Studies towards the Development of Novel HIV-1 Integrase Inhibitors

THESIS

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ABSTRACT

The project has focused on the preparation of several series of compounds designed as potential HIV-1 integrase inhibitors. Various 2-nitrobenzaldehydes have been reacted with two activated alkenes, methyl vinyl ketone (MVK) and methyl acrylate, under Baylis-Hillman conditions to afford α-methylene-β-hydroxylalkyl derivatives in moderate to excellent yields. The reactions were conducted using the tertiary amine catalysts, 1,4-diazabicyclo[2.2.2]octane (DABCO) or 3-hydroxyquinuclidine (3-HQ) with chloroform as solvent, and yields were optimised by varying the catalyst, reagent concentrations and the reaction time. Reductive cyclization of the Baylis-Hillman adducts *via* catalytic hydrogenation, using 10% palladium-on-carbon catalyst in ethanol, afforded quinoline and quinoline *N*-oxide derivatives. In some cases "acyclic" reduction products were also isolated.

Reaction of the Baylis-Hillman MVK adducts with HCl, has resulted in effective nucleophilic (S_N') displacement of the hydroxyl group to afford allylic chloride derivatives. Direct substitution of these chloro derivatives by secondary or primary amines, followed by catalytic hydrogenation gave quinoline derivatives containing a 3-aminomethyl substituent.

The Baylis-Hillman ester adducts obtained from reaction with methyl acrylate were treated directly with various amines to give diastereomeric conjugate addition products. Reactions with piperazine gave *N,N'*-disubstituted piperazine products. The piperidine derivatives have been dehydrated to give cinnamate esters in moderate yields.

The products, which have all been satisfactorily characterised by elemental (HRMS) and spectroscopic (1- and 2-D NMR) analysis, constitute a "library" of compounds for *in silico* and *in vitro* studies as potential HIV integrase inhibitors.

ACKNOWLEDGMENTS

Firstly, I would like to thank my supervisor Professor Perry Kaye for all his help and guidance. His constant support, encouragement and patience have helped me to overcome the problems I encountered during the course of two years. It's a privilege to work under his supervision.

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1. INTRODUCTION

1.1 The Human Immunodeficiency Virus – Type 1 (HIV-1)

The Human Immunodeficiency Virus (HIV) is a member of the lentivirus genus of the family *Retroviridae* and is categorised into two types, type 1 and type 2.[1] The human retrovirus is associated with the disease, Acquired Immunodeficiency Syndrome (AIDS), in which HIV damages the immune system, leaving the body susceptible to infection from a wide range of bacteria, fungi and viruses. AIDS is one of the most serious and widespread diseases facing us and is an important challenge for chemotherapy in the 21st century. HIV-type 1 (HIV-1) is the predominant virus in the world, while the type 2 virus is restricted to countries in West Africa.[1] The statistics indicate that, since the early 1980's when the HIV virus was discovered and the AIDS name was defined, approximately 20 million people had died and 33 million people were infected with the disease in 2006.[2]

The HIV virion (Figure 1) consists of an envelope, within which the corn-shaped proteinaceous capsid, surrounds the virus genome. The capsid and envelope contain proteins which aid entry into the cytoplasm of host cell.
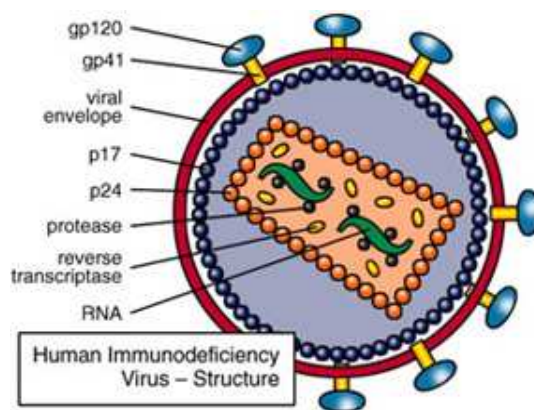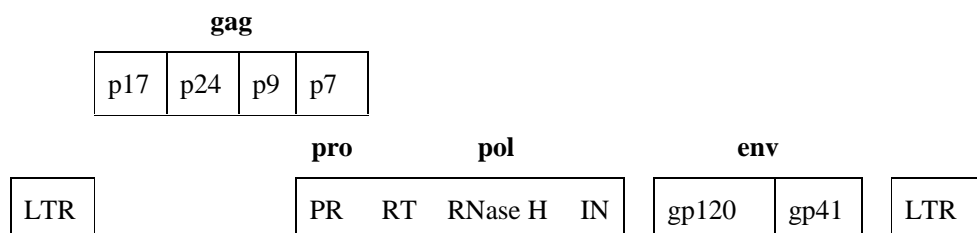

Fig. 1: The structure of an HIV virion (Reproduced with permission).[3]

The life cycle (*i.e.* the cycle of infection) of HIV-1 starts with the attachment of a glycoprotein (gp-120) protuberance of a free virion (Fig. 2) to a helper T-cell (also known as T-lymphocyte or CD4 cells) at a $CD4^+$ receptor site.[1, 4] The envelope of the virion and the membrane of the cell fuse together and the nucleocapsid within the virus enters the cell. Within the host cell the two single-stranded RNA genomes from the nucleocapsid are reverse-transcribed into double-stranded proviral DNA which is then carried into the host cell nucleus and integrated with its chromosomal DNA (cDNA). The integrated viral DNA is transcribed into messenger RNA (mRNA) and carried out of the nucleus into the cytoplasm. Some of the mRNA becomes the genomes of progeny virions and some are translated into large protein molecules which are proteolysed into smaller functional components. After assembly of these viral proteins with the viral RNAs, enzymes, and some cell membranes, the new immature virion acquires its envelope by budding at the cell surface and leaves the infected host cell. The released viral particles become infectious after proteolytic mutation by the protease enzyme.[5]

The genomic structure of the HIV-1 retrovirus is encoded with three types of genes: *gag*, *pol* and *env* (Fig. 3). The name *gag* comes from *g*roup-specific *a*nti*g*en; the protein encoded in this gene is highly conserved and forms the capsid of the retrovirion.[6] The *gag* gene is followed by the *pol* gene; *pol* is short for *pol*ymerase (also known as Gag/Pol protein) which affords three functional enzymes: reverse transcriptase (RT), ribonuclease H (RNase H) and integrase (IN). The overlap region of the *gag* and *pol* gene is known as *pro* and it encodes a *pr*otease (PR) protein that is required for the processing of *gag* and *pol*. The protease is self-cleaved from 5'-end of the *pol* precursor and it cleaves the RT, RNase H and IN apart from each other.[4] The fourth gene, *env*, encodes the envelope glycoprotein present at the surface of the retrovirion. These four types of gene are present in all retroviruses and are always found in this order in the genome. There are various other genes present in the HIV genome, and they are known as auxiliary genes. These genes have many roles in transporting viral components within the cell, controlling viral gene expression, and modifying the body's immune response.[7]

HIV Life Cycle and Anti-HIV Drug Targets
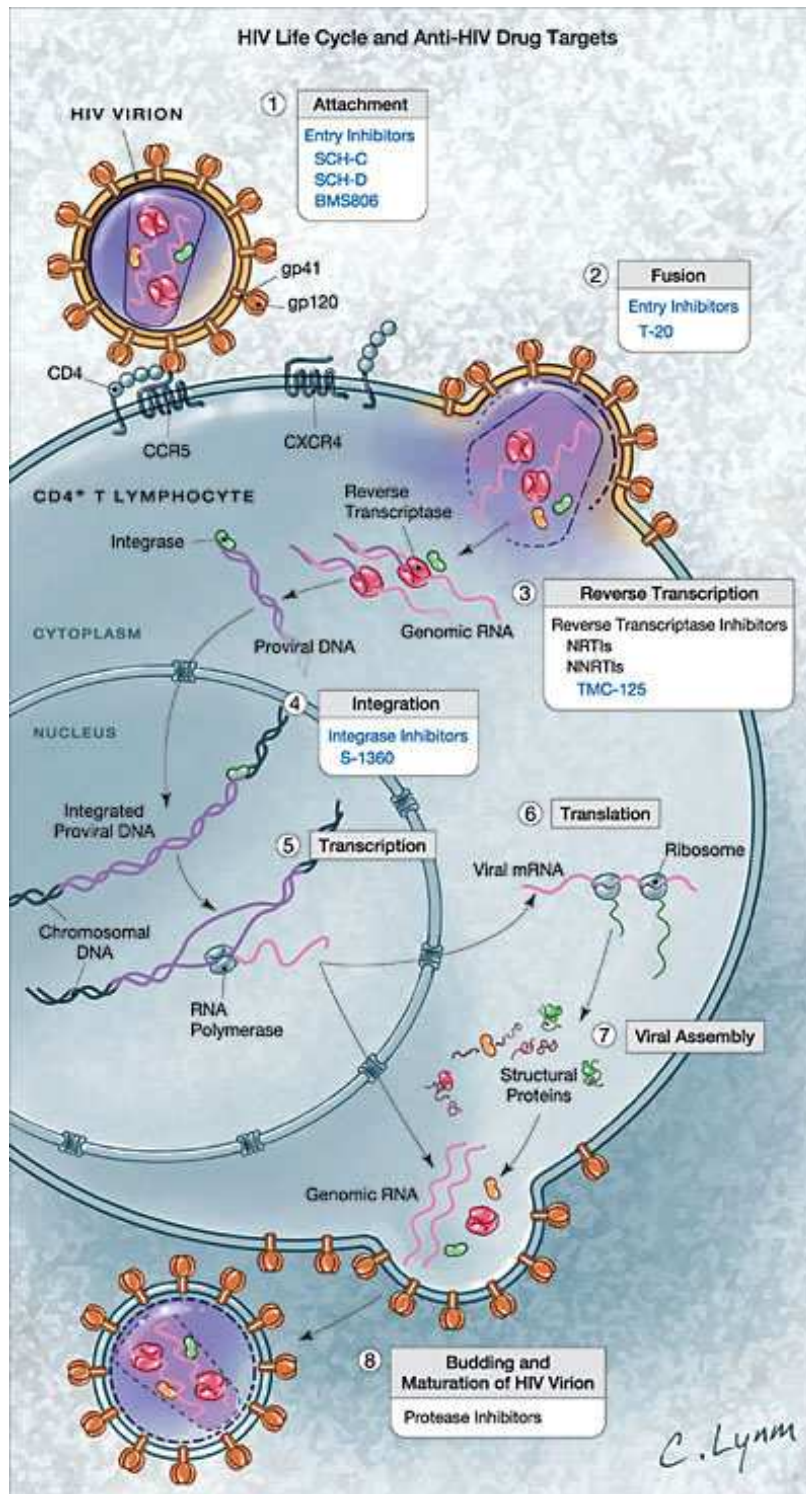
Figure 2: Cartoon of the HIV-1 life cycle: The orange circle is the HIV-1 virion ready to infect a healthy cell. The uninfected host cell is the large blue circle and the small blue circle inside it is the cell nucleus (reproduced with permission).[8]

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                    gag
            ┌─────┬─────┬─────┬─────┐
            │ p17 │ p24 │ p9  │ p7  │
            └─────┴─────┴─────┴─────┘

                   pro        pol              env
  ┌─────────┐    ┌──────────────────────────┐ ┌──────────────┐ ┌─────────┐
  │  LTR    │    │ PR   RT   RNase H    IN   │ │ gp120 │ gp41 │ │  LTR    │
  └─────────┘    └──────────────────────────┘ └──────────────┘ └─────────┘
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Figure 3: Schematic representation of the genetic organization of the HIV-1 genome.[4]

1.1.1   The enzymes as potential antiretroviral (ARV) targets

Every enzyme in the HIV retrovirion has its own role in the HIV life cycle. It is therefore important to design and synthesise drugs that will stop the functioning of these enzymes and, as a consequence, stop the life cycle and hence stop the HIV infection. In order to do so, an enormous research effort has been concentrated on understanding the structure and function of the virus, the pathway of infection and the development of drug resistance. While attention is now is being given to inhibiting virion entry (*i.e.* fusion), the major potential targets in the HIV life cycle for designing antiviral drugs have been the reverse transcription, proteolysis and integration steps, and the enzymes responsible for each of these steps, RT, PR and IN, respectively. The application of ARV drugs in combination therapy, known as highly active antiretroviral therapy (HAART), has significantly reduced the mortality and morbidity rate of HIV/AIDS. Attention has also been given to dual inhibitors, *i.e.* single drugs inhibiting multiple steps in the HIV life cycle. A recent article describes attempts to synthesise an inhibitor for both RT and IN enzymes.[9]

The *pro* gene encodes the **protease enzyme (PR)**, which is responsible for the cleavage of the gag/pol polyproteins into structural and functional proteins essential for viral replication. The structure of the protease enzyme (Fig. 4) shows that the polypeptide exists in a homodimeric form,[1] in which the active enzyme has a crystallographic two-fold (C_2) rotational symmetry. Each monomeric chain supplies one aspartic acid (Asp-25 and Asp-25') moiety in the active site between the two monomers. On "top" of the cavity are the two highly flexible β-hairpins (called "flaps"). Tóth *et al.*[10] proposed that, when the polyprotein enters and docks into the

active site, hydrogen bonds form between the substrate flaps and the aspartic residues, and the protease conformation changes from semi-open to a closed conformation. As the conformation changes from open to closed, the two flaps extend over the polyprotein substrate and proteolysis occurs, cleaving the polyprotein. In fact, the enzyme functions as "a pair of molecular scissors". To stop this from happening, inhibitors are required. The role of an inhibitor is to enter the active site, bind to the protease and block the active site such that the natural polyprotein substrates cannot enter.
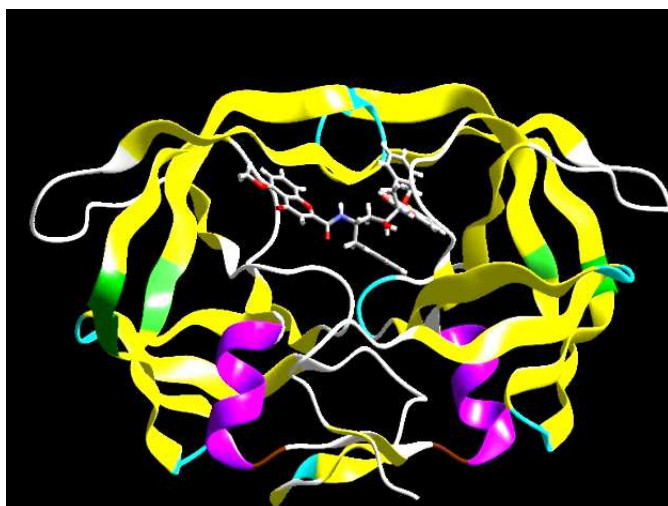
Fig. 4: A ribbon representation of HIV-1 PR; the yellow ribbons represent β-sheets, the white regions represent β-coils and the pink ribbons represent the alpha helices. A protease inhibitor (Ritonavir) is attached to the receptor cavity (active site). **PDB code: 1HXW.**

There are currently a number of protease inhibitors (PRIs) available for clinical treatment, Ritonavir is a well known and popular inhibitor used in the treatment of HIV patients. It is a peptidic isostere that mimics the structure of the natural substrate and was approved for clinical use by the Food and Drug Administration (FDA) in March, 1996.[11]

Norvir (Ritonavir)
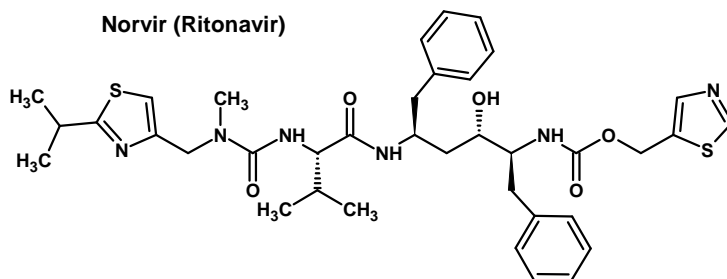
The **reverse transcriptase (RT) enzyme** catalyses an essential step in the replication of HIV-1, *i.e.* the synthesis of proviral DNA from retroviral RNA. RT exists in an asymmetric heterodimeric form composed of two subunits of identical sequences named p66 and p51.[6] The p66 subunit contains a triad of aspartic acid residues in the active site and exhibits DNA polymerase and RNase H activities, whereas the p51 subunit lacks these activities but provides structural support for the p66 subunit to upload onto the template primer.[1, 4]

The reverse transcription process involves two activities; firstly, a DNA strand is manufactured from the RNA template of the virus (a process called DNA polymerizaition) to give hybrid double-stranded DNA and template RNA. The RNA strand is then destroyed by RNase H to give a so-called first-strand DNA or minus-strand DNA.[6, 12] Secondly, the minus-strand DNA acts as a template for DNA polymerization for the synthesis of the plus-strand DNA. This gives rise to the double-stranded proviral DNA suitable for integration (Section 1.1.2). DNA synthesis takes place shortly after the virion enters the host cell and the proviral DNA can be detected within a few hours after infection. There are two categories of RT inhibitors (RTIs), nucleoside (NRTIs) and non-nucleoside (NNRTIs). The NRTIs are inhibitors that mimic the peptide structure of the major contacts between the p66 and p51 subunits of RT to prevent heterodimer formation.[2] The NNRTIs bind to the RT enzyme in a hydrophobic cavity adjacent to the polymerase catalytic site, where they are thought to inhibit chain elongation.[13]

Another area of focus in the search for ARV drugs is inhibiting fusion of the HIV virion with the host cell and entrance of the virion into the cell. There are three steps in the entrance process: i) the HIV-1 gp-120 attaches to a CD4 cell; ii) gp-120 binds to the cell co-receptor (CCR5 or CXCR4); and iii) gp41-mediated membrane fusion.[14-17] Each of these steps represents a unique drug target in designing ARV drugs and the general name for drugs

inhibiting these steps is entry inhibitors or, sometimes, fusion inhibitors. The gp-41 consists of three domains: an extracellular domain (ectodomain), a transmembrane domain and an intracellular domain (endodomain). The ectodomain contains three functional regions; the first region, fusion peptide (FP), consists of conserved hydrophobic residues that are essential for the penetration of the cell membrane. It has been confirmed that replacement of the conserved hydrophobic residues deflects gp41 fusion activity.[18-22] The gp120 and gp41 proteins change their conformation after the attachment to the CD4 cell and co-receptor, bringing the viral and cellular membranes into close proximity and leading to membrane fusion.[16, 23] The first approved fusion inhibitor, Enfuvirtide (T20), has been shown to be effective in patients who fail to respond to RTIs and PRIs.[24]

At present, there are five classes of drugs approved by the FDA for use with HIV patients, *viz.*, nucleoside reverse transcriptase inhibitors (13), non-nucleoside reverse transcriptase inhibitors (4), protease inhibitors (11), fusion inhibitors (2) and an integrase inhibitor.[25] Despite the fact that there are many anti-HIV drugs approved by the FDA, the rate of morbidity and mortality continues world-wide, although recent data suggests that mortality rates may be levelling out or even dropping slightly.[26] This indicates that clinical treatment of the HIV/AIDS infection is not sufficient to control viral replication and the formation of resistant strains. In order to keep the viral count of patients, who have developed resistance against RTIs and PRIs, as low as possible, new inhibitors need to be introduced. The HIV-1 integrase enzyme offers an important target for intervention by such inhibitors.

1.1.2   The integrase enzyme and inhibitors

The HIV IN enzyme is endcoded within the *pol* gene and translated as a polyprotein; IN is released from the polyprotein by protease cleavage to give the 288 amino acid protein (32kDa). The IN polypeptide chain folds into three distinct domains: the N-terminal domain, the Centre core domain, and C-terminal domain (Fig. 5a-c).[27] The complete 3-D structure of IN is not fully understood but it has been suggested that IN exists as a tetramer in human cells. The N-terminal domain (NTD) contains a catalytically important histidine-histidine-cystine-cystine (HHCC) motif coordinated with a single zinc ion.[27] Zinc binding is believed to

stabilise folding of the IN protein and to be involved in protein multimerization. The centre core domain (CCD) contains three catalytic acidic amino acids: aspartic acid (Asp) 64 (D), Asp 116 (D), and glutamic acid 152 (E), *i.e.* the DDE motif. Mutation of any of these residues abolishes IN enzymatic activity and prohibits virus replication. The motif forms an active site within IN and is coordinated with a divalent metal ion, usually Mg^{2+} or Mn^{2+}, which is required for catalytic activity for DNA integration.[7] The centre core domain is also known as the catalytic core domain as the role of the active site in this domain is to bind to the viral DNA.
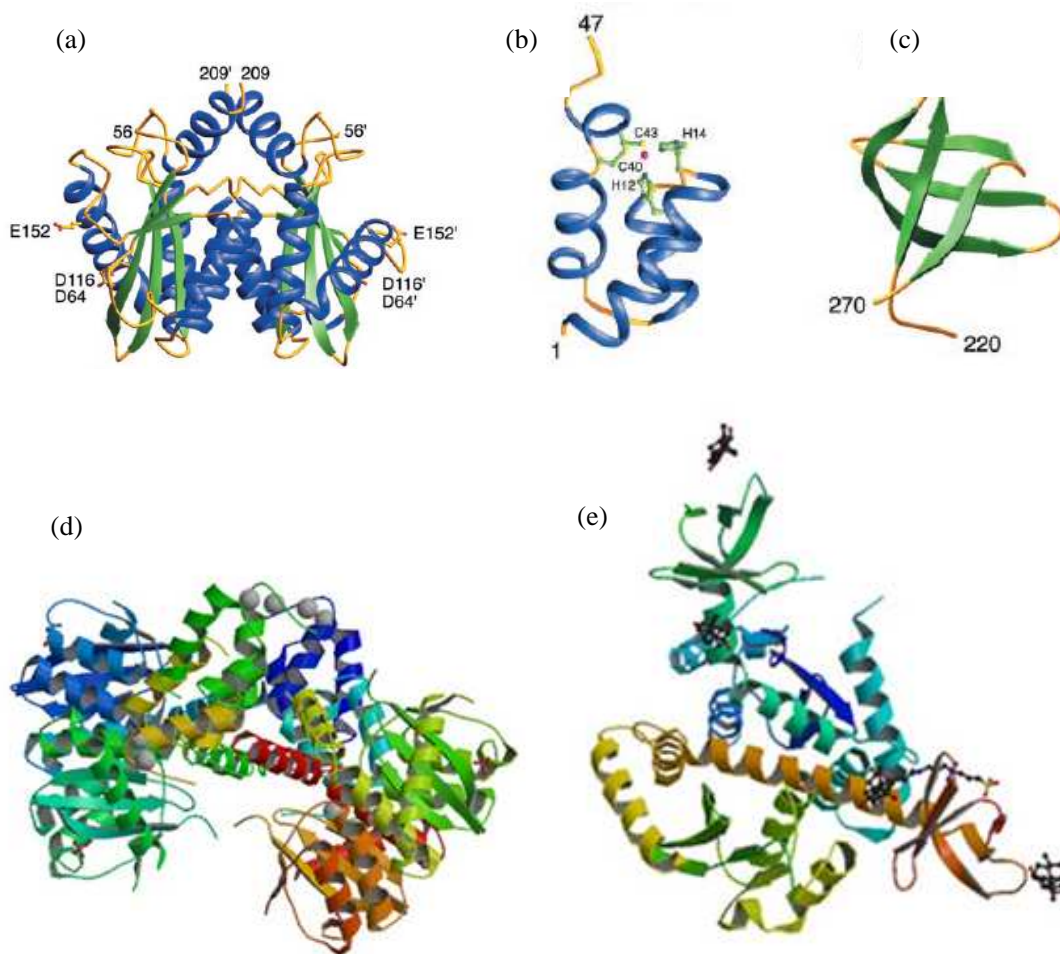
Figure 5: a) The catalytic core domain (CCD): three acidic residues D64, D116 and E152, (the DDE motif); b) N-terminal domain (NTD) with four residues, H12, H14, C40, and C43 (green sticks), and a zinc atom (red sphere); c) C-terminal domain (CTD).[28] d) crystal structure of CCD-NTD (**PDB code: 1K6Y**); and e) CCD-CTD (**PDB code: 1EX4**) (Reproduced with permission).

The C-terminal domain (CTD) exhibits a very strong DNA binding activity and its role is to bind to the host DNA. All three domains have a dimeric structure and any alteration of the structure deactivates the activity of IN. While a crystal structure of the complete IN enzyme has yet to be determined, X-ray crystal structures of NTD with CCD and CTD with CCD have been determined (Fig. 5d and 5e).[29] The IN enzyme binds to viral DNA, host DNA and the substrates during the catalytic process, and it is believed that the enzyme itself undergoes extensive conformational changes to permit favourable interactions.[30]

HIV-1 integrase catalyses the integration of viral DNA with the host cell DNA in the cell nucleus, to give a complete DNA chain. While there are two main catalytic steps in the integration mechanism, there is a crucial step before the catalytic steps, *viz.*, binding of IN to the viral DNA's long terminal region (LTR) to give a stable viral DNA-IN complex.[11] The first catalytic step, which then follows, is the 3' processing (3'-P) step (Fig. 6), involving hydrolysis of dinucleotides from the 3' end of the viral DNA LTR. The removal of the two nucleotides, the GT bases adjacent to highly conserved CA dinucleotides, occurs when a nucleophile (usually H_2O) attacks the LTRs to expose a 3' hydroxyl end (CA-3-OH). The removal takes place in the cytoplasm of the $CD4^+$ T cell within the pre-integration complex (PIC). This complex contains host proteins and viral proteins, including IN, nucleocapsids, RT, and matrix and viral DNA.[31] The HIV PIC enters the cell nucleus through a nuclear pore (nuclear translocation); however the mechanism of this translocation has not been fully elucidated.
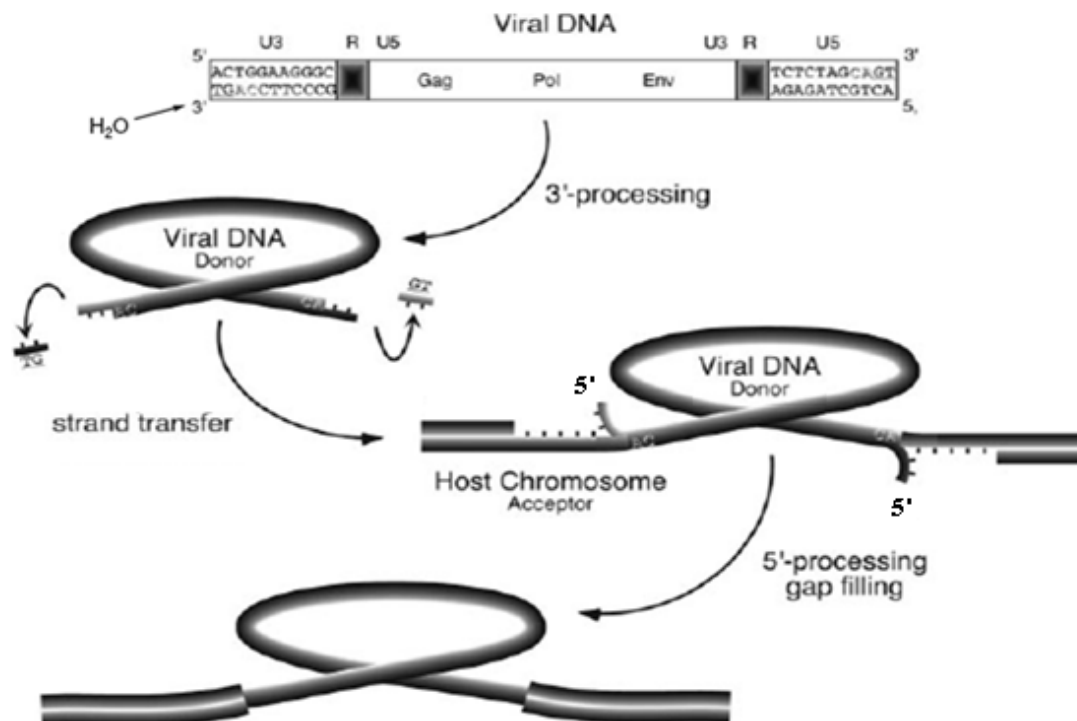
Figure 6: The viral DNA and cDNA integration mechanism within the cell nucleus (reproduced with permission).[32]

The second catalytic step, the strand transfer (ST) step, is a transesterification reaction and this takes place in the cell nucleus. The water molecule removes the TG dinucleotides from the 3' end of the viral DNA. The 3'-OH group of the viral DNA acts as a nucleophile and attacks the 5'-phosphate end of the host DNA, leaving 5'-end of the viral DNA with an overhang of two nucleotides and a 5-base single-strand gap. The host cell DNA repair enzyme removes the overhanging nucleotides and repairs the single-strand gap to afford a complete integrated doubled-strand DNA.[28] It was thought that although 3' processing and strand transfer steps are very similar at a chemical level, the way the active site of IN interacts with the DNA substrate must differ for 3' processing and strand transfer.

Design and development of INIs began over 20 years ago and, while there are many potential INIs undergoing development, few are in clinical trials and there were no INIs available for HIV treatment before 12 October 2007, when the first integrase inhibitor, Raltegravir (brand name Isentress, Fig. 7), was approved by the FDA.[11] This inhibitor was designed to inhibit the

strand transfer step and is manufactured by Merck.

Lataillade and Kozal [31] have described various classes of pre-clinical and clinical drugs that inhibit different steps in the integration mechanism; there are pyrano-dipyrimidines (PDPs, preventing the viral DNA-IN binding process *in vitro*); styrylquinolines (SQLs); carbazole derivatives; β-diketo acids (DKAs) and their derivatives,[33, 34] naphthyridines, fungal polyketides, and antimicrobial peptides (ST inhibitors); oxadiazoles (nuclear import inhibitors); and caffeine-related methylxanthines (host cell DNA repair protein inhibitors). Further classes of INIs have been summarised by Iyer *et al.*[35] and these include chicoric acid, coumarins, aromatic sulfonamides, arylamides, naphthalene-based, curcumins,[36] salicyhydrazines, and many others (Fig. 8). The dual IN and RT inhibitor, V-165, inhibits viruses which show resistance against entry and RT inhibitors. This inhibitor targets both IN and RT steps; however it is believed that IN is the primary antiviral target for this inhibitor.[32]
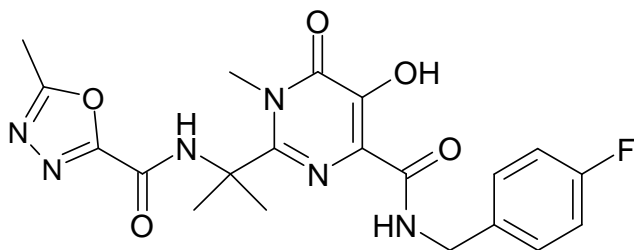

Figure 7: Raltegravir (brand name: Isentress; or MK-0518 before approval)
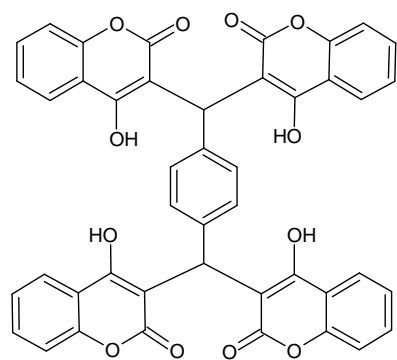
hydroxycoumarins
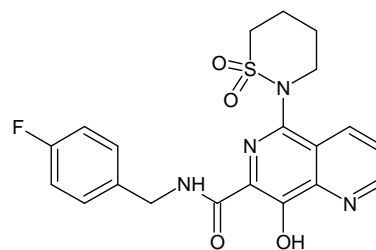
L-870,810 (naphthyridine carboxamide)
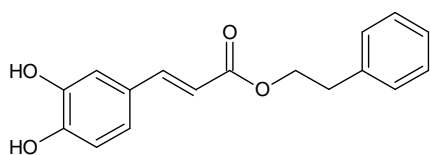
CAPE (L-chicoric acid derivitives)
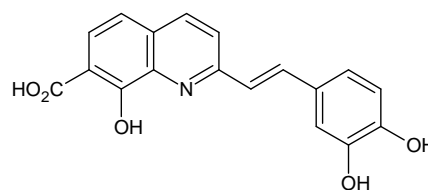
KHD16 (Styrylquinoline)
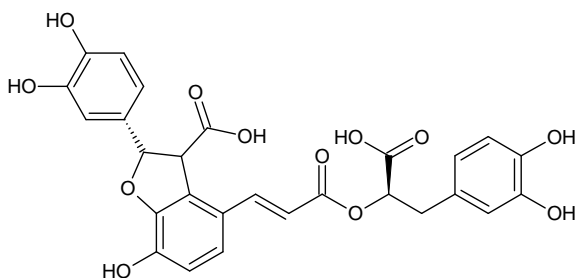
Lithospermic acid (natural product)
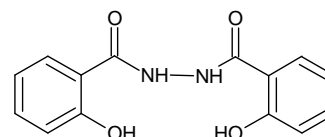
salicylhydrazine
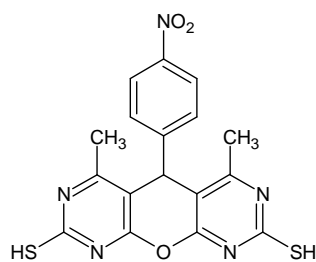
V-165 (Dual inhibitor)

**Figure 8**: Classes of HIV-1 integrase inhibitors.

Quinolinonyl DKA derivatives are examples of bifunctional inhibitors, effective against both ST and 3'-P steps. Research has shown that the bi-DKA moiety may not be as efficient as the mono-DKA quinolinonyl systems. This hypothesis has been proved by Di Santo and his colleagues,[26, 37] who synthesized and evaluated the antiviral activities of mono-DKA quinolinonyl compounds relative to bi-DKA quinolinonyl derivatives. 3-Acetyl-4(1*H*)-quinolinones **2a-h**, prepared by reacting aniline derivatives **1a-h** with ethyl orthoformate and ethyl acetoacetate (Scheme 1), were alkylated with 4-fluorobenzyl bromide in alkaline medium (K_2CO_3) to give the *N*-substituted quinolones **3a-h.** The acetyl derivatives **2a-h** and **3a-h** undergo sodium ethoxide catalysed Claisen condensation with diethyl oxalate to give the ethyl esters **4a-h**, which were hydrolysed with sodium hydroxide to afford the acid derivatives **5a-h**.[26, 37]
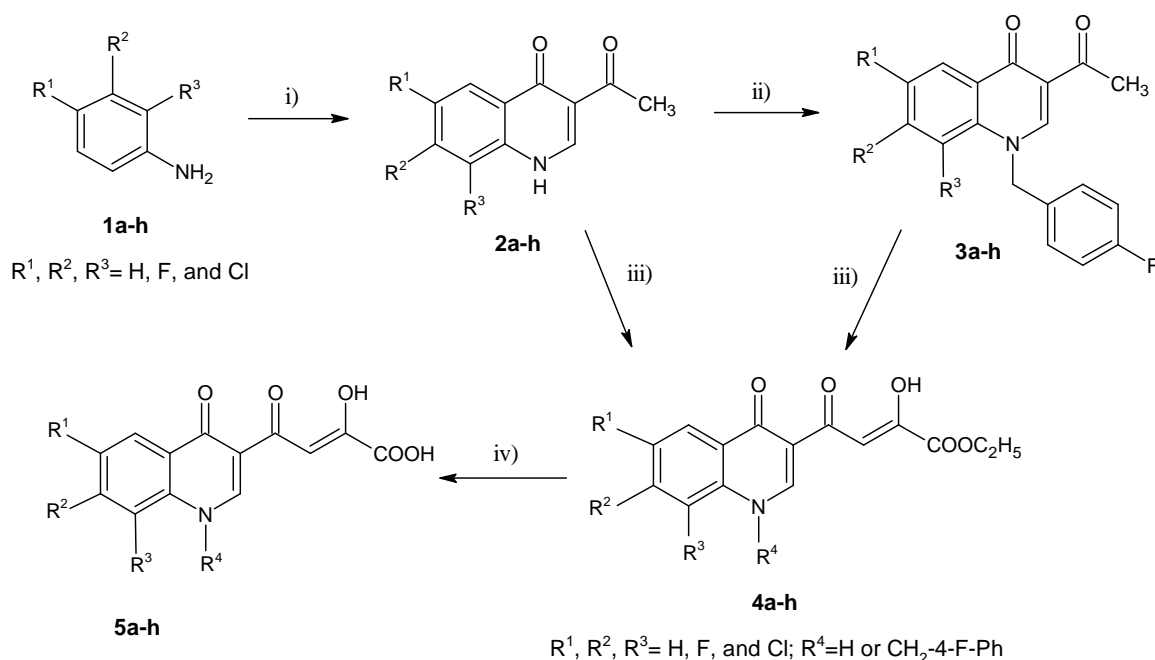
Reagents and conditions: i) ethyl orthoformate, ethyl acetoacetate, Dowtherm A, 95-254°C, 8h; ii) 4-fluorobenzyl bromide, K_2CO_3, DMF, 100°C, 1h; iii) diethyl oxalate, C_2H_5ONa, THF, r.t., 2h; iv) 1M-NaOH, THF/CH_3OH, r.t., 40min.

