SYNTHETIC AND SPECTROSCOPIC STUDIES OF INDOLIZINE DERIVATIVES

THESIS

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ABSTRACT

The crystalline compound resulting from thermal cyclization of the Baylis-Hillman product, methyl 3-hydroxy-2-methylene-3-(2-pyridyl)propanoate, has been identified as the indolizine derivative, methyl indolizine-2-carboxylate, and this approach involving the reaction of pyridine-2-carboxaldehydes and acrylate analogues has been established as a general route to 2-substituted indolizines. The ease of cyclization of the Baylis-Hillman products to indolizines has been shown to increase by converting the hydroxy group to an acetoxy group, and a range of acetylated Baylis-Hillman products were prepared and cyclized to the corresponding 2-substituted indolizines, generally in good overall yield. In the reaction of pyridine-2-carboxaldehyde and methyl vinyl ketone, the intermediate cyclized readily and directly to the corresponding indolizine. One- and two-dimensional ^{1}H and ^{13}C NMR analysis of the 2-substituted indolizine products has permitted complete assignment of all ^{1}H and ^{13}C NMR signals, as well as the measurement of all coupling constants for these compounds.

A kinetic and mechanistic study has been conducted on the Baylis-Hillman reaction using ^{1}H NMR spectroscopy. A range of substrates has been examined and the reaction has been found to be third-order overall. A mechanism involving an addition - elimination sequence is proposed, which fits the kinetic data and accounts for observed substituent effects.

Reaction of *N,N*-dimethylacrylamide with pyridine-2-carboxaldehyde in the presence of the tertiary amine catalyst, DABCO, in chloroform, yielded an unexpected product which has been identified by single crystal X-ray diffraction analysis as 1-(2,2,2-trichloro-1-hydroxyethyl)pyridine.

Attempted extension of the general indolizine route to the preparation of chromene systems by reacting salicylaldehyde with methyl acrylate in the presence of DABCO, also led to an unexpected, crystalline material, identified by single crystal X-ray diffraction analysis as the coumarin derivative, 3-[(2-formylphenoxy)methyl]-coumarin.

A series of chloroquine analogues have been prepared from indolizine-2-carboxylic acid, pyrrolo[1,2-*a*]quinoline-2-carboxylic acid and imidazo[1,2-*a*]pyridine-2-carboxylic acid by reaction with suitable amines in the presence of the coupling reagent 1,1′-carbonyldiimidazole. This route has been shown to be a vast improvement on earlier procedures and has provided access to both secondary and tertiary indolizine-2-carboxamides. A range of *N,N*-dialkylindolizine-2-carboxamides have been prepared by this route, and the influence of substituents on their N-CO rotational energy barriers has been determined using variable temperature ^{1}H and ^{13}C NMR techniques.

Intercalation with natural DNA by both chloroquine and the synthesized chloroquine analogues has been examined using UV spectrophotometry, and ^{1}H and ^{31}P NMR spectroscopy. The pyrrolo[1,2-*a*]quinolines have been shown to be DNA intercalators with binding affinities similar to that of the known antimalarial intercalator, chloroquine. In a preliminary study the synthesis of a short oligonucleotide has been undertaken and changes have been observed in the ^{1}H and ^{31}P NMR spectra of the oligonucleotide on addition of the intercalator, chloroquine.

CONTENTS

ACKNOWLEDGEMENTS

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Finally, I would like to thank AECI Ltd , the FRD and Rhodes University for financial support.

ABBREVIATIONS

This list includes the less common abbreviations.

BOC - *t*-butoxy carbonyl

COSY - ^{1}H-^{1}H correlated spectroscopy

DBU - 1,8-diazabicyclo[5.4.0]undec-7-ene

DIBAL-H - Diisobutylaluminium hydride

HETCOR - ^{1}H-^{13}C correlated spectroscopy

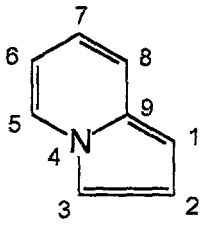
INADSY - ^{13}C-^{13}C correlated spectroscopy

LiHMDS - lithium hexamethyldisilazide

NOESY - nuclear Overhauser effect spectroscopy

1. INTRODUCTION

Indolizine **1** was discovered by Angeli in 1890,[1] and first prepared by Scholtz[2] in 1912. Several different names have been used for this compound in the past, including pyrrocoline, pyrindole, 8-pyrrolopyridine and pyrrolo[1,2-*a*]pyridine. The name indolizine has been adopted by Chemical Abstracts, and the numbering is shown in structure **1**. The perhydroindolizine **2** is known as indolizidine, and is the alkaloid δ-coniceine.



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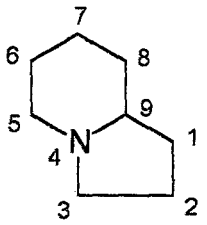

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1.1 INDOLIZINE CHEMISTRY

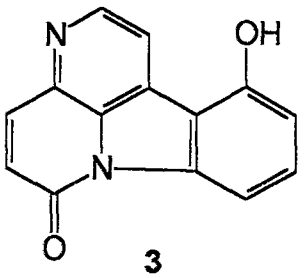
1.1.1 OCCURRENCE OF INDOLIZINES

The discrete indolizine nucleus has not been found to be naturally occurring, but a few alkaloids containing indolizine as part of a more complex structure have been isolated, for example amarorine **3** and derivatives from *Amaroria soulameoides*,[3] and canthine-6-one-3*N*-oxide **4** and derivatives from *Ailanthus altissima*.[4]

In contrast to the indolizines, there are many examples of naturally occurring indolizidines. Alkaloids of the *Elaeocarpus* species represent a group of about 20 similar indolizidine natural products,[5] while the fungus *Rhizoctonia leguminicola* produces two toxic indolizidine alkaloids, slaframine **5** and swainsonine **6**.[6-9] Castanospermine **7**, an amino sugar derivative, was isolated from seeds of the tree *Castanospermium australe*[10]

and a number of indolizidine alkaloids have been isolated from the skin extracts of the *Dendrobatidae* family of neotropical arrow poison frogs.[11-13]

A large number of these indolizidines are biologically active and this aspect will be discussed in Section 1.1.2.4.



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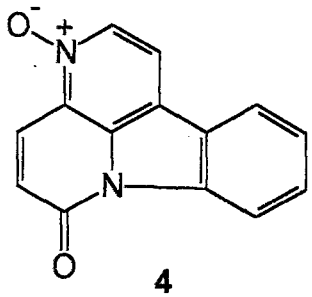

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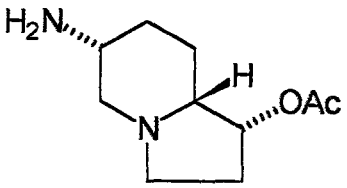

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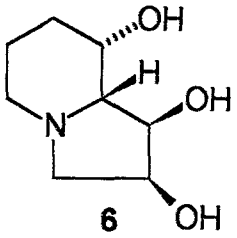

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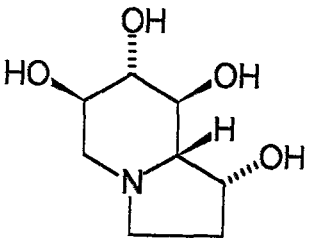

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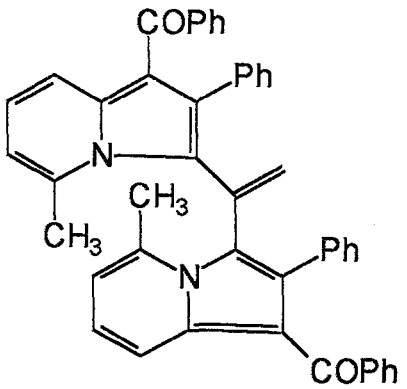
1.1.2 PROPERTIES OF THE INDOLIZINE NUCLEUS

1.1.2.1 PHYSICAL AND SPECTRAL PROPERTIES

Indolizine has a delocalized 10π-electron system.[14] X-ray crystal structure determinations have been carried out on compounds **8** and **9**, and these have shown the indolizine ring system to be almost planar.[14]



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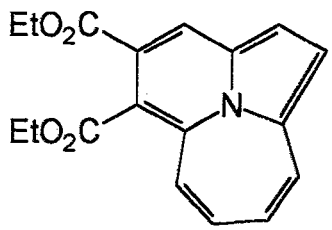

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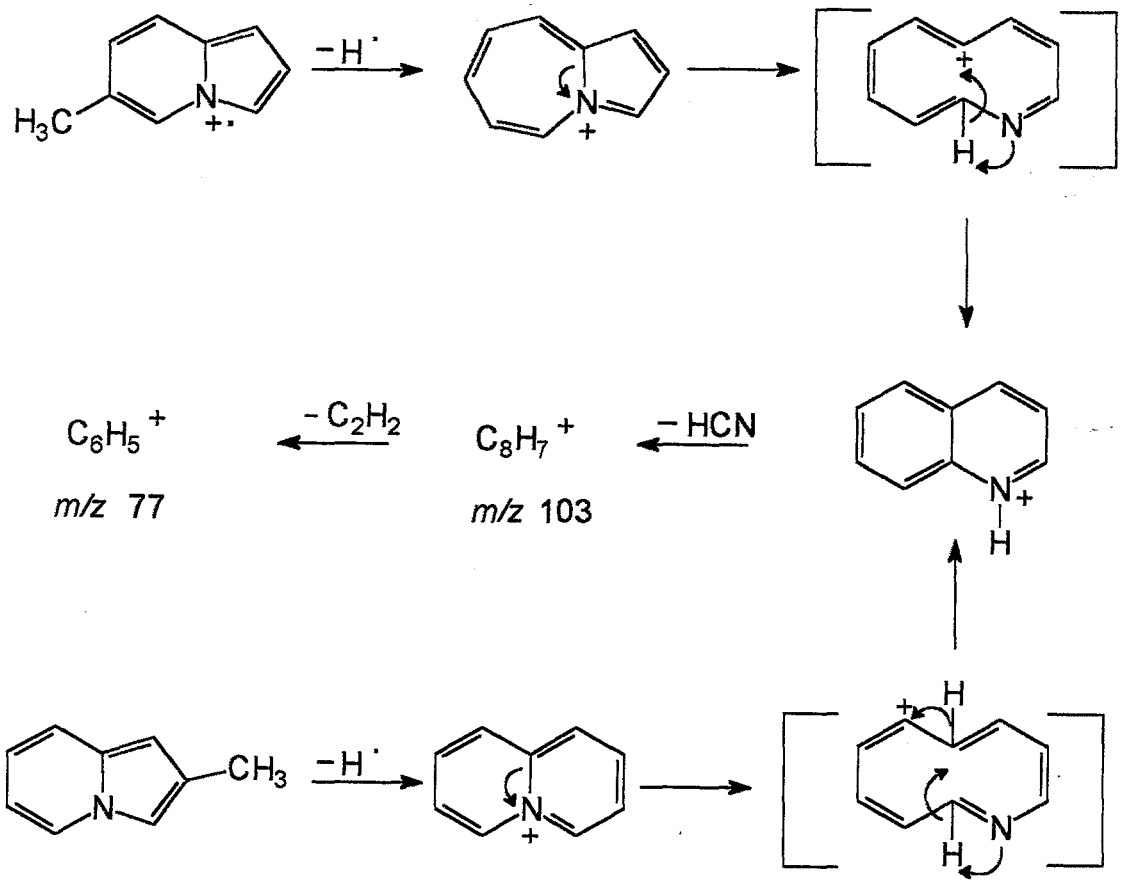
Absorption-emission characteristics of indolizines were studied by Lerner *et al.*[15,16] and the absorption spectrum of indolizine shows 8 maxima between 390 and 235nm. Indolizines have unusually high room temperature fluorescence quantum yields and relatively long fluoresence lifetimes, and these properties have led to interest in possible biological applications (Section 1.1.2.4).

The mass spectrum of indolizine shows that the molecular ion is also the base peak (*m/z* 117). The first obvious loss is of HCN and H_2CN, followed by loss of C_2H_2 to give fragments at *m/z* 90, 89 and 64 respectively. The mass spectra of isomeric monomethylindolizines are very similar to each other, irrespective of the point of substitution, and the fragmentation patterns of two of these compounds are illustrated in Scheme 1.[17] A recent study on the mass-spectral fragmentation of indolizines has been

conducted by Bobrovskii.[18]

The first detailed ^1H NMR study of indolizines was carried out by Black *et al.*[19] and this initial study was extended by Dainis[20-22] who published a number of additional examples. ^{13}C NMR studies have been conducted by Jones *et al.*;[23] Pugmire *et al.*,[24,25] and a more recent NMR study has been published by Abarca *et al.*[26] A more detailed discussion of our own investigation of the NMR spectra of indolizines can be found in Section 2.1.2.2.

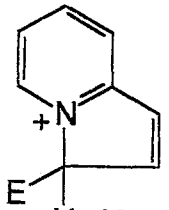


SCHEME 1

1.1.2.2 CHEMICAL PROPERTIES

The bridgehead nitrogen atom of indolizine influences the properties of both rings, giving the five-membered ring the characteristics of electron-rich pyrroles, and the six-membered ring the characteristics of electron-deficient pyridines.[14]

Most reactants for indolizine synthesis are chosen so as to achieve functionality at the desired positions, in the initial step. Relatively simple functional group conversions are then carried out to achieve the required end products. Far less common is the addition of functional groups to an existing indolizine, unsubstituted at the desired positions.



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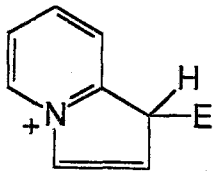

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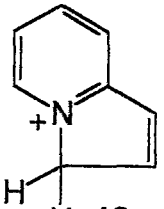
(i) Electrophilic substitution

Indolizines fall into the class of compounds known as π-excessive heterocycles, they readily undergo electrophilic substitution in the 5-membered ring, but are resistant to nucleophilic attack. Molecular orbital, as well as frontier electron density calculations[27,28] predict that the site most susceptible to electrophilic substitution is position 3, followed by position 1. The Wheland intermediates **10** and **11** show that the aromaticity of the pyridine ring is not lost in either case. Experimental evidence supports these predictions, and all reported electrophilic substitutions have occurred at one or both of these positions. Delocalization of the nitrogen lone pair renders indolizine more reactive than benzene towards electrophilic agents.[29]

(a) Protonation

NMR studies show that protonation generally occurs at position 3 to give the conjugate acid **12,** if this position is unsubstituted.[30-33] In 3-substituted indolizines protonation may still occur at C-3, or at C-1 (**13**), depending on the nature of the substituent at C-3, as well as the substituents at positions 1,2 and 5. In 1,3-disubstituted indolizines, where the substituents are the same, protonation occurs exclusively at C-3.[30] Deuterium exchange has also been shown to occur preferentially at positions 3 and 1.[34] Armarego[32] determined the pK_a values of selected indolizines from their UV spectra, and found the pK_a value of indolizine to be 3.9, while that of 2-methylindolizine was found to be 5.9. Although indolizines are relatively weakly basic, they readily form salts with mineral acids.[35]



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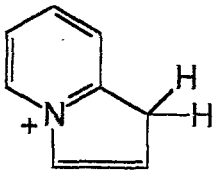

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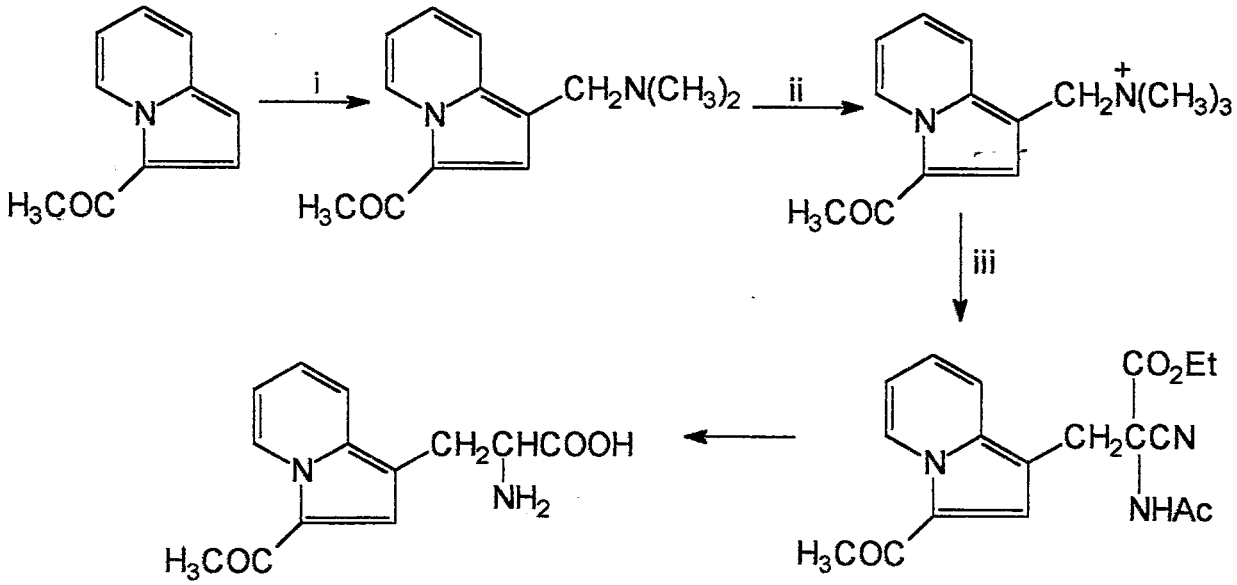
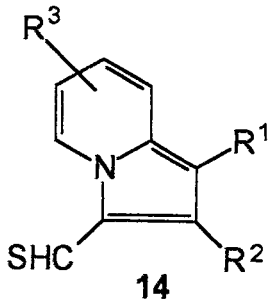
(b) Alkylation

Alkyl indolizines are most conveniently prepared from the appropriate pyridyl precursors, virtually any substitution pattern being achievable in this way. However, alkylation of indolizines *via* the Mannich reaction has been carried out in a number of cases,[36-41] as illustrated in Scheme 2.



SCHEME 2

Reagents : (i) CH_2O, $(CH_3)_2NH$
(ii) CH_3I
(iii) $CNCH(NHAc)CO_2Et$



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(c) Acylation

Acylation of indolizines occurs on heating with acid anhydrides in the presence of the sodium salts of the corresponding acids. The product formed initially is the 3-acyl derivative but, on further heating, the 1,3-disubstituted product is obtained.[29,42] Acyl derivatives may also be formed from acid chlorides and esters.[14,43,44] The Vilsmeier reaction, applied to indolizines, has generally yielded the corresponding indolizine-3-aldehydes.[45] McKenzie and Reid[46] prepared the thioaldehyde derivatives **14**, by solvolysis of the Vilsmeier salts with sodium hydrogen sulphide.

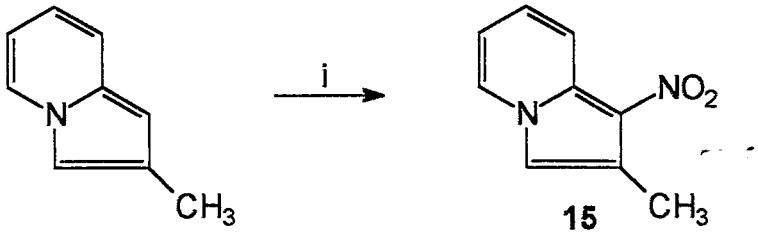
(d) Nitration, Nitrosation and Diazo coupling

Nitration of indolizines is often accompanied by oxidation, and usually results in a mixture of isomers. Borrows *et al.*[47] found that nitration of 2-methylindolizine with nitric acid in concentrated sulphuric acid, yielded predominantly the 1-nitro derivative **15** (Scheme 3). Indolizine and 2-methylindolizine, when treated with nitric acid in excess acetic anhydride at low temperature, yielded the corresponding 3-nitroindolizines.[48]

Nitration of 2-phenylindolizine with a two-fold excess of nitric acid in sulphuric acid yielded 2-(4-nitrophenyl)indolizine and 1-nitro-2-(4-nitrophenyl)indolizine, together with 20% of an unidentified dinitro compound, thought to be 3-nitro-2-(4-nitrophenyl)indolizine.[49]

Direct nitrosation of 2-substituted indolizines leads to the formation of 3-nitroso derivatives[48,50,51] in good yield; when the 3 position is occupied, nitrosation occurs at position 1.

Azo derivatives of indolizines are readily prepared since the arenediazonium ion is a good electrophile,[14,44] and some of these compounds have been used as dyes. Aminoindolizines are unstable, and therefore have not been used to prepare diazonium salts. 1-Nitroindolizines, however, have been shown to give diazonium salts **16** on treatment with



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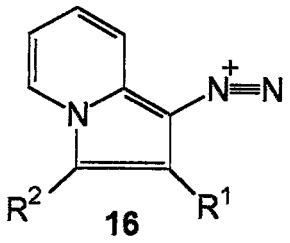
SCHEME 3

Reagents : (i) HNO_3, H_2SO_4



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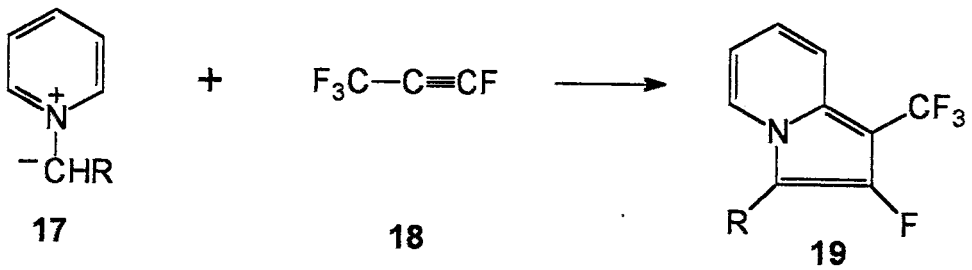


17 18 19

SCHEME 4

nitric oxide. These salts couple readily with phenoxide anions to give the expected azo dyes.[52]

(e) Halogenation

Very little work has been carried out on the halogenation of indolizines, and the only successful reaction to date appears to be iodination. 3-Acetyl-2-phenylindolizine, when treated with iodine in ethanolic solution, yielded 1,3-diiodo-2-phenylindolizine and, in the presence of sodium acetate, yielded 3-acetyl-1-iodo-2-phenylindolizine.[53] Fluorine derivatives **19** have been prepared from pyridinium methylides **17** and perfluoropropene **18** (Scheme 4).[54]

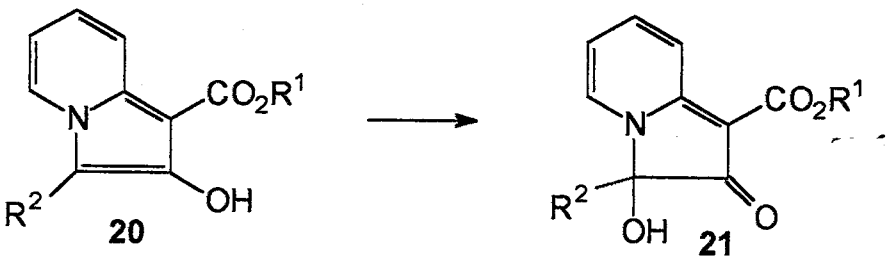
(ii) Oxidation

Oxidation of the indolizine ring system occurs readily, and in many instances results in ring cleavage.[14,44] Electron-withdrawing substituents on the 5-membered ring stabilize indolizine in the presence of oxidants: for example, indolizine-1,2,3-tricarboxylic acid is not oxidized when treated with nitric or chromic acids.[29] In a few cases the indolizine nucleus remains intact during oxidation and Bowers and Brown[55] have described the oxidation of the hydroxyindolizine **20**, in the presence of potassium ferricyanide, to give the hydroxyketone **21** (Scheme 5).

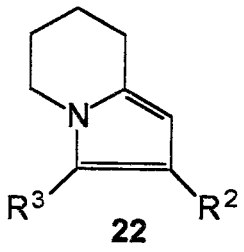
(iii) Reduction

Borrows *et al.*[56] and Dainis[57] investigated the reduction of indolizines, and found that substituted indolizines could be reduced to 5,6,7,8-tetrahydroindolizines **22** in the presence of platinum oxide, raney nickel or copper chromite.

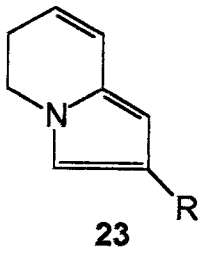
Reduction of indolizine with sodium in liquid ammonia has been shown to give a mixture of 5,6-dihydroindolizine **23** and 5,6,7,8-tetrahydroindolizine **22** (R^2=Me, R^3=H). Selective hydrogenation of the 5-membered ring occurs with palladium-on-charcoal, in



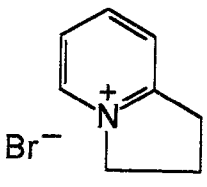
SCHEME 5

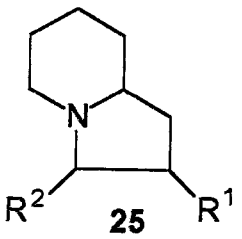


R^2 = Me, Ph
R^3 = Ac



R = Me





the presence of aqueous hydrobromic acid, to yield 1,2-dihydro-3*H*-indolizinium bromide **24**.[58] Complete reduction to indolizidine **25** can be achieved using raney nickel and copper chromite under more vigorous conditions.[56]

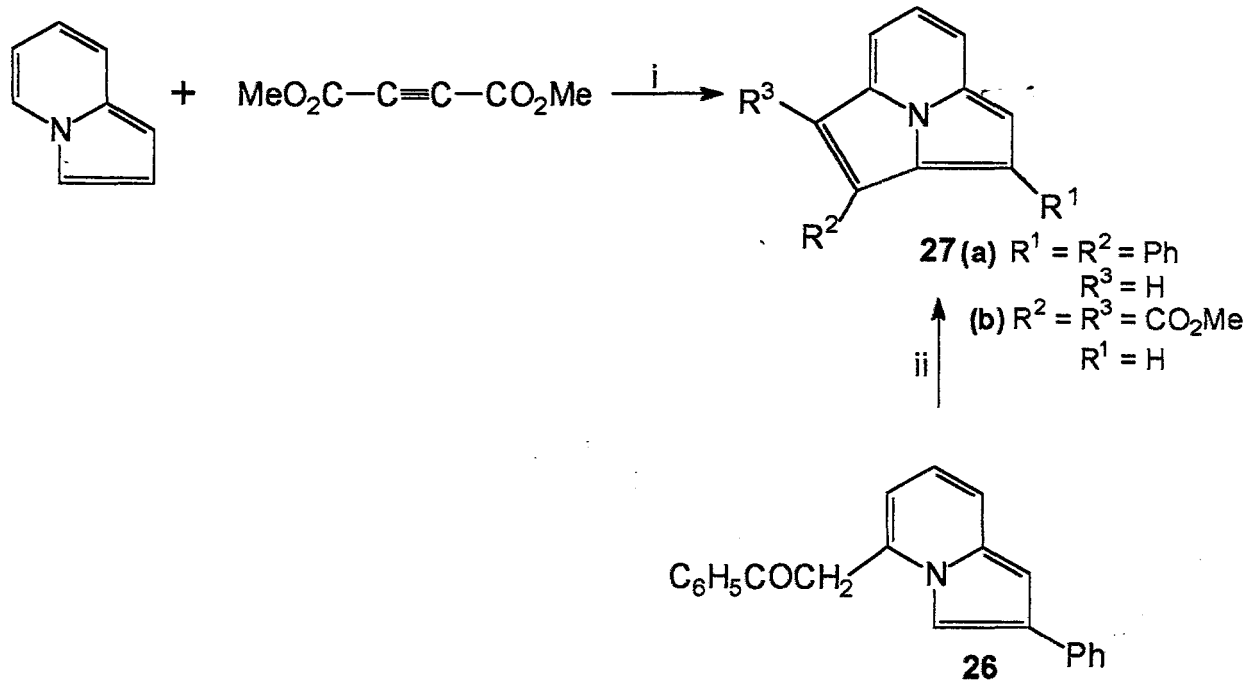
(iv) Cycloaddition

These reactions result in the fusion of one or more rings to the indolizine system. 5-Methyl-2-phenylindolizine was reacted with butyllithium and *N,N*-dimethylbenzamide to give the phenacyl derivative **26** which, on heating in the presence of acetic acid, yielded the [2.2.3]cyclazine **27a** (Scheme 6).[59]

Indolizine also reacts with dimethyl acetylenedicarboxylate in the presence of palladium-on-carbon to give [2.2.3]cyclazine **27b** (Scheme 6).[60] There are many other examples of indolizine cycloadditions in the literature.[61-67]

(v) Reactivity of ring substituents

3-Nitroso-, 3-nitro-, and 3-azoindolizines have been reduced to the corresponding amines in the presence of palladium-on-charcoal and hydrazine hydrate.[48,68] Treatment of amides with lithium aluminium hydride also yielded amines.[69] LAH reduction of a 2-methylindolizine-3-aldehyde yielded 2,3-dimethylindolizine[46], while similar treatment of esters yielded the hydroxymethyl derivatives. More vigorous conditions resulted in formation of the alkyl derivatives.[70] Preparation of amides from esters and the appropriate alkylamines has been carried out, but in low yields.[69-72] Holland and Nayler[73] prepared amides *via* the 3-carbonyl chloride, but all attempts to prepare a similar carbonyl chloride in position 2 failed.[69] An efficient approach to indolizine-2-carboxamides was established in the present study, and will be discussed in Section 2.2.1. Recently Renard and Gubin[74] reported the first regioselective lithiation of 2-phenylindolizine at position 5. This permits the introduction of many different substituents in this position, including alkyl, aryl or trimethylsilyl groups.



SCHEME 6

Reagents : (i) Pd/ C, toluene
(ii) AcOH, heat

1.1.2.3 SYNTHESIS OF INDOLIZINES

The first known synthesis of indolizine was carried out by Scholtz[2] in 1912. Subsequently, many more efficient syntheses were developed and continue to be developed to the present day. These syntheses can be divided into a number of different types on the basis of where bond formation occurs,[14] and these are discussed below. Methods for the construction of the indolizine nucleus have been extensively reviewed.[1,14,29,35,42,44,75,76]

1.1.2.3.1 SYNTHESES INVOLVING FORMATION OF ONE BOND

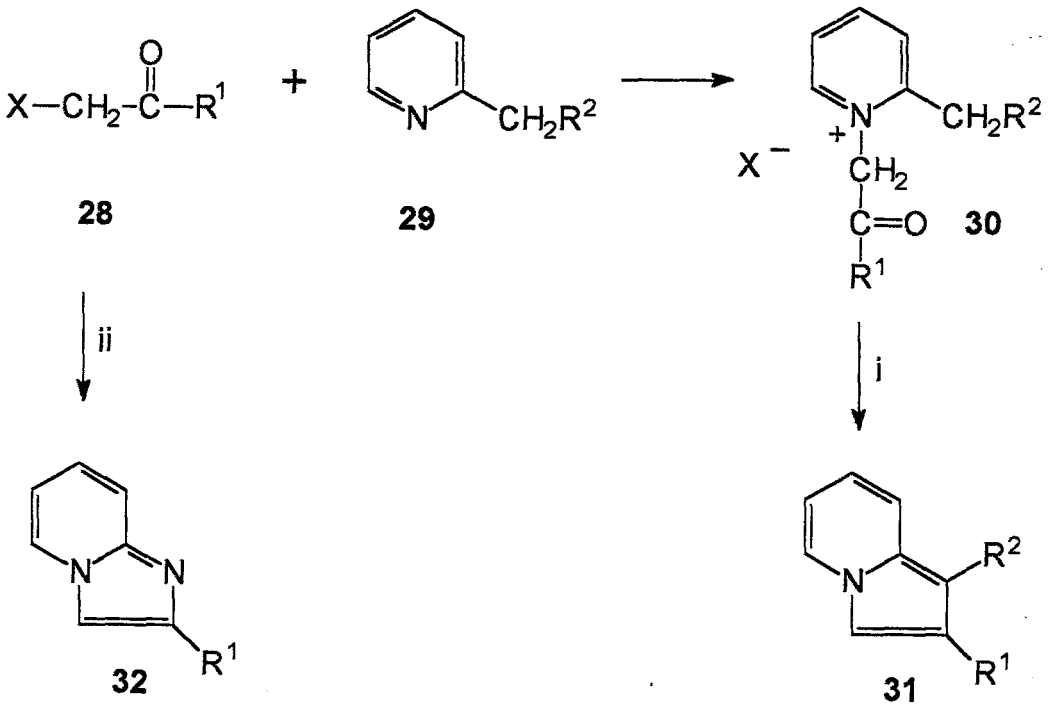
(i) The Tschitschibabin and related reactions : 1,2-bond formation

The original reactions were carried out by Tschitschibabin[77] in 1927 and involved the reaction of α-halogenated ketones **28** with 2-picoline or its derivatives **29** (Scheme 7). The resulting quaternary salts **30** were converted into indolizines **31** by heating with aqueous sodium hydrogen carbonate. The synthesis is analogous to Tschitschibabin's earlier synthesis of imidazo[1,2-*a*]pyridines **32** from 2-aminopyridines and α-haloketones **28**.[35] Azaindolizines with more than one nitrogen atom in the 6-membered ring can also be synthesized using this procedure by replacing 2-picoline with a suitable aza-analogue.[78]

This approach is particularly useful for the synthesis of 2-alkyl or 2-arylindolizines,[51,79,80] but is of no use for the synthesis of indolizines unsubstituted in the 5-membered ring.[80] Modifications to the original synthesis have broadened the scope of this reaction considerably. Borrows and Holland[80] replaced the α-bromoketone with ethyl bromopyruvate **28** (R^1=CO_2Et, X=Br) and successfully synthesized indolizine-2-carboxylic acid **31** (R^1=COOH, R^2=H) in 30% yield. Bragg and Wibberley[81,82] successfully employed ethyl 2-pyridylacetate **29** (R^2=CO_2Et) and related compounds instead of 2-picoline in reactions with a range of α-bromo carbonyl compounds **28**, making it possible to synthesize directly indolizines with ester, acyl or cyano groups in

positions 1 or 3. The reaction of α-bromoesters with ethyl 2-pyridylacetate **29** yielded the first 2-hydroxyindolizines to be reported. A mechanism for the Tschitschibabin reaction involving an aldol-type condensation was suggested by Bragg and Wibberley.[82] Reaction of bromoacetaldehyde **28** (R^1=H, X=Br) with 2-cyanomethylpyridine **29** (R^2=CN) and 2-nitromethylpyridine hydrate **29** (R^2=NO_2) yielded 1-cyano- **31** (R^2=CN) and 1-nitroindolizine **31** (R^2=NO_2) respectively.[83]

The Tschitschibabin reaction has been widely employed for the synthesis of substituted indolizines since its discovery[1,35,76] and continues to be the synthesis of choice in a large number of cases.[71,72,84-86]

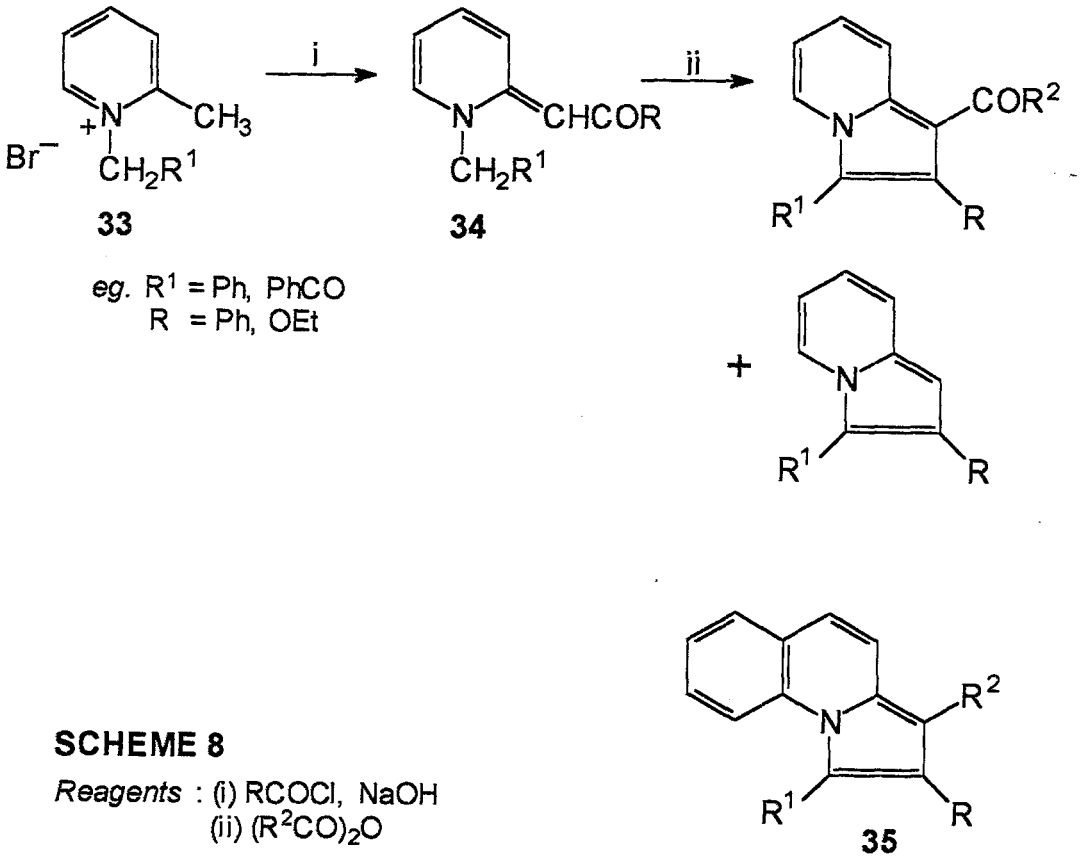


SCHEME 7

Reagents : (i) $NaHCO_3$, heat
(ii) 2-aminopyridine

(ii) 2,3-Bond formation

Pyridinium salts, in the presence of acetic and other anhydrides, have been shown to cyclize to give indolizines by 2,3-bond formation.[14,29,44] The cyclization occurs *via* an intramolecular aldol-type condensation of suitable acyl- or ethoxycarbonyl-methines **34** on heating in acetic anhydride (Scheme 8).[87,88] The reaction sequence starts from a quaternary salt **33** which is acylated and dehydrobrominated in one step to give the methine **34**.[88] Methines in which either the *N*-methylene or the side-chain carbonyl group is not suitably activated, for example the 1-ethylmethines **34** ($R^1=CH_3$), give unexpected indolizine products as a result of competing reaction pathways.[20-22,88] This approach is particularly useful for the preparation of 1,2,3-trisubstituted indolizines. The same approach was employed by Melton *et al.*[87] for the synthesis of pyrrolo[1,2-*a*]quinolines **35** from the corresponding methines (Scheme 8).

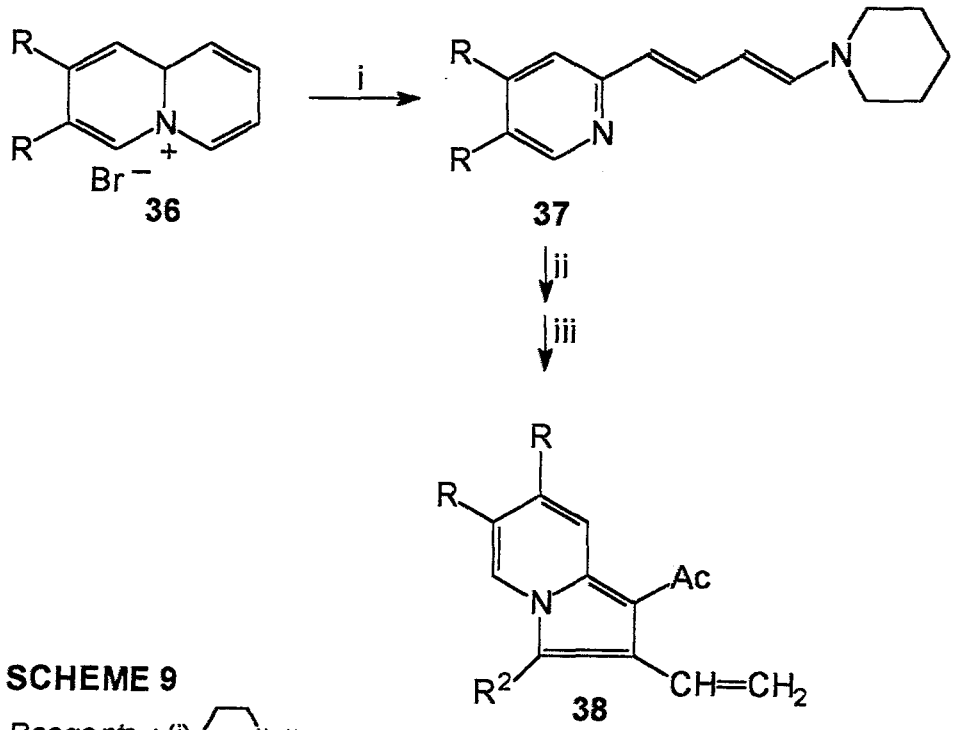


SCHEME 8

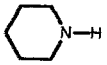
Reagents : (i) RCOCl, NaOH
(ii) $(R^2CO)_2O$

Secondary amines such as piperidine can cleave quinolizinium salts **36**, resulting in tetramethine compounds **37** which can be converted into indolizines **38** (Scheme 9).[89]

Tominaga *et al.*[67] prepared 1-cyano-2-methylthioindolizines by heating pyridinium salts in the presence of triethylamine or potassium carbonate.



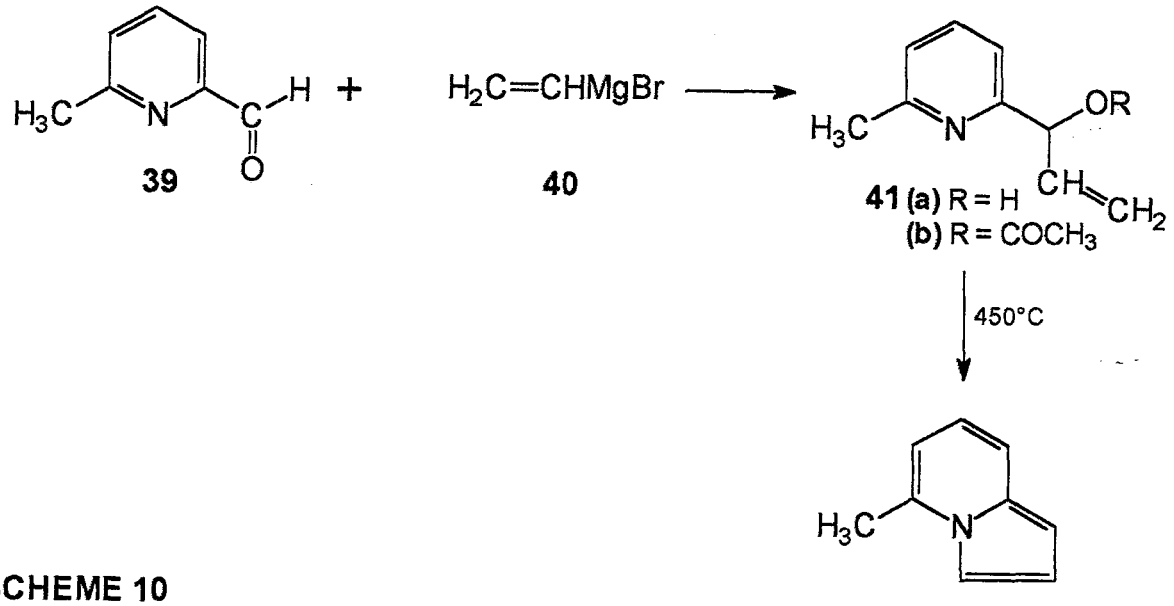
SCHEME 9

Reagents : (i) 
(ii) R^1X
(iii) Ac_2O, Et_3N

(iii) 3,4-Bond formation

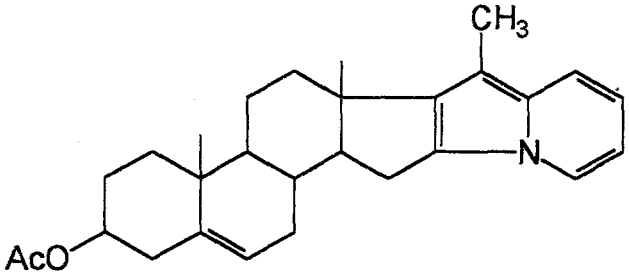
This route has proved particularly useful for the synthesis of indolizines unsubstituted in the 5-membered ring. Boekelheide and Windgassen[90] developed a synthesis of 5-methylindolizine **42** which involved treatment of 6-methyl-2-pyridinecarboxaldehyde **39** with vinyl magnesium bromide **40** to give the allyl alcohol **41a** in 68% yield. This alcohol was converted to the acetate **41b**, and subsequent pyrolysis at 450°C, yielded 5-methylindolizine **42** in 30% yield (Scheme 10).

An analogous cyclization was carried out by Heer and Hoffmann[91] by treating a steroidal alcohol with phosphorous tribromide and methanol, to yield the highly substituted indolizine **43** in 50% yield.



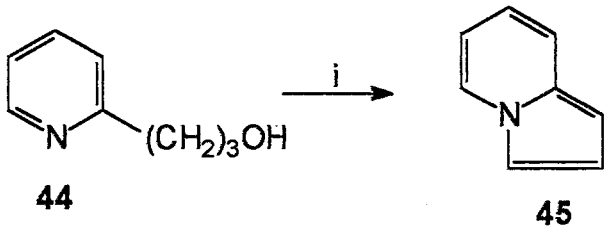
39 40 41 (a) R = H, (b) R = $COCH_3$ 42

SCHEME 10



43

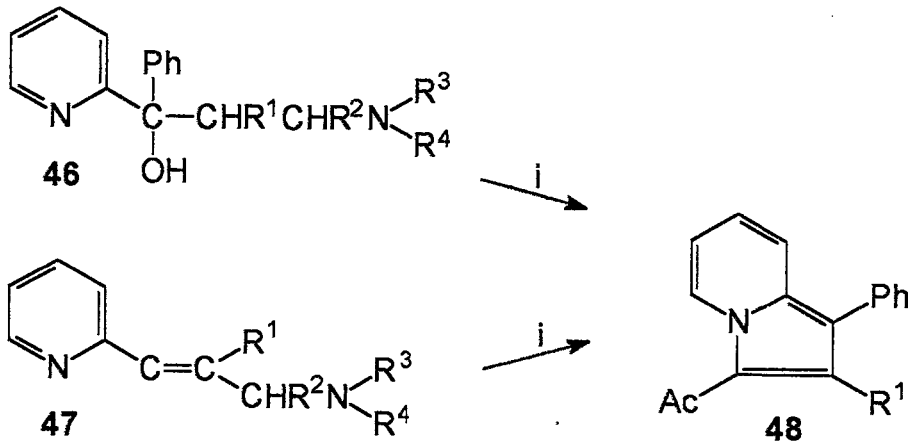
The 2,3-bond formation approach has also been used in several syntheses of the parent indolizine. Thus, indolizine **45** was synthesized in 50% yield by heating 3-(2-pyridyl)-1-propanol **44** in the presence of a dehydrogenation catalyst (Scheme 11).[90] Treatment of compound **44** with tosyl chloride followed by heating of the resulting salt with rhodium on alumina at 200°C gave indolizine **45** in 60% yield.[92] Boekelheide and Feely[93] acetylated the *N*-oxide of **44** with acetic anhydride, and then heated the diacetoxy product to obtain indolizine **45** in 35% overall yield (Scheme 11).



44 **45**

SCHEME 11

Reagents : (i) Pd/ C or TsCl / Rhodium on alumina



46 **47** **48**

SCHEME 12

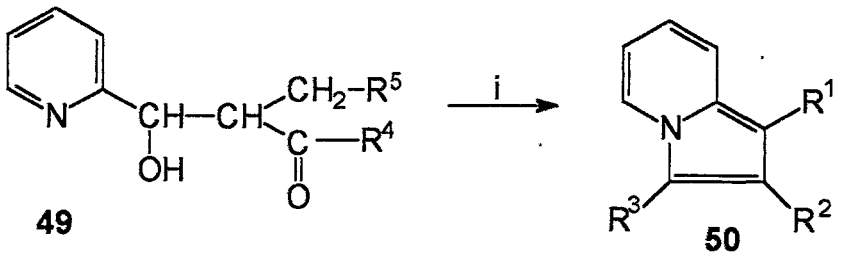
Reagents : (i) Ac_2O, heat

Barrett[94] found that treating pyridylalkanols, such as compound **46**, and pyridylalkenes, such as **47**, with boiling acetic anhydride led to the corresponding 1-arylindolizines **48** (Scheme 12). Extension of this method for the synthesis of 1-alkylindolizines has been reported.[95]

Cyclization of the aldol products **49** of pyridine-2-carboxaldehyde and β-dimethylamino- (or β-hydroxy-) ketones led to a novel synthesis of 2-acylindolizines **50** (Scheme 13).[96] Indolizine derivatives **52a** and **52b** were also obtained from the Perkin reaction of 2-pyridinecarboxaldehyde, the expected 3-(2-pyridyl)acrylic acids **51** not being isolated (Scheme 14).[97] Treatment of 2-hydroxy-5-azachromans **53** with sodium acetate in excess acetic anhydride yielded 8-acetoxyindolizines **54** (Scheme 15).[98]

The first indolizine synthesis by Scholtz,[2] which is discussed in detail in a number of reviews[1,14,29,35,44,76] also involves 3,4-bond formation. This synthesis involves reaction of 2-picoline with acetic anhydride, and a large number of indolizines have been synthesized by this route. The Scholtz synthesis is no longer popular as a result of the low yields obtained in many cases.

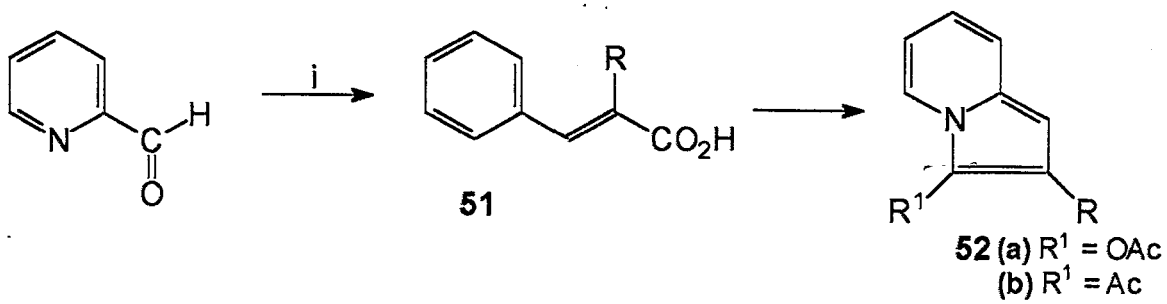
A number of azaindolizines have been synthesized utilizing the 3,4-bond formation route. An example of this is given in Scheme 16, where cyclization of the amino compound **55** gives rise to an imidazo[1,5-*a*]pyridine **56**. Uchida and Matsumoto[1] discuss further examples.



49 **50**

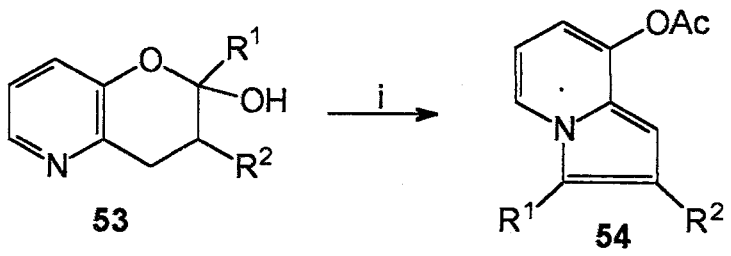
SCHEME 13

Reagents : (i) Ac_2O, heat



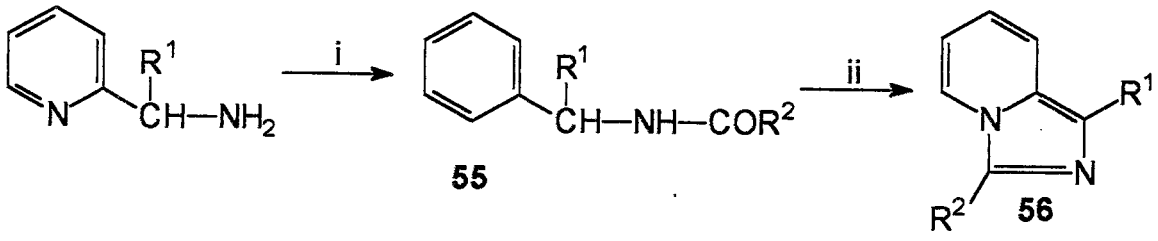
SCHEME 14

Reagents : (i) Ac_2O, KOAc



SCHEME 15

Reagents : (i) NaOAc, Ac_2O



SCHEME 16

Reagents : (i) R^2COOH

(ii) $POCl_3$, benzene, heat

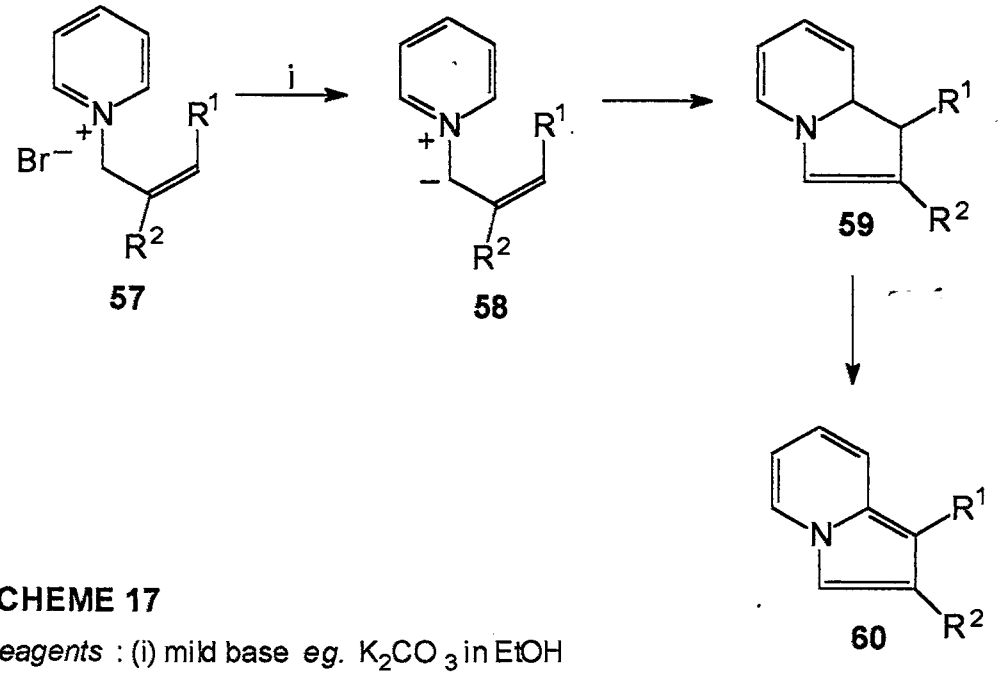
(iv) 1,5-Dipolar cyclizations : 9,1-bond formation

In the presence of mild base, pyridinium *N*-allylides **58**, which are readily accessible by dehydrohalogenation of the corresponding pyridinium salts **57**, have been shown in the presence of mild base to yield indolizines **60** *via* 1,5-dipolar cyclizations (Scheme 17).[67,99-106] The pyridinium *N*-allylides may react intramolecularly as 1,5-dipoles, or may react intermolecularly as 1,3-dipoles.[101,103] The predominance of 1,3- or 1,5-dipolar cyclization is influenced by the substituents on the double bond. Increased stability of ylides favours 1,5-cyclization[104] while increased steric hindrance, for example in pyridinium allylides with geminal substituents, favours 1,3-dipolar cycloaddition, which is discussed below.[103]

The 1,5-cyclization seems to proceed *via* a symmetry-allowed electrocyclic ring closure of the ylide intermediate **58**, followed by loss of two hydrogen atoms to yield indolizines.[102] In certain cases the dihydroindolizines **59** have been isolated and stored in an inert atmosphere for varying lengths of time.[100]

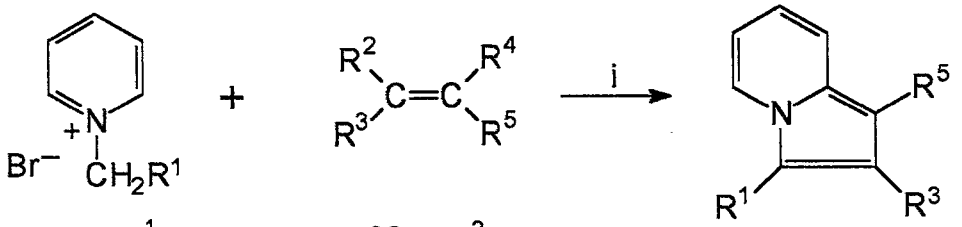
A number of indolizines have been successfully prepared using this method. Tamura *et al.*[102] synthesized 1-benzoyl-2-phenylindolizine **60** (R^1=COPh, R^2=Ph) from the pyridinium salt **57** (R^1=COPh, R^2=Ph) obtained from pyridine and 4-bromo-1,2-diphenyl-2-buten-1-one. 1-Acetyl- **63a** and 1-aroylindolizines **63b** have been prepared by the reaction of aromatic amine *N*-ylides **61a** and **61b** with methoxyethylene compounds **62a** and **62b** (Scheme 18).[105] Synthesis of indolizine-3-carbonitrile derivatives **63c** by reaction of *N*-cyanomethylpyridinium *N*-ylides **61c** with suitably substituted ethylene compounds, has been reported. Similar reactions have been used to synthesize 1-nitroindolizines.[67,107]

Reaction of diphenylcyclopropenone **64** with pyridine or substituted pyridine derivatives also leads to indolizines (Scheme 19).[108-110] These reactions are thought to involve 1,5-dipolar intermediates.[1]



SCHEME 17

Reagents : (i) mild base *eg.* K_2CO_3 in EtOH



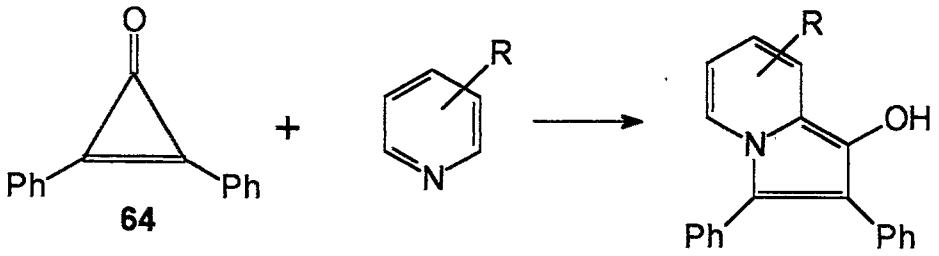
61(a) $R^1 = CO_2Et$
(b) $R^1 = CO_2Et$
(c) $R^1 = CN$

62(a) R^2 = OMe
R^3, R^4 = H
R^5 = COMe
(b) R^2 = OMe
R^3, R^4 = H
R^5 = COPh
(c) R^2, R^3 = SMe
R^4 = H
$R^5 = NO_2$

63(a)
(b)
(c)

SCHEME 18

Reagents : (i) Et_3N



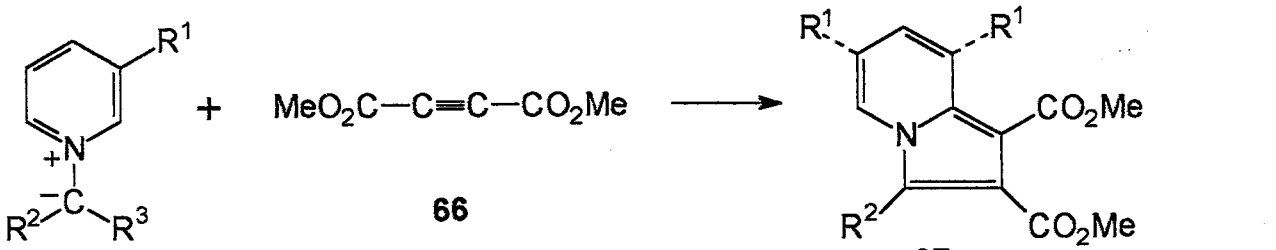
64

SCHEME 19

1.1.2.3.2 SYNTHESES INVOLVING FORMATION OF TWO BONDS

(i) 1,3-Dipolar cycloadditions

Pyridinium ylides act as 1,3-dipoles, and reaction with suitable dipolarophiles leads to indolizine derivatives *via* a cycloaddition - elimination sequence.[111] Boekelheide and Fahrenholtz[112] treated pyridinium phenacylide **65a** with dimethyl acetylenedicarboxylate (DMAD) **66** in the presence of a palladium-on-charcoal catalyst and obtained dimethyl 3-benzoylindolizine-1,2-dicarboxylate **67a** in 20% yield (Scheme 20). Henrick *et al.*[113] later showed the dehydrogenation catalyst to be unnecessary and higher yields were obtained under milder conditions.



65 (a) R^1, R^3 = H; R^2 = COPh
(b) R^1 = H; R^2, R^3 = CN
(c) R^1 = Me; R^2, R^3 = CN
(d) R^1, R^3 = H; R^2 = COMe
(e) R^1, R^3 = H; R^2 = CO_2Et
(f) R^1 = H; R^2 = CN; R^3 = COOR
(g) R^1, R^3 = H; R^2 = SO_2–*p*–Tol

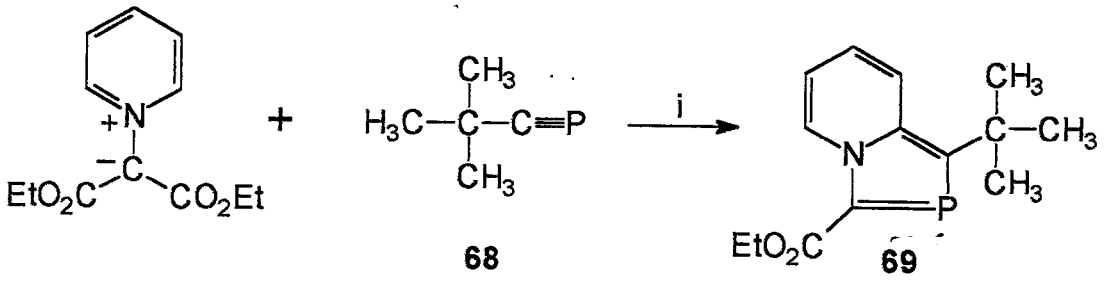
67 (a) - (g)
(c) R^1 = 8 - Me or 6 - Me
(g) R^2 = H

SCHEME 20

DMAD has commonly been employed as the dipolarophile in reactions with various pyridinium ylides. Pyridinium dicyanomethylide **65b**, prepared from pyridine and tetracyanoethylene oxide,[111] reacts with DMAD to give dimethyl 3-cyanoindolizine-1,2-dicarboxylate **67b**, while 3-substituted pyridinium dicyanomethylides **65c** react with DMAD to yield a mixture of 8-substituted and 6-substituted indolizine derivatives **67c** (Scheme 20).[114] Other pyridinium ylides which have been reacted with DMAD to yield indolizines include pyridinium-acetonylide **65d**, pyridinium-ethoxycarbonylmethylide **65e**,[113] cyanocarbalkoxy-pyridinium-methylides **65f**[115] and 1-pyridinium-arylsulphonyl-methylides **65g**.[116]

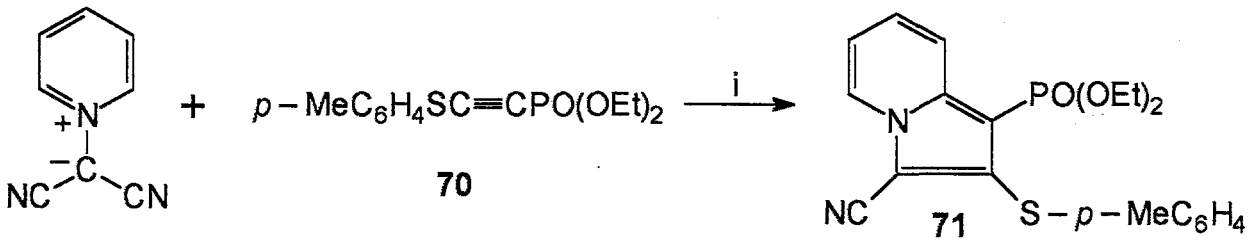
Various dipolarophiles may be employed in this reaction, leading to a wide range of indolizine derivatives. Bergstraßer *et al.*[117] used the phosphaalkyne **68** for the synthesis of phosphaindolizines **69** (Scheme 21), while Acheson and Ansell[118] used diethyl *p*-tolylthioethynylphosphonate **70** to synthesize indolizinylphosphonates **71** (Scheme 22). Phenylsulphinylethene **72** and bis(trimethylsilyl)ethyne **73** serve as acetylene equivalents in 1,3-dipolar cycloadditions to yield various indolizines.[119]
Cycloaddition reactions of 1,2,3-triphenylcyclopropene **74** with pyridinium dicyanomethylides lead to 1,2,3-triphenylindolizines **75** and **76**, together with cyanotriphenyl-*4H*-quinolizines **77** and **78** (Scheme 23).[120,121]

The scope of 1,3-dipolar cycloaddition reactions for the preparation of indolizines and azaindolizines has been reviewed.[1,29,44]



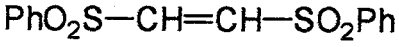
SCHEME 21

Reagents : (i) Toluene, 140°C, pressure



SCHEME 22

Reagents : (i) Toluene, heat



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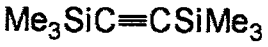

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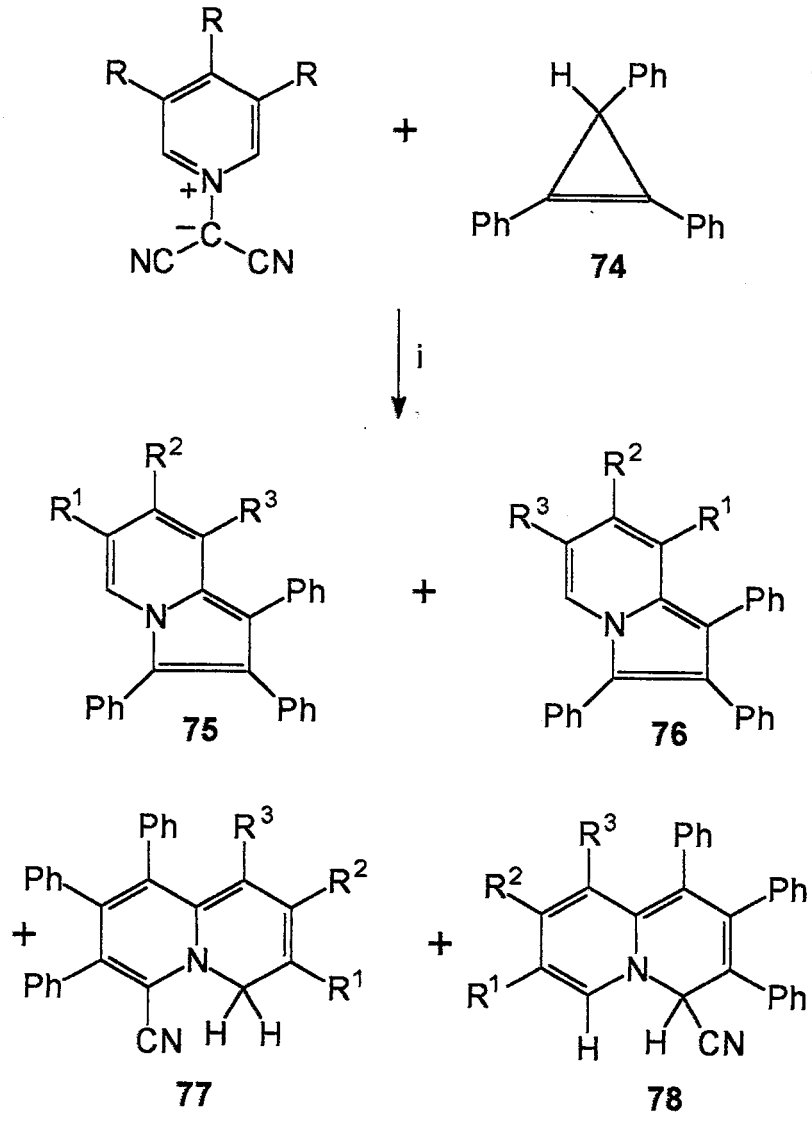
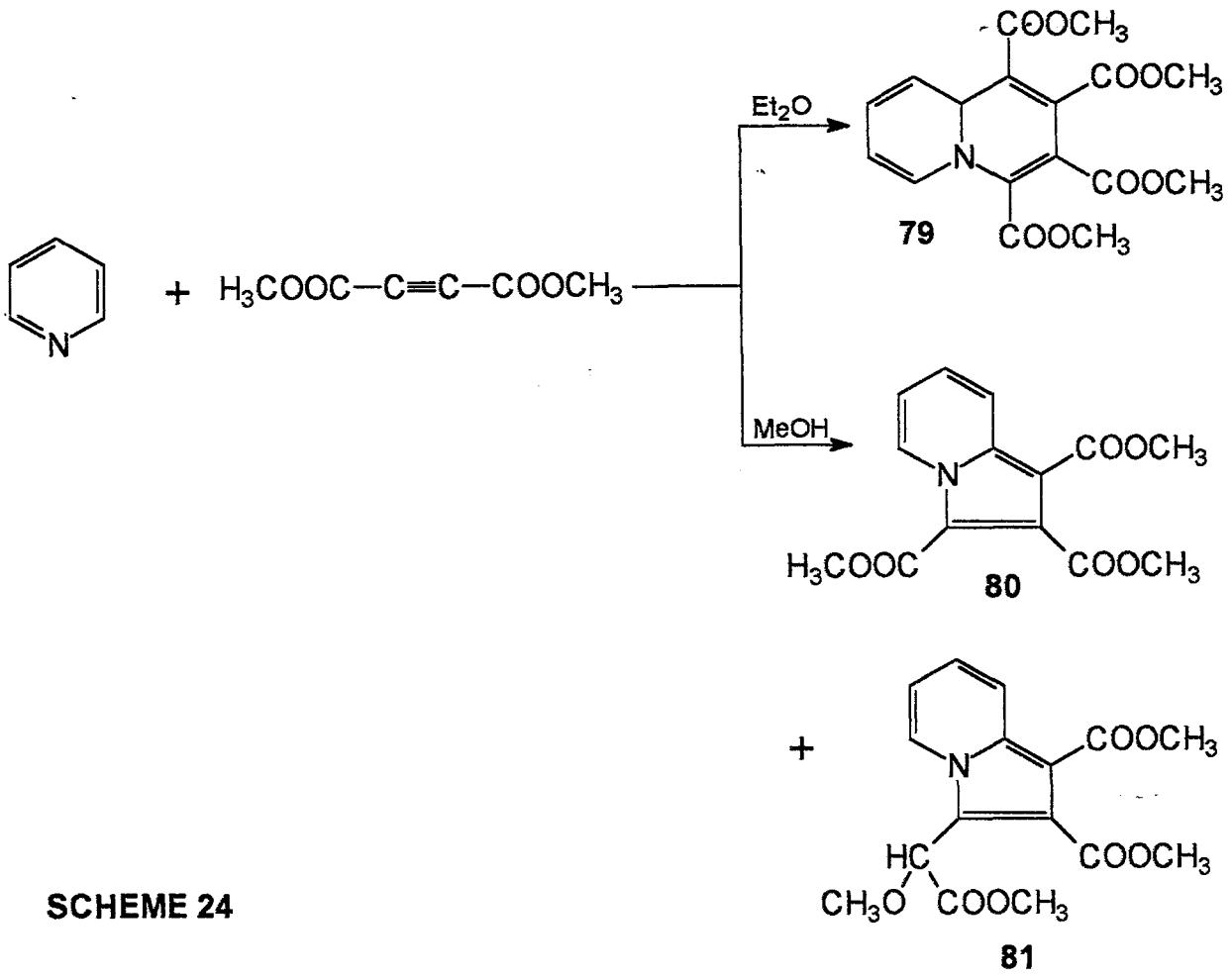


SCHEME 23

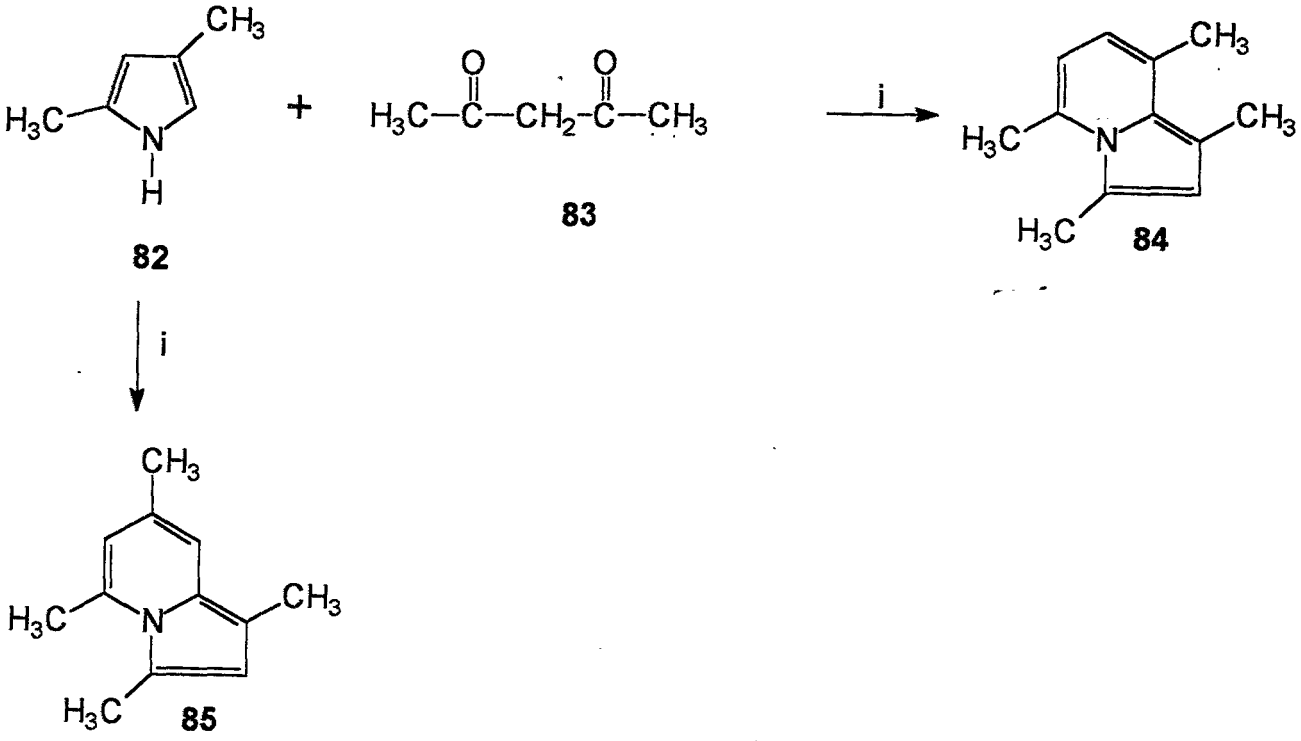
Reagents : (i) DMF, heat

(ii) Diels-Alder synthesis

Diels and his co-workers carried out extensive work on the reactions of pyridine and its homologues with DMAD. They found that, in ethereal solution, the reaction of pyridine with DMAD yielded at least three products,[35] one of which was the tetramethyl ester of *4H*-quinolizine-1,2,3,4-tetracarboxylic acid **79** (Scheme 24). Treatment of this tetramethyl ester with bromine, followed by hydrolysis or oxidation with nitric or chromic acid, yielded trimethyl indolizine-1,2,3-tricarboxylate **80**. Diels *et al.* found that in methanol the same reactants yielded trimethyl indolizine-1,2,3-tricarboxylate **80** directly, together with compound **81**, which could be oxidized to give **80** (Scheme 24).[29] Wiley and Knabeschuh,[122] however, obtained trimethyl indolizine-1,2,3-tricarboxylate **80** directly from reaction of pyridine with DMAD in ether, presumably due to oxidation taking place during the course of the reaction. The products obtained from these reactions thus appear to vary according to the reaction conditions employed. Reactions of pyridines with acetylenic and olefinic compounds to give indolizine esters are discussed by Uchida and Matsumoto.[1] This route to indolizines has not been widely employed in recent years.

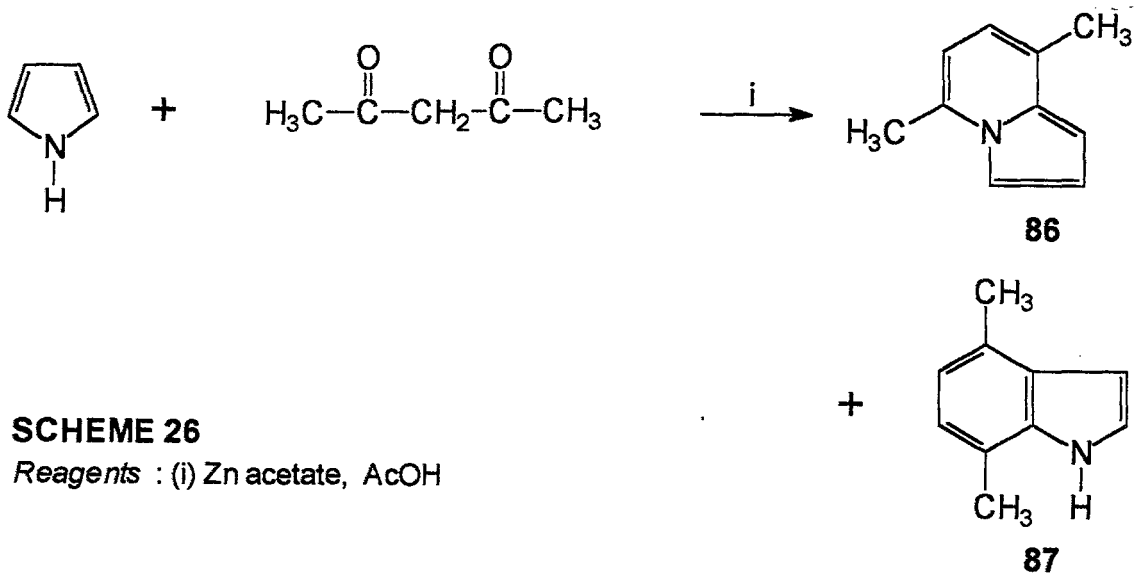

+ H3COOC—C≡C—COOCH3
Et2O
MeOH
COOCH3
COOCH3
COOCH3
COOCH3
79
H3COOC
80
+
HC
CH3O
81

SCHEME 24



SCHEME 25

Reagents : (i) Zn acetate, AcOH



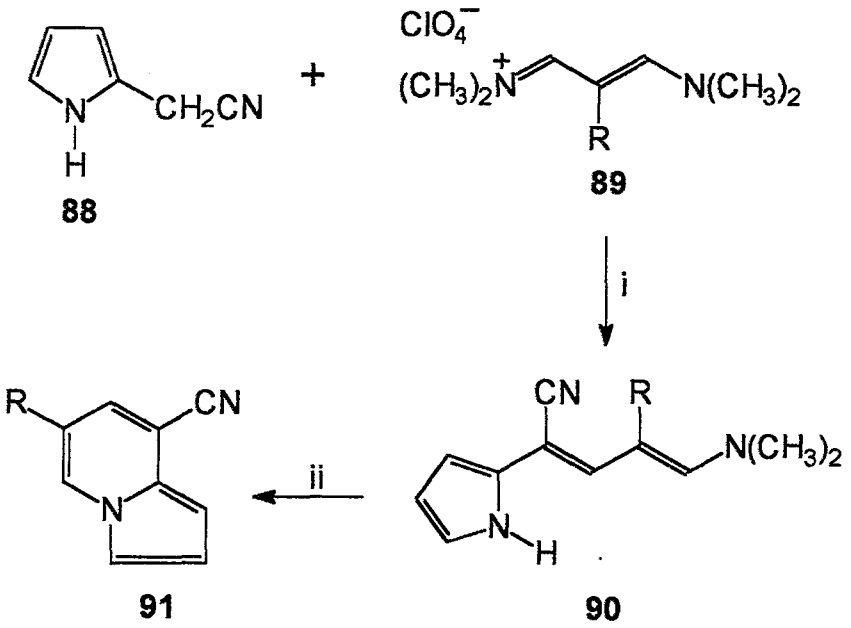
SCHEME 26

Reagents : (i) Zn acetate, AcOH

1.1.2.3.3 OTHER SYNTHESES OF INDOLIZINES

(i) Indolizines from pyrroles

There are a few examples in the literature of indolizine formation from pyrroles. Saxton[123] obtained 1,3,5,8-tetramethylindolizine **84** from the reaction of 2,4-dimethylpyrrole **82** and acetylacetone **83** in the presence of zinc acetate and acetic acid (Scheme 25), while self-condensation of 2,4-dimethylpyrrole **82** in the presence of zinc acetate and acetic acid yielded 1,3,5,7-tetramethylindolizine **85**. Reaction of pyrrole and acetylacetone under the same conditions led to a mixture of 5,8-dimethylindolizine **86** (13%) and 4,7-dimethylindole **87** (Scheme 26).[124,125] *N*-unsubstituted pyrrole-2-acetonitrile **88** condenses with perchlorate salts **89** to form 5-dimethylamino-2-(2-pyrrolyl)-2,4-pentadienonitriles **90**, thermolysis of which leads to indolizine-8-carbonitriles **91** (Scheme 27).[126]

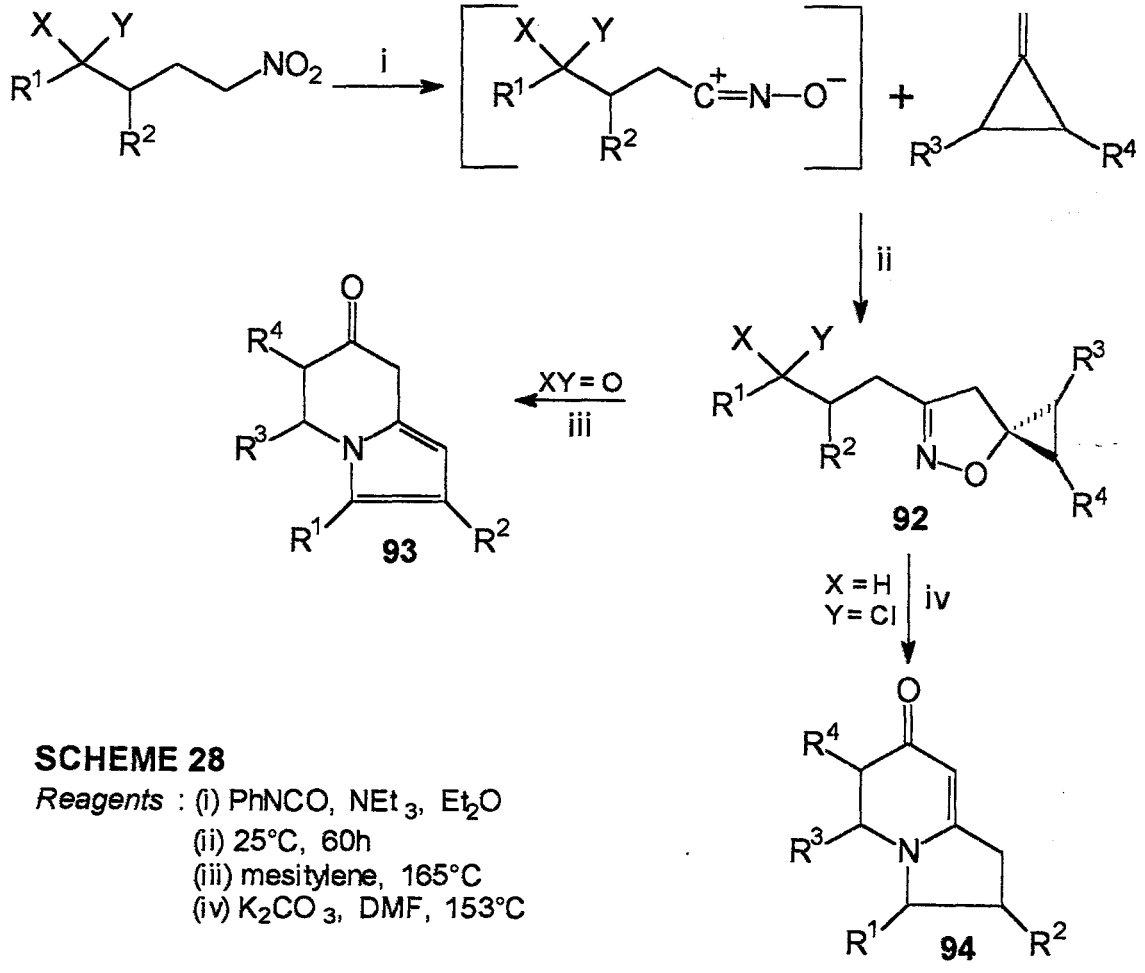


SCHEME 27

Reagents : (i) $NaOCH_3$, 50 - 70°C
(ii) 160 - 220°C

(ii) Miscellaneous syntheses

Certain indolizine syntheses do not appear to fall into any of the previous categories and these are included in this section. Goti and co-workers[127,128] describe the thermal rearrangement-annulation of isoxazoline-5-spirocyclopropanes **92** to yield various partially saturated indolizine derivatives **93** and **94** (Scheme 28). Wilson[129] found that reaction of furan and ammonia over an alumina catalyst at 400°C gave small amounts of indolizine.

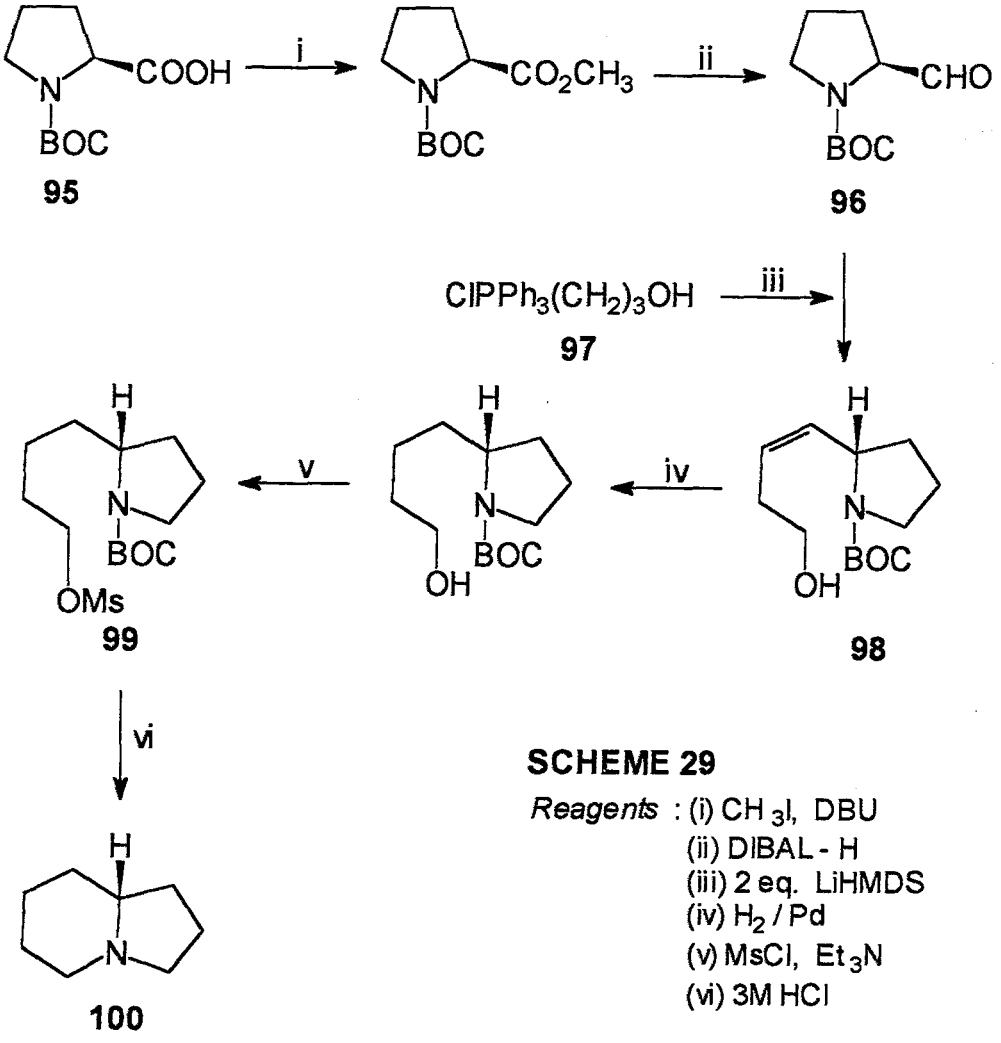


SCHEME 28

Reagents : (i) PhNCO, NEt_3, Et_2O
(ii) 25°C, 60h
(iii) mesitylene, 165°C
(iv) K_2CO_3, DMF, 153°C

1.1.2.3.4 Indolizidine synthesis

Much interest has been shown in recent years in the synthesis of indolizidines, as a result of the large range of biological activities exhibited by this group. The syntheses are regularly reviewed[9] and only one example will be discussed here. Sibi and Christensen[130] described a synthesis of the parent indolizidine, δ-coniceine **100**, from the amino acid precursor L-prolinal **95** (Scheme 29). The phosphorane **97** was treated with 2 equivalents of LiHMDS, followed by addition of the aldehyde **96** to give **98** as a cis/trans mixture. Hydrogenation of the olefin **98** was followed by mesylation of the resulting primary alcohol to give **99**. Deprotection and cyclization yielded the final product **100** in an overall yield of 42%.



SCHEME 29

Reagents : (i) CH_3I, DBU
(ii) DIBAL - H
(iii) 2 eq. LiHMDS
(iv) H_2 / Pd
(v) MsCl, Et_3N
(vi) 3M HCl

1.1.2.4 APPLICATIONS OF INDOLIZINES AND DERIVATIVES

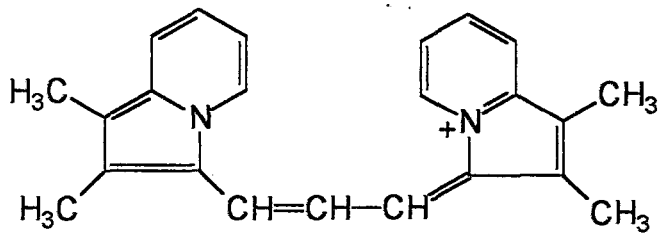
Much interest has been shown over the years in the use of indolizine derivatives as dyestuffs,[76,131] and many studies have been conducted on their incorporation into azo dyes. Mono- and poly-methine dyes incorporating indolizines, for example compound **101**, are used as photographic sensitizing and fabric brightening agents.[14,76] Access to a new class of dyes incorporating an indolizine structural unit **104** has recently been developed, utilizing the oxidative coupling reaction of oxoindolizinium salts **102** and active methylene compounds **103** (Scheme 30).[132]

Many indolizidines are naturally occurring, and they have been found to exhibit a wide range of biological activities.

Slaframine **5** is a mycotoxin responsible for the disease, "black patch", in ruminants consuming feed contaminated with *Rhizoctonia leguminicola*. This alkaloid has potential as a stimulator of parasympathomimetic exocrine glands. There is also significant interest in its use to improve efficiency of feed utilization in ruminants, and lactation and growth in sheep and cattle.[8,9]

Swainsonine **6** and castanospermine **7** are potent inhibitors of certain glycosidase enzymes and, as such, have potential use as antimetastatic or immunomodulatory agents.[130,133] Castanospermine **7** has been shown to inhibit replication of a number of viruses, including the human immunodeficiency virus (HIV),[10,130,134] while certain of the indolizidine alkaloids found in skin extracts of the *Dendrobatidae* family of poison frogs are considered to be potential partial agonists for nicotinic acetyl choline receptors.[11]

There are many more examples of biologically active indolizidines, and a number of these are discussed in a recent review article by Michael.[9]



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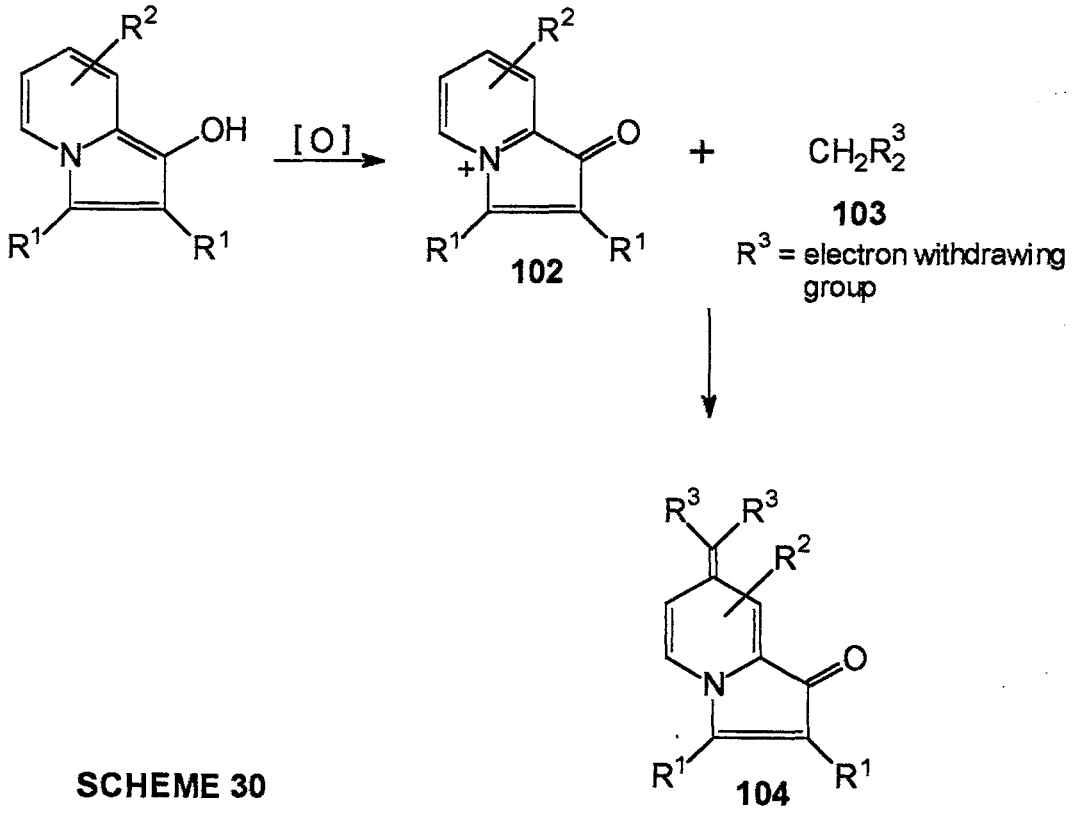
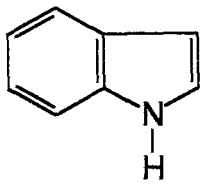


SCHEME 30

The indolizine system has received attention with respect to its potential biological activity. Indole **105** forms the basis of many biological compounds, and replacement of the indole ring with indolizine could conceivably give rise to compounds with pharmacological activity. Carbon and Brehm[41] synthesized 1-indolizinealanine as a possible tryptophan antimetabolite, and Cardellini *et al.*[135] prepared indolizine-1-acetic acid, the structural analogue of heteroauxin (indole-3-acetic acid), and found it to have appreciable auxin-like activity. The Mannich bases **106** were prepared from 2-phenylindolizines as possible bufotenine **107** analogues. Bufotenine is known to have pronounced central nervous system activity and biological evaluation of the indolizine analogues revealed central nervous system (CNS) depressant activity.[37-40] Partially saturated aminoalkylindolizines **108** have also been shown to be highly CNS active *in vivo*.[136]



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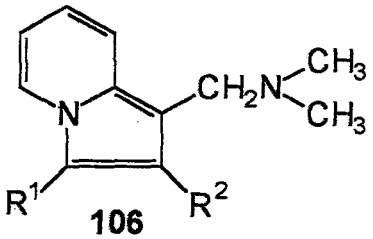

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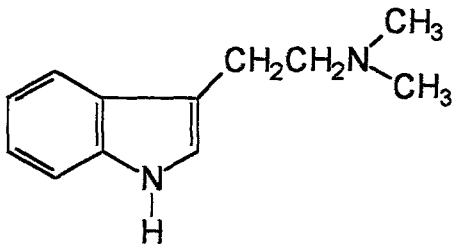
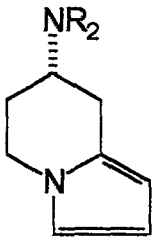
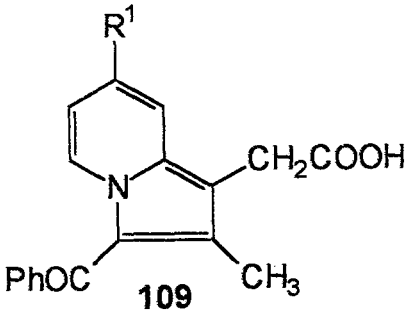


107



108

Another group of indole-analogues shown to have significant biological activity are certain indolizine-1-acetic acids **109**, which are similar to indomethacin **110**, a known anti-inflammatory agent. The indolizine-1-acetic acids exhibit anti-inflammatory as well as analgesic activity.[86] A series of *N'*-substituted hydrazides of indolizine-2-carboxylic acid **111** showed antimonoamine oxidase activity;[137] diethyl indolizine-1,2-dicarboxylate showed significant antineoplastic activity in Ehrlich ascites carcinoma;[138] certain 2-(*N*-alkylaminomethyl)indolizines **112** exhibited weak anti-inflammatory, anti-Parkinson and hypoglycaemic activity;[69] and indeno[2,1-*b*]indolizine derivatives **113** were shown to be analgesic and antiacid agents.[139]



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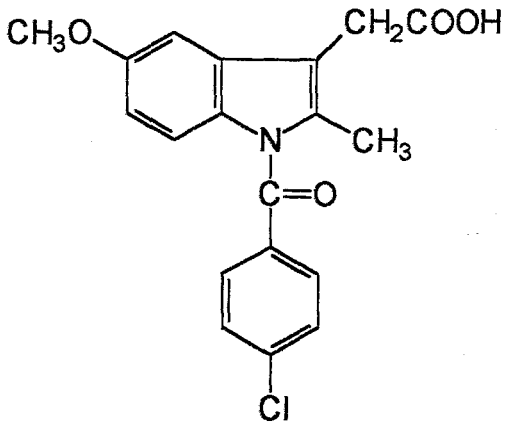

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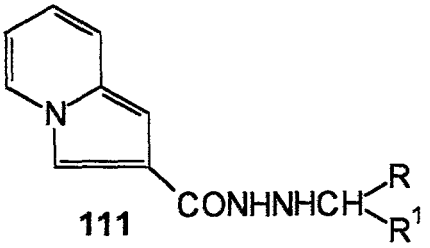

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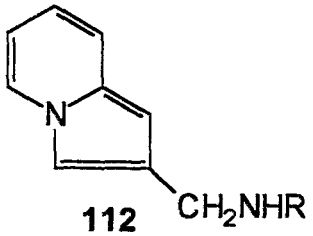

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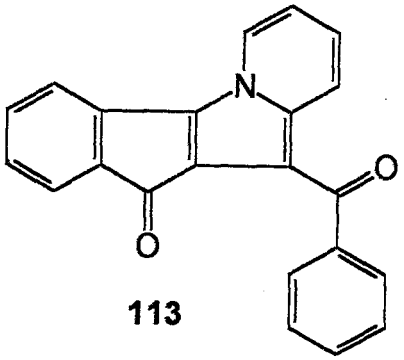
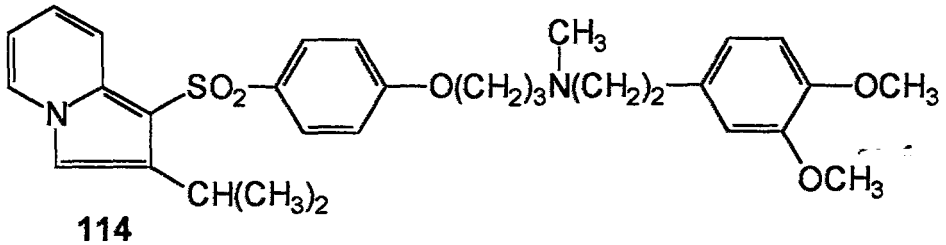


113

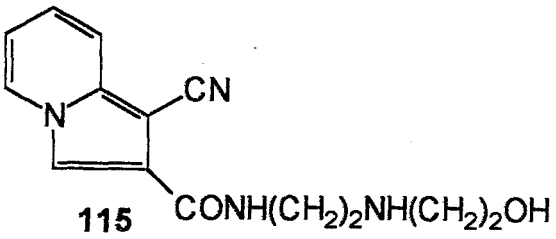
Multi-drug resistant cells are characterized by reduced drug accumulation. This can be reversed to some extent by treatment of these cells with "chemosensitizers", which increase intracellular drug accumulation. One group of chemosensitizers are the calcium channel blockers and a newly discovered calcium channel blocker is the indolizine sulphone **114**, which has been shown to be a more potent chemosensitizer than verapamil, one of the most active calcium channel blockers.[140,141]

Compounds labelled with a fluorophore can be used as markers of biological states, or as indicators of either biochemical processes or the presence of specific chemicals in biological systems.[72] Indolizines, as a result of their high fluorescence quantum yields, are a good choice as fluorophores. Mahon *et al.*[72] synthesized indolizine carboxylic acids, esters and carboxamides, for example **115**, which can be linked to the biologically active part of a probe molecule and, using this approach, prepared fluorescent probes to test for hypoxia in solid tumours.[71]

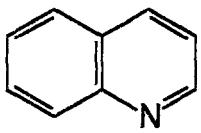
Many biologically active compounds act *via* selective DNA intercalation and, although there are no reports of intercalation studies of indolizines, these systems do have the flat aromatic structure characteristic of intercalating drugs such as antimalarials, antitumour drugs and many others. Indolizine has some similarity to the quinoline systems **116** typically found as antimalarials, the differences being the location of the heterocyclic nitrogen and the size of the heterocyclic ring. It is therefore conceivable that suitably substituted indolizines could act in the same way as intercalating quinoline antimalarials.


CH3
SO2
O(CH2)3N(CH2)2
OCH3
OCH3
N
CH(CH3)2

114


CN
N
CONH(CH2)2NH(CH2)2OH

115


N

116

1.2 DNA INTERCALATION

1.2.1 LIGAND-DNA INTERACTIONS

1.2.1.1 DNA STRUCTURE

The double-helix structure (Fig. 1) of DNA was first proposed by Watson and Crick,[142] on the basis of X-ray diffraction patterns, produced from wet DNA fibres, and molecular modelling. The polynucleotide chains run antiparallel, and hydrogen bonding between specific base pairs holds the chains together. The base pairs are stacked on one another with their planes almost perpendicular (6° off perpendicular) to the helix axis. Strong van der Waals interactions exist between the base pairs, each of which is rotated by 36° with respect to the next, allowing 10 base pairs to be accommodated per helix turn. The base pairs are packed closely within the helix, the distance between each base pair in the helix direction being 0.34nm. The phosphate-deoxyribose backbones are situated on the outside of the helix, in contact with the aqueous environment.

