Studies towards the synthesis of Novel, Coumarin-based HIV-1 Protease Inhibitors

Thesis

Submitted in fulfillment of the

requirements for the degree

of

MASTERS OF SCIENCE

of Rhodes University

by

THOMPHO JASON RASHAMUSE
B.Sc (University of Venda)
B.Sc Hons (University of Limpopo)

January 2008

Department of chemistry

Rhodes University

Grahamstown

ABSTRACT

A series of the Baylis-Hillman adducts have been obtained by reacting protected *O*-benzylated and unprotected substituted salicylaldehydes with methyl acrylate or *tert*-butyl acrylate, respectively, using DABCO as catalyst. Treatment of the Baylis-Hillman adducts with HCl in a mixture of acetic acid and acetic anhydride afforded the corresponding 3-(chloromethyl)coumarin derivatives with yields of up to 94%. Similar use of HI afforded the corresponding 3-(iodomethyl)coumarins but, depending on the reaction time, the reduced 3-methyl analogues could also be obtained.

Arbuzov reactions of the 3-(halomethyl)coumarin derivatives have been undertaken to afford 4-phosphorylated and 1'-phosphorylated derivatives, regioselectivity being dependent on the halide-leaving group. The 3-(chloromethyl)coumarin derivatives have been subjected to nucleophilic (S_N) attack by benzylamine to give the corresponding 3-[(benzylamino)methyl]coumarin derivatives in yields of up to 74%. Further treatment of the 3-[(benzylamino)methyl]coumarin derivatives with chloroacetyl chloride afforded the chloroacetamide derivatives, which exhibit hindered rotation about the amine C(O)-N bond. The acetamide derivatives have also been subjected to Arbuzov reaction conditions to afford the phosphorylated derivatives in yields of up to 86%.

In a preliminary modelling study, hydrolysed analogues of the synthesized phosphorylated derivatives have been docked into the active site of the HIV-1 protease enzyme using the Cerius-2 Ligandfit software module to provide an insight into potential receptor-ligand hydrogen bonding interactions.

ACKNOWLEGEMENTS

I wish to express my gratitude to my supervisor, Prof P.T. Kaye, for his guidance, valuable ideas, and excellent support during the course of this study. It was a privilege and I feel honored to have worked under his supervision. I have gained much invaluable experience through this study and his help has been appreciated.

To my mom, dad, sisters, brothers, aunt, grandma and all my relatives, I thank you very much guys for your love, guidance, encouragement, moral and financial support through my entire studies. You are the best guys.

Special thanks to Dr Rosa Klein for insightful discussions and critical reading of my work. It would not have been possible without her assistance and guidance.

I would like to thank Mr. Andy Soper for technical support with the NMR spectrometer, Mr. Kevin Lobb for his help with molecular modelling studies, Mr. Aubrey Sonemann for collecting low-resolution mass spectra and Dr Johan Jordaan (North-West University, Potchefstroom Campus) for collecting high-resolution MS data. I wish to thank Rhodes University Chemistry Department staff and secretaries for all their help.

I owe thanks to my F22 lab colleagues for making the time in the laboratory enjoyable and all the chemistry postgraduate students.

I would like to thank my honours supervisor, Prof A.C Gelebe for his guidance and encouragement.

I would like to thank all my friends especially from Gavin Relly Postgraduate village and Oakdene House for kind help during my stay.

I dedicate my life to God almighty. Lastly, I would like to dedicate special thanks to the National Research Foundation for financial assistance during my studies.

TABLE OF CONTENTS

Page

1. INTRODUCTION

1.1. A brief overview of HIV/AIDS

The Human Immunodeficiency virus (HIV) is a retrovirus that causes Acquired Immunodeficiency Syndrome (AIDS). The virus has a single-stranded RNA genome which replicates to double stranded DNA by a catalytic reverse transcriptase. A unique feature of HIV is that the virus first attaches to the surface of the T-helper lymphocyte cells *via* glycoprotein120 (gp120) before infecting them. These cells play a crucial role in the immune system by coordinating the action of the rest of the immune system. The virus uses the energy and the nutrients of lymphocyte cells to make copies of itself before destroying the cell. The reduction in the number of the T-helper cells seriously weakens the immune system.[1,2]

Today, AIDS remains a serious health problem worldwide. In December 2006 it was estimated that 3.6–6.6 million people were suffering from HIV/AIDS. While more than 2.9 million had died since the beginning of that year and an estimated 4.3 million are infected every year.[3]

1.2. Structure of HIV-1

A schematic drawing of the matured virion is shown in **Figure 1**.[4] The shape of virion is spherical and its length is about 100 nm.[5]

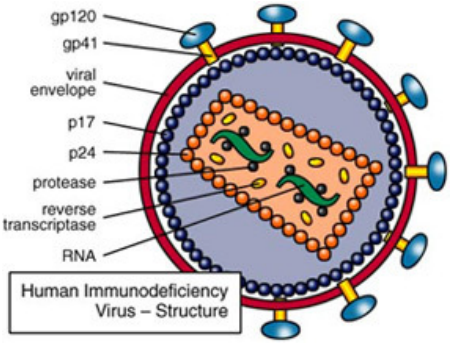


Figure 1. Schematic drawing of the mature virion[4] (re-produced by permission).

The virion consists of nine genes; the gap, pol and env genes have the necessary information required to produce structural protein, while the tat, rev, nef, vif, vpr and vpu genes are responsible for reproduction of the virus and infecting the host cells. The virion has a bilayer membrane that is derived from the infected host cell. The outer surface is surrounded by many little spikes, comprising glycoproteins (gp120) that are anchored to the transmembrane protein (gp41). The glycoprotein plays an important role in the entry of the HIV into the host cell. The conical capsid located at the centre of the virion contains the genetic material. Two copies of the viral RNA genome are within the capsid, which also contains the three enzymes essential for HIV replication, *viz*., reverse transcriptase (RT), protease (PR) and integrase.[5,6,7]

1.3. Life cycle of HIV-1

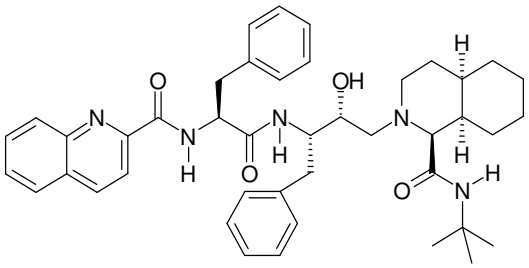
Once HIV enters the body, it attaches to the CD4 receptors [*via* glycoprotein 120 (gp120)] and other co-receptor cells within the system, allowing the HIV-1 to fuse into the cell and release genetic RNA and essential enzymes.[7,8] The HIV-1 enzyme, reverse transcriptase, transcribes the single-stranded RNA into a double-stranded viral DNA molecule, which migrates into the host-cell nucleus where it is integrated into the host genome by the enzyme, integrase. The proviral DNA is transcribed into mRNA and the viral proteins are produced in the cytoplasm. Finally, the enzyme, protease (PR), cleaves viral polyproteins to afford mature proteins, permitting budding and release of new virions so spreading the disease. [8,9,10,11]

1.4. The current strategies for HIV-1 treatment

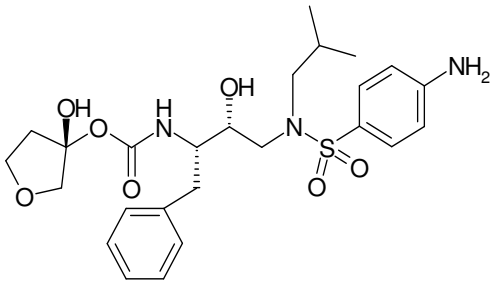
A number of anti-viral therapies have been developed, but the problems of drug resistance and cross-resistant mutants remain. There are several HIV inhibitors, which work by interfering with different stages of the life cycle of the virus; these include fusion or entry inhibitors, [12] protease inhibitors (PIs) [9,13] and reverse transcriptase inhibitors (RTIs).[14,15] The fusion inhibitors work by binding to HIV glycoprotein 120,

which prevents fusion with the cell. The RTIs are used to inhibit the viral reverse transcriptase needed to convert viral RNA to DNA. At present several RTIs have been developed and are in clinical use. The main classes of RTIs are nucleoside reverse transcriptase inhibitors (NRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTI).

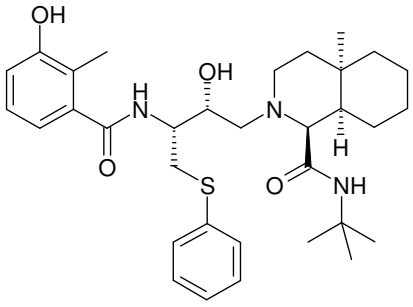
A numbers of HIV-1 protease inhibitors are currently available. These compounds prevent cleavage of long polypeptide chains and formation of new virions. The PIs work by binding to the active site of HIV-1 protease. At present, the Food and Drugs Administration (FDA) in the United States have approved seven drugs as inhibitors for HIV-1 PR.



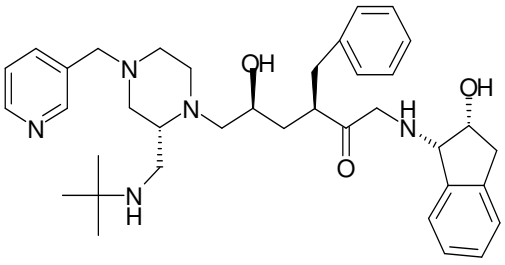
Saquinavir (K_i= 0.12nM) [16,17]



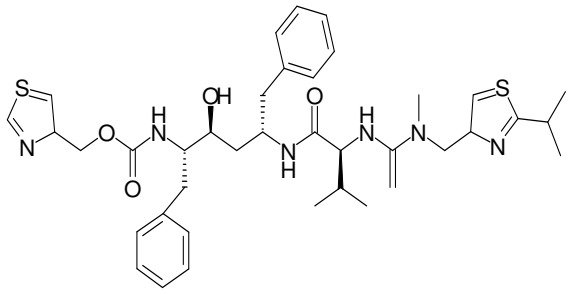
Amprenavir (K_i=0.6nM) [18]



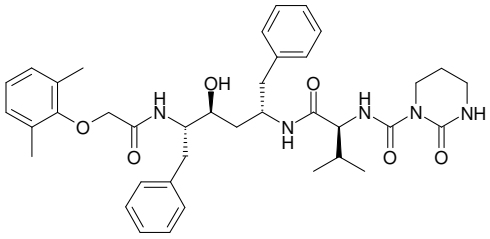
Nelfinavir (K_i=2nM) [19,20]



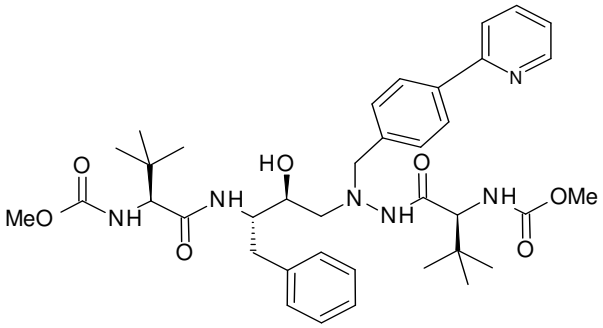
Indinavir (K_i=0.56nM) [21]



Ritonavir (K_i=0.01nM) [21,22]



Lopinavir (K_i=0.003nM) [21,22]



Atazanavir[23]

FDA approved HIV-1 protease inhibitors with their inhibition potentials (K_i).

In 1995, Saquiniavir was approved by the FDA.[16,17] This was followed by Indinavir [21] in 1996, Nelfinavir [19,20] in 1997, Amprenavir [16] in 1999 and Lopinavir [22] in September 2000. The latest HIV-1 protease inhibitor, Atazanavir [23] was developed by Boehringer Ingelheim and was approved by the FDA in 2003. Although these inhibitors are now available, there is still a demand for new HIV-1 protease inhibitors because the present inhibitors have undesirable side effects and the virus is beginning to develop resistance to them.

1.5. HIV-1 protease enzyme

By definition, HIV-1 protease is an enzyme that cleaves long polypeptide chains into smaller proteins, which are essential for the formation of new HIV virions. The HIV-1 protease enzyme (HIV-1 PR) is a homo-dimer containing two identical polypeptide chains of 99 amino acids each. Aspartyl residues are involved in the cleavage mechanism and, hence, the protein is an aspartyl protease enzyme. HIV-1 protease has one active site, and the structure of the enzyme is shown in Figure 2.

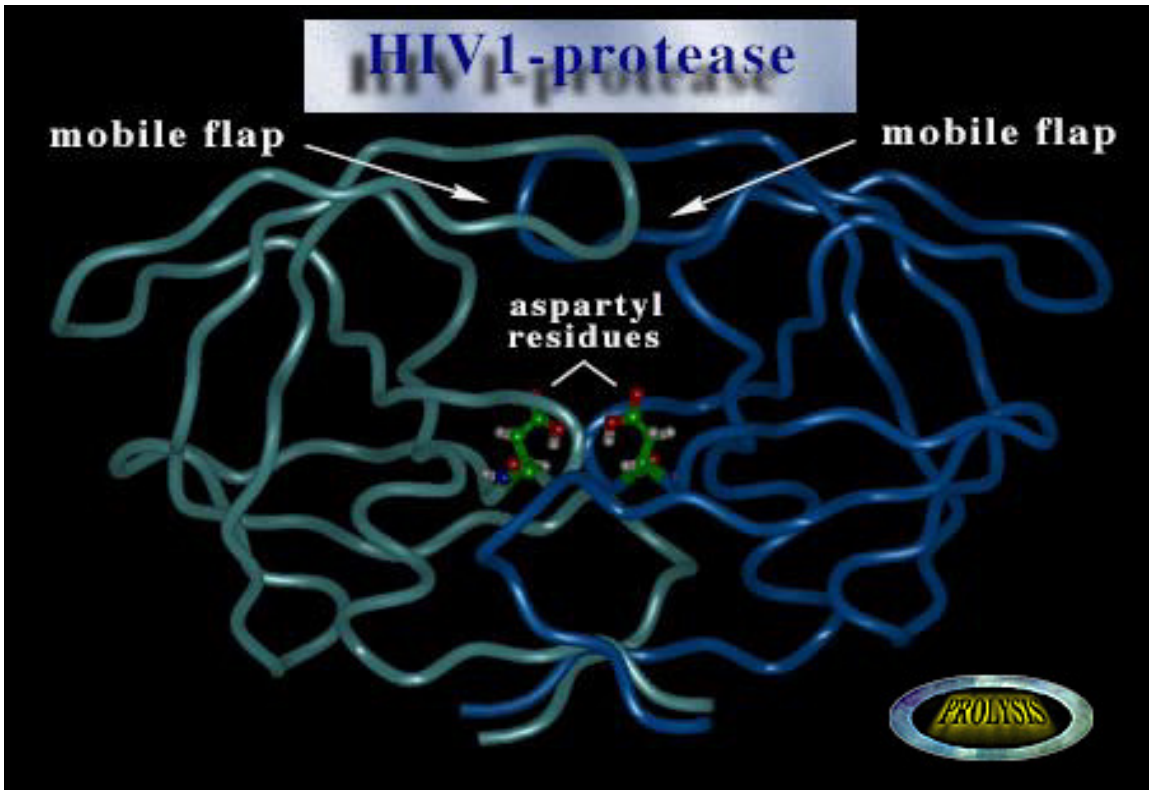


Figure 2. Structure of HIV-1 protease enzyme[25] (re-produced by permission).

The active site is located at the base of the receptor cavity and contains two essential aspartyl residues (Asp-25 and Asp-25'). The cavity is surrounded by two flexible flaps, which are involved in substrate binding and product release. These flaps contain two isoleucine residues, Ile-50 and Ile-50', which interact through hydrogen bonding with an activated water molecule. [10, 24, 26]

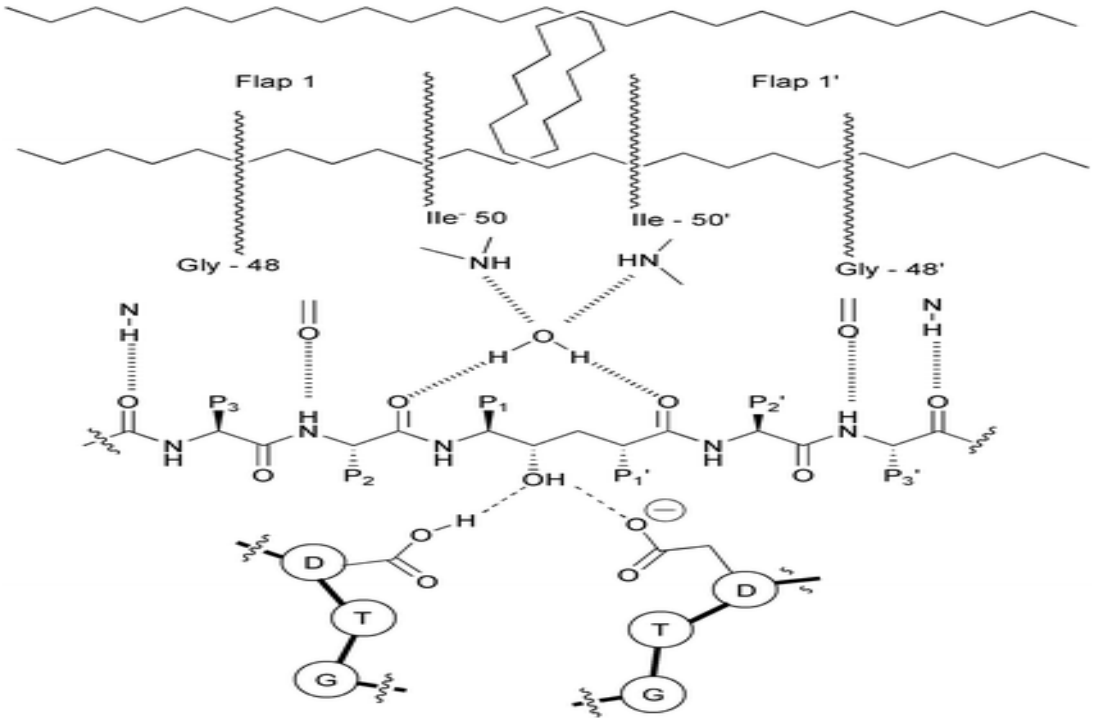


Figure 3. Schematic representation of HIV-1 PR with a water molecule binding a peptide substrate *via* two hydrogen bonds.[10]

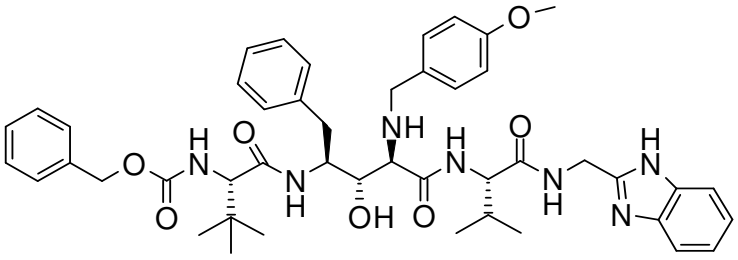
The water molecule at the base of the receptor cavity is responsible for binding the substrate as shown in Figure 3. HIV-1 protease has sub-sites, which are mostly hydrophobic, and structurally equivalent and these are identified by the symbols, S1, S′1, S2, S′2 *etc.*).[10,24,26]

The synthetic HIV-1 protease inhibitors need to fulfil the following design criteria; they must: - i) be non-cleavable; ii) be capable of bonding to the aspartyl residues and the structural water molecule; iii) be hydrophobic; and iv) should exhibit low K_i values.[27,28]

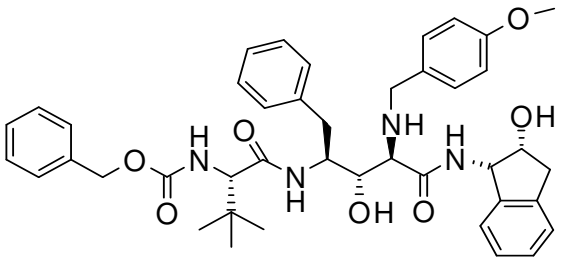
1.6. Different compounds with anti HIV-1 activity

The investigation of libraries aimed at finding inhibitors of HIV-1 protease led to the discovery of 2-hetero-substituted 4-amino-3-hydroxy-5-phenylpentanoic acid

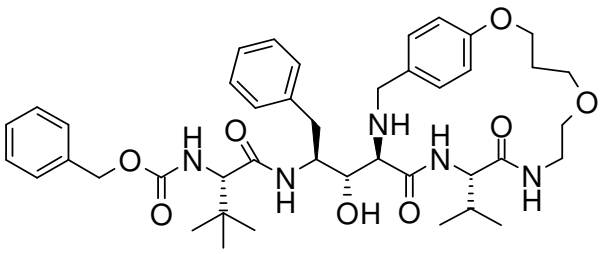
derivatives.[29] Compound **A** with a benzimidizole moiety at the P3'-subsite has been shown to inhibit the protease enzyme with inhibitory activity (K_i) of 3nM and anti-viral activity (IC_{50}) of less than 30nM. However, uptake of compound **A** by animals shows low oral bioavailability. The challenge to increase oral bioavailability led to the discovery of compound **B**, which was achieved by introducing 1(*S*)-amino-2(*R*)-hydroxyindane at the C-terminus of the inhibitor. Compound **B** also exhibits improved anti-viral activity (IC_{50} < 242nM).



Compound **A** (K_i = 3 nM and IC_{50} < 30nM)



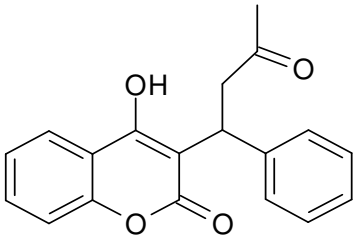
Compound **B** (K_i = 9.5 nM and IC_{50} < 242nM)



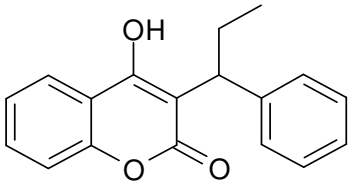
Compound **C** (K_i = 7.9 nM and IC_{50} < 21nM)

The aim of keeping oral activity while improving anti-viral activity led to the development of the peptidomimetic inhibitor **C** containing ether–type macrocycles.

Compound **C** has been found to have good anti-HIV-1 PR activity (IC_{50} < 21nM) without affecting the oral bioavailability.



Compound **D**

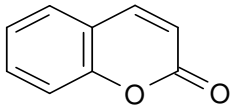


Compound **E**

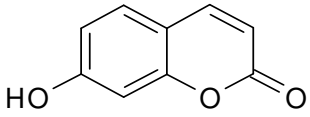
Further investigation led to the discovery of compounds containing a 3-substituted-4-hydroxycoumarin moiety, such as warfarin **D**[19] and phenprocoumon **E**[30]. However, compound **D** is waekly active against HIV-PR. The phenprocoumon **E**, however, is more active against the protease enzyme with an inhibition potential of 1 μM. The hydroxyl group on phenprocoumon **E** has the potential to hydrogen-bond with the catalytic aspartic residues, while the carbonyl group of the coumarin nucleus hydrogen-bonds with the Ile-50 residue of protease enzyme.

1.7. A brief overview of coumarins and their biological activity

Compounds containing the coumarin moiety (2*H*-1-benzopyran-2-one) **1** constitute an important class of heterocycles, many examples of which are found in nature. Coumarin itself was first isolated in 1822 from the tonka bean.[31] Coumarin and its derivatives have been isolated from sweet clover, bison grass and woodruff.[32,33]

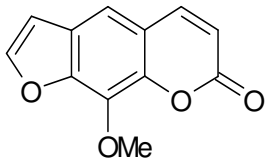


Coumarin **1**

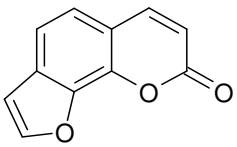


Umbelliferone **2**

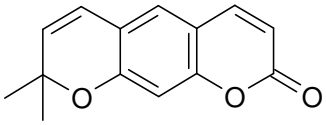
Coumarins can be divided into four sub-types: i) simple coumarins which are hydroxylated, alkoxylated or alkylated on the benzene ring (*e.g.* umbelliferone **2**);[32,34] ii) Furanocoumarins, which contain a five-membered furan ring attached to the coumarin moiety and which are sub-divided into the linear furanocoumarins (*e.g.* xanthotoxin **3**) and the angular furanocoumarins (*e.g.* angeligin **4**);[32,35] iii) pyranocoumarins, containing a six-membered ring attached to the coumarin moiety (*e.g.* seselin **5** and xanthyletin **6**);[32,36] and iv) coumarins with substituents in the pyrone ring (*e.g.* warfarin **7**).[37]



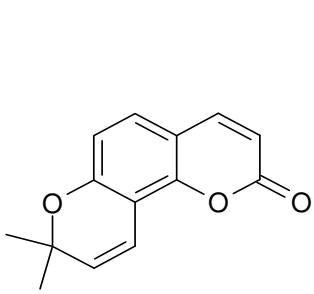
xanthotoxin **3**



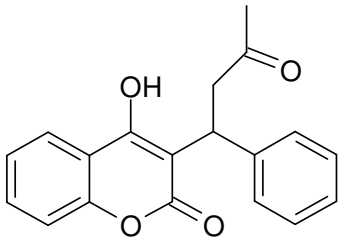
angeligin **4**



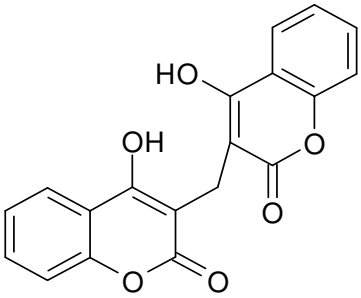
seselin **5**



Xanthyletin **6**



Warfarin **7**



Dicoumarol **8**

Many compounds containing a coumarin moiety have also been found to exhibit useful and multi-biological activities, including anti-inflammatory,[38] antifungal,[39] antimicrobial[40] and anti-HIV properties.[36] As indicated earlier, 4-hydroxycoumarin derivatives,[42] have the potential to hydrogen-bond *via* the 4-hydroxyl group with the catalytic aspartic residues in the HIV-1 protease active site. [42]

1.8. Some naturally occurring coumarins

Warfarin **7** is a naturally occurring compound containing the 4-hydroxy coumarin moiety. It has been isolated from woodruff as well as from lavender and is used to prevent clotting of blood in the veins, lungs or heart.[43,44] Another well-known natural compound containing the coumarin nucleus is 7-hydroxycoumarin, also known as umbelliferone **2**,[45] which is found in a variety of plants, such as carrots, coriander and garden angelica. It has been used as a sunscreen, a fluorescence indicator and as a dye indicator.[45,46,47] Dicoumarol **8** is another natural occurring compound containing the coumarin nucleus, and is known for causing sweet clover disease in cattle.[48,49] It has been isolated from sweet clover hay and used as an anticoagulant.[50]

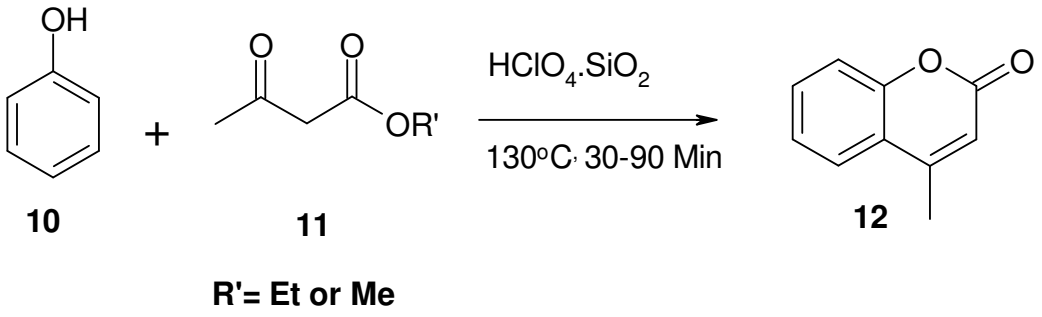
Many synthetic compounds, which contain the coumarin moiety, are well known for their odour, stability to alkali, and availability. They are widely used in perfume, soaps and detergents.[51] Coumarin was once used as a food flavourant, but was banned by the FDA due to carcinogenicity.[52]

1.9. Methods of coumarin synthesis

Because of their varied biological activities, the preparation of coumarin and its derivatives has attracted the attention of organic chemists. Numerous methods have been developed for the their synthesis, including the Pechmann condensation,[53,54] the Perkin reaction,[55,56] the Knoevenagel condensation,[57,58] the Wittig reaction[56,59] and the Baylis-Hillman reaction.[56,60,61]

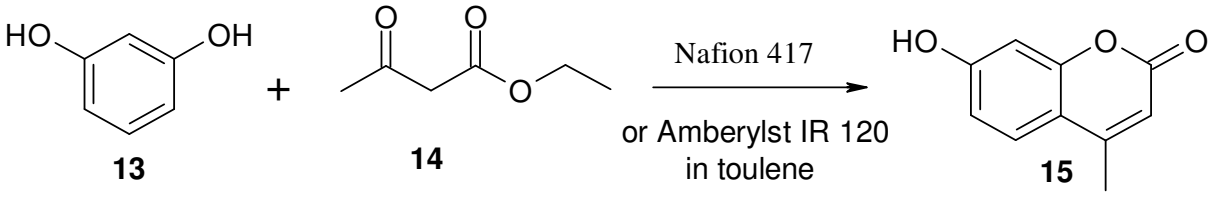
1.9.1. Pechmann condensation

In the Pechmann reaction, a substituted phenol is condensed with a β–keto ester, such as ethyl acetoacetate or methyl acetoacetate, under solvent-free conditions. This approach commonly employs heterogeneous catalysts such as $HClO_4.SiO_2$.[53] The products are typically isolated in yields ranging from 65 to 97%. The method offers several advantages, *viz*., the low cost of the catalyst (which can be recovered), the generally high yield of products and fast reaction times.



Scheme 2

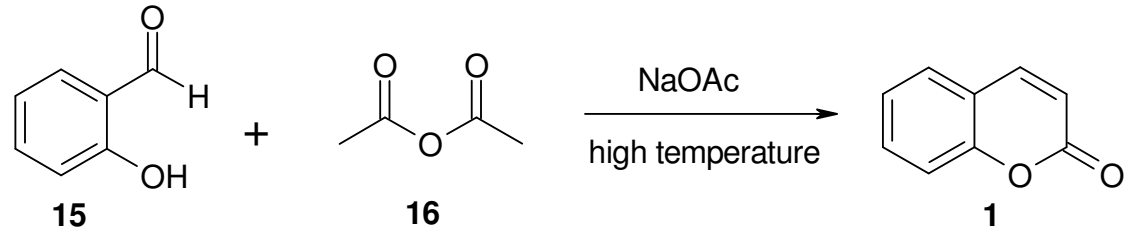
Thimons *et al*.[54] have also reported the synthesis of substituted coumarins from substituted phenols and ethyl acetoacetate *via* the Pechmann condensation, using toluene as solvent in the presence of acidic catalysts, such as Amberlyst IR120 or Nafion 417 (Scheme 3). A disadvantage of this methodology, however, is the low yield of products obtained.



Scheme 3

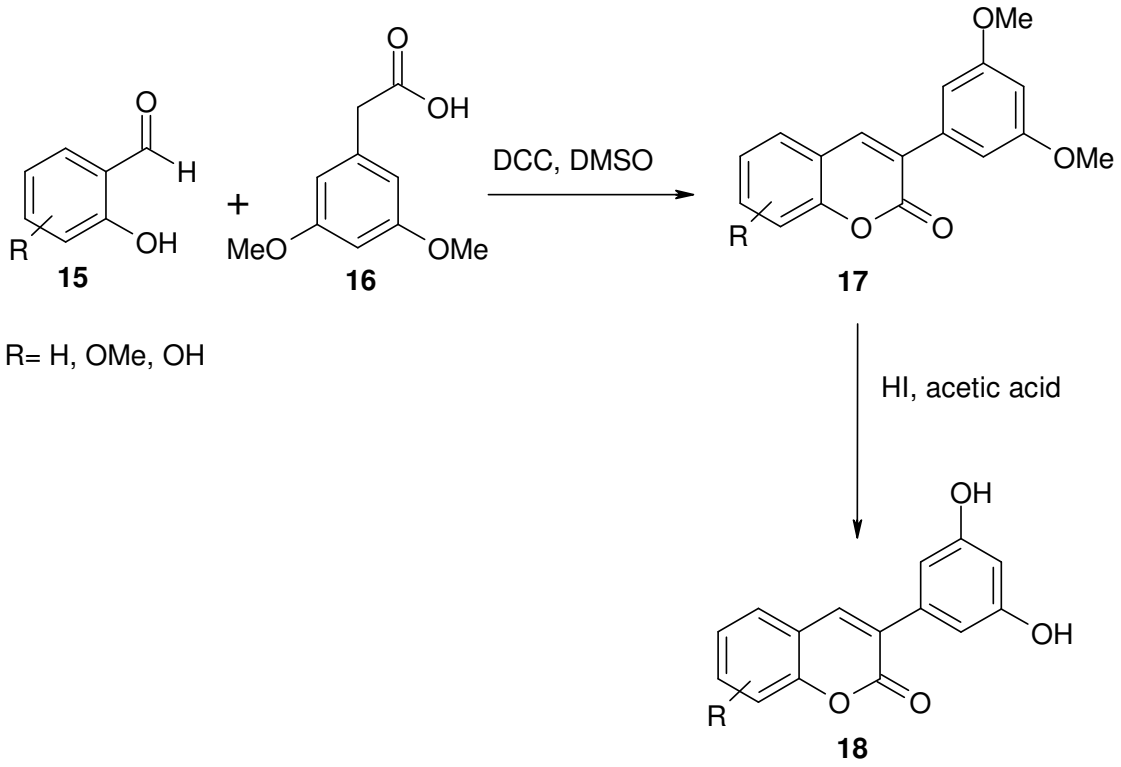
1.9.2. Perkin reaction

The Perkin reaction is another useful approach for the synthesis of coumarin and its derivatives. This reaction involves heating salicylaldehyde with acetic anhydride in a 1:2 molar ratio using sodium acetate as catalyst at high temperature (Scheme 4). Although the coumarin is obtained in low yield and is accompanied by the formation of tarry materials, the Perkin approach does offer the advantage of not forming the isomeric chromene. [56]



Scheme 4

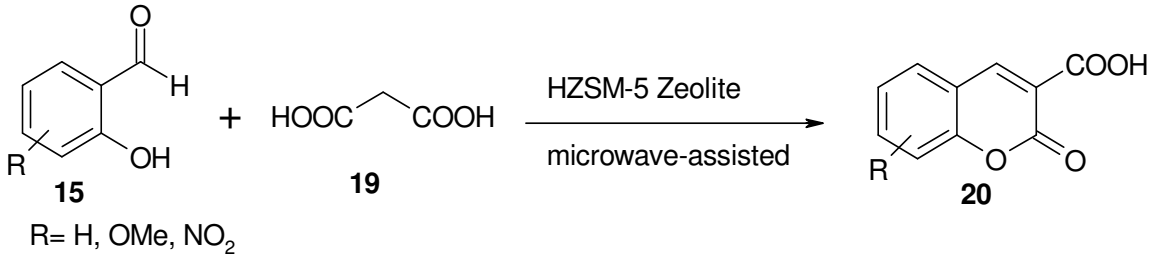
Santana *et al*.[55] have also reported the preparation of coumarin derivatives by reacting substituted salicylaldehydes with 3,5-dimethoxyphenylacetic acid using *N,N*'-dicyclohexylcarbodiimide in DMSO *via* a Perkin-type reaction. The methoxy groups were then hydrolyzed using HI in acetic anhydride to give di- or trihydroxy derivatives with reasonable yields up to 79% as illustrated in Scheme 5**.**



Scheme 5

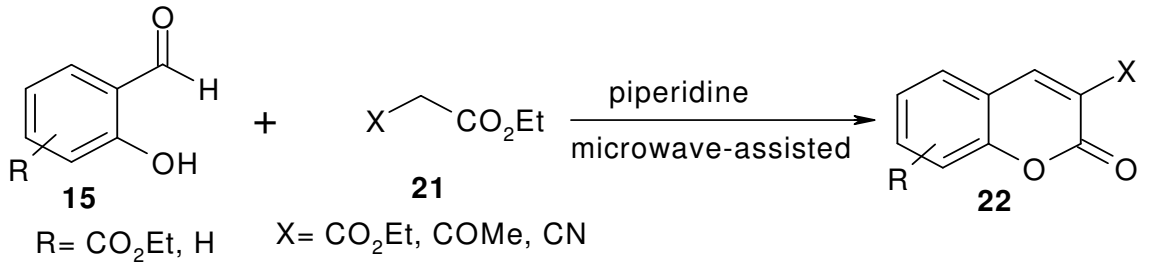
1.9.3. Knoevenagel condensation

In general, the Knoevenagel condensation reaction occurs between a substituted salicyladehyde and activated methylene compounds in the presence of an amine catalyst. However, Heravi *et al*.[57] has reported the preparation of coumarin derivatives *via* microwave-assisted Knoevenagel reactions using 2-hydroxybenzaldehydes or hydroxynaphthaldehyde with malonic acid on microwave irradiation in solvent-free conditions (Scheme 6). The method employs the HZSM-5 zeolite as a catalyst, and affords isolated products in yields of up to 97%.



Scheme 6

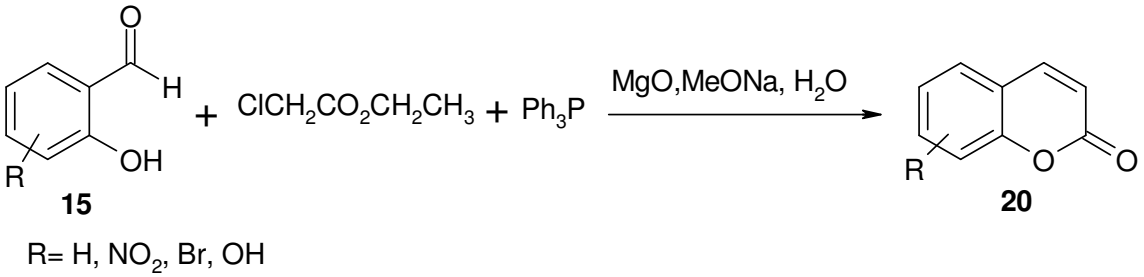
Bagdol[58] has also reported the preparation of coumarins by reacting salicylaldehyde and its derivatives with substituted ethyl acetate *via* a microwave-assisted Knoevenagel reaction (Scheme 7)**.** This approach employed piperidine as catalyst under solvent-free conditions, and offers several advantages, such as lower reaction time and high yields of product.



Scheme 7

1.9.4. Wittig reaction

The Wittig reaction has also been used to synthesise coumarin derivatives.[56] This approach involves reaction of an aromatic aldehyde or ketone with a phosphonate or phosphorus ylide. For example, when a substituted salicylaldehyde **15**, triphenylphosphine and ethyl chloroacetate were reacted in the presence of MgO and sodium methoxide, the coumarin products were isolated in excellent yields of up to 90% (Scheme 8).[59]

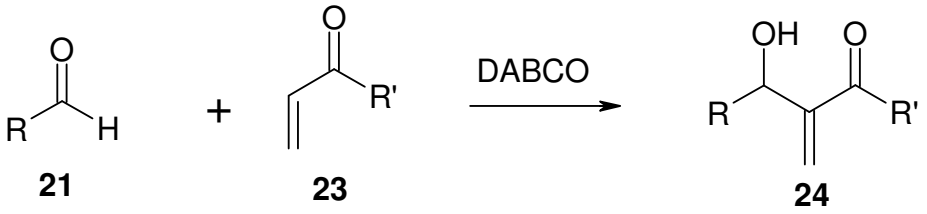


R= H, NO_2, Br, OH

Scheme 8

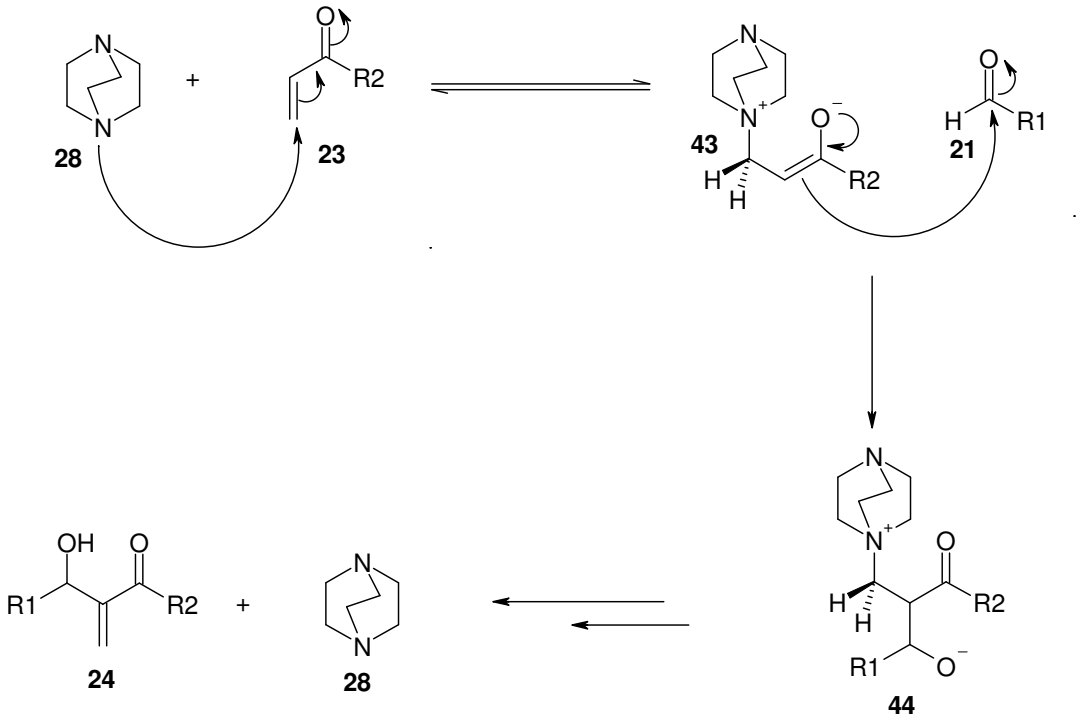
1.9.5. Baylis-Hillman Reaction

The Baylis–Hillman reaction provides another useful approach to the synthesis of coumarin derivatives. Previous members of our group have applied Baylis-Hillman methodology in the synthesis of quinolines,[62] indolizines,[63] 2*H*-1-thiochromenes[60] and coumarins.[56,60,61] This method involves C-C bond formation between an activated alkene and an aldehyde in the presence of a catalyst, such as DABCO, to give allylic alcohol products (Scheme 9).[64] A common disadvantage of Baylis-Hillman methodology, however, is the long reaction time. It can take up to 3 weeks to obtain reasonable yields.



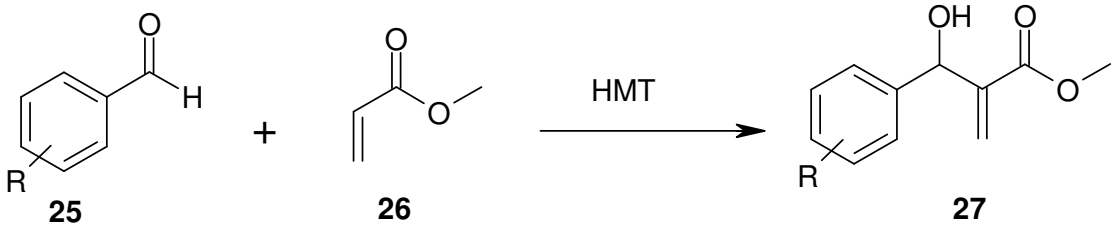
Scheme 9

Bode[65] investigated the mechanism of the Baylis-Hillman reaction. A key step is believed to be the formation of a zwitterionic enolate species by nucleophilic addition of the catalyst to the double bond of the alkene; this is followed by addition of the reacting enolate species to the aldehyde in the rate-determining step. Proton transfer and elimination of catalyst then affords the Baylis-Hillman product.[61] The reaction was shown to be first-order in the aldehyde, the alkene and catalyst and third-order overall (Scheme 10).



