Studies of the Effects of Biological Catalysts and Cyclodextrins on Chemical Transformations

A Thesis Submitted in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

By

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2001

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ACKNOWLEDGMENTS

The author wishes to express his sincere gratitude to Prof. Chris Easton for his supervision and guidance. Thanks also to Drs Greg Simpson and Paul Savage for their assistance during my PhD.

Gratitude is also expressed to my fellow Easton Group members, postgraduate students, post doctoral fellows and academics.

I would also like to take the opportunity to thank Dr Kitty Lee for her proof reading, Super Tech James Kelly for his assistance with HPLC and other things, Ms Connie Lee for providing some of the crystal structures that appear in this thesis and Dr Tony Willis for preparing the remaining crystal structures. Thanks also to Drs Richard Webster and Graham Heath for their assistance with the electrochemistry that appears in this thesis.

Special thanks go to my wife, Fiona, and my parents and outlaws for their love and support during this time.

This thesis is dedicated to their efforts on my behalf.

STATEMENT

This work contains no material that has been accepted for the award of any other degree or diploma in any university or other tertiary institution, and to the best of my knowledge, contains no material previously published or presented by another person, except where due reference has been made in the text.

I give my consent to this copy of my thesis, when deposited in the University Library, being available for loan or photocopying.

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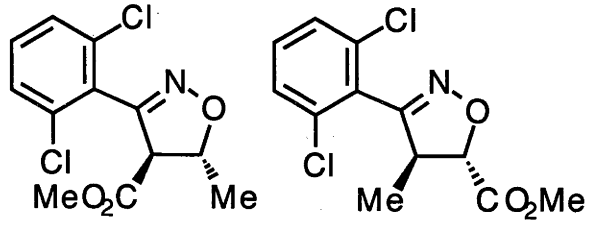
PUBLICATIONS

C. J. Easton, G. A. Heath, C. M. Hughes, C. K. Y. Lee, G. P. Savage, G. W. Simpson, E. R. T. Tiekink, G. J. Vuckovic, R. D. Webster; *J. Chem. Soc., Perkin Trans. 1*, 2001, 1168.

CORRIGENDUM

Page 35, Line 1	"Ramo Rao" should read "Rama Rao".
Page 59, Line 1	"rational" should read "rationale".
Page 70, Line 8	"(*R*)-2-phenoxypropionic (*R*)-(**46**)" should read "(*R*)-2-phenoxypropionic acid (*R*)-(**46**)".
Page 76, Line 6 and elsewhere	The correct nomenclature for the ester **47** is phenoxypropionate (**47**).
Page 78, Line 19	"sampled" should read "sample".
Page 84, Line 4	"pentet" should read "quintet".
Page 94, Lines 6-8	"pentet" should read "quintet".
Page 94, Scheme 64	Isoxazolines **99** and **101** should be drawn as shown below.



99 **101**

Page 97, Line 12	Last five words of this sentence should be deleted.
Page 105, Line 25	"former" should read "formed".
Page 115, Line 9	"it" should read its".
Page 119, Line 23	"enantioselectivity" should read "enantioselectively".

ABSTRACT

The preferential extraction of the isoxazolines **12**, **14** and **21** into chloroform from mixtures containing the isoxazolines **11** and **12**, **13** and **14**, and **20** and **21** in 20% aqueous ethanol solution in the presence of β-CD has been investigated. It was established that the isoxazolines **12** and **21** are more soluble in the aqueous ethanol solution than their regioisomers, the isoxazolines **11** and **20**, in the absence of β-CD. In the presence of β-CD, the solubility of the isoxazolines **11** and **20** increased significantly while the solubility of the isoxazoline **21** increased slightly and the solubility of the isoxazoline **12** did not increase. This suggests the isoxazolines **11** and **20** form more stable complexes with β-CD than do the isoxazolines **12** and **21**. An equilibrium between each isoxazoline and β-CD will exist in the 20% aqueous ethanol solution. The ratio of isoxazolines free in solution and available for extraction by chloroform will depend on their solubility in the aqueous ethanol solution and the selectivity of binding by the cyclodextrin. The isoxazolines **12** and **21** are less readily complexed by β-CD and are much more soluble in aqueous ethanol than the isoxazolines **11** and **20**, as a consequence the equilibria establish so that the ratio of the isoxazolines in each aqueous ethanol solution is enriched with the isoxazoline **12** or **21**. When the aqueous ethanol solution is extracted with chloroform, the isoxazolines quickly pass into the chloroform layer, in a ratio reflecting that in the aqueous ethanol, thus resulting in the preferential extraction of the isoxazolines **12** and **21**.

However, when the absolute solubility of each isoxazoline in aqueous ethanol is relatively high and the binding exhibited by the cyclodextrin is not selective, as is the case with the isoxazolines **22** and **23**, there is no preferential extraction of one isomer over the other.

Furthermore it was shown that when a mixture of the isoxazolines **13** and **14** is present in a solution containing β-CD, the isoxazoline **14** forms an insoluble complex with the cyclodextrin and precipitates out of solution. Therefore, the preferential extraction of the

isoxazoline **14** observed cannot proceed *via* the equilibrium suggested above but rather the isoxazoline **14** is extracted directly from the complex into chloroform.

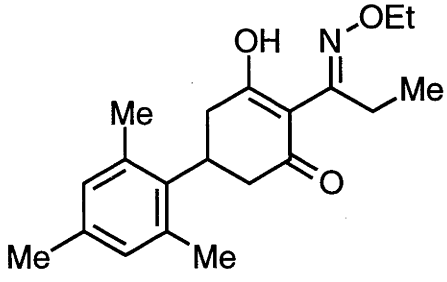
Whilst conducting experiments in which 20% aqueous ethanol solutions containing β-CD and a mixture of the isoxazolines **11** and **12** were extracted with chloroform, it was found that a white precipitate formed at the interface of the two phases. The precipitate was identified as the β-CD-chloroform complex. This observation has been developed into a convenient method for separating β-CD from mixtures of cyclodextrins. This separation technique relies on a difference in solubilities of the complexes formed between chloroform and α- and β-CD. The solubility of the α-CD-chloroform complex is 0.8g/100 ml, while the solubility of the β-CD-chloroform complex is 0.07g/100 ml. The organic solvent was introduced either by diffusing chloroform vapour over the aqueous solution in a desiccator or by direct addition of chloroform into the aqueous solution. In experiments containing different proportions and concentrations of α- and β-CD the percentage recovery of β-CD ranged from 16-86%.

It was shown that BSA does not catalyse the hydrolysis of aryloxypropionates in pH 11.5 phosphate buffer enantioselectively. Furthermore, it was shown that the addition of β-CD does not accelerate the rate of hydrolysis or increase the enantioselectivity observed. An investigation of this work has shown that the BSA binds preferentially to the (*S*)-enantiomers of the acids **46** and **48**. After the solutions are quenched with ethanol, the BSA/(*S*)-acid complexes precipitate and are removed through filtration, thus enriching the solutions with the (*R*)-enantiomers of the acids **46** and **48**. However, complexation between BSA and (*S*)-esters **45** and **47** was not discounted as an explanation for the enantioselectivity seen in the hydrolysis although this is difficult to probe as the esters **45** and **47** are readily hydrolysed in pH 11.5 phosphate buffer. These experiments discredit the suggestion of Kamal *et al.*[90] that BSA complexes with the esters **45** and **47** and catalyses the preferential hydrolysis of the (*R*)-enantiomers.

The biochemical ring opening of isoxazoles by yeast has been mimicked electrochemically with a variety of isoxazoles. In doing so a general electrochemical procedure has been developed for this process. On electrolysis, the 4-carbonyl-substituted isoxazoles **56** and **58**, the 4-methoxycarbonyl-substituted isoxazole **93** and the 4-cyano-substituted isoxazole **95**, undergo N-O bond cleavage to give the ring opened species in 61, 66, 56 and 74% yield, respectively. The 5-carbonyl-substituted isoxazoles **60** and **61**, the 5-methoxycarbonyl-substituted isoxazoles **92** and **98** and the 5-cyano-substituted isoxazole **94** have larger reduction potentials than their regioisomers and regioisomeric analogues and afforded multiple product mixtures on electrolysis. Both phenylisoxazoles **96** and **97** afforded multiple product mixtures on electrolysis. Therefore, it was shown that an electron-withdrawing and conjugating group, such as a carbonyl group, at C-4 on the isoxazole ring facilitates the cleavage of the N-O bond to give the corresponding imine in good yield. Isoxazoles with electron-withdrawing and conjugating groups at C-5 did undergo electrolysis but were more resistant to reduction and gave complex product mixtures. Isoxazoles with a non electron-withdrawing but conjugating substituent at either C-4 or C-5, such as a phenyl group, give multiple product mixtures, however in this case, the 5-substituted isoxazole is less resistant to electrochemical reduction. This behaviour under electrochemical conditions was rationalised by considering the structure of the starting materials and the electron distribution in the initial reduction products, the radical anions. The 4-substituted isoxazoles **56**, **58**, **93** and **95** were found to have longer N-O bonds and shorter adjacent C-O bonds and formed more stable radical anions on electrolysis than the corresponding 5-substituted regioisomers and regioisomeric analogues. This suggested the substituent on C-4 was conjugated with the ring oxygen. This conjugation allows electron flow from the radical anion, through the isoxazole ring to the N-O bond which then undergoes homolysis to give the ring opened imine. A similar ring opening mechanism is impossible for the 5-substituted isoxazoles.

INTRODUCTION

In the early 1980's ICI research developed a new herbicide for use in wheat and barley. This herbicide was marketed in Australia and overseas under the tradename of GRASP **1**.[1]



1

GRASP **1** created much interest in the agricultural chemicals industry as it exhibited low acute toxicity to birds, fish and earthworms.[1] It was also found to be non-mutagenic and non-teratogenic in rats and rabbits.[1] GRASP **1** is synthesised industrially *via* a five step process with an overall yield of 44% and requires the presence of an aldehyde moiety with a bulky unreactive group as an initial synthetic building block.[1] The synthetic strategy is somewhat restrictive as it limits the preparation of structural analogues *via* this process.

The ongoing success of GRASP **1** and the inflexibility of its synthesis prompted CSIRO Molecular Science to undertake a pilot project which would investigate the production of structural analogues of GRASP **1** centring around the β-enamino ketone moiety. It was thought that this could be performed by exploring nitrile oxide cycloaddition chemistry. The preparation of bicyclic isoxazoles was of particular interest. Bicyclic isoxazoles are prepared by the oxidation of isoxazolines and the β-enamino ketones could then be prepared *via* reductive ring opening. Further functionalisation of the β-enamino ketones would lead to structural analogues of GRASP **1**.

The initial aim of the research presented here was to investigate work published concerning the effect of β-cyclodextrin (β-CD) on the regioselectivity of nitrile oxide cycloadditions with a view to applying such methods to the synthesis of crop protection chemicals.

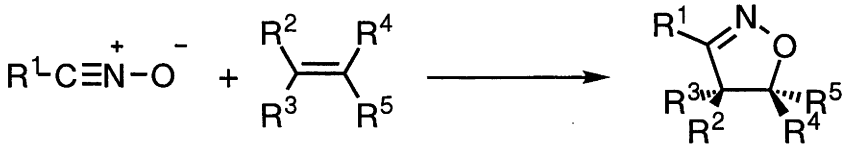
1.1 Nitrile oxide cycloaddition chemistry

1.1.1 Introduction

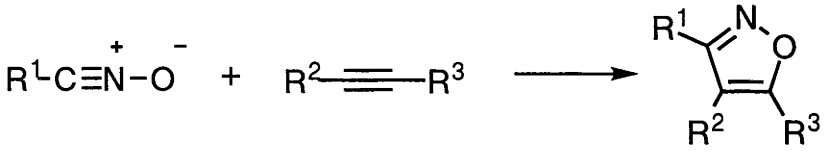
Nitrile oxide cycloadditions resulting in the formation of Δ^2-isoxazolines and isoxazoles have attracted much interest since the pioneering work of Werner and Buss[2] in 1894, Wieland[3,4] in 1907 and Quilico *et al.*[5,6] in 1950. These processes were characterised by Huisgen[7,8,9] as members of the broad class of [3+2] cycloadditions. The intense interest in this area of research has led to a variety of reviews[10-18] being published.

1.1.2 Synthesis

Isoxazolines are easily prepared *via* 1,3-dipolar cycloaddition reactions between nitrile oxides and substituted alkenes (Scheme 1). Similarly, isoxazoles are prepared *via* cycloadditions between nitrile oxides and alkynes (Scheme 2).



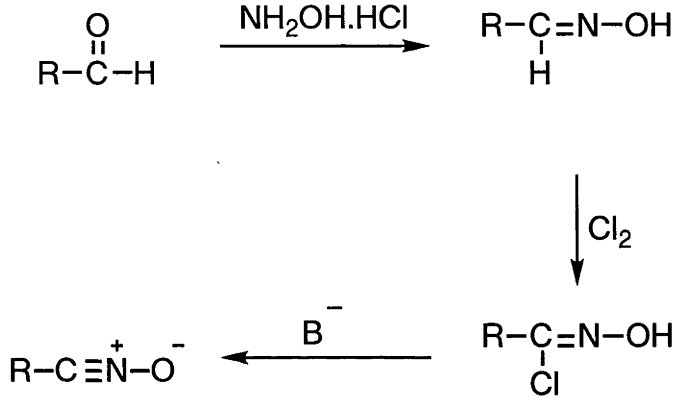
Scheme 1



Scheme 2

There are several methods for the synthesis of nitrile oxides reported in the literature.[10-18] The most common *in situ* generation method for the synthesis of nitrile oxides was established by Werner and Buss[2] in 1894. This method involves the chlorination of a suitably substituted aldoxime followed by the dehydrohalogenation of the resulting

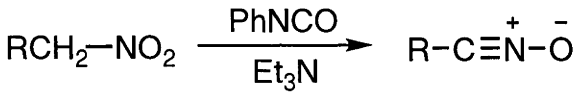
hydroximinoyl chloride using base (Scheme 3). The chlorination of aldoximes has been achieved by a variety of chlorinating agents such as chlorine,[19] nitrosyl chloride,[20] *N*-chlorosuccinimide,[21] hypochlorite,[22,23] chloroamine-T (*N*-chloro-*N*-sodio-4-methylbenzenesulfonamide),[24] 1-chlorobenzotriazole,[25] iodobenzene dichloride,[26] and hydrogen chloride in DMF/OXONE.[27]



Scheme 3

In their pioneering work Werner and Buss[2] used sodium carbonate as the base to facilitate the dehydrohalogenation reaction. In more recent times tertiary amines, particularly triethylamine, have been used in place of carbonate.[28-30] The dehydrohalogenation reaction has also been promoted by aluminium oxide,[31] florisil,[31] molecular sieves,[32] hexabutylditin,[33] bis(tributyltin)oxide,[34] tetraphenyltin,[34] tributyltin hydride,[35] and alkali metal fluorides.[36] Nitrile oxides have been synthesised *via* the hydroximinoyl bromide using brominating agents such as hypobromite,[37] sodium bromite with a catalytic amount of tributyltin chloride,[38] and *N*-bromosuccinimide.[39] Other variations have included the thermal dehydrohalogenation of the hydroximinoyl halide[40-42] and thermolysis has been employed to generate the nitrile oxide from *O*-ethoxycarbonylaldoxime.[43] Nitrile oxides have also been obtained from the corresponding aldoxime through electrolysis in the presence of sodium chloride[44,45] and by oxidation with dimethyl dioxirane[46] or mercuric acetate.[47]

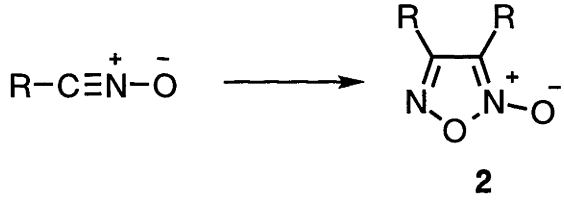
Another popular *in situ* nitrile oxide generation method is the dehydration of primary nitroalkanes using phenyl isocyanate and a catalytic amount of triethylamine (Scheme 4). This reaction is known as the Mukaiyama method.[48]



Scheme 4

A variety of dehydrating agents have been used in place of phenyl isocyanate; these include phosphorus oxychloride,[49] chloroformate esters,[50] aryl[50,51] and alkyl sulfonyl chlorides,[52] and acetic acid and anhydride.[52] It is often advantageous to use the Mukaiyama method for the preparation of nitrile oxides from substrates such as sulfides because of their tendency to be oxidised.[53]

Nitrile oxides will readily self-condense to form furoxans **2** (Scheme 5). This tendency to form dimers is greatly reduced by generating the nitrile oxide *in situ*.[8]



2

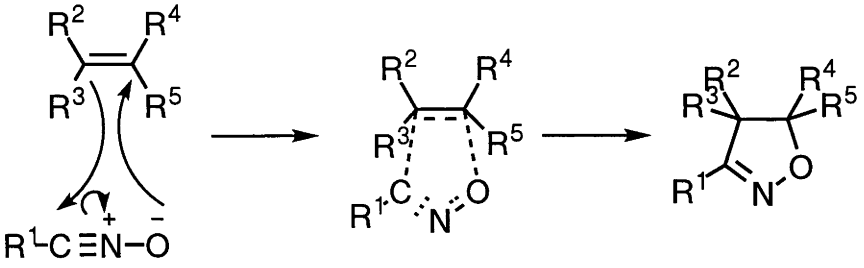
Scheme 5

Aryl nitrile oxides are more resistant to dimerisation than aliphatic and acyl nitrile oxides and usually have half lives of several hours. Dimerisation of aryl nitrile oxides is impeded by bulky groups at the 2- and 6-positions and by electron-donating groups.[37] For example, aryl nitrile oxides such as 2,6-dichloro- and 2,4,6-trimethylbenzonitrile oxide are sufficiently stable to be stored.[11]

1.1.3 Mechanism

The reactions between nitrile oxides and alkenes and alkynes are 1,3-dipolar cycloadditions and their mechanism has been the subject of numerous investigations. Several mechanisms have been proposed for the cycloaddition reaction. It is thought that the reaction proceeds *via* a one-step concerted process[54,55] (as shown in Scheme 6 for

alkenes) but other investigations have suggested that a stepwise mechanism *via* a zwitterionic intermediate **3** or *via* a diradical **4** may also be possible.



Scheme 6

3 **4**

Although there is no direct proof for any of these mechanistic possibilities, there is considerable evidence to suggest that the cyclisation is concerted. The configuration of the alkene is retained in the cycloadduct[54,55] and reaction thermodynamics exhibit moderate enthalpy of activation and strongly negative entropy of activation, as expected for a concerted process.

1.1.4 Reactivity

Reactivity of nitrile oxide cycloadditions varies greatly and rates of reaction range over several orders of magnitude. Cycloaddition reactivities have been rationalised on the basis of the Sustmann frontier molecular orbital (FMO) theory.[56-65] According to Sustmann, cycloadditions can be divided into the following three categories (Figure 1):

Type I: The cycloaddition involves interaction between the lowest unoccupied molecular orbital (LUMO) of the alkene or alkyne (herein-after referred to as the dipolarophile) and the highest occupied molecular orbital (HOMO) of the nitrile oxide.

Type II: The cycloaddition involves both interaction between the LUMO of the dipolarophile and the HOMO of the nitrile oxide and between the HOMO of the dipolarophile and the LUMO of the nitrile oxide.

Type III: The cycloaddition involves interaction between the HOMO of the dipolarophile and the LUMO of the nitrile oxide.

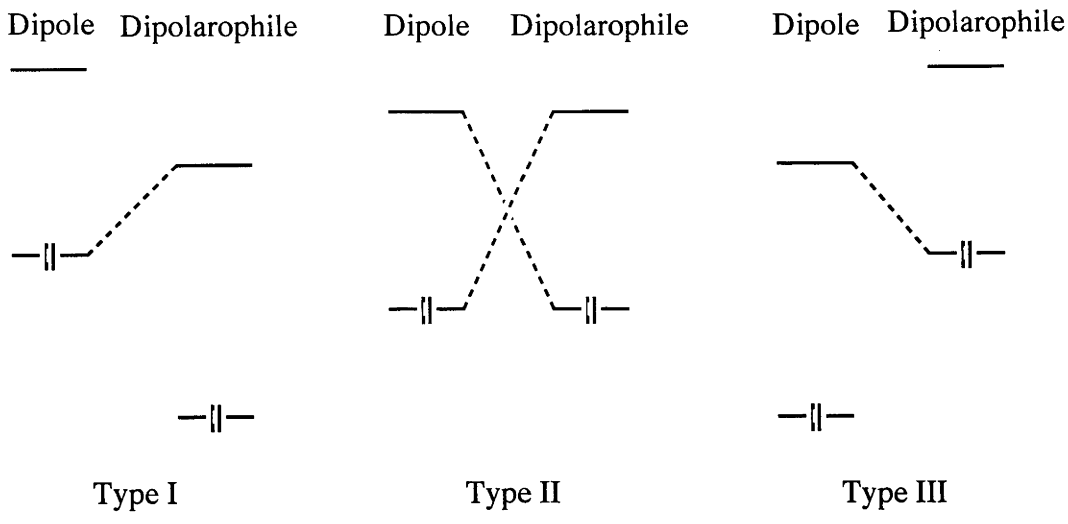


Figure 1: Sustmann classification of the FMOs for the interaction of dipolarophiles and nitrile oxides.

In each of the three categories, the reactivity is inversely proportional to the energy difference between the interacting orbitals.[66,67] Electron-donating substituents increase the FMO energies of the dipolarophile, decreasing the reactivity in Type I systems and increasing the reactivity in Type III systems. Electron-withdrawing substituents lower the dipolarophile's FMO energies thus increasing the reactivity of Type I systems and decreasing the reactivity of Type III systems. In Type II systems, the effect of the dipolarophile's substituents depends on which orbital interaction becomes dominant by substitution. With substituents of different types, each moderates the effect of the other. An increase in reactivity of Type I, Type II and Type III systems results from conjugating substituents on the dipolarophile because substituents of this type raise the dipolarophile's HOMO and lower its LUMO. Consequently, the reactivity of a dipolarophile is increased by the addition of a carbonyl group. The effect of substituents on the nitrile oxide can be rationalised in a similar way. Electron-withdrawing substituents increase the reactivity of Type III systems while electron-donating substituents increase the reactivity of Type I systems. Type III cycloaddition is therefore favoured with benzenesulfonyl and acyl nitrile oxides. Although simplistic, the relative ease with which nitrile oxides dimerize

has been used as a comparative standard to compare the reactivity of dipolarophiles.[58, 68,69]

The steric effect of an alkyl substituent on a dipolarophile serves to decrease reactivity. A conjugating substituent will have a rate enhancing effect which is greater than the retarding steric effect. The reactivity of disubstituted dipolarophiles is generally lower than that of mono-substituted dipolarophiles although the electronic effects of the substituents may affect reactivity. *cis*-Alkenes tend to be less reactive than the corresponding *trans*-isomers because there is then greater steric compression during a cycloaddition. Tri-substituted alkenes are even less reactive.

1.1.5 Regioselectivity

In cycloaddition reactions between nitrile oxides and unsymmetrical dipolarophiles the regioselectivity must be considered. Reactions involving monosubstituted dipolarophiles will give the 5-substituted regioisomer almost exclusively.[70] In most cases cycloadditions involving 1,1-disubstituted and trisubstituted alkenes the oxygen of the nitrile oxide will become attached to the more sterically hindered end of the double bond.[59,71] The regioselectivity of nitrile oxide cycloaddition reactions is governed by electronic and steric effects. In practice steric effects generally predominate over electronic effects, but with 1,2-disubstituted dipolarophiles a mixture of regioisomers is usually obtained. The isomer that is favoured electronically will be the predominant isomer. For example, electron withdrawing substitutents such as carbonyl groups on the dipolarophile are known to direct the cycloaddition reaction such that the carbonyl group is located at the 4-position of the cycloadduct ring.[72-78] Alternatively, electron donating groups such as alkoxy groups promote the formation of a cycloadduct with the substituent in the 5-position of the ring.[79]

1.2 Effect of β-CD on the regioselectivity of nitrile oxide cycloadditions

The control of regioselectivity has generated much interest in nitrile oxide cycloaddition chemistry.[18] For example, cyclodextrins have been used to influence the outcome of such reactions.

β-CD is a cyclic oligomer consisting of seven α-D-glucopyranose units and is a cup shaped molecule as a result of which it is often represented as a truncated cone (Figure 2).[80,81] Cyclodextrins contain a hydrophobic cavity[81] and are known to form inclusion complexes with a wide variety of hydrophobic molecules.[82] This characteristic has made cyclodextrins popular models for the study of enzymatic systems.

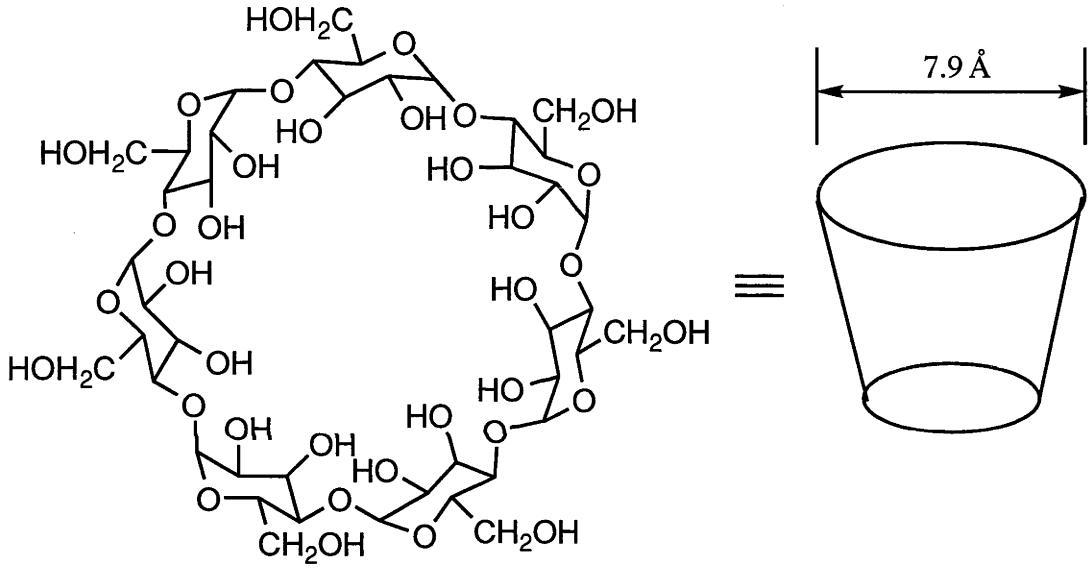
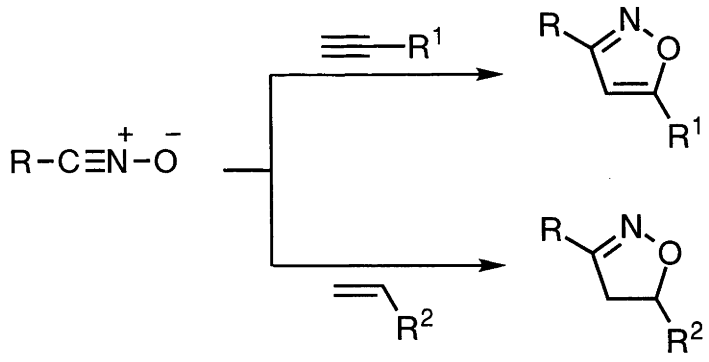


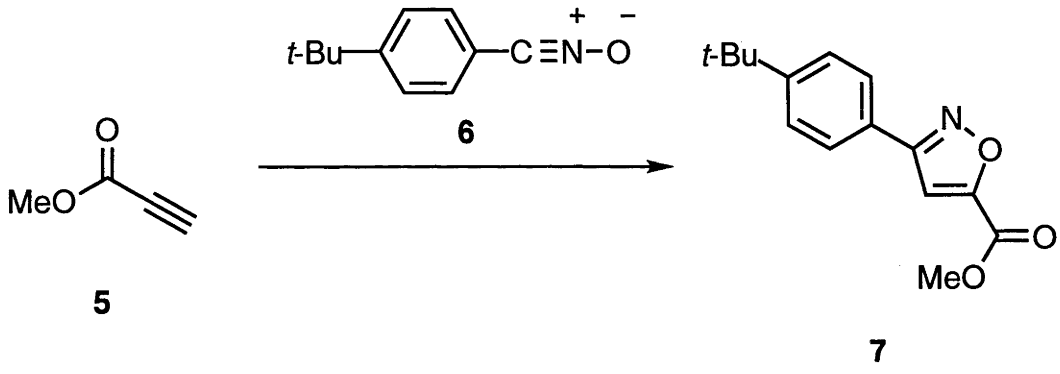
Figure 2: β-CD

Meyer *et al.*[83] have reported the use of β-CD to reverse the regioselectivity of nitrile oxide cycloadditions. As discussed earlier, nitrile oxides undergo cycloadditions with monosubstituted alkenes and alkynes to give 3,5-disubstituted isoxazolines and 3,5-disubstituted isoxazoles, respectively (Scheme 7).



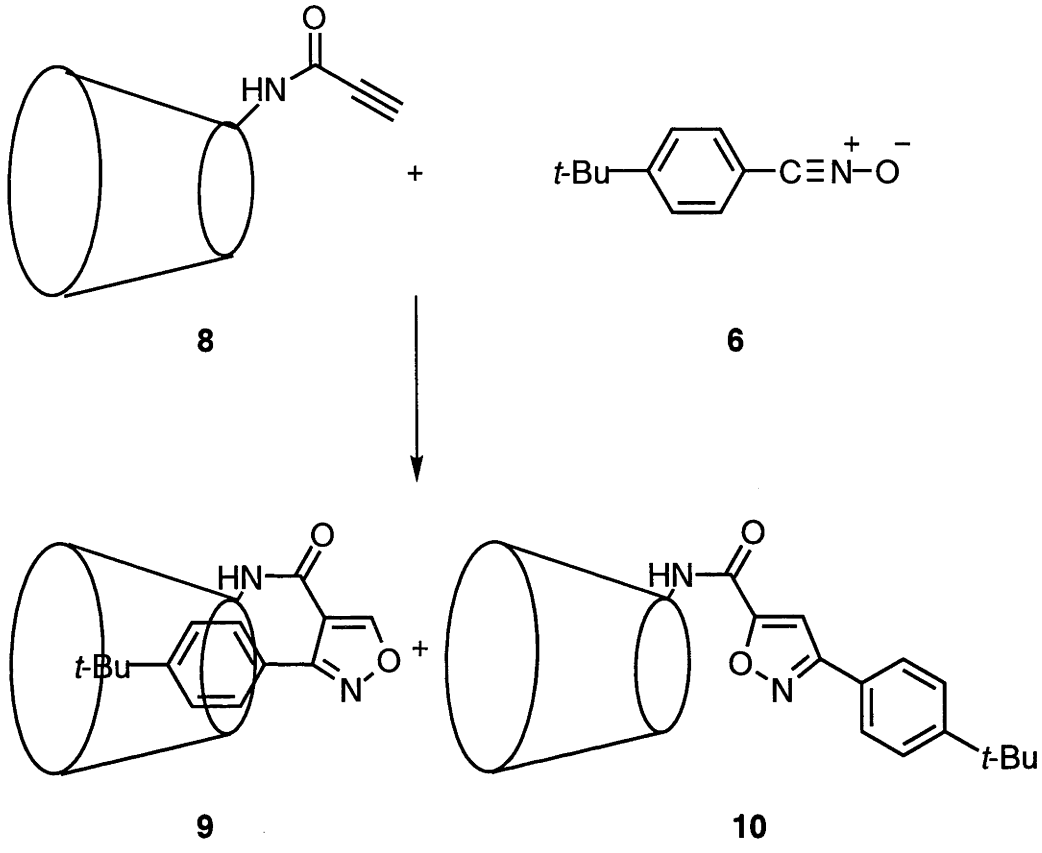
Scheme 7

For example, the cycloaddition between the nitrile oxide **6** and methyl propynoate (**5**) gives the 5-substituted regioisomer **7**, exclusively (Scheme 8).



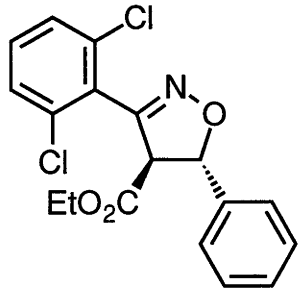
Scheme 8

Meyer *et al.*[83] reported tethering various dipolarophiles to β-CD and reacting the resulting modified cyclodextrins with a variety of aryl nitrile oxides in water. For example in an analogous reaction to that shown in Scheme 7, the cycloaddition between the nitrile oxide **6** and the modified cyclodextrin **8** results in the formation of the isoxazoles **9** and **10** in a ratio of 15:1 (Scheme 9). Therefore, the cyclodextrin affects a reversal of the expected regioselectivity in the cycloaddition reaction. The reversal of regioselectivity is achieved because the nitrile oxide includes within the hydrophobic cavity of the cyclodextrin thus fixing its orientation with respect to the dipolarophile and promoting the formation of the 3,4-disubstituted isoxazole. The small amount of the isoxazole **10** present probably results from the reaction between compound **8** and uncomplexed nitrile oxide **6**.

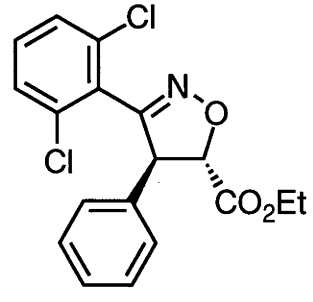


Scheme 9

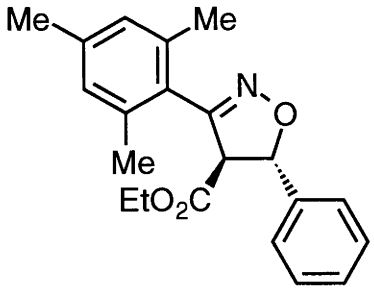
In the early 1990's another series of papers[84-88] was published concerning the influence of β-CD on nitrile oxide cycloadditions. These papers detailed 1,3-dipolar cycloadditions between cinnamic esters and a variety of nitrile oxides in the presence of bakers' yeast and β-CD in aqueous ethanol. Specifically, the compounds discussed were the isoxazolines **11**, **12**, **13** and **14**.



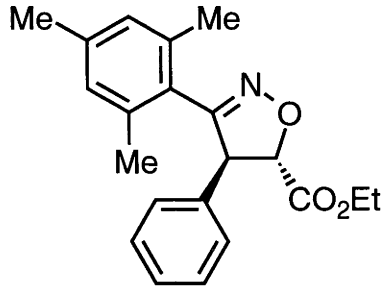
(±)-**11**



(±)-**12**

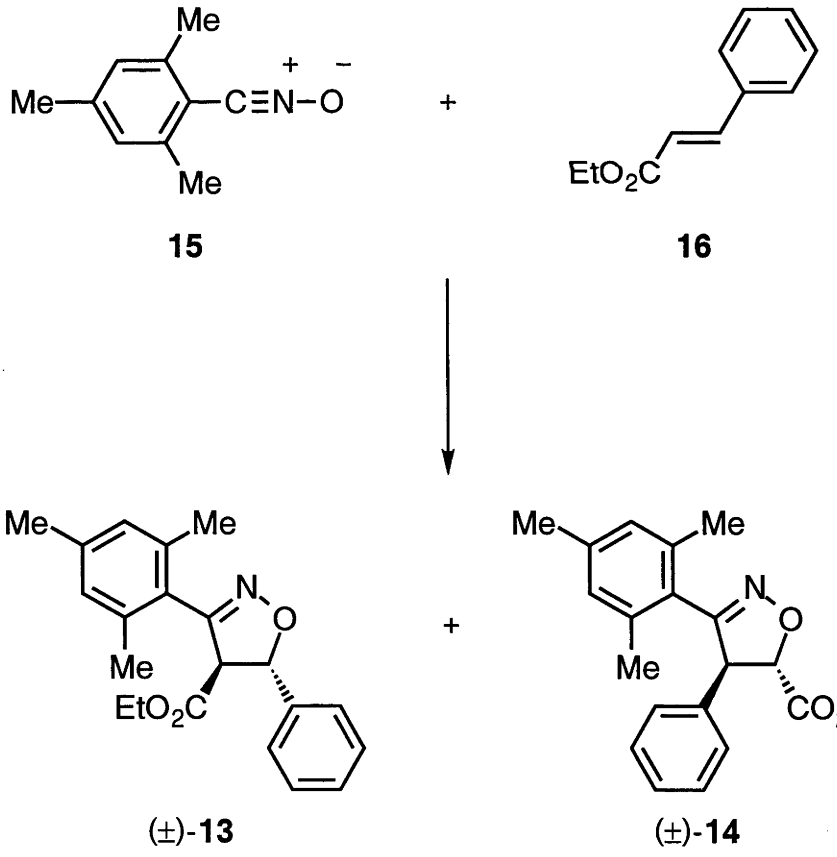


(±)-**13**



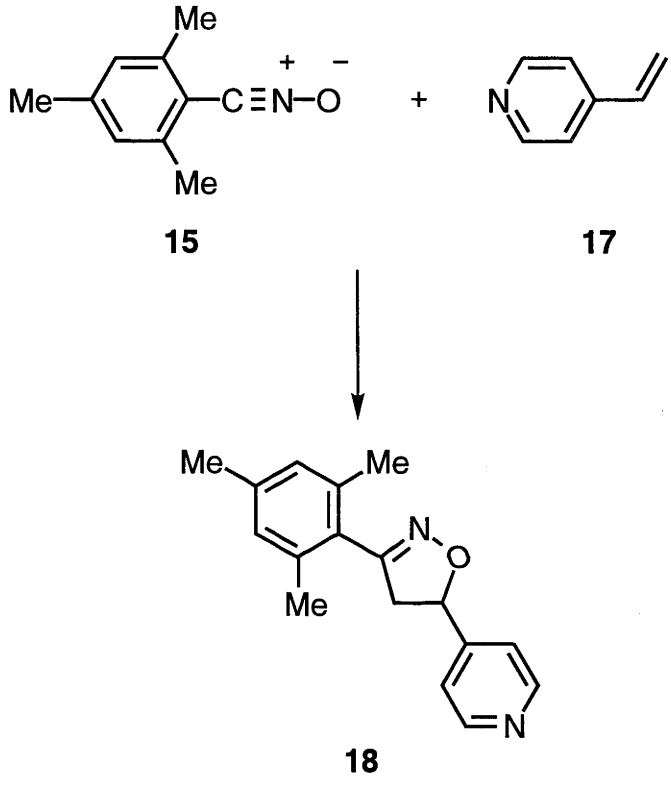
(±)-**14**

Rama Rao *et al.*[84-88] reported that the reaction between ethyl cinnamate (**16**) and 2,4,6-trimethylbenzonitrile oxide (**15**) in the presence of bakers' yeast produced a mixture of the isoxazolines **13** and **14** in the ratio 65:35 (Scheme 10). Bakers' yeast was required to facilitate the cycloaddition and the addition of β-CD altered the regioselectivity of the reaction. When the reaction was repeated with the addition of a molar equivalent of β-CD, the isoxazoline **14** was obtained exclusively.



Scheme 10

Rama Rao *et al.* did not propose a mechanism for the interaction between bakers' yeast and β-CD with ethyl cinnamate (**16**) and 2,4,6-trimethylbenzonitrile oxide (**15**). However, in a subsequent paper[85] a mechanism was proposed for the reaction between 4-vinylpyridine (**17**) and 2,4,6-trimethylbenzonitrile oxide (**15**) in the presence of bakers' yeast and β-CD (Scheme 11) with the implication that the cycloaddition between ethyl cinnamate (**16**) and 2,4,6-trimethylbenzonitrile oxide (**15**) occurs in a similar fashion.



Scheme 11

Accordingly, the aryl moiety of the nitrile oxide **15** is bound to the active site of an enzyme from the bakers' yeast. Likewise the pyridine moiety of 4-vinylpyridine (**17**) is included within the hydrophobic cavity of the cyclodextrin (Figure 3). The authors suggested that the binding of the two reactants in such a way fixes the geometry and thus promotes the exclusive formation of the isoxazoline **18**.

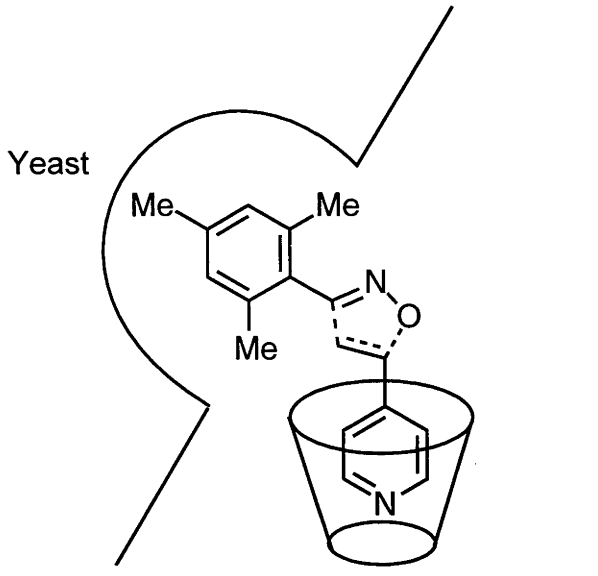


Figure 3: Proposed mechanism for the interaction of β-CD and bakers' yeast during the formation of the isoxazoline **18**.

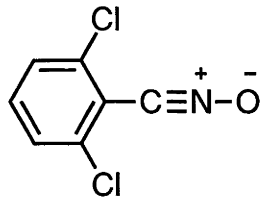
As mentioned earlier, the regioselectivity of nitrile oxide cycloadditions involving mono-substituted alkenes is governed by steric effects such that the 5-substituted regioisomer is formed almost exclusively.[70] Therefore, on that basis the isoxazoline **18** is expected to form exclusively regardless of any influence exerted by bakers' yeast or β-CD on the cycloaddition. Similarly, the isoxazoline **13** is expected to be the predominant regioisomer on electronic grounds from the cycloaddition between ethyl cinnamate (**16**) and the nitrile oxide **15**.[72-78] Furthermore, it seemed unlikely that the reagents, once included within the cavities of the yeast and cyclodextrin, would come together in a way that is conducive to cycloaddition. Therefore, it seemed unlikely that bakers' yeast or β-CD was influencing the cycloadditions in the way suggested by Rama Rao *et al.* This prompted a re-investigation of the claims made by Rama Rao *et al.*

Hughes *et al.*[89] compared the ratios of regioisomers resulting from the cycloaddition between the cinnamate **16** and the nitrile oxide **15** in the presence of bakers' yeast, in the presence of bakers' yeast and β-CD, and in the absence of both bakers' yeast and β-CD. The results obtained are presented in Table 1.

	Bakers' Yeast, No β-CD	Bakers' Yeast, with β-CD	No Bakers' Yeast or β-CD
Ratio of **13**:**14**	59:41	60:40	61:39

Table 1: Ratios of regioisomers **13** and **14** from cycloadditions containing bakers' yeast and no β-CD, bakers' yeast and β-CD and no bakers' yeast or β-CD.

A similar experiment was conducted for the cycloaddition between 2,6-dichlorobenzonitrile oxide (**19**) and ethyl cinnamate (**16**) to produce the isoxazolines **11** and **12**. The results obtained are presented in Table 2.



19

	Bakers' Yeast, No β-CD	Bakers' Yeast, with β-CD	No Bakers' Yeast or β-CD
Ratio of **11**:**12**	94:6	97:3	87:13

Table 2: Ratios of regioisomers **11** and **12** from cycloadditions containing bakers' yeast and no β-CD, bakers' yeast and β-CD and no bakers' yeast or β-CD.

These initial results prompted the following conclusions. Contrary to Rama Rao's claims, the presence of bakers' yeast is not required for the cycloaddition reactions to proceed in good yield. Furthermore, the presence or absence of β-CD has little or no effect on the ratio of regioisomers produced.

Hughes *et al.* suggested that, given the aromatic nature of the compounds, the change in regioisomer ratios in the presence and absence of β-CD observed by Rama Rao *et al.* might result from selective product complexation rather than the cyclodextrin influencing the regioselectivity of the cycloaddition. Rama Rao *et al.* conducted these cycloadditions

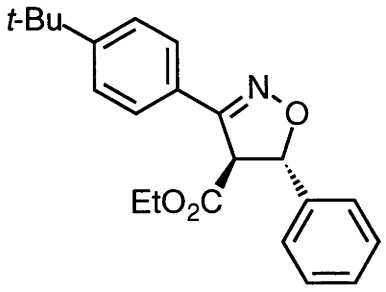
in aqueous ethanol solutions which were extracted with chloroform to isolate the mixture of isoxazolines produced. If β-CD had a greater affinity for one regioisomer, that compound would be retained in solution and its extraction into chloroform would be retarded. If during the extraction one regioisomer moved freely into the chloroform layer while the passage of the other regioisomer was retarded, the ratio of regioisomers extracted would be altered. For the above hypothesis to hold, additional extractions of the aqueous ethanol solution with organic solvent would be progressively enriched with the regioisomer for which the cyclodextrin had the greater affinity. Hughes *et al.*[89] prepared an aqueous ethanol solution containing β-CD and a known ratio of the isoxazolines **13** and **14**. The solution was extracted initially with chloroform, then three times with ethyl acetate and the ratios of regioisomers in each extraction were determined by ^{1}H nmr spectroscopy. The results obtained are presented in Table 3.

	Ratio of isomers **13**:**14**
Initial regioisomeric ratio	47:53
Chloroform extraction	21:79
1st Ethyl acetate extraction	25:75
2nd Ethyl acetate extraction	77:33
3rd Ethyl acetate extraction	100:0

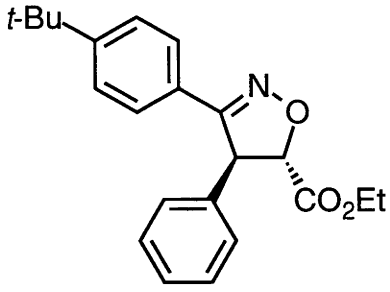
Table 3: Ratios of the isoxazolines **13** and **14** obtained from each solvent extraction of an aqueous ethanol solution initially containing a 47:53 mixture.

There was enrichment of the isoxazoline **13** with each subsequent solvent extraction until only compound **13** was obtained in the final extract. These results suggested that β-CD was retarding the extraction of the isoxazoline **13**, presumably by the formation of an inclusion complex.

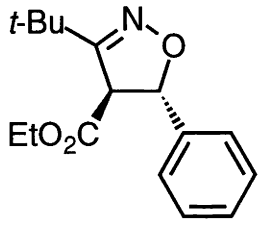
To test the generality of this extraction effect, additional isoxazolines containing alkyl and aryl substituents at C-3 on the isoxazoline ring were synthesised. The compounds prepared were the isoxazolines **20**, **21**, **22** and **23**.



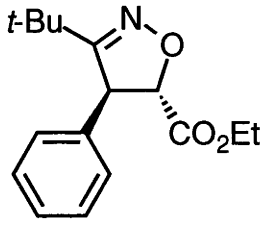
(±)-**20**



(±)-**21**



(±)-**22**



(±)-**23**

Hughes *et al.* incubated the isoxazolines **11** and **12**, **13** and **14**, **20** and **21**, and **22** and **23** in aqueous ethanol in the presence of β-CD under Rama Rao's reaction conditions. The mixtures were then extracted twice with chloroform and the ratios of regioisomers in the extracts were determined by ^{1}H nmr spectroscopy.

Isomers	Initial Ratio#	Extraction Ratio*
11 and **12**	87:13	80:20
13 and **14**	47:53	21:79
20 and **21**	71:29	37:63
22 and **23**	48:52	48:52

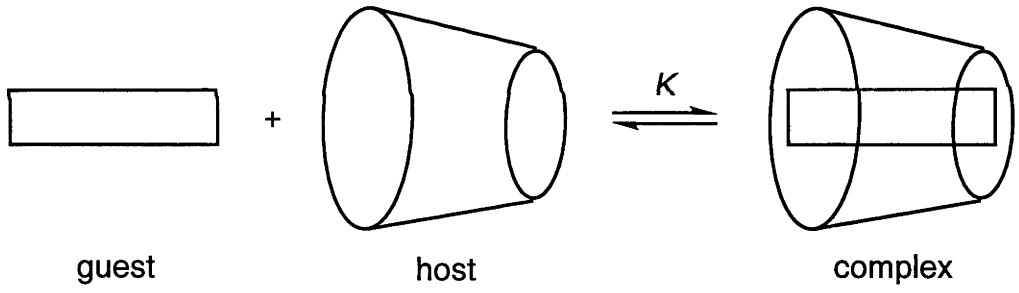
Table 4: Initial and final ratios of the isoxazolines **11** and **12**, **13** and **14**, **20** and **21**, and **22** and **23** from the extraction experiments in aqueous ethanol in the presence of β-CD under Rama Rao's reaction conditions.
Ratio of isoxazolines present in the aqueous ethanol solution prior to extraction with chloroform. * Ratio of isoxazolines present in the combined organic fractions after extraction of the aqueous ethanol solution with chloroform.

The results obtained clearly showed that the isoxazolines **12**, **14** and **21** are preferentially extracted while there was no difference between the initial and final ratios for the isomers

22 and **23** (Table 4). Therefore, these results confirm that β-CD is influencing the ratio of regioisomers extracted and not the ratio of regioisomers produced during the cycloaddition.

The aim of this area of work was now to determine how β-CD was influencing the ratio of regioisomeric isoxazolines extracted from these cycloadditions. Hughes *et al.* had suggested the effect observed by Rama Rao *et al.* might be due to selective binding between one regioisomer in each isoxazoline pair and β-CD. Therefore, to determine the validity of this hypothesis, it was necessary to determine the extent of complexation between the isoxazolines **11**, **12**, **13**, **14**, **20**, **21**, **22** and **23** and β-CD in aqueous ethanol.

The extent to which a host binds a guest to form a complex can be expressed as an association constant or binding constant, *K* (Scheme 12).



Scheme 12

$$K = \frac{[\text{complex}]}{[\text{guest}].[\text{host}]} \qquad (1)$$

The binding constant, *K*, can be determined from equation (1) once the concentrations of complex, guest and host are known. The studies of the binding constants, *K*, for the complexes formed between the isoxazolines **11**, **12**, **13**, **14**, **20**, **21**, **22** and **23** and β-CD in aqueous ethanol and how β-CD is influencing the ratio of regioisomeric isoxazolines extracted from these cycloadditions are described in Chapter One of the Results and Discussion.

1.3 The effect of bovine serum albumin (BSA) and β-CD on the hydrolysis of aryloxypropionates

In a similar system to that proposed by Rama Rao *et al.* where β-CD and bakers' yeast are simultaneously binding substrates and co-ordinating their interaction in a cycloaddition. Kamal *et al.*[90] have suggested that BSA and β-CD simultaneously affect both the extent and enantioselectivity of the hydrolysis of aryloxypropionates. As discussed earlier, cyclodextrins are known to form inclusion complexes with a wide variety of hydrophobic species in aqueous solution.[82] This characteristic has led to the use of cyclodextrins to control and influence chemical transformations, particularly enzyme catalysed processes. An overview of the influence of cyclodextrins on enzyme catalysed reactions is presented below.

1.3.1 The use of cyclodextrins with enzymes

Enzymes have become more frequently used in organic synthesis because of their safety, ease of handling and the mild conditions under which they operate. However, aspects of their biology limit their use in organic synthesis.

An enzyme catalysed reaction may be considered in terms of the reaction represented in Scheme 13 where E represents an enzyme, S a substrate and P the product of the enzyme catalysed reaction.[91]

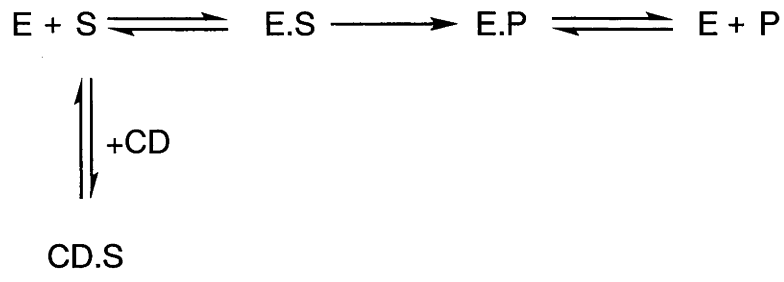
$$E + S \rightleftharpoons E.S \longrightarrow E.P \rightleftharpoons E + P$$

Scheme 13

The catalytic activity of an enzyme is based on effective complexation between it and the substrate to form the Michaelis complex, E.S. Cyclodextrins have been used to influence enzyme catalysed reactions in the following ways.

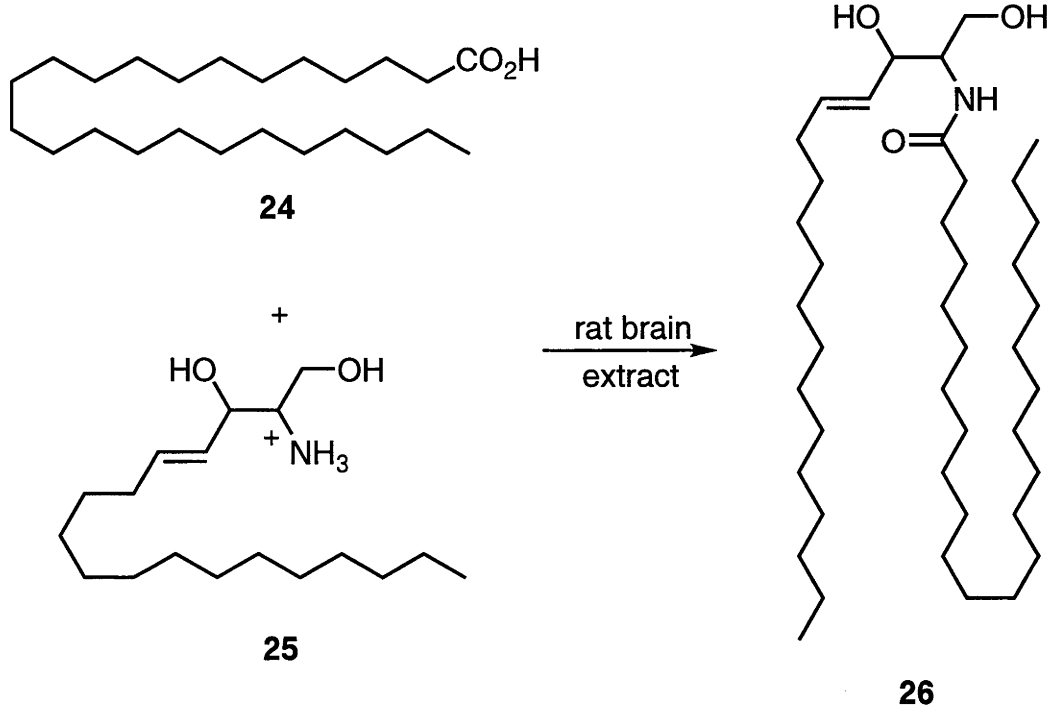
1.3.1.1 Increasing substrate availability

Enzyme catalysed processes are normally carried out in aqueous solution where the solubility of substrates is often too low for organic synthesis to be feasible because large scale reactions would be required. Cyclodextrins have been used to overcome these problems by acting as substrate reservoirs (Scheme 14). The total amount of substrate available to the enzyme is increased through its complexation with cyclodextrin.



Scheme 14

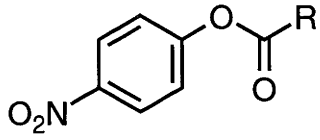
One of the earliest references to the use of cyclodextrins to increase the availability of substrates in enzymatic systems focussed on the preparation of the ceramide **26** from a reaction between *n*-tetracosanoic acid (**24**) and sphingosine (**25**) catalysed by rat brain extract (Scheme 15).



Scheme 15

The acid **24** has a solubility of less than 10^{-6} mol dm^{-3} in water but inclusion within α-cyclodextrin (α-CD) increases its solubility by two orders of magnitude.[92] The increase in solubility of the acid **24** on complexation with α-CD results in a twenty fold increase in the rate of reaction to give compound **26**.[92,93]

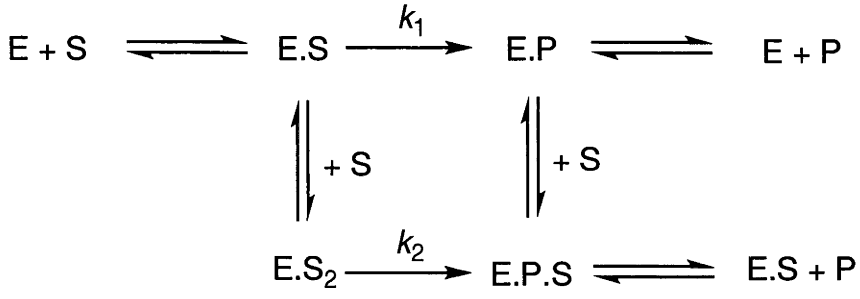
The use of cyclodextrins to increase the availability of substrates for enzyme catalysed reactions has not been limited to natural substrates. The solubility of the esters **27** and **28** has been increased several hundred fold by addition of β-CD, thus increasing the availability of the esters **27** and **28** for hydrolysis by a variety of hydrolases.[94]



27 R = $(CH_2)_3Me$
28 R = $(CH_2)_7Me$

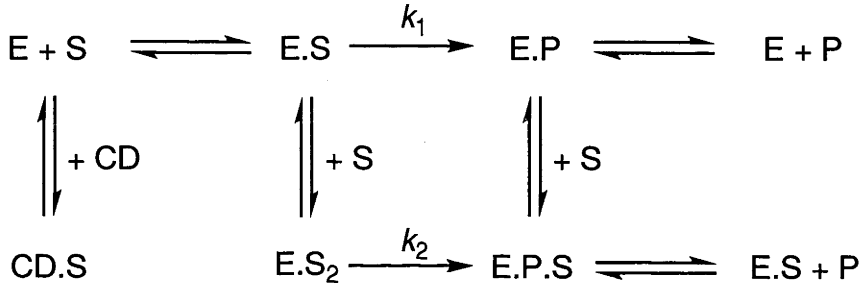
1.3.1.2 Limiting substrate inhibition

Substrate inhibition is a key form of metabolic control that prevents the wasteful digestion of an abundant substrate and the accumulation of the corresponding product. Substrate inhibition is the result of a second substrate molecule binding to the enzyme and decreasing its activity.[95,96] Substrate inhibition can be described as shown in Scheme 16, where $k_1 > k_2$.



