Synthesis of Novel Coumarin Derivatives as Potential Inhibitors of HIV-1 Protease

THESIS

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by

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ABSTRACT

This research has focused on the development of novel coumarin derivatives containing peptide-like side chains as potential HIV-1 protease inhibitors. The reaction of various salicylaldehyde derivatives with *tert*-butyl acrylate in the presence of 1,4-diazabicyclo[2.2.2]octane (DABCO) has afforded a series of Baylis-Hillman adducts in moderate yield. Cyclisation of the adducts in the presence of HCl afforded the corresponding 3-(chloromethyl)coumarin derivatives, which have been reacted with various amine hydrochlorides in the presence of Proton Sponge® to afford a series of novel 3-(aminomethyl)coumarin derivatives, which were fully characterised by NMR and HRMS methods. Various approaches to the introduction of hydroxyl or amino groups at the C-4 position of coumarin and the 3-(chloromethyl)coumarin derivatives have been explored; these have included dihydroxylation of the coumarin double bond, and the synthesis of 4-benzylaminocoumarin derivatives as potential intermediates. The Vilsmeier-Haack and Mannich reactions have also been investigated as possible methods of introducing the desired peptide-like functionality.

Computer modelling of selected structures has indicated that some of the novel 3-(aminomethyl)coumarin derivatives may exhibit activity as inhibitors of HIV-1 protease. The planned enzyme inhibition assays were unfortunately precluded by the aqueous insolubility of the selected compounds.

Three ^{13}C NMR chemical shift algorithms, *viz.*, Modgraph Neural Network, Modgraph HOSE and ChemWindow, have been applied to selected compounds prepared in this study. The Modgraph Neural Network algorithm was found, in all cases, to provide the most accurate correlations with the experimentally-determined chemical shifts.

ACKNOWLEDGMENTS

To the Original Chemist, my creator God, be glory and thanks for His grace and love towards me. Everything that we can ever discover and investigate was put in place by Him and bears testimony to His glory.

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Abbreviations used in this thesis

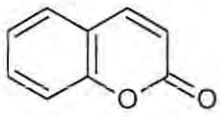
DABCO	1,4-diazabicyclo[2.2.2]octane
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DMAP	4-Dimethylaminopyridine
DMF	*N,N*-Dimethylformamide
HSQC	Heteronuclear Spin Quantum Correlation
NBS	*N*-bromosuccinimide
NMR	Nuclear Magnetic Resonance
THF	Tetrahydrofuran

CONTENTS

1. INTRODUCTION

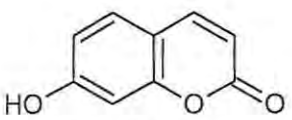
1.1 Coumarins: A brief overview

Coumarin itself, or 2*H*-1-benzopyranone **1**, is a benzannulated, oxygen-containing, heterocyclic compound. It consists of a 2*H*-pyran-2-one ring and a fused benzene ring, and, as a result, has both aromatic and aliphatic properties. In most cases, the heterocyclic ring displays reactivity characteristic of aliphatic compounds.[1]

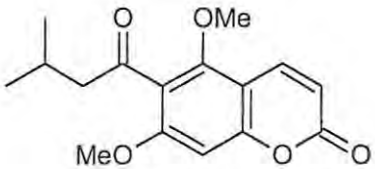


coumarin **1**

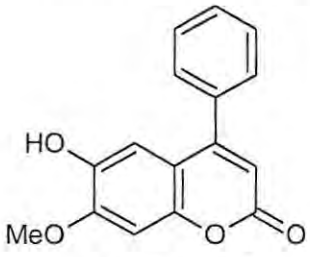
Coumarin is a natural product found in various plants, including sweet clover (*Melilotus sp.*) and the tonka bean (*Dipteryx odorata*).[1] Once used as a food flavourant, concerns about its toxicity and carcinogenicity have led to its banning by the FDA.[2] Many natural products contain the coumarin nucleus. Common examples include umbelliferone **2**, angelicone **3**, dalbergin **4** and mammein **5**.[1] Furocoumarins are also common natural products, and examples include psoralen **6** and aflatoxin G **7**.[1] The 4-hydroxycoumarin derivatives are an important class of compounds, as many have medicinal applications. Novobiocin **8**,[3] an antibiotic which is produced by *Streptomyces spheroides*, contains a 4-hydroxycoumarin moiety. Dicoumarol **9**, another 4-hydroxycoumarin derivative, is a powerful anticoagulant, and is the cause of 'sweet clover disease' in cattle. Warfarin **10**, a synthetic coumarin, is also an anticoagulant, and has found application as a rodenticide.[1]



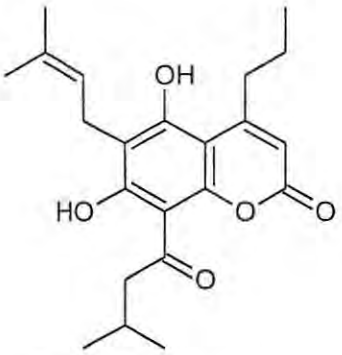
umbelliferone **2**



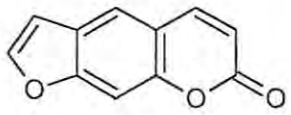
angelicone **3**



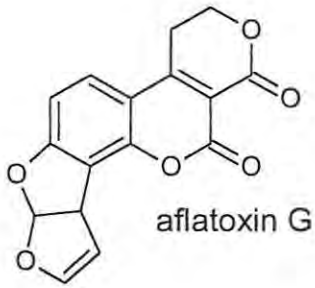
dalbergin **4**



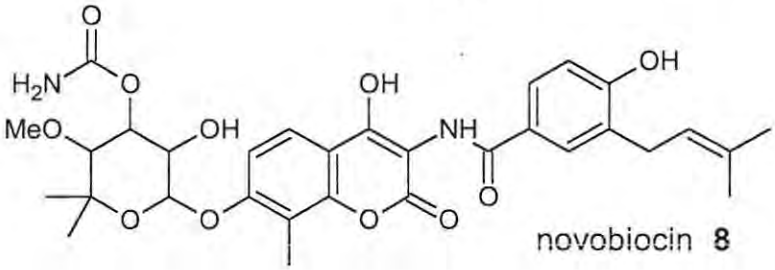
mammein **5**



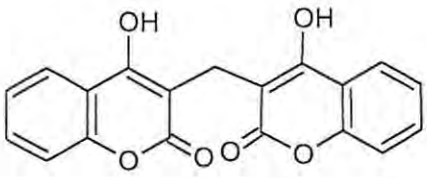
psoralen **6**



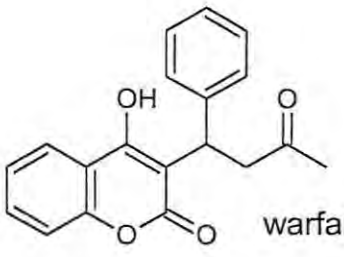
aflatoxin G **7**



novobiocin **8**



dicoumarol **9**



warfarin **10**

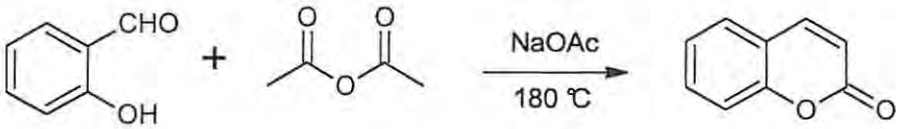
According to Murray,[4] most naturally occurring coumarins have an oxygen substituent at C-7. Umbelliferone **2** is thus regarded as the parent system in both a biosynthetic and in a structural sense. The simpler coumarins are considered to be formed biosynthetically from the corresponding cinnamic acids, produced in turn *via* the shikimic acid pathway. More complex structures such as angelicone **3** and mammein **5**, which contain isoprenoid substituents, are considered to be mevalonate-derived.[4]

1.2 Methods of Coumarin Synthesis

A number of reactions which produce coumarins have been developed. These include the Perkin, Pechmann, Wittig, Knoevenagel, Claisen, Michael and Baylis-Hillman reactions. The use of these reactions to synthesise coumarins is outlined in the following section. This brief review is by no means comprehensive but merely serves to highlight the wide range of available synthetic approaches to the coumarin nucleus.

1.2.1 Perkin Reaction

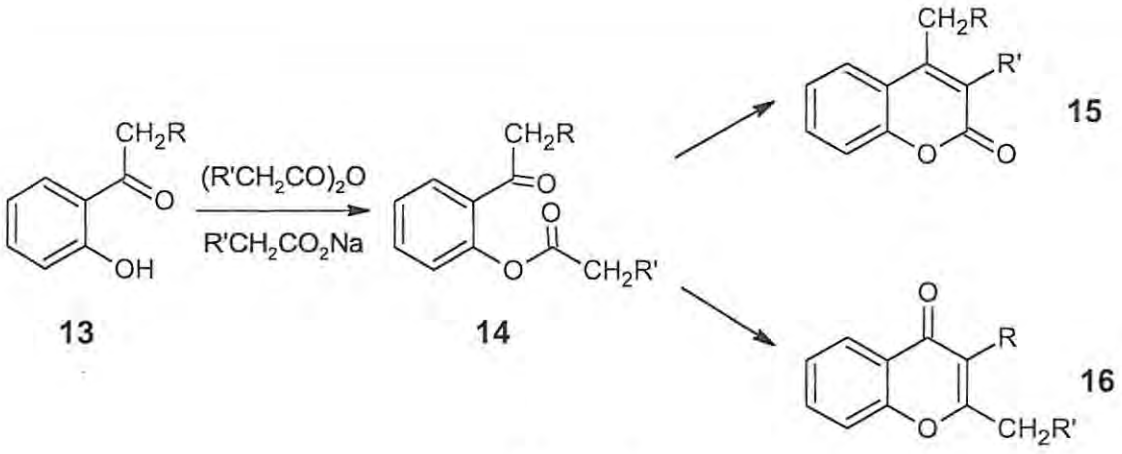
Perkin's original synthesis of coumarins, discovered in the mid-19th century, involves the reaction of salicylaldehyde **11** with acetic anhydride in the presence of sodium acetate (Scheme 1).[1] The mechanism is considered to involve addition of the enolate of acetic anhydride to the aldehyde, forming an aldol-type product, which then undergoes intramolecular acylation, elimination of acetic acid, hydrolysis and, finally, intramolecular esterification to give the coumarin product.[5]



11 **1**

Scheme 1

A variant of the Perkin reaction, the Kostanecki-Robinson reaction, can also be used to make 4-substituted coumarins **15** (Scheme 2). However, this reaction has the disadvantage of forming chromones **16** as well as coumarins,[1] depending on the influence of the substitution patterns of the starting material on which way the intermediate cyclises.

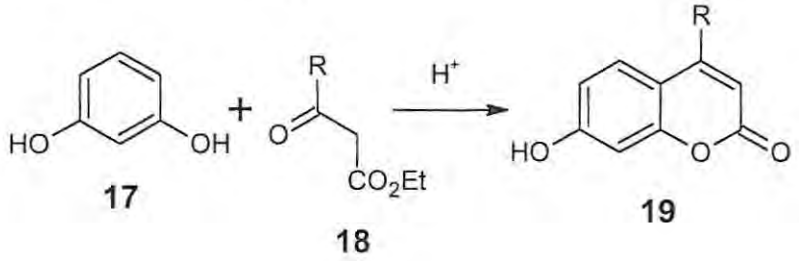


Scheme 2

1.2.2 Pechmann Reaction

Von Pechmann's coumarin synthesis involves condensation of a β-keto ester **18** with resorcinol **17** or other similarly-substituted phenols. This provides access to 4-substituted coumarins **19** (Scheme 3),[1] and is considered to proceed *via* electrophilic attack by the β-keto ester at the *ortho*-position of the resorcinol. Dehydration and intramolecular acylation then gives the coumarin.[7]

Generally, the source of H^+ in the Pechmann reaction is H_2SO_4 or other mineral acids, but trifluoroacetic acid has also been used successfully.[6] The use of cation exchange resins to catalyse the Pechmann reaction was first reported in 1960 by John and Israelstam.[7] These catalysts facilitate purification of the product and are recoverable. Recent research indicates that solid acid catalysts, such as zeolites,[8] and clay catalysts, such as montmorillonite, are also useful catalysts for this reaction.[9]

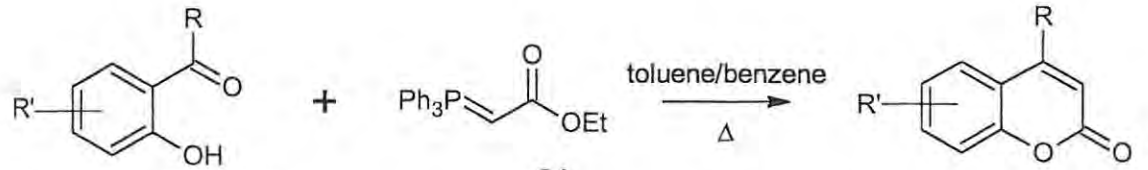


Scheme 3

1.2.3 Wittig Reaction

The Wittig reaction generally involves the treatment of an aldehyde or ketone with a

phosphorus ylide or with a phosphonate (in which case it is known as the *Horner-Wadsworth-Emmons* modification of the Wittig reaction) to yield an alkene.[10] An application of the Wittig reaction utilises various substituted salicylaldehydes **11** or *o*-hydroxyacetophenones **20** to synthesise coumarins **23** in moderate to excellent yields by reaction of these with the ylide, ethoxycarbonylmethylenetriphenylphosphorane **21** (Scheme 4).[11] The same reaction has also recently been carried out with *in situ* generation of the ylide species, in solventless conditions, under microwave irradiation.[12] A variation of the Wittig reaction which utilises an ester, instead of the usual aldehyde or ketone species, involves the reaction of keteneylidene(triphenyl)phosphorane **23** with salicylate esters **24** to afford 4-alkoxycoumarins **25** in good yields (Scheme 5).[13]



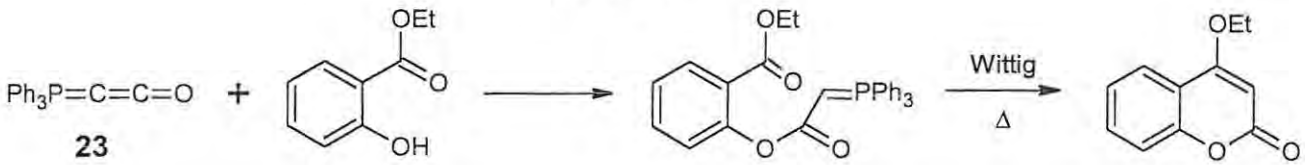
11: R = H, R' = H, Me

20: R = Me , R' = H, Me, OMe

21

22

Scheme 4



23

24

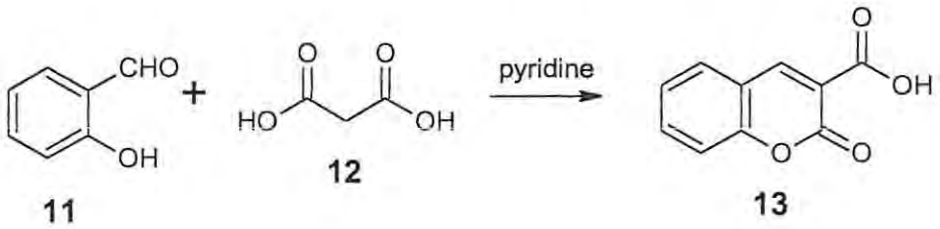
25

Scheme 5

1.2.4 Knoevenagel Reaction

The Knoevenagel reaction, although similar to the Perkin reaction in some respects, proceeds under much milder conditions.[14] It entails a condensation between an aldehyde or ketone and a species with an active methylene group, and is normally activated by an organic base.[15] Knoevenagel originally proposed that the purpose of the base (an amine) was to form an imine with the aldehyde, which was then condensed with malonic acid.[15] The discovery that pyridine was also an effective catalyst for the reaction led to the mechanism proposed by Hann and Lapworth,[16] who suggested that the function of the base was to abstract a proton from the methylene group of the malonic acid, to generate an enolate species, which could then attack the aldehyde. Salicylaldehyde **11** and malonic acid **12** (which contains the active methylene group) thus react in the presence of an

organic base, *e.g.* aniline or pyridine, to form coumarin-3-carboxylic acid **13** (Scheme 6).[17] The yield for the Knoevenagel reaction was improved by Woods and Sapp by using malononitrile instead of malonic acid.[18] Woods and Johnson reported the synthesis of 4-hydroxycoumarin-3-carboxylic acids from salicylic acid and malonic acid in the presence of trifluoroacetic acid,[19] which is a departure from the usual basic conditions of the Knoevenagel reaction, but is assumed to proceed by a similar mechanism to that proposed by Hann and Lapworth.

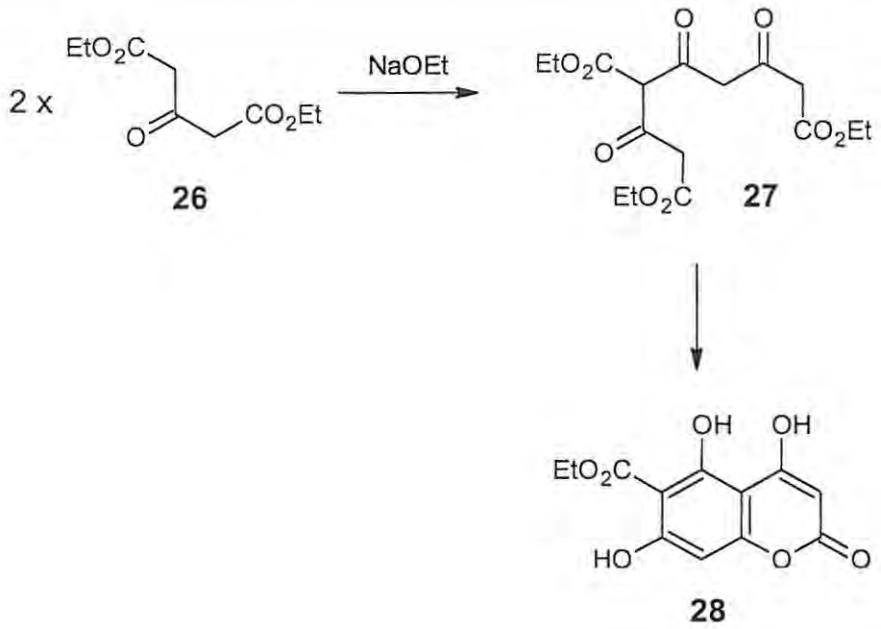


Scheme 6

Recent advances in the chemistry of the Knoevenagel reaction include the synthesis of coumarins under solvent-free conditions,[20] under microwave irradiation,[21] and in the presence of metal catalysts such as Ti(O-*i*-Pr)$_4$.[22]

1.2.5 Claisen Condensation

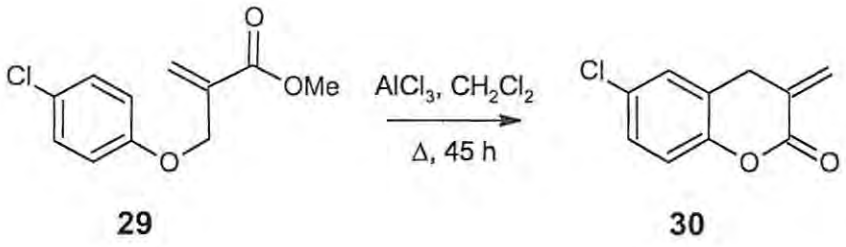
Condensation of diethyl 3-oxopentanedioate **26** in the presence of a strong base affords 6-ethoxycarbonyl-4,5,7-trihydroxycoumarin **28**. This reaction is presumed to occur in two steps; the first is an intermolecular Claisen condensation between two molecules of diethyl 3-oxopentanedioate to yield the intermediate **27**, which then undergoes intramolecular condensation (Dieckmann condensation), followed by tautomerism and intramolecular acyl substitution to yield the coumarin (Scheme 7).[23]



Scheme 7

1.2.6 Claisen Rearrangement

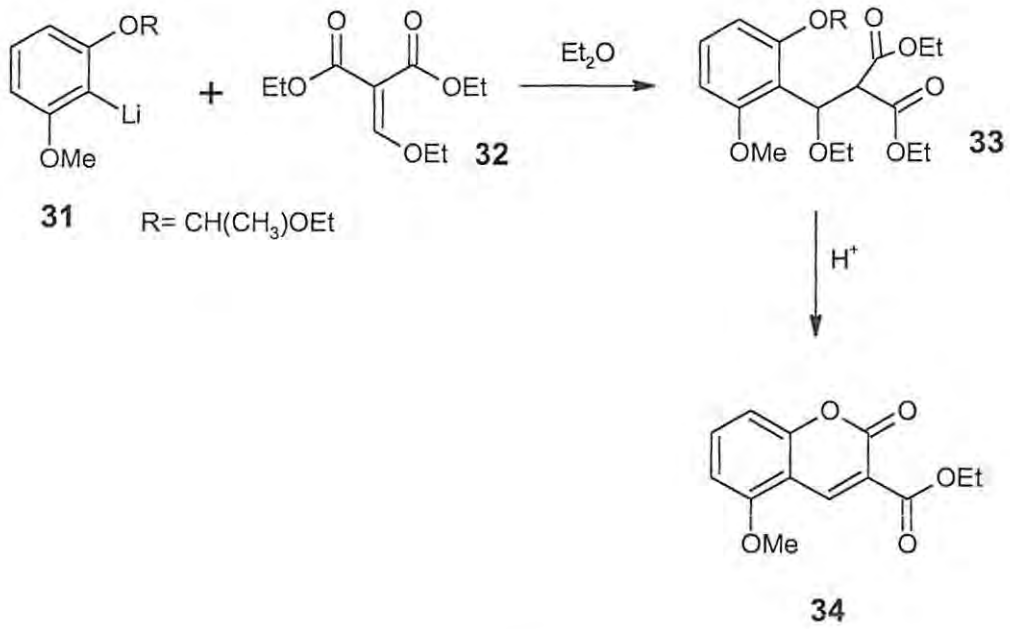
The Claisen rearrangement can also be used to synthesise coumarin derivatives. This rearrangement of allylic aryl ethers occurs on heating, with or without a catalyst, to give rise to *o*-substituted phenols.[10] An example of this reaction is the rearrangement of methyl aryloxymethacrylates **29** in the presence of a Lewis acid such as $AlCl_3$ (Scheme 8).[24] The mechanism is considered to involve a [3,3] sigmatropic shift,[10] which is presumably followed by intramolecular acyl substitution.



Scheme 8

1.2.7 Michael Reaction

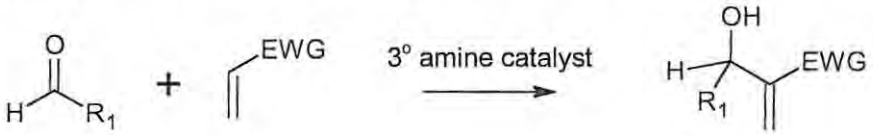
The Michael addition has also been used in the synthesis of coumarin derivatives. For example, o*rtho*-lithiation of protected resorcinol, and subsequent Michael addition of the lithiated species **31** to a suitable α,β-unsaturated ester **32** affords, *via* mild hydrolysis, the coumarin derivative **34** (Scheme 9).[25]



Scheme 9

1.2.8 Baylis-Hillman Reaction

The Baylis-Hillman reaction[26] is a carbon-carbon bond-forming reaction which involves nucleophilic addition of an intermediate zwitterionic species to an aldehyde **35**; the nucleophilic zwitterion is generated by conjugate addition of the catalyst to an activated alkene **36** (Scheme 10). Most often, this catalyst is a tertiary amine such as 1,4-diazabicyclo[2.2.2]octane (DABCO) **38** or quinuclidine **39**.[27]



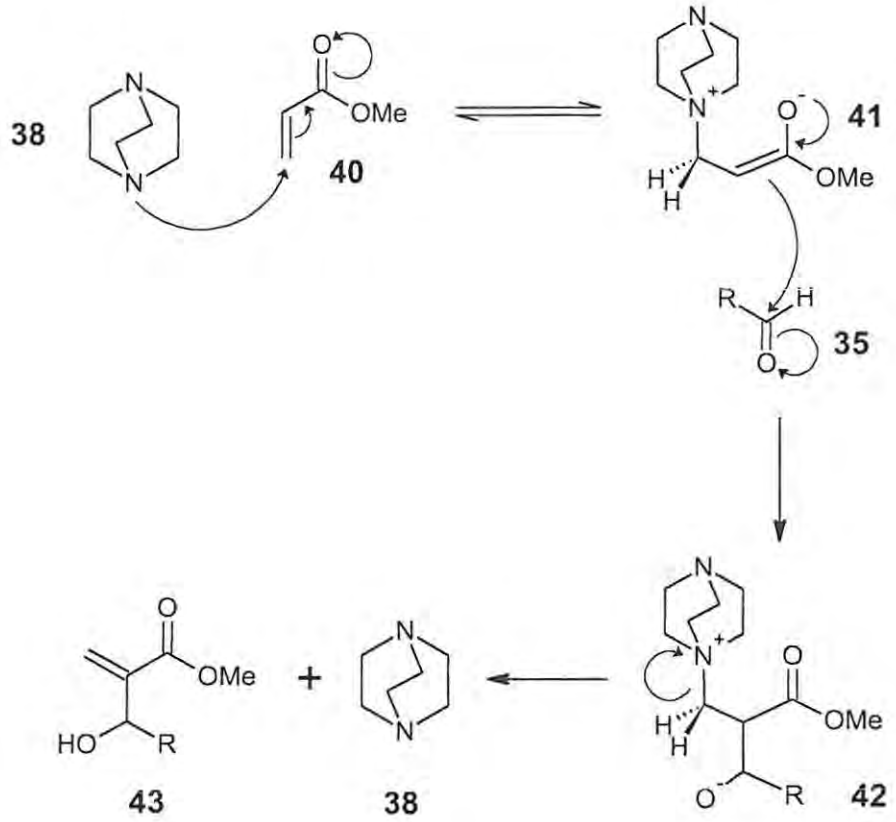
35 **36** **37**

Scheme 10

DABCO **38** quinuclidine **39**

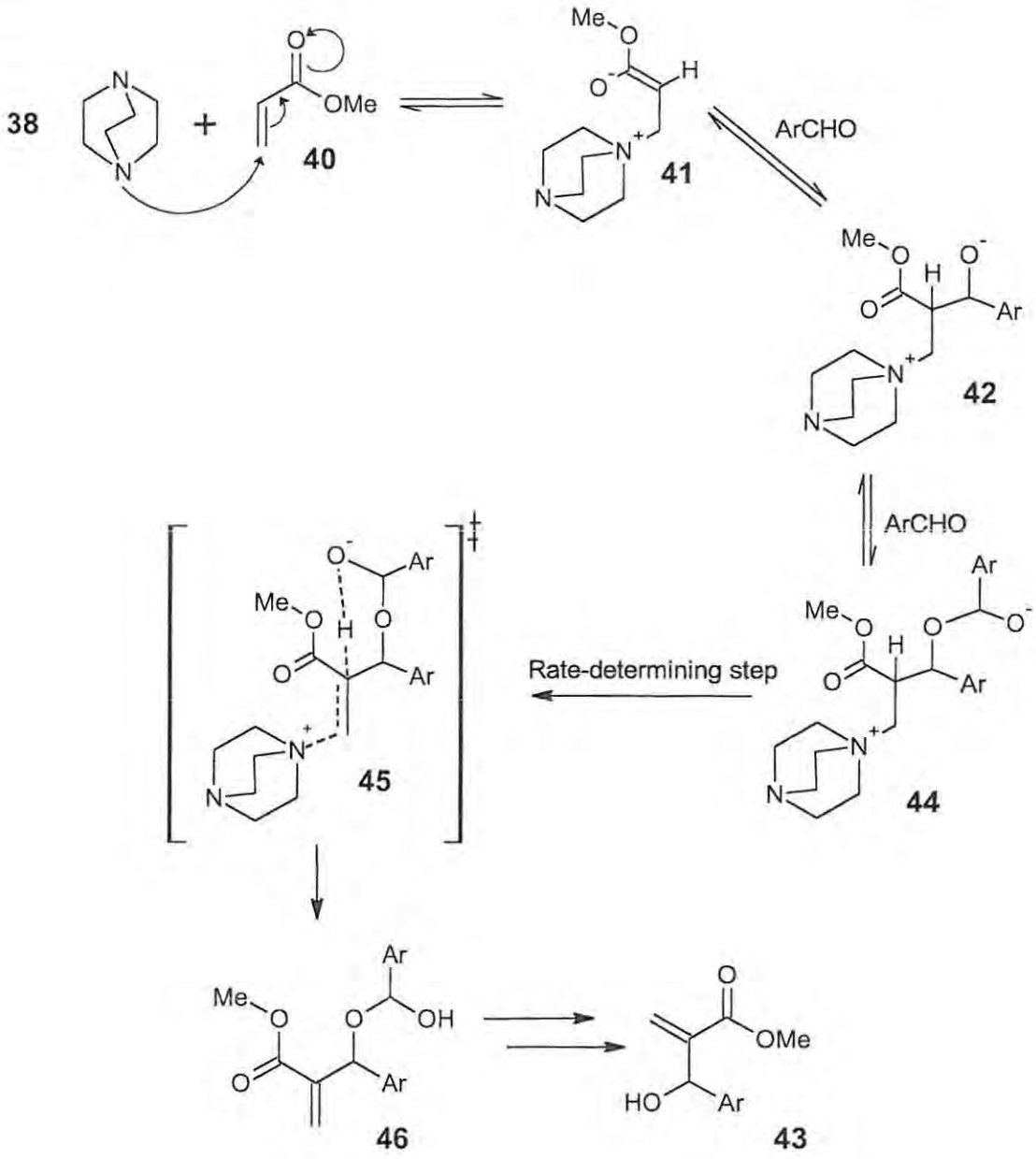
The mechanism of the Baylis-Hillman reaction has been investigated in our group by Bode,[28] using pyridine-4-carboxaldehyde and various alkyl acrylates as starting materials, and a variety of tertiary amine catalysts, including DABCO, quinuclidine and 3-hydroxyquinuclidine. The first step involves the conjugate addition of the catalyst to the

acrylate double bond, forming a zwitterionic enolate species **41**. This species then attacks the aldehyde **35** in the rate-determining step of the reaction. Elimination of the catalyst and concurrent proton transfer affords the Baylis-Hillman adduct **43** (Scheme 11).[28] According to this study, the reaction is first-order in catalyst, aldehyde and alkene, and third-order overall.



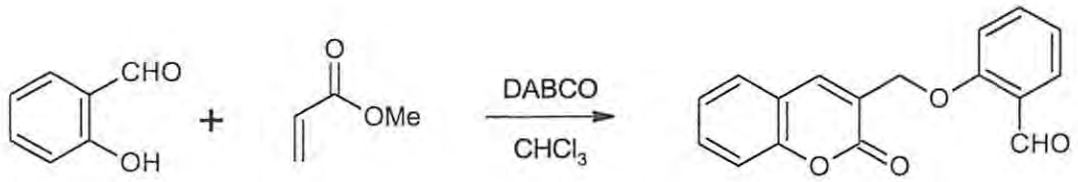
Scheme 11

Recent work, however, reported by McQuade *et al.* has shown that the reaction is in fact second-order in aldehyde and first-order in catalyst and alkene. Their proposed mechanism is outlined below (Scheme 12). The first two steps are identical with those proposed by Bode and Kaye (the addition of the catalyst **38** to the acrylate **40** to form the zwitterion **41**, and the attack at the aldehyde carbon by the zwitterion to give the intermediate **42**. The mechanisms differ in that McQuade's mechanism involves the attack of a second aldehyde molecule by the intermediate **42** to give a second intermediate **44**. This species undergoes intramolecular proton transfer and loss of the catalyst molecule to give the hemiacetal intermediate **46** which then loses the extra aldehyde moiety to afford the conventional Baylis-Hillman adduct **43**.[29,30]



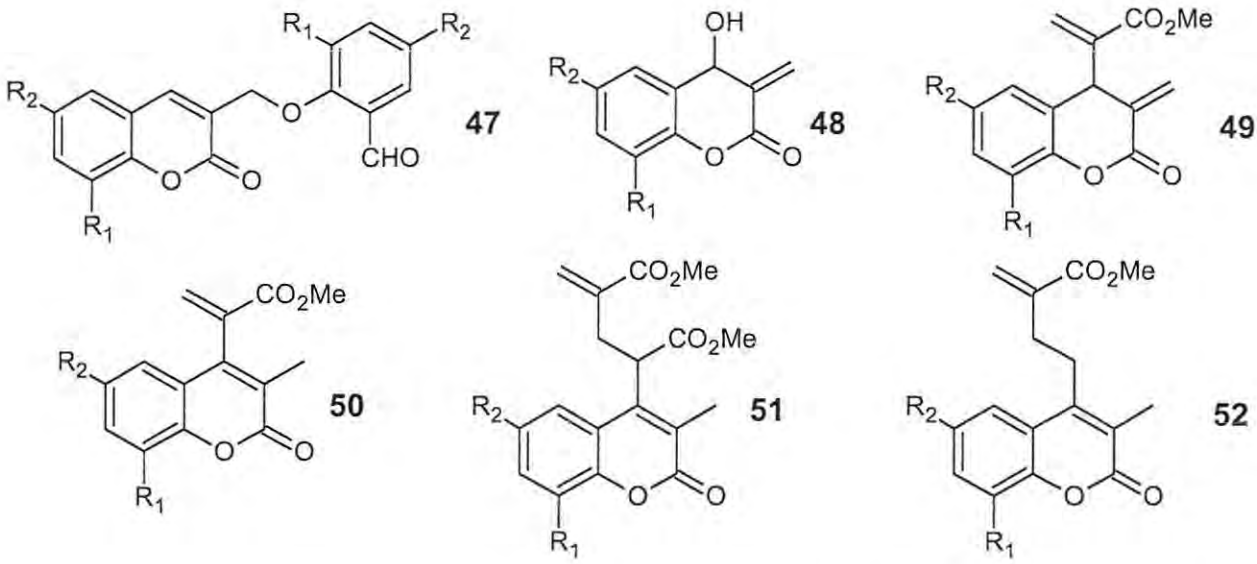
Scheme 12

Previous work in our group has demonstrated the use of the Baylis-Hillman reaction in the synthesis of coumarin derivatives. Bode originally isolated a coumarin derivative **47** from of the DABCO-catalysed Baylis-Hillman reaction between salicylaldehyde **11** and methyl acrylate **40** (Scheme 13).[31] This coumarin was not the expected Baylis-Hillman adduct, and its isolation prompted further investigation into the formation of coumarins *via* the Baylis-Hillman reaction. These studies led to the formation of complex mixtures of chromene and coumarin derivatives, which were separated by careful chromatography to yield eight different classes of systems, including the coumarin derivatives **47-52**.[32]

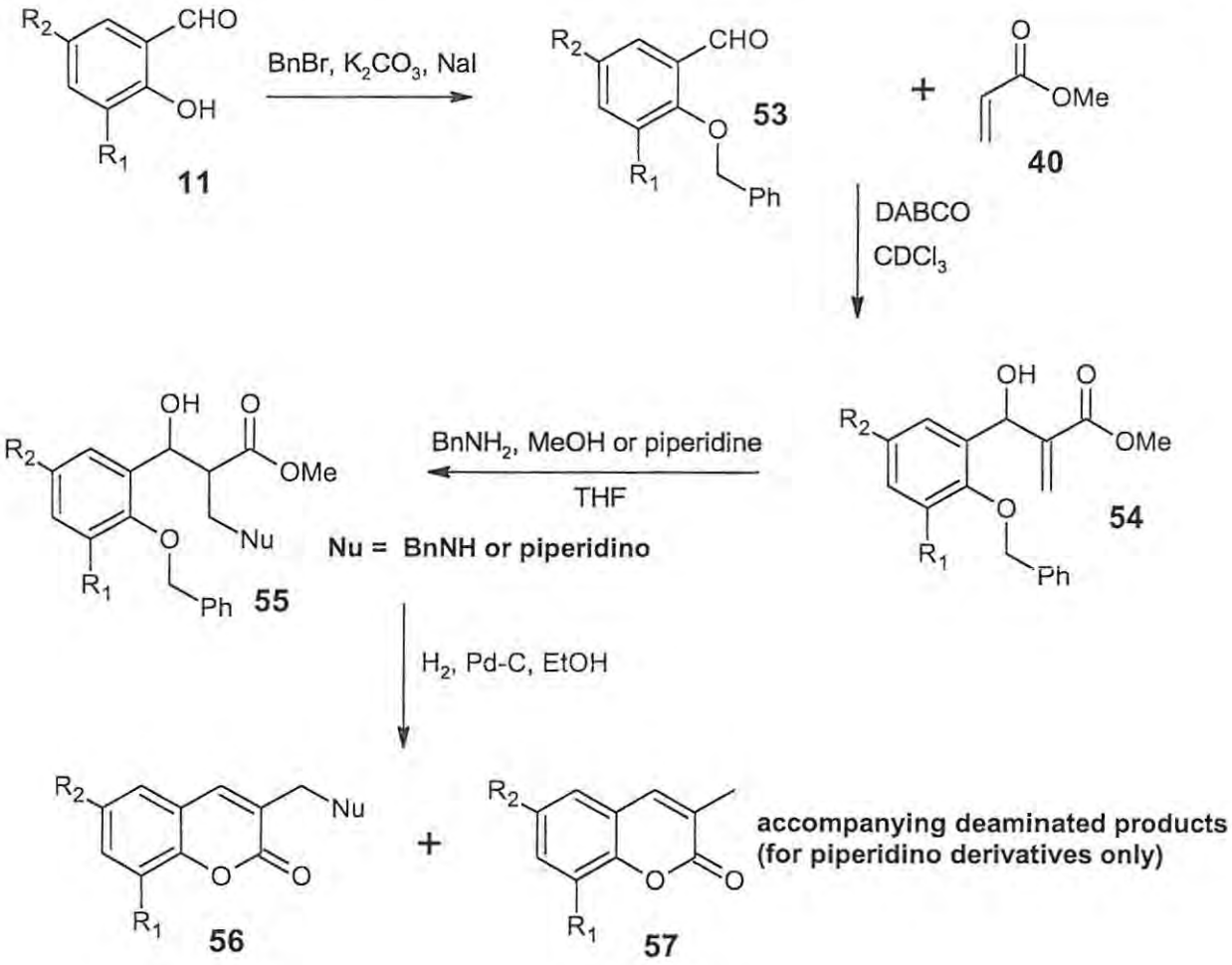


11 **40** **47**

Scheme 13

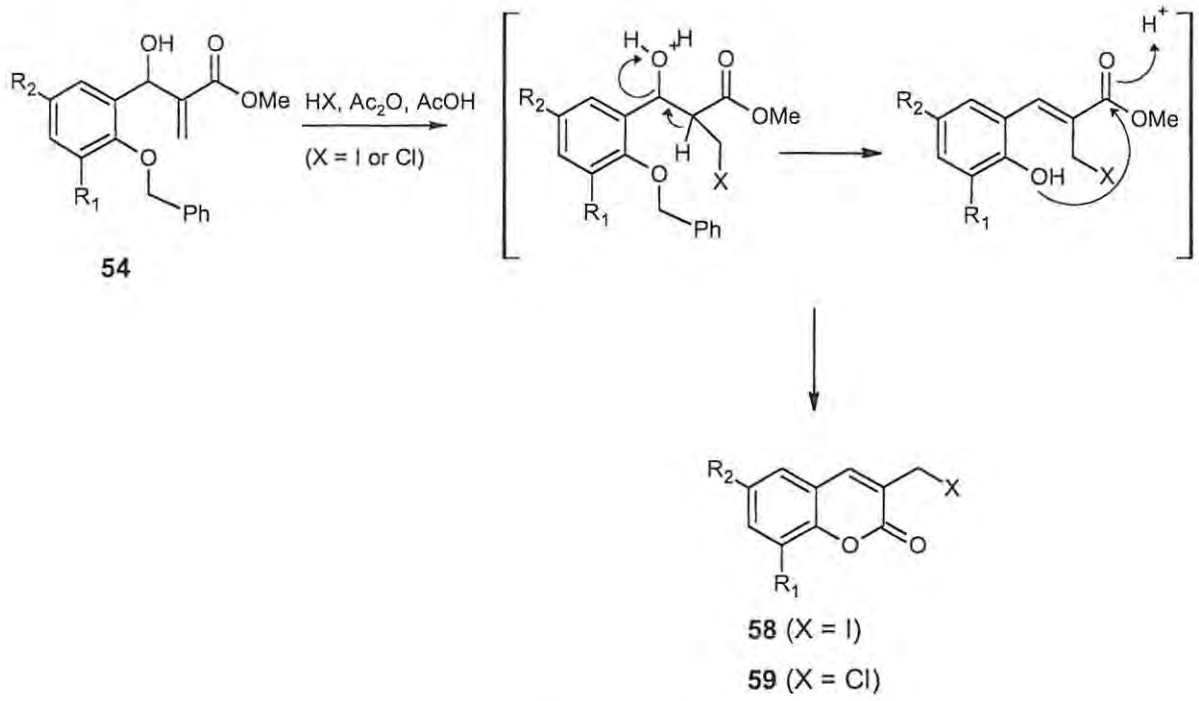


It was found that the regioselectivity of the cyclisation of the Baylis-Hillman intermediate (and thus the selectivity of chromene or coumarin formation) can be controlled. Thus, coumarins can be accessed by protecting the salicylaldehyde phenolic group before the Baylis-Hillman reaction, giving the benzyl ether **53**, and intercepting the electrophilic vinylic centre of the Baylis-Hillman adduct **54** with a suitable nucleophile; subsequent deprotection of the phenolic group by hydrogenolysis (Scheme 14) then permits cyclisation to the coumarin derivatives **56** and **57**.[33]

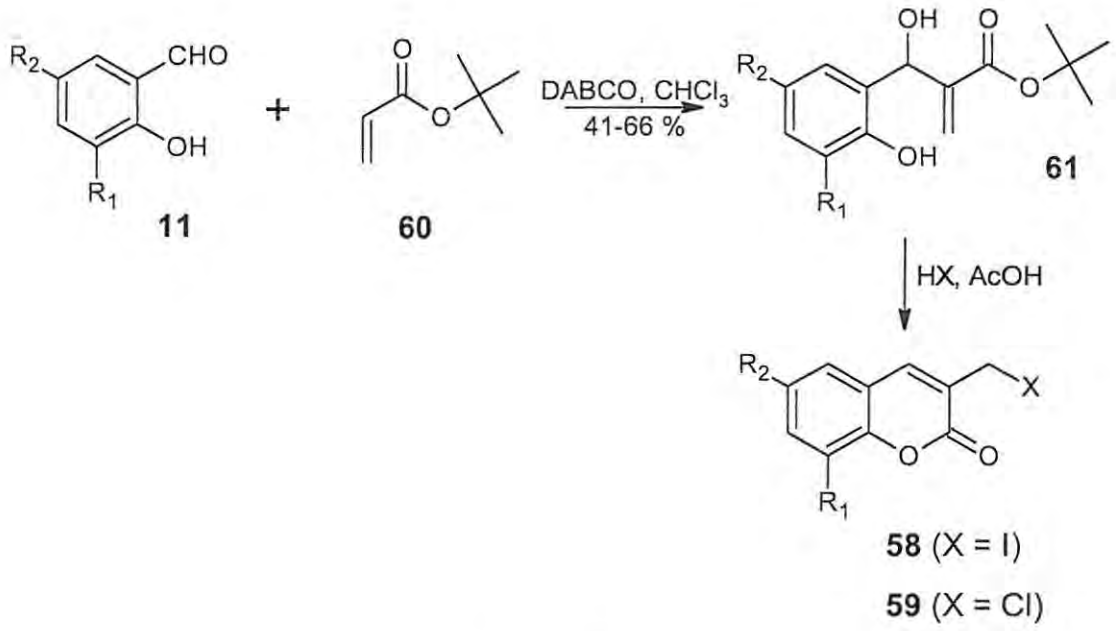


Scheme 14

It was then found that the nucleophilic interception step could be eliminated by treating the protected Baylis-Hillman adduct **54** with hydriodic acid or hydrochloric acid. The acid presumably adds to the unsaturated ester moiety, cleaves the benzyl ether and facilitates dehydration to afford the coumarin derivatives **58** and **59** (Scheme 15). Addition of the halogen acid to the double bond seems to inhibit cyclisation to the chromene.[34] A further improvement was achieved by eliminating the need to protect the salicylaldehyde phenolic moiety. When the Baylis-Hillman reaction was performed with *t*-butyl acrylate **60** and an unprotected salicylaldehyde derivative **11**, the resulting adduct **61** proved to be stable and isolable, and was formed in reasonable yields (41-66 %). Cyclisation of the Baylis-Hillman adduct was achieved by reacting it with HCl or HI in AcOH. If HCl was used, the 3-chloromethylcoumarin derivatives **59** were formed in 86-98 % yield (Scheme 16), whereas when HI was used, the 3-iodomethylcoumarin derivatives **58** were formed in only 17 % yield.[35] Thus, the Baylis-Hillman reaction can be used to form 3-substituted coumarin derivatives in moderate yields without the need to use any protecting groups.

