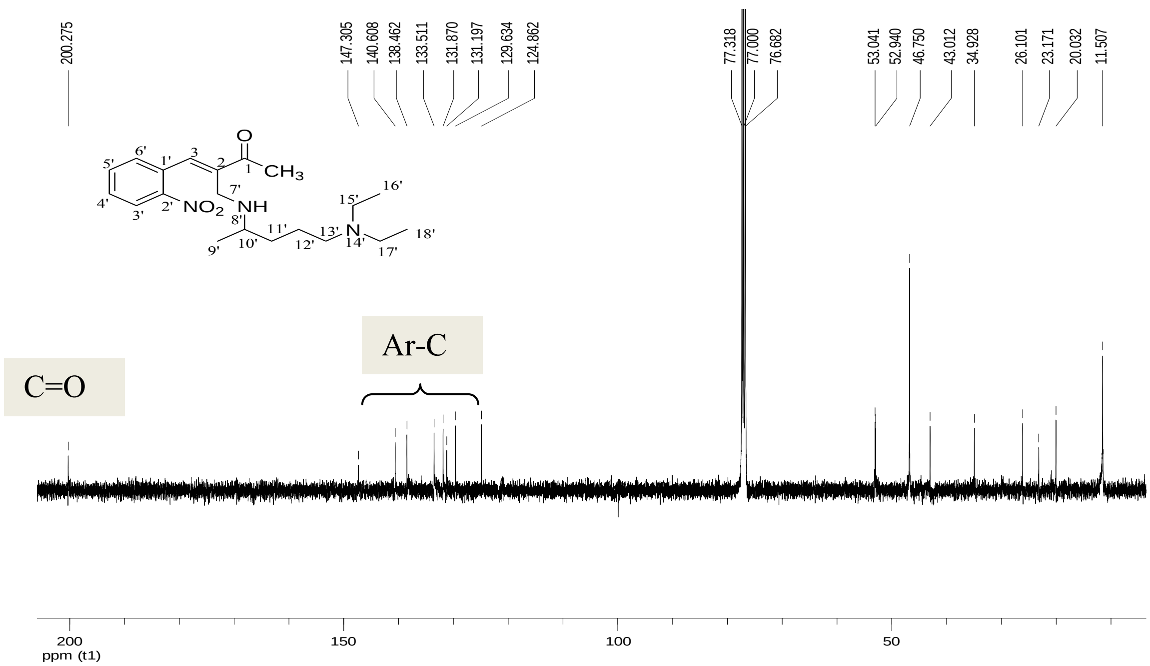


**Figure 2.63.** 100MHz $^{13}$C NMR spectrum of compound **103a** in CDCl$_3$.

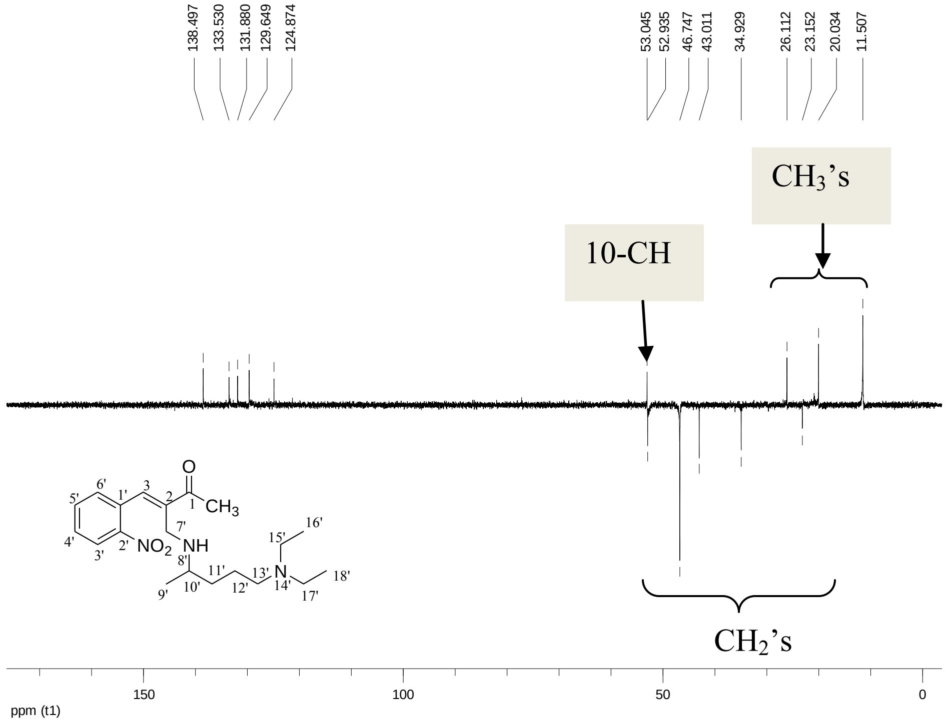


**Figure 2.64** DEPT 135 spectrum of compound **103a** in $CDCl_3$**.**

### 2.3.7 Antiplasmodial Bioassay of Compound 99b.

**Table 2.7.** Antiplasmodial bioassay results.

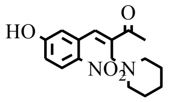
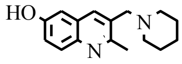
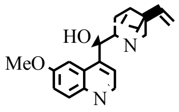
| | Antimalarial actigvity - FCR3 strain | | | Haemolytic activity (RBC toxicity) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| compound | Mean $IC_{50}$ value (µM) | | | Mean $IC_{50}$ value (µM) | Mean @ 100µM | | | | | Mean @ 50µM | | | Safety Index |
| | $IC_{50}$ | s.e. | n | $IC_{50}$ | s.e. | n | % Haemolysis | s.d. | n | % Haemolysis | s.d. | n | |
|  | 22.376 | 1.315 | 4 | N/A | 9.359 | | | 0.446 | 3 | 3.751 | 0.466 | 3 | >4.469 |
|  | 12.928 | 0.729 | 4 | N/A | 0.561 | | | 0.142 | 3 | 0.428 | 0.202 | 3 | >7.735 |
|  | 0.126 | 0.010 | 5 | | | | 0.544 | 0.331 | 6 | 0.629 | 0.102 | 5 | >794.132 |

Table 2.7 shows the results of the antimalarial bioassays carried out, for the author, on the initially isolated 3-(aminomethyl)quinoline **99b** and its precursor, **98b.** These results are significant in that they provide the basis for the continuation of this study, as information gathered from the literature had suggested that only the 2- and 4-amino-substituted quinoline analogues effectively complex with hematin, and exhibit antimalarial activity.[15] It was necessary to establish whether the targeted 3-aminomethylquinolines, which although structurally different from 3-amino-substituted quinoline analogues, would show antimalarial activity or not. As shown in Table 2.7, the antimalarial activity of compounds **98b** and **99b** was tested against the FCR 3 strain of plasmodium. Quinine, a well known, frontline antimalarial was used as the standard and it is evident that

compound **99b** with $IC_{50}$ value of 12.9 μM, shows some level of antimalarial activity. The $IC_{50}$ value 22.4 μM, of the precursor **98b** is indicative of antimalarial activity. It should be noted that the amino side-chain could also contribute significantly to antiplasmodial activity and that the piperidine moiety might well be relatively ineffective. This reasoning informed our determination to access 3-aminomethylquinoline containing the chloroquine side chain.

From Table 2.7, the percentage haemolysis for compound **99b** (0.428%) is comparable with that of quinine (0.629%), which implies that the antimalarial activity of compound **99b** is not based on haemolysis, although the assay procedure to ascertain the antimalarial mechanism of this compound was not available. This result confirms our expectation that the structural difference between the 3-(aminomethyl)quinolines and their 3-aminoquinoline analogues, may result in a difference in therapeutic activity. There is however, a significant difference in the safety index of compound **99b** (> 7.7) and quinine (>794.1).

In summary, the bioassay result has provided substantial evidence to support the antimalarial potential of the 3-(aminomethyl)quinolines. Therefore, in spite of the difficulties encountered, efforts towards the synthesizing a full range of the 3-(aminomethyl)quinoline analogues, especially those with an amino side chain like that of chloroquine and, perhaps, the “quinine side chain” as well were considered worth pursuing.

## 2.4. MOLECULAR MODELLING AND DOCKING STUDIES.

The structure of the "parent" cinnamate ester-AZT conjugate **89a** was modelled, conformational search was conducted on VEGA ZZ,[113] to obtain the most stable conformation, and geometry optimized using Gaussian03 at the B3LYP/6-31 G(d) level.[90] Figure 2.65 illustrates the geometry optimized and the line structure of the cinnamate ester-AZT conjugate **89a.**

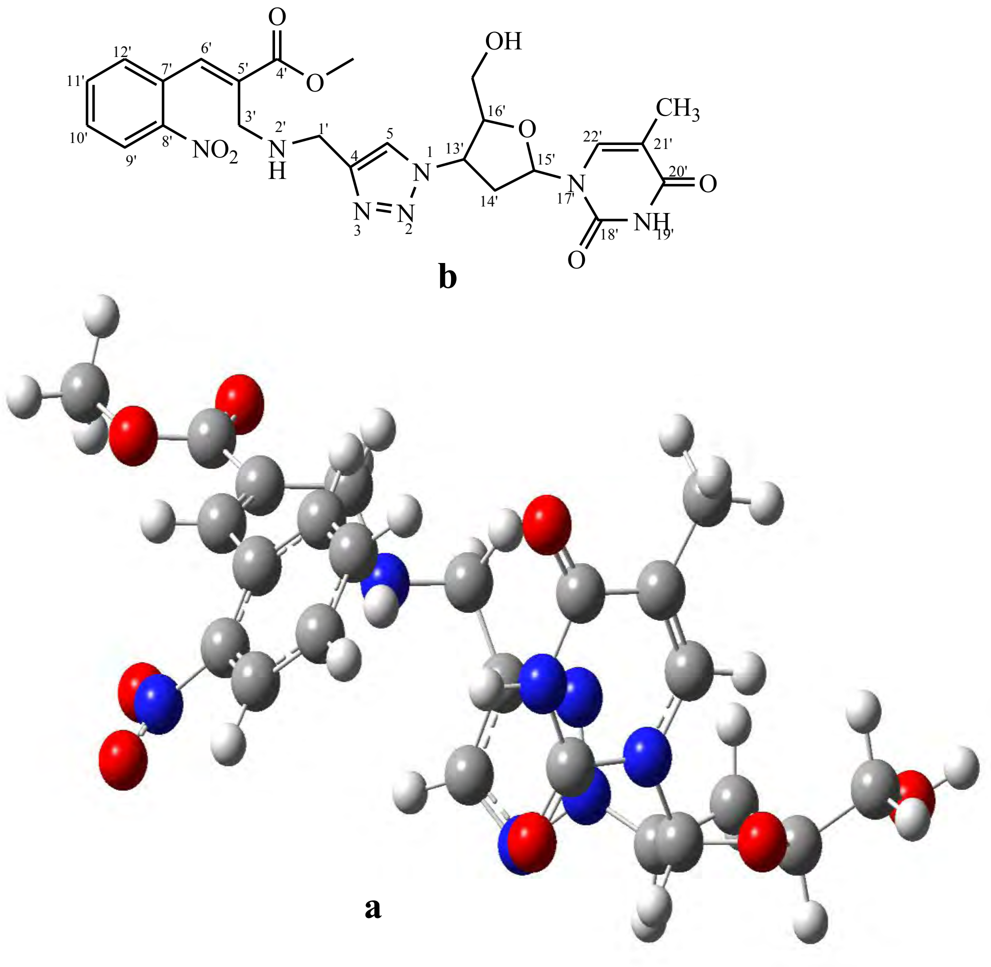


**Figure 2.65.** (**a**) Geometry optimized structure, and (**b**) line structure of cinnamate ester-AZT conjugate **89a.**

These ligands **89a-h**, were designed as potential dual-action HIV-1 IN and RT inhibitors. *In silico* docking studies of the parent system **89a** in the active sites of the respective enzymes HIV-1 IN and RT were therefore undertaken. The X-ray crystallographic protein structures of the HIV-1 integrase enzyme, (1QS4)[95] and the reverse transcriptase enzyme (1IKW) [96] were obtained from the protein databank (pdb). The ligand, **89a** was docked into the active sites of these enzymes using Autodock version 4.[114-115] The docking study was aimed at exploring the interactions of this cinnamate ester-AZT conjugate **89a**, with the active-site residues and compare its docking modes with those of known inhibitors. Figure 2.66 illustrates a balloon representation of the integrase enzyme and the docked ligand **89a** superimposed on 5-CITEP, fitting within the active site of enzyme, and showing potential hydrogen-bonding interactions with amino acid residues in the active site. While the AZT moiety clearly extends beyond the enzyme active-site, the cinnamate ester moiety of the ligand **89a** appears to be reasonably well accommodated within the active-site.

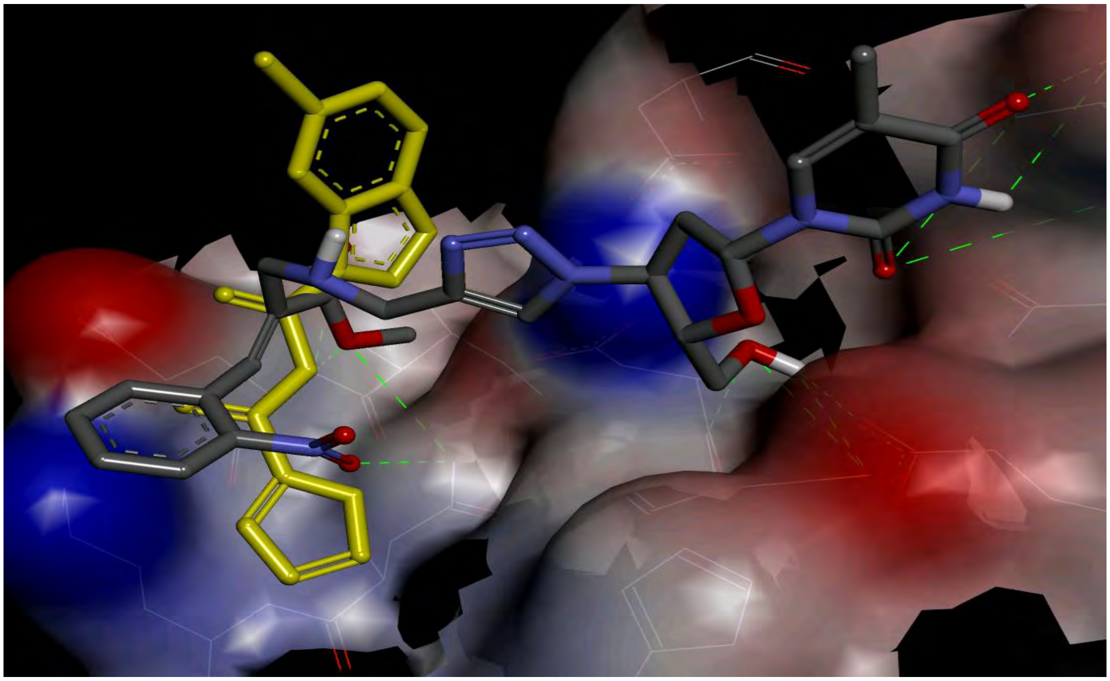


**Figure 2.66**. Balloon representation of 1QS4 integrase enzyme;[95] 5-CITEP (yellow) superimposed with ligand **89a** (atoms coloured gray for carbon, blue for nitrogen and red for oxygen) and showing potential hydrogen-bonding interactions (dotted green lines) with amino acid residues in and around the active site.

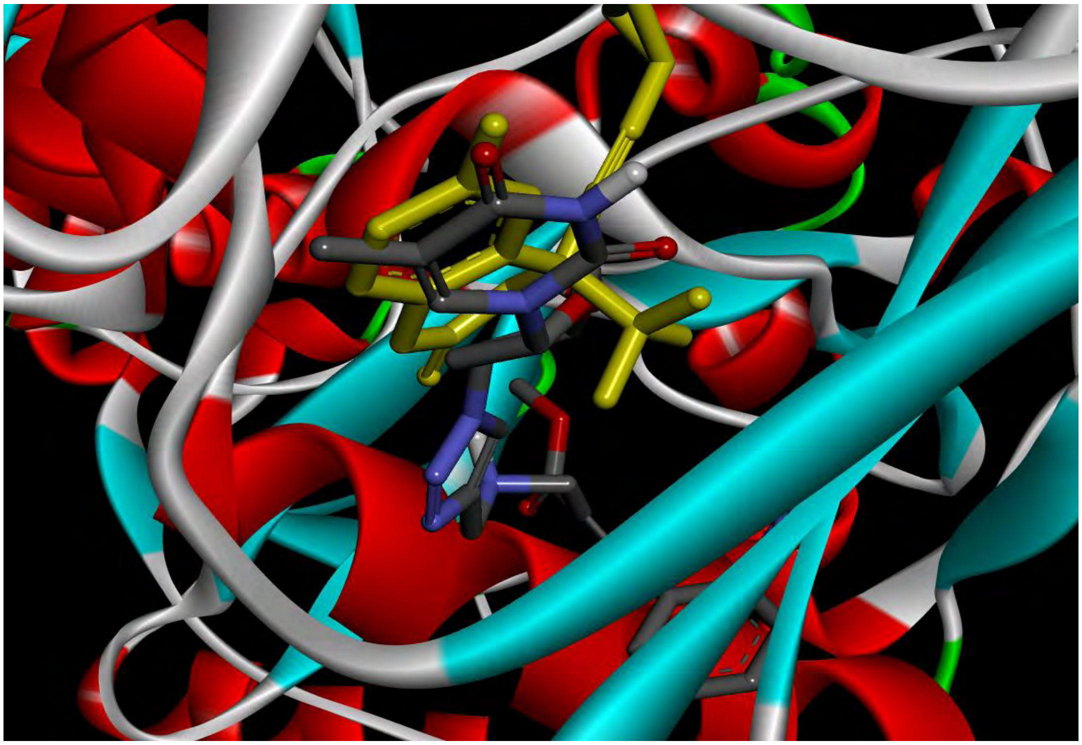


**Figure 2.67**. Ribbon representation of the HIV-1 RT enzyme 1IKW,[96] with the superimposed structures of Efavirenz (yellow) and ligand **89a**, (coloured by atom: gray for carbon, blue for nitrogen and red for oxygen).

In line with our dual-action drug strategy, the ligand **89a** was also docked into the active site of the HIV-1 reverse transcriptase enzyme, (1IKW).[96] Figures 2.67 and 2.68 depict graphically the ligand **89a** bound into the active site and nicely aligned (in Figure 2.67) with the superimposed structure of efavirenz, a well known non-nucleoside reverse transcriptase inhibitor (NNRTI). Figure 2.68 also shows the potential hydrogen-bonding interactions between the ligand and amino acid residues in the binding pocket of the enzyme.

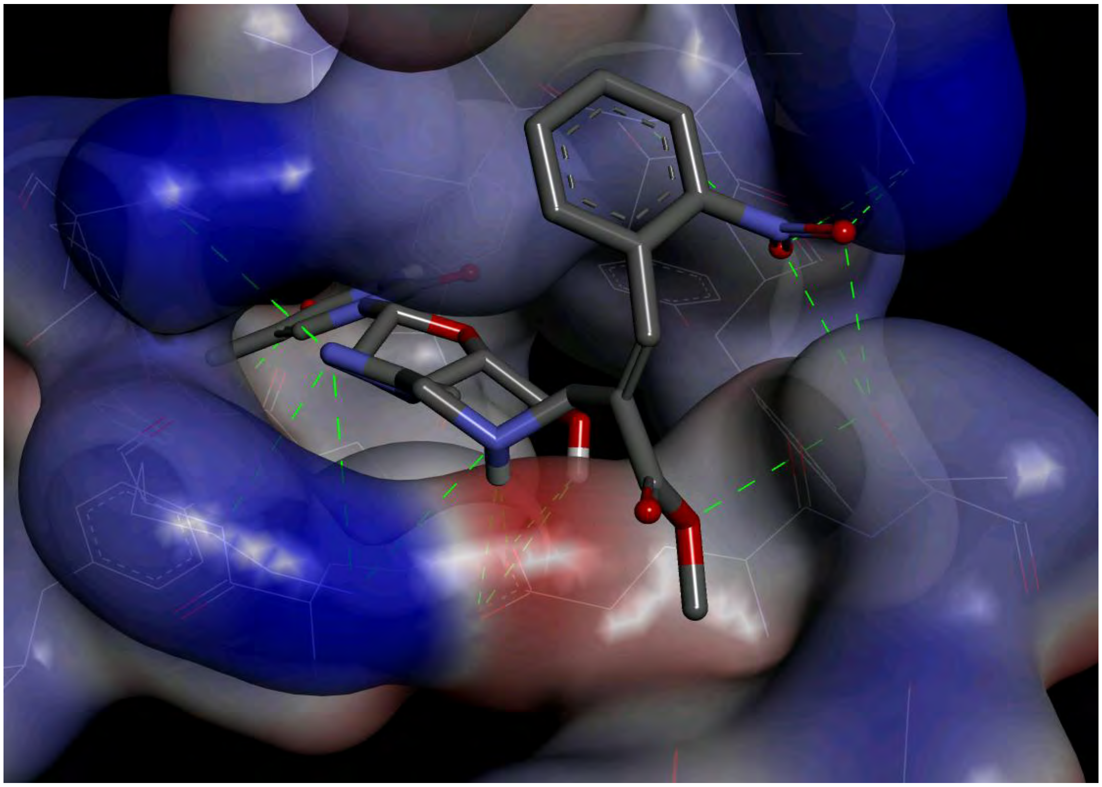
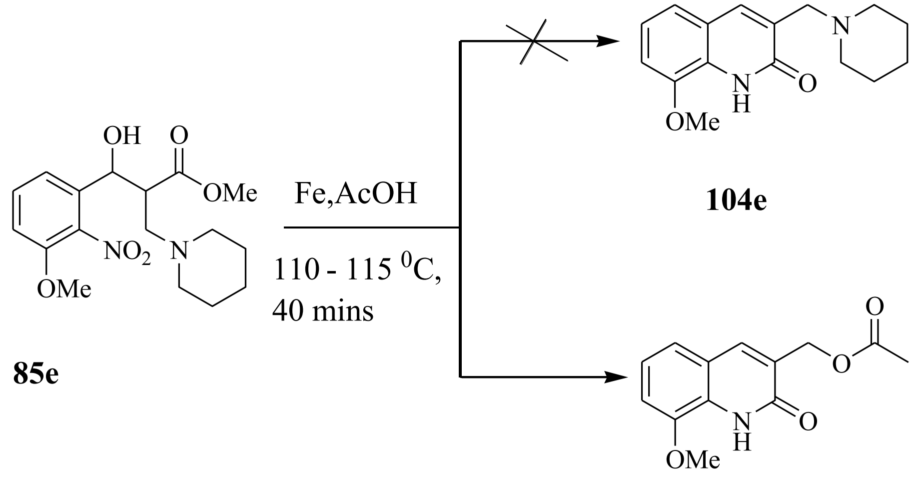


**Figure 2.68.** Graphical representation of ligand **89a** bound in the active-site of the HIV-1 RT enzyme (1IKW)[96] showing potential hydrogen-bonding marked in dotted green lines interactions with amino acid residues.

## 2.5. SYNTHESIS OF QUINOLONES AND QUINOLINES.

### 2.5.1. Synthesis of 3-aminomethyl-substituted quinolones.

One of the goals of this study has been to synthesize quinolone derivatives as potential antimalarial agents. 2-Quinolones have, in fact been reported to have exhibited some antimalarial activity.[93a] Adapting the method reported by Basavaiah *et al.,* [94] the 3-piperidino β-hydroxy ester **85e** was refluxed with acetic acid in the presence of iron powder (Scheme 2.26). Surprisingly, instead of the expected 3-[(piperidin-1-yl)methyl]-2-quinolone **104e**, the reaction produced 3-(acetoxymethyl)-8-methoxy-2-quinolone **112e**, the formation of which is attributable to acid-catalysed displacement of the piperidinyl moiety by the acetoxy group from the acetic acid used in the reaction.



**112e**

**Scheme 2.26**.

The $^1$H NMR spectrum of compound **112e** (Figure 2.69) reveals the acetoxy methyl singlet at 2.13 ppm, the aromatic methoxy singlet at 3.95 ppm, and the methylene singlet at 5.14 ppm. The signals for the four aromatic protons appear between 6.95 and 7.75 ppm, and the amino proton resonates as a broad singlet at 9.50 ppm. The $^{13}$C NMR spectrum (Figure 2.70) reveals the quinolone carbonyl signal at 170.7 ppm, the eight aromatic carbon signals between 110.0 and 160.9 ppm, the methylene carbon signal at

61.4 ppm, the aromatic methoxy carbon signal at 55.9 ppm and the acetoxy methyl carbon signal at 20.9 ppm.

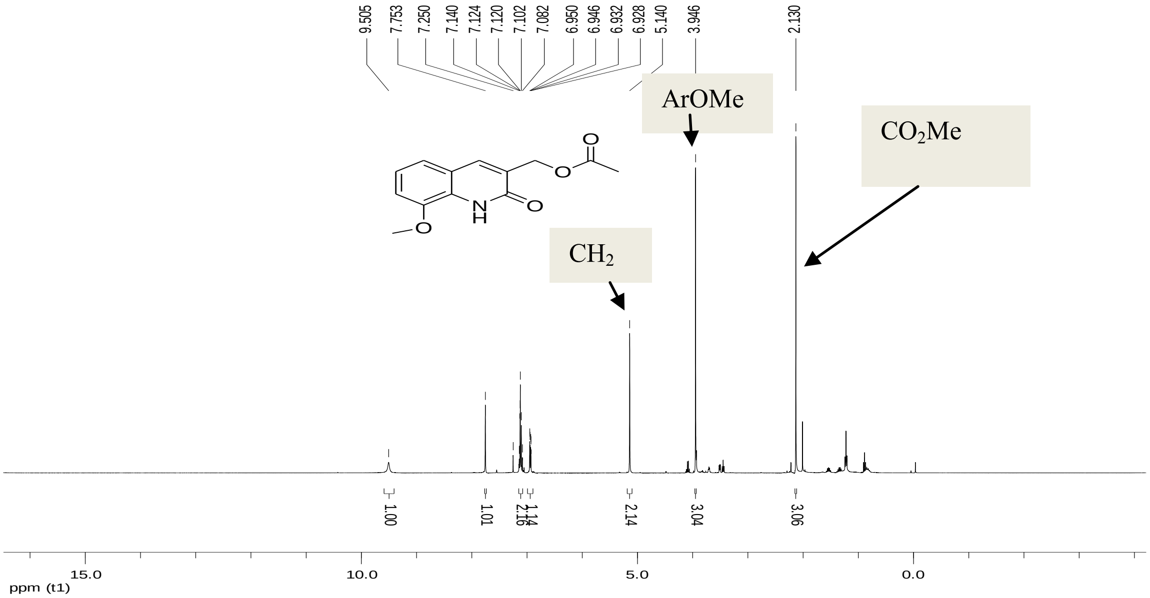


**Figure 2.69.** 400 MHz $^1$H NMR spectrum of 3-(acetoxymethyl)-8-methoxy-2-quinolone **112e** in CDCl$_3$**.**

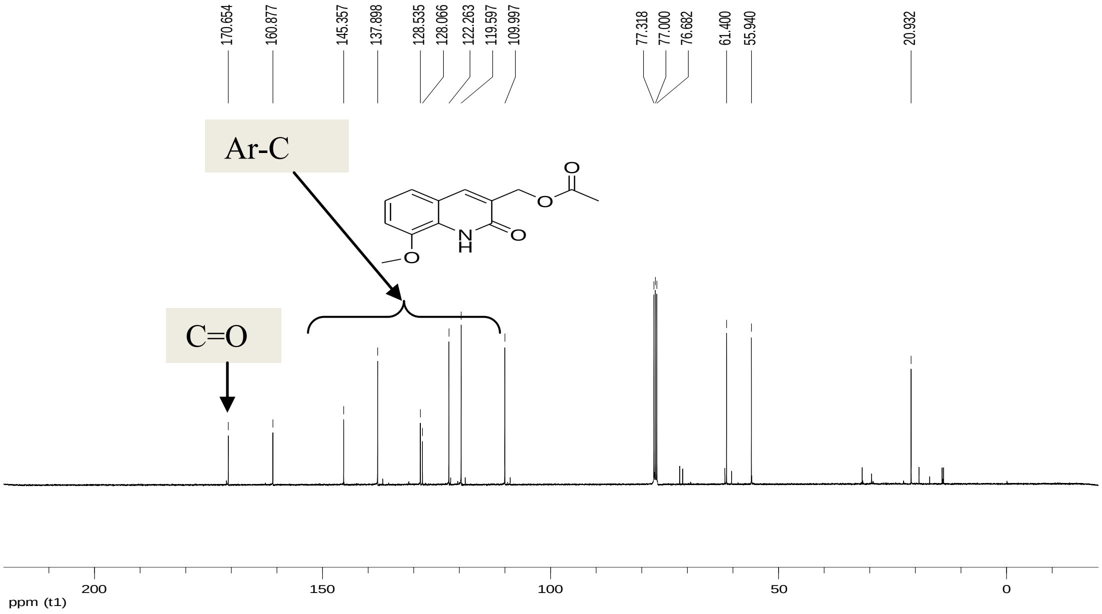
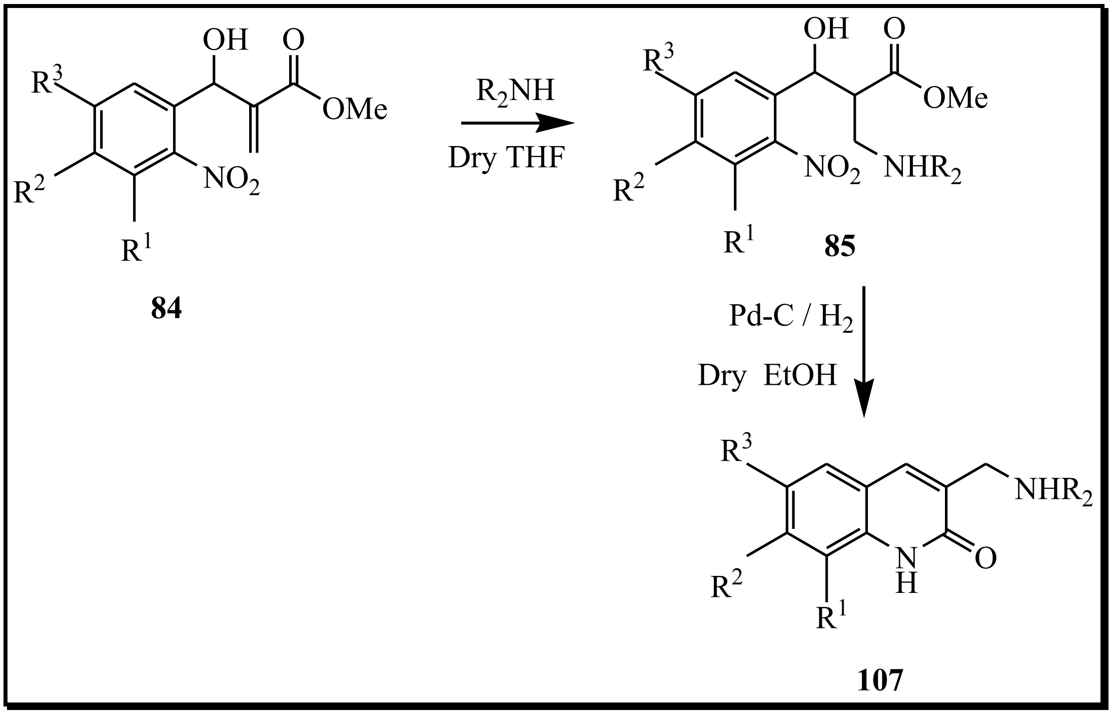
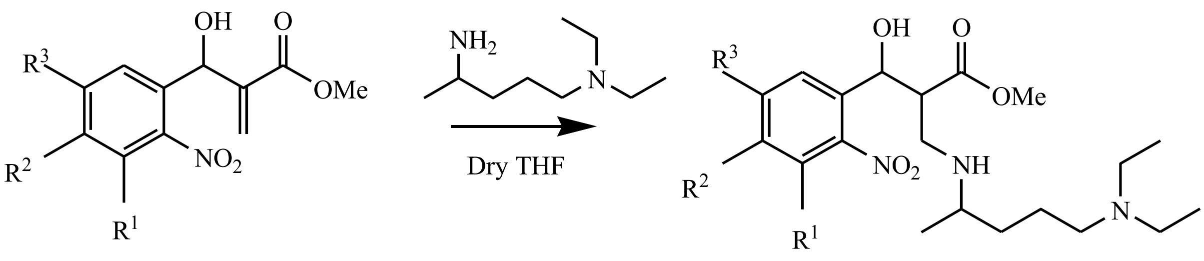


**Figure 2.70.** 100 MHz $^{13}$C NMR spectrum of 3-acetoxymethyl-8-methoxy-2-quinolone **112e** in CDCl$_3$**.**

Following this unsuccessful attempt to access the 3-(piperidinomethyl)-2-quinolone **104,** a different approach to the synthesis of quinolones with 3-aminomethyl substituents was explored. The strategy adopted is outlined in Strategic Plan (IV) Scheme 2.27 below.[93b] This involved adding the amine of interest, *via* a conjugate addition reaction, to the BH esters **84a,c-h** and effecting reductive cyclization with hydrogen gas and 10% Pd-C as catalyst in anhydrous ethanol. It was expected that the use of ethanol as solvent, instead of acetic acid, might obviate the displacement of the amine moiety by the acetate group, as illustrated in Scheme 2.26.



**Scheme 2.27.** ***STRATEGIC PLAN IV:*** Synthesis of 3-aminomethyl-substituted quinolones.



**84a,c,d,f-h**

**106a,c,d,f-h**

|  | $R^1$ | $R^2$ | $R^3$ |
|---|---|---|---|
| a | H | H | H |
| c | H | $-OCH_2O-$ |  |
| d | H | OMe | OMe |
| f | H | H | Cl |
| g | H | Cl | H |
| h | H | H | OMe |

**Scheme 2.28.** Reaction of BH-derived adducts with 2-amino-5-diethylaminopentane.

The first step in the synthesis of the 3-aminomethyl-substituted 2-quinolones involved the addition of the primary amine, 2-amino-5-diethyaminopentane to the BH esters **84**. As outlined in Scheme 2.28, the usual conjugate addition reaction protocol often used in our group was employed with some modification.[93b] Thus, to a solution of the BH ester **84a** (1 eq.) in anhydrous THF [basified with triethylamine (0.1 eq.)] was added 2-amino-5-diethyaminopentane (1 eq.) and the mixture left to stir in a stoppered flask. After 24 – 48 h, all volatiles were removed *in vacuo* to afford the crude material, purification of which was achieved by flash chromatography on basified silica gel. The conjugate addition products **106a,c,d,f-h** were isolated as diastereomeric mixtures (as revealed by the $^1$H NMR analysis) in excellent yields as shown in Table 2.8. The occurrence of the conjugate addition products as diastereomeric mixtures as explained earlier, is attributable to the presence of three stereogenic centres (C2 and C3 and C10'); thus there is the possibility of affording up to four diastereomeric pairs of enantiomers (*i.e.* $2^3$ stereoisomeric products). Of course, no enantioselectivity is expected from the reactions of the racemic BH adducts **84**, with the racemic amine.

**Table 2.8.** Yields of isolated BH-derived α-aminomethyl-β-hydroxyesters.

| α-Aminomethyl-β-hydroxy ester. | $R^1$ | $R^2$ | $R^3$ | Yield |
|---|---|---|---|---|
| **106a** | H | H | H | 87% |
| **106c** | H | -OCH$_2$O- | | 88% |
| **106d** | H | OMe | OMe | 92% |
| **106f** | H | H | Cl | 97% |
| **106g** | H | Cl | H | 94% |
| **106h** | H | H | OMe | 90% |

The $^1$H NMR spectrum of the mixture of diastereomers **106a** (Figure 2.71) followed the pattern described for product **85a** (Figure 2.6), showing the 3-methine proton signals between 5.5 and 6.0 ppm, integration of which permitted the determination of the diastereomeric ratio in each case. The doublet at 5.74 ppm ($J$ = 9.2 Hz), corresponds to the major diastereomer. The reactions favoured the formation of two of the four diastereomeric pairs of enantiomers, one of which is clearly dominant. The broad multiplet (a "hump-like" signal) at *ca.* 4.70 ppm is assigned to the one hydroxy and two NH protons. The $^{13}$C NMR spectrum (Figure 2.72) clearly reveals the presence of a pair of carbonyl signals at 172.8 and 178.1 ppm with the former belonging to the major diastereomer, and confirming the formation of two diastereomeric systems. Most of the other carbon signals are also doubled. Five pairs of methylene carbons are observed in the DEPT135 spectrum (Figure 2.73), corresponding to five types of methylene group present in the α-aminomethyl-β-hydroxy ester **106a**. The signal assignments are supported by the HSQC data (Figure 2.74).

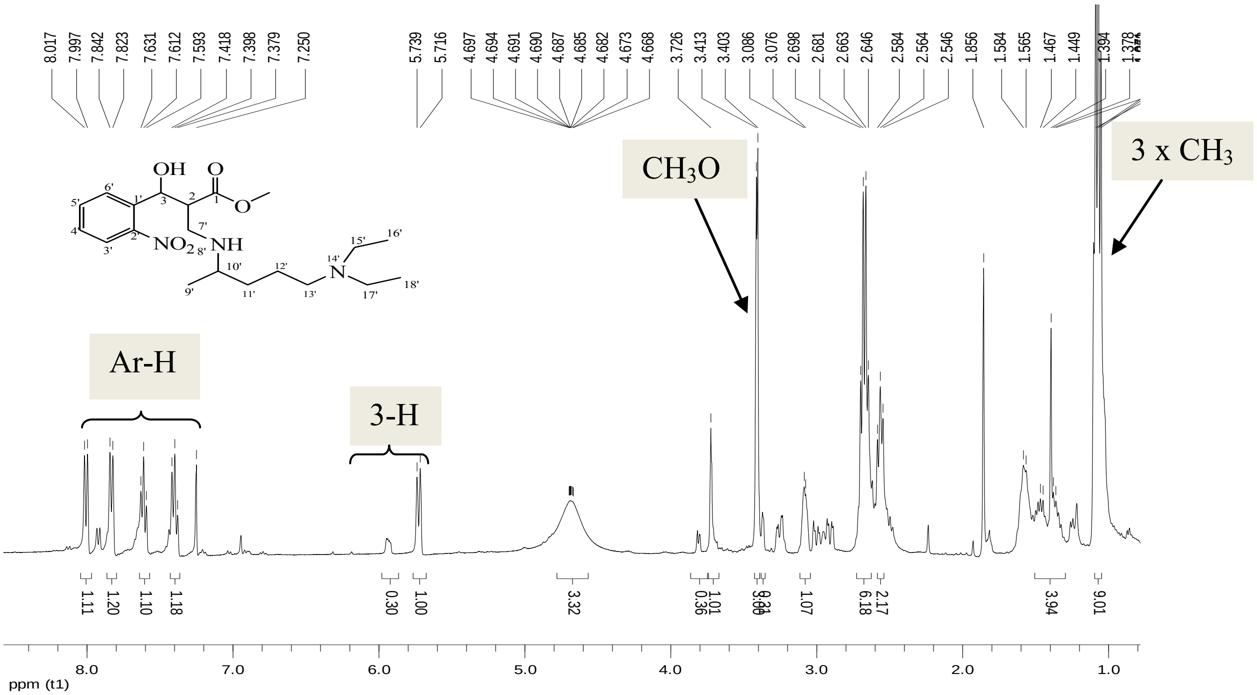


**Figure 2.71.** 400MHz $^1$H NMR spectrum of methyl 3-[(4-diethylamino-1-methylbutylamino)-methyl]-3-hydroxy-3-(2- nitrophenyl)propanoate **106a** in $CDCl_3$.

