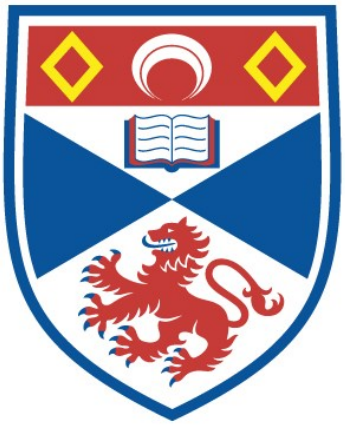
N-OXIDES FROM SUBSTITUTED O-NITROANILINES

Colin Stuart French

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



1998

N-Oxides From Substituted *o*-Nitroanilines

A thesis by

Colin Stuart French

Submitted for the degree of Doctor of Philosophy in the University of St Andrews

March 1998

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Declaration

I, Colin Stuart French, hereby certify that this thesis has been composed by me, that it is an accurate representation of the work undertaken by me in the University of St Andrews since my admission as a research student in October 1994, and that it has not been accepted in any previous application for any higher degree or professional qualification.

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I hereby certify that Colin Stuart French has fulfilled the regulations appropriate to the degree of Ph.D.

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Dedication

This thesis is dedicated to my parents, to my gran, and to my nephew Stephen.

Contents

Acknowledgements

My gratitude is extended to several people. Firstly, to my boss, Dr David Smith, for his special brand of encouragement, supervision and friendship over the last few years.

Secondly, to the E.P.S.R.C. for the funding of this project, and also for funds which enabled me to attend the 16th I.S.H.C. International Congress on Heterocyclic Chemistry in Bozeman, Montana in 1997. Thanks also to the technical staff of the School of Chemistry: Mrs Melanja Smith for lots of bubbly advice on NMR spectroscopy, Mrs Sylvia Williamson for microanalyses and Mr Colin Millar for mass spectra. I would like to thank Mandy Parsons for carrying out some preliminary experiments (as detailed in Chapter Five) carried out under my supervision for her Senior Honours Project in 1997.

On a more personal note, many many thanks are due to some close friends: Dr Iain Greig, who put up with me for three years in the same flat, and took so much time to dash up mountains, drink malt whisky and race round Europe with me; Arwel Lewis, who also did the above (although he doesn't like whisky, and managed to wriggle out of a particularly ghastly train journey across Bulgaria), and on whom I took out my many frustrations on the squash court; Nicola Davidson and Doug Short for a splendid environment in which to spend my last six months. Special thanks to Neil Anderson for an enlightening friendship, who also has had to put up with my many frustrations, and without whom I would probably be a gibbering wreck. I am also indebted to Arwel and to Nicola for the use of their computers for the production of this thesis - thank you!

Members of my research group also deserve my thanks for a lighthearted atmosphere in the lab, especially the banter of Rick White, Dr András Kotschy (who also very kindly put me up in Budapest), Karen Muirhead, Colin Morton and Simon Martyr.

Finally, I would like to thank my parents for putting up with me for so long.

Abstract

The aim of this project was to investigate the cyclisation reactions of some *o*-nitroaryl derivatives of α-amino acids, with a view to the synthesis of potentially biologically active heterocyclic compounds.

Chapter One is concerned with an overview of the synthesis of heterocyclic *N*-oxides, mainly *via* the cyclisations of *ortho*-substituted nitroaromatics. Firstly, the properties of heterocyclic *N*-oxides are considered, then both reductive and non-reductive methods of their synthesis by cyclisation reactions are explored. After a discussion of intramolecular condensations leading to cyclisation, the possibilities for alternative mechanisms for these reactions are deliberated.

Chapter Two begins with an introduction to the specific cyclisation reactions of *o*-nitroaryl- and 2,4-dinitrophenyl-amino acids. The preparation and cyclisation reactions of *o*-nitrophenyl derivatives of α-amino acid esters are then described with emphasis on the implications for the mechanism of these cyclisation reactions.

Chapter Three discusses the related cyclisations of *N*-alkyl-*o*-nitroanilines which have no activating group (for example, an ester) on the *N*-alkyl chain. The mechanistic implications of this are explored, in the context of the cyclisations discussed in Chapter Two.

In Chapter Four, the synthesis of benzimidazole and quinoxaline acyclic nucleoside analogues is briefly described. These have the potential to be biologically active compounds.

CHAPTER ONE

Introduction

1.1 General Properties of Heterocyclic *N*-oxides

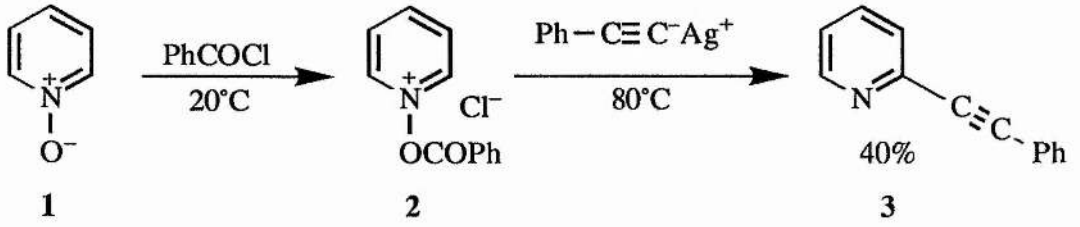
Most of the *N*-oxides described in later chapters of this thesis are those of substituted benzimidazoles; it seems appropriate, however, first to consider in outline some general characteristics of the *N*-oxide as a functional group.

The simplest tertiary amine *N*-oxide, trimethylamine *N*-oxide, is commercially available and used as an oxidising reagent, for example in the oxidation of alkyl halides to aldehydes[1], and the oxidation of alkylboranes to alcohols[2]. However, this introduction is concerned mainly with heteroaromatic rather than aliphatic *N*-oxides.

A comparison of *N*-oxides and their parent heterocycles generally shows a marked difference in their properties. The polarity of the N–O bond imparts a greater dipole moment to the *N*-oxide, although the difference in dipole moment is less than the difference between the dipole moments of aliphatic amines and their *N*-oxides. For example, the dipole moments (μ) of triethylamine and its *N*-oxide are 0.65 D and 5.02 D respectively, and those of pyridine and its *N*-oxide are 2.20 D and 4.24 D respectively. The N–O bond is comparatively unstable, and so reduction is a relatively straightforward process (for instance, by the use of catalytic hydrogenation over Raney nickel, or phosphorus trichloride), although the heteroaromatic oxides are more resistant to reduction than their aliphatic counterparts because of the resonance effects involving the oxygen lone pair of electrons and the aromatic π-system.

Interestingly, at the same time as supplying electrons to the ring, the oxygen also exerts a strong electron-withdrawing inductive effect on the ring, thereby increasing the reactivity of the ring to nucleophilic attack[3]. Pyridine *N*-oxide has increased reactivity towards electrophilic substitution relative to pyridine itself - substitution occurs mainly in the 4-position[3], whereas in pyridine itself, electrophilic substitution occurs (albeit with difficulty) in the 3-position. The *N*-oxide of pyridine (**1**) can also be used as an auxiliary

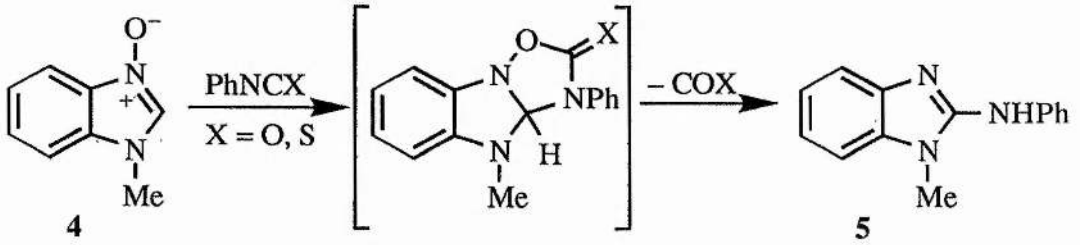
for promoting nucleophilic substitution at the 2- or 4-position; nucleophilic attack at the 2-position is facilitated by the formation of the *O*-benzoyl derivative (**2**), where elimination follows addition and the *N*-benzoate moiety becomes a leaving group. For example, reaction of this *O*-benzoyl derivative with silver phenylacetylide (as a carbanionic nucleophile) gives 1-phenyl-2-(2-pyridyl)acetylene (**3**) (Scheme 1.1)[4].



1 **2** **3**

Scheme 1.1

N-Oxides can also undergo 1,3-dipolar cycloadditions[5]. With 1-methylbenzimidazole 3-oxide (**4**), the adducts with isocyanates and isothiocyanates are unstable and decompose to the 2-substituted benzimidazoles (**5**) (Scheme 1.2).

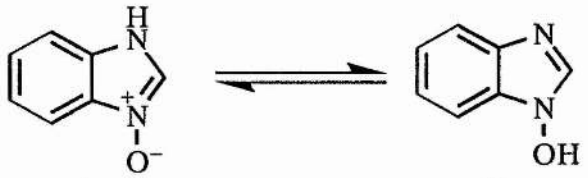


4 **5**

Scheme 1.2

Tautomerism is a factor to be considered when contemplating the structure of heterocyclic *N*-oxides. For example, 1-unsubstituted benzimidazole *N*-oxides are tautomeric with *N*-hydroxybenzimidazoles; the position of the equilibrium is solvent-dependent[6]. With benzimidazole *N*-oxide, the predominant tautomer in aqueous solution is the *N*-oxide form (**6**), but as the polarity of the solvent decreases, the predominance of the *N*-hydroxy form (**7**) increases[7]. The situation is similar with 2-hydroxypyridine *N*-oxide (**8**) (1-hydroxy-2-pyridone) - in this case, each tautomer is strongly intramolecularly

hydrogen bonded and the evidence (such as infra-red and ultra-violet spectroscopy) suggests that it exists mainly as 1-hydroxy-2-pyridone (**9**) in aqueous solution[8]. In the literature, the *N*-oxide and *N*-hydroxy forms are used more or less interchangeably, and the reader should be aware of the equilibria (Scheme 1.3) with the alternative tautomeric structures[9].



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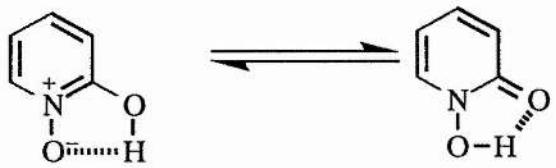

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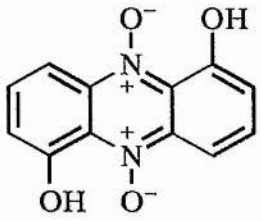
Scheme 1.3

1.2 Biological and Pharmaceutical Applications

There is much potential for the biological and therapeutic application of heterocyclic *N*-oxides - naturally-occurring examples of these include iodinin (**10**) and aspergillic acid (**11**) below. Iodinin[10,11] was isolated from *Chromobacterium iodinum* in 1938[12], and was found to possess antibacterial activity. Aspergillic acid[13] was isolated in 1943[14], and although this was found to be so toxic as to preclude its therapeutic use as an antibiotic, it is nevertheless an example of a biologically active heterocyclic *N*-oxide, and as such is a useful model for drug (or biocide) design. In some instances, the *N*-oxide can have a far greater therapeutic effect than the parent heterocycle. For example, (**13**) has a much greater selective affinity than (**12**) for the AMPA (α-amino-3-hydroxy-5-methylisoxazole-4-propionate) receptor[15], which is of great importance for the treatment of neurological conditions such as Parkinson's, Huntingdon's and Alzheimer's

diseases in which the excessive stimulation of excitatory amino acid receptors such as the AMPA receptor is involved.



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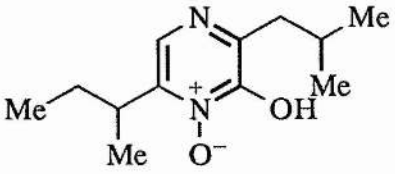

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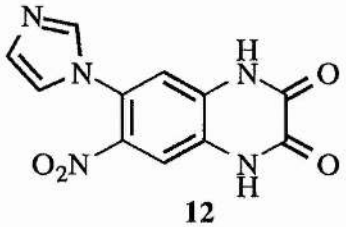

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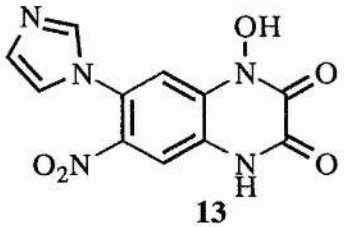


13

2,5,6-Trisubstituted benzimidazole *N*-oxides are known to be antihelmintic agents[16] (helminthiasis is the infestation of the gastrointestinal tract with helmintic parasitic worms), and 5,6-dimethylbenzimidazole is of interest since it was discovered to be a constituent part of vitamin B_{12}[17].

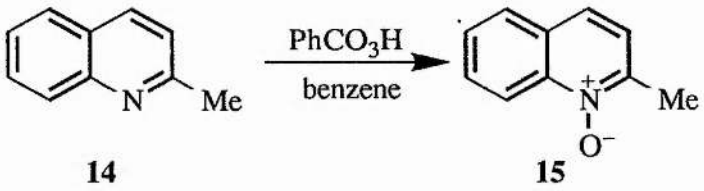
1.3 Synthetic Strategies

The synthesis of heterocyclic *N*-oxides has been the target of much research for many decades; various synthetic strategies are considered below.

1.3.1 Direct *N*-oxidation

The most obvious route to such *N*-oxides would appear to be the direct oxidation of the parent heterocycles by reaction with (for example) hydrogen peroxide in warm glacial acetic acid, or with a peroxyacid such as peroxybenzoic acid or monoperoxyphthalic

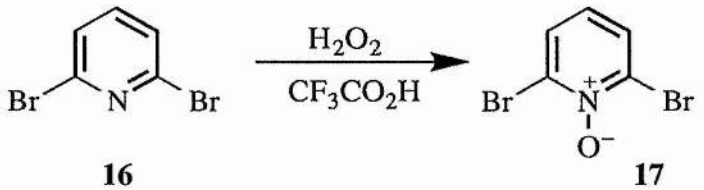
acid[3]. For example, quinaldine (**14**) is oxidised in "very good" yield to quinaldine *N*-oxide (**15**) in benzene with peroxybenzoic acid at room temperature (Scheme 1.4)[18].



14 **15**

Scheme 1.4

Peroxytrifluoroacetic acid has been used for those heterocycles which are more weakly nucleophilic and therefore more resistant to oxidation; for instance 2,6-dibromopyridine (**16**) fails to oxidise with peroxybenzoic or peroxyacetic acids (its reduced nucleophilicity is due to the negative inductive and steric effects of the two flanking bromine atoms), but the oxidation to (**17**) proceeds in good yield with 30% hydrogen peroxide in warm trifluoroacetic acid (Scheme 1.5)[19].

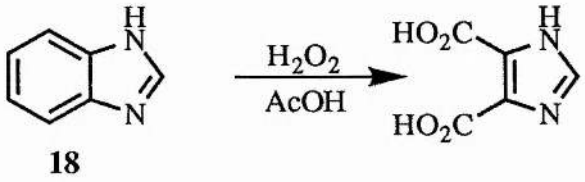


16 **17**

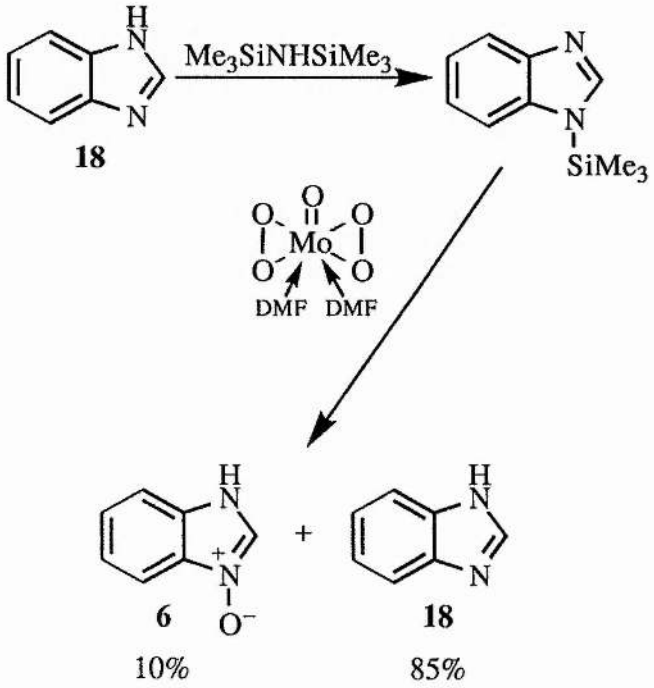
Scheme 1.5

However, benzimidazole (**18**) is resistant to oxidation by this method[20] - reaction with hydrogen peroxide in warm glacial acetic acid leads to cleavage of the benzene ring (Scheme 1.6)[21]. *N*-Methylbenzimidazole is oxidised (with hydrogen peroxide in acetic acid) to *N*-methylbenzimidazolone[22], so again, the *N*-oxide is inaccessible by direct oxidation. Only one direct oxidation of benzimidazole (after trimethylsilylation using hexamethyldisilazane) has been found in the literature, utilising a molybdenum complex in *N*,*N*-dimethylformamide (Scheme 1.7)[23], but this gives only a 10% yield of the *N*-oxide (**6**), and so cannot be regarded as a viable synthetic route; no later modifications of this procedure have been reported to date. In fact, benzimidazole *N*-oxide is even

known to be deoxygenated under apparently oxidising conditions (formic acid and hydrogen peroxide)[24], although this is probably due to the reducing properties of formic acid predominating over the oxidising power of peroxyformic acid in this instance.



Scheme 1.6

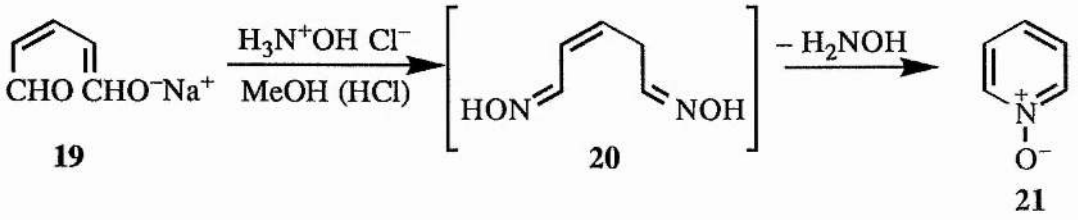


Scheme 1.7

One must therefore turn to another route in order to prepare benzimidazole *N*-oxides - *i.e.* cyclisation of an appropriately substituted benzene derivative (see section 1.5.2, below). This cyclisation strategy also has applications for the synthesis of many other benzo-fused heterocyclic *N*-oxides, and may indeed be more generally useful as a route to otherwise inaccessible systems. The following sections will therefore consider the synthetic utility of cyclisation reactions leading to heterocyclic *N*-oxides in general.

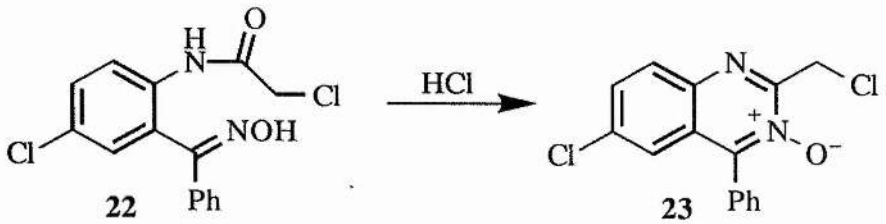
1.3.2 Hydroxylamine reactions

There are several classes of cyclisation reactions which lead to heterocyclic *N*-oxides. The cyclisations of hydroxylamine derivatives are one such class. For example, when glutaconic aldehyde (as its anion, **19**) and hydroxylamine are heated in acidic methanol, pyridine *N*-oxide (**21**) is formed *via* the dioxime (**20**) (Scheme 1.8)[25].



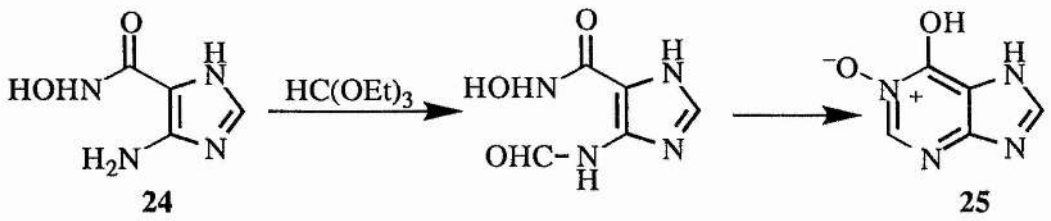
Scheme 1.8

More simply, direct nucleophilic attack of a hydroxylamine group on a carbonyl group (for example, the dichloroacetanilide **22**), followed by dehydration, will lead to an *N*-oxide (in this case, **23**) (Scheme 1.9)[26].



Scheme 1.9

The purine *N*-oxide (**25**) has been synthesised by the intramolecular nucleophilic attack of a hydroxylamino group on the carbonyl carbon of a formamide, which is obtained by the intermolecular formylation of the amine (**24**) with triethyl orthoformate (Scheme 1.10)[27].



24 25

Scheme 1.10

1.4 Use of the Nitro Group

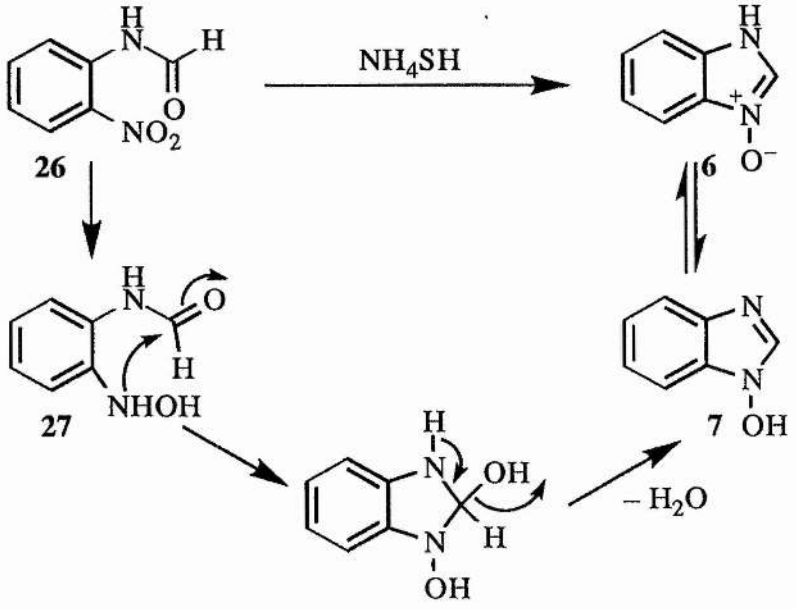
In aromatic chemistry, the use of the nitro group as a precursor to other functional groups such as NO, NH_2, NHOH, -N=N- etc, is well authenticated[28], as is its use both as an activating group[29] and as a leaving group[30,31] in aromatic nucleophilic substitution. However, chemistry of the nitro group as a functional group in its own right is not particularly extensive unless the functional group transformation involves intramolecular interactions of the nitro group with (for example) *ortho*-substituents on a benzene ring.

There are numerous types of intramolecular transformations involving nitro groups, most of which lead to cyclisation. These reactions have been reviewed twice in the literature, in 1964[32] and 1972.[33] The purpose of this section is to collect together some of the work in this area, with a view to a mechanistic consideration of these interactions. The emphasis will be mainly on the reactions of *N*-substituted *o*-nitroanilines with base, although some photolytic, thermolytic and reductive processes will be considered, along with the transformations of some other *ortho*-substituted nitrobenzenes.

1.4.1 Reductive Transformations

The first synthesis of benzimidazole *N*-oxide (**6**) was carried out by the partial reduction of *o*-nitroformanilide (**26**) with ammonium sulphide (Scheme 1.11) by von Niementowski[34]. The assumption is that the reduction of the nitro group proceeds as far

as the hydroxylamine (**27**); there then follow nucleophilic attack of the latter on the carbonyl group, and a final dehydration.

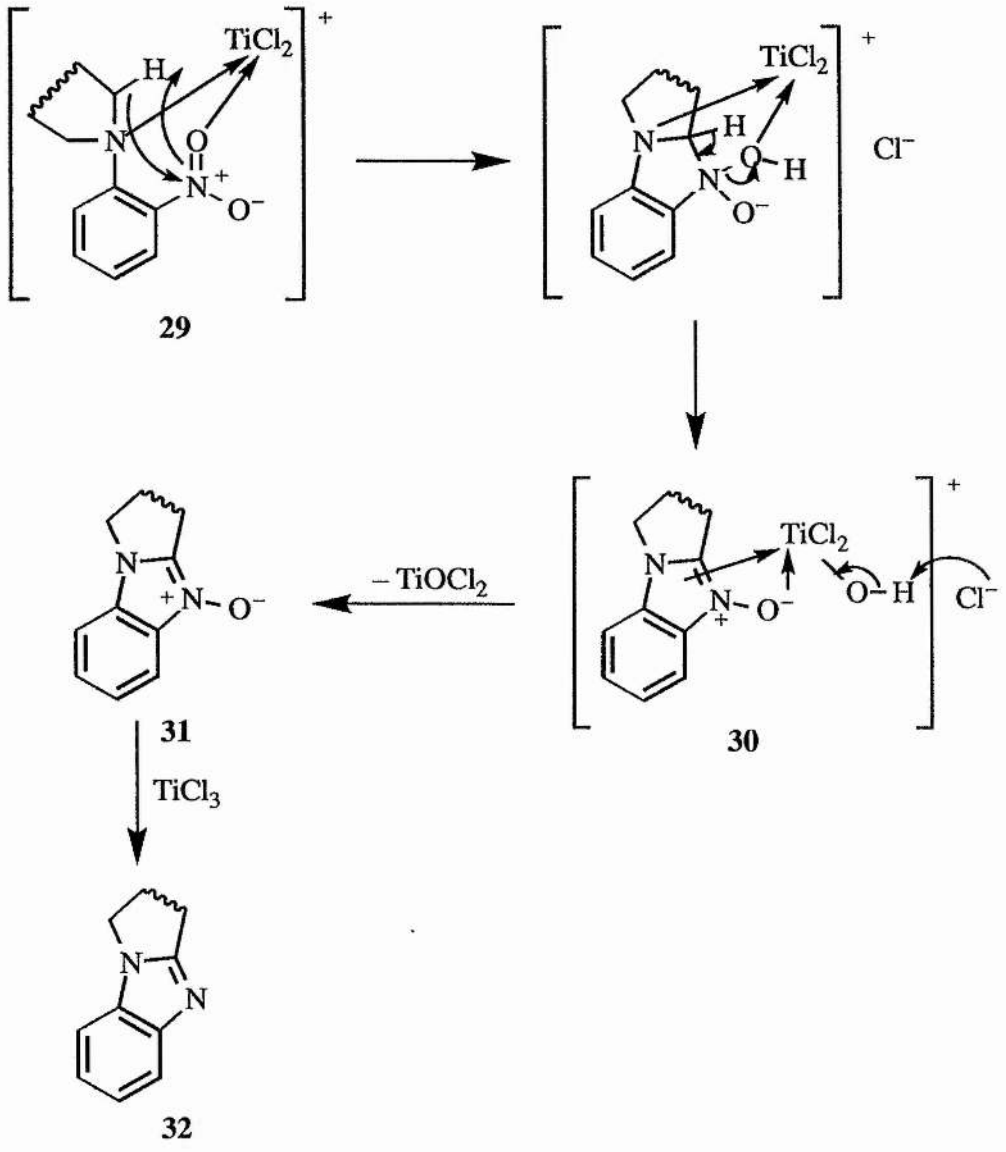


Scheme 1.11

Tricyclic benzimidazole *N*-oxides (**31**) have also been synthesised by the reductive cyclisation of aromatic nitro compounds (**29**) using titanous chloride ($TiCl_3$) in hot acid solution[35]. When an extra molar equivalent of reducing agent is used, the *N*-oxide undergoes deoxygenation and the parent benzimidazole (**32**) is recovered (Scheme 1.12). It was originally suggested[36] that the function of the reducing agent was to furnish the nitroso compound for the cyclisation to occur. However, when the corresponding amine is oxidised specifically to the nitroso compound (using Caro's acid[37,38], H_2SO_5), cyclisation to the benzimidazole fails. The reaction is more likely to proceed through the π-complexed intermediate (**30**), with the titanous chloride acting both as a chelating agent to align the interacting groups favourably, and as a reducing agent in the final step (this reduction was demonstrated separately to consume one mole of reducing agent).

One inherent problem with reductive cyclisations for the synthesis of *N*-oxides in general is in ensuring that reduction of the nitro group does not go beyond the level of the

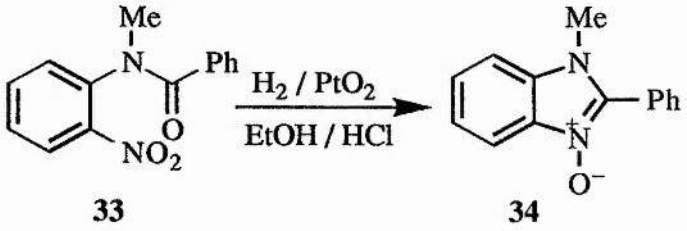
hydroxylamine to give the primary amine, or indeed that further reduction of the *N*-oxide does not take place. The difficulty involved in controlling the level of reduction tends to mean either that *N*-oxide yields are lower, or that mixtures of products are obtained.



Scheme 1.12

However, various methodologies have been employed to give fairly reasonable yields[9]. Catalytic hydrogenation of an appropriate starting material over platinum or palladium in the presence of at least one equivalent of acid (usually HCl) gives the desired benzimidazole *N*-oxide. For example, reaction of *N*-methyl-2-nitrobenzanilide (**33**) with

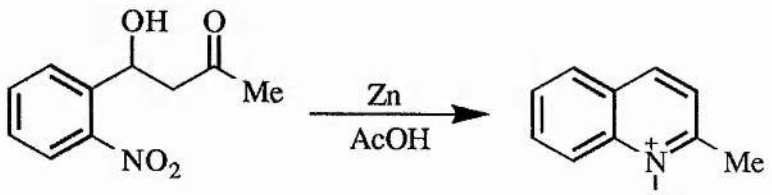
hydrogen over platinum oxide in ethanolic hydrogen chloride furnishes 1-methyl-2-phenylbenzimidazole 3-oxide (**34**) (Scheme 1.13) in 50% overall yield[6]. Absence of the acid (or its presence in only catalytic amount) leads to the complete reduction of the nitro group to the *o*-aminoanilide and recovery of some of the parent benzimidazole. When the oxygen is protonated, attack by the hydroxylamino group on the carbonyl group is presumably more favourable than further reduction to the amine.



33 **34**

Scheme 1.13

Some examples of other heterocycles prepared by reductive processes involving nitro groups are outlined below. The synthesis of quinaldine *N*-oxide (**15**) by the direct oxidation of quinaldine has already been referred to (Section 1.3.1); its synthesis by the reduction (with zinc in acetic acid) of 4-hydroxy-4-(*o*-nitrophenyl)butan-2-one (**35**) has also been described[39] (Scheme 1.14), although the yield is not specified, and the correct structure for the product was not established until later[19].

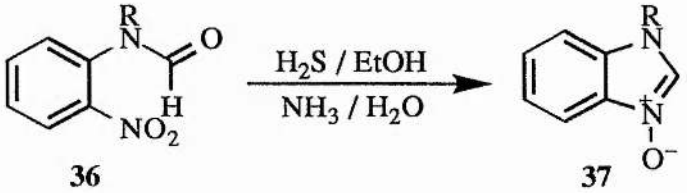


35 **15**

Scheme 1.14

Because quinaldine *N*-oxides are accessible by direct oxidation, this type of reductive cyclisation is of little practical use, but for benzo-fused azole *N*-oxides, which are generally not accessible by direct oxidation, cyclisation becomes the only route possible.

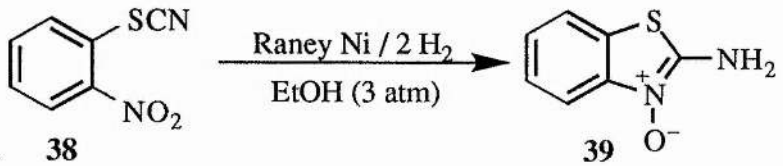
1-Substituted benzimidazole 3-oxides (**37**) can be prepared by reductive cyclisation of the appropriate formanilide derivative (**36**) with hydrogen sulphide in ethanolic aqueous ammonia at room temperature (Scheme 1.15)[40] in a manner similar to that of von Niementowski[34].



36 **37**

Scheme 1.15

Sulphur-containing heterocycles are accessible by reductive cyclisation methods. Benzothiazole *N*-oxides (**39**) have been prepared by the reduction of *o*-nitrophenyl thiocyanate (**38**) with Raney nickel and 2 equivalents of hydrogen (Scheme 1.16)[41]. In this case, the product is the 2-amino substituted heterocycle, which arises from the nucleophilic attack of the hydroxylamine intermediate on the thiocyanate carbon.

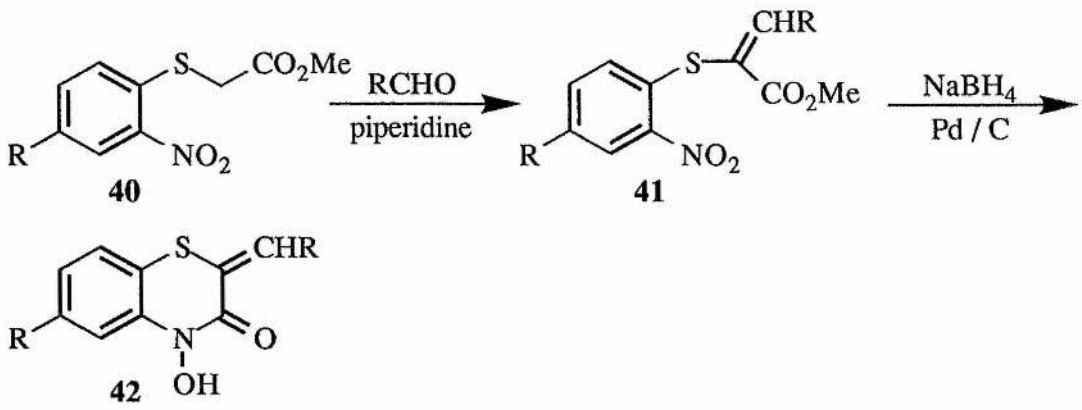


38 **39**

75-88%

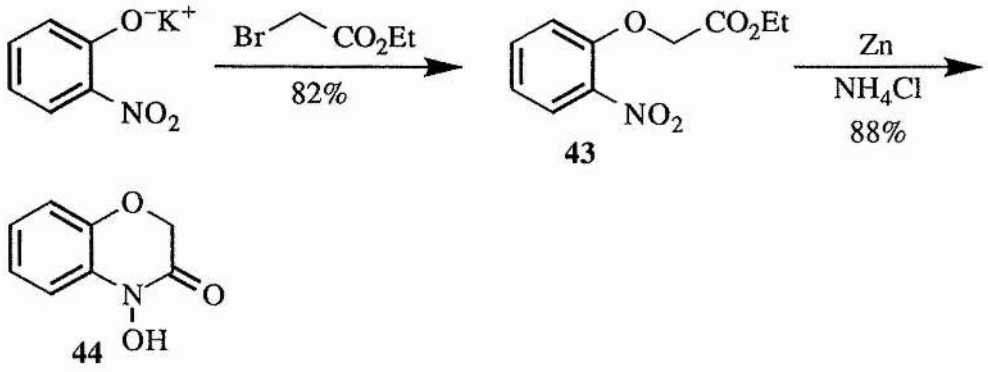
Scheme 1.16

Benzothiazine *N*-oxides (**42**) are obtained by the catalytic transfer hydrogenation of the adduct (**41**) of the thioacetic acid derivative (**40**) with an aldehyde using sodium borohydride and palladium/charcoal (Scheme 1.17)[42].



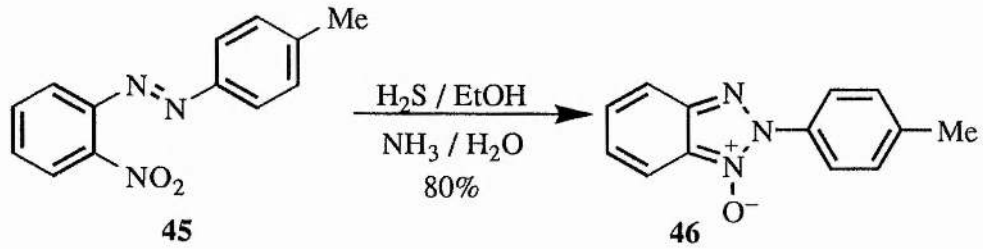
Scheme 1.17

An example of an oxygen-containing heterocycle synthesised by reductive cyclisation is the 4-hydroxybenzoxazin-3-one (**44**) (Scheme 1.18)[43], produced from the reaction of ethyl *o*-nitrophenoxyacetate (**43**) with zinc and ammonium chloride.



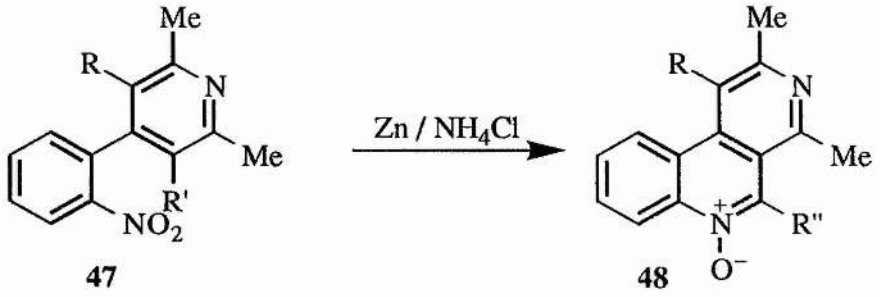
Scheme 1.18

Benzotriazole 1-oxides (**46**) are accessible from azobenzene derivatives (**45**) - again, by reduction with hydrogen sulphide in ethanolic aqueous ammonia (Scheme 1.19)[44,45].



Scheme 1.19

Cyclisations can also be effected between *ortho*-nitro groups and *ortho'*-substituents in an adjacent ring - for example in the 2-nitro-2'-substituted biaryls (**47**) shown in Scheme 1.20[46], in which the reduction to the pyrido[3,4-*c*]quinoline 6-oxide (**48**) is effected by zinc and ammonium chloride in aqueous ethanol, although the yields in most cases are relatively low (25-35%).



47 **48**

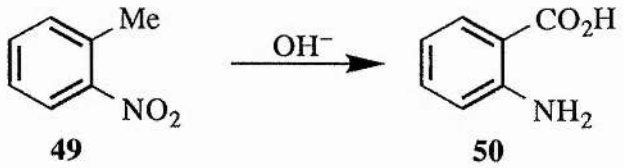
R = CO_2Et, CN, H, COMe
R' = CO_2Et, CN, COMe

R" = OH, Me, NH_2

Scheme 1.20

1.4.2 Intramolecular Redox Processes

Some examples of this type of transformation do not actually involve cyclisation, but illustrate the process of simultaneous oxidation and reduction of *ortho*-substituted side chains. For example, under alkaline conditions (potassium hydroxide) and with heat, *o*-nitrotoluene (**49**) is converted into anthranilic acid (**50**) (Scheme 1.21)[47,48].

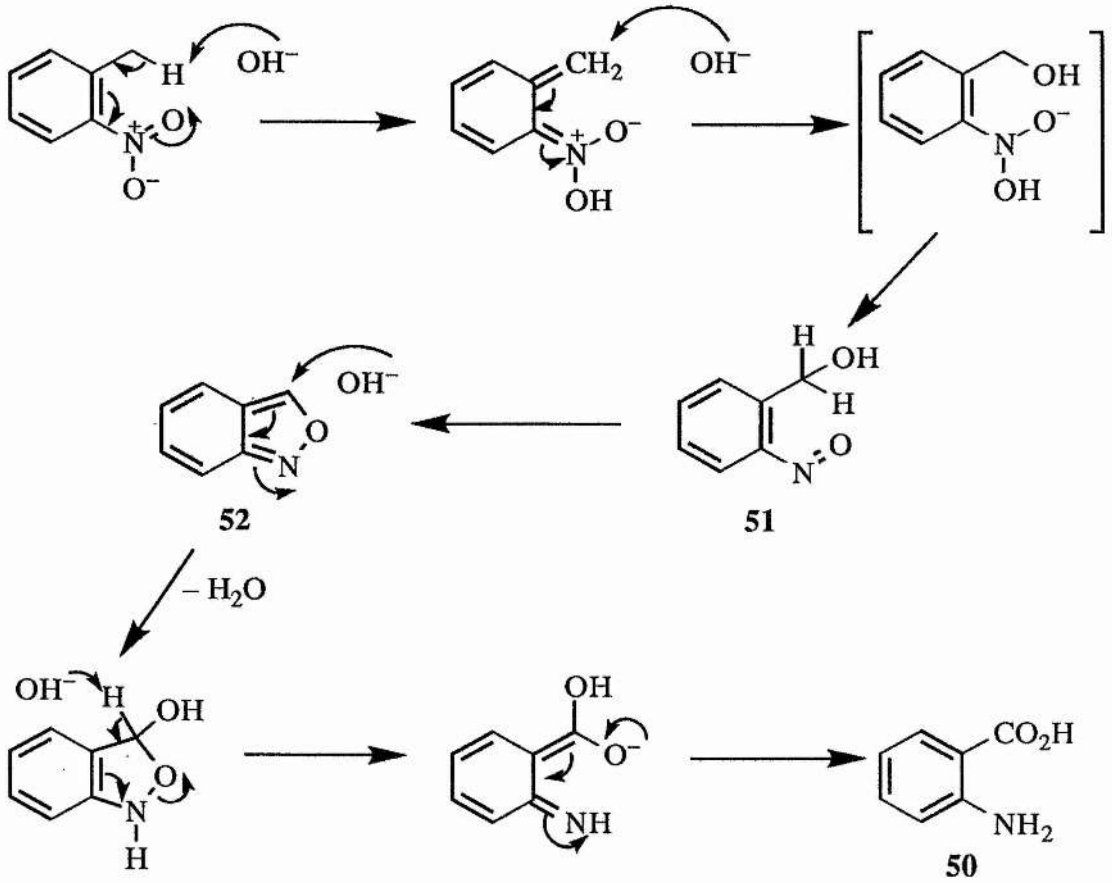


49 **50**

Scheme 1.21

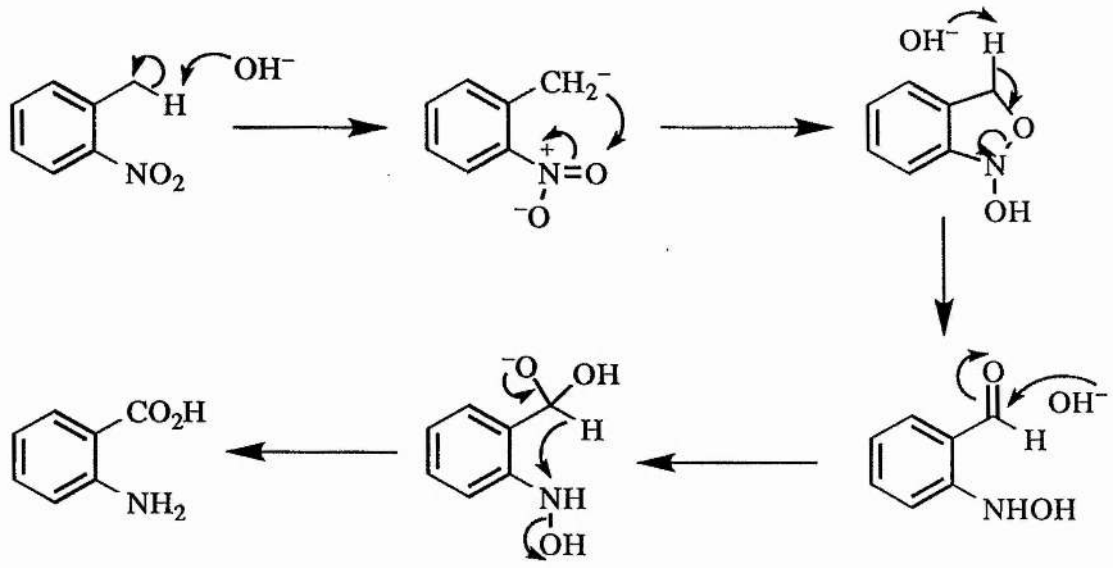
A plausible mechanism for this would appear to involve the stepwise reduction of the nitro group with concomitant oxidation of the methyl group, catalysed by OH^-. Kukhtenko proved by ^{18}O studies[49] (using $K^{18}OH$ and $H_2{}^{18}O$) that one oxygen in the

CO_2H group comes from the nitro group, and the other from the reaction medium. The mechanism for this transformation proposed by Scholl in 1913[50] was later extended by Loudon and Tennant[32] and proceeds to anthranilic acid (**50**) *via* *o*-nitrosobenzyl alcohol (**51**) and then anthranil (**52**) (Scheme 1.22).