Scheme 1

13

The approved INI belongw to the DKA class, a very potent class of disintegration drugs which serve as lead compounds in the search for new INIs. There are numerous DKA ARV drugs in the different phases of clinical trials. Figure 9 show the structure of three DKA derivatives, which inhibit the strand transfer step in the HIV-1 integration mechanism. Full structures of IN enzymes with or without an inhibitor attached are not available, but *in silico* docking of 5-CITEP into the X-ray determined structure of the CCD led to the discovery of a new open space, the "trench", which is adjacent to the active site and which 5-CITEP occupies.[38]
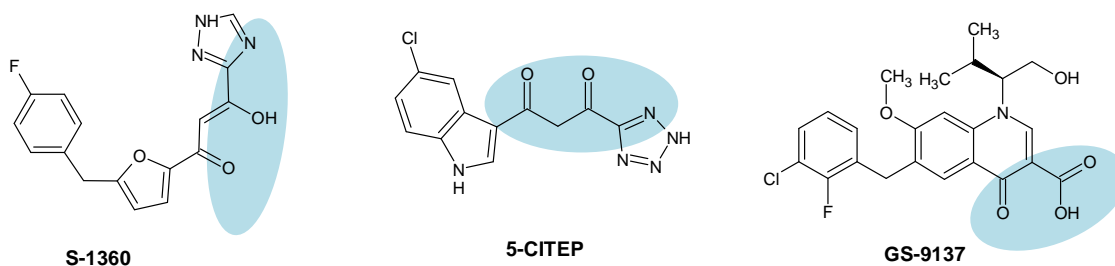
Figure 9: DKA strand transfer inhibitors in clinical trials: the blue circles indicate the diketo acid moiety.

Kawasuji *et al.*[39, 40] have proposed a two-metal binding model for the HIV-IN inhibition mechanism, while Merchard *et al.*[29] have suggested a similar mechanism, which is shown in Figure 10. The catalytic route is outlined with orange arrows and the inhibition route by the blue arrow. In stages A and B, the Mg^{2+} metal ion coordinates with the carboxylic groups of the acidic residues of the DDE catalytic triad of the enzyme within the active site. Each of the Mg^{2+} ions binds to a carboxylic oxygen from two different residues and to the phosphate oxygens of a coordinated viral DNA molecule. Water acts as a nucleophile attacking the phosphate ion of the viral DNA (stages B and C) to give a 3'-OH terminal fragment which then attacks the host DNA-Mg^{2+} complex at the phosphate centre (stage D) to give an integrated host DNA and viral DNA chain and metal-residue complex (stage E).
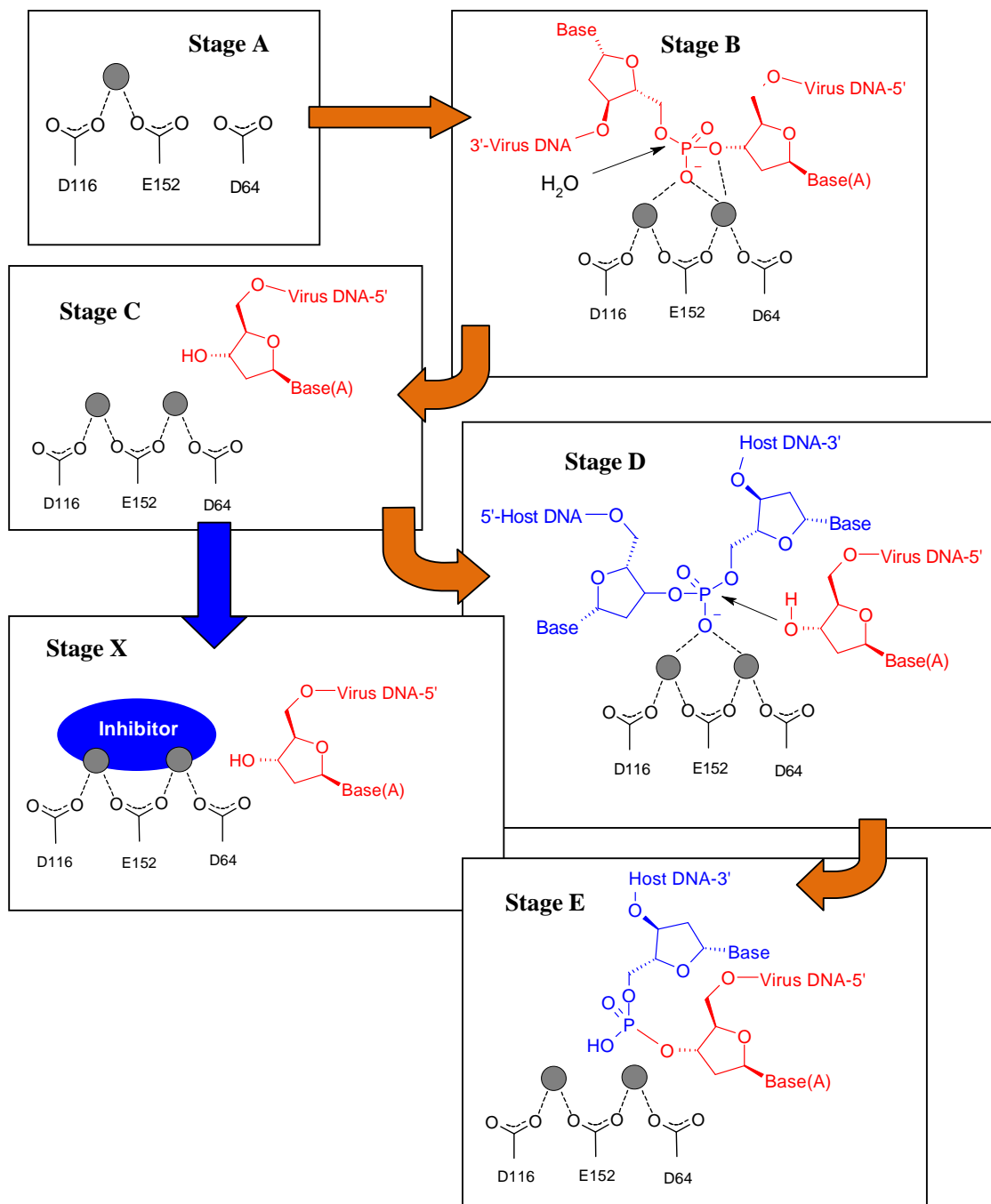
Figure 10: The two-metal binding HIV IN catalytic and inhibition mechanism, showing:- orange arrows (stages A to E) for the catalytic mechanism and a blue arrow (stage C to X) for IN inhibition; the viral DNA (in red) and the host DNA (in blue); the two metal cofactors as grey spheres; and the three IN enzyme amino acid residues (D116, D64, and E152 motif) of the DDE catalytic triad in the CCD active site in black (Reproduced with permission).[40]

Inhibition of the integration process occurs in stage X, when the inhibitor chelates with the metal ions, prohibiting coordination of host DNA and hence inhibiting integration. Discovery of the binding site within the catalytic domain of IN and formulation of the mechanism is the result of many years of research, using computer modelling, three-dimensional (3-D) database searching[41, 42] and QSAR analysis.[35] Numerous organic molecules were examined for potency as INIs, permitting a pharmacophore for INI drugs to be established.

1.1.3 Styrylquinolines as HIV-1 integrase inhibitors

The styrylquinolines (SQL) constitute a class of heterocyclic antiviral compounds which contain an ethylenic spacer connected to the quinoline moiety and an ancillary aromatic nucleus. The method typically used to generate SQLs involves a Perkin-type condensation which is slightly different to the usual Perkin condensation method. The usual method involves reaction between an aldehyde and an enolate anion derived from an ester, ketone, aldehyde or anhydride in the present of a base (sodium acetate or a tertiary amine) with or without solvent.[43-45] Chandrasekhar *et al.*[44] proposed a revised mechanism for the reaction (Scheme 2). Thus, reaction between benzaldehyde **6** and acetic anhydride **7** generate a *gem*-diacetate **8** in high yield; an α-proton is then removed by the acetate ion, and cyclization affords intermediate **9**. Electron delocalization activates the double bond of **9** to attack another benzaldehyde molecule **6** to afford intermediate **10**; transfer of the acetyl group leads to intermediate **11** and elimination of benzaldehyde and acetate ion finally yields the cinnamic acid **13**.
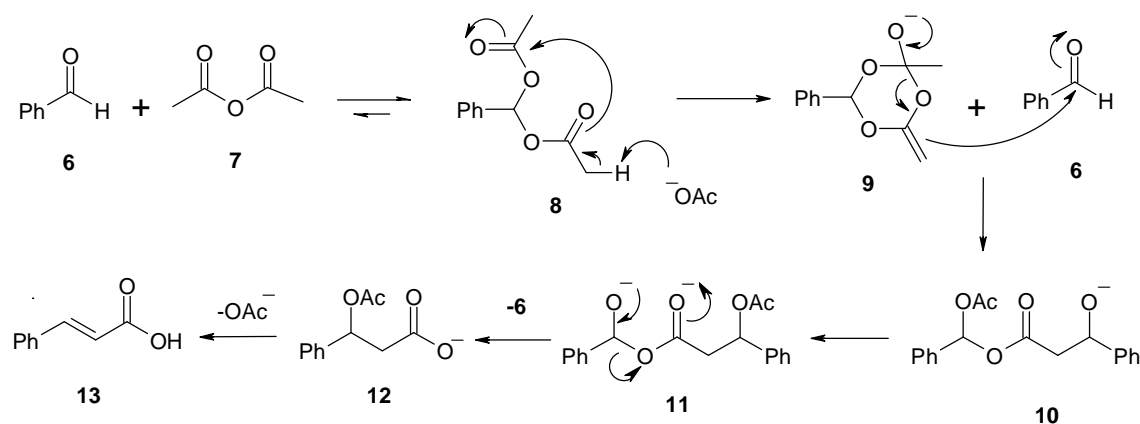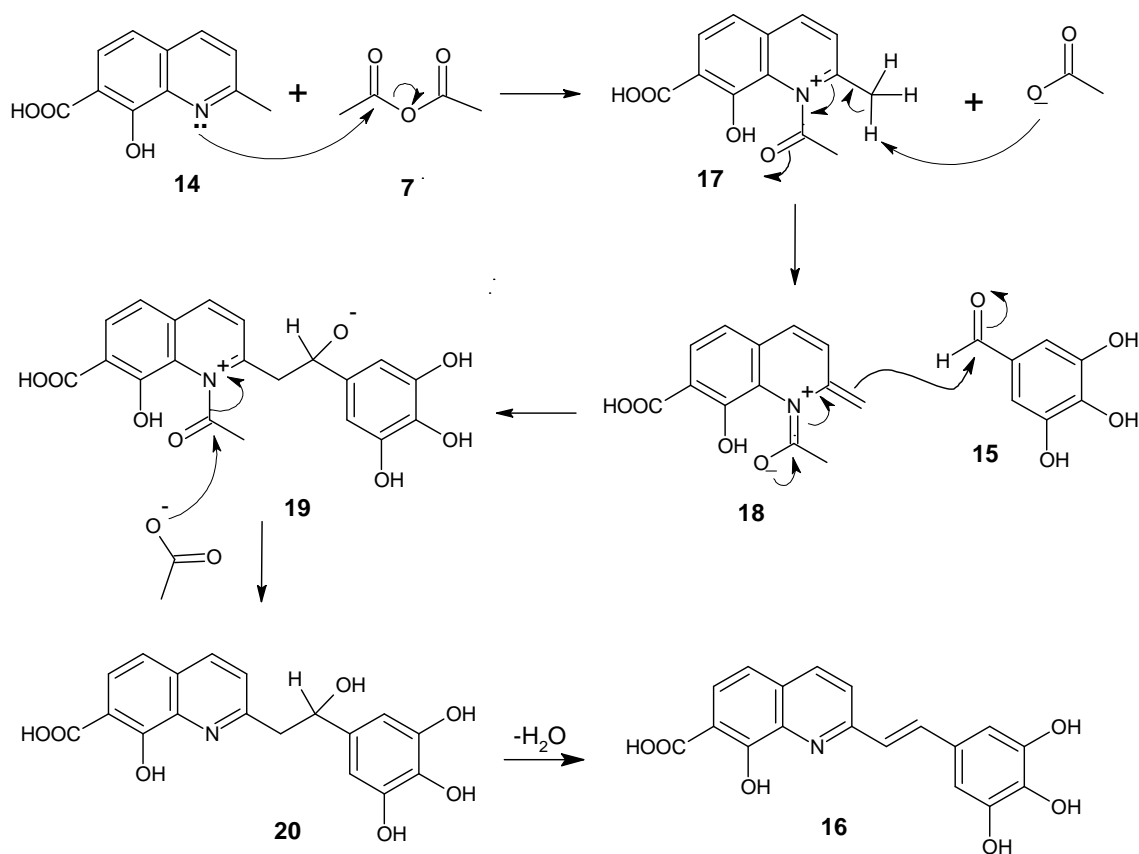

Scheme 2

Application of the Perkin-type condensation in the synthesis of styrylquinolines (*e.g.* **16**) involves reaction of α-methylquinolines (*e.g.* **14**) with a benzaldehyde (*e.g.* **15**) in the absence of catalyst (Scheme 3) but typically in the presence of acetic anhydride.[44] A proposed mechanism for this reaction is illustrated in Scheme 4. Thus, acetic anhydride is proposed to dissociate on reaction with the quinoline derivative **14**; the released acetate anion then acts as a base removing a proton from the quinoline α-methyl group to generate the nucleophilic resonance-stabilised anion **18**, which attacks the benzaldehyde **15**. The acetate anion also attacks the carbonyl carbon of the *N*-acetyl group in intermediate **19** to regenerate acetic anhydride. Protonation and dehydration of the resulting alcohol **20** then affords SQL **16**.
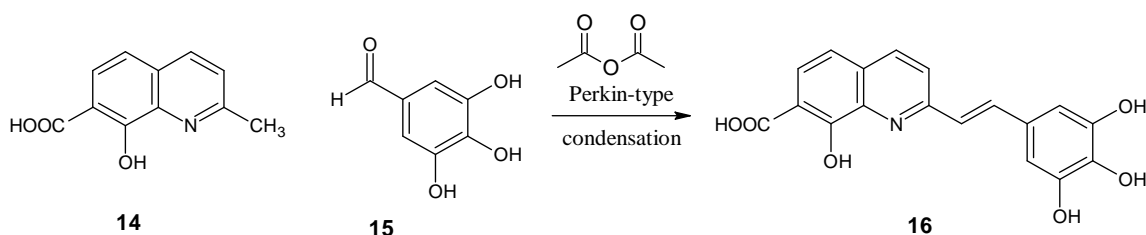
Scheme 3

The SQL drugs act as HIV-1 integrase inhibitors, inhibiting the 3' processing step by competing with the HIV's LTR substrates, and to a lesser extent by inhibiting the nuclear translocation step. Researchers has explored numerous SQL derivatives with the aim of identifying the most active integrase inhibitors by monitoring their IC_{50} values.[46-48] Based on the results, they have concluded that the substituents on the quinoline ring and ancillary aromatic ring are critical features for high antiviral activity. The styrylquinoline derivatives that exhibit high inhibition activity (*i.e.* low IC_{50} values) contain a COOH group at position 7 and an OH group at position 8 of the quinoline moiety and a polyhydroxy ancillary aromatic ring. (*E*)-8-Hydroxy-2-[2-(3,4,5-trihydroxyphenyl)ethenyl]-7-quinolinecarboxylic acid **16** (Scheme 3) was found to be the most potent HIV-1 integrase inhibitor in *in vitro* experiments, blocking viral replication in cell cultures, with an IC_{50} value of 0.3µM without exhibiting cytotoxicity.[46] (Other values have been reported over the years: 2.4µM in 2000[47] and 1.2µM in 2005[48])

Scheme 4

Although, the first HIV-1 integrase inhibitor drug approved by FDA was a diketo acid, and β-diketo acid derivatives appear to be the leading class of contenders as potential drugs, the styrylquinoline **16** also exhibits high anti-IN activity and shows a common sub-structure with the napthyridine carboxamide L-870810 (a potential INI in clinical trial) (Fig.11). These latter comounds exhibit a common hydrophilic domain with three heteroatoms (N or O, within the blue circle), permitting chelation with the two metal ions (generally Mg^{2+}) in the IN CCD.

Kawasuji *et al.*[40] used computer modelling methods to explore the docking of 2-hydroxy-3-heteroaryl acrylic acid derivatives (HHAAs) into the IN active site and identified two crucial active sites: a hydrophilic domain and a hydrophobic domain. The tautomeric hydrophilic
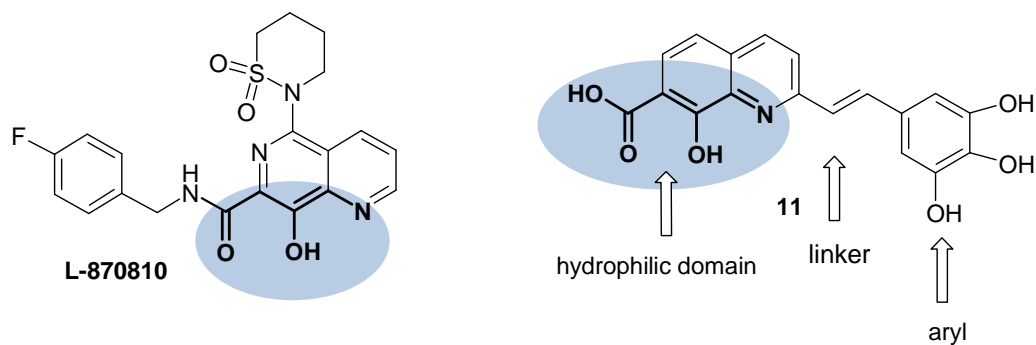
18

Figure 11

domain of the ligand (the blue-shaded motif; Scheme 5) permits chelation of the two metal ions *via* the enolate species, where X and Y represent heteroatoms that donate a lone-pair of electrons. The keto group is enolised and then ionised to give a negatively charged oxygen (enolate anion) enhancing the ability of the delocatlised system to coordinate to two metal ions at the same time. Application of the proposed two-metal ion binding mechanism (Fig 10) to the HHAA compounds is illustrated in Scheme 5 (in red). This model has also been used on the SQL compound **16** (in green), to illustrate the possible interaction between the enzyme and the SQL.
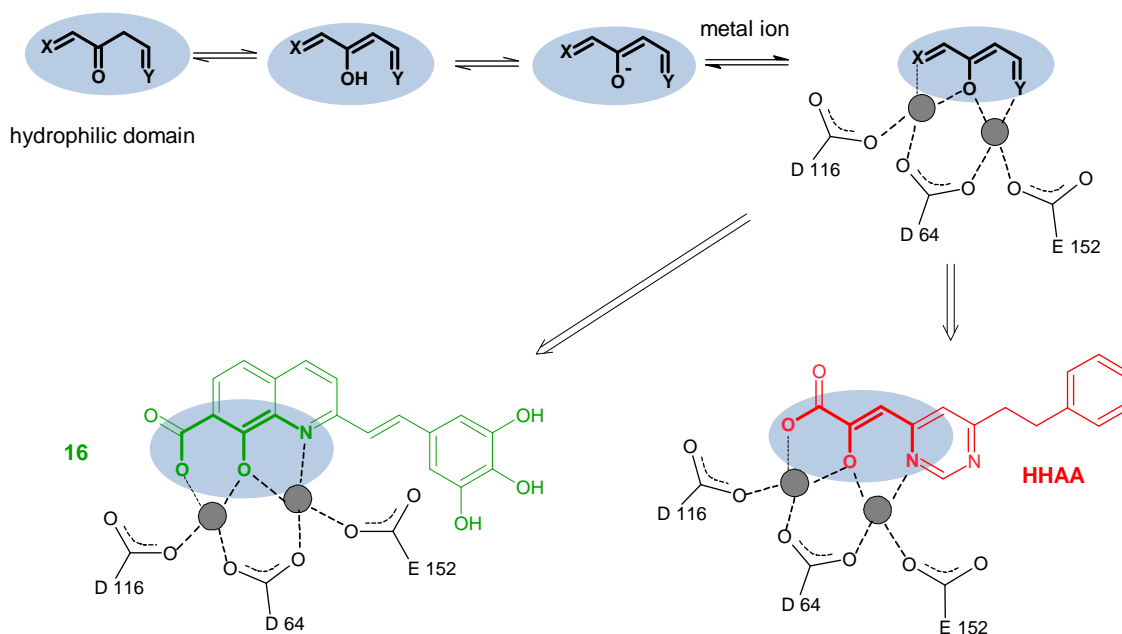
Scheme 5

1.1.4   Aims of the present investigation

The aim of this project has been to synthesise novel compounds that could act as HIV-1 integrase inhibitors. More specifically, the research has included the following objectives:

i.   Use of Baylis-Hillman (BH) methodology to synthesise a series of α-methylene-β-hydroxycarbonyl compounds from various *o*-nitrobenzaldehydes and methyl vinyl ketone (MVK) or methyl acrylate.

ii. Nucleophilic substitution (S_N') on and aza-Michael addition to the BH-MVK adducts using various nucleophiles.

iii. Reductive cyclization of the BH-MVK adducts and BH-MVK addition products to afford quinoline derivatives.

iv. The formation of cinnamate esters from BH-methyl acrylate adducts as potential HIV-1 integrase inhibitors.

2   DISCUSSION

In the discussion, which follows, attention will given to the synthesis of Baylis-Hillman products (**Section 2.1**) and their reductive cyclization to quinoline derivatives (**Section 2.2**), The 2-methyl quinolines obtained using MVK have been subjected to Perkin-type condensation to afford styrylquinolines (**Section 2.4**). The Baylis-Hillman adducts obtained using MVK and methyl acrylate have been elaborated further by:- i) substitution reactions (**Section 2.5**); ii) nucleophilic addition (**Section 2.6**) and; iii) dehydration to give cinnamate esters (**Section 2.7**). Attention has also been given to the synthesis of bis-styrylpyridine derivatives (**Section 2.8**).
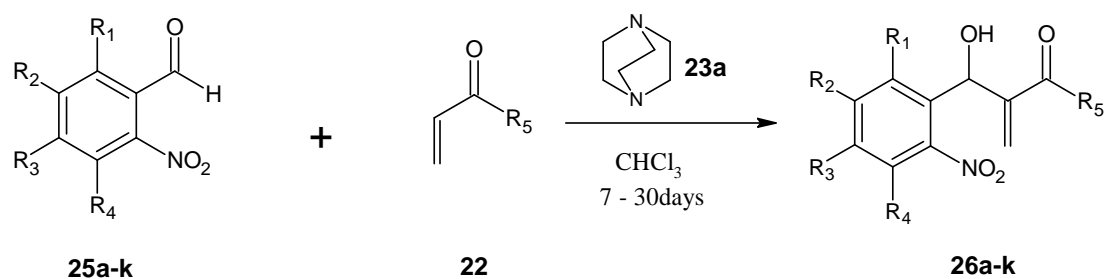
2.1   Synthesis of Baylis-Hillman products

The Baylis-Hillman reaction is so named after the scientists A. B. Baylis and M. E. D. Hillman who, in 1972, patented the use of tertiary amines as effective catalysts for the coupling of ethyl acrylate or acrylonitrile with acetaldehyde.[49] Morita[50] had previously reported similar transformations using phosphine catalysts and, hence, the reaction is also known as the Morita-Baylis-Hillman (MBH) reaction. The Baylis-Hillman (BH) reaction has been used extensively to couple activated alkenes with aldehydes and other suitable electrophiles, and application of the reaction in the synthesis of various heterocyclic systems has been one of the main foci in our research group. The Baylis-Hillman reaction is a useful method for forming a new carbon-carbon single bond between an aldehyde **21** and, for example, an α,β-unsaturated carboxyl derivative **22** to yield an α-methylene-β-hydroxycarbonyl derivative **24**. This reaction requires an unhindered nucleophilic tertiary amine catalyst **23,** most commonly,: 1,4-diazabicyclo[2.2.2]octane (DABCO) **23a**, 4-dimethylaminopyridine (DMAP) **23b**, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) **23c**, 3-hydroxyquinuclidine (3-HQ) **23d** and imidazole **23e** (Scheme 6).
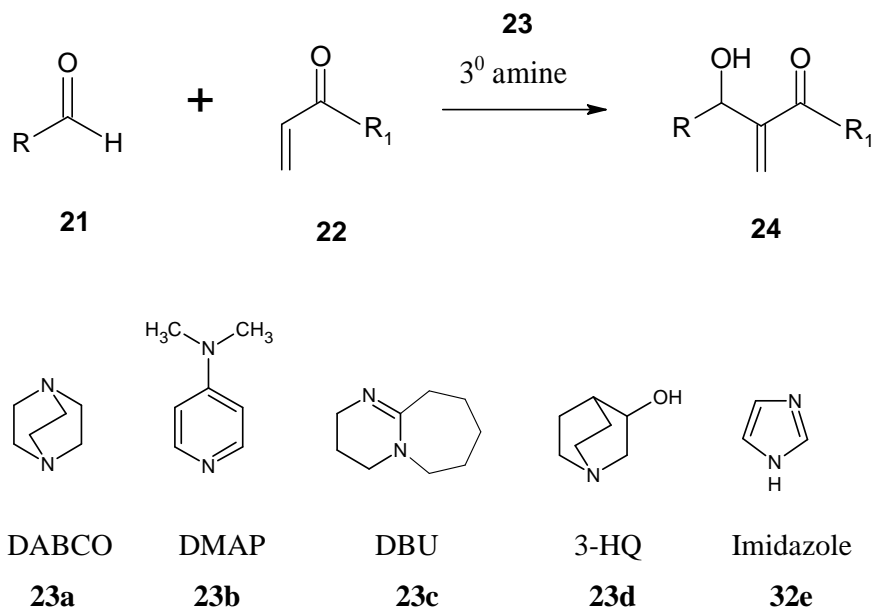
Scheme 6

The aldehydes chosen for the BH reactions in this study were the 2-nitrobenzaldehydes **25a-k**, while methyl vinyl ketone (MVK) and methyl acrylate were used as the activated alkenes. The different reactions undertaken and the corresponding products and isolated yields are summarised in Scheme 7 and Table 1. The reactions were run at atmospheric pressure and room temperature. The reaction times ranged from 2 to 30 days with chloroform being used as the solvent and DABCO **23a** as the catalyst. At the end of each reaction, the solvent was removed under reduced pressure and the residue chromatographed, by flash chromatography, to give the desired product. For the MVK adducts the DABCO was removed by washing a solution of the reaction mixture in EtOAc with 10% hydrochloric acid. The yields of the Baylis-Hillman products **26a-k** varied considerably but, in a number of cases, the yields were very good (Table 1). In general, the Baylis Hillman ester adducts **26g-k** were obtained in higher yields and within shorter experimental times (2 to 7 days) than the MVK derivatives **26a-f** (7 to 30 days).

Scheme 7

Table 1: Yields of Baylis-Hillman products **26a-k** (Scheme 7).

|	R^1	R^2	R^3	R^4	R^5	% Yield
a	H	H	H	H	CH_3	45
b	H	OH	H	H	CH_3	89
c	H	OCH_2O		H	CH_3	27
d	H	OCH_3	OCH_3	H	CH_3	58[a]
e	H	H	H	OCH_3	CH_3	40
f	H	Cl	H	H	CH_3	88
g	H	H	H	H	OCH_3	96
h	H	H	H	OCH_3	OCH_3	98
i	Cl	H	H	H	OCH_3	91
j	H	OCH_2O		H	OCH_3	54
k	H	H	NO_2	H	OCH_3	96

[a] Crude yield.

The mechanism of the BH reaction has been studied by several research groups,[51] including our own;[52, 53] the currently accepted details are illustrated in Scheme 8. The catalytic cycle begins by conjugate addition of DABCO **23a** to the activated alkene **22**, generating a zwitterionic intermediate **27**. The adol-type addition of intermediate **27** to **25** leads to the enolate anion **28**, which undergoes proton transfer to afford the resonance-stabilised anion **29**.

Elimination of DABCO *via* an E1$_{CB}$ process then gives the β-hydroxy-α-methylene product **26**.
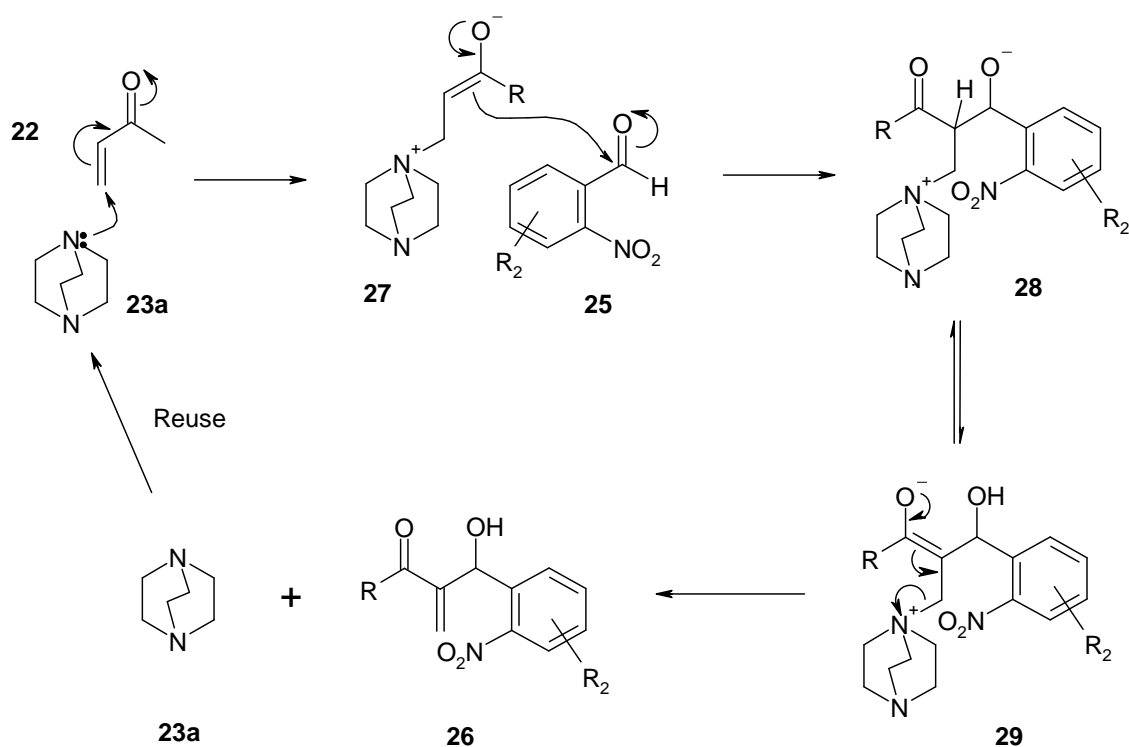

Scheme 8

The Baylis-Hillman products **26a-k** were fully characterised by NMR, IR and high- and low-resolution MS analysis. There are a number of significant features one should look for in the ^{1}H NMR spectrum of a BH adduct which indicate formation of the product, *viz.*, i) two singlets at *ca.* 6 ppm corresponding to the two vinylic protons and ii) a singlet due to the 4-methine proton in the same region. The ^{1}H NMR spectrum of adduct **26b** (Fig. 12) shows a methyl singlet at 2.30ppm and the three significant signals at 5.60 and 6.16 ppm (vinylic protons) and at 6.19 ppm (4-methine proton). The vinylic protons are diastereotopic and give rise to two discrete signals. The signals between 6.50 ppm and 8 ppm are due to the three aromatic protons, while the two hydroxyl protons resonate as a doublet at 5.81 and a broad singlet at 10.90 ppm.

24
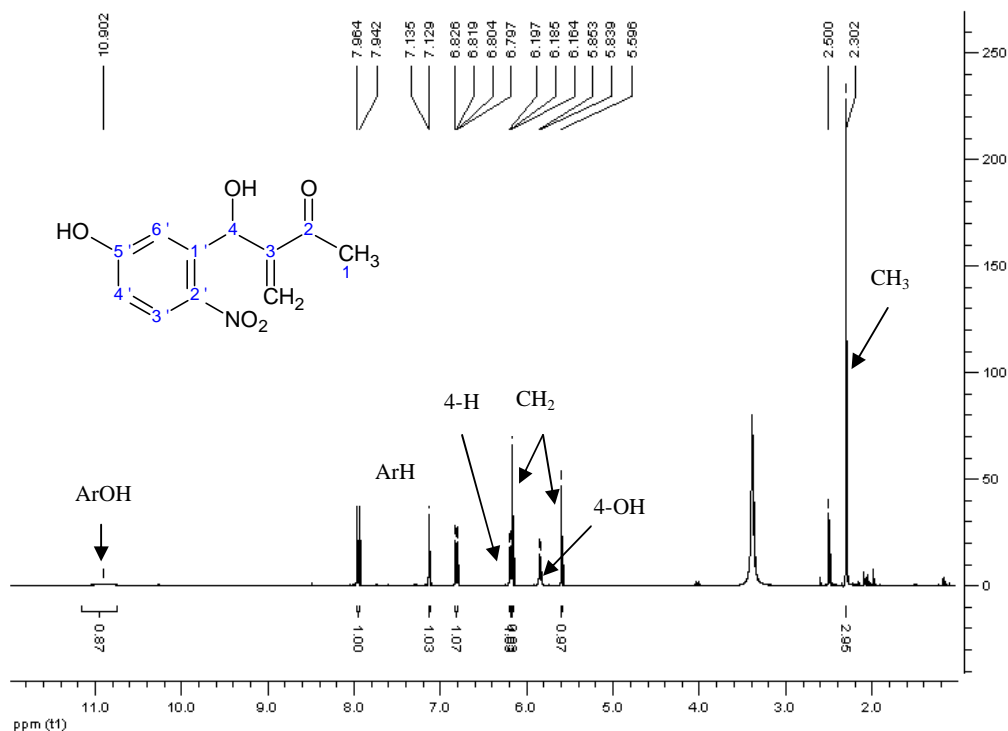
Figure 12: 400MHz ^{1}H NMR spectrum of 4-hydroxy-3-methylene-4-(5-hydroxy-2-nitrophenyl)butan-2-one **26b** in CDCl$_3$.