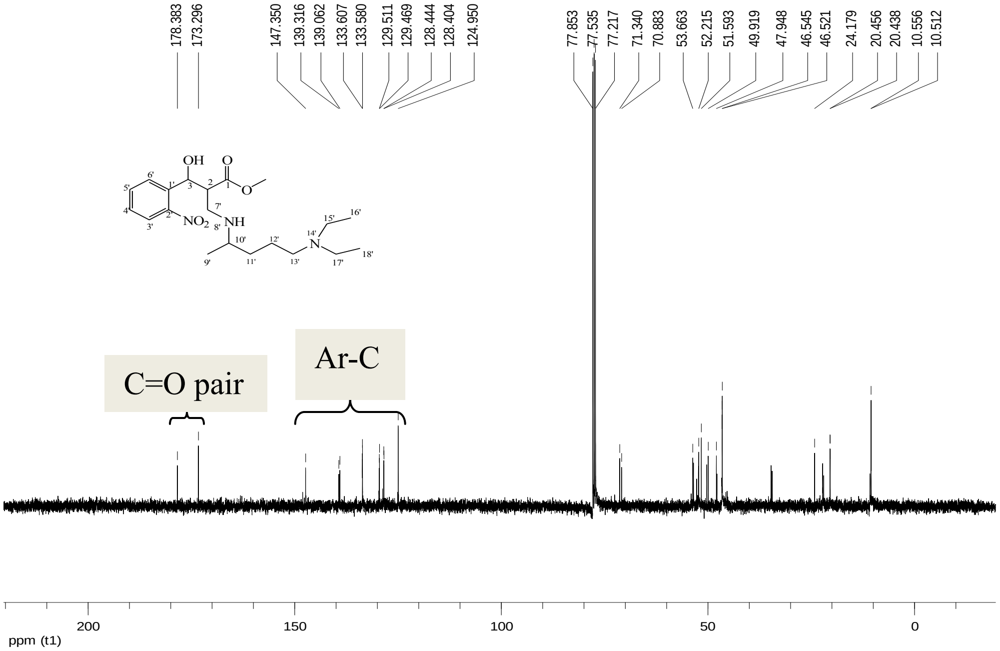


**Figure 2.72.** 100MHz $^{13}$C NMR spectrum of methyl 3-[(4-diethylamino-1-methylbutylamino)- methyl]-3-hydroxy-3-(2-nitrophenyl)propanoate **106a** in $CDCl_3$.

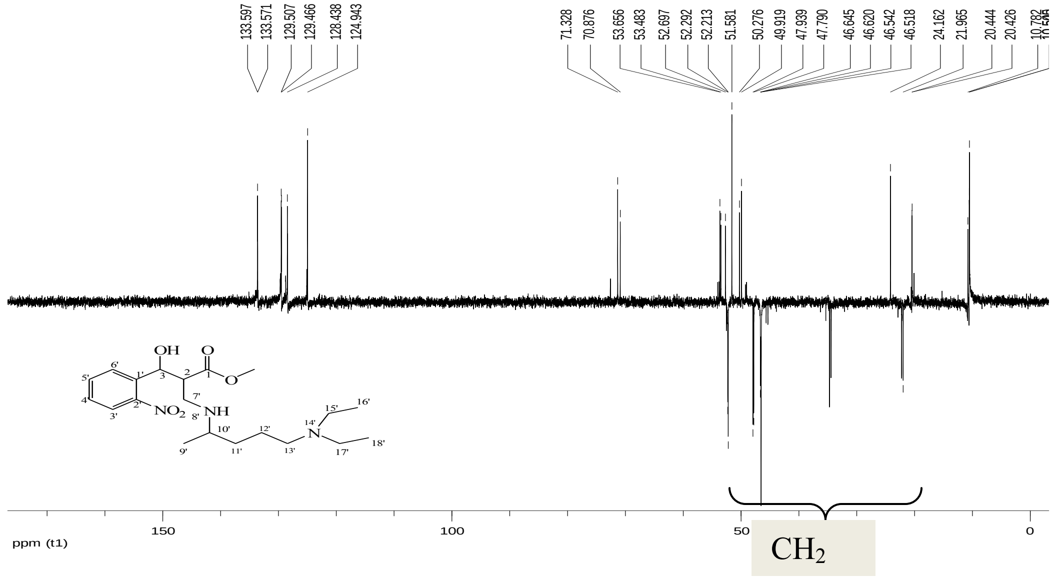


**Figure 2.73.**DEPT 135 spectrum of methyl 3-[(4-diethylamino-1-methylbutylamino)-methyl]-3-hydroxy-3-(2-nitrophenyl)propanoate **106a** in $CDCl_3$.

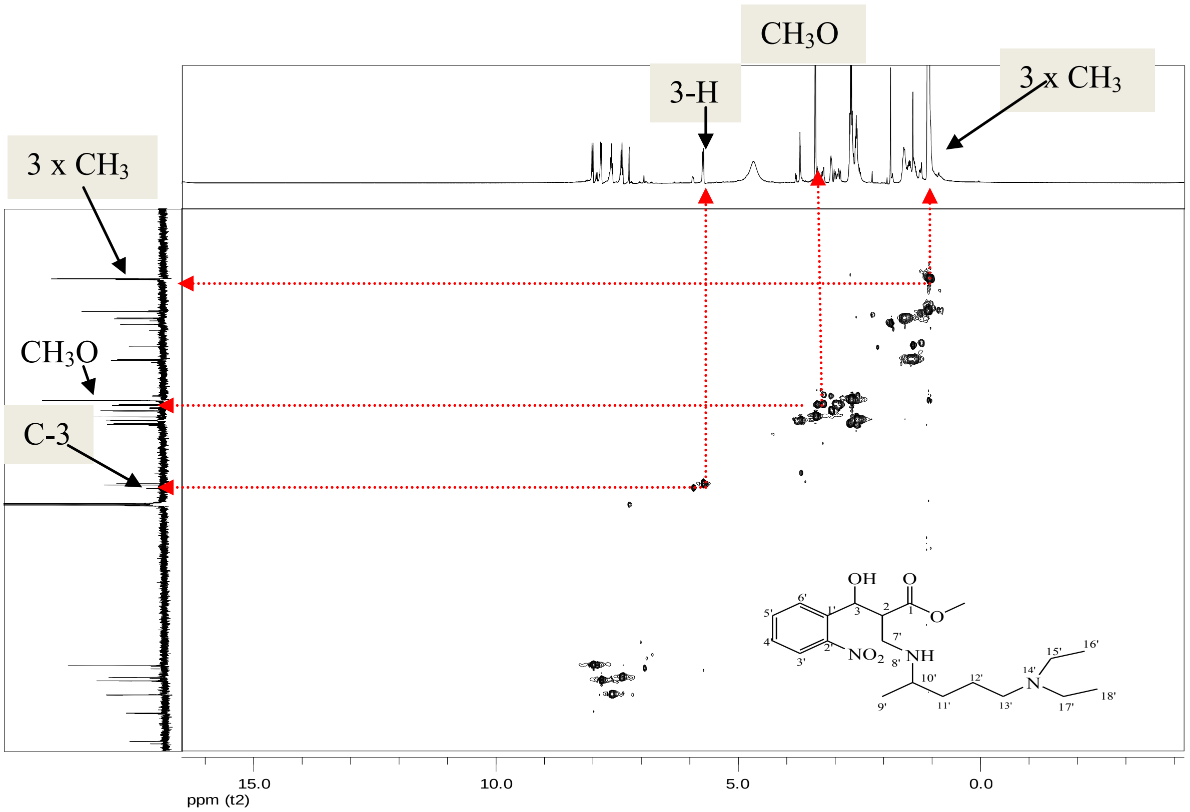
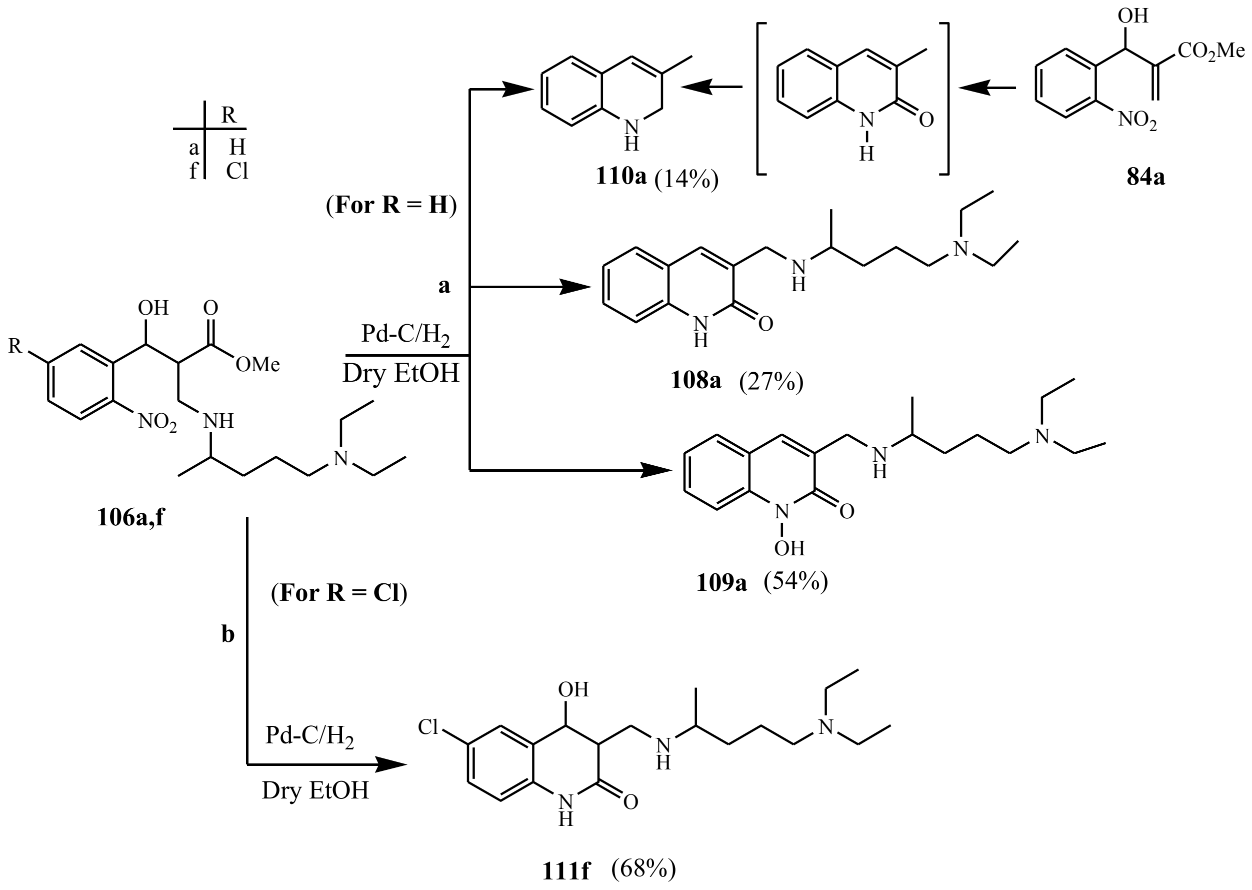


**Figure 2.74.**HSQC spectrum of methyl 3-[(4-diethylamino-1-methylbutylamino)methyl]-3-hydroxy-3-(2-nitrophenyl)propanoate **106a** in $CDCl_3$.

The diastereomeric mixtures **106a**,**f** were subjected to catalytic hydrogenation using a Pd-C catalyst in anhydrous ethanol (Scheme 2.29). Purification of products from the reaction of the the parent system **106a** by flash chromatography on Discovery DSC-Diol, basified and conditioned overnight with basic eluent, afforded the desired 3-aminomethyl-2-quinolone **108a** (27%), the *N*-hydroxy analogue **109a** (54%) and the 1,2-dihydro-3-methylquinoline **110a** (14%). Reaction of the 5-chloro substrate **106f**, however afforded 6-chloro-4-hydroxy-3,4-dihydro-2-quinolone **111f** in 68% yield.



**Scheme 2.29.** Reductive cyclization of aminomethyl BH-derived ester **106a**,**f** to quinolone derivatives.

Product formation involves reduction of the nitro group to an amine group, followed by intramolecular cyclization to the corresponding 2-quinolone *via* nucleophilic attack at the carbonyl carbon. The incomplete hydrogenation of the nitro group in substrate **106a,** is presumed to lead to the *N*-hydroxy derivative **109a**, while the 1,2-dihydro-3-methylquinoline **110a** may arise from reductive cyclization of the BH ester **84a**, present as a contaminant in the substrate **106a**, followed by reduction of the resulting 2-quinoline as indicated in Scheme 2.29. For the purpose of this discussion, spectra of the target quinolone **108a** are described; the experimental data for the other products are detailed in the experimental section (Chapter 3).

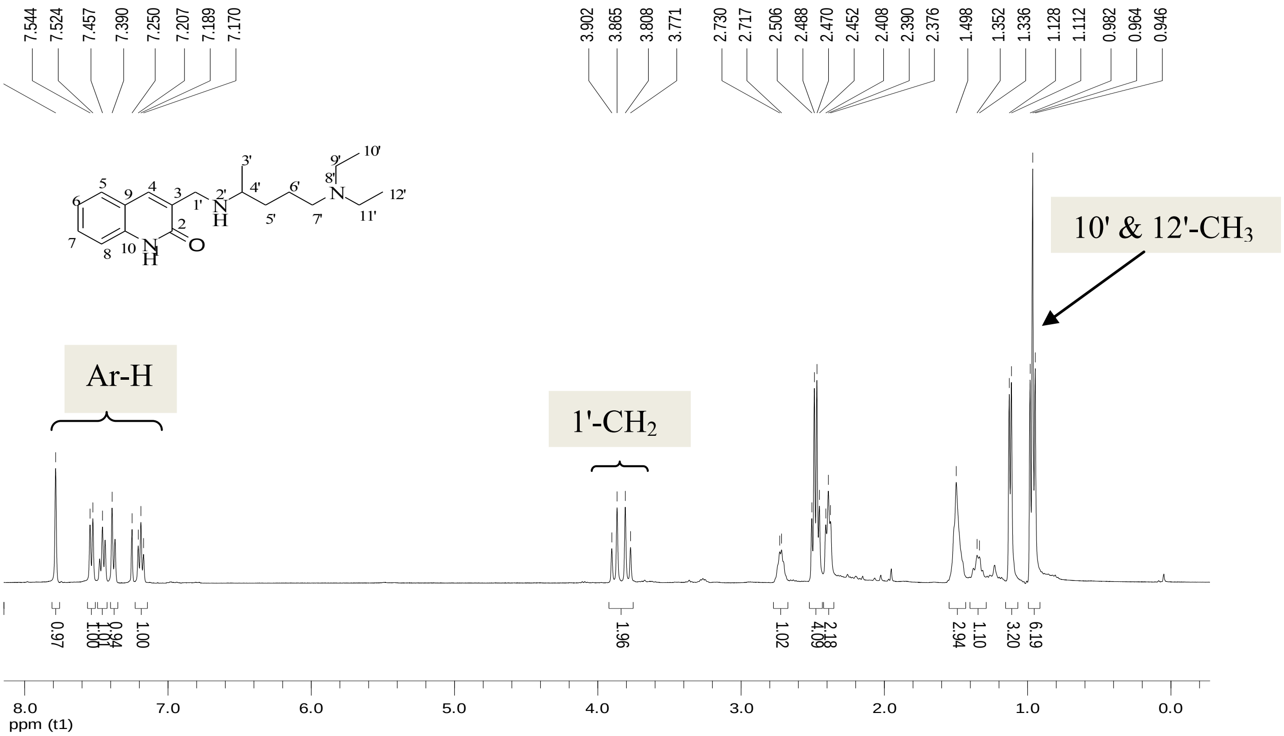


**Figure 2.75.** 400MHz $^1$H NMR spectrum of 3-[(4-diethylamino-1-methylbutylamino)-methyl]-2-quinolone **108a** in $CDCl_3$.

The formation of the quinolone **108a** is supported by the $^1$H NMR spectrum (Figure 2.75), which shows the presence of five aromatic protons, instead of the four in the diastereomeric substrate **106a**. The reductive cyclization and dehydration clearly leads to the loss of two of the three chiral centres (C-2 and C-3) present in the substrate **106a**, leaving C-4' as the only stereogenic centre. The triplet at 0.98 ppm corresponds to the terminal 10' and 12' methyl groups, while the diastereotropic 1'-aminomethylene protons resonate as a pair of doublets at 3.90 and 3.81 ppm ($J$ = 14.8 Hz).

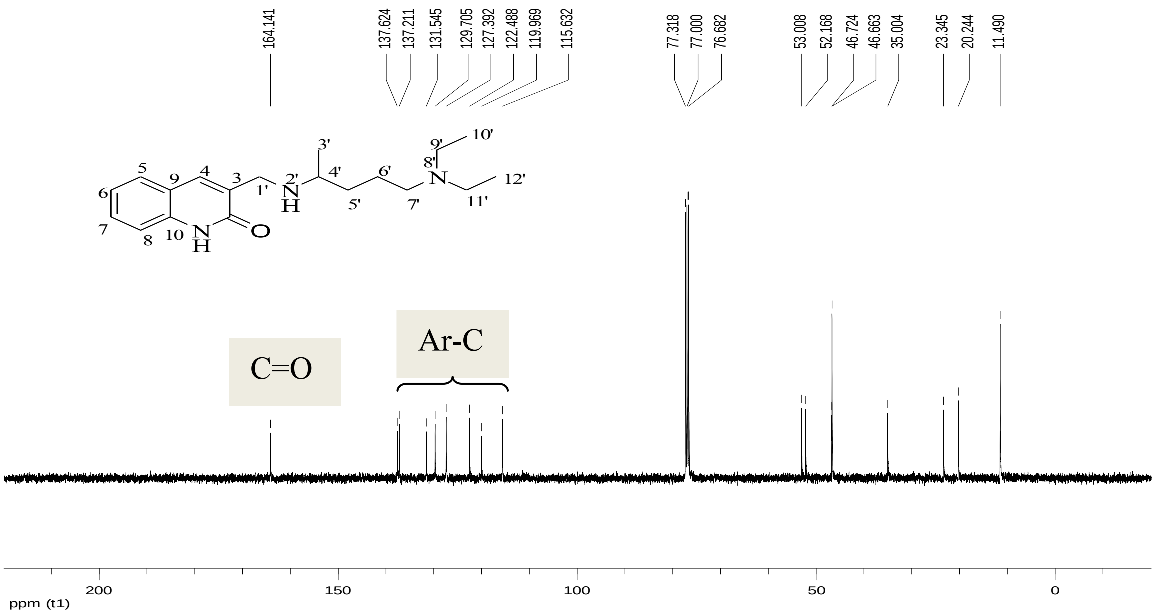


**Figure 2.76.** 100MHz $^{13}$C NMR spectrum of 3- [(4-diethylamino-1-methyl-butylamino)methyl]-2-quinolone **108a** in CDCl$_3$.

The $^{13}$C NMR spectrum (Figure 2.76) reveals the carbonyl signal at 163.1 ppm, a shift downfield relative to the chemical shift of the carbonyl signal in the ester substrate at 170.0 ppm (Figure 2.72). The eight aromatic carbon signals appear between 115 and 137.6 ppm, while the DEPT 135 spectrum (Figure 2.77) clearly reveals the methyl and 4'-methine carbon signals at 11.5, 20.2 and 53.0 ppm. The five methylene carbon signals are seen between 23.4 and 53.0 ppm. The assignment of these signals is confirmed by the HSQC data (Figure 2.78). Dehydration of the β-hydroxy ester **106** (Scheme 2.29) would lead to cinnamate esters containing the chloroquine side-chain – compounds which might exhibit both HIV-1 integrase inhibition and antimalarial activity. Unfortunately, time constraints did not permit this possibility to be explored nor reductive cyclization of the remaining β-hydroxy esters to the corresponding 2-quinolones.

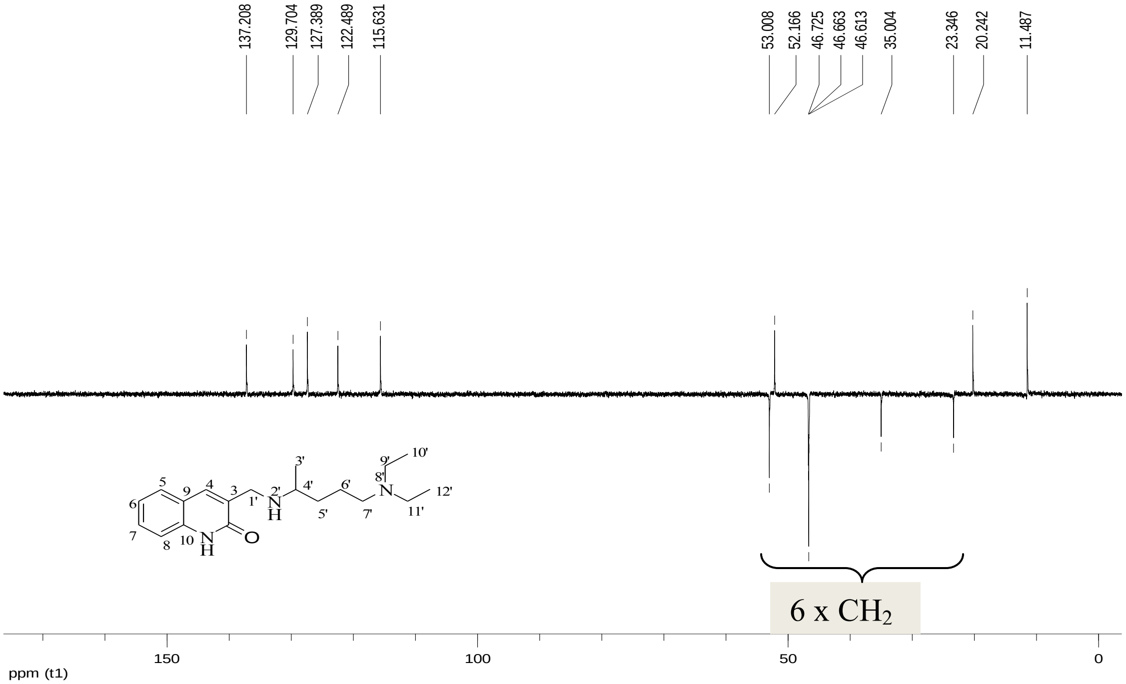


**Figure 2.77.** DEPT 135 spectrum of 3-[(4-diethylamino-1-methylbuylamino)methyl]-2-quinolone **108a** in CDCl$_3$.

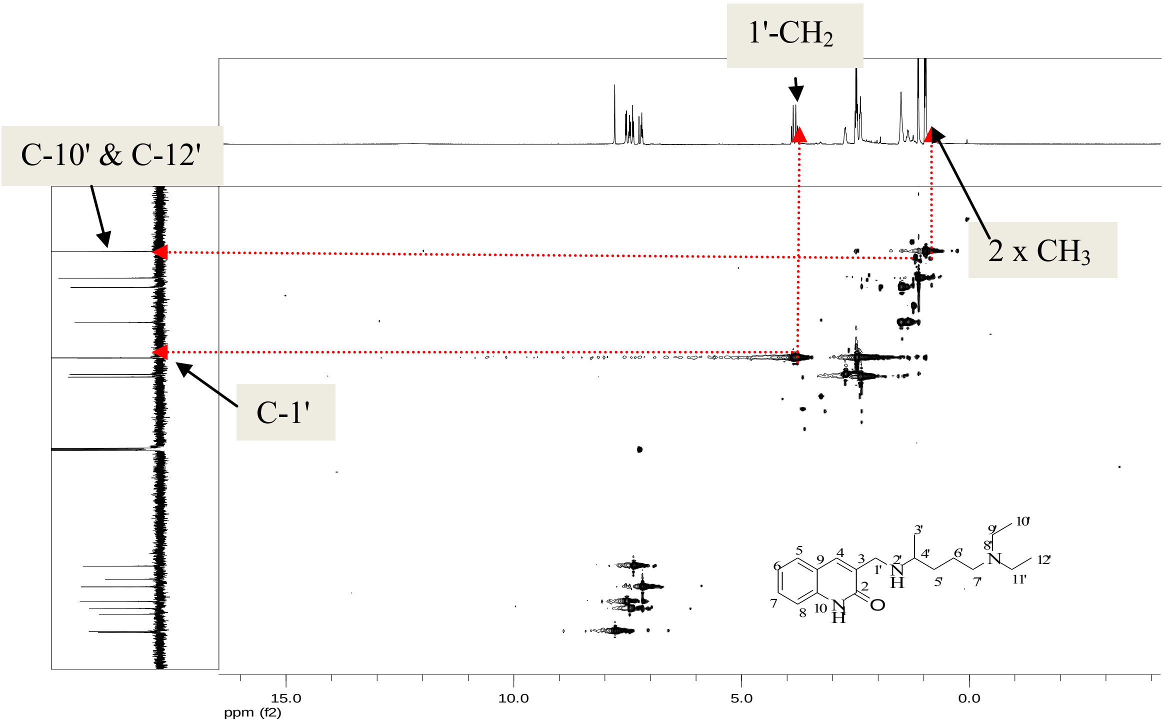
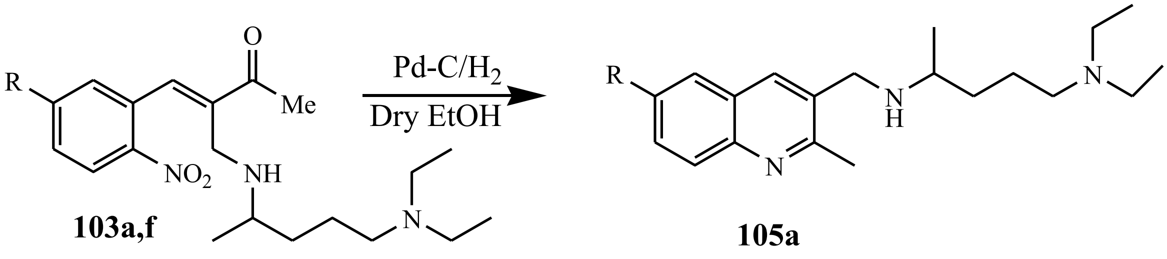


**Figure 2.78.** HSQC spectrum of 3-[(4-diethylamino-1-methylbutylamino)methyl]-2-quinolone **108a** in CDCl$_3$.

## 2.5.2. Synthesis of 3-(aminomethyl)-substituted quinolines.

Encouraged by the availability of the 4-(diethylamino)butylamino derivatives **103a,f** (Section 2.3.6) and by the isolation of the 3-(piperidin-1-ylmethyl)-2-quinoline **99b** (Section 2.3.4.3) and its apparent antimalarial activity, we explored the possibility of generating quinoline derivatives containing the chloroquine side-chain. Catalytic hydrogenation of the α-aminomethyl α,β-unsaturated ketone **103a,** using Pd-C in anhydrous ethanol, permitted access to the 3-amino-substituted quinoline **105a** in moderate yield (58%).



| | R |
|---|---|
| a | H |
| b | Cl |

**Scheme 2.30.** Reductive cyclization to the α-(aminomethyl)-2-quinoline **105a**.

The $^1$H NMR spectrum (Figure 2.79) reveals a triplet at 1.00 ppm for the terminal 10' and 12' methyl groups while the 1'-aminomethylene protons resonate as a pair of doublets at 3.96 and 3.86 ($J$ = 13.6 Hz ppm) and the five aromatic proton signals expected in the product are evident between 7.02 and 8.72 ppm. The $^{13}$C NMR spectrum (Figure 2.80) reveals the absence of the carbonyl signal, thus supporting the reductive cyclization. The $^{13}$C NMR spectrum also revealed the expected nine aromatic carbon signals between 119.6 and 146.3 ppm, while the DEPT 135 spectrum (Figure 2.81) clearly shows four positive signals corresponding to the 10'- and 12'- (equivalent methyl groups), the aromatic methyl, 3'- methyl and the 4'-methine carbons at 11.4, 14.4, 20.5 and 53.2 ppm, respectively; the six methylene carbon signals are evident as five negative signals between 23.3 and 53.0 ppm (9'- and 11'- methylene are equivalent). The assignment of these signals is supported by the HSQC spectrum (Figure 2.82), depicting correlations between carbon and proton signals. Compound **105a** is significant in that it is a quinoline derivative bearing the chloroquine side-chain at position 3.

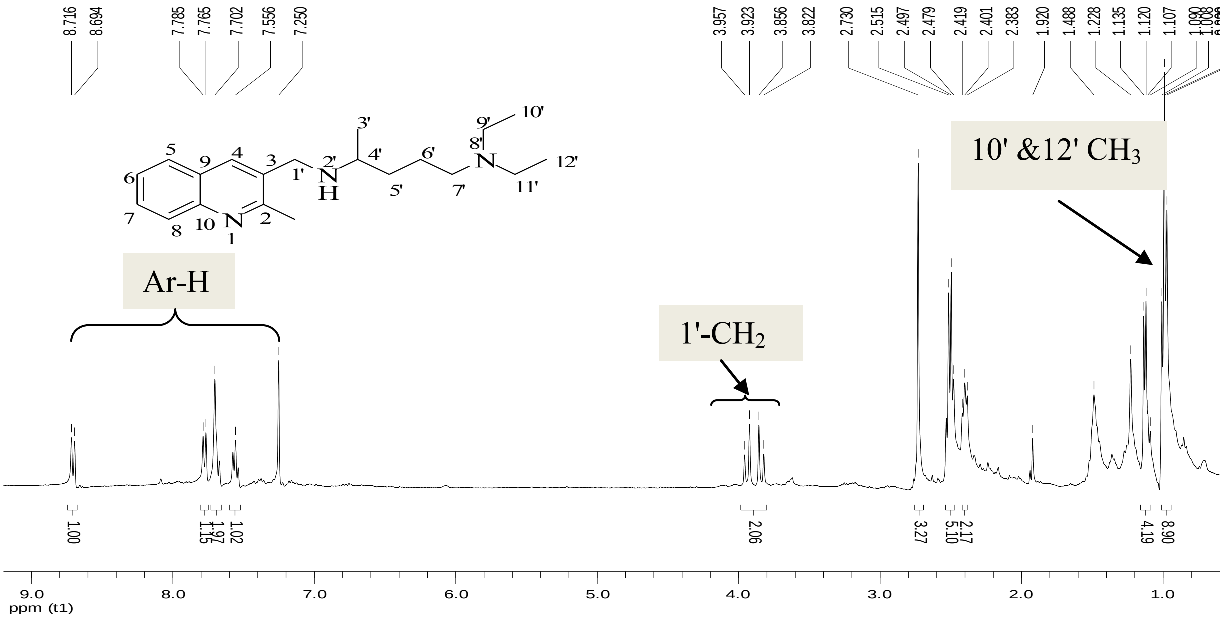


**Figure 2.79.** 400MHz $^{1}$H NMR spectrum of 3-[(4-diethylamino-1-methylbutylamino)]methyl-2-methylquinoline **105a** in $CDCl_3$.

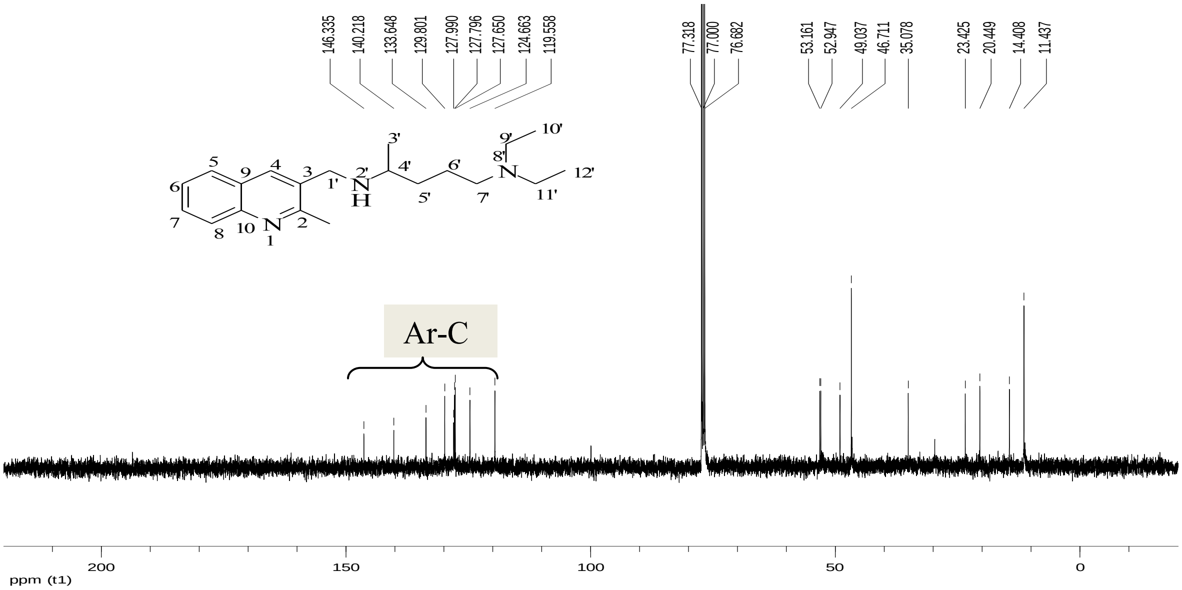


**Figure 2.80.**100MHz $^{13}$C NMR spectrum of 3-[(4-diethylamino-1-methyaminobutylamino)-methyl]-2-methylquinoline **105a** in $CDCl_3$.

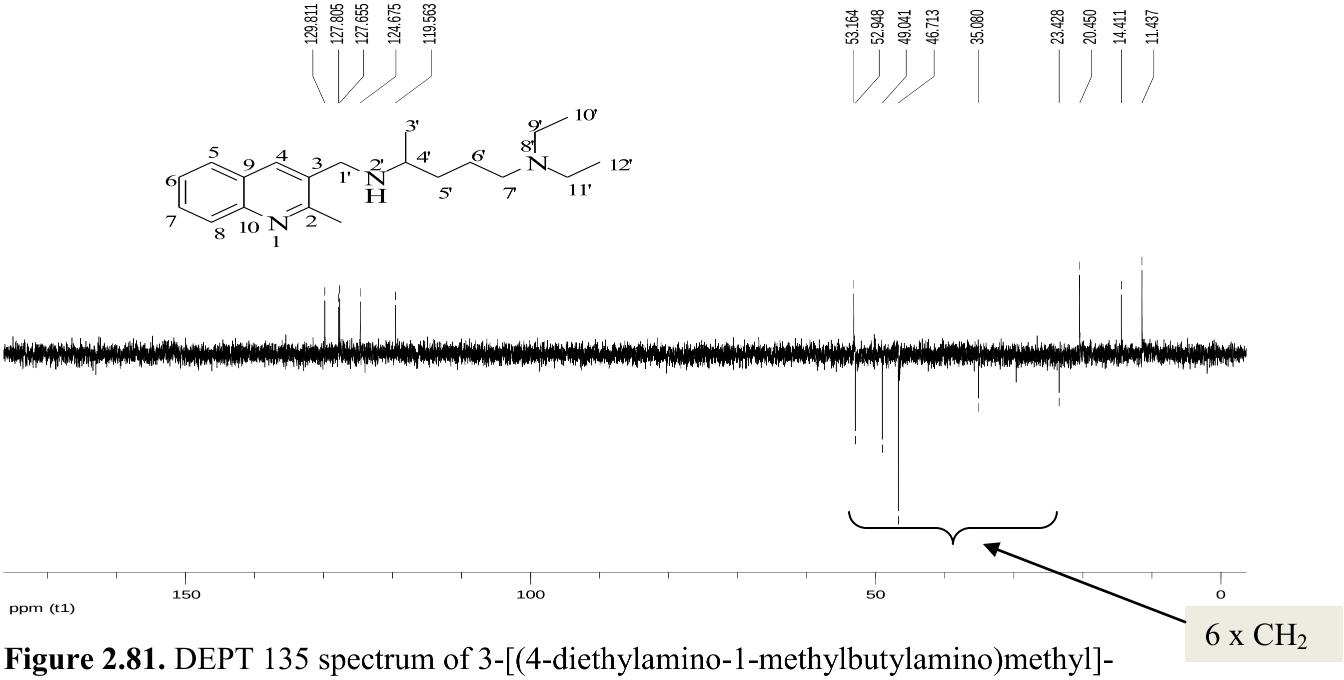


**Figure 2.81.** DEPT 135 spectrum of 3-[(4-diethylamino-1-methylbutylamino)methyl]-2-methylquinoline **105a** in CDCl$_3$.

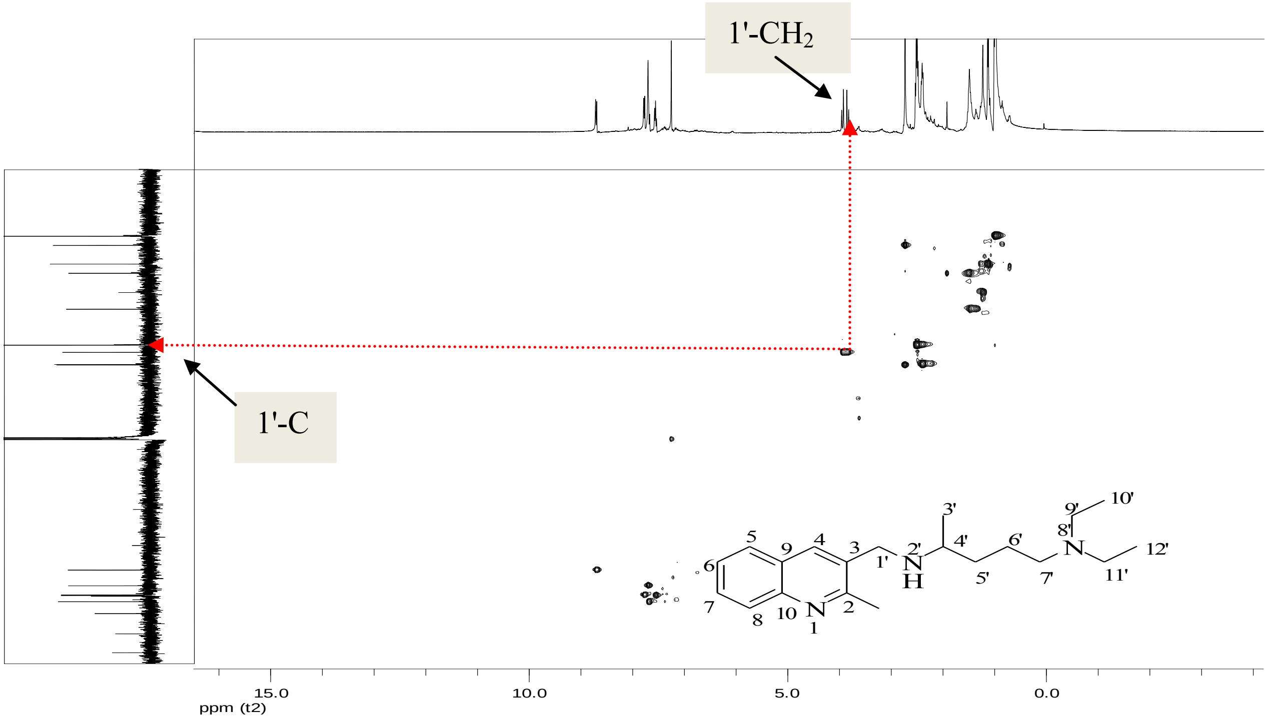


**Figure 2.82.** HSQC spectrum of 3-methylamino-[4-diethlamino-1-methylbutylamino)methyl]-2-methylquinoline **105a** in CDCl$_3$.