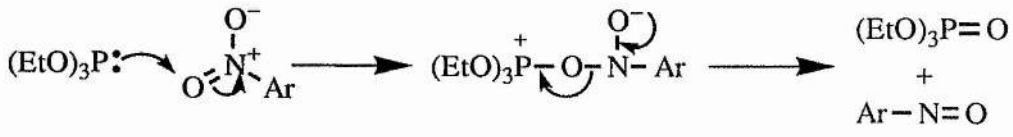
Scheme 1.22

The ^{18}O studies could also be accommodated by the mechanism outlined in Scheme 1.23, where the oxygen of the nitro group undergoes nucleophilic attack by the initial *o*-nitrophenylmethylene anion. The final step involves a hydride transfer. Again, in this mechanism, only one oxygen is derived from the nitro group, and the other from the reaction medium.



Scheme 1.23

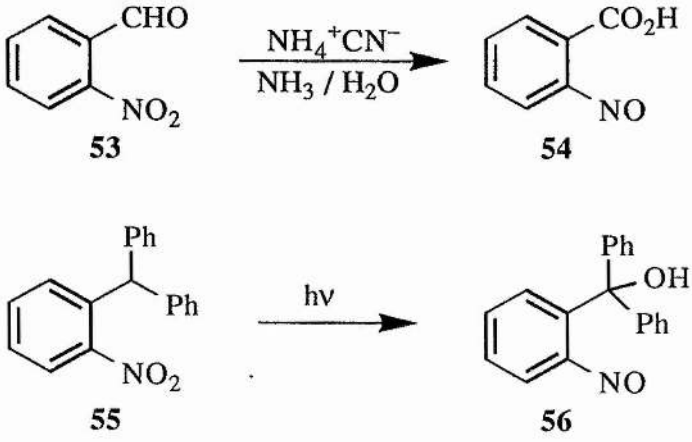
This phenomenon of attack on the oxygen of the nitro group has been rationalised by Buck[51], with the claim that "the environment of the nitrogen is completely saturated with electrons... and nucleophilic attack is consequently no longer possible on the nitrogen, and should occur, if at all, on the oxygen". An example given of this is shown in Scheme 1.24, with the reduction of nitroaryl groups to nitrosoaryl groups with triethyl phosphite. This statement, however, is curious when coupled with Buck's observation that "condensations of this type [*i.e.* with nitrogen as the electrophilic centre] are observed only in the case of biphenyl derivatives, where intramolecular condensation to form a six-membered ring is sterically favoured". There are many examples of what appear to be straightforward condensations with the nitrogen as the electrophilic centre (see Section 1.5).



Scheme 1.24

It has also been suggested[52], however, that this reaction could proceed by a mechanism involving radical intermediates, although a detailed mechanism was not outlined in the communication. This postulation was made in the light of the observation of the spontaneous formation of radical anions of some nitroaromatics in basic solution: for example, *p*- and *o*-nitrotoluene have been shown by ESR spectroscopy to form radicals with potassium *t*-butoxide in *t*-butanol[52].

o-Nitrobenzaldehyde (**53**) is converted, both photochemically[53], and chemically in the presence of ammonium cyanide and aqueous ammonia[54], into *o*-nitrosobenzoic acid (**54**) (Scheme 1.25). The reaction is claimed[55] to be general for *o*-nitrobenzene derivatives bearing an α–hydrogen; the rearrangement of the nitro compound (**55**) to the *o*-nitrosobenzyl alcohol (**56**) is one such case[56].

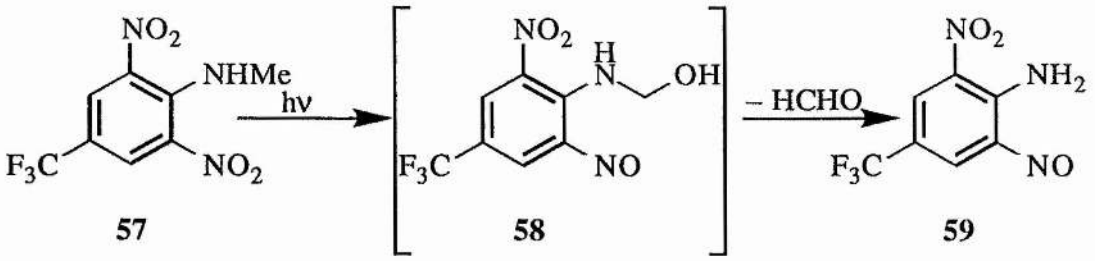


53 **54** **55** **56**

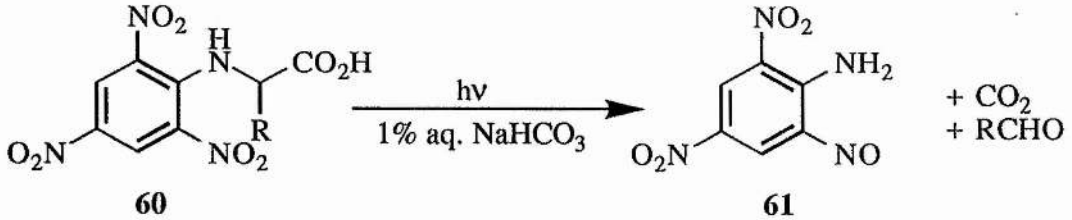
Scheme 1.25

Similarly, the photolytic conversion of *N*-(2,6-dinitro-4-trifluoromethyl)-*N*-methylaniline (**57**) to the *N*-unsubstituted nitrosoaniline (**59**) is envisaged as proceeding through the intermediate (**58**) (Scheme 1.26)[57], where the methyl group has been oxidised to the alcohol, and the nitro group reduced to nitroso. Extrusion of formaldehyde then follows. The photolytic cleavage of the trinitrophenylamino acid derivatives (**60**) to 2,4-dinitro-6-nitrosoaniline (**61**) (Scheme 1.27)[58] probably also proceeds through a similar intermediate derived from an intramolecular redox process. The amino acid derivatives

involved were those of glycine, phenylglycine, leucine, isoleucine and glutamic acid. That of tryptophan (R = 3-indolylmethyl) reacted only reluctantly, and gave unidentifiable products.



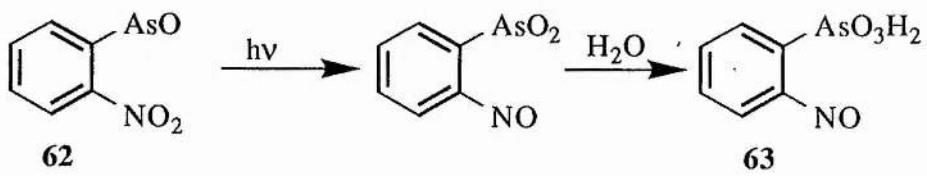
Scheme 1.26



R = H, Ph,
$CH_2CH(CH_3)_2$,
$CH(CH_3)Et$,
$CH_2CH_2CO_2H$

Scheme 1.27

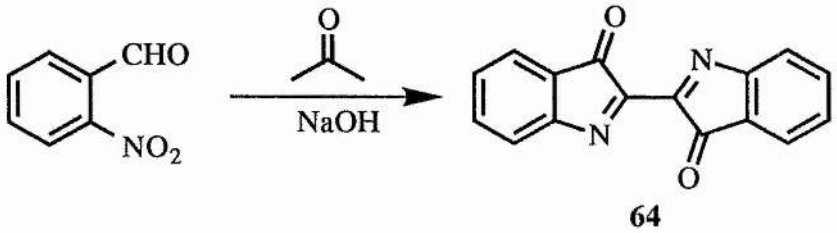
Another redox process involving interaction between a nitro group and an *ortho*-substituent is the formation of *o*-nitrosophenylarsonic acid (**63**) from *o*-nitrophenylarsenoxide (**62**) (Scheme 1.28)[59].



Scheme 1.28

Intramolecular redox processes which lead to cyclisations include the Baeyer-Drewsen synthesis of indigo[60] (**64**) (Scheme 1.29), which involves the condensation of

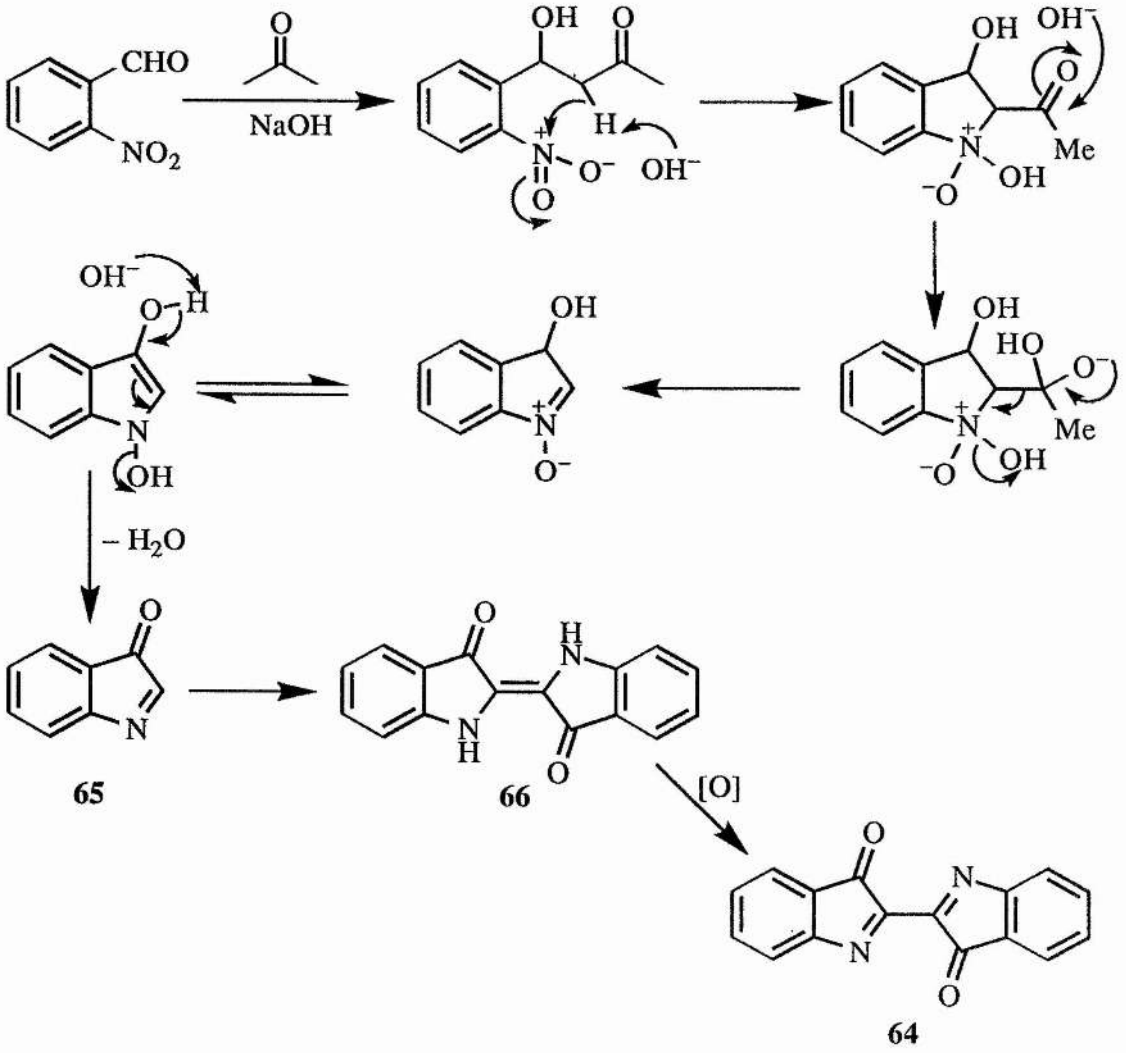
o-nitrobenzaldehyde with acetone in the presence of sodium hydroxide, and then cyclisation by some sort of redox process to indoxyl (**65**). A credible mechanism is shown in Scheme 1.30; the dimerisation of (**65**) to 2,2'-bisindoxyls (**66**) is known[61], and the oxidation of (**66**) to indigo is also known to occur readily in air[62] (although this is impossible for 2-substituted indoxyls).



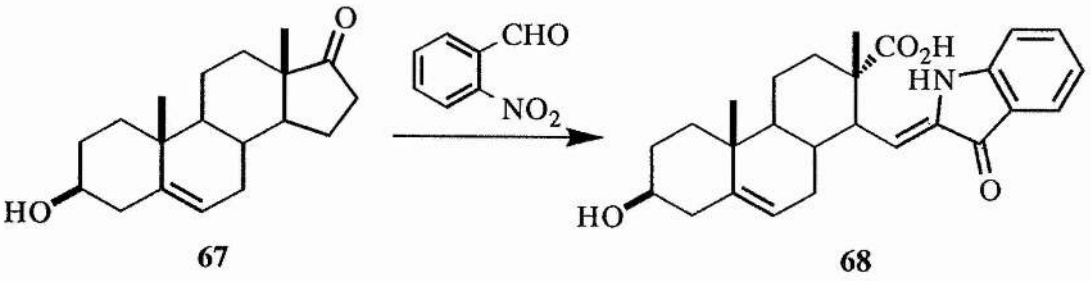
64

Scheme 1.29

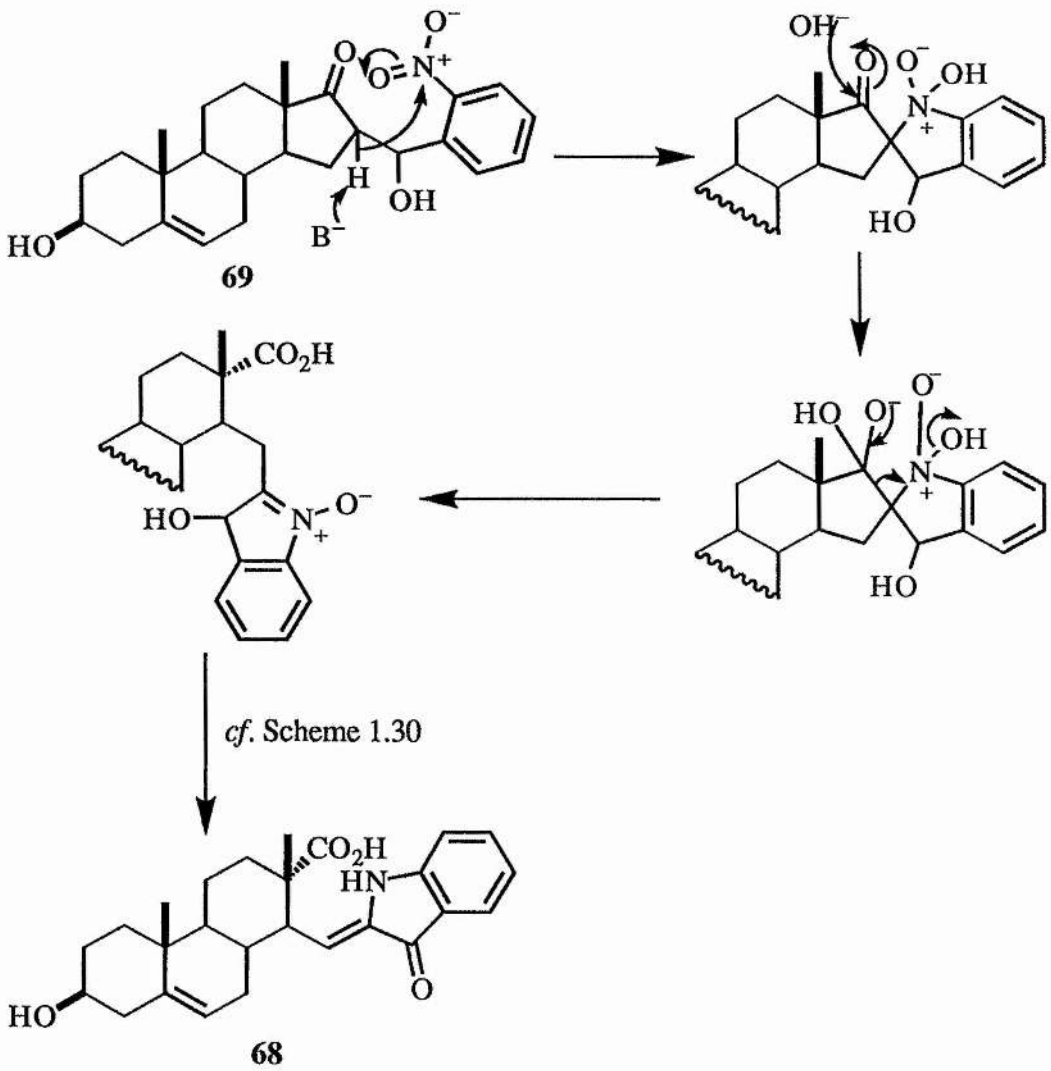
It was found that the reaction of the steroid (**67**) (5-androsten-3β-ol-17-one) with *o*-nitrobenzaldehyde in basic solution does not give the expected steroidal 16-*o*-nitrobenzylidene condensation product; instead the steroidal indoxyl compound (**68**) is isolated (Scheme 1.31)[63]. This reaction probably proceeds through the hydroxy compound (**69**) and follows the same mechanism (Scheme 1.32)[64] as that postulated for the Baeyer-Drewsen reaction in Scheme 1.30, although without the dimerisation step. This reaction is general for 17-ketosteroids; with simple ketones the reaction leads to more complex products.



Scheme 1.30

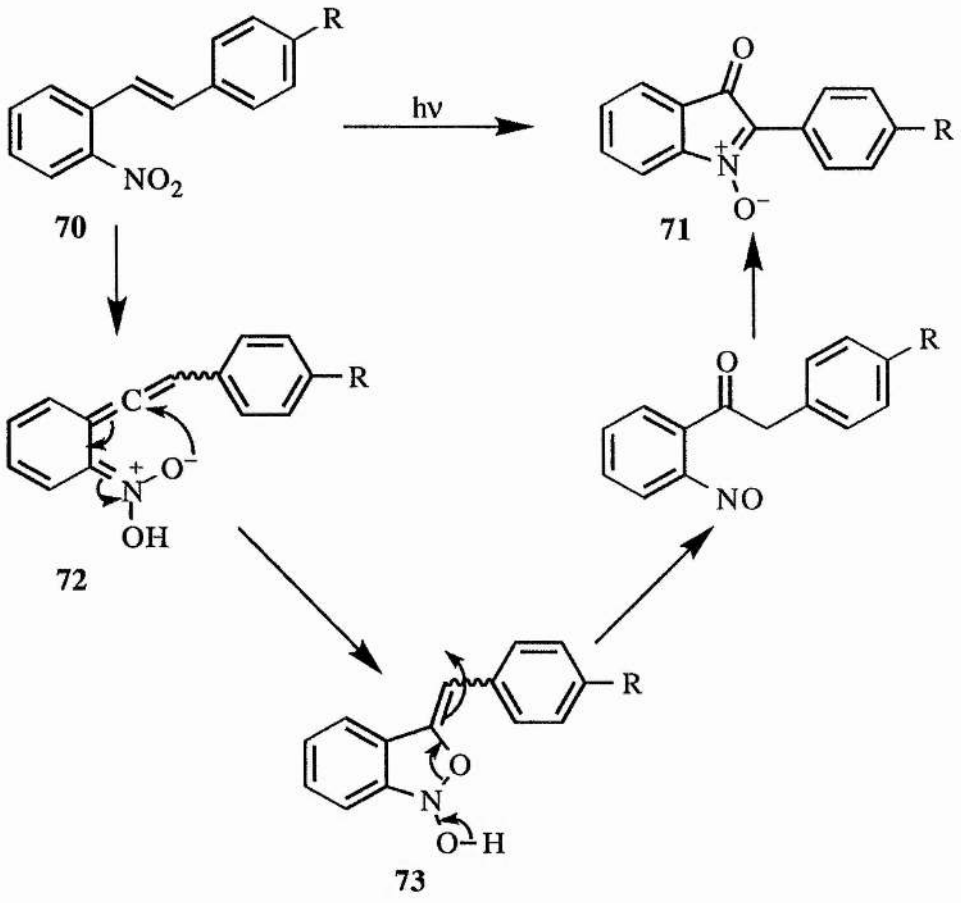


Scheme 1.31



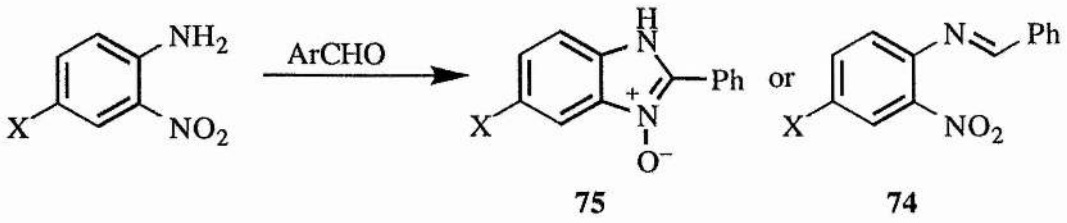
Scheme 1.32

During an investigation of the photochemistry of *trans* *o*-nitrostilbenes (**70**)[65], it was found that instead of the expected isomerisation to the *cis*-isomers upon illumination, the isatogens (**71**) were recovered. The authors suggest that transfer of the oxygen from the nitro group to the carbon atom occurs as a direct result of the irradiation, and the subsequent oxidation/reduction occurs as a "thermal dark reaction" following the irradiation. Alternatively, an *aci*-nitro intermediate (**72**) can be envisaged, followed by cyclisation to the 5 membered ring (**73**) (Scheme 1.33); this cyclisation is a favoured process with regard to Baldwin's rules for cyclisation[66].



Scheme 1.33

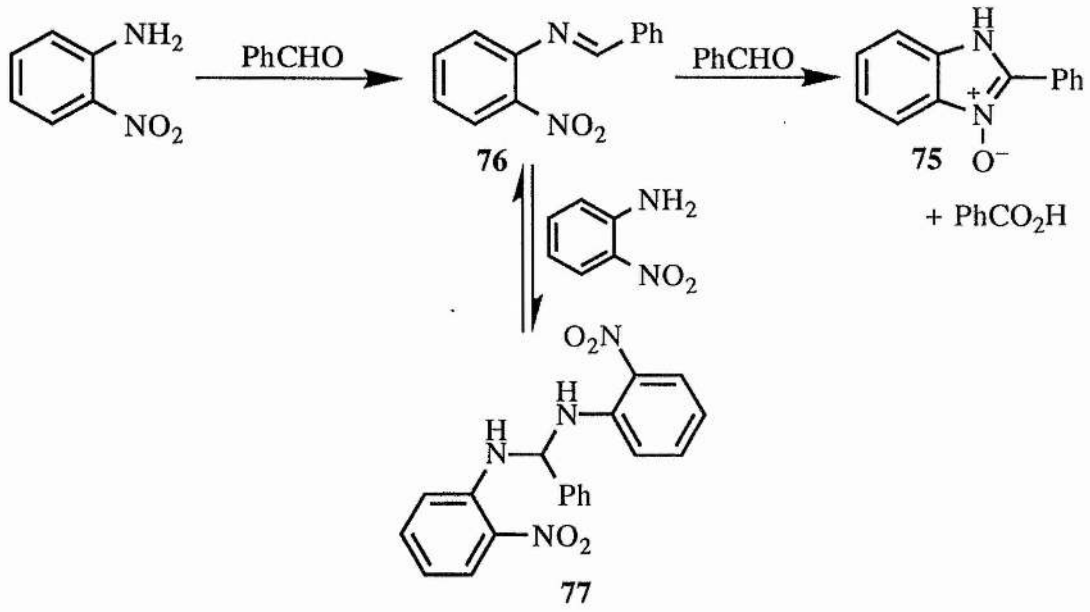
Benzimidazole *N*-oxides are obtained when *o*-nitroanilines are heated with excess of an aldehyde (for example, benzaldehyde). Indeed, in an attempted synthesis of *N*-benzylidene-*o*-nitroaniline (**74**), by heating *o*-nitroaniline with benzaldehyde as solvent, the heterocyclic *N*-oxide (**75**) instead may be isolated (Scheme 1.34)[67]. This seems to depend on the addition of xylene (b.p. ~140°C) as an inert co-solvent to aid in the removal of water from the reaction mixture by distillation. However, when toluene (b.p. 110 °C) is used as the co-solvent, the anil is obtained in satisfactory yield, suggesting that use of the higher boiling co-solvent favours cyclisation. The reaction has also been carried out in refluxing *p*-xylene or tetralin to give the parent benzimidazole in 18-94% yield[68].



75 **74**

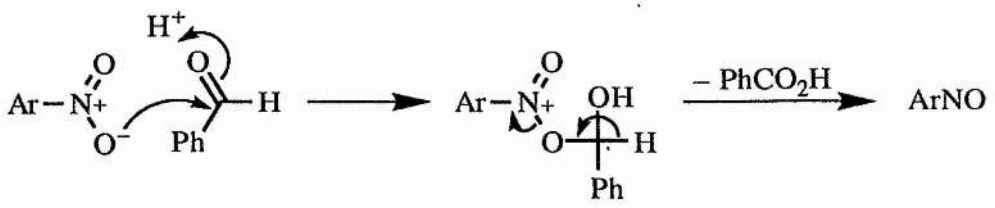
Scheme 1.34

This reaction is thought[69] to proceed by initial formation of the expected *o*-nitroanil (**76**) (Scheme 1.35). The anil reacts readily with another molecule of *o*-nitroaniline giving (**77**); the excess of aldehyde is necessary to suppress this side reaction. The aldehyde, however, also functions as a reducing agent, giving presumably the *o*-nitrosoanil (and benzoic acid; *cf.* Scheme 1.36); such anils are known to cyclise spontaneously to benzimidazole *N*-oxides[70]. Prolonged reaction at even higher temperatures leads to further reduction and the production of the parent 2-phenylbenzimidazole.



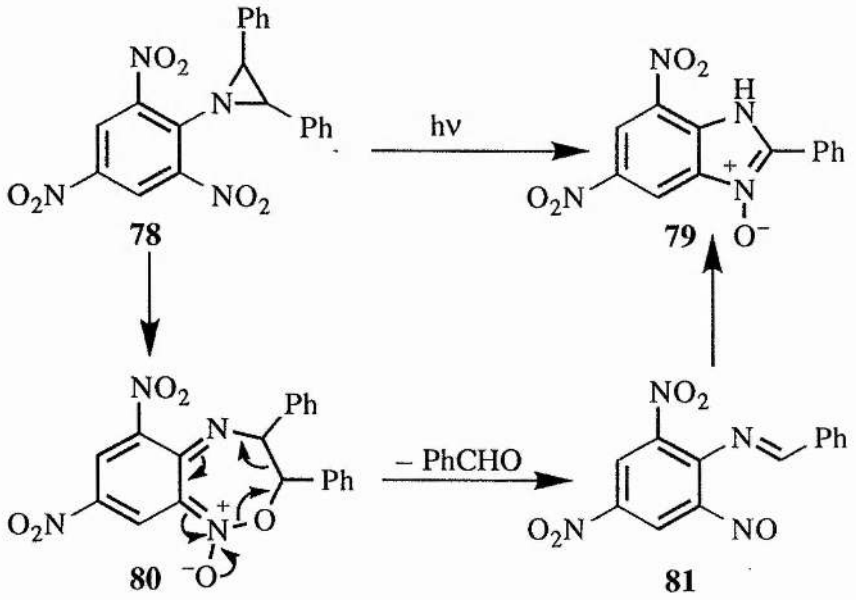
76 **75** **77**

Scheme 1.35

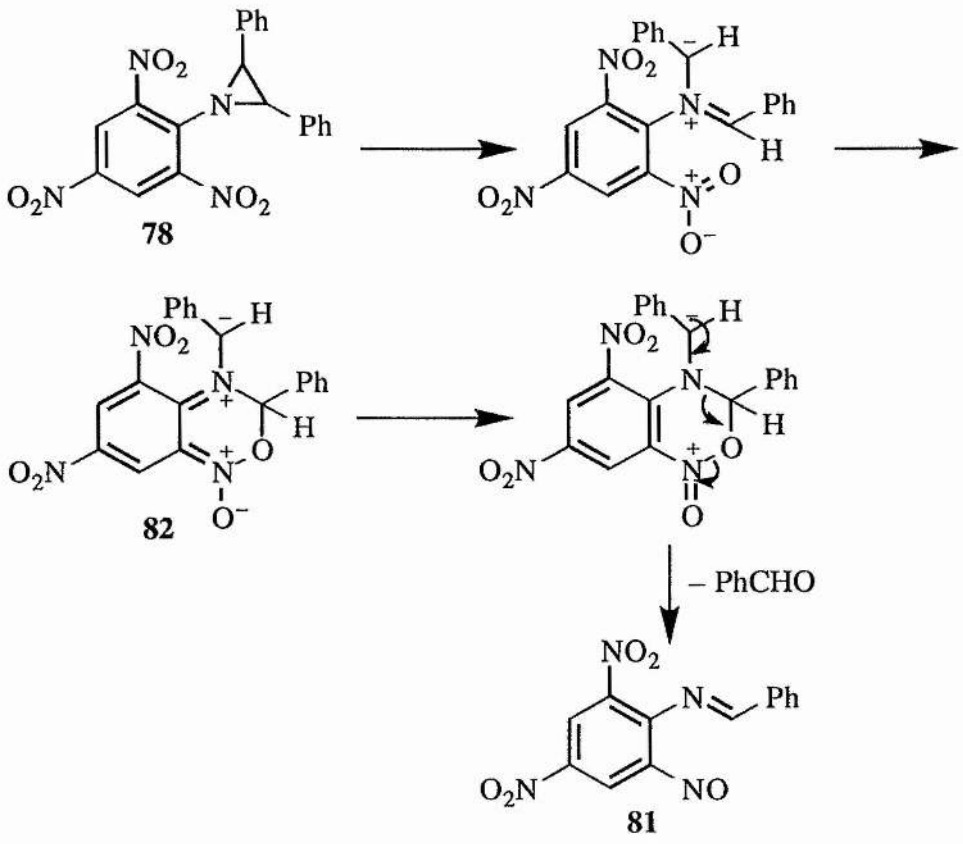


Scheme 1.36

The *N*-picrylaziridine (**78**) is cyclised photolytically to 5,7-dinitro-2-phenylbenzimidazole 3-oxide (**79**) (Scheme 1.37)[71] in very high yield. The strained aziridine ring is presumed by the authors to cleave, giving a benzoxadiazepine intermediate (**80**), which collapses with the extrusion of benzaldehyde to the *o*-nitrosoanil (**81**); recyclisation to the product then follows. However, Sukumaran *et al.*[72] propose that this reaction occurs by an electrocyclic mechanism (Scheme 1.38). Electrocyclic ring opening of the aziridine ring is followed by electrocyclisation to the benzoxadiazine intermediate (**82**), which then loses benzaldehyde to give the *o*-nitrosoanil; recyclisation then occurs as before. The same product is isolated if one of the phenyl groups is replaced by benzoyl; in this case benzoic acid is the co-product.



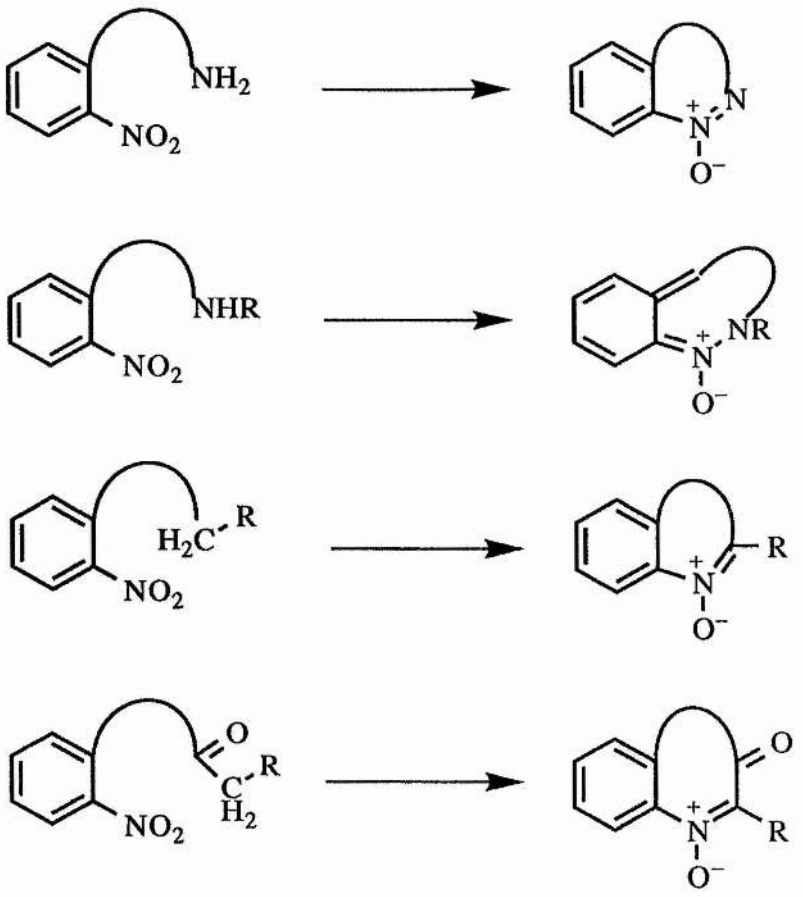
Scheme 1.37



Scheme 1.38

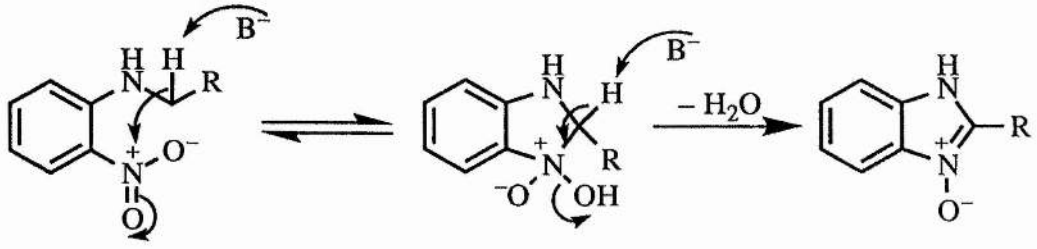
1.5 Condensation Reactions

There are many examples of base-induced cyclisations which appear to occur as a straightforward condensation of the intramolecular aldol type, whereby the nitrogen of the nitro group acts as the electrophilic centre in the manner of the carbon of a carbonyl group. A general representation of these reactions can be shown as in Scheme 1.39 which shows three types of cyclisation. The first involves an amine as the intramolecular nucleophile (if the amine is secondary, then the benzenoid character of the six-membered ring is lost, although the heterocycle is still aromatic as a 10 π system). The second type of cyclisation has as the intramolecular nucleophile a methylene group activated by a terminal electron-withdrawing R group, and the third a methylene group activated by a non-terminal carbonyl group.



Scheme 1.39

The generally accepted mechanism for this type of reaction[9] is outlined in Scheme 1.40 for the formation of benzimidazole *N*-oxides, involving nucleophilic attack of the methylene anion, and subsequent dehydration.



Scheme 1.40

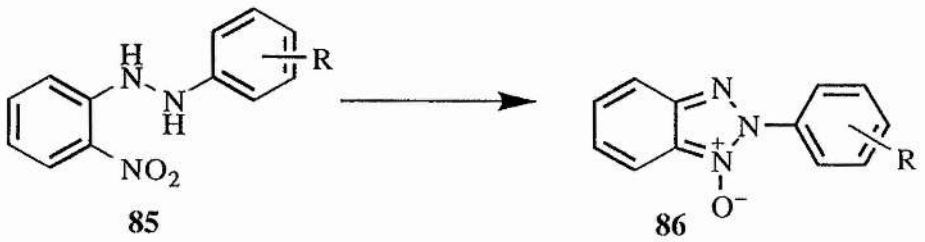
This mechanism appears to be quite satisfactory for a substantial number of cyclisation reactions in which a nucleophilic centre in an *ortho* side chain reacts with the nitrogen of the nitro group to effect a cyclisation.

1.5.1 Condensations Involving Nitrogen Nucleophiles

There are several examples of condensations involving nitrogen as the internal nucleophile. The nitrogen can be part of several different functional groups, the simplest probably being hydrazino: *o*-nitrophenylhydrazine (**83**) cyclises to 1-hydroxy-benzotriazole (**84**) (Scheme 1.41). The synthesis of 1-hydroxybenzotriazole (which has widespread applications as a coupling reagent for peptide synthesis[73]) was the first reported heterocyclisation of an *o*-nitrophenylamine[74,75,76]. The substituted *o*-nitrophenylhydrazine (**85**) cyclises both in ethanolic hydrogen chloride[77] and with acetic anhydride[78] to the 2-substituted benzotriazole 1-oxide (**86**) (Scheme 1.42); apparently condensation of the β–amino group with the nitro group is followed by removal of the α–amino proton to effect the dehydration.

83 **84**

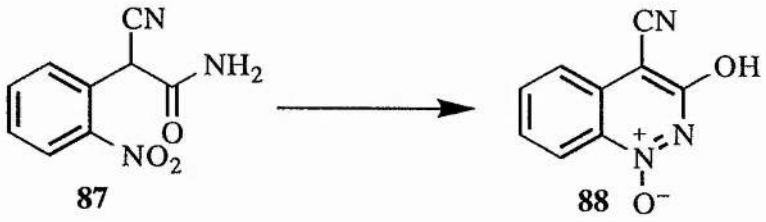
Scheme 1.41



85 **86**

Scheme 1.42

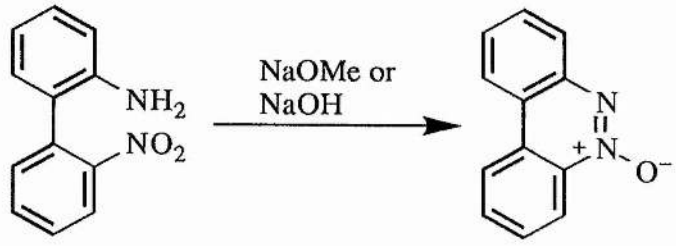
An intriguing condensation (in aqueous sodium hydroxide) between the amino group of certain *o*-nitrophenylacetamides (**87**) and the nitro group leads to cinnoline *N*-oxides (**88**) (Scheme 1.43)[79,32].



87 **88**

Scheme 1.43

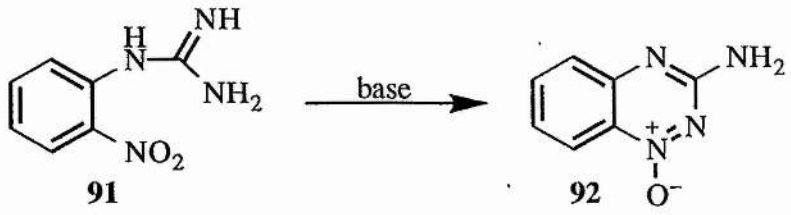
The amine nucleophile can also be on an adjacent ring. Benzo[*c*]cinnoline *N*-oxides (**90**) are the result of the reaction of 2-nitro-2'-aminobiphenyls (**89**)[80] with sodium methoxide or sodium hydroxide in methanol (Scheme 1.44).



89 **90**

Scheme 1.44

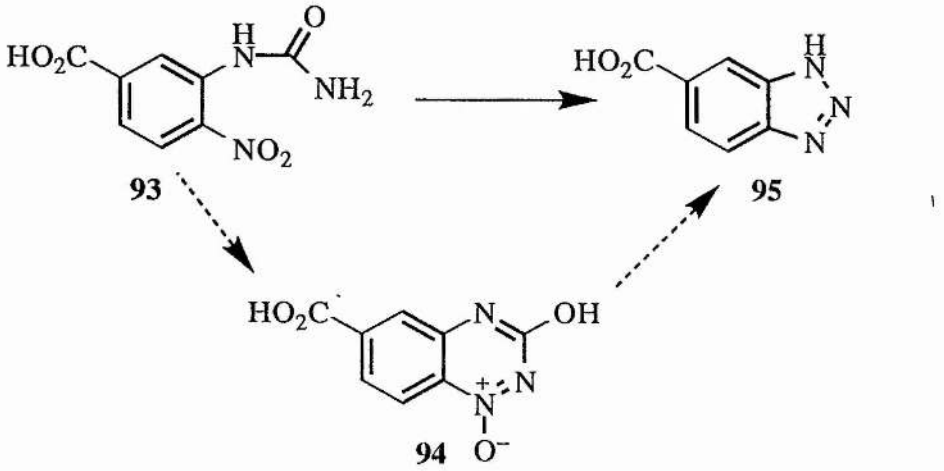
3-Aminobenzotriazine-1-oxides (**92**) are obtained from the reaction of *o*-nitrophenylguanidines (**91**) with aqueous sodium hydroxide[81], or with potassium *t*-butoxide in tetrahydrofuran[82] (Scheme 1.45); the corresponding 1,4-dioxides are then accessible by direct oxidation with hydrogen peroxide.



91 **92**

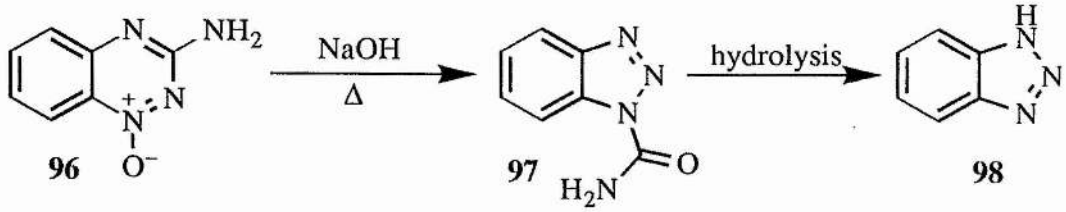
Scheme 1.45

An interesting variation of this reaction is the reaction of 4-nitro-3-ureidobenzoic acid (**93**) with hot potassium hydroxide[83]. The expected 3-hydroxybenzo-1,2,4-triazine 1-oxide (**94**) is not observed; instead the benzotriazole (**95**) is isolated. However, this is considered[84] to arise from a rearrangement of the initially formed (**94**) and subsequent hydrolysis of the rearrangement product to the observed benzotriazole (Scheme 1.46). Evidence for this was obtained by observing the rearrangement (Scheme 1.47) of 3-aminobenzo-1,2,4-triazine 1-oxide (**96**) to the 1-carboxamide (**97**). Subsequent hydrolysis of the rearrangement product leads to benzotriazole (**98**).



93 **95** **94**

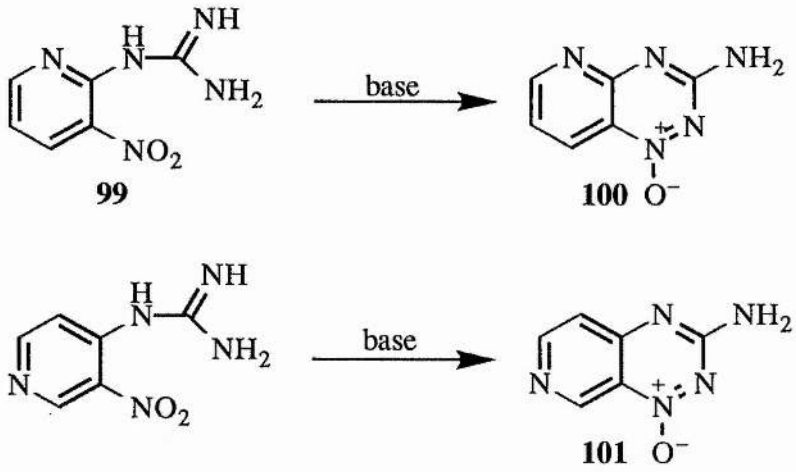
Scheme 1.46



96 **97** **98**

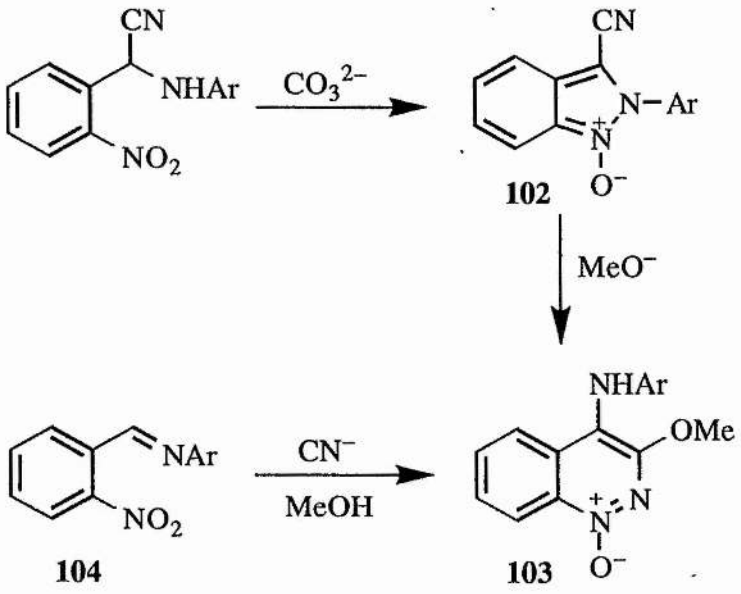
Scheme 1.47

This rearrangement can be kept to a minimum if the conditions are chosen carefully (Scheme 1.48). Pyrido[2,3-*e*]-1,2,4-triazine 1-oxides (**100**) are obtained without rearrangement occurring by refluxing the guanidinopyridine (**99**) in aqueous potassium carbonate for several hours[85]. Similarly, pyrido[4,3-*e*]-1,2,4-triazine 1-oxides (**101**) are obtained from the appropriate starting materials, again in refluxing aqueous potassium carbonate, although this time high yields are achieved after only 5 minutes[86].