DNA has been shown to exhibit a high degree of polymorphism when exposed to differing environmental conditions. The DNA structure studied by Watson and Crick is known as the B form, and exists under conditions of high humidity.[143] Fibre diffraction studies and studies on single crystals[144] have yielded much information about the various polymorphs. The major forms are the A, B, C, D and Z forms of DNA, the last being a high-salt form which differs from the others in being a left-handed helix.[145-147] Fibre X-ray diffraction studies make the assumption that the helices are regular, with constant rotational and translational helix parameters, the helix parameters given above are thus average values and do not take into account local variations in structure. Single-crystal X-ray structure analysis of short DNA sequences, as carried out by Dickerson,[148] yields detailed information on the helix structure, and has shown extensive local variations in, for example, the helix twist angle.

A consequence of the different geometric features of the DNA polymorphs is that the surface of the helices are significantly different in the different polymorphs. The twist of the helix gives rise to two grooves on the helix surface, known as the major and minor groove, and these grooves differ markedly in dimension from polymorph to polymorph. The size of the grooves is of fundamental importance with regard to the binding of ligands to DNA, as discussed in the following sections.[146,149]

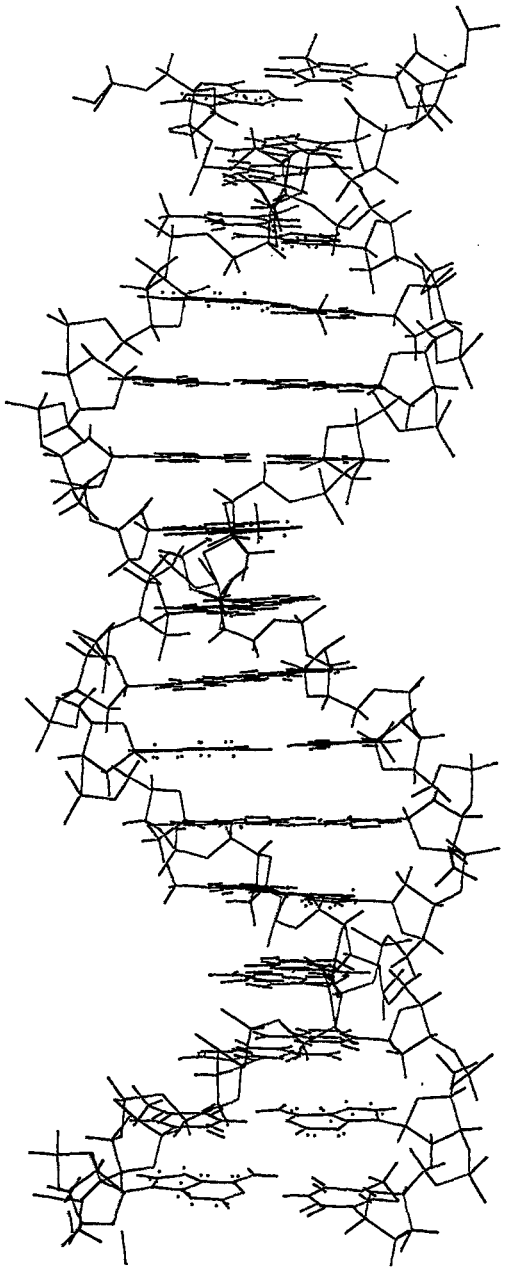


Figure 1 DNA double helix

1.2.1.2 TYPES OF LIGAND-DNA INTERACTIONS

There are many different kinds of interactions between ligands and DNA, including covalent bond formation (*e.g.* alkylation) and various reversible interactions, which are the subject of this discussion. Broadly speaking there are three different types of reversible interactions which can occur between ligands and DNA: (i) non-specific electrostatic interaction with the external phosphate groups; (ii) groove-binding interactions with either the major or minor groove and; (iii) intercalation between base pairs.[146,150]

The non-specific electrostatic interactions have been shown to occur with, for example, lithium and sodium ions,[146] and with planar aromatic cations, such as proflavine **117**, which can stack along the anionic DNA sugar-phosphate chain in a non-specific manner known as outside stacking.[150] The outside stacking of planar cations such as proflavine **117**, which are also intercalators, occurs at higher concentrations of ligand.

Groove-binding can involve steric, electrostatic and hydrogen-bonding interactions, and can occur in the major or minor groove. In B-DNA, the greater width of the major groove allows it to bind some prokaryotic gene regulatory proteins, and certain regulatory oligonucleotides have been shown to exhibit binding specificity for the major groove.[146,150] Most small groove-binding ligands are minor-groove specific, probably because the width of the groove matches the width of these ligands, and they can be stabilized by van der Waals and hydrophobic interactions with the groove walls. The groove-binders have all been shown to be specific for AT base pairs, and examples of these ligands are the antiviral compound netropsin **118**, and the anti-parasitic compound, berenil **119**.[146,150] Further information on groove-binding compounds can be found in a review by Zimmer and Wähnert.[151]

The third type of reversible interaction to be discussed is intercalation. This is by far the most widely studied type of ligand-DNA interaction, because of the wide range of

biological activities exhibited by ligands falling into this group.[152-156] The study of intercalation has yielded much information about the structure of DNA, as well as an increased understanding of the mode of action of many intercalating drugs.[149]

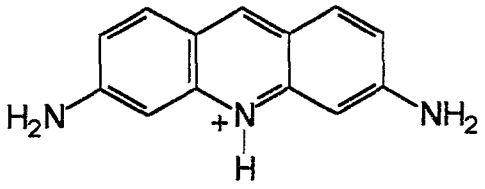
Lerman[157] was the first to propose that planar aromatic compounds such as proflavine **117** could be sandwiched between base pairs in the DNA double helix, a phenomenon which he called intercalation. He observed a number of physical changes in the DNA on binding, *viz.*, increased viscosity, a decreased sedimentation coefficient, and considerable changes in the X-ray diffraction patterns of fibres of the ligand-DNA complex. The intercalation hypothesis was the only explanation which fully accounted for the physical changes observed. Further studies by Lerman[158] on the binding of acridines to DNA, using fluorescence and flow dichroism techniques showed that both the base pairs and the acridine were almost perpendicular to the helix axis.

The physical changes which take place on intercalation of a ligand into DNA are now well documented. There is a length increase in the double helix as a result of the bases moving apart to accommodate a 0.34nm thick planar aromatic molecule, and this increase results in changes in intrinsic viscosity and the sedimentation coefficient.[152-154] The intercalating ligand lies in van der Waals contact with the base pairs above and below, and occupies the same space as an extra base pair. In practice the observed length increase is slightly less than the expected 0.34nm per intercalated molecule.[150,153] The close contact between the π-orbitals of the ligand and the base pairs allows stabilization of the complex *via* hydrophobic and charge-transfer forces.[152] Hydrogen-bonding may also occur between suitable groups on the ligand and the charged oxygen atoms in the phosphate groups of the backbone.[154] In order for the ligand to intercalate, the base pairs must separate (unstack) vertically, and this distorts the sugar-phosphate backbone, destroying the regular helical structure, as observed in X-ray diffraction studies.[159] The helix, in fact, unwinds at the site of intercalation; the resulting decrease in the angle of rotation between one base pair and the next, is termed the intercalation unwinding angle. When intercalating compounds are added to superhelical DNA, the helix is unwound and the natural right-handed superhelical turns are removed until no superhelical structure

remains. Addition of further intercalating drug causes the formation of a left-handed superhelical structure.[153] This became a method whereby the unwinding angle of a ligand could be accurately determined, using viscometric titrations, or sedimentation experiments.

Traditionally, drugs have been classified as intercalators if the afore-mentioned physical changes occur on DNA-ligand binding, *i.e.*, helix extension, changes in X-ray fibre diffraction patterns and helix unwinding. More recent techniques, for example NMR, now play a greater role in studies of intercalation, and new rules to classify intercalation, incorporating older and more modern techniques, have been suggested.[160]

A discussion on the techniques used to study DNA intercalation follows in Section 1.2.3.



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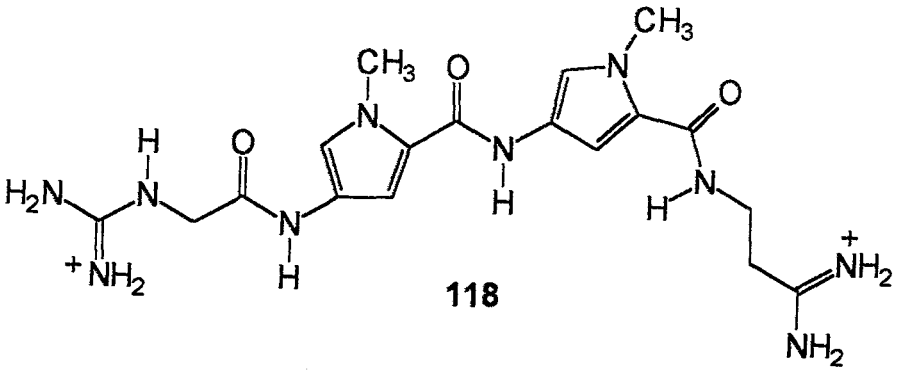

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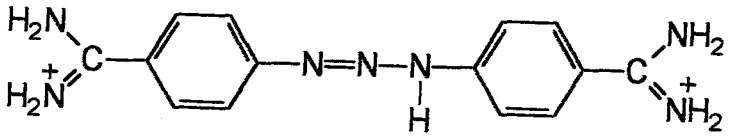


119

1.2.1.3 QUANTITATIVE ANALYSIS OF LIGAND-DNA INTERACTIONS

Quantitative analysis of ligand-DNA interactions is hindered by the fact that in any given mixture of DNA and ligand, there is likely to be more than one kind of interaction taking place. This leads to certain ambiguities in the analysis of binding data. Peacocke and Skerrett[161] studied the interaction of proflavine **117** with DNA using a spectrophotometric method, as well as equilibrium dialysis. They obtained similar, non-linear binding curves for both methods and a curved Scatchard plot,[162] which in most cases indicates a heterogeneity of binding sites.[155,163] The different binding processes were designated I and II, with I being a strong interaction associated with intercalation, and II being a weaker interaction due to non-specific stacking of the ligand molecules along the sugar-phosphate backbone.[155,156] Type I binding occurs at low levels of ligand, up to a ligand:base pair ratio of about 0.4, and has an association constant of 3×10^6 mol^{-1} for proflavine **117**. Type II binding, which occurs in ligand:base pair ratios of up to about one, has an association constant an order of magnitude less than for type I, and is greatly reduced by increasing the ionic strength of the environment.[153] The external binding is found to contribute only about 7% to the total binding when the salt concentration is high (0.2M Na^+).[164]

Analysis of binding data for a large number of intercalators showed that even when type II binding was essentially eliminated, Scatchard plots showed curvature,[165] and a saturation ratio higher than one ligand per two DNA base pairs could not be obtained.[153] This gave rise to the principle of neighbour exclusion, whereby each base pair is a potential binding site, but once a ligand binds, a neighbouring binding site is effectively eliminated. A possible explanation for this is that the binding of a ligand at one site changes the sugar conformation at an adjacent site, thus restricting binding at that site.[150,152,153] Studies with bis-intercalators, *i.e.* molecules with two intercalating aromatic systems connected by a linking chain, have generally supported the neighbour exclusion principle, although there are a few exceptions.[153]

The analyses mentioned thus far assume a single intrinsic binding constant to DNA, irrespective of the nucleotide sequence. This is an oversimplification, as it has been shown that many ligands show a preference for a particular base pair or sequence. Most intercalators show either no binding preference or a GC base pair preference.[150,156] Base sequence, together with external environment, dictates which DNA polymorph predominates for any particular DNA polymer. Different polymorphs have different major and minor groove dimensions, which are crucial to ligand-DNA interactions. Any internal binding site is accessed *via* the major or minor groove, and many intercalators have side chains which need to be accommodated in either the major or minor groove, if intercalation is to occur.[146]

Analysis of binding data obtained from DNA-ligand interactions is thus by no means simple, and is open to misinterpretation. A further difficulty arises from the fact that binding data obtained from experiments conducted at different ionic strengths or pH's, cannot be compared.

1.2.2 INTERCALATING DRUGS

The intercalating drugs will be discussed in two sections. Firstly, the classical intercalators which have been widely studied; more recent additions to this group displaying similar pharmacological activity will be touched on briefly. Secondly, attention will be focussed specifically on the antimalarials.

1.2.2.1 THE CLASSICAL INTERCALATORS

The interaction of acridines and ethidium bromide **120** with DNA has been widely studied and most intercalation studies, particularly the original investigations, have been conducted on these compounds.[157,158,164,166-173]

Ethidium bromide **120** is a phenanthridine trypanocide, which displays other pharmacological properties, such as antiviral and antibacterial activity.[152,154,172] Acridines such as proflavine **117** are antiviral and antitumour agents, and both acridines and ethidium bromide are active *in vivo* as a result of their interference with the structure and function of DNA. Both compounds inhibit DNA and RNA synthesis and, in the case of ethidium, the extent of inhibition of RNA polymerase activity is directly proportional to the amount of ethidium bound to the DNA template.[152]

Ethidium bromide **120** has been used to study intercalation into encapsulated DNA in bacteriophages,[174] as well as to determine the number of superhelical turns in closed circular DNA of the tumour virus SV40.[175] Aminoacridines have been shown to cause frameshift mutations in phage T4 DNA, and this property was used in the experiments which first established the triplet nature of the genetic code.[176]

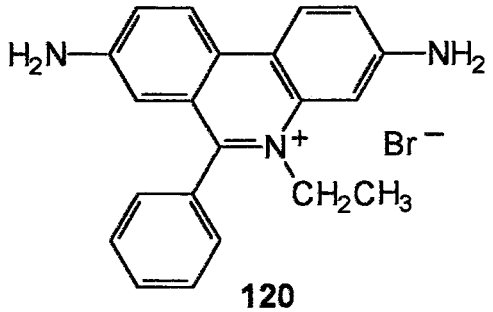
The anthracyclines **121** are antitumour antibiotics, and members of this group include daunomycin, adriamycin and nogalamycin. These antibiotics penetrate cells *in vivo* and become localized in the nucleus, where they change the morphology and biochemical

reactions of the chromatin.[153] They inhibit DNA and RNA synthesis, most likely by inhibiting DNA and RNA polymerases, and there is a good correlation between antileukaemic activity of daunomycin derivatives and their ability to inhibit RNA polymerase.[152] Daunomycin and nogalamycin have also been shown to inhibit helicase enzymes in *Escherichia coli*.[177]

Actinomycin **122** exhibits antibiotic and antitumour activity, but because of poor selective toxicity is employed only as an antitumour agent, with very limited use. This compound has, however, found widespread use as a biochemical tool to inhibit DNA-directed RNA synthesis.[152,153] The association and dissociation kinetics of the actinomycin-DNA complex are a great deal slower than for other intercalators.[178] This could perhaps account for its inhibition of RNA polymerase at low drug concentrations, as the slow dissociation of the drug would block the progress of the enzyme along the DNA molecule. Actinomycin displays an almost absolute base specificity for GC base pairs, which is attributed to the presence of the pentapeptide rings, since the chromophore alone shows no base pair preference.[149]

Examples of other antitumour drugs which exert their biological activity through intercalation are ellipticine, miracil D, and the bis-intercalators echinomycin and triostin A.[150,152]

There is continuing interest in the development of intercalating drugs like the benzothiopyranoindazoles **123**, which have anti-cancer activity, but lower cardiotoxicity.[179]



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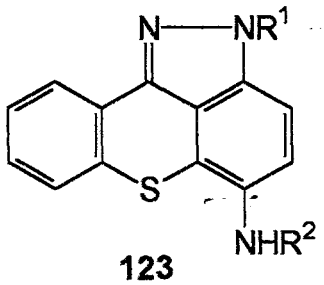

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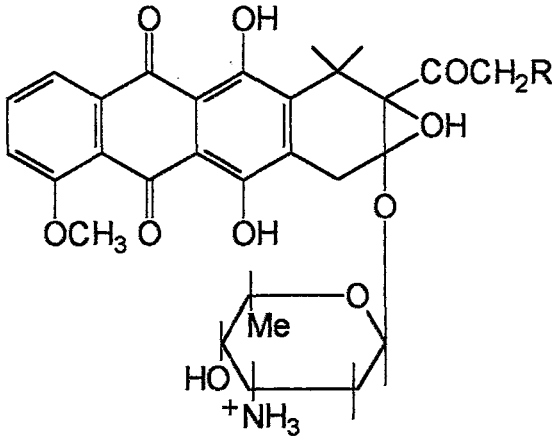

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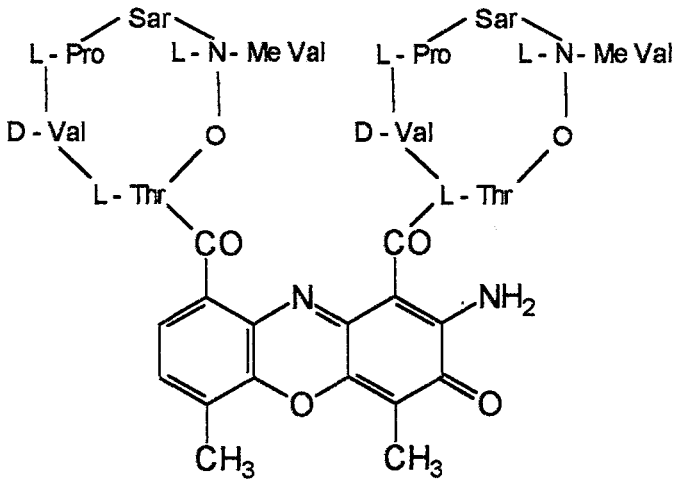

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1.2.2.2 THE ANTIMALARIALS

Many antimalarials are planar aromatic compounds which have been shown to bind to DNA by intercalation. There has been some dispute as to whether this property accounts fully for their antimalarial activity, and this will be discussed in Section 1.2.2.2(iii). In order to fully appreciate the proposed mechanisms of action of the antimalarials, it is necessary to be familiar with the life cycle of the malarial parasite.

(i) The malarial parasite

Malaria in humans is caused by five species of protozoan parasites of the genus *Plasmodium*,[180] of which *P. falciparum* is the most deadly. Infection results from the bite of an infected female *Anopheles* mosquito, which injects sporozoites into the blood (Fig. 2). These enter liver cells and undergo asexual reproduction (exoerythrocytic schizogony), which gives rise to mature tissue schizonts, called merozoites, in which the nuclei and cytoplasm of the parasites have divided. During this time no symptoms of the disease are apparent. Merozoites are released into the bloodstream by rupture of the infected liver cells, where they invade red blood cells to begin a new cycle of asexual reproduction (erythrocytic schizogony). Others re-enter healthy liver cells and undergo further cycles of asexual division. These forms are believed to be responsible for the typical relapses which occur even after an apparent cure has been achieved. The merozoites which enter the red blood cells, now called trophozoites, multiply until the red cell ruptures, releasing merozoites which begin new cycles of erythrocytic schizogony.

Rupture of all the infected red cells is essentially synchronous, and this gives rise to the periodic fevers characteristic of the disease and results in anaemia. The parasites use vast amounts of glucose and excrete lactic acid, resulting in hypoglycaemia and muscle and joint pains. Infected red blood cells become sticky, causing blockage of capillaries.

Some daughter merozoites, instead of initiating new cycles of schizogony, differentiate into male and female forms called gametocytes. When the host is again bitten by a

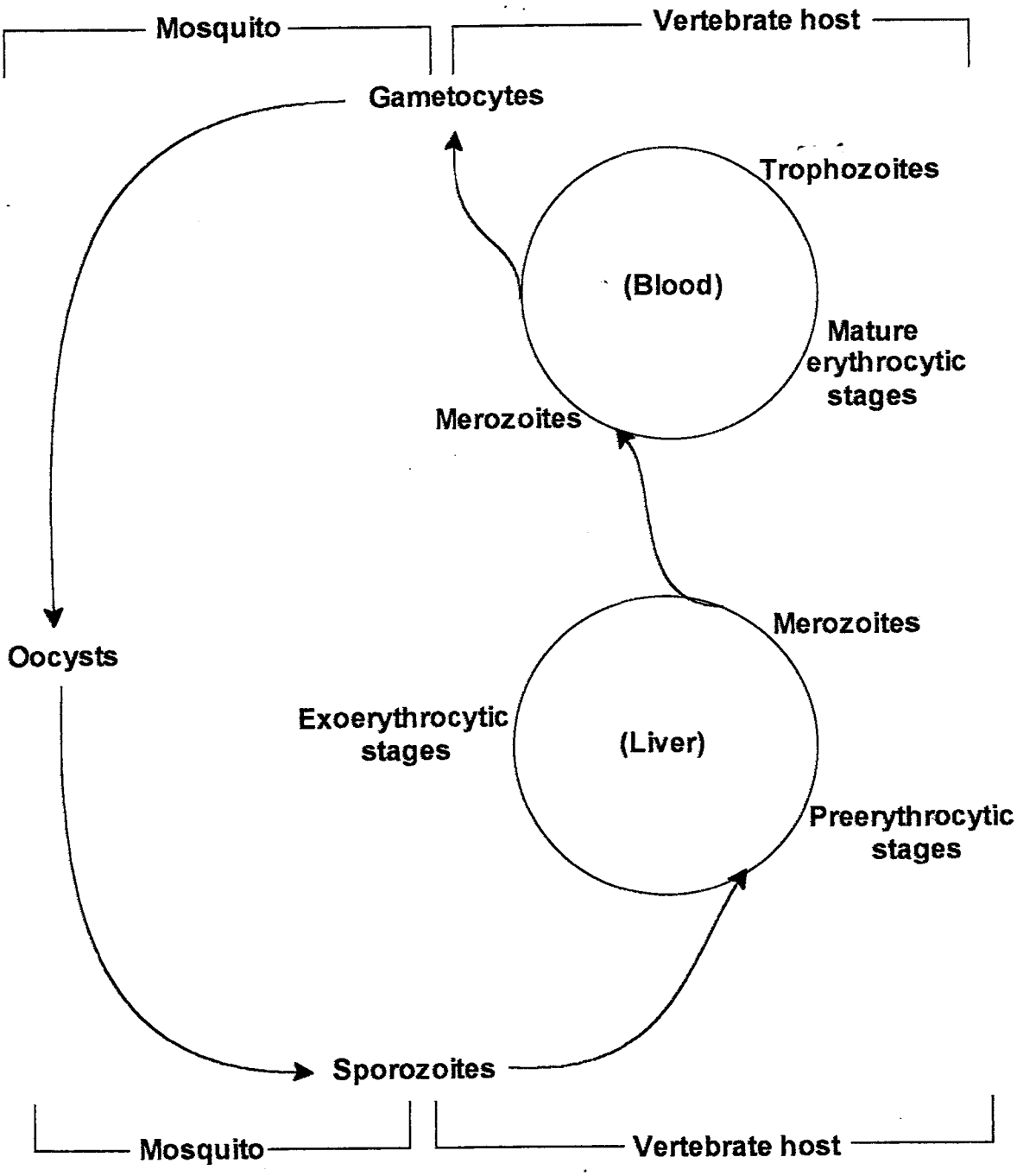


FIGURE 2 Life cycle of the malaria parasite.

mosquito, the gametocytes enter the stomach of the insect, where fertilization occurs. The resulting ookinete develops into an oocyst, which ruptures and releases sporozoites. These enter the salivary glands of the mosquito, and are released into a human host when the mosquito takes a blood meal.[180-184]

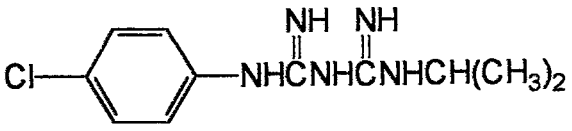
(ii) Drug treatment of malaria

No drugs are known that will kill sporozoites before they enter liver cells, this is largely due to the short time period between entry of the sporozoites into the bloodstream, and their entry into liver cells. There are a few existing drugs which act on the asexual parasites in tissue cells, prior to erythrocytic infection. Examples of these include chlorguanide **124** and primaquine **125**.[181] The vast majority of antimalarial drugs are blood schizonticides, *i.e.*, they are active against the asexual erythrocytic forms of the parasite. These drugs are responsible for the prevention of clinical symptoms and are often also used for prophylaxis, examples include chloroquine **126** and mefloquine **127**.[184,185]

The earliest of the antimalarials was quinine **128**, an alkaloid first extracted from the bark of the cinchona tree in 1600, and still in use to the present day.[184,186] In 1934, chloroquine **126**, a 4-aminoquinoline, was synthesized as a quinine analogue and has been the most widely used of all antimalarials because of its effectiveness, non-toxicity and low cost.[185] Since the synthesis of chloroquine **126**, a large number antimalarials have been developed and these fall into two major categories : those based on a quinoline structure, for example mefloquine **127** and primaquine **125**, and those known as antimetabolites, for example pyrimethamine **129** and chlorguanide **124**.[181,184] The large number of effective antimalarials available led the World Health Organization to declare in 1955 that malaria would soon be eradicated.[183] Unfortunately, the last few decades have shown the emergence of drug-resistant strains, and in certain regions of the world (*e.g.*, Thailand) it appears that man is once again completely at the mercy of *P. falciparum*.[186] Today the number of malarial cases is growing worldwide, with an estimated 270 million people

being infected each year, of which 2 million die and 100 million experience acute illness.[183]

The current widespread resistance of malarial parasites to chloroquine **126**, traditionally a mainstay of antimalarial treatment, has led to renewed interest in the search for new antimalarial drugs.[187]



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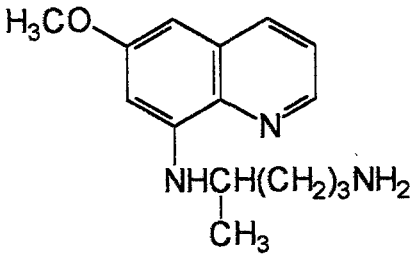

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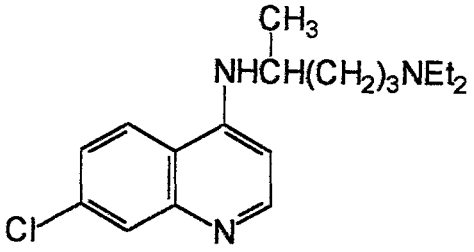

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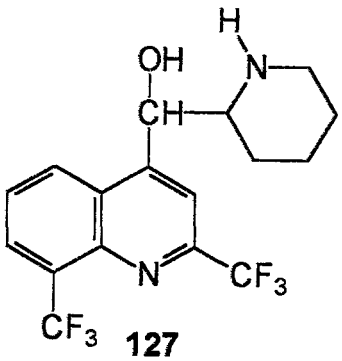

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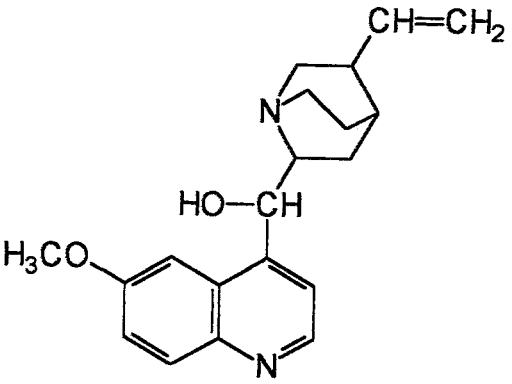

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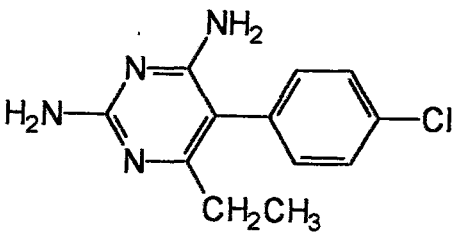

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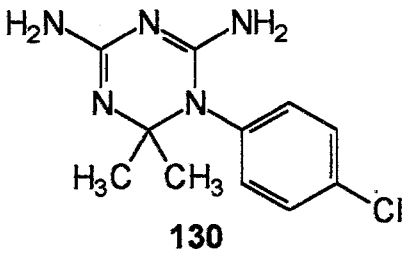
(iii) The mode of action of antimalarials

A brief discussion of the mode of action of the antimetabolites will be followed by a more detailed account of the proposed mechanisms of action of chloroquine, the most widely studied of the quinoline antimalarials.

The group of antimetabolites (antifolics) includes the sulphonamides and sulphones, which are most often used in combination with other antimalarials, as well as the two most extensively used drugs in this class, pyrimethamine **129** and chlorguanide (Proguanil) **124**. The antifolics are too slow-acting as schizontocides to be used in the treatment of acute malarial attacks,[181] and thus their major role has been in prophylaxis. The antimetabolites exert their antimalarial activity by inhibiting the enzyme dihydrofolate reductase (DHFR). Chlorguanide as such is not active, but is converted into the active form, cycloguanil **130**, *in vivo*. Inhibition of DHFR decreases the pool of tetrafolate cofactors which are necessary for the synthesis of purines, methionine and thymidylate, and thus DNA synthesis is inhibited; this, as well as the shortage of folate cofactors, is lethal to the parasite. These drugs are selective as a result of their great affinity for the parasite enzyme, for example pyrimethamine has a 2000-fold greater affinity for the parasite enzyme than for the host enzyme.[186]



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The mode of action of chloroquine **126** has not yet been completely elucidated, although there are a number of sites in the cell at which chloroquine exerts an effect. There are, at present, three proposed mechanisms of action for the antimalarial activity of chloroquine: DNA intercalation, lysosome accumulation, and binding to ferriprotoporphyrin IX.[188-190]

Parker and Irvin[191] first observed, in 1952, that chloroquine could bind to nucleic acids, and they suggested that this could be responsible for the antimalarial activity of the drug. Kurnick and Radcliffe[192] observed that chloroquine binding to DNA caused an increase in the viscosity of the DNA and a reduction in the sedimentation constant; both of these observations are consistent with intercalation, as first described by Lerman.[157] Many studies of the chloroquine-DNA complex followed, and these provided further evidence that the DNA-chloroquine interaction involved intercalation. Thus, O'Brien *et al.*[193] used flow dichroism to show that the chloroquine ring, when complexed with DNA, was situated parallel to the DNA bases, suggesting intercalation. Allison *et al.*[194] obtained similar results to those obtained by Kurnick and Radcliffe[192] with regard to viscosity and sedimentation and, in addition, found that chloroquine exhibited GC base pair specificity. Cohen and Yielding[195] and O'Brien *et al.*[196] found that chloroquine inhibited DNA polymerase, and to a lesser extent RNA polymerase, probably by binding to the DNA primer, and suggested that these observations could provide a possible molecular basis for chloroquine action. The antimalarials, quinacrine and quinine, showed the same trend, *i.e.*, greater inhibition of DNA polymerase than of RNA polymerase.[196] This result, in addition to the findings of Estensen *et al.*[197] that quinine was also most likely an intercalator, led to proposals that these antimalarials shared the same mode of action, *viz.*, inhibition of DNA synthesis by intercalation.

Several other theories gained popularity, as more information was gathered on the interaction of chloroquine with intact parasites in host red blood cells. Chloroquine is concentrated by malarial parasites up to 600 times the external concentration.[189] It was suggested that chloroquine accumulates in parasite acid food vacuoles as a result of the

weak base effect, *i.e.*, unprotonated drug is membrane-permeable and moves into the acidic vacuole, where it becomes protonated, and effectively trapped.[190,198,199] The influx of weak base results in an increase in pH and a consequent disruption of processes such as lysosomal proteolysis and receptor-mediated endocytosis, leading to parasite death.[199] However, other workers have shown that substantial alkalinization does not occur at the therapeutic dose of the drug.[198] It has been suggested that there must be an active transport mechanism for importing chloroquine into the parasite from the erythrocyte stroma, as the weak base effect cannot account for the extent of accumulation observed, particularly in the case of weak monoprotic bases, such as quinine.[200]

Intraerythrocytic malarial parasites feed on their host cell erythrocyte stroma, as the main source of amino acids. Haemoglobin is digested inside the food vacuoles, and the released amino acids are utilized for protein synthesis. The other component of haemoglobin released is the haem moiety, ferriprotoporphyrin IX (FPIX), which is toxic to the parasite and which it is unable to degrade. The haem is co-ordinated by the parasite into a crystalline matrix called haemozoin (malarial pigment), which is a polymer of haems, linked by an iron-carboxylate bond between the ferric ion of one haem and a side-group oxygen of another. This haemozoin is associated with various proteins *in vivo*, and the complex is not toxic to the parasite.[201,202]

It has been shown that chloroquine can bind to FPIX, and that the complex is toxic to biological membranes, and results in lysis of the malarial parasites.[190,199] This was suggested as a possible mode of action for chloroquine and related antimalarials, but it has been shown that the presence of cellular proteins inhibits the lytic effect of FPIX and FPIX-chloroquine complexes;[203] the former studies were conducted in protein-free extracts.

Slater and Cerami[204] have isolated a haem polymerase from extracts of *P. falciparum*, and this enzyme appears to be responsible for the formation of haemozoin from FPIX. They found that this enzyme was inhibited by quinoline antimalarials such as chloroquine and

quinine, and the level of inhibition correlated well with antimalarial activity. The toxic haem would thus remain in the vacuole, possibly blocking further haemoglobin degradation and resulting in cell lysis. This presents a possible mode of action for chloroquine, although ultrastructural studies showed no detectable damage to the vacuolar membrane of the parasite after exposure to chloroquine.[198]

There has been a recent resurgence in interest in the original hypothesis of chloroquine acting primarily by intercalation. Krogstad and Schlesinger[199] determined the binding affinity of chloroquine for DNA, and suggested the affinity was not great enough to cause occupation of sufficient sites on the DNA to kill the parasite, at the drug concentrations used for malaria treatment. The binding affinity of chloroquine for DNA has, however, been re-evaluated, and found to be highly salt-dependent. The value varies by approximately 100-fold at different salt concentrations,[188] and thus earlier reported association constants could be misleading. Chloroquine has also been shown to bind preferentially to GC sites on DNA, and thus could selectively accumulate on specific genes and inhibit their expression.[189] Structure-activity relationship studies of chloroquine analogues, supported the intercalation hypothesis.[205-207]

The exact nature of the antimalarial activity of chloroquine remains unknown, but the preceding studies show that it is extremely complex, and most likely involves a combination of the various mechanisms discussed.

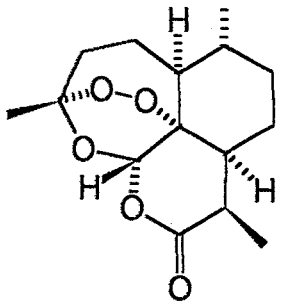
(iv) New antimalarials

The success of chloroquine as an antimalarial in the 1940s and 1950s led to the optimistic prediction that malaria could soon be eradicated, or at least well controlled.[186] This prediction has been proved wrong by the appearance of drug-resistant malarial strains all over the world.

Chloroquine resistant parasites accumulate reduced quantities of the drug, and this is thought to be the basis of their resistance. These parasites have an efflux mechanism,

which releases chloroquine 40 to 50 times faster than in sensitive strains, but certain drugs, such as verapamil, vinblastine and daunomycin can reverse this effect to some extent by blocking the efflux mechanism.[200,208,209]

As a result of the problem of drug-resistant malarial strains, the development of new antimalarials is essential, although resistance is likely to develop to any novel antimalarial. Much research is being directed towards the development of an antimalarial based on the structure of qinghaosu **131**, the active component of an ancient herbal fever remedy,[210-217] as well as towards synthesizing analogues of existing antimalarials such as chloroquine, which will be active against drug-resistant strains.[218-226]



131

1.2.3 INTERCALATION STUDIES

A large number of methods have been used over the years to determine the binding modes of ligands with DNA. Peacocke and Skerret[161] used UV absorption spectroscopy to probe the interaction of proflavine **117** with DNA. Absorption spectroscopy has proved to be particularly useful in following the intercalation of ligands into DNA; the spectrum of the chromophore shifts to longer wavelengths and the maximum absorption is reduced in magnitude (hypochromism), as intercalation occurs. This results from strong interaction of the electronic states of the ligand with the electronic states of the DNA bases. The strength of this electronic interaction decreases as the cube of the distance separating the ligand and base, and thus strong hypochromism is considered, by some workers, to be a definitive indication of intercalation.[227] Using this method, the total concentration of bound drug in a drug-DNA mixture can be estimated.[155,228] Many studies of DNA-ligand interactions have made use of spectrophotometric methods, including fluorescence, often in combination with other methods.[165,166,168,169,178,229-231]

Equilibrium dialysis has also been used to estimate bound drug in a drug-DNA mixture, as well as to determine base specificity of ligands.[161,230,232-234]

The physical changes which take place in the DNA on intercalation can be monitored in a number of ways, including viscosity measurements,[165,229,235,236] sedimentation experiments,[237,238] electrophoresis[239] and X-ray diffraction.[157,167,240-244] Linear flow and electric dichroic techniques are used to establish the orientation of the bound ligand chromophore[158,166,169,245,246] relative to the DNA bases.

Kinetic studies of the binding process have been monitored by difference spectroscopy,[174] temperature-jump relaxation studies[164,171,173] and stopped-flow spectrophotometry.[247,248]

NMR techniques have become important in the study of ligand-DNA interactions since the advent of high resolution instruments, and the synthesis of short oligonucleotide sequences have made detailed NMR analyses of binding possible. These techniques will be discussed in detail in Sections 2.3.1.2 and 2.3.2.

1.3 AIMS OF THE INVESTIGATION

The Baylis-Hillman reaction,[249] which involves coupling of α,β-unsaturated vinyl systems and aldehydes, or other suitable electrophiles, possesses enormous synthetic potential because it results in carbon-carbon bond formation (see Section 2.1.1).

The Baylis-Hillman adduct obtained by coupling pyridine-2-carboxaldehyde with methyl acrylate was found to undergo thermal rearrangement to give a crystalline product, which we subsequently identified as an indolizine derivative.[250] Indolizine derivatives have been shown to exhibit a wide range of biological activities (Section 1.1.2.4), and the similarity of indolizine to quinoline raises the possibility that indolizine derivatives could act as chloroquine analogues. To our knowledge, no intercalation studies have been conducted on indolizine derivatives, although their planar structure makes them potential candidates for intercalation.

The present investigation has involved the following objectives:

(1) A complete kinetic and mechanistic study of the Baylis-Hillman reaction.

(2) An exploration of the generality of the Baylis-Hillman cyclization route to indolizines.

(3) Preparation of indolizine-carboxamides as chloroquine analogues.

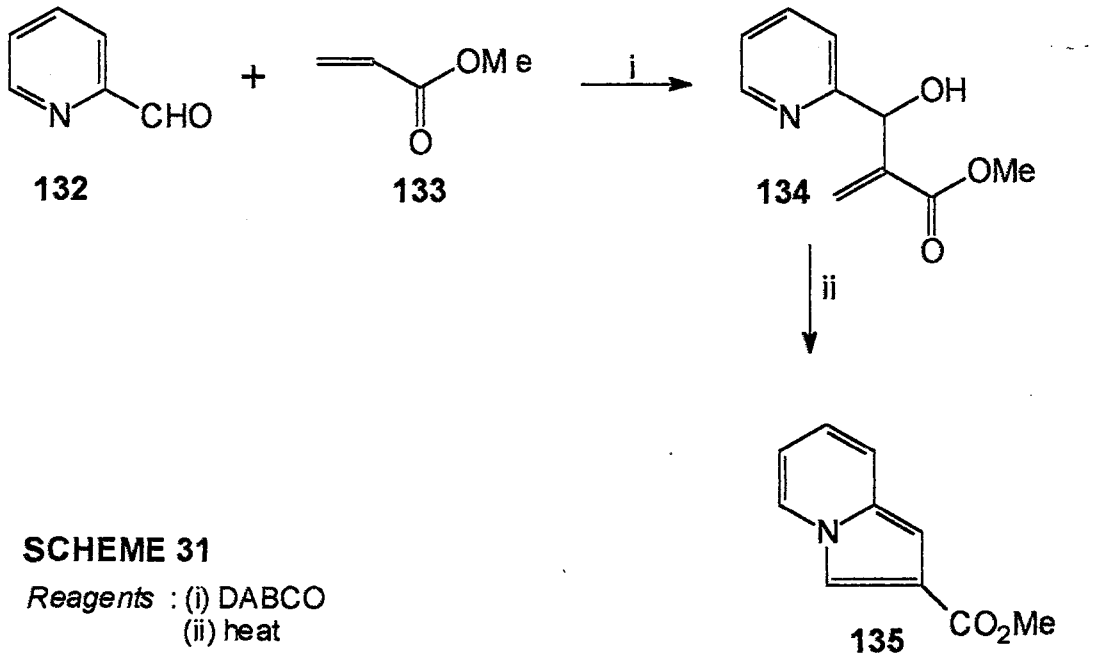
(4) A dynamic NMR study of indolizine-carboxamides to explore substituent effects on N-CO rotational barriers.

(5) DNA intercalation studies on selected indolizine-carboxamides using UV and NMR techniques.

2. DISCUSSION

2.1 PREPARATION OF INDOLIZINES *VIA* BAYLIS-HILLMAN INTERMEDIATES

The Baylis-Hillman reaction[249] involves the coupling of α,β-unsaturated vinyl systems with electrophiles, such as aldehydes; the reaction is catalyzed by tertiary amines such as 3-hydroxyquinuclidine or diazabicyclo[2.2.2]octane (DABCO). The Baylis-Hillman reaction between pyridine-2-carboxaldehyde **132** and methyl acrylate **133**, catalyzed by DABCO, yielded a product **134**, attempted distillation of which gave a compound we have shown[250] to be the indolizine derivative **135** (Scheme 31). In the discussion which follows, attention will be focussed on our research into the preparation of Baylis-Hillman products as indolizine precursors (Section 2.1.1), a mechanistic study of the Baylis-Hillman reaction (Section 2.1.1), the cyclization of these precursors to give indolizine derivatives (Section 2.1.2), and an extension of the Baylis-Hillman approach which gave rise to an unexpected product (Section 2.1.3).



SCHEME 31

Reagents : (i) DABCO
(ii) heat

2.1.1 THE BAYLIS-HILLMAN REACTION

The reaction was first described by Baylis and Hillman[251] in the patent literature in 1972. They reacted α,β-unsaturated esters, nitriles, amides and ketones with a number of aldehydes, using cyclic tertiary amines as catalysts. Carbon-carbon bond formation is of fundamental importance in organic synthesis and, therefore, there has been much interest in the Baylis-Hillman reaction, which constructs such a bond at the α-position of activated alkenes,[252] in fact, the alkene can be viewed as a vinyl anion equivalent. The total synthesis of natural products necessitates the preparation of multifunctional intermediates, and the Baylis-Hillman reaction has been widely employed for the synthesis of necic acid synthons, and other natural product intermediates.[253-266]

The catalyst used in most of these reactions is DABCO, but a number of other compounds also successfully catalyze the reaction, for example quinuclidine,[249] 3-hydroxyquinuclidine,[267] triethylamine,[268] tricyclohexylphosphine,[269] tributylphosphine with triethylaluminium[270] and a rhodium(I) hydride complex.[271]

The most commonly employed α,β-unsaturated vinyl systems are the acrylate esters, but a number of other systems couple with aldehydes to give the expected Baylis-Hillman products. Examples of these include methyl vinyl ketone[254,272,273] and other alkyl ketones,[274] acrylonitrile,[268,272,275,276] diethyl vinyl phosphonate,[277] phenyl vinyl sulphone,[278-280] ethyl 2,3-butadienoate,[281] acrolein[268] and acrylamide, but in very low yield.[268]

Aldehydes may be replaced in the reaction by substrates such as α-keto esters,[257,282,283] tosylimines of aromatic aldehydes,[284] and benzylidenecarbamate,[285] but this is less common than variation of the acrylate.

The reactions are conducted at room temperature and are usually slow, typically requiring several days, but the less reactive substrates may take weeks. This problem is diminished

when using electrophilic heterocyclic aldehydes or 3-hydroxyquinuclidine instead of DABCO,[267] or when the reaction is conducted under high pressures.[268]

The Baylis-Hillman reaction is thus extremely versatile, and there is continued interest in its applications in synthesis.[252,286-289]

2.1.1.1 PREPARATION OF BAYLIS-HILLMAN PRODUCTS

In order to explore the generality of the cyclization reaction of pyridine-2-carboxaldehyde - acrylate adducts,† such as **134**, it was necessary to prepare a range of these adducts, employing various acrylate and pyridine-2-carboxaldehyde derivatives.

We initially explored the possibility of preparing a range of pyridine-2-carboxaldehydes. Unfortunately, there are very few reported methods for the synthesis of pyridine-2-carboxaldehydes, and most of those methods appearing in the literature suffer the serious drawback of being extremely low yielding, as discussed by Iqbal *et al.*[290] We decided in any event to attempt the aldehyde synthesis, and initially 5-nitro-2-picoline **136** was prepared from 2-chloro-5-nitropyridine by reaction with Na[291] or NaH[292] and diethylmalonate, with a view to preparing substituted pyridine-2-carboxaldehydes from the appropriately substituted 2-picolines. However, attempted oxidation of 2-picoline using SeO_2 in dioxan,[293] and iodine in DMSO[294] failed, as did attempts to prepare the aldehyde from picoline by bromination-hydrolysis using *n*-BuLi and Br_2[295]. The SeO_2 oxidation attempted above was an adapted procedure of Chandler *et al.*,[293] who prepared phenanthroline aldehydes using this procedure. The I_2/DMSO procedure was used by Markovac *et al.*[294] for the synthesis of pyridine-2-carboxaldehydes from picolines, however, in most cases the aldehyde was not isolated, but directly reacted to form pyridine-2-aldoximes. From our experience, it seems that pyridine-2-carboxaldehyde **132** is extremely sensitive to oxidation, and this is the reason for the low yields obtained in

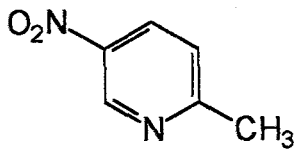
†The Baylis-Hillman product is in fact a substitution product, but it will be referred to as an adduct throughout this discussion, for the sake of convenience.

the literature procedures, and the reason for the failure of our attempted preparations. In all the preparations we attempted, the required product was shown to be present by ^{1}H NMR spectroscopy, but the major product in all cases was shown, by the presence of a strong hydroxyl band in the IR spectra, to be pyridine-2-carboxylic acid. A very low-yielding reaction at this stage would decrease the value of the entire synthetic sequence and for this reason, we decided to proceed with our study using the commercially available pyridine-2-carboxaldehyde, 6-methyl-2-pyridinecarboxaldehyde and quinoline-2-carboxaldehyde.

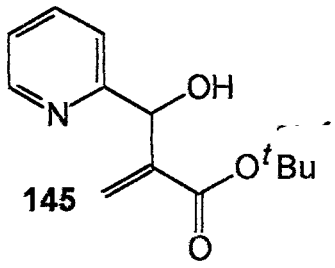
The only acrylates which we required but which were not commercially available were isopropyl acrylate **137a** and *tert*-butyl acrylate **137b** [which were prepared from acryloyl chloride and the appropriate alcohol[296] (Scheme 32)], and *N,N*-dimethylacrylamide **138** [which was prepared from acrylic acid and *N,N*-dimethylcarbamoyl chloride[297] (Scheme 33)].

Using the appropriate precursors a range of Baylis-Hillman adducts (**134** and **139-144**) were prepared[267] in yields ranging from good to excellent (see Table 1). The reactions were performed in $CHCl_3$, and catalyzed either by DABCO or 3-hydroxyquinuclidine, the yields obtained from both catalysts being similar. With hydroxyquinuclidine, however, the reactions were complete within 1 day, in contrast to the 3 days required for the DABCO catalyzed reactions. Basavaiah and Gowriswari[273] have reported that their reactions using methyl vinyl ketone were cleaner in more dilute reaction mixtures. This was also found to be true in our work and, on average, reactant concentrations for Baylis-Hillman reactions involving methyl vinyl ketone were five times more dilute than for the other substrates.

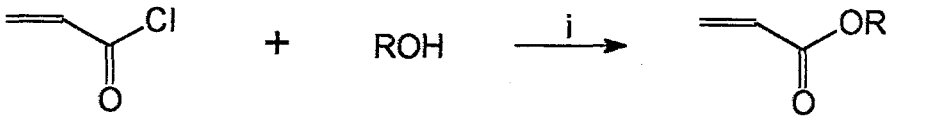
The adducts **134** and **139-144** were fully characterized by spectroscopic (^{1}H and ^{13}C NMR, IR) and elemental analysis (high resolution mass spectrometry), and representative ^{1}H, ^{13}C, HETCOR and COSY NMR spectra of compound **139** are included in Appendix 5.1. The progress of the Baylis-Hillman reaction can be monitored using ^{1}H NMR spectroscopy, by observing the disappearance of the aldehyde proton peak, and observing a change in the vinyl signals during the course of the reaction (Fig. 3).



136



145

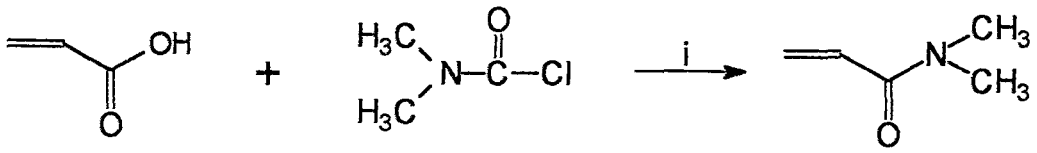


137a R = ^{i}Pr

b R = ^{t}Bu

SCHEME 32

Reagents : (i) *N,N* - dimethylaniline, hydroquinone, Et_2O

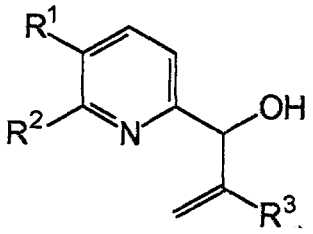


138

SCHEME 33

Reagents : (i) Et_3N, hydroquinone, PhCl

TABLE 1 Comparative yields (%) of Baylis-Hillman products



Compound	R^1	R^2	R^3	Purified Yield† (%)
134	H	H	CO_2Me	94
139	H	H	CO_2Et	96
140	H	H	$CO_2{}^iPr$	51
141	H	Me	CO_2Me	94
142	H	H	COMe	81‡
143	$-(CH)_4-$		CO_2Me	98
144	H	H	CN	92

† Yields are quoted for DABCO-catalyzed reactions
‡ 5% of indolizine **159** was isolated together with this compound

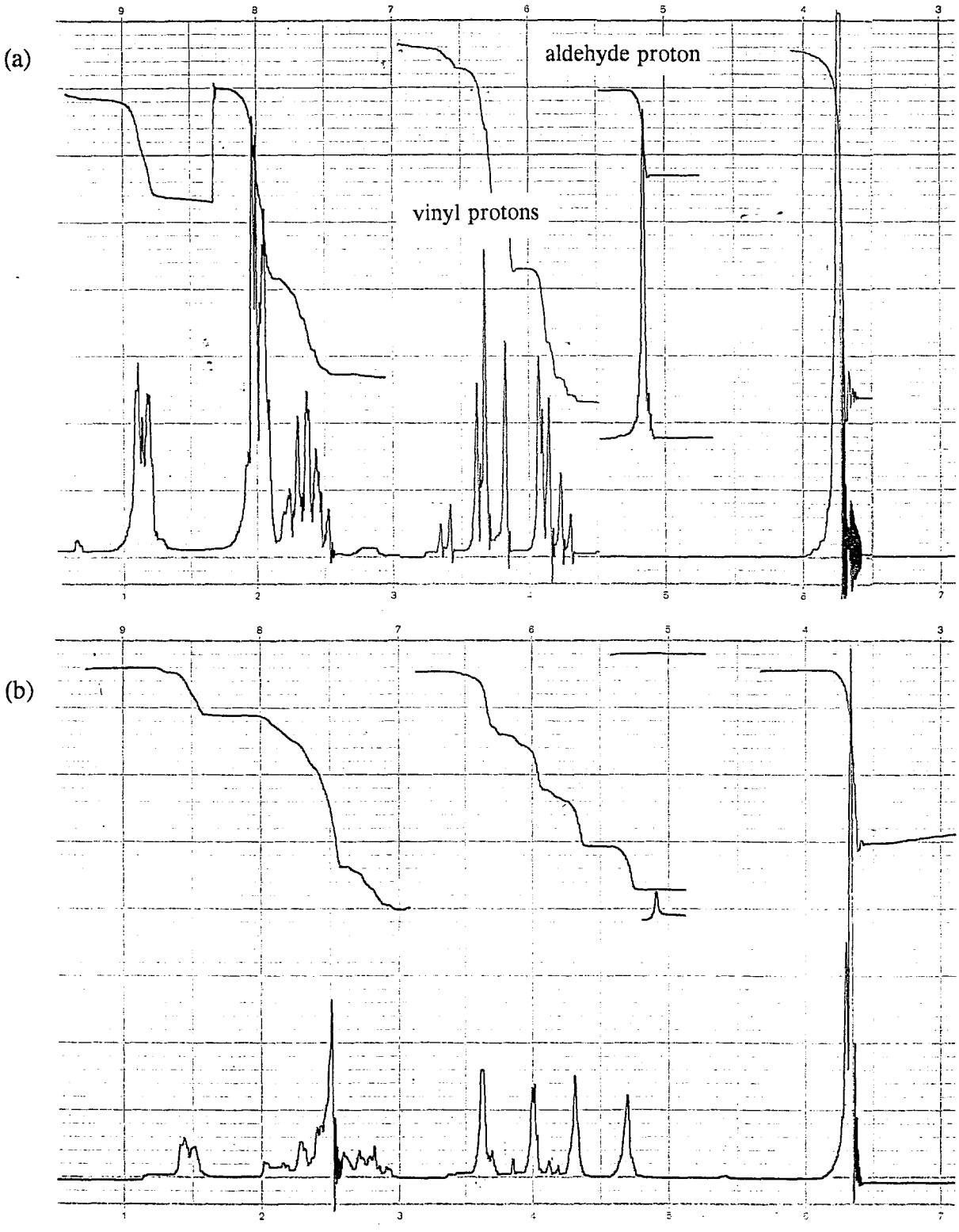


Figure 3 ^{1}H NMR spectra of the pyridine-2-carboxaldehyde - methyl acrylate reaction mixture : (a) at the start of reaction; and (b) after completion of the reaction.

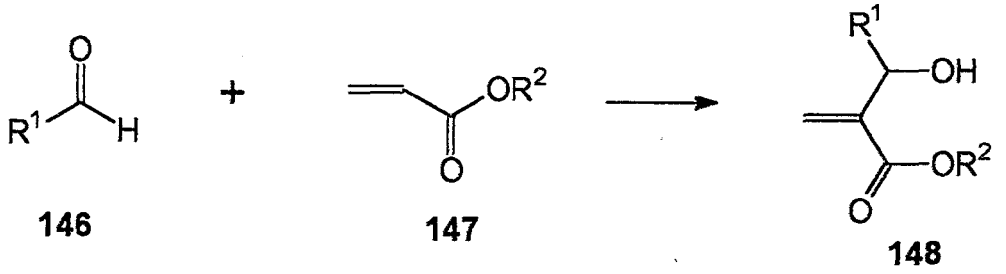
Although the *tert*-butyl product **145** was successfully isolated from the Baylis-Hillman reaction, the result was not always reproducible. A number of the acrylate systems which we examined failed to undergo the Baylis-Hillman reaction with pyridine-2-carboxaldehyde **132** altogether; these included acrylic acid, acryloyl chloride, acrylamide and acrolein. In the case of acryloyl chloride, the entire reaction mixture turned black and tarry, possibly as a result of polymerization. A similar result was obtained with acrolein, even in the presence of hydroquinone at 0°C and with the dropwise addition of acrolein. These two substrates are probably too reactive to be employed in this reaction although Hill and Isaacs[268] successfully coupled acrolein with less electrophilic aldehydes such as acetaldehyde, and in the presence of the less reactive catalyst, triethylamine. Acrylamide and acrylic acid, on the other hand, are probably too unreactive to undergo reaction. This conclusion is supported, in the case of acrylamide, by the fact that Hill and Isaacs[268] obtained only 5% of coupled product when they reacted acrylamide with acetone under 5kbar pressure for 1000 min. They concluded that, qualitatively, the acrylamides are the least reactive of the acrylates employed.

The replacement of pyridine-2-carboxaldehyde **132** with 2-hydroxyacetophenone in a reaction with methyl acrylate in the presence of 3-hydroxyquinuclidine yielded no product, even after heating at 100°C. This is in accordance with previously recorded results[249] which indicate that ketones are generally unreactive at atmospheric pressure, and have only successfully been employed at pressures of 5kbar.[268]

The reaction of *N,N*-dimethylacrylamide **138** with pyridine-2-carboxaldehyde **132** in $CHCl_3$, in the presence of DABCO, yielded an unexpected product, and the structure of this compound was determined by single X-ray diffraction analysis (Section 2.1.1.3).

2.1.1.2 KINETIC AND MECHANISTIC STUDY OF THE BAYLIS-HILLMAN REACTION

Although many workers have proposed a tentative mechanism for the Baylis-Hillman reaction,[249,253,266,268,296] no thorough kinetic investigation has been carried out by any group, prior to our study. This is probably due to problems associated with following the kinetics of this reaction, which arise from (a) the duration of the reaction, typically days, and (b) the need to incorporate solvent into the reaction mixture to allow rate constants to be computed, which further slows the reaction. These problems were overcome in the present study by using highly reactive heterocyclic aldehydes, *viz.*, pyridine-2- and pyridine-4-carboxaldehyde, and by replacing DABCO with 3-hydroxyquinuclidine, these measures having been shown previously[267] to accelerate the reaction dramatically. The consequent reduction in reaction time from days to hours made a ^{1}H NMR kinetic study feasible, and the reactions were followed by monitoring the integral ratios of adequately resolved aldehyde **146** and adduct **148** (Scheme 34) heteroaromatic signals (Fig. 4). The reactants were prewarmed to the probe temperature (37°C) prior to initiation of the reaction and the results, in each case, represent the mean of duplicate determinations. The reactions involving pyridine-2-carboxaldehyde were followed until 80% of the starting material was consumed. When pyridine-4-carboxaldehyde was used the reactions proceeded almost to completion, and were monitored until solidification of the reaction mixture occurred.



146 **147** **148**

SCHEME 34

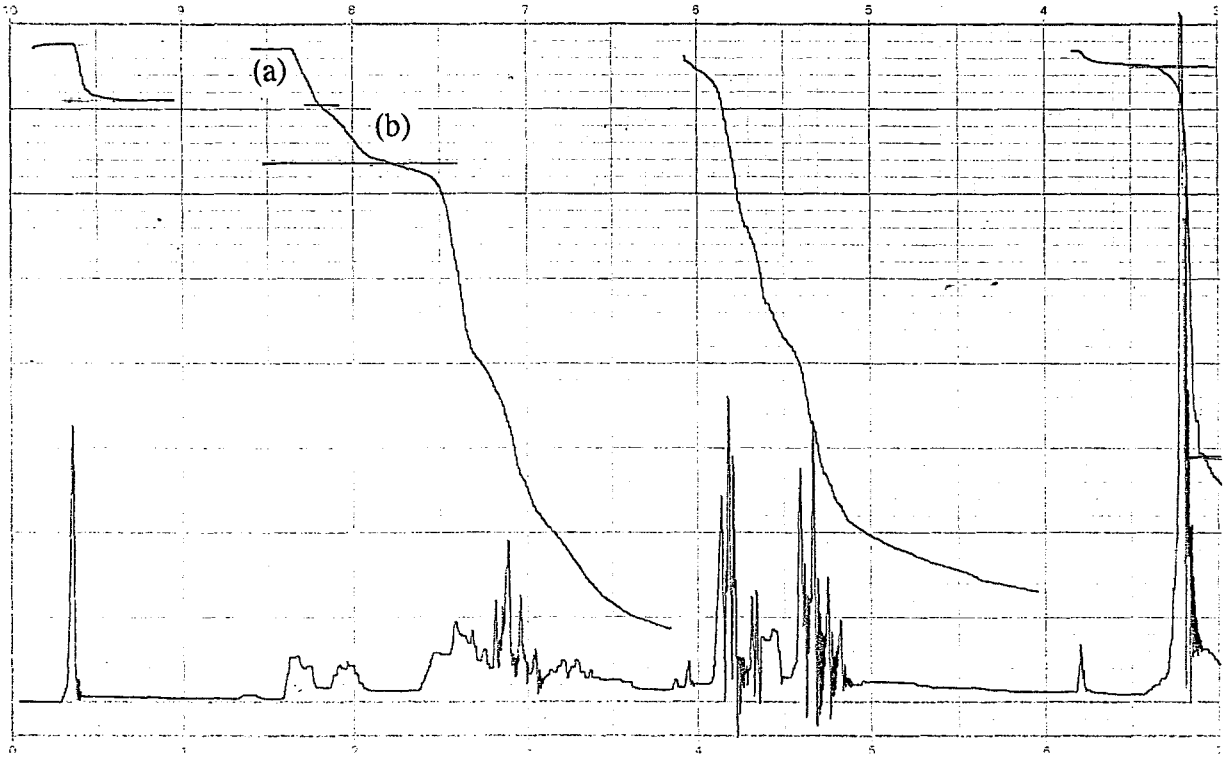


Figure 4 ^{1}H NMR spectrum of the pyridine-4-carboxaldehyde - methyl acrylate reaction mixture indicating : (a) the integral of the starting material followed; and (b) the integral of the product followed.

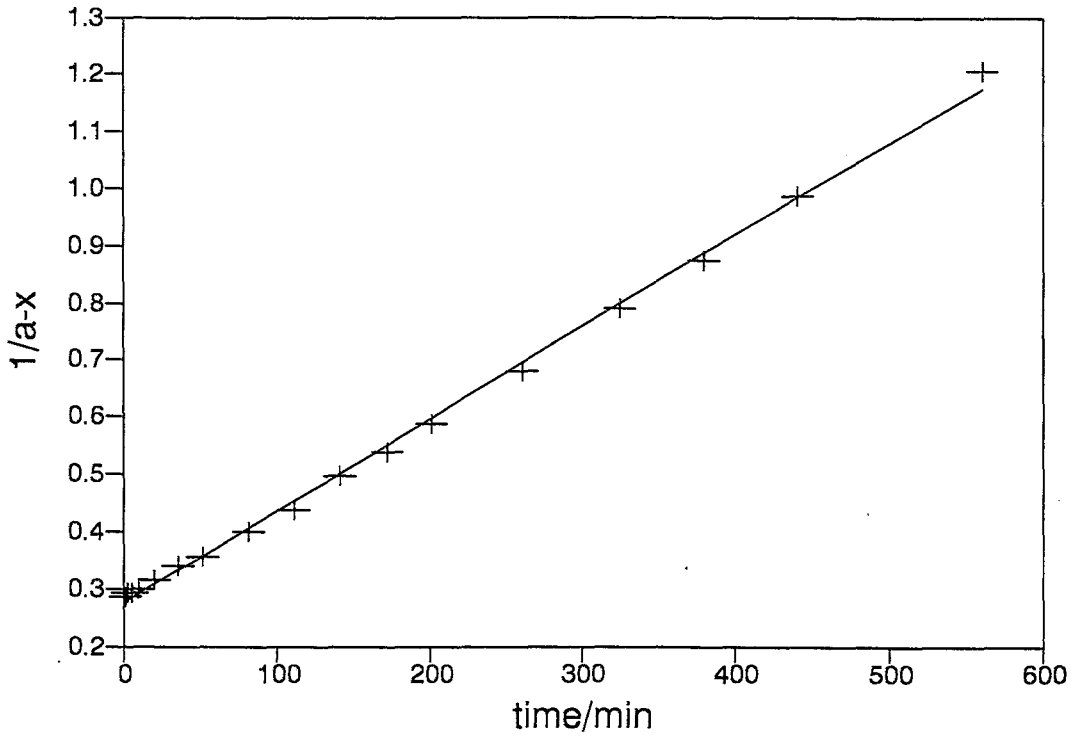


Figure 5 Linear pseudo second-order plot for the DABCO-catalyzed reaction of pyridine-4-carboxaldehyde and methyl acrylate.

Reaction systems were selected to examine the effects of varying:- the 3° amine catalyst; the substrate substituents, R^1 and R^2 (Scheme 34); and the component concentrations. For given concentrations of 3° amine, the reactions exhibit linear second-order plots (Fig. 5). Plots (with correlation coefficients of greater than 0.98) of 1/a-x *versus* t for a=b, and ln a-x/b-x *versus* t for $a \neq b$ gave straight lines over the initial 50-70% of each reaction, with slopes equal to k_a or $k_a(a-b)$ respectively, where

a = initial concentration of aldehyde

b = initial concentration of acrylate

x = concentration of product at time t

The experimental data are consistent with third-order kinetics overall (equation 1) or, assuming the concentration of 3° amine to be constant, pseudo second-order kinetics (equation 2).

$$\text{Rate} = k_{obs}\,[A]\,[B]\,[C] \qquad (1)$$

$$\text{Rate} = k_a\,[A]\,[B] \qquad (2)$$

where $k_a = k_{obs}\,[C]$

$$\text{Rate} = k_2K_1\,[A]\,[B]\,[C] \qquad (3)$$

Where,

A = aldehyde

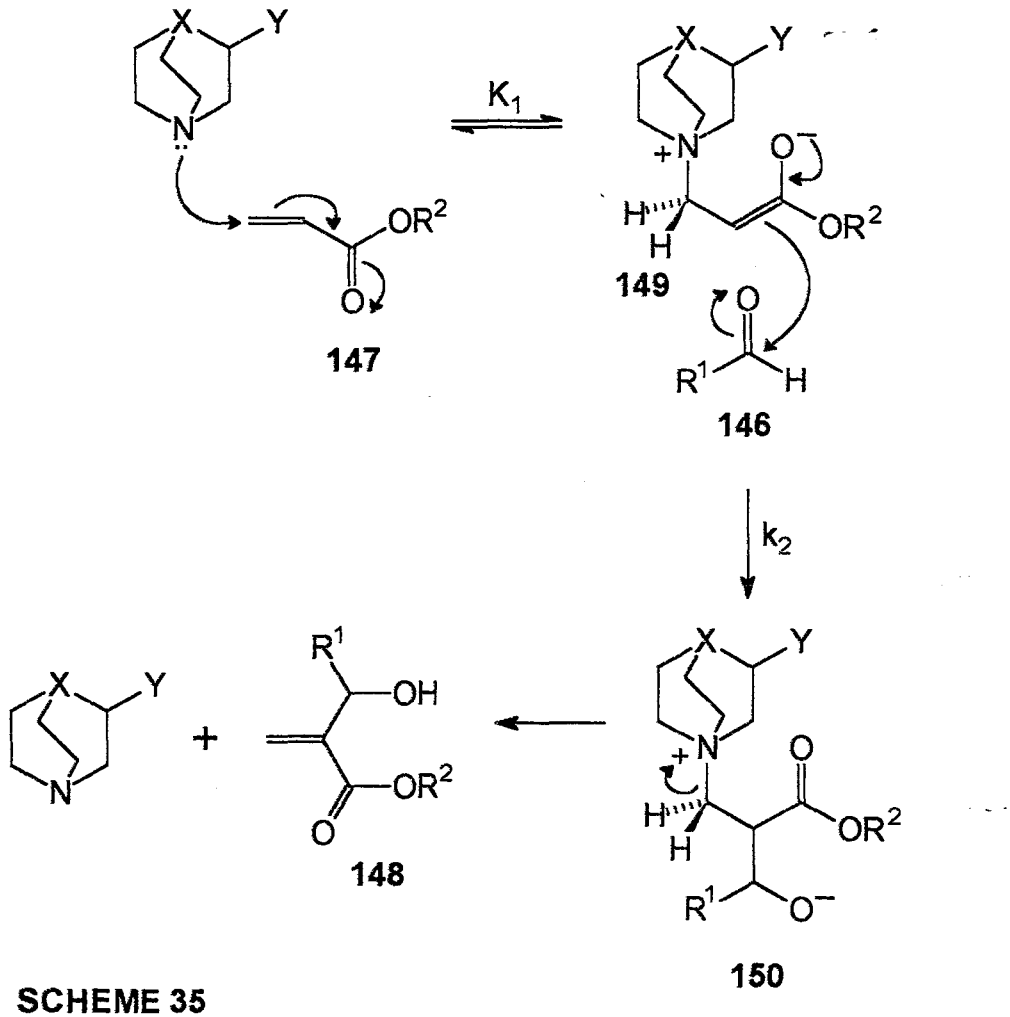
B = acrylate

C = tertiary amine

The third order rate constants (k_{obs}) are shown in Table 2.

The proposed mechanism, shown in Scheme 35, fits the experimentally determined rate equations, and is in agreement with earlier mechanistic suggestions of Drewes and Roos[249] and Hoffmann and Rabe.[253] The overall mechanism involves an addition - elimination sequence, which is initiated by nucleophilic attack of the 3° amine on the acrylate substrate **147** to give a short-lived dipolar enolate **149**. This enolate then attacks the aldehyde **146** to form an intermediate adduct **150**. The rate of formation of **150** may be expressed in terms of equation 3 which, for $k_{obs} = k_2K_1$, is identical to the

experimentally determined rate equation 1, and this step is considered to be rate-determining. The final coupled product **148** is obtained by rapid proton transfer, and subsequent elimination of the catalyst.



SCHEME 35

X = N, CH
Y = H, OH

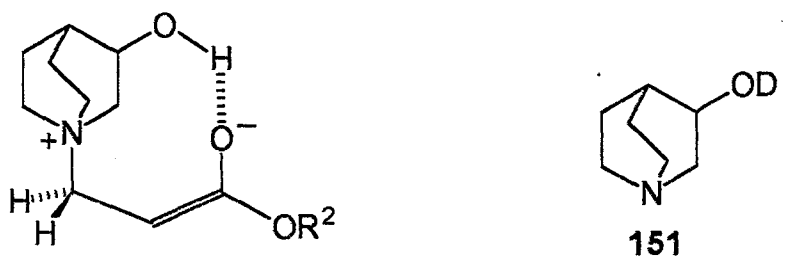
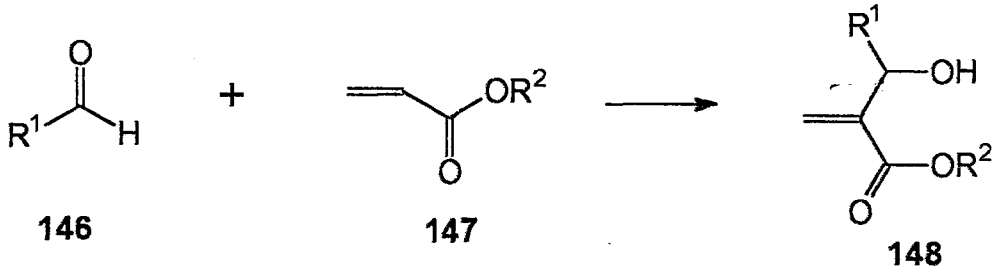


FIGURE 6

TABLE 2 Kinetic data for the synthesis of the adducts (**148**) from acrylate esters (**147**) and pyridine-2- or pyridine-4-carboxaldehydes (**146**) in $CDCl_3$.



146 **147** **148**

Entry	R^1	R^2	Catalyst	Molal conc/mol.kg^{-1} (146):(147):catalyst	k_{obs}[a]/mol^{-2}kg^{2}s^{-1}
1	pyr-4	Me	Hq[b]	1.82 : 1.82 : 0.10	(1.42 ± 0.04) x10^{-3}
2	pyr-4	Et	Hq	1.82 : 1.82 : 0.10	(9.03 ± 0.01) x10^{-4}
3	pyr-4	Pr^i	Hq	1.82 : 1.82 : 0.10	(4.60 ± 0.14) x10^{-4}
4	pyr-4	Me	Hq	3.63 : 1.82 : 0.10	(1.32 ± 0.02) x10^{-3}
5	pyr-4	Me	Hq	1.82 : 1.82 : 0.19	(1.42 ± 0.06) x10^{-3}
6	pyr-4	Me	Hq	1.82 : 3.63 : 0.10	(9.77 ± 0.10) x10^{-4}
7	pyr-4	Me	D-Hq[c]	1.82 : 1.82 : 0.10	(1.09 ± 0.06) x10^{-3}
8	pyr-4	Me	DABCO[d]	3.63 : 3.63 : 0.18	(1.51 ± 0.02) x10^{-4}
9	pyr-4	Me	Hq	3.63 : 3.63 : 0.19	(1.11 ± 0.09) x10^{-3}
10	pyr-2	Me	Hq	1.82 : 1.82 : 0.10	(2.89 ± 0.04) x10^{-4}

[a]Mean of duplicate determinations. [b]Hq is 3-hydroxyquinuclidine.

[c]D-Hq is monodeuteriated 3-hydroxyquinuclidine.

[d]DABCO is 1,4-diazabicyclo[2.2.2]octane.

The rate constants k_{obs} in Table 2 indicate that variation of the aldehyde substituent (R^1; entries 1 and 10), as well as variation of the alkyl substituent (R^2; entries 1, 2 and 3) results in changes in reaction rate. Entries 1, 2 and 3 show that use of those acrylate esters with a greater electron-releasing inductive effect, leads to a reduction in reaction rate. This may be attributed to relative destabilization of the dipolar enolate **149**, and a consequent decrease in the equilibrium constant, K_1 (equation 3). Entries 1 and 10 show that use of pyridine-2-carboxaldehyde instead of pyridine-4-carboxaldehyde, also leads to a reduction in reaction rate. The rate for the reaction of pyridine-3-carboxaldehyde could not be accurately determined, as a result of overlapping signals in the ^{1}H NMR spectrum, but the rate was comparable to that of pyridine-4-carboxaldehyde. These effects presumably reflect the differing electrophilicities of the aldehydes. Substitution of 3-hydroxyquinuclidine for DABCO increases the rate constant ten-fold (entries 8 and 9), and this rate enhancement may be rationalized in terms of hydrogen-bonding stabilization[267] of the dipolar intermediate **149** (Fig. 6) and a consequent increase in the equilibrium constant, K_1 (equation 3). Entries 1 and 7 show the small kinetic isotope effect (k_H/k_D = 1.3) which is observed with the use of deuteriated 3-hydroxyquinuclidine **151** (prepared by stirring 3-hydroxyquinuclidine with methanol-d_4), and this supports the proposed hydrogen-bonding model (Fig. 6). Doubling the proportions of non-polar reactants leads to a decrease in rate constant (compare entries 1 and 5 with 4, 6 and 9), and this is attributed to a decrease in the overall polarity of the concentrated solutions which were used to ensure convenient reaction times.

The possibility that the mechanism could involve an electron-transfer process was explored by conducting a reaction (comparable to entry 1, Table 2) in the probe of an EPR spectrometer. Three scans, using various gain, modulation amplitude and microwave power settings failed to provide any evidence for the presence of radical species, and this type of mechanism is thus considered unlikely.

Another possible mechanism, which would fit the experimental data, involves abstraction of the α-proton from the acrylate by the tertiary amine, now acting as a base, to produce

a vinyl carbanion. This vinyl carbanion could add to the aldehyde to give the desired adduct, with regeneration of the catalyst. Such a mechanism has been proposed[296] for the reaction of vinyl carbanions of acrylate esters with aldehydes. However, under Baylis-Hillman conditions this mechanism is considered highly unlikely for a number of reasons: primarily, because the reaction conditions employed are extremely mild and there are generally very few competing side reactions. This is in direct contrast to the reaction conditions used to generate the vinyl carbanions, in the afore-mentioned example. These carbanions were generated from acrylates using the strong bases lithium diisopropylamide (LDA) or lithium tetramethylpiperidine (LTMP).[249] There are practical difficulties associated with generating vinyl carbanions under these harsh conditions, notably the ease with which the acrylates undergo polymerization, a problem not usually experienced in the Baylis-Hillman reaction.

Another factor supporting the nucleophilic mechanism (Scheme 35), is that addition of methanol to the reaction mixture appears to enhance the reaction rate.[267] If the vinyl carbanion mechanism were operative, the tertiary amine catalyst would be expected to preferentially abstract the more acidic methanol proton, thus slowing or halting the reaction.

Of all the mechanisms suggested, we propose that the nucleophilic addition - elimination mechanism, shown in Scheme 35, best fits the kinetic data and other experimental evidence.

After publication of our mechanistic proposals,[298] other workers[299] presented results which support our general findings.

2.1.1.3 STRUCTURAL DETERMINATION OF 1-(2,2,2-TRICHLORO-1-HYDROXYETHYL)PYRIDINE

The Baylis-Hillman reaction of pyridine-2-carboxaldehyde **132** with *N,N*-dimethyl-acrylamide **138** in $CHCl_3$, in the presence of DABCO or 3-hydroxyquinuclidine, is expected to be slow as a result of the low reactivity of acrylamides in this reaction. This low reactivity can be explained in terms of nitrogen lone-pair delocalization (Fig. 7) which reduces the electrophilicity of the vinyl system and, thus, inhibits formation of the dipolar enolate (Fig. 7, see also Scheme 35, p. 72). The reactants were thus allowed to stand at room temperature for 1 month, after which time crystals became visible in the reaction vessel.

^1H NMR analysis of these crystals clearly indicated the absence of methyl and vinyl proton peaks (Fig. 8), and the infrared spectrum (Fig. 9) showed no carbonyl to be present.

The crystalline product was finally identified by single crystal X-ray diffraction analysis (Fig. 10 and Fig. 11) as 1-(2,2,2-trichloro-1-hydroxyethyl)pyridine **152** (Scheme 36).

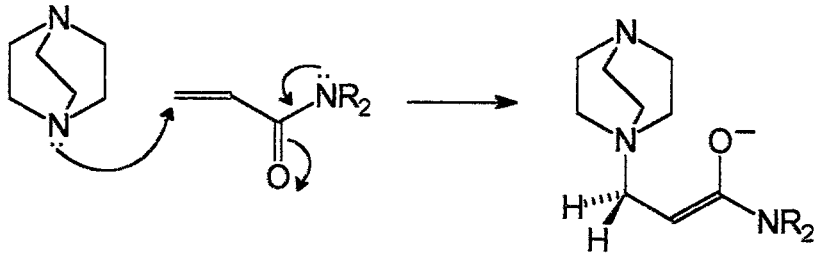
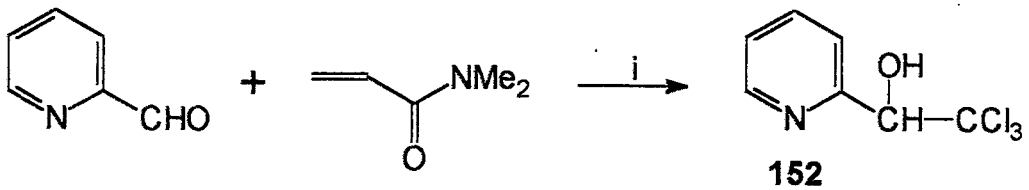


FIGURE 7



152

SCHEME 36

Reagents : (i) DABCO, $CHCl_3$

Figure 8 ^{1}H NMR spectrum of 1-(2,2,2-trichloro-1-hydroxyethyl)pyridine **152**.

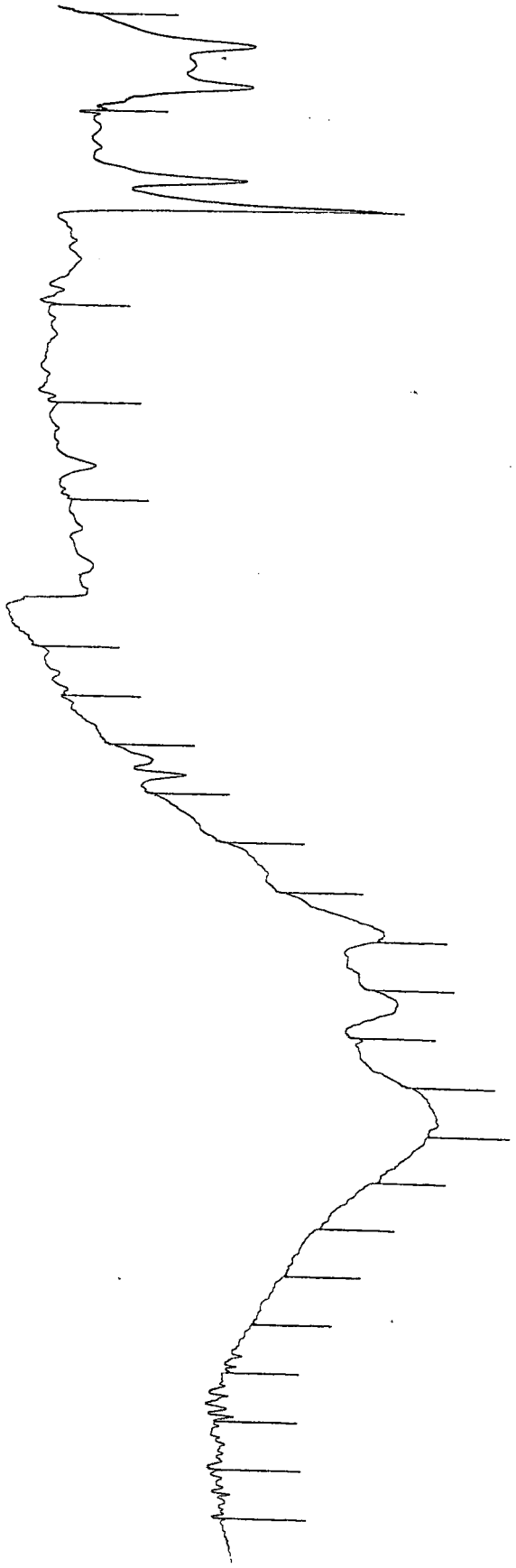


Figure 9 IR spectrum of 1-(2,2,2-trichloro-1-hydroxyethyl)pyridine **152**.

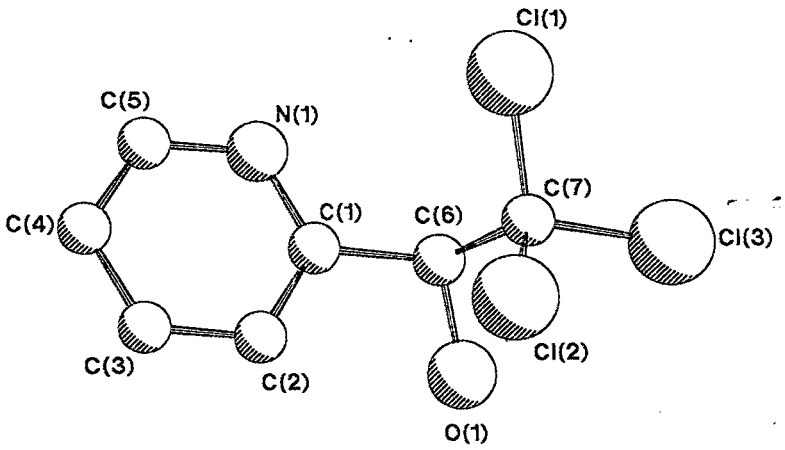


Figure 10 X-ray crystal structure of 1-(2,2,2-trichloro-1-hydroxyethyl)pyridine **152** showing crystallographic numbering.

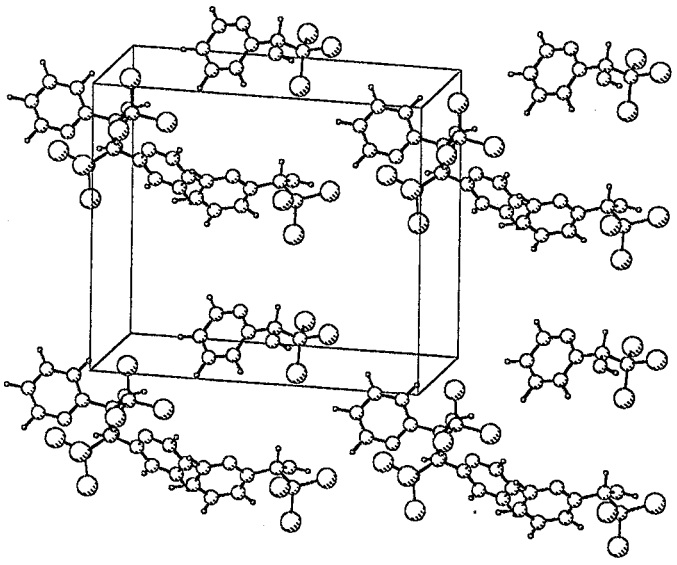


Figure 11 Packing diagram of 1-(2,2,2-trichloro-1-hydroxyethyl)pyridine **152**.

X-ray analysis shows 8 molecules to be present in the unit cell, and indicates that no significant hydrogen-bonding occurs between molecules. Some decay of the crystal was observed during data collection, presumably as a result of the presence of chlorine atoms. Detailed crystal data, including tables of bond lengths, anisotropic temperature factors, and fractional co-ordinates can be found in Appendix 5.2 (Tables 13-17).

This unexpected product results from reaction of pyridine-2-carboxaldehyde **132** with the solvent, chloroform, but was not found to be present in any of the other Baylis-Hillman reaction mixtures, even after standing for equal lengths of time. A number of reactions were set up in an attempt to determine the necessary components for this reaction with the solvent to occur. The absence of tertiary amine catalyst or *N,N*-dimethylacrylamide resulted in no product formation, and no product was formed when *N,N*-dimethylacrylamide was replaced with acrylamide. It would thus appear that for this unusual reaction to occur, tertiary amine catalyst and *N,N*-dimethylacrylamide must be present with the chloroform and pyridine-2-carboxaldehyde. Further investigation is necessary to determine the exact course of this reaction.

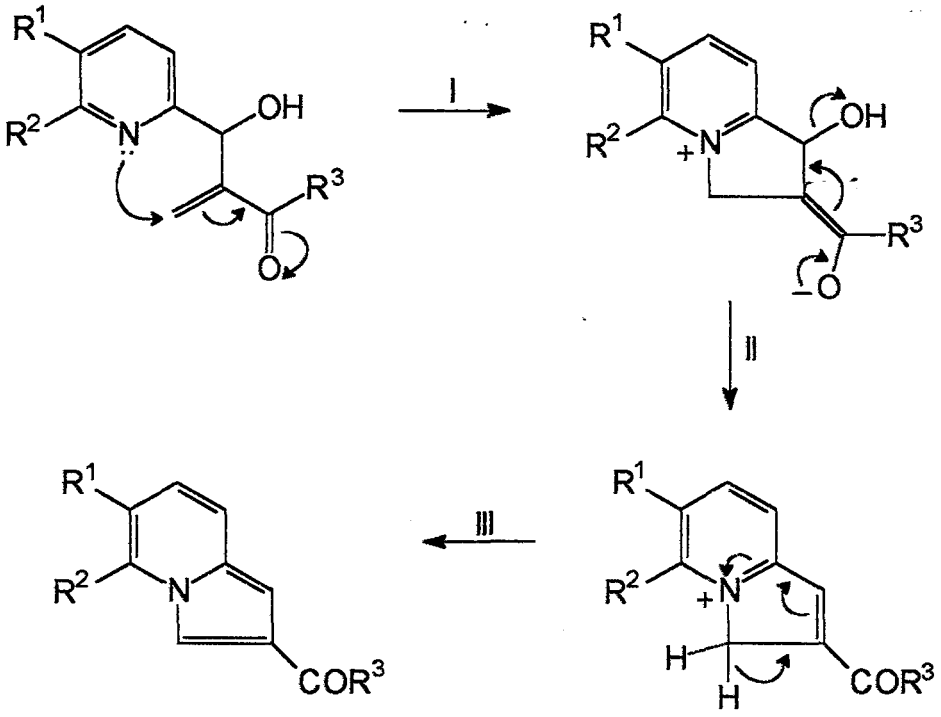
1-(2,2,2-Trichloro-1-hydroxyethyl)pyridine **152** has previously been prepared by the condensation of trimethylsilyl-2-pyridine with chloral, in the presence of a Lewis acid. Interestingly, this compound has been shown to exhibit tranquilizing activity, similar to that of meprobamate, and was particularly promising as it showed no convulsant properties.[300]

2.1.2 PREPARATION OF INDOLIZINES

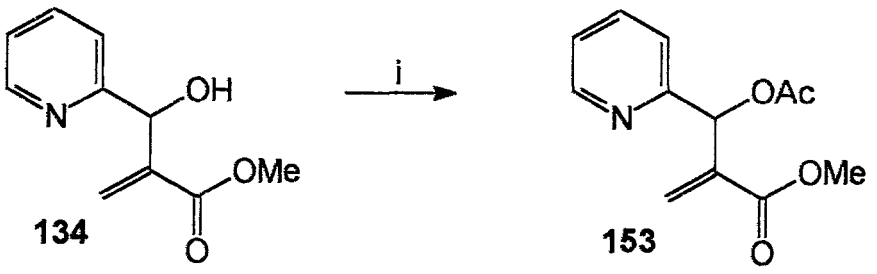
2.1.2.1 CYCLIZATION OF BAYLIS-HILLMAN INTERMEDIATES

The conversion of methyl 3-hydroxy-2-methylene-3-(2-pyridyl)propanoate **134** into the crystalline methyl indolizine-2-carboxylate **135** (Scheme 31, p. 61) was found to occur at approximately 140°C. Various methods were employed in order to obtain the most efficient conversion of adduct **134** to indolizine **135**, including:- heating in a Kügelrohr under vacuum to distil off the product; refluxing in xylene; refluxing with sicapent C in toluene; and heating the adduct without solvent in an oil bath at 140°C. The last method gave the best result, with a 22% conversion to product. However, heating compound **134** at this temperature yielded a large proportion of charred material together with the product and, thus, a method was sought to facilitate cyclization at a lower temperature.

Thermal cyclization was presumed to follow the addition - elimination sequence detailed in Scheme 37 and, clearly, conversion of the hydroxy function into a better leaving group would facilitate the process. The leaving group chosen was acetate, and a number of acetylation procedures were carried out in order to maximize the efficiency of this reaction (Scheme 38). For example, adduct **134** was reacted with acetyl chloride in THF/pyridine, to yield 48% of the acetylated adduct **153**; the adduct **134** was heated in the presence of Ac_2O, triethylamine and DMAP to yield 58% of the acetylated product; and adduct **134** was treated with *n*-BuLi, followed by acetyl chloride, yielding a large number of products in addition to the desired acetylated adduct **153**. The most efficient acetylation procedure found was to heat the adduct **134** in the presence of Ac_2O at 100°C for 30 min; this procedure yielded 78% of the desired product **153**, while similar heating in the presence of Ac_2O and glacial acetic acid (1:1) yielded only 35% of the required product.



SCHEME 37



Reagents	Yield (%)
CH_3COCl, THF, pyridine	48
Ac_2O, Et_3N, DMAP	58
n-BuLi, CH_3COCl, THF	not isolated
Ac_2O, glacial AcOH	35
Ac_2O	78

SCHEME 38

Heating of the acetylated derivative, methyl 3-acetoxy-2-methylene-3-(2-pyridyl)-propanoate **153**, was carried out at different temperatures while the reaction was monitored by TLC, to determine the optimal temperature required for cyclization to the indolizine product **135**. A temperature of 120°C for 1h proved necessary for maximum conversion of acetylated adduct to indolizine, a conversion of 68% being obtained in this way. Thus, conversion of the Baylis-Hillman adduct **134** to indolizine **135** *via* the acetylated adduct **153** proceeded in greater than 50% yield - a vast improvement on the direct conversion, which yielded only 22% product.

The success of this approach with adduct **134** led us to attempt this route with the other Baylis-Hillman adducts **139-144**. The acetylated adducts **153-158** were obtained in good yield and, in some cases, acetylation was accompanied by direct cyclization to the corresponding indolizines, as shown in Table 3. The acetylated adducts **153-158**, all of which are new compounds, were fully characterized by spectroscopic (^{1}H and ^{13}C NMR and IR) and elemental analysis (high-resolution mass spectrometry), and representative ^{1}H and ^{13}C NMR spectra of compound **153** are included in Appendix 5.1. The acetylation reaction can easily be monitored using ^{1}H NMR spectroscopy, by following the disappearance of the hydroxyl proton peak, and the appearance of the acetate signal (Fig. 12).

All attempts to isolate the acetylated intermediate of compound **142** failed, and instead 2-acetylindolizine **159** was obtained directly during the acetylation procedure. In fact, 5% of 2-acetylindolizine **159** was even isolated together with its hydroxy precursor **142** from the room temperature reaction of pyridine-2-carboxaldehyde with methyl vinyl ketone. Heating the hydroxy compound **142** at 100°C for 40 min yielded 2-acetylindolizine **159** directly, in 53% yield. The obvious ease of cyclization, in this case, may be attributed to the greater electrophilicity of the vinyl ketone system in step I (Scheme 37; R^3=Me) relative to the α,β-unsaturated ester moiety, in which alkyl-*O* lone-pair delocalization (a; Fig. 13) may be expected to reduce π-delocalization (b).

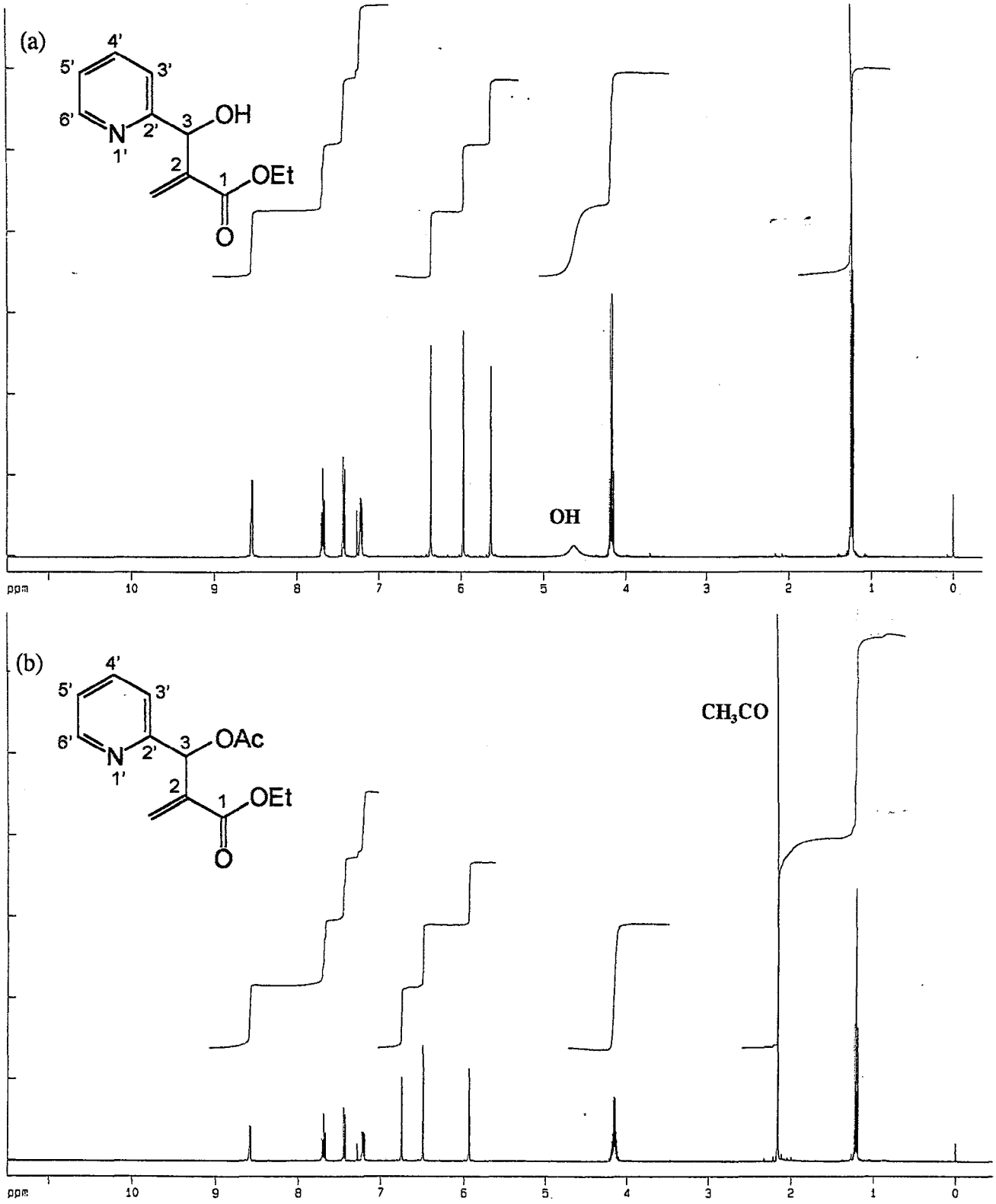
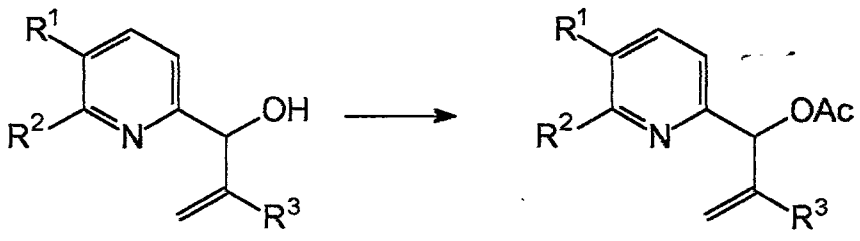


Figure 12 ^{1}H NMR spectra of : (a) ethyl 3-hydroxy-2-methylene-3-(2-pyridyl)propanoate **139**; and (b) ethyl 3-acetoxy-2-methylene-3-(2-pyridyl)propanoate **154**.

TABLE 3 Comparative yields (%) of acetylated Baylis-Hillman products



Compound	R^1	R^2	R^3	Purified Yield (%)
153	H	H	CO_2Me	78
154	H	H	CO_2Et	70
155	H	H	$CO_2{}^iPr$	62
156	H	Me	CO_2Me	52 (36)‡
157	-$(CH)_4$-		CO_2Me	57 (26)‡
158	H	H	CN	58

‡ Yield of the corresponding indolizine derivative obtained during acetylation

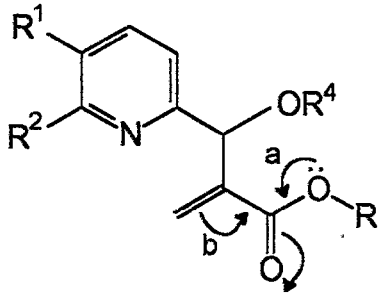


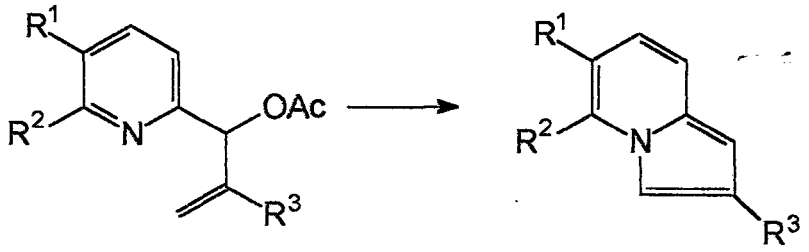
FIGURE 13

The indolizines **135** and **159-164** were prepared by heating their respective acetoxy precursors at 120°C for 1h; the yields are shown in Table 4. Some of the cyclization reactions proceeded in lower yield than the others. Several attempts to increase the extent of cyclization, in the case of indolizine **160**, were met with failure. Thus, direct cyclization of the hydroxy compound **139** by heating at 145°C for 1h, followed by heating at 155°C for 1h, yielded only 10% of indolizine **160**. Heating the acetylated compound **154** at 120°C for 2h, instead of 1h, led to no increase in yield, and heating at 100°C for 4h yielded only 26% of the indolizine product. An attempt was made to transform the hydroxyl group into a better leaving group than acetate, namely a tosyl or mesyl group. The adduct **139** proved to be resistant to treatment with TsCl in pyridine or THF, and treatment with NaH, followed by TsCl, yielded a large number of products, as shown by TLC. An attempted mesylation using MsCl and KOH in THF,[301] yielded none of the desired product. The original acetylation thus provided the best means of converting adduct **139** into indolizine **160**.

In a remarkably similar approach, Boekelheide and Windgassen[90] found it necessary to heat 3-acetoxy-3-(6-methyl-2-pyridyl)propene to 450°C to obtain 5-methylindolizine in 30% yield (Scheme 10, p. 18), cyclization presumably involving direct allylic displacement (S_N') of the acetoxy group. The relative ease of cyclization of the α,β-unsaturated carbonyl and carbonitrile substrates used in our study, may be attributed to the enhanced electrophilicity of the vinyl system, and the involvement of an intramolecular conjugate addition step.

The success of the cyclization of Baylis-Hillman adducts to yield indolizines led us to explore the applicability of this route to the preparation of pyrrolo[1,2-*a*]pyrazines **170** (Scheme 39). Thus, pyrazine-2-carboxylic acid **165** was esterified to give methyl pyrazine-2-carboxylate **166**, which was reduced with $LiAlH_4$ to pyrazine-2-carboxaldehyde **167**.[302] The aldehyde **167** was then reacted with methyl acrylate under Baylis-Hillman conditions to give adduct **168** in 56% yield. Heating this compound at 120°C for 45 min, and at 150°C for 1h, yielded none of the expected cyclized product

TABLE 4 Comparative yields (%) of indolizines from their acetoxy precursors



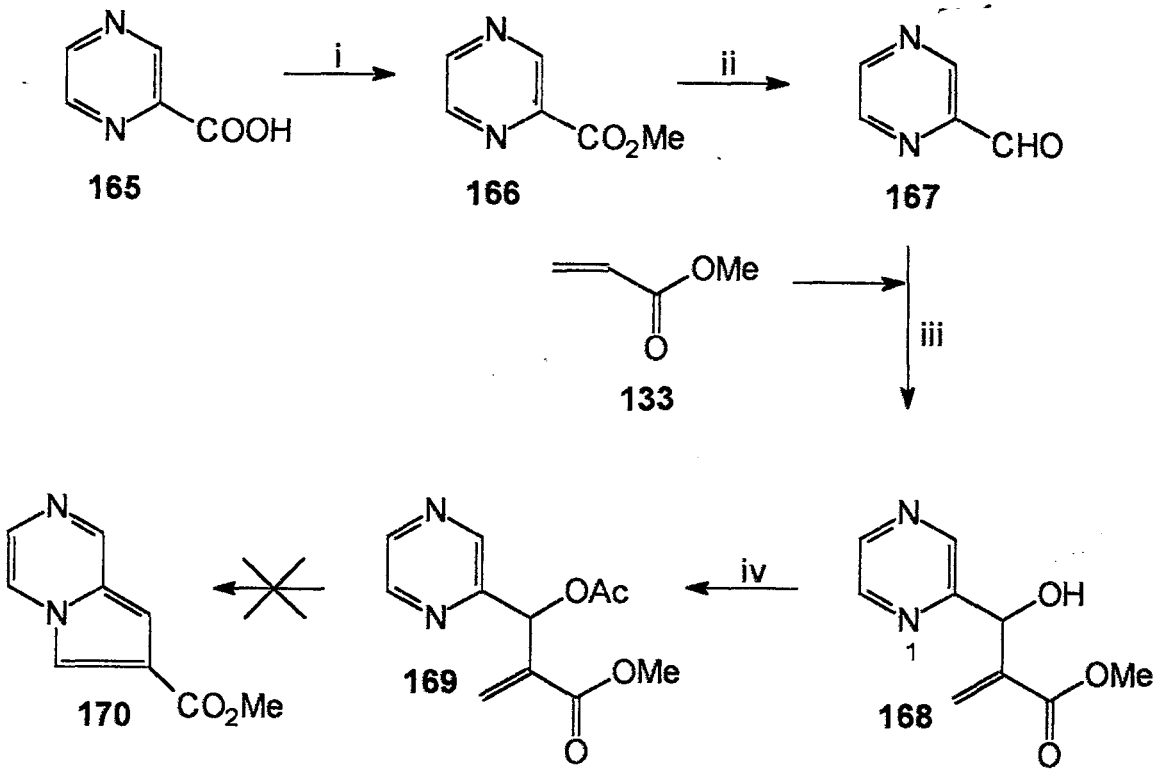
Compound	R^1	R^2	R^3	Purified Yield (%)
135	H	H	CO_2Me	68
159	H	H	COMe	53†
160	H	H	CO_2Et	38
161	H	H	$CO_2{}^iPr$	26
162	H	Me	CO_2Me	84
163	-$(CH)_4$-		CO_2Me	86
164	H	H	CN	32

† Obtained by direct cyclization of the hydroxy precursor **142**

170, as shown by TLC. Acetylation of **168** was carried out to give compound **169** in 22% yield, and heating of this compound with Ac_2O at 120°C still yielded no cyclized product. It would thus appear that the nitrogen at position 1 in pyrazine **168** is not sufficiently nucleophilic, as a result of the presence of the second ring nitrogen, to initiate the intramolecular conjugate addition step (Step 1, Scheme 37, p. 82) necessary for formation of the cyclized product.