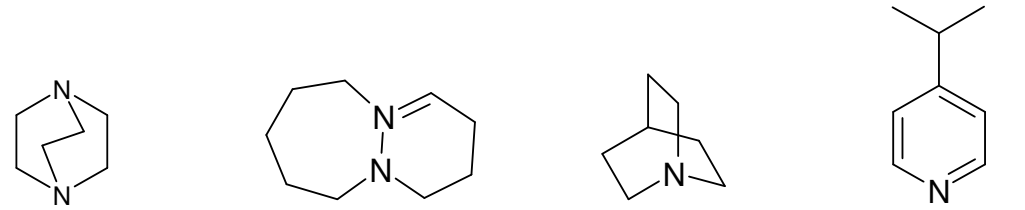
Scheme 10

De Souza *et al.*[66] have reported the use of hexamethylethylenetetraamine (HMT) as a catalyst to synthesise the Baylis-Hillman adducts (Scheme 11). HMT has been shown to improve the yields and reduces the time. Other tertiary amine catalysts such as DBU **29**, quinuclidine **30** and DMAP **31** have also been applied in the Baylis-Hillman reaction

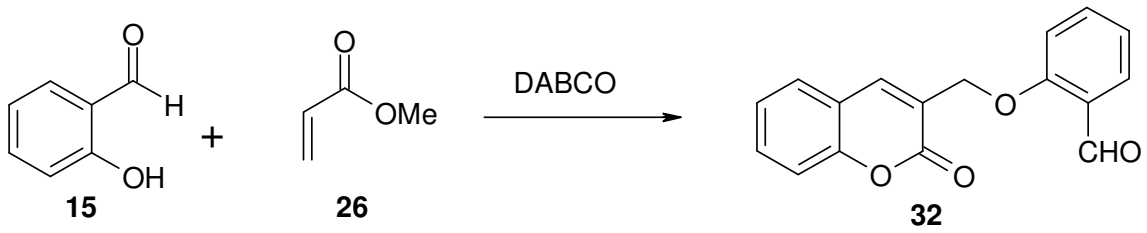


R = NO_2, H, OMe, OH

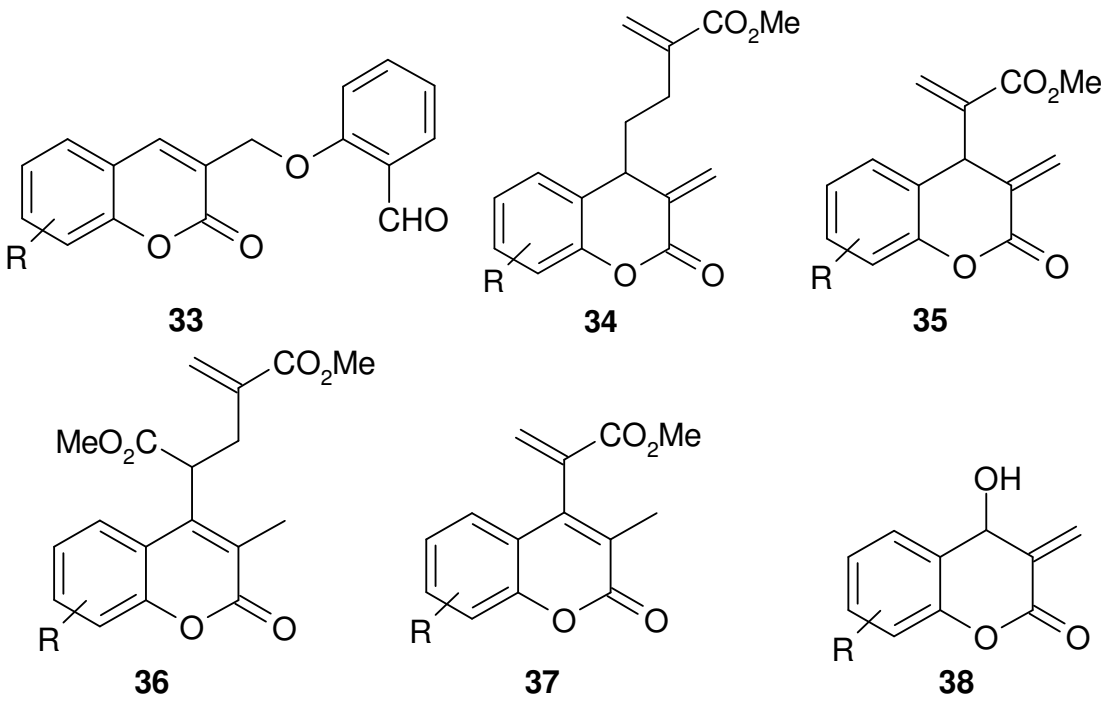
Scheme 11



Bode [65] had reported that reacting salicylaldehyde with methyl acrylate in the presence of DABCO didn't give the expected Baylis-Hillman adduct but gave, instead, the coumarin derivative **32** (Scheme 12). Robinson,[67] on re-visiting these reactions, demonstrated formation of various chromene and coumarin derivatives. Careful separation by flash column chromatography led to the isolation of eight different classes of products, *e.g.* **34-38**, as well as products **33** corresponding to product **32**, isolated by Bode.



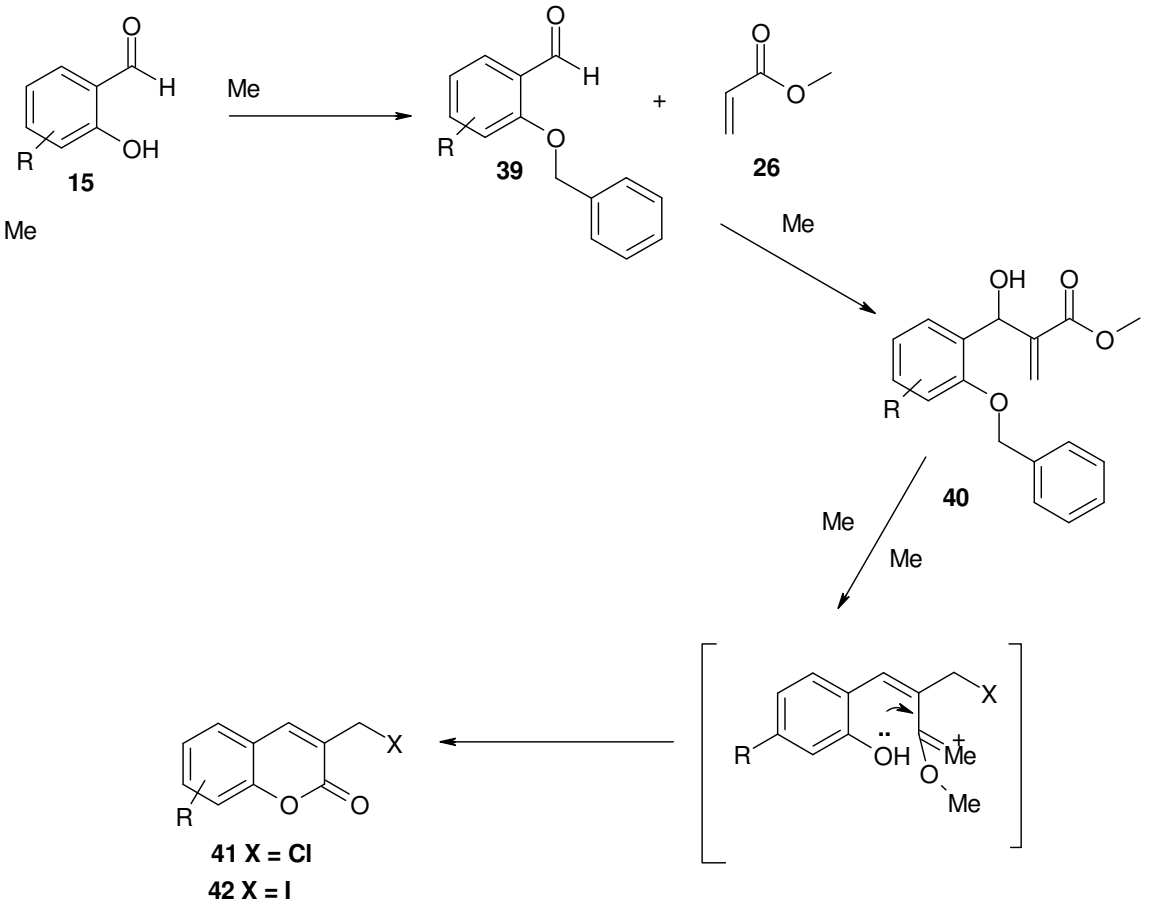
Scheme 12



33 **34** **35**

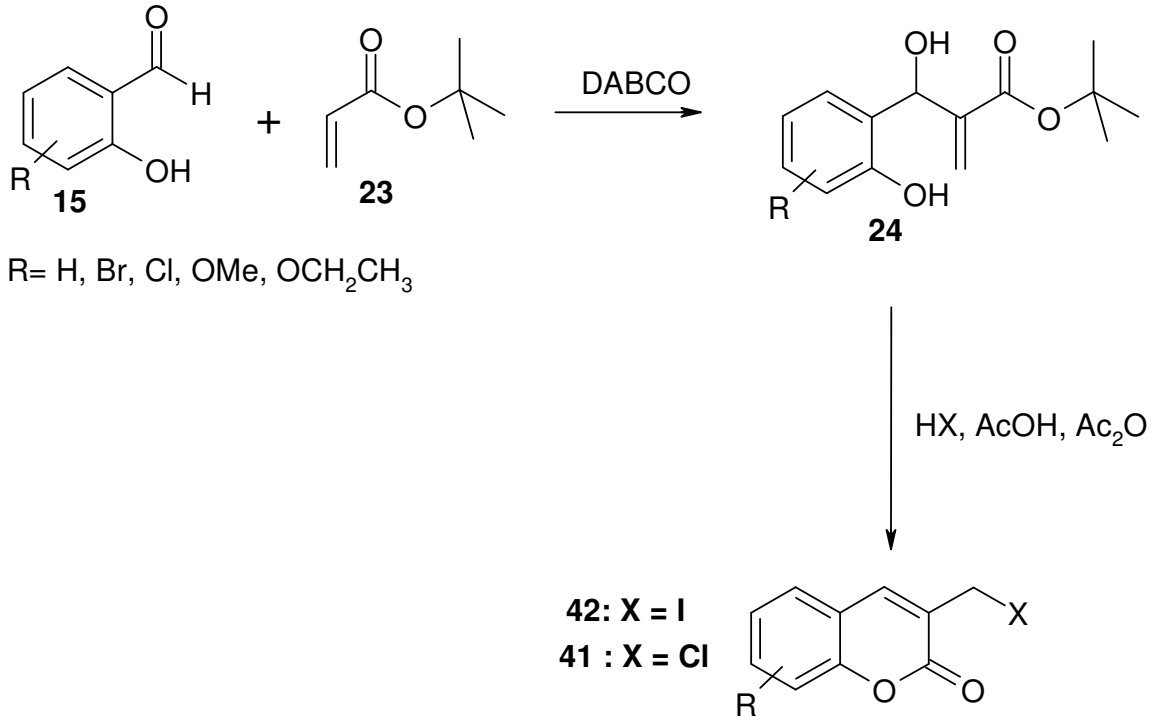
36 **37** **38**

The regioselectivity of the cyclisation could be controlled to form coumarin by protecting the salicylaldehyde phenolic group before reacting it with the activated alkene as the benzyl ether.[61] Treatment of the resulting benzyl ether with methyl acrylate in the presence of DABCO afforded the Baylis-Hillman adducts **40**. The reaction of HI or HCl with the α,β-unsaturated adducts **40** and acid-catalysed acylation gave the desired coumarin derivatives **41** and **42** (Scheme 13).



Scheme 13

Musa[68] synthesized coumarin derivatives using Baylis-Hillman methodology by reacting salicylaldehyde derivatives **15** and *t*-butyl acrylate **23** using DABCO **28** as catalyst. The resulting Baylis-Hillman adducts were obtained in yields of up to 60%. The Baylis-Hillman adducts were then reacted with HCl or HI in acetic acid to form the coumarin derivatives. The use of HCl gave 3-(chloromethyl)coumarin derivatives **41** in good yields (86-90%), whereas use of HI gave the corresponding 3-(iodomethyl)coumarin derivatives **42** with low yields (17%) as shown in Scheme 14.

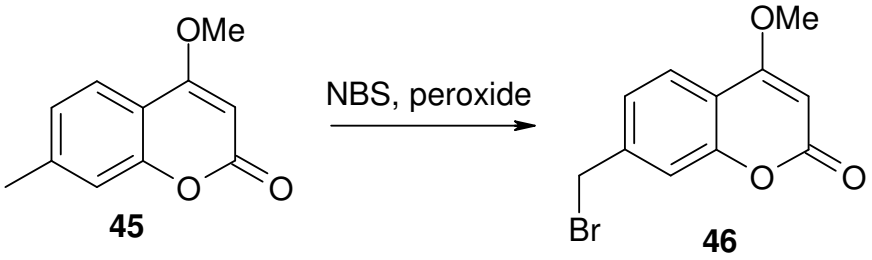


Scheme 14

1.10. Reactivity of coumarins

Coumarin and its derivatives are highly reactive. Because the coumarin moiety is aliphatic, it is likely to undergo ring-opening at the acyl centre or conjugate addition at the carbon-carbon double bond.[69,70] The carbonyl lactone group of the coumarin nucleus is characterized by an IR absorption band at *ca.*1700 cm^{-1}.[71] The presence of a methyl group at C-4 or C-6 makes the coumarin nucleus more reactive, and can result in the coumarin nucleus undergoing halogenation as well as condensation with the aldehydes.[71] Carbon-6 on the aromatic ring can undergo electrophilic attack, *e.g.* sulfonation or Friedel-crafts acylation leading to the formation 6-substituted derivatives. Electrophilic attack at C-3 can only occur under forcing conditions unless a C-4 substituent, such as a hydroxyl group directs the incoming group to the C-3 position.[72,73] The nucleophilic attack on the coumarin nucleus depends on the nature of the nucleophile. Thus, strong nucleophiles, such as secondary amines, are likely to open the ring through attack at the acyl centre, whereas weak nucleophiles attack the C-4 position (*e.g.* bromination).[71]

A methyl substituent on the coumarin nucleus may react differently, depending on the position of attachment. For example, a methyl group attached to C-6 or C-4 is more reactive than a methyl groups at the C-3 or C-5 positions.[71,74] A methyl substituent on the pyran moiety of coumarin appears to be stable under bromination conditions whereas bromination is observed on the 7-methyl group attached to the benzene ring (Scheme 15).[75]



45 **46**

Scheme 15. Bromination of a 7-methyl group.[75]

1.11. Objectives of the present Study

The proposed research formed part of a group programme aimed at preparing compounds that might inhibit the HIV-1 protease enzyme, and the project was expected to build on methodology developed by previous members of the group. Specific objectives of the research have included the following.

1. Application of the Baylis-Hillman methodology in the synthesis of coumarin derivatives.
2. Application of the Arbuzov reaction to the 3-(halomethyl)coumarin products to access phosphorylated derivatives.
3. Preliminary computer modelling studies to explore binding of the synthetic products in the HIV-1 protease receptor cavity.

2. DISCUSSION.

In this discussion, attention will be given to the following.

(i) Synthesis of 3-(halomethyl)coumarin derivatives *via* cyclisation of *O*-benzylated Baylis-Hillman products. (**Section 2.1**)

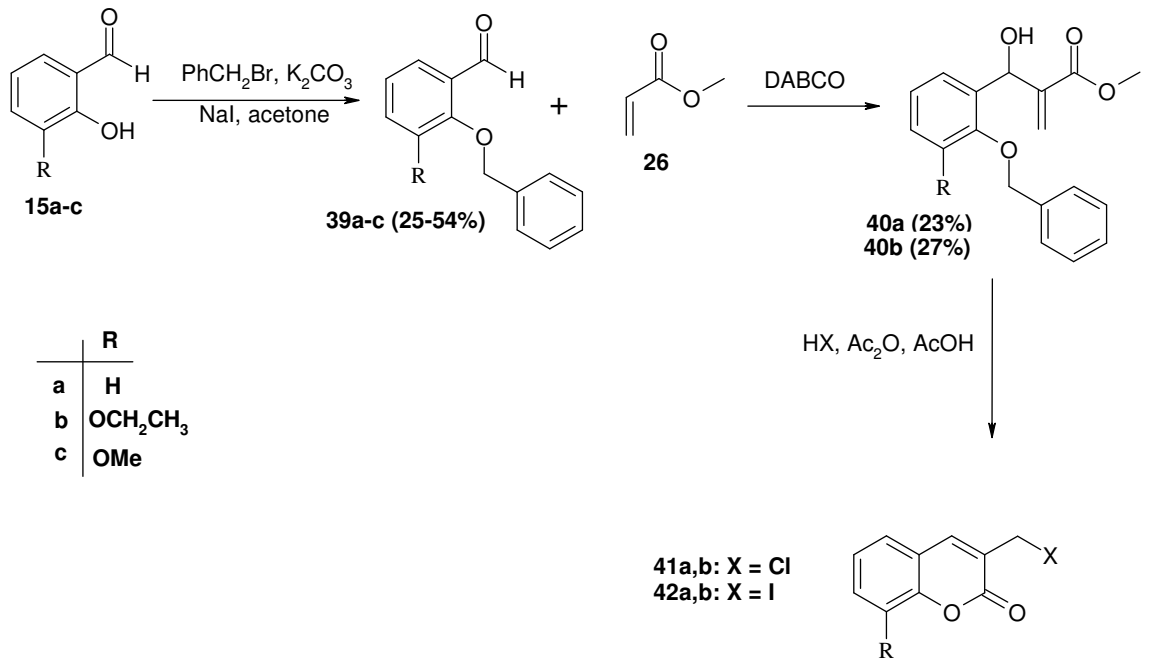
(ii) Synthesis of 3-(halomethyl)coumarin derivatives *via* cyclisation of unprotected Baylis-Hillman adducts. (**Section 2.2**)

(iii) Nucleophilic substitution reactions of the 3-(halomethyl)coumarin derivatives. (**Section 2.3**)

(iv) Molecular modelling of selected structures. (**Section 2.4**)

2.1. Synthesis of 3-(halomethyl)coumarin derivatives *via* cyclisation of *O*-benzylated Baylis-Hillman adducts

The study was begun by demonstrating the preparation of *O*-benzylated Baylis-Hillman adducts by first protecting the salicylaldehyde phenolic hydroxyl group as a benzyl ether group before reacting with methyl acrylate (Scheme 16). In the research conducted by Musa,[60] the use of a protecting group was found to afford coumarin derivatives directly during cyclisation of these Baylis-Hillman adducts and to inhibit the formation of unwanted chromene derivatives. The advantage of using ethers as a protecting group is that they are relatively inert but cleavable on heating with concentrated hydroiodic acid or hydrochloric acid.[61] The formation of the known benzyl ethers was achieved by reacting the substituted salicylaldehydes **15a-c** with benzyl bromide in the presence of potassium hydroxide and sodium iodide, using acetone as solvent. Chromatography on silica gel and work-up afforded the corresponding salicylaldehyde benzyl ethers **39a-c** in reasonable yields of up to 54%. The salicylaldehyde benzyl ethers were analyzed by NMR spectroscopy. Thus, the ^{1}H NMR spectrum of 2-benzyloxybenzaldehyde **39a** (Figure 4) shows the two benzylic protons resonating as a singlet at 5.18 ppm and the aldehydic proton as a singlet at 10.52 ppm. The aromatic region shows the signals corresponding to the 9 aromatic protons.



Scheme 16

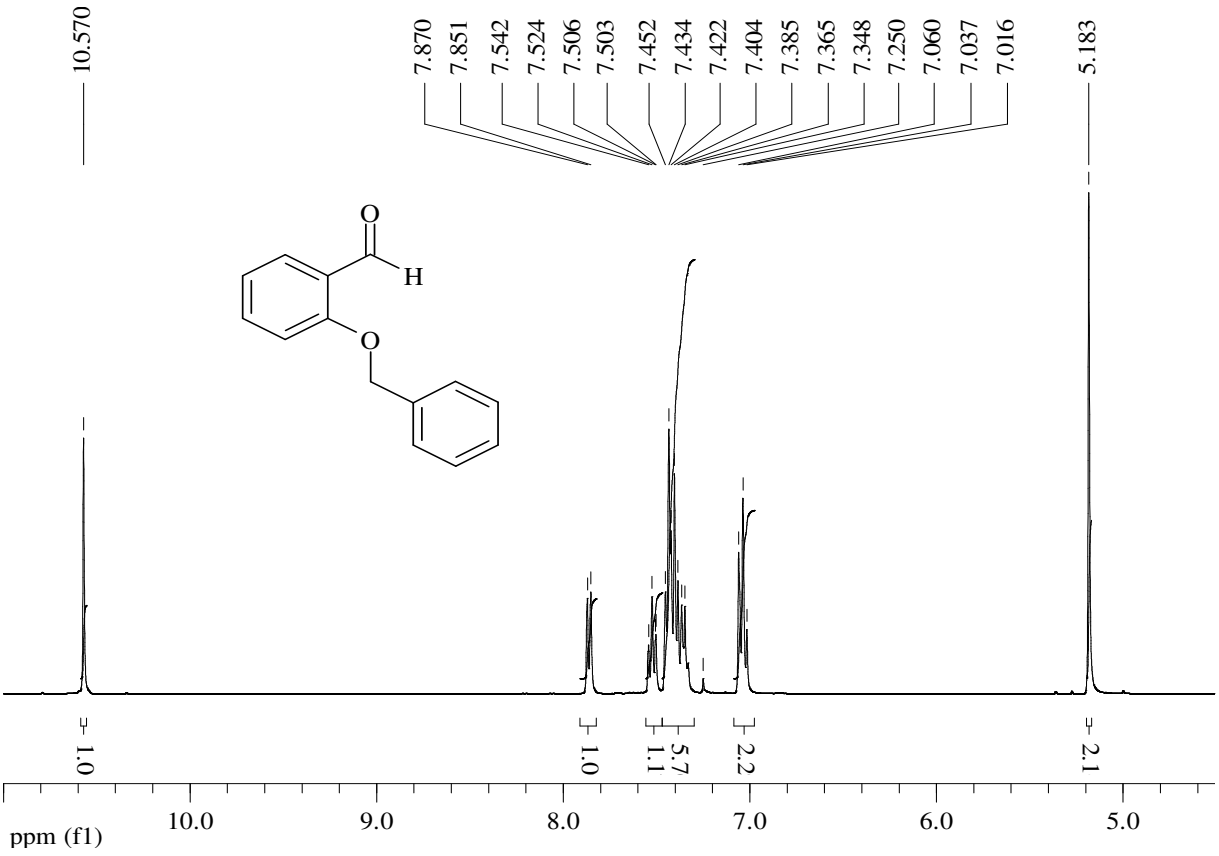


Figure 4. 400MHz ^{1}H NMR spectrum of benzyl ether **39a** in $CDCl_3$.

The 2-benzyloxybenzaldehydes **39a,b** were then reacted with methyl acrylate **26** in the presence of a catalytic quantity of DABCO using $CHCl_3$ as solvent.[60] The mixtures were stirred in stoppered reaction flasks for 3 weeks to afford the corresponding *O*-benzylated Baylis-Hillman adducts **40a**,**b** which were isolated by flash chromatography. The chromatographed products were obtained in low yield (< 27%) and were analyzed by NMR spectroscopy. The ^{1}H NMR spectrum of the Baylis-Hillman adduct **40a** (Figure 5) exhibits a singlet at 3.71 ppm that corresponds to the methyl signal and a broad signal at 3.41 ppm corresponding to the hydroxy group. The two benzylic protons resonate as a singlet at 5.08 ppm, whereas the two vinylic protons resonate as two singlets at 5.69 and 6.28 ppm, respectively. The singlet at 5.94 ppm corresponds to the methine proton, while the signals in the aromatic region correspond to the nine aromatic protons.

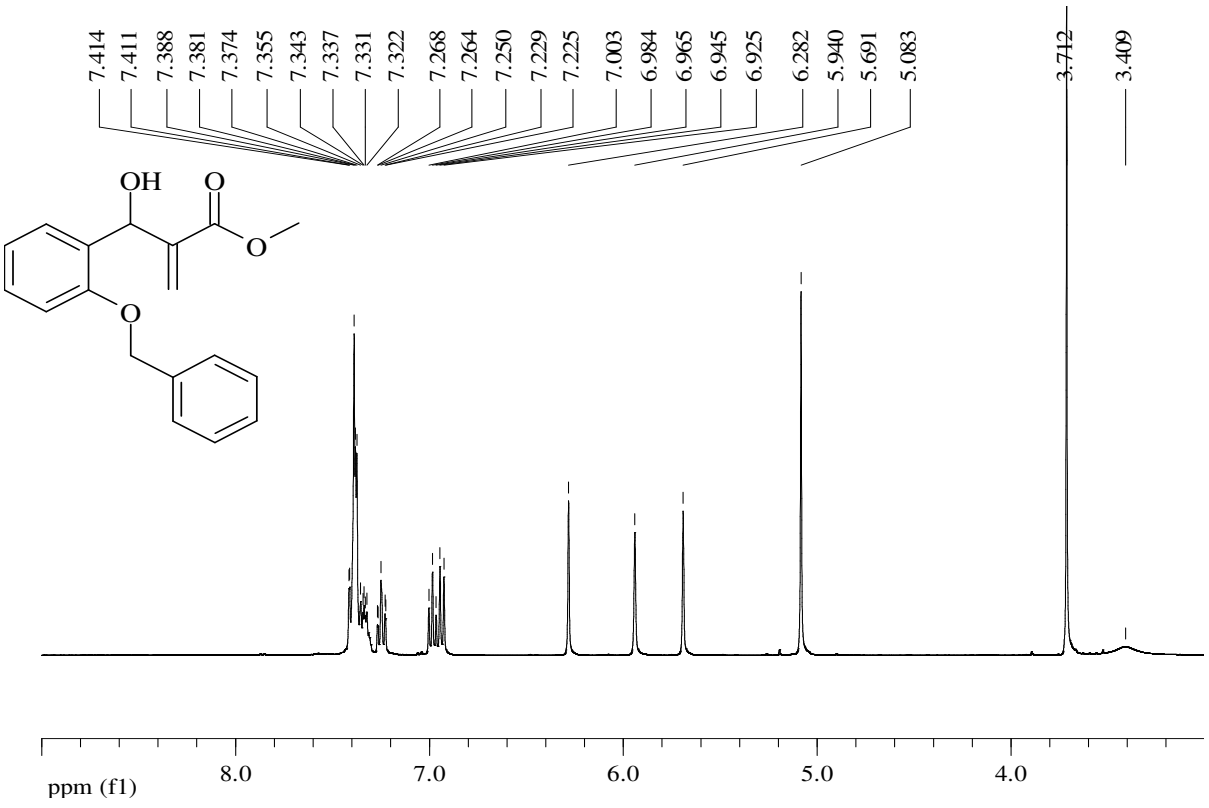
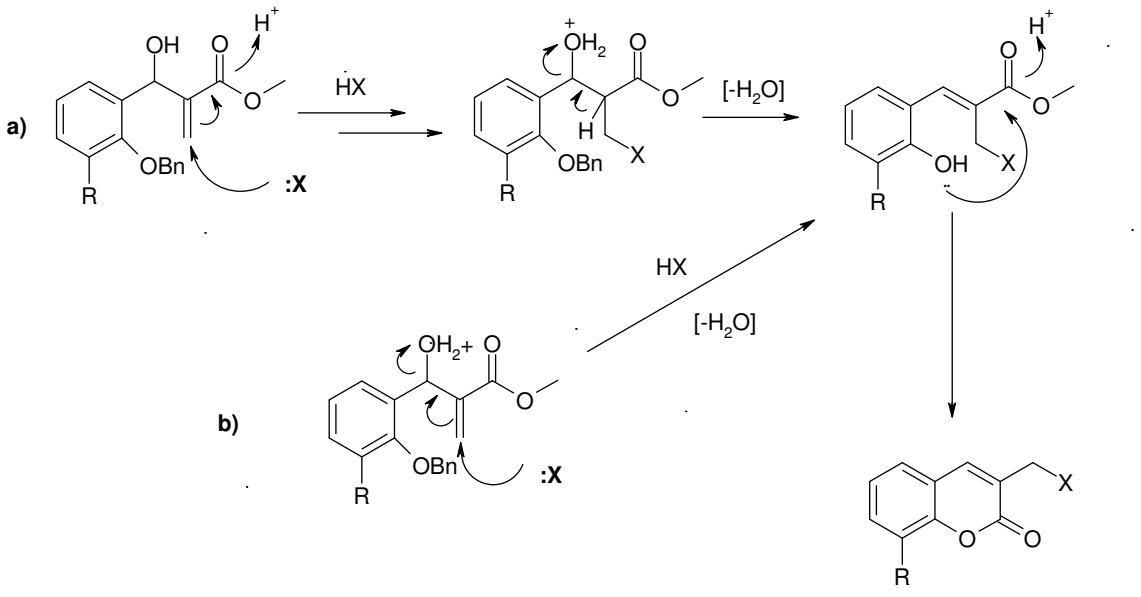


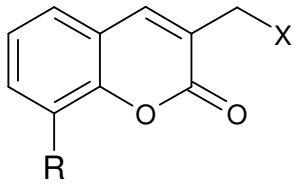
Figure 5. 400MHz ^{1}H NMR spectrum of compound **40a** in $CDCl_3$.

The *O*-benzylated Baylis-Hillman adducts **40a,b** were then treated with HCl or HI in the presence of acetic acid and acetic anhydride to form the 3-(halomethyl)coumarin derivatives as illustrated in the Scheme 16. The use of HCl gave the 3-(chloromethyl)coumarin derivatives **41a,b** in good yields of up to 83%, whereas use of HI gave the corresponding 3-(iodomethyl)coumarin derivatives **42a,b** in yields of up to 65% (Table 1). The ^{1}H NMR spectrum of 3-(iodomethyl)coumarin **42a** (Figure 7a) reveals the iodomethylene protons resonating as a singlet at 4.37 ppm, while the chloromethylene protons in 3-(chloromethyl)coumarin **41a** resonate at 4.53 ppm (Figure 6a). The ^{13}C NMR spectrum of 3-(iodomethyl)coumarin **42a** (Figure 7b) shows the iodomethylene carbon resonating upfield of TMS at -1.5 ppm, whereas the chloromethylene carbon in 3-(chloromethyl)coumarin **41a** resonates downfield at 40.9 ppm (Figure 6b). Likely mechanisms for the formation of the 3-(halomethyl)coumarin derivatives are outlined in Scheme 17. The first step in path (a) involves conjugate addition of halogen acid to the α,β-unsaturated ester followed by elimination of water. Cleavage of the benzyl ether by the halogen acid then exposes the phenolic hydroxyl group, which attacks the ester group; acyl substitution then affords the coumarin. In an alternative pathway (b), acid-catalysed allylic displacement (S_N'), followed by cyclisation affords the phenolic intermediate.



Scheme 17. Mechanisms for the formation of 3-(halomethyl)coumarin derivatives **41** and **42**. Path a) conjugate addition-elimination, followed by acyl substitution; Path b) S_N' reaction followed by cyclization.

Table 1. Yields obtained for the 3-(halomethyl)coumarin derivatives (Scheme 15).



Compounds	R	X	Yield/ %
41a	H	Cl	83
41b	OCH_2CH_3	Cl	70
42a	H	I	65
42b	OCH_2CH_3	I	62

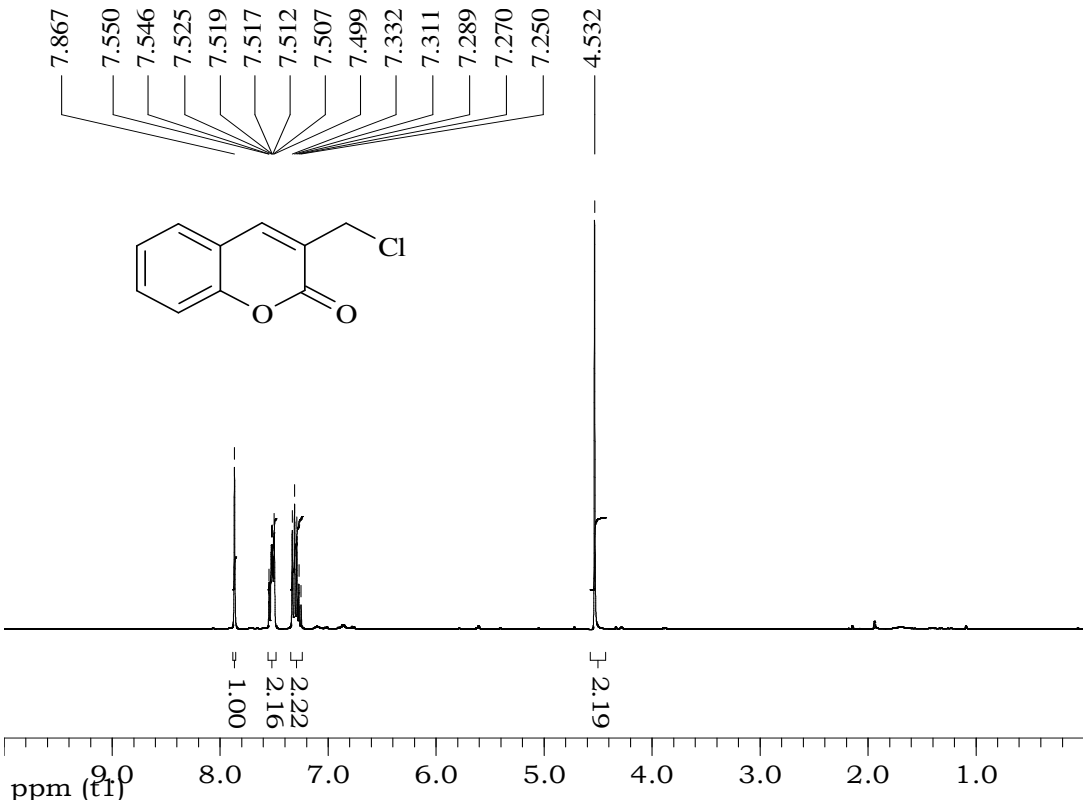


Figure 6a. 400MHz ^{1}H NMR spectrum of 3-(chloromethyl)coumarin **41a** in $CDCl_3$.

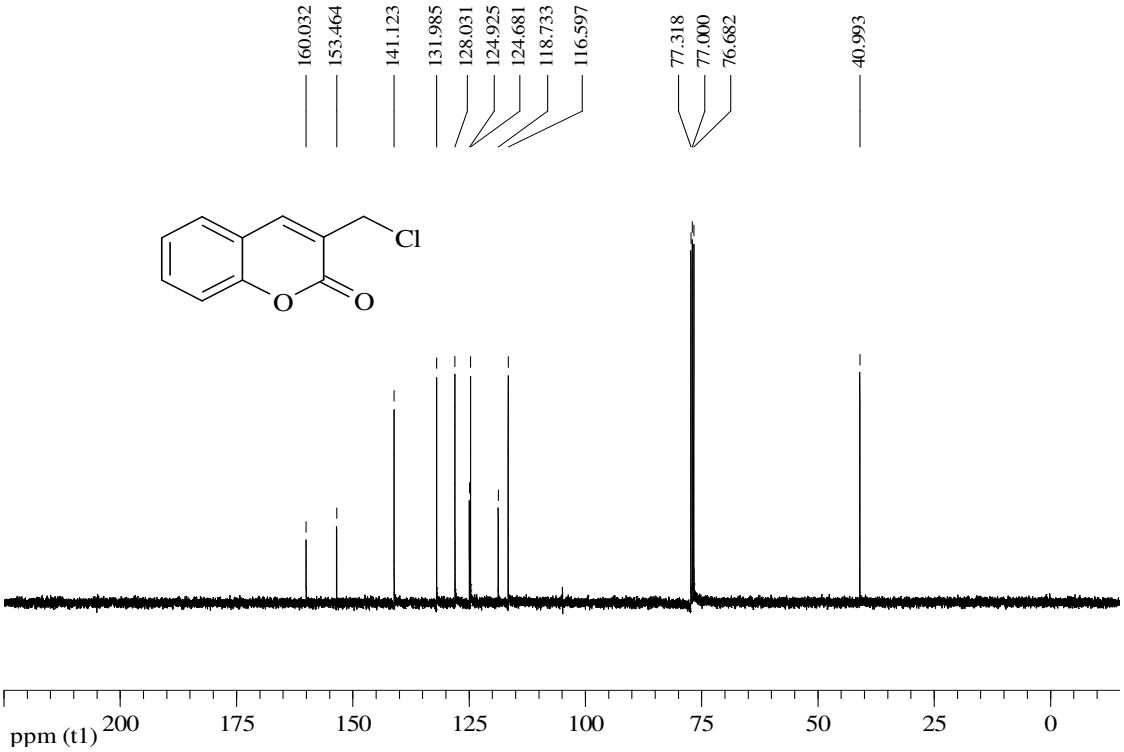


Figure 6b. 100MHz ^{13}C NMR spectrum of 3-(chloromethyl)coumarin **41a** in $CDCl_3$.

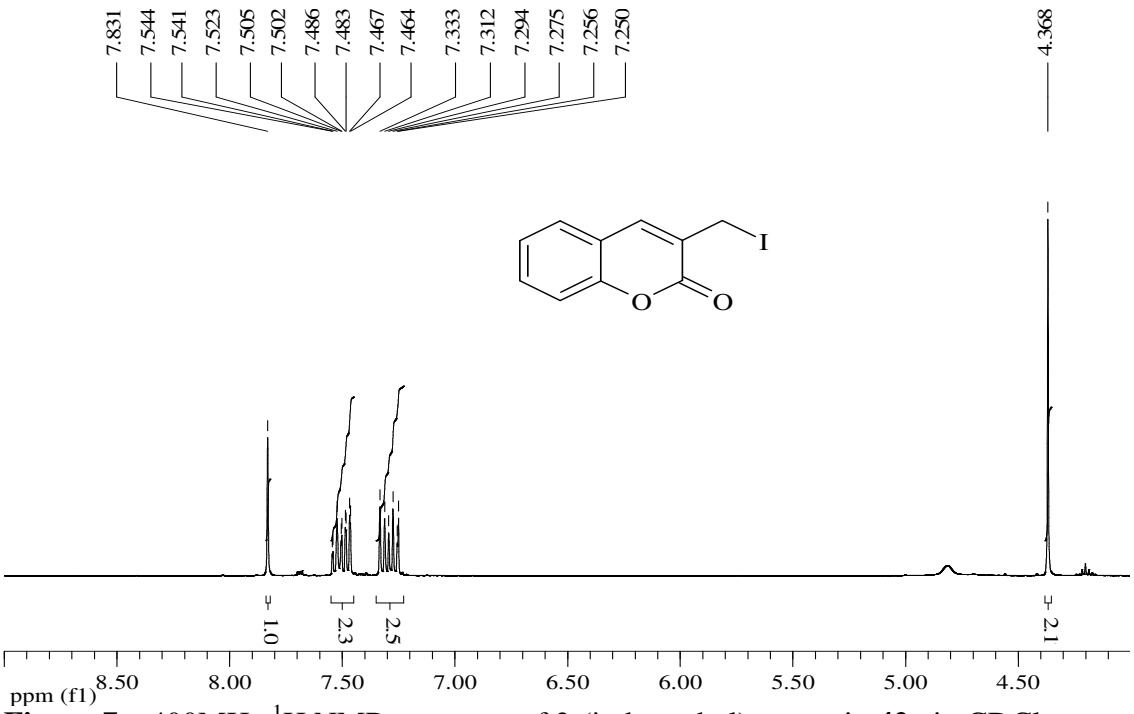


Figure 7a. 400MHz ^{1}H NMR spectrum of 3-(iodomethyl)coumarin **42a** in $CDCl_3$.

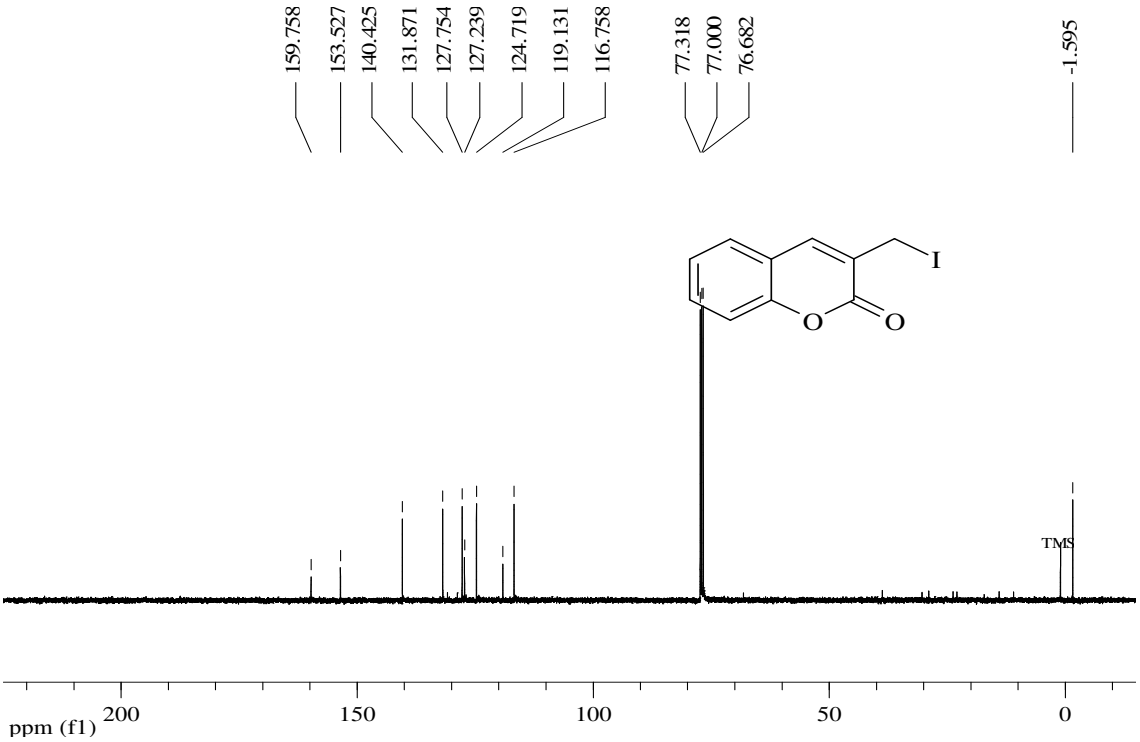
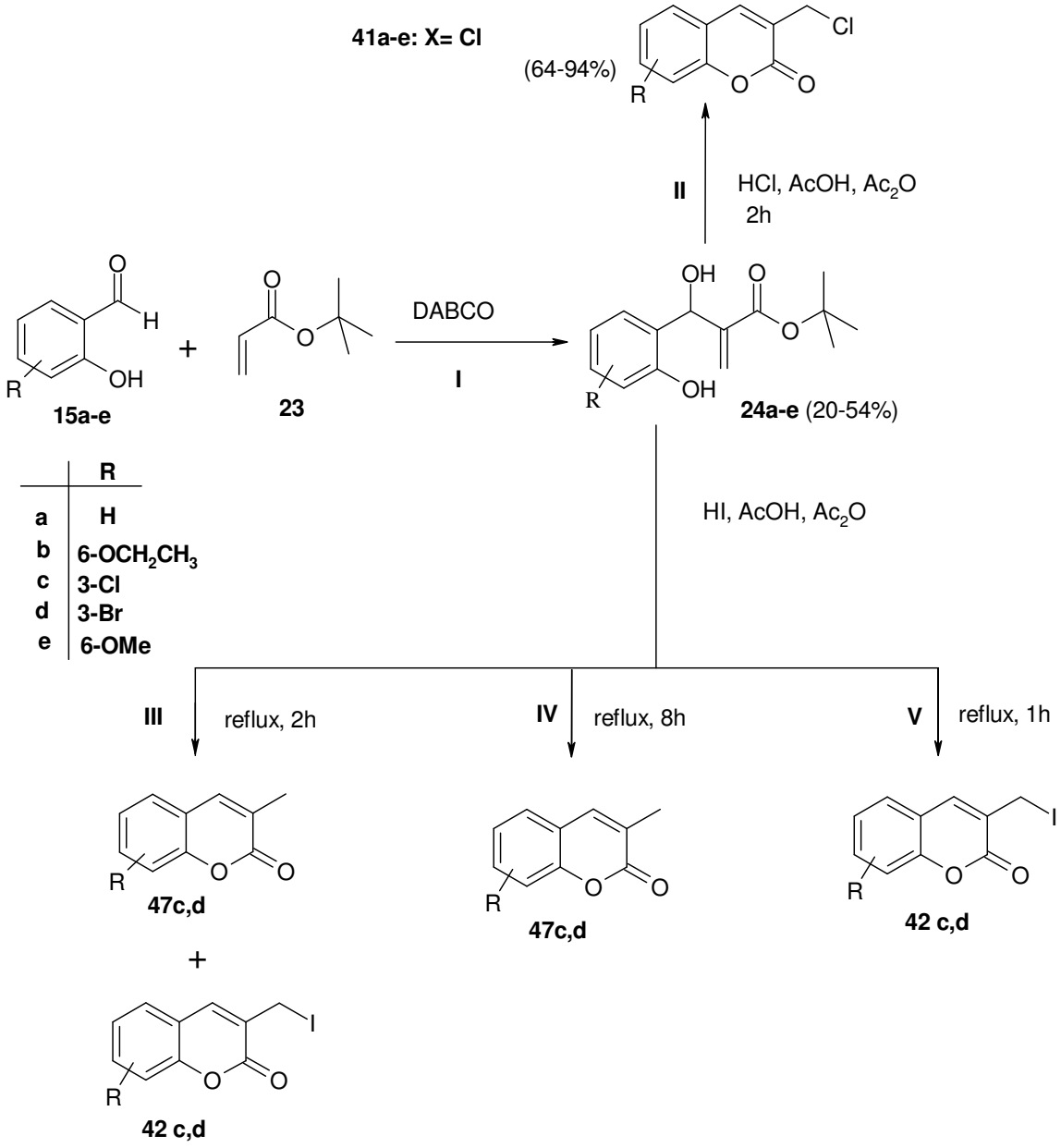


Figure 7b. 100MHz ^{13}C NMR spectrum of 3-(iodomethyl)coumarin **42a** in $CDCl_3$.