Unfortunately, it seems the catalytic hydrogenation of compound **103f** not only effected reductive cyclization to the quinoline but also replaced the chlorine by hydrogen, affording the same 3-aminomethylquinoline **105a.** Isolation of the 6-chloro-2-quinolone derivative **111f** (Scheme 2.29) suggests that it should be possible to modify the conditions to minimize loss of chlorine during reductive cyclization. This, however, remains a challenge for investigation. Reductive cyclization of the 5-chloro analogue **103f** (Scheme 2.29) was considered particularly important because it was expected to afford a product containing both the chloroquine side-chain at position 3 and the 6-chloroquinoline moiety (isomeric with the 7-chloroquinoline moiety) present in chloroquine **2**. However, HRMS analysis of the isolated product failed to indicate the presence of chlorine, and examination of the NMR data indicated the product to be the parent system.

## 2.6. CONCLUSIONS.

This study has been concerned with the successful application of Baylis-Hillman (BH) methodology in the synthesis of novel heterocyclic derivatives as potential lead compounds for the development of therapeutic agents. More particularly, attention has been given to the synthesis of α-substituted cinnamate esters as HIV-1 integrase (IN) inhibitors, cinnamate ester-AZT conjugates as dual-action HIV-1 integrase-reverse transcriptase (IN-RT) inhibitors and 3-(aminomethyl)- quinolines and –quinolones as antimalarial agents. In all, over eighty compounds have been prepared, more than fifty of which are new, and two papers containing aspects of this work have thus far been published.[81,87]

2-Nitrobenzaldehydes with various ring substituents have been reacted under BH conditions using methyl acrylate and methyl vinyl ketone (MVK) as activated alkenes, and 1,4-diazabicyclo[2,2,2]octane (DABCO) or 3-hydroxyquinuclidine (3-HQ) as catalysts. Some of the BH reactions were conducted under microwave conditions which gave higher yields in shorter reaction time (within minutes instead of days or weeks). The BH β-hydroxy esters, derived from the reaction of 2-nitrobenzaldehydes and methyl acrylate, were obtained in yields ranging between 37% and 91%, while the BH β-hydroxy ketones derived from 2-nitrobenzaldehyde and MVK were isolated in 18% to 80% yields.

The BH esters were treated with the amines, piperidine and 2-amino-5-diethylaminopentane to afford diastereomeric conjugate addition products. Pd-C-catalysed reductive cyclization of which, gave 2-quinolones in moderate yields. Dehydration of the diastereomeric products using EDC appeared to give the desired cinnamate esters but isolation of the products proved to be extremely difficult. Hence, a change of approach to the cinnamate esters was conceived, which involved treating the MBH β-hydroxy esters with an *in situ*-generated Vilsmeier-Haack reagent to afford α-chloromethyl derivatives of the cinnamate esters. The Vilsmeier-Haack reagent proved to be highly stereoselective and gave products in good to excellent yields and purification of products was straight-forward. Reaction of these (α-chloromethyl)cinnamates with propargylamine afforded a series of α- [(propynylamino)methyl]cinnamate esters as potential HIV-1 integrase inhibitors, in some cases, together with the isomeric 3-aryl-2-

methylene-3-(propynylamino)propanoate ester arising from allylic ($S_N'$) rather than direct ($S_N$) displacement of chloride.

The terminal alkyne group in the α-[(propynylamino)methyl]cinnamate esters provided the opportunity for the copper(I)-catalysed 1,3-dipolar Huisgen cycloaddition 'click chemistry' reaction with the azide group in AZT to afford cinnamate ester-AZT conjugates – novel heterocyclics with potential as dual-action HIV-1 integrase-reverse transcriptase (IN-RT) inhibitors. The parent cinnamate ester – AZT conjugate **89a** was modelled *in silico*., and a conformational search was conducted using Vega ZZ[113] programme to obtain the most stable conformation, The structure, at the DFT level using the Gaussian '03 programme,[90] and its docking into the HIV-1 IN and RT enzyme active-sites docking were explored using the Autodock 4.2 programme.[114,115] X-ray crystal structures of the integrase (1QS4) and the reverse transcriptase (1IKW) enzymes were obtained from the RCSB Protein Data Bank (PDB) and the ligand **89a** was docked separately into each of the active sites. The docking data show that the ligand fits reasonably within each active site, and also revealed potential hydrogen-bonding interactions with amino acid residues in the respective receptor cavities.

DABCO-catalysed reactions between 2-nitrobenzaldehydes and MVK typically afforded the expected BH β-hydroxy ketonic adducts, with competition products being isolated in some cases, *i.e.* the "MVK-dimer" and diastereomeric bis-MVK adducts. The isolation and full characterization of these side-products provided confirmation of their formation in a previous study and have been published as a journal article.[81]

Reaction of the MVK-derived BH ketones with *in situ*.-generated HCl afforded α-chloromethyl α,β-unsaturated ketones in essentially quantitative yields, based on NMR assessment. Treatment of these α-chloromethyl ketones with amines gave substitution products in low to moderate yields, but, in some instances, as intractable mixtures. Several attempts were made to react piperidine with the α-chloromethyl ketones but unfortunately, after much effort, only one α-piperidinylmethyl derivative **98b** could be isolated, and subsequently reductively cyclized to 3-(piperidinylmethyl)quinoline **99b**. The quinoline derivative **99b** and its precursor **98b** were submitted for antimalarial bioassay against quinine as the standard; the 3-(piperidinylmethyl)quinoline **99b** exhibited some antimalarial activity, while its precursor **98b** showed very weak activity. The positive result obtained for **99b** supports the idea that the small structural difference between these 3-(aminomethyl)quinolines and their 3-aminoquinoline analogues may be significant, since the 3-aminoquinolines do not show antimalarial activity.[14]

While there are a number of well established synthetic pathways to quinolines, [16-18] and quinolones,[46-48] the Morita-Baylis-Hillman approach seemed well-suited to access 3-aminomethyl-substituted analogues and has been the focus of this investigation.

Consequently, in spite of variable yields and the frustrating difficulties encountered in isolating and purifying most of the products, we persevered and succeeded in obtaining three 3-(aminomethyl)- substituted quinolines and four 3-substituted-2-quinolones. It is clear, however, that there is room for improvement in the synthetic and separation protocols. It is also possible that functional elaboration of BH-derived 3-hydroxymethyl substituted quinolines,[94] might offer a more efficient access to the targeted 3-(aminomethyl)quinolines.

Reactions of another secondary amine, piperazine, with the BH-derived α-chloromethyl ketones afforded bis-substituted piperazines, which have $C_2$ symmetry axis and might therefore serve as HIV-1 protease inhibitors. Generally, isolation of these conjugate reaction products was very difficult and, in some instances, impossible. Successful access to quinolines derivatives finally came as a consolation towards the end of the project, as we were able to isolate two additional novel quinoline derivatives.

Access to 3-(aminomethyl)-2-quinolones was attempted using Fe/acetic acid to reductively cyclize 3-hydroxy-3-(2-nitrophenyl)-2-[(piperidin-1-yl)methyl]propanoate-to-3-[(piperidinyl)methyl]-1*H*-quinoline-2-one, but 3-acetoxyquinolone was isolated instead. Again, successful access to 2-quinolones was eventually achieved using Pd-C catalyst.

While this research has resulted in the successful production of potentially interesting BH-derived compounds, several avenues can be identified for future investigation. These include the following –

i) Optimising the reaction conditions and isolation methods used to access the 3-(aminomethyl)quinoline and 2-quinolone derivatives.

ii) Exploring the use of BH-derived 3-hydroxymethylquinolines as a more efficient alternative to to access 3-(aminomethyl)quinolines.

iii) Biological screening of the cinnamate esters as HIV-1 IN inhibitors, the cinnamate ester-AZT conjugates as dual-action HIV-1 IN-RT inhibitors, the quinolines and quinolones as antimalarials and the bis-substituted piperazine derivatives as HIV-1 integrase inhibitors and or protease inhibitors.

# 3. EXPERIMENTAL.

## GENERAL DIRECTIONS.

All $^1H$ and $^{13}C$ NMR spectra were recorded on Bruker AMX 400 MHz or Biospin 600 MHz spectrometers at 303K and were calibrated using solvent signals ($\delta_{H:}$ 7.25 ppm for $CDCl_3$, 2.50 ppm for DMSO-$d_6$ and 3.31 ppm (quintuplet) for methanol-$d_4$; $\delta c$ 77.0 ppm for $CDCl_3$, 39.4 ppm for DMSO-$d_6$ and 49.05 for methanol-$d_4$). IR spectra were recorded on a Perkin-Elmer FT-IR Spectrum 100 spectrometer (neat). Melting points were determined using a Reichert hot-stage apparatus and are uncorrected. High resolution mass spectra (HRMS) were recorded on Waters API-Q-TOF Ultima instrument (University of Stellenbosch).

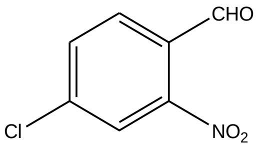
All chemical reagents were used as supplied by the manufacturer. Solvents were dried according to methods described by Perrin and Armarego[106] and Vogel.[107] Hence THF and $Et_2O$ were dried over sodium wire and distilled from sodium, using benzophenone as indicator, under nitrogen; $CH_2Cl_2$ was washed with conc. $H_2SO_4$, dried over $CaCl_2$ and distilled from $CaH_2$; *N*,*N*-dimethylformamide was pre-dried and distilled from 3Å molecular sieves under reduced pressure; ethanol and methanol were dried by reaction with magnesium turnings and iodine and distilled from the resulting magnesium alkoxide under nitrogen.

Flash chromatography was carried out using Merck silica gel 60 [230-430 mesh (particle size 0.040-0.063 mm)], sometimes basified using aqueous ammonia or triethylamine; basic alumina and Discovery® DCS-Diol were also used. Reverse-phase column chromatography was carried out using Sep-Pak columns (C18 Cartridges) and preparative layer chromatography was carried out using silica gel 60 $PF_{254}$. HPLC was carried out on a Partisil 10 Magnum 6 normal phase column using a Spectra-Physics P100 isocratic pump and a Waters K1410 differential refractometry detector. Thin layer chromatography (TLC) was done on pre-coated Merck silica gel $F_{254}$ plates on aluminum, visualization was executed by inspection under UV light (254/365nm) or following exposure to iodine.

Conformational search were conducted using Vega ZZ[113] to determine the most stable conformations, which were then optimized using Gaussian 03.[90] Docking simulation studies were done using Autodock 4.2, [114,115] and docked structures were visualized using Discovery Studio (DS) Visulaizer. [116]

## 3.1. PREPARATION OF 2-NITROBENZALDEHYDE PRECURSORS

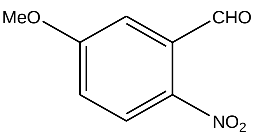
### 4-Chloro-2-nitrobenzaldehyde 81g



**Method 1.** A solution of 4-chloro-2-nitrobenzyl alcohol **82a** (1.9 g, 10 mmol), in dichloromethane (20 mL) was added rapidly at room temperature to a well-stirred suspension of pyridinium chlorochromate (PCC) (3.2 g, 15 mmol) in dichloromethane (15 mL) in an open glass beaker. After 2 h, the resulting black mixture was dispersed in dry diethyl ether (175 mL); the ethereal solution was then decanted and the residue was washed twice with diethyl ether. The combined ethereal solutions were filtered through Celite and concentrated *in vacuo* to afford a dark green oil which crystallized on standing. Recrystallization from hexane gave, as a yellow powder, 4-chloro-2-nitrobenzaldehyde **81g** (1.39 g, 75%), m.p. 66-68 °C (lit.[62] *ca* 50 – 60 °C); $\upsilon_{max}$ (ATR)/cm$^{-1}$ 1691 (C=O), 1519 and 1342 ($NO_2$); $\delta_H$ (400 MHz; $CDCl_3$) 7.54 (1H, d, *J* = 7.2 Hz, 6-H), 7.93 (1H, d, *J* = 7.2 Hz, 5-H), 8.08 (1H, s, 3-H) and 10.36 (1H, s, CHO); $\delta_C$ (100 MHz; $CDCl_3$) 124.7 (C-6), 129.3 (C-5), 130.9 (C-3), 134.0 (C-1), 140.1 (C-2), 150.0 (C-4) and 186.7 (C=O).

**Method 2.** A solution of 4-chloro-2-nitrotoluene **79a** (1.05 g, 6.11 mmol) in DMF-DMA (2.18 g, 2.43 mL 18.3 mmol) was heated at 170 °C (external oil bath temperature) for 20 h, the progress of the reaction being monitored by TLC. The brick-red reaction mixture containing the enamine **80a** was cooled to room temperature and then added dropwise to a solution of $NaIO_4$ (3.9 g, 18 mmol) in 50 % aqueous THF (50 mL); the mixture was stirred for 3 h or until the reaction was judged complete as the colour fades away. The insoluble white precipitate ($NaIO_3$) was removed by filtration and washed with EtOAc. The combined filtrate and washings were washed in turn with saturated aqueous $NaHCO_3$ (3 x 20 mL) and then dried over anhydrous $MgSO_4$. The resulting organic solution was concentrated *in vacuo* and the residue flash chromatographed [on silica gel; elution with $CH_2Cl_2$-hexane (1:1)] to afford, as a yellow powder, 4-chloro-2-nitrobenzaldehyde **81g** (0.27 g, 18 %), m.p. 80-82 °C.[63]

**5-methoxy-2-nitrobenzaldehyde 81h**



**Method 1.** A solution of 3-methyl-4-nitroanisole (1.03 g, 6.16 mmol) in DMF-DMA (2.94 g, 3.28 mL, 24.6 mmol) was irradiated in the microwave apparatus at 170 watts and 150 °C for 3 h, the reaction progress being monitored by TLC. The brick-red reaction mixture containing the enamine was cooled to room temperature and added dropwise to a solution of $NaIO_4$ (3.95 g, 18.5 mmol) in 50 % aqueous THF (30 mL); the mixture was stirred for 3 h or until the reaction was judged to be complete as the colour fades away. The insoluble white precipitate ($NaIO_3$) was removed by filtration and washed with EtOAc. The combined filtrate and washings were washed in turn with saturated aqueous $NaHCO_3$ (3 x 15mL) and then dried over anhydrous $MgSO_4$. Concentration *in vacuo* and flash chromatography [on silica gel; eluting with $CH_2Cl_2$-hexane (1:1)] afforded, as a bright yellow powder, 5-methoxy-2-nitrobenzaldehyde **81h** (0.7 g, 68%), m.p. 80-82 °C (lit.[63] 82-83 °C) (Found $M^+$: 181.0378. Calc for $C_8H_7NO_4$, $MH^+$: 181.0378); $\upsilon_{max}$ (ATR)/$cm^{-1}$ 1689 (C=O), 1577 and 1282 ($NO_2$); $\delta_H$ (400 MHz; $CDCl_3$) 3.94 (3H, s, $OCH_3$), 7.14 (1H, dd, $J$ = 9.2 and 2.6 Hz, 4-H), 7.30 (1H, d, $J$ = 2.6 Hz, 6-H), 8.14 (1H, d, $J$ = 9.2 Hz, 3-H) and 10.45 (1H, s, CHO); $\delta_C$ (100 MHz; $CDCl_3$) 56.3 (O*C*$H_3$), 113.3 (C-6), 118.5 (C-4), 127.2 (C-3), 134.3 (C-1), 142.3 (C-2), 164.0 (C-5) and 188.4 (C=O).

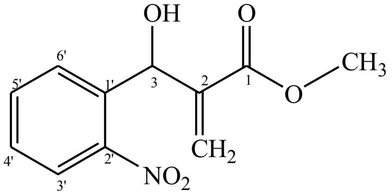
**Method 2.** A solution of 3-methyl-4-nitroanisole (2.57 g, 15.4 mmol) in DMF-DMA (7.32 g, 8.20 mL, 61.4 mmol) was heated at 170 °C (external oil bath temperature) for 20 h, reaction progress being monitored by TLC. The brick-red reaction mixture containing the enamine was cooled to room temperature and added dropwise to a solution of $NaIO_4$ (9.9 g, 46 mmol) in 50 % aqueous THF (50 mL) and the mixture was stirred for 3 h or until the reaction was judged to be complete as the colour fades away. The insoluble material $NaIO_3$ was removed by filtration and washed with EtOAc. The combined filtrate and washings were washed in turn with saturated aqueous $NaHCO_3$ solution (3 x 20 mL) and then dried over anhydrous $MgSO_4$. The resulting organic solution was concentrated *in vacuo* and flash chromatographed [on silica gel; eluting with $CH_2Cl_2$-hexane (1:1)] to afford, as a bright yellow powder, 5-methoxy-2-nitrobenzaldehyde (0.7 g, 63 %), m.p. 80-82 °C.

**Method 3.** To a stirred aqueous solution of 5-hydroxy-2-nitrobenzaldehyde **81b** (10.58 g, 63.30 mmol) and dimethyl sulphate (31.94 g, 23.96 mL, 253.2 mmol) in a two-necked flask, fitted with dropping funnel and reflux condenser, was added drop-wise 30% aqueous NaOH (45 mL) to maintain an alkaline pH (between 9-10). The mixture was warmed on a water bath to 40 °C; however, the temperature went above 40 °C and heating was stopped immediately and the reaction mixture cooled using cold water. The reaction mixture was left to stir for 5 h at 40 °C and pH 9 – 10, and then cooled to 10 °C. The resulting precipitate was filtered off under suction to give a filter cake which was washed with cold water (3 x 30 mL) and dried in the air (fume hood) to give as a yellow solid, 5-methoxy-2-nitrobenzalidehyde (0.7 g, 63 %), m.p. 80-82 °C.

**CAUTION:** *Dimethyl sulphate is a pathogenic substance. As a precaution, an open beaker of concentrated ammonia solution was placed in the fume-cupboard, to mop-up any* $(Me)_2SO_4$ *vapour or spills.*[63]

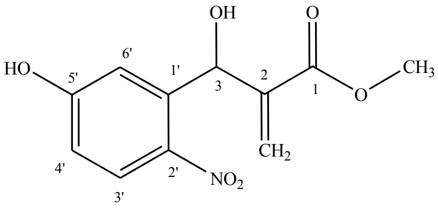
## 3.2. SYNTHESIS OF BAYLIS-HILLMAN ESTERS.

**Methyl 3-hydroxy-2-methylene-3-(2-nitrophenyl)propanoate 84a**



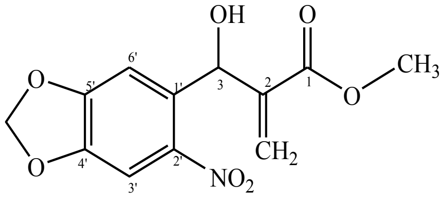
To a solution of 2-nitrobenzaldehyde **81a** (3.0 g, 19.9 mmol), methyl acrylate **83** (2.7 mL, 2.6 g, 30 mmol) in $CHCl_3$ (0.6 mL) was added DABCO (0.12 g, 1.0 mmol). The mixture was left to stir at room temperature in a stoppered flask for 2 days, the progress of reaction being monitored daily by TLC. Excess solvent and unreacted methyl acrylate were evaporated from the crude mixture *in vacuo,* and the residue flash chromatographed [on silica gel eluting; with EtOAc-hexane (1:3)] to afford, as a lemon-green oil, methyl 3-hydroxy-2-methylene-3-(2-nitrophenyl)propanoate **84a** (3.9 g, 84%) (lit.[74] cited as solid, m.p. 36-38°C and oil[102] ); $\upsilon_{max}$ (ATR)/cm$^{-1}$ 3443 (OH) and 1711 (C=O); $\delta_H$ (400 MHz; $CDCl_3$) 3.61 (3H, s, $CH_3$), 3.88 (1H, br s, CHO*H*), 5.63 and 6.23 (2H, 2 x s, C=C*$H_2$*), 6.12 (1H, s, C*H*OH), 7.38 (1H, t, *J* = 7.6 Hz, 4'-H), 7.56 (1H, t, *J* = 7.6 Hz, 5'-H), 7.67 (1H, d, *J* = 8.0 Hz, 6'-H) and 7.83 (1H, d, *J* = 8.0 Hz, 3'-H ); $\delta_C$ (100 MHz: $CDCl_3$**)** 52.0 ($CH_3$), 67.3 (C-3), 124.3 (C-3'), 126.1 (C=*C*$H_2$), 128.5 (C-4'), 128.7 (C-6'), 133.2 (C-5'), 136.1 (C-1'), 140.9 (*C*=$CH_2$), 148.1 (C-2') and 166.2 (C=O).

### Methyl 3-hydroxy-3-(5-hydroxy-2-nitrophenyl)2-methylene-propanoate 84b.



To a solution of 5-hydroxy-2-nitrobenzaldehyde **81b** (5.0 g, 30 mmol), methyl acrylate (7.8 mL, 7.5g; 87 mmol) in $CHCl_3$-MeOH (1:1): (20.0 mL) was added DABCO (0.5 g, 4 mmol). The mixture was left to stir at room temperature in a stoppered flask for 6 weeks, the progress of reaction being monitored regularly by TLC. Excess solvent and unreacted methyl acrylate were evaporated from the crude mixture *in vacuo,* and the residue flash chromatographed [on silica gel eluting with EtOAc-hexane (2:3)] to afford, as a reddish-brown solid, *methyl 3-hydroxy-3-(5-hydroxy-2-nitrophenyl)-2-methylene propanoate* **84b** (2.7 g, 37%), m.p. 121-123 °C (lit.[69]cited without full set of data) (Found M-1: 252.0525. Calc. for $C_{11}H_{10}NO_6$, M-H: 252.0508); $\upsilon_{max}$ (ATR)/$cm^{-1}$ 3346 (OH) and 1684 (C=O); $\delta_H$ (400 MHz; $CDCl_3$) 3.43 (1H, br, CHO*H*), 3.68 (3H, s, $CH_3$), 6.02 and 6.19 (2H, 2 x d, *J* = 5.0 Hz, C=$CH_2$), 6.11 (1H, s, C*H*OH), 6.85 (1H, d, *J* = 8.8 Hz, 4'-H), 7.16 (1H, d, *J* = 2.0 Hz, 6'-H), 7.98 (1H, d, *J* = 8.8 Hz, 3'-H) and 10.88 (1H, br, Ar-OH ); $\delta_C$ (100 MHz; $CDCl_3$) 51.8 ($CH_3$), 65.6 (C-3), 114.7 (C-6'), 114.9 (C-4'), 124.3 (C=*C*$H_2$), 127.7 (C-3'), 139.0 (C-1'), 141.5 (C-2'), 143.1 (*C*=$CH_2$), 162.4 (C-5') and 165.8 (C=O).

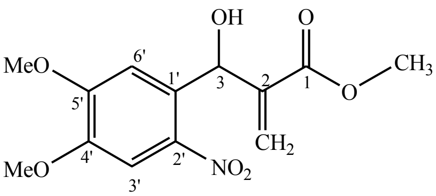
### Methyl 3-hydroxy-2-methylene-3-(4,5-methylenedioxy-2-nitrophenyl)propanoate 84c.



The procedure described for the preparation of methyl 3-hydroxy-2-methylene-3-(2-nitrophenyl)propanoate **84a** was followed, using 3,4-methylenedioxy-2-nitrobenzaldehyde **81c** (5.4 g; 28 mmol), methyl acrylate (7.5 mL, 7.2 g, 83 mmol) and DABCO (0.34 g, 3.1 mmol) in $CHCl_3$ (10 mL) and stirring for approximately 4 weeks.

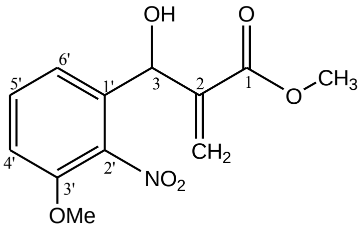
The crude product was purified by flash chromatography [on silica gel; eluting with chloroform-EtOAc-hexane (1:1:3)] to afford, as a pale yellow powder, methyl 3-hydroxy-2-methylene-3-(4,5-methylenedioxy-2-nitrophenyl)- propanoate **84c** (4.9 g, 63%), m.p. 120-122 °C (lit.[73] 128-129 °C); $\upsilon_{max}$ (ATR)/cm$^{-1}$ 3488 (OH) and 1709 (C=O); $\delta_H$ (400 MHz; $CDCl_3$) 3.43 (1H, br s, CHO*H*), 3.74 (3H, s, $CH_3$ ), 5.69 and 6.33 (2H, 2 x s, C=C*H*$_2$), 6.11 (2H, s, OC*H*$_2$O), 6.17 (1H, s, C*H*OH), 7.16 (1H, s, 6'-H) and 7.49 (1H, s, 3'-H); $\delta_C$ (100 MHz, $CDCl_3$) 52.2 ($CH_3$), 67.7 (C-3), 103.1 ($OCH_2O$), 105.5 (C-3'), 107.7 (C-6'), 126.2 (C=*C*H$_2$), 133.6 (C-1'), 140.8 (*C*=CH$_2$), 142.2 (C-2'), 147.3 (C-5'), 152.2 (C-4') and 166.5 (C=O).

**Methyl 3-hydroxy-3-(4,5-dimethoxy-2-nitrophenyl)-2-methylenepropanoate 84d.**



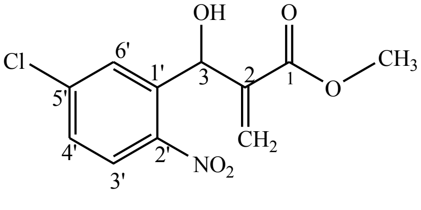
The procedure described for the synthesis of methyl 3-hydroxy-2-methylene-3-(2-nitrophenyl)- propanoate **84a** was followed, using 4,5-dimethoxy-2-nitrobenzaldehyde **81d** (4.9 g, 23 mmol), methyl acrylate (6.4 mL, 6.1 g, 71 mmol), DABCO (0.43 g 3.9 mmol) in $CHCl_3$ (8 mL). . The crude product was purified by flash chromatography [on silica gel; eluting with chloroform-EtOAc-hexane (3:1:1)] to afford, as a golden-brown solid, methyl 3-hydroxy-3-(3-methoxy-2-nitrophenyl)-2-methylenepropanoate **84e** (4.5 g, 66%), m.p. 72-74 °C (lit.[101] cited as brownish-oil); $\upsilon_{max}$ (ATR)/cm$^{-1}$ 3301 (OH) and 1721 (C=O); $\delta_H$ (400 MHz; $CDCl_3$) 3.71 (3H, s, $CO_2CH_3$ ), 3.92 (6H, d, *J* = 8.8 Hz, 2x $OCH_3$), 5.50 (1H, s, C*H*OH), 6.21 and 6.25 (2H, 2 x s, C=CH$_2$), 7.21 (1H, s, 6'-H) and 7.56 (1H, s, 3'-H); $\delta_C$ (100 MHz, $CDCl_3$) 51.7 (CO$_2$*C*H$_3$), 55.8 and 55.9. (2 x $ArOCH_3$), 66.6 (C-3'), 107.4 (C-6'), 109.6 (C-3'), 125.1 (C=*C*H$_2$), 131.3 (C-1'), 139.5 (*C*=CH$_2$), 141.5 (C-2'), 147.5 (C-5'), 152.2 (C-4') and 166.5 (C=O).

**Methyl 3-hydroxy-3-(3-methoxy-2-nitrophenyl)-2-methylenepropanoate 84e.**



The procedure described for the synthesis of methyl 3-hydroxy-2-methylene-3-(2-nitrophenyl)- propanoate **84a** was followed, using 3-methoxy-2-nitrobenzaldehyde **81e** (2.8 g, 15 mmol), methyl acrylate **83** (2.1 mL, 2.0 g, 23 mmol), DABCO (0.1 g, 0.8 mmol) in $CHCl_3$ (2 mL). The mixture was left to stir at room temperature in a stoppered flask for seven days. The crude product was purified by flash chromatography [on silica gel; eluting with EtOAc-hexane (1:1)] to afford, as a leafy-green solid, *methyl 3-hydroxy-3-(3-methoxy-2-nitrophenyl)-2-methylene-propanoate* **84e** (3.7 g, 91%), m.p. 114-116 °C (lit.$^3$ 112-114 °C) (Found M: 266.0658. Calc. for $C_{12}H_{12}NO_6$. M-H: 266.0665); $\upsilon_{max}$ (ATR)/cm$^{-1}$ 3483 (OH) and 1709 (C=O); $\delta_H$ (400 MHz; $CDCl_3$) 3.28 (1H, d, *J* = 3.2 Hz, CHO*H*), 3.69 (3H, s, $CO_2CH_3$ ), 3.88 (3H, s, $ArOCH_3$*)*, 5.63 (1H, d, *J* = 3.6 Hz, C*H*OH)), 5.86 and 6.40 (2H, 2 x s, C=$CH_2$), 7.00 (1H, d, *J* = 8.0 Hz, 6'-H), 7.10 (1H, d, *J* = 7.6 Hz, 4'-H) and 7.43 (1H, t, *J* = 8.0 Hz, 5'-H); $\delta_C$ (100 MHz; $CDCl_3$**)** 52.1 ($CO_2$*C*$H_3$), 56.5 ($ArOCH_3$), 68.3 (C-3), 112.2 (C-4'), 119.3 (C-6'), 127.3 (C=*C*$H_2$), 131.3 (C-5'), 134.4 (*C*=$CH_2$), 139.6 (C-3'), 140.4 (C-1'), 150.9 (C-2') and 166.1 (C=O).

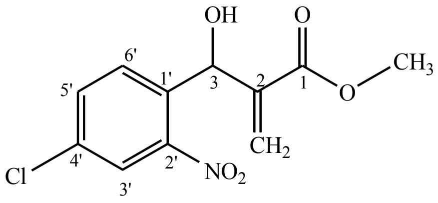
**Methyl 3-(5-chloro-2-nitrophenyl)-3-hydroxy-2-methylenepropanoate 84f.**



The procedure described for the synthesis of methyl 3-hydroxy-2-methylene-3-(2-nitrophenyl)-propanoate **84a** was followed, using 5-chloro-2-nitrobenzaldehyde **81f** (5.1 g, 28 mmol), methyl acrylate (3.8 mL, 3.6 g, 42 mmol), DABCO (0.2 g, 1 mmol) in

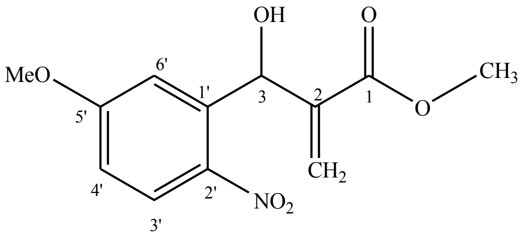
$CHCl_3$ (9 mL). The mixture was left to stir at room temperature in a stoppered flask for seven days. The crude product was purified by flash chromatography [on silica gel; eluting with EtOAc-hexane (7:1)] to afford, as a golden-brown solid, methyl 3-(5-chloro-2-nitrophenyl)-3-hydroxy-2-methylenepropanoate **84f** (4.1 g, 55%), m.p. 64-66 °C (lit.$^5$ cited as brownish-oil); $\upsilon_{max}$ (ATR)/cm$^{-1}$ 3468 (OH) and 1709 (C=O); $\delta_H$ (400 MHz; $CDCl_3$) 3.63 (3H, d, *J* = 2.4 Hz, $CO_2CH_3$), 4.21 (1H, br s, CHO*H*), 5.59 (1H, s, C*H*OH), 6.14 and 6.21 (2H, 2 x s, C=$CH_2$), 7.33 (1H, d, *J* = 8.4 Hz, 3'-H), 7.67 (1H, s, 6'-H) and 7.83 (1H, dd, *J* = 2 and 8.8 Hz, 4'-H); $\delta_C$ (100 MHz; $CDCl_3$**)** 52.0 ($CO_2$*C*$H_3$), 66.5 (C-3), 125.9 (C-3'), 126.2 (C=*C*$H_2$), 128.4 (C-4'), 128.8 (C-6'), 138.5 (C-5'), 139.6 (C-1'), 140.7 (*C*=$CH_2$), 145.8 (C-2') and 165.9 (C=O).

**Methyl 3-(4-chloro-2-nitrophenyl)-3-hydroxy-2-methylenepropanoate 84g.**



The procedure described for the synthesis of methyl 3-hydroxy-2-methylene-3-(2-nitrophenyl)- propanoate **84a** was followed, using 4-chloro-2-nitrobenzaldehyde **81g** (0.7 g, 4 mmol), methyl acrylate (0.4 mL, 0.4 g, 6 mmol), DABCO (0.02 g, 0.18mmol) in $CHCl_3$ (0.5 mL). The mixture was left to stir at room temperature in a stoppered flask for six days. The crude product was purified by flash chromatography [on silica gel; eluting with EtOAc-hexane (1:3)] to afford, as a brown viscous oil which crystallized on standing, *methyl 3-(4-chloro-2-nitrophenyl)-3-hydroxy-2-methylenepropanoate* **84g** (0.77 g, 78 %), m.p. 78-80 °C (Found M$^+$ + 23: 294.0151. Calc. for $C_{11}H_{10}NO_5NaCl$: 294.0145); $\upsilon_{max}$ (ATR)/cm$^{-1}$ 3478 (OH) and 1709 (C=O); $\delta_H$ (400 MHz; $CDCl_3$) 3.47 (1H, br s, CHO*H*), 3.72 (3H, s, $CO_2CH_3$), 5.71 (1H, s, C*H*OH), 6.14 and 6.35 (2H, 2 x s, C=$CH_2$), 7.61 (1H, d, *J* = 9.2 Hz, 6'-H), 7.71 (1H, d, *J* = 8.4 Hz, 5'-H) and 7.93 (1H, s, 5'-H); $\delta_C$ (100 MHz; $CDCl_3$**)** 52.1 ($CO_2$*C*$H_3$), 66.6 (C-3), 124.3 (C-3'), 126.3 (C=*C*$H_2$), 130.1 (C-4'), 133.2 (C-6'), 134.0 (C-5'), 134.8 (C-1'), 140.6 (*C*=$CH_2$), 148.1 (C-2') and 166.1 (C=O).

**Methyl 3-hydroxy-3-(5-methoxy-2-nitrophenyl)-2-methylenepropanoate 84h.**



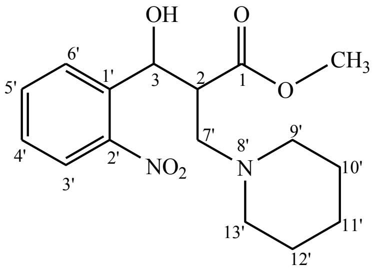
The procedure described for the synthesis of methyl 3-hydroxy-2-methylene-3-(2-nitrophenyl)- propanoate **84a** was followed, using 5-methoxy-2-nitrobenzaldehyde **81h** (5.5 g, 30 mmol), methyl acrylate (4.1 mL, 4.0 g, 45 mmol), DABCO (0.2 g, 2 mmol) in $CHCl_3$ (19 mL). The mixture was left to stir at room temperature in a stoppered flask for seven days. The crude product was purified by flash chromatography [on silica gel; eluting with EtOAc-hexane (1:3)] to afford, as a yellowish solid, *methyl 3-hydroxy-3-(5-methoxy-2-nitrophenyl)-2-methylene-propanoate* **84h** (6.5 g, 80%), m.p. 69-71 °C (Found M – OH: 250.O719, Calc. for $C_{12}H_{12}NO_5$: 250.0715); $\upsilon_{max}$ (ATR)/cm$^{-1}$ 3341 (OH) and 1719 (C=O); $\delta_H$ (400 MHz; $CDCl_3$) 3.60 (1H, br, s, CHO*H*), 3.74 (3H, s, $CO_2CH_3$ ), 3.88 (3H, s, $ArOCH_3$), 5.56 (1H, s, C*H*OH), 6.30 and 6.27 (2H, 2 x d, *J* = 7.6 and 2.8 Hz, C=$CH_2$), 6. 90 (1H, dd, *J* = 9.2 and 2.8 Hz, 4'-H), 7.27 (1H, d, *J* = 2.4 Hz, 6'-H), and 8.08 (1H, d, *J* = 7.6 Hz, 3'-H); $\delta_C$ (100MHz; $CDCl_3$) 52.2 ($CO_2$*C*$H_3$), 55.9 ($ArOCH_3$), 66.8 (C-3), 113.4 (C-6'), 113.6 (C-4'), 126.1 (C=*C*$H_2$), 127.7 (C-3'), 139.5 (C-1'), 140.7 (*C*=$CH_2$), 141.0 (C-2'), 163.7 (C-5') and 166.6 (C=O).

## 3.3. REACTIONS OF BH ESTERS WITH AMINES.

**General Method:** A solution of the Morita-Baylis-Hillman ester (1 eq.) and the specified amine (1.5 eq.) in anhydrous THF was stirred in a stoppered flask at room temperature for periods which varied according to the reactants involved. The solution was then concentrated *in vacuo* and the crude product purified by flash chromatography to afford the aminated product. $^1$H NMR and $^{13}$C NMR data are cited for the major product in each case.

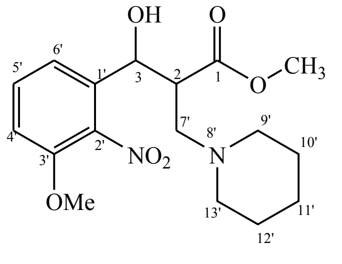
### 3.3.1. Reactions with piperidine.