The indolizines **159-164** are all new compounds, which were fully characterized by spectroscopic (^1H and ^{13}C NMR and IR) and elemental analysis (CHN analysis or high-resolution mass spectrometry), and representative ^1H, ^{13}C, HETCOR and COSY NMR spectra of compound **164** are included in Appendix 5.1. The conversion of acetoxy precursors to the corresponding indolizines is accompanied by disappearance of the vinyl proton peaks, and the appearance of a number of signals in the aromatic region of the ^1H NMR spectrum, as illustrated in Fig. 14.

The cyclization of Baylis-Hillman adducts thus provides a convenient and relatively efficient route to 2-carbonyl- and 2-cyano-indolizines. The approach is limited only by the availability of suitably substituted pyridine-2-carboxaldehydes, and the necessity of using acrylate systems which undergo Baylis-Hillman reaction. These results have recently been published.[303]



SCHEME 39

Reagents : (i) MeOH, HCl gas
(ii) $LiAlH_4$
(iii) DABCO
(iv) Ac_2O, heat

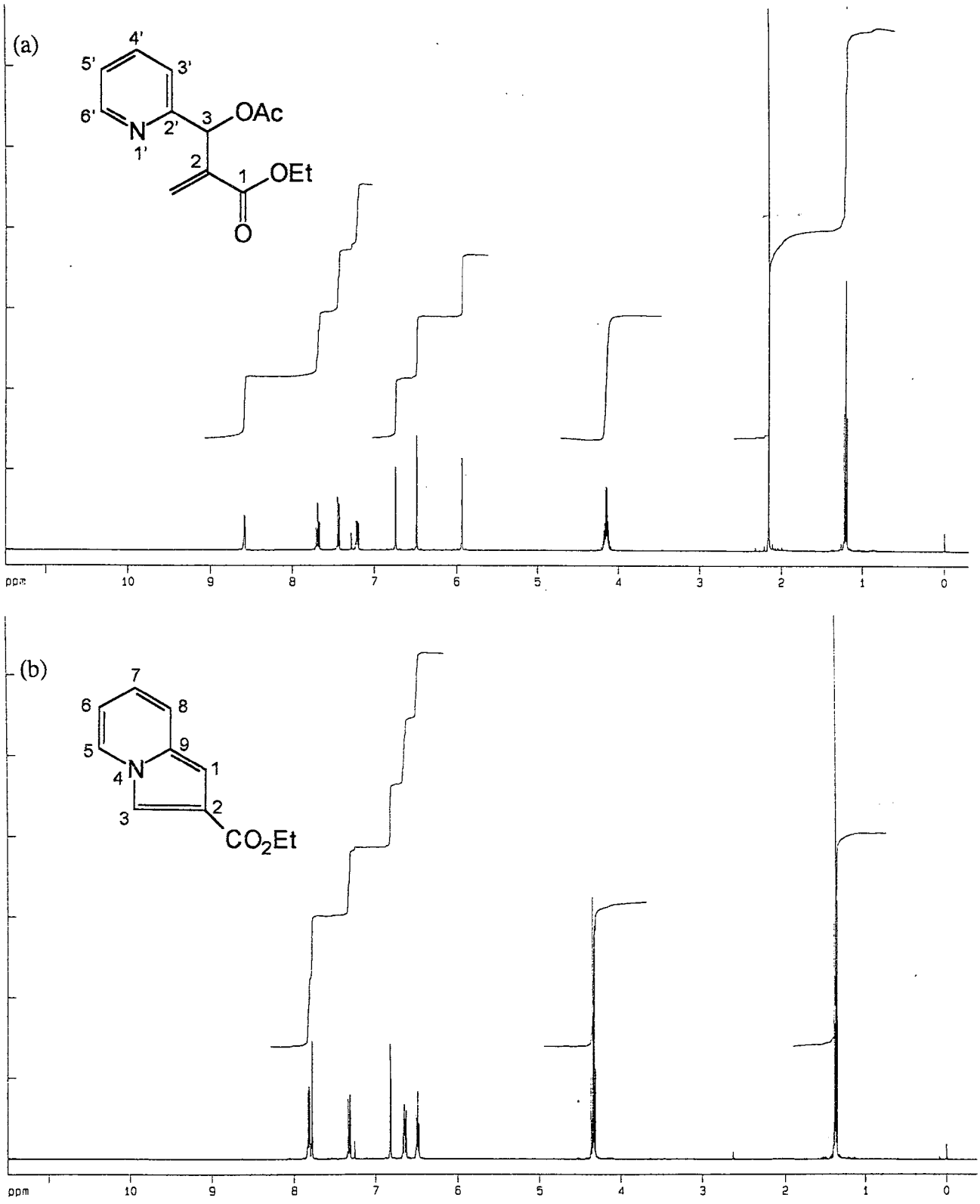


Figure 14 ^{1}H NMR spectra of : (a) ethyl 3-acetoxy-2-methylene-3-(2-pyridyl)propanoate **154**; and (b) ethyl indolizine-2-carboxylate **160**.

2.1.2.2 AN NMR STUDY OF INDOLIZINE DERIVATIVES

Several one-dimensional, and largely lowfield, NMR spectroscopic studies of a relatively small number of indolizines have been published.[19,23-26,304] The availability of the seven 2-substituted indolizines **135** and **159-164** provided us with an opportunity to extend these earlier studies.

Black *et al.*[19] were the first workers to publish a complete assignment of the ^{1}H NMR spectrum of indolizine. Their assignments were based on spin-decoupling experiments, and on the spectrum of the deuteriated derivative, indolizine-1,3-d_2.

Our own ^{1}H NMR assignments are based on coupling patterns and constants obtained from both one-dimensional and two-dimensional correlation (COSY) spectroscopy. Representative ^{1}H NMR (Fig. 15) and COSY spectra (Fig. 16) of methyl indolizine-2-carboxylate **135** illustrate the trends observed for the series. The spectrum of 5-methylindolizine **162** removed any possible ambiguity in the assignment of protons 5-H and 8-H, as in this compound the 5-H proton is absent and the missing signal must therefore be the signal resulting from this proton. The 400MHz ^{1}H NMR data for indolizines **135**, **159-162** and **164** are summarized in Table 5, and are essentially consistent with those published for other indolizine systems.[19,26] A remarkable feature of the ^{1}H NMR spectra of indolizines is the extensive long-range ^{1}H-^{1}H coupling. Examples of this can be seen in the COSY spectrum of methyl indolizine-2-carboxylate **135** (Fig. 16), which shows, for example, that:- 8-H couples with both 3-H and 5-H; 7-H couples very weakly with 1-H; and 1-H couples with 5-H. These particularly long-range couplings are not observed in the one-dimensional ^{1}H NMR spectrum, but are readily observed in the COSY spectrum. The other indolizines studied showed very similar coupling patterns.

By treating the protons in indolizine as a close-coupled seven-spin system, Crews *et al.*[304] used computer simulation of 100MHz data to derive values for all twenty-one of the possible couplings. At 400MHz, direct measurement of some of the smaller coupling constants becomes feasible, by the use of resolution enhanced spectroscopy. Fig. 17

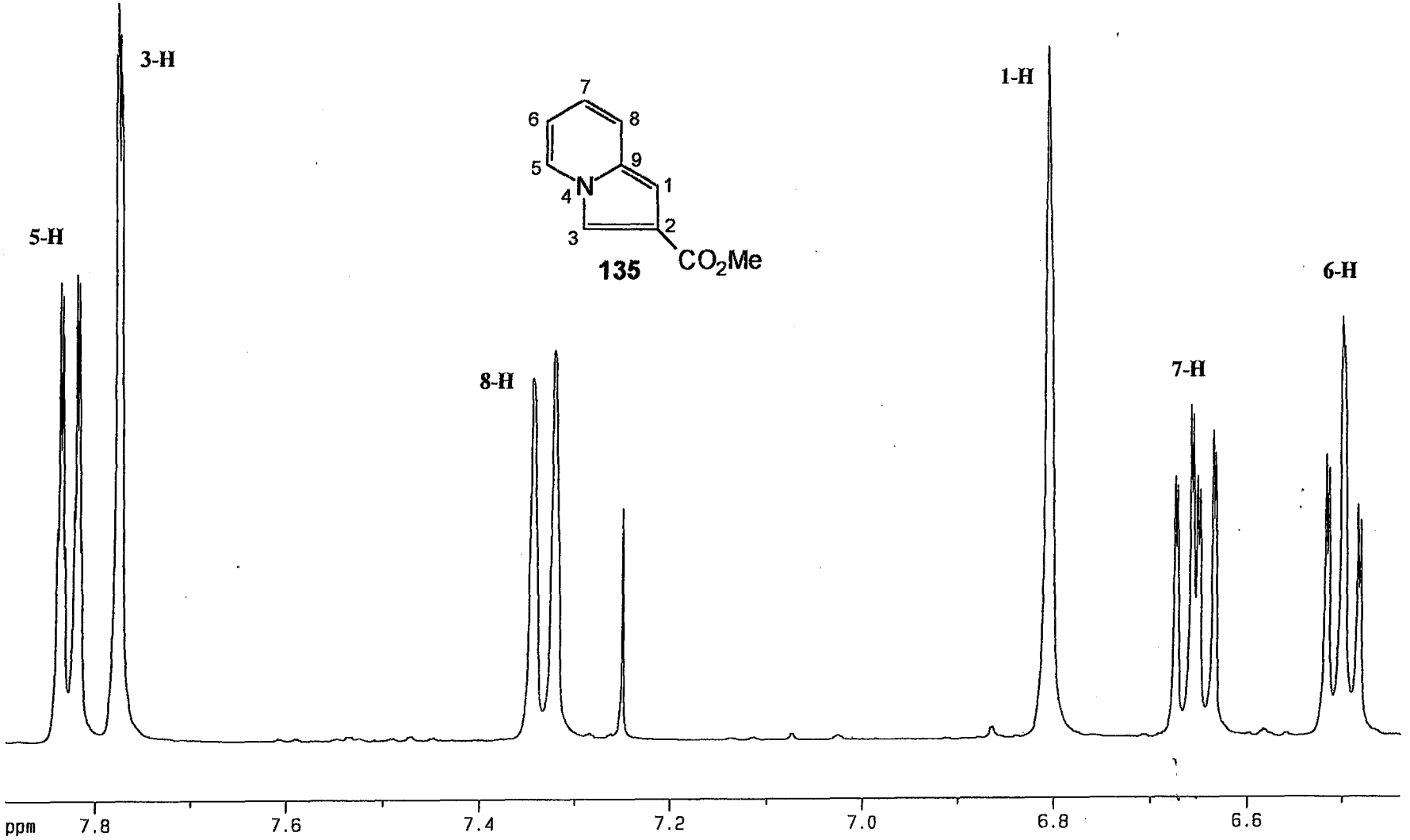


Figure 15 ^{1}H NMR spectrum of methyl indolizine-2-carboxylate **135** showing the aromatic region.

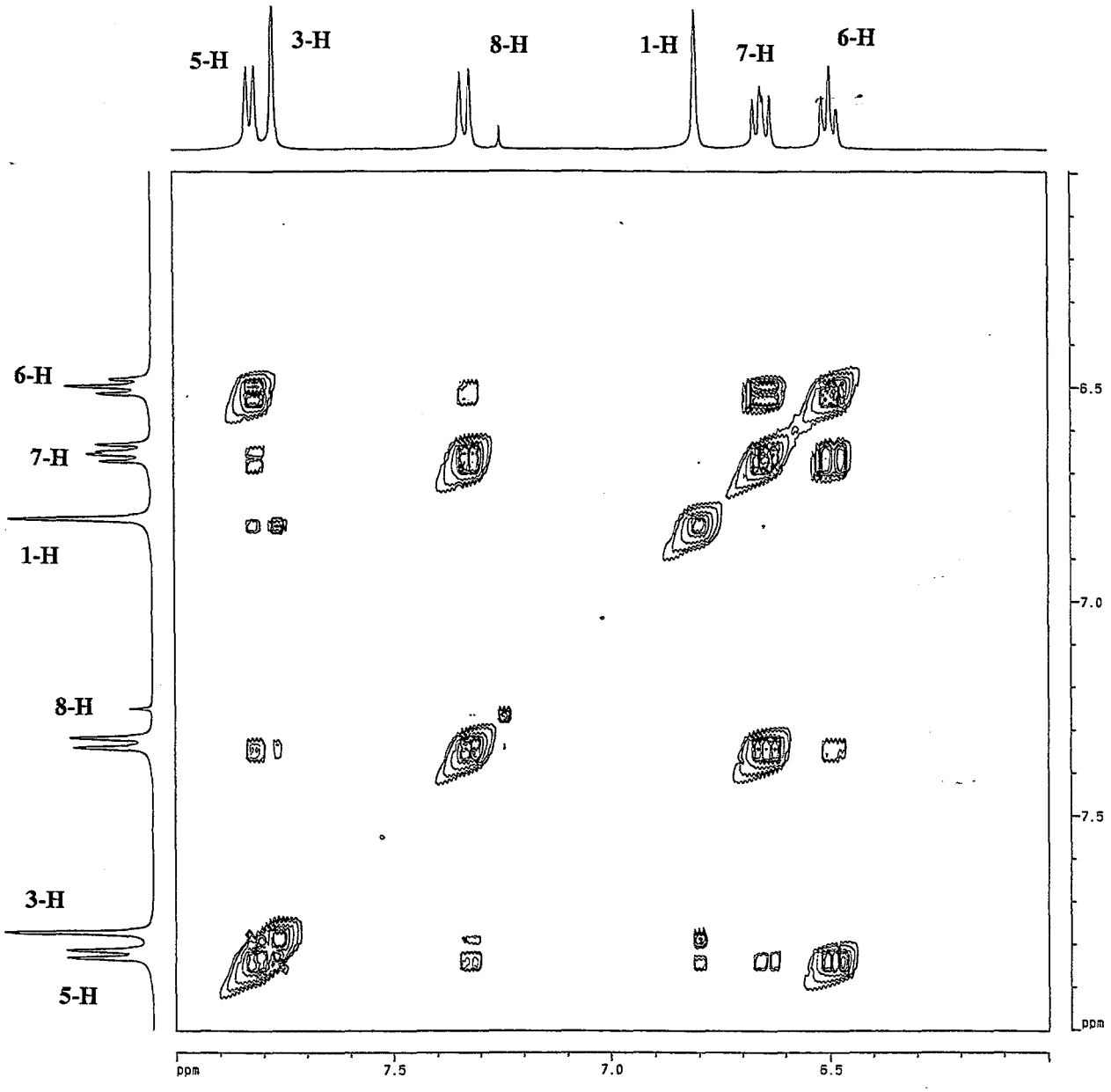


Figure 16 COSY spectrum of methyl indolizine-2-carboxylate **135**.

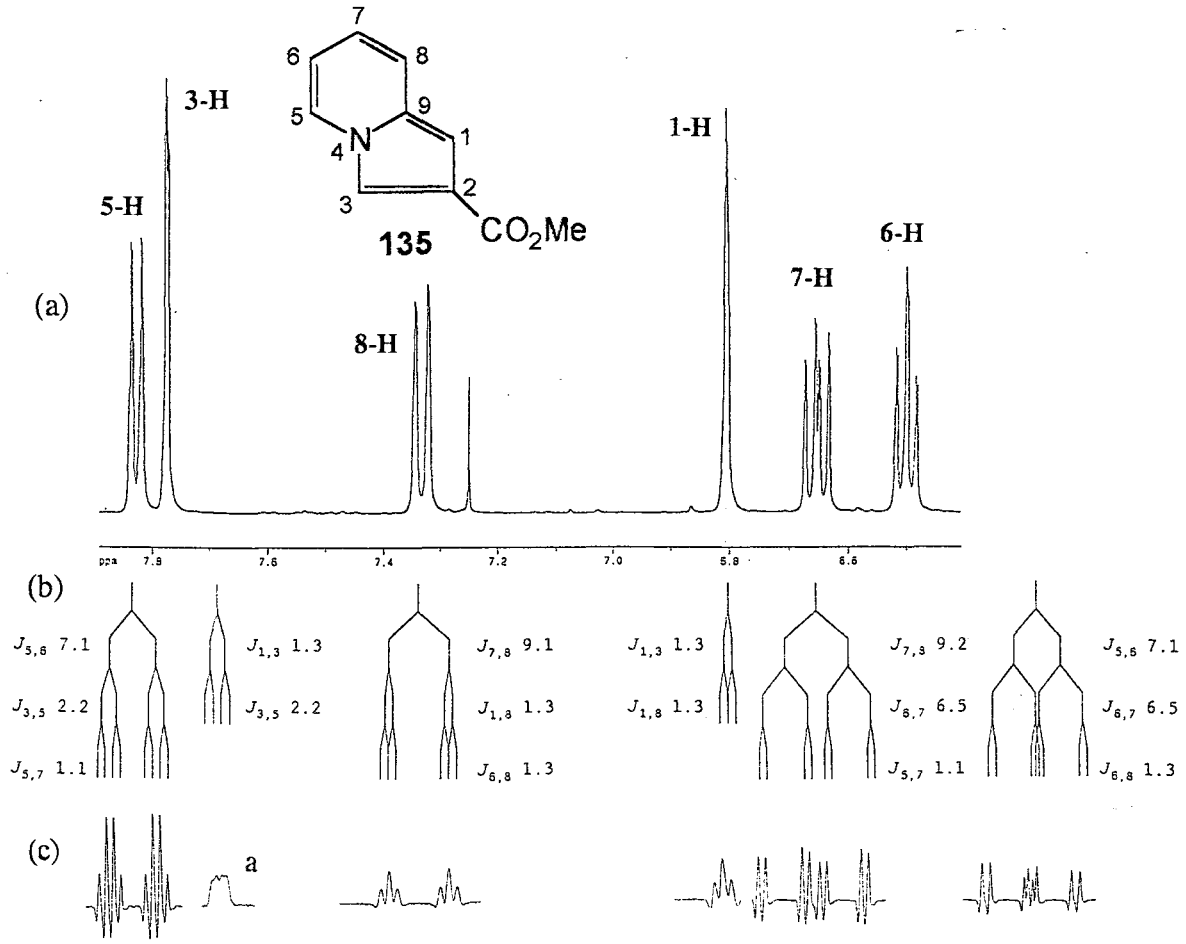


Figure 17 ^1H-^1H coupling between the aromatic protons in methyl indolizine-2-carboxylate **135** : (a) ^1H NMR spectrum; (b) analysis of splitting patterns with the corresponding coupling constants in Hz; and (c) resolution enhanced multiplets.

[a] Resolution of the dd was poor, *J* values correspond to coupling constant values measured for 1-H and 5-H nuclei.

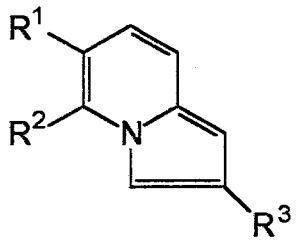
shows the resolution enhanced spectrum of methyl indolizine-2-carboxylate **135**, which illustrates the various ^{1}H-^{1}H couplings observed using this technique. The assignments are supported by correlation spectroscopy (COSY) data. The measured *J* values, in fact, correspond reasonably well with those derived for indolizine by Crews *et al.*[304]

A number of workers have investigated the ^{13}C NMR characteristics of indolizines and their aza-derivatives,[23-25] and, in a few cases, ^{15}N chemical shifts have also been measured.[305] Pugmire *et al.*[24] used ^{13}C NMR spectroscopy to determine the extent to which the free electron pair on the bridgehead nitrogen contributes to the delocalized electronic structures. They showed that the bridgehead nitrogen behaves in a similar fashion to a pyrrole-type nitrogen, where significant delocalization of the lone pair occurs.

Our ^{13}C NMR assignments are based on two-dimensional heteronuclear correlation spectroscopy (HETCOR), and correlate well with other published values.[23-26] Table 6 summarizes the ^{13}C NMR data for indolizines **135**, **159-162** and **164**, and representative ^{13}C NMR (Fig. 18) and HETCOR spectra (Fig. 19) of ethyl indolizine-2-carboxylate **160** show the general trends observed for the series. In the case of the quinoline derivative **163**, COSY and HETCOR analyses failed to permit unambiguous assignments of the signals, necessitating application of a multiple-quantum coherence (INADSY) experiment (Fig. 20). A combination of the resulting ^{13}C-^{13}C coupling and HETCOR data (Fig. 21) finally provided the basis for the assignments detailed in Table 7 (the numbering following Mosby[35]). These results are discussed in a recent publication.[303]

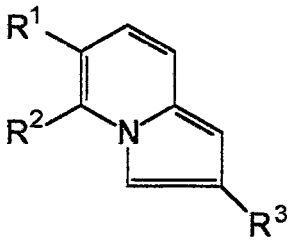
The chemical shift values of indolizine derivatives are consistent with those expected for π-excessive systems, *i.e.*, from the chemical shifts, it may be deduced that indolizines possess a ring current, resulting in deshielding of the aromatic signals. The relative deshielding of the aromatic signals of the 6-membered ring, however, is less for indolizine than for its pyridine precursors, as shown in Table 8, which compares the chemical shifts of ethyl indolizine-2-carboxylate **160** with its hydroxy **139** and acetoxy **154** precursors. One of the reasons for this is that the deshielding effect of the nitrogen

TABLE 5 ^{1}H NMR chemical shift data for indolizines **135**, **159-162** and **164**



Compound	1	3	5	6	7	8
135	6.81	7.77	7.83	6.50	6.65	7.33
159	6.74	7.71	7.79	6.47	6.63	7.30
160	6.83	7.79	7.83	6.49	6.65	7.34
161	6.81	7.77	7.82	6.48	6.64	7.32
162	6.88	7.68	-	6.36	6.65	7.27
164	6.67	7.66	7.87	6.62	6.77	7.36

TABLE 6 ^{13}C NMR chemical shift data for indolizines **135**, **159-162** and **164**



Compound	1	2	3	5	6	7	8	9
135	100.4	119.6	115.8	125.3	112.2	118.1	120.2	132.8
159	99.3	128.4	115.1	125.4	112.5	118.3	120.3	133.0
160	100.3	120.0	115.7	125.2	112.1	118.0	120.2	132.7
161	100.4	120.5	115.7	125.2	112.1	117.9	120.2	132.7
162	100.8	119.3	112.8	133.3	111.2	118.4	117.8	132.9
164	102.5	97.4	117.5	125.1	113.0	119.4 and 119.7		132.7

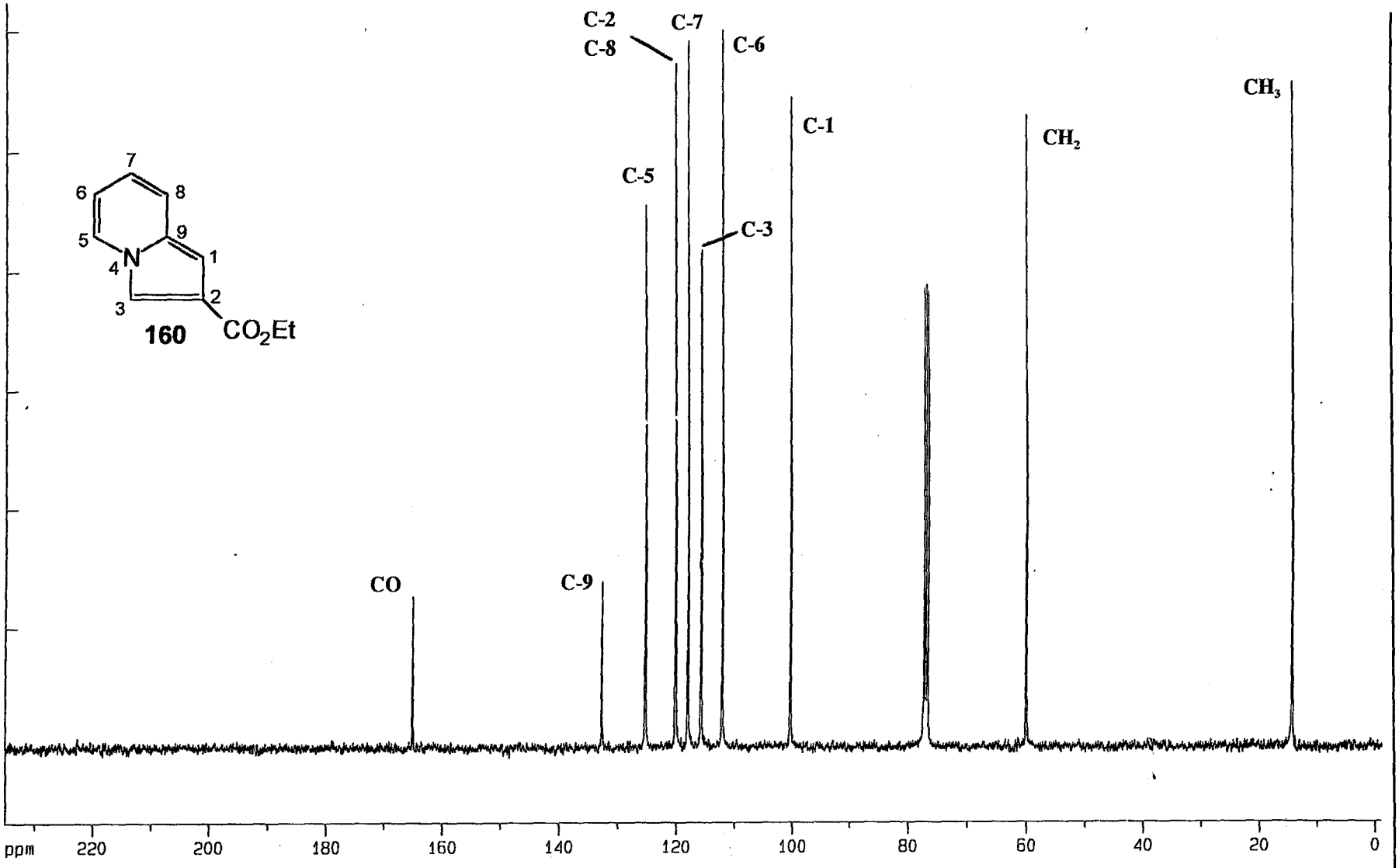


Figure 18 ^{13}C NMR spectrum of ethyl indolizine-2-carboxylate **160**.

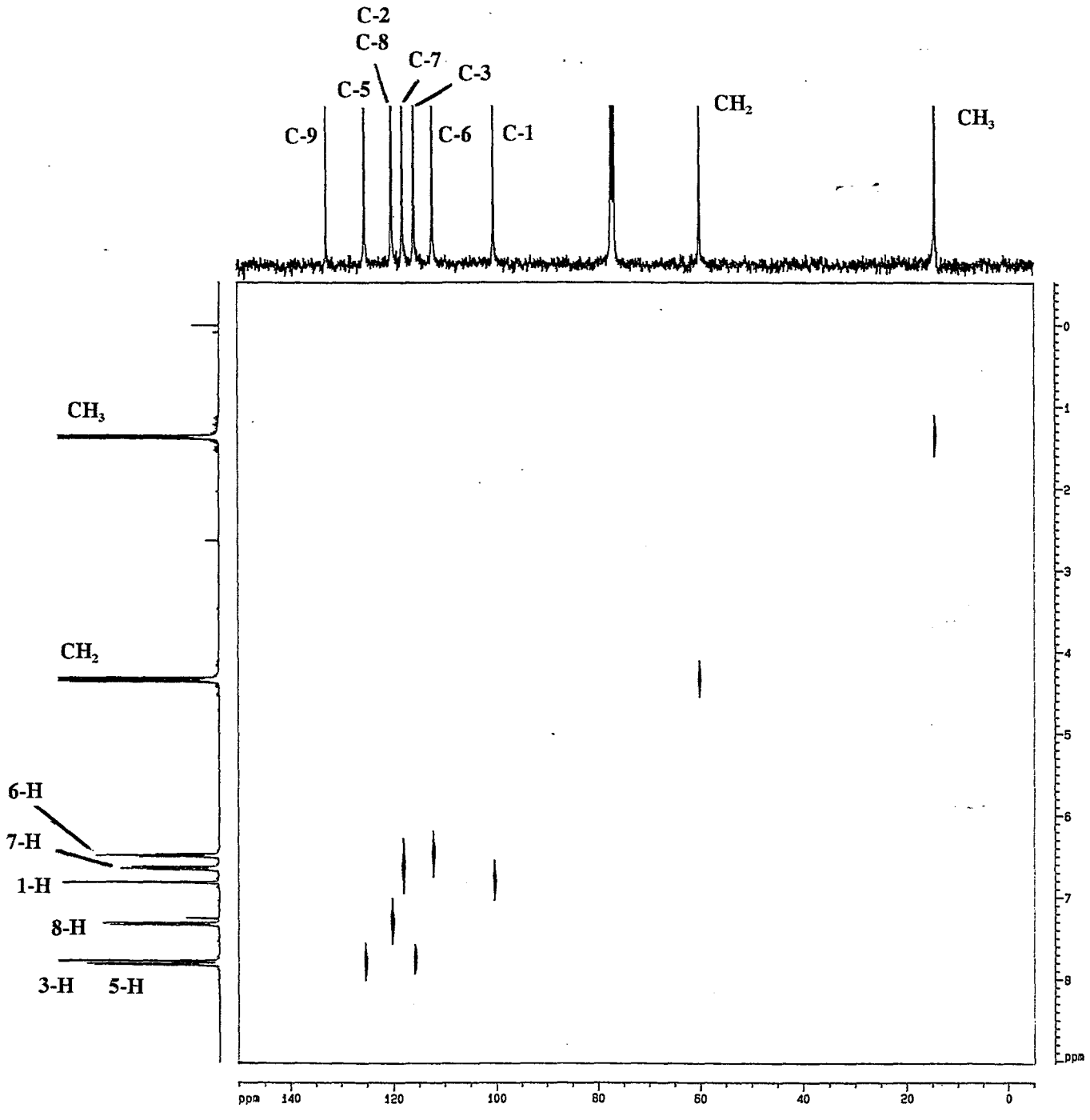


Figure 19 HETCOR spectrum of ethyl indolizine-2-carboxylate **160**.

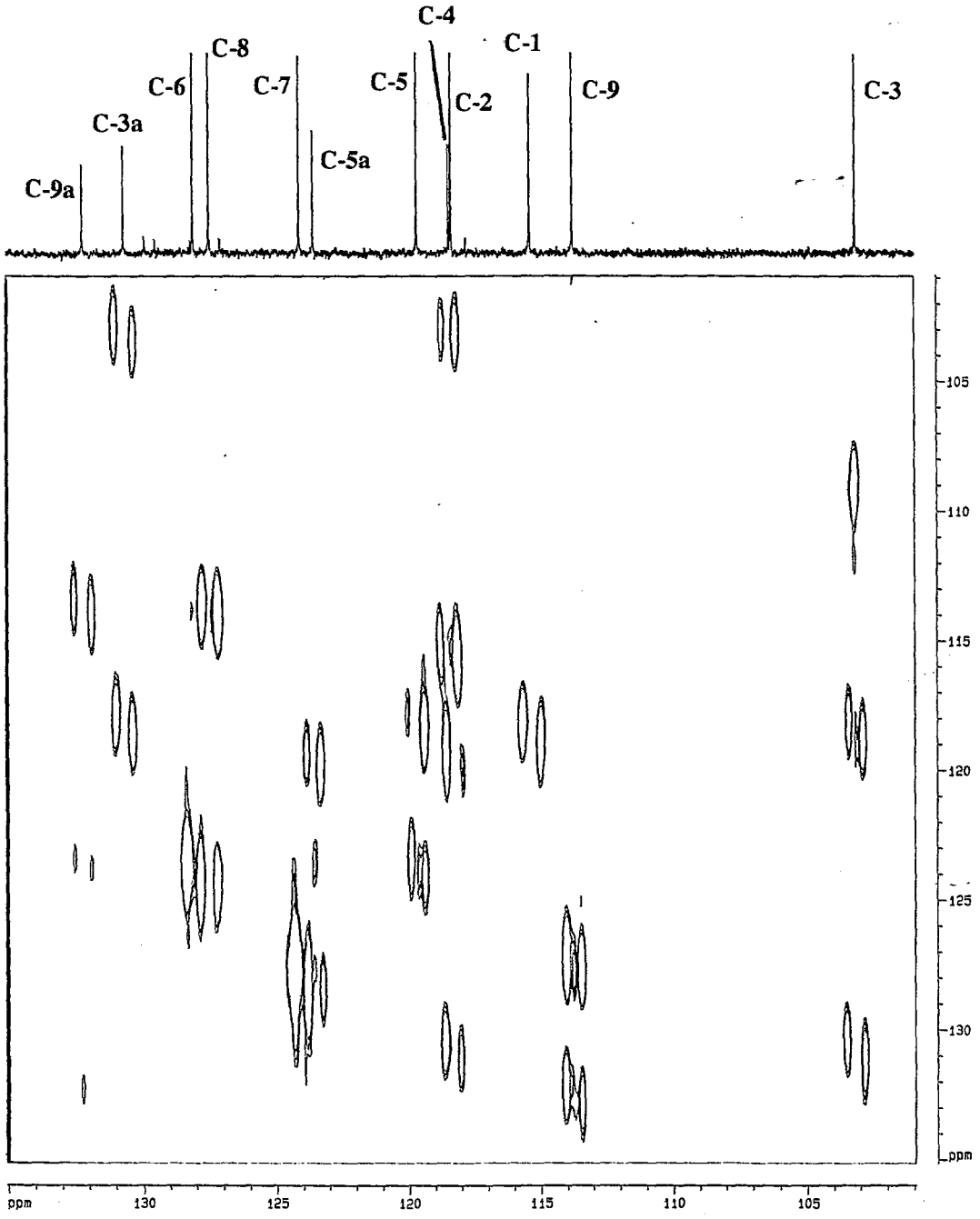
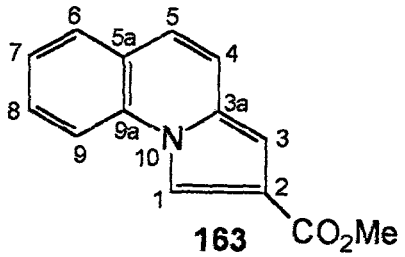


Figure 20 INADSY spectrum of methyl pyrrolo[1,2-*a*]quinoline-2-carboxylate **163**.



163

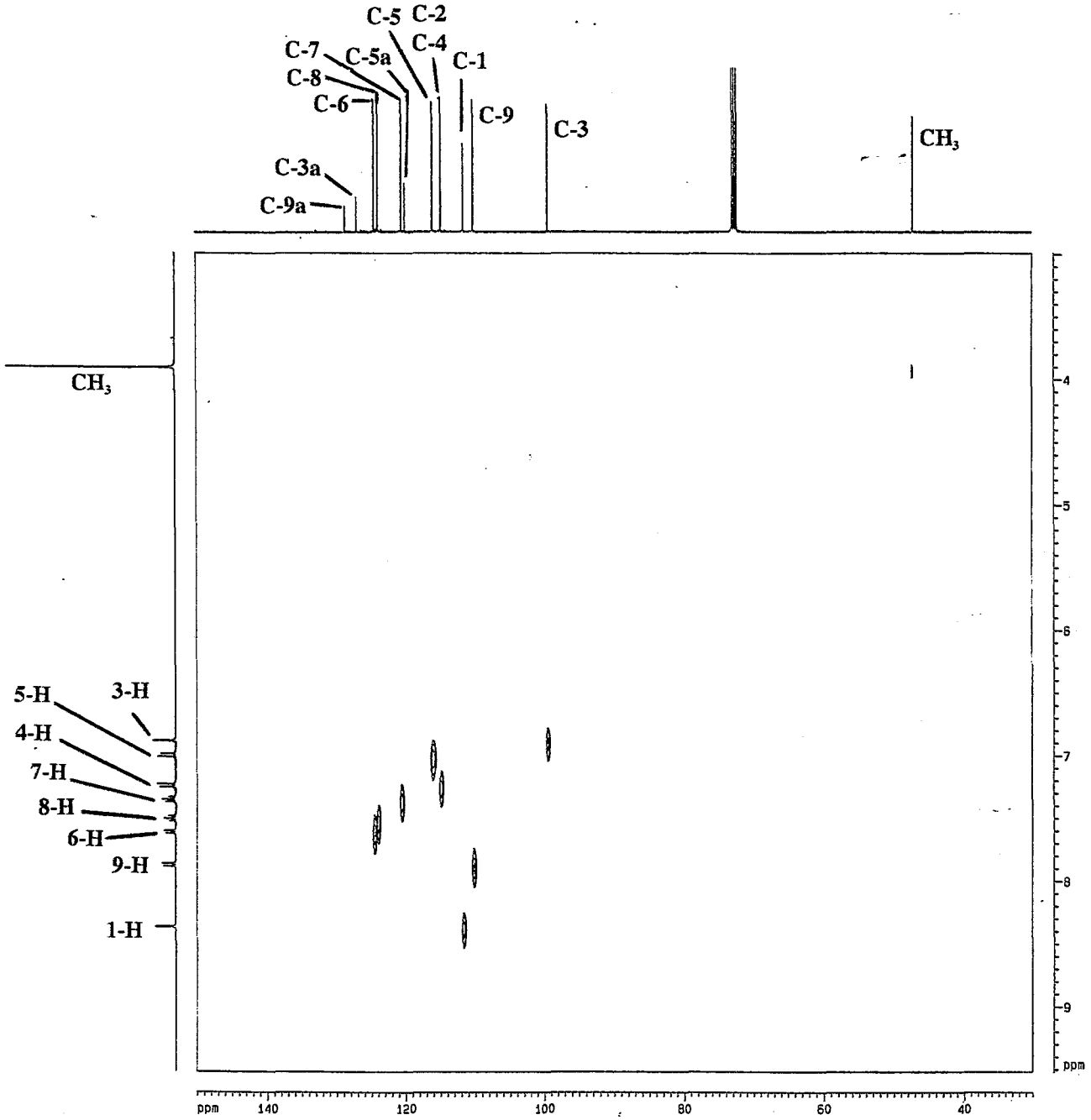
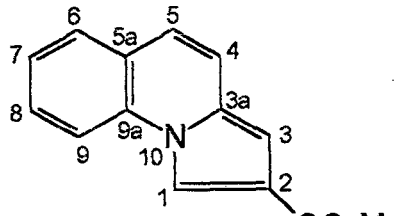


Figure 21 HETCOR spectrum of methyl pyrrolo[1,2-*a*]quinoline-2-carboxylate **163**.

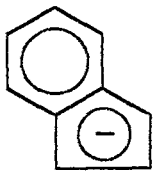
is decreased in the case of indolizine compared with pyridine because of extensive delocalization of the nitrogen lone pair in indolizine. In fact, Pugmire *et al.*[24] showed that the ^{13}C chemical shifts for indolizine and the isoelectronic indenyl anion **171**, which possesses no heteroatom, were very similar, the only exception being at position C-3 in the 5-membered ring adjacent to the heteroatom where, in the case of indolizine, the signal was shifted downfield by 21.1ppm.

TABLE 7 ^{1}H and ^{13}C chemical shift assignments for methyl pyrrolo[1,2-*a*]quinoline-2-carboxylate **163**



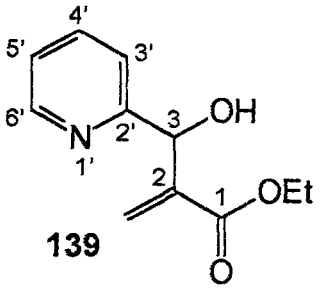
163

Position	^{1}H	^{13}C
1	8.38	115.7
2	-	118.87
3	6.88	103.5
3a	-	131.2
4	7.24	118.90
5	7.00	120.1
5a	-	124.1
6	7.61	128.6
7	7.36	124.6
8	7.51	128.0
9	7.88	114.3
9a	-	132.8



171

TABLE 8 ^{1}H and ^{13}C NMR chemical shift data for ethyl indolizine-2-carboxylate **160** and its acetoxy **154** and hydroxy **139** precursors



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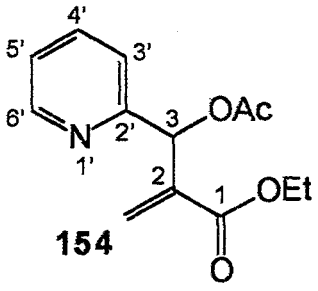

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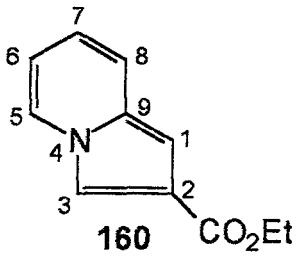


160

Compound	Nucleus‡	1	2	3	5	6	7	8
139	^{1}H	5.53	-	5.84 6.24	8.37	7.06	7.53	7.30
	^{13}C	72.0	141.9	126.1	148.0	122.3	136.5	121.0
154	^{1}H	5.89	-	6.45 6.75	8.58	7.22	7.69	7.45
	^{13}C	73.9	138.2	127.3	149.4	123.0	136.6	122.7
160	^{1}H	6.83	-	7.79	7.83	6.49	6.65	7.34
	^{13}C	100.3	120.0	115.7	125.2	112.1	118.0	120.2

‡ For comparative purposes, nuclei are numbered to reflect their correspondence with the indolizine product **160**.

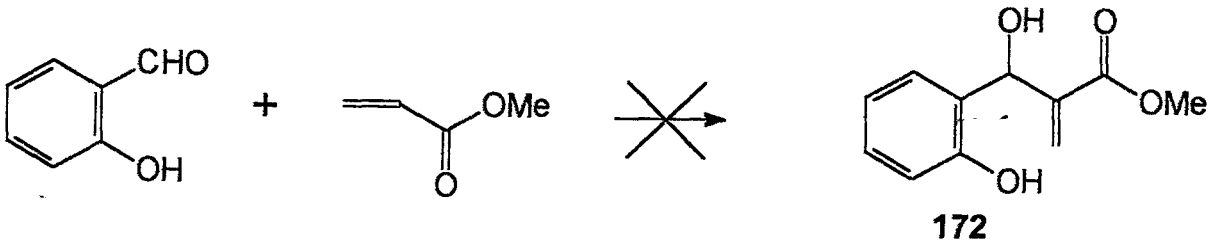
2.1.3 EXTENSIONS OF THE BAYLIS-HILLMAN REACTION

The cyclization of Baylis-Hillman adducts prepared from pyridine-2-carboxaldehyde has been shown to provide a convenient route to indolizine derivatives and, in a projected extension of this approach, synthesis of the Baylis-Hillman adduct **172** of salicylaldehyde and methyl acrylate was expected to provide access to chromene systems **173** (Scheme 40). Treatment of salicylaldehyde with methyl acrylate in the presence of DABCO afforded a colourless crystalline compound as the sole product. Spectroscopic analysis of this product showed that it was not the expected adduct **172**. The expected hydroxyl band in the IR spectrum (Fig. 22) was absent, and two carbonyl bands were present instead of the expected one. The ^{1}H NMR spectrum (Fig. 23) showed the presence of an aldehydic proton and the absence of a methyl ester, and the ^{13}C NMR spectrum (Fig. 24) showed many more peaks than expected for the adduct **172**. The compound was finally identified as the coumarin derivative **174** by single crystal X-ray diffraction analysis.

2.1.3.1 STRUCTURAL DETERMINATION OF THE COUMARIN DERIVATIVE 174

The crystalline product obtained from reaction of salicylaldehyde and methyl acrylate was relatively insoluble in all the solvents tried, including chloroform, DMSO, acetone, carbon tetrachloride, hexane, ethyl acetate, methanol and ethanol. This insolubility made recrystallization impossible, and the crystals obtained directly from the reaction mixture were used for collection of X-ray data. The crystal structure and packing diagrams for 3-[(2-formylphenoxy)methyl]coumarin **174** are shown in Figures 25 and 26. Detailed crystal data, including fractional coordinates, bond lengths, anisotropic temperature factors and mean plane calculations can be found in Appendix 5.2 (Tables 18-23).

From the X-ray diffraction analysis it is apparent that the molecule is almost completely planar, with atom C(8) exhibiting the greatest deviation (*ca.* 0.1Å) from the molecular



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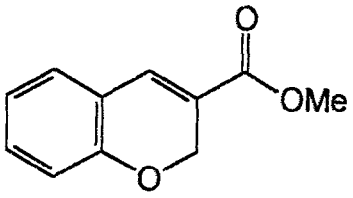
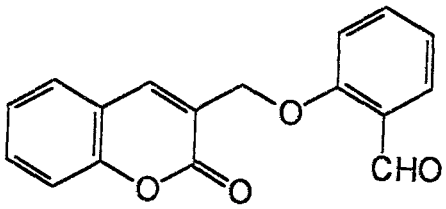


173

SCHEME 40



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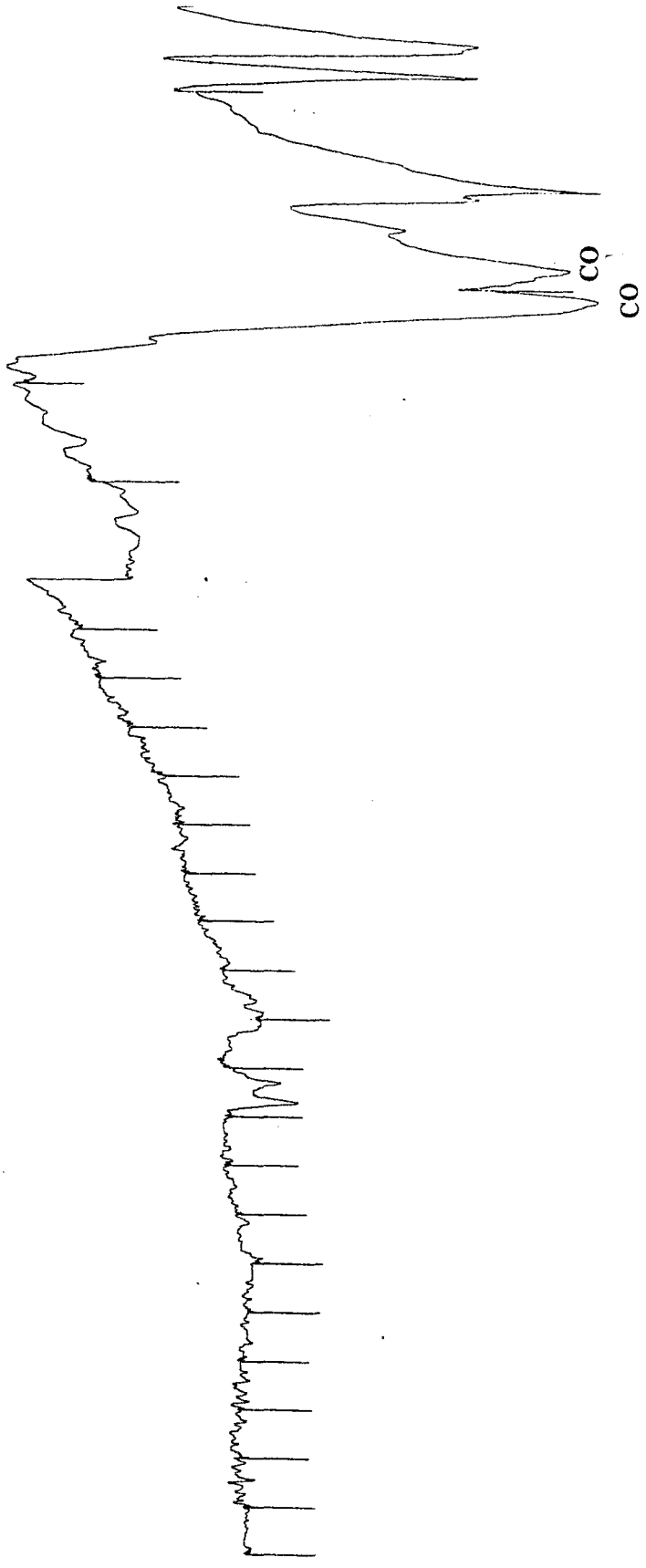


Figure 22 IR spectrum of 3-[(2-formylphenoxy)methyl]coumarin **174**.

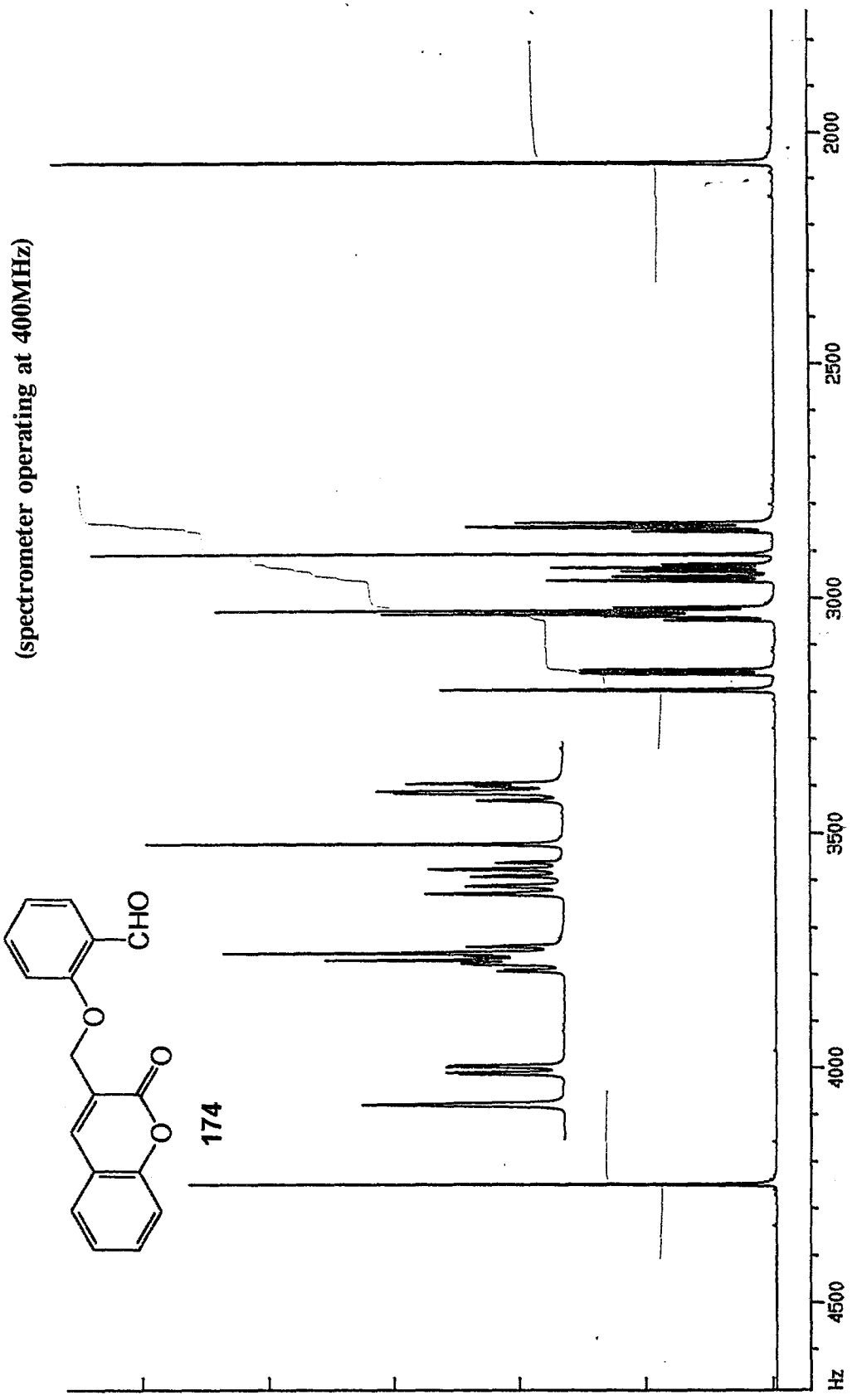


Figure 23 ^{1}H NMR spectrum of 3-[(2-formylphenoxy)methyl]coumarin 174.

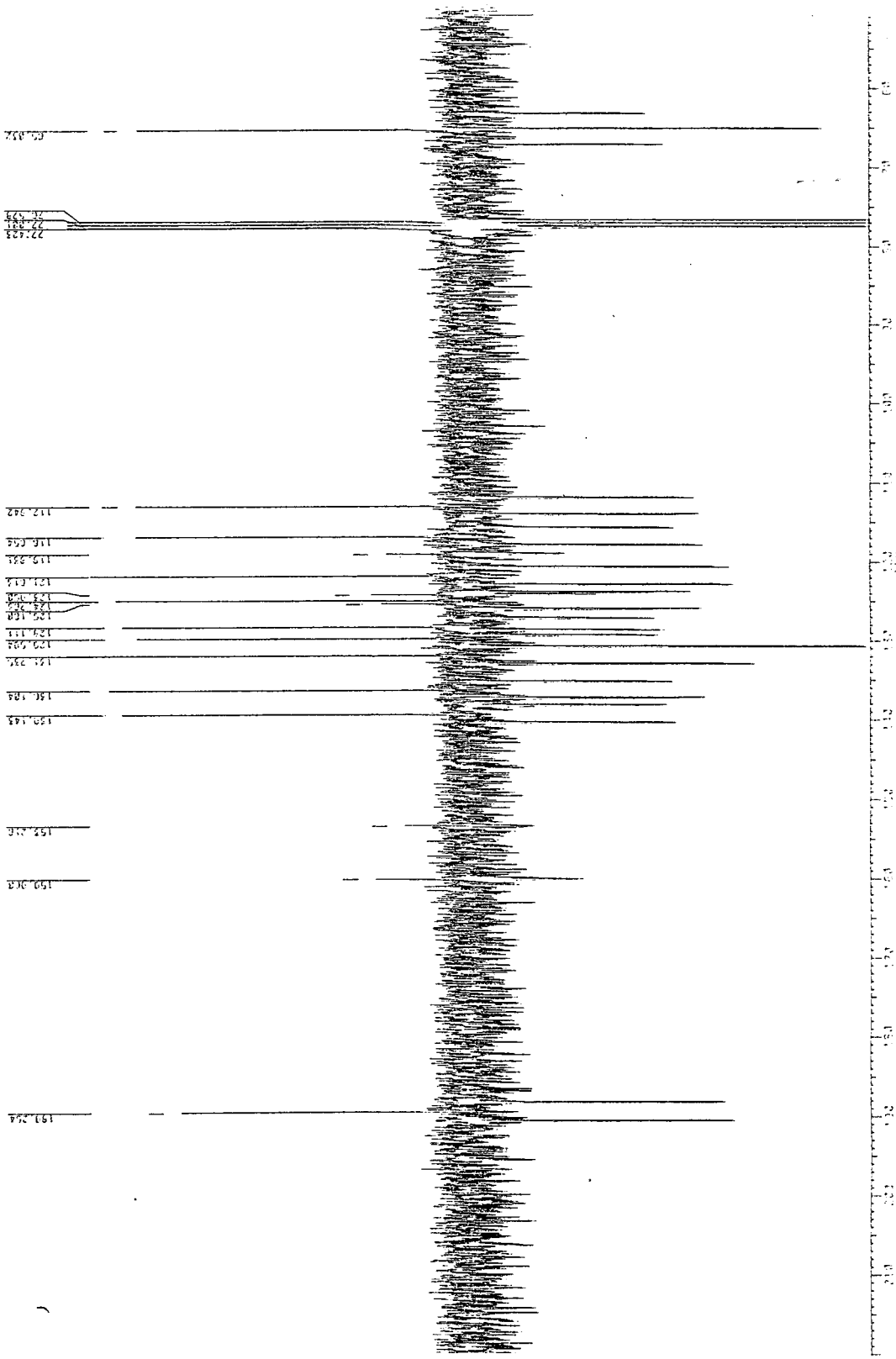


Figure 24 ^{13}C NMR spectrum of 3-[(2-formylphenoxy)methyl]coumarin **174**.

plane; the unit cell is thus effectively only one atom thick. The angle between the normals to the two phenyl rings is 2.7°. Intermolecular interactions are the likely cause of the remarkable planarity. The crystal is a Schmidt β-structure, as shown by the short *a* axis, in which sheet-based packing is favoured by C-H...O hydrogen bonding. There are a number of planar oxygen-containing aromatics which show this type of packing, and examples include ethers, coumarins, and quinones.[306] The C-H...O contacts have H...O distances which are less than the sum of their van der Waals radii, *viz.*, 2.6Å, and these contacts are illustrated by dashed lines in Fig. 26(b). Pairs of centrosymmetrically related molecules, which are held together by six carbonyl-O...H interactions, are linked by similar interactions between adjacent pairs. The sheets are inclined at 32.5° to the *yz* plane, and have a van der Waals thickness of 4.55Å.

Analogous reactions using 5-bromosalicylaldehyde and 2-hydroxy-5-methoxy-benzaldehyde instead of salicylaldehyde, did not yield the corresponding coumarin derivatives.

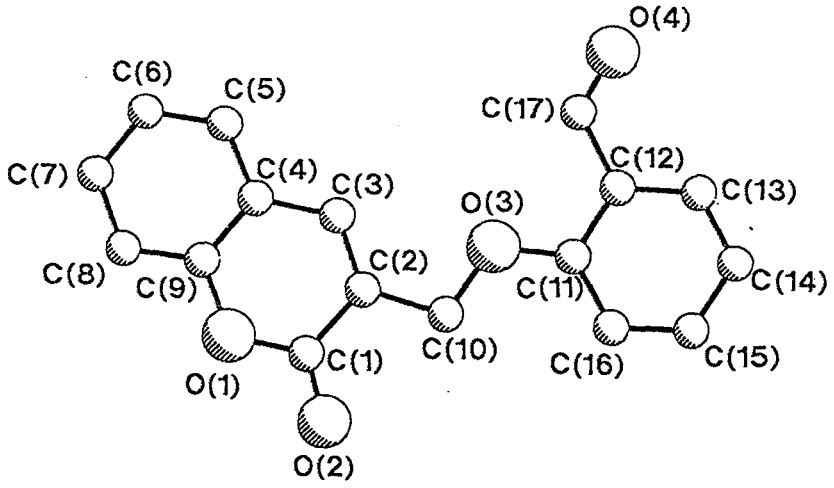
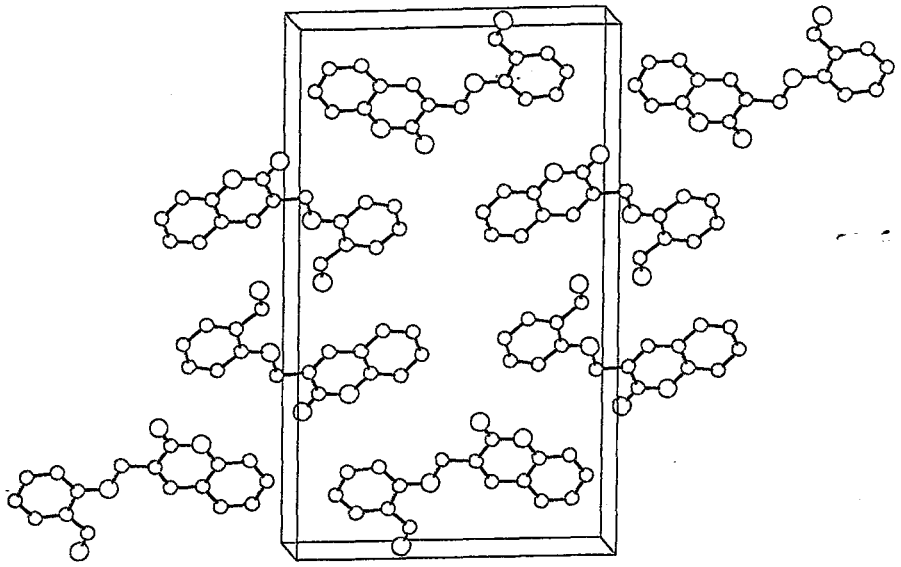
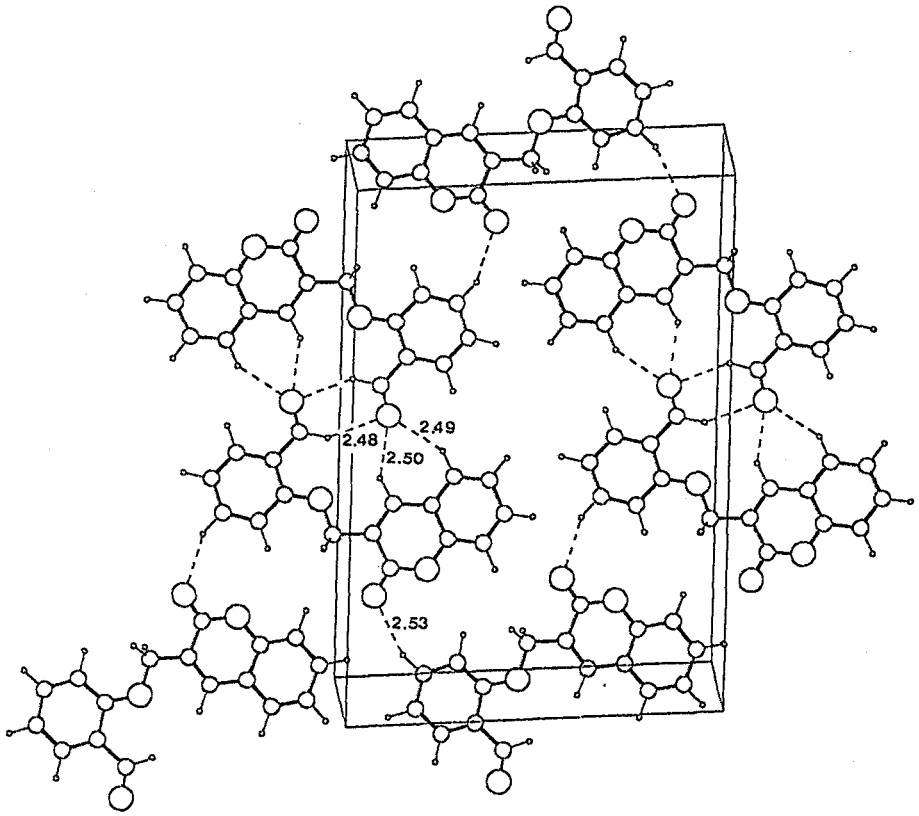


Figure 25 X-ray crystal structure of 3-[(2-formylphenoxy)methyl]coumarin **174** showing crystallographic numbering.



(a) Packing diagram of 3-[(2-formylphenoxy)methyl]coumarin **174**.



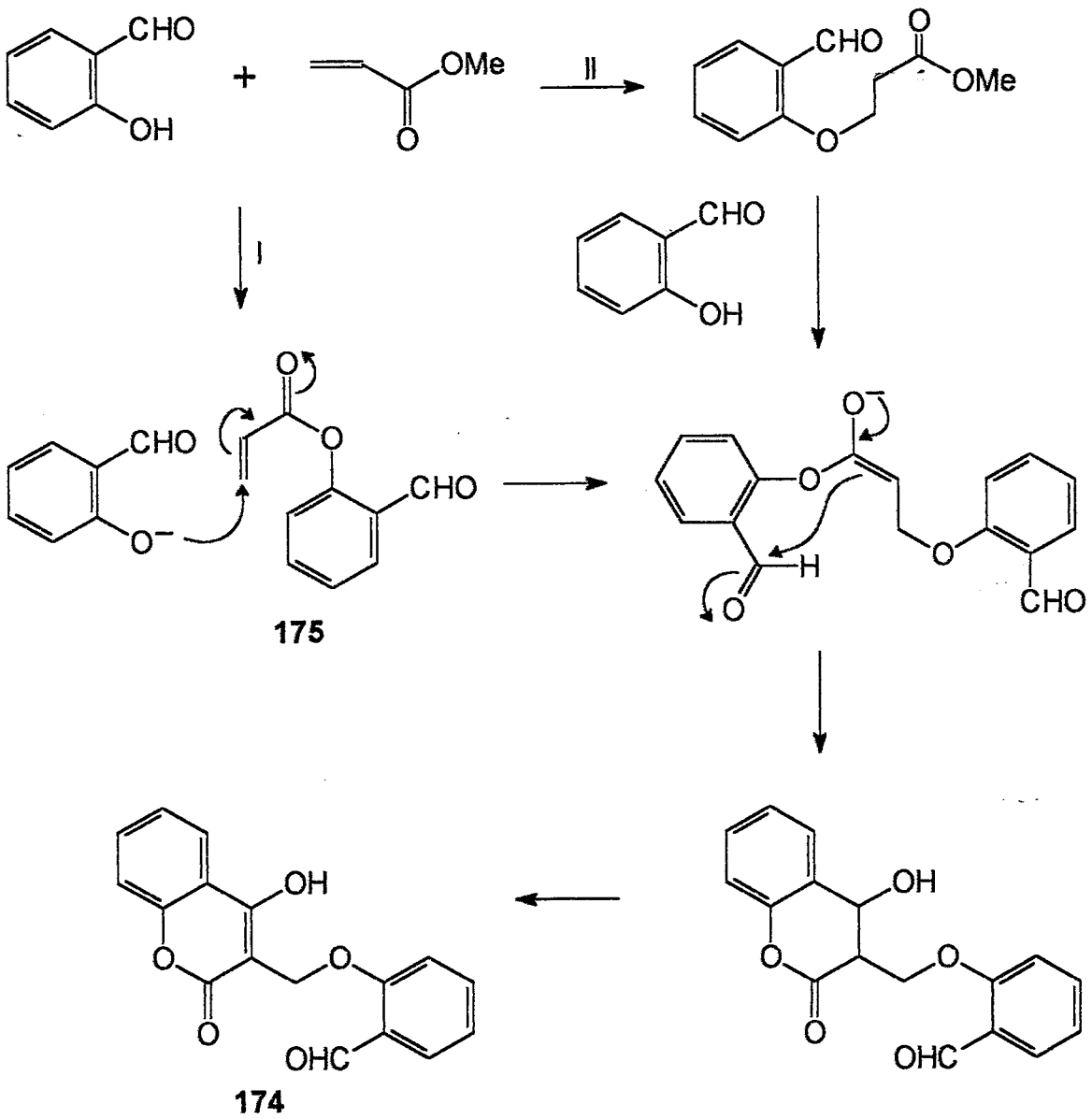
(b) Packing diagram of 3-[(2-formylphenoxy)methyl]coumarin **174** with the short H...O contacts indicated by dashed lines and the distances shown in Å.

Figure 26

2.1.3.2 A POSSIBLE MECHANISM FOR THE FORMATION OF 3-[(2-FORMYLPHENOXY)METHYL]COUMARIN 174

Reaction of salicylaldehyde with methyl acrylate does not occur in the absence of a catalyst. The reaction is catalyzed by both DABCO and 3-hydroxyquinuclidine, but not by the non-nucleophilic base, "proton sponge" [1,8-bis(dimethylamino)naphthalene]. On the basis of this information, a number of possible approaches which would account for the formation of **174** are outlined in Schemes 41 and 42. Compound **175** was prepared,[307] as a possible intermediate, and reacted with salicylaldehyde in the presence of DABCO but, even after one week, only starting material was present, thus excluding route I (Scheme 41) as a possible mechanism for the formation of the coumarin derivative **174**. Route II (Scheme 41) involves (i) conjugate addition; (ii) transesterification; and (iii) an aldol-type condensation while Scheme 42 outlines an alternative sequence involving (i) Baylis-Hillman hydroxyalkylation; (ii) intramolecular transesterification; (iii) dehydration; and (iv) nucleophilic substitution. This last-mentioned sequence is supported by the fact that the reaction is not catalyzed by a non-nucleophilic base.

This interesting transformation, which has been published,[308] is being further investigated in our research group.


CHO
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+
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175
O⁻
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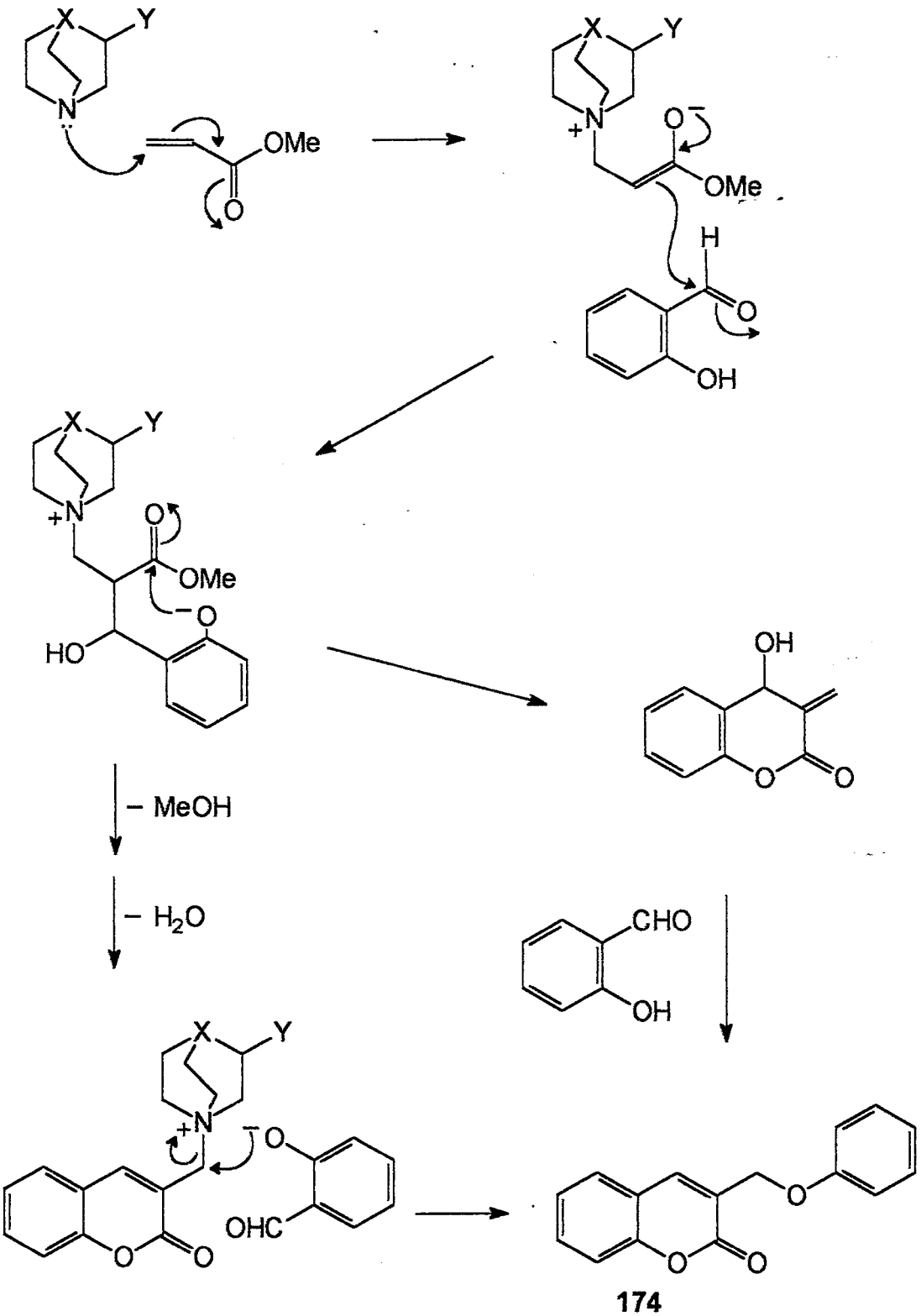
OHC
174

SCHEME 41



174

SCHEME 42

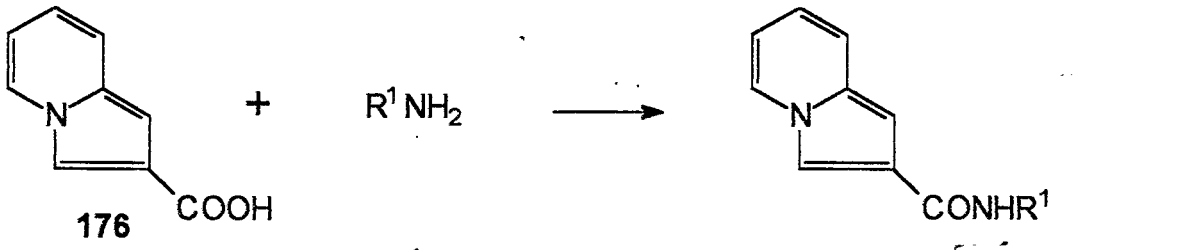
2.2 SYNTHESIS AND CONFORMATIONAL ANALYSIS OF INDOLIZINE-CARBOXAMIDES

2.2.1 PREPARATION OF INDOLIZINE-2-CARBOXAMIDES

The preparation of chloroquine analogues necessitated the introduction of amino side-chains to the 5-membered ring of the 2-substituted indolizines. The commercial availability of 3-diethylaminopropylamine **178** and 2-amino-5-diethylaminopentane **179** made preparation of the amides **180** and **181** (Scheme 43) the most convenient route for introduction of these amino side-chains. An alternative approach which we considered involving preparation of 2-chloromethylindolizine **183**, and subsequent reaction of this compound with amine **178** or **179**, was unsuccessful as a result of the failure of 2-hydroxymethylindolizine **182** (prepared by $LiAlH_4$ reduction of methyl indolizine-2-carboxylate[70]) to undergo reaction with $SOCl_2$ to give the desired chloro-product **183** (Scheme 44).

The most obvious route to the amides was by reaction of indolizine-2-carbonyl chloride with amines **178** and **179**. The first step required hydrolysis of methyl indolizine-2-carboxylate **135** to indolizine-2-carboxylic acid **176**, and this was achieved in 72% yield using 8M-H_2SO_4 and glacial acetic acid.[309] A better procedure, employing KOH in EtOH, was subsequently found,[81] which quantitatively converted methyl indolizine-2-carboxylate **135** to the corresponding acid **176**. This procedure was also used for the conversion of methyl pyrrolo[1,2-*a*]quinoline-2-carboxylate **163** to the corresponding acid **177** in 94% yield. Attempted conversion[310] of indolizine-2-carboxylic acid **176** to indolizine-2-carbonyl chloride failed - a result which is consistent with earlier attempts by De and Saha[69] at a similar conversion. This is in contrast with the ready formation of the 3-carbonyl chloride,[70] albeit using a different approach.

The only literature procedure available for the preparation of indolizine-carboxamides was that initially reported by De and Saha,[69] who heated methyl indolizine-2-carboxylate and the appropriate alkylamine in a sealed tube for 12-16h. Mahon *et al.*[72] and Hodgkiss *et*

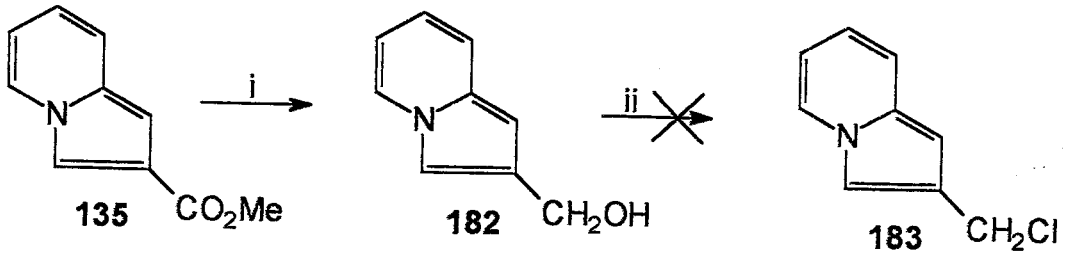


176

178 $R^1 = (CH_2)_3NEt_2$ **180** $R^1 = (CH_2)_3NEt_2$

179 $R^1 = CH(CH_3)(CH_2)_3NEt_2$ **181** $R^1 = CH(CH_3)(CH_2)_3NEt_2$

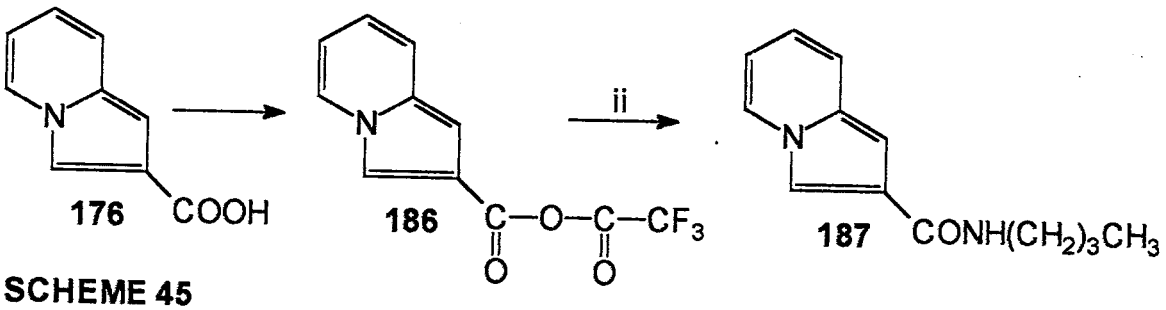
SCHEME 43



135 **182** **183**

SCHEME 44

Reagents : (i) $LiAlH_4$
(ii) $SOCl_2$



176 **186** **187**

SCHEME 45

Reagents : (i) $(CF_3CO)_2O$, THF
(ii) *n* - butylamine

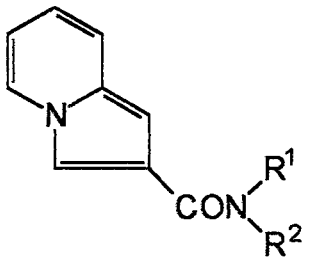
al.[71] reacted diethyl indolizine-1,2-dicarboxylate with ammonia, and with the aliphatic amine, 2-[(2-aminoethyl)amino]ethanol, following a slightly modified procedure of De and Saha,[69] and obtained the corresponding indolizine-2-carboxamides; the latter carboxamide in only 40% yield. De and Saha[69] did not report their yields, but a repetition of their synthesis using methyl indolizine-2-carboxylate **135** and ethylamine, in our hands yielded only 30% of 2-(*N*-ethylcarboxamido)indolizine **184** after heating for 16h at 100°C, and 37% after heating for 53h at 100°C (Table 9). Heating methyl indolizine-2-carboxylate **135** with 3-diethylaminopropylamine **178** and 2-amino-5-diethylaminopentane **179** for 13h at 100°C and for 25h at 150°C, yielded carboxamides **180** and **181** in 60% and 14% yield respectively (Table 9). Reaction of *tert*-butylamine with methyl indolizine-2-carboxylate **135** yielded only starting materials, even after heating for 53h at 100°C.

An attempt to prepare *N,N*-dimethylindolizine-2-carboxamide **185** by stirring methyl indolizine-2-carboxylate **135** with 33% dimethylamine in EtOH at room temperature for 3 days yielded only 2% of the desired product (Table 9). These reagents were then heated in an autoclave at 150°C at 15 bar for 8h, but the reaction was abandoned when TLC showed a large number of products to be present after heating. A similar procedure was employed for the synthesis of 2-carbamoylindolizine by Jones and Stanyer,[70] who reacted methyl indolizine-2-carboxylate and ammonia in methanol at 155°C for 65h under pressure, and obtained the product in 46% yield. It would thus appear that this procedure proceeds in low yield in the case of primary amines, but is entirely unsuccessful in the case of secondary amines.

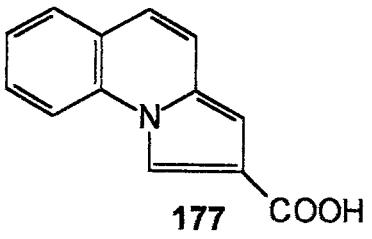
The length of time required for these reactions, and the low yields obtained, particularly in the case of branched amines (such as **179**), led us to attempt the development of a general procedure for the preparation of indolizine-carboxamides, as a practical route was obviously not available.

The first approach adopted was to react indolizine-2-carboxylic acid **176** with trifluoroacetic anhydride to yield the mixed anhydride **186** (Scheme 45), followed

TABLE 9 Indolizine-2-carboxamides prepared by less successful routes



Compound	R^1	R^2	Yield (%)
180	$(CH_2)_3NEt_2$	H	60
181	$CH(CH_3)(CH_2)_3NEt_2$	H	14
184	Et	H	37
185	Me	Me	2
187	*n*-Bu	H	7



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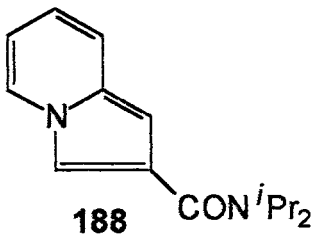

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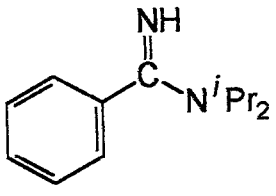

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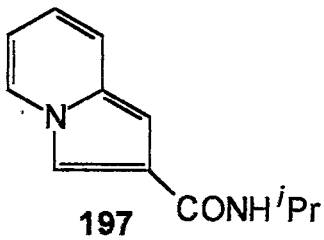


197

immediately by reaction with an amine to yield the desired product. The amine used was *n*-butylamine, the expected product being 2-(*N*-*n*-butylcarboxamido)indolizine **187** (Scheme 45). A number of different experimental procedures were employed (*e.g.* the reaction was performed:- at room temperature without solvent; at 0°C in THF; and with refluxing in THF) but the maximum yield of **187** obtained by this route was 7% (Table 9).

The second approach adopted was the use of a well known reagent for the formation of peptide bonds, *N,N'*-dicyclohexylcarbodiimide. Thus, indolizine-2-carboxylic acid **176** was reacted with *n*-butylamine in the presence of *N,N'*-dicyclohexylcarbodiimide at room temperature, as described by Sheehan and Hess,[311] but even after 24h only starting material was shown to be present (TLC).

A number of other approaches were then attempted for the preparation of 2-(*N,N*-diisopropylcarboxamido)indolizine **188**. Lithium diisopropylamide (LDA) was prepared from diisopropylamine and butyllithium, and reacted with methyl indolizine-2-carboxylate **135**. A number of products were found to be present, none of which was the desired amide **188**. Another possible approach to amide **188** was considered, *viz.*, reaction of indolizine-2-carbonitrile with LDA, followed by hydrolysis. This approach was tested using benzonitrile and lithium diisopropylamide. The product obtained was the imine **189**, which, unfortunately, proved to be resistant to acid hydrolysis; attempted hydrolysis using $NaNO_2/HCl$[312] yielded a large number of products (TLC) and was thus abandoned.

Another general approach was then explored. This involved reaction between indolizine-2-carboxylic acid **176** and an amine, in the presence of the coupling reagent, 1,1'-carbonyldiimidazole (CDI).[313,314] 3-Diethylaminopropylamine **178** was added to a complex of indolizine-2-carboxylic acid **176** and CDI, and reacted for 15min before quenching the reaction. This led to a 79% yield of compound **180**, after column chromatography. Reaction with the branched amine **179**, was also successful, yielding

45% of amide **181** after 15 min reaction time, and 71% yield after 2 days reaction at room temperature. The final test of this route came with the attempted reaction of indolizine-2-carboxylic acid **176** with secondary amines. To our knowledge, tertiary indolizine-2-carboxamides have not previously been prepared, and this route proved successful for the preparation of a number of these compounds.

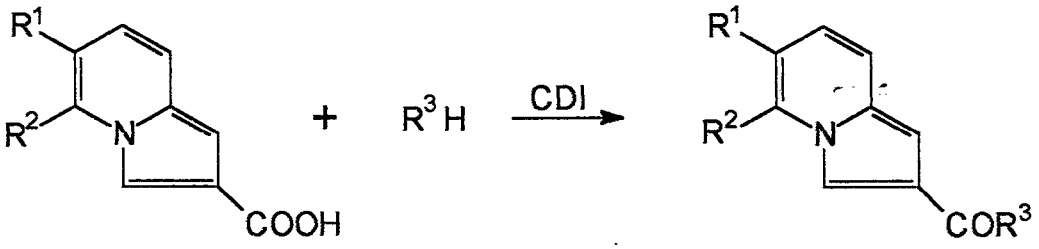
This route thus proved to be the general route we were looking for, and the amides synthesized using the CDI procedure are shown in Table 10. A number of symmetrically substituted *N,N*-dialkylamides were also prepared with a view to exploring N-CO rotational barriers in these compounds.

In the case of 2-(*N,N*-dimethylcarboxamido)indolizine **185**, dimethylamine hydrochloride was used in reaction with the acid, instead of the free amine. As shown by the preparation of compound **181**, increasing the reaction time leads to an increased yield. In the case of compounds **191** and **194**, reaction was carried out for only 15 min, prior to quenching, and it is likely that longer reaction times would lead to higher yields.

Attempted preparation of 2-(*N,N*-diisopropylcarboxamido)indolizine **188** by this route was not successful, however, presumably because the amine is highly sterically hindered. The only product obtained from this reaction was 4% of 2-(*N*-isopropylcarboxamido)indolizine **197**, possibly as a result of contamination of the diisopropylamine with traces of isopropylamine, although GLC analysis of the diisopropylamine showed no clear evidence of such contamination.

One of the factors which may affect intercalation of a ligand into DNA is its basicity. However, because of its pyrrole-type bridgehead nitrogen, indolizine is only very weakly basic and, in order to test the importance of basicity for intercalation, the imidazo[1,2-*a*]-pyridine-carboxamides **202** and **203** were required (Scheme 46). The second ring nitrogen in these compounds may be readily protonated, making these ring systems suitably basic. Ethyl imidazo[1,2-*a*]pyridine hydrobromide **200** was prepared[315] from ethyl bromopyruvate[316] **198** and 2-aminopyridine **199**, and hydrolyzed (following the same

TABLE 10 Indolizine-2-carboxamides prepared by the 1,1′-carbonyldiimidazole route



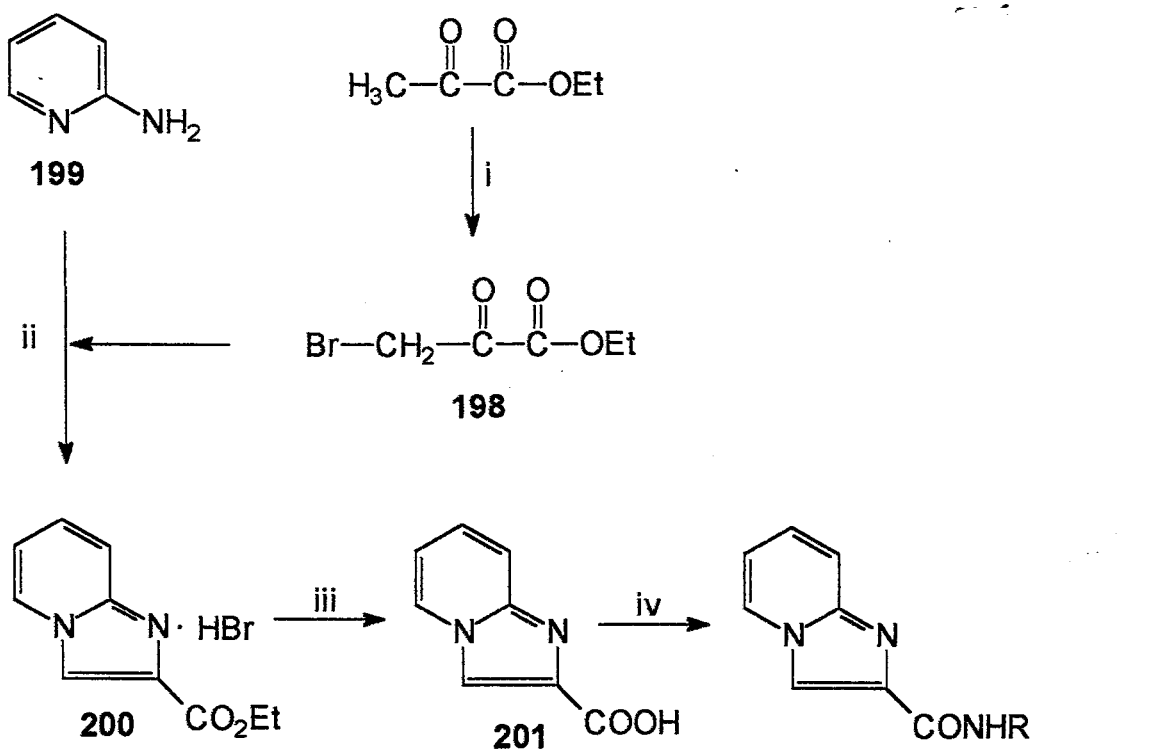
Compound	R^1	R^2	R^3	Yield (%)
180	H	H	$NH(CH_2)_3NEt_2$	79
181	H	H	$NHCH(CH_3)(CH_2)_3NEt_2$	71
185	H	H	NMe_2	87
187	H	H	$NH(CH_2)_3CH_3$	63
190	H	H	$NH(CH_2)_2CH_3$	72
191	H	H	NEt_2	45
192	H	H	$\overline{N(CH_2)_3CH_2}$	80
193	H	H	$\overline{N(CH_2)_4CH_2}$	70
194	$-(CH)_4-$		NEt_2	25
195	$-(CH)_4$		$NH(CH_2)_3NEt_2$	92
196	$-(CH_4)-$		$NHCH(CH_3)(CH_2)_3NEt_2$	57

procedure used for the indolizine esters) to give imidazo[1,2-*a*]pyridine-2-carboxylic acid **201**. Reaction of this carboxylic acid with amines **178** and **179**, in the presence of CDI, yielded the required products **202** and **203** in 58% and 54% yield respectively.

The carboxamides **180**, **181**, **195**, **196**, **202** and **203** were purified by flash chromatography on silica gel using CH_2Cl_2:MeOH:ammonia (20:4:1) as eluant, as they failed to move in the regular eluants, hexane and EtOAc. The chromatographed product, in each case, contained a small proportion of residual contaminant, shown by NMR spectroscopy (^1H and ^{13}C) to be imidazole, which results from the breakdown of CDI during reaction. Separation of imidazole from the desired products, using this purification procedure could not be achieved, although various eluant combinations were tried.

An alternative chromatographic purification procedure was attempted using Sephadex LH-20 gel, which is suitable for use with organic solvents. When the eluant is made up of a single component, in this case MeOH, separation is achieved by gel filtration. Solvent was pumped through the column using a peristaltic pump, and a successful separation of **180** from imidazole was achieved. A drawback of this procedure is the small quantities which can be separated at one time, and the length of time required for each separation. A more efficient alternative was found to be separation on a semi-preparative reverse-phase HPLC column, eluting with MeOH, and carboxamides **180**, **181**, **195**, **196**, **202** and **203** were successfully purified by HPLC.

Compounds **180**, **181**, **185**, **191-197**, **202** and **203** are all new compounds, which were fully characterized by spectroscopic (^1H and ^{13}C NMR and IR) and elemental analysis (high-resolution mass spectrometry). Representative ^1H, ^{13}C, HETCOR and COSY NMR spectra of indolizine-2-carboxamide **191**, pyrrolo[1,2-*a*]quinoline-2-carboxamide **194** and imidazo[1,2-*a*]pyridine **202** are included in Appendix 5.1.



SCHEME 46

Reagents : (i) Br_2
(ii) EtOH, heat
(iii) KOH, EtOH
(iv) CDI, RNH_2

2.2.2 CONFORMATIONAL ANALYSIS OF INDOLIZINE-2-CARBOXAMIDES

Conformational analysis involves a study of the energetics and the origin of conformational preference in various molecules; a conformer having a specific geometry which corresponds to an energy minimum. Conformational changes may include rotations about single bonds, bond angle deviations, bond length variations, and so on. Most rotations about single bonds are accompanied by very small energy fluctuations, and bond rotations are generally too rapid for the individual conformers to be detected.[317] Certain formal single bonds possess significant double bond character, and the energy of activation for rotation is intermediate between that for formal single and double bonds. An example of this is the N-CO bond of amides, and rotation about this bond is often sufficiently slow for individual conformers to be detected.[318] The double bond character of this formal single bond (**204**, Fig. 27) arises from the contribution of canonical form **205** to the ground state of amides.[319]

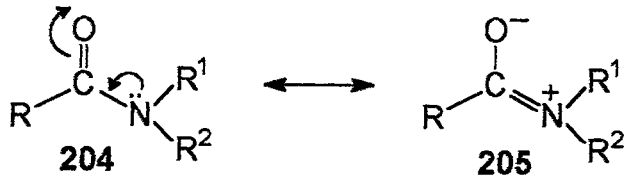


FIGURE 27

A number of techniques are available for the detection of conformers in dynamic equilibrium; these include IR, UV and NMR spectroscopy. There are two main factors which influence the choice of detection method: (a) the average life-time of the species must be much greater than the inverse frequency of the radiation it is absorbing, and (b) each conformer to be detected must possess different absorption characteristics and be present in high enough concentration to be detected. UV and IR spectroscopy can be

used to monitor species with very short lifetimes, 10^{-12} and 10^{-8}s respectively, but a major problem with these techniques is that often the different conformers possess very similar absorption characteristics. This is often not the case for NMR spectroscopy, where the major drawback is the relatively long conformational life-time (10^{5}s) required for detection.[317]

The majority of conformational studies are carried out by NMR spectroscopy, one of the earliest such studies being conducted on *N,N*-dimethylformamide.[319] The double bond nature of the amide N-CO bond has a number of consequences: (a) the nitrogen substituents are geometrically and chemically non-equivalent, even when $R^1=R^2$ (**203**, Fig. 27); (b) long-range spin coupling may occur between R and R^1 or R^2 (Fig. 27); (c) the amide occupies a stiff, approximately planar framework, which corresponds to an energy minimum; and (d) a large barrier to rotation about the amide bond exists,[319] usually between 50 and 100kJmol^{-1}.[317]

The large rotational barrier means that NMR spectra of symmetrically substituted *N,N*-dialkylamides often show separate resonances for the two identical alkyl groups at room temperature.[317] The anisotropy of the magnetic susceptibility of the amide group is the principal cause of this and, consequently, the protons nearest to the anisotropic group *i.e.* those α to the nitrogen, will usually be shifted most.[319] Shielding generally occurs in conical regions extending above and below the plane of the amide group, and deshielding occurs in the plane of the amide group.[319]

Another result of the rigidity of the planar amide framework is that it leads to the possibility of other slow rotations in amides. For example, in the *ortho*-substituted benzamides, rotation of the aryl-nitrogen bond is hindered as a result of steric hindrance.[319] Rotational barriers may be determined for each bond in systems where simultaneous rotation about more than one bond occurs, provided the site-exchange for each process is slow enough.[320]

A number of different methods for the analysis of NMR data to determine the heights of

rotational barriers have been used; these include line shape analysis, approximate methods, an equilibration method and the spin-echo method.[319] Full analyses of the spectra of interchanging conformers can be highly complex, and the approximate methods are valuable as they allow relatively simple equations to be applied to systems, provided they satisfy certain criteria. Thus, simplified equations can be applied in the case of symmetrically substituted *N,N*-dialkylamides, where conformational interchange is accompanied by the exchange of magnetic nuclei which are only slightly coupled, and where the exchange occurs between equally populated pairs of sites.[318] Allerhand *et al.*[321] discuss the possible errors associated with these approximations. These errors occur particularly when the simplified equations are applied to more complex systems. These authors also describe experimental errors, such as temperature fluctuations and magnetic field drifts, as a possible source of inaccuracy in the calculation of rotational barriers. With the advent of high resolution NMR and finely-controlled temperature units experimental error is now likely to play a smaller role.

In indolizine-2-carboxamides (Scheme 47, R=R*), simultaneous rotation about the N-CO and C(2)-CO bonds leads to two equivalent pairs of almost planar conformers (Ia≡IIa and Ib≡IIb, Scheme 47), corresponding to the conformational energy minima. In *N,N*-dialkylbenzamides, it was found[322] that steric interaction between the alkyl groups and the *ortho* hydrogen atoms led to a twisting of the alkyl groups slightly out of the N-CO plane, as well as a twisting of the aromatic system relative to the carboxamide plane. A very similar situation probably exists with the indolizine-2-carboxamides as molecular modelling (Fig. 28) of an indolizine-2-carboxamide and a benzamide shows that the distances between the alkyl groups of the carboxamide and the hydrogens at positions 1 and 3 in indolizine, and in the *ortho* positions in the benzamide, are very similar (when the aromatic ring and the carboxamide are planar), and thus similar steric interactions are likely to exist, resulting in similar conformations. Rotation about the C(2)-CO bond in indolizine-2-carboxamides is expected to be fast relative to the NMR time scale, as even in the sterically hindered *ortho*-substituted benzamides, C(1)-CO rotational barriers are less than 60kJmol^{-1}.[319] Rotation about the N-CO bond, however, should be

sufficiently inhibited by delocalization [as is the case with most amide N-CO bonds, (Fig. 29); *i.e.* k_a, $k_{a'}$ « k_b, $k_{b'}$ (Scheme 47)] to allow analysis using dynamic NMR methods.

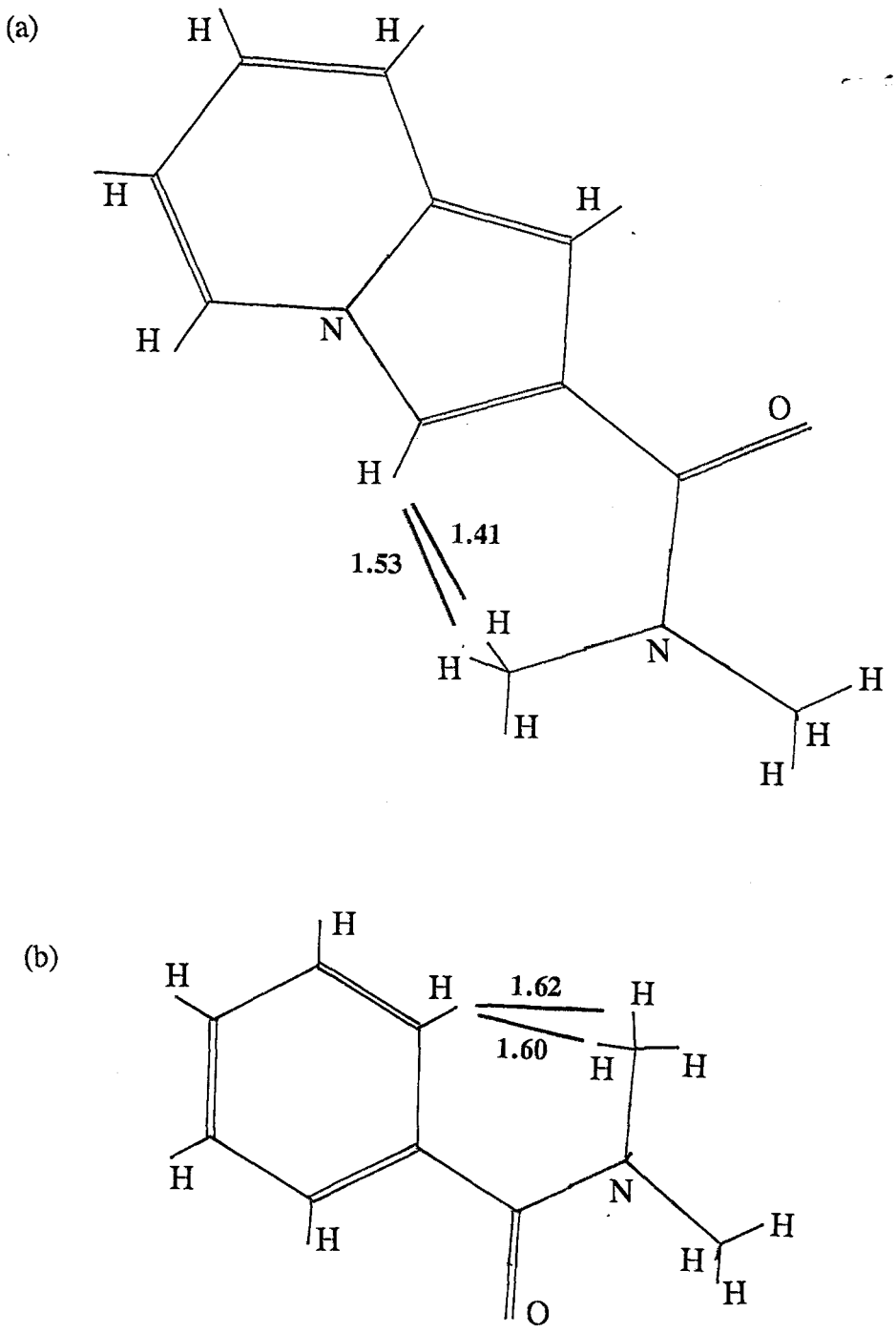
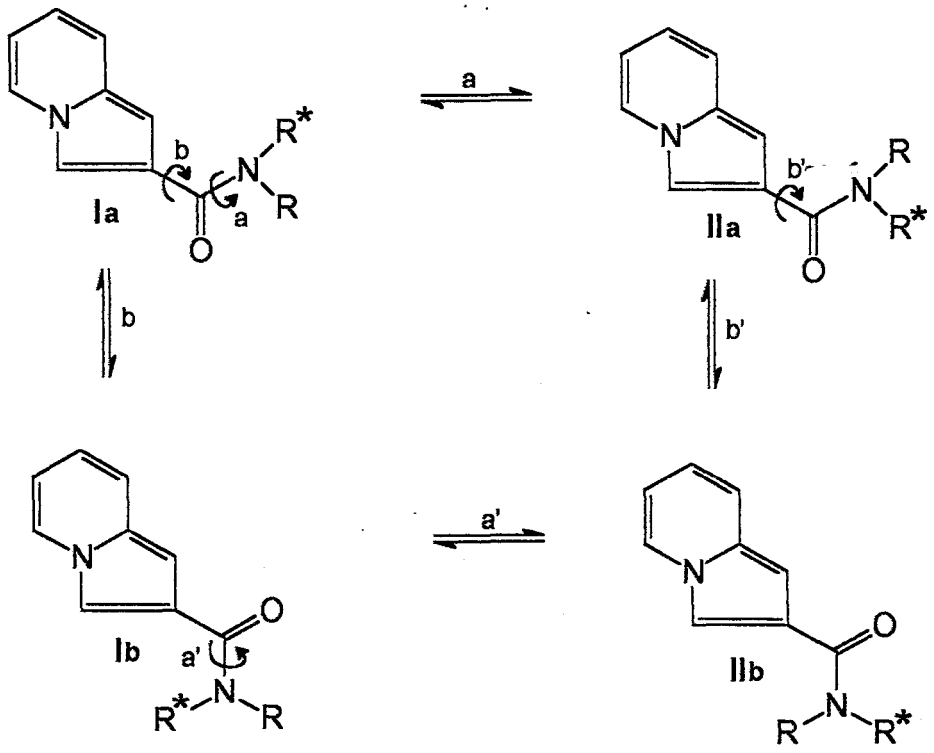


Figure 28 Molecular models of : (a) an indolizine-2-carboxamide; and (b) a benzamide, with distances between atoms shown in Å.