Scheme 1.48

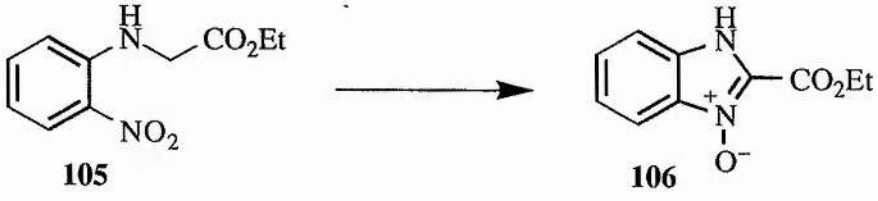
A final example of an amine (secondary in this instance) as a nucleophile is the cyclisation in carbonate to the 2*H*-indazole 1-oxide (**102**) (Scheme 1.49)[87]. Interestingly, further reaction of this with methoxide leads to the cinnoline *N*-oxide (**103**), by addition of methanol to the nitrile triple bond, ring opening and recyclisation. Similarly, reaction of the Schiff base (**104**) with cyanide in methanol gives the same product, presumably *via* a similar mechanism.



Scheme 1.49

1.5.2 Condensations Involving Carbanionic Nucleophiles

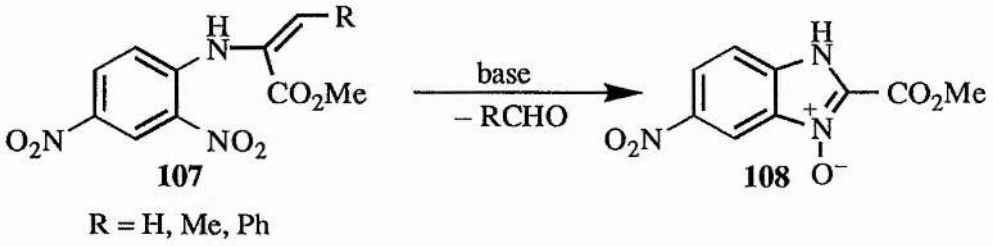
There follow examples of cyclisations which appear to involve the nucleophilic attack of a carbanionic centre on the nitrogen of the nitro group. These include the base-induced cyclisation of the glycine ester derivative (**105**) to the benzimidazole *N*-oxide (**106**)[88] (Scheme 1.50).



Scheme 1.50

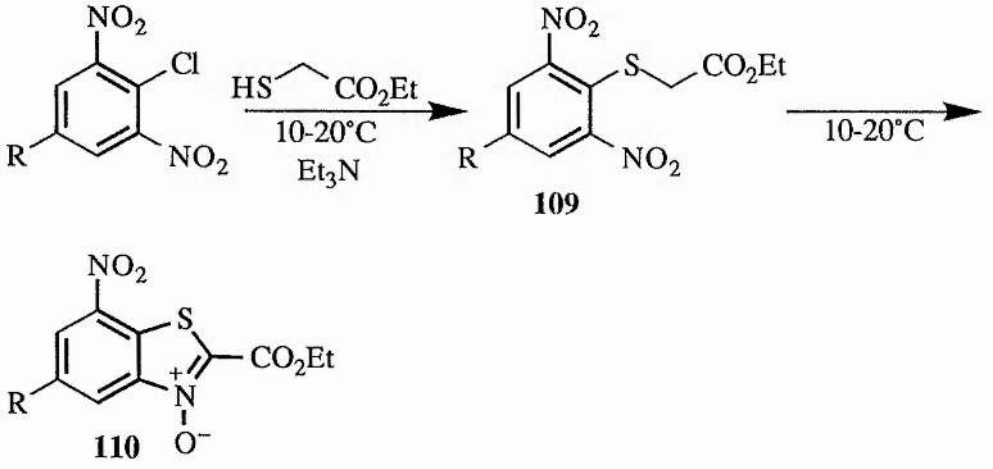
The unsaturated ester derivatives (**107**) react with base with the extrusion of an aldehyde to give methyl benzimidazole *N*-oxide-2-carboxylates (**108**). (Scheme 1.51)[89]. Interestingly, the rate of cyclisation decreases as the level of substitution on the alkene increases; the acrylate (R = H) cyclises faster then the crotonate (R = Me), which in turn cyclises faster than the cinnamate (R = Ph). This may be envisaged as an extension of

the glycine cyclisation in Scheme 1.50 above, whereby the alkenyl side chain is lost by addition of water across the double bond, and then retro-aldol scission occurs with loss of aldehyde to give the glycine derivative, which then cyclises under the reaction conditions (alternatively, the scission could occur after the cyclisation step).



Scheme 1.51

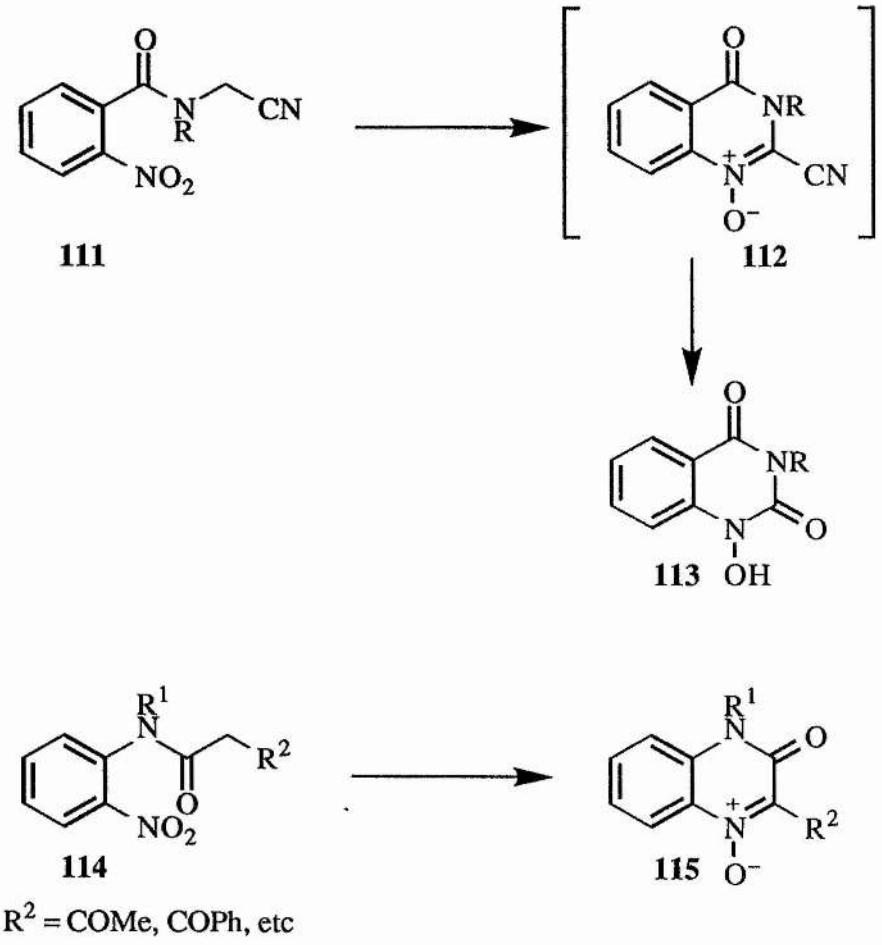
In the benzene series, *ortho*-substituents can be modified to vary the heterocyclic system obtained. (Arylthio)acetic acid derivatives (**109**) cyclise readily under basic conditions to the *N*-oxides of the benzothiazole series (Scheme 1.52)[90]. Here, in the reaction of ethyl thiolacetate with chloro-2,6-dinitrobenzene derivatives, the reaction proceeds to the ethyl 4-nitrobenzothiazole 1-oxide-2-carboxylate (**110**), again presumably *via* an aldol-type condensation.



Scheme 1.52

o-Nitrobenzamido-acetonitriles (**111**) react with sodium ethoxide in ethanol to give 1-hydroxyquinazoline-2,4-diones (**113**)[91,92]. The initial intermediate, however, is

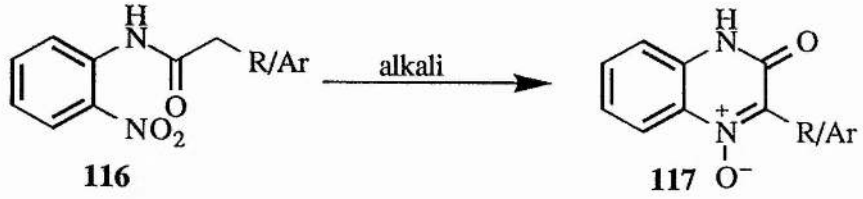
presumed to be the product (**112**) expected from a direct condensation between the active methylene and the nitrogen of the nitro group (Scheme 1.53), although this has not been isolated. Acetanilides (**114**) with electron withdrawing groups as substituents cyclise in basic conditions to quinoxalin-3-one 1-oxides (**115**)[93], again apparently as a result of a simple aldol-type condensation.



Scheme 1.53

The *o*-nitroacetanilide derivative (**116**; R = CN) is cyclised to 2-cyanoquinoxalin-3-one 1-oxide (**117**; R = CN) in aqueous alkali (Scheme 1.54)[94]. For those analogues with aryl groups in place of the cyano group, it is found[95] that the presence of electron-withdrawing groups on the aryl group facilitates formation of the anion, and so therefore has a profound effect on the ease of cyclisation, whereas substituents on the anilide ring have little effect. The mechanism was proposed[96] to proceed by formation of the

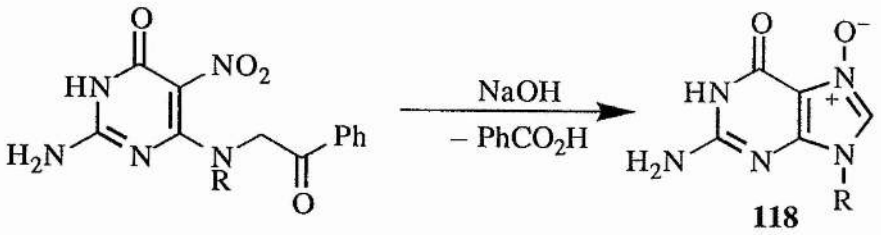
methylene anion and its nucleophilic attack on the nitrogen of the nitro group followed by dehydration.



116 **117**

Scheme 1.54

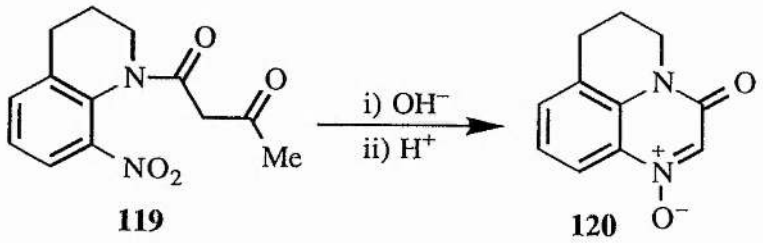
A similar type of condensation occurs to give purine *N*-oxides (**118**) (with loss of benzoic acid) (Scheme 1.55)[97], although the cyclisation does not work when R = H. According to the authors, this is "probably due to destabilisation of the phenacyl carbanion by the adjacent NH group", although this is presumably because the most acidic hydrogen in the molecule is the NH proton; the corresponding anion is well delocalised and the methylene carbanion is more unlikely to form.



118

Scheme 1.55

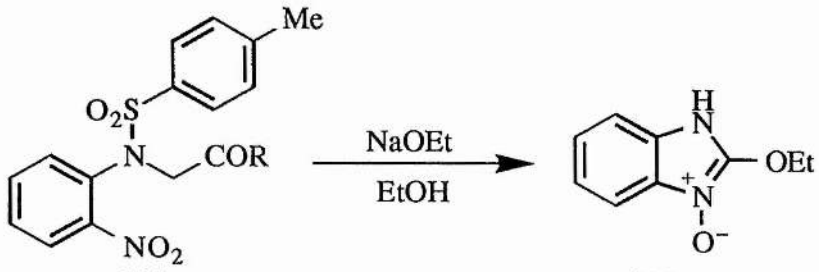
The tricyclic quinoxaline derivatives (**120**) are the products of the reaction of the acetoacetanilide (**119**) with sodium hydroxide in pyridine (Scheme 1.56)[98].



119 **120**

Scheme 1.56

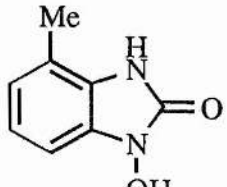
2-Alkoxybenzimidazole *N*-oxides (**122**) are obtained by the reaction of *N*-phenacyl- (or *N*-acetonyl-) *N*-(toluene-*p*-sulphonyl)-*o*-nitroanilines (**121**) with sodium alkoxides in the appropriate alcohol; the 2-substituent is derived from the alkoxide (Scheme 1.57)[99]. Interestingly, when 6-methyl-2-nitroaniline derivatives (i.e. with a second group *ortho* to the amino group) are cyclised, 1-hydroxy-4-methylbenzimidazolone (**123**) is isolated. The mechanistic implications of this will be discussed in Chapter 3.



121

R = Ph, Me

122

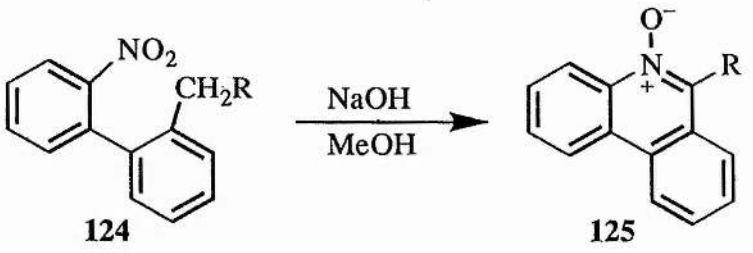


123

Scheme 1.57

Interactions of nitro groups with *ortho* substituents on an adjacent ring were described briefly in Section 1.4.1, in the context of reductive cyclisations. Condensation of an active methylene group with an *o*'-nitro group, for example in biphenyl derivatives, leads to the formation of tricyclic *N*-oxides. Phenanthridine *N*-oxides (**125**) can be prepared by reaction of the appropriately substituted biphenyl (**124**) with methanolic sodium hydroxide (Scheme 1.58)[100]. When R = COPh, *un*substituted phenanthridine 5-oxide and sodium benzoate are isolated. 6-Benzoylphenanthridine 5-oxide is apparently formed initially, the benzoyl substituent being subsequently displaced by nucleophilic attack of hydroxide ion. Direct *N*-oxidation to 6-benzoylphenanthridine 5-oxide (with peroxyacetic acid as the oxidising agent) was also unsuccessful. When R = Ph, the

phenyl group is presumably not sufficiently electron-withdrawing, and no cyclisation takes place[80].

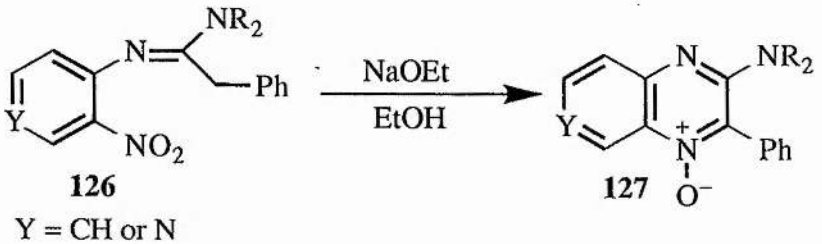


124 **125**

R = CN, CO_2Me, $CONH_2$

Scheme 1.58

The cyclisation strategy for *N*-oxide synthesis has utility when a direct oxidation method would give uncertain results (*i.e.* uncertainty as to which nitrogen might be oxidised). The synthesis of quinoxaline 1-oxides and pyrido[3,4-*b*]-pyrazine 4-oxides (**127**) can be effected unambiguously by cyclisation of *o*-nitrophenylacetamidines (**126**) (Scheme 1.59)[101] with sodium ethoxide in ethanol. The adjacent acetamidine moiety is probably sufficient activation for the methylene group for a simple condensation mechanism to be considered.



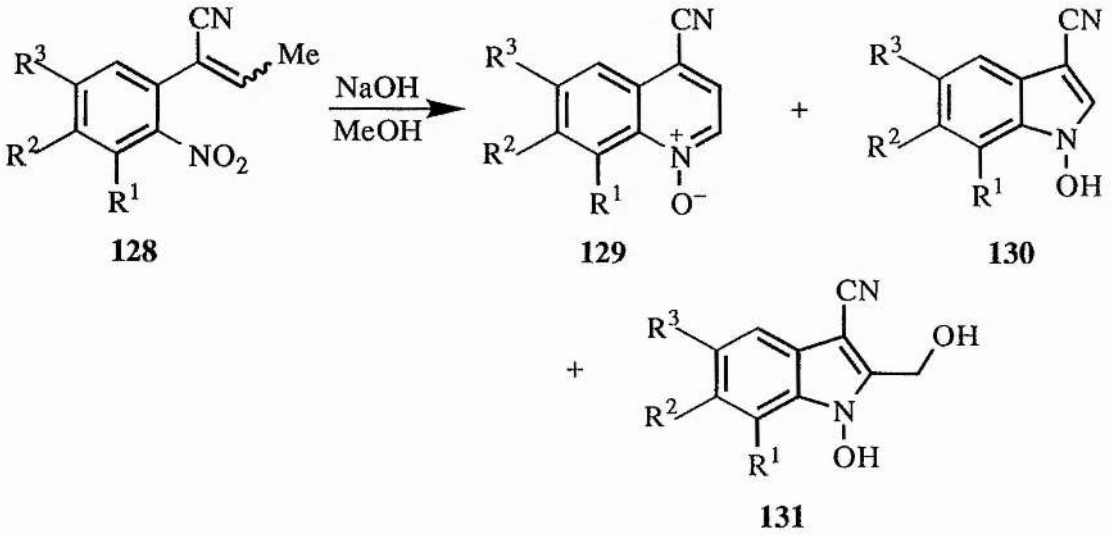
126 **127**

Y = CH or N

Scheme 1.59

Varying the conditions of a reaction can sometimes have a profound effect on its course. For instance, cyclisation of the *o*-nitrophenylallyl derivatives (**128**) (Scheme 1.60)[102] leads to quinoline 1-oxides (**129**) and 1-hydroxyindoles (**130** and **131**). Altering the basicity of the reaction medium gives a mixture of products, depending on the chosen conditions. The more electron-withdrawing the substituents R^1 - R^3 on the benzene ring,

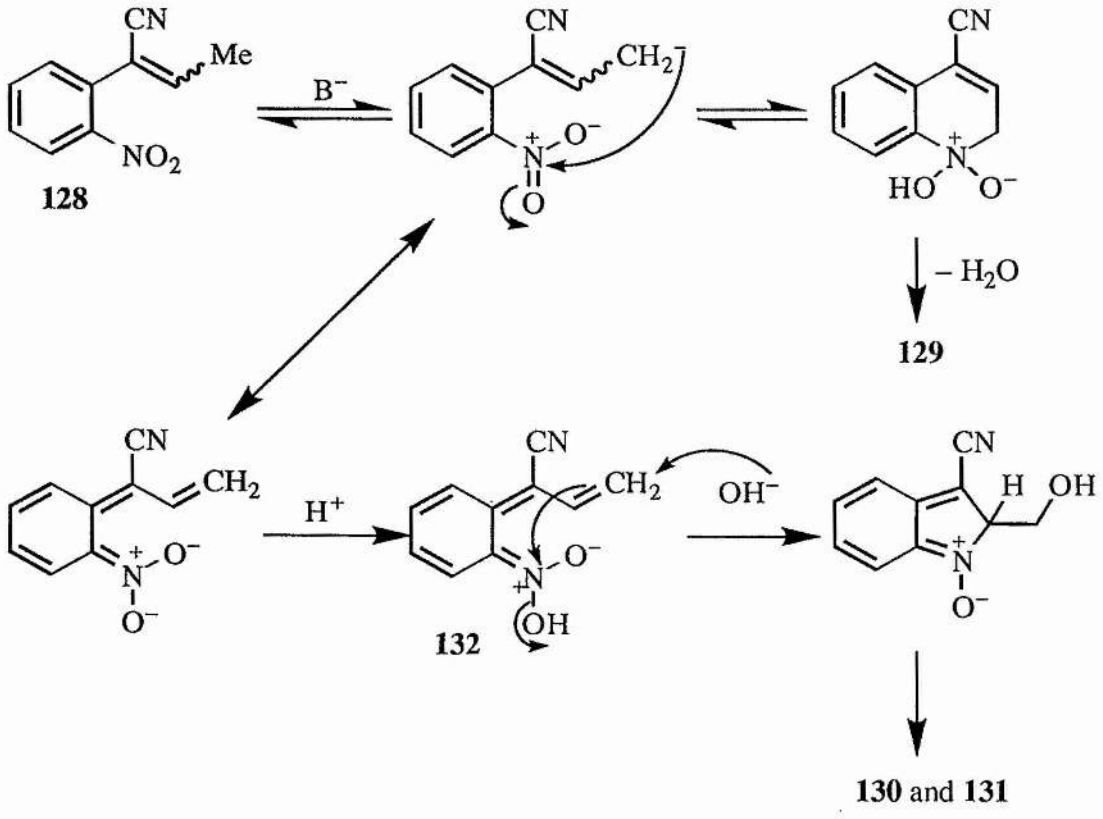
the more acidic are the methyl hydrogens, and so the formation of the quinoline *N*-oxides is apparently more favoured (for example, with Cl, Br, SO_2Ph). Conversely, electron-donating substituents such as OMe and SMe increase the proportion of the 1-hydroxyindoles isolated. Indeed, the reaction of those less acidic nitriles requires the addition of dimethyl sulphoxide to increase the basicity of the reaction medium (by increasing the activity of the hydroxide ions). For a given starting material, increasing the basicity of the reaction medium increases the proportion of the 6-membered ring formed.



Scheme 1.60

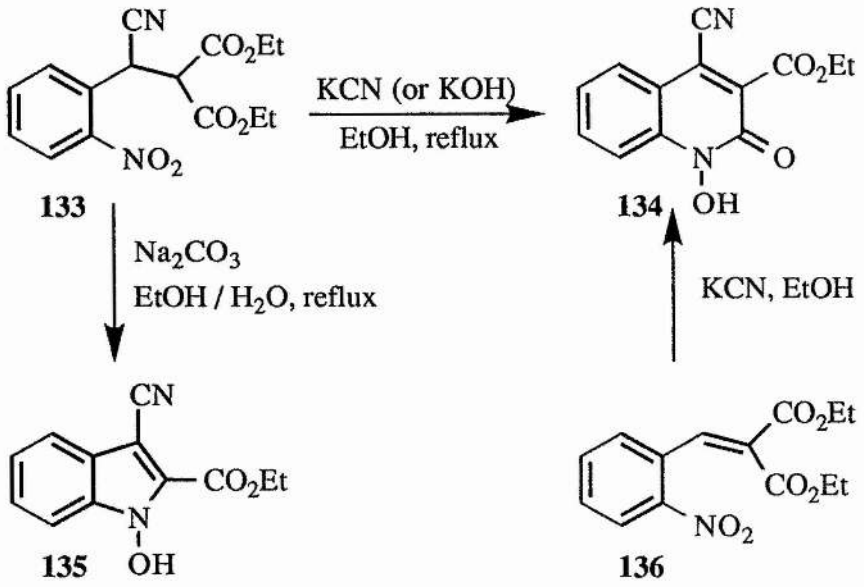
The authors propose a rationalisation (Scheme 1.61) of these observations, taking into account the fact that no interconversion of the three products is observed. The cyclisation step leading to the 6-membered ring (**129**) could perhaps be irreversible under strongly basic conditions, whereas protonation to give the *aci*-nitro intermediate (**132**) happens under less basic conditions, with subsequent cyclisation leading to the 5-membered ring. The cyclisation step is considered by the authors to be a cycloaddition, although the Michael-type addition shown in the Scheme is perhaps more likely. The mechanistic pathway to the 2-unsubstituted indole (**130**) is unclear although the one-carbon unit is

presumably lost as formaldehyde by a retro-aldol cleavage, as formaldehyde has been detected by trapping as its dimedone derivative.



Scheme 1.61

1-Hydroxy-2-quinolones (**134**) are obtained from the reaction of diethyl (α-cyano-*o*-nitrobenzyl)malonate (**133**) with either potassium cyanide or potassium hydroxide in ethanol[103]. However, this requires a strongly alkaline environment: if the conditions employed are less basic (for example by using sodium carbonate in aqueous ethanol), or the starting material is less reactive (with hydrogen in place of the nitrile group), the course of the reaction is altered and the 1-hydroxyindole (**135**) is formed. The vinylogous analogue (**136**) of the nitrile is also cyclised with potassium cyanide to (**134**) (Scheme 1.62), although (**136**) probably first adds HCN across the double bond to give (**133**) as the initial intermediate.

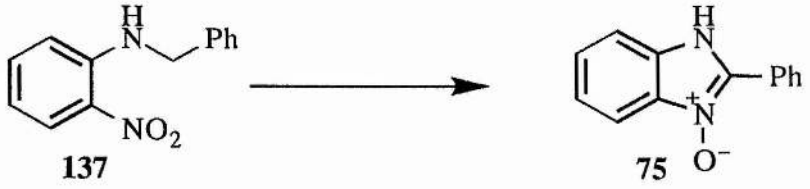


Scheme 1.62

1.6 Alternative Mechanisms?

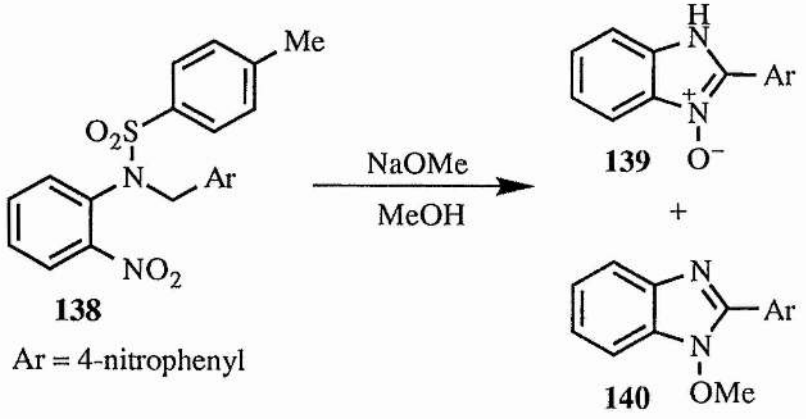
Rationalising the foregoing reactions by invoking a straightforward aldol-type condensation (*cf.* Scheme 1.40) appears to be satisfactory in many cases. However, there are several classes of cyclisation which appear to involve attack on the nitro group by a less obviously nucleophilic centre; *i.e.* activation of the methylene group appears to be insufficient for generation of a carbanion under the reaction conditions employed.

For instance, the feeble activation of the methylene group in *N*-benzyl-*o*-nitroaniline (**137**) appears to be overcome as cyclisation to 2-phenylbenzimidazole *N*-oxide (**75**) occurs under alkaline conditions with sodium hydroxide (Scheme 1.63)[69]. Cyclisation fails, however, for the *N*-methyl analogue, even under more drastic conditions (sodium hydride)[6]. The reason for this is unclear if a simple condensation mechanism operates.



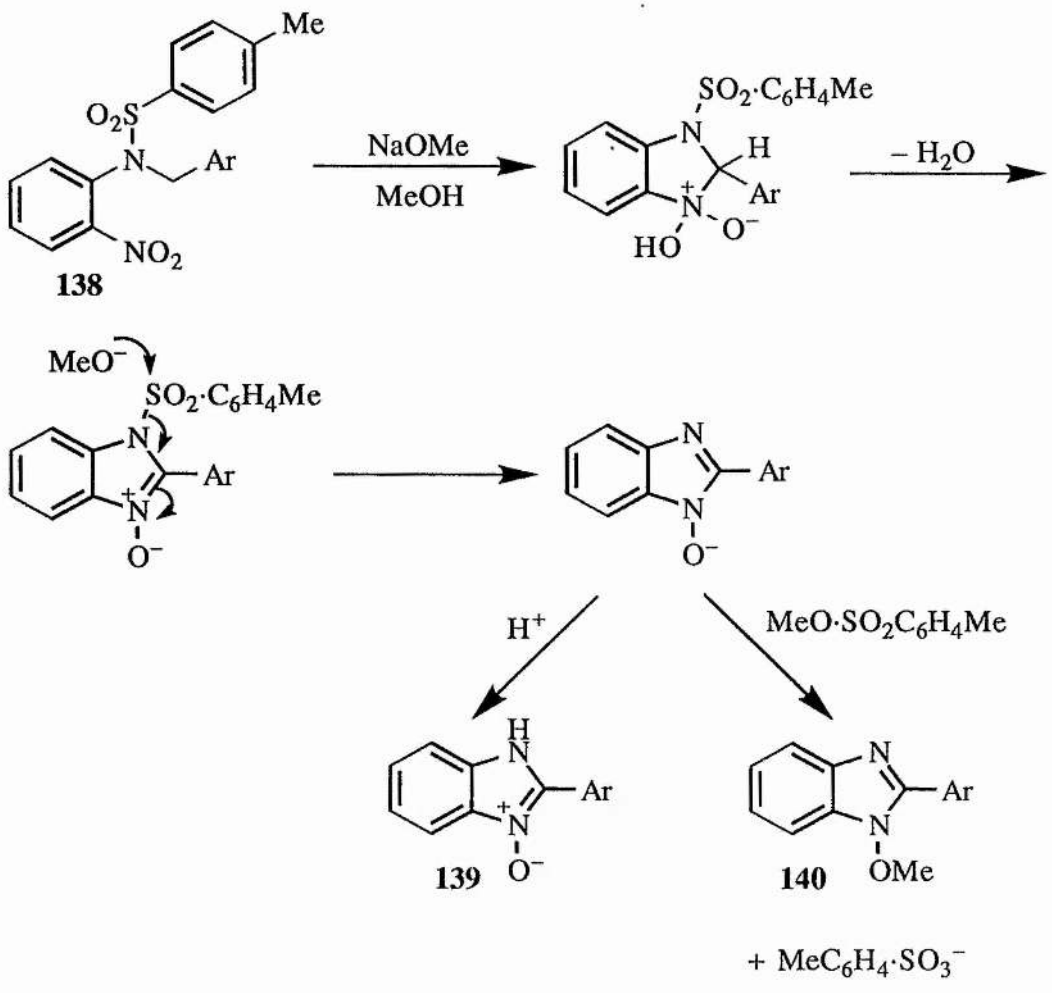
Scheme 1.63

Attempted syntheses of *o*-nitroanils by the elimination of toluene-*p*-sulphinic acid from *N*-arylmethyl-*N*-(toluene-*p*-sulphonyl)-*o*-nitroanilines (**138**) have proved unsuccessful; instead the 2-arylbenzimidazole 1-oxide (**139**) is isolated (Scheme 1.64)[104]. Again, activation of the methylene carbon is provided only by an aryl group. The co-product in this reaction is a 1-methoxy-2-arylbenzimidazole (**140**). Presumably this arises from methylation of the *N*-oxide by methyl toluene-*p*-sulphonate, which in turn is generated by nucleophilic attack on the sulphur by methoxide ion after the cyclisation has taken place (Scheme 1.65).



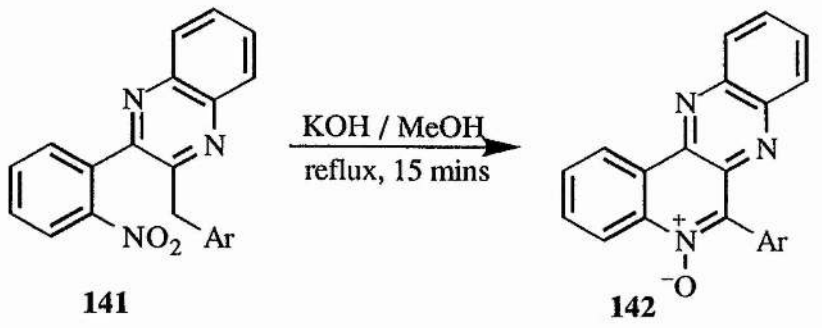
Scheme 1.64

Later work by Smith *et al.*[105] showed that cyclisation of the *N*-tosylated starting materials (**138**) with sodium methoxide in methanol depends on the extent of methylene activation: for the *N*-benzyl derivative, no cyclisation occurs, but for the *N*-(*p*-nitrobenzyl) derivative, the activation is presumably sufficient for reaction to occur.



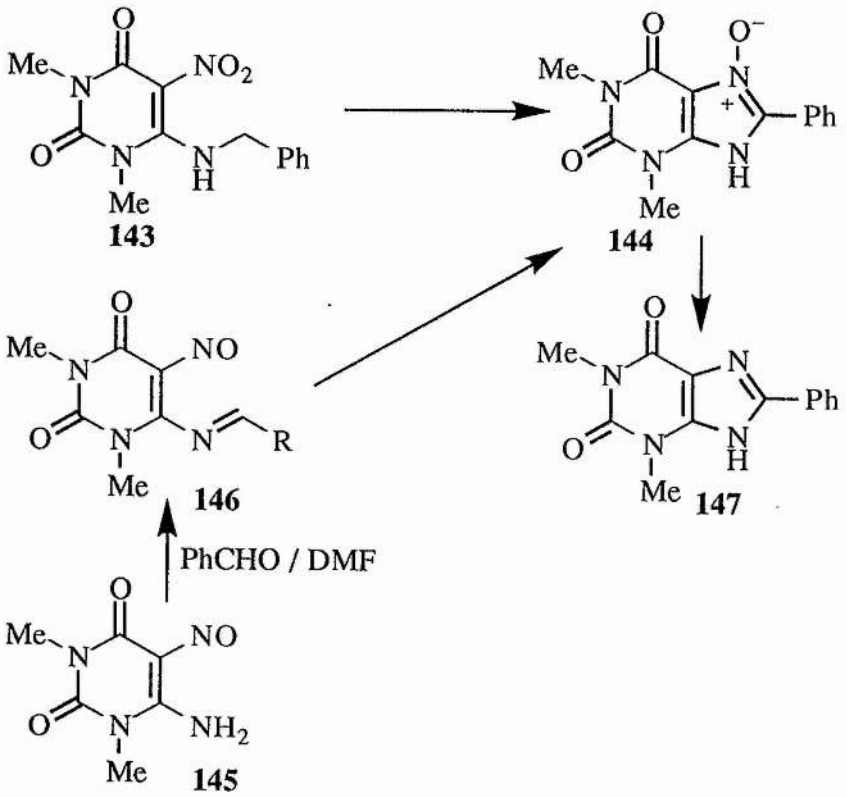
Scheme 1.65

An apparently simple condensation between an active methylene group and a nitro group in an adjacent ring [i.e. 2-benzyl-3-(*o*-nitrophenyl)quinoxaline (**141**) furnishes the tetracyclic quinolino[3,4-*b*]quinoxaline 5-oxide (**142**) (Scheme 1.66)[106], although here the acidity of the methylene protons is perhaps enhanced by the two flanking aryl groups, and condensation with the nitro group is sterically favoured. However, a comparison with the phenanthridine synthesis (Scheme 1.58) shows that in that instance, activation of the methylene group of (**124**; R = Ph) by two flanking phenyl groups was insufficient for cyclisation to take place. This discrepancy is intriguing in that the only difference in activation between (**124**) and (**141**) is that one of the aryl groups is quinoxalino instead of phenyl.



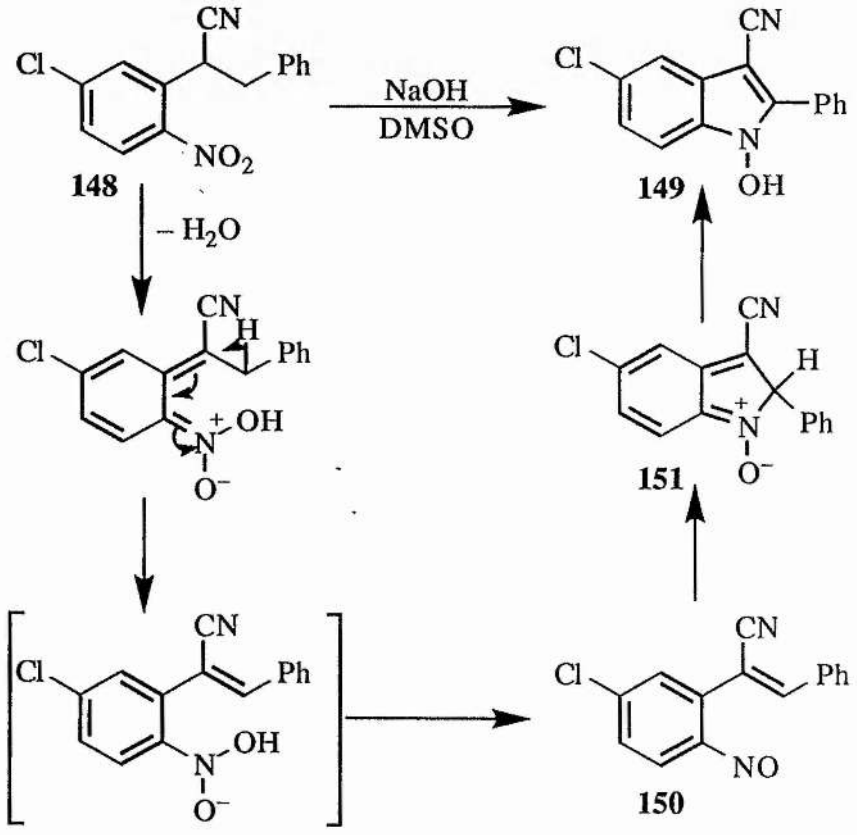
Scheme 1.66

Xanthine *N*-oxides (**144**) can be obtained by cyclisation of the nitrouracil (**143**) with warm ethanolic potassium carbonate (Scheme 1.67)[107]. The nitrosouracil derivative (**146**) is a presumed intermediate in the reaction of the 4-amino-5-nitrosouracil (**145**) by heating with benzaldehyde in *N*,*N*-dimethylformamide to give (**144**) and the parent xanthine (**147**)[108], the cyclisation occurring in a fashion analogous to the spontaneous cyclisation of *o*-nitrosoanils to benzimidazole *N*-oxides[70].

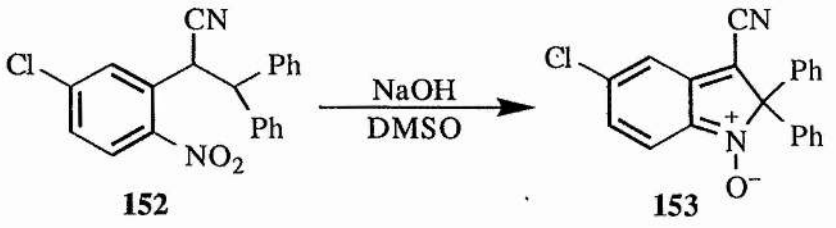


Scheme 1.67

1-Hydroxyindoles (**149**) are obtained by the reaction of an appropriately substituted *o*-(benzylcyanomethyl)nitrobenzene (**148**) with sodium hydroxide in dimethyl sulphoxide (Scheme 1.68)[109], the postulated course of the reaction involving a nitroso intermediate (**150**). The crucial step is thought to involve dehydration to give (**150**) (although no mechanism is proposed for this, a plausible route is included in the Scheme), and then electrocyclisation to a nitrone intermediate (**151**) followed by tautomerisation. Cyclisation still occurs if there is extra substitution on the β-carbon; in this case [the cyclisation of (**152**) to (**153**); Scheme 1.69] the carbon at the 2-position of the indole (**153**) remains tetrahedral. This lends support to the proposed mechanism, which in any case is unlikely to be a simple condensation involving the nitro group.



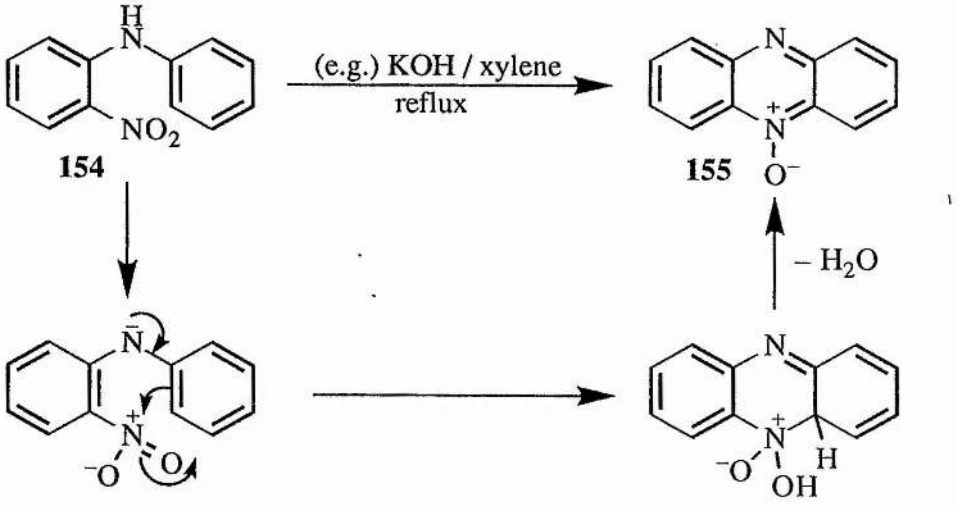
Scheme 1.68



152 **153**

Scheme 1.69

Phenazines (**155**) and their *N*-oxides are obtained by heating 2-nitrodiphenylamines (**154**) in basic solution[110]. Relatively high reaction temperatures are required (for example, refluxing xylene or decalin), although the proportion of *N*-oxide decreases with increasing reaction temperature. In this case, the reaction is tantamount to electrophilic aromatic substitution (Scheme 1.70). The deoxygenation appears to be a thermal process, which would explain the decreased proportion of *N*-oxide obtained at higher temperatures, although in decalin the deoxygenation process could also be facilitated by the oxidation of the solvent to tetralin or naphthalene.

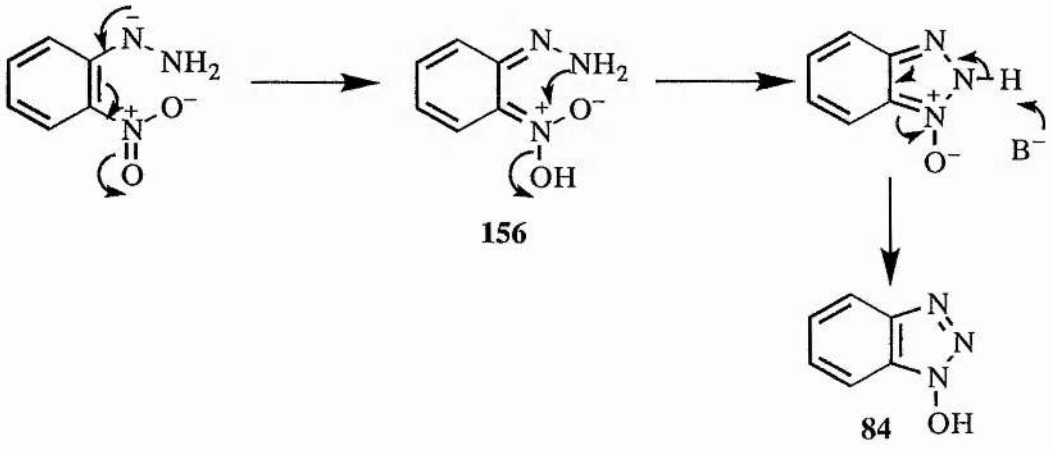


154 **155**

Scheme 1.70

In the context of the alternative mechanisms proposed for some of these cyclisation reactions, it is useful now to reconsider some of the apparently simple condensation mechanisms proposed for some of the reactions in Sections 1.5.1 and 1.5.2.