Of the products obtained from reactions with MVK, the Baylis-Hillman MVK adduct **26b**, was isolated after 30 days in the highest percentage yield (89%). Initially the starting material **25b** was immiscible with CHCl$_3$ but, after 8 days, the solid dissolved. The reaction was monitored using thin layer chromatography (TLC) and, after 30 days, TLC analysis indicated complete consumption of the reactant **25b**. The expected 11 signals for the adduct (**26b**) were observed in the ^{13}C NMR spectrum (Fig. 13) with the methyl carbon signal at 26.0 ppm and carbonyl signal at 198.0 ppm. The DEPT 135 NMR spectrum (Fig. 14) shows the presence of the vinylic methylene carbon signal at 125.0 ppm and the 4-methine carbon signal at 64.9 ppm.. A clear correlation between the 4-hydroxy and 4-methine protons (circled) is evident in the COSY NMR spectrum (Fig. 15) confirming the coupling observed for their respective signals in the ^{1}H NMR spectrum.

25
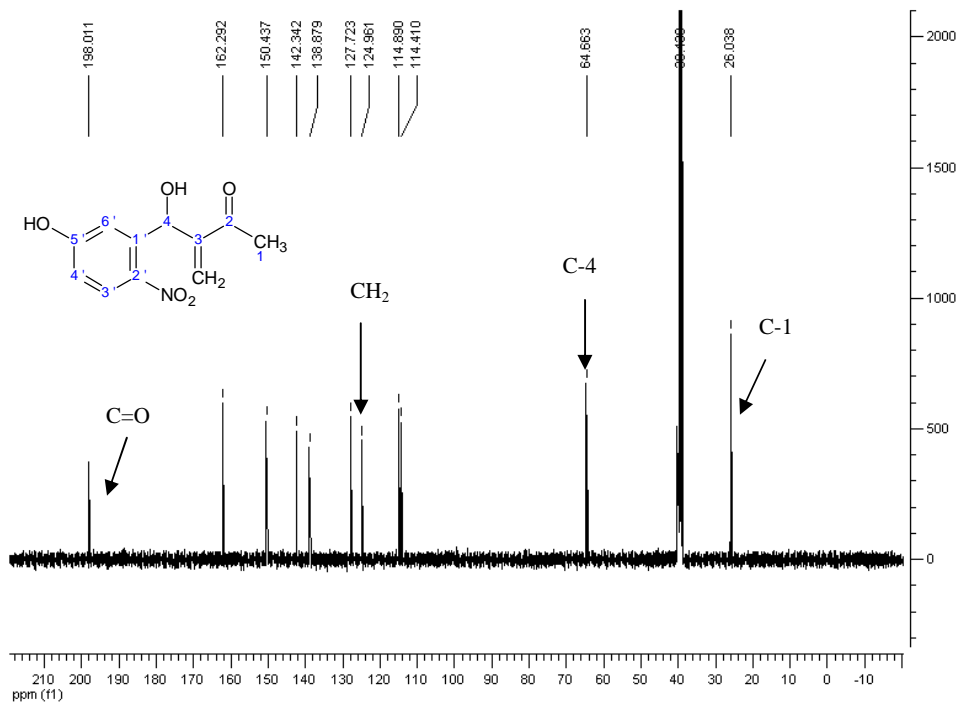
Figure 13: 100MHz ^{1}H NMR spectrum of 4-hydroxy-4-(5-hydroxy-2-nitrophenyl)-3-methylenebutan-2-one **26b** in DMSO-d_6.
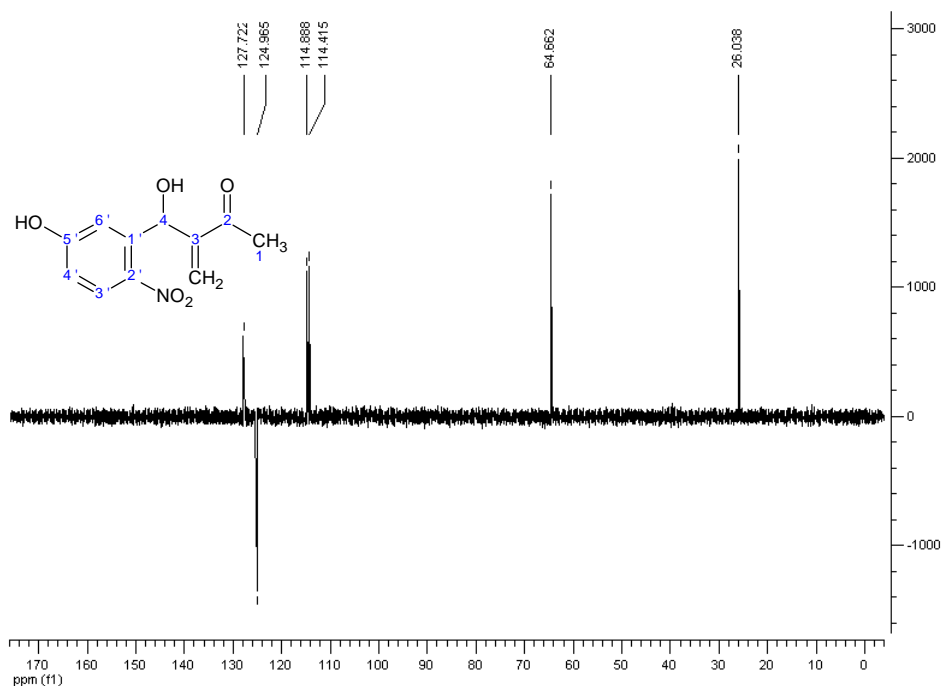
Figure 14: DEPT 135 NMR spectrum of 4-hydroxy-4-(5-hydroxy-2-nitrophenyl)-3-methylenebutan-2-one **26b** in DMSO-d_6.
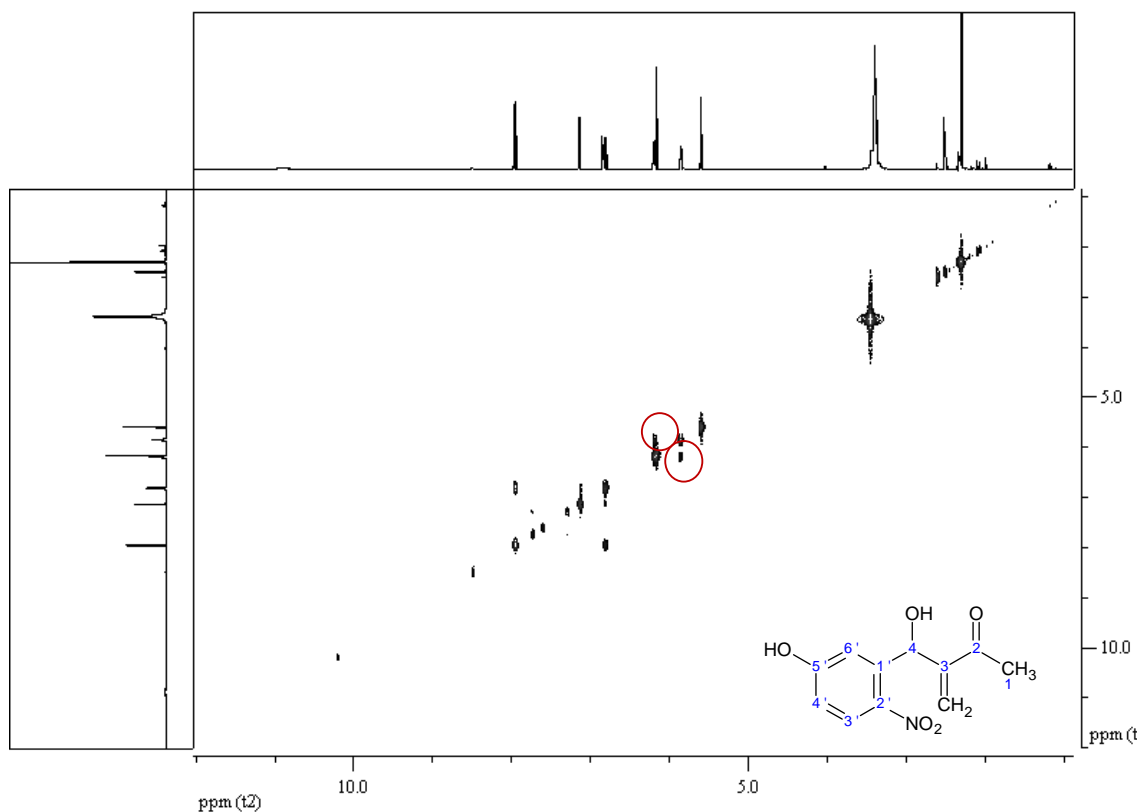
Figure 15: 400MHz COSY NMR spectrum of 4-hydroxy-4-(5-hydroxy-2-nitrophenyl)-3-methylenebutan-2-one **26b** in DMSO-d_6.

To synthesise compounds **26a-f**, the reactants, nitrobenzaldehyde, MVK and DABCO, were used in the ratio 1:1.5:0.5.[54] In some cases, additional MVK and DABCO were added if the TLC analysis indicated that the reaction was no longer proceeding and a large amount of starting material was still present. The yield reported in Table 1 for compound **26d** is the crude yield before any form of purification. It was observed that if the crude mixture was left for a period of time, reversion to the 2-nitrobenzaldehyde **25d** and MVK precursors occurred.[55] This reversion was evident from ^{1}H NMR spectra, in which the intensities of the characteristic vinylic and 4-methine proton signals began to decrease over time. This phenomenon was also observed with the BH adduct **26c**.

It had been reported in a previous study in our group[52, 53] that BH reactions using MVK may not only give the desired β-hydroxy-α-methylene product (e.g., **26a-k**), but also the methyl vinyl ketone dimer **30** and the bis-MVK adduct **31** (Scheme 9). Similar observations have

been reported by Shi *et al.*.[56, 57] The MVK dimer and bis-MVK adduct were, in fact, obtained in some cases in the present study. Figure 16 illustrates the ^{1}H NMR spectrum of the MVK dimer **30**, revealing the two methyl singlets at 2.10 and 2.30ppm, the methylene multiplets at *ca.* 2.54 ppm and the vinylic signals at 5.80 and 5.99 ppm. The ^{1}H NMR spectrum of a mixture of the MVK dimer **30e** and the bis-MVK adduct **31e** is illustrated in Figure 17. The signals arising from the bis-MVK adduct **31e** are visible at 2.22 and 2.56 ppm for the 4-methylene protons, *ca.* 3.9 ppm for the OMe and 8-OH protons, and at 3.09 and 4.66 ppm for the 5- and 6-methine protons, respectively.
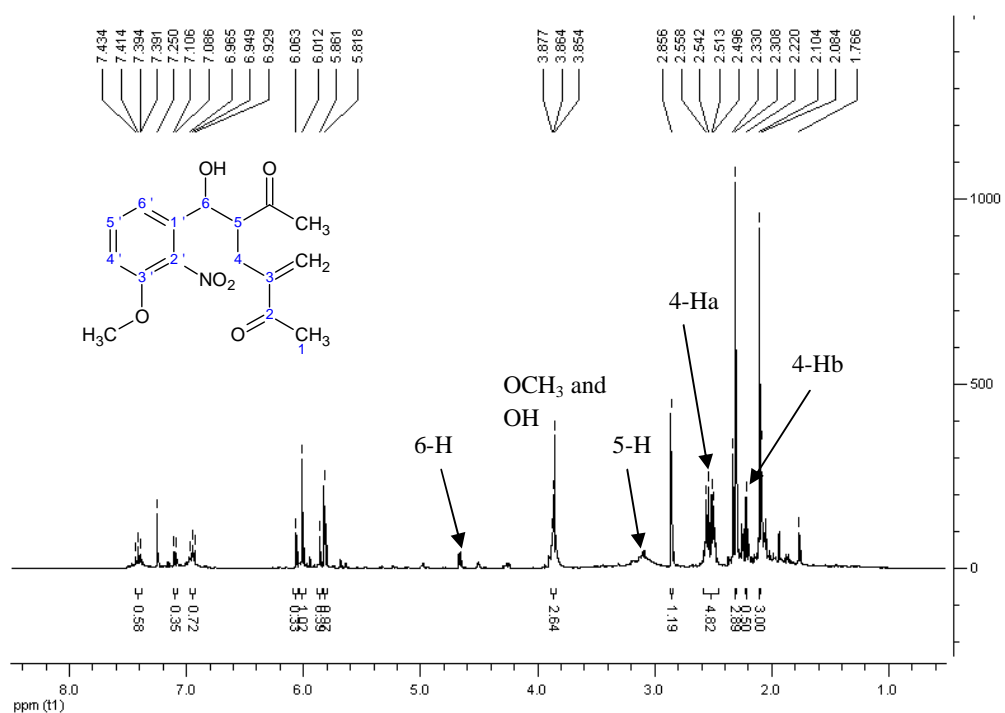

25	R=
c	4,5-OCH$_2$O
d	4,5-dimethoxy
e	3-OMe
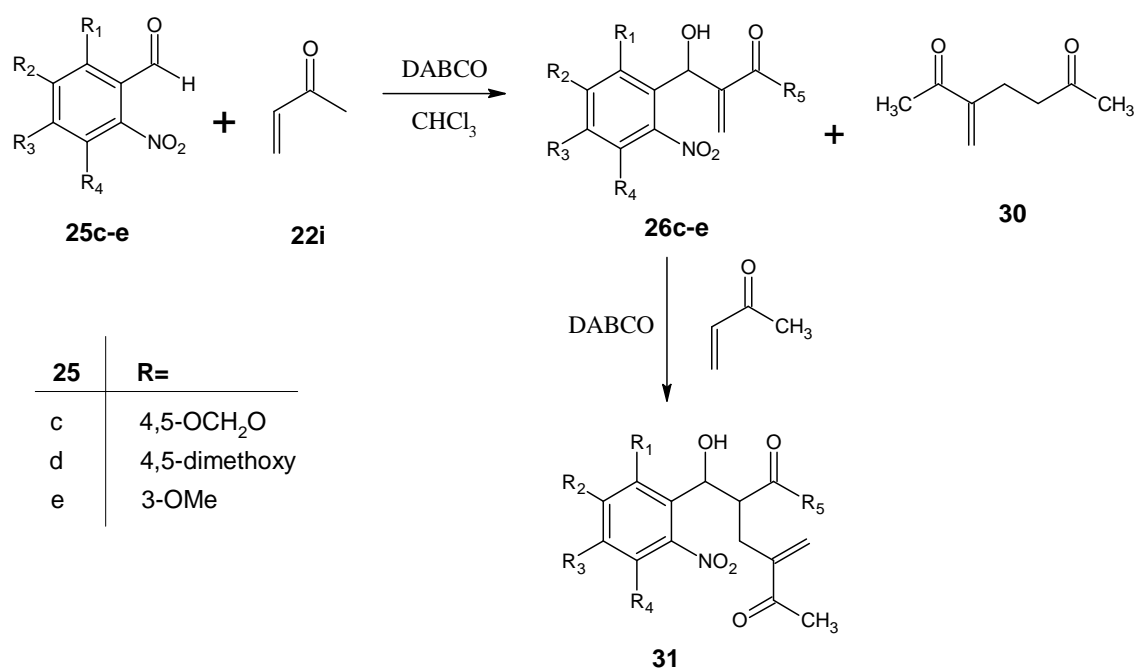
Scheme 9
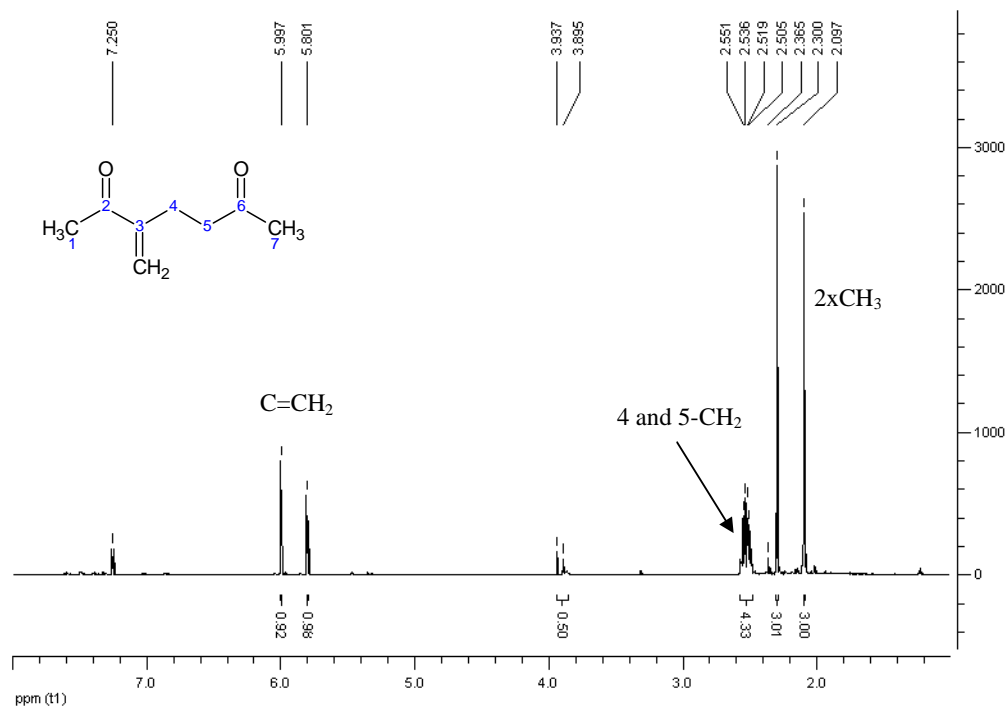
Figure 16: 400MHz ^{1}H NMR spectrum of the MVK dimer (3-methyleneheptan-2,6-dione) **30** in CDCl$_3$.

Figure 17: 400MHz ^{1}H NMR spectrum of a mixture of the MVK dimer **30** and 5-acetyl-6-hydroxy-6-(3-methoxy-2-nitrophenyl)-3-methylene-2-hexanone **31e** in CDCl$_3$.

29

The Baylis-Hillman reaction was the first step in the proposed synthesis of the styrylquinoline derivatives and, therefore, it was crucial to optimise the yields of the BH adducts. This was attempted by varying the catalyst, the solvent, the reactant concentrations and reaction times. The first approach was to change the solvent from chloroform to dichloromethane and to cool the mixture (*ca.* 0°C) during the addition of reactants to give cleaner products.[58] The reaction was carried out using 2-nitrobenzaldehyde **25a** and MVK **22i** in the presence of DABCO, and terminated after 7 days. This increased the yield of the reaction from 45% to 60% and purification of the product was much simpler.

Scheme 10 outlines three further approaches to optimising the reaction conditions. Firstly, DMAP was used as catalyst and DMF as solvent; Shi *et al.*[56] had reported that the reaction of *o*-nitrobenzaldehyde with DMAP as the Lewis base took place in 20 hours in DMF to give the BH adduct **26a** in 83% yield. The same reaction conditions were therefore adopted using the *o*-nitrobenzaldehydes **25a** and **25c**. The reactions were terminated after 20 hours, but the product yields (5-23%, Table 2) were not as promising.

25a,c,d   **22i**   **26a,c,d**
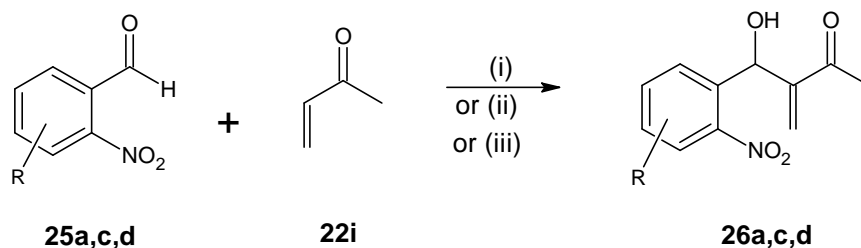
i) DMAP, DMF, 20h, r.t.

ii) Imidazole, 1M satd. aq. NaHCO$_3$, THF, 18h, r.t..

iii) *p*-Nitrophenol, triphenylphosphine, THF, 20h, r.t..

Scheme 10

Table 2: Yield of of Baylis-Hillman products **26a,c,d.**

| R	Method	Product	Yield/%
H	i	**26a**	23
4,5-OCH$_2$O-	i	**26c**	5
H	ii	**26a**	22
H	iii	**26a**	43
4,5-diOCH$_3$	Iii	**26d**	27

The second approach involved following the method reported by Luo *et al.*,[59] who used aqueous THF and 1M aq. NaHCO$_3$ as the reaction medium and imidazole as catalyst, to accelerate the reaction and produce relatively high yields (*ca.* 88%). Davies *et al.*[60] have found that water can play a crucial role in the acceleration of the Baylis-Hillman reactions. However, application of this method gave the product **26a** in only 23% yield. The last variation examined was the Morita-Baylis-Hillman reaction where the catalyst used was a phosphine rather than a tertiary amine. The reaction in THF was co-catalysed by triphenylphosphine and nitrophenol, and was terminated after 20 hours to afford products **26a** and **26d** in 27 % and 43% yield, respectively. This method also gave a relatively low yield compared to the 98% yield reported by Shi.[61]

Other attempts to increase the yields involved altering the catalyst from DABCO to 3-HQ **23d**. It was found that reactions conducted using 3-HQ proceeded more quickly than when using DABCO. The Baylis-Hillman products **26b,c,f-k** were prepared again under the same reaction conditions as before, but replacing DABCO with 3-HQ.[51, 62] It was observed that the reactions were completed 1 to 2 days faster than when DABCO was used, but the products were isolated in similar yields to previous preparations using DABCO (see Table 1).

## 2.2   Synthesis of quinoline derivatives

Many compounds possessing a quinoline moiety are known to exhibit high biological activities against malarial, fungal, bacterial and protozoan infections.[63-66] These compounds include chloroquine, as an antimalarial drug, and 8-hydroxyquinoline and its derivatives as antifungal drugs. The method adopted for the synthesis of quinoline derivatives in our research group has involved the reductive cyclization of *o*-nitrobenzaldehyde derived BH products. The reaction takes place in a cascade of three steps, hydrogenation, cyclization and dehydration.

The Baylis-Hillman adducts were first dissolved in 50mL of ethanol, the air inside the reaction vessel was extracted under reduced pressure and argon introduced into the vessel – the process being repeated three times before the addition of the 10% palladium-on-carbon catalyst. Thereafter, the vessel was flushed out three times with argon as before, and, finally, the hydrogen gas was introduced into the vessel. The hydrogenation reaction may proceed *via* two different pathways. The reaction outlined in Scheme 11, shows that the nitro group of the substrate **26** is reduced to the amino derivative **32**, the nitrogen lone pair electron may then attack the methylene carbon (path **I**) or the carbonyl carbon (path **II**) to give tetrahydroquinolines **33** or the tetrahedral intermediates **34**, respectively. In the systems **32** examined here, cyclization was found to be regioselective, with path **II** being favoured.[53] Once formed, the intermediate **34** may undergo nucleophilic acyl substitution to products **36**, **37** and **38** when the substrate is an ester (R^1=OMe) or nucleophilic addition to products **35** when R^1 is ketone (R^1=Me).
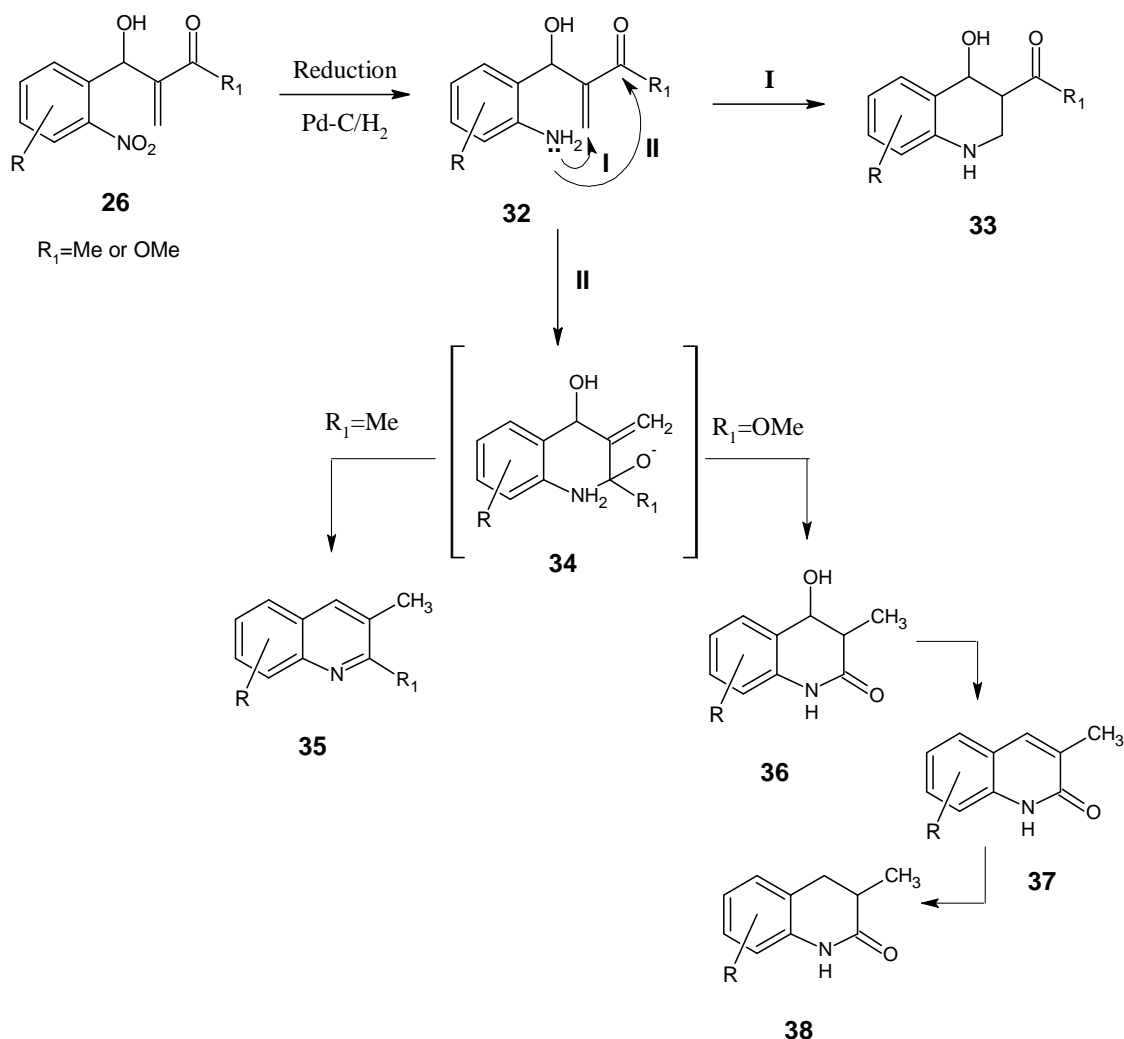
Scheme 11

One of the aims of the project was to synthesise quinoline derivatives and, hence, the reaction pathway to product **35** needed to be followed. The Baylis-Hillman products **26a** and **26b** were used in this study. The reaction was terminated after 3 hours stirring under H_2 and the solvent was removed under reduced pressure. Following the procedure reported by Pakade[52] CH_2Cl_2 was added to the crude mixture; however, a brown precipitate formed as the residue mixed with CH_2Cl_2. The precipitate was filtered off and both the solid and filtrate were analysed by NMR spectroscopy (see Figures 18 and 19).
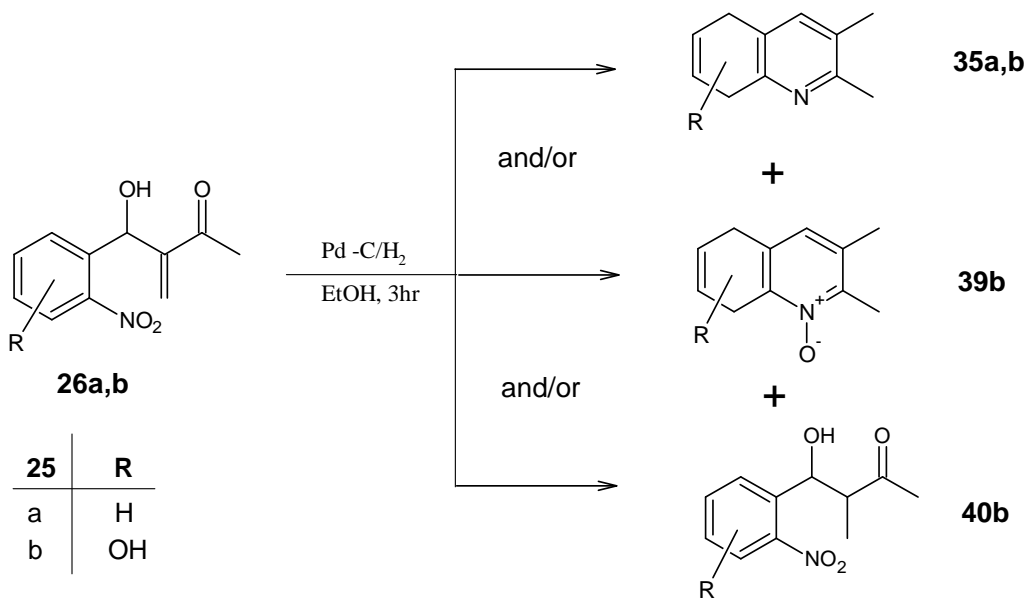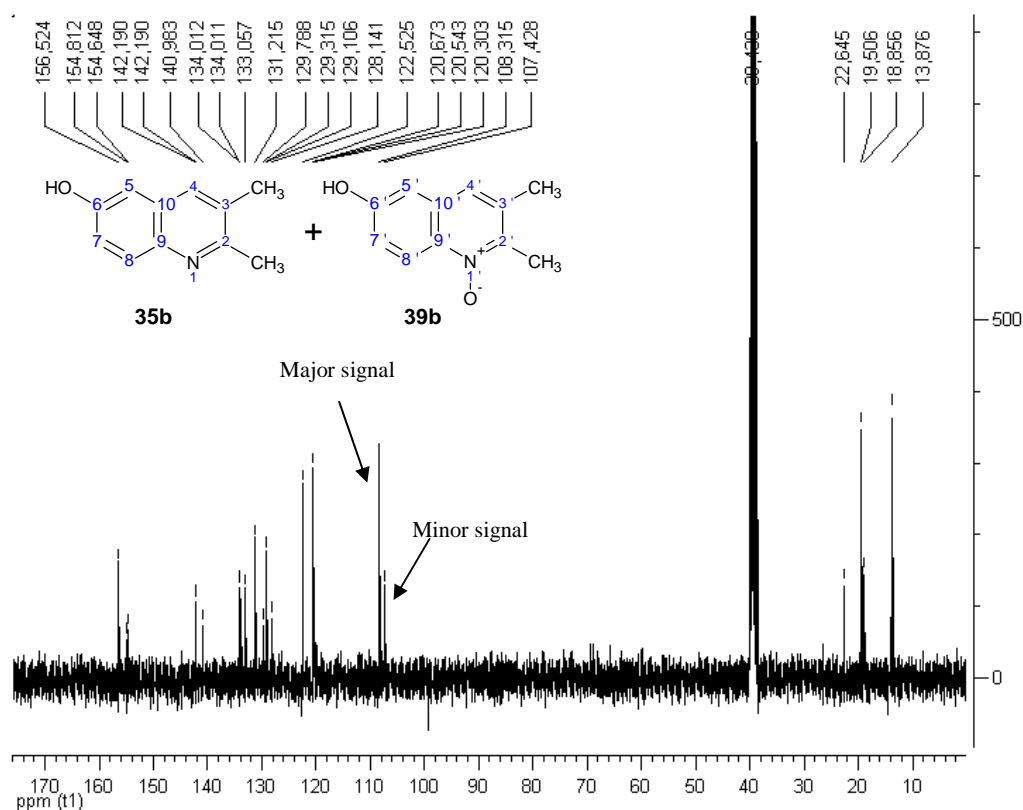
Scheme 12
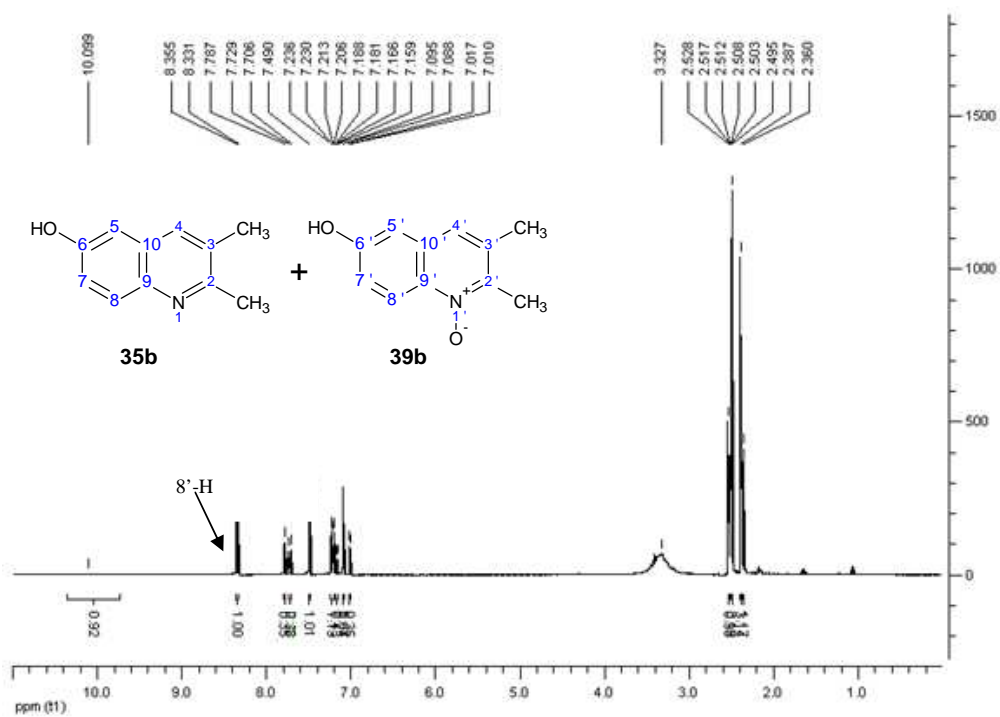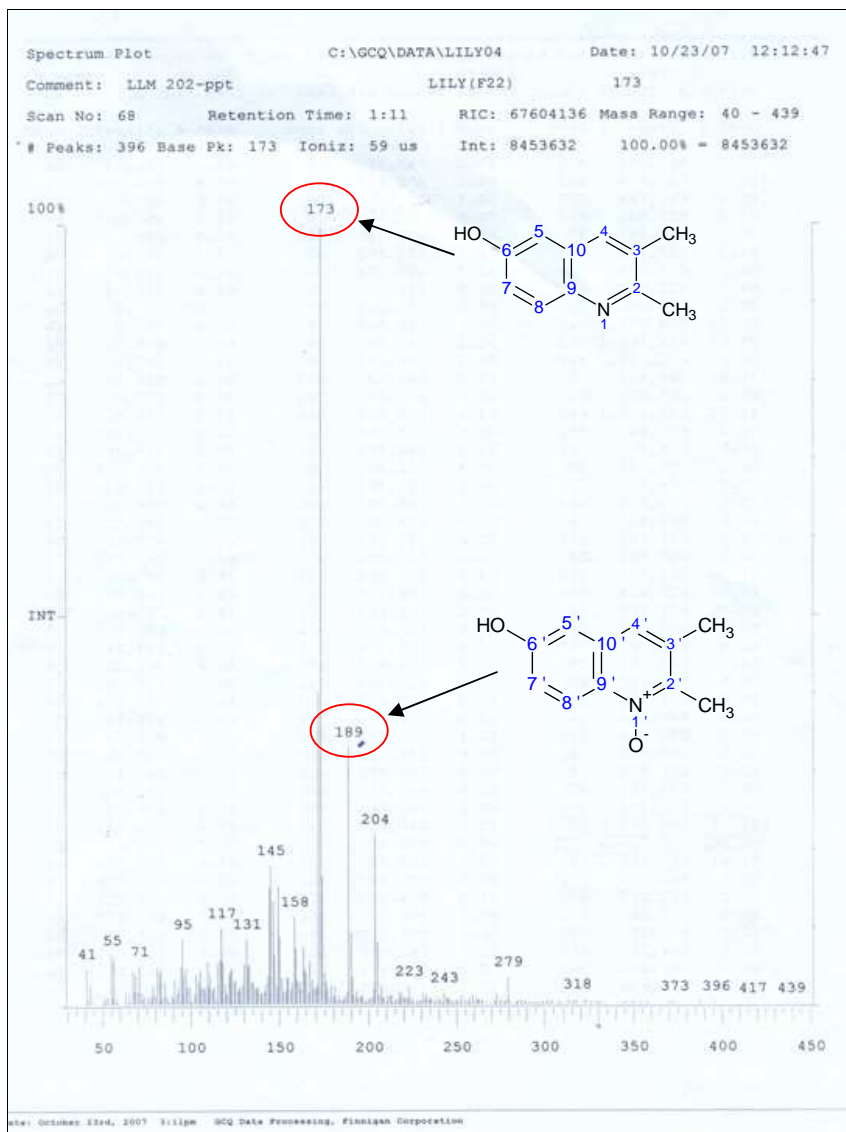

Figure 18: 400MHz ^{1}H NMR spectrum of the isolated solid, comprising a mixture of 6-hydroxy-2,3-dimethylquinoline **35b** and 6-hydroxy-2,3-dimethylquinoline-*N*-oxide **39b**, in DMSO-d_6.