SCHEME 47

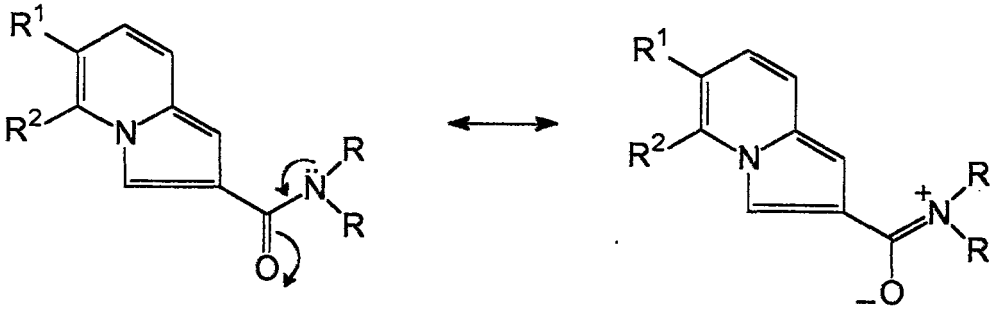


FIGURE 29

This was, in fact, found to be the case, and variable temperature ^{1}H and ^{13}C NMR techniques were employed to explore substituent effects on the N-CO rotational barriers of the symmetrically substituted indolizine-2-carboxamides **185** and **191-194**, prepared as described in Section 2.2.1. To our knowledge, no previous conformational studies on indolizine-carboxamides have been conducted, but certain preferred-conformation studies have been carried out on indolizine-3-aldehydes,[323] 3-acetylindolizines[36] and 1- and 3-thioformylindolizines.[46]

An important difference between indolizine-carboxamides and other carboxamides, such as the benzamides, is that rotation of the amide group in symmetrically substituted benzamides involves site-exchange between a pair of equivalent, almost planar conformers, while in symmetrically substituted indolizine-2-carboxamides, the situation is more complex in that rotamer types a and b (Scheme 47) are non-equivalent. The measured rates of site-exchange may thus be viewed as a combination of the individual rates, k_a[Ia] and $k_{a'}$[Ib].[327]

Thus, $k_{obs} = k_a[Ia] + k_{a'}[Ib]$

Because the indolizine-2-carboxamides studied satisfy the necessary criteria for use of the simplified equations, these were employed to calculate the rotational energy barriers. In a variable temperature experiment, three distinct spectral types are observed when slow site-exchange occurs : (i) above coalescence temperature, T_C, a single, often broad peak is observed, which sharpens with increasing temperature, (ii) at T_C, two peaks are just discernible, and (iii) below T_C two peaks are clearly evident, but broadened, and these peaks become sharper and the separation between them increases with decreasing temperature, until maximum separation is achieved.

The various approximate equations in use relate to characteristics such as peak separation and the width of coalesced lines. The equations employed in the present study to calculate the energy of activation (ΔG^*, equation 1) and the first-order rate constant (k, equation 2) were those used by Smith *et al.*[320] and Lai and Chen.[324]

$$\Delta G^* = RT_C (22.96 + \ln T_C / \Delta\nu_C) \quad (1)$$

$$\ln k = \ln (k_b T / h) - \Delta G^* / RT \quad (2)$$

where T_C = coalescence temperature

R = gas constant k_b = Boltzmann constant

h = Planck constant T = 298K

$\Delta\nu_C$ = frequency separation at coalescence

Thus, plots of frequency separation ($\Delta\nu$) *versus* temperature (T) (Fig. 30) were extrapolated to obtain the frequency separation at coalescence ($\Delta\nu_C$), in order to calculate ΔG^* from equation 1. A typical set of spectra obtained from the variable temperature experiments are shown in Fig. 31. Measurement of $\Delta\nu$ becomes inaccurate when approaching coalescence and, thus, only values measured when peak resolution was good, *i.e.* at lower temperatures, were included in the $\Delta\nu$ *vs* T plots. The results of the variable temperature experiments are shown in Table 11. The ^{1}H NMR signals for the CH_2 groups adjacent to the nitrogen in compound **193** were not well resolved and, consequently, the ^{13}C signals were followed in this case. Compound **194** was investigated using both the ^{1}H and ^{13}C NMR data, in order to compare the results obtained from the two different methods.

The experimentally determined rotational barriers (ΔG^*) fall between the values 58.8 and 67.4kJmol^{-1}, which is within the typical range for amide rotational barriers (50 - 100kJmol^{-1}).[317] The value of ΔG^* for 2-(*N,N*-dimethylcarboxamido)indolizine (entry 1) is similar to, but slightly lower than that reported for *N,N*-dimethylbenzamide,[325,326] and for the 2-substituted 4-oxo-4*H*-chromene analogue.[327] The lower rotational barrier observed for the indolizine-2-carboxamide could be attributed to greater competitive delocalization[319] in the case of the relatively electron-rich aromatic indolizine. This would decrease the double bond character of the N-CO bond relative to the less electron-rich benzene, and the electron-withdrawing chromene system. The same effect is observed with the *N,N*-dialkylamides, which have lower rotational barriers than the *N,N*-dialkylacetamides, as a result of competitive delocalization[319] (Fig. 32).

The lower rotational energy barrier observed for 2-(*N,N*-diethylcarboxamido)indolizine

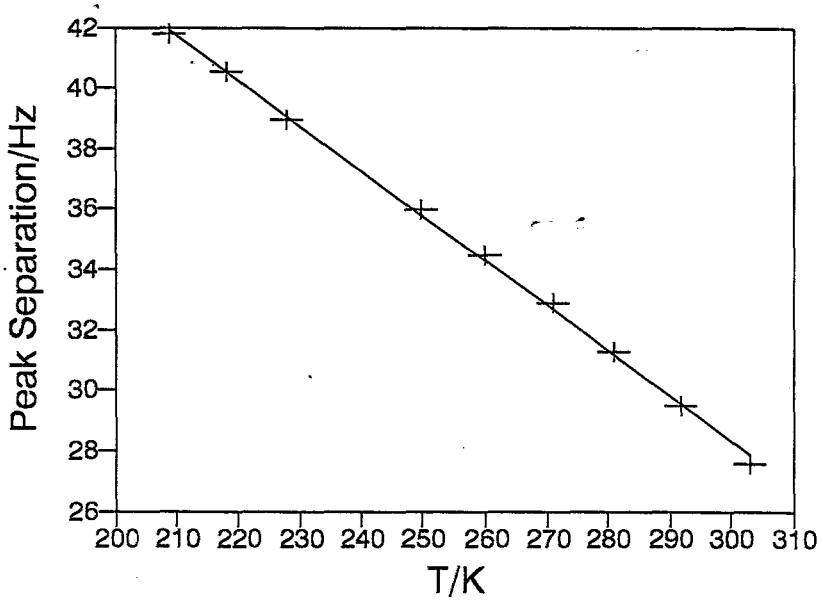


Figure 30 A plot of peak separation *versus* temperature for carboxamide **192**.

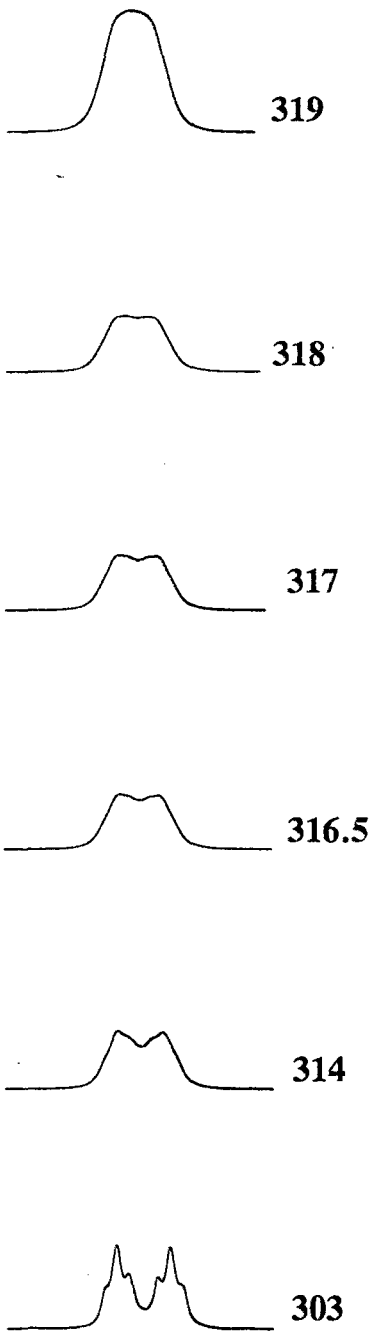
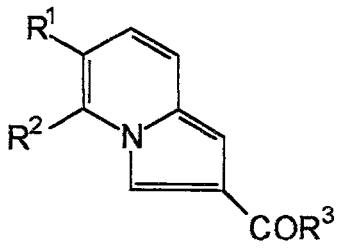


Figure 31 Variable temperature spectra obtained for carboxamide **192**, at the indicated temperatures (K).

TABLE 11 Data from dynamic NMR study of indolizine-2-carboxamides[a]



Compound	R^1	R^2	R^3	T_C[b]/K	$\Delta\nu_C$[c]/Hz	ΔG^{*}[d]/kJmol^{-1}	k_{298}[e]/s^{-1}
185	H	H	NMe_2	298	48.9 ± 0.6	61.4 ± 0.3	109
191	H	H	NEt_2	265	4.2 ± 0.3	59.7 ± 0.3	210
192	H	H	$N(CH_2)_3CH_2$	318	25.6 ± 0.4	67.4 ± 0.3	10
193[†]	H	H	$N(CH_2)_4CH_2$	319	538.2 ±0.9	59.5 ± 0.2	230
194	$-(CH)_4-$		NEt_2	260	3.3 ± 1.7	59.1 ± 1.8	272
194[†]	$-(CH)_4-$		NEt_2	308	302.7 ±1.1	58.8 ± 0.2	301

[a]Variable temperature 400MHz ^{1}H NMR spectra recorded in $CDCl_3$.

[b]Coalescence temperature (± 0.5K). [c]Frequency separation at coalescence.

[d]Free energy of activation for N-CO rotation; $\Delta G^{*} = RT_C\ (22.96 + \ln T_C/\Delta\nu_C)$.

[e]First-order rate constant at 298 K for N-CO rotation;

$\ln k = \ln (k_bT/h) - \Delta G^{*}/RT$.

Where : R = gas constant

k_b = Boltzmann constant

h = Planck constant

T = 298 K

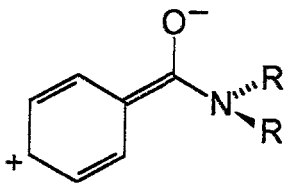


FIGURE 32

(entry 2), relative to the dimethyl derivative (entry 1) follows the same trend as that observed for the *N,N*-dialkylbenzamides[326] and the general trend that larger *N*-alkyl groups have smaller rotational barriers.[319]

Comparing entries 1 and 5 it may be seen that similar values were obtained for the diethyl derivatives of indolizine-2-carboxamide and pyrrolo[1,2-*a*]quinoline-2-carboxamide. This is not unexpected as the second six-membered ring is not likely to affect delocalization in the carboxamide moiety. Entries 5 and 6 show that the values of the rotational energy barrier for compound **194**, calculated from ^{13}C and ^{1}H NMR data, agree within the quoted error margins. Thus, ^{1}H and ^{13}C methods appear to be comparable in terms of accuracy. The highest rotational energy barrier was observed for the pyrrolidine derivative (entry 3), which was found to be significantly higher than that for the piperidine derivative (entry 4). This is in accordance with results obtained for simple pyrrolidine and piperidine compounds, where the five-membered ring systems consistently have rotational barriers which are 8-12kJmol^{-1} higher than for the six-membered rings.[319] In heterocyclic amides such as **192** and **193**, additional conformational equilibria may be established through nitrogen inversion and ring reversal. The ring inversion barriers are expected to be slightly lower than the amide rotational barrier, while the nitrogen inversion barriers are expected to be very much lower.[322] The pyrrolidine group in compound **192** is expected to adopt an almost planar arrangement, with the whole group being approximately co-planar with the CO plane. Thus, the nitrogen atom readily adopts an sp^2-type arrangement, which permits effective lone-pair delocalization and this causes the higher rotational barrier observed for this compound. The piperidine group in compound **193** is expected to exist largely in a chair conformation, and a trigonal arrangement of the nitrogen is less favoured than in the pyrrolidine derivative, resulting in a significantly lower rotational barrier.

2.3 INTERCALATION STUDIES

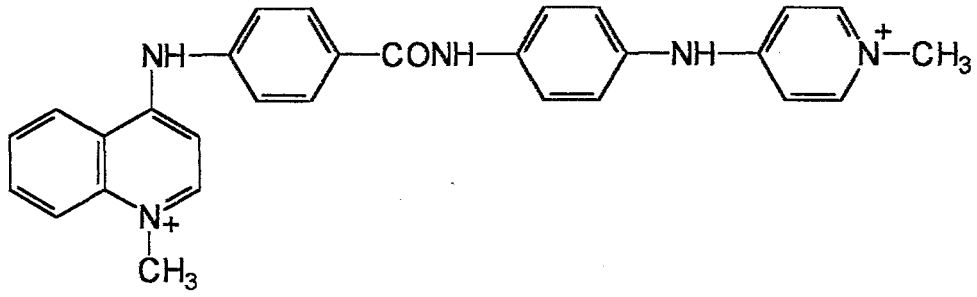
2.3.1 STUDIES OF INTERCALATION WITH NATURAL DNA

As discussed in Section 1.2.3, a large number of methods have been used to study ligand-DNA interactions. One of the earliest methods used was spectrophotometry,[161] where the UV absorption characteristics of a ligand were shown to change on binding to DNA. The most recent technique employed for these studies, which is now commonly used, is NMR spectroscopy and both of these techniques have been used in the present study to probe the interaction of chloroquine **126** and various indolizine-2-carboxamides with natural DNA.

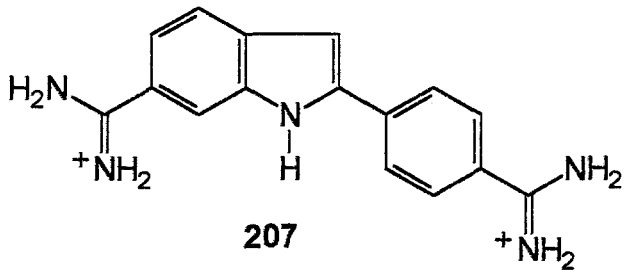
2.3.1.1 SPECTROPHOTOMETRIC STUDIES

The absorption spectrum of a ligand which intercalates into DNA is altered[228] as a result of strong interaction between the electronic states of the ligand and the DNA bases.[227] This interaction causes a decrease in the maximum absorption (hypochromism) of the ligand, and a shift of the bound ligand spectrum, usually to longer wavelengths.[228] In order for significant electronic interactions to occur, the ligand and DNA bases must be in very close proximity and, thus, observation of strong hypochromism is considered very strong evidence for intercalation.[219,227] However, Braithewaite and Baguley[328] observed changes in the absorption spectrum of a quinoline derivative **206** on addition of DNA, but did not classify this compound as an intercalator because it caused no viscosity changes to the DNA. The DNA binding of certain ligands is extremely complex since they may exhibit both groove-binding in AT-rich regions of DNA and act as intercalators in GC-rich regions. For these types of compounds (*e.g.* **207**) a clear distinction between modes of binding is not possible, and the experimental evidence will reflect both modes of binding.[329] This could perhaps also be the case for the quinoline derivative **206** studied by Braithewaite and Baguley,[328] as this compound possesses certain structural similarities to compound **207**, notably the presence of a two-ring planar aromatic system connected

to a one-ring system. As a result of such complexities, Long and Barton[160] suggested that the proposal of an intercalative interaction should not be concluded on the basis of any one of the possible criteria alone. However, no intercalator which does not cause spectral changes has ever, to our knowledge, been reported and, thus, any ligand which displays no spectral changes on addition of DNA may be considered to be non-intercalating.



206



207

In the present study, the UV absorption spectra of chloroquine **126** and the indolizine-2-carboxamides **180**, **181**, **195**, **196**, **202** and **203** in buffer (pH 7.0) were recorded and, in each case, increasing amounts of calf thymus DNA were added to the cuvette containing a fixed amount of ligand, with corrections being made for the increasing volume. The absorption spectra of carboxamides **180**, **181**, **202** and **203** showed no change on addition of DNA, and thus it was concluded that these compounds are not DNA intercalators. The absorption spectra of carboxamides **195** and **196** and chloroquine **126**, however, showed the hypochromism and shift to longer wavelengths characteristic of most intercalators. This was taken as strong evidence for the classification of

compounds **195** and **196** as intercalators, an assumption which was confirmed in complementary studies (Section 2.3.1.2).

The binding of chloroquine **126** to DNA has previously been monitored by absorption spectrophotometry;[191,194,330-332] some of these studies being used simply as qualitative indicators of binding to DNA, and some being used as quantitative measures of binding constants. There are many pitfalls associated with the calculation of binding constants, as discussed in Section 1.2.1.3, and care must be taken not to draw too many conclusions from simple binding studies. Comparison of binding data obtained at different salt concentrations or pH's can not accurately be compared and, thus, in this study the binding of chloroquine **126** to DNA using absorption spectrophotometry was reinvestigated to permit comparison with carboxamides **195** and **196**. The binding of chloroquine to DNA has been shown to be highly salt-dependent, with a 100-fold variation in affinity at different salt concentrations[188,189] and thus only comparisons made using identical experimental conditions are valid. Chloroquine **126** has previously been shown to obey Beer's Law at 343nm over the appropriate concentration range required for this study,[330] and carboxamides **195** and **196** were both shown to obey Beer's Law at 336nm over the required concentration range. For each of the compounds, the wavelength at which absorbance changes were measured fell outside of the absorbance wavelengths of natural DNA. Figures 33, 34 and 35 show the result of adding increasing amounts of calf thymus DNA to carboxamides **195** and **196**, and chloroqine, respectively. The progressive decrease in the absorption maxima and small shift to higher wavelengths are clearly visible; the decrease in absorption continues until a limit is reached, which represents the spectrum of completely bound ligand. The addition of further DNA beyond this point causes no further spectral changes to occur. As can be seen from the figures, all the spectra in a given series pass through a common point, called an isosbestic point. This occurs most commonly when there are only two forms of the ligand, in this case free and bound. However, the existence of an isosbestic point can not be considered proof that only two forms are present. If two different forms of bound ligand are present, having different spectra but always being present in the same ratio, an isosbestic point will still be observed.[228]

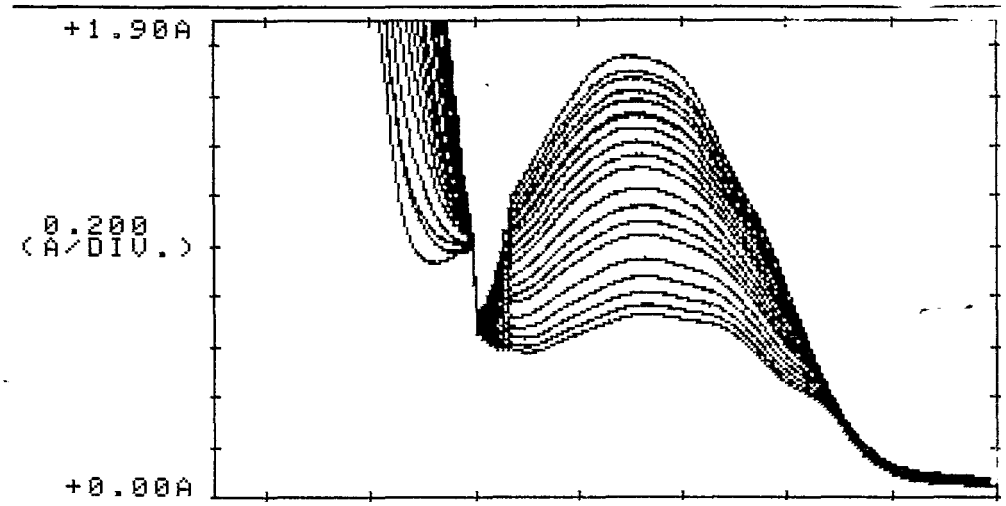


Figure 33 Changes in the UV spectrum of carboxamide **195** on addition of DNA.

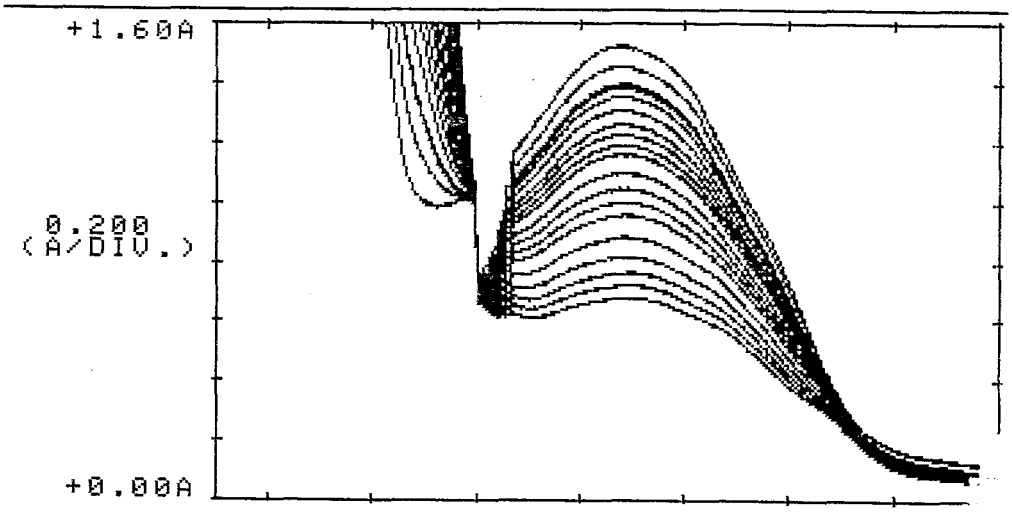


Figure 34 Changes in the UV spectrum of carboxamide **196** on addition of DNA.

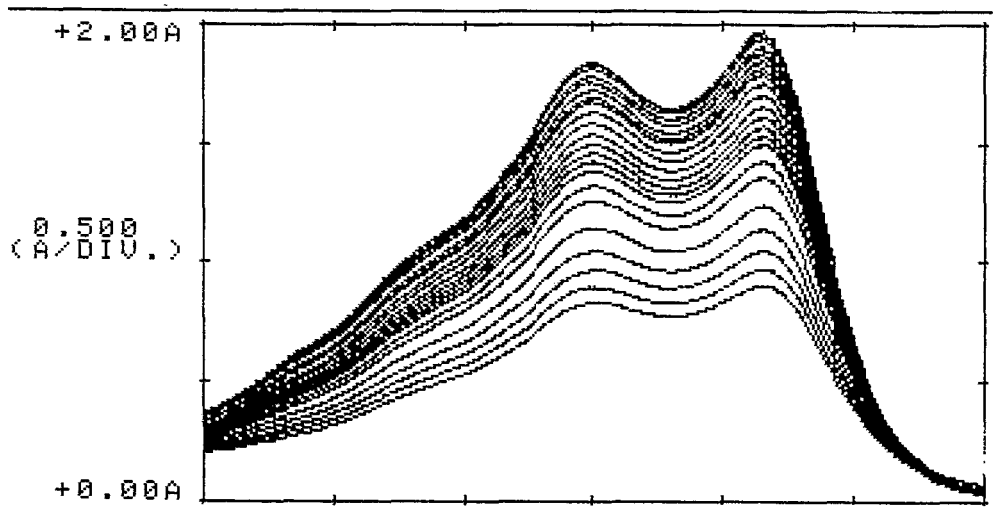


Figure 35 Changes in the UV spectrum of chloroquine **126** on addition of DNA.

In the case of chloroquine **126**, the absorption maximum followed at 343nm is the highest maximum of the spectrum, and a relatively low concentration of chloroquine (6.3 x 10^{-6}M) gave an absorbance reading which fell into a suitable range for measurement. In the case of carboxamides **195** and **196**, the secondary absorption maxima at 336nm were followed since the highest maxima fall into the same absorbance range as DNA, and are thus not suitable for monitoring. Significantly higher concentrations of ligand (3.0 x 10^{-4}M) were thus required to ensure absorbance changes suitable for measurement.

In order to estimate the binding constant for each ligand, the fraction of total ligand bound, α (equation 1) was plotted against DNA concentration, expressed as base pairs per litre. The concentration of DNA was determined spectrophotometrically, with ξ_{260} = 6600M^{-1} nucleotide.[330,333]

$$\alpha = D_1 - D \,/\, D_1 - D_2 = \Delta A \,/\, \Delta A_{max} \qquad (1)$$

where D_1 = absorbance of free ligand

D_2 = absorbance of bound ligand

D = observed absorbance of the mixture

These experiments were conducted in duplicate, and the results are shown in Figures 36, 37 and 38 for carboxamides **195** and **196**, and chloroquine **126**, respectively.

Binding results are often presented in the form of Scatchard[162] plots,[330,333-335] which are plots of bound ligand per base pair / free ligand *versus* bound ligand per base pair.[333] From our experimental results, chloroquine **126** gave a curved Scatchard plot (Fig. 39), as previously observed by other workers.[330] This is indicative of:- more than one class of binding site; ligand-ligand interaction; or the ligand covering more than one binding-site.[334] Cohen and Yielding[330] ascribed the curved Scatchard plot to heterogeneity of binding sites since, in a similar study, they obtained linear Scatchard plots when using apurinic or apyrimidic nucleic acids. The calculation of binding data from curved Scatchard plots is open to significant error, as extrapolation does not lead to a unique solution, but represents only one set of possible values which fit the experimental data.[330] Data from the carboxamides **195** and **196** could not be converted into meaningful

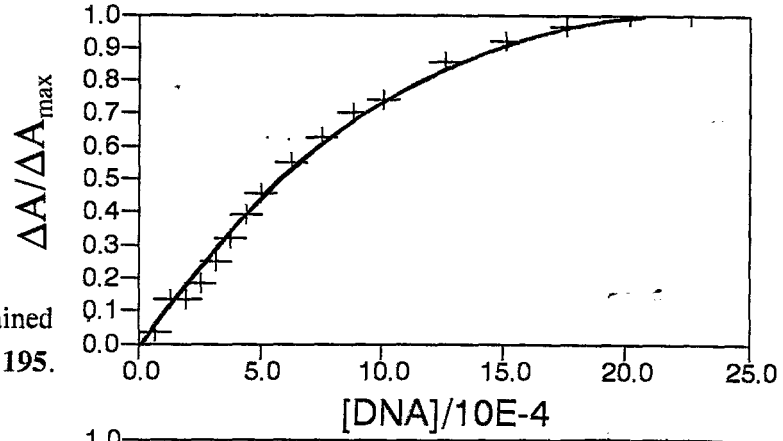


Figure 36 Binding curve obtained for carboxamide **195**.

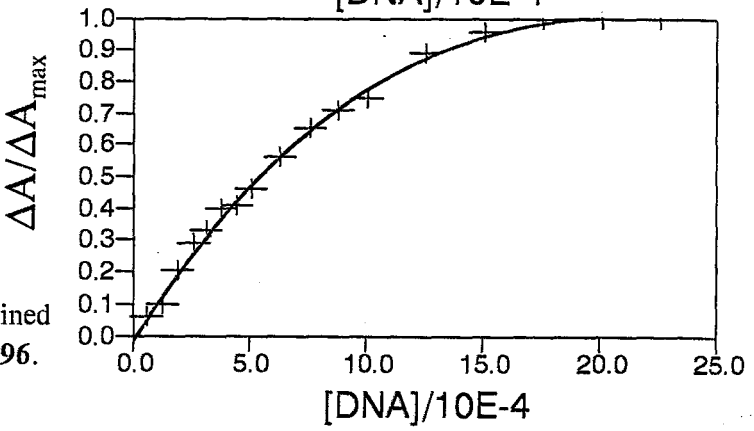


Figure 37 Binding curve obtained for carboxamide **196**.

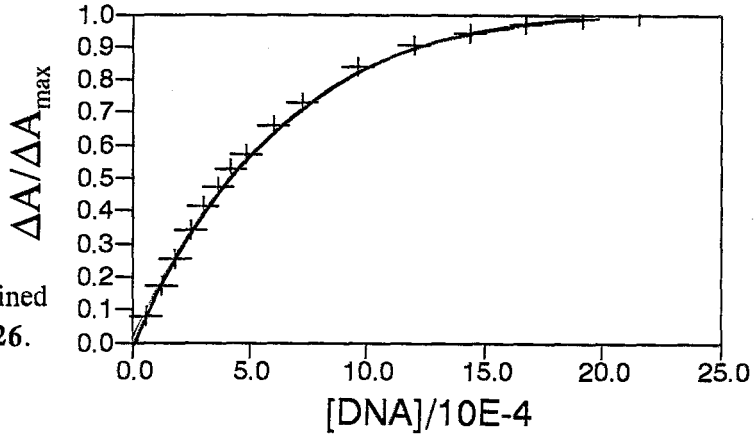


Figure 38 Binding curve obtained for chloroquine **126**.

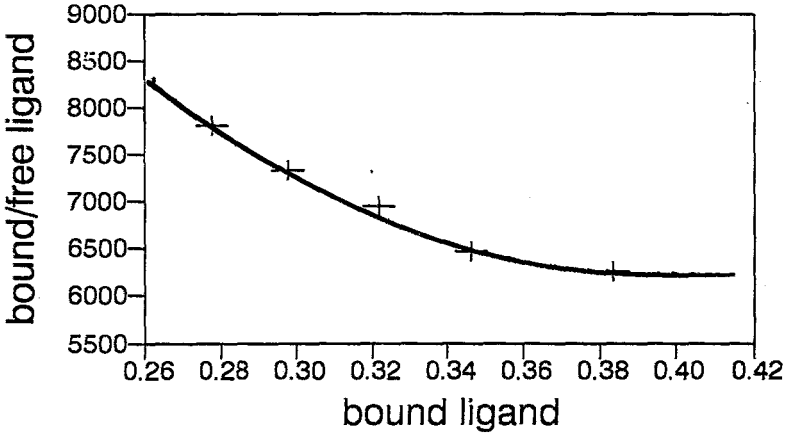


Figure 39 Scatchard plot for chloroquine **126**.

Scatchard plots, possibly as a result of one of the reasons mentioned above, or as a result of the fact that absorbance was measured at a secondary maximum, which is significantly smaller than the absolute maximum. The correspondingly small absorbance changes on addition of DNA, compared with those for chloroquine, could lead to large errors on conversion of the carboxamide data to Scatchard plots.

Approximate binding constants were thus obtained by reading, from the plots shown in Figures 36, 37 and 38, the DNA concentration when 50% of the ligand was bound (see equations 2 and 3).

Thus, K_{int} = [complex] / [DNA - complex][free ligand] (2)

When 50% of the ligand is bound,

[complex] = [free ligand]

So, K_{int} = 1 / [DNA - 50% ligand] (3)

The results obtained for the three ligands studied are shown in Table 12, and this shows that the binding affinities of all three compounds are very similar, with that of compound **195** being slightly smaller than for chloroquine **126** and compound **196** due to the increased length of the amino side-chain in the two last-mentioned compounds.

TABLE 12 DNA binding affinities of chloroquine and compounds **195** and **196**

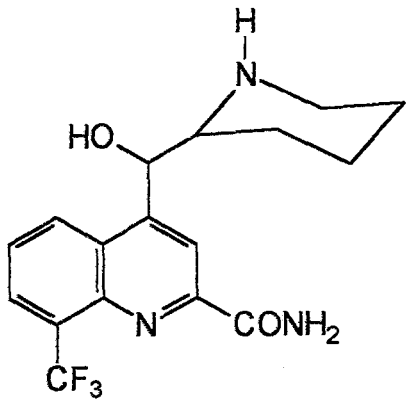
Compound	K_{int}
195	$(2.2 \pm 0.2) \times 10^3$
196	$(2.9 \pm 0.3) \times 10^3$
chloroquine	$(2.7 \pm 0.1) \times 10^3$

O'Brien and Hahn[206] showed that optimal binding of chloroquine analogues to DNA should occur when the distance between the amino groups of the side-chain spans that between two DNA phosphates across the minor groove, a distance of approximately 10.5Å. When one takes into account the ionic radii of the nitrogens in the side-chain and the phosphates of DNA, the ideal distance between the side-chain nitrogens should be approximately 7.5Å *i.e.* 4 atoms. O'Brien and Hahn[206] showed a correlation between the number of atoms between the side-chain nitrogens and antimalarial activity in a group of chloroquine analogues, with the greatest antimalarial activity, and by inference the greatest binding, occurring when there were 4 atoms between the side-chain nitrogens, which is the case with compound **196** and chloroquine.

The fact that the binding constant obtained for chloroquine was similar to that for compounds **195** and **196** is somewhat unexpected in view of the fact that the planar area of the three-ring systems is significantly larger than that of the two-ring chloroquine, thus allowing for a greater area of interaction between ligand and DNA base pairs, which would be expected to lead to a higher binding affinity.[238] Allison *et al.*[194] accounted for the greater binding affinity of acridines over chloroquine in this way, by comparing the approximately 40Å^2 planar area of an acridine ring with the approximately 28Å^2 planar area of a quinoline ring, proposing that the former compound could interact more effectively with the 50Å^2 planar area of a DNA base pair, thus making intercalation more favourable.

The smaller area of the two-ring indolizine and imidazo[1,2-*a*]pyridine systems could, of course, account for the failure of indolizine-2-carboxamides **180** and **181**, and imidazo[1,2-*a*]pyridine-2-carboxamides **202** and **203** to undergo intercalation. For certain quinoline-carboxamides, such as compound **208**, space-filling models show that the carboxamide group can be rotated into the plane of the quinoline ring forming a planar system which is capable of intercalation.[153] The two-ring carboxamides used in the present study also perhaps lack other substituents on the ring, which could encourage intercalation. In the case of chloroquine, intercalation may depend on protonation of the nitrogen and the presence of the chlorine substituent, which is thought to interact

favourably with the 2-amino group of guanine, thus causing intercalation to be energetically favourable.[194,206] The presence of the proton is thought to aid hydrogen-bonding with DNA, further favouring intercalation.[231] The presence of the proton alone, however, is not enough to cause intercalation, as evidenced by the failure of imidazo[1,2-*a*]pyridines **202** and **203**, which are expected to be protonated at pH 7, to undergo intercalation.



208

2.3.1.2 NMR STUDIES

The advent of high-resolution NMR instruments has enabled the complex interaction between ligands and DNA to be monitored using this technique, the advantage of which is that signals from both the ligand and the DNA can be monitored. NMR experiments with natural DNA samples initially proved to be difficult, because of the extremely broad peaks obtained. This broadening is due to the dipolar interactions between protons in the slowly tumbling high molecular weight polymer.[336] Development of sonication and enzymatic digestion techniques, which reduce the size of the DNA polymer to a few hundred base pairs in length, have made meaningful studies on natural DNA possible, as this reduces the line-widths by allowing the molecules to tumble rapidly enough to average out the dipolar interactions.[337]

There are a number of different NMR experiments which can be carried out on natural DNA, or on natural DNA-ligand complexes, and these include:- monitoring of the

exchangeable imino protons (Fig. 40) which resonate between 10 and 15ppm downfield of sodium 3-(trimethylsilyl)propanesulphonate-d_6;[337-341] following the ligand proton spectrum on addition of increasing amounts of natural DNA;[329,342-344] monitoring the ^{31}P spectrum of natural DNA;[345-352] using ^{23}Na spin-lattice relaxation rate measurements;[353,354] following ^{13}C NMR signals of natural DNA fragments;[355,356] and using solid-state ^{1}H and ^{13}C NMR spectroscopy.[357]

In the present study, calf thymus DNA was sonicated in a high salt buffer [(0.01M PIPES,‡‡ 1mM EDTA, 0.5M NaCl, pH 7.0) to maintain the DNA in a double-stranded state] until polyacrylamide gel electrophoresis (Fig. 41) showed the average length of the double-stranded DNA fragments to be approximately 350 base pairs. After phenol extraction, precipitation and centrifugation, the DNA was resuspended in buffer (0.1M NaCl, 0.01M $MgCl_2$, 0.01M Na cacodylate, pH 7.0). This buffer was chosen as it is believed to disfavour non-specific outside stacking (electrostatic interactions) relative to the intercalative binding mode.[339]

Monitoring the chemical shifts of aromatic protons of a ligand, on addition of sonicated DNA, is a sensitive and direct method to distinguish intercalation from other modes of binding.[329] The concentration of ligand used in these experiments is generally low, in order to minimize ligand dimerization. Many planar aromatic compounds have the tendency to dimerize in solution, and the extent of dimerization is unique to each ligand. Both binding of DNA to ligands and ligand dimerization cause upfield shifts in these NMR experiments. On addition of DNA to dimerized intercalator, two competing effects are observed on the chemical shift of the aromatic protons. Intercalation of monomers causes an upfield shift to occur, while dissociation of the dimers, which occurs as the monomer concentration is reduced, causes downfield shifts of the same protons. The observed shifts for both phenomena are small, and experimental error is high when dimerization occurs.[342]

‡‡PIPES is 1,4-piperazinebis(ethanesulphonic acid)

Dimerization occurs more readily at lower temperatures, and a method to test for the extent of dimerization is to compare the spectrum of ligand obtained at lower temperatures and that obtained at higher temperatures. If the chemical shifts of the ligand protons do not alter significantly from one spectrum to the next, then significant dimerization is not considered to be occurring at the lower temperature. Thus, spectra of 5mM solutions of each of the ligands studied by this method (chloroquine **126** and the carboxamides **195** and **196**) were run at 303K and at 333K, and compared to ensure that significant shifts were not occurring. The ligands all showed insignificant shifts, and it was thus concluded that dimerization was not occurring to any significant extent at 303K, and the experiments were conducted on 5mM solutions at 303K.

The ^{1}H NMR spectra of the carboxamides **195** and **196** and chloroquine **126** were monitored as increasing amounts of sonicated calf thymus DNA were added. Representative spectra of each set are shown in Figures 42, 43 and 44, with the ligand-DNA base pair ratio shown next to each spectrum. The full set of spectra obtained for each compound examined, can be found in Appendix 5.3. It is evident, in each case, that significant upfield shifts of the aromatic proton signals occurred, together with broadening of these signals. Both of these phenomena are characteristic of intercalative binding, and do not occur with any other mode of binding.[342] Thus, this confirms that the carboxamides **195** and **196** bind to DNA by intercalative means. The upfield shifts of the aromatic protons are due to the ring current effects of the DNA base pairs, and depend on their overlap with the intercalating ring system. The observed broadening of the signals is the result of severely restricted mobility of the intercalated ligands.[342] The ^{1}H NMR signals of the side chain nuclei of the 3 ligands studied in this way, showed no shifts on addition of DNA, a result which confirms that the side-chains are located outside of the helix and are thus not subject to ring current effects.

For each compound, the shifts of all the aromatic protons were comparable, with no one signal showing a significantly larger shift than any other. The average maximum shift observed for the aromatic protons of chloroquine which, to our knowledge, has never been studied by this means before, was 0.18ppm. Each of the two carboxamides **195** and **196** showed maximum shifts of approximately 0.28ppm. The broadening observed in the

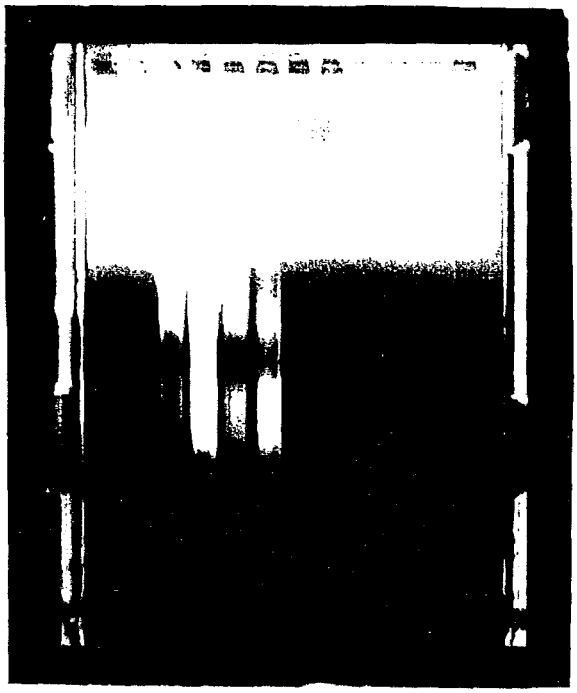


Figure 41 Polyacrylamide gel electrophoresis showing from left to right : reference markers, and calf thymus DNA after 15min, 30min, 45min and 1h sonication time.

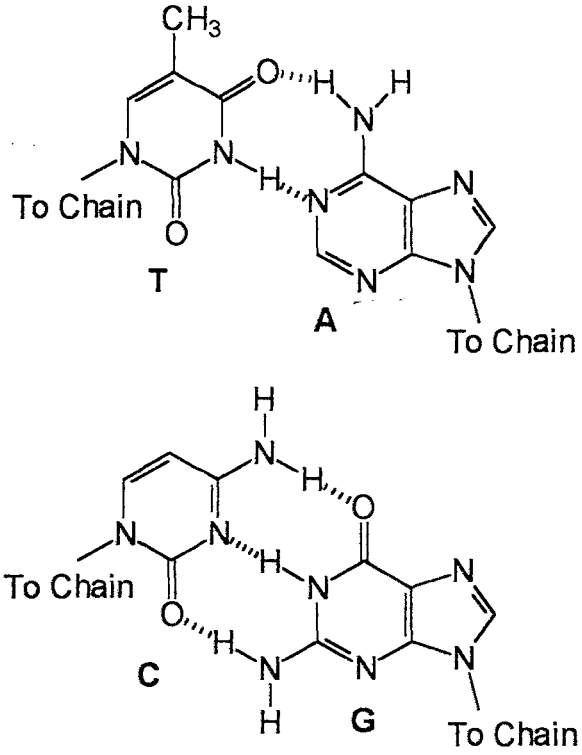


Figure 40 DNA base pairs showing the imino protons, which are involved in hydrogen-bonding.

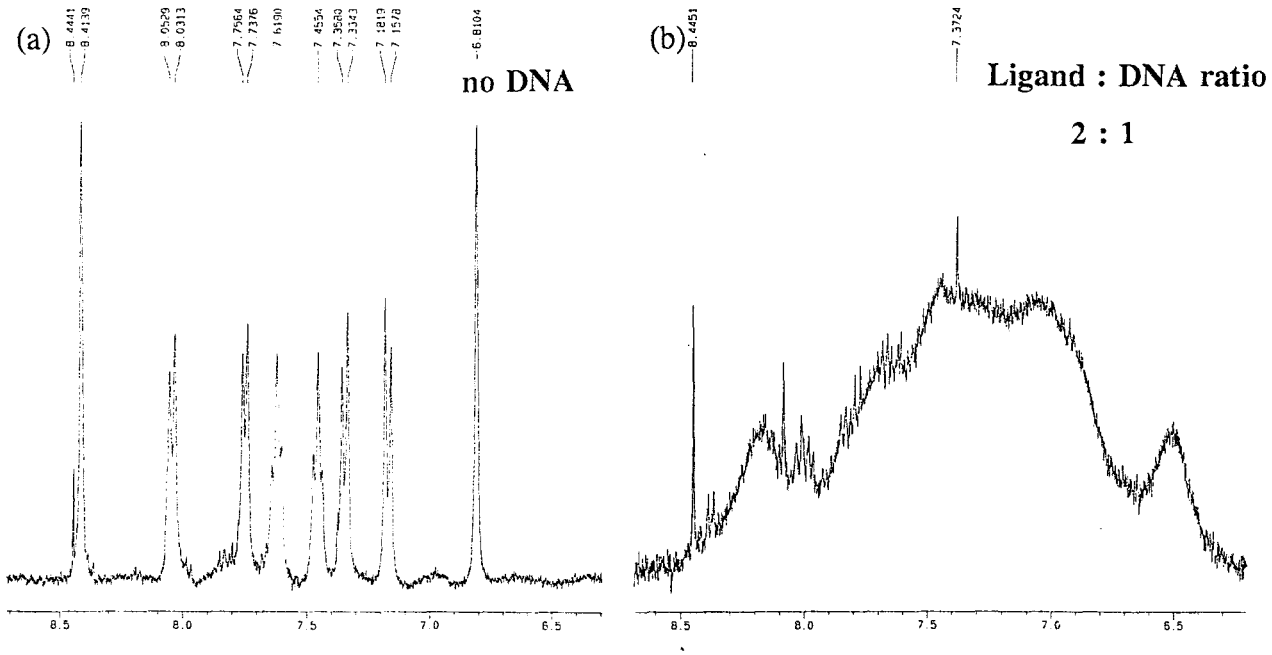


Figure 42 ^{1}H NMR spectra of carboxamide **195** : (a) before addition of DNA; and (b) after addition of DNA.

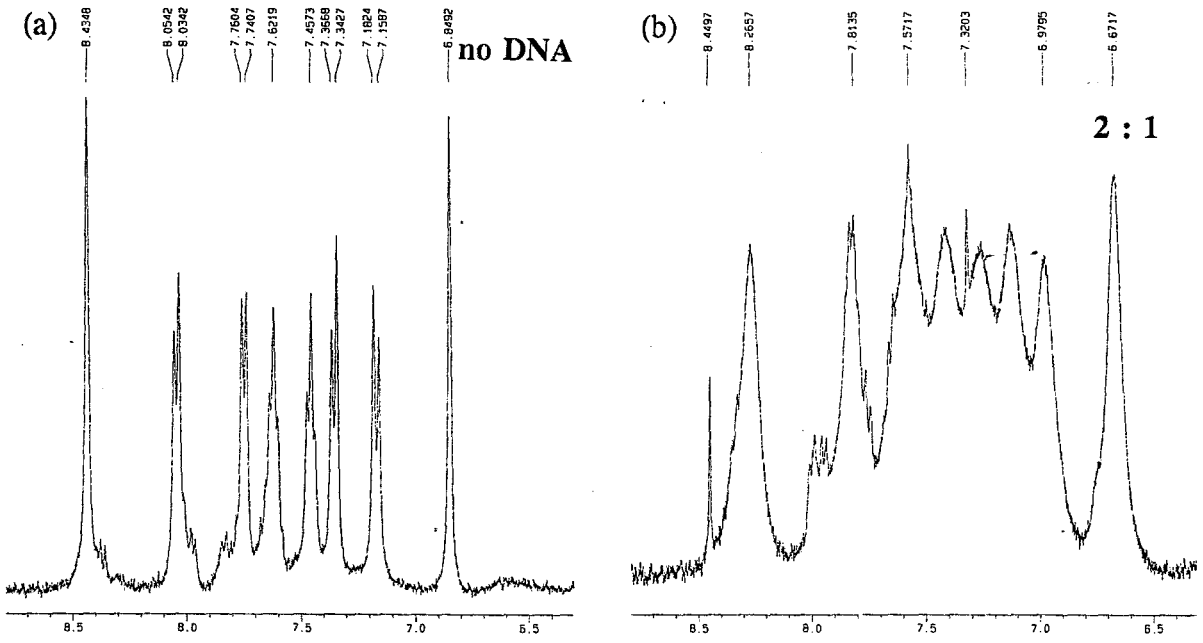


Figure 43 ^{1}H NMR spectra of carboxamide **196** : (a) before addition of DNA; and (b) after addition of DNA.

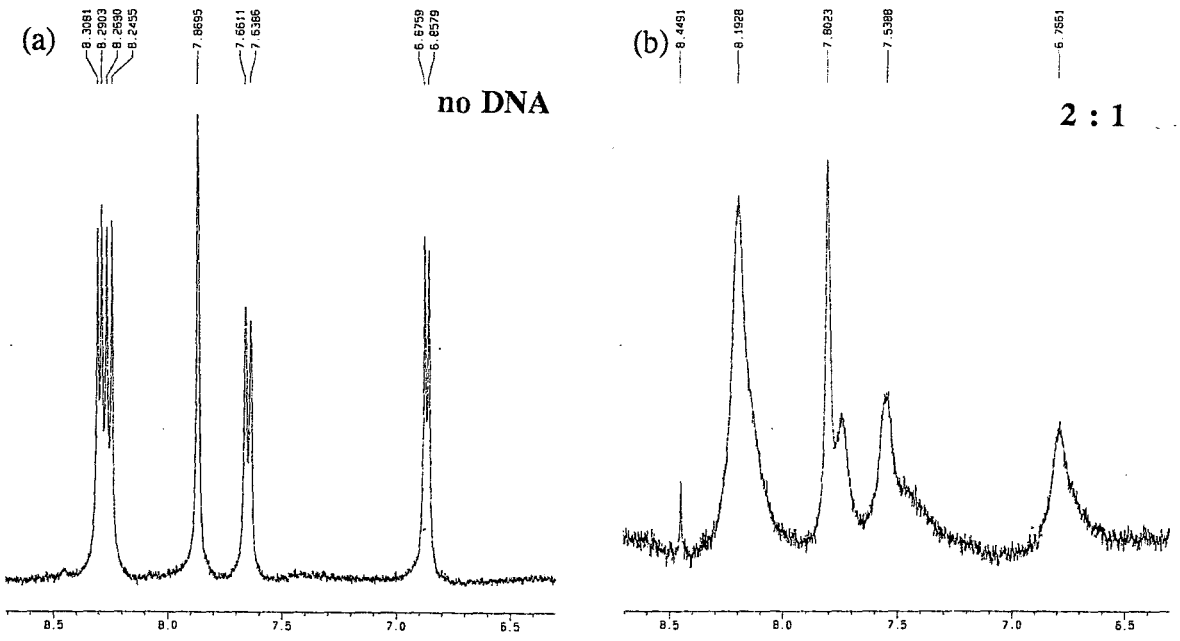


Figure 44 ^{1}H NMR spectra of chloroquine **126** : (a) before addition of DNA; and (b) after addition of DNA.

case of compound **195** (Fig. 42) was severe and, at higher DNA concentrations, very few meaningful peaks could be observed; a similar result was obtained by Chandrasekaran *et al.*[342] in the case of quinacrine.

A second NMR method used to probe the intercalation of these compounds, was monitoring of the exchangeable imino protons of the DNA, which fall at the lowfield end of the spectrum (between 10 and 15ppm) and which are easily followed without interference from ligand signals. The signals occurring in this lowfield region comprise two partially resolved envelopes, which are due to a number of overlapping resonances from the GC and AT hydrogen-bonded imino protons. The higher field peak at ~12.7ppm represents the GC base pair protons and the lower field peak at ~13.8ppm represents the AT base pair protons. Since these protons will undergo deuterium exchange, the experiments must be conducted in water (H_2O), with only enough D_2O present for a lock-signal to be obtained. Water suppression techniques are thus essential for the success of this method.[341] The binding of chloroquine has previously been studied in this way by Feigon *et al.*[339] whose results were very similar to those obtained in the present study.

The carboxamides **180**, **195**, **196** and **202** and chloroquine **126** were added in increasing amounts to calf thymus DNA, and the effect on the imino protons was monitored. Representative spectra for chloroquine **126**, **195**, **196** and **202**, with the ligand-DNA ratios indicated, are shown in Figures 45, 46, 47 and 48 respectively (the full sets of spectra can be found in Appendix 5.3). The intercalators all showed upfield shifts of the imino protons, while the carboxamides **180** and **202** showed no shift, a result which supports our earlier finding by spectrophotometric means that these compounds are not intercalators. Figure 45 shows that there is a much greater shift of the GC envelope than of the AT envelope on addition of chloroquine, a result which indicates that chloroquine exhibits significant GC specificity. The maximum shift of the AT envelope, which we observed on chloroquine binding, was 0.15ppm, while that for the GC envelope was 0.51ppm. This GC specificity of chloroquine has been observed using other methods,

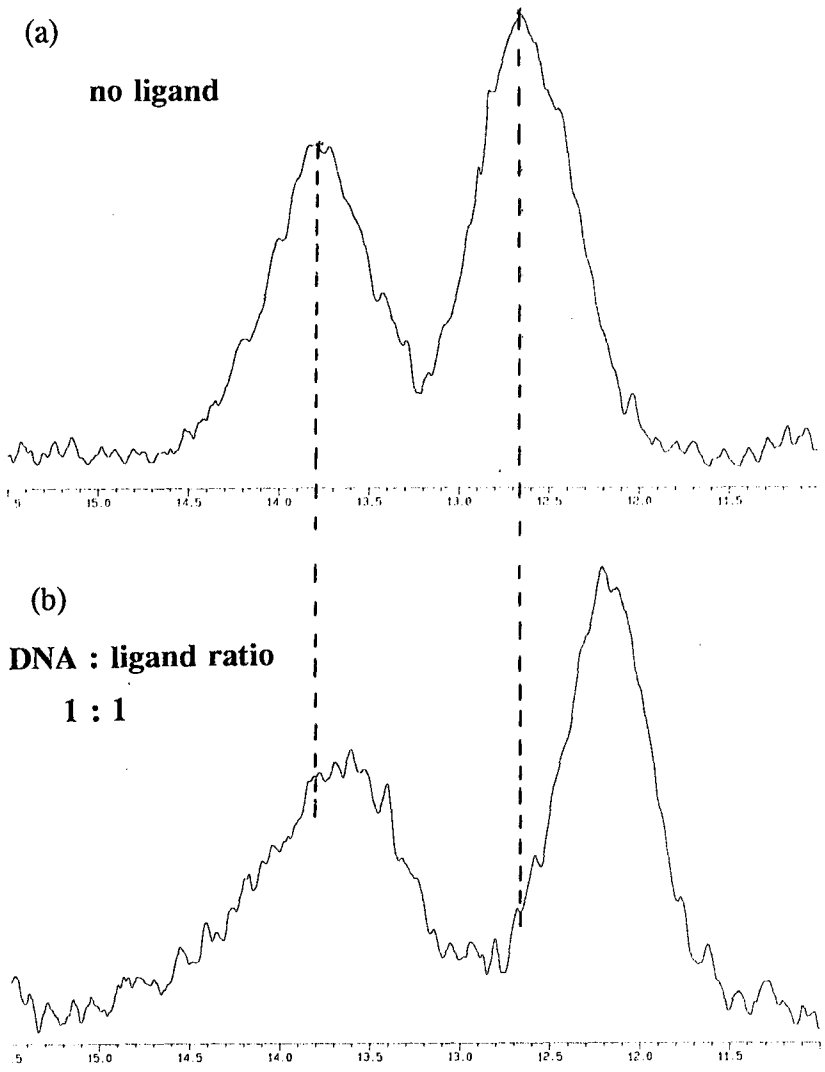


Figure 45 ^{1}H NMR spectra of the DNA imino protons : (a) before addition of ligand; and (b) after addition of chloroquine **126**.

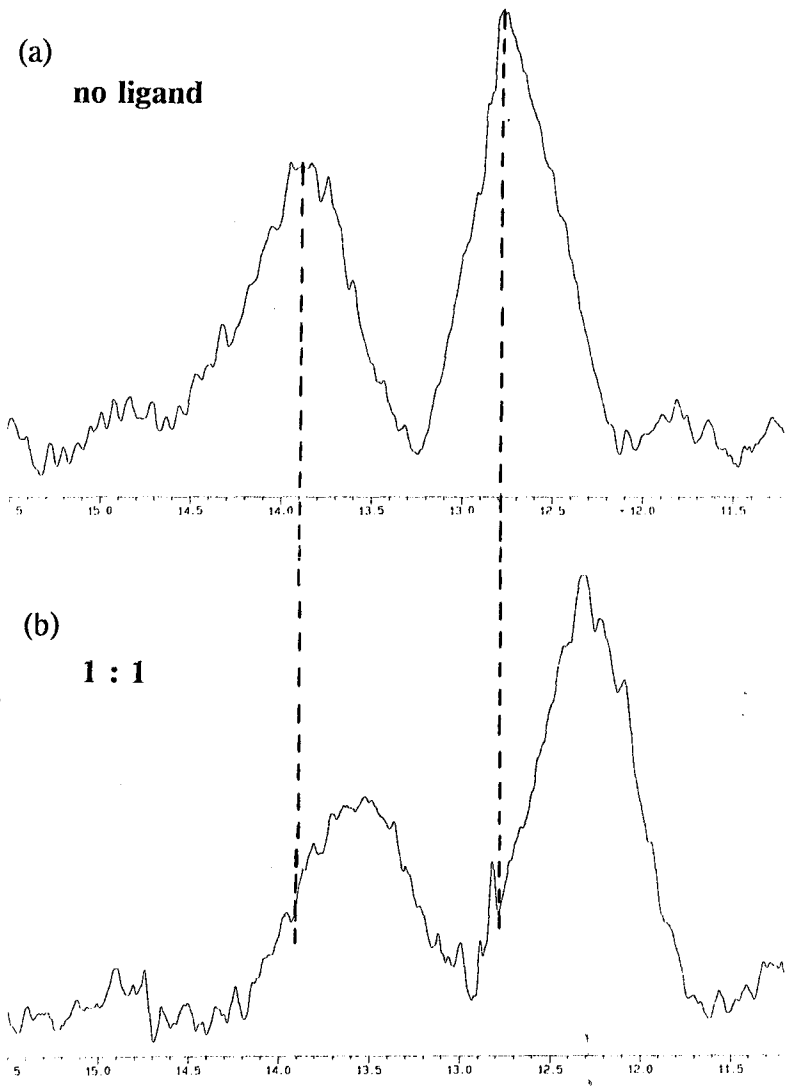


Figure 46 ^{1}H NMR spectra of the DNA imino protons : (a) before addition of ligand; and (b) after addition of carboxamide **195**.

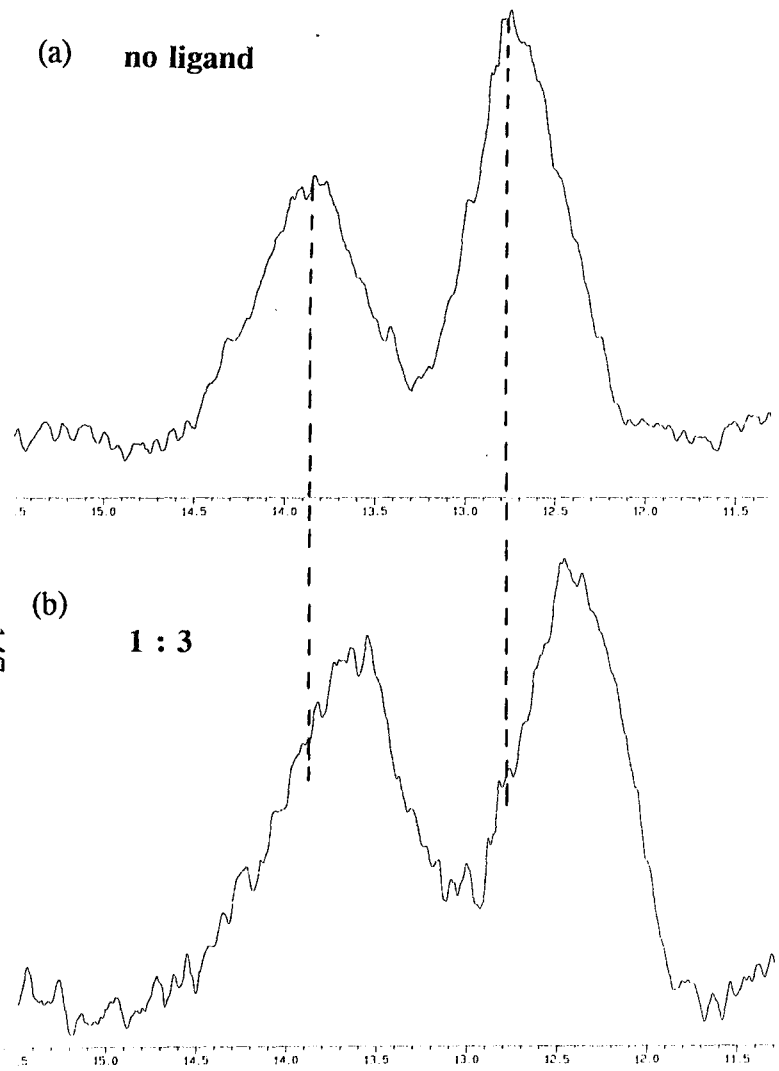


Figure 47 ^{1}H NMR spectra of the DNA imino protons : (a) before addition of ligand; and (b) after addition of carboxamide **196**.

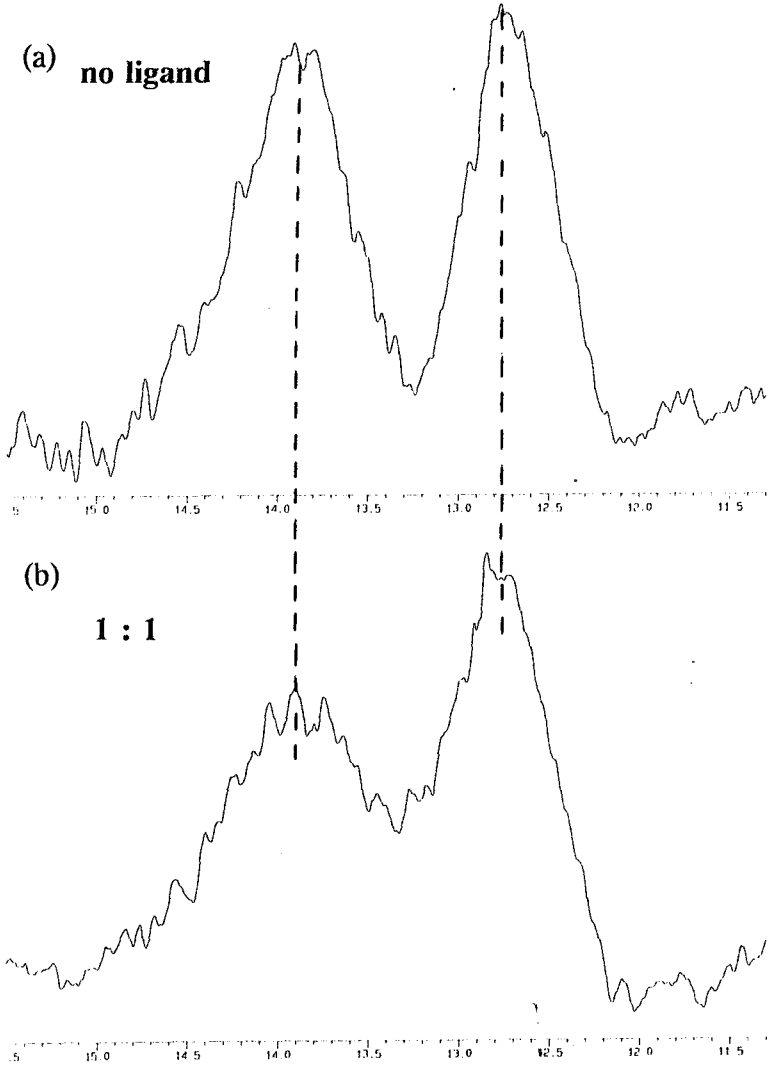


Figure 48 ^{1}H NMR spectra of the DNA imino protons : (a) before addition of ligand; and (b) after addition of carboxamide **202**.

including absorption spectroscopy. Allison *et al.*[194] showed that the absorption spectrum of chloroquine did not change on addition of DNA not containing GC base pairs. Each of the compounds **195** and **196** showed a slightly greater shift of the GC envelope than of the AT envelope, typically 0.46ppm and 0.34ppm respectively, but this difference is not considered to be significant, indicating that these intercalators exhibit no base pair specificity.

The upfield shifts of the DNA imino protons observed in the presence of intercalating ligands is a result of ring current effects on the DNA base pairs. The ring current effect initially exerted by neighbouring base pairs is replaced by that of the intercalating ligand. Ring current shifts from these ligands are generally considered to be greater than for those due to a base pair and, hence, the upfield shift.[339] Broadening of the imino proton signals on binding of a ligand can occur as a result of kinetic effects. Generally, significant broadening of the imino signals on binding of an intercalator indicates that exchange of the ligand among different DNA binding sites is slow, while intercalators which do not significantly change the breadth of the imino signals are considered to undergo fast exchange.[358] From our own results, it would appear that chloroquine **126** and the carboxamides **195** and **196** undergo fast site exchange.

Most of the NMR techniques used to study ligand-DNA interactions monitor changes in the spectrum of either the DNA or the ligand, which are caused by close interaction of the ligand chromophore and the DNA bases. ^{31}P NMR spectroscopy, however, monitors structural change[160] which occurs on intercalation of a ligand into DNA and, thus, is an important complementary method to the other NMR techniques. ^{31}P NMR spectroscopy conveniently monitors conformational changes of the phosphodiester backbone which occur upon binding of intercalating drugs, and much detailed work in this area has been conducted by Gorenstein and co-workers.[350,359-363]

Thus, the ^{31}P NMR spectrum of sonicated DNA was monitored on addition of increasing amounts of compounds **195**, **196**, **181** and chloroquine **126**, and the results obtained are

illustrated by representative spectra, shown in Figures 49, 50, 51 and 52, together with the ligand-DNA ratios. The full sets of spectra obtained can be found in Appendix 5.3. The ^{31}P NMR spectrum of DNA shows only one, averaged phosphate signal at ~4.38ppm upfield of the reference signal (trimethyl phosphate) for all the phosphates present. Figures 49, 50 and 52 show that addition of the intercalators **195**, **196** and chloroquine **126** to DNA caused a small downfield shift of this averaged signal, while Figure 51 shows that addition of the non-intercalator **181** produced no visible shift. This is in accordance with results obtained for other intercalators.[351,356] The observed downfield shift was small, 0.15ppm in the case of compound **196** and 0.26ppm for both compound **195** and chloroquine **126**. This downfield shift, observed on intercalation, has been explained in terms of the torsional angles in the sugar phosphate backbone undergoing a change as the helix unwinds to allow intercalation to take place.[351] Experimental evidence has confirmed this hypothesis, as the magnitude of the chemical shift change at saturation of DNA with a ligand was found to be linearly related to the DNA base pair unwinding angle for that ligand.[345]

The ^{31}P chemical shift observed on intercalation of a ligand is extremely ligand specific, and varies considerably from one ligand to another. Jones and Wilson[351] compared the unwinding angles of ethidium bromide (26°), quinacrine (17°) and daunorubicin (10°) with their ^{31}P NMR chemical shifts, which were found to be 0.62ppm, 0.40ppm and 0.16ppm, respectively. The unwinding angle of chloroquine has been measured, and found to be highly salt-dependent, varying from 9° at low salt concentrations to around 17° at high salt concentrations.[364] ^{31}P NMR studies on chloroquine binding to DNA do not appear to have been conducted prior to our study. The value of 0.26ppm which we obtained for the ^{31}P NMR shift of DNA on saturation with chloroquine is intermediate between the shifts observed for quinacrine and daunorubicin.[351] This would seem to imply an unwinding angle for chloroquine of between 10° and 17° under the experimental conditions employed. Our results also imply a very similar unwinding angle (10-17°) for compound **195**. The ^{31}P NMR shift observed for compound **196** would seem to indicate a significantly lower unwinding angle, but this shift value is not considered

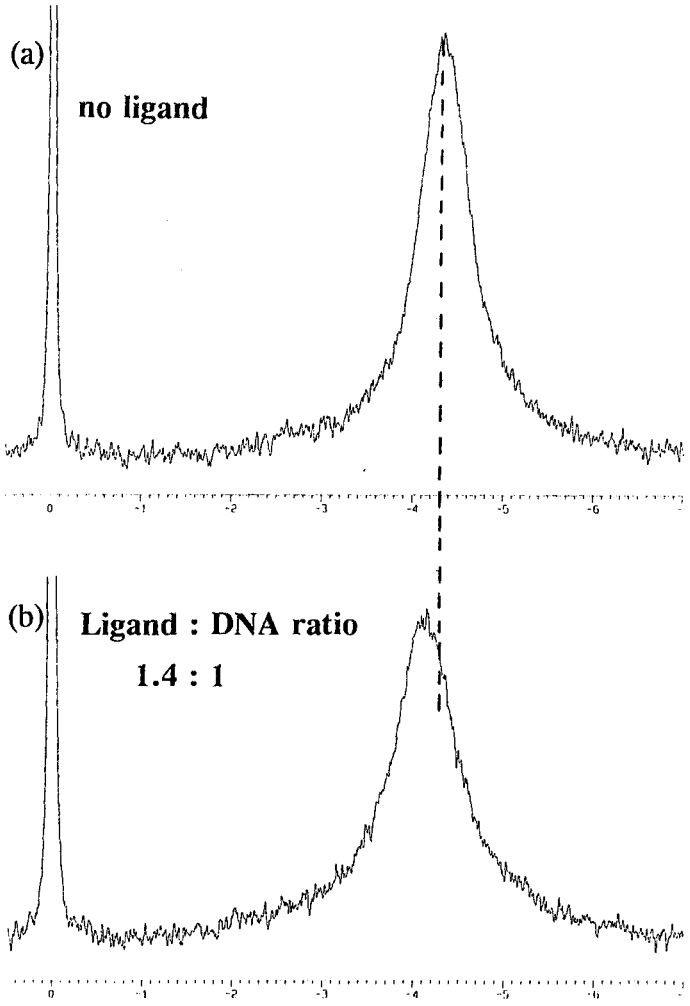


Figure 49 ^{31}P NMR spectra of calf thymus DNA : (a) before addition of ligand; and (b) after addition of carboxamide **195**.

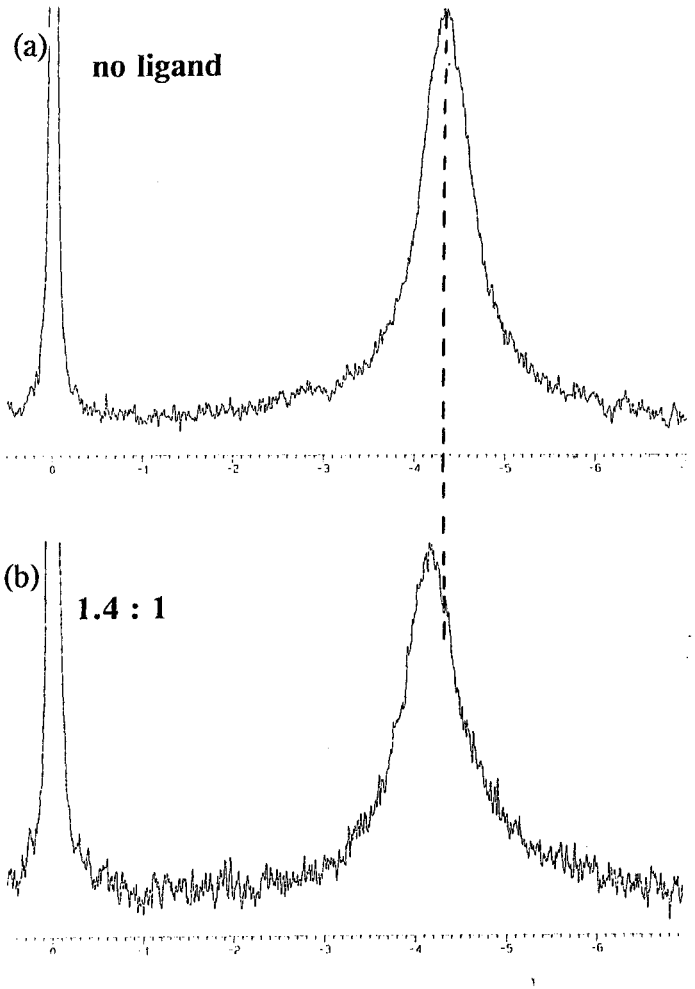


Figure 50 ^{31}P NMR spectra of calf thymus DNA : (a) before addition of ligand; and (b) after addition of carboxamide **196**.

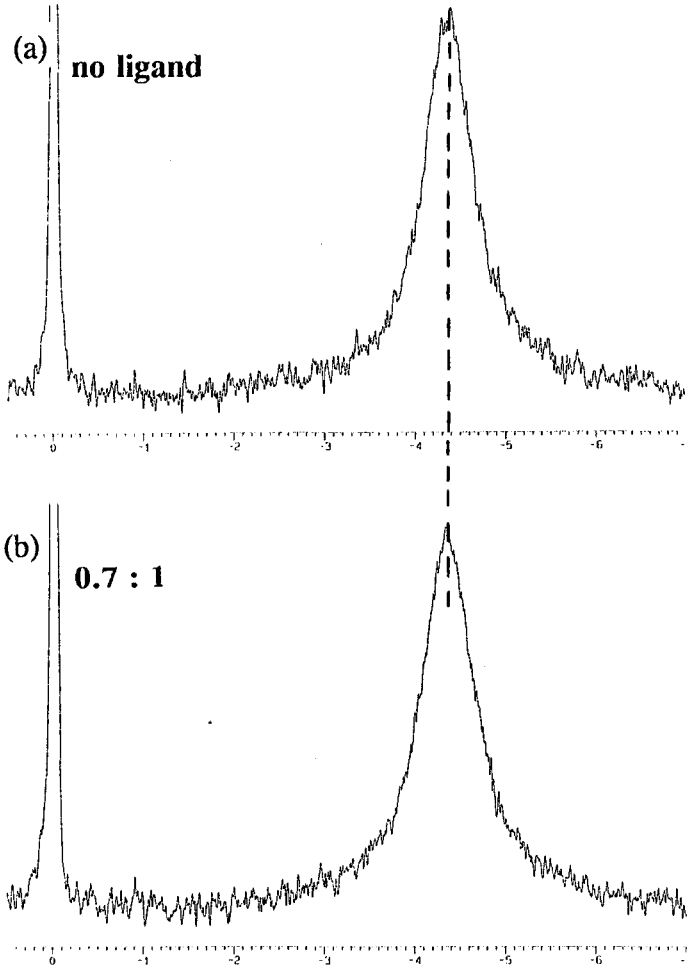


Figure 51 ^{31}P NMR spectra of calf thymus DNA : (a) before addition of ligand; and (b) after addition of carboxamide **181**.

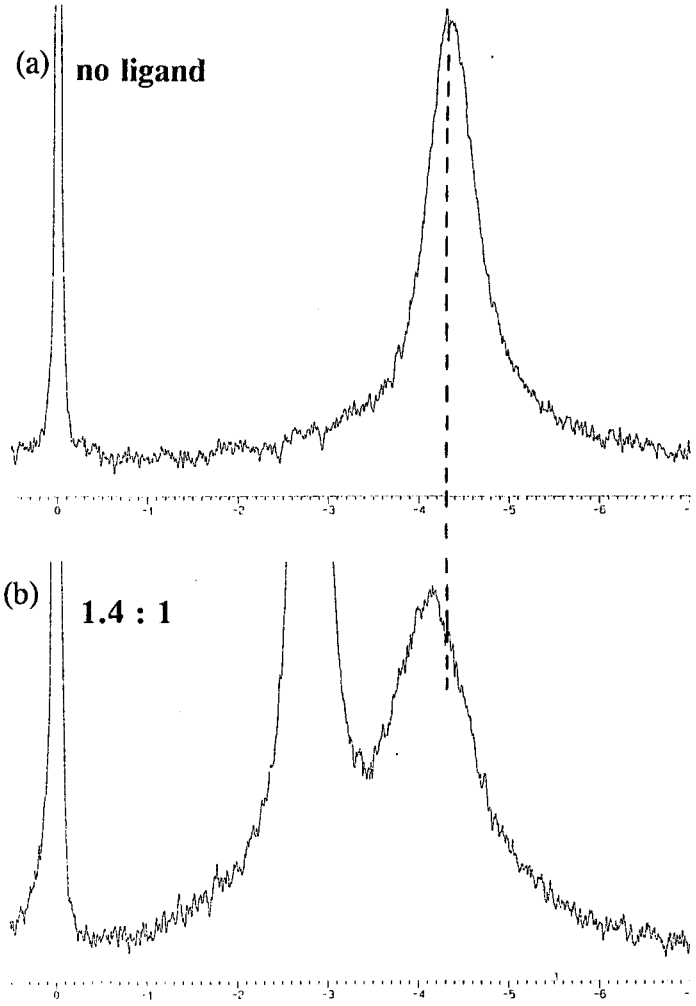


Figure 52 ^{31}P NMR spectra of calf thymus DNA : (a) before addition of ligand; and (b) after addition of chloroquine **126**.

to be the maximum shift value obtainable, as significant problems with the low solubility of this compound in aqueous solution were experienced. The fact that the intercalating drugs shift the ^{31}P NMR signal as a single peak, and do not result in the appearance of new signals, suggests that these ligands are in fast or intermediate exchange among DNA binding sites.[345] This supports the similar finding obtained by monitoring the imino protons.

DNA-ligand interactions have thus been studied using a number of different techniques, which together have provided conclusive evidence that compounds **195** and **196** are intercalators, while compounds **180**, **181**, **202** and **203** are not. Chloroquine-DNA interactions have also been studied by a number of methods not previously used to study this compound.

The combination of techniques used to monitor ligand-DNA interactions in this study provide an effective screening process to determine which compounds bind to DNA by intercalation, and which bind by some other mode. These methods also provide some details of binding such as whether the ligand exhibits base specificity and whether fast or slow exchange between different DNA binding sites is occurring.

It would appear from the literature, and from our own studies, that generally for intercalation to occur, at least a three-ring chromophore is necessary, except when a two-ring chromphore possesses a side-chain which will interact very favourably with certain groups on the DNA base pairs. In the case of chloroquine, this group is chlorine, which is also probably responsible for its GC specificity[206] and, in the case of quinine, either the methoxy or hydroxy group, or both, may encourage intercalation. In general the DNA binding affinity of these two-ring systems is found to be lower than for three-ring systems,[365] but this in no way implies that all three-ring, planar compounds are DNA intercalators.[229,366] The exact positioning and size of side-chains in both two-ring and three-ring systems is important, as certain substituents can effectively block intercalation by steric hindrance,[366] or favour an alternative binding mode[229] for energetic reasons.

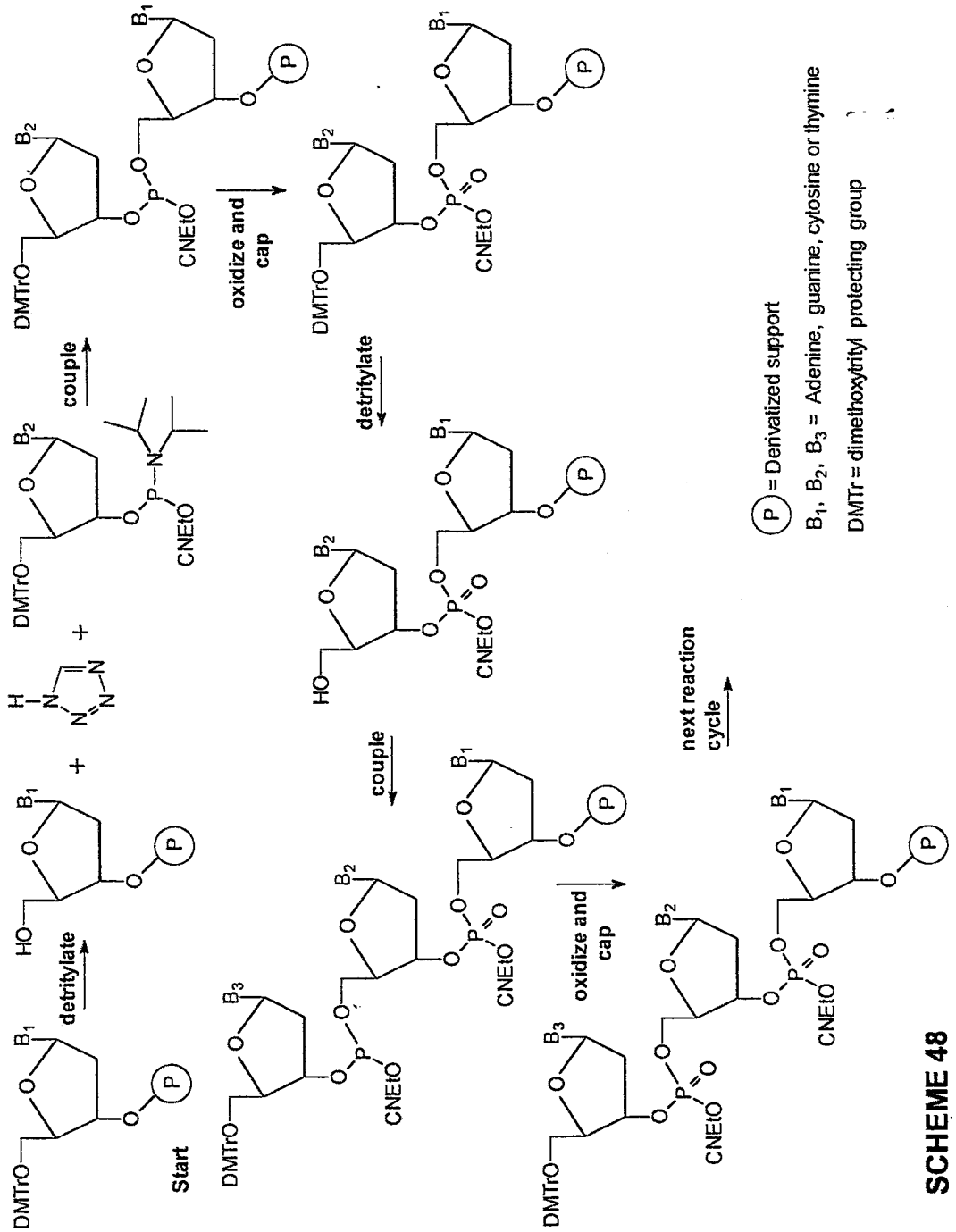
2.3.2 SYNTHESIS OF AN OLIGONUCLEOTIDE

A logical extension of studies on natural DNA fragments is the study of ligand interactions with specific, known sequences of DNA, from which very detailed information of the interaction can be obtained. NMR spectroscopy of oligonucleotides has been made possible by the advent of convenient DNA synthetic methods, and by advances in NMR technology.[367] The chemical synthesis of oligonucleotides has been discussed in a number of reviews[368-370] and the current literature contains many examples of improvements to the existing solid phase method of synthesis.[371-373]

As part of our interest in the synthesis and DNA interactions of potentially biologically active compounds, we conducted a preliminary study on the synthesis of a short oligonucleotide, and its interaction with chloroquine, with a view to extending this study, in the future, to compounds of known or potential biological activity, which is dependent on interaction with DNA.

The oligonucleotide selected for synthesis was d(5′-GCATGC)$_2$, the major advantage of this sequence being that it is palindromic, which greatly simplifies the ^{1}H NMR spectrum. The synthesis, known as the phosphite triester method, employs β-cyanoethyl-*N,N*-diisopropyl phosphoramidites,[374] and is outlined in Scheme 48. The synthesis was carried out on a semi-manual Cruachem DNA synthesizer where the solid support is held in a column to which the individual phosphoramidites are introduced manually, and through which reagents, controlled from a central manifold, are pumped under pressure. A major problem associated with this synthesis is that the phosphoramidites are extremely moisture-sensitive and even a small amount of water in any of the reagents leads to a dramatic drop in yield of the final product. The necessity of introducing these phosphoramidites manually, and thus exposing them to air, reduces the efficiency of the synthesis, and this was one of the major problems we encountered.

At the end of a synthesis, the finished sequence is removed from the solid support and deprotected. The purification procedure which we used was reverse-phase HPLC [eluting


Start
detritylate
couple
oxidize and cap
detritylate
couple
oxidize and cap
next reaction cycle
DMTrO
HO
CNEtO
B1
B2
B3
P
= Derivatized support
B1, B2, B3 = Adenine, guanine, cytosine or thymine
DMTr = dimethoxytrityl protecting group

SCHEME 48

with 0.1M triethyl ammonium acetate buffer (pH 7.0)].[375]

A second major problem which we encountered was the insolubility of certain of the HPLC fractions, which made examination by ^{1}H NMR spectroscopy difficult. All the fractions were examined by ^{1}H NMR spectroscopy, and one fraction appeared to contain the correct sequence. This fraction was concentrated and the oligonucleotide redissolved in D_2O containing 0.2M NaCl (pH 6), this automatically results in duplex formation as the strands are self-complementary.[367] This oligonucleotide has been synthesized by other workers[376-378] and thus assignments of the aromatic ^{1}H resonances were based on these previous assignments.[§§§] To the synthetic oligonucleotide d(5'-GCATGC)$_2$ was added chloroquine **126**, a known intercalator, to examine any shifts resulting from intercalation. Fig 53 shows the spectrum of the oligonucleotide, with the aromatic proton assignments, before and after addition of chloroquine. Expanded spectra, which more clearly show the small shifts observed, can be found in Appendix 5.3. The observed shifts can be rationalized in terms of interaction between the chloroquine chromophore and the oligonucleotide base pairs, which are in close proximity as a result of intercalation.

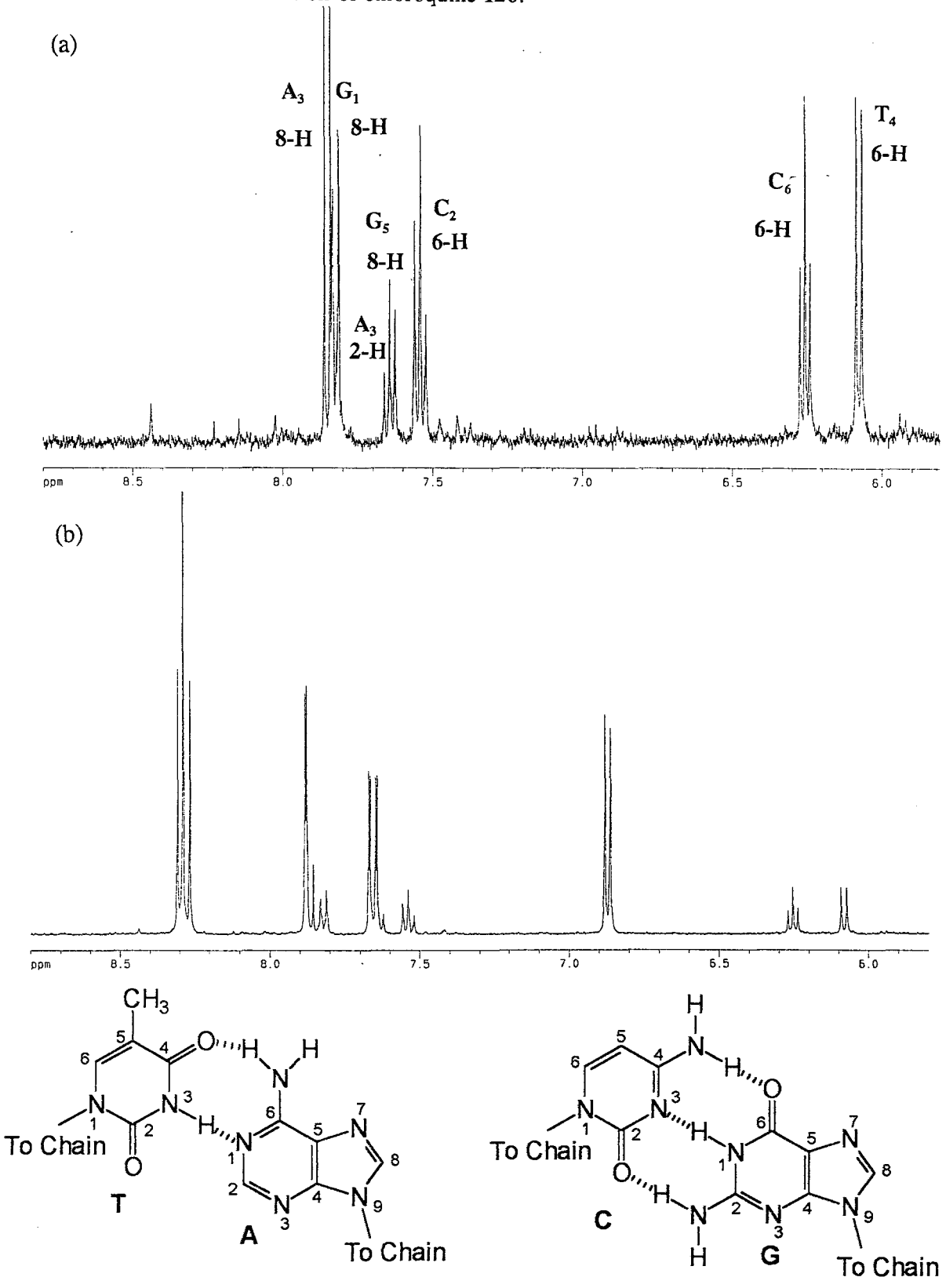
The ^{31}P NMR spectra of the oligonucleotide alone, and in the presence of chloroquine, are shown in Fig. 54. This figure shows that two of the resolved phosphate peaks showed small upfield shifts, while the third showed a small downfield shift. This is indicative of changes to the phosphodiester backbone, which would result on intercalative binding of chloroquine to the oligonucleotide.

The conclusions drawn in this preliminary oligonucleotide study are necessarily tentative, and simply reflect our first foray into a new area of research.

This relatively new field of study has already produced a great deal of information regarding DNA structure[384-390] and DNA-ligand interactions,[391-401] and promises to yield much information in the future.

[§§§]Assignments of synthetic oligonucleotide resonances are based on a sequential assignment procedure, using two-dimensional NOESY and COSY spectra, similar to that employed in the assignment of protein resonances.[379-383]

Figure 53 ^{1}H NMR spectra of the oligonucleotide : (a) before addition of ligand; and (b) after addition of chloroquine **126**.



DNA base pairs, showing the numbering system

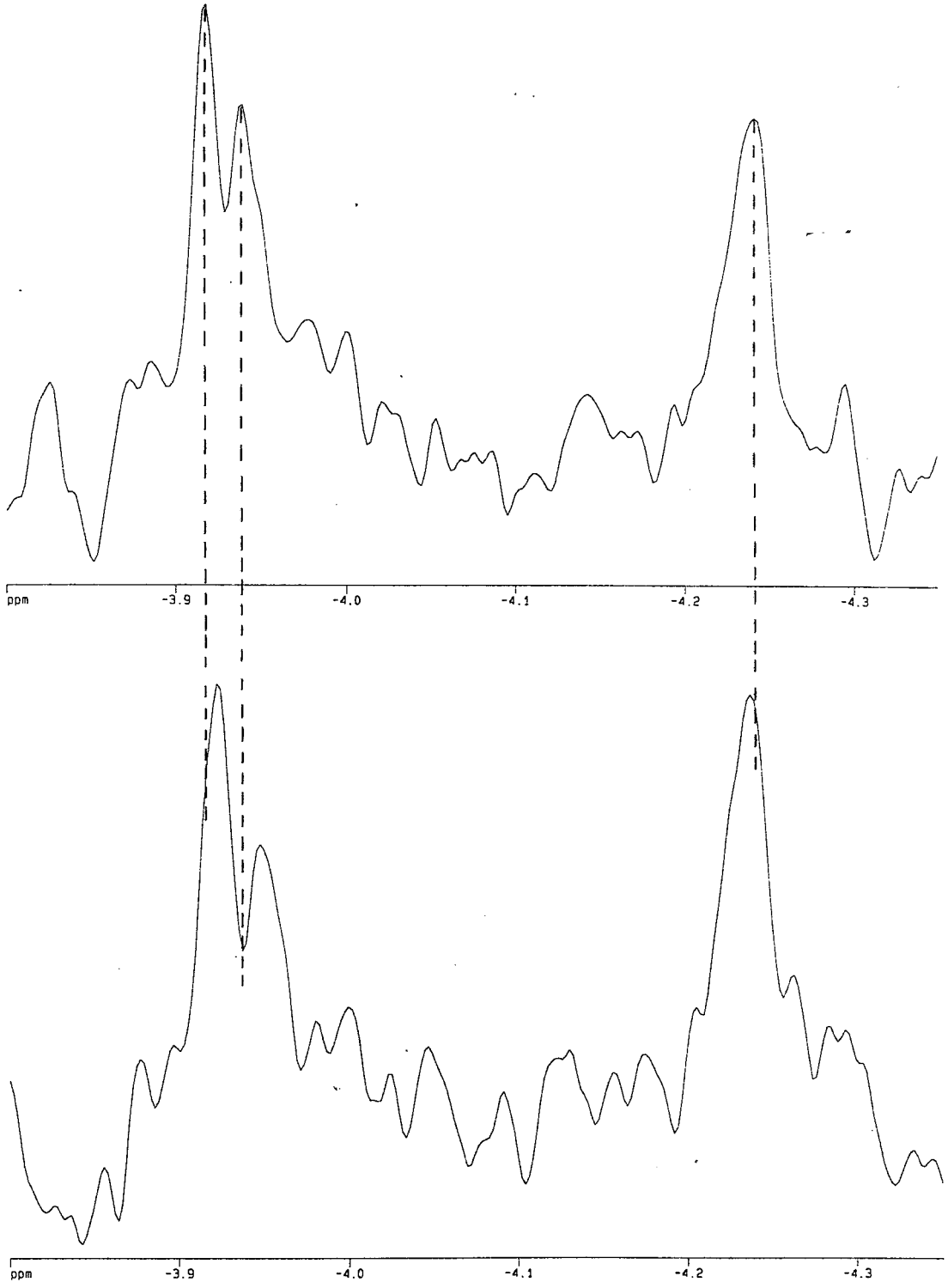


Figure 54 ^{31}P NMR spectra of the oligonucleotide : (a) before addition of the ligand; and (b) after addition of chloroquine **126**.

2.4 CONCLUSIONS

The results of this investigation have led to the development of efficient synthetic routes to indolizine derivatives and provided useful insights into their spectroscopic properties and DNA-intercalation potential.

The discovery that thermal cyclization of the product from the Baylis-Hillman reaction of pyridine-2-carboxaldehyde with methyl acrylate gives rise to an indolizine derivative,[250] has led to a general route for the preparation of 2-substituted indolizines from pyridine-2-carboxaldehydes and appropriate acrylate systems.[303] This route generally gives indolizines in good overall yield, and the approach is limited only by the availability of substituted pyridine-2-carboxaldehydes. The cyclization is facilitated by conversion of the hydroxy group of the Baylis-Hillman product to an acetoxy group, this intermediate step leading to greater overall yields than those obtained by direct cyclization of the hydroxy precursors.

A mechanistic sequence for the Baylis-Hillman reaction has been proposed, based on a detailed ^{1}H NMR kinetic investigation.[298] The proposed mechanism involves an addition - elimination sequence which starts with initial nucleophilic attack by the tertiary amine catalyst on the acrylate system to give a short-lived dipolar enolate; attack by this enolate on the aldehyde leads to an intermediate adduct, and finally to the Baylis-Hillman product by proton transfer and elimination of the tertiary amine.

Indolizine-2-carboxamides have previously been prepared only in low yields, and this study has established an efficient synthesis of indolizine-2-carboxamides, including previously inaccessible tertiary amides. This synthetic route, which involves reaction of indolizine carboxylic acids with amines in the presence of the coupling reagent 1,1'-carbonyldiimidazole, has been successfully used to prepare a number of symmetrically substituted *N,N*-dialkylindolizine-2-carboxamides which, in turn, have been subjected to variable temperature ^{1}H and ^{13}C NMR analysis to determine substituent effects on the

N-CO rotational barriers.

Intercalation studies on indolizinecarboxamides, pyrrolo[1,2-*a*]quinolinecarboxamides and imidazo[1,2-*a*]pyridinecarboxamides showed that only the pyrrolo[1,2-*a*]quinolines intercalate between the base pairs of double-stranded DNA. This is most likely due to the fact that the two-ring indolizine and imidazo[1,2-*a*]pyridine derivatives lack other ring substituents, which have been shown in certain cases to encourage intercalation. An additional factor preventing intercalation of indolizines may be the fact that the ring nitrogen can not easily be protonated at physiological pH (at which intercalation studies are conducted) - apparently a desirable feature for encouraging DNA intercalation.
The pyrrolo[1,2-*a*]quinolinecarboxamides were shown to bind to DNA with a similar binding affinity to the known intercalator and antimalarial agent chloroquine. Thus, the pyrrolo[1,2-*a*]quinolinecarboxamides might exhibit antimalarial activity, but a biological evaluation is necessary to determine whether they do possess this activity, as many factors other than binding to DNA influence biological activity.

Aspects of this research with potential for further development include:

(1) Synthesis of substituted indolizine-2-, imidazo[1,2-*a*]pyridine-2- and pyrrolo[1,2-*a*]quinoline-2-carboxamides to determine the influence of substituents on intercalation. From these studies, the importance of basicity to intercalation could also be determined.

(2) Synthesis, and complete characterization of customized oligonucleotides by high-field NMR spectroscopy to facilitate a molecular-level examination of intercalation processes. Such studies would assist in the design of novel biologically active compounds, whose mode of action involves DNA intercalation.

3. EXPERIMENTAL

3.1 GENERAL

All melting points were determined using a Kofler hot-stage apparatus, and the values are uncorrected. Most of the ^{1}H NMR spectra were run at 400MHz on a Bruker AMX400 spectrometer, a 500MHz spectrum was run on a Bruker WM500 spectrometer, and the 60MHz spectra on a Perkin-Elmer R12 spectrometer. The proton noise decoupled ^{13}C 100MHz, DEPT and the two-dimensional NMR (COSY, HETCOR and INADSY) spectra were all run on a Bruker AMX400 spectrometer. In most cases, the NMR sample was dissolved in $CDCl_3$, but in some cases D_2O was used. Spectra recorded in $CDCl_3$ were calibrated on the chloroform signal at 7.25ppm for ^{1}H and 77.00 for ^{13}C, while ^{1}H NMR spectra run in D_2O were calibrated using sodium 3-(trimethylsilyl)propanesulphonate-d_6 at 0.0ppm.

IR spectra were recorded on a Perkin-Elmer 180 or a Beckman IR 4260 spectrometer, using KBr discs, liquid films or Nujol mulls.

UV spectra were recorded on a Shimadzu UV-160A spectrophotometer using matching quartz cuvettes with a 1cm light path.

Low-resolution mass spectra were obtained using a Hewlett Packard 5988A mass spectrometer, and high-resolution mass spectra using a Varian Mat 212 or a Kratos MS 80RF mass spectrometer.

GLC analyses were performed on a Hewlett Packard 5980A gas chromatograph using flame ionization detection. Semi-preparative HPLC separations were achieved using a Spectra-Physics P100 HPLC with a Spectra-Physics UV100 detector set to 280nm. The column used was a 250 x 10mm Maxsil 5 C18 reverse phase column.

Flash chromatography[402] was carried out on Merck Silica gel 60 [particle size 0.040-0.063mm (230-400 mesh)]. In many cases where purification was achieved using flash chromatography, preparative thin layer chromatography was used for the separation of

smaller quantities of the material; this was carried out using Merck Silica gel 60 PF_{254}. Routine thin layer chromatography (TLC) was performed on precoated Merck Silica gel F_{254} plates, with visualization of compounds being achieved by inspection under UV light or by exposure to iodine vapour.

Solvents were dried using procedures described by Perrin and Armarego[403] :

(1) Ether and THF were dried over Na wire, and distilled from Na wire under N_2 with benzophenone as an indicator.

(2) 1,2-Dichloroethane, xylene, chlorobenzene and dichloromethane were distilled from P_2O_5.

(3) DMF was dried over $MgSO_4$.

(4) Pyridine was dried over KOH overnight and distilled from fresh KOH.

The numbering of indolizines, and other compounds, used for quoting NMR data follows the conventions used in the preceding sections. Pyridyl derivatives are numbered with the pyridine atoms being denoted by "primed" notation, for example 2′-H, with the numbering following that of the parent pyridine.

3.2 PREPARATIVE PROCEDURES

3.2.1 PREPARATION OF INDOLIZINE PRECURSORS AND DERIVATIVES

Pyridine-2-carboxaldehyde (**132**). -

Attempted preparation 1. A mixture of 2-picoline (2.0g, 21mmol) and SeO_2 (5.7g, 52mmol) in dioxan (150ml), containing 4% water, was heated under reflux for 2h and then filtered through celite while hot. The filtrate separated into two layers on standing and the top dioxan layer was dried over $CaCl_2$ and $MgSO_4$. The solvent was removed *in vacuo* to yield a crystalline material, shown by IR spectroscopy to be pyridine-2-carboxylic acid.

Attempted preparation 2.[294] Iodine (6.5g, 25mmol) and 2-picoline (2.3g, 25mmol) were mixed at room temperature to form a complex which became crystalline on cooling in an ice bath. The solid complex was dissolved in DMSO (3ml) and dropped into an excess of DMSO (22ml) preheated to 130°C. The mixture was stirred and heated slowly to 160°C. Stirring was continued for 15min at this temperature whereafter the mixture was cooled and neutralized with saturated aqueous $NaHCO_3$. Half of this material was continually extracted with ether for 3h and the other half was extracted with ether (4 x 50ml) in a separating funnel. Drying of the separate ether extracts with $MgSO_4$, followed by removal of the solvent *in vacuo* yielded a dark brown viscous oil in both cases. ^1H NMR spectroscopy indicated the presence of a small proportion of the desired aldehyde, IR spectroscopy showed the majority of material to be pyridine-2-carboxylic acid.

Attempted preparation 3. To a previously flame-dried, nitrogen-flushed 250ml four-necked round bottomed flask fitted with a septum, thermometer, dropping funnel and a condensor attached to a N_2 line, was added a solution of 2-picoline (1.49g, 1.58ml, 16mmol) in dry THF. This solution was cooled to -20°C in an ice-$CaCl_2$-liquid N_2 bath and 1.6M-*n*-BuLi (10ml, 16mmol) was added slowly by means of

syringe, with the temperature maintained below -20°C. This mixture was allowed to stir at -20°C for 45min, after which a solution of Br_2 (2.56g, 0.83ml, 16mmol) in dry THF (5ml) was added dropwise maintaining the temperature below 0°C. This was allowed to stir for 30min at 0°C whereafter a second equivalent of *n*-BuLi was added at -20°C as before, followed by a second equivalent of Br_2. After the final addition, stirring was continued at 0°C for 30min whereafter a solution of NaOH (1.40g, 35mmol) in water (10ml) was added and allowed to stir overnight at room temperature. The contents of the four-necked flask was poured into ice water (100ml) and the aqueous layer extracted with Et_2O (2 x 75ml). After drying of the organic layer ($MgSO_4$), solvent was removed *in vacuo* and IR spectroscopy showed the presence of a small proportion of desired material. The large number of products present (TLC) indicated that this procedure was not viable for the preparation of pyridine-2-carboxaldehyde.