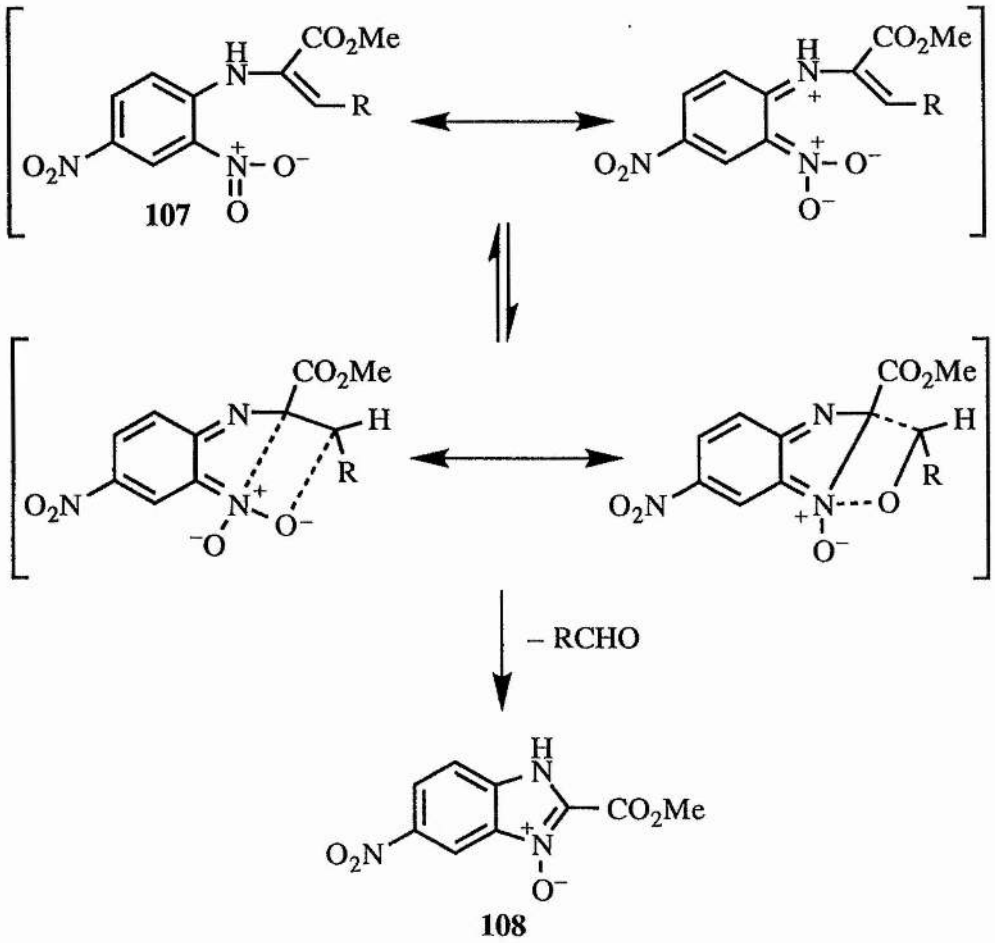
The mechanism of the formation of 1-hydroxybenzotriazole (**84**) from the cyclisation of *o*-nitrophenylhydrazine (**83**) (Scheme 1.41) has received considerable attention. Kinetic studies[111] have suggested that a monoanion is formed during the reaction. The most stable monoanion is that of the α-NH, as this is stabilised by the *ortho* nitro group. The formation of an *aci*-nitro intermediate (**156**) is envisaged, followed by nucleophilic attack of the β-NH_2 on the nitro group, and subsequent dehydration and tautomerisation (Scheme 1.71). However, formation of the β-monoanion has not been ruled out, and the possibility of a redox process, involving cyclisation of an intermediate *o*-nitrosoazobenzene, has also been tentatively suggested[111].



Scheme 1.71

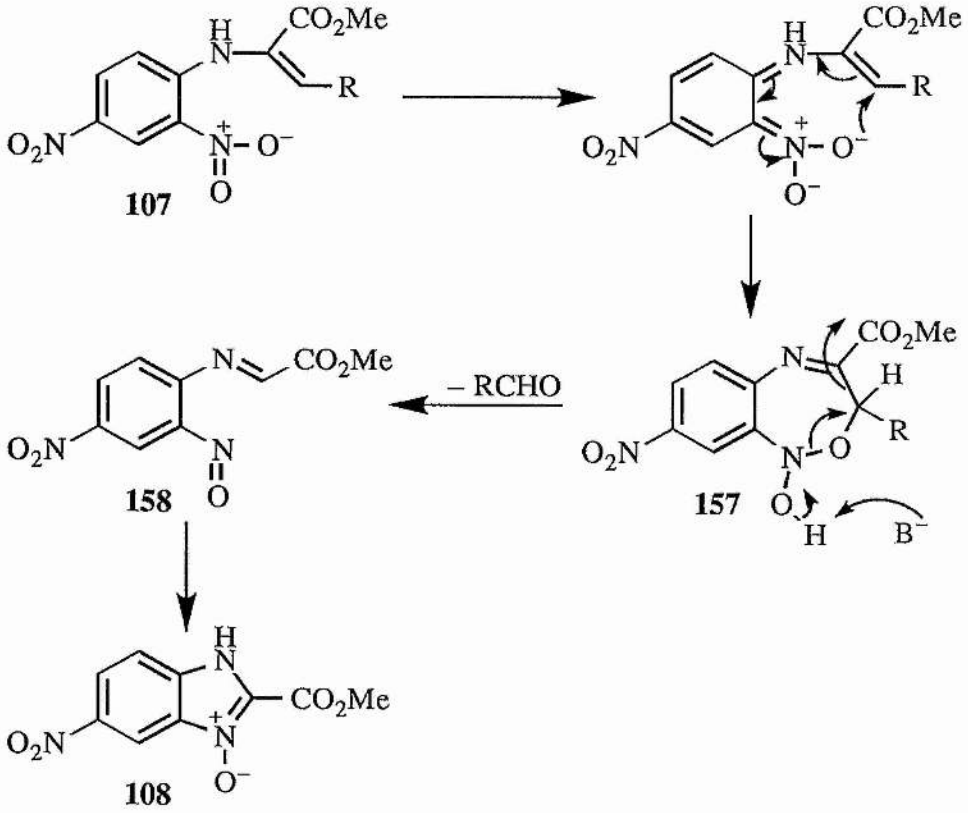
The cyclisation of the unsaturated ester derivatives (**107**) (Scheme 1.51)[88] is proposed by the authors to proceed by a concerted mechanism (Scheme 1.72) because of the fact that they could not detect any intermediates during the course of the reaction.

However, another mechanism which would seem to be more likely can be envisaged wherein a 7-membered ring intermediate (**157**) is formed which then collapses to the *o*-nitrosoanil (**158**) with concomitant loss of aldehyde; the *o*-nitrosoanil then cyclises spontaneously to the benzimidazole *N*-oxide (**108**) (Scheme 1.73).



Scheme 1.72

In conclusion, then, it is apparent that there is considerable scope for a reappraisal of the presumed mechanisms for some intramolecular cyclisations involving nitro groups and *ortho* side chains.


CO2Me
H
N
R
O2N
N+
O−
O
107
CO2Me
H
N
R
O2N
N+
O−
O−
CO2Me
N
H
R
O2N
N
O
157
O
H
B−
– RCHO
N
CO2Me
O2N
N
O
158
H
N
CO2Me
O2N
N+
O−
108

Scheme 1.73

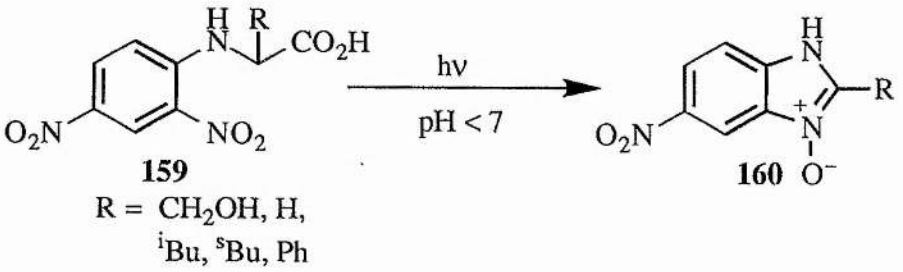
CHAPTER TWO

Cyclisations of Amino Acid Derivatives

2.1 Introduction

The research carried out for this thesis is concerned mostly with the cyclisation reactions of derivatives of amino acids. This type of reaction was introduced in Section 1.5.2 (page 32), with regard to condensations involving carbanionic nucleophiles. The mechanism of the cyclisation of the glycine derivative in Scheme 1.50 would appear to be a straightforward condensation of the methylene-derived carbanion with the nitro group, but as explored in Chapter 1, this is open to question, as are the mechanisms of cyclisations of various other *o*-nitroanilines.

The photochemical decomposition of *N*-(*o*-nitrophenyl) derivatives of amino acids in the solid state has been known for quite some time[112,113] and results in decarboxylation to give the corresponding *N*-alkyl-2,4-dinitroaniline[114]. The *N*-(2,4-dinitrophenyl) (henceforth DNP-) derivatives of α-amino acids (**159**) cyclise in aqueous solution by photolysis at pH<7 to give the 2-substituted benzimidazole *N*-oxides (**160**) (Scheme 2.1).



159
R = CH_2OH, H, ^{i}Bu, ^{s}Bu, Ph

160

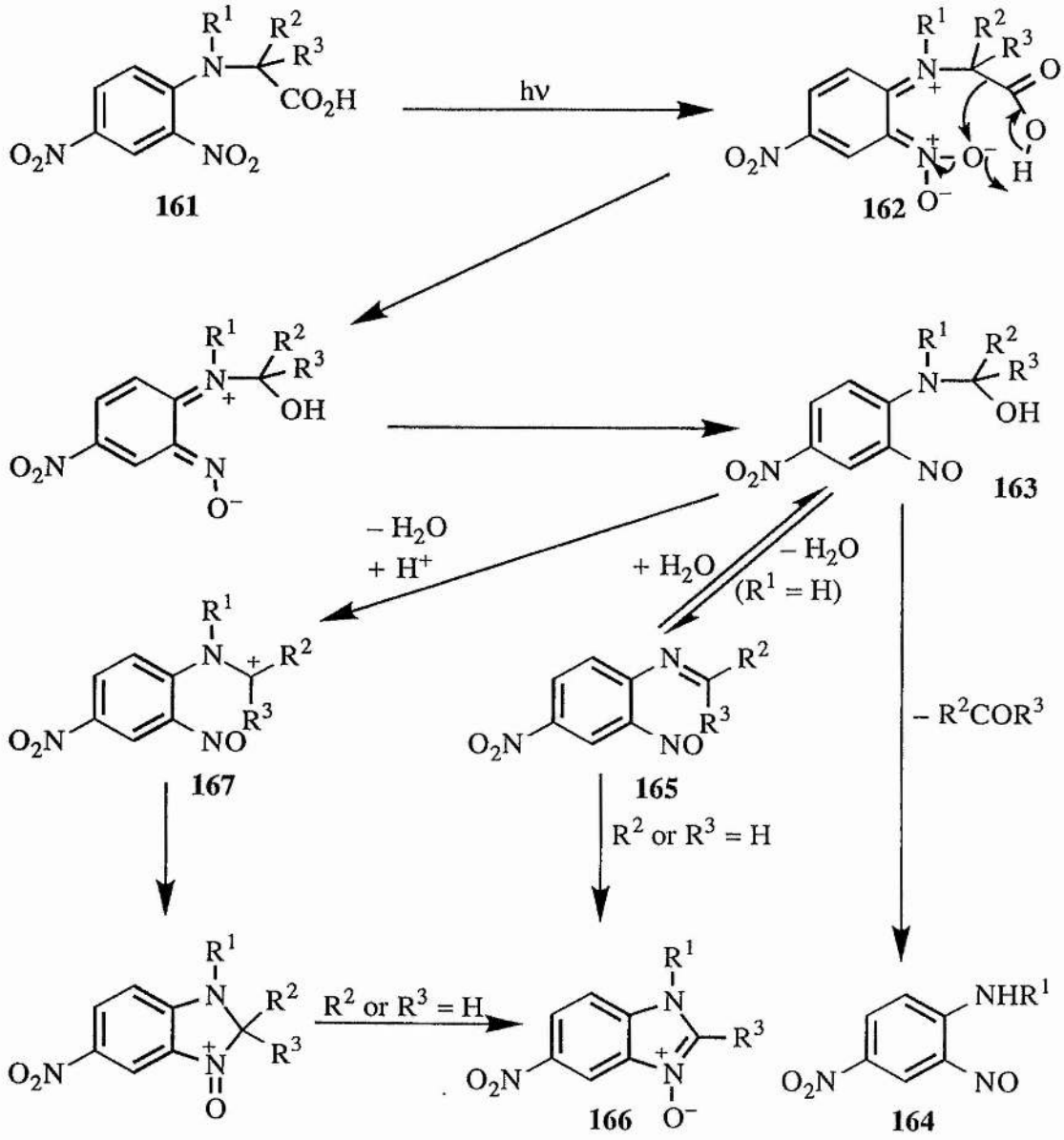
Scheme 2.1

Later work by Pollitt[115] on the photolysis of these compounds in solution showed that this was the case between pH 2 and pH 5, but at pH values above and below these values, the main product was 4-nitro-2-nitrosoaniline (**164**; R^1 = H)), with the amino acid side chain having been cleaved off. Extension of this work[116,117] showed that the reaction is general for DNP-α-amino acids with an α-hydrogen. The benzimidazole *N*-oxides thus formed are stable to further irradiation. *N*-(2,4-Dinitrophenyl)-β-amino

acids and DNP-peptides (also DNP-tryptophan) are decomposed only slowly by light at high temperatures.

Mechanistic proposals by Neadle and Pollitt[117] to account for the apparent pH dependency of the reaction involve the intermediacy of the substituted *N*-hydroxymethyl-2-nitroso-4-nitroaniline (**163**), arising from the decarboxylation of the photo-induced excited state of the starting material (**162**) (Scheme 2.2). Dependency on pH seems reasonable, because the decarboxylation is more likely to occur if the carboxy group is protonated. The intermediate (**163**) then has several possibilities for further reaction, depending on conditions. Direct hydrolysis would lead to the 2-nitroso-4-nitroaniline (**164**) with loss of the disubstituted α-carbon as a ketone or aldehyde. If R = H, reversible dehydration leading to the anil (**165**) would then, if R^2 = H also, result in cyclisation to the 5-nitro-1*H*-benzimidazole 3-oxide (**166**). For sarcosine, however, this route is not feasible since the dehydration step could not occur. Another route could be envisaged whereby the carbocation (**167**) is formed; where R^2 = H, this would then cyclise to the 5-nitro-1*H*-benzimidazole 3-oxide (**166**).

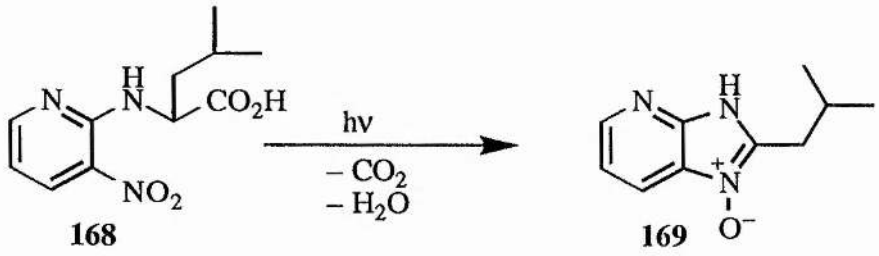
The authors suggest that this is probably the route which operates at very low pH. For the sarcosine derivative, at pH 0 in the dark after rapid photolysis, formation of 1-methyl-5-nitro-1*H*-benzimidazole 3-oxide occurs as a secondary reaction, the initial reaction occurring as a result of the photolysis being formation of 4-nitro-2-nitroso-*N*-methylaniline and extrusion of formaldehyde. This is presumably the same reaction as that described by Russell[118], whereby reaction of 4-nitro-2-nitrosoaniline with an aldehyde furnishes 2-substituted-5-nitro-1*H*-benzimidazole 3-oxides, the 2-substitution depending on the aldehyde used.



Scheme 2.2

The authors also note that in the case of the threonine derivative (**159**) [where R = CH(Me)OH], a mixture of two benzimidazole *N*-oxides (**160**) was isolated, *viz.* where R = CH(Me)OH and where R = H. It was established that no interconversion between the two products occurred, which implied that cleavage of the side chain took place before the cyclisation step.

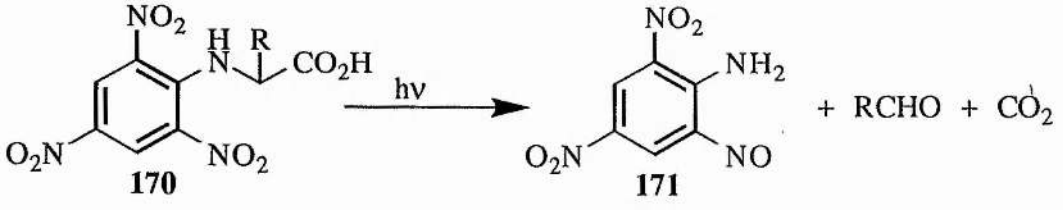
Photolysis of the corresponding pyridylamino acid derivatives (**168**) leads as expected to 2-substituted 3*H*-imidazo[4,5-*b*]pyridine 1-oxides (**169**)[119] with loss of carbon dioxide and water (Scheme 2.3).



168 **169**

Scheme 2.3

Photolysis of *N*-(2,4,6-trinitrophenyl)amino acids (**170**) fails to yield any isolable heterocyclic product. In weakly basic aqueous methanol, photolysis leads only to isolation of 2-nitroso-4,6-dinitroaniline (**171**) and an aldehyde, with loss of carbon dioxide[120] (Scheme 2.4). Photolysis of these compounds in dilute hydrochloric acid was previously reported[121] to lead to picramide (2,4,6-trinitroaniline).

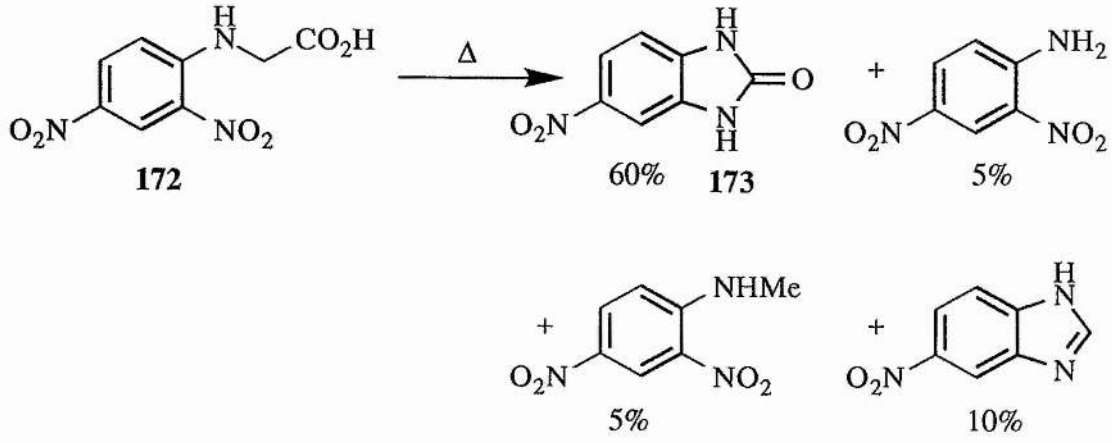


170 **171**

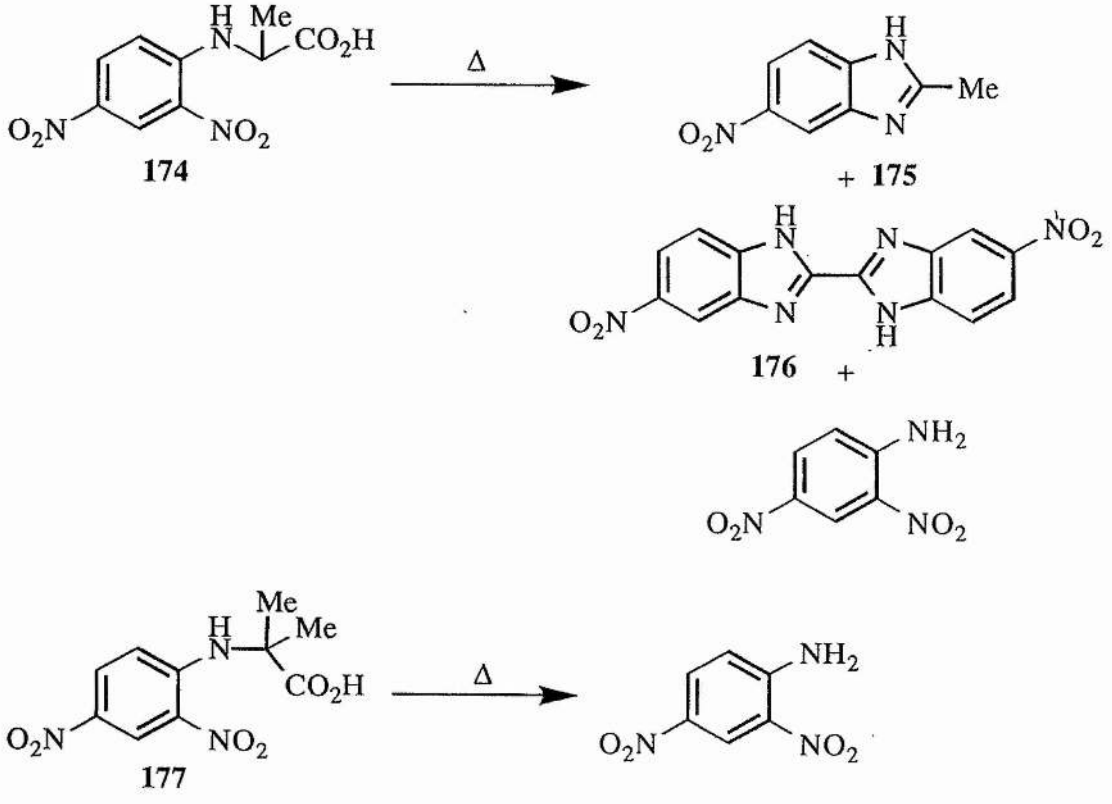
Scheme 2.4

On the other hand, thermolyses of the same starting materials give different mixtures of products[122]; in no case is the benzimidazole *N*-oxide isolated. For example, pyrolysis of DNP-glycine (**172**) at 200 °C gives four isolable products, the main product being 5-nitrobenzimidazolone (**173**) (Scheme 2.5). *N*-Methyl or *N*-phenyl substitution appears not to have a significant effect on product distribution, although α-substitution, for example in the case of DNP-alanine, (**174**) gives mostly 2-methyl-5(6)-nitrobenzimidazole (**175**), along with some of the 2,2'-bibenzimidazolyl (**176**) and

2,4-dinitroaniline (Scheme 2.6). The formation of the bibenzimidazolyl demonstrates the loss of the two methyl groups, which is unusual; the mechanism of this cleavage is unclear. Thermolysis of the corresponding (α,α-disubstituted) 2-methylalanine derivative (**177**) gives 2,4-dinitroaniline as the only identifiable product.

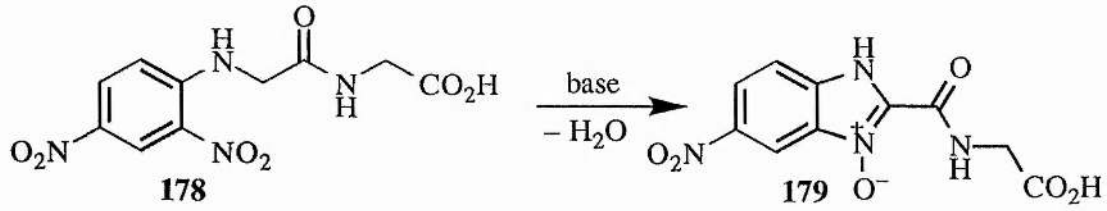


Scheme 2.5



Scheme 2.6

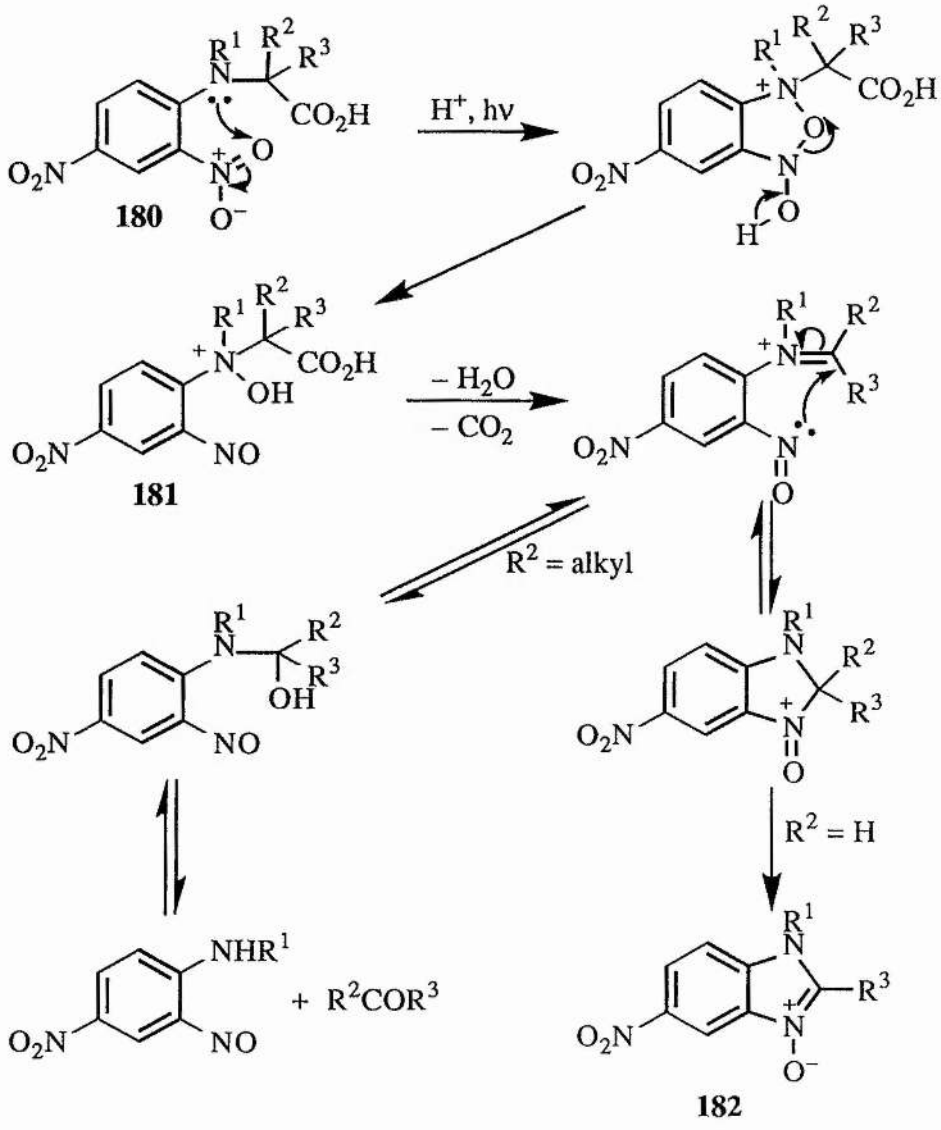
Luetzow and Vercellotti reported[89] the cyclisation of DNP-glycine methyl ester (**105**) under basic conditions to the benzimidazole *N*-oxide (**106**) in 1967 (see Section 1.5.2, on page 32). The same sort of cyclisation arose during the work of Ljublinskaya and Stepanov on peptide sequencing[123]. They demonstrated that DNP-glycylglycine (**178**) cyclised under the basic conditions employed in the sequencing process (triethylamine with carbonate buffer at pH 8.3) to the corresponding 2-substituted benzimidazole *N*-oxide (**179**) (Scheme 2.7). It was also shown that the cyclisation only occurred when the *N*-terminal amino acid was glycine.



178 **179**

Scheme 2.7

An alternative mechanism which has been proposed[124,125] to account for the cyclisations in acidic media of the DNP-derivatives of α-substituted amino acids (**180**) to the 2-substituted benzimidazole *N*-oxides (**182**), involves nucleophilic attack by the amine nitrogen on the oxygen of the nitro group with quenching of the positive charge on the nitrogen of the nitro group. Ring opening to give the nitroso compound (**181**) is followed by decarboxylation and re-cyclisation to the benzimidazole *N*-oxide (Scheme 2.8). The mechanism is diverted when the amino acid is α,α-disubstituted - in this case, the product is the *o*-nitrosoaniline and the alkyl side chains are lost as the appropriate ketone.

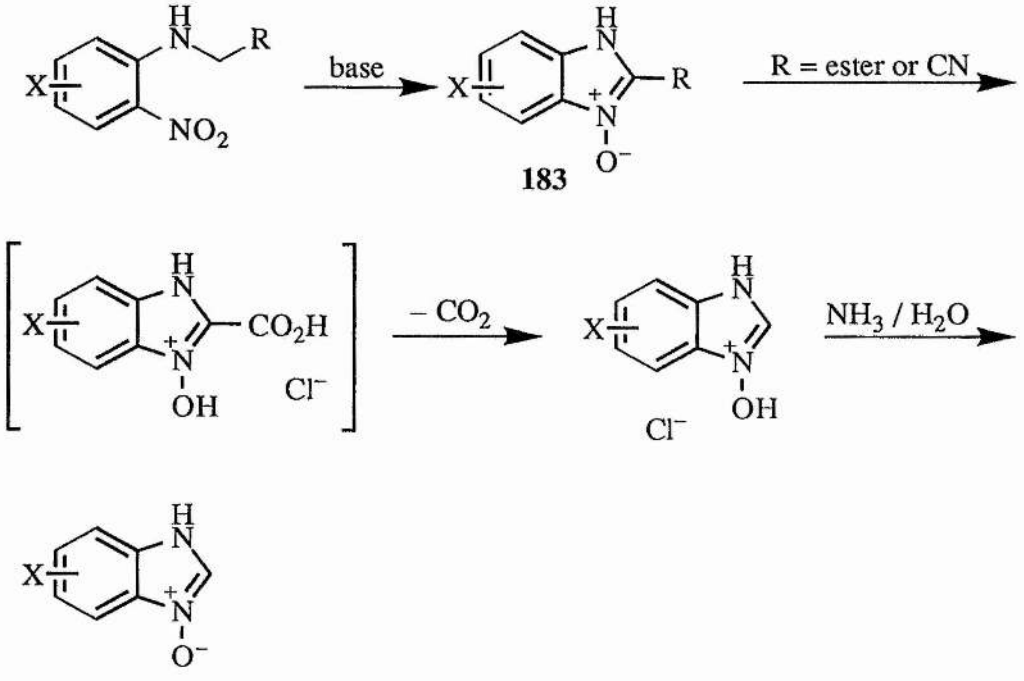


Scheme 2.8

The base-induced cyclisation reaction can be further exploited as a synthetic method leading to 2-unsubstituted benzimidazole *N*-oxides. The cyclisation of amino acid derivatives (including esters and nitriles) under basic conditions has been shown to be a viable route[88,126,127,128]. A general outline of this route is shown in Scheme 2.9 below. The 2-substituted *N*-oxides (**183**) are hydrolysed in either acidic or basic media; it is presumed that the carboxylic acid undergoes spontaneous decarboxylation. Acidic hydrolysis leads directly to the hydrochloride salts of the 2-unsubstituted *N*-oxides,

which can be purified and then the free *N*-oxide isolated by basification using aqueous ammonia.

It should be noted that in the starting materials, the amino nitrogen is secondary; when the nitrogen is tertiary, however, the reaction sequence results in a completely different set of products (Section 2.2).

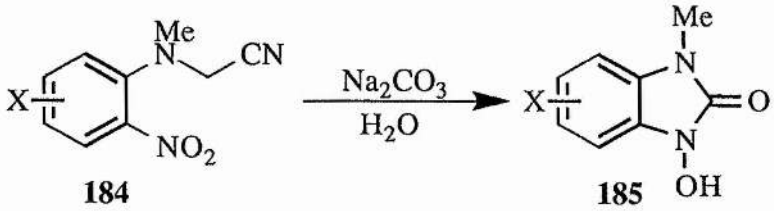


Scheme 2.9

2.2 Mechanistic Discussion

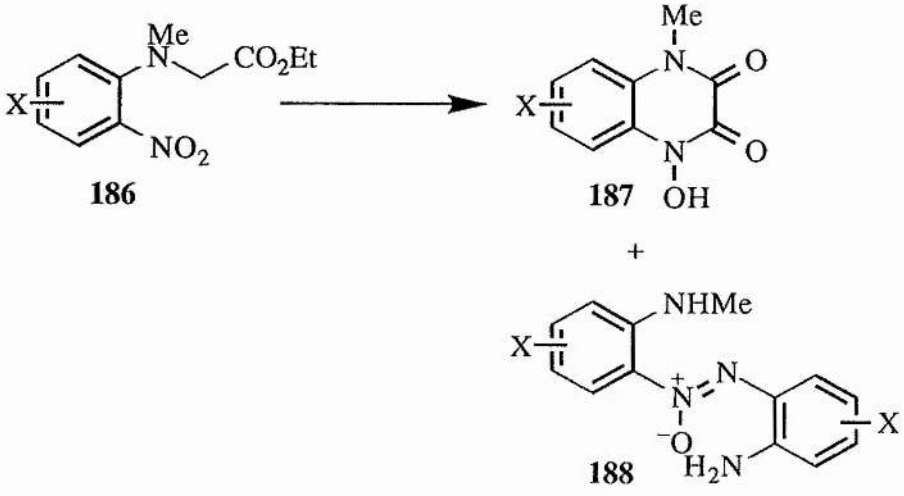
As mentioned above, the amino nitrogen of the starting material in Scheme 2.9 is secondary. When this is tertiary, as in the case of the nitrile (**184**), it was found[128,129] that cyclisation under basic conditions gives the *N*-hydroxybenzimidazolone (**185**) instead of the expected 1-methyl-1*H*-benzimidazole 3-oxide (Scheme 2.10). For the corresponding sarcosine esters (**186**), however, reaction with potassium carbonate affords the 1-hydroxyquinoxaline-2,3(1*H*,4*H*)-dione (**187**), along with some of the

unusual azoxybenzene derivative (**188**)[129] (Scheme 2.11). The structures of both of these were confirmed by X-ray crystallography[130].



184 **185**

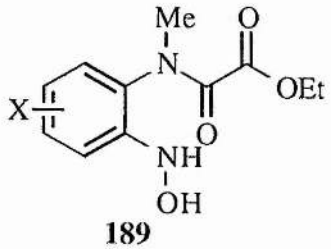
Scheme 2.10



186 **187** **188**

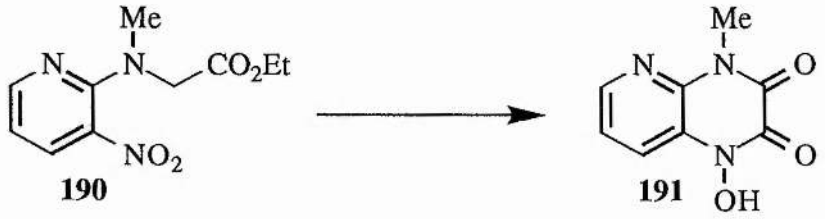
Scheme 2.11

Apparently, the reaction proceeds *via* an internal redox type of mechanism, whereby the CH_2 becomes oxidised and the nitro group becomes reduced to the hydroxylamine. This would imply an intermediate such as the *o*-hydroxylamino anilido ester (**189**), the ester carbonyl of which would then undergo nucleophilic attack by the hydroxylamine to give the 1-hydroxyquinoxaline-2,3-dione (**187**).



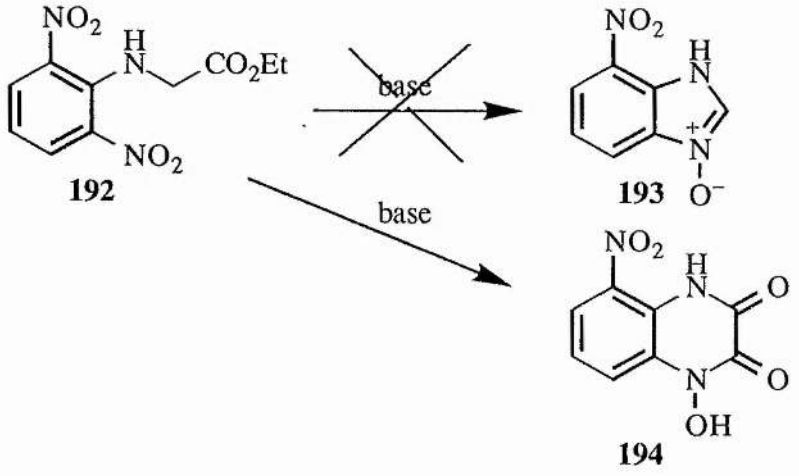
189

The same reaction path is apparently followed by the corresponding nitropyridyl-sarcosine derivative (**190**), which is cyclised to furnish 1-hydroxy-4-methyl-pyrido[2,3-*b*]pyrazine-2,3(1*H*,4*H*)-dione[131] (**191**) (Scheme 2.12).



Scheme 2.12

Previously we have seen a deviation from the course of the "normal" cyclisation when glycine is replaced by sarcosine. However, it is highly significant that the reaction of *N*-(2,6-dinitrophenyl)glycine ethyl ester (**192**) with base (Scheme 2.13) as opposed to that of the *N*-(2,4-dinitrophenyl) derivative does not give the expected 7-nitro-1*H*-benzimidazole 3-oxide (**193**). Instead 1-hydroxy-5-nitroquinoxaline-2,3-dione (**194**) is isolated[132]. Here we now have a deviation from the "normal" reaction with a glycine derivative.



Scheme 2.13

Evidently the difference in the course of the cyclisation is due to the presence of the second *ortho*-nitro group. It is interesting to consider whether the effect of this second

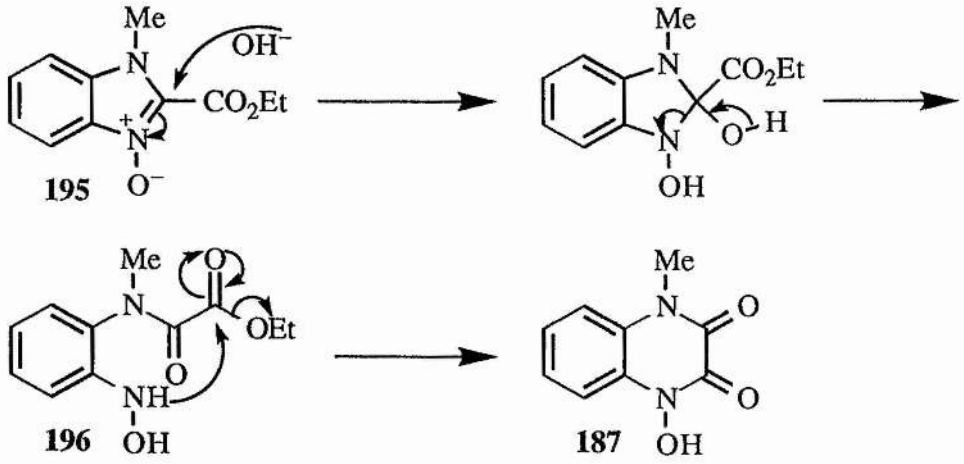
nitro group is steric, perhaps making the NH less accessible to the base, or electronic, perhaps affecting the acidity of the amino proton.

Whereas in Scheme 2.13, the benzimidazole *N*-oxide (**193**) is *not* formed, other 6-substituted derivatives have been cyclised with base and their products investigated[132]. The 6-acetamido- and 6-methyl- glycine derivatives both cyclise "normally" to the benzimidazole *N*-oxide; however, the 6-chloro and 6-trifluoromethyl analogues both give a mixture of the 1-hydroxyquinoxaline-2,3-dione, the benzimidazole *N*-oxide and the azoxy compound (**188**). So it would seem that steric factors may not be responsible for the deviation from the "normal" reaction pathway.

Acidity of the NH appears also to be ruled out, because *N*-(2,4-dinitrophenyl) and *N*-(2,6-dinitrophenyl)glycine esters are cyclised to the benzimidazole *N*-oxide and the hydroxyquinoxaline/benzimidazole *N*-oxide mixture respectively. One would expect the acidity of the NH to be similar in both these cases (both having the same electron-withdrawing groups *ortho*/*ortho* or *ortho*/*para* to the amino substituent), yet the two cyclisations proceed differently. What is clear, however, is that 6-substitution diverts the mechanism to a varying extent.

Nevertheless, the NH does appear to be significant in determining the course of the reaction. The previously accepted condensation mechanism for these cyclisations is shown in Scheme 1.40 (page 27). However, as represented there, the amino hydrogen is involved only after the cyclisation is complete, so it is unclear how *N*-substitution in the case of sarcosine derivatives could affect the course of the reaction so profoundly as to give the quinoxaline-2,3-dione instead of the benzimidazole *N*-oxide. On the basis of this mechanism, one might expect the product of the sarcosine cyclisation to be the 1-methylbenzimidazole 3-oxide (**195**), but this is not the case. It could be supposed that (**195**) is an intermediate in the reaction, which reacts further to give (**196**) (Scheme 2.14). In the absence of an acidic hydrogen, nucleophilic attack could occur at C-2 and

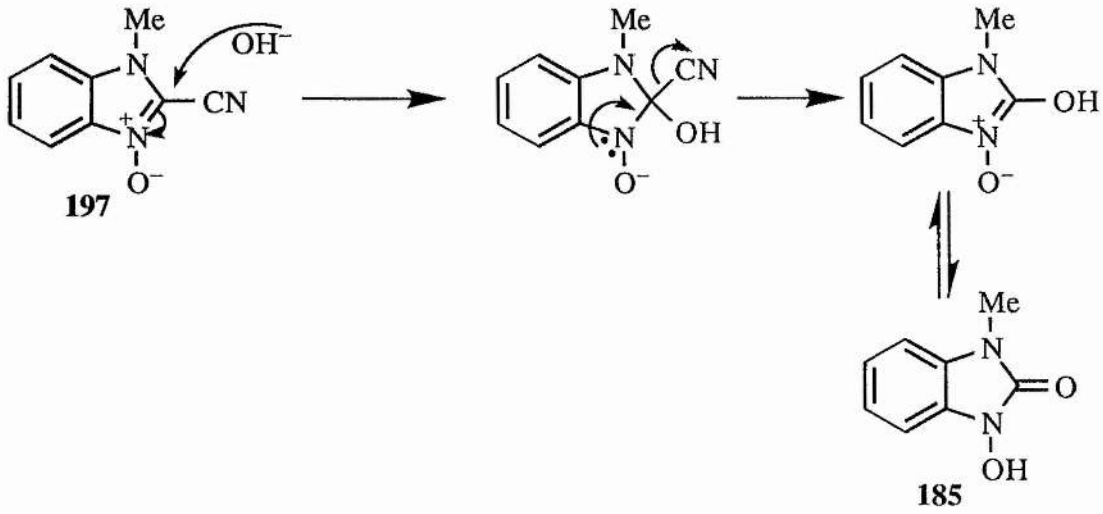
ring opening might follow; the resultant hydroxylamine would then attack the ester carbonyl to give (**187**).



Scheme 2.14

However, the reaction of ethyl 1-methyl-1*H*-benzimidazole-2-carboxylate 3-oxide (**195**) with sodium hydroxide is known[133] to lead to complete loss of the ester function and not to ring opening, so some other explanation of the anomalous behaviour is required.

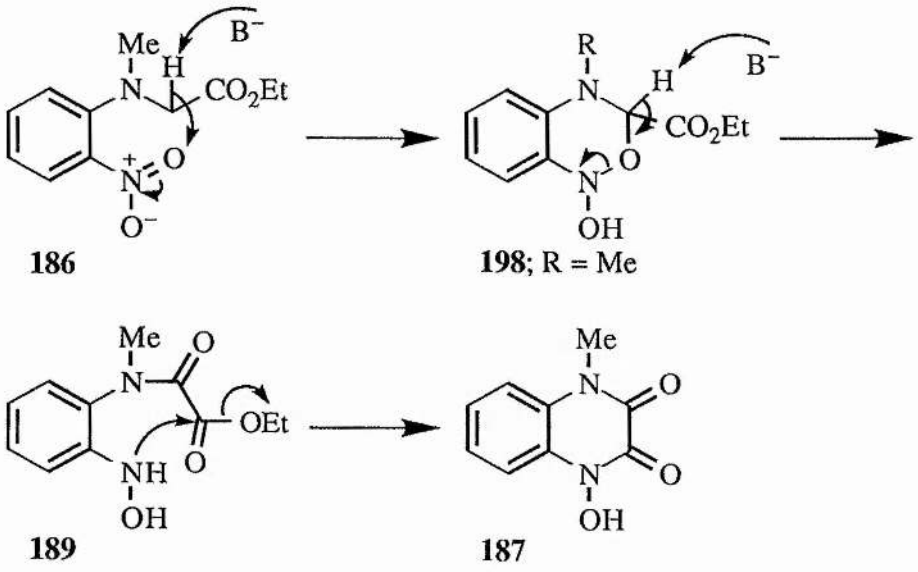
Formation of the benzimidazolone (**185**) could be rationalised similarly, where nucleophilic attack on the 2-cyano-1-methyl-1*H*-benzimidazole 3-oxide (**197**) would lead to (**185**); Scheme 2.15. The non-methylated benzimidazole oxide is not really a genuine *N*-oxide, but a tautomeric system with an acidic hydrogen, so deprotonation occurs in preference to nucleophilic attack and the benzimidazolone is not observed.