2.2. Synthesis of 3-(halomethyl)coumarin derivatives *via* cyclisation of unprotected Baylis-Hillman adducts

Musa and Kaye [68] have reported that the use of *tert*-butyl acrylate **23** as an activated alkene permits direct cyclisation to coumarin derivatives without protecting the phenolic hydroxyl group. Attention was then turned to the synthesis the Baylis-Hillman adducts **24a-e** using this approach. This was achieved by reacting the substituted salicylaldehydes **15a-e** with *tert*-butyl acrylate **23** in the presence of DABCO using $CHCl_3$ as solvent (Scheme 18). The mixtures were stirred at room temperature for periods ranging from 5 days to 14 days, affording the Baylis-Hillman adducts in the yields of up to 57%, after isolation by flash chromatography. The ^1H NMR spectrum of *tert*-butyl 3-hydroxy-3-(2-hydroxyphenyl)-2-methylenepropanoate **24a** (Figure 8) shows the *tert*-butyl singlet at 1.50 ppm, while the two vinylic protons resonate as singlets at 5.49 and 6.23 ppm, respectively. The methine proton resonates as a singlet at 5.69 ppm and the attached hydroxylic proton resonates as a singlet at 4.34 ppm. The singlet at 8.12 ppm corresponds to the phenolic proton.


41a-e: X= Cl
(64-94%)
Cl
R
O
O
II
HCl, AcOH, Ac2O
2h
O
H
OH
R
15a-e
+
O
O
23
DABCO
I
OH
O
O
OH
R
24a-e (20-54%)
HI, AcOH, Ac2O
R
a H
b 6-OCH2CH3
c 3-Cl
d 3-Br
e 6-OMe
III
reflux, 2h
IV
reflux, 8h
V
reflux, 1h
47c,d
+
47c,d
42 c,d
42 c,d

Scheme 18

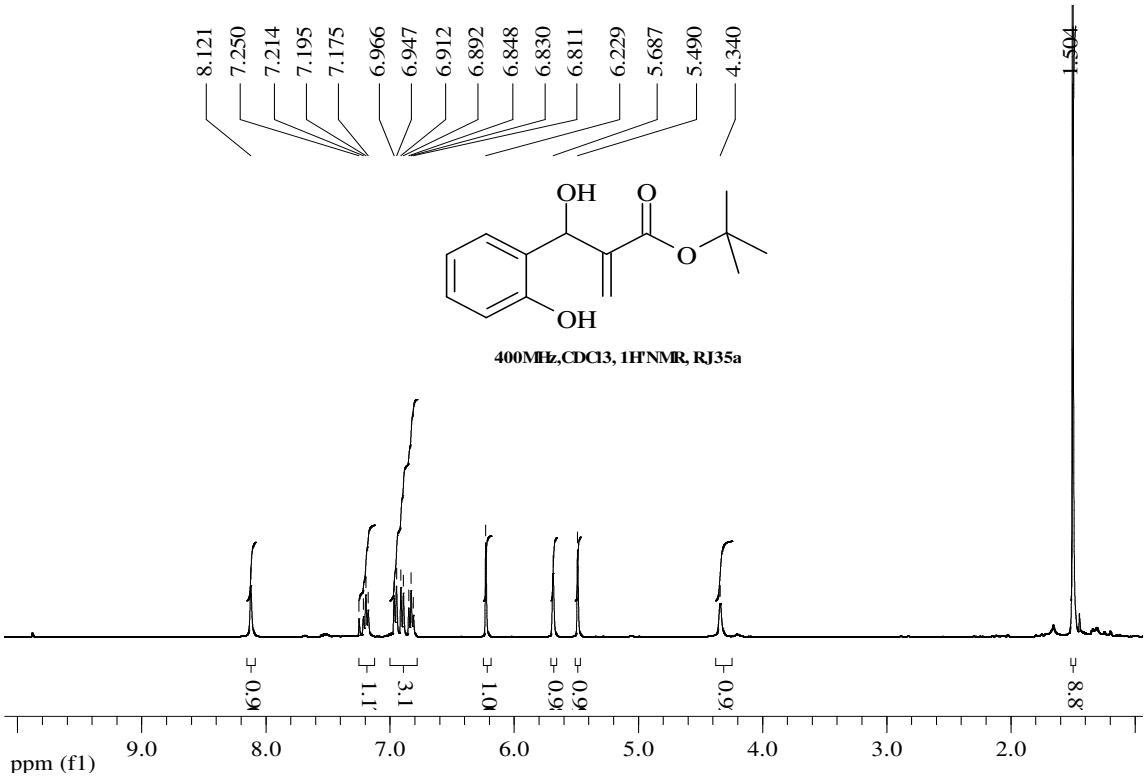
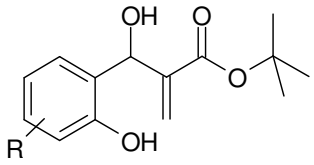


Figure 8. 400 MHz ^{1}H NMR spectrum of compound **24a** in $CDCl_3$.

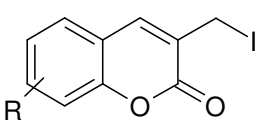
Table 2. Yields obtained for the Baylis-Hillman products **24a-e** (Scheme 18).



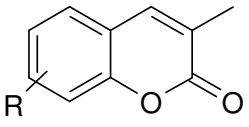
Compound	R	Yield/ %
24a	H	23
24b	3-OCH_2CH_3	20
24c	5-Cl	54
24d	5-Br	50
24e	3-OCH_3	25

The Baylis-Hillman adducts **24a-e** were then reacted under acidic conditions as outlined in Scheme 18. When the adducts were refluxed for 2 hours with hydrochloric acid in a mixture of acetic acid and acetic anhydride, the 3-(chloromethyl)coumarin derivatives **41a-e** were obtained in yields of up to 94% (pathway II). The adducts were also reacted with hydriodic acid under similar conditions, but for different periods of time. Refluxing for 2 hours resulted in a mixture of two products, which were isolated by flash column chromatography, *viz*., the expected 3-(iodomethyl)coumarin **42c** and **d** and the reduced 3-methyl analogues **47c** and **d** (Scheme 18, pathway III: Figure 9). When the reaction mixtures were refluxed for 8 hours, the reduced analogues **47c** and **d** were obtained as the sole products. Presumably, as reaction times increase, the initial iodomethyl products are converted to the methyl analogues with HI acting as a reducing agent. When compounds **24c** and **d** were reacted for 1h, the required 3-(iodomethyl)coumarin **42c** and **d** were obtained as the sole products. The ^{1}H NMR spectrum of 6-bromo-3-methylcoumarin **47d** (Figure 10a) reveals the methyl proton singlet at 2.20 ppm, while the spectrum of the 6-bromo-3-(iodomethyl)coumarin precursor **42d** (Figure 11a) reveals the iodomethyl signal at 4.53 ppm. The methyl carbon in compound **47d** resonates at 17.2 ppm, while the iodomethyl carbon resonates upfield at -2.3 ppm (see Figures 10b and 11b), respectively. The DEPT135 spectrum of compound **47d** confirms the methyl carbon assignment as a positive signal, whereas the iodomethyl signal in compound **42d** is observed as negative methylene signal (see Figures 10c and 11c).

Table 3. Yields obtained for the synthesis of coumarin derivatives **42a-d** and **47c-d** using HI (Scheme 18).



42a-d



47c-d

Substrate	R	Refluxing period /h	Yields of **42**/ %	Yields of **47**/ %
24a	H	2	60	-
24b	8-OCH_2CH_3	2	62	-
24c	6-Cl	1	61	-
		2	52	45
		8	91	-
24d	6-Br	1	58	-
		2	58	43
		8	80	-

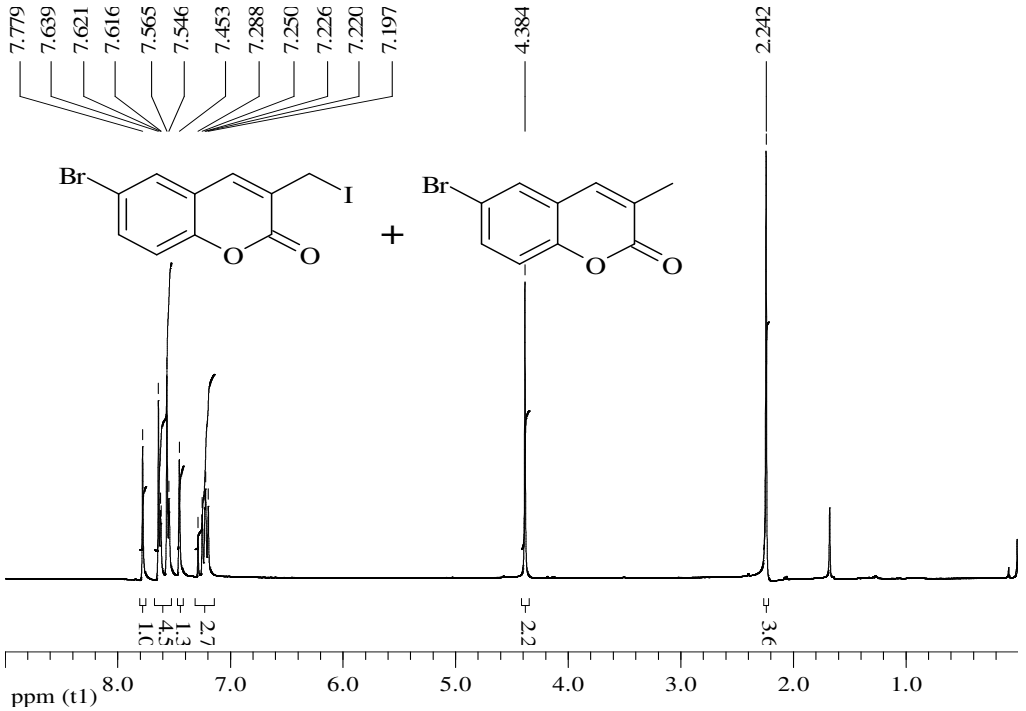


Figure 9. 400MHz ^{1}H NMR spectrum of the mixture of **42d** and **47d** in $CDCl_3$.

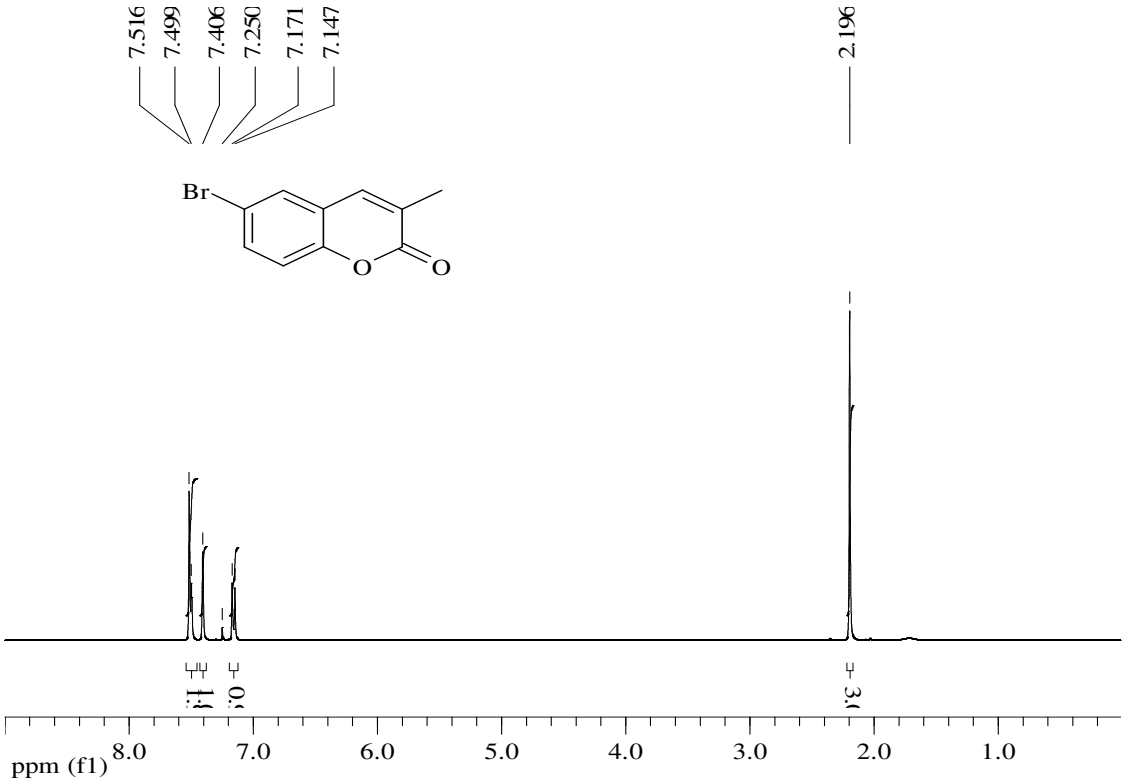


Figure 10a: 400MHz ^{1}H NMR spectrum of 6-bromo-3-methylcoumarin **47d** in $CDCl_3$.

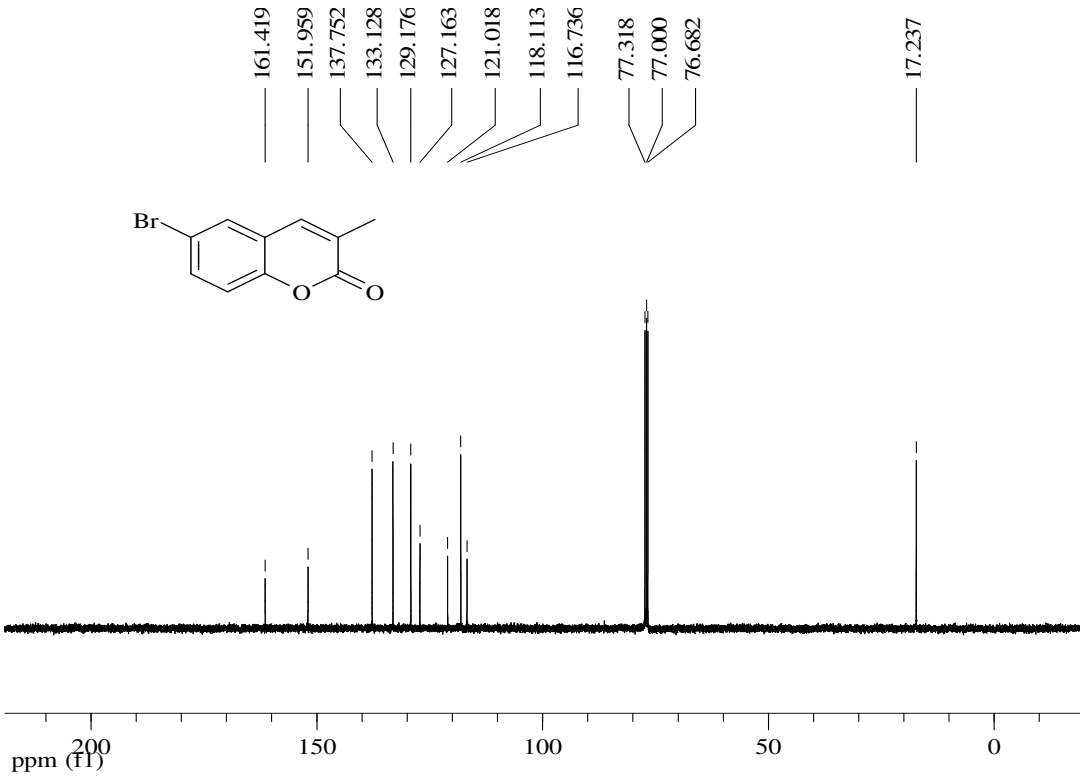


Figure 10b. 100MHz ^{13}C NMR spectrum of 6-bromo-3-methylcoumarin **47d** in $CDCl_3$.

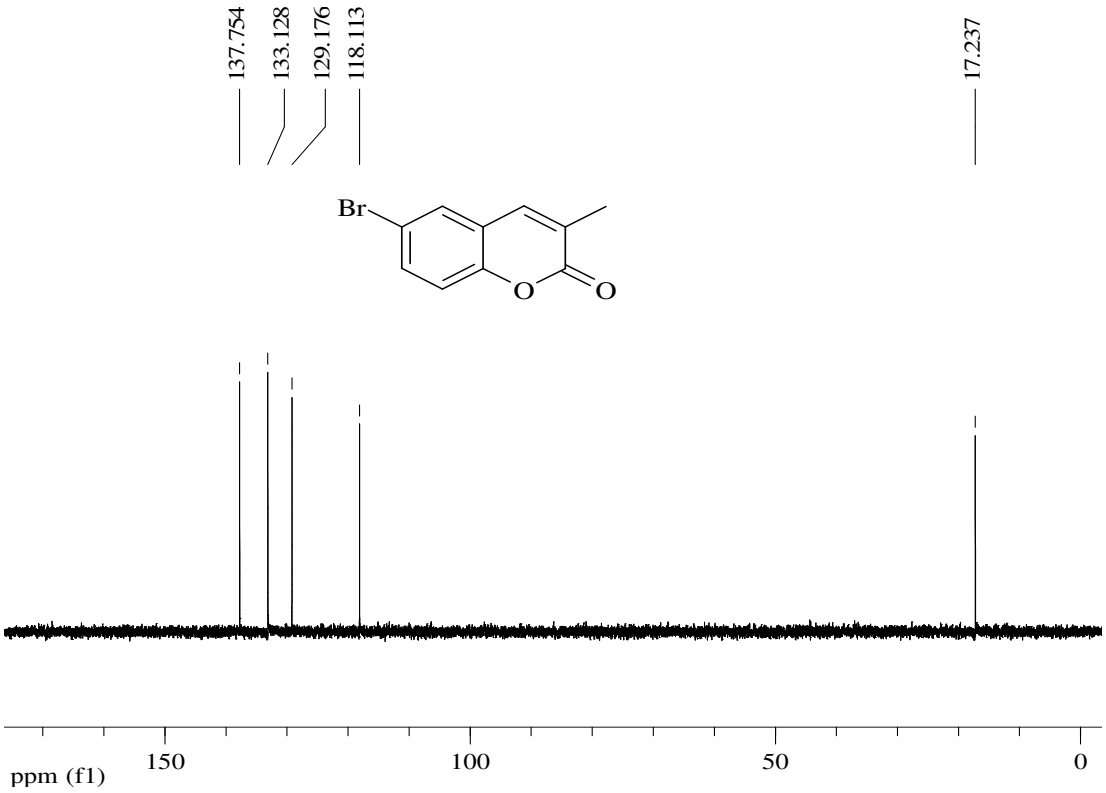


Figure 10c. DEPT135 spectrum of 6-bromo-3-methylcoumarin **47d** in $CDCl_3$.

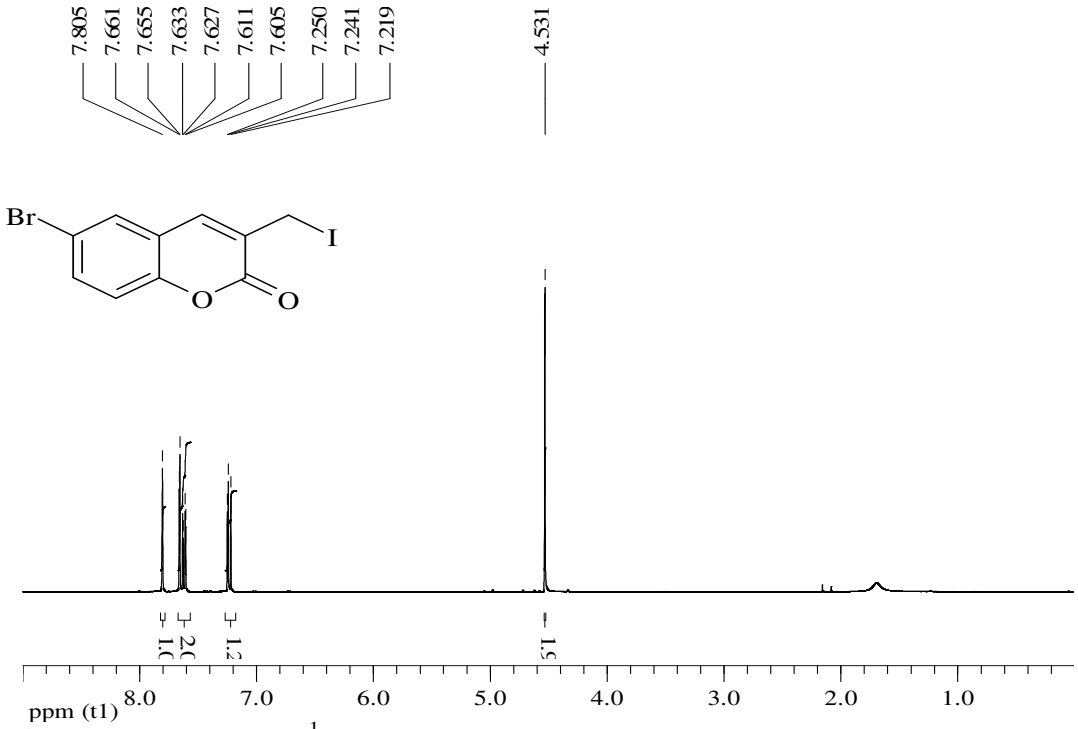


Figure 11a. 400MHz ^1H NMR spectrum of 6-bromo-3-(iodomethyl)coumarin **42d** in $CDCl_3$.

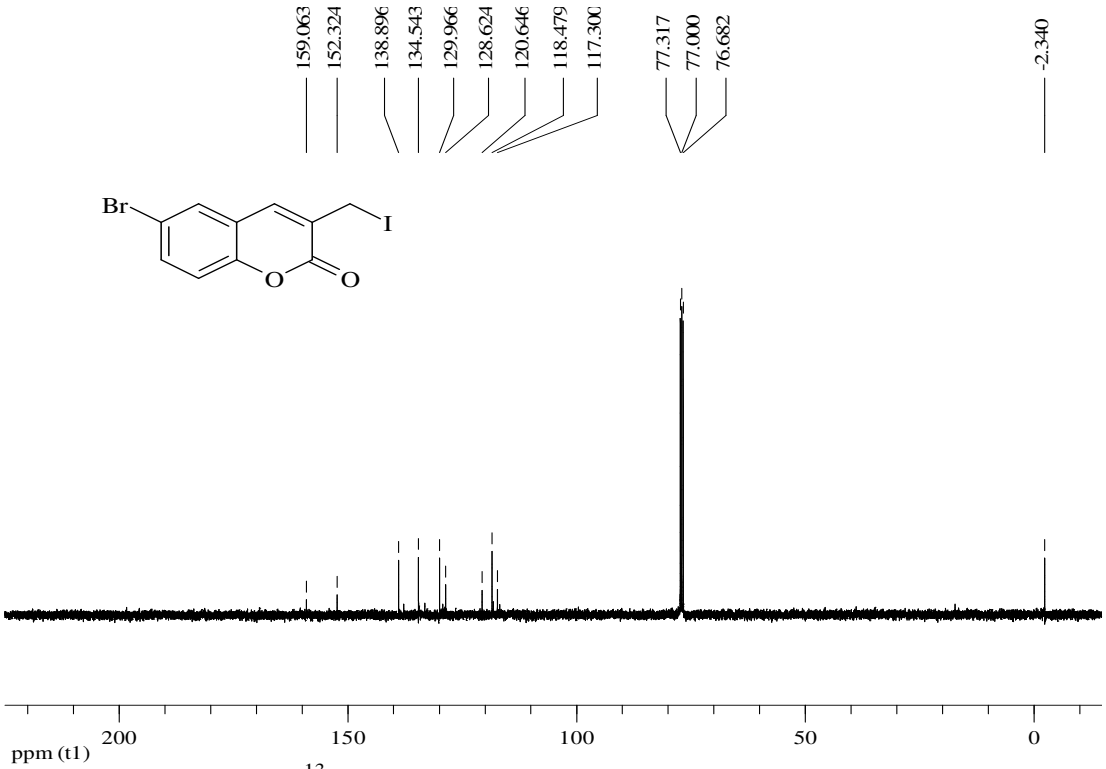


Figure 11b. 100MHz ^{13} C NMR spectrum of 6-bromo-3-(iodomethyl)coumarin **42d** in $CDCl_3$.

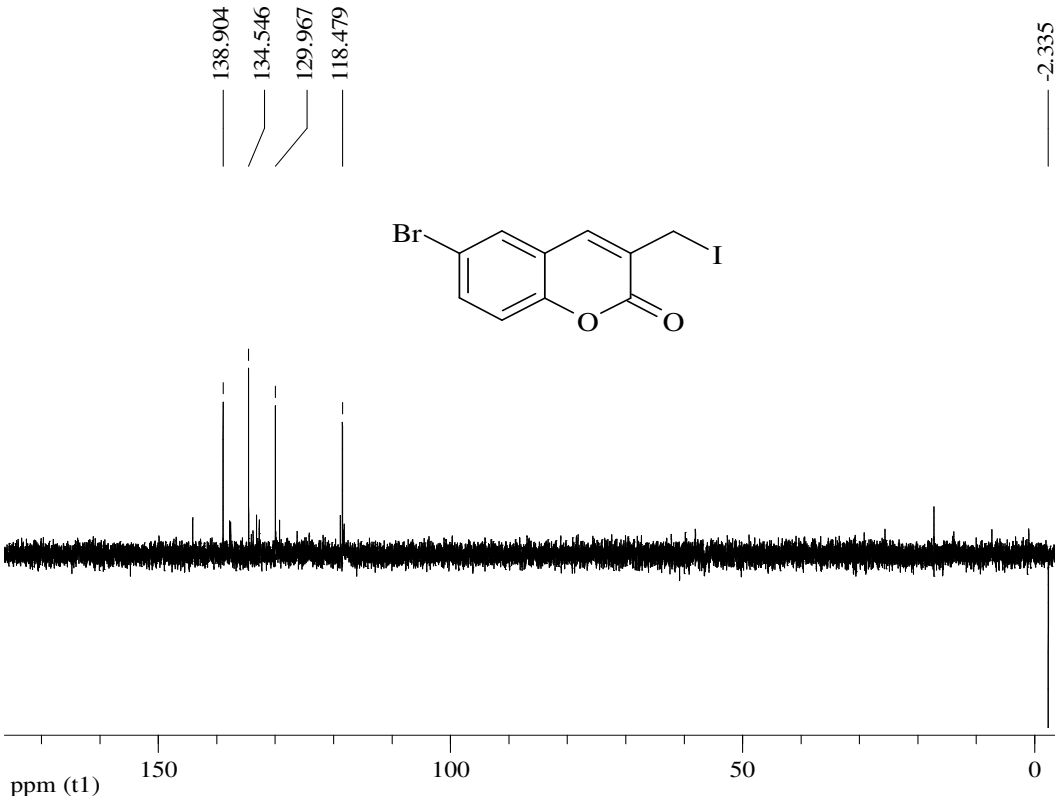


Figure 11c. DEPT135 spectrum of 6-bromo-3-(iodomethyl)coumarin **42d** in $CDCl_3$.

2.3. Nucleophilic substitution of the 3-(halomethyl)coumarin derivatives

A unique feature of the 3-(halomethyl)coumarin system is its potential for attack by a nucleophile at one or more of three electrophilic centres (C-2, C-4 or C-1') as shown in Figure 12. Attack at C-2, the electrophilic carbonyl centre, may result in acyl substitution and ring-opening, while attack at the C-4 centre could involve either allylic substitution of the halide X (S_N') or conjugate addition. Displacement of a leaving group X could also be effected by direct (S_N) substitution at C-1'.

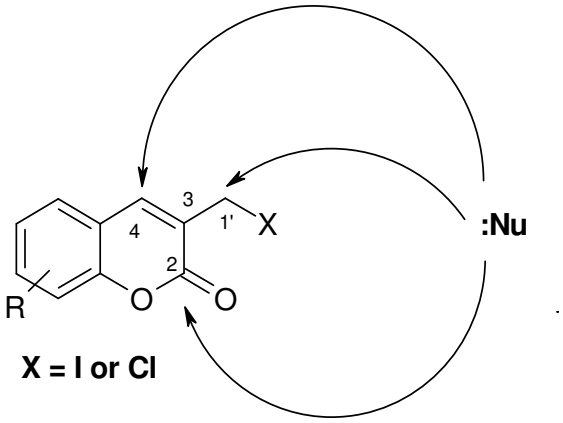


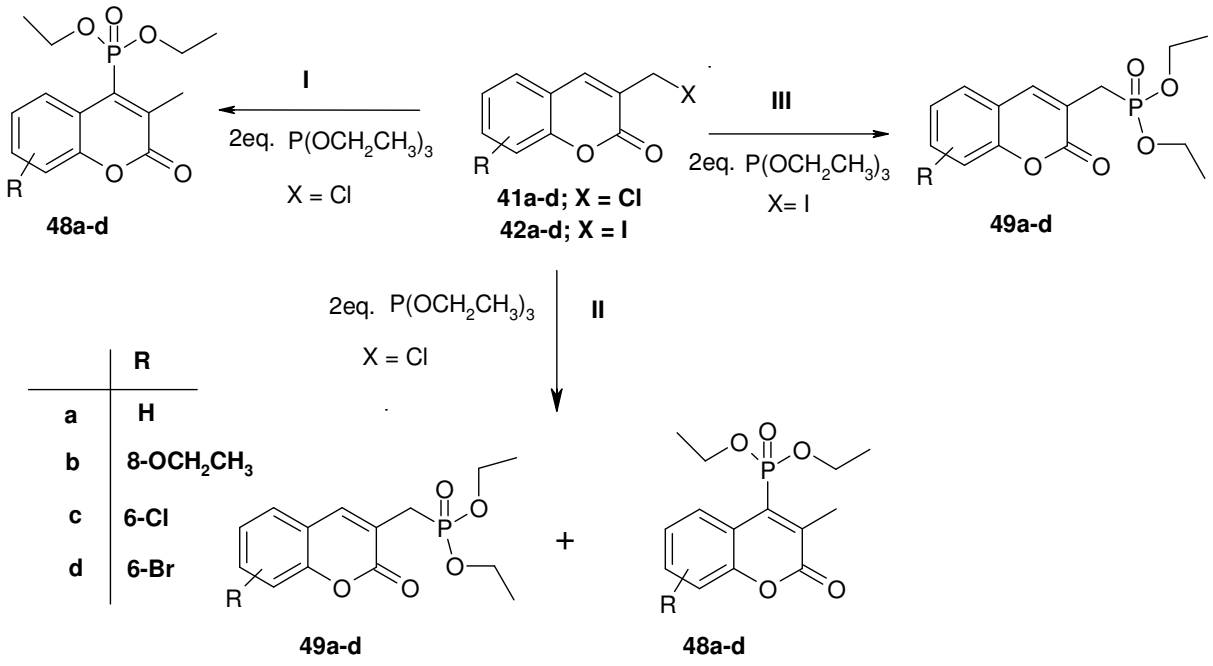
Figure 12. Possible ways in which a nucleophile may attack the 3-(halomethyl)coumarin derivatives.

2.3.1 Arbuzov reactions of 3-(halomethyl)coumarin products

Attention was given to the preparation of phosphonate derivatives *via* the Michaelis-Arbuzov reaction as illustrated in Scheme 19.[76] This synthetic approach permits the formation of a new C-P bond between a trivalent phosphorus reagent and alkyl halide derivatives.[76,77,78]

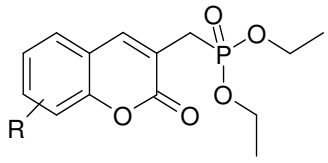
When the 3-(chloromethyl)coumarin derivatives **41a-d** were heated with two equivalents of triethylphosphite under solvent-free conditions in air at 120-130^{o}C (pathway I, Scheme 18),[78] the unexpected 4-phosphorylated products **48a-d** were obtained in yields of up to 68% (Table 4), with no trace of the expected 1'-phosphorylated products **49a-d**. Literature reviews indicate that the phosphorous group at the C-1' position can be obtained when the reactions are carried out under nitrogen.[78,79] The 3-

(chloromethyl)coumarin derivatives **41a-d** were therefore treated with 2 equivalents of triethylphosphite under the same conditions as those used in pathway I except that the reaction was conducted under nitrogen (pathway II). Flash chromatography of the isolated material afforded both 1'-phosphorylated products **49a-d** in yields of up to 85% and the 4-phosphorylated products **48a-d** in yields of up to 24%. In a similar way, treatment of the 3-(iodomethyl)coumarins **42a-d** with 2 equivalents of triethylphosphite under same conditions as those described for pathway II led to formation of the 1'-phosphorylated products **49a-d** with no trace of the 4-phosphorylated analogues **48a-d** (pathway III). A possible explanation for the formation of **48a-d** and **49a-d**, using 3-(chloromethyl)coumarin derivatives **42** (pathway II) as starting material, is that chloride is a poorer leaving group than iodide. Therefore, the reaction rates for the chloro analogues are lower, permitting the incoming nucleophile (phosphorous group) to attack at both the C-1' and C-4 positions, leading to the formation of the isomeric products **48a-d** and **49a-d**. Also, the more electronegative Cl atom may increase the electrophilicity of the vinylic centre (C-4) *via* an electro-withdrawing inductive effect.

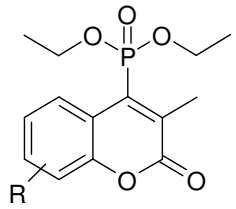


Scheme 19

Table 5: Yields obtained for the synthesis of 1'-phosphorylated products **48a-d** and 4-phosphorylated products **49a-d**.



49

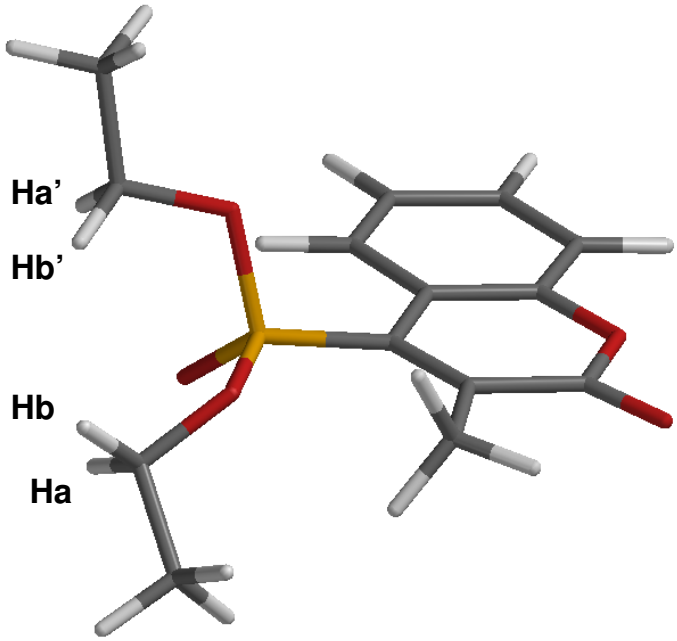


48

Substrate	R	X	Yields of **49**/% (under N_2)	Yields of **48**/% (under N_2)	Yields of **48**/% (in air)
41a	H	Cl	43	24	60
41b	8-OCH_2CH_3	Cl	85	12	45
41c	6-Cl	Cl	55	10	68
41d	6-Br	Cl	53	8	52
42a	H	I	61	-	-
42b	8-OCH_2CH_3	I	85	-	-
42c	6-Cl	I	55	-	-
42d	6-Br	I	43	-	-

IR and NMR spectroscopy were used to analyse all compounds. The ^{1}H NMR spectrum of diethyl (6-chloro-3-methyl-2-oxo-2*H*-chromen-4-yl)phosphonate **48c** (Figure 13a) shows the 3-methyl group resonating as a doublet at 2.56 ppm due to coupling with phosphorous ($J_{P,H}$ = 3.2Hz). The ^{1}H NMR spectrum of the isomeric diethyl [(6-chloro-2-oxo-2*H*-chromen-3-yl)methyl]phosphonate **49c** (Figure 14a) reveals a doublet, corresponding to the methylene protons attached to phosphorous, at 3.15 ppm with a large coupling constant ($J_{P,H}$ = 22 Hz). Furthermore, the aromatic region of the spectrum of compound **48c** reveals the presence of three protons, while the spectrum of isomer **49c** reveals the presence of 4 aromatic protons. The methylene protons of compound **48c** resonate as a pair of multiplets at 4.17 and 4.25 ppm. This is attributed to the

diastereotopicity of the geminal protons (Ha and Hb; Ha' and Hb') in each methylene group in the preferred non-planar conformation illustrated below.



Equilibrium geometry calculated at the semi-empirical AM1 level.

The ^{13}C NMR spectrum of compound **48c** (Figure 13b) reveals two methyl carbon signals as doublets at *ca.* 16 ppm, while the spectrum of isomer **49c** (Figure 14b) reveals doublets corresponding to the ester- and 3-methyl carbons at *ca.* 16.3 and 16.5 ppm, respectively. The coupling between the methyl and methylene protons of the two ethoxy groups in compounds **48c** and **49c** was evident from their COSY spectra, while the HSQC spectra of these compounds show the correlations between protons and carbons (see Figures **13d, e** and **14d, e** respectively). The DEPT 135 of spectrum of compound **48c** supports the presence of two equivalent methylene groups coupled to phosphorus, whereas the methylene signals observed in the DEPT spectrum of compound **49c**, reflects the presence of the two different types of the methylene groups; the $J_{P,C}$ coupling constant for the methylene carbon attached directly to phosphorous (resonating at *ca.*26 ppm) exhibits the larger value ($J_{P,C}$ = 139Hz) (see Figures 13c and 14c). The IR spectrum of compound **48c** (Figure 13g) clearly confirms the presence of the phosphoryl (P=O) and

the carbonyl group (C=O) absorption bands at 1256 and 1740 cm^{-1}, respectively, while the bands for the corresponding regioisomer **49c** (Figure 14g) were observed at 1260 and 1737 cm^{-1}, respectively. The ^{31}P NMR chemical shifts provide useful information about nature of the phosphorous groups in the isomers **48c** and **49c**. The ^{31}P NMR spectrum of compound **48c** (Figure 13f) shows a phosphorous signal at 13.56 ppm, while the phosphorous attached to an sp^2 carbon resonates at 24.87 ppm in the spectrum of compound **49c** (Figure 14f).

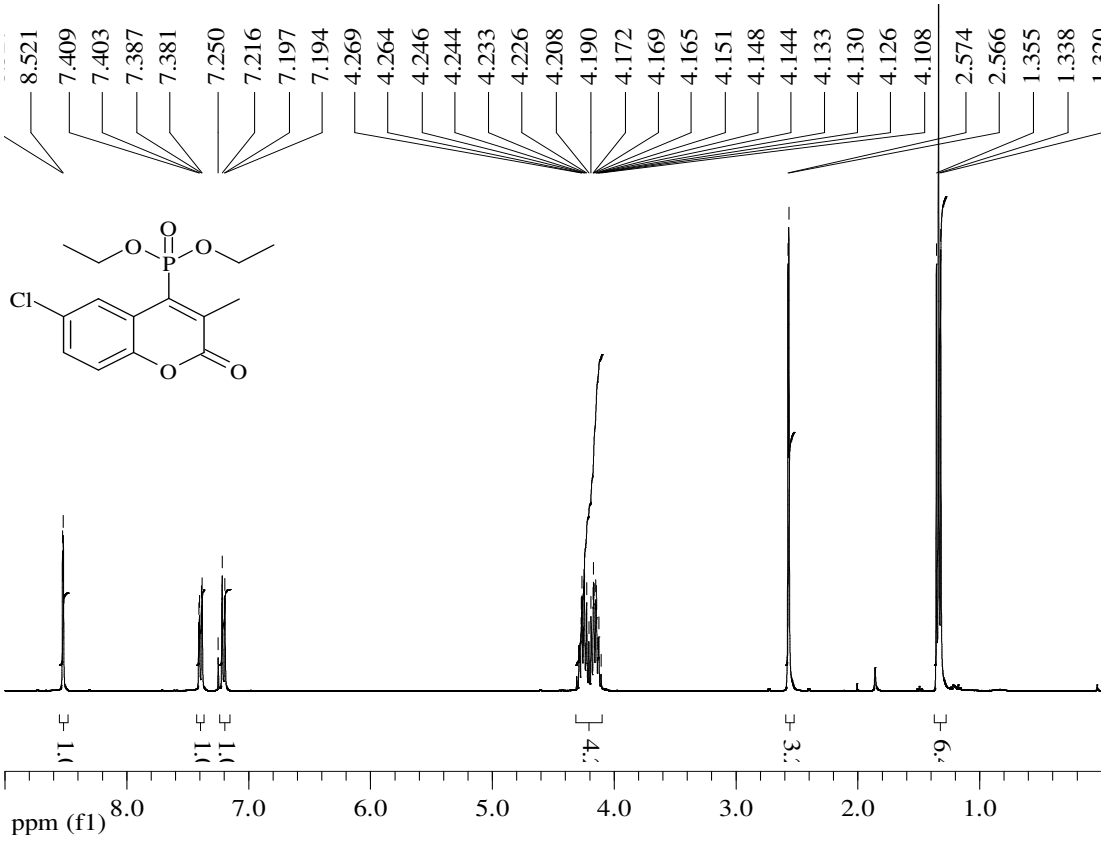


Figure 13a. 400MHz ^1H NMR spectrum of compound **48c** in $CDCl_3$.

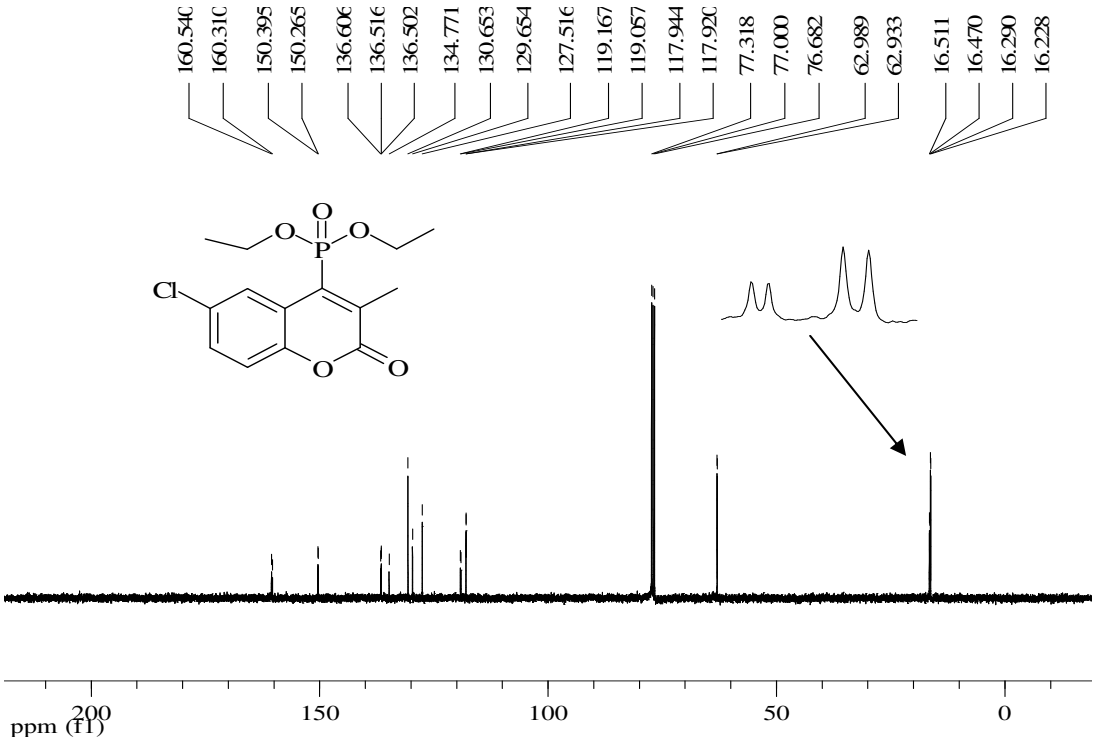


Figure 13b. 100MHz ^{13}C NMR spectrum of compound **48c** in $CDCl_3$.

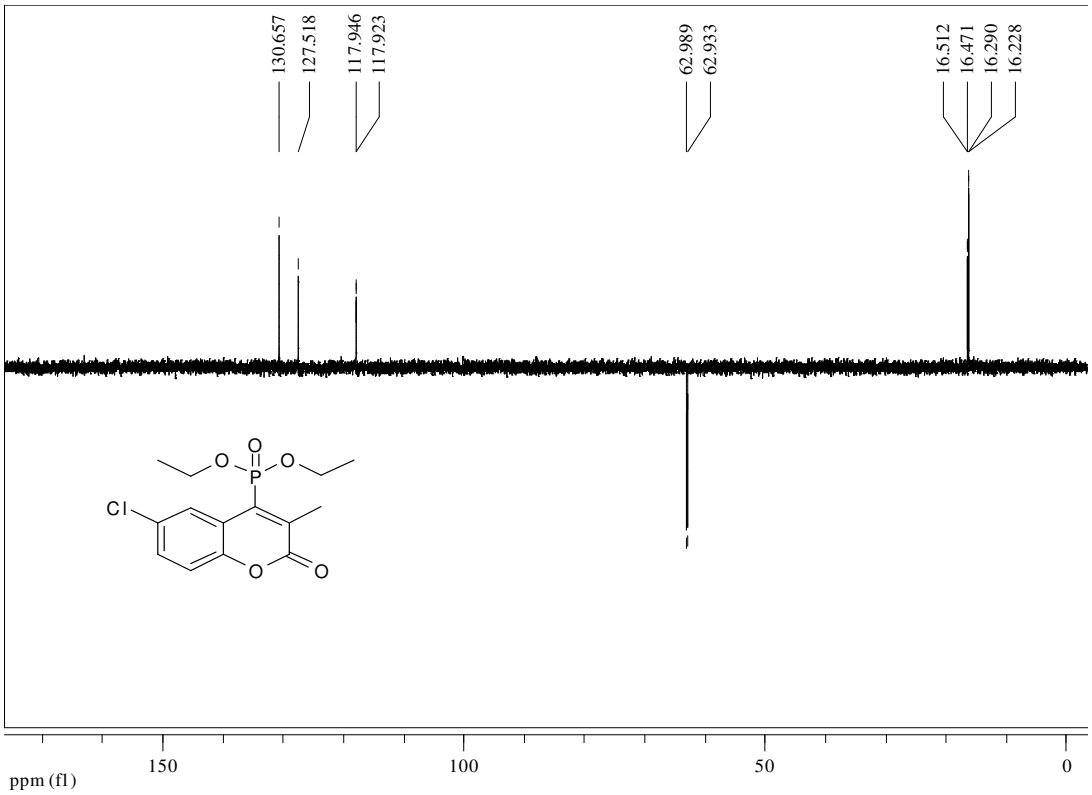


Figure 13c. DEPT135 spectrum of compound **48c** in $CDCl_3$.