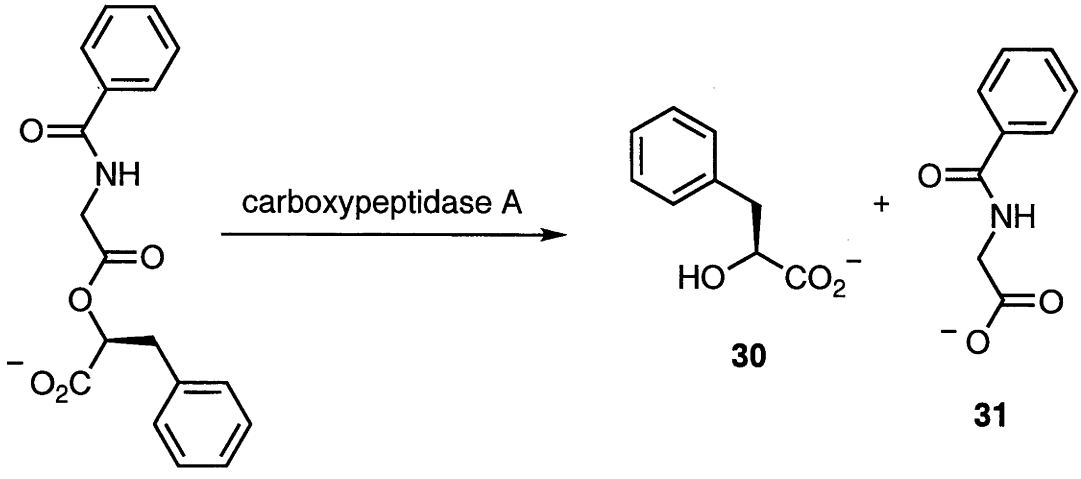
Scheme 16

At low substrate concentrations, the binary enzyme-substrate complex, E.S, dominates such that reaction rate increases with increasing substrate concentration. At higher substrate concentrations, the ternary complex $E.S_2$, forms and the reaction rate decreases. Therefore, to maintain significant enzyme activity, a low substrate concentration must be maintained. In a system which exhibits substrate inhibition, low concentrations of substrate are required to ensure that enzyme activity is retained. What this means for preparative organic synthesis is large scale reactions are required. In a similar fashion to that described above for increased substrate availability, cyclodextrins have been used to overcome this problem by complexing with the substrate and reducing the amount free in solution, thus acting as accessible substrate reservoirs (Scheme 17).



Scheme 17

The hydrolysis of (*S*)-*N*-benzoylglycyl-β-phenyllactate (**29**) by carboxypeptidase A to give β-phenyllactate (**30**) and (*S*)-*N*-benzoylglycine (**31**) displays substrate inhibition (Scheme 18).[97]



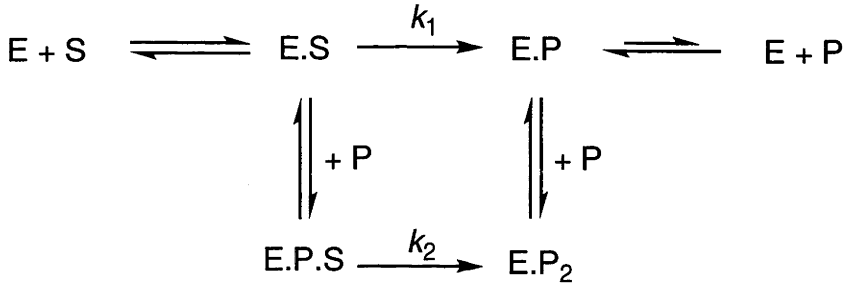
29 **30** **31**

Scheme 18

The addition of β-CD to the carboxypeptidase A catalysed hydrolysis reaction limits the extent of substrate inhibition and results in a two and a half fold increase in the rate of hydrolysis of the substrate **29**.[98]

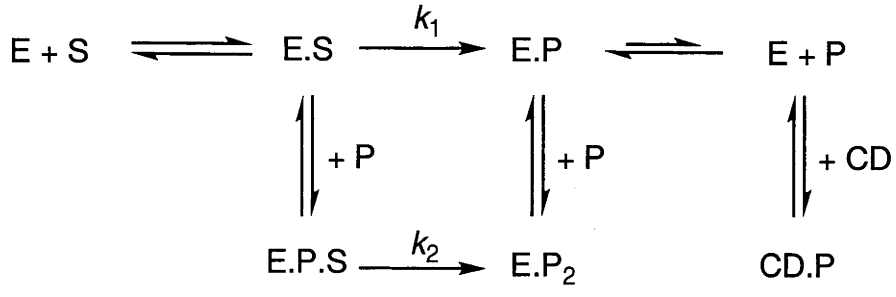
1.3.1.3 Limiting product inhibition

Product inhibition is another regulatory mechanism which prevents wasteful formation of a product by reducing the enzyme's activity. As shown in Scheme 19, where $k_1 > k_2$, product inhibition results from either binding of product to the enzyme-substrate complex reducing its catalytic activity, or through reduced extent of product dissociation.



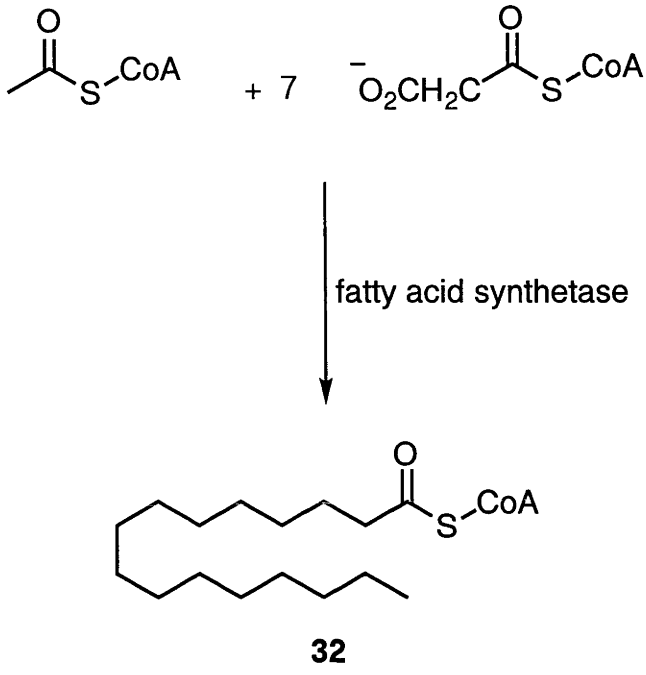
Scheme 19

In addition to maintaining enzyme activity by limiting the extent of substrate inhibition through inclusion of the substrate, cyclodextrins can be used in a similar way to limit the extent of product inhibition. The cyclodextrin includes the product within it's cavity thus reducing the amount free in solution to inhibit the enzyme's activity, increasing the rate of digestion of the substrate and hence the extent of reaction (Scheme 20).



Scheme 20

Bloch *et al.*[99,100] published the earliest example of cyclodextrins being used to limit product inhibition. Palmitoyl CoA **32** is formed in reactions catalysed by fatty acid synthetase (Scheme 21) derived from *Mycobacterium smegmatis* and *Mycobacterium phlei*. It is also a potent inhibitor of fatty acid synthetase activity.[101]



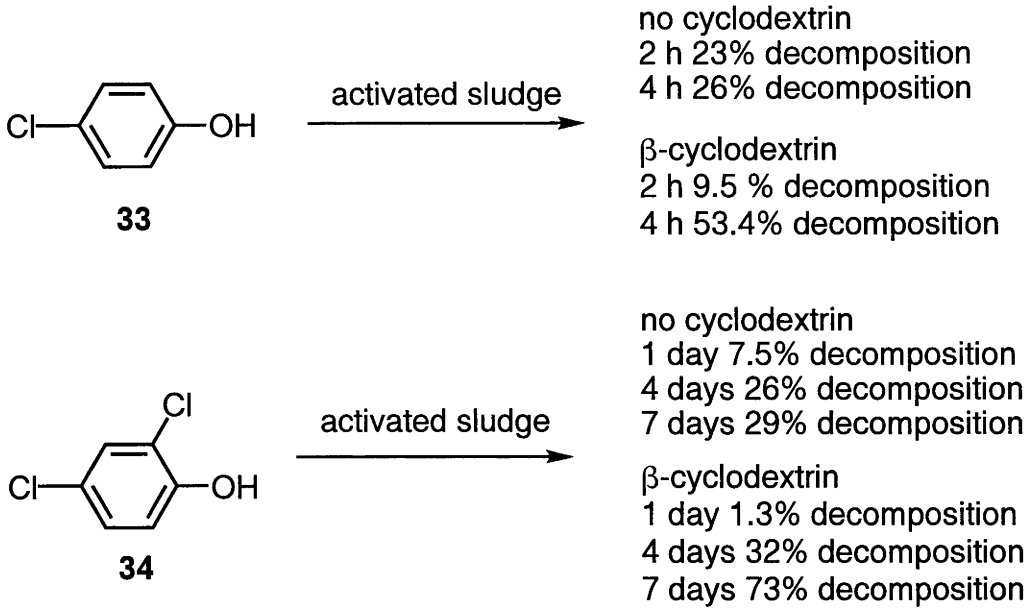
32

Scheme 21

The addition of β-CD and other modified cyclodextrins to the fatty acid synthetase catalysed reaction increases the enzyme's activity through inclusion of the inhibitor **32**, as illustrated in Scheme 20.

1.3.1.4 Other effects of selective guest binding

While the activity of an enzyme may be reduced in the presence of a high substrate concentration through substrate inhibition, in whole cell systems high substrate concentrations may also be toxic to the cells. Cyclodextrins have been used to address this problem by reducing the amount of substrate free in solution, through complexation, while the complexed substrate remains accessible. An example of such an application is the use of β-CD to limit substrate toxicity in the decomposition of chlorophenols in industrial waste water by activated sludge (Scheme 22).



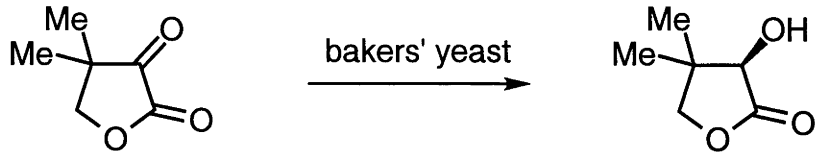
Scheme 22

The activated sludge has little activity after either two hours or four days in the presence of *ca.* 2.5×10^{-4} mol dm^{-3} of either the chlorophenol **33** or the dichlorophenol **34**, respectively.[102] The addition of β-CD limits the substrate toxicity because it forms inclusion complexes with both the chlorophenol **33** and the dichlorophenol **34** and reduces the effective concentration of each substrate in solution.[103] The initial rate of decomposition is slowed by β-CD because it limits substrate toxicity through inclusion of the substrate, such that the activity of the sludge is maintained for a longer period of time.

Another application of cyclodextrins in enzyme catalysed processes is substrate masking.[100] The effect of cyclodextrin is simply to lower the concentration of free substrate and therefore the extent of formation of the Michaelis complex, E.S, thus decreasing catalytic activity. This method has been used to limit undesirable processes. The extent to which an enzyme's activity is retarded can be predicted by considering the binding constant of a given cyclodextrin-substrate complex and the kinetic characteristics of the enzyme catalysed process.[104]

An example of substrate masking is the reduction of ketopantolactone (**35**) to (*R*)-pantolactone (**36**) by bakers' yeast. This process is catalysed by a number of the

dehydrogenases present (Scheme 23). The most enantioselective hydrogenase has the lowest dissociation constant for the enzyme-substrate complex, therefore, the addition of β-CD increases the enantioselectivity of the conversion by lowering the amount of compound **35** free in solution, thus increasing the enantioselectivity of the reduction without appreciable loss in yield.

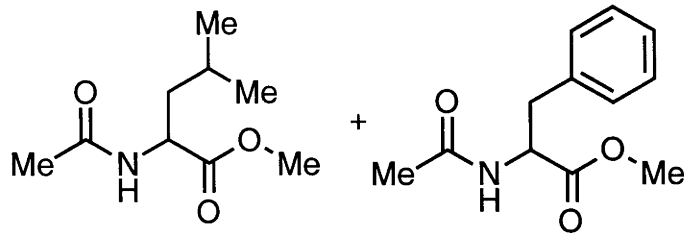


35 **36**

no cyclodextrin 41%, 73% ee
β-cyclodextrin 39%, 93% ee

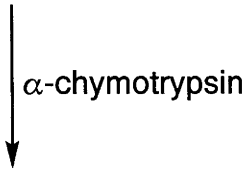
Scheme 23

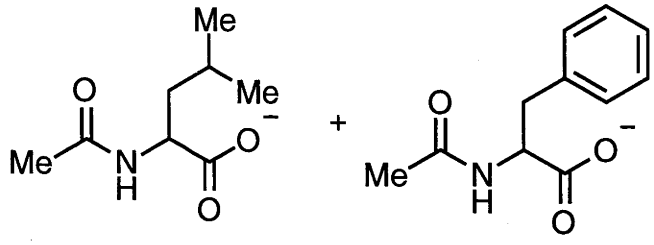
When a mixture of substrates is digested by an enzyme, a mixture of products is produced, some of which may be undesirable. Cyclodextrins have been used to affect the digestion of a mixture of substrates through selective complexation of one of the substrates. For example, in the hydrolysis of the esters **37** and **38** by α-chymotrypsin (Scheme 24), the addition of α-CD increases the proportion of compound **39** in the product mixture as a result of selective complexation of substrate **38**.[105]



37 **38**

10 : 1



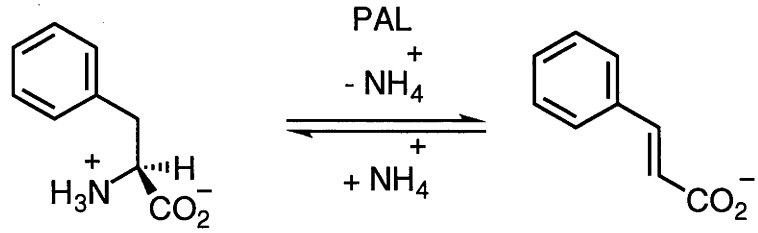


39 **40**

no cyclodextrin	1	:	2.7
α-cyclodextrin	3.1	:	1

Scheme 24

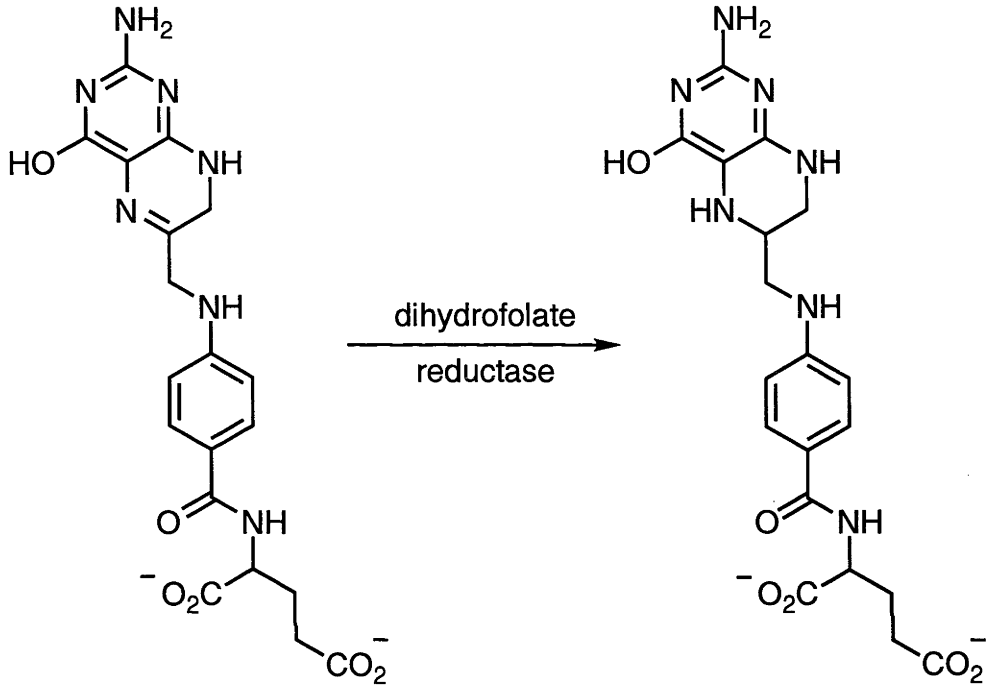
Cyclodextrins have also been used to promote product formation in enzyme catalysed processes in which the formation of the enzyme-product complex from the Michaelis complex is reversible. This has been applied in the (*S*)-phenylalanine ammonia lyase (PAL) catalysed conversion of phenylalanine (**41**) to the cinnamate **42** (Scheme 25).[98] At equilibrium, the ratio of phenylalanine (**41**) to the cinnamate **42** is 1:8.5 in the absence of cyclodextrin. This ratio changes to 1:23 and <1:100 on addition of α- and β-CD, respectively, due to complexation of the cinnamate **42** by the cyclodextrins.



41 **42**

Scheme 25

Finally, cyclodextrins have been used to improve the efficiency of enzyme catalysed reactions by sequestering inhibitors. Palmitoyl CoA inhibits, through complexation, a large number of functionally diverse enzymes.[99,100,106] The inhibition of dihydrofolate reductase by palmitoyl CoA is reduced by β-CD (Scheme 26).[107] β-CD complexes the palmitoyl CoA, reducing its concentration in solution and thus reducing its inhibitory effect on dihydrofolate reductase.



43 **44**

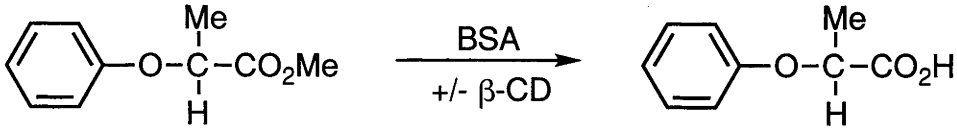
Additive	Relative enzyme activity
-	1
Palmitoyl CoA	0.22
Palmitoyl CoA + β-CD	0.40

Scheme 26

As discussed earlier, Rama Rao *et al.* suggested that β-CD and bakers' yeast were simultaneously binding substrates and co-ordinating their interaction in a cycloaddition. Kamal *et al.*[90] have suggested a similar interaction between BSA and β-CD that affects both the extent and enantioselectivity of the hydrolysis of a variety of aryloxypropionates.

The biological importance of (*R*)-phenoxypropionic acids is well established. The racemates are used widely in agriculture because of their herbicidal activity[108] which stems mainly from the (*R*)-enantiomer.

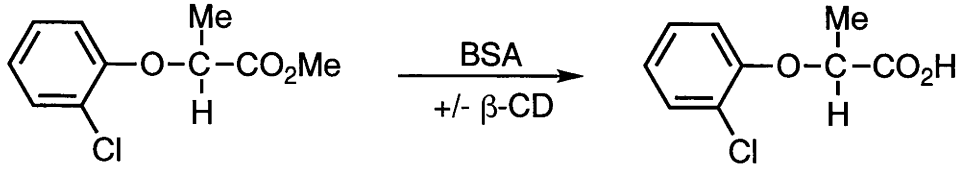
Kamal *et al.* suggested that the addition of β-CD not only improved the enantioselectivity and but also the efficiency of the aryloxypropionate hydrolysis reaction catalysed by BSA.



45 **46**

Scheme 27

For example, Kamal *et al.* reported that the hydrolysis of the methyl ester **45** conducted in pH 11.5 phosphate buffer after 8 h in the presence of BSA gave the corresponding acid **46** in a conversion of 43% and an enantiomeric excess of 66% in favour of the (*R*)-enantiomer (Scheme 27). Kamal *et al.* repeated the hydrolysis of the methyl ester **45** in the presence of BSA and 0.2 molar equivalents of β-CD in pH 11.5 phosphate buffer and found that after 4 h there was a 62% conversion to the acid **46** and the enantiomeric excess had increased to 89% in favour of the (*R*)-enantiomer. This result is intriguing because an enantiomeric excess of 89% from a 62% conversion is not theoretically possible without epimerisation of the starting material.



47 **48**

Scheme 28

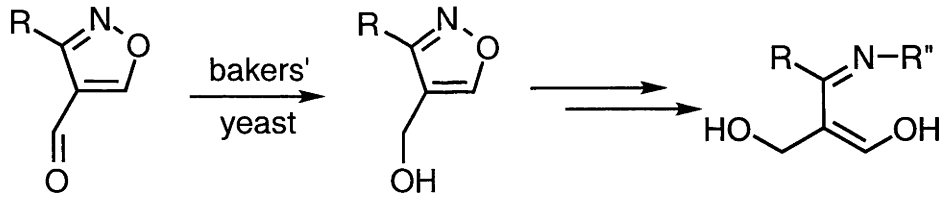
Similar trends were experienced in the hydrolysis of the chlorinated analogue **47** (Scheme 28). After 6 h of hydrolysis under the reaction conditions described above for the methyl

ester **45**, 36% of the ester **47** had been converted to the corresponding acid **48** with an enantiomeric excess of 57% in favour of the (*R*)-enantiomer in the presence of BSA. The hydrolysis of the ester **47**, repeated in the presence of both BSA and 0.2 molar equivalents of β-CD, resulted in 47% conversion to the acid **48** in 2 h and an enantiomeric excess of 87% in favour of the (*R*)-enantiomer.

Even though the hydrolysis reactions were not repeated in the absence of BSA, Kamal *et al.* concluded that the BSA was catalysing the hydrolysis of the aryloxypropionates enantioselectively such that the (*R*)-phenoxypropionic acids were produced preferentially. Furthermore, the presence of β-CD in these reactions apparently promoted a faster and more enantioselective hydrolysis. The authors stated that negligible hydrolysis of the aryloxypropionates was observed with β-CD alone, implying that β-CD serves to enhance the action of BSA rather than participating in the hydrolysis reaction directly.[90] Enantioselective enhancement of reactions by β-CD has been observed before, but not to such a large extent and not *via* direct interaction between an enzyme and cyclodextrin. Furthermore, it seems highly unlikely that β-CD, BSA and the substrate could come together in an orientation which would promote enantioselective hydrolysis. The industrial importance of aryloxypropionates, particularly to agriculture, and an interest in the influence of cyclodextrins on chemical transformations prompted an investigation into this area of work. A re-investigation of the work outlined by Kamal *et al.* is described in Chapter Three of the Results and Discussion.

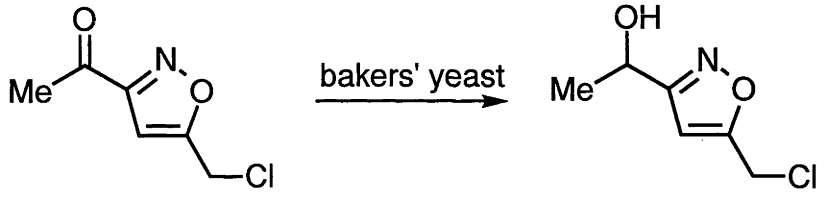
1.4 The reductive ring opening of isoxazoles

As discussed earlier, CSIRO Molecular Science had undertaken a pilot project to investigate production of structural analogues of GRASP **1** centring around the β-enamino ketone moiety. One synthetic approach considered was to exploit nitrile oxide cycloaddition chemistry to produce 4-acyl isoxazoles that could be ring opened to produce analogues of GRASP. Bakers' yeast catalysed reduction of the 4-acyl group would give the hydroxy-diketone analogues of GRASP (Scheme 29).



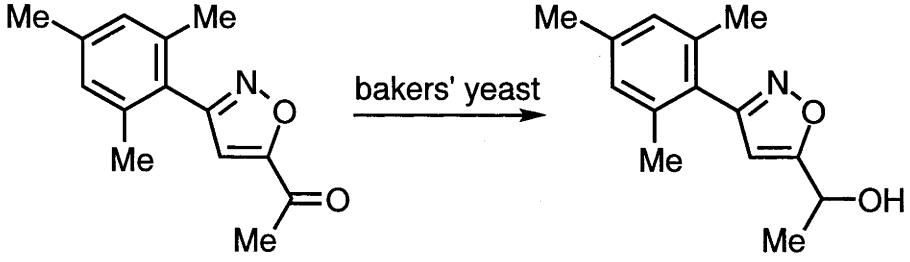
Scheme 29

The use of fermenting bakers' yeast (sp. *Saccharomyces cerevisiae*) to facilitate synthetic transformations in organic chemistry is well established.[109,110] Of particular importance has been the use of bakers' yeast in the reduction of carbonyl compounds to the corresponding alcohols. For example, the isoxazoles **49** and **51** are converted to the corresponding alcohols **50** and **52** in 78% and 75% yield, respectively (Schemes 30 and 31).[109,110]



49 **50**

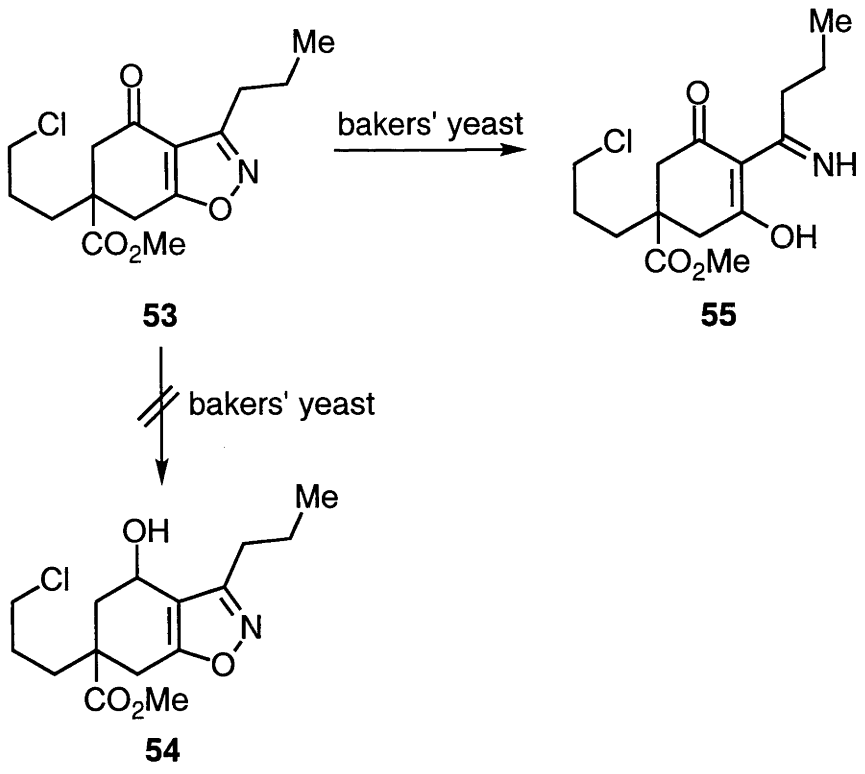
Scheme 30



51 **52**

Scheme 31

However, investigations conducted at the CSIRO found that the bakers' yeast catalysed reduction of the isoxazole **53** did not, as expected, produce the corresponding alcohol **54** but instead gave the ring opened imine **55** (Scheme 32).



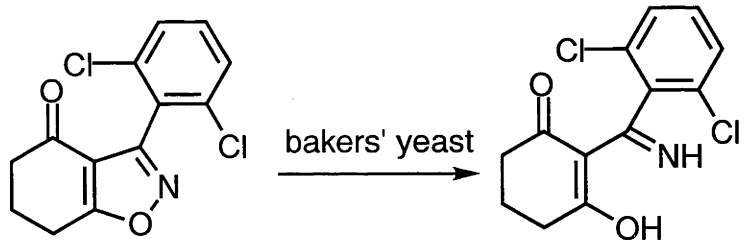
53 **55** **54**

Scheme 32

The cleavage of the isoxazole N-O bond can be facilitated by a number of reagents including Grignard reagents[111] and molybdenum hexacarbonyl[112] but the most popular method is by hydrogenolysis over platinum,[113] palladium[114] or Raney nickel.[115] However, these methods are unsuitable when other sensitive groups or catalyst poisons are present in the molecule.

To test the generality of the yeast catalysed isoxazole ring opening process and its application to the synthesis of GRASP analogues, Hughes *et al.*[116] carried out a series of experiments in which isoxazoles were reduced under fermenting yeast conditions.

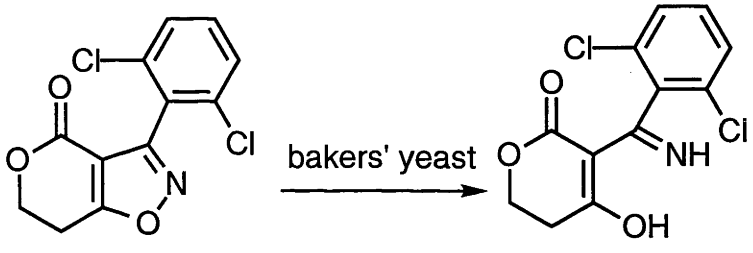
The isoxazole **56** was added to a fermenting yeast solution and the mixture was incubated for twenty four hours. After purification, the ring opened imine **57** was obtained in 23% yield (Scheme 33).



56 **57**

Scheme 33

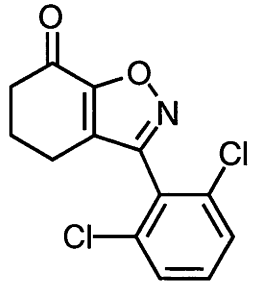
The structurally related isoxazole **58** was incubated with yeast and after purification gave the ring opened imine **59** in 21% yield (Scheme 34).



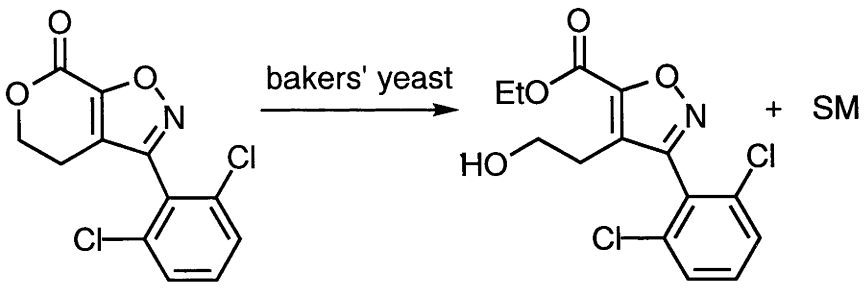
58 **59**

Scheme 34

By contrast, the isoxazole **60**, the regioisomer of the isoxazole **56**, when incubated with yeast returned starting material. The regioisomer of the isoxazole **58**, the isoxazole **61**, when incubated with yeast gave predominantly starting material and a small amount of the transesterification product **62** (Scheme 35).



60

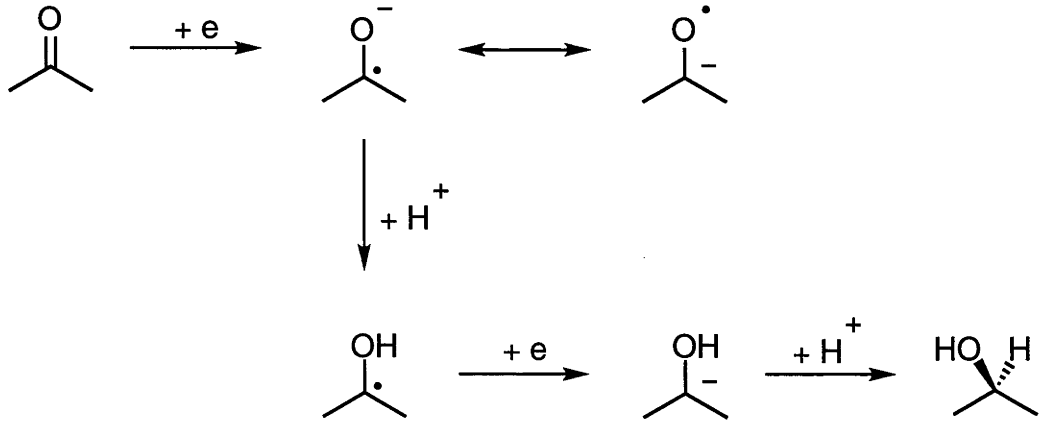


61 **62**

Scheme 35

When considering the isoxazoles which underwent ring opening with yeast and those that did not, an obvious difference between the two groups of compounds is the position of the carbonyl substituent on the isoxazole ring. The compounds, which in the presence of yeast, undergo the ring opening, compounds **53**, **56** and **58**, have a carbonyl group in the 4-position of the isoxazole ring, while the compounds which do not ring open, compounds **60** and **61**, have a carbonyl group in the 5-position. Clearly the position of the carbonyl group is an important factor in the yeast catalysed N-O bond cleavage process.

It is commonly understood that yeast catalysed transformations of aldehydes and ketones proceed *via* an electron transfer mechanism (Scheme 36). It therefore seemed plausible that the process by which bakers' yeast ring opens isoxazoles might be similar.



Scheme 36

With this in mind an electrochemical investigation of the reductive ring opening of isoxazoles was conducted. In doing so, it was hoped that this would provide a greater understanding of the mechanism by which yeast catalyses the reductive ring opening of isoxazoles and might provide an alternative, higher yielding method for the preparation of such compounds. Previous work[116] had demonstrated the substituent type and position on the isoxazole ring is important to the success of the ring opening process. Therefore, it was expected that the reduction of a series of 4- and 5-substituted isoxazoles containing a variety of substituent types under electrochemical conditions might also provide valuable insight into the structure-reactivity relationships present in these systems. Furthermore, the use of electrochemistry to facilitate the ring opening process might be more efficient and produce imines in a higher yield than was experienced with fermenting bakers' yeast.

The investigations into the electrochemical ring opening of isoxazoles are described in Chapter Four of the Results and Discussion.

RESULTS AND DISCUSSION CHAPTER ONE

The Effect of Bakers' Yeast and β-CD on Nitrile Oxide Cycloadditions

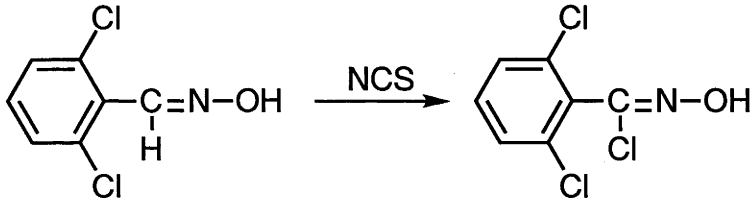
As discussed in the Introduction, Ramo Rao and co-workers[84-88] claimed that bakers' yeast was required to promote cycloadditions between aryl nitrile oxides and cinnamates in an aqueous ethanol solution and that the addition of β-CD to these reactions altered the ratio of regioisomers produced. An investigation of these systems[89] found that bakers' yeast was not required for the cycloaddition reactions to proceed and the presence of β-CD did not influence the regioisomeric outcome of the cycloadditions. Furthermore, it was found that β-CD was interacting with the products of the cycloadditions, not the reactants, and thus influencing the ratio of the isomers isolated.

This evidence prompted the following hypothesis. It was thought that the ratio of regioisomers isolated from these reactions was affected by differences in the extent of complexation between the β-CD and the aryl substituents on the cycloadducts. As discussed in the Introduction, as a consequence of the hydrophobic nature of their annuli, cyclodextrins are capable of forming inclusion complexes with a variety of guests in aqueous solution. A measure of the extent of complexation between a cyclodextrin and a guest is the stability constant, *K*. By calculating the stability constants for each cycloadduct-cyclodextrin inclusion complex, it was expected that a comparison of the strength of binding could be made, and that the effects of cyclodextrins on the ratios of products isolated from the cycloaddition reactions could be rationalised.

2.1 Synthesis of the Isoxazolines 11, 12, 13, 14, 20, 21, 22 and 23

It was first necessary to synthesise compounds **11**, **12**, **13**, **14**, **20**, **21**, **22** and **23** because they were not available from the previous work and had not previously been fully characterised.

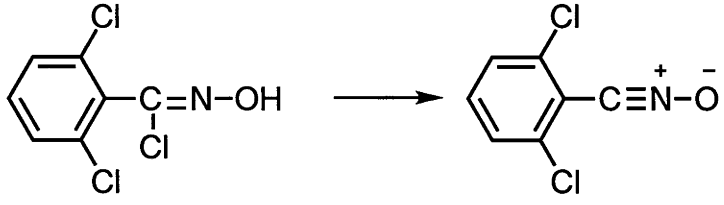
The synthesis of the isoxazolines **11** and **12** began with the conversion of the commercially available aldoxime **63** to the corresponding hydroximinoyl chloride **64** by treatment with *N*-chlorosuccinimide in DMF (Scheme 37) as previously reported by Lui.[21]



63 **64**

Scheme 37

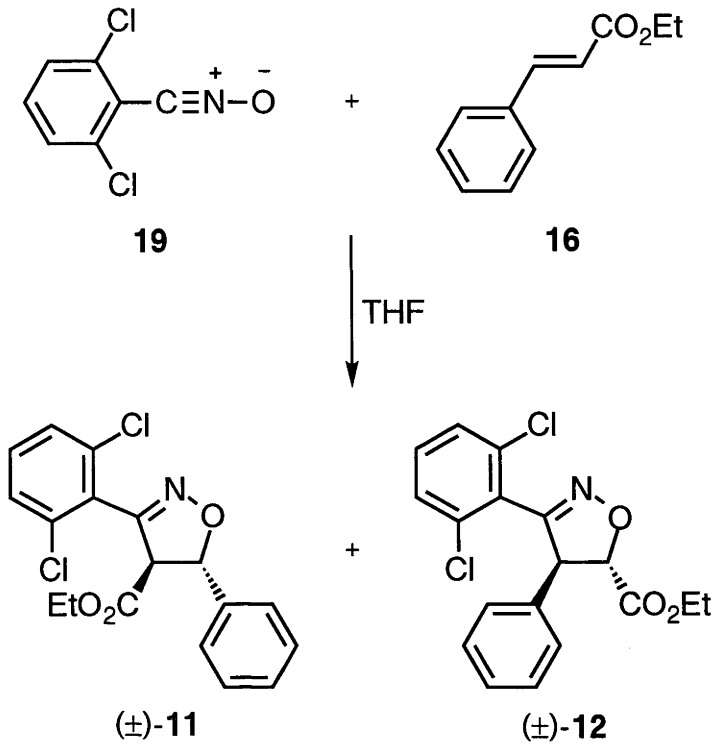
The hydroximinoyl chloride **64** was treated with triethylamine to generate the corresponding nitrile oxide **19** *in situ* (Scheme 38) which underwent a 1,3-dipolar cycloaddition reaction with ethyl cinnamate (**16**) to give a regioisomeric mixture of the isoxazolines **11** and **12** in a ratio of 85:15 as determined by ^{1}H nmr spectroscopy (Scheme 39). The nitrile oxide **19** is generated *in situ* to limit the possibility of dimerisation occurring.[37]



64 **19**

Scheme 38

The isoxazolines **11** and **12** were separated by flash column chromatography on silica and isolated in 41% and 8% yield, respectively.



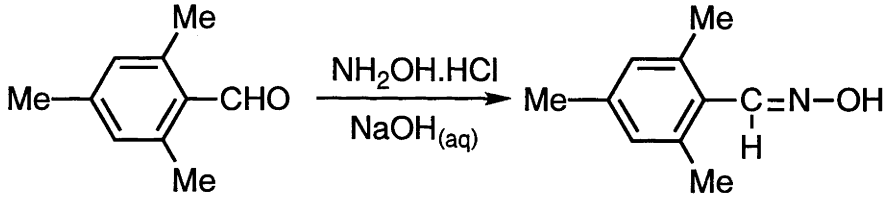
Scheme 39

The regioisomers were distinguished by comparing the chemical shifts of their C-4 and C-5 isoxazoline ring protons using ^{1}H nmr spectroscopy. The ^{1}H nmr spectrum of the isoxazoline **11** exhibits doublets at δ 4.57 and δ 6.24 with coupling constants of 9 Hz which correspond to the C-4 and C-5 protons, respectively. The chemical shifts and the coupling constants are consistent with the ester substituent being attached to C-4 and the phenyl substituent being attached to C-5.[117,118] The structure of the isoxazoline **11** had been confirmed by X-ray crystallographic analysis.[119] The ^{1}H nmr spectrum of the isoxazoline **12** exhibits doublets at δ 5.24 and δ 5.28 with coupling constants of 5.5 Hz corresponding to the C5 and C4 ring protons, respectively. The chemical shifts and the coupling constants are consistent with the phenyl substituent being attached to C-4 and the ester substituent being attached to C-5.[117,118]

The regioselectivity of nitrile oxide cycloadditions is governed by electronic and steric effects. In practice steric effects generally predominate over electronic effects, but with 1,2-disubstituted alkenes a mixture of regioisomers is usually obtained, and the isomer

that is favoured electronically will be the predominant isomer. For example, electron withdrawing substitutents such as carbonyl groups on the alkene are known to direct the cycloaddition reaction such that the carbonyl group is located at the 4-position of the cycloadduct ring.[72-78] Alternatively, electron donating groups such as alkoxy groups promote the formation of a cycloadduct with the alkoxy group in the 5-position of the ring.[79] Accordingly, the carbonyl group of the alkene **16** polarises this species such that the oxygen of the nitrile oxide **19** reacts with the carbon adjacent to the phenyl group, thus producing the isoxazoline **11** as the predominant isomer.

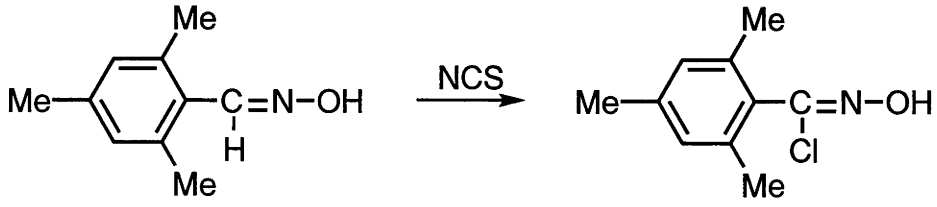
The synthesis of the isoxazolines **13** and **14** began with the conversion of 2,4,6-trimethylbenzaldehyde (**65**) to the corresponding aldoxime **66** by addition of hydroxylamine hydrochloride and sodium hydroxide in aqueous ethanol (Scheme 40).[21]



65 **66**

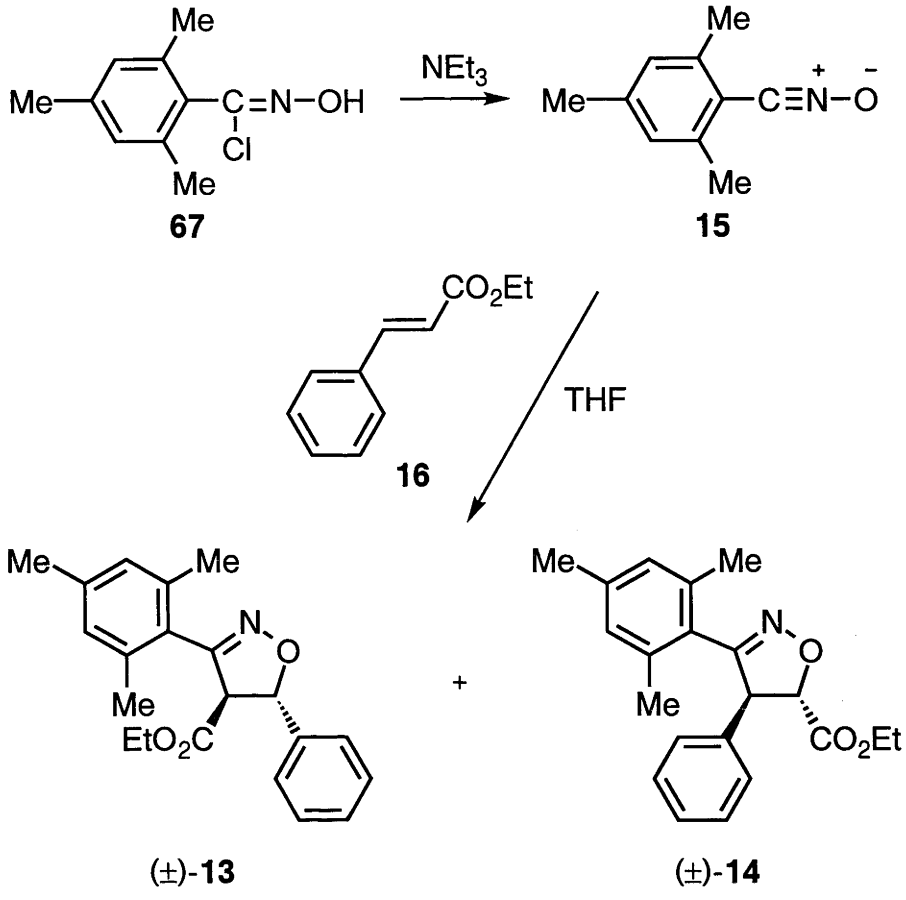
Scheme 40

The aldoxime **66** was treated with *N*-chlorosuccinimide to give the corresponding hydroximinoyl chloride **67** (Scheme 41). The hydroximinoyl chloride **67** was treated with triethylamine to produce the corresponding nitrile oxide **15** *in situ* which underwent a cycloaddition with ethyl cinnamate (**16**) in dry tetrahydrofuran to give a regioisomeric mixture of the isoxazolines **13** and **14** in the ratio of 50:50 as determined by ^{1}H nmr spectroscopy (Scheme 42).



66 **67**

Scheme 41

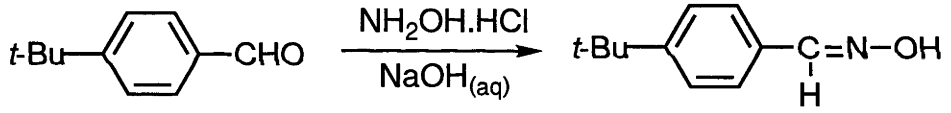


Scheme 42

The isoxazolines **13** and **14** were separated by flash column chromatography on silica and obtained in yields of 49% and 29%, respectively. The ^{1}H nmr spectrum of the isoxazoline **13** exhibits a doublet at δ 4.37 corresponding to the C-4 proton and a doublet at δ 6.11 corresponding to the C-5 proton; both with coupling constants of 9.5 Hz. The chemical shifts and coupling constants are consistent with the ester substituent being attached to C-4 and the phenyl substituent being attached to C-5.[117,118] The ^{1}H nmr spectrum of the isoxazoline **14** exhibits a doublet at δ 4.82 corresponding to the C-5 proton and a doublet at δ 5.33 corresponding to the C-4 proton; both with coupling constants of 4 Hz. The chemical shifts and the coupling constants are consistent with the phenyl substituent being attached to C-4 and the ester substituent being attached to C-5.[117,118]

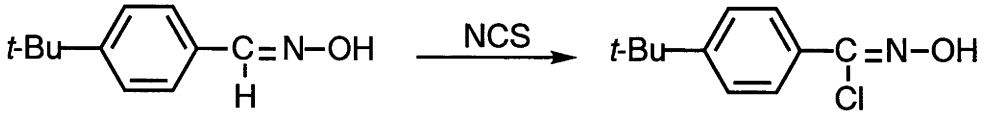
Typically, cycloadditions between nitrile oxides and 1,2-disubstituted alkenes result in regioisomeric mixtures, with the isomer that is favoured electronically being the predominant isomer. Surprisingly, the cycloaddition between the nitrile oxide **15** and the cinnamate **16** consistently gave a near 1:1 mixture of the isoxazolines **13** and **14**. Based on the polarity of the reagents, the isoxazoline **13** was expected to be the predominant isomer from the cycloaddition. The fact that there is a near 1:1 mixture of regioisomers resulting from this reaction suggests that the cycloaddition is not straightforward but is complicated by electronic and steric factors influencing the regioselectivity.

The synthesis of the isoxazolines **20** and **21** began with the conversion of 4-(*tert*-butyl)benzaldehyde (**68**) to the corresponding aldoxime **69** by addition of hydroxylamine hydrochloride and sodium hydroxide in aqueous ethanol (Scheme 43).[21] The aldoxime **69** was treated with *N*-chlorosuccinimide in DMF to give the corresponding hydroximinoyl chloride **70** (Scheme 44).



68 **69**

Scheme 43

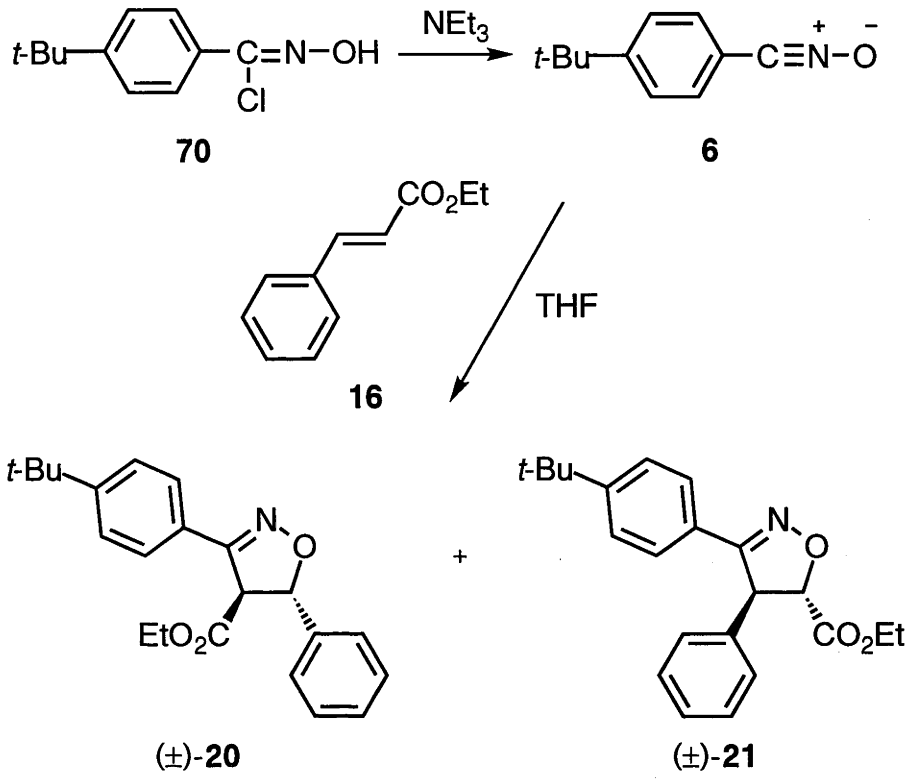


69 **70**

Scheme 44

The hydroximinoyl chloride **70** was treated with triethylamine to produce the corresponding nitrile oxide **6** *in situ* which then underwent a cycloaddition with ethyl cinnamate (**16**) to give a regioisomeric mixture of the isoxazolines **20** and **21** in the ratio of 73:27 as determined by ^{1}H nmr spectroscopy (Scheme 45). The isoxazolines **20** and **21** were separated by flash column chromatography on silica and were subsequently

recrystallised from ethyl acetate/hexane and obtained in yields of 47% and 11%, respectively.

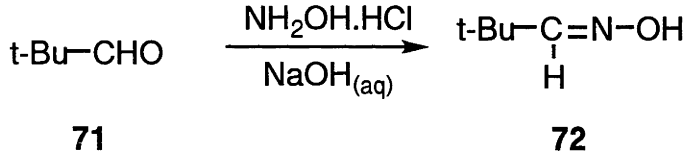


Scheme 45

The regioisomers were distinguished by the chemical shifts of their C-4 and C-5 protons in the ^{1}H nmr spectrum. The ^{1}H nmr spectrum of the isoxazoline **20** exhibits a doublet at δ 4.42 corresponding to the C-4 proton and a doublet at δ 5.96 corresponding to the C-5 proton; both have coupling constants of 6 Hz. The chemical shifts and the coupling constants are consistent with the ester substituent being attached to C-4 and the phenyl substituent being attached to C-5.[117,118] The ^{1}H nmr spectrum of the isoxazoline **21** exhibits a doublet at δ 4.93 corresponding to the C-5 proton and a doublet at δ 5.01 corresponding to the C-4 proton; both have coupling constants of 4 Hz. The chemical shifts and the coupling constants are consistent with the phenyl substituent being attached to C-4 and the ester substituent being attached to C-5.[117,118]

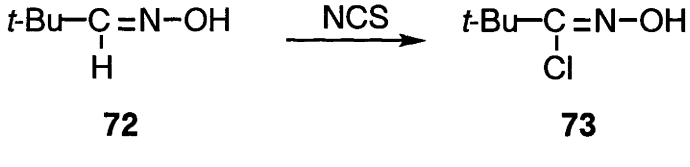
The isoxazoline **20** is the predominant isomer because the electron withdrawing carbonyl group on the alkene directs the cycloaddition reaction such that it is located at the 4-position of the cycloadduct ring.[72-78]

Finally, the synthesis of the isoxazolines **22** and **23** began with the conversion of pivaldehyde (**71**) to the corresponding aldoxime **72** by addition of hydroxylamine hydrochloride and sodium hydroxide in aqueous ethanol (Scheme 46).[21] Compound **72** was treated with *N*-chlorosuccinimide to give the corresponding hydroximinoyl chloride **73** as a pale yellow oil (Scheme 47).



71 **72**

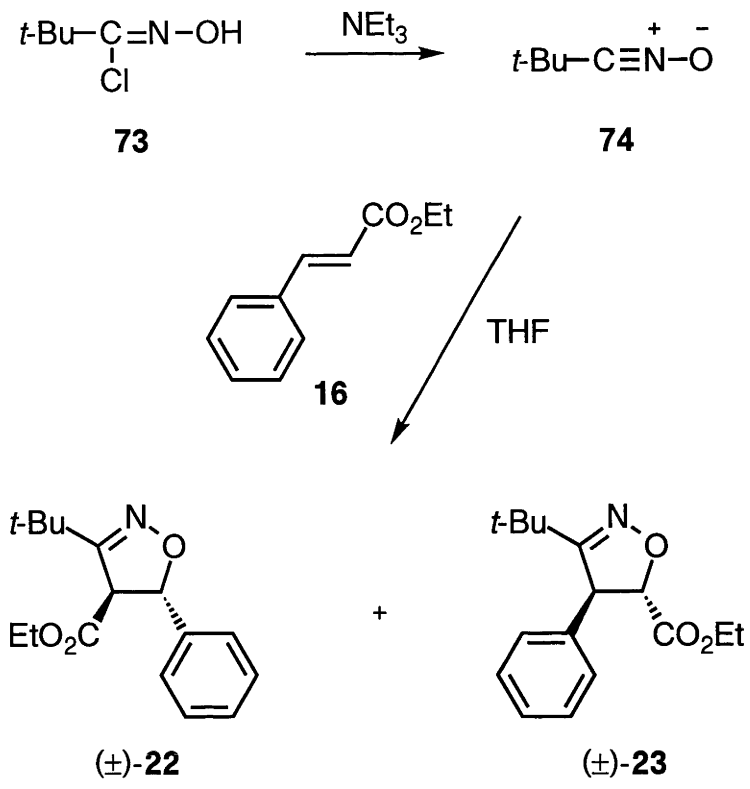
Scheme 46



72 **73**

Scheme 47

The hydroximinoyl chloride **73** was treated with triethylamine to form the corresponding nitrile oxide **74** *in situ* which then underwent a cycloaddition with ethyl cinnamate (**16**) to give a regioisomeric mixture of the isoxazolines **22** and **23** in the ratio of 74:26 as determined by ^{1}H nmr spectroscopy (Scheme 48).



Scheme 48

The isoxazolines **22** and **23** were separated by flash column chromatography on silica and the isoxazoline **22** was subsequently recrystallised from ethyl acetate/hexane and obtained in 36% yield. The isoxazoline **23** was isolated as a pale yellow oil in 8% yield.

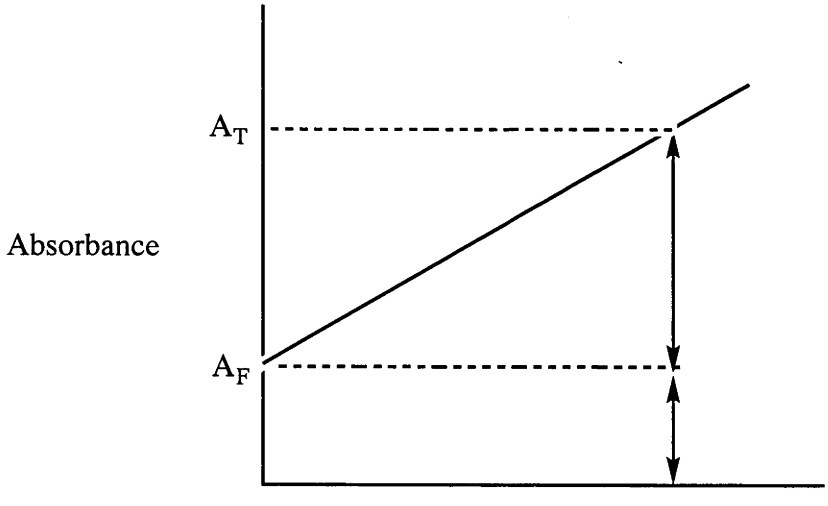
The regioisomers were distinguished by the chemical shifts of their C-4 and C-5 protons in their ^{1}H nmr spectra. The ^{1}H nmr spectrum of the isoxazoline **22** exhibits a doublet at δ 3.99 corresponding to the C-5 proton and a doublet at δ 5.76 corresponding to the C-4 proton; both with coupling constants of 6.5 Hz. The chemical shifts and coupling constants are consistent with the ester substituent being attached to C-4 and the phenyl substituent being attached to C-5.[117,118] The ^{1}H nmr spectrum of the isoxazoline **23** exhibits a doublet at δ 4.55 corresponding to the C5 proton and a doublet at δ 4.77 corresponding to the C4 proton; both with coupling constants of 3 Hz. The chemical shifts and the coupling constants are consistent with the phenyl substituent being attached to C-4 and the ester substituent being attached to C-5.[117,118]

The isoxazoline **22** is the predominant isomer because the electron withdrawing carbonyl group on the alkene directs the cycloaddition reaction such that it is located at the 4-position of the cycloadduct ring.[72-78]

Having prepared the isoxazolines **11**, **12**, **13**, **14**, **20**, **21**, **22** and **23**, a method was now needed to determine the stability constants of the inclusion complexes formed between these compounds and β-CD.

2.2 Methods for Determining Inclusion Complex Stability Constants

Previous work[120] has demonstrated the use of a UV-visible technique for the calculation of stability constants of cyclodextrin inclusion complexes. This method involves measuring the absorbance of a series of β-CD solutions of varying concentration which have been saturated with the appropriate guest. The plot of the absorbance against the concentration of cyclodextrin in each solution then produces a graph similar to that shown in Figure 4.