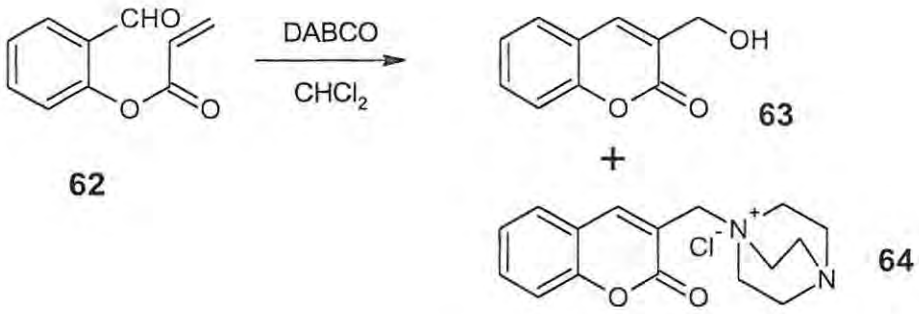


Scheme 15



Scheme 16

The Baylis-Hillman reaction has also been used to synthesise coumarins, using a completely different approach. Drewes *et al.* have reported an intramolecular Baylis-Hillman reaction of the acrylate ester **62**, which affords both 3-hydroxymethylcoumarin **63** and a quaternary ammonium salt **64** (Scheme 17).[36]



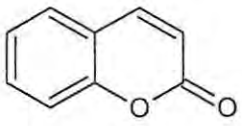
Scheme 17

1.2.9 Other synthetic approaches to coumarins

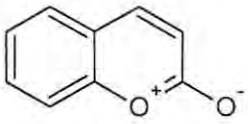
Many other reactions have been used to synthesise coumarin derivatives. Examples include the use of directed *ortho*-metallation (DoM) of protected phenols,[37] the use of Pd(II) catalysts to cyclise aryl propynoates to coumarins,[38,39] tellurium-catalysed cyclisations of salicylaldehyde-derived precursors,[40] and even the use of second-generation Grubbs catalysts to synthesise coumarins by ring-closing metathesis.[41]

1.3 Reactivity of the coumarin nucleus

The coumarin nucleus **1** contains both an aromatic ring and an unsaturated 2-pyranone ring, but complete aromaticity for the whole molecule would only be possible if coumarin existed as a betaine structure **65**. However, IR spectra of coumarin systems exhibit carbonyl frequencies characteristic of lactones.[42] The chemical reactivity of coumarin also indicates that the pyranone ring is aliphatic, and can thus react at the carbon-carbon double bond or at the lactone carbonyl carbon. The double bond is conjugated to the carbonyl group and to the aromatic ring and, while nucleophilic attack generally occurs at the carbonyl carbon, it may also occur at C-4 due the conjugation of the double bond with the carbonyl group. Electrophilic attack will generally occur at C-6 (on the aromatic ring), although, under more forcing conditions, it can also occur at C-3 (on the heterocyclic ring).[42] For example, sulfonation of coumarin occurs first at C-6, and under more forcing conditions at C-3 as well. Nitration follows the same pattern.[43] However, when a 4-hydroxy substituent is present, electrophilic substitution occurs preferentially at C-3. Electrophilic attack by formaldehyde yields the compound dicoumarol [3,3'-methylene-bis-(4-hydroxycoumarin)] **10** *via* substitution at C-3.[1]



1



65

As indicated above, nucleophilic attack occurs at C-4 or C-2. Weak nucleophiles such as bisulfite, cyanide and methoxide add at C-4, while stronger nucleophiles are able to open the lactone.[1] In the presence of an hydroxide ion, the lactone opens to give *cis-o*-hydroxycinnamic acid (coumarinic acid). The *cis*-acid is not stable, and rapidly regenerates the coumarin under acidic[44] or even mildly basic conditions.[1] If the *cis*-acid is left in strongly basic conditions for extended periods of time,[1] or heated in the presence of a strong base,[44] it isomerises to give *trans-o*-hydroxycinnamic acid, which is stable and cannot cyclise back to coumarin.

1.4 AIDS, HIV and Current Treatment Strategies: a brief overview

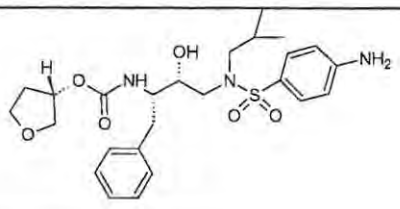
AIDS was first reported by the US Centre for Disease Control in 1981.[45] In 1988, it was reported that a retrovirus called HIV (Human Immunodeficiency Virus) was responsible for the syndrome. Statistics released by UNAIDS in December 2004 estimated a total number of 39.4 million people infected with HIV worldwide, and the total number of AIDS deaths was estimated to be 3.1 million in that year. Approximately 25 million of these cases were in sub-Saharan Africa.

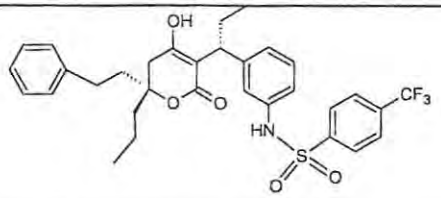
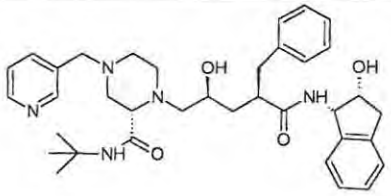
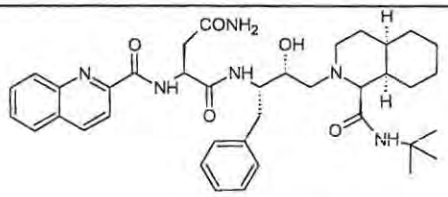
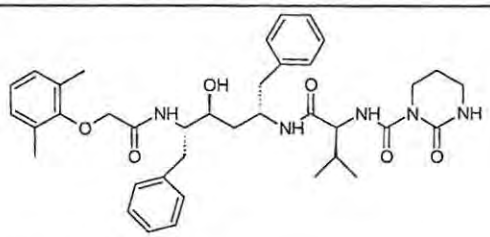
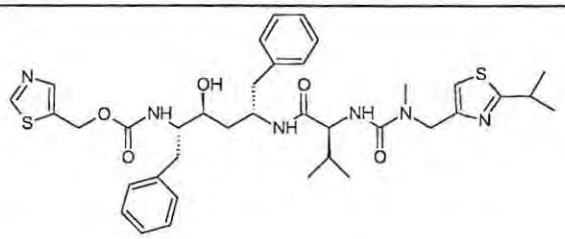
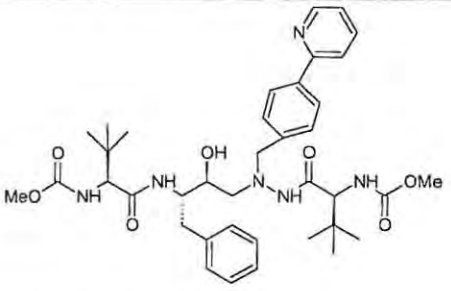
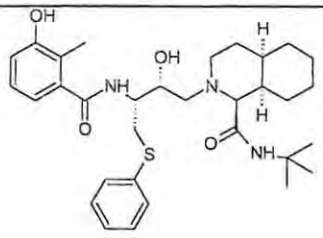
1.4.1 The life-cycle of HIV

Upon entering the cell, viral RNA is reverse-transcribed to DNA by the reverse transcriptase enzyme, and viral DNA enters the $CD4^+$ cell nucleus, where it is integrated into the cell's genetic material by the integrase enzyme. Viral DNA is then transcribed into mRNA, which is translated into viral polyproteins. These polyproteins are cleaved into mature proteins by the protease enzyme (HIV-PR). Viral RNA and the proteins then form new virions, which are released into the bloodstream to infect other $CD4^+$ cells.[45]

Current treatment strategies for HIV focus on the interruption of the life cycle of the virus. Targets for such intervention include the reverse transcriptase (RT) and protease (PR) enzymes. A number of RT inhibitors have been developed and are in clinical use at present; these are divided into two broad classes: the Nucleotide Reverse Transriptase Inhibitors (NRTIs) and the non-Nucleotide Reverse Transcriptase Inhibitors (nNRTIs). Protease inhibitors (PIs) inhibit the action of the protease enzyme, which is responsible for cleaving viral polyproteins into mature proteins.[45] At present there are several commercially available PIs, most of which are peptide-type inhibitors, which mimic the polyproteins normally cleaved by the enzyme. One non-peptide inhibitor is now in clinical use, *viz.* Tipranavir.[46] Table 1 shows the PIs which are currently in use.

Table 1. Commercially available HIV-1 PR inhibitors. [45, 47]

Brand Name	Generic Name	Structure
Agenerase®	amprenavir	

Aptivus®	tipranavir[48]	
Crixivan®	indinavir	
Invirase®	saquinavir	
Kaletra®	lopinavir + ritonavir	
Lexiva®	fosamprenavir	Prodrug of amprenavir
Norvir®	ritonavir	
Reyataz®	atazanavir[49]	
Viracept®	nelfinavir	

Most treatment strategies involve the use of one NRTI, one nNRTI and one PI. Such a combination of HIV drugs is known as Highly Active Antiretroviral Treatment (HAART).

1.4.2 The HIV-1 Protease Enzyme

The protease enzyme of HIV-1 (HIV-1 PR) comprises a pair of 99-amino acid protein chains, and may be classified as an aspartyl protease enzyme.[45] It is a dimer with C_2 symmetry, and contains only one active site (Fig. 1). The mechanism by which the enzyme is considered to cleave proteins is outlined in Scheme 18.[45]

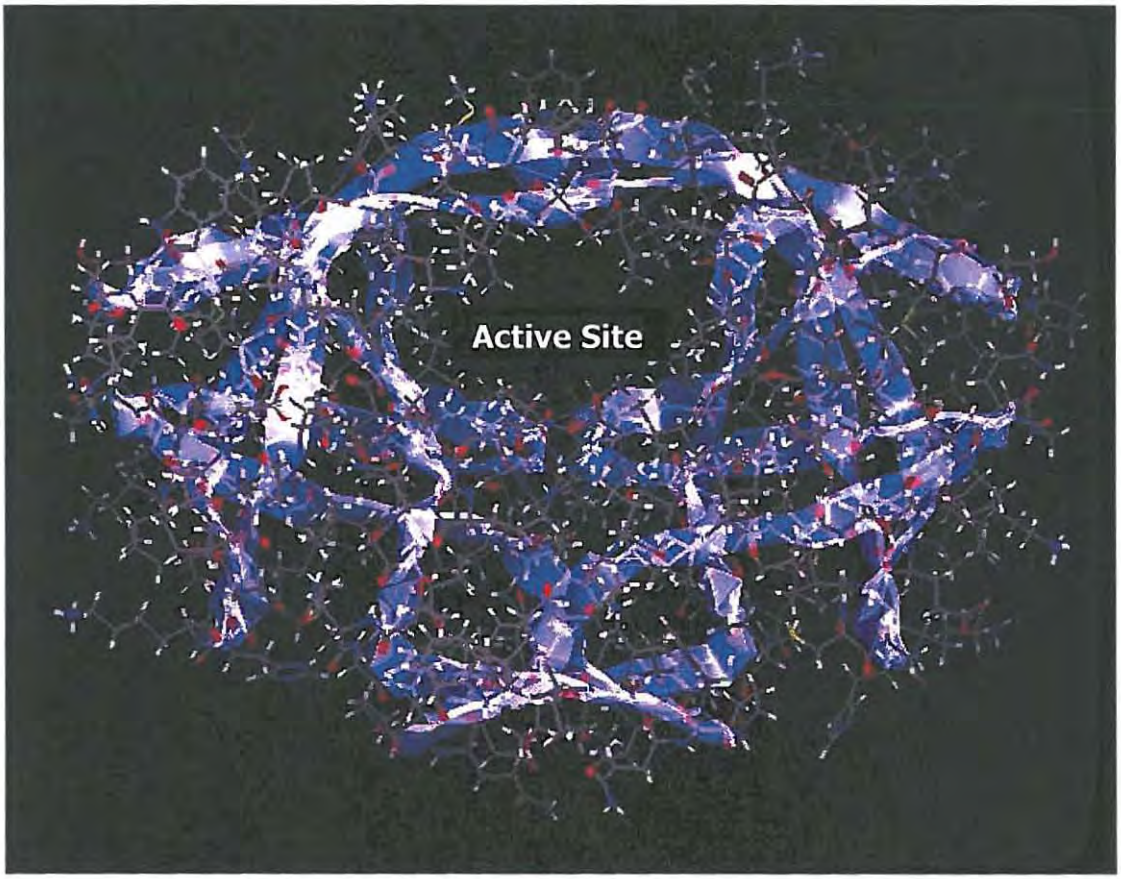
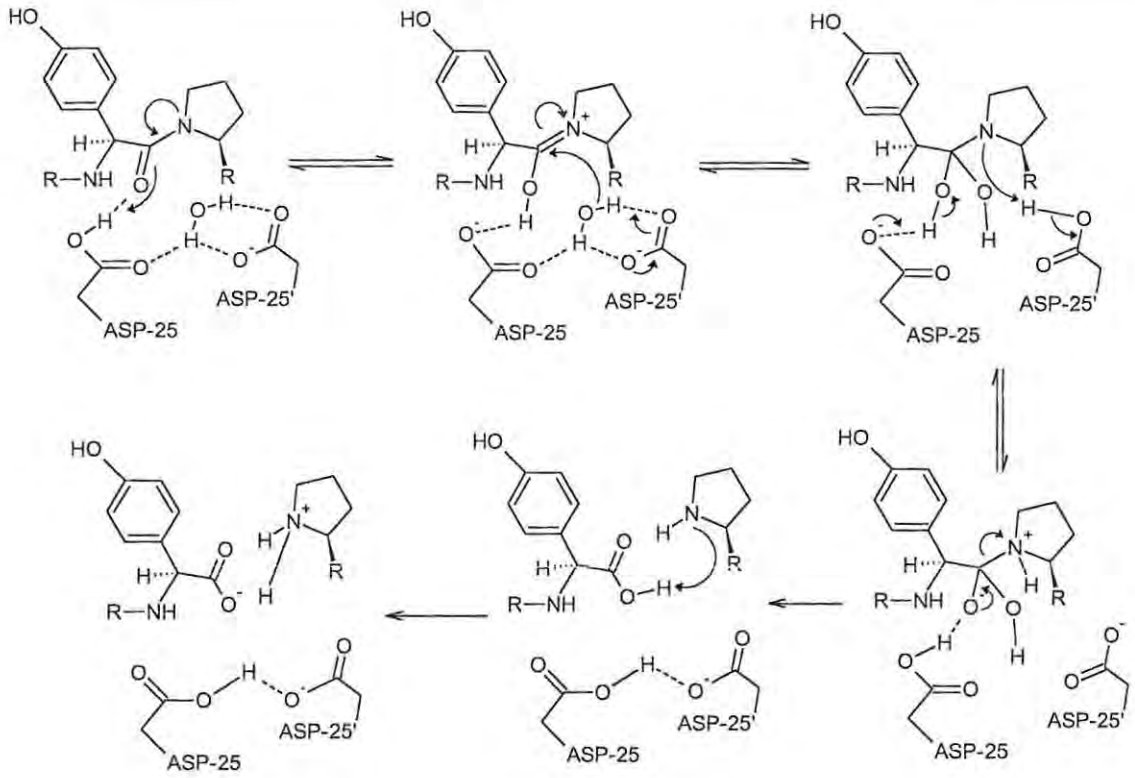


Figure 1. The HIV-1 protease enzyme (NMR structure, determined when the enzyme was complexed with inhibitor DMP323; downloaded from Protein Data Bank[50])



Scheme 18

The active site of the enzyme contains the two catalytic aspartyl residues, Asp-25 and Asp-25', at the base of the cavity. The cavity is covered by glycine-rich flaps, which contain two isoleucine residues, Ile-50 and Ile-50'. These residues provide crucial hydrogen-bonding to a structural water molecule, which is held in the cavity and which hydrogen-bonds to the protein substrate (Fig. 2). A number of subsites have also been identified, and these are identified by the nomenclature, S_1, S_2, *etc.*, and are mostly hydrophobic in nature.

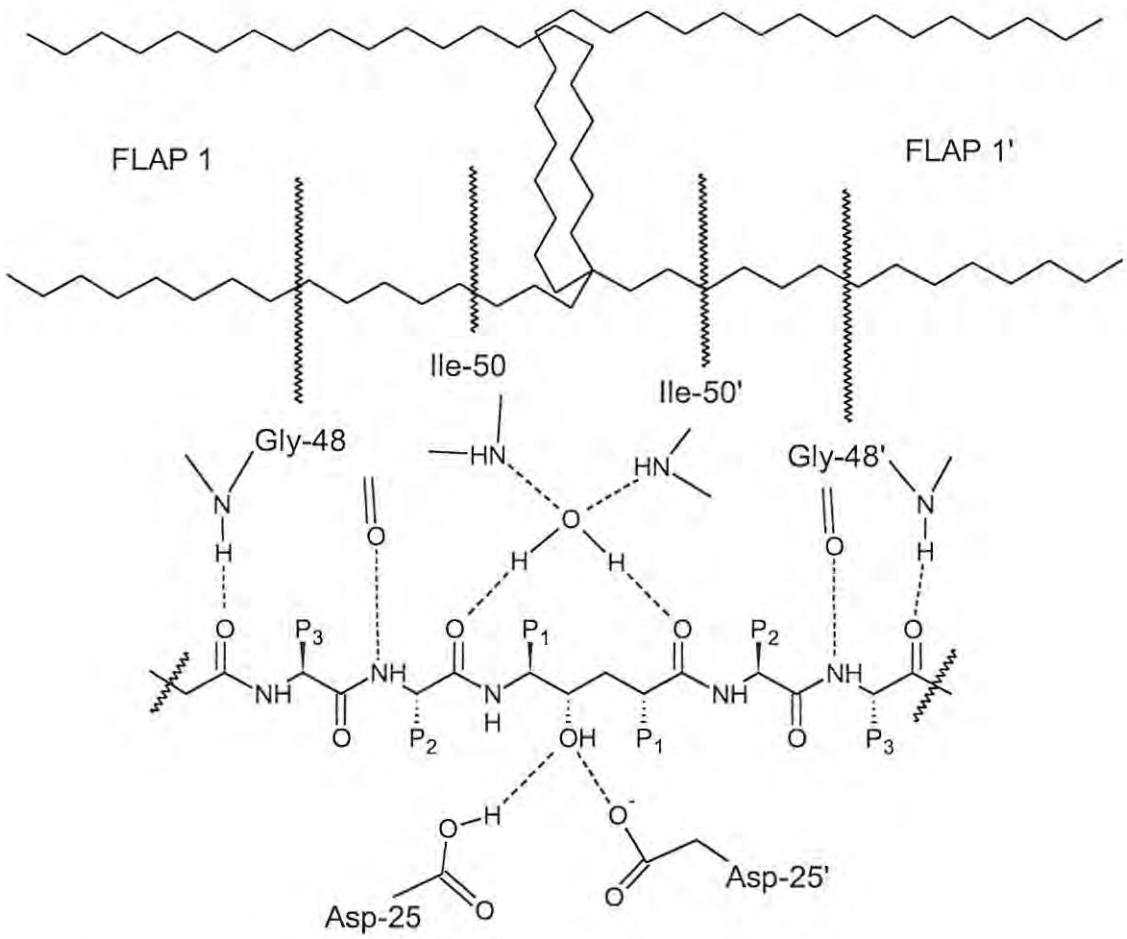
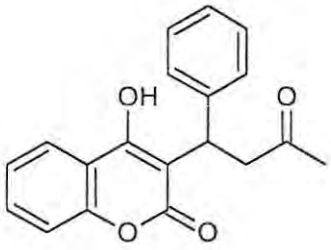


Figure 2. Schematic view of the active site of HIV-1 PR, with a peptide positioned in the active site.[45]

The design of any protease inhibitor must take into account the presence of the structural water molecule and the nature of the subsites of the receptor cavity. Inhibitors must be non-cleavable, capable of H-bonding to the aspartyl residues and the structural water molecule (or capable of replacing it and H-bonding to the Ile-50 and Ile-50' residues) and should contain hydrophobic functionalities that will interact with and fill the subsites. Peptidomimetic inhibitors such as Ritonavir fulfil these criteria, and are potent inhibitors of HIV-1 PR. However, the severe side-effects and poor oral bioavailability of these peptide-based inhibitors led to the design of non-peptide based inhibitors as alternative treatment strategies. These non-peptide based inhibitors have included cyclic urea derivatives,[51] and 4-hydroxycoumarin/4-hydroxypyranone based inhibitors.[48]

1.5 Development of coumarin- and 2-pyranone-based inhibitors of HIV-1 PR

High-volume broad screening of a large library of dissimilar compounds by a number of pharmaceutical companies in the early 1990s led to the discovery of warfarin **10**, a coumarin derivative, as a weak inhibitor of HIV-1 PR.[52,53,54] Further similarity-based searching of compound libraries revealed that phenprocoumon **66** also inhibits HIV-1 PR, with improved inhibitory activity (K_i = 1μM*).[52]



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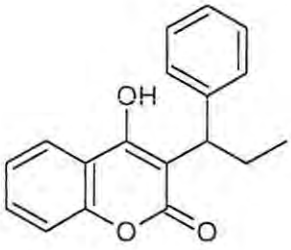


66

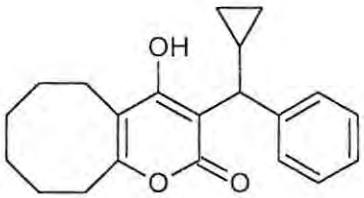
A crystal structure of the phenprocoumon-HIV-1 PR complex indicated good hydrogen bonding between the lactone oxygens of the coumarin moiety and the Ile-50 residues, and also between the 4-OH group of the coumarin and the Asp-25 residues of the enzyme.[52] The ethyl and phenyl rings in the C-3 substituent tend, more or less, to fill the S_1 and S_2 subsites of the enzyme receptor cavity. The position of the benzene ring of the coumarin moiety in the S_1' subsite, however, does not allow for the introduction of substituents that might be able to fill the S_2' subsite.[52] This limitation led to the development of compounds based on the 4-hydroxy-2-pyranone ring, as this moiety would still have the hydrogen-bonding properties of 4-hydroxycoumarin system.[52] In further developments, series of compounds with slightly differing substituents were synthesised and screened for inhibitory activity.

It was noted that amide moieties in peptidic protease inhibitors showed useful hydrogen bonding to the Gly-48 and Asp-29 residues in the protease enzyme, and that the amide groups allowed hydrophobic groups to adopt the favourable orientation required to fill the S_3 subsite of the enzyme. By analogy, 4-hydroxy-2-pyranones with carboxamide-containing substituents were designed (*e.g.* compound **67**); these displayed increased potency as inhibitors.[55] Replacement of the coumarin benzene ring by a cycloalkyl ring

* K_i = dissociation constant of the enzyme-inhibitor complex

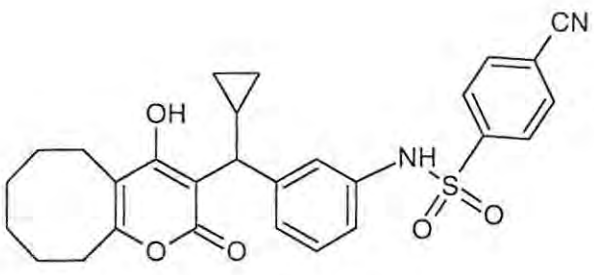
(*e.g.* a cyclooctyl ring) also afforded increased potency (*e.g.* compound **68**), as this allowed better orientation of substituents in the S' subsites.[56] Further work indicated that the replacement of the amide carbonyl with a sulfonyl group increased hydrogen bonding to the enzyme, and sulfonamide-containing 4-hydroxy-2-pyranones (*e.g.* compound **69**) were found to display improved inhibitory activity.[57,58] An inhibitor incorporating both the cyclooctyl ring and the sulfonamide grouping provided the best K_i values (K_i = 0.8 nM) of all compounds tested up to that point. Various other alkyl and aryl substituents at position 6 on the pyranone ring also gave good inhibition of HIV-1.[48]

67 K_i = 1.4 nM

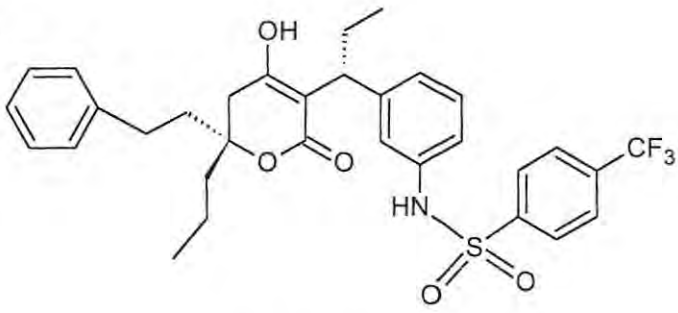


68 K_i = 15 ± 5 nM

The research efforts aimed at finding clinically useful coumarin- and pyranone-based inhibitors culminated in the design of Tipranavir **70**. This inhibitor incorporates the 5,6-dihydropyranone ring, the sulfonamide grouping and alkyl and aryl substituents at position 6. This compound inhibits HIV-1 PR with K_i = 8 pM, and has an IC_{90} value of 100nM in antiviral cell culture.[48] In 2005, Tipranavir was approved by the FDA for commercial use, and is the first non-peptidic inhibitor of HIV-1 PR to be used commercially for the treatment of HIV-AIDS.



69 K_i = 0.8 nM



70 K_i = 8 pM

1.6 Aims of the current study

The aims of this work have been focussed on the application of Baylis-Hillman methodology, developed in the group, to access coumarin derivatives as potential inhibitors of the HIV-1 protease enzyme. However, other synthetic approaches to these compounds have also been explored. Computer modelling of potential drug-enzyme interactions, as well as enzyme inhibition assays of the synthesised compounds were expected to provide some indication of their potential as inhibitors of HIV-1 PR.

Specific objectives of this work have included the following.

i) The preparation of a range of coumarin derivatives using Baylis-Hillman and other methodologies.
ii) Elaboration of the coumarin derivatives to include peptidomimetic and other moieties.
iii) Computer modelling of potential inhibitor-enzyme interactions, using the Cerius^2 LigandFit module.
iv) Enzyme inhibition assays of representative compounds.
v) Application of NMR prediction modules to confirm/support signal assignments of the synthesised compounds.

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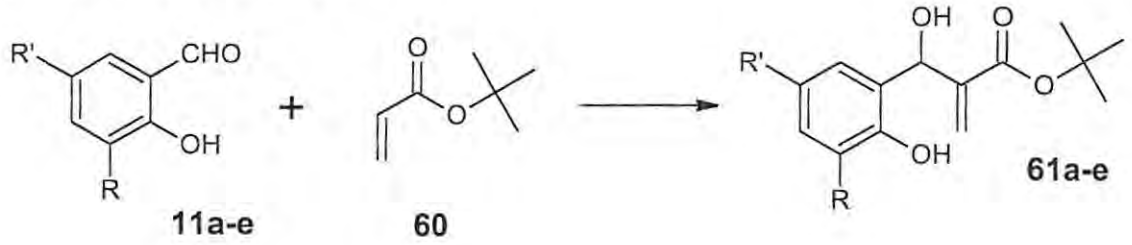
2. DISCUSSION

2.1 Synthesis of Coumarin Derivatives using Baylis-Hillman Methodology

The synthesis of a series of coumarin derivatives was achieved using the approach developed by Musa.[35] Various substituted salicylaldehydes **11a-e** were employed together with *tert*-butyl acrylate **60** as starting materials (Scheme 19). DABCO **38** was used as the Baylis-Hillman catalyst, and the reactions were carried out in chloroform. The rate of these reactions is very slow, and the reaction mixtures were left to stir at room temperature for anything from 14 to 28 days.



	R	R'
a	H	H
b	OEt	H
c	OMe	H
d	H	Br
e	H	Cl

Scheme 19

The Baylis-Hillman adducts **61a-e** have been isolated previously by column chromatography, but, in the present study, the formation of the adducts **61a-c** was confirmed by ^{1}H NMR analysis of the crude products, which were used without further purification in the next step of the synthesis. In the case of the adducts **61d** and **61e**, the Baylis-Hillman products precipitated out of the reaction solution and were simply washed with chloroform to purify them (**61d:** 54 %; **61e:** 22 %). NMR analysis of the precipitates (in DMSO-d_6) confirmed that these were, in fact, the expected Baylis-Hillman adducts. In the ^{1}H NMR spectrum of adduct **61e** (Fig. 3), the singlet at 1.31 ppm integrates for nine protons, and corresponds to the nine *tert*-butyl protons. Aromatic signals at 6.79 (doublet) and 7.09 ppm (multiplet) integrate to one and two protons respectively and correspond to the three aromatic protons. The one proton singlets at 5.64 and 6.05 ppm are due to the two vinylic protons, while the singlet at 5.67 ppm correlates to the C-3 methine proton. The two hydroxyl protons do not resonate as discrete signals but rather as two very broad

signals at 5.52 and 9.73 ppm, corresponding to the aliphatic and phenolic hydroxy protons respectively.

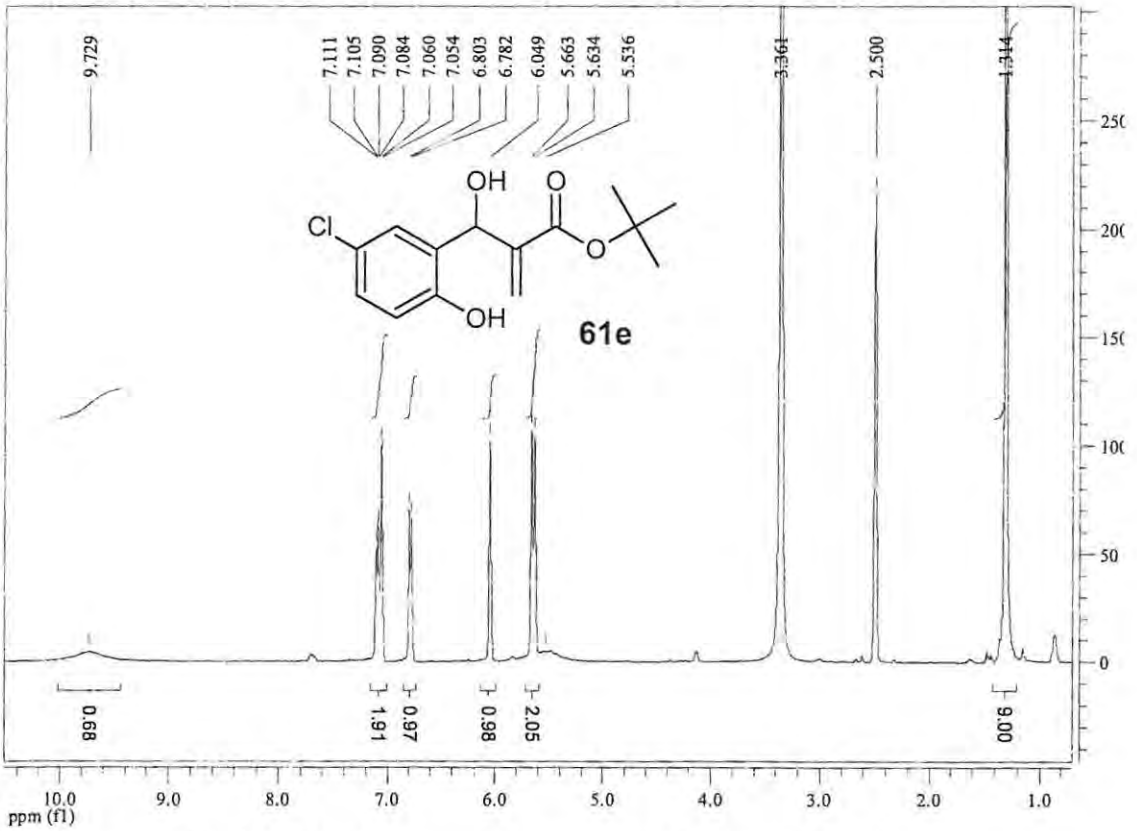
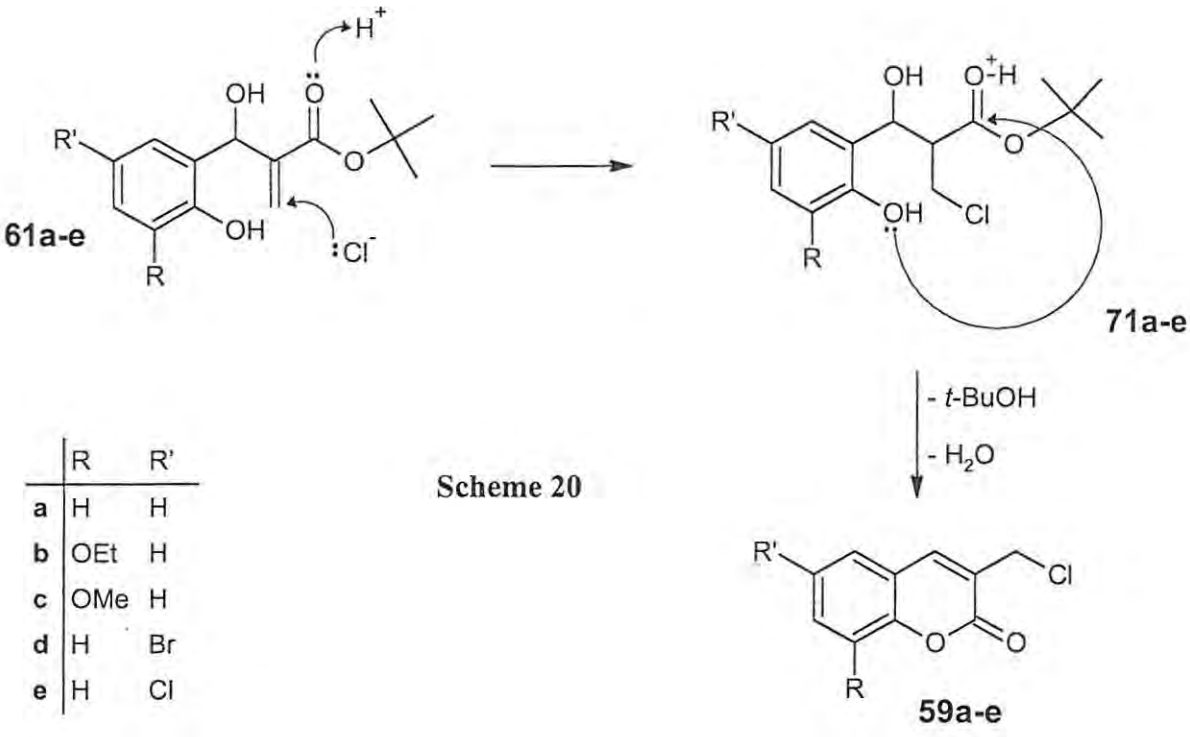


Figure 3. 400 MHz ^{1}H NMR spectrum of compound **61e** in DMSO-d_6.