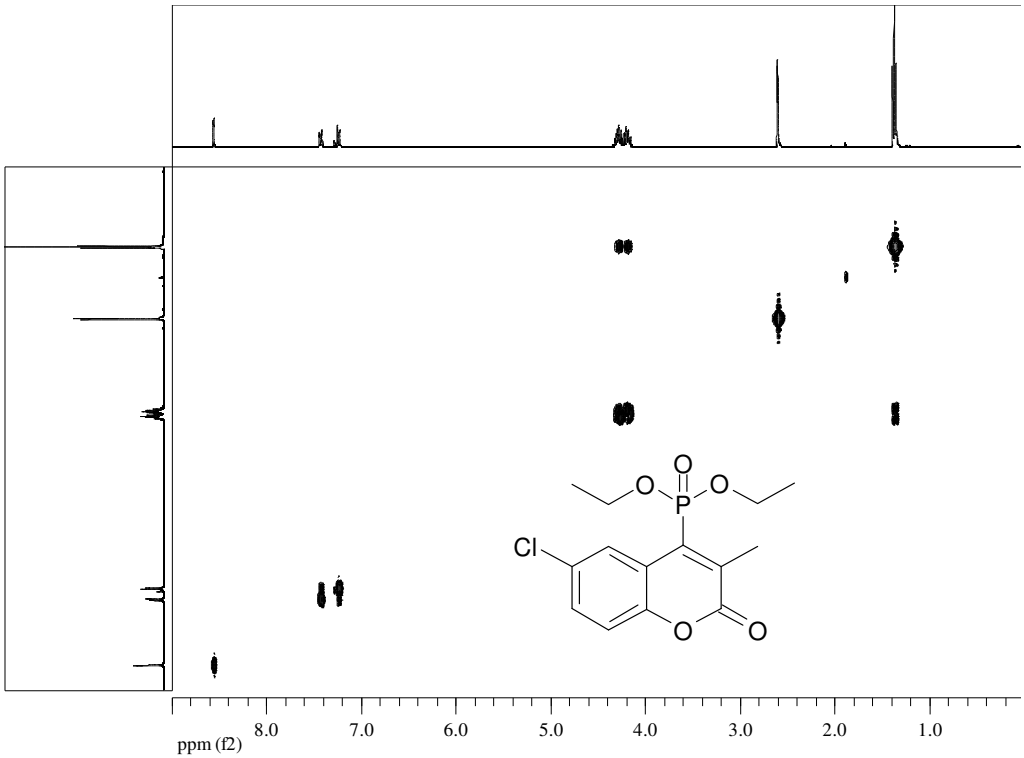


Figure 13d. 400MHz COSY spectrum of compound **48c** in $CDCl_3$.

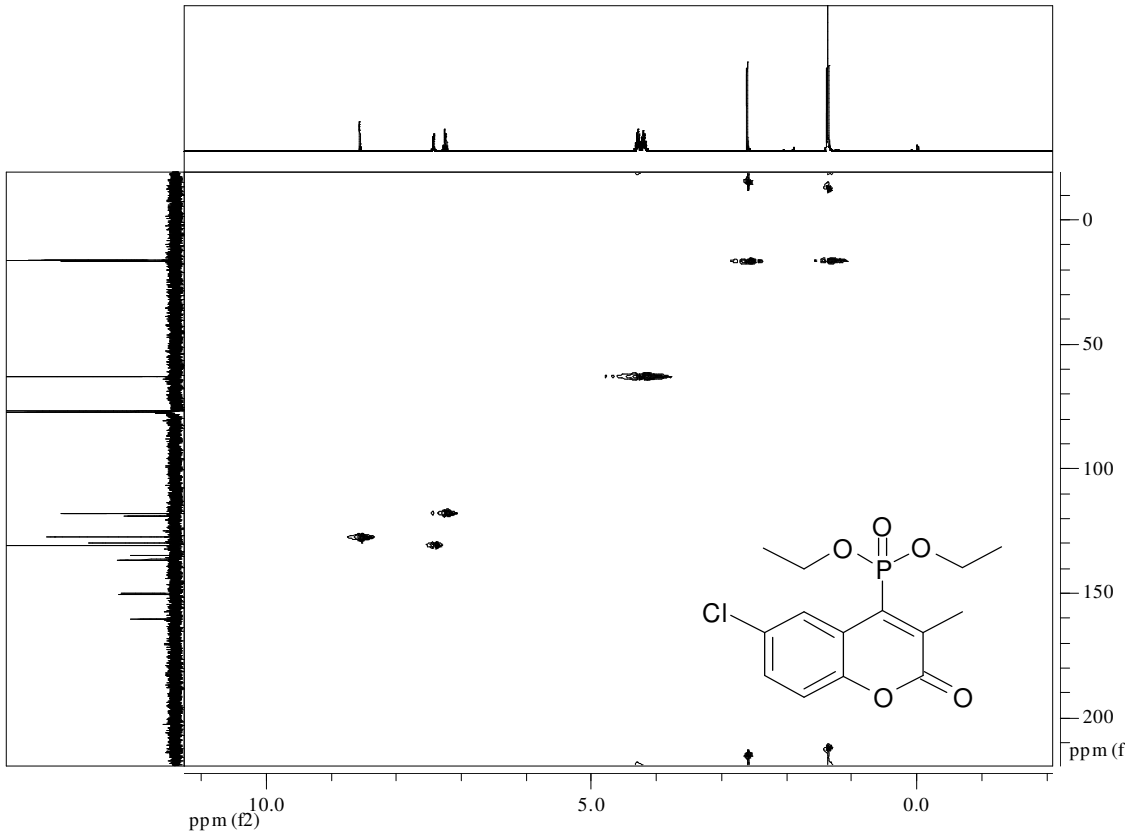


Figure 13e. HSQC spectrum of compound **48c** in $CDCl_3$.

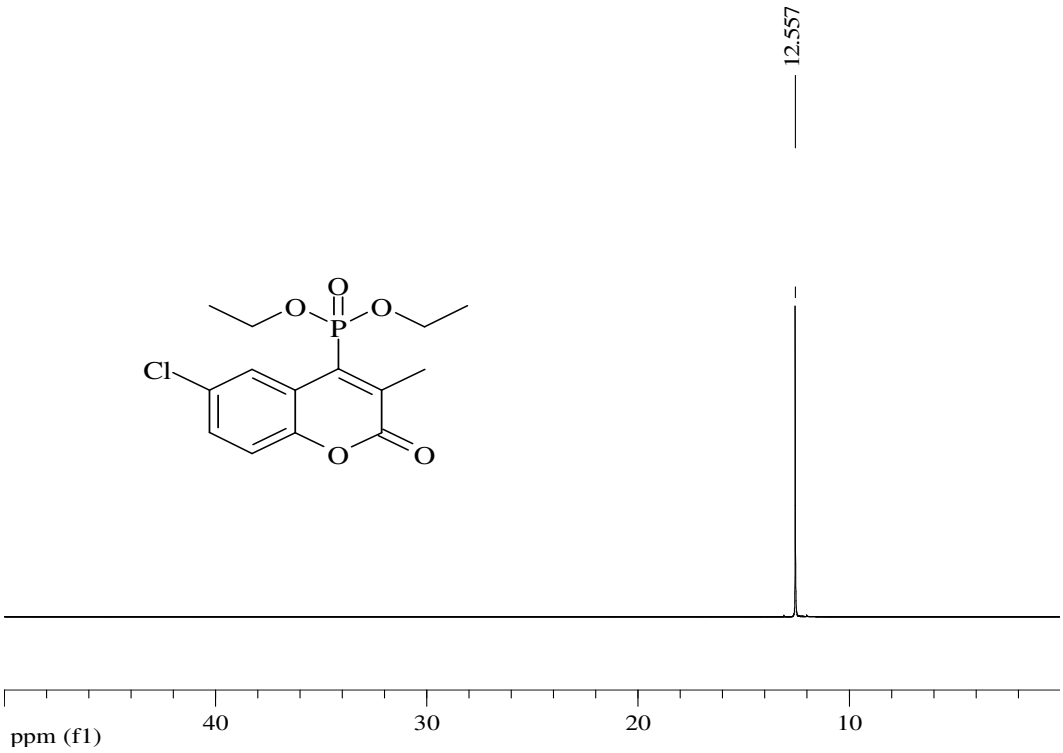


Figure 13f. 162MHz ^{31}P NMR spectrum of compound **48c** in $CDCl_3$.

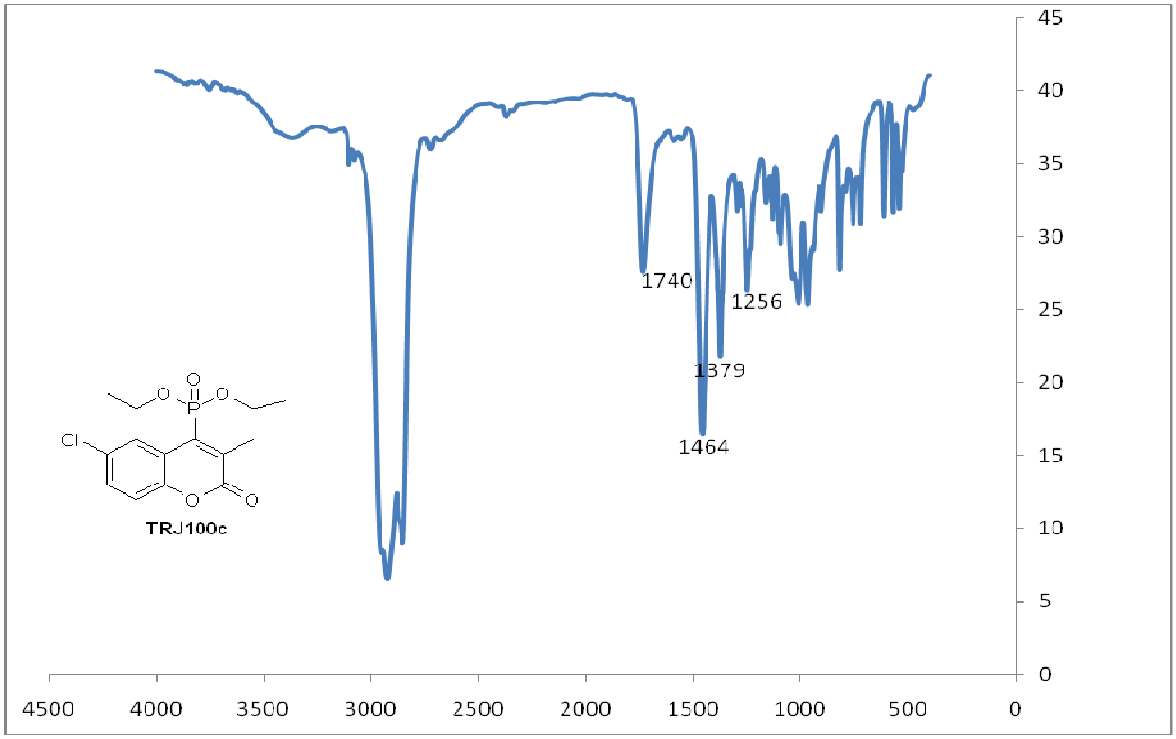


Figure 13g. IR spectrum of compound **48c** (nujol mull).

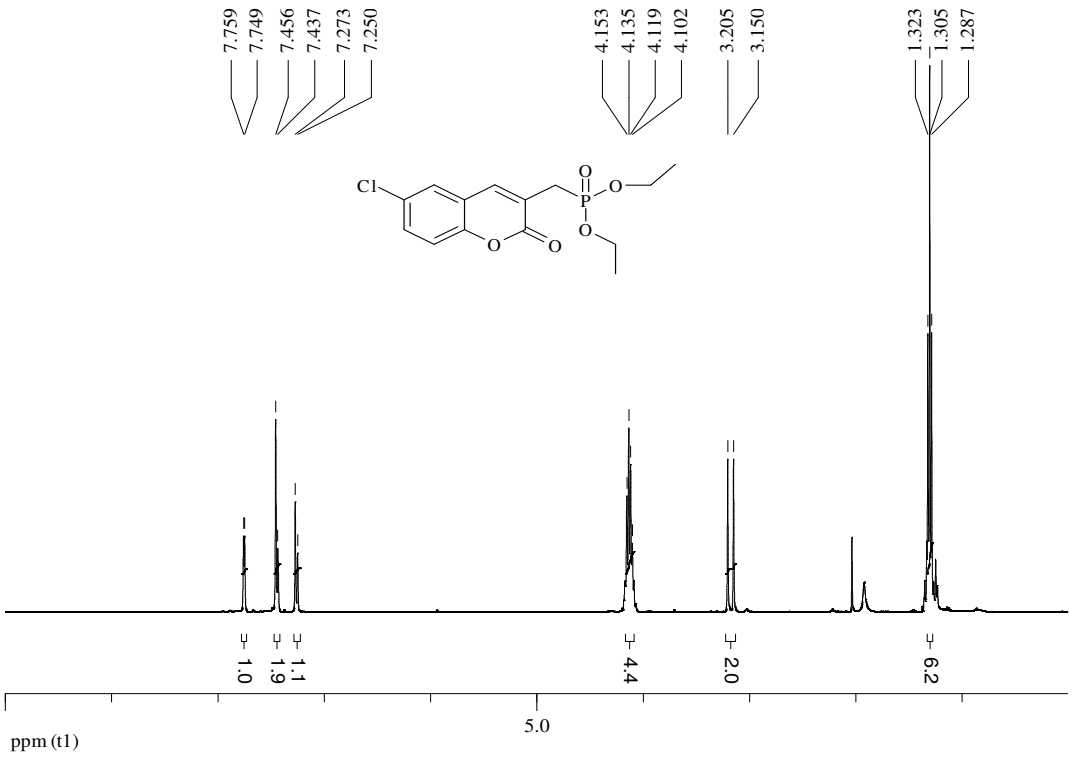


Figure 14a. 400MHz ^{1}H NMR spectrum of compound **49c** in $CDCl_3$.

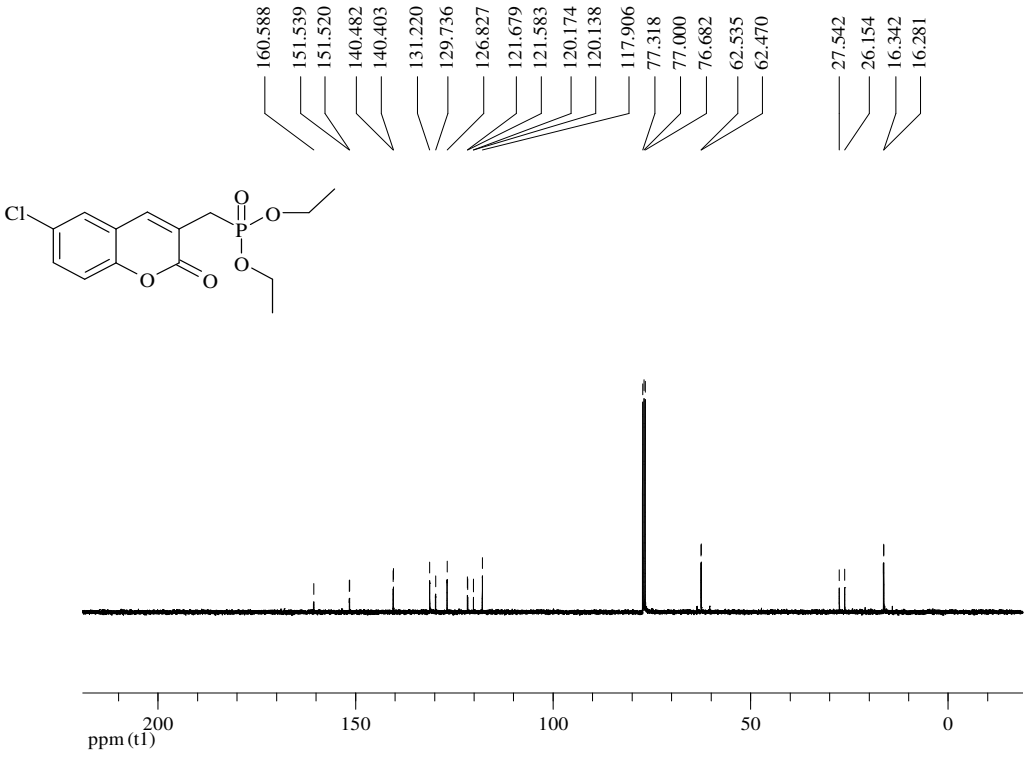


Figure 14b. 100MHz ^{13}C NMR spectrum of compound **49c** in $CDCl_3$.

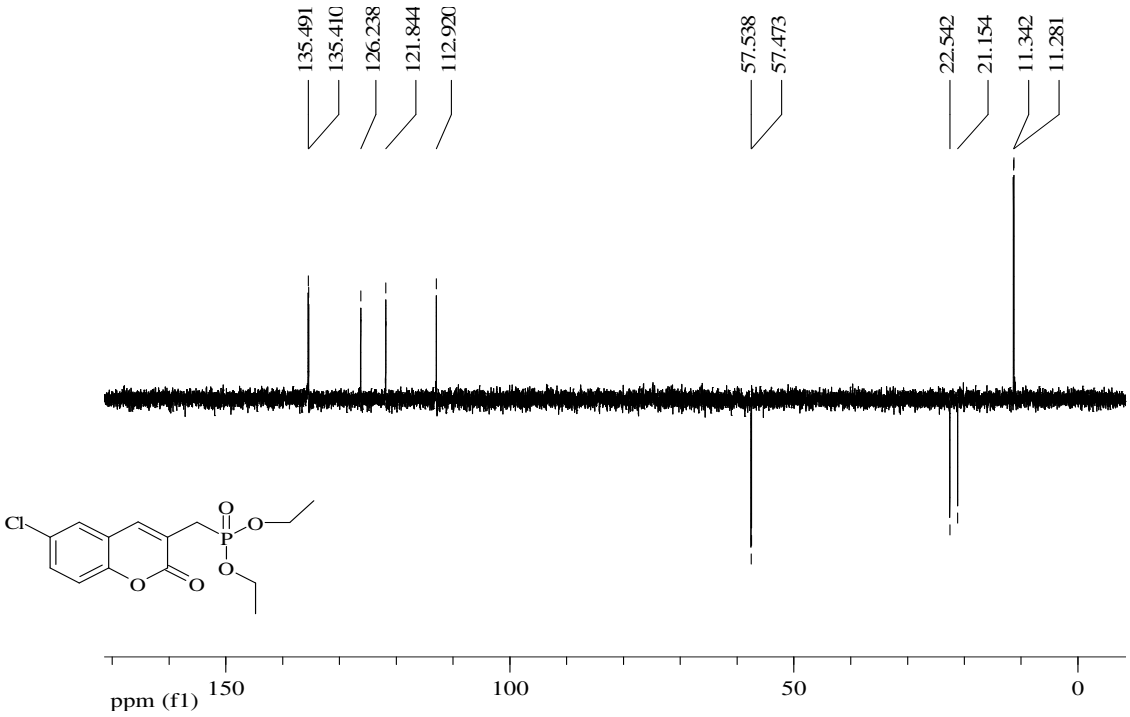


Figure 14c. DEPT135 spectrum of compound **49c** in $CDCl_3$.

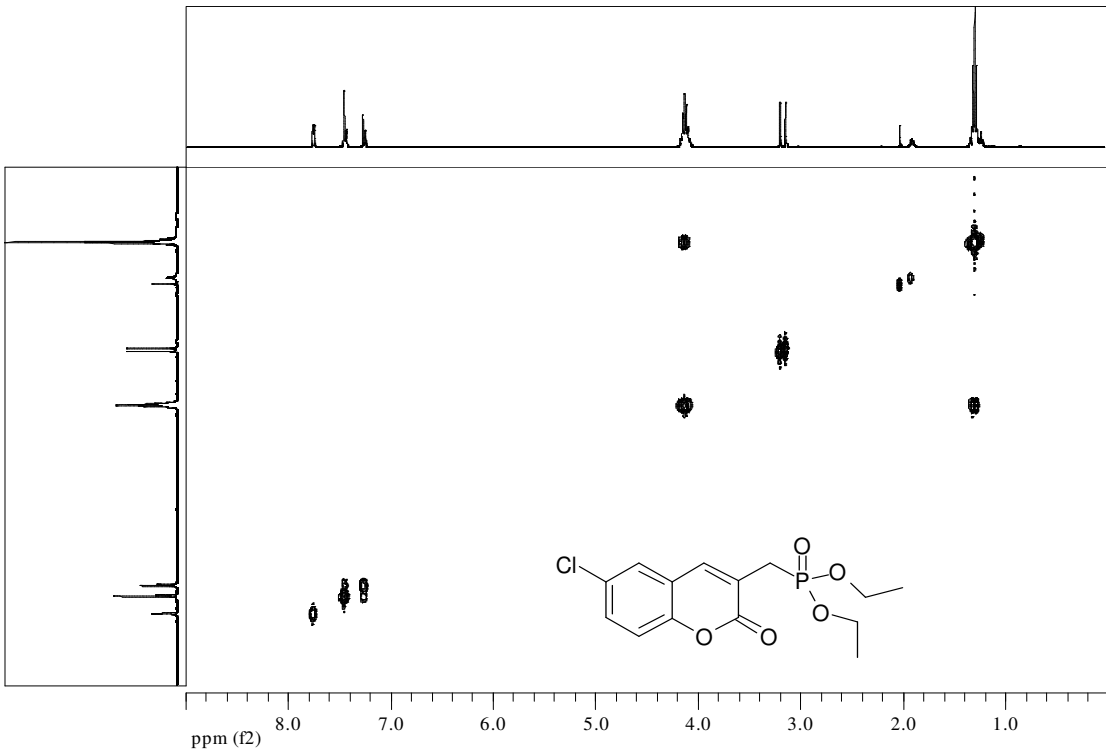


Figure 14d. 400MHz COSY spectrum of compound **49c** in $CDCl_3$.

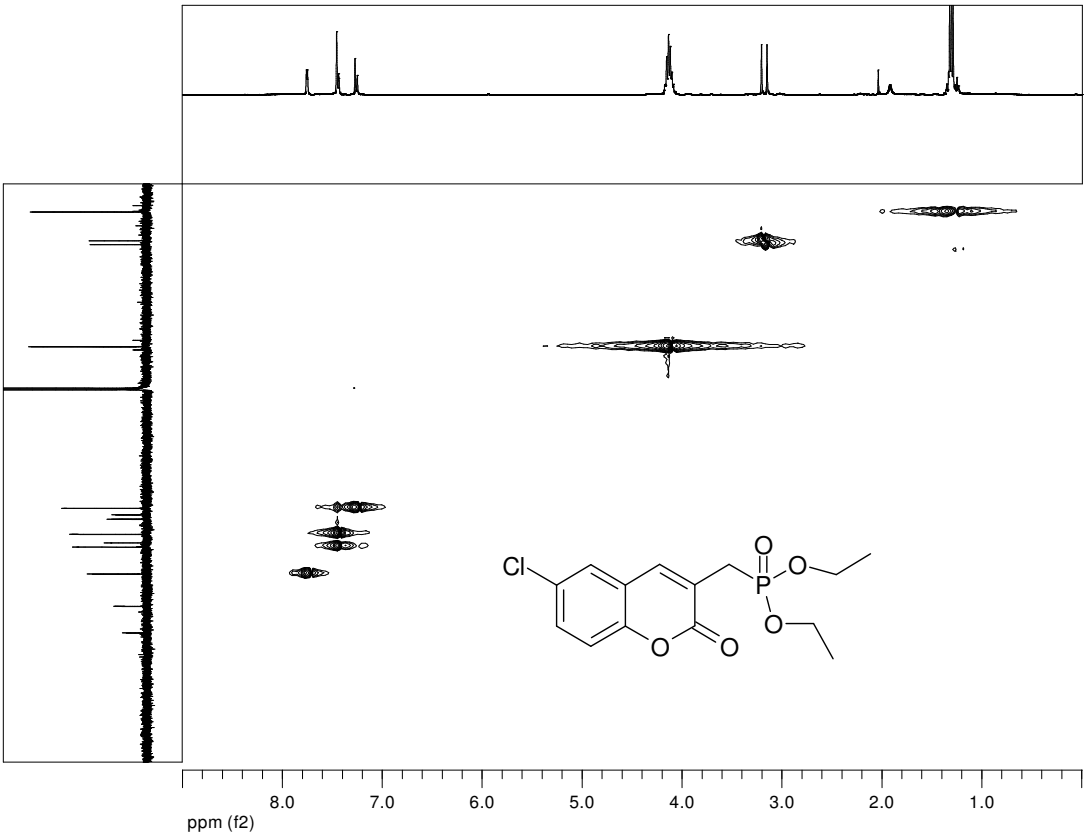


Figure 14e. HSQC spectrum of compound **49c** in $CDCl_3$.

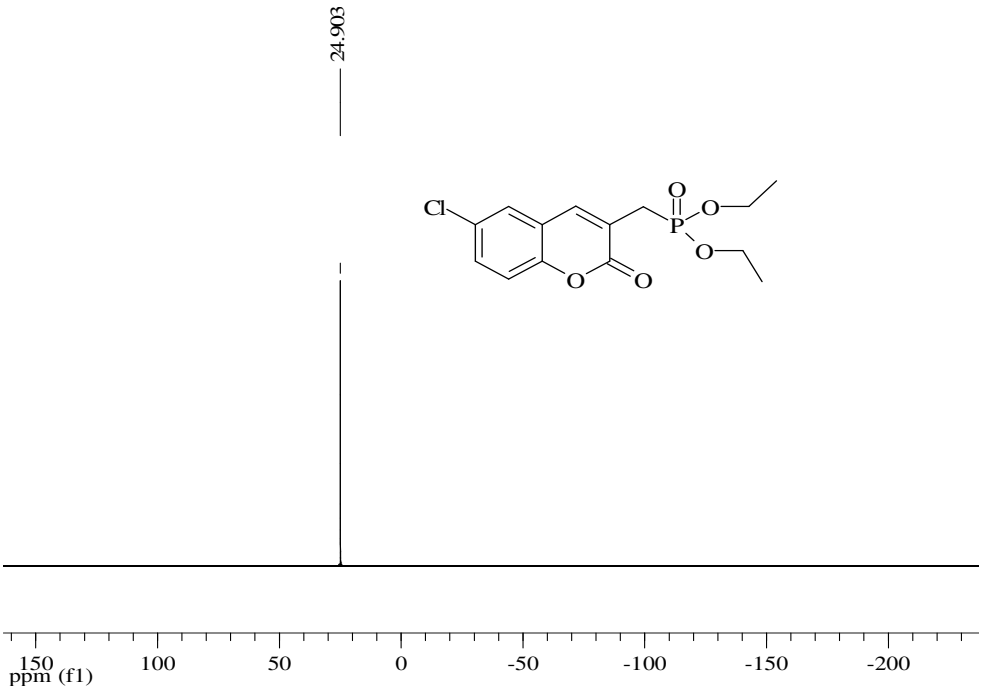


Figure 14f. 162MHz ^{31}P NMR spectrum of compound **49c** in $CDCl_3$.

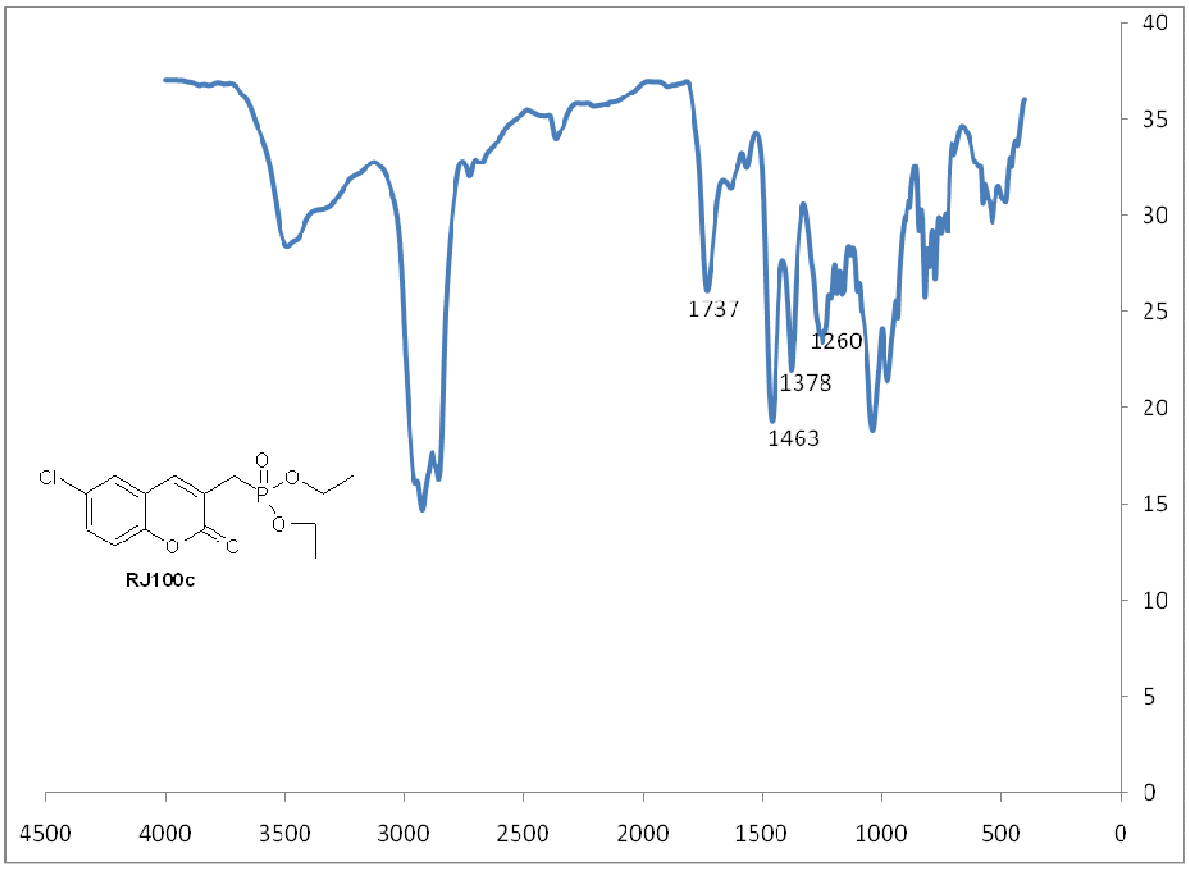
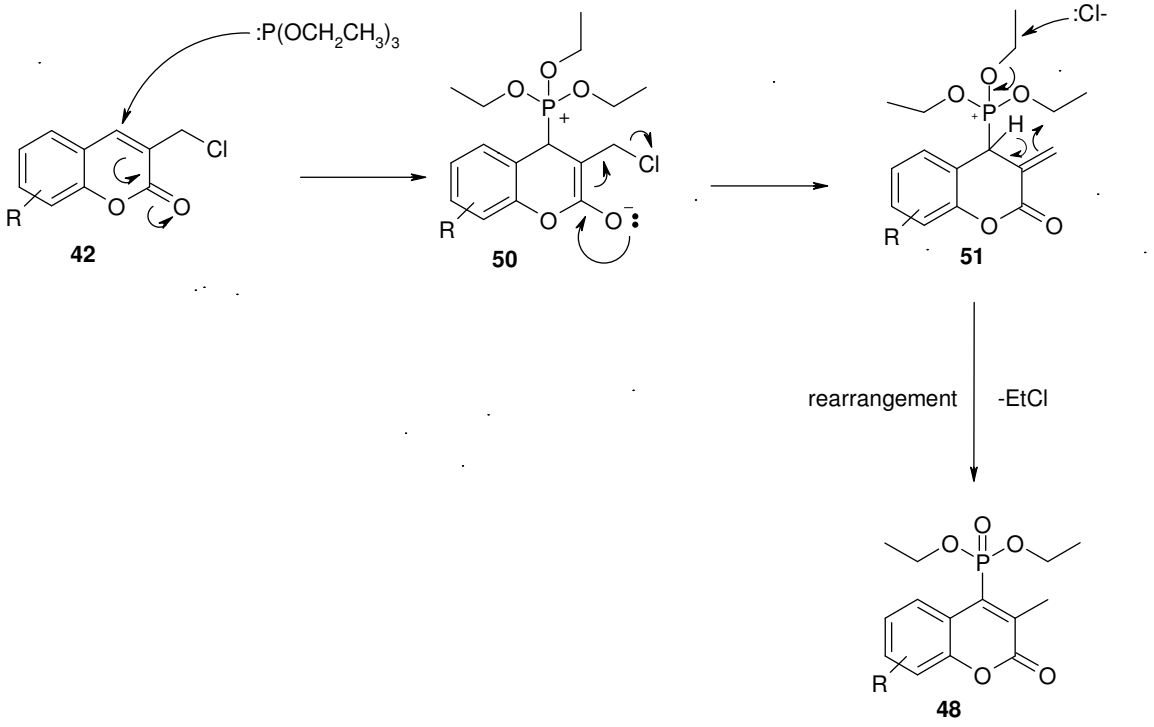


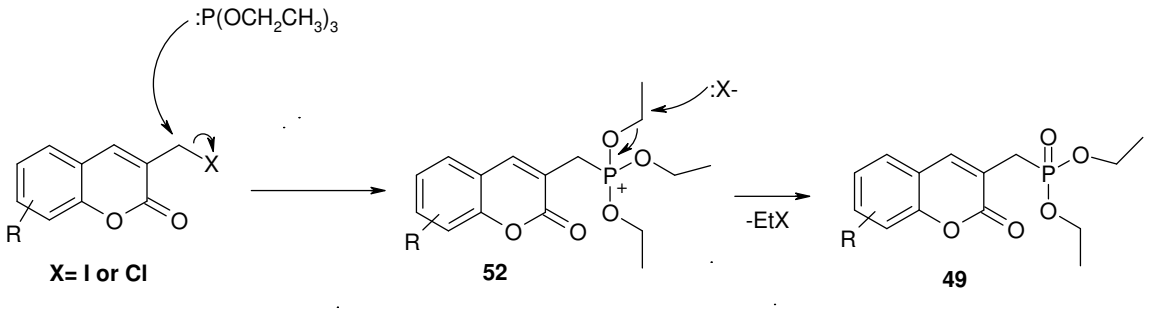
Figure 14g. IR spectrum of compound of compound **49c** (nujol mull).

The expected mechanism for the formation of the 4-phosphorylated products **48** is outlined in Scheme 20. The first step is believed to involve conjugate addition of $P(OEt)_3$ to the α,β-unsaturated carbonyl system, followed by displacement of halide, resulting in the formation of a phosphonium intermediate **51**. The halide ion then attacks one of the *O*-ethyl groups in an S_N reaction, and rearrangement of the double bond then affords the 3-methyl derivatives **48** in what is effectively an allylic Arbuzov reaction. However, the possibility of the allylic displacement of Cl^- by $P(OEt)_3$ in an S_N' reaction to give the intermediate **51** in the one step cannot be excluded.



Scheme 20: Mechanism for the formation of the 3-methyl derivatives **48**.[65,66]

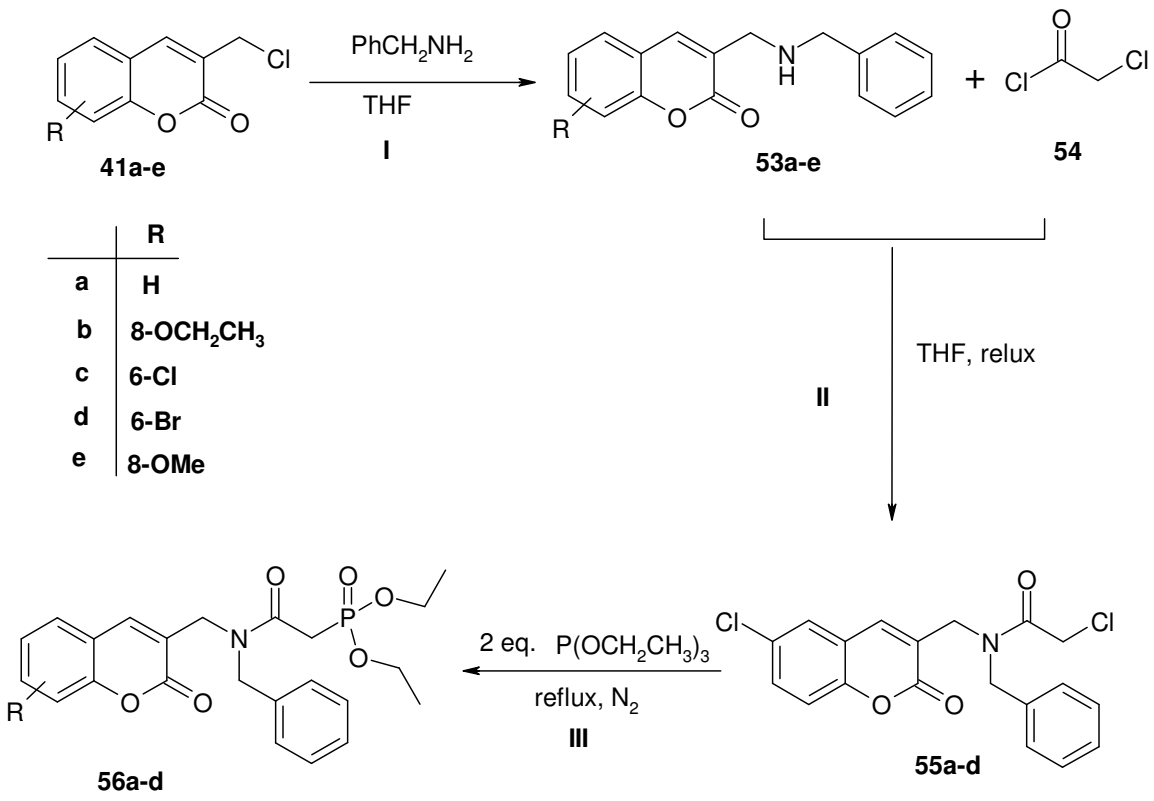
The formation of the 1'-phosphorylated systems **49**, on the other hand, is expected to involve the 'normal' Arbuzov pathway illustrated in the Scheme 21. This reaction proceeds *via* an S_N mechanism in which the alkyl halide is attacked by nucleophilic $P(OEt)_3$ resulting in the formation of a phosphonium salt intermediate **52**. The halide ion then attacks one of *O*-ethyl groups of the phosphonium intermediate to afford the 1'-phosphorylated derivatives **49**.[81]



Scheme 21: Mechanism for the formation of the phosphorylated derivatives **49**.[81]

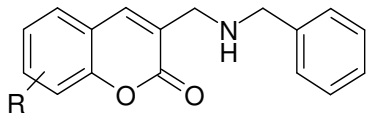
2.3.2. Synthesis of 3-[(benzylamino)methyl]coumarin derivatives

C-N bond formation is one of the fundamental transformations in organic chemistry.[81] Generally the reaction of an alkyl halide with a primary amine is a widely used approach for the preparation of the secondary amines.[78,81,82] The nucleophilic amine attacks the alkyl halide leading to the formation of a secondary amine. Heating or simply stirring in a suitable solvent, depending on the amine used, can achieve the reaction. In the present study, the 3-(chloromethyl)coumarin derivatives **41a-e** were reacted with benzylamine using THF as solvent as shown in Scheme 22.[81] The mixtures were stirred at room temperature in a stoppered reaction flask for 4 hours and after evaporation of the solvent *in vacuo*, the 3-[(benzylamino)methyl]coumarin derivatives **53a-e** were isolated by flash chromatography. The products were obtained in yields of up to 74% (Table 6). The ^{1}H NMR spectrum of 3-[(benzylamino)methyl]coumarin **53a** (Figure 15a) shows a broad signal at 1.99 ppm corresponding to the amino proton while the two methylene groups resonate as singlets at 3.74 and 3.84 ppm. The ^{13}C NMR spectrum reveals the two-methylene carbon signals at 53.2 and 48.4 ppm and the carbonyl carbon signal at 161.4 ppm (Figure 15b).



Scheme 22

Table 6. Yields obtained for the 3-[(benzylamino)methyl]coumarin derivatives **53a-e.**



Compound	R	Yield/ %
53a	H	74
53b	8-OCH_2CH_3	61
53c	6-Cl	64
53d	6-Br	35
53e	8-OCH_3	73

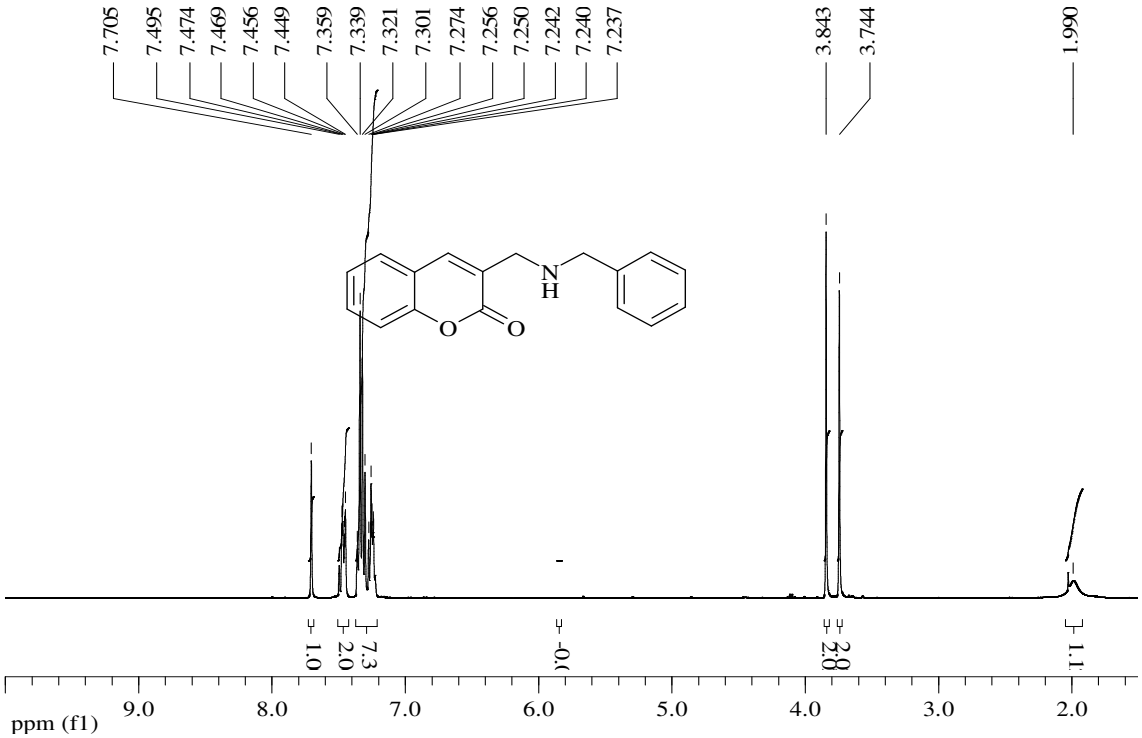


Figure 15**a**. 400MHz ^{1}H NMR spectrum of compound **53a** in $CDCl_3$.

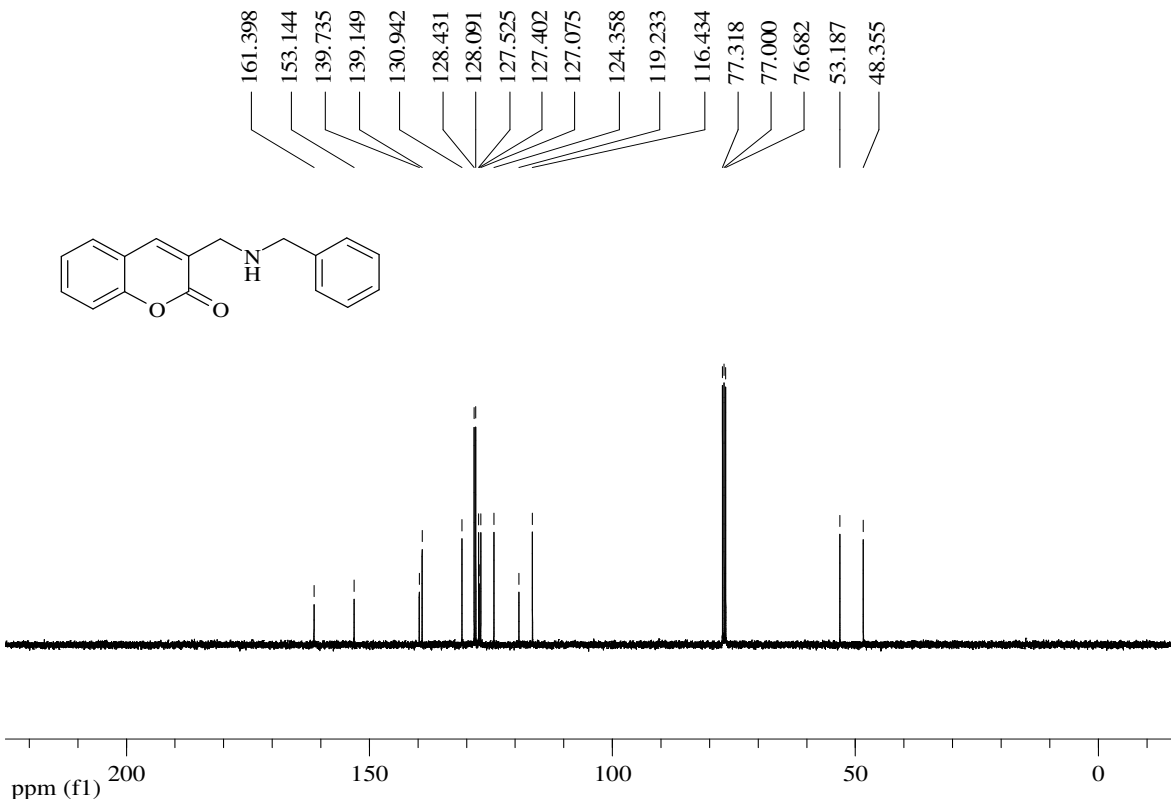


Figure 15b. 100MHz ^{13}C NMR spectrum of compound **53a** in $CDCl_3$.