Scheme 2.15

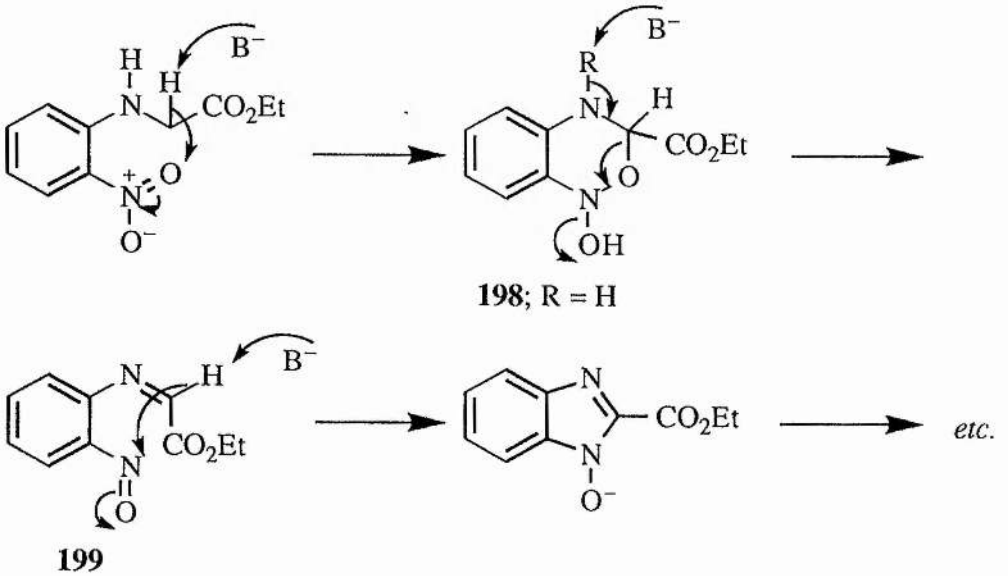
If the cyclisation mechanism involves removal of the amino hydrogen *before* the cyclisation step, then the difference in the course of the cyclisation between the glycine and sarcosine derivatives could be rationalised whereby a common intermediate [a 2,1,4-benzoxadiazine (**198**)] is formed in both cases by initial removal of the α-hydrogen and nucleophilic attack by the resultant carbanionic centre on the *oxygen* of the nitro group.

In the sarcosine-derived benzoxadiazine intermediate there is no amino hydrogen available for abstraction by base, so the second α-hydrogen may then be removed instead with concomitant ring-opening to give the hydroxylamine (**199**). This nucleophile would then be expected to attack the ester carbonyl carbon to form the quinoxaline-2,3-dione (Scheme 2.16).



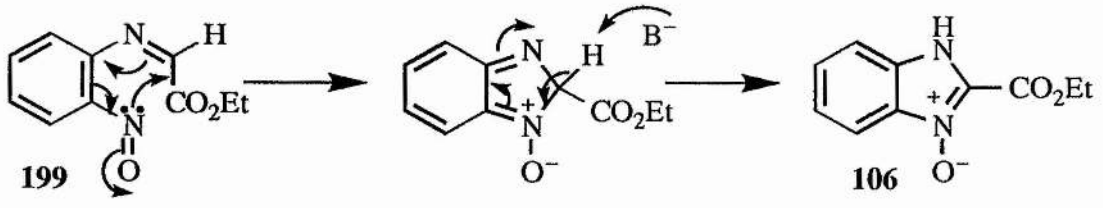
Scheme 2.16

The above mechanism can also be applied to the "normal" cyclisation of the glycine derivatives (ester and nitrile) (Scheme 2.17). In this case, instead of the second α-hydrogen being abstracted by the base, the amino hydrogen is removed to give the *o*-nitrosoanil intermediate (**199**), which then cyclises to the benzimidazole *N*-oxide (isolated as the hydrochloride in the work-up procedure).



Scheme 2.17

Alternatively, given that the α-proton of the *o*-nitrosoanil (**199**) is unlikely to be particularly acidic, the lone pair of electrons on the nitrogen of the nitroso group could be involved in an electrocyclic process breaking up the aromaticity of the benzene ring which would then be followed by tautomerisation to give the product (**106**) directly (Scheme 2.18).



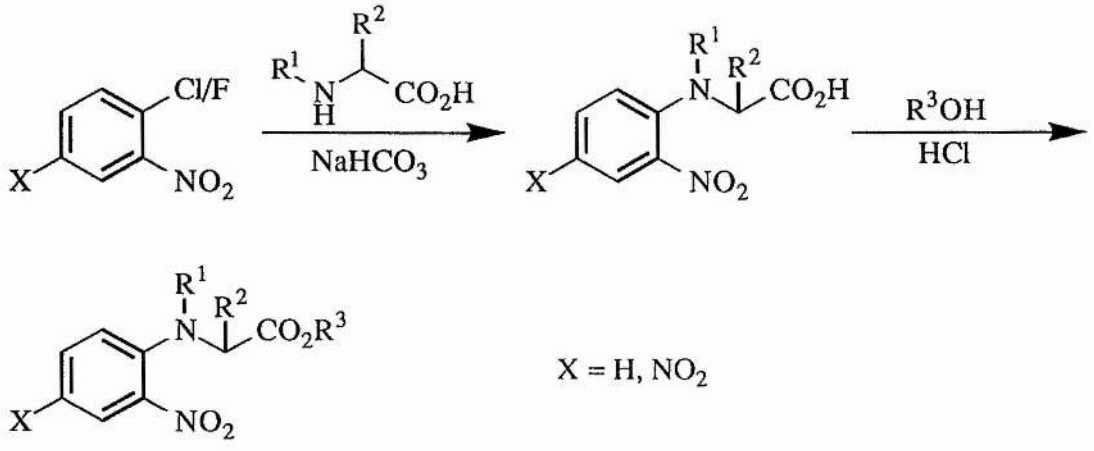
Scheme 2.18

2.3 Results and Discussion

The intention of the following work was to investigate the reactions of *N*-(*o*-nitrophenyl) derivatives of α-substituted amino acid esters with base, the hope being to elucidate the mechanism of the cyclisation reaction so that perhaps some intermediates could be isolated. It should be noted that either the racemic mixture (*R*,*S*) or the single (*S*)-enantiomer of the amino acids were used, depending solely on their availability (specified in the Experimental section). It was not considered important which of these was used, as the configuration of the stereogenic centre would not be retained during cyclisation, and the geometry was not expected to affect the course of the reaction.

Synthesis of the DNP derivatives of the amino acids was generally carried out by reacting the amino acid with either chloro- or fluoro-2,4-dinitrobenzene in refluxing aqueous ethanol; esterification of the acid was carried out by heating under reflux in the appropriate dry alcohol containing dry hydrogen chloride gas (Scheme 2.19). The 2-nitrophenyl derivatives were prepared similarly, although only 2-fluoronitrobenzene

was used as the starting material since 2-chloronitrobenzene is not reactive enough towards these particular nucleophiles.



Scheme 2.19

Given the relative ease with which DNP-amino acids are prepared by the well-established method of Sanger[134], and their usefulness in peptide sequencing, it is perhaps surprising that in fact very few of the esters prepared during the course of this work have previously been reported in the literature.

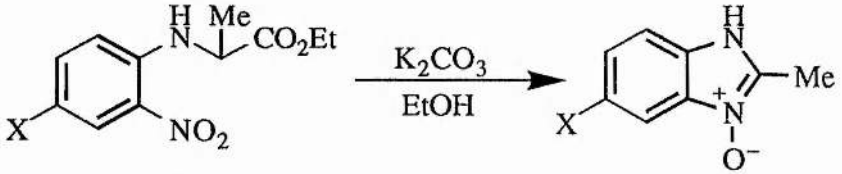
2.3.1 Alanine Derivatives

The alanine derivatives described below were prepared in order to probe further the reactions of amino acid derivatives with base - it was thought that perhaps the cyclisation might be intercepted at some stage because of the fact that there was one less α-hydrogen to be involved in the mechanism and therefore a diversion of the reaction pathway might occur.

Synthesis of both the DNP and *N*-(2-nitrophenyl) derivatives of alanine and their ethyl esters (**200a** and **b**) proved straightforward. However, the melting point obtained for DNP-alanine ethyl ester (**200b**) (105 °C) was much higher than that quoted in the literature (60 °C)[135], which was curious given that all the other data were consistent with

the proposed structure. It is possible that this discrepancy could be due to a different crystalline form being obtained.

Reaction of both the DNP and *N*-(2-nitrophenyl) derivatives of alanine ethyl ester with ethanolic potassium carbonate resulted in isolation of the corresponding 2-methyl-1*H*-benzimidazole 3-oxides (**201a** and **b**) (Scheme 2.20) in 30-40% yield. The ester group is lost in preference to the α-methyl group. The reaction of the *o*-nitrophenyl derivative also yielded some *N*-(2-nitrophenyl)alanine, resulting from simple hydrolysis of the ester to acid.



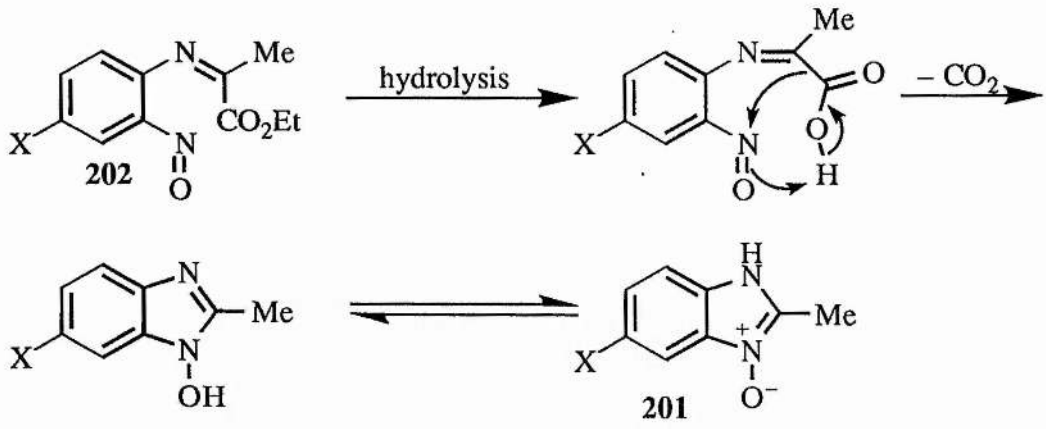
200a; X = H
200b; X = NO_2

201a; X = H
201b; X= NO_2

Scheme 2.20

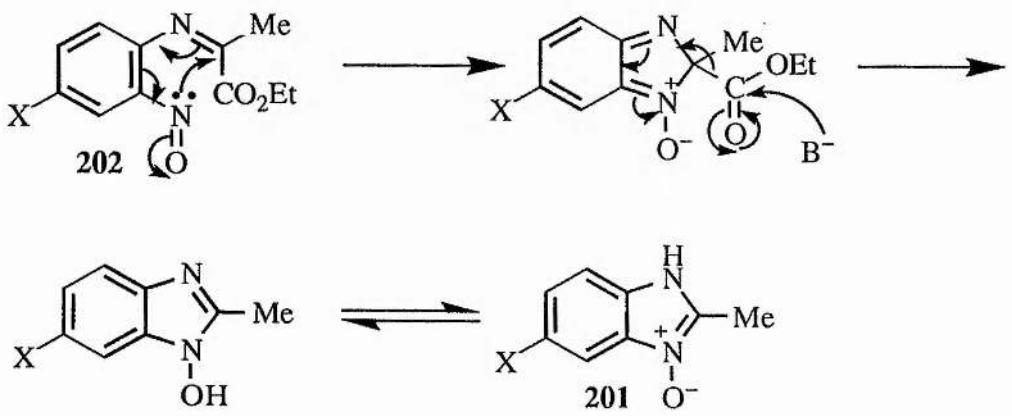
A simple condensation mechanism now seems even more unlikely than in the case of the glycine derivatives, because the α-methyl group, having a positive inductive effect, will make formation of the methine-derived carbanion less facile - in other words, the carbanion would be less stable than in the glycine case. There is ample precedent for methyl groups attached to the carbanionic centre making compounds less acidic[136]. There is little doubt that the most acidic hydrogen in (**200**) should be the amino proton, especially given the considerable mesomeric effect of the *ortho*-nitro group, in addition to the *para*-nitro group in (**200b**).

This result could be rationalised by a simple extension of the proposed mechanism of Scheme 2.17. Formation of the *o*-nitrosoanil (**202**) could be followed by ester hydrolysis and decarboxylation with concomitant cyclisation (Scheme 2.21).



Scheme 2.21

However, it is in fact not necessary to invoke hydrolysis of the ester at all. Instead, cyclisation of the *o*-nitrosoanil (**202**) by an electrocyclic process could be envisaged (*cf.* Scheme 2.18), followed by nucleophilic attack on the ester carbonyl leading to the product (**201**) (Scheme 2.22). It is unlikely that the ester group is lost before cyclisation, because a carbanion would consequently have no stability - it is known that *N*-ethyl-*o*-nitroaniline does not react under these conditions[137]. The ester group is retained in the case of the glycine analogue because of the presence of the remaining acidic proton instead of the methyl group in the tetrahedral 2-position.



Scheme 2.22

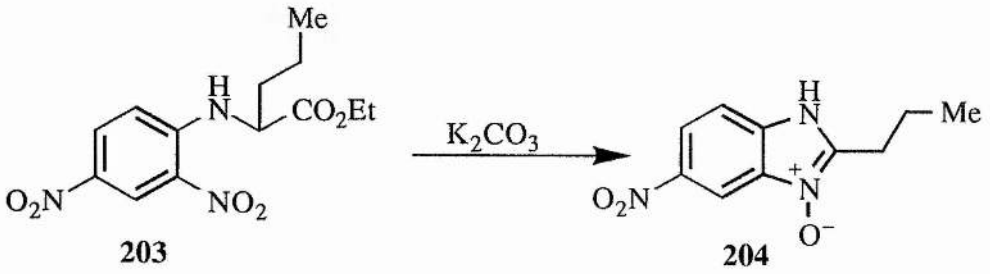
Although the yields for this process are not particularly high (30-40%), they are acceptable in light of the fact that this is the most useful route towards 2-alkyl

benzimidazole *N*-oxides, and is reliable. The other alternative is reductive cyclisation of *o*-nitroanilides, but as discussed in Section 1.4.1, this route is less reliable because it is difficult to control the extent to which the nitro group is reduced, and it is also not compatible with the presence of other reducible groups.

Attempted reaction of the esters with triethylamine as the base in ethanol resulted only in recovery of starting material.

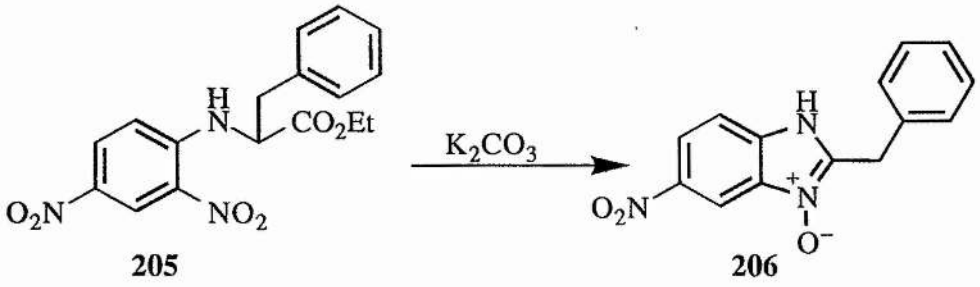
2.3.2 Norvaline and Phenylalanine Derivatives

Similarly to the cyclisation of the alanine derivatives, both DNP-norvaline and DNP-phenylalanine ethyl esters (**203**) and (**205**) are cyclised to the corresponding 2-propyl- and 2-benzyl-1*H*-benzimidazole 3-oxides respectively [(**204**) and (**206**); Schemes 2.23 and 2.24]. It is likely here that the same mechanism applies as for the alanine cyclisations, although again it is not entirely clear at what stage the ester group is lost, or indeed whether ester hydrolyis occurs. Ester hydrolysis would be expected in the basic conditions of the reaction if there were water present, but dry ethanol (dried by its reaction with magnesium and iodine) was used as the solvent for the cyclisation reactions, so whether there would be enough water present for this to occur is questionable.



203 **204**

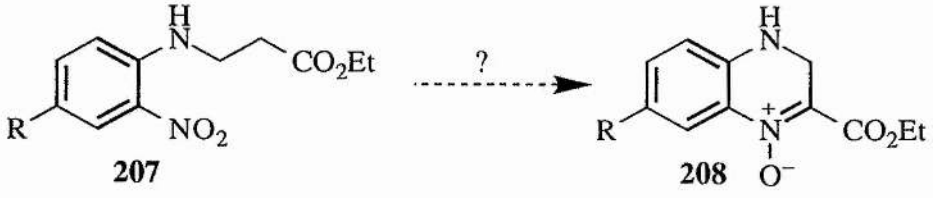
Scheme 2.23



Scheme 2.24

2.3.3 β-Alanine Derivatives

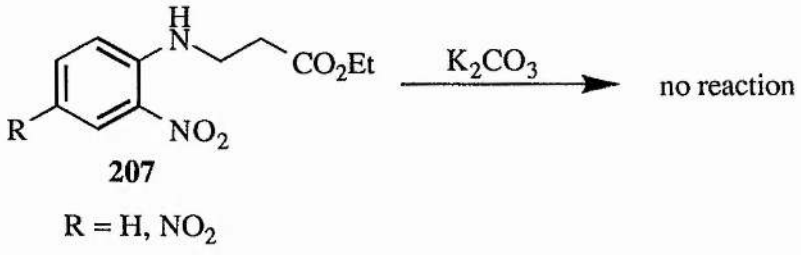
Synthesis of the β-alanine derivatives (**207**) was undertaken in order to assess whether separating the acidic methylene group from the amino group would lead to a six-membered ring being formed [*i.e.* giving a quinoxaline derivative (**208**); Scheme 2.25], as would be expected if the simple condensation mechanism were operating.



Scheme 2.25

However, reaction of both the DNP- and *N*-(2-nitrophenyl) derivatives of β-alanine ethyl ester with ethanolic potassium carbonate resulted only in isolation of a mixture of the unchanged ester and its hydrolysis product [for DNP-, 76% of the acid and 17% of the ester; for *N*-(2-nitrophenyl)-, 39% of the acid and 42% of the ester], with no cyclised products being found (Scheme 2.26). These results suggest that the extra methylene group separating the amino and ester functionalities in β-alanine prevents any reaction from occurring other than hydrolysis of the ester. This in turn implies that a simple condensation mechanism may not be operating in these types of reactions since there is no obvious difference in the potential acidity of the CH_2 groups between (**207**) and its glycine analogue. It could be suggested, however, that cyclisation does not take place

due to there being less of a driving force for the process as the quinoxaline derivative (**208**) would not have the extra stability of an aromatic system.

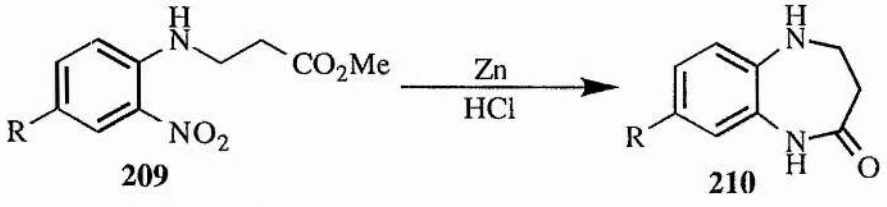


207

R = H, NO_2

Scheme 2.26

Interestingly, β-alanine derivatives have been cyclised to heterocyclic products (Scheme 2.27), although this procedure involves reduction. For example, *N*-(*o*-nitrophenyl)-β-alanine methyl esters (**209**) are cyclised under dissolving metal reduction conditions (heating with zinc in hydrochloric acid) to give 8-substituted-1,2,3,4-tetrahydro-3*H*-1,5-benzodiazepin-2-ones (**210**)[138]. The yields for this process depend on the R group: for R = H, 48%; R = OMe, 51%; R = NO_2, 79%. Once again, no *N*-oxide is obtained by this reductive procedure.



209 **210**

R = H, OMe, NO_2

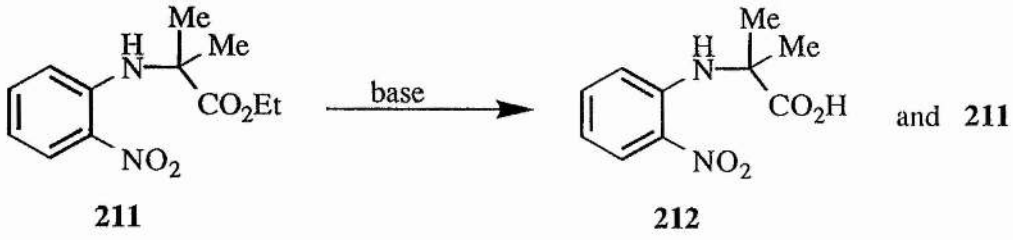
Scheme 2.27

2.3.4 2-Methylalanine Derivatives

Given that the cyclisation reaction is considerably diverted when the NH is not present (*i.e.* the sarcosine derivatives discussed on page 58), the 2-methylalanine derivatives (**211**) were prepared and cyclisation attempted in order to see if a corresponding

diversion occurred when the α-position (bearing two methyl groups) instead of the amine was blocked from reaction. The likelihood of there being a reaction between the amine and the *o*-nitro group was therefore probed.

The *N*-(2-nitrophenyl) derivative of 2-methylalanine ethyl ester (**211**) was prepared in the usual manner, but again, no reaction with potassium carbonate occurred (Scheme 2.28), and the starting material was recovered in 90% yield, along with 10% of the acid (**212**) resulting from hydrolyis of the ester. Reaction with sodium ethoxide as the base similarly yielded both the starting ester (40%) and the acid (60%). Clearly, the presence of at least one α-hydrogen is required for any sort of cyclisation to take place, the only reaction observed in this case being simple hydrolysis of the ester, and no obvious reaction between the amine and the nitro group was observed.



211 **212**

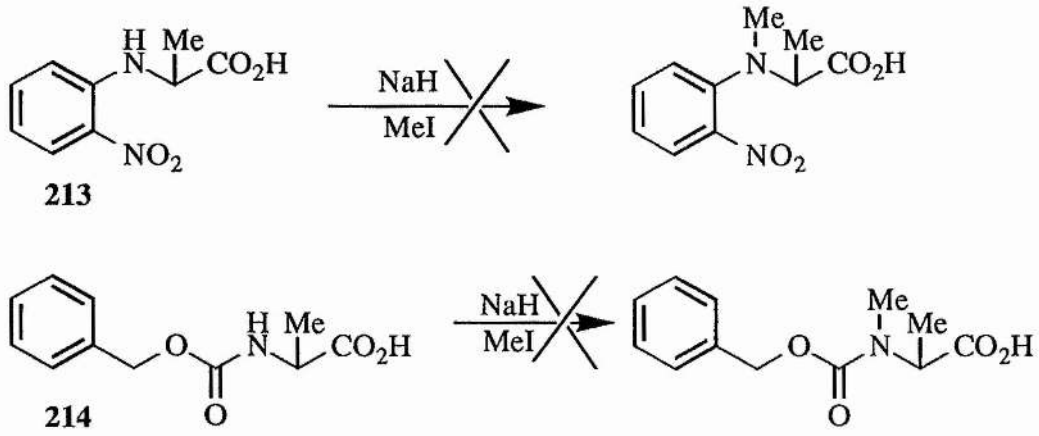
Scheme 2.28

2.3.5 *N*-Methylalanine Derivatives

It was desirable to determine the effect on cyclisation of having methyl groups on both the nitrogen and the α-carbon. *N*-Methylalanine is available commercially, but is prohibitively expensive, so a synthesis was undertaken from alanine itself.

Attempts were made to *N*-methylate directly both *N*-(2-nitrophenyl)alanine (**213**) (by reaction with methyl iodide and sodium hydride) and *N*-benzyloxycarbonylalanine (**214**) (by the method of McDermott and Benoiton[139], also using methyl iodide and sodium hydride), but no more than 20% methylation could be achieved [in the case of the

N-benzyloxycarbonyl-protected amino acid (**214**)], and no methylation at all took place with *N*-(2-nitrophenyl)alanine (**213**) (Scheme 2.29). These failures were presumably due to the poor nucleophilicity of the amino group (because of the negative mesomeric effect of the *o*-nitro group in one case, and in the other because the amino nitrogen is part of a carbamate group).



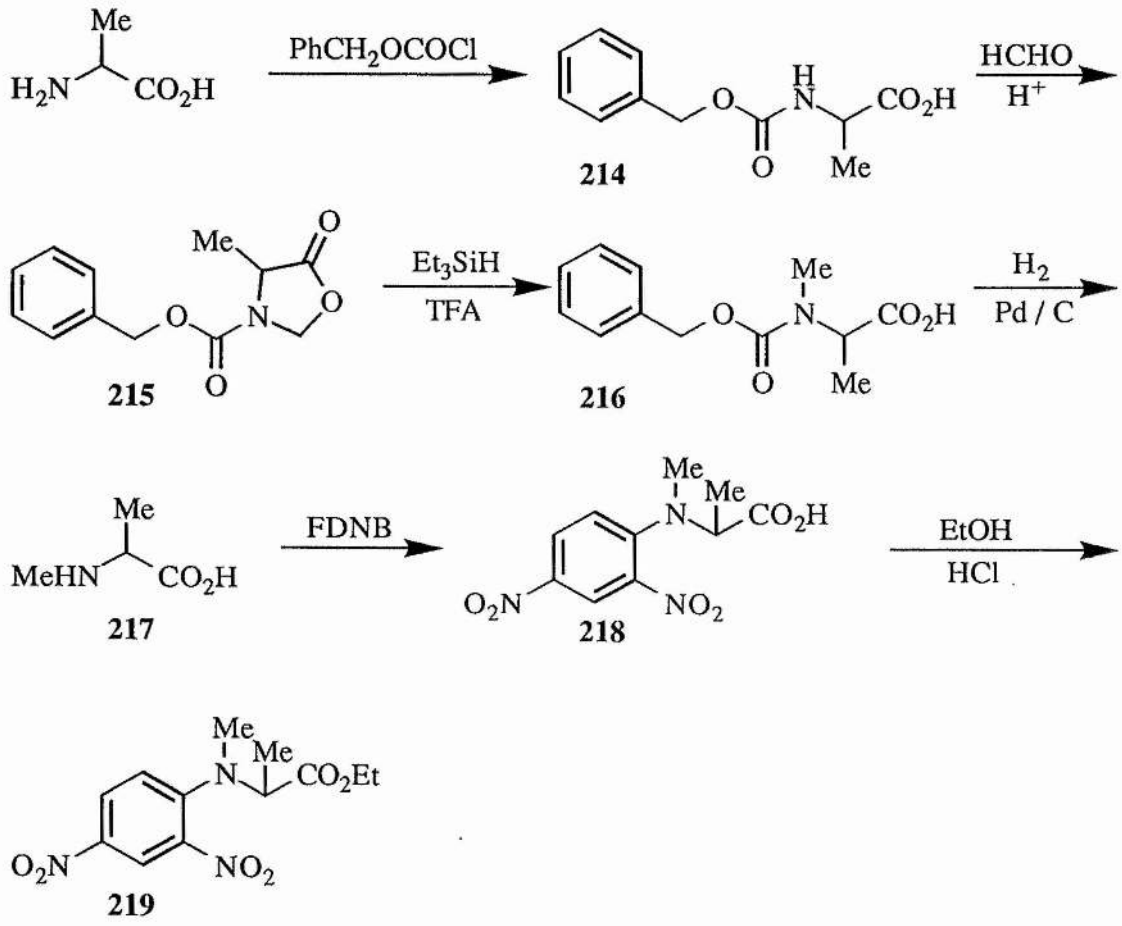
Scheme 2.29

The synthesis therefore followed the procedure of Freidinger *et al.*[140] [substituting the benzyloxycarbonyl group (Z) for the fluorenylmethoxycarbonyl (Fmoc) group], outlined in Scheme 2.30.

Alanine was Z-protected (by reacting with benzyl chloroformate in pyridine[141]), then reacted with paraformaldehyde (in refluxing toluene with a catalytic amount of *p*-toluenesulphonic acid and removal of water using Dean-Stark apparatus) to give the oxazolidinone (**215**). Reductive cleavage of this heterocycle by ionic hydrogenation (using triethylsilane as the hydride donor and trifluoroacetic acid as the proton donor)[142] resulted in the Z-protected *N*-methylated amino acid (**216**). Deprotection to give *N*-methylalanine (**217**) was effected by hydrogenolysis (over palladium/charcoal).

Reaction with fluoro-2,4-dinitrobenzene was then followed by esterification, although a number of problems were encountered in the purification of the ester, for example

hydrolytic loss of the amino acid side chain to give 2,4-dinitrophenol. Distillation of the reaction mixture residue (which was an oil) also appeared to result in hydrolysis of the ester, as the proportion of starting material present in the distillate was greatly increased. Purification was finally achieved by recrystallising the distillate from ethanol, although the yield by this stage was very low (6%).



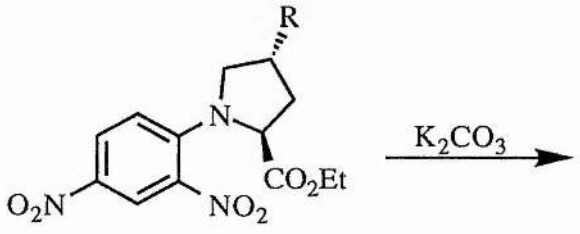
Scheme 2.30

Reaction of DNP-*N*-methylalanine ethyl ester (**219**) with ethanolic potassium carbonate was carried out under the normal conditions, but unfortunately only a complex mixture of compounds (at least 9 spots were observed by tlc) was recovered, none of the components of which could be identified.

2.3.6 Proline Derivatives

These derivatives were prepared as cyclic analogues of *N*-methylalanine, with a view to the preparation of tricyclic heterocycles. Difficulties were encountered in the purification of DNP-proline ethyl ester (**221**); the *trans*-4-hydroxyproline analogue (**222**) proved to be easier to handle. The hydroxyl group would also lend itself to the introduction of further functionality in any heterocyclic products formed.

However, reaction of both the DNP-proline derivatives (**221**) and (**222**) with ethanolic potassium carbonate (Scheme 2.31) similarly failed to yield any identifiable cyclised products. Compound (**221**) was also reacted with sodium ethoxide, but again, no products were isolable from the reaction mixture.

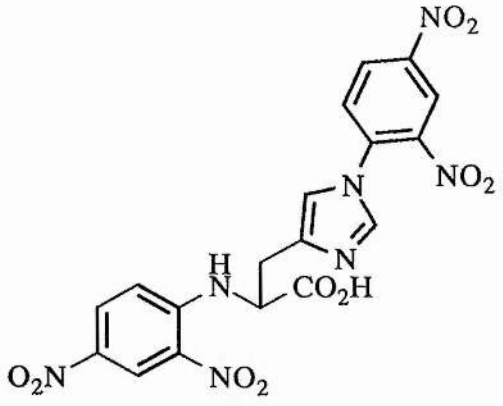


221; R = H
222; R = OH

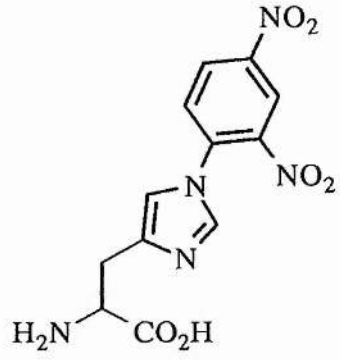
Scheme 2.31

2.3.7 Histidine Derivatives

N(α)-(2,4-Dinitrophenyl)histidine ethyl ester (**225**) was prepared as before. The mono-substituted product was obtained without difficulty by using one equivalent of fluoro-2,4-dinitrobenzene; neither the bis-DNP compound (**223**) nor the *N*(1-(2,4-dinitro-phenyl) compound (**224**) was observed. Evidently the amine nitrogen was more nucleophilic than the imidazole nitrogen.

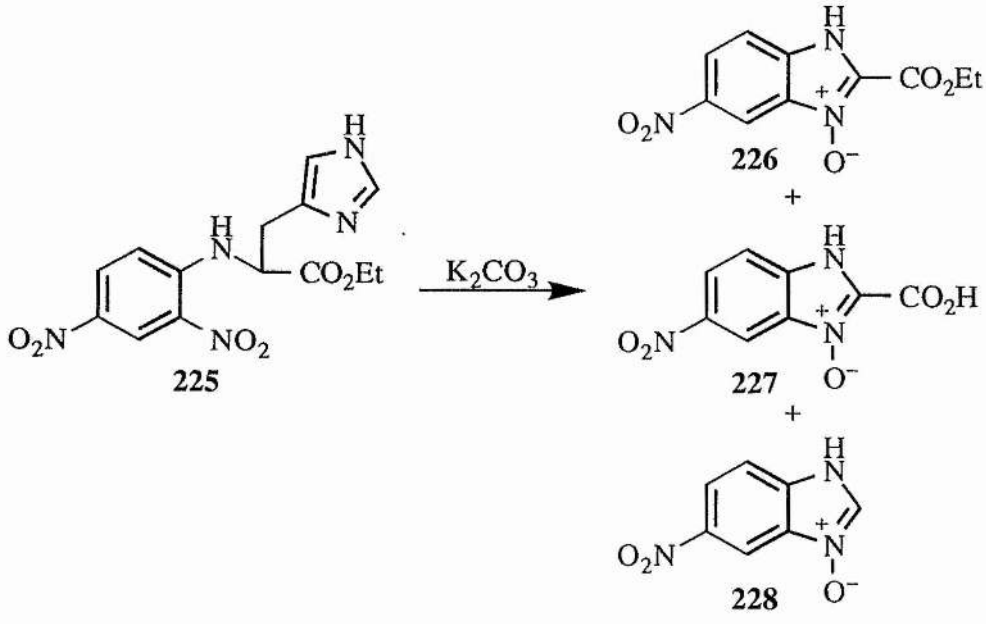


223



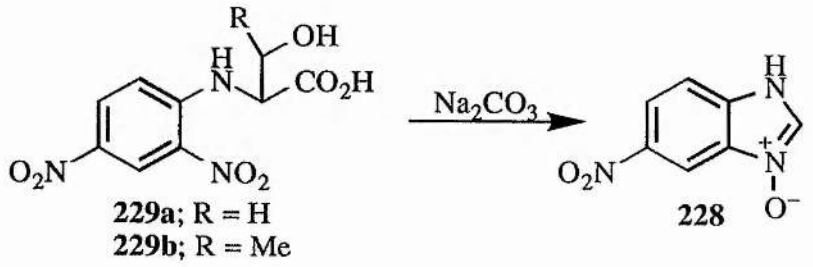
224

Cyclisation with potassium carbonate in ethanol produced a mixture of 5-nitro-1*H*-benzimidazole-2-carboxylic acid 3-oxide (**227**) and the corresponding ethyl ester (**226**) , as well as some 2-unsubstituted benzimidazole *N*-oxide (**228**, with complete loss of the imidazolylmethyl side chain from the products (Scheme 2.32). One experiment yielded 30% of the ester and 38% of the acid; another yielded 26% of the ester, 47% of the acid, and 6% of the 2-unsubstituted product.

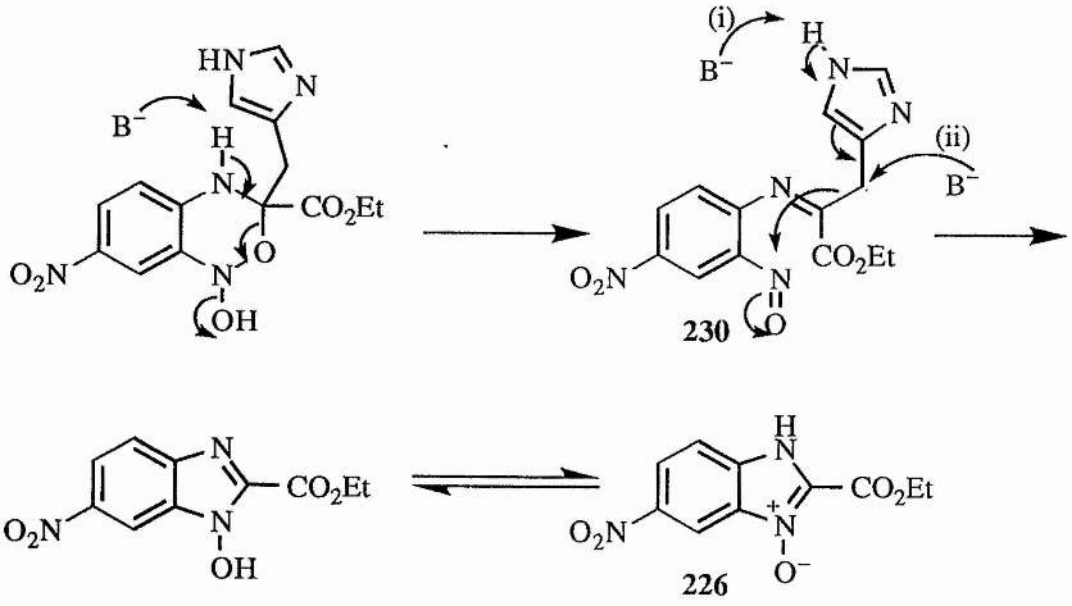


Scheme 2.32

The imidazolyl side chain was presumed to be cleaved in a manner analogous to that of the serine and threonine derivatives (**229a** and **b**) described by Luetzow and Vercellotti[89]. They propose that DNP-serine undergoes a retro-aldol cleavage under the basic conditions of the reaction to give the glycine derivative, which then cyclises to the benzimidazole *N*-oxide (**228**) (Scheme 2.33). However, the imidazolylmethyl side chain in this reaction of the histidine derivative could presumably also be cleaved at some other stage during the cyclisation process, for example from the *o*-nitrosoanil intermediate (**230**) by abstraction of the imidazole NH [route (i)] as shown in Scheme 2.34. Alternatively, nucleophilic attack by the base on the methylene carbon could displace the heterocycle as a leaving group [route (ii)].



Scheme 2.33



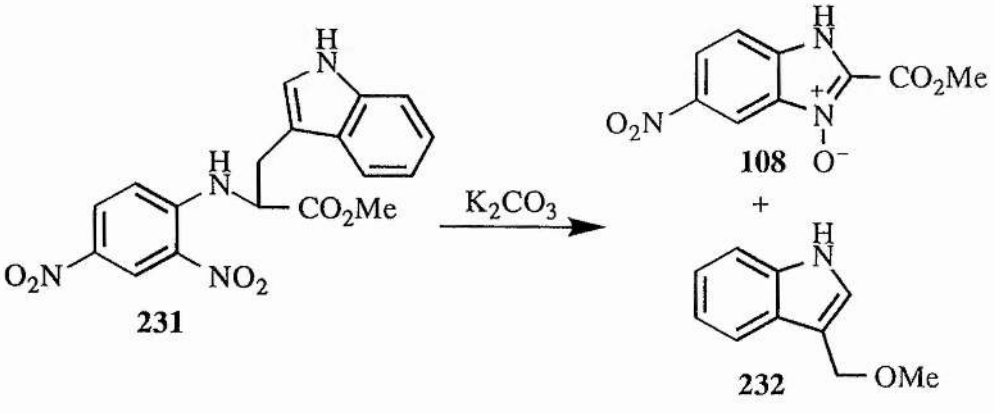
Scheme 2.34

Unfortunately, however, the imidazole-containing moiety was never successfully isolated - its presence in a small amount of impure oil was detected, but identification proved elusive.

2.3.8 Tryptophan Derivatives

It was hoped that cyclisation of tryptophan derivatives would lead to benzimidazole *N*-oxides with the indole moiety in position 2 of the heterocycle. Synthesis of DNP-tryptophan was straightforward, but esterification proved to be problematic, in that only very low yields of its hydrochloride salt could be obtained, from a complex mixture of products separated by chromatography. *N*-(2,4-Dinitrophenyl)tryptophan methyl ester (**231**) was therefore prepared directly from fluoro-2,4-dinitrobenzene and tryptophan methyl ester hydrochloride in 98% yield.

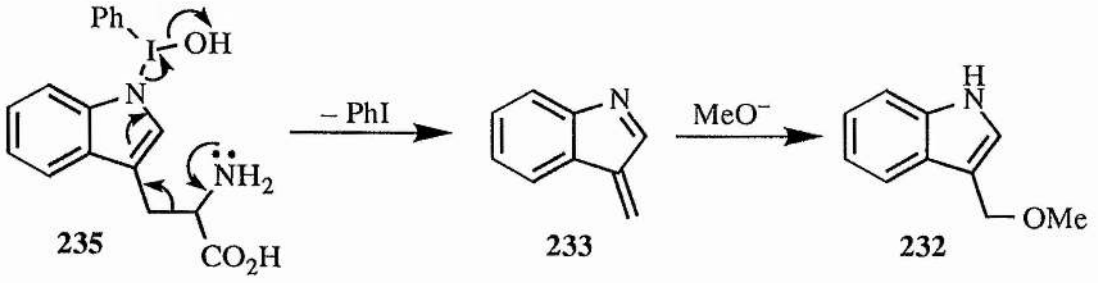
Reaction of DNP-tryptophan methyl ester with methanolic potassium carbonate resulted in isolation of methyl 5-nitro-1*H*-benzimidazole-2-carboxylate 3-oxide (**108**) in 53% yield (Scheme 2.35). No cyclised product containing the indole moiety could be found; further investigation, however, led to recovery of 3-(methoxymethyl)indole (**232**) (in 69% yield) as a buff-coloured water-insoluble solid.



Scheme 2.35

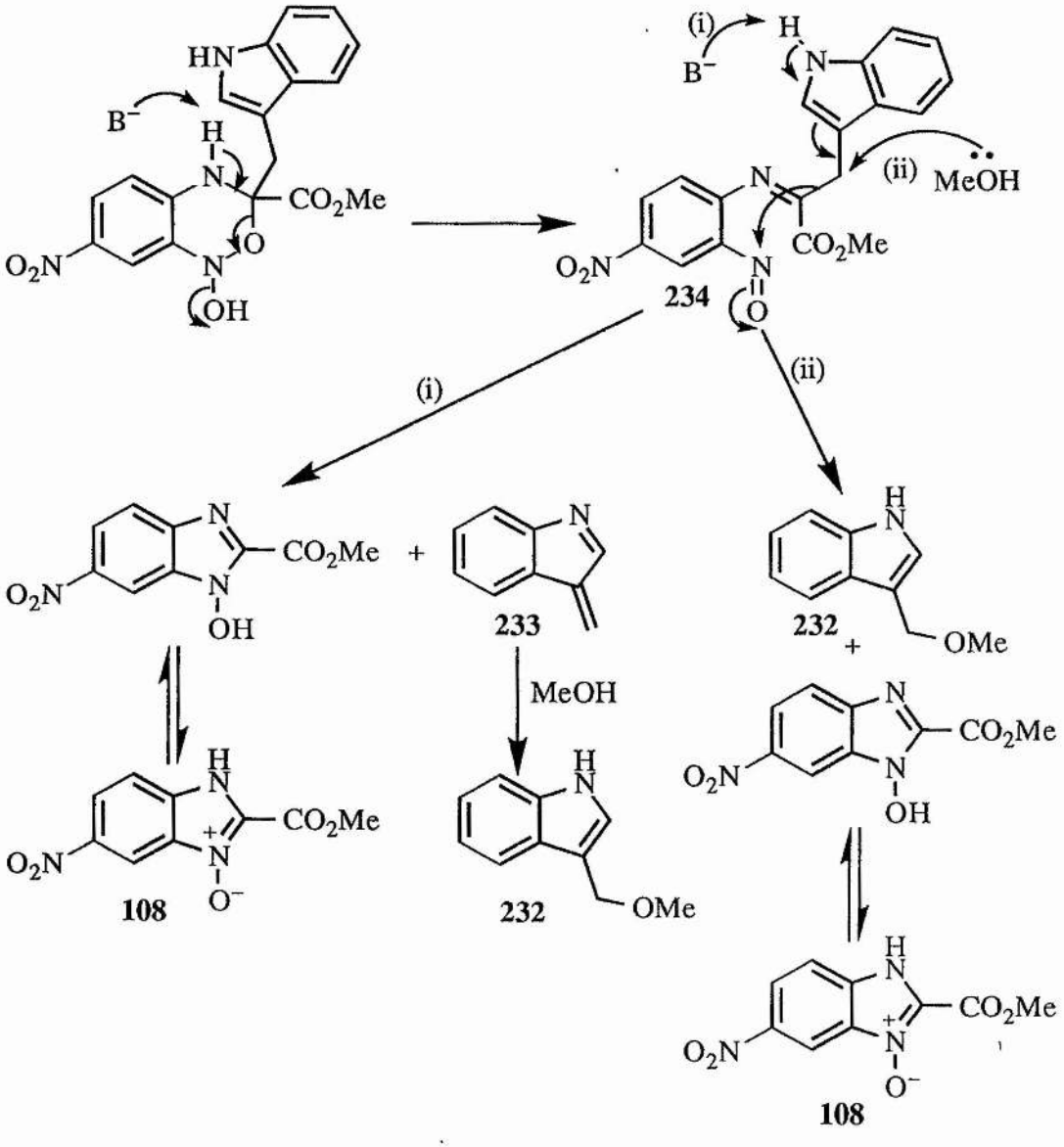
Cleavage of the amino acid side chain could be rationalised by a retro-aldol scission similar to that envisaged for the histidine (**225**), serine (**229a**) and threonine (**229b**) derivatives. The final co-product would then arise from nucleophilic Michael-like attack of methanol on the exocyclic double bond of (**233**) [if route (i) is followed]. If route (ii) is followed, nucleophilic attack of methanol on the CH_2 of the side chain of the *o*-nitrosoanil (**234**) would lead to loss of the product (**108**) as a leaving group and 3-(methoxymethyl)indole (**232**) directly (Scheme 2.37). The fact that the yields for the benzimidazole and indole fragments are not greatly dissimilar suggests that the fragmentation occurs at a late stage in the mechanism as depicted, rather than (as suggested by Luetzow and Vercellotti) as an initial step with the formation of the glycine derivative which then undergoes cyclisation.