In the case of the adducts **61a-c**, the solvent and excess *tert*-butyl acrylate were removed, and the resulting crude mixtures were used as starting material for the next step of the respective syntheses. This involved refluxing the substrate with acetic acid and concentrated hydrochloric acid for 2 h to give the 3-(chloromethyl)coumarin derivatives **59a-c**, together with the corresponding salicylaldehyde precursors **11a-c**. Flash chromatography afforded the pure 3-(chloromethyl)coumarin derivatives, albeit in poor yield (18-26 % for the two steps combined). However, the second step involving conversion from the Baylis-Hillman adducts to the coumarin derivatives was virtually quantitative, as evidenced by the ^{1}H NMR spectrum of the crude products following cyclisation. The decision to perform the second step on the crude mixture was motivated by the easier separation of starting materials from the coumarin derivatives than from the initial Baylis-Hillman adducts. The pure Baylis-Hillman adducts **61d** and **61e** were cyclised similarly, yielding the respective 3-(chloromethyl)coumarins **59d** and **59e** as grey precipitates, in good yield (81 % and 83 %, respectively). Since chromene formation seems to be inhibited under these conditions, the cyclisation probably occurs following the

initial addition of HCl. Intramolecular acyl substitution with elimination of *t*-butanol and water yields the coumarin derivatives **59** (Scheme 20). Competitive addition of nucleophilic chloride and nucleophilic DABCO (if DABCO is still present) may occur. If DABCO is added to the vinylic carbon, the *retro*-Baylis-Hillman reaction occurs, resulting in starting materials (the salicylaldehyde and *tert*-butyl acrylate). However, when HCl is added, irreversible cyclisation of the protonated species **71** is favoured to form the coumarin derivatives **59a-e**.[59] Initial attempts to perform the Baylis-Hillman reaction on 5-nitro- and 3,5-dinitrosalicylaldehyde led to the formation of yellow precipitates within a short period of time. A similar effect was observed by Robinson,[32] and was ascribed to the electron-withdrawing effect of the nitro groups rendering the phenolic proton sufficiently acidic to form a salt with DABCO. Robinson, in fact, identified the yellow precipitates as being the phenolate salts of DABCO and the nitrosalicylaldehydes. In the present study, the precipitates were not characterised.



	R	R'
a	H	H
b	OEt	H
c	OMe	H
d	H	Br
e	H	Cl

Scheme 20

The structures of the coumarin derivatives **59a-e** were all confirmed by ^{1}H and ^{13}C NMR analysis, and the ^{1}H NMR spectrum of the derivative **59a** is illustrated in Fig. 4. The signal at 4.55 ppm correlates to the two exocyclic C-1' methylene protons, while the singlet at 7.88 ppm corresponds to the C-4 vinylic proton. The remaining signals in the ^{1}H NMR spectrum can be assigned to the four aromatic protons of the coumarin system.

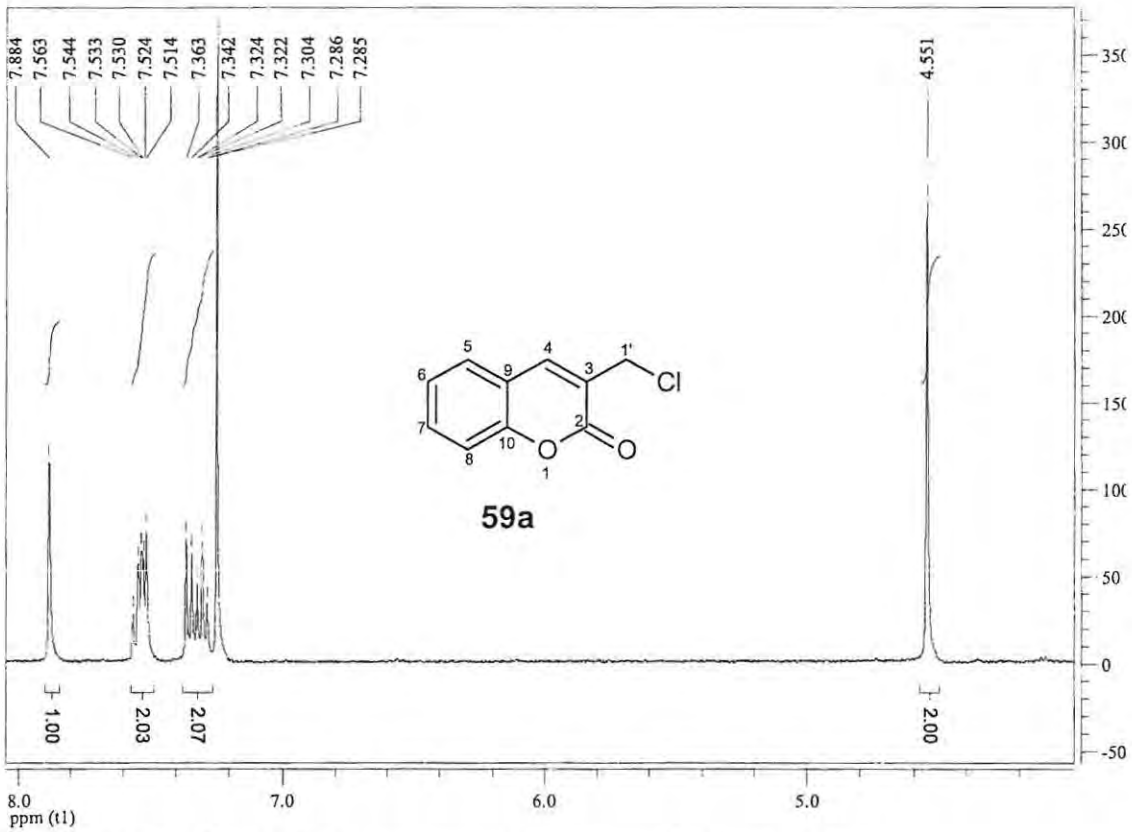
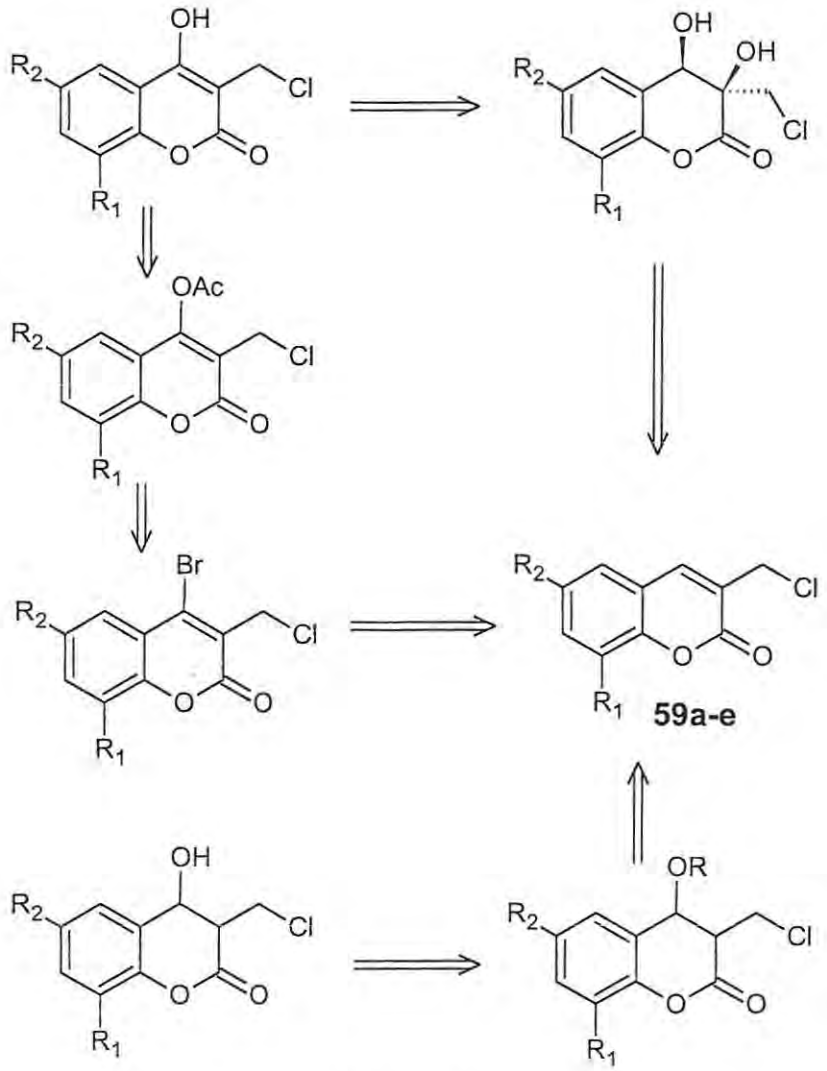


Figure 4. 400 MHz ^{1}H NMR spectrum of compound **59a** in $CDCl_3$.

2.2 Functionalisation of coumarin and 3-(chloromethyl)coumarin derivatives

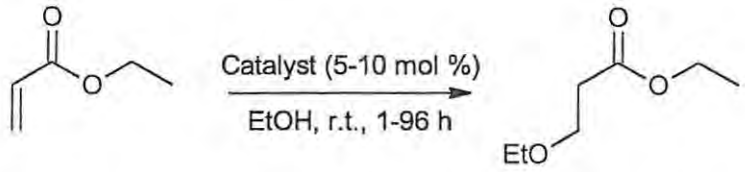
All coumarin and pyranone derivatives which are active against HIV-1 PR are characterised by the presence of a hydroxy group at position 4.[52-58] Thus, a means of introducing this group in the 3-(chloromethyl)coumarin derivatives synthesised by Baylis-Hillman methodology was sought. Possibilities included: (i) hydroalkoxylation,[60,61] followed by hydrogenolysis; (ii) bromination at C-4,[1,62-64] followed by acetylation and then hydrolysis; (iii) dihydroxylation of the double bond,[72] followed by controlled dehydration, which would be expected to yield a 4-hydroxy isomer with a conjugated endocyclic double bond (Scheme 21). Exploratory studies were undertaken using coumarin **1** itself as a model substrate.



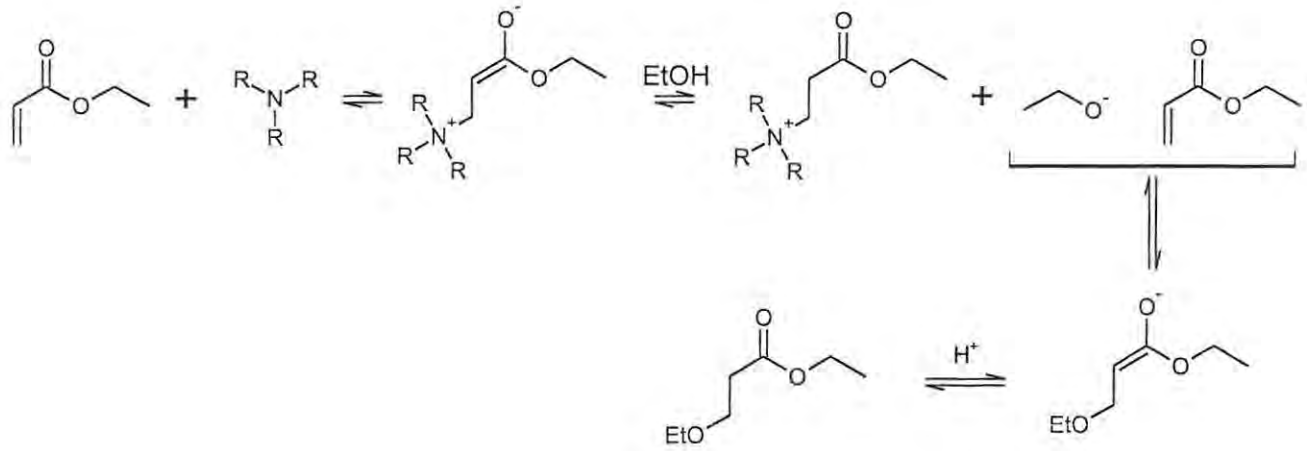
Scheme 21

Attempted hydroalkoxylation of the α,β–unsaturated double bond

Examples of the base-catalysed hydration and hydroalkoxylation of activated alkenes in the literature led us to attempt this approach to the functionalisation of the 4-position of the coumarin derivatives **59a-e**. Stewart *et al.*[60] have reported the phosphine-catalysed hydration and hydroalkoxylation of activated alkenes (Scheme 22), while Murtagh and coworkers[61] describe similar reactions catalysed by tertiary amines such as 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU); these methods have the advantage of not being moisture or air-sensitive – shortcomings exhibited by the phosphine-catalysed reactions. In the latter approach, conjugate addition of the tertiary amine to the alkene results, initially, in an enolate, which is protonated by the solvent, *e.g.* ethanol or methanol; use of DBU as the tertiary amine has been found to give the highest yields.[61] The resulting alkoxide anion then attacks a second molecule of the alkene, which on protonation yields the hydroalkoxylated alkene as a conjugate addition product (Scheme 23).[61]



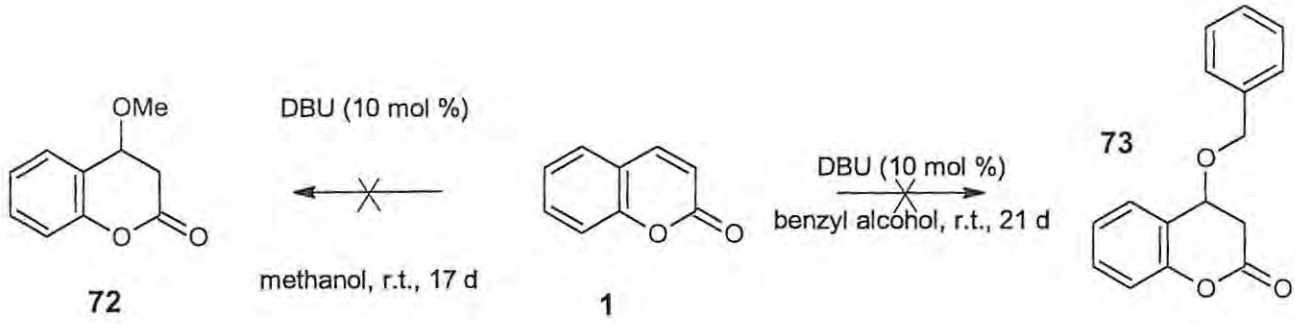
Scheme 22



Scheme 23

Since coumarin **1** contains an α,β-unsaturated carbonyl group, Murtagh's method was attempted, using first benzyl alcohol, and then methanol as the solvent and DBU as the

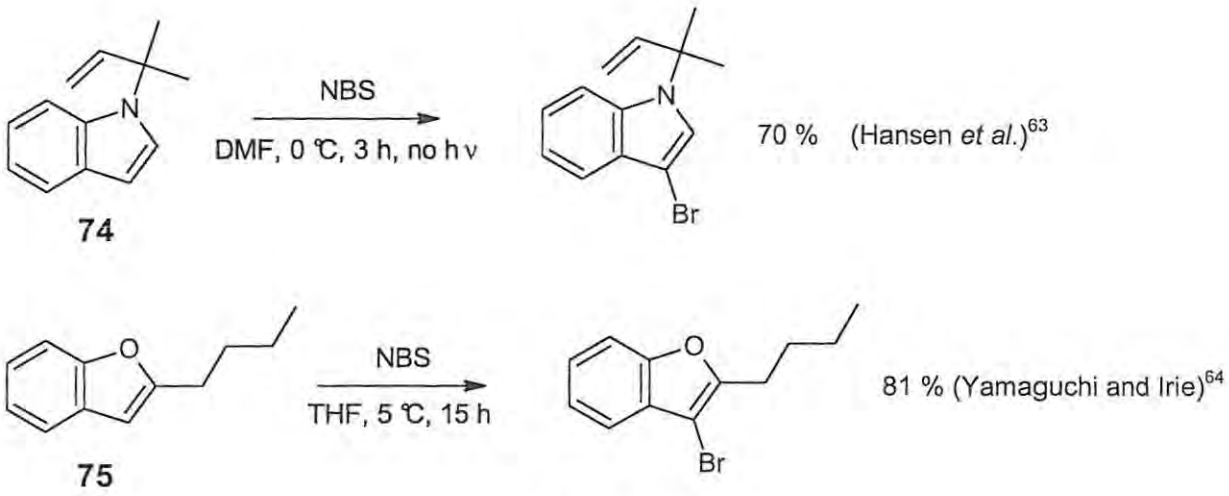
catalyst (Scheme 24). Although the reaction mixtures were stirred for several weeks, no product formation was observed at all, and this approach was abandoned.



Scheme 24

Attempted bromination of coumarin at position 4

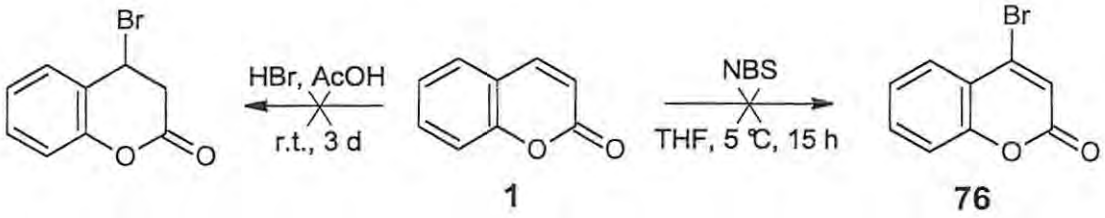
The next approach to be explored for functionalising the 4-position of coumarin and its derivatives involved selective bromination at C-4. Dibromination of the coumarin double bond occurs easily,[62] but the 3,4-dibromocoumarin product is unstable and decomposes readily to 3-bromocoumarin.[1] Hydrobromination of the activated double bond using HBr, on the other hand, might be expected to form the 4-bromo isomer. The 4-position is also benzylic, and there examples in the literature of selective substitutive bromination at an unsaturated benzylic position using *N*-bromosuccinimide (NBS) as a source of bromine. Hansen *et al.*[63] have reported the selective bromination of an indole **74** at position 3 using NBS, while Yamaguchi and Irie[64] successfully brominated 2-butylbenzofuran **75** at position 3 (Scheme 25); no competing allylic bromination was reported.



Scheme 25

These two examples of bromination at an unsaturated benzylic position led us to attempt similar bromination of coumarin **1** using NBS (Scheme 26). Some product formation was, in fact, observed by TLC; however, chromatography of the crude mixture afforded material which proved unstable. The NBS approach presumably involves electrophilic addition and, in coumarin, the alkene moiety is probably more activated towards nucleophilic attack, which means that this method is unlikely to be an effective means of bromination at position C-4.

Conjugate addition of HBr to the α,β-unsaturated carbonyl group was also attempted, in the expectation that protonation of the carbonyl oxygen would facilitate nucleophilic conjugate addition by the bromide ion. Coumarin **1** in acetic acid was treated with a solution of HBr in acetic acid. After 3 days, however, no product appeared to have formed and the starting material was recovered.

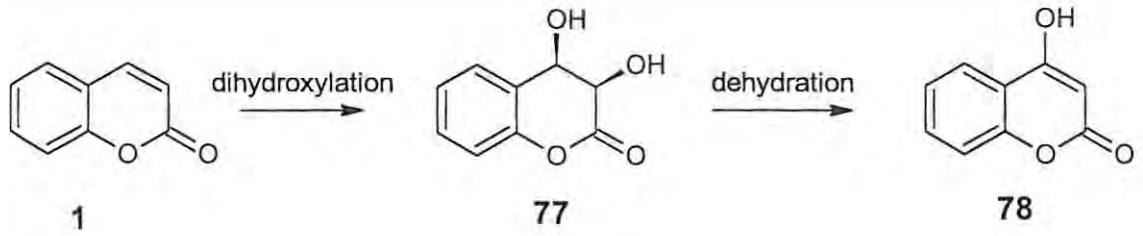


1 **76**

Scheme 26

Dihydroxylation of coumarin and 3-(chloromethyl)coumarin derivatives

A third approach to the functionalisation of coumarin **1** [and, hence, the 3-(chloromethyl)coumarin derivatives **59a-e**] involved dihydroxylation of the double bond to afford the diol **77**, to be followed by dehydration to yield the 4-hydroxycoumarin target **78** (Scheme 27).

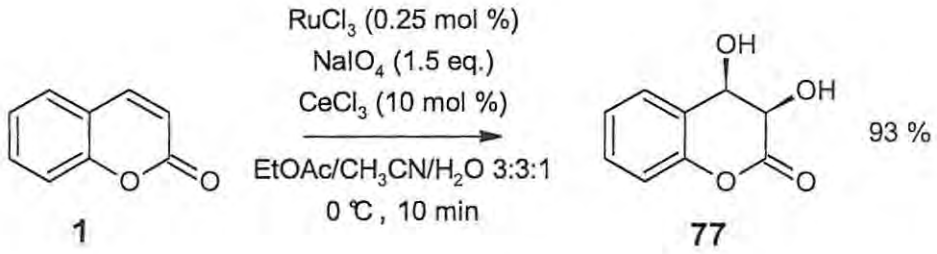


Scheme 27

The dihydroxylation of alkenes can generally be accomplished by a number of methods. $KMnO_4$,[65] OsO_4[66] and RuO_4[67] are all recognised reagents for accomplishing this transformation, and all achieve *syn*-dihydroxylation.[68] The osmium-mediated reaction can be effected more economically by the use of a stoichiometric oxidant such as H_2O_2, *N*-methylmorpholine-*N*-oxide, *tert*-butylhydroperoxide or $K_3Fe(CN)_6$.[68]

Asymmetric dihydroxylation (Sharpless dihydroxylation) may be achieved using OsO_4, a stoichiometric oxidant and a suitable chiral ligand.[69] *anti*-Dihydroxylation can be carried out by first forming the epoxide (using H_2O_2 and HCOOH,[68] or *m*-chloroperbenzoic acid[70]), which may be opened to give the *anti*-dihydroxylated product. However, these reactions all depend on the nucleophilicity of the π-electron-rich alkene moiety. In coumarin systems, the double bond is conjugated to the lactone carbonyl group and, hence, is deactivated towards electrophilic attack.

A $RuCl_3$-$CeCl_3$-$NaIO_4$ catalyst system (in which the catalytic species RuO_4 is generated *in situ*) has been developed by Plietker and Niggemann for the dihydroxylation of deactivated alkenes, including coumarin **1**, the dihydroxylation product of which (**77**) was obtained in 93 % yield after 10 min (Scheme 28).[71,72] Thus it was decided to attempt the dihydroxylation using this system. The toxicity of OsO_4 was also a motivating factor in the decision to use the ruthenium-based catalytic system.

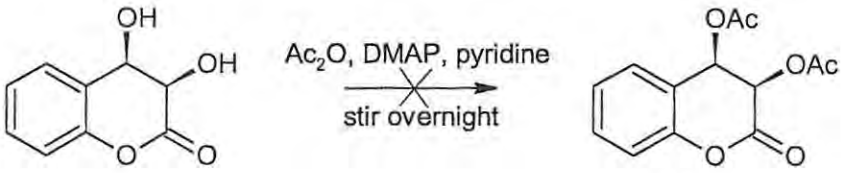


Scheme 28

It was decided to repeat this reaction on coumarin **1** (as a model system) before applying the method to the 3-(chloromethyl)coumarins **59a-e**. However, the yields were disappointing (< 5 %). A number of sources of error were suggested by Plietker;[73] these included the presence of any bases or carboxylic acids (such as the *o*-hydroxycinnamic acid, formed by ring-opening of the coumarin lactone) or contamination of the $NaIO_4$ with NaOH. Although the formation of *o*-hydroxycinnamic acid is not likely (coumarin is more stable in its lactone form, and the free acid cyclises to the lactone spontaneously[44]), the coumarin starting material was purified by neutralisation, recrystallisation and finally silica-gel chromatography. However, these measures proved unsuccessful. Finally, a new supply of $NaIO_4$ was used, and this led to the successful transformation to the diol 77.

Plietker and Niggemann reported the purification of the product, (3*S*,4*S*)-3,4-dihydroxychroman-2-one **77** by flash chromatography on silica. All our attempts to purify the product by this means, however, led to decomposition of the diol on silica. The presence of the diol product was nevertheless confirmed by TLC and ^{1}H NMR analysis of the crude product mixture, the latter indicating a yield of *ca.* 83 %.

Protection strategies were then explored in an attempt to stabilise the diol **77**. It was decided to protect the diol by forming the diacetate **81**, as shown in Scheme 29. The diol **77** was treated with a 1:1 mixture of acetic anhydride and pyridine in the presence of catalytic amounts of 4-dimethylaminopyridine (DMAP) to afford a complex mixture of products that did not appear, on ^{1}H NMR analysis, to contain the expected diacetylated product.

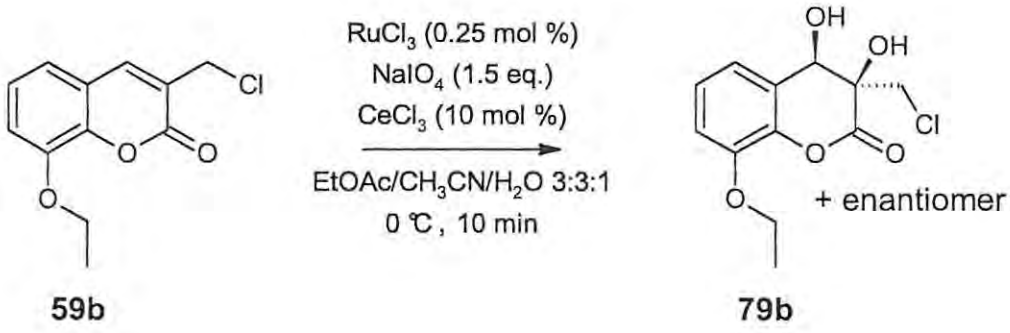


77 **81**

Scheme 29

Given the success of the RuO_4-catalysed dihydroxylation of coumarin **1**, it was decided to apply the method to the dihydroxylation of 3-chloromethyl-8-ethoxycoumarin **59b** (Scheme 30). The same procedure was followed as for coumarin **1**, and ^{1}H NMR analysis

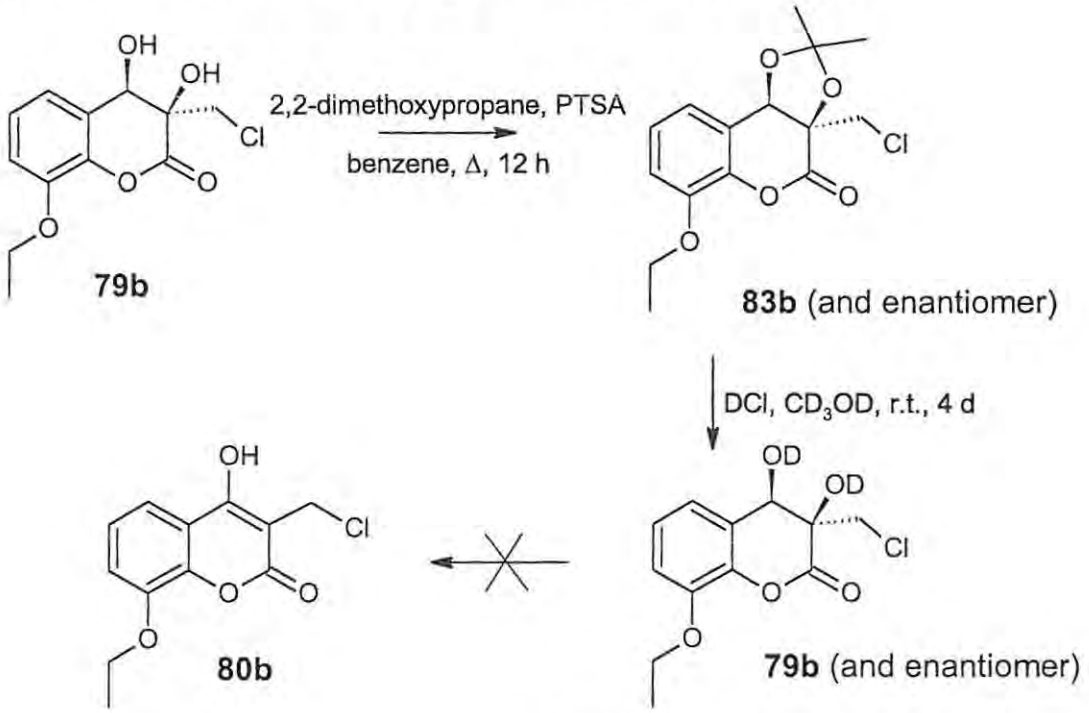
of the crude mixture confirmed the formation of the desired diol **79b** in *ca.* 40 % yield.



59b **79b**

Scheme 30

Given the failure of the attempted diacetylation of the dihydroxy coumarin derivative **77**, a second protection strategy was explored, *viz.* ketalisation. The ketal **83b** was formed by reacting the diol **79b** with 2,2-dimethoxypropane, in the presence of *p*-toluenesulfonic acid (PTSA) as the catalyst (Scheme 31).[74] This reaction proved successful, yielding the protected diol, (3*R*,4*R*)-3-chloromethyl-3,4-*O*-isopropylidene-8-ethoxycoumarin **83b** (and its enantiomer), which was separated from the starting material (**59b**) by HPLC. The structure of the protected coumarin diol was confirmed by NMR spectroscopy and its molecular formula confirmed by high-resolution mass spectrometry.



79b **83b** (and enantiomer)

80b **79b** (and enantiomer)

Scheme 31

The ^{1}H NMR spectrum (Fig. 6) exhibits two singlets at 1.44 and 1.55 ppm which represent

the diastereotopic isopropylidene methyl groups. The triplet at 1.46 ppm and the quartet at 4.14 ppm are characteristic of the 8-ethoxy group, while the pair of doublets at 3.81 ppm corresponds to the diastereotopic C-1' methylene protons and the singlet at 5.17 ppm corresponds to the C-4 methine proton. The HSQC spectrum (Fig. 7) confirmed these signal assignments. The carbons resonating at 26.3 and 27.2 ppm correlate with the methyl proton signals at 1.44 and 1.55 ppm, while the ^{13}C signal at 76.1 ppm correlates to the C-4 methine proton signal. The carbons which resonate between 110 and 130 ppm correlate to the aromatic proton signals between 7.00 and 7.18 ppm, while the carbon signals at shifts greater than 127 ppm correspond to the quaternary aromatic, aliphatic and carbonyl carbons, as evidenced by the lack of correlation with any proton signals.

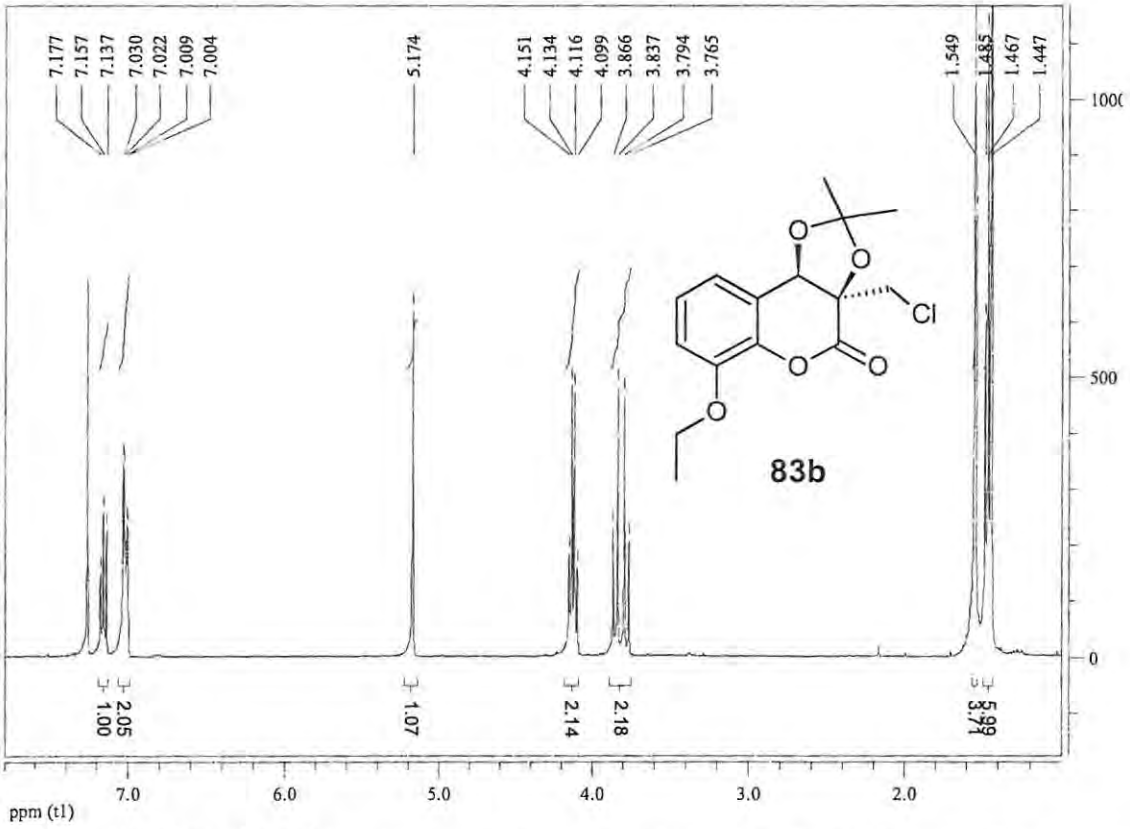


Figure 6. 400 MHz ^1H NMR spectrum of protected coumarin diol **83b** and its enantiomer in $CDCl_3$.

Having separated the protected diol from the starting material successfully, deprotection was attempted. This was carried out as an NMR-scale experiment in order to monitor the deprotection carefully. It was uncertain whether the acid-catalysed deprotection conditions would be sufficient to effect dehydration of the diol at the same time, and so this method was chosen. The protected diol **83b** was dissolved in CD_3OD, and a catalytic amount of DCl (33 wt. % solution in D_2O) was added (Scheme 31). This experiment showed that deprotection was accomplished after 4 days, at room temperature. The ^1H NMR stackplot,

which shows the progress of the reaction, is shown in Fig. 8. The first spectrum was recorded before the addition of DCl. Spectra were then recorded every 200 s for 2000 s; thereafter spectra were recorded 3 h, 5 h, 2 days, 4 days and 1 week after the reaction was started. The large signal at 5.8 ppm corresponds to the DCl, while the signal at 3.31 ppm is the solvent signal. The loss of the diastereotopic isopropylidene methyl signals, and the steady growth of a signal at 2.1 ppm (which corresponds to acetone) indicated that the deprotection was successful. All other signals shifted slightly during the course of the reaction.

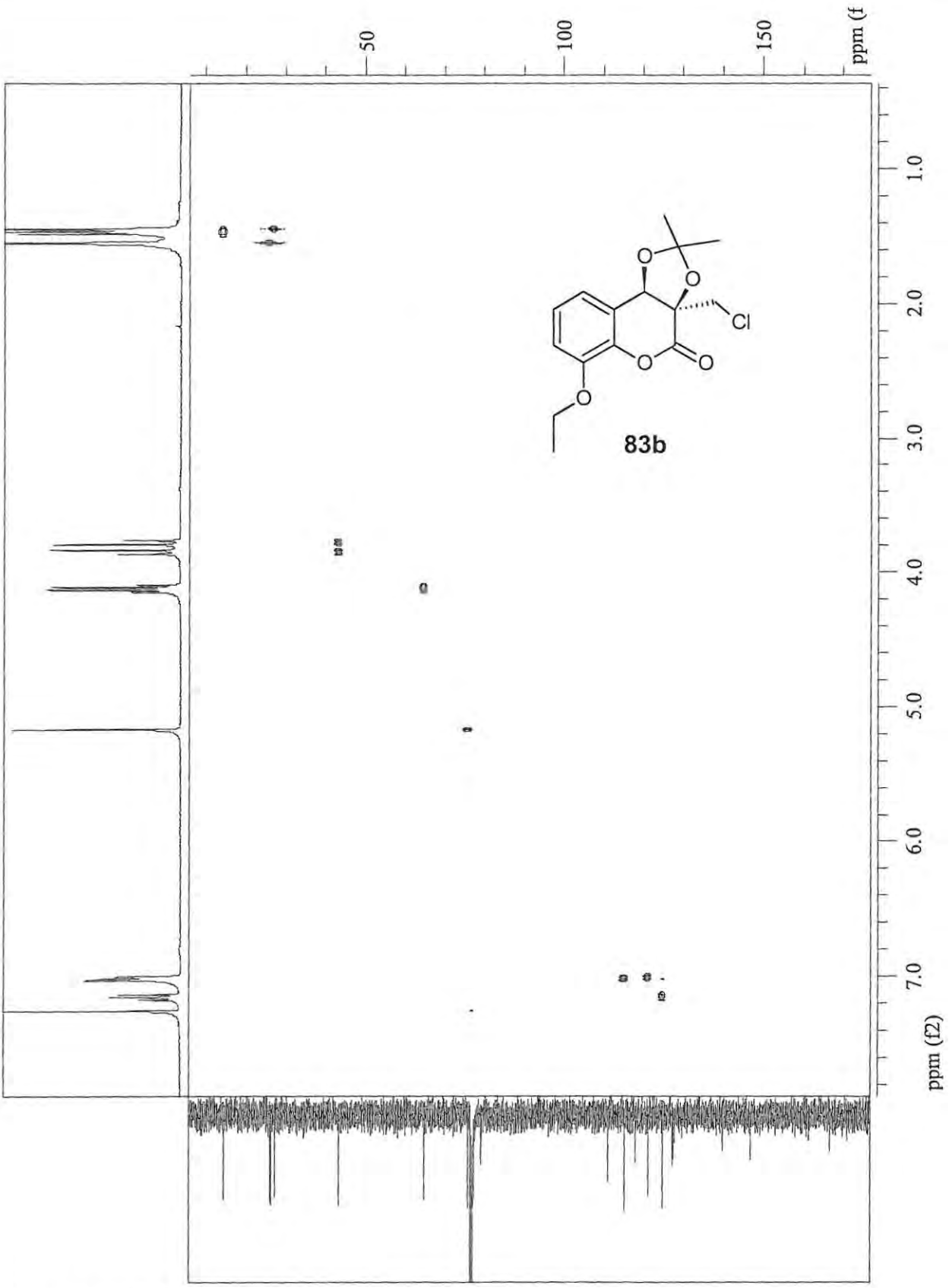


Figure 7. HSQC spectrum of protected coumarin diol **83b** in $CDCl_3$.