2.3.3. Synthesis of chloroacetamide derivatives

The 3-[(benzylamino)methyl]coumarin derivatives **53a-d** were then reacted with chloroacetyl chloride **54** using THF as solvent (step II, Scheme 22). The mixtures were refluxed under nitrogen for 45 minutes to afford the amide derivatives **55a-d** in yields of up to 72%. NMR and IR spectroscopy were used to characterize the chloroacetamide derivatives **55a-d**. However, the ^{1}H NMR and ^{13}C NMR spectra were complicated by the presence of additional signals. These were attributed to hindered rotation about the C-N amide bond. When samples were run at 50^{o}C, the signals began to broaden and then coalesce because the internal rotation became fast relative to the NMR time-scale. The resistance to rotation about the C-N bond arises from the delocalisation illustrated in Figure 16; the partial double-bond character of the C-N bond presents a barrier to internal rotation. Nuclei in different environments about the amide group may then exhibit different chemical shifts. At higher temperature, the internal rotation rate increases and the signals begin to broaden and then coalesce.[83,84,85]

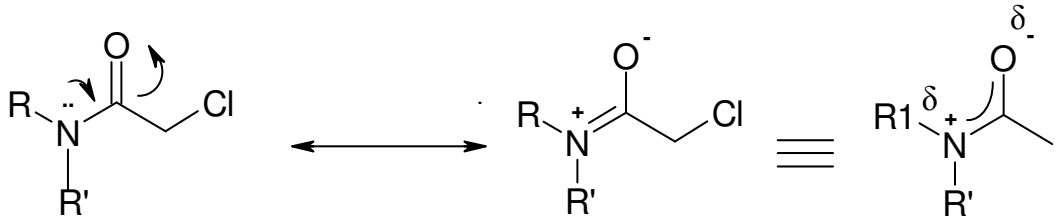
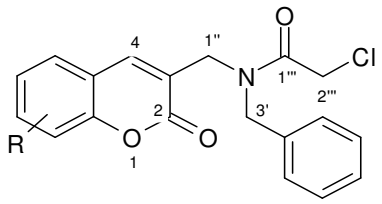


Figure 16. Resonance in the amide system resulting in the partial double-bond character of the C-N bond.[85]

Table 7. Yields obtained for the *N*-benzyl-2-chloro-*N*-[(2-oxo-2*H*-chromen-3-yl)methyl]-acetamide derivatives **55a-d**.



Compound	R	Yields/ %
55a	H	64
55b	8-OCH_2CH_3	72
55c	6-Cl	70
55d	6-Br	56

The ^{1}H NMR spectrum of chloroacetamide **55d** in DMSO-d_6 at 30^{o}C (Figure 17a) exhibits two singlets at 4.59 and 4.71 ppm corresponding to the 1"-methylene protons and two singlets, corresponding to the 3'-methylene protons, at 4.27 and 4.37 ppm reflecting the presence of rotamers. The chloromethylene protons resonate as overlapping singlets at 4.58 ppm. But these signals coalesce at 100^{o}C; the 1"-methylene group resonates at 4.39 ppm whereas the 3"-methylene group resonates at 4.63 ppm. The ^{13}C NMR spectrum (Figure 17c) and the DEPT 135 of compound **55d** (Figure 17d) clearly illustrate the presence of additional signals due to the presence of the rotameric products. The IR spectrum of compound **55d** clearly reveals the absorption bands of the two carbonyl groups (C=O) at 1660 and 1723 cm^{-1} (Figure 17h). Although complicated by the presence of signals corresponding to both rotamers, the COSY, HSQC and HMBC spectra were consistent with the assigned structures (Figure 17e-g).

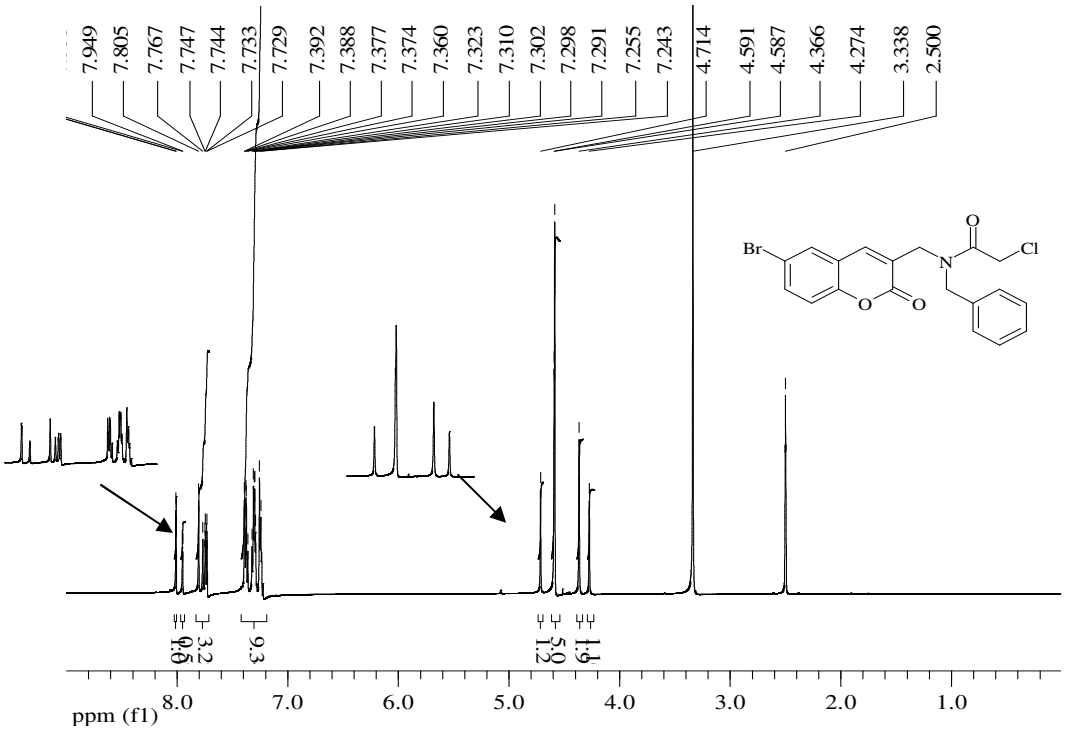


Figure 17a. 400MHz ^{1}H NMR spectrum of compound **55d** in DMSO-d_6 at 30^{o}C.

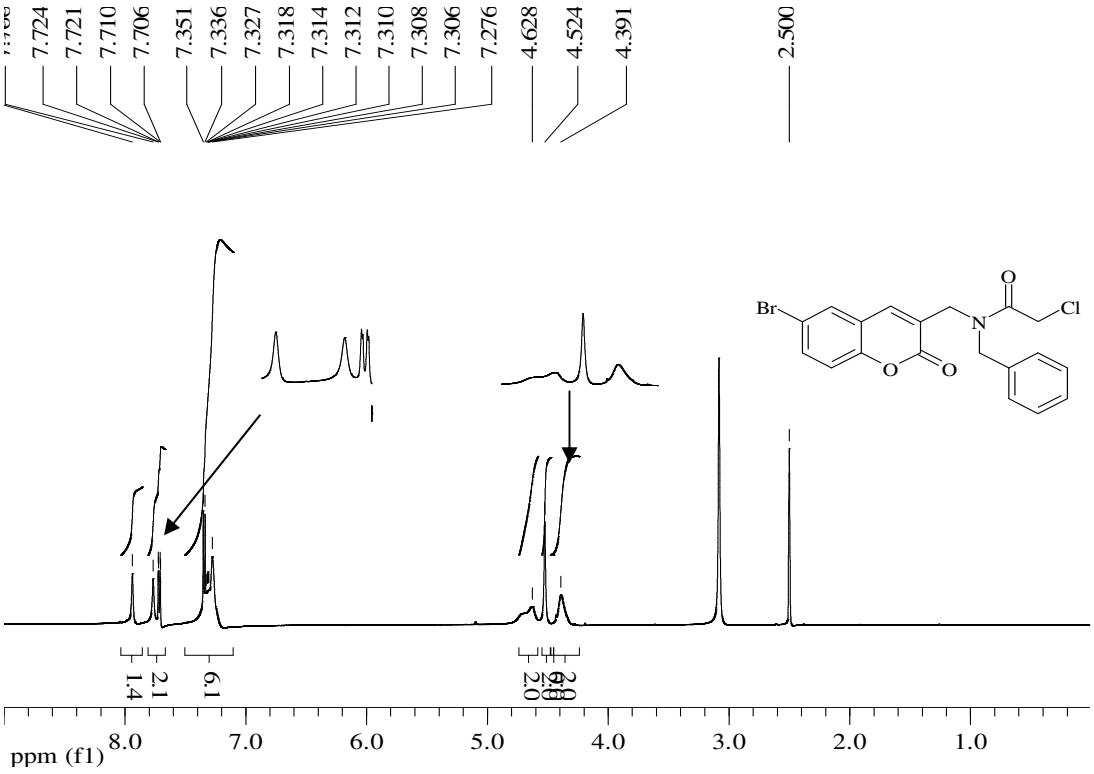


Figure 17b. 400MHz ^{1}H NMR spectrum of compound **55d** in DMSO-d_6 at 100^{o}C.

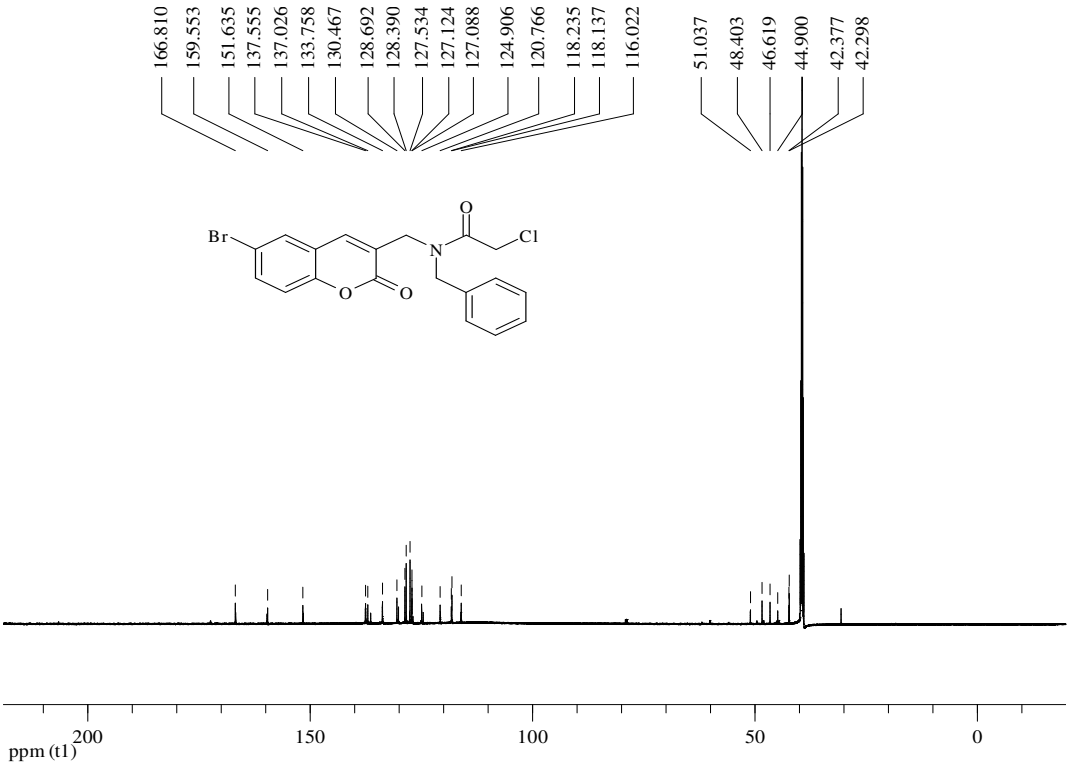


Figure 14c. 100MHz ^{13}C NMR spectrum of compound **55d** in DMSO-d_6.

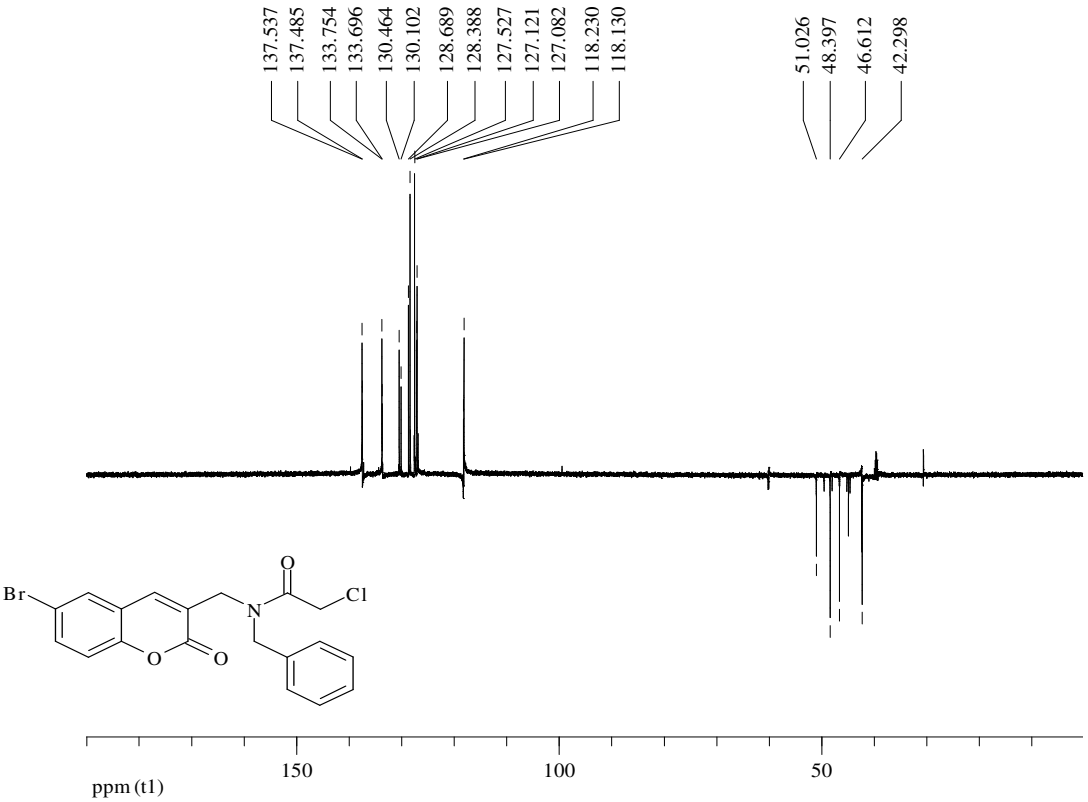


Figure 17d. DEPT135 spectrum of compound **55d** in DMSO-d_6.

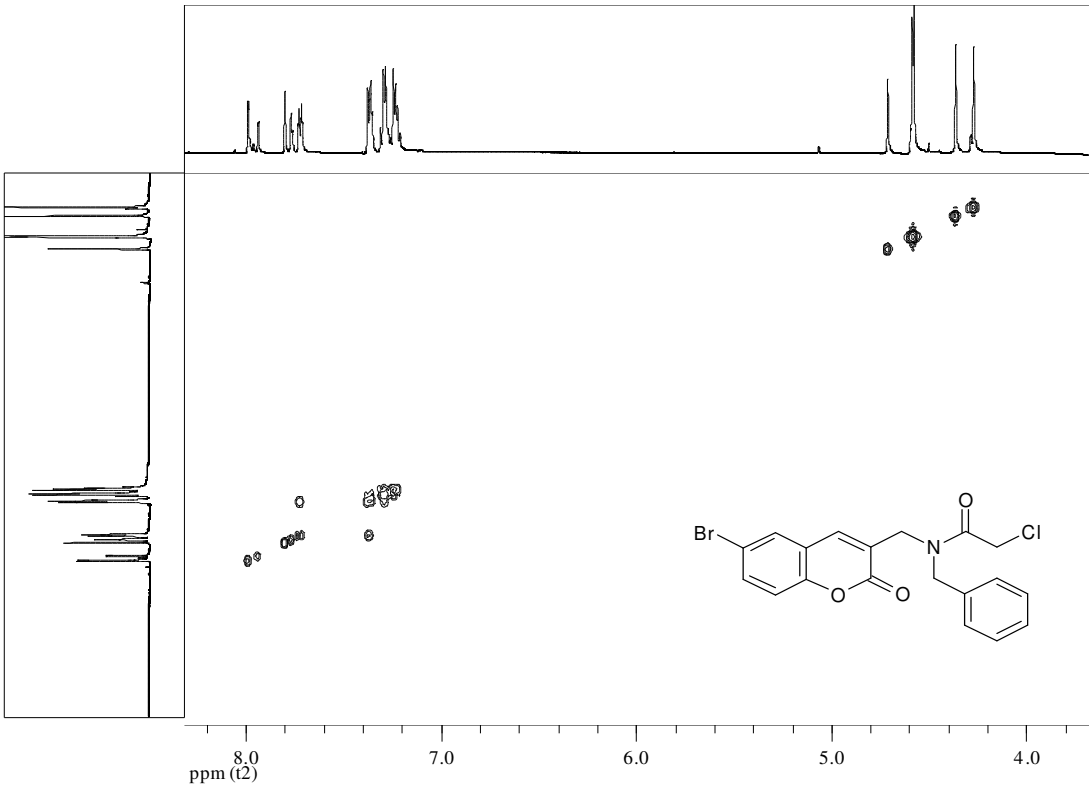


Figure 17e. 400MHz COSY spectrum of compound **55d** in DMSO-d_6.

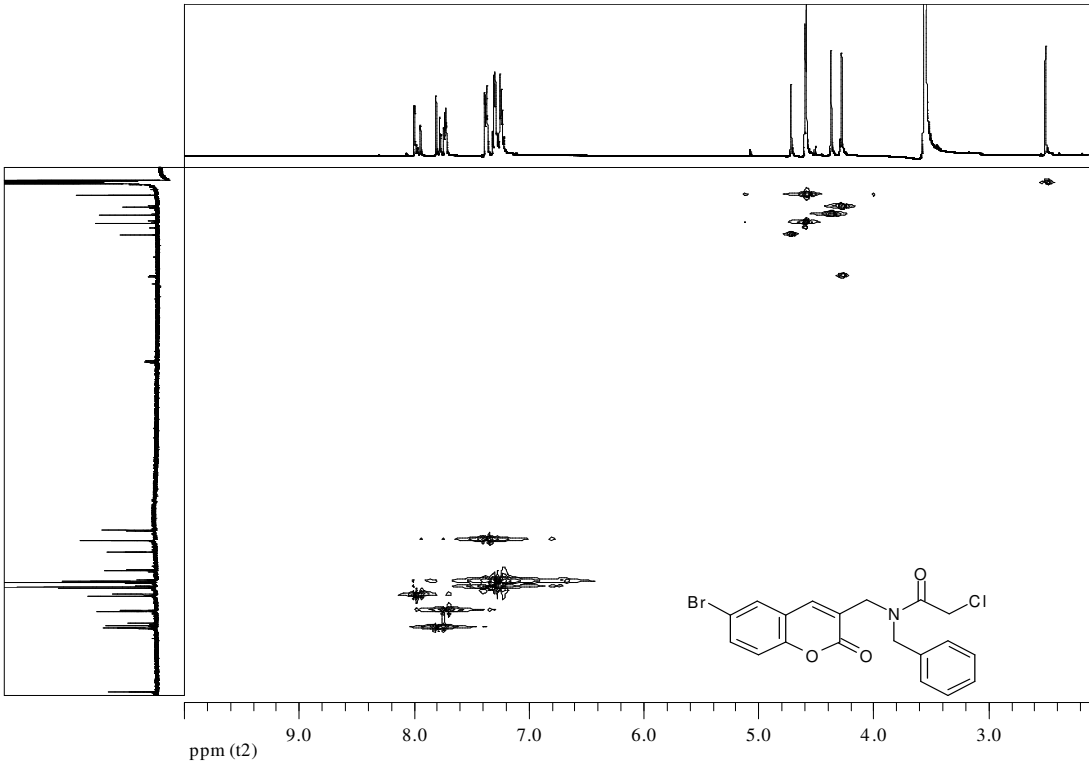


Figure 17**f**. HSQC spectrum of compound **55d** in DMSO-d_6.

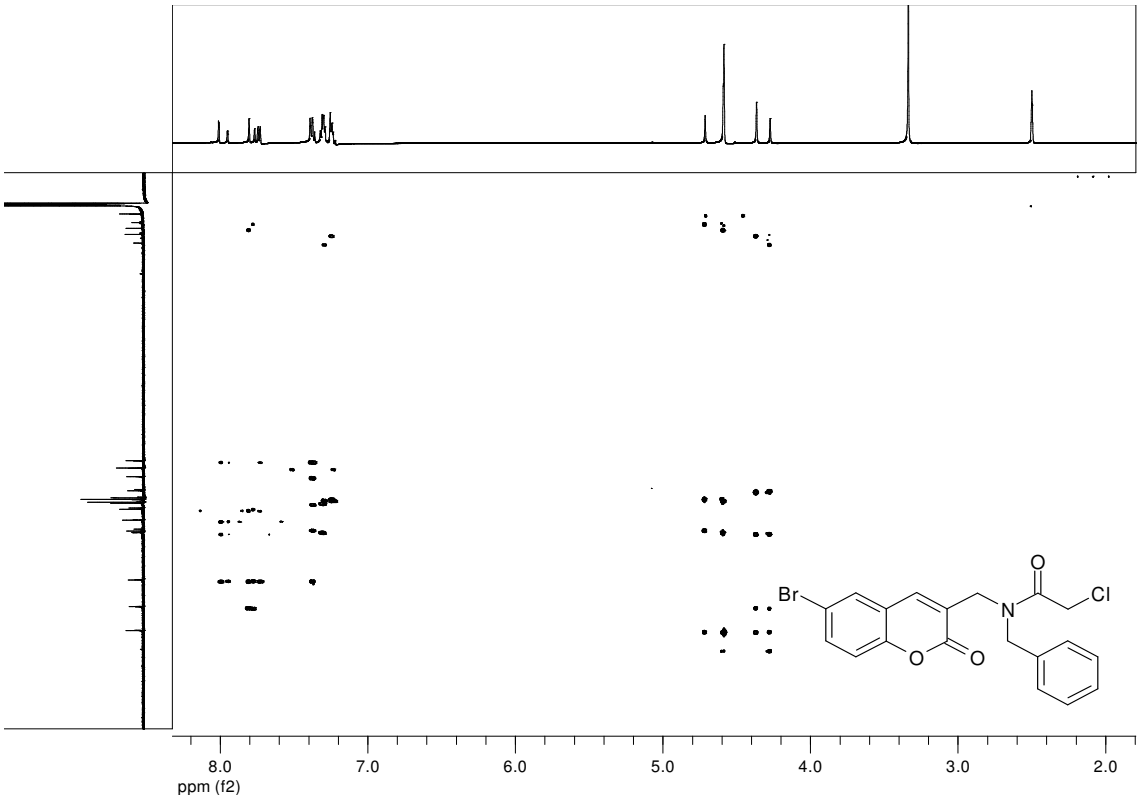


Figure 17g. HMBC spectrum of compound **55d** in DMSO-d_6.

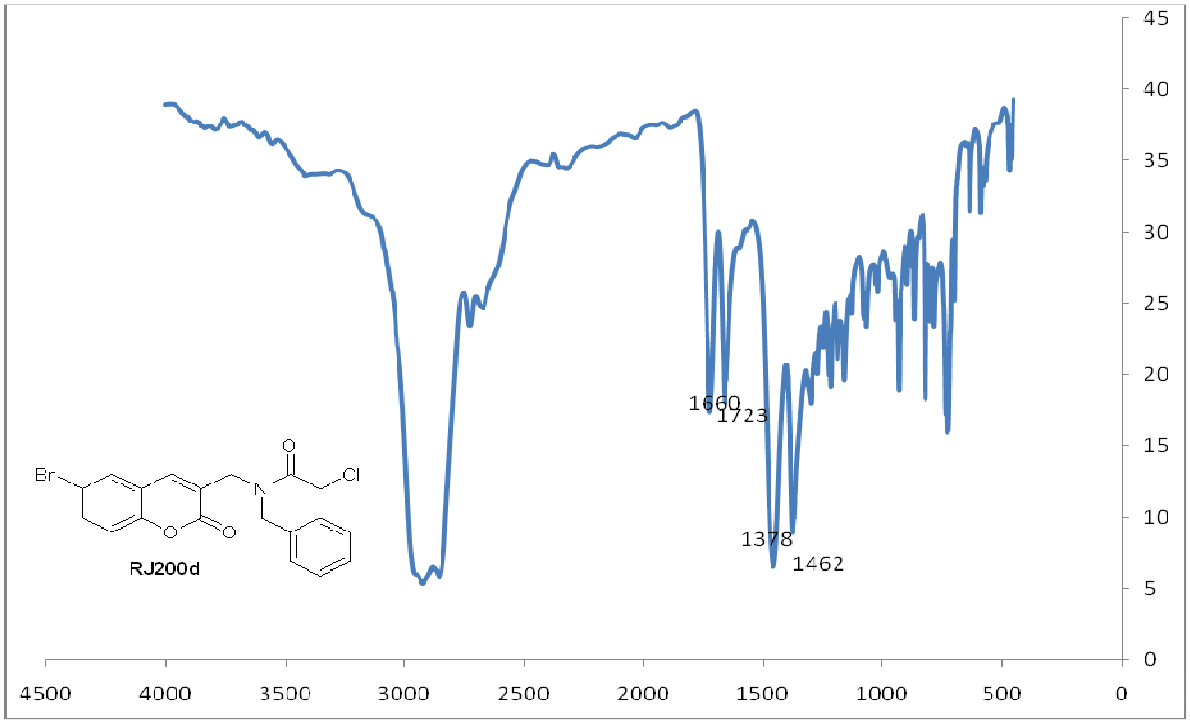
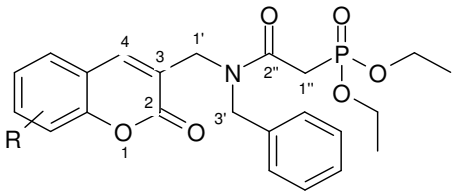


Figure 17h. IR spectrum of compound **55d**-(nujol mull).\

2.3.4. Arbuzov reactions of chloroacetamide derivatives

The chloroacetamide derivatives **55a-d** were then reacted with 2 equivalents of triethyl phosphite in Michaelis–Arbuzov reactions (pathway III, Scheme 22).[78,79] The mixtures were heated under nitrogen at 120-130^{o}C. Isolation by flash chromatography afforded the phosphorylated derivatives **56a-d** in good yields of up to 86% (Table 8).

Table 8. Yields of phosphorylated products **56a-d**.



Compound	R	Yields/ %
56a	H	60
56b	8-OCH_2CH_3	77
56c	6-Cl	77
56d	6-Br	86

The compounds were characterised using NMR and IR spectroscopy. The ^{1}H NMR spectra (*e.g.* Figure 18) of the phosphorylated derivatives **56** show additional signals, which are attributed to hindered rotation about the amide C-N bond. ^{1}H NMR and ^{13}C NMR spectra are illustrated in Figures **18a** and **18b**, respectively. The ^{1}H NMR spectrum of compound **56d** shows doublet at *ca.* 3.08 ppm corresponding to the methylene group attached directly to phosphorous, while the 1'-methylene protons resonate at 4.48 ppm and the 3'- methylene protons at 4.82 ppm. The ^{13}C NMR spectrum of compound **56d** reveals signals for the methylene carbons attached directly to phosphorus at 32.9 and 34.2

ppm, while the 1'- and 3'-methylene carbons resonate at 45.9 and 52.8 ppm, respectively. The DEPT 135 spectrum of compound **56d** (Figure 18c) confirms the methylene carbons as negative signals. The coupling between methyl and methylene protons of the two ethoxy groups in compound **56d** was evident from their COSY spectra, while the HSQC and HMBC spectra of these compounds show the correlations between protons and carbons (Figure 18d-f).

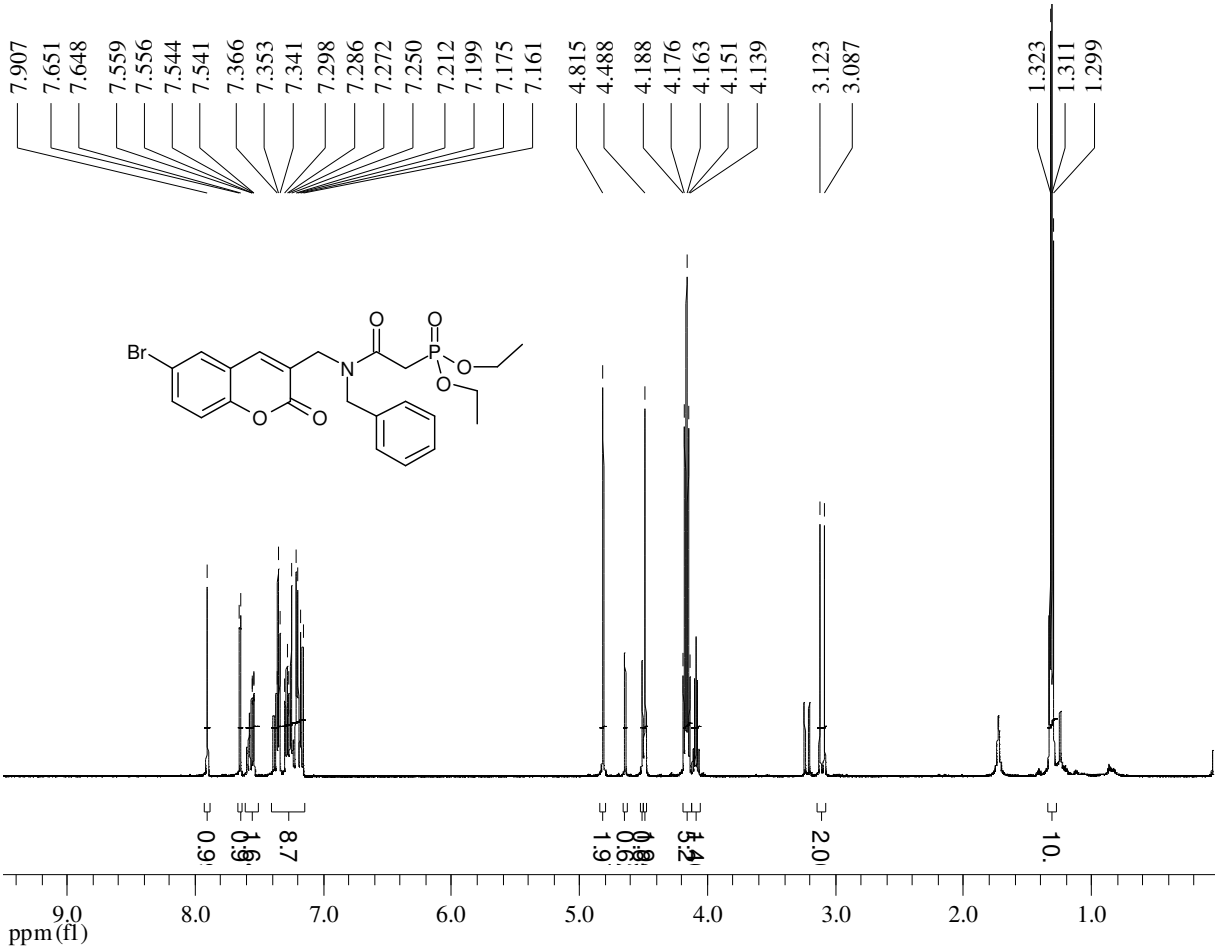


Figure 18a. 400MHz ^{1}H NMR spectrum of compound **56d** in $CDCl_3$.

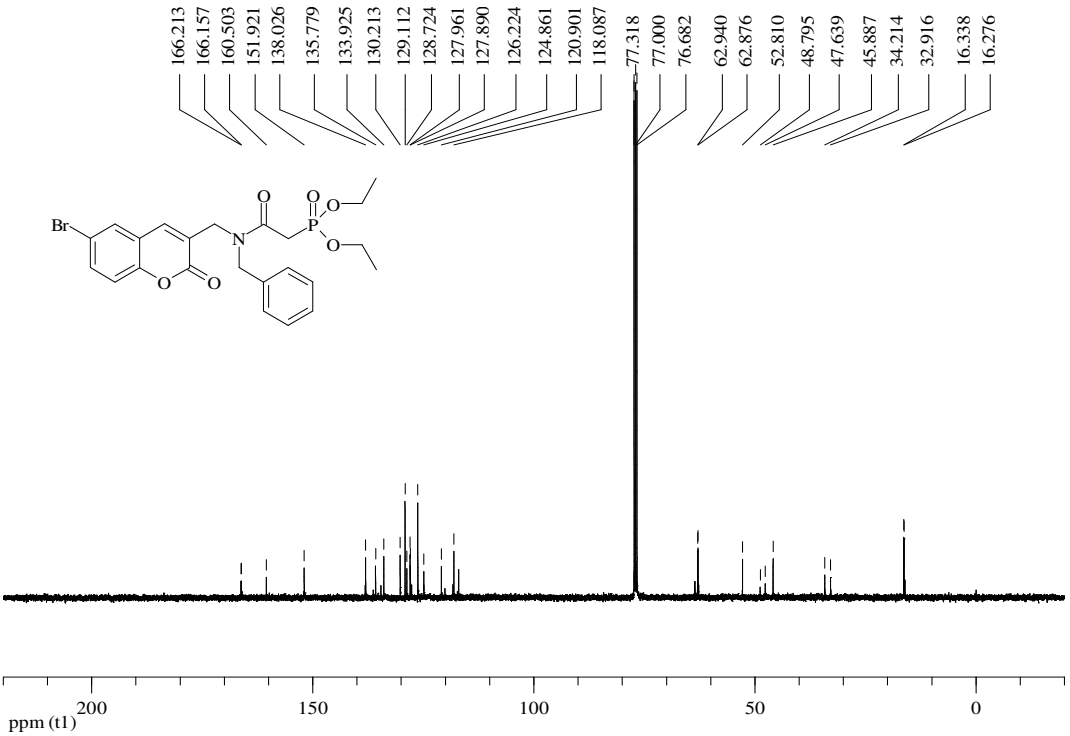


Figure 18b. 100MHz ^{13}C NMR spectrum of compound **56d** in $CDCl_3$.

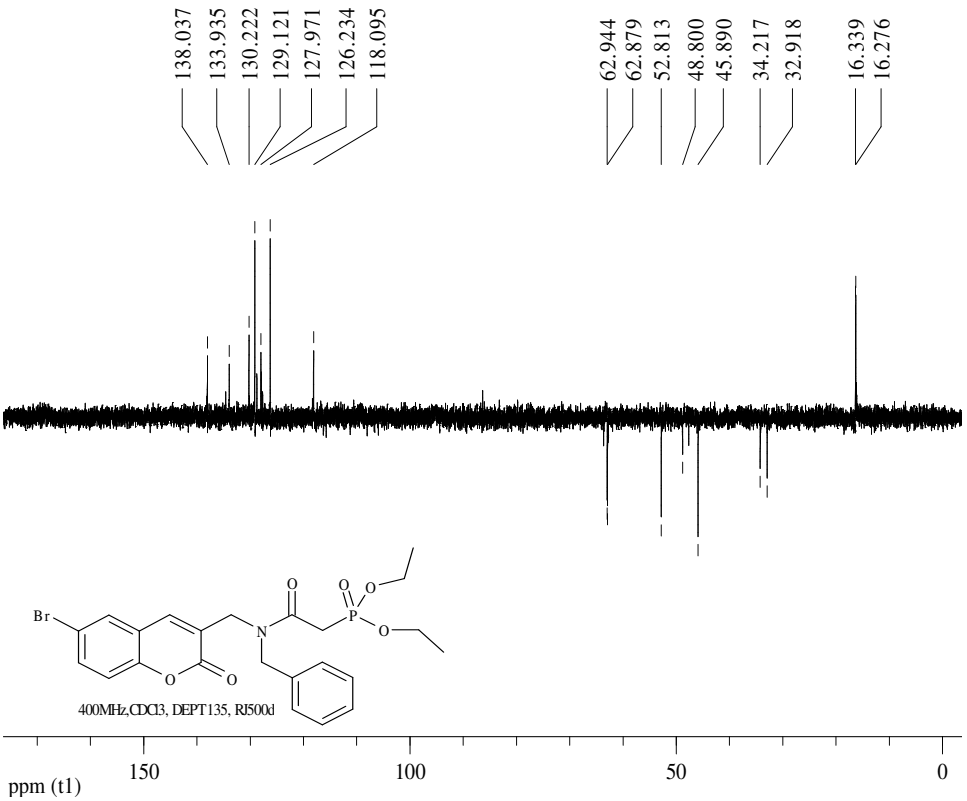


Figure 18c. DEPT135 spectrum of compound **56d** in $CDCl_3$.

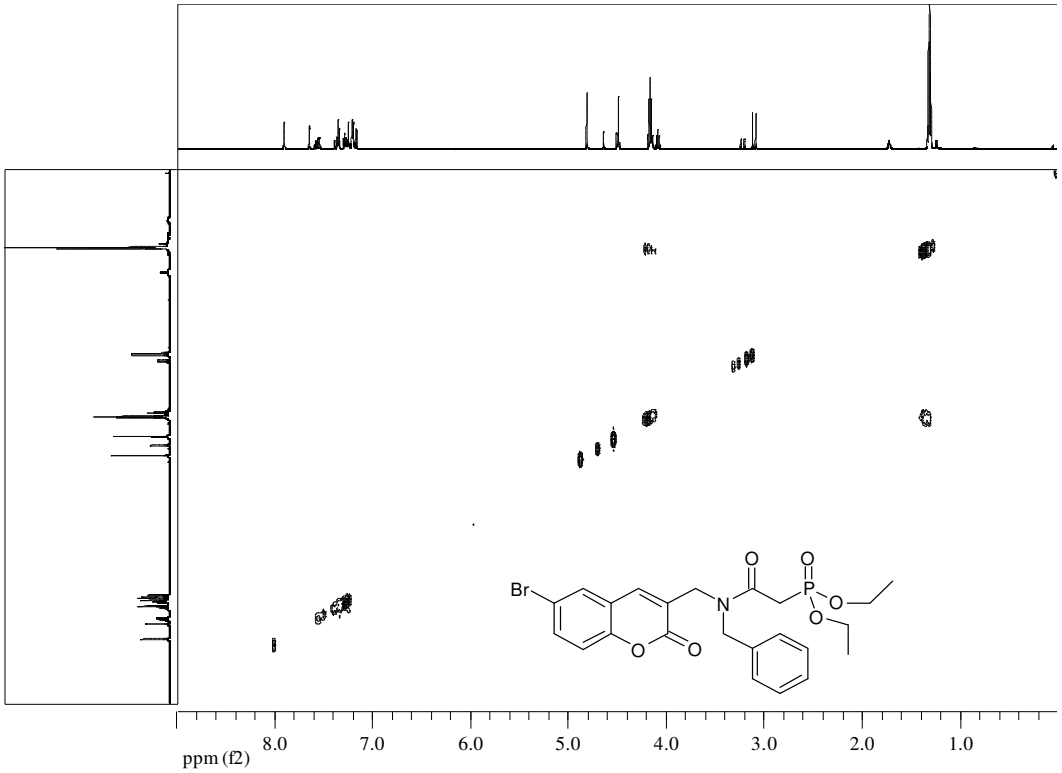


Figure 18d. 400MHz COSY spectrum of compound **56d** in $CDCl_3$.

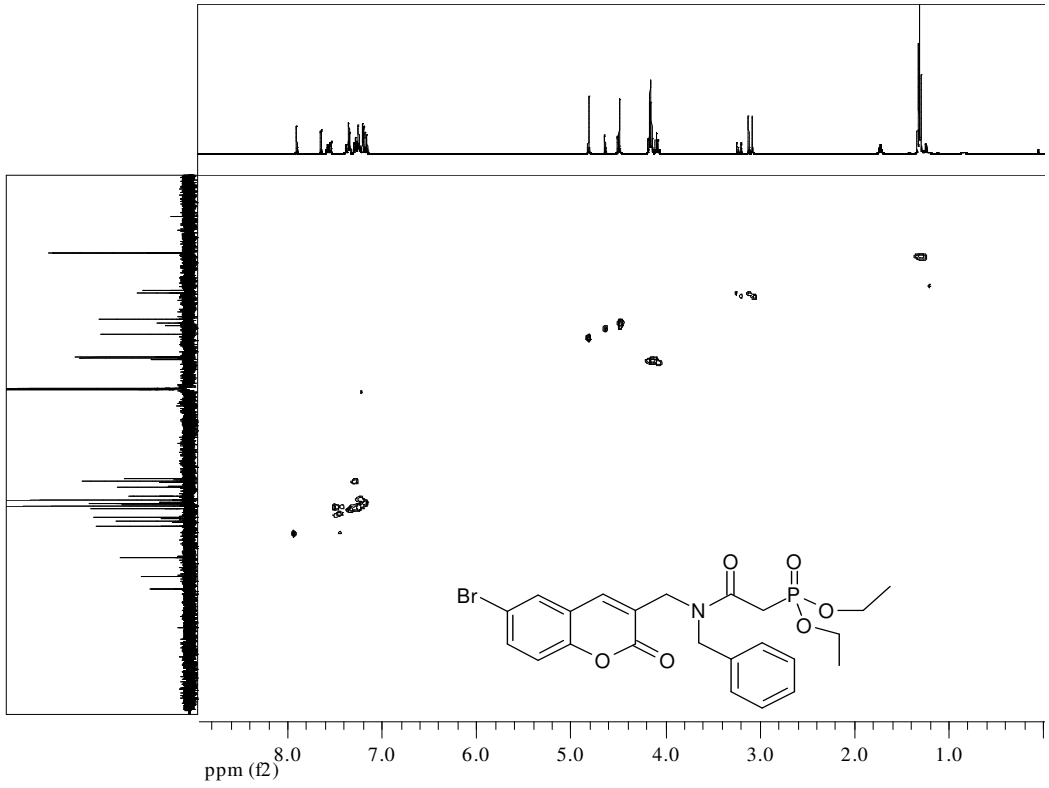


Figure 18e. HSQC spectrum of compound **56d** in $CDCl_3$.

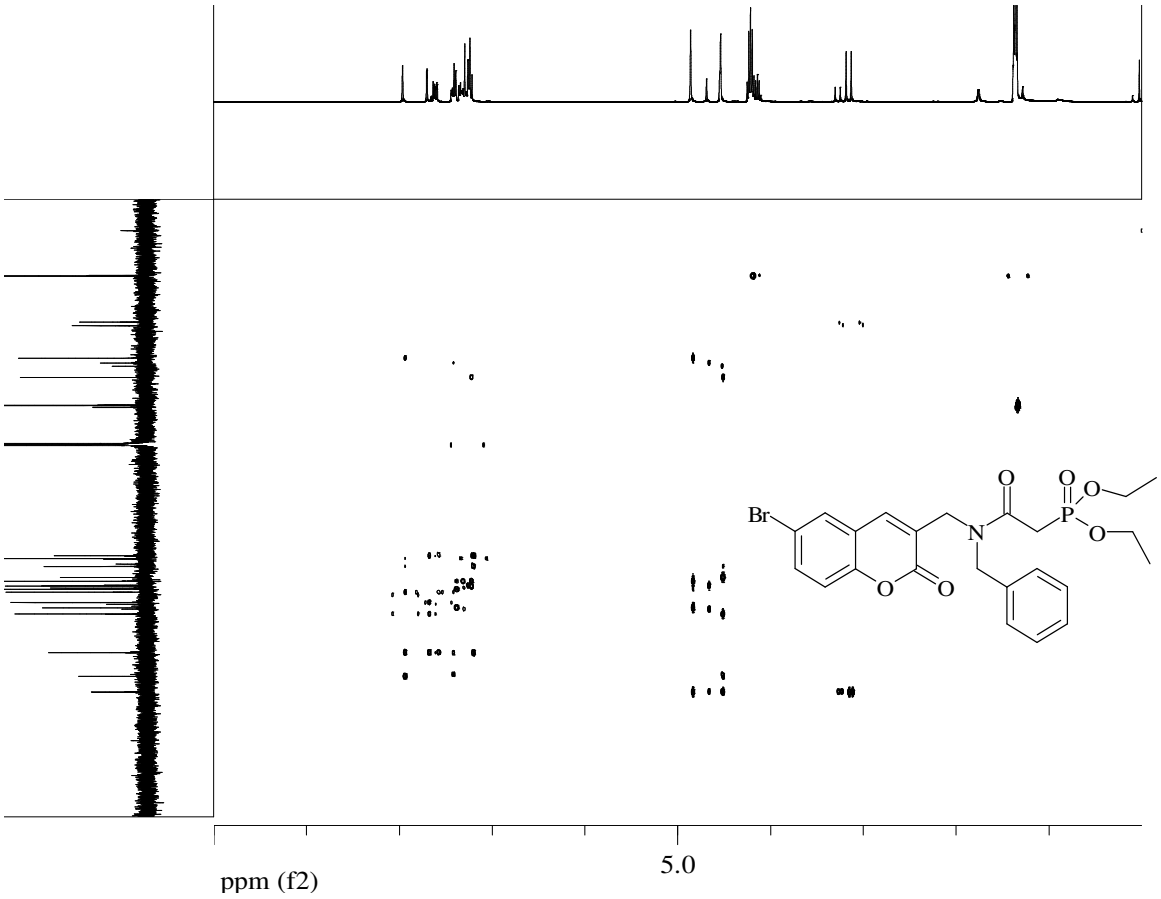
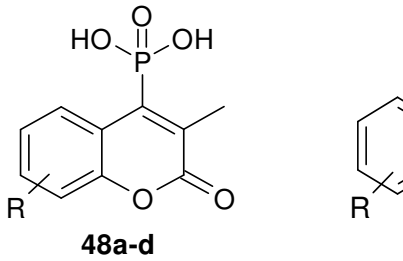


Figure 18f. HMBC spectrum of compound **56d** in $CDCl_3$.