Methyl 3-hydroxy-2-methylene-3-(2-pyridyl)propanoate (**134**).[267] -

Method 1. A solution of methyl acrylate (0.83g, 9.7mmol), pyridine-2-carboxaldehyde (0.98g, 9.3mmol) and DABCO (0.05g, 0.45mmol) in $CHCl_3$ (0.7ml) was allowed to stand at room temperature in a stoppered flask for 3d. The crude mixture was chromatographed (flash chromatography on silica gel; elution with EtOAc) to afford methyl 3-hydroxy-2-methylene-3-(2-pyridyl)propanoate (**134**) (1.69g, 94%); ν_{max} (thin film)/cm^{-1} 3400 and 1720; δ_H(60MHz;$CDCl_3$) 3.75 (3H, s, CH_3), 4.80 (1H, br s, OH), 5.73 (1H, s, C*H*OH), 6.06 and 6.44 (2H, 2 x s, C=CH_2), 7.14-7.97 (3H, m, ArH) and 8.50-8.79 (1H, m, ArH).

Alternative procedure. Employing the same method as above, methyl acrylate (5.0g, 0.058mol), pyridine-2-carboxaldehyde (5.9g, 0.055mol) and 3-hydroxyquinuclidine (0.34g, 1.34mmol) in $CHCl_3$ (4ml) were reacted for 1d to yield methyl 3-hydroxy-2-methylene-3-(2-pyridyl)propanoate (**134**) (9.78g, 92%).

Methyl indolizine-2-carboxylate (**135**). - The preparative procedures for this compound follow those for compound **153**.

Methyl 3-acetoxy-2-methylene-3-(2-pyridyl)propanoate (**153**). -

Method 1. Methyl 3-hydroxy-2-methylene-3-(2-pyridyl)propanoate (**134**) (1.0g, 5.2mmol) was heated with Ac_2O (5ml) at 100°C for 30min in a flask fitted with a reflux condensor and a drying tube. The cooled mixture was poured into $NaHCO_3$-ice; the remaining mixture was stirred for 30min and then extracted with Et_2O (2 x 100ml). The organic layer was washed sequentially with aqueous $NaHCO_3$ (100ml) and brine (100ml), and dried over $MgSO_4$. The solvent was evaporated and the residue chromatographed [flash chromatography on silica gel; elution with EtOAc-hexane (6:4)] to afford *methyl 3-acetoxy-2-methylene-3-(2-pyridyl)propanoate* (**153**) as a colourless oil (0.78g, 78%) (Found: M^+-$C_2H_4O_2$ 175.063. $C_{10}H_9NO_2$ requires *M*, 175.063); ν_{max} (thin film)/cm^{-1} 1750 and 1730; δ_H(400MHz;$CDCl_3$) 2.13 (3H, s, CH_3CO), 3.68 (3H, s, CH_3O), 5.94 (1H, s, CHOAc), 6.46 and 6.71 (2H, 2 x s, $C=CH_2$), 7.19 (1H, ddd, $J_{5',6'}$ 4.9, $J_{4',5'}$ 7.6 and $J_{3',5'}$ 1.0, 5'H), 7.42 (1H, d, $J_{3',4'}$ 7.8, 3'-H), 7.66 (1H, td, $J_{3',4'}$ and $J_{4',5'}$ 7.7 and $J_{4',6'}$ 1.8, 4'-H) and 8.55 (1H, d, $J_{5',6'}$ 4.7, 6'-H);δ_C(100MHz;$CDCl_3$) 21.0 (CH_3CO), 51.9 (CH_3O), 73.9 (CHOAc), 122.7 (C-3'), 123.0 (C-5'), 127.5 ($C=CH_2$), 136.6 (C-4'), 138.0 ($C=CH_2$), 149.5 (C-6'), 156.9 (C-2') and 165.4 and 169.6 (2xCO); *m/z* 175 (M^+-$C_2H_4O_2$, 54%) and 117 (100%).

Method 2. Methyl 3-hydroxy-2-methylene-3-(2-pyridyl)propanoate (**134**) (1.0g, 5.2mmol) was dissolved in dry THF (30ml) and pyridine (0.63ml, 7.8mmol) in a round-bottomed flask fitted with a dropping funnel and a condensor fitted with a drying tube. Acetyl chloride (0.56ml, 7.8mmol) was added dropwise through the dropping funnel and the reaction mixture was allowed to reflux for 1h. After cooling of the reaction mixture, aqueous $NaHCO_3$ was added until the pH was above 7. This aqueous mixture was extracted with ether (2 x 100ml) and the combined ethereal layers were dried over $MgSO_4$. Evaporation of the solvent afforded the crude *methyl*

3-acetoxy-2-methylene-3-(2-pyridyl)propanoate (**153**) (0.58g,48%).

Method 3. To methyl 3-hydroxy-2-methylene-3-(2-pyridyl)propanoate (**134**) (1.0g, 5.2mmol) in a round-bottomed flask fitted with a condensor were added Ac_2O (0.98ml, 10.4mmol), triethylamine (1.45ml, 10.4mmol) and DMAP (0.064g, 0.52mmol). The mixture was heated at 60°C for 30min after which it was poured into ice water (20ml). Aqueous $NaHCO_3$ was added until the solution was basic. After stirring for 20min the resulting mixture was extracted with ether (2 x 100ml) and the combined ethereal extracts were washed with saturated aqueous $NaHCO_3$ (75ml) and brine (75ml). Drying of the organic layer over $MgSO_4$ was followed by removal of solvent to afford crude *methyl 3-acetoxy-2-methylene-3-(2-pyridyl)propanoate* (**153**) (0.71g, 58%).

Method 4. Methyl 3-hydroxy-2-methylene-3-(2-pyridyl)propanoate (**134**) (1.21g, 6.3mmol) was heated at 100°C for 30min in the presence of Ac_2O (2.5ml) and glacial acetic acid (2.5ml) in a flask fitted with a reflux condensor and a drying tube. The cooled mixture was poured into $NaHCO_3$-ice; the remaining mixture was stirred for 30min and then extracted with Et_2O (2 x 100ml). The organic layer was washed sequentially with aqueous $NaHCO_3$ (100ml) and brine (100ml), and dried over $MgSO_4$. Evaporation of the solvent afforded the crude *methyl 3-acetoxy-2-methylene-3-(2-pyridyl)propanoate* (**153**) (0.51g, 35%).

Attempted preparation. A solution of methyl 3-hydroxy-2-methylene-3-(2-pyridyl)propanoate (**134**) (1.0g, 5.2mmol) in dry THF (20ml) in a three-necked round-bottomed flask fitted with a septum, thermometer and condensor attached to a N_2 line was cooled to 0°C in a salt-ice bath. *n*-BuLi (4ml, 6mmol) was added dropwise with a syringe, not allowing the temperature of the reaction mixture to rise above 0°C. After stirring the resulting mixture for 30min, acetyl chloride (0.37ml, 5.2mmol) was added slowly with a syringe and stirring continued for an additional 2h at low temperature. The reaction was quenched with water (25ml) and extracted with EtOAc

(2 x 75ml). The combined organic layers were dried over $MgSO_4$ and the solvent evaporated. TLC showed a number of different products to be present in addition to the expected *methyl 3-acetoxy-2-methylene-3-(2-pyridyl)propanoate* (**153**).

Methyl indolizine-2-carboxylate (**135**). -

Method 1. Methyl 3-acetoxy-2-methylene-3-(2-pyridyl)propanoate (**153**) (0.51g, 2.2mmol) was heated at 120°C for 1h and the resulting mixture was chromatographed [flash chromatography on silica gel; elution with EtOAc-hexane (3:7)] to yield methyl indolizine-2-carboxylate (**135**) as yellowish crystals (0.26g, 68%), m.p. 99-100°C (from hexane) (lit.,[80] 97-99°C) (Found: C,68.4; H,5.1; N,8.1. Calc. for $C_{10}H_9NO_2$: C,68.6; H,5.2; N,8.0%); ν_{max} (KBr)/cm^{-1} 1715; δ_H(400MHz;$CDCl_3$) 3.87 (3H, s, CH_3), 6.50 (1H, td, $J_{6,7}$ and $J_{5,6}$ 6.8 and $J_{6,8}$ 1.2, 6-H), 6.65 (1H, ddd, $J_{6,7}$ 6.5, $J_{7,8}$ 9.1 and $J_{5,7}$ 1.0, 7-H), 6.81 (1H, s, 1-H), 7.33 (1H, d $J_{7,8}$ 9.1, 8-H), 7.77 (1H, m, 3-H) and 7.83 (1H, dq, $J_{5,6}$ 7.1 and $J_{5,7}$ 1.0, 5-H); δ_C(100MHz;$CDCl_3$) 51.4 (CH_3), 100.4 (C-1), 112.2 (C-6), 115.8 (C-3), 118.1 (C-7), 119.6 (C-2), 120.2 (C-8), 125.3 (C-5), 132.8 (C-9) and 165.5 (CO); *m/z* 175 (M^+, 99%) and 117 (100%).

Alternative procedure. Methyl 3-hydroxy-2-methylene-3-(2-pyridyl)propanoate (**134**) (3.61g, 19mmol) was heated in a flask fitted with a reflux condensor at 140°C for 1.5h. The crude material was chromatographed [flash chromatography on silica gel; elution with EtOAc-hexane (3:7)] to afford methyl indolizine-2-carboxylate (**135**) (0.71g, 22%).

5-Nitro-2-picoline (**136**). -

Method 1.[291] Sodium sand was prepared by heating sodium (3g) in dry xylene in a flange flask fitted with a mechanical stirrer and a reflux condensor leading to a N_2 line. When the sodium became molten, rapid stirring with the mechanical stirrer was carried out, and as the oil bath cooled (at ~110°C) vigorous stirring was resumed until the Na resolidified. To this sodium sand (3g, 0.13mol) was added dropwise

diethyl malonate (20.7ml, 0.14mol), the temperature being maintained below 30°C in an ice bath throughout the addition. To this slurry was added 2-chloro-5-nitropyridine (20.7g, 0.13mol) in dry xylene (10ml), after which the reaction mixture was refluxed for 1h. After cooling of the mixture, aqueous 50% H_2SO_4 (170ml) was added and the xylene layer was removed. To the acidic layer was added H_2O (85ml), and this aqueous solution was refluxed for 7h. After cooling, neutralization with aqueous KOH was carried out and ether extracts (5 x 100ml) of this aqueous material were combined, dried over $MgSO_4$ and the solvent evaporated to yield crude material. This material was chromatographed [flash chromatography on silica gel; elution EtOAc-hexane (4:6)] to yield 5-nitro-2-picoline (**136**) (1.64g, 9%).

Alternative procedure.[292] To a slurry of NaH (3.1g, 0.13mol) in dry Et_2O (220ml) in a three-necked round-bottomed flask fitted with a thermometer, dropping funnel and condensor attached to a N_2 line, was added dropwise diethyl malonate (20.5g, 0.13mol) in dry Et_2O (20ml). When evolution of hydrogen subsided, 2-chloro-5-nitropyridine (20g, 0.13mol) was added in powder form. This thick mixture was stirred manually to aid mixing, and after the addition, Et_2O was removed by distillation and the remaining red, tarry residue was heated to 110°C for 1h. 12N-H_2SO_4 (150ml) was added and the resulting mixture was refluxed for 9h. The reaction mixture was neutralized with NaOH and extracted with Et_2O (2 x 100ml). The ether extracts were dried and the solvent removed *in vacuo* to give crude material which was chromatographed [flash chromatography on silica gel; elution EtOAc-hexane (4:6)] to yield 5-nitro-2-picoline (**136**) (2.12g, 12%); δ_H(60MHz;$CDCl_3$) 2.74 (3H, s, CH_3), 7.50 (1H, d, ArH), 8.50 (1H, dd, ArH) and 9.41 (1H, d, ArH).

Isopropyl Acrylate (**137a**).[296] - Acryloyl chloride (10.0g, 0.11mol) was added dropwise to a stirred solution of isopropyl alcohol (9.0g, 0.15mol), *N,N*-dimethylaniline (13.4g, 0.11mol) and hydroquinone (0.5g) in dry ether (190ml) in a two-necked round-bottomed flask fitted with a dropping funnel and a condensor with a drying tube. The reaction mixture was heated under reflux for 2.5d after which the liquid was decanted

from the solid and washed with 2.5**M**-HCl (2 x 20ml) and 1**M**-NaOH (20ml). The organic layer was dried over $MgSO_4$, and CuCl (0.2g) was added prior to distillation of the ether at atmospheric pressure. Distillation at a water pump yielded isopropyl acrylate (**137a**) (6.1g, 49%), b.p. 68-72°C/77mmHg (lit.,[296] 47-49°C/67mmHg); ν_{max} (thin film)/cm^{-1} 2980 and 1725; δ_H(60MHz;$CDCl_3$) 1.23 and 1.33 (2Ĥ ,2́ x s, 2 x CH_3), 4.81-5.51 [1H, sept, C*H*$(CH_3)_2$] and 5.66-6.78 (3H, m, CH_2=CH).

tert-*Butyl Acrylate* (**137b**).[296] - Acryloyl chloride (21.2g, 19.0ml, 0.23mol) was added dropwise to a stirred solution of *N,N*-dimethylaniline (28.3g, 29.6ml, 0.23mol), *tert*-butanol (22.25g, 28.5ml, 0.3mol) and hydroquinone (0.50g) in dry ether (200ml). The resulting mixture was boiled under reflux for 3d after which the liquid was decanted from the solid and washed with 2**M**-HCl (2 x 20ml), 1**M**-NaOH (20ml), and dried over $MgSO_4$. Cu(I)Cl (0.5g) was added to the ethereal solution and the ether was removed by distillation at atmospheric pressure. Distillation under reduced pressure yielded *tert*-butyl acrylate (**137b**) (16.45g, 56%), b.p. 26-29°C/18mm Hg (lit.,[296] 27-29°C/20mm Hg); ν_{max} (thin film)/cm^{-1} 2980 and 1720; δ_H(60MHz;$CDCl_3$) 1.52 [9H, s, $(CH_3)_3C$)] and 5.63-6.60 (3H, m, CH_2=CH).

N,N-*dimethylacrylamide* (**138**).[297] -

Method 1. Acrylic acid (10.0g, 0.14mol), *N,N*-dimethylcarbamoyl chloride (15.1g, 0.14mol), triethylamine (15.6g, 0.16mol) and hydroquinone (0.5g) were refluxed in dry chlorobenzene in a round-bottomed flask fitted with a condensor and a drying tube for 3h. Solid material was removed by filtration after addition of sufficient hexane for mobilization of the solidified reaction mixture. The filtrate was applied to a short silica column and chlorobenzene was removed by elution with EtOAc-MeOH (91:9) and the solvent was evaporated *in vacuo* at a temperature not exceeding 25°C. Distillation of the resulting oil in the presence of hydroquinone afforded *N,N*-dimethylacrylamide (**138**) (3.94g, 28%), b.p. 88-89°C/24mmHg (lit.,[297] 63-66°C/6mmHg); ν_{max} (thin film)/cm^{-1} 3480 and 1720; δ_H(400MHz;$CDCl_3$) 2.70 and

2.80 (2H, 2 x s, 2 x CH_3), 5.36 and 5.97 (2H, 2 x dd, CH_2=CH) and 6.32 (1H, m, CH_2=C*H*); *m/z* 99 (M^+, 40%) and 98 (100%).

Attempted preparation 1.

N,N-*dimethylamine hydrochloride*[404] - HCl gas, generated by dropping concentrated hydrochloric acid into concentrated sulphuric acid, was bubbled through a solution of dimethylamine in absolute ethanol (50ml) at 0°C until the reaction mixture solidified. The ethanol was removed *in vacuo* and the slurry washed three times with ether. After the ether washes, solvent was removed from the slurry under vacuum and one more ether wash of the slurry was conducted, followed by final drying *in vacuo* to yield *N,N*-dimethylamine hydrochloride.

To a precooled (-5°C) mixture of *N,N*-dimethylamine hydrochloride (1.7g, 20.9mmol) in dry pyridine (25ml) in a three-necked flask fitted with a condensor, thermometer and dropping funnel under a nitrogen atmosphere, was added dropwise acryloyl chloride (0.94g, 0.84ml, 10.4mmol). The reaction was allowed to stir at 0°C for 2h and at room temperature for a further 20h, after which the reaction mixture was poured into 2M-HCl and allowed to stand for 30min. The aqueous layer was extracted with EtOAc (2 x 100ml) and the extract washed with aqueous $NaHCO_3$ and brine, and dried over $MgSO_4$. Evaporation of the solvent *in vacuo* revealed none of the expected product to be present.

Attempted preparation 2. To a mixture of *N,N*-dimethylamine hydrochloride (1.7g, 20.9mmol), *N,N*-dimethylaniline (1.33ml, 10.4mmol) and hydroquinone (0.05g) in dry ether (25ml) under a nitrogen atmosphere was added dropwise acryloyl chloride (0.94g, 0.84ml, 10.4mmol) in dry ether (5ml). The reaction was stirred at room temperature for 30min and then refluxed for 1h. ^{1}H NMR spectroscopy of the reaction mixture after this time showed none of the expected product to be present.

Ethyl 3-hydroxy-2-methylene-3-(2-pyridyl)propanoate (**139**).[267] -

Method 1. A solution of ethyl acrylate (2.90g, 29mmol), pyridine-2-carboxaldehyde (2.95g, 28mmol) and DABCO (0.15g, 1.34mmol) in $CHCl_3$ (2ml) was allowed to stand at room temperature in a stoppered flask for 3d. The crude mixture was chromatographed [flash chromatography on silica gel; elution with EtOAc-hexane (7:3)] to afford ethyl 3-hydroxy-2-methylene-3-(2-pyridyl)propanoate (**139**) (5.56g, 96%) (Found: M^+ 207.090. Calc. for $C_{11}H_{13}NO_3$: *M*, 207.090); ν_{max} (thin film)/cm^{-1} 3375, 2980 and 1710; δ_H(400MHz;$CDCl_3$) 1.08 (3H, t, CH_3), 4.03 (2H, q, CH_2), 4.90 (1H, br s, OH), 5.53 (1H, s, C*H*OH), 5.84 and 6.24 (2H, 2 x s, C=CH_2), 7.06 (1H, ddd, 5′-H), 7.30 (1H, d, 3′-H), 7.53 (1H, td, 4′-H) and 8.37 (1H, d, 6′-H); δ_C(100MHz;$CDCl_3$) 13.7 (CH_3), 60.4 (CH_2), 72.0 (CHOH), 121.0 (C-3′), 122.3 (C-5′), 126.1 (C=*C*H_2), 136.5 (C-4′), 141.9 (*C*=CH_2), 148.0 (C-6′), 159.8 (C-2′) and 165.8 (CO); *m/z* 207 (M^+, 6%) and 190 (100%).

Alternative procedure. Employing the same method as above, ethyl acrylate (2.90g, 0.029mol), pyridine-2-carboxaldehyde (2.95g, 0.028mol) and 3-hydroxyquinuclidine (0.17g, 1.34mmol) in $CHCl_3$ (2ml) were reacted for 1d to yield ethyl 3-hydroxy-2-methylene-3-(2-pyridyl)propanoate (**139**) (2.65g, 46%).

Isopropyl 3-hydroxy-2-methylene-3-(2-pyridyl)propanoate (**140**). - A solution of isopropyl acrylate (**137a**) (3.31g, 29mmol), pyridine-2-carboxaldehyde (2.95g, 28mmol) and DABCO (0.15g, 1.34mmol) in $CHCl_3$ (2ml) was allowed to stand at room temperature in a stoppered flask for 3d. The crude mixture was chromatographed [flash chromatography on silica gel; elution with EtOAc-hexane (4:6)] to afford *isopropyl 3-hydroxy-2-methylene-3-(2-pyridyl)propanoate* (**140**) as a yellow oil (3.17g, 51%) (Found: M^+ 221.104. $C_{12}H_{15}NO_3$ requires *M*, 221.105); ν_{max} (thin film)/cm^{-1} 3390 (br), 2980 and 1715; δ_H(400MHz;$CDCl_3$) 1.10 and 1.12 (6H, 2 x d, *J* 6.3, 2 x CH_3), 4.95 {2H, septet [*J* 6.3, C*H*(CH_3)$_2$] and overlapping br s (OH)}, 5.55 (1H, s, C*H*OH), 5.87 and 6.28 (2H, 2 x s, C=CH_2), 7.12 (1H, dd, $J_{5',6'}$ 5.3 and

$J_{4',5'}$ 7.0, 5'-H), 7.35 (1H, d, $J_{3',4'}$ 7.9, 3'-H), 7.60 (1H, td, $J_{4',5'}$ and $J_{3',4'}$ 7.7 and $J_{4',6'}$ 1.6, 4'-H), 8.45 (1H, d, $J_{5',6'}$ 4.7, 6'-H); δ_C(100MHz;$CDCl_3$) 21.5 (2 x CH_3), 68.1 [$(CH_3)_2CH$], 72.2 (CHOH), 121.1 (C-3'), 122.3 (C-5'), 126.1 (C=CH_2), 136.6 (C-4'), 142.3 (C=CH_2), 148.1 (C-6'), 159.8 (C-2') and 165.5 (CO); *m/z* 221 (M^+, 1%) and 78 (100%).

Methyl 3-hydroxy-2-methylene-3-(6-methyl-2-pyridyl)propanoate (**141**). -
Method 1. A solution of methyl acrylate (2.50g, 29mmol), 6-methyl-2-pyridinecarboxaldehyde (3.39g, 28mmol) and 3-hydroxyquinuclidine (0.17g, 1.34mmol) in $CHCl_3$ (2ml) was allowed to react at room temperature. After 1d crystals appeared and a number of crops were collected and dried to afford *methyl 3-hydroxy-2-methylene-3-(6-methyl-2-pyridyl)propanoate* (**141**) as white crystals (5.42g, 94%), m.p. 84-85°C (from hexane) (Found: C, 63.5; H, 6.1; N, 6.6. $C_{11}H_{13}NO_3$ requires : C, 63.8; H, 6.3; N, 6.8%); ν_{max} (KBr)/cm^{-1} 3130 (br), 2860 and 1715; δ_H(400MHz;$CDCl_3$) 2.52 (3H, s, CH_3Ar), 3.73 (3H, s, CH_3O), 5.21 (1H, d, *J* 5.9, OH), 5.58 (1H, d, *J* 5.7, C*H*OH), 5.92 and 6.31 (2H, 2 x s, C=CH_2), 7.03 (1H, d, $J_{4',5'}$ 7.6, 5'-H), 7.14 (1H, d, $J_{3',4'}$ 7.7, 3'-H) and 7.52 (1H, t, $J_{4',5'}$ and $J_{3',4'}$ 7.7, 4'-H); δ_C(100MHz;$CDCl_3$) 24.2 (CH_3Ar), 51.8 (CH_3O), 71.2 (CHOH), 118.0 (C-3'), 122.1 (C-5'), 126.6 (C=CH_2), 137.0 (C-4'), 142.2 (C=CH_2), 157.0 (C-6'), 158.3 (C-2') and 166.6 (CO); *m/z* 207 (M^+, 5%) and 190 (100%).

Alternative procedure. Employing the same method as above, methyl acrylate (0.83g, 9.7mmol), 6-methyl-2-pyridinecarboxaldehyde (1.13g, 9.3mmol) and DABCO (0.05g, 0.45mmol) in $CHCl_3$ (0.7ml) were reacted for 3d to yield *methyl 3-hydroxy-2-methylene-3-(6-methyl-2-pyridyl)propanoate* (**141**) (1.60g, 83%).

4-Hydroxy-3-methylene-4-(2-pyridyl)-2-butanone (**142**). - A solution of methyl vinyl ketone (1.40g, 20mmol), pyridine-2-carboxaldehyde (2.14g, 20mmol) and 3-hydroxyquinuclidine (0.14g, 1.1mmol) in $CHCl_3$ (11ml) was allowed to stand at room

temperature in a stoppered flask for 1d. The crude mixture was chromatographed (flash chromatography on silica gel; elution with EtOAc) to afford *4-hydroxy-3-methylene-4-(2-pyridyl)-2-butanone* (**142**) as a yellow oil (2.86g, 81%); ν_{max} (thin film)/cm^{-1} 3350 (br) and 1685; δ_H(400MHz;$CDCl_3$) 2.19 (3H, s, CH_3), 4.99 (1H, br s, OH), 5.60 (1H, s, C*H*OH), 6.03 (1H, d, *J* 0.9, C=CH_2), 6.10 (1H, s, C=CH_2), 7.05 (1H, dd, $J_{5',6'}$ 5.0 and $J_{4',5'}$ 7.3, 5'-H), 7.30 (1H, d, $J_{3',4'}$ 7.9, 3'-H), 7.52 (1H, td, $J_{4',5'}$ and $J_{3',4'}$ 7.7 and $J_{4',6'}$ 1.8, 4'-H) and 8.37 (1H, d, $J_{5',6'}$ 4.6, 6'-H); δ_C(100MHz;$CDCl_3$) 26.1 (CH_3), 70.9 (CHOH), 121.2 (C-3'), 122.2 (C-5'), 126.4 (C=*C*H_2), 136.5 (C-4'), 147.9 (C-6'), 149.7 (*C*=CH_2), 159.9 (C-2') and 199.3 (CO); *m/z* 177 (M^+, 18%) and 78 (100%). 2-Acetylindolizine (**159**) (0.16g, 5%) was isolated together with compound (**142**).

Methyl 3-hydroxy-2-methylene-3-(2-quinolyl)propanoate (**143**). -

Method 1. A solution of methyl acrylate (0.63g, 7.3mmol), 2-quinoline-carboxaldehyde (1.10g, 7mmol) and DABCO (0.04g, 0.34mmol) in $CHCl_3$ (1ml) was allowed to stand at room temperature in a stoppered flask for 4d. The crude mixture was chromatographed [flash chromatography on silica gel; elution with EtOAc-hexane (5:5)] to afford *methyl 3-hydroxy-2-methylene-3-(2-quinolyl)propanoate* (**143**) as a yellow oil (1.67g, 98%) (Found: M^+ 243.089. $C_{14}H_{13}NO_3$ requires *M*, 243.089); ν_{max} (thin film)/cm^{-1} 3400 (br), 2970 and 1735; δ_H(400MHz;$CDCl_3$) 3.71 (3H, s, CH_3), 5.50 (1H, br s, OH), 5.77 (1H, s, C*H*OH), 5.97 and 6.36 (2H, 2 x s, C=CH_2), 7.43 (1H, d, $J_{3',4'}$ 8.3, 3'-H), 7.51 (1H, m, 6'-H), 7.68 (1H, m, 7'-H), 7.77 (1H, dd, $J_{5',6'}$ 8.3 and $J_{5',7'}$ 1.0, 5'-H), 8.07 (1H, d, $J_{7',8'}$ 8.6, 8'-H) and 8.09 (1H, d, $J_{3',4'}$ 8.4, 4'-H); δ_C(100MHz;$CDCl_3$) 51.8 (CH_3), 71.8 (CHOH), 118.8 and 126.5 (2 x ArC), 127.5 (C=*C*H_2), 127.5 (ArC), 127.6 (*C*=CH_2), 128.7, 129.7, 137.0, 141.7, 146.3 and 159.3 (6 x ArC) and 166.5 (CO); *m/z* 243 (M^+, 11%) and 226 (100%).

Alternative procedure. Employing the same method as above, methyl acrylate (2.50g, 0.029mol), 2-quinolinecarboxaldehyde (4.0g, 0.026mol) and 3-hydroxy-

quinuclidine (0.17g, 1.34mmol) in $CHCl_3$ (4ml) were reacted for 3d to yield *methyl 3-hydroxy-2-methylene-3-(2-quinolyl)propanoate* (**143**) (5.26g, 83%)

3-Hydroxy-2-methylene-3-(2-pyridyl)propanenitrile (**144**). - A solution of acrylonitrile (1.54g, 29mmol), pyridine-2-carboxaldehyde (2.95g, 28mol) and DABCO (0.15g, 1.34mmol) in $CHCl_3$ (2ml) was allowed to stand at room temperature in a stoppered flask for 4d. The crude mixture was chromatographed (flash chromatography on silica gel; elution with EtOAc) to afford *3-hydroxy-2-methylene-3-(2-pyridyl)-propanenitrile* (**144**) as colourless crystals (4.14g, 92%), m.p. 66-67°C (from hexane) (Found: M^+ 160.064. $C_9H_8N_2O$ requires *M*, 160.064); ν_{max} (thin film)/cm^{-1} 3200 (br), 2225 and 1600; δ_H(400MHz;$CDCl_3$) 5.27 (2H, 2 x overlapping s, C*H*OH and OH), 6.05 and 6.22 (2H, 2 x s, $C=CH_2$), 7.29 (1H, m, 5'-H), 7.39 (1H, d, 3'-H), 7.76 (1H, m, 4'-H) and 8.57 (1H, m, 6'-H); δ_C(100MHz;$CDCl_3$) 72.8 (CHOH), 116.7 (CN), 121.2 (C-3'), 123.7 (C-5'), 125.8 ($C=CH_2$), 130.9 ($C=CH_2$), 137.5 (C-4'), 148.5 (C-6') and 156.0 (C-2'); *m/z* 160 (M^+, 2%) and 143 (100%).

tert-*Butyl 3-hydroxy-2-methylene-3-(2-pyridyl)propanoate* (**145**). - *tert*-Butyl acrylate (**137b**) (1.35g, 10.5mmol), pyridine-2-carboxaldehyde (1.07g, 10mmol) and DABCO (0.056g, 0.5mmol) in $CHCl_3$ (0.5ml) were allowed to react at room temperature for 2 months. Evaporation of the solvent was followed by chromatography [flash chromatography on silica gel; elution with EtOAc-hexane (5:5)] to give tert-*butyl 3-hydroxy-2-methylene-3-(2-pyridyl)propanoate* (**145**) (0.46g, 20%); δ_H(60MHz;$CDCl_3$) 1.18 [9H, s, $(CH_3)_3C$], 4.54 (1H, br s, OH), 5.33 (1H, s, C*H*OH), 5.70 and 6.03 (2H, 2 x d, $CH_2=C$), 6.85-7.98 (3H, m, ArH) and 8.33 (1H, m, ArH).

Unsuccessful Baylis-Hillman reactions.

A number of substrates failed to undergo the Baylis-Hillman reaction with pyridine-2-carboxaldehyde in reactions set up identically to those described above. These

substrates were acrylic acid, acrylamide and acryloyl chloride. Reaction of methyl acrylate with 2-hydroxyacetophenone under Baylis-Hillman conditions also failed to occur.

3-Hydroxy-2-methylene-3-(2-pyridyl)propanal. -

Attempted preparation. To a solution of pyridine-2-carboxaldehyde (1.48g, 14mmol), 3-hydroxyquinuclidine (0.09g, 0.67mmol) and hydroquinone (0.08g) in $CHCl_3$ (1ml) at 0°C was added dropwise acrolein (0.79g, 0.94ml, 14mmol). The mixture rapidly thickened to form a black sludge and ^1H NMR spectroscopy showed no desired product to be present, most likely polymerization occurred even at low temperature and in the presence of hydroquinone.

Deuteriated 3-hydroxyquinuclidine (**151**). - A solution of 3-hydroxyquinuclidine (1g, 7.9mmol) in CD_3OD (1ml) was stirred for 20min, after which time the solvent was removed *in vacuo* and an additional 1ml portion of CD_3OD added. The process was repeated until no hydroxyl signal was visible in the ^1H NMR spectrum of the deuteriated product.

1-(2,2,2-Trichloro-1-hydroxyethyl)pyridine (**152**). -

Method 1. A solution of *N,N*-dimethylacrylamide (**138**) (1.44g, 15mmol), pyridine-2-carboxaldehyde (1.48g, 4mmol) and 3-hydroxyquinuclidine (0.09g, 0.7mmol) in $CHCl_3$ (2ml) was allowed to stand at room temperature in a stoppered flask for 1 month. The crude mixture was chromatographed [flash chromatography on silica gel; elution with EtOAc-hexane (5:5)] to afford 1-(2,2,2-trichloro-1-hydroxyethyl)pyridine (**152**) (0.87g, 28%), m.p. 107-108°C (from hexane) (lit.,[300] 110°C, from ether:CH_2Cl_2) (Found: C, 37.6; H, 2.8; N, 5.9. Calc. for $C_7H_6Cl_3NO$: C, 37.1; H, 2.7; N, 6.2%); ν_{max} (KBr)/cm^{-1} 3060 and 1600; δ_H(400MHz;$CDCl_3$) 5.22 (1H, d, C*H*OH), 5.98 (1H, d, OH), 7.38 (1H, ddd, ArH), 7.67 (1H, d, ArH), 7.77 (1H, td, ArH) and 8.64 (1H, d, ArH); δ_C(100MHz;$CDCl_3$) 82.0 (CHOH), 102.1 (CCl_3), 124.4, 125.0, 136.4, 147.9 and 152.6 (5 x ArC); *m/z* 226 (M^+, 6%) and 83 (100%).

Alternative procedure. Employing the same method as above, *N,N*-dimethylacrylamide (**138**) (2.88g, 0.029mol), pyridine-2-carboxaldehyde (2.95g, 0.028mol) and DABCO (0.15g, 1.34mmol) in $CHCl_3$ (2ml) were reacted for 2 weeks to yield 1-(2,2,2-trichloro-1-hydroxyethyl)pyridine (**152**) (0.40g, 6%).

Two reactions similar to that described above were set up, one without *N,N*-dimethylacrylamide (**138**), and one without catalyst. In both cases no product resulted, even after a number of months.

Methyl 3-acetoxy-2-methylene-3-(2-pyridyl)propanoate (**153**). - The preparative procedures for this compound can be found after those for compound **134**, beginning on page 164.

Ethyl 3-acetoxy-2-methylene-3-(2-pyridyl)propanoate (**154**). - Ethyl 3-hydroxy-2-methylene-3-(2-pyridyl)propanoate (**139**) (1.56g, 7.5mmol) was reacted and treated following the procedure described for the preparation of methyl 3-acetoxy-2-methylene-3-(2-pyridyl)propanoate (**153**) with the exception that heating was carried out for 40min. The crude mixture was chromatographed [flash chromatography on silica gel; elution with EtOAc-hexane (5:5)] to afford *ethyl 3-acetoxy-2-methylene-3-(2-pyridyl)propanoate* (**154**) as a yellow oil (1.32g, 70%) (Found: M^+ 249.099. $C_{13}H_{15}NO_4$ requires *M*, 249.100); ν_{max} (thin film)/cm^{-1} 1745 and 1720; δ_H(400MHz;$CDCl_3$) 1.17 (3H, t, *J* 7.2, CH_3CH_2), 2.11 (3H, s CH_3CO), 4.11 (2H, m, CH_2), 5.89 (1H, s, CHOAc), 6.45 and 6.75 (2H, 2 x s, $C=CH_2$), 7.22 (1H, ddd, $J_{5',6'}$ 4.8, $J_{4',5'}$ 7.5 and $J_{3',5'}$ 1.0, 5'-H), 7.45 (1H, d, $J_{3',4'}$ 7.8, 3'-H), 7.69 (1H, td, $J_{4',5'}$ and $J_{3',4'}$ 7.7 and $J_{4',6'}$ 1.8, 4'-H), 8.58 (1H, d, $J_{5',6'}$ 4.4, 6'-H); δ_C(100MHz;$CDCl_3$) 13.9 (CH_3CH_2), 21.0 (CH_3CO), 60.8 (CH_2O), 73.9 (CHOAc), 122.7 (C-3'), 123.0 (C-5'), 127.2 ($C=CH_2$), 136.6 (C-4'), 138.2 ($C=CH_2$), 149.4 (C-6'), 157.0 (C-2') and 164.9 and 169.5 (2 x CO); *m/z* 189 (M^+-$C_2H_4O_2$, 66%) and 117 (100%).

Ethyl 3-tosyloxy-2-methylene-3-(2-pyridyl)propanoate. -

Attempted preparation 1. A solution of ethyl 3-hydroxy-2-methylene-3-(2-pyridyl)-propanoate (**139**) (0.50g, 2.4mmol) and tosyl chloride (0.46g, 2.4mmol) in dry pyridine (20ml) was stirred at 4°C for 20h and then refluxed for 2.5h. TLC showed largely starting material to be present.

Attempted preparation 2. A solution of ethyl 3-hydroxy-2-methylene-3-(2-pyridyl)-propanoate (**139**) (0.33g, 1.59mmol) and tosyl chloride (0.33g, 1.75mmol) in dry THF (10ml) was stirred at room temperature for 2d and then refluxed for 2.5h. Once again TLC showed predominantly starting materials to be present.

Attempted preparation 3. To a previously flame-dried, nitrogen-flushed four-necked round-bottomed flask fitted with a septum, thermometer, dropping funnel and reflux condensor attached to a N_2 line, was added NaH (0.28g, 5.8mmol) in dry THF (25ml). To this was added ethyl 3-hydroxy-2-methylene-3-(2-pyridyl)propanoate (**139**) (1.0g, 4.8mmol) through the dropping funnel, and the resulting mixture was stirred for 1h at room temperature. Tosyl chloride (1.01g, 5.3mmol) in dry THF (10ml) was added dropwise by syringe to this mixture and stirring was continued for an additional 1.5h. TLC of the reaction mixture at this stage showed a large number of compounds to be present and thus the reaction was abandoned.

Ethyl 3-mesyloxy-2-methylene-3-(2-pyridyl)propanoate. -

Attempted preparation. To ethyl 3-hydroxy-2-methylene-3-(2-pyridyl)propanoate (**139**) (1.0g, 4.8mmol) in dry THF (10ml) in a two-necked round-bottomed flask fitted with a dropping funnel and a reflux condensor leading to a N_2 line, was added powdered KOH (0.39g, 6.9mmol). This mixture was cooled to 0°C in an ice bath, and mesyl chloride (0.63g, 5.5mmol) in dry THF (5ml) was added dropwise through the dropping funnel. The resulting mixture was stirred at 0°C for 5h, at room temperature for 12h and heated under reflux for 3h. TLC of the mixture at this stage showed mainly starting material to be present.

Isopropyl 3-acetoxy-2-methylene-3-(2-pyridyl)propanoate (**155**). - Isopropyl 3-hydroxy-2-methylene-3-(2-pyridyl)propanoate (**140**) (1.0g, 4.5mmol) was reacted and treated following the procedure described for the preparation of methyl 3-acetoxy-2-methylene-3-(2-pyridyl)propanoate (**153**). The crude mixture was chromatographed [flash chromatography on silica gel; elution with EtOAc-hexane (4:6)] to afford *isopropyl 3-acetoxy-2-methylene-3-(2-pyridyl)propanoate* (**155**) as a yellow oil (0.74g, 62%) (Found: M^+ - $C_2H_4O_2$ 203.094. $C_{12}H_{13}NO_2$ requires *M*, 203.094); ν_{max} (thin film)/cm^{-1} 1745 and 1715; δ_H(400MHz;$CDCl_3$) 1.03 and 1.09 (6H, 2 x d, *J* 6.3, 2 x CH_3), 2.04 (3H, s, CH_3CO), 4.91 [1H,sept, *J* 6.3, $CH(CH_3)_2$], 5.77 (1H, s, CHOAc), 6.35 and 6.63 (2H, 2 x s, $C=CH_2$), 7.12 (1H, ddd, $J_{4',5'}$ 7.4, $J_{5',6'}$ 4.9 and $J_{3',5'}$ 1.1, 5'-H), 7.34 (1H, d, $J_{3',4'}$ 7.9, 3'-H), 7.60 (1H, td, $J_{4',5'}$ and $J_{3',4'}$ 7.7 and $J_{4',6'}$ 1.8, 4'-H), 8.48 (1H, dd, $J_{5',6'}$ 4.8 and $J_{4',6'}$ 0.7, 6'-H); δ_C(100MHz;$CDCl_3$) 20.6 (CH_3CO), 21.2 and 21.3 (2 x CH_3), 68.1 [$(CH_3)_2CH$], 73.7 (*C*HOAc), 122.3 (C-3'), 122.6 (C-5'), 126.5 ($C=CH_2$), 136.2 (C-4'), 138.6 ($C=CH_2$), 149.1 (C-6'), 157.0 (C-2') and 164.1 and 169.1 (2 x CO); *m/z* 203 (M^+ - $C_2H_4O_2$, 31%) and 161 (100%).

Methyl 3-acetoxy-2-methylene-3-(6-methyl-2-pyridyl)propanoate (**156**). - Methyl 3-hydroxy-2-methylene-3-(6-methyl-2-pyridyl)propanoate (**141**) (0.70g, 3.4mmol) was reacted and treated following the procedure described for the preparation of methyl 3-acetoxy-2-methylene-3-(2-pyridyl)propanoate (**153**). The crude mixture was chromatographed [flash chromatography on silica gel; elution with EtOAc-hexane (3:7)] to afford *methyl 3-acetoxy-2-methylene-3-(6-methyl-2-pyridyl)propanoate* (**156**) as a yellow oil (0.44g, 52%) (Found: M^+ - $C_2H_3O_2$ 190.087. $C_{11}H_{12}NO_2$ requires *M*, 190.087); ν_{max} (thin film)/cm^{-1} 1750 and 1730; δ_H(400MHz;$CDCl_3$) 2.10 (3H, s, CH_3CO), 2.48 (3H, s, CH_3Ar), 3.67 (3H, s, CH_3O), 5.78 (1H, s, CHOAc), 6.41 and 6.67 (2H, 2 x s, $C=CH_2$), 7.03 (1H, d, $J_{4',5'}$ 7.7, 5'-H), 7.15 (1H, d, $J_{3',4'}$ 7.7, 3'-H), 7.53 (1H, t, $J_{4',5'}$ and $J_{3',4'}$ 7.7, 4'-H); δ_C(100MHz;$CDCl_3$) 21.0 and 24.4 (2 x CH_3), 51.8 (CH_3O), 74.0 (*C*HOAc), 119.0 (C-3'), 122.5 (C-5'), 127.6 ($C=CH_2$), 136.6 (C-4'), 138.4 ($C=CH_2$), 156.2 (C-6'), 158.2 (C-2') and 165.5 and 169.5 (2 x CO); *m/z*

190 (M^+ - $C_2H_3O_2$, 19%) and 83 (100%). *Methyl 5-methylindolizine-2-carboxylate* (**162**) (0.23g, 36%) was isolated together with compound (**156**).

Methyl 3-acetoxy-2-methylene-3-(2-quinolyl)propanoate (**157**). - Methyl 3-hydroxy-2-methylene-3-(2-quinolyl)propanoate (**143**) (1.73g, 7.1mmol) was reacted and treated following the procedure described for the preparation of methyl 3-acetoxy-2-methylene-3-(2-pyridyl)propanoate (**153**). The crude mixture was chromatographed [flash chromatography on silica gel; elution with EtOAc-hexane (3:7)] to afford *methyl 3-acetoxy-2-methylene-3-(2-quinolyl)propanoate* (**157**) as a yellow oil (1.15g, 57%) (Found: M^+ 285.100. $C_{16}H_{15}NO_4$ requires *M*, 285.100); ν_{max} (thin film)/cm^{-1} 1750 and 1730; δ_H(400MHz;$CDCl_3$) 2.16 (3H, s, CH_3CO), 3.69 (3H, s, CH_3O), 5.92 (1H, s, CHOAc), 6.49 and 6.90 (2H, 2 x s, $C=CH_2$), 7.52 [2H, td ($J_{6',7'}$ and $J_{5',6'}$ 8.0 and $J_{6',8'}$ 1.1, 6'-H) overlapping d ($J_{3',4'}$ 8.6, 3'-H)], 7.68 (1H, m, 7'-H), 7.78 (1H, dd, $J_{5',6'}$ 8.1 and $J_{5',7'}$ 1.1, 5'-H), 8.07 (1H, d, $J_{7',8'}$ 8.3, 8'-H) and 8.14 (1H, d, $J_{3',4'}$ 8.4, 4'-H); δ_C(100MHz;$CDCl_3$) 21.01 (CH_3CO), 52.0 (CH_3O), 74.5 (*C*HOAc), 119.9 and 126.7 (2 x ArC), 127.46 ($C=CH_2$), 127.53 ($C=CH_2$), 127.9, 129.5, 129.6, 136.7, 138.2, 147.6 and 157.2 (7 x ArC) and 165.5 and 169.6 (2 x CO); *m/z* 285 (M^+, 0.6%) and 226 (100%). *Methyl pyrrolo[1,2-a]quinoline-2-carboxylate* (**163**) (0.41g, 26%) was isolated together with compound (**157**).

3-Acetoxy-2-methylene-3-(2-pyridyl)propanenitrile (**158**). - 3-Hydroxy-2-methylene-3-(2-pyridyl)propanenitrile (**144**) (1.0g, 6.2mmol) was reacted and treated following the procedure described for the preparation of methyl 3-acetoxy-2-methylene-3-(2-pyridyl)propanoate (**153**). The crude mixture was chromatographed [flash chromatography on silica gel; elution with EtOAc-hexane (5:5)] to afford *3-acetoxy-2-methylene-3-(2-pyridyl)propanenitrile* (**158**) as a yellow oil (0.72g, 58%) (Found: M^+ 202.074. $C_{11}H_{10}N_2O_2$ requires *M*, 202.074); ν_{max} (thin film)/cm^{-1} 2225 and 1750; δ_H(400MHz;$CDCl_3$) 2.14 (3H, s, CH_3CO), 6.08 and 6.11 (2H, 2 x s, $C=CH_2$), 6.33 (1H, s, C*H*OAc), 7.22 (1H, dd, $J_{5',6'}$ 4.8 and $J_{4',5'}$ 7.6, 5'-H), 7.43 (1H, d, $J_{3',4'}$ 7.7,

3′-H), 7.70 (1H, td, $J_{4',5'}$ and $J_{3',4'}$ 7.7 and $J_{4',6'}$ 1.2, 4′-H) and 8.55 (1H, d, $J_{5',6'}$ 4.9, 6′-H); δ_C(100MHz;$CDCl_3$) 20.7 (CH_3), 75.1 (*C*HOAc), 115.8 (CN), 121.1 (C-3′), 121.4 (*C*=CH_2), 123.6 (C-5′), 133.5 (C=*C*H_2), 137.1 (C-4′), 149.5 (C-6′), 154.8 (C-2′) and 169.0 (CO); *m/z* 202 (M^+, 0.3%) and 143 (100%).

4-Acetoxy-3-methylene-4-(2-pyridyl)-2-butanone. -

Attempted preparation. 4-Hydroxy-3-methylene-4-(2-pyridyl)-2-butanone (**142**) (1.05g, 5.9mmol) was heated at 100°C for 30min in the presence of Ac_2O (5ml). TLC showed a number of compounds to be present, 2-acetylindolizine (**159**) being the major component. For this reason, direct cyclization of 4-hydroxy-3-methylene-4-(2-pyridyl)-2-butanone (**142**) was carried out.

2-Acetylindolizine (**159**). - 4-Hydroxy-3-methylene-4-(2-pyridyl)-2-butanone (**142**) (1.01g, 5.7mmol) was heated at 100°C for 40min in a round-bottomed flask fitted with a reflux condensor. The resulting mixture was chromatographed (flash chromatography on silica gel; elution with EtOAc) to yield 2-acetylindolizine (**159**) as colourless crystals (0.48g, 53%), m.p. 123-124°C (from EtOAc) (lit.,[96] 127°C); ν_{max} (KBr)/cm^{-1} 1675; δ_H(400MHz;$CDCl_3$) 2.48 (3H, s, CH_3), 6.47 (1H, td, $J_{5,6}$ and $J_{6,7}$ 6.8 and $J_{6,8}$ 1.1, 6-H), 6.63 (1H, ddd, $J_{7,8}$ 9.2, $J_{6,7}$ 6.5 and $J_{5,7}$ 1.0, 7-H), 6.74 (1H, s, 1-H), 7.30 (1H, d, $J_{7,8}$ 9.2, 8-H), 7.71 (1H, m, 3-H) and 7.79 (1H, dd, $J_{5,6}$ 7.1 and $J_{5,7}$ 1.0, 5-H); δ_C(100MHz;$CDCl_3$) 27.5 (CH_3), 99.3 (C-1), 112.5 (C-6), 115.1 (C-3), 118.3 (C-7), 120.3 (C-8), 125.4 (C-5), 128.4 (C-2), 133.0 (C-9) and 194.8 (CO).

Ethyl indolizine-2-carboxylate (**160**). -

Method 1. Ethyl 3-acetoxy-2-methylene-3-(2-pyridyl)propanoate (**154**) (0.48g, 1.9mmol) was heated at 120°C for 1h and the resulting mixture was chromatographed [flash chromatography on silica gel; elution with EtOAc-hexane (5:5)] to yield *ethyl indolizine-2-carboxylate* (**160**) as a yellow oil (0.14g, 38%) (Found: M^+ 189.079. $C_{11}H_{11}NO_2$ requires *M*, 189.079); ν_{max} (thin film)/cm^{-1} 2975 and 1710;

δ_H(400MHz;$CDCl_3$) 1.38 (3H, t, *J* 7.2, CH_3), 4.35 (2H, q, *J* 7.1, CH_2), 6.49 (1H, td, $J_{6,7}$ and $J_{5,6}$ 6.8 and $J_{6,8}$ 1.1, 6-H), 6.65 (1H, ddd, $J_{6,7}$ 6.6, $J_{7,8}$ 9.1 and $J_{5,7}$ 1.0, 7-H), 6.83 (1H, s, 1-H), 7.34 (1H, d, $J_{7,8}$ 9.1, 8-H), 7.79 (1H, m, 3-H) and 7.83 (1H, dd, $J_{5,6}$ 7.1 and $J_{5,7}$ 1.0, 5-H); δ_C(100MHz;$CDCl_3$) 14.4 (CH_3), 60.1 (CH_2), 100.3 (C-1), 112.1 (C-6), 115.7 (C-3), 118.0 (C-7), 120.0 (C-2), 120.2 (C-8), 125.2 (C-5), 132.7 (C-9) and 165.1 (CO); *m/z* 189 (M^+, 52%) and 117 (100%).

Method 2. Similar reactions to that above were carried out with varying reaction times and temperatures. Heating ethyl 3-acetoxy-2-methylene-3-(2-pyridyl)propanoate (**154**) for 1h at 120°C yielded 37% of the purified product while heating at 100°C for 4h yielded 26% of the purified *ethyl indolizine-2-carboxylate* (**160**).

Method 3. Ethyl 3-hydroxy-2-methylene-3-(2-pyridyl)propanoate (**139**) (4.15g, 20mmol) was heated in a Kügelrohr at 145°C for 1h and at 155°C for 1h. The crude mixture was chromatographed [flash chromatography on silica gel; elution with EtOAc-hexane (3:7)] to yield *ethyl indolizine-2-carboxylate* (**160**) (0.36g, 10%).

Isopropyl indolizine-2-carboxylate (**161**). - Isopropyl 3-acetoxy-2-methylene-3-(2-pyridyl)propanoate (**155**) (0.40g, 1.5mmol) was heated at 120°C for 1h and the resulting mixture was chromatographed [flash chromatography on silica gel; elution with EtOAc-hexane (4:6)] to afford *isopropyl indolizine-2-carboxylate* (**161**) as a yellow oil (0.08g, 26%) (Found: M^+ 203.094. $C_{12}H_{13}NO_2$ requires *M*, 203.094); ν_{max} (thin film)/cm^{-1} 2980 and 1705; δ_H(400MHz;$CDCl_3$) 1.35 (6H, d, *J* 6.3, 2 x CH_3), 5.24 [1H, sept, *J* 6.3, $(CH_3)_2CH$], 6.48 (1H, td, $J_{6,7}$ and $J_{5,6}$ 6.8 and $J_{6,8}$ 1.1, 6-H), 6.64 (1H, ddd, $J_{7,8}$ 9.1, $J_{6,7}$ 6.5 and $J_{5,7}$ 1.0, 7-H), 6.81 (1H, s, 1-H), 7.32 (1H, d, $J_{7,8}$ 9.1, 8-H), 7.77 (1H, m, 3-H) and 7.82 (1H, dd, $J_{5,6}$ 7.1 and $J_{5,7}$ 1.0, 5-H); δ_C(100MHz;$CDCl_3$) 22.0 (2 x CH_3), 67.3 [$(CH_3)_2CH$], 100.4 (C-1), 112.1 (C-6), 115.7 (C-3), 117.9 (C-7), 120.2 (C-8), 120.5 (C-2), 125.2 (C-5), 132.7 (C-9) and 164.6 (CO); *m/z* 203 (M^+, 20%) and 161 (100%).

Methyl 5-methylindolizine-2-carboxylate (**162**). - Methyl 3-acetoxy-2-methylene-3-(6-methyl-2-pyridyl)propanoate (**156**) (0.57g, 2.3mmol) was heated at 120°C for 1h and the resulting mixture was chromatographed [flash chromatography on silica gel; elution with EtOAc-hexane (3:7)] to afford *methyl 5-methylindolizine-2-carboxylate* (**162**) as a yellow oil (0.36g, 84%) (Found: M^+ 189.079. $C_{11}H_{11}NO_2$ requires *M*, 189.079); ν_{max} (thin film)/cm^{-1} 2950 and 1720; δ_H(400MHz;$CDCl_3$) 2.42 (3H, s, CH_3Ar), 3.86 (3H, s, CH_3O), 6.36 (1H, d, $J_{6,7}$ 6.6, 6-H), 6.65 (1H, dd, $J_{6,7}$ 6.6 and $J_{7,8}$ 9.0, 7-H), 6.88 (1H, m, 1-H), 7.27 (1H, d, $J_{7,8}$ 9.3, 8-H) and 7.68 (1H, m, 3-H); δ_C(100MHz;$CDCl_3$) 18.2 (CH_3Ar), 51.2 (CH_3O), 100.8 (C-1), 111.2 (C-6), 112.8 (C-3), 117.8 (C-8), 118.4 (C-7), 119.3 (C-2), 132.9 (C-9), 133.3 (C-5) and 165.6 (CO); *m/z* 189 (M^+, 100%).

Methyl pyrrolo[1,2-a]quinoline-2-carboxylate (**163**). - Methyl 3-acetoxy-2-methylene-3-(2-quinolyl)propanoate (**157**) (1.15g, 4.0mmol) was heated at 120°C for 1h and the resulting mixture was chromatographed [flash chromatography on silica gel; elution with EtOAc-hexane (3:7)] to afford *methyl pyrrolo[1,2-a]quinoline-2-carboxylate* (**163**) as colourless crystals (0.77g, 86%), m.p. 109-110°C (from hexane) (Found: M^+ 225.079. $C_{14}H_{11}NO_2$ requires *M*, 225.079); ν_{max} (KBr)/cm^{-1} 1710; δ_H(400MHz;$CDCl_3$) 3.89 (3H, s, CH_3), 6.88 (1H, m, 3-H), 7.00 (1H, d, $J_{4,5}$ 9.4, 5-H), 7.24 (1H, d, $J_{4,5}$ 9.4, 4-H), 7.36 (1H, td, $J_{6,7}$ and $J_{7,8}$ 7.5 and $J_{7,9}$ 1.1, 7-H), 7.51 (1H, m, 8-H), 7.61 (1H, dd, $J_{6,7}$ 7.8 and $J_{6,8}$ 1.4, 6-H), 7.88 (1H, d, $J_{8,9}$ 8.3, 9-H) and 8.38 (1H, m, 1-H); δ_C(100MHz;$CDCl_3$) 51.4 (CH_3), 103.5 (C-3), 114.3 (C-9), 115.7 (C-1), 118.87 (C-2), 118.90 (C-4), 120.1 (C-5), 124.1 (C-5a), 124.6 (C-7), 128.0 (C-8), 128.6 (C-6), 131.2 (C-3a), 132.8 (C-9a) and 165.3 (CO); *m/z* 225 (M^+, 100%).

2-Cyanoindolizine (**164**). - 3-Acetoxy-2-methylene-3-(2-pyridyl)propanenitrile (**158**) (0.31g, 1.5mmol) was heated at 120°C for 1h and the resulting mixture was chromatographed [flash chromatography on silica gel; elution with EtOAc-hexane (5:5)] to afford *2-cyanoindolizine* (**164**) as colourless crystals (0.07g, 32%), m.p.

67.5-69°C (from hexane) (Found: C, 76.2; H, 4.3; N, 20.0. $C_9H_6N_2$ requires C, 76.0; H, 4.25; N, 19.7%); ν_{max} (KBr)/cm^{-1} 3110 and 2210; δ_H(400MHz;$CDCl_3$) 6.62 (1H, td, $J_{6,7}$ and $J_{5,6}$ 6.8 and $J_{6,8}$ 1.1, 6-H), 6.67 (1H, s, 1-H), 6.77 (1H, ddd, $J_{6,7}$ 6.6, $J_{7,8}$ 9.2 and $J_{5,7}$ 0.9, 7-H), 7.36 (1H, d, $J_{7,8}$ 9.2, 8-H), 7.66 (1H, m, 3-H) and 7.87 (1H, dd, $J_{5,6}$ 7.1 and $J_{5,7}$ 1.0, 5-H); δ_C(100MHz;$CDCl_3$) 97.4 (C-2), 102.5 (C-1), 113.0 (C-6), 116.4 (CN), 117.5 (C-3), 119.4 and 119.7 (C-7 and C-8), 125.1 (C-5) and 132.7 (C-9); *m/z* 142 (M^+, 100%).

Methyl pyrazine-2-carboxylate (**166**). - HCl gas, produced by dropping concentrated HCl into concentrated H_2SO_4, was bubbled through methanol (60ml) for 2h after which pyrazine-2-carboxylic acid (6.0g, 0.048mol) was added and the solution allowed to stir at room temperature for 2d. Volatiles were removed *in vacuo* and ether (100ml) was added to the residue. Solid $NaHCO_3$ was added until effervescence ceased, the ethereal solution was decanted from any solid material and 3 further portions of ether were added to the solid material in order to extract all the product from the solid residue. The combined ether extracts were dried ($MgSO_4$) and the solvent removed *in vacuo* to yield methyl pyrazine-2-carboxylate (**166**) (6.07g, 92%), m.p. 58°C (lit.,[302] 59°C); ν_{max} (KBr)/cm^{-1} 1720; δ_H(60MHz;$CDCl_3$) 4.04 (1H, s, CH_3), 8.83 (2H, m, ArH) and 9.33 (1H, m, ArH).

Pyrazine-2-carboxaldehyde (**167**).[302] - To a solution of methyl pyrazine-2-carboxylate (**166**) (2.40g, 17.4mmol) in dry THF (70ml) in a three-necked round-bottomed flask fitted with a thermometer, septum and condensor under N_2 at -70°C, was added $LiAlH_4$ (0.33g, 8.69mmol) in dry THF (25ml) dropwise over 40min. After the addition of $LiAlH_4$, the reaction was allowed to stir at -70°C for 45min after which glacial acetic acid (4ml) was added to quench the reaction. Volatiles were removed *in vacuo*, water (50ml) added to the residue, and the pH adjusted to 4. The aqueous layer was extracted with $CHCl_3$ (4 x 50ml), the combined organic extracts washed with aq. $NaHCO_3$ (100ml) and then dried ($MgSO_4$). The solvent was removed *in*

vacuo and the resulting oil was purified by chromatography [flash chromatography on silica gel; elution with EtOAc-hexane (5:5)] to yield pyrazine-2-carboxaldehyde (**167**) (0.53g, 28%); ν_{max} (thin film)/cm^{-1} 1720; δ_H(60MHz;$CDCl_3$) 8.94 (2H, m, ArH), 9.25 (1H, m, ArH) and 10.18 (1H, s, CHO).

Methyl 3-hydroxy-2-methylene-3-(2-pyrazinyl)propanoate (**168**). - A solution of methyl acrylate (0.14g, 1.67mmol), pyrazine-2-carboxaldehyde (**167**) (0.18g, 1.67mmol) and 3-hydroxyquinuclidine (0.01g, 0.08mmol) in $CHCl_3$ (1.5ml) was allowed to stand at room temperature in a stoppered flask for 1d. The crude mixture was chromatographed (flash chromatography on silica gel; elution with EtOAc) to afford *methyl 3-hydroxy-2-methylene-3-(2-pyrazinyl)propanoate* (**168**) (0.18g,56%); δ_H(60MHz;$CDCl_3$) 3.71 (3H, s, CH_3), 4.77 (1H, br s, OH), 5.65 (1H, s, C*H*OH), 6.04 and 6.38 (2H, 2 x s, C=CH_2), 8.47 (2H, m, ArH) and 8.77 (1H, m, ArH).

Methyl 3-acetoxy-2-methylene-3-(2-pyrazinyl)propanoate (**169**). - Methyl 3-hydroxy-2-methylene-3-(2-pyrazinyl)propanoate (**168**) (0.15g, 0.77mmol) was heated with Ac_2O (3ml) at 120°C for 10h in a flask fitted with a reflux condensor and a drying tube. The cooled mixture was poured into $NaHCO_3$-ice; the remaining mixture was stirred for 30min and then extracted with Et_2O (2 x 60ml). The organic layer was washed sequentially with aqueous $NaHCO_3$ (60ml) and brine (60ml), and dried over $MgSO_4$. The solvent was evaporated and the residue chromatographed [flash chromatography on silica gel; elution with EtOAc-hexane (7:3)] to afford *methyl 3-acetoxy-2-methylene-3-(2-pyrazinyl)propanoate* (**169**) (0.04g,22%); δ_H(60MHz;$CDCl_3$) 2.14 (3H, s, CH_3CO), 3.70 (3H, s, CH_3O), 4.05 (1H, s, CHOAc), 4.55 and 4.79 (2H, 2 x s, C=CH_2), 8.55 (2H, m, ArH) and 8.78 (1H, m, ArH).

3-[(2-Formylphenoxy)methyl]coumarin (**174**). -

Method 1. A solution of methyl acrylate (1.81g, 21mmol), salicylaldehyde (2.44g, 20mmol) and DABCO (0.112g, 1mmol) in $CHCl_3$ (1ml) was allowed to stand at room

temperature in a stoppered flask. After 3d crystalline material began to deposit and several crops were collected at the pump, washed with acetone and dried to afford *3-[(2-formylphenoxy)methyl]coumarin* (**174**) (0.54g,19%), m.p. 180-182°C; (Found: M^+ 280.073. $C_{17}H_{12}O_4$ requires *M*, 280.073); ν_{max} (thin film)/cm^{-1} 1715 and 1675; δ_H(400MHz;$CDCl_3$) 5.13 (2H, s, CH_2O), 7.03-7.13 (2H, m, ArH), 7.23-7.38 (2H, m, ArH), 7.49-7.60 (3H, m, ArH), 7.81-7.88 (1H, dd, ArH), 7.96 (1H, s, ArH) and 10.58 (1H, s, CHO); δ_C(100MHz;$CDCl_3$) 65.0 (CH_2O), 112.8, 116.7, 118.8, 121.6, 124.0, 124.8, 125.2, 128.1, 129.6, 131.8, 136.2, 139.2, 153.2 and 160.0 (14xArC), 160.1 (CO) and 189.3 (CHO).

Alternative procedure. Employing the same method as above, methyl acrylate (1.81g, 21mmol), salicylaldehyde (2.44g, 20mmol) and 3-hydroxyquinuclidine (0.13g, 1mmol) in $CHCl_3$ (1ml) were reacted for 2 weeks to yield *3-[(2-formylphenoxy)-methyl]coumarin* (**174**) (0.09g, 3%).

Attempted preparation 1. A similar reaction to that described above was set up reacting salicylaldehyde (2.44g, 20mmol) and methyl acrylate (1.81g, 21mmol) in $CHCl_3$ (1ml), without the tertiary amine catalyst. No product resulted, even after standing for a number of weeks at room temperature.

Attempted preparation 2. Salicylaldehyde (2.44g, 20mmol), methyl acrylate (1.81g, 21mmol) and 1,8-bis(dimethylamino)naphthalene (0.21g, 1mmol) in $CHCl_3$ (1ml) were allowed to react at room temperature for 4 weeks. No crystals appeared, and TLC showed only starting materials to be present.

2-Formylphenyl acrylate (**175**). - Triethylamine (5.6g, 55mmol) was added slowly to a cooled, stirred solution of salicylaldehyde (5.6g, 4.9ml, 46mmol) in dry dichloromethane (100ml) in a three-necked round-bottomed flask fitted with a dropping funnel, thermometer and condensor with a drying tube. To this was added dropwise acryloyl chloride (5.0g, 55mmol) in dry dichloromethane (25ml). The

reaction flask was removed from the ice bath and stirring was continued at room temperature for 2h until TLC showed no salicylaldehyde to be present. The reaction mixture was washed with water (2 x 100ml) and 0.5N-HCl (100ml) followed by drying over $MgSO_4$ and removal of the solvent *in vacuo*. Distillation under reduced pressure in the presence of hydroquinone (0.3g) yielded *2-formylphenyl acrylate* (**175**) (2.47g, 31%), b.p. 148-150°C/17mmHg (Found: C,67.7; H,4.4. $C_{10}H_8O_3$ requires: C,68.2; H,4.6%); ν_{max} (thin film)/cm^{-1} 1750 and 1705; δ_H(400MHz;$CDCl_3$) 6.11 (1H, dd, CH_2=CH), 6.39 (1H, dd, CH_2=C*H*), 6.68 (1H, dd, CH_2=CH), 7.25 (1H, d, ArH), 7.40 (1H, t, ArH), 7.65 (1H, m, ArH), 7.92 (1H, dd, ArH) and 10.15 (1H, s, CHO); δ_C(100MHz;$CDCl_3$) 123.3 and 126.4 (CH=CH_2), 127.1 (ArC), 128.1 (ArC), 130.3 (ArC), 133.7(ArC), 135.3(ArC), 151.8(ArC) and 164.1 and 188.4 (2xCO); *m/z* 176 (M^+, 3%) and 55(100%).

Attempted preparation of coumarin 174 from compound 175. Salicylaldehyde (1.22g, 10mmol), 2-formylphenyl acrylate (**175**) (1.76g, 10mmol) and DABCO (0.006g, 0.5mmol) in $CHCl_3$ were allowed to stand at room temperature for 1 week. TLC showed only starting material to be present.

Attempted preparation of a dibromo derivative of coumarin 174. 5-Bromosalicylaldehyde (5.63g, 28mmol), methyl acrylate (2.50g, 29mmol) and 3-hydroxyquinuclidine (0.17g, 1.34mmol) in $CHCl_3$ (8ml) were allowed to react at room temperature for 2 weeks. TLC showed only starting materials to be present.

Attempted preparation of a dimethoxy derivative of coumarin 174. 2-Hydroxy-5-methoxybenzaldehyde (4.26g, 28mmol), methyl acrylate (2.50g, 29mmol) and 3-hydroxyquinuclidine (0.17g, 1.34mmol) in $CHCl_3$ (2ml) were allowed to react at room temperature for 1 week, after which time a pebble-like material became visible in the reaction vessel. This material was extremely insoluble and could not be identified, but was not thought to be the desired material.

Indolizine-2-carboxylic acid (**176**).[81] -

Method 1. Methyl indolizine-2-carboxylate (**135**) (1.0g, 5.7mmol) was added to a solution of KOH (2.0g, 36mmol) in EtOH (40ml) in a round-bottomed flask fitted with a reflux condensor, and the mixture was refluxed for 16h. Sufficient water was added to solubilize the solid material present in the reaction mixture and the pH was adjusted with dilute HCl to 2-3. The aqueous layer was extracted with EtOAc (2 x 100ml), the organic layer dried ($MgSO_4$) and the solvent removed *in vacuo* to afford indolizine-2-carboxylic acid (**176**) as cream-coloured crystals (0.92g, 100%), m.p. 220-222°C (from EtOH) (lit.,[80] 240-241°C); ν_{max} (KBr)/cm^{-1} 2920, 2580 and 1670; δ_H(400MHz;$CDCl_3$/DMSO) 2.92 (1H, br s, OH), 6.13 (1H, td, $J_{5,6}$ and $J_{6,7}$ 6.8 and $J_{6,8}$ 1.3, 6-H), 6.27 (1H, ddd, $J_{7,8}$ 9.2, $J_{6,7}$ 6.5 and $J_{5,7}$ 1.0, 7-H), 6.35 (1H, s, 1-H), 6.94 (1H, d, $J_{7,8}$ 9.1, 8-H), 7.42 (1H, d, $J_{1,3}$ 0.9, 3-H) and 7.55 (1H, dd, $J_{5,6}$ 7.0 and $J_{5,7}$ 1.1, 5-H).

Alternative procedure. Methyl indolizine-2-carboxylate (**135**) (2.0g, 11.4mmol), 8M-H_2SO_4 (5ml) and glacial acetic acid (10ml) were refluxed for 12h. After cooling, water (20ml) was added and the mixture was allowed to stand overnight. The resulting precipitate was removed by filtration, and basification of the filtrate with $NaHCO_3$ produced further precipitate which was collected and added to the first precipitate obtained. Any remaining water was removed from the precipitate with a vacuum pump to afford indolizine-2-carboxylic acid (**176**) (1.30g, 72%).

Pyrrolo[1,2-a]quinoline-2-carboxylic acid (**177**). - Methyl pyrrolo[1,2-*a*]quinoline-2-carboxylate (**163**) (2.14g, 9.5mmol) and KOH (3.40g, 61mmol) were dissolved in EtOH (70ml) and refluxed for 16h. Water (70ml) was added and the pH adjusted to 4, followed by extraction of the aqueous layer with Et_2O (2 x 150ml). The organic extracts were washed with water (150ml) and brine (150ml) and dried over $MgSO_4$. Evaporation of the solvent yielded pyrrolo[1,2-a]quinoline-2-carboxylic acid (**177**) as a yellow solid (1.88g, 94%), m.p. 234-236°C (from EtOH); ν_{max} (KBr)/cm^{-1}

2850 (br) and 1675; δ_H(400MHz;$CDCl_3$/DMSO) 2.75 (1H, br s, OH), 6.32 (1H, d, $J_{1,3}$ 1.3, 3-H), 6.52 (1H, d, $J_{4,5}$ 9.4, 5-H), 6.75 (1H, d, $J_{4,5}$ 9.4, 4-H), 6.85 (1H, t, $J_{6,7}$ and $J_{7,8}$ 7.5, 7-H), 7.01 (1H, td, $J_{7,8}$ and $J_{8,9}$ 7.8 and $J_{6,8}$ 1.2, 8-H), 7.13 (1H, d, $J_{6,7}$ 6.9, 6-H), 7.49 (1H, d, $J_{8,9}$ 8.3, 9-H) and 7.93 (1H, s, 1-H).

Indolizine-2-carbonyl chloride. -

Attempted preparation. Indolizine-2-carboxylic acid (**176**) (0.94g, 5.8mmol), $SOCl_2$ (0.55ml, 7.6mmol), DMF (0.11ml, 1.5mmol) and 1,2-dichloroethane (6ml) were refluxed for 1h in a flask fitted with a reflux condensor leading to a N_2 line. The dichloroethane was removed *in vacuo* and an additional portion (6ml) added to the residue. After thorough swirling of the flask contents, the dichloroethane was once again removed *in vacuo*. IR spectroscopy showed that the desired carbonyl chloride was not present.

2-(N-*diethylaminopropylcarboxamido)indolizine* (**180**).§ -

Method 1. Indolizine-2-carboxylic acid (**176**) (0.83g, 5.2mmol) was dissolved in dry DMF (10ml) in a round-bottomed flask fitted with a condensor and a drying tube. 1,1'-carbonyldiimidazole (1.35g, 8.3mmol) was added to the stirred solution, and the resulting mixture was heated at 40°C for 5min, after which time gas evolution had ceased. Once the reaction reached room temperature, 3-diethylaminopropylamine (**178**) (2.03g, 2.46ml, 15.6mmol) was added and stirring was continued for 15min, after which the reaction was quenched with water (7ml). Volatiles were removed *in vacuo* and to the residue was added 1M Na_2CO_3 (50ml). Extraction with EtOAc (2 x 80ml) was followed by washing of the organic layer with water (80ml) and brine (80ml). Solvent was removed *in vacuo* after drying over $MgSO_4$. The crude material was chromatographed [flash chromatography on silica gel; elution with CH_2Cl_2-MeOH-ammonia (20:4:1)] to afford *2-(N-diethylaminopropylcarboxamido)indolizine* (**180**) as a yellow oil (1.12g, 79%) (Found: M^+ 273.184. $C_{16}H_{23}N_3O$ requires *M*,

§ These compounds are named following the convention of reference 69 but systematically they are indolizine-2-carboxamides.

273.184); ν_{max} (thin film)/cm^{-1} 2960, 1625 and 1550; δ_H(400MHz;$CDCl_3$) 0.96 (6H, t, *J* 7.2, 2 x CH_3), 1.65 (2H, quin, *J* 6.2, ($CH_2CH_2CH_2$), 2.47 (6H, m, 2 x CH_3CH_2 and CH_2NEt_2), 3.44 (2H, q, *J* 5.7, $CONHCH_2$), 6.37 (1H, td, $J_{5,6}$ and $J_{6,7}$ 6.8 and $J_{6,8}$ 1.0, 6-H), 6.50 (1H, s, 1-H), 6.55 (1H, dd, $J_{6,7}$ 6.7 and $J_{7,8}$ 8.7, 7-H), 7.21 (1H, d, $J_{7,8}$ 8.7, 8-H), 7.66 (1H, m, 3-H), 7.73 (1H, d, $J_{5,6}$ 7.0, 5-H) and 8.11 (1H, br s, NH); δ_C(100MHz;$CDCl_3$) 11.2 (2 x CH_3), 25.2 ($CH_2CH_2CH_2$), 39.8 and 52.4 (CH_2NH and CH_2NEt_2), 46.7 (2 x CH_3CH_2), 97.2 (C-1), 111.4 (C-6), 113.8 (C-3), 117.8 (C-7), 119.7 (C-8), 124.2 (C-2), 125.3 (C-5), 132.6 (C-9) and 164.7 (CO); *m/z* 273 (M^+, 72%) and 86 (100%). Further purification of this compound by semi-preparative HPLC (elution with MeOH) was carried out prior to spectral analysis in order to remove a lingering minor impurity, found to be imidazole. An alternative purification procedure employing a cross-linked Sephadex gel was also carried out, but HPLC proved to be the most effective purification procedure.

Alternative procedure. Methyl indolizine-2-carboxylate (**135**) (0.50g, 2.9mmol) and 3-diethylaminopropylamine (**178**) (4ml, 3.30g, 25mmol) were heated in a sealed vial at 100°C for 13h and at 150°C for 25h. The reaction mixture was poured into water and extracted with EtOAc (2 x 60ml). The organic layer was washed with water (60ml), dried over $MgSO_4$ and the solvent evaporated. The residue was dissolved in 0.1M-HCl and extracted with EtOAc to remove any starting material. The aqueous layer was then basified with NaOH and re-extracted with EtOAc. The organic layer was washed with water, dried over $MgSO_4$ and evaporated down to yield crude *2-(N-diethylaminopropylcarboxamido)indolizine* (**180**) (0.48g, 60%). This product was purified by chromatography [gravity neutral alumina column; elution with $CHCl_3$-MeOH (99:1)].

2-(N-2-amino-5-diethylaminopentylcarboxamido)indolizine (**181**). -

Method 1. Indolizine-2-carboxylic acid (**176**) (0.50g, 3.1mmol) in dry DMF (7ml), 1,1'-carbonyldiimidazole (0.81g, 5.0mmol) and 2-amino-5-diethylaminopentane (**179**)

(1.47g, 1.80ml, 9.3mmol) were reacted and treated following the procedure described for the preparation of 2-(*N*-diethylaminopropylcarboxamido)indolizine (**180**) except that the reaction was allowed to proceed for 2d prior to quenching. The crude mixture was chromatographed [flash chromatography on silica gel; elution with CH_2Cl_2-MeOH-ammonia (20:4:1)] to afford *2-(N-2-amino-5-diethylaminopentyl-carboxamido)indolizine* (**181**) as a yellow oil (0.67g, 71%) (Found: M^+ 301.217. $C_{18}H_{27}N_3O$ requires *M*, 301.215); ν_{max} (thin film)/cm^{-1} 2965, 1625 and 1550; δ_H(400MHz;$CDCl_3$) 1.00 (6H, t, *J* 7.2, 2 x CH_3CH_2), 1.23 (3H, d, *J* 6.6, CH_3CH), 1.55 [4H, m, $CH(CH_2)_2CH_2N$], 2.46 (2H, m, CH_2NEt_2), 2.53 (4H, q, *J* 7.1, 2 x CH_3CH_2), 4.29 (1H, m, CH_3CH), 6.09 (1H, d, *J* 7.9, NH), 6.49 (1H, td, $J_{5,6}$ and $J_{6,7}$ 6.8 and $J_{6,8}$ 0.9, 6-H), 6.56 (1H, s, 1-H), 6.66 (1H, dd, $J_{6,7}$ 6.5 and $J_{7,8}$ 8.7, 7-H), 7.30 (1H, d, $J_{7,8}$ 9.1, 8-H), 7.73 (1H, m, 3-H) and 7.84 (1H, d, $J_{5,6}$ 6.7, 5-H); δ_C(100MHz;$CDCl_3$) 10.4 (2 x CH_3CH_2), 21.3, 22.7 and 34.5 [CH_3CH and $CH(CH_2)_2CH_2$], 44.9 and 52.4 (CHNH and CH_2NEt_2), 46.7 (2 x CH_3CH_2), 97.4 (C-1), 111.7 (C-6), 114.0 (C-3), 118.1 (C-7), 119.8 (C-8), 124.1 (C-2), 125.4 (C-5), 132.8 (C-9) and 164.3 (CO); *m/z* 301 (M^+, 48%) and 86 (100%). Further purification of this compound by semi-preparative HPLC (elution with MeOH) was carried out prior to spectral analysis in order to remove a lingering minor impurity, found to be imidazole. Extending the reaction time in the above procedure from 15min to 2d led to an increase in yield from 45% to 71%.

Alternative procedure. Methyl indolizine-2-carboxylate (**135**) (0.50g, 2.9mmol) and 2-amino-5-diethylaminopentane (**179**) (4ml, 3.27g, 21mmol) were heated in a sealed tube at 100°C for 13h and at 150°C for 25h. The reaction mixture was poured into water, cooled, and filtered to remove the starting material. The filtrate was acidified and extracted with EtOAc to remove residual starting material. Basification was followed by re-extraction with EtOAc. The organic layer was washed with water and dried over $MgSO_4$. Evaporation of the solvent yielded *2-(N-2-amino-5-diethylamino-pentylcarboxamido)indolizine* (**181**) (0.12g, 14%).

2-Hydroxymethylindolizine (**182**).[70] - A solution of methyl indolizine-2-carboxylate (**135**) (0.5g, 2.9mmol) in dry ether (40ml) was added slowly with a syringe to a suspension of $LiAlH_4$ (0.11g, 2.9mmol) in dry ether (10ml) in a three-necked round-bottomed flask fitted with a septum, thermometer and a condensor attached to a N_2 line. The solution was stirred at room temperature for 30min after which it was allowed to boil briefly (5min). EtOAc (10ml), water (10ml) and dilute HCl (5N, 5ml) were added and the pH adjusted with ammonia to 5-6. The ethereal layer was separated off and the aqueous layer further extracted with ether (2 x 60ml) after which the combined ethereal layers were dried ($MgSO_4$) and the solvent removed *in vacuo*. The resulting solid material was chromatographed [flash chromatography on silica gel; elution with EtOAc-hexane (6:4)] to afford 2-hydroxy-methylindolizine (**182**) (0.21g, 49%); δ_H(60MHz;$CDCl_3$) 1.98(1H, br s, OH), 4.78 (2H, s, CH_2), 6.30-6.84 (3H, m, ArH), 7.16-7.46 (2H, m, ArH) and 7.66-7.96 (1H, m, ArH).

2-Chloromethylindolizine (**183**). -

Attempted preparation. To a solution of 2-hydroxymethylindolizine (**182**) (0.2g, 1.4mmol) in dry 1,2-dichloroethane (50ml) under N_2 in a two-necked round-bottomed flask fitted with a dropping funnel and a reflux condensor, was added dropwise $SOCl_2$ (0.13ml, 1.8mmol) in dry 1,2-dichloroethane (5ml). The resulting mixture was refluxed for 1.5h and then chromatographed after removal of the solvent [flash chromatography on silica gel; elution with EtOAc-hexane (7:3)] to yield two fractions, neither of which was the desired product.

2-(N-ethylcarboxamido)indolizine (**184**).[69] - Methyl indolizine-2-carboxylate (**135**) (0.5g, 2.9mmol) and ethylamine (2ml, 31mmol) were heated in a sealed tube in a steam bath for 53h. The reaction mixture was poured into water and this aqueous layer was extracted with EtOAc (2 x 80ml). The organic layer was washed with water (80ml) and brine (80ml) and dried over $MgSO_4$. Solvent was removed *in vacuo* and the residue chromatographed [flash chromatography on silica gel; elution with

EtOAc-hexane (5:5)] to afford 2-(*N*-ethylcarboxamido)indolizine (**184**) (0.20g, 37%); ν_{max} (thin film)/cm^{-1} 3330 and 1635; δ_H(400MHz;$CDCl_3$) 1.18 (3H, t, *J* 7.3, CH_3), 3.44 (2H, m, CH_2), 6.41 (1H, t, $J_{5,6}$ 6.8, 6-H), 6.62 (3H, m, 7-H, 1-H and NH), 7.24 (1H, d, $J_{7,8}$ 9.1, 8-H) and 7.73 (2H, m, 3-H and 5-H).

*2-(*N-t-*butylcarboxamido)indolizine.* -

Attempted preparation. Methyl indolizine-2-carboxylate (**135**) (0.50g, 2.9mmol) and *tert*-butylamine (2ml, 19mmol) were heated in a sealed tube in a steam bath for 53h. The reaction mixture was poured into water, cooled, and the solid material collected. This material was shown to be methyl indolizine-2-carboxylate (**135**) (0.5g *i.e.* 100% recovery of starting material).

*2-(*N,N-*dimethylcarboxamido)indolizine* (**185**). -

Method 1. Indolizine-2-carboxylic acid (**176**) (0.20g, 1.2mmol) in pyridine (3ml) and dry DMF (4ml), 1,1′-carbonyldiimidazole (0.32g, 2mmol) and dimethylamine hydrochloride (0.60g, 7.4mmol) were reacted and treated following the procedure described for the preparation of 2-(*N*-diethylaminopropylcarboxamido)indolizine (**180**) except the reaction proceeded for 1d prior to quenching. The crude mixture was chromatographed [flash chromatography on silica gel; elution with EtOAc-hexane (9:1)] to afford *2-(*N,N-*dimethylcarboxamido)indolizine* (**185**) as a brown solid (0.20g, 87%), m.p. 80-83°C (crude, as attempts at recrystallization resulted in the formation of a black, tarry material) (Found: $\mathbf{M}^+$ 188.093. $C_{11}H_{12}N_2O$ requires *M*, 188.095); ν_{max} (KBr)/cm^{-1} 1605; δ_H(400MHz;$CDCl_3$) 3.16 (6H, br s, 2 x CH_3), 6.47 (1H, td, $J_{5,6}$ and $J_{6,7}$ 6.8 and $J_{6,8}$ 1.2, 6-H), 6.54 (1H, s, 1-H), 6.65 (1H, ddd, $J_{6,7}$ 6.5, $J_{7,8}$ 9.1 and $J_{5,7}$ 1.0, 7-H), 7.31 (1H, d, $J_{7,8}$ 9.0, 8-H), 7.55 (1H, m, 3-H), 7.83 (1H, dq, $J_{5,6}$ 7.0 and $J_{5,7}$ 1.0, 5-H); δ_C(100MHz;$CDCl_3$) 35.7 and 39.4 (2 x CH_3), 99.4 (C-1), 111.4 (C-6), 113.9 (C-3), 117.8 (C-7), 119.6 (C-8), 123.3 (C-2), 125.1 (C-5), 132.0 (C-9) and 167.5 (CO); *m/z* 188 (M^+, 50%) and 144 (100%).

Attempted preparation 1. Methyl indolizine-2-carboxylate (**135**) (1.0g, 5.7mmol) was dissolved in 33% dimethylamine in EtOH (15.6ml, 0.114mol) and allowed to stir at room temperature in a stoppered flask for 3d. Solvent was removed *in vacuo* and the residue was chromatographed [flash chromatography on silica gel; elution with EtOAc-hexane (8:2)] to afford a mixture of components which were further purified by preparative TLC (development with EtOAc) to afford *2-(N,N-dimethyl-carboxamido)indolizine* (**185**) (0.02g, 2%).

Attempted preparation 2. Methyl indolizine-2-carboxylate (**135**) (0.50g, 2.9mmol) and 33% dimethylamine in EtOH (20ml, 0.15mol) were heated in an autoclave at 150°C at a pressure of 15bar for 8h. TLC showed a large number of components to be present, and therefore this method was abandoned.

2-(N-n-butylcarboxamido)indolizine (**187**). -

Method 1. Indolizine-2-carboxylic acid (**176**) (0.20g, 1.24mmol) in dry DMF (4ml), 1,1'-carbonyldiimidazole (0.32g, 2mmol) and *n*-butylamine (0.27g, 0.37ml, 3.7mmol) were reacted and treated following the procedure described for the preparation of 2-(*N*-diethylaminopropylcarboxamido)indolizine (**180**). The crude material was chromatographed [flash chromatography on silica gel; elution with EtOAc-hexane (6:4)] to afford 2-(*N-n*-butylcarboxamido)indolizine (**187**) as a yellow oil which solidified (0.17g, 63%), m.p. 75-77°C (from hexane) (lit.,[69] 70-72°C); ν_{max} (thin film)/cm^{-1} 3310 and 1640; δ_H(400MHz;$CDCl_3$) 0.91 (3H, t, *J* 7.4, CH_3), 1.37 (2H, m, CH_3CH_2), 1.56 (2H, m, $CH_3CH_2CH_2$), 3.42 (2H, m, CH_2NH), 6.30 (1H, br s, NH), 6.45 (1H, td, $J_{5,6}$ and $J_{6,7}$ 6.8 and $J_{6,8}$ 1.1, 6-H), 6.59 (1H, s, 1-H), 6.63 (1H, ddd, $J_{6,7}$ 6.5, $J_{7,8}$ 9.0 and $J_{5,7}$ 0.7, 7-H), 7.27 (1H, d, $J_{7,8}$ 9.1, 8-H), 7.72 (1H, m, 3-H) and 7.79 (1H, dd, $J_{5,6}$ 7.1 and $J_{5,7}$ 0.8, 5-H); *m/z* 216 (M^+, 55%) and 117 (100%).

Attempted preparation 1. Trifluoroacetic anhydride (0.37ml, 2.7mmol) was added to indolizine-2-carboxylic acid (**176**) (0.54g, 3.4mmol) in a flask fitted with a reflux

condensor and a drying tube. There was insufficient liquid to dissolve the indolizine-2-carboxylic acid (**176**) and to solve this problem dry THF (4ml) and an additional portion of trifluoroacetic anhydride (0.37ml, 2.7mmol) were added. This reaction mixture was heated at 70°C for 30min and after cooling, *n*-butylamine (2ml, 20mmol) was added. The resulting mixture was stirred at room temperature for 1d. Volatiles were removed *in vacuo* and the remaining residue chromatographed [flash chromatography on silica gel; elution with EtOAc-hexane (6:4)] to afford 2-(*N*-*n*-butylcarboxamido)indolizine (**187**) (0.05g, 7%).

The above preparation was repeated in dry THF at 0°C under N_2, and in dry THF under reflux in a N_2 atmosphere but the yield could not be increased.

Attempted preparation 2. To a solution of indolizine-2-carboxylic acid (**176**) (0.5g, 3.1mmol) in CH_2Cl_2 (30ml), was added *n*-butylamine (0.31ml, 3.1mmol). *N,N'*-dicyclohexylcarbodiimide (0.70g, 3.4mmol) was added to this stirred mixture. Stirring was continued for an additional 24h at room temperature, after which time acetic acid was added to quench the reaction. Insoluble material was removed by filtration and the filtrate extracted with EtOAc (80ml) after being adjusted to pH 7. The organic layer was dried ($MgSO_4$) and evaporated to yield indolizine-2-carboxylic acid (**176**) (0.30g, 60%) and a trace of the desired product, shown to be present by TLC.

*2-(*N,N-*diisopropylcarboxamido)indolizine* (**188**). -

Attempted preparation 1. Indolizine-2-carboxylic acid (**176**) (0.20g, 1.2mmol) in dry DMF (14ml), 1,1'-carbonyldiimidazole (0.32g, 2mmol) and diisopropylamine (0.72g, 7.1mmol) were reacted and treated following the procedure described for the preparation of 2-(*N*-diethylaminopropylcarboxamido)indolizine (**180**) except the reaction proceeded for 5d prior to quenching. The crude mixture was separated by preparative TLC [development with EtOAc-hexane (7:3)] to afford mostly recovered

starting material (**176**) (0.17g, 85%) together with *2-(N-isopropylcarboxamido)-indolizine* (**197**) (0.01g, 4%) (Found: M^+ 202.111. $C_{12}H_{14}N_2O$ requires *M*, 202.111); δ_H(400MHz;$CDCl_3$) 1.25 (6H, d, *J* 6.5, 2 x CH_3), 4.29 [1H, m, $(CH_3)_2CH$], 5.81 (1H, br s, NH), 6.52 (2H, m, 1-H and 6-H), 6.67 (1H, dd, $J_{6,7}$ 6.6 and $J_{7,8}$ 9.0, 7-H), 7.31 (1H, d, $J_{7,8}$ 9.1, 8-H), 7.74 (1H, s, 3-H) and 7.85 (1H, d, $J_{5,6}$ 6.9, 5-H); δ_C(100MHz;$CDCl_3$) 23.0 (2 x CH_3), 41.4 (CH), 96.9 (C-1), 111.8 (C-6), 114.0 (C-3), 118.2 (C-7), 119.8 (C-8), 124.2 (C-2), 125.4 (C-5) and 132.9 (C-9) and 164.0 (CO); *m/z* 202 (M^+, 37%) and 117 (100%).

Attempted preparation 2. Diisopropylamine (0.58g, 0.80ml, 5.7mmol) was dissolved in dry THF (20ml) in a two-necked round-bottomed flask fitted with a septum and a reflux condensor leading to a N_2 line, and the solution was cooled to -78°C in an acetone-liquid N_2 bath. *n*-BuLi (3.9ml, 6.3mmol) was added dropwise and the resulting mixture allowed to stir for 15min at low temperature. Methyl indolizine-2-carboxylate (**176**) (0.50g, 2.9mmol) in dry THF was added dropwise, and after this addition, the reaction was allowed to warm to room temperature and was refluxed for 1h. After stirring overnight at room temperature the reaction was quenched with aqueous $NaHCO_3$ and extracted with EtOAc (2 x 60ml). The combined organic layers were dried ($MgSO_4$) and evaporated and the residue chromatographed [flash chromatography on silica gel; elution with EtOAc-hexane (5:5)]. NMR spectroscopy of the collected fractions showed no desired product to be present.

N,N-*diisopropylbenzamide*. -

Attempted preparation. To a three-necked round-bottomed flask fitted with a thermometer, septum and condensor leading to a N_2 line, was added diisopropylamine (1.4ml, 10mmol) dissolved in dry THF (10ml). This solution was cooled in an acetone-liquid N_2 bath to -78°C, and *n*-BuLi (7.3ml, 11mmol) was added dropwise. The resulting mixture was allowed to stir at -78°C for 45min, after which time

benzonitrile (1.02ml, 10mmol) was added, and stirring continued for an additional 2h. The reaction was quenched at -78°C with 1M-HCl in THF (12ml) and stirred at low temperature for 30min and at room temperature overnight. Water (50ml) was added and the aqueous layer extracted with EtOAc (2 x 75ml). The combined organic extracts were dried over $MgSO_4$ and the solvent removed *in vacuo* to yield a yellow oil, shown by ^1H NMR spectroscopy to be *N,N*-diisopropylbenzimine (**189**) (2.0g, 98%); δ_H(400MHz;$CDCl_3$) 1.29 [12H, d, 2 x $(CH_3)_2$], 3.59 [2H, sept, 2 x C*H*$(CH_3)_2$], 5.25 (1H, br s, NH), 7.22 (2H, m, ArH) and 7.30 (3H, m, ArH).

Attempted hydrolysis of *N,N*-diisopropylbenzimine (189). -

Attempted hydrolysis 1. *N,N*-diisopropylbenzimine (**189**) (0.43g, 2.1mmol) was placed in a three-necked round-bottomed flask fitted with a thermometer, dropping funnel and a reflux condensor attached to a N_2 line, together with conc. HCl (150ml). To this was added dropwise a solution of $NaNO_2$ (0.51g, 7.4mmol) in water (5ml) through the dropping funnel, maintaining the mixture at room temperature. The resulting mixture was allowed to stir at room temperature for 1h, after which urea (3.0g, 50mmol) in water (6ml) was added dropwise over 10min, to quench the reaction. Stirring was continued for 15min after quenching and the aqueous mixture was extracted with EtOAc (2 x 150ml). The combined organic extracts were washed sequentially with aqueous $NaHCO_3$ (100ml) and brine (100ml), and dried over $MgSO_4$. A large number of products were shown to be present by TLC, and thus this route was abandoned.

Attempted hydrolysis 2. Treatment of *N,N*-diisopropylbenzimine (**189**) with 10M-HCl yielded the original starting materials benzonitrile and diisopropylamine.

*2-(*N-n-*propylcarboxamido)indolizine* (**190**). - Indolizine-2-carboxylic acid (**176**) (0.20g, 1.2mmol) in dry DMF (4ml), 1,1'-carbonyldiimidazole (0.32g, 2mmol) and *n*-propylamine (0.22g, 0.31ml, 3.7mmol) were reacted and treated following the

procedure described for the preparation of 2-(*N*-diethylaminopropyl-carboxamido)indolizine (**180**). The crude mixture was chromatographed [flash chromatography on silica gel; elution with EtOAc-hexane (7:3)] to afford 2-(*N-n*-propylcarboxamido)indolizine (**190**) as white crystals (0.18g, 72%), m.p. 100-102°C (from hexane) (lit.,[69] 78-80°C)[‡] ; ν_{max} (KBr)/cm^{-1} 3310 and 1635; δ_H(400MHz;$CDCl_3$) 0.95 (3H, t, *J* 7.4, CH_3), 1.62 (2H, sextet, *J* 7.3, CH_3CH_2), 3.38 (2H, m, CH_2NH), 6.23 (1H, br s, NH), 6.47 (1H, td, $J_{5,6}$ and $J_{6,7}$ 6.8 and $J_{6,8}$ 1.1, 6-H), 6.58 (1H, s, 1-H), 6.65 (1H, ddd, $J_{6,7}$ 6.6, $J_{7,8}$ 9.1 and $J_{5,7}$ 0.8, 7-H), 7.29 (1H, d, $J_{7,8}$ 9.1, 8-H), 7.73 (1H, m, 3-H) and 7.81 (1H, dd, $J_{5,6}$ 7.0 and $J_{5,7}$ 0.9, 5-H); *m/z* 202 (M^+, 63%) and 144 (100%).

2-(N,N-diethylcarboxamido)indolizine (**191**). - Indolizine-2-carboxylic acid (**176**) (0.50g, 3.1mmol) in dry DMF (7ml), 1,1'-carbonyldiimidazole (0.81g, 5.0mmol) and diethylamine (0.48g, 0.68ml, 6.6mmol) were reacted and treated following the procedure described for the preparation of 2-(*N*-diethylaminopropyl-carboxamido)indolizine (**180**). The crude mixture was chromatographed [flash chromatography on silica gel; elution with EtOAc-hexane (7:3)] to afford *2-(N,N-diethylcarboxamido)indolizine* (**191**) as a beige solid (0.30g, 45%), m.p. 98-99°C (from hexane) (Found: M^+ 216.124. $C_{13}H_{16}N_2O$ requires *M*, 216.126); ν_{max} (KBr)/cm^{-1} 1600; δ_H(400MHz;$CDCl_3$) 1.23 (6H, t, *J* 7.1, 2 x CH_3), 3.55 (4H, q, *J* 7.1, 2 x CH_2), 6.48 (1H, td, $J_{5,6}$ and $J_{6,7}$ 6.8 and $J_{6,8}$ 1.2, 6-H), 6.51 (1H, s, 1-H), 6.66 (1H, ddd, $J_{6,7}$ 6.6, $J_{7,8}$ 9.1 and $J_{5,7}$ 0.9, 7-H), 7.32 (1H, d, $J_{7,8}$ 9.0, 8-H), 7.54 (1H, m, 3-H) and 7.85 (1H, dd, $J_{5,6}$ 7.0 and $J_{5,7}$ 1.0, 5-H); δ_C(100MHz;$CDCl_3$) 13.8 (2 x CH_3), 40.0 and 42.8 (2 x CH_2), 98.5 (C-1), 111.2 (C-6), 113.2 (C-3), 117.7 (C-7), 119.5 (C-8), 123.9 (C-2), 125.1 (C-5), 132.1 (C-9) and 166.9 (CO); *m/z* 216 (M^+, 39%) and 117 (100%).

[‡]The reason for the discrepancy between the literature melting point value and the melting point determined in our investigation is not known, as spectroscopic evidence clearly indicates that the correct product is present in our case.

1-(Indolizin-2-ylcarbonyl)pyrrolidine (**192**). - Indolizine-2-carboxylic acid (**176**) (0.50g, 3.1mmol) in dry DMF (7ml), 1,1'-carbonyldiimidazole (0.81g, 5.0mmol) and pyrrolidine (0.66g, 0.77ml, 9.3mmol) were reacted and treated following the procedure described for the preparation of 2-(*N*-diethylaminopropyl-carboxamido)indolizine (**180**) except the reaction proceeded for 30min prior to quenching. Evaporation of the solvent after drying ($MgSO_4$) yielded *1-(indolizin-2-ylcarbonyl)pyrrolidine* (**192**) as a brown oil which solidified (0.53g, 80%), m.p. 104-105°C (crude, as all attempts at recrystallization led to the formation of black, tarry material) (Found: $\mathbf{M}^+$ 214.111. $C_{13}H_{14}N_2O$ requires *M*, 214.111); ν_{max} (KBr)/cm^{-1} 1590; δ_H(400MHz;$CDCl_3$) 1.91 [4H, m, $CH_2(CH_2)_2CH_2$], 3.68 [4H, m, $(CH_2)N(CH_2)$], 6.46 (1H, td, $J_{5,6}$ and $J_{6,7}$ 6.8 and $J_{6,8}$ 1.1, 6-H), 6.63 (2H, m, 1-H and 7-H), 7.30 (1H, d, $J_{7,8}$ 9.1, 8-H), 7.66 (1H, m, 3-H) and 7.83 (1H, dd, $J_{5,6}$ 7.0 and $J_{5,7}$ 1.0, 5-H); δ_C(100MHz;$CDCl_3$) 24.1 and 26.5 [$CH_2(CH_2)_2CH_2$], 46.6 and 48.7 [$(CH_2)N(CH_2)$], 99.5 (C-1), 111.5 (C-6), 114.5 (C-3), 117.7 (C-7), 119.7 (C-8), 124.1 (C-2), 125.1 (C-5), 132.1 (C-9) and 164.9 (CO); *m/z* 214 (M^+, 32%) and 117 (100%).

1-(Indolizin-2-ylcarbonyl)piperidine (**193**). - Indolizine-2-carboxylic acid (**176**) (0.50g, 3.1mmol) in dry DMF (7ml), 1,1'-carbonyldiimidazole (0.81g, 5.0mmol) and piperidine (0.79g, 0.92ml, 9.3mmol) were reacted and treated following the procedure described for the preparation of 2-(*N*-diethylaminopropylcarboxamido)indolizine (**180**) except the reaction proceeded for 30min prior to quenching. Evaporation of the solvent after drying ($MgSO_4$) yielded *1-(indolizin-2-ylcarbonyl)piperidine* (**193**) as a yellow oil which solidified (0.50g, 70%), m.p. 139-141°C (from hexane) (Found: $\mathbf{M}^+$ 228.126. $C_{14}H_{16}N_2O$ requires *M*, 228.126); ν_{max} (KBr)/cm^{-1} 1605; δ_H(400MHz;$CDCl_3$) 1.63 [6H, m, $CH_2(CH_2)_3CH_2$], 3.68 [4H, t, *J* 5.3, $(CH_2)N(CH_2)$], 6.47 (2H, m, 1-H and 6-H), 6.66 (1-H, td, $J_{6,7}$ 6.5, $J_{7,8}$ 9.1 and $J_{5,7}$ 1.0, 7-H), 7.31 (1H, d, $J_{7,8}$ 9.0, 8-H), 7.50 (1H, m, 3-H) and 7.84 (1H, dd, $J_{5,6}$ 7.0 and $J_{5,7}$ 1.0, 5-H); δ_C(100MHz; $CDCl_3$) 24.8 [$(CH_2)_2CH_2(CH_2)_2$], 26.2 ($CH_2CH_2CH_2CH_2CH_2$), 43.5 and 48.5

[(CH_2)N(CH_2)], 98.8 (C-1), 111.3 (C-6), 113.3 (C-3), 117.9 (C-7), 119.5 (C-8),123.5 (C-2), 125.2 (C-5), 132.1 (C-9) and 166.4 (CO); *m/z* 228 (M^+,35%) and 117 (100%).

*2-(*N,N-*Diethylcarboxamido)pyrrolo[1,2-*a*]quinoline* (**194**). - Pyrrolo[1,2-*a*]quinoline-2-carboxylic acid (**177**) (0.66g, 3.1mmol) in dry DMF (7ml), 1,1'-carbonyl-diimidazole (0.81g, 5.0mmol) and diethylamine (0.68g, 0.96ml, 9.3mmol) were reacted and treated following the procedure described in the preparation of 2-(*N*-diethylaminopropylcarboxamido)indolizine (**180**). The crude mixture was chromatographed [flash chromatography on silica gel; elution with EtOAc-hexane (6:4)] to afford *2-(*N,N-*diethylcarboxamido)pyrrolo[1,2-*a*]quinoline* (**194**) as a yellow oil which solidified (0.21g, 25%), m.p. 81-83°C (Found: M^+ 266.140. $C_{17}H_{18}N_2O$ requires *M*, 266.142); ν_{max} (KBr)/cm^{-1} 2370 and 1605; δ_H(400MHz;$CDCl_3$) 1.22 (6H, t, *J* 7.1, 2 x CH_3), 3.54 (4H, q, *J* 7.1, 2 x CH_2), 6.57 (1H, d, $J_{1,3}$ 1.5, 3-H), 6.92 (1H, d, $J_{4,5}$ 9.4, 5-H), 7.18 (1H, d, $J_{4,5}$ 9.3, 4-H), 7.24 (1H, td, $J_{6,7}$ and $J_{7,8}$ 7.5 and $J_{7,9}$ 1.0, 7-H), 7.41 (1H, td, $J_{7,8}$ and $J_{8,9}$ 7.8 and $J_{6,8}$ 1.4, 8-H), 7.53 (1H, dd, $J_{6,7}$ 7.8 and $J_{6,8}$ 1.2, 6-H), 7.77 (1H, d, $J_{8,9}$ 8.3, 9-H) and 8.10 (1H, d, $J_{1,3}$ 1.2, 1-H); δ_C(100MHz;$CDCl_3$) 13.5 (2 x CH_3), 40.3 and 42.4 (2 x CH_2), 101.9 (C-3), 113.4 (C-1), 114.0 (C-9), 118.5 (C-4), 119.7 (C-5), 123.0 and 123.7 (C-2 and C-5a), 124.0 (C-7), 127.8 (C-8), 128.4 (C-6), 130.3 (C-3a), 132.7 (C-9a) and 166.5 (CO); *m/z* 266 (M^+, 0.1%) and 86 (100%).