The ^{1}H NMR spectrum of the solid showed the presence of two products with overlapping signals in the aromatic region, while the ^{13}C NMR spectrum gave a much clearer picture. In the latter spectrum (Fig. 19), for example, the signal at 108.3 ppm is accompanied by a adjacent but less intense signal at 107.4 ppm. This phenomenon is evident across the spectrum with each major signal being accompanied by a minor signal corresponding to the major and minor products. Isolation of the two products was not considered practicable, since TLC analysis showed the presence of only a single spot and attempts to separate the two products by changing the elution solvent were unsuccessful. It was concluded that the two products comprising the solid must have similar polarity, the two products were presumed to be 6-hydroxy-2,3-dimethylquinoline **35b** and the corresponding quinoline *N*-oxide **39b**. This assumption was supported by low resolution MS analysis which indicated the presence of molecular ions for both **35b** (*m*/z 173 g.mol^{-1}, Fig. 20) and **39b** (*m*/z 189g.mol^{-1}).

Figure 19: 100MHz ^{13}C NMR spectrum of the isolated solid, comprising a mixture of 6-hydroxy-2,3-dimethylquinoline **35b** and 6-hydroxy-2,3-dimethylquinoline-*N*-oxide **39b**, in DMSO-*d${}_6$*.

35

Figure 20: GCQ low resolution mass spectrum of the isolated solid, comprising a mixture of 6-hydroxy-2,3-dimethylquinoline **35b** and 6-hydroxy-2,3-dimethylquinoline-*N*-oxide **39b**.

It was previously observed [52, 53] that the 2,3-dimethylquinoline derivatives **35** and **39** were not the only products obtained in this reaction, but that the "acyclic" derivatives **40** could also be isolated. This was reported by Klaas[53] and Pakade[52]. The formation of *N*-oxide **39** is attributed to the incomplete reduction prior to cyclization. Klaas[53] and Pakade[52], working in our group had shown that the *N*-oxides isolated in these reactions can be readily reduced to the corresponding quinolines using phosphorus tribromide in DMF.

The hydrogenation reaction proved to be less straightforward than expected, since the reaction produced more than one product, and therefore an alternative method was examined. Basavaiah *et al.* [67] reported a one-pot reaction for the synthesis of quinolines from Baylis-Hillman products by making use of Fe powder and acetic acid. Applying this method to substrate **26c**, the reaction mixture was refluxed for 30 minutes to afford the quinoline derivative **44c**, as a white solid in 20% isolated yield. The reaction sequence is outlined in Scheme 13. Thus, the nitro compound **26c** was reduced to the aniline derivative **41c** before cyclization to intermediate **42c**; acetic acid then attacked the methylene carbon in an acid-catalysted S_N1 process (illustrated by **43b**) to generate quinoline product **44c**.
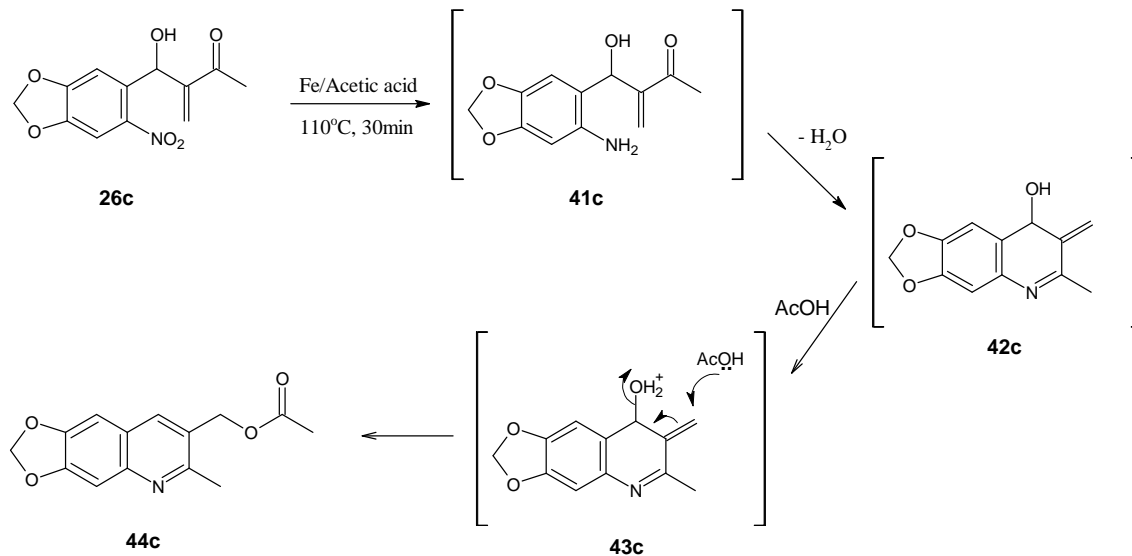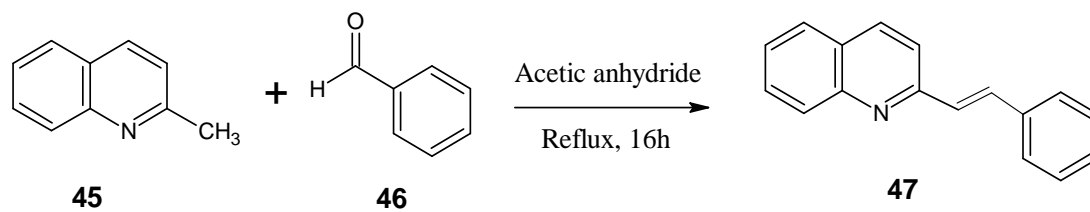

Scheme 13

The ^{1}H NMR spectrum of compound **44c** (Fig. 21) indicated there were two methyl signals at 2.11 and 2.65 ppm, two methylene signals at 5.20 and 6.07 ppm, and three aromatic proton signals (7-8 ppm). This clearly indicated that the 3-acetoxymethyl-2-methyl-6,7-(methylenedioxy)quinoline **44c** had been formed. Removal of the acetyl group can be achieved by methanolysis using K_2CO_3 in methanol.[67]
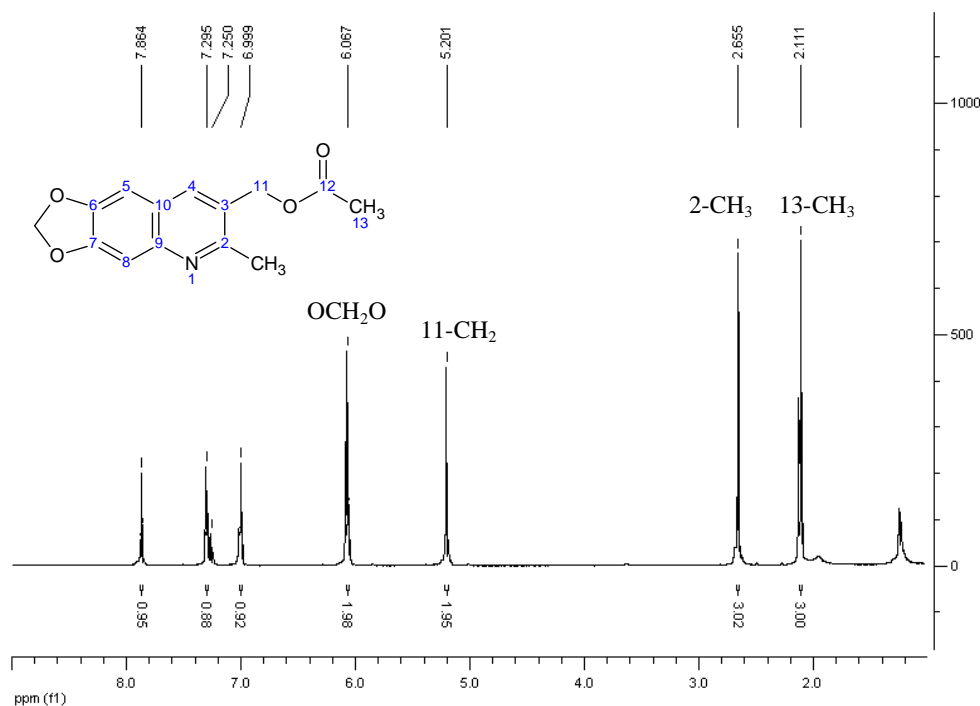
Figure 21: 400MHz ^{1}H NMR spectrum of 3-acetoxymethyl-2-methyl-6,7-(methylenedioxy)-quinoline **44c** in CDCl$_3$.

2.3   Perkin-type condensation of quinolines to styrylquinolines

The final step in the synthesis of styrylquinoline compounds as potential HIV-1 integrase inhibitors was the Perkin-type condensation reaction, the mechanism of which is outlined in Scheme 4 (p.21, Section 1.1.3). A trial reaction was carried out using quinaldine (2-methylquinoline) **45**, as a model system, and benzaldehyde **46** (Scheme 14) in the presence of acetic anhydride, which acted as both a catalyst (see Scheme 2, p.19) and a solvent. The mixture was refluxed for 16 hours. A black crystal, which slowly formed during the reaction, was removed and recrystalized from chloroform-hexane to afford (*E*)-2-styrylquinoline **47** as a yellow powder in 30% isolated yield. The ^{1}H NMR spectrum of compound **47** (Fig. 22B) showed no evidence of the methyl signal at 2.70 ppm which is characteristic of the quinaldine spectrum (Fig. 22A). The spectrum of 2-styrylquinoline **47** was expanded to facilitate assignment of all the expected (Fig. 23).

38

Scheme 14
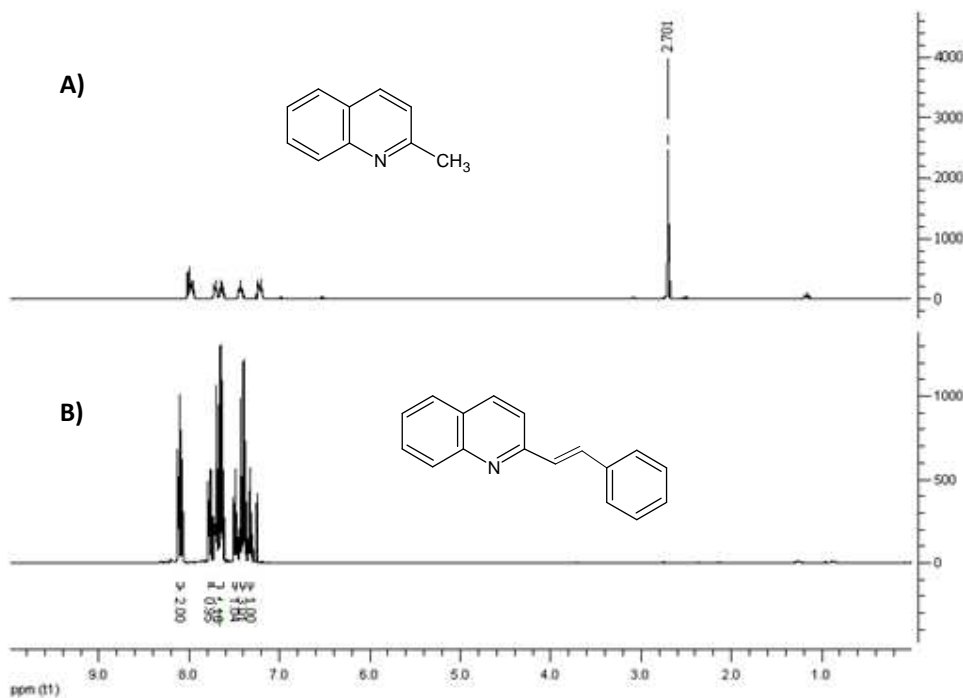
Figure 22: 400MHz ^{1}H NMR spectrum of A) quinaldine **45** and B) (*E*)-2-styrylquinoline **47** in CDCl$_3$.

The Perkin-type condensation is an effective method for the synthesis of styryl-like compounds. However, the disadvantages of this method are the long reaction time (ranging from 12 hours to 4 days), low yield and excess of reagent (benzaldehyde) required.[52, 53, 65, 66, 68-70] Microwave assisted organic synthesis (MAOS) is an alternative to the traditional approach. Musiol and his colleagues[64-66] were able to synthesize styrylquinoline using a microwave-assisted reaction in which the substituted quinaldine and aldehyde derivatives were irradiated in a microwave oven for 4 to 6 minutes at different power levels and temperatures, which

were determined based on the reagents used. This method apparently solved the problems experienced using normal reflux.
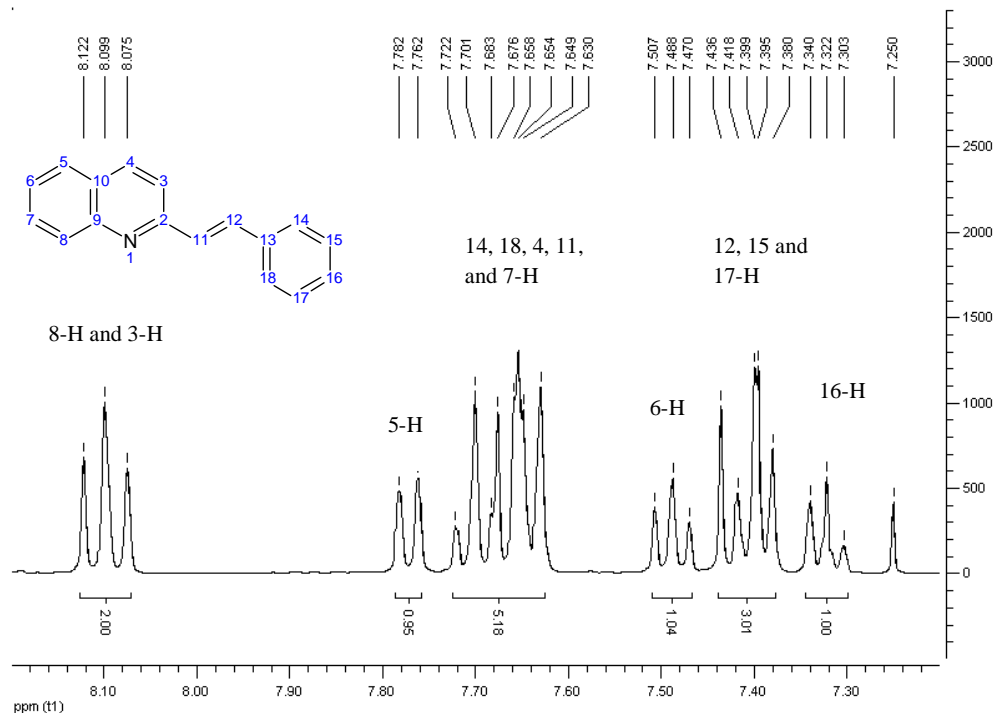

Figure 23: 100MHz ^{13}C NMR spectrum of (*E*)-2-styrylquinoline **47** in CDCl$_3$, expanded to facilitate assignment.

The same reagents detailed in Scheme 14 were used for the microwave reaction. Acetic anhydride was mixed with the reactants **45** and **46** in a reaction vessel, which was inserted into the microwave reactor and the mixture subjected to the reaction conditions listed in Table 3. The results showed a much higher yield (60-80%) and TLC analysis of the mixtures from all three reactions indicated the absence of competition product. Optimization of the microwave reaction, including power and temperature settings, and its application in the preparation of the styrylquinolines targeted in the present study will be the focus of future work.

Table 3: Microwave reaction conditions for the reaction of compounds **45** and **46**.

	Power (W)	Temperature (°C)	Time (min)
1)	150	120	5
2)	200	150	7
3)	300	178	5

The synthesis of quinoline derivatives was not as simple as expected, therefore alternative pathways to the styrylquinoline were considered, including condensation of Baylis-Hillman products **26a** (R=H) and **26c** (R=4,5-OCH$_2$O-) with benzaldehyde **46** to afford **48a,c** followed by cyclization to afford styrylquinoline derivatives **49a,c** (Scheme 15). This reaction was conducted using both the conventional thermal reaction system and a microwave-assisted reaction. However, the using both approaches were disappointing with formation of none of the desired product **49a,c**.

i) Acetic anhydride, 16h, 150°C
ii) Microwave irradiation, 150W, 150KPa, 10min.

Scheme 15

41
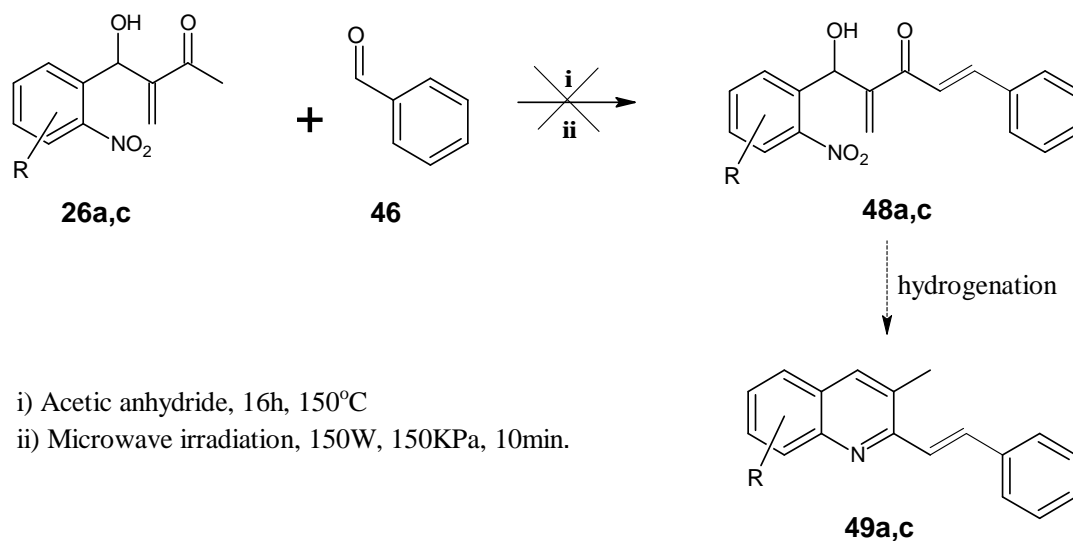
## 2.4   Substitution reactions of Baylis-Hillman products

The vinylic group in the 2-nitrophenyl Baylis-Hillman products **26** may undergo two different reactions, *viz*., conjugate addition or nuclecophilic substitution with rearrangement (S_N') (Scheme 16) depending on the R^2 group. When R^2 was methyl, the substrate **26** was first reacted with HCl. The chlorinated S_N1 product **52** could then be reacted with nitrogen nucleophiles, *via* direct S_N' reaction, and then cyclised to give quinoline derivatives **54**. With the ester (R^2=OMe), however, conjugate addition by nitrogen nucleophilies, followed by dehydration afforded the corresponding prodcuts **51**.
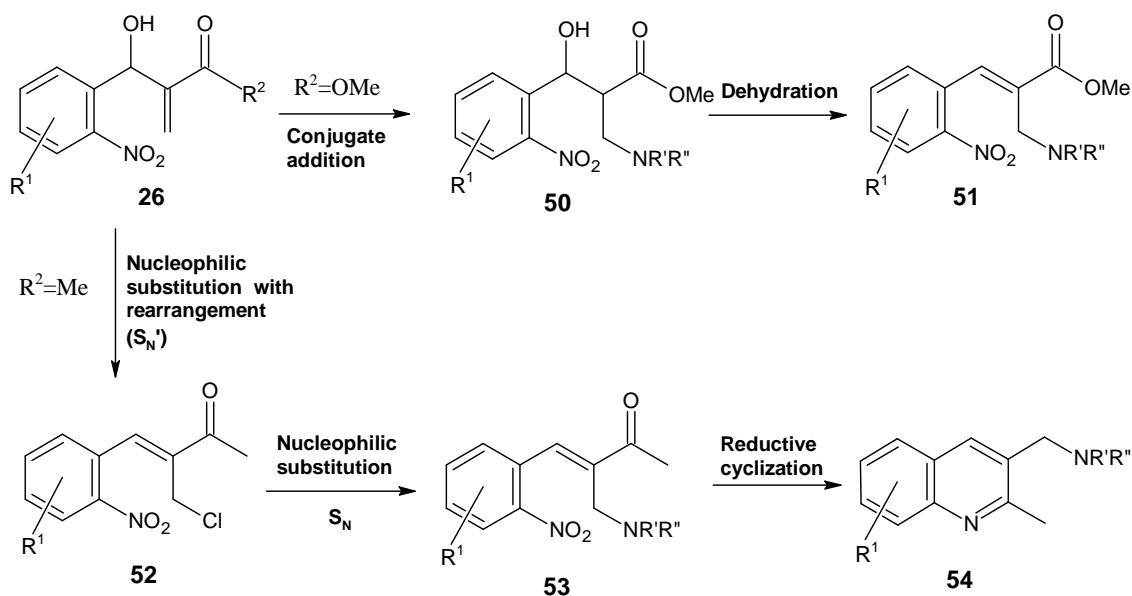
Scheme 16

Thus, treatment of the Baylis-Hillman products **26b,c,f** with HCl (generated from acetyl chloride in ethanol, Scheme 17), which attacked the vinylic carbon, gave 3-chloromethyl-4-(2-nitrophenyl)but-3-en-2-one **52** (Scheme 17). This is an exothermic reaction and, therefore, the reaction vessel was placed on ice to avoid overheating. The reaction vessel was open to the atmosphere, with a condenser attached to prevent loss of the mixture in the event of over-heating. The chloromethyl ketones **52b,c,f** were isolated in extremely high yield. 3-Chloromethyl-4-(4,5-methylene-2-nitrophenyl)but-3-en-2-one **52c** was obtained as a clean

product without any purification, in quantitative yield (Table 4). The vinylic proton signals characteristic of the substrate **26c** were absent in the ^{1}H NMR spectrum of the product **52c** (Fig. 24), confirming the transformation. However, the HRMS analyses with ESI (electrospray ionization) and EI (electron ionization) has failed to detect the molecular ion of the products **52b,c,f**. This warrants further exploration.
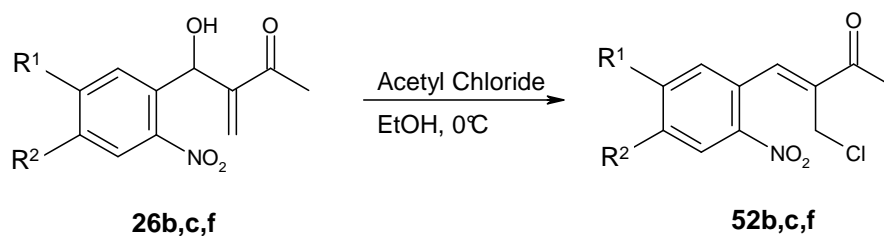
Scheme 17

Table 4: Chlorinated Baylis-Hillman products **52b,c,f** .

|	R^1	R^2	Yield (%)
52b	OH	H	94
52c	OCH$_2$O		100
52f	Cl	H	94
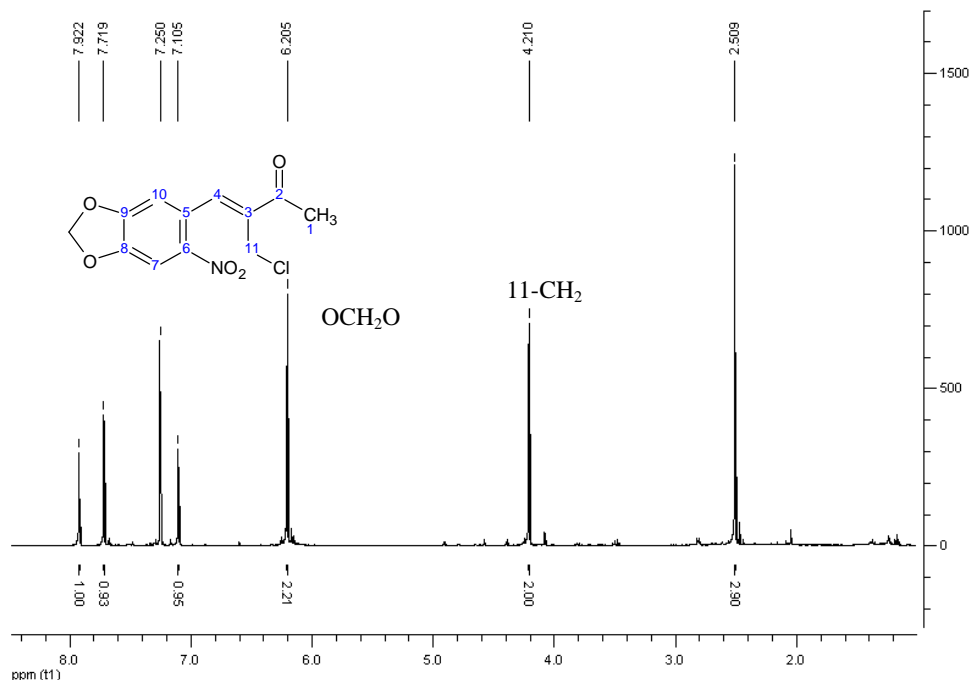
Figure 24: 400MHz ^{1}H NMR spectrum of 3-chloromethyl-4-(4,5-methylene-2-nitrophenyl)but-3-en-2-one **52c** in CDCl$_3$.

2.5   Conjugate addition reactions of Baylis-Hillman products with amine nucleophiles

The nucleophilic addition of primary or secondary amines to the Baylis-Hillman products may be achieved *via* two different routes (Scheme 18). When R^2 is methyl, direct addition of an amine to the Baylis-Hillman products **26** is not possible,[55] and must be preceded by substitution with chloride, as described above. Direct conjugate addition of the nucleophile to the vinylic β-carbon is only possible when R^2 is an OMe. The primary and secondary amines selected for the methyl ester Baylis-Hillman products **26g-k** were piperidine **56**, piperazine **60** and L-proline methyl ester hydrochloride **57**, as shown in Scheme 18 and 19. The reactions with piperidine and piperazine were carried in THF, whereas methanol (containing triethylamine to neutralise the hydrochloride salt) was used as the solvent for the L-proline methyl ester hydrochloride reaction. The reaction mixtures were stirred for 3 to 7 days to give, as diastereoisomeric mixtures, the products listed in Table 5.
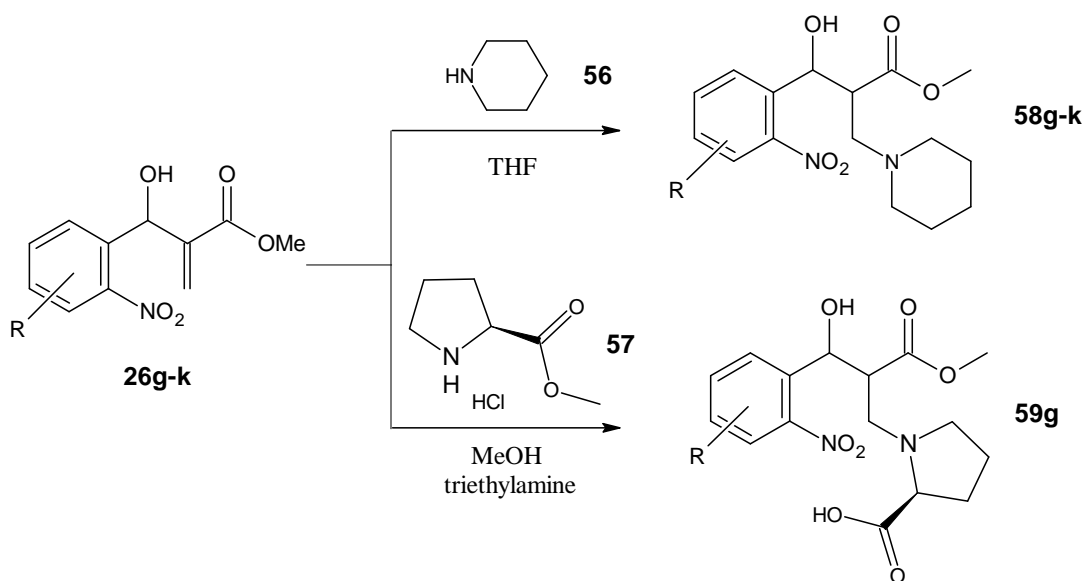
Scheme 18

Table 5: Percentage yields of the compounds **58g-59g** (Scheme 18).

|	**R**	**58**	**59**
g	H	74	54[a]
h	3-OMe	76	—[b]
i	6-Cl	61	—[b]
j	4,5-OCH$_2$O-	95	—[b]
k	4-NO$_2$	57	—[b]

[a] As a mixture of four diastereomers.
[b] Reaction not performed.

In the NMR spectra of the diastereoisomeric mixture **58g – 59g**, the 3-methine signals at *ca.* 5 – 6 ppm were diagnostic of the desired products - a separate signal for each diastereomer. Integration of these signals permitted the ratio of two diastereomers to be determined in each case. In the ^{1}H NMR spectrum of **58g** (Fig. 25), the two doublets at 5.30 and 5.79 ppm exhibited coupling constants of 8.8Hz and 4.0Hz, for the major and minor diastereomers, respectively, which their relative integrals indicated a diastereomeric ratio of 2:1. In the ^{13}C NMR spectrum (Fig. 26) the total number of carbon signals was almost double the total

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number of carbons expected for either diastereomer and almost every major signal was accompanied by a minor signal. The DEPT 135 spectrum (Fig. 27) showed four pairs of methine signals which corresponds to the four aromatic methine carbons and one pair of methylene signals corresponding to C-10. The C-11/15 and C-12/14 methylene signals for each diastereomeric pair overlap at 26.3 and 55.1 ppm, respectively. The 10-methylene carbon signals, however, are well separated.
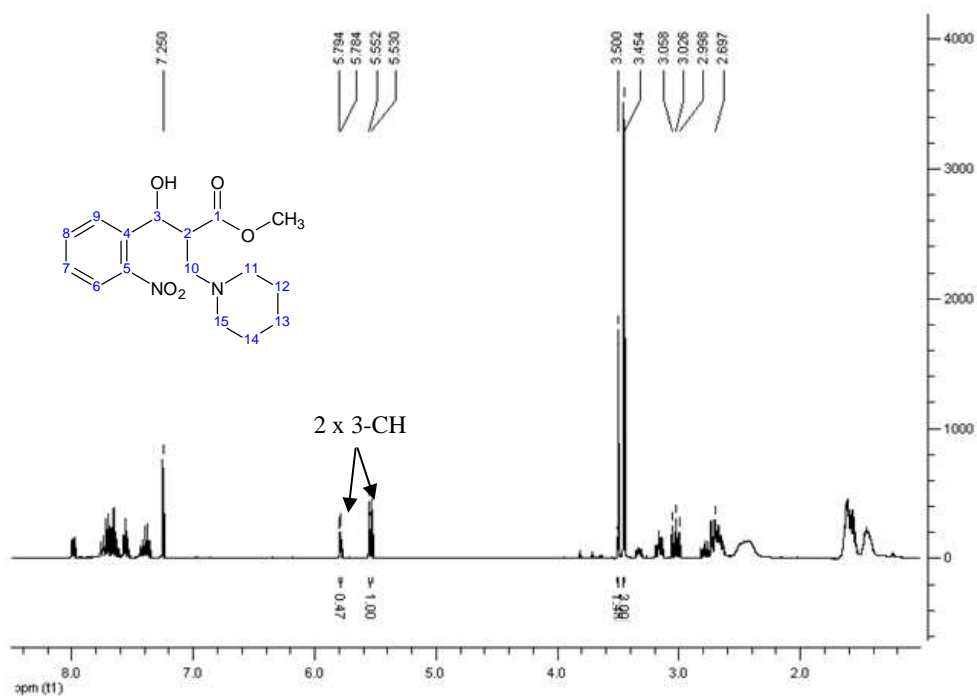
Figure 25: 400MHz ^{1}H NMR spectrum of a mixture of diastereomers of methyl 3-hydroxy-3-(2-nitrophenyl)-2-[(piperidin-1-yl)methyl]propanoate **58g** in CDCl$_3$.