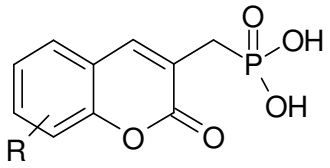
2.4. Molecular modelling studies of phosphorylated derivatives

Each of the phosphorylated derivatives was modelled as the corresponding hydrolysed analogues by replacing the ethoxy groups by hydroxyl groups. The assumption is that esters would be hydrolysed *in vivo*. Docking of these analogues into the HIV receptor cavity was explored using the Ligandfit module in the Accelrys Cerius^2 platform on an SG-O^2 computer, and potential hydrogen-bonding interactions between the enzyme and these ligands were indentified (Scheme 19). An HIV-1 protease X-ray diffraction structure containing ritonavir was used in the molecular modelling studies, and was downloaded from the Cambridge Crystallographic protein data bank (Figure 19).[86] After removing ritonavir from the enzyme structure, the energy-minimised, phosphorylated derivatives were docked into the active site using the Cerius^2 Ligandfit module. Application of the Ligandfit module involves a 4-step process, consisting of "Site

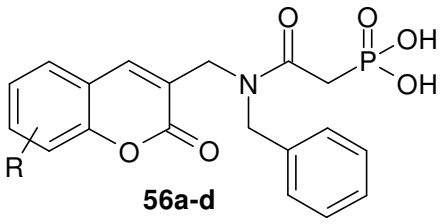
search", "Conformation search", "Ligand fitting" and "Ligand scoring".[87] The best-fit conformers were examined for their possible hydrogen-bonding interactions with the enzyme receptor and/or structural water molecules, and their docking scores, van der Waals and ligand scores are shown in Table 9. The hydrogen-bonding interactions between the enzyme receptor in the presence of structural water molecules and ligand **56d** are shown in Figures 20 and 21. Possible hydrogen-bonding interactions are indicated between: - i) a phosphoryl oxygen and two water molecules; ii) a water molecule and the amide nitrogen; iii) the carbonyl oxygen and a water molecule; and iv) the hydrogen of a hydroxyl group attached to phosphorous with the oxygen of a water molecule. Potential hydrogen-bonding interactions in the case of ligand **48d** are illustrated in Figures 22 and 23. Such interactions are indicated between: - i) a water molecule and the bromine; ii) a water molecule and the coumarin ring oxygen; and iii) an Ile-50 (A) amide hydrogen with a hydroxyl oxygen attached to phosphorous. Possible hydrogen-bonding interactions between the ligand **49d** and the enzyme receptor are shown in Figures 24 and 25. These involve interactions between: - i) the Gly-49 (A) residue and a hydrogen of a hydroxyl group attached to phosphorous; ii) Gly-48 (B) with the hydrogen of a hydroxyl group attached to phosphorous; and iii) a structural water molecule and the oxygen of the coumarin ring.



48a-d



49a-d



56a-d

	R
a	H
b	8-OCH_2CH_3
c	6-Cl
d	6-Br

Scheme 19. Representative phosphorylated derivatives selected for docking in the HIV-1 protease active site.

Table 9. Docking scores, Ligandscores and van der Waals energies for selected ligands in the HIV-1 protease active site.

Ligands	Docking score/ kcal.mol^{-1}	Ligand score/ kcal/mol^{-1}	van der Waals/ energy/kcal.mol^{-1}
56a-OH	33.421627	2.95	-29.79
56b-OH	35.307972	0.85	-7.09
56c-OH	17.006662	1.80	-24.27
56d-OH	11.487108	2.27	5.17
49a-OH	11.223932	1.44	-14.13
49b-OH	31.294571	1.13	-10.76
49c-OH	11.433449	1.23	-4.30
49d-OH	15.306229	1.39	-24.89
48a-OH	2.190857	-0.83	25.73
48b-OH	26.244118	1.36	-22.61
48c-OH	5.299529	-0.29	2.54
48d-OH	9.531050	1.42	-1.49

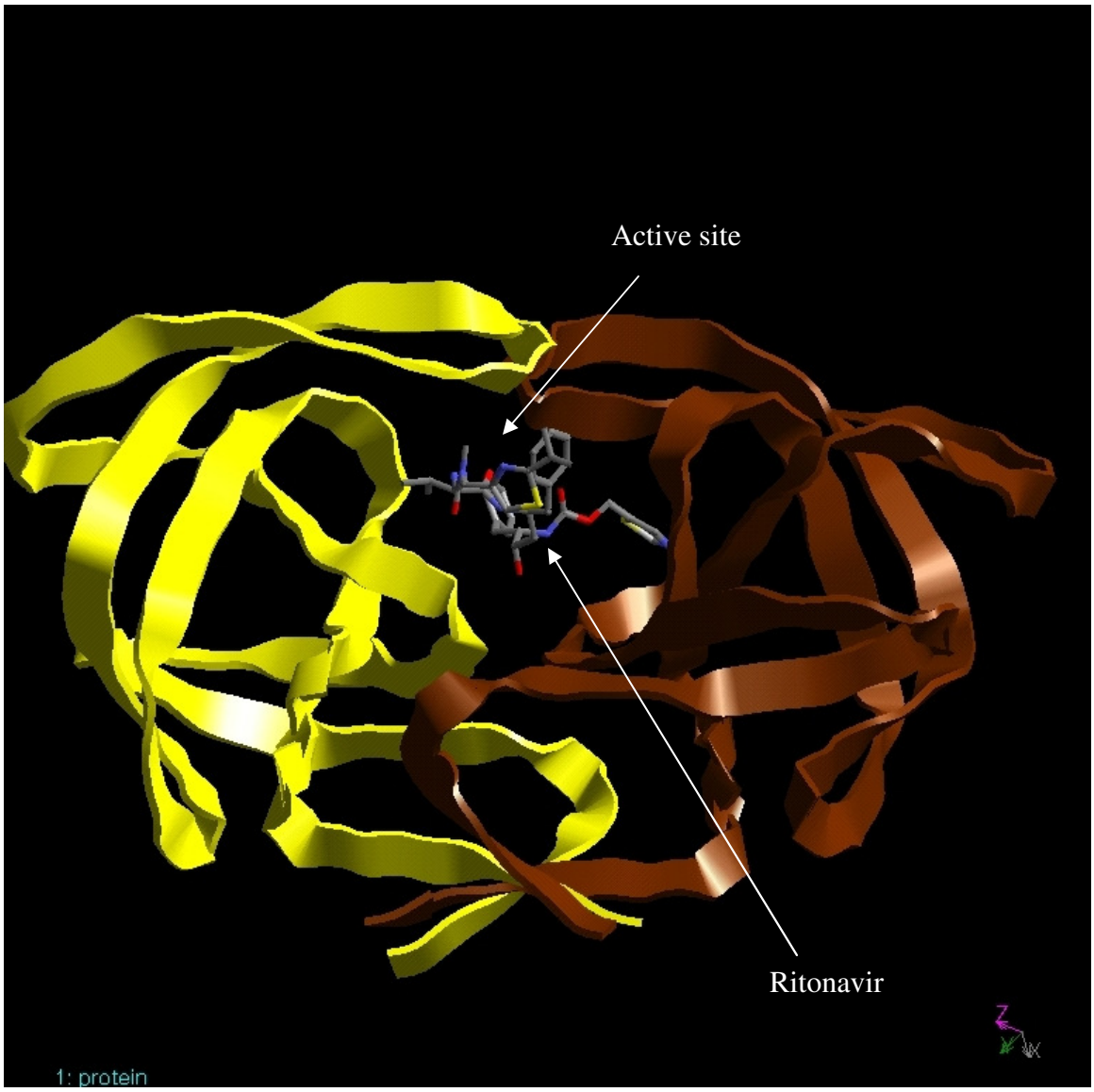


Figure 19. Ribbon representation of the X-ray crystal structure of HIV-1 protease showing ritonavir in the binding cavity as reported by Kempf *et al*.[86]

The phosphorylated analogues **48a-d**, **49a-d** and **56a-d** clearly have the ability to fit within the HIV-1 protease enzyme receptor cavity, and the potential to bind through the hydrogen-bonding interactions with structural water molecules and receptors subsites, such as Gly-48, Gly-49 and Ile-50. The next step in this research programme is expected to involve enzyme-inhibition and binding studies of the hydrolysed compounds.

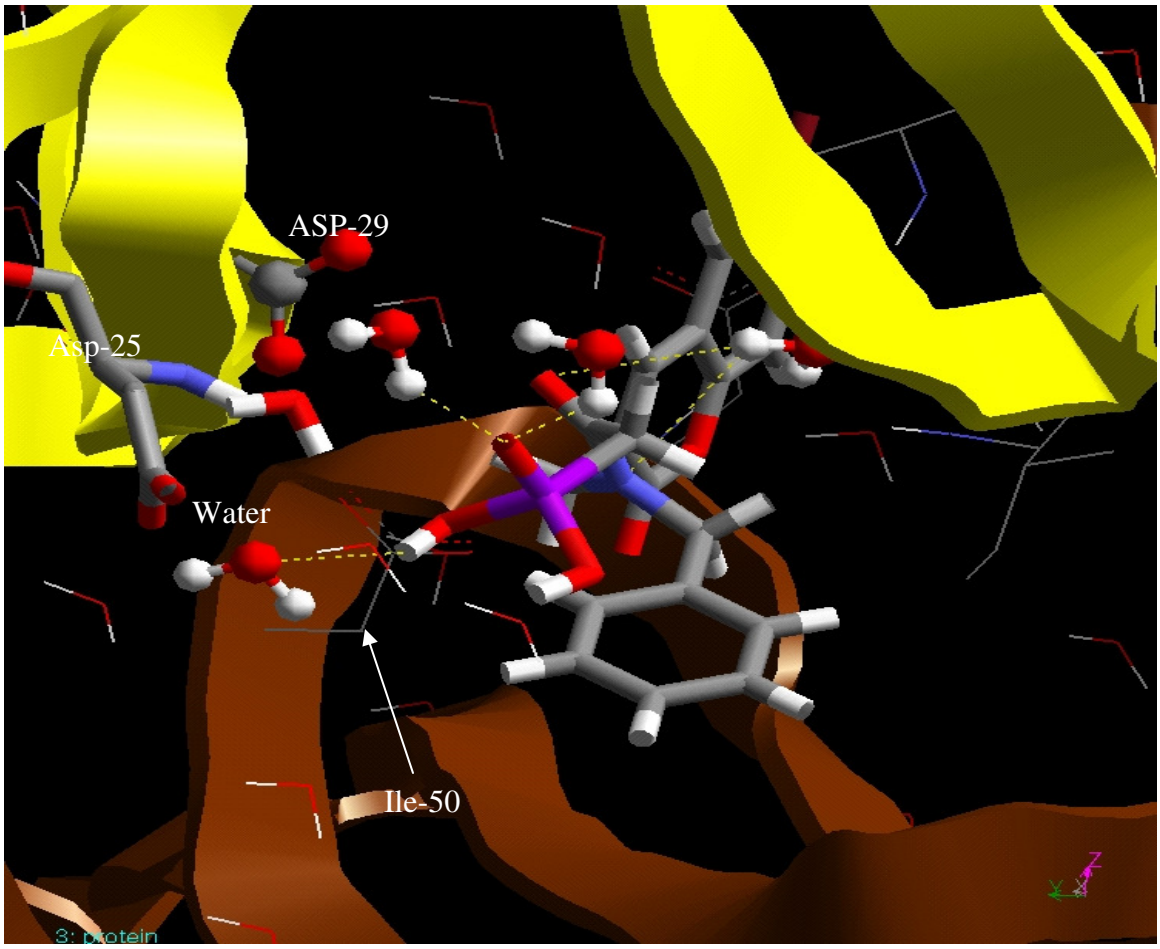


Figure 20: The best-fit conformation of ligand **56d-OH** docked in the active site of HIV-1 PR. The hydrogen-bonding interactions are represented by yellow dashed lines and reflect separations of less than 3.5Å.

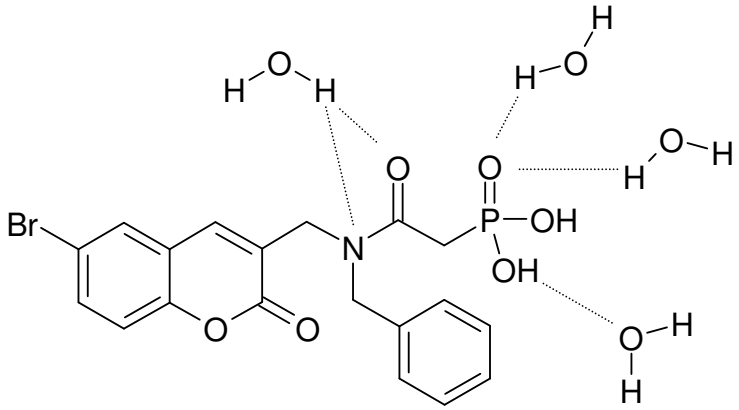


Figure 21: A simplified schematic diagram of ligand **56d-OH** and structural water molecules in the active site of the HIV-1 PR enzyme showing potential hydrogen-bonding interactions.

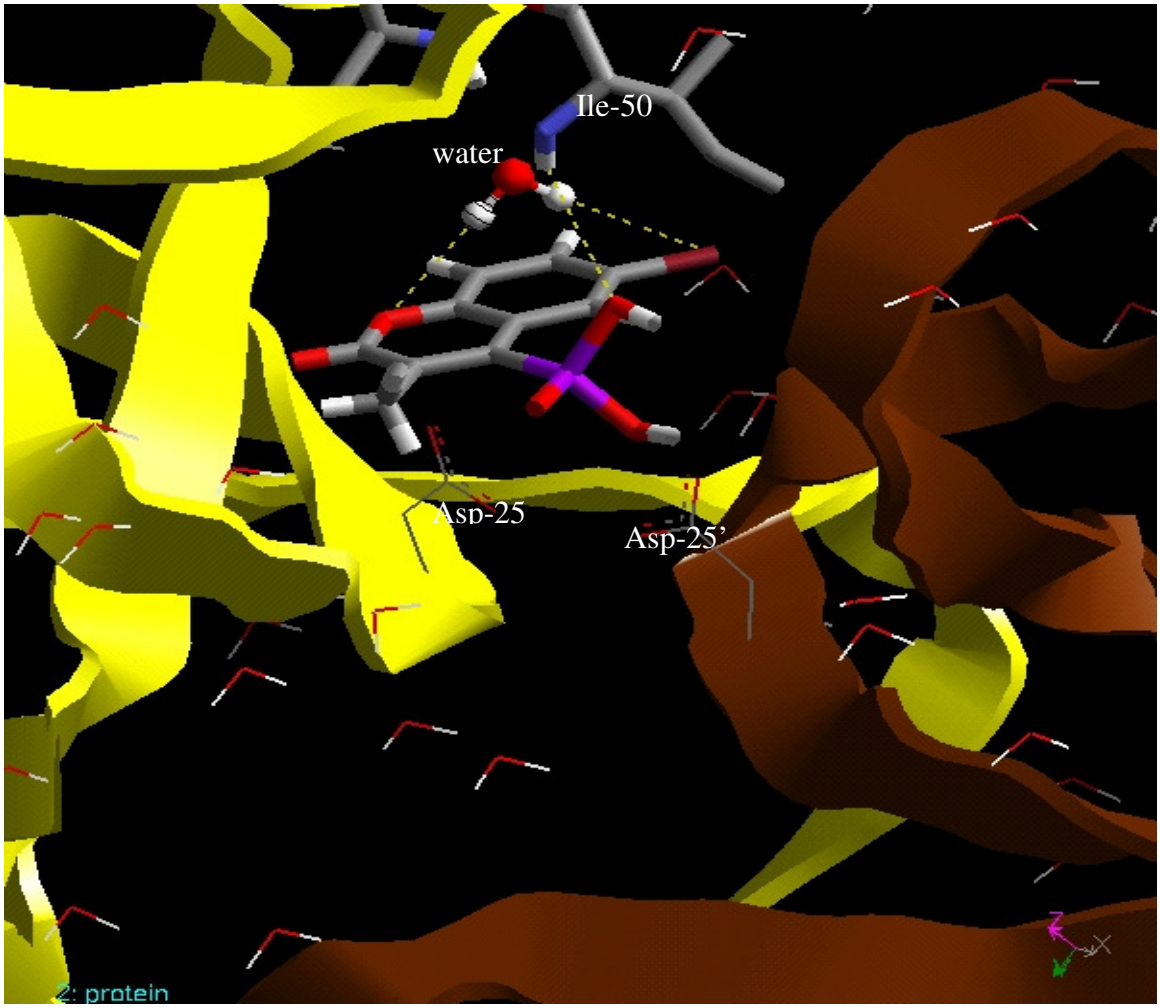


Figure 22: The best-fit conformation of ligand **48d-OH** docked in the active site of HIV-1 PR. The hydrogen-bonding interactions are represented by yellow dashed lines and reflect separations of less than 3.5Å.

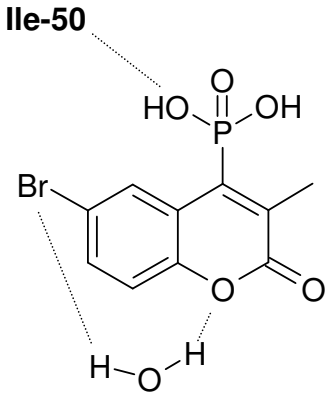


Figure 23: A simplified schematic diagram of potential hydrogen-bonding interactions between ligand **48d-OH**, the active site of HIV-1 PR enzyme and a structural water molecule.

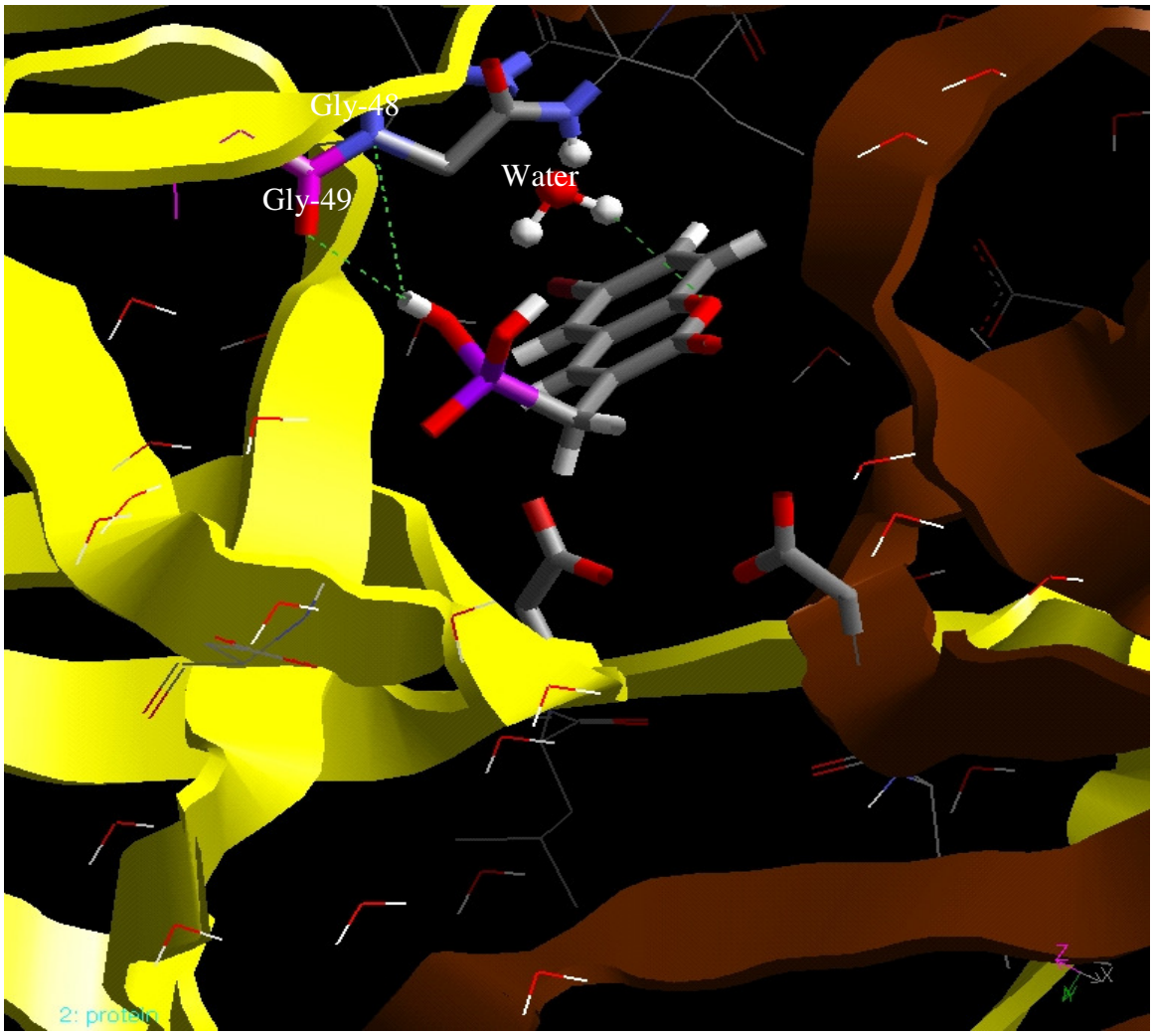


Figure 24: The best-fit conformation of ligand **49d-OH** docked in the active site of HIV-1 PR. The hydrogen-bonding interactions are represented by green dashed lines and reflect separations of less than 3.5Å.

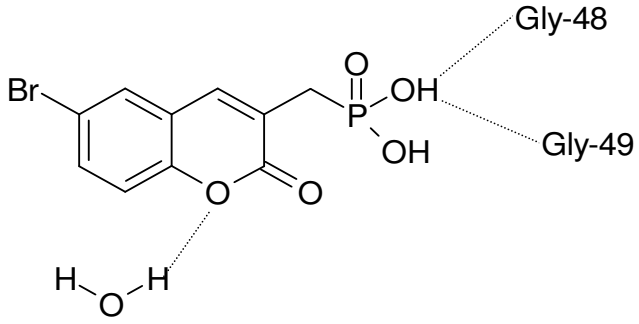


Figure 25: A simplified schematic diagram showing potential hydrogen-bond interactions between ligand **49d-OH**, the active site of HIV-1 PR and a structural water molecule.

2.5. Conclusions

The cyclisation of unprotected Baylis-Hillman adducts have successfully afforded a series of the 3-(halomethyl)coumarin derivatives directly, without protecting the salicylaldehyde phenolic hydroxyl group. However, treatment of the halogen-substituted, unprotected Baylis-Hillman adducts with HI afforded the 6-halogeno-3-(iodomethyl)coumarin derivatives and the unexpected 3-methyl analogues, the product distribution depending on the reaction times. The application of the Arbuzov reaction to the 3-(halomethyl)coumarin derivatives has afforded both the 1'-phosphorylated derivatives and the unexpected 4-phosphorylated analogues. The regioselectivity of phosphorylation appears to depend on the nature of the leaving group (Cl or I) and the reaction conditions. Nucleophilic attack on the 3-(chloromethyl)coumarins by benzylamine gave the 3-[(benzylamino)methyl]coumarin derivatives, while treatment of these products with chloroacetyl chloride afforded the corresponding chloroacetamides as mixtures of rotamers arising from hindered rotation about the C(O)-N amide bond. At high temperature (*ca*. 100^{o}C) the relevant ^{1}H NMR signals at 4.39 and 4.63 ppm were broadened and coalesced because the rate of internal rotation had increased. Modelling studies of hydrolysed analogues of the phosphorylated compounds have indicated their capacity to interact with the active site of the HIV-1 protease enzyme through hydrogen bonding with the structural water molecules and/or receptor subsites.

The various objectives of the present study have been successfully achieved and future work based in this area of research is expected to include the following.

1. Preparation of the 4-hydroxylated-3-(halomethyl)coumarin derivatives by oxidizing the Baylis-Hillman adducts prior to cyclisation.
2. X-ray crystallographic analyses of the *N*-benzyl-2-chloro-*N*-[(2-oxo-2*H*-chromen-3-yl)methyl]acetamides and their phosphorylated derivatives to establish solid-state conformational preferences.

3. Variable temperature NMR studies of internal rotation in the acetamide derivatives in DMSO-d_6 at different temperatures to explore the rotational barriers.

3. EXPERIMETAL

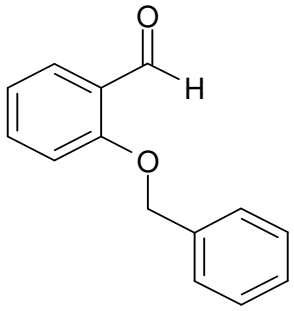
3.1. General

NMR spectra were recorded on Bruker AMX 400 and Biospin 600 spectrometers at 303K in DMSO-d_6 or $CDCl_3$ and calibrated using solvent signals [7.25 ($CHCl_3$) and 2.50 ppm (DMSO-d_6) for ^{1}H NMR; 77.0 ($CDCl_3$) and 34.5 (DMSO-d_6) for ^{13}C NMR]. ^{31}P NMR spectra were taken using phosphoric acid (H_3PO_4) as an internal reference. Melting points were measured using a Kofler hot stage apparatus and are uncorrected. Flash column chromatography was performed using Merck Silica gel 60 [particle size 0.040-0.063 mm (230-400 mesh)] and MN Kieselgel 60 (particle size 0.063-0.200 mm) and TLC was run on pre-coated Merck silica gel F_{254} plates viewed under UV light (254/365 nm) or following exposure to iodine vapour. Infrared spectra were obtained on a Perkin Elmer FT-IR Spectrum 2000 spectrometer using nujol mulls. Low-resolution (EI) mass spectra were obtained on a Finnigan-Mat GCQ mass spectrometer and high-resolution (EI) mass spectra on a VG70-SEQ Micromass double-focussing magnetic sector spectrometer (Potchefstroom University Mass Spectrometry Unit).

The reagents used in the present study were supplied by Aldrich, and used without further purification. Solvents were distilled under nitrogen and stored over type 3A molecular sieves following methodology described by Perrin and Armarego.[88] THF was distilled from sodium using benzophenone as indicator, and acetone was distilled from 3A molecular sieves.

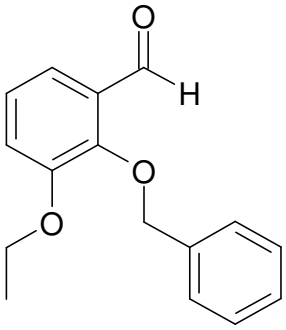
3.2. Synthetic procedures

3.2.1. Preparation of the salicylaldehyde benzyl ethers



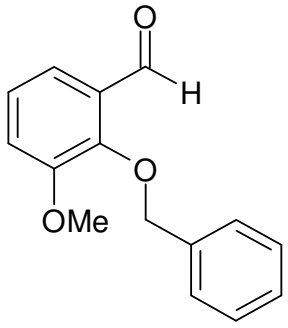
2-Benzyloxybenzaldehyde **39a**[60]

To a mixture of salicylaldehyde (2.5g, 21mmol), benzyl bromide (2.5ml, 21mmol), anhydrous K_2CO_3 (16.9g, 0.12mol) and NaI (18.5g, 0.12mol) was added distilled acetone (40ml), and the mixture was boiled under reflux for 12h. Water (20ml) was then added and the organic aqueous layer extracted with $CHCl_3$ (2 x 100ml). The combined organic extracts were then washed with brine and dried (anhydrous Na_2SO_4), filtered and concentrated *in vacuo* to give a dark brown oil. Separation using flash column chromatography [on silica gel; elution with ethyl acetate-hexane (1:9)] afforded 2-benzyloxybenzaldehyde **39a** as yellow crystals (1.2g, 27%); m.p. 43-46^oC (lit.[60] 42-44^oC); ν_{max} (nujol)/cm^{-1} 1697 (C=O); δ_H (400 MHz; $CDCl_3$) 5.18 (2H, s, CH_2), 7.02-7.45 (7H, series of multiplets, Ar-H), 7.51 (1H, t, *J* = 7.2Hz, Ar-H), 7.85 (1H, d, *J* = 7.6Hz, Ar-H) and 10.57 (1H, s, CHO); δ_C (100 MHz; $CDCl_3$) 70.4 (CH_2), 113.0, 120.9, 125.1, 127.2, 128.2, 128.3, 128.6, 135.8, 136.0 and 161.0 (Ar-C) and 189.6 (C=O).



2-Benzyloxy-3-ethoxybenzaldehyde **39b** [60]

The procedure described for the synthesis of 2-benzyloxybenzaldehyde **39a** was followed, using 3-ethoxysalicylaldehyde (2.0g, 7mmol), benzyl bromide (2.0ml, 17 mmol), anhydrous K_2CO_3 (10.4g, 73mmol) and NaI (11.3g, 73mmol) in distilled acetone (80ml). Work-up and chromatography afforded 2-benzyloxy-3-ethoxybenzaldehyde **39b** as a pale orange crystals (1.89g, 42%); m.p. 36-39^oC (lit.[60] 36-38^oC); ν_{max} (nujol)/cm^{-1} 1693 (C=O); δ_H (400 MHz; $CDCl_3$) 1.49 (3H, t, *J* =7.0Hz, CH_2CH_3), 4.11 (2H, q, *J* = 7.1Hz, OCH_2CH_3), 5.19 (2H, s, CH_2Ph), 7.10-7.41 (8H, series of overlapping signals, Ar-H) and 10.26 (1H, s, CHO); δ_C (100 MHz; $CDCl_3$) 14.8 (CH_3), 64.6 (CH_2), 76.2 (CH_2Ph), 118.9, 119.1, 124.1, 128.4, 128.5, 128.6, 130.3, 136.5, 151.2 and 152.3 (Ar-C) and 190.2 (C=O).

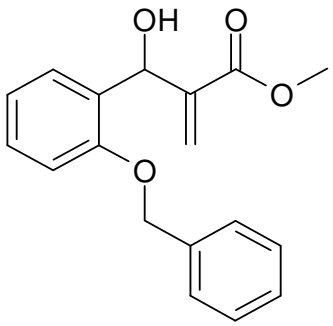


2-Benzyloxy-3-methoxybenzaldehyde **39c** [60]

The procedure described for the synthesis of 2-benzyloxybenzaldehyde **39a** was followed, using 3-methoxysalicylaldehyde (1.19g, 8mmol), benzyl bromide (1ml,

8mmol), anhydrous K_2CO_3 (6.5g, 0.03mol) and NaI (7.1g, 0.03mmol) in distilled acetone (40ml). Work-up and chromatography afforded 2-benzyloxy-3-methoxybenzaldehyde **39c** as a pale yellow crystals (1.01g, 54%); m.p. 37-40^oC (lit.[60] 36-38^oC); ν_{max} (nujol)/cm^{-1} 1688 (C=O); δ_H (400 MHz; $CDCl_3$) 3.93 (3H, s, OCH_3), 5.17 (2H, s, CH_2Ph), 7.12 (2H, series of overlapping multiplets, Ar-H), 7.34 (6H, series of overlapping signals, Ar-H) and 10.3 (1H, s, CHO); δ_C (100 MHz; $CDCl_3$) 56.1 (OCH_3), 76.3 (CH_2Ph), 118.0, 119.0, 124.2, 128.5, 128.6, 130.3, 130.8, 136.4, 151.0 and 153.0 (Ar-C) and 190.2 (C=O).

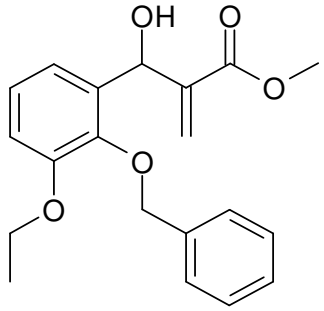
3.2.2. Preparation of *O*-benzylated Baylis-Hillman adducts



Methyl 3-(2-benzyloxyphenyl)-3-hydroxy-2-methylenepropanoate **40a** [60]

A mixture of 2-benzyloxybenzaldehyde **39a** (1.0g, 5.0mmol), methyl acrylate (2.4ml, 5mmol) and DABCO (0.91g, 3mmol) in $CHCl_3$ (1.25ml) was stirred in a stoppered reaction flask for three weeks. The mixture was concentrated in *vacuo* to give a brown oil, which was purified by flash column chromatography [on silica gel; elution with hexane-EtOAc (9:1)] to afford methyl 3-(-2-benzyloxyphenyl)-3-hydroxy-2-methylenepropanoate **40a** as a pale yellow oil (0.32g, 23%); ν_{max} (thin film)/cm^{-1} 3508 (OH) and 1725 (C=O); δ_H (400 MHz; $CDCl_3$) 3.42 (1H, br s, OH), 3.71 (3H, s, OCH_3), 5.08 (2H, s, OCH_2Ph), 5.69 and 6.28 (2H, 2 x s, $C=CH_2$), 5.94 (1H, s, CH), 6.93-7.00 (2H, overlapping multiplets, Ar-H), 7.22-7.41 (7H, series of overlapping multiplets, Ar-H); δ_C (100 MHz; $CDCl_3$) 51.8 (OCH_3), 68.5 ($CHOH$), 70.2 (OCH_2), 111.9, 121.0, 125.8,

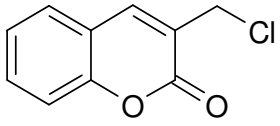
127.3, 127.8, 128.0, 128.6, 128.8, 129.6, 136.7, 141.4 and 155.7 ($C=CH_2$ and Ar-C) and 167.0 (C=O).



Methyl 3-(2-benzyloxy-3-ethoxyphenyl)-3-hydroxy-2-methylenepropanoate **40b** [60]

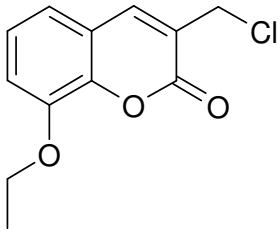
The procedure described for the synthesis of methyl 3-(2-benzyloxyphenyl)-3-hydroxy-2-methylenepropanoate **40a** was followed, using 2-benzyloxy-3-ethoxybenzaldehyde **39b** (1.52g, 6mmol), methyl acrylate (1.2ml, 12mmol) and DABCO (1.25g, 3mmol) in $CDCl_3$ (1.25ml). Work up and chromatography afforded methyl 3-(2-benzyloxy-3-ethoxyphenyl)-3-hydroxy-2-methylenepropanoate **40b** as a pale yellow oil (0.538g, 27%); ν_{max} (thin film)/cm^{-1} 3515 (OH) and 1719 (C=O); δ_H (400 MHz; $CDCl_3$) 1.45 (3H, t, *J* = 7Hz, CH_2CH_3), 3.59 (1H, s, OH), 3.69 (3H, s, OCH_3), 4.07 (2H, q, *J* = 7Hz, OCH_2CH_3), 5.09 (2H, s, OCH_2Ph), 5.72 and 6.28 (2H, 2 x s, $C=CH_2$) 5.87 (1H, s, CHOH) and 6.88-7.47 (8H, series of multiplets, Ar-H); δ_C (100 MHz; $CDCl_3$) 15.0 (OCH_2CH_3), 51.8 (OCH_3), 64.3 (OCH_2CH_3), 67.9 (CHOH), 74.6 (OCH_2Ph), 113.2, 119.3, 124.1, 125.8, 128.0, 128.3, 128.4, 135.1, 137.7, 141.7, 145.4 and 151.8 ($C=CH_2$ and Ar-C) and 166.8 (C=O).

3.2.3. Cyclisation of *O*-benzylated Baylis-Hillman adducts



3-*(Chloromethyl)coumarin* **41a**[60]

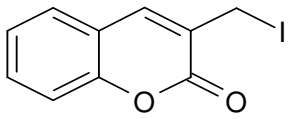
Conc. HCl (4ml) was added to a solution of methyl 3-(2-benzyloxyphenyl)-3-hydroxy-2-methylenepropanoate **40a** (0.531g, 2mmol) in AcOH (2ml). The mixture was boiled under reflux for 2h, allowed to cool to room temperature and then poured into ice-cooled water (20ml). Stirring for *ca*. 30min gave a precipitate, which was filtered off and washed with hexane to afford 3-(chloromethyl)coumarin **41a** as a grey solid (0.287g, 83%); m.p. 108-110^{o}C (lit.[60] 108-110^{o}C); ν_{max} (nujol)/cm^{-1} 1713 (C=O); δ_H (400 MHz; $CDCl_3$) 4.54 (2H, s, CH_2Cl), 7.28-7.53 (4H, series of multiplets, Ar-H) and 7.87 (1H, s, 4-H); δ_C (100 MHz; $CDCl_3$) 41.0 (CH_2), 116.6, 118.8, 124.70, 125.0; 128.0, 132.0, 141.1 and 153.45 (Ar-C) and 160.1 (C=O).



3-(Chloromethyl)-8-ethoxycoumarin **41b**[60]

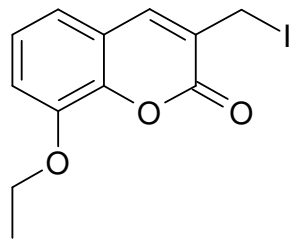
The procedure described for the synthesis of 3-(chloromethyl)coumarin **41a** was followed, using conc. HCl (4ml) and a solution of methyl 3-(2-benzyloxy-3-ethoxyphenyl)-3-hydroxy-2-methylenepropanoate **40b** (1.21g, 4mmol) in AcOH (1ml) and Ac_2O (1ml). Work-up afforded, as a pale pink solid, 3-(chloromethyl)-8-

ethoxycoumarin **41b** (0.59g, 70%); m.p. 121-124°C (lit.[60] 122-124°C); ν_{max} (nujol)/cm^{-1} 1709 (C=O); δ_H (400 MHz; $CDCl_3$) 1.45 (3H, t, *J* = 7Hz, CH_2CH_3), 4.14 (2H, q, *J* = 7Hz, OCH_2CH_3), 4.52 (2H, s, CH_2Cl), 7.04-7.07 (2H, m, Ar-H), 7.16 (1H, t, Ar-H) and 7.83 (1H, s, 4-H); δ_C (100 MHz; $CDCl_3$) 14.7 (CH_2CH_3), 41.0 (CH_2Cl), 65.0 (OCH_2CH_3), 115.2, 119.3, 119.5, 124.5, 125.1, 141.3, 143.4 and 146.5 (Ar-C) and 159.7 (C=O).



3-(Iodomethyl)coumarin **42a**[60]

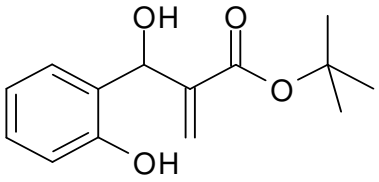
Conc. HI (4ml) was added to a solution of methyl 3-(2-benzyloxyphenyl)-3-hydroxy-2-methylenepropanoate **40a** (0.46g, 2mmol) in a mixture of AcOH (2ml). The mixture was boiled under reflux for 2h, allowed to cool to room temperature and then poured into ice-cooled water (10ml). Stirring for *ca*. 30min gave a precipitate, which was filtered off and washed with hexane to afford 3-(iodomethyl)coumarin **42a** as a grey solid (0.287g, 65%); m.p. 150-153°C (lit.[60] 150-152°C); ν_{max} (nujol)/cm^{-1} 1710 (C=O); δ_H (400 MHz; $CDCl_3$) 4.37 (2H, s, CH_2I), 7.28-7.53 (4H, series of overlapping multiplets, Ar-H) and 7.84 (1H, s, 4-H); δ_C (100 MHz; $CDCl_3$) –1.5 (CH_2I), 116.8, 119.1, 124.7, 127.2, 127.8, 131.9, 140.3 and 153.5 (Ar -C) and 159.8 (C=O).



8-Ethoxy-3-(iodomethyl)coumarin **42b**[60]

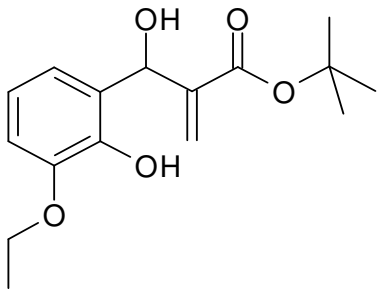
The procedure described for the synthesis of 3-(iodomethyl)coumarin **42a** was followed, using conc. HI (4ml) and methyl 3-(2-benzyloxy-3-ethoxyphenyl)-3-hydroxy-2-methylenepropanoate **40b** (0.312g, 1mmol) in a mixture of AcOH (5ml). Work-up afforded, as a pale yellow solid, 3-(iodomethyl)-8-ethoxycoumarin **42b** (0.187g, 62%); m.p. 119-122°C (lit.[60] 120-122); ν_{max} (nujol)/cm^{-1} 1718 (C=O); δ_H (400 MHz; $CDCl_3$) 1.47 (3H, t, *J* = 6.8Hz, CH_2CH_3), 4.14 (2H, q, *J* = 7Hz, OCH_2CH_3), 4.36 (2H, s, CH_2I), 7.01 (2H, overlapping multiplets, Ar-H), 7.15 (1H, t, *J* = 8Hz, Ar-H) and 7.79 (1H, s, 4-H). δ_C (100 MHz; $CDCl_3$) –1.5 (CH_2I), 14.7 (CH_2CH_3), 65.1 (OCH_2CH_3), 115.1, 119.1, 119.8, 124.5, 127.4, 140.7, 143.4 and 146.5 (Ar-C) and 159.4 (C=O).

3.2.4. Synthesis of Baylis-Hillman products using *tert*-butyl acrylate.



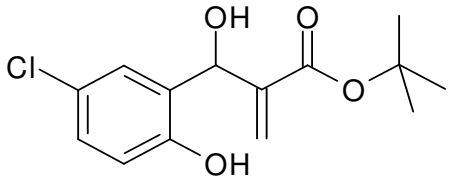
tert-*Butyl 3-hydroxy-3-(2-hydroxyphenyl)-2-methylenepropanoate* **24a**[60]

A mixture of salicylaldehyde (5.0ml, 41mmol), *tert*-butyl acrylate (10.5ml, 115mmol) and DABCO (4.3g, 39mmol) in $CHCl_3$ (15ml) was stirred in a stoppered reaction flask for 14 days. The mixture was concentrated *in vacuo* to give a dark brown oil, which was purified using flash column chromatography [on silica gel; elution with hexane-EtOAc (9:1)] to afford *tert*-butyl 3-hydroxy-3-(2-hydroxyphenyl)-2-methylenepropanoate **24a** as a pale yellow oil which later crystallized (2.56g, 25%); m.p.107-111°C (lit.[60] 108-110°C); ν_{max} (nujol)/cm^{-1}: 3442 (OH) and 1728 (C=O); δ_H (400 MHz; $CDCl_3$) 1.50 [9H, s, $C(CH_3)_3$], 4.35 (1H, s, OH), 5.50 and 6.23 (2H, 2x s, $C=CH_2$), 5.69 (1H, s, CHOH), 6.81-7.21 (4H, series of multiplets, Ar-H) and 8.12 (1H, s, ArOH); δ_C (100 MHz; $CDCl_3$) 28.0 [$C(CH_3)_3$], 73.6 (CHOH), 82.6 [$C(CH_3)_3$], 117.5, 119.8, 124.1, 124.2, 127.8, 129.5, 140.9 and 155.9 ($C=CH_2$ and Ar-C) and 166.7 (C=O).