Cleavage of the tryptophan side chain in this manner has been previously described[143] in the literature. Reaction of tryptophan itself with methanolic potassium hydroxide and $PhI(OAc)_2$ (a source of hypervalent iodine) to give (**235**) results in formation of 3-(methoxymethyl)indole (**232**) and iodobenzene. This β-cleavage of the side chain was claimed by the authors to have been previously unobserved in chemical systems, although known in biological systems. The mechanism they suggest (Scheme 2.36) also involves the nucleophilic attack of methanol on the exocyclic double bond of (**232**). However, the authors do not describe the fate of the amino acid residue.



235 233 232

Scheme 2.36

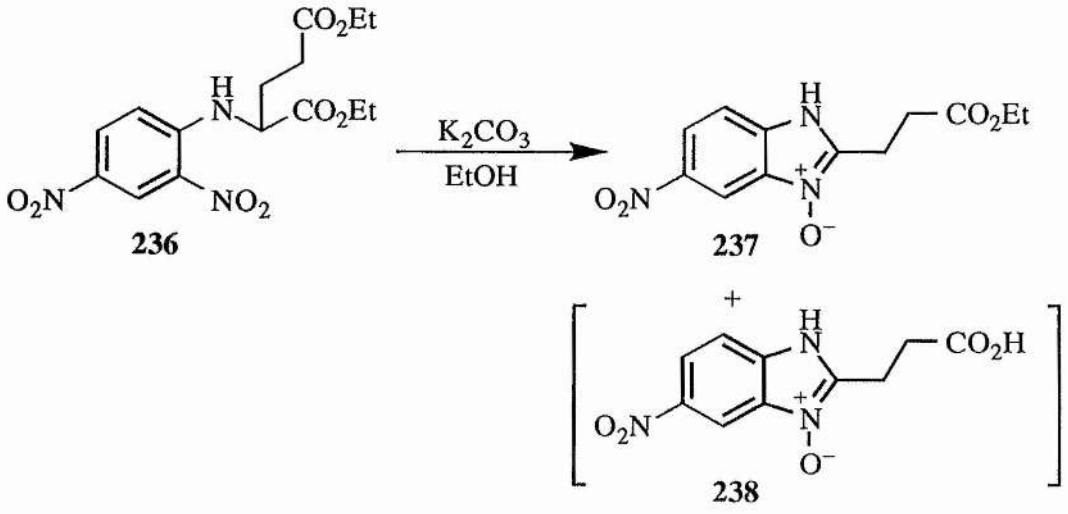


Scheme 2.37

2.3.9 Glutamic Acid Derivatives

Diethyl *N*-(2,4-dinitrophenyl)glutamate (**236**) was obtained from the hygroscopic DNP-acid as a viscous yellow oil. Reaction with ethanolic potassium carbonate (Scheme 2.38) resulted in retention of the α-substituent in the cyclised product, which was almost entirely ethyl 3-(5-nitro-3-oxido-1*H*-benzimidazol-2-yl)propionate (**237**) and a very small amount of the corresponding propionic acid (**238**) which was detected by ^{1}H NMR spectroscopy in one of the isolated fractions. When the reaction was carried out on a

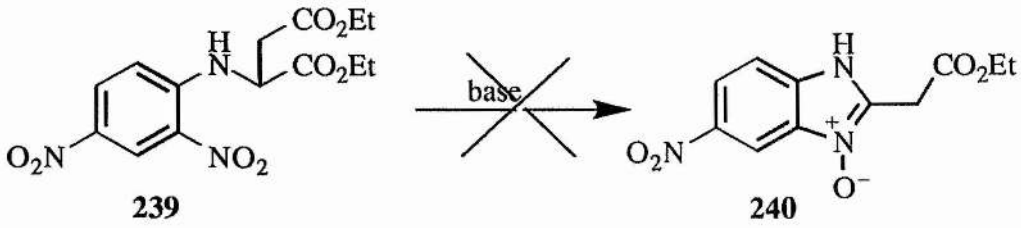
small scale, only the ester (**237**) was isolated (in 70% yield). The mechanism for this cyclisation is again presumably similar to that postulated for the alanine derivatives in Scheme 2.21. However, given the high yield of the ester obtained, this would tend to suggest that hydrolysis of the α-ester is not in fact an important process in rationalising the loss of the ester group, and so presumably this makes the other mechanism proposed (Scheme 2.22) more likely than that requiring hydrolysis of the ester followed by spontaneous decarboxylation (Scheme 2.21).



Scheme 2.38

2.3.10 Aspartic Acid Derivatives

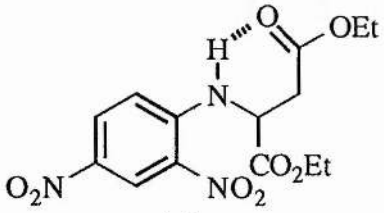
It was expected that cyclisation of diethyl DNP-aspartate (**239**) would result in ethyl 3-(5-nitro-3-oxido-1*H*-benzimidazol-2-yl)acetate (**240**) (Scheme 2.39) analogously to the cyclisation of the corresponding glutamate derivative (**236**), but reaction with ethanolic potassium carbonate both with and without heating failed to yield any isolable products other than 63% recovery of the starting material from one attempt. Reaction with sodium ethoxide as the base was similarly unfruitful. It is unclear as to why this should be the case, especially in view of the fact that the glutamic acid derivative cyclises with relative ease.



239 **240**

Scheme 2.39

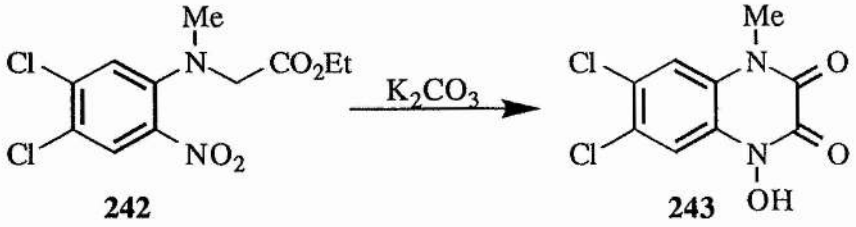
It is conceivable that the amino proton is intramolecularly hydrogen bonded to the β-ester carbonyl oxygen in a six-membered ring (**241**), which could account for the lack of reactivity by rendering the amino proton less able to become involved in the reaction. The corresponding glutamyl derivative (**236**) would be unlikely to exhibit such strong hydrogen bonding because in this case it would be a less stable seven-membered ring, and so cyclisation therefore occurs readily.



241

2.3.11 Sarcosine Derivatives

The cyclisation of DNP-sarcosine derivatives (**186**) has already been discussed (Section 2.2) - *i.e.* the anomalous cyclisation to 1-hydroxy-4-methylquinoxaline-2,3(1*H*,4*H*)-diones (**187**). The 4,5-dichloro analogue (**242**) has also been prepared (bearing in mind the work of Townsend with respect to the synthesis of antiviral compounds- see Section 4.2) and cyclised to the corresponding 6,7-dichloro-1-hydroxy-4-methylquinoxaline-2,3(1*H*,4*H*)-dione (**243**) (Scheme 2.40). However, although ^{1}H NMR data were consistent with the proposed structure, not enough material was recovered to carry out a full characterisation.

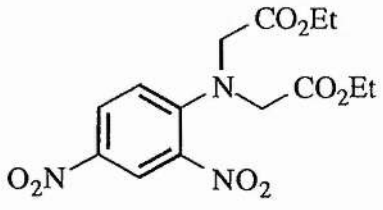


242 243

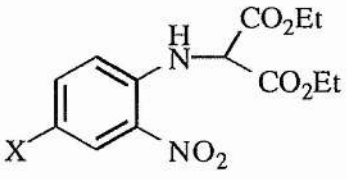
Scheme 2.40

2.3.12 Other Amino Acid Derivatives

Attempted syntheses of both diethyl 2,4-dinitrophenyliminodiacetate (**244**) and the DNP- and *N*-(*o*-nitrophenyl)- derivatives of diethyl aminomalonate (**245**) proved unsuccessful. Cyclisation of diethyl 2,6-dinitrophenyliminodiacetate would have been of interest in order to determine whether a "double" cyclisation could be effected to give the tricyclic heterocycle (**246**) in the manner of the sarcosine cyclisations to 1-hydroxy-4-methylquinoxaline-2,3-diones (**187**) (Sections 2.2 and 2.3.11). Synthesis of the diethyl iminodiacetate derivative (**244**) was attempted both *via* the acid with subsequent esterification, and by direct reaction of fluoro-2,4-dinitrobenzene with diethyl iminodiacetate (attempted preparation of the 2,4-DNP derivative was undertaken initially to see if a monocyclisation would occur with the one *ortho*-nitro group). The acid was prepared successfully, but the ester could not be isolated from the esterification reaction mixture.

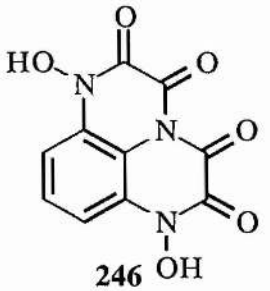


244

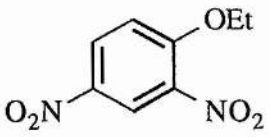


245

X = NO_2, H



246



247

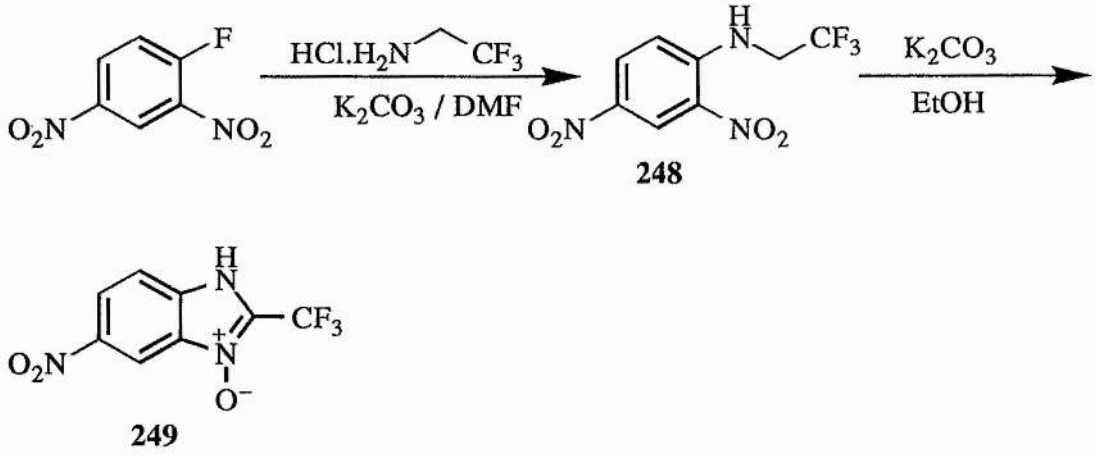
Direct reaction of fluoro-2,4-dinitrobenzene with diethyl iminodiacetate presented difficulties. The product was isolated (in very low yield) by distillation from the reaction mixture residue (b.p. 210 °C/0.5 mmHg), but attempted purification of the yellow solid by recrystallisation from ethanol led simply to nucleophilic displacement of the iminodiacetate moiety by ethanol to leave 2,4-dinitrophenetole (**247**). Diethyl iminodiacetate was also recovered by distillation (b.p. 160 °C/0.5 mmHg). It has been noted in the literature that the imino group of such amino acids displays poor nucleophilic reactivity[144], so the difficulties encountered with this reaction are perhaps not surprising, and evidently diethyl iminodiacetate is a particularly good leaving group (ethanol not being a particularly strong nucleophile).

Neither attempt at the synthesis of diethyl DNP- or diethyl *N*-(*o*-nitrophenyl)amino-malonate (**245**) led to isolation of any product - only intractable mixtures were obtained.

2.3.13 Trifluoroethyl Derivatives

Variously substituted 2-trifluoromethylbenzimidazole *N*-oxides are of interest as acaricidal agents[145]. It was thought that by using the trifluoromethyl group in place of the ester group the methylene carbon should be activated sufficiently for cyclisation to the benzimidazole *N*-oxide to occur. The DNP-2',2',2'-trifluoroethyl compound (**248**) was therefore prepared by heating fluoro-2,4-dinitrobenzene with 2,2,2-trifluoroethylamine hydrochloride and potassium carbonate in *N*,*N*-dimethylformamide. Cyclisation took place when (**248**) was reacted with ethanolic potassium carbonate in the usual manner to

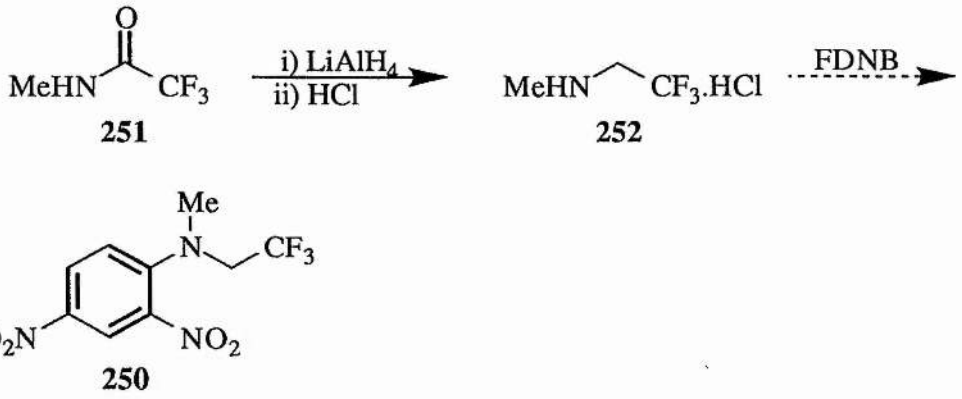
give 5-nitro-2-trifluoromethyl-1*H*-benzimidazole 3-oxide (**249**) in 74% yield as a buff-coloured solid (Scheme 2.41). This route is a simple procedure, lending itself to the synthesis of analogues substituted in the benzene ring, and also *O*-alkylated derivatives (see Chapter 4).



Scheme 2.41

The mechanism for this cyclisation is presumably the same as that for the glycine analogue - *i.e.* a combination of Schemes 2.17 and 2.18, simply substituting the trifluoromethyl group for the ester group.

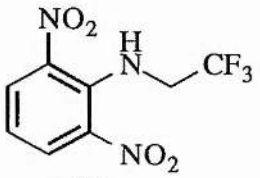
Attempts were made at the preparation of *N*-methyl-*N*-(2,4-dinitrophenyl)-2',2',2'-trifluoroethylamine (**250**) in order to see whether a diversion of the cyclisation analogous to that of the glycine and sarcosine ester cyclisations (pages 57-58) occurred. However, synthesis of this starting material in a pure enough form proved to be unsuccessful. *N*-Methyl-2,2,2-trifluoroethylamine hydrochloride (**252**) was prepared by the literature procedure[146] - *i.e.* by reduction with lithium aluminium hydride of *N*-methyl-2,2,2-trifluoroacetamide (**251**) followed by bubbling hydrogen chloride gas through an ethereal solution of the free amine to precipitate the hydrochloride. This was subsequently reacted with fluoro-2,4-dinitrobenzene in *N*,*N*-dimethylformamide with potassium carbonate (Scheme 2.42). Even after heating at 120 °C for 66 hours, however, starting material still remained and attempts at purification of the product were unsuccessful.



251 **252** **250**

Scheme 2.42

Synthesis of *N*-(2,6-dinitrophenyl)-2',2',2'-trifluoroethylamine (**253**) was also attempted, again to make comparisons with the anomalous reaction of the correspondingly substituted glycine derivative (Section 2.2). However, again after heating the reactants together in *N*,*N*-dimethylformamide at 110 °C for over 140 hours, and after the addition of further portions of amine and carbonate, starting material (*i.e.* fluoro-2,6-dinitrobenzene) was still present as detected by thin layer chromatography.

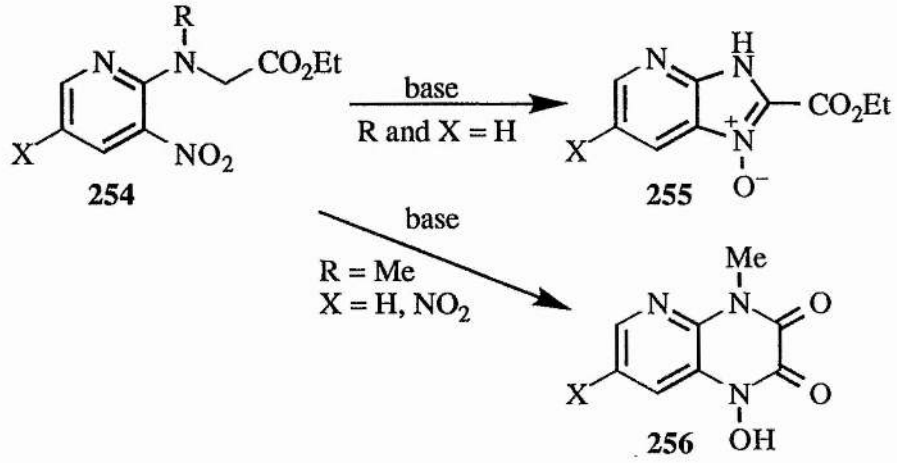


253

2.3.14 Pyridyl Amino Acid Derivatives

The reaction of the *o*-nitropyridyl glycine ester (**254**; R = H) with base proceeds analogously to that of the phenyl analogues[131,147] (Scheme 2.43) to give ethyl 3*H*-imidazo[4,5-*b*]pyridine-2-carboxylate 1-oxide (**255**) and its corresponding 2-unsubstituted analogue. Similarly, the 3,5-dinitro-2-pyridylsarcosine analogue (**254**; R = Me) cyclises to give 1-hydroxy-4-methylpyrido[2,3-*b*]pyrazine-2,3(1*H*,4*H*)-dione (**256**). The only difference between the cyclisations of amino acid derivatives of the

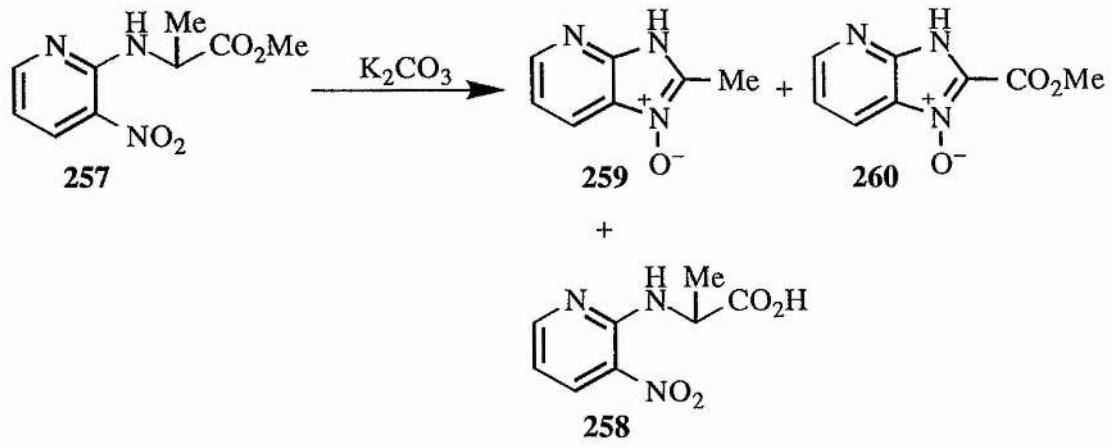
nitrophenyl and nitropyridyl series was that the 3,5-dinitro-2-pyridylglycine derivative (**254**; R = H, X = NO_2) failed to yield any identifiable products.



Scheme 2.43

The alanine analogue (**257**) was prepared in order to establish whether this cyclisation of *N*-(*o*-nitroaryl)amino acid esters was general for the pyridyl series as well as for the phenyl series. *N*-(3-Nitro-2-pyridyl)alanine methyl ester (**257**) was prepared directly from the reaction of 2-chloro-3-nitropyridine and alanine methyl ester hydrochloride with methanolic triethylamine in 73% yield. Reaction of the ester with methanolic potassium carbonate (Scheme 2.44) led to recovery of the hydrolysis product of the starting ester [*i.e.* (**258**)] in 19% yield, and 2-methyl-3*H*-imidazo[4,5-*b*]pyridine 1-oxide (**259**) in 18% yield. A similar amount of material was recovered as a brown oil, along with a very small amount of fine yellow needles, tentatively identified as the ester (**260**) by ^{1}H NMR spectroscopy, although not enough material was isolated to confirm this assignment. The brown oil was intractable.

First indications are, therefore, that the cyclisation reaction applies in the pyridyl series in addition to the phenyl series; work is currently under way by an Honours undergraduate (P. Nevin) to confirm this with the cyclisations of other pyridyl-amino acid derivatives.



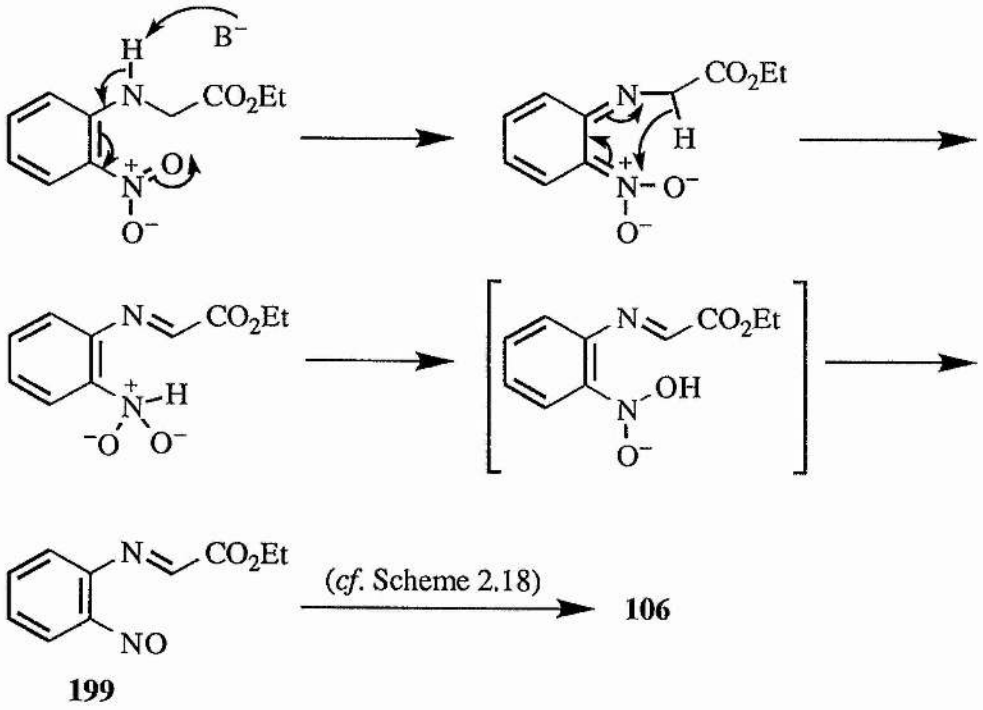
257 259 260 258

Scheme 2.44

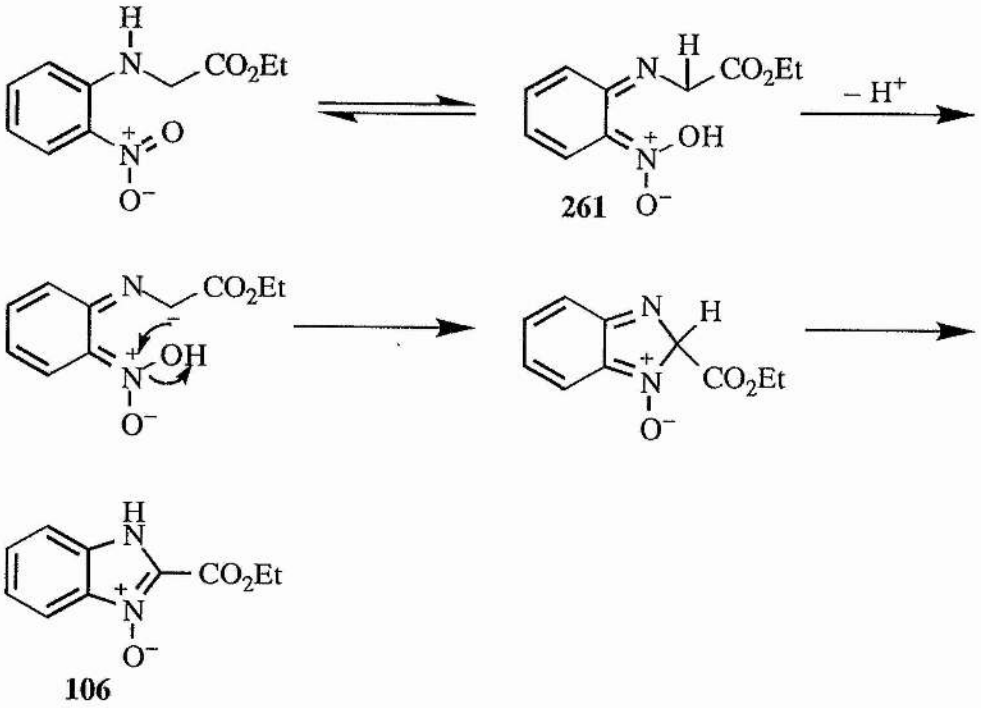
2.4 Conclusions

There are several possible mechanisms for these types of cyclisation which have been mooted so far. Doubt has already been cast on the validity of the simple condensation mechanism of Scheme 1.40. A mechanism involving a benzoxadiazine intermediate (**198**) has been proposed (Schemes 2.16 and 2.17), which can rationalise some of the results obtained for which the condensation mechanism appears to be insufficient (in addition to accounting for the simple cyclisations themselves).

Two other mechanisms can also be envisaged. In the first, initial deprotonation of the amine is followed either by a 1,5-sigmatropic hydrogen shift or an intramolecular hydride transfer on to the nitrogen of the nitro group leading to an *o*-nitrosoanil intermediate (**199**) (Scheme 2.45). The second involves the formation of an *aci*-nitro intermediate (**261**), which then undergoes deprotonation at the α-carbon (the hydrogen of which is now effectively allylic and therefore acidic), followed by cyclisation to (**106**) (Scheme 2.46). It should be noted, however, that the *o*-nitrosoanil (**199**) could also be formed *via* the benzoxadiazine route (Scheme 2.17); its further reaction (which is known to occur spontaneously[70])to the benzimidazole *N*-oxide (**106**) could be as shown in Scheme 2.18 on page 64.

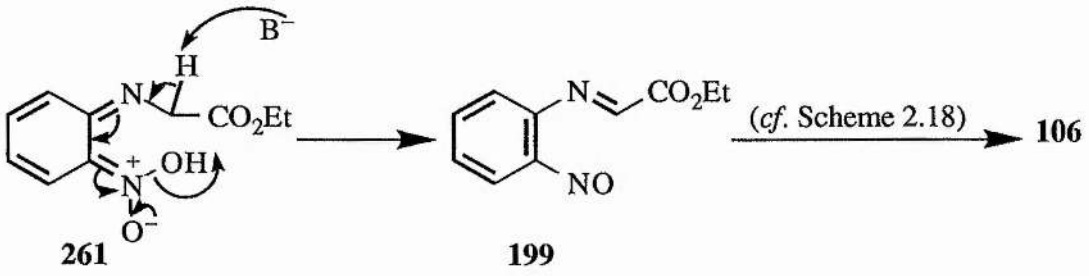


Scheme 2.45



Scheme 2.46

A combination of these two mechanisms shown above could involve formation of the *aci*-nitro intermediate (**261**) followed by re-aromatisation of the benzene ring with concomitant loss of water to furnish the *o*-nitrosoanil (**199**) (Scheme 2.47).



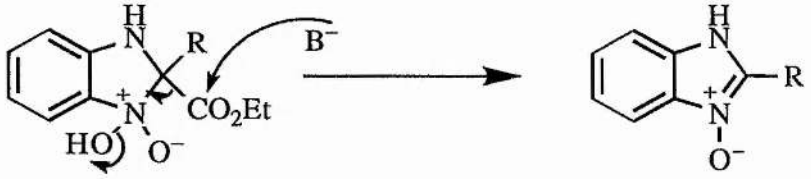
Scheme 2.47

Clearly, these mechanisms which involve abstraction of the amino proton apply only when the amino nitrogen is secondary - otherwise, the mechanism must be diverted somehow, as is observed with the (*N*-methylamino)acetonitriles (**184**) and the sarcosine derivatives (**186**). The mechanism is apparently also diverted when there is a second *ortho*-nitro group in the starting ester (or nitrile) which restricts the reactivity of the amino hydrogen by intramolecular hydrogen bonding.

Certainly one α-hydrogen is required for cyclisation to occur, given that the 2-methylalanine derivative (**211**) failed to react with base. Those amino acid derivatives with an α-hydrogen and an α-substituent which is not labile towards nucleophiles or bases [*i.e.* those derivatives of alanine (**200**), norvaline (**203**), phenylalanine (**205**) and glutamic acid (**236**)] cyclise to give the corresponding 2-substituted benzimidazole *N*-oxides directly, with loss of the ester group.

The question as to whether one or other of the previously published mechanisms is sufficient to explain the results of this research must now be addressed - it would be desirable to have a "unifying" mechanism which could account for all the types of cyclisation which have been observed. Whereas the simple aldol-type condensation

mechanism (with the nitrogen of the nitro group as the electrophile) is adequate for simple cases, it is unlikely to account for these cyclisations of α-substituted amino acid derivatives, because this would require the presence of two α-hydrogens (Scheme 1.40). This could be modified, however, by invoking attack by base on the ester group, which would give the product with loss of OH^- (Scheme 2.48). When R = H, in the case of the glycine derivative, the ester group would be retained because the α-hydrogen is removed preferentially.



R = Me, Bz, $CH_2CH_2CO_2Et$, $CH_2CH_2CH_3$

Scheme 2.48

However, the amino proton of the starting material is clearly more acidic than the α-hydrogen, especially given the positive inductive effect of the alkyl substituent and the mesomeric effect of the *ortho*-nitro group. Intermediacy of the benzoxadiazine intermediate (**198**) and subsequent ring-opening to the *o*-nitrosoanil (**202**) is conceivable (*cf.* Scheme 2.17), followed by an electrocyclic recyclisation and loss of the ester group (Scheme 2.22), although again this necessitates the removal of the α-hydrogen prior to the more acidic amino hydrogen.

In order to avoid this apparent paradox, although the *o*-nitrosoanil (**202**) is a likely intermediate which then cyclises (*cf.* Scheme 2.22), it would seem more likely to arise from the *aci*-nitro compound (**261**; 2-substituted) (*cf.* Schemes 2.45 and 2.47) because the CH, being allylic, is now acidic.

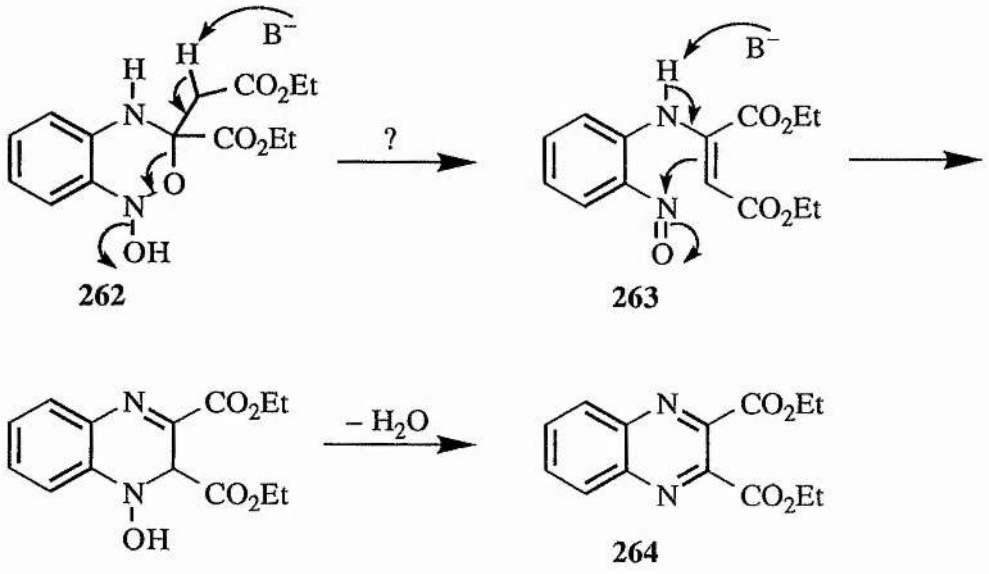
So we now have a mechanism which can account for the cyclisations of glycine and α-substituted amino acid derivatives to benzimidazole *N*-oxides, one which involves an *aci*-nitro intermediate formed by the initial removal by base of the most acidic hydrogen in the molecule (the amino hydrogen). This would lead to the key *o*-nitrosoanil intermediate which then spontaneously cyclises to the observed product.

It seems that in the absence of an accessible acidic amino proton [*i.e.* either due to its absence (in the sarcosine derivatives) or to its intramolecular hydrogen bonding to a second *ortho* nitro group], the hydrogen α- to the electron-withdrawing substituent is removed by base instead, being then the most acidic proton in the molecule. This leads to cyclisation to the proposed benzoxadiazine intermediate, then ring opening and recyclisation to the appropriate product, depending on the remaining substituents. As discussed on page 61 (Scheme 2.14), the other possibility here is that the benzimidazole *N*-oxide is formed first, but this is known to react further with base to lead to complete loss of the ester function[133].

If the α-substituent interacts with the amino hydrogen in such a way as to suppress its reactivity [for example through hydrogen bonding, as depicted for the aspartic acid derivative (**241**) (Section 2.3.10)], then cyclisation also appears to be suppressed, although admittedly with aspartic acid, the outcome of the reaction was not established. The mechanism of this suppression of cyclisation may well be the same as that proposed for causing diversion of the cyclisation of *N*-(2,6-dinitrophenyl) derivatives [*i.e.* intramolecular hydrogen bonding of the amino proton; structure (**241**)]. However, the outcome is different because there is only one α-hydrogen present, and formation of the anilido ester intermediate [(**189**); NH for NMe] which would have led to the quinoxaline-2,3-dione is no longer possible.

One could, however, visualise another possible cyclisation path for the aspartic acid derivatives, involving the formation of an α,β-unsaturated intermediate (**263**) from the

benzoxadiazine intermediate (**262**) (Scheme 2.49). The intermediate (**263**) might then be expected to lose the amino proton and undergo a Michael-type condensation with the nitroso group to form the quinoxaline (**264**). No such product has been observed, and even if (**262**) is formed (reversibly) from (**239**), it may be that the driving force of the stability of the aromatic quinoxaline system is not sufficient to overcome the intramolecular hydrogen bonding of the amino proton with the β-ester group for this process to occur. This would explain why no heterocyclic products were observed.

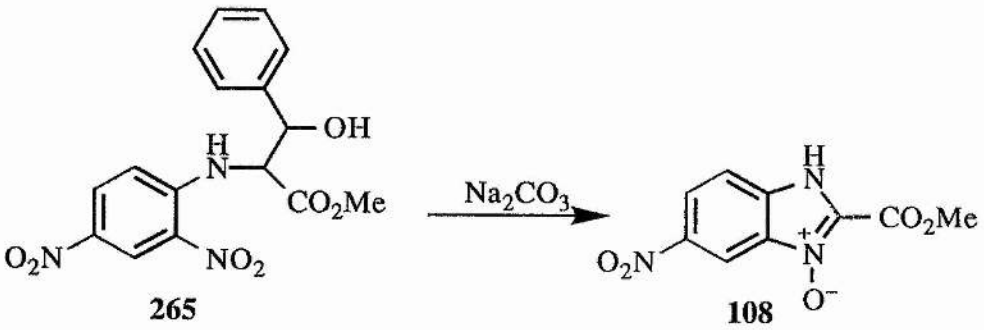


Scheme 2.49

In summary, for those cyclisations which cannot be accounted for by the *aci*-nitro - *o*-nitrosoanil mechanism of Scheme 2.47, the invoking of the benzoxadiazine intermediate appears to be necessary. However, it is now considered unlikely that the benzoxadiazine mechanism is involved in the cyclisations of the glycine and other α-substituted amino acid derivatives because of the presence of the more acidic amino hydrogen in these cases.

When the side chain of the amino acid is labile to nucleophiles or bases (*i.e.* histidine and tryptophan, along with the serine and threonine derivatives of Luetzow and Vercellotti[89]),

a base-induced scission takes place, and the α-substituent is lost altogether, the ester group remaining in the 2-position of the benzimidazole *N*-oxide product. This cleaved side chain was isolated as 3-methoxymethylindole (**233**) from the tryptophan cyclisation, whereas Luetzow and Vercellotti isolated the side chain in the cyclisation of DNP-β-phenylserine methyl ester (**265**) to methyl 5-nitro-1*H*-benzimidazole-2-carboxylate 3-oxide (**108**) as benzaldehyde (Scheme 2.50).



265 **108**

+ PhCHO

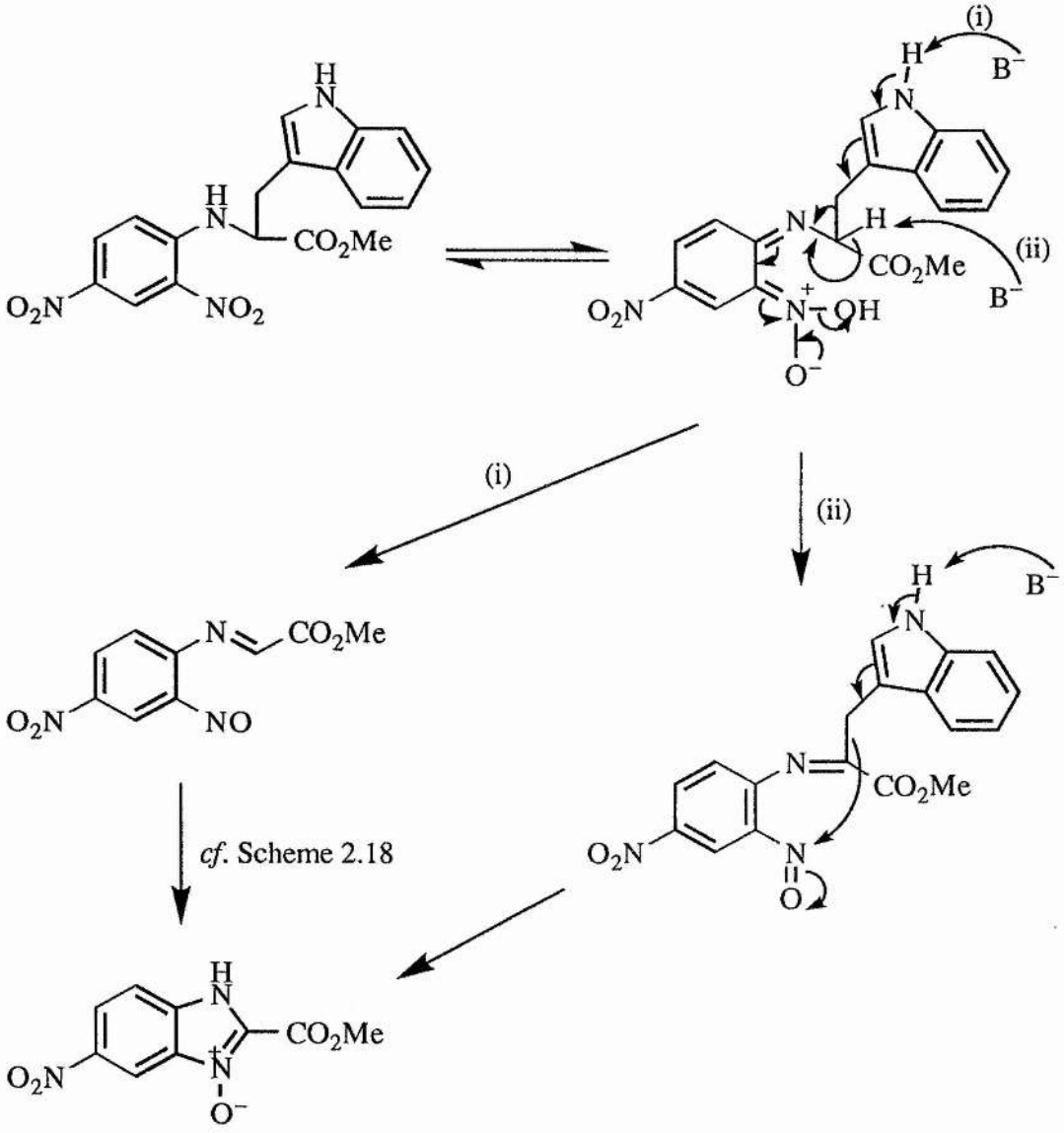
Scheme 2.50

Presumably the serine and threonine derivatives (**229**) also led to loss of the side chains as the corresponding aldehydes, formaldehyde and acetaldehyde. Although detection of these was not reported, it was inferred from the cyclisation reactions of the α,β-unsaturated amino acid derivatives (**107**) (Scheme 1.52), from which the aldehydes were detected, and presumed to arise from the addition of water across the double bond and subsequent retro-aldol cleavage.

Although the imidazole moiety could not be recovered from the histidine cyclisation, it was presumed that a similar sort of cleavage took place.

In the light of the foregoing discussion on the likely mechanisms for these cyclisations, it is apparent that there are several possible stages at which the labile side chain could be cleaved. Luetzow and Vercellotti suggest that it is cleaved initially to give the glycine

derivative, which then cyclises. However, it is more conceivable that the reaction initially proceeds *via* the *aci*-nitro intermediate (due to the acidity of the amino hydrogen), whereafter the side chain could be cleaved at several points (two of which are depicted in Scheme 2.51 with the tryptophan derivative as an example).

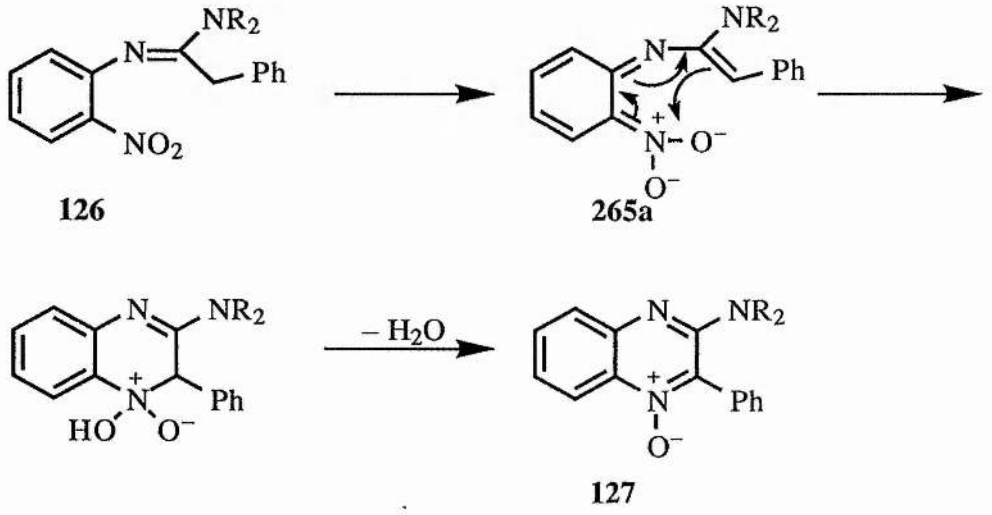


Scheme 2.51

In conclusion, then, it is difficult to reconcile all the results of the various cyclisations under the umbrella of one unifying mechanism. It is thought that the most appropriate way of rationalising them is by invoking an *aci*-nitro intermediate [for example, (**261**)]

where there is an accessible and acidic amino nitrogen, or by invoking a benzoxadiazine intermediate [for example, (**198**)] where there is not.