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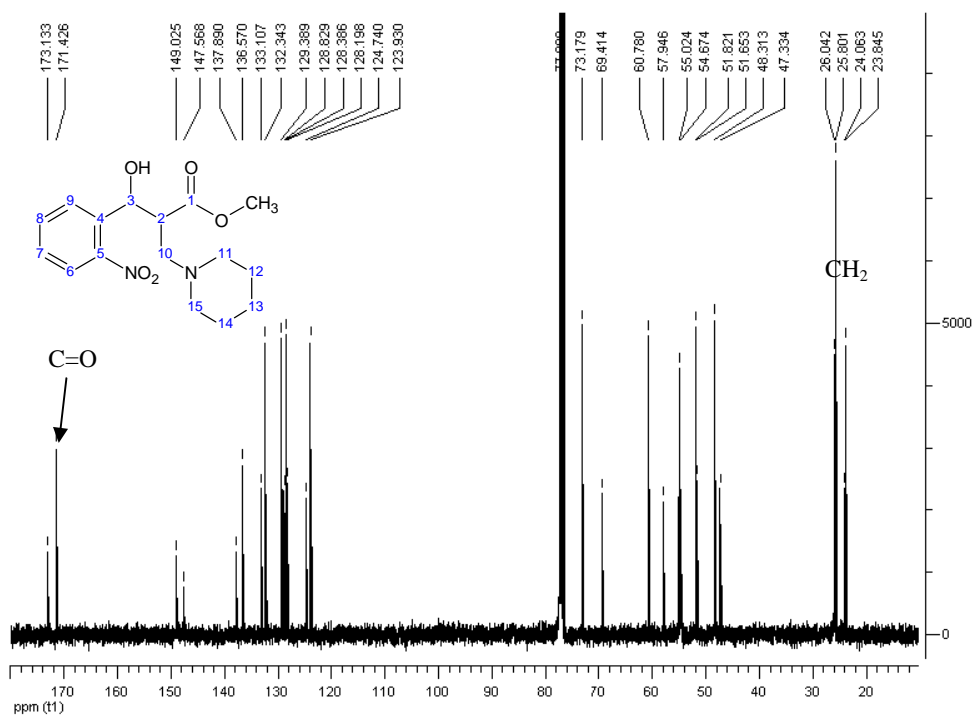
Figure 26: 100MHz ^{13}C NMR spectrum of a mixture of diastereomers of methyl 3-hydroxy-3-(2-nitrophenyl)-2-[(piperidin-1-yl)methyl]propanoate **58g** in CDCl$_3$.
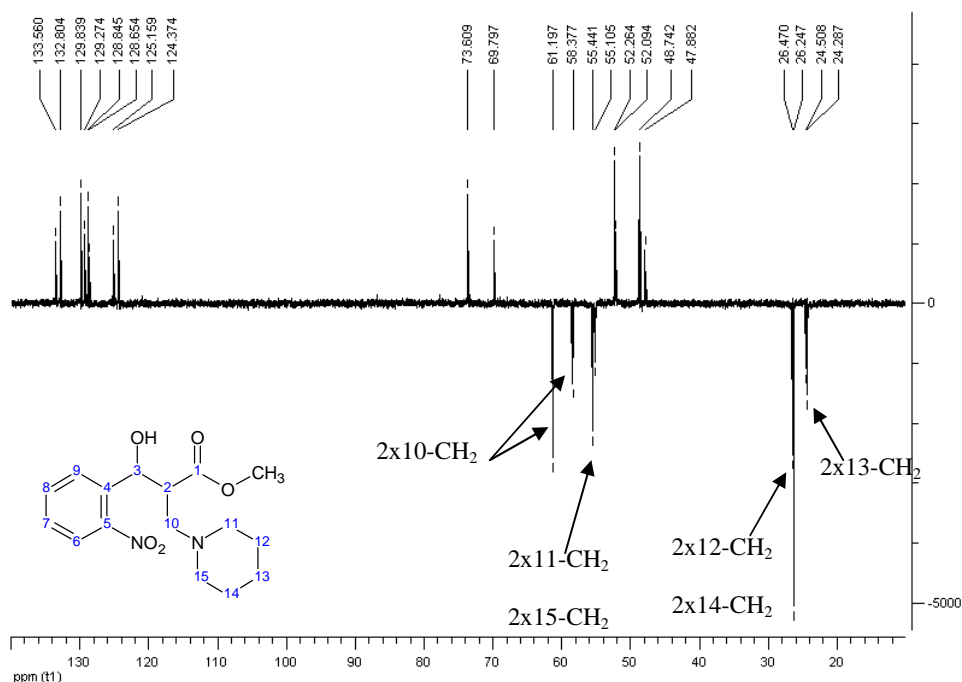
Figure 27: 100MHz DEPT 135 NMR spectrum of a mixture of diastereomers of methyl 3-hydroxy-3-(2-nitrophenyl)-2-[(piperidin-1-yl)methyl]propanoate **58g** in CDCl$_3$.

The piperidine derivatives **61g-k** were obtained in moderate to good yields, but the piperazine reactions had an unwanted outcome (see Table 6). Reaction of 1eq. of the Baylis-Hillman adducts **26g-k** with 1.5eq. of piperazine **60** was expected to form the mono-substituted methyl piperizinyl-propanoate esters **61g-k**. The ^{1}H NMR spectrum (Fig. 28), while indicating the presence of a contaminant, appeared to be compatible with such a conclusion. However, the results from HRMS indicated otherwise. The MS spectrum (Fig. 29) contains a peak at *m*/z 324 which corresponds to M+1 for the mono-substituted products **61g** (R=H) as well as a base peak at *m*/z 560 which corresponds to the disubstituted piperazine **62g** (Scheme 19). It was unclear whether both of the products were present or only the disubstituted **62g**, and a DOSY (*D*iffusion-*O*rdered *S*pectroscopy) experiment was run to confirm the results.
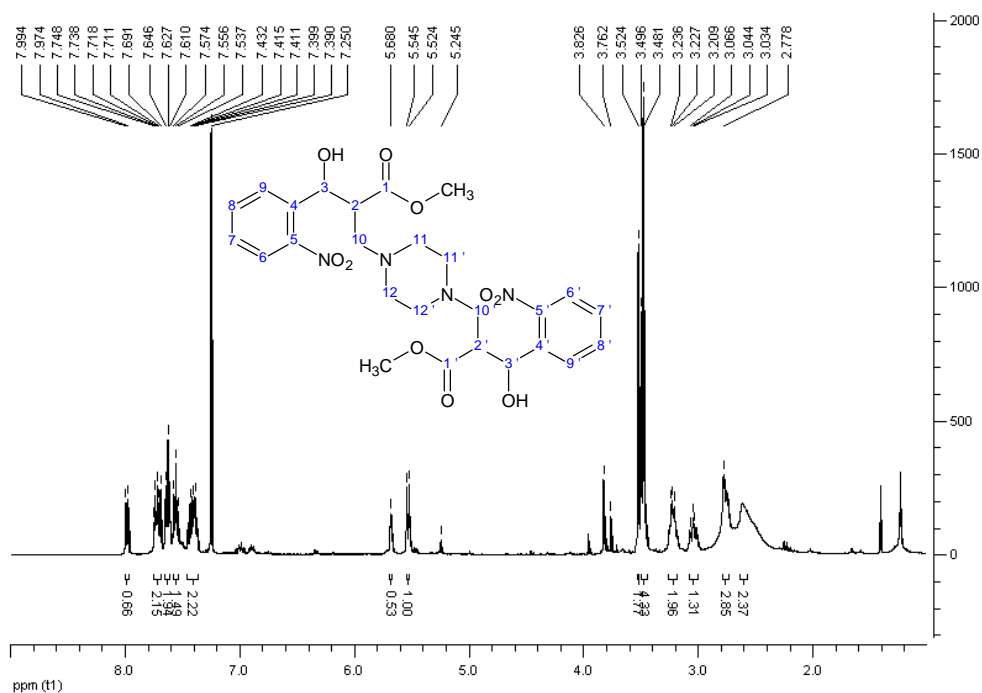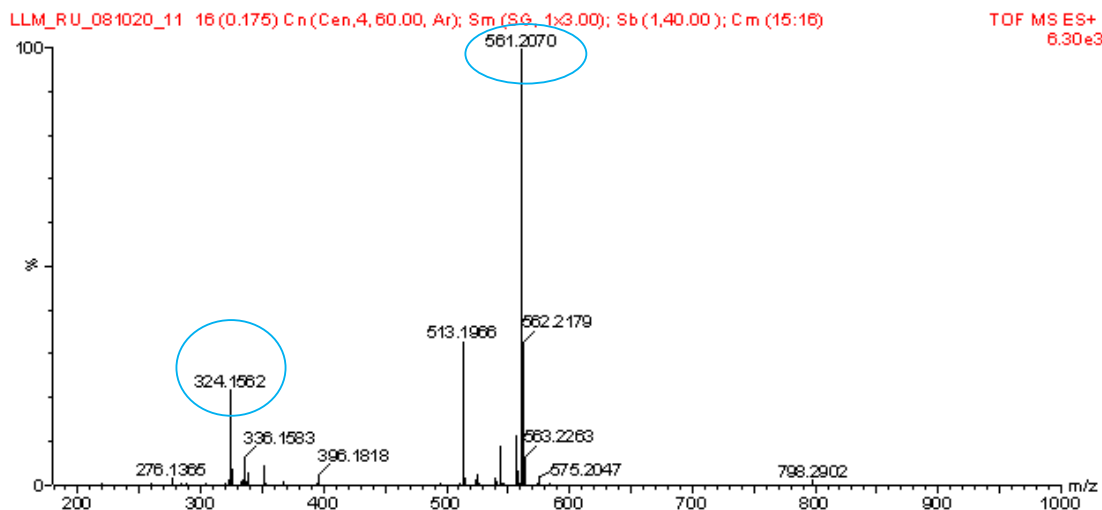

Scheme 19

48
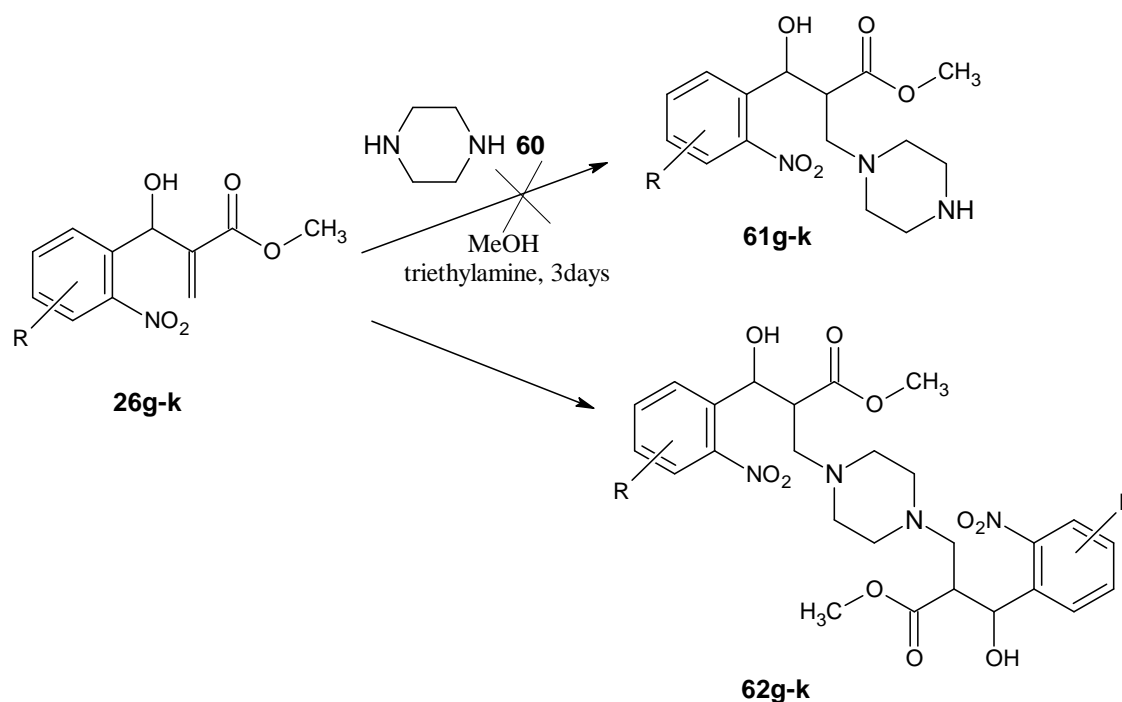
Table 6: Percentage yield of compounds **62g-k** (Scheme 19).

	R	62
g	H	24
h	3-OMe	4.3
i	6-Cl	4.3
j	4,5-OCH$_2$O-	6
k	4-NO$_2$	—— [a]

[a] The product was not found in any of the fractions collected.
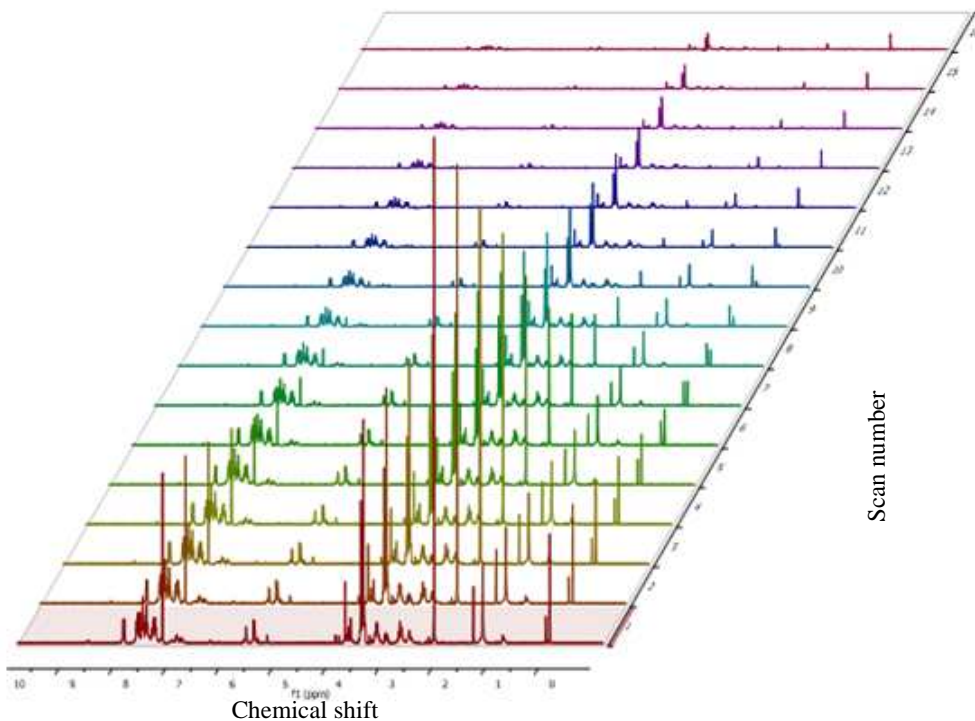

Figure 28: 400MHz ^{1}H NMR spectrum of *N,N'*-bis[2-carbomethoxy-3-hydroxy-3-(2-nitrophenyl)]piperazine **62g** in CDCl$_3$.
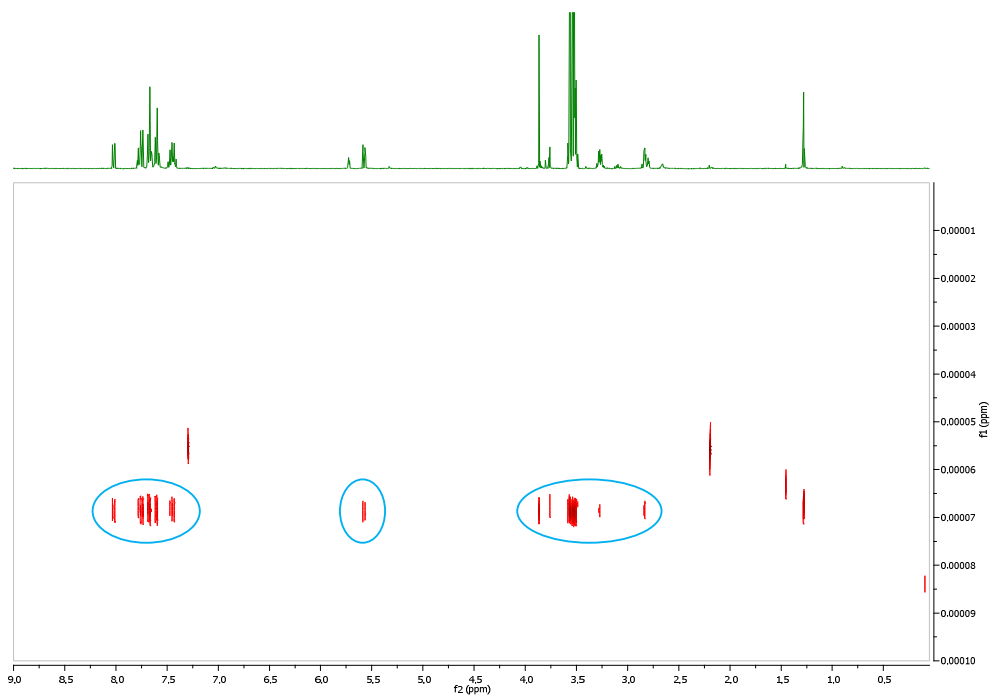
Figure 29: HRMS spectrum of *N,N'*-bis[2-carbomethoxy-3-hydroxy-3-(2-nitrophenyl)]-piperazine **62g** using electrospray ionization plus source.

The DOSY experiment is a pseudo-chromatographic 2-D NMR experiment where the pulsed field gradient (PFG) and the deuterated solvent serve as the "mobile phase". The separation occurs when two or more compounds in a mixture show a difference in size and shape, which affects their mobility and their diffusion coefficients.[71-74] Figure 30 is the original data from the DOSY experiment, where the ^{1}H NMR spectrum of **61g** was run over number of scans (y-axis) with increments in the field gradient intensity. The 2-D DOSY spectrum (Fig. 31) indicated that the solute was a single product and, not a mixture of mono- and di-substituted piperazines. All the signal peaks in the blue circles in the spectrum have the same diffusion coefficients; the signals outside the blue circles correspond to the deuterated chloroform and impurities in the CDCl₃. The DOSY experiment was only conducted on **62g** and **62j** and, in both cases, the results indicated the formation of the bis-substituted piperazines. The HRMS data for the products of the reaction of piperazine with Baylis-Hillman adducts **26g-i**, which were all obtained in very low yield (≤6%), indicated the presence of the corresponding disubstituted piperazines **62h, 62i,** and **62k**.

Figure 30: DOSY experiment scans of *N,N'*-bis[2-carbomethoxy-3-hydroxy-3-(2-nitrophenyl)]piperazine **62g** with chemical shift on x-axis and scan number on the y-axis.


Figure 31: 2-D DOSY spectrum of *N,N'*-bis[2-carbomethoxy-3-hydroxy-3-(2-nitrophenyl)]piperazine **62g** in CDCl₃.

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In the conjugate addition of L-proline methyl ester hydrochloride to Baylis-Hillman derivative **26g**, a base was needed to free the nucleophilic amino group from the hydrochloride salt. Two different bases were examined, proton sponge and triethylamine. The reaction with proton sponge was deemed unsuccessful after stirring for 14 days. However, chromatography of the crude product (with triethylamine in the mobile phase), revealed the presence of four stereoisomers (**59g$_{1-4}$**), which were characterized using NMR spectroscopy and elemental analysis.

The chlorinated Baylis-Hillman products **52b,c,f** were also treated with various amines (**56, 57, 63,** and **64**) as illustrated in Scheme 20. The reactions were conducted in either THF or methanol (with triethylamine to neutralise the hydrochloride salts). The reactions were terminated after 3-7 days and the crude products chromatographed to afford the expected products in moderate to excellent yields (Table 7). In these reactions, it seems that direct (S$_N$) substitution proceeds smoothly and in the ^{1}H NMR spectrum of compound **65b**, for example, (Fig. 32) the allylic methylene signal appears as a singlet at 3.09 ppm; the vinylic 4-methine proton was the most deshielded giving rise to a signal at 7.76 ppm.
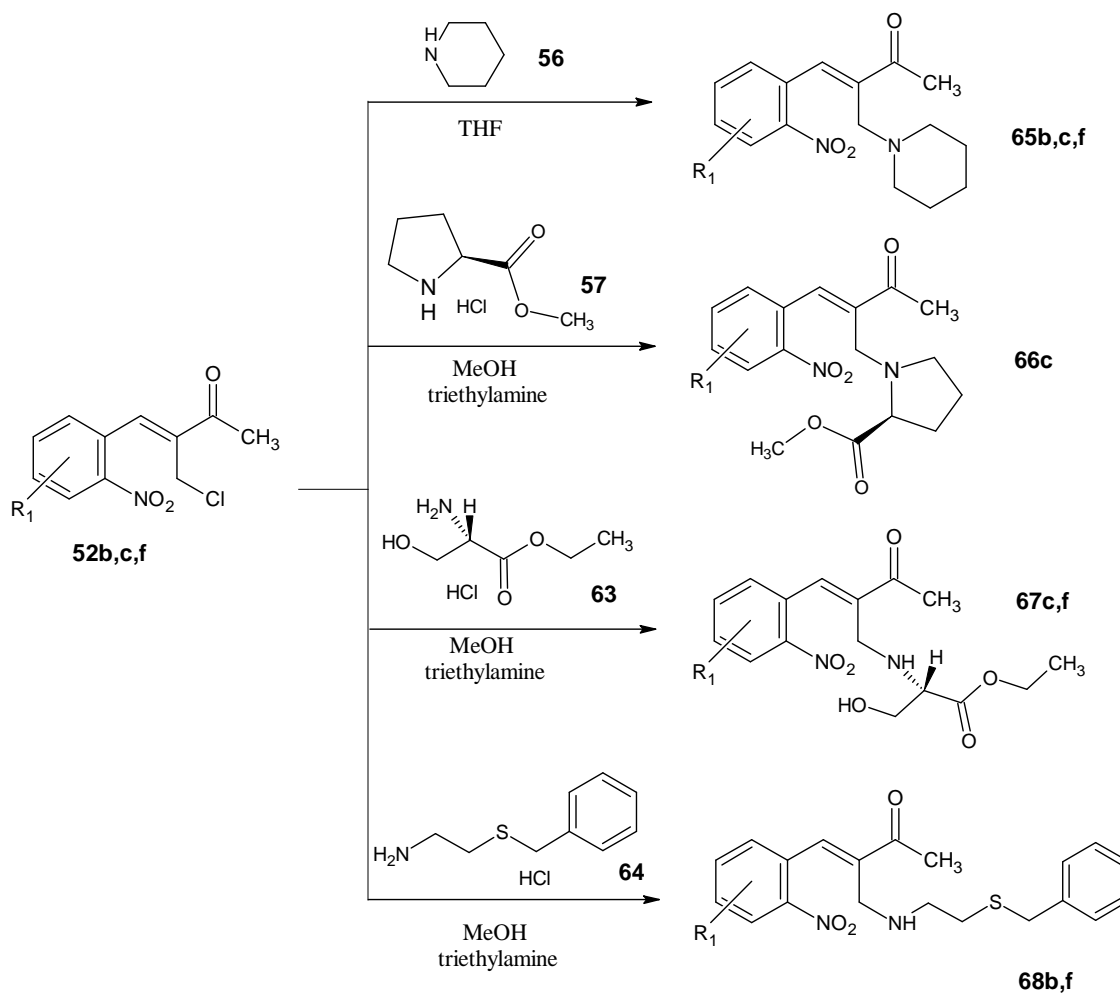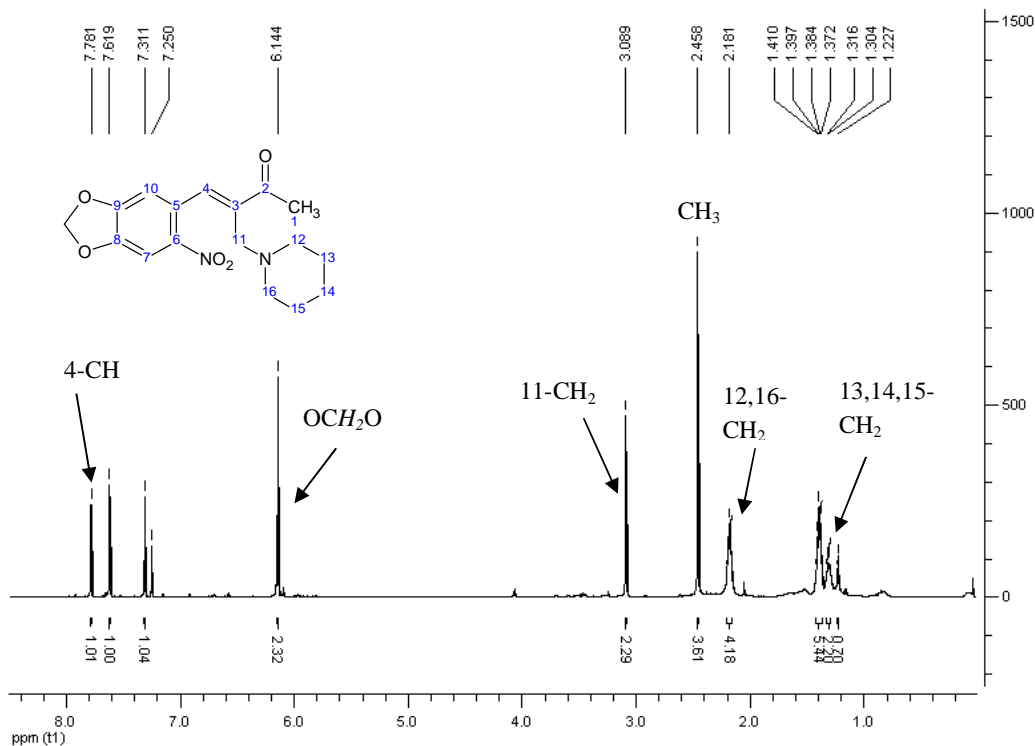
Scheme 20

Table 7: Yields of the nucleophilic substitution products **65**-**68.**

|	R	65	66	67	68
b	5-OH	79	—^{a}	—^{a}	20
c	4,5-OCH$_2$O-	86	57	52	—^{a}
f	5-Cl	90	—^{a}	36	—^{b}

^{a} Reaction not performed.
^{b} Reaction attempted but no product obtained after chromatography.

Figure 32: 400MHz ^{1}H NMR spectrum of 4-(4,5-methylenedioxy-2-nitrophenyl)-3[(piperidin-1-yl)methyl]but-3-en-2-one **65c** in CDCl$_3$.

2.6 Reductive cyclization of Baylis-Hillman products to quinoline derivatives

The products **65c-67c**, derived from BH adducts **26c**, were treated with 10% Pd-C and H$_2$ for 1.5 hours in ethanol afford 2-methylquinoline derivatives **69c-71c.** 2-Methyl-6,7-methylenedioxy-3-[(piperidin-1-yl)methyl]quinoline **69c** was isolated in 12% yield, after purification, while products **70c-71c** were obtained in crude yields of 18-23% (Scheme 21). The most deshielded signal in the ^{1}H NMR spectrum of **65c** (Fig. 32) at 7.78 ppm corresponds to the 4-H nucleus whereas in the spectrum of **69c** it is the 8-H nucleus at 8.08 ppm (Fig. 33). In the ^{13}C NMR spectrum (Fig. 34), the carbonyl signal at 199.9 ppm (in the spectrum of the substrate **65c**) has disappeared, indicating that the cyclization has occurred. However, for the synthesis of **70c** and **71c**, both reactions gives a complex mixture and no purification were done due to time constraints.
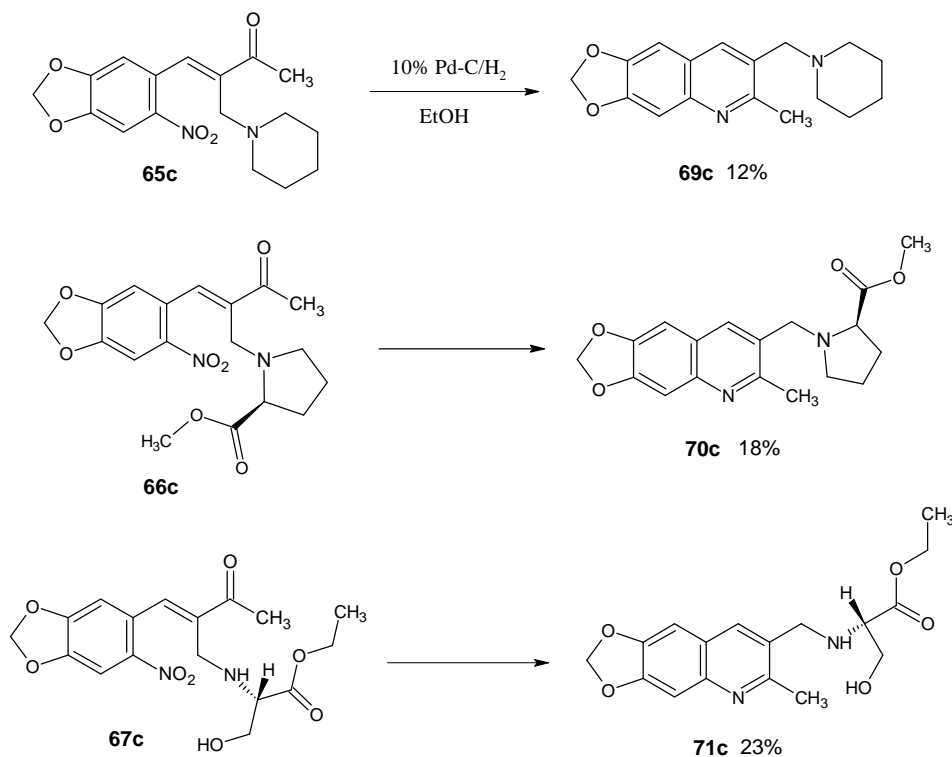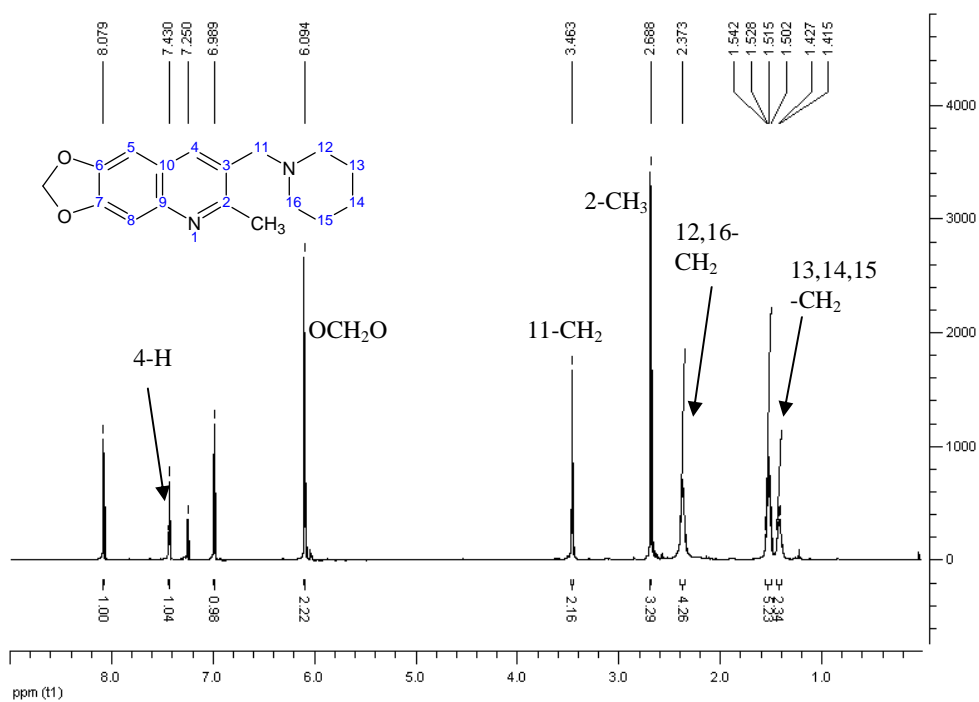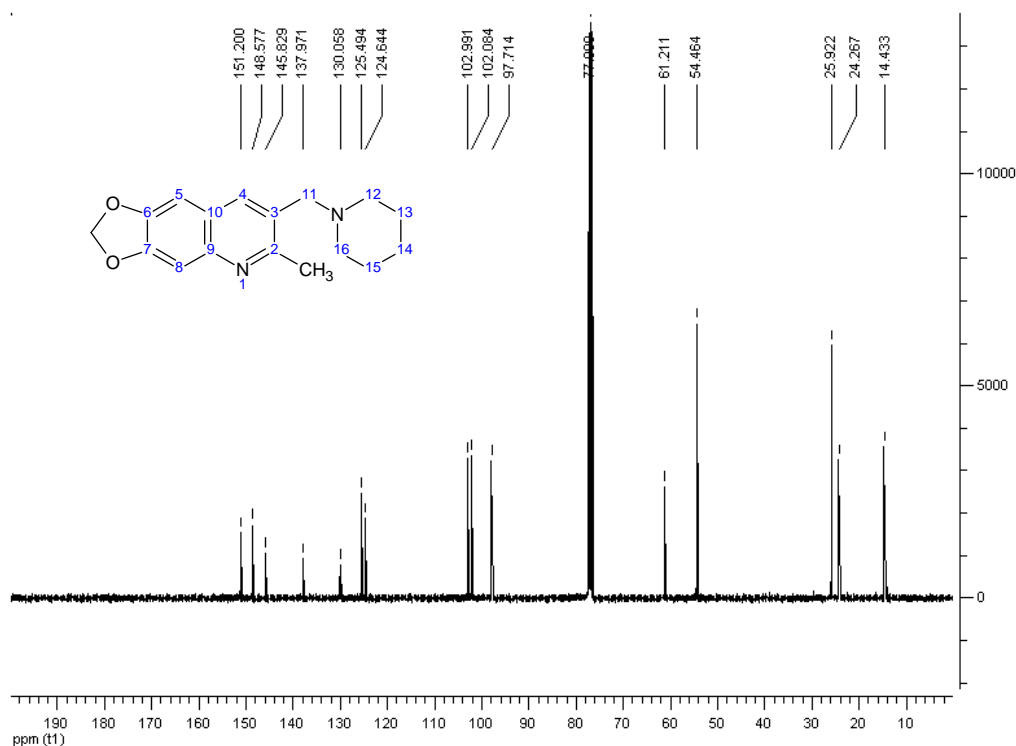
Scheme 21

Figure 33: 400MHz ^{1}H NMR spectrum of 2-methyl-6,7-methylenedioxy-3-[(piperidin-1-yl)methyl] quinoline **69c** in CDCl$_3$.
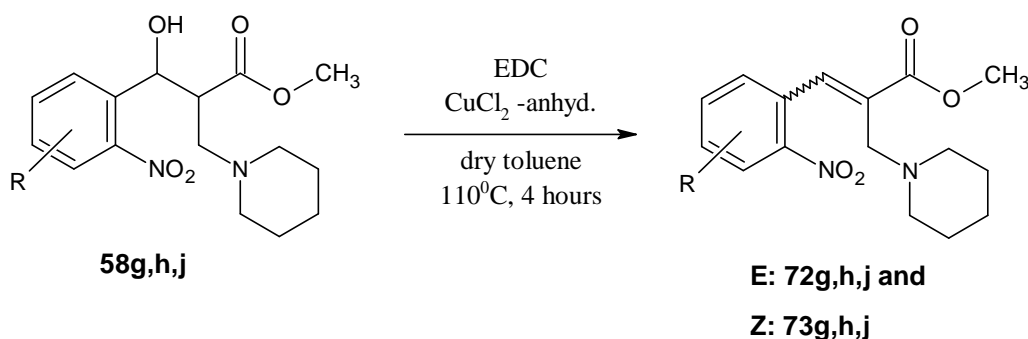
Figure 34: 100MHz ^{13}C NMR spectrum of 2-methyl-6,7-methylenedioxy-3-[(piperidin-1-yl)methyl] quinoline **69c** in CDCl$_3$.

2.7   Dehydration of Baylis-Hillman products to afford cinnamate esters

The second approach to potential HIV-1 integrase inhibitor was explored, namely, the synthesis of cinnamate esters from BH derivatives. Sai *et al.* [75] have published a paper on a stereoselective method to synthesise (*E*)- and (*Z*)-α-substituted cinnamates using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) as a dehydrating agent. The reaction proceeds *via* an E$_2$ elimination reaction of *syn*- and *anti*-α-substituted-β-hydroxyesters. They reported that *syn*-α-substituted-β-hydroxyesters will undergo E2 elimination reaction to give a mixture of (*E*)- and (*Z*)-α-substituted cinnamates whereas *anti*-α-substituted-β-hydroxyesters react stereoselectively to give, (*E*)-α-substituted cinnamates *(Z:E*, 1:99).[75] A possible explanation for this phenomenon is that the α-proton of the β-hydroxyester is too acidic for E$_2$ elimination to compete with E1cB elimination. The most effective reaction conditions, concluded Shi *et al.*, involved 1eq. of β-hydroxyester, 2eq. of EDC and 0.1eq. of CuCl$_2$ reacting in refluxing toluene for 2 hours. Applying this method, dehydration of the methyl 3-hydroxy-3-(2-

nitrophenyl)-2-[(piperidin-1-yl)methyl]propanoate esters **58g,h,j** was effected by refluxing in toluene with EDC for 4 hours to afford the diastereoisomeric mixture (*E*)-**72** and (*Z*)-**73** in 8-50% yield (Scheme 22, Table 8). In the ^{1}H NMR spectrum of **72g** and **73g** (Fig. 33) the signals corresponding to 3-vinylic proton at 5.53 and 5.77 ppm and 2-methine proton at 3.16 and 3.31 ppm have disappeared and new 3-vinylic proton signals appeared at 7.26 and 7.97 ppm. In the reaction of **58g,h,j** with EDC the (*E*)-cinnamate esters **72** are the main products (**72g**:**73g** :: 1:2,**72h**:**73h** :: 1:7, and **72j**:**73j** :: 1:4).