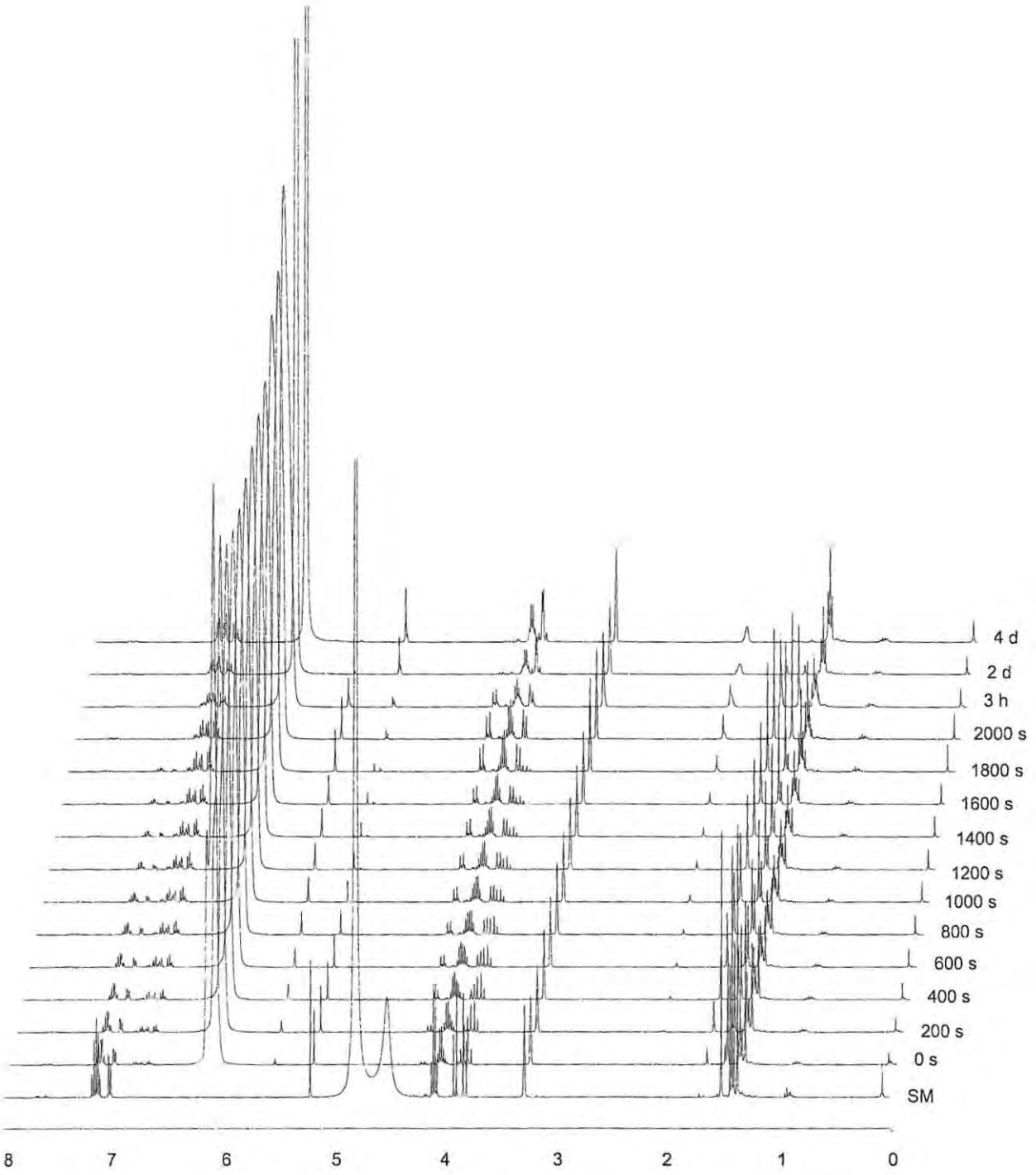


Figure 8. 400 MHz ^{1}H NMR spectra of the deprotection of **83b**, catalysed by DCl in MeOD. 'SM' = starting material. Horizontal axis = δ_H (ppm).

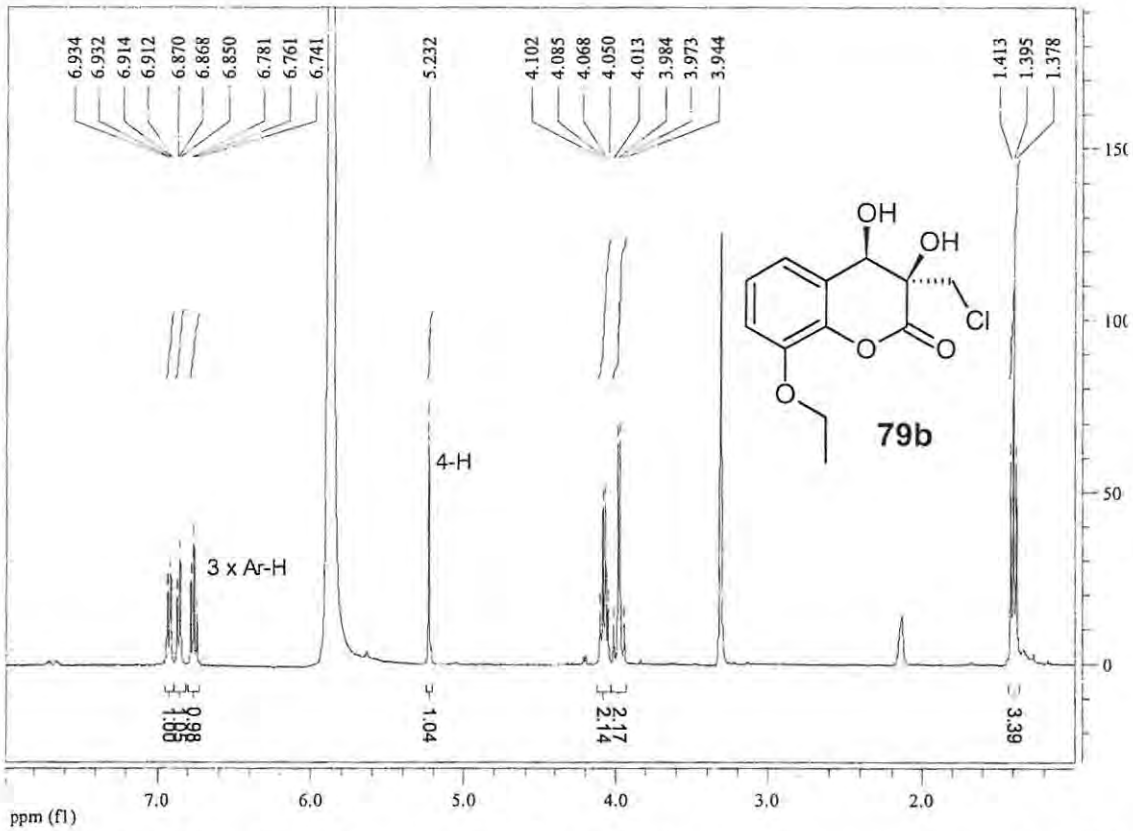
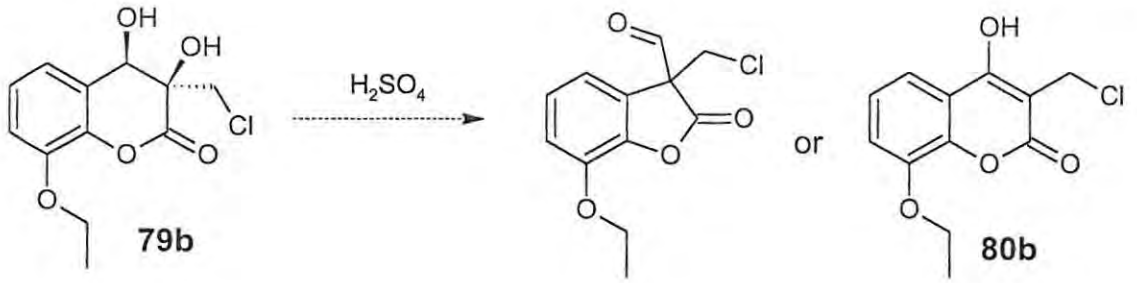


Figure 9. 400 MHz ^{1}H NMR spectrum of the dihydroxylated coumarin derivative **79b** in CD_3OD, after removal of the ketal by acid-catalysed hydrolysis.

In the event, no dehydration of the coumarin diol **79b** to the 4-hydroxycoumarin derivative **80b** was observed under these conditions, even after the reaction tube was left standing for 2 weeks. The presence of the 4-H methine proton signal at 5.23 ppm indicated that the diol was still present, as this proton would be lost if dehydration to an endocyclic double bond were to occur. If dehydration to an exocyclic double bond had occurred, the signals corresponding to the diastereotopic exocyclic C-1' methylene protons would have disappeared. However, these signals were still present, as a pair of doublets at 3.98 ppm. The quartet at 4.07 ppm and the triplet at 1.39 ppm represent the 8-ethoxy group.

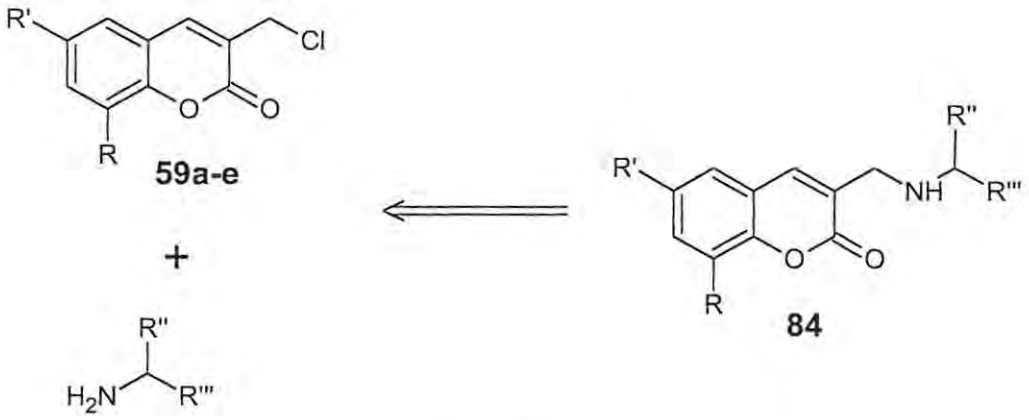
Dehydration of 1,2-diols (to yield enols) is generally accomplished by acid catalysis. When H_2SO_4 is used, there is the possibility of a pinacol rearrangement occurring, which, in this case, would contract the 6-membered pyranone ring to a five-membered furanone ring, and introduce an aldehyde moiety into the molecule (Scheme 32).[75] The resulting benzofuranone would still have hydrogen-bonding capabilities, and could, in fact, provide access to HIV-PR inhibitors. The chloromethyl moiety would still be present, allowing for the introduction of suitable amine groups.



Scheme 32

2.3 Synthesis of 3-aminomethyl coumarins

One of the aims of this project was the introduction of peptide-like groups to the simple coumarin nucleus (Scheme 33). The chlorine atom at position C-1' in the 3-(chloromethyl)coumarin synthons **59a-e** provided an ideal opportunity for nucleophilic substitution by suitable amine moieties to afford aminated target molecules **84**. It has already been established[76] that both carbon and nitrogen nucleophiles substitute only at this position, without any attack at either C-2 (the carbonyl carbon) or C-4 (the β-carbon). It was thus anticipated that the displacement of chloride by these amines would present no real difficulties.



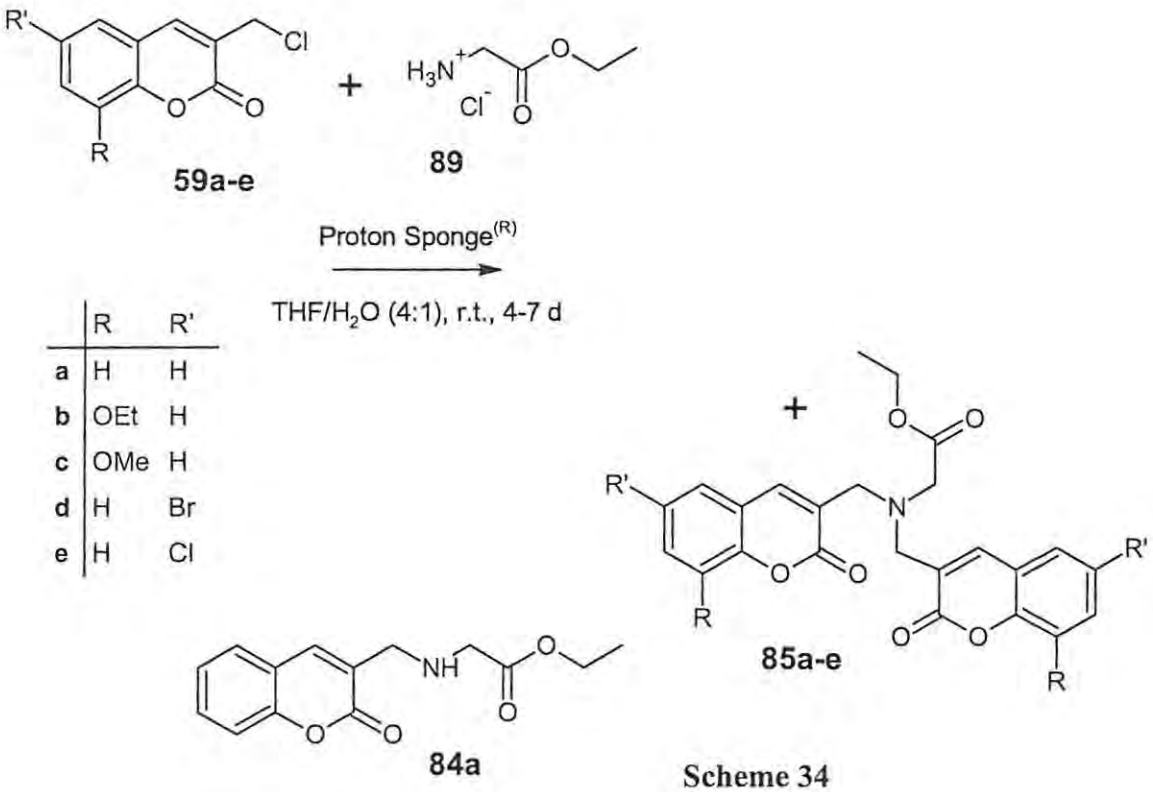
Scheme 33

However, the one obstacle not anticipated was the difficulty encountered in neutralising the amines used (glycine ethyl ester, L-serine ethyl ester and *S*-benzylcysteamine), as they were available only as hydrochloride salts. Neutralisation with $NaHCO_3$ or NaOH was first attempted *in situ*, either in a solvent mixture such as THF/H_2O, or in a biphasic system, *e.g.* $EtOAc/H_2O$. In both of these solvent systems, solubility of the 3-(chloromethyl)coumarin substrate presented no problems, but attempts to neutralise the amine hydrochlorides *in situ* led to insoluble reaction products which could not be successfully characterised. Isolation of the free amine by first neutralising (*e.g.* with NaOH) and then extracting the amine into a suitable organic solvent, *e.g.* EtOAc, was also unsuccessful, as the volatility of the amine meant that it was lost when the solvent was removed *in vacuo*.

Both of these approaches having failed, a completely different strategy was employed. Proton Sponge® [1,8-bis(dimethylamino)naphthalene] is a very strong base (pK_a = 12.34)[77] and is not very nucleophilic due to its steric bulk. This base has found synthetic

application in phosphorylation reactions,[78] but has not (to our knowledge) been used for the deprotonation of problematic amine hydrochlorides. Thus, Proton Sponge®, the requisite amine hydrochloride and the 3-(chloromethyl)coumarin substrate **59** were dissolved in a THF/H_2O (4:1) solvent mixture, and the resulting solution was stirred at room temperature for 4-7 days. Extraction with EtOAc yielded the crude aminated coumarin products.

The 3-(chloromethyl)coumarin derivatives **59a-e** were treated with glycine ethyl ester hydrochloride **89** in the presence of Proton Sponge® and, in each case, chromatography (flash chromatography or HPLC) yielded both the monocoumarinated (**84a-e**) and the dicoumarinated (**85a-e**) amino products (Scheme 34). The ratio of **84b** to **85b** (based on integration of ^{1}H NMR spectra of crude product mixtures) was estimated at 2:1, despite the large excesses of amine hydrochloride (2 eq.) and Proton Sponge® (2.4 eq.) used. All the compounds formed in these reactions are new compounds, and have been fully characterised using NMR spectroscopy and high-resolution mass spectrometry (providing elemental composition). Figures 10 and 11 show the ^{1}H NMR and HSQC spectra of compound **84a**, while Fig. 12 shows the ^{1}H NMR spectrum of **85e**.



Scheme 34

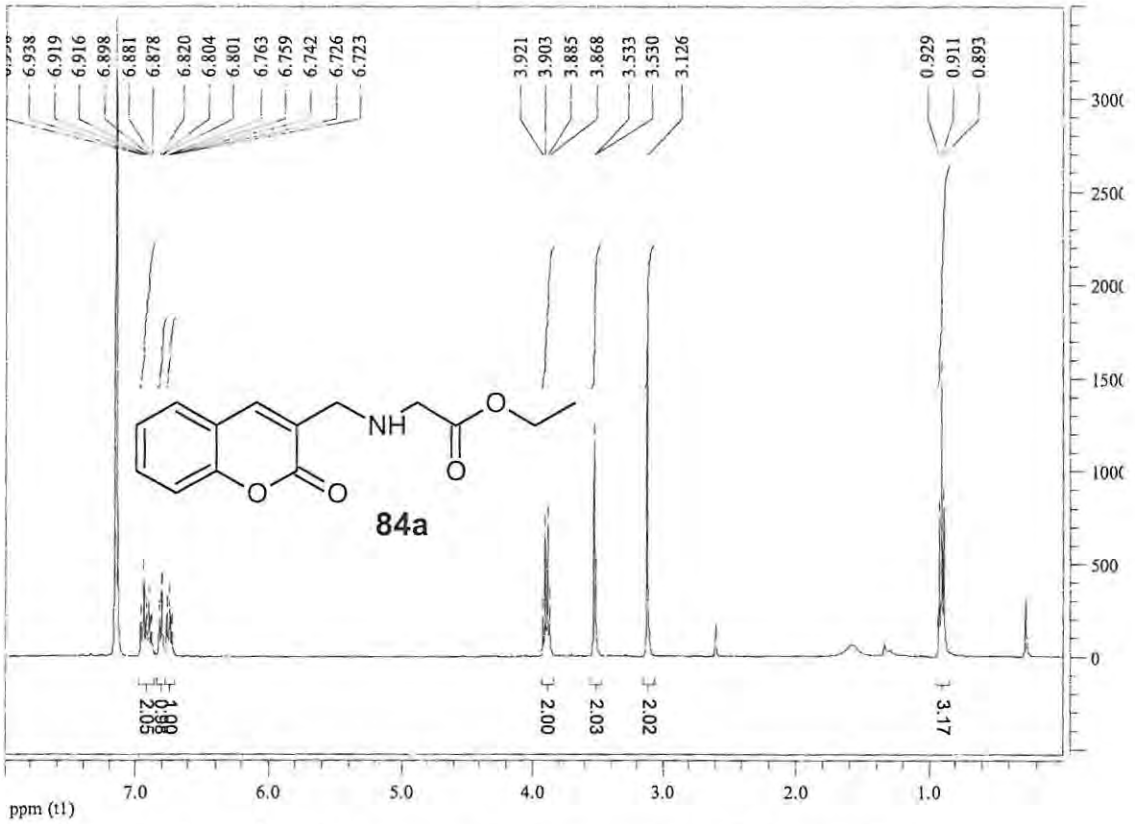


Figure 10. 400 MHz ^{1}H NMR spectrum of compound **84a** in benzene-d_6.

The triplet at 0.91 ppm and the quartet at 3.89 ppm in the ^{1}H NMR spectrum of compound **84a** (Fig. 10) are characteristic of the ethyl ester moiety, while the singlets at 3.13 and 3.53 ppm are due to the two methylene groups attached to the amine nitrogen. The remaining signals between 6.55 and 6.95 ppm correspond to the coumarin aromatic. Although the proton signal corresponding to the 4-H nucleus is obscured by the benzene solvent signal, this correlation can be seen in the HSQC spectrum (Fig. 11). The two signals at 160.5 and 171.9 ppm in the ^{13}C spectrum are characteristic of ester carbonyls, and correspond to the lactone and ethyl ester carbonyl carbons, respectively. The signals at 48.2 and 50.7 ppm correlate to the two methylene groups adjacent to the nitrogen, while the signals at 14.2 and 60.6 ppm correlate to the ethyl ester protons. Signals between 116 and 152 ppm correspond to the aromatic and vinylic carbons. The 4-H proton signal, which cannot be seen in the proton spectrum due to the benzene signal, nevertheless correlates to a carbon signal at 137.5 ppm, which was used to confirm the presence of the 4-H signal beneath the benzene solvent signal.

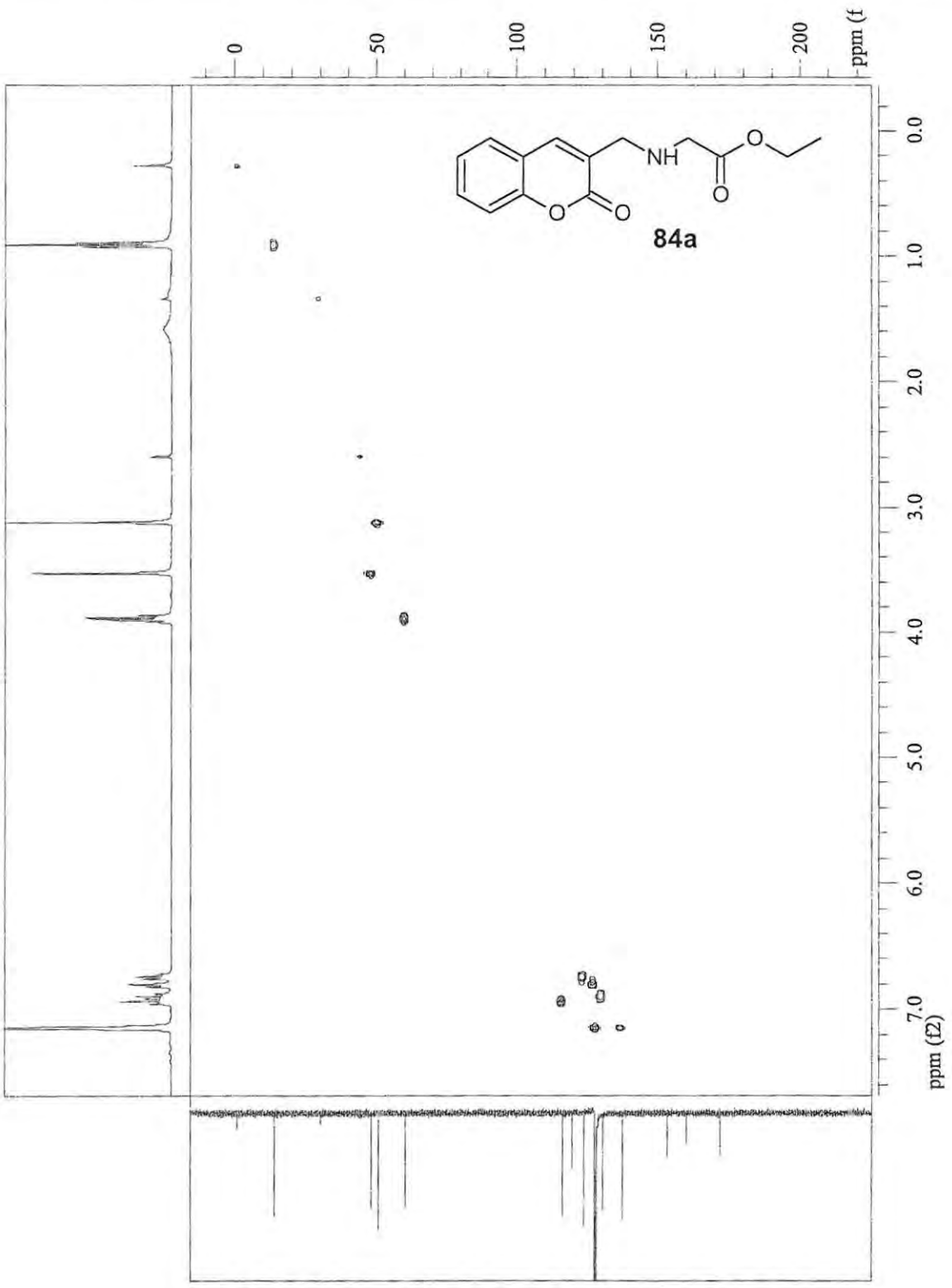


Figure 11. HSQC spectrum of compound **84a** in benzene-d_6.

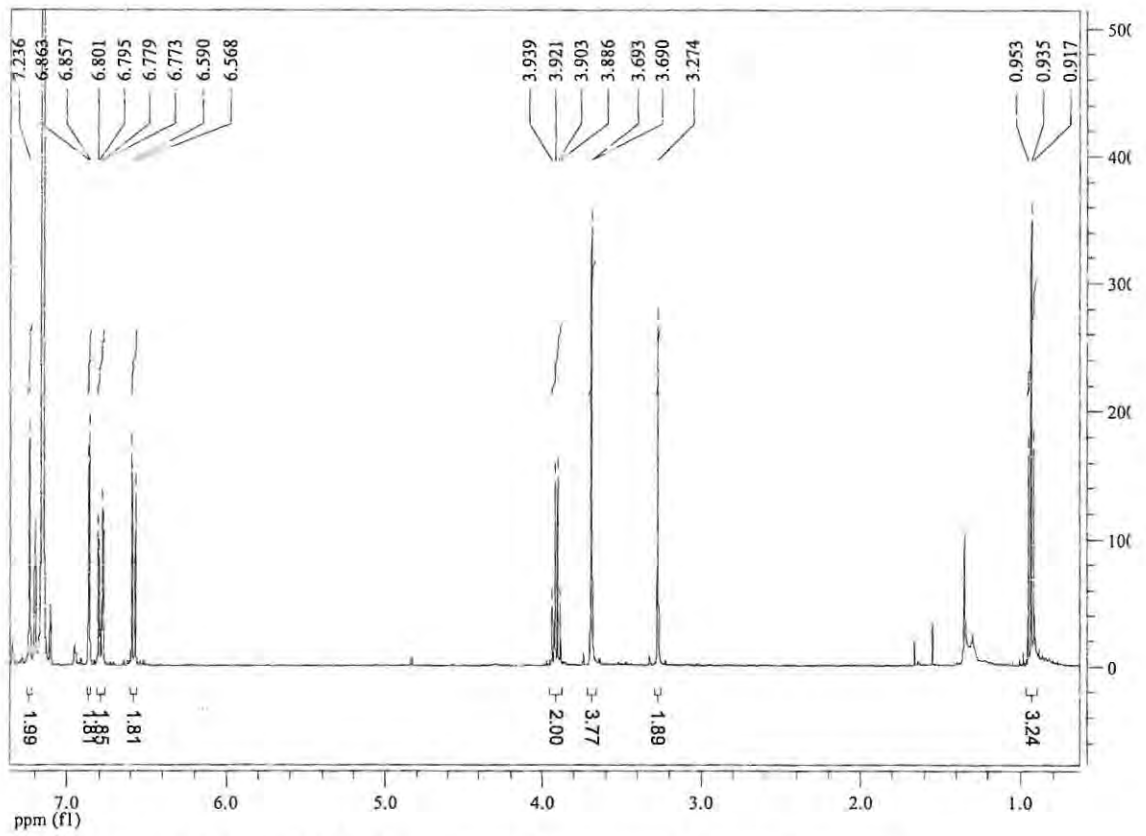
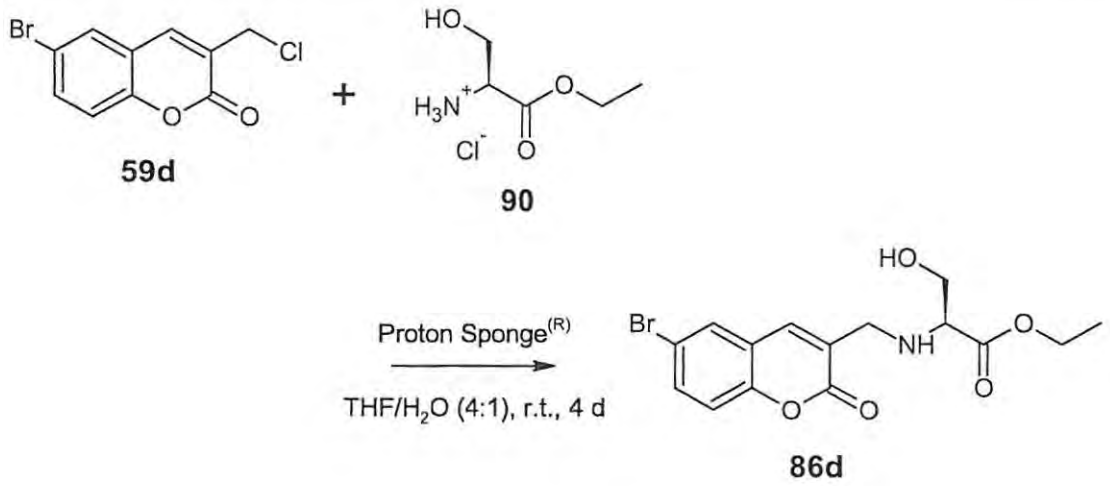


Figure 12. 400 MHz ^{1}H NMR spectrum of compound **85e** in benzene-d_6.

Although the spectra of the dicoumarinated glycine ethyl esters **85a-e** are very similar to those of the monocoumarinated compounds **84a-e**, the difference in the number of protons corresponding to the signal at 3.69 ppm [four protons in this spectrum of compound **85e** (Fig. 12), as opposed to two protons in the spectrum of compound **84a** (Fig. 10)] is indicative of the fact that there are two of these identical methylene groups attached to the amine nitrogen.

The 3-(chloromethyl)coumarin derivative **59d** was also reacted with L-serine ethyl ester hydrochloride **90** (Scheme 35). In this reaction, very little of the dicoumarinated amine was formed; its presence was detected by TLC, but none was isolated. The monocoumarinated amine **86d** was obtained in 92 % yield after radial chromatographic separation, and a small amount of the compound was purified further by HPLC for analytical purposes. This particular compound may be of interesting synthetic significance, as it is the first generated from the coumarin nucleus (in this work) with a chiral centre. This compound's structure was confirmed by full spectroscopic analysis, and the ^{1}H NMR spectrum of the compound is illustrated in Fig. 13.



Scheme 35

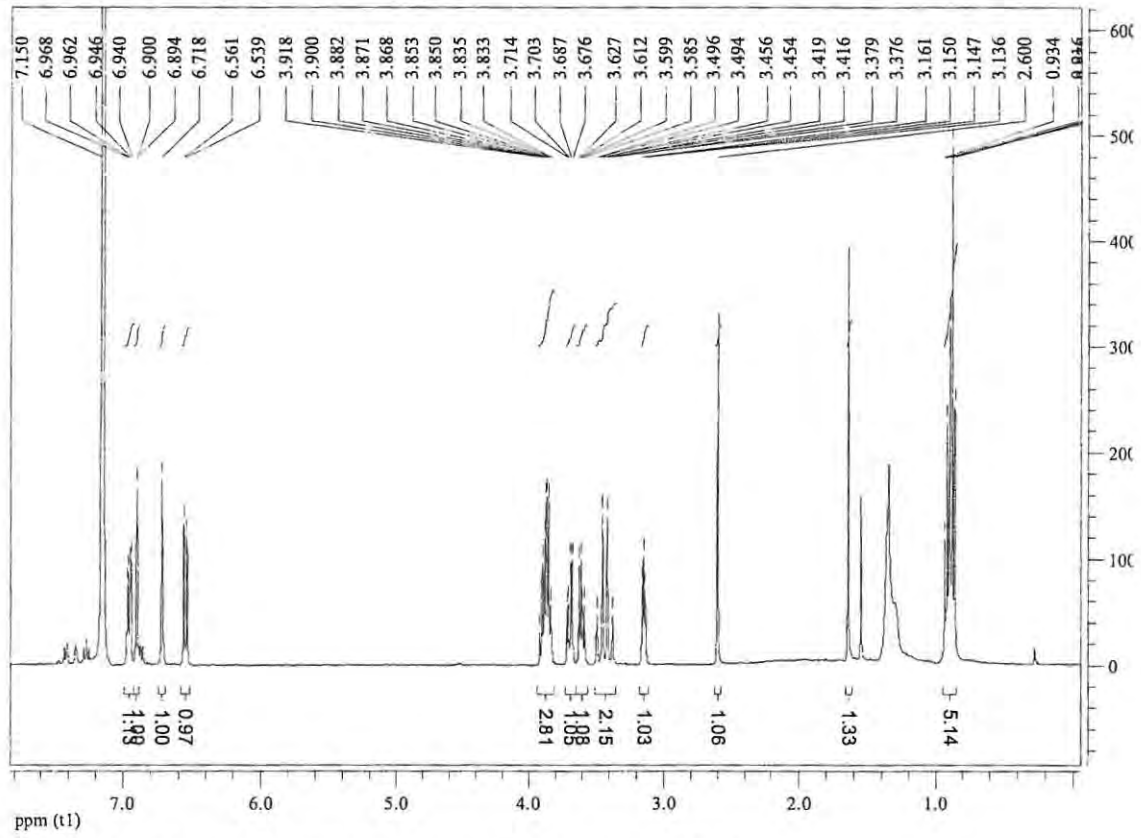


Figure 13. 400 MHz ^{1}H NMR spectrum of compound **86d** in benzene-d_6.

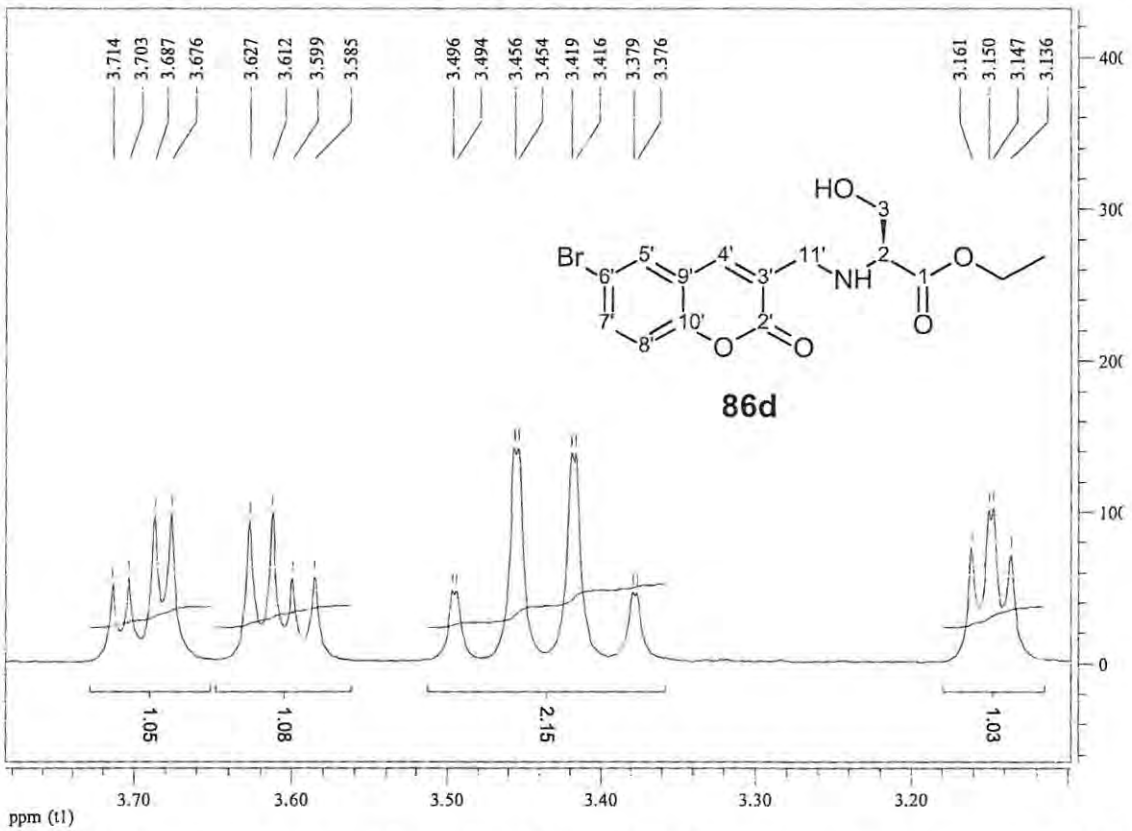


Figure 14. Expanded region of the 400 MHz ^{1}H NMR spectrum of compound **86d** in benzene-d_6.

The complex coupling observed in the ^{1}H NMR spectrum between 3.0 and 4.0 ppm is worthy of further discussion. The double doublet at 3.15 ppm has coupling constants of 4.5 and 5.6 Hz, while the double doublets at 3.60 and 3.69 ppm have coupling constants of 5.7, 10.8 Hz and 4.3, 10.8 Hz respectively. Thus the proton represented by the signal at 3.15 ppm couples to both protons at 3.60 and 3.69 Hz; these in turn couple with each other (J = 10.8 Hz). These couplings led to the assignment of the signal at 3.15 ppm to the methine proton (2-H) at the chiral centre, and the two signals at 3.60 and 3.69 to the two methylene protons on the carbon adjacent to the chiral centre. The two double doublets at 3.39 and 3.47 ppm have strong coupling with each other (J = 16.0 Hz) and also long-range coupling (J = 1.0 Hz) with the proton at C-2. The signals at 3.39 and 3.47 ppm have thus been assigned to the protons on C-11'. The HSQC spectrum (Fig. 15) supports these assignments; both proton signals at 3.39 and 3.47 ppm correlate to the same carbon signal (47.3 ppm), and both proton signals at 3.60 and 3.69 Hz correlate to the carbon signal at 62.8 ppm.