A_F= absorbance due to free guest in solution

A_T - A_F = absorbance due to complexed guest in solution.

Figure 4: Graph of absorbance against [CD] used to determine the absorbances of free and complexed guest.

The absorbance due to the free guest is represented by the value A_F. The absorbance due to the complexed guest is represented by the value A_T-A_F and will change as the concentration of the cyclodextrin changes. The concentrations that correspond to the absorbance values A_F and A_T-A_F can then be calculated from equation (2).[121]

$$C = \frac{A}{\varepsilon} \qquad (2)$$

Where ε = molar extinction coefficient (M^{-1} cm^{-1})

C = concentration (M)

These values can then be used with equation (1) to calculate the stability constant, *K*, of the guest-cyclodextrin complex.

$$K = \frac{[\text{complex}]}{[\text{guest}]\,[\text{cyclodextrin}]} \qquad (1)$$

The major advantage of this method is that direct measurement of free and bound guest concentrations is expected to give more accurate values for the binding constants.[122] However, the UV-Vis method does have some limitations. To successfully determine stability constants *via* this method, the guest must be active in the UV-Vis region of the spectrum and its molar extinction coefficient when free and complexed by cyclodextrin must be known or it should be possible for it to be calculated. The guest must have limited solubility in the solvent in which the studies are carried out and this solubility should increase in the presence of cyclodextrin within a measurable range. It is also necessary that the solutions of guest and cyclodextrin used to calculate the stability constants be saturated with guest and free of undissolved guest prior to absorbances being determined. Finally, the complex that results from the inclusion of the guest by cyclodextrin must be sufficiently soluble to stay in solution.

In order to use this method to calculate the stability constant of the complex formed between β-CD and each isoxazoline, it was therefore necessary to determine the molar extinction coefficient, ε, for each isoxazoline, first in the absence of β-CD. The solvent system used by Rama Rao *et al.* was aqueous ethanol, so the molar extinction coefficients needed to be determined in this solvent system. It was thought that the isoxazole **11** might first be dissolved in a small quantity of water to which ethanol would be added to give a 20% aqueous ethanol solution. A quantity of the isoxazoline **11** (~5mg) was added to water (5 ml) but did not dissolve. Alternatively, a stock solution of the isoxazoline **11** (~5mg) was prepared in ethanol (5 ml) and an attempt made to dilute this solution to the required concentration by the addition of water. When the water was added, the solution turned cloudy indicating that the dissolved isoxazoline was precipitating out of solution. This suggested that the isoxazoline **11** has limited solubility in the aqueous ethanol solvent, which is one of the requirements for the UV-Vis method to be successful. To ensure that a molar extinction coefficient for the isoxazoline **11** could be obtained, neat ethanol was used as a solvent. While a compromise it was thought that the molar extinction coefficient in ethanol would be a reasonable approximation of the molar extinction coefficient for the same compound in aqueous ethanol. The molar extinction coefficient for the isoxazoline **11** was calculated to be 24454 M^{-1} cm^{-1} at the λ_{max} of 220 nm. The UV-Vis experiments described below were well advanced at the time that the molar extinction coefficient for the isoxazoline **11** was calculated. The difficulties with the UV-Vis method discussed below indicated that this analytical method was unsuitable for determining the concentrations of free and complexed guest in solution required for stability constants calculations. Therefore, no further calculations of molar extinction coefficient were conducted.

Prior to determining the absorbances of the isoxazolines **11**, **12**, **13**, **14**, **20**, **21**, **22** and **23**, it was necessary to prepare a series of buffered aqueous ethanol solutions containing β-CD. The cycloaddition reactions studied by Rama Rao *et al.* were all conducted in 18.5% ethanol-pH 7.2 phosphate buffer solution. The cyclodextrin solutions used in these studies were prepared by serial dilution and each experiment was

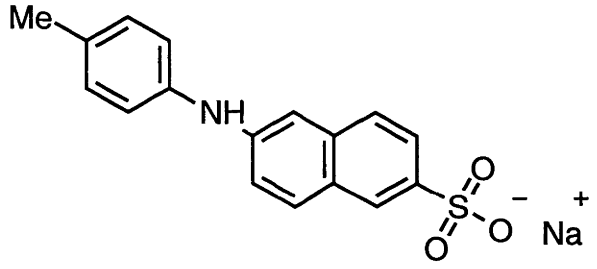
conducted numerous times, so, for convenience all the following experiments were conducted in 20% ethanol-pH 7.2 phosphate buffer solution. Measurements were made at a wavelength of 220 nm because this was determined to be the λ_{max} of the isoxazoline **11**.

A stock solution of β-CD in 20% ethanol-0.05 M pH 7.2 phosphate buffer was prepared. A serial dilution of the stock solution gave four solutions of varying cyclodextrin concentration. A control solution which contained no β-CD was also included in the experiments. The isoxazolines **11**, **12**, **13**, **14**, **20** and **21** were added to the cyclodextrin solutions and the resulting suspensions were sonicated for several hours. The isoxazolines **22** and **23** were not included in these preliminary experiments. An excess of cycloadduct was maintained in each cyclodextrin solution by ensuring that undissolved material remained after sonication. The sonicated solutions were equilibrated at 30 °C, filtered through 0.2 μm syringe filters to remove excess cycloadduct and the UV absorbances were determined for each solution at a wavelength of 220 nm. To ensure the solutions were saturated, more cycloadduct was added and the process repeated until no further change in the absorbance was observed. Graphs of absorbance as a function of cyclodextrin concentration were thus obtained for the isoxazolines **11**, **12**, **13**, **14**, **20** and **21**.

The graphs of absorbance as a function of cyclodextrin concentration obtained for the isoxazolines **12**, **14** and **21** were reproducible and exhibited good correlation coefficients. However, the graphs obtained for the isoxazolines **11**, **13** and **20** did not exhibit the same reproducibility or linearity. The most concerning problem was that a number of the graphs exhibited negative y-intercepts. This would imply a negative concentration of guest in solution which is clearly impossible. The specific problem with this method is therefore that the y-intercept can not be determined accurately because the solubility of the isoxazolines is too low in aqueous ethanol in the absence of cyclodextrin. There are three most probable reasons for these unreliable results. Firstly, the sonication of the solutions may lead to the formation of supersaturated solutions. Secondly, the

solutions may not be truly homogenous and contain small amounts of undissolved isoxazoline and finally, there may be a problem with the analytical method. Clearly this analytical method was unsuitable for determining the concentrations of free and complexed guest in solution required for stability constants calculations. Therefore, an alternative method was needed.

A well known method for determining stability constants of cyclodextrin inclusion complexes is the sodium 6-(*p*-toluidino)-2-naphthalenesulfonate (**75**) (TNS) displacement method.[123] TNS fluoresces once included within the hydrophobic cavity of β-CD. If the stability constants of the guest-cyclodextrin complex and the TNS-cyclodextrin complex are similar, the guest will displace the TNS from the cavity and cause a reduction in the observed fluorescence intensity.



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This change in fluorescence on addition of the guest can be used to calculate the stability constant, K, for the guest-cyclodextrin complex. Typically, the cyclodextrin concentration used in these experiments is *ca.* 10^{-5} mol dm^{-3} and for there to be a significant reduction in the fluorescence, the guest needs to displace a substantial portion of the TNS from the cavity of the cyclodextrin. Based on equation (1) for there to be a 50% reduction in fluorescence, the guest would need to displace half of the TNS from the cavity of the cyclodextrin. Therefore, the guest concentration must be equal to the reciprocal of the stability constant for the guest-cyclodextrin complex. So if the stability constant for the guest-cyclodextrin complex is between 1000 and 10000 M^{-1}, the concentration of guest in these experiments would need to be between 10^{-3} to 10^{-4} mol

dm^{-3} to be successful. However, preliminary experiments indicated the solubilities of the cycloadducts to be much less then 10^{-4} mol dm^{-3} suggesting this method is unsuitable for determining the stability constants in this system. Once again an alternative method for determining the solubilities of the isoxazolines **11**, **12**, **13**, **14**, **20**, **21**, **22** and **23** in the absence and presence of β-CD in 20% aqueous ethanol needed to be found.

Lehn *et al.*[124] and Laufer *et al.*[125] have both published methods for determining stability constants of cyclodextrin inclusion complexes using nmr spectroscopy. Complexation of a guest within the cavity of β-CD causes a change in the chemical environment of that guest which may result in a change in the chemical shift in part or all of the guest's nmr spectrum. This variation may be either upfield or downfield depending on the proximity of the guest's protons to either the protons or oxygens within the β-CD cavity. If the chemical shift difference is determined across a series of cyclodextrin concentrations and those chemical shift values are plotted against the β-CD concentration, a curve is produced. Non-linear regression analysis of that curve combined with equations (1) and (3) can then be used to determine the stability constant of the inclusion complex.

$$K = \frac{[\text{complex}]}{[\text{guest}]\,[\text{cyclodextrin}]} \qquad (1)$$

$$\delta_{observed} = \frac{([\text{guest}]\,\delta_{free} + [\text{complex}]\,\delta_{included})}{([\text{guest}] + [\text{complex}])} \qquad (3)$$

To test the viability of the nmr method, a saturated solution of the isoxazoline **11** in deuterated 20% aqueous ethanol solution was prepared and a ^1H nmr spectrum was acquired. The resulting spectrum did not contain any signals that could be attributed to the isoxazoline **11** suggesting it has a very low solubility in this particular solvent system. Deuterated 20% aqueous ethanol was therefore not a suitable solvent for these experiments.

Given that the polarity of methanol is approximately midway between that of ethanol and water,[126] it was thought a solution of 50% aqueous methanol could be used as an alternative solvent system. Although a compromise, it was hoped that the isoxazolines would be more soluble in this solvent and allow stability constants to be determined using the ^{1}H nmr method described above. The preliminary investigations were conducted using the isoxazolines **11** and **12**. Analysis of the ^{1}H nmr spectra obtained for the isoxazoline **11** in deuterated 50% aqueous methanol in the presence and absence of β-CD indicated that the aromatic protons and the C-4 and C-5 protons had experienced a very small change in chemical shift. Analysis of the ^{1}H nmr spectra obtained for the isoxazoline **12** in deuterated 50% aqueous methanol in the presence and absence of β-CD did not show a difference in chemical shift. The change in chemical shift observed in the ^{1}H nmr spectrum of the isoxazoline **11** suggested there might be a change in the chemical environment of the aromatic, C-4 and C-5 protons on inclusion within the cyclodextrin. The next step was to determine the chemical shift differences across a range of cyclodextrin concentrations. To each nmr tube was added a *ca.* 5×10^{-7} M solution of the isoxazoline **11**. The required amount of β-CD was added to each nmr tube so that there was a series of solutions containing between 0 and 15 equivalents of cyclodextrin. The ^{1}H nmr spectrum of each solution was obtained and the chemical shift of the C5-H for each solution graphed against the cyclodextrin concentration. As mentioned above, the plot of chemical shift change against cyclodextrin concentration usually produces a curve from which non-linear regression analysis is used to determine the stability constant of the inclusion complex. However, the graph of the chemical shift change against cyclodextrin concentration for the isoxazoline **11** gave a straight line suggesting the chemical shift change observed in the ^{1}H nmr spectra in the above experiments either resulted from something other than complexation by the cyclodextrin or the isoxazoline-cyclodextrin complex has a low stability constant. To investigate whether the change in chemical shift observed for the isoxazoline **11** in the presence of β-CD is due to complexation, the chemical shift change for a solution containing half the isoxazoline **11** and β-CD concentrations was determined. From equation (1) above, if the cyclodextrin and isoxazoline concentrations are halved, the concentration of the complex formed will

be reduced by a factor of four. From equation (3), if the concentration of the guest and cyclodextrin are reduced by a factor of two and as a result the concentration of the complex is reduced by a factor of four, then the change in chemical shift observed will therefore be reduced by a factor of two. The chemical shift observed for the C-5 proton on the isoxazoline **11** (5×10^{-4} M) in the absence of β-CD is 6.21 ppm. In the presence of β-CD (8×10^{-3} M) the chemical shift observed for the same proton on the isoxazoline **11** is 6.05 ppm, giving a chemical shift difference of 0.16 ppm. On that basis, if the change in chemical shift experienced in these experiments is due to complexation, repeating the experiment with half the original guest and β-CD concentration should result in a change in chemical shift of 0.08 ppm, giving a chemical shift for the C-5 proton of the isoxazoline **11** of approximately 6.13 ppm. However, when the experiment with half the original guest and β-CD concentration (2.5×10^{-4} and 4×10^{-3} M, respectively) was repeated the chemical shift observed for the C-5 proton of the isoxazoline **11** is 6.01 ppm or a change in chemical shift of 0.2 ppm. This would suggest that the chemical shift difference observed in these experiments is not due to complexation of the isoxazoline **11** by β-CD. Therefore, the ^{1}H nmr method was not suitable for determining stability constants in this case.

The ^{1}H nmr method was not pursued further because of the problems discussed above and ideally, to be directly relevant, a 20% aqueous ethanol solvent system should be used to determine the solubilities and stability constants of the complexes formed between the isoxazolines **11**, **12**, **13**, **14**, **20**, **21**, **22** and **23** and β-CD.

As discussed above, to calculate a stability constant for a guest-cyclodextrin inclusion complex, it is necessary to know the concentration of the guest, the concentration of the complex and the cyclodextrin concentration at equilibrium. Once determined, these values can be substituted into equation (1) and the stability constant calculated. The most significant problem encountered in determining the solubilities of the isoxazolines **11**, **12**, **13**, **14**, **20**, **21**, **22** and **23** in 20% aqueous ethanol by the methods discussed above had been that the quantities of the isoxazolines in solution are below the limits of

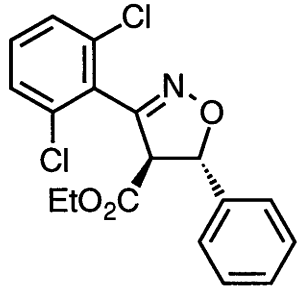
detection of the analytical methods used. It was thought that this problem could be overcome by preparing saturated solutions of each isoxazoline on a large scale, followed by extraction of the isoxazolines into an organic solvent. This procedure could then be repeated at a known cyclodextrin concentration. This would allow the concentrations of free guest and complex to be determined. To address the problems associated with supersaturation, non-homogenous solutions and the analytical method, the solutions were equilibrated for a longer period of time, after which they were filtered more extensively through smaller porosity filters, and the analysis was conducted by HPLC.

Saturated solutions of each regioisomeric pair of the isoxazolines **11**, **12**, **13**, **14**, **20**, **21**, **22** and **23** in aqueous ethanol were prepared and equilibrated at 30 °C for 24 h. The cloudy suspensions were filtered, firstly through a 0.2 μm syringe filter, then through a 0.02 μm syringe filter, to remove excess isoxazoline, and the resulting solutions were extracted with ethyl acetate. The ethyl acetate was removed under reduced pressure and the residue was taken up in acetonitrile to which a standard had been added. The isoxazoline **11** was used as a standard for the experiments involving the isoxazolines **20**, **21**, **22** and **23** and the isoxazoline **20** was used as a standard for the experiments involving the isoxazolines **11**, **12**, **13** and **14**. To enable the quantities of each isoxazoline present in the HPLC samples to be calculated, HPLC response ratios or peak areas per mole of isoxazoline were determined. This was achieved by injecting a known amount of each pair of isoxazolines and determining the resulting peak area for each regioisomer. The relative and absolute solubilities of the isoxazolines **11** and **12**, **13** and **14**, **20** and **21**, and **22** and **23** in 20% aqueous ethanol in the absence of β-CD were determined by HPLC and are presented in Table 5. The experiments and calculations of solubilities were carried out in triplicate and were found to be reproducible within experimental error.

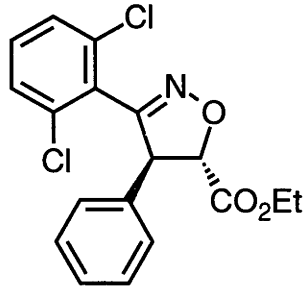
Isoxazoline	Solubility (mol dm^{-3})	Relative Solubility
11	1.1×10^{-5}	1:1.6 **11:12**
12	1.8×10^{-5}	
13	2.8×10^{-6}	1:6 **13:14**
14	1.7×10^{-5}	
20	9.6×10^{-9}	1:510 **20:21**
21	4.9×10^{-6}	
22	6.7×10^{-4}	2.7:1 **21:23**
23	2.5×10^{-4}	

Table 5: Relative and absolute solubilities for the isoxazolines **11**, **12**, **13**, **14**, **20**, **21**, **22** and **23** in 20% aqueous ethanol in the absence of β-CD at 30 °C.

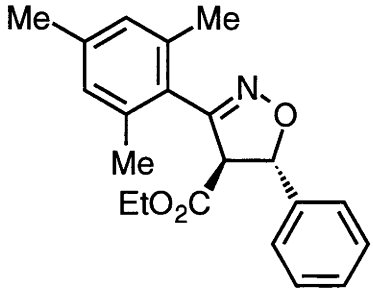
The solubilities of the isoxazolines **11**, **12**, **13**, **14**, **20**, **21**, **22** and **23** shown in Table 5 clearly show that the decision to abandon the TNS displacement method was correct. As discussed above for there to be a substantial reduction in fluorescence, an isoxazoline concentration of between 10^{-3} and 10^{-4} M was required for the method to be successful. However, as indicated in Table 5, only the isoxazolines **22** and **23** have solubilities within this range while the solubilities of the isoxazolines **11**, **12**, **13**, **14**, **20** and **21** are well below 10^{-4} M.



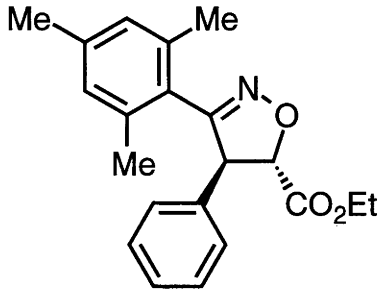
(±)-**11**



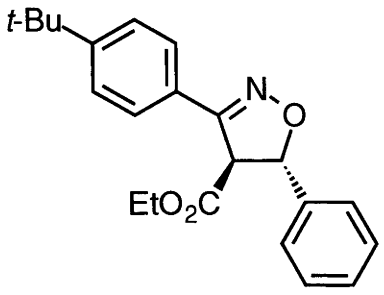
(±)-**12**



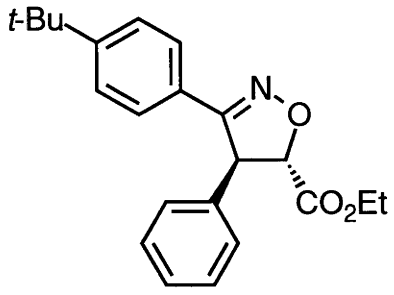
(±)-**13**



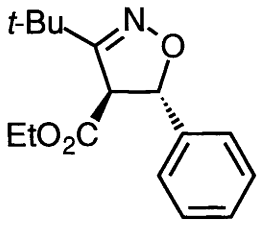
(±)-**14**



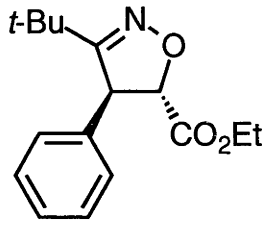
(±)-**20**



(±)-**21**



(±)-**22**



(±)-**23**

The solubilities of the isoxazolines **12**, **14** and **21** in the aqueous ethanol solution, as detailed in Table 5, were found to be significantly higher than those of their corresponding regioisomers, the isoxazolines **11**, **13** and **20**. The solubility of the isoxazoline **22** in the aqueous ethanol solution was found to be higher than that of the isoxazoline **23**, a reversal of the trend experienced for the other three regioisomeric pairs.

The solubility of a compound in an aqueous solution depends on its extent of solvation by water molecules. Presumably, in these cases it would be the extent of solvation of the ester groups on the isoxazolines **11**, **12**, **13**, **14**, **20**, **21**, **22** and **23** that would be responsible for their aqueous solubility. The ester moieties of the isoxazolines **11**, **13** and **20** are less readily solvated because the approach of the water molecules is hindered by the large aryl substituent at C-3 on the isoxazoline ring. As a result, the isoxazolines **11**, **13** and **20** are less soluble in the aqueous ethanol solution than their corresponding regioisomers. However, the isoxazoline **23** is more soluble in aqueous ethanol than its regioisomer, compound **22**. This is a reversal of the trend observed above for the other three pairs of isoxazolines and occurs because the *tert*-butyl moiety at C-3 on the isoxazolines **22** and **23** does not hinder the solvation by water of the ester moiety at C-4

as much as the bulky aryl substituents have in the isoxazolines **11**, **13** and **20**. Another trend identified in these experiments was that the isoxazolines **22** and **23** were found to be the most soluble in aqueous ethanol. The isoxazolines **11** and **12** were the next most soluble, followed by the isoxazolines **13** and **14** and finally the least soluble were the isoxazolines **20** and **21**.

The solubilities of the isoxazolines **11**, **12**, **13**, **14**, **20**, **21**, **22** and **23** in the presence of β-CD were determined. This was achieved by the method described above in a 0.05 M solution of β-CD in 20% aqueous ethanol. In these experiments the isoxazolines in the aqueous ethanol solutions were not extracted into ethyl acetate, rather the samples were injected directly onto the HPLC, allowing the quantities of isoxazolines present in the samples to be determined directly. The relative and absolute solubilities of the isoxazolines **11** and **12**, **13** and **14**, **20** and **21** and **22** and **23** in 20% aqueous ethanol in the presence of β-CD are presented in Table 6. The experiments and calculations of solubilities were carried out in triplicate and were found to be reproducible within experimental error.

Isoxazoline	Solubility (mol dm^{-3})	Relative Solubility
11	9.9×10^{-5}	5.5:1 **11:12**
12	1.8×10^{-5}	
13	5.2×10^{-5}	274:1 **13:14**
14	1.9×10^{-7}	
20	1.1×10^{-5}	1:9.1 **20:21**
21	1.0×10^{-4}	
22	4.1×10^{-3}	2.4:1 **22:23**
23	1.7×10^{-3}	

Table 6: Relative and absolute solubilities for the isoxazolines **11**, **12**, **13**, **14**, **20**, **21**, **22** and **23** at 30 °C in 0.05 M β-CD/20% aqueous ethanol solution.

As indicated in Table 6, the apparent solubility of the isoxazoline **14** was substantially less in the presence of β-CD than in its absence. It was thought that in the presence of β-

CD, the resulting complex formed between the cyclodextrin and the isoxazoline **14** has a low solubility and as such is filtered out of solution. To test this hypothesis, the relative and absolute solubilities of the isoxazolines **13** and **14** were determined at a reduced cyclodextrin concentration. In this case the quantity of cyclodextrin in the experiment was reduced by 75%. The results obtained are detailed in Table 7.

Isoxazoline	Solubility (mol dm^{-3})	Relative Solubility
13	1.3×10^{-5}	23:1 **13:14**
14	5.7×10^{-7}	

Table 7: Relative and absolute solubilities for the isoxazolines **13** and **14** at 30 °C in 0.013 M β-CD/20% aqueous ethanol solution.

2.3 Discussion of Results

The apparent solubility of the isoxazoline **14** in the presence of a reduced concentration of β-CD did indeed increase, however, it was still well below the absolute solubility of the isoxazoline **14** in aqueous ethanol in the absence of β-CD. In the presence of an excess of the isoxazoline **14**, β-CD would continue to complex with the isoxazoline **14** and precipitate until all of the cyclodextrin had been removed from solution. Therefore, once all the cyclodextrin is removed through complexation, the amount of the isoxazoline **14** present in solution would be equal to its absolute solubility in 20% aqueous ethanol, which is 1.7×10^{-5} mol dm^{-3}. The result above clearly shows that the isoxazoline **14** was not in excess in this experiment. However, this evidence does suggest that the complex formed between the isoxazoline **14** and β-CD has a low solubility in the aqueous ethanol solution and as such is filtered out prior to analysis of the samples by HPLC. By contrast, when the concentration of β-CD is reduced by a factor of four, the apparent solubility of the isoxazoline **13** also falls by a factor of four to 1.3×10^{-5} mol dm^{-3}. This suggests the complex formed between the isoxazoline **13** and β-CD is more soluble in aqueous ethanol and as such remains in solution. Further experiments with excess isoxazoline **14** would be pointless because the insoluble complex would precipitate out of solution removing the cyclodextrin and ultimately producing a solution

saturated with guest but in the absence of cyclodextrin. It cannot be discounted that similar complex precipitation is not occurring to some extent in the experiments conducted with the isoxazolines **11**, **12**, **20**, **21**, **22** and **23**. However, in the experiments involving the isoxazolines **11**, **12**, **20**, **21**, **22** and **23**, the presence of β-CD does increase the solubility of the isoxazolines. The precipitation of complex from solution may in fact be responsible for the inaccuracies experienced in the UV-Vis experiments described earlier.

As discussed in the Introduction, when Hughes *et al.*[89] incubated the isoxazolines **11** and **12**, **13** and **14**, **20** and **21**, and **22** and **23** in aqueous ethanol in the presence of β-CD under Rama Rao's reaction conditions and extracted these solutions with chloroform, the isoxazolines **12**, **14**, and **21** were preferentially extracted while no preferential extraction was observed with the isoxazolines **22** and **23**. The greatest preferential extraction was experienced with the mixture of the isoxazolines **20** and **21**, followed by the isoxazolines **13** and **14**, and then the isoxazolines **11** and **12**.

As shown in Table 5, the isoxazoline **21** is more soluble in the aqueous ethanol solution than its regioisomer, the isoxazoline **20**, in the absence of β-CD. In the presence of β-CD, the solubility of the isoxazoline **20** increased significantly while the solubility of the isoxazoline **21** increased slightly. This suggests the isoxazoline **20** forms a more stable complex with β-CD than does the isoxazoline **21**. An equilibrium between each isoxazoline and β-CD will exist in the 20% aqueous ethanol solution (Figure 5). The ratio of isoxazolines free in solution and available for extraction by chloroform will depend on their solubility in the aqueous ethanol solution and the selectivity of binding by the cyclodextrin. The isoxazoline **21** is less readily complexed by β-CD and is approximately 500 times more soluble in aqueous ethanol than the isoxazoline **20**, as a consequence the equilibria establish so that the ratio of the isoxazolines **20** and **21** in the aqueous ethanol solution is enriched with the isoxazoline **21**. When the aqueous ethanol solution is extracted with chloroform, the isoxazolines quickly pass into the chloroform

layer, in a ratio reflecting that in the aqueous ethanol, thus resulting in the preferential extraction of the isoxazoline **21**.

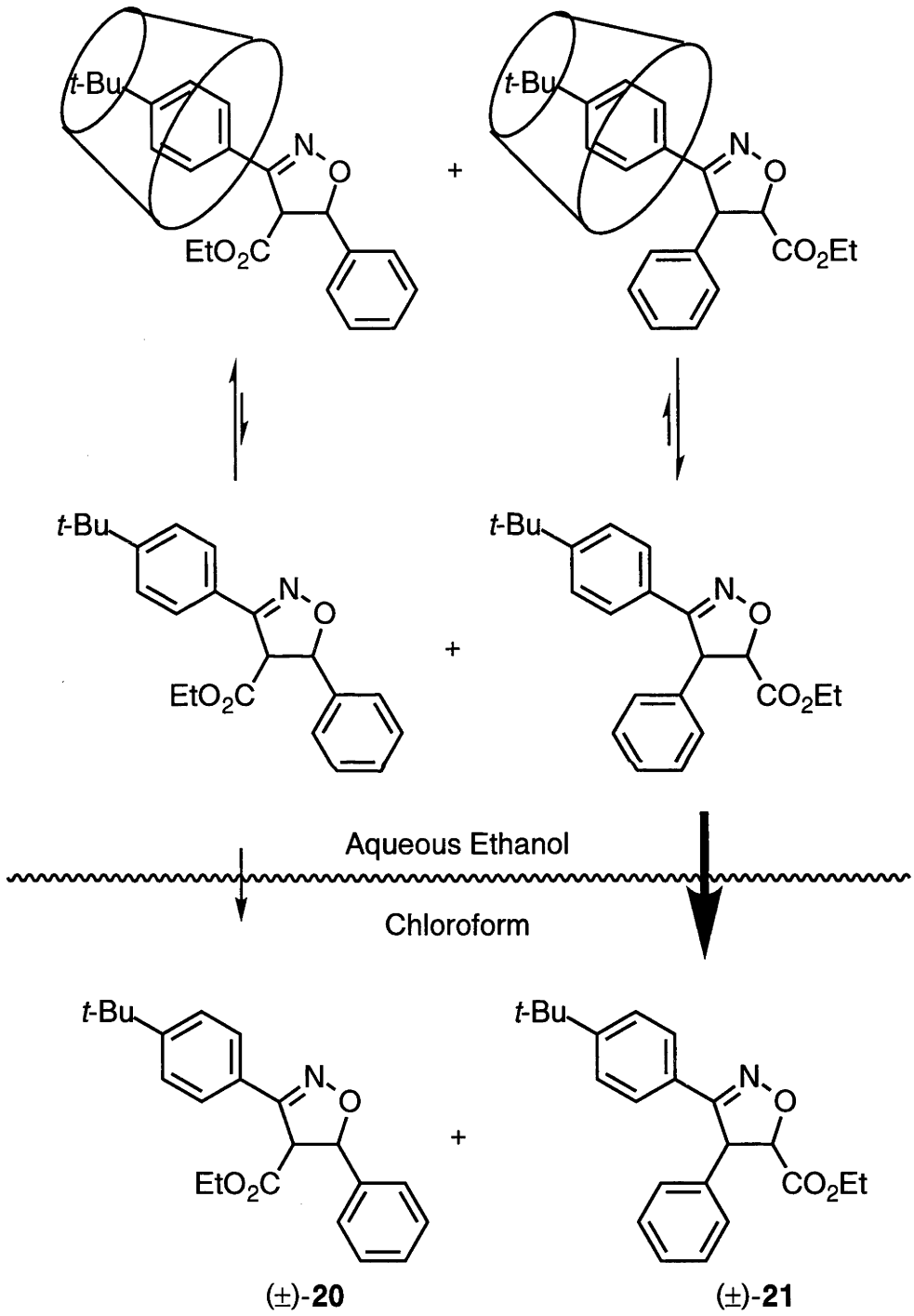


Figure 5: Preferential extraction of the isoxazoline **21** from a mixture of the isoxazolines **20** and **21** in the presence of β-CD.

A similar rational can be used to explain the preferential extraction of the isoxazoline **12** from a mixture of the isoxazolines **11** and **12**. The isoxazoline **12** is slightly more soluble in the aqueous ethanol solution than its regioisomer, the isoxazoline **11**, in the absence of β-CD. In the presence of β-CD, the solubility of the isoxazoline **11** increased significantly while the solubility of the isoxazoline **12** does not increase. This suggests the isoxazoline **11** forms a more stable complex with β-CD than does the isoxazoline **12**. As discussed above, an equilibrium will exist between each isoxazoline and β-CD in the 20% aqueous ethanol solution and the ratio of isoxazolines free in solution and available for extraction by chloroform will depend on the their solubility in the aqueous ethanol solution and the selectivity of binding by the cyclodextrin. The isoxazoline **12** is less readily complexed by β-CD and is approximately 2 times more soluble in aqueous ethanol than the isoxazoline **11**, as a consequence the equilibria establish so that the ratio of the isoxazolines **11** and **12** in the aqueous ethanol solution is enriched with the isoxazoline **12**. When the aqueous ethanol solution is extracted with chloroform, the non-complexed isoxazolines quickly pass into the chloroform layer thus resulting in the preferential extraction of the isoxazoline **12**. The extent to which the isoxazoline **12** is preferentially extracted from a mixture of the isoxazolines **11** and **12** is less than the selectivity experienced for the isoxazolines **20** and **21** because the absolute solubilities of the isoxazolines **11** and **12** in aqueous ethanol are higher and the difference in relative solubilities is much smaller than that seen with the absolute and relative solubilities for the isoxazolines **20** and **21**.

The isoxazoline **22** is slightly more soluble in the aqueous ethanol solution than its regioisomer, the isoxazoline **23**, in the absence of β-CD. In the presence of β-CD, the solubilities of the isoxazolines **22** and **23** both increased significantly, however, the isoxazoline **22** is still the more soluble of the two compounds. This suggests the isoxazoline **22** forms a similarly stable complex with β-CD to that of the isoxazoline **23**. As discussed above, an equilibrium will exist in the 20% aqueous ethanol solution between each isoxazoline and β-CD and the ratio of isoxazolines free in solution and available for extraction by chloroform will depend on the their solubility in the aqueous

ethanol solution and the selectivity of binding by the cyclodextrin. However in this example, the absolute solubility of each isoxazoline in aqueous ethanol is relatively high and the binding exhibited by the cyclodextrin is not selective, so there is no preferential extraction of one isomer over the other.

However, the explanation presented above for the preferential extraction of one isoxazoline from a mixture of isoxazolines observed initially by Rama Rao *et al.*[84-88] and later by Hughes *et al.*[89] does have limitations. For example, previous experiments discussed in this Chapter have shown that when a mixture of the isoxazolines **13** and **14** are present in a solution containing β-CD, the isoxazoline **14** forms an insoluble complex with the cyclodextrin and precipitates out of solution. Therefore, the preferential extraction of the isoxazoline **14** observed cannot proceed *via* the equilibrium suggested in Figure 5. To explain the preferential extraction of compound **14**, it is thought that the complex between the isoxazoline **14** and β-CD precipitates and then migrates into the organic layer where the isoxazoline **14** is extracted directly into chloroform.

It cannot be stated with certainty that either explanation is solely responsible for the extraction effect observed in these systems. For example, in the extraction experiments involving the isoxazolines **11** and **12**, and **20** and **21** it is possible that solid complexes may be formed that, once present in the organic layer, allow the isoxazoline to be abstracted directly by chloroform. However, there is no experimental evidence to indicate this. Similarly, it cannot be completely discounted that some of the isoxazoline **14**-β-CD complex may remain in the aqueous layer and facilitated the extraction of the isoxazoline **14** *via* the mechanism detailed in Figure 5.

In conclusion, Rama Rao *et al.*[84-88] suggested that bakers' yeast was required for the cycloaddition reactions to occur and the addition of β-CD reversed the regioselectivity observed. However, Hughes *et al.*[89] and work presented in this Chapter has shown that the cycloaddition reaction proceeded in good yield in the absence of bakers' yeast and that the regioisomeric resolution observed in the presence of β-CD results from a difference in

relative solubilities of the isoxazoline regioisomers in the aqueous ethanol solution and the selectivity of complexation of each isoxazoline by β-CD.

RESULTS AND DISCUSSION CHAPTER TWO

β-CD-Chloroform Complexation

During the investigations into the viability of using a UV method for determining the stability constants of the complexes formed between the isoxazolines **11**, **12**, **13**, **14**, **20**, **21**, **22** and **23** and β-CD discussed in Chapter One, it was found that when an aqueous solution containing β-CD was mixed with chloroform an insoluble material formed at the interface. This work involved monitoring the extraction of isoxazolines from aqueous solution by positioning the UV beam through the chloroform layer and monitoring the change in absorbance. To do this chloroform was added to a UV cell. The aqueous ethanol solution containing a mixture of isoxazolines and β-CD was carefully layered over the chloroform and the cell was placed in the spectrometer. After short periods of time a white precipitate formed at the interface of the two phases. The water solubilities of β-CD and other CDs are well known and had not been exceeded,[127] therefore, it was thought that the most likely explanation for the formation of the precipitate was complexation between β-CD and chloroform. To test this hypothesis, the precipitate was collected by filtration, dried under vacuum and its structure determined by analysis of its ^{1}H nmr spectrum taken in deuterated DMSO. Analysis of the ^{1}H nmr spectrum indicated that the precipitate isolated from the UV cell was β-CD. Furthermore, there was no evidence of the presence of chloroform in the nmr sample suggesting that it is removed from the cavity of the CD during the drying process. An unsuccessful attempt was made to grow and isolate a sample of the crystalline material for X-ray analysis. The crystals developed and grew in the presence of chloroform, however, once removed for this atmosphere the crystals quickly become opaque and crack. Analysis of the ^{1}H nmr spectrum indicated no evidence of the presence of chloroform in the dried sample.

French *et al.*[128] have studied a variety of CD precipitants including halogenated hydrocarbons such as chloroform to isolate CDs from aqueous solutions. α-, β- and γ-CD were prepared by the *macerans* amylase catalysed digestion of starch. The yield and the proportion of each CD was controlled by varying the enzymolysis conditions such that each CD could be prepared preferentially. After enzymolysis, the CDs were removed from the aqueous solutions as the insoluble complexes of either chloroform, toluene or trichloroethylene. French *et al.* determined the solubility of the α-CD-chloroform complex to be 0.8g/100 ml of aqueous solution and the solubility of the β-CD-chloroform complex to be 0.07g/100 ml of aqueous solution.

To further investigate the phenomenon observed in the work described in Chapter One, chloroform was added to a series of solutions containing either α-CD or β-CD. Two methods of introducing chloroform into the aqueous solutions were investigated.

The first involved layering the aqueous solution directly onto the chloroform layer. The required volume of chloroform was added to a test tube. To this was carefully added milliQ water containing the CD and the resulting 2-phase solution was allowed to stand undisturbed for several days. After this time, a white precipitate formed at the water-chloroform interface in the samples which contained β-CD. It was collected by filtration and analysed by ^{1}H nmr spectroscopy. The quantities of CD used in each experiment and the results obtained are presented in Table 8.

Mass of CD (g)	Volume of chloroform (ml)	Volume of milli Q water (ml)	Mass of material recovered (g)	[CD] mg/ml	Yield
0.05 α-CD	10	10	0	5	0%
0.10 α-CD	10	10	0	10	0%
0.15 α-CD	10	10	0	15	0%
0.05 β-CD	10	10	0.008	5	16%
0.10 β-CD	10	10	0.012	10	12%
0.15 β-CD	10	10	0.049	15	33%

Table 8: Percentage recovery of α-CD and β-CD from aqueous CD solutions layered on chloroform

A modest recovery of precipitate occurred in samples containing β-CD while no precipitate was recovered from samples containing α-CD. Analysis of the recovered β-CD by ^{1}H nmr in d^{6}DMSO showed that the isolated product was free of chloroform and not altered by the complexation. The amount of α-CD and β-CD recovered from these experiments is less than might have been expected, based on the solubility limits of the chloroform complexes determined by French *et al.*[128] However, the selective recovery of β-CD is consistent with the lower solubility recorded for the chloroform complex of that species.

The second method of introducing chloroform involved diffusing its vapour over the aqueous solution in a desiccator. Aqueous solutions of α-CD and β-CD were prepared as shown in Table 9 and were placed in a desiccator containing a beaker of chloroform. The desiccator was placed under vacuum and left for several days. Any precipitate formed was then collected by filtration, dried and analysed by ^{1}H nmr spectroscopy. The quantities of CD recovered are shown in Table 9.

Mass of CD (g)	Volume of milli Q water (ml)	Mass of material recovered (g)	[CD] mg/ml	Yield
0.25 α-CD	50	0	5	0%
0.50 α-CD	50	0	10	0%
0.75 α-CD	50	0	15	0%
0.25 β-CD	50	0.149	5	60%
0.50 β-CD	50	0.428	10	86%
0.75 β-CD	50	0.588	15	78%

Table 9: Percentage recovery of α-CD and β-CD from aqueous CD solutions exposed to a atmosphere of chloroform.

Again, precipitate was only recovered from solutions which contained β-CD while no precipitate was recovered from samples containing α-CD. Analysis of the recovered β-CD by ^{1}H nmr in d^{6}DMSO showed that the isolated product was free of chloroform and not altered by the complexation.

It seemed likely that similar experiments could be used to separate mixtures of α-CD and β-CD in aqueous solution. Aqueous solutions containing mixtures of α-CD and β-CD were prepared as shown in Table 10. These solutions were placed in a desiccator, a beaker of chloroform was added and the desiccator was placed under vacuum and left for several days. Any white precipitate was then collected by filtration and dried. The purity of the material was assessed by ^{1}H nmr spectroscopy and the composition of the solids isolated was determined by HPLC. The results are presented in Table 10.

Mass of α-CD (g)	Mass of β-CD (g)	Volume of water (ml)	Mass recovered	Composition	Yield %
0.125	0.125	50	0.073 g	β-CD	58
0.25	0.25	50	0.111 g	β-CD	44
0.50	0.50	50	0.322 g	β-CD	64
0.75	0.75	50	0.593 g	β-CD	79
2.00	0.50	50	1.053 g	Mix	29 β-CD 71 α-CD
0.25	0.50	50	0.285 g	β-CD	57

Table 10: Percentage recovery of α-CD and β-CD from aqueous solutions containing mixtures of α-CD and β-CD exposed to an atmosphere of chloroform.

Once again analysis of the recovered material by ^{1}H nmr in d^{6}DMSO showed that the isolated product was free of chloroform and not altered by the complexation, suggesting that, once the complex is removed from the solution saturated by the solvent, the chloroform readily leaves the cavity of the CD.

The separations observed above result from the differences in the solubilities of the complexes formed between α-CD and β-CD and chloroform. For a CD precipitate to form there must firstly be sufficient quantities of both chloroform and CD present in solution so that the equilibrium shown in Figure 6 lies mostly to the right.

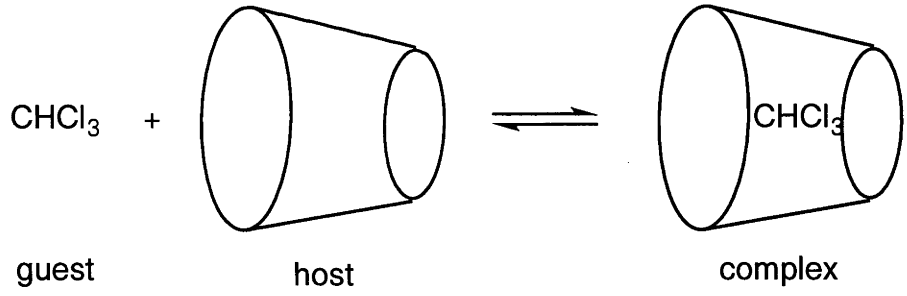


Figure 6: Equilibrium of the complexation between chloroform and CD.

When the equilibrium position has been reached, a precipitate will begin to form when there is enough complex present to exceed its solubility. As indicated above the α-CD-chloroform complex is approximately 11 times more soluble in aqueous solution than the β-CD-chloroform complex. Therefore in the experiments described above, precipitates were isolated from only those solutions in which sufficient quantities of chloroform, β-CD and in one case α-CD were present for enough complex to form so that the solubilities of the β-CD-chloroform complex and the α-CD-chloroform complex were exceeded.

A possible application of this work could be in the industrial separation of α-, β- and γ-CD. These CDs are produced industrially as mixtures by the enzyme CD glucosyltransferase.[129] β-CD is isolated from these mixtures either by enzymatic digestion of the other CDs or by precipitation using aromatic hydrocarbons. Neither method is ideal. For example, enzymatic digestion leads to the loss of α- and γ-CD while aromatic hydrocarbons are difficult to completely remove from the cavity of the CDs once the separation is complete. Therefore, a useful industrial method for the separation of β-CD from mixtures of other CDs could be developed utilising chloroform. This method would not result in the loss of non-target CDs such as α- and γ-CD as is the case during enzymatic digestion. Furthermore, chloroform readily leaves the cavity once the complex is removed from the chloroform atmosphere.

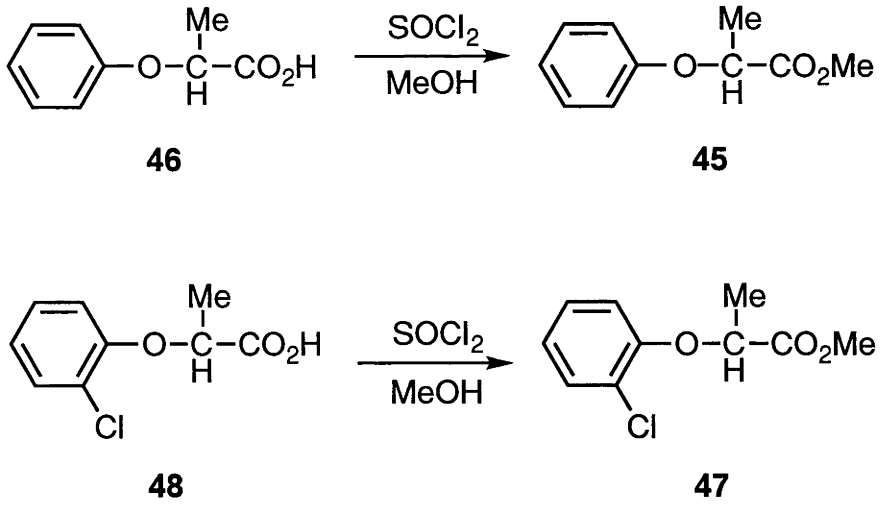
RESULTS AND DISCUSSION CHAPTER THREE

The Effect of BSA and β-CD on the Hydrolysis of Aryloxypropionates

As discussed in Chapter One, Rama Rao *et al.*[84-88] suggested that β-CD and bakers' yeast were simultaneously binding reactants and co-ordinating their interaction in a cycloaddition. Similarly, Kamal *et al.*[90] have suggested that BSA and β-CD simultaneously affect both the extent and enantioselectivity of the hydrolysis of aryloxypropionates. As discussed in the Introduction, Kamal *et al.*[90] reported that BSA catalysed the hydrolysis of a variety of aryloxypropionates enantioselectively. Furthermore, the addition of β-CD to these reactions not only served to improve the enantioselectivity, but also to accelerate the rate of hydrolysis. There are numerous reports of β-CD[130] and modified cyclodextrins[131] acting enantioselectively, but not to the extent suggested by Kamal *et al.*[90] In addition, it was difficult to see how BSA and β-CD would jointly affect the reactions of aryloxypropionates. These concerns and those put forward in the Introduction, as well as the conclusion that Rama Rao's claims could not be substantiated, prompted a re-investigation of the work of Kamal *et al.*[90]

4.1 Synthesis of the Phenoxypropionates 45 and 47

Kamal *et al.*[90] investigated the hydrolysis of a wide variety of aryloxypropionates, including the methyl esters **45** and **47**. Therefore, these compounds were chosen to re-investigate the influence of BSA and β-CD. The commercially available acids **46** and **48** were converted to the corresponding methyl esters **45** and **47** in methanol that had been acidified by treatment with thionyl chloride (Scheme 49).

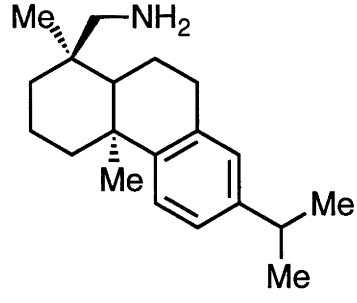


Scheme 49

The phenoxypropionates **45** and **47** were obtained as pale yellow oils in 100% yield in each case. All physical and spectroscopic data obtained for compounds **45** and **47** are consistent with those reported.[132]

4.2 Synthesis of Enantiopure (R)-2-Phenoxypropionic Acid (R)-(46)

To allow the stereochemistry of the enantiomers produced from the hydrolysis of the methyl ester **45** to be accurately assigned by HPLC it was necessary to prepare an enantiopure sample of the acid **46**. Two methods were considered to produce enantiopure (*R*)-2-phenoxypropionic acid (*R*)-(**46**). The use of amines as chiral resolving agents is well documented in the literature. In fact, Kamal *et al.*[90] cited a literature article in which dehydroabietylamine (**76**) had been successfully used to resolve racemic 2-phenoxypropionic acid **46**.[133]



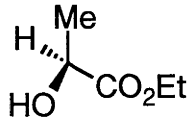
76

This method relies on a difference in physical properties of the diasteromeric salts resulting from the complexation of the racemate with the chiral amine. In this case the

dehydroabietylamine-(*R*)-2-phenoxypropionic acid complex has a lower solubility in aqueous methanol than the (*S*)-enantiomer complex and forms crystals.

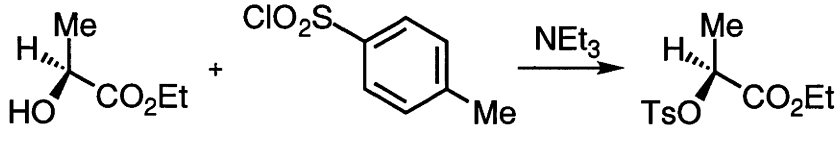
However, addition of dehydroabietylamine (**76**) to racemic 2-phenoxypropionic acid (**46**) resulted in only a very low yield of crystalline material. No further attempts were made to produce (*R*)-2-phenoxypropionic acid (*R*)-(**46**) *via* this method.

Alternatively, (*R*)-2-phenoxypropionic acid (*R*)-(**46**) can be synthesised stereoselectivly from (*S*)-ethyl lactate (**77**).[134]



77

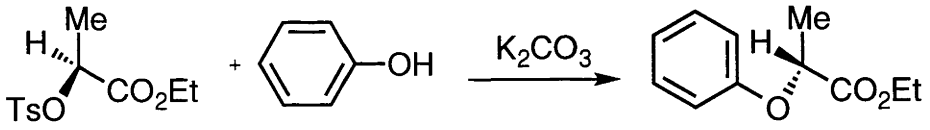
(*S*)-Ethyl lactate (**77**) was stirred with *p*-toluenesulfonyl chloride (**78**) in the presence of base to give the (*S*)-tosylate **79** as a pale yellow oil in 58% yield (Scheme 50). The spectroscopic data obtained for compound **79** are consistent with those reported.[135]



77 **78** **79**

Scheme 50

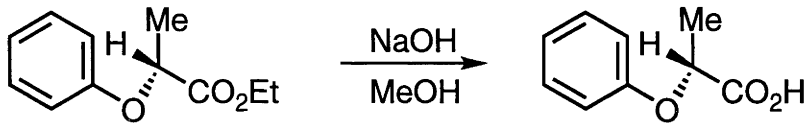
The (*S*)-tosylate **79** underwent an S_N2 displacement reaction with phenol **80** in the presence of potassium carbonate to give the crude propionate **81** in 92% yield (Scheme 51). The S_N2 displacement reaction proceeds with complete inversion of stereochemistry at the chiral centre.[136] Therefore, (*S*)-tosylate **79** is converted to the (*R*)-propionate **81**. The spectroscopic data obtained for compound **81** are consistent with those reported.[137]



79 **80** **81**

Scheme 51

The (*R*)-propionate **81** was hydrolysed to the corresponding (*R*)-2-phenoxypropionic acid (*R*)-(**46**) with 10 M NaOH in 45% yield (Scheme 52).



81 (*R*)-**46**

Scheme 52

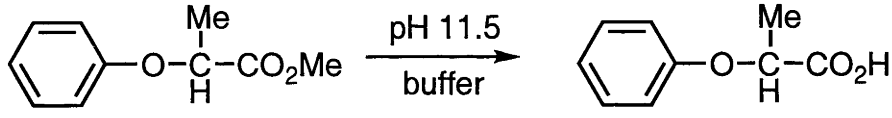
The ^{1}H nmr spectrum of the acid (*R*)-(**46**) obtained is consistent with that reported for the racemate.[138]

The (*S*)-ethyl lactate (**77**) used in the first step of this synthesis contained 1% (*R*)-ethyl lactate which would have been carried through the reactions described above to give a small amount of (*S*)-2-phenoxypropionic acid (*S*)-(**46**). This small amount of (*S*)-2-phenoxypropionic acid (*S*)-(**46**) was removed from the (*R*)-2-phenoxypropionic (*R*)-(**46**) by fractional crystallisation with *n*-propylamine. The *n*-propylamine complexes with the (*R*)-phenoxypropionic acid (*R*)-(**46**) to form insoluble crystals. The crystals were collected and treated with HCl to give (*R*)-2-phenoxypropionic acid (*R*)-(**46**) in 46% yield. The spectral data for (*R*)-2-phenoxypropionic acid (*R*)-(**46**) are identical to those obtained for the racemic compound **46** above. Analysis by HPLC using a chiral AGP column indicated only one enantiomer was present. As the synthetic sequence was performed using (*S*)-ethyl lactate (**77**) it is assumed that as a consequence of the chemistry the enantiomer produced is (*R*)-2-phenoxypropionic acid (*R*)-(**46**).

4.3 Investigations into the Hydrolysis of Aryloxypropionates

A series of experiments was conducted to determine the role of β-CD, BSA and the pH 11.5 phosphate buffer in the hydrolysis of the racemic ester **45** under the conditions described by Kamal *et al.*[90] The hydrolysis reactions were repeated in the absence of BSA and β-CD, in the presence of BSA, in the presence of β-CD and in the presence of both BSA and β-CD.

The methyl ester **45** (~ 300 mg) was added to 0.01 M pH 11.5 phosphate buffer (20 ml) with and without BSA and β-CD and the mixtures were stirred vigorously at 37 °C (Scheme 53). Given that the ester **45** has a low solubility in the phosphate buffer, the mixtures were stirred vigorously to ensure the ester **45** was mixed as thoroughly as possible in the aqueous solutions.



45 **46**

Scheme 53

Samples of the reaction mixtures were taken at 0 min and again after 24 h and to each was added an equal volume of ethanol followed by analysis by HPLC. The purpose of adding ethanol to the samples was two-fold. Firstly, because the ester **45** is only sparingly soluble in the phosphate buffer, the addition of ethanol is necessary to ensure that all available ester **45** is in solution prior to the samples being analysed. This ensures an accurate representation of the ester **45** content in the samples and hence indicates the true extent of hydrolysis. Secondly, ethanol was added to denature the BSA protein so that it would precipitate out of solution prior to analysis. The running solvent used in the analysis of samples was 40% methanol/pH 6 phosphate buffer, so there was a possibility that, once injected, the methanol in the HPLC running solvent would cause any BSA in the samples to precipitate out of solution and block the HPLC system. Even though BSA was not present in all of the reactions, ethanol was added to each to maintain consistency.

It was difficult to draw detailed conclusions from the results obtained from these experiments for two reasons. Firstly, all reactions stopped after approximately 15% of the ester **45** had been hydrolysed. Presumably, this occurs because the acid **46** formed from the hydrolysis of the ester **45** exceeds the buffering capacity of the phosphate buffer resulting in a lowering of the pH and as a consequence a slowing in the rate of hydrolysis. This was confirmed by monitoring the pH of the hydrolysis of the ester **45** in the absence of both BSA and β-CD. It was found that the pH of the reaction solution drops from 11.5 to 9 within 45 min, indicating that, under these conditions the hydrolysis of the ester **45** stops well before the reaction is sampled at 24 h. Secondly, the hydrolysis reactions are heterogenous because the ester **45** is sparingly soluble in the buffer solution. This makes accurate sampling difficult.

In order to increase the extent of reaction the quantity of the ester **45** in each reaction was reduced from 300 to 30 mg so as to reduce the potential for the buffering capacity of the phosphate buffer to be exceeded. Similarly, the ionic strength of the phosphate buffer was increased from 0.01 M to 0.1 M. To ensure the sampling technique was consistent throughout the duration of the experiment an external standard was added to each sample taken from the reaction mixture. The reactions were also stirred vigorously to ensure even distribution of the ester **45** throughout the reaction mixture.

The hydrolysis of racemic methyl 2-phenoxypropionate (**45**) (30 mg) was conducted in pH 11.5 phosphate buffer (20 ml) in the absence of both β-CD and BSA, in the presence of β-CD, in the presence of BSA and finally in the presence of both β-CD and BSA. For example, four separate heterogenous solutions of methyl 2-phenoxypropionate (**45**) in 0.1 M pH 11.5 phosphate buffer were prepared. The first solution was placed under a nitrogen atmosphere and stirred vigorously at 37 °C. To the second solution was added 0.2 mole equivalents with respect to the ester **45** of β-CD, to the third solution was added 0.03 mole equivalents with respect to the ester **45** of BSA and to the fourth solution was added 0.2 mole equivalents with respect to the ester **45** of β-CD and 0.03 mole equivalents with respect to the ester **45** of BSA. These three remaining solutions

were placed under a nitrogen atmosphere and stirred vigorously at 37°C. Each reaction was sampled at 0, 9, 18, 27 and 36 min. The samples were added to an equal volume of ethanol, a standard was added and each sample was filtered and analysed by HPLC with the results detailed in Table 11. The standard added to each sample prior to analysis was 1-naphthoic acid. To enable the quantities of the acid **46** present in the HPLC samples to be calculated, HPLC response ratios or peak areas per mole of acid **46** and standard were determined. This was achieved by injecting a known amount of the acid **46** and the 1-naphthoic acid standard and determining the resulting peak area for each compound.

Time (min)	No β-CD or BSA		With β-CD		With BSA		With β-CD and BSA	
	% Acid	% Ester	% Acid	% Ester	% Acid	% Ester	% Acid	% Ester
0	3	97	3	97	0	100	0	100
9	26	74	31	56	2	97	3	97
18	38	56	41	37	5	86	5	91
27	43	43	45	30	6	85	7	91
36	45	40	45	30	8	84	9	84

Table 11: The extent of hydrolysis of the ester **45** in the absence of both BSA and β-CD, in the presence of β-CD, in the presence of BSA and in the presence of both BSA and β-CD.

The hydrolysis of the ester **45** stopped after approximately 45% had been consumed suggesting that, despite reducing the amount of ester **45** and increasing the buffer strength, the buffering capacity of the phosphate buffer is still being exceeded. However, the results clearly show the hydrolysis of the phenoxypropionate **45** proceeds in the absence of both β-CD and BSA. The extent of hydrolysis in the absence of β-CD and BSA after 36 min was found to be 45%. It is not particularly surprising that a considerable amount of hydrolysis takes place in such a basic reaction medium. After 36 min in the presence of β-CD the extent of hydrolysis was also found to be 45%. The slight differences in rate at 9, 18 and 27 min can be attributed to sampling errors which occur as a result of the heterogeneous nature of the reaction mixture. The extent of hydrolysis after 36 min is similar in both the presence and the absence of β-CD, thus β-

CD has little or no effect on the extent or the rate of hydrolysis. In the presence of BSA the extent of hydrolysis after 36 min is 8%, while in the presence of both BSA and β-CD the extent of hydrolysis after 36 min is 9%. This suggests that the addition of BSA actually impedes hydrolysis and confirms that β-CD has little effect on the extent or rate of reaction. Presumably, the BSA precludes the access of the base to the ester **45** and thus slows the hydrolysis reaction considerably.