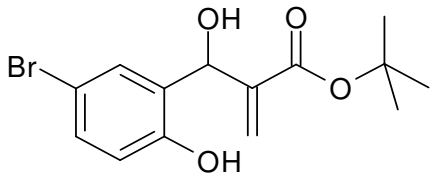
tert-*Butyl 3-hydroxy-3-(3-ethoxy-2-hydroxyphenyl)-2-methylenepropanoate* **24b**[60]

The procedure described for the synthesis of *tert*-butyl 3-hydroxy-3-(2-hydroxyphenyl)-2-methylenepropanoate **24b** was followed, using 3-ethoxysalicylaldehyde (2.0g, 12mmol), *tert*-butyl acrylate (2.5ml, 17mmol) and DABCO (0.86g, 7.7mmol) in $CHCl_3$ (3ml). Work-up and chromatography [on silica gel; elution with EtOAc-hexane (1:8)] afforded *tert*-butyl 3-hydroxy-3-(3-ethoxy-2-hydroxyphenyl)-2-methylenepropanoate **24b** as a pale yellow oil (0.68g, 20%); ν_{max} /cm^{-1} 3502 (OH) and 1714 (C=O); δ_H (400 MHz; $CDCl_3$) 1.41 [12H, overlapping s and t, $C(CH_3)_3$ and CH_2CH_3], 3.60 (1H, d, *J* = 5.6Hz, OH), 4.07 (2H, q, *J* = 6.9Hz, OCH_2CH_3), 5.66 and 6.22 (2H, 2 x s, $C=CH_2$), 5.81 (1H, d, *J* = 5.2Hz, CHOH), 6.43 (1H, s, ArOH) and 6.78-6.84 (3H, series of overlapping signals, Ar-H); δ_C (100 MHz; $CDCl_3$) 14.9 (CH_2CH_3), 28.0 [$C(CH_3)_3$], 64.6 (OCH_2CH_3), 69.4 (CHOH), 81.6 [$C(CH_3)_3$], 111.4, 119.5, 119.6, 125.1, 126.6, 142.2, 143.7 and 146.1 ($C=CH_2$ and Ar-C) and 166.1 (C=O).



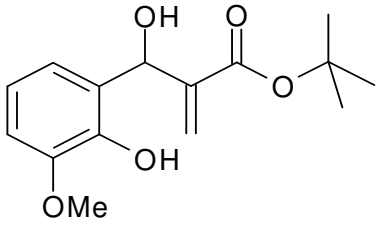
tert-*Butyl 3-(5-chloro-2-hydroxyphenyl)-3-hydroxy-2-methylenepropanoate* **24c**[60]

The procedure described for the synthesis of *tert*-butyl 3-hydroxy-3-(2-hydroxyphenyl)-2-methylenepropanoate **24a** was followed, using 5-chlorosalicylaldehyde (7.02g, 46mmol), *tert*-butyl acrylate (12ml, 95mmol) and DABCO (8.8g, 33mmol) in $CHCl_3$ (8ml) and stirring for 4 days. The reaction mixture was filtered through a layer of silica gel. Crystallization from $CHCl_3$ afforded *tert*-butyl 3-(5-chloro-2-hydroxyphenyl)-3-hydroxy-2-methylenepropanoate **24c** as white crystals (7.25g, 56%); m.p. 185-187^oC (lit.[60] 186-188); ν_{max} (nujol)/cm^{-1} 3309 (OH) and 1686 (C=O); δ_H (400 MHz; DMSO-d_6) 1.32 [9H, s, $C(CH_3)_3$], 5.52 (1H, s, CHO*H*), 5.64 and 6.06 (2H, 2 x s, $C=CH_2$), 5.67 (1H, C*H*OH), 6.78 (1H, d, *J* = 8.4Hz, Ar-H), 7.05-7.11 (2H, multiplets, Ar-H) and 9.71 (1H, s, ArOH); δ_C (100 MHz; DMSO-d_6) 27.5 [$C(CH_3)_3$], 64.5 (CHOH), 80.1 [$C(CH_3)_3$], 116.5, 122.0, 123.0, 126.9, 127.5, 131,3 144.8 and 153.3 ($C=CH_2$ and Ar-C) and 165.0 (C=O).



tert-*Butyl 3-(5-bromo-2-hydroxyphenyl)-3-hydroxy-2-methylenepropanoate* **24d**[60]

The procedure described for the synthesis of *tert*-butyl 3-hydroxy-3-(2-hydroxyphenyl)-2-methylenepropanoate **24a** was followed, using 5-bromosalicylaldehyde (2g, 10mmol), *tert*-butyl acrylate (2.1ml, 15mmol) and DABCO (0.86g, 7.7mmol) in $CHCl_3$ (3ml) and stirring for 4 days. The reaction mixture was filtered through a layer of silica gel. Crystallization from $CHCl_3$ afforded *tert*-butyl 3-(5-bromo-2-hydroxyphenyl)-3-hydroxy-2-methylenepropanoate **24d** as white crystals (1.75g, 53%); m.p. 185-187^oC (lit.[60] 186-188^oC); ν_{max} (nujol)/cm^{-1} 3304 (OH) and 1688 (C=O); δ_H (400 MHz; DMSO-d_6) 1.32 [9H, s, $C(CH_3)_3$], 5.48 (1H, br s, OH), 5.65 and 6.05 (2H, 2 x s, $C=CH_2$), 5.66 (1H, s, C*H*OH), 6.74 (1H, d, *J* = 8.4Hz, Ar-H), 7.19 -7.22 (2H, m, Ar-H) and 9.70 (1H, br, ArOH); δ_C (100 MHz; DMSO-d_6) 27.4 [$C(CH_3)_3$], 64.5 (CHOH), 80.0 [$C(CH_3)_3$], 109.6, 117.1, 122.8, 129.7, 130.3, 131.8, 144.8 and 153.7 ($C=CH_2$ and Ar-C) and 164.9 (C=O).

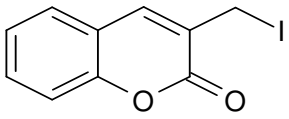


tert-*Butyl 3-hydroxy-3-(2-hydroxy-3-methoxyphenyl)-2-methylenepropanoate***24e**[60]

The procedure described for the synthesis of *tert*-butyl 3-hydroxy-3-(2-hydroxyphenyl)-2-methylenepropanoate **24a** was followed, using 3-methoxysalicylaldehyde (4.32g, 28mmol), *tert*-butyl acrylate (7.3ml, 57mmol) and DABCO (2.58g, 23mmol) in $CDCl_3$ (12ml). Work-up and chromatography [on silica gel; elution with EtOAc-hexane (1:6)] afforded *tert*-butyl 3-hydroxy-3-(2-hydroxy-3-methoxy-phenyl)-2-methylenepropanoate **24e** as a brown-yellow oil (1.69g, 25%); ν_{max} /cm^{-1} 3482 (OH) and 1717 (C=O); δ_H (400 MHz; $CDCl_3$) 1.43 [9H, $C(CH_3)_3$], 3.90 (1H, s, OH), 3.86 (3H, s, CH_3) 5.65 and 6.21 (2H, 2x s, $C=CH_2$), 5.81 (1H, s, C*H*OH) and 6.80 (3H, series of overlapping signals, Ar-H); δ_C (100 MHz, $CDCl_3$): 27.8 [$C(CH_3)_3$], 55.9 (OCH_3), 69.3 (CHOH), 81.4 [$C(CH_3)_3$], 110.3, 119.3, 119.6, 124.8, 126.5, 142.2, 143.6 and 147.0 ($C=CH_2$ and Ar-C) and 165.9 (C=O).

3.2.5. Cyclisation of Baylis-Hillman adducts

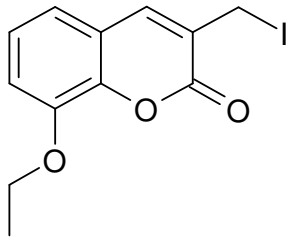
3.2.5.1. *Synthesis of 3-(iodomethyl)coumarin derivatives*



3-(Iodomethyl)coumarin **42a**[60]

Conc. HI (10ml) was added to a solution of *tert*-butyl 3-hydroxy-3-(2-hydroxyphenyl)-2-methylenepropanoate **24a** (0.50g, 2.0mmol) in a mixture of AcOH (5ml) and Ac_2O (5ml). The mixture was boiled under reflux for 2h, allowed to cool to room temperature and then poured into ice-cooled water (10ml). Stirring for *ca*. 30min gave a precipitate, which

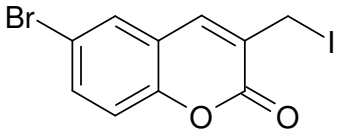
was filtered off and washed with hexane to afford 3-(iodomethyl)coumarin **42a** as a grey solid (0.35g, 60%); m.p. 148-151°C (lit.[61] 150-152°C); ν_{max} (nujol)/cm^{-1} 1710 (C=O); δ_H (400 MHz; $CDCl_3$) 4.37 (2H, s, CH_2I), 7.25-7.52 (4H, series of overlapping multiplets, Ar-H) and 7.83 (1H, s, 4-H); δ_C (100 MHz; $CDCl_3$) -1.6 (CH_2I), 116.8, 119.1, 124.7, 127.2, 127.8, 131.9, 140.4 and 153.5 (Ar -C) and 159.8 (C=O).

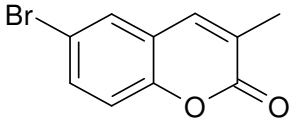


8-Ethoxy-3-(iodomethyl)coumarin **42b**[60]

The procedure described for the synthesis of 3-(iodomethyl)coumarin **42a** was followed, using conc. HI (10ml) and *tert*-butyl 3-ethoxy-3(-2-hydroxyphenyl)-3-hydroxy-2-methylenepropanoate **24b** (0.58g, 2mmol) in a mixture of AcOH (5ml) and Ac_2O (5ml). Work-up afforded, as a pale yellow solid, 3-(iodomethyl)-8-methoxycoumarin **42b** (0.29g, 62%); m.p. 120-123°C (lit.[60] 120-122°C); ν_{max} (nujol)/cm^{-1} 1718 (C=O); δ_H (400 MHz; $CDCl_3$) 1.46 (3H, t, *J* = 6.8Hz, CH_2CH_3), 4.14 (2H, q, *J* = 7Hz, OCH_2CH_3), 4.36 (2H, s, CH_2I), 7.01-7.07 (2H, overlapping multiplets, Ar-H), 7.15 (1H, t, Ar-H) and 7.80 (1H, s, 4-H); δ_C (100 MHz; $CDCl_3$) -1.5 (CH_2I), 14.7 (CH_2CH_3), 65.1 (OCH_2CH_3), 115.1, 119.1, 119.9, 124.6, 127.4, 140.7, 143.4 and 146.6 (Ar-C) and 159.4 (C=O).

3.2.5.2. *Optimisation Studies of reactions of compound* 24**c** *and d* with HI.



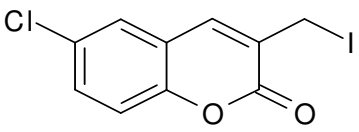


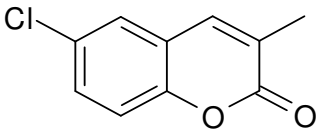
6-Bromo-3-(iodomethyl)coumarin **42d** [60] *6-Bromo-3-methylcoumarin* **47d**

Conc. HI (10ml) was added to a solution of *tert*-butyl 3-(5-bromo-2-hydroxyphenyl)-3-hydroxy-2-methylenepropanoate **24d** (0.61g, 1.0mmol) in a mixture of AcOH (5ml) and Ac_2O (5ml). The mixture was boiled under reflux for 2h, allowed to cool to room temperature and then poured into ice-cooled water (10ml). Stirring for ca. 30min gave a precipitate, which was filtered off and washed with hexane. The products were separated by flash column chromatography [on silica gel; elution with ethyl acetate-hexane (1:5)] to afford two fractions.

Fraction 1: 6-Bromo-3-(iodomethyl)coumarin **42d** as a grey solid (0.349g, 52%); m.p. 147-149^{o}C (lit.[60] 148-150^{o}C); ν_{max} (nujol)/cm^{-1} 1722 (C=O); δ_H (400 MHz; $CDCl_3$) 4.40 (2H, s, CH_2I), 7.27 (1H, s, Ar-H); 7.47-7.66 (2H, series of overlapping signals, Ar-H) and 7.80 (1H, s, 4-H); δ_C (100 MHz; $CDCl_3$) –2.2 (CH_2I), 117.3, 118.5, 120.6, 128.6, 130.0, 134.5, 139.9 and 152.3 (Ar-C) and 159.1 (C=O).

Faction 2: 6-Bromo-3-methylcoumarin **47d** as a grey solid (0.189g, 43%); m.p. 154-156^{o}C; ν_{max} (nujol)/cm^{-1} 1729 (C=O); δ_H (400 MHz; $CDCl_3$) 2.21 (3H, s, CH_3), 7.17 (1H, m, Ar-H), 7.53 (2H, s, Ar-H) and 7.51 (1H, s, 4-H); δ_C (100 MHz; $CDCl_3$) 17.3 (CH_3), 116.8, 118.2, 121.1, 127.2, 129.2, 133.2, 137.8 and 152.0 (Ar-C) and 161.5 (C=O).





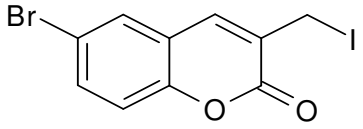
6-Chloro-3-(Iodomethyl)coumarin **42c** [60] *6-Chloro-3-methylcoumarin* **47c**

The procedure described for the synthesis of 6-bromo-3-(iodomethyl)coumarin **42d** and 6-bromo-3-methyl-2H-chromen-2-one **47d** was followed, using conc. HI (10ml) and *tert*-

butyl 3-(5-chloro-2-hydroxyphenyl)-3-hydroxy-2-methylenepropanoate **24c** (0.52g, 2mmol) in a mixture of AcOH (5ml) and Ac_2O (5ml). Work-up and chromatography [on silica gel; elution with ethyl acetate-chloroform-hexane (1:1:3) afforded two fractions.

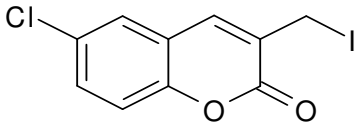
Fraction 1: 6-Chloro-3-(iodomethyl)coumarin **42c** as a yellow solid (0.308g, 52%); 186-189^oC (lit.[60] 188-190); ν_{max} (nujol)/cm^{-1} 1723 (C=O); (400 MHz; $CDCl_3$) 4.35 (2H, s, CH_2I), 7.28 (1H, s, Ar-H) 7.45 (2H, series of overlapping signals, Ar-H) and 7.75 (1H, s, 4-H); δ_C (100 MHz; $CDCl_3$) -2.2 (CH_2I), 118.2, 120.1, 126.1, 128.5, 130.0, 131.7, 139.0 and 151.8 (Ar-C) and 159.2 (C=O).

Fraction 2: 6-Chloro-3-methylcoumarin **47c** as pale yellow solid (0.162g, 45%); m.p 1128-132^oC (lit.[60] 158-160); ν_{max} (nujol)/cm^{-1} 1731 (C=O); δ_H (400 MHz; $CDCl_3$) 2.21 (3H, s, CH_3), 7.26 and 7.42 (3H, m, Ar-H), and 7.40 (1H, s, 4-H); δ_C (100 MHz; $CDCl_3$) 17.3 (CH_3), 117.9, 120.5, 126.2, 127.2, 129.4, 130.4, 137.9 and 151.6 (Ar-C) and 161.5 (C=O).



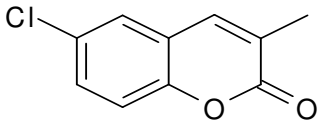
6-Bromo-3-(iodomethyl)coumarin **42d**[60]

Conc. HI (10ml) was added to a solution *tert*-butyl 3-(5-bromo-2-hydroxyphenyl)-3-hydroxy-2-methylenepropanoate **24d** (0.51g, 2mmol) in a mixture of AcOH (5ml) and Ac_2O (5ml). The mixture was boiled under reflux for 1h, allowed to cool to room temperature and then poured into ice-cooled water (10ml). Stirring for *ca.* 30min gave a precipitate, which was filtered off and washed with hexane 6-bromo-3-(iodomethyl)coumarin **42d** a grey solid (0.33g, 58%).



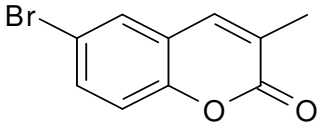
6-Chloro-3-(iodomethyl)coumarin **42c**[60]

The procedure described for the synthesis of 6-bromo-3-(iodomethyl)coumarin **42d** was followed, using conc. HI (10ml) and *tert*-butyl 3-(5-chloro-2-hydroxyphenyl)-3-hydroxy-2-methylenepropanoate **24c** (0.37g, 1.0mmol) in a mixture of AcOH (5ml) and Ac_2O (5ml). Work-up and afforded 6-chloro-3-(iodomethyl)coumarin **42c** as a grey solid (0.23g, 61%).



6-Chloro-3-methylcoumarin **47c**

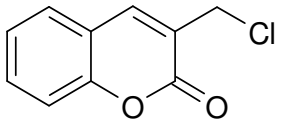
Conc. HI (10ml) was added to a solution of *tert*-butyl 3-hydroxy-3-(5-chloro-2-hydroxyphenyl)-2-methylenepropanoate **24a** (0.38g, 1mmol) in a mixture of AcOH (5ml) and Ac_2O (5ml). The mixture was boiled under reflux for 8h, allowed to cool to room temperature and then poured into ice-cooled water (10ml). Stirring for *ca*. 30min gave a precipitate, which was filtered off and washed with hexane to afford, as a pale yellow solid, 6-chloro-3-methylcoumarin **47c** (0.237g, 91%).



6-Bromo-3-methylcoumarin **47d**

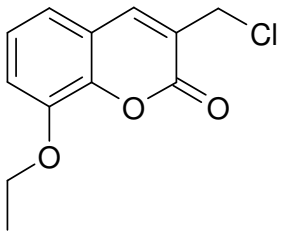
The procedure described for the synthesis of 6-chloro-3-methylcoumarim **47c** was followed, using conc. HI (10ml) and a solution of *tert*-butyl 3-(5-bromo-2-hydroxyphenyl)-3-hydroxy-2-methylenepropanoate **24d** (0.41g, 2mmol) in AcOH (5ml) and AcOH (5ml). Work-up afforded 6-bromo-3-methylcoumarin **47d** as a grey solid (0.298g, 80%).

3.2.5.3. *Synthesis of 3-(chloromethyl)coumarin derivatives*



6-(Chloromethyl)coumarin **41c**[60]

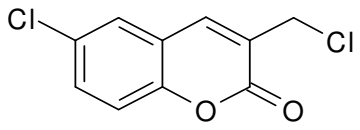
Conc. HCl (10ml) was added to a solution of *tert*-butyl 3-hydroxy-3-(2-hydroxyphenyl)-2-methylenepropanoate **24a** (2.02g, 81mmol) in AcOH (10ml). The mixture was boiled under reflux for 2h, allowed to cool to room temperature and then poured into ice-cooled water (20ml). Stirring for *ca*. 30min gave a precipitate, which was filtered off and washed with hexane to afford 3-(chloromethyl)coumarin **41a** as a grey solid (1.02g, 65%); m.p. 108-110°C (lit.[60] 108-110°C); ν_{max} (nujol)/cm^{-1} 1713 (C=O); δ_H (400 MHz; $CDCl_3$) 4.53 (2H, s, CH_2Cl), 7.27-7.55 (4H, series of multiplets, Ar-H) and 7.87 (1H, s, 4-H); δ_C (100 MHz; $CDCl_3$) 41.0 (CH_2), 116.6, 118.7, 124.7, 124.9; 128.0, 132.0, 141.1 and 153.5 (Ar-C) and 160.0 (C=O).



3-(Chloromethyl)-8-ethoxycoumarin **41b**[60]

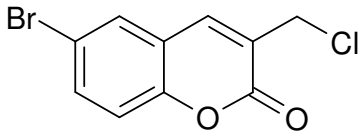
The procedure described for the synthesis of 3-(chloromethyl)coumarin **41a** was followed, using conc. HCl (10ml) and a solution of *tert*-butyl 3-(8-ethoxy-2-hydroxyphenyl)-3-hydroxy-2-methylenepropanoate **24b** (0.55g, 2mmol) in AcOH (5ml). Work-up afforded, as a pale pink solid, 3-(chloromethyl)-8-ethoxycoumarin **41** (0.299g, 74%); m.p. 122-125°C (lit.[60] 122-124°C); ν_{max} (nujol)/cm^{-1} 1709 (C=O); δ_H (400 MHz;

$CDCl_3$) 1.46 (3H, t, *J* = 7Hz, CH_2CH_3), 4.14 (2H, q, *J* = 7Hz, OCH_2CH_3), 4.53 (2H, s, CH_2Cl), 7.05-7.07 (2H, m, Ar-H), 7.17 (1H, t, *J* = 8Hz Ar-H) and 7.83 (1H, s, 4-H); δ_C (100 MHz; $CDCl_3$) 14.7 (CH_2CH_3), 41.0 (CH_2Cl), 65.0 (OCH_2CH_3), 115.2, 119.3, 119.5, 124.5, 125.1, 141.3, 143.4 and 146.5 (Ar-C) and 159.7 (C=O).



6-Chloro-3-(chloromethyl)coumarin **41c**[60]

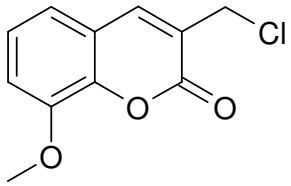
The procedure described for the synthesis of 3-(chloromethyl)coumarin **41a** was followed, using conc. HCl (10ml) and a solution of *tert*-butyl 3-(5-chloro-2-hydroxyphenyl)-3-hydroxy-2-methylenepropanoate **24d** (2.32g, 8mmol) in AcOH (8ml) Work-up afforded 6-chloro-3-(chloromethyl)coumarin **41c** as a pale pink solid (1.50g, 80%); m.p. 110-114°C (lit.[60] 112-114°C); ν_{max} (nujol)/cm^{-1} 1722 (C=O); δ_H (400 MHz; $CDCl_3$) 4.52 (2H, s, CH_2Cl), 7.27 (1H, s, Ar-H), 7.45-7.48 (2H, overlapping multiplets, Ar-H) and 7.80 (1H, s, 4-H); δ_C (100 MHz; $CDCl_3$) 40.8 (CH_2), 118.0, 119.7, 126.2, 127.2, 129.9, 131.8, 139.7, and 151.7 (Ar-C) and 159.4 (C=O).



6-Bromo-3-(chloromethyl)coumarin **41d**[60]

The procedure described for the synthesis of 3-(chloromethyl)coumarin **41a** was followed, using conc. HCl (10ml) and a solution of *tert*-butyl 3-(5-bromo-2-hydroxyphenyl)-3-hydroxy-2-methylenepropanoate **24d** (1.52g, 1.0mmol) in AcOH (6ml) Work-up afforded 6-bromo-3-(chloromethyl)coumarin **41d** as a pale pink solid

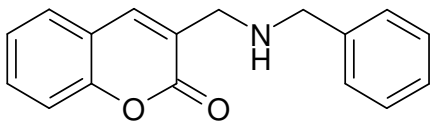
(1.21g, 95%); m.p. 113-115°C (lit.[60] 112-114 °C); ν_{max} (nujol)/cm^{-1} 1722 (C=O); δ_H (400 MHz; $CDCl_3$) 4.53 (2H, s, CH_2Cl), 7.21 (1H, d, Ar-H), 7.61-7.65 (2H, m, Ar-H) and 7.80 (1H, s, 4-H); δ_C (100 MHz; $CDCl_3$) 40.7 (CH_2), 117.2, 118.3, 120.24, 126.2, 130.2, 134.6, 139.5, and 152.3 (Ar-C) and 159.3 (C=O).



3-(Chloromethyl)-8-methoxycoumarin **41e**[60]

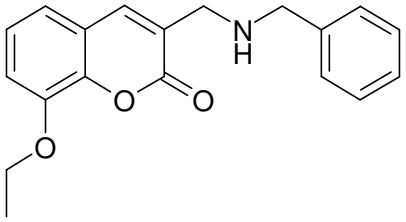
The procedure described for the synthesis of 3-(chloromethyl)coumarin **41a** was followed, using conc. HCl (10ml) and a solution of *tert*-butyl 3-(8-methoxy-2-hydroxyphenyl)-3-hydroxy-2-methylenepropanoate **24e** (1.02g, 4mmol) in AcOH (8ml). Work-up afforded 3-(chloromethyl)-8-methoxycoumarin **41e** as a pale grey solid (0.54g, 65%); m.p. 144-148°C (lit.[60] 146-148°C); ν_{max} (nujol)/cm^{-1} 1709 (C=O); δ_H (400 MHz; $CDCl_3$) 3.94 (3H, s, OCH_3), 4.53 (2H, s, CH_2Cl), 7.06 (2H, d, *J* = 8 Hz, Ar-H), 7.19 (1H, t, *J* = 8 Hz, Ar-H) and 7.84 (1H, s, 4-H); δ_C (100 MHz; $CDCl_3$) 40.9 (OCH_3), 56.2 (CH_2Cl), 113.8, 119.3, 124.5, 125.1, 141.2, 143.1 and 147.0 (Ar-C) and 159.5 (C=O).

3.2.6. Synthesis of benzylamine derivative from 3-(chloromethyl)coumarin derivatives.



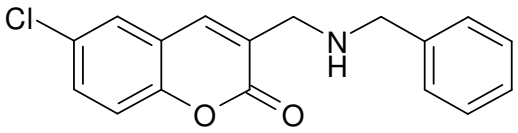
3-(Benzylaminomethyl)coumarin **53a**[54]

A mixture of 3-(chloromethyl)coumarin **42a (**0.31g, 1.2mmol) and benzylamine (0.34ml) in THF (6ml) was stirred in a stoppered reaction flask for 4 hours. The mixture was concentrated in *vacuo* and separated by flash chromatography [on silica gel; elution with $CHCl_3$-EtOAc (4:1)] to afford 3-(benzylaminomethyl)coumarin **53a** as a pale yellow solid (0.31g, 74%); m.p. 70-74°C (lit.[54] 70-74°C); ν_{max} (nujol)/cm^{-1} 3324 (N-H) and 1720 (C=O); δ_H (400 MHz; $CDCl_3$) 1.99 (1H, br s, NH), 3.74 (2H, s, 1'-CH_2), 3.84 (2H, s, CH_2Ph), 7.23-7.36 (7H, series of overlapping signals, Ar-H), 7.44-7.50 (2H, m, Ar-H,) and 7.71 (1H, s, 4-H); δ_C (100 MHz; $CDCl_3$) 48.4 and 53.2 (2xCH_2), 116.4, 119.2, 124.4, 127.1, 127.4, 127.5, 128.1, 128.4, 130.9, 139.2, 139.7 and 153.1 (Ar-C) and 161.4 (C=O).



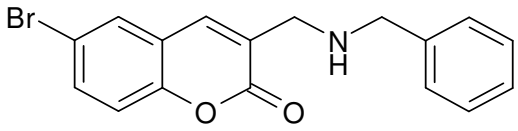
3-(Benzylaminomethyl)-8-ethoxycoumarin **53c**[54]

The procedure described for the synthesis of 3-(benzylaminomethyl)coumarin **53a** was followed, using 8-ethoxy-3-(chloromethyl)coumarin **42b** (0.2g, 9mmol) and benzylamine (0.15ml) in THF (4ml). Work-up and chromatography [on silica gel, elution with $CHCl_3$-EtOAc 4:1)] afforded 3-(benzylaminomethyl)-8-ethoxycoumarin **53b** as a pale yellow solid (0.19g, 73%); m.p. 97-101°C (lit.[54] 98-102); ν_{max}(nujol)/cm^{-1} 3425 (NH) and 1714 (C=O); δ_H (400 MHz; $CDCl_3$) 1.47 (3H, t, J = 7 \Hz, CH_2CH_3), 2.10 (1H, br s, NH), 3.75 (2H, s, 1'-CH_2), 3.83 (2H, s, CH_2Ph), 4.15 (2H, q, J = 6.8 \Hz, OCH_2CH_3), 7.01-7.36 (8H, series of multiplets, Ar-H) and 7.68 (1H, s, 4-H); δ_C (100 MHz; $CDCl_3$) 14.7 (CH_2CH_3), 48.4 and 53.1 (2xCH_2N), 65.0 (OCH_2CH_3), 114.3, 119.0, 120.0, 124.2, 127.1, 127.4, 128.1, 128.4, 139.5, 139.7, 143.1 and 146.4 (Ar-C) and 161.1 (C=O).



3-(Benzylaminomethyl)-6-chlorocoumarin **53c**[54]

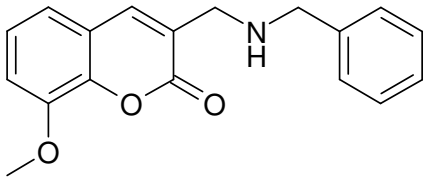
The procedure described for the synthesis of 3-(benzylaminomethyl)coumarin **53a** was followed, using 6-chloro-3-(chloromethyl)coumarin **42c** (0.84g, 4mmol) and benzylamine (0.76ml) in THF (8ml). Work-up and chromatography afforded 3-(benzylaminomethyl)-6-chlorocoumarin **53c** as a yellow solid (0.71g, 64%), m.p. 106-109^{o}C (lit.[54] 106-110); ν_{max} (nujol)/cm^{-1} 3429 (NH) and 1704 (C=O); δ_H (400 MHz; $CDCl_3$) 2.00 (1H, br s, NH), 3.74 (2H, 1'-CH_2), 3.84 (2H, s, CH_2Ph), 7.24-7.43 (8H, series of multiplets, Ar-H) and 7.65 (1H, s, 4-H); δ_C (100 MHz; $CDCl_3$) 48.2 and 53.3 (2 x CH_2), 117.9, 120.3, 126.8, 127.2, 128.1, 128.5, 128.9, 129.6, 130.8, 137.8, 139.6 and 151.5 (Ar-C) and 160.8 (C=O).



3-(Benzylaminomethyl)-6-bromocoumarin **53d**[54]

The procedure described for the synthesis of 3-(benzylaminomethyl) coumarin **53a** was followed, using 6-bromo-3-(chloromethyl)coumarin **42d** (0.27g, 1mmol) and benzylamine (0.21ml) in THF (6ml). Work-up and chromatography [on silica gel; elution with $CHCl_3$-EtOAc (4:1)] afforded 3-(benzylaminomethyl)-6-bromocoumarin as a pale yellow solid **53d** (0.114g, 35%); m.p 107-110^{o}C (lit.[54] 106-110^{o}C); ν_{max} (nujol)/cm^{-1} 3326 (NH) and 1723 (C=O); δ_H (400 MHz; $CDCl_3$) 1.85 (1H, br s, NH), 3.74 (2H, 1'-CH_2), 3.84 (2H, s, CH_2Ph), 7.19-7.34 (6H, series of multiplets, Ar-H) and 7.55-7.59 (2H, m, Ar-H) and 7.65 (1H, s, 4H); δ_C (100 MHz; $CDCl_3$) 48.2 and 53.2 (2xCH_2), 117.0,

118.2, 120.8, 127.2, 128.1, 128.5, 128.9, 129.8, 133.7, 137.7, 139.6 and 152.0 (Ar-C) and 160.7 (C=O).



3-(Benzylaminomethyl)-8-methoxycoumarin **53e**[54]

The procedure described for the synthesis of 3-(benzylaminomethyl) coumarin **53a** was followed, using 3-methoxy-3-(chloromethyl)coumarin **42e** (0.2g, 1mmol) and benzylamine (0.15ml) in THF (4ml). Work-up and chromatography [on silica gel; elution with $CHCl_3$-EtOAc (4:1)] afforded 3-(benzylaminomethyl)-8-methoxycoumarin **53e** as a pale yellow oil (0.19g, 73%); ν_{max}(nujol) /cm^{-1} 3448 (NH) and 1716 (C=O); δ_H (400 MHz; $CDCl_3$) 2.15 (1H, br s, NH), 3.72 (2H, s, 1'-CH_2), 3.81 (2H, s, CH_2Ph), 3.91 (3H, s, OCH_3), 7.00 (2H, d, *J* = 8Hz, Ar-H), 7.14-7.33 (6H, series of multiplets, Ar-H) and 7.66 (1H, s, 4-H); δ_C (100 MHz; $CDCl_3$) 48.2 and 53.1 (2 x CH_2), 56.1 (OCH_3), 112.8, 118.9, 119.8, 124.2, 127.0, 127.4, 128.0, 128.3, 139.3, 139.6, 142.6 and 146.9 (Ar-C) and 160.8 (C=O).

3.2.7. Arbuzov reactions of 3-(halomethyl)coumarin products

3.2.7.1. *Optimisation Studies of Arbuzov reactions*

Experimental procedure 1

To 3-(chloromethyl)coumarin **42a** (0.35g, 1.3mmol) was added triethyl phosphite (0.40ml) and the mixture was boiled under reflux for 4 hours. Upon completion of the reaction, as monitored by TLC, the mixture was separated by flash column chromatography [on silica gel; elution with ethyl acetate-hexane (3:1)] to afford *diethyl (3-methyl-2-oxo-2*H*-chromen-4-yl)phosphonate* **48a** as a yellow solid (0.318g, 60%)

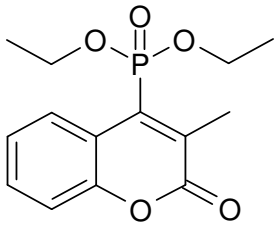
Experimental procedure 2

To 3-(chloromethyl)coumarin **42a** (0.823g, 4.3mmol) was added triethyl phosphite (0.70ml) and the mixture was refluxed under nitrogen for 4 hours. Upon completion of the reaction, as monitored by TLC, the mixture was separated by flash column chromatography [on silica gel; elution with ethyl acetate-hexane (3:1)] to afford *diethyl (3-methyl-2-oxo-2*H-*chromen-4-yl)phosphonate* **48a** as a yellow solid (0.236g, 16%) and *diethyl [(2-oxo-2*H-*chromen-3-yl)methyl]phosphonate* **49a** as a brown oil (0.401g, 43%)

Experimental procedure 3

To 3-(iodomethyl)coumarin **41a** (0.34g, 1.2mmol) was added triethyl phosphite (0.40ml) and the mixture was refluxed under nitrogen for 4 hours. Upon completion of the reaction, as monitored by TLC, the mixture was separated by flash column chromatography [on silica gel; elution with ethyl acetate-hexane (3:1)] to afford *diethyl [(2-oxo-2*H-*chromen-3-yl) methyl]phosphonate* **49a** as a brown oil (0.215g, 61%).

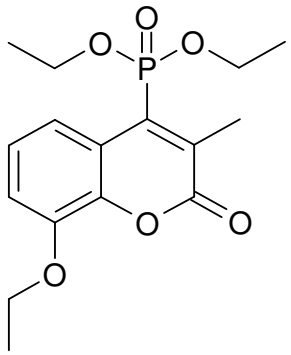
Experimental procedure 1



*Diethyl (3-methyl-2-oxo-2*H-*chromen-4-yl)phosphonate* **48a**

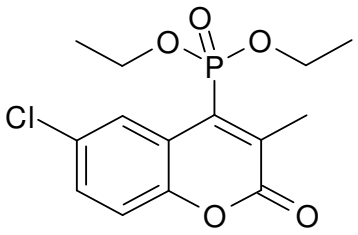
To 3-(chloromethyl)coumarin **42a** (0.35g, 1.3mmol) was added triethyl phosphite (0.42ml) and the mixture was boiled under reflux for 4 hours. Upon completion of the reaction, as monitored by TLC, the mixture was separated by flash column chromatography [on silica gel; elution with ethyl acetate-hexane (3:1)] to afford *diethyl (3-methyl-2-oxo-2*H-*chromen-4-yl)phosphonate* **48a** as a yellow solid (0.318g, 60%); (Found M^+: 296.082484. $C_{14}H_{17}O_5P$ requires *M*: 296.081362); m.p. 47-49^oC; ν_{max} (nujol)/cm^{-1} 1240 (P=O) and 1734 (C=O); δ_H (400 MHz; $CDCl_3$) 1.33 (6H, t, *J* = 7Hz, 2 x CH_2CH_3), 2.60 (3H, d, *J* = 3.2Hz, CH_3), 4.12 and 4.26 (4H, 2 x m, 2 x CH_2OP), 7.25 (2H, m, Ar-H), 7.44 (1H, t, *J* = 8.4Hz, Ar-H) and 8.50 (1H, s, Ar-H); δ_C (100 MHz; $CDCl_3$)

14.7 (Ar-OCH_2CH_3), 16.2 (CH_3), 16.3 (d, $J_{P,C}$ = 6.1Hz, 2 x CH_2CH_3), 62.8 (d, $J_{P,C}$ = 5.5Hz, 2 x CH_2OP), 116.7, 118.2, 124.2, 128.0, 130.6, 137.4 and 152.0 (Ar-C), 135.6 (d, $J_{P,C}$ = 12Hz, C-4) and 161.1 (C=O); *m/z* 296 (M+1, 100%).



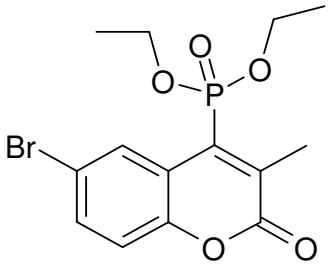
*Diethyl (8-ethoxy-3-methyl-2-oxo-2*H*-chromen-4-yl)phosphonate* **48b**

The procedure described in *experimental procedure 3* for the synthesis of **48a** was followed using 3-(chloromethyl)-8-ethoxycoumarin **42b** (0.25g, 1.2mmol) and triethyl phosphite (0.35ml). Work-up and chromatography [elution with ethyl acetate-hexane (1:3)] afforded *diethyl (8-ethoxy-3-methyl-2-oxo-2*H*-chromen-4-yl)phosphonate* **48b** as a pale yellow solid (0.186g, 52%); (Found M^+: 340.106262. $C_{16}H_{21}O_6P$ requires *M*: 340.107577); m.p. 42-45°C; ν_{max} (nujol)/cm^{-1} 1260 (P=O) and 1709 (C=O); δ_H (400 MHz; $CDCl_3$) 1.30 (6H, t, *J* = 7.2Hz, 2 x OCH_2CH_3), 1.45 (3H, t, *J* = 7Hz, Ar-OCH_2CH_3), 2.59 (3H, d, *J* = 3.2Hz, CH_3), 4.10-4.27 (6H, m, 2 x CH_2OP and 1 x Ar-OCH_2CH_3), 7.00 (1H, d, *J* = 8 Hz, Ar-H), 7.13 (1H, t, *J* = 8.2Hz, Ar-H) and 8.03 (1H, d, *J* = 8.4Hz, Ar-H); δ_C (100 MHz; $CDCl_3$) 14.7 (Ar-OCH_2CH_3), 16.3 (1 x CH_3), 16.4 (d, $J_{P,C}$ = 6.2Hz, 2 x OCH_2CH_3), 62.7 (d, $J_{P,C}$ = 5.5Hz, 2 x CH_2OP), 65.0 (Ar-OCH_2CH_3), 114.0, 118.8, 119.3, 123.6, 137.5, 142.2 and 146.3 (Ar-C), 135.8 (d, $J_{P,C}$ = 11Hz, C-4) and 160.6 (C=O).



*Diethyl (6-chloro-3-methyl-2-oxo-2*H*-chromen-4-yl)phosphonate* **48c**

The procedure described in *experimental procedure 3* for the synthesis of **48a** was followed using 6-chloro-3-(chloromethyl)coumarin **42c** (0.21g, 1.1mmol) and triethyl phosphite (0.30ml). Work-up and chromatography [elution with ethyl acetate-hexane (1:3)] afforded *diethyl (6-chloro -3-methyl-2-oxo-2*H*-chromen-4-yl)phosphonate* **48d** as a pale yellow solid (0.26g, 68%); (Found M^+: 330.042192. $C_{14}H_{16}ClO_5P$ requires *M*: 330.042390); m.p. 76-78^oC; ν_{max} (nujol)/cm^{-1} 1255 (P=O) and 1730 (C=O); δ_H (400 MHz; $CDCl_3$) 1.32 (6H, t, *J* = 7Hz, 2 x CH_2CH_3), 2.57 (3H, d, *J* = 3.2Hz, CH_3), 4.11 and 4.31 (4H, m, 2 x CH_2OP), 7.19 (1H, d, *J* = 8.8Hz, Ar-H), 7.38 (1H, dd, *J* = 8.8 Hz, Ar-H) and 8.52 (1H, d, *J* = 2.4 Hz, Ar-H); δ_C (100 MHz; $CDCl_3$) 16.3 (1 x CH_3), 16.5 (d, $J_{P,C}$ = 6.2Hz, 2 x OCH_2CH_3), 63.0 (d, $J_{P,C}$ = 5.6Hz, 2 x CH_2OP), 117.9, 119.1, 127.5, 129.7, 130.7, 134.8 and 150.4 (Ar-C), 136.6 (d, $J_{P,C}$ = 10Hz, C-4) and 160.5 (C=O); *m/z* 330 (M+1, 100%).

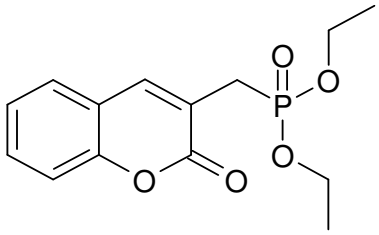


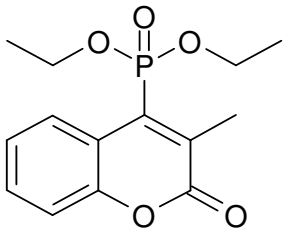
*Diethyl (6-bromo-3-methyl-2-oxo-2*H*-chromen-4-yl)phosphonate* **48d**

The procedure described in *experimental procedure 3* for the synthesis of **48a** was followed using 6-bromo-3-(chloromethyl)coumarin **42d** (0.35g, 1,2mmol) and triethyl phosphite (0.42ml). Work-up after chromatography [elution with ethyl acetate-hexane (1:3)] afforded *diethyl (6-bromo-3-methyl-2-oxo-2*H*-chromen-4-yl)phosphonate* **48d** as a

yellow solid (0.31g, 65%) (Found M^+: 373.989683. $C_{14}H_{16}BrO_5P$ requires *M*: 373.991873); m.p. 77-79^oC; ν_{max} (nujol)/cm^{-1} 1240 (P=O) and 1734 (C=O); δ_H (400 MHz; $CDCl_3$) 1.34 (6H, t, *J* = 7.2Hz, 2 x CH_2CH_3), 2.59 (3H, d, *J* = 3.2Hz, CH_3), 4.16 and 4.26 (4H, 2 x m, 2 x CH_2OP), 7.15 (1H, d, *J* = 8.8Hz, Ar-H), 7.54 (1H, dd, *J* = 8.8Hz, Ar-H) and 8.69 (1H, s, Ar-H); δ_C (100 MHz; $CDCl_3$) 16.3 (CH_3), 16.6 (d, $J_{P,C}$ = 6.1Hz, 2 x OCH_2CH_3), 63.1 (d, $J_{P,C}$ = 5.5Hz, 2 x CH_2OP), 117.2, 118.3, 119.6, 130.6, 133.5, 134.9 and 150.8 (Ar-C), 136.6 (d, $J_{P,C}$ = 10Hz,C-4) and 160.3 (C=O); *m/z* 374 (M+1, 95%) and 379 (100%).

Experimental procedure 2





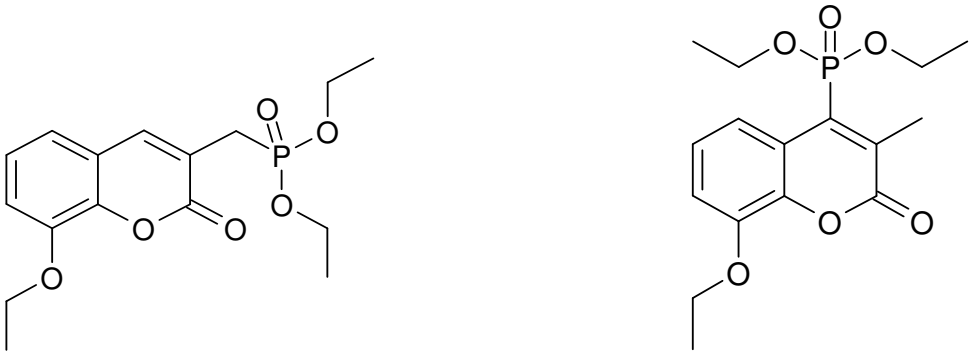
Diethyl [(2-oxo-2H-chromen-3-yl)methyl]phosphonate **49a** and *diethyl (3-methyl-2-oxo-2*H*-chromen-4-yl)phosphonate* **48a**

To 3-(chloromethyl)coumarin **42a** (0.823g, 4.3mmol) was added triethyl phosphite (1.4ml) and the mixture was refluxed under nitrogen for 4 hours. Upon completion of the reaction, as monitored by TLC, the mixture was then separated by flash column chromatography [on silica gel; elution with ethyl acetate-hexane (3:1)] to afford two fractions.

Fraction 1: *diethyl (3-methyl-2-oxo-2*H*-chromen-4-yl)phosphonate* **48a** as a pale yellow solid (0.236g, 16%).