*2-(*N-*diethylaminopropylcarboxamido)pyrrolo[1,2-*a*]quinoline* (**195**). - Pyrrolo[1,2-*a*]-quinoline-2-carboxylic acid (**177**) (0.33g, 1.6mmol) in dry DMF (4ml), 1,1'-carbonyldiimidazole (0.41g, 2.5mmol) and 3-diethylaminopropylamine (**178**) (0.61g, 0.74ml, 4.7mmol) were reacted and treated following the procedure described in the preparation of 2-(*N*-diethylaminopropylcarboxamido)indolizine (**180**) except the reaction was allowed to proceed for 2d prior to quenching. The crude mixture was chromatographed [flash chromatography on silica gel; elution with CH_2Cl_2:MeOH: ammonia (20:4:1)] to afford *2-(*N-*diethylaminopropylcarboxamido)-*

pyrrolo[1,2-a]quinoline (**195**) as a viscous yellow oil (0.48g, 92%) (Found: M^+ 323.202. $C_{20}H_{25}N_3O$ requires *M*, 323.200); ν_{max} (thin film)/cm^{-1} 3290, 2960 and 1625; δ_H(400MHz;$CDCl_3$) 1.07 (6H, t, *J* 7.2, 2 x CH_3), 1.76 (2H, quin, *J* 6.0, $CH_2CH_2CH_2$), 2.59 (6H, m, 2 x CH_3CH_2 and CH_2NEt_2), 3.55 (2H, q, *J* 5.7, $CONHCH_2$), 6.65 (1H, d, $J_{1,3}$ 1.1, 3-H), 6.97 (1H, d, $J_{4,5}$ 9.4, 5-H), 7.21 (1H, d, $J_{4,5}$ 9.4, 4-H), 7.30 (1H, t, $J_{6,7}$ and $J_{7,8}$ 7.4, 7-H), 7.46 (1H, td, $J_{7,8}$ and $J_{8,9}$ 7.8 and $J_{6,8}$ 1.0, 8-H), 7.58 (1H, d, $J_{6,7}$ 7.7, 6-H), 7.83 (1H, d, $J_{8,9}$ 8.3, 9-H), 8.25 (1H, br s, NH) and 8.34 (1H, s, 1-H); δ_C(100MHz;$CDCl_3$) 11.5 (2 x CH_3), 25.2 ($CH_2CH_2CH_2$), 40.2 (CH_2NH), 46.9 and 52.8 (2 x CH_3CH_2 and CH_2NEt_2), 100.6 (C-3), 113.8 (C-1), 114.3 (C-9), 118.9 (C-4), 120.0 (C-5), 123.6 and 124.0 (C-2 and C-5a), 124.3 (C-7), 128.1 (C-8), 128.6 (C-6), 131.1 (C-3a), 133.1 (C-9a) and 164.5 (CO); *m/z* 323 (M^+, 8%) and 86 (100%).

The product was further purified by semi-preparative HPLC; elution with MeOH.

*2-(*N*-2-amino-5-diethylaminopentylcarboxamido)pyrrolo[1,2-a]quinoline* (**196**). - Pyrrolo[1,2-*a*]quinoline-2-carboxylic acid (**177**) (0.66g, 3.1mmol) in DMF (7ml), 1,1'-carbonyldiimidazole (0.81g, 5.0mmol) and 2-amino-5-diethylaminopentane (**179**) (1.47g, 1.80ml, 9.3mmol) were reacted and treated following the prodedure described in the preparation of 2-(*N*-diethylaminopropylcarboxamido)indolizine (**180**) except the reaction was allowed to proceed for 2d prior to quenching. The crude mixture was chromatographed [flash chromatography on silica gel; elution with CH_2Cl_2:MeOH: ammonia (20:4:1)] to afford *2-(*N*-2-amino-5-diethylaminopentylcarboxamido)-pyrrolo[1,2-a]quinoline* (**196**) as a viscous yellow oil (0.62g, 57%) (Found: M^+ 351.231. $C_{22}H_{29}N_3O$ requires *M*, 351.231); ν_{max} (thin film)/cm^{-1} 3290, 2960 and 1620; δ_H(400MHz;$CDCl_3$) 1.00 (6H, t, *J* 7.2, 2 x CH_3CH_2), 1.25 (3H, d, *J* 6.6, CH_3CH), 1.57 (4H, m, $CH(CH_2)_2CH_2N$), 2.46 (2H, m, CH_2NEt_2), 2.52 (4H, q, *J* 7.2, 2 x CH_3CH_2), 4.23 (1H, m, CH_3CH), 6.19 (1H, d, *J* 8.2, NH), 6.66 (1H, d, $J_{1,3}$ 1.5, 3-H), 6.99 (1H, d, $J_{4,5}$ 9.4, 5-H), 7.22 (1H, d, $J_{4,5}$ 9.4, 4-H), 7.32 (1H, td, $J_{6,7}$ and $J_{7,8}$ 7.5 and $J_{7,9}$ 1.0, 7-H), 7.48 (1H, td, $J_{7,8}$ and $J_{8,9}$ 7.8 and $J_{6,8}$ 1.5, 8-H), 7.60 (1H, dd,

$J_{6,7}$ 7.8 and $J_{6,8}$ 1.3, 6-H), 7.86 (1H, d, $J_{8,9}$ 8.3, 9-H) and 8.34 (1H, d, $J_{1,3}$ 1.3, 1-H); δ_C(100MHz;$CDCl_3$) 11.3 (2 x CH_3CH_2), 21.1 (CH_3CH), 23.6 and 35.0 [$CH(CH_2)_2CH_2$], 45.2 (CH_3CH), 46.8 and 52.7 (2 x CH_3CH_2 and CH_2NEt_2), 100.5 (C-3), 113.9 (C-1), 114.4 (C-9), 118.8 (C-4), 120.2 (C-5), 123.4 and 124.0 (C-2 and C-5a), 124.4 (C-7), 128.2 (C-8), 128.7 (C-6), 131.2 (C-3a), 133.0 (C-9a) and 164.1 (CO); *m/z* 352 (M^+, 0.1%) and 86 (100%).
The product was further purified by semi-preparative HPLC; elution with MeOH.

Ethyl bromopyruvate (**198**).[316] - Bromine (23.97g, 7.73ml, 0.15mol) was added dropwise to ethyl pyruvate (17.42g, 16.64ml, 0.15mol) which had been heated to 70°C in a three-necked round-bottomed flask fitted with a condensor, thermometer and dropping funnel. The reaction mixture was stirred for a further 30min after addition of the bromine. Distillation under reduced pressure yielded ethyl bromopyruvate (**198**) (26.50g, 91%), b.p. 76-78°C/2mmHg (lit.,[316] 98-100°C/10mmHg) ; ν_{max} (thin film)/cm^{-1} 3480, 2980 and 1730; δ_H(400MHz;$CDCl_3$) 1.34 (3H, t, *J* 7.2, CH_3), 4.29 (2H, s, CH_2Br) and 4.32 (2H, q, *J* 7.1, CH_2CH_3).

Ethyl imidazo[1,2-a]pyridine-2-carboxylate hydrobromide (**200**).[315] - *2-Amino-1-carbethoxycarbonylmethylpyridinium bromide* - Ethyl bromopyruvate (**198**) (11.7g, 0.07mol) was added dropwise to a stirred solution of 2-aminopyridine (5.6g, 0.06mol) in 1,2-dimethoxyethane (50ml). The temperature of the mixture was maintained below 50°C throughout the addition. Stirring was continued for an additional 30min whereafter filtration of the reaction mixture yielded 2-amino-1-carbethoxycarbonylmethylpyridinium bromide (17.0g, 98%), m.p. 139-141°C (lit.,[315] 134-136°C); ν_{max} (KBr)/cm^{-1} 3150, 1745 and 1660; δ_H(400MHz;D_2O) 1.34 (3H, t, *J* 7.2, CH_3), 4.38 (2H, q, *J* 7.2, CH_3CH_2), 4.79 and 5.21 (2H, 2 x d, *J* 14.3, CH_2),[††]

[††] The two doublets resulting from the methylene protons can be rationalized in terms of this compound existing as a hemiaminal, with the adjacent chiral centre resulting in diastereotopicity of the methylene protons.

7.17 (2H, m, ArH), 8.10 (1H, t, *J* 8.1, ArH) and 8.17 (1H, d, *J* 6.5, ArH). 2-Amino-1-carbethoxycarbonylmethylpyridinium bromide (15g, 0.05mol) was refluxed in EtOH (400ml) for 2h after which the volume of EtOH was reduced to 80ml and ether (80ml) added. Filtration of the resulting mixture yielded ethyl imidazo[1,2-*a*]-pyridine-2-carboxylate hydrobromide (**200**) (10.48g, 77%), m.p. 172-174°C (lit.,[315] 174.5-175.5°C from ethanol-ether); ν_{max} (Nujol mull)/cm^{-1} 3400 and 1720; δ_H(400MHz;D_2O) 1.45 (3H, t, *J* 7.2, CH_3), 4.54 (2H, q, *J* 7.2, CH_3CH_2), 7.54 (1H, td, $J_{5,6}$ and $J_{6,7}$ 7.0 and $J_{6,8}$ 0.9, 6-H), 7.93 (1H, dd, $J_{7,8}$ 9.2 and $J_{6,8}$ 0.7, 8-H), 8.06 (1H, m, 7-H), 8.69 (1H, s, 3-H) and 8.74 (1H, d, $J_{5,6}$ 6.9, 5-H).

Imidazo[1,2-a]pyridine-2-carboxylic acid (**201**). - Ethyl imidazo[1,2-*a*]pyridine-2-carboxylate hydrobromide (**200**) (10g, 37mmol) was added to a solution of KOH (14g, 0.25mol) in EtOH (350ml) in a round-bottomed flask fitted with a reflux condensor, and the mixture was refluxed for 16h. Water was added and the pH adjusted to pH 6 with HCl. Evaporation of the water yielded imidazo[1,2-*a*]pyridine-2-carboxylic acid (**201**) (5.1g, 86%), m.p. 219-222°C; ν_{max} (KBr)/cm^{-1} 3090 (br) and 1725; δ_H(400MHz;D_2O) 7.52 (1H, t, $J_{5,6}$ and $J_{6,7}$ 6.8, 6-H), 7.92 (1H, d, $J_{7,8}$ 9.1, 8-H), 8.05 (1H, m, 7-H), 8.57 (1H, s, 3-H) and 8.72 (1H, d, $J_{5,6}$ 6.8, 5-H).

2-(N-diethylaminopropylcarboxamido)imidazo[1,2-a]pyridine (**202**). - Imidazo[1,2-*a*]-pyridine-2-carboxylic acid (**201**) (5.5g, 34mmol) in dry DMF (20ml), 1,1'-carbonyldiimidazole (7g, 55mmol) and 3-diethylaminopropylamine (**178**) (13.41g, 16.25ml, 0.10mol) were reacted and treated following the procedure described in the preparation of 2-(*N*-diethylaminopropylcarboxamido)indolizine (**180**). The crude mixture was chromatographed [flash chromatography on silica gel; elution with CH_2Cl_2:MeOH:ammonia (20:4:1)] to afford *2-(N-diethylaminopropylcarboxamido)-imidazo[1,2-a]pyridine* (**202**) as a yellow oil (5g, 54%) (Found: M^+ 274.179. $C_{15}H_{22}N_4O$ requires *M*, 274.179); ν_{max} (thin film)/cm^{-1} 2960, 1650 and 1560; δ_H(400MHz;$CDCl_3$) 0.97 (6H, t, *J* 7.2, 2 x CH_3), 1.71 (2H, quin, *J* 6.8,

$CH_2CH_2CH_2$), 2.47 (6H, m, 2 x CH_3CH_2 and CH_2NEt_2), 3.46 (2H, q, *J* 6.7, CH_2NH), 6.74 (1H, td, $J_{5,6}$ and $J_{6,7}$ 6.8 and $J_{6,8}$ 1.0, 6-H), 7.13 (1H, m, 7-H), 7.46 (1H, dd, $J_{7,8}$ 9.2 and $J_{6,8}$ 0.6, 8-H), 7.96 (1H, br s, NH) and 8.07 (2H, m, 3-H and 5-H); δ_C(100MHz;$CDCl_3$) 11.5 (2 x CH_3), 26.7 ($CH_2CH_2CH_2$), 38.2 (CH_2NH), 46.7 and 51.3 (2 x CH_3CH_2 and CH_2NEt_2), 113.0 (C-6), 113.8 (C-3), 118.0 (C-8), 125.5 (C-7), 126.3 (C-5), 140.3 and 144.3 (C-2 and C-9) and 162.6 (CO); *m/z* 274 (M^+, 6%) and 86 (100%).

The product was further purified by semi-preparative HPLC; elution with MeOH.

*2-(*N*-2-amino-5-diethylaminopentylcarboxamido)imidazo[1,2-*a*]pyridine* (**203**). - Imidazo[1,2-*a*]pyridine-2-carboxylic acid (**201**) (5.5g, 34mmol) in dry DMF (20ml), 1,1'-carbonyldiimidazole (7g, 55mmol) and 2-amino-5-diethylaminopentane (16.16g, 19.76ml, 0.10mol) were reacted and treated following the procedure described in the preparation of 2-(*N*-diethylaminopropylcarboxamido)indolizine (**180**). The crude mixture was chromatographed [flash chromatography on silica gel; elution with CH_2Cl_2:MeOH:ammonia (20:4:1)] to afford *2-(*N*-2-amino-5-diethylaminopentyl-carboxamido)imidazo[1,2-*a*]pyridine* (**203**) as a yellow oil (6g, 58%) (Found: M^+ 302.212. $C_{17}H_{26}N_4O$ requires *M*, 302.211); ν_{max} (thin film)/cm^{-1} 2960, 1630 and 1560; δ_H(400MHz;$CDCl_3$) 0.88 (6H, td, *J* 7.1 and 1.9, 2 x CH_3CH_2), 1.17 (3H, dd, *J* 6.5 and 1.5, CH_3CH), 1.47 [4H, m, $CH(CH_2)_2CH_2N$], 2.28-2.43 (6H, m, 2 x CH_3CH_2 and CH_2NEt_2), 4.12 (1H, m, CH_3CH), 6.72 (1H, t, $J_{5,6}$ and $J_{6,7}$ 6.3, 6-H), 7.13 (2H, m, 7-H and NH), 7.44 (1H, d, $J_{7,8}$ 9.0, 8-H) and 8.05 (2H, m, 5-H and 3-H); δ_C(100MHz;$CDCl_3$) 11.5 (2 x CH_3CH_2), 20.9 (CH_3CH), 23.6 and 34.8 [$CH(CH_2)_2$], 44.7 (CH_3CH), 46.6 and 52.5 (2 x CH_3CH_2 and CH_2NEt_2), 113.0 (C-6), 113.9 (C-3), 117.8 (C-8), 125.6 (C-7), 126.3 (C-5), 140.2 and 144.2 (C-9 and C-2) and 161.8 (CO); *m/z* 302 (M^+, 4%) and 86 (100%).

The product was further purified by semi-preparative HPLC; elution with MeOH.

3.2.2 PREPARATION OF THE OLIGONUCLEOTIDE d(5'-GCATGC)$_2$

The synthesis of the oligonucleotide d(5'-GCATGC)$_2$ was carried out on a Cruachem PS100 semi-manual DNA synthesizer. The protocol followed was the CE (β-cyanoethyl) phosphoramidite protocol.

The synthesis requires extremely dry solvents and reagents because the phosphoramidites are very moisture-sensitive, and thus on the two days preceding a synthesis, drying and distillation of these solvents and reagents was carried out:
(i) Acetonitrile, THF, and 2,6-lutidine were refluxed over CaH_2 for 2h under N_2, and then distilled from CaH_2. The first 80ml fraction collected, in each case, was discarded and the following fraction was collected and transferred to a dry vessel, which was then sealed with Parafilm.
(ii) Acetic anhydride was refluxed over $CaCl_2$ for 2h under N_2, and then distilled under reduced pressure into a dry vessel, which was then sealed with Parafilm.

On the day before a synthesis, each phosphoramidite required for the synthesis was weighed out into oven-dried 5ml dark serum bottles. For a 10μmol synthesis of the above oligonucleotide, 37.5mg of DMT-thymidine,[###] 2 x 42mg of *i*Bu-DMT-deoxyguanosine, 43mg of Bz-DMT-deoxyadenosine[####] and 42mg of Bz-DMT-deoxycytidine CE phosphoramidites were weighed into serum bottles, which were immediately closed with septa. Five portions of 17.5mg tetrazole were also weighed into 5ml dark serum bottles fitted with septa. These serum bottles were all vented with needles, placed in a desiccator, and evacuated by pumping with a vacuum pump for 5h.

On the day of the synthesis, nitrogen was bled into the evacuated desiccator. The

[###] DMT is dimethoxytrityl

[####] Bz is benzoyl

phosphoramidites and tetrazole were dissolved in anhydrous acetonitrile, and the venting needles were replaced with nitrogen balloons to maintain a N_2 atmosphere over the phosphoramidite and tetrazole solutions. Oven-dried syringes, one for each of the phosphoramidite serum bottles, and one for tetrazole were placed in a desiccator, ready for use.

To the reaction column was transferred 250mg of Bz-DMT-deoxycytidine controlled pore glass. The synthesis is conducted in the 3′→5′ direction, and thus the nucleotide attached to the controlled pore glass is that at the 3′ end of the required oligonucleotide. The reagents were made up and transferred to the appropriate reservoirs of the DNA synthesizer and the entire system was then sealed and pressurized, ready for synthesis to begin.

The synthesis was carried out following the protocol outlined in the Cruachem manual,[405] and a schematic representation of the synthesis can be found in Section 2.3.2 (Scheme 48, p. 154).

The first deoxyribonucleoside is attached through its 3′-hydroxyl group to an inert insoluble support, which also acts as a 3′-blocking device. Each cycle effectively involves initial deprotection of the 5′-hydroxyl group of the phosphoramidite already attached to the support using 3% dichloroacetic acid in 1,2-dichloroethane, followed by manual injection of the next phosphoramidite in anhydrous acetonitrile (activated by mixing with tetrazole solution immediately prior to injection). This is followed by washing with acetonitrile and capping of any free 5′-hydroxyl groups which failed to undergo coupling with Ac_2O/DMAP/THF, followed by oxidation with iodine of the 3′-5′ internucleotide phosphite-triester linkage to the more stable 3′-5′-phosphotriester linkage. Washing with acetonitrile and deprotection of the newly added phosphoramidite with 3% dichloroacetic acid in 1,2-dichloroethane completes one full cycle. The entire cycle is repeated for each phosphoramidite added, the timing of each step is crucial to a successful synthesis.

Following the synthesis, the controlled pore glass (to which the oligonucleotide is attached) was transferred to a tube containing concentrated ammonium hydroxide, and the mixture allowed to stand for 20min at room temperature. This step removes the oligonucleotide from the controlled pore glass support. Deprotection of the bases and cleavage of the cyanoethyl ester were achieved by heating the tightly sealed tube containing the oligonucleotide and ammonium hydroxide for 17h at 50°C. After degassing, the solution was evaporated to dryness *in vacuo*.

The crude oligonucleotide was dissolved in water and separation was achieved on a semi-preparative reverse-phase HPLC column, eluting with triethyl ammonium acetate buffer (pH 7.0). This buffer is chosen because it is volatile and no subsequent desalting steps are necessary. The fractions obtained were examined by ^{1}H NMR spectroscopy to determine which fraction was most likely to contain the required oligonucleotide. After identification of the most promising fraction, final deprotection was carried out by allowing the residue to stand for 20min at room temperature in acetic acid-water (4:1). A description of the NMR experiments carried out on this fraction can be found in Section 3.6.

3.3 KINETIC STUDIES

Kinetic studies were conducted using a Perkin-Elmer R12 60MHz spectrometer. Reactants were weighed into a 1ml volumetric flask in the order : pyridine-carboxaldehyde, tertiary amine catalyst and then $CDCl_3$. These reactants were pre-warmed to probe temperature (37°C) in a controlled-temperature water bath, in the tightly stoppered volumetric flask. After 4min equilibration time, the reaction was initiated by the addition of pre-weighed acrylate. The resulting reaction mixture was immediately transferred to an NMR tube and placed in the probe of the NMR spectrometer. A spectrum was then run immediately, and spectra were run at regular intervals over the duration of the reaction.

A typical reaction mixture contained 0.107g pyridine-4-carboxaldehyde, 0.086g methyl acrylate, 0.550g $CDCl_3$ and 0.007g of 3-hydroxyquinuclidine, with the duration of the reaction being approximately 6h. The data obtained gave linear second-order kinetic plots, and these are shown on the following pages. Rate constant calculations were carried out as discussed in Section 2.1.1.2. In each case, experiments were repeated until the results from duplicate runs fell within acceptable limits of error. The third-order rate constants (the reactions being pseudo second-order), together with their error limits (deviations from the mean) are shown in Table 2 (Section 2.1.1.2, p. 73), and represent the means from duplicate determinations.

A kinetic experiment was conducted in the probe of a Varian E-line Century EPR spectrometer operating at 5.5GHz. Three different settings were used:

(i) Gain 10 x 10^3 x 10, modulation amplitude 1G p-p and microwave power 10dβ attenuation.

(ii) Gain 1.6 x 10^4 x 10, modulation amplitude 0.1G p-p and microwave power 10dβ attenuation.

(iii) Gain and modulation amplitude as in (ii), and microwave power 20dβ attenuation.

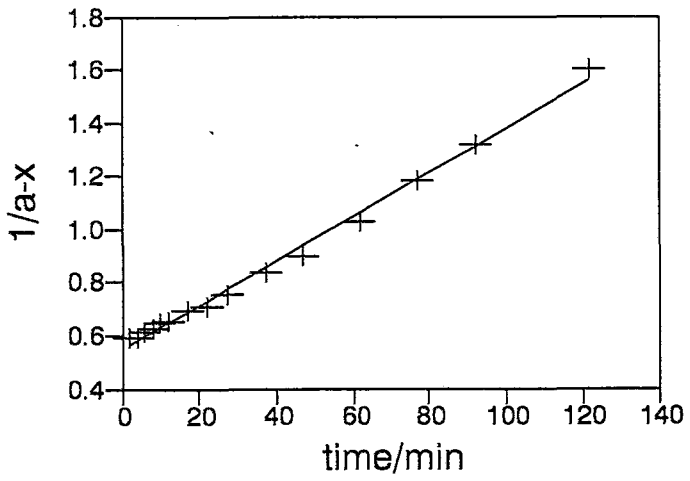
No EPR signal indicative of a radical species was observed.

Run 1 Pyr-4 : MeAc : Hq (1 : 1 : 0.05)

No.	t	ratio	a-x	1/a-x	y(calc)
1	2	0.929	1.689	0.592	0.566
2	4	0.925	1.682	0.595	0.582
3	6	0.897	1.631	0.613	0.599
4	8	0.879	1.598	0.626	0.615
5	10	0.852	1.549	0.646	0.632
6	12	0.841	1.529	0.654	0.648
7	17	0.789	1.434	0.697	0.690
8	22	0.774	1.407	0.711	0.731
9	27	0.726	1.320	0.758	0.773
10	37	0.657	1.194	0.837	0.856
11	47	0.611	1.111	0.900	0.938
12	62	0.537	0.976	1.024	1.063
13	77	0.467	0.849	1.178	1.187
14	92	0.418	0.760	1.316	1.311
15	122	0.343	0.624	1.604	1.560

Regression Output:

Constant	0.5490
Std Err of Y Est	0.0239
R Squared	0.9943
No. of Observations	15
Degrees of Freedom	13
X Coefficient(s)	0.0083
Std Err of Coef.	0.0002

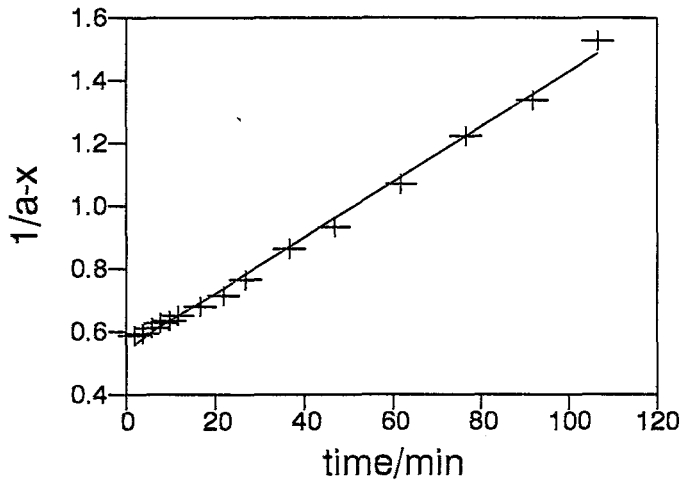


Run 1

No.	t	ratio	a-x	1/a-x	y(calc)
1	2	0.941	1.711	0.585	0.559
2	4	0.926	1.683	0.594	0.577
3	6	0.905	1.645	0.608	0.595
4	8	0.879	1.598	0.626	0.612
5	10	0.87	1.582	0.632	0.630
6	12	0.851	1.547	0.646	0.647
7	17	0.813	1.478	0.677	0.691
8	22	0.771	1.402	0.713	0.735
9	27	0.719	1.307	0.765	0.779
10	37	0.639	1.162	0.861	0.867
11	47	0.592	1.076	0.929	0.954
12	62	0.517	0.940	1.064	1.086
13	77	0.45	0.818	1.222	1.218
14	92	0.412	0.749	1.335	1.350
15	107	0.361	0.656	1.524	1.481

Regression Output:

Constant	0.5418
Std Err of Y Est	0.0203
R Squared	0.9958
No. of Observations	15
Degrees of Freedom	13
X Coefficient(s)	0.0088
Std Err of Coef.	0.0002

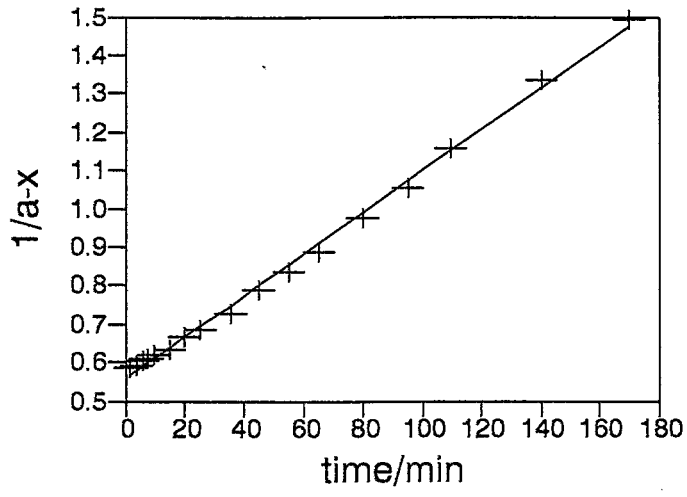


Run 2 Pyr-4 : EtAc : Hq (1 : 1 : 0.05)

No.	t	ratio	a-x	1/a-x	y(calc)
1	2	0.938	1.705	0.586	0.567
2	4	0.932	1.694	0.590	0.578
3	6	0.908	1.651	0.606	0.589
4	8	0.899	1.634	0.612	0.600
5	10	0.888	1.614	0.619	0.611
6	15	0.866	1.574	0.635	0.638
7	20	0.825	1.500	0.667	0.665
8	25	0.801	1.456	0.687	0.692
9	35	0.755	1.373	0.729	0.746
10	45	0.698	1.269	0.788	0.800
11	55	0.659	1.198	0.835	0.854
12	65	0.622	1.131	0.884	0.908
13	80	0.564	1.025	0.975	0.989
14	95	0.523	0.951	1.052	1.071
15	110	0.476	0.865	1.156	1.152
16	140	0.412	0.749	1.335	1.314
17	170	0.368	0.669	1.495	1.476

Regression Output:

Constant	0.5566
Std Err of Y Est	0.0159
R Squared	0.9969
No. of Observations	17
Degrees of Freedom	15
X Coefficient(s)	0.0054
Std Err of Coef.	7.76E-05

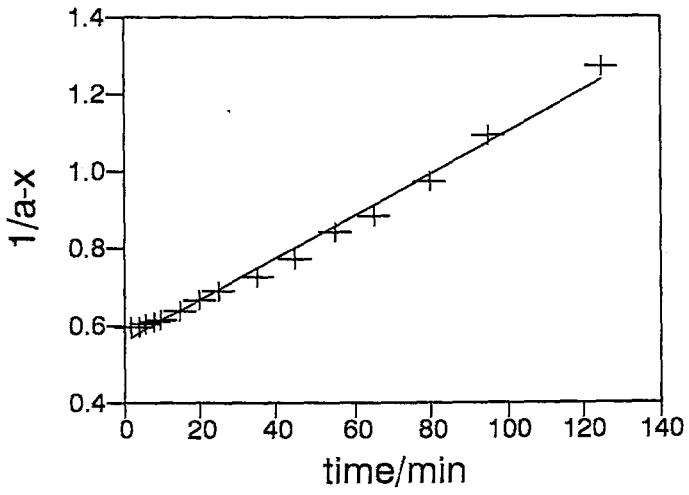


Run 2

No.	t	ratio	a-x	1/a-x	y(calc)
1	2	0.929	1.689	0.592	0.566
2	4	0.929	1.689	0.592	0.577
3	6	0.910	1.654	0.604	0.588
4	8	0.908	1.651	0.606	0.599
5	10	0.897	1.631	0.613	0.610
6	15	0.865	1.573	0.636	0.637
7	20	0.828	1.505	0.664	0.664
8	25	0.804	1.462	0.684	0.691
9	35	0.760	1.382	0.724	0.745
10	45	0.714	1.298	0.770	0.800
11	55	0.657	1.194	0.837	0.854
12	65	0.623	1.133	0.883	0.908
13	80	0.566	1.029	0.972	0.990
14	95	0.505	0.918	1.089	1.071
15	125	0.434	0.789	1.267	1.234

Regression Output:

Constant	0.5556
Std Err of Y Est	0.0202
R Squared	0.9910
No. of Observations	15
Degrees of Freedom	13
X Coefficient(s)	0.0054
Std Err of Coef.	0.0001

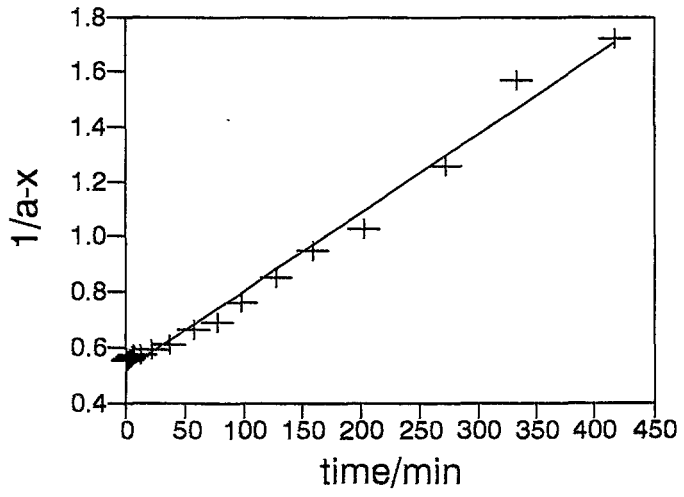


Run 3 Pyr-4 : iPrAc : Hq (1 : 1 : 0.05)

No.	t	ratio	a-x	1/a-x	y(calc)
1	2	0.990	1.800	0.556	0.523
2	4	0.980	1.782	0.561	0.529
3	6	0.969	1.762	0.568	0.535
4	8	0.966	1.756	0.569	0.540
5	13	0.956	1.738	0.575	0.555
6	23	0.928	1.687	0.593	0.583
7	38	0.904	1.643	0.608	0.626
8	58	0.828	1.505	0.664	0.683
9	78	0.795	1.445	0.692	0.740
10	98	0.721	1.311	0.763	0.797
11	128	0.644	1.171	0.854	0.882
12	158	0.580	1.054	0.948	0.968
13	203	0.535	0.973	1.028	1.096
14	273	0.437	0.794	1.259	1.295
15	333	0.351	0.638	1.567	1.466
16	418	0.320	0.582	1.719	1.708

Regression Output:

Constant	0.5175
Std Err of Y Est	0.0431
R Squared	0.9875
No. of Observations	16
Degrees of Freedom	14
X Coefficient(s)	0.0028
Std Err of Coef.	8.58E-05



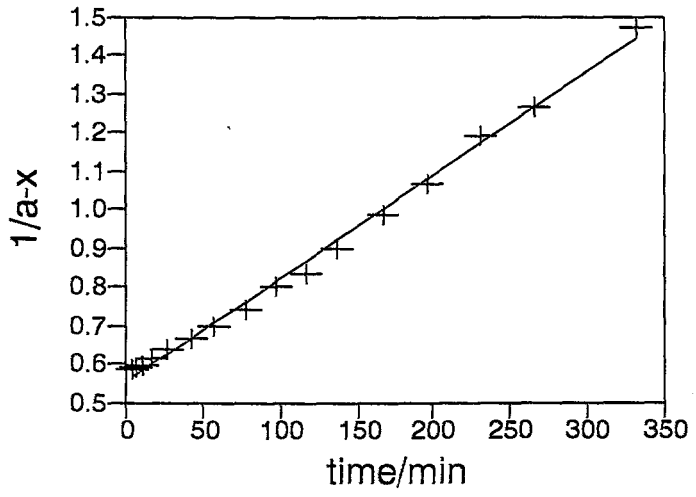
Run 3

No.	t	ratio	a-x	1/a-x	y(calc)
1	5	0.942	1.713	0.584	0.566
2	7	0.932	1.694	0.590	0.571
3	12	0.921	1.674	0.597	0.585
4	17	0.898	1.633	0.613	0.598
5	27	0.866	1.574	0.635	0.625
6	42	0.829	1.507	0.664	0.665
7	57	0.789	1.434	0.697	0.705
8	77	0.746	1.356	0.737	0.759
9	97	0.687	1.249	0.801	0.812
10	117	0.660	1.200	0.833	0.866
11	137	0.612	1.113	0.899	0.919
12	167	0.557	1.013	0.988	0.999
13	197	0.517	0.940	1.064	1.080
14	232	0.462	0.840	1.191	1.173
15	267	0.434	0.789	1.267	1.267
16	332	0.374	0.680	1.471	1.441

Regression Output:

Constant	0.5525
Std Err of Y Est	0.0186
R Squared	0.9957
No. of Observations	16
Degrees of Freedom	14

X Coefficient(s)	0.0027
Std Err of Coef.	4.71E-05

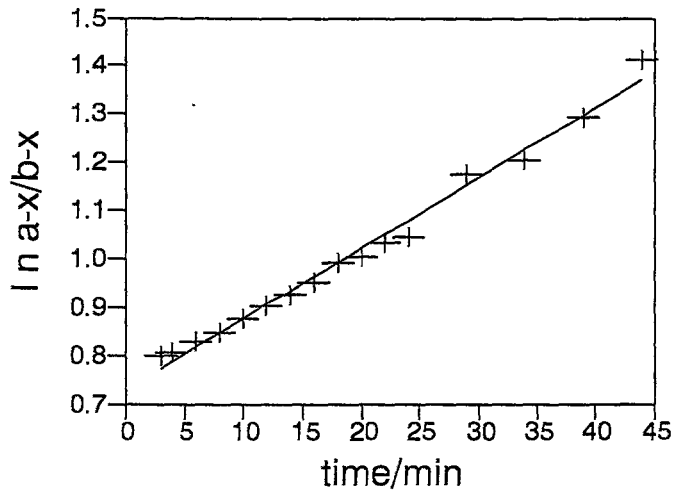


Run 4 Pyr-4 : MeAc : Hq (2 : 1 : 0.05)

No.	t	ratio	a-x	x	b-x	lna-x/b-x	y(calc)
1	3	0.910	3.309	0.327	1.491	0.797	0.774
2	4	0.904	3.287	0.349	1.469	0.805	0.788
3	6	0.888	3.229	0.407	1.411	0.828	0.817
4	8	0.875	3.182	0.454	1.364	0.847	0.847
5	10	0.856	3.112	0.524	1.294	0.877	0.876
6	12	0.842	3.062	0.574	1.244	0.901	0.905
7	14	0.829	3.014	0.622	1.196	0.924	0.934
8	16	0.815	2.963	0.673	1.145	0.951	0.963
9	18	0.795	2.891	0.745	1.073	0.991	0.992
10	20	0.790	2.872	0.764	1.054	1.002	1.021
11	22	0.776	2.822	0.814	1.004	1.034	1.050
12	24	0.771	2.803	0.833	0.985	1.046	1.079
13	29	0.723	2.629	1.007	0.811	1.176	1.152
14	34	0.714	2.596	1.040	0.778	1.205	1.225
15	39	0.689	2.505	1.131	0.687	1.293	1.298
16	44	0.661	2.403	1.233	0.585	1.412	1.370

Regression Output:

Constant	0.7302
Std Err of Y Est	0.0203
R Squared	0.9884
No. of Observations	16
Degrees of Freedom	14
X Coefficient(s)	0.0145
Std Err of Coef.	0.0004

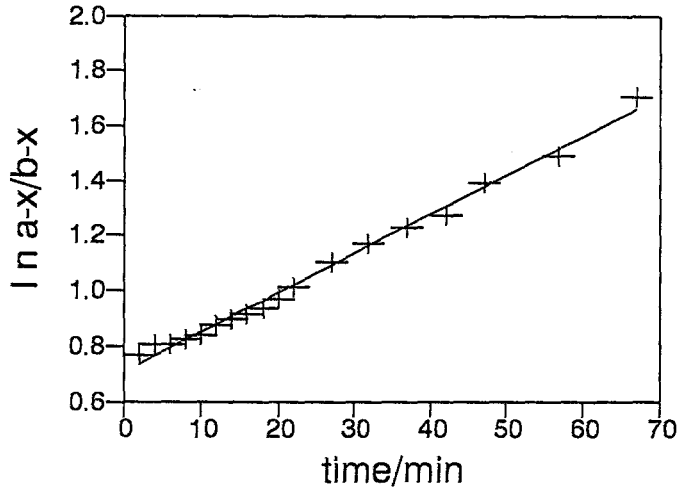


Run 4

No.	t	ratio	a-x	x	b-x	lna-x/b-x	y(calc)
1	2	0.936	3.403	0.233	1.585	0.764	0.737
2	4	0.905	3.291	0.345	1.473	0.804	0.765
3	6	0.902	3.280	0.356	1.462	0.808	0.793
4	8	0.890	3.236	0.400	1.418	0.825	0.822
5	10	0.883	3.211	0.425	1.393	0.835	0.850
6	12	0.856	3.112	0.524	1.294	0.877	0.879
7	14	0.844	3.069	0.567	1.251	0.898	0.907
8	16	0.835	3.036	0.600	1.218	0.913	0.935
9	18	0.822	2.989	0.647	1.171	0.937	0.964
10	20	0.806	2.931	0.705	1.113	0.968	0.992
11	22	0.785	2.854	0.782	1.036	1.013	1.020
12	27	0.747	2.716	0.920	0.898	1.107	1.091
13	32	0.726	2.640	0.996	0.822	1.167	1.162
14	37	0.707	2.571	1.065	0.753	1.228	1.233
15	42	0.694	2.523	1.113	0.705	1.275	1.304
16	47	0.665	2.418	1.218	0.600	1.394	1.375
17	57	0.646	2.349	1.287	0.531	1.487	1.517
18	67	0.611	2.222	1.414	0.404	1.706	1.659

Regression Output:

Constant	0.7083
Std Err of Y Est	0.0239
R Squared	0.9925
No. of Observations	18
Degrees of Freedom	16
X Coefficient(s)	0.0142
Std Err of Coef.	0.0003

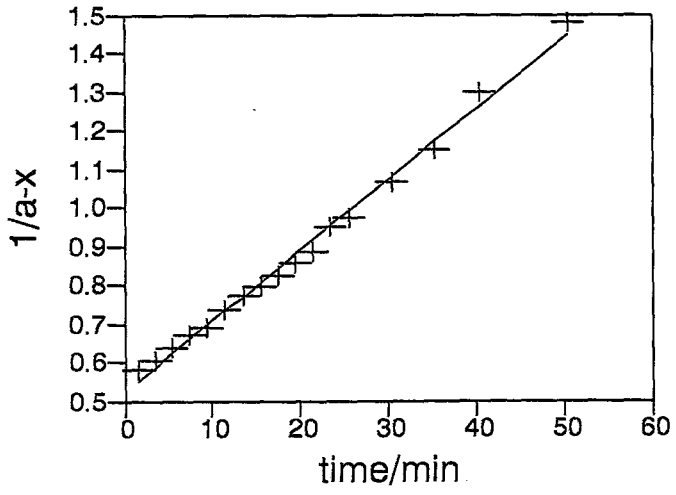


Run 5 Pyr-4 : MeAc : Hq (1 : 1 : 0.1)

No.	t	ratio	a-x	1/a-x	y(calc)
1	1.5	0.943	1.714	0.583	0.550
2	3.5	0.907	1.649	0.606	0.587
3	5.5	0.863	1.569	0.637	0.623
4	7.5	0.819	1.489	0.672	0.660
5	9.5	0.800	1.454	0.688	0.696
6	11.5	0.750	1.364	0.733	0.733
7	13.5	0.714	1.298	0.770	0.769
8	15.5	0.692	1.258	0.795	0.806
9	17.5	0.667	1.213	0.825	0.842
10	19.5	0.642	1.167	0.857	0.879
11	21.5	0.624	1.134	0.881	0.915
12	23.5	0.581	1.056	0.947	0.952
13	25.5	0.565	1.027	0.974	0.988
14	30.5	0.517	0.940	1.064	1.080
15	35.5	0.478	0.869	1.151	1.171
16	40.5	0.424	0.771	1.297	1.262
17	50.5	0.372	0.676	1.479	1.445

Regression Output:

Constant	0.5228
Std Err of Y Est	0.0220
R Squared	0.9928
No. of Observations	17
Degrees of Freedom	15
X Coefficient(s)	0.0183
Std Err of Coef.	0.0004

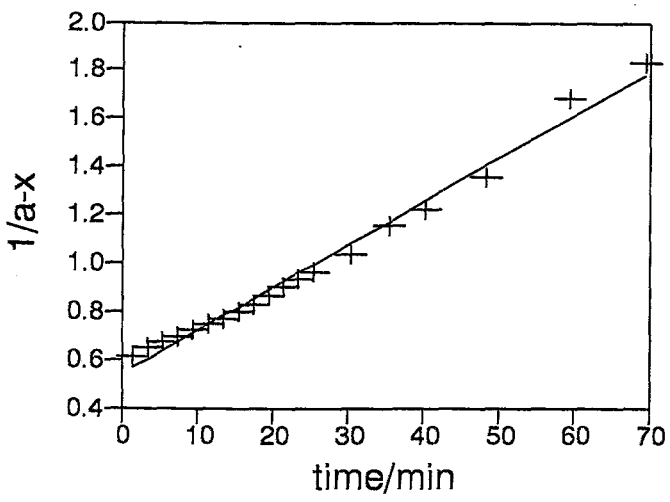


Run 5

No.	t	ratio	a-x	1/a-x	y(calc)
1	1.5	0.894	1.625	0.615	0.565
2	3.5	0.852	1.549	0.646	0.601
3	5.5	0.817	1.485	0.673	0.637
4	7.5	0.789	1.434	0.697	0.672
5	9.5	0.762	1.385	0.722	0.708
6	11.5	0.738	1.342	0.745	0.744
7	13.5	0.716	1.302	0.768	0.780
8	15.5	0.686	1.247	0.802	0.815
9	17.5	0.664	1.207	0.828	0.851
10	19.5	0.635	1.154	0.866	0.887
11	21.5	0.612	1.113	0.899	0.923
12	23.5	0.588	1.069	0.935	0.958
13	25.5	0.574	1.044	0.958	0.994
14	30.5	0.529	0.962	1.040	1.083
15	35.5	0.476	0.865	1.156	1.173
16	40.5	0.451	0.820	1.220	1.262
17	48.5	0.406	0.738	1.355	1.405
18	59.5	0.327	0.594	1.682	1.602
19	69.5	0.300	0.545	1.834	1.780

Regression Output:

Constant	0.5384
Std Err of Y Est	0.0392
R Squared	0.9877
No. of Observations	19
Degrees of Freedom	17
X Coefficient(s)	0.0179
Std Err of Coef.	0.0005

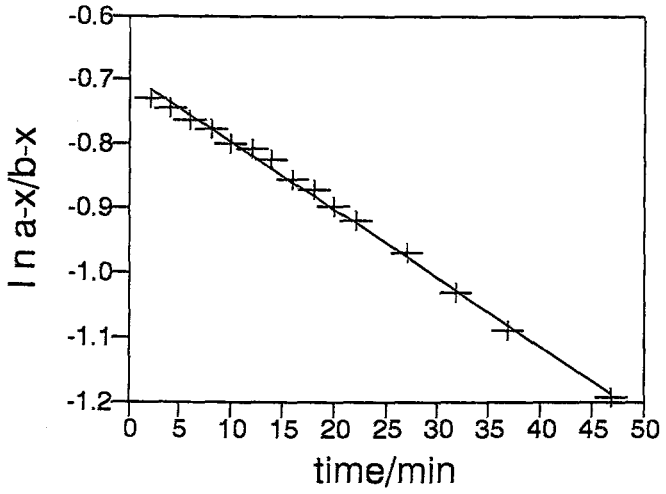


Run 6 Pyr-4 : MeAc : Hq (1 : 2 : 0.05)

No.	t	ratio	a-x	x	b-x	lna-x/b-x	y(calc)
1	2.17	0.933	1.696	0.122	3.514	-0.728	-0.715
2	4	0.906	1.647	0.171	3.465	-0.744	-0.734
3	6	0.871	1.583	0.235	3.401	-0.765	-0.755
4	8	0.851	1.547	0.271	3.365	-0.777	-0.776
5	10	0.816	1.483	0.335	3.301	-0.800	-0.797
6	12	0.805	1.463	0.355	3.281	-0.807	-0.818
7	14	0.779	1.416	0.402	3.234	-0.826	-0.840
8	16	0.741	1.347	0.471	3.165	-0.854	-0.861
9	18	0.718	1.305	0.513	3.123	-0.872	-0.882
10	20	0.687	1.249	0.569	3.067	-0.898	-0.903
11	22	0.663	1.205	0.613	3.023	-0.920	-0.924
12	27	0.610	1.109	0.709	2.927	-0.971	-0.976
13	32	0.554	1.007	0.811	2.825	-1.031	-1.029
14	37	0.506	0.920	0.898	2.738	-1.091	-1.081
15	47	0.435	0.791	1.027	2.609	-1.194	-1.186

Regression Output:

Constant	-0.6923
Std Err of Y Est	0.0088
R Squared	0.9961
No. of Observations	15
Degrees of Freedom	13
X Coefficient(s)	-0.0105
Std Err of Coef.	0.0002

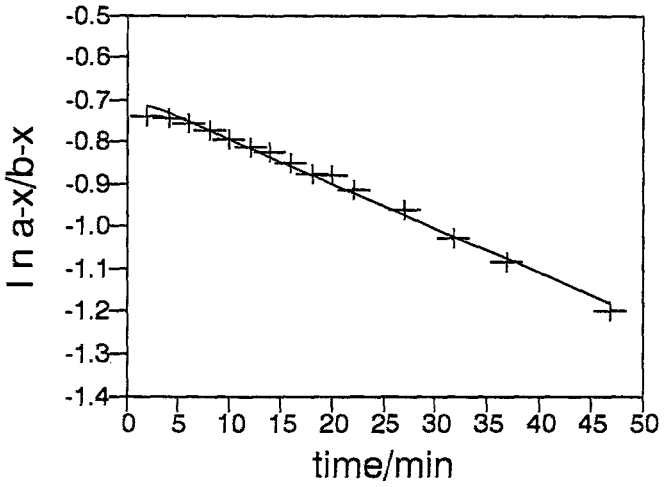


Run 6

No.	t	ratio	a-x	x	b-x	lna-x/b-x	y(calc)
1	2	0.914	1.662	0.156	3.480	-0.739	-0.713
2	4	0.906	1.647	0.171	3.465	-0.744	-0.733
3	6	0.885	1.609	0.209	3.427	-0.756	-0.754
4	8	0.854	1.553	0.265	3.371	-0.775	-0.775
5	10	0.825	1.500	0.318	3.318	-0.794	-0.796
6	12	0.796	1.447	0.371	3.265	-0.814	-0.817
7	14	0.781	1.420	0.398	3.238	-0.824	-0.838
8	16	0.745	1.354	0.464	3.172	-0.851	-0.858
9	18	0.714	1.298	0.520	3.116	-0.876	-0.879
10	20	0.708	1.287	0.531	3.105	-0.881	-0.900
11	22	0.667	1.213	0.605	3.031	-0.916	-0.921
12	27	0.621	1.129	0.689	2.947	-0.959	-0.973
13	32	0.557	1.013	0.805	2.831	-1.028	-1.025
14	37	0.51	0.927	0.891	2.745	-1.085	-1.077
15	47	0.433	0.787	1.031	2.605	-1.197	-1.181

Regression Output:

Constant	-0.6918
Std Err of Y Est	0.0123
R Squared	0.9922
No. of Observations	15
Degrees of Freedom	13
X Coefficient(s)	-0.0104
Std Err of Coef.	0.0003

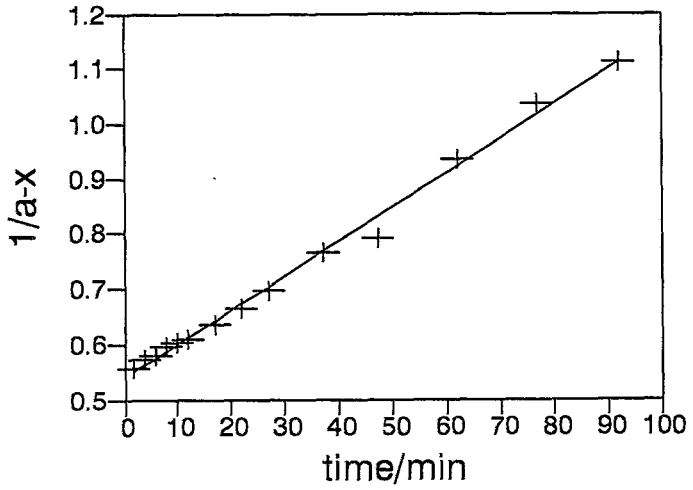


Run 7 Pyr-4 : MeAc : D-Hq (1 : 1 : 0.05)

No.	t	ratio	a-x	1/a-x	y(calc)
1	2	0.985	1.791	0.558	0.550
2	4	0.961	1.747	0.572	0.562
3	6	0.951	1.729	0.578	0.574
4	8	0.922	1.676	0.597	0.587
5	10	0.911	1.656	0.604	0.599
6	12	0.905	1.645	0.608	0.612
7	17	0.867	1.576	0.634	0.643
8	22	0.826	1.502	0.666	0.674
9	27	0.789	1.434	0.697	0.705
10	37	0.720	1.309	0.764	0.767
11	47	0.695	1.264	0.791	0.829
12	62	0.588	1.069	0.935	0.923
13	77	0.531	0.965	1.036	1.016
14	92	0.496	0.902	1.109	1.109

Regression Output:

Constant		0.5371
Std Err of Y Est		0.0146
R Squared		0.9940
No. of Observations		14
Degrees of Freedom		12
X Coefficient(s)	0.0062	
Std Err of Coef.	0.0001	

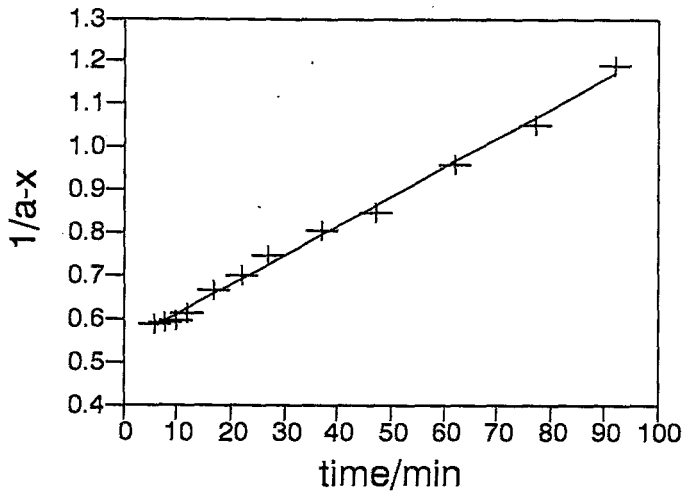


Run 7

No.	t	ratio	a-x	1/a-x	y(calc)
1	6	0.938	1.705	0.586	0.581
2	8	0.931	1.693	0.591	0.594
3	10	0.925	1.682	0.595	0.608
4	12	0.899	1.634	0.612	0.622
5	17	0.828	1.505	0.664	0.656
6	22	0.786	1.429	0.700	0.691
7	27	0.739	1.344	0.744	0.725
8	37	0.685	1.245	0.803	0.794
9	47	0.650	1.182	0.846	0.863
10	62	0.573	1.042	0.960	0.966
11	77	0.523	0.951	1.052	1.070
12	92	0.462	0.840	1.191	1.173

Regression Output:

Constant	0.5394
Std Err of Y Est	0.0137
R Squared	0.9957
No. of Observations	12
Degrees of Freedom	10
X Coefficient(s)	0.0069
Std Err of Coef.	0.0001



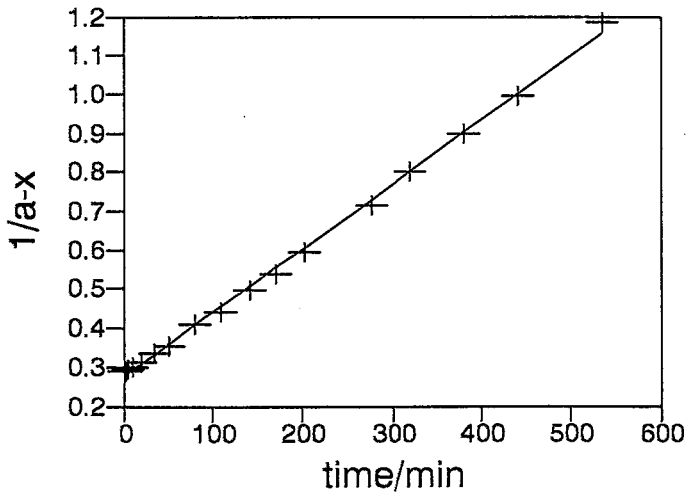
Run 8 Pyr-4 : MeAc : DABCO (conc) (1 : 1 : 0.05)

No.	t	ratio	a-x	1/a-x	y(calc)
1	2	0.953	3.465	0.289	0.277
2	4	0.943	3.429	0.292	0.281
3	6	0.932	3.389	0.295	0.284
4	11	0.918	3.338	0.300	0.292
5	21	0.876	3.185	0.314	0.309
6	36	0.815	2.963	0.337	0.333
7	51	0.781	2.840	0.352	0.358
8	81	0.675	2.454	0.407	0.407
9	111	0.620	2.254	0.444	0.457
10	141	0.551	2.003	0.499	0.506
11	171	0.510	1.854	0.539	0.556
12	201	0.462	1.680	0.595	0.605
13	276	0.386	1.403	0.713	0.729
14	321	0.344	1.251	0.799	0.803
15	381	0.306	1.113	0.899	0.902
16	441	0.276	1.004	0.996	1.000
17	536	0.232	0.844	1.185	1.157

Regression Output:

Constant	0.2741
Std Err of Y Est	0.0121
R Squared	0.9982
No. of Observations	17
Degrees of Freedom	15

X Coefficient(s)	0.0016
Std Err of Coef.	1.79E-05

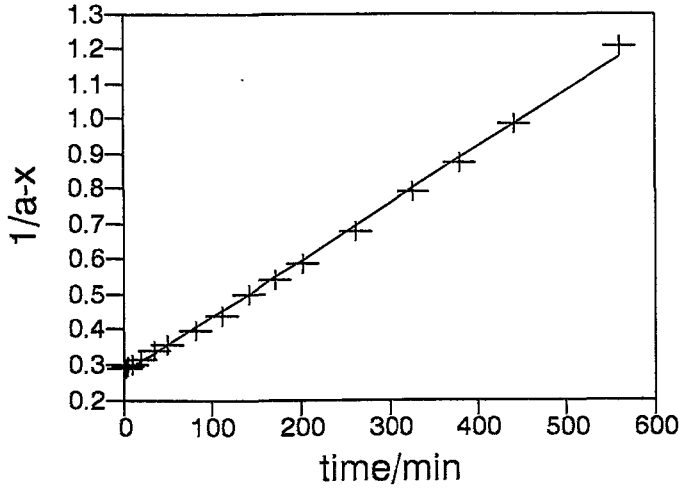


Run 8

No.	t	ratio	a-x	1/a-x	y(calc)
1	2	0.957	3.480	0.287	0.278
2	4	0.943	3.429	0.292	0.281
3	6	0.936	3.403	0.294	0.284
4	11	0.917	3.334	0.300	0.292
5	21	0.872	3.171	0.315	0.308
6	36	0.812	2.952	0.339	0.332
7	51	0.775	2.818	0.355	0.357
8	81	0.689	2.505	0.399	0.405
9	111	0.628	2.283	0.438	0.453
10	141	0.553	2.011	0.497	0.501
11	171	0.510	1.854	0.539	0.550
12	201	0.468	1.702	0.588	0.598
13	261	0.404	1.469	0.681	0.694
14	326	0.348	1.265	0.790	0.799
15	381	0.315	1.145	0.873	0.887
16	441	0.279	1.014	0.986	0.984
17	561	0.228	0.829	1.206	1.177

Regression Output:

Constant	0.2746
Std Err of Y Est	0.0123
R Squared	0.9982
No. of Observations	17
Degrees of Freedom	15
X Coefficient(s)	0.0016
Std Err of Coef.	1.78E-05

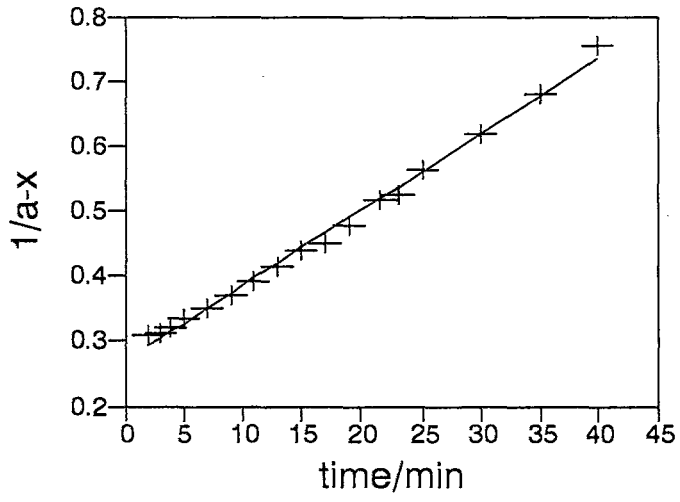


Run 9 Pyr-4 : MeAc : Hq (conc) (1 : 1 : 0.05)

No.	t	ratio	a-x	1/a-x	y(calc)
1	2	0.893	3.247	0.308	0.292
2	3	0.881	3.203	0.312	0.304
3	4	0.858	3.120	0.321	0.315
4	5	0.825	3:000	0.333	0.327
5	7	0.784	2.851	0.351	0.350
6	9	0.742	2.698	0.371	0.374
7	11	0.703	2.556	0.391	0.397
8	13	0.665	2.418	0.414	0.420
9	15	0.626	2.276	0.439	0.444
10	17	0.609	2.214	0.452	0.467
11	19	0.576	2.094	0.477	0.491
12	21.5	0.532	1.934	0.517	0.520
13	23	0.523	1.902	0.526	0.537
14	25	0.487	1.771	0.565	0.561
15	30	0.444	1.614	0.619	0.619
16	35	0.405	1.473	0.679	0.677
17	40	0.364	1.324	0.756	0.736

Regression Output:

Constant	0.2685
Std Err of Y Est	0.0099
R Squared	0.9949
No. of Observations	17
Degrees of Freedom	15
X Coefficient(s)	0.0117
Std Err of Coef.	0.0002

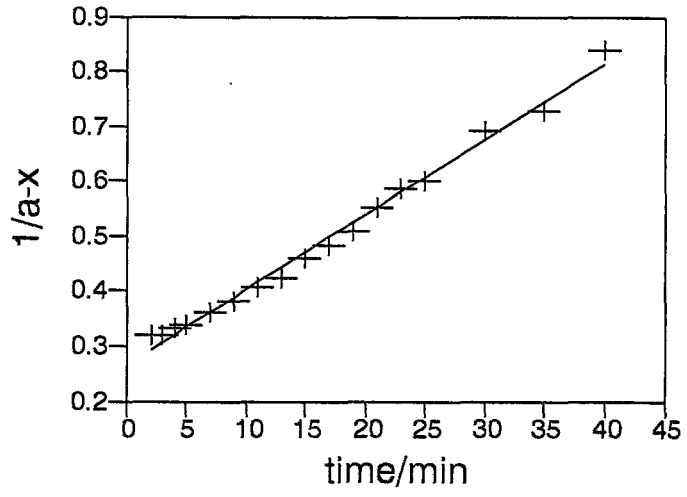


Run 9

No.	t	ratio	a-x	1/a-x	y(calc)
1	2	0.861	3.131	0.319	0.293
2	3	0.863	3.138	0.319	0.307
3	4	0.831	3.022	0.331	0.321
4	5	0.811	2.949	0.339	0.334
5	7	0.761	2.767	0.361	0.362
6	9	0.718	2.611	0.383	0.389
7	11	0.677	2.462	0.406	0.416
8	13	0.650	2.363	0.423	0.444
9	15	0.597	2.171	0.461	0.471
10	17	0.570	2.073	0.483	0.499
11	19	0.541	1.967	0.508	0.526
12	21	0.500	1.818	0.550	0.553
13	23	0.467	1.698	0.589	0.581
14	25	0.457	1.662	0.602	0.608
15	30	0.397	1.443	0.693	0.676
16	35	0.377	1.371	0.730	0.745
17	40	0.327	1.189	0.841	0.813

Regression Output:

Constant		0.2659
Std Err of Y Est		0.0155
R Squared		0.9909
No. of Observations		17
Degrees of Freedom		15
X Coefficient(s)	0.0137	
Std Err of Coef.	0.0003	

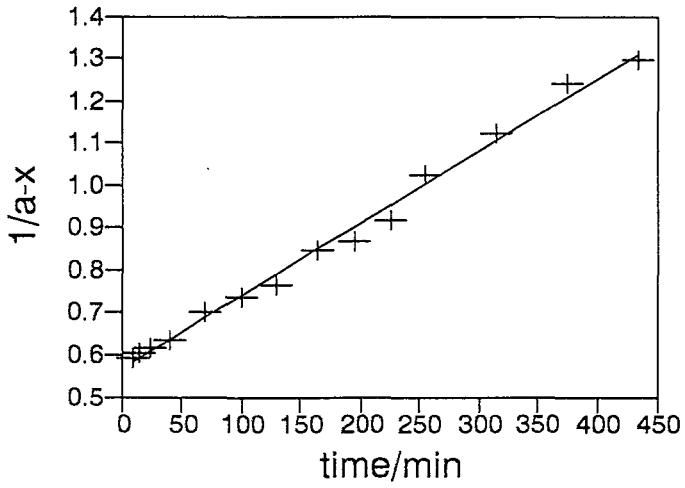


Run 10 Pyr-2 : MeAc : Hq (1 : 1 : 0.05)

No.	t	ratio	a-x	1/a-x	y(calc)
1	10	0.927	1.685	0.593	0.584
2	15	0.909	1.653	0.605	0.593
3	25	0.892	1.622	0.617	0.610
4	40	0.870	1.582	0.632	0.636
5	70	0.784	1.425	0.702	0.687
6	100	0.748	1.360	0.735	0.738
7	130	0.718	1.305	0.766	0.789
8	165	0.651	1.184	0.845	0.849
9	195	0.634	1.153	0.868	0.900
10	225	0.598	1.087	0.920	0.952
11	255	0.536	0.974	1.026	1.003
12	315	0.490	0.891	1.123	1.105
13	375	0.444	0.807	1.239	1.208
14	435	0.425	0.773	1.294	1.310

Regression Output:

Constant	0.5674
Std Err of Y Est	0.0209
R Squared	0.9928
No. of Observations	14
Degrees of Freedom	12
X Coefficient(s)	0.0017
Std Err of Coef.	4.19E-05

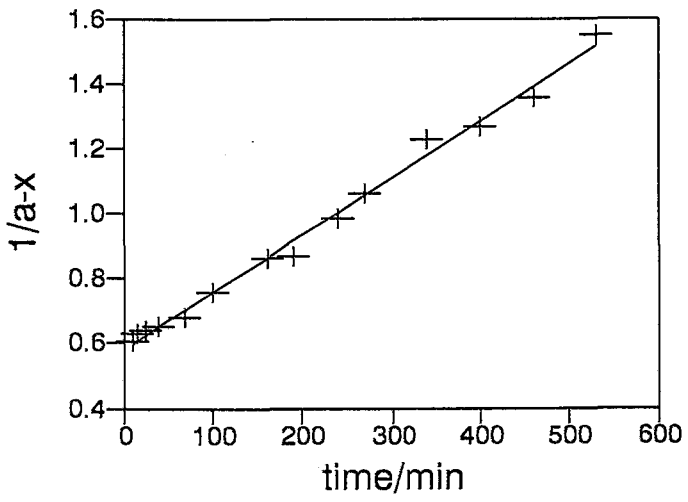


Run 10

No.	t	ratio	a-x	1/a-x	y(calc)
1	10	0.907	1.649	0.606	0.598
2	15	0.870	1.582	0.632	0.607
3	25	0.860	1.563	0.640	0.625
4	40	0.841	1.529	0.654	0.651
5	70	0.807	1.467	0.682	0.704
6	100	0.727	1.322	0.757	0.756
7	160	0.638	1.160	0.862	0.862
8	190	0.636	1.156	0.865	0.914
9	240	0.560	1.018	0.982	1.002
10	270	0.519	0.944	1.060	1.055
11	340	0.448	0.814	1.228	1.178
12	400	0.434	0.789	1.267	1.283
13	460	0.406	0.738	1.355	1.389
14	532	0.355	0.645	1.549	1.515

Regression Output:

Constant	0.5807
Std Err of Y Est	0.0279
R Squared	0.9924
No. of Observations	14
Degrees of Freedom	12
X Coefficient(s)	0.0018
Std Err of Coef.	4.42E-05



3.4 SINGLE CRYSTAL X-RAY DIFFRACTION ANALYSES

The structures of compounds **152** and **174** were determined using single crystal X-ray diffraction analysis. Compound **174** proved to be very sparingly soluble in most solvents and, thus, recrystallization was not possible and crystals obtained directly from the reaction mixture were used for data collection. Compound **152** was easily recrystallized from hexane but crystals obtained directly from the reaction mixture proved to be of the necessary quality for the collection of X-ray data, and were used directly.

The summarized crystal data for compounds **152** and **174** can be found in Tables 13 and 18, respectively (Appendix 5.2). Data collection was carried out using a CAD4 diffractometer operating in the ω-2θ mode with scan width = 0.8 + 0.35 tan θ, a variable ω scan speed (max = 6° min^{-1}) and using graphite monochromated Mo-K_α radiation.

Direct methods,[406] followed by full-matrix least-squares refinement were used for structure analysis, with all the non-hydrogen atoms anisotropic and hydrogen atoms in calculated positions with common isotropic temperature factors.

For compound **152** the refinement converged to R = 0.065 (114 parameters), R_w was 0.065 [$w = 0.594/ (\sigma^2F + F^2)$].

In the case of compound **174**, the refinement converged to R = 0.073 (193 parameters), R_w was 0.101 [$w = 0.102/ (\sigma^2F + F^2)$].

The plotting program PLUTO[407] was used to obtain molecular structure and packing diagrams, viewed in the direction of minimum overlap.

Tables of fractional co-ordinates for hydrogen and non-hydrogen atoms, as well as tables of bond lengths and angles and anisotropic temperature factors can be found in Tables 14-17 for compound **152** and Tables 19-22 for compound **174** (Appendix 5.2). For compound **174**, a table of least-squares planes can also be found in Appendix 5.2 (Table 23).

3.5 NMR CONFORMATIONAL STUDIES

Variable temperature ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AMX400 spectrometer operating at 400MHz for ^{1}H and 100MHz for ^{13}C nuclei. The samples were made up in $CDCl_3$ solution, thus setting the minimum possible temperature for collection in the region of 210K, and the maximum in the region of 335K. The ^{1}H NMR data were collected using an inverse 5mm probe, and the ^{13}C NMR data using a broad band 5mm probe. Samples were allowed to equilibrate for 5 minutes at each new temperature prior to data collection. No problems relating to insolubility of material were experienced at low temperature.

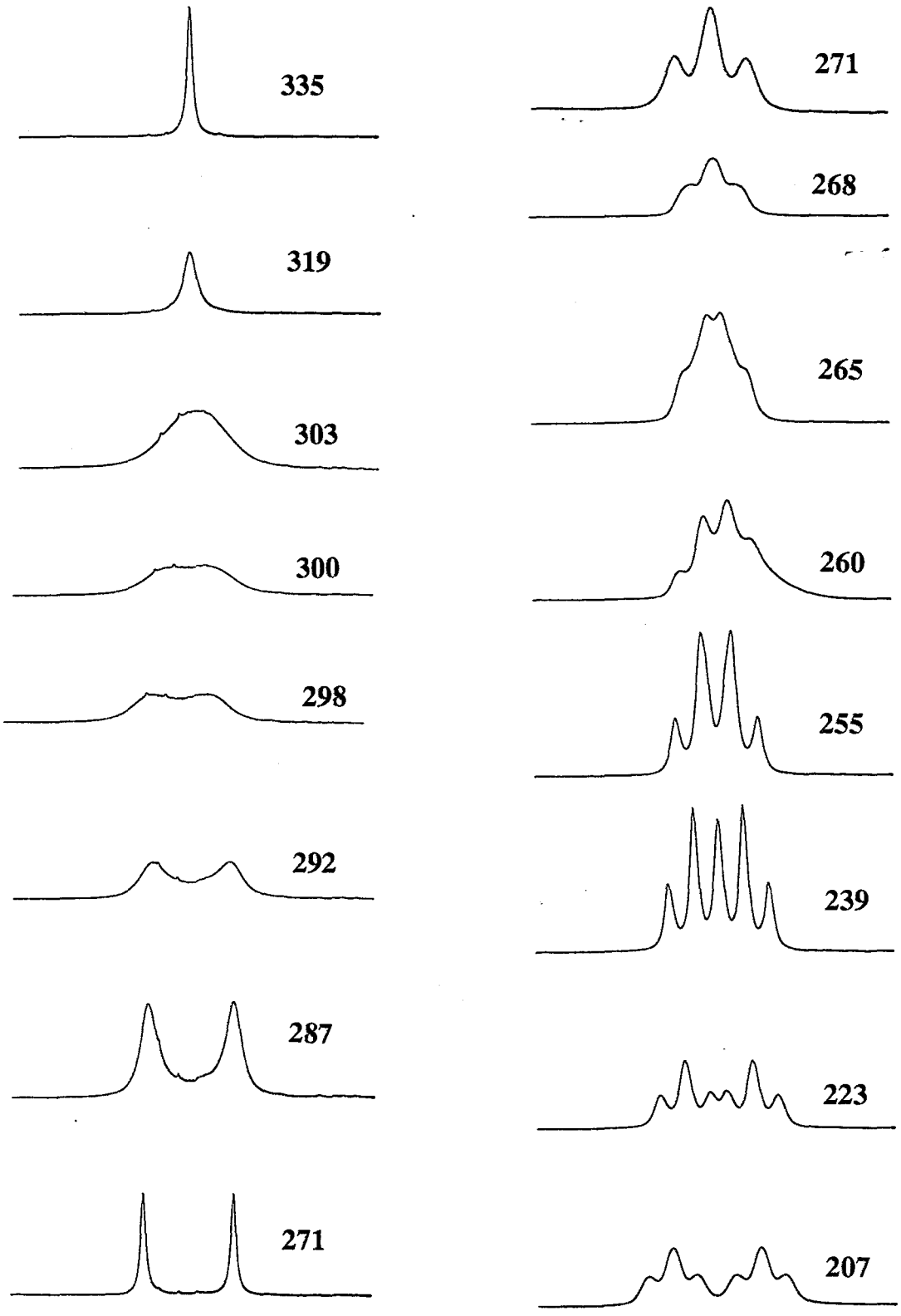
The NMR probe temperatures were accurately determined by performing variable temperature experiments on calibration samples of 4% methanol in methanol-d_6 between 193K and 303K, and 80% ethylene glycol in DMSO-d_6 between 303K and 373K. The peak separations between the CH_2 signal and the OH signal in the calibration samples were measured at various temperatures over the appropriate ranges, and an accurate measurement of a particular probe temperature was obtained by reading from a calibration curve[408] of peak separation (CH_2 and OH) against actual temperature. Errors resulting from inaccurate measurement of the probe temperature were thus minimized, and the overall error in the probe temperature is estimated to be $\pm$0.5K.

Coalescence temperatures (T_C) were obtained from the variable temperature spectra shown on the following pages, and are estimated to be correct within $\pm$0.5K. The estimated overall error in T_C (*viz.*, $\pm$1K) is thus the sum of the contributing errors.

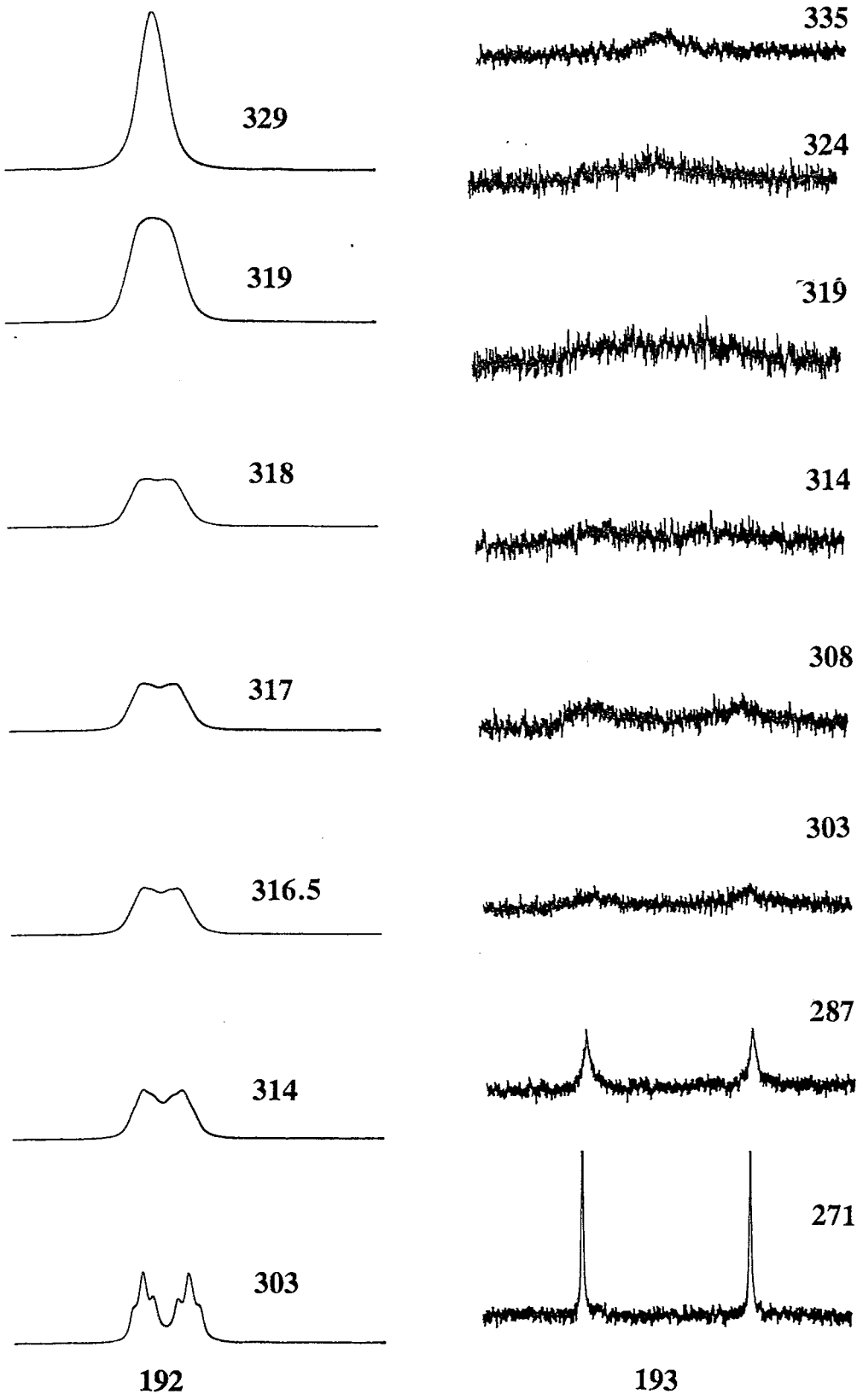
Frequency separations at coalescence ($\Delta\nu_C$) were determined by extrapolating plots of frequency separation ($\Delta\nu$) against temperature (T) and these plots are shown on the following pages, following the variable temperature spectra. The values of frequency separation ($\Delta\nu$) become more difficult to measure as coalescence approaches, as a result of peak broadening, and a departure from linearity is observed in the plots of $\Delta\nu$ *versus*

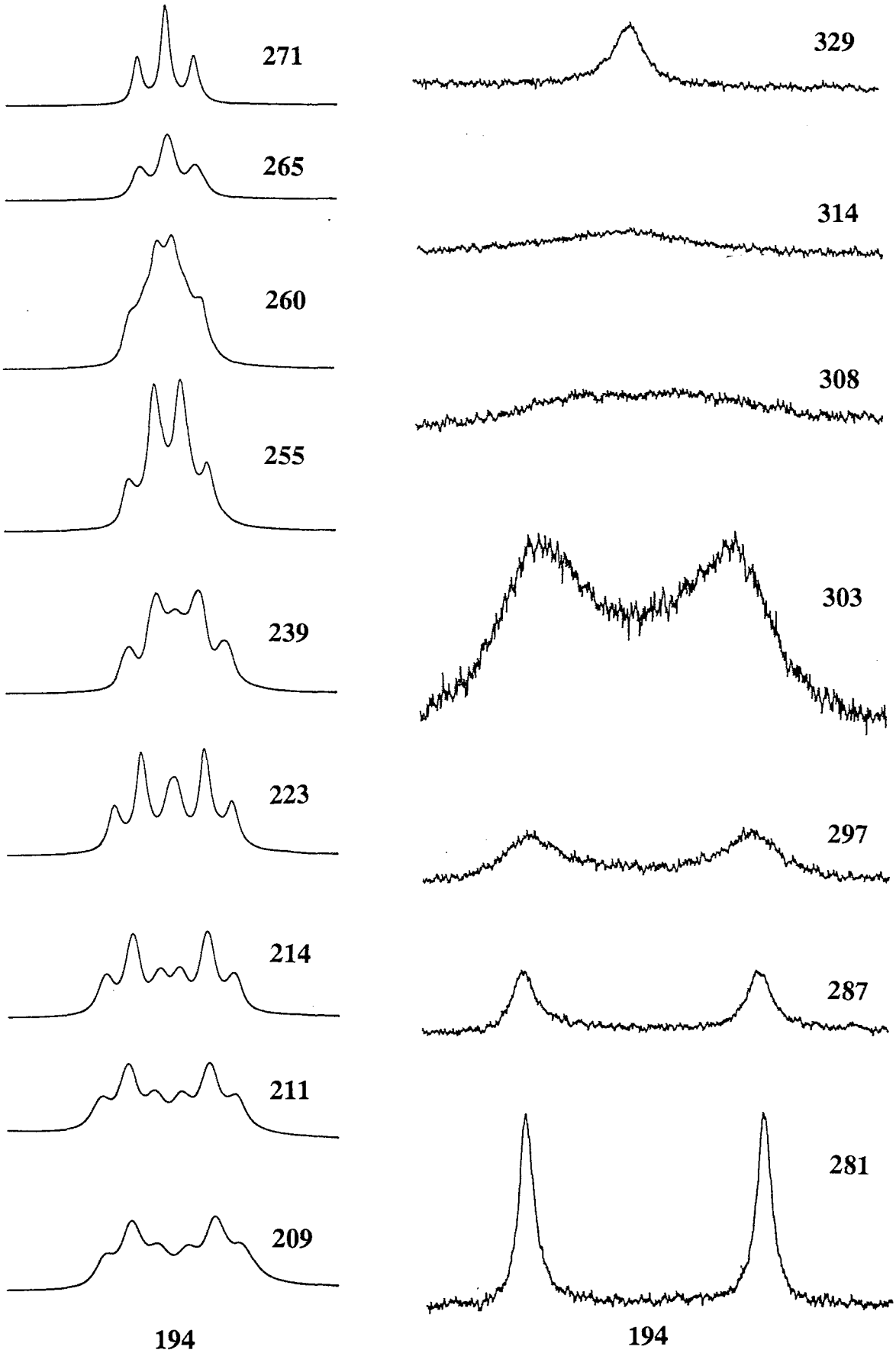
T: these points have been excluded and the linear portion of the plots, obtained at lower temperatures, was used for extrapolation purposes. This is believed to lead to a more accurate determination of peak separation at coalescence ($\Delta\nu_C$).[324] In all the plots used for extrapolation purposes, the correlation coefficients were extremely satisfactory and, in all but one case, they were greater than 0.99 (see the following pages). The lowest correlation coefficient was 0.98, in the case of compound **185**, where broad peaks made accurate measurement of peak separation more difficult.

An estimation of the errors in the $\Delta\nu_C$ values was made in each case, based on the possible error of extrapolation. Rotational energy barriers (ΔG^*) were calculated from coalescence temperature (T_C) and the frequency separation at coalescence ($\Delta\nu_C$), as described in Section 2.2.2, p. 128. The estimated error in ΔG^* values was calculated from the contributing errors in T_C and $\Delta\nu_C$, and Table 11 (Section 2.2.2, p. 130) shows the rotational energy barriers together with the calculated errors.



Variable temperature spectra obtained for carboxamides **185** and **191-194** (temperatures are shown in Kelvin).


329
319
318
317
316.5
314
303
192
335
324
319
314
308
303
287
271
193

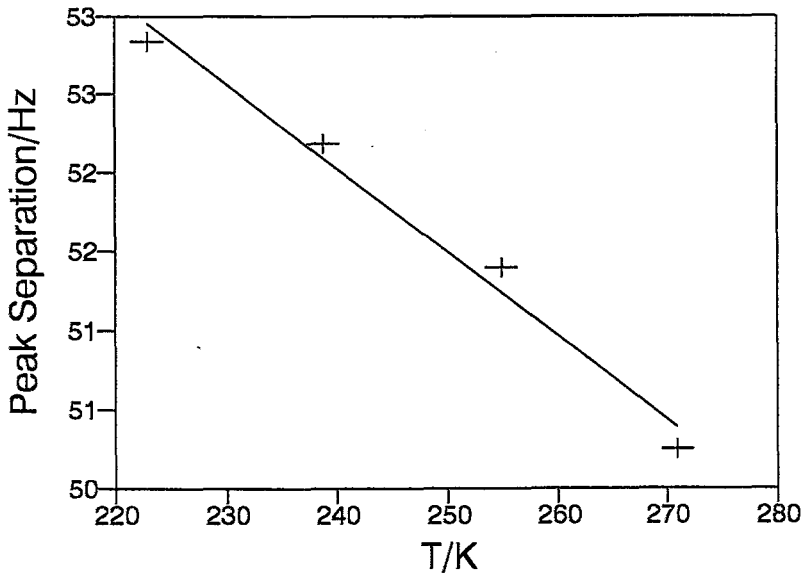

271
265
260
255
239
223
214
211
209
194
329
314
308
303
297
287
281
194

Compound 185

T/K	v/Hz	y(calc)
223	52.83	52.95
239	52.19	52.09
255	51.4	51.24
271	50.24	50.38

Regression Output:

Constant	64.8795
Std Err of Y Est	0.1871
R Squared	0.9812
No. of Observations	4
Degrees of Freedom	2
X Coefficient(s)	-0.0535
Std Err of Coef.	0.0052

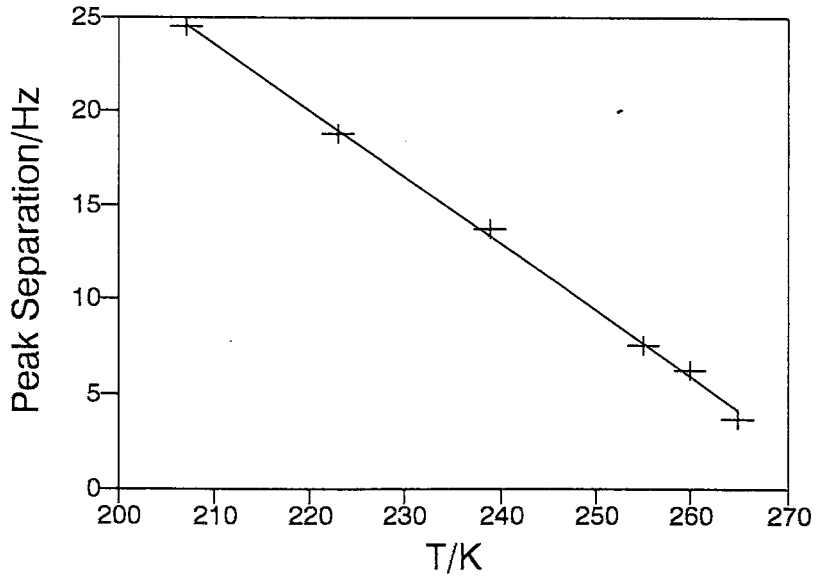


Compound 191

T/K	v/Hz	y(calc)
207	24.5	24.60
223	18.79	18.96
239	13.76	13.32
255	7.6	7.68
260	6.27	5.91
265	3.69	4.15

Regression Output:

Constant	97.56294
Std Err of Y Est	0.380558
R Squared	0.998221
No. of Observations	6
Degrees of Freedom	4
X Coefficient(s)	-0.3525
Std Err of Coef.	0.007441



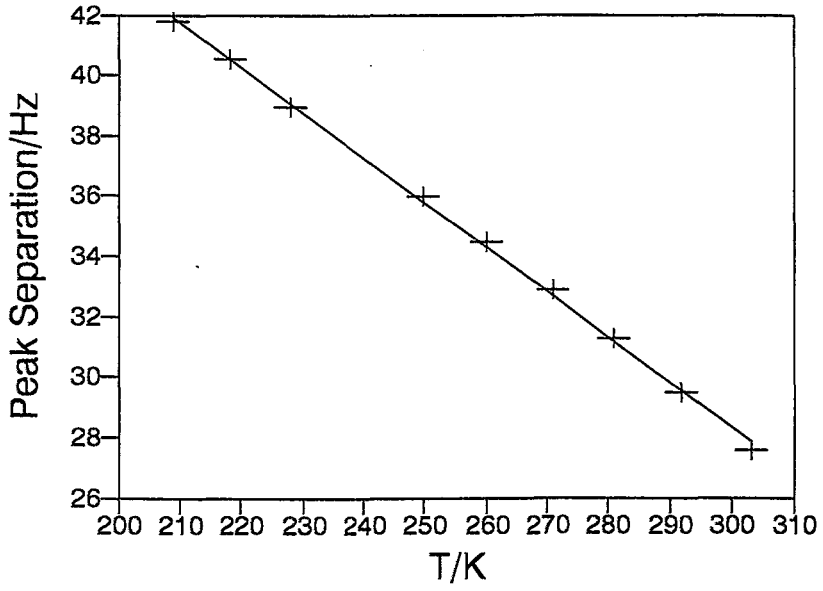
Compound 192

T/K	v/Hz	y(calc)
209	41.79	41.90
218	40.53	40.56
228	38.93	39.07
250	35.94	35.79
260	34.48	34.30
271	32.88	32.66
281	31.27	31.17
292	29.48	29.53
303	27.59	27.89

Regression Output:

Constant	73.05648
Std Err of Y Est	0.184708
R Squared	0.998785
No. of Observations	9
Degrees of Freedom	7

X Coefficient(s)	-0.14906
Std Err of Coef.	0.001965



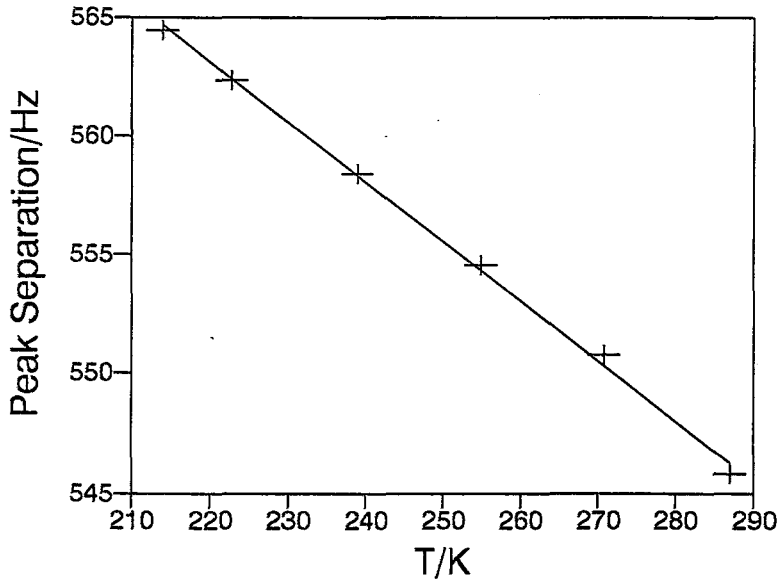
Compound 193

T/K	ν/Hz	y(calc)
214	564.47	564.65
223	562.34	562.38
239	558.38	558.35
255	554.54	554.32
271	550.75	550.29
287	545.77	546.26

Regression Output:

Constant	618.5641
Std Err of Y Est	0.365331
R Squared	0.997881
No. of Observations	6
Degrees of Freedom	4

X Coefficient(s)	-0.25194
Std Err of Coef.	0.005805

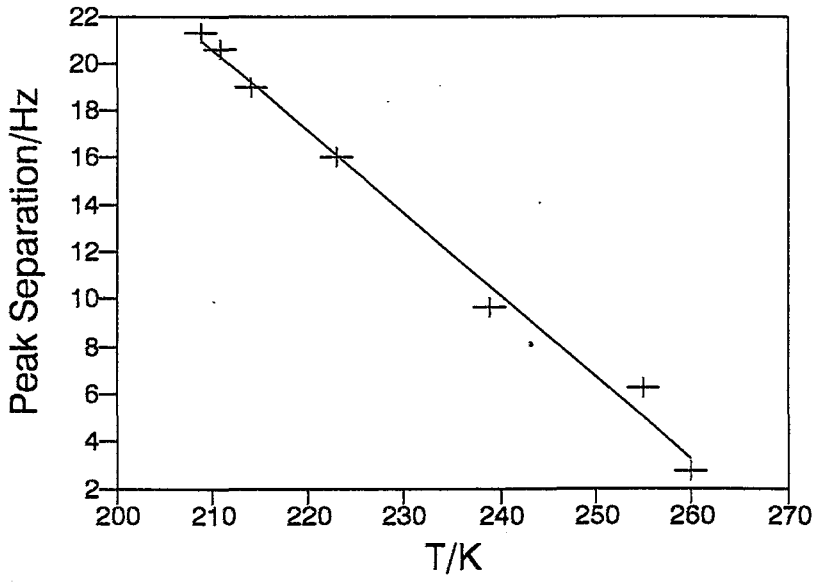


Compound 194 (proton NMR)

T/K	v/Hz	y(calc)
209	21.25	20.99
211	20.61	20.30
214	18.98	19.25
223	16.06	16.13
239	9.63	10.56
255	6.24	5.00
260	2.73	3.26

Regression Output:

Constant	93.64253
Std Err of Y Est	0.76598
R Squared	0.99113
No. of Observations	7
Degrees of Freedom	5
X Coefficient(s)	-0.34761
Std Err of Coef.	0.014706

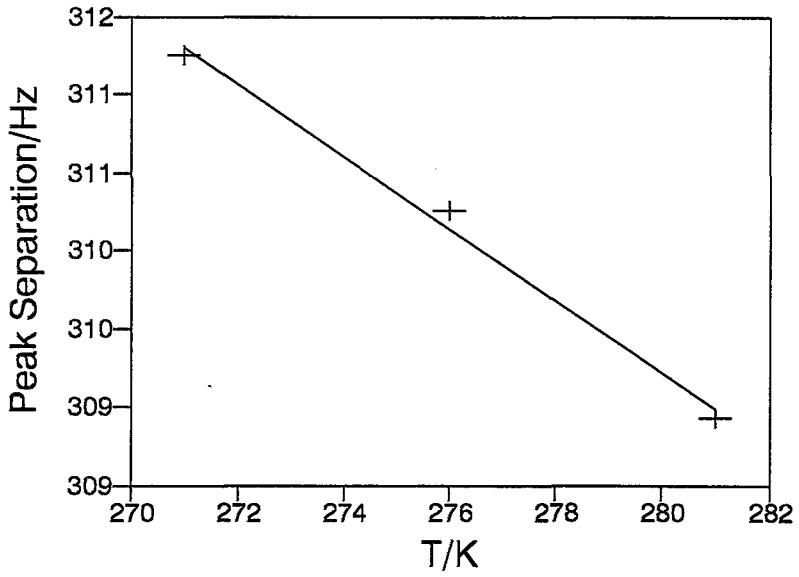


Compound 194 (carbon 13 NMR)

T/K	v/Hz	y(calc)
271	311.25	311.30
276	310.25	310.14
281	308.93	308.98

Regression Output:

Constant	374.1753
Std Err of Y Est	0.130639
R Squared	0.993698
No. of Observations	3
Degrees of Freedom	1
X Coefficient(s)	-0.232
Std Err of Coef.	0.018475



3.6 INTERCALATION STUDIES

The natural DNA used for the intercalation studies was calf thymus DNA (sodium salt) and the chloroquine used was in the form of the diphosphate salt.

UV STUDIES

DNA for the UV studies was dissolved in buffer (0.01M PIPES, 1mM EDTA, 0.05M NaCl, pH 7.0) by stirring at 4°C for 3d. The total concentration of the DNA solution was determined spectrophotometrically to be 2.4 x 10^{-3} M in base pairs using the expression: $A = \xi cl$

where A = absorbance

ξ = molar extinction coefficient (for calf thymus DNA = 6600M^{-1} nucleotide)

c = concentration

l = light path length

Solutions of chloroquine and compounds **180**, **181**, **195**, **196**, **202** and **203** were made up in the same buffer as the DNA to concentrations of 6.3 x 10^{-6}M for chloroquine and 3.0 x 10^{-4}M for the other compounds. The experiments were conducted by first obtaining UV spectra of the ligand alone in buffer, followed by spectra of the ligand solution with increasing amounts of calf thymus DNA added. The reference solution against which all the samples were blanked, was pure buffer. The DNA was transferred to the cuvette containing ligand solution using an auto-pipette, and mixing was achieved using a magnetic stirrer bar in the cuvette. Corrections to the absorbance readings were made, in each case, to compensate for the volume increase.

The UV spectra of compounds **180**, **181**, **202** and **203** showed no change on addition of DNA. The data obtained, and the resulting plots for chloroquine and compounds **195** and **196** are shown at the end of this section, as are the Beer's Law plots for compounds **195** and **196**. These plots exhibited linearity for both compounds over the appropriate concentration range. Experiments were repeated until results obtained from duplicate

runs fell within reasonable limits. The binding constants determined using this method are quoted in Table 12 (Section 2.3.1.1, p. 138) together with error margins, and these results represent the means of duplicate determinations.

NMR STUDIES

The natural calf thymus DNA was sonicated prior to use in the NMR studies.

Preparation of sonicated DNA

Preparation of reagents[409]

Phenol is used to extract any protein which may be present in the DNA sample. Prior to use, phenol must be equilibrated to a pH > 7.8 to prevent the DNA from partitioning into the organic phase.

Phenol (100g) was melted at 70°C and hydroxyquinoline (0.1g) was added. To the melted phenol was added buffer (0.5M Tris-Cl, pH 8.0; 100ml) and the mixture stirred for 15min. After separation of the layers, the aqueous phase was removed and buffer (0.1M Tris-Cl, pH 8.0; 100ml) was added to the organic layer. Stirring for 15min was followed once again by removal of the aqueous layer and addition of further buffer (0.1M Tris-Cl, pH 8.0; 100ml). These extractions were repeated until the pH of the phenolic phase was greater than 7.8. After removal of the final aqueous phase, buffer (0.1M Tris-Cl, pH 8.0; 1ml) containing β-mercaptoethanol (0.05ml) was added to the organic layer. A mixture of equilibrated phenol, chloroform, and isoamyl alcohol (25:24:1) was used for the extraction of protein from the DNA sample.

Preparation of DNA sample

Calf thymus DNA (250mg) was dissolved in a high salt buffer (0.01M PIPES, 1mM EDTA, 0.5M NaCl, pH 7; 10ml) by stirring for 2d at 4°C. The solution was saturated with N_2 and sonicated, with stirring, for a total of 1.5h. A Vibra cell sonicator was used at 50% power, pulsing on for 30s and off for 30s. The sample was cooled in an ice-bath, and the temperature maintained at < 12°C throughout sonication. After sonication

the sample was centrifuged (4000g for 10min), and the supernatant passed twice through a 0.45μm Millipore filter to remove any particulate material. Any protein present in the sample was removed by two extractions with an equal volume of equilibrated phenol:chloroform:isoamyl alcohol (25:24:1). An ether extraction of the aqueous layer was followed by ethanol precipitation with two volumes of a 2M solution of ammonium acetate in ethanol at 4°C. The precipitate was collected by centrifugation (10 000g for 10min) and resuspended in buffer (0.1M NaCl, 0.01M $MgCl_2$, 0.01M Na cacodylate, pH 7; 10ml). The sample was analysed by polyacrylamide gel electrophoresis in a 1% gel, and the average length of the DNA was found to be 300-400 base pairs. The final DNA concentration was determined by UV spectrophotometry, and found to be 11mM in base pairs.

NMR experiments

The NMR experiments were conducted using a Bruker AMX400 spectrometer. Three sets of NMR experiments were performed using natural, sonicated DNA and these are described separately.

(i) ^{1}H NMR spectroscopy of intercalator protons

These experiments monitored changes in the chemical shifts of the intercalator protons on addition of increasing amounts of DNA. Solutions of the ligands (5mM) were prepared using the buffer used above (0.1M NaCl, 0.01M $MgCl_2$, 0.01M Na cacodylate, pH 7, but made up in D_2O). These experiments were conducted at 303K using an inverse 5mm probe and, in each case, the volume of sample used was 0.8ml. Thus, appropriate volumes of DNA solution in buffer (corresponding to the required DNA : ligand ratios) were transferred by auto-pipette to a series of small vials, and the water was evaporated. The transfer of DNA to the ligand solution in an NMR tube was achieved by removing the contents of the NMR tube with an extended pasteur pipette, and placing it in the appropriate DNA vial. Thorough mixing was followed by transfer of the ligand-DNA solution back to the NMR tube, using the pasteur pipette. In this way it was ensured that the ligand solution was not diluted, and loss of material was minimized by use of

small vials with a small surface area. This process was repeated for the entire sequence of vials, for each ligand. The DNA base pair : ligand molar ratios used were 1 : 10, 2, 1.2 and 0.6. The number of scans required to obtain reasonable spectra at these low concentrations was 2000, and with a delay time of 1s the duration of each run was 2h. The ligands investigated in this way were compounds **195**, **196** and chloroquine. The resulting spectra can be found in Section 2.3.1.2 (Figs 42-44, pp. 143 and 144).

(ii) ^{1}H NMR analysis of DNA imino protons

The concentration of the DNA solution used for these experiments was 11mM in base pairs, and the buffer used was that described above, made up in 90% water and 10% D_2O. The total volume of DNA solution used, in each case was 0.67ml.

The experiments were conducted by initially running a spectrum of DNA alone, and then spectra of the DNA with increasing amounts of ligand present. The ligand : DNA base pair molar ratios used were 1 : 25, 8.3, 4.1, 3 and 1. The same transfer procedure was employed for these experiments as described for the previous experiments [(i) above], with the appropriate amounts of ligand (dissolved in methanol), in this case, being transferred to a series of vials, using an auto-pipette, followed by evaporation of the methanol. The ligands used in this experiment were chloroquine and compounds **181**, **195**, **196** and **202**. The resulting spectra can be found in Section 2.3.1.2 (Figs 45-48, pp. 146 and 147).

The presence of such a large proportion of water in the samples made the use of a water suppression technique essential, and the 1-3-3-1 pulse sequence[410,411] was used to achieve this. The probe used for these experiments was an inverse 5mm probe, and the probe temperature was 312K. The short delay time between pulses (300ms) takes into account the rapid relaxation of the rigid DNA polymer. The number of scans necessary to obtain reasonable spectra was 2000, the duration of each run being 35min. In each case 5Hz line-broadening was used.

(iii) ^{31}P NMR analysis of DNA

In these experiments changes in the ^{31}P spectrum of DNA were monitored on addition of increasing amounts of ligand. The probe used for the experiments was a broad band 5mm probe in a Bruker AMX400 spectrometer, which operates at a field of 160MHz for ^{31}P nuclei. The DNA concentration used was the same as that used for the second set of NMR experiments [(ii) above], *viz.*, 11mM in base pairs. The buffer was the same as that used above, made up in D_2O, and the sample volumes, in each case, were 0.7ml. The ^{31}P spectrum of DNA was initially run in the absence of any ligand, followed by spectra of the DNA with increasing amounts of ligand added. The transfer procedure used was the same as that described in the preceding experiments [(i) and (ii) above], and the ratios of DNA base pairs : ligand used were 1 : 0.3, 0.7 and 1.4. The number of scans required for satisfactory spectra was 1000, with the duration of each run being 1h 45min. The experiments were conducted at 318K with a delay time of 5s, and line-broadening of 2Hz. The ligands investigated using this approach were compounds **181**, **195**, **196** and chloroquine, and the resulting spectra can be found in Section 2.3.1.2 (Figs 49-52, pp. 150 and 151).