The hydrolysis of methyl 2-phenoxypropionate (**45**) was repeated in the absence of both BSA and β-CD, in the presence of β-CD, in the presence of BSA and in the presence of both BSA and β-CD and the enantioselectivity of each reaction was determined at 18 and 36 min. The samples were quenched with ethanol, filtered and analysed by HPLC with the results detailed in Table 12.

	Ratio of *R/S* enantiomers of acid **46**			
Time (min)	No β-CD or BSA	With β-CD	With BSA	Both β-CD and BSA
18	50:50	50:50	53:47	52:48
36	50:50	50:50	53:47	52:48

Table 12: Ratio of *R/S* enantiomers of the acid **46** resulting from the hydrolysis of the ester **45** in the absence of both BSA and β-CD, in the presence of β-CD, in the presence of BSA and in the presence of both BSA and β-CD.

The results presented in Table 12 show that β-CD has no effect on the ratio of enantiomers of the acid **46** produced from the hydrolysis of the ester **45**. However, in both reactions in which BSA is present, there is a greater proportion of the (*R*)-acid **46** isolated from the reaction mixture. There are three possible explanations. As suggested by Kamal *et al.*[90], the BSA complexes with the (*R*)-ester **45** and promotes its hydrolysis preferentially. However, this is unlikely given the results shown in Table 11 where the addition of BSA to the hydrolysis of the ester **45** leads to a reduced extent of reaction, suggesting Kamal's hypothesis was incorrect. Another possible explanation is BSA may

complex preferentially with the (*S*)-ester **45**, blocking the approach of the base and thus resulting in preferential hydrolysis of the (*R*)-ester **45**. Alternatively, BSA may complex preferentially with the (*S*)-acid **46**, removing it from solution during the workup and thus resulting in a greater proportion of the (*R*)-acid **46** isolated from the reaction mixture. It would be difficult to determine whether complexation takes place between the ester **45** and BSA because the ester is unstable under the basic reaction conditions. However, complexation by BSA of the acid **46** can be studied.

To test the latter hypothesis, solutions containing 1.5, 2.2 and 3.2 mg of the phenoxypropionic acid **46** were prepared in pH 11.5 phosphate buffer (3 ml) and to each was added BSA (500 mg). It was hoped that reducing the concentration of the acid **46** would result in a greater proportion of the (*R*)-acid **46** isolated from these solutions. The solutions were placed under a nitrogen atmosphere and equilibrated at 37 °C. After 15 min a sampled from each solution was taken. To maintain consistency with previous experiments ethanol was added to the samples taken and the resulting solutions were filtered and analysed by HPLC. The results are detailed in Table 13.

Initial [acid] (mM)	*R/S* Ratio	ee (%)
2.1	68:32	36
4.3	64:36	28
6.4	61:39	22

Table 13: *R/S* Ratios of the acid **46** isolated from 2.1, 4.3 and 6.4 mM solutions of the phenoxypropionic acid **46** in pH 11.5 phosphate buffer in the presence of BSA.

The results presented in Table 13 suggest that the BSA binds preferentially to the (*S*)-enantiomer of the acid **46**. After the solution is quenched with ethanol, the BSA/(*S*)-acid **46** complex precipitates and is removed through filtration, thus enriching the solution with the (*R*)-enantiomer of the acid **46**. However, complexation between BSA and (*S*)-ester **45** cannot be discounted as an explanation for the enantioselectivity seen in the

hydrolysis but this would be difficult to probe as the ester **45** is readily hydrolysed in pH 11.5 phosphate buffer. This experiment further discredits the suggestion of Kamal *et al.*[90] that BSA complexes with the ester **45** and catalyses the preferential hydrolysis of the (*R*)-enantiomer.

In an effort to determine the generality of the selectivity exhibited by BSA in the hydrolysis of the phenoxypropionate **45**, the hydrolysis of the phenoxypropanoate **47** was investigated. The hydrolysis of racemic methyl 2-(2-chlorophenoxy)propanoate (**47**) (30 mg) was conducted in pH 11.5 phosphate buffer (20 ml) in the absence of both β-CD and BSA, in the presence of β-CD, in the presence of BSA and finally in the presence of both β-CD and BSA. The results obtained for the extent of the reaction observed are presented in Table 14.

Time (min)	No β-CD or BSA		With β-CD		With BSA		With β-CD and BSA	
	% Acid	% Ester	% Acid	% Ester	% Acid	% Ester	% Acid	% Ester
0	3	97	2	98	0	99	0	100
9	12	44	14	69	2	132	3	85
18	21	65	24	105	5	106	7	82
27	29	53	33	43	7	97	10	74
36	-	-	39	44	8	96	11	72

Table 14: The extent of hydrolysis of the ester **47** in the absence of both BSA and β-CD, in the presence of β-CD, in the presence of BSA and in the presence of both BSA and β-CD.

The entries in Table 14 for the percentage of ester remaining that are greater than 100% can be attributed to sampling errors which occur as a result of the heterogeneous nature of the reaction mixture. The hydrolysis of the phenoxypropanoate **47** stopped after approximately 40% had been consumed suggesting that, despite reducing the amount of the ester **47** and increasing the buffer strength, the buffering capacity of the phosphate buffer is still being exceeded. However as was the case in the hydrolysis of the ester **45** discussed above, the results clearly show the hydrolysis of the phenoxypropionate **47**

proceeds in the absence of both β-CD and BSA. The extent of hydrolysis in the absence of β-CD and BSA after 27 min was found to be 29% while after 27 min in the presence of β-CD the extent of hydrolysis was found to be 33%. This small difference in extent of reaction can be attributed to sampling errors which occur as a result of the heterogeneous nature of the reaction mixture. The extent of hydrolysis after 27 min is similar in both the presence and the absence of β-CD, thus β-CD has little or no effect on the extent and the rate of hydrolysis. In the presence of BSA the extent of hydrolysis after 36 min is 8%, while in the presence of both BSA and β-CD the extent of hydrolysis after 36 min is 11%. This suggests that, as was the case in the hydrolysis of the ester **45**, the addition of BSA actually impedes hydrolysis of the ester **47** and confirms that β-CD has little effect on the extent or rate of reaction. Presumably, the BSA precludes the access of the base to the ester **47** and thus slows the hydrolysis reaction considerably.

The hydrolysis of methyl 2-(2-chlorophenoxy)propanoate (**47**) was repeated in the absence of both BSA and β-CD, in the presence of β-CD, in the presence of BSA and in the presence of both BSA and β-CD and the enantioselectivity of each reaction was determined at 18 and 36 min. The samples were quenched with ethanol, filtered and analysed by HPLC with the results detailed in Table 15.

	Ratio of *R/S* enantiomers of acid **48**			
Time (min)	No β-CD or BSA	With β-CD	With BSA	Both β-CD and BSA
18	50:50	50:50	53:47	53:47
36	50:50	50:50	52:48	55:45

Table 15 Ratio of *R/S* enantiomers of the acid **48** resulting from the hydrolysis of the ester **47** in the absence of both BSA and β-CD, in the presence of β-CD, in the presence of BSA and in the presence of both BSA and β-CD.

The results presented in Table 15 show that β-CD has no effect on the ratio of enantiomers of the acid **48** produced from the hydrolysis of the ester **47**. However as was the case in the hydrolysis of the ester **45**, in both reactions in which BSA is present,

there is a greater proportion of the (*R*)-acid **48** isolated from the reaction mixture. There are three possible explanations. As suggested by Kamal *et al.*[90], the BSA complexes with the (*R*)-ester **47** and promotes its hydrolysis preferentially. However, this is unlikely given the results shown in Table 14 where the addition of BSA to the hydrolysis of the ester **47** leads to a reduced extent of reaction, suggesting Kamal's hypothesis is incorrect. Another possible explanation is BSA may complex preferentially with the (*S*)-ester **47**, blocking the approach of the base and thus resulting in preferential hydrolysis of the (*R*)-ester **47**. Alternatively, BSA may complex preferentially with the (*S*)-acid **48**, removing it from solution during the workup and thus resulting in a greater proportion of the (*R*)-acid **48** isolated from the reaction mixture. As discussed with the hydrolysis of the ester **45**, it would be difficult to determine whether complexation takes place between the ester **47** and BSA because the ester is unstable under the basic reaction conditions. However, complexation by BSA of the acid **48** can be studied.

To test the latter hypothesis, solutions containing 3.8, 7.6 and 10.9 mg of the phenoxypropionic acid **48** were prepared in pH 11.5 phosphate buffer (10 ml) and to each was added BSA (1.7 g). As was the case with the acid **46**, it was hoped that reducing the concentration of the acid **48** would result in a greater proportion of the (*R*)-acid **48** isolated from these solutions. The solutions were placed under a nitrogen atmosphere and equilibrated at 37 °C. After 15 min a sampled from each solution was taken. Again to maintain consistency with previous experiments ethanol was added to the samples taken and the resulting solutions were filtered and analysed by HPLC. The results are detailed in Table 16.

Initial [acid] (mM)	R/S Ratio	ee (%)
1.9	96:4	92
3.8	92:8	84
5.2	77:23	54

Table 16: *R/S* Ratios of the acid **48** isolated from 1.9, 3.8 and 5.2 mM solutions of the phenoxypropionic acid **48** in pH 11.5 phosphate buffer in the presence of BSA.

The results presented in Table 16 suggest that the BSA binds preferentially to the (*S*)-enantiomer of the acid **48**. As was the case with the racemate of the acid **45**, after ethanol is added to the solution, the BSA/(*S*)-acid **48** complex precipitates and is removed through filtration, thus enriching the solution with the (*R*)-enantiomer of the acid **48**. However, complexation between BSA and (*S*)-ester **48** cannot be discounted as an explanation for the enantioselectivity seen in the hydrolysis but this would be difficult to probe as the ester **47** is readily hydrolysed in pH 11.5 phosphate buffer. As was the case in the hydrolysis of the ester **45**, this experiment also discredits the suggestion of Kamal *et al.*[90] that BSA complexes with the ester **47** and catalyses the preferential hydrolysis of the (*R*)-enantiomer.

The selectivity exhibited by BSA towards the (*S*)-enantiomers of the acids **46** and **48** is not surprising given that there are many examples in the literature in which BSA has been used in the enantiomeric separation of a wide variety of compounds. For example, D,L-tryptophan and D,L-5-hydroxytryptophan have been resolved by chromatography using a BSA-agarose column.[139]

Kamal *et al.*[90] claimed that the BSA was catalysing the hydrolysis of the esters **45** and **47** enantioselectivly. Furthermore, the addition of β-CD resulted in an increase in hydrolytic efficiency and enantiomeric excess observed in these reactions. The authors attributed this increase in efficiency to an interaction between BSA and β-CD.

However, the work presented in this chapter has shown that the hydrolysis of the esters **45** and **47** proceeds in the absence of both BSA and β-CD. The addition of β-CD to the hydrolysis of the esters **45** and **47** has little or no effect on the efficiency or extent of reaction, nor does it affect the enantioselectivity experienced. The addition of BSA to the hydrolysis results in reduced extent of reaction, presumably by hindering access of the base to the esters **45** and **47**. However, the addition of BSA to the hydrolysis does alter the ratio of enantiomers of the product acids **46** and **48**. It seems likely that BSA does this by complexing preferentially with the (*S*)-acids **46** and **48**, removing them from

solution and thus enriching the reaction mixture with the (*R*)-acids **46** and **48**, although, BSA complexing preferentially with the (*S*)-esters **45** and **47**, blocking the approach of the base and thus resulting in preferential hydrolysis of the (*R*)-esters **45** and **47** can not be discounted.

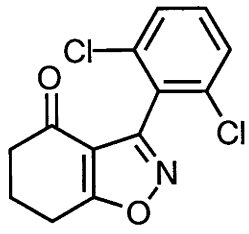
RESULTS AND DISCUSSION CHAPTER FOUR

Electrochemical Reductive Ring Opening of Isoxazoles

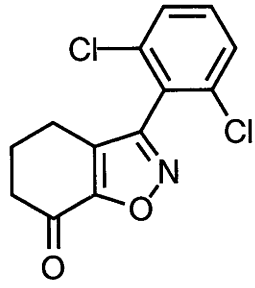
Chapter One detailed an investigation into a reported effect of yeast on nitrile oxide cycloadditions. As discussed in the Introduction, CSIRO Molecular Science had investigated the production of structural analogues of GRASP **1** utilising bakers' yeast. This work centred around the β-enamino ketone moiety and exploited nitrile oxide cycloaddition chemistry to produce 4-acylisoxazoles. Ring opening followed by the bakers' yeast catalysed reduction of the 4-acyl group could give hydroxy-diketone analogues of GRASP. However, these investigations found that the bakers' yeast catalysed reduction of certain isoxazoles did not, as expected, produce the corresponding alcohols but instead gave the ring opened imines. An interest in the influence of bakers' yeast on nitrile oxide cycloadditions and the uniqueness of the bio-chemical transformation observed prompted an investigation of these results. It is commonly understood that yeast catalysed transformations proceed *via* an electron transfer mechanism. With this in mind an electrochemical investigation of the reductive ring opening of isoxazoles was conducted.

5.1 Synthesis of the Isoxazoles 56, 58, 60 and 61

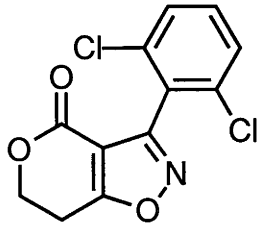
The earlier investigations into the baker's yeast facilitated reductive ring opening of isoxazoles,[116] discussed in the Introduction, were conducted using the pairs of regioisomers **56** and **60**, and **58** and **61**. Therefore, these compounds were chosen to determine if the same transformation could be carried out electrochemically. They were synthesised as reported previously.[140]



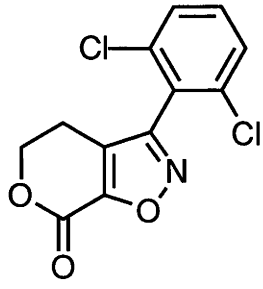
56



60

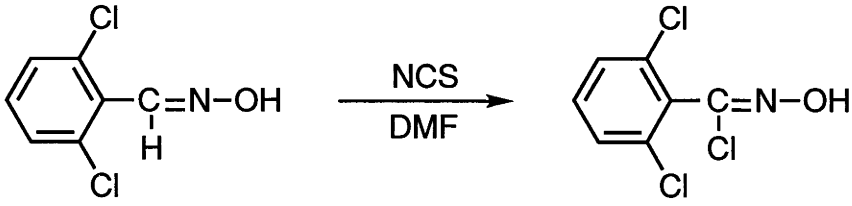


58



61

The synthesis began with preparation of 2,6-dichlorobenzohydroximinoyl chloride (**64**), the precursor of the nitrile oxide **19** required for the subsequent cycloaddition reactions. Commercially available 2,6-dichlorobenzaldoxime (**63**) was treated with *N*-chlorosuccinimide in DMF to give the corresponding hydroximinoyl chloride **64** in 78% yield (Scheme 54).

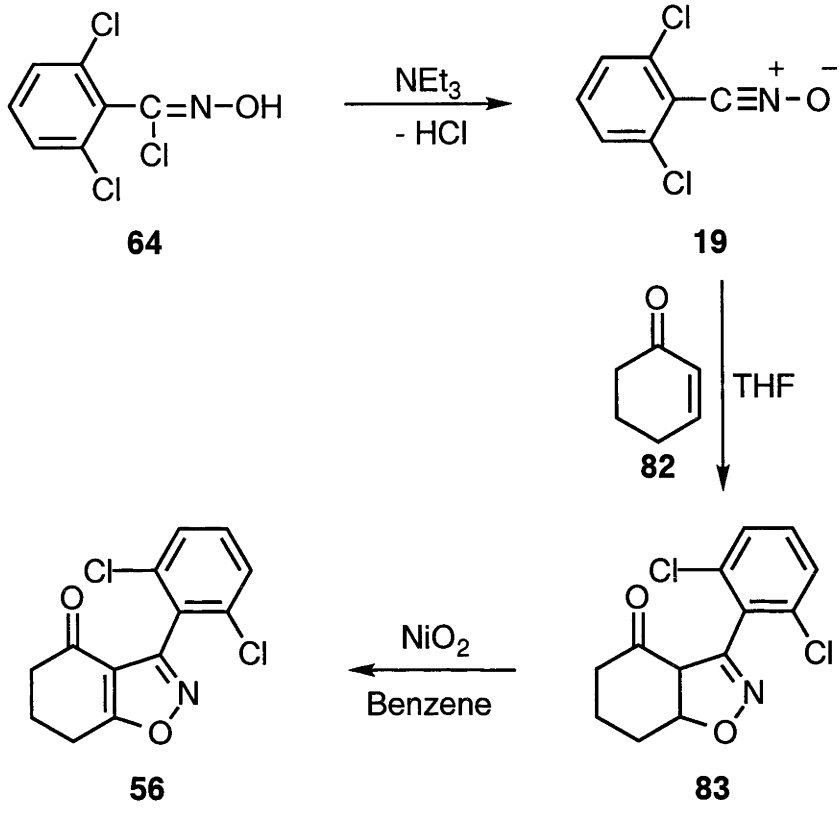


63 **64**

Scheme 54

2,6-Dichlorobenzohydroximinoyl chloride (**64**) was added to a solution of cyclohex-2-en-1-one (**82**) and triethylamine in THF. The triethylamine induces a dehydrohalogenation reaction, converting the hydroximinoyl chloride **64** to the corresponding 2,6-dichlorobenzonitrile oxide (**19**), which then undergoes a 1,3-dipolar cycloaddition with cyclohex-2-en-1-one (**82**), to give the isoxazoline **83** in 40% yield

(Scheme 55). The nitrile oxide **19** was generated *in situ* so as to limit the possibility of its dimerisation.[87]



Scheme 55

The structure of the isoxazoline **83** was confirmed by comparison of its ^{1}H nmr spectrum with literature data.[119,140] Specifically, the C-4 and C-5 isoxazoline ring protons exhibited a doublet at δ 4.41 with a coupling constant of 11 Hz, and a doublet of triplets at δ 5.26 with coupling constants of 11 and 4.5 Hz, respectively. By comparison, the corresponding signals for the possible regioisomeric cycloadduct would be expected to be much closer in chemical shift.[118] Such signals were not detected.

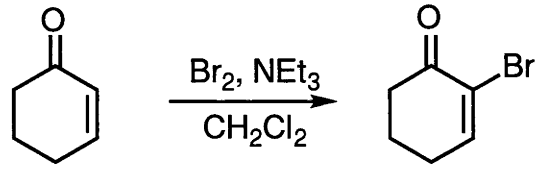
A mixture of regioisomers usually results from a 1,3-dipolar cycloaddition between a nitrile oxide and a 1,2-disubstituted alkene. However, an electron-withdrawing substituent directs the cycloaddition such that it is generally located at the 4-position of the predominant cycloadduct.[72-78] Accordingly, the carbonyl group of the alkene **82** polarises this species such that the oxygen of the nitrile oxide **19** reacts at C-3 of

cyclohex-2-en-1-one (**82**), thus producing the isoxazoline **83** as the predominant product.

The isoxazoline **83** was oxidised to the corresponding isoxazole **56**, in 49% yield, using nickel peroxide (Scheme 55). The ^{1}H nmr spectrum of the isoxazole **56** exhibits a pentet at δ 2.30, a triplet at δ 2.56, a triplet at δ 3.14 and a multiplet at δ 7.33-7.44. All physical and spectroscopic data obtained for the isoxazoline **56** are consistent with those reported.[119,140]

To prepare the isoxazole **60** *via* a 1,3-dipolar cycloaddition, it was necessary to effect a reversal of regioselectivity. This was achieved by adding a steric auxiliary to the alkene to increase the steric bulk, as cycloadditions involving 1,1-disubstituted and 1,2,2-trisubstituted alkenes are predominantly controlled by steric effects.[57,71] In these cases the oxygen of the nitrile oxide reacts with the more sterically hindered end of the dipolarophile.

An α-bromo substituent was introduced onto the cycloalkene **82** as the steric auxiliary.[119,140] Cyclohex-2-en-1-one (**82**) in dichloromethane was stirred with bromine. The slow addition of base gave the brominated compound **84** in 63% yield (Scheme 56).

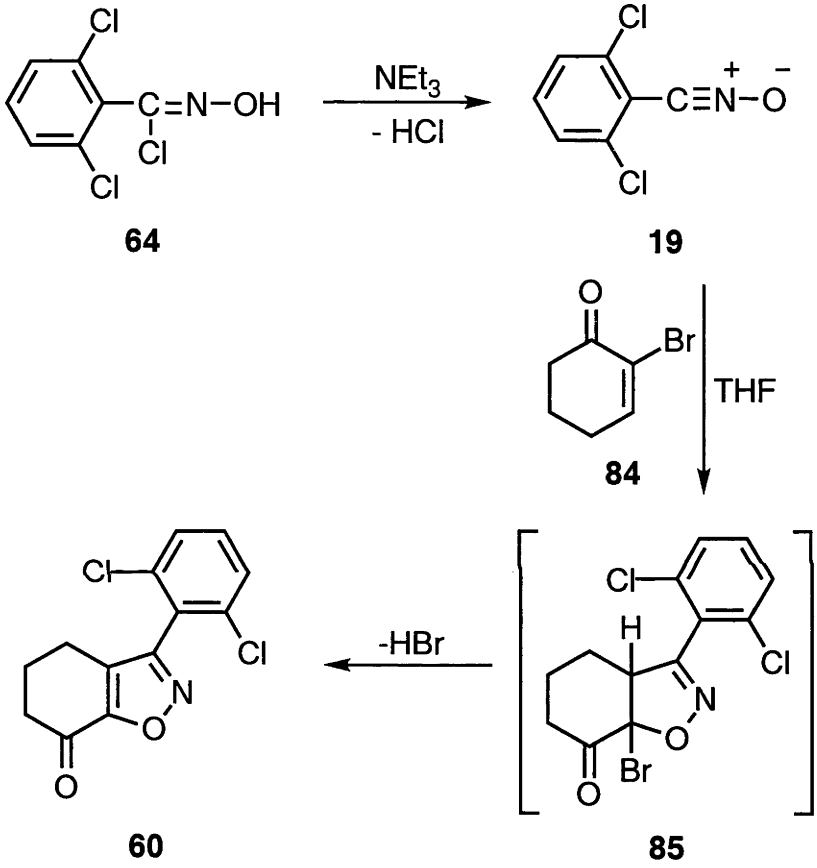


82 **84**

Scheme 56

The isoxazole **60** was prepared *via* reaction between the *in situ* generated 2,6-dichlorobenzonitrile oxide (**19**) and the bromocyclohexenone **84** (Scheme 57). Presumably, the cycloaddition reaction results in formation of the intermediate

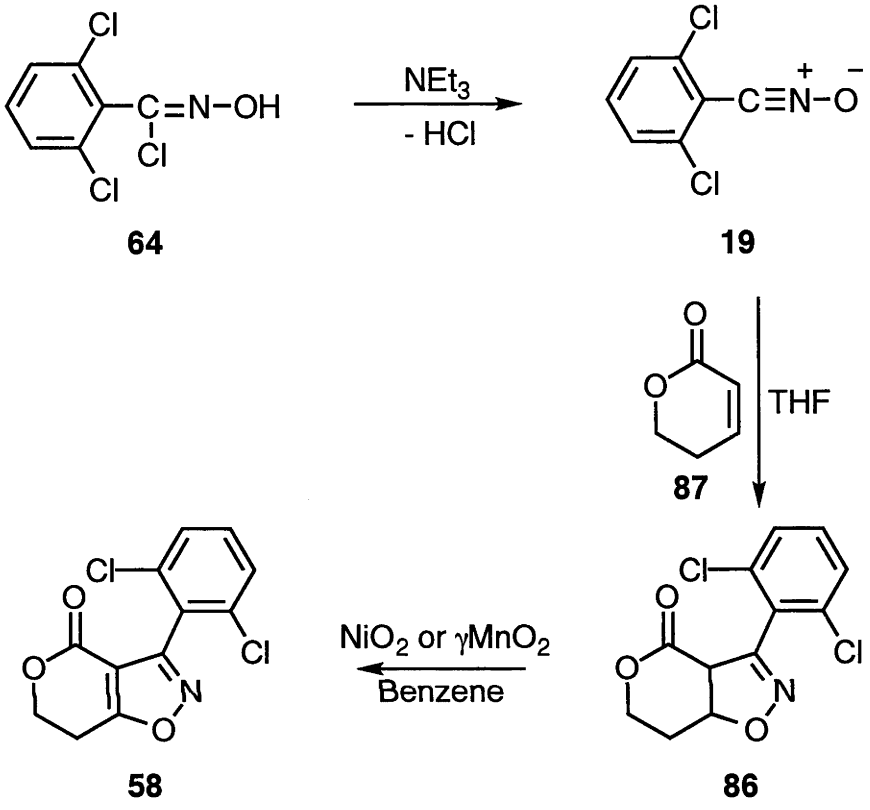
bromoisoxazoline **85**, which undergoes spontaneous dehydrobromination to give the isoxazole **60**, in 53% yield. The ^{1}H nmr spectrum of the isoxazole **60** is characteristic and different from that of the regioisomer **56**. It exhibits a multiplet at δ 2.23, a triplet at δ 2.61, a triplet at δ 2.70 and a multiplet at δ 7.37-7.46. All physical and spectroscopic data obtained for the isoxazole **60** are consistent with those reported in the literature.[119,140]



Scheme 57

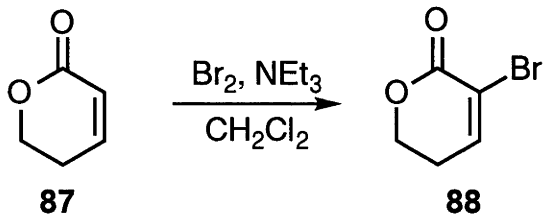
The methodology described above for preparation of compounds **56** and **60** was also used to prepare compounds **58** and **61**. The isoxazoline **86** was prepared in 64% yield *via* a cycloaddition between 2,6-dichlorobenzonitrile oxide (**19**) and commercially available 5,6-dihydro-2*H*-pyran-2-one (**87**) (Scheme 58). Comparison of its ^{1}H nmr spectrum with literature data was used to confirm the structure of the isoxazoline **86**.[119,140] The spectrum exhibits a doublet of doublets of doublets at δ 4.71 with coupling constants of 3, 11 and 11 Hz, corresponding to the isoxazoline C-5 proton, and

a doublet at δ 4.75 with a coupling constant of 11 Hz, corresponding to the isoxazoline C-4 proton. The isoxazoline **86** was oxidised to the corresponding isoxazole **58**, using nickel peroxide or γ-activated manganese dioxide, and isolated in 32 and 38% yield, respectively.

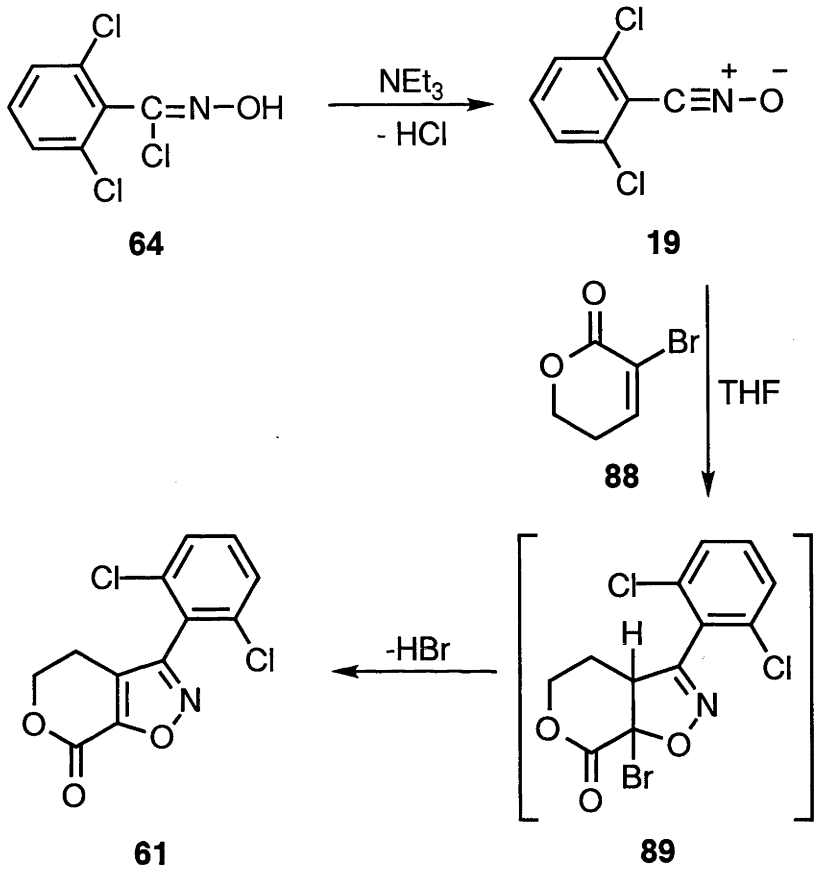


Scheme 58

As described above for synthesis of the bromocyclohexenone **84**, 5,6-dihydro-2*H*-pyran-2-one (**87**) was stirred with bromine and treated with base to give the corresponding α-brominated pyranone **88**, in 100% yield (Scheme 59). The bromide **88** underwent a 1,3-dipolar cycloaddition with 2,6-dichlorobenzonitrile oxide (**19**) to give the isoxazole **61**, in 26% yield, presumably through the isoxazoline **89** which spontaneously eliminates hydrogen bromide (Scheme 60).



Scheme 59



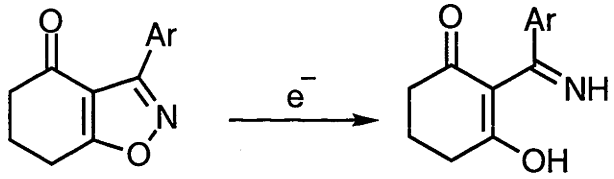
Scheme 60

5.2 Electrolysis of the Isoxazoles 56, 60, 58 and 61

Having obtained the regioisomeric pairs of isoxazoles **56** and **60**, and **58** and **61**, they were reduced under electrochemical conditions.

The electrolysis experiments were carried out using a mercury working electrode, a platinum counter electrode, and a Ag/AgCl reference electrode, in acetonitrile solutions containing *ca.* 0.1 M tetrabutylammonium tetrafluoroborate, under nitrogen. For each isoxazole the reduction potential was first determined using a polarogram. Controlled potential electrolyses were then carried out at or just above the reduction potential until each compound was completely consumed.

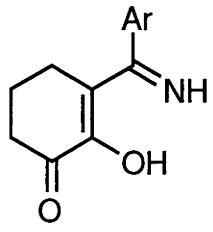
The isoxazole **56** has a reduction potential of -2.2 V. On controlled potential electrolysis, TLC of the reaction mixture after less than an hour indicated that the starting material was completely consumed and showed an intense UV active spot corresponding to a discrete electrolysis product. This was separated from the electrolyte by flash column chromatography, followed by preparative TLC, to give the ring opened product, the imine **57** (Scheme 61), in 61% yield. The ^{1}H nmr spectrum of the imine **57** exhibits a broad singlet at δ 6.02 corresponding to the imine proton and a broad singlet at δ 12.02 corresponding to the hydroxyl proton. The structure of the imine **57** was confirmed by comparison of its physical and spectral data with literature values.[116,119]



56 **57**

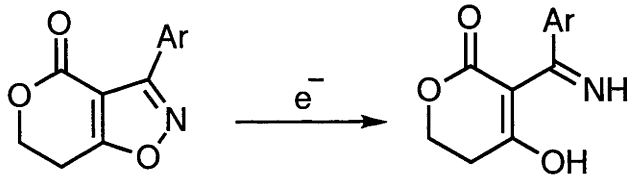
Scheme 61

The isoxazole **60** has a reduction potential of -2.5 V. Analysis of the reaction mixture resulting from controlled potential electrolysis indicated that the isoxazole **60** was completely consumed in less than one hour. TLC showed several UV active spots of equal intensity, indicating that several products had been formed during the reaction. Despite extensive chromatography, discrete products were neither obtained nor identified by analysis using ^{1}H nmr and MS. There was no evidence to suggest formation of the imine **90**.



90

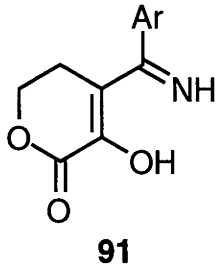
The reduction potential of the isoxazole **58** is -2.1 V. TLC of the controlled potential electrolysis indicated that the starting material had been consumed completely after less than one hour and a discrete product had been formed. After chromatography, the imine **59** (Scheme 62) was isolated in 66% yield. The product was identified as the imine **59** by comparing its physical and spectral data with literature values.[116,119] The ^{1}H nmr spectrum of the imine **59** exhibits a broad singlet at δ 6.08 corresponding to the imine proton and a broad singlet at δ 11.50 corresponding to the hydroxyl proton.



58 **59**

Scheme 62

The polarogram of the isoxazole **61** showed a reduction potential of -2.5 V. Analysis of the controlled potential electrolysis reaction mixture indicated that the starting material had been completely consumed after one hour. As with the electrolysis of the isoxazole **60**, TLC of this reaction mixture showed several UV active spots of equal intensity suggesting several products were present. Extensive chromatography of the reaction mixture failed to give discrete products, and analysis using ^{1}H nmr and MS did not lead to identification of any species. There was no evidence to suggest formation of the imine **91**.



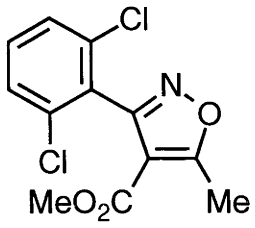
91

The results obtained from electrolysis of the isoxazoles **56, 58, 60** and **61** parallel those from the yeast catalysed reduction of these compounds. The isoxazoles **56** and **58** give

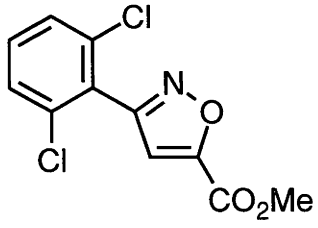
the corresponding imines **57** and **59** either on treatment with yeast or electrolysis. In contrast, the isoxazoles **60** and **61** do not produce the imines **90** and **91**, respectively. Compounds **60** and **61** are inert to N-O bond cleavage on treatment with yeast. They do undergo electrolysis but at higher reduction potentials than those of their corresponding regioisomers **56** and **58**, and on reduction they give complex product mixtures.

These experiments show that the electrochemical reactions of the isoxazoles **56, 58, 60** and **61** are a suitable chemical model for the reactions of these compounds with yeast. However, the electrochemical reactions of the isoxazoles **56** and **58** are more efficient. The yields of the imines **57** and **59** are 61 and 66% when produced electrochemically, compared with 23 and 21% from the yeast induced reactions.

The obvious difference between the regioisomeric pairs of isoxazoles **56** and **60**, and **58** and **61** is the position of the carbonyl substituent with respect to the isoxazole ring. In order to investigate the importance of this structural relationship to the electrochemical ring opening process, other isoxazoles with carbonyl substituents at either the 4- or 5-position of the isoxazole ring were considered. Compounds containing cyano substituents were also considered because, like carbonyl substituents, cyano groups have conjugating and electron withdrawing characteristics. Compounds containing phenyl substituents were also included to determine the effect of conjugating but not electron withdrawing substituents. Other isoxazoles containing carbonyl substituents in the 3- and 5-positions of the isoxazole ring are reduced to the corresponding alcohols on treatment with yeast.[141,142] A dicarbonyl substituted isoxazole was therefore included in this investigation to probe its behaviour under electrochemical conditions. Therefore, the isoxazoles **92, 93, 94, 95, 96, 97** and **98** were identified as suitable compounds for further study.



93



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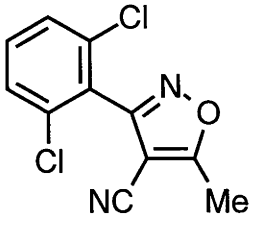

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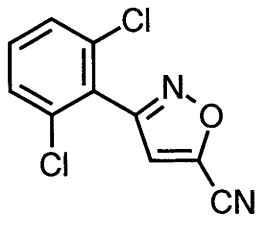

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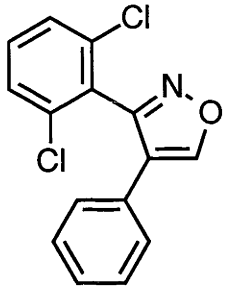

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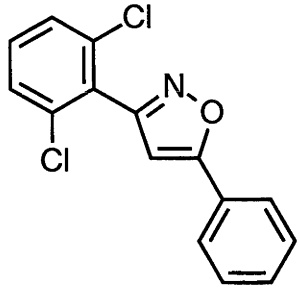

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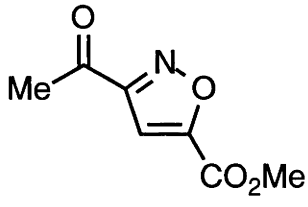
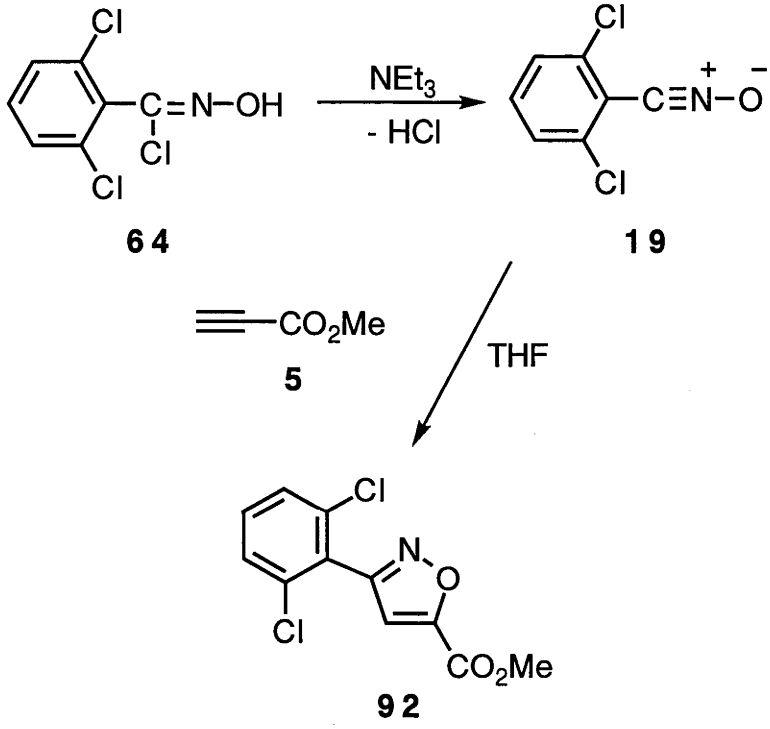


98

5.3 Synthesis of the Isoxazoles 92, 93, 94, 95, 96, 97 and 98

Whereas the isoxazoles **56** and **58** were obtained by nitrile oxide cycloadditions with alkenes followed by oxidation of the corresponding intermediate isoxazolines **83** and **86**, isoxazoles may also be formed directly from cycloadditions between nitrile oxides and alkynes. This approach is preferable where the alkyne is readily accessible because it eliminates a synthetic step. Therefore, the isoxazole **92** was prepared *via* a 1,3-dipolar cycloaddition between the nitrile oxide **19** and methyl propynoate (**5**), in 27% yield (Scheme 63). The ^{1}H nmr spectrum of the isoxazole **92** exhibits a singlet at δ 7.08

which is characteristic of the isoxazole C-4 proton. By comparison, the signal for the isoxazole C-5 proton of the possible regioisomeric cycloadduct would be expected around δ 9.00.[143] Such a signal was not seen. The structure of the isoxazole **92** was confirmed by X-ray crystallographic analysis (Figure 7). Compound **92** is produced without the possible regioisomer because the regioselectivity of cycloadditions between monosubstituted alkynes and nitrile oxides is controlled by steric effects such that the 5-substituted regioisomer is formed predominantly.[70]



Scheme 63

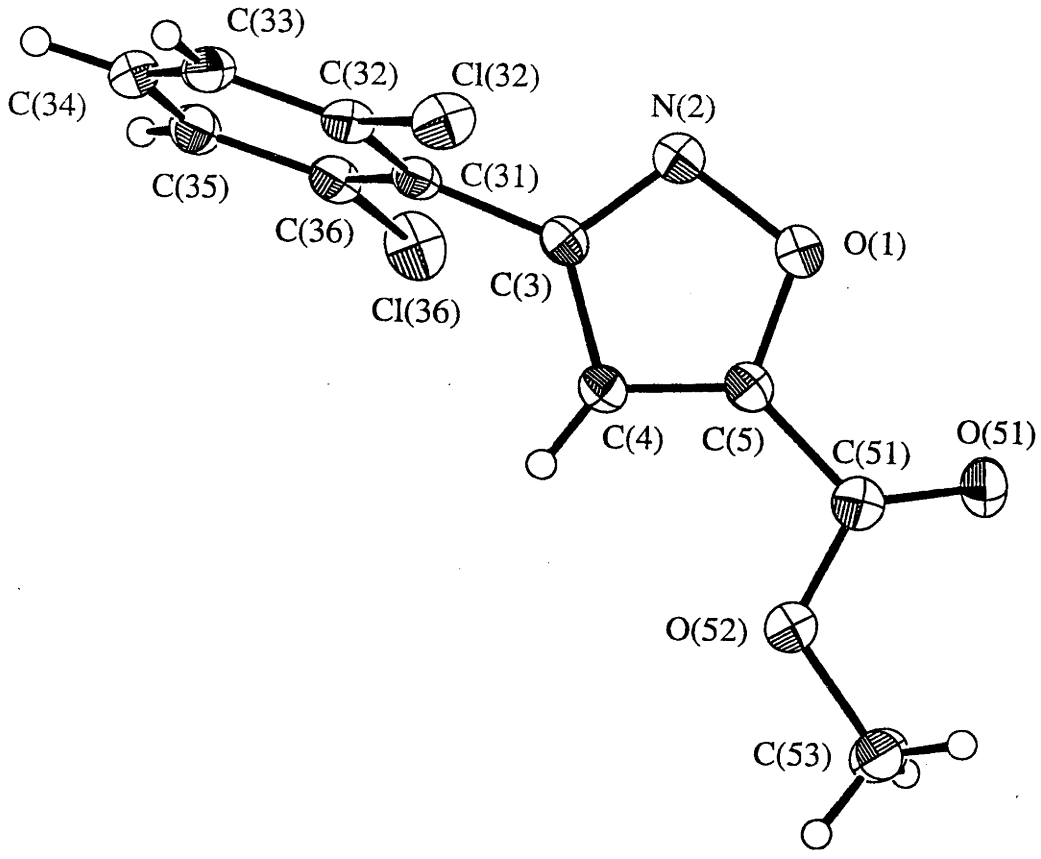
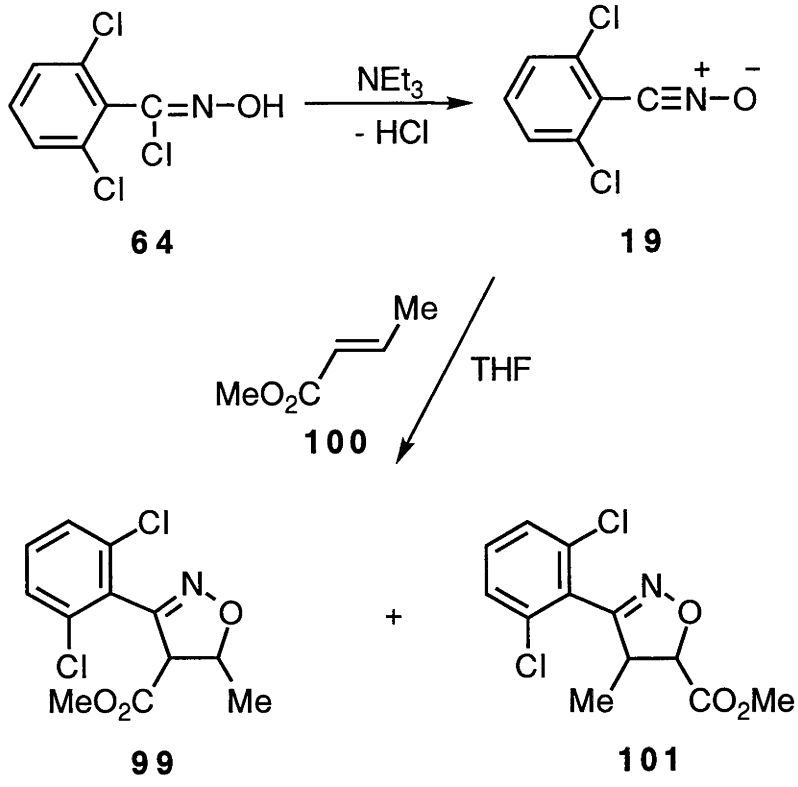


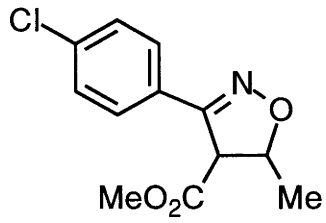
Figure 7: Crystal structure of the isoxazole **92**

Compound **93** has been prepared previously by Hanson *et al.*,[144] *via* the general procedure of Quilico and Fusco,[145] by condensing 2,6-dichlorobenzohydroximinoyl chloride (**64**) with methyl sodioacetoacetate. Rather than using this procedure, nitrile oxide cycloaddition chemistry was used to prepare the isoxazole **93**. In principle, a cycloaddition between methyl 2-butynoate and the nitrile oxide **19** would give the isoxazole **93**, but alternatively, it was prepared *via* the isoxazoline **99** because *trans*-methyl crotonate (**100**) was more readily available. The cycloaddition between the nitrile oxide **19** and *trans*-methyl crotonate (**100**) gave a regioisomeric mixture of the isoxazolines **99** and **101** in a ratio of 7:1 (Scheme 64). This ratio was determined by analysis of the ^{1}H nmr spectrum of the reaction mixture.



Scheme 64

The isoxazoline **99** was separated from its regioisomer **101** by flash column chromatography and was obtained in 41% yield. The structures of the isoxazolines **99** and **101** were confirmed by comparing their ^{1}H nmr spectra with literature data for the isoxazolines **102** and **103**.[146] The ^{1}H nmr spectrum of the isoxazoline **99** exhibits a doublet at δ 4.17 with a coupling constant of 7 Hz corresponding to the C-4 proton and an apparent pentet at δ 5.34 with a coupling constant of 7 Hz corresponding to the C-5 proton. The ^{1}H nmr spectrum of the minor isomer, the isoxazoline **101**, exhibits an apparent pentet at δ 4.06 with a coupling constant of 5.5 Hz corresponding to the C-4 proton and a doublet at δ 4.86 with a coupling constant of 5.5 Hz corresponding to the C-5 proton. These C4 and C5 isoxazoline ring proton coupling constants are characteristic of a *trans* relationship because *cis* coupling constants are typically between 11 and 12 Hz.[146] In nitrile oxide cycloadditions, the configuration of the alkene is retained in the product isoxazoline.[18] Therefore, the *trans*-isoxazolines **99** and **101** result from a cycloaddition involving *trans*-methyl crotonate (**100**).



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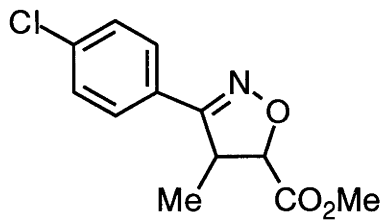
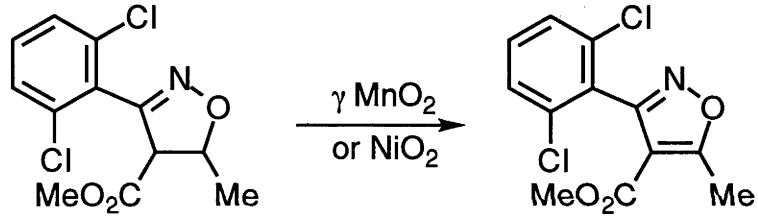


103

The isoxazoline **99** is the major regioisomer formed in the cycloaddition because the carbonyl group of the alkene **100** polarises this species and directs the regioselectivity of the reaction. While mixtures usually result from cycloadditions between 1,2-disubstituted alkenes and nitrile oxides, an electron-withdrawing substituent will direct the cycloaddition such that it is generally located at the 4-position of the predominant cycloadduct.[72-78]



99 **93**

Scheme 65

The isoxazoline **99** was oxidised to the corresponding isoxazole **93**, using nickel peroxide or γ-activated manganese dioxide, and isolated in 53 and 40% yield, respectively (Scheme 65). The structure of the isoxazole **93** was confirmed by comparison of its physical data with those reported[115] and by X-ray crystallographic analysis (Figure 8).

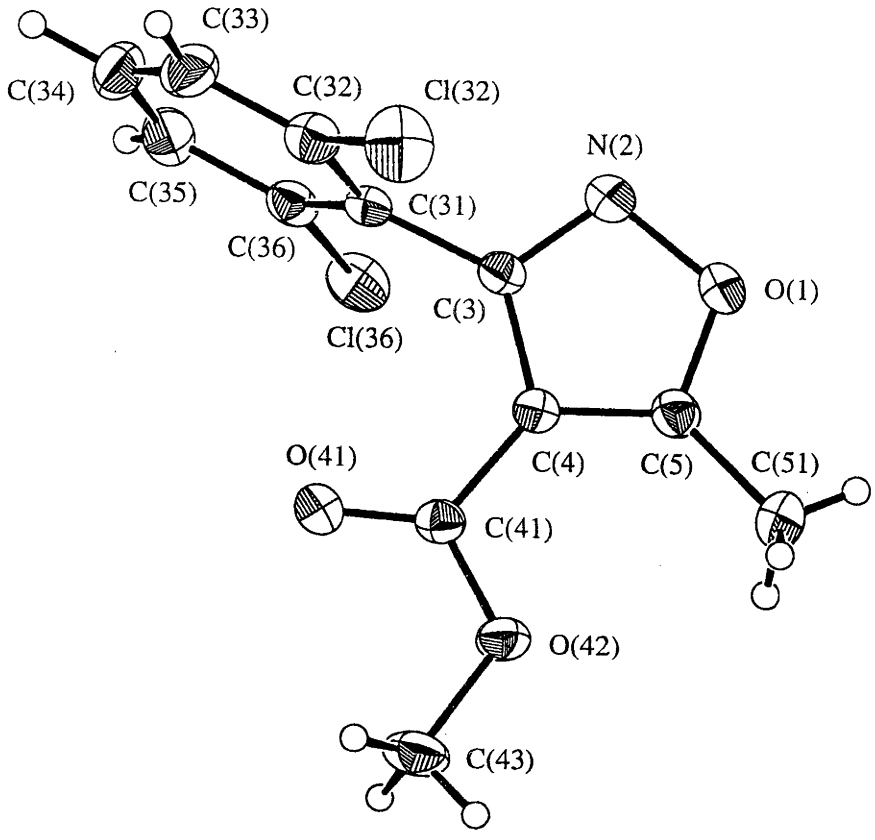
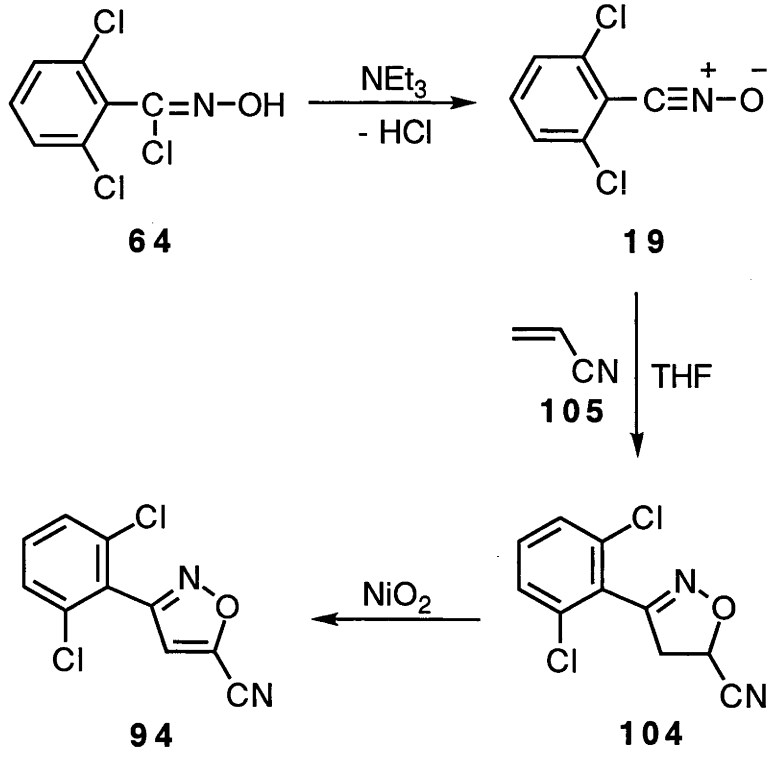


Figure 8: Crystal structure of the isoxazole **93**

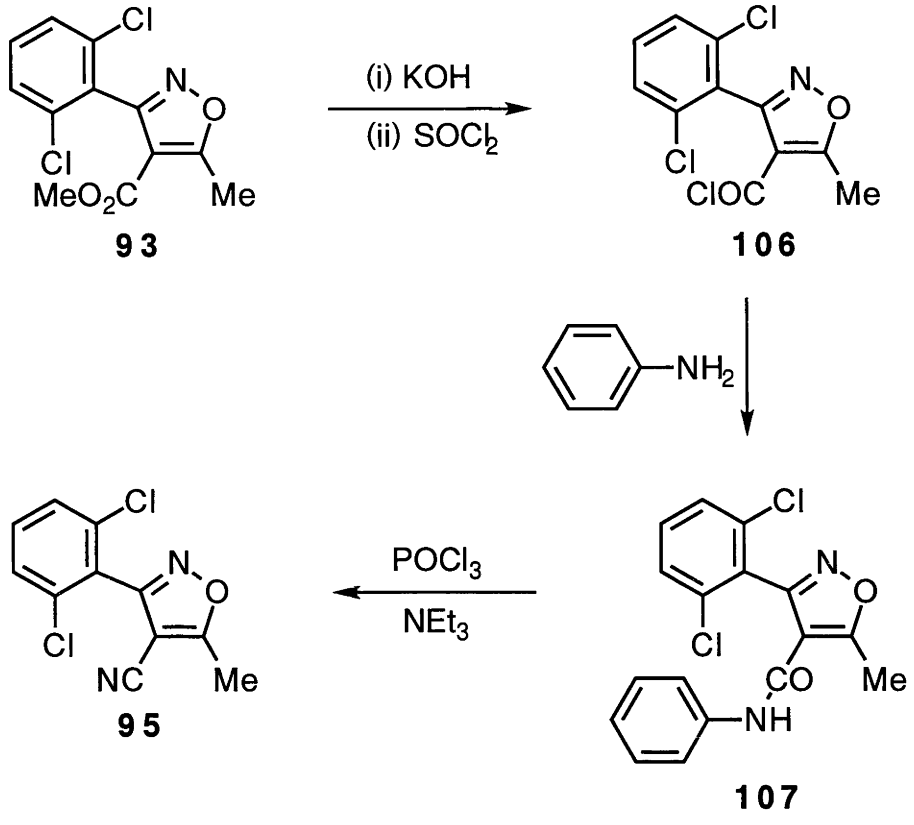
The methodology described above for the preparation of compounds **92** and **93** was also used to prepare compounds **94** and **95**. The isoxazole **94** has been prepared previously by Micetich[147] by reacting 2,6-dichlorobenzonitrile oxide (**19**) with 2-chloroacrylonitrile. However, the isoxazole **94** was prepared here *via* the isoxazoline **104**, following a literature procedure,[87] because acrylonitrile (**105**) was more readily available. Acrylonitrile (**105**) underwent a 1,3-dipolar cycloaddition with the nitrile oxide **19** to give the isoxazoline **104**, in 47% yield (Scheme 66). The structure of the isoxazoline **104** was confirmed by comparison of its ^{1}H nmr spectrum with literature data.[87] The spectrum exhibits a doublet of doublets at δ 3.62 with coupling constants of 5.5 and 17 Hz, and a doublet of doublets at δ 3.74 with coupling constants of 11 and 17 Hz, corresponding to the two C-4 protons, and a doublet of doublets at δ 5.47 with coupling constants of 5.5 and 11 Hz, corresponding to the C-5 proton. There was no indication of formation of the regioisomeric alternative cycloadduct. This is consistent with the regioselectivity expected for nitrile oxide cycloadditions to monosubstituted

alkenes.[70] The isoxazoline **104** was converted to the isoxazole **94** in 45% yield with nickel peroxide. The structure of the isoxazole **94** was confirmed by comparison of its ^{1}H nmr spectrum with literature data.[147] It exhibits a singlet at δ 7.15 which is characteristic of the isoxazole C-4 proton. By comparison, the signal for the isoxazole C-5 proton of the possible regioisomeric cycloadduct would be expected around δ 9.00.[142] This signal is not observed. All other physical and spectral data for the isoxazole **94** are consistent with those reported.[147] This evidence confirms the structure of the precursor isoxazoline **104**.