Fraction 2: *diethyl [(2-oxo-2*H*-chromen-3-yl)methyl]phosphonate* **49a** as a pale brown oil (0.401g, 43%); (Found M^+: 296.3079819. $C_{14}H_{17}O_5P$ requires *M*: 296.081362); ν_{max} (nujol)/cm^{-1} 1240 (P=O) and 1734 (C=O); δ_H (400 MHz; $CDCl_3$) 1.28 (6H, t, *J* =7 Hz, 2 x

OCH_2CH_3), 3.18 (2H, d, $J_{P,H}$= 22Hz, CH_2P), 4.10 (4H, q, J = 4.3Hz, 2 x CH_2OP), 7.26-7.51 (4H, series of multiplets, Ar-H) and 7.82 (1H, d, $J_{P,C}$ = 4.4Hz, 4-H); δ_C (100 MHz; $CDCl_3$) 16.3 (d, $J_{P,C}$ = 6.2Hz, 2 x OCH_2CH_3), 26.7 (d, $J_{P,C}$= 139Hz, CH_2P), 62.4 (d, $J_{P,C}$ = 6.6Hz, 2 x CH_2OP), 116.4, 119.1, 120.2, 124.4, 127.6, 131.2, 141.7, and 153.2 (Ar-C) and 161.1 (C=O); *m/z* 296 (M+1, 90%) and 160 (100%).



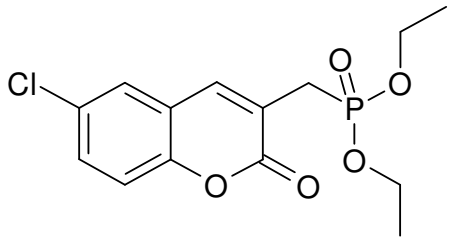
*Diethyl [(8-ethoxy-2-oxo-2*H*-chromen-3-yl)methyl]phosphonate* **49b** and *diethyl (8-ethoxy-3-methyl-2-oxo-2*H*-chromen-4-yl)phosphonate* **48b**

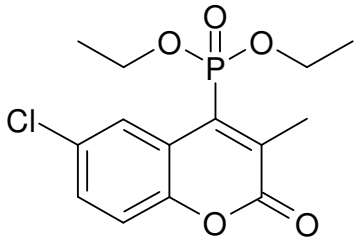
The procedure described in *experimental procedure 2* for the synthesis of compounds **48a** and **49a** was followed using 8-ethoxy-3-(chloromethyl)coumarin **42b** (0.86g, 3.6mmol) and triethyl phosphite (1.2ml). Work-up and chromatography [on silica gel; elution with ethyl acetate-hexane (1:3)] afforded two fractions.

Fraction 1: *diethyl (8-ethoxy-3-methyl-2-oxo-2*H*-chromen-4-yl)phosphonate* as a pale yellow crystals, **48b** (0.174g, 18%).

Fraction 2: *diethyl [(8-ethoxy-2-oxo-2*H*-chromen-3-yl)methyl]phosphonate* **49b** as a pale yellow solid (0.83g, 85%); (Found M^+: 340.107526. $C_{16}H_{21}O_6P$ requires *M*: 340.107526); m.p. 53-56^{o}C; ν_{max} (nujol)/cm^{-1} 1258 (P=O) and 1724 (C=O); δ_H (400 MHz; $CDCl_3$) 1.24 (6H, t, J = 7Hz, 2 x OCH_2CH_3), 1.43 (3H, t, J = 6.8Hz, Ar-OCH_2CH_3), 3.11 (2H, d, $J_{P,H}$ = 20Hz, CH_2P), 4.07 (6H, m, 2 x CH_2OP and 1 x Ar-OCH_2CH_3), 7.27 (2H, dd, J = 4.6Hz, Ar-H), 7.11 (1H, t, J = 7.8Hz, Ar-H) and 7.74 (1H, d, $J_{P,C}$ = 4.4Hz, 4-H); δ_C (100 MHz; $CDCl_3$) 14.6 (Ar-OCH_2CH_3), 16.3 (d, $J_{P,C}$= 6.1Hz, 2

x OCH_2CH_3), 26.6 (d, $J_{P,C}$ = 139Hz, CH_2P), 62.4 (d, $J_{P,C}$ = 6.5Hz, 2 x CH_2OP), 64.9 (Ar-OCH_2CH_3), 114.5, 119.0, 119.9, 120.3, 124.3, 142.0, 143.0 and 146.3 (Ar-C) and 160.8 (C=O); *m/z* 340 (M+1, 100%).



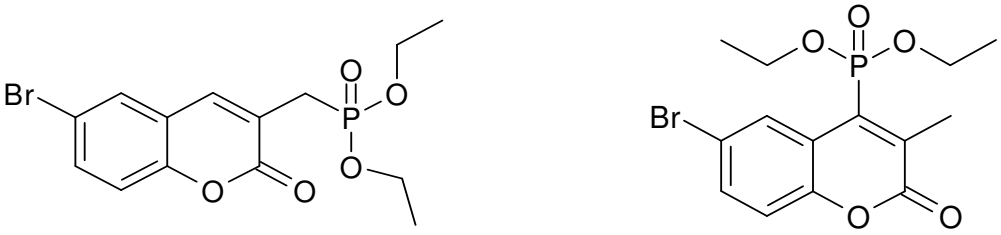


*Diethyl [(6-chloro-2-oxo-2*H*-chromen-3-yl)methyl]phosphonate* **49c** *and diethyl (6-chloro-3-methyl-2-oxo-2*H*-chromen-4-yl)phosphonate* **48c**

The procedure described in *experimental procedure 2* for the synthesis of compounds **48a** and **49a** was followed using 6-chloro-3-(chloromethyl)coumarin **42c** (0.81g, 3.5mmol) and triethyl phosphite (1.2ml). Work-up and chromatography [on silica gel; elution with ethyl acetate-hexane (1:3)] afforded two fractions.

Fraction 1: *diethyl (6-chloro-3-methyl-2-oxo-2*H*-chromen-4-yl)phosphonate* **48c** as a pale yellow solid (0.12g, 10%).

Fraction 2: *diethyl [(6-chloro-2-oxo-2*H*-chromen-3-yl)methyl]phosphonate* **49c** as a yellow solid (0.61g, 53%); (Found M^+: 330.042081. $C_{14}H_{16}ClO_5P$ requires *M*: 330.012390); m.p. 72-74^oC; ν_{max} (nujol)/cm^{-1} 1260 (P=O) and 1725 (C=O); δ_H (400 MHz; $CDCl_3$) 1.29 (6H, t, *J* = 7.2Hz, 2 x OCH_2CH_3), 3.15 (2H, d, $J_{P,H}$= 22Hz, CH_2P), 4.10 (4H, m, $J_{P,H}$ = 6.8Hz, 2 x CH_2OP), 7.27 (1H, s, Ar-H), 7.44 and 7.47 (2H, 2 x s, Ar-H) and 7.75 (1H, d, $J_{P,C}$ = 4Hz, 4-H); δ_C (100 MHz; $CDCl_3$) 16.3 (d, $J_{P,C}$ = 6.1Hz, 2 x CH_2CH_3), 26.8 (d, $J_{P,C}$ = 139Hz, CH_2P), 62.5 (d, $J_{P,C}$ = 6.5Hz, 2 x CH_2OP), 117.9, 120.1, 121.6, 126.8, 129.7, 131.2, 140.4, 151.5 (Ar-C) and 161.3 (C=O); *m/z* 330 (M+1, 80%) and 109 (100%).



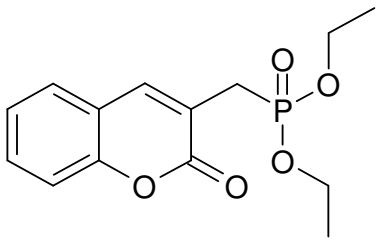
*Diethyl [(6-bromo-2-oxo-2*H*-chromen-3-yl)methyl]phosphonate* **49d** and *diethyl (6-bromo-3-methyl-2-oxo-2*H*-chromen-4-yl)phosphonate* **48d**

The procedure described in *experimental procedure 2* for the synthesis of **48a** and **49a** was followed using 6-bromo-3-(chloromethyl)coumarin **42d** (1.05g, 4.0mmol) and triethyl phosphite (1.3ml). Work-up and chromatography [on silica gel; elution with ethyl acetate-hexane (1:3)] afforded two fractions.

Fraction1: *diethyl (6-bromo-3-methyl-2-oxo-2*H*-chromen-4-yl)phosphonate* **48d** as a yellow solid (0.12g, 8%).

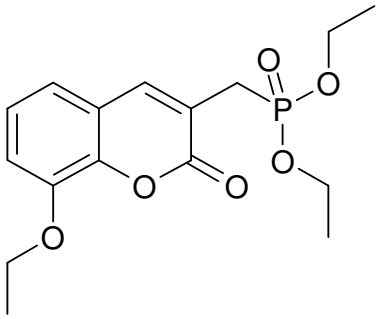
Fraction 2: *diethyl [(6-bromo-2-oxo-2*H*-chromen-3-yl)methyl]phosphonate* **49d** as a pale brown oil (0.761g, 53%); (Found M^+: 373.014356. $C_{14}H_{16}BrO_5P$ requires *M*: 373.010978); ν_{max} (nujol)/cm^{-1} 1260 (P=O) and 1725 (C=O); δ_H (400 MHz; $CDCl_3$) 1.28 (6H, t, *J* = 7.2Hz, 2 x CH_2CH_3), 3.15 (2H, d, $J_{P,H}$ = 22Hz, CH_2P), 4.10 (4H, q, *J* = 6.8Hz, 2x CH_2OP), 7.27-7.45 (3H, series of multiplets, Ar-H) and 7.74 (1H, d, $J_{P,C}$ = 6.1Hz, 4-H); δ_C (100 MHz; $CDCl_3$) 16.9 (d, $J_{P,C}$ = 6.1Hz, 2 x OCH_2CH_3), 27.5 (d, $J_{P,C}$ = 139Hz, CH_2P), 62.4 (d, $J_{P,C}$ = 6.1Hz, 2 x CH_2OP), 118.5, 120.8, 122.3, 127.5, 130.4, 131.9, 141.1, and 152.2 (Ar-C) and 161.2 (C=O); *m/z* 376 (M+1, 70%) and 109 (100%).

Experimental procedure 3



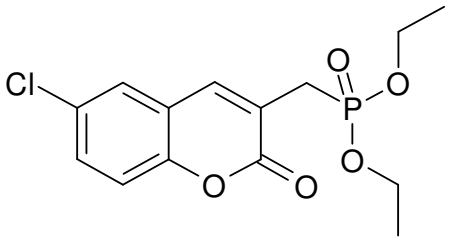
Diethyl [(2-oxo-2H-chromen-3-yl)methyl]phosphonate **49a**

To 3-(iodomethyl)coumarin **41a** (0.35g, 1.2mmol) was added triethyl phosphite (0.40ml) and the mixture was refluxed under nitrogen for 4 hours. After completion of the reaction, as monitored by TLC, the mixture was separated by flash column chromatography [on silica gel; elution with ethyl acetate-hexane (3:1)] to afford *diethyl [(2-oxo-2*H*-chromen-3-yl)methyl]phosphonate* **49a** as a brown oil (0.22g, 61%).



*Diethyl [(8-ethoxy-2-oxo-2*H*-chromen-3-yl)methyl]phosphonate* **49b**

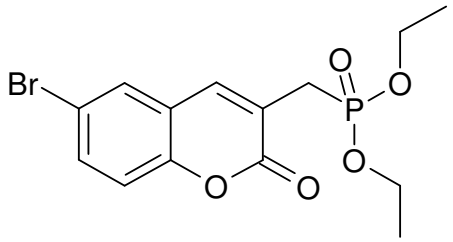
The procedure described in *experimental procedure 3* for the synthesis of compound **41a** was followed using 8-ethoxy-3-(iodomethyl)coumarin **41b** (0.42g, 1.2mmol) and triethyl phosphite (0.40ml). Work-up and chromatography [on silica gel; elution with ethyl acetate-hexane (1:3)] afforded *diethyl [(8-ethoxy-2-oxo-2*H*-chromen-3-yl)methyl]phosphonate* **49b** as a yellow solid (0.172g, 40%).



*Diethyl [(6-chloro-2-oxo-2*H*-chromen-3-yl)methyl]phosphonate* **49c**

The procedure described in *experimental procedure 3* for the synthesis of compound **41a** was followed using 6-chloro-3-(iodomethyl)coumarin **41c** (0.421g, 1.3mmol) and triethyl phosphite (0.44ml). Work-up and chromatography [on silica gel; elution with ethyl

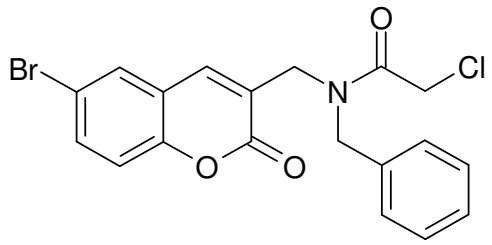
acetate-hexane (1:3)] afforded as a yellow solid *diethyl [(6-chloro-2-oxo-2*H-*chromen-3-yl)methyl]phosphonate* **49c** as a yellow solid (0.234g, 54%).



*Diethyl [(6-bromo-2-oxo-2*H-*chromen-3-yl)methyl]phosphonate* **49d**

The procedure described in *experimental procedure 3* for the synthesis of compound **49a** was followed using 6-bromo-3-(iodomethyl)coumarin **41d** (0.423g, 1.2mmol) and triethyl phosphite (0.40ml). Work-up and chromatography [on silica gel; elution with ethyl acetate-hexane (1:3)] afforded *diethyl [(6-bromo-2-oxo-2*H-*chromen-3-yl)methyl]phosphonate* **49d** as a brown oil (0.172g, 40%).

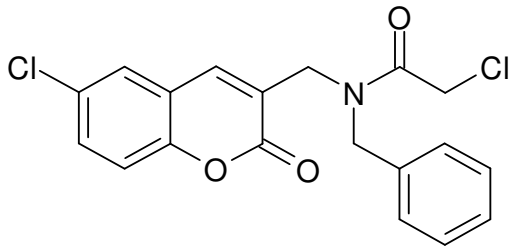
3.2.8. Acetylation of 3-(benzylaminomethyl)coumarin derivatives



N-*Benzyl-2-bromo*-N-*[(6-chloro-2-oxo-2*H-*chromen-3-yl)methyl]acetamide* **55d†**

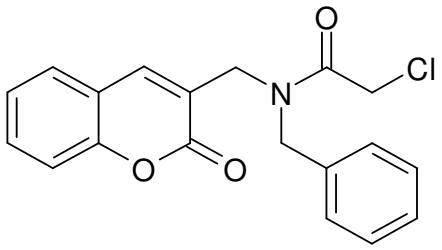
To 3-[(benzylamino)methyl]-6-bromocoumarin (0.49g, 1.4mmol) and chloroacetyl chloride (0.32ml, 2mmol) in THF (6ml) were added.[60] The mixture was refluxed at 100-110^{o}C under nitrogen for 45 minutes, and the resulting solution was allowed to cool to room temperature. Crystallization from ethanol afforded N-*benzyl-2-bromo*-N-*[(6-chloro-2-oxo-2*H-*chromen-3-yl)methyl]acetamide* **55d** as a white solid (0.34g, 56%); (Found M^+: 418.990988. $C_{19}H_{15}BrClNO_3$ requires *M*: 418.992382); m.p. 109-111^{o}C; ν_{max} (nujol)/cm^{-1} O-C=O (1721) and N-C=O (1658); δ_H (400 MHz; DMSO-d_6) 4.27-4.71 (6H,

series of signals, 3 x CH_2), 7.23-7.80 (8H, overlapping multiplets, Ar-H) and 7.99 (1H, s, 4-H); *m/z* 421 (M+1, 18%) and 182 (100%).



N-*Benzyl-2-chloro*-N-*[(6-chloro-2-oxo-2*H-*chromen-3-yl)methyl]acetamide* **55c**†

The procedure described for the synthesis of compound **55d** was followed using 3-[(benzylamino)methyl]-6-chlorocoumarin **53c** (0.18g, 0.6mmol) and chloroacetyl chloride (0.13ml, 1.2mmol) in THF (4ml). Crystallization from ethanol afforded N-*benzyl-2-chloro*-N-*[(6-chloro-2-oxo-2*H-*chromen-3-yl)methyl]acetamide* **55c** as a white solid (0.156g, 70%); (Found M^+: 377.040222. $C_{19}H_{15}Cl_2NO_3$ requires *M*: 377.039949); m.p. 120-122^{o}C; ν_{max} (nujol)/cm^{-1} O-C=O (1721) and N-C=O (1658); δ_H (400 MHz; $CDCl_3$) 4.17-4.45 (6H, series of signals, 3 x CH_2), 7.27-7.48 (8H, overlapping multiplets, Ar-H), 7.74 (1H, s, 4-H); *m/z* 377 (M+1, 13%) and 91 (100%).

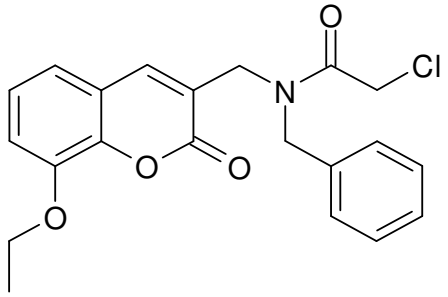


N-*benzyl-2-chloro*-N-*[(2-oxo-2*H-*chromen-3-yl)methyl]acetamide* **55a**†

The procedure described for the synthesis of compound **55d** was followed using 3-[(benzylamino)methyl]coumarin **53a** (0.35g, 1.3mmol) and chloroacetyl chloride (0.30ml, 2.6mmol) in THF (6ml). Crystallization from ethanol afforded N-*benzyl-2-chloro*-N-*[(2-oxo-2*H-*chromen-3-yl)methyl]acetamide* **55a** as a white solid (0.29g, 64%);

† *^{13}C data not cited due to multiplicity of signals arising from rotamers at 30^{o}C.*

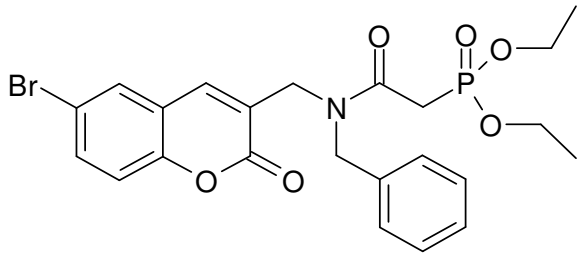
(Found M^+: 341.083861. $C_{19}H_{16}ClNO_3$ requires *M*: 341.081871); m.p. 98-100^oC; ν_{max} (nujol)/cm^{-1} O-C=O (1721) and N-C=O (1658); δ_H (400 MHz; $CDCl_3$) 4.15-4.45 ((6H, series of signals, 3 x CH_2), 7.23-7.57 (9H, overlapping multiplets, Ar-H) and 7.84 (1H, s, 4-H); *m/z* 341 (M+1, 22%) and 182 (100%); *m/z* 385 (M+1, 25%) and 182 (100%).



N-*benzyl-2-chloro*-N-*[(8-ethoxy-2-oxo-2*H-*chromen-3-yl)methyl]acetamide* **55b†**

The procedure described for the synthesis of compound **55d** was followed using 3-[(benzylamino)methyl]-8-ethoxycoumarin **53b** (0.54g, 1.7mmol) and chloroacetyl chloride (0.4ml, 4mmol) in THF (8ml). Chromatography [elution with ethyl acetate-hexane (1:4)] afforded N-*benzyl-2-chloro*-N-*[(8-ethoxy-2-oxo-2*H-*chromen-3-yl)methyl]acetamide* **55b** as a brown oil (0.48g, 72%); (Found M^+: 385.108324. $C_{21}H_{20}ClNO_4$ requires *M*: 385.980888); ν_{max} (nujol)/cm^{-1} O-C=O (1721) and N-C=O (1658); δ_H (400 MHz; DMSO-d_6): 1.38 (3H, t, *J* = 6.6Hz, OCH_2CH_3), 4.17 (2H, q, *J* = 6.8Hz, OCH_2CH_3), 4.17-4.59 (6H, series of signals, 3 x CH_2), 7.26-7.38 (8H, overlapping multiplets, Ar-H) and 7.78 (1H, s, 4-H); *m/z* 385 (M+1, 25%) and 182 (100%).

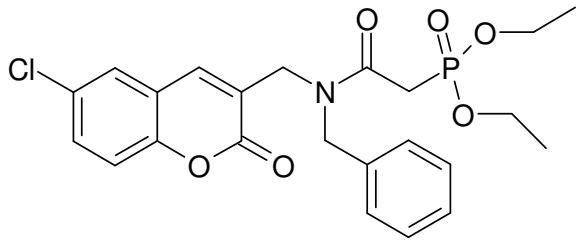
3.2.9. Arbuzov reactions of chloroacetamide derivatives



Diethyl N-*benzyl*-N-*[(6-bromo-2-oxo-2*H-*chromen-3-yl)methyl]carbamoylmethylphosphonate* **56d**‡

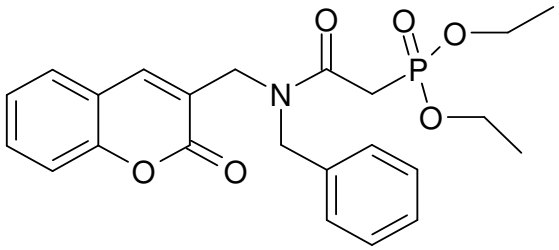
To *N*-benzyl-2-chloro-*N*-[(6-bromo-2-oxo-2*H*-chromen-3-yl)methyl]acetamide **55d** (0.157g, 0.4mmol) was added triethyl phosphite (0.2ml, 0.8mmol) and the mixture was refluxed under nitrogen for 4 hours and then allowed to cool to room temperature. The crude product was isolated by flash chromatography [on silica gel; elution with ethyl acetate-hexane (1:2)] to afford *diethyl* N-*benzyl*-N-*[(6-bromo-2-oxo-2*H-*Chromen-3-yl)methyl]carbamoylmethylphosphonate* **56d** as a pale yellow solid (0.168g, 86%); (Found M^+: 521.058999. $C_{23}H_{25}BrNO_6P$ requires *M*: 521.060287); m.p. 137-139^oC; ν_{max} (nujol)/cm^{-1} O-C=O (1721), N-C=O (1658) and 1244 (P=O); (400 MHz; $CDCl_3$) 1.32 (6H, overlapping triplets, *J* = 7Hz, 2 x OCH_2CH_3), 3.10 (2H, d, $J_{P,H}$ = 22Hz, CH_2P), 4.17 (2H, overlapping multiplets, 2 x CH_2OP), 4.50 and 4.83 (4H, 2 x s, 2 x CH_2N), 7.19-7.60 (7H, m, Ar-H), 7.67 (1H, s, Ar-H), and 7.93 (1H, s, 4-H); δ_C (100 MHz; $CDCl_3$) 16.3 (d, $J_{P,C}$ = 6.2Hz, 2 x OCH_2CH_3), 33.6 (d, $J_{P,C}$ = 140Hz, CH_2P), 45.9 and 52.8 (2 x CH_2N), 62.9 (d, $J_{P,C}$ = 6.4Hz, 2 x CH_2OP), 117.0, 118.0, 120.9, 124.2, 126.2, 127.9, 128.7, 129.1, 130.2, 133.9, 135.7, 138.0 and 151.9 (Ar-C), 160.5 and 166.2 (C=O); *m/z* 523 (M+1, 16%) and 106 (100%).

‡ *^{13}C data cited for major rotamer.*



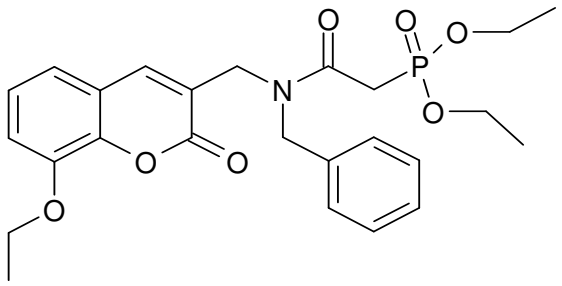
Diethyl N-*benzyl*-N-*[(6-chloro-2-oxo-2*H-*chromen-3-yl)methyl]carbamoylmethylphosphonate* **56d**

The procedure described for the synthesis of compound **56d** was followed using *N*-benzyl-2-chloro-*N*-[(6-chloro-2-oxo-2*H*-chromen-3-yl)methyl]acetamide **55c** (0.46g, 1.2mmol) and triethyl phosphite (0.40ml, 2mmol). Chromatography and afforded *diethyl* N-*benzyl*-N-*[(6-chloro-2-oxo-2*H-*chromen-3-yl)methyl]carbamoylmethylphosphonate* **56d** as a yellow solid (0.455g, 77%) m.p. 165-167^{o}C; (Found M^{+}: 477.111852. $C_{23}H_{25}ClNO_6P$ requires *M*: 477.110804); ν_{max} (nujol)/cm^{-1} O-C=O (1721), N-C=O (1658) and 1244 (P=O); (400 MHz; $CDCl_3$) 1.35 (6H, overlapping triplets, 2 x OCH_2CH_3), 3.14 (2H, d, $J_{P,H}$= 22Hz, CH_2P), 4.09 (4H, overlapping multiplets, 2 x CH_2OP), 4.55 and 4.87 (4H, 2 x s, 2 x CH_2N), 7.27-7.49 (7H, m, Ar-H), 7.56 (1H, s, 10-H) and 7.93 (1H, s, 4-H); δ_C (100 MHz; $CDCl_3$) 16.3 (d, $J_{P,C}$ = 6.2Hz, 2x OCH_2CH_3), 33.6 (d, $J_{P,C}$ = 130Hz, CH_2P), 45.9 and 52.8 (2 x CH_2N), 62.9 (d, $J_{P,C}$ = 6.5Hz, 2 x CH_2OP) 117.8, 120.4, 124.9, 126.2, 127.2, 127.9, 128.7, 129.1, 129.7, 131.1, 135.8, 138.1 and 151.4 (Ar-C), 160.6 and 166.2 (C=O); *m/z* 477 (M+1, 10%) and 106 (100%) .



Diethyl N-*benzyl*-N-*[(2-oxo-2*H-*chromen-3-yl)methyl]carbamoylmethylphosphonate* **56a**

The procedure described for the synthesis of compound **56d** was followed using *N*-benzyl-2-chloro-*N*-[(2-oxo-2*H*-chromen-3-yl)methyl]acetamide **55a** (0.16g, 0.5mmol) and triethyl phosphite (0.2ml, 0.9mmol). Chromatography and afforded *diethyl* N-*benzyl*-N-*[(2-oxo-2*H*-chromen-3-yl)methyl]carbamoylmethylphosphonate* **56a** as a yellow solid (0.134g, 60%); (Found M^+: 443.152637. $C_{23}H_{26}NO_6P$ requires *M*: 443.149776); m.p. 138-140^oC; ν_{max} (nujol)/cm^{-1}: O-C=O (1721), N-C=O (1658) and 1244 (P=O); (400 MHz; $CDCl_3$) 1.31 (6H, overlapping triplets, 2 x OCH_2CH_3), 3.10 (2H, d, $J_{P,H}$ = 22Hz, CH_2P), 4.09 (2H, overlapping signals, 2 x CH_2OP), 4.51 and 4.85 (4H, 2 x s, 2 x CH_2N), 7.22-7.67 (9H, overlapping multiplets, Ar-H) and 7.98 (1H, s, 4-H); δ_C (100 MHz; $CDCl_3$) 16.3 (d, $J_{P,C}$ = 6.3Hz, 2 x OCH_2CH_3), 33.6 (d, $J_{P,C}$ = 130Hz, CH_2P), 45.8 and 52.7 (2 x CH_2N), 62.9 (d, $J_{P,C}$ = 6.6Hz, 2 x CH_2OP), 116.3, 119.3, 123.5 124.4, 126.2, 128.0, 128.7, 129.1, 131.2, 135.9, 139.6 and 153.1 (Ar-C), 161.3 and 166.1 (C=O); *m/z* 443 (M+1, 10%) and 106 (100%).



Diethyl N-*benzyl*-N-*[(8-ethoxy-2-oxo-2*H*-chromen-3-yl)methyl]carbamoylmethylphosphonate* **56d**

The procedure described for the synthesis of compound **56d** was followed using *N*-benzyl-2-chloro-*N*-[(8-ethoxy-2-oxo-2*H*-chromen-3-yl)methyl]acetamide **55b** (0.30g, 0.8mmol) and triethyl phosphite (0.26ml, 2mmol). Chromatography and afforded *diethyl* N-*benzyl*-N-*[(8-ethoxy-2-oxo-2*H*-chromen-3-yl)methyl]carbamoylmethylphosphonate* **56d** as a brown oil (0.19g, 77%); (Found M^+: 487.174816. $C_{25}H_{30}NO_7P$ requires *M*, 487.175991); ν_{max} (nujol)/cm^{-1} O-C=O (1721), N-C=O (1658) and 1244 (P=O); (400 MHz; $CDCl_3$) 1.28 (6H, overlapping triplets, 2 x OCH_2CH_3), 1.46 (3H, t, *J* = 7Hz, Ar-

OCH_2CH_3), 3.1 (2H, d, $J_{P,H}$ = 22Hz, CH_2P), 4.17 (6H, overlapping multiplets, 2 x CH_2OP and Ar-OCH_2CH_3), 4.50 and 4.82 (4H, 2 x s, 2 x CH_2N), 7.10-7.43 (8H, overlapping multiplets, Ar-H), 7.92 (1H, s, 4-H); δ_C (100 MHz; $CDCl_3$) 14.7 (Ar-OCH_2CH_3), 16.3 (d, $J_{P,C}$ = 4.2Hz, 2 x OCH_2CH_3), 27.4 (d, $J_{P,C}$ = 298Hz, CH_2P), 45.8 and 52.7 (2 x CH_2N), 62.8 (d, $J_{P,C}$ = 4.3Hz, 2 x CH_2OP), 65.0 (Ar-OCH_2CH_3), 119.5, 120.1, 124.2, 124.6, 126.3; 127.9, 128.0, 128.7, 129.1, 136.0, 139.8 and 146.3 (Ar-C), 160.9 and 166.1 (C=O); *m/z* 487 (M+1, 24%) and 106 (100%).

3.2.10. Computer Docking

The Accelrys Cerius ^{2} software package was run on an SGI O^2 platform. The Universal forcefield was used as a default setting. The structures were drawn using the 3D sketcher on the visualizer, cleaned, energy-minimised and saved in MSI format. The HIV-1 protease enzyme containing ritonavir was downloaded from the protein data bank.[86] Ritonavir was removed using the protein tools module and structure based options were used to dock the ligand into the active site of the enzyme. The docking, van der Waals' and Ligandfit scores were recorded.

4. REFERENCES

1. Adriana, S.; Russell, B.; Catarina, S.; Rui, S.; Manuela, D.; Alice, R.; Margarida, L.; Francisco, A.; Rui, M. M. V.; Ana, E. S. *Clinical Immunology*, **2007,** *125*, 67–75.
2. Casseb, J. *Elsevier Science*, **2002**, *58*(6), 519-520.
3. http://data.unaids.org/pub/EpiReport/2006/2006_EpiUpdate_en.pdf.
4. Reproduced by permission. http://www.avert.org/virus.htm.
5. Greene, W.C. *Scientific American*, **1997**, *269*, 98-105.
6. Alterman, M. *PhD Thesis, Uppsala University,* **2001**.
7. Robin A.W. *IUBMB Life*, **2002**, *53(4)*, 201-205.
8. Pierre, L. B.; Proudfoot, J. *Boehringer Ingelheim Pharmaceuticals Inc*.; pp1-6.
9. Brik, A.; Wong, C. *Org. Biomol.Chem.*, **2003**, *1*, 5-14.
10. Turner, B.G.; Summers, M.F.; *J. Mol. Biol.,* **1996**, *272*, 872-877.
11. Ratner, L.; *Prospect Drug Discovery Des*. **1993**, *1*, 2-22.
12. Cal Cohen, M.D. *The Internet's Most Comprehensive HIV/AIDS Resource*, **2000,** 1-16.
13. Provasi, D.; Vasile, F.; Ottolina, G.; Longhi, R.; Tiana, G. *Proteins: Structure, Function, and Bioinformatics*, **2006**, *62(4)*, 928-933.
14. De Clercg, E. *Rev. med. Virol*. **2000**, *10,* 255-277.
15. Stefania, P.; Fausto, B.; Giulia, C.; Reynel, C.; Amalia, B.; Giovanni, M.; Giuseppe, G. *Elsevier Science*, **2007**, 76, 99–103.
16. Roberts, N.A.; Martin, J. A.; Kinchington, D.; Broadhurst, A. V.; Ducan, I. B. Galpin, S. A.; Handa, B. K.; Kay, J.; Kröhn, A.; Lambert, R. W.; Merrett, J. H.; Parkes, K. E. B.; Redshaw, S.; Ritchie, A. J.; Talyer, D. L.; Thomas, G. J.; Machin, P. J. *Science,* **1990**, *248*, 358-364.
17. Winstona, A.; Backb, D.; Fletchera, C.; Robinson, L.; Unsworth, J.; Tolowinska, I.; Schutzc, M.; Pozniaka, A. L.; Gazzarda, B.; Boffitoa, M. *AIDS,* **2006**, *20*, 1401–1406.
18. Zhan, K.; Wu, E.; Patcik, A.K.; Kerr, B.; Zobras, M.; Lankford, A. kobayashi, T.; Maeda, Y.; Shetty, B.; Webber, S. *J. Am. Soc. Microbiology*., **2001**, 45, 1086–1093.
19. Kaldor, W. S.; Kalish, J. V.; Davies, J. F.; Shetty, B. V.; Fritz, E. J.; Appelt, K.;

Burgess, J. A.; Campanale, M. K.; Chirgadze, N. Y.; Calwson, D. K.; Dessman, B. A.; Hatch, D. S.; Khalil, D. A.; Kosa, B. M.; Lubbehusen, P. P.; Muesing, M. A.; Patick, A. K.; Reich, H. S.; Su, S. K.; Talock, H. J. *J. Med. Chem*., **1997**, *40*, 3979-3985.

20. Vacca, J. P.; Dorsey, B. D.; Schleif, W. A.; Levin, R. B.; Mcdaniel, S. L.; Zugay, J.; Quintero, J. C.; Blahy, O. M.; Roth, E.; Sardana, V. V.; Schlabach, A. G.; Garham, P. I.; Condra, J. H.; Gotlib, L.; Holloway, M. K.; Lin, J.; Chen, I. W.; Vastag, K.; Ostovic, D.; Anderson, P. S.; Emini E. A.; Huff, J. R. *Proc. Natl. Acad. Sci. USA*, **1994**, *91*, 4096- 4100.
21. Kempf, D. J.; Marsh, K. C.; Denisssens, J. F.; Mcdonald, E.; Vasavanonda, S.; Flentge, C. A.; Green, B. E.; Fino, L.; Parks, C. K.; Kongs, X. P.; Wideburg, N. E.; Salsivar, A.; Ruiz, L.; Kati, W. M.; Sham, H. L.; Robins, T.; Srewardt, K. D.; Hsu, A.; Paltrner, J. J.; Leonard, J. M.; Norbeck, D. W. *Proc. Natl. Acad. Sci*., USA, **1995**, *92*, 2484-2488.
22. Stoner, E. J.; Cooper, A. J.; Dickman, D. A.; Loaczkowski, L.; Lallaman, j. E.; Liu, J.; Oliver-Shaffer, P. A.; Patel, K. M.; Paterson, J. B.; Plata, D. J. Rivey, D. A.; Sham, H. L.; Stengel. P. J; Tien, J. *J. Org. Proc. Res. Dev*. **2000**, *4*, 264-269.
23. Ly, T.; Ruiz, M. *Clin. Infect. Dis.,* **2007***, 44*, 6.
24. Levy, Y.; Caflisch, A.; Onuchic, N.J.; Wolynes, P. G. *J. Mol. Biol.* **2004**, 340, 67–79.
25. http://delphi.phys.univ-tours.fr/Prolysis/Images/hvp.jpeg.
26. Hornak, V.; Okur, A.; Rizzo, R. C.; Simmerling, C.; *PNAS. Org*., **2006**, *103*(4), 915-2920.
27. Ding, L. *Biochem188.standfor. Edu*. **1999**.
28. Ekegren, J. K.; Gising, J.; Wallberg, H.; Larhed, M.; Samuelsson, B.; Hallberg, A. *Org. Biomol. Chem*., **2006**, *4*, 3040–3043.
29. Ettmayer, P.; Billich, A.; Hetcht, P.; Rosenwirth, B.; Gstach, H. *J. Med. Chem*., **1996**, *39*, 3291-3299.
30. Kirkiacharian, S.; Thuy, T. D.; Sicsic, S.; Bakhchinian, R.; Kurkjian, R.; Tonnaire, T. *Farmaco*. **2002**, *57*, 703-708.

31. Gleye,C; Lewin, G.; Laurens, A.; Jullian, J Loiseau, P.; Bories, C.; Hocquemiller, R. *J. Nat. Prod.,* **2003**, *66,* 690-692

32. http://en.wikipedia.org/wiki/Coumarin.

33. Ojala, T. *PhD Thesis*, *University of Helsink*., **2001**.

34. Ramesh, B.; Viswanathan, P.; Pagalendi, V. S.; *J. Pharm*., **2007**, *566*, 231-239.

35. http://www.life.uiuc.edu/berenbaum/newpage1.htm.

36. Magiatis, P.; Melliou, E.; Skatsounis, A.; Mitaku, S.; Renard, P.; Pierre´, A.; Atassi, A. *J. Nat. Prod.,* **1998**, *61,* 982-986.

37. http://en.wikipedia.org/wiki/Warfarin.

38. Kontogiorgis, C.; Hadjipavlou-Litina, D. *Journal of Enzyme Inhibition and Medicinal Chemistry*, **2003**, *18(1),* 63–69.

39. Mouri, T.; Yano, T.; Kochi, S.; Ando, T.; Hori, M. *J. Pestic. Sci.,* **2005**, *30*, 209–213.

40. Mutlu, D. A.; Dilek, D. E. *Turk. J. Chem.* **2003**, *27*, 757 -764.

41. Singh, I. P.; Bharate, S. B.; Bhutani, K. K. *Current Science*, **2005***, 89*, 2.

42. Kostova, I.; Raleva, S.; Genova, P.; Argirova, R. *Bioinorganic Chemistry and Applications*, **2006**, *68274*, 1-9.

43. Thaisrivongs, S.; Tomich, P. K.; Watenpaugh, K. D.; Chong, K. Howe, W.; Yang, C.; Strohbach, J.; Tureer, S.; McGrath, J.; Bohanon, M.; Lynn, J.; Mulichak, A.; Spinelli, P.; Hinshaw, R.; Pagano, P.; Moon, J.; Ruwart, M.; Wilkinson, K.; Rush, B.; Zipp, G.; Dalga, R.; Schwende, F.; Howard, G.; Padbury, G.; Toth, L.; Zhao, Z.; Koeplinger, K.; Kakuk, T.; Cole, S.; Zaya, R.; Piper, R.; Jeffrey**,** P. *J. Med. Chem*., **1994**, *37*, 3200-3204.

44. Bell, R. G.; Caldwell, P. T. *Biochemistry*, **1973**, *12*, 1759-1762.

45. Lima, O. A.; Polonskwi, J.; *Phytochemstry*, **1973**, *12*, 913-916

46. Dean, F. M. *Naturally Occurring Oxygen Ring Compounds*, **1963**, Butterworths, London, p. 337

47. Joule, J. A.; Mills, K.; Smith, G. F. *Heterocyclic chemistry,* **2000**, 4th edition, Blackwell Science, Oxford, UK, p. 60.8

48. Stahman, M. A.; Huebner, C. F.; Link, K. P. *J Biol. Chem*., **1941**, *138,* 513.

49. Conlin, G.; Gear, J. *J. Nat. Prod*., **1993**, *56 (8*), 1402-140.

50. Davies, E. G.; Ashton, W. M. *Journal of Science of food and Agriculture,* **2006**, *11(15)*, 733-738.
51. Gottlieb, O. R.; Herrmann, K.; Murray, R. D. H.; Ohloff, G.; Pattenden, G. *Progress in the Chemistry of Organic Natural Products, Springer-Verlag Wein,* **1978**, Pp. 200-427.
52. Felter, S.P.; Vassallo, J.D.; Carlton, B.D.; Daston, G.P. *Food and Chemical Toxicology,* **2006**, 44, 462–475.
53. Maheswar. M.; Vidavalur; S.; Guri, L.; Vasantha, D.; Yerra K. R.; Rao, C.V. *J. Mol. Cat*., **2006**, *255*, 49-52.
54. Thimons, M.; Chua, C.A.; Achalabun, M. *J. Chem. Ed.;* **1998**, *75*, 12.
55. Vilar, S.; Quezada, E.; Santana, L.; Uriarte, E.; Fraiz, Y.N.; Alcaide, C; Cano, E.; Orallo, F. *Bioorganic and Medicinal Chemistry Letters,* **2006**, 16, 257-261.
56. Musa, M. A. *PhD Thesis, Rhodes University*., 2003.
57. Heravi, M. M.; Hekmatshoar, R.; Emamgholizadeh, M. *Phosphorus, Sulfur Silicon Relat. Elem*., **2004**, 179, 1893-1896.
58. Bogdal, D.; *J. Chem. Res.,* **1998**, 468-46.
59. Shockravi, A.; Valizadeh, H.; Heravi, M. M., *Phosphorus, Sulfur Silicon. Relat. Elem*., **2003**, 178, 501-504.
60. Kaye, P.T.; Musa, M.A. *Synthesis,* **2002**, 2701-2706.
61. Kaye, P.T. *South African Journal of Science,* **2004**, 100, 545- 547.
62. Familoni, O.B.; Kaye, P.T.; Klaas, P.J. *J. Chem. Soc.*, 1**998**, 2563.
63. Bode M.L. Kaye, P.T. *J. Chem. Soc*., *Perkin Trans1*, **1990**, 2612.
64. Kaye, P.T.; Nocanda, X. W. Synthesis., **2001**, 2389.
65. Bode, M. L.; Kaye, P.T.; Klass, P. J. *J. Chem. Soc*., **1998,** 2563.
66. De Souza, R. O.; Bruno, A. M.; Lŭcia, C. S. A.; Vasconcellos, M. L. A. *Journal of Synthetic Organic Chemistry,* **2004**, *10*, 1595-1600.
67. Kaye, P. T.; Robinson, R. S. *Synth. Commun*. **1996**, *26*, 2085.
68. Kaye, P. T.; Musa, M. A. *Synthesis,* **2003**, 531-534.
69. Freid, J.; Elderfied, R. C. *J. Org. Chem.*, **1941**, *6*, 577.
70. Livingstone, R.; Miller, D.; Morris, S. *J. Chem. Soc*., **1960**, 2157.
71. Prey. V.; Kerres, B.; Berbalk, H. *Monatsh. Chem*., **1960**, *91*, 774.

72. Katriktzky, A.; Rees, C. W. *Comprehensive Heterocyclic Chemistry*, **1984**, Vol. 8, Pp. 689-670.
73. De, A.; Pooupelin, J. P. Saint-Ruf, G. *Bull. Soc. Chim. Fr*., **1974**, 1917.
74. Mashelker, U. C.; Usqaonkar, R. N. *Indian Chem*., **1979**, *18(B)*, 362.
75. Lecocq, J.; Buu-Hoi, N. P.; *Hebd. Sciences. Acad. Sci*., **1947**, *224*, 937.
76. Wafaa M. A.; Ashraf A. S. *Tetrahedron*, **1999**, *55*, 1477-4790.
77. Gandavaram, S. P.; Manubolu, M.; Kachi, R.; Kishore, K.R.; Obulam, V. S. R.; Cirandur, S.R**.** *Arkivoc.*, **2006**, *xvi*, 128-135.
78. Goodman, S.N.; Jacobsen, E.N.; *Adv. Catal*., **2002**, *344*, 953-956.
79. Fall, Y.; Teran, C. Teijeira, M; Satana, L; Uriarte, E. *Synthesis,* **2000**, 643.
80. Ianni, A.; Waldvogel, S. R. *Synthesis.*, **2006**, 2103-2112.
81. Gawande, M.B.; Deshpande, S.S.; Satam, J.R.; Jayaram, R.V. *Catal. Commun*., **2007**, *8*, 576-582.
82. Singh, O. M.; Singh, S.J.; Kim, S. M.; Lee**,** S. G**.** *Bull. Korean Chem. Soc*., **2007,** *28***,** 115-117.
83. Licea, R. Q.; Valladares, J. F. C.; Quintero, A, C.; Padilla, C. R.; Guerra1, R. T.; Flores, R. G.; Waksman, N. *Molecules*., **2002**, *7*, 662-673.
84. Kim, Y. J.; Streitwieser, A.; Chow, A.; Fraenkel, G. *Org. Lett*., **1999**, *13*, 2069-2071.
85. Kemnitz, C. R. Loewen, M. J. *J. Am. Chem. Soc*., **2007,** *129(9)*, 2521-2528.
86. Kempf, D. J.; Marsh, K. C.; Denissen, J. F.; McDonald, E.; Vasavanonda, S.; Flentge, C. A.; Green, B. E.; Fino, L.; Park, C. H.; Kong, X. P. *Proc. Nat. Acad. Sci*. USA, **1995**, *92*, 2484.
87. http://www.accerlrys.com/doc/life/cerius46/ligandfit.
88. Perrin, D. D.; Amarego, W. L. F. *Purification of Laboratory Chemicals*, Pergamon Press, Oxford, 3rd EDN., **1988**.

Studies towards the Synthesis of Novel, Coumarin-based HIV-1 Protease Inhibitors

Thesis

Submitted in fulfilment of the

requirements for the degree

of

MASTER OF SCIENCE

of Rhodes University

by

THOMPHO JASON RASHAMUSE
B.Sc (University of Venda)
B.Sc Hons (University of Limpopo)

January 2008

Department of Chemistry

Rhodes University

Grahamstown