In the above three sets of experiments, the temperatures were chosen, in each case, to minimize line-widths, and appropriate delay times were selected in order to minimize collection time while ensuring sufficient relaxation of the nuclei. The ^{1}H NMR spectra were calibrated on sodium 3-(trimethylsilyl)propanesulphonate-d_6, and the ^{31}P spectra on trimethyl phosphate (both at 0.0ppm).

NMR analysis of the synthetic oligonucleotide d(5'-GCATGC)$_2$

The ^{1}H NMR spectrum of a fraction thought to contain the oligonucleotide d(5'-GCATGC)$_2$ was obtained at 303K, in a 0.2M NaCl D_2O solution at pH 6. The number of scans necessary to obtain a reasonable spectrum was 3000, and the delay time used was 300ms. A small quantity of chloroquine was then added to the oligonucleotide solution, and the spectrum re-run to observe any shifts of the DNA protons resulting

from intercalation. The results from this experiment are shown in Fig. 53 (Section 2.3.2, p. 156).

A ^{31}P spectrum of the oligonucleotide was also run at 303K. The number of scans necessary was 4600, and a delay time of 2.5s was used. The spectrum was re-run after the addition of chloroquine to observe shifts occurring as a result of intercalation. These results can be found in Fig. 54 (Section 2.3.2, p. 157).

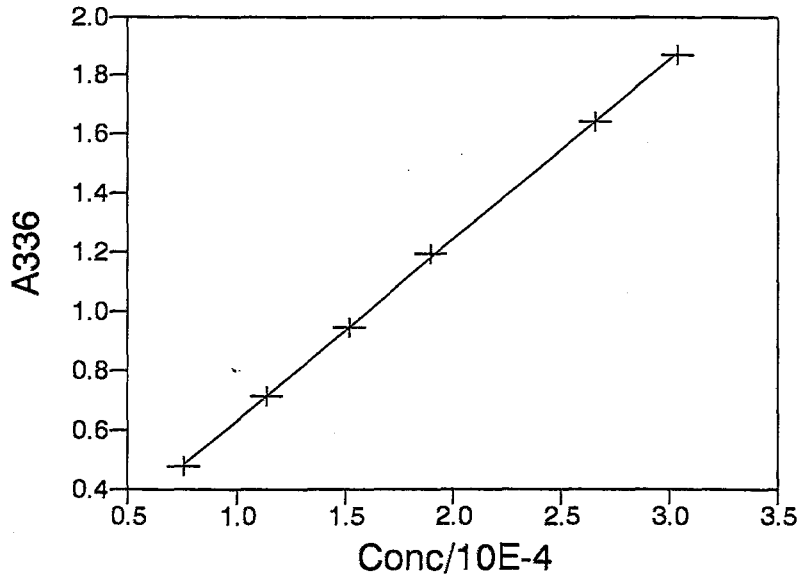
The spectra of the oligonucleotide were calibrated on the same standards used for the NMR studies on natural DNA.

Beer's Law plot for compound 195

Conc10E-4	A336	y(calc)
0.76	0.474	0.481
1.14	0.713	0.714
1.52	0.946	0.947
1.9	1.196	1.180
2.66	1.647	1.645
3.04	1.869	1.878

Regression Output:

Constant	0.015447
Std Err of Y Est	0.010083
R Squared	0.999721
No. of Observations	6
Degrees of Freedom	4
X Coefficient(s)	0.6127329
Std Err of Coef.	0.0051221

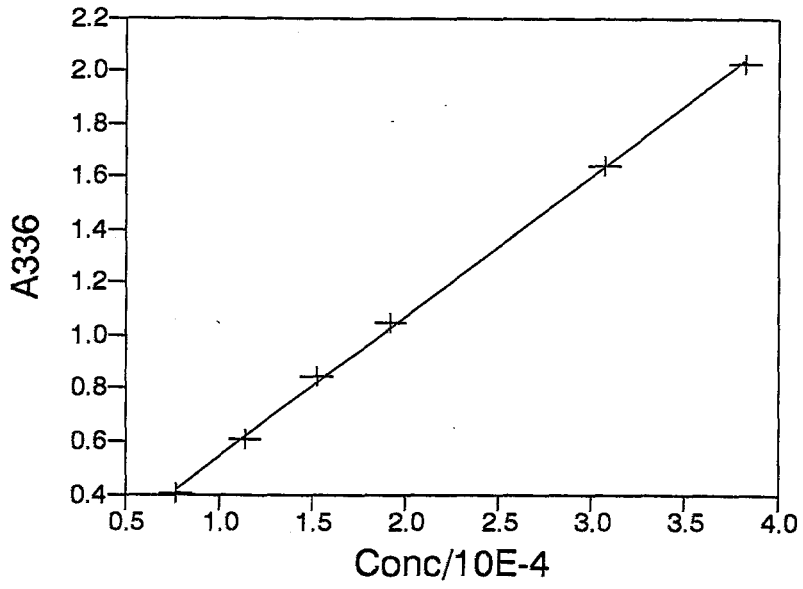


Beer's Law plot for compound 196

Conc10E-4	A336	y(calc)
0.77	0.406	0.418
1.15	0.604	0.619
1.53	0.84	0.821
1.92	1.043	1.027
3.07	1.642	1.636
3.83	2.026	2.039

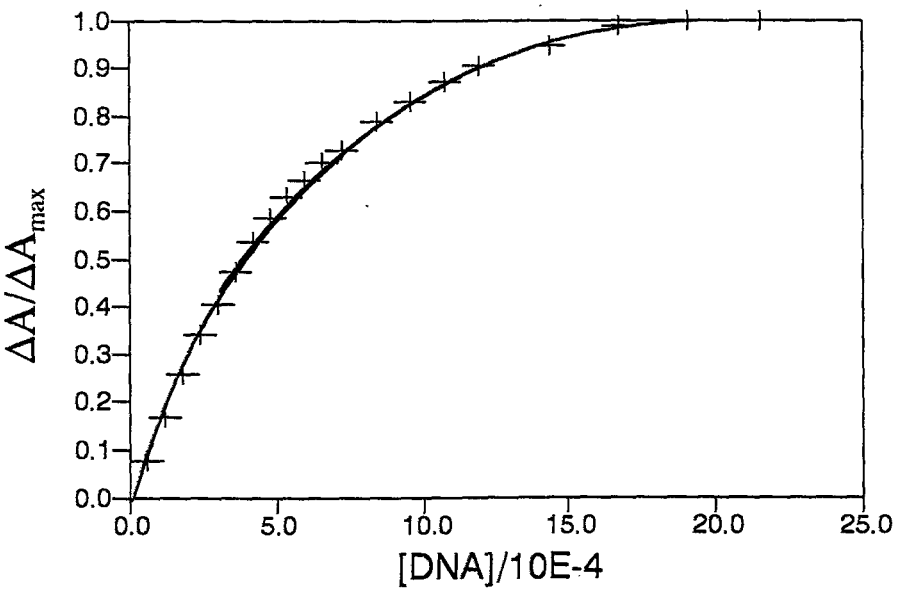
Regression Output:

Constant	0.010313
Std Err of Y Est	0.017352
R Squared	0.999382
No. of Observations	6
Degrees of Freedom	4
X Coefficient(s)	0.5296759
Std Err of Coef.	0.0065847



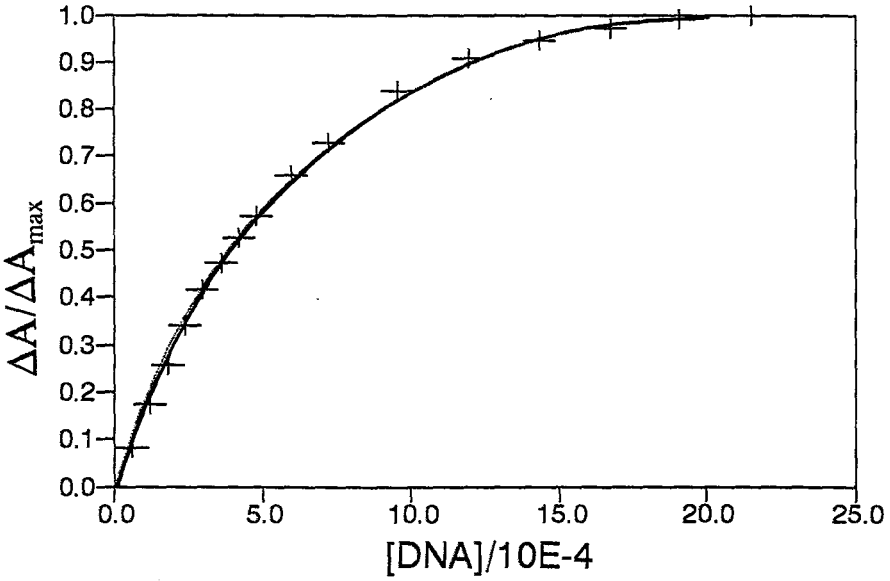
Chloroquine (1)

Abs	dA	[DNA] 10E-4	A/Amax
2.390	0.000	0.000	0.000
2.311	0.079	0.598	0.078
2.221	0.169	1.200	0.168
2.130	0.260	1.790	0.258
2.047	0.343	2.390	0.340
1.982	0.408	2.990	0.404
1.916	0.474	3.590	0.470
1.850	0.540	4.190	0.535
1.800	0.590	4.780	0.585
1.755	0.635	5.380	0.629
1.721	0.669	5.980	0.663
1.683	0.707	6.580	0.701
1.656	0.734	7.180	0.728
1.597	0.793	8.370	0.786
1.553	0.837	9.570	0.830
1.512	0.878	10.760	0.870
1.478	0.912	11.960	0.904
1.435	0.955	14.350	0.947
1.396	0.994	16.740	0.985
1.381	1.009	19.140	1.000
1.381	1.009	21.530	1.000



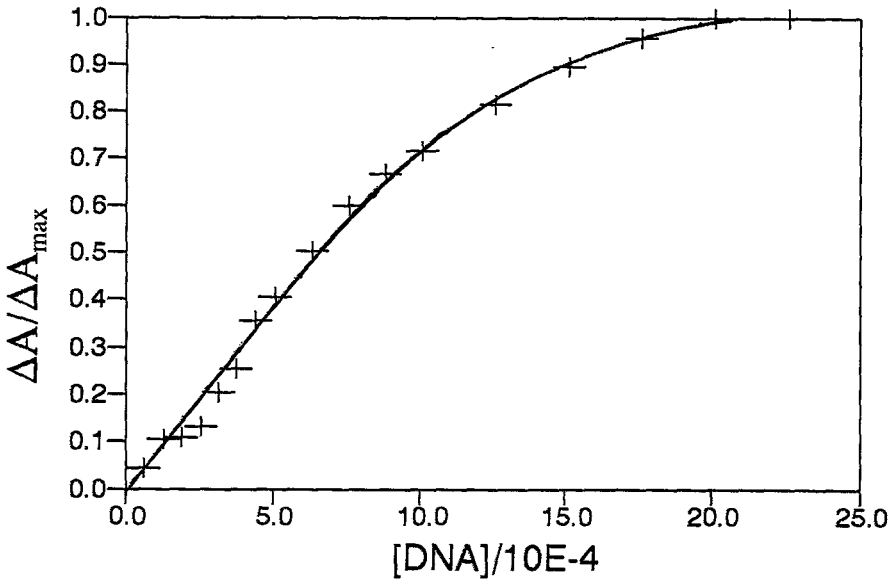
Chloroquine (2)

Abs	dA	[DNA] 10E-4	A/Amax
2.392	0.000	0.000	0.000
2.308	0.084	0.598	0.081
2.211	0.181	1.200	0.174
2.126	0.266	1.790	0.256
2.037	0.355	2.390	0.341
1.960	0.432	2.990	0.415
1.898	0.494	3.590	0.475
1.844	0.548	4.190	0.526
1.796	0.596	4.780	0.573
1.704	0.688	5.980	0.661
1.632	0.760	7.180	0.730
1.519	0.873	9.570	0.839
1.449	0.943	11.960	0.906
1.408	0.984	14.350	0.945
1.380	1.012	16.740	0.972
1.363	1.029	19.140	0.989
1.351	1.041	21.530	1.000



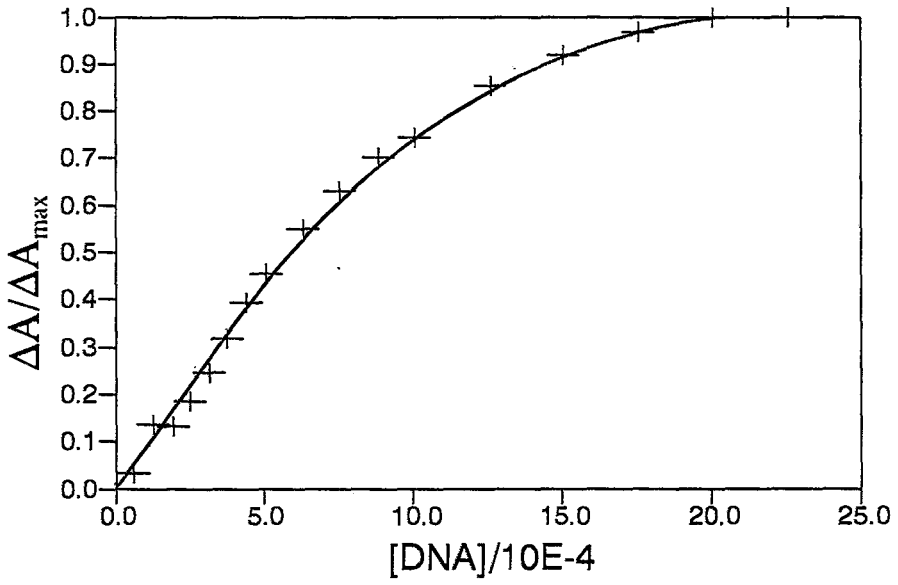
Compound 195 (1)

Abs	dA	[DNA] 10E-4	A/Amax
1.726	0.000	0.000	0.000
1.711	0.015	0.629	0.045
1.691	0.035	1.260	0.106
1.690	0.036	1.890	0.109
1.683	0.043	2.520	0.130
1.658	0.068	3.150	0.205
1.642	0.084	3.770	0.254
1.608	0.118	4.400	0.357
1.592	0.134	5.030	0.405
1.559	0.167	6.290	0.505
1.528	0.198	7.550	0.598
1.505	0.221	8.810	0.668
1.489	0.237	10.060	0.716
1.456	0.270	12.580	0.816
1.429	0.297	15.100	0.897
1.409	0.317	17.610	0.958
1.395	0.331	20.130	1.000
1.395	0.331	22.640	1.000



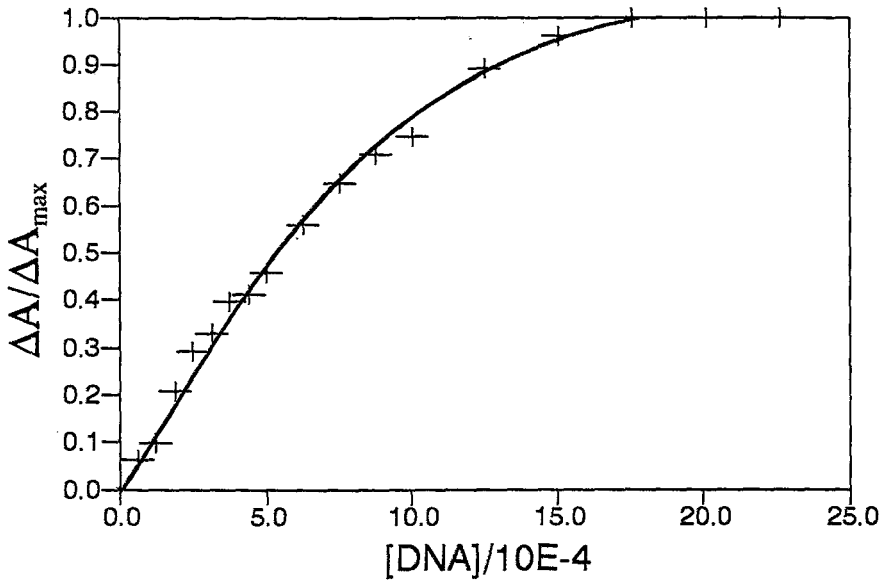
Compound 195 (2)

Abs	dA	[DNA] 10E-4	A/Amax
1.727	0.000	0.000	0.000
1.715	0.012	0.629	0.034
1.680	0.047	1.260	0.135
1.681	0.046	1.890	0.132
1.663	0.064	2.520	0.183
1.641	0.086	3.150	0.246
1.616	0.111	3.770	0.318
1.590	0.137	4.400	0.393
1.568	0.159	5.030	0.456
1.535	0.192	6.290	0.550
1.508	0.219	7.550	0.628
1.482	0.245	8.810	0.702
1.468	0.259	10.060	0.742
1.429	0.298	12.580	0.854
1.406	0.321	15.100	0.920
1.390	0.337	17.610	0.966
1.378	0.349	20.130	1.000
1.378	0.349	22.640	1.000



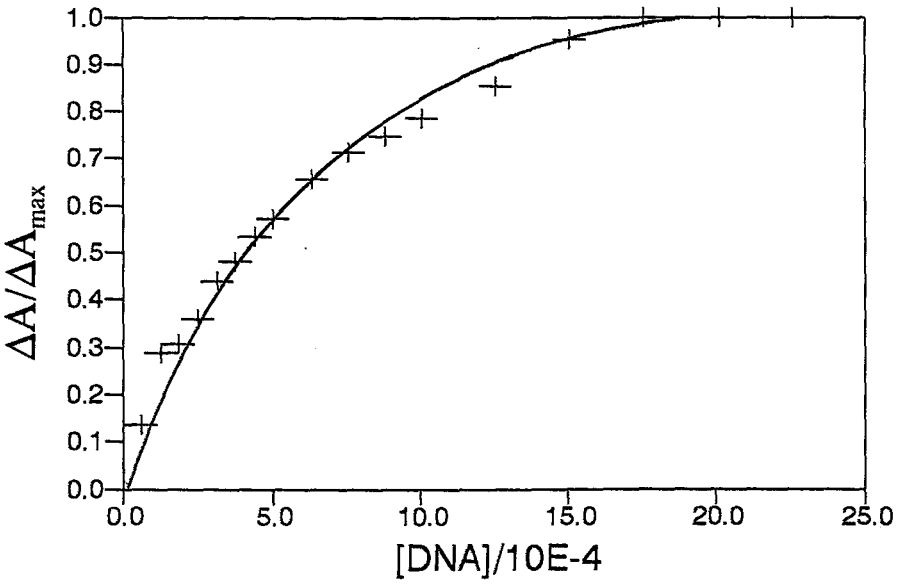
Compound 196 (1)

Abs	dA	[DNA] 10E-4	A/Amax
1.464	0.000	0.000	0.000
1.452	0.012	0.629	0.062
1.445	0.019	1.260	0.098
1.424	0.040	1.890	0.206
1.408	0.056	2.520	0.289
1.400	0.064	3.150	0.330
1.387	0.077	3.770	0.397
1.384	0.080	4.400	0.412
1.375	0.089	5.030	0.459
1.355	0.109	6.290	0.562
1.338	0.126	7.550	0.650
1.326	0.138	8.810	0.711
1.319	0.145	10.060	0.747
1.291	0.173	12.580	0.892
1.278	0.186	15.100	0.959
1.270	0.194	17.610	1.000
1.270	0.194	20.130	1.000
1.270	0.194	22.640	1.000



Compound 196 (2)

Abs	dA	[DNA] 10E-4	A/Amax
1.465	0.000	0.000	0.000
1.438	0.027	0.629	0.134
1.407	0.058	1.260	0.287
1.403	0.062	1.890	0.307
1.392	0.073	2.520	0.361
1.376	0.089	3.150	0.441
1.368	0.097	3.770	0.480
1.357	0.108	4.400	0.535
1.349	0.116	5.030	0.574
1.333	0.132	6.290	0.654
1.321	0.144	7.550	0.713
1.314	0.151	8.810	0.748
1.306	0.159	10.060	0.787
1.293	0.172	12.580	0.852
1.273	0.192	15.100	0.951
1.263	0.202	17.610	1.000
1.263	0.202	20.130	1.000
1.263	0.202	22.640	1.000



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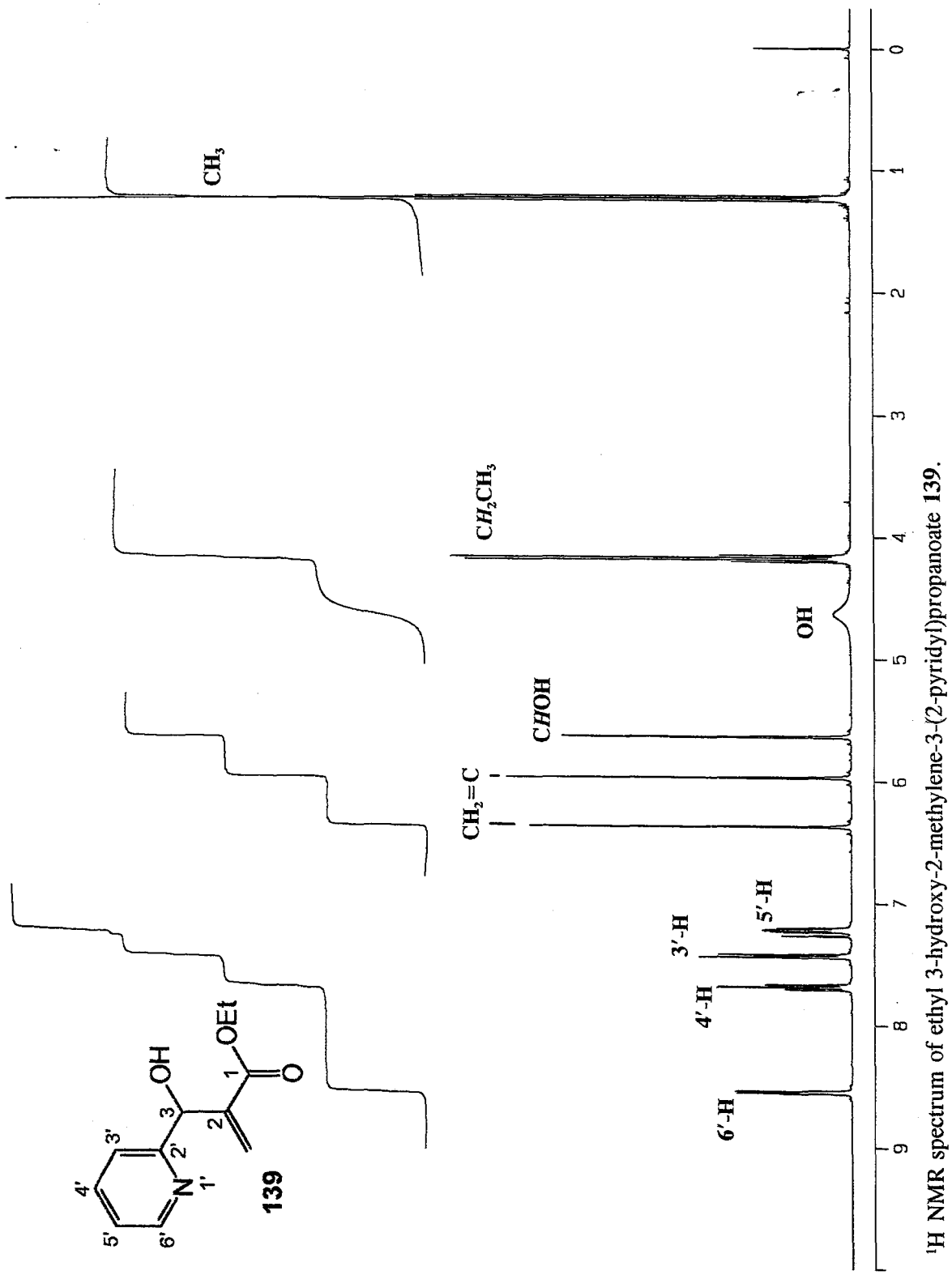
5. APPENDICES

APPENDIX 5.1 NMR SPECTRA

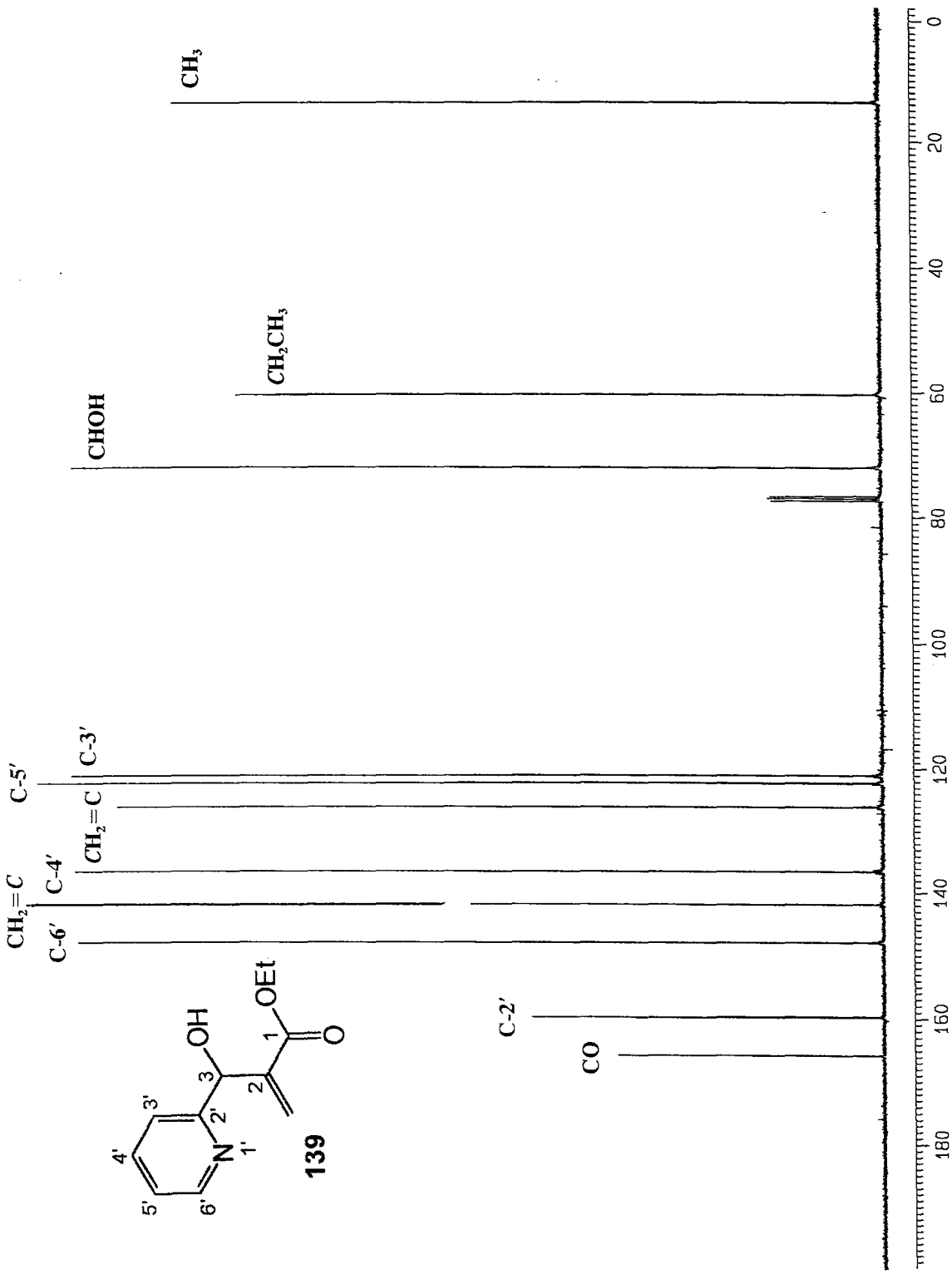
APPENDIX 5.2 CRYSTALLOGRAPHIC DATA

APPENDIX 5.3 NMR SPECTRA FROM DNA STUDIES

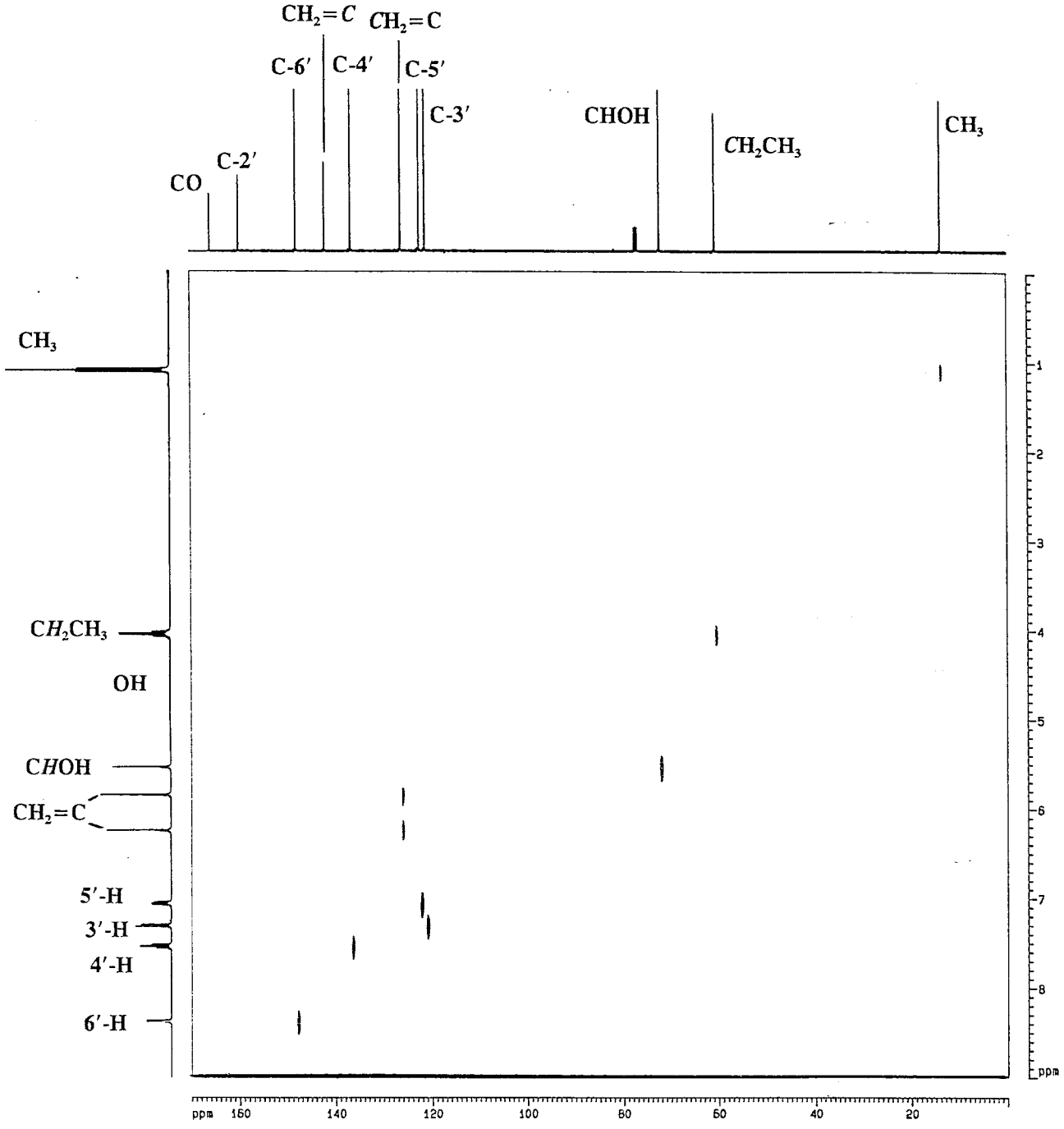
5.1 NMR SPECTRA



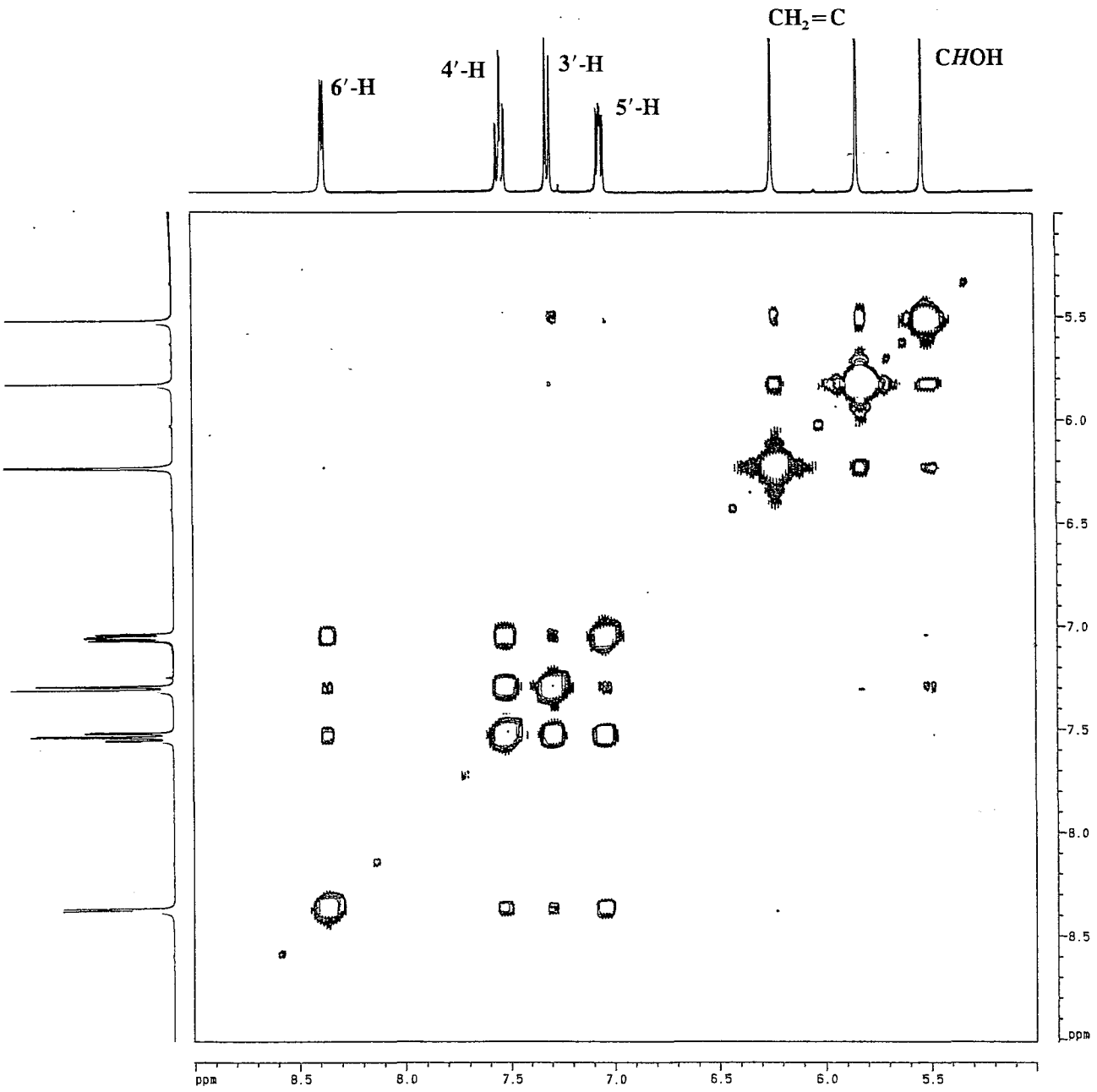
^{1}H NMR spectrum of ethyl 3-hydroxy-2-methylene-3-(2-pyridyl)propanoate **139**.



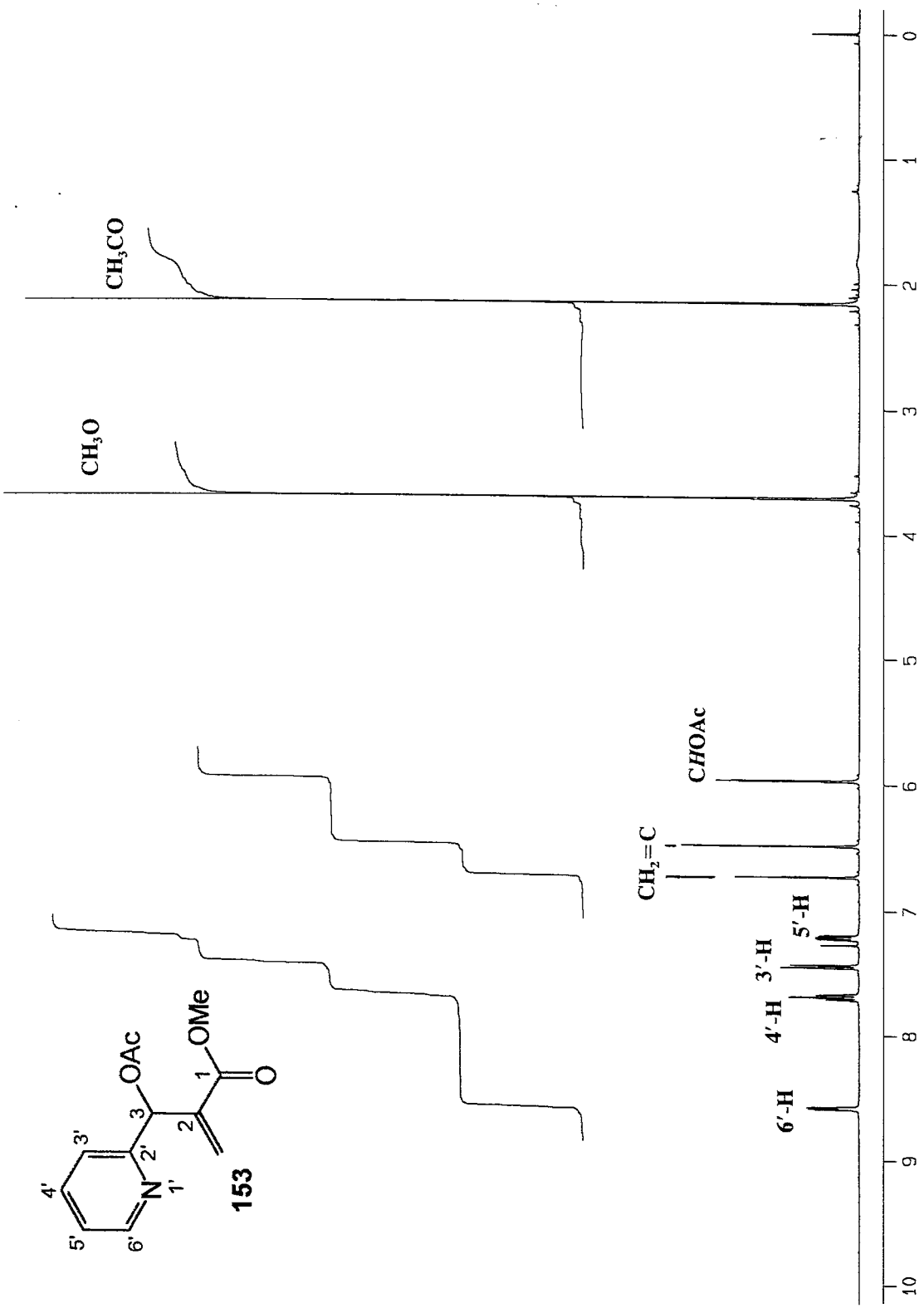
^{13}C NMR spectrum of ethyl 3-hydroxy-2-methylene-3-(2-pyridyl)propanoate **139**.



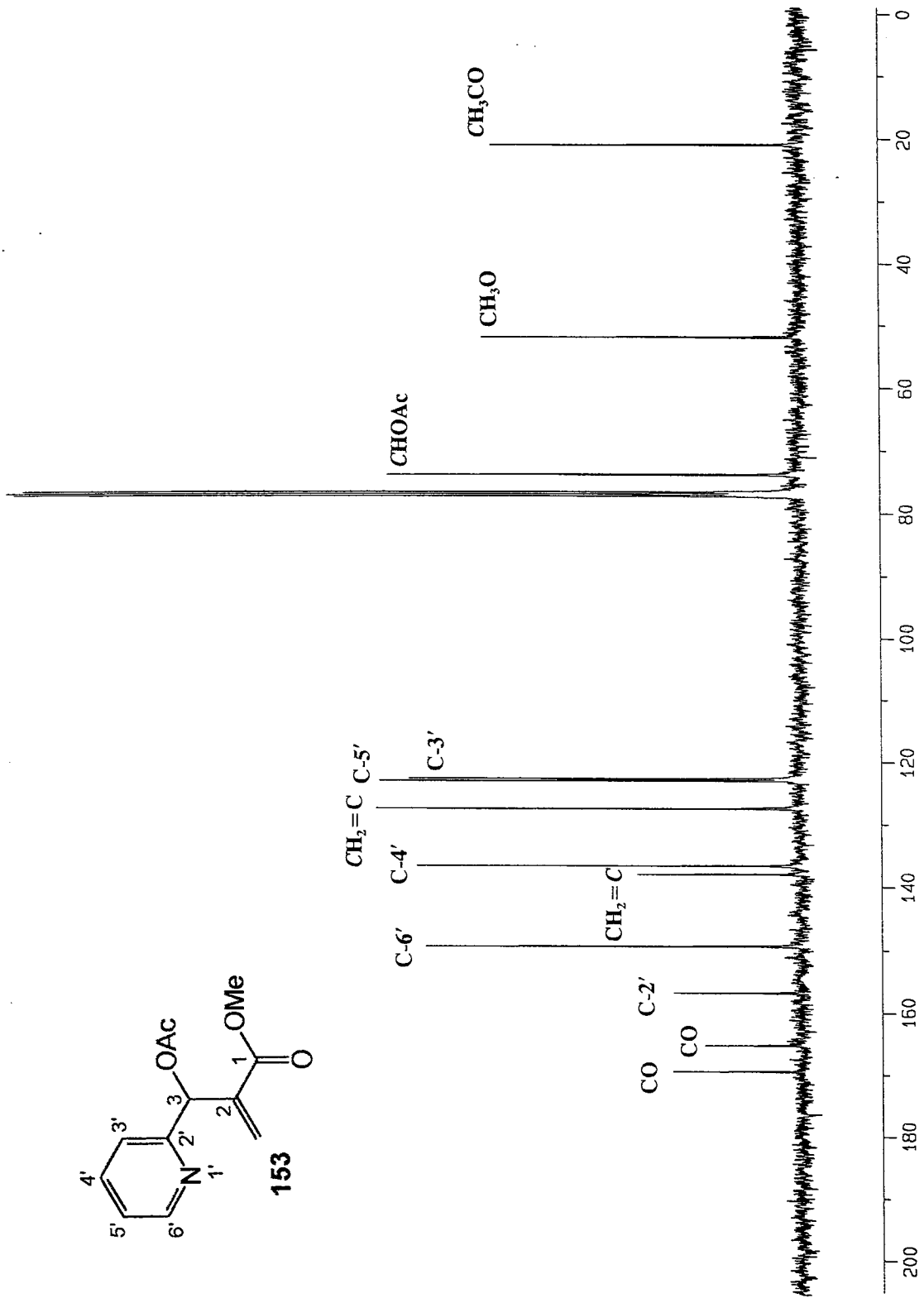
HETCOR spectrum of ethyl 3-hydroxy-2-methylene-3-(2-pyridyl)propanoate **139**.



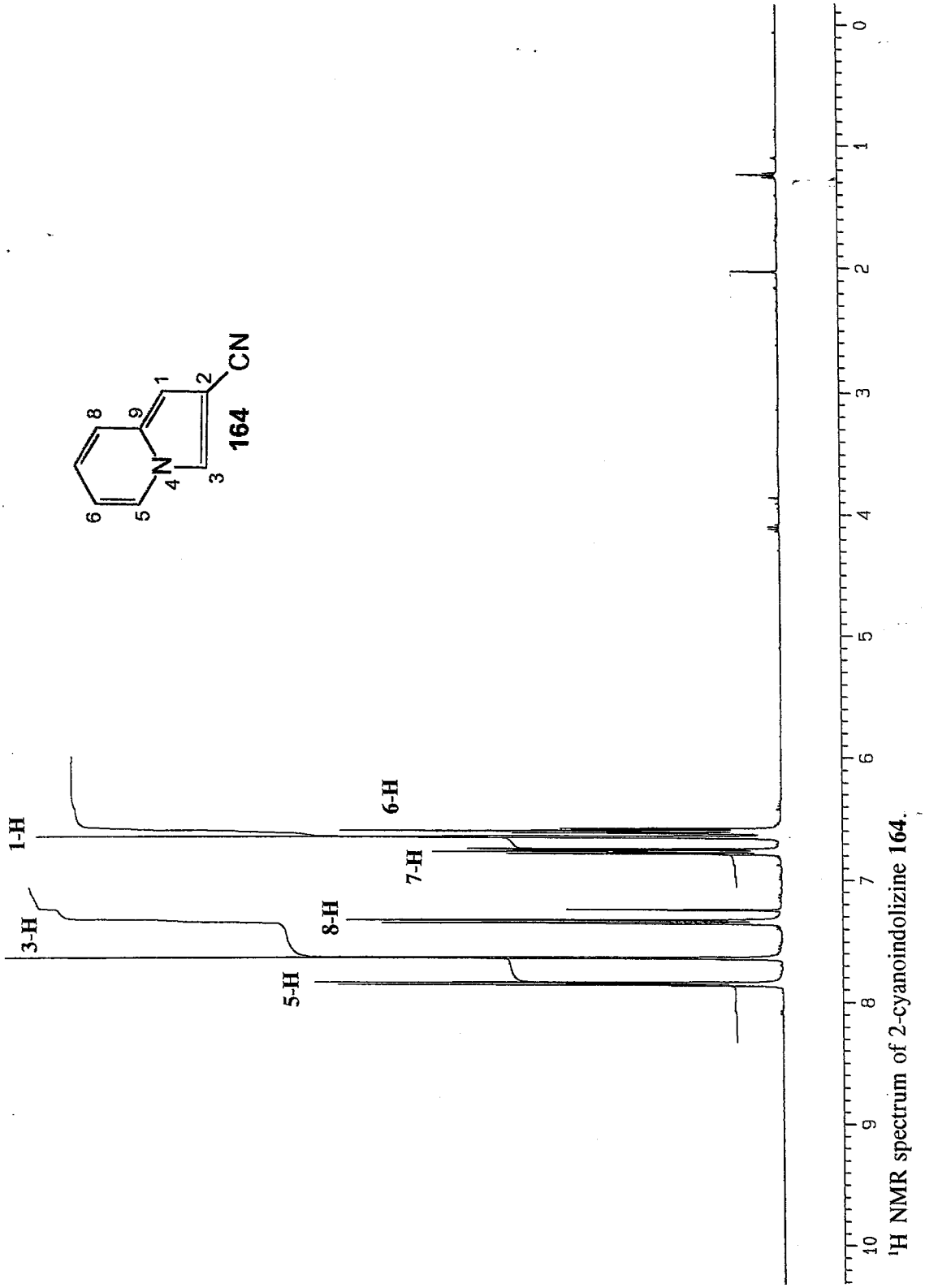
COSY spectrum of ethyl 3-hydroxy-2-methylene-3-(2-pyridyl)propanoate **139**.



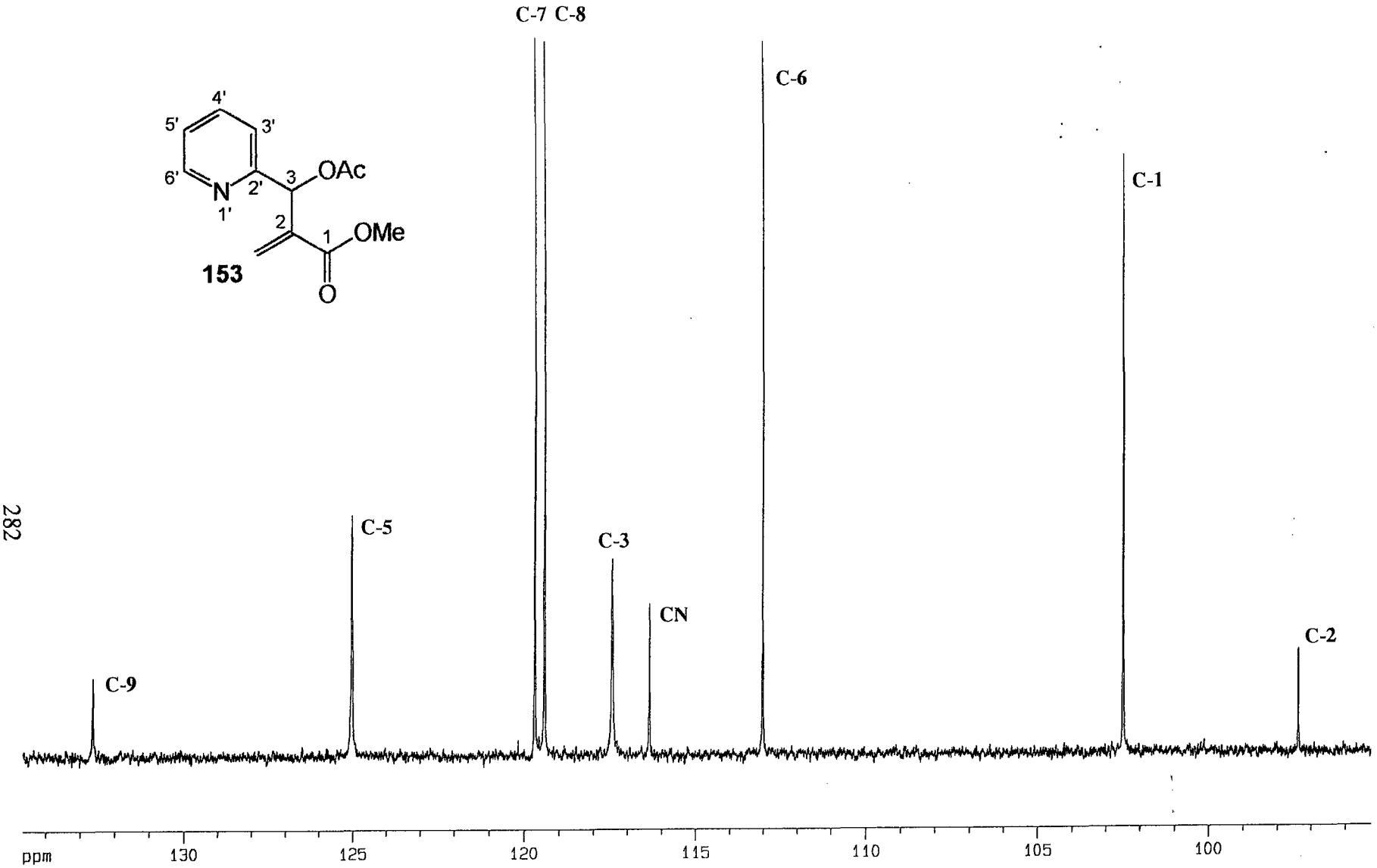
^{1}H NMR spectrum of methyl 3-acetoxy-2-methylene-3-(2-pyridyl)propanoate **153**.



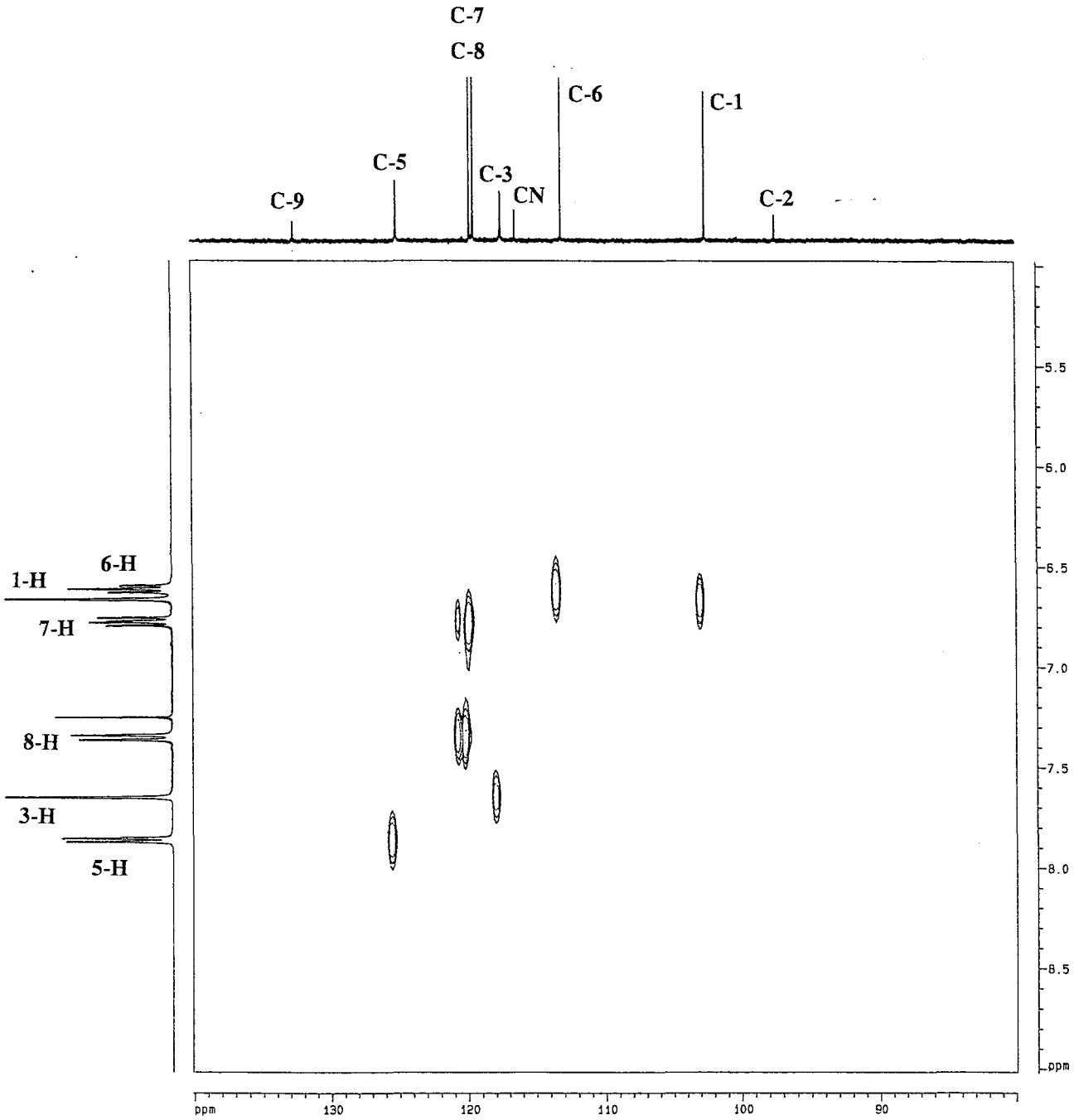
^{13}C NMR spectrum of methyl 3-acetoxy-2-methylene-3-(2-pyridyl)propanoate **153**.



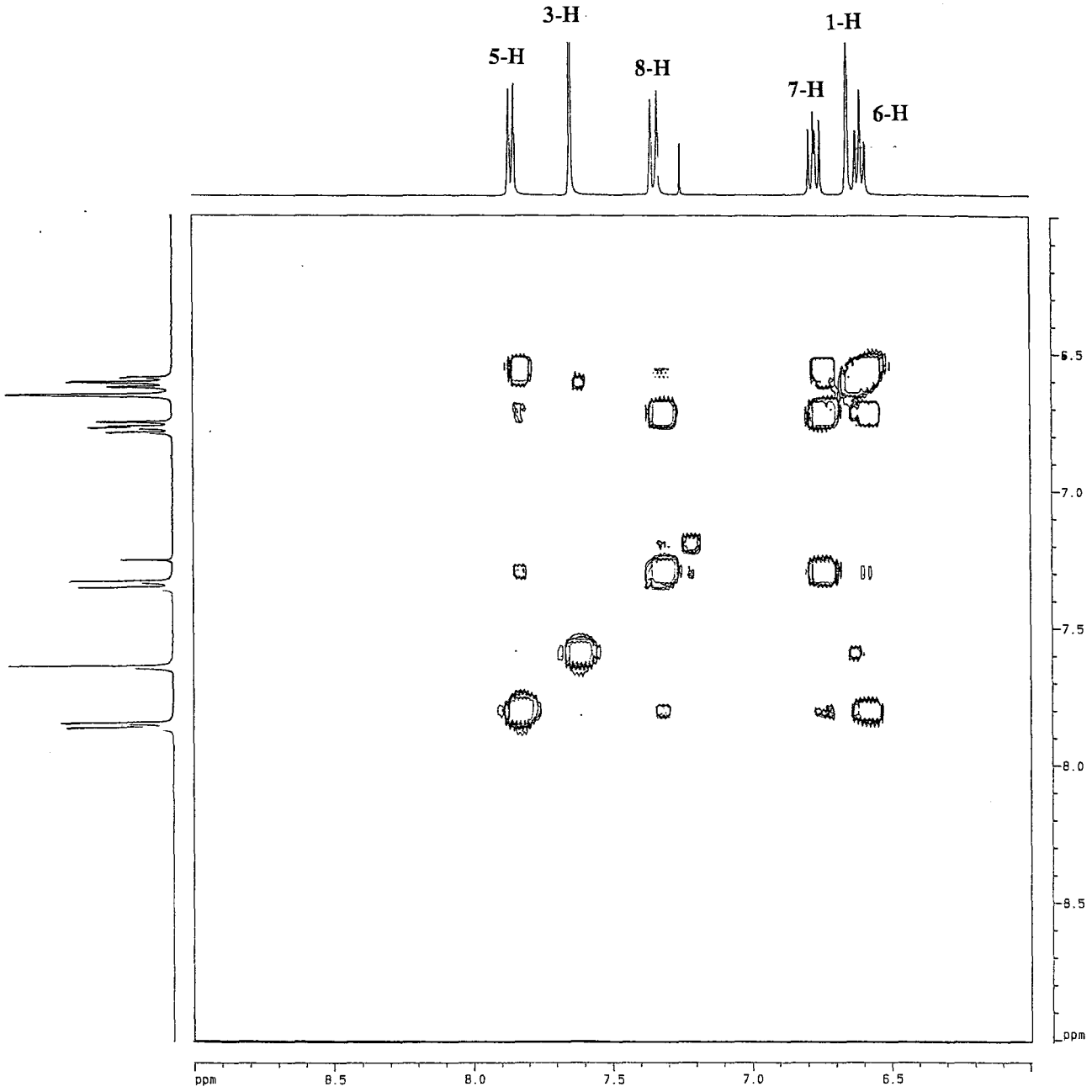
^{1}H NMR spectrum of 2-cyanoindolizine **164**.



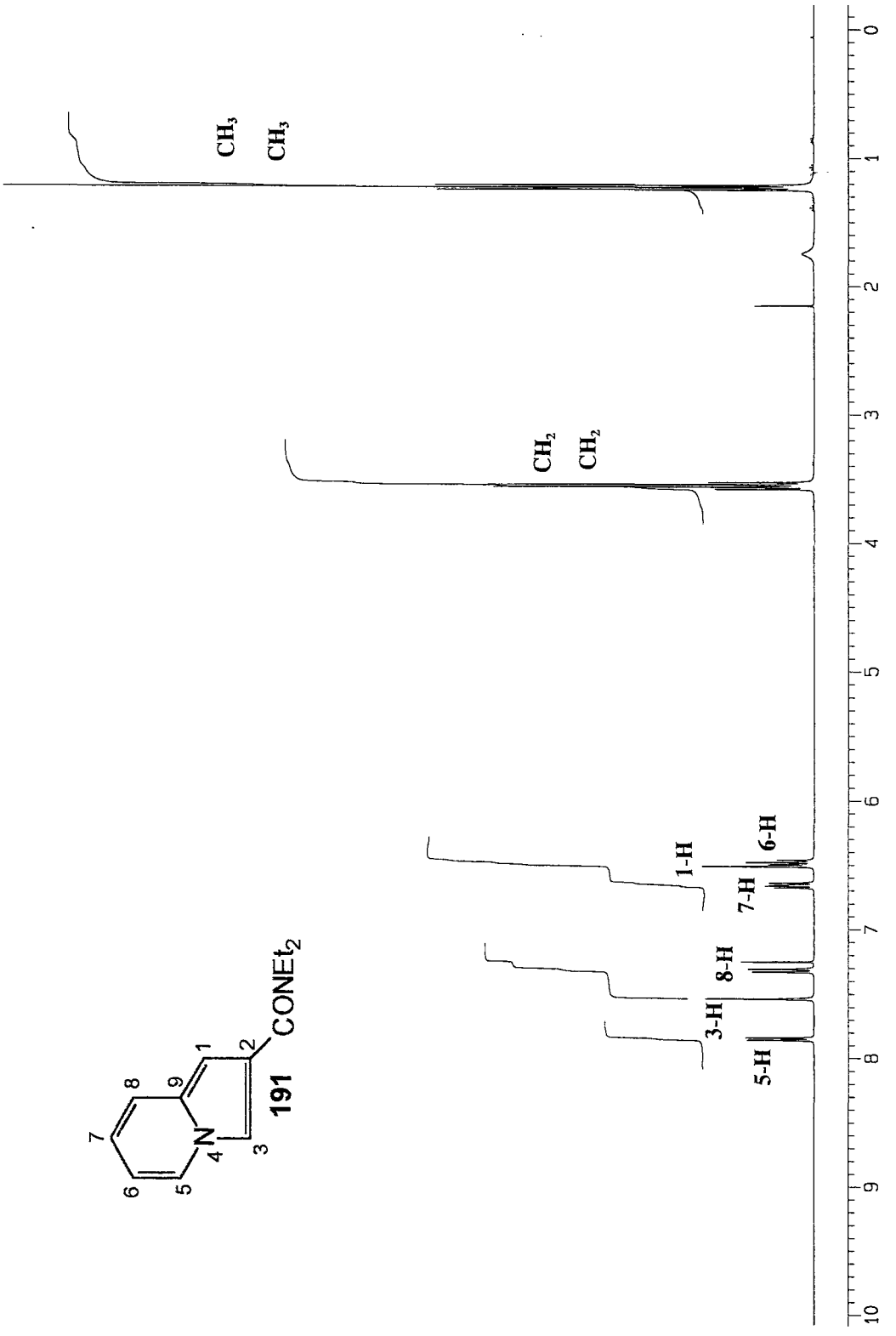
^{13}C NMR spectrum of 2-cyanoindolizine **164**.



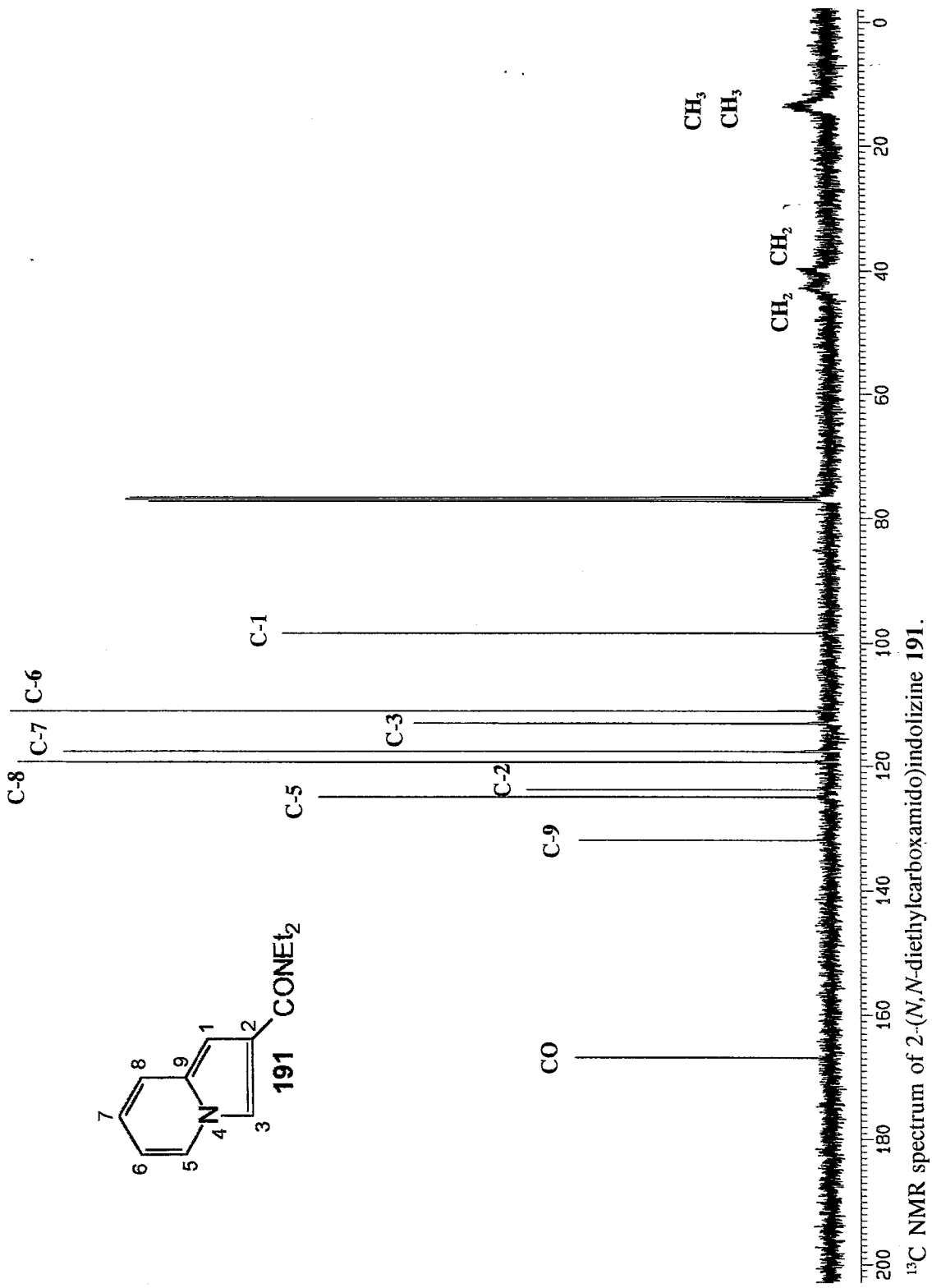
HETCOR spectrum of 2-cyanoindolizine **164**.



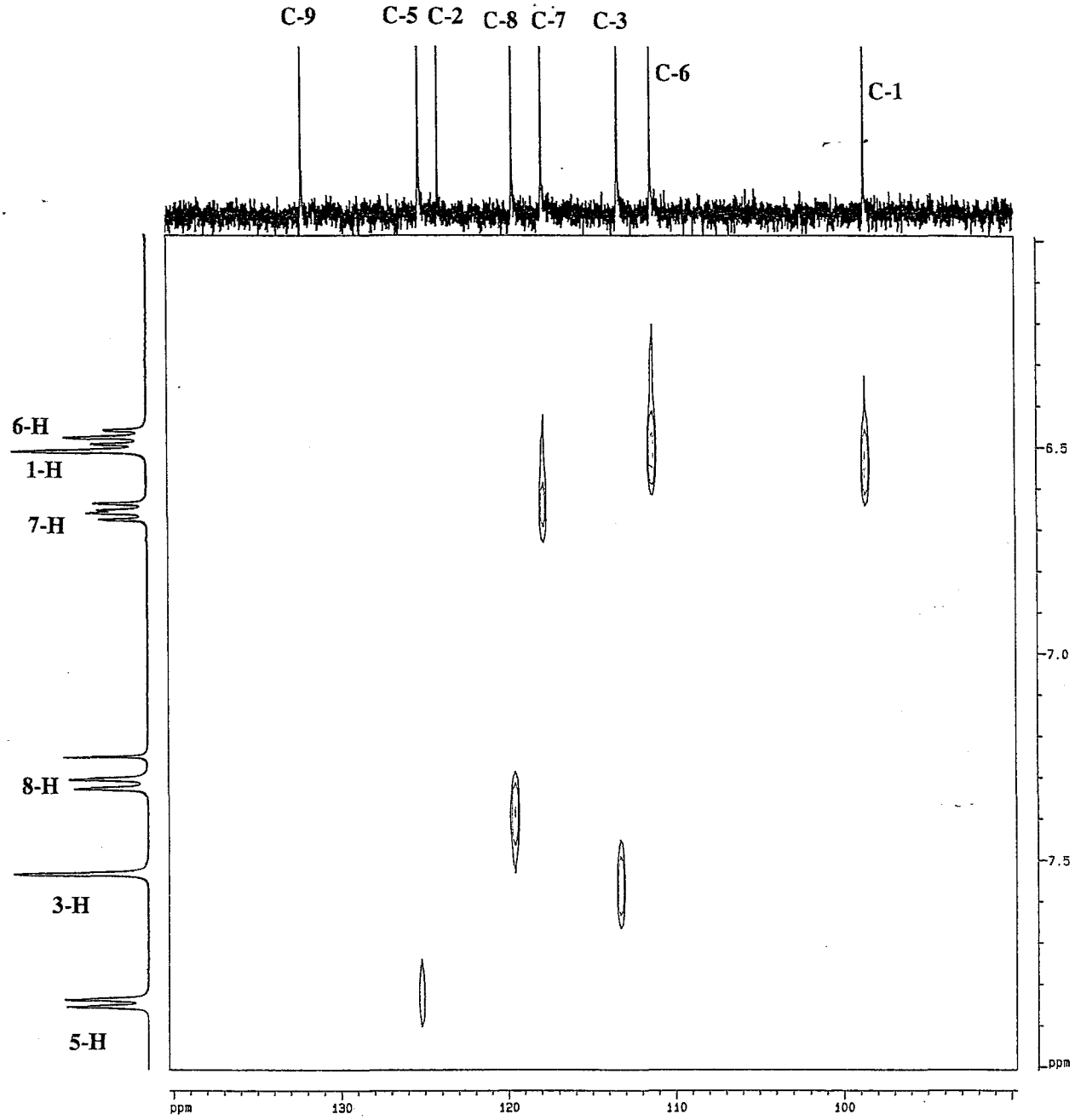
COSY spectrum of 2-cyanoindolizine **164**.



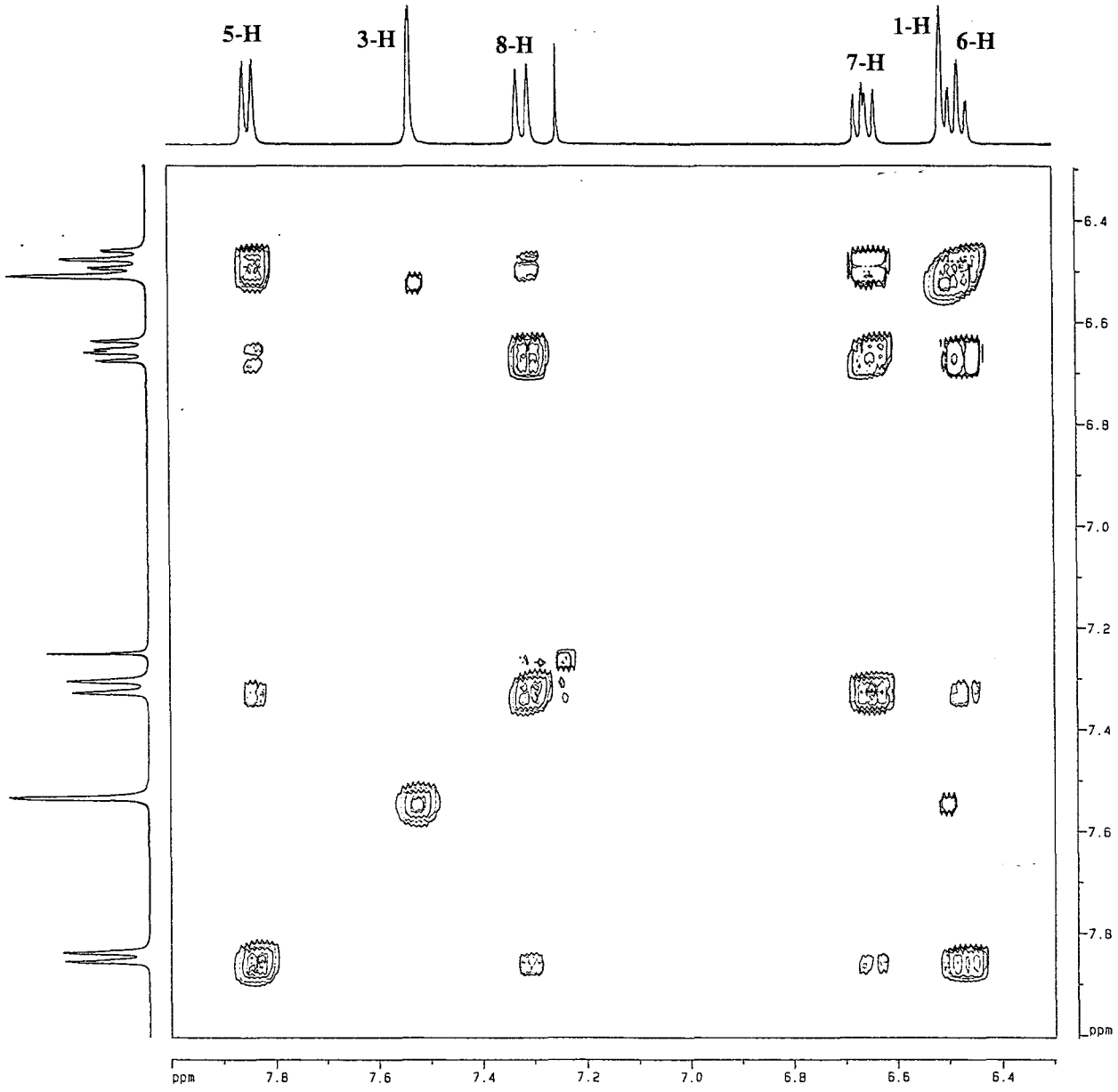
^{1}H NMR spectrum of 2-(*N*,*N*-diethylcarboxamido)indolizine **191**.



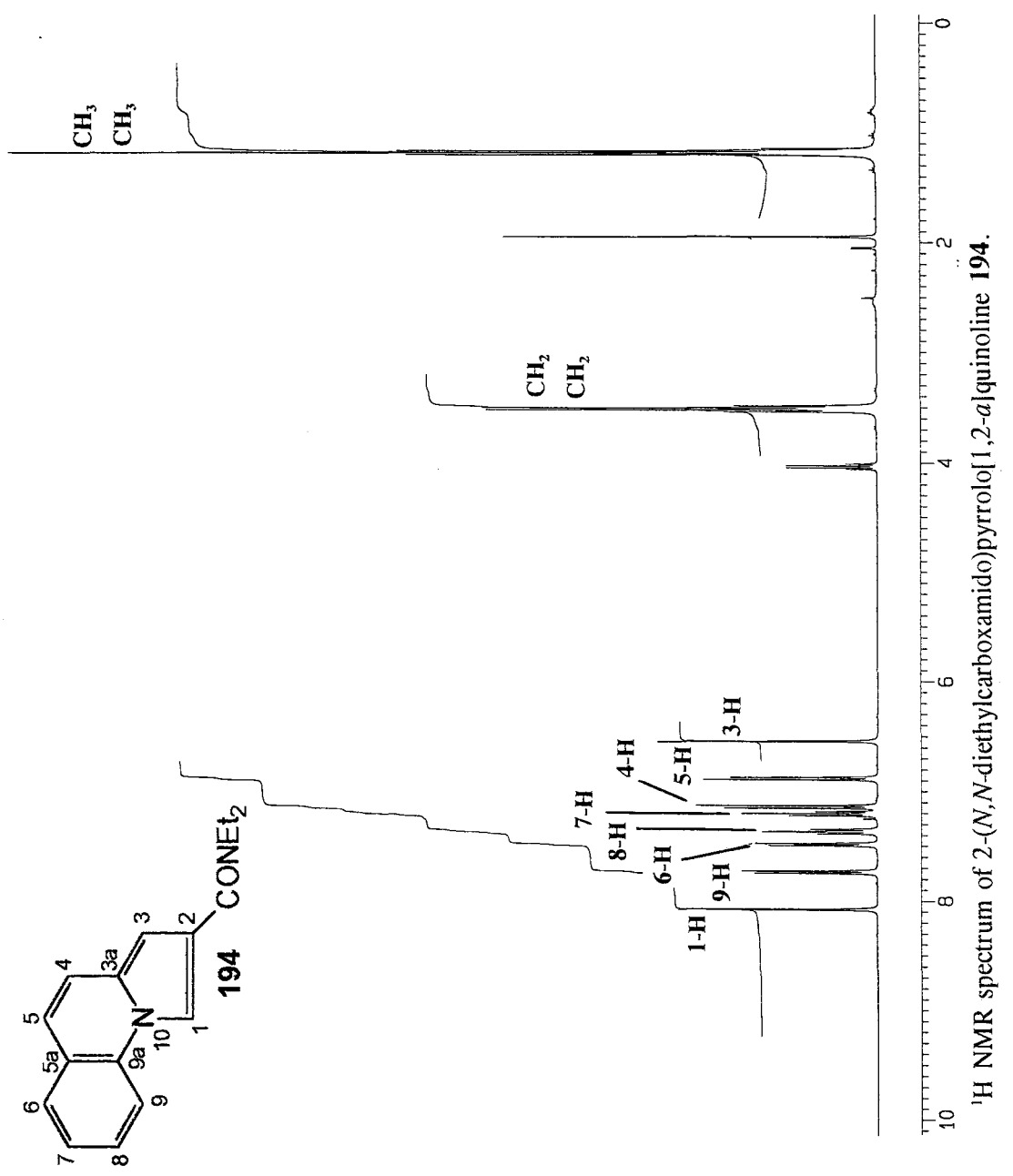
^{13}C NMR spectrum of 2-(*N*,*N*-diethylcarboxamido)indolizine **191**.



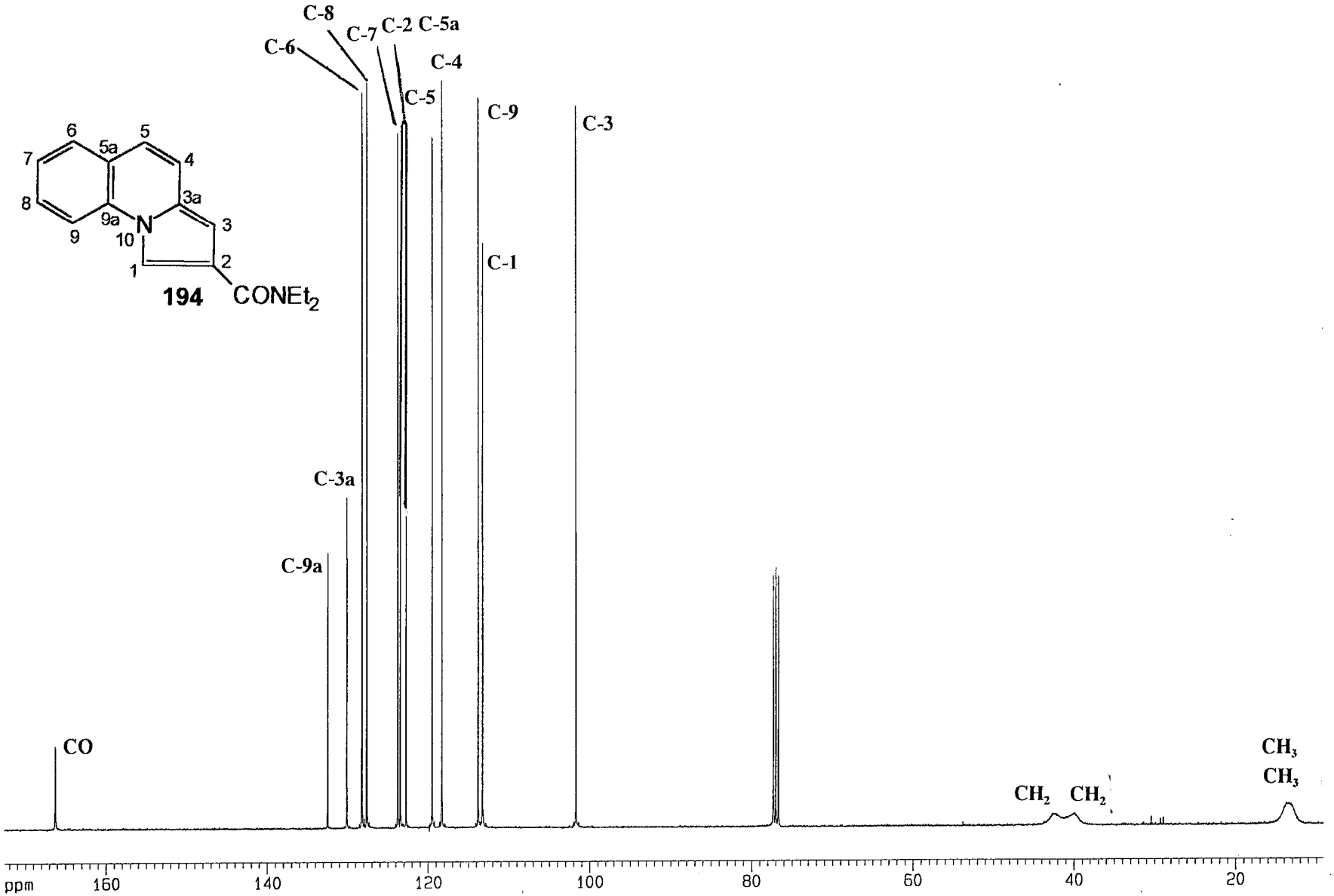
HETCOR spectrum of 2-(*N,N*-diethylcarboxamido)indolizine **191**.



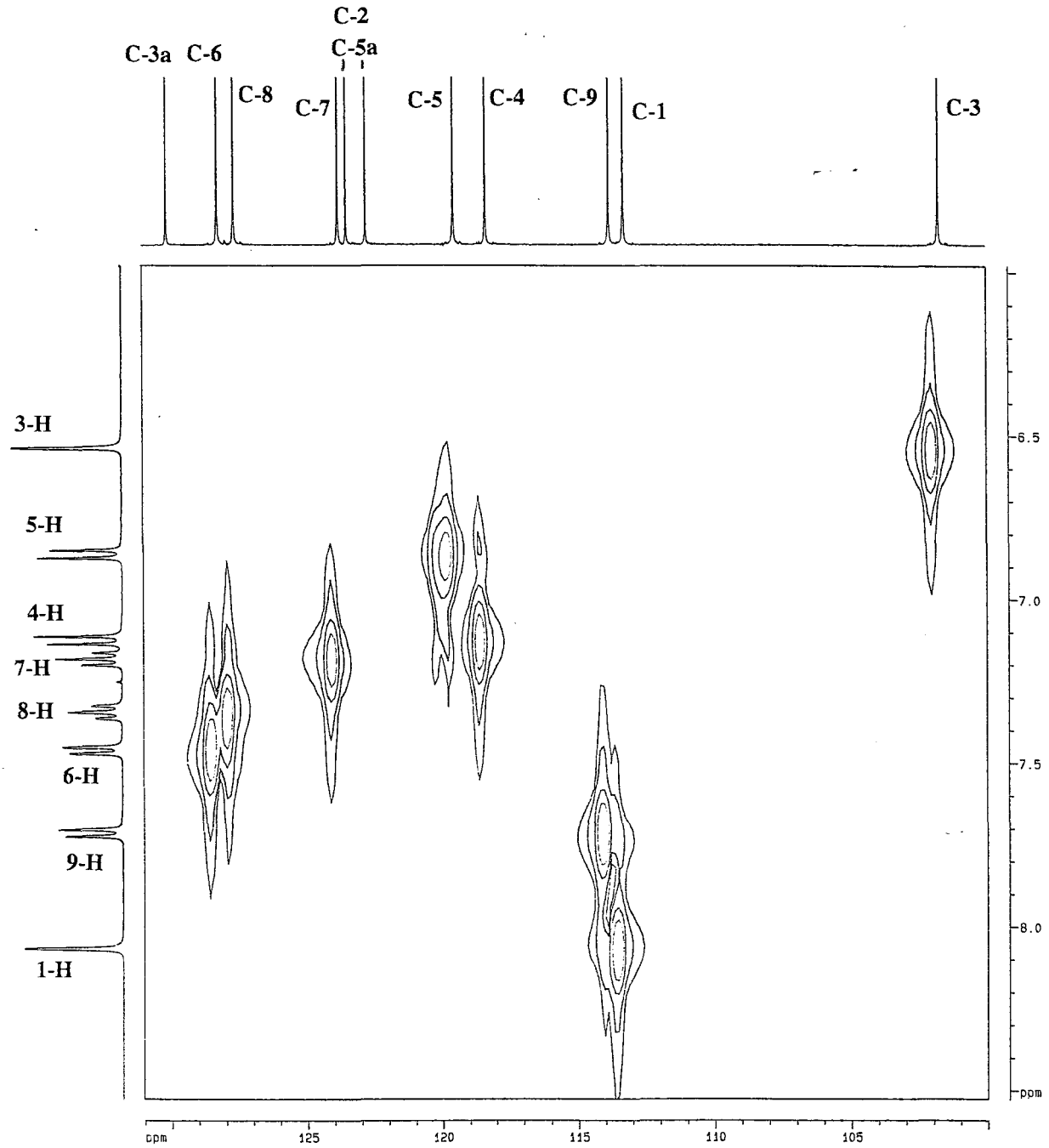
COSY spectrum of 2-(*N*,*N*-diethylcarboxamido)indolizine **191**.



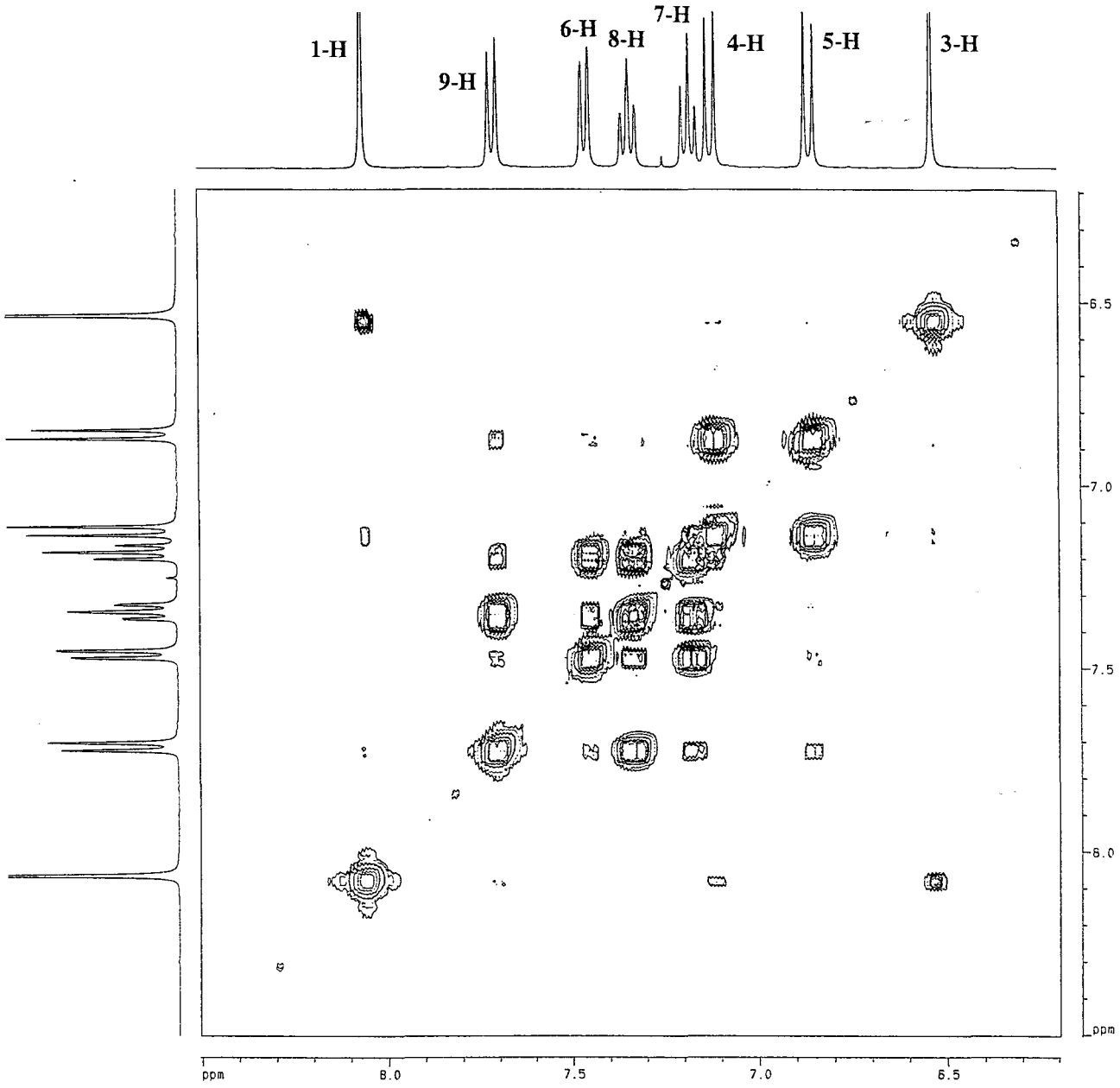
^{1}H NMR spectrum of 2-(*N*,*N*-diethylcarboxamido)pyrrolo[1,2-*a*]quinoline **194**.



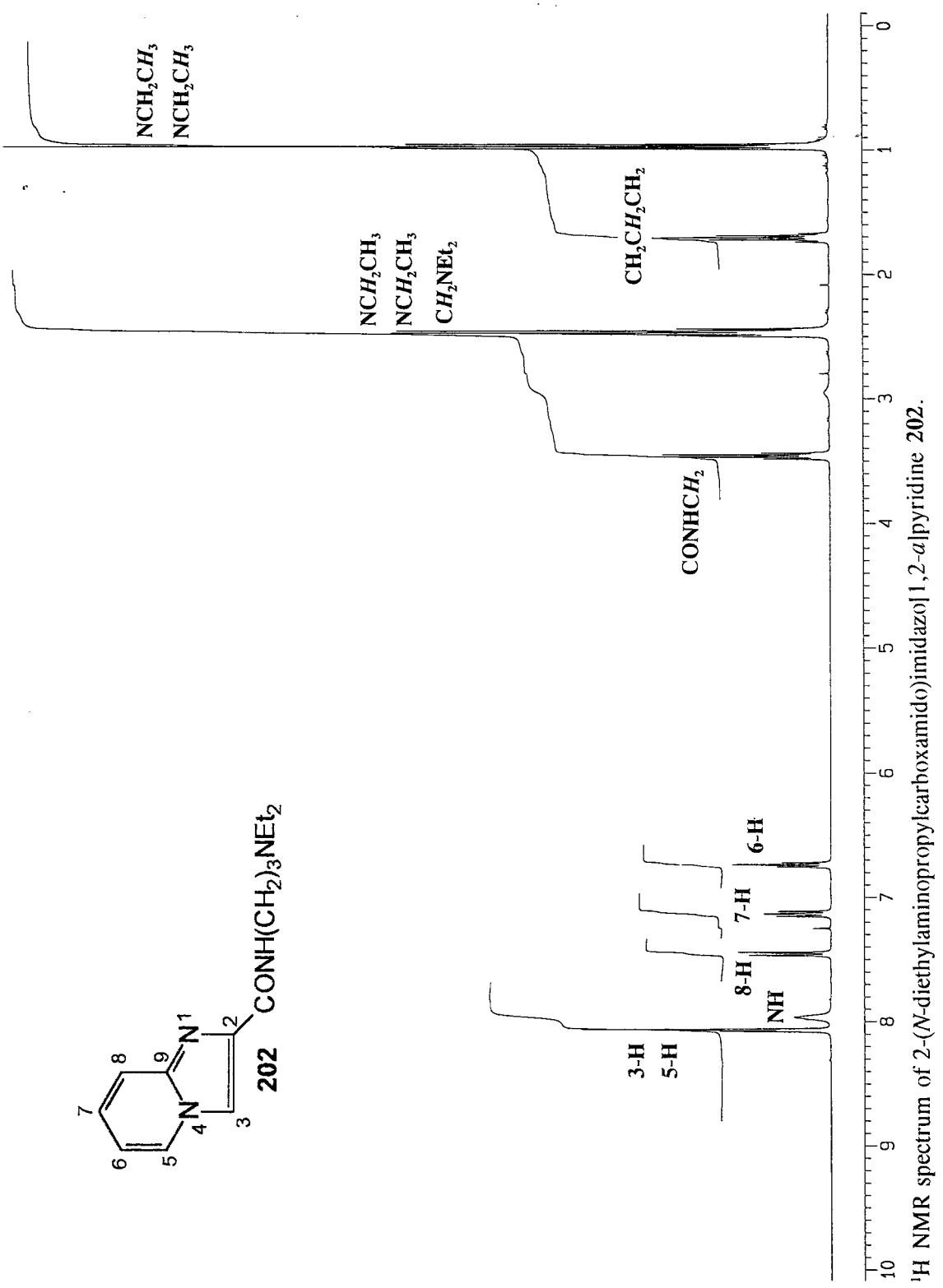
^{13}C NMR spectrum of 2-(*N,N*-diethylcarboxamido)pyrrolo[1,2-*a*]quinoline **194**.



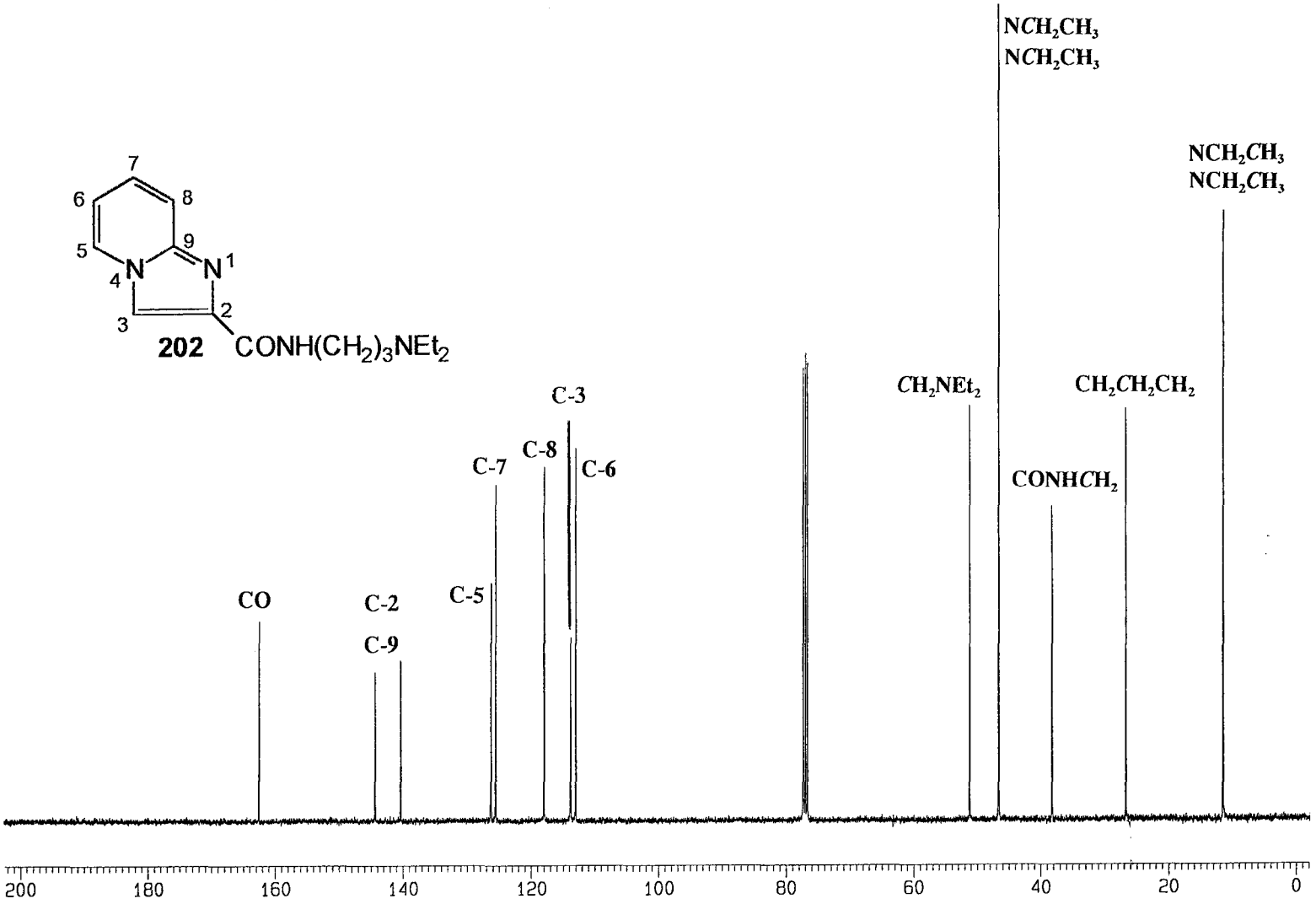
HETCOR spectrum of 2-(*N*,*N*-diethylcarboxamido)pyrrolo[1,2-*a*]quinoline **194**.



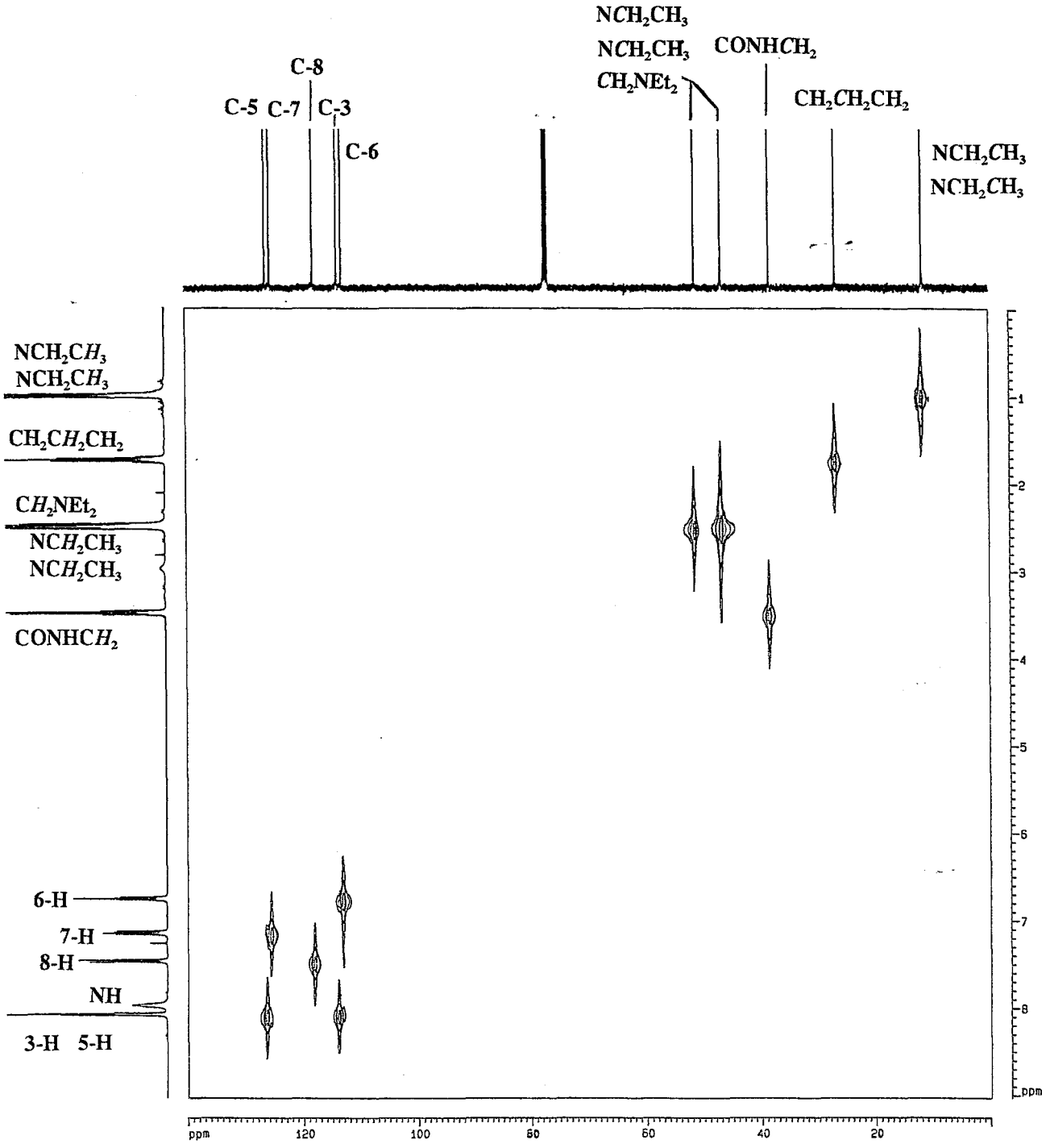
COSY spectrum of 2-(*N,N*-diethylcarboxamido)pyrrolo[1,2-*a*]quinoline **194**.



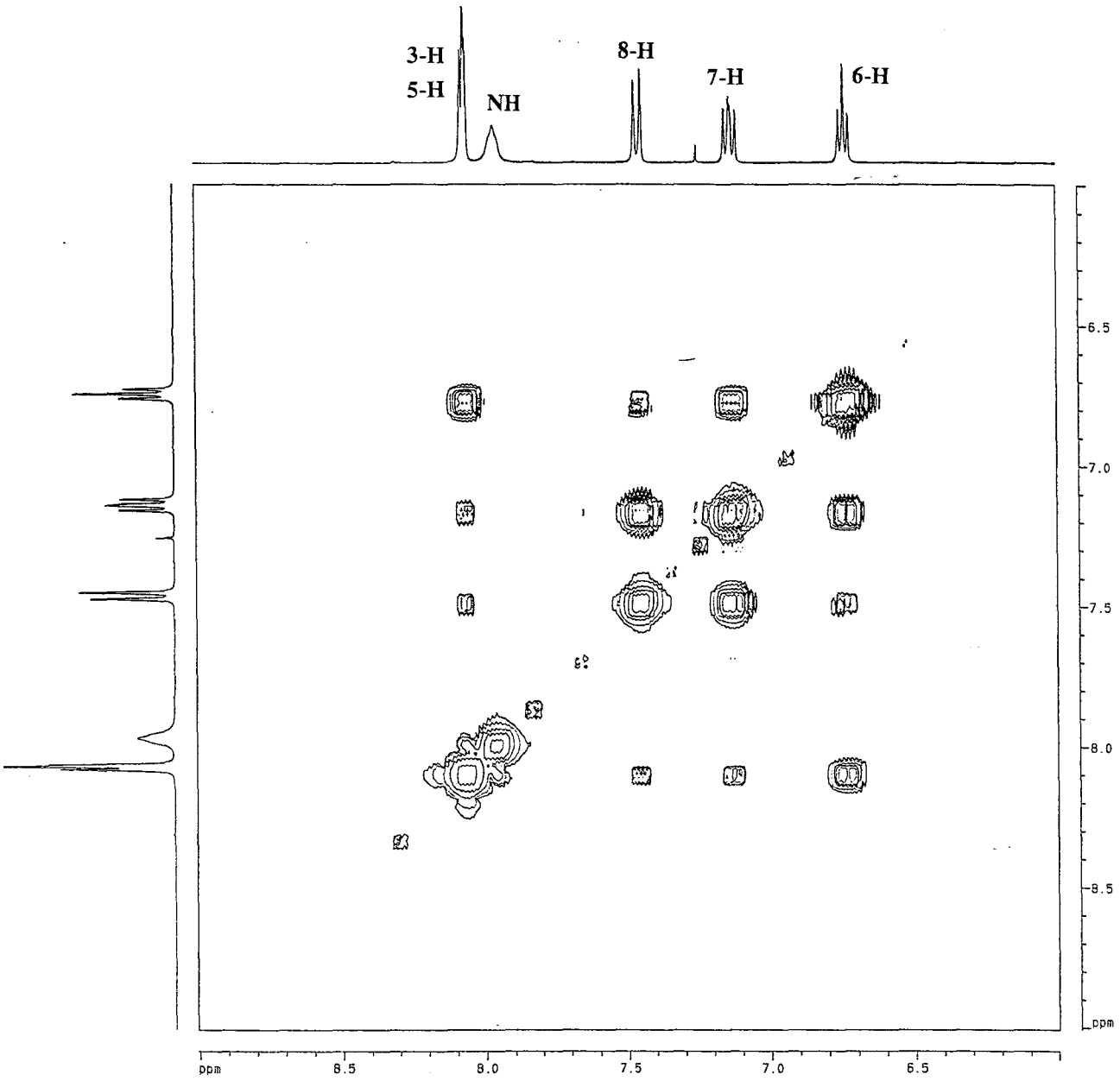
^{1}H NMR spectrum of 2-(*N*-diethylaminopropylcarboxamido)imidazo[1,2-*a*]pyridine **202**.