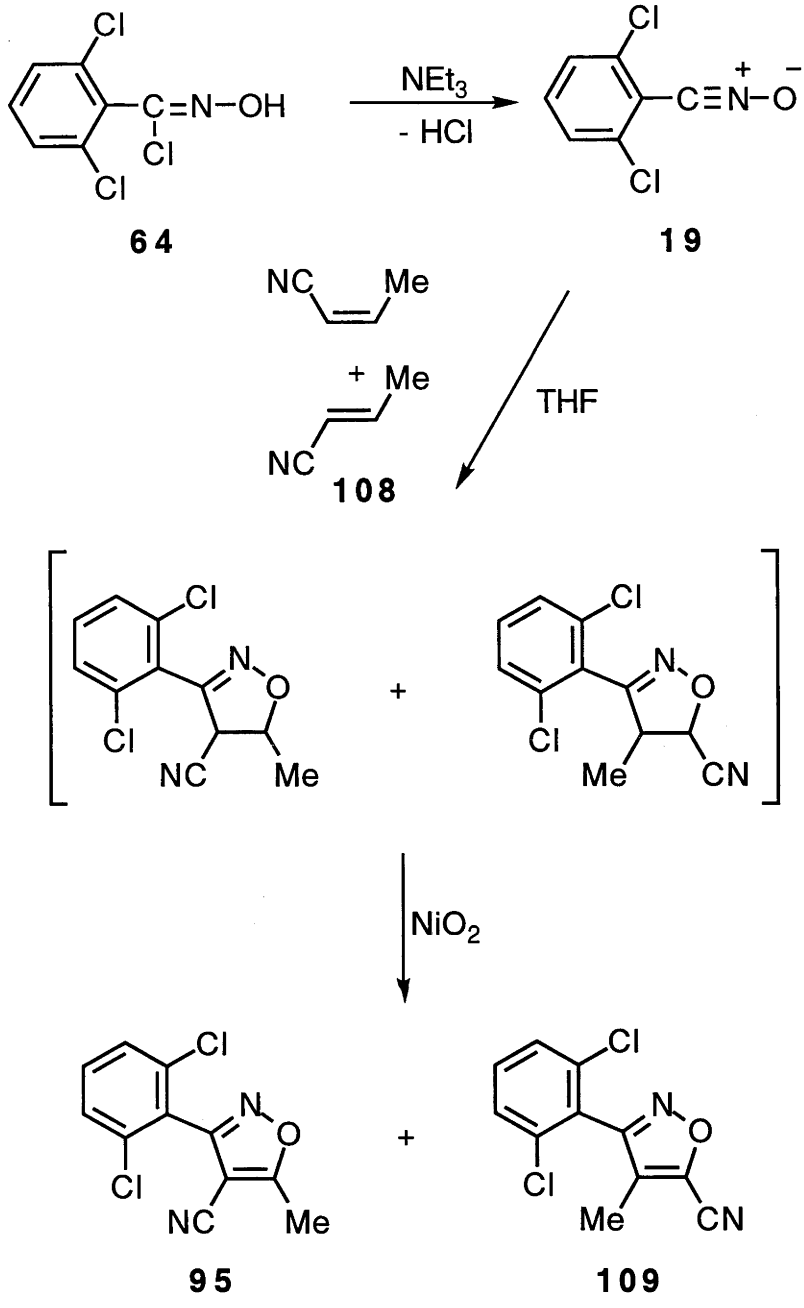
Scheme 66

The isoxazole **95** has been prepared from the ester **93** by McGregor *et al.*,[115] *via* the procedure shown in Scheme 67. The isoxazole **93** was hydrolysed with potassium hydroxide to give the corresponding acid, which reacted with thionyl chloride to form the acid chloride **106**. The acid chloride **106** was treated with aniline to give the corresponding anilide **107**, which was converted to the corresponding nitrile **95** *via* an amide dehydration reaction.



Scheme 67

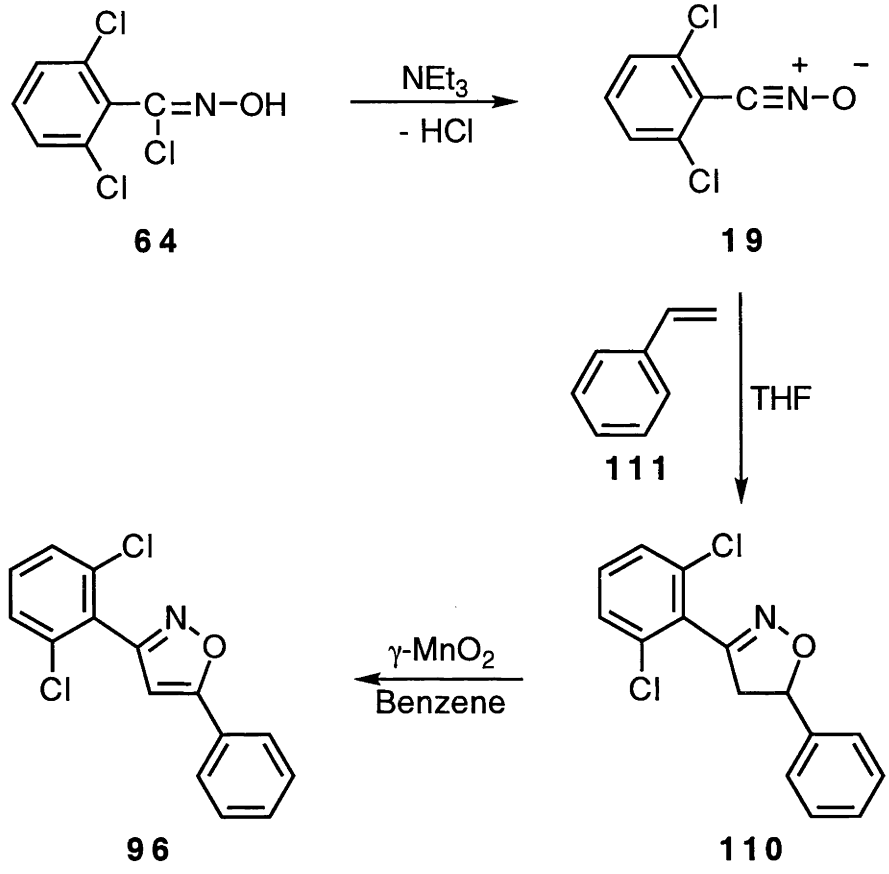
This procedure was not followed because it was more practical to produce the isoxazole **95** *via* nitrile oxide cycloaddition chemistry using crotononitrile (**108**) as a dipolarophile. Accordingly, in an analogous procedure to that used to prepare the isoxazoline **99**, crotononitrile (**108**) underwent a 1,3-dipolar cycloaddition with the nitrile oxide **19** to give a mixture of isoxazolines (Scheme 68). This mixture was isolated in 75% yield. A mixture results from this cycloaddition because crotononitrile (**108**) is a 1:1.5 mixture of the *cis* and *trans* isomers, as determined by analysis of the ^{1}H nmr spectrum. The isoxazolines proved difficult to separate chromatographically, so the mixture was treated with nickel peroxide to give the corresponding isoxazoles **95** and **109**. The isoxazole **95** was isolated in 10% yield by recrystallisation. The structure of the isoxazole **95** was confirmed by comparison of its physical and spectral data with those reported in the literature.[115]



Scheme 68

The isoxazole **96** has been previously prepared by Dondoni[148] *via* a cycloaddition between the nitrile oxide **19** and phenylacetylene, and by Bianchetti[149] *via* a performic acid promoted oxidation and elimination of a phenylthioisoxazoline. The isoxazole **96** was prepared here *via* the isoxazoline **110** because styrene **111** was more readily available than phenylacetylene. Compound **110** has been prepared previously by Fukumoto[150] *via* a 1,3-dipolar cycloaddition between the nitrile oxide **19** and styrene

(**111**). Accordingly, the nitrile oxide **19** and styrene (**111**) afforded the desired product **110**, in 91% yield (Scheme 69).

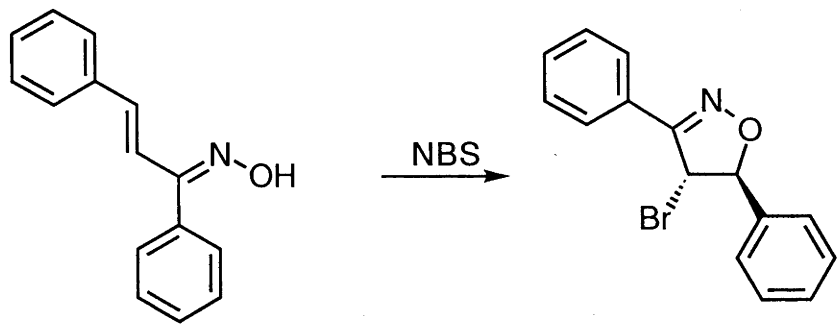


Scheme 69

The ^{1}H nmr spectrum of the isoxazoline **110** is consistent with literature values.[149] It exhibits a pair of doublets of doublets at δ 3.28 and δ 3.73 with coupling constants of 9 and 17 Hz, and 11 and 17 Hz, respectively, corresponding to the two C-4 protons, and a doublet of doublets at δ 5.82 with coupling constants of 9 and 11 Hz, corresponding to the C-5 proton. There were no additional peaks in the ^{1}H nmr spectrum that could be attributed to the possible regioisomer. All other physical and spectral data for the isoxazoline **110** are consistent with those reported.[150] Compound **110** was obtained as the sole regioisomer because cycloadditions between nitrile oxides and monosubstituted alkenes are controlled by steric effects such that the 5-substituted compound predominates.[70] The isoxazoline **110** was oxidised with γ-activated manganese dioxide to the corresponding isoxazole **96** in 11% yield. The structure of the isoxazole **96** was

confirmed by comparison of its ^{1}H nmr spectrum with literature data.[148] The ^{1}H nmr spectrum of the isoxazole **96** exhibits a singlet at δ 6.63 which is characteristic of the isoxazole C-4 proton. By comparison, the signal for the C-5 proton of the regioisomer, the isoxazole **97**, occurs at δ 8.90 as described below.[149] This signal was not observed. All other physical and spectral data for the isoxazole **96** are consistent with those reported in the literature.[148] In turn this evidence confirms the structure of the precursor isoxazoline **110**.

The isoxazole **97** has been previously prepared by Bianchetti[149] *via* a performic acid promoted oxidation and elimination of a phenylthioisoxazoline. This procedure was not used to prepare the isoxazole **97** because the phenylthioisoxazoline required was not readily available. Instead, β-bromostyrene (**112**) was used. In a method analogous to that employed for the preparation of the isoxazoles **60** and **61**, β-bromostyrene (**112**) underwent a cycloaddition with the nitrile oxide **19** to give a mixture of isoxazoline and isoxazole cycloadducts (Scheme 71). The ^{1}H nmr spectrum of the mixture obtained after flash column chromatography exhibits a pair of doublets at δ 5.57 and δ 6.08, both with coupling constants of 3.5 Hz, a singlet at δ 6.63 and a singlet at δ 8.79. The singlets at δ 6.63 and δ 8.79 were attributed to the isoxazoles **96** and **97** because these chemical shifts are characteristic of the C4 proton of the isoxazole **96**[147] as discussed above and the C5 proton of the isoxazole **97**.[149] Hansen *et al.*,[151] have prepared the related isoxazoline **113** by reacting the oxime **114** with *N*-bromosuccinimide (Scheme 70). The ^{1}H nmr spectrum of the isoxazoline **113** exhibits a pair of doublets at δ 5.33 and δ 6.01, both with coupling constants of 2 Hz, corresponding to the C4 and C5 protons. Therefore, the doublets at δ 5.57 and δ 6.08 observed in the spectrum of the product mixture discussed above are consistent with the C4 and C5 protons of the isoxazoline **115**. The ratio of the isoxazoles **97** and **96**, and the isoxazoline **115**, in the mixture was 7.3:5:1 as determined by analysis of the ^{1}H nmr spectrum.



114 (±)-**113**

Scheme 70

After extensive chromatography, the target isoxazole **97** was isolated in 7% yield. The structure of the isoxazole **97** was confirmed by comparison of its ^{1}H nmr spectrum with literature data[149] and by X-ray crystallographic analysis (Figure 9). The low yield of the isoxazole **97** may be attributed to the formation of side products discussed above and losses incurred during chromatography and purification.

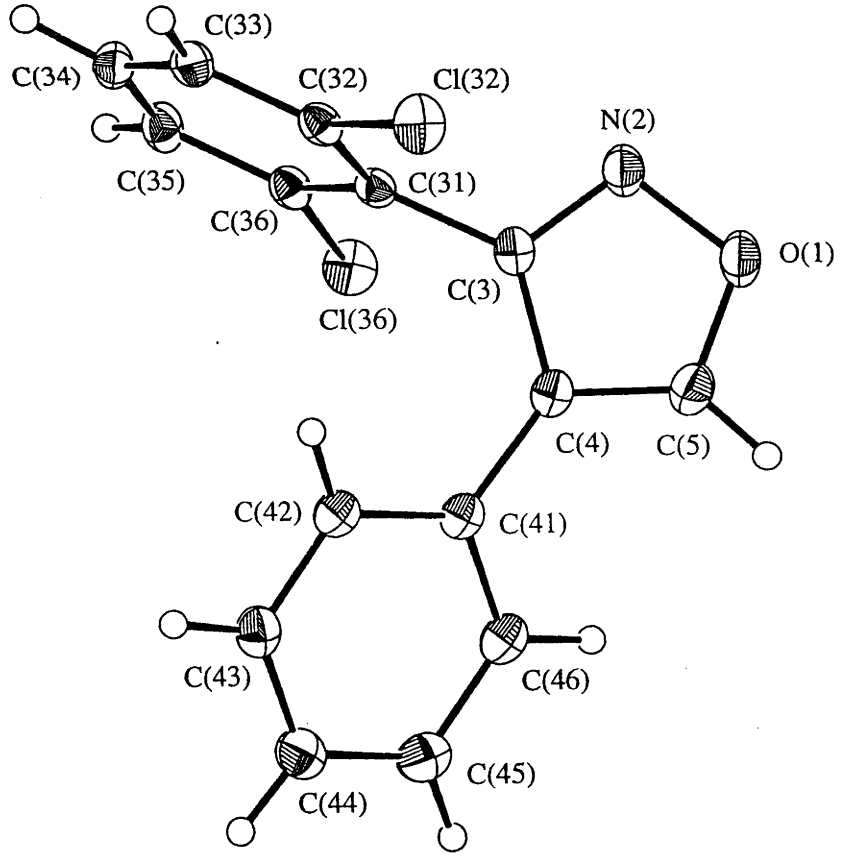
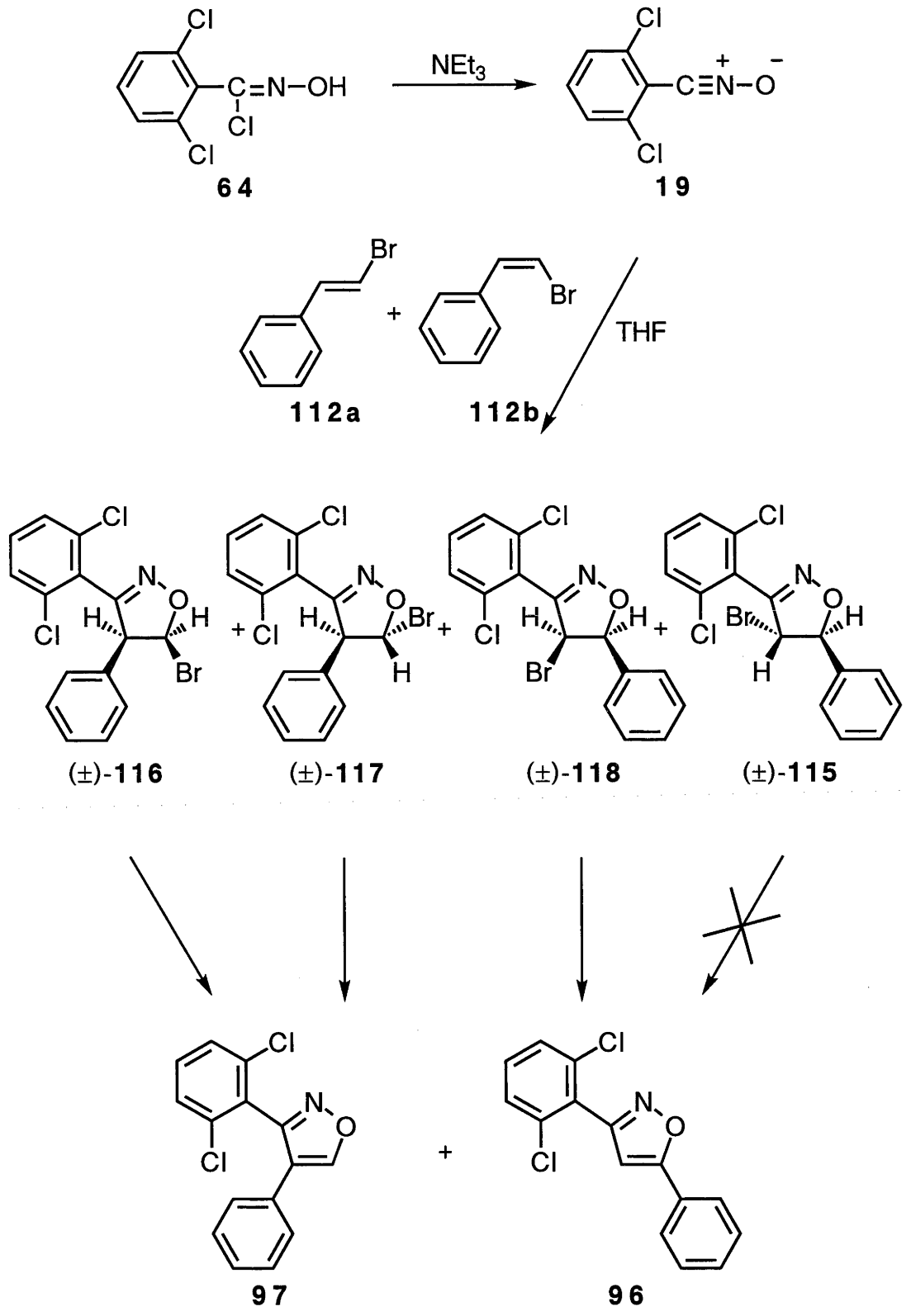


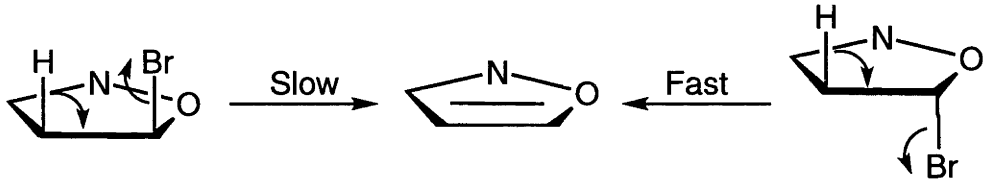
Figure 9: Crystal structure of the isoxazole **97**



Scheme 71

A mixture of products results from the cycloaddition because the β-bromostyrene (**112**) used was a mixture of the *cis* and *trans* isomers **112b** and **112a**. *trans*-Alkenyl protons

have coupling constants between 12 and 18 Hz while *cis*-alkenyl protons have coupling constants between 6 and 12 Hz.[152] The ^{1}H nmr spectrum of β-bromostyrene (**112**) exhibits a pair of doublets at δ 6.76 and δ 7.13 with coupling constants of 14 Hz, which are therefore attributable to the *trans*-isomer **112a**, and a pair of doublets at δ 6.43 and δ 7.68 with coupling constants of 8 Hz, which correspond to the *cis*-isomer **112b**. There was a 4:1 ratio of *trans*-β-bromostyrene (**112a**) to *cis*-β-bromostyrene (**112b**). In nitrile oxide cycloadditions, the configuration of an alkene is retained in the product isoxazoline.[18] Therefore, the reaction between the nitrile oxide **19** and a 4:1 mixture of *trans*- and *cis*-β-bromostyrene (**112a**) and (**112b**) presumably results in the formation of the isoxazolines **116**, **117**, **118** and **115**. Presumably, elimination of hydrogen bromide to form the corresponding isoxazoles **96** and **97** occurs much faster when the hydrogen to be removed and the leaving group adopt an *anti*-periplanar geometry rather than a *syn*-periplanar geometry (Scheme 72).[153]

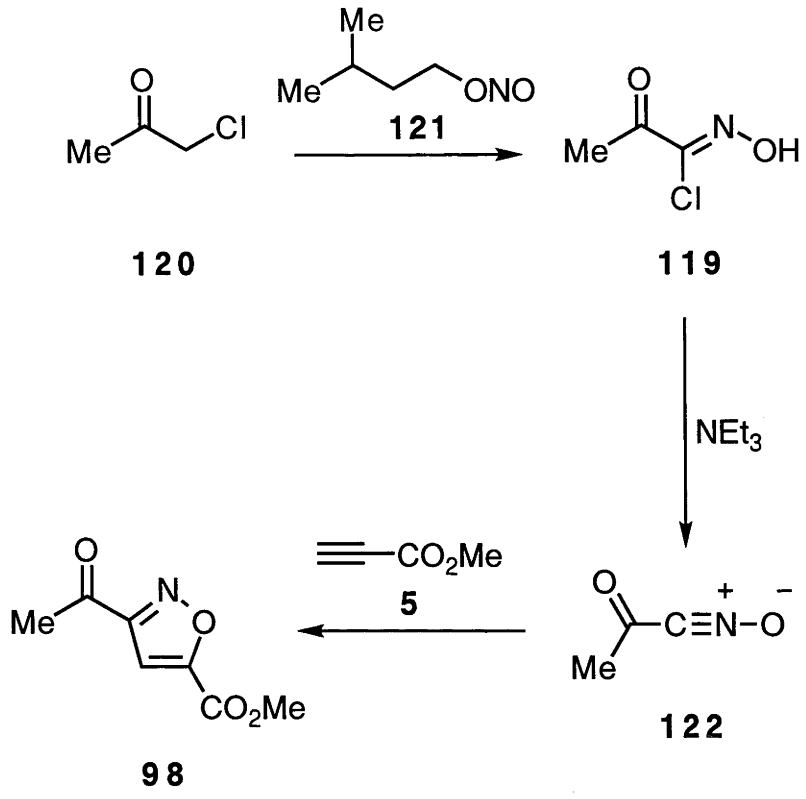


Scheme 72

The isoxazolines **117** and **118** both have *anti*-periplanar geometry with respect to the benzylic hydrogen and bromine substituent and therefore readily eliminate hydrogen bromide to form the corresponding isoxazoles **96** and **97**. Although the benzylic hydrogen and bromine have *syn*-periplanar geometry in the isoxazoline **116**, the hydrogen adjacent to the imine is probably sufficiently acidic that it is readily removed by base and the elimination reaction proceeds to produce the corresponding isoxazole **97**. By contrast, the isoxazoline **115** is stable under the reaction conditions because its C-5 hydrogen is less acidic and that it and the bromine substituent exhibit *syn*-periplanar geometry.

The immediate products of the cycloaddition between the β-bromostyrenes **112a** and **112b** and the nitrile oxide **19**, the 4-phenylisoxazolines **116** and **117**, and the 5-phenylisoxazolines **118** and **115** are produced in an approximate 1:1 ratio. In contrast to the cycloaddition involving styrene (**111**) which resulted in the formation of predominantly the 5-phenylisoxazoline **110**, the effect of the bromine is to increase the steric bulk on the β-carbon of the dipolarophiles **112a** and **112b** such that in the cycloaddition the oxygen of the nitrile oxide **19** is equally likely to react with either end of the dipolarophile thus producing an approximate 1:1 mixture of 4-and 5-substituted isoxazolines.

The isoxazole **98** had not been prepared previously, but was obtained *via* a cycloaddition between the hydroximinoyl chloride **119** and methyl propynoate (**5**). Chloroacetone (**120**) reacted with isopentyl nitrite (**121**) under acidic conditions to give the acyl hydroximinoyl chloride **119**,[154] in 14% yield (Scheme 73). In a procedure analogous to that used to prepare the isoxazole **92**, the hydroximinoyl chloride **119** was treated with triethylamine to produce the corresponding nitrile oxide **122** which underwent a cycloaddition with the alkyne **5** to give the 3,5-disubstituted isoxazole **98**, in 22% yield (Scheme 73). The ^{1}H nmr spectrum of the isoxazole **98** exhibits a singlet at δ 7.26 which is characteristic of the isoxazole C-4 proton. By comparison, the signal for the isoxazole C-5 proton of the possible regioisomeric product would be expected around δ 9.00.[143] This signal is not observed. The low yield obtained for the isoxazole **98** was attributed to dimerisation of the nitrile oxide **122** during the cycloaddition and losses of product during the purification. Compound **98** is produced without the possible regioisomer because, as stated above, the regioselectivity of cycloadditions involving monosubstituted alkynes and nitrile oxides is controlled by steric effects such that the 5-substituted regioisomer is former predominantly.[70]

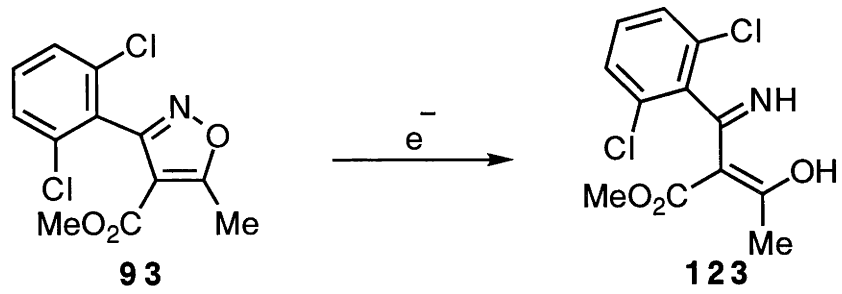


Scheme 73

Having prepared the isoxazoles **92**, **93**, **94**, **95**, **96**, **97** and **98**, they were electrolysed following the procedure detailed above for the electrolysis of the isoxazoles **56**, **58**, **60** and **61**.

5.4 Electrolysis of the Isoxazoles 92, 93, 94, 95, 96, 97 and 98

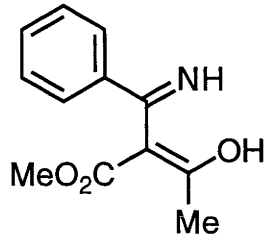
The isoxazole **93** has a reduction potential of -2.3 V. TLC of the reaction mixture after one hour of controlled potential electrolysis showed one intense UV active spot, indicating a discrete product had been formed during the reaction, and the starting material was completely consumed. This product was isolated from the reaction mixture by flash column chromatography, follow by preparative TLC. The ring opened product, the imine **123** (Scheme 74) was obtained in 56% yield.[155]



93 **123**

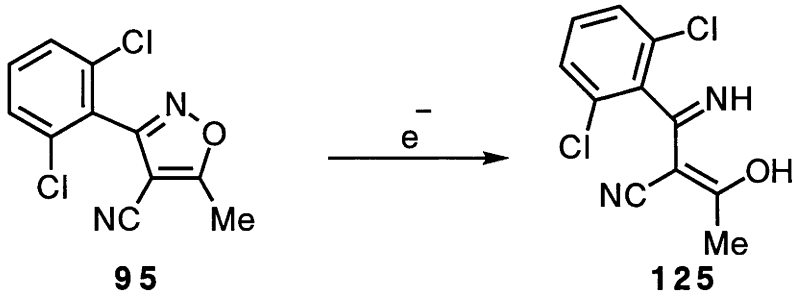
Scheme 74

The ^{1}H nmr spectrum of the imine **123** exhibits a broad singlet at δ 5.50 corresponding to the imine proton and a broad singlet at δ 11.35 corresponding to the hydroxyl proton. The structure of the imine **123** was confirmed by comparison of its physical data with literature values[115] and by comparing its ^{1}H nmr spectrum with literature data for the imine **124**.[156]



124

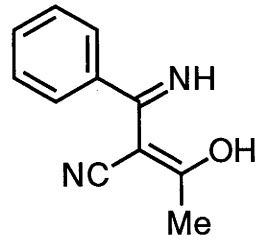
The isoxazole **95** has a reduction potential of -1.4 V. After controlled potential electrolysis for one hour, TLC showed a discrete UV active spot, indicating a single product had formed and the starting material had been completely consumed. The product was separated from the electrolyte by flash column chromatography, followed by preparative TLC. The imine **125** (Scheme 75) was isolated in 74% yield.



95 **125**

Scheme 75

The ^{1}H nmr spectrum of the imine **125** exhibits a broad singlet at δ 5.87, corresponding to the imine proton and a broad singlet at δ 10.80, corresponding to the hydroxyl proton. The structure of the imine **125** was confirmed by comparison of its physical data with literature values[115] and by comparing its ^{1}H nmr spectrum with literature data for the imine **126**.[157]



126

The reduction potentials of the isoxazoles **92, 94, 96, 97** and **98** are -2.4, -1.7, -2.0, -2.4 and -1.4 V, respectively. TLC of the controlled potential electrolyses of the isoxazoles **92, 94, 96, 97** and **98**, after one hour, each showed complete consumption of starting material and the presence of three to seven UV active spots of equal intensity, indicating several products had been formed in each reaction. This is consistent with TLC of the electrolysis mixtures of the isoxazoles **60** and **61** for which extensive chromatography failed to isolate discrete products, and analysis using ^{1}H nmr and MS did not lead to identification of any species. Therefore, the isolation of discrete species from the mixtures resulting from the electrolysis of the isoxazoles **92, 94, 96, 97** and **98** was not pursued further.

These reactions show the 4-alkoxycarbonyl- and 4-cyano-substituted isoxazoles **93** and **95** gave the ring opened species **123** and **125**, in 56 and 74% yield, respectively, while the 5-alkoxycarbonyl- and 5-cyano-substituted isoxazoles **92** and **94** were more resistant to reduction and gave multiple product mixtures. Both the phenylisoxazoles **96** and **97** gave multiple product mixtures, with the 4-substituted regioisomer **97** being the more resistant to electrolysis. The 3,5-dicarbonylisoxazole **98** also failed to give a discrete ring opened product.

The results obtained for electrolysis of the isoxazoles **92**, **93**, **94**, **95** and **98** parallel those from the electrolysis of the isoxazoles **56**, **58**, **60** and **61**. On electrolysis, the 4-carbonyl-substituted isoxazoles **56** and **58**, the 4-methoxycarbonyl-substituted isoxazole **93** and the 4-cyano-substituted isoxazole **95**, underwent N-O bond cleavage to give the ring opened species in 61, 66, 56 and 74% yield, respectively. The 5-carbonyl-substituted isoxazoles **60** and **61**, the 5-methoxycarbonyl-substituted isoxazoles **92** and **98** and the 5-cyano-substituted isoxazole **94** have greater reduction potentials than their regioisomers and regioisomeric analogues and afforded multiple product mixtures on electrolysis. Both phenylisoxazoles afforded multiple product mixtures on electrolysis. Therefore, a carbonyl- or cyano-substituent at the 4-position of the isoxazole ring leads to ring opening while a similar substituent at the 5-position does not. A phenyl substituent, regardless of its position on the isoxazole ring, does not facilitate ring opening during electrolysis. Therefore, the presence of an electron-withdrawing and conjugating substituent in the 4-position of the isoxazole ring facilitates the electrochemical ring opening process. An isoxazole containing an electron-withdrawing and conjugating substituent in the 5-position is more resistant than its regioisomer or regioisomeric analogue to electrochemical reduction and on electrolysis affords a multiple product mixture. The electrolysis of an isoxazole with a non electron-withdrawing but conjugating substituent in either the 4- or 5-position gives multiple product mixtures, however in this case, the 5-substituted isoxazole is less resistant to electrochemical reduction.

5.5 Discussion of Results

These substituent effects indicate that an electron-withdrawing and conjugating group at the 4-position of an isoxazole makes the ring susceptible to N-O bond cleavage, possibly as a result of conjugation of the ring oxygen with the 4-substituent through C-4 and C-5. This behaviour under electrochemical conditions can be rationalised by considering the structure of the starting materials and the electron distribution in the initial reduction products, the radical anions.

5.5.1 Structure of the starting materials

In a conjugated system, single bonds experience shortening due to increased double bond character.[158] On that basis, if there is conjugation through the isoxazole ring system between the electron-withdrawing substituent at C-4 and the ring oxygen, there would be a shortening of the C-O bond and an increase in the N-O bond length due to deformation of the 5-membered ring. The C-O and N-O bond lengths obtained from the crystal structures of the isoxazoles **56, 60, 58, 61, 93** and **92** are shown in Figure 10.

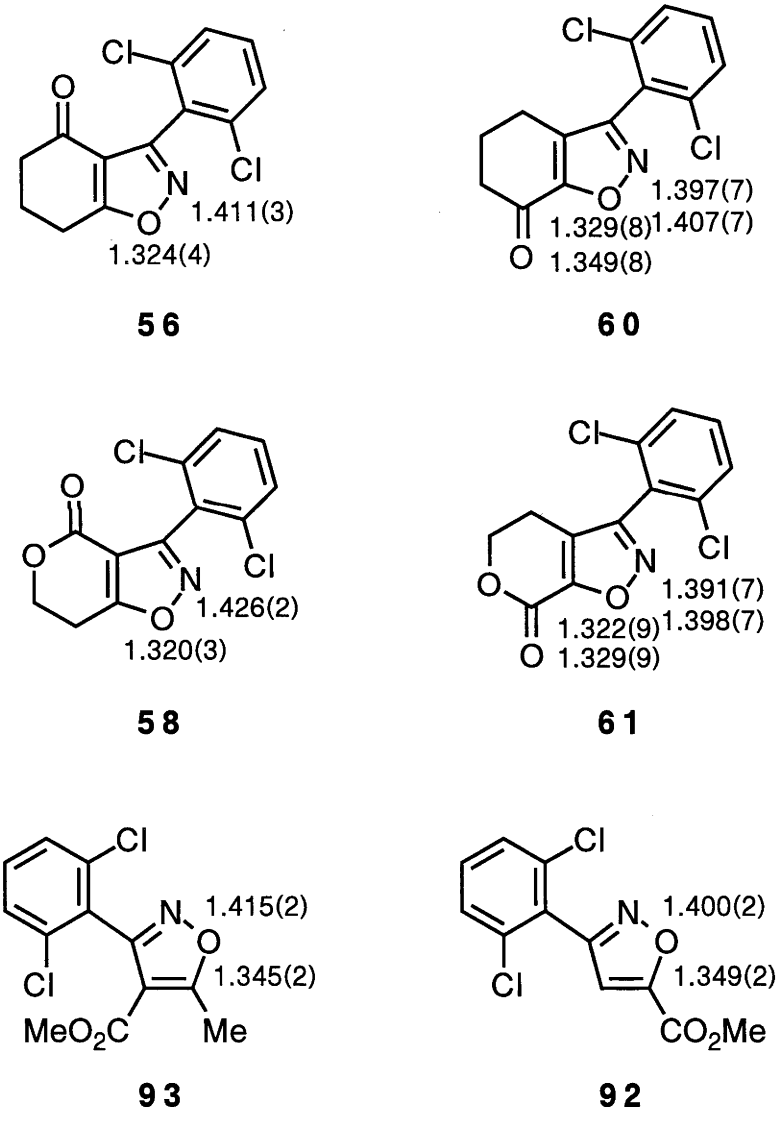
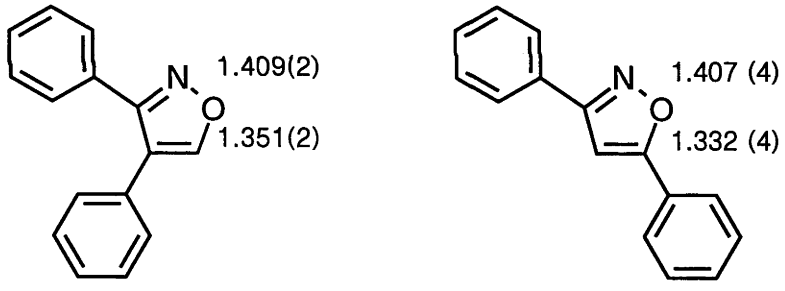


Figure 10: C-O and N-O bond lengths for the isoxazoles **56, 60, 58, 61, 93** and **92**

A comparison of the crystallographic data for the 4- and 5-substituted isoxazoles indicates shorter C-O bonds and longer N-O bonds in the 4-substituted isoxazoles **56, 58** and **93**

than in the 5-substituted isoxazoles **60**, **61** and **92**. The 4-substituted isoxazoles **56**, **58** and **93** undergo N-O bond homolysis under electrochemical conditions to give the corresponding imines **57**, **59** and **123**. A shortening of the C-O bond indicates the electron-withdrawing group at C-4 is conjugated through the ring to the oxygen resulting in a stronger C-O bond and the elongation of the adjacent N-O bond suggests it is weaker and thus more likely to undergo homolysis in the 4-acyl- and 4-alkoxycarbonyl-substituted compounds **56**, **58** and **93** than in their corresponding regioisomers.

Suitable crystals of the isoxazoline **97** were obtained for crystallographic analysis, however, all attempts at preparing crystals of the isoxazole **96** were unsuccessful. For the purposes of comparison, it was necessary to have a pair of regioisomers. Therefore, the crystallographic data for the structurally analogous compounds, the diphenyl isoxazoles **127** and **128** were used (Figure 11). The crystallographic data for the isoxazoles **127** and **128** were obtained from the literature.[159,160]



127 **128**

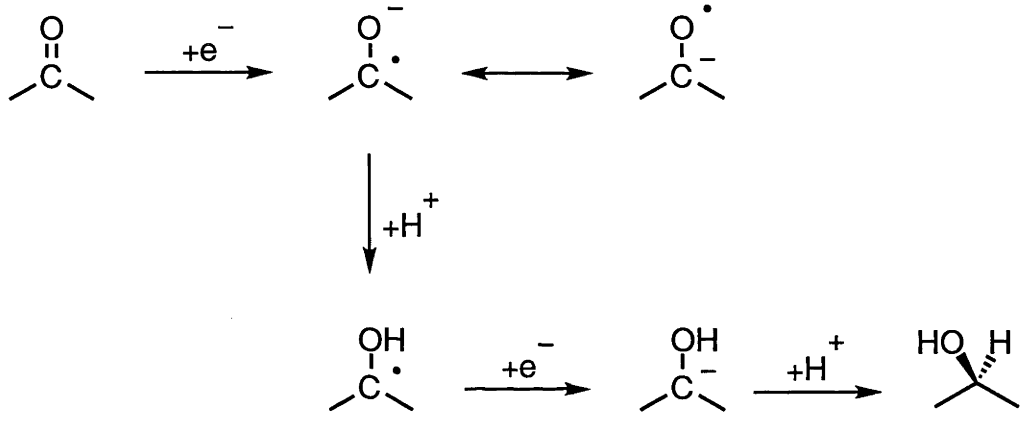
Figure 11: C-O and N-O bond lengths for the isoxazoles **127** and **128**

The C-O bond contraction trend experienced above for the isoxazoles **56** and **60**, **58** and **61** and **93** and **92**, is reversed in the isoxazolines **127** and **128**. The 5-phenylisoxazole **128** has a shorter C-O bond length than its regioisomer, the isoxazole **127**, while there is no difference in the N-O bond lengths for the 4- and 5-phenylsubstituted compounds. This suggest the phenyl group in the 5-phenylisoxazole **128** is conjugated through the ring with the oxygen and that both the 4- and 5-phenylisoxazoles **127** and **128** have N-O bonds of similar strength.

Therefore the isoxazoles which under electrochemical conditions display ring opening experience N-O bond elongation that results from the deformation of the isoxazole ring as a result of conjugation of the electron withdrawing substituent positioned at C-4 through the ring to the oxygen.

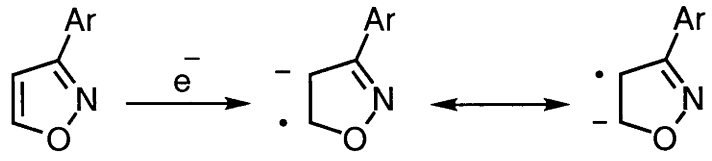
5.5.2 Electron Distribution in the Radical Anion Intermediates

The mechanism which has been proposed for the yeast-catalysed reduction of ketones to alcohols is presented in Scheme 76.[119]



Scheme 76

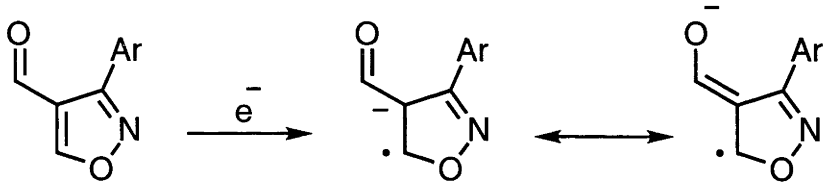
The carbonyl substituent undergoes an electron transfer to form the radical anion. This is then protonated followed by sequential transfer of an electron and a proton to give the alcohol. It seems likely that, under similar conditions, an acyl-isoxazole undergoes the initial electron transfer to the carbonyl group to form the radical anion in analogy with the first step of the mechanism outlined in Scheme 76. The reaction is diverted from the formation of the alcohol because the radical anion is stabilised through conjugation with the isoxazole ring. The dominant resonance contributor of a radical anion formed on an isoxazole ring is **129** because positioning the unpaired spin density, rather than the negative charge, next to the electron rich oxygen is more favourable (Scheme 77).



129

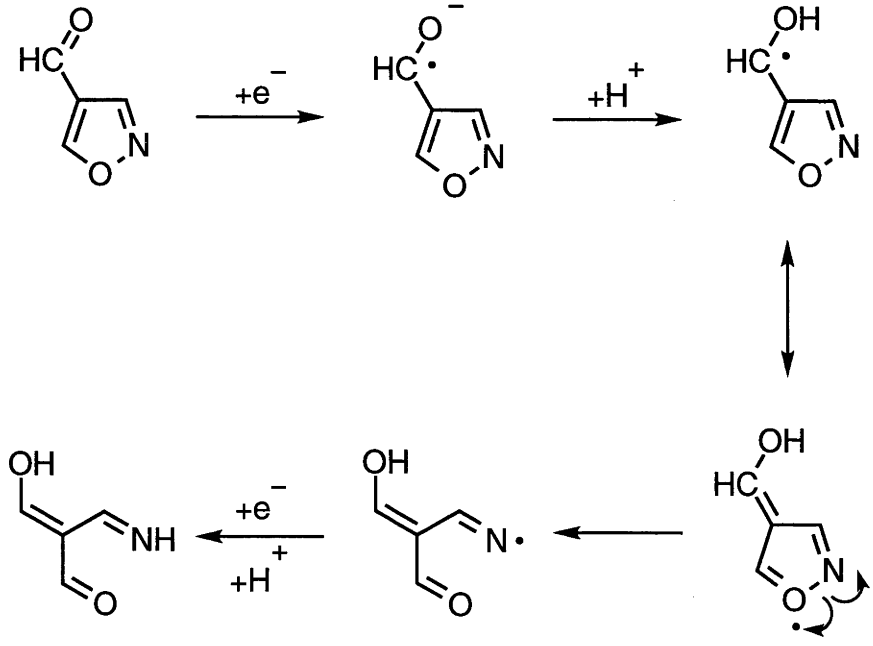
Scheme 77

The addition of a carbonyl or cyano group at the 4-position further stabilises the negative charge through conjugation (Scheme 78).



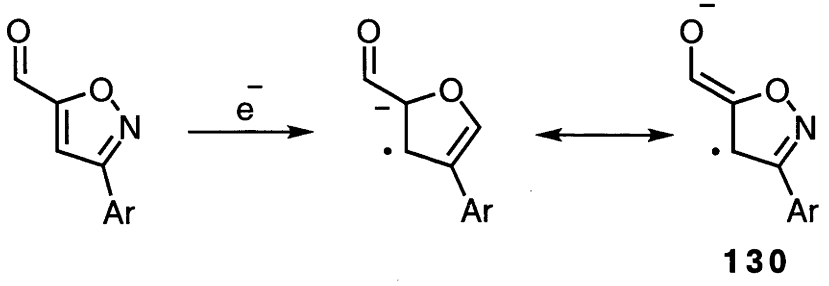
Scheme 78

At this point, the reaction is diverted from the reduction of the carbonyl group as the radical anion is stabilised through resonance with the isoxazole ring. The delocalised radical undergoes N-O bond homolysis to produce the nitrogen centred radical which, following subsequent electron and proton transfer, produces the imine (Scheme 79).



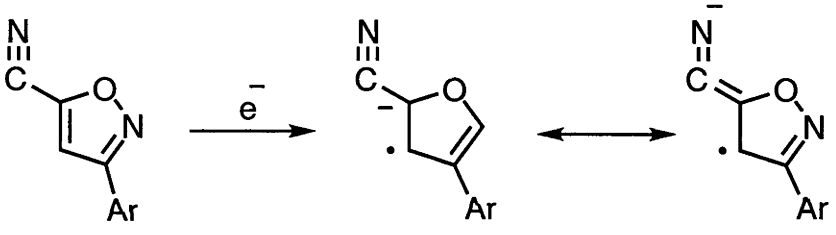
Scheme 79

In contrast, the dominant resonance contributor for the radical anions formed from the 5-carbonyl and 5-cyano substituted isoxazoles are **130** and **131** because the negative charge is stabilised through conjugation with the electron-withdrawing substituent at C-5 (Schemes 80 and 81).



130

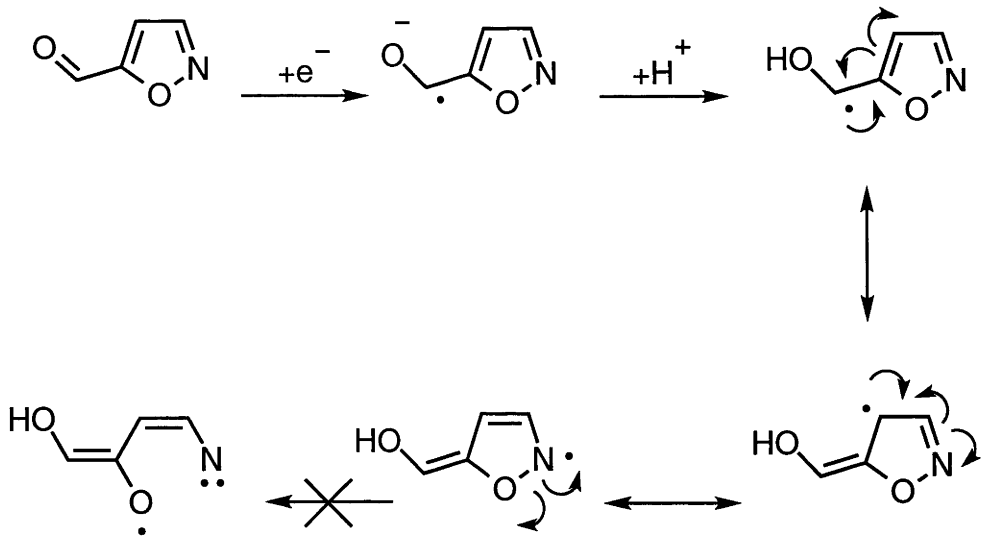
Scheme 80



131

Scheme 81

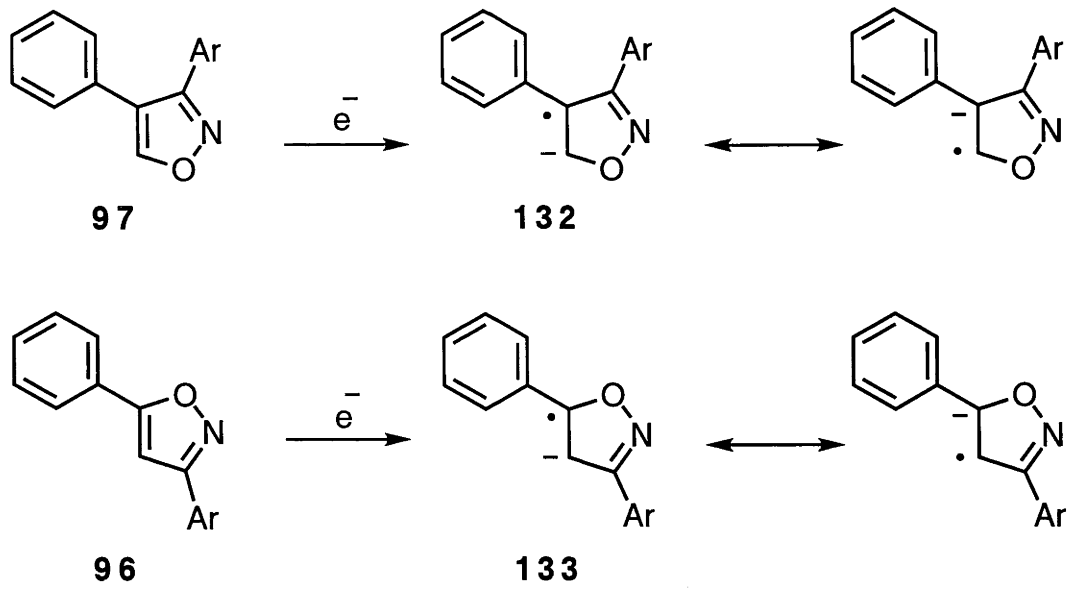
The delocalised radical is unlikely to undergo N-O bond homolysis because that would result in the formation of an unstable electron deficient nitrene (Scheme 82).



Scheme 82

A reduction potential is an indication of how readily a compound is reduced, which is directly related to the stability of the initial reduction product. Therefore, with respect to a pair of isoxazole regioisomers, the isomer which produces the more stable radical anion will have a lower reduction potential. On that basis, the 4-carbonyl and 4-cyano substituted isoxazoles **56**, **58**, **93** and **95** would have lower reduction potentials than their regioisomers and regioisomeric analogues because of the stabilisation through conjugation leading to a more stable product. The isoxazoles **56**, **58**, **93** and **95** do indeed have lower reduction potentials than the isoxazoles **60**, **61**, **92** and **94**.

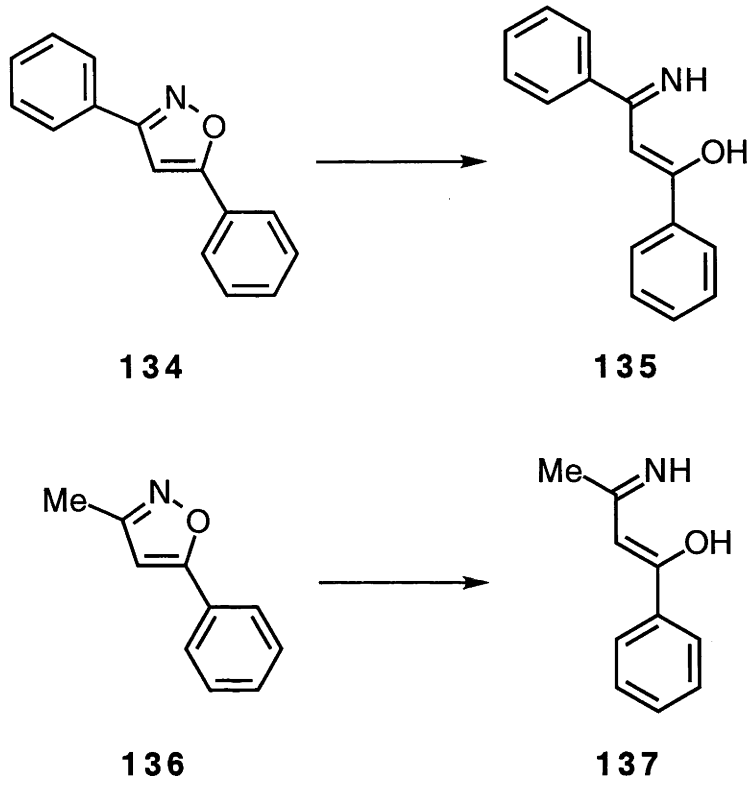
The 5-phenyl isoxazole **96** was reduced at a potential of -2.0 V while it regioisomer, isoxazole **97** has a reduction potential of -2.4 V, a reversal of the reduction potential trend observed above.



Scheme 83

The dominant resonance contributor for the radical anion of the 4-phenylisoxazole **97** is **132** because the spin is strongly delocalised by resonance with the phenyl ring (Scheme 83). For the 5-phenylisoxazole **96** radical anion the dominant resonance contributor is **133** because the spin is strongly delocalised by the phenyl ring and the unpaired spin density is also positioned adjacent to a electron rich oxygen. On that basis it was expected that **133** would be a more stable radical anion than **132** and as such, the isoxazole **96** has a lower reduction potential than its regioisomer, the isoxazole **97**.

The electrochemical reductions of isoxazoles **96** and **97** both gave complex mixtures of products. However, Lund[161] and Markova[162] have accomplished the ring opening of a number of 3,5-biphenyl isoxazoles and 5-phenyl isoxazoles under electrochemical conditions (Scheme 84).



134 **135**

136 **137**

Scheme 84

For example, Lund has reported the successful electrochemical ring opening of the 5-phenylisoxazoles **134** and **136** to their corresponding imines **135** and **137** in yields of 74% and 83%, respectively. There is no evidence in the literature to suggest that a 4-phenyl substituted isoxazole has been ring opened electrochemically. The investigations detailed above are therefore consistent with those published by Lund and Markova. Clearly, the 2,6-dichloro substituent is in some way influencing the electron distribution in the radical anion resulting from the electrolysis of the isoxazole **96** such that no discrete ring opened product is produced.

In conclusion, the electrochemical reduction of isoxazoles can be considered a chemical model for the reductive ring opening of the isoxazoles by yeast. Furthermore, the reductive ring opening of isoxazoles with yeast and under electrochemical conditions is dependent on the position and electron-withdrawing and conjugation characteristics of the substituents on the isoxazole ring. The mechanism by which the isoxazole ring opening is thought to occur is outlined in Scheme 79 and is a diversion of the mechanism by

which yeast converts carbonyl groups into alcohols. This research has also shown that the electrochemical reduction of isoxazoles is a viable and environmentally friendly method for their ring-opening.

CONCLUSION

Rama Rao *et al.*[84-88] suggested that bakers' yeast was required for some nitrile oxide cycloaddition reactions to occur and that the addition of β-CD reversed the regioselectivity observed for these processes. However, Hughes *et al.*[89] and work presented here have shown that the cycloaddition reactions proceed in good yield in the absence of bakers' yeast and that the isomeric resolution observed in the presence of β-CD results from a difference in relative solubilities of the isoxazoline regioisomers in the aqueous ethanol solution and the selectivity of complexation of each isoxazoline by β-CD.

The complexation of chloroform by β-CD has been shown to be a viable method for the isolation of β-CD for a mixture of cyclodextrins. This separation technique relies on a difference in solubilities of the complexes formed between chloroform and α- and β-CD.

In Chapter One, a method for the reverse phase extraction of isoxazolines using simple sugars has been investigated. This method could be developed as a separation technique for regioisomers containing substituents capable of complexing with β-CD and that have low but different solubilities in aqueous solvent systems. Ideally, β-CD would be immobilised on a solid support in a chromatography column through which a mixture could be passed and the regioisomers separated. This method of separation would be advantageous because it does not rely on the use of organic solvents.

In Chapter Two a practical method for the separation of cyclodextrin mixtures has also been developed. It is envisaged that this method could be extended to the isolation of β-CD from industrial mixtures of α, β and γ-CD produced by the enzyme cyclodextrin glucosyltransferase.

The hydrolysis of aryloxypropionates in pH 11.5 phosphate buffer is not catalysed enantioselectivity by BSA. Furthermore, the addition of β-CD neither increases the

efficiency, nor does it enhance the enantioselectivity of the hydrolysis reaction. The hydrolysis is promoted by the reaction medium. BSA was shown to bind selectively to the (*S*)-enantiomer of the corresponding aryloxypropionic acid and remove it from solution during the work up procedure. Therefore, the remaining solution was enriched with (*R*)-aryloxypropionic acid such that analysis of this solution gave the impression that the (*R*)-aryloxypropionate had been hydrolysed enantioselectivity by BSA. The addition of β-CD to the reaction mixture was found to have little or no effect on the extent of hydrolysis of the aryloxypropionate or its efficiency.

The work presented in Chapter Three lays the foundation for the development of a practical method for the enantioselective separation of aryloxypropionates using BSA. BSA could be immobilised on a solid support in a chromatography column through which a mixture could be passed and the enantiomers separated.

The ring opening of isoxazoles catalysed by bakers' yeast has been mimicked electrochemically. Furthermore, it was found that the type and position of the substituents on the isoxazole ring are important to the success of both the yeast catalysed and electrochemical ring opening processes. Electron-withdrawing groups such as acyl, alkoxycarbonyl or cyano at the 4-position on the isoxazole ring promote N-O bond homolysis to give the corresponding ring opened product while isoxazoles with similar groups at the 5-position are more resistant to electrolysis and give complex product mixtures. The 4-acyl-, 4-alkoxycarbonyl- and 4-cyano-substituted isoxazoles are susceptible to N-O bond cleavage because the resulting radical anions are stabilised through conjugation of the ring-oxygen with the 4-substituent. Electrolysis of an isoxazole with a phenyl substituent in either the 4- of 5-position on the isoxazole ring does not facilitate clean ring opening, however in this case the 5-substituted compound is less resistant to reduction. Chapter Four describes a synthetically viable, high yielding, efficient and environmentally friendly method for the ring opening of isoxazoles.

EXPERIMENTAL

GENERAL

Proton nuclear magnetic resonance (^{1}H nmr, 300 MHz) and carbon nuclear magnetic resonance (^{13}C nmr, 75 MHz) spectra were recorded on a Varian Gemini 300 spectrometer in deuterated chloroform ($CDCl_3$) using tetramethylsilane as an internal standard δ 0 ppm. Chemical shifts are quoted as δ in parts per million downfield of the internal standard. Multiplicities are abbreviated to: s, singlet; d, doublet; t, triplet; q, quartet; p, pentet; m, multiplet; b, broad.

Elemental analyses were performed by the Microanalytical Unit, Research School of Chemistry, The Australian National University, Canberra, Australia.

Thin layer chromatography (TLC) was performed on Merck Kieselgel $60F_{254}$ silica on aluminium backed plates. Flash column chromatography was performed using Merck Kieselgel 60 silica (230-400 mesh ASTM) using ethyl acetate and hexane as eluants unless otherwise specified.

High performance liquid chromatography (HPLC) was performed on a Waters HPLC system with dual 510 pumps, a 486 UV detector, a 410 refractive index detector and a Rheodyne manual injector or Waters Autoinjector using eluants and columns as specified. The system was controlled by a Digital Electronic Corporation Data Station running Millennium 2010 chromatography software.

Electron impact (ei) mass spectra were recorded on either a VG Autospec double focussing trisector mass spectrometer operating at 70 eV or a Vacuum Generators ZAB2-SEQ mass spectrometer. Electrospray (es) mass spectra were recorded on a VG Quatro 2 triple quadrupole mass spectrometer. Fast atom bombardment (FAB) mass spectra were recorded on a vacuum Generators ZAB 2HF mass spectrometer.

Infrared (IR) spectra were recorded on a Perkin-Elmer 1600 FTIR spectrometer either as nujol mulls or neat liquids between sodium chloride plates unless otherwise specified.

Melting points were determined on a Kofler hot-stage melting point apparatus under a Reichert microscope and are uncorrected.