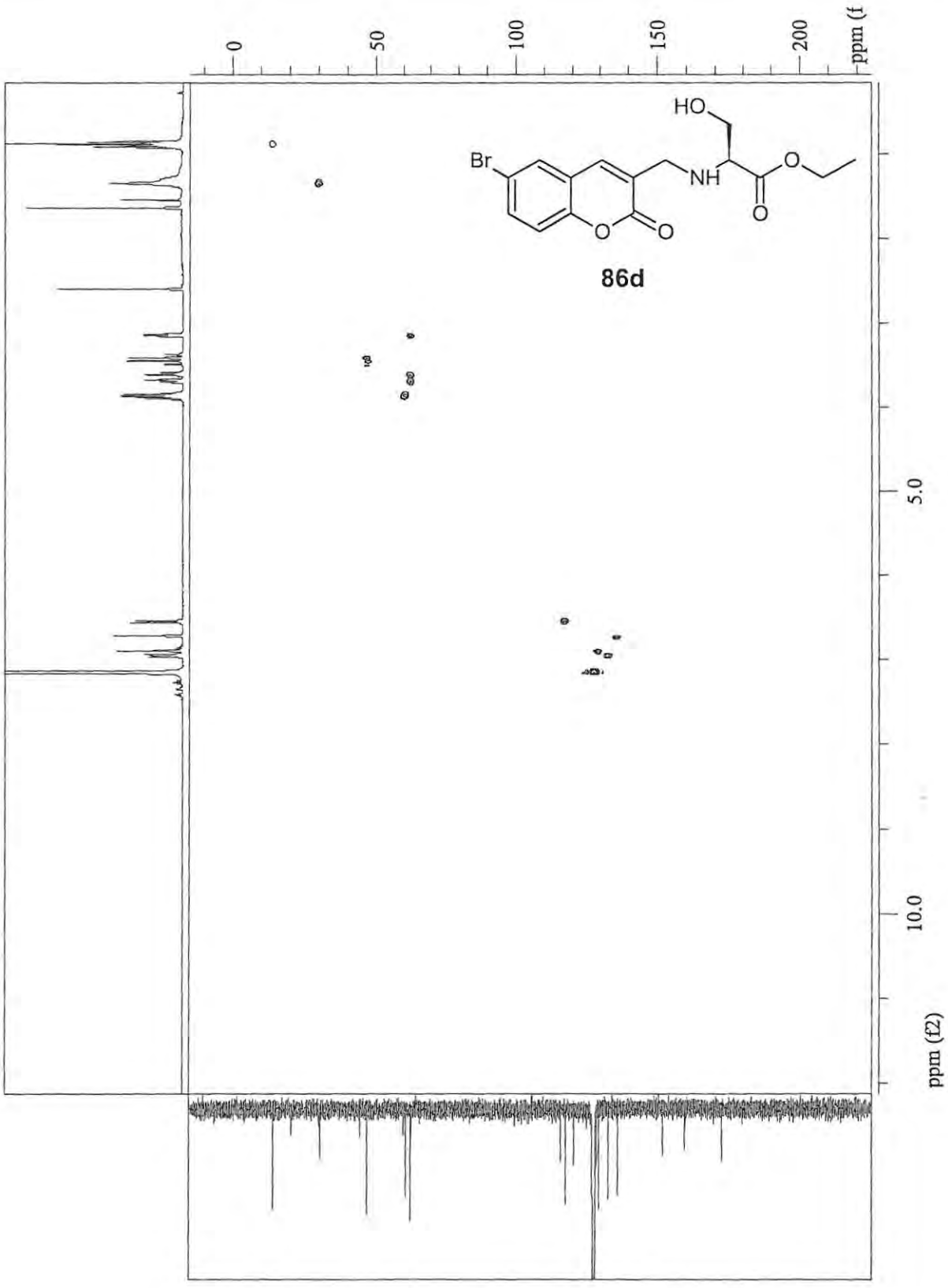
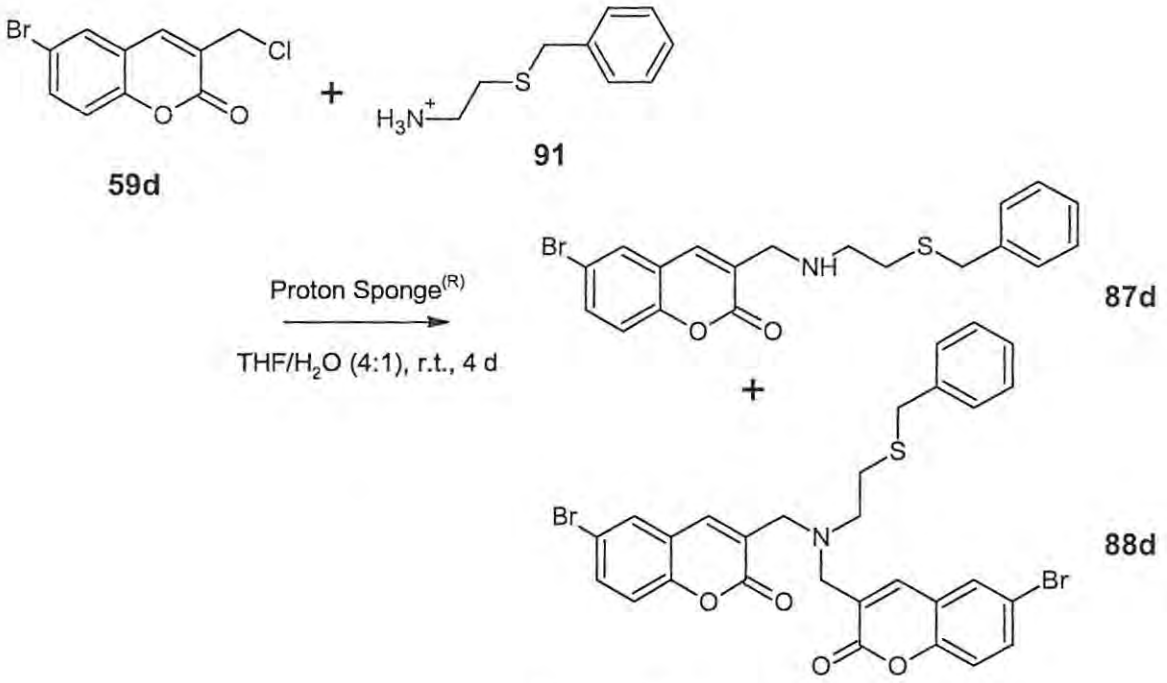


Figure 15. HSQC spectrum of compound **86d** in benzene-d_6.

The 3-(chloromethyl)coumarin derivative **59d** was also reacted with *S*-benzylcysteamine hydrochloride **91** (Scheme 36) and, in this case, both the monocoumarinated and the dicoumarinated amines were formed (**87d** and **88d** respectively). Again, these are novel compounds and have been fully characterised using NMR spectroscopy and high-resolution mass spectrometry. No competing attack at either C-4 or C-2 of the coumarin nucleus was observed in any of these reactions, supporting the earlier observations.[76]



Scheme 36

The signals in the ^{1}H NMR spectrum of compound **87d** (Fig. 16) were assigned as follows. The two triplets at 2.34 and 2.44 ppm correspond to the two methylene groups between the sulfur and nitrogen atoms, while the two singlets at 3.36 and 3.43 correspond to the methylene groups attached to the coumarin ring and to the phenyl ring, respectively. The series of aromatic signals between 6.55 and 7.18 ppm correspond to the eight aromatic protons and the C-4 vinylic proton. The ^{1}H NMR spectrum of compound **88d** (Fig. 17) is very similar, with the exception that the singlet at 3.30 ppm integrates for four protons instead of two, as in the spectrum of **87d**. This singlet at 3.30 ppm thus corresponds to the four C-1' exocyclic methylene protons. Likewise, the aromatic signals in this spectrum integrate to 13 protons, the extra four protons being due to the presence of the second coumarin moiety.

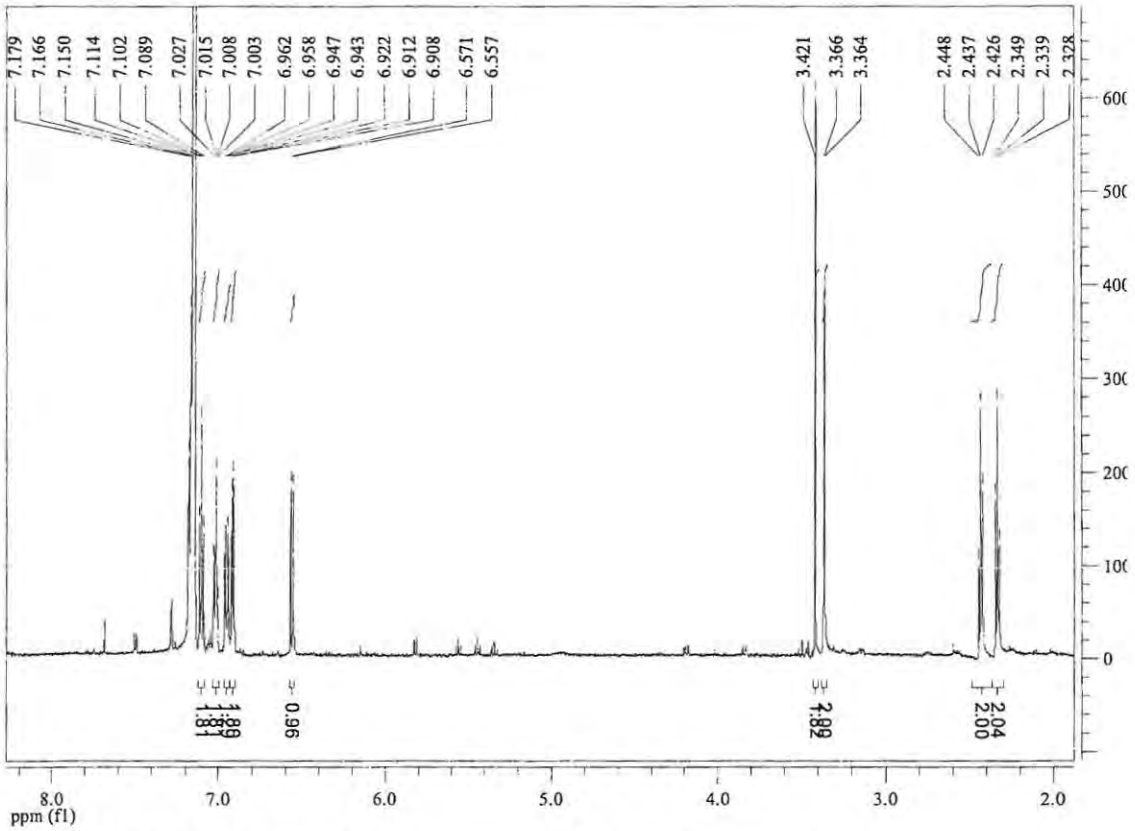


Figure 16. ^{1}H NMR spectrum of compound **87d** in benzene-d_6.

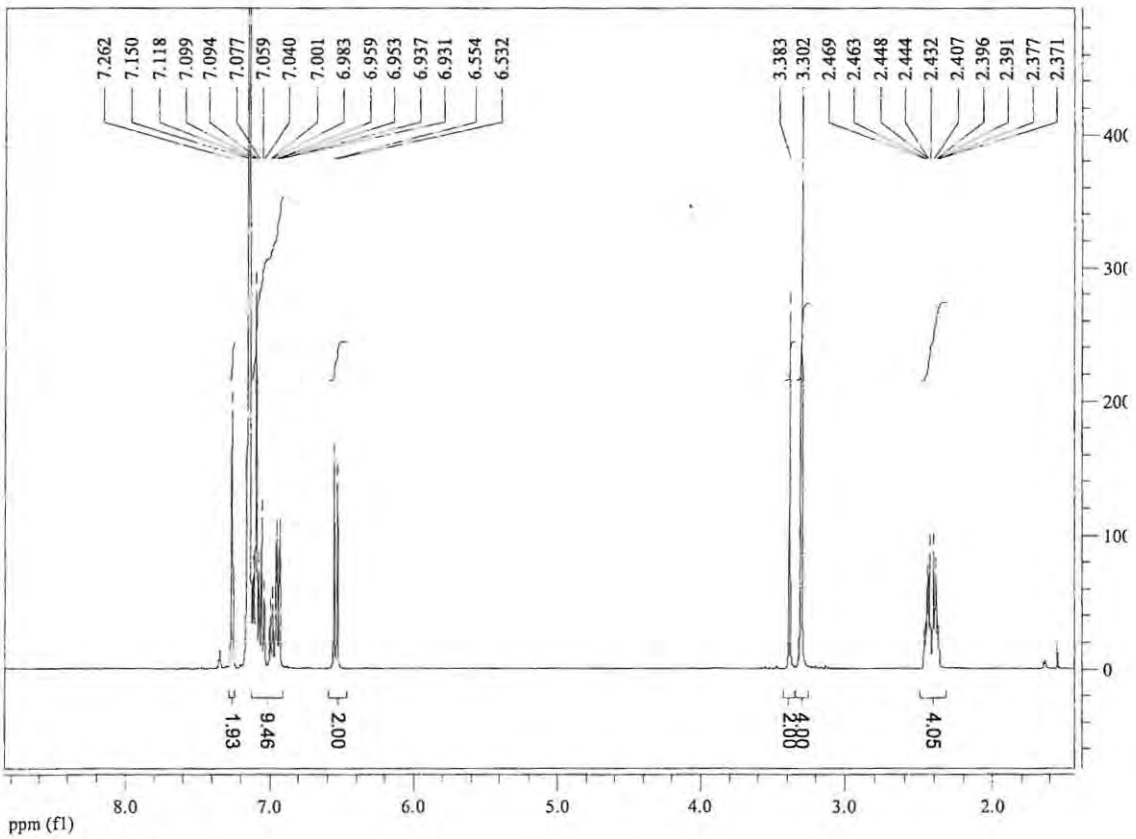
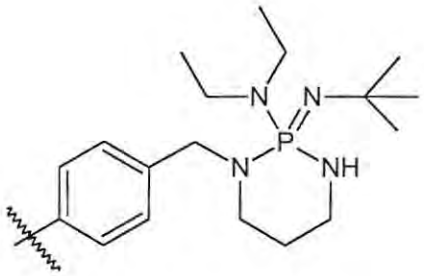


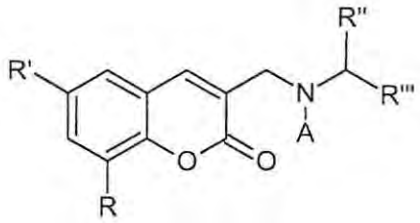
Figure 17. ^{1}H NMR spectrum of compound **88d** in benzene-d_6.

Some difficulty was encountered in removing excess Proton Sponge® from the crude product mixtures; it was not always easily separated from the monocoumarinated amines (**84a-e**, **86d** and **87d**) as it 'tails' badly on silica (in TLC, column chromatography and HPLC). Perhaps the use of a polymer-supported strong, non-nucleophilic base, such as polymer-supported 2-*tert*-butylimino-2-diethylamino-1,3-dimethylperhydro-1,3,2-diaza-phosphorine **92** (PBEMP), which has a pK_{BH+} of 27.5,[79] would simplify purification of the products.



Polymer-supported BEMP **92**

Table 3. List of Novel Compounds synthesised by reaction of amine hydrochlorides with 3-(chloromethyl)coumarin derivatives **59a-e** in the presence of Proton Sponge®.



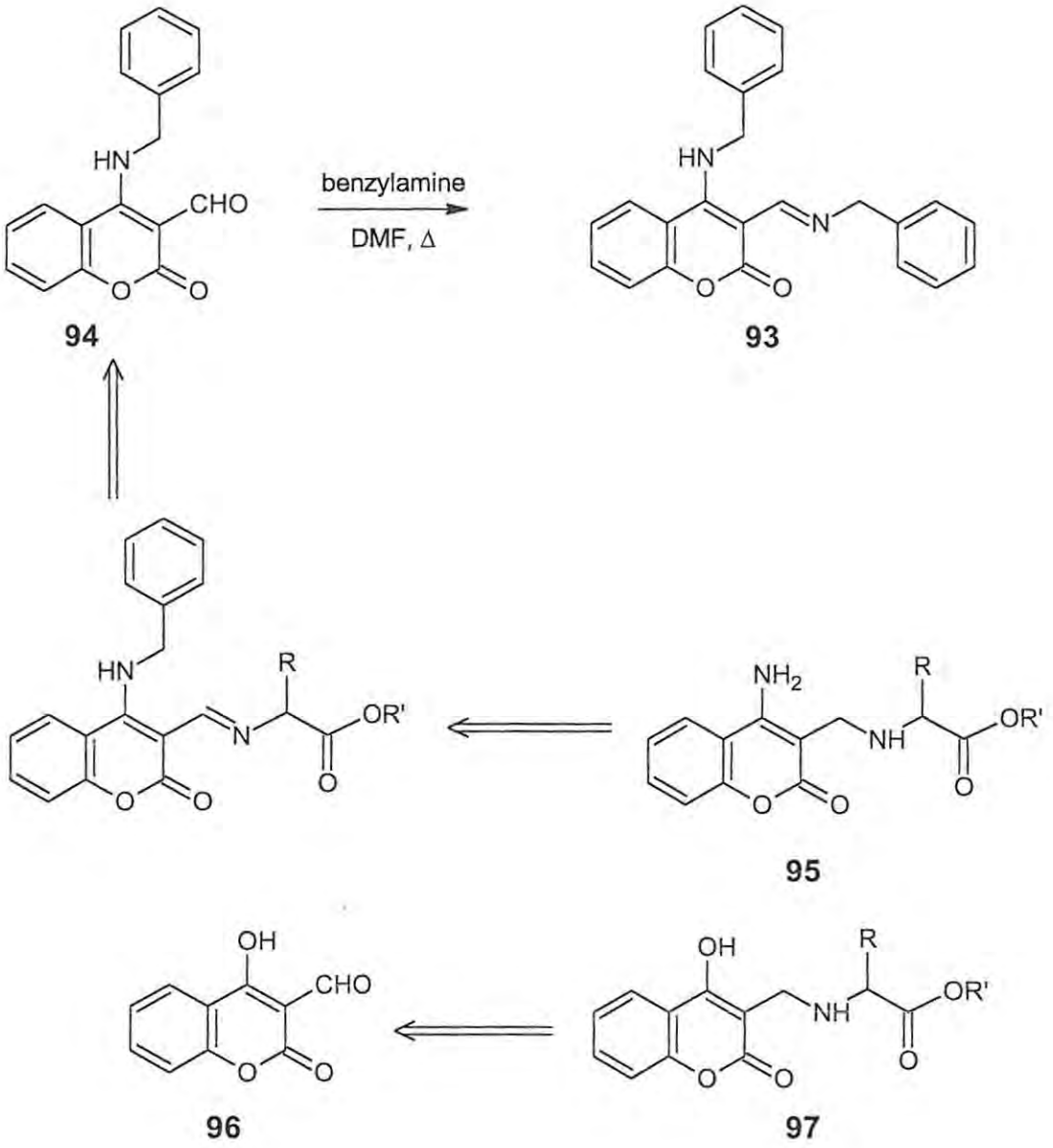
Compound	R	R'	R''	R'''	A	Yield (%)
84a	H	H	H	CO_2Et	H	45
84b	OEt	H	H	CO_2Et	H	22
84c	OMe	H	H	CO_2Et	H	39
84d	H	Br	H	CO_2Et	H	72
84e	H	Cl	H	CO_2Et	H	44
85a	H	H	H	CO_2Et	Coumaryl	32
85b	OEt	H	H	CO_2Et	Coumaryl	6
85c	OMe	H	H	CO_2Et	Coumaryl	18
85d	H	Br	H	CO_2Et	Coumaryl	6
85e	H	Cl	H	CO_2Et	Coumaryl	28
86d	H	Br	CH_2OH	CO_2Et	H	92
87d	H	Br	H	CH_2SCH_2Ph	H	46
88d	H	Br	H	CH_2SCH_2Ph	Coumaryl	12

2.4 Synthetic Approaches to 4-hydroxycoumarin derivatives

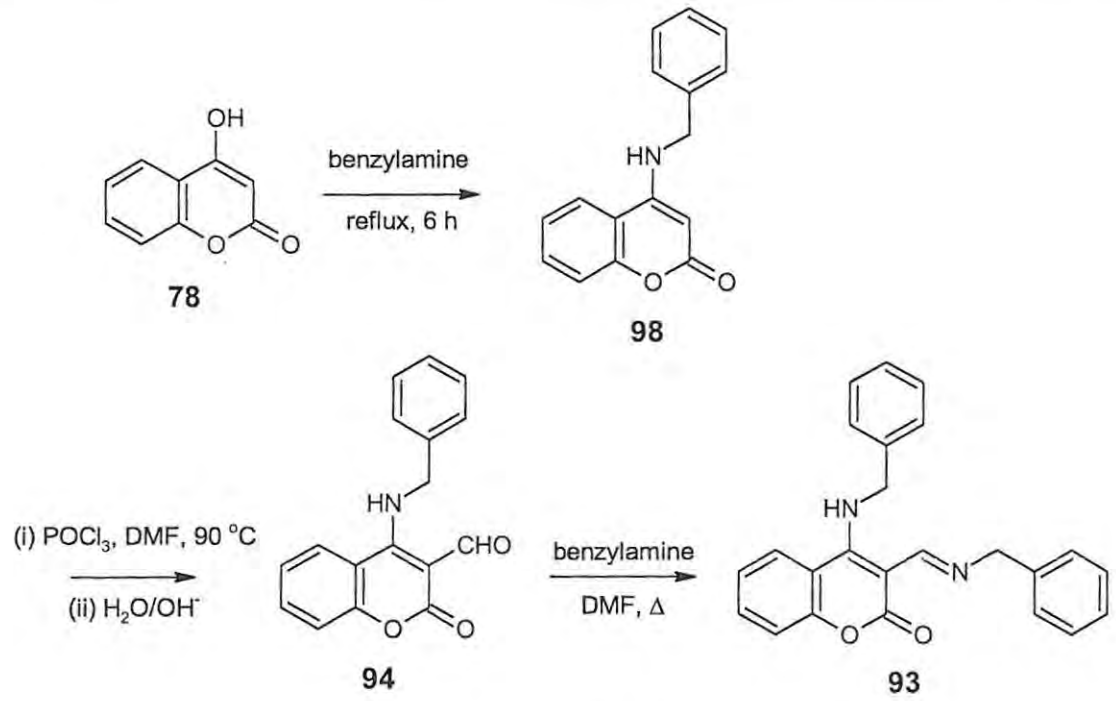
Coumarin derivatives which show useful activity in inhibiting HIV-1 PR are characterised by the presence of a hydroxy group at position 4.[52-58] Consequently, we have sought to develop synthetic approaches to 4-hydroxycoumarin derivatives which are functionalised at position 3 with amino acid-type moieties. 4-Aminocoumarin derivatives are expected to have similar hydrogen-bonding capabilities, and so attention has also been given to synthetic approaches to 4-aminocoumarin derivatives.

Ivanov *et al.*[80] reported a means of synthesising 3-alkylimino-4-(alkylamino)coumarins such as **93** by reacting a suitable primary amine (*e.g.* benzylamine) with 3-formyl-4-(alkylamino)coumarins, such as 4-benzylamino-3-formylcoumarin **94,** by heating the 3-formyl coumarin derivative and the amine in DMF for 6 h. Although Ivanov *et al.* made use of aliphatic and aromatic amines, we anticipated that this procedure could be extended to include amino acid derivatives as substrates for reaction with suitable 3-formylcoumarin derivatives, thus providing access to coumarin derivatives such as **95** and **97**, with peptide-like groups at position C-3, and a group at C-4 (either –OH or $–NH_2$) capable of hydrogen bonding to the Asp-25 residues in the HIV-1 PR active site (Scheme 37).

Tabaković *et al.*,[81] have reported the synthesis of 4-benzylamino-3-formylcoumarin **94** from 4-benzylaminocoumarin **98,** which they synthesised from 4-hydroxycoumarin **78**. (Scheme 38). We achieved the synthesis of 4-benzylaminocoumarin **98** following their procedure, by boiling 4-hydroxycoumarin **78** under reflux in a large excess of benzylamine for 45 min (Scheme 38). The yield reported by Tabaković *et al.* was 67 %. When we carried out this reaction, the yield, after recystallisation, was 58 %. Tabaković *et al.* did not report NMR data for the aminocoumarin **98**;[81] however, a later paper by Conlin and Gear[82] reports ^{1}H NMR chemical shifts which are consistent with those obtained in our analysis (see Fig. 18). Thus the signal at 4.52 ppm was assigned to the two methylene protons adjacent to the nitrogen atom, the singlet at 5.05 ppm to 3-H, and the various aromatic signals between 7.26 and 8.13 were assigned to the nine aromatic protons in the molecule. The broad signal at 8.38 ppm was assigned to the amine proton. Ivanov *et al.* carried out similar reactions with alkylamines, and established that the optimum amine:4-hydroxycoumarin molar ratio was 10:1.[83] More recently, Ivanov and Stoyanov reported the microwave-assisted reaction of benzylamine with 4-hydroxycoumarin, in a molar ratio of 1:1.2, achieving 98 % yield in 2 min, under 850 W microwave irradiation.[84]



Scheme 37



Scheme 38

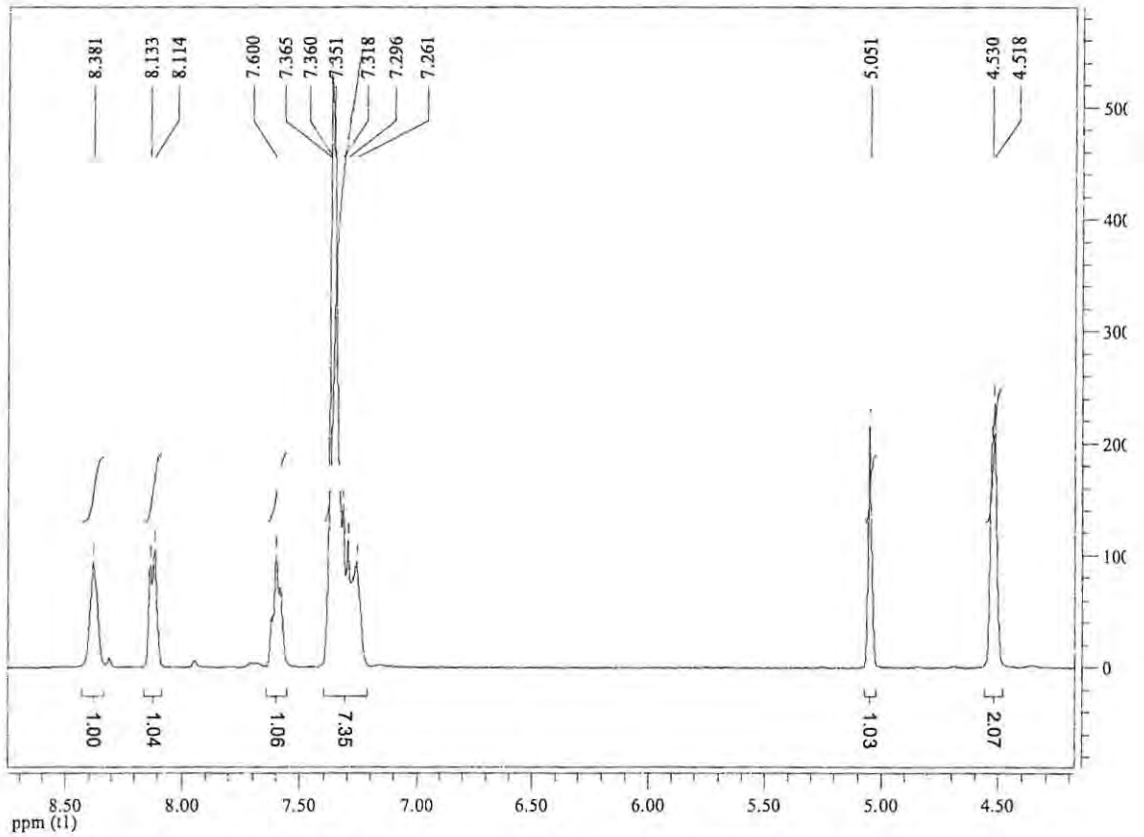
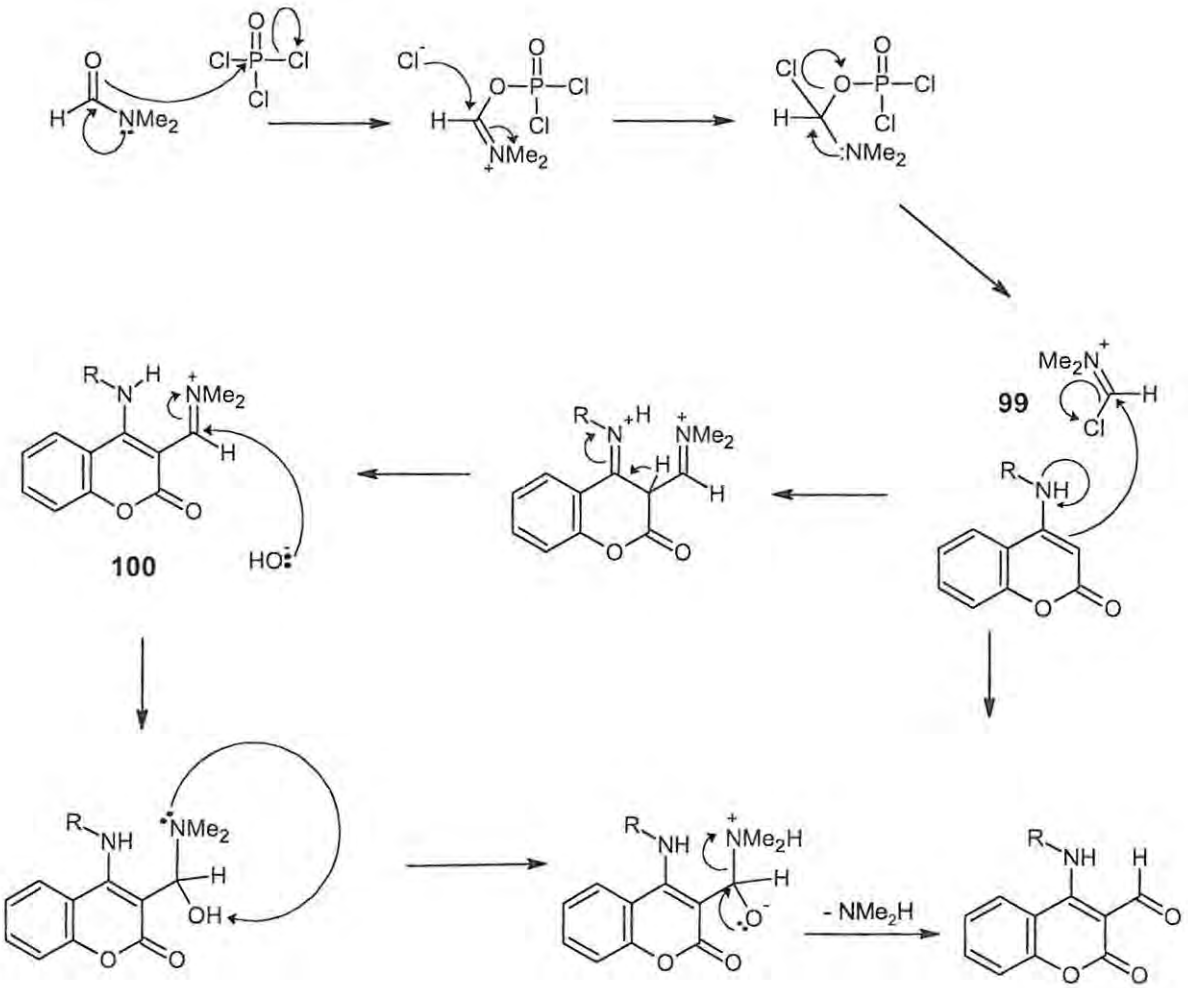


Figure 18: 400 MHz ^{1}H NMR spectrum of compound **98** in DMSO-d_6.

Having synthesised 4-benzylaminocoumarin **98** in reasonable yield, the next step in the synthetic sequence outlined by Ivanov *et al.*[80] was the formylation of **98** under Vilsmeier-

Haack conditions. This reaction involves the formation of an iminium species **99** by the reaction of $POCl_3$ with a suitable formamide (*e.g.* DMF or *N*-methyl-*N*-phenylformamide)[85]; this ion then attacks the double bond of the coumarin to yield intermediate **100**, alkaline hydrolysis of which gives the 3-formyl coumarin derivative (Scheme 39).



Scheme 39

We achieved the formylation of 4-benzylaminocoumarin **98** in 54 % yield following the procedure outlined by Tabaković *et al.*[81] (Scheme 39). NMR spectroscopy confirmed the structure of the product (Fig. 19). Thus, the signal at 5.06 ppm was assigned to the methylene protons adjacent to the nitrogen atom, a broad singlet at 12.31 ppm was assigned to the amine proton, a sharp singlet at 10.17 ppm indicated the presence of the aldehyde, and the aromatic signals correspond to the nine aromatic protons present in compound **94**.

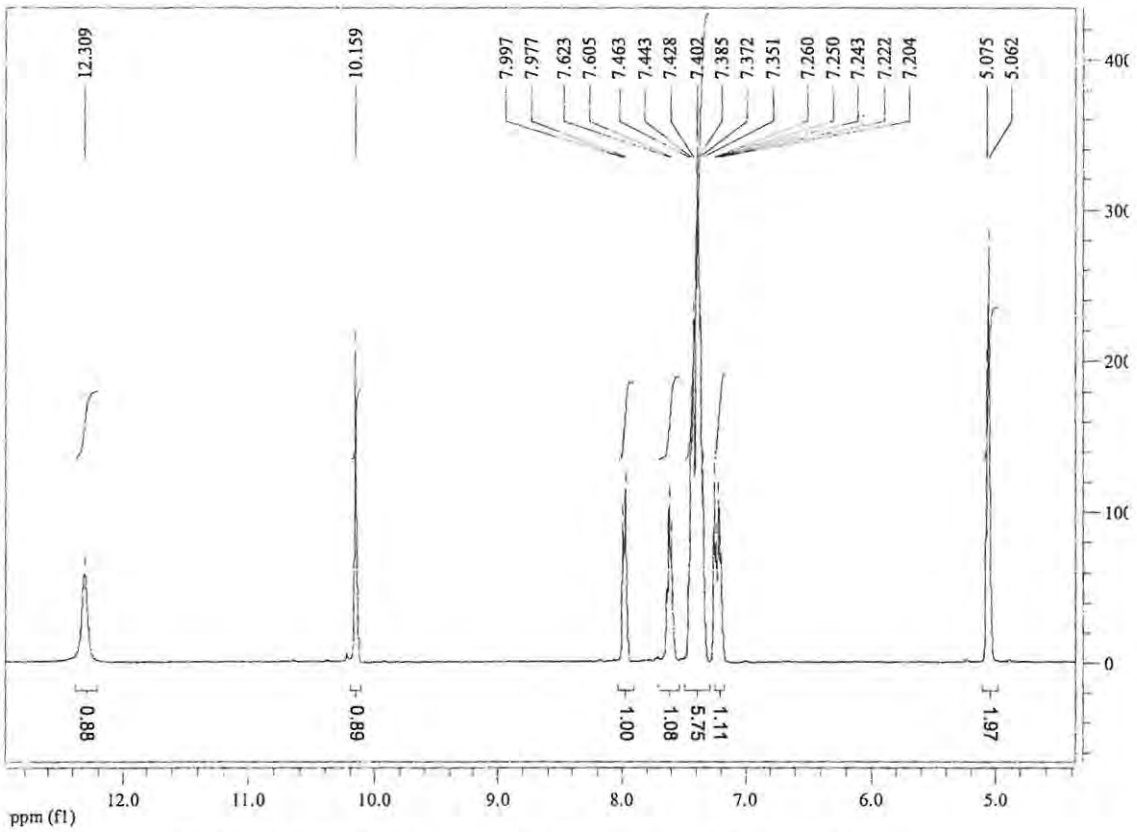
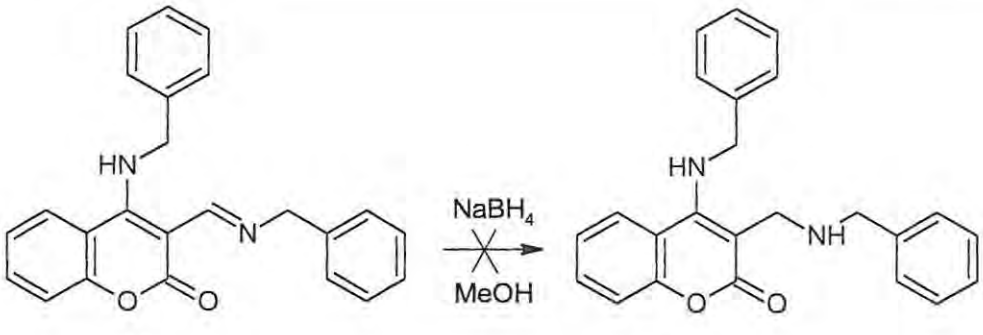


Figure 19. 400 MHz ^{1}H NMR spectrum of compound **94** in $CDCl_3$.

4-benzylamino-3-formylcoumarin **94** was then reacted with benzylamine in DMF, following the procedure reported by Ivanov *et al.*,[80] to yield 4-benzylamino-3-[(*N*-benzylimino)methyl]coumarin **93** in 54 % yield and in sufficient purity not to require recrystallisation (Scheme 39).

The reduction of 4-benzylamino-3-[(*N*-benzylimino)methyl]coumarin **93** to the amine **101** was attempted, using $NaBH_4$ as the reducing agent (Scheme 40), but ^{1}H NMR analysis of the crude product indicated that only starting material was present. Time did not permit repetition of the reduction; a different solvent such as isopropanol (as $NaBH_4$ is slowly decomposed by methanol)[86] or a different reducing agent such as sodium cyanoborohydride might prove more effective. Stronger reducing agents, such as $LiAlH_4$, are known to open the lactone ring and reduce the coumarin double bond,[44] so these are not likely to be suitable for carrying out the required reduction selectively. The formation of imines from 4-benzylamino-3-formylcoumarin **94** (and subsequent reduction to the amines) using amino acid derivatives such as glycine ethyl ester hydrochloride, could also be explored.

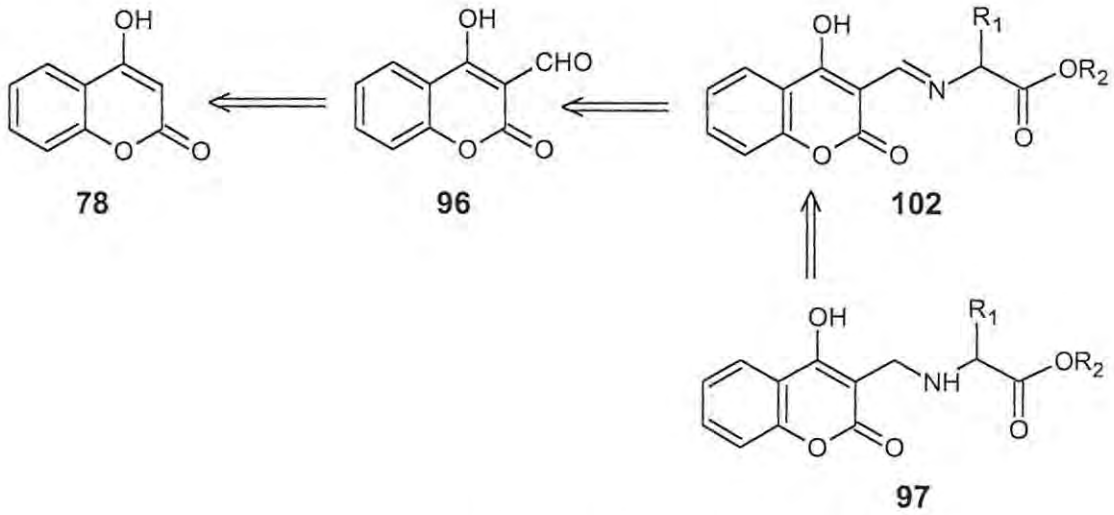


93 **101**

Scheme 40

Attempted formylation of 4-hydroxycoumarin

Given the success of the formylation of 4-benzylaminocoumarin **98** under Vilsmeier-Haack conditions, it was decided to attempt the formylation of 4-hydroxycoumarin in an analogous manner, as this approach might be expected to provide access to the 4-hydroxycoumarin derivatives **97** following the retrosynthetic analysis outlined in Scheme 41.