^{13}C NMR spectrum of 2-(*N*-diethylaminopropylcarboxamido)imidazo[1,2-*a*]pyridine **202**.



HETCOR spectrum of 2-(*N*-diethylaminopropylcarboxamido)imidazo[1,2-*a*]pyridine **202**.



COSY spectrum of 2-(*N*-diethylaminopropylcarboxamido)imidazo[1,2-*a*]pyridine **202**.

5.2 CRYSTALLOGRAPHIC DATA

Table 13 Crystal Data for $C_7H_6NOCl_3$

Formula	$C_7H_6NOCl_3$
Molar Mass	226.49
Crystal system	orthorhombic
Space group	Pbca
a(Å)	16.795 (6)
b(Å)	7.550 (1)
c(Å)	14.798 (4)
V(Å^3)	1876.42
Z	8
D_c(g.cm^{-1})	1.6035 (3)
F(000)	912
μ(cm^{-1})	0.85
Number of reflections $(2 < \theta < 30°)$	3120
Observed reflections $[I > \sigma(I)]$	2318
R	0.0646
R_W	0.0650
w^{-1}	$0.5939(\Sigma^2 \mid F \mid + 0.000737 \mid F \mid^2)^{-1}$
$N_{parameters}$	114

Table 14 Fractional Coordinates ($x10^4$) and Equivalent Isotropic Temperature Factors ($Å^2, x10^3$) for Non-Hydrogen Atoms

atom	x/a	y/b	z/c	U_{eq}
Cl(1)	4163(1)	490(1)	6497(1)	68(1)
Cl(2)	4111(1)	-1907(1)	5001(1)	58(1)
Cl(3)	3071(1)	-2439(2)	6508(1)	80(1)
O(1)	4512(1)	-4715(2)	6460(1)	49(1)
N(1)	5914(1)	-1357(3)	6945(1)	42(1)
C(1)	5518(1)	2480(3)	6407(2)	36(1)
C(2)	5879(2)	-3295(4)	5671(2)	46(1)
C(3)	6667(2)	-2951(4)	5485(2)	56(2)
C(4)	7077(2)	-1793(4)	6045(2)	60(2)
C(5)	6683(2)	-1043(4)	6751(2)	54(2)
C(6)	4670(1)	-2933(3)	6666(2)	38(1)
C(7)	4044(1)	-1751(3)	6188(2)	43(1)

$$U_{eq} = (1/3)\ \Sigma_i\Sigma_j\ U_{ij}a_i^*a_j^*(\mathbf{a}_i.\mathbf{a}_j)$$

Table 15 Fractional Coordinates ($x10^4$) for Hydrogen Atoms for $C_7H_6NOCl_3$

atom	x/a	y/b	z/c	U
H(1)	4366(22)	-5216(44)	6957(23)	65(10)
H(2)	5544(2)	-4193(4)	5249(2)	70(4)
H(3)	6959(2)	-3574(4)	4918(2)	70(4)
H(4)	7696(2)	-1494(4)	5917(2)	70(4)
H(5)	7008(2)	-140(4)	7181(2)	70(4)
H(6)	4619(1)	-2688(3)	7383(2)	70(4)

Table 16 Bond Lengths (Å) and Angles (°) for $C_7H_6NOCl_3$

Cl(1)-C(7)	1.764(3)	C(1)-C(6)	1.514(3)
Cl(2)-C(7)	1.764(3)	C(2)-C(3)	1.375(4)
Cl(3)-C(7)	1.779(3)	C(3)-C(4)	1.387(4)
N(1)-C(1)	1.340(3)	C(4)-C(5)	1.361(4)
N(1)-C(5)	1.344(4)	C(6)-O(1)	1.405(3)
C(1)-C(2)	1.390(3)	C(6)-C(7)	1.550(3)

C(1)-N(1)-C(5)	117.5(2)	O(1)-C(6)-C(1)	109.8(2)
C(1)-C(2)-C(3)	119.5(3)	O(1)-C(6)-C(7)	108.9(2)
C(1)-C(6)-C(7)	113.1(2)	Cl(1)-C(7)-Cl(2)	108.3(1)
C(2)-C(1)-C(6)	120.6(2)	Cl(1)-C(7)-Cl(3)	108.4(1)
C(2)-C(3)-C(4)	118.5(3)	Cl(1)-C(7)-C(6)	110.9(2)
C(3)-C(4)-C(5)	118.7(3)	Cl(2)-C(7)-Cl(3)	107.7(1)
N(1)-C(1)-C(2)	121.9(2)	Cl(2)-C(7)-C(6)	111.9(2)
N(1)-C(1)-C(6)	117.3(2)	Cl(3)-C(7)-C(6)	109.5(2)
N(1)-C(5)-C(4)	123.9(3)		

Table 17 Anisotropic Temperature Factors (Å,x10^3) for $C_7H_6NOCl_3$

atom	U(11)	U(22)	U(33)	U(23)	U(13)	U(12)
Cl(1)	76(1)	49(1)	79(1)	-17(1)	-25(1)	21(1)
Cl(2)	72(1)	62(1)	41(1)	1(1)	-13(1)	6(1)
Cl(3)	40(1)	112(1)	88(1)	27(1)	3(1)	3(1)
O(1)	62(1)	39(1)	47(1)	1(1)	5(1)	-7(1)
N(1)	46(1)	38(1)	42(1)	-3(1)	-5(1)	4(1)
C(1)	41(1)	32(1)	36(1)	3(1)	-1(1)	6(1)
C(2)	52(2)	44(1)	44(1)	-4(1)	4(1)	9(1)
C(3)	56(2)	54(2)	58(2)	2(1)	17(1)	13(1)
C(4)	44(1)	53(2)	85(2)	9(2)	10(2)	3(1)
C(5)	45(1)	48(1)	68(2)	0(1)	-6(1)	1(1)
C(6)	43(1)	38(1)	35(1)	0(1)	-1(1)	4(1)
C(7)	43(1)	44(1)	42(1)	-1(1)	-4(1)	2(1)

Table 18 Crystal Data for $C_{17}H_{12}O_4$

Formula	$C_{17}H_{12}O_4$
Molar Mass	280.29
Crystal system	monoclinic
Space group	$P2_1/c$
a(Å)	3.919(1)
b(Å)	14.266(3)
c(Å)	23.812(4)
β(°)	91.55(5)
V(Å^3)	1330.80(3)
Z	4
D_c(g.cm^{-1})	1.3992(3)
F(000)	584
μ(cm^{-1})	0.60
Number of reflections $(2 < \theta < 30°)$	2509
Observed reflections $[I > \sigma(I)]$	1941
R	0.0726
R_W	0.1007
w^{-1}	$0.1017(\Sigma^2 \mid F \mid + 0.0775 \mid F \mid ^2)^{-1}$
$N_{parameters}$	193

Table 19 Fractional Coordinates ($x10^4$) and Equivalent Isotropic Temperature Factors ($Å^2, x10^3$) for Non-Hydrogen Atoms

atom	x/a	y/b	z/c	U_{eq}
O(1)	571(6)	2051(2)	2803(1)	53(1)
O(2)	-369(6)	649(2)	2447(1)	60(1)
O(3)	5074(5)	-641(1)	3762(1)	50(1)
O(4)	10574(7)	-1266(2)	5121(1)	66(1)
C(1)	916(8)	1100(2)	2826(1)	45(1)
C(2)	2818(7)	694(2)	3297(1)	41(1)
C(3)	4016(8)	1247(2)	3721(1)	45(1)
C(4)	3533(7)	2242(2)	3701(1)	43(1)
C(5)	4643(8)	2857(2)	4123(2)	53(1)
C(6)	4163(9)	3808(2)	4067(2)	60(1)
C(7)	2517(10)	4162(2)	3585(2)	62(1)
C(8)	1393(9)	3578(2)	3161(2)	57(1)
C(9)	1856(7)	2622(2)	3227(1)	44(1)
C(10)	3268(7)	-348(2)	3266(1)	43(1)
C(11)	5758(7)	-1572(2)	3816(1)	40(1)
C(12)	7602(7)	-1822(2)	4310(1)	39(1)
C(13)	8364(7)	-2767(2)	4409(1)	47(1)
C(14)	7350(9)	-3439(2)	4027(2)	55(1)
C(15)	5594(8)	-3184(2)	3541(2)	51(1)
C(16)	4795(8)	-2261(2)	3432(1)	47(1)
C(17)	8784(9)	-1103(2)	4714(1)	50(1)

$$U_{eq} = (1/3)\ \Sigma_i\Sigma_j\ U_{ij}a_i^*a_j^*(\mathbf{a}_i.\mathbf{a}_j)$$

Table 20 Bond Lengths (Å) and Angles (°) for $C_{17}H_{12}O_4$

O(1)-C(1)	1.364(4)	O(1)-C(9)	1.381(4)
O(2)-C(1)	1.206(4)	O(3)-C(10)	1.422(3)
O(3)-C(11)	1.361(3)	C(1)-C(2)	1.452(4)
C(2)-C(3)	1.355(4)	C(2)-C(10)	1.499(4)
C(3)-C(4)	1.433(4)	C(4)-C(5)	1.396(4)
C(4)-C(9)	1.400(4)	C(5)-C(6)	1.376(5)
C(6)-C(7)	1.396(5)	C(7)-C(8)	1.372(5)
C(8)-C(9)	1.385(5)	C(11)-C(16)	1.388(4)
C(11)-C(12)	1.410(4)	C(13)-C(14)	1.373(5)
C(13)-C(12)	1.400(4)	C(14)-C(15)	1.379(5)
C(15)-C(16)	1.376(4)	C(12)-C(17)	1.471(4)
C(17)-O(4)	1.203(4)		

C(1)-O(1)-C(9)	121.6(2)	C(10)-O(3)-C(11)	117.3(2)
O(1)-C(1)-O(2)	117.6(2)	O(3)-C(11)-C(16)	125.4(3)
O(2)-C(1)-C(2)	124.1(3)	C(16)-C(11)-C(12)	119.7(3)
C(1)-C(2)-C(10)	114.6(3)	C(13)-C(14)-C(15)	120.0(3)
C(2)-C(3)-C(4)	120.6(3)	C(11)-C(16)-C(15)	119.8(3)
C(3)-C(4)-C(9)	118.0(3)	C(11)-C(12)-C(17)	121.0(3)
C(4)-C(5)-C(6)	120.7(3)	C(12)-C(17)-O(4)	123.9(3)
C(6)-C(7)-C(8)	121.2(3)	C(7)-C(8)-C(9)	118.4(3)
O(1)-C(9)-C(4)	121.0(3)	O(1)-C(9)-C(8)	117.0(3)
C(4)-C(9)-C(8)	122.0(3)	O(3)-C(10)-C(2)	107.9(2)
O(1)-C(1)-C(2)	118.4(3)	O(3)-C(11)-C(12)	114.9(2)
C(1)-C(2)-C(3)	120.3(3)	C(14)-C(13)-C(12)	120.4(3)
C(3)-C(2)-C(10)	125.1(3)	C(14)-C(15)-C(16)	121.1(3)
C(3)-C(4)-C(5)	124.1(3)	C(11)-C(12)-C(13)	119.0(3)
C(5)-C(4)-C(9)	117.9(3)	C(13)-C(12)-C(17)	120.0(3)
C(5)-C(6)-C(7)	119.7(3)		

Table 21 Anisotropic Temperature Factors (Å,x10^3) for $C_{17}H_{12}O_4$

atom	U(11)	U(22)	U(33)	U(23)	U(13)	U(12)
O(1)	68(1)	45(1)	46(1)	8(1)	-18(1)	3(1)
O(2)	73(2)	57(2)	49(1)	-1(1)	-24(1)	-4(1)
O(3)	67(1)	36(1)	45(1)	1(1)	-22(1)	5(1)
C(1)	50(2)	46(2)	38(1)	6(1)	-6(1)	0(1)
C(2)	43(2)	44(2)	35(2)	8(1)	-3(1)	2(1)
C(3)	43(2)	49(2)	42(2)	10(1)	-7(1)	1(1)
C(4)	41(2)	48(2)	40(2)	5(1)	-4(1)	-2(1)
C(5)	57(2)	43(2)	58(2)	-3(1)	-6(1)	3(1)
C(6)	70(2)	44(2)	65(2)	-4(2)	-7(2)	4(2)
C(7)	76(2)	38(2)	72(2)	7(2)	2(2)	7(2)
C(8)	64(2)	47(2)	59(2)	13(2)	-6(2)	2(1)
C(9)	47(2)	43(2)	44(2)	7(1)	-3(1)	1(1)
C(10)	43(2)	47(2)	39(2)	4(1)	-12(1)	1(1)
C(11)	38(1)	39(2)	42(2)	2(1)	-4(1)	-1(1)
C(13)	50(2)	44(2)	48(2)	2(1)	-5(1)	3(1)
C(14)	62(2)	34(2)	70(2)	0(1)	2(2)	6(1)
C(15)	55(2)	42(2)	55(2)	-12(1)	3(1)	-8(1)
C(16)	54(2)	45(2)	42(2)	-4(1)	-7(1)	-2(1)
C(12)	42(1)	36(1)	39(2)	0(1)	-3(1)	0(1)
C(17)	63(2)	44(2)	44(2)	-1(1)	-17(1)	2(1)
O(4)	84(2)	56(1)	55(1)	-4(1)	-30(1)	8(1)

Table 22 Fractional Coordinates ($x10^4$) for Hydrogen Atoms for $C_{17}H_{12}O_4$

atom	x/a	y/b	z/c	U
H(3)	5340(8)	935(2)	4078(1)	70(4)
H(5)	5891(8)	2583(2)	4499(2)	70(4)
H(6)	5058(9)	4280(2)	4394(2)	70(4)
H(7)	2123(10)	4909(2)	3544(2)	70(4)
H(8)	164(9)	3859(2)	2786(2)	70(4)
H(10A)	4703(7)	-528(2)	2900(1)	40(5)
H(10B)	804(7)	-687(2)	3241(1)	40(5)
H(13)	9757(7)	-2967(2)	4787(1)	70(4)
H(14)	7933(9)	-4168(2)	4108(2)	70(4)
H(15)	4830(8)	-3717(2)	3241(2)	70(4)
H(16)	3431(8)	-2073(2)	3048(1)	70(4)
H(17)	8005(9)	-386(2)	4642(1)	93(14)

Table 23 Least-squares planes

Deviations from the Mean Plane 1 Defined By C_{11}-C_{17}

O(1)	-0.05	C(8)	0.104
O(2)	-0.15	C(9)	-0.03
O(3)	-0.05	C(10)	-0.03
O(4)	0.12	C(11)	-0.02
C(1)	-0.10	C(12)	-0.01
C(2)	-0.07	C(13)	-0.01
C(3)	-0.08	C(14)	0.00
C(4)	-0.07	C(15)	0.01
C(5)	-0.10	C(16)	0.01
C(6)	-0.06	C(17)	0.02
C(7)	0.01		

Equation of Plane 1
(0.8599) XO + (0.1047) YO + (-0.4995) ZO = -2.7863
(3.4218) X + (1.4935) Y + (-11.8951) Z = -2.7863

Table 23 cont.

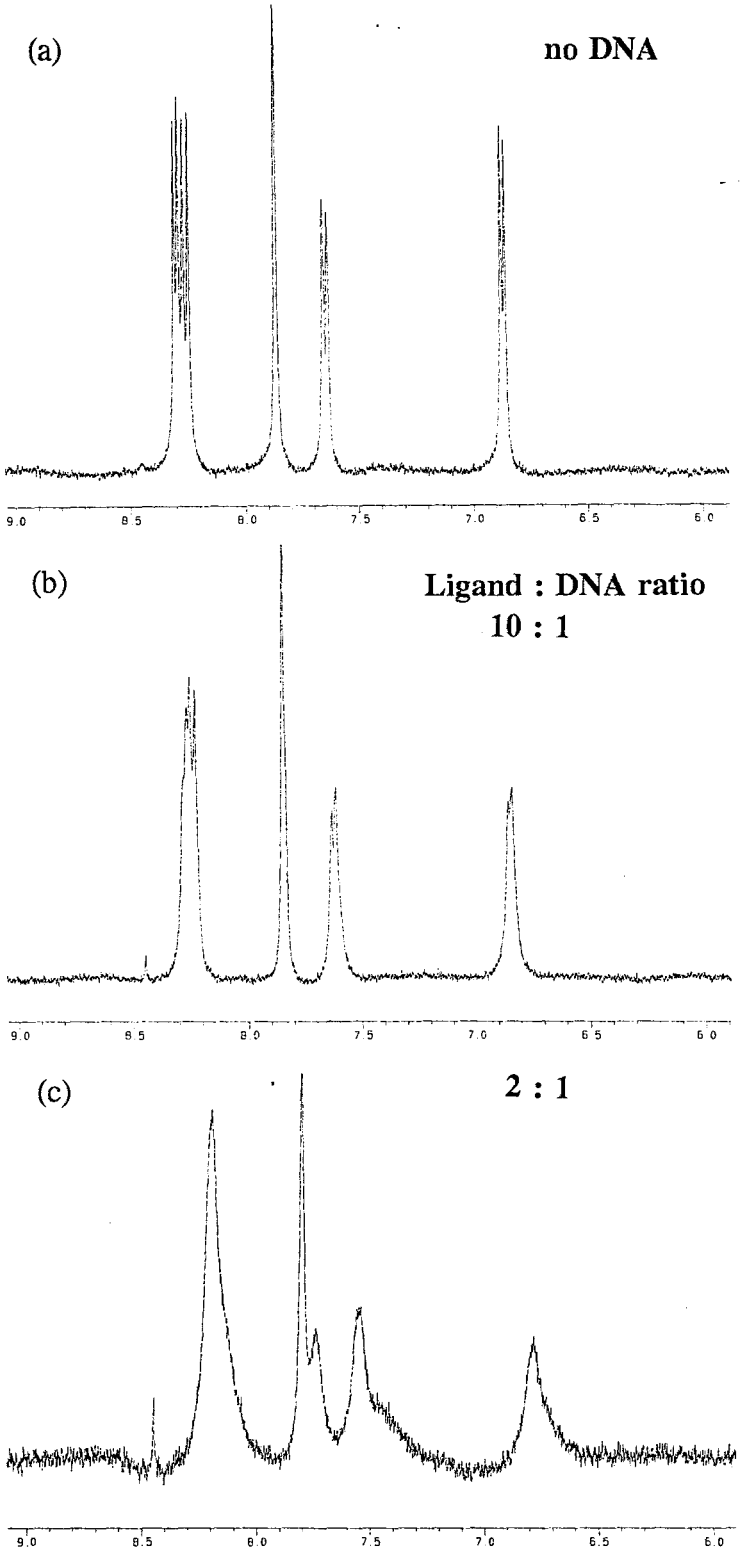
Deviations from the Mean Plane 2 Defined By C_4-C_9

O(1)	-0.06	C(8)	0.01
O(2)	-0.15	C(9)	-0.01
O(3)	0.16	C(10)	0.11
O(4)	0.51	C(11)	0.23
C(1)	-0.07	C(12)	0.30
C(2)	0.03	C(13)	0.35
C(3)	0.04	C(14)	0.34
C(4)	0.01	C(15)	0.30
C(5)	0.00	C(16)	0.24
C(6)	0.00	C(17)	0.35
C(7)	0.00		

Equation of Plane 2
(0.8815) XO + (0.0791) YO + (-0.4656) ZO = -2.6209
(3.5025) X + (1.1278) Y + (-11.0867)Z = -2.6209

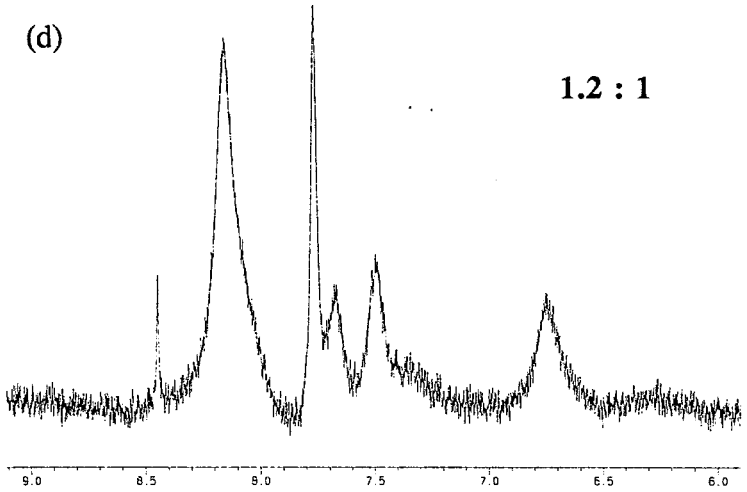
Angle between normals to plane 1 and 2 = 2.73°

5.3 NMR SPECTRA FROM DNA STUDIES

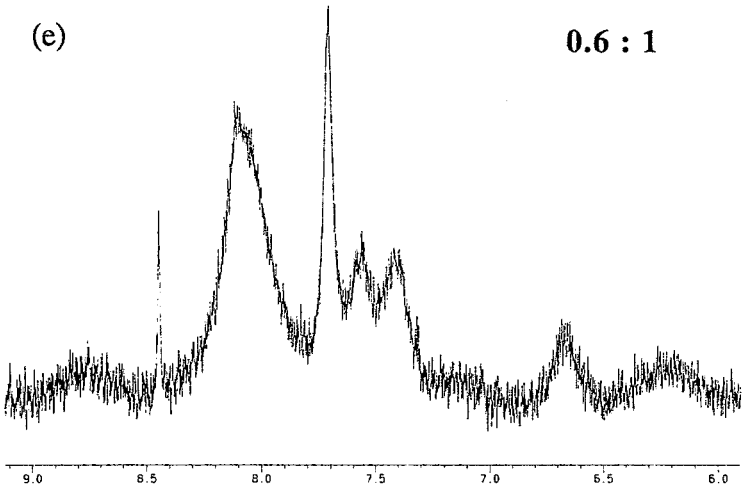


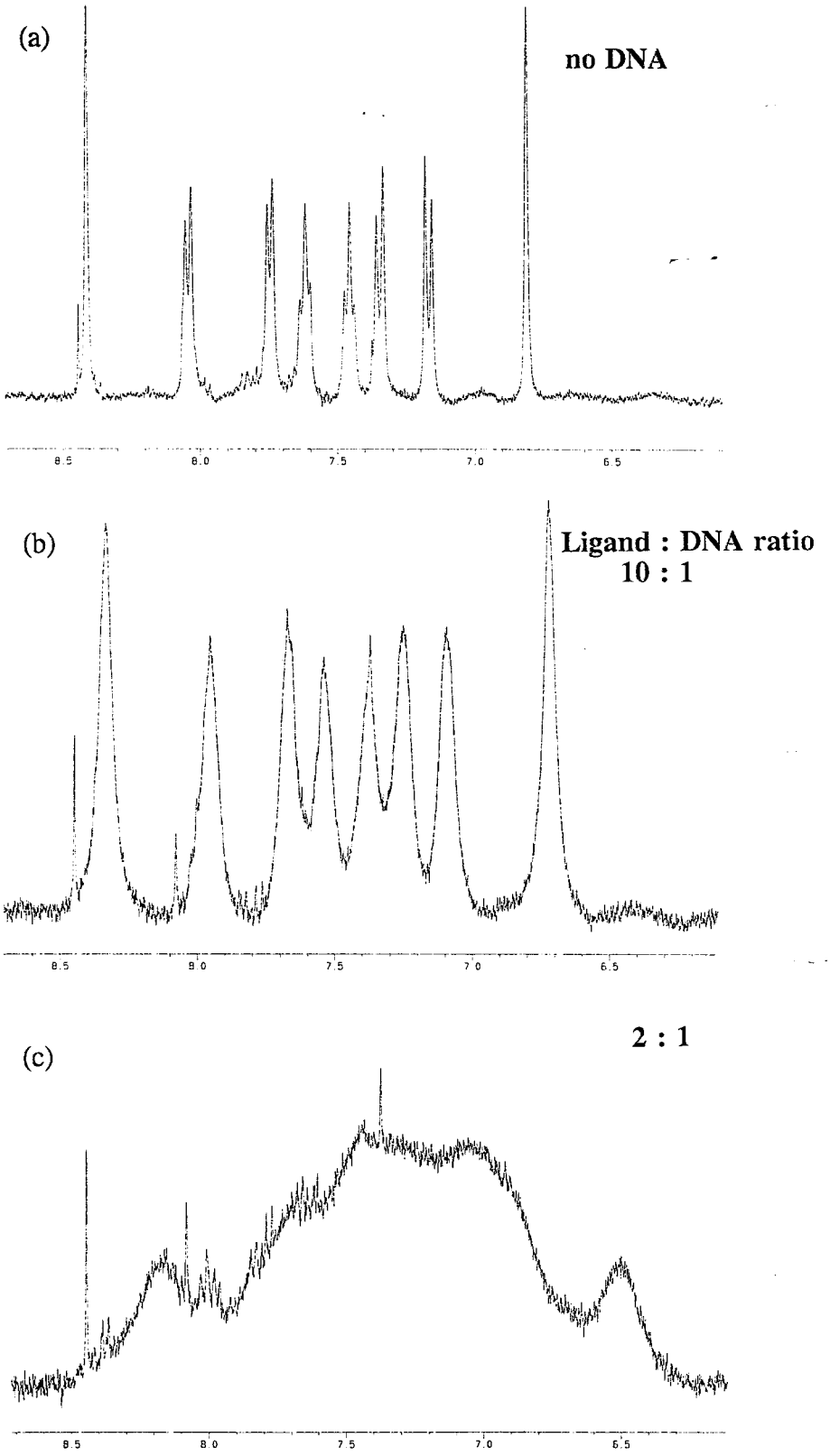
^{1}H NMR spectra of chloroquine **126** showing the aromatic region : (a) before addition of DNA; and (b)-(e) after addition of increasing amounts of DNA.

(d)



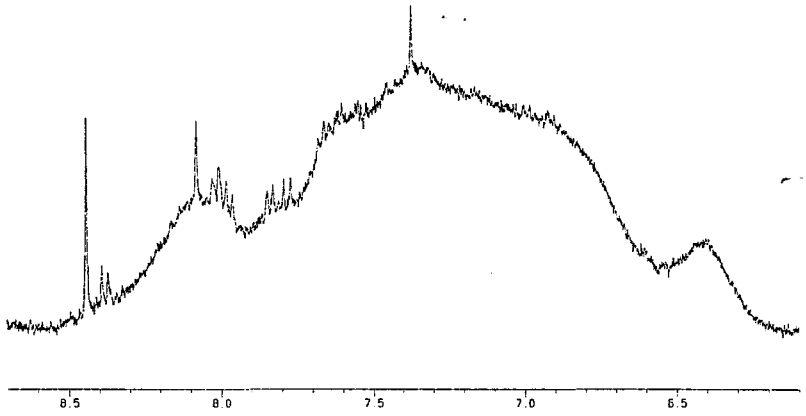
(e)

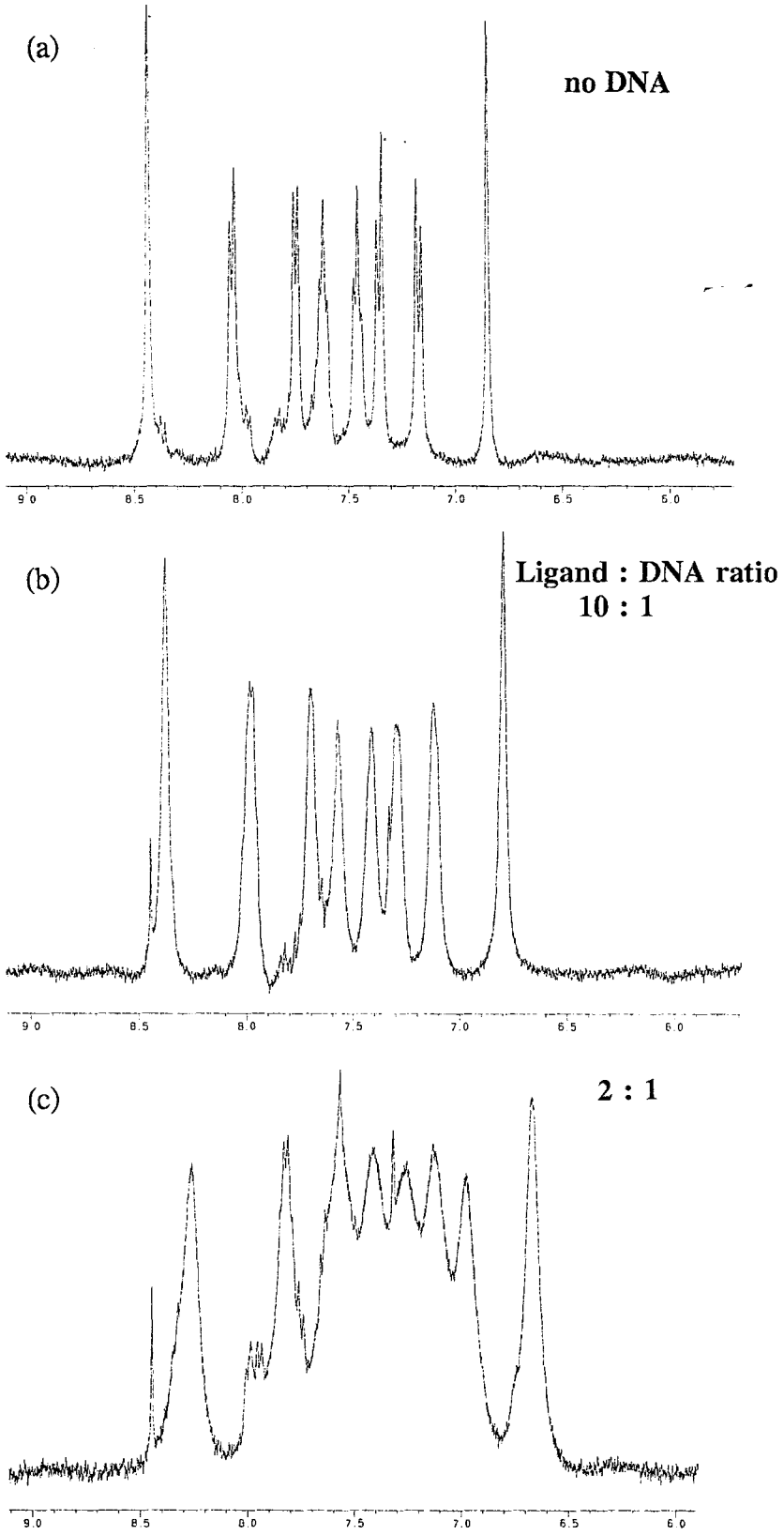




^{1}H NMR spectra of carboxamide **195** showing the aromatic region : (a) before addition of DNA; and (b)-(d) after addition of increasing amounts of DNA.

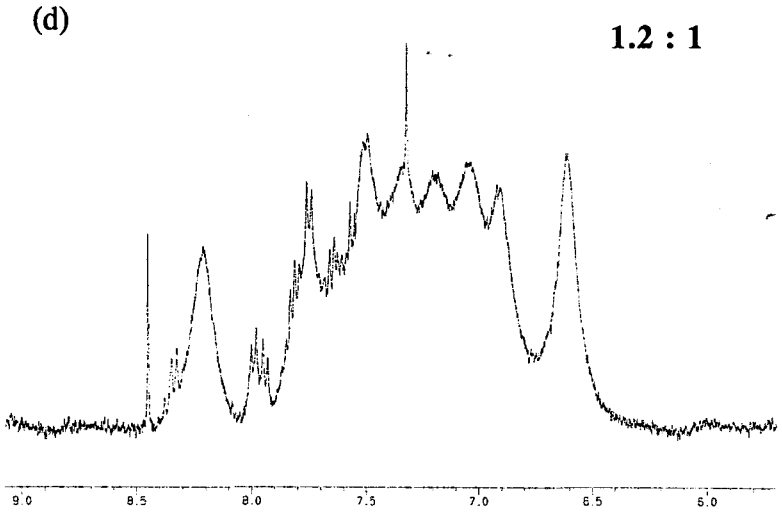
(d) **1.2 : 1**

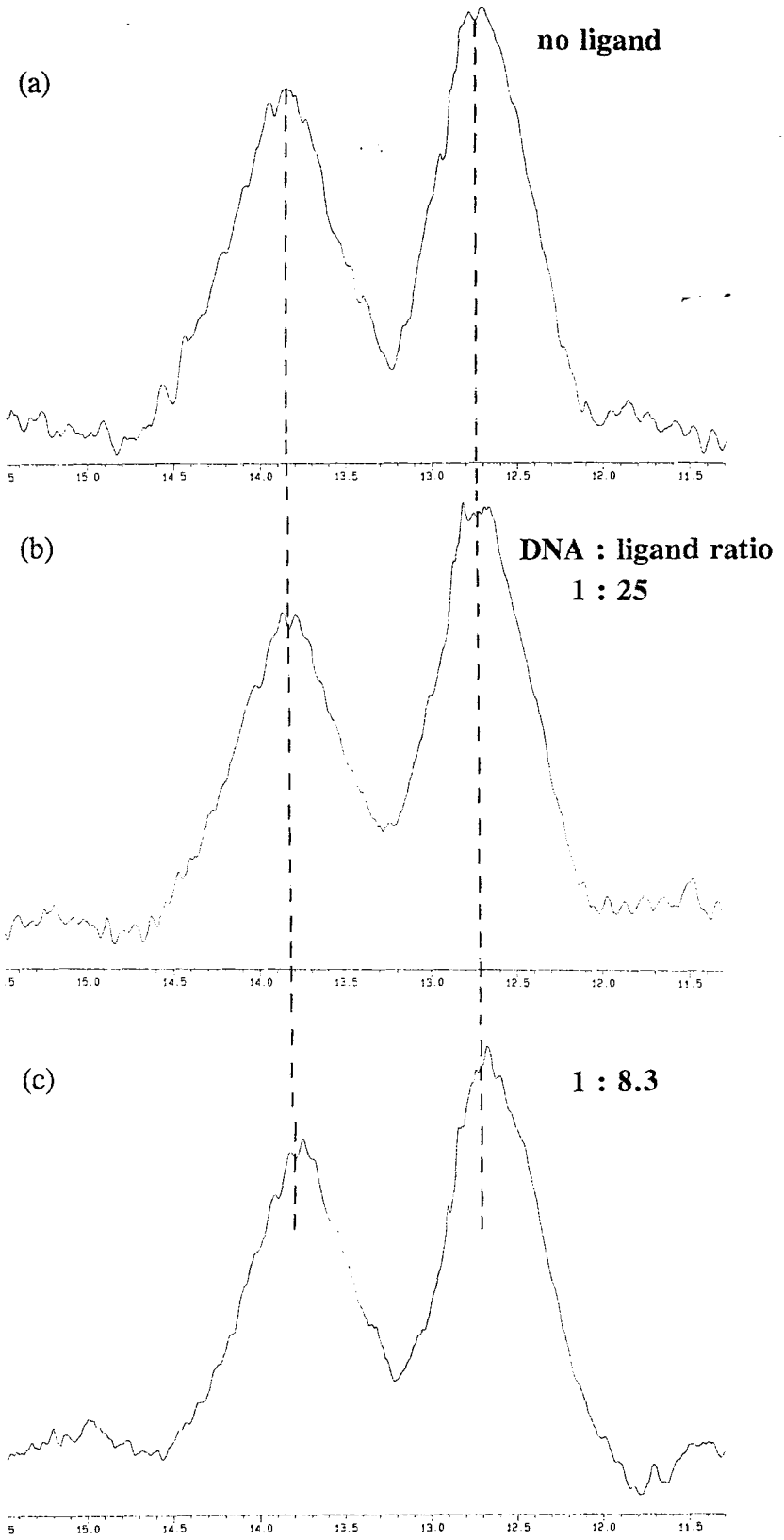




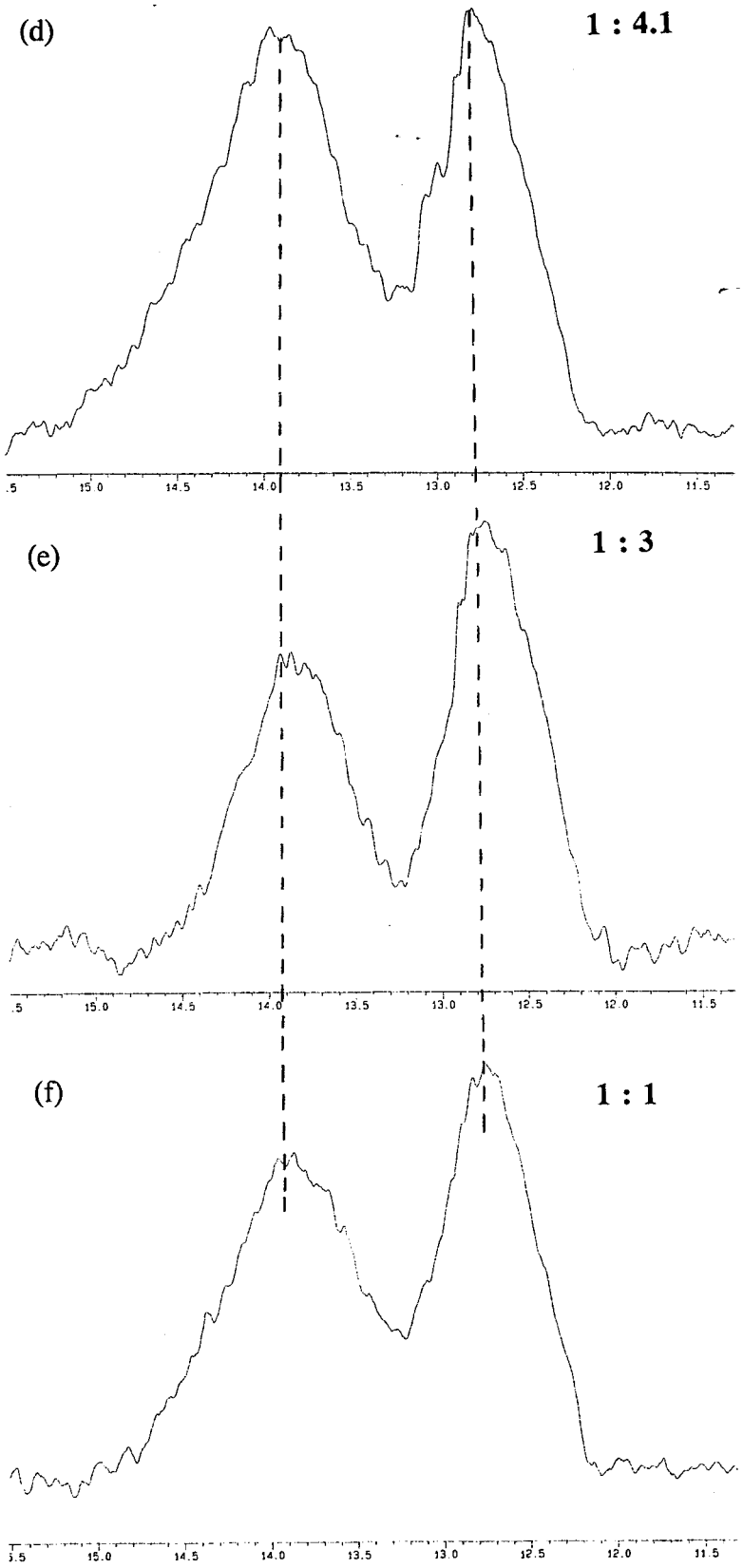
^{1}H NMR spectra of carboxamide **196** showing the aromatic region : (a) before addition of DNA; and (b)-(d) after addition of increasing amounts of DNA.

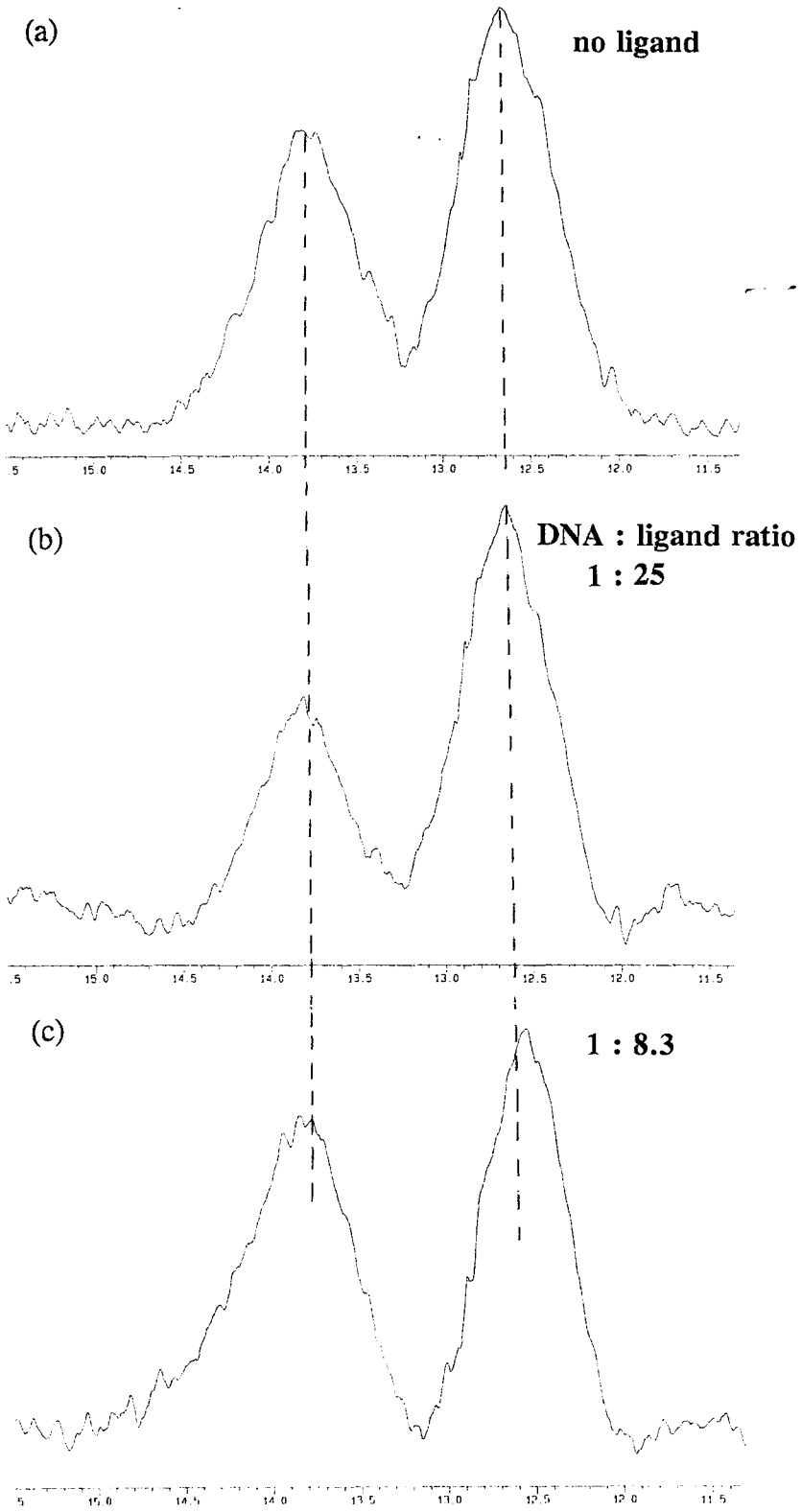
(d)



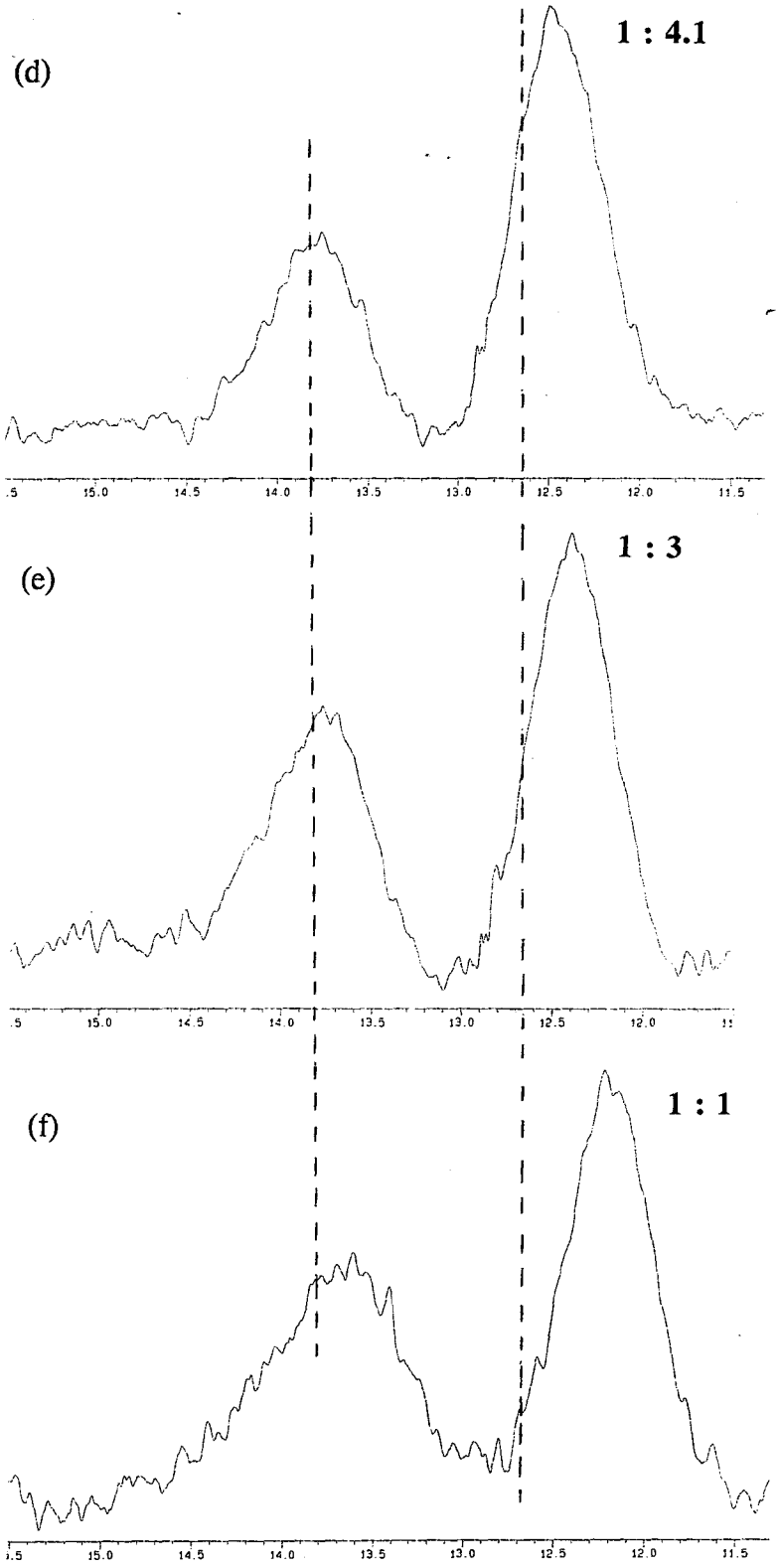


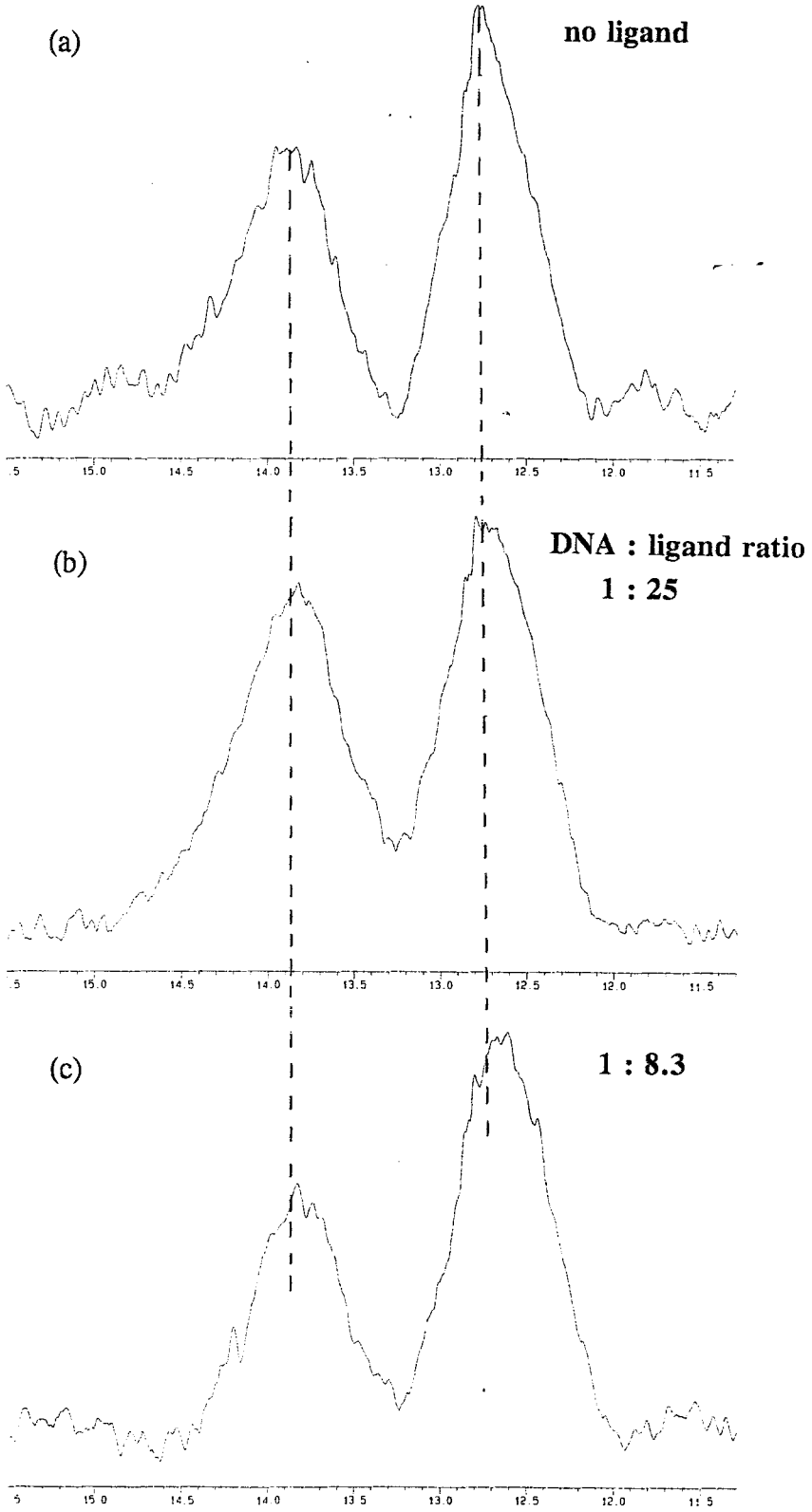
^{1}H NMR spectra of DNA imino protons : (a) before addition of ligand; and (b)-(f) after addition of increasing amounts of carboxamide **181**, a non-intercalator.


(d)
1 : 4.1
15.0
14.5
14.0
13.5
13.0
12.5
12.0
11.5
(e)
1 : 3
15.0
14.5
14.0
13.5
13.0
12.5
12.0
11.5
(f)
1 : 1
15.0
14.5
14.0
13.5
13.0
12.5
12.0
11.5

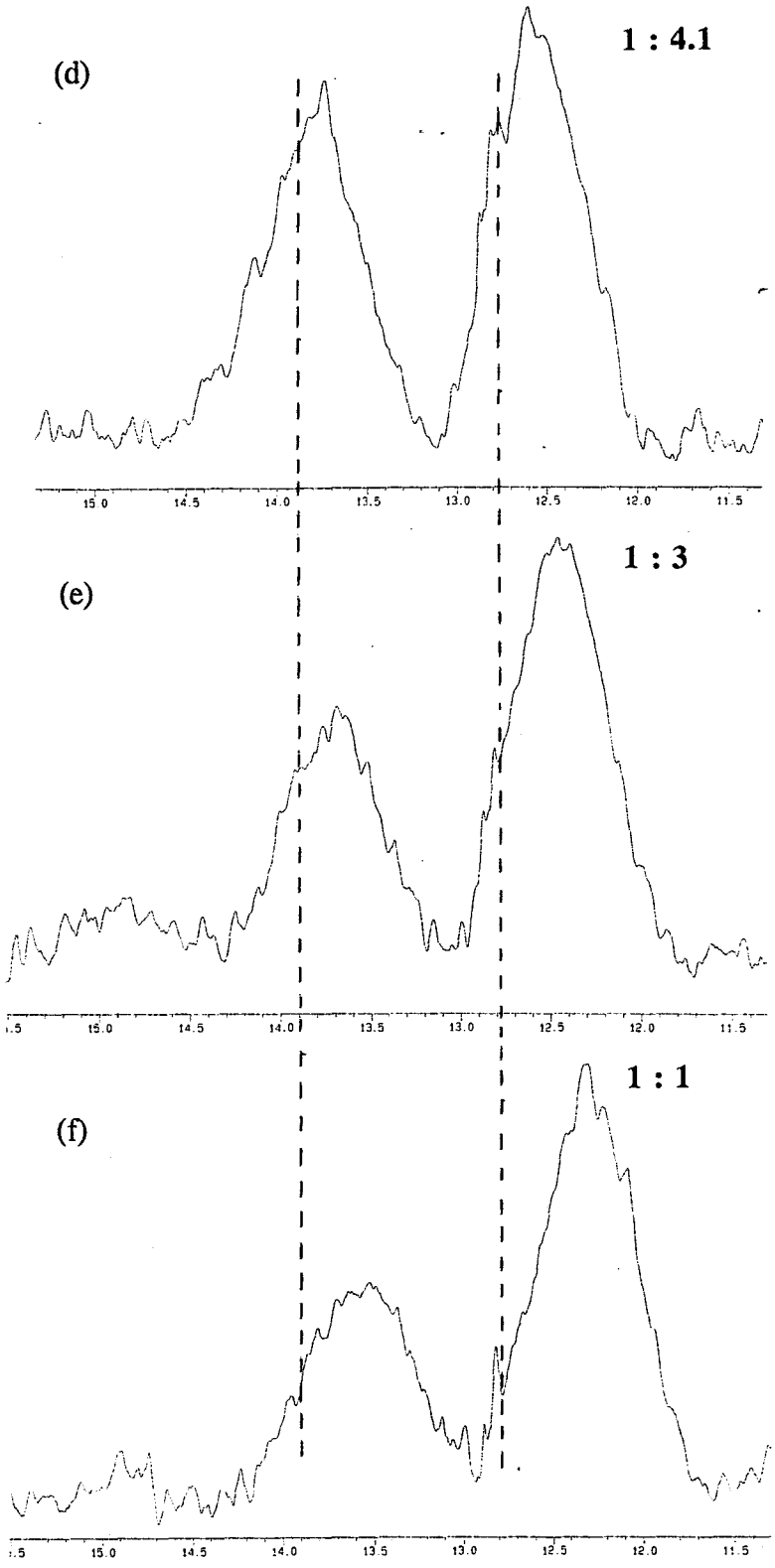


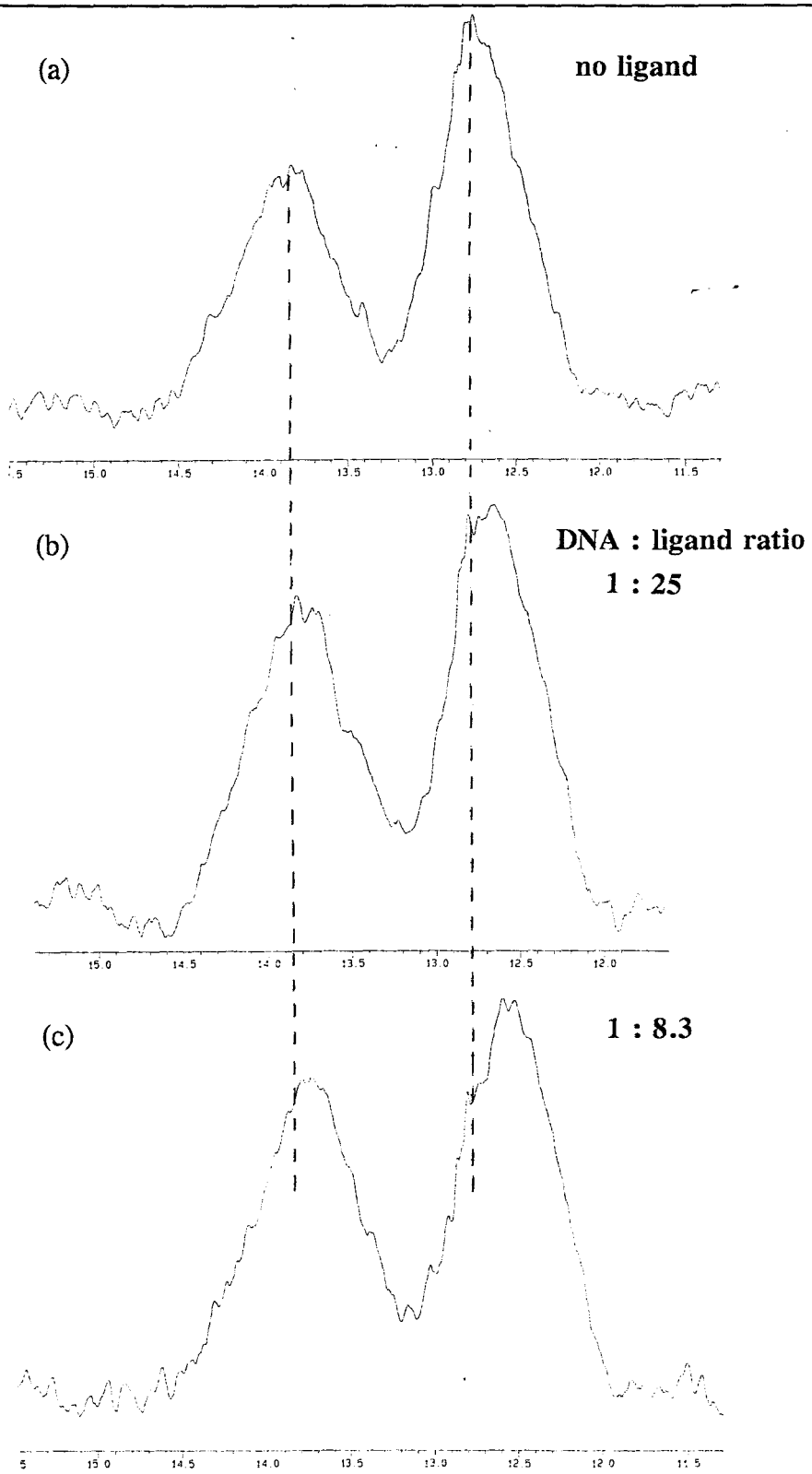
^{1}H NMR spectra of DNA imino protons : (a) before addition of ligand; and (b)-(f) after addition of chloroquine **126**, an intercalator.


(d)
1 : 4.1
15.0
14.5
14.0
13.5
13.0
12.5
12.0
11.5
(e)
1 : 3
15.0
14.5
14.0
13.5
13.0
12.5
12.0
(f)
1 : 1
15.0
14.5
14.0
13.5
13.0
12.5
12.0
11.5

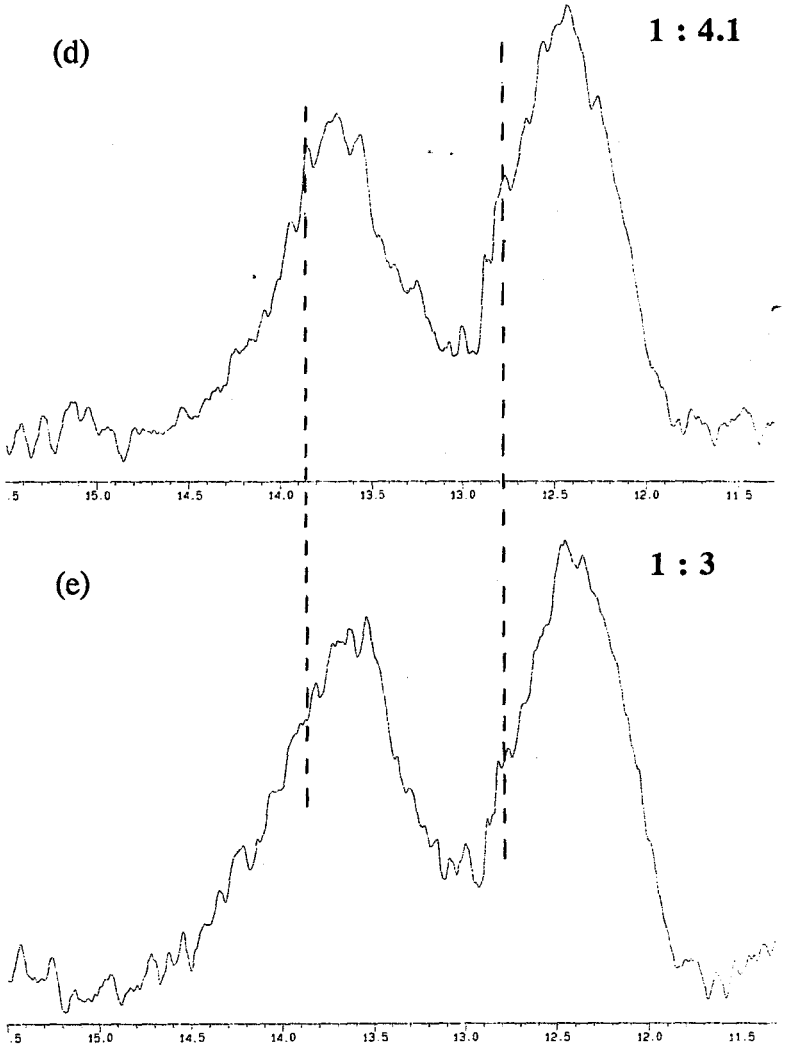


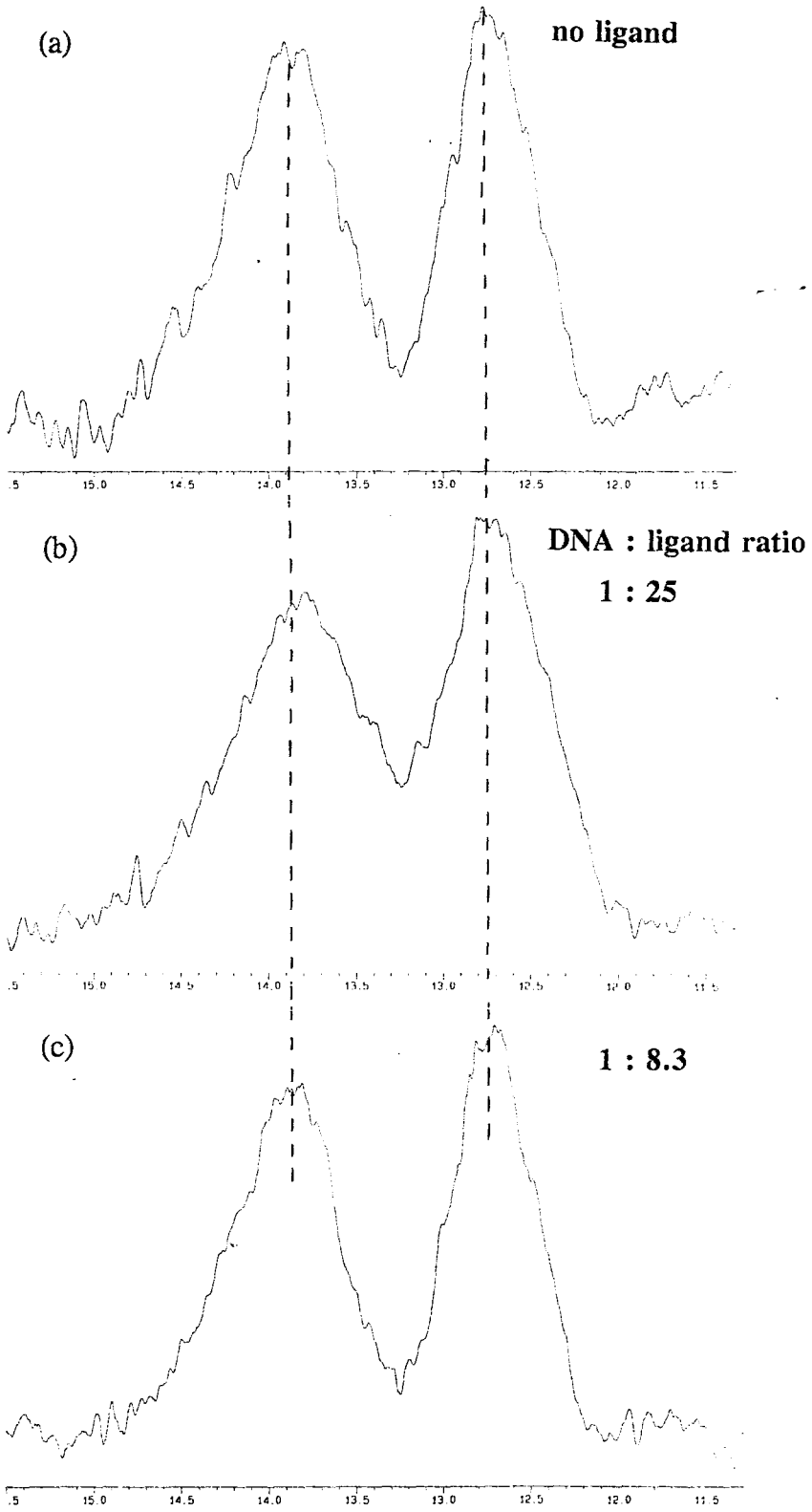
^{1}H NMR spectra of DNA imino protons : (a) before addition of ligand; and (b)-(f) after addition of increasing amounts of carboxamide **195**, an intercalator.


(d)
1 : 4.1
15.0
14.5
14.0
13.5
13.0
12.5
12.0
11.5
(e)
1 : 3
15.0
14.5
14.0
13.5
13.0
12.5
12.0
11.5
(f)
1 : 1
15.0
14.5
14.0
13.5
13.0
12.5
12.0
11.5

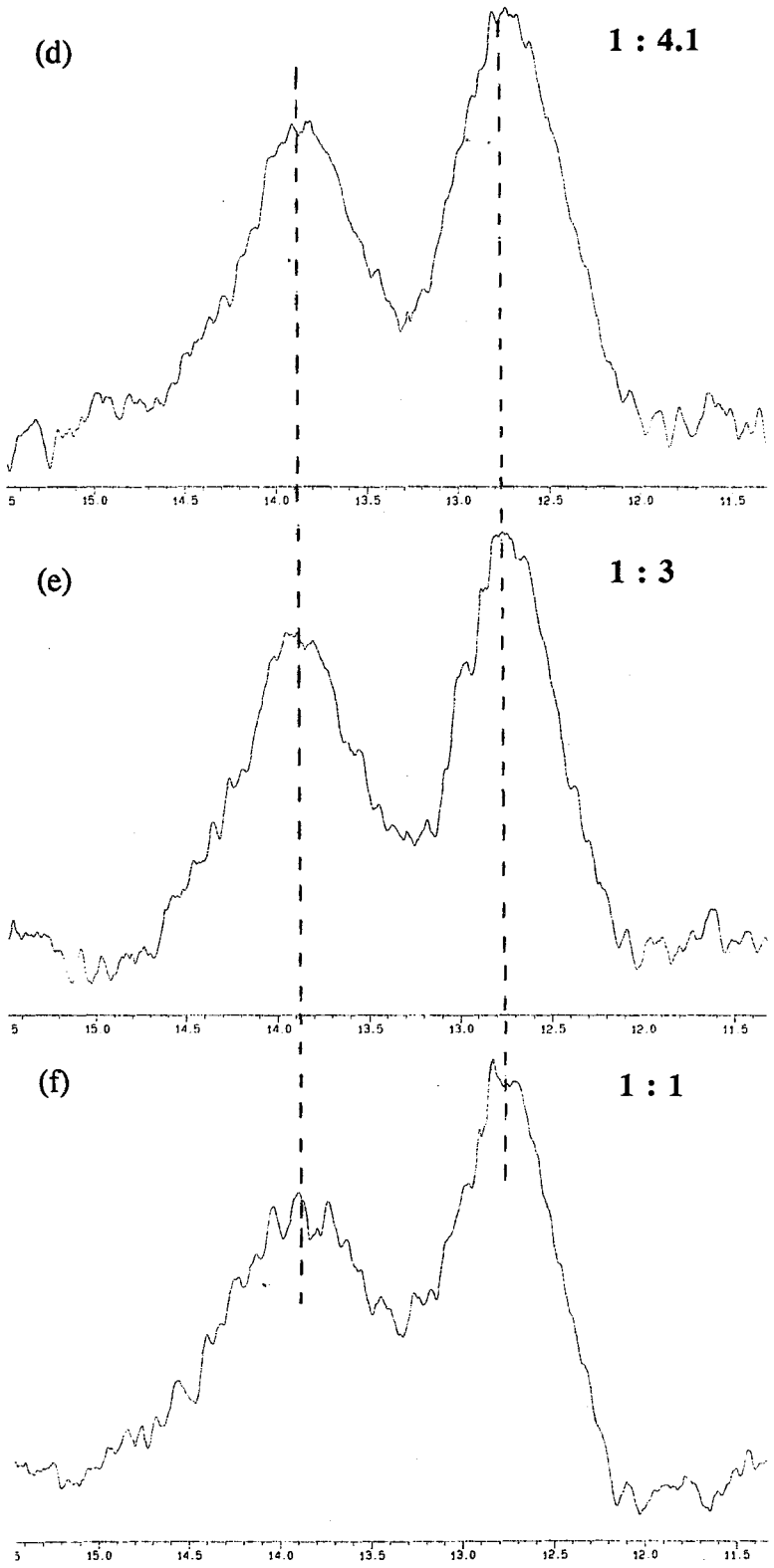


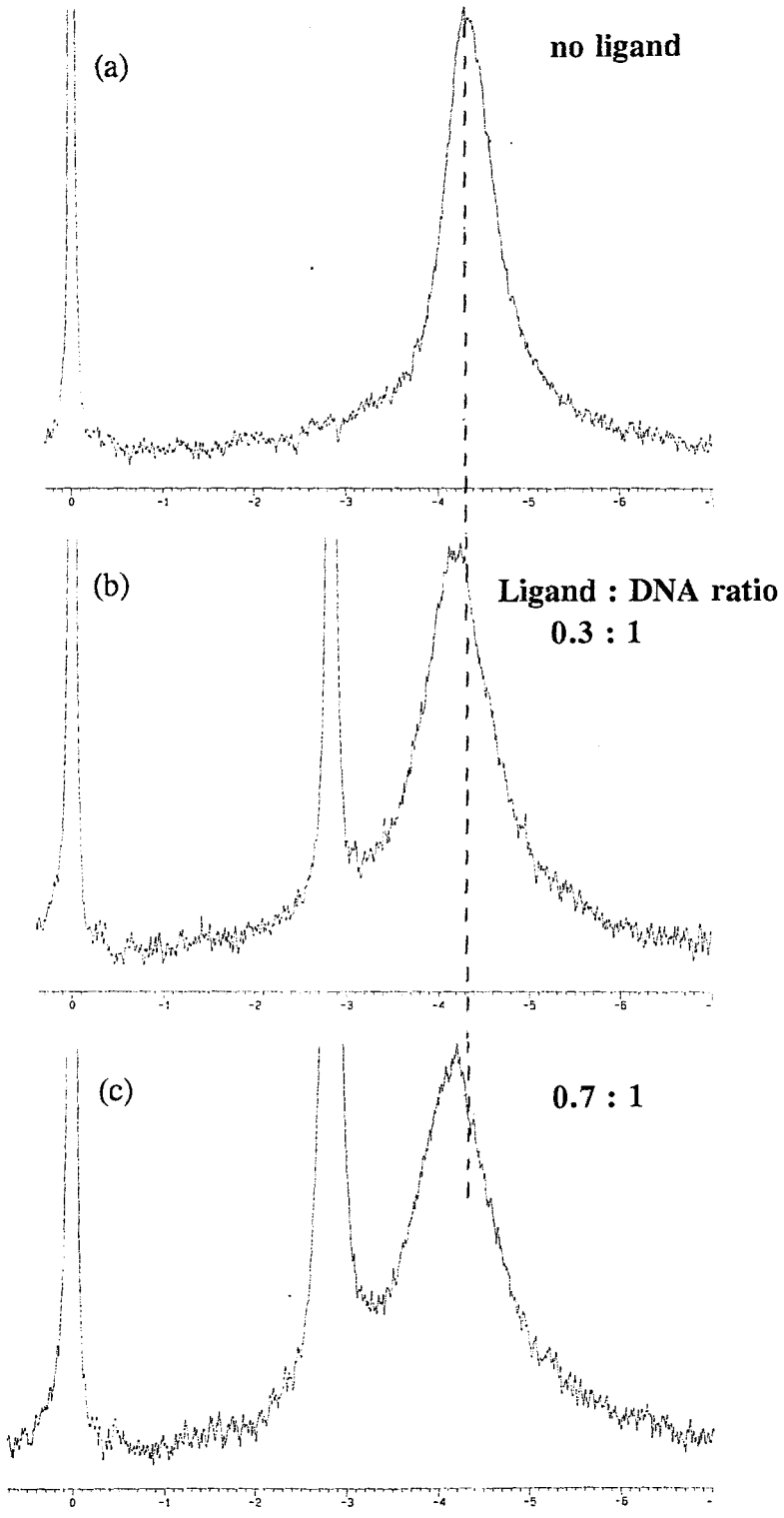
^{1}H NMR spectra of DNA imino protons : (a) before addition of ligand; and (b)-(e) after addition of increasing amounts of carboxamide **196**, an intercalator.


(d)
1 : 4.1
15.0
14.5
14.0
13.5
13.0
12.5
12.0
(e)
1 : 3
15.0
14.5
14.0
13.5
13.0
12.5
12.0
11.5

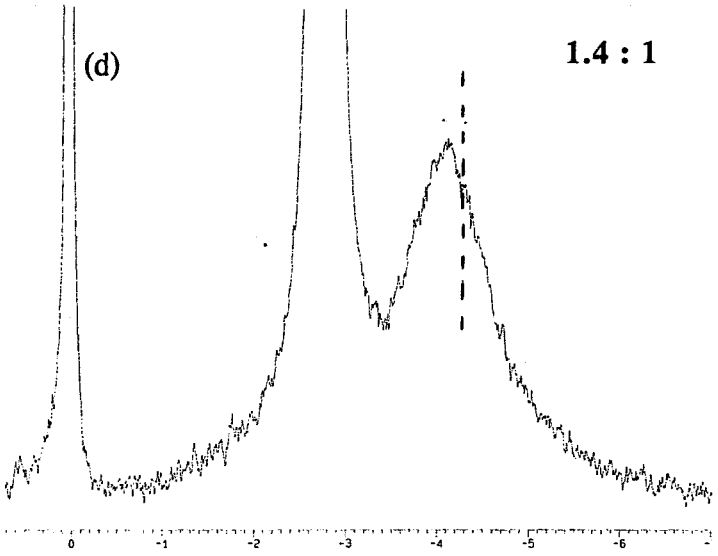


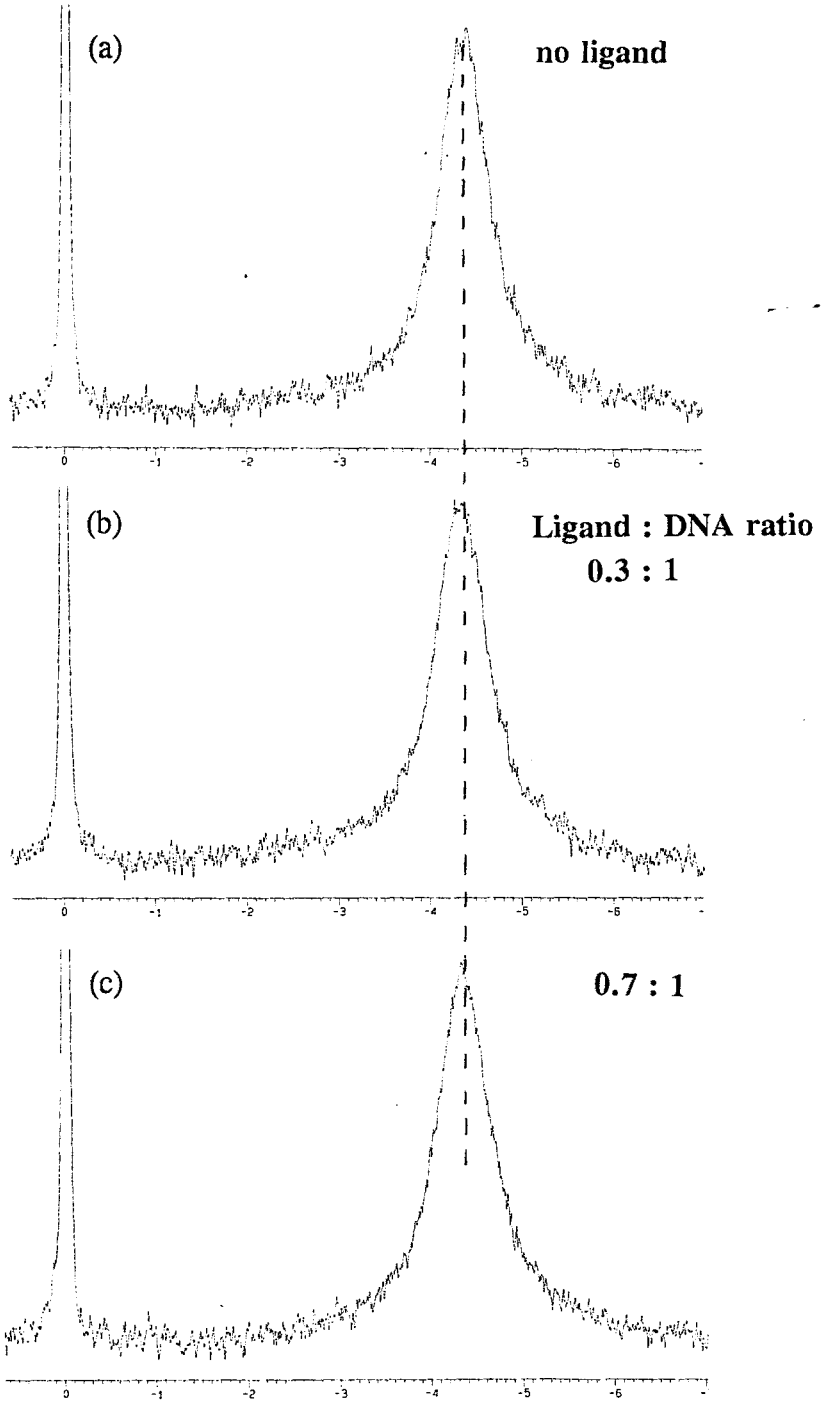
^{1}H NMR spectra of DNA imino protons : (a) before addition of ligand; and (b)-(f) after addition of increasing amounts of carboxamide **202**, a non-intercalator.


(d)
1 : 4.1
(e)
1 : 3
(f)
1 : 1
5
15.0
14.5
14.0
13.5
13.0
12.5
12.0
11.5

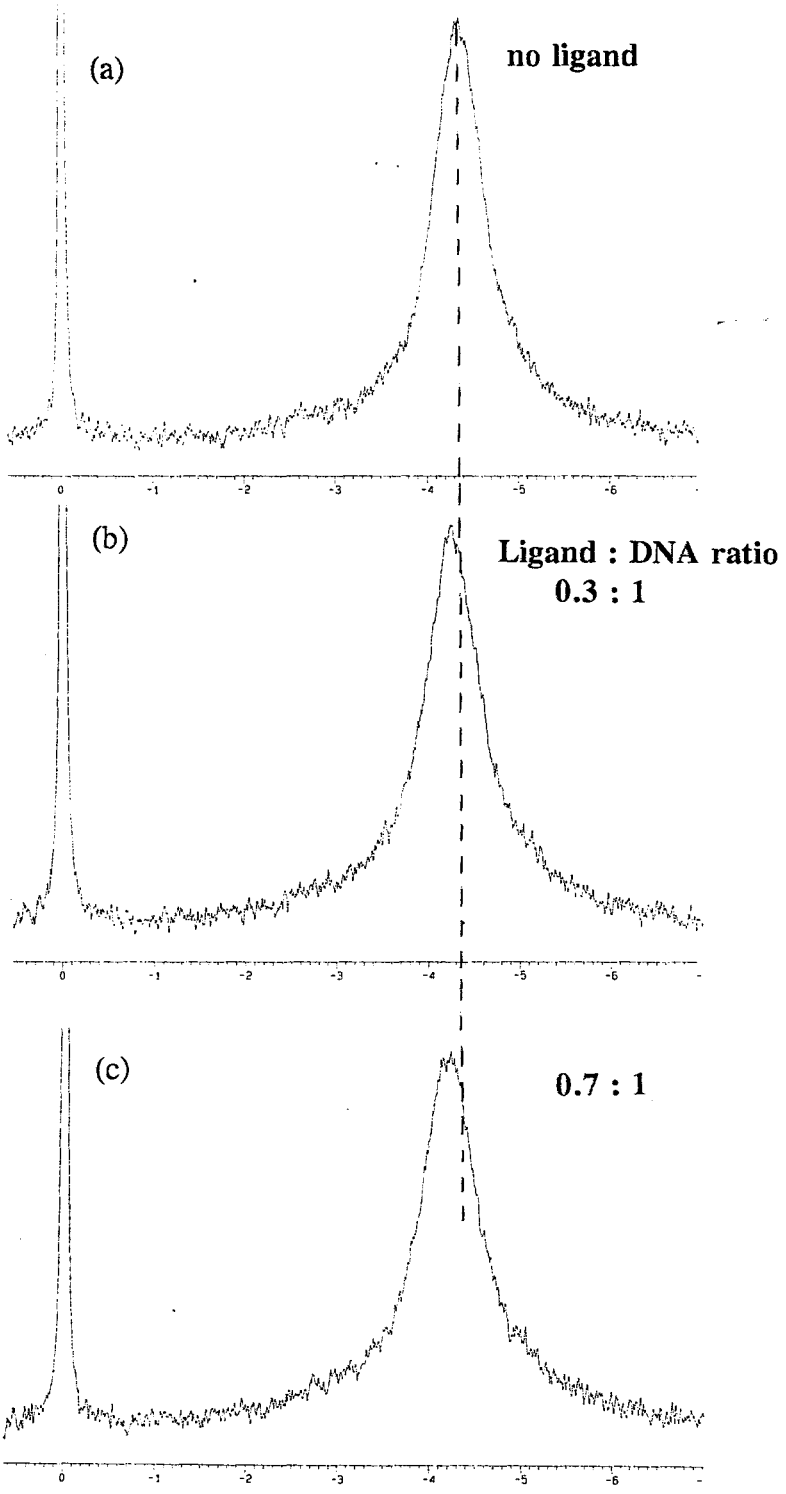


^{31}P NMR spectra of DNA : (a) before addition of ligand; and (b)-(d) after addition of increasing amounts of chloroquine **126**, an intercalator.

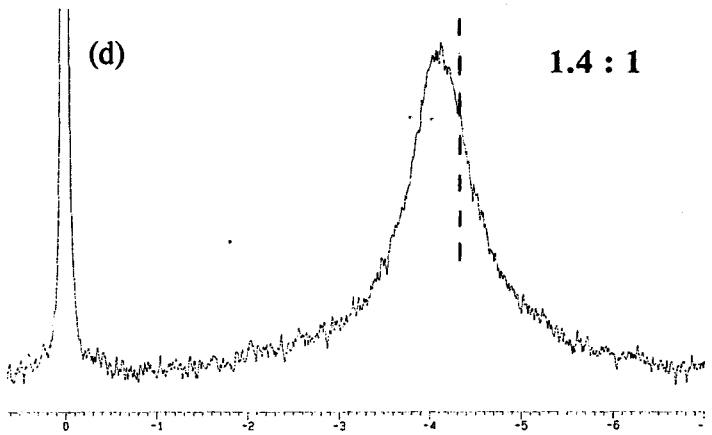

(d)
1.4 : 1
0
-1
-2
-3
-4
-5
-6

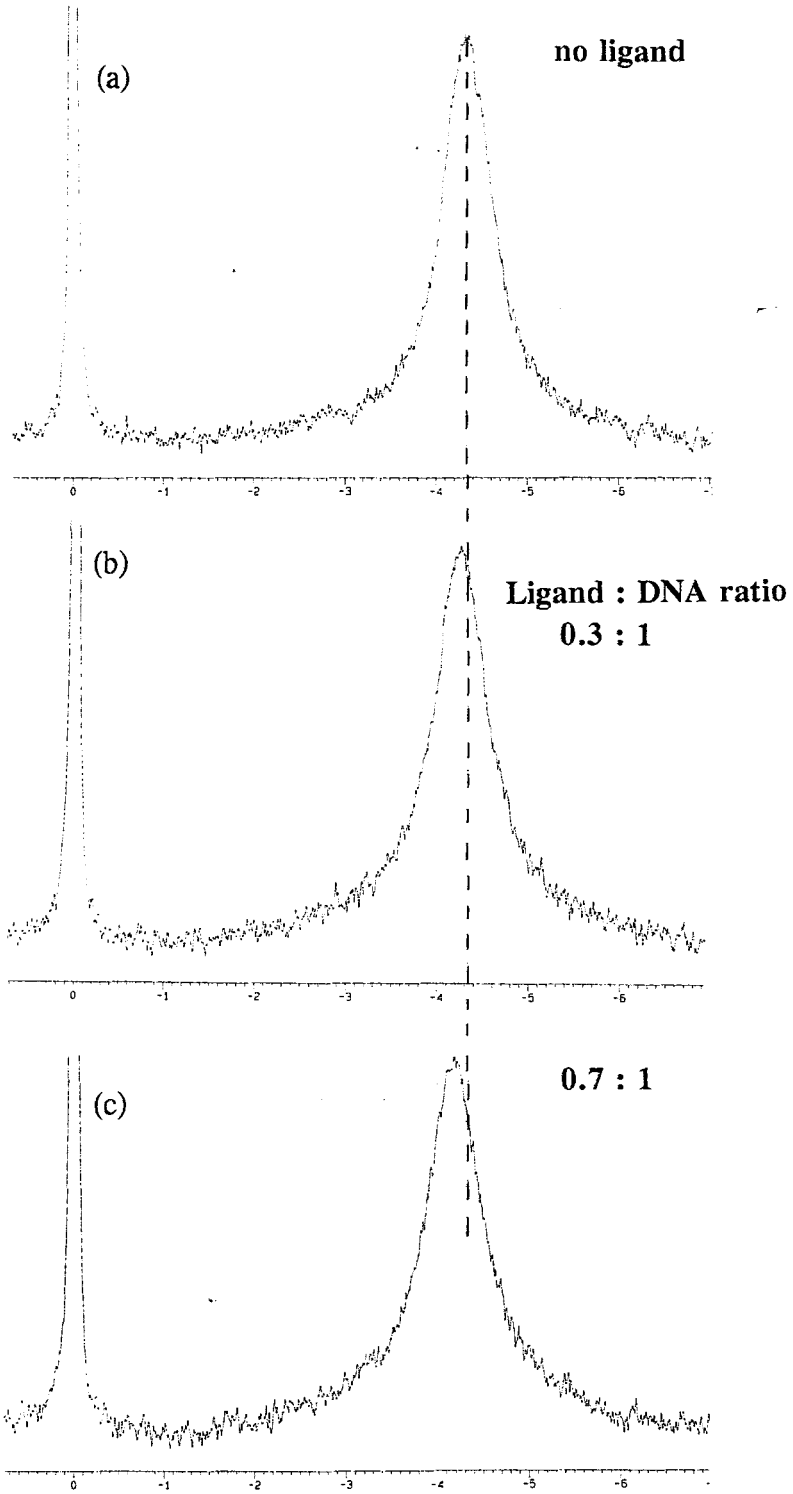


^{31}P NMR spectra of DNA : (a) before addition of ligand; and (b) and (c) after addition of increasing amounts of carboxamide **181**, a non-intercalator.

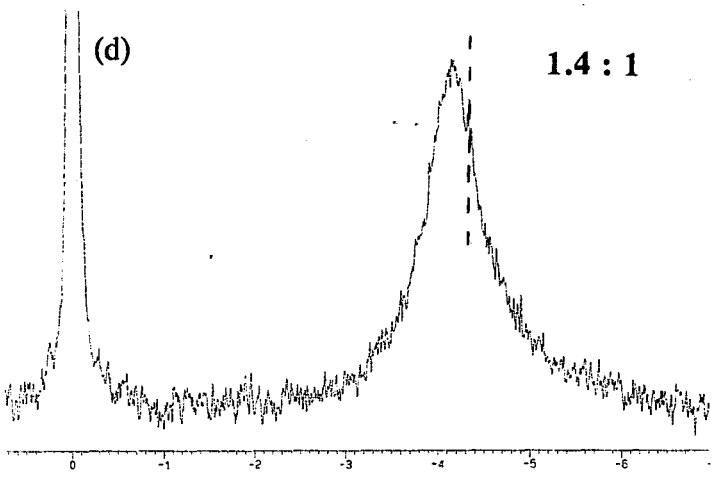


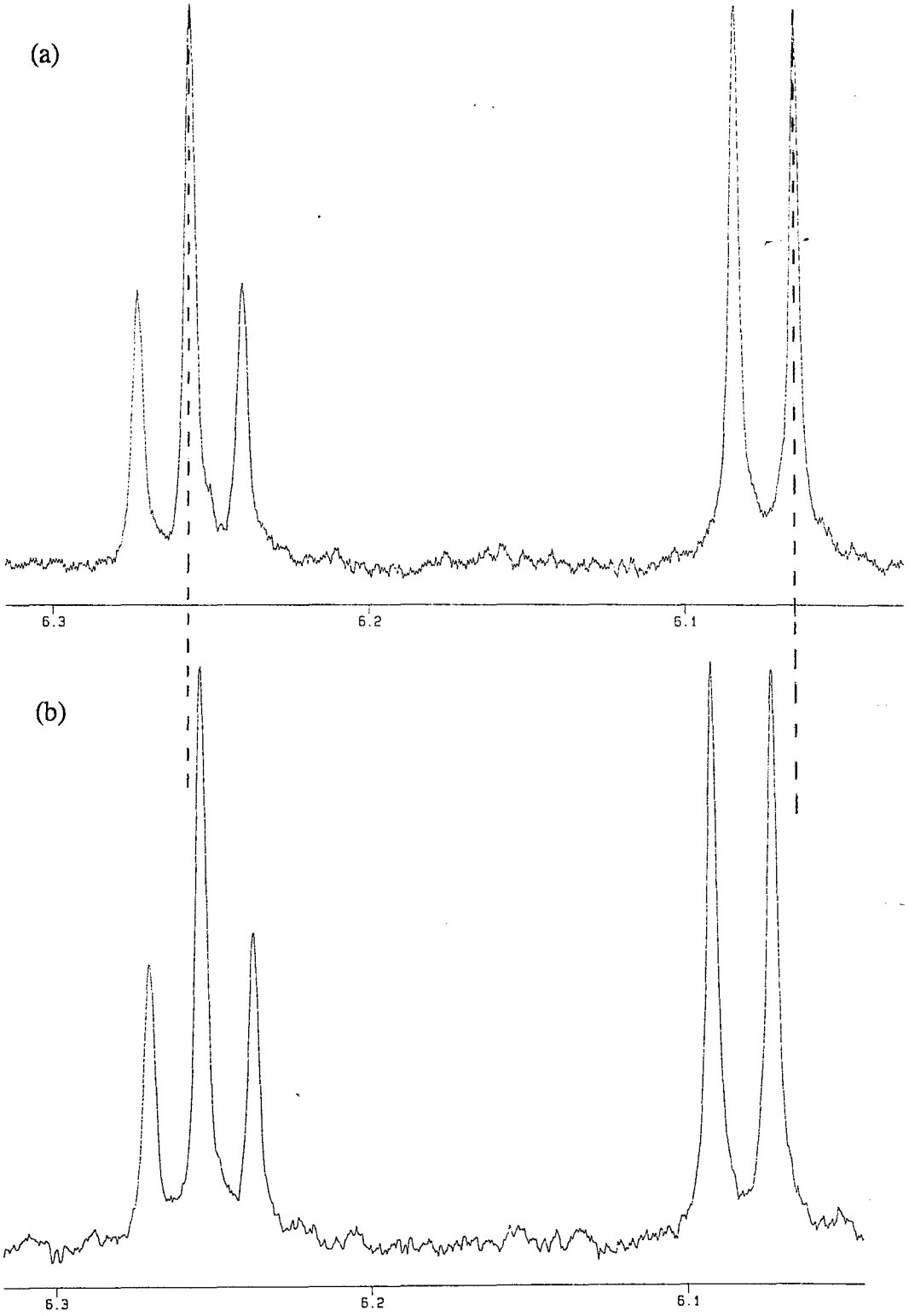
^{31}P NMR spectra of DNA : (a) before addition of ligand; and (b)-(d) after addition of increasing amounts of carboxamide **195**, an intercalator.


(d)
1.4 : 1
0
-1
-2
-3
-4
-5
-6

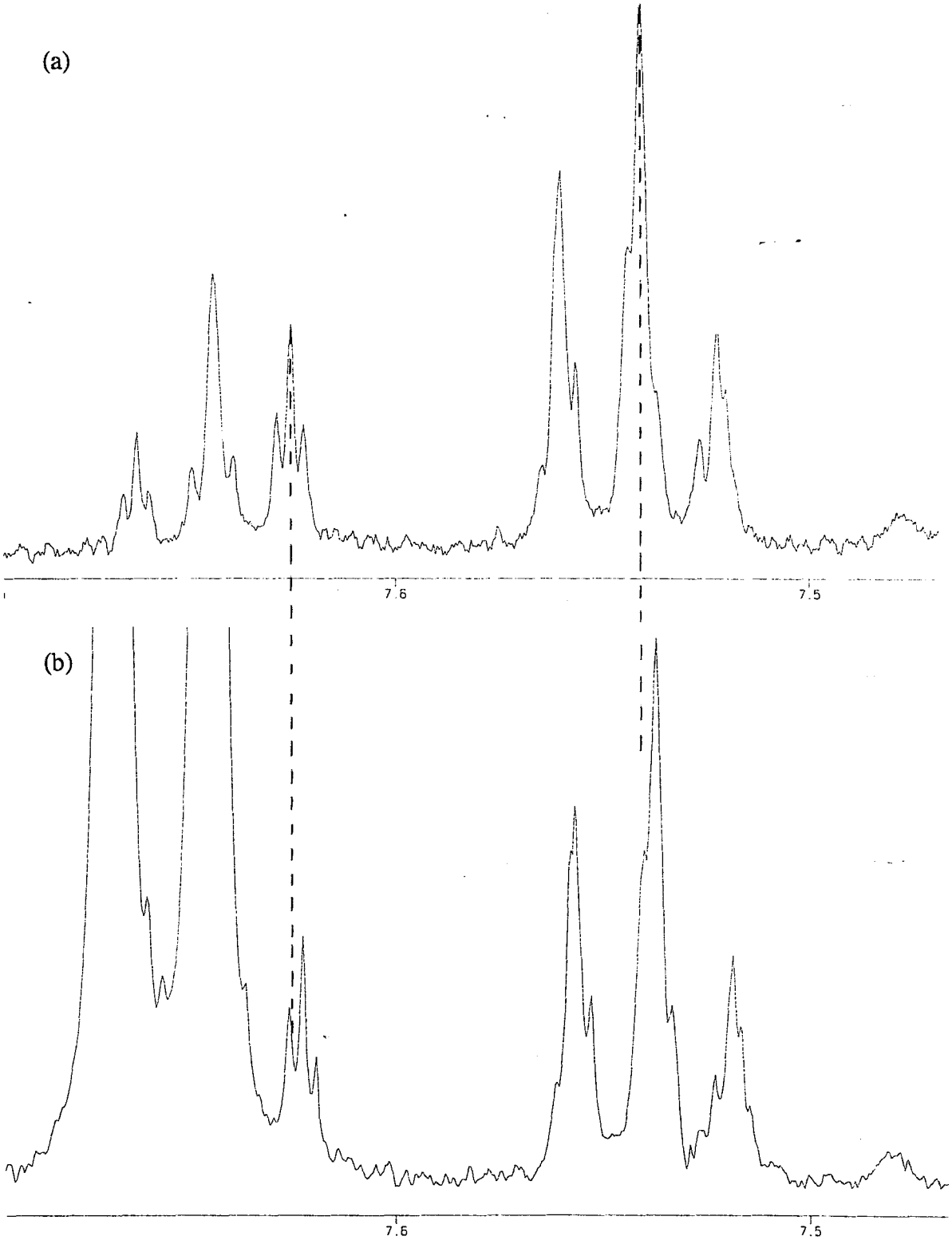


^{31}P NMR spectra of DNA : (a) before addition of ligand; and (b)-(d) after addition of increasing amounts of carboxamide **196**, an intercalator.

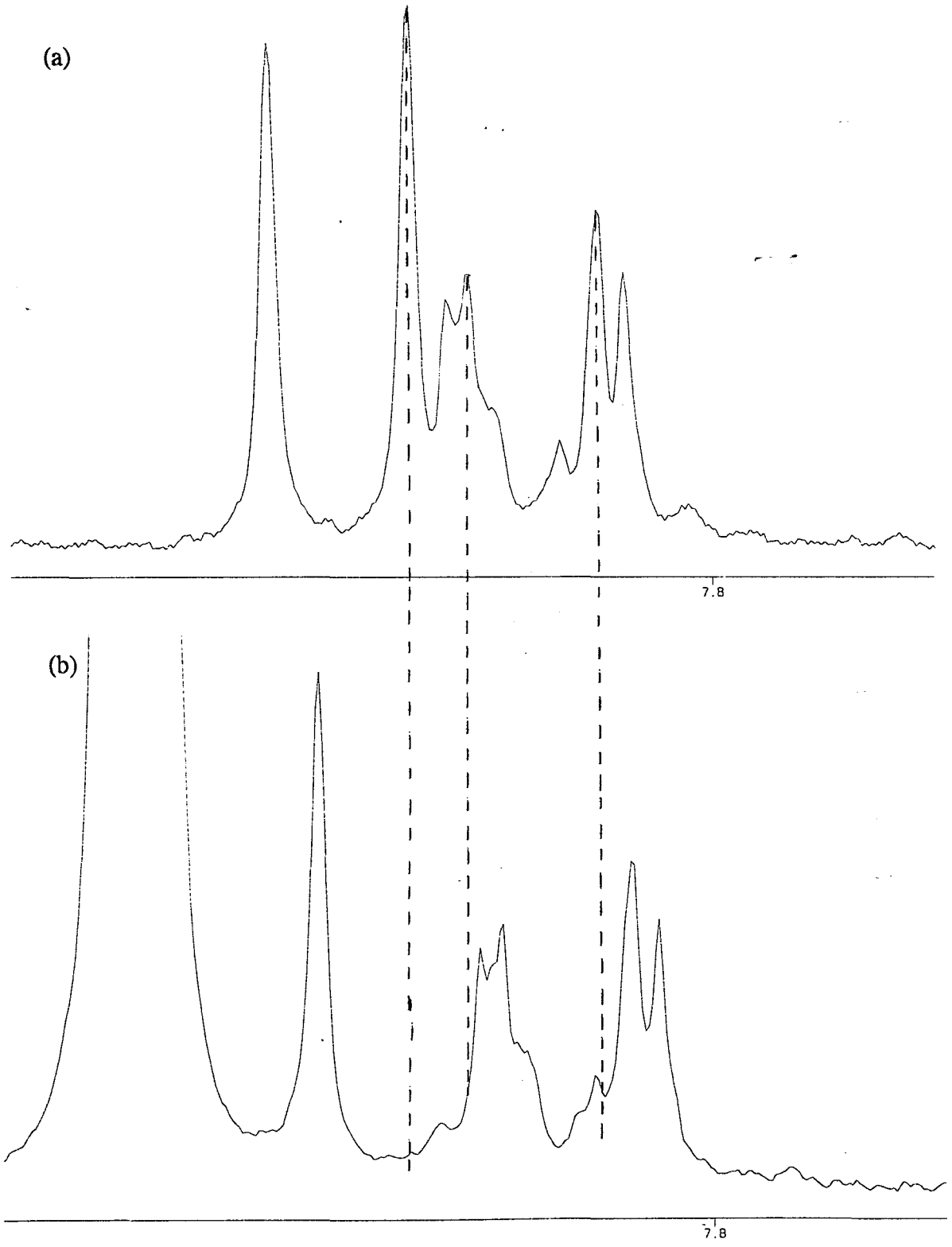

(d)
1.4 : 1
0
-1
-2
-3
-4
-5
-6



^{1}H NMR spectrum of the oligonucleotide showing a portion of the aromatic region : (a) before addition of ligand; and (b) after addition of chloroquine **126**, an intercalator.



^{1}H NMR spectrum of the oligonucleotide showing a portion of the aromatic region : (a) before addition of ligand; and (b) after addition of chloroquine **126**, an intercalator.



^{1}H NMR spectrum of the oligonucleotide showing a portion of the aromatic region : (a) before addition of ligand; and (b) after addition of chloroquine **126**, an intercalator.