Solvents used were analytical reagent (AR) grade and were not purified further unless otherwise stated. Anhydrous tetrahydrofuran was obtained by distillation over potassium metal. Drying and other purification of organic solvents and reagents was performed using standard laboratory procedures.[163]

CHAPTER ONE EXPERIMENTAL

2,6-Dichlorobenzohydroximinoyl chloride (64). To a stirred solution of 2,6-dichlorobenzoaldoxime (**63**) (5.00 g, 26.3 mmol) in *N,N*-dimethylformamide (22 ml) was added *N*-chlorosuccinimide (3.51 g, 26.3 mmol). The *N*-chlorosuccinimide addition resulted in a slight temperature decrease after which the reaction became exothermic but was kept between 25-35 °C using an ice bath. After the cessation of the exothermic reaction the solution was poured into ice/water (100 ml) and the mixture was extracted twice with diethyl ether (2 × 50 ml). The combined organic fractions were washed with water (3 × 50 ml), and dried ($MgSO_4$), and the solvent was removed under reduced pressure to give a colourless solid. The product was recrystallised from diethyl ether/*n*-hexane to give 2,6-dichlorobenzohydroximinoyl chloride (**64**) as large, rectangular, colourless crystals (4.60 g, 78%), m.p. 89-91 °C (lit.[119] m.p. 91-92 °C). ^{1}H nmr 7.31-7.41 (3H, m, aromatic protons), 8.41 (1H, s, Ar-C(Cl)=NO**H**).

***trans*-3-(2,6-Dichlorophenyl)-5-phenyl-4,5-dihydroisoxazole-4-carboxylic acid ethyl ester (11) and *trans*-3-(2,6-dichlorophenyl)-4-phenyl-4,5-dihydroisoxazole-5-carboxylic acid ethyl ester (12).** To a solution of 2,6-dichlorobenzohydroximinoyl chloride (**64**) (3.67 g, 16.3 mmol) in dry tetrahydrofuran (30 ml) was added, dropwise and with stirring, a solution of *trans*-ethyl cinnamate (**16**) (4.77 g, 27.1 mmol) and triethylamine (3.2 ml, 22.9 mmol) in dry tetrahydrofuran (60 ml). The mixture was stirred at room temperature for 1 h and then heated to reflux for 3 h. The cooled solution was concentrated under reduced pressure and the residue was taken up in chloroform. The solution was washed with water (3 × 50 ml), dried ($MgSO_4$) and concentrated under reduced pressure to give a crude mixture of the isoxazolines **11** and **12** in the ratio 85:15 as determined by ^{1}H nmr spectroscopy. The isoxazolines **11** and **12** were separated from the crude product mixture by flash column chromatography (ethyl acetate/*n*-hexane, 1:5) and each was recrystallised from ethyl acetate/*n*-hexane.

Isoxazoline **11**: (2.42 g, 41%) m.p. 85-87 °C (lit.[119] m.p. 88-89 °C). ^{1}H nmr 1.00 (3H, t, *J* 7 Hz, CH_3-CH_2-), 4.08 (2H, m, CH_3-CH_2-), 4.57 (1H, d, *J* 9 Hz, C4-**H**), 6.24 (1H, d, *J* 9 Hz, C5-**H**), 7.29-7.49 (8H, m, aromatic hydrogens). *Anal.* Calcd. for $C_{18}H_{15}Cl_2NO_3$: C 59.36, H 4.15, N 3.85. Found: C 59.59, H 4.20, N 3.98%. Mass spectrum (ei) *m/z* 367, 365, 363, (2, 8, 12; M^+), 192 (100), 148, 146 (11, 37).

Isoxazoline **12**: (0.48 g, 8%) m.p. 86-87 °C (lit.[119] m.p. 87 °C). ^{1}H nmr 1.34 (3H, t, *J* 7 Hz, CH_3-CH_2-), 4.32 (2H, q, *J* 7 Hz, CH_3-CH_2-), 5.24 (1H, d, *J* 5.5 Hz, C5-**H**), 5.28 (1H, d, *J* 5.5 Hz, C4-**H**), 7.22-7.46 (8H, m, aromatic hydrogens). *Anal.* Calcd. for $C_{18}H_{15}Cl_2NO_3$: C 59.36, H 4.15, N 3.85. Found: C 59.39, H 4.40, N 3.87%.

2,4,6-Trimethylbenzoaldoxime (66). To 2,4,6-trimethylbenzaldehyde (**65**) (2.00 g, 13.5 mmol) in water (4 ml), ethanol (4 ml) and ice (6 ml) was added hydroxylamine hydrochloride (1.03 g, 14.8 mmol). To this mixture was added 50% sodium hydroxide solution (2.70 ml) with stirring. The mixture was stirred for 1.5 h, washed with diethyl ether (1 × 20 ml) to remove any neutral impurities, and acidified with concentrated hydrochloric acid and extracted twice with diethyl ether (2 × 20 ml). The combined organic extracts were dried ($MgSO_4$) and the solvent was removed under reduced pressure. The resulting colourless solid was recrystallised from aqueous ethanol to yield 2,4,6-trimethylbenzoaldoxime (**66**) as small, colourless, trapezoid crystals (0.61 g, 28%), m.p. 124-125 °C (lit.[21] m.p. 125-127 °C). ^{1}H nmr 2.25 (1H, bs, CH=NO**H**), 2.30 (3H, s, CH_3), 2.39 (6H, s, $(CH_3)_2$), 6.90 (2H, s, aromatic protons), 8.43 (1H, s, Ar-C**H**=NOH).

2,4,6-Trimethylbenzohydroximinoyl chloride (67). To a stirred solution of 2,4,6-trimethylbenzoaldoxime (**66**) (0.61 g, 3.73 mmol) in *N,N*-dimethylformamide (13 ml) was added *N*-chlorosuccinimide (2.05 g, 15.4 mmol). The *N*-chlorosuccinimide addition resulted in a slight temperature decrease after which the reaction became exothermic but was kept between 25-35 °C. After the cessation of the exothermic reaction the solution was poured into ice/water (12 ml) and the mixture was extracted with

diethyl ether (2 × 10 ml). The combined organic fractions were washed with water (3 × 20 ml), and dried ($MgSO_4$) and the solvent was removed under reduced pressure to give a colourless solid. The product was recrystallised from diethyl ether/*n*-hexane to give 2,4,6-trimethylbenzohydroximinoyl chloride (**67**) as a pale yellow powder (0.62 g, 84%), m.p. 49-63 °C (lit.[21] m.p. 61-69 °C). ^{1}H nmr 2.28 (6H, s, $(CH_3)_2$), 2.41 (3H, s, CH_3), 6.86 (2H, s, aromatic protons), 8.31 (1H, s, C(Cl)=NOH).

***trans*-3-(2,4,6-Trimethylphenyl)-5-phenyl-4,5-dihydroisoxazole-4-carboxylic acid ethyl ester (13) and trans-3-(2,4,6-trimethylphenyl)-4-phenyl-4,5-dihydroisoxazole-5-carboxylic acid ethyl ester (14).** To a solution of 2,4,6-trimethylbenzohydroximinoyl chloride (**67**) (6.00 g, 30.4 mmol) in dry tetrahydrofuran (40 ml) was added, dropwise and with stirring, a solution of ethyl *trans*-cinnamate (**16**) (5.74 g, 32.6 mmol) and triethylamine (5.32 ml, 38.2 mmol) in dry tetrahydrofuran (70 ml). The mixture was stirred at room temperature for 1 h and then heated to reflux for 3 h. The cooled solution was concentrated under reduced pressure and the residue taken up in chloroform. The solution was washed with water (3 × 50 ml), dried (magnesium sulphate) and concentrated under reduced pressure to give the crude mixture of the isoxazolines **13** and **14** in the ratio of 50:50 as determined by ^{1}H nmr spectroscopy. The isoxazolines **13** and **14** were separated from the crude product mixture by flash column chromatography (ethyl acetate/*n*-hexane, 1:5) and each was recrystallised from ethyl acetate/*n*-hexane.

Isoxazoline **13**: (5.02 g, 49%) m.p. 85-87 °C (lit.[119] m.p. 87-89 °C). ^{1}H nmr 0.93 (3H, t, *J* 7 Hz, CH_3-CH_2-), 2.22 (6H, s, Ar-$(CH_3)_2$), 2.28 (3H, s, Ar-CH_3), 4.00 (2H, m, CH_3-CH_2-), 4.37 (1H, d, *J* 9.5 Hz, C4-**H**), 6.11 (1H, d, *J* 9.5 Hz, C5-**H**), 7.36-7.47 (7H, m, aromatic protons). *Anal.* Calcd. for $C_{21}H_{23}NO_3$: C 74.75, H 6.87, N 4.15. Found: C 74.88, H 6.90, N 4.08%. Mass spectrum (ei) *m/z* 337 (100; M^+), 192 (58), 186 (67), 104 (96).

Isoxazoline **14**: (2.99 g, 29%) m.p. 83-85 °C (lit.[119] m.p. 85-86 °C). ^{1}H nmr 1.35 (3H, t, *J* 7 Hz, CH_3-CH_2-), 1.98 (3H, s, Ar-CH_3), 2.25 (6H, s, Ar-$(CH_3)_2$), 4.33 (2H, q, *J* 7 Hz, CH_3-CH_2-), 4.82 (1H, d, *J* 4 Hz, C5-**H**), 5.33 (1H, d, *J* 4 Hz, C4-**H**), 7.14-7.18 (2H, m, aromatic protons). *Anal.* Calcd. for $C_{21}H_{23}NO_3$: C 74.75, H 6.87, N 4.15. Found: C 74.35, H 6.66, N 3.74%. Mass spectrum (ei) *m/z* 337 (7; M^+), 264 (100; M^+-$CO_2CH_2CH_3$), 161 (70).

4-*tert*-Butylbenzoaldoxime (69). To 4-*tert*-butylbenzaldehyde (**68**) (7.76 g, 47.8 mmol) in water (11 ml), ethanol (11 ml) and ice (19 ml) was added hydroxylamine hydrochloride (3.7 g, 53.2 mmol). To the mixture was added 50% sodium hydroxide solution (8.6 ml) with stirring. The mixture was stirred for 1.5 h, washed with diethyl ether (1 × 30 ml) to remove any neutral impurities, acidified with concentrated hydrochloric acid and extracted with diethyl ether (2 × 30 ml). The combined organic extracts were dried ($MgSO_4$) and the solvent was removed under reduced pressure. The resulting colourless solid was recrystallised from aqueous ethanol to yield 4-*tert*-butylbenzoaldoxime (**69**) as colourless, needle like crystals (4.37 g, 52%), m.p. 104-106 °C (lit.[119] m.p. 105-106 °C). ^{1}H nmr 1.32 (9H, s, $(CH_3)_3$, 7.40 (2H, d, *J* 6.5 Hz, aromatic protons), 7.52 (2H, d, *J* 6.5 Hz, aromatic protons), 8.14 (1H, bs, C**H**=NOH), 8.48 (1H, s, Ar-CH=NO**H**).

4-*tert*-Butylbenzohydroximinoyl chloride (70). To a stirred solution of 4-*tert*-butylbenzoaldoxime (**69**) (1.00 g, 5.6 mmol) in *N,N*-dimethylformamide (8 ml) was added *N*-chlorosuccinimide (0.75 g, 5.6 mmol). The *N*-chlorosuccinimide addition resulted in a slight temperature decrease after which the reaction became exothermic but was kept between 25-35 °C. After the cessation of the exothermic reaction the solution was poured into ice/water (15 ml) and the mixture was extracted with diethyl ether (2 × 10 ml). The combined organic fractions were washed with water (3 × 10 ml), and dried (calcium sulfate), and the solvent was removed under reduced pressure to give 4-*tert*-butylbenzohydroximinoyl chloride (**70**) as a pale yellow powder (0.92 g, 77%), m.p. 69-73 °C (lit.[119] m.p. 70-76 °C). ^{1}H nmr 1.32 (9H, s, $(CH_3)_3$), 7.43 (2H, d, *J* 6.5 Hz,

aromatic protons), 7.76 (2H, d, *J* 6.5 Hz, aromatic protons), 8.49 (1H, s, Ar-C(Cl)=NO**H**). Mass spectrum (FAB) *m/z* : 214, 212 (32, 85; M^+ + H), 163, 161 (21, 99), 56 (100).

***trans*-3-(1,1-Dimethylethylphenyl)-5-phenyl-4,5-dihydroisoxazole-4-carboxylic acid ethyl ester (20) and *trans*-3-(1,1-dimethylethylphenyl)-4-phenyl-4,5-dihydroisoxazole-5-carboxylic acid ethyl ester (21).** To a solution of 4-*tert*-butylbenzohydroximinoyl chloride (**70**) (1.00 g, 4.7 mmol) in dry tetrahydrofuran (15 ml) was added, dropwise and with stirring, a solution of ethyl *trans*-cinnamate (**16**) (0.83 g, 4.7 mmol) and triethylamine (0.60 ml, 4.3 mmol) in dry tetrahydrofuran (30 ml). The mixture was stirred at room temperature for 1 h and then heated to reflux for 3 h. The cooled solution was concentrated under reduced pressure and the residue was taken up in chloroform. The solution was washed with water (3 × 40 ml), dried ($MgSO_4$) and concentrated under reduced pressure to give a crude mixture of the isoxazolines **20** and **21** in the ratio 73:27 as determined by ^1H nmr spectroscopy. The isoxazolines **20** and **21** were separated from the crude product mixture by flash column chromatography (ethyl acetate/*n*-hexane, 1:20) and each was recrystallised from ethyl acetate/*n*-hexane.

Isoxazoline **20**: (0.78 g, 47%) m.p. 77-79 °C (lit.[119] m.p. 78-79 °C). ^1H nmr 1.24 (3H, t, *J* 7 Hz, CH_3-CH_2-), 1.32 (9H, s, Ar-$(CH_3)_3$), 4.24 (2H, q, *J* 7 Hz, CH_3-CH_2), 4.42 (1H, d, *J* 6 Hz, C4-**H**], 5.96 (1H, d, *J* 6 Hz, C5-**H**), 7.35-7.39 (7H, m, aromatic hydrogens), 7.67 (2H, d, *J* 8.5 Hz, Ar-H_2). *Anal.* Calcd. for $C_{22}H_{25}NO_3$: C 75.19, H 7.17, N 3.99. Found: C 75.28, H 7.45, N 3.85%. Mass spectrum (ei) *m/z* 351 (46; M^+), 336 (39, M^+-CH_3) 192 (48), 160 (36), 146 (58), 105 (100), 77 (43). HR mass spect. $C_{22}H_{25}NO_3$ requires M+, *m/z* 351.1834; found M^+, *m/z* 351.1793.

Isoxazoline **21**: (0.18 g, 11%) m.p. 94-96 °C (lit.[119] m.p. 96-97 °C). ^1H nmr 1.26 (9H, s, Ar-$(CH_3)_3$), 1.30 (3H, t, *J* 7 Hz, CH_3-CH_2-), 4.27 (2H, q, *J* 7 Hz, CH_3-CH_2), 4.93 (1H, d, *J* 4 Hz, C5-**H**), 5.01 (1H, d, *J* 4 Hz, C4-**H**), 7.26-7.35 (7H, m, aromatic

hydrogens), 7.56 (2H, d, *J* 8.5 Hz, Ar-$\mathbf{H}_2$). *Anal.* Calcd. for $C_{22}H_{25}NO_3$: C 75.19, H 7.17, N 3.99. Found: C 75.41, H 6.92, N 3.97%. Mass spectrum (ei) *m/z* 351(32; M^+), 278 (100), 114 (30), 91(44). HR mass spect. $C_{22}H_{25}NO_3$ requires M^+, *m/z* 351.1834; found M+, *m/z* 351.1766.

2,2-Dimethyl-propanal oxime (72). To pivaldehyde (**71**) (2.00 g, 23.2 mmol) in water (3 ml), ethanol (3 ml) and ice (5 ml) was added hydroxylamine hydrochloride (1.78 g, 25.6 mmol). To this mixture was added 50% sodium hydroxide solution (2.4 ml) with stirring. The mixture was stirred for 1.5 h, washed with diethyl ether (1 × 5 ml) to remove any neutral impurities, acidified with concentrated hydrochloric acid and extracted with diethyl ether (2 × 10 ml). The combined organic extracts were dried (calcium sulfate) and the solvent was removed under reduced pressure. The resulting colourless solid was recrystallised from aqueous ethanol to yield 2,2-dimethyl-propanal oxime (**72**) as a pale yellow oil (2.15 g, 92%). ^{1}H nmr 1.11 (9H, s, $(\mathbf{CH}_3)_3$), 1.95 (1H, bs, CH=NO**H**), 4.80 (1H, s, C**H**=NOH). I.R. (nujol): 3396, 2976 cm^{-1}.

***N*-Hydroxy-2,2-dimethyl propanimidoyl chloride (73).** To a stirred solution of oxime (**72**) (1.00 g, 9.9 mmol) in *N,N*-dimethylformamide (8 ml) was added *N*-chlorosuccinimide (1.32 g, 9.9 mmol). The *N*-chlorosuccinimide addition resulted in a slight temperature decrease after which the reaction became exothermic but was kept between 25-35 °C. After the cessation of the exothermic reaction the solution was poured into ice/water (15 ml) and extracted with diethyl ether (2 × 20 ml). The combined organic fractions were washed with water (2 × 30 ml) and dried (calcium sulfate) and the solvent was removed under reduced pressure to give *N*-hydroxy-2,2-dimethyl propanimidoyl chloride (**73**) as a pale yellow oil (0.73 g, 54%). ^{1}H nmr 1.25 (9H, s, $(\mathbf{CH}_3)_3$), 8.44 (1H, s, C(Cl)=NO**H**).

***trans*-3-(1,1-Dimethylethyl)-5-phenyl-4,5-dihydroisoxazole-4-carboxylic acid ethyl ester (22) and *trans*-3-(1,1-dimethylethyl)-4-phenyl-4,5-dihydroisoxazole-5-carboxylic acid ethyl ester (23).** To a solution of the

hydroximinoyl chloride (**73**) (0.88 g, 6.49 mmol) in dry tetrahydrofuran (7 ml) was added, dropwise and with stirring, a solution of ethyl *trans*-cinnamate (**16**) (1.14 g, 6.47 mmol) and triethylamine (1.05 ml, 7.53 mmol) in dry tetrahydrofuran (14 ml). The mixture was stirred at room temperature for 1 h and then heated to reflux for 3 h. The cooled solution was concentrated under reduced pressure and the residue was taken up in chloroform. The solution was washed with water (3 × 50 ml), dried ($MgSO_4$) and concentrated under reduced pressure to give a crude mixture of the isoxazolines **22** and **23** in the ratio 74:26 as determined by ^1H nmr spectroscopy. The isoxazolines **22** and **23** were separated from the crude product mixture by flash column chromatography (ethyl acetate/*n*-hexane, 1:20) and the isoxazoline **22** was recrystallised from ethyl acetate/*n*-hexane. The isoxazoline **23** was isolated as a viscous yellow oil.

Isoxazoline **22**: (0.64 g, 36%) m.p. 45-46 °C (lit.[119] m.p. 49 °C). ^1H nmr 1.24 (9H, s, $(CH_3)_3$-), 1.33 (3H, t, *J* 7 Hz, CH_3-CH_2-), 3.99 (1H, d, *J* 6.5 Hz, C5-**H**), 4.29 (2H, q, *J* 7 Hz, CH_3-CH_2-), 5.76 (1H, d, *J* 6.5 Hz, C4-**H**), 7.28-7.41 (5H, m, aromatic hydrogens). *Anal.* Calcd. for $C_{16}H_{21}NO_3$: C 69.79, H 7.69, N 5.09. Found: C 69.66, H 7.72, N 5.01%. Mass spectrum (ei) *m/z* 202 (16; M^+-$CO_2CH_2CH_3$), 192 (68), 146 (44), 105 (100), 57 (74; $(CH_3)_3$). HR mass spect. $C_{16}H_{21}NO_3$-$C_2H_5O_2$ requires M^+-$C_2H_5O_2$, *m/z* 202.1319; found M^+-$C_2H_5O_2$, *m/z* 202.1273.

Isoxazoline **23**: (0.15 g, 8%) ^1H nmr 1.06 (9H, s, $(CH_3)_3$-), 1.25 (3H, t, *J* 7 Hz, CH_3-CH_2-), 4.27 (2H, q, *J* 7 Hz, CH_3-CH_2), 4.55 (1H, d, *J* 3 Hz, C5-**H**), 4.77 (1H, d, *J* 3 Hz, C4-**H**), 7.26-7.39 (5H, m, aromatic hydrogens). *Anal.* Calcd. for $C_{16}H_{21}NO_3$: C 69.79, H 7.69, N 5.09. Found: C 69.70, H 7.61, N 4.85%. Mass spectrum (ei) *m/z* 202 (25; M+-$CO_2CH_2CH_3$), 57 (100); $(CH_3)_3$). HR mass spect. $C_{16}H_{21}NO_3$-$C_2H_5O_2$ requires M^+-$C_2H_5O_2$, *m/z* 202.1319; found M^+-$C_2H_5O_2$, *m/z* 202.1252.

UV response ratios for *trans*-3-(2,6-Dichlorophenyl)-5-phenyl-4,5-dihydroisoxazole-4-carboxylic acid ethyl ester (11) and *trans*-3-(2,6-dichlorophenyl)-4-phenyl-4,5-dihydroisoxazole-5-carboxylic acid ethyl

ester (12). A mixture of the isoxazolines **11** (2.91×10^{-3} mg, 7.99×10^{-3} mmol) and **12** (2.19×10^{-3} mg, 6.01×10^{-3} mmol) was dissolved in acetonitrile (5 ml). A sample (3 μl) of this solution was analysed by HPLC using a Waters Symmetry® C_{18} 3.5 μm (75 mm × 4.6 mm) column monitored at 220 nm and eluted with 70% acetonitrile/water at a flow rate of 1.1 ml/min. The isoxazoline **11** (4.79×10^{-9} mol) gave a peak area of 4360511 mV.s and the isoxazoline **12** (3.61×10^{-9} mol) gave a peak area of 3640188 mV.s.

Preparation of the *trans*-3-(2,6-dichlorophenyl)-5-phenyl-4,5-dihydroisoxazole-4-carboxylic acid ethyl ester (11) standard. *trans*-3-(2,6-Dichlorophenyl)-5-phenyl-4,5-dihydroisoxazole-4-carboxylic acid ethyl ester (**11**) (10.4 mg, 2.855×10^{-2} mmol) was dissolved in HPLC Grade acetonitrile (5 ml).

Preparation of the *trans*-3-(1,1-dimethylethylphenyl)-5-phenyl-4,5-dihydroisoxazole-4-carboxylic acid ethyl ester (20) standard. *trans*-3-(1,1-Dimethylethylphenyl)-5-phenyl-4,5-dihydroisoxazole-4-carboxylic acid ethyl ester (**20**) (11.1 mg, 3.158×10^{-2} mmol) was dissolved in HPLC Grade acetonitrile (5 ml).

Preparation of the β-cyclodextrin-11% aqueous ethanol standard solution. To a solution of 11% aqueous ethanol (200 ml) was added β-cyclodextrin (11.51g, 0.01 mol). The solution was heated gently until all the cyclodextrin dissolved.

Determination of absolute and relative solubilities of *trans*-3-(2,6-dichlorophenyl)-5-phenyl-4,5-dihydroisoxazole-4-carboxylic acid ethyl ester (11) and *trans*-3-(2,6-dichlorophenyl)-4-phenyl-4,5-dihydroisoxazole-5-carboxylic acid ethyl ester (12) in 20% aqueous ethanol at 30 °C in the absence of β-cyclodextrin. To a solution of 11% aqueous ethanol (363 ml) was added dropwise with stirring ethanol (37 ml) containing the isoxazoline **11** (15.9 mg, 4.37×10^{-2} mmol) and the isoxazoline **12** (12.1 mg, 3.32×10^{-2} mmol). The cloudy suspension was equilibrated at 30 °C overnight after which it

was filtered twice through 0.2 μm syringe filters (Sartorius Minisartat®) at 28 °C. The filtrate was filtered again though 0.02 μm syringe filters (Whatman® Anotop 25 Plus) at 28 °C and the resulting filtrate was extracted with chloroform (100 ml × 3). The chloroform was removed under reduced pressure and the residue was taken up in acetonitrile (HPLC grade, 500 μl) and the *trans*-3-(1,1-dimethylethylphenyl)-5-phenyl-4,5-dihydroisoxazole-4-carboxylic acid ethyl ester (**20**) standard added (100 μl). A sample (10 μl) was analysed by HPLC using a Waters Symmetry® C_{18} 3.5 μm (75 mm × 4.6 mm) column monitored at 220 nm and eluted with 70% acetonitrile/water at a flow rate of 1.1 ml/min and the absolute and relative solubilities were determined by comparison of peak areas with those determined above for known quantities of the isoxazolines **11** and **12**. This experiment was repeated in triplicate.

	Isoxazoline **11** (mol/dm^{-3})	Isoxazoline **12** (mol/dm^{-3})	Relative Solubility **11:12**
Experiment 1	1.4×10^{-5}	2.2×10^{-5}	1:1.6
Experiment 2	0.8×10^{-5}	1.2×10^{-5}	1:1.5
Experiment 3	1.2×10^{-5}	1.9×10^{-5}	1:1.6
Average	1.1×10^{-5}	1.8×10^{-5}	1:1.6

UV response ratios for *trans*-3-(2,4,6-trimethylphenyl)-5-phenyl-4,5-dihydroisoxazole-4-carboxylic acid ethyl ester (13) and trans-3-(2,4,6-trimethylphenyl)-4-phenyl-4,5-dihydroisoxazole-5-carboxylic acid ethyl ester (14). A mixture of the isoxazolines **13** (2.58×10^{-3} g, 7.65×10^{-3} mmol) and **14** (3.02×10^{-3} g, 8.95×10^{-3} mmol) was dissolved in acetonitrile (5 ml). A sample (3 μl) of this solution was analysed by HPLC using a Waters Symmetry® C_{18} 3.5 μm (75 mm × 4.6 mm) column monitored at 220 nm and eluted with 70 % acetonitrile/water at a flow rate of 1.1 ml/min. The isoxazoline **13** (4.59×10^{-9} mol) gave a peak area of 5954359 mV.s and the isoxazoline **14** (5.37×10^{-9} mol) gave a peak area of 4963998 mV.s.

Determination of absolute and relative solubilities of *trans*-3-(2,4,6-trimethylphenyl)-5-phenyl-4,5-dihydroisoxazole-4-carboxylic acid ethyl

ester (13) and trans-3-(2,4,6-trimethylphenyl)-4-phenyl-4,5-dihydroisoxazole-5-carboxylic acid ethyl ester (14) in 20% aqueous ethanol at 30 °C in the absence of β-cyclodextrin. To a solution of 11% aqueous ethanol (90 ml) was added dropwise with stirring ethanol (10 ml) containing the isoxazoline **13** (34.9 mg, 1.03×10^{-1} mmol) and the isoxazoline **14** (40.9 mg, 1.21×10^{-1} mmol). The cloudy suspension was equilibrated at 30 °C overnight after which it was filtered twice through 0.2 μm syringe filters (Sartorius Minisartat®) at 28 °C. The filtrate was filtered again though 0.02 μm syringe filters (Whatman® Anotop 25 Plus) at 28 °C and the resulting filtrate was extracted with chloroform (100 ml × 3). The chloroform was removed under reduced pressure and the residue was taken up in acetonitrile (HPLC grade, 500 μl) and the *trans*-3-(1,1-dimethylethylphenyl)-5-phenyl-4,5-dihydroisoxazole-4-carboxylic acid ethyl ester (**20**) standard added (100 μl). A sample (1 μl) was analysed by HPLC using a Waters Symmetry® C_{18} 3.5 μm (75 mm × 4.6 mm) column monitored at 220 nm and eluted with 70% acetonitrile/water at a flow rate of 1.1 ml/min and the absolute and relative solubilities were determined by comparison of peak areas with those determined above for known quantities of the isoxazolines **13** and **14**. This experiment was repeated in triplicate.

	Isoxazoline **13** (mol/dm^{-3})	Isoxazoline **14** (mol/dm^{-3})	Relative Solubility **13**:**14**
Experiment 1	2.7×10^{-6}	1.5×10^{-5}	1:5.6
Experiment 2	3.2×10^{-6}	1.9×10^{-5}	1:5.9
Experiment 3	2.5×10^{-6}	1.7×10^{-5}	1:6.8
Average	2.8×10^{-6}	1.7×10^{-5}	1:6.1

UV response ratios for *trans*-3-(1,1-dimethylethylphenyl)-5-phenyl-4,5-dihydroisoxazole-4-carboxylic acid ethyl ester (20) and *trans*-3-(1,1-dimethylethylphenyl)-4-phenyl-4,5-dihydroisoxazole-5-carboxylic acid ethyl ester (21). A mixture of the isoxazolines **20** (4.34×10^{-3} g, 1.23×10^{-2} mmol) and **21** (7.65×10^{-4} g, 2.18×10^{-3} mmol) was dissolved in acetonitrile (5 ml). A sample (3 μl) of this solution was analysed by HPLC using a Waters Symmetry® C_{18} 3.5 μm (75 mm × 4.6 mm) column monitored at 220 nm and eluted with 70% acetonitrile/water at

a flow rate of 1.1 ml/min. The isoxazoline **20** (7.38×10^{-9} mol) gave a peak area of 5791248 mV.s and the isoxazoline **21** (1.31×10^{-9} mol) gave a peak area of 868196 mV.s.

Determination of absolute and relative solubilities of *trans*-3-(1,1-dimethylethylphenyl)-5-phenyl-4,5-dihydroisoxazole-4-carboxylic acid ethyl ester (20) and *trans*-3-(1,1-dimethylethylphenyl)-4-phenyl-4,5-dihydroisoxazole-5-carboxylic acid ethyl ester (21) in 20% aqueous ethanol at 30 °C in the absence of β-cyclodextrin. To a solution of 11% aqueous ethanol (363 ml) was added dropwise with stirring ethanol (37 ml) containing the isoxazoline **20** (28.7 mg, 8.17×10^{-2} mmol) and the isoxazoline **21** (5.06 mg, 1.44×10^{-2} mmol). The cloudy suspension was equilibrated at 30 °C overnight after which it was filtered twice through 0.2 μm syringe filters (Sartorius Minisartat®) at 28 °C. The filtrate was filtered again though 0.02 μm syringe filters (Whatman® Anotop 25 Plus) at 28 °C and the resulting filtrate was extracted with chloroform (100 ml × 3). The chloroform was removed under reduced pressure and the residue was taken up in acetonitrile (HPLC grade, 500 μl) and the *trans*-3-(2,6-dichlorophenyl)-5-phenyl-4,5-dihydroisoxazole-4-carboxylic acid ethyl ester (**11**) standard added (100 μl). A sample (10 μl) was analysed by HPLC using a Waters Symmetry® C_{18} 3.5 μm (75 mm × 4.6 mm) column monitored at 220 nm and eluted with 70% acetonitrile/water at a flow rate of 1.1 ml/min and the absolute and relative solubilities were determined by comparison of peak areas with those determined above for known quantities of the isoxazolines **20** and **21**. This experiment was repeated in triplicate.

	Isoxazoline **20** (mol/dm^{-3})	Isoxazoline **21** (mol/dm^{-3})	Relative Solubility **20:21**
Experiment 1	1.2×10^{-8}	6.0×10^{-6}	1:500
Experiment 2	7.2×10^{-9}	5.4×10^{-6}	1:750
Experiment 3	-	3.3×10^{-6}	-
Average	9.6×10^{-9}	4.9×10^{-6}	1:510

UV response ratios for *trans*-3-(1,1-dimethylethyl)-5-phenyl-4,5-dihydroisoxazole-4-carboxylic acid ethyl ester (22) and *trans*-3-(1,1-

dimethylethyl)-4-phenyl-4,5-dihydroisoxazole-5-carboxylic acid ethyl ester (23). A mixture of the isoxazolines **22** (3.95×10^{-3} g, 1.43×10^{-2} mmol) and **23** (1.05×10^{-3} g, 3.81×10^{-3} mmol) was dissolved in acetonitrile (5 ml). A sample (3 μl) of this solution was analysed by HPLC using a Waters Symmetry® C_{18} 3.5 μm (75 mm × 4.6 mm) column monitored at 220 nm and eluted with 70% acetonitrile/water at a flow rate of 1.1 ml/min. The isoxazoline **22** (8.58×10^{-9} mol) gave a peak area of 3212449 mV.s and the isoxazoline **23** (2.29×10^{-9} mol) gave a peak area of 997556 mV.s.

Determination of absolute and relative solubilities of *trans*-3-(1,1-dimethylethyl)-5-phenyl-4,5-dihydroisoxazole-4-carboxylic acid ethyl ester (22) and *trans*-3-(1,1-dimethylethyl)-4-phenyl-4,5-dihydroisoxazole-5-carboxylic acid ethyl ester (23) in 20% aqueous ethanol at 30 °C in the absence of β-cyclodextrin. To a solution of 11% aqueous ethanol (90 ml) was added dropwise with stirring ethanol (10 ml) containing the isoxazoline **22** (49.8 mg, 1.81×10^{-1} mmol) and the isoxazoline **23** (13.2 mg, 4.79×10^{-2} mmol). The cloudy suspension was equilibrated at 30 °C overnight after which it was filtered twice through 0.2 μm syringe filters (Sartorius Minisartat®) at 28 °C. The filtrate was filtered again though 0.02 μm syringe filters (Whatman® Anotop 25 Plus) at 28 °C and the resulting filtrate was extracted with chloroform (100 ml × 3). The chloroform was removed under reduced pressure and the residue was taken up in acetonitrile (HPLC grade, 500 μl) and the *trans*-3-(2,6-dichlorophenyl)-5-phenyl-4,5-dihydroisoxazole-4-carboxylic acid ethyl ester (**11**) standard added (100 μl). A sample (10 μl) was analysed by HPLC using a Waters Symmetry® C_{18} 3.5 μm (75 mm × 4.6 mm) column monitored at 220 nm and eluted with 70 % acetonitrile/water at a flow rate of 1.1 ml/min. The absolute and relative solubilities were determined by comparison of peak areas with those determined above for known quantities of the isoxazolines **22** and **23**. This experiment was repeated in triplicate.

	Isoxazoline **22** (mol/dm^{-3})	Isoxazoline **23** (mol/dm^{-3})	Relative Solubility **22:23**
Experiment 1	6.8×10^{-4}	2.5×10^{-4}	2.7:1
Experiment 2	6.8×10^{-4}	2.5×10^{-4}	2.7:1
Experiment 3	6.6×10^{-4}	2.4×10^{-4}	2.8:1
Average	6.7×10^{-4}	2.5×10^{-4}	2.7:1

General procedure for the determination of absolute and relative solubilities in 20% aqueous ethanol in the presence of β-cyclodextrin. To a solution of 11% aqueous ethanol containing β-cyclodextrin (9 ml) was added dropwise with stirring ethanol (1 ml) containing the mixture of isoxazolines detailed below. The cloudy suspension was equilibrated in a waterbath at 30 °C overnight after which it was filtered twice through 0.2 μm syringe filters (Sartorius Minisartat®) and then though 0.02 μm syringe filters (Whatman® Anotop 25 Plus) at 28 °C. To this solution (500 μl) was added the standard (100 μl) and a sample (10 μl) was analysed by HPLC using a Waters Symmetry® C_{18} 3.5 μm (75 mm × 4.6 mm) column monitored at 220 nm and eluted with 70 % acetonitrile/water at a flow rate of 1.1 ml/min. The absolute and relative solubilities were determined by comparison of peak areas with those determined above for known quantities of the isoxazolines. These experiments were repeated in triplicate.

Determination of absolute and relative solubilities of *trans*-3-(2,6-dichlorophenyl)-5-phenyl-4,5-dihydroisoxazole-4-carboxylic acid ethyl ester (11) and *trans*-3-(2,6-dichlorophenyl)-4-phenyl-4,5-dihydroisoxazole-5-carboxylic acid ethyl ester (12) in 0.05 M β-cyclodextrin/20% aqueous ethanol at 30 °C.

The isoxazoline mixture: The isoxazoline **11** (28.8 mg, 7.90×10^{-2} mmol) and the isoxazoline **12** (21.7 mg, 5.96×10^{-2} mmol) were dissolved in ethanol (3.5 ml). The standard used in these experiments was *trans*-3-(1,1-dimethylethylphenyl)-5-phenyl-4,5-dihydroisoxazole-4-carboxylic acid ethyl ester (**20**).

	Isoxazoline **11** (mol/dm^{-3})	Isoxazoline **12** (mol/dm^{-3})	Relative Solubility **11:12**
Experiment 1	1.0×10^{-4}	1.5×10^{-5}	6.7:1
Experiment 2	1.0×10^{-4}	2.6×10^{-5}	3.8:1
Experiment 3	9.8×10^{-5}	1.4×10^{-5}	7.0:1
Average	9.9×10^{-5}	1.8×10^{-5}	5.5:1

Determination of absolute and relative solubilities of *trans*-3-(2,4,6-trimethylphenyl)-5-phenyl-4,5-dihydroisoxazole-4-carboxylic acid ethyl ester (13) and trans-3-(2,4,6-trimethylphenyl)-4-phenyl-4,5-dihydroisoxazole-5-carboxylic acid ethyl ester (14) in 0.05 M β-cyclodextrin/20% aqueous ethanol at 30 °C.

The isoxazoline mixture: The isoxazoline **13** (28.0 mg, 8.30×10^{-2} mmol) and the isoxazoline **14** (32.8 mg, 9.72×10^{-2} mmol) were dissolved in ethanol (3.5 ml). The standard used in these experiments was *trans*-3-(1,1-dimethylethylphenyl)-5-phenyl-4,5-dihydroisoxazole-4-carboxylic acid ethyl ester (**20**).

	Isoxazoline **13** (mol/dm^{-3})	Isoxazoline **14** (mol/dm^{-3})	Relative Solubility **13:14**
Experiment 1	5.5×10^{-5}	2.0×10^{-7}	275:1
Experiment 2	5.2×10^{-5}	2.0×10^{-7}	260:1
Experiment 3	4.9×10^{-5}	1.6×10^{-7}	306:1
Average	5.2×10^{-5}	1.9×10^{-7}	274:1

Determination of absolute and relative solubilities of *trans*-3-(1,1-dimethylethylphenyl)-5-phenyl-4,5-dihydroisoxazole-4-carboxylic acid ethyl ester (20) and *trans*-3-(1,1-dimethylethylphenyl)-4-phenyl-4,5-dihydroisoxazole-5-carboxylic acid ethyl ester (21) in 0.05 M β-cyclodextrin/20% aqueous ethanol at 30 °C.

The isoxazoline mixture: The isoxazoline **20** (42.9 mg, 1.22×10^{-1} mmol) and the isoxazoline **21** (7.58 mg, 2.16×10^{-2} mmol) were dissolved in ethanol (3.5 ml). The standard used in these experiments was *trans*-3-(2,6-dichlorophenyl)-5-phenyl-4,5-dihydroisoxazole-4-carboxylic acid ethyl ester (**11**).

	Isoxazoline **20** (mol/dm^{-3})	Isoxazoline **21** (mol/dm^{-3})	Relative Solubility **20:21**
Experiment 1	1.2×10^{-5}	1.0×10^{-4}	1:8.3
Experiment 2	1.0×10^{-5}	1.0×10^{-4}	1:10.0
Experiment 3	1.1×10^{-5}	1.0×10^{-4}	1:9.1
Average	1.1×10^{-5}	1.0×10^{-4}	1:9.1

Determination of absolute and relative solubilities of *trans*-3-(1,1-dimethylethyl)-5-phenyl-4,5-dihydroisoxazole-4-carboxylic acid ethyl ester (22) and *trans*-3-(1,1-dimethylethyl)-4-phenyl-4,5-dihydroisoxazole-5-carboxylic acid ethyl ester (23) in 0.05 M β-cyclodextrin/20% aqueous ethanol at 30 °C.

The isoxazoline mixture: The isoxazoline **22** (89.2 mg, 3.24×10^{-1} mmol) and the isoxazoline **23** (23.7 mg, 8.61×10^{-2} mmol) were dissolved in ethanol (3.5 ml). The standard used in these experiments was *trans*-3-(2,6-dichlorophenyl)-5-phenyl-4,5-dihydroisoxazole-4-carboxylic acid ethyl ester (**11**).

	Isoxazoline **22** (mol/dm^{-3})	Isoxazoline **23** (mol/dm^{-3})	Relative Solubility **22:23**
Experiment 1	4.2×10^{-3}	1.7×10^{-3}	2.5:1
Experiment 2	3.9×10^{-3}	1.6×10^{-3}	2.4:1
Experiment 3	4.2×10^{-3}	1.7×10^{-3}	2.5:1
Average	4.1×10^{-3}	1.7×10^{-3}	2.4:1

Determination of absolute and relative solubilities of *trans*-3-(2,4,6-trimethylphenyl)-5-phenyl-4,5-dihydroisoxazole-4-carboxylic acid ethyl ester (13) and trans-3-(2,4,6-trimethylphenyl)-4-phenyl-4,5-dihydroisoxazole-5-carboxylic acid ethyl ester (14) in 0.013 M β-cyclodextrin/20% aqueous ethanol at 30 °C. To a solution of 11% aqueous ethanol (9 ml) containing β-cyclodextrin (0.138 g, 1.22×10^{-4} mol) was added dropwise with stirring ethanol (1 ml) containing the isoxazoline **13** (9.8 mg, 2.90×10^{-2} mmol) and the isoxazoline **14** (11.1 mg, 3.29×10^{-2} mmol). The cloudy suspension was equilibrated at 30 °C overnight after which it was filtered twice through 0.2 μm syringe

filters (Sartorius Minisartat®) and then though 0.02 μm syringe filters (Whatman® Anotop 25 Plus) at 28 °C. To this solution (500 μl) was added the *trans*-3-(1,1-dimethylethylphenyl)-5-phenyl-4,5-dihydroisoxazole-4-carboxylic acid ethyl ester (**20**) standard (100 μl) and a sample (10 μl) was analysed by HPLC using a Waters Symmetry® C_{18} 3.5 μm (75 mm × 4.6 mm) column monitored at 220 nm and eluted with 70 % acetonitrile/water at a flow rate of 1.1 ml/min. The absolute and relative solubilities were determined by comparison of peak areas with those determined above for known quantities of the isoxazolines **13** and **14**. This experiment was repeated in triplicate.

	Isoxazoline **13** (mol/dm^{-3})	Isoxazoline **14** (mol/dm^{-3})	Relative Solubility **13**:**14**
Experiment 1	1.3×10^{-5}	6.2×10^{-7}	21:1
Experiment 2	1.3×10^{-5}	5.3×10^{-7}	25:1
Experiment 3	1.3×10^{-5}	5.5×10^{-7}	24:1
Average	1.3×10^{-5}	5.7×10^{-7}	23:1

CHAPTER TWO EXPERIMENTAL

Recovery of CD from aqueous cyclodextrin solutions layered on chloroform. Aqueous solutions of α-CD and β-CD were prepared as shown in the table below and carefully layered onto chloroform in a test tube. The resulting 2-phase solutions were allowed to stand undisturbed for several days. After this time, any white precipitate that formed was collected by filtration and analysed by ^{1}H nmr spectroscopy.

Mass of CD (g)	Volume of chloroform (ml)	Volume of milli Q water (ml)	Mass of material recovered (g)	[CD] mg/ml	Yield
0.05 α-CD	10	10	0	5	0%
0.10 α-CD	10	10	0	10	0%
0.15 α-CD	10	10	0	15	0%
0.05 β-CD	10	10	0.008	5	16%
0.10 β-CD	10	10	0.012	10	12%
0.15 β-CD	10	10	0.049	15	33%

Recovery of CD from aqueous cyclodextrin solutions exposed to a atmosphere of chloroform. Aqueous solutions of α-CD and β-CD were prepared as shown in the table below and were placed in a desiccator containing a beaker of chloroform. The desiccator was placed under vacuum and left for several days. Any precipitate that formed was then collected by filtration, dried and analysed by ^{1}H nmr spectroscopy.

Mass of CD (g)	Volume of milli Q water (ml)	Mass of material recovered (g)	[CD] mg/ml	Yield
0.25 α-CD	50	0	5	0%
0.50 α-CD	50	0	10	0%
0.75 α-CD	50	0	15	0%
0.25 β-CD	50	0.149	5	60%
0.50 β-CD	50	0.428	10	86%
0.75 β-CD	50	0.588	15	78%

Recovery of α-CD and β-CD from aqueous solutions containing mixtures of CD exposed to a atmosphere of chloroform. Aqueous solutions of α-CD and β-CD were prepared as shown in the table below. These solutions were placed in a desiccator, a beaker of chloroform was added and the desiccator was placed under vacuum and left for several days. Any white precipitate that formed was then collected by filtration and dried. The purity of the material was assessed by ^{1}H nmr spectroscopy and the composition of the sample analysed by high performance liquid chromatography (HPLC) using a Waters Carbohydrate Analysis (300mm × 3.9mm) column monitored by a refractive index detector and eluted with 70% acetonitrile/water at 1.5ml/min.

Mass of α-CD (g)	Mass of β-CD (g)	Volume of water (ml)	Mass recovered	Composition	Yield %
0.125	0.125	50	0.073 g	β-CD	58
0.25	0.25	50	0.111 g	β-CD	44
0.50	0.50	50	0.322 g	β-CD	64
0.75	0.75	50	0.593 g	β-CD	79
2.00	0.50	50	1.053 g	Mix	61 β-CD 38 α-CD
0.25	0.50	50	0.285 g	β-CD	57

CHAPTER THREE EXPERIMENTAL

2-Phenoxypropionic acid methyl ester (45). To a cooled solution of 2-phenoxypropionic acid (**46**) (5.00 g, 30.0 mmol) in methanol (100 ml) was added dropwise and with stirring thionyl chloride (21.7 ml, 297 mmol). The reaction was stirred overnight at room temperature. The methanol was removed under reduced pressure and the residue taken up in methanol (100 ml) and the solvent was again removed under reduced pressure. The crude product was purified by flash column chromatography (ethyl acetate/*n*-hexane, 1:5) to give 2-phenoxypropionic acid methyl ester (**45**) as a yellow oil (5.41 g, 100%).[132] ^{1}H nmr 1.61 (3H, d, *J* 7 Hz, $CHCH_3$), 3.75 (3H, s, CO_2CH_3), 4.77 (1H, q, *J* 7 Hz, CH-CH_3), 6.87 (2H, apparent d, aromatic protons), 6.96 (1H, apparent t, aromatic protons), 7.26 (2H, apparent t, aromatic protons).

2-(2-Chlorophenoxy)propionic acid methyl ester (47). To a cooled solution of 2-(2-chlorophenoxy)propionic acid (**48**) (5.00 g, 24.9 mmol) in methanol (100 ml) was added dropwise and with stirring thionyl chloride (18.2 ml, 250 mmol). The reaction was stirred overnight at room temperature. The solvent was removed under reduced pressure and the residue was taken up in methanol (100 ml) and the solvent was again removed under reduced pressure. The crude product was purified by flash column chromatography (ethyl acetate/*n*-hexane, 1:5) to give 2-(2-chlorophenoxy)propionic acid methyl ester (**46**) as a pale yellow oil (5.34 g, 100%).[132] ^{1}H nmr 1.69 (3H, d, *J* 7 Hz, $CHCH_3$), 3.77 (3H, s, CO_2CH_3), 4.77 (1H, q, *J* 7 Hz, $CHCH_3$), 6.85 (1H, apparent d, aromatic protons), 6.94 (1H, apparent t, aromatic protons), 7.21 (1H, apparent t, aromatic protons), 7.38 (1H, apparent d, aromatic protons).

Ethyl (*S*)-2-(tosyloxy)propanoate (79). To a stirred solution of (*S*)-ethyl lactate (**77**) (2.50 g, 21.2 mmol), triethylamine (12.5 ml, 89.7 mmol) and a catalytic amount of

DMAP in dry dichloromethane (90 ml) was added dropwise *p*-toluenesulphonyl chloride (**78**) (8.80 g, 46.2 mmol) in dry dichloromethane (90 ml). The reaction was stirred at room temperature for 5 h, poured into water (200 ml) and extracted with dichloromethane. The organic layer was washed with dilute HCl, aqueous NaCl, dried ($MgSO_4$) and concentrated under reduced pressure to give ethyl (*S*)-2-(tosyloxy)propanoate (**79**) as a pale yellow oil (3.36 g, 58%).[135] ^{1}H nmr 1.21 (3H, t, *J* 7 Hz, CH_2CH_3), 1.51 (3H, d, *J* 6.5 Hz, $CHCH_3$), 2.45 (3H, s, Ar-CH_3), 4.14 (2H, q, *J* 7 Hz, CH_2CH_3), 4.93 (1H, q, *J* 6.5 Hz, $CHCH_3$), 7.35 (2H, d, *J* 8 Hz, aromatic protons), 7.82 (2H, d, *J* 8 Hz, aromatic protons).

(*R*)-2-Phenoxypropionic acid ethyl ester (81). To a solution of ethyl (2S)-2-[(4-methylphenyl)sulfonyl]-propionic acid methyl ester (**79**) (3.36 g, 12.3 mmol) in acetonitrile (35.0 ml) was added phenol (**80**) (1.30 g, 13.8 mmol) and potassium carbonate (2.04 g, 14.8 mmol). The solution was refluxed for 4 h under a nitrogen atmosphere. The precipitate was filtered using a Büchner funnel and washed with ether. The organic layer was concentrated under reduced pressure and purified by flash cloumn chromatography (dichloromethane/*n*-hexane, 9:1) to give (*R*)-2-phenoxypropionic ethyl ester (**81**) as a colourless solid (2.20 g, 92%), m.p.79-82 °C.[137] ^{1}H nmr 1.23 (3H, t, *J* 7 Hz, CH_2CH_3), 1.61 (3H, d, *J* 7 Hz, $CHCH_3$), 4.21 (1H, q, *J* 7 Hz, CH_2CH_3), 4.74 (1H, q, *J* 7 Hz, $CHCH_3$), 6.88 (2H, d, *J* 2 Hz, aromatic protons), 6.96 (1H, t, *J* 2 Hz, aromatic protons), 7.26 (2H, t, *J* 2 Hz, aromatic protons).

(*R*)-2-Phenoxypropionic acid *R*-(46). To (*R*)-2-Phenoxypropionic acid ethyl ester (**81**) (2.20 g, 11.3 mmol) dissolved in methanol (20.0 ml) was added 10M NaOH (20.0 ml). The reaction was refluxed for 1 h. The methanol was removed under reduced pressure and the residue dissolved in water and extracted with ether. The aqueous layer was acidified with concentrated hydrochloric acid and extracted into ether, dried ($MgSO_4$) and concentrated under reduced pressure to give (*R*)-2-phenoxypropionoic acid (**46**) as a colourless solid (0.84 g, 45%), m.p. 82-85 °C.[138] ^{1}H nmr 1.69 (3H, d, *J* 7 Hz,

$CHCH_3$), 4.84 (1H, q, *J* 7 Hz, $CHCH_3$), 6.93 (2H, d, *J* 7 Hz, aromatic protons), 7.04 (1H, t, *J* 7 Hz, aromatic protons), 7.34 (2H, t, *J* 7 Hz, aromatic protons).

Purification of (*R*)-2-Phenoxypropionic acid *R*-(46). To (*R*)-2-phenoxypropionic acid *R*-(**46**) (7.74 g, 46.6 mmol) dissolved in ethyl acetate (18.0 ml) was added *n*-propylamine (10.8 ml, 131 mmol). The solution was allowed to cool slowly overnight. The resulting crystals were decomposed with 1 M HCl and extracted into diethyl ether, dried ($MgSO_4$), and concentrated under reduced pressure to give (*R*)-2-phenoxypropionic acid *R*-(**46**) as a colourless solid (3.57 g, 46%). The physical and spectral data of this sample were identical to those obtained for compound **46** above. Analysis by HPLC using a chiral AGP column eluted with phosphate buffer (pH 5.6) at a flow rate of 1.1 ml/min showed only the *R*-enantomer was obtained exclusively.

Preparation of 40 % Methanol/pH 6 Phosphate Buffer. In deionised water (2.00 L) was dissolved disodium hydrogen orthophosphate (0.20 g) and potassium dihydrogen orthophosphate (6.05 g). The actual pH of the solution was found to be 6.02. Methanol (1.33 L) was added to make a 40 % solution.

Preparation of pH 11.5 Phosphate Buffer. In deionised water (2.00 L) was dissolved trisodium orthophosphate (38.1 g). The solution was adjusted to pH 11.5 by addition of concentrated HCl.

Preparation of pH 7 Phosphate Buffer. In deionised water (1.00 L) was dissolved disodium hydrogen orthophosphate (2.08 g) and potassium dihydrogen orthophosphate (1.40 g). The actual pH of the solution was found to be 7.02.

Preparation of pH 5.6 Phosphate Buffer. In deionised water (1.00 L) was dissolved disodium hydrogen orthophosphate (0.43 g) and potassium dihydrogen orthophosphate (8.67 g). The actual pH of the solution was found to be 5.6.

UV response ratio for 2-phenoxypropionic acid (46). 2-Phenoxypropionic acid (**46**) (0.1015 g, 6.12×10^{-4} mol) was dissolved in methanol (100 ml) to produce a 6.12 mM standard solution. 1-Naphthoic acid (0.1043 g, 6.06×10^{-4} mol) was dissolved in methanol (100 ml) to produce a 6.06 mM standard solution. A 1:1 mixture of the 1-naphthoic acid standard solution (200 μl) and the 2-phenoxypropionic acid methyl ester standard solution (200 μl) was made and a sample (25 μl) analysed by HPLC using a Alltech Hypersil BDS C18 5 μm (250 mm × 4.6 mm) column monitored at 260 nm and eluted with 40 % methanol/pH 6 phosphate buffer at a flow rate of 1.1 ml/min. The peak area ratio of compound (**46**):1-naphthoic acid standard was found to be 1: 2.95.

UV response ratio for 2-(2-chlorophenoxy)propionoic acid (48). 2-(2-Chlorophenoxy)-propionic acid (**48**) (0.1215 g, 6.06×10^{-4} mol) was dissolved in methanol (100 ml) to produce a 6.06 mM standard solution. 1-Naphthoic acid (0.1043 g, 6.06×10^{-4} mol) was dissolved in methanol (100 ml) to produce a 6.06 mM standard solution. A 1:1 mixture of the 1-naphthoic acid standard solution (200 μl) and the 2-(2-chlorophenoxy)propionic acid methyl ester standard solution (200 μl) was made and a sample (25 μl) analysed by HPLC using a Alltech Hypersil BDS C18 5 μm (250 mm × 4.6 mm) column monitored at 260 nm and eluted with 40 % methanol/pH 6 phosphate buffer at a flow rate of 1.1 ml/min. The peak area ratio of compound (**46**):1-naphthoic acid standard was found to be 1: 3.78.

General hydrolysis procedure. To a round bottom flask containing pH 11.5 phosphate buffer (20.0 ml) was added as required β-CD (0.20 mol equivalents) and/or BSA (0.03 mol equivalents). To this suspension was added the ester (0.03 g) and the flask was placed under a nitrogen atmosphere and stirred vigorously at 37 °C. The heterogenous reactions were sampled at 0 min, 9 min, 18 min, 27 min and 36 min. At these times, a reaction sample (1000 μl) was added to ethanol (1000 μl) and 1-

naphthanoic acid standard (500 μl). The samples were filtered (Sartorius, Minisart, 0.2 mm syringe filter unit) and analysed by HPLC using a Alltech Hypersil BDS C18 5 μm (250 mm × 4.6 mm) column monitored at 260 nm and eluted with 40 % methanol/pH 6 phosphate buffer at a flow rate of 1.1 ml/min.

Hydrolysis of 2-phenoxypropionic acid methyl ester (45) expressed as % conversion to acid **46**.

Time (min)	No β-CD or BSA		With β-CD		With BSA		With β-CD and BSA	
	% Acid	% Ester	% Acid	% Ester	% Acid	% Ester	% Acid	% Ester
0	3	97	3	97	0	100	0	100
9	26	74	31	56	2	97	3	97
18	38	56	41	37	5	86	5	91
27	43	43	45	30	6	85	7	91
36	45	40	45	30	8	84	9	84

Hydrolysis of 2-(2-chlorophenoxy)propionic acid methyl ester (47) expressed as % conversion to acid **48**.

Time (min)	No β-CD or BSA		With β-CD		With BSA		With β-CD and BSA	
	% Acid	% Ester	% Acid	% Ester	% Acid	% Ester	% Acid	% Ester
0	3	97	2	98	0	99	0	100
9	12	44	14	69	2	132	3	85
18	21	65	24	105	5	106	7	82
27	29	53	33	43	7	97	10	74
36	-	-	39	44	8	96	11	72

General procedure for determining the enantioselectivity in the ester hydrolysis reaction. To a round bottom flask containing pH 11.5 phosphate buffer (20.0 ml) was added as required β-CD (0.20 mol equivalents) and/or BSA (0.03 mol

equivalents). To this suspension was added the ester (0.03 g) and the flask was placed under a nitrogen atmosphere and stirred vigorously at 37 °C. The reaction was sampled at 18 min and 36 min. At these times, a reaction sample (1000 μl) was added to ethanol (3000 μl) and pH 7 phosphate buffer (3000 μl). The solution was filtered and analysed by HPLC using a ChemTech Chiral-AGP 5 μm (100 mm × 4 mm) column monitored at 260 nm and eluted with pH 5.6 phosphate buffer at a flow rate of 1.1 ml/min.

Determination of enantioselectivity in the hydrolysis of 2-phenoxypropionic acid methyl ester (45) expressed as the ratio of (*R*)-enantiomer to (*S*)-enantiomer of the acid **46**.

Time (min)	No β-CD or BSA	with β-CD	With BSA	With β-CD & BSA
18	50:50	50:50	53:47	52:48
36	50:50	50:50	53:47	52:48

Determination of enantioselectivity in the hydrolysis of 2-(2-chlorophenoxy)propionic acid methyl ester (47) expressed as the ratio of (*R*)-enantiomer to (*S*)-enantiomer of the acid **48**.

Time (min)	No β-CD or BSA	with β-CD	With BSA	With β-CD & BSA
18	50:50	50:50	53:47	53:47
36	50:50	50:50	52:48	55:45

Preparation of a 2-phenoxypropionic acid (46) standard solution. To pH 11.5 phosphate buffer (500 μl) was added racemic 2-phenoxypropionic acid (**46**) (10.6 mg, 6.38×10^{-2} mmol).