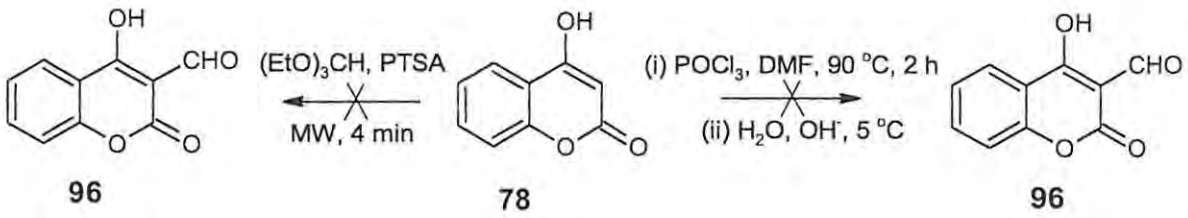


78 **96** **102** **97**

Scheme 41

Four synthetic approaches to 3-formyl-4-hydroxycoumarin **96** were found in the literature.[87-90] Two of these methods were explored: the first, a low-yielding (19 %) Vilsmeier-Haack reaction, involves use of *N*-methyl-*N*-phenylformamide as reactant and chlorobenzene or dioxane as solvent.[87] It was decided to attempt this reaction using available reagents and the same conditions used for the synthesis of 4-benzylamino-3-formylcoumarin **94** (Scheme 42). However, all that was obtained in this reaction was a

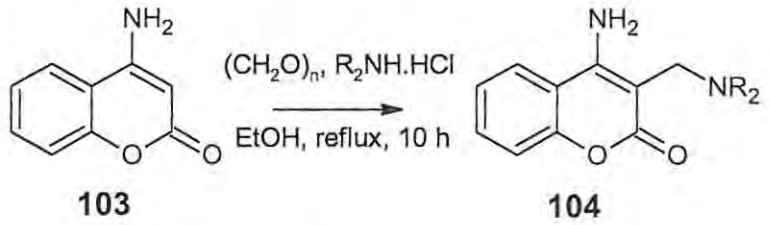
dark-brown insoluble solid, presumably produced by decomposition of the substrate at the elevated (90 °C) temperature. The second approach involved the reaction of 4-hydroxycoumarin **78** with triethyl orthoformate under microwave irradiation (2 min at 240 W, followed by 2 min at 180 W) in the presence of an acid catalyst.[88] This reaction was attempted twice, but both attempts yielded nothing but starting material.



Scheme 42

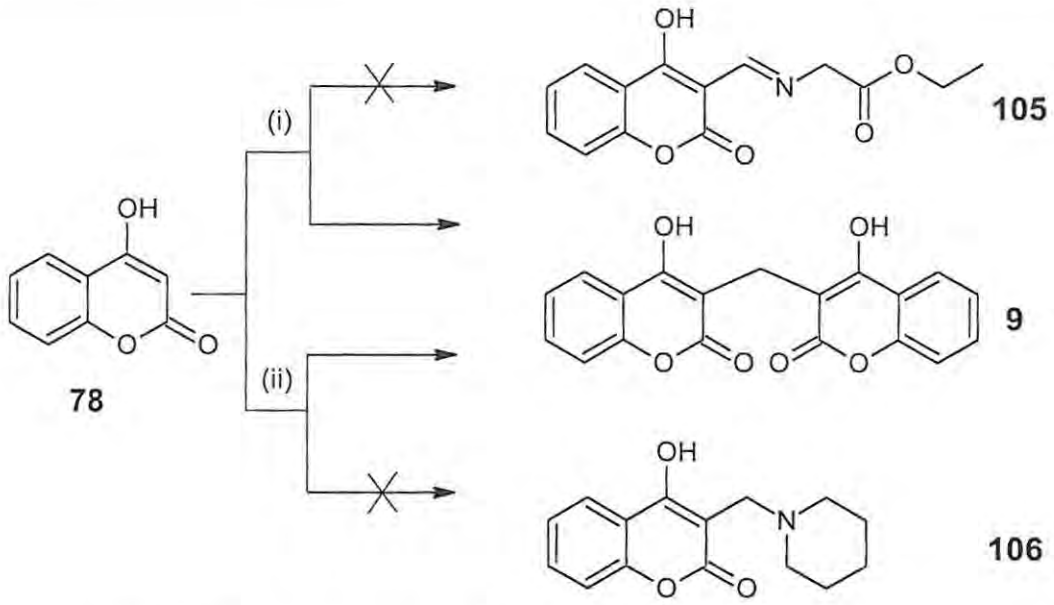
Attempted Mannich reactions on 4-hydroxycoumarin

Ivanov *et al.* successfully used the Mannich reaction to prepare 3-alkylaminomethyl-4-aminocoumarin derivatives **104** from 4-aminocoumarin **103**, paraformaldehyde and secondary alkyl amine hydrochloride salts, by refluxing in anhydrous ethanol for 10 h.[80] These reactions yielded the corresponding 3-alkylaminomethyl-4-aminocoumarin derivatives **104** in yields of 61-84 % (Scheme 43).



Scheme 43

Based on this work, it was decided to attempt a similar reaction using glycine ethyl ester hydrochloride, 4-hydroxycoumarin **78** and paraformaldehyde in anhydrous ethanol (Scheme 44). However, after 24 h reflux, dicoumarol **10** had precipitated out of solution, and none of the expected Mannich base **105** had formed.



(i) $(CH_2O)_n$, glycine ethyl ester.HCl, EtOH, reflux, 12 h

(ii) $(CH_2O)_n$, piperidine.HCl, EtOH, reflux, 15 h

Scheme 44

This approach was repeated using piperidine hydrochloride **107**, but, again, dicoumarol **9** was the only product obtained – a result consistent with earlier observations by Robertson and Link.[91] Based on their conclusions, it seems likely that these reactions failed because amine *hydrochlorides* were used. Robertson and Link have reported the Mannich reaction of primary amines with formaldehyde (as formalin) and 4-hydroxycoumarin, with yields ranging from 49 to 77 %. They also achieved the Mannich reactions of secondary amines such as dimethylamine and piperidine with 4-hydroxycoumarin, *in the absence of an acid catalyst.* In a similar manner, Pigini and co-workers synthesised 4-aminomethyl-3-hydroxycoumarins by the Mannich reaction of free amines (not hydrochloride salts) with 3-hydroxycoumarin.[92] This is in contrast to Ivanov's method for Mannich reactions of 4-aminocoumarin with secondary amine hydrochlorides, which are in effect, acid-catalysed Mannich reactions.

It certainly seems that the use of the Mannich reaction to prepare 3-aminomethyl-4-hydroxycoumarin derivatives should be explored further. Robertson and Link reported the facile synthesis of primary and secondary aminomethyl-4-hydroxycoumarins by this method, which required little or no purification of the products. The difficulty which must be overcome is the neutralisation of the amine hydrochloride salts of the primary amines (perhaps with Proton Sponge® as described earlier), as the acidic conditions lead to

formation of dicoumarol instead of the desired Mannich base. Polymer-supported bases could be especially useful, as they should facilitate isolation of the products.

2.5 Application of NMR shift prediction programmes

The ^{13}C NMR chemical shifts of selected compounds (**59a, 61e, 79b, 83b, 84a, 85a** and **86d**) were investigated using three different NMR prediction programmes. These were the Modgraph Neural Network algorithm, the Modgraph HOSE (Hierarchically Ordered Spherical Description of Environment) algorithm and the ChemWindow ^{13}C prediction facility. These predictions were used, in some cases, to support the ^{13}C signal assignments for the compounds. The accuracy with which each method was able to predict the experimentally-determined chemical shift values was also assessed.

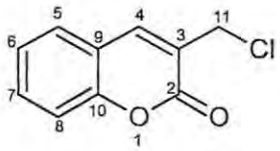
The Modgraph ^{13}C NMR prediction system utilises two different algorithms, and presents the data for both, making recommendations as to which algorithm's prediction for a particular nucleus is likely to be more reliable. The Neural Network algorithm is generally considered to be more reliable than the HOSE algorithm. The HOSE algorithm, developed by Bremser,[93] considers each carbon nucleus in the context of the atoms one bond away; this 'shell' of nuclei one bond away is matched against carbon nuclei with similar 'shells' in a database of known compounds with experimentally determined chemical shifts. Once the first 'shell' has been considered, the atoms two bonds away are considered; these constitute the second shell, and are matched against similar shells in the database, and so on. Thus each carbon's chemical shift is predicted by comparing it to carbons in similar chemical environments in the database of known compounds. This method is only considered to be reliable if at least three shells are used to make a prediction.[94] If compounds are not well-represented in the database by similar shells, then the Neural Network algorithm, which is more general, is better at predicting chemical shifts accurately.

The chemical shift predictions for each of the selected compounds are presented below in tabular format. Although Modgraph applies solvent corrections to ^{1}H NMR predictions, no such facility is available for the ^{13}C NMR predictions. The predicted values have been compared to the experimentally-determined values by calculating RMS values. In all cases, the Neural Network algorithm provided the best predictions overall, as measured by the RMS values. However, in some cases, the other algorithms (HOSE, ChemWindow) provided better predictions for specific carbon nuclei. For compound **59a**, the predictions were generally fairly accurate for all three algorithms, with the exception of nuclei C-4 and

C-11 (the exocyclic methylene carbon); all three algorithms were quite inaccurate in predicting the chemical shifts of these nuclei. The predictions for the C-4 nucleus in the other molecules evaluated were also widely varied. All three algorithms had difficulty prediciting the chemical shifts of the nuclei in compound **83b**; the inaccuracies were particularly apparent in the chemical shift predictions for the chiral centres C-3 and C-4. The predictions for the chiral centres in compound **79b** were slightly more accurate, however. In general the predictions for compound **61e** were fairly accurate; the same can be said for the 3-(aminomethyl)coumarins **84a**, **85a** and **86d**.

Table 4. Experimental (100 MHz) and Predicted ^{13}C NMR Chemical Shift Data (δ/ppm) for 3-(chloromethyl)coumarin **59a** (Experimental values obtained in $CDCl_3$ solution).*

Nucleus	A	B	C	D
C2	160.1	159.4	161.4	162.0
C3	125.0	128.7	131.9	130.9
C4	141.1	133.9	123.2	135.3
C5	128.0	128.4	127.9	126.6
C6	124.7	124.8	120.9	125.2
C7	132.0	131.7	132.4	128.1
C8	116.7	117.3	116.8	121.3
C9	118.8	121.6	118.2	127.8
C10	153.5	153.3	149.8	150.8
C11	41.0	46.8	45.3	45.1
RMS values		**3.3**	**6.5**	**4.6**



* A = Experimentally-determined ^{13}C Chemical Shift values, B = Modgraph Neural Network values, C = Modgraph HOSE values, D = ChemWindow values.

Table 5. Experimental (100 MHz) and Predicted ^{13}C NMR Chemical Shift Data (δ/ppm) for *t*-butyl 3-(5-chloro-2-hydroxyphenyl)-3-hydroxy-2-methylenepropanoate **61e** (Experimental values obtained in DMSO-d_6 solution).*

Nucleus	A	B	C	D
C1	164.9	167.1	167.0	165.0
C2	144.8	141.9	137.1	142.8
C3	64.5	63.2	74.9	69.1
C4	122.0	126.9	126.9	122.1
C5	80.0	82.1	80.9	73.6
C6	27.5	28.4	28.4	29.1
C7	27.5	28.4	28.4	29.1
C8	27.5	28.4	28.4	29.1
C9	131.3	129.0	135.1	127.4
C10	153.3	154.4	152.9	155.2
C11	116.5	116.8	115.0	117.2
C12	127.5	131.3	130.1	127.6
C13	122.9	125.1	125.5	126.5
C14	126.8	129.1	128.5	130.1
RMS values		**2.3**	**4.1**	**2.9**

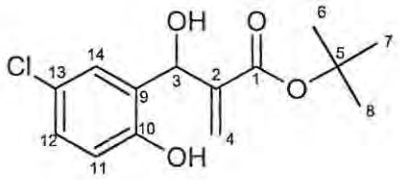
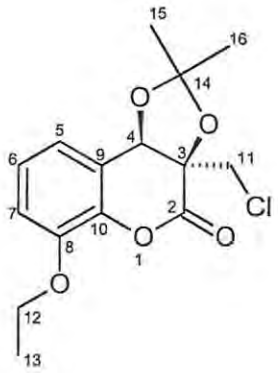


Table 6. Experimental (100 MHz) and Predicted ^{13}C NMR Chemical Shift Data (δ/ppm) for (3*R*,4*R*)-3-chloromethyl-3,4-*O*-isopropylidene-8-ethoxycoumarin **83b** (Experimental values obtained in $CDCl_3$ solution).*

Nucleus	A	B	C	D
C2	166.6	no value	171.0	171.5
C3	79.5	91.5	84.4	102.5
C4	76.1	76.3	81.0	83.7
C5	121.5	119.3	127.0	120.7
C6	125.2	128.5	125.6	125.0
C7	115.5	118.6	115.7	111.4
C8	147	146.2	154.1	150.8
C9	111.4	117.6	130.3	134.6
C10	118.2	143.5	152.9	139.0
C11	43.4	50.6	45.2	42.6
C12	65	64.5	65.5	65.1
C13	14.7	13.8	14.7	14.3
C14	127.5	111.3	108.6	97.6
C15	27.2	27.2	27.3	28.6
C16	27.2	27.2	27.3	28.6
RMS values		**8.8**	**11.7**	**12.9**



* A = Experimentally-determined ^{13}C Chemical Shift values, B = Modgraph Neural Network values, C = Modgraph HOSE values, D = ChemWindow values.

Table 7. Experimental (100 MHz) and Predicted ^{13}C NMR Chemical Shift Data (δ/ppm) for (3*R*,4*R*)-3-chloromethyl-3,4-dihydroxy-8-ethoxycoumarin **79b** (Experimental values obtained in MeOD-d_4 solution).*

Nucleus	A	B	C	D
C2	173.1	174.1	171.0	171.5
C3	83.4	72.7	77.3	96.6
C4	73.5	77.2	72.7	78.3
C5	120.1	120.3	131.9	120.7
C6	122.1	128.1	125.6	125.0
C7	113.5	117.8	115.7	111.4
C8	147.7	145.4	154.1	150.8
C9	126.4	120.5	127.0	134.6
C10	145.5	143.0	152.9	139.0
C11	49.7	54.2	46.2	44.5
C12	65.8	64.5	65.5	65.1
C13	15.2	13.8	14.7	14.3
RMS values		4.6	5.1	5.5

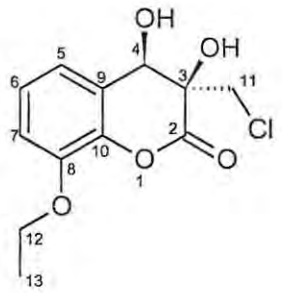
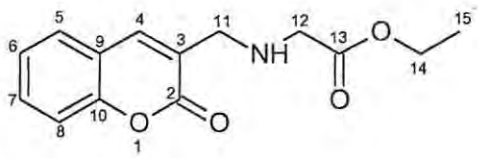


Table 8. Experimental (100 MHz) and Predicted ^{13}C NMR Chemical Shift Data (δ/ppm) for ethyl *N*-[(2*H*-1-benzopyran-2-on-3-yl)methyl]glycinate **84a** (Experimental values obtained in benzene-d_6 solution).*

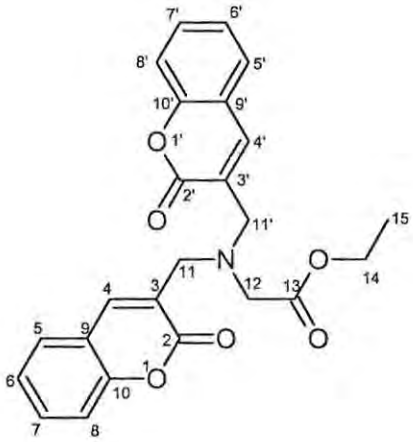
Nucleus	A	B	C	D
C2	160.5	162.7	161.4	162.0
C3	127.5	125.9	131.9	130.4
C4	137.5	136.0	123.2	132.9
C5	128.4	128.4	127.9	126.6
C6	123.8	121.6	118.2	125.2
C7	130.5	131.7	132.4	128.1
C8	116.4	117.3	116.8	121.3
C9	119.7	124.8	120.9	127.8
C10	153.6	153.3	149.8	150.8
C11	50.7	45.6	49.5	49.0
C12	48.4	51.8	48.0	52.9
C13	171.9	171.4	169.4	171.0
C14	60.5	61.2	61.2	59.2
C15	14.2	14.7	14.1	13.6
RMS values		2.4	4.5	3.4



* A = Experimentally-determined ^{13}C Chemical Shift values, B = Modgraph Neural Network values, C = Modgraph HOSE values, D = ChemWindow values.

Table 10. Experimental (100 MHz) and Predicted ^{13}C NMR Chemical Shift Data (δ/ppm) for ethyl *N,N*-bis[(2*H*-1-benzopyran-2-on-3-yl)methyl]glycinate **85a** (Experimental values obtained in benzene-d_6 solution).**

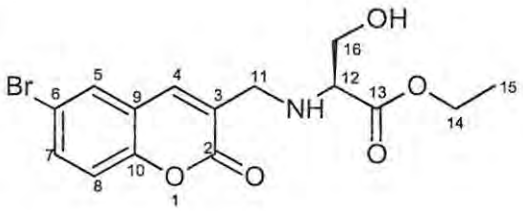
Nucleus	A	B	C	D
C2	160.0	162.4	161.4	162.0
C3	130.2	129.9	131.9	130.4
C4	138.6	138.0	123.2	132.9
C5	127.5	128.4	127.9	126.6
C6	118.0	124.8	120.9	125.2
C7	133.6	131.7	132.4	128.1
C8	116.6	117.3	116.8	121.3
C9	121.0	121.6	118.2	127.8
C10	152.5	153.3	149.8	150.8
C11	53.7	53.6	55.0	53.8
C12	55.1	57.6	53.4	57.7
C13	170.1	171.5	171.2	171.0
C14	60.5	61.2	59.3	59.2
C15	14.2	14.7	14.1	13.6
C2'	160.0	162.4	161.4	162.0
C3'	130.2	129.9	131.9	130.4
C4'	138.6	138.0	123.2	132.9
C5'	127.5	128.4	127.9	126.6
C6'	118.0	124.8	120.9	125.2
C7'	133.6	131.7	132.4	128.1
C8'	116.6	117.3	116.8	121.3
C9'	121.0	121.6	118.2	127.8
C10'	152.5	153.3	149.8	150.8
C11'	53.7	53.6	55.0	53.8
RMS values		**2.3**	**4.8**	**4.0**



* A = Experimentally-determined ^{13}C Chemical Shift values, B = Modgraph Neural Network values, C = Modgraph HOSE values, D = ChemWindow values.

Table 9. Experimental (100 MHz) and Predicted ^{13}C NMR Chemical Shift Data (δ/ppm) for ethyl (2*S*)-*N*-[(2*H*-1-benzopyran-2-on-3-yl)methylamino]3-hydroxypropanoate **86d** (Experimental values obtained in benzene-d_6 solution).*

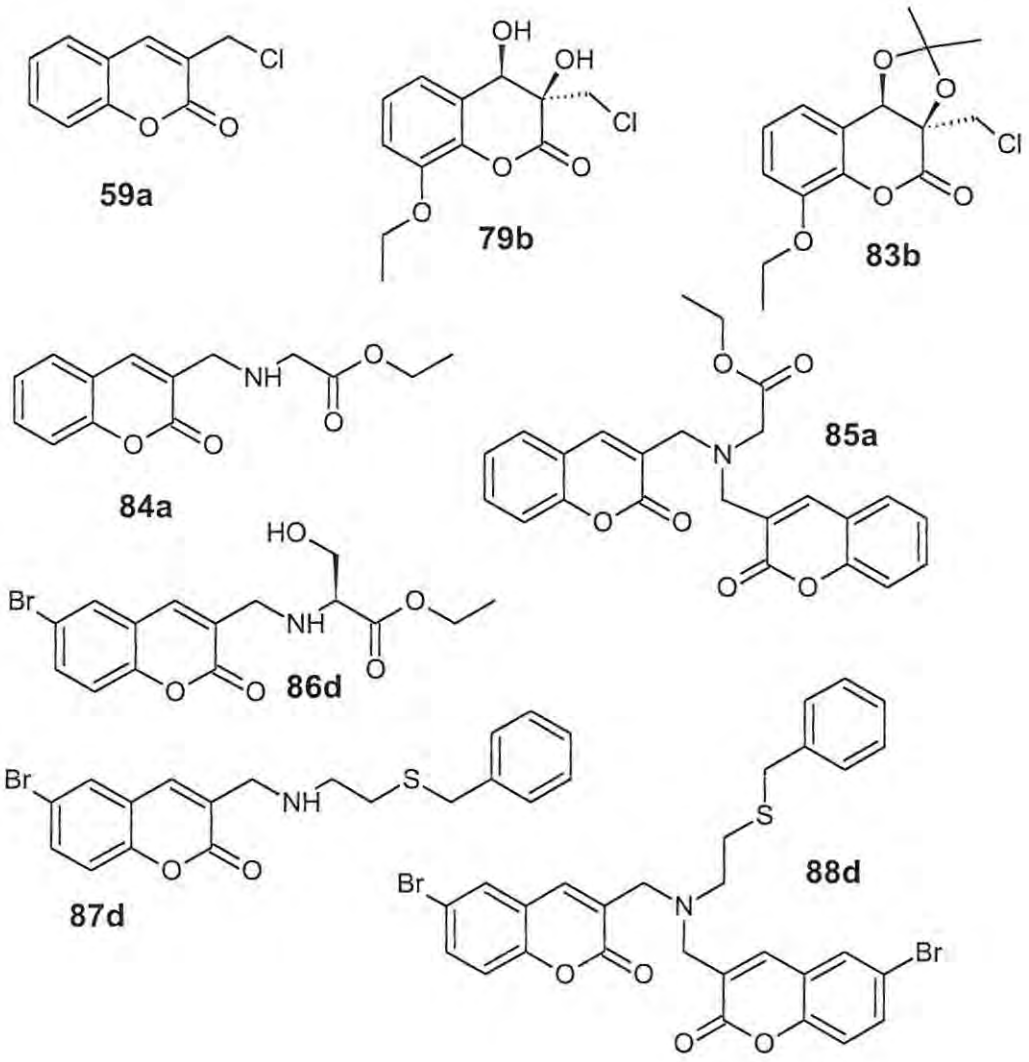
Nucleus	A	B	C	D
C2	159.9	163.5	161.4	162.0
C3	128.9	124.4	131.9	130.4
C4	130	135.7	123.2	132.9
C5	136.4	129.8	130.0	129.9
C6	121	121.2	116.0	119.8
C7	133.3	134.4	133.0	131.4
C8	118.1	120.1	116.2	123.5
C9	116.4	120.5	119.6	130.0
C10	152.3	153.1	151.6	149.8
C11	47.3	44.3	50.2	46.8
C12	62.9	62.6	76.6	64.7
C13	172.7	173.0	171.3	172.0
C14	61.1	61.8	61.3	59.5
C15	14.1	14.7	14.0	13.6
C16	62.8	62.7	62.0	64.8
RMS values		**3.1**	**4.7**	**4.4**



* A = Experimentally-determined ^{13}C Chemical Shift values, B = Modgraph Neural Network values, C = Modgraph HOSE values, D = ChemWindow values.

2.6 Computer Modelling of Enzyme-Inhibitor Interactions

The Cerius[2] LigandFit Module was used to investigate enzyme-inhibitor interactions of selected compounds synthesised in the present study, *viz.*, **59a, 79b, 83b, 84a, 85a** and **86d, 87d** and **88d**.

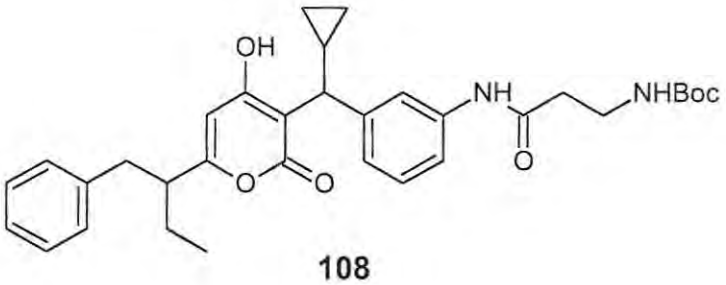


The ligand fitting process used in the LigandFit Module consists of four steps: these are **Site Search, Conformational Search, Ligand Fitting** and **Ligand Scoring**.[95] The **Site Search** step is used to define the protein binding site. There are two methods of doing this; the first is based on the protein shape, and searches for suitable cavities in the protein's 3D structure, while the second is based on the shape of a ligand already docked in the protein. If the second method (Docked Ligand) is to be used, then a structure of the protein-ligand complex is needed (this is usually determined experimentally, using X-ray crystallography or NMR). The **Conformational Search** step searches for stable conformations of the

ligand, using a Monte Carlo method. Only torsion angles are randomised in this search; bond lengths and bond angles are not altered. The **Ligand Fitting** step fits newly-generated conformations of the ligand into the protein binding site. This is an iterative process, whereby successive conformations of the ligand, and the various possible orientations of these conformations in the binding site, are examined. A docking score for each possible combination of conformation and orientation is computed. The docking score is described as "the negative value of the non-bonded intermolecular energy between the ligand and the protein."[95] If the docking score for a particular orientation is better than the previous value, that orientation is saved, and the process is repeated until the maximum number of trials is reached. Once this has happened, Rigid Body Minimisation is applied to the saved conformations to optimise the docking scores further. **Ligand Scoring** is then carried out. This process computes a general score for a particular conformation of a ligand, called LIGSCORE. The three descriptors[95] used to calculate LIGSCORE are:

i) **vdW** – a softened Lennard-Jones 6-9 potential;
ii) **C+pol** – a count of the buried polar surface area between the protein and the ligand. It involves attractive protein-ligand interactions; and
iii) **Totpol^2** – a count of the buried polar surface area between protein and ligand, involving attractive and repulsive protein-ligand interactions.

The protein model used for this work was downloaded from the Protein Data Bank (PDB code 2UPJ), and is a crystallographically-determined structure of HIV-1 PR complexed with the synthetic inhibitor **108**.[55] This protein model was chosen because the synthetic inhibitor **108** has structural characteristics such as the lactone ring and the amide-type groups, which are similar to the synthesised compounds **84a, 85a, 86d** and **87d**. Because the enzyme is held rigid during ligand fitting (to simplify calculations) it is necessary to use a protein model which is complexed with a similar ligand to those being evaluated. This ensures that the active site has a shape and size similar to that which would be adopted by the enzyme if it were complexed with the ligand being fitted.



108

The selected ligands were constructed using the Cerius^2 Model Builder, and docked using the LigandFit module. (Details of the parameters used for this process can be found in the Experimental section.) LIGSCOREs were computed for each conformation of each ligand, and the 100 best fits reported. The results are presented in Table 11. Ligand **88d** was found, by this method, to have the most promising interactions with the HIV-1 PR enzyme. The hydrogen-bonding interactions between ligand **88d** and the HIV-1 PR active site residues were also calculated; hydrogen-bonds which are less than 3.5 Å in length are shown in Fig. 20 and Fig. 21. Hydrogen-bonding is predicted between Ile-50, Ile-50' and the lactone oxygen of **88d**, and also between Asp-29', Arg-8 and the bromine atoms of **88d**.

For the sake of comparison, the 4-hydroxy analogues of the synthesised compounds **59a, 84a, 85a, 86d, 87d** and **88d** were also fitted into the active site of HIV-1 PR. It was decided to compare the LIGSCOREs of these (theoretical) compounds with the LIGSCOREs of the synthesised compounds. The results are presented in Table 12. Ligand **85a–4OH** (the 4-OH analogue of compound **85a**) was found, by this method, to have the most promising interaction with HIV-1 PR; the hydrogen-bonding interactions (less than 3.5 Å in length) between ligand **85a–4OH** and the HIV-1 PR active site residues were also calculated, and are shown in Fig. 22 and Fig. 23. Ligand **88d–4OH** also scored very well; this compound scored well in both the 4-H and 4-OH form, suggesting that it has other structural characteristics besides the presence or absence of the 4-OH group, that enable it to interact favourably with the enzyme's active site. The introduction of the hydroxyl groups resulted in higher ligand scores for all the compounds, confirming that the introduction of these groups to the synthesised coumarins may result in increased potency as inhibitors of the protease enzyme.

Table 11. LIGSCOREs for selected synthesised compounds.

Compound	Average LIGSCORE	Number of conformations reported	Highest LIGSCORE	Lowest LIGSCORE
79b	2.90	2	**3.17**	2.62
93	2.81	14	**3.60**	2.07
59a	0.98	3	**0.88**	1.15
83b	1.75	2	**1.76**	1.74
84a	1.86	10	**2.23**	1.57
85a	3.00	32	**4.00**	2.20
86d	2.62	11	**2.86**	1.91
87d	2.03	9	**2.34**	1.77
88d	3.40	68	**4.79**	2.31

Table 12. LIGSCOREs for 4-OH analogues of synthesised compounds.

Compound	Average LIGSCORE	Number of conformations reported	Highest LIGSCORE	Lowest LIGSCORE
59a-4OH	1.50	2	**2.00**	1.00
84a-4OH	2.42	6	**2.87**	1.72
85a-4OH	3.95	54	**5.72**	2.80
86d-4OH	2.71	14	**3.52**	1.63
87d-4OH	2.95	9	**3.38**	2.21
88d-4OH	4.01	78	**5.56**	2.52

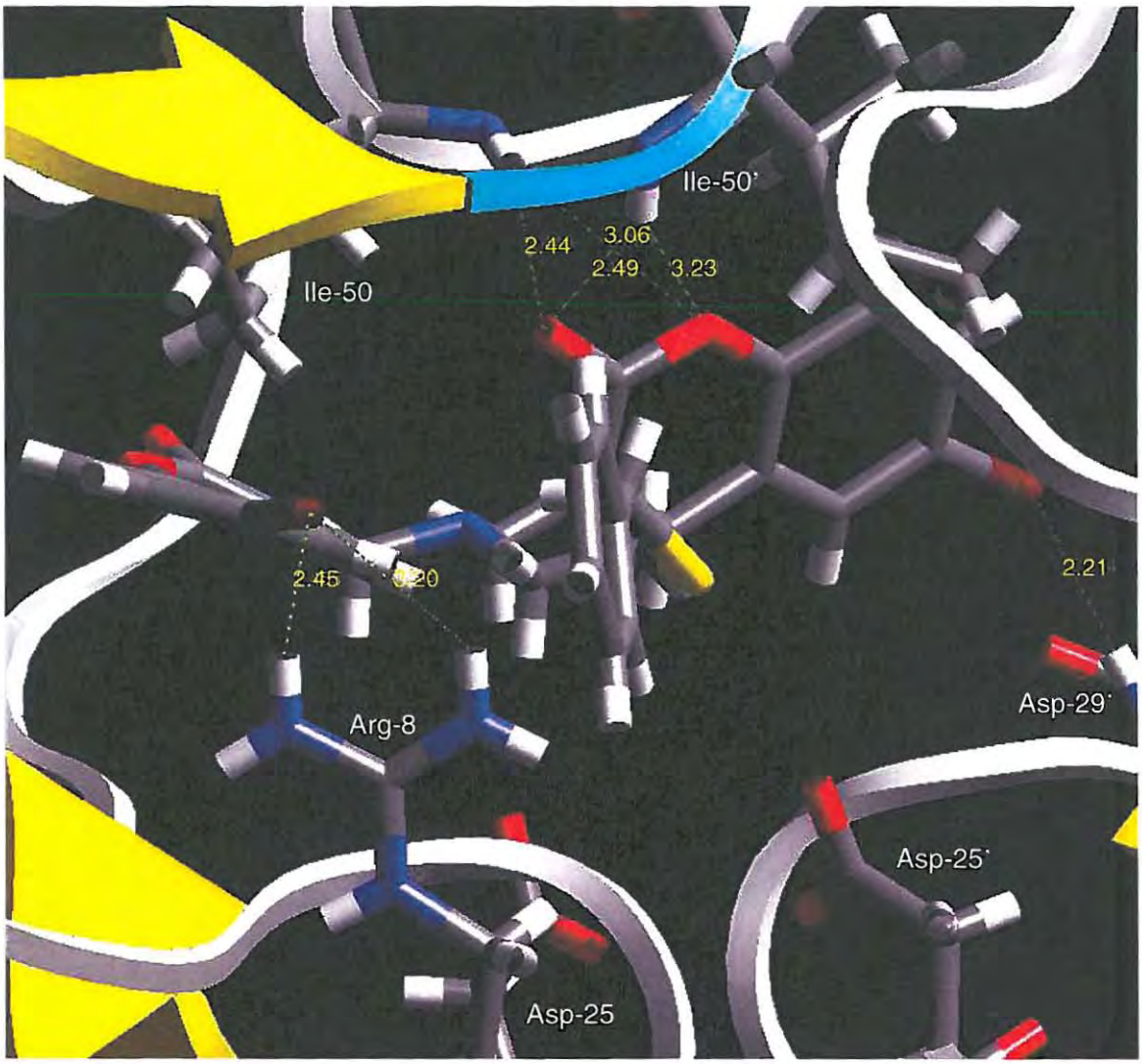


Figure 20. Compound **88d** docked in the active site of HIV-1 PR. Hydrogen-bonding interactions are indicated by dashed yellow-green lines. Hydrogen-bonding distances are given in angstroms. Residues which have hydrogen-bonding interactions with the inhibitor at a distance of less than 3.5 Å have been shown; all other residues have been omitted for clarity.

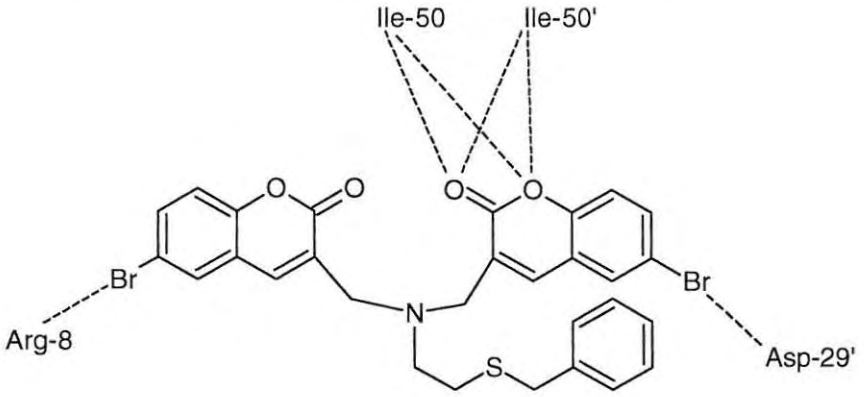


Figure 21. A simplified schematic diagram showing the hydrogen-bonding interactions between the inhibitor **88d** and the active site of HIV-1 PR, as determined by LigandFit.

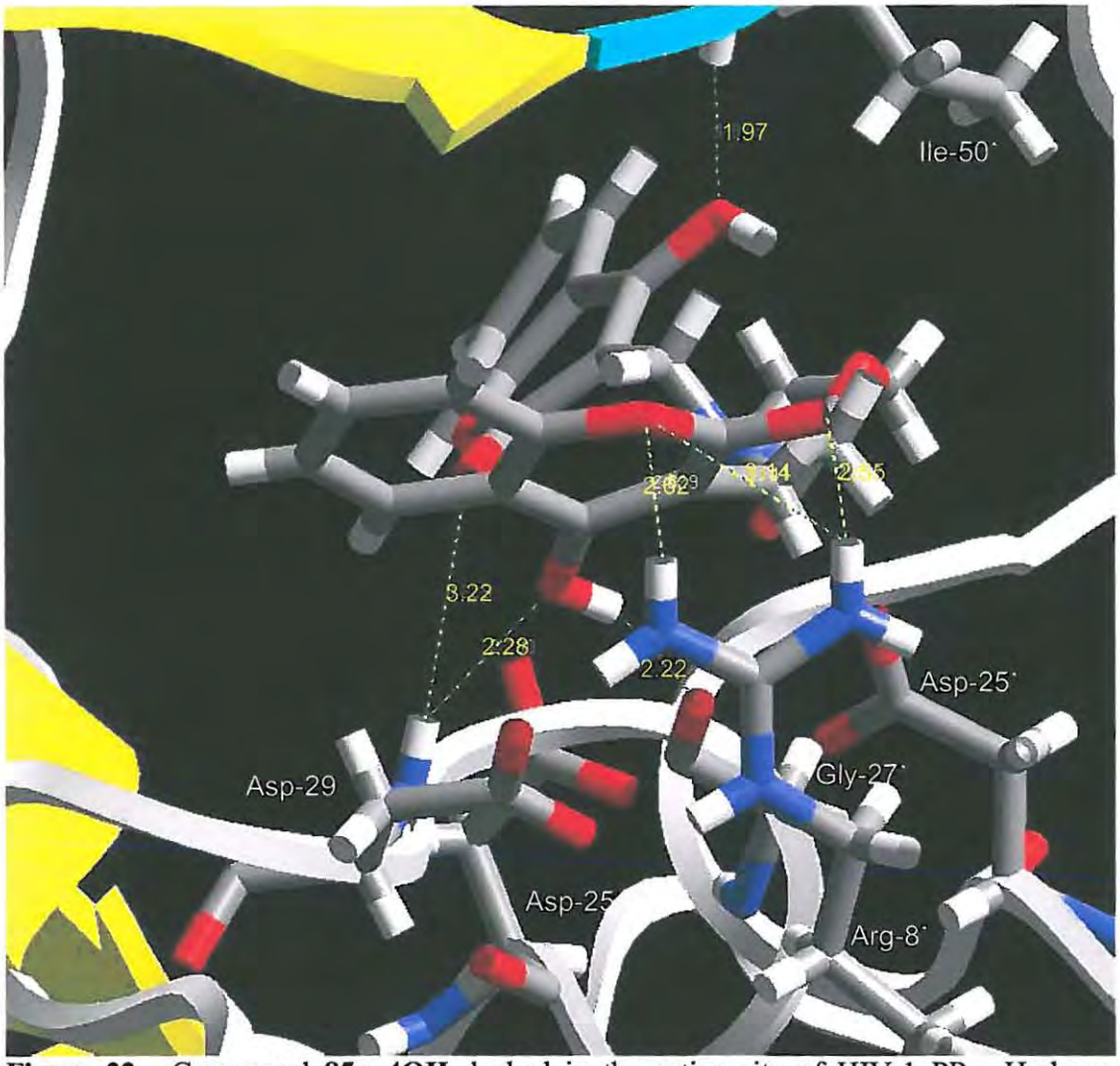


Figure 22. Compound **85a–4OH** docked in the active site of HIV-1 PR. Hydrogen-bonding interactions are indicated by dashed yellow-green lines. Hydrogen-bonding distances are given in angstroms. Residues which have hydrogen-bonding interactions with the inhibitor at a distance of less than 3.5 Å have been shown; all other residues have been omitted for clarity.