This can be applied successfully to some of the cyclisations of the *o*-nitroarylamines discussed in Chapter One. For example, the cyclisation of the acetamidine (**126**) (Scheme 1.59) could be envisaged as proceeding through the *aci*-nitro intermediate (**265a**), followed by an electrocyclic re-aromatisation/cyclisation step and dehydration to give the product (**127**) (Scheme 2.52), rather than a simple methylene anion condensation. The nitrosoanil is not formed because of the lack of an appropriate removable hydrogen.



Scheme 2.52

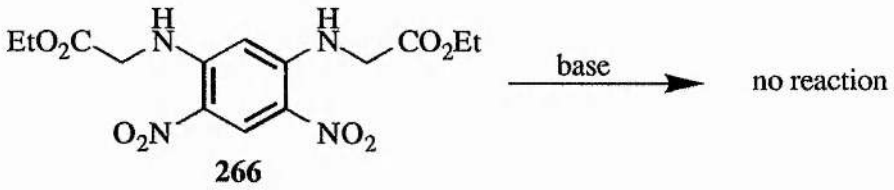
Similarly, the cyclisation of the nitrouracil (**143**) to the xanthine *N*-oxide (**144**) (Scheme 1.67) presumably proceeds *via* the nitroso intermediate (**146**), which in turn is formed from the *aci*-nitro intermediate. This again takes account of the fact that the most acidic hydrogen in the molecule is clearly the amino hydrogen, and so the simple condensation mechanism is unlikely to be occurring.

Finally, then, as a route to 2-substituted benzimidazole *N*-oxides, the base-induced cyclisation of *N*-(*o*-nitrophenyl)amino acid esters is particularly useful when the

2-substituent required is non-labile, and does not interfere with the amino hydrogen of the starting material (for example, by intramolecular hydrogen bonding).

2.5 Potential Tricyclic Systems

The attempted cyclisation (Scheme 2.53) of *N,N*-(4,6-dinitrophenylene)bis-glycine ethyl ester (**266**)[126] failed to lead to any cyclised products; unreacted starting material was all that was recovered. The second amino group attached to the benzene ring apparently deactivates the system sufficiently to prevent cyclisation from occurring.



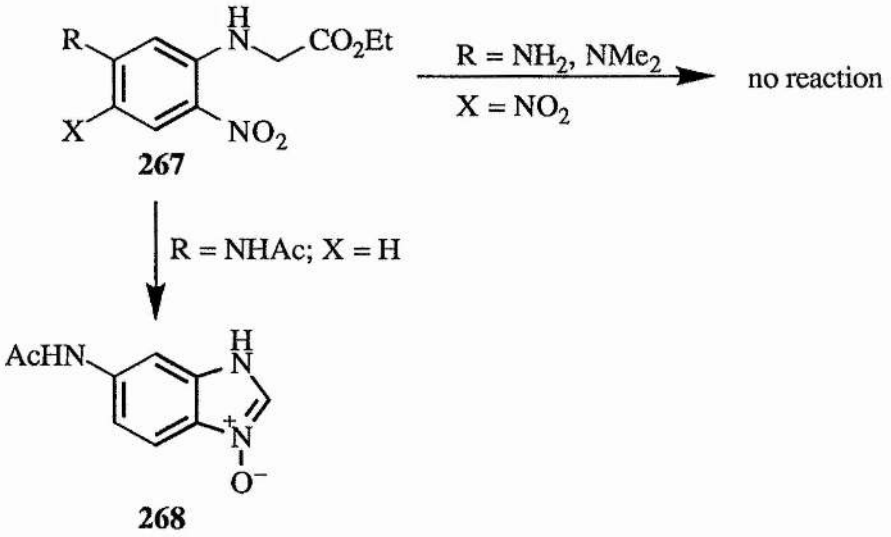
266

Scheme 2.53

Other glycine derivatives with an amino group in position 5 of the benzene ring were also found to give largely unreacted starting material - for example, (**267**), where R = NH_2 or NMe_2 (Scheme 2.54). The amino derivative gives only the ester hydrolysis product, whereas the dimethylamino derivative gives only recovery of starting material. So primary, secondary and tertiary amino groups in this position all have the same negative effect on cyclisation. However, when the acetamido group is used instead (although with only one nitro group), cyclisation then proceeds to the benzimidazole *N*-oxide (**268**) in 67% yield[126].

Rationalisation of this appears to be a straightforward consideration of the electrophilicity of the nitro group - in the cases where there is an amino (electron-donating) group in position *para* to the nitro group, cyclisation is suppressed because of the reduced electrophilicity of the nitro group, whereas cyclisation succeeds with an acetamido (electron-withdrawing) group in the *para* position. With the nitro group less

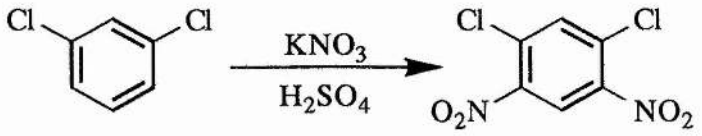
electrophilic, its condensation with the methylene anion becomes more unlikely, along with attack by the methylene carbanion on the oxygen of the nitro group to give the benzoxadiazine. This accords with the mechanism proposed in Scheme 2.47, because the *aci*-nitro intermediate would be less likely to form.



Scheme 2.54

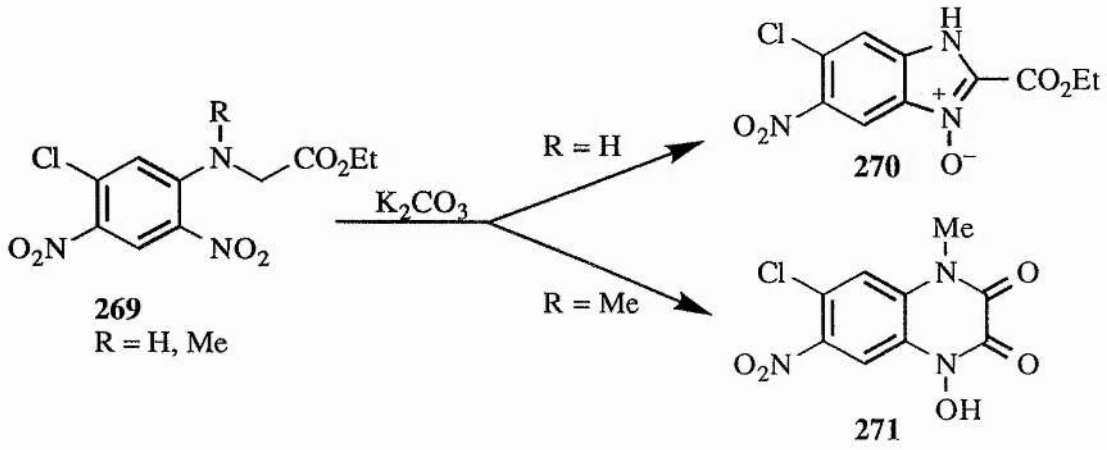
A variety of starting materials was prepared with a view to the synthesis of tricyclic products, either from the "one-pot" cyclisation of two amino acid side chains on to two *ortho* nitro groups, or from the cyclisation of a monosubstituted starting material, subsequent reaction of another amino acid with another leaving group *ortho* to the second nitro group, and then a second cyclisation step. The 1,3-substitution pattern of the two amino acid residues was employed so that the disposition of the two nitro groups *ortho*/*para* to each of the chlorine atoms would enhance the reactivity of the chlorines towards the amine nucleophiles.

Firstly, 1,3-dichloro-4,6-dinitrobenzene was prepared by the literature method[148]: *i.e.* nitration of *m*-dichlorobenzene with potassium nitrate in concentrated sulphuric acid (Scheme 2.55).



Scheme 2.55

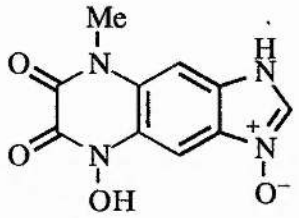
The mono-substituted *N*-(5-chloro-2,4-dinitrophenyl) derivatives (**269**) were prepared in the usual manner using one equivalent of the acid, and subsequent esterification. The acids in each case were isolated in 86-90% yield, with none of the bis-substituted products being formed, and the esters were cyclised to the benzimidazole *N*-oxide (**270**) and the 1-hydroxyquinoxaline-2,3-dione (**271**) respectively (Scheme 2.56).



Scheme 2.56

However, attempts to gauge the reactivity of the remaining chlorine atom towards nucleophiles proved unsuccessful, since neither glycine nor piperidine reacted with (**270**) or (**271**). One attempt was made at the synthesis of 6-fluoro-1-hydroxy-4-methyl-7-nitroquinoxaline-2,3-dione (**271**; F for Cl) in order to facilitate the nucleophilic attack of another amino acid (fluorine being more reactive than chlorine as a leaving group in aromatic nucleophilic substitution). However, although the ester (**269**; R = Me; F for Cl) was successfully prepared in 60% overall yield, the quinoxalinedione could not prepared in sufficient purity for further reaction, although it was identified by ^{1}H NMR

spectroscopy. The hope was to have been able to prepare "unsymmetrical" tricyclic systems such as (**272**) by this method.

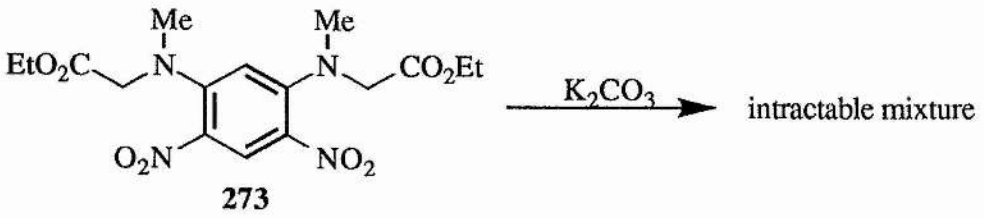


272

The result of Smith *et al.*[126] was confirmed by preparing the disubstituted glycine compound (**266**). This was carried out in 87% yield by reacting two equivalents of the glycine ethyl ester in dimethyl sulphoxide with sodium hydrogen carbonate with 1,3-dichloro-4,6-dinitrobenzene. Attempted cyclisation of this with ethanolic potassium carbonate led, as expected, only to recovery of starting material (55%) and the corresponding bis-acid (19%) from hydrolysis of the ester.

The disubstituted sarcosine diester (**273**) was similarly prepared, albeit in much lower yield (42%). Each attempted cyclisation of (**273**), however, led simply to production of intractable mixtures (Scheme 2.57), from which no products could be identified other than some starting material and the diacid thereof. It therefore would appear that formation of the quinoxalinedione is also inhibited by the presence of an amino group in 5-position of the starting material (although this was only attempted with another tertiary amino group).

It was concluded, therefore, that because no tricyclic systems could be isolated, this was not a viable route to such molecules. From a mechanistic point of view, the only rationalisation of this would appear to be the reduced electrophilicity of the nitro group, as already suggested previously, but otherwise, it is unconvincing as to whether this result is particularly informative about the mechanism.



273

Scheme 2.57

CHAPTER THREE

Cyclisations of Non-Activated *o*-Nitroanilines

3.1 Introduction

While this research was in progress, two pieces of work appeared in the literature, both of which were of obvious mechanistic relevance to the work in the preceding chapter, and both of which also seemed to be rather difficult to account for. Firstly, an Argentinian group had cyclised *o*-nitroaniline derivatives in basic conditions to 2-substituted benzimidazole *N*-oxides, but significantly, the *N*-substituent of the *o*-nitroaniline was not activated by an electron-withdrawing group[149]. This posed an interesting mechanistic problem, especially given the mildness of the reaction conditions, and that it had been found (Section 2.3.2) that an activating group adjacent to the α-carbon was required for cyclisation (Scheme 2.25). *N*-(2,6-Dinitrophenyl)-*n*-butylamine (**274**) reacted with 0.2 M sodium hydroxide in 60% dioxan/water to give 7-nitro-2-*n*-propyl-1*H*-benzimidazole 3-oxide (**275**) in almost quantitative yield (Scheme 3.1).

274 **275**

Scheme 3.1

The second *ortho*-nitro group is apparently necessary for the cyclisation to take place. The authors report that reaction of the *N*-(2,4-dinitrophenyl) analogue under the same conditions (and also with 0.01 M sodium hydroxide) gives quantitative recovery of 2,4-dinitrophenol. They also report that the *N*-(*o*-nitrophenyl) analogue does not react at all, although a very small amount of an unidentified product was observed after heating under reflux for 65 hours. In the case of the picryl (2,4,6-trinitrophenyl) derivative, reaction with 0.2 M sodium hydroxide leads to quantitative recovery of picric acid, but at 0.01 M concentration, the dinitrobenzimidazole *N*-oxide is isolated in

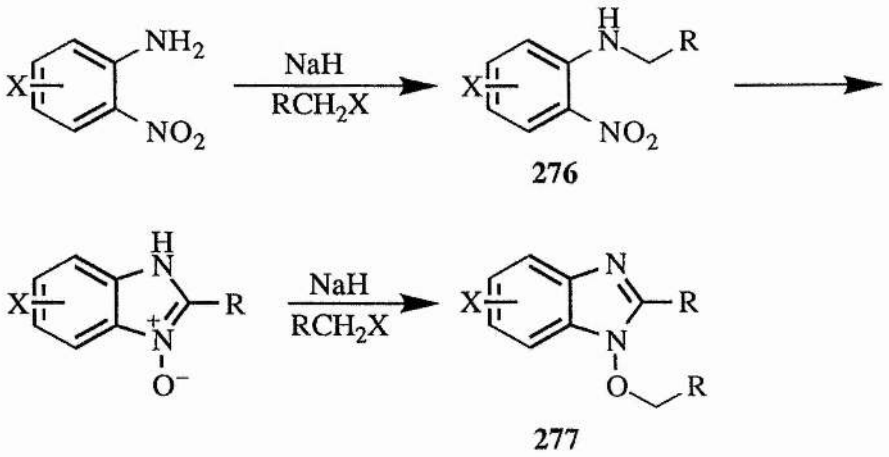
46% yield, along with 10% of picric acid. The concentration of base is therefore also apparently critical in determining the outcome of the reaction.

In their preliminary communication, de Vargas and Cañas do not venture a mechanism to account for this cyclisation reaction, but they do suggest that the reason for isolation of the corresponding phenol in some of the reactions is due to straightforward nucleophilic substitution of the amino substitutent by hydroxide ion. This occurs in preference to cyclisation both when the base concentration is high, and when the amine is not sufficiently reactive for cyclisation to occur (*i.e.* when only one nitro group is present). When three nitro groups are present, the ring is activated so much towards nucleophilic substitution by hydroxide that the cyclisation reaction can compete with formation of picric acid only at low concentrations of base. However, there is no explanation of why the second *ortho*-nitro group in place of a *para*-nitro group should be critical.

Evidently the presence of the second *ortho*-nitro group is mechanistically significant, despite the fact that it remains unchanged in the final product of the reaction. This lends support to the hypothesis (page 67) that in the case of the cyclisation of the 2,4-dinitrophenyl norvaline derivative (**203**), loss of the ester group does not occur first. If this were the case, then no cyclisation would be expected (given the evidence above) to take place with the 2,4-dinitrophenyl compound.

Secondly, another group which has been working on the cyclisations of unactivated *N*-alkyl-*o*-nitroanilines is that of Gardiner at UMIST. They have developed a synthesis of 2-substituted-*O*-alkylated benzimidazole *N*-oxides (**277**) (for the preparation of potential HIV-1 inhibitors) from the tandem reactions of an *o*-nitroaniline with an alkyl halide and sodium hydride. The first step (*N*-alkylation) is followed by cyclisation of the resultant *N*-alkyl-*o*-nitroaniline (**276**) and then by *O*-alkylation of the ensuing heterocyclic *N*-oxide with another equivalent of the alkyl halide (Scheme 3.2)[150,151,152].

The *O*-alkylation of benzimidazole *N*-oxides (along with 1-hydroxyquinoxaline-2,3-diones) will be discussed further in Chapter 4.



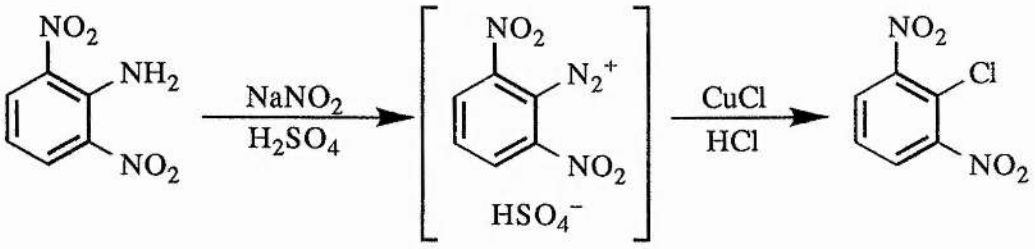
Scheme 3.2

Similarly to the cyclisation of the *n*-butylamine derivative (**274**) in Scheme 3.1, the cyclisation takes place even when the methylene adjacent to the amine is not particularly acidic (for example when R = H or ethyl), and so one must suppose that the mechanism is such that formation of the methylene anion (if, indeed, it is formed at all) is driven by the facility of the subsequent cyclisation step to give the heteroaromatic product.

However, Stacy *et al.*[6] had earlier noted that reaction of *N*-benzyl-*N*-methyl-*o*-nitroaniline with sodium hydride did not lead to 3-methyl-2-phenylbenzimidazole 1-oxide as desired or expected, and in fact in a variety of basic media (such as sodium hydroxide-methanol, sodium ethoxide-ethanol, and sodium hydroxide-dioxan-water) no reaction was observed. This could possibly be attributed to the presence of the tertiary, as opposed to secondary, nitrogen, since it has been found that various *N*-methylated *o*-nitroanilines fail to react with bases under conditions where their secondary counterparts have undergone cyclisation (for example, Schemes 2.2 and 2.8 and Section 2.3.4).

3.2 Results and Discussion

Preparation of 2,6-dinitroanilines was undertaken by reaction of the appropriate amine nucleophile with chloro-2,6-dinitrobenzene. This itself was prepared from 2,6-dinitroaniline by the method of Gunstone and Tucker[153] (Scheme 3.3): the aniline was diazotised with sodium nitrite in concentrated sulphuric acid and the resultant diazonium salt (**278**) then reacted *in situ* with cuprous chloride in concentrated hydrochloric acid to give the product in 80% yield.



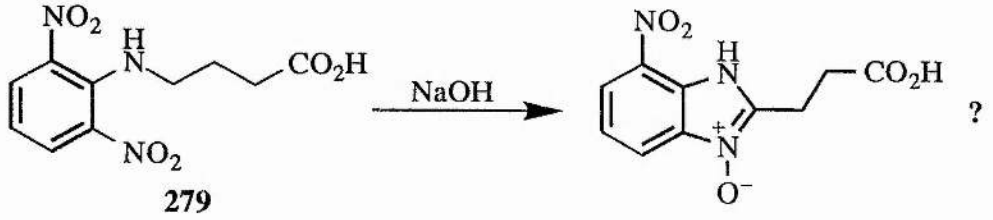
278

Scheme 3.3

Because the communication of de Vargas and Cañas[149] contained no experimental details, the reaction of *N*-(2,6-dinitrophenyl)-*n*-butylamine (**274**) (which was prepared in 70% yield by heating the amine with chloro-2,6-dinitrobenzene in *N*,*N*-dimethylformamide) with sodium hydroxide in dioxan/water was repeated and their result [*i.e.* almost quantitative isolation of 7-nitro-2-*n*-propyl-benzimidazole 3-oxide (**275**)] confirmed by ^{1}H and ^{13}C NMR spectroscopy.

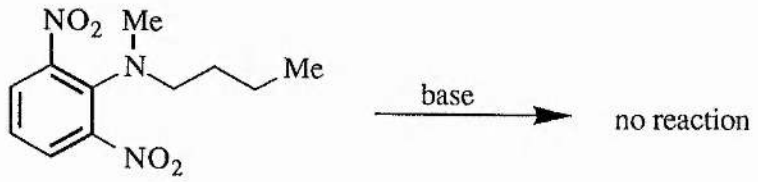
The γ-aminobutyric acid analogue (**279**) was prepared and cyclisation attempted under the same conditions as those of de Vargas and Cañas. Although no products could be isolated from the reaction mixture, ^{1}H NMR spectroscopy of the acetone extract did indicate that cyclisation had probably occurred, the most likely heterocyclic product being the 2-substituted benzimidazole *N*-oxide. This conclusion was reached because the three resonances in the ^{1}H NMR spectrum corresponding to the $CH_2CH_2CH_2$

moiety of the starting material (a triplet, a quartet and a quintet) had collapsed to two triplets at δ = 2.92 and 3.25, the integrals of each resonance corresponding to two hydrogens, indicating a CH_2CH_2 moiety. The aromatic substitution pattern was also no longer symmetrical (as in the starting material), although the peaks corresponding to the cyclised product could not be distinguished from those of the various impurities present.



279

In order to see whether any deviation from the reaction course would occur, the similarly prepared *N*-methyl analogue (**280**) was also reacted under the same conditions, but this time no reaction took place - the starting material was recovered quantitatively after heating under reflux for 2 hours (Scheme 3.4). Reaction of (**280**) with ethanolic potassium carbonate also resulted in quantitative recovery of starting material, even after heating the reaction mixture under reflux for 18 hours. Presumably the presence of the *N*-methyl group is responsible for blocking the cyclisation reaction, which implies that the NH is involved somehow in the mechanism.

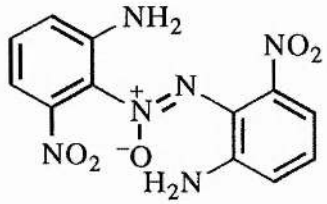


280

Scheme 3.4

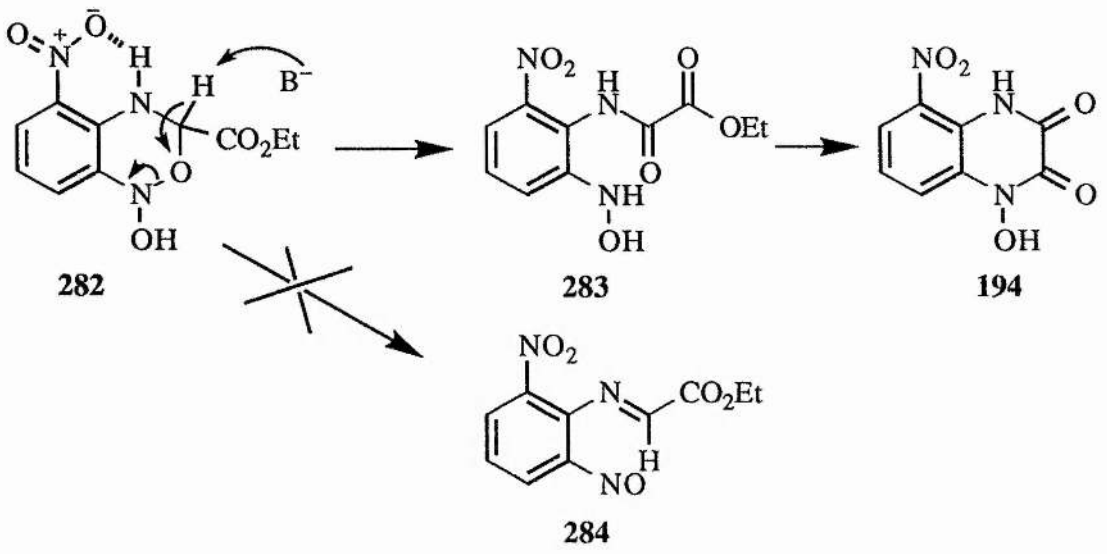
In considering a plausible mechanistic explanation for these results, it is useful now to reconsider the cyclisations of *N*-(2,6-dinitrophenyl)amino acid esters. The cyclisation of the glycine ester derivative (**192**) to 1-hydroxy-5-nitroquinoxaline-2,3-dione (**194**) has already been discussed (Section 2.2; Scheme 2.13). However, this reaction was

initially observed[154] to give only a very low yield (8%) of the heterocycle along with a small amount of 2,2'-diamino-3,3'-dinitroazoxybenzene (**281**) (which was identified by mass spectrometry), with no other products being isolated[132], and so it was thought that perhaps some other products had been missed in the course of working up the reaction. The cyclisation was therefore repeated, but it was found that increasing the reaction time (stirring at room temperature for 20 hours instead of 2.5 hours) merely increased the yield of 1-hydroxy-5-nitroquinoxaline-2,3-dione to 37%, and again, no additional products were identified.



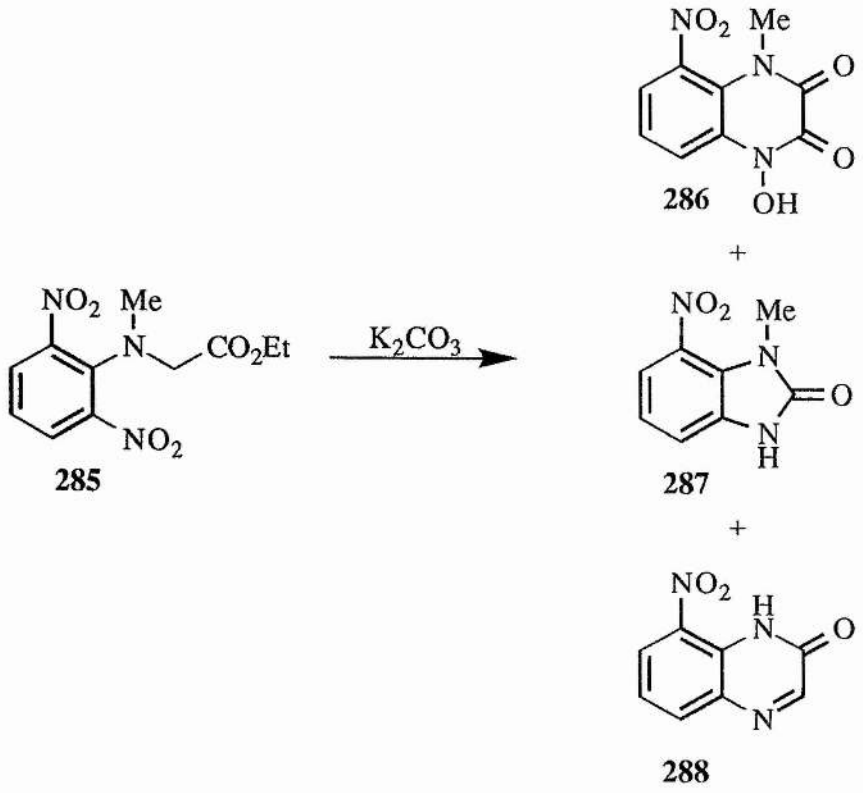
281

One possible rationalisation of the formation of the quinoxalinedione instead of the benzimidazole *N*-oxide could be the intramolecular hydrogen bonding of one of the *ortho*-nitro groups to the NH of the glycine residue. If the 2,1,4-benzoxadiazine (**282**) is an intermediate in the reaction (as proposed in Section 2.4), then because the NH is hydrogen bonded to the second *ortho*-nitro group, removal of the second α-hydrogen by the base would occur in preference to removal of the amino hydrogen (Scheme 3.5). The anilido ester intermediate (**283**) thus formed [instead of the *o*-nitrosoanil (**284**), which would have led to the benzimidazole *N*-oxide], would then lead (by intramolecular nucleophilic attack of the hydroxylamine on the ester carbonyl) to 1-hydroxy-5-nitroquinoxaline-2,3-dione (**194**).



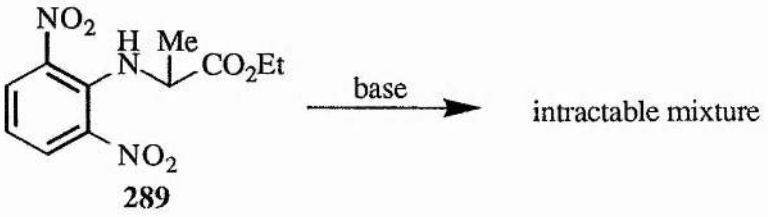
Scheme 3.5

The sarcosine analogue (**285**) is cyclised to a mixture of products[131] (Scheme 3.6), the major product being 1-hydroxy-4-methyl-5-nitroquinoxaline-2,3-dione (**286**) (19%), along with some (presumably small amounts) 1-methyl-7-nitrobenzimidazol-2-one (**287**) and 8-nitroquinoxalin-2-one (**288**) in unspecified yields.



Scheme 3.6

N-(2,6-Dinitrophenyl)alanine ethyl ester (**289**) was prepared and cyclisation attempted, but each attempt at this reaction resulted merely in intractable mixtures from which no products could be identified (Scheme 3.7), so no parallels with the *N*-(2,4-dinitrophenyl)alanine ester (**200b**) cyclisation could be drawn. Having said that, perhaps intramolecular hydrogen bonding of the NH to the second *ortho*-nitro group in an identical fashion to that shown in Scheme 3.5 with the reaction of the *N*-(2,6-dinitrophenyl)glycine ester could prevent formation of the *o*-nitrosoanil (**284**) expected (*cf.* Scheme 2.21 on page 67) so that no cyclisation could take place as the second α-hydrogen is no longer present to allow formation of the alternative anilido ester intermediate (**283**).



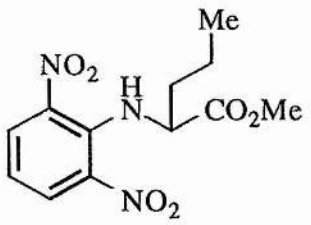
289

Scheme 3.7

Given the failure of this cyclisation, it is useful to note that the synthesis of 2-alkyl-5-nitrobenzimidazole 3-oxides [for example, (**201**), (**204**) and (**206**)] reported in Chapter 2 is complemented by the method of de Vargas and Cañas for the preparation of 2-alkyl-7-nitrobenzimidazole 3-oxides [for example, (**275**)], which seem to be inaccessible from the cyclisation of *N*-(2,6-dinitrophenyl)-α-substituted amino acid esters.

The cyclisation of *N*-(2,6-dinitrophenyl)norvaline methyl ester (**290**) was attempted in order to gauge whether the longer alkyl chain would have an effect on the cyclisation. However, no products could be identified from the reaction, although the ^{1}H NMR spectrum of one residue (isolated in very small yield) indicates that cyclisation of some sort has occurred because the aromatic resonances indicate an unsymmetrically

substituted benzene ring, and the alkyl CH resonance of the starting material has also disappeared.



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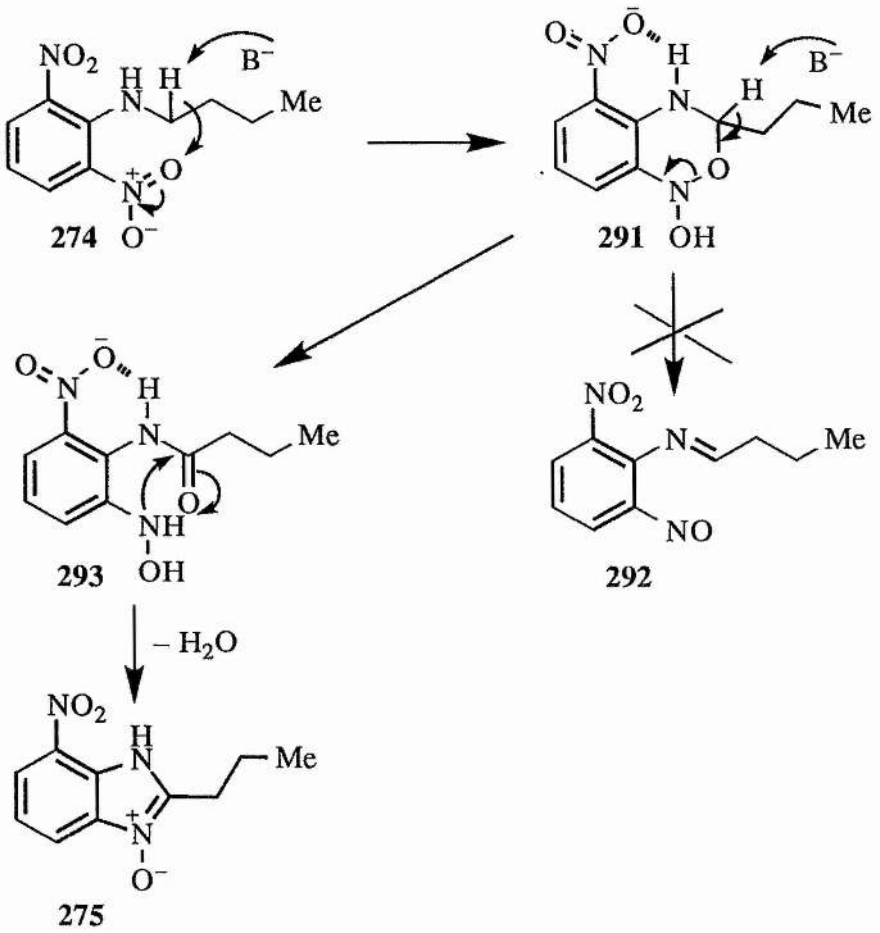
Some possible reasons for the course of the glycine reaction were discussed on pages 59-60. It seems appropriate at this stage to expand on the argument in order to attempt to understand the results of the cyclisation of the non-activated anilines with a second *ortho*-nitro group.

Let it be supposed that the cyclisation of *N*-(2,6-dinitrophenyl)-*n*-butylamine (**274**) occurs initially in the same manner as the corresponding glycine derivative. The same hydrogen-bonded benzoxadiazine intermediate (**291**) could be envisaged, where abstraction of the amino proton to give the *o*-nitrosoanil (**292**) is inhibited; the second α-hydrogen is then removed and the anilide (**293**) is formed. Condensation between the hydroxylamine and the anilide carbonyl would then follow and lead to the observed benzimidazole *N*-oxide (**275**) (Scheme 3.8).

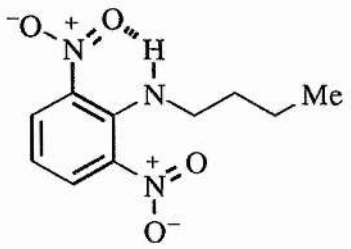
However, given that in the absence of the hydrogen bonding provided by the second *ortho*-nitro group the *o*-nitrosoanil [(**292**); 4-nitro for 6-nitro] is likely to be formed instead, this fails to explain sufficiently the observed results because the *o*-nitrosoanil [derived from *N*-(2,4-dinitrophenyl)-*n*-butylamine] could also be expected to cyclise to the benzimidazole *N*-oxide, for example by an electrocyclic process (*cf.* Scheme 2.18 on p. 64).



Scheme 3.8

Going one step further back in the postulated mechanism, it is possible that the benzoxadiazine intermediate itself does not form unless the second *ortho*-nitro group is present. It could be supposed that the intramolecular hydrogen bonding holds the molecule (of the starting material) in such a configuration (**294**) that the α-carbon is in close proximity to the one of the oxygens of the "first" *ortho*-nitro group (*i.e.* the one which will become integrated into the heterocycle). The activation energy for the hydrogen abstraction/cyclisation process would presumably therefore not be as high, and the extra activation which would be provided by an electron-withdrawing group such as an ester or nitrile may not be required. Cyclisation to the benzoxadiazine therefore occurs, whereas in the non-activated *and* non-hydrogen bonded case, the activation energy for the initial cyclisation is too high. No cyclised products are

therefore observed without the second *ortho*-nitro group. Once the benzoxadiazine intermediate is formed, it would seem unlikely that the *o*-nitrosoanil would form (Scheme 3.8), because of the hydrogen bonding hindering the amino hydrogen abstraction. Instead the hydroxylaminoanilide (**293**) would lead, by condensation between the hydroxylamine and carbonyl groups, to the observed benzimidazole *N*-oxide.



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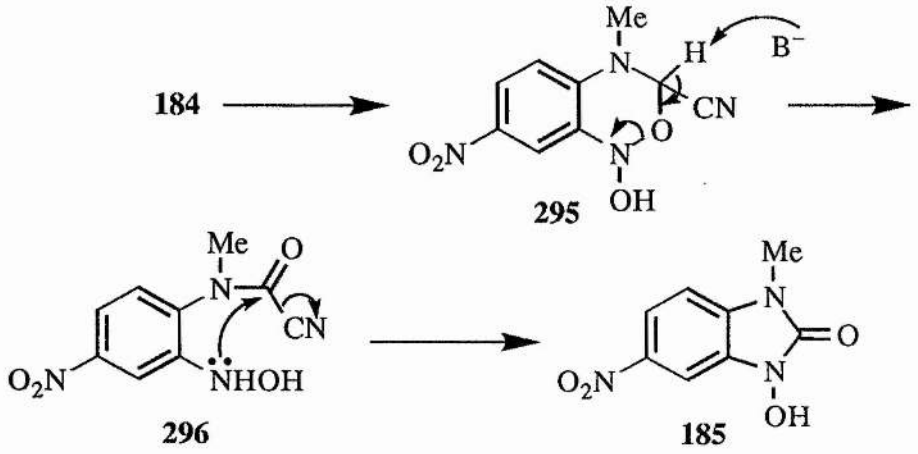
3.3 Conclusions

In summary, then, there is a balance between the relative acidities of the amino hydrogen and the α-hydrogen, the outcome of which determines the course of reaction with base. This balance in turn seems to depend on such factors as intramolecular hydrogen bonding, the presence or absence of electron-withdrawing groups, and the spatial relationship between the α-carbon and the *ortho*-nitro group. If the amino hydrogen is in some way hindered (or indeed absent, as in the case of sarcosine derivatives), then formation of the *o*-nitrosoanil intermediate (**284**) or (**292**) [by way of an *aci*-nitro intermediate such as (**261**)] is prevented. Formation of the benzimidazole *N*-oxide *may* also then be prevented, depending on whether or not an ester group is present β- to the amine. Presence of the ester group leads to formation of the quinoxaline-2,3-dione; absence of a leaving group such as ester [for example, in the *n*-butylamine derivative (**274**)] leads to formation of the benzimidazole *N*-oxide.

This could also apply to the reaction of the (*N*-methylamino)acetonitrile (**184**) to give the benzimidazolone (**185**) (Scheme 2.10). The benzoxadiazine intermediate (**295**) can

again be envisaged, which then ring opens to give the anilido nitrile (**296**). Intramolecular nucleophilic attack of the hydroxylamine on the carbonyl, with cyanide as a leaving group, would result directly in the observed benzimidazolone (Scheme 3.9).



Scheme 3.9

In conclusion, the combination of mechanisms proposed in Section 2.4 to rationalise the cyclisations of α-amino acid derivatives also seems able to account for the cyclisations of the non-activated *o*-nitroanilines described in this chapter. The different products arising from the cyclisations of the *N*-(2,6-dinitrophenyl) derivatives of glycine ethyl ester (**192**) and *n*-butylamine (**274**) [*i.e.* the quinoxalinedione (**194**) and the benzimidazole *N*-oxide (**275**)] can also be rationalised in this manner.

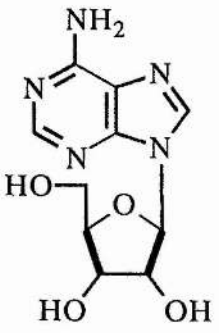
CHAPTER FOUR

Potential Biological Activity

4.1 Introduction

The potential for derivatives of benzimidazole *N*-oxides and 1-hydroxyquinoxaline-2,3-diones to be biologically active is considerable. Firstly, *O*-alkylated benzimidazole *N*-oxides are acyclic analogues of nucleosides and as such are potential antiviral compounds.

Purine nucleoside analogues [*i.e.* analogues of adenosine (**297**) and guanosine (**298**)] such as acyclovir[155] (**299**) and famciclovir[156] (**300**) are well known. Acyclovir (marketed by Glaxo-Wellcome under the brand name Zovirax) is used for the treatment of cold sores (caused by the herpes simplex virus, HSV), and also successfully treats chickenpox and shingles (which are caused by the varicellazoster virus, VZV). The antiviral action of acyclic nucleoside analogues like acyclovir involves incorporation of the drug into the growing nucleic acid chain of the virus. This causes termination of chain growth because of the lack of a complete sugar moiety, (chain extension normally occurs from the 3'-oxygen of the ribose sugar ring) and the virus is thus unable to replicate. Famciclovir is oxidised and deacetylated *in vivo* to the active form of the drug, which then acts in the same manner as acyclovir. 9-Alkoxypurines (*i.e.* with the oxygen of the acyclic moiety attached directly to the nitrogen) have been prepared and demonstrated to be potent inhibitors of HSV-1, HSV-2 and VZV. For example, 9-(2-hydroxy)ethoxyguanine (**301**) is more potent than acyclovir[157].



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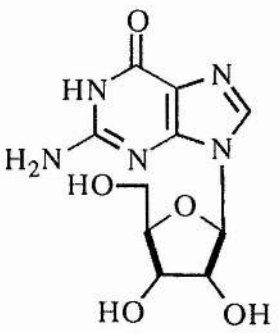

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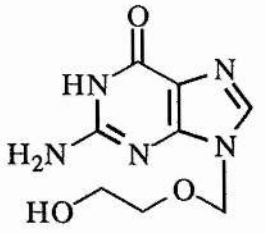

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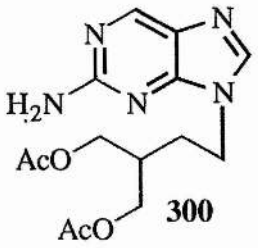

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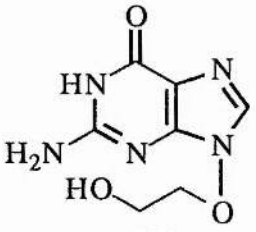
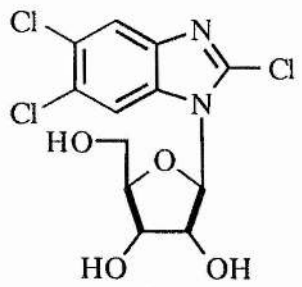


301

A benzimidazole nucleoside which has been prepared and biologically tested by Townsend *et al.*, *viz.* 2,5,6-trichloro-1-(β-D-ribofuranosyl)benzimidazole (TCRB) (**302**) has been discovered to have pronounced antiviral properties. For example, TCRB displays potent and selective activity against human cytomegalovirus (HCMV)[158], a virus which is particularly dangerous for immunocompromised patients, such as those with AIDS or who are undergoing chemotherapy or radiotherapy. Previously mentioned[150,151,152] (Section 3.1) are 2-substituted 1-alkoxybenzimidazoles (**277**) which are modest inhibitors of HIV reverse transcriptase. These results indicate that the pyrimidine ring of the heterocycle is not essential for biological activity, and the simpler benzimidazole-derived analogues are therefore viable synthetic targets.



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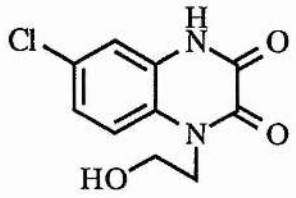
Secondly, many derivatives of quinoxaline-2,3-diones are biologically active compounds. Several are involved in antagonistic interactions with neurotransmitter receptors in the central nervous system (CNS), and as such are of interest for the combatting of diseases of the CNS such as Huntingdon's, Parkinson's and Alzheimer's (for example, compound **13**, Section 1.2). Other biologically active compounds include 6-chloro-1-(2-hydroxyethyl)quinoxaline-2,3-dione (**303**) which is useful as a tranquilliser[159], 6-nitro-1-*p*-tolylquinoxaline-2,3-dione (**304**) which demonstrates insecticidal properties[160], and 6,7-dinitroquinoxaline-2,3-dione (**305**) which also displays excitatory amino acid pharmacology[161,162]. The 6,7-disubstituted 5-nitroquinoxaline-2,3-diones (**306**) are efficacious in treating or preventing neuronal loss associated with (for example) stroke, ischaemia and hypoglycaemia, as well as the neurodegenerative diseases mentioned above and Down's Syndrome[163]. Other simple quinoxaline derivatives are variously active as antibacterials, fungicides and anti-inflammatory drugs[164], and 6,7-dimethyl-1-ribitylquinoxaline-2,3-dione has been shown to be a bacterial degradation product of riboflavin[165] [vitamin B_2; (**307**)].



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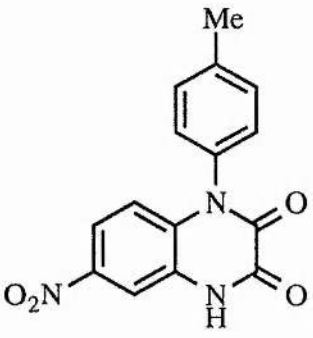

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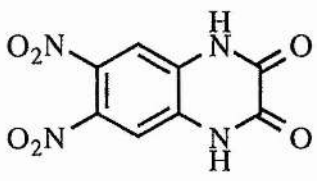

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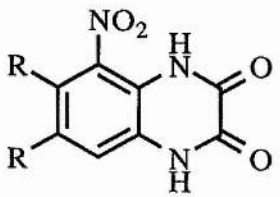
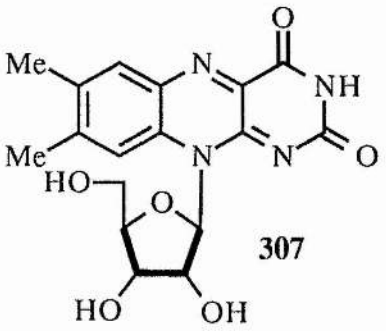


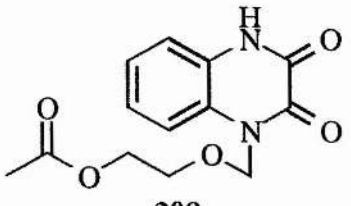
306

R = haloalkyl, alkyl, alkoxy, CN, halogen



307

Quinoxalinedione derivatives have also been prepared as potential antiviral compounds, for example 1-(2-acetyl)ethoxymethylquinoxaline-2,3-dione (**308**), although these were found to be inactive against HSV-1, HSV-2, vaccinia virus and vesicular stomatitis virus[166].



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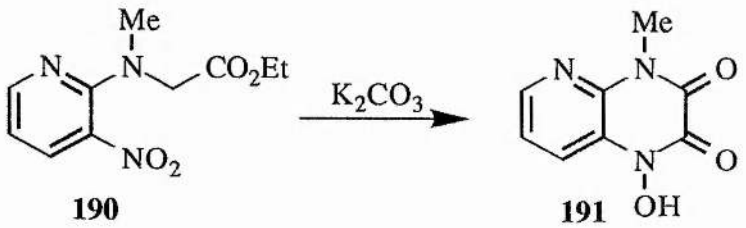
4.2 Results and Discussion

The biological targets of this project were benzimidazole and quinoxaline-2,3-dione analogues of acyclic nucleosides such as acyclovir. It should be noted that acyclovir and most of the other biologically active compounds shown above are not *N*-alkoxy heterocycles, but *N*-alkoxymethyl heterocycles. In view of the proven antiviral activity of compounds (**301**) and TCRB (**302**), it was decided to *O*-alkylate the benzimidazole *N*-oxides and 1-hydroxyquinoxaline-2,3-diones {and pyrido[2,3-*b*]pyrazines (**191**), prepared by the cyclisation of the pyridyl sarcosine ester (**190**); Scheme 4.1[131]} obtained from the cyclisation reactions of *o*-nitroanilines described in Chapter 2.



190 **191**

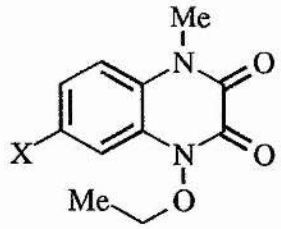
Scheme 4.1

It might be supposed that in heterocycles such as benzimidazole *N*-oxides and 1-hydroxyquinoxaline-2,3-diones, there are other potentially nucleophilic atoms other than the oxygen of the *N*-oxide moiety. However, it was found that alkylation occurred

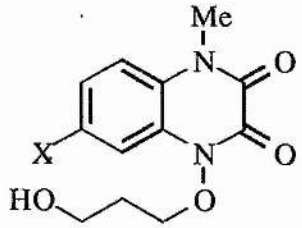
exclusively on the oxygen. This was carried out by heating the *N*-oxide dissolved in *N*,*N*-dimethylformamide with an alkyl halide and one equivalent of triethylamine. Isolation of the products (**311**) to (**318**) was generally carried out simply by dilution of the cooled reaction mixture with water and filtration of the resultant buff-coloured product. Yields were variable, but generally in the region of 40-70%.

Each heterocyclic *N*-oxide was first reacted with ethyl iodide as a simple model reaction, then the 3-hydroxypropyl derivatives were prepared as analogues of acyclovir. 3-Bromopropan-1-ol was acquired commercially, and was converted to 3-iodopropan-1-ol in order to provide a more electrophilic reagent. The transformation was accomplished by heating 3-bromopropan-1-ol with sodium iodide in acetone[167]; sodium bromide, being insoluble in acetone, precipitates out of the reaction mixture and drives the equilibrium of the displacement reaction towards the iodoalcohol.

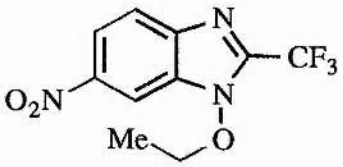
This route towards acyclic nucleoside analogues is particularly useful, since the alkylation occurs with a variety of nitrogen heterocyclic *N*-oxides, and also accommodates a variety of side chains (some more of which were prepared by Ritchie[168]. Biological evaluation of the nucleoside analogues prepared here is still awaited. Assays will be undertaken initially to establish activity against HSV-1 and -2, and HCMV.



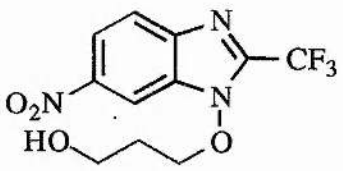
311; X = H
312; X = NO_2



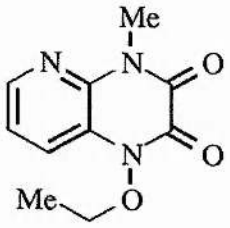
313; X = H
314; X = NO_2



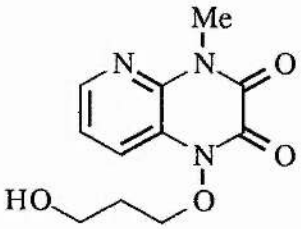
315



316



317



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CHAPTER FIVE

Experimental Section

5.1 Preamble

Melting points were determined on an Electrothermal 9100 apparatus and are uncorrected; NMR spectra were carried out either on a Varian Gemini 200 spectrometer (^1H at 200 MHz; ^{13}C at 50.31 MHz) or a Varian Gemini 300 spectrometer (^1H at 300 MHz; ^{13}C at 75.4 MHz) in DMSO-d_6 (δ values are quoted relative to DMSO at δ = 2.49 ppm) unless otherwise stated (in $CDCl_3$, δ values are quoted relative to tetramethylsilane as an internal standard at δ = 0 ppm). Mass spectra were carried out either on a VG 70-250 SE or a Kratos MS-50 spectrometer, and were generated under electron impact.

"Ether" refers to diethyl ether, "petrol" refers to petroleum ether of boiling point 40-60 °C and "HCl" refers to concentrated hydrochloric acid of specific gravity 1.18. "Dry HCl" refers to hydrogen chloride gas, generated as required by dropping concentrated hydrochloric acid into concentrated sulphuric acid, and drying the gas over glass beads coated in concentrated sulphuric acid. "Tlc" refers to thin layer chromatography, carried out on silica gel coated plates.

5.2 Chapter Two Experimental

***N*-(2-Nitrophenyl)-(*RS*)-alanine ethyl ester (200a).** A solution of (*RS*)-alanine (5.34 g, 60 mmol) in water (80 cm^3) with sodium hydrogen carbonate (15.12 g, 180 mmol) was added to a stirred solution of fluoro-2-nitrobenzene (7.05 g, 50 mmol) in ethanol (150 cm^3) at room temperature. The mixture was heated under reflux for 5 h, concentrated to dryness under reduced pressure, then partitioned between water and ether. The aqueous layer was acidified with HCl, and the bright yellow crystals of *N*-(2-nitrophenyl)-(*RS*)-alanine filtered off (6.41 g, 61%); m.p. 144-5 °C (lit.[122] 144 °C). δ_H 1.49 (3H, d, CH_3), 4.49 (1H, quintet, CH), 6.75 (1H, t, 4-H), 7.02 (1H, d, 6-H), 7.58 (1H, t, 5-H), 8.12 (1H, dd, 3-H), 8.37 (1H, d, NH). $J_{CHCH_3} = J_{CHNH}$ 7.5, $J_{3,4} = J_{4,5} = J_{5,6}$ 10, $J_{3,5} = J_{4,6}$ 2 Hz. *m/z* 210 ($M^{+\cdot}$, 43%), 165 (100), 149 (15), 118 (35), 104 (20), 91 (34), 77 (24), *etc.*

A solution of *N*-(2-nitrophenyl)-(*RS*)-alanine (6.36 g, 30.3 mmol) in ethanol (100 cm^3) containing dry HCl (3.5 g) was heated under reflux for 5 h. The solution was cooled to room temperature, but no precipitation occurred, so the solution was concentrated under reduced pressure to 50 cm^3 and poured into vigorously stirred ice/water (100 cm^3). The water/ethanol was removed under reduced pressure and the residue partitioned between water and ether. The ether layer was dried over $MgSO_4$ and concentrated under reduced pressure to give an orange oil (6.5 g, 90%); b.p. 166 °C/0.1 mmHg. (Found: C, 55.4; H, 6.3; N, 11.75. $C_{11}H_{14}N_2O_4$ requires C, 55.5; H, 5.9; N, 11.8%.) δ_H ($CDCl_3$) 1.30 (3H, t, CH_2CH_3), 1.62 (3H, d, $CHCH_3$), 4.25 (2H, q, CH_2), 4.29 (1H, quintet, CH), 6.68-6.72 (2H, m, 4- and 6-H), 7.44 (1H, t, 5-H), 8.20 (1H, dd, 3-H), 8.32 (1H, d, NH). $J_{CH_2CH_3}$ 7.5, J_{CHCH_3} 7, $J_{3,4}$ 10, $J_{3,5}$ 2.5, $J_{4,5}$ 7.5 Hz. δ_C 14.1 (CH_2CH_3), 18.5 ($CHCH_3$), 51.4 (CH), 61.7 (CH_2), 113.9 (C-6), 116.2 (C-4), 127.1 (C-3), 132.8 (C-2), 136.3 (C-5), 144.0 (C-1), 172.8 (CO).

2-Methyl-1*H*-benzimidazole 3-oxide (201a). Potassium carbonate (1.94 g, 14 mmol) was added to a stirred solution of *N*-(2-nitrophenyl)-(*RS*)-alanine ethyl ester (2.38 g, 10 mmol) in ethanol (50 cm^3) and the mixture heated under reflux for 6 h. After cooling to room temperature, the reaction mixture was filtered, the filtrate was concentrated under reduced pressure to dryness, the residue taken up in water and acidified (HCl). The pale yellow product was filtered off (0.37 g, 25%); m.p. 236-240 °C (lit.[22] 238 °C). δ_H 2.50 (3H, s, CH_3), 7.11-7.26 (2H, m, 5- and 6-H), 7.40-7.54 (2H, m, 4- and 7-H). δ_C 12.2 (CH_3), 108.6 (C-4), 118.9 (C-7), 121.4 (C-6), 122.0 (C-5), 132.4 (C-3a), 137.9 (C-7a), 148.2 (C-2).

***N*-(2,4-Dinitrophenyl)-(*RS*)-alanine ethyl ester (200b).** A solution of (*RS*)-alanine (5.34 g, 60 mmol) in water (80 cm^3) containing sodium hydrogen carbonate (15.12 g, 180 mmol) was added to a stirred solution of choro-2,4-dinitrobenzene (10.12 g, 50 mmol) in ethanol (170 cm^3) at room temperature. The mixture was heated under reflux for 6 h, concentrated under reduced pressure to dryness, the residue dissolved in water and extracted with ether. The aqueous layer was acidified (HCl), and the resultant oil was crystallised by stirring for 1 h. *N*-(2,4-Dinitrophenyl)-(*RS*)-alanine was recrystallised from propan-2-ol/water to give 11.40 g (89%), m.p. 176-7 °C (lit.[169,170] 178 °C and 180 °C respectively). δ_H 1.53 (3H, d, CH_3), 4.69 (1H, quintet, CH), 7.21 (1H, d, 6-H), 8.30 (1H, dd, 5-H), 8.88 (1H, d, 3-H), 8.99 (1H, d, NH). J_{CHCH_3} 7.5, $J_{5,6}$ 10, $J_{3,5}$ 3 Hz. δ_C 18.0 (CH_3), 51.1 (C-CH_3), 115.9 (C-6), 116.0 (C-5), 123.8 (C-2), 130.4 (C-3), 135.7 (C-4), 147.1 (C-1), 173.4 (CO).

A solution of *N*-(2,4-dinitrophenyl)-(*RS*)-alanine (4.07 g, 16 mmol) in ethanol (60 cm^3) containing dry HCl (1.5 g) was heated under reflux for 5.5 h. The solution was cooled to room temperature and the yellow ester filtered off and recrystallised twice from ethanol, giving 3.73 g (82%) of yellow crystals; m.p. 105 °C (lit.[135] 60 °C). δ_H ($CDCl_3$) 1.25 (3H, t, CH_2CH_3), 1.56 (3H, d, $CHCH_3$), 4.24 (2H, q, CH_2), 4.82 (1H, quintet, CH), 7.23 (1H, d, 6-H), 8.31 (1H, dd, 5-H), 8.90 (1H, d, 3-H), 8.93 (1H, s, NH). $J_{CH_2CH_3}$ 7.5, J_{CHCH_3} 7.5, $J_{3,5}$ 2.5, $J_{5,6}$ 10 Hz. δ_C 14.3 (CH_2CH_3), 17.9

($CHCH_3$), 51.2 (CH), 61.9 (CH_2), 116.0 (C-6), 123.8 (C-5), 130.4 (C-2), 130.5 (C-3), 135.9 (C-4), 147.1 (C-1), 171.8 (CO).

2-Methyl-5-nitro-1*H*-benzimidazole 3-oxide (201b). Potassium carbonate (1.10 g, 8 mmol) was added to a suspension of *N*-(2,4-dinitrophenyl)-(*RS*)-alanine ethyl ester (1.42 g, 5 mmol) in ethanol (50 cm^3), and the mixture stirred at room temperature overnight. Mostly starting material remained (by tlc), so the reaction mixture was heated under reflux for 5 h, then filtered. The filtrate was concentrated under reduced pressure to dryness, partitioned between water and ethyl acetate, and the aqueous layer acidified (HCl) to furnish 2-methyl-5-nitro-1*H*-benzimidazole 3-oxide (0.32 g; 33%); m.p. 277-9 °C (dec.; from ethanol) (lit.[116] 290-4 °C). δ_H 2.60 (3H, s, CH_3), 7.74 (1H, d, 7-H), 8.10 (1H, dd, 6-H), 8.31 (1H, d, 4-H). $J_{6,7}$ 9, $J_{4,6}$ 2 Hz. δ_C 12.5 (CH_3), 105.4 (C-4), 117.5 (C-7), 119.1 (C-6), 131.5 (C-3a), 142.2 (C-5), 142.6 (C-7a), 154.1 (C-2). *m/z* 193 ($M^{+\cdot}$, 100%), 177 (42), 147 (17), 131 (20) *etc.*

Reaction of *N*-(2,4-dinitrophenyl)-(*RS*)-alanine ethyl ester (200b) with triethylamine. *N*-(2,4-Dinitrophenyl)-(*RS*)-alanine ethyl ester (2.83 g, 10 mmol) and triethylamine (1.1 g, 11 mmol) in ethanol (50 cm^3) were heated under reflux for 7 h. The mixture was cooled to room temperature, and the yellow precipitate filtered off (1.67 g, 59% of starting material; confirmed by m.p., ^1H NMR spectroscopy and tlc). The mother liquor was concentrated under reduced pressure and the residue partitioned between water and ether. The ether layer afforded 0.24 g of a yellow solid; m.p. 107-108 °C, also identified as starting material. The aqueous layer was acidified (HCl), but no precipitation occurred, and no residue remained after removing the water under reduced pressure.

***N*-(2,4-Dinitrophenyl)-(*RS*)-norvaline ethyl ester (203).** A solution of chloro-2,4-dinitrobenzene (4.66 g, 23 mmol) and (*RS*)-norvaline (2.93 g, 25 mmol) in ethanol (80 cm^3) had added to it a solution of sodium hydrogen carbonate (6.30 g,

75 mmol) in water (40 cm^3) and the mixture heated under reflux for 10 h, concentrated under reduced pressure to dryness and taken up as much as possible in water. The water-insoluble yellow solid was filtered off and dried *in vacuo* to give 3.66 g (56%) of *N*-(2,4-dinitrophenyl)-(*RS*)-norvaline. δ_H 0.80 (3H, t, CH_3), 1.25 (2H, CH_2CH_3), 1.75 (2H, m, $CHCH_2$), 3.97 (1H, q, CH), 7.07 (1H, d, 6-H), 8.17 (1H, dd, 5-H), 8.86 (1H, d, 3-H), 9.78 (1H, d, NH). $J_{CH_2CH_3}$ 7, J_{CHCH_2} 5, J_{NHCH} 6, $J_{5,6}$ 10, $J_{3,5}$ 3 Hz. δ_C 14.2 (CH_3), 17.7 (CH_2CH_3), 33.7 ($CHCH_2$), 57.4 (CH), 116.1 (C-5), 124.1 (C-3), 129.3 (C-6), 130.0 (C-2), 134.0 (C-4), 146.7 (C-1), 172.1 (CO).

N-(2,4-Dinitrophenyl)-(*RS*)-norvaline (2.26 g, 8 mmol) in ethanol (40 cm^3) had dry HCl bubbled through it for 40 min. The mixture was heated under reflux for 6 h, cooled to room temperature, filtered, and the filtrate concentrated under reduced pressure to dryness. The oil was triturated with petrol and the yellow ester filtered off and recrystallised from ethanol/water (stirring was needed to crystallise the oil). Yield 1.73 g (69%); m.p. 58-59 °C. δ_H ($CDCl_3$) 1.00 (3H, t,$CH_2CH_2CH_3$), 1.31 (3H, t, ester CH_3), 1.49 (2H, sextet, $CH_2CH_2CH_3$), 1.99 (2H, m, $CHCH_2$), 4.27 (2H, q, ester CH_2), 4.32 (1H, q, CH), 6.81 (1H, d, 6-H), 8.28 (1H, dd, 5-H), 8.89 (1H, d, NH), 9.18 (1H, d, 3-H). $J_{CH_2CH_3}$ 7, J_{CHNH} 6, $J_{3,5}$ 3, $J_{5,6}$ 9 Hz. δ_C 13.5 (ester CH_3), 14.0 ($CH_2CH_2CH_3$), 18.5 ($CH_2CH_2CH_3$), 34.2 ($CHCH_2$), 56.0 (CH), 62.1 (ester CH_2), 114.0 (C-5), 124.3 (C-3), 130.4 (C-6), 131.1 (C-2), 136.7 (C-4), 147.4 (C-1), 171.0 (CO). The ester was cyclised without further purification.

2-Propyl-5-nitro-1*H*-benzimidazole 3-oxide (204). A suspension of *N*-(2,4-dinitrophenyl)-(*RS*)-norvaline ethyl ester (0.62 g, 2 mmol) and potassium carbonate (0.35 g, 2.5 mmol) in dry ethanol (20 cm^3) was stirred at room temperature for 2 h, then heated under reflux for 2.5 h, filtered, and the filtrate concentrated under reduced pressure to dryness. The residue was dissolved in water, extracted with ethyl acetate, and the aqueous phase acidified (HCl). The acidic solution was extracted into ethyl acetate, dried ($MgSO_4$) and concentrated under reduced pressure to a pale buff-coloured solid (0.15 g, 34%); m.p. 161-162 °C. δ_H 0.97 (3H, t, CH_3), 1.83 (2H, sex,

CH_2CH_3), 2.89 (2H, t, CCH_2), 7.73 (1H, d, 7-H), 8.06 (1H, dd, 6-H), 8.29 (1H, d, 4-H). $J_{CH_2CH_3} = J_{CH_2CH_2} = 7$, $J_{6,7}$ 9, $J_{4,6}$ 2 Hz. δ_C 13.7 (CH_3), 19.8 (CH_2CH_3), 27.4 (CCH_3), 105.3 (C-4), 117.3 (C-7), 119.2 (C-6), 131.5 (C-3a), 142.3 (C-5), 142.6 (C-7a), 157.0 (C-2). Attempts at further purification for microanalysis were unsuccessful.

***N*-(2,4-Dinitrophenyl)-(*S*)-phenylalanine ethyl ester (205).** A warmed solution of *(S)*-phenylalanine (3.63 g, 22 mmol) and sodium hydrogen carbonate (5.04 g, 60 mmol) in water (60 cm^3) was added to a stirred solution of fluoro-2,4-dinitrobenzene (3.72 g, 20 mmol) in ethanol (80 cm^3) and the mixture heated under reflux for 5 h. The reaction mixture was concentrated under reduced pressure to dryness, partitioned between water and ether, and the aqueous layer acidified (HCl). The resultant oil was extracted into ether, dried ($MgSO_4$) and concentrated under reduced pressure to yield 5.79 g (87%) of *N*-(2,4-dinitrophenyl)-(*S*)-phenylalanine; m.p. 50-2 °C (lit.[171].186 °C) [this discrepancy was overlooked, given the consistency of the NMR data, and the correct analysis for the ester (**205**) below]. δ_H 3.26 (1H, dd, CH_2^a), 3.45 (1H, dd, CH_2^b), 4.62 (1H, dt, NCH), 6.70 (1H, d, 6-H), 7.2-7.4 (5H, m, Ph-H), 8.19 (1H, dd, 5-H), 8.89 (1H, d, NH), 9.12 (1H, d, 3-H). J_{CH_2} 14, $J_{CHCH_2^a}$ 7.5, $J_{CHCH_2^b}$ 5, $J_{3,5}$ 2, $J_{5,6}$ 9 Hz. δ_C 38.3 (CH_2), 57.1 (CH), 113.9 (C-6), 124.1 (C-3), 127.9 (C-4'), 129.1 and 129.2 (C-2' and -6'; and C-3' and -5'), 130.2 (C-5), 131.1 (C-1'), 134.4 (C-2), 136.8 (C-4), 147.0 (C-1), 174.7 (CO).

A solution of *N*-(2,4-dinitrophenyl)-(*S*)-phenylalanine (3.97 g, 12 mmol) in ethanol (50 cm^3) containing dry HCl (6 g) was heated under reflux for 7 h. The solution was cooled to room temperature and the yellow crystals filtered off; 3.71 g (86%); m.p. 115 °C (from ethanol) [lit.[172] 105-7 °C (from benzene)]. (Found: C, 56.8; H, 5.0; N, 11.7. $C_{17}H_{17}N_3O_6$ requires C, 56.8; H, 4.8; N, 11.7%.) δ_H ($CDCl_3$) 1.24 (3H, t, CH_3), 3.21 (1H, dd, $CHCH_2^a$), 3.35 (1H, dd, $CHCH_2^b$), 4.25 (2H, q, CH_2CH_3), 4.57 (1H, dt, CH), 6.69 (1H, d, 6-H), 7.2-7.3 (5H, m, Ph-H), 8.18 (1H, dd, 5-H), 8.90 (1H, d, NH), 9.10 (1H, d, 3-H). $J_{CH_2CH_3}$ 7, J_{CH_2} 14, $J_{CHCH_2^a}$ 7.5, $J_{CHCH_2^b}$ 5,

$J_{5,6}$ 10, $J_{3,5}$ 3 Hz. δ_C 14.0 (CH_3), 38.5 ($CHCH_2$), 57.5 (CH), 62.3 (CH_2CH_3), 114.0 (C-6), 124.2 (C-3), 127.8 (C-4'), 129.1 and 129.3 (C-2',6' and C-3',5'), 130.2 (C-5), 131.1 (C-1'). 134.8 (C-2), 136.8 (C-4), 147.2 (C-1), 170.2 (CO).

2-Benzyl-5-nitro-1*H*-benzimidazole 3-oxide (206). A mixture of *N*-(2,4-dinitrophenyl)-(*S*)-phenylalanine ethyl ester (0.72 g, 2 mmol) and potassium carbonate (0.30 g, 2.2 mmol) in dry ethanol (25 cm^3) was stirred at room temperature for 19 h, then under reflux for 1.5 h. The mixture was cooled to room temperature, filtered, and the filtrate concentrated under reduced pressure to dryness. The residue was partitioned between water and ethyl acetate and the aqueous layer acidified (HCl), extracted with ethyl acetate and the organic layer dried ($MgSO_4$) and concentrated under reduced pressure to give 0.27 g (50%) of product; m.p. 240-243 °C. δ_H 4.30 (2H, s, CH_2), 7.2-7.4 (5H, m, Ph-H), 7.73 (1H, d, 7-H), 8.05 (1H, dd, 6-H), 8.28 (1H, d, 4-H), 12.43 (1H, br s, NH). ^1H NMR data are consistent with the proposed structure; however, attempts at further purification of the title compound were unsuccessful and a fuller characterisation was not achieved.

***N*-(2-Nitrophenyl)-β-alanine ethyl ester (207a).** A solution of β-alanine (2.23 g, 25 mmol) and sodium hydrogen carbonate (3.86 g, 46 mmol) in water (40 cm^3) was added to a stirred solution of fluoro-2-nitrobenzene (2.82 g, 20 mmol) in ethanol (80 cm^3) at room temperature. The reaction mixture was heated under reflux for 5.5 h, then concentrated under reduced pressure to dryness and partitioned between water and ether. The aqueous layer was acidified (HCl) and the yellow *N*-(2-nitrophenyl)-β-alanine filtered off; 3.28 g (78%); m.p. 145-6 °C (lit.[173] 144-5 °C). δ_H 2.60 (2H, t, CH_2CO), 3.55 (2H, br q, CH_2NH), 6.68 (1H, t, 4-H), 7.08 (1H, d, 6-H), 7.54 (1H, t, 5-H), 8.05 (1H, dd, 3-H), 8.20 (1H, br s, NH). $J_{CH_2CH_2}$ 5.5, $J_{3,4} = J_{5,6} = 8.5$, $J_{3,5}$ 1.5 Hz. δ_C 33.5 (NCH_2), 38.5 (CH_2CO), 114.6 (C-6), 115.6 (C-4), 126.5 (C-3), 131.3 (C-2), 136.9 (C-5), 145.1 (C-1), 173.3 (CO).

A solution of *N*-(2-nitrophenyl)-β-alanine (1.47 g, 7 mmol) in ethanol (40 cm^3) containing dry HCl (2.4 g) was heated under reflux for 6.5 h, then concentrated under reduced pressure to dryness. The residue was recrystallised from ethanol to give 1.52 g (91%) of product; m.p. 69-70 °C (lit.[174] 66-68.5 °C). δ_H ($CDCl_3$) 1.28 (3H, t, CH_3), 2.71 (2H, t, CH_2CO), 3.65 (2H, q, CH_2NH), 6.67 (1H, t, 4-H), 6.87 (1H, d, 6-H), 7.42 (1H, t, 5-H), 8.18 (1H, d, 3-H). $J_{CH_2CH_3}$ 7.1, $J_{CH_2CH_2}$ 6.7, $J_{3,4} = J_{5,6} = 8.5$ Hz. δ_C 14.1 (CH_2CH_3), 33.8 (NCH_2), 38.4 (CH_2CO), 61.0 (CH_2CH_3), 113.3 (C-6), 115.5 (C-4), 126.9 (C-3), 132.0 (C-2), 136.2 (C-5), 144.9 (C-1), 171.3 (CO).

Reaction of *N*-(2-nitrophenyl)-β-alanine ethyl ester (207a) with potassium carbonate. *N*-(2-Nitrophenyl)-β-alanine ethyl ester (0.95 g, 4 mmol) and potassium carbonate (0.83 g, 6 mmol) in ethanol (40 cm^3) were heated under reflux for 8 h, then cooled and filtered. The filtrate was concentrated under reduced pressure to dryness and partitioned between water and ethyl acetate. The aqueous layer was acidified (HCl) and the yellow precipitate was collected (0.33 g, 39%) and identified by ^1H NMR spectroscopy as *N*-(2-nitrophenyl)-β-alanine; m.p. 145-7 °C. The ethyl acetate layer was dried ($MgSO_4$) and concentrated under reduced pessure to give 0.40 g (42%) of the starting material; m.p. 69-70 °C.

***N*-(2,4-Dinitrophenyl)-β-alanine ethyl ester (207b).** A solution of β-alanine (4.01 g, 45 mmol) and sodium hydrogen carbonate (7.56 g, 90 mmol) in water (80 cm^3) was added to a stirred solution of chloro-2,4-dinitrobenzene (8.10 g, 40 mmol) in ethanol (160 cm^3) at room temperature. The mixture was heated under reflux for 6.5 h, concentrated under reduced pressure to dryness, and partitioned between water and ether. The aqueous layer was acidified (HCl) and the yellow *N*-(2,4-dinitrophenyl)-β-alanine filtered off (10.14 g, 99%); m.p. 146-7 °C (from ethanol) (lit.[173,175] 144-5 °C and 142-3 °C respectively). δ_H 2.67 (2H, t, CH_2CO), 3.71 (2H, q, $NHCH_2$), 7.28 (1H, d, 6-H), 8.26 (1H, dd, 5-H), 8.84 (1H, d, 3-H), 8.92 (1H, t, NH). $J_{CH_2CH_2}$ 7, $J_{5,6}$ 10, $J_{3,5}$ 3 Hz.

A solution of *N*-(2,4-dinitrophenyl)-β-alanine (3.83 g, 15 mmol) in ethanol (70 cm^3) containing dry HCl (2.4 g) was heated under reflux for 7 h. The solution was cooled to room temperature, and the resultant oil crystallised by stirring in ethanol; yield: 4.08 g (96%); m.p. 72-3 °C (from ethanol). (Found: C, 46.2; H, 4.4; N, 14.5. $C_{11}H_{13}N_3O_6$ requires C, 46.6; H, 4.6; N, 14.8%.) δ_H ($CDCl_3$) 1.29 (3H, t, CH_3), 2.78 (2H, t, CH_2CO), 3.76 (2H, q, $NHCH_2$), 4.21 (2H, q, CH_2CH_3), 6.98 (1H, d, 6-H), 8.29 (1H, dd, 5-H), 8.80 (1H, br s, NH), 9.13 (1H, d, 3-H). $J_{CH_2CH_3}$ 7.1, $J_{CH_2CH_2}$ 6.4, $J_{5,6}$ 9.5, $J_{3,5}$ 2.8 Hz. δ_C 14.2 (CH_3), 33.5 (CH_2CO), 39.0 ($NHCH_2$), 61.4 (CH_2CH_3), 113.6 (C-3), 124.4 (C-6), 130.4 (C-5), 130.7 (C-2), 136.2 (C-4), 148.0 (C-1), 170.9 (CO).

Reaction of *N*-(2,4-dinitrophenyl)-β-alanine ethyl ester (207b) with potassium carbonate. Potassium carbonate (1.10 g, 8 mmol) was added to a suspension of *N*-(2,4-dinitrophenyl)-β-alanine ethyl ester (1.42 g, 5 mmol) in ethanol (50 cm^3), and the mixture heated under reflux for 11.5 h then filtered. The solid was taken up in water, acidified (HCl), and the yellow precipitate filtered off (0.70 g, 55%) and identified by ^1H NMR spectroscopy as *N*-(2,4-dinitrophenyl)-β-alanine; m.p. 138-43 °C. The reaction mixture filtrate was concentrated under reduced pressure to dryness, partitioned between water and ethyl acetate, the aqueous layer acidified (HCl) and a further crop (0.27 g, 21%) of the acid collected; m.p. 145-7 °C (overall yield 76%). The ethyl acetate layer was dried ($MgSO_4$) and concentrated under reduced pressure to give a residue which was crystallised from ethanol and identified as starting material (0.24 g) by ^1H NMR spectroscopy.

***N*-(2-Nitrophenyl)-2-methylalanine ethyl ester (211).** A suspension of 2-aminoisobutyric acid (4.12 g, 40 mmol) and sodium hydrogen carbonate (10.08 g, 120 mmol) in water (70 cm^3) was added to a stirred solution of fluoro-2-nitrobenzene (4.94 g, 35 mmol) in ethanol (100 cm^3) at room temperature. The mixture was heated under reflux for 6.5 h, concentrated under reduced pressure to dryness, then partitioned

between water and ether. The aqueous layer was acidified with HCl and the yellow *N*-(2-nitrophenyl)-2-methylalanine filtered off; m.p. 140-1 °C (lit.[122] 142 °C). δ_H 1.59 (6H, s, 2 x CH_3), 6.76 (2H, m, 4- and 6-H), 7.56 (1H, t, 5-H), 8.13 (1H, d, 3-H), 8.39 (1H, s, NH). $J_{3,4}$ 10, $J_{4,5}$ 10, $J_{5,6}$ 8 Hz.

A solution of *N*-(2-nitrophenyl)-2-methylalanine (1.70 g, 7.6 mmol) in ethanol (20 cm^3) containing dry HCl (2.3 g) was heated under reflux for 5.5 h. The reaction mixture was cooled, evaporated to dryness under reduced pressure, the residue partitioned between water and ether, and the ether layer dried over $MgSO_4$ and concentrated under reduced pressure to an oil (1.40 g, 73%); b.p. 141 °C/0.1 mmHg. (Found: C, 56.8; H, 6.6; N, 11.35. $C_{12}H_{15}N_2O_4$ requires C, 57.1; H, 6.4; N, 11.1%.) δ_H 1.20 (3H, t, CH_2CH_3), 1.68 (6H, s, 2 x CH_3), 4.19 (2H, q, CH_2CH_3), 6.59 (1H, d, 6-H), 6.68 (1H, t, 4-H), 7.38 (1H, t, 5-H), 8.23 (1H, d, 3-H), 8.41 (1H, s, NH). $J_{CH_2CH_3}$ 6.8, $J_{3,4} = J_{5,6} = 7.4$, $J_{4,5}$ 7.3 Hz. δ_C 14.0 (CH_2CH_3), 26.2 (2 x CCH_3), 57.2 [$C(CH_3)_2$], 61.8 (CH_2), 115.2 (C-6), 116.0 (C-4), 127.3 (C-3), 133.0 (C-2), 135.7 (C-5), 143.6 (C-1), 175.0 (CO).

Reaction of *N*-(2-nitrophenyl)-2-methylalanine ethyl ester (211) with potassium carbonate. Potassium carbonate (0.34 g, 2.5 mmol) was added to a stirred solution of *N*-(2-nitrophenyl)-2-methylalanine ethyl ester (0.50 g, 2 mmol) in ethanol (20 cm^3) and the mixture stirred at room temperature for 1.5 h. No reaction occurred, so the mixture was heated under reflux for 12 h, concentrated under reduced pressure to dryness, partitioned between water and ether, and the aqueous layer acidified with HCl. The aqueous layer was extracted into ethyl acetate, washed with water, dried over $MgSO_4$ and concentrated under reduced pressure to give 70 mg of a yellow solid, which was identified by ^1H NMR spectroscopy as the acid resulting from hydrolysis of the starting material. The ether layer was concentrated under reduced pressure to yield starting material (0.45 g, 90%) (identified by ^1H NMR spectroscopy).

Reaction of *N*-(2-nitrophenyl)-2-methylalanine ethyl ester (211) with sodium ethoxide. A solution of *N*-(2-nitrophenyl)-2-methylalanine ethyl ester (0.50 g, 2 mmol) in ethanol (15 cm^3) had added to it sodium ethoxide [from Na (0.058 g, 2.5 mmol) in ethanol (6 cm^3)], and the solution heated under reflux for 6.5 h. The reaction mixture was concentrated under reduced pressure to dryness and partitioned between water and ether. The ether layer afforded 0.20 g of an orange oil which was identified as starting material, and the aqueous layer, upon acidification (HCl) and extraction into ethyl acetate, yielded 0.27 g of the starting acid (m.p. 140-1 °C, and by ^1H NMR spectroscopy).

***N*-Benzyloxycarbonyl-(*RS*)-alanine (214).** To a solution of (*RS*)-alanine (8.9 g, 100 mmol) in 4 M sodium hydroxide solution (4 g, 100 mmol in 25 cm^3 of water) cooled to 0 °C was added benzyl chloroformate (17.9 g, 105 mmol) and 4 M sodium hydroxide solution (25 cm^3) alternately and portionwise over 30 min with stirring and cooling. The mixture was warmed to room temperature, stirred for 2 h, then extracted with ether. The aqueous phase was acidified (HCl) and extracted with ether; the ether was dried ($MgSO_4$) and concentrated under reduced pressure to a white solid (14.55 g, 65%) which was recrystallised from ether/petrol; m.p. 113-4 °C (lit.[176] 112-3 °C). δ_H ($CDCl_3$) 1.46 (3H, d, CH_3), 4.43 (1H, m, CH), 5.15 (2H, br s, CH_2), 5.34 (1H, d, NH), 7.35 (5H, s, 5 x ArH), 10.29 (1H, br s, CO_2H). J_{CHNH} 7 Hz. δ_C 18.9 (CH_3), 50.0 (CH), 67.7 (CH_2), 128.7, 128.8, 129.1 (C-2 and -6; C-3 and -5; and C-4), 136.5, (C-1), 156.4 (CON), 178.3 (CO).

Attempted direct synthesis of *N*-benzyloxycarbonyl-*N*-methyl-(*RS*)-alanine (216). *N*-Benzyloxycarbonyl-(*RS*)-alanine (1.12 g, 5 mmol) and methyl iodide (2.5 cm^3, 40 mmol)) were dissolved in tetrahydrofuran (20 cm^3) and cooled to 0 °C (under nitrogen). Sodium hydride (80% dispersion in oil; 0.45 g, 15 mmol) was added cautiously with gentle stirring, then the suspension stirred at room temperature for 24 h. Ethyl acetate (25 cm^3) was added followed by water dropwise to consume the

excess sodium hydride. The solution was evaporated to dryness and the residue partitioned between water and ether. The ether layer was washed with aqueous sodium hydrogen carbonate, the combined aqueous extracts acidified (HCl) and the product extracted into ethyl acetate. The ethyl acetate was washed with water, aqueous sodium thiosulphate (5% solution) then water, dried ($MgSO_4$) and concentrated under reduced pressure to an oil which was further dried at the oil pump to yield a white solid which was recrystallised from ether/petrol. ^1H NMR spectroscopy showed that only 20% methylation had taked place.

Attempted synthesis of *N*-(2-nitrophenyl)-*N*-methyl-(*RS*)-alanine. *N*-(2-Nitrophenyl)-(*RS*)-alanine (0.42 g, 2 mmol) and methyl iodide (1.0 cm^3, 2.27 g, 16 mmol) were dissolved in dry tetrahydrofuran (20 cm^3) and cooled to 0 °C. Sodium hydride (80% dispersion in oil; 0.18 g, 6 mmol) was added cautiously with gentle stirring, then the suspension was stirred at room temperature for 5 days. Ethyl acetate was added, followed by water dropwise to destroy excess sodium hydride. The solution was evaporated to dryness, the residue partitioned between water and ether, the ether washed with aqueous 5% sodium hydrogen carbonate, then the combined aqueous extracts acidified (HCl) and extracted into ethyl acetate. The ethyl acetate was washed with water, aqueous 5% sodium thiosulphate. At this stage, the product moved into the aqueous layer, so this was acidified (HCl), extracted into ethyl acetate, washed with water, dried ($MgSO_4$) and concentrated under reduced pressure to a yellow solid, which by ^1H NMR spectroscopy was entirely starting material.

3-(Benzyloxycarbonyl)-4-methyl-5-oxazolidinone (215). *N*-Benzyl-oxycarbonyl-(*RS*)-alanine (14.50 g, 65 mmol) suspended in toluene (600 cm^3) had added to it paraformaldehyde (13.5 g) and *p*-toluenesulphonic acid (1.35 g). The mixture was heated under reflux for 30 min [with azeotropic water removal (Dean-Stark apparatus)], then cooled, washed with 1 M aqueous sodium hydrogen carbonate solution, dried ($MgSO_4$) and concentrated under reduced pressure. Recrystallisation of

the residue from propan-2-ol gave 13.60 g (89%) of white crystals; m.p. 63-4 °C. δ_H ($CDCl_3$) 1.58 (3H, d, CH_3), 4.32 (1H, q, $CHCH_3$), 5.20-5.30 (2H, m, CH_2Ph), 5.50 (2H, s, NCH_2O), 7.41 (5H, s, aromatics). J_{CHCH_3} 7 Hz. δ_C 16.6 (CH_3), 50.6 (ox-C-4), 67.8 (CH_2Ph), 77.3 (ox-C-2), 128.3, 128.6 and 128.7 (C-2' and 6'; C-3' and 5'; and C-4'), 135.5 (C-1'), 172.9 (2 x CO).

***N*-(Benzyloxycarbonyl)-*N*-methyl-(*RS*)-alanine (216).** 3-(Benzyloxycarbonyl)-4-methyl-5-oxazolidinone (9.40 g, 40 mmol) was dissolved in dichloromethane (200 cm^3), then trifluoroacetic acid (200 cm^3) and triethylsilane (13.92 g, 120 mmol) were added. The solution was stirred at room temperature for 22 h, then concentrated under reduced pressure to an oil. The triethylsilyl residues were removed by azeotroping the oil with toluene, and the toluene by azeotroping with petrol; the title compound persisted as an oil (9.48 g; 100%), which was not further purified. δ_H ($CDCl_3$) 1.47 (3H, d, $CHCH_3$), 2.93 (3H, s, NCH_3), 4.7-4.9 (1H, m, CH), 5.17 (2H, s, CH_2), 7.37 (5H, s, Ar*H*), 6.6-6.9 (1H, br s, CO_2H). J_{CHCH_3} 7 Hz.

***N*-Methyl-(*RS*)-alanine (217).** A solution of *N*-benzyloxycarbonyl-*N*-methyl-(*RS*)-alanine (9.48 g, 40 mmol) in ethanol (130 cm^3) containing 10% palladium on charcoal (0.82 g) was stirred under an atmosphere of hydrogen gas overnight. The catalyst was filtered off and washed with water, the filtrate concentrated under reduced pressure to dryness and the white solid recrystallised from ethanol (2.44 g, 59%); m.p. 257-8 °C (lit.[177,178] 315-7 °C and 260 °C respectively). δ_H (D_2O) 1.33 (3H, d, $CHCH_3$), 2.55 (3H, s, NCH_3), 3.47 (1H, q, CH). J_{CHCH_3} 7 Hz.

***N*-Methyl-*N*-(2,4-dinitrophenyl)-(*RS*)-alanine (218).** A solution of *N*-methyl-(*RS*)-alanine (2.62 g, 22 mmol) in water (40 cm^3) containing sodium hydrogen carbonate (3.78 g, 44 mmol) was added to a stirred solution of fluoro-2,4-dinitrobenzene (3.91 g, 21 mmol) in ethanol (80 cm^3) at room temperature. The mixture was heated under reflux for 6 h, concentrated under reduced pressure to dryness,

redissolved in water and extracted with ether. The aqueous layer was acidified (HCl) and the yellow product (4.92 g, 87%) filtered off and recrystallised from ethanol; m.p. 162 °C (from ethanol). (Found: C, 44.9; H, 4.0; N, 15.7. $C_{10}H_{11}N_3O_6$ requires C, 44.6; H, 4.1; N, 15.6%.) δ_H 1.47 (3H, d, $CHCH_3$), 2.79 (3H, s, NCH_3), 4.60 (1H, q, CH), 7.31 (1H, d, 6-H), 8.26 (1H, dd, 5-H), 8.60 (1H, d, 3-H). J_{CHCH_3} 7, $J_{5,6}$ 9.5, $J_{3,5}$ 1.5 Hz. δ_C 15.0 ($CHCH_3$), 36.4 (NCH_3), 59.0 (CH), 119.5 (C-6), 123.6 (C-3), 127.8 (C-5), 136.2 and 136.6 (C-2 and -4), 149.4 (C-1), 172.2 (CO).

***N*-Methyl-*N*-(2,4-dinitrophenyl)-(*RS*)-alanine ethyl ester (219).** A solution of *N*-(2,4 dinitrophenyl)-(*RS*)-alanine (4.84 g, 18 mmol) in dry ethanol (80 cm^3) containing dry HCl was heated under reflux for 6.5 h, then cooled to room temperature and concentrated under reduced pressure to an oil; b.p. 200 °C/0.8 mmHg (Kugelrohr). The distillate was dissolved in ethyl acetate, washed with aqueous sodium hydrogen carbonate to remove remaining *N*-methyl-*N*-(2,4-dinitrophenyl)-(*RS*)-alanine, dried ($MgSO_4$) and concentrated under reduced pressure. The residue was recrystallised from ethanol; m.p. 82-83 °C. δ_H ($CDCl_3$) 1.34 (3H, t, CH_2CH_3), 1.74 (