**Methyl 3-hydroxy-3-(2-nitrophenyl)-2-[(piperidin-1-yl)methyl]propanoate 85a**



A mixture of methyl 3-hydroxy-2-methylene-3-(2-nitrophenyl)propanoate **84a** (0.57 g, 2.4 mmol), piperidine (0.5 mL, 5 mmol) and anhydrous THF (5 mL) was stirred in a stoppered flask for 2 days. Excess solvent and unreacted piperidine were evaporated *in vacuo* and the residue was purified by flash chromatography [on silica gel; eluting with hexane-EtOAc (1:1)] to afford, as a yellow solid, comprising a 2:1 diastereomeric mixture, of methyl 3-hydroxy-2-methylene-3-(2-nitrophenyl)-2-[(piperidi-1-nyl)methy]-propanoate **86a** (0.6 g, 77%), m.p. 73-75 °C (lit.[103] 76-78 °C) (Found M+H: 323.1609. Calc. for $C_{16}H_{23}N_2O_5$, 323.1607); $\upsilon_{max}$ (ATR)/cm$^{-1}$ 3396 (OH) and 1716 (C=O); $\delta_H$ (400 MHz; $CDCl_3$) 1.44 (2H, m, 11'-$CH_2$), 1.51 (4H, m, 10'- and 12'-$CH_2$), 2.43 (4H, m, 9'- and 13'-$CH_2$), 2.78 (2H, m, 7'-$CH_2$), 3.02 (1H, t, *J* = 11.8 Hz, 2-H), 3.14 (1H, m, CHO*H*), 3.43 (3H, s, $CO_2CH_3$), 5.53 (1H, d, *J* = 8.8 Hz, 3-H), 7.35 (1H, m, 4'-H), 7.55 (1H, t, *J* = 7.6 Hz, 5'-H) and 7.63 – 7.69 (2H, m, 3'- and 6'-H); $\delta_C$ (100 MHz; $CDCl_3$) 23.8 (C-11'), 25.8 (C-2), 26.0 (C-10' and -12'), 51.6 ($CO_2\mathit{C}H_3$), 54.9 (C-9' and 13'), 60.6 (C-7'), 73.0 (C-3), 123.8 (C-6'), 128.3 (C-4'), 129.3 (C-5'), 132.2 (C-3'), 136.6 (C-1'), 149.0 (C-2') and 171.4 (C=O).

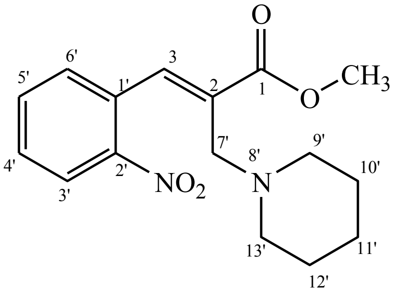
**Methyl 3-hydroxy-3-(3-methoxy-2-nitrophenyl)-2-[(piperidin-1-yl)methyl]propanoate 85e.**



The general method was followed, using methyl 3-hydroxy-3-(3-methoxy-2-nitrophenyl)2-methylene-propanoate, **84e** (1.4 g, 5.2 mmol), piperidine (0.8 mL, 8 mmol) and anhydrous THF (5 mL). After 3 days, the mixture was concentrated *in vacuo* and the crude product purified by flash chromatography [on silica gel; eluting with 5% THF in DCM) to afford a brown oil, comprising (4:3) diastereomeric mixture of *methyl 3-hydroxy-3-(3-methoxy-2-nitrophenyl)-2-[(piperidin-1-yl)methyl]propanoate* **85e** (4.18 g, 81%) (cited as brick red solid.[73]) (Found: M+1, 353.1720. Calc. for $C_{17}H_{25}N_2O_6$, 353.1713); $\upsilon_{max}$ (ATR)/cm$^{-1}$ 3365 (OH) and 1744 (C=O); $\delta_H$ (400 MHz; $CDCl_3$), 1.34 – 1.46 (6H, m, 10'-, 11'- and 12'-$CH_2$), 2.33 (4H, m, 9'- and 13'-$CH_2$), 2.55 (2H, m, 7'-$CH_2$), 3.09 (1H, m, 2-H), 3.55 (3H, s, $CO_2CH_3$), 3.65 (1H, br s, CHO*H*), 3.81 ( 3H, s, $ArOCH_3$), 4.98 (1H, d, *J* = 4.4 Hz, 3-H), 6.93 (1H, d, *J* = 7.6 Hz, 6'-H), 7.06 (1H, d, *J* = 7.6 Hz, 4'-H) and 7.38 (1H, t, *J* = 7.2 Hz, 5'-H); $\delta_C$ (100 MHz; $CDCl_3$); 23.9 (C-11'), 25.8 (C-10' and -12'), 30.1 (C-2), 48.7 (C-3), 51.6 ($CO_2CH_3$), 54.5 (C-9' and -13'), 56.2 ($ArOCH_3$), 69.0 (C-7'), 111.5 (C-4'), 118.8 (C-6'), 128.0 (C-5'), 130.6 (C-1'), 135.2 (C-2'), 150.3 (C-3') and 173.2 (C=O).

## 3.4. DEHYDRATION TO CINNAMATE ESTERS.

### Methyl (E)- (2-nitrophenyl) 2-[(piperidin-1-ynyl)methyl]-3-propenoate 86a.

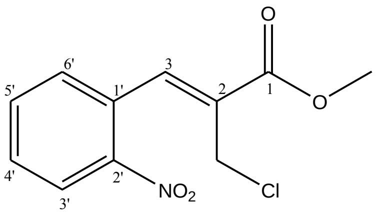


To a refluxing solution of methyl 3-hydroxy-3-(2-nitrophenyl)-2-[(piperidin-1-yl)methyl]propanoate **85a (**0.24 g, 0.8 mmol) in anhydrous toluene (10 mL) was added anhydrous $CuCl_2$ (0.01 g, 0.08 mmol) and EDC (0.27 mL, 1.5 mmol), under nitrogen. After 4 hours, the mixture was cooled to room temperature, diluted with water (15 mL) to quench the reaction and extracted with EtOAc (15 mL x 3). The combined organic layers were washed sequentially with aqueous citric acid, aqueous $NaHCO_3$ and brine, and then dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. The crude residue obtained was purified by flash chromatography [on neutral alumina; eluting with hexane-EtOAc (11:1)] to afford, a yellow transparent oil, comprising a diastereomeric mixture (*ca.*3:1), (*E:Z ca.* 3:1) of *methyl 2-(2-nitrophenyl)-[(piperidin-1-ynylamino)methyl]-3-propenoate* **86a** (0.04 g, 15%) [(Found: M+H, 305.1501 Calc. for $C_{16}H_{21}N_2O_4$, 305.1496); $\upsilon_{max}$ (ATR)/cm$^{-1}$ 1720 (C=O); $\delta_H$ (400 MHz; $CDCl_3$), 1.54 (6H, m, 10'-, 11'- and 12'-$CH_2$), 1.80 (4H, m, 9'- and 13'-$CH_2$), 2.72 (3H, br s, $CO_2CH_3$), 3.68 (2H, s, 7'-$CH_2$), 7.65 (1H, m, 6'-H), 7.75 (1H, m, 4'-H), 7.85 (1H, m, 3'-H), 8.20 (1H, s, 3-H) and 8.32 (1H, d, *J* = 5.6 Hz, 5'-H); $\delta_C$ (100 MHz; $CDCl_3$); 24.2 (C-10' and -11') , 25.9 (C-12'), 51.5 (C-9' and -13'), 52.1 (C-7'), 54.3 ($CO_2CH_3$), 124.4 (C-6'), 128.4 (C-4'), 130.5 (C-5'), 131.2 (C-1'), 131.9 (C-2), 133.0 (C-5'), 147.7 (C-2'), and 167.9 (C=O).

## 3.5. SYNTHESIS OF (Z) ALLYL CHLORIDES.

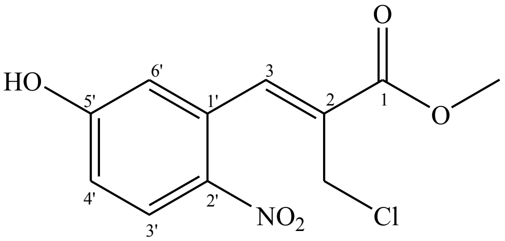
**General Procedure:**[74] Phosphorus oxychloride ($POCl_3$) (2 eq.) was added to DMF and the resulting mixture was stirred at room temperature for 10 mins. $CH_2Cl_2$ was then added, followed by a solution of the Baylis-Hillman adduct (**84**) (1 eq.) in $CH_2Cl_2$, in one portion. The mixture was stirred at room temperature and the reaction monitored (TLC) until completion. Water was added, and the organic phase was separated and washed with brine, dried over anhydrous $MgSO_4$ and concentrated *in vacuo* to give the crude product. Purification by flash chromatography [on silica gel; eluting with hexane: EtOAc (5:1)] afforded the desired product (**87**). The different solid products varied in colour from light to intensely deep yellow.

### Methyl (*Z*)-2-(chloromethyl)-3-(2-nitrophenyl)propenoate 87a.



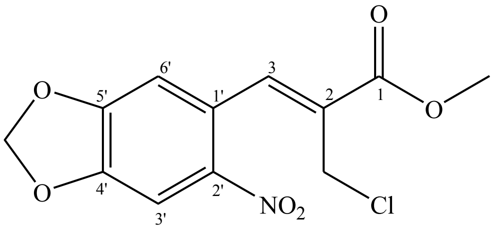
The general procedure was followed using $POCl_3$ (0.61 g, 4.0 mmol) in DMF (0.6 mL), $CH_2Cl_2$ (20 mL) and a solution of methyl 3-hydroxy-2-methylene-3-(2-nitrophenyl)propanoate **84a** (0.47 g, 2.0 mmol) in $CH_2Cl_2$ (5.0 mL). Work-up and flash chromatography gave, as an off-white solid, methyl **(***Z***)-**2-(chloromethyl)-4-(2-nitrophenyl)propenoate **87a** (0.31 g, 61%), m.p 53-55 $^{\circ}$C (lit.[74,11] 47-49 $^{\circ}$C); (Found M+1:256.0377. Calc. for $C_{11}H_{11}NO_4Cl$, M+1: 256.0371); $\upsilon_{max}$ (ATR)/cm$^{-1}$ 1712 (C=O), $\delta_H$ (400 MHz; $CDCl_3$ ), 3.88 (3H, s, $OCH_3$), 4.13 (2H, s, $CH_2Cl$), 7.61 (1H, t, *J* = 7.6 Hz, 4'-H), 7.66 (1H, d, *J* = 7.2 Hz, 6'-H), 7.76 (1H, t, *J* = 7.4 Hz, 5'-H), 8.02 ( 1H, s, 3-H) and 8.20 (1H, d, *J* = 8.0 Hz, 3'-H); $\delta_C$ (100 MHz; $CDCl_3$ ), 38.5 ($CH_2Cl$), 52.6 ($OCH_3$), 125.1 (C-4'), 129.7 ( C-2), 130.1 (C-6'), 130.2 (C-1'), 130.6 (C-5'), 134.0 (C-3), 140.3 (C-3'), 147.2 (C-2') and 165.6 (C=O).

### Methyl (*Z*)-2-(chloromethyl)-3-(5-hydroxy-2-nitrophenyl)propenoate 87b.



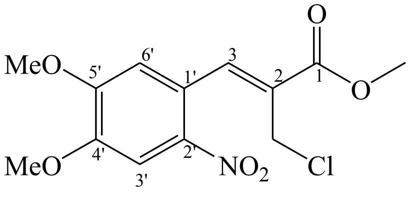
The general procedure was followed using $POCl_3$ (2.4 g, 16 mmol) in DMF (2.3 mL), $CH_2Cl_2$ (70 mL) and a solution of methyl 3-hydroxy-3-(5-hydroxy-2-nitrophenyl)-2-methylene-propanoate **84b** (2.0 g, 7.8 mmol) in $CH_2Cl_2$ (15.0 mL). Work-up and flash chromatography gave, as a brown solid, methyl **(***Z***)-***2-(chloromethyl)-4-(5-hydroxy-2-nitrophenyl)propenoate* **87b** (1.5 g, 72%), m.p 110-112 $^{\circ}$C (Found M+1: 272.0327. Calc for $C_{11}H_{11}NO_5Cl$: 272.0326); $\upsilon_{max}$ (ATR)/cm$^{-1}$ 1679 (C=O); $\delta_H$ (400 MHz; methanol-$d_4$ ), 3.94 (3H, s, $OCH_3$), 4.32 (2H, s, $CH_2Cl$), 6.99 (1H, s, 4'-H), 7.01 (1H, s, 6'-H), 8.13 (1H, s, 3-H) and 8.21 (1H, d, *J* = 8.0 Hz, 3'-H); $\delta_C$ (100 MHz; methanol-$d_4$ ), 39.3 ($CH_2Cl$), 53.0 ($OCH_3$), 117.2 (C-4'), 117.4 ( C-3'), 129.1 (C-6'), 130.1 (C-1'), 134.3 (C-5'), 140.4 (C-2), 143.0 (C-3), 164.2 (C-5') and 167.4 (C=O).

**Methyl (*Z*)-2-(chloromethyl)-3-(4,5-methylenedioxy-2-nitrophenyl)propenoate 87c.**



The general procedure was followed using $POCl_3$ (2.4 g, 17 mmol) in DMF (2.5 mL), $CH_2Cl_2$ (105 mL) and a solution of methyl 3-hydroxy-2-methylene-3-(4,5-methylenedioxy-2-nitrophenyl)propanoate **84c** (2.4 g, 8.4 mmol) in $CH_2Cl_2$ (20 mL). Work-up, and crystallization from EtOAc, afforded as a bright yellow solid, *methyl (Z)-2-(chloromethyl)-3-(4,5-methylenedioxy-2-nitrophenyl)propenoate* **87c**, (1.33 g, 53%), m.p. 197-199 °C (Found $M^+$+23: 338.0414. Calc. for $C_{13}H_{14}NO_6NaCl$: M+Na,338.0408) (Found: C, 47.95, H, 3.55; N, 4.6. Calc. for $C_{13}H_{14}ClNO_6$: C, 48.10, H, 3.36, N, 4.67%); $\upsilon_{max}$ (ATR)/cm$^{-1}$ 1712 (C=O); $\delta_H$ (400 MHz; DMSO-$d_6$) 3.82 (3H, s, $OCH_3$), 4.32 (2H, s, $CH_2Cl$), 6.32 (2H, s , $OCH_2O$), 7.04 (1H, s, 6'-H), 7.84 (1H, s, 3'-H) and 8.00 (1H, s, 3-H); $\delta_C$ (100 MHz; DMSO-$d_6$) 38.9 ($CH_2Cl$), 52.4 ($OCH_3$), 104.0 ($OCH_2O$),105.4 (C-6'), 108.5 (C-3), 126.2 (C-2), 128.1 (C-1'), 141.3 (C-4’), 141.7 (C-3), 148.5 (C-5'), 152.2 (C-2') and 165.3 (C=O).

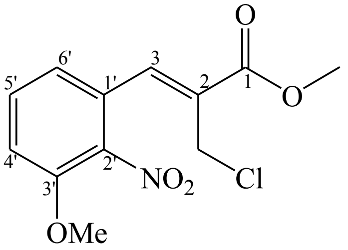
**Methyl (*Z*)-2-(chloromethyl)-3-(4,5-dimethoxy-2-nitrophenyl)propenoate 87d.**



The general procedure was followed using $POCl_3$ (2.75 g, 18.0 mmol) in DMF (2.7 mL), $CH_2Cl_2$ (120 mL) and a solution of methyl 3-(4,5-dimethoxy-2-nitrophenyl)-3-hydroxy-2-methylene-propanoate **84d** (2.7 g, 9.0 mmol) in $CH_2Cl_2$ (15.0 mL). Work-up and flash chromatography gave as a bright-yellow solid, *methyl (Z)-2-(chloromethyl)-3-(4,5-dimethoxy-2-nitrophenyl)propenoate* **87d**; (8.6 g, 96%), m.p. 139-141 °C, (Found M+Na: 338.0414. Calc for $C_{13}H_{14}NO_6NaCl$. 338.0407); $\upsilon_{max}$ (ATR)/cm$^{-1}$ 1707 (C=O) , $\delta_H$ (400

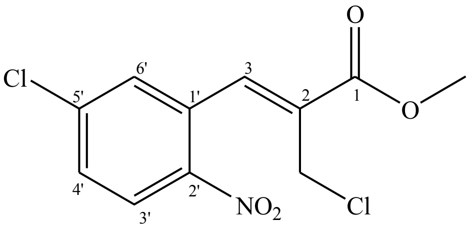
MHz; $CDCl_3$ ); 3.88 (3H, s, $OCH_3$), 4.00 (6H, d, *J* = 8.4 Hz, 2 x $ArOCH_3$), 4.28 (2H, s, $CH_2Cl$), 7.17 (1H, s, 3'-H), 7.76 (1H, s, 6'-H) and 8.13 ( 1H, s, 3-H); $\delta_C$ (100 MHz; $CDCl_3$ ) 39.3 ($CH_2Cl$), 52.6 ($OCH_3$), 56.5 and 56.8 ( 2 x $ArOCH_3$), 108.0 (C-3'), 112.0 ( C-6'), 124.6 (C-4'), 128.7 (C-5'), 140.1 (C-2), 141.5 (C-3), 149.4 (C-1'), 153.4 (C-2') and 165.8 (C=O).

**Methyl *(Z)*-2-(chloromethyl)-3-(3-methoxy-2-nitrophenyl)propenoate 87e.**



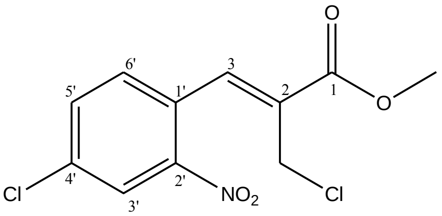
The general procedure was followed using $POCl_3$ (4.1 g, 27 mmol) in DMF (4.0 mL), $CH_2Cl_2$ (120 mL) and a solution of methyl 3-hydroxy-3-(3-methoxy-2-nitrophenyl)-2-methylene-propanoate **84e** (3.6 g, 13.3 mmol) in $CH_2Cl_2$ (30 mL). Work-up and flash chromatography gave, as an off-white solid, *methyl* (Z)*-2-(chloromethyl)-3-(3-methoxy-2-nitrophenyl)propenoate* **87e**; (2.3 g, 61%), m.p. 88-90°C (Found M+1: 286.0484. Calc. for $C_{12}H_{13}NO_5Cl$, 286.0482); $\upsilon_{max}$ (ATR)/$cm^{-1}$ 1722 (C=O) , $\delta_H$ (400 MHz; $CDCl_3$ ) 3.84 (3H, s, $ArOCH_3$), 3.91 (3H, s, $OCH_3$), 4.29 (2H, s, $CH_2Cl$), 7.13 (1H, d, *J* = 8.4 Hz, 4'-H), 7.24 (1H, d, *J* = 8.0 Hz, 6'-H), 7.51 (1H, t, *J* = 8.0 Hz, 5'-H) and 7.63 (1H, s, 3-H); $\delta_C$ (100 MHz; $CDCl_3$ ); 38.3 ($CH_2Cl$), 52.6 ($OCH_3$), 56.5 ($ArOCH_3$), 113.7 (C-4'), 120.7 ( C-4), 128.0 (C-2), 131.6 (C-6'), 133.0 (C-1'), 135.7 (C-3), 140.3 (C-2'), 147.2 (C-2'), and 165.6 (C=O).

### Methyl (*Z*)-2-(chloromethyl)-3-(5-chloro-2-nitrophenyl)propenoate 87f.



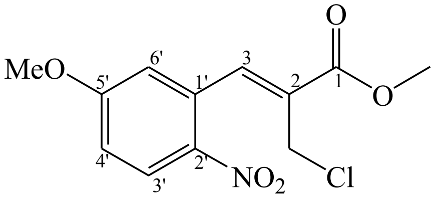
The general procedure was followed using $POCl_3$ (2.20 g, 14.3 mmol) in DMF (2.2 mL), $CH_2Cl_2$ (110 mL) and a solution of methyl 3-(5-chloro-2-nitrophenyl)-3-hydroxy-2-methylene-3-propanoate **84f** (1.95 g, 7.2 mmol) in $CH_2Cl_2$ (10 mL). Work-up and flash chromatography [on silica gel; eluting with hexane: EtOAc (5:1)] afforded, as bright-yellow crystals, *methyl (Z)-2-(chloromethyl)-3-(5-chloro-2-nitrophenyl)propenoate* **87f** (2.1 g, 82%), m.p. 74-76 °C, (Found M+1: 291.0060 Calc. for $C_{11}H_{11}NO_4Cl_2$. 291.0065); $\upsilon_{max}$ (ATR)/cm$^{-1}$ 1717 (C=O); $\delta_H$ (400 MHz; $CDCl_3$) 3.87 (3H, s, $OCH_3$), 4.19 (2H, s, $CH_2Cl$), 7.56 (1H, d, *J* = 8.4 Hz, 4'-H), 7.62 (1H, s, 6'-H), 8.00 ( 1H, s, 3-H) and 8.17 (1H, d, *J* = 8.8 Hz, 3'-H); $\delta_C$ (100 MHz; $CDCl_3$ ), 38.1 ($CH_2Cl$), 52.7 ($OCH_3$), 126.6 (C-4’), 130.1 ( C-6'), 130.3 (C-3'), 130.4 (C-2), 132.0 (C-3'), 139.0 (C-1), 140.5 (C-5'), 145.4 (C-2') and 165.2 (C=O).

### Methyl (*Z*)-2-(chloromethyl)-3-(4-chloro-2-nitrophenyl)propenoate 87g.



The general procedure was followed using $POCl_3$ (3.0 g, 20 mmol) in DMF (3.0 mL), $CH_2Cl_2$ (95 mL) and a solution of methyl 3-(4-chloro-2-nitrophenyl)-3-hydroxy-2-methylenepropanoate **84g** (2.66 g, 9.8 mmol) in $CH_2Cl_2$ (15 mL). Work-up and flash chromatography [on silica gel; eluting with hexane-EtOAc (6:1)] afforded, as bright-yellow crystals, *methyl* (Z)*-2-(chloromethyl)-3-(4-chloro-2-nitrophenyl)propenoate* **87g** (2.39 g, 84%), m.p. 60-62 °C (Found: C, 45.21; H, 3.47; N, 4.71. Calc. for $C_{11}H_9NO_4Cl_2$: C, 45.54; H, 3.13; N, 4.84%); $\upsilon_{max}$ (ATR)/cm$^{-1}$ 1714 (C=O); $\delta_H$ (400 MHz; $CDCl_3$) 3.88 (3H, s, $OCH_3$), 4.20 (2H, s, $CH_2Cl$), 7.64 (1H, d, *J* = 8.4 Hz, 6'-H), 7.73 (1H, d, *J* = 8.4 Hz, 5'-H), 8.00 (1H, s, 3-H) and 8.19 (1H, s, 3'-H); $\delta_C$ (100 MHz; $CDCl_3$) 38.3 ($CH_2Cl$), 52.7 ($OCH_3$), 125.4 (C-6'), 128.5 (C-4'), 130.4 (C-1'), 131.7 (C-3'), 134.1 (C-5'), 136.1 (C-2), 139.5 (C-3), 147.6 (C-2') and 165.4 (C=O).

**Methyl (*Z*)-2-(chloromethyl)-3-(5-methoxy-2-nitrophenyl)propenoate 87h.**



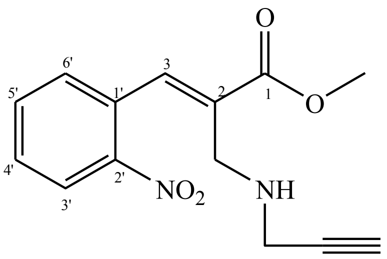
The general procedure was followed using $POCl_3$ (2.60 g, 17.0 mmol) in DMF (1.6 mL), $CH_2Cl_2$ (60 mL) and a solution of methyl 3-hydroxy-3-(5-methoxy-2-nitrophenyl)-2-methylene-propanoate **84h** (2.31 g, 8.6 mmol) in $CH_2Cl_2$ (10 mL). Work-up and flash chromatography [on silica gel; eluting with hexane: EtOAc (6:1)] afforded, as bright yellow crystals, *methyl* (Z)*-2-(chloromethyl)-3-(5-methoxy-2-nitrophenyl)propenoate* **87h** (2.0g, 81%), m.p. 83-85°C, (Found: C, 50.53; H, 4.66; N, 4.89. Calc. for $C_{12}H_{12}NO_5Cl$: C, 50.45; H, 4.23; N, 4.90%); $\upsilon_{max}$ (ATR)/cm$^{-1}$ 1714 (C=O); $\delta_H$ (400 MHz; $CDCl_3$); 3.88 (3H, s, $OCH_3$), 3.93 (3H, s, $ArOCH_3$), 4.25 (2H, s, $CH_2Cl$), 7.02 (1H, d, *J* = 9.2 Hz, 4'-H), 7.11 (1H, s, 6'-H), 8.12 ( 1H, s, 3-H) and 8.23 (1H, d, *J* = 9.2 Hz, 3'-H); $\delta_C$ (100 MHz; $CDCl_3$) 38.9 ($CH_2Cl$), 52.7 ($OCH_3$), 56.2 ($ArOCH_3$), 114.6 (C-4'), 115.7 ( C-6'), 127.7 (C-3'), 129.1 (C-2), 132.9 (C-1'), 140.1 (C-2'), 141.3 (C-3), 163.7 (C-5'), and 165.7 (C=O).

## 3.6. REACTIONS OF α-(CHLOROMETHYL) ESTERS.

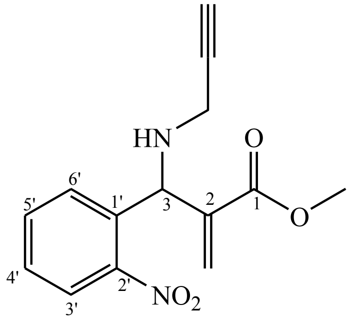
**General Method**: A solution of the allyl chloride (**87**) (1eq.) and the propargylamine (2 eq.) in anhydrous THF[105] was stirred in a stoppered flask at room temperature for periods which varied according to the reactants involved. The solution was then concentrated *in vacuo* and the crude product purified by flash chromatography to afford the aminated product.

**Methyl (*E*)- 2-[-3-(2-nitrophenyl)(prop-2-ynylamino)methyl] propenoate 88a.**

**methyl 3-(2-nitrophenyl)-2-methylene-3-(prop-2-ynylamino)propanoate 90a.**



**88a**



**90a**

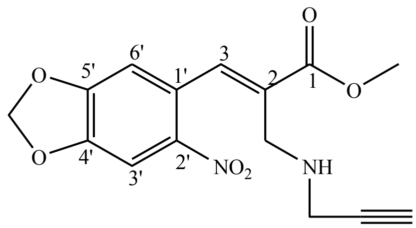
The general method was followed, using methyl (*Z)*-2-(chloromethyl)-3-(2-nitrophenyl)propenoate **87a** (0.40 g, 1.6 mmol.) and propargylamine (0.17 g, 3.2 mmol) in dry THF (2.0 mL). After 48 h, TLC indicated that the starting material had been consumed. Work-up and flash chromatography [on silica gel; eluting with EtOAc-hexane (1:1) ] afforded two fractions.

**Fraction 1:** as light lemon-yellow crystals, *methyl* (E*)-2-[-3-(2-nitrophenyl)(prop-2-ynylamino)methyl] propenoate* **88a** (0.25 g, 56%), m.p. 53-55 °C (Found M+1: 275.1025. Calc. for $C_{14}H_{15}N_2O_4$, 275.1032) $\nu_{max}$ (ATR)/cm$^{-1}$ 1718 (C=O); $\delta_H$ (400 MHz; $CDCl_3$) 1.82 (1H, br s, NH), 2.02 (1H, s, C≡CH), 3.33 (2H, d, *J* = 2 Hz, C$H_2$NH ), 3.48

(2H, s, C$\mathit{H}_2$C≡CH), 3.84 (3H, s, $CH_3$), 7.54 (1H, t, *J* = 7.6 Hz, 4'-H), 7.61(1H, d, *J* = 7.6 Hz, 6'-H), 7.67 (1H, t, *J* = 7.4 Hz, 5'-H), 8.04 ( 1H, s, 3-H) and 8.15 (1H, d, *J* = 8.4 Hz, 3'-H); $\delta_C$ (100 MHz; $CDCl_3$ ) 37.6 and 44.9 (2 x $NCH_2$), 52.3 ($CO_2\mathit{C}H_3$), 71.7 and 81.4 (C≡CH), 124.8 (C-4'), 129.4 ( C-6), 131.1 (C-1'), 131.2 (C-2), 131.7 (C-5'), 133.5 (C-3), 139.0 (C-3'), 147.6 (C-2') and 167.5 (C=O).

**Fraction 2:** as off-white solid, *Methyl 3-(2-nitrophenyl)-2-methylene-3-(prop-2-ynylamino)propanoate* **90a** ( > 10 %. Estimation from $^1$H NMR analysis); $\delta_H$ (400 MHz; $CDCl_3$) 2.22 (1H, s, C≡CH), 3.33 (2H, NHC$\mathit{H}_2$), 3.64 (3H, s, $CO_2CH_3$ ), 5.39 and 6.38 (2H, 2 x s, C=$CH_2$), 5.87 (1H, s, 3-H), 7.56 (1H, t, *J* = 7.6 Hz, 6'-H) and 7.67 ( 1H, d, *J* = 8.0 Hz, 5'-H), 7.82 (1H, d, *J* = 8.0 Hz, 3'-H) and 8.16 (1H, t, *J* = 8.8 Hz, 4'-Hz); $\delta_C$ (100 MHz; $CDCl_3$) 36.5 ($NHCH_2$), 52.1 ($CO_2\mathit{C}H_3$), 55.8 (CH-NH), 72.1 and 80.9 (C≡CH), 124.2 (C-6'), 126.7 (C=$CH_2$), 128.3 (C-4'), 129.6 (C-5'), 132.7(C-3'), 134.9 (C-1'), 140.1 (C-2'), and 166.0 (C=O).

### Methyl (*E*)-3-(4,5-methylenedioxy-2-nitrophenyl)-2-[(prop-2-ynylamino)-methyl]-propenoate 88c.

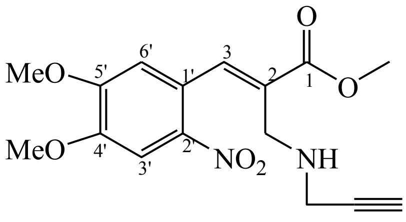


The general method was followed, using methyl (*Z)*-2-(chloromethyl)-3-(4,5-methylenedioxy-2-nitrophenyl)propenoate **87c** (0.55 g, 1.8 mmol.) and propargylamine (0.21 g, 3.7 mmol) in dry DMF (4.0 mL). After 72 h, TLC indicated that the starting material had been consumed. DCM (25 mL) was added, followed by $H_2O$ (2 x 10 mL). The organic phase was washed with brine (2 x 10 mL), dried over anhydrous $MgSO_4$ and concentrated *in vacuo* to afford the crude product. Flash chromatography [on silica gel; eluting with EtOAc-hexane (1:1)] afforded, as light lemon- yellow crystals, *methyl* (E)-*3-(4,5-methylenedioxy-2-nitrophenyl)-2-[(prop-2-ynylamino)methyl]-propenoate* **88c** (0.45 g, 78%), m.p. 70-72 °C (Found M+1: 319.0917. Calc. for $C_{15}H_{15}N_2O_6$. 319.0925);

$\nu_{max}$ (ATR)/cm$^{-1}$ 1689 (C=O); $\delta_H$ (400 MHz; $CDCl_3$) 1.22 (1H, br, s, NH), 2.09 (1H, t, *J* = 2.4 Hz, C≡*C*H), 3.35 (2H, d, *J* = 2.4 Hz, C*H*$_2$NH), 3.47 (2H, s, C*H*$_2$C≡CH), 3.81 (3H, s, $CH_3$), 6.13 (2H, s , $OCH_2O$), 7.08 (1H, s, 6'-H), 7.62 (1H, s, 3'-H), 7.95 (1H, s, 3-H); $\delta_C$ (100 MHz; $CDCl_3$) 37.5 and 44.9 (2 x $NCH_2$), 52.2 ($OCH_3$), 71.4 and 81.4 (C≡*C*H), 103.3 ($OCH_2O$), 105.5 (C-6'), 110.2 ( C-3'), 127.7 (C-1'), 130.4 (C-2), 139.5 (C-3), 142.0 (C-2'), 148.2 (C-5'), 152.0 (C-4') and 167.5 (C=O).

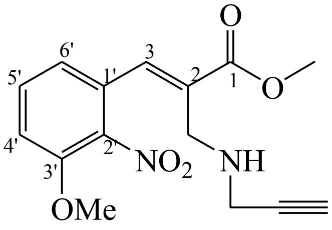
NOTE: Anhydrous DMF was used as the solvent as methyl (*Z*)-2-(chloromethyl)-3-(4,5-methylenedioxy-2- nitrophenyl)propenoate **87c** was insoluble in anhydrous THF.

### Methyl (*E*)-3-(4,5-dimethoxy-2-nitrophenyl) 2-[(prop-2-ynylamino)methyl]-propenoate 88d.

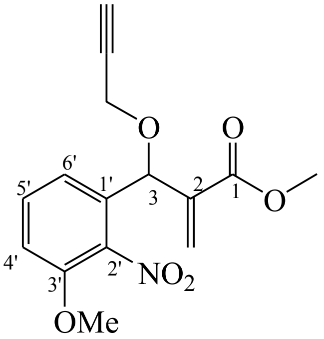


The general method was followed, using methyl (*Z)*-2-(chloromethyl)-3-(4,5-dimethoxy-2-nitrophenyl)propenoate **87d** (0.32 g, 1.0 mmol.) and propargylamine (0.17 g, 3.2 mmol) in dry THF (2.0 mL). After 48 h, TLC indicated that the starting material has been consumed. Work-up and flash chromatography [on silica gel; eluting with EtOAc-hexane (1:1)] afforded, as light lemon-yellow crystals, *methyl* (E)-*3-(4,5-dimethoxy-2-nitrophenyl)-2-[(prop-2-ynylamino)-methyl]propenoate* **88d** (0.30 g, 86%), m.p. 111-113 °C, (Found M + 1: 335.1253. Calc. for $C_{16}H_{19}N_2O_6$: 335.1243); $\nu_{max}$ (ATR)/cm$^{-1}$ 1702 (C=O); $\delta_H$ (400 MHz; $CDCl_3$) 2.09 (1H, s, C≡CH), 2.27 (1H, br, s, NH), 3.37 (2H, d, *J* = 2.0 Hz, C*H*$_2$NH ), 3.43 (2H, s, C*H*$_2$C≡CH), 3.81 (3H, s, $OCH_3$), 3.96 (6H, d, *J* = 5.6 Hz, 2 x $ArOCH_3$), 7.31 (1H, s, 3'-H), 7.70 (1H, s, 6'-H) and 8.14 (1H, s, 3-H); $\delta_C$ (100 MHz; $CDCl_3$ ) 38.5 and 46.1 (2 x $NCH_2$), 52.7 ($CO_2$*C*$H_3$), 56.8 and 57.1 (2 x $ArOCH_3$) , 72.1 and 81.8 (C≡CH), 108.1 (C-3'), 113.7 ( C-6'), 126.1 (C-4'), 130.4 (C-5'), 140.6( C-2), 140.7 (C-3), 149.5 (C-1'), 153.7 (C-2') and 168.2 (C=O).

**Methyl (*E*)-3-(3-methoxy-2-nitrophenyl)-2-[(prop-2-ynylamino)methyl]propenoate 88e and methyl 3-(3-methoxy-2-nitrophenyl)-2-methylene-3-(prop-2-ynylamino)-propanoate 90e.**



**88e**



**90e**

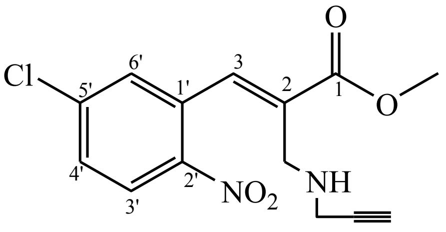
The general method was followed, using methyl (*Z*)-2-(chloromethyl)-3-(3-methoxy-2-nitrophenyl)propenoate **87e** (0.3 g, 1 mmol.) and propargylamine (0.1 g, 2.0 mmol) in anhydrous THF (1.0 mL). After 4 days, TLC indicated that the starting material had been consumed. Work-up and flash chromatography [on silica gel; eluting with EtOAc-hexane (1:1)] afforded two fractions.

**Fraction 1**: as a light lemon-yellow solid, *methyl* (E)-*3-(3-methoxy-2-nitrophenyl)-2-[(prop-2-ynylamino)methyl]propenoate* **88e** (0.12 g, 40%), m.p. 66-68 °C (Found M+1: 307.1098. Calc. for $C_{15}H_{17}N_2O_5$ 305.1137); $\nu_{max}$ (ATR)/cm$^{-1}$ 1711 (C=O); $\delta_H$ (400 MHz; methanol-$d_4$) 1.31 (1H, s, C≡CH), 2.48 (1H, s br, NH), 3.34 (2H, s, C$H_2$NH), 3.59 (2H, s, C$H_2$C≡CH), 3.84 ($CO_2CH_3$), 3.96 ($ArOCH_3$), 7.19 (1H, d, *J* = 7.6 Hz, 4'-H), 7.32 (1H, d, *J* = 8.4 Hz, 6'-H), 7.59 (1H, t, *J* = 8.4 Hz, 5'H) and 7.62 (1H, s, 3-H); $\delta_C$ (100 MHz; methanol-$d_4$) 38.0 and 45.3 (2 x $NCH_2$), 53.0 ($CO_2CH_3$), 57.3 ($ArOCH_3$) 73.3 and 81.8 (C≡CH) , 114.8 (C-4'), 122.7 ( C-6'), 129.6 (C-2), 132.7 (C-5'), 135.9 (C-3), 136.0 (C-1'), 142.0 (C-2'), 152.5 (C-3') and 168.5 (C=O).

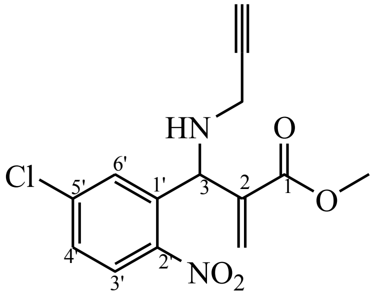
**Fraction 2**: as an off-white solid, *methyl 3-(3-methoxy-2-nitrophenyl)-2-methylene-3-(prop-2-ynylamino)propanoate* **90e** (0.33g, 48 %,), m.p. 72-74 °C (Found M+1: 307.1129. Calc. for $C_{15}H_{17}N_2O_5$ 305.1137); $\upsilon_{max}$ (ATR)/cm$^{-1}$ 1714 (C=O); $\delta_H$ (400 MHz; $CDCl_3$) 2.06 (1H, br, s, NH), 2.27 (1H, s, C≡CH), 3.36 (2H, d, *J* = 2.4 Hz, NHC$H_2$), 3.70 (3H, s, $CO_2CH_3$), 3.88 (3H, s, $ArOCH_3$), 4.91 (1H, s, 3-H), 5.96 and 6.46 (2H, 2 x s, C=$CH_2$), 6.99 (1H, d, *J* = 8.4 Hz, 6'-H) 7.11 (1H, d, *J* = 8.0 Hz, 4'-H) and 7.42 (1H, t, *J* =

8.2 Hz, 5’-H); $\delta_C$ (100 MHz; $CDCl_3$**)** 36.2 ($NHCH_2$), 51.9 (CHNH), 56.3 ($CO_2CH_3$), 56.3 ($ArOCH_3$), 72.1 and 80.9 (C≡CH), 111.8 (C-6'), 120.2 (C-5'), 127.4 ($C=CH_2$), 130.8 (C-2), 133.3 ($C=CH_2$), 139.1 (C-1'), 141.2 (C-3'), 150.7 (C-2') and 165.9 (C=O).

### Methyl (*E)*-3-(5-chloro-2-nitrophenyl)-2-[(prop-2-ynylamino)methyl]propenoate 88f and methyl 3-(5-chloro-2-nitrophenyl)-2-methylene-3-(prop-2-ynylamino)-propanoate 90f.



**88f**



**90f**

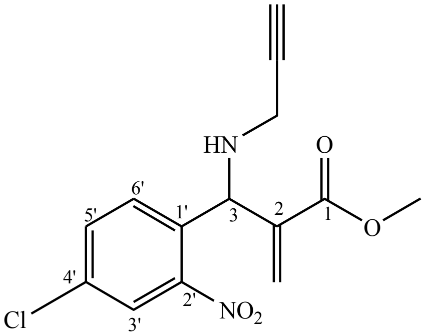
The general method was followed, using methyl (*Z)*-2-(chloromethyl)-3-(5-chloro-2-nitrophenyl)propenoate **87f** (0.53 g, 1.8 mmol.) and propargylamine (0.20 g, 3.6 mmol) in anhydrous THF (2.0 mL). After 48 h, TLC indicated that the starting material had been consumed. Work-up and preparative thin layer chromatography [on silica gel; eluting with EtOAc-hexane (1:1)] afforded, two fractions.

**Fraction 1:** as light yellow solids, *methyl* (E*)-3-(5-chloro-2-nitrophenyl)-2-[(prop-2-ynylamino)methyl]propenoate* **88f** (0.20 g, 36%), m.p. 59-61 °C; (Found M+1: 309.0649 Calc. for $C_{14}H_{14}N_2O_4Cl$. 309.0642); $\upsilon_{max}$ (ATR)/cm$^{-1}$ 1709 (C=O); $\delta_H$ (400 MHz; $CDCl_3$) 0.85 (1H, s, C≡CH), 2.12 (2H, s, C*H*$_2$NH), 2.14 (1H, s, br, NH), 3.28 (2H, s, $CH_2C≡CH$), 3.80 ($CO_2CH_3$), 7.42 (1H, s, 4'-H), 7.44 (1H, s, 6'-H), 7. 90 (1H, s, 3-H) and 8.08 (1H, d, *J* = 8.8 Hz, 3'-H); $\delta_C$ (100 MHz; $CDCl_3$ ) 40.7 and 48.4 (2 x $NCH_2$), 52.3 ($CO_2CH_3$), 73.0 and 76.9 (C≡CH), 126.2 (C-3'), 129.2 ( C-6'), 131.7 (C-4'), 131.9 (C-1'), 132.8 (C-2), 138.1 (C-3), 139.8 (C-5'), 145.8 (C-2') and 167.3 (C=O).

**Fraction 2:** as a brown wax, *methyl 3-(5-chloro-2-nitrophenyl)-2-methylene-3-(prop-2-ynylamino)propanoate* **90f** (2.2g, 40%), m.p. 39-41 °C (Found M+1: 309.0642 Calc. for $C_{14}H_{14}N_2O_4Cl$. 309.0637); $\upsilon_{max}$ (ATR)/cm$^{-1}$ 1714 (C=O); $\delta_H$ (400 MHz; $CDCl_3$) 1.92

(1H, s, br, NH), 2.24 (1H, s, C≡CH), 3 39 (2H, s, NHC*H*$_2$), 3.67 (3H, s, $CO_2CH_3$ ), 5.43 (1H, s, 3-H), 5.85 and 6.40 (2H, 2 x s, C=CH$_2$), 7.36 (1H, dd, *J* = 2.0 and 8.4 Hz, 3'-H), 7.70 (1H, d, *J* = 2.0 Hz, 6'-H) and 7.79 (1H, d, *J* = 8.4 Hz, 4'-H); $\delta_C$ (100 MHz, $CDCl_3$**)** 36.5 (NHCH$_2$), 52.1 (CO$_2$*C*H$_3$), 55.7 (C-3), 72.2 and 80.9 (C≡CH), 125.9 (C-3'), 127.1 (C=*C*H$_2$), 128.5 (C-4'), 129.9 (C-6'), 137.5 (C-2), 139.3 (C-1'), 139.8 (C-5'), 147.9 (C-2') and 165.9 (C=O).

**Methyl 3-(4-chloro-2-nitrophenyl)-2-methylene-3-(prop-2-ynylamino)propanoate 90g.**

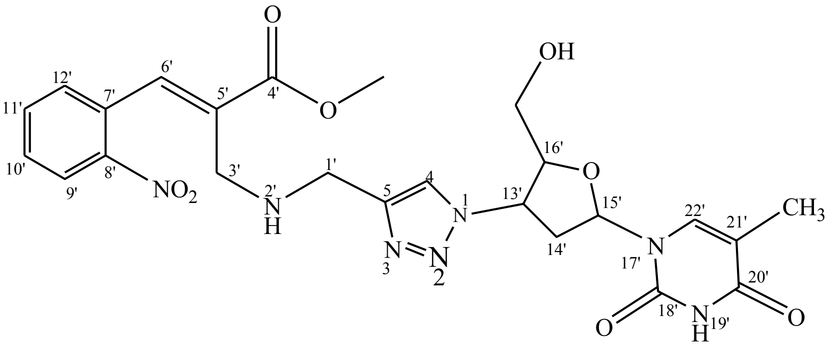


The general method was followed, using methyl (*Z)*-2-(chloromethyl)-3-(4-chloro-2-nitrophenyl)propenoate **87g** (0.53 g, 1.8 mmol.) and propargylamine (0.20 g, 3.6 mmol) in anhydrous THF (2.0 mL). After 48 h, TLC indicated that the starting material had been consumed. Work-up and preparative layer chromatography [on silica gel; eluting with EtOAc-hexane (1:1)] afforded, as a transparent yellow oil, *methyl 3-(4-chloro-2-nitrophenyl)-2-methylene-3-(prop-2-ynylamino)propanoate* **90g** (0.25 g, 41%) (Found M+1: 309.0653 Calc. for $C_{14}H_{14}N_2O_4Cl$. 309.0642); $\nu_{max}$ (ATR)/cm$^{-1}$ 1714 (C=O); $\delta_H$ (400 MHz; $CDCl_3$) 1.90 (1H, br s, NH), 2.24 (1H, d, *J* = 2.4 Hz, C≡CH), 3.38 (2H, s, NHCH$_2$), 3.67 (3H, s, $CO_2CH_3$), 5.37 (1H, s, 3-H), 5.88 and 6.40 (2H, 2 x s, C=CH$_2$), 7.53 (1H, d, *J* = 8.4 Hz, 6'=H), 7.67 (1H, d, *J* = 8.8 Hz, 5'-H) and 7.79 (1H, s, 3'-H); $\delta_C$ (100 MHz, $CDCl_3$**)** 36**.**5 (NHCH$_2$), 52.1 ($CO_2CH_3$), 55.6 (C-3), 72.2 (C≡CH), 81.0 (C≡CH), 124.3 (C-6'), 127.0 (C=*C*H$_2$), 131.2 (C-5'), 132.8 (C-3'), 133.7 (C-6'), 134.0 (C-1'), 140.0 (*C*=CH$_2$), 150.1 (C-2') and 165.9 (C=O).

## 3.7. SYNTHESIS OF CINNAMATE ESTER-AZT CONJUGATES.

**General Method:** To a solution of 3'-azido-3'-deoxythymidine (AZT); (1 eq.) in $H_2O$-THF (1:1) was added the propargylamine derivative **88** (1 eq.), sodium ascorbate (0.2 eq.) and copper sulphate pentahydrate ($CuSO_4.5H_2O$; 0.03 eq.). After stirring at room temperature for 24 h, the mixture was extracted with $CH_2Cl_2$, and the organic phase was washed sequentially with $H_2O$ and brine, and then dried over anhydrous $MgSO_4$. The solution was concentrated *in vacuo* to give the crude product which was purified by flash chromatography [on silica gel; eluting with EtOAc, followed by MeOH-EtOAc (2:3)] to afford the cinnamate ester-AZT conjugate **89**.

**Cinnamate ester–AZT conjugate 89a**†



The general method was followed using 3'-azido-3'-deoxythymidine (AZT) (0.16 g, 0.59 mmol) in $H_2O$-THF (1:1; 7.5 mL), methyl *(Z)*-2-[3-(2-nitrophenyl)(propynylamino)methyl]propenoate **88a** (0.16 g, 0.59 mmol), sodium ascorbate (0.02 g, 0.12 mmol) and $CuSO_4.5H_2O$ (5 mg, 18 μmol). Work-up and flash chromatography afforded, as lemon-yellow flakes, the *cinnamate ester-AZT conjugate* **89a** (0.14 g, 44%), m.p. 106-108 °C (Found M+1: 542.1998. Calc. for $C_{24}H_{28}N_7O_8$: 542.1998); $\upsilon_{max}$ (ATR)/$cm^{-1}$ 3321 (OH) and 1682 (C=O); $\delta_H$ (400 MHz; methanol-$d_4$) 1.24 (1H, t, $J$ = 7.0 Hz, $CH_2OH$), 1.88 ( 3H, s, 21'-$CH_3$), 1.99 (1H, s, $CH_2NHCH_2$), 2.68

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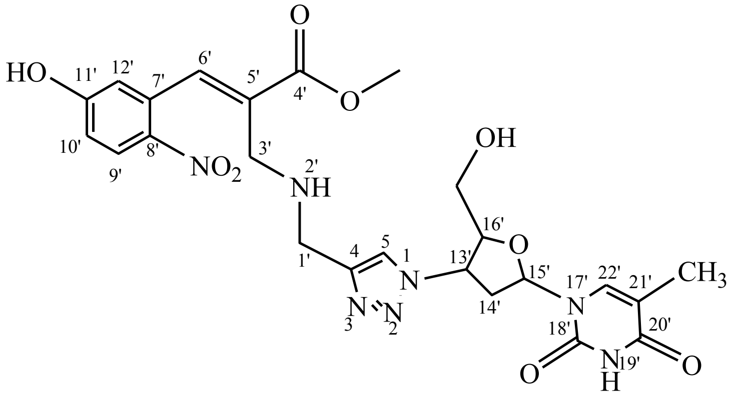
† **Systematic name:** *4{[2-methoxycarbonyl-3-(2-nitrophenyl)-2-propenylamino]methyl}-1-[(2*S*,3*R*,5*R*)-5-(5-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)-2-(hydroxymethyl)tetrahydrofuran-3-yl]-1H-1,2,3-triazole*.

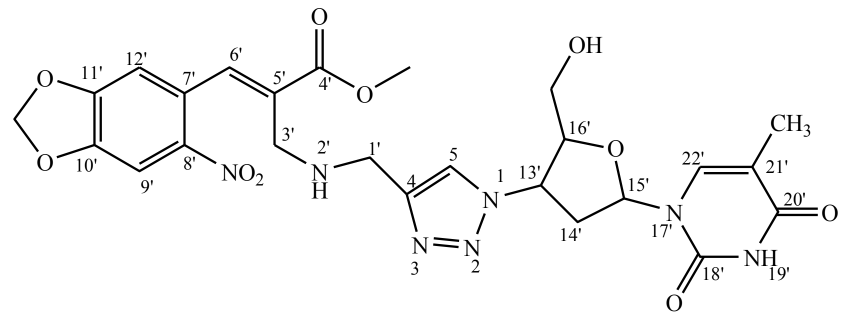
- 2.81 (2H, 2 x m, 14'-NCHC*H*$_2$), 3.43 and 3.74 (4H, 2 x s, 2 x NCH$_2$), 3.71 and 3.88 (2H, m, C*H*$_2$OH), 3.83 (3H, s, OCH$_3$), 4.28 (1H, m, 16'-H), 5.34 (1H, m, 13'-H), 6.46 (1H, t, *J* = 6.60 Hz, 15'-H), 7.48 (1H, d, *J* = 7.60 Hz, Ar-H), 7.62 (1H, dd *J* = 8.40 and 0.80 Hz, Ar-H), 7.68 (1H, t, *J* = 7.60 Hz, Ar-H), 7.83 (1H, s, Ar-H), 7.88 (1H, d, *J* = 0.80 Hz, Ar-H), 8.01 (1H, s, Ar-H) and 8.17 (1H, dd, *J* = 8.00 and 0.80 Hz, 6'-H); $\delta_C$ (100 MHz; methanol-*d*$_4$ ) 10.0 (21'-CH$_3$), 36.5 (14'-CH$_2$), 41.7 and 43.1 (2 x CH$_2$NH), 50.4 (CO$_2$*C*H$_3$), 58.5 (d, C-13'), 59.7 (CH$_2$OH), 83.9 (d, C-16'), 84.2 (d, C-15'), 109.2 (d, Ar-C), 121.3 (d, Ar-C), 123.5 (d, Ar-C), 128.6 (d, Ar-C), 129.4 (d, Ar-C), 129.6 (s, Ar-C), 130.0 (d, Ar-C), 132.4 (d, Ar-C), 135.7 (d, Ar-C), 138.2 (d, Ar-C), 144.6 (s, Ar-C) and 146.5 (s, Ar-C), 149.8, 163.9 and 166.4 (3 x C=O).

**Cinnamate ester–AZT conjugate 89b.**



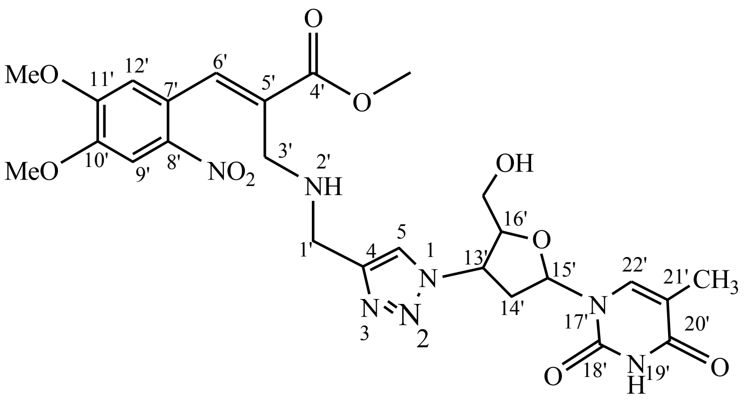
The general method was followed using AZT (0.48 g, 1.8 mmol) in H$_2$O-THF (1:1; 8.0 mL), methyl *(Z)*-3-(5-hydroxy-2-nitrophenyl)-2-[(propynylamino)methyl]propenoate **88b** (0.52 g, 1.8 mmol), sodium ascorbate (0.07 g, 0.4 mmol) and CuSO$_4$.5H$_2$O (10 mg, 5.0 μmol). Work-up and flash chromatography afforded, as lemon-yellow flakes, the *cinnamate ester-AZT conjugate* **89b** (0.4 g, 42%), m.p. 168-170 °C; $\upsilon_{max}$ (ATR)/cm$^{-1}$ 3320 (OH) and 1676 (C=O); $\delta_H$ (400 MHz; DMSO-*d*$_6$ ) 1.81 (3H, s, 21'-CH$_3$), 2.40 (2H, s, 3'-CH$_2$), 2.58 (2H, m, 14'-CH$_2$), 3.60 (2H, m, C*H*$_2$OH), 3.67 (1H, br s, CH$_2$O*H*), 3.70 (5H, br s, CO$_2$CH$_3$ and 1'-CH$_2$), 4.04 (1H, m, 13'-H), 5.21 (1H, m, 16'-H), 6.37 (1H, t, *J* = 6.4 Hz, 15'-H), 6.63 (1H, s, 22'-H), 6.89 (1H, d, *J* = 8.8 Hz, 10'-H), 7.53 (1H, s, Ar-H), 7.82 (1H, s, Ar-H), 8.08 (1H, s, Ar-H) and 11.34 (1H, br s, 19'-NH); $\delta_C$ (100 MHz; DMSO-$d_6$) 13.1 (21'-$CH_3$), 37.9 (14'-$CH_2$), 47.8 and 49.5 (3'- and 1'-$CH_2$), 52.8 ($CO_2CH_3$), 60.0 (C-13'), 61.6 ( $CH_2OH$), 84.8 (C-16'), 85.3 (C-15'), 110.5 (s, Ar-C), 116.7 (d, Ar-C), 118.6 (d, Ar-C), 123.2 ( d, Ar-C), 128.7 ( d, Ar-C), 131.0 (s, Ar-C), 134.4 (s, Ar-C), 137.1 (d, Ar-C), 139.4 (s, Ar-C), 140.4 (d, Ar-C), 144.4 (s, Ar-C), 151.3 (s, Ar-C) and 163.5, 164.6 and 168.3 (3 x C=O).

**Cinnamate ester–AZT conjugate 89c.**



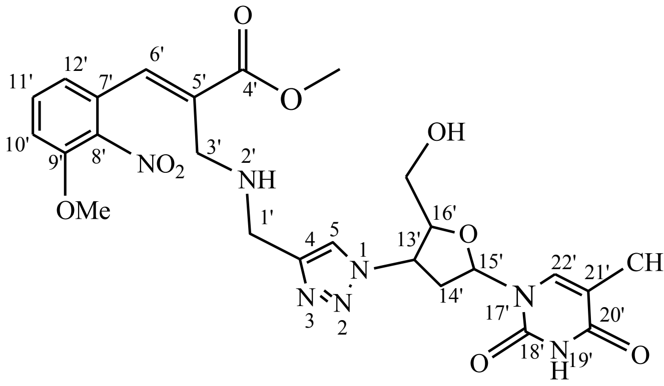
The general method was followed using AZT (0.17 g, 0.62 mmol) in $H_2O$-THF (1:1; 8.0 mL), methyl (*Z*)-3-(4,5-methylenedioxy-2-nitrophenyl)-2-[(propynylamino)methyl])-propenoate **88c** (0.20 g, 0.62 mmol), sodium ascorbate (0.03 g, 0.12 mmol) and $CuSO_4.5H_2O$ (7 mg, 19 μmol). Work-up and flash chromatography afforded, as lemon-yellow flakes, the *cinnamate ester-AZT conjugate* **89c** (0.22 g, 62%), m.p. 102-104 °C (Found M + 1: 586.1887. Calc. for $C_{25}H_{28}N_7O_{10:}$ , 586.1893)**;** $\upsilon_{max}$ (ATR)/cm$^{-1}$ 3321 (OH) and 1682 (C=O); $\delta_H$ (400 MHz; methanol-$d_4$) 1.93 (3H, s, 21'-$CH_3$), 2.77 and 2.90 (2H, m, 14'-$CH_2$), 3.35 and 3.38 (2H, 2 x m, $CH_2OH$), 3.35 (2H, m, 3'-$CH_2$), 3.81 (1H, m, 13'-H), 3.82 (2H, m, 1'-$CH_2$), 3.87 (3H, s, $CO_2CH_3$), 5.52 (1H, m, 16'-H), 6.29 (2H, d, *J* = 4.0 Hz, $OCH_2O$), 6.49 (1H, m, 15'-H), 7. 69 (1H, d, *J* = 4.0 Hz, Ar-H), 7.69 (1H, d, *J* = 4.4 Hz, Ar-H), 7.93 (2H, overlapping s, Ar-H) and 7.98 (1H, d, *J* = 4.0 Hz, Ar-H), $\delta_C$ (100 MHz; methanol-$d_4$); 14.5 (21'-$CH_3$), 39.0 (14'-$CH_2$), 44.2 and 45.7 (3'- and 1'-$CH_2$), 52.9 ($CO_2CH_3$), 61.1 (C-13'), 61.6 ($CH_2OH$), 86.5 (C-16'), 86.8 (C-15'), 105.3 ($OCH_2O$), 106.4 (d, Ar-C), 110.8 (d, Ar-C), 111.7 (s, Ar-C), 123.8 (d,Ar-C), 128.6 (s, Ar-C), 131.3 (s, Ar-C), 138.2 (d, Ar-C), 141.3 (d, Ar-C), 143.3 (s, Ar-C), 150.0 (s, Ar-C), 152.3 (s, Ar-C), 153.7 (s, Ar-C) and 166.4, 169.0 and 173.0 (3 x C=O).

**Cinnamate ester–AZT conjugate 89d.**



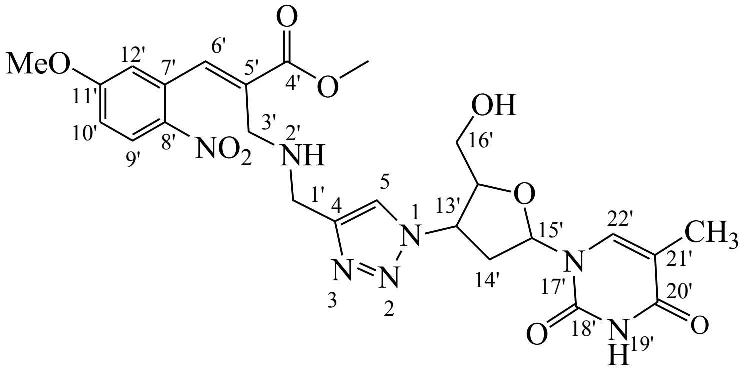
The general method was followed using AZT (0.14 g, 0.55 mmol) in $H_2O$-THF (1:1; 8.0 mL), methyl (*Z*)-3-(4,5-dimethoxy-2-nitrophenyl)-2-[(propynylamino)methyl])-propenoate **88d** (0.18 g, 0.55 mmol), sodium ascorbate (0.02 g, 0.11 mmol) and $CuSO_4.5H_2O$ (5 mg, 17 μmol). Work-up and flash chromatography afforded, as lemon-yellow flakes, the *cinnamate ester-AZT conjugate* **89d** (0.23 g, 70%), m.p. 123-125 °C (Found M + 1: 602.2225. Calc. for $C_{26}H_{32}N_7O_{10}$: 601.2211); $\upsilon_{max}$ (ATR)/cm$^{-1}$ 3317 (OH) and 1687 (C=O); $\delta_H$ (400 MHz; DMSO-$d_6$ ) 1.81 (3H, s, 21'-$CH_3$), 2.65 (2H, m, 14'-$CH_2$), 3.40 (4H, m, 1'- and 3'-$CH_2$), 3.56 (1H, d, *J* = 3.2 Hz, 13'-H), 3.74 (2H, s, C*H*$_2$OH), 3.77 (3H, s, $CO_2CH_3$), 3.83 and 3.89 (6H, 2 x s, 2 x $ArOCH_3$), 4.13 (1H, d, *J* = 3.2Hz, 16'-H), 5.32 (2H, m, CHO*H* and 2'-N*H*), 6.40 (1H, t, *J* = 6.4 Hz, 15'-H), 7.43 (1H, s, Ar-H), 7.74 (1H, s, Ar-H), 7.83 (1H, s, Ar-H), 7.91 (1H, s, Ar-H), 8.05 (1H, s, Ar-H); $\delta_C$ (100 MHz; DMSO-$d_6$ ) 12.2 (21'-$CH_3$), 37.0 (14'-$CH_2$), 43.9 and 45.2 (C-1' and C-3'), 52.1 ($CO_2$*C*$H_3$), 56.1 and 56.3 (2 x $ArOCH_3$), 58.9 (C-13'), 60.6 (*C*$H_2$OH), 83.8 (C-16'), 84.5 (C-15'), 107.7 (d, Ar-C), 109.6 (s, Ar-C), 113.1 (d, Ar-C), 122.1 (d, Ar-C), 124.9 (s, Ar-C), 130.3 (s, Ar-C), 136.2 (d, Ar-C), 138.8 (d, Ar-C), 139.9 (s, Ar-C), 146.1 (s, Ar-C), 148.5 (s, Ar-C), 150.4 (s, Ar-C) and 152.8, 163.7 and 167.4 (3 x C=O).

## Cinnamate ester – AZT conjugate 89e.



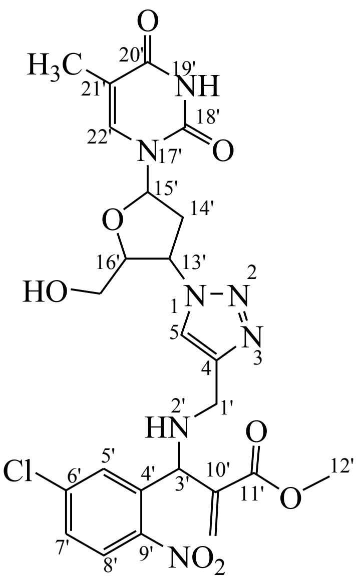
The general method was followed using AZT (0.38 g, 1.4 mmol) in $H_2O$-THF (1:1; 8.4 mL), methyl (*Z*)-3-(3-methoxy-2-nitrophenyl)-2-[(propynylaminomethyl])propenoate **88e** (0.42 g, 1.4 mmol), sodium ascorbate (0.07 g, 0.3 mmol) and $CuSO_4.5H_2O$ (18 mg, 40 μmol). Work-up and flash chromatography afforded, as lemon-yellow flakes, the *cinnamate ester-AZT conjugate* **89e** (0.22 g, 91%), m.p. 110-112 °C (Found M+1: 572.2105. Calc. for $C_{25}H_{30}N_7O_9$); $\upsilon_{max}$ (ATR)/cm$^{-1}$ 3321 (OH) and 1682 (C=O); $\delta_H$ (400 MHz; methanol-*d*$_4$ ) 1.91 (3H, s, 21'-$CH_3$), 2.74 – 2.84 (2H, m, 14'-$CH_2$), 3.37 (4H, m, C*H*$_2$OH, and 3'-$CH_2$), 3.51 (2H, m, 1'-$CH_2$), 3.79 ( 1H, m, 13'-H), 3.82 (3H, s, $CO_2CH_3$), 3.93 (3H, s, $ArOCH_3$), 5.41 (1H, m, 16'-H), 6.50 (1H, t, *J* = 6.4 Hz, 15'-H), 7.07 (1H, d, *J* = 7.6 Hz, Ar-H), 7.31 (1H, d, *J* = 8.8 Hz, Ar-H), 7.52 (1H, t, *J* = 8.4 Hz, Ar-H), 7.59 (1H, s, Ar-H), and 7.93 (1H, d, *J* = 8.4 Hz, Ar-H); $\delta_C$ (100 MHz; methanol-*d*$_4$) 11.6 (21'-$CH_3$), 38.1 (14'-$CH_2$) 43.2 and 44.7 (1'- and 3'-$CH_2$), 52.1 ($CO_2CH_3$), 56.4 ($ArOCH_3$), 60.1 (C-13'), 60.3 ($CH_2OH$), 85.5 (C-16'), 85.7 (C-15'), 110.7 (s, Ar-C), 113.8 (d, Ar-C), 121.5 ( d, Ar-C), 123.1 (d, Ar-C), 128.5 (s, Ar-C'), 131.8 (d, Ar-C), 134.8 (d, Ar-C), 134.9 (s, Ar-C), 137.3 (d, Ar-C), 140.7 (s, Ar-C), 146.9 (s, Ar-C), 151.4 (s, Ar-C) and 165.4, 167.4 and 171.4 (3 x C=O).

**Cinnamate ester–AZT conjugate 89h.**



The general method was followed using AZT (0.6 g, 2.2 mmol) in $H_2O$-THF (1:1; 10 mL), methyl (*Z*)-3-(5-methoxy-2-nitrophenyl)-2-[(propynylamino)methyl])propenoate **88h** (0.68 g, 2.2 mmol), sodium ascorbate (0.09 g, 0.5 mmol) and $CuSO_4.5H_2O$ (17 mg, 67 μmol). Work-up and flash chromatography afforded, as lemon-yellow flakes, the *cinnamate ester-AZT conjugate* **89h** (0.6 g, 44%), m.p. 102-104 °C (Found M + 1: 572.2105. Calc. for $C_{25}H_{30}N_7O_9$: 572.2105); $\upsilon_{max}$ (ATR)/cm$^{-1}$ 3392 (OH) and 1684 (C=O); $\delta_H$ (400 MHz; methanol-*d*$_4$) 1.93 (3H, s, 21'-$CH_3$), 2.90 and 2.75 (2H, 2 x m, 14'-$CH_2$), 3.34 and 3.50 (4H, 2 x s, 1'- and 3'-$CH_2$), 3.80 (3H, m, $CH_2OH$, and 13'-H), 3.87 (3H, s, $CO_2CH_3$), 5.38 (1H, m, 16'-H), 6.50 (1H, m, 15'-H), 7.03 (1H, s, Ar-H), 7.14 (1H, dd, *J* = 9.2 and 2 Hz, Ar-H), 7.87 (1H, s, Ar-H), 7.92 (1H, s, Ar-H) and 8.26 (1H, d, *J* = 8.8 Hz, Ar-H); $\delta_C$ (100 MHz; methanol-*d*$_4$) 12.5 (21'-$CH_3$), 39.0 (14'-$CH_2$), 44.1 and 45.7 (1'- and 3'-$CH_2$), 52.9 ($CO_2CH_3$), 57.1 ($ArOCH_3$), 61.0 (C-13'), 62.2 ($CH_2OH$), 86.4 (C-16'), 86.7 (C-15'), 111.7 (s, Ar-C), 115.8 (d, Ar-C), 117.1 (d, Ar-C), 123.8 (d, Ar-C), 128.8 (s, Ar-C), 131.5 (s, Ar-C), 134.8 (s, Ar-C), 138.2 (d, Ar-C), 141.6 (s, Ar-C), 147.1 (s, Ar-C), 152.3 (s, Ar-C) and 165.1, 166.4 and 169.0 (3 x C=O).

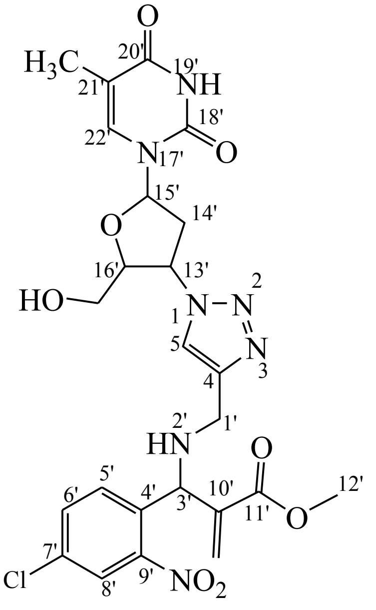
**MBH ester–AZT conjugate 113f**



The general method was followed using AZT (0.50 g, 1.6 mmol) in $H_2O$-THF (1:1; 10 mL), a mixture containing (4:1) methyl (*Z*)-3-(5-chloro-2-nitrophenyl)-2-[(propynylamino)methyl])propenoate **88f** and methyl 2-methylene-3-(2-propnynylamino)-3-(5-chloro-2-nitrophenyl)propanoate **90f** (0.58 g, 1.6 mmol), sodium ascorbate (0.07 g, 0.4 mmol) and $CuSO_4.5H_2O$ (14 mg, 60 μmol). Work-up and flash chromatography afforded, as lemon-yellow flakes, the BH *ester-AZT conjugate* **113f** (0.5 g, 29 %), m.p. 81-83 °C (Found M + 1: 576.1617. Calc. for $C_{24}H_{27}N_7O_8Cl$: 576.1610); $\upsilon_{max}$ (ATR)/cm$^{-1}$ 3398 (OH) and 1689 (C=O); $\delta_H$ (400 MHz; methanol-*d*$_4$ ) 1.93 (3H, s, 21'-$CH_3$), 2.90 and 2.75 (2H, 2 x m, 14'-$CH_2$), 3.82 -3.98 (4H, m, $CH_2OH$ and 1'-$CH_2$), 4.40 (1H, d, *J* = 1.6 Hz, 13'-H), 5.35 and 6.45 (2H, 2 x s, C=$CH_2$) 5.98 (1H, s, 3'-H), 5.47 (1H, t, *J* = 3.2 Hz, 16'-H), 6.52 (1H, t, *J* = 5.2 Hz, 15'-H), 7.51 (1H, d, *J* = 5.20 Hz, Ar-H), 7.89 (1H, d, *J* = 7.60 Hz, Ar-H), 7.94 (1H, s, Ar-H) and 8.06 (1H, s, Ar-H); (100 MHz; methanol-*d*$_4$) 12.6 (21'-$CH_3$), 39.1 (14'-$CH_2$), 43.3 ($CH_2OH$), 52.7 ($CO_2CH_3$), 56.9 (C-13'), 60.8 (C-3'), 62.1 ($NCH_2$), 86.4 ( C-15'), 86.7 (C-16'), 111.7 (s, Ar-C), 124.1 (d, Ar-C), 127.3 (d, Ar-C), 127.4 (C=$CH_2$), 129.7 (d, Ar-C), 130.6 (d, Ar-C), 138.3 (d, Ar-

C), 139.4 (s, Ar-C), 140.1 (s, Ar-C), 141.8 (*C*=$CH_2$), 147.6 (s, Ar-C), 149.5 (s, Ar-C), 152.3 (s, Ar-C) and 166.3, 167.6 and 173.0 (3 x C=O).

**MBH ester–AZT conjugate 113g.**

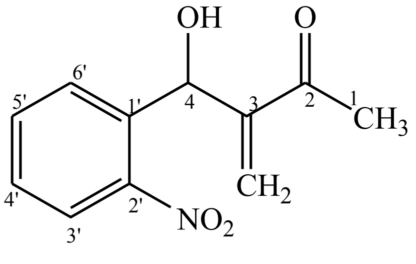


The general method was followed using AZT (0.23 g, 0.9 mmol) in $H_2O$-THF (1:1; 5 mL), a mixture containing (4:1) methyl (*Z*)-3-(4-chloro-2-nitrophenyl)-2-[(propynylamino)methyl]propenoate **88g** and methyl 3-(4-chloro-2-nitrophenyl)-2-methylene-3-(2-propnynylamino)propanoate **90g** (0.28 g, 0.9 mmol), sodium ascorbate (0.04 g, 0.2 mmol) and $CuSO_4.5H_2O$ (7 mg, 30 μmol). Work-up and flash chromatography afforded, as lemon-yellow flakes, the BH *ester-AZT conjugate* **113g** (0.07 g, 15 %), m.p. 74-76 °C (Found M + 1: 576.1609. Calc. for $C_{24}H_{27}N_7O_8Cl$: 576.1610); $\upsilon_{max}$ (ATR)/$cm^{-1}$ 3398 (OH) and 1689 (C=O); $\delta_H$ (400 MHz; methanol-$d_4$ ) 1.96 (3H, s, 21'-$CH_3$), 2.82 and 2.69 (2H, 2 x m, 14'-$CH_2$), 3.84 -3.87 (4H, m, $CH_2OH$ and 1'-$CH_2$), 4.30 (1H, s, 13'-H), 5.18 (1H, s, 13'-H), 5.35 and 6.40 (2H, 2 x s, C=$CH_2$), 5.93 (1H, d, *J* = 2.4 Hz, 3'-H), 6.42 (1H, s, 16'-H), 6.43 (1H, d, *J* = 4.8 Hz, 15'-H), 7.38 (1H, d, *J* = 8.4 Hz, Ar-H), 7.64 (1H, s, Ar-H), 7.69 (1H, dd, *J* = 8.4 and 1.6 Hz, Ar-H), 7.95 (1H, s, Ar-H), and 8.12 (1H, s, Ar-H); (100 MHz; methanol-$d_4$) 12.6 (21'-$CH_3$), 39.1

(14'-$CH_2$), 43.2 ($CH_2OH$), 52.6 ($CO_2CH_3$), 56.6 (C-13'), 61.0 (C-3'), 62.1($NCH_2$), 86.5 ( C-15'), 86.7 (C-16'), 124.2 (d, Ar-C), 111.7 (s, Ar-C), 124.5 (d, Ar-C), 125.3 (d, Ar-C), 126.0 (d, Ar-C), 127.3 ($C{=}CH_2$), 132.3 (d, Ar-C), 133.9 (d, Ar-C), 138.3 (d, Ar-C), 139.7 (d, Ar-C), 141.7 (s, Ar-C) 141.8 ($C{=}CH_2$), 145.9 (s, Ar-C), 147.7 (s, Ar-C), 149.3 (s, Ar-C), 152.3 (s, Ar-C) and 166.4, 169.0 and 173.1 (3 x C=O).

## 3.8. SYNTHESIS OF MVK-DERIVED BH ADDUCTS.

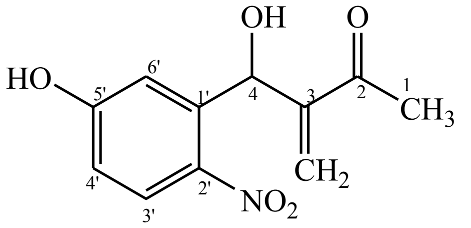
**4-Hydroxy-3-methylene-4-(2-nitrophenyl)butan-2-one 92a.**



**Method 1.** To a solution of 2-nitrobenzaldehyde **81a (**1.0 g, 6.2mmol) and MVK **91** (0.59 mL, 0.49 g, 7.0 mmol) in chloroform (0.5 mL) was added DABCO (0.04 g, 0.3 mmol). The mixture was stirred at room temperature in a stoppered flask for ten days, the progress of the reaction being monitored daily by TLC. Excess solvent and unreacted MVK were evaporated from the crude mixture *in vacuo*, and the residue was purified by flash chromatography [on silica gel; eluting with EtOAc-hexane (2:3)] to afford, as hygroscopic golden-brown crystals, 4-hydroxy-3-methylene-4-(2-nitrophenyl)butan-2-one **92a** (0.41 g, 28%), m.p. 78-80 °C (lit.[68] 79.5-82.5 °C); $\upsilon_{max}$ (ATR)/$cm^{-1}$ 3443 (OH) and 1709 (C=O); $\delta_H$ (400 MHz; $CDCl_3$) 2.37 (3H, s, $CH_3$), 3.50 (1H, d, $J$ = 3.4 Hz, OH), 5.78 and 6.16 (2H, 2 x s, $CH_2$), 6.21 (1H, d, $J$ = 3.4 Hz , 4-H), 7.45 (1H, t, d, $J$ =8.2 and 1.2 Hz, 4'-H), 7.64 (1H, t, d, $J$ = 8.0 and 0.8 Hz, 5'-H), 7.77 (1H, d, $J$ = 7.6 Hz, 6'-H) and 7.97 (1H, dd, $J$ = 8.0 and 0.8 Hz, 3'-H); $\delta_C$ (100 MHz; $CDCl_3$ ) 25.9 (C-1), 67.2 (C-4), 124.5 (C-3'), 126.4 ($C{=}CH_2$ ), 128.4 (C-4'), 128.8 (C-6'), 133.4 (C-5'), 136.5 (C-1'), 147.9 (C-2'), 149.0 ($C{=}CH_2$ ) and 199.7 (C=O).

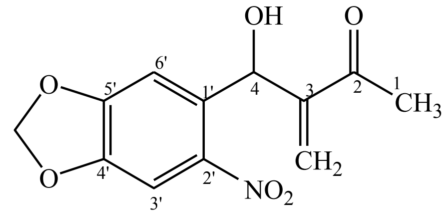
**Method 2.** A mixture of 2-nitrobenzaldehyde **81a** (0.52 g, 3.4 mmol), MVK **91** (0.30 mL, 0.25 g, 3.6 mmol) and DABCO (0.02 g, 0.2 mmol) in a stoppered 8 mL sample tube was irradiated with stirring in a microwave reactor for 30 min. at 25 °C and 150 Watts power. The progress of the reaction was monitored every 10 min. by TLC. Excess MVK was evaporated from the crude mixture *in vacuo* and the residue was purified by flash chromatography [on silica gel; eluting with EtOAc-hexane-MeOH (14:85:1)] to afford a golden-brown viscous oil, which crystallized on standing, to give 4-hydroxy-3-methylene-4-(2-nitrophenyl)butan-2-one, **92a** (0.37 g, 54%).

### 4-Hydroxy-4-(5-hydroxy-2-nitrophenyl)-3-methylenebutan-2-one 92b.



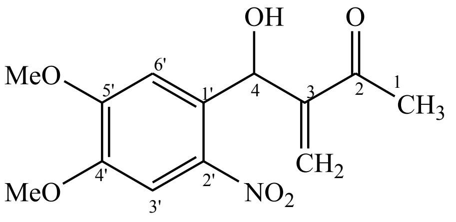
To a solution of 5-hydroxy-2-nitrobenzaldehyde **81b** (2.9 g, 17 mmol) and MVK **91** (1.60 mL, 1.4 g, 33 mmol), in a methanol-chloroform mixture (1:2; 5.5 mL) was added 3-hydroxyquinuclidine (3HQ) (0.16 g, 0.58 mmol). The mixture was left to stir at room temperature in a stoppered flask for *ca* six weeks, the progress of reaction being monitored regularly by TLC. Excess solvent and unreacted MVK were evaporated from the crude mixture *in vacuo,* and the residue was purified by flash chromatography [on silica gel; eluting with EtOAc-hexane (2:3)] to afford colourless crystals (which became light brown on standing) of 4-hydroxy-4-(5-hydroxy-2-nitrophenyl)-3-methylenebutan-2-one **92b**, (2.27 g, 51%), m.p. 148-150 °C (lit.[73]150–152 °C ); $\upsilon_{max}$ (ATR) / $cm^{-1}$ 3356 (OH) and 1673 (C=O); $\delta_H$ (400 MHz; DMSO-$d_6$) 2.30 (3H, s, $CH_3$), 5.60 (1H, s, 4-H), 5.85 (1H, d, *J* = 4.0 Hz, OH), 6.16 and 6.19 (2H, 2 x overlapping s, $CH_2$), 6.83 (1H, dd, *J* = 8.8 and 2.8 Hz, 4'-H), 7.14 (1H, d, *J* = 2.4 Hz, 6'-H), 7.96 (1H, d, *J* = 8.8 Hz, 3'-H) and 10.9 (1H, br s, 5'-OH); δc (100 MHz; DMSO-$d_6$) 26.0 (C-1), 64.7 ( C-4), 114.4 (C-4'), 114.9 (C-3'), 124.9 (C=*C*$H_2$), 127.7 (C-6'), 138.9 (C-5'), 142.4 (C-1'), 150.4 (C-2'), 162.3 (*C*=$CH_2$) and 198.0 (C=O).

**4-Hydroxy-3-methylene-4-(4,5-methylenedioxy-2-nitrophenyl)butan-2-one 92c.**



The procedure described for the synthesis of 4-hydroxy-3-methylene-4-(2-nitrophenyl)butan-2-one **81a** was followed, using 4,5-methylenedioxy-2-nitrobenzaldehyde **55c** (3.7 g,19 mmol), MVK **91** (2.5 mL, 2.0 g, 29 mmol), 3HQ (0.13 g, 1.0 mmol) in $CHCl_3$ (5 mL). The mixture was left to stir at room temperature in a stoppered flask for *ca* six weeks, the progress of reaction being monitored regularly by TLC. Excess solvent and unreacted MVK were evaporated from the crude mixture *in vacuo,* and the residue was purified by flash chromatography [on silica gel; eluting with EtOAc-hexane (1:3)] to afford as a brownish yellow, waxy solid (which slowly crystallized on standing), 4-hydroxy-3-methylene-4-(4,5-methylenedioxy-2-nitrophenyl)butan-2-one **92c**, (1.36 g, 26%), m.p. 94-96 °C (lit.[101]100-103 °C); $\upsilon_{max}$ (ATR)/cm$^{-1}$ 3356 (OH) and 1673 (C=O); $\delta_H$ (400 MHz; $CDCl_3$) 2.37 (3H, s, $CH_3$ ), 3.50 (1H, d, *J* = 1.6 Hz, OH), 5.75 (1H, d, *J* = 0.8 Hz, 4-H), 6.11 (2H, s, $OCH_2O$), 6.12 and 6.18 (2H, 2 x s, C=$CH_2$), 7.20 (1H, s, 6'-H) and 7.52 (1H, s, 3'-H); $\delta_C$ (100 MHz; $CDCl_3$**)** 25.9 (C-1), 67.4 (C-4), 103.0 ($OCH_2O$), 105.5 (C-3'), 107.7 (C-6'), 125.9 (C-*C*$H_2$), 134.2 (C-1'), 141.8 (C-2'), 147.2 (C-7'), 149.1 (*C*=$CH_2$), 152.2 (C-4') and 199.8 (C=O).

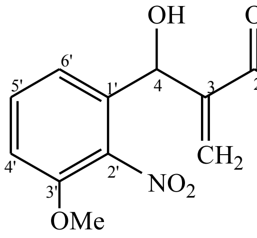
**4-(4,5-Dimethoxy-2-nitrophenyl)-4-hydroxy-3-methylenebutan-2-one 92d.**

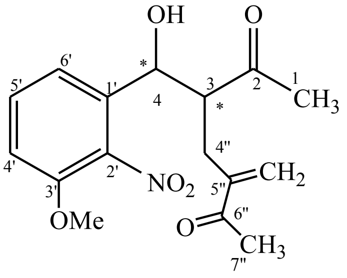


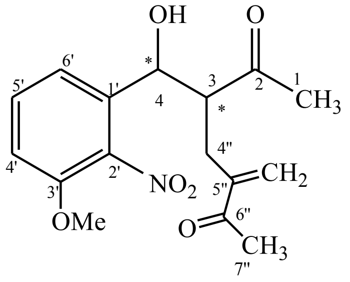
**Method 1.** The procedure described for the synthesis of 4-hydroxy-3-methylene-4-(2-nitrophenyl)butan-2-one **81a** was followed, using 4,5-dimethoxy-2-nitrobenzaldehyde **55d** (1.01 g, 4.78 mmol), MVK **91** (0.60 mL, 0.50 g, 7.1 mmol) and DABCO (0.03 g, 0.25mmol) in $CHCl_3$ (3 mL) and stirring for *ca* six weeks. The crude product was purified by flash chromatography [on silica gel; eluting with EtOAc-hexane (2:1)] to afford as a golden-brown oil which solidified on standing to a hard wax, 4-(4,5-dimethoxy-2-nitrophenyl)-4-hydroxy-3-methylenebutan-2-one **92d** (0.77 g, 16%), m.p. 66-68 °C (cited as a yellowish-brown oil);[73] $\upsilon_{max}$ (ATR)/cm$^{-1}$ 3353 (OH) and 1706 (C=O); $\delta_H$ (400 MHz; $CDCl_3$) 2.35 (3H, s , $CH_3$), 5.54 (1H, s, C*H*OH), 6.02 and 6.28 (2H, 2x s, C=$CH_2$), 3.91 and 3.95 (6H, 2 x s, 2x Ar$OCH_3$), 7.28 (1H, s, 6'-H) and 7.60 (1H, s, 3'-H); $\delta_C$ (100 MHz; $CDCl_3$**)** 25.9 (C-1), 56.3 and 56.4 (2 x Ar$OCH_3$ ), 67.3 (C-4), 107.9 (C-6'), 109.9 (C-3'), 125.7 (C=*C*$H_2$), 131.9 (C-1'), 139.6 (C-2’), 147.8 (C-5'), 149.5 (C-4’), 153.4 (*C*=$CH_2$) and 200.1 (C=O).

**Method 2.** The procedure described for was also followed, using 4,5-dimethoxy-2-nitrobenzaldehyde **81d** (2.1 g; 9.9 mmol), MVK (1.2 mL, 1.0 g, 15 mmol), DABCO (0.11 g, 0.99 mmol) in $CHCl_3$ (1.5 mL). The mixture was irradiated in a microwave reactor at 250 Watts and 75 °C for *ca*. 30 min, the progress of reaction being monitored by TLC. Excess solvent and unreacted MVK were evaporated from the crude mixture *in vacuo,* and the residue was purified by flash chromatography [on silica gel; eluting with EtOAc-hexane (30:70)] to afford as a golden brown oil, 4-(4,5-dimethoxy-2-nitrophenyl)-4-hydroxy-3-methylenebutan-2-one **92d** (0.60 g, 22%).

### 4-Hydroxy-4-(3-methoxy-2-nitrophenyl)-3-methylenebutan-2-one 92e, 5-Acetyl-6-hydroxy-6-(3-methoxy-2-nitrophenyl)-3-methylene-2-hexanone97e$_{syn}$ and 5-Acetyl-6-hydroxy-6-(3-methoxy-2-nitrophenyl)-3-methylene-2-hexanone 97e$_{anti}$.







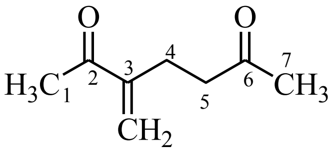
The procedure described for the synthesis of 4-hydroxy-3-methylene-4-(2-nitrophenyl)butan-2-one **92a** was followed, using 3-methoxy-2-nitrobenzaldehyde **81e** (2.35 g, 13.0 mmol), MVK **91** (1.90 mL, 1.59 g, 22.8 mmol), DABCO (0.7 g, 0.7 mmol) in $CHCl_3$ (3 mL). The mixture was left to stir for *ca*. 2 days, the progress of reaction being monitored daily by TLC. Excess solvent and unreacted MVK were evaporated from the crude mixture *in vacuo*, and the residue was purified by flash chromatography [on silica gel; eluting with EtOAc-hexane (1:3)] to afford two fractions.

**Fraction 1:** 4-hydroxy-4-(3-methoxy-2-nitrophenyl)-3-methylenebutan-2-one, as a viscous, light-brown transparent oil, **92e** (1.30 g, 40%), (cited as oil);[73] $\nu_{max}$ (ATR)/cm$^{-1}$ 3407 (OH) and 1671 (C=O); $\delta_H$ (400 MHz; $CDCl_3$) 2.23 (3H, s, $CH_3$), 3.72 (1H, d, $J$ = 4.8 Hz, OH), 3.78 (3H, s, $ArOCH_3$), 5.57 (1H, d, $J$ = 3.6 Hz, 4-H), 5.92 and 6.17 (2H, 2x s, $C{=}CH_2$), 6.93 (1H, d, $J$ =8.4 Hz, 6'-H ), 7.01 (1H, d, $J$ =7.6 Hz, 4'-H), and 7.34 (1H, t, $J$ = 8.2 Hz, 5'-H); $\delta_C$ (100 MHz; $CDCl_3$) 25.8 (C-1), 56.2 ($OCH_3$), 67.1 (C-4), 111.9 (C-6'), 119.2 (C-4'), 127.2 ($C{=}\mathit{C}H_2$), 131.0 (C-5'), 134.3 (C-1'), 139.9 (C-2'), 147.5 ($\mathit{C}{=}CH_2$), 150.4 (C-3') and 199.3 (C=O).

**Fraction 2:** A dark-brown oil (0.84 g, *ca*. 20%), comprising a pair of diastereomers (1:3; from NMR analysis) which were separated by HPLC, eluting with EtOAc-hexane (1:1)] to afford:- (i) *5-Acetyl-6-hydroxy-6-(3-methoxy-2-nitrophenyl)-3-methylene-2-hexanone* **97e**$_{syn}$**:** (Found M + 23: 344.1099. Calc. for $C_{16}H_{19}NO_6Na$: 344.1110); $\nu_{max}$ (ATR)/cm$^{-1}$ 3412 (OH), 1706 and 1671 (2 x C=O); $\delta_H$ (400 MHz; $CDCl_3$) 2.07 and 2.24 (6H, 2 x s, 1- and 7''-$CH_3$), 2.61 (2H, d, *J* = 4.0 Hz, 4''-$CH_2$), 3.22 (1H, dd, *J* = 12.0 and 4.0 Hz, 3-H), 3.38 (1H, s, OH), 3.87 (3H, d, *J* = 4.0 Hz, $ArOCH_3$), 5.00 (1H, d, *J* = 4.0 Hz, 4-H), 5.68 and 5.94 ( 2H, 2 x s, C=C*H*$_2$), 6.98 (1H, d, *J* = 8.0 Hz, 4'-H), 7.18 (1H, d, *J* = 8.0 Hz, 6'-H) and 7.42 (1H, t, *J* = 8.0 Hz, 5'-H); $\delta_C$ (100 MHz; $CDCl_3$) 25.7 (C-1), 27.5 ( C-4''), 31.1 (C-7''), 55.6 (C-3), 56.5 (ArO*C*H$_3$), 68.8 (C-4), 112.0 (C-5'), 120.0 (C-6'), 127.6 (C=*C*H$_2$),130.9 (*C*=CH$_2$), 134.2 (C-4''), 140.0 (C-1'), 146.0 (C-3'), 150.9 (C-2') , 199.5 and 211.6 ( 2 x C=O).

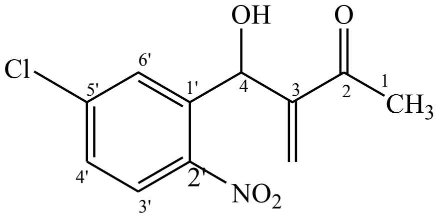
*5-Acetyl-6-hydroxy-6-(3-methoxy-2-nitrophenyl)-3-methylene-2-hexanone* **97e**$_{anti}$**:** (Found M + 23: 344.1111. Calc. for $C_{16}H_{19}NO_6Na$: 344.1110); $\nu_{max}$ (ATR)/cm$^{-1}$ 3427 (OH), 1709 and 1671 (2 x C=O); $\delta_H$ (400 MHz; $CDCl_3$) 2.08 and 2.33 (6H, 2 x s, 1- and 7''-$CH_3$), 2.38 (1H, m, 3-H), 2.57 and 3.12 (2H, 2 x dd, *J* = 13.2 and 6.4Hz, 4''-$CH_2$), 3.86 (3H, s, $ArOCH_3$), 4.00 (1H, br s, OH), 4.68 (1H, s, 4-H), 5.86 and 6.06 (2H, 2 x s, C=C*H*$_2$), 6.95 (1H, d, *J* = 8.4 Hz, 4'-H), 7.10 (1H, d, *J* = 8.0 Hz, 6'-H) and 7.43 (1H, t, *J* = 8.2 Hz, 5'-H); $\delta_C$ (100 MHz; $CDCl_3$) 25.6 (C-1), 30.8 ( C-4''), 31.9 (C-7''), 54.9 (C-3), 56.4 (ArO*C*H$_3$), 69.5 ( C-4), 111.8 (C-5'), 112.0 ( C-6'), 118.9 (C-6'), 128.6 (C=*C*H$_2$), 131.2 (C-4'), 135.4 (*C*=CH$_2$), 139.9 (C-4'), 140.1 (C-1'), 145.0 (C-3'), 150.6 (C-2') , 199.3 and 213.2 (2 x C=O).

**3-Methyleneheptan-2,6-dione 96.**



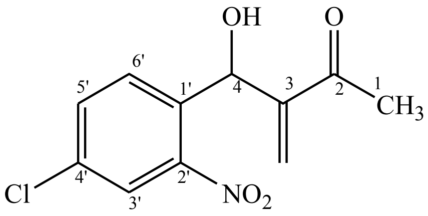
The MVK dimer **96** was isolated (0.76 g, *ca*.26%), as a competition product with some of the Morita-Baylis-Hillman adducts **92**, as a light yellow oil; $\nu_{max}$ (ATR)/cm$^{-1}$ 1711 and 1671 (2 x C=O); $\delta_H$ (400 MHz; CDCl$_3$) 1.74 and 1.94 (6H, 2x s, 2x CH$_3$), 2.23 (4H, m, 4- and 5-CH$_2$), 5.46 and 5.70 (2H, 2x s, C=CH$_2$); $\delta_C$ (100 MHz; CDCl$_3$) 24.3 and 28.9 (C-1 and C-7), 25.0 and 41.4 (C-4 and C-5), 125.1 (C=*C*H$_2$), 147.0 (C-3), 198.3 and 206.6 (2 x C=O).

**4-(5-Chloro-2-nitrophenyl)4-hydroxy-3-methylenebutan-2-one 92f.**



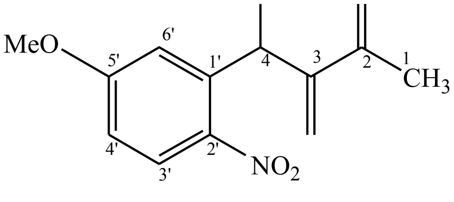
The procedure described for the synthesis of 4-hydroxy-3-methylene-4-(2-nitrophenyl)butan-2-one **92a** was followed, using 5-chloro-2-nitrobenzaldehyde **81f** (5.0 g, 27 mmol), MVK **91** (4.5 mL, 3.8 g, 54 mmol), DABCO (0.2g, 1.4 mmol) in CHCl$_3$ (1.5 mL). The mixture was left to stir for *ca* six days, the progress of reaction being monitored daily by TLC. Excess solvent and unreacted MVK were evaporated from the crude mixture *in vacuo,* and the residue was purified by flash chromatography [on silica gel; eluting with EtOAc-hexane (1:3)] to afford, as yellow, transparent crystals, 4-(5-chloro-2-nitrophenyl)-4-hydroxy-3-methylenebutan-2-one **92f** (5.5 g, 80%), m.p. 73-75 °C, (lit. [73] 75-78°C); $\upsilon_{max}$ (ATR)/cm$^{-1}$ 3351 (OH) and 1661 (C=O); $\delta_H$ (400 MHz; CDCl$_3$) 2.37 (3H, s, CH$_3$), 3.48 (1H, br s, CHO*H*), 5.76 (1H, s, C*H*OH), 6.15 and 6.22 (2H, 2 x s, C=CH$_2$) , 7.40 (1H, t, *J* = 6.8 Hz, 4'-H), 7.77 (1H, s, 6'-H), 7.95 (1H, d, *J* = 8.8 Hz, 3'-H); $\delta_C$ (100 MHz; CDCl$_3$) 25.9 (C-1), 67.2 (C-4), 126.2 (C-6'), 126.5 (C=*C*H$_2$), 128.6 (C-4'), 129.1 (C-3'), 138.7 (C-1'), 140.3 (C-5'), 146.1 (C-2'), 148.0 (*C*=CH$_2$) and 199.7 (C=O).

### 4-(4-Chloro-2-nitrophenyl)-4-hydroxy-3-methylenebutan-2-one 92g.



The procedure described for the synthesis of 4-hydroxy-3-methylene-4-(2-nitrophenyl)butan-2-one **92a** was followed, using 4-chloro-2-nitrobenzaldehyde **81g** (0.51 g, 2.8 mmol), MVK **66** (0.34mL, 0.29 g, 4.2 mmol), DABCO (0.02 g, 0.14 mmol) in $CHCl_3$ (0.5 mL). The mixture was left to stir for *ca*. 7 days, the progress of reaction being monitored daily by TLC. Excess solvent and unreacted MVK were evaporated from the crude mixture *in vacuo,* and the residue was purified by flash chromatography [on silica gel; eluting with EtOAc-hexane (1:3)] to afford, as a golden brown, transparent oil, 4-(4-chloro-2-nitrophenyl)-4-hydroxy-3-methylenebutan-2-one, **92g** (0.31 g, 44%); $\upsilon_{max}$ (ATR)/$cm^{-1}$ 3412 (OH) and 1671 (C=O); $\delta_H$ (400 MHz; $CDCl_3$) 2.26 (3H, s, $CH_3$), 4.16 (1H, br s, CHO*H*), 5.77 (1H, s, C*H*OH), 6.09 and 6.11 (2H, 2 x s, C=$CH_2$), 7.51 (1H, d, *J* = 8.8 Hz, 5'-H), 7.64 (1H, d, *J* =7.6 Hz, 6'-H) and 7.82 (1H, d, *J* = 2.0 Hz, 3'-H); $\delta_C$ (100 MHz; $CDCl_3$) 25.7 (C-1), 66.0 (C-4), 124.2 (C-6'), 126.4 (C=*C*$H_2$), 130.0 (C-5'), 133.1 (C-3'), 133.7 (C-3), 135.4 (C-1'), 147.0 (C-4'), 148.7 (C-2') and 199.2 (C=O).

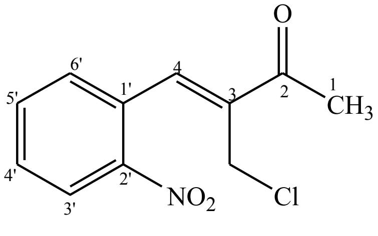
### 4-Hydroxy-4-(5-methoxy-2-nitrophenyl)-3-methylenebutan-2-one 92h.



The procedure described for the synthesis of 4-hydroxy-3-methylene-4-(2-nitrophenyl)butan-2-one **92a** was followed, using 5-methoxy-2-nitrobenzaldehyde **81h** (1.03 g, 5.7 mmol), MVK (0.71 mL, 0.60 g, 8.5 mmol), DABCO (0.05 g, 0.28 mmol) in $CHCl_3$ (1.5 mL). The mixture was left to stir for *ca.* 5 days, the progress of reaction being monitored daily by TLC. Excess solvent and unreacted MVK were evaporated from the crude mixture *in vacuo,* and the residue was purified by flash chromatography [on silica gel; eluting with EtOAc-hexane (1:3)] to afford, as a golden-brown, transparent oil, 4-hydroxy-4-(5-methoxy-2-nitrophenyl)-3-methylenebutan-2-one*,* **92h** (1.29 g, 91%), (cited without full data set [65,66]); $\upsilon_{max}$ (ATR)/cm$^{-1}$ 3437 (OH) and 1671 (C=O); $\delta_H$ (400 MHz; $CDCl_3$) 1.92 (1H, s, CHO*H*), 2.27 (3H, s, $CH_3$), 3.80 (3H,s, $ArOCH_3$), 5.55 (1H, s, C*H*OH), 6.00 and 6.22 (2H, 2 x s, $C{=}CH_2$), 6.80 (1H, dd, $J$ = 9.2 and 2.4 Hz, 4'-H), 7.23 (1H, d, $J$ = 2.0 Hz, 6'-H) and 8.00 (1H, d, $J$ = 9.2 Hz, 3'-H); $\delta_C$ (100 MHz; $CDCl_3$) 25.6 (C-1), 55.7 ($OCH_3$), 66.7 (C-4), 112.8 (C-6'), 113.5 (C-4'), 125.5 (C=*C*$H_2$), 127.4 (C-3'), 140.1 (C-1'), 140.2 (C-3), 149.3 (C-5'), 163.5 (C-2') and 199.3 (C=O).

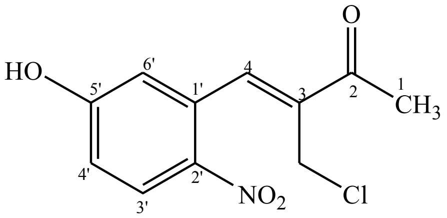
## 3.9. REACTIONS OF KETONES WITH HCl.

### (*Z*)-3-(Chloromethyl)-4-(2-nitrophenyl)but-3-en-2-one 93a.



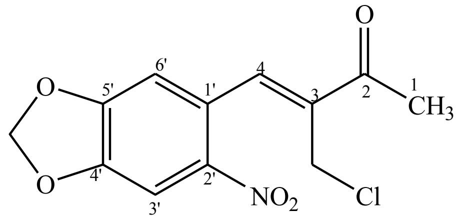
A solution of 4-hydroxy-3-methylene-4-(2-nitrophenyl)butan-2-one **92a** (0.11 g, 0.50 mmol) in ethanol (0.5 mL) was added to ethanolic HCl [generated *in situ,* by adding acetyl chloride (0.5 mL) dropwise to anhydrous ethanol (1.0 mL) at 0 °C in a two-necked flask fitted with a reflux condenser and a dropping funnel, the mixture and was left to stir for 5 min.]. Stirring was continued at room temperature overnight. Excess solvent and unreacted HCl were evaporated *in vacuo* to afford, as a light-brown powder, (*Z*)-3-(chloromethyl)-4-(2-nitrophenyl)but-3-en-2-one **93a** (0.12 g, 99%), m.p. 121-122 °C, (lit.[102] 120-122°C); $\upsilon_{max}$ (ATR)/cm$^{-1}$ 1671 (C=O); $\delta_H$ (400 MHz; $CDCl_3$) 2.51 (3H, s, $CH_3$), 4.17 (2H, s, $CH_2Cl$), 7.63 (1H, t, *J* = 7.6 Hz, 5'-H), 7.70 (1H, d, *J* = 7.6 Hz, 6'-H), 7.78 (1H, t, *J* = 7.2 Hz, 4'-H), 8.00 (1H, s, 4-H) and 8.23 (1H, d, *J* = 8.4 Hz, 3'-H); $\delta_C$ (100 MHz; $CDCl_3$) 26.6 (C-1), 37.6 ($CH_2Cl$), 124.7 (C-1') 125.2 (C-6'), 130.2 (C-4'), 130.5 (C-5') 134.1 (C-3'), 137.8 (C-2'), 140.2 (C-4), 147.0 (C-3) and 196.7 (C=O).

### *(Z)*-3-(Chloromethyl)-4-(5-hydroxy-2-nitrophenyl)but-3-en-2-one 93b.



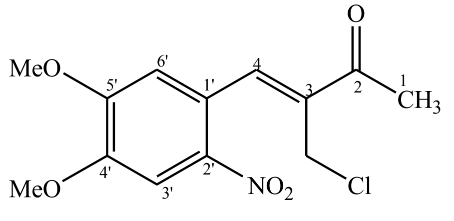
A solution of 4-hydroxy-4-(5-hydroxy-2-nitrophenyl)-3-methylenebutan-2-one **92b** (0.82 g, 3.3 mmol) in ethanol (2 mL) was added to ethanolic HCl [generated *in situ* by adding acetyl chloride (4.5 mL) dropwise to anhydrous ethanol (2.3 mL) at 0 °C in a two-necked flask fitted with a reflux condenser and a dropping funnel and left to stir for 5 min.]. Stirring was continued at room temperature overnight. Excess solvent and unreacted HCl were evaporated *in vacuo* to afford, as brown crystals, (Z)-*3-(chloromethyl)-4-(5-hydroxy-2-nitrophenyl)but-3-en-2-one* **93b** (0.57 g, 92%), m.p. 147-148 °C (lit.[73]144 °C but without a full set of data); (Found M-1: 254.0225. Calc. for $C_{11}H_9ClNO_4$. 254.0220); $\upsilon_{max}$ (ATR)/cm$^{-1}$ 3270 (OH) and 1660 (C=O); $\delta_H$ (400 MHz; DMSO-$d_6$) 2.46 (3H, s, $CH_3$ ), 4.23 (2H, s, $CH_2Cl$), 6.91 (1H, d, *J* = 2.4 Hz, 6'-H), 7.03 (1H, dd, *J* = 8.8 and 2.0 Hz, 4'-H), 8.16 (1H, s, 4-H), 8.21 (1H, d, *J* = 8.8 Hz, 3'-H) and 11.30 (1H, br s, OH); $\delta_C$ (100 MHz; DMSO-$d_6$) 25.9 (C-1), 37.5 ($CH_2Cl$), 116.0 (C-6'), 116.2 (C-4'), 128.0 (C-3'), 133.2 (C-2'), 135.5 and 138.7 (C-3 and C-1'), 142.7 (C-4), 162.8 (C-5') and 197.0 (C=O)

### (*Z*)-3-(Chloromethyl)-4-(4,5-methylenedioxy-2-nitrophenyl)but-3-en-2-one 93c.



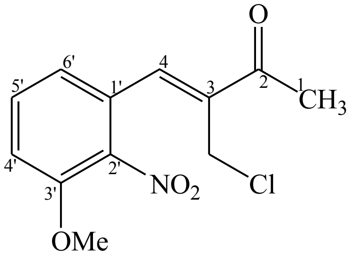
A solution of 4-hydroxy-3-methylene-4-(4,5-methylenedioxy-2-nitrophenyl)butan-2-one **92c** (0.66 g, 2.5 mmol) in ethanol (2 mL) was added to the ethanolic HCl [generated *in situ* by adding acetyl chloride (3.0 mL) dropwise to anhydrous ethanol (6.0 mL) at 0 °C in a two-neck flask fitted with a reflux condenser and a dropping funnel, and the mixture was left to stir for 5 min.]. Stirring was continued at room temperature overnight. Excess solvent and unreacted HCl were evaporated *in vacuo* to afford, as black wax which crystallized on standing, (Z)-*3-(chloromethyl)-4-(4,5-methylene-2-nitrophenyl)but-3-en-2-one* **93c** (0.74 g, > 99%), m.p. 146-148 °C (reported as black wax, without a full data set[73]); (Found M + H: 284.0236. Calc. for $C_{12}H_{10}ClNO_5$. 284.0226); $\upsilon_{max}$ (ATR)/cm$^{-1}$ 1714 (C=O); $\delta_H$ (400 MHz; $CDCl_3$ ) 2.45 (3H, s, $CH_3$), 4.16 (2H, s, $CH_2Cl$), 6.16 (2H, s , $OCH_2O$), 7.02 (1H, s, 6'-H), 7.64 (1H,s, 3'-H) and 7.88 (1H, s, 4-H); $\delta_C$ (100 MHz; $CDCl_3$ ) 25.9 (C-1), 37.0 ($CH_2Cl$), 103.6 ($OCH_2O$), 105.7 (C-3'), 108.8 (C-6'), 126.7 (C-1'), 137.0 (C-3), 140.9 (C-4), 143.0 (C-2'), 148.8 (C-5'), 152.4 (C-4') and 196.6 (C=O).

### (*Z*)-3-(Chloromethyl)-4-(4,5-dimethoxy-2-nitrophenyl)but-3-en-2-one 93d.



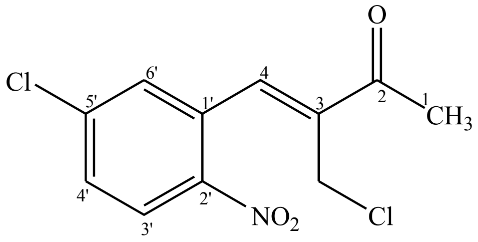
A solution of 4-(4,5-dimethoxy-2-nitrophenyl)-4-hydroxy-3-methylene-butan-2-one **92d** (0.13 g, 0.47 mmol) in ethanol (1.0 mL) was added to ethanolic HCl [generated *in situ* by adding acetyl chloride (1.5 mL) dropwise to anhydrous ethanol (2.0 mL) at 0 °C in a two-necked reaction flask fitted with a reflux condenser and a dropping funnel, and the mixture was left to stir for 5 min.]. Stirring was continued at room temperature overnight. Excess solvent and unreacted HCl were evaporated in *vacuo* to afford, as dark-brown crystals, (Z)-*3-(chloromethyl)-4-(4,5-dimethoxy-2-nitrophenyl)but-3-en-2-one* **93d** (0.14 g, > 99%), m.p. 48-50 $^{\circ}$C (reported as black oil without a full data set[73]); $\upsilon_{max}$ (ATR)/cm$^{-1}$ 1722 (C=O); $\delta_H$ (400 MHz; CDCl$_3$) 2.50 (3H, s, CH$_3$), 4.00 (6H, d, *J* = 8.0 Hz, 2 x OCH$_3$), 4.22 (2H, s, CH$_2$Cl), 7.22 (1H, s, 4-H), 7.77 (1H, s, 6'-H), and 8.03 (1H, s, 3'-H); $\delta_C$ (100 MHz; CDCl$_3$**)** 26.1 (C-1), 37.7 (CH$_2$Cl), 56.4 and 56.5 (2 x ArOCH$_3$ ), 108.2 (C-6'), 110.3 (C-3'), 132.4 (C-1'), 139.8 (C-3), 141.7 (C-4), 146.9 (C-2'), 148.2 (C-4'), 153.1 (C-5') and 202.0 (C=O).

### (*Z*)-3-(Chloromethyl-4-(3-methoxy-2-nitrophenyl)but-3-en-2-one 93e.



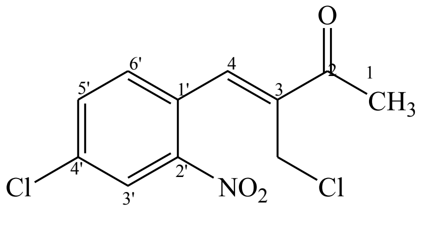
A solution of 4-hydroxy-4-(3-methoxy-2-nitrophenyl)-3-methylenebutan-2-one **92e** (0.67 g, 2.7 mmol) in ethanol (1.0 mL) was added to ethanolic HCl [generated *in situ* by adding acetyl chloride (1.0 mL) dropwise to anhydrous ethanol (2.0 mL) at 0 °C in a two-necked flask fitted with a reflux condenser and a dropping funnel, and the mixture was left to stir for 5 min.]. Stirring was continued at room temperature overnight. Excess solvent and unreacted HCl were evaporated *in vacuo* to afford, as dark-brown crystals *3-(chloromethyl-4-(3-methoxy-2-nitrophenyl)but-3-en-2-one* **93e** (0.73 g, > 99%), m.p. 66-68 $^{0}$C (Found M$^{+}$: 269.0427. Calc. for C$_{12}$H$_{12}$NO$_4$Cl, 269.0455); $\upsilon_{max}$ (ATR)/cm$^{-1}$ 1671 (C=O); $\delta_H$ (400 MHz; CDCl$_3$ ) 2.42 (3H, s, CH$_3$), 3.93 (3H, s, OCH$_3$), 4.25 (2H, s, CH$_2$Cl), 7.14 (1H, d, *J* = 8.4 Hz, 6'-H), 7.27 (1H, d, *J* = 8.0 Hz, 4'-H), 7.49 (1H, s, 4-H) and 7.55 ( 1H, t, *J* = 8.0 Hz, 5'-H); $\delta_C$ (100 MHz; CDCl$_3$) 25.9 (C-1), 36.9 (CH$_2$Cl), 56.6 (OCH$_3$), 113.9 (C-6'), 120.8 (C-4'), 121.8 (C-1'), 128.2 (C-3), 131.8 (C-5'), 135.5 (C-4), 140.9 (C-2'), 151.4 (C-3') and 196.3 (C=O).

### (Z)-3-(Chloromethyl)-4-(5-chloro-2-nitrophenyl)but-3-en-2-one 93f.



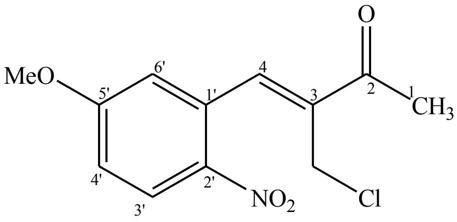
A solution of 4-(5-chloro-2-nitrophenyl)-4-hydroxy-3-methylenebutan-2-one **92f** (0.22 g, 0.85 mmol) in ethanol (1.0 mL) was added to ethanolic HCl [generated *in situ* by adding acetyl chloride (1.5 mL) dropwise to anhydrous ethanol (3.5mL) at 0 °C in a two-necked flask fitted with a reflux condenser and a dropping funnel, and left to stir for 5 min.]. Stirring was continued at room temperature overnight. Excess solvent and unreacted HCl were evaporated in *vacuo* to afford, as light-brown, transparent crystals, (Z)-*3-(chloromethyl)-4-(5-chloro-2-nitrophenyl)but-3-en-2-one*, **93f** (0.23 g, . 99%), m.p. 102-103 $^0$C (reported as black oil without full data set[102]), (Found M+1: 274.0032. Calc. for $C_{11}H_{10}NO_3Cl_2$: 274.0038); $\upsilon_{max}$ (ATR)/cm$^{-1}$ 1671 (C=O) , $\delta_H$ (400 MHz; CDCl$_3$ ) 2.53 (3H, s, CH$_3$ ), 4.18 (2H, s, CH$_2$Cl), 7.59 (1H, d, *J* =8.8 Hz, 3'-H), 7.69 (1H, s, 6'-H), 7.91 (1H, s, 4-H) and 8.21 ( 1H, d, *J* = 8.8 Hz, 3'-H); $\delta_C$ (100MHz; CDCl$_3$ ) 26.0 (C-1), 36.7 (CH$_2$Cl), 126.7 (C-3'), 130.2 (C-4'), 130.5 (C-6'), 132.0 (C-1'), 138.5 (C-3), 138.6 (C-4), 140.8 (C-2'), 145.4 (C-5') and 196.3 (C=O).

### (Z)-3-(Chloromethyl)-4-(4-chloro-2-nitrophenyl)but-3-en-2-one 93g.



A solution of 4-(4-chloro-2-nitrophenyl)-4-hydroxy-3-methylenebutan-2-one **92g** (0.40 g, 1.6 mmol) in ethanol (1.0 mL) was added to ethanolic HCl [generated *in situ* by adding acetyl chloride (1.5 mL) dropwise to anhydrous ethanol (1.5 mL) at 0 °C in a two-necked flask fitted with a reflux condenser and a dropping funnel, and the mixture was left to stir for 5 min.]. Stirring was continued at room temperature overnight. Excess solvent and unreacted HCl were evaporated *in vacuo* to afford, as a dark-brown oil, (Z)-*3-(chloromethyl)-4-(4-chloro-2-nitrophenyl)but-3-en-2-one* **93g** (0.42 g, > 99%) (Found M: 274.0032. Calc. for $C_{11}H_{10}NO_3Cl_2$: 274.0038); $\upsilon_{max}$ (ATR)/cm$^{-1}$ 1671 (C=O) , $\delta_H$ (400 MHz; $CDCl_3$ ) 2.49 (3H, s, C*H*$_3$ ), 4.14 (2H, s, $CH_2Cl$), 7.66 (1H, d, *J* = 7.2 Hz, 5'-H), 7.72 (1H, s, 6'-H), 7.90 (1H, s, 4-H) and 8. 19 ( 1H, s, 3'-H); $\delta_C$ (100 MHz; $CDCl_3$) 25.9 (C-1), 36.8 ($CH_2Cl$), 125.4 (C-3'), 128.5 (C-4'), 131.6 (C-6'), 134.1 (C-5'), 136.1 (C-1'), 138.1 (C-2'), 138.9 (C-6'), 147.3 (C-4) and 196.3 (C=O).

**(*Z*)-3-(Chloromethyl)-4-(5-methoxy-2-nitrophenyl)but-3-en-2-one 93h.**



A solution of 4-hydroxy-4-(5-methoxy-2-nitrophenyl)-3-methylenebutan-2-one **92h (**0.91 g, 3.6 mmol) in anhydrous ethanol (2.5 mL) was added to ethanolic HCl [generated *in situ* by adding acetyl chloride (3 mL) dropwise to anhydrous ethanol (3.5 mL) at 0°C in a two-necked flask fitted to a reflux condenser and a dropping funnel, and the mixture was left to stir for 5min.]. Stirring was continued at room temperature overnight. Excess solvent and unreacted HCl were evaporated in *vacuo* to afford, as a dark-brown oil, (Z)-*3-(chloromethyl)-4-(5-methoxy-2-nitrophenyl)but-3-en-2-one*, **93h** (0.36 g, > 99%), m.p. 84-86 °C (Found M+H: 270.0528. Calc. for $C_{12}H_{12}NO_4Cl$. 270.0533); $\upsilon_{max}$ (ATR)/cm$^{-1}$ 1709 (C=O) , $\delta_H$ (400 MHz; $CDCl_3$ ) 2.47 (3H, s, $CH_3$ ), 3.89 (3H, s, $ArOCH_3$), 4.16 (2H, s, $CH_2Cl$), 7.00 (1H, d, *J* = 9.2 Hz, 4'-H), 7.11 (1H, s, 6'-H), 8.00 (1H, s, 4-H) and 8. 20 (1H, d, *J* = 9.2 Hz, 3'-H); $\delta_C$ (100 MHz; $CDCl_3$ ) 25.8 (C-1), 37.1 ($CH_2Cl$), 56.2

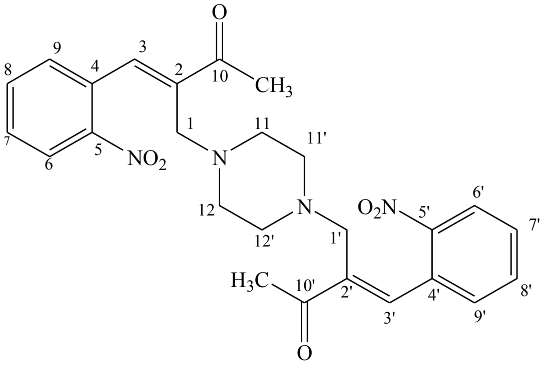
($OCH_3$), 114.5 (C-4'), 115.5 (C-3'), 127.6 (C-6'), 132.8 (C-5'), 136.9 (C-1'), 139.7 (C-2'), 141.2 (C-4), 163.6 (C-3) and 196.6 (C=O).

## 3.10. CONJUGATE ADDITION OF AMINES.

**General Method:** A solution of the Baylis-Hillman chloromethyl derivatives **93** and the specified amine in anhydrous THF was stirred in a stoppered flask at room temperature for periods which varied according to the reactants involved. The solution was then concentrated *in vacuo* and the crude product purified by flash chromatography to afford the aminated product.

### 3.10.1. Reactions with piperazine.

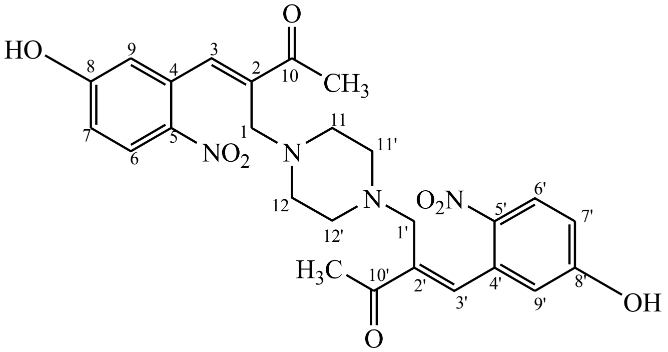
***N*,*N*'-Bis{[(*E*)-4-(2-nitrophenyl)but-3-en-2-on-3-yl]methyl-1,4-iperazine 94a.**



The general method was followed, using equimolar amounts of *(Z)-3-(*chloromethyl)-4-(2-nitrophenly)but-3-en-2-one **93a** (1.00 g, 4.2 mmol) and piperazine (0.36 g, 4.2 mmol) in anhydrous THF (2.0 mL). After 5 days, the solvent was removed *in vacuo* and the crude product was purified by flash chromatography [on silica gel; eluting with EtOAc-hexane (1:1)] followed by triturating with EtOAc-hexane (1:1) to afford, as bright-yellow crystals, N,N'*-bis{[*(E)*-4-(2-nitrophenyl)but-3-en-2-on-3-yl]methyl}-1,4-piperazine* **94a** (0.19 g, 19%), m.p. 174-176 °C (Found M+1**:** 493.2103. Calc. for $C_{26}H_{29}N_4O_6$: 493.2087); $\nu_{max}$ (ATR)/cm$^{-1}$ 1654 (C=O); $\delta_H$ (400 MHz; $CDCl_3$) 2.01 (8H, s, 4 x $CH_2$), 2.44 (6H, s, 2 x $CH_3$), 3.04 (4H, s, 1- and 1'–$CH_2$), 7.53 (4H, m, 7-, 7'-, 8- and 8'- H), 7.64 (2H, m, 9- and 9'-H), 7.81 (2H, s, 3- and 3'-H) and 8.13 (2H, d, *J* = 8.0 Hz ,6- and 6'-H); $\delta_C$ (100 MHz; $CDCl_3$) 26.5 ( 2 x $CH_3$), 52.5 ( 4 x $CH_2$), 52.6 ( C-1 and

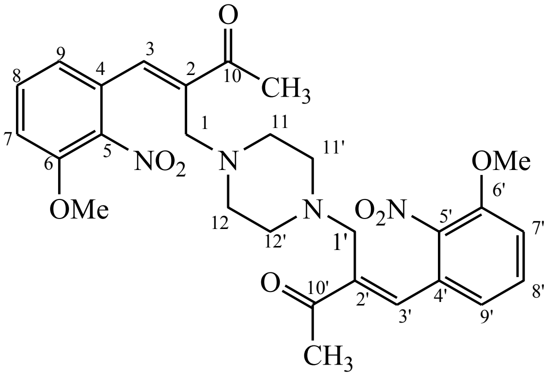
C-1'), 124.6 (C-7 and C-7'), 129.1 ( C-8 and C-8'), 131.0 (C-9 and C-9'), 131.9 (C-2 and C-2') 133.2 (C-3 and C-3'), 138.8 (C-6 and C-6'), 139.3 (C-4 and C-4'), 147.5 (C-5 and C-5') and 199.7 (C-10 and C-10').

**_N,N'_-Bis{[(_E_)-[4-(5-hydroxy-2-nitrophenyl)but-3-en-2-on-3-yl]methyl}-1,4-piperazine 94b.**



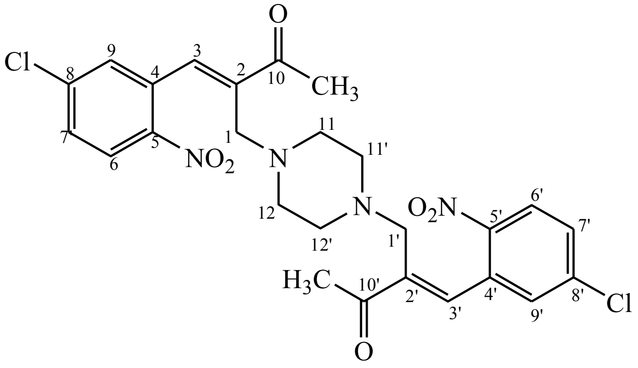
The general method was followed, using equimolar amounts of (Z)-3-(chloromethyl)-4-(5-hydroxy-2-nitrophenly)but-3-en-2-one **93b** (0.6 g, 0.2 mmol.) and piperazine (2 mg, 0.2 mmol) in anhydrous THF (1.5 mL). After 2 days, the solvent was removed *in vacuo* and the crude product was purified by flash chromatography [on silica gel; eluting with EtOAc-hexane (1:1)] followed by triturating with EtOAc-hexane (1:1) to afford, as bright-yellow crystals, N,N'*-bis{[*(E)*-4-(5-hydroxy-2-nitrophenyl)but-3-en-2-on-3-yl]methyl}-1,4-piperazine* **94b** (0.02 g, > 5%), m.p. 184-186 °C $\nu_{max}$ (ATR)/cm$^{-1}$ 1653 (C=O); $\delta_H$ (400 MHz; methanol-*d*$_4$) 1.24 (8H, s, 4 x NCH$_2$), 2.54 (6H, s, 2 x CH$_3$), 4.21 (4H, s, 1- and 1' -CH$_2$), 7.03 (4H, d, *J* = 9.2 Hz, 7-, 7'-, 8- and 8'- H), 7.09 (2H, d, *J* = 1.6 Hz, 9- and 9'-H), 8.03 (2H, s, 3- and 3'-H) and 8.24 (2H, d, *J* = 9.2 Hz, 6- and 6'-H ) $\delta_C$ (100 MHz; methanol-*d*$_4$) 26.5 ( 2 x CH$_3$), 50.5 (4 x *C*H$_2$), 52.8 (C-1 and C-1'), 117.2 (C-7 and C-7'), 117.8 (C-9 and C-9'), 128.9 (C-6 and C-6'), 133.0 (C-2 and C-2') 138.8 (C-4 and C-4'), 139.7 (C-5 and C-5'), 142.0 (C-3 and C-3'), 161.3 (C-8 and C-8') and 199.0 (C-10 and C-10').

**_N_,_N'_-Bis{[(_E_)-4-(3-methoxy-2-nitrophenyl)but-3-en-2-on-3-yl]methyl}-1,4-piperazine 94e.**



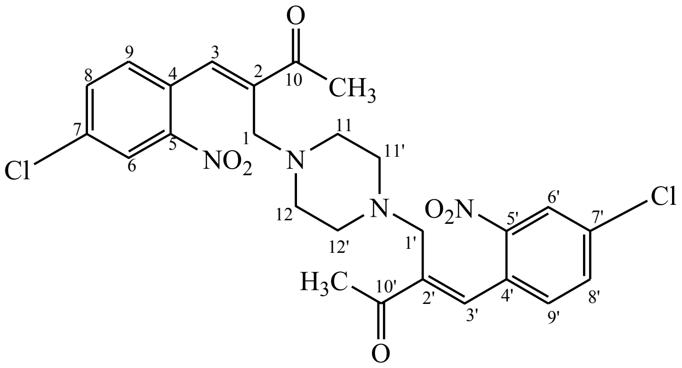
The general method was followed, using equimolar amounts of (*Z*)-3-(chloromethyl)-4-(3-methoxy-2-nitrophenyl)but-3-en-2-one **93e** (0.32 g, 1.2mmol.) and piperazine (0.10 g, 1.2 mmol) in anhydrous THF (2.0 mL). After 4 days, the solvent was removed *in vacuo* and the crude product was purified by flash chromatography [on silica gel; eluting with EtOAc-hexane (1:1)] followed by triturating with EtOAc-hexane (1:1) to afford, as a pale-white fluffy solid, N,N'-*bis{[*(E)-*4-(3-methoxy-2-nitrophenyl)but-3-en-2-on-3-yl]methyl}-1,4-piperazine* **94e** (0.17 g, 50%), m.p 204-206 °C (Found: C, 60.9; H, 6.2; N, 9.8. Calc. for $C_{28}H_{32}N_4O_8$: C, 60.9; H, 5.8; N, 10.1%); $\nu_{max}$ (ATR)/cm$^{-1}$ 1669 (C=O); $\delta_H$ (400 MHz; CDCl$_3$) 2.24 (8H, br, 4 x CH$_2$), 2.35 (6H, s, 2 x CH$_3$), 3.16 (4H, s, 1- and 1' -CH$_2$), 3.90 (6H, s, 2 x ArOCH$_3$) 7.04 (2H, d, *J* = 8.0 Hz, 7- and 7'-H), 7.20 (2H, d, *J* = 7.6 Hz, 9- and 9'-H), 7.32 (2H, s, 3- and 3'-H) and 8.43 (2H, t, *J* = 8.0 Hz, 8- and 8'-H ); $\delta_C$ (100 MHz; CDCl$_3$) 26.8 (2 x CH$_3$), 52.7 (4 x *C*H$_2$), 53.1 (C-1 and C-1'), 56.5 (2 x ArOCH$_3$), 112.6 (C-7 and C-7'), 122.0 (C-8 and C-8'), 129.4 (C-2 and C-2'), 131.0 (C-9 and C-9'), 133.1 (C-3 and C-3'), 140.5 (C-4 and C-4'), 142.8 (C-6 and C-6'), 150.9 (C-5 and C-5') and 199.8 (C-10 and C-10').

### *N*,*N*'-Bis{[(*E*)-4-(5-chloro-2-nitrophenyl)but-3-en-2-on-3-yl]methyl}-1,4-piperazine **94f.**



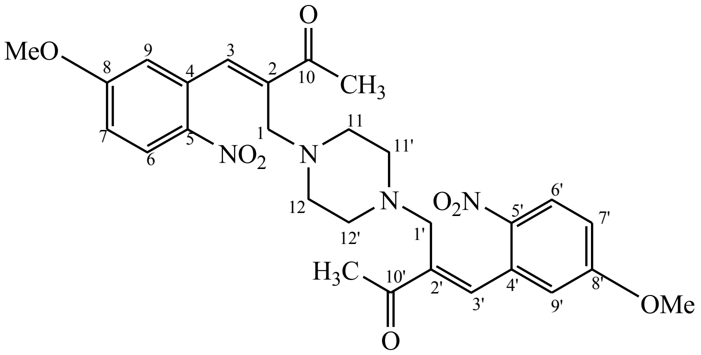
The general method was followed, using equimolar amounts of (*Z*)-3-(chloromethyl)-4-(5-chloro-2-nitrophenyl)but-3-en-2-one **93f** (0.34 g, 1.2 mmol.) and piperazine (0.10 g, 1.2 mmol) in anhydrous THF (2.0 mL). After 3 days, the solvent was removed *in vacuo* and the crude product was purified by flash chromatography [on silica gel; eluting with EtOAc-hexane (1:1)] followed by triturating with EtOAc-hexane (1:1) to afford, as bright-yellow crystals, N,N'-*bis{[*(E)*-(4-(5-chloro-2-nitrophenyl)but-3-en-2-on-3-yl]methyl]-1,4-piperazine* **94f** (0.04 g, 13%), m.p. 197-199 °C (Found: C, 55.5; H, 4.8; N, 9.9. Calc. for $C_{24}H_{26}N_4O_6Cl_2$: C, 55.6; H, 4.7; N, 10.0%); $\nu_{max}$ (ATR)/cm$^{-1}$ 1686 (C=O); $\delta_H$ (400 MHz; $CDCl_3$) 2.09 (8H, br s, 4 x C*H*$_2$), 2.46 (6H, d, *J* = 1.6 Hz, 2 x $CH_3$), 3.06 (4H, s, 1- and 1' -$CH_2$), 7.47 (2H, overlapping m, 9- and 9'-H), 7.83 (4H, overlapping m, 6- and 6'-H as well as 7 and 7'-H), and 8.10 (2H, overlapping m, 3- and 3'-H), $\delta_C$ (100 MHz; $CDCl_3$); 26.3 (2 x $CH_3$), 52.2 (4 x $CH_2$), 52.5 (C-1 and C-1'), 126.0 (C-7 and C-7'), 129.0 (C-6 and C-6'), 131.5 (C-9 and C-9'), 133.5 (C-2 and C-2'), 138.0 (C-3 and C-3'), 139.7 (C-4 and C-4') 140.1 (C-5 and C-5'), 145.9 (C-8 and C-8'), and 199.1 (C-10 and C-10').

**N,N'-Bis{[(E)-4-(4-chloro-2-nitrophenyl)but-3-en-2-on-3-yl]methyl}-1,4-piperazine 94g.**



The general method was followed, using equimolar amounts of (*Z*)-3-(chloromethyl)-4-(4-chloro-2-nitrophenyl)but-3-en-2-one **93g** (0.31 g, 1.1 mmol.) and piperazine (0.09 g, 1.1 mmol) in anhydrous THF (2.0 mL). After 6 days, the solvent was removed *in vacuo* and the crude product was purified by flash chromatography [on silica gel; eluting with EtOAc-hexane (1:1)] followed by triturating with EtOAc-hexane (1:1) to afford, as a bright-yellow powdery solid, N,N'-*bis{[*(E)-*4-(4-chloro-2-nitrophenyl)but-3-en-2-on-3yl]methyl}-1,4-piperazine* **94g** (0.06 g, 8.8%), m.p. 139-140 °C (Found M+1: 561.1281**.** Calc. for $C_{26}H_{27}N_4O_6Cl_2$: 561.1308) $\nu_{max}$ (ATR)/cm$^{-1}$ 1689 (C=O); $\delta_H$ (400 MHz; $CDCl_3$) 2.06 (8H, d, *J* = 16 Hz, 4 x C*H*$_2$), 2.45 (6H, s, 2 x $CH_3$), 3.05 (4H, s, 1- and 1'–$CH_2$), 7.61 (4H, 2 x d, *J* = 8.0 and 8.0Hz, 8- and 8'-H as well as 9 and 9'-H ), 7.75 (2H, s, 6- and 6'-H), and 8.12 (2H, s, 3- and 3'-H); $\delta_C$ (100 MHz; $CDCl_3$) 26.4 (2 x $CH_3$), 52.4 (4 x $CH_2$), 52.5 (C-1 and C-1'), 124.8 (C-8 and C-8'as well as C-9 and C-9'), 130.2 (C-2 and C-2'), 132.3 ( C-6 and C-6'), 133.3 (C-3 and C-3'), 135.0 (C-4 and C-4'), 137.7 (C-5 and C-5'), 139.9 (C-7 and C-7') and 199.2 (C-10 and C-10').

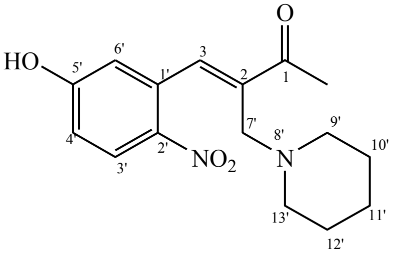
### ***N,N'*-Bis{(*E*)-4-(5-methoxy-2-nitrophenyl)but-3-en-2-on-3-yl]-methyl}-1,4-piperazine 94h.**



The general method was followed, using equimolar amounts of (*Z*)-3-(chloromethyl)-4-(5-methoxy-2-nitrophenyl)but-3-en-2-one **93h** (1.41 g, 2.6 mmol.) and piperazine (0.22g, 2.6 mmol) in dry THF (3.0 mL). After five days, the solvent was removed *in vacuo* and the crude product was purified by flash chromatography [on silica gel; eluting with EtOAc-hexane-methanol (45:45:10) followed by triturating with EtOAc-hexane (1:1)] to afford, as a pale-yellow fluffy solid, N,N'-*bis{*(E)-(*4-(5-methoxy-2-nitrophenyl)but-3-en-2-on-3-yl]methyl}-1,4 piperazine* **94h** (21 mg, 2%), m.p. 207-209 °C (Found M+1: 553.2305. Calc. for $C_{28}H_{33}N_4O_8$: 553.2398); $\nu_{max}$ (ATR)/cm$^{-1}$ 1663 (C=O); $\delta_H$ (400 MHz; CDCl$_3$); 2.31 (8H, br, 4 x C*H*$_2$), 2.49 (6H, d, *J* = 10.4 Hz, 2 x CH$_3$), 3.16 (4H, s, 1- and 1' -CH$_2$), 3.90 (6H, s, 2 x ArOCH$_3$), 7.00 (2H, d, *J* = 8.8 Hz, 7- and 7'-H), 7.02 (2H, d, *J* = 2.0 Hz, 9- and 9'-H), 7.90 (2H, s, 3- and 3'-H) and 8.21 (2H, d, *J* = 9.2 Hz, 6- and 6'-H ); $\delta_C$ (100 MHz; CDCl$_3$) 26.9 (2 x CH$_3$), 45.5 (C-1 and C-1'), 53.1 (4 x CH$_2$), 56.6 (2 x ArOCH$_3$), 114.3 (C-6 and C-6'), 116.4 (C-7 and C-7'), 127.9 (C-8 and C-8'), 134.8 (C-2 and C-2'), 138.8 (C-4 and C-4'), 140.5 (C-9 and C-9'), 142.8 (C-3 and C-3'), 163.7 (C-2 and C-2') and 200.0 (C-10 and C-10').

## 3.10.2. REACTION WITH PIPERIDINE.

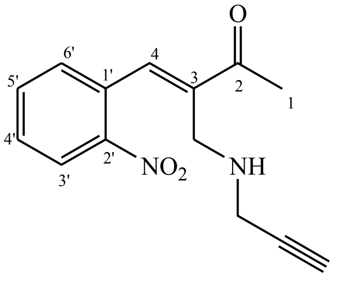
### (*E*)-4-(5-Hydroxy-2-nitrophenyl)-3-[(piperidin-1-yl)methyl]but-3-en-2-one 95b.



The general method was followed, using 3-chloromethyl-4-(5-hydroxy-2-nitrophenyl)but-3-en-2-one **93b** (1.0 g, 4.0 mmol), piperidine (0.8 mL, 8.0 mmol) and anhydrous THF (1.5 mL). After 24 hours, the reaction was terminated, work-up and purification of the crude product by flash chromatography [on silica gel; eluting with 5% MeOH in $CHCl_2$] afforded, as a dirty-green fluffy solid, (E)-*4-(5-hydroxy-2-nitrophenyl)-3-[(piperidin-1-yl)methyl]but-3-en-2-one* **95b** (0.3 g, 23%), m.p. 114 – 116 °C (Found M+1: 305.1516. Calc. for $C_{16}H_{21}N_2O_4$: 305.1501); $\nu_{max}$ (ATR)/cm$^{-1}$ 3561 (OH) and 1734 (C=O); $\delta_H$ (400 MHz; DMSO-*d*$_6$) 1.27 (6H, m, 10'-, 11'- and 12'- $CH_2$). 2.08 (4H, m, 9'- and 13'- $CH_2$), 2.41 (3H, s, $CH_3$), 2.84 (1H, br s, OH), 3.06 ( 2H, s, 7'-$CH_2$), 6.73 (1H, s, *J* = 2.0 Hz, 6'-H), 6.88 (1H, dd, *J* = 8.8 and 2.4 Hz, 4'-H), 7.82 (1H, s, 3'-H) and 8.10 (1H, d, *J* = 9.2 Hz, 3-H); $\delta_C$ (100MHz; DMSO-*d*$_6$ ) 21.6 (C-11'), 25.4 (C-10' and C-12'), 26.6 ($CH_3$), 53.5 (C-9' and C-13'), 116.4 (C-6'), 116.8 (C-4'), 128.1 (C-3'), 133.4 (C-2), 138.3 (C-1'), 139.2 (C-2'), 163. 3 (C-5') and 199.1 (C=O).

## 3.10.3. REACTION WITH PROPARGYLAMINE.

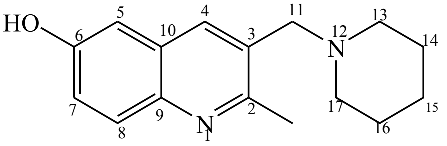
### (*E*)-4-(2-Nitrophenyl)-3-[(propynylamino)methyl]but-3-en-2-one 102a.



The general method was followed using 3-chloromethyl-4-(5-hydroxy-2-nitrophenyl)]but-3-en-2-one **93a**, (1.1 g, 4.5 mmol), propargylamine, (0.43 mL, 6.7 mmol) in anhydrous THF (2.5 mL). After 5 days, the reaction was terminated, work-up and flash chromatography [on silica gel; eluting with EtOAc-hexane (1:1)] followed by preparative HPLC with the same solvent system and subsequent trituration with hexane-EtOAc (1:1) afforded as a yellow solids, *4-(2-nitrophenyl)-3-[(propynylamino)methyl]but-3-en-2-one* **102a,** (46 mg, 4%), m.p 55 – 57 °C; $\nu_{max}$ (ATR)/cm$^{-1}$ 1704 (C=O); $\delta_H$ (400 MHz; $CDCl_3$) (3H, m, $CH_3$), 1.41 ( 1H, s, C≡CH), 2.01 and 3.23 (4H, 2x s, $CH_2NCH_2$), 2.42 (3H, s, $CH_3$), 4.12 (1H, m, NH), 7.32 (1H, d, *J* = 7.6 Hz, 6'-H), 7.49 (1H, t, *J* = 7.6Hz, 4'-H), 7.62 (1H, t, *J* = 7.0 Hz, 5'-H), 7.79 (1H, s, 4-H), 8.12 (1H, d, *J* = 8.0 Hz, 3'-H); $\delta_C$ (100 MHz; $CDCl_3$) 26.7 ($CH_3$), 40.2 and 48.2 ($CH_2NCH_2$), 72.6 and 77.0 (C≡CH), 124.7 (C-4'), 129.2 (C-6'), 131.4 (C-1'), 131.8 (C-5'), 133.4 (C-3'), 138.5 (C-4), 138.7 (C-3), 147.5 (C-2') and 199.5 (C=O).

## 3.12 REDUCTIVE CYCLIZATION.

**6-Hydroxy-2-methyl-3-[(piperidin-1-yl)methyl]quinoline 99b.**

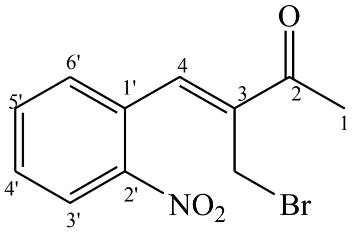


To a solution of 4-(5-hydroxy-2-nitrophenyl)-3-[(piperidin-1-yl)methyl]but-3-en-2-one **95b** (0.95 g, 3.1 mmol) in absolute ethanol (20 mL), 10% Pd-C catalyst (1.2 g) was added and the reaction mixture was stirred under a hydrogen atmosphere for 1.5 hours. The reaction mixture was filtered over a bed of Celite to remove the catalyst and give a purple filtrate, which was then concentrated *in vacuo* to afford, as a dark-brown solid, *6-hydroxy-2-methyl-3-[(piperidin-1-yl)methyl]quinoline* **99b** (0.44 g, 55 %), m.p. 204 – 206 $^0$C (Found M + 1: 257.1654. Calc. for $C_{16}H_{21}N_2O_4$: 257.1564), $\nu_{max}$ (ATR)/cm$^-$ 3387 (OH); $\delta_H$ (400 MHz; DMSO-*d*$_6$) 1.55 (2H, m, 15-$CH_2$), 1.67 (4H, m, 14-, and 16-$CH_2$), 2.35 (3H, s, 2-$CH_3$), 2.98 (6H, m, 11-, 13-, and 17-$CH_2$), 7.02 (1H, d, *J* = 2.8 Hz, 7-H), 7.19 (1H. dd, *J* = 8.8 and 2.4 Hz, 7-H ), 7.72 (1H, d, *J* = 9.2 Hz, 8-H), and 7.79 (1H, s, 4-

H), $\delta_C$ (100 MHz; DMSO-$d_6$) 19.0 (ArCH$_3$), 21.7 (C-15), 22.1 (C-14 and -16), 43.4 (C-11, -13 and -17), 107.5 (C-5), 120.4 (C-7), 128.2 (C-3), 129.2 (C-8), 129.9 (C-9), 133.2 (C-4), 141.0 ( C-2). 154.8 (C-10) and 155.0 (C-6).

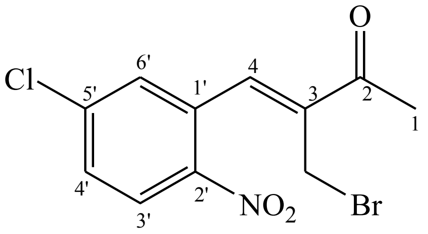
## 3.13. REACTIONS OF BH KETONES WITH HBr.

### (*Z*)-3-(Bromomethyl)-4-(2-nitrophenyl)but-3-en-2-one 104a.



A solution of 4-hydroxy-3-methylene-4-(2-nitrophenyl)butan-2-one **92a** (1.1 g, 4.4 mmol) in ethanol (2.0 mL) was added to ethanolic HBr [generated *in situ* by adding acetyl bromide (1.5 mL, 8.8 mmol) dropwise to dry ethanol (5.0 mL) at 0°C, in a two-necked flask fitted with a reflux condenser and a dropping funnel, and the mixture was left to stir for 5 min.]. Stirring was continued at room temperature overnight. Excess solvent and unreacted HBr were evaporated *in vacuo* to afford crude material which was purified by flash chromatography [on silica gel; eluting with EtOAc-hexane (1:3)] to afford, as light-brown powder, (Z)-*3-(bromomethyl)-4-(2-nitrophenyl)but-3-en-2-one* **104a** (0.80 g, *ca*. 64%), m.p. 118-120 °C (Found M + 1: 285.9902. Calc. for $C_{11}H_{10}BrNO_3$: 285.9922); $\upsilon_{max}$ (ATR)/cm$^{-1}$ 1671 (C=O); $\delta_H$ (400 MHz; CDCl$_3$) 2.53 (3H, s, CH$_3$), 4.07 (2H, s, CH$_2$Br), 7.63 (1H, t, *J* =7.6 Hz, 4'-H), 7.80 (2H, m, 5'- and 6'-H), 7.95 (1H, s, 4-H) and 8.25 (1H, d, *J* = 8.0 Hz, 3'-H); $\delta_C$ (100 MHz; CDCl$_3$) 23.9 (CH$_2$Br), 26.1 (C-1), 125.3 (C-4') 129.9 (C-6'), 130.1 (C-5'), 130.4(C-1') 134.1 (C-3'), 138.1 (C-3), 139.5 (C-4), 147.0 (C-2') and 196.6 (C=O).

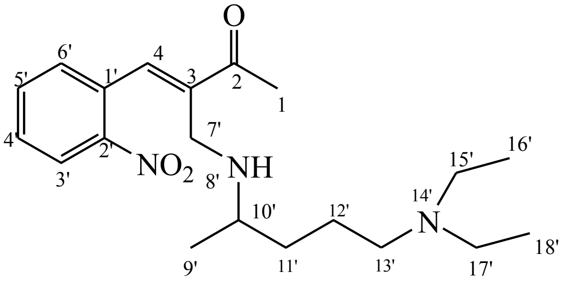
### (*Z*)-3-(Bromomethyl)-4-(5-chloro-2-nitrophenyl)but-3-en-2-one 104f.



A solution of 4-(5-chloro-2-nitrophenyl)-4-hydroxy-3-methylenebutan-2-one **92f** (1.2 g, 4.7 mmol) in ethanol (3.0 mL) was added to ethanolic HBr [generated *in situ* by adding acetyl bromide (2.0 mL, 9.4 mmol) dropwise to dry ethanol (6.0 mL) at 0°C in a two-necked flask fitted with a reflux condenser and a dropping funnel, and the mixture was left to stir for 5 min.]. Stirring was continued at room temperature overnight. Excess solvent and unreacted HBr were evaporated *in vacuo* to afford crude material which was purified by flash chromatography [on silica gel; eluting with EtOAc-hexane (1:3)] to afford, as light-brown powder (Z)-*3-(bromomethyl)-4-(5-chloro-2-nitrophenyl)but-3-en-2-one* **104f** (1.1 g, 89%), m.p. 82 – 84 °C (Found M+1: 317.9533. Calc.for $C_{11}H_{10}BrClNO_3$. 317.9528); $\upsilon_{max}$ (ATR)/cm$^{-1}$ 1671 (C=O); $\delta_H$ (400 MHz; $CDCl_3$) 2.52 (3H, s, $CH_3$ ), 4.06 (2H, s, $CH_2Br$), 7.59 (1H, d, *J* = 8.8 and 1.6 Hz, 4'-H), 7.72 (1H, d, *J* = 1.6 Hz, 6'-H), 7.87 (1H, s, 4-H) and 8.22 (1H, d, *J* = 8.8 Hz, 3'-H); $\delta_C$ (100 MHz; $CDCl_3$) 23.4 ($CH_2Br$), 26.0 (C-1), 126.8 (C-4') 129.8 (C-6'), 129.8 (C-3'), 132.1 (C-1'), 138.0 (C-4) 138.6 (C-3), 140.8 (C-5'), 145.2 (C-2') and 196.2 (C=O).

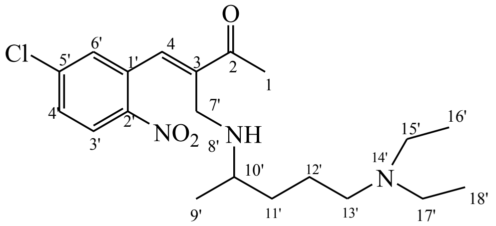
## 3.14. REACTIONS WITH 2-AMINO-5-DIETHYLAMINOPENTANE.

### (*E*)-3-[(5-Diethylamino-1-methylpentylamino)methyl]-4-(2-nitrophenyl)]but-3-en-2-one 103a



To a solution of 3-bromomethyl-4-(2-nitrophenyl)]but-3-en-2-one **104a** (0.4 g, 2.0 mmol), in anhydrous THF (2.0 mL), was added 2-amino-5-(diethylamino)pentane (0.57 mL, 3.0 mmol) and the mixture was left to stir in a stoppered flask at room temperature. After 7 days, the reaction was terminated, work-up and flash chromatography [on basified silica gel; eluting with 5% triethylamine in hexane-EtOAc (1:1)] afforded, as brown wax, (E)-*3-[(5-diethylamino-1-methylpentylamino)methyl]-4-(2-nitrophenyl)]but-3-en-2-one* **103a,** (0.2 g, 36%); (Found M+1: 362.2446. Calc. for $C_{20}H_{32}N_3O_3$, 362.2444) $\nu_{max}$ (ATR)/cm$^{-1}$ 1716 (C=O); $\delta_H$ (400MHz; $CDCl_3$) 0.9 (9H, s, 3 x $CH_3$), 1.23 (4H, m, 11'- and 12'-$CH_2$), 1.41 (1H, br s, NH), 2.36 (3H, m, 1-$CH_3$), 2.42 (1H, 10'-H), 2.48 (6H, m, 13'- 15'- and 17'-$CH_2$), 3.34 (2H, m, 7'-$CH_2$), 7.54 (1H, m, 6'-H), 7.62 (1H, d, *J* = 0.8 Hz, 4'-H), 7.80 (1H, m, 5'-H), 7.89 (1H, s, 3'-H) and 8.18 (1H, m, 4-H), $\delta_C$ (100 MHz; $CDCl_3$); 11.5 (16'- and 18'-$CH_3$), 20.0 (C-9'), 23.2 (C-12'), 26.1 (1-$CH_3$), 34.9 (11'-$CH_2$) 43.0 (7'-$CH_2$), 46.8 (15'- and 17'-$CH_2$), 52.9 (13'-$CH_2$) 53.1 (10'-CH), 124.9 (C-6'), 129.7 (C-4'), 131.2 (C-3), 131.9 (C-5'), 133.5 (C-3'), 138.5 (C-4), 140.6 (C-2'), 147.5 (C-5') and 200.3 (C=O).

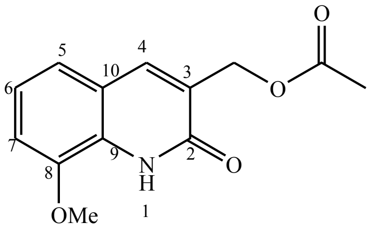
### (*E*)-3-[-4-(5-chloro-2-nitrophenyl)(5-Diethylamino-1-methylpentylamino)methyl]but-3-en-2-one 103f



To a solution of 3-bromomethyl-4-(5-chloro-2-nitrophenyl)]but-3-en-2-one, **104**f (0.59 g, 1.85 mmol), in anhydrous THF (4.0 mL) and triethylamine (1.0 mL) was added 2-amino-5-diethylaminopentane (0.72 mL, 3.7 mmol) and left to stir in a stoppered flask at room temperature. After 2 days, the reaction was terminated, work-up and flash chromatography [on basified silica gel conditioned overnight with the eluent; eluting sequentially with 5% triethylamine in hexane-EtOAc (1:1), followed by 10% methanol in the same eluent], afforded, as reddish-brown oil, *(*E*)-3-[-4-(5-chloro-2-nitrophenyl)(5-Diethylamino-1-methylpentylamino)methyl]but-3-en-2-one* **103f** (0.4 g, 58%); (Found M + 1: 396.2047. Calc. for $C_{20}H_{31}N_3O_3$: 396.2054) $\nu_{max}$ (ATR)/cm$^{-1}$ 1740 (C=O); $\delta_H$ (400MHz; CDCl$_3$) 1.01 (9H, dd, *J* = 14.4 and 7.2 Hz, 3 x CH$_3$), 1.23 (4H, m, 11'- and 12'-CH$_2$), 1.37 (1H, m, 10'-H), 2.43 (2H, t, *J* = 7.4 Hz, 13'-CH$_2$), 2.47 (3H, s, 1-H), 2.56 (5H, m, N-H, 15'- and 17'-CH$_2$), 3.31 (2H, 2 x d, *J* = 11.6 and 11.2 Hz, 7'-CH$_2$), 7.51 (1H, d, *J* = 8.8 Hz, 4'-H), 7.81 (1H, s, 6'-H), 8.04 (1H, s, 4-H) and 8.18 (1H, d, *J* = 8.8 Hz, 3'-H), $\delta_C$ (100 MHz; CDCl$_3$); 11.3 (16'- and 18'-CH$_3$), 20.0 (C-10'), 23.0 (C-12'), 26.0 (1-CH$_3$), 35.0 (11'-CH$_2$) 43.0 (7'-CH$_2$), 46.6 (15'- and 17'-CH$_2$), 52.8 (13'-CH$_2$) 53.5 (9'-CH$_3$), 126.3 (C-6'), 129.7 (C-4'), 131.9 (C-4), 132.9 (C-3), 137.1 (C-3'), 140.2 (C-1'), 141.2 (C-2'), 145.5 (C-5') and 200.0 (C=O).

# 3.15. REDUCTIVE CYCLIZATION TO QUINOLONE DERIVATIVES.

### 3-(Acetoxymethyl)- 8-methoxy-(1*H*)-2-quinolone 112e.

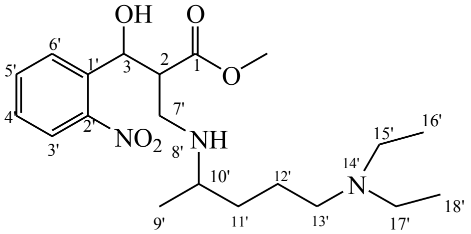


To a solution of methyl 3-hydroxy-3-(3-methoxy-2-nitrophenyl)-2-[(piperidin-1-yl)methyl]propanoate **85e** (1.0 g, 0.3 mmol) in acetic acid (5 mL) at 112 °C was added electrolytic iron powder (0.3 g, 6.0 mmol), and the resulting mixture was stirred for 40 min. at 110-115°C. The mixture was cooled to room temperature, poured into ice-water (50 mL), and the acidic mixture was neutralized by adding saturated aqueous $NaHCO_3$ solution until the pH became alkaline. The cloudy mixture was filtered, and the filtrate was washed with EtOAc (3 x 20 mL) and the organic layers were combined, washed with brine (3 x 10 mL), dried over anhydrous $MgSO_4$ and then concentrated *in vacuo* to afford, as lemon-green powder, 3-(acetoxymethyl-8-methoxy(1*H*)-2-quinolone, **112e** (30 mg, 12%), m.p 163-165 °C (lit. [93] 167-169 °C), $\nu_{max}$ (ATR)/$cm^{-1}$ 1605 and 1641 (2 x C=O); $\delta_H$ (400 MHz; $CDCl_3$) 2.13 (3H, s, $OCH_3$), 3.01 (3H, s, $ArOCH_3$), 5.14 (2H, s, $CH_2$), 6.95 (1H, dd, $J$ = 7.2 and 0.8 Hz, 7-H), 7.14 (2H, m, 5- and 6-H), 9.51 (1H, s, 4-H) and 9.51 (1H, br s, NH); $\delta_C$ (100 MHz; $CDCl_3$) 20.9 ($OCH_3$), 55.9 ($ArOCH_3$), 61.4 ($CH_2$), 110.0 ( C-7), 119.6 (C-5), 122.3 (C-6), 122.3 (C-9), 128.1 (C-10), 128.5 (C-4), 137.9 (C-3), 145.4 (C-8), 160.9 (2-C=O), 170.7 (C=O).

### 3.3.2. Reactions with 2-amino-5-diethylaminopentane.

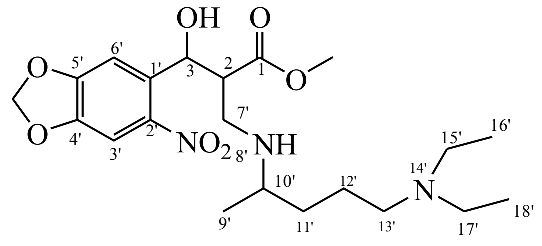
**General Method:** To a solution of the BH adduct **82** (1 eq.) in anhydrous THF basified with triethylamine (0.1 eq.) was added 2-amino-5-diethylaminopentane (1 eq.) and the mixture was left to stir in a stoppered flask for periods which varied according to the reactant involved. Excess solvent and unreacted amine were evaporated *in vacuo* and the residue was purified by flash chromatography on silica gel afforded products as diastereomeric mixture. Diastereomeric ratio were determined from the $^1$H NMR integral data and $^1$H and $^{13}$C NMR data are cited for the major diastereomer.

### Methyl 3-[(4-diethylamino-1-methylbutylamino)-methyl-3-hydroxy-3-(2-nitrophenyl)]-propanoate 106a.



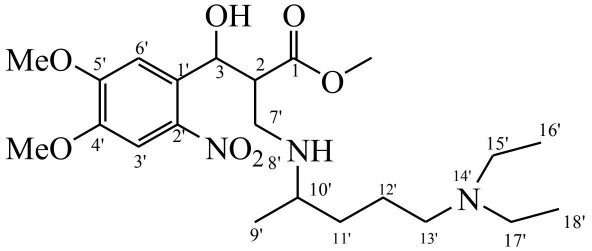
The general method was followed, using methyl 3-hydroxy-2-methylene-3-(2-nitrophenyl)propanoate **59a** (1.2 g, 4.9 mmol) in anhydrous THF (2 mL) and triethylamine ( 0.7 mL), and 2-amino-5-diethylaminopentane (0.78 g, 1.0 mL, 4.9 mmol). After stirring in a stoppered flask for 2 days, excess solvent and unreacted amine were evaporated *in vacuo* and the residue was purified by flash chromatography [on silica gel, basified and conditioned overnight with 5% triethylamine in hexane-EtOAc (1:1); eluting sequentially with 5% triethylamine in hexane-EtOAc (1:1)], followed by 5% methanol in the initial eluent] to afford, as a yellowish-brown, transparent oil, a (3:1) diastereomeric mixture of *methyl 3-[(4-diethylamino-1-methylbutylamino)methyl]-3-hydroxy-3-(2-nitrophenyl)propanoate* **106a**, (1.7 g, 87%); (Found M+1: 396.2494. Calc. for $C_{20}H_{34}N_3O_5$. 396.2496) $\upsilon_{max}$ (ATR)/cm$^{-1}$ 1729 (C=O); $\delta_H$ (400 MHz; CDCl$_3$) 1.09 (9H, overlapping multiplets, 9'-, 16'- and 18'-CH$_3$), 1.36 (4H, overlapping multiplets, 11'- and 12'-CH$_2$), 2.58 (2H, t, $J$ = 7.6 Hz, 7'-CH$_2$), 2.70 (6H, overlapping multiplets, 13', 15'-, and 17'- CH$_2$), 3.09 (1H, m, 10'-H), 3.41 (3H, s, CO$_2$CH$_3$), 3.73 (1H, broad signal, 2-H), 4.70 (3H, br s, 2 x NH and OH), 5.74 (1H, d, $J$ = 9.2 Hz, 3-H), 7.42 (1H, overlapping multiplets, 4'-H), 7.63 (1H, t, *J* = 7.6 Hz, 5'-H), 7.84 (1H, d, *J* = 7.6 Hz, 6'-H) and 8.02 (1H, d, *J* = 8.0 Hz, 3'-H); $\delta_C$ (100 MHz; $CDCl_3$) 10.3 (16'- and 18'-$CH_3$), 20.0 (9'-$CH_3$), 22.1 (11'-$CH_2$), 34.4 (12'-$CH_2$), 46.2 (15'- and 17'-$CH_2$), 47.7 (7'-$CH_2$), 49.9 (C-2), 51.6 ($CO_2$*C*$H_3$), 52.1 (13'-$CH_2$), 53.7 (C-10'), 71.3 (C-3), 124.9 (d, Ar-C), 128.0 (d, Ar-C), 129.0 (d, Ar-C), 133.6 (d, Ar-C), 138.5 (s, Ar-C), 146.8 (s, Ar-C) and 172.8 (C=O).

### Methyl 3[(4-diethylamino-1-methylbutylamino)methyl]-3-hydroxy-3-(4,5-methylenedioxy-2-nitrophenyl)propanoate 106c.



The procedure described for the synthesis of methyl 3-[(4-diethylamino-1-methylbutylamino)methyl]-3-hydroxy-3-(2-nitrophenyl) propanoate **106a** was followed, using methyl 3-hydroxy-2-methylene-3-(4,5-methylenedioxy-2-nitrophenyl)propanoate **82c** (1.3 g, 4.5 mmol) in anhydrous THF (2 mL) and triethylamine (0.7 mL), 2-amino-5-diethylaminopentane (0.7 g, 0.9 mL, 5 mmol). After stirring in a stoppered flask for 2 days, excess solvent and unreacted amine were evaporated *in vacuo* and the residue was purified by flash chromatography [on silica gel, basified and conditioned overnight with 5% triethylamine in hexane-EtOAc (1:1); eluting sequentially with 5% triethylamine hexane-EtOAc (1:1), followed by 5% methanol in the initial eluent] to afford, as a yellowish-brown, transparent oil, a (2:1) diastereomeric mixture of *methyl 3[(4-diethylamino-1-methylbutylamino)methyl]-3-hydroxy-3-(4,5-methylenedioxy-2-nitrophenyl)propanoate* **106c** (1.7 g, 88%) (Found M+1: 440.2398. Calc. for $C_{21}H_{34}N_3O_7$. 440.2397); $\upsilon_{max}$ (ATR)/$cm^{-1}$ 1729 (C=O); $\delta_H$ (400 MHz; $CDCl_3$) 1.0 (9H, overlapping multiplets, 9'-, 16'- and 18'-$CH_3$), 1.45 (4H, m, 11'- and 12'-$CH_2$), 2.40 (2H, t, *J* = 7.2 Hz, 7’-$CH_2$), 2.50 (6H, overlapping multiplets, 13', 15'- and 17'-$CH_2$), 3.05 (1H, m, 10'-H), 3.48 (3H, s, $CO_2CH_3$), 3.75 (1H, m, 2-H), 5.66 (1H, m, 3-H), 6.08 (2H, m, $OCH_2O$), 7.31 (1H, m, 6'-H) and 7.53 (1H, s, 3'-H); $\delta_C$ (100 MHz; $CDCl_3$) 11.3 (16'- and 18'-$CH_3$), 20.0 (9'-$CH_3$), 23.6 (11'-$CH_2$), 35.3 (12'-$CH_2$), 46.6 (15'-and 17'-$CH_2$), 48.0 (C-7'), 49.4 (C-2), 51.2 ($CO_2CH_3$), 52.2 (C-10'), 52.7 (13'-$CH_2$), 72.5 (C-3), 105.1 (C-6'), 107.7 (C-3'), 125.5 ($OCH_2O$), 136.8 (1'-C), 138.8 (C-5'), 140.7 (C-4'), 146.7 (C-2') and 172.9 (C=O).

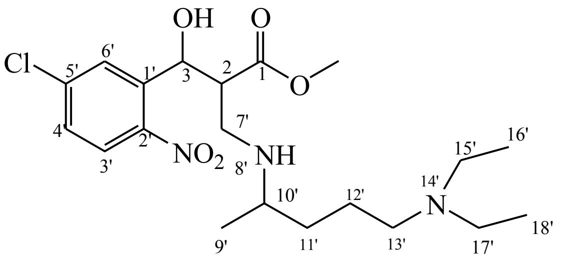
### Methyl 3-[(4-diethylamino-1-methylbutylamino)methyl]-3-hydroxy-3-(4,5-dimethoxy-2-nitrophenyl)propanoate 106d.



The procedure described for the synthesis of methyl 3-[(4-diethylamino-1-methylpentylaminomethyl]-3-hydroxy-3-(2-nitrophenyl)propanoate **106a** was followed, using methyl 3-(4,5-dimethoxy-2-nitrophenyl)-3-hydroxy-2-methylenepropanoate **82d** (0.7 g, 2 mmol) in anhydrous THF (1 mL) and triethylamine (0.3 mL), and 2-amino-5-diethylaminopentane (0.4 g, 0.4 mL, 2.5 mmol). After stirring in a stoppered flask for 3 days, excess solvent and unreacted amine were evaporated *in vacuo* and the residue was purified by flash chromatography [on silica gel, basified and conditioned overnight with 5% triethylamine in hexane-EtOAc (1:1); eluting sequentially with 5% triethylamine hexane-EtOAc (1:1), followed by 5% methanol in the initial eluent] to afford, as a yellow, transparent oil, a (2:1) diastereomeric mixture of *methyl-3-[(4-diethylamino-1-methylbutylamino)methyl]-3-hydroxy-3-(4,5-dimethoxy-2-nitrophenyl)-propanoate* **106d** (0.9 g, 92%); (Found M+1: 456.2711. Calc. for $C_{22}H_{38}N_3O_7$. 456.2710); $\upsilon_{max}$ (ATR)/cm$^{-1}$ 2962 (OH) and 1729 (C=O); $\delta_H$ (400 MHz; $CDCl_3$) 1.00 (9H, m, 9'-, 16'- and 18'-$CH_3$), 1.47 (4H, 2 x m, 11'- and 12'-$CH_2$), 2.43 (2H, t, *J* = 7.2 Hz, 7’-$CH_2$), 2.53 (6H, m, 13', 15'- and 17'-$CH_2$), 2.89 (1H, br s, OH), 3.05 (1H, m, 10'-H), 3.40 (3H, s, $CO_2CH_3$), 3.73

(1H, overlapping multiplets, 2-H), 4.90 (6H, overlapping singlets, 2 x $ArOCH_3$), 5.05 (2H, br s, 2 x NH), 5.75 (1H, br signal, 3-H), 7.28 (1H, s, 6'-H) and 7.62 (1H, s, 3'-H); $\delta_C$ (100 MHz; $CDCl_3$**)** 11.0 (16'- and 18'-$CH_3$), 20.5 (9'-$CH_3$), 22.7 (11'-$CH_2$), 34.4 (12'-$CH_2$), 46.5 (15'- and 17'-$CH_2$), 47.9 (7'-$CH_2$), 48.0 (C-2), 51.0 ($CO_2\mathit{C}H_3$), 52.2 (C-10'), 52.5 (13'-$CH_2$), 56.1 and 56.3 (2 x $ArOCH_3$), 72.3 (C-3), 107.5 (C-6'), 109.9 (C-3'), 134.9 (C-1'), 139.9 (C-4'), 147.6 (C-2'), 153.2 (C-5') and 172.9 (C=O).

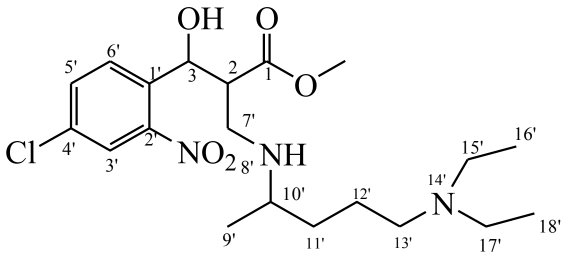
### Methyl 3-(5-chloro-2-nitrophenyl)-3-[(4-diethyl-amino-1-methylbutyl-amino)-methyl]-3-hydroxypropanoate 106f.



The procedure described for the synthesis of methyl 3-[(4-diethylamino-1-methylbutylamino)methyl]-3-hydroxy-3-hydroxy-3-(2-nitrophenylpropanoate **106a** was followed, using methyl 3-hydroxy-2-methylene-3-(5-chloro-2-nitrophenyl)propanoate **82f** (1.4 g, 5.3 mmol) in anhydrous THF (2 mL) and triethylamine (0.7 mL), and 2-amino-5-diethylaminopentane (0.8 g, 1 mL, 5 mmol). After stirring in a stoppered flask for 3 days, excess solvent and unreacted amine were evaporated *in vacuo* and the residue was purified by flash chromatography [on silica gel, basified and conditioned overnight with 5% triethylamine in hexane-EtOAc (1:1); eluting sequentially with 5% triethylamine hexane-EtOAc (1:1), followed by 5% methanol in the initial eluent] to afford, as a yellow, transparent oil, a (2:1) diastereomeric mixture of *methyl 3-(5-chloro-2-nitrophenyl)-3-[(4-diethylamino-1-methylbutylamino)methyl]-3-hydroxypropanoate* **106f** (2.2 g, 97%) (Found M+1: 430.2108. Calc. for $C_{20}H_{33}N_3O_5Cl$. 430.2109); $\upsilon_{max}$ (ATR)/$cm^{-1}$ 1729 (C=O); $\delta_H$ (400 MHz; $CDCl_3$) 0.98 (9H, overlapping multiplets, 9'-, 16'-

and 18'-CH$_3$), 1.43 (4H, 2 x m, 11'- and 12'-CH$_2$), 2.40 (2H, t, *J* = 6.8 Hz, 7'-CH$_2$), 2.50 (6H, overlapping multiplets, 13', 15'- and 17'-CH$_2$), 3.02 (1H, m, 10'-H), 3.47 (3H, s, CO$_2$CH$_3$), 3.63 (1H, m, 2-H), 5.01 (1H, m, 3-H), 7.39 (1H, m, 6'-H), 7.55 (1H, d, *J* = 8.8 Hz, 4'-H) and 8.0 (1H, d, *J* = 8.8 Hz, 3'-H); $\delta_C$ (100 MHz; CDCl$_3$) 11.2 (2 x CH$_3$), 20.0 (9'-CH$_3$), 22.8 (11'-CH$_2$), 34.4 (12'-CH$_2$), 46.5 (15'- and 17'-CH$_2$), 47.6 (7'-CH$_2$), 50.3 (C-2), 51.3 (CO$_2$*C*H$_3$), 52.7 (13'-CH$_2$), 53.4 (C-10'), 71.5 (C-3), 126.1 (C-6'), 129.3 (C-4'), 130.3 (C-3'), 136.3 (C-1'), 140.7 (C-5'), 147.3 (C-2') and 172.4 (C=O).

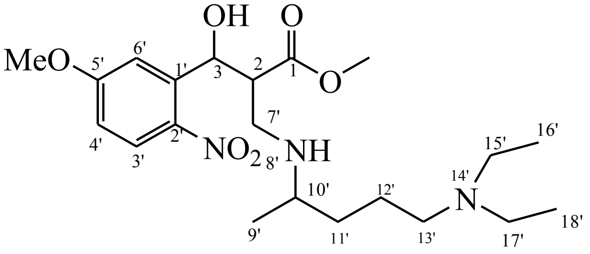
**Methyl 3-(4-chloro-2-nitrophenyl)-3-[(4-diethylamino-1-methylbutylamino)-methyl]-3-hydroxypropanoate 106g.**



The procedure described for the synthesis of methyl 3-[(4-diethylamino-1-methylbutylamino)methyl]-3-hydroxy-3-(2-nitrophenylpropanoate **106a** was followed, using methyl 3-(4-chloro-2-nitrophenyl)-3-hydroxy-2-methylene-propanoate **82g** (0.6 g, 0.8 mmol) in anhydrous THF (1 mL) basified with triethylamine (0.1 mL), and 2-amino-5-diethylaminopentane (0.3 g, 0.5 mL, 0.8 mmol), After stirring in a stoppered flask for 3 days, excess solvent and unreacted amine were evaporated *in vacuo* and the residue was purified by flash chromatography [on silica gel, basified and conditioned overnight with 5% triethylamine in hexane-EtOAc (1:1); eluting sequentially with 5% triethylamine hexane-EtOAc (1:1), followed by 5% methanol in the initial eluent] to afford, as a yellow, transparent oil, a (2:1) diastereomeric mixture of *methyl 3-(4-chloro-2-nitrophenyl)-3[(4-diethylamino-1-methylbutylamino)methyl]-3-hydroxypropanoate* **106g** (0.3 g, 87%) (Found M+1: 430.2110. Calc. for C$_{20}$H$_{33}$N$_3$O$_5$Cl: 430.2109); $\upsilon_{max}$

(ATR)/cm$^{-1}$ 1726 (C=O); $\delta_H$ (400 MHz; $CDCl_3$) 0.93 (9H, overlapping multiplets, 9'-, 16'- and 18'-$CH_3$), 1.39 (4H, overlapping multiplets, 11'- and 12'-$CH_2$), 2.32 (2H, m, 7'-$CH_2$), 2.45 (6H, overlapping multiplets, 13', 15'- and 17'- $CH_2$), 2.96 (1H, m, 10'-H), 3.39 (3H, s, $CO_2CH_3$), 3.63 (1H, m, 2-H), 5.59 (1H, m, 3-H), 7.53 (1H, d, $J$ = 8.4 Hz, 6'-H), 7.75 (1H, d, $J$ = 8.4 Hz, 5'-H) and 7.93 (1H, s, 3'-H); $\delta_C$ (100 MHz; $CDCl_3$**)** 11.2 (2 x $CH_3$), 19.8 (9'-$CH_3$), 23.0 (11'-$CH_2$), 34.6 (12'-$CH_2$), 46.6 (15'- and 17'-$CH_2$), 47.6 (7'-$CH_2$), 49.5 (C-2), 50.9 ($CO_2CH_3$), 52.6 (13'-$CH_2$), 53.4 (C-10'), 70.9 (C-3), 124.1 (C-6'), 130.4 (C-5'), 132.8 (C-3'), 133.4 (C-1'), 137.2 (C-4'), 147.0 (C-2') and 172.4 (C=O).

**Methyl 3-[(4-diethylamino-1-methylbutylamino)methyl]-3-hydroxy-3-(5-methoxy-2-nitrophenyl)propanoate 106h.**

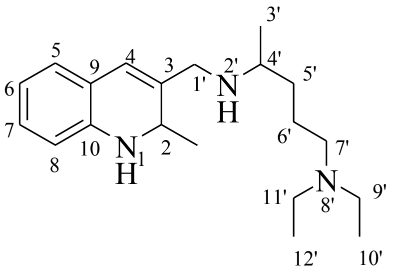


The procedure described for the synthesis of methyl 3-[(4-diethylamino-1-methylbutylamino)methyl]-3-hydroxy-3-(2-nitrophenyl)propanoate **106a** was followed, using methyl 3-hydroxy -3-(5-methoxy-2-nitrophenyl)-2-methylenepropanoate **82g** (0.7 g, 3 mmol) in anhydrous THF (2 mL) and triethylamine (0.3 mL), was added 2-amino-5-diethylaminopentane (0.4 g, 0.5 mL, 3 mmol). After stirring in a stoppered flask for 2 days, excess solvent and unreacted amine were evaporated *in vacuo* and the residue was purified by flash chromatography [on silica gel, basified and conditioned overnight with 5% triethylamine in hexane-EtOAc (1:1); eluting sequentially with 5% triethylamine hexane-EtOAc (1:1), followed by 5% methanol in the initial eluent] to afford, as a dirty-green oil, a (2:1) diastereomeric mixture of *methyl 3-[(4-diethylamino-1-methylbutylamino)methyl]-3-hydroxy-3-(5-methoxy-2-nitrophenyl)propanoate* **106g** (1.0 g, 90%) (Found M+1: 426.2598. Calc. for $C_{21}H_{36}N_3O_6$: 426.2604); $\upsilon_{max}$ (ATR)/cm$^{-1}$

1728 (C=O); $\delta_H$ (400 MHz; $CDCl_3$) 0.94 (9H, overlapping multiplets, 9'-, 16'- and 18'-$CH_3$), 1.41 (4H, 2 x m, 11'- and 12'-$CH_2$), 2.38 (2H, m, 7'-$CH_2$), 2.48 (6H, m, 13', 15'-, and 17'-$CH_2$), 2.98 (1H, m, 10'-H), 3.35 (3H, m, $CO_2CH_3$), 3.68 (1H, m, 2-H), 3.81 (3H, overlapping signals, $ArOCH_3$), 5.01 (2H, br s, 2 x NH), 5.71 (1H, br s, 3-H), 6.74 (2H, overlapping multiplets, 4' and 6'-H) and 8.01 (1H, d, *J* = 9.2 Hz, 3'-H); $\delta_C$ (100 MHz; $CDCl_3$) 10.7 (2 x $CH_3$), 19.7 (9'-$CH_3$), 22.5 (11'-$CH_2$), 34.4 (12'-$CH_2$), 46.3 (15'- and 17'-$CH_2$), 47.5 (7'-$CH_2$), 50.8 ($CO_2CH_3$), 52.0 (C-2), 52.2 (13'-$CH_2$), 53.2 (C-10'), 55.6 ($ArOCH_3$), 71.0 (C-3), 112.6 (C-6'), 113.4 (C-4'), 127.2 (C-3'), 139.5 (C-1'), 142.2 (C-2'), 163.4 (C-5') and 172.6 (C=O).

## Reductive cyclization products.

### 3-[(5-Diethylamino-1-methylbutylamino)methyl]-2-methyl-quinoline 105a.

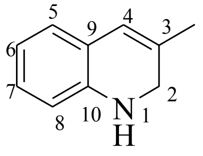


**Method 1**. To a solution of 3-[(4-diethylamino-1-methylamino)methyl]-4-(2-nitrophenyl)but-3-en-2-one **103a (**0.2 g, 0.5 mmol) in anhydrous ethanol (8 mL), 10% Pd-C catalyst (0.9 g) was added and the reaction mixture was stirred under a hydrogen atmosphere. After 3 hours, the reaction mixture was filtered through a bed of Celite to remove the catalyst. The filtrate was concentrated *in vacuo* to afford the crude product, which was then purified by flash chromatography [on a Discovery DSC diol column, basified and conditioned overnight with 5% triethylamine (TEA) in EtOAc-hexane (1:1); eluting sequentially with 5% TEA in EtOAc-hexane (1:1), followed by the addition of

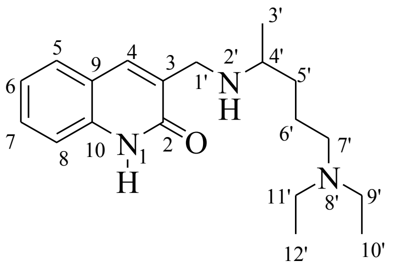
5% methanol to the initial eluent] to afford, as a brown oil, *3-[(5-diethylamino-1-methylbutylamino)methyl]-2-methyl-quinoline* **105a** (0.1 g, 54%) (Found M + 1: 314.2593. Calc. for $C_{20}H_{32}N_3$: 314.2596); $\delta_H$ (400MHz; $CDCl_3$) 1.01 (9H, overlapping multiplets, 3 x $CH_3$), 1.13 (4H, m, 5'- and 6'-$CH_2$), 2.42 (2H, t, *J* = 7.2 Hz, 7'-$CH_2$), 2.53 (5H, m, 4'-H, 9'- and 11'-$CH_2$), 2.73 (3H, m, 2-$CH_3$), 3.84 and 3.94 (2H, 2 x d, *J* = 13.6 Hz, 7'-$CH_2$), 7.58 (1H, t, *J* = 7.6 Hz, Ar-H), 7.70 (2H, m, 2 x Ar-H), 7.79 (1H, d, *J* = 8.0 Hz, Ar-H) and 8.72 (1H, d, *J* = 8.8 Hz, Ar-H); $\delta_C$ (100 MHz; $CDCl_3$) 11.4 (10'- and 12'-$CH_3$), 14.4 (2-$CH_3$), 20.5 (3'-$CH_3$), 35.1 (5'-$CH_2$), 46.7 (1'-$CH_2$), 49.0 (9'- and 11'-$CH_2$), 53.0 (7'-$CH_2$), 53.2 (C-4'), 119.6 (d, Ar-C), 124.7 (d. Ar-C), 127.7 (d, Ar-C), 127.8 (d, Ar-C), 128.8 (s, Ar-C), 129.8 (d, Ar-C), 133.7 (s, Ar-C), 140.2 (s, Ar-C) and 146.3 (s, Ar-C).

**Method 2.** To a solution of 4-(5-chloro-2-nitrophenyl)-3-[(-4-diethylamino-1-methylamino)methyl]but-3-en-2-one **103f (**0.2 g, 0.6 mmol) in anhydrous ethanol (12 mL), 10% Pd-C catalyst (1.0 g) was added and the reaction mixture was stirred under a hydrogen atmosphere. After 3 hours, the reaction mixture was filtered through a bed of Celite to remove the catalyst. The filtrate was concentrated *in vacuo* to afford the crude product, which was then purified by flash chromatography [on a Discovery DSC diol column, basified and conditioned overnight with 5% triethylamine (TEA) in EtOAc-hexane (1:1); eluting sequentially with 5% TEA in EtOAc-hexane (1:1), followed by the addition of 5% methanol to the initial eluent] to afford, as a brown oil, *3-[(5-diethylamino-1-methylbutylamino)methyl]-2-methyl-quinoline* **105a** (0.2 g, 86%). $^1$H and $^{13}$C NMR data are identical with those obtained from method 1; absence of the Cl atom in the product was observed during the HRMS experiment and confirmed by the NMR data.

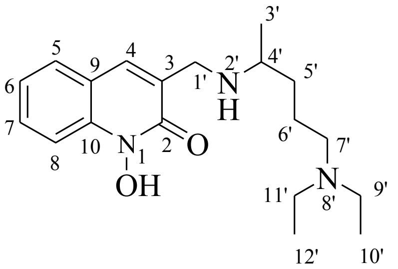
### 3-Methyl-1,2-dihydroquinoline 110a,
### 3-[(4-diethylamino-1-methylbutylamino)methyl]-1*H*-2-quinolone 108a and
### 3-[(4-diethylamino-1-methylbutylamino)methyl]-1-hydroxy-2-quinolone 109a.



**110a**



**108a**



**109a**

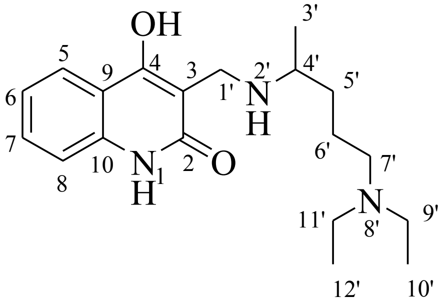
To a solution of methyl 3-[(4-diethylamino-1-methylbutylamino)methyl]-3-hydroxy-(2-nitrophenyl)propanoate **106a (**0.8 g, 2 mmol) in anhydrous ethanol (10 mL), 10% Pd-C catalyst (1.3 g) was added and the reaction mixture was stirred under a hydrogen atmosphere. After 3 hours, the reaction mixture was filtered through a bed of Celite to remove the catalyst. The filtrate was concentrated *in vacuo* to afford the crude product, which was then purified by flash chromatography [on a Discovery DSC diol column, basified and conditioned overnight with 5% triethylamine (TEA) in EtOAc-hexane (1:1); eluting sequentially with 5% TEA in EtOAc-hexane (1:1), followed by the addition of 5% methanol to the initial eluent to afford three fractions.

**Fraction I:** as a yellowish-brown oil, 3-methyl-1,2-dihydroquinoline **110a** (0.14 g, 14%); $\nu_{max}$ $\delta_H$ (400MHz; $CDCl_3$) 2.28 (3H, s, Ar-$CH_3$), 2.61 (2H, s, 2-$CH_2$), 7.19 (1H, t, *J* = 7.2 Hz, Ar-H), 7.36 (1H, d, *J* = 8.0 Hz, Ar-H), 7.45 (1H, t, *J* = 7.6 Hz, Ar-H), 7.50 (1H, d, *J* = 8.0 Hz, Ar-H) and 7.64 (1H, s, Ar-H); $\delta_C$ (100 MHz; $CDCl_3$) 29.7 (2-$CH_2$), 40.9 (Ar-$CH_3$), 115.5 (d. Ar-C), 120.3 (s, Ar-C), 122.4 (d, Ar-C), 126.9 (d, Ar-C), 129.3 (d, Ar-C), 130.1 (s, Ar-C), 137.4 (d, Ar-C) and 164.4 (s, Ar-C).

**Fraction II:** as a pinkish oil which solidified to a wax upon standing, *3-[(5-diethylamino-1-methylbutylamino)methyl]-1*H*-2-quinolone* **108a** (0.22 g, 27%) (Found M + 1: 316.2385. Calc. for $C_{19}H_{30}N_3O$: 316.2389) $\nu_{max}$ (ATR)/cm$^{-1}$ 1653 (C=O); $\delta_H$ (400MHz; $CDCl_3$) 0.98 (6H, t, *J* = 7.2 Hz, 2 x $CH_3$), 1.13 (3H, d, *J* = 7.2 Hz, 3'-$CH_3$), 1.35 and 1.50 (4H, m, 5'- and 6'-$CH_2$), 2.41 (2H, t, *J* = 7.2 Hz, 7'-$CH_2$), 2.51 (4H, m, 9'- and 11'-$CH_2$), 2.73 (1H, m, 4'-H), 3.79 and 3.88 (2H, 2 x d, *J* = 14.8 Hz, 1'-$CH_2$), 7.19 (1H, t, *J* = 7.2 Hz, Ar-H), 7.39 (1H, d, *J* = 8.4 Hz, Ar-H), 7.48 (1H, t, *J* = 7.6 Hz, Ar-H) and 7.54 (1H, d, *J* = 8.0 Hz, Ar-H); $\delta_C$ (100 MHz; $CDCl_3$) 9.6 (10'- and 12'-$CH_3$), 20.2 (3'-$CH_3$), 23.0 (6'-$CH_2$), 32.2 (5'-$CH_2$), 46.7 (9'- and 11'-$CH_2$), 46.7 (1'-$CH_2$), 52.2 (C-4'), 53.0 (7'-$CH_2$), 115.6 (d, Ar-C), 120.0 (s, Ar-C), 122.5 (d, Ar-C), 127.4 (d, Ar-C), 129.7 (d, Ar-C), 131.6 (s, Ar-C), 137.2 (d, Ar-C), 137.6 (s, Ar-C) and 164.1 (C=O).

**Fraction III:** as a light green oil, *3-[(4-diethylamino-1-methylbutylamino)methyl]-1-hydroxy-2-quinolone* **109a** (0.41 g, 54%) (Found M + 1: 332.2331. Calc. for $C_{19}H_{30}N_3O_2$: 332.2332) $\nu_{max}$ (ATR)/cm$^{-1}$ 1632 (C=O); $\delta_H$ (400MHz; $CDCl_3$) 1.16 (6H, t, *J* = 5.2 Hz, 2 x $CH_3$), 1.39 (3H, d, *J* = 5.6 Hz, 3'-$CH_3$), 1.70-1.86 ( 4H, overlapping multiplets, 5'- and 6'-$CH_2$), 2.0 (1H, br s, NH), 2.79 (2H, t, *J* = 8.0 Hz, 7'-$CH_2$), 2.87 (4H, overlapping multiplets, 9'- and 11'-$CH_2$), 3.11 (1H, m, 4'-H), 4.06 and 4.10 (2H, 2 x d, *J* = 7.0 Hz, 1'-$CH_2$), 6.81 (1H, m, Ar-H), 7.09 (1H, s, Ar-H), 7.37 (2H, overlapping multiplets, Ar-H), 7.48 (1H, br s, NOH) and 7.71 (1H, t, *J* = 7.2 Hz, Ar-H); $\delta_C$ (100 MHz; $CDCl_3$) 9.5 (10'- and 12'-$CH_3$), 17.6 (3'-$CH_3$), 21.1 (6'-$CH_2$), 32.1 (5'-$CH_2$), 45.8 (9'- and 11'-$CH_2$), 46.9 (1'-$CH_2$), 51.4 (7'-$CH_2$), 52.8 (C-4'), 113.6 (d, Ar-C), 118.5 (s. Ar-C), 122.0 (d, Ar-C), 127.3 (d, Ar-C), 129.9 (d, Ar-C), 133.7 (d, Ar-C), 139.4 (s, Ar-C), 158.6 (s, Ar-C) and 177.5 (C=O).

### 6-Chloro-3-[(4-diethylamino-1-methylbutylamino)methyl]-4-hydroxy-3,4-dihydro-2-quinolone 111f.



To a solution of methyl 3-(5-chloro-2-nitrophenyl)-2-[(4-diethylamino-1-methylbutylamino)methyl]-3-hydroxypropanoate **106f (**0.4 g, 0.8 mmol) in anhydrous ethanol (6 mL), 10% Pd-C catalyst (1.0 g) was added and the reaction mixture was stirred under a hydrogen atmosphere. After 3 hours, the reaction mixture was filtered through a bed of Celite to remove the catalyst. The filtrate was concentrated *in vacuo* to afford the crude product, which was then purified by flash chromatography [on a Discovery DSC diol column, basified and conditioned overnight with 5% triethylamine (TEA) in EtOAc-hexane (1:1); eluting sequentially with 5% TEA in EtOAc-hexane (1:1), followed by addition of 5% methanol to the initial eluent] to afford, as a brick-red oil, a (3:1) diastereomeric mixture of 6-chloro-*3-[(4-diethylamino-1-methylbutylamino)methyl]-4-hydroxy-3,4-dihydro-2-quinolone* **111f** (0.21 g, 68%); $\nu_{max}$ (ATR)/cm$^{-1}$ 1651 (C=O); $^{1}$H and $^{13}$C NMR data cited for the major isomer: $\delta_H$ (400MHz; CDCl$_3$) 1.02 (6H, overlapping multiplets, 2 x CH$_3$), 1.11 (3H, d, *J* = 5.2 Hz, 3'-CH$_3$), 1.33 and 1.48 (4H, overlapping multiplets, 5'- and 6'-CH$_2$), 2.42 (2H, t, *J* = 4.8 Hz, 7'-CH$_2$), 2.52 (4H, dd, *J* = 11.6 and 4.8 Hz, 9'- and 11'-CH$_2$), 2.67 (1H, br s, CHO*H*), 3.05 (1H, br s, 3-H), 3.42 (1H, m, 4'-H), 3.45 (2H, m, 1'-CH$_2$), 5.73 (1H, s, C*H*OH), 7.40 (1H, m, Ar-H), 7.86 (1H, s, Ar-H) and 8.02 (1H, d, *J* = 8.8 Hz, Ar-H); $\delta_C$ (100 MHz; CDCl$_3$) 11.5 (10'- and 12'-CH$_3$), 20.1 (3'-CH$_3$), 23.5 (6'-CH$_2$), 34.9 (5'-CH$_2$), 46.7 (9'- and 11'-CH$_2$), 48.2 (1'-CH$_2$), 49.0 (C-3'), 52.8 (C-4'), 71.8 (C-4), 126.6 (d, Ar-C), 128.0 (d. Ar-C), 129.5 (d, Ar-C), 140.1 (s, Ar-C), 140.7 (s, Ar-C), 139.4 (s, Ar-C), 144.9 (s, Ar-C) and 172.5 (C=O).

Data provided by Prof. M R Caira,
Dept of Chemistry, U C T, June 2010

## 3.16. Brief Report: X-Ray Analysis of Compound 89a.

**Crystal Data and Details of the Structure Determination for *N*,*N'*-Bis{(*E*)-2-carbomethoxy-3-[4-(2-nitrophenyl)but-3-en-2-on-3-yl]methyl-1,4-piperazine 69a. (R = 0.04)**

### Crystal Data

| | |
|---|---|
| Formula | C26 H28 N4 O6 |
| Formula Weight | 492.52 |
| Crystal System | Monoclinic |
| Space group | $P2_1/n$ (No. 14) |
| a, b, c [Angstrom] | 9.1228(2) 14.5994(4) 9.7531(2) |
| alpha, beta, gamma [deg] | 90 104.630(2) 90 |
| V [Ang**3] | 1256.87(5) |
| Z | 2 |
| D(calc) [g/cm**3] | 1.301 |
| Mu(MoKa) [ /mm ] | 0.094 |
| F(000) | 520 |
| Crystal Size [mm] | 0.12 x 0.14 x 0.18 |

### Data Collection

| | |
|---|---|
| Temperature (K) | 173 |
| Radiation [Angstrom] | MoKa 0.71073 |
| Theta Min-Max [Deg] | 3.5, 27.5 |
| Dataset | -11: 11 ; -18: 18 ; -12: 12 |
| Tot., Uniq. Data, R(int) | 5488, 2826, 0.015 |
| Observed data [I > 2.0 sigma(I)] | 2131 |

### Refinement

| | |
|---|---|
| Nref, Npar | 2826, 164 |
| R, wR2, S | 0.0402, 0.1119, 1.03 |

w = 1/[\s^2^(Fo^2^)+(0.0586P)^2^+0.1868P] where P=(Fo^2^+2Fc^2^)/3

| | |
|---|---|
| Max. and Av. Shift/Error | 0.00, 0.00 |
| Min. and Max. Resd. Dens. [e/Ang^3] | -0.24, 0.13 |

*NOTE*: This structure has been published[87] and relevant data deposited in the Cambridge Crystallographic Data Centre (CCDC).

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