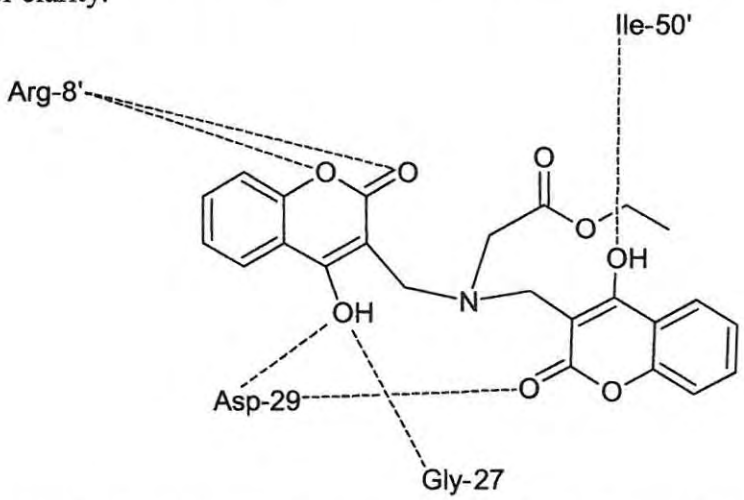
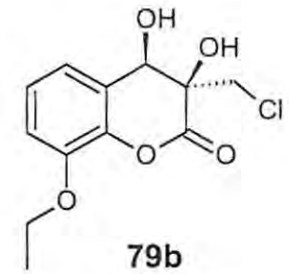


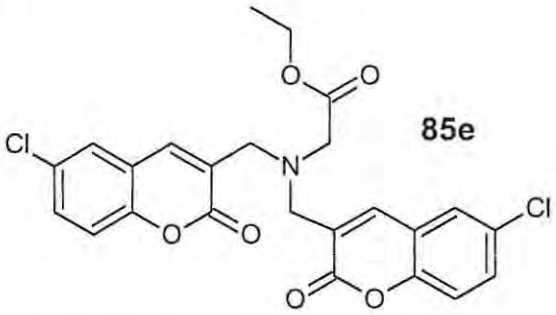
Figure 23. A simplified schematic diagram showing the hydrogen-bonding interactions between the inhibitor **85a – 4OH** and the active site of HIV-1 PR, as determined by LigandFit.

2.7 Enzyme Inhibition Assays

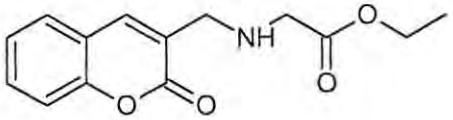
Representative compounds were selected for enzyme inhibition assays. These compounds (**79b, 84a, 85e** and **87d**) are representative of the different classes of compounds synthesised, and it was hoped that the results of these enzyme inhibition assays would give an indication of the inhibitory potential of the compounds synthesised.



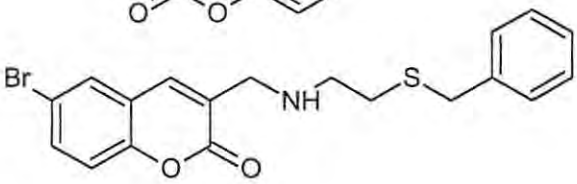
79b



85e



84a



87d

The HIV-1 PR enzyme was obtained from BACHEM and prepared in an aqueous buffer solution. Likewise, the substrate (HIV Protease Substrate III, H-His-Lys-Ala-Arg-Val-Leu-*p*-nitro-Phe-Glu-Ala-Nle-Ser-NH_2)[96] was prepared in aqueous buffer. The substrate dependence assay was performed effectively, with UV detection of the reactions, at 300 nm. However, when it was attempted to dissolve the inhibitors (**79b, 84a, 85e** or **87d**) in DMSO or MeOH, prior to dilution with the aqueous buffer, the compounds proved insoluble in both DMSO and MeOH, at 0.1 mg/mL. Unfortunately the assays had to be abandoned at this point. However, if hydrochloride salts of compounds **84a, 85e** and **87d** were to be prepared, these would probably prove soluble, allowing assessment of the inhibitory activity of these compounds.

2.8 Conclusions and Recommendations for Future Work

The various aims identified at the outset of the study have largely been achieved. Thus, Baylis-Hillman methodology has been successfully applied in the synthesis of the coumarin derivatives **59a-e**. The simple coumarin nucleus has been extended with peptide-like groups by the substitution of suitable amino acid esters, affording the novel compounds **84a-e, 85a-e, 86d, 87d** and **88d.** These compounds were fully characterised by NMR spectroscopy and mass spectrometry. The functionalisation at C-4 of the coumarin **1** and the coumarin derivative **59b** was attempted using a variety of synthetic approaches. Dihydroxylation of coumarin **1** and 3-(chloromethyl)-8-ethoxycoumarin **59b** was achieved, although time constraints did not permit dehydration to the 4-hydroxycoumarin derivative **80b** to be explored. Approaches to 4-hydroxycoumarin derivatives using 4-hydroxycoumarin **78** as starting material were investigated, and the 4-benzylaminocoumarin derivatives **93, 94** and **98** were prepared, but a number of attempts at preparing 3-formyl-4-hydroxycoumarin **96**, and 4-hydroxycoumarin Mannich bases proved unsuccessful. Computer modelling of possible enzyme-inhibitor interactions has been carried out, and has indicated that the compounds **85a** and **88d** could demonstrate potency as inhibitors of HIV-1 PR. NMR chemical shift prediction modules have been used to confirm ^{13}C NMR chemical shift assignments of selected synthesised compounds, and the efficacy of these modules has been assessed. Finally, while enzyme inhibition assays were attempted, the insolubility of the synthesised compounds proved problematic, preventing completion of the assays.

Recommendations for future work include the following.

i) Exploration of synthetic approaches to Baylis-Hillman-derived 4-hydroxycoumarin derivatives, either by dihydroxylation and subsequent dehydration of the 3-(chloromethyl)coumarin derivatives, or by oxidation of the Baylis-Hillman products prior to cyclisation to the coumarin derivatives.

ii) Further investigation into 3-(aminomethyl)coumarin derivatives, with regard to extension of the peptide-like chain, and possible functionalisation of the coumarin aromatic ring.

iii) Synthesis of 3-methylaminocoumarins using amino acid ester substrates, from 3-formylcoumarin derivatives.

iv) Exploration of the usefulness of the Mannich reaction in the preparation of 4-hydroxycoumarin analogues.

v) Preparation of hydrochloride salts of compounds synthesised in this study to enhance aqueous solubility, and thus facilitate enzyme inhibition assays.

3. EXPERIMENTAL

3.1 General details

The reagents used in this project were supplied by Aldrich, and were used without further purification. Thin layer chromatography was carried out using Merck silica gel 60 PF_{254} plates, while flash chromatography was carried out using Merck silica gel 60 (particle size 0.040 – 0.063 mm). High Performance Liquid Chromatography was carried out using a Partisil 10 column and an R401 refractive index detector.

Low resolution mass spectra were obtained at Rhodes University on a Finnegan Mat GCQ spectrometer. High resolution mass spectra were acquired by Mr Tommie van der Merwe at the University of the Witwatersrand. NMR spectra were recorded on a Bruker 400 MHz AVANCE spectrometer or a Bruker Biospin 600 MHz spectrometer, and were referenced using solvent signals (δ_H: 7.26 ppm for $CDCl_3$, 2.50 ppm for DMSO-d_6, 3.31 ppm for MeOD and 7.15 ppm for C_6D_6; δ_C: 77.0 ppm for $CDCl_3$, 39.43 ppm for DMSO-d_6, 49.05 ppm for MeOD and 128.02 ppm for C_6D_6). A Perkin-Elmer FT-IR Spectrum 2000 spectrometer was used to record all IR spectra, using KBr discs, nujol mulls or thin films. Melting points were determined using a Reichert 281313 hot-stage apparatus, and are uncorrected.

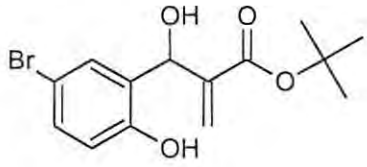
Solvents were purified according the methods described by Perrin and Armarego.[97] Hence, ethyl acetate and hexane were distilled before use. Chloroform was washed with 10 % aqueous NaOH, then with brine, and then dried ($MgSO_4$) before use. EtOH was distilled from magnesium ethoxide, generated *in situ* from Mg turnings with I_2 as catalyst. Likewise, MeOH was distilled from magnesium methoxide, generated from Mg turnings with I_2 catalyst, THF was distilled from sodium wire and benzophenone, DMF was distilled under reduced pressure after standing over 3A molecular sieves overnight, and pyridine was used after standing over 3A molecular sieves for one hour.

Table 12. List of compounds prepared.

Compound	**Page No.**
3,3'-methylene-bis(4-hydroxycoumarin) **9**	99
3-(chloromethyl)coumarin **59a**	80
3-chloromethyl-8-ethoxycoumarin **59b**	81
3-chloromethyl-8-methoxycoumarin **59c**	81
6-bromo-3-(chloromethyl)coumarin **59d**	82
6-chloro-3-(chloromethyl)coumarin **59e**	83
t-Butyl 3-(5-bromo-2-hydroxyphenyl)-3-hydroxy-2-methylenepropanoate **61d**	79
t-Butyl 3-(5-chloro-2-hydroxyphenyl)-3-hydroxy-2-methylenepropanoate **61e**	79
(3*R*,4*R*)-3,4-dihydroxychroman-2-one **77**	84
(3*R*,4*R*)-3-chloromethyl-3,4-dihydroxy-8-ethoxycoumarin **79b**	85
(3*R*,4*R*)-3-chloromethyl-3,4-*O*-isopropylidene-8-ethoxycoumarin **83b**	85
Ethyl *N*-[(2*H*-1-benzopyran-2-on-3-yl)methyl]glycinate **84a**	88
Ethyl *N*-[(8-ethoxy-2*H*-1-benzopyran-2-on-3-yl)methyl]glycinate **84b**	89
Ethyl *N*-[(8-methoxy-2*H*-1-benzopyran-2-on-3-yl)methyl]glycinate **84c**	90
Ethyl *N*-[(6-bromo-2*H*-1-benzopyran-2-on-3-yl)methyl]glycinate **84d**	91
Ethyl *N*-[(6-chloro-2*H*-1-benzopyran-2-on-3-yl)methyl]glycinate **84e**	92
Ethyl *N,N*-bis[(2*H*-1-benzopyran-2-on-3-yl)methyl]glycinate **85a**	88
Ethyl *N,N*-bis[(8-ethoxy-2*H*-1-benzopyran-2-on-3-yl)methyl]glycinate **85b**	89
Ethyl *N,N*-bis[(8-methoxy-2*H*-1-benzopyran-2-on-3-yl)methyl]glycinate **85c**	90
Ethyl *N,N*-bis[(6-bromo-2*H*-1-benzopyran-2-on-3-yl)methyl]glycinate **85d**	91
Ethyl *N,N*-bis[(6-chloro-2*H*-1-benzopyran-2-on-3-yl)methyl]glycinate **85e**	92
Ethyl (2*S*)-*N*-[(2*H*-1-benzopyran-2-on-3-yl)methylamino]3-hydroxypropanoate **86d**	93
2-Benzylthio-*N*-[(6-bromo-2*H*-1-benzopyran-2-on-3-yl)methyl]ethylamine **87d**	94
2-benzylthio-*N,N*-bis[(6-bromo-2*H*-1-benzopyran-2-on-3-yl)methyl]ethylamine **88d**	94
4-Benzylamino-3-benzyliminomethylcoumarin **93**	97
4-Benzylamino-3-formylcoumarin **94**	96
4-Benzylaminocoumarin **98**	96
Piperidine Hydrochloride **107**	99

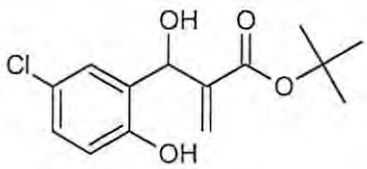
3.2 Synthesis of Baylis-Hillman-derived coumarins

***t-Butyl 3-(5-bromo-2-hydroxyphenyl)-3-hydroxy-2-methylenepropanoate* 61d**



A solution of 5-bromosalicylaldehyde (5.0 g, 25 mmol), *t*-butyl acrylate (5.05 g, 39.4 mmol) and DABCO (2.33 g, 20.7 mmol) in $CHCl_3$ (8.7 mL) was sealed in a round-bottomed flask and stirred at room temperature for 25 days. The precipitated solid was filtered off in a sintered glass funnel, yielding *tert*-butyl 3-(5-bromo-2-hydroxyphenyl)-3-hydroxy-2-methylenepropanoate **61d** as a white powder (4.33 g, 54 %); m.p. 189-191 °C (lit.[35] 186-188 °C); ν_{max} (KBr)/cm^{-1}: 3299, 3165 (2 x OH) and 1685 (C=O); δ_H (400 MHz, DMSO-d_6) 1.31 [9H, s, $C(CH_3)_3$], 5.49 (1H, br s, OH), 5.64 and 6.05 (2H, 2 x s, $C=CH_2$), 5.65 (1H, s, C*H*OH), 6.75 (1H, d, J = 8.5 Hz, Ar-H), 7.20 (2H, m, Ar-H) and 9.72 (1H, br s, Ar-OH); δ_C (100 MHz, DMSO-d_6) 27.5 [$C(CH_3)_3$], 64.5 (CHOH), 80.0 [$C(CH_3)_3$], 109.6, 117.1, 122.9, 129.7, 130.4, 131.8, 144.8 and 153.8 (C=C and Ar-C) and 164.9 (C=O); *m/z* 328 [M^+ (^{79}Br), 10 %] and 211 (100).

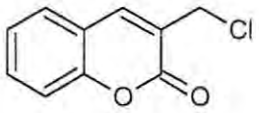
***t-Butyl 3-(5-chloro-2-hydroxyphenyl)-3-hydroxy-2-methylenepropanoate* 61e**



A solution of 5-chlorosalicylaldehyde (2.0 g, 12.8 mmol), *t*-butyl acrylate (2.59 g, 20.2 mmol) and DABCO (1.19 g, 10.6 mmol) in $CHCl_3$ (4.5 mL) was sealed in a round-bottomed flask and stirred at room temperature for 26 days. The precipitated solid was filtered off, yielding tert-*butyl 3-(5-chloro-2-hydroxyphenyl)-3-hydroxy-2-methylenepropanoate* **61e** as a white powder (786 mg, 22 %); m.p. 171-173 °C (Found: $\mathbf{M^+}$, 284.08336. $C_{14}H_{17}O_4{}^{35}Cl$ requires ***M***,

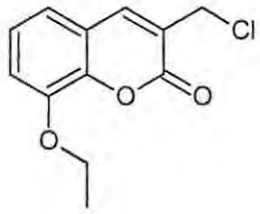
284.08154); ν_{max} (KBr)/cm^{-1}: 3299, (OH) and 1716 (C=O); δ_H (400 MHz, DMSO-d_6) 1.32 [9H, s, C(CH$_3$)$_3$], 5.52 (1H, br s, OH), 5.64 and 6.05 (2H, 2 x s, C=CH$_2$), 5.67 (1H, s, C*H*OH), 6.79 (1H, d, J = 8.4 Hz, Ar-H), 7.09 (2H, m, Ar-H) and 9.73 (1H, br s, Ar-OH); δ_C (100 MHz, DMSO-d_6) 27.5 [C(*C*H$_3$)$_3$], 64.5 (CHOH), 80.0 [*C*(CH$_3$)$_3$], 116.5, 122.0, 122.9, 126.8, 127.5, 131.3, 144.8 and 153.3 (C=C and Ar-C), 164.9 (C=O); *m/z* 284 (M$^+$, 17 %) and 210 (100).

***3-Chloromethylcoumarin* 59a**



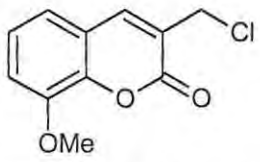
A solution of salicylaldehyde (7.5 g, 61 mmol), *t*-butyl acrylate (12.2 g, 97 mmol) and DABCO (5.7 g, 51 mmol) in $CHCl_3$ (21 mL) was sealed in a round-bottomed flask and stirred at room temperature for 18 d. The crude reaction mixture was shown, by ^{1}H NMR analysis, to contain the Baylis-Hillman adduct **61a**, and was used without further purification. The solvent and *t*-butyl acrylate were removed *in vacuo*, and AcOH (34 mL) and HCl (11-M, 67.5 mL) were added to the mixture, which was boiled and stirred under reflux for 2 h. After cooling to room temperature, ice-water (100 mL) was added, and stirring continued for a further 12 h. The precipitated solid was filtered off and then dissolved in $CHCl_3$. The resulting solution was dried ($MgSO_4$), and the $CHCl_3$ removed *in vacuo*. The crude product was purified by flash chromatography [on silica gel; elution with hexane:EtOAc (9:1)] to afford 3-(chloromethyl)coumarin **59a** as beige crystals (3.12 g, 26 % yield for both steps); m.p. 116-120 °C (lit.[34] 108-110 °C); ν_{max} (nujol)/cm^{-1}: 1690 (C=O); δ_H (400 MHz, $CDCl_3$) 4.55 (2H, s, CH_2Cl), 7.29-7.56 (4H, series of multiplets, Ar-H) and 7.88 (1H, s, 4-H); δ_C (100 MHz, $CDCl_3$) 41.0 (CH_2) 116.7, 118.8, 124.7, 125.0, 128.0, 132.0, 141.1 and 153.5 (C=C and Ar-C), and 160.1 (C=O); *m/z* 194 (M$^+$, 34 %) and 159 (100).

***3-Chloromethyl-8-ethoxycoumarin* 59b**



A solution of 3-ethoxysalicylaldehyde (6.81 g, 41 mmol), *t*-butyl acrylate (8.31 g, 65 mmol) and DABCO (3.83 g, 34 mmol) in $CHCl_3$ (14 mL) was sealed in a round-bottomed flask and stirred at room temperature for 26 d. The crude reaction mixture was shown, by ^1H NMR analysis, to contain the Baylis-Hillman intermediate **61b**, and was used without further purification. The solvent and *t*-butyl acrylate were removed *in vacuo*, and AcOH (22.5 mL) and HCl (11-M, 45 mL) were added to the mixture, which was boiled and stirred under reflux for 2 h. After cooling to room temperature, ice-water (120 mL) was added, and stirring continued for a further 12 h. The precipitated solid was filtered off and then dissolved in $CHCl_3$. The resulting solution was dried ($MgSO_4$), and the $CHCl_3$ removed *in vacuo*. The crude product was purified by flash chromatography [on silica gel; elution with hexane:EtOAc (19:1, then 9:1)] to afford 3-chloromethyl-8-ethoxycoumarin **59b** as beige crystals (2.03 g, 21 % yield for both steps); m.p. 128-129 °C (lit.[34] 122-124 °C); ν_{max} (nujol)/cm^{-1}: 1694 (C=O); δ_H (400 MHz, $CDCl_3$) 1.50 (3H, t, J = 7.0 Hz, OCH_2CH_3), 4.19 (2H, q, J = 7.0 Hz, OCH_2CH_3), 4.56 (2H, s, CH_2Cl), 7.08 (2H, d, J = 7.8 Hz, Ar-H), 7.21 (1H, t, J = 7.8 Hz, Ar-H), 7.86 (1H, s, 4-H); δ_C (100 MHz, $CDCl_3$) 14.7 (OCH_2CH_3), 41.0 (CH_2Cl), 65.1 (OCH_2CH_3), 115.2, 119.3, 119.5, 124.5, 125.1, 141.3, 143.4 and 146.5 (C=C and Ar-C), and 159.7 (C=O); *m/z* 238 (M^+, 72 %) and 175 (100).

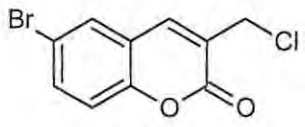
***3-Chloromethyl-8-methoxycoumarin* 59c**



A solution of 3-methoxysalicylaldehyde (2.0 g, 13 mmol), *t*-butyl acrylate (2.67 g, 20 mmol) and DABCO (1.23 g, 11 mmol) in $CHCl_3$ (4.3mL) was sealed in a round-bottomed flask and

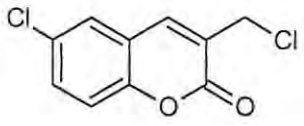
stirred at room temperature for 27 d. The crude reaction mixture was shown, by ^{1}H NMR analysis, to contain the Baylis-Hillman intermediate **61c**, and was used without further purification. The solvent and *t*-butyl acrylate were removed *in vacuo*, and AcOH (8 mL) and HCl (11-M, 16 mL) were added to the mixture, which was boiled and stirred under reflux for 2 h. After cooling to room temperature, ice-water (50 mL) was added, and stirring continued for a further 12 h. The precipitated solid was filtered off and then dissolved in $CHCl_3$. The resulting solution was dried ($MgSO_4$), and the $CHCl_3$ removed *in vacuo*. The crude product was purified by flash chromatography [on silica gel; elution with hexane:EtOAc (9:1)] to afford 3-chloromethyl-8-methoxycoumarin **59c** as beige crystals (518 mg, 18 % yield for both steps); m.p. 141-145 °C (lit.[34] 146-148 °C); ν_{max} (nujol)/cm^{-1}: 1720 (C=O); δ_H (400 MHz, $CDCl_3$) 3.97 (3H, s, OCH_3), 4.56 (2H, s, CH_2Cl), 7.10 (2H, d, J = 7.9 Hz, Ar-H), 7.24 (1H, t, Ar-H) and 7.87 (1H, s, 4-H); δ_C (100 MHz, $CDCl_3$) 41.0 (CH_2), 56.3 (OCH_3), 113.9, 119.4, 119.4, 124.6, 125.2, 141.2, 143.2 and 147.2 (C=C and Ar-C), and 159.6 (C=O); *m/z* 224 (M^+, 56 %) and 189 (100).

***6-Bromo-3-chloromethylcoumarin* 59d**



t-Butyl 3-(5-bromo-2-hydroxyphenyl)-3-hydroxy-2-methylenepropanoate **61d** (4.0 g, 12 mmol) was added to AcOH (12.5 mL) and HCl (11-M, 25 mL), and the resulting mixture was boiled and stirred under reflux for 2 h. After cooling to room temperature, ice-water (70 mL) was added, and the mixture allowed to stir for a further 1.5 h. The precipitated solid was filtered off and dissolved in $CHCl_3$. The solution was dried ($MgSO_4$) and the solvent removed *in vacuo* to yield 6-bromo-3-chloromethylcoumarin **59d** as a beige solid (2.7 g, 81 %); m.p. 121-122 °C (lit.[34] 116-118 °C); ν_{max} (nujol)/cm^{-1}: 1720 (C=O); δ_H (400 MHz, $CDCl_3$) 4.55 (2H, s, CH_2Cl), 7.23-7.67 (3H, series of multiplets, Ar-H) and 7.82 (1H, s, 4-H); δ_C (100 MHz, $CDCl_3$) 40.8 (CH_2), 117.3, 118.4, 120.3, 126.3, 130.3, 134.7, 139.6 and 152.3 (C=C and Ar-C), and 159.4 (C=O); *m/z* 272 [M^+ (^{79}Br), 40 %] and 239 (100).

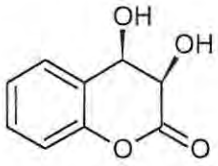
***6-Chloro-3-chloromethylcoumarin* 59e**



t-Butyl 3-(5-chloro-2-hydroxyphenyl)-3-hydroxy-2-methylenepropanoate **61e** (786 mg, 2.76 mmol) was added to AcOH (3 mL) and HCl (11M, 5.5 mL)and the resulting mixture stirred under reflux for 2 h. After cooling to room temperature, ice-water (20 mL) was added, and the mixture allowed boiled and stirred for a further 1.5 h. The precipitated solid was then filtered off and dissolved in $CHCl_3$. The resulting solution was dried (anhydrous $MgSO_4$) and the solvent removed *in vacuo* to yield 6-chloro-3-chloromethylcoumarin **59e** as a grey powder (522 mg, 83 %); m.p. 119-120 °C (lit.[34] 112-114 °C); ν_{max} (nujol)/cm^{-1}: 1723 (C=O); δ_H (400 MHz, $CDCl_3$) 4.55 (2H, s, CH_2Cl), 7.30 (1H, d, J = 8.7 Hz, Ar-H), 7.51 (2H, m, Ar-H) and 7.82 (1H, s, 4-H); δ_C (100 MHz, $CDCl_3$) 40.8 (CH_2) 118.1, 119.8, 126.3, 127.3, 130.0, 131.9, 134.4 and 139.6 (C=C and Ar-C), and 159.5 (C=O); *m/z* 228 (M^+, 51 %) and 193 (100).

3.3 Functionalisation of position C-4 of coumarin 1 and of 3-chloromethyl-8-ethoxycoumarin 59b

(3R,4R)-3,4-dihydroxychroman-2-one 77



$NaIO_4$ (642 mg, 3 mmol) was placed in a 25 mL round-bottom flask. Deionised water (0.9 mL) was added, and the flask was heated gently for 20 min. $CeCl_3.7H_2O$ (75 mg, 0.2 mmol) was then added, and the mixture was stirred until a bright yellow solid formed. The flask was then cooled to 0 °C. CH_3CN (3 mL) was added, and the mixture stirred for 2 min, after which time $RuCl_3$ (50 μL of 0.1 M aq. solution, equivalent to 0.01 mmol) was added. On stirring, the organic layer became bright yellow due to the formation of RuO_4. Coumarin **1** (292 mg, 2 mmol) dissolved in EtOAc (3 mL) was added and the mixture stirred for 10 min. TLC at this time showed the formation of the desired product. The mixture was allowed to stir for a further 3 h, during which time the reaction was monitored by TLC and more product was observed to form. Na_2SO_4 (1 g) was then added, the mixture filtered and the filter cake washed with EtOAc (5 x 10 mL). The filtrate was washed with satd. aq. Na_2SO_3 solution (3 mL) and the organic layer separated and dried ($MgSO_4$). The solvents were removed *in vacuo*, to yield the crude (3*R*,4*R*)-3,4-dihydroxychroman-2-one **77** as a white solid (285 mg, 83 %).

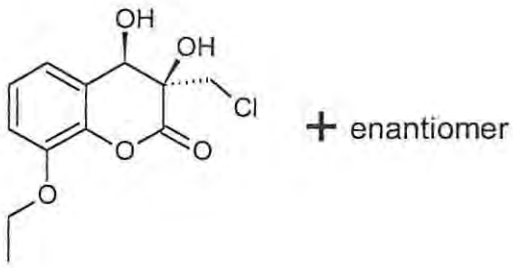
Initial attempts to purify this crude material by chromatography on silica gel led to the loss of the product.

Attempted Diacetylation of crude (3*R*,4*R*)-3,4-dihydroxychroman-2-one 77

Pyridine (2.5 mL), acetic anhydride (2.5 mL) and 4-dimethylaminopyridine (DMAP) (20 mg) were added to the crude product, and the mixture was stirred overnight at room temperature. The mixture was purified by HP20 chromatography as follows. Water was added dropwise to the mixture until a precipitate was just beginning to form (ca. 5 mL was added). The mixture

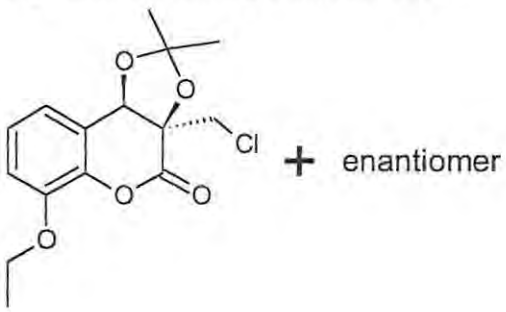
was passed through a 10 mm column containing 8 mL HP20 beads. More water was added to the eluted mixture until a precipitate was just beginning to form, and the mixture was passed through the column again. The same procedure was repeated three more times. The products were stripped off the column as follows. The column was first washed with water (20 mL), which was collected as the first fraction. The second column wash consisted of 1:1 water:acetone mixture, and the third wash consisted just of acetone. Solvents were removed *in vacuo* from the three fractions collected by this stripping method, and ^{1}H NMR analysis of the fractions indicated that the diacetylation attempt was unsuccessful.

***3-chloromethyl-3,4-dihydroxy-8-ethoxycoumarin* 79b**



The procedure for the dihydroxylation of coumarin **1** was followed, using 3-chloromethyl-8-ethoxycoumarin **59b** (477 mg, 2 mmol) as starting material. Workup afforded the crude product (430 mg) as a yellow oil, which later crystallised, ^{1}H NMR analysis of which showed the presence of *3-chloromethyl-3,4-dihydroxy-8-ethoxycoumarin* **79b** (*ca.* 33 %). This was not purified by chromatography, as the diol **77** was lost when this method of purification was attempted.

***3-chloromethyl-3,4-O-isopropylidene-8-ethoxycoumarin* 83b**



The crude product from the dihydroxylation of **59b** (430 mg) was dissolved in benzene (50 mL), and 2,2-dimethoxypropane (143 mg, 1.38 mmol) and *p*-toluenesulfonic acid (5 mg, 0.03 mmol) were added. The flask was fitted with a Dean-Stark trap and the mixture refluxed for 12 h. On cooling, the reaction mixture was neutralised with aq. K_2CO_3, and the organic layer was separated and dried ($MgSO_4$). Evaporation of the solvent *in vacuo* gave a yellow-brown oil which later crystallised (390 mg). A portion (80 mg) of the crude product was chromatographed [HPLC, on a Partisil 10 column, elution with hexane:EtOAc (3:1)] to afford *3-chloromethyl-3,4-O-isopropylidene-8-ethoxycoumarin* **83b** as a white solid (33 mg, *ca.* 26 % after both steps, *i.e.* dihydroxylation and ketalisation), m.p. 100-102 °C; (Found: M^+, 312.07401. $C_{15}H_{17}O_5{}^{35}Cl$ requires *M*, 312.07645); ν_{max} (thin film)/cm^{-1} 1773 (C=O); δ_H (400 MHz, MeOD) 1.44 [3H, s, $C(CH_3)$], 1.46 (3H, t, J = 7.0 Hz, OCH_2CH_3), 1.55 [3H, s, $C(CH_3)$], 3.81 (2H, dd, J = 12, 30 Hz, CH_2Cl), 4.05 (2H, q, J = 7.0 Hz, OCH_2CH_3), 5.17 (1H, s, 4-H), 7.01(2H, m, Ar-H) and 7.16 (1H, t, J = 7.8 Hz, Ar-H); δ_C (100 MHz, MeOD) 14.7 (OCH_2CH_3), 26.3, 27.2 [$C(CH_3)_2$], 43.4 (CH_2Cl), 65.0 (OCH_2CH_3), 76.1 (C-4), 79.5 (C-3), 111.4, 115.5, 118.2, 121.5, 125.2 and 147.0 (Ar-C), 127.5 [$C(CH_3)_2$] and 166.6 (C=O).

Deprotection of 3-chloromethyl-3,4-*O*-isopropylidene-8-ethoxycoumarin 83b

In a dry NMR tube, (3*R*,4*R*)-3-chloromethyl-3,4-*O*-isopropylidene-8-ethoxycoumarin **83b** (10 mg, 0.03 mmol) was dissolved in MeOD (600 μL), and an initial ^1H NMR spectrum was recorded. DCl (33 wt. % solution in D_2O) (60 μL) was added and a spectrum recorded every 200 s for the first 40 min. After this time, spectra were recorded at various intervals over the period of 2 weeks. The mixture was then decanted onto 3 A molecular sieves and filtered; solvents were then removed *in vacuo* from the filtrate, to afford *(3R,4R)-3-chloromethyl-3,4-dihydroxy-8-ethoxycoumarin* **79b** as a beige solid (9 mg, 100 %); ν_{max} (thin film)/cm^{-1}: 3373 (OH) and 1738 (C=O); δ_H (400 MHz, CD_3 OD) 1.40 (3H, t, J = 7.0 Hz, OCH_2CH_3), 3.95 (2H, dd, J = 4.6, 16.1 Hz, CH_2Cl), 4.05 (2H, q, J = 6.9 Hz, OCH_2CH_3), 5.21 (1H, s, 4-H), 6.74 (1H, t, J = 7.9 Hz, Ar-H), 6.83 (1H, dd, J = 0.9, 8.0 Hz, Ar-H) and 6.89 (1H, dd, J = 0.8, 7.7 Hz, Ar-H); δ_C (100 MHz, MeOD) 15.2 (OCH_2CH_3), 49.7 (CH_2Cl), 65.8 (OCH_2CH_3), 73.5 (C-4), 83.4 (C-3), 113.5, 120.1, 122.1, 126.4, 145.5 and 147.7 (Ar-C), and 173.1 (C=O).

Attempted hydrobromination of coumarin

Coumarin **1** (4.0 g, 27 mmol) was dissolved in glacial acetic acid (30 mL). HBr (33 wt. % solution in CH_3COOH; 5.9 mL, 33 mmol) was added dropwise. The mixture was stirred for 3 d, after which time it was poured into ice-water and the white precipitate was filtered off. ^1H NMR analysis of this precipitate indicated that it was the starting material, coumarin **1**.

Attempted benzyloxylation of coumarin

Coumarin **1** (1.46 g, 10 mmol) and DBU (152 mg, 1 mmol) were dissolved in benzyl alcohol (7.78 mL, 27 mmol), and the resulting mixture stirred for 21 d at room temperature. TLC analysis after this time did not indicate any product formation.

Attempted methoxylation of coumarin

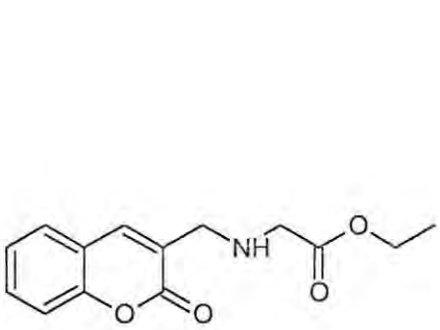
Coumarin **1** (1.46 g, 10 mmol) and DBU (152 mg, 1 mmol) were dissolved in methanol (20 mL), and the mixture stirred for 17 d at room temperature. TLC analysis after this time did not indicate any product formation.

Attempted bromination of coumarin

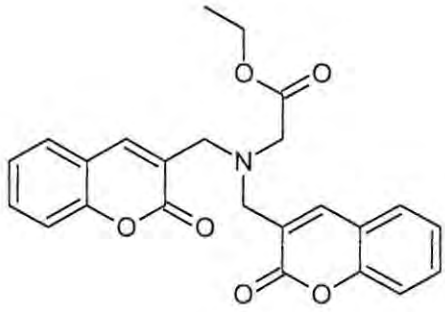
Coumarin **1** (0.5 g, 3 mmol) and *N*-bromosuccinimide (0.91 g, 5.1 mmol) were dissolved in THF (5 mL) at 5 °C. The mixture was stirred for 4 h, after which time Et_2O (10 mL) was added, and the mixture stirred overnight. The solid white precipitate (found by ^1H NMR analysis to be succinimide) which formed was filtered off, and the filtrate washed with aqueous $Na_2S_2O_3$ and dried (Na_2SO_4). Solvents were removed *in vacuo* to yield a yellow oil, which later crystallised (650 mg). Initial TLC analysis suggested product formation, but when repeated 6 days later (prior to chromatography), the product appeared to have decomposed. The reaction was repeated, but no product formation was observed.

3.4 Synthesis of coumarinated amines

***Ethyl* N-*[(2H-1-benzopyran-2-on-3-yl)methyl]glycinate* 84a** and ***ethyl* N,N-*bis[(2H-1-benzopyran-2-on-3-yl)methyl]glycinate* 85a**



84a

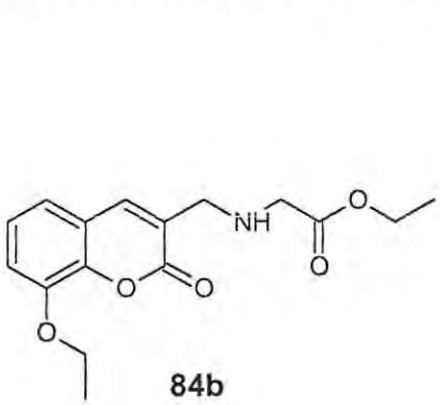


85a

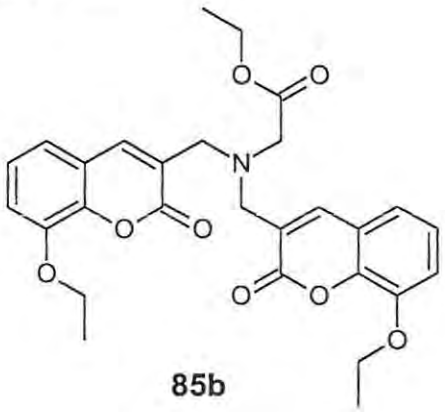
3-(Chloromethyl)coumarin **59a** (97 mg, 0.5 mmol), glycine ethyl ester hydrochloride (209 mg, 1.5 mmol) and Proton Sponge® (343 mg, 3.2 mmol) were dissolved in a mixture of THF (8 mL) and H_2O (2 mL). The mixture was sealed in a reaction tube and stirred for 7 d. Extraction with EtOAc (10 mL), drying ($MgSO_4$), and subsequent removal of the solvent *in vacuo* yielded the crude product (205 mg), which was purified by flash chromatography [on silica gel; elution with hexane:EtOAc (2:1)] to afford two fractions. Fraction (i): *ethyl* N-*[(2H-1-benzopyran-2-on-3-yl)methyl]glycinate* **84a** as a brown oil (59 mg, 45 %) (Found: $\mathbf{M}^+$, 261.10049. $C_{14}H_{15}O_4N$ requires ***M***, 261.10011), ν_{max} (thin film)/cm^{-1}: 3343 (NH), 1722 (C=O) and 1609 cm (C=C); δ_H (400 MHz, C_6D_6) 0.92 (3H, t, J = 7.1 Hz, CH_2CH_3), 3.13 (2H, s, CH_2NH), 3.53 (2H, d, J = 1.3 Hz, $NHCH_2$), 3.91 (2H, q, J = 7.1 Hz, CH_2CH_3), 6.72 (1H, m, Ar-H), 6.81 (1H, m Ar-H), 6.91 (2H, m Ar-H) and 7.15 (1H, s, 4-H); δ_C (100 MHz, C_6D_6) 14.2 (CH_2CH_3), 48.4 (CH_2N), 50.7 (CH_2N), 60.5 (CH_2CH_3), 116.4, 119.7, 123.8, 127.5, 128.2, 130.5, 137.5 and 153.6 (Ar-C and C=C), 160.5 (coumaryl C=O) and 171.9 (glycinate C=O), and Fraction (ii): *ethyl* N,N-*bis[(2H-1-benzopyran-2-on-3-yl)methyl]glycinate* **85a** as beige crystals (34 mg, 32 %); m.p. 133-134 °C (Found: $\mathbf{M}^+$, 419.13656. $C_{24}H_{21}O_6N$ requires ***M***, 419.13689), ν_{max} (nujol)/cm^{-1}: 1727 (C=O) and 1698 (C=O); δ_H (400 MHz, C_6D_6) 0.93 (3H, t, J = 7.1 Hz, CH_2CH_3), 3.28 (2H, s, $NHCH_2C$=O), 3.69 (4H, s, 2 x CH_2NH), 3.92 (2H, q, J = 7.1 Hz, OCH_2CH_3), 6.79 (2H, dt, J = 1.1, 7.6 Hz, Ar-H), 6.91-7.01 (6H, series of multiplets, Ar-H) and 7.35 (2H, s, 2 x 4-H); δ_C (100 MHz, C_6D_6) 14.2 (OCH_2CH_3), 53.7 (2 x CH_2N), 55.1

(C=OCH_2N), 60.5 (OCH_2CH_3), 116.6, 118.0, 121.0, 127.5, 130.2, 133.6, 138.6, 152.5, (Ar-C and C=C), 160.0 (coumaryl C=O) and 170.1 (glycinate C=O).