General procedure for determining the enantioselectivity exhibited by BSA in a 2.9 mM, 4.3 mM and 6.4 mM 2-phenoxypropionic acid (46) solution. To a round bottom flask containing the required amount of the 2-phenoxypropionic acid

(**46**) was added pH 11.5 phosphate buffer (2.9 ml) containing BSA (500 mg, 7.35×10^{-3} mmol). The reaction was placed under a nitrogen atmosphere and stirred at 37°C for 15 min. After which time the reaction mixture (500 μl) was added to ethanol (500 μl), filtered and analysed by HPLC using a ChemTech Chiral-AGP 5 μm (100 mm × 4 mm) column monitored at 260 nm and eluted with pH 5.6 phosphate buffer at a flow rate of 1.1 ml/min.

Initial [acid] (mM)	*R/S* Ratio	ee (%)
2.9	68:32	36
4.3	64:36	28
6.4	61:39	22

General procedure for determining the enantioselectivity exhibited by BSA in a 1.9 mM, 3.8 mM and 5.43 mM 2-(2-chlorophenoxy)propionic acid (48) solution. To a round bottom flask containing the required amount of 2-(2-chlorophenoxy)propionic acid (**48**) was added pH 11.5 phosphate buffer (10 ml) containing BSA (1.7 g, 0.025 mmol). The reaction was placed under a nitrogen atmosphere and stirred at 37°C for 15 min. After which the reaction mixture (1000 μl) was added to ethanol (1000 μl), filtered and analysed by HPLC using a ChemTech Chiral-AGP 5 μm (100 mm × 4 mm) column monitored at 260 nm and eluted with pH 5.6 phosphate buffer at a flow rate of 1.1 ml/min.

Initial [acid] (mM)	R/S Ratio	ee (%)
1.9	96:4	92
3.8	92:8	84
5.2	77:23	54

CHAPTER FOUR EXPERIMENTAL

***cis*-3-(2,6-Dichlorophenyl)-5,6,7,7a-tetrahydro-1,2-benzisoxazol-4(3a*H*)-one (83).** A solution of 2,6-dichlorobenzohydroximinoyl chloride (**64**) (1.00 g, 4.45 mmol) in dry tetrahydrofuran (3.60 ml) was added dropwise to a stirred solution of cyclohex-2-enone (**82**) (0.43 g, 4.47 mmol) and triethylamine (0.68 ml, 4.88 mmol) in dry tetrahydrofuran (7.26 ml). The solution was stirred at room temperature for 1h and heated to reflux for 3 h, after which it was concentrated under reduced pressure and the resulting residue taken up in chloroform. The solution was washed with water (3 × 20.0 ml), dried ($MgSO_4$) and was concentrated. The crude product was purified by flash column chromatography (ethyl acetate/*n*-hexane, 1:1) and recrystallised from ethyl acetate/*n*-hexane to give the *cis*-3-(2,6-dichlorophenyl)-5,6,7,7a-tetrahydro-1,2-benzisoxazol-4(3a*H*)-one (**83**) as large, rhomboid, colourless crystals (0.51 g, 40%, m.p. 149-151 °C (Lit[119,140] m.p. 150-151 °C). ^{1}H nmr 1.98-2.56 (6H, m, C3-**H**$_2$, C4-**H**$_2$ and C5-**H**$_2$), 4.41 (1H, d, *J* 11 Hz, C1-**H**), 5.26 (1H, dt, *J* 4.5 Hz, *J* 11 Hz, C6-**H**), 7.27-7.39 (3H, m, aromatic hydrogens).

3-(2,6-Dichlorophenyl)-6,7-dihydro-1,2-benzisoxazol-4(5*H*)-one (56).

Method 1: Synthesis by dehydrogenation of cis*-3-(2,6-dichlorophenyl)-5,6,7,7a-tetrahydro-1,2-benzisoxazol-4(3a*H*)-one (83) with nickel peroxide.*

cis-3-(2,6-Dichlorophenyl)-5,6,7,7a-tetrahydro-1,2-benzisoxazol-4(3a*H*)-one (**83**) (3.00 g, 10.6 mmol) was refluxed overnight with nickel peroxide (30.0 g) in benzene (300 ml). The resulting mixture was filtered through celite and the solvent removed under reduced pressure to yield 3-(2,6-dichlorophenyl)-6,7-dihydro-1,2-benzisoxazol-4(5*H*)-one (**56**) as a white solid (1.46 g, 49 %), mp 203-205 °C (Lit[119,140] m.p. 205-207 °C). ^{1}H nmr 2.30 (2H, p, *J* 6.5 Hz, C4-**H**$_2$), 2.56 (2H, t, *J* 6.5 Hz, C5-**H**$_2$), 3.14 (2H, t, *J* 6.5 Hz, C3-**H**$_2$), 7.33-7.44 (3H, m, aromatic hydrogens).

Method 2 Synthesis by dehydrogenation of cis-*3-(2,6-dichlorophenyl)-5,6,7,7a-tetrahydro-1,2-benzisoxazol-4(3a*H*)-one (83) with γ-activated manganese dioxide.*

cis-3-(2,6-Dichlorophenyl)-5,6,7,7a-tetrahydro-1,2-benzisoxazol-4(3a*H*)-one (**83**) (1.00 g, 3.50 mmol) was refluxed overnight with γ-activated manganese dioxide (5.00 g) in benzene (50.0 ml). The resulting mixture was filtered through celite and the solvent was removed under reduced pressure to yield 3-(2,6-dichlorophenyl)-6,7-dihydro-1,2-benzisoxazol-4(5*H*)-one (**56**) as a white solid (0.40 g, 40%). The physical and spectral data of this sample were identical to those obtained above.

cis-3-(2,6-Dichlorophenyl)-3a,6,7,7a-tetrahydro-4*H*-pyrano[3,4-*d*]isoxazol-4-one (86) A solution of 2,6-dichlorobenzohydroximinoyl chloride (**64**) (1.14 g, 5.10 mmol) in dry tetrahydrofuran (7.70 ml) was added dropwise to a stirred solution of 5,6-dihydro-2*H*-pyran-2-one (**87**) (0.50 g, 5.10 mmol) and triethylamine (0.80 ml, 5.70 mmol) in dry tetrahydrofuran (15.0 ml). The solution was stirred at room temperature for 1 h and heated to reflux for 3 h, after which it was concentrated under reduced pressure and the resulting residue taken up in chloroform. The solution was washed with water (3 × 20.0 ml), dried ($MgSO_4$) and concentrated under reduced pressure. The crude product was then purified by flash column chromatography using (ethyl acetate/*n*-hexane, 2:3) and recrystallised from ethyl acetate/*n*-hexane to give *cis*-3-(2,6-dichlorophenyl)-3a,6,7,7a-tetrahydro-4*H*-pyrano[3,4-*d*]isoxazol-4-one (**86**) as large, rhomboid, colourless crystals (0.94 g, 64%), m.p. 161-163 °C (Lit[119,140] m.p. 159-162 °C) ^{1}H nmr 2.28 (2H, m, C5-$\mathbf{H}_2$), 4.48 (1H, m, C1-**H**), 4.71 (1H, ddd, *J* 3 Hz, *J* 11 Hz, *J* 11 Hz, C5-**H**), 4.75 (1H, d, *J* 11 Hz, C4-**H**), 5.33 (1H, m, C6-**H**), 7.32-7.42 (3H, m, aromatic protons).

3-(2,6-Dichlorophenyl)-6,7-dihydro-4*H*-pyrano[3,4-*d*]isoxazol-4-one (58)

Method 1: Synthesis by dehydrogenation of cis-*3-(2,6-dichlorophenyl)-3a,6,7,7a-tetrahydro-4*H*-pyrano[3,4-*d*]isoxazol-4-one (86) with nickel peroxide.*

cis-3-(2,6-Dichlorophenyl)-3a,6,7,7a-tetrahydro-4*H*-pyrano[3,4-*d*]isoxazol-4-one (**86**) (0.50 g, 1.75 mmol) was refluxed overnight with nickel peroxide (5.00 g) in benzene (50.0 ml). The resulting mixture was filtered through celite and the solvent removed under reduced pressure to yield 3-(2,6-dichlorophenyl)-6,7-dihydro-4*H*-pyrano[3,4-*d*]isoxazol-4-one (**58**) as a white solid (0.16 g, 32%), mp 174-176 °C (Lit[119,140] m.p. 175-177 °C). ^{1}H nmr 3.34 (2H, t, *J* 6 Hz, C5-$\mathbf{H_2}$), 4.69 (2H, t, *J* 6 Hz, C4-$\mathbf{H_2}$), 7.39-7.47 (3H, m, aromatic protons).

*Method 2: Synthesis by dehydrogenation of cis-3-(2,6-dichlorophenyl)-3a,6,7,7a-tetrahydro-4H-pyrano[3,4-*d*]isoxazol-4-one (**86**) with γ-activated manganese dioxide.*

cis-3-(2,6-Dichlorophenyl)-3a,6,7,7a-tetrahydro-4*H*-pyrano[3,4-*d*]isoxazol-4-one (**86**) (1.00 g, 3.50 mmol) was refluxed overnight with γ-activated manganese dioxide (5.00 g) and benzene (100 ml). The resulting mixture was filtered through celite and the solvent was removed under reduced pressure to yield 3-(2,6-dichlorophenyl)-6,7-dihydro-4*H*-pyrano[3,4-*d*]isoxazol-4-one (**58**) as a white solid (0.38 g, 38%). The physical and spectral data of this sample were identical to those obtained above.

2-Bromocyclohexen-2-en-1-one (84). To a stirred solution of cyclohex-2-en-1-one (**82**) (1.00 g, 10.4 mmol) in dichloromethane (36.5 ml) was added over 10 min a solution of bromine (1.67 g, 10.5 mmol) in dichloromethane (10.4 ml) and the mixture was stirred for 2 h. Triethylamine (1.46 ml, 10.5 mmol) was then added over 5 min and the mixture stirred for a further 3 h. The reaction was poured into water (42.0 ml) and the organic layer separated. The aqueous fraction was extracted twice with dichloromethane. The combined organic fractions were dried ($MgSO_4$) and the solvent removed under reduced pressure. The crude product was recrystallised from aqueous methanol to give 2-bromocyclohexen-2-en-1-one (**84**) as large colourless crystals (1.14 g, 63%), m.p. 71-73.5 °C (Lit[119,140] m.p. 72-75 °C). ^{1}H nmr 2.08 (2H, m, C5-$\mathbf{H_2}$), 2.46 (2H, m, C4-$\mathbf{H_2}$), 2.65 (2H, m, C6-$\mathbf{H_2}$), 7.45 (1H, t, *J* 4.5 Hz, C3-**H**).

3-(2,6-Dichlorophenyl)-5,6-dihydro-1,2-benzisoxazol-7(4*H*)-one (60). A solution of 2,6-dichlorobenzohydroximinoyl chloride (**64**) (1.00 g, 4.45 mmol) in dry tetrahydrofuran (7.70 ml) was added dropwise to a stirred solution of 2-bromocyclohex-2-en-1-one (**84**) (0.76 g, 4.34 mmol) and triethylamine (0.70 ml, 5.00 mmol) in dry tetrahydrofuran (15.4 ml). The solution was stirred at room temperature for 1 h and heated to reflux for 3 h, after which it was concentrated under reduced pressure and the resulting residue taken up in chloroform. The solution was washed with water (3 × 20.0 ml), dried ($MgSO_4$) and concentrated under reduced pressure. The crude product was then purified by flash column chromatography using (ethyl acetate/*n*-hexane, 1:1) and recrystallised from ethyl acetate/*n*-hexane to give 3-(2,6-dichlorophenyl)-5,6-dihydro-1,2-benzisoxazol-7(4*H*)-one (**60**) as pale orange crystals (0.65 g, 53%), m.p. 107.5-110 °C (Lit[119,140] m.p. 108.5-110.5 °C). ^{1}H nmr 2.23 (2H, m, *J* 6 Hz, C4-$\mathbf{H_2}$), 2.61 (2H, t, *J* 6 Hz, C5-$\mathbf{H_2}$), 2.70 (2H, t, *J* 6 Hz, C3-$\mathbf{H_2}$), 7.37-7.46 (3H, m, aromatic protons).

3-Bromo-5,6-dihydro-2*H*-pyran-2-one (88). To a stirred solution of 5,6-dihydro-2*H*-pyran-2-one (**87**) (0.98 g, 10.0 mmol) in dichloromethane (35.0 ml) was added over 10 min a solution of bromine (1.60 g, 10.0 mmol) in dichloromethane (10.0 ml) and the mixture was stirred for 2 h. Triethylamine (1.40 ml, 10.0 mmol) was added over 5 min and the mixture stirred for a further 3 h. The reaction was poured into water (40.0 ml) and the organic layer separated. The aqueous fraction was extracted twice with dichloromethane. The combined organic fractions were dried ($MgSO_4$) and the solvent removed under reduced pressure. The crude product was recrystallised from aqueous methanol to give 3-bromo-5,6-dihydro-2*H*-pyran-2-one (**88**) as large colourless crystals (1.77 g, 100%), m.p. 31-33°C (Lit[119,140] m.p. 32-34 °C). ^{1}H nmr 2.59 (2H, dt, *J* 4.5 Hz, *J* 6 Hz, C5-$\mathbf{H_2}$), 4.49 (2H, t, *J* 6 Hz, C6-$\mathbf{H_2}$), 7.29 (1H, t, *J* 4.5 Hz, C4-**H**).

3-(2,6-Dichlorophenyl)-4,5-dihydro-7*H*-pyrano[4,3-*d*]isoxazol-7-one (61). A solution of 2,6-dichlorobenzohydroximinoyl chloride (**64**) (1.00 g, 4.45 mmol) in dry tetrahydrofuran (5.00 ml) was added dropwise to a stirred solution of 3-bromo-5,6-dihydro-2*H*-pyran-2-one (**88**) (0.55 g, 3.11 mmol) and triethylamine (0.40

ml, 2.87 mmol) in dry tetrahydrofuran (10.0 ml). The solution was stirred at room temperature for 1 h and heated to reflux for 3 h, after which it was concentrated under reduced pressure and the resulting residue taken up in chloroform. The solution was washed with water (3 × 10.0 ml), dried ($MgSO_4$) and concentrated under reduced pressure. The crude product was purified by flash column chromatography using (ethyl acetate/*n*-hexane, 2:3) and recrystallised from ethyl acetate/*n*-hexane to give 3-(2,6-dichlorophenyl)-4,5-dihydro-7*H*-pyrano[4,3-*d*]isoxazol-7-one (**61**) as pale orange crystals (0.23 g, 26%), m.p. 105-108°C (Lit[119,140] m.p. 106-107 °C). ^{1}H nmr 2.89 (2H, t, *J* 6 Hz, C5-H_2), 4.68 (2H, t, *J* 6 Hz, C4-H_2), 7.43-7.47 (3H, m, aromatic protons).

Nickel peroxide. To a stirred solution of nickel sulphate hexahydrate (10.0 g, 38.0 mmol) in water (23.0 ml) was added a mixture of 6 % sodium hypochlorite (18.5 ml, 14.9 mmol) and sodium hydroxide (2.60 g, 65.0 mmol). The mixture was stirred at room temperature for 0.5 h. The resulting black precipitate was collected by vacuum filtration and washed with water. The nickel peroxide was then azeotroped with benzene (65.0 ml) to remove the remaining water. The solvent was removed under reduced pressure and the black solid was placed in a vacuum desiccator and powdered after 48 h to give nickel peroxide (3.23 g, 57%).

γ-Activated manganese dioxide. To a stirred solution of manganese sulphate monohydrate (10.0 g, 59.2 mmol) in water (190 ml) at 60 °C was added a solution of potassium permanganate (6.96 g, 44.0 mmol) in water (131 ml). The brown suspension was stirred at 60 °C for 1 h, filtered and washed with water. The precipitate was dried to constant weight at 65 °C to yield γ-activated manganese dioxide as a dark brown amorphous powder (3.81 g, 74%)

3-(2,6-Dichlorophenyl)isoxazole-5-carboxylic acid methyl ester (92). To a solution of 2,6-dichlorobenzohydroximinoyl chloride (**64**) (1.00 g, 4.45 mmol) in dry tetrhydrofuran (10.0 ml) was added, dropwise and with stirring, a solution of methyl

propiolate (**5**) (0.48 g, 5.70 mmol) and triethylamine (0.81 ml, 5.81 mmol) in dry tetrahydrofuran (20.0 ml). The reaction was stirred at room temperature for 1 h and heated to reflux for 3 h, after which it was concentrated under reduced pressure and the residue taken up in chloroform. The solution was washed with water (3 × 30.0 ml), dried ($MgSO_4$) and concentrated under reduced pressure. The crude product was recrystalised from ethyl acetate/*n*-hexane to yield 3-(2,6-dichlorophenyl)isoxazole-5-carboxylic acid methyl ester (**92**) (0.33 g, 27%), m.p. 114-116 °C. ^{1}H nmr 4.03 (3H, s, OCH_3), 7.08 (1H, s, C4-**H**), 7.38-7.47 (3H, m, aromatic protons); (Found 270.9803. $C_{11}H_7Cl_2NO_3$ requires 270.9806); *m/z* 275 (M^+, 9%), 273 (M^+, 30), 271 (M^+, 38), 216 (31), 214 (80), 212 (100), 186 (34), 184 (46); ^{13}C nmr 51.9, 52.9, 111.1, 127.8, 128.1, 131.3, 131.5, 135.4, 163.2. The structure was confirmed by X-ray crystallographic analysis (Appendix 1).

***trans*-3-(2,6-Dichlorophenyl)-5-methyl-4,5-dihydroisoxazole-4-carboxylic acid methyl ester (99).** To a solution of 2,6-dichlorobenzohydroximinoyl chloride (**64**) (1.00 g, 4.45 mmol) in dry tetrahydrofuran (15.0 ml) was added, dropwise and with stirring, a solution of methyl crotonate (**100**) (0.53 g, 5.29 mmol) and triethylamine (0.81 ml, 5.81 mmol) in dry tetrahydrofuran (15.0 ml). The reaction was stirred at room temperature for 1 h and heated to reflux for 3 h, after which it was concentrated under reduced pressure and the residue taken up in chloroform. The solution was washed with water (3 × 20.0 ml), dried ($MgSO_4$) and concentrated under reduced pressure to give a crude mixture of isoxazolines in a ratio of 7:1. The regioisomers were separated by flash column chromatography (ethyl acetate/*n*-hexane, 1:5) and the 4-carboxymethyl isomer was recrystallised from ethyl acetate/*n*-hexane to give methyl 3-(2,6-dichlorophenyl)-5-methyl-4,5-dihydroisoxazole-4-carboxylic acid methyl ester (**99**) (0.52 g, 41%), m.p. 92-93°C. ^{1}H nmr 1.55 (3H, d, *J* 6.5 Hz, C5-CH_3), 3.64 (3H, s, C4-OCH_3), 4.17 (1H, d, *J* 7 Hz, C4-**H**), 5.34 (1H, p, *J* 7 Hz, C5-**H**), 7.28-7.41 (3H, m, aromatic protons). ^{13}C nmr 13.4, 51.7, 109.3, 127.7, 127.9, 131.0, 135.3, 158.6, 161, 5, 175.6.

3-(2,6-Dichlorophenyl)-5-methylisoxazole-4-carboxylic acid methyl ester (93).

Method 1: Synthesis by dehydrogenation of 3-(2,6-dichlorophenyl)-5-methyl-4,5-dihydroisoxazole-4-carboxylic acid methyl ester (99) with nickel peroxide.

3-(2,6-Dichlorophenyl)-5-methyl-4,5-dihydroisoxazole-4-carboxylic acid methyl ester (**99**) (0.19 g, 0.66 mmol) was refluxed overnight with nickel peroxide (1.80 g) in benzene (20 ml). The mixture was filtered through celite and the solvent was removed under reduced pressure. The resulting colourless solid was recrystallised from ethyl acetate/*n*-hexane to yield 3-(2,6-dichlorophenyl)-5-methylisoxazole-4-carboxylic acid methyl ester (**93**) as a white solid (0.10 g, 53%), m.p. 112-115 °C (Lit[164] m.p. 115 °C). ^{1}H nmr 2.80 (3H, s, C5-C**H**$_3$), 3.69 (3H, s, C4-OC**H**$_3$), 7.34-7.43 (3H, m, aromatic protons).

Method 2: Synthesis by dehydrogenation of 3-(2,6-dichlorophenyl)-5-methyl-4,5-dihydroisoxazole-4-carboxylic acid methyl ester (99) with γ-activated manganese dioxide.

3-(2,6-Dichlorophenyl)-5-methyl-4,5-dihydroisoxazole-4-carboxylic acid methyl ester (**99**) (0.20 g, 0.69 mmol) was refluxed overnight with γ-activated manganese dioxide (1.00 g) in benzene (20 ml). The mixture was filtered through celite and the solvent was removed under reduced pressure to yield 3-(2,6-dichlorophenyl)-5-methylisoxazole-4-carboxylic acid methyl ester (**93**) as a white solid (0.08 g, 40%). The physical and spectral data of this sample were identical to those obtained above. The structure was confirmed by X-ray crystallographic analysis (Appendix 2).

5-Cyano-3-(2,6-dichlorophenyl)-4,5-dihydroisoxazole (104). To a solution of 2,6-dichlorobenzonitrile oxide (**19**) (1.00 g, 5.31 mmol) in dry tetrahydrofuran (6.00 ml) was added, dropwise and with stirring, a solution of acrylonitrile (**105**) (0.26 g, 4.90 mmol) in dry tetrahydrofuran (10.0 ml). The reaction was heated to reflux for 1 h. The solution was concentrated under reduced pressure and the residue taken up in chloroform. The solution was washed with water (3 × 10.0 ml), dried ($MgSO_4$)

concentrated under reduced pressure and purified by flash column chromatography (ethyl acetate/*n*-hexane, 1:5) to give 5-cyano-3-(2,6-dichlorophenyl)-4,5-dihydroisoxazole (**104**) exclusively as a viscous yellow oil (0.55 g, 47%).[87] ^{1}H nmr 3.62 (1H, dd, *J* 5.5 Hz, *J* 17 Hz, C4-**H**), 3.74 (1H, dd, *J* 11 Hz, *J* 17 Hz, C4-**H**), 5.47 (1H, dd, *J* 5.5 Hz, *J* 11 Hz, C5-**H**), 7.36-7.44 (3H, m, aromatic protons).

5-Cyano-3-(2,6-dichlorophenyl)-isoxazole (94). 3-(2,6-dichlorophenyl)-4,5-dihydroisoxazole-5-carbonitrile (**104**) (0.52g, 2.16 mmol) was refluxed overnight with nickel peroxide (5.00g) in benzene (50 ml). The mixture was filtered through celite and the solvent was removed under reduced pressure. The resulting colourless solid was recrystallised from ethyl acetate/*n*-hexane to yield 5-cyano-3-(2,6-dichlorophenyl)-isoxazole (**94**) as a white solid (0.23 g, 45%), mp 114-117 °C (Lit[147] m.p. 115-117 °C). ^{1}H nmr 7.15 (1H, s, C4-**H**), 7.29-7.36 (3H, m, aromatic protons).

4-Cyano-3-(2,6-dichlorophenyl)-5-methylisoxazole (95). To a solution of 2,6-dichlorobenzonitrile oxide (**19**) (1.00 g, 5.32 mmol) in dry tetrahydrofuran (6.0 ml) was added, dropwise and with stirring, a solution of crotononitrile (**108**) (0.32 g, 4.77 mmol) in dry tetrahydrofuran (10.0 ml). The reaction was heated to reflux for 1 h. The solution was concentrated under reduced pressure and the residue taken up in chloroform. The solution was washed with water (3 × 20.0 ml), dried ($MgSO_4$) and concentrated under reduced pressure and purified by flash column chromatography (ethyl acetate/*n*-hexane, 1:5) to give a mixture of isoxazoline cycloadducts. To this was added nickel peroxide (10.0 g) and benzene (100 ml) and the mixture was refluxed overnight. The mixture was filtered through celite and the solvent was removed under reduced pressure. The resulting colourless solid was recrystallised from ethyl acetate/*n*-hexane to yield 4-cyano-3-(2,6-dichlorophenyl)-5-methylisoxazole (**95**) as a white solid (0.12 g, 10%), mp 98-100 °C (Lit[115] m.p. 99-100 °C). ^{1}H nmr 2.75 (3H, s, C5-C**H**$_3$), 7.43-7.46 (3H, m, aromatic protons).

***trans*-3-(2,6-Dichlorophenyl)-4,5-dihydro-5-phenylisoxazole (110).** To a solution of 2,6-dichlorobenzohydroximinoyl chloride (**64**) (1.00 g, 4.45 mmol) in dry tetrahydrofuran (10.0 ml) was added, dropwise and with stirring, a solution of styrene (**111**) (0.55 g, 5.28 mmol) and triethylamine (0.80 ml, 5.74 mmol) in dry tetrahydrofuran (20.0 ml). The reaction was stirred at room temperature for 1 h and then heated to reflux for 3 h, after which it was concentrated under reduced pressure and the residue taken up in chloroform. The solution was washed with water (3 × 20.0 ml), dried ($MgSO_4$) and concentrated under reduced pressure. The crude product was purified by flash cloumn chromatography (ethyl acetate/*n*-hexane, 1:20) and recrystalised from ethyl acetate/*n*-hexane to yield 3-(2,6-dichlorophenyl)-5-phenyl-4,5-dihydroisoxazole (**110**) as a colourless powder (1.18 g, 91%), m.p. 63-65 °C (Lit[150] m.p. 64-65 °C). ^{1}H nmr 3.28 (1H, dd, *J* 9 Hz, *J* 17 Hz, C4-**H**), 3.73 (1H, dd, *J* 11 Hz, *J* 17 Hz, C4-**H**), 5.82 (1H, dd, *J* 9 Hz, *J* 11 Hz, C5-**H**), 7.23-7.48 (8H, m, aromatic protons).

3-(2,6-Dichlorophenyl)-5-phenylisoxazole (96). 3-(2,6-Dichlorophenyl)-5-phenyl-4,5-dihydroisoxazole (**110**) (0.50 g, 1.71 mmol) was refluxed overnight with γ-activated manganese dioxide (2.5 g) in benzene (50 ml). The mixture was filtered through celite and the solvent was removed under reduced pressure to yield 3-(2,6-dichlorophenyl)-5-phenylisoxazole (**96**) as a white solid (0.057 g, 11%), m.p. 75-76°C (Lit[148] m.p. 75-76 °C). ^{1}H nmr 6.63 (1H, s, C4-**H**), 7.30-7.52 (6H, m, aromatic protons), 7.86 (2H, d, *J* 8 Hz, aromatic protons).

3-(2,6-Dichlorophenyl)-4-phenylisoxazole (97). To a solution of 2,6-dichlorobenzohydroximinoyl chloride (**64**) (3.40 g, 15.1 mmol) in dry tetrhydrofuran (16.0 ml) was added, dropwise and with stirring, a solution of β-bromostyrene (**112**) (2.75 g, 15.0 mmol) and triethylamine (2.20 ml, 15.8 mmol) in dry tetrahydrofuran (32.0 ml). The reaction was stirred at room temperature for 1 h and heated to reflux for 3 h, after which it was concentrated under reduced pressure and the residue taken up in chloroform. The solution was washed with water (3 × 40 ml), dried ($MgSO_4$) and concentrated under reduced pressure. The reaction mixture was chromatographed (ethyl

acetate/*n*-hexane, 1:20) to give a *ca.* 7.3:5:1 mixture of the title compound **97**, 3-(2,6-dichlorophenyl)-5-phenylisoxazole (**96**) and *trans*-4-bromo-3-(2,6-dichlorophenyl)-4,5-dihydro-5-phenylisoxazole (**115**). This mixture was recrystallised twice from ethyl acetate/*n*-hexane to yield 3-(2,6-dichlorophenyl)-4-phenylisoxazole (**97**) as a colourless solid (0.30 g, 7%), m.p. 89-90°C (Lit[149] m.p. 90-92 °C). ^{1}H nmr 7.16-7.43 (8H, m, aromatic protons), 8.73 (1H, s, C5-**H**). The structure was confirmed by X-ray crystallographic analysis (Appendix 3).

1-Chloro-1-(hydroxyimino)acetone (119). To chloroacetone (**120**) (10.0 g, 108 mmol) in ether (120 ml) containing concentrated hydrochloric acid (2.00 ml) was added isopentyl nitrite (**121**) (13.6 g, 116 mmol) in small portions. The reaction was stoppered with a calcium chloride drying tube and stirred overnignt. The solvent was removed under reduced pressure and the resulting residue was recrystallised from carbon tetrachloride to give 1-chloro-1-(hydroxyimino)acetone (**119**) as colourless powder (1.80 g, 14%), m.p. 103-105 °C (Lit[154] m.p. 107-108 °C) ^{1}H nmr 2.51 (3H, s, CH_3), 8.65 (1H, s, NO**H**).

3-Acetylisoxazole-5-carboxylic acid methyl ester (98). To a solution of 1-chloro-1-(hydroxyimino)acetone (**119**) (0.50 g, 4.11 mmol) in dry tetrhydrofuran (25.0 ml) was added, dropwise and with stirring, a solution of methyl propiolate (**5**) (0.69 g, 8.21 mmol) and triethylamine (0.63 ml, 4.52 mmol) in dry tetrahydrofuran (100 ml). The reaction was stirred at room temperature for 1 h and then heated to reflux for 3 h. The solution was concentrated under reduced pressure and the residue taken up in chloroform. The solution was washed with water (3 × 40.0 ml), dried ($MgSO_4$) and concentrated under reduced pressure. The crude product was purified by flash column chromatography (ethyl acetate/*n*-hexane, 1:20) and recrystalised from ethyl acetate/*n*-hexane to yield 3-acetylisoxazole-5-carboxylic acid methyl ester (**98**) as a colourless solid (0.15 g, 22%), m.p. 89-90°C. ^{1}H nmr 2.71 (3H, s, C(O)CH_3), 3.99 (3H, s, -OCH_3), 7.26 (1H, s, C4-**H**), ^{13}C nmr 27.5, 53.2, 107.7, 156.6, 161.6, 162, 3, 190.9, [Found

170.0451 ($M+H^+$). $C_7H_7NO_4$ requires 170.0453 ($M+H^+$)], *m/z* 169(M^+, 5%), 154 (100), 138 (31).

Purification of acetonitrile for electrochemistry.

Step1: Reflux acetonitrile over anhydrous aluminum chloride (15 g/L) for 1 h followed by rapid distillation.

Step 2: Reflux acetonitrile over potassium permanganate (10 g/L) and litium carbonate (10g/L) for 15 min followed by rapid distillation.

Step 3: Reflux acetonitrile over potassium bisulfate (15 g/L) for 1 h followed by rapid distillation.

Step 4: Reflux acetonitrile over calcium hydride (2 g/L) for 1 h followed by careful fractional distillation retaining the middle 80% fraction.

Purification of mercury for electrochemistry.

Recycled mercury was washed successively with concentrated nitric acid, water, acetone and electrochemical grade acetonitrile.

General electrolysis procedure.

Cyclic voltammetric and controlled potential electrolysis experiments were conducted with a PAR model 273A potentiostat/galvanostat controlled through a PC with standard PAR electrochemical software. Cyclic voltammograms were performed at room temperature (20 - 25 °C) using a 1 mm diameter planar mercury coated Pt working electrode in conjunction with a Pt auxiliary electrode and an Ag/Ag^+ (0.05 M $AgNO_3$ in MeCN) reference electrode. The reference electrode was calibrated relative to the ferrocene/ferrocinium redox couple.

Controlled potential electrolysis experiments were conducted in a two compartment electrolytic cell separated with a porosity no. 5 (1.0 - 1.7 μm) sintered glass frit. The working electrode consisted of a Pt mesh coated in mercury and the auxiliary electrode was an identical size Pt mesh that was symmetrically arranged with respect to the

working electrode. The Ag/Ag^+ reference electrode (same as above) was positioned so that the glass frit at the tip of the salt bridge was within 2 mm of the surface of the working electrode. The volume of both the working and auxiliary electrode compartments were approximately 30.0 ml. The auxiliary and working electrode compartments were degassed with solvent saturated argon for 30 minutes before the electrolysis was commenced and the purging continued until the completion of the electrolysis. For some experiments where large quantities of materials were reduced (0.05 - 0.1 M), the electrolysis cell was cooled to 0 °C using a Lauda variable temperature methanol bath, to avoid excessive heating effects associated with the high currents passed (0.1 - 0.5 A). In all cases the concentration of supporting electrolyte was 0.2 M, which was at least double the concentration of analyte.

The general procedure for performing the synthetic scale electrolysis experiments involved first obtaining the potential for the reduction by performing a cyclic voltammogram with a 1 mm planar mercury coated Pt electrode that was placed in the working electrode compartment. The constant potential electrolysis was then commenced using the large mercury coated mesh electrode and at varying intervals the electrolysis was paused and cyclic voltammograms performed until none of the starting material could be electrochemically detected.

At the completion of the electrolysis, the solution in the working electrode compartment was drained through the base of the cell into a round bottom flask and the acetonitrile removed by rotary evaporator.

2-[(2,6-Dichlorophenyl)iminomethyl]-3-hydroxycyclohex-2-en-1-one (57). To the electrochemical cell was added 3-(2,6-dichlorophenyl)-6,7-dihydro-1,2-benzisoxazol-4(5*H*)-one (**56**) (0.30 g, 1.06 mmol) and a controlled potential electrolysis was conducted *via* the general electrochemical method described. The crude product was purified by flash column chromatography (ethyl acetate/*n*-hexane, 1.5:1), followed by preparative TLC (ethyl acetate/*n*-hexane, 1.5:1) to yield 2-[(2,6-

dichlorophenyl)iminomethyl]-3-hydroxycyclohex-2-en-1-one (**57**) as a colourless powder (0.184 g, 61%), m.p. 228-231°C (Lit[119,140] m.p. 229-232 °C). ^{1}H nmr 1.95 (2H, t, *J* 6.5 Hz, C5-$\mathbf{H}_2$), 2.41 (2H, t, *J* 6.5 Hz, C6-$\mathbf{H}_2$), 2.65 (2H, t, *J* 6.5 Hz, C4-$\mathbf{H}_2$), 6.02 (1H, bs, **NH**), 7.23-7.37 (3H, m, aromatic protons), 12.02 (1H, bs, **OH**).

3-[(2,6-Dichlorophenyl)iminomethyl]-5,6-dihydro-4-hydroxy-2*H*-pyran-2-one (59). To the electrochemical cell was added 3-(2,6-dichlorophenyl)-6,7-dihydro-4*H*-pyrano[3,4-*d*]isoxazol-4-one (**58**) (0.30 g, 1.06 mmol) and a controlled potential electrolysis was conducted *via* the general electrochemical method described. The crude product was purified by flash column chromatography (ethyl acetate/*n*-hexane, 1.5:1), followed by preparative TLC (ethyl acetate/*n*-hexane, 1.5:1) to yield 3-[(2,6-dichlorophenyl)iminomethyl]-5,6-dihydro-4-hydroxy-2*H*-pyran-2-one (**59**) as a colourless powder (0.20 g, 66%), m.p. 216-218°C (Lit[119,140] m.p. 216-218 °C). ^{1}H nmr 2.75 (2H, t, *J* 6 Hz, C5-$\mathbf{H}_2$), 4.37 (2H, t, *J* 6 Hz, C6-$\mathbf{H}_2$), 6.08 (1H, bs, **NH**), 7.29-7.39 (3H, m, aromatic protons), 11.50 (1H, bs, **OH**).

α-Acetyl-β-amino-2,6-dichlorocinnamic acid methyl ester (123). To the electrochemical cell was added 3-(2,6-dichlorophenyl)-5-methylisoxazole-4-carboxylic acid methyl ester (**93**) (0.23 g, 0.80 mmol) and a controlled potential electrolysis was conducted *via* the general electrochemical method described. The crude product was purified by flash column chromatography (ethyl acetate/*n*-hexane, 1.5:1), followed by preparative TLC (ethyl acetate/*n*-hexane, 1.5:1) to yield α-acetyl-β-amino-2,6-dichlorocinnamic acid methyl ester (**123**) as a colourless powder (0.13 g, 56%), m.p. 155-157 °C (Lit[115] m.p. 155-156.5 °C). ^{1}H nmr 2.49 (3H, s, $\mathbf{CH}_3$), 3.46 (3H, s, $\mathbf{CO_2CH_3}$), 5.50 (1H, bs, **NH**), 7.29-7.40 (3H, m, aromatic protons), 11.35 (1H, bs, **OH**).

α-Acetyl-β-amino-2,6-dichlorocinnamonitrile (125). To the electrochemical cell was added 4-cyano-3-(2,6-dichlorophenyl)-5-methylisoxazole (**95**) (0.08 g, 0.32 mmol) and a controlled potential electrolysis was conducted *via* the general

electrochemical method described. The crude product was purified by flash column chromatography (ethyl acetate/*n*-hexane, 1.5:1), followed by preparative TLC (ethyl acetate/*n*-hexane, 1.5:1) to yield α-acetyl-β-amino-2,6-dichlorocinnamonitrile (**125**) as a colourless powder (0.06 g, 74%), m.p. 229-232 °C (Lit[115] m.p. 231-232 °C). ^{1}H nmr 2.44 (3H, s, C**H**$_3$), 5.87 (1H, bs, N**H**), 7.36-7.47 (3H, m, aromatic protons), 10.80 (1H, bs, O**H**).

Electrolysis of the isoxazoles 58, 61, 92, 94, 96, 97 and 98. To the electrochemical cell were added the isoxazoles **58**, **61**, **92**, **94**, **96**, **97** and **98** and a controlled potential electrolysis was conducted *via* the general electrochemical procedure described above. Under these conditions the isoxazoles **58**, **61**, **92**, **94**, **96**, **97** and **98** gave complex product mixtures from which it was not practical to isolate discrete species.

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Appendix One

Sample: gjv4

Compound: $C_{11}H_7Cl_2NO_3$

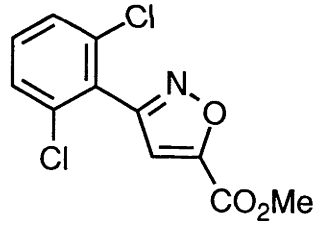
X-ray Structure Report

for

George J. Vuckovic and Christopher J. Easton

by

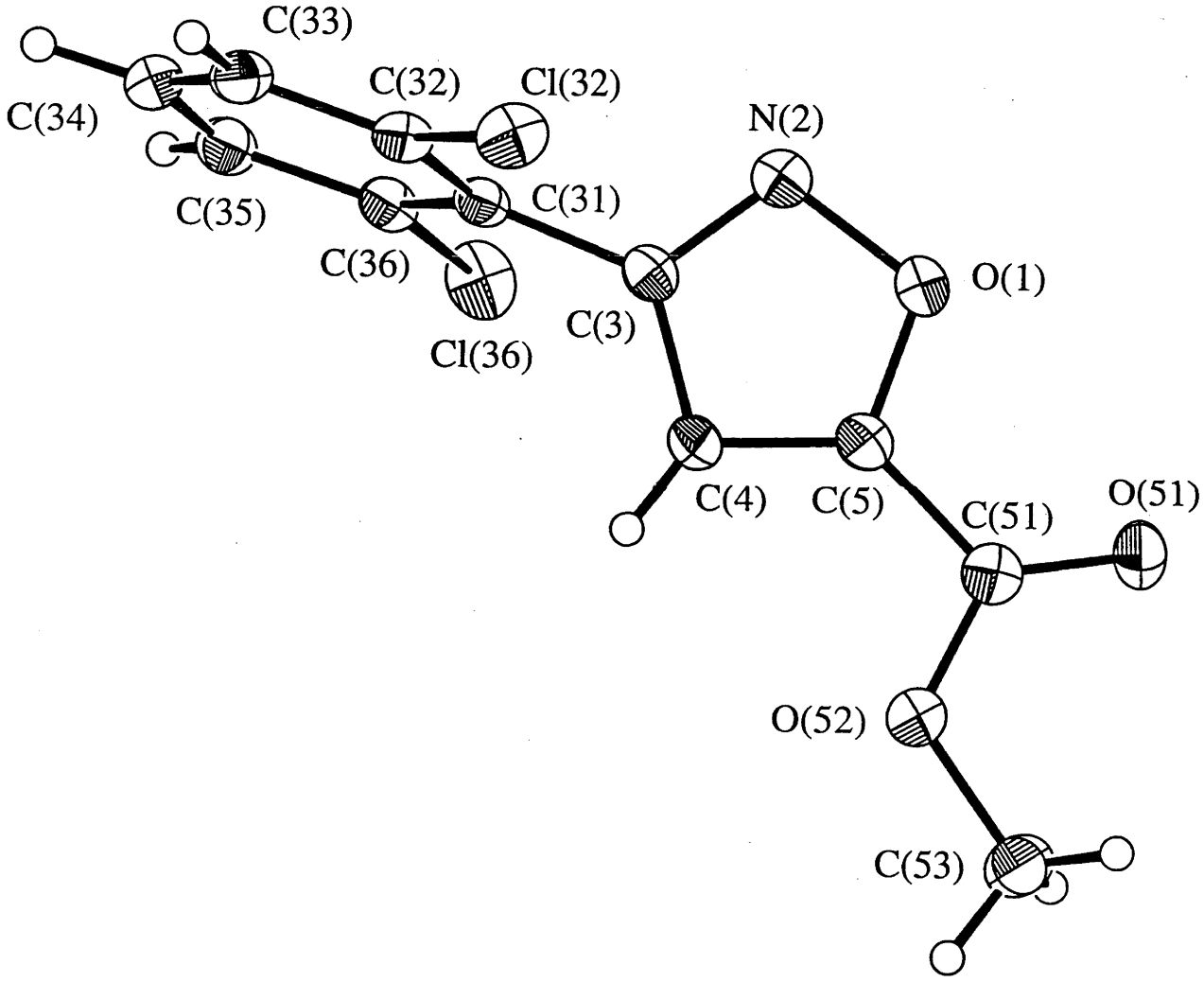
Anthony C. Willis



Research School of Chemistry, Institute of Advanced Studies

Australian National University, Canberra, ACT 0200, Australia

Monday, 19th June, 2000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C(53)

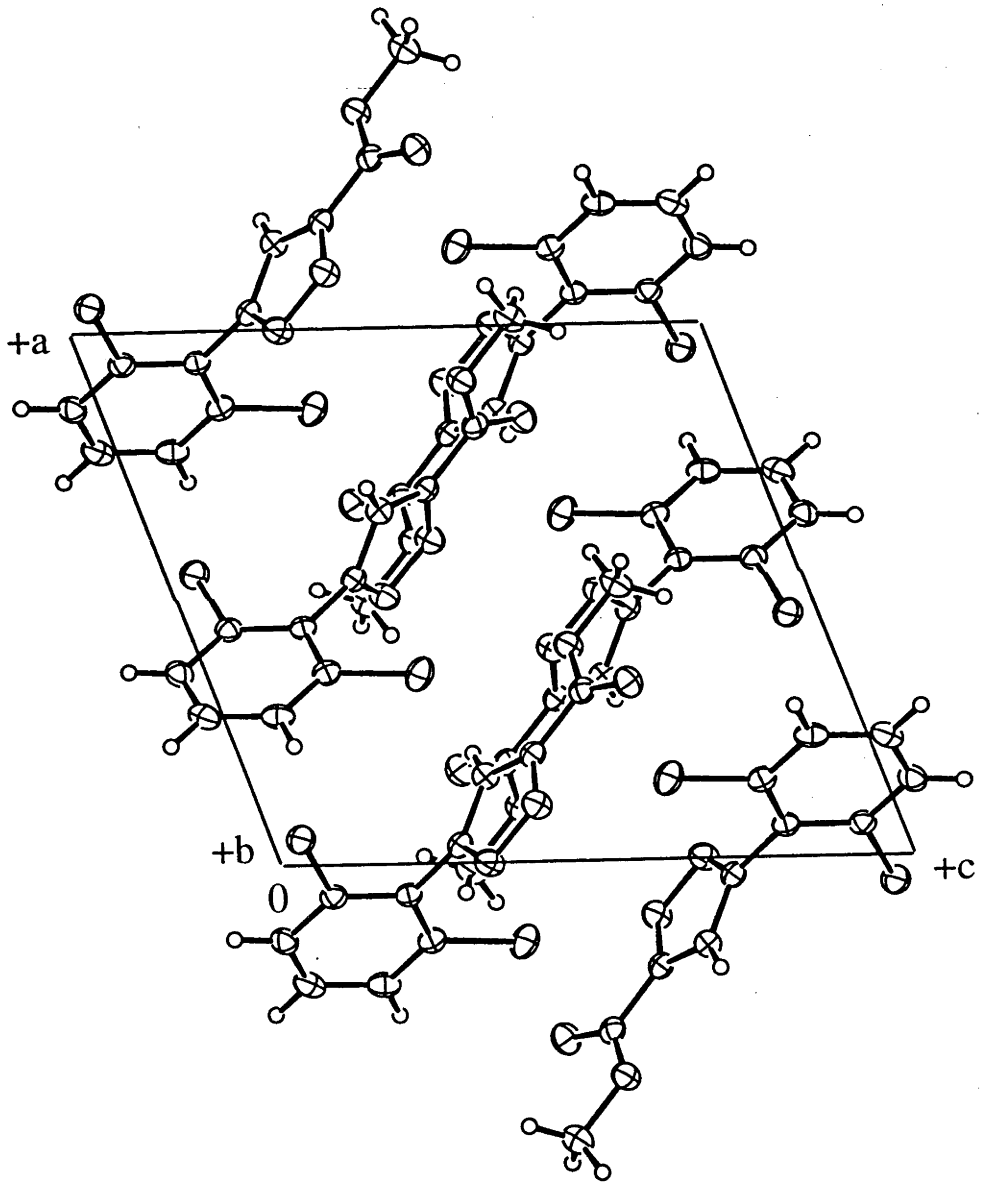
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Figure Captions for $C_{11}H_7Cl_2NO_3$

ɡure 1. Thermal ellipsoid diagram of $C_{11}H_7Cl_2NO_3$ with labelling of selected atoms. Ellipsoids ɔw 30% probability levels. Hydrogen atoms are drawn as circles with small radii.

ɡure 2. Unit cell diagram of $C_{11}H_7Cl_2NO_3$ projected down the b axis. Ellipsoids show 30% ɔbability levels. Hydrogen atoms are drawn as circles with small radii.

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           for George J. Vuckovic and Christopher J. Easton
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 C(3) C(31)  C(32) 122.0(2) . . . yes
 C(3) C(31)  C(36) 121.0(2) . . . yes
 C(32) C(31)  C(36) 116.9(2) . . . yes
 Cl(32) C(32)  C(31) 118.7(2) . . . yes
 Cl(32) C(32)  C(33) 119.3(2) . . . yes
 C(31) C(32)  C(33) 122.0(2) . . . yes
 C(32) C(33)  C(34) 119.3(2) . . . yes
 C(32) C(33)  H(2) 120(1) . . . no
 C(34) C(33)  H(2) 121(1) . . . no
 C(33) C(34)  C(35) 120.7(2) . . . yes
 C(33) C(34)  H(3) 121(1) . . . no
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 C(34) C(35)  C(36) 119.2(2) . . . yes
 C(34) C(35)  H(4) 119(1) . . . no
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 C(31) C(36)  C(35) 121.9(2) . . . yes
 O(51) C(51)  O(52) 126.0(2) . . . yes
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 O(52) C(51)  C(5) 108.9(2) . . . yes
 O(52) C(53)  H(5) 112(2) . . . no
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 H(6) C(53)  H(7) 112(2) . . . no
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 N(2)      C(53)     3.476(4)    . 2_646 no
 C(32)     C(34)     3.549(3)    . 3_675 no
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 O(1) N(2) C(3) C(31) . . . . -178.5(2) no
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 O(1) C(5) C(51) O(51) . . . . -5.1(3) no
 O(1) C(5) C(51) O(52) . . . . 176.7(2) no
 O(51) C(51) O(52) C(53) . . . . -4.5(3) no
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 O(52) C(51) C(5) C(4) . . . . -7.7(3) no
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 N(2) O(1) C(5) C(51) . . . . 176.5(2) no
 N(2) C(3) C(4) C(5) . . . . -0.5(2) no
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 C(3) C(4) C(5) C(51) . . . . -175.5(2) no
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 C(3) C(31) C(36) C(35) . . . . 176.5(2) no
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 C(4) C(3) C(31) C(36) . . . . -78.6(3) no
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 C(5) C(51) O(52) C(53) . . . . 173.7(2) no
 C(31) C(32) C(33) C(34) . . . . 0.6(3) no
 C(31) C(36) C(35) C(34) . . . . 0.6(3) no
 C(32) C(31) C(36) C(35) . . . . -0.6(3) no
 C(32) C(33) C(34) C(35) . . . . -0.5(3) no
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 C(33) C(34) C(35) C(36) . . . . -0.1(3) no
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;
                    Table of Least-Squares Planes
                   -------------------------------

              ------------- Plane number  1 ---------------

              Atoms Defining Plane      Distance      esd
                       O(1)   (    1)    -0.0007      0.0015
                       N(2)   (    1)     0.0022      0.0019
                       C(3)   (    1)    -0.0026      0.0018
                       C(4)   (    1)     0.0025      0.0021
                       C(5)   (    1)    -0.0005      0.0018

              Additional Atoms          Distance
                       C(31)  (    1)    -0.0335
                       C(51)  (    1)    -0.0841
                       H(1)   (    1)    -0.0001

              Mean deviation from plane is 0.0017  angstroms
               Chi-squared:         5.0

              ------------- Plane number  2 ---------------

              Atoms Defining Plane      Distance      esd
```

```
          C(31)   (    1)     0.0015       0.0018
          C(32)   (    1)     0.0017       0.0019
          C(33)   (    1)    -0.0041       0.0021
          C(34)   (    1)     0.0016       0.0021
          C(35)   (    1)     0.0025       0.0021
          C(36)   (    1)    -0.0039       0.0019

 Additional Atoms              Distance
          Cl(32)  (    1)     0.0453
          Cl(36)  (    1)    -0.0340
          C(3)    (    1)     0.0711
          H(2)    (    1)     0.0248
          H(3)    (    1)     0.0265
          H(4)    (    1)     0.0076

 Mean deviation from plane is 0.0025  angstroms
  Chi-squared:        11.5

 Dihedral angles between least-squares planes
              plane  plane   angle
                2      1     80.59

 ---------- Plane number  3 ----------

 Atoms Defining Plane          Distance       esd
          O(51)   (    1)    -0.0025       0.0015
          O(52)   (    1)    -0.0019       0.0014
          C(5)    (    1)    -0.0029       0.0018
          C(51)   (    1)     0.0105       0.0019

 Additional Atoms              Distance
          C(53)   (    1)    -0.1346
          O(1)    (    1)     0.0759
          N(2)    (    1)    -0.0328
          C(3)    (    1)    -0.1759
          C(4)    (    1)    -0.1561