58g,h,j → EDC, CuCl$_2$ -anhyd., dry toluene, 110^{0}C, 4 hours → **E: 72g,h,j and** **Z: 73g,h,j**

Scheme 22

Table 8: Yields of dehydrated diastereomeric compounds **72-73g,h,j** (Scheme 22).

	R=	% Yields
g	H	50
h	2-OMe	16
j	4,5-OCH$_2$O-	8

The mechanism for the dehydration reaction of the *syn-* and *anti-*α-substituted-β-hydroxyesters (***anti-*74** and ***syn-*75**) is outlined in Scheme 23. Formation of intermediates **75i and 75ii** result from reacting EDC and CuCl$_2$ with ***anti-*74** and ***syn-*75**. The dimethyl amino group in EDC facilitates the deprotonation step. The intermediates **75i and 75ii** eliminate EDC to give the products (*E*)-**72** and (*Z*)-**72**, respectively.

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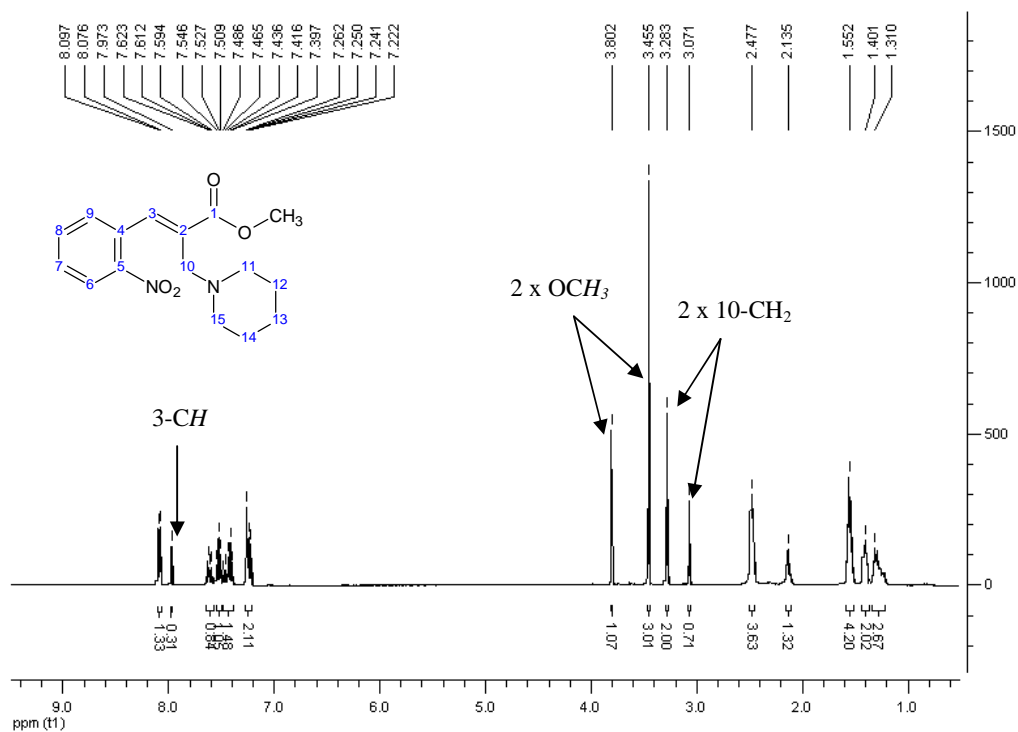
Figure 35: 400MHz ^{1}H NMR spectrum of methyl (2*E*)-3-(2-nitrophenyl)-2[(piperidin-1-yl)methyl]-prop-2-enoate **72g** and methyl (2*Z*)-3-(2-nitrophenyl)-2[(piperidin-1-yl)methyl]-prop-2-enoate **73g** in CDCl$_3$.

Scheme 23

58
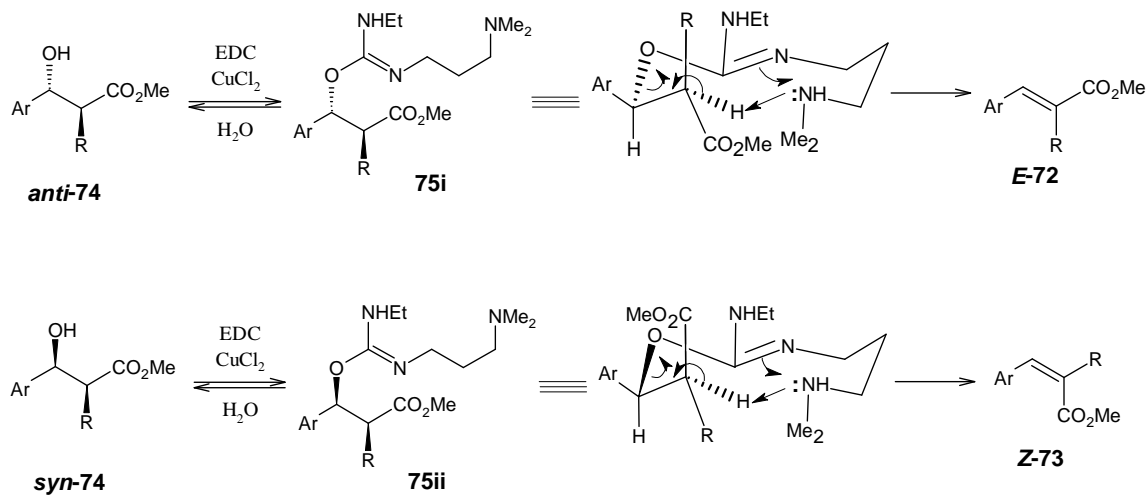
2.8   Conclusion

The reaction of a range of 2-nitrobenzaldehydes with methyl vinyl ketone and methyl acrylate has successfully led to the isolation of the corresponding Baylis-Hillman products in moderate to excellent yields. Other by-products were also formed in some reactions, including the MVK dimer and bis-MVK products. Reductive cyclization of the Baylis-Hillman adducts *via* catalytic hydrogenation was found to afford the expected quinoline derivatives as the major products with the quinoline *N*-oxides being isolated as the minor products. Another method of cyclization to afford quinolines was examined, *viz*., one-pot reductive cyclization using Fe/acetic acid, but this approach afforded the required products in low yield. Thermal Perkin-type condensation reactions of 2-methylquinolines with benzaldehyde in the presence of acetic anhydride proved to be time-consuming and, therefore, microwave-assisted reactions were explored, but the results were disappointing.

Conjugate addition of secondary or primary amines to the Baylis-Hillman methyl acrylate adducts produced mixtures of stereoisomers, while the reaction with piperazine resulted in the formation of *N,N'*-disubstituted products instead of the expected mono-substituted analogues. Cinnamate esters have been identified as potential HIV-1 integrase inhibitors and, in this study, the mono-substituted amino derivatives were dehydrated using EDC and $CuCl_2$ in refluxing to toluene to access several novel cinnamate ester derivatives. S_N' displacement of the hydroxyl group in the MVK-derived Baylis-Hillman adducts by HCl, followed by direct (S_N) substitution of chloride by various amino compounds proceeded smoothly and excellent yields were obtained for both reactions. However, attempts to reductively cyclize these products were not successful.

Many of the compounds prepared in this study are new, and future work is expected to involve computer modelling and biological assays to explore their antiretroviral activities.

3. EXPERIMENTAL

3.1 General

NMR spectra were recorded on Bruker AMX 400MHz or 600MHz spectrometers in $CDCl_3$ or DMSO-d_6. Spectra run in $CDCl_3$ were calibrated using the residual $CHCl_3$ signal at 7.25 ppm for ^1H and the $CDCl_3$ 77.00 ppm for ^{13}C; spectra run in DMSO-d_6 were calibrated using the residual DMSO signals at 2.5 ppm for ^1H and the DMSO-d_6 signal at 39.4 ppm for ^{13}C. Infrared spectra were recorded on a Perkin Elmer FT-IR spectrum 2000 spectrometer, using nujol mulls or KBr discs. Melting points were determined using a Reichert 281313 hot-stage apparatus and are uncorrected

Low-resolution mass spectra were carried out on a Finnigan-Mat GCQ mass spectrometer using the electron ionisation (EI) mode, and the high-resolution mass spectra on a Waters API Q-TOF Ultima instrument with electrospray ionisation (ESI+) source (Stellenbosch University; Central Analytical Facility). Microwave reactions were conducted using a CEM Focused Microwave^{TM} Discover reactor.

Chemical reagents were used as supplied by Sigma-Aldrich, without further purification. Flash chromatography was carried out using Separations silica gel 60 (particle sizes 0.040-0.063mm) and preparative layer chromatography was conducted using Merck silica gel 60 PF_{254}. Thin layer chromatography (TLC) was conducted using Merck aluminium plates, pre-coated with silica gel 60 F_{254}.

3.2 Preparation of Baylis-Hillman products

General Method: To a solution of 2-nitrobenzaldehyde (1eq.) and methyl vinyl ketone (or methyl acrylate) (1.5eq.) in CHCl$_3$, was added DABCO (or 3-HQ) (0.05eq.), and the reaction mixture was stirred in a stoppered flask at room temperature for periods which varied according to the 2-nitrobenzaldehyde used in the reaction. The solvent and excess MVK (or methyl acrylate) were removed *in vacuo* and the crude product was purified by flash chromatography or preparative layer chromatography.

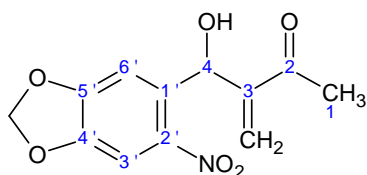*4-Hydroxy-3-methylene-4-(2-nitrophenyl)butan-2-one* **26a**
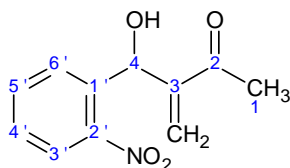
The general procedure was followed, using 2-nitrobenzaldehyde (4.98g, 33.0mmol), methyl vinyl ketone (4.1mL, 50mmol), DABCO (0.19g, 1.7mmol) in CHCl$_3$ (1mL) and the mixture was stirred for 7 days. The crude product was purified by flash chromatography on silica gel [elution with hexane-EtOAc (3:1)] to afford, as red-brown crystals, 4-hydroxy-3-methylene-4-(2-nitrophenyl)butan-2-one **26a** (3.28g, 45%), m.p. 78-80°C (lit.[52] 80-81°C); v_{max} (KBr)/cm^{-1} 3345 (OH) and 1665 (C=O); δ_H (400MHz; CDCl$_3$) 2.35 (3H, s, CH$_3$), 3.54 (1H, br d, J=3.2Hz, OH), 5.58 and 6.16 (2H, 2xs, C=CH$_2$), 6.20 (1H, d, J=3.2Hz, C*H*OH), 7.44 (1H, t, J=7.8Hz, 4'-H), 7.63 (1H, t, J=7.6Hz, 5'-H), 7.76 (1H, d, J=8Hz, 6'-H), and 7.94 (1H, d, J=8Hz, 3'-H); δ_C (100MHz; CDCl$_3$) 26.0 (C-1), 67.4 (C-4), 124.6 (C-3'), 126.5 (C=*C*H$_2$), 128.5 (C-4'), 128.8 (C-6'), 133.5 (C-5'), 136.4 (C-1'), 147.9 (C-2'), 148.8 (*C*=CH$_2$) and 199.8 (C=O); *m/z* 222 (**M+1**, 40%) and 161 (100%).

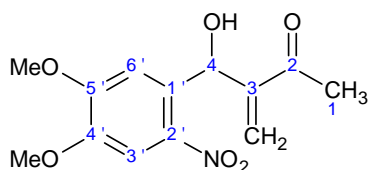*4-Hydroxy-4-(5-hydroxy-2-nitrophenyl)-3-methylenebutan-2-one* **26b**
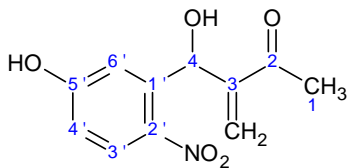
The general procedure was followed, using 5-hydroxy-2-nitrobenzaldehyde (3.0g, 18mmol), methyl vinyl ketone (4.3mL, 52mmol), DABCO (0.10g, 0.90mmol) in $CHCl_3$ (15mL) and stirred for 34 days. The crude product was purified by flash chromatography on silica gel [elution with hexane-EtOAc (1:1)] to afford, as a yellow solid, 4-hydroxy-4-(5-hydroxy-2-nitrophenyl)-3-methylenebutan-2-one **26b** (4.2g, 89%), m.p. 150-152°C (lit.[52] 131-133°C); ν_{max} (KBr)/cm^{-1} 3358 (OH) and 1626 (C=O); δ_H (400MHz; DMSO-d_6) 2.30 (3H, s, CH$_3$), 5.60 and 6.16 (2H, 2xs, C=CH$_2$), 5.81 (1H, d, J=5.0Hz, OH), 6.19 (1H, d, J=4.8Hz, C*H*OH), 6.81 (1H, dd, J=8.8Hz and 2.8Hz, 4'-H), 7.13 (1H, d, J=2Hz, 6'-H), 7.94 (1H, d, J=6.4Hz, 3'-H) and 10.85 (1H, br s, 5'-OH); δ_C (100MHz; DMSO-d_6) 26.0 (C-1), 64.7 (C-4), 114.4 (C-4'), 114.9 (C-6'), 125.0 (C=*C*H$_2$), 127.7 (C-3'), 138.9 (C-2'), 142.3 (C-1'), 150.4 (*C*=CH$_2$), 162.3 (C-5') and 19.0 (C=O); *m/z* 238 (**M+1**, 4%) and 191 (100%)

4-Hydroxy-3-methylene-4-(4,5-methylenedioxy-2-nitrophenyl)butan-2-one **26c**

The general procedure was followed, using 3,4-methylenedioxy-6-nitrobenzaldehyde (2.0g, 12mmol), methyl vinyl ketone (1.30mL, 15.6mmol), DABCO (0.06g, 0.5mmol) in $CHCl_3$ (4mL) and stirred for 7 days. The crude product was purified by flash chromatography on silica gel [elution with hexane-EtOAc-CHCl$_3$ (1:1:3)] to afford, as a yellow solid, 4-hydroxy-3-methylene-4-(4,5-methylenedioxy-2-nitrophenyl)butan-2-one **26c** (0.9g, 27%), m.p. 98-99 (lit.[53] 100-103°C); ν_{max} (KBr)/cm^{-1} 3466 (OH) and 1624 (C=O); δ_H (400MHz, CDCl$_3$) 2.37 (3H, s, CH$_3$), 3.54 (1H, br s, OH), 5.75 and 6.12 (2H, 2xs, C=CH$_2$), 6.11 (2H, s, OCH$_2$O), 6.18 (1H, s, C*H*OH), 7.19 (1H, s, 6'-H) and 7.51 (1H, s, 3'-H); δ_C (100MHz, CDCl$_3$) 26.0 (C-1), 67.4 (C-4), 103.0 (OCH$_2$O), 105.5 (C-3'), 107.7 (C-6'), 126.1 (C=*C*H$_2$), 134.2 (C-1'), 141.8 (C-2'), 147.2(C-7'), 149.1 (*C*=CH$_2$), 152.29 (C-4') and 199.49 (C=O); *m/z* 266 (**M+1**, 14%) and 188 (100%).

4-(4,5-Dimethoxy-2-nitrophenyl)-4-hydroxy-3-methylenebutan-2-one 26d

The general procedure was followed, using 4,5-dimethoxy-2-nitrobenzaldehyde (2.0g, 8.9mmol), methyl vinyl ketone (1.5mL, 13mmol), DABCO (0.11g, 0.90mmol) in $CHCl_3$ (4mL) and stirred for 7 days. The crude product was purified by flash chromatography on silica gel [elution with hexane-EtOAc-$CHCl_3$ (1:1:3)] to afford, as a yellow-brown oil, 4-(4,5-dimethoxy-2-nitrophenyl)-4-hydroxy-3-methylenebutan-2-one **26d** (1.4g, 89%) (lit.[52] yellowish-brown oil); v_{max} (KBr)/cm^{-1} 3449 (OH) and 1712 (C=O); δ_H (400MHz; $CDCl_3$) 2.37 (3H, s, CH_3), 3.40 (1H, br s, OH), 3.93 and 4.11 (6H, 2xs, 4'- and 5'-OCH_3), 5.57 and 6.04 (2H, 2xs, C=CH_2), 6.30 (1H, s, C*H*OH), 7.30 (1H, s, 6'-H) and 7.62 (1H, s, 3'-H); δ_C (100MHz; $CDCl_3$) 26.1 (C-1), 56.8 and 56.8 (2xOCH_3), 68.2 (C-4), 108.5 (C-6'), 110.5 (C-3'), 126.1 (C=CH_2), 134.8 (C-1'), 145.0 (C-2'), 147.9 (C-5'), 149.6 (C-4'), 153.7 (*C*=CH_2) and 200.5 (C=O); *m/z* 281 (**M+1**, 12%), and 43 (100%).

4-Hydroxy-4-(3-methoxy-2-nitrophenyl)-3-methylenebutan-2-one 26e and *5-acetyl-6-hydroxy-6-(3-methoxy-2-nitrophenyl)-3-methylene-2-hexanone* 31e
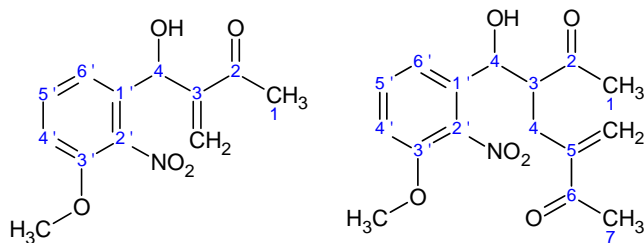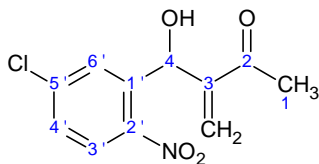

The general procedure was followed, using 3-methoxy-2-nitrobenzaldehyde (1.2g, 6.9mmol), methyl vinyl ketone (0.86mL, 10mmol), DABCO (38mg, 0.34mmol) and $CHCl_3$ (2mL). The crude product was purified by flash chromatography on silica gel [elution with hexane-EtOAc (1.5:1)] to afford,

Faction 1: as a brown oil, 4-hydroxy-4-(3-methoxy-2-nitrophenyl)-3-methylenebutan-2-one **26e** (0.69g, 40%) (lit.[52] reddish-brown oil); v_{max} (KBr)/cm^{-1} 3449 (OH) and 1712 (C=O); δ_H (400MHz; $CDCl_3$) 2.34 (3H, s, CO.CH_3), 2.86 (1H, br s, OH), 3.87 (3H, s, ArOCH_3), 5.64 (1H, d, *J*=1.2Hz, 4-H), 5.95 and 6.22 (2H, 2xs, C=CH_2), 6.99 (1H, d, *J*=8.4Hz, ArH), 7.11

(1H, d, *J*=8Hz, ArH), and 7.42 (1H, t, *J*=8Hz, 5'-H); δ_C (100MHz; CDCl$_3$) 26.0 (C-1), 56.3 (OCH$_3$), 67.8 (C-4), 111.9 (C-4'), 119.2 (C-6'), 127.8 (C=*C*H$_2$), 131.1 (C-5'), 134.3 (C-1'), 139.9 (C-2'), 147.4 (*C*=CH$_2$), 150.6 (C-3') and 199.7 (C=O); *m/z* 205 (**M$^+$-NO$_2$**, 10%) and 125 (100%).

Fraction 2: as a reddish-brown oil, 5-acetyl-6-hydroxy-6-(3-methoxy-2-nitrophenyl)-3-methylene-2-hexanone **31e** (10%); ν_{max} (KBr)/cm^{-1} 3433 (OH) and 1624 (C=O); δ_H (400MHz; CDCl$_3$) 2.08 and 2.33 (6H, 2xs, 1- and 8-CH$_3$), 2.22 and 2.56 (2H, 2xm, 5-CH$_2$), 3.09 (1H, m, 3-H), 3.86 (4H, m, OCH$_3$ and OH), 4.66 (1H, m, 4-H), 5.86 and 6.06 (2H, 2 x s, C=CH$_2$), 6.93 (1H, d, *J*=8Hz, 6'-H), 7.09 (1H, d, *J*=8Hz, 4'-H) and 7.41 (1H, t, , *J*=8Hz, 5'-H); δ_C (100MHz; CDCl$_3$) 25.6 (C-1), 40.0 (C-4), 31.9 (C-8), 55.1 (C-6), 56.5 (OCH$_3$), 69.6 (C-5), 111.9 (C5'), 119.0 (C-6'), 128.8 (C=*C*H$_2$), 131.3 (C-4'), 135.4 (C-2'), 140.2 (C-1'), 145.0 (C-3'), 150.5 (C-3), 211.5 and 213.4 (2xC=O).

4-(5-Chloro-2-nitrophenyl)-4-hydroxy-3-methylenebutan-2-one **26f**

The general procedure was followed, using 5-chloro-2-nitrobenzaldehyde (5.5g, 2.7mmol), methyl vinyl ketone (0.33mL, 4.1mmol), DABCO (16mg, 0.14mmol) in CHCl$_3$ (0.6mL) and stirred for 6 days. The crude product was purified by flash chromatography on silica gel [elution with hexane-EtOAc (3:1)] to afford, as a red solid, 4-(5-chloro-2-nitrophenyl)-4-hydroxy-3-methylenebutan-2-one **26f** (0.61g, 88%), m.p. 75-78°C (lit.[52] 72-74°C); ν_{max} (KBr)/cm^{-1} 3449 (OH) and 1692 (C=O); δ_H (400MHz; CDCl$_3$) 2.37 (3H, s, CH$_3$), 3.54 (1H, br s, CHO*H*), 5.76 and 6.16 (2H, 2xs, C=CH$_2$), 6.23 (1H, s, C*H*OH), 7.42 (1H, dd, *J*= 2.0 and 8.4Hz, 4'-H), 7.77 (1H, d, *J*=2.4Hz, 6'-H) and 7.95 (1H, d, *J*=8.8Hz, 3'-H); δ_C (100MHz; CDCl$_3$) 26.0 (C-1), 67.1 (C-4), 126.2 (C-6'), 126.7 (C=*C*H$_2$), 128.6 (C-4'), 129.1 (C-3'), 138.7 (C-1'), 140.3 (C-5'), 146.0 (C-2'), 148.5 (*C*=CH$_2$) and 199.7 (C=O); *m/z* 256 [**M+1** (^{35}Cl), 0.98%] and 178 (100%).

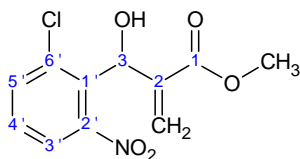*Methyl 3-hydroxy-2-methylene-3-(2-nitrophenyl)propanoate* **26g**
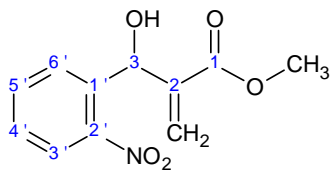
The general procedure was followed, using 2-nitrobenzaldehyde (3.0g, 20mmol), methyl acrylate (2.7mL, 30mmol), DABCO (0.12g, 1.1mmol) and CHCl$_3$ (0.6mL). The reaction mixture was stirred for 2 days and the crude product was purified by flash chromatography on silica gel [elution with hexane-EtOAc (3:1)] to afford, as a pale-green oil which slowly crystallized to a pale-green solid, methyl 3-hydroxy-2-methylene-3-(2-nitrophenyl)propanoate **26g** (4.6g, 96%), m.p. 36-38°C (lit.[76] cited as oil); v_{max} (KBr)/cm^{-1} 3471 (OH) and 1710 (C=O); δ_H (400MHz; CDCl$_3$) 3.49 (1H, br s, OH), 3.71 (3H, s, OCH$_3$), 5.71 and 6.34 (2H, 2xs, C=CH$_2$), 6.18 (1H, s, C*H*OH), 6.45 (1H, t, *J*= 8Hz, 4'-H), 7.63 (1H, t, *J*=7.6Hz, 5'-H), 7.73 (1H, d, *J*=7.6Hz, 6'-H) and 7.92 (1H, d, *J*=8Hz, 3'-H); δ_C (100MHz; CDCl$_3$) 52.2 (OCH$_3$), 67.6 (C-3), 124.54 (C-3'), 126.5 (C=*C*H$_2$), 128.7 (C-4'), 128.9 (C-6'), 122.4 (C-5'), 136.1 (C-1'), 140.7 (*C*=CH$_2$), 128.3 (C-2') and 166.4 (C=O); *m/z* 238 (**M+1**, 10%) and 78 (100%).

Methyl 3-hydroxy-3-(3-methoxy-2-nitrophenyl)-2-methylenepropanoate **26h**
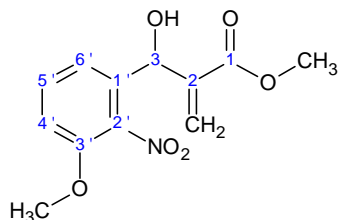
The general procedure was followed, using 3-methoxy-2-nitrobenzaldehyde (3.0g, 17mmol), methyl acrylate (2.2mL, 25mmol), DABCO (1.1g, 0.83mmol) and CHCl$_3$ (1mL). The reaction mixture was stirred for 3 days and the crude product was purified by flash chromatography on silica gel [elution with hexane-EtOAc (1:1)] to afford, as a brown solid, methyl 3-hydroxy-3-(3-methoxy-2-nitrophenyl)-2-methylenepropanoate **26h** (4.33g, 98%), m.p. 112-114°C (lit.[76] 108-109°C); v_{max} (KBr)/cm^{-1} 3485 (OH) and 1706 (C=O); δ_H (400MHz; CDCl$_3$) 3.30 (1H, br s, OH), 3.69 (3H, s, CO$_2$CH$_3$), 3.88 (3H, s, OCH$_3$), 5.63 (1H, s, C*H*OH), 5.86 and 6.40 (2H, 2xs, C=CH$_2$), 6.98 (1H, d, *J*=8.4Hz, 4'-H), 7.08 (1H, d, *J*=7.6Hz, 6'-H) and 7.41 (1H, t, *J* =8Hz, 5'-H); δ_C (100MHz; CDCl$_3$) 52.1 (CO$_2$*C*H$_3$), 56.5 (OCH$_3$), 68.2 (C-3), 112.2 (C-4'),

119.3 (C-6'), 127.3 (C=CH_2), 131.3 (C-5'), 134.3 (C-1'), 139.6 (C=CH$_2$), 140.4 (C-2'), 150.8 (C-3') and 166.1 (C=O); *m/z* 268 (**M+1**, 4.4%) and 221 (100%).

Methyl 3-(6-chloro-2-nitrophenyl)-3-hydroxy-2-methylenepropanoate **26i**

The general procedure was followed, using 6-chloro-2-nitrobenzaldehyde (0.10g, 0.54mmol), methyl acrylate (0.07mL, 0.8mmol), DABCO (3.6mg, 0.03mmol) and CHCl$_3$ (0.3mL), the reaction being completed in 5 days. The crude product was purified by flash chromatography on silica gel [elution with hexane-EtOAc (3:1)] to afford, as a yellowish-brown semi-solid, methyl 3-(6-chloro-2-nitrophenyl)-3-hydroxy-2-methylenepropanoate **26i** (134mg, 91%) (lit.[76] cited as solid, m.p. 99-101°C); v_{max} (KBr)/cm^{-1} 3335 (OH) and 1704 (C=O); δ_H (400MHz; CDCl$_3$) 3.60 (1H, d, *J*=6.8Hz, OH), 3.74 (3H, s, OCH$_3$), 5.74 and 6.45 (2H, 2xs, C=CH$_2$), 6.17 (1H, d, C*H*OH), 7.39 (1H, t, *J*=7.8Hz, 4'-H), 7.52 (1H, d, *J*=7.6Hz, 5'-H) and 7.60 (1H, d, *J*=8Hz, 3'-H); δ_C (100MHz; CDCl$_3$) 52.2 (OCH$_3$), 69.0 (C-3), 122.9 (C-5'), 127.9 (C=CH_2), 129.4 (C-4'), 131.6 (C-1'), 133.4 (C-3'), 135.6 (C-6'), 137.9 (C=CH$_2$), 151.5 (C-2') and 166.33 (C=O); *m/z* 272 [**M+1** (^{35}Cl), 38%], and 155 (100%),

Methyl 3-hydroxy-2-methylene-3-(4,5-methylenedioxy-2-nitrophenyl)propanoate **26j**
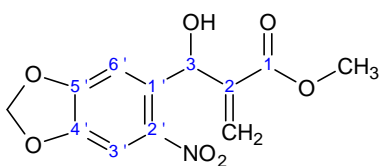
The general procedure was followed, using 3,4-methylenedioxy-2-nitrobenzaldehyde (3.1g, 15mmol), methyl acrylate (2.1mL, 23mmol), DABCO (86mg, 0.77mmol) and CHCl$_3$ (5mL), and the reaction was terminated after 3 weeks. The crude product was purified by flash chromatography on silica gel [elution with hexane-EtOAc (3:1)] to afford, as a pale-yellow solid, methyl 3-hydroxy-2-methylene-3-(4,5-methylenedioxy-2-nitrophenyl)-propanoate **26j** (2.4g, 54%), m.p. 128-129°C (lit.[76] 121-123°C); v_{max} (KBr)/cm^{-1} 3486 (OH) and 1716 (C=O); δ_H (400MHz; CDCl$_3$) 3.45 (1H, d, *J*=4.4Hz, OH), 3.74 (3H, s, OCH$_3$), 5.68 and 6.32 (2H, 2xs, C=CH$_2$), 6.11 (2H, s, OCH$_2$O), 6.17 (1H, d, *J*=3.2Hz, C*H*OH), 7.15 (1H, s, 6'-H) and 7.48

(1H, s, 3'-H); δ_C (100MHz; CDCl$_3$) 52.2 (OCH$_3$), 67.7 (C-3), 103.1 (OCH$_2$O), 105.5 (C-3'), 107.7 (C-6'), 126.1 (C=CH$_2$), 133.6 (C-1'), 140.9 (C=CH$_2$), 142.2 (C-2'), 147.3 (C-5'), 152.2 (C-4') and 166.5 (C=O); *m/z* 281 (**M$^+$**, 2%) and 176 (100%).

Methyl 3-(2,4-dinitrophenyl)-3-hydroxy-2-methylenepropanoate **26k**
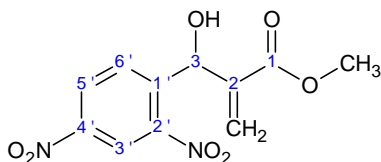
The general procedure was followed, using 2,4-dinitrobenzaldehyde (0.55g, 2.8mmol), methyl acrylate (0.34mL, 4.2mmol), 3-HQ (16mg, 0.13mmol) and CHCl$_3$ (0.5mL). The reaction mixture was stirred for 7 days and the crude product was purified by flash chromatography on silica gel [elution with hexane-EtOAc (3:1)] to afford, as a red solid, methyl 3-(2,4-dinitrophenyl)-3-hydroxy-2-methylenepropanoate **26k** (0.8g, 96%), m.p. 84-86°C (lit.[53] 82-85°C); v_{max} (KBr)/cm^{-1} 3449 (OH) and 1724 (C=O); δ_H (400MHz; CDCl$_3$), 3.59 (1H, br s, OH), 3.74 (3H, s, OCH$_3$), 5.74 and 6.39 (2H, 2xs, C=CH$_2$), 6.28 (1H, s, CHOH), 8.02 (1H, d, *J*=8.8Hz, 6'-H), 8.46 (1H, dd, *J*=2.0 and 8.8Hz, 5'-H) and 8.77 (1H, d, *J*=2.4Hz, 3'-H); δ_C (100MHz; CDCl$_3$) 52.4 (OCH$_3$), 67.5 (C-3), 120.0 (C-3'), 127.3 (C-5'), 127.4 (C=CH$_2$), 130.7 (C-6'), 130.8 (C-2), 142.7 (C-1'), 147.2 (C-4'), 148.1 (C-2') and 166.0 (C=O); *m/z* 283 (**M+1**, 9%) and 149 (100%).

3-Methyleneheptan-2,6-dione **30c-e**
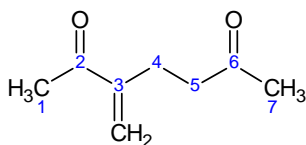
The MVK dimer **30** was isolated as a competition product together with each of the Baylis-Hillman adducts **26c,d,e** as a reddish-brown oil; v_{max} (KBr)/cm^{-1} 1698 (C=O); δ_H (400MHz; CDCl$_3$) 2.10 and 2.30 (6H, 2xs, 2xCH$_3$), 2.54 (4H, m, 4- and 5-CH$_2$) and 5.80 and 6.00 (2H, 2xs, C=CH$_2$); δ_C (100MHz; CDCl$_3$) 25.3 (C-7), 42.4 (C-1), 56.4 and 56.5 (C-4 and C-5), 126.2 (C=CH$_2$), 140.0 (C-3), 199.0 and 207.6 (2xC=O).

3.3 Hydrogenation of Baylis-Hillman products

Attempted preparation of *2,3-dimethylquinoline* **35a**
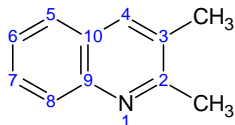
To a solution of 4-hydroxy-3-methylene-4-(2-nitrophenyl)butan-2-one **26a** (1.0g, 4.5mmol) in ethanol (50mL), 10% Pd-C catalyst (0.16g) was added and the resulting mixture was stirred under a hydrogen atmosphere for 1.5 hours. The reaction mixture was filtered to remove catalyst and concentrated *in vacuo*. The crude mixture was re-dissolved in CH_2Cl_2 and dried with anhydrous $MgSO_4$.[52] The solvent was evaporated *in vacuo* and the ^1H NMR spectrum of the solid showed that the reaction had been unsuccessful.
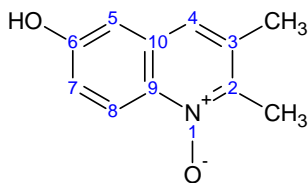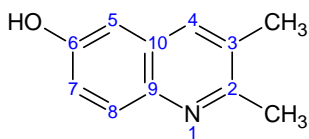
6-Hydroxy-2,3-dimethylquinoline **35b** and *6-hydroxy-2,3-dimethylquinoline-N-oxide* **39b**

To a solution of 4-hydroxy-3-methylene-4-(5-hydroxy-2-nitrophenyl)butan-2-one **26b** (1.3g, 5.7mmol) in ethanol (50mL), 10% Pd-C catalyst (0.24g) was added and the resulting mixture was stirred under hydrogen for 3 hours. The reaction mixture was filtered to remove the catalyst and the filtrate concentrated *in vacuo*. The crude mixture was re-dissolved in CH_2Cl_2 and dried with anhydrous $MgSO_4$.[52] The residue was dissolved in ethyl acetate from which a brown precipitate formed and was filtered off. The brown precipitate was shown by NMR analysis to comprise a mixture of 6-hydroxy-2,3-dimethylquinoline **35b** [δ_H (400MHz; DMSO-d_6) 2.34 (3H, s, 3-CH_3), 2.51 (3H, s, 2-CH_3), 7.01 (1H, d, *J*=2.4Hz, 5-H), 7.17 (1H, dd, *J*=2.4 and 8.8Hz, 7-H), 7.71 (1H, d, *J*=9.2Hz, 8-H), 7.78 (2H, s, 4-H) and 9.98 (OH); δ_C (100MHz; DMSO-d_6) 19.0 (3-CH_3), 22.8 (2-CH_3), 107.5 (C-5), 120.4 (C-7), 128.2 (C-9), 129.9 (C-8), 129.9 (C-3), 133.2 (C-4), 141.0 (C-2), 154.7 (C-6) and 154.9 (C-10); *m/z* 173 (**M**$^+$, 100%)] and 6-hydroxy-2,3-dimethylquinoline-*N*-oxide **39b** (1.10g) [δ_H (400MHz; DMSO-d_6) 2.63 (3H, s, 3-CH_3), 2.48 (3H, s, 2-CH_3), 7.08 (1H, d, *J*=2.4Hz, 5-H), 7.20 (1H, dd,

J=2.4 and 9.6Hz, 7-H), 7.48 (1H, s, 4-H), 8.33 (1H, d, *J*=9.2Hz, 8-H) and 9.98 (OH); δ_C (100MHz; DMSO-d_6) 14.0 (2-CH$_3$), 19.6 (3-CH$_3$), 108.4 (C-5), 120.7 (C-8), 120.7 (C-7), 122.7 (C-4), 131.3 (C-3), 134.0 (C-10), 142.4 (C-2) and 156.5 (C-9); *m/z* 189 (**M**$^+$, 33%)].