***Ethyl* N-*[(8-ethoxy-2H-1-benzopyran-2-on-3-yl)methyl]glycinate* 84b** and ***ethyl* N,N-*bis[(8-ethoxy-2H-1-benzopyran-2-on-3-yl)methyl]glycinate* 85b**



84b

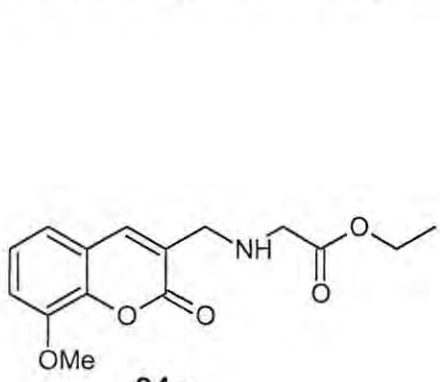


85b

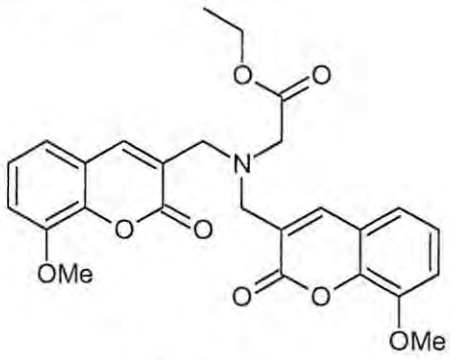
3-Chloromethyl-8-ethoxycoumarin **59b** (46 mg, 0.2 mmol), glycine ethyl ester hydrochloride (54 mg, 0.4 mmol) and Proton Sponge® (100 mg, 0.47 mmol) were dissolved in a mixture of THF (2 mL) and H_2O (1 mL). TLC monitoring of the reaction showed complete consumption of starting material after 3 d. EtOAc was added to extract the organic-soluble product, and the organic phase was then dried ($MgSO_4$) and the solvents removed *in vacuo*, to yield a brown oil (52 mg). Chromatography of this oil [HPLC on a Partisil 10 column; elution with hexane:EtOAc (1:1)] afforded two fractions. Fraction (i): *ethyl* N-*[(8-ethoxy-2H-1-benzopyran-2-on-3-yl)methyl]glycinate* **84b** as a brown oil (13 mg, 22 %); (Found: $\mathbf{M}^+$, 305.12830. $C_{16}H_{19}O_5N$ requires ***M***, 305.12632), ν_{max} (thin film)/cm^{-1}: 3349 (NH), 1715 (C=O) and 1607 (C=C); δ_H (400 MHz, C_6D_6) 0.91 (3H, t, J = 7.1 Hz, $COOCH_2CH_3$), 1.13 (3H, t, J = 7.0 Hz, OCH_2CH_3), 3.11 (2H, s, CH_2NH), 3.52 (2H, s, $NHCH_2$), 3.62 (2H, q, J = 7.0 Hz, OCH_2CH_3), 3.88 (2H, q, J = 7.1 Hz, $COOCH_2CH_3$), 6.55 (2H, d, J = 7.9 Hz, Ar-H), 6.78 (1H, t, J = 7.9 Hz, Ar-H) and 7.21 (1H, s, 4-H); δ_C (100 MHz, C_6D_6) 14.2 and 14.7 (2 x CH_2CH_3), 48.4 (CH_2N), 50.7 (CH_2N), 60.5 ($COOCH_2CH_3$), 64.9 (OCH_2CH_3), 114.3, 119.2, 120.6, 123.7, 127.9, 137.8, 143.8, 146.9 (Ar-C and C=C), 160.4 (coumaryl C=O) and 171.9 (glycinate C=O)and Fraction (ii): *ethyl* N,N-*bis[(8-ethoxy-2H-1-benzopyran-2-on-3-yl)methyl]glycinate* **85b** as beige crystals (3 mg, 6 %); m.p. 126-128 °C, (Found: $\mathbf{M}^+$, 507.18993. $C_{28}H_{29}O_8N$ requires ***M***, 507.18932), ν_{max} (thin film)/cm^{-1}: 1715 (C=O) and 1609 (C=C); δ_H (400 MHz,

C_6D_6) 0.93 (3H, t, J = 7.1 Hz, $COOCH_2CH_3$), 1.13 (6H, t, J = 7.0 Hz, 2 x OCH_2CH_3), 3.27 (2H, s, $NHCH_2C{=}O$), 3.62 (4H, q, J = 7.0 Hz, OCH_2CH_3), 3.73 (4H, s, 2 x CH_2NH), 3.89 (2H, q, J = 7.1 Hz, $COOCH_2CH_3$), 6.52 (2H, dd, J = 1.2, 7.9 Hz, Ar-H), 6.69 (2H, dd, J = 1.3, 7.8 Hz, Ar-H), 6.75 (2H, t, J = 7.9 Hz, Ar-H) and 7.60 (2H, s, 2 x 4-H); δ_C (100 MHz, C_6D_6) 14.2 ($COOCH_2CH_3$), 14.7 (2 x OCH_2CH_3), 53.5 (2 x CH_2N), 55.3 ($C{=}OCH_2NH$), 60.3 ($COOCH_2CH_3$), 64.9 (2 x OCH_2CH_3), 114.6, 119.6, 120.6, 123.8, 126.7, 127.9, 140.3, 144.1 and 146.8 (Ar-C and C=C), 160.5 (coumaryl C=O) and 170.9 (glycinate C=O).

***Ethyl* N-*[(8-methoxy-2H-1-benzopyran-2-on-3-yl)methyl]glycinate* 84c** and ***ethyl* N,N-*bis[(8-methoxy-2H-1-benzopyran-2-on-3-yl)methyl]glycinate* 85c**



84c

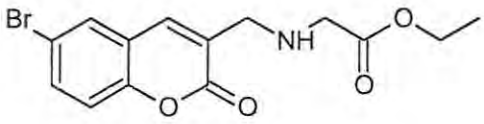


85c

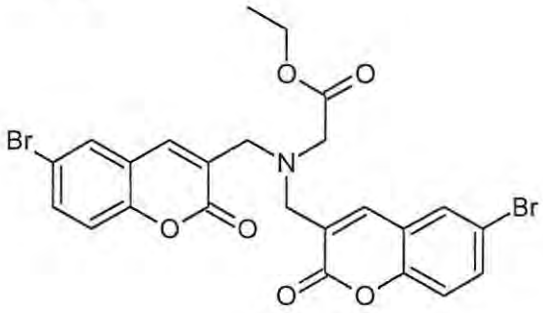
3-Chloromethyl-8-methoxycoumarin **59c** (112 mg, 0.5 mmol), glycine ethyl ester hydrochloride (209 mg, 1.5 mmol) and Proton Sponge® (343 mg, 3.2 mmol) were dissolved in a mixture of THF (8 mL) and H_2O (2 mL). The mixture was sealed in a reaction tube and stirred for 7 d. Extraction with EtOAc (10 mL), drying ($MgSO_4$), and subsequent removal of the solvent *in vacuo* yielded the crude product (214 mg), which was purified by flash chromatography [on silica gel; elution with hexane:EtOAc (2:3)] to afford two fractions. Fraction (i): *ethyl* N-*[(8-methoxy-2*H*-1-benzopyran-2-on-3-yl)methyl]glycinate* **84c** as a brown oil (57 mg, 39 %) (Found: $\mathbf{M}^+$, 291.10956. $C_{15}H_{17}O_5N$ requires M, 291.11067), ν_{max} (thin film)/cm^{-1}: 3339 (NH), 1722 (C=O) and 1609 (C=C); δ_H (400 MHz, C_6D_6) 0.92 (3H, t, J = 7.1 Hz, CH_2CH_3), 3.14 (2H, s, $ArCH_2NH$), 3.36 (3H, s, OCH_3), 3.54 (2H, d, J = 1.3 Hz, $NHCH_2CO$.), 3.91 (2H, q, J = 7.1 Hz, CH_2CH_3), 6.50 (2H, dd, J = 1.0, 8.0 Hz, Ar-H), 6.57 (1H, dd, J = 1.0, 7.8 Hz, Ar-H), 6.78 (1H, t, J = 8.0 Hz, Ar-H) and 7.24 (1H, s, 4-H); δ_C (100 MHz, C_6D_6) 14.2 (CH_2CH_3), 48.4 (CH_2N), 50.7 (CH_2N), 55.8 (OCH_3), 60.5 (CH_2CH_3), 113.0,

119.2, 120.4, 123.8, 127.9, 138.0, 143.5 and 147.5 (Ar-C and C=C), 160.4 (coumaryl C=O) and 171.9 (glycinate C=O), and Fraction (ii): *ethyl* N,N-*bis[(8-methoxy-2*H-*1-benzopyran-2-on-3-yl)methyl]glycinate* **85c** as beige crystals (21 mg, 18 %); m.p. 169-170 °C (Found: $\mathbf{M}^+$, 479.15964. $C_{26}H_{25}O_8N$ requires ***M***, 479.15802), ν_{max} (nujol)/cm^{-1}: 1697 (C=O); δ_H (400 MHz, C_6D_6) 0.93 (3H, t, J = 7.1 Hz, CH_2CH_3), 3.23 (2H, s, $NHCH_2CO$.), 3.29 (6H, s, 2 x OCH_3), 3.74 (4H, s, 2 x CH_2NH), 3.89 (2H, q, J = 7.1 Hz, OCH_2CH_3), 6.42 (2H, d, J = 7.8 Hz, 2 x Ar-H), 6.69 (4H, m, 4 x Ar-H) and 7.59 (2H, s, 2 x 4-H); δ_C (100 MHz, C_6D_6) 14.2 (OCH_2CH_3), 53.5 (2 x CH_2N), 55.3 ($CO.CH_2N$), 55.8 (2 x OCH_3), 60.3 (OCH_2CH_3), 113.2, 119.5, 120.5, 123.8, 126.8, 140.2, 143.9 and 147.5 (Ar-C and C=C), 160.5 (coumaryl C=O) and 171.0 (glycinate C=O).

***Ethyl* N-*[(6-bromo-2*H-*1-benzopyran-2-on-3-yl)methyl]glycinate* 84d** and ***ethyl* N,N-*bis[(6-bromo-2*H-*1-benzopyran-2-on-3-yl)methyl]glycinate* 85d**



84d

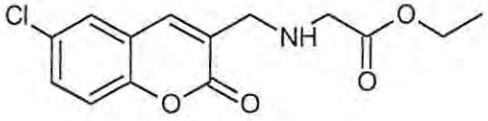


85d

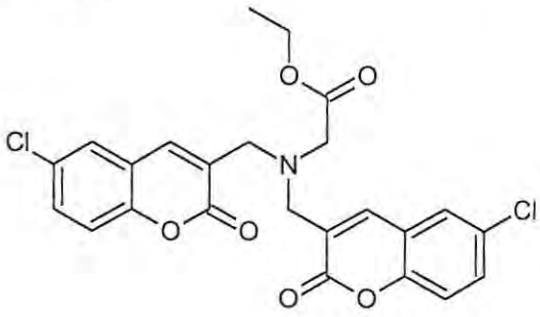
6-Bromo-3-chloromethylcoumarin **59d** (137 mg, 0.5 mmol), glycine ethyl ester hydrochloride (209 mg, 1.5 mmol) and Proton Sponge® (429 mg, 2 mmol) were dissolved in a mixture of THF (8 mL) and H_2O (2 mL). The mixture was sealed in a reaction tube and stirred for 4 d. H_2O (5 mL) was added, followed by extraction with EtOAc (10 mL); this was dried ($MgSO_4$), and subsequent removal of the solvent *in vacuo* yielded 405 mg crude product, which was purified by radial chromatography [1 mm silica gel plate; elution with hexane:EtOAc (3:2)] to afford two fractions. A portion of Fraction (i) was purified further [HPLC on a Partisil 10 column; elution with hexane:EtOAc (3:2)], to afford *ethyl* N-*[(6-bromo-2*H-*1-benzopyran-2-on-3-yl)methyl]glycinate* **84d** as brown crystals (123 mg, 72 %); m.p. 96-97 °C (Found: $\mathbf{M}^+$, 339.00950. $C_{14}H_{14}O_4N^{79}Br$ requires ***M***, 339.01062), ν_{max} (thin film)/cm^{-1}: 1696 (C=O) and

1605 (C=C); δ_H (400 MHz, C_6D_6) 0.92 (3H, t, J = 7.2 Hz, CH_2CH_3), 3.08 (2H, s, CH_2NH), 3.45 (2H, d, J = 1.3 Hz, $NHCH_2$), 3.91 (2H, q, J = 7.1 Hz, CH_2CH_3), 6.55 (2H, d, J = 8.8 Hz, Ar-H), 6.84 (1H, d, J = 2.2 Hz, Ar-H), 6.88 (1H, s, 4-H) and 6.95 (1H, dd, J = 2.3, 8.8 Hz, Ar-H); δ_C (100 MHz, C_6D_6) 14.2 (CH_2CH_3), 48.2 (CH_2N), 50.7 (CH_2N), 60.6 (CH_2CH_3), 116.4, 118.0, 121.2, 129.2, 129.9, 133.1, 136.1 and 152.2 (Ar-C and C=C), 159.8 (coumaryl C=O) and 171.9 (glycinate C=O), and Fraction (ii) from radial chromatography: *ethyl* N,N-*bis[(6-bromo-2*H*-1-benzopyran-2-on-3-yl)methyl]glycinate* **85d** as white crystals (9 mg, 6 %); m.p. 159-163 °C, ν_{max} (thin film)/cm^{-1}: 1715 cm^{-1} (C=O) and 1607 (C=C); δ_H (400 MHz, C_6D_6) 0.93 (3H, t, J = 7.1 Hz, CH_2CH_3), 3.28 (2H, s, $NHCH_2C$=O), 3.69 (4H, s, 2 x CH_2NH), 3.92 (2H, q, J = 7.1 Hz, OCH_2CH_3), 6.51 (2H, d, J = 8.8 Hz, Ar-H), 6.92 (2H, dd, J = 2.3, 8.8 Hz, Ar-H), 7.01 (2H, d, J = 2.2 Hz, Ar-H) and 7.35 (2H, s, 2 x 4-H); δ_C (100 MHz, C_6D_6) 14.2 (OCH_2CH_3), 53.7 (2 x CH_2N), 55.1 (C=OCH_2N), 60.5 (OCH_2CH_3), 116.6, 118.0, 121.0, 127.5, 130.2, 133.6, 138.6 and 152.5 (Ar-C and C=C), 160.0 (coumaryl C=O) and 170.1 (glycinate C=O).

***Ethyl* N-*[(6-chloro-2*H*-1-benzopyran-2-on-3-yl)methyl]glycinate* 84e** and ***ethyl* N,N-*bis[(6-chloro-2*H*-1-benzopyran-2-on-3-yl)methyl]glycinate* 85e**



84e

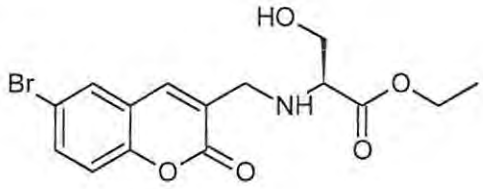


85e

6-Chloro-3-chloromethylcoumarin **59e** (115 mg, 0.5 mmol), glycine ethyl ester hydrochloride (209 mg, 1.5 mmol) and Proton Sponge® (343 mg, 3.2 mmol) were dissolved in a mixture of THF (8 mL) and H_2O (2 mL). The mixture was sealed in a reaction tube and stirred for 7 d. Extraction with EtOAc (10 mL), drying ($MgSO_4$), and subsequent removal of the solvent *in vacuo* yielded the crude product (569 mg), which was purified by flash chromatography [on silica gel; elution with hexane:EtOAc (2:1)] to afford two fractions. Fraction (i): *ethyl* N-*[(6-chloro-2*H*-1-benzopyran-2-on-3-yl)methyl]glycinate* **84e** as beige crystals (65 mg, 44 %); m.p.

97-99 °C (Found: $\mathbf{M}^+$, 295.06260. $C_{20}H_9O_2N$ requires ***M***, 295.06333), ν_{max} (thin film)/cm^{-1}: 3349 (NH), 1715 (C=O) and 1607 (C=C); δ_H (400 MHz, C_6D_6) 0.92 (3H, t, *J* = 7.2 Hz, CH_2CH_3), 3.10 (2H, s, CH_2NH), 3.47 (2H, d, *J* = 1.2 Hz, $NHCH_2$), 3.91 (2H, q, *J* = 6.9 Hz, CH_2CH_3), 6.63 (1H, d, *J* = 8.7 Hz, Ar-H), 6.72 (1H, d, *J* = 2.3 Hz, Ar-H), 6.82 (1H, dd, *J* = 2.4, 8.8 Hz, Ar-H) and 6.92 (1H, s, 4-H); δ_C (100 MHz, C_6D_6) 14.2 (CH_2CH_3), 48.3 (CH_2N), 50.7 (CH_2N), 60.6 (CH_2CH_3), 117.7, 120.7, 126.9, 129.0, 129.2, 130.3, 136.3 and 151.8 (Ar-C and C=C), 159.9 (coumaryl C=O) and 171.9 (glycinate C=O), and Fraction (ii): *ethyl* N,N-*bis[(6-chloro-2*H*-1-benzopyran-2-on-3-yl)methyl]glycinate* **85e** as beige crystals (34 mg, 28 %); m.p. 167-168 °C (Found: $\mathbf{M}^+$, 487.05911. $C_{24}H_{19}O_6N^{35}Cl_2$ requires ***M***, 487.05894), ν_{max} (thin film)/cm^{-1}: 1701 (C=O) and 1607 (C=C); δ_H (400 MHz, C_6D_6) 0.94 (3H, t, *J* = 7.1 Hz, CH_2CH_3), 3.28 (2H, s, $NHCH_2C{=}O$), 3.69 (4H, s, 2 x CH_2NH), 3.91 (2H, q, *J* = 7.1 Hz, OCH_2CH_3), 6.58 (2H, d, *J* = 8.8 Hz, Ar-H), 6.79 (2H, dd, *J* = 2.5, 8.8 Hz, Ar-H), 6.86 (2H, d, *J* = 2.5 Hz, Ar-H) and 7.24 (2H, s, 2 x 4-H); δ_C (100 MHz, C_6D_6) 14.2 (OCH_2CH_3), 53.6 (2 x CH_2N), 55.1 ($C{=}OCH_2N$), 60.5 (OCH_2CH_3), 117.1, 120.5, 127.1, 127.6, 129.2, 130.8, 138.6 and 152.1 (Ar-C and C=C), 160.1 (coumaryl C=O) and 170.9 (glycinate C=O).

***Ethyl (2S)-N-[(2H-1-benzopyran-2-on-3-yl)methylamino]3-hydroxypropanoate* 86d**

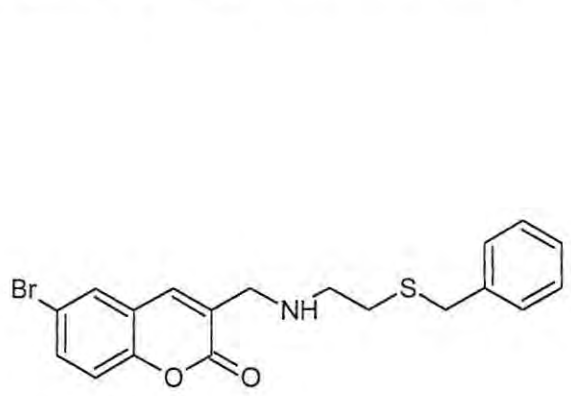


6-Bromo-3-chloromethylcoumarin **59d** (137 mg, 0.5 mmol), L-serine ethyl ester hydrochloride (254 mg, 1.5 mmol) and Proton Sponge® (429 mg, 2 mmol) were dissolved in a mixture of THF (8 mL) and H_2O (2 mL). The mixture was sealed in a reaction tube and stirred for 4 d. H_2O (5 mL) was added, followed by extraction with EtOAc (10 mL); this was dried ($MgSO_4$), and subsequent removal of the solvent *in vacuo* yielded 351 mg crude product, which was purified by radial chromatography [1 mm silica gel plate; elution with hexane:EtOAc (3:2)] to afford *ethyl (2S)-N-[(2H-1-benzopyran-2-on-3-yl)methylamino]3-hydroxypropanoate* **86d** as brown crystals (170 mg, 92 %); m.p. 100-105 °C; (Found: $\mathbf{M}^+$, 369.02210. $C_{15}H_{16}O_5N^{79}Br$ requires ***M***, 369.02118), ν_{max} (nujol)/cm^{-1}: 3278 (OH), 1734, 1707 (2 x C=O); δ_H (400 MHz,

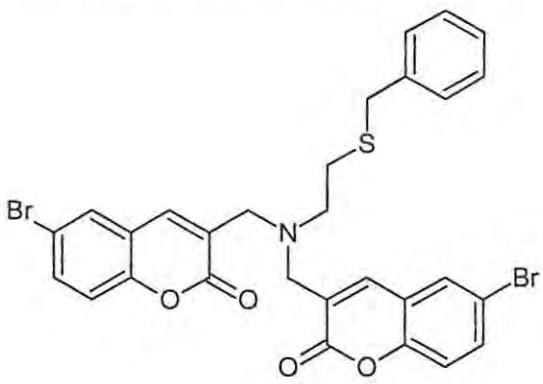
C_6D_6) 0.88 (3H, t, J = 7.1 Hz, OCH_2CH_3), 2.60 (1H, s, N-H), 3.15 (1H, dd, J = 1.1, 5.5 Hz, CHC=O), 3.39 and 3.47 (2H, 2 x dd, J = 1.0, 16.0 Hz, CH_2NH), 3.60 (1H, dd, J = 5.7, 10.8 Hz, CH_aOH), 3.69 (1H, dd, J = 4.3, 10.8 Hz, CH_bOH), 3.87 (2H, q, J = 7.1 Hz, OCH_2CH_3), 6.55 (1H, d, J = 8.7 Hz, Ar-H), 6.72 (1H, s, 4-H), 6.89 (1H, d, J = 2.2 Hz, Ar-H), 6.95 (1H, d, J = 2.3, 8.7 Hz, Ar-H); δ_C (100 MHz, C_6D_6) 14.1 (OCH_2CH_3), 47.3 (CH_2NH), 61.1 (OCH_2CH_3), 62.8 (CH_2OH), 62.9 (CHC=O), 116.4, 118.1, 121.0, 128.9, 130.0, 133.3, 136.4 and 152.3 (Ar-C and C=C), 159.9 (coumaryl C=O) and 172.7 (L-serinyl C=O).

Note: When attempting to measure $[\alpha]_D$ for **86d**, no optical rotation was observed. However, when europium (III) tris[3-(heptafluoropropylhydroxymethylene)-D-camphorate] chiral shift reagent (1 mg) was added to the NMR sample of **86d**, no resolution of enantiomers was observed. Thus it was concluded that **86d** was enantiomerically pure, but had an optical rotation too small to be observed accurately by the Perkin-Elmer polarimeter used to measure $[\alpha]_D$. alternatively, the amino acid moiety may have undergone racemization in the presence of proton sponge.

2-benzylthio-N-[(6-bromo-2H-1-benzopyran-2-on-3-yl)methyl]ethylamine **87d** and ***2-benzylthio-N,N-bis[(6-bromo-2H-1-benzopyran-2-on-3-yl)methyl]ethylamine*** **88d**



87d



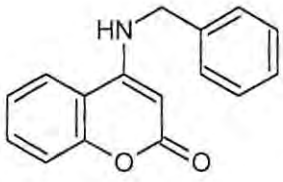
88d

6-Bromo-3-chloromethylcoumarin **59d** (137 mg, 0.5 mmol), *S*-benzylcysteamine hydrochloride (306 mg, 1.5 mmol) and Proton Sponge® (429 mg, 2 mmol) were dissolved in a mixture of THF (8 mL) and H_2O (2 mL). The mixture was sealed in a reaction tube and stirred for 4 d. H_2O (5 mL) was added, followed by extraction with EtOAc (10 mL); this was dried ($MgSO_4$), and subsequent removal of the solvent *in vacuo* yielded 340 mg crude product,

which was purified by radial chromatography [1 mm silica gel plate; elution with hexane:EtOAc (3:2)] to afford two fractions. A portion (20 mg) of Fraction (i) (yield after radial chromatography = 94 mg, 46 %) was purified further [HPLC on a Partisil 10 column; elution with hexane:EtOAc (1:1)], to afford *2-benzylthio*-N-*[(6-bromo-2*H-*1-benzopyran-2-on-3-yl)methyl]ethylamine* **87d** as brown crystals; m.p. 99-101 °C (Found: $\mathbf{M}^+$, 403.02367. $C_{19}H_{18}O_2NS^{79}Br$ requires ***M***, 403.02416), ν_{max} (thin film)/cm^{-1}: 1710 (C=O), 1604 (C=C); δ_H (600 MHz, C_6D_6); 2.34 and 2.44 (2 x 2H, 2 t, J = 6.2 Hz, NCH_2CH_2S), 3.36 (2H, s, CH_2N), 3.43 (2H, s, CH_2S) and 6.55 – 7.18 (9H, series of multiplets, Ar-H and 4-H); δ_C (150 MHz, C_6D_6); 31.9 (CH_2CH_2S), 36.3 (SCH_2Ph), 2 x 48.2 (2 x CH_2N), 116.4, 118.0, 121.2, 127.2, 128.7, 129.2, 133.1, 136.4, 139.0 and 152.2 (Ar-C and C=C), and 159.9 (C=O). Fraction (ii): *2-benzylthio*-N,N-*bis[(6-bromo-2*H-*1-benzopyran-2-on-3-yl)methyl]ethylamine* **88d** as white crystals (18 mg, 12 %); m.p. 131-132 °C (Found: $\mathbf{M}^+$, 284.08336. $C_{14}H_{17}O_4{}^{35}Cl$ requires ***M***, 284.08154), ν_{max} (nujol)/cm^{-1}: 1701 (C=O); δ_H (400 MHz, C_6D_6); 2.41 (4H, m, NCH_2CH_2S), 3.30 (4H, s, 2 x CH2N), 3.38 (2H, s, CH2S), 6.55-7.12 (11H, series of multiplets, Ar-H) and 7.26 (2H, s, 2 x 4-H); δ_C (100 MHz, C_6D_6) 29.5 (CH_2CH_2S), 36.7 (SCH_2Ph), 53.6 and 54.2 (2 x CH_2N), 116.6, 118.1, 121.0, 127.9, 128.1, 128.4, 128.7, 129.1, 130.1, 133.6, 138.3 and 152.4 (Ar-C and C=C), and 159.9 (C=O).

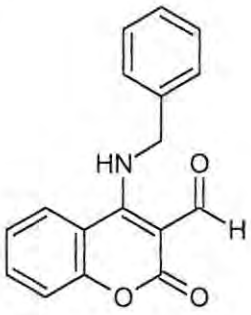
3.5 Synthesis of 4-hydroxycoumarin derivatives

***4-Benzylaminocoumarin* 98**



A mixture of 4-hydroxycoumarin (22.7 g, 0.14 mol) and benzylamine (98 g, 0.92 mol) was refluxed for 45 min. After cooling, MeOH (300 mL) and 0.2 M aq. NaOH (250 mL) were added, and the mixture stirred for 20 min. The precipitated solid was filtered off and recrystallised from DMF to yield 4-benzylaminocoumarin **98** as pale yellow crystals (20.3 g, 58%); m.p. 246-247 °C (lit.[81] 240-242 °C); ν_{max} (nujol)/cm^{-1}: 3277 (NH) and 1663 (C=O); δ_H (400 MHz, DMSO-d_6) 4.52 (2H, d, J = 5.8 Hz, CH_2N), 5.05 (1H, s, 3-H), 7.26-7.36 (7H, m, Ar-H), 7.6 (1H, t, J = 7.6 Hz, Ar-H), 8.11 (1H, d, J = 8.0 Hz, Ar-H) and 8.36 (1H, t, J = 5.6 Hz, NH); δ_C (100 MHz, DMSO-d_6) 45.4 (CH_2), 82.4 (C=*C*H), 114.4, 116.9, 122.3, 123.4, 126.9, 127.0, 128.5, 131.9, 137.6, 153.0 and 153.1 (Ar-H and *C*=CH), and 161.3 (C=O); *m/z* 251 (M^+, 100 %).

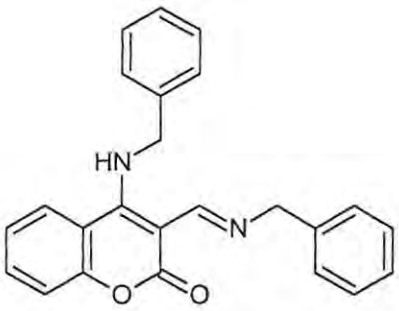
***4-Benzylamino-3-formylcoumarin* 94**



Phosphorus oxychloride (2.5 mL, 35 mmol) was added dropwise to DMF (30 mL, 0.31 mol) under nitrogen over a period of 10 min, maintaining the temperature of below 5 °C. The mixture was stirred for 15 min, after which 4-benzylaminocoumarin **98** (2.0 g, 7.9 mmol) was added. The mixture was heated at 105 °C for 2 h. After cooling, the mixture was poured into ice-water (400 mL) and the pH adjusted to pH 10 with 0.2 M aq. NaOH. The precipitated

solid was filtered off and recrystallised from absolute ethanol to yield 4-benzylamino-3-formylcoumarin **94** as pale pink crystals (840 mg, 38 %); m.p. 202-208 °C (Lit.[81] 280.3 °C)*; ν_{max} (nujol)/cm^{-1}: 1694 (aldehyde C=O), 1625 (C=C); δ_H (400 MHz, $CDCl_3$) 5.07 (2H, d, J = 5.8 Hz, CH_2), 7.20-8.00 (9H, series of multiplets, Ar-H), 10.17 (1H, s, CHO) and 12.31 (1H, br s, NH); δ_C (100 MHz, $CDCl_3$) 51.3 (CH_2), 97.0, 113.8, 118.9, 123.7, 127.0, 127.5, 128.6, 129.4, 134.6, 135.6, 155.5, 160.0 (Ar-H and C=C), 162.7 (lactone C=O) and 192.0 (CHO); *m/z* 279 (M^+, 41 %) and 250 (100).

***4-Benzylamino-3-benzyliminomethylcoumarin* 93**



A stirred solution of 4-benzylamino-3-formylcoumarin **94** (558 mg, 2 mmol) and benzylamine (1.158 g, 10.8 mmol) in DMF (4 mL) was heated at 100 °C for 6 h. After cooling, the white precipitated solid was filtered off, and found to be 4-benzylamino-3-benzyliminomethylcoumarin **93**, which was sufficiently pure not to need recrystallisation (400 mg, 54 %); m.p. 169-171 °C (lit.[80] 165-166 °C); ν_{max} (nujol)/cm^{-1}: 1689 (C=O) and 1602 (C=N); δ_H (400 MHz, C_6D_6) 4.23 and 4.27 (4H, 2 x s, 2 x CH_2N), 6.88-7.45 (14H, series of multiplets, Ar-H), 9.18 (1H, s, HC=N) and 12.96 (1H, br s, NH); δ_C (100 MHz, C_6D_6) 51.1 (CH_2NH), 64.8 (*C*H_2N=C), 94.2, 115.5, 118.4, 122.5, 126.7, 126.9, 127.9, 128.1, 128.4, 128.6, 129.0, 132.2, 137.5, 140.2 and 155.5 (Ar-H and C=C), 156.5 (C=N) and 162.8 (lactone C=O) [some aromatic signals were partially obscured by solvent peaks and thus difficult to assign]; *m/z* 368 (M^+, 9 %) and 278 (100).

* It seems likely that some solvent (either DMF or ethanol) was still present when the melting point was measured, thus accounting for the significant difference between the literature value and the experimental value.

Attempted reduction of 4-benzylamino-3-benzyliminomethylcoumarin 93

4-Benzylamino-3-benzyliminomethylcoumarin **93** (300 mg, 0.8 mmol) was placed in a dry 25 mL round-bottomed flask under nitrogen. Dry MeOH (5 mL) was added, followed by $NaBH_4$ (60 mg, 1.6 mmol), and the mixture was stirred at 0 °C for 20 min, and then allowed to warm to room temperature and stirred overnight. The solvent was removed *in vacuo* and the organic material extracted with EtOAc. The organic solvent was removed *in vacuo*, yielding a light beige solid, ^1H NMR analysis of which indicated that no product had formed, *i.e.* the beige-white solid was starting material.

Attempted Synthesis of 3-formyl-4-hydroxycoumarin 96

Method 1:

4-Hydroxycoumarin **78** (1.00 g, 6.2 mmol), triethyl orthoformate (7 mL, 42 mmol) and *p*-toluenesulfonic acid (0.02 g, 0.1 mmol) were placed in a 50 mL beaker and covered with a stemless funnel. The beaker was placed in a Defy Microwave Oven[†] and irradiated at Power level 2 for 1 min, then Power level 1.5 for 1 min; the process was repeated once. On cooling, a yellow solid precipitated out. Excess solvent was removed with a Pasteur pipette, and the residue was taken up in 0.15 N Na_2CO_3 (20 mL), which was then acidified with aqueous HCl to pH 4. The material was then extracted with Et_2O (2 x 20 mL) and the solvent removed *in vacuo* to yield a yellow solid, which, on NMR analysis, was shown to be starting material.

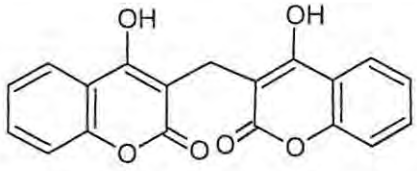
Method 2:

Phosphorus oxychloride (5 mL, 58 mmol) was added dropwise to DMF (50 mL, 0.5 mol) under nitrogen over a period of 15 min, maintaining the temperature of below 5 °C. The mixture was stirred for 15 min, after which 4-hydroxycoumarin **78** (2.1 g, 13 mmol) was added. The mixture was heated at 90 °C for 2 h. After cooling, the mixture was poured into ice-water (400 mL) and the pH adjusted to pH 10 with 0.2 M aq. NaOH. Filtration yielded dark-brown, intractable insoluble solids.

[†] DEFY Microwave Oven, Model DM129/DM0309, 1000 W maximum output level. Actual wattage of 'Power level 1.5' and 'Power level 2' not given.

Attempted Mannich Reactions on 4-hydroxycoumarin

Attempted Mannich reaction with 4-hydroxycoumarin and glycine ethyl ester hydrochloride



4-Hydroxycoumarin (1.0 g, 6.1 mmol), glycine ethyl ester hydrochloride (1.72 g, 12.3 mmol) and paraformaldehyde (369 mg, 12.3 mmol) were dissolved in dry EtOH (60 mL) and refluxed under argon for 24 h. The resulting white precipitate was then filtered off, and found, by ^{1}H NMR analysis, to be 3,3'-methylene-bis(4-hydroxycoumarin) or dicoumarol **9** (1.027 g, 100 %); m.p. 292-294 °C (lit.[98] 290-292 °C); ν_{max} (nujol)/cm^{-1}: 1653 (C=O); δ_H (400 MHz, $CDCl_3$) 3.84 (2H, s, CH_2), 7.37-8.01 (8H, series of multiplets, Ar-H), 11.31 (2H, s, 2 x OH); δ_C (100 MHz, $CDCl_3$) 19.9 (CH_2), 102.9 (C-4), 116.4, 116.7, 124.0, 124.8, 132.6, 152.3 and 164.4 (Ar-C and C-3), and 168.7 (C=O); *m/z* 336 (M^+, 100 %).

Preparation of piperidine hydrochloride 107

Piperidine hydrochloride was prepared by stirring piperidine (5.59 g, 65 mmol) and NH_4Cl (1.72 g, 33 mmol) vigorously in a 50 mL beaker covered with a stemless funnel. The mixture was heated gently for the first 30 min and then stirred at room temperature for 12 h. EtOAc was then added and the white solid filtered off and washed with EtOAc (5 x 20 mL). The solid was then dried under vacuum for 2 h, to yield, as a white crystalline solid, piperidine hydrochloride **107** (1.93 g, 48 %); m.p. 249-250 °C (lit.[98] 245-248 °C).

Mannich Reaction with 4-hydroxycoumarin and piperidine hydrochloride

Piperidine hydrochloride **107** (486 mg, 4 mmol), 4-hydroxycoumarin **78** (319 mg, 2 mmol) and paraformaldehyde (120 mg, 4 mmol) were placed in a 100 mL flask. The flask was fitted with a condenser and purged with nitrogen. Dry EtOH (20 mL) was added and the mixture was refluxed for 15 h under argon. The white precipitate was then filtered off, and found, by

^{1}H NMR analysis, to be 3,3'-methylene-bis(4-hydroxycoumarin) **9** (220 mg, 65 %); m.p. 292-294 °C (lit.[98] 290-292 °C).

3.6 Details of LigandFit Parameters and Commands used

Table 13. Details of LigandFit parameters used for fitting synthesised compounds **59a, 79b, 83b, 84a, 85a, 86d, 87d** and **88d**.

Protein model:	2UPJ	
LigandFit Preferences:		
Autocharge Ligand Atoms	Y	
Auto Adjust Hydrogens	Y	
Perform Flexible Fit	Y	
Monte Carlo Parameters:		
No. of trials	10000	
Max no of conformers saved:	100	
Ligand Internal Energy:		
Include Electrostatic Energy	Y	
Interaction Energy:		
Use Soft Potential Energy	Y	
Approximated by	Trilinear interpolation value	
Apply Penalties for ligand atoms outside binding site:	N	
Energy Grid Preferences:		
Extension from site:	3	A
Nonbond cutoff distance	10	A
Dielectric constant	1	
Output:		
Perform Clustering	Y	
Method	Leader	
Choice of Clusters	Use threshold	
Distance Threshold	1.5	
Member selection method	Best Member	
Compute RMS with starting conformer	N	
Energy Minimise Ligands in Protein	Y	
Max no of iterations	500	

Table 14. Details of LigandFit parameters used for fitting 4-OH analogues of synthesised compounds, *i.e.* **59a-OH, 79b-OH, 83b-OH, 84a-OH, 85a-OH, 86d-OH, 87d-OH** and **88d-OH**.

Protein model:	2UPJ	
LigandFit Preferences:		
Autocharge Ligand Atoms	Y	
Auto Adjust Hydrogens	Y	
Perform Flexible Fit	Y	
Monte Carlo Parameters:		
No. of trials	10000	
Max no of conformers saved:	100	
Ligand Internal Energy:		
Include Electrostatic Energy	Y	
Interaction Energy:		
Use Soft Potential Energy	Y	
Approximated by	Trilinear interpolation value	
Apply Penalties for ligand atoms outside binding site:	N	
Energy Grid Preferences:		
Extension from site:	3	A
Nonbond cutoff distance	10	A
Dielectric constant	1	
Output:		
Perform Clustering	Y	
Method	Leader	
Choice of Clusters	Use threshold	
Distance Threshold	1.5	
Member selection method	Best Member	
Compute RMS with starting conformer	N	
Energy Minimise Ligands in Protein	Y	
Max no of iterations	500	

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