 Mean deviation from plane is 0.0044  angstroms
  Chi-squared:        37.9

 Dihedral angles between least-squares planes
              plane  plane   angle
                3      1      6.79
                3      2     84.01
;
#------------------------------------------------------------------------------
#===END
```

Appendix Two

Sample: gjv3a

Compound: $C_{12}H_9Cl_2NO_3$

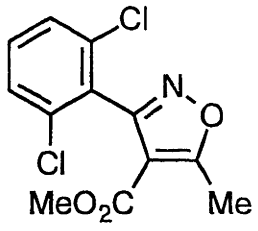
X-ray Structure Report

for

George J. Vuckovic and Christopher J. Easton

by

Anthony C. Willis



Research School of Chemistry, Institute of Advanced Studies

Australian National University, Canberra, ACT 0200, Australia

Wed Jan 19 2000

Experimental

Data Collection

A colourless block-shaped crystal of $C_{12}H_9Cl_2NO_3$ having approximate dimensions of 0.40 × 0.30 × 0.20 mm was mounted on a glass fibre. All measurements were made on a Nonius KappaCCD area detector with graphite monochromated Mo-Kα radiation.

Cell constants and an orientation matrix for data collection corresponded to a primitive monoclinic cell with dimensions:

a = 11.8632(3) $\AA$
b = 8.9185(2) $\AA$ β = 111.844(1)°
c = 13.3160(3) $\AA$
V = 1307.70(5) $\AA^3$

For Z = 4 and F.W. = 286.11, the calculated density is 1.45 g/cm^3. The systematic absences of:

h0l: l ≠ 2n
0k0: k ≠ 2n

uniquely determine the space group to be:

$P2_1/c$ (#14)

The data were collected at a temperature of -72 ± 1°C to a maximum 2θ value of 60.1°. Data were collected with oscillations of 2.0°/frame at a rate of 10 sec/°. The crystal-to-detector distance was 25.00 mm with the detector at the zero swing position. Multiple scan sets were collected to give at least 4-fold redundancy on 90% of the unique data.

Data Reduction

The intensities of a total of 31388 reflections were collected and processed with the use of the computer programs Denzo and Scalepak.[1]

The linear absorption coefficient, μ, for Mo-Kα radiation is 4.9 cm^{-1}. A multiscan absorption correction was applied which resulted in transmission factors ranging from 0.906 to 0.851.[2] Equivalent reflections were merged (R_{int} = 0.050) to yield 3814 unique reflections. The data were corrected for Lorentz and polarization effects.

Structure Solution and Refinement

The structure was solved by direct methods[3] and expanded using Fourier techniques[4]. The non-hydrogen atoms were refined anisotropically. The hydrogen atoms coordinates were refined but their isotropic B's were

held fixed: restraints needed to be applied to the H–C–H angles about C(51). The final cycle of full-matrix least-squares refinement[5] was based on 2294 observed reflections ($I > 2.0\sigma(I)$) and 190 variable parameters and converged (largest parameter shift was 0.05 times its esd) with unweighted and weighted agreement factors of:

$$R = \Sigma||Fo| - |Fc||/\Sigma|Fo| = 0.042$$

$$R_w = \sqrt{(\Sigma w(|Fo| - |Fc|)^2/\Sigma wFo^2)} = 0.050$$

The standard deviation of an observation of unit weight[6] was 1.29. The weighting scheme was based on counting statistics and included a factor (p = 0.040) to downweight the intense reflections. Plots of $\Sigma w(|Fo| - |Fc|)^2$ versus $|Fo|$, reflection order in data collection, $\sin\theta/\lambda$ and various classes of indices showed no unusual trends. The maximum and minimum peaks on the final difference Fourier map corresponded to 0.25 and -0.33 $e^-/Å^3$, respectively.

Neutral atom scattering factors were taken from Cromer and Waber[7]. Anomalous dispersion effects were included in Fcalc[8]; the values for $\Delta f'$ and $\Delta f''$ were those of Creagh and McAuley[9]. The values for the mass attenuation coefficients are those of Creagh and Hubbel[10]. Calculations were performed using the crystallographic software packages maXus[11] and teXsan[12] operating on in-house computers and the Silicon Graphics Power Challenge computer of the Australian National University Supercomputer Facility.

References

(1) Otwinowski, Z. and Minor, W, (1997). In Methods in Enzymology, 276, edited by C.W. Carter, Jr. & R.M. Sweet pp. 307-326, New York:Academic Press.

(2) Blessing, R. H.; Acta Crystallogr., Section A, 51, 33-37 (1995). Blessing, R. H.; J. Appl. Crystallogr., 30, 421-426 (1997).

(3) SIR92: Altomare, A., Cascarano, M., Giacovazzo, C., Guagliardi, A., Burla, M.C., Polidori, G. and Camalli, M. (1994). J. Appl. Cryst., 27, 435.

(4) DIRDIF94: Beurskens, P.T., Admiraal, G., Beurskens, G., Bosman, W.P., de Gelder, R., Israel, R. and Smits, J.M.M. (1994). The DIRDIF-94 program system, Technical Report of the Crystallography Laboratory, University of Nijmegen, The Netherlands.

(5) Least-Squares:

Function minimized: $\Sigma w(|Fo| - |Fc|)^2$

(6) Standard deviation of an observation of unit weight:

$$\sqrt{\Sigma w(|Fo| - |Fc|)^2/(No - Nv)}$$

where: No = number of observations

Nv = number of variables

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(11) maXus: Mackay, S., Gilmore, C. J.,Edwards, C., Stewart, N. & Shankland, K. (1999). maXus Computer Program for the Solution and Refinement of Crystal Structures. Nonius, The Netherlands, MacScience, Japan & The University of Glasgow.

(12) teXsan: Single Crystal Structure Analysis Software. Version 1.8. Molecular Structure Corporation, 3200 Research Forest Drive, The Woodlands, TX 77381, USA (1997).

EXPERIMENTAL DETAILS

A. Crystal Data

Empirical Formula	$C_{12}H_9Cl_2NO_3$
Formula Weight	286.11
Crystal Colour, Habit	colourless, block
Crystal Dimensions	0.40 × 0.30 × 0.20 mm
Crystal System	monoclinic
Lattice Type	Primitive
Lattice Parameters	a = 11.8632(3)Å b = 8.9185(2) Å c = 13.3160(3) Å β = 111.844(1)°
	V = 1307.70(5) Å^3
Space Group	$P2_1/c$ (#14)
Z value	4
D_{calc}	1.453 g/cm^3
F_{000}	584.00
μ(MoKα)	4.94 cm^{-1}

B. Intensity Measurements

Diffractometer	Nonius KappaCCD
Radiation	MoKα (λ = 0.71069 $\AA$) graphite monochromated
Detector Aperture	65 mm × 65 mm
Data Images	2.00°/frame; 10 sec/°
Detector Position	25.00 mm
$2\theta_{max}$	60.1°
No. of Reflections Measured	Total: 31388 Unique: 3814 (R_{int} = 0.050)
Corrections	Lorentz-polarization Absorption (trans. factors: 0.906 - 0.851)

C. Structure Solution and Refinement

Structure Solution	Direct Methods (SIR92)
Refinement	Full-matrix least-squares
Function Minimized	$\Sigma w(\|Fo\| - \|Fc\|)^2$
Least Squares Weights	$w = [\sigma_c^2(Fo) + \frac{p^2}{4}Fo^2]^{-1}$
p-factor	0.040
Anomalous Dispersion	All non-hydrogen atoms
No. Observations (I>2.0σ(I))	2294
No. Variables	190
Reflection/Parameter Ratio	12.07
Residuals: R; Rw	0.042 ; 0.050
Goodness of Fit Indicator	1.29
Max Shift/Error in Final Cycle	0.05
Maximum peak in Final Diff. Map	0.25 $e^-/\AA^3$
Minimum peak in Final Diff. Map	-0.33 $e^-/\AA^3$

Table 1. Atomic coordinates and B_{iso}/B_{eq} for $C_{12}H_9Cl_2NO_3$

atom	x	y	z	B_{eq}
Cl(32)	-0.00631(5)	0.02407(8)	0.24990(5)	5.96(2)
Cl(36)	0.39972(4)	0.10275(6)	0.16553(4)	4.44(1)
O(1)	0.3606(1)	-0.0115(1)	0.47754(9)	4.17(3)
O(41)	0.2276(1)	-0.2602(1)	0.1562(1)	4.15(3)
O(42)	0.3165(1)	-0.4051(1)	0.3019(1)	3.82(3)
N(2)	0.2979(1)	0.0856(2)	0.3906(1)	3.91(4)
C(3)	0.2627(1)	0.0012(2)	0.3046(1)	2.84(3)
C(4)	0.2998(1)	-0.1507(2)	0.3302(1)	2.78(3)
C(5)	0.3606(2)	-0.1513(2)	0.4396(1)	3.33(4)
C(31)	0.1906(1)	0.0709(2)	0.1997(1)	2.86(3)
C(32)	0.0660(2)	0.0928(2)	0.1681(2)	3.70(4)
C(33)	-0.0015(2)	0.1636(2)	0.0724(2)	4.62(5)
C(34)	0.0553(2)	0.2131(2)	0.0056(2)	4.87(5)
C(35)	0.1772(2)	0.1932(2)	0.0328(2)	4.26(4)
C(36)	0.2444(2)	0.1227(2)	0.1292(1)	3.18(3)
C(41)	0.2765(1)	-0.2741(2)	0.2526(1)	2.97(3)
C(43)	0.2965(2)	-0.5347(2)	0.2314(2)	4.82(5)
C(51)	0.4217(2)	-0.2667(2)	0.5205(2)	4.44(5)
H(1)	-0.081(2)	0.181(2)	0.056(2)	5.5452
H(2)	0.006(2)	0.261(2)	-0.063(2)	5.8470
H(3)	0.218(2)	0.223(2)	-0.007(2)	5.1593
H(4)	0.212(2)	-0.550(2)	0.189(2)	5.7810
H(5)	0.311(2)	-0.615(3)	0.281(2)	5.7810
H(6)	0.360(2)	-0.543(2)	0.193(2)	5.7810

Table 1. Atomic coordinates and B_{iso}/B_{eq} for $C_{12}H_9Cl_2NO_3$ (continued)

atom	x	y	z	B_{eq}
H(7)	0.458(2)	-0.229(2)	0.580(2)	5.3630
H(8)	0.366(1)	-0.341(2)	0.527(2)	5.3630
H(9)	0.477(2)	-0.320(2)	0.501(2)	5.3630

$$B_{eq} = \frac{8}{3}\pi^2(U_{11}(aa^*)^2 + U_{22}(bb^*)^2 + U_{33}(cc^*)^2 + 2U_{12}aa^*bb^*\cos\gamma + 2U_{13}aa^*cc^*\cos\beta + 2U_{23}bb^*cc^*\cos\alpha)$$

Table 2. Anisotropic Displacement Parameters for $C_{12}H_9Cl_2NO_3$

atom	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
Cl(32)	0.0562(3)	0.1028(5)	0.0794(4)	-0.0062(3)	0.0389(3)	0.0009(4)
Cl(36)	0.0527(3)	0.0603(3)	0.0656(3)	-0.0062(2)	0.0335(3)	-0.0103(2)
O(1)	0.0732(9)	0.0466(7)	0.0344(7)	0.0066(6)	0.0150(6)	-0.0060(5)
O(41)	0.0634(8)	0.0459(7)	0.0397(7)	0.0059(6)	0.0090(6)	-0.0069(6)
O(42)	0.0537(8)	0.0296(6)	0.0532(8)	0.0032(5)	0.0099(6)	-0.0026(5)
N(2)	0.065(1)	0.0409(9)	0.0390(8)	0.0075(7)	0.0159(7)	-0.0028(6)
C(3)	0.0397(9)	0.0335(8)	0.0378(8)	0.0001(7)	0.0179(7)	-0.0031(6)
C(4)	0.0370(8)	0.0332(8)	0.0370(8)	-0.0003(7)	0.0155(7)	-0.0009(7)
C(5)	0.049(1)	0.0403(9)	0.0393(8)	0.0004(8)	0.0196(8)	-0.0006(7)
C(31)	0.0426(8)	0.0276(8)	0.0383(9)	0.0006(7)	0.0149(7)	-0.0035(6)
C(32)	0.0456(9)	0.044(1)	0.051(1)	-0.0008(8)	0.0179(8)	-0.0039(8)
C(33)	0.049(1)	0.051(1)	0.062(1)	0.008(1)	0.0056(9)	0.0005(9)
C(34)	0.073(1)	0.046(1)	0.050(1)	-0.002(1)	0.004(1)	0.008(1)
C(35)	0.076(1)	0.042(1)	0.044(1)	-0.0138(9)	0.0229(9)	0.0013(8)
C(36)	0.0490(9)	0.0317(9)	0.0424(9)	-0.0058(7)	0.0196(7)	-0.0057(7)
C(41)	0.0338(8)	0.0338(8)	0.0445(9)	0.0009(7)	0.0137(7)	-0.0025(7)
C(43)	0.062(1)	0.035(1)	0.080(2)	-0.001(1)	0.019(1)	-0.017(1)
C(51)	0.068(1)	0.055(1)	0.041(1)	0.006(1)	0.014(1)	0.0072(9)

The general temperature factor expression:

$$\exp(-2\pi^2(a^{*2}U_{11}h^2 + b^{*2}U_{22}k^2 + c^{*2}U_{33}l^2 + 2a^*b^*U_{12}hk + 2a^*c^*U_{13}hl + 2b^*c^*U_{23}kl))$$

Table 3. Bond Lengths (Å) Involving Non-Hydrogen Atoms of $C_{12}H_9Cl_2NO_3$

atom	atom	distance	atom	atom	distance
Cl(32)	C(32)	1.731(2)	Cl(36)	C(36)	1.732(2)
O(1)	N(2)	1.415(2)	O(1)	C(5)	1.345(2)
O(41)	C(41)	1.202(2)	O(42)	C(41)	1.338(2)
O(42)	C(43)	1.451(3)	N(2)	C(3)	1.303(2)
C(3)	C(4)	1.426(3)	C(3)	C(31)	1.476(3)
C(4)	C(5)	1.365(3)	C(4)	C(41)	1.464(3)
C(5)	C(51)	1.471(3)	C(31)	C(32)	1.391(3)
C(31)	C(36)	1.396(3)	C(32)	C(33)	1.380(3)
C(33)	C(34)	1.374(4)	C(34)	C(35)	1.366(4)
C(35)	C(36)	1.384(3)			

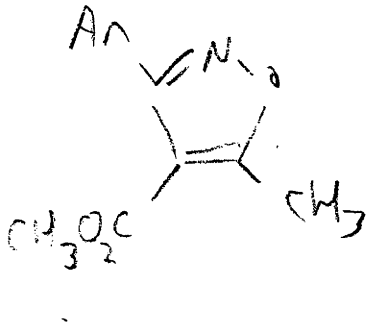


Table 4. Bond Lengths (Å) Involving Hydrogen Atoms of $C_{12}H_9Cl_2NO_3$

atom	atom	distance	atom	atom	distance
C(33)	H(1)	0.90(2)	C(34)	H(2)	0.98(3)
C(35)	H(3)	0.89(2)	C(43)	H(4)	0.96(3)
C(43)	H(5)	0.94(3)	C(43)	H(6)	1.06(3)
C(51)	H(7)	0.82(2)	C(51)	H(8)	0.96(2)
C(51)	H(9)	0.93(2)			

Table 5. Bond Angles (°) Involving Non-Hydrogen Atoms of $C_{12}H_9Cl_2NO_3$

atom	atom	atom	angle	atom	atom	atom	angle
N(2)	O(1)	C(5)	109.5(1)	C(41)	O(42)	C(43)	115.8(2)
O(1)	N(2)	C(3)	105.4(2)	N(2)	C(3)	C(4)	111.6(2)
N(2)	C(3)	C(31)	118.2(2)	C(4)	C(3)	C(31)	130.1(2)
C(3)	C(4)	C(5)	104.6(2)	C(3)	C(4)	C(41)	125.8(2)
C(5)	C(4)	C(41)	129.5(2)	O(1)	C(5)	C(4)	108.8(2)
O(1)	C(5)	C(51)	116.3(2)	C(4)	C(5)	C(51)	134.9(2)
C(3)	C(31)	C(32)	121.5(2)	C(3)	C(31)	C(36)	121.8(2)
C(32)	C(31)	C(36)	116.7(2)	Cl(32)	C(32)	C(31)	118.6(2)
Cl(32)	C(32)	C(33)	119.3(2)	C(31)	C(32)	C(33)	122.0(2)
C(32)	C(33)	C(34)	119.3(2)	C(33)	C(34)	C(35)	120.8(2)
C(34)	C(35)	C(36)	119.6(2)	Cl(36)	C(36)	C(31)	118.9(2)
Cl(36)	C(36)	C(35)	119.4(2)	C(31)	C(36)	C(35)	121.6(2)
O(41)	C(41)	O(42)	123.8(2)	O(41)	C(41)	C(4)	124.4(2)
O(42)	C(41)	C(4)	111.8(2)				

Table 6. Bond Angles (°) Involving -Hydrogen Atoms of $C_{12}H_9Cl_2NO_3$

atom	atom	atom	angle	atom	atom	atom	angle
C(32)	C(33)	H(1)	120(2)	C(34)	C(33)	H(1)	121(2)
C(33)	C(34)	H(2)	118(2)	C(35)	C(34)	H(2)	121(2)
C(34)	C(35)	H(3)	124(2)	C(36)	C(35)	H(3)	116(2)
O(42)	C(43)	H(4)	112(2)	O(42)	C(43)	H(5)	102(2)
O(42)	C(43)	H(6)	113(1)	H(4)	C(43)	H(5)	100(2)
H(4)	C(43)	H(6)	119(2)	H(5)	C(43)	H(6)	108(2)
C(5)	C(51)	H(7)	111(2)	C(5)	C(51)	H(8)	112(1)
C(5)	C(51)	H(9)	112(1)	H(7)	C(51)	H(8)	109(2)*
H(7)	C(51)	H(9)	108(2)*	H(8)	C(51)	H(9)	105(2)*

* restrained during refinement

Table 7. Torsion Angles (°) Involving Non-Hydrogen Atoms of $C_{12}H_9Cl_2NO_3$

atom	atom	atom	atom	angle	atom	atom	atom	atom	angle
Cl(32)	C(32)	C(31)	C(3)	-4.7(3)	Cl(32)	C(32)	C(31)	C(36)	177.5(1)
Cl(32)	C(32)	C(33)	C(34)	-177.6(2)	Cl(36)	C(36)	C(31)	C(3)	0.6(2)
Cl(36)	C(36)	C(31)	C(32)	178.5(1)	Cl(36)	C(36)	C(35)	C(34)	-178.0(2)
O(1)	N(2)	C(3)	C(4)	-0.2(2)	O(1)	N(2)	C(3)	C(31)	178.2(2)
O(1)	C(5)	C(4)	C(3)	-0.1(2)	O(1)	C(5)	C(4)	C(41)	179.3(2)
O(41)	C(41)	O(42)	C(43)	-0.6(3)	O(41)	C(41)	C(4)	C(3)	3.8(3)
O(41)	C(41)	C(4)	C(5)	-175.5(2)	O(42)	C(41)	C(4)	C(3)	-176.3(2)
O(42)	C(41)	C(4)	C(5)	4.4(3)	N(2)	O(1)	C(5)	C(4)	-0.1(2)
N(2)	O(1)	C(5)	C(51)	-179.5(2)	N(2)	C(3)	C(4)	C(5)	0.2(2)
N(2)	C(3)	C(4)	C(41)	-179.3(2)	N(2)	C(3)	C(31)	C(32)	-82.1(2)
N(2)	C(3)	C(31)	C(36)	95.7(2)	C(3)	N(2)	O(1)	C(5)	0.2(2)
C(3)	C(4)	C(5)	C(51)	179.2(2)	C(3)	C(31)	C(32)	C(33)	177.1(2)
C(3)	C(31)	C(36)	C(35)	-177.5(2)	C(4)	C(3)	C(31)	C(32)	96.0(2)
C(4)	C(3)	C(31)	C(36)	-86.3(3)	C(4)	C(41)	O(42)	C(43)	179.6(2)
C(5)	C(4)	C(3)	C(31)	-178.0(2)	C(31)	C(3)	C(4)	C(41)	2.6(3)
C(31)	C(32)	C(33)	C(34)	0.6(3)	C(31)	C(36)	C(35)	C(34)	0.1(3)
C(32)	C(31)	C(36)	C(35)	0.4(3)	C(32)	C(33)	C(34)	C(35)	-0.1(4)
C(33)	C(32)	C(31)	C(36)	-0.7(3)	C(33)	C(34)	C(35)	C(36)	-0.2(4)
C(41)	C(4)	C(5)	C(51)	-1.4(4)					

Table 8. Non-bonded Contacts out to 3.60 Å Involving Non-Hydrogen Atoms of $C_{12}H_9Cl_2NO_3$

atom	atom	distance	ADC	atom	atom	distance	ADC
Cl(32)	O(42)	3.539(2)	2	Cl(32)	C(43)	3.581(3)	2
Cl(36)	O(42)	3.232(2)	65502	Cl(36)	C(43)	3.563(3)	65502
O(1)	O(1)	3.141(3)	65603	O(1)	C(5)	3.399(2)	65603
O(1)	C(51)	3.575(3)	65603	O(41)	C(34)	3.271(3)	3
O(41)	C(33)	3.335(3)	3	O(41)	C(51)	3.424(3)	54404
N(2)	C(35)	3.399(3)	4	N(2)	C(51)	3.485(3)	65603
C(32)	C(34)	3.519(3)	3	C(33)	C(34)	3.504(3)	3
C(33)	C(33)	3.505(5)	3	C(35)	C(43)	3.479(3)	56501
C(36)	C(43)	3.309(3)	56501				

The ADC (atom designator code) specifies the position of an atom in a crystal. The 5-digit number shown in the table is a composite of three one-digit numbers and one two-digit number: TA (first digit) + TB (second digit) + TC (third digit) + SN (last two digits). TA, TB and TC are the crystal lattice translation digits along cell edges a, b and c. A translation digit of 5 indicates the origin unit cell. If TA = 4, this indicates a translation of one unit cell length along the a-axis in the negative direction. Each translation digit can range in value from 1 to 9 and thus ± 4 lattice translations from the origin (TA=5, TB=5, TC=5) can be represented.

The SN, or symmetry operator number, refers to the number of the symmetry operator used to generate the coordinates of the target atom. A list of symmetry operators relevant to this structure are given below.

For a given intermolecular contact, the first atom (origin atom) is located in the origin unit cell and its position can be generated using the identity operator (SN=1). Thus, the ADC for an origin atom is always 55501. The position of the second atom (target atom) can be generated using the ADC and the coordinates of the atom in the parameter table. For example, an ADC of 47502 refers to the target atom moved through symmetry operator two, then translated -1 cell translations along the a axis, +2 cell translations along the b axis, and 0 cell translations along the c axis.

An ADC of 1 indicates an intermolecular contact between two fragments (eg. cation and anion) that reside in the same asymmetric unit.

Symmetry Operators:

(1)	X,	Y,	Z	(2)	-X,	1/2+Y,	1/2-Z
(3)	-X,	-Y,	-Z	(4)	X,	1/2-Y,	1/2+Z

Table 9. Least Squares Planes for $C_{12}H_9Cl_2NO_3$

Plane number 1

Atoms defining plane	Distance
O(1)	-0.001(2)
N(2)	0.001(2)
C(3)	-0.001(2)
C(4)	0.001(2)
C(5)	0.000(2)

Additional Atoms	Distance
C(31)	-0.041
C(41)	0.014
C(51)	-0.013

Plane number 2

Atoms defining plane	Distance
C(31)	0.002(2)
C(32)	-0.004(2)
C(33)	0.002(2)
C(34)	0.002(2)
C(35)	-0.002(2)
C(36)	-0.001(2)

Additional Atoms	Distance
Cl(32)	-0.067
Cl(36)	0.047
C(3)	0.059

Plane number 3

Atoms defining plane	Distance
O(41)	0.000(2)
O(42)	0.000(1)
C(4)	0.000(2)
C(41)	-0.001(2)

Additional Atoms	Distance
C(3)	-0.074
C(5)	0.082
C(43)	-0.011

Summary

plane	mean deviation	χ^2
1	0.0007	1.0
2	0.0021	7.5
3	0.0003	0.2

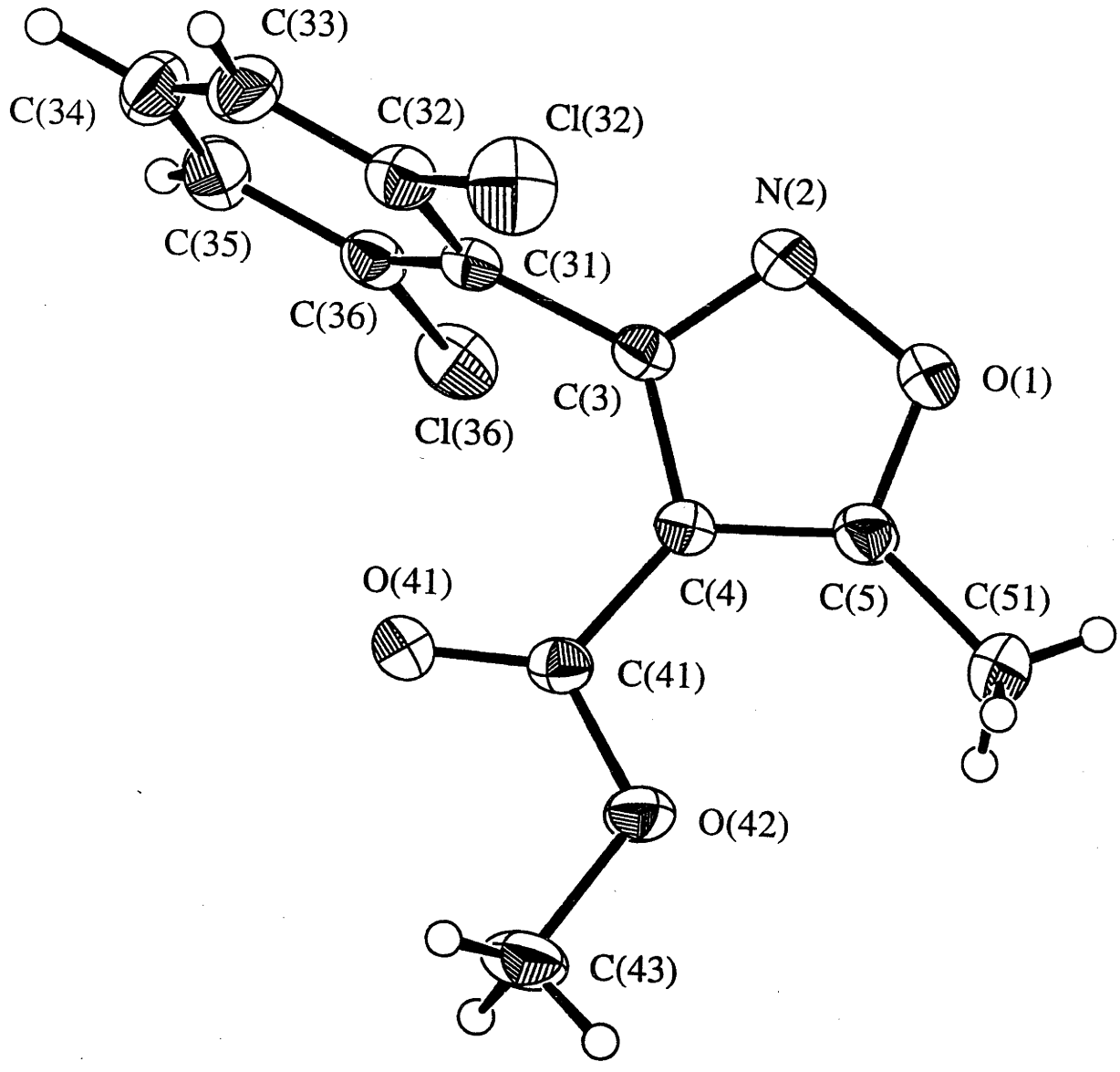
Dihedral angles between planes (°)

plane	1	2
2	83.78	
3	4.06	84.08

Figure Captions for $C_{12}H_9Cl_2NO_3$

Figure 1. Thermal ellipsoid diagram of $C_{12}H_9Cl_2NO_3$ with selected atom labelling. Ellipsoids show 30% probability levels; hydrogen atoms are drawn as circles with small radii.

Figure 2. Unit cell diagram for $C_{12}H_9Cl_2NO_3$ projected down the b axis. Ellipsoids show 30% probability levels; hydrogen atoms are drawn as circles with small radii.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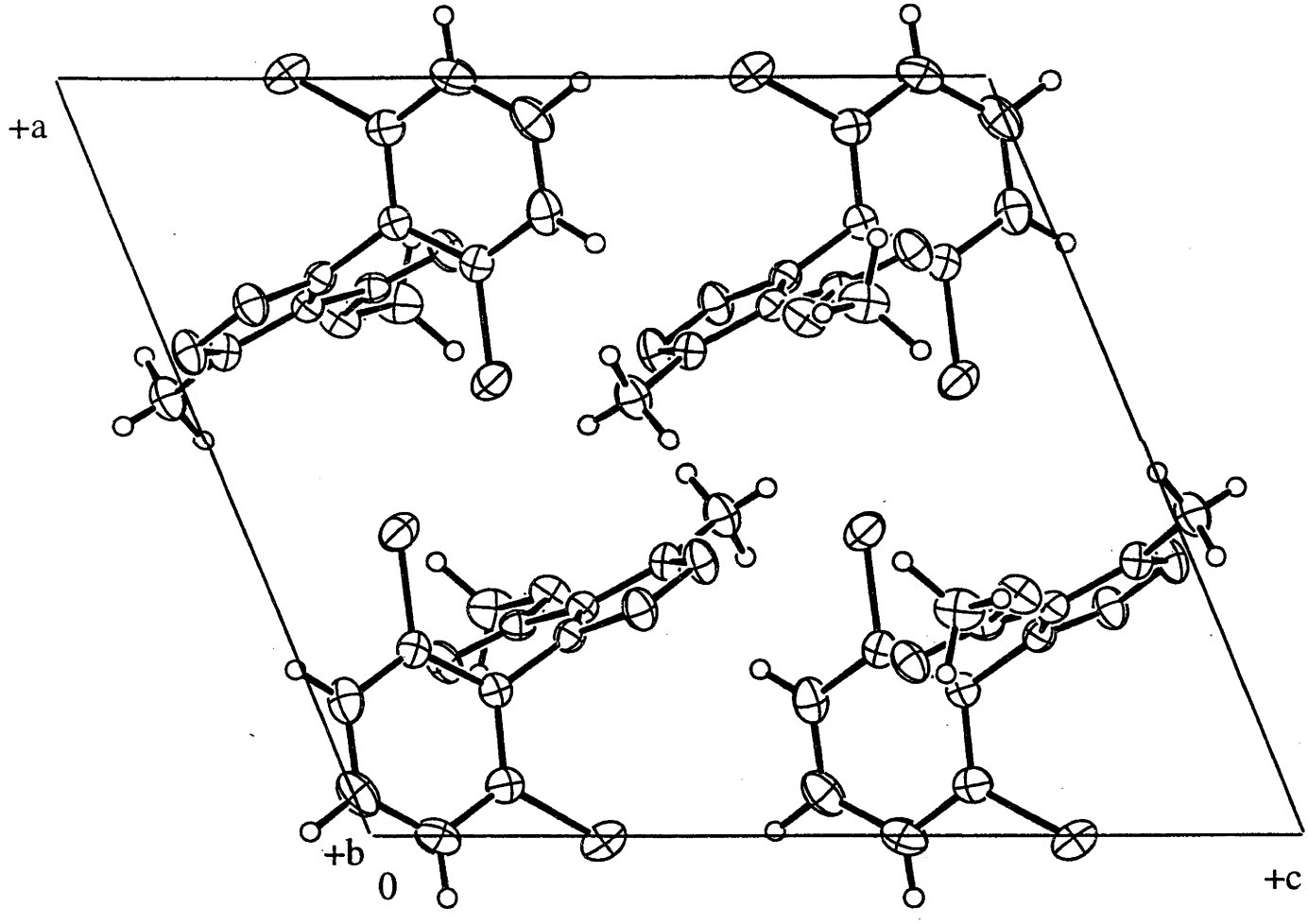
Cl(32)
N(2)
C(35)
C(31)
C(36)
C(3)
O(1)
Cl(36)
O(41)
C(4)
C(5)
C(51)
C(41)
O(42)
C(43)


+a
+b
0
+c

Appendix Three

Sample: gjv1

Compound: $C_{15}H_9Cl_2NO$

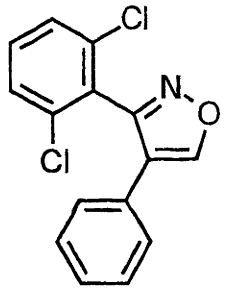
X-ray Structure Report

for

George J. Vuckovic and Christopher J. Easton

by

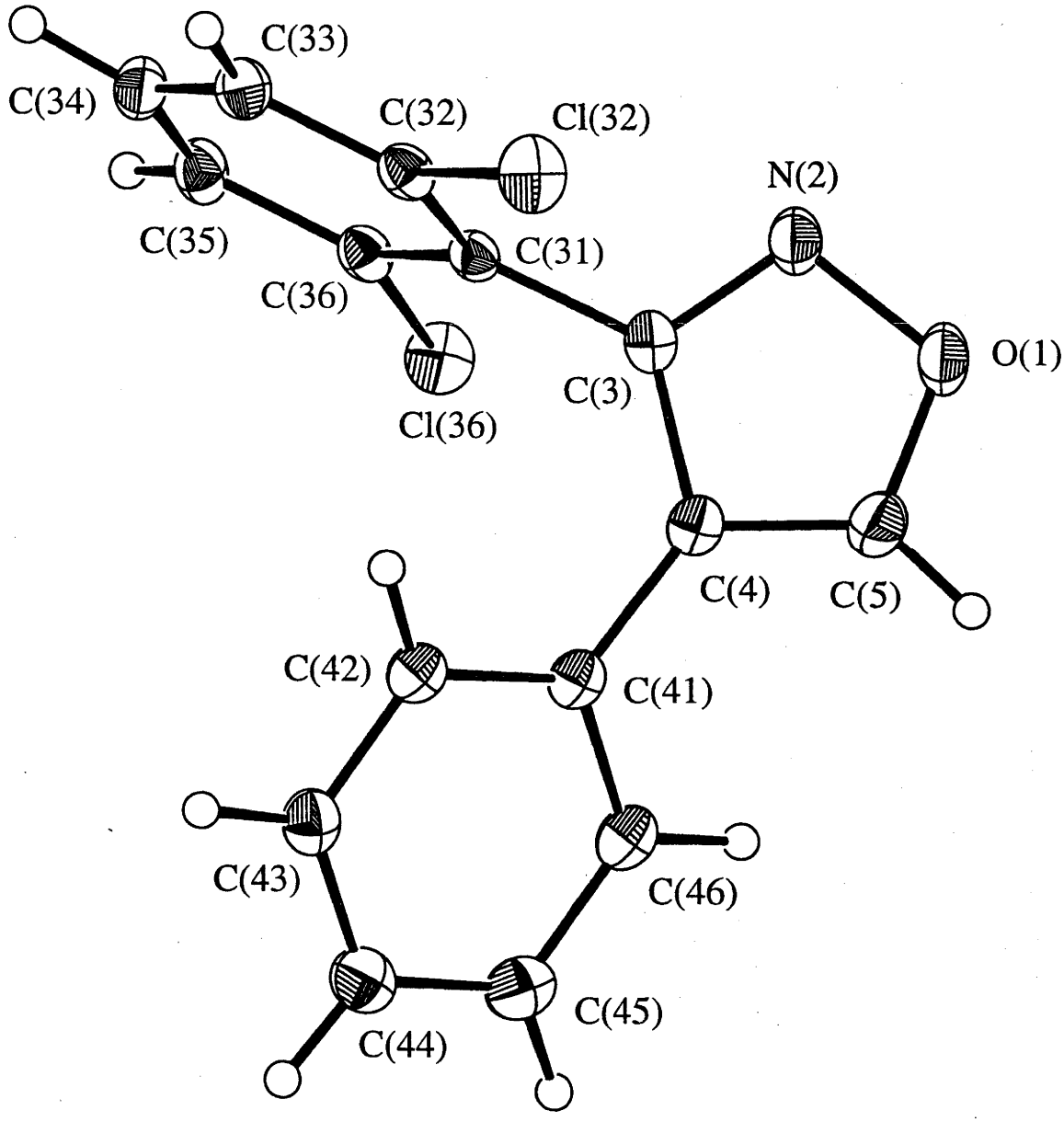
Anthony C. Willis



Research School of Chemistry, Institute of Advanced Studies

Australian National University, Canberra, ACT 0200, Australia

Tue Oct 19 1999


C(33)
C(34)
C(32)
Cl(32)
N(2)
C(35)
C(31)
C(36)
O(1)
C(3)
Cl(36)
C(4)
C(5)
C(42)
C(41)
C(43)
C(46)
C(45)
C(44)

Experimental

Data Collection

A colourless platelet crystal of $C_{15}H_9Cl_2NO$ having approximate dimensions of 0.37 x 0.23 x 0.08 mm was mounted on a quartz fibre. All measurements were made on a Rigaku AFC6R diffractometer with graphite monochromated Cu-Kα radiation and a rotating anode generator.

Cell constants and an orientation matrix for data collection, obtained from a least-squares refinement using the setting angles of 25 carefully centred reflections in the range $107.55 < 2\theta < 109.46°$, corresponded to a primitive monoclinic cell with dimensions:

$$a = 10.221(3)\ \AA$$
$$b = 9.528(3)\ \AA \qquad \beta = 93.90(2)°$$
$$c = 13.631(3)\ \AA$$
$$V = 1324.4(7)\ \AA^3$$

For Z = 4 and F.W. = 290.15, the calculated density is 1.46 g/cm^3. The systematic absences of:

$$h0l:\ h+l \neq 2n$$
$$0k0:\ k \neq 2n$$

uniquely determine the space group to be:

$$P2_1/n\ (\#14)$$

The data were collected at a temperature of -85 $\pm$ 1°C using the ω-2θ scan technique to a maximum 2θ value of 120.1°. Omega scans of several intense reflections, made prior to data collection, had an average width at half-height of 0.30° with a take-off angle of 6.0°. Scans of $(1.20 + 0.30 \tan\theta)°$ were made at a speed of 16.0°/min (in omega). The weak reflections ($I < 10.0\sigma(I)$) were rescanned (maximum of 4 scans) and the counts were accumulated to ensure good counting statistics. Stationary background counts were recorded on each side of the reflection. The ratio of peak counting time to background counting time was 2:1. The diameter of the incident beam collimator was 1.0 mm, the crystal to detector distance was 400 mm, and the detector aperture was 7.0 x 7.0 mm (horizontal x vertical).

Data Reduction

The intensities of a total of 4154 reflections were collected. Three representative reflections were measured after every 150 reflections to monitor orientation and decay. Over the course of data collection, the standards decreased by 7.1%. A linear correction factor was applied to the data to account for this phenomenon.

The linear absorption coefficient, μ, for Cu-Kα radiation is 43.2 cm^{-1}. An analytical absorption correction was applied which resulted in transmission factors ranging from 0.40 to 0.70. Equivalent reflections were merged ($R_{int} = 0.052$) to yield 1981 unique data. The data were corrected for Lorentz and polarization

effects.

Structure Solution and Refinement

The structure was solved by direct methods[1] and expanded using Fourier techniques[2]. The non-hydrogen atoms were refined anisotropically. All hydrogen atoms were observed in a difference electron density map. The hydrogen atom coordinates were refined but their isotropic B's were held fixed. The final cycle of full-matrix least-squares refinement[3] was based on 1767 observed reflections ($I > 2.0\sigma(I)$) and 199 variable parameters and converged (largest parameter shift was 0.02 times its esd) with unweighted and weighted agreement factors of:

$$R = \Sigma||Fo| - |Fc||/\Sigma|Fo| = 0.032$$

$$R_w = \sqrt{(\Sigma w(|Fo| - |Fc|)^2/\Sigma wFo^2)} = 0.033$$

The standard deviation of an observation of unit weight[4] was 1.72. The weighting scheme was based on counting statistics and included a factor (p = 0.020) to downweight the intense reflections. Plots of $\Sigma w(|Fo| - |Fc|)^2$ versus $|Fo|$, reflection order in data collection, sin θ/λ and various classes of indices showed no unusual trends. The maximum and minimum peaks on the final difference Fourier map corresponded to 0.21 and -0.32 $e^-/\AA^3$, respectively.

Neutral atom scattering factors were taken from Cromer and Waber[5]. Anomalous dispersion effects were included in Fcalc[6]; the values for Δf' and Δf" were those of Creagh and McAuley[7]. The values for the mass attenuation coefficients are those of Creagh and Hubbel[8]. All calculations were performed using the teXsan[9] crystallographic software package of Molecular Structure Corporation operating on an in-house IRIS 4D/310VGX computer and the Silicon Graphics Power Challenge computer of the Australian National University Supercomputer Facility.

References

(1) SIR92: Altomare, A., Cascarano, M., Giacovazzo, C., Guagliardi, A., Burla, M.C., Polidori, G. and Camalli, M. (1994). J. Appl. Cryst., 27, 435.

(2) DIRDIF94: Beurskens, P.T., Admiraal, G., Beurskens, G., Bosman, W.P., de Gelder, R., Israel, R. and Smits, J.M.M. (1994). The DIRDIF-94 program system, Technical Report of the Crystallography Laboratory, University of Nijmegen, The Netherlands.

(3) Least-Squares:

Function minimized: $\Sigma w(|Fo| - |Fc|)^2$

where w = $\frac{1}{\sigma^2(Fo)} = [\sigma_c^2(Fo) + \frac{p^2}{4}Fo^2]^{-1}$

$\sigma_c(Fo)$ = e.s.d. based on counting statistics

p = p-factor

(4) Standard deviation of an observation of unit weight:

$$\sqrt{\Sigma w(|Fo| - |Fc|)^2/(No - Nv)}$$

where: No = number of observations

Nv = number of variables

(5) Cromer, D. T. & Waber, J. T.; "International Tables for X-ray Crystallography", Vol. IV, The Kynoch Press, Birmingham, England, Table 2.2 A (1974).

(6) Ibers, J. A. & Hamilton, W. C.; Acta Crystallogr., 17, 781 (1964).

(7) Creagh, D. C. & McAuley, W.J .; "International Tables for Crystallography", Vol C, (A.J.C. Wilson, ed.), Kluwer Academic Publishers, Boston, Table 4.2.6.8, pages 219-222 (1992).

(8) Creagh, D. C. & Hubbell, J.H.; "International Tables for Crystallography", Vol C, (A.J.C. Wilson, ed.), Kluwer Academic Publishers, Boston, Table 4.2.4.3, pages 200-206 (1992).

(9) teXsan: Single Crystal Structure Analysis Software. Version 1.8. Molecular Structure Corporation, 3200 Research Forest Drive, The Woodlands, TX 77381, USA (1997).

EXPERIMENTAL DETAILS

A. Crystal Data

Empirical Formula	$C_{15}H_9Cl_2NO$
Formula Weight	290.15
Crystal Colour, Habit	colourless, plate
Crystal Dimensions	0.37 × 0.23 × 0.08 mm
Crystal System	monoclinic
Lattice Type	Primitive
No. of Reflections Used for Unit Cell Determination (2θ range)	25 (107.6 - 109.5°)
Omega Scan Peak Width at Half-height	0.30°
Lattice Parameters	a = 10.221(3)Å b = 9.528(3) Å c = 13.631(3) Å β = 93.90(2)°
	V = 1324.4(7) Å^3
Space Group	$P2_1/n$ (#14)
Z value	4
D_{calc}	1.455 g/cm^3
F_{000}	592.00
μ(CuKα)	43.23 cm^{-1}

B. Intensity Measurements

Diffractometer	Rigaku AFC6R
Radiation	CuKα (λ = 1.54178 Å) graphite monochromated
Take-off Angle	6.0°
Detector Aperture	7.0 mm horizontal 7.0 mm vertical
Crystal to Detector Distance	400 mm
Voltage, Current	50kV, 180mA
Temperature	-85.0°C
Scan Type	ω-2θ
Scan Rate	16.0°/min (in ω) (up to 4 scans)
Scan Width	(1.20 + 0.30 tan θ)°
$2\theta_{max}$	120.1°
No. of Reflections Measured	Total: 4154 Unique: 1981 (R_{int} = 0.052)
Corrections	Lorentz-polarization Absorption (trans. factors: 0.3987 - 0.7024) Decay (7.09% decline)

C. Structure Solution and Refinement

Structure Solution	Direct Methods (SIR92)
Refinement	Full-matrix least-squares
Function Minimized	$\Sigma w(\|Fo\| - \|Fc\|)^2$
Least Squares Weights	$w = [\sigma_c^2(Fo) + \frac{p^2}{4}Fo^2]^{-1}$
p-factor	0.020
Anomalous Dispersion	All non-hydrogen atoms
No. Observations (I>2.00σ(I))	1767
No. Variables	199
Reflection/Parameter Ratio	8.88
Residuals: R; Rw	0.032 ; 0.033
Goodness of Fit Indicator	1.72
Max Shift/Error in Final Cycle	0.02
Maximum peak in Final Diff. Map	0.21 $e^-/\AA^3$
Minimum peak in Final Diff. Map	-0.32 $e^-/\AA^3$

Table 1. Atomic coordinates and B_{iso}/B_{eq} for $C_{15}H_9Cl_2NO$

atom	x	y	z	B_{eq}
Cl(32)	0.52473(5)	0.29125(6)	0.31278(4)	3.49(1)
Cl(36)	0.93204(5)	0.34547(6)	0.58486(4)	3.34(1)
O(1)	0.7283(1)	-0.0449(2)	0.4604(1)	3.40(3)
N(2)	0.6786(2)	0.0876(2)	0.4839(1)	3.05(4)
C(3)	0.7533(2)	0.1786(2)	0.4418(1)	2.33(4)
C(4)	0.8541(2)	0.1119(2)	0.3901(1)	2.41(4)
C(5)	0.8317(2)	-0.0250(2)	0.4049(2)	3.09(5)
C(31)	0.7241(2)	0.3293(2)	0.4512(1)	2.15(4)
C(32)	0.6198(2)	0.3924(2)	0.3966(1)	2.38(4)
C(33)	0.5903(2)	0.5326(2)	0.4055(1)	2.75(5)
C(34)	0.6662(2)	0.6149(2)	0.4708(2)	3.01(5)
C(35)	0.7710(2)	0.5573(2)	0.5258(1)	2.86(5)
C(36)	0.7990(2)	0.4165(2)	0.5156(1)	2.36(4)
C(41)	0.9579(2)	0.1759(2)	0.3342(1)	2.39(4)
C(42)	0.9349(2)	0.2970(2)	0.2785(1)	2.70(4)
C(43)	1.0312(2)	0.3512(2)	0.2224(2)	2.97(5)
C(44)	1.1520(2)	0.2857(2)	0.2229(2)	3.30(5)
C(45)	1.1765(2)	0.1665(3)	0.2785(2)	3.28(5)
C(46)	1.0801(2)	0.1116(2)	0.3339(1)	2.79(4)
H(5)	0.874(2)	-0.111(3)	0.390(2)	3.7080
H(33)	0.520(2)	0.570(2)	0.372(2)	3.3005
H(34)	0.640(2)	0.711(3)	0.476(2)	3.6166
H(35)	0.822(2)	0.616(3)	0.569(1)	3.4336
H(42)	0.851(2)	0.337(2)	0.277(1)	3.2345

Table 1. Atomic coordinates and B_{iso}/B_{eq} for $C_{15}H_9Cl_2NO$ (continued)

atom	x	y	z	B_{eq}
H(43)	1.009(2)	0.437(2)	0.184(1)	3.5598
H(44)	1.215(2)	0.330(3)	0.184(2)	3.9563
H(45)	1.260(2)	0.124(3)	0.284(2)	3.9343
H(46)	1.096(2)	0.026(3)	0.373(2)	3.3490

$$B_{eq} = \frac{8}{3}\pi^2(U_{11}(aa^*)^2 + U_{22}(bb^*)^2 + U_{33}(cc^*)^2 + 2U_{12}aa^*bb^*\cos\gamma + 2U_{13}aa^*cc^*\cos\beta + 2U_{23}bb^*cc^*\cos\alpha)$$

Table 2. Anisotropic Displacement Parameters for $C_{15}H_9Cl_2NO$

atom	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
Cl(32)	0.0479(3)	0.0454(4)	0.0372(3)	-0.0067(2)	-0.0120(2)	-0.0035(2)
Cl(36)	0.0397(3)	0.0464(3)	0.0393(3)	-0.0018(2)	-0.0096(2)	-0.0016(2)
O(1)	0.0580(9)	0.0252(8)	0.0463(8)	-0.0054(7)	0.0060(7)	0.0010(7)
N(2)	0.049(1)	0.028(1)	0.0390(9)	-0.0049(8)	0.0056(8)	-0.0002(8)
C(3)	0.037(1)	0.027(1)	0.0235(9)	-0.0038(9)	-0.0034(8)	0.0005(9)
C(4)	0.038(1)	0.028(1)	0.0243(9)	0.0008(9)	-0.0024(8)	-0.0012(9)
C(5)	0.050(1)	0.030(1)	0.038(1)	0.002(1)	0.003(1)	-0.003(1)
C(31)	0.031(1)	0.028(1)	0.0234(9)	-0.0026(8)	0.0064(8)	0.0010(8)
C(32)	0.033(1)	0.034(1)	0.0240(9)	-0.0055(9)	0.0031(8)	0.0002(9)
C(33)	0.039(1)	0.033(1)	0.032(1)	0.003(1)	0.0034(9)	0.008(1)
C(34)	0.053(1)	0.026(1)	0.038(1)	0.000(1)	0.012(1)	0.001(1)
C(35)	0.047(1)	0.030(1)	0.032(1)	-0.010(1)	0.0050(9)	-0.0049(9)
C(36)	0.033(1)	0.032(1)	0.0250(9)	-0.0049(8)	0.0031(8)	0.0013(8)
C(41)	0.036(1)	0.031(1)	0.0234(9)	-0.0002(9)	-0.0021(8)	-0.0056(9)
C(42)	0.036(1)	0.034(1)	0.032(1)	0.0028(9)	-0.0003(8)	-0.0017(9)
C(43)	0.046(1)	0.034(1)	0.032(1)	-0.003(1)	0.0016(9)	-0.001(1)
C(44)	0.044(1)	0.043(1)	0.038(1)	-0.009(1)	0.005(1)	-0.008(1)
C(45)	0.035(1)	0.045(1)	0.043(1)	0.000(1)	-0.001(1)	-0.011(1)
C(46)	0.041(1)	0.034(1)	0.030(1)	0.003(1)	-0.0039(9)	-0.005(1)

The general temperature factor expression:

$$\exp(-2\pi^2(a^{*2}U_{11}h^2 + b^{*2}U_{22}k^2 + c^{*2}U_{33}l^2 + 2a^*b^*U_{12}hk + 2a^*c^*U_{13}hl + 2b^*c^*U_{23}kl))$$

Table 3. Bond Lengths (Å) Involving Non-Hydrogen Atoms of $C_{15}H_9Cl_2NO$

atom	atom	distance	atom	atom	distance
Cl(32)	C(32)	1.741(2)	Cl(36)	C(36)	1.739(2)
O(1)	N(2)	1.406(2)	O(1)	C(5)	1.354(2)
N(2)	C(3)	1.313(2)	C(3)	C(4)	1.436(3)
C(3)	C(31)	1.473(3)	C(4)	C(5)	1.343(3)
C(4)	C(41)	1.479(3)	C(31)	C(32)	1.394(3)
C(31)	C(36)	1.398(3)	C(32)	C(33)	1.376(3)
C(33)	C(34)	1.384(3)	C(34)	C(35)	1.378(3)
C(35)	C(36)	1.381(3)	C(41)	C(42)	1.393(3)
C(41)	C(46)	1.391(3)	C(42)	C(43)	1.387(3)
C(43)	C(44)	1.383(3)	C(44)	C(45)	1.379(3)
C(45)	C(46)	1.383(3)			

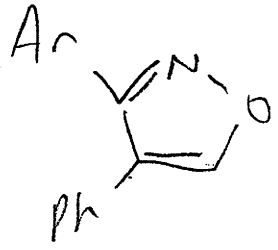


Table 4. Bond Lengths ($\AA$) Involving Hydrogen Atoms of $C_{15}H_9Cl_2NO$

atom	atom	distance	atom	atom	distance
C(5)	H(5)	0.95(2)	C(33)	H(33)	0.90(2)
C(34)	H(34)	0.96(2)	C(35)	H(35)	0.94(2)
C(42)	H(42)	0.94(2)	C(43)	H(43)	0.99(2)
C(44)	H(44)	0.96(2)	C(45)	H(45)	0.94(2)
C(46)	H(46)	0.98(2)			

Table 5. Bond Angles (°) Involving Non-Hydrogen Atoms of $C_{15}H_9Cl_2NO$

atom	atom	atom	angle	atom	atom	atom	angle
N(2)	O(1)	C(5)	108.1(1)	O(1)	N(2)	C(3)	105.3(2)
N(2)	C(3)	C(4)	112.3(2)	N(2)	C(3)	C(31)	118.6(2)
C(4)	C(3)	C(31)	129.1(2)	C(3)	C(4)	C(5)	102.7(2)
C(3)	C(4)	C(41)	129.4(2)	C(5)	C(4)	C(41)	127.9(2)
O(1)	C(5)	C(4)	111.6(2)	C(3)	C(31)	C(32)	121.7(2)
C(3)	C(31)	C(36)	121.9(2)	C(32)	C(31)	C(36)	116.4(2)
Cl(32)	C(32)	C(31)	118.9(2)	Cl(32)	C(32)	C(33)	118.6(2)
C(31)	C(32)	C(33)	122.4(2)	C(32)	C(33)	C(34)	119.3(2)
C(33)	C(34)	C(35)	120.3(2)	C(34)	C(35)	C(36)	119.4(2)
Cl(36)	C(36)	C(31)	118.9(2)	Cl(36)	C(36)	C(35)	118.9(2)
C(31)	C(36)	C(35)	122.2(2)	C(4)	C(41)	C(42)	121.4(2)
C(4)	C(41)	C(46)	119.7(2)	C(42)	C(41)	C(46)	118.8(2)
C(41)	C(42)	C(43)	120.6(2)	C(42)	C(43)	C(44)	119.8(2)
C(43)	C(44)	C(45)	120.2(2)	C(44)	C(45)	C(46)	120.1(2)
C(41)	C(46)	C(45)	120.5(2)				

Table 6. Bond Angles (°) Involving Hydrogen Atoms of $C_{15}H_9Cl_2NO$

atom	atom	atom	angle	atom	atom	atom	angle
O(1)	C(5)	H(5)	113(1)	C(4)	C(5)	H(5)	136(1)
C(32)	C(33)	H(33)	121(2)	C(34)	C(33)	H(33)	119(2)
C(33)	C(34)	H(34)	116(1)	C(35)	C(34)	H(34)	124(1)
C(34)	C(35)	H(35)	119(1)	C(36)	C(35)	H(35)	122(1)
C(41)	C(42)	H(42)	118(1)	C(43)	C(42)	H(42)	121(1)
C(42)	C(43)	H(43)	117(1)	C(44)	C(43)	H(43)	123(1)
C(43)	C(44)	H(44)	116(1)	C(45)	C(44)	H(44)	124(1)
C(44)	C(45)	H(45)	122(1)	C(46)	C(45)	H(45)	118(1)
C(41)	C(46)	H(46)	119(1)	C(45)	C(46)	H(46)	121(1)

Table 7. Torsion Angles (°) Involving Non-Hydrogen Atoms of $C_{15}H_9Cl_2NO$

atom	atom	atom	atom	angle	atom	atom	atom	atom	angle
Cl(32)	C(32)	C(31)	C(3)	-1.8(2)	Cl(32)	C(32)	C(31)	C(36)	178.4(1)
Cl(32)	C(32)	C(33)	C(34)	-179.1(1)	Cl(36)	C(36)	C(31)	C(3)	1.1(2)
Cl(36)	C(36)	C(31)	C(32)	-179.1(1)	Cl(36)	C(36)	C(35)	C(34)	179.6(1)
O(1)	N(2)	C(3)	C(4)	-0.3(2)	O(1)	N(2)	C(3)	C(31)	178.7(2)
O(1)	C(5)	C(4)	C(3)	-0.3(2)	O(1)	C(5)	C(4)	C(41)	179.5(2)
N(2)	O(1)	C(5)	C(4)	0.1(2)	N(2)	C(3)	C(4)	C(5)	0.3(2)
N(2)	C(3)	C(4)	C(41)	-179.5(2)	N(2)	C(3)	C(31)	C(32)	-76.1(2)
N(2)	C(3)	C(31)	C(36)	103.6(2)	C(3)	N(2)	O(1)	C(5)	0.1(2)
C(3)	C(4)	C(41)	C(42)	-36.5(3)	C(3)	C(4)	C(41)	C(46)	145.9(2)
C(3)	C(31)	C(32)	C(33)	179.2(2)	C(3)	C(31)	C(36)	C(35)	-179.0(2)
C(4)	C(3)	C(31)	C(32)	102.7(2)	C(4)	C(3)	C(31)	C(36)	-77.5(3)
C(4)	C(41)	C(42)	C(43)	-176.5(2)	C(4)	C(41)	C(46)	C(45)	177.1(2)
C(5)	C(4)	C(3)	C(31)	-178.5(2)	C(5)	C(4)	C(41)	C(42)	143.7(2)
C(5)	C(4)	C(41)	C(46)	-33.8(3)	C(31)	C(3)	C(4)	C(41)	1.6(3)
C(31)	C(32)	C(33)	C(34)	0.0(3)	C(31)	C(36)	C(35)	C(34)	-0.3(3)
C(32)	C(31)	C(36)	C(35)	0.8(3)	C(32)	C(33)	C(34)	C(35)	0.5(3)
C(33)	C(32)	C(31)	C(36)	-0.7(3)	C(33)	C(34)	C(35)	C(36)	-0.4(3)
C(41)	C(42)	C(43)	C(44)	-1.0(3)	C(41)	C(46)	C(45)	C(44)	-0.1(3)
C(42)	C(41)	C(46)	C(45)	-0.6(3)	C(42)	C(43)	C(44)	C(45)	0.4(3)
C(43)	C(42)	C(41)	C(46)	1.1(3)	C(43)	C(44)	C(45)	C(46)	0.2(3)

Table 8. Non-bonded Contacts out to 3.60 $\AA$ Involving Non-Hydrogen Atoms of $C_{15}H_9Cl_2NO$

atom	atom	distance	ADC	atom	atom	distance	ADC
Cl(32)	Cl(36)	3.4418(7)	45404	O(1)	C(34)	3.309(3)	54501
O(1)	C(46)	3.370(2)	75603	N(2)	C(44)	3.502(3)	45504
C(32)	C(34)	3.541(3)	66603	C(33)	C(33)	3.328(4)	66603
C(33)	C(34)	3.506(3)	66603	C(33)	C(41)	3.545(3)	65502
C(33)	C(42)	3.553(3)	65502				

The ADC (atom designator code) specifies the position of an atom in a crystal. The 5-digit number shown in the table is a composite of three one-digit numbers and one two-digit number: TA (first digit) + TB (second digit) + TC (third digit) + SN (last two digits). TA, TB and TC are the crystal lattice translation digits along cell edges a, b and c. A translation digit of 5 indicates the origin unit cell. If TA = 4, this indicates a translation of one unit cell length along the a-axis in the negative direction. Each translation digit can range in value from 1 to 9 and thus ± 4 lattice translations from the origin (TA=5, TB=5, TC=5) can be represented.

The SN, or symmetry operator number, refers to the number of the symmetry operator used to generate the coordinates of the target atom. A list of symmetry operators relevant to this structure are given below.

For a given intermolecular contact, the first atom (origin atom) is located in the origin unit cell and its position can be generated using the identity operator (SN=1). Thus, the ADC for an origin atom is always 55501. The position of the second atom (target atom) can be generated using the ADC and the coordinates of the atom in the parameter table. For example, an ADC of 47502 refers to the target atom moved through symmetry operator two, then translated -1 cell translations along the a axis, +2 cell translations along the b axis, and 0 cell translations along the c axis.

An ADC of 1 indicates an intermolecular contact between two fragments (eg. cation and anion) that reside in the same asymmetric unit.

Symmetry Operators:

(1)	X,	Y,	Z	(2)	1/2-X,	1/2+Y,	1/2-Z
(3)	-X,	-Y,	-Z	(4)	1/2+X,	1/2-Y,	1/2+Z

Table 9. Least Squares Planes for $C_{15}H_9Cl_2NO$

Plane number 1

Atoms defining plane	Distance
O(1)	0.000(2)
N(2)	0.001(2)
C(3)	-0.002(2)
C(4)	0.002(2)
C(5)	-0.001(2)
Additional Atoms	**Distance**
C(31)	-0.029
C(41)	0.011
H(5)	0.057

Plane number 2

Atoms defining plane	Distance
C(31)	-0.004(2)
C(32)	0.002(2)
C(33)	0.002(2)
C(34)	-0.004(2)
C(35)	0.000(2)
C(36)	0.003(2)
Additional Atoms	**Distance**
Cl(32)	0.035
Cl(36)	0.020
C(3)	-0.023
H(33)	-0.040
H(34)	-0.036
H(35)	0.008

Plane number 3

Atoms defining plane	Distance
C(41)	-0.004(2)
C(42)	0.006(2)
C(43)	-0.003(2)
C(44)	-0.001(2)
C(45)	0.003(2)
C(46)	0.000(2)

Additional Atoms	Distance
C(4)	-0.072
H(42)	-0.040
H(43)	-0.010
H(44)	0.020
H(45)	0.072
H(46)	-0.005

Summary

plane	mean deviation	χ^2
1	0.0012	2.2
2	0.0027	18.2
3	0.0029	16.9

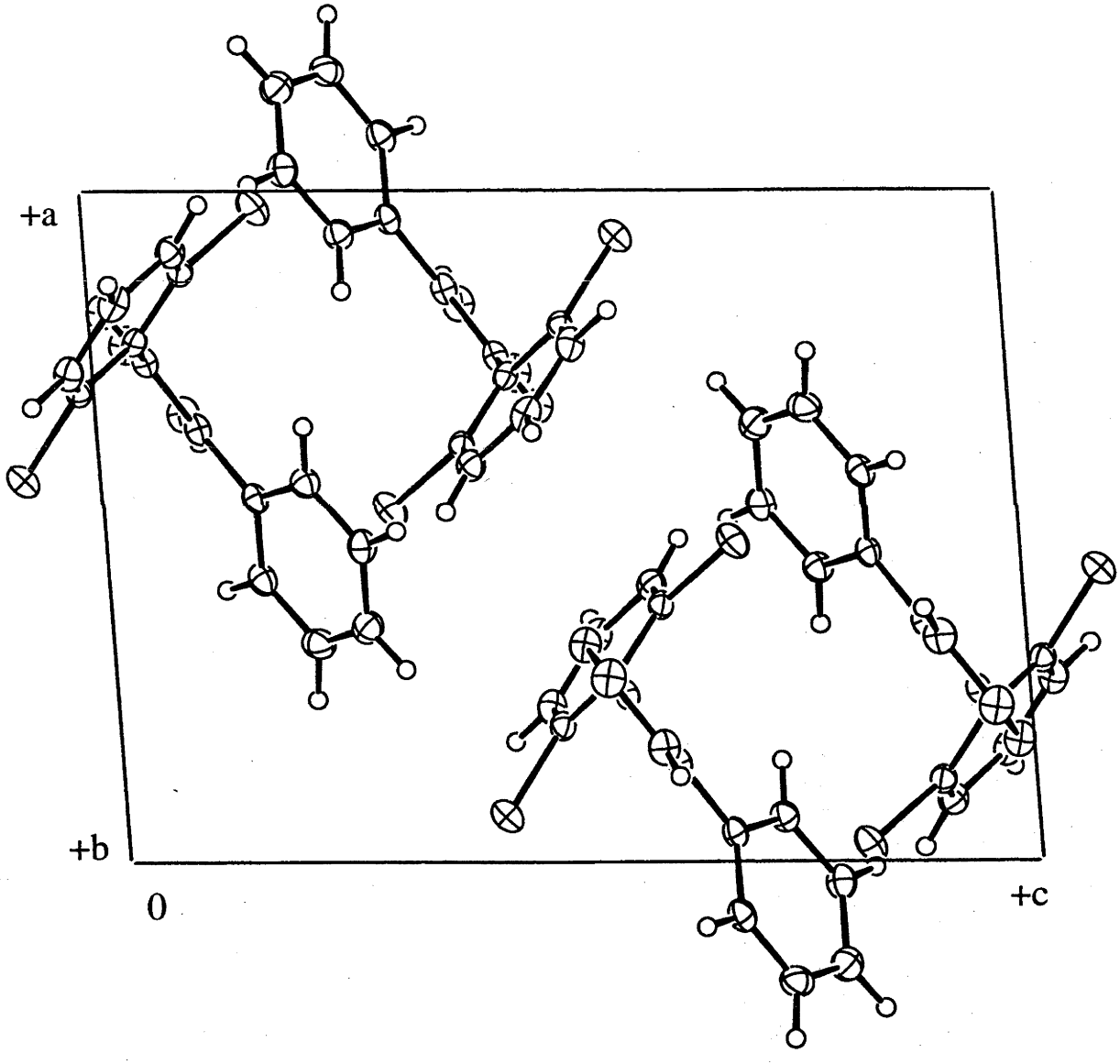
Dihedral angles between planes (°)

plane	1	2
2	103.28	
3	35.29	104.85

Figure Captions for $C_{15}H_9Cl_2NO$

Figure 1. Thermal ellipsoid diagram of $C_{15}H_9Cl_2NO$ with selected atom labelling. Ellipsoids show 30% probability levels. Hydrogen atoms are drawn as circles with small radii.

Figure 2. Unit cell diagram for $C_{15}H_9Cl_2NO$ projected down the b axis. Ellipsoids show 30% probability levels. Hydrogen atoms are drawn as circles with small radii.


+a
+b
0
+c