3-Acetoxymethyl-2-methyl-6,7-methylenedioxyquinoline **44c**
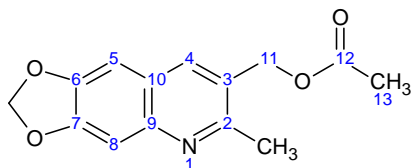
A mixture of 4-hydroxy-3-methylene-4-(4,5-methylenedioxy-2-nitrophenyl)butan-2-one **26c** (0.11g, 0.42mol) in acetic acid (2.6mL), was heated to 110°C. Iron powder (0.14g, 2.5mmol) was added and the resulting mixture refluxed for 1.5 hours. The excess acetic acid was removed under reduced pressure; the residue was dispersed in EtOAc (15mL), and the dispersion stirred for 2 min and filtered to remove the iron powder. The insoluble iron powder was washed with EtOAc (2x10mL). The filtrate and washings were combined and concentrated *in vacuo*. Flash chromatography on silica gel (elution with hexane-EtOAc [1.5:1]) afforded, as a light-yellow powder, *3-acetoxymethyl-2-methyl-6,7-methylenedioxyquinoline* **44c** (20mg, 20%) (Found **M**$^+$: 259.0848. Calc. for C$_{14}$H$_{13}$NO$_4$, *M*: 259.0844); δ_H (600MHz, CDCl$_3$) 2.12 (3H, s, CH$_3$CO), 2.66 (3H, s, 2-CH$_3$), 5.21 (2H, s, OCH$_2$), 6.08 (2H, s, OCH$_2$O), 7.01 (1H, s, 5-H), 7.30 (1H, s, 8-H) and 7.87 (1H, s, 4-H); δ_C (100MHz, DMSO-d_6) 20.9 (*C*H$_3$CO), 22.4 (2-CH$_3$), 64.1 (OCH$_2$), 101.6 (OCH$_2$O), 102.6 (C-5), 105.2 (C-8), 123.4 (C-9), 125.8 (C-3), 135.5 (C-4), 145.8 (C-10), 147.5 (C-7), 150.9 (C-6), 155.5 (C-2) and 170.8 (C=O); *m/z* 259 (M$^+$, 0.14%) and 144 (100%).

3.4 Perkin-type condensation of quinolines to styrylquinolines

(E) 2-Styrylquinoline **47**
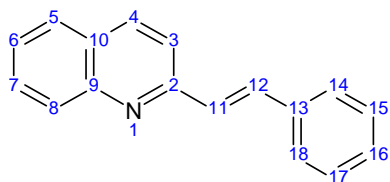
To a solution of quinaldine (1.0mL, 7.4mmol), in acetic anhydride (12mL) was added benzaldehyde (1.5mL, 15mmol). The reaction mixture was refluxed for 16 hours and then concentrated *in vacuo*. The black crystals, which slowly crystallised out were recrystallised from chloroform-hexane to afford, as a yellow powder, (*E*) 2-styrylquinoline **47** (0.51g, 30%),m.p. 92-94°C (lit.[77] 97-100°C); v_{max} (KBr)/cm^{-1} 1594 (N=C); δ_H (400MHz; CDCl$_3$) 7.32 (1H, t, *J*=7.2Hz, 16-H), 7.39 (3H, m, 12-, 15- and 17-H), 7.49 (1H, t, *J*=7.2Hz, 6-H), 7.63-7.71 (5H, m, 4-, 7-, 11-, 14- and 18-H), 7.78 (1H, d, *J*=8Hz, 5-H) and 8.10 (2H, m, 3- and 8-H); δ_C (100MHz; CDCl$_3$) 119.2 (C-4), 126.2 (C-6), 127.3 (C-14 and C-18), 127.3 (C-13), 127.5 (C-5), 128.6 (C-16), 128.8 (C-15 and C-17), 129.0 (C-12), 129.2 (C-8), 129.7 (C-7), 134.5 (C-11), 136.4 (C-3), 136.5 (C-10), 148.2 (C-2), and 156.0 (C-9); *m/z* 231 (M$^+$, 48%) and 230 (100%).

Attempted preparation of *5-hydroxy-4-methylene-5-(2-nitrophenyl)-1-phenylpent-1-en-3-one* 48a
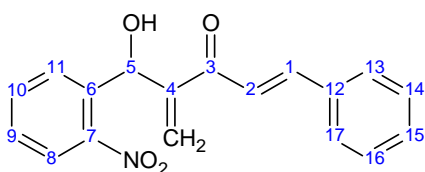
Method A: A solution of 4-hydroxy-3-methylene-4-(2-nitrophenyl)butan-2-one **26a** (0.1g, 0.4mmol), benzaldehyde (0.08mL, 0.8mmol) and acetic anhydride (1.2mL) was refluxed under N$_2$ for 16 hours. The crude mixture was added to a mixture of crushed ice and saturated aq. NaHCO$_3$ (20mL) and the resulting mixture stirred overnight before extracting with ethyl acetate (10mL). The organic layer was dried with anhydrous MgSO$_4$ and concentrated *in vacuo*, but ^{1}H and ^{13}C NMR analysis of the residue indicated that compound **48a** was not present.

Method B: A solution of 4-hydroxy-3-methylene-4-(2-nitrophenyl)butan-2-one **26a** (0.1g, 0.4mmol), benzaldehyde (0.08mL, 0.8mmol) and acetic anhydride (1.2mL) was treated with microwave radiation (150W) with maximum pressure of 150KPa for 10 minutes. The resulting solution was cooled and stirred with crushed ice and saturated aq. NaHCO$_3$ (20mL) overnight. The mixture was extracted ethyl acetate and the organic layer dried with anhydrous MgSO$_4$ and concentrated *in vacuo*, but ^{1}H NMR analysis indicated that the desired product **48a** had not formed.

Attempted preparation of *5-hydroxy-4-methylene-5-(4,5-methylenedioxy-2-nitrophenyl)-1-phenylpent-1-en-3-one* **48c**
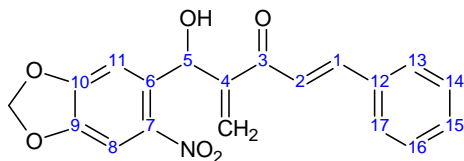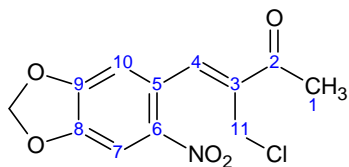

A solution of 4-hydroxy-3-methylene-4-(4,5-methylenedioxy-2-nitrophenyl)butan-2-one **26c** (0.10g, 0.38mmol), benzaldehyde (0.08mL, 0.8mmol) and acetic anhydride (1.2mL) was refluxed under N_2 for 16 hours. The crude mixture was added to crushed ice and saturated aq. $NaHCO_3$ (20mL) was added and the resulting mixture was stirred overnight before extracting with ethyl acetate (10mL). The organic layer was dried with anhydrous $MgSO_4$ and was concentrated *in vacuo*, but ^{1}H and ^{13}C NMR analysis of the residue indicated that the reaction had not been successful.

3.5 Substitution reactions of Baylis-Hillman products

3-Chloromethyl-4-(5-hydroxy-2-nitrophenyl)but-3-en-2-one **52b**
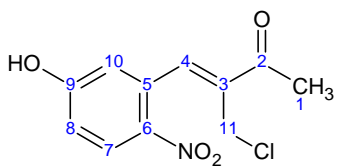
Acetyl chloride (7mL) was added dropwise to dry ethanol (12mL), cooled in ice, and the mixture was stirred for 5 minutes before adding 4-hydroxy-4-(5-hydroxy-2-nitrophenyl)-3-methylenebutan-2-one **26b** (0.96g, 4.1mmol). The resulting mixture was stirred for 10 hours and the excess HCl and solvent were evaporated *in vacuo* to afford, as a yellow-brown solid, *3-chloromethyl-4-(5-hydroxy-2-nitrophenyl)but-3-en-2-one* **52b** (0.97g, 94%), m.p. 141-144°C; ν_{max} KBr/cm^{-1} 3279 (OH) and 1662 (C=O); δ_H (400MHz; CDCl$_3$) 2.44 (3H, s, CH$_3$), 4.18 (2H, s, 11-CH$_2$), 6.86 (1H, d, *J*=2Hz, 10-H), 6.98 (1H, dd, *J*=2.4 and 9.2Hz, 8-H), 8.12 (1H, s, 4-H), 8.17 (1H, d, *J*=9.2Hz, 7-H) and 8.18 (1H, s, OH); δ_C (100MHz; CDCl$_3$); δ_C (100MHz; CDCl$_3$) 26.3 (C-1), 37.8 (C-11), 112.4 (C-10), 116.7 (C-8), 128.5 (C-7), 133.6 (C-6), 136.0 (C-3), 138.7 (C-5), 143.3 (C-4), 163.2 (C-9) and 197.8 (C=O).

3-Chloromethyl-4-(4,5-methylenedioxy-2-nitrophenyl)but-3-en-2-one **52c**

Acetyl chloride (18mL) was added dropwise to dry ethanol (30mL), cooled in ice, and the mixture was stirred for 5 minutes before adding 4-hydroxy-3-methylene-4-(4,5-methylenedioxy-2-nitrophenyl)butan-2-one **52c** (3.0g, 11mmol). The resulting mixture was stirred overnight and the excess HCl and ethanol were evaporated *in vacuo* to afford, as a black wax, *3-chloromethyl-4-(4,5-methylenedioxy-2-nitrophenyl)but-3-en-2-one* **58c** (3.20g, 100%); v_{max} KBr/cm^{-1} 1664 (C=O); δ_H (400MHz; CDCl$_3$) 2.50 (3H, s, COCH$_3$), 4.21 (2H, s, 11-CH$_2$), 6.21 (2H, s, -OCH$_2$O-), 7.11 (1H, s, 10-H), 7.72 (1H, s, 7-H), and 7.92 (1H, s, 4-H); δ_C (100MHz; CDCl$_3$) 26.0 (C-1), 37.1 (C-11), 103.6 (-OCH$_2$O-), 106.0 (C-7), 109.03 (C-10), 126.9 (C-5), 137.2 (C-3), 140.9 (C-4), 141.6 (C-6), 148.8 (C-9), 152.5 (C-8), and 196.8 (C=O).
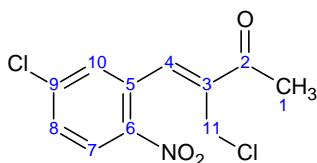
3-Chloromethyl-4-(5-chloro-2-nitrophenyl)but-3-en-2-one **52f**

Acetyl chloride (20mL) was added dropwise to dry ethanol (40mL), cooled in ice, and the mixture was stirred for 5 minutes before adding 4-(5-chloro-2-nitrophenyl)-4-hydroxy-3-methylenebutan-2-one **26f**. The resulting mixture was stirred for 10 hours and the excess HCl and solvent were evaporated *in vacuo* to afford, as a black oil, *3-chloromethyl-4-(5-chloro-2-nitrophenyl)but-3-en-2-one* **52f** (3.8g, 94%); v_{max} KBr/cm^{-1} 1677 (C=O); δ_H (400MHz; CDCl$_3$) 2.51 (3H, s, CH$_3$), 4.17 (2H, s, 11-CH$_2$), 7.56 (1H, dd, *J*=1.0 and 8.8Hz, 8-H), 7.67 (1H, d, *J*=1.2Hz, 10-H), 7.91 (1H, s, 4-H) and 8.18 (1H, d, *J*=8.8Hz, 7-H); δ_C (100MHz; CDCl$_3$) 26.0 (CH$_3$), 36.7 (C-11), 126.7 (C-7), 130.2 (C-8), 130.4 (C-10), 131.9 (C-5), 138.3 (C-3), 138.8 (C-4), 140.8 (C-6), 145.2 (C-9) and 196.3 (C=O).

## 3.6 Conjugate addition reactions of Baylis-Hillman adducts

General Method: A solution of the Baylis-Hillman product (1eq.), and the specified amine (1.5eq.) in dry THF or methanol 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* to afford the aminated product. ^{1}H NMR and ^{13}C NMR data are cited for the major diastereoisomeric product in each case.

Methyl 3-hydroxy-3-(2-nitrophenyl)-2-[(piperidin-1-yl)methyl]propanoate **58g**
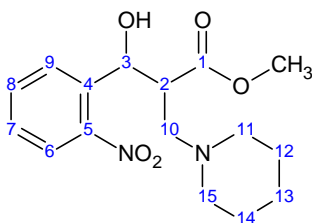
The general method was followed, using methyl 3-hydroxy-2-methylene-3-(2-nitrophenyl)-propanoate **26g** (1.40g, 6.32mmol), piperidine (0.54mL, 5.5mmol) and dry THF (5mL). After 3days, the mixture was concentrated *in vacuo* and the crude product purified by flash chromatography on silica gel [elution with hexane-EtOAc (3:1)] to afford a yellow solid, comprising a 2:1 diastereomeric mixture of methyl 3-hydroxy-3-(2-nitrophenyl)-2-[(piperidin-1-yl)methyl]propanoate **58g** (1.5g, 74%), m.p. 59-61 °C (lit.[76] 76-78°C); v_{max} (KBr)/cm^{-1} 3473 (OH) and 1714 (C=O); δ_H (400MHz; CDCl$_3$) 1.44 (2H, m, 13-CH$_2$), 1.54-1.64 (4H, m, 12- and 14-CH$_2$), 2.45 (4H, m, 11- and 15-CH$_2$), 2.70 and 3.01 (2H, 2xm, 10-CH$_2$), 3.16 (1H, m, 2-CH), 3.44 (3H, s, CH$_3$), 5.52 (1H, d, *J*=8.8Hz, 3-H), 7.38 (1H, m, 7-H), 7.55 (1H, t, *J*=7.6Hz, 8-H) and 7.60-7.75 (2H, m, 9- and 6-H); δ_C (100MHz; CDCl$_3$) 23.8 (C-13), 25.8 (C-12 and C-14), 48.3 (C-2), 51.8 (CH$_3$), 55.0 (C-11 and C-15), 60.7 (C-10), 73.1 (C-3), 124.7 (C-6), 128.4 (C-7), 129.8 (C-9), 132.3 (C-8), 136.5 (C-4), 149.0 (C-5) and 171.4 (C=O); *m/z* 323 (**MH$^+$**, 2.5%) and 98 (100%).

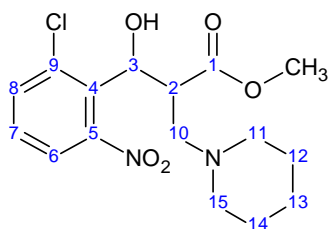*Methyl 3-hydroxy-3-(3-methoxy-2-nitrophenyl)-2-[(piperidin-1-yl)methyl]propanoate* **58h**
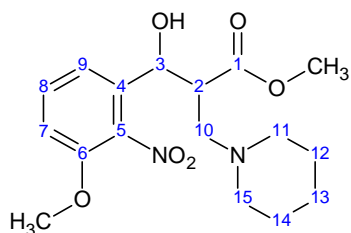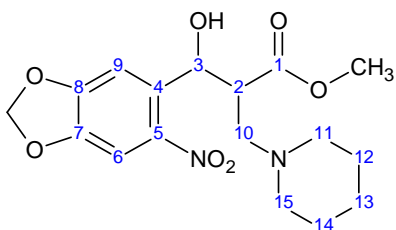

The general method was followed, using methyl 3-hydroxy-2-methylene-3-(3-methoxy-2-nitrophenyl)propanoate **26h** (1.3g, 4.9mmol), piperidine (0.43mL, 4.2mmol) and dry THF (5mL). After 3 days, the mixture was concentrated *in vacuo* and the crude product purified by flash chromatography on silica gel (elution with hexane-EtOAc [1:1]) to afford, as a brick-red wax, a 5:3 diastereomeric mixture of methyl 3-hydroxy-3-(3-methoxy-2-nitrophenyl)-2-[(piperdin-1-yl)methyl]propanoate **58h** (1.1g, 76%, lit.[76] cited as brown oil) (Found **M+1**: 353.1724. Calc. for $C_{17}H_{25}N_2O_6$: MH^+, 353.1713); v_{max} (KBr)/cm^{-1} 3362 (OH) and 1743 (C=O); δ_H (400MHz; CDCl$_3$) 1.38-1.62 (6H, m, 12-, 13- and 14-CH$_2$), 2.40 (4H, m, 11- and 15-CH$_2$), 2.68 and 2.98 (2H, m, 10-CH$_2$), 3.28 (1H, m, 2-H), 3.46 (3H, s, CO$_2$CH$_3$), 3.85 (3H, s, Ar-OCH$_3$), 5.05 (1H, m, 3-H), 6.91-6.95 (2H, m, 7- and 9-H) and 7.32 (1H, t, *J*=8.4Hz, 8-H); δ_C (100MHz; CDCl$_3$) 23.8 (C-13), 25.7 (C-12 and C-14), 47.5 (C-2), 51.8 (CO$_2$CH$_3$), 54.8 (C-11 and C-15), 56.4 (Ar-OCH$_3$), 61.0 (C-10), 75.2 (C-3), 111.9 (C-7), 120.0 (C-9), 130.4 (C-8), 134.9 (C-4), 140.3 (C-4), 150.8 (C-6) and 171.4 (C=O).

Methyl 3-(6-chloro-2-nitrophenyl)-3-hydroxy-2-[(piperidin-1-yl)methyl]propanoate **58i**

The general method was followed, using methyl 3-(6-chloro-2-nitrophenyl)-3-hydroxy-2-methylenepropanoate **26i** (0.50g, 1.9mmol), piperidine (0.27mL, 2.8mmol) and dry THF (5mL). After 3 days, the mixture was concentrated *in vacuo* and the crude product purified by flash chromatography on silica gel [elution with hexane-EtOAc (3:2)] to afford, as a black oil, a 10:7 diastereomeric mixture of *methyl 3-(6-chloro-2-nitrophenyl)-3-hydroxy-2-[(piperidin-1-yl)methyl]propanoate* **58i** (0.4g, 61%) [Found **M**+1 (^{35}Cl): 357.1209. Calc. for

74

$C_{16}H_{22}N_2O_5Cl$: MH^+, 357.1217); ν_{max} KBr/cm^{-1} 3331 (OH) and 1741 (C=O); δ_H (400MHz; CDCl$_3$) 1.52 (6H, m, 12-, 13- and 14-CH$_2$), 2.49 and 2.59 (2H, 2xm, 10-CH$_2$), 3.12 (4H, m, 11- and 12-CH$_2$), 3.54 (1H, m, 2-H), 3.70 (3H, s, OCH$_3$), 5.59 (1H, d, J=8.4Hz, 3-H), 7.31 (1H, t, J=8Hz, 7-H), 7.44 (1H, d, J=8Hz, 6-H) and 7.52 (1H, d, J=8Hz, 8-H); δ_C (100MHz; CDCl$_3$) 25.7 (C-11 and C-15), 47.9 (C-12 and C-14), 48.5 (C-2), 52.0 (OCH$_3$), 54.5 (C-13), 57.6 (C-10), 70.3 (C-3), 122.8 (C-6), 128.7 (C-7), 132.9 (C-8), 133.2 (C-4), 134.9 (C-9), 151.3 (C-5) and 173.7 (C=O).

Methyl 3-hydroxy-3-(4,5-methylenedioxy-2-nitrophenyl)-2-[(piperidin-1-yl)methyl]propanoate 58j

The general method was followed, using methyl 3-hydroxy-2-methylene-3-(4,5-methylenedioxy-2-nitrophenyl)propanoate **26j** (0.51g, 1.8mmol), piperidine (0.27mL, 2.7mmol) and dry THF (5mL). After 2 days, the solvent was removed *in vacuo* and the crude product was purified by flash chromatography on silica gel [elution with hexane-EtOAc (3:2)] to afford, as a brown wax, a 4:1 diastereomeric mixture of methyl 3-hydroxy-3-(4,5-methylenedioxy-2-nitrophenyl)-2-[(piperidin-1-yl)methyl]-propanoate **58j** (0.63g, 95%), (lit.[76] m.p. 102-105°C); ν_{max} (KBr)/cm^{-1} 3338 (OH) and 1713 (C=O); δ_H (400MHz; CDCl$_3$) 1.44 (2H, m, 13-CH$_2$), 1.59 (4H, m, 12- and 14-CH$_2$), 2.50 (4H, m, 11- and 15-CH$_2$), 2.72 and 3.01 (2H,m, 10-CH$_2$), 2.94 (1H, m, 2-H). 3.47 (3H, s, CH$_3$), 5.62 (1H, d, J=8Hz, 3-H), 6.06 (2H, s, OCH$_2$O), 7.17 (1H, s, 9-H) and 7.27 (1H, s, 6-H); δ_C (100MHz; CDCl$_3$) 23.8 (C-11 and C-15), 25.7 (C-12 and C-14), 48.6 (C-2), 51.9 (CH$_3$), 54.6 (C-13), 60.2 (C-10), 72.1 (C-3), 103.8 (OCH$_2$O), 104.7 (C-6), 107.9 (C-9), 134.4 (C-4), 142.5 (C-5), 147.0 (C-8), 152.5 (C-7) and 171.2 (C=O); m/z 367(**M+1**, 2.8%) and 98 (100%).

Methyl 3-(2,4-dinitrophenyl)-3-hydroxy-2-[(piperidin-1-yl)methyl]propanoate **58k**
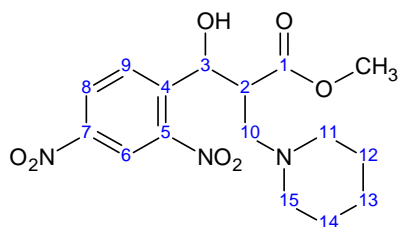
The general method was followed, using methyl 3-hydroxy-2-methylene-3-(2,4-dinitrophenyl)propanoate **26k** (0.90g, 3.2mmol), piperidine (0.47mL, 4.8mmol) and dry THF (5mL). After 3 days, the solvent was removed *in vacuo* and the crude product was purified by flash chromatography on silica gel [elution with hexane-EtOAc (3:1)] to afford, as a reddish-brown oil, a 5:2 diastereomeric mixture of methyl 3-(2,4-dinitrophenyl)-3-hydroxy-2-[(piperidin-1-yl)methyl]propanoate **58k** (0.67g, 57%), (lit.[76] cited as brown oil) (Found **M+1**: 368.1465. Calc. for $C_{16}H_{22}N_3O_7$: MH^+, 368.1458); ν_{max} (KBr)/cm^{-1} 3317 (OH) and 1723 (C=O); δ_H (400MHz; CDCl$_3$) 1.47 (2H, m, 13-CH$_2$), 1.62 (4H, m, 12- and 14-CH$_2$), 2.43 and 2.70 (2H, 2xm, 11- and 15-CH$_2$), 2.77 and 3.06 (2H, 2xm, 10-CH$_2$), 3.14 (1H, m, 2-H), 3.49 (3H, s, CH$_3$), 5.61 (1H, d, *J*=8.8Hz, 3-H), 7.91 (1H, d, *J*=8.8Hz, 9-H), 8.37 (1H, dd, *J*=8.8 and 1.6Hz, 8-H) and 8.57 (1H, d, *J*=1.6Hz, 6-H); δ_C (100MHz; CDCl$_3$) 23.7 (C-13), 25.7 (C-12 and C-14), 47.8 (C-2), 52.1 (CH$_3$), 54.8 (C-11 and C-15), 60.7 (C-10), 73.0 (C-3), 119.6 (C-6), 126.4 (C-8), 131.2 (C-9), 140.3 (C-7), 143.6 (C-4), 148.8 (C-5) and 170.9 (C=O).

Methyl 2-[(2-carbomethoxypyrrolidin-1-yl)methyl]-3-hydroxy-3-(2-nitrophenyl)propanoate **59g**
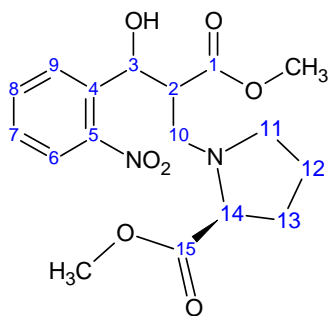
Method A: To a solution of methyl 3-hydroxy-2-methylene-3-(2-nitrophenyl)propanoate **26g** (0.54g, 2.3mmol) and L-proline methyl ester hydrochloride (0.35g, 2.2mmol) in THF (5mL) was added proton sponge (0.67g, 3.1mmol), and the resulting mixture stirred in a stoppered

flask at room temperature for 14 days. The solvent was removed *in vacuo* and the residue was washed with ethyl acetate. The insoluble proton sponge was filtered off and the ethyl acetate was evaporated from the filtrate, but ^{1}H NMR analysis of the residue showed that the desired product **59g** not formed.

Method B: A solution of methyl 3-hydroxy-2-methylene-3-(2-nitrophenyl)propanoate **26g** (0.54g, 2.3mmol), L-proline methyl ester hydrochloride (0.71g, 4.3mmol) and triethylamine (0.28mL, 2.0mmol) in methanol (5mL) was stirred for 7 days, and the reaction mixture was then concentrated *in vacuo*. Addition of ethyl acetate gave a reddish-yellow solid, which was filtered off; the filtrate was concentrated *in vacuo* and chromatographed by flash chromatography on silica gel [elution with hexane-EtOAc (3:1)] to afford three fractions comprising diastereomers of *methyl 2-[(2-carbomethoxypyrrolidin-1-yl)methyl]-3-hydroxy-3-(2-nitrophenyl)propanoate* **59g**

Fraction 1: Diastereomer **59g$_1$** as a yellow oil (89mg, 11%), (Found **M+1**: 367.1505. Calc. for $C_{17}H_{23}N_2O_7$: MH^+, 367.1505); v_{max} KBr/cm^{-1} 3424 (OH) and 1732 (C=O); δ_H (400MHz; CDCl$_3$) 1.90 (3H, m, 12-CH$_2$ and 13-CH$_A$), 2.21 (1H, m, 13-CH$_B$), 2.35 (1H, q, *J*=8.2Hz, 11-CH$_A$), 2.56 (1H, dd, *J*=3.2Hz, 10-CH$_A$), 3.25 (3H, s, 1-OCH$_3$), 3.33 (3H, m, 2-CH, 10-CH$_B$ and 14-CH), 3.50 (1H, m, 11-CH$_A$), 3.75 (3H, s, 15-OCH$_3$), 5.98 (1H, d, *J*=2.8Hz, 3-H), 7.39 (1H, t, *J*=8.6Hz, 7-H), 7.61 (1H, t, *J*=8.2Hz, 8-H), 7.87 (1H, d, *J*=8Hz, 9-H) and 8.01 (1H, d, *J*=8Hz, 6-H); δ_C (100MHz; CDCl$_3$) 23.5 (C-12), 29.3 (C-13), 49.2 (C-2), 51.0 (1-OCH$_3$), 52.3 (15-OCH$_3$) 52.4 (C-10), 53.2 (C-11), 65.7 (C-14), 66.7 (C-3), 124.4 (C-6), 127.7 (C-7), 128.7 (C-9), 133.2 (C-8), 138.2 (C-5), 147.0 (C-4), 171.9 and 176.1 (2xC=O).

Fraction 2: as a yellow oil (0.31mg, 37%), comprising a mixture of two diastereoisomers **59g$_{2,3}$** (NMR data cited for major stereoisomer) (Found **M+1**: 367.1512. Calc for $C_{17}H_{23}N_2O_7$: MH^+, 367.1505); v_{max} KBr/cm^{-1} 3410 (OH) and 1739 (C=O); δ_H (400MHz; CDCl$_3$) 1.81 (2H, m, 12-CH$_2$), 2.06 (2H, m, 11-CH$_2$), 2.57 (1H, q, *J*=7.6Hz, 13-CH$_A$), 3.02-3.35 (5H, m, 14-H, 2-H, 10-CH$_2$, and 13-CH$_B$), 3.45 (3H, s, 1-OCH$_3$), 3.64 (3H, s, 15-OCH$_3$), 5.62 (1H, d, *J*=7.2Hz, 3-H), 7.30 (1H, m, 8-H), 7.50 (1H, m, 7-H) and 7.71 (2H, d, *J*=8Hz, 6-H and 9-H); δ_C (100MHz; CDCl$_3$) 23.3 (C-12), 29.0 (C-11), 50.0 (C-2), 51.7 (2xOCH$_3$), 52.9 (C-13), 53.9 (C-10), 65.6 (C-14), 70.0 (C-3), 123.8 (C-6), 128.1 (C-8), 129.1 (C-9), 132.5 (C-7), 136.8 (C-5), 148.3 (C-4), 172.3 (C-1) and 174.0 (C-15).

***Fraction 3*:** Diastereomer **59g$_4$** as pale yellow oil (69mg, 6%), (Found **M+1**: 367.1509. Calc for C$_{17}$H$_{23}$N$_2$O$_7$: *MH$^+$*, 367.1505); v_{max} KBr/cm^{-1} 3450 (OH) and 1730 (C=O);δ_H (400MHz; CDCl$_3$) 1.86-2.01 (4H, m, 12- and 13-CH$_2$), 2.54 (1H, q, *J*=7.6-11.6Hz, 11-H$_A$), 2.85 (1H, dd, *J*=4.8Hz, 10-H$_A$), 3.15 (2H, m, 11-H$_B$ and 2-H), 3.30 (1H, m, 10-H$_B$), 3.39 (1H, m, 14-H), 3.50 (3H, s, 1-OCH$_3$), 3.67 (15-OCH$_3$), 5.45 (1H, d, *J*=3.2Hz, 3-H), 7.40 (1H, t, *J*=7.6Hz, 8-H), 7.61 (1H, t, *J*=8Hz, 7-H), 7.10 (1H, d, *J*=7.6Hz, 9-H) and 7.95 (1H, d, *J*=8Hz, 6-H); δ_C (100MHz; CDCl$_3$) 23.4 (C-12), 29.0 (C-13), 50.1 (C-2), 51.6 (2xOCH$_3$), 53.1 (C-11), 54.5 (C-10), 54.3 (C-14), 69.2 (C-3), 124.6 (C-6), 138.3 (C-8), 128.4 (C-9), 133.3 (C-7), 138.2 (C-5), 147.3 (C-4), 173.9 (C1) and 174.0 (C15).

N,N'-*Bis[2-carbomethoxy-3-hydroxy-3-(2-nitrophenyl)propyl]-1,4-piperazine* 62g
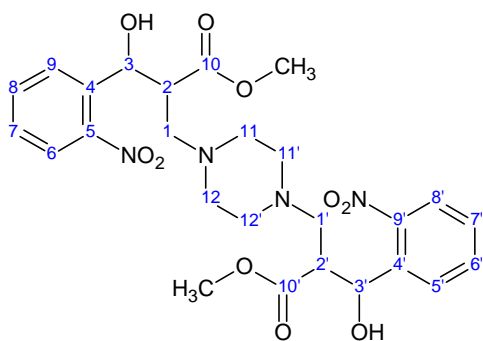
The general method was followed, using methyl 3-hydroxy-2-methylene-3-(2-nitrophenyl)-propanoate **26g** (1.30g, 5.48mmol), piperazine (0.41g, 4.78mmol) and dry THF (5mL). After 24 hours the solvent was removed *in vacuo* and the crude product was purified by flash chromatography on silica gel (elution with hexane-EtOAc [1:1]) to afford, as a yellowish-green solid, a 2:3 diastereomeric mixture of N,N'-*bis[2-carbomethoxy-3-hydroxy-3-(2-nitrophenyl)propyl]-1,4-piperazine* **62g** (0.74g, 24%), m.p. 78-82°C (Found **M+1**: 561.2214. Calc. for C$_{26}$H$_{33}$N$_4$O$_{10}$, *MH$^+$*: 561.2197); v_{max} KBr/cm^{-1} 3353 (OH) and 1732 (C=O); δ_H (400MHz; CDCl$_3$)$^{\#}$ 2.77 and 3.03 (4H, 2xm, 1- and 1'-CH$_2$), 3.26 (2H, m, 2- and 2'-H), 3.50 (6H, s, 2xOCH$_3$), 3.78 (8H, m, 11-, 11'-, 12- and 12'-CH$_2$), 5.54 (2H, d, *J*=5.2Hz, 3- and 3'-H), 7.40 (2H, t, *J*=4.8Hz, 8- and 8'-H), 7.56 (2H, t, *J*=5.0Hz, 7- and 7'-H), 7.62 (2H, d, *J*=4.4Hz, 9- and 9'-H) and 7.75 (2H, d, *J*=5.6Hz, 6- and 6'-H); δ_C (100MHz; CDCl$_3$) 47.9 (C-2 and C-2'), 52.0 (2xOCH$_3$), 52.1 (C-11, C-11', C-12 and C-12'), 59.2 (C-1 and C-1'), 73.3

(C-3 and C-3'), 124.3 (C-6 and C-6'), 128.6 (C-8 and C-8'), 129.3 (C-9 and C-9'), 132.4 (C-7 and C-7'), 136.1 (C-5 and C-5'), 149.0 (C-4 and C-4') and 171.7 (2xC=O).

NMR data cited for the major product.

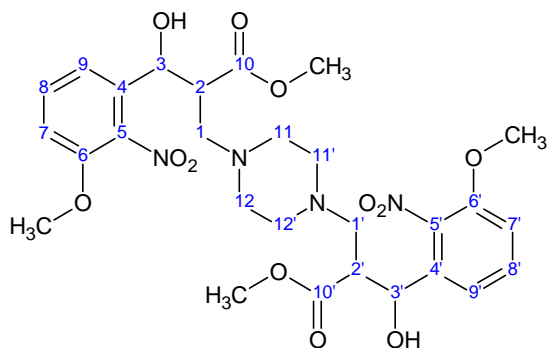**N,N'-*Bis[2-carbomethoxy-3-hydroxy-3-(3-methoxy-2-nitrophenyl)propyl]-1,4-piperazine* 62h**

The general method was followed, using methyl 3-hydroxy-2-methylene-3-(3-methoxy-2-nitrophenyl)propanoate **26h** (1.31g, 4.86mmol), piperazine (0.37g, 4.3mmol) and dry THF (5mL). After 6 hours the mixture was concentrated *in vacuo* and the crude product purified by flash chromatography on silica gel (elution with hexane-EtOAc [3:1]) to afford, as a yellow oil, a 5:3 diastereomeric mixture of N,N'-*bis[2-carbomethoxy-3-hydroxy-3-(3-methoxy-2-nitrophenyl)propyl]-1,4-piperazine* **62h** (0.12g, 4.3%) (Found **M+1**: 621.2392. Calc. for $C_{28}H_{37}N_4O_{12}$, MH^+: 621.2408); v_{max} KBr/cm^{-1} 3358 (OH) and 1730 (C=O); δ_H (400MHz; CDCl$_3$)$^{\#}$ 2.14-2.74 (10H, m, 1- and 1'-CH$_A$, 11-, 11'-, 12- and 12'-CH$_2$), 3.04 (2H, m, 1- and 1'-CH$_B$), 3.28 (2H, m, 2- and 2'-CH), 3.61(6H, 2xm, 1- and 1'-OCH$_3$), 3.85 (6H, m, 2xAr-OCH$_3$), 5.07 (2H, 2xm, 3- and 3'-CH), 6.87 (2H, 2xm, 9- and 9'-H), 6.94 (2H, m, 7- and 7'-H) and 7.31 (2H, 2xm, 8- and 8'-H); δ_C (100MHz; CDCl$_3$) 47.3 (C-2 and C-2'), 51.9 (1-and 1'-OCH$_3$), 56.6 (C-11, C-11', C-12 and C-12'), 56.4 (6- and 6'-OCH$_3$), 59.7 and 60.0 (C-10 and C-10'), 75.3 (C-3 and C-3'), 112.0 (C-7 and C-7'), 119.9 (C-9 and C-9'), 130.4 (C-8 and C-8'), 134.5 (C-4 and C-4'), 140.2 (C-5 and C-5'), 151.0 (C-6 and C-6') and 171.2 (2xC=O).
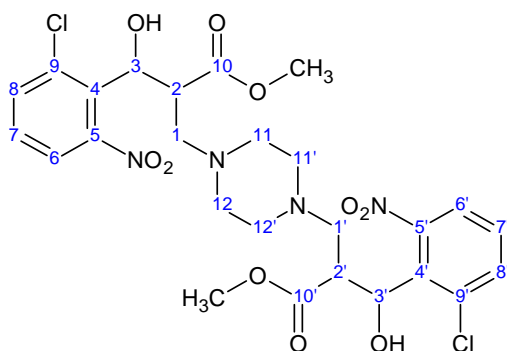
NMR data cited for the major product.

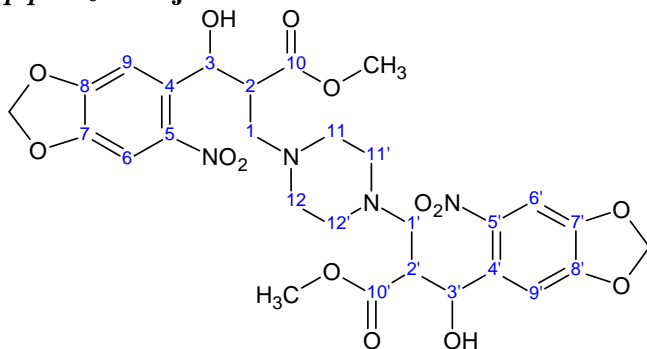**N,N'-Bis [2-carbomethoxy-3-hydroxy-3-(6-chloro-2-nitrophenyl)propyl]-1,4-piperazine 62i**

The general method was followed, using methyl 3-(6-chloro-2-nitrophenyl)-3-hydroxy-2-methylenepropanoate **26i** (0.50g, 1.9mmol), piperazine (0.24g, 2.8mmol) and dry THF (5mL). After 3 days the solvent was removed *in vacuo* and the crude product was purified by flash chromatography on silica gel [elution with hexane-EtOAc (3:2)] to afford, as a yellow solid, a 2:1 diastereomeric mixture of homodimers of N,N'-*bis[2-carbomethoxy-3-hydroxy-3-(6-chloro-2-nitrophenyl)propyl]-1,4-piperazine* **62i** (30mg, 3%), (Found **M+1**: 629.1357. Calc. for $C_{26}H_{31}N_4O_{10}Cl_2$, MH^+: 629.1417); ν_{max} KBr/cm^{-1} 3375 (OH) and 1728 (C=O); δ_H (400MHz; CDCl$_3$)$^{\#}$ 2.15 and 2.40 (8H, 2xm, 11-, 11'-, 12- and 12'-CH$_2$), 2.54 (4H, m, 1- and 1'-CH$_2$), 3.50 (6H, m, 2xOCH$_3$), 3.59 (2H, m, 2- and 2'-H), 5.58 (2H, m, 3- and 3'-H), 7.29-7.57 (6H, m, ArH); δ_C (100MHz; CDCl$_3$) 44.3 (C-2 and C-2'), 48.8 (2xOCH$_3$) 52.2 (C-11, C-11', C-12 and C-12'), 56.6 (C-1 and C-1'), 73.7 (C-3 and C-3'), 123.0 (2xArC), 128.9 (2xArC), 133.1 (C-8 and C-8'), 134.9 (C-4 and C-4'), 151.5 (C-9 and C-9'), 170.8 (C-5 and C-5') and 173.4 (2x C=O).

NMR data cited for the major product.

N,N'-Bis[2-carbomethoxy-3-hydroxy-3-(4,5-methylenedioxy-2-nitrophenyl)propyl]-1,4-piperazine 62j

The general method was followed, using methyl 3-hydroxy-2-methylene-3-(4,5-methylenedioxy-2-nitrophenyl)propanoate **26j** (0.51g, 1.83mmol), piperazine (0.23g, 2.6mmol) and dry THF (5mL). After 2 days the solvent was removed *in vacuo* and the crude product was purified using preparative thin layer chromatography on silica gel [elution with hexane-EtOAc (3:1)] to afford, as a yellow wax, a 4:1 diastereomeric mixture of N,N'-*bi-[2-carbomethoxy-3-hydroxy-3-(4,5-methylenedioxy-2-nitrophenyl)propyl]-1,4-piperazine* **62j** (40mg, 3%), (Found **M+1**: 649.1995. Calc. for $C_{28}H_{33}N_4O_{14}$, MH^+: 649.1988); ν_{max} KBr/cm^{-1} 3349 (OH) and 1730 (C=O); δ_H (400MHz; CDCl$_3$)$^{\#}$ 1.23 (8H, m, 11-, 11'-, 12- and 12'-CH$_2$), 2.77 and 2.99 (4H, 2xm, 1- and 1'-CH$_2$), 2.99 (2H, m, 2- and 2'-H), 3.54 (6H, m, 2xOCH$_3$), 5.62 (2H, m, 3- and 3'-H), 6.09 (4H, m, 2xOCH$_2$O), 7.15 (2H, s, 9- and 9'-H) and 7.33 (2H, s, 6- and 6'-H); δ_C (100MHz; CDCl$_3$) 30.1 (C-11, C-11', C-12 and C-12'), 48.8 (C-2 and C-2'), 52.5 (2xOCH$_3$), 59.1 (C-1 and C-1'), 72.3 (C-3 and C-3'), 103.3 (2xOCH$_2$O), 105.5 (C-6 and C-6'), 108.3 (C-9 and C-9'), 134.3 (C-5 and C-5'), 142.9 (C-4 and C-4'), 147.6 (C-8 and C-8'), 152.1 (C-7 and C-7') and 172.1 (2xC=O).

NMR data cited for the major product.

4-(5-Hydroxy-2-nitrophenyl)-3-[(piperidin-1-yl)methyl]but-3-en-2-one **65b**
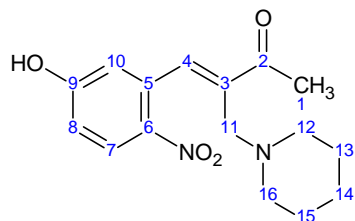
The general method was followed, using 3-chloromethyl-4-(5-hydroxy-2-nitrophenyl)but-3-en-2-one **52b** (0.5g, 2mmol), piperidine (0.25mL, 2.9mmol) and dry THF (5mL). The reaction was terminated after 6 days, and work-up and purification of the crude product by flash chromatography [elution with EtOAc] afforded, as a reddish-brown wax, *4-(5-hydroxy-2-nitrophenyl)-3-[(piperidin-1-yl)methyl]but-3-en-2-one* **65b** (0.52g, 86%)   (Found **M+1**: 305.1508. Calc. for $C_{16}H_{21}N_2O_4$, MH^+: 305.1501); ν_{max} KBr/cm^{-1} 3560 (OH) and 1732 (C=O); δ_H (400MHz; CDCl$_3$) 1.27 (6H, m, 13-, 14- and 15-CH$_2$), 2.08 (4H, m, 12- and 16-CH$_2$), 2.41 (3H, s, CH$_3$), 3.06 (2H, s, 11-CH$_2$), 2.84 (1H, br s, OH), 6.76 (1H, d, J=2.4Hz, 10-H), 6.88 (1H, dd, J=8.8 and 2.4Hz, 8-H), 7.83 (1H, s, 4-H) and 8.10 (1H, d, J=9.2Hz, 7-H); δ_C (100MHz; CDCl$_3$) 23.6 (C-14), 25.4 (C-13 and C-15), 26.6 (C-1), 53.1 (C-11), 53.5 (C-12

and C-16), 115.6 (C-8), 116.9 (C-10), 127.6 (C-7), 135.0 (C-6), 137.5 (C-5), 138.1 (C-3), 139.3 (C=CH), 163.5 (C-9) and 199.6 (C=O).
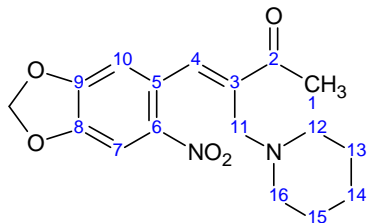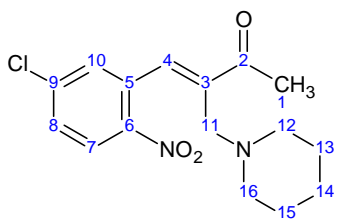
4-(4,5-Methylenedioxy-2-nitrophenyl)-3-[(piperidin-1-yl)methyl]but-3-en-2-one **65c**

The general method was followed, using 3-chloromethyl-4-(4,5-methylenedioxy-2-nitrophenyl)but-3-en-2-one **52c** (0.81g, 2.8mmol), piperidine (0.42mL, 4.2mmol) and dry THF (5mL). After 3 days, the mixture was concentrated *in vacuo* and the crude product purified by flash chromatography on silica gel (elution with hexane-EtOAc [3:2]) to afford, as a reddish-brown wax, *4-(4,5-methylenedioxy-2-nitrophenyl)-3-[(piperidin-1-yl)methyl]but-3-en-2-one* **65c** (0.75g, 79%) (Found **M+1**: 333.1465. Calc. for $C_{17}H_{21}N_2O_5$, MH^+: 333.1450); v_{max} KBr/cm^{-1} 1669 (C=O); δ_H (400MHz; CDCl$_3$) 1.30 (2H, m, 14-CH$_2$) 1.38 (4H, m, 13- and 15-CH$_2$), 2.18 (4H, m, 12- and 16-CH$_2$), 2.46 (3H, s, CH$_3$), 3.09 (2H, s, 11-CH$_2$), 6.14 (2H, s, OCH$_2$O), 7.31 (1H, s, 10-H), 7.62 (1H, s, 7-H) and 7.78 (1H, s, 4-H); δ_C (100MHz; CDCl$_3$) 24.1 (C-14), 26.0 (C-13 and C-15), 26.6 (C-1), 53.1 (C-11), 53.9 (C-12 and C-16), 103.2 (OCH$_2$O), 105.3 (C-7), 110.5 (C-10), 128.7 (C-6), 139.1 (C-4), 139.2 (C-3), 141.9 (C-5), 147.8 (C-9), 151.7 (C-8) and 200.0 (C=O).

4-(5-Chloro-2-nitrophenyl)-3-[(piperidin-1-yl)methyl]but-3-en-2-one **65f**

The general method was followed, using 3-chloromethyl-4-(5-chloro-2-nitrophenyl)but-3-en-2-one **52f** (0.8g, 3mmol), piperidine (0.43mL, 4.4mmol), and dry THF (5mL). The reaction was terminated after 6 days; work-up and purification of the crude product by flash chromatography [elution with hexane-EtOAc (3:2)] afforded, as a reddish-brown wax, *4-(5-chloro-2-nitrophenyl)-3-[(piperidin-1-yl)methyl]but-3-en-2-one* **65f** (0.85g, 90%) (Found

M+1: 323.1161. Calc. for $C_{16}H_{20}N_2O_3Cl$, MH^+: 323.1162); v_{max} KBr/cm^{-1} 1673 (C=O); δ_H (400MHz; CDCl$_3$) 1.30-1.40 (6H, m, 13-, 14- and 15-CH$_2$), 2.16 (4H, m, 12- and 16-CH$_2$), 2.48 (3H, s, CH$_3$), 3.07 (2H, s, 11-CH$_2$), 7.44 (1H, dd, *J*=2.4 and 8.4Hz, 8-H), 8.0 (1H, s, 4-H), 7.94 (1H, d, *J*=2.4Hz, 10-H) and 8.08 (1H, d, *J*=8.8Hz, 7-H); δ_C (100MHz; CDCl$_3$) 24.0 (C-14), 26.0 (C-12 and C-15), 26.4 (C-1), 53.1 (C-11), 53.9 (C-12 and C-16), 125.9 (C-7), 128.9 (C-8), 131.8 (C-10), 133.7 (C-5), 137.4 (C-4), 139.6 (C-9), 140.8 (C-3), 145.8 (C-6) and 199.4 (C=O).

3-[(2-Carbomethoxypyrrolidin-1-yl)methyl]-4-(4,5-methylenedioxy-2-nitrophenyl)but-3-en-2-one **66c**
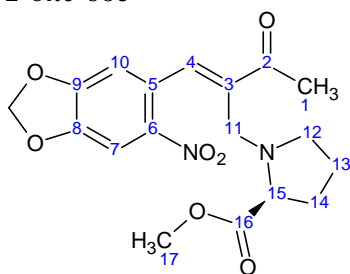
The general method was followed, using 3-chloromethyl-4-(4,5-methylenedioxy-2-nitrophenyl)but-3-en-2-one **52c** (0.80g, 2.8mmol), L-proline methyl ester hydrochloride (0.93g, 5.6mmol), triethylamine (0.79mL, 5.6mmol) and methanol (5mL). After stirring for 7 days, work-up and flash chromatography of the crude product on silica gel [elution with hexane-EtOAc (1:1)] afforded, as a brown oil, *3-[(2-carbomethoxypyrrolidin-1-yl)methyl]-4-(4,5-methylenedioxy-2-nitrophenyl)but-3-en-2-one* **66c** (0.61g, 57%) (Found **M+1**: 377.1342. Calc. for $C_{18}H_{21}N_2O_7$, MH^+: 377.1349); v_{max} KBr/cm^{-1} 1734 (C=O); δ_H (400MHz; CDCl$_3$) 1.77 (3H, m, 13-CH$_2$ and 14-CH$_A$), 1.99 (1H, m, 14-CH$_B$), 2.29 and 2.87 (2H, 2xm, 12-CH$_2$), 2.47 (2H, s, 1-CH$_3$), 3.15 (1H, m, 15-CH), 3.34 and 3.52 (2H, 2xd, *J*=12.4Hz, 11-CH$_2$), 3.61 (3H, s, 17-CH$_3$), 6.17 (2H, s, OCH$_2$O), 7.33 (1H, s, 10-H), 7.64 (1H, s, 7-H) and 7.80 (1H, s, 4-H); δ_C (100MHz; CDCl$_3$) 23.2 (C-13), 26.4 (C-1), 29.6 (C-14), 48.0 (C-11), 51.5 (C-17), 53.0 (C-12), 64.7 (C-15), 103.23 (OCH$_2$O), 105.5 (C-7), 110.9 (C-10), 128.1 (C-6), 138.7 (*C*=CH), 139.2 (C=*C*H), 141.9 (C-5), 148.1 (C-9), 151.9 (C-8), 174.7 (C-16) and 199.8 (C-2).
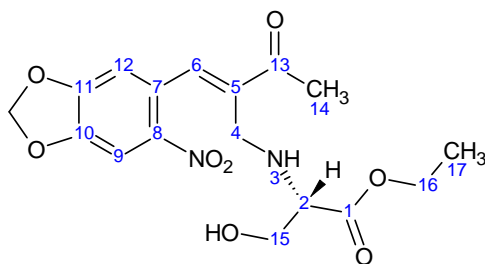
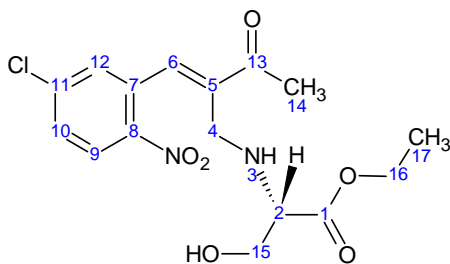***Ethyl 5-acetyl-2-hydroxymethyl-6-(4,5-methylenedioxy-2-nitrophenyl)-3-aza-5-hexenoate 67c***

The general method was followed, using 3-chloromethyl-4-(4,5-methylenedioxy-2-nitrophenyl)but-3-en-2-one **52c** (0.6g, 2.1mmol), L-serine ethyl ester hydrochloride (0.72g, 4.2mmol) and triethylamine (0.60mL, 4.2mmol) in methanol (5mL). After 4 days, work-up and purification of the crude product by flash chromatography [elution with hexane-EtOAc (1:1)] afforded, as a reddish-brown wax, *ethyl 5-acetyl-6-(4,5-methylenedioxy-2-nitrophenyl)-2-hydroxymethyl-3-aza-5-hexenoate* **67c** (0.4g, 52%) (Found **M+1**: 381.1331. Calc. for $C_{17}H_{21}N_2O_8$, MH^+: 381.1298); δ_H (400MHz; CDCl$_3$) 1.23 (3H, m, 17-CH$_3$), 2.46 (3H, s, 14-CH$_3$), 2.74 (2H, m, 15-OH and NH), 3.21 (1H, m, 2-H), 3.34 and 3.47 (2H, 2xm, 4-CH$_2$), 3.55 and 3.68 (2H, 2xm, 15-CH$_2$), 4.08 (2H, m, 16-CH$_2$), 6.16 (1H, s, OCH$_2$O), 7.00 (1H, s, 12-H), 7.67 (1H, s, 9-H) and 7.83 (1H, s, 6-H); δ_C (100MHz; CDCl$_3$) 14.1 (C-17), 25.0 (C-14), 43.5 (C-4), 62.2 (C-15), 62.5 (C-2), 63.2 (C-16), 103.5 (OCH$_2$O), 105.7 (C-9), 109.7 (C-12), 127.6 (C-10), 138.8 (C-11), 140.0 (C-6), 141.6 (C-8), 148.5 (C-7), 152.2 (C-5), 172.4 and 200.0 (2xC=O).

Ethyl 5-acetyl-6-(5-chloro-2-nitrophenyl)-2-hydroxymethyl-3-aza-5-hexenoate 67f

The general method was followed, using 3-chloromethyl-4-(5-chloro-2-nitrophenyl)but-3-en-2-one **52f** (0.41g, 1.5mmol), L-serine ethyl ester hydrochloride (0.50g, 3.0mmol), and triethylamine (0.41mL, 3.0mmol) in methanol (5mL). After 7 days, work-up and purification of the crude product by flash chromatography [elution with EtOAc] afforded, as a reddish-brown wax, *ethyl 5-acetyl-6-(5-chloro-2-nitrophenyl)-2-hydroxymethyl-3-aza-5-hexenoate*

67f (0.21g, 36%), (Found **M+1**: 371.1023. Calc. for $C_{16}H_{20}N_2O_6Cl$, *MH*[+]: 371.1010); v_{max} KBr/cm[-1] 3349 (OH) and 1728 (C=O); δ_H (400MHz; CDCl$_3$) 1.21 (3H, m, 17-CH$_3$), 2.47 (3H, s, 14-CH$_3$), 2.77 (2H, br s, 15-OH and NH), 3.19 (1H, m, 2-H), 3.29 and 3.44 (2H, 2xm, 4-CH$_2$), 3.56 and 3.66 (2H, 2xm, 15-CH$_2$), 4.13 (2H, m, 16-CH$_2$), 7.50 (1H, d, *J*=8.8Hz, 10-H), 7.61 (1H, s, 12-H), 7.81 (1H, s, 6-H) and 8.13 (1H, d, *J*=8.8Hz, 9-H); δ_C (100MHz; CDCl$_3$) 14.1 (C-17), 26.0 (C-14), 43.6 (C-4), 61.2 (C-16), 62.4 (C-15), 62.6 (C-2), 126.5 (C-9), 129.7 (C-10), 131.1 (C-12), 132.7 (C-8), 137.7 (C-6), 140.2 and 140.3 (C-5 and C-7), 145.4 (C-11), 172.3 and 199.5 (2xC=O).

2-Acetyl-1-(5-hydroxy-2-nitrophenyl)-8-phenyl-4-aza-7-thia-1-octene **68b**

The general method was followed, using 3-chloromethyl-4-(5-hydroxy-2-nitrophenyl)but-3-en-2-one **58b** (0.93g, 3.3mmol), *S*-benzylcysteamine hydrochloride (1.3g, 6.6mmol), triethylamine (0.92mL, 6.6mmol) and methanol (5mL). After 7 days, work-up and purification of the crude product by flash chromatography on silica gel [elution with hexane-EtOAc (3:2)] afforded reddish-brown wax (0.24g), but ^{1}H NMR analysis showed only a trace amount of the desired product 2-acetyl-1-(5-hydroxy-2-nitrophenyl)-8-phenyl-4-aza-7-thia-1-octene **68b**; no purification was performed due to time constraints.

Attempted preparation of *2-Acetyl-1-(5-chloro-2-nitrophenyl)-8-phenyl-4-aza-7-thia-1-octene* **68f**
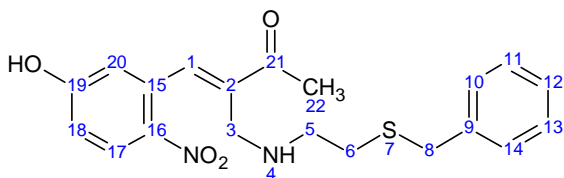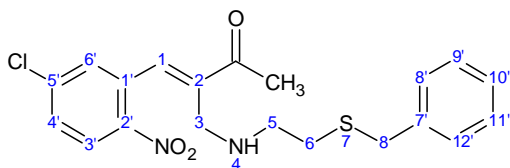

The general method was followed, using 3-chloromethyl-4-(5-chloro-2-nitrophenyl)but-3-en-2-one **52f** (0.81g, 3.0mmol), *S*-benzylcysteamine hydrochloride (1.2g, 5.9mmol) and triethylamine (0.83mL, 5.9mmol) in methanol (5mL). After 4 days, work-up and purification of the crude product by flash chromatography (elution with EtOAc) gave a reddish-brown

wax, but ^{1}H NMR analysis of the wax failed to indicate the presence of the expected product **68f**.

3.7 Reductive cyclization of Baylis-Hillman products to quinoline derivatives.

2-Methyl-6,7-methylenedioxy-3-[(piperidin-1-yl)methyl]quinoline **69c**
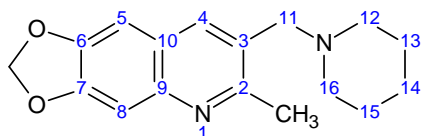
To a solution of 4-(4,5-methylenedioxy-2-nitrophenyl)-3-[(piperidin-1-yl)methyl]but-3-en-2-one **65c** (0.2g, 0.6mmol) and ethanol (50mL), 10% Pd-C catalyst (24mg, 0.18mmol) was added and the reaction mixture was stirred under a hydrogen atmosphere for 1.5 hours. The reaction mixture was filtered and the filtrate concentrated *in vacuo* to afford, as a light yellow solid, *2-methyl-6,7-methylenedioxy-3-[(piperidin-1-yl)methyl]quinoline* **69c** (21mg, 12%), m.p. 201-203°C (Found **M+1**: 285.1649. Calc. for $C_{17}H_{21}N_2O_2$, *MH*$^+$: 285.1603); δ_H (400MHz; CDCl$_3$) 1.43 (2H, m, 14-CH$_2$), 1.53 (4H, m, 13- and 15-CH$_2$), 2.38 (4H, m, 12- and 16-CH$_2$), 2.70 (3H, s, CH$_3$), 3.47 (2H, s, 11-CH$_2$), 6.11 (2H, s, OCH$_2$O), 7.00 (1H, s, 5-H), 7.44 (1H, s, 4-H) and 8.10 (1H, s, 8-H); δ_C (100MHz; CDCl$_3$) 14.4 (CH$_3$), 24.3 (C-14), 25.9 (C-13 and C-15), 54.5 (C-12 and C-16), 61.2 (C-11), 97.7 (C-8), 102.1 (OCH$_2$O), 103.0 (C-5), 124.6 (C-9), 125.5 (C-4), 130.1 (C-2), 138.0 (C-10), 145.8 (C-3), 148.6 (C-7) and 151.2 (C-6).

Attempted preparation of *3-[(2-carbomethoxy-1-pyrrolidinyl)]-2-methyl-6,7-methylene-dioxyquinoline* **70c**
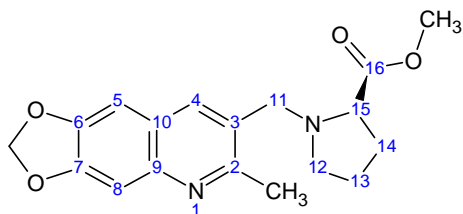
The procedure described for the synthesis of 2-methyl-6,7-methylenedioxy-3-[(piperidin-1-yl)methyl]quinoline **69c** was followed, using 3-[(2-carbomethoxypyrrolidin-1-yl)methyl]-4-(4,5-methylenedioxy-2-nitrophenyl)but-3-en-2-one **66c** (0.2g, 0.5mmol), 10% Pd-C catalyst (18mg, 0.16mmol), ethanol (50mL) and hydrogen gas. The reaction was terminated after

stirring for 3 hours; the reaction mixture was filtered and the filtrate concentrated *in vacuo* to afford a brown oil (crude yield, 18%). The TLC analysis showed the presence of starting material and other components and the ^{1}HNMR spectrum indicated the presence of a complex mixture, which could not be purified due to time constraints.

Attempted preparation of *3-[(1-carbethoxy-2-hydroxyethylamino)methyl]-2-methyl-6,7-methylenedioxyquinoline* **71c**
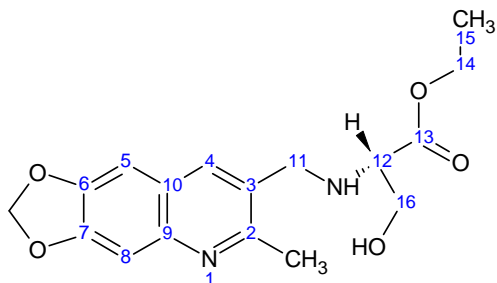
The procedure described for the synthesis of 3-[(1-carbethoxy-3-hydroxy-2-propylamino)methyl]2-methyl-6,7-methylenedioxyquinoline **69c** was followed, using 3-[(1-carbethoxy-3-hydroxy-2-propylamino)methyl]-4-(4,5-methylenedioxy-2-nitrophenyl)but-3-en-2-one **67c** (0.2g, 0.5mmol), 10% Pd-C catalyst (28mg, 0.26mmol), ethanol (50mL) and hydrogen gas. The reaction was terminated after stirring for 1.5 hours; the reaction mixture was filtered and filtrate concentrated *in vacuo* to afford a reddish-brown oil (crude yield, 23%). TLC analysis revealed multiple components and the ^{1}HNMR spectrum indicated the presence of a complex mixture, which could not be purified due to the time constraints.

3.8 Dehydration of Baylis-Hillman products to afford cinnamate esters

Methyl (E)-3-(2-nitrophenyl)-2-[(piperidin-1-yl)methyl]prop-2-enoate **72g** and *methyl (Z)-3-(2-nitrophenyl)-2-[(piperidin-1-yl)methyl]prop-2-enoate* **73g**
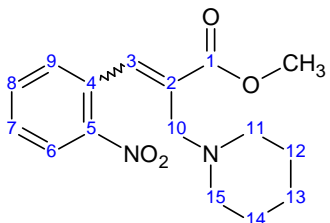
A mixture of methyl 3-hydroxy-3-(2-nitrophenyl)-2-[(piperidin-1-yl)methyl]propanoate **58g** (0.22g, 0.62mmol), EDC (2.7mL, 1.2mmol), copper(II) chloride (0.90mg, 0.06mmol) and dry

toluene (10mL) was boiled under reflux for 3 hours. The reaction was quenched with water and the resulting mixture extracted with ethyl acetate. The organic layer was washed sequentially with aqueous citric acid, aqueous sodium hydrogen carbonate and brine, and dried with anhydrous $MgSO_4$. The EtOAc was removed *in vacuo* and the residue was chromatographed [preparative layer chromatography; elution with hexane-EtOAc (3:1)] to afford, as a yellow oil, a 1:2.7 mixture of the *E* and *Z* distereoisomers of *methyl 3-(2-nit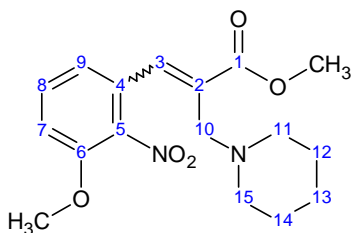rophenyl)-2-[(piperidin-1-yl)methyl]prop-2-enoate* **72g** and **73g** (95mg, 50%) (Found **M+1**: 305.1501. Calc. for $C_{16}H_{21}N_2O_4$, MH^+: 305.1501); ν_{max} KBr/cm^{-1} 1717 (C=O); δ_H (400MHz; CDCl$_3$)$^\#$ 1.41 (2H, m, 13-CH$_2$), 1.55 (4H, m, 12- and 14-CH$_2$), 2.48 (4H, m, 11- and 15-CH$_2$), 3.28 (2H, s, 10-CH$_2$), 3.46 (3H, s, CH$_3$), 2.22 (1H, d, *J*=7.6Hz, 9-H), 7.26 (1H, s, C=CH), 7.42 (1H, t, *J*=7.8Hz, 7-H), 7.53 (1H, t, *J*=7.4Hz, 8-H), and 8.08 (1H, d, *J*=8Hz, 6-H); δ_C (100MHz; CDCl$_3$) 24.2 (C-13), 26.0 (C-12 and C-14), 51.4 (CH$_3$), 54.4 (C-11 and C-15), 61.3 (C-10), 124.4 (C-6), 128.3 (C-7), 130.5 (C-9), 131.7 (C-2), 133.0 (C-8), 133.4 (C-5), 133.7 (C=*C*H), 147.0 (C-4) and 168.0 (C=O).

NMR data cited for the major product.

***Methyl (E)-3-(3-methoxy-2-nitrophenyl)-2-[(piperidin-1-yl)methyl]prop-2-enoate* 72h** and
***methyl (Z)-3-(3-methoxy-2-nitrophenyl)-2-[(piperidin-1-yl)methyl]prop-2-enoate* 73h**

The procedure described for the synthesis of *E* and *Z* distereoisomers of methyl 3-(2-nitrophenyl)-2-[(piperidin-1-yl)methyl]prop-2-enoate **72g** and **73g** was followed, using methyl 3-hydroxy-3-(3-methoxy-2-nitrophenyl)-2-[(piperdin-1-yl)methyl]propanoate **58h** (0.20g, 0.57mmol), EDC (0.25mL, 1.7mmol), copper(II) chloride (23mg, 0.17mmol) and toluene (10mL). After work up, the crude product was purified by preparative layer chromatography [elution with hexane-EtOAc (3:1)] to afford, as a reddish-brown oil, a 1:7.2 mixture of the *E* and *Z* distereoisomers of *methyl 3-(3-methoxy-2-nitrophenyl)-2-[(piperidin-1-yl)methyl]prop-2-enoate* **72h** and **73h** (30mg, 16%) (Found **M+1**: 335.1592. Calc. for $C_{17}H_{23}N_2O_5$, MH^+: 335.1607); ν_{max} KBr/cm^{-1} 1728 (C=O); δ_H (400MHz; CDCl$_3$)$^\#$ 1.14 (2H, m, 13-CH$_2$), 1.53 (4H, m, 12- and 14-CH$_2$), 2.39 (4H, m, 11- and 15-CH$_2$), 3.32 (2H, s, 10-CH$_2$),

3.57 (3H, s, CO$_2$CH$_3$), 3.39 (3H, s, Ar-OCH$_3$), 6.79 (1H, s, 4-H), 6.83 (1H, d, *J*=7.6Hz, 9-H), 8.94 (1H, d, *J*=8.4Hz, 7-H), and 7.39 (1H, t, *J*=8.0Hz, 8-H) ; δ$_C$ (100MHz; CDCl$_3$) 24.2 (C-13), 25.9 (C-12 and C-14), 51.7 (CO$_2$*C*H$_3$), 54.7 (C-11 and C-15), 56.4 (Ar-OCH$_3$), 61.7 (C-10), 111.7 (C-7), 120.6 (C-9), 128.4 (C=*C*H), 130.7 (C-8), 131.0 (C-5),136.5 (*C*=CH), 140.0 (C-4), 150.8 (C-6) and 168.1 (C=O).

NMR data cited for the major product.

Methyl (E)-3-(4,5-methylenedioxy-2-nitrophenyl)-2-[(piperidin-1-yl-)methyl]prop-2-enoate 72j and **methyl (Z)-3-(4,5-methylenedioxy-2-nitrophenyl)-2-[(piperidin-1-yl-)methyl]prop-2-enoate 73j**
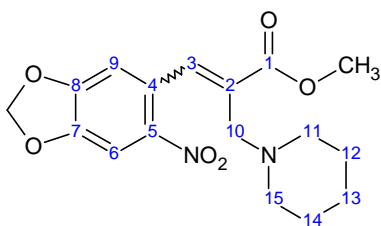

The procedure described for the synthesis of *E* and *Z* disterioisomers of methyl 3-(2-nitrophenyl)-2-[(piperidin-1-yl)methyl]prop-2-enoate **72g** and **73g** was followed, using methyl 3-hydroxy-3-(4,5-methylenedioxy-2-nitrophenyl)-2-[(piperidin-1-yl)methyl]propan-oate **58j** (0.20g, 0.56mmol), EDC (0.36mL, 1.6mmol), copper(II) chloride (15mg, 0.11mmol) and tolutene (10mL). After work up, the crude product was purified by preparative layer chromatography [elution with hexane-EtOAc (3:2)] to afford, as a light yellow oil, a 1:3.9 mixture of the *E* and *Z* disterioisomers of *methyl 3-(4,5-methylenedioxy-2-nitrophenyl)-2-[(piperidin-1-yl)methyl]prop-2-enoate* **72j** and **73j** (15mg, 8%) (Found **M+1**: 349.1394. Calc. for C$_{17}$H$_{21}$N$_2$O$_6$, *MH$^+$*: 349.1400); *ν*$_{max}$ KBr/cm^{-1} 1719 (C=O); δ$_H$ (400MHz; CDCl$_3$)$^{#}$ 1.24 (2H, m, 13-CH$_2$), 1.57 (4H, m, 12- and 14-CH$_2$), 2.50 (4H, m, 11- and 15-CH$_2$), 3.30 (2H, s, 10-CH$_2$), 3.56 (3H, s, OCH$_3$), 6.10 (2H, s, OCH$_2$O), 6.62 (1H, s, 9-H), 7.22 (1H, s, C=CH) and 7.62 (1H, s, 6-CH); δ$_C$ (100MHz; CDCl$_3$) 25.9 (C-12 and C-14), 29.7 (C-13), 51.7 (OCH$_3$), 54.4 (C-11 and C-15), 61.3 (C-10), 103.1 (OCH$_2$O), 105.2 (C-6), 109.2 (C-9), 130.3 (C-5), 131.7 (*C*=CH), 135.0 (C=*C*H), 141.1 (C-4), 147.5 (C-8), 151.8 (C-7) and 167.9 (C=O).

NMR data cited for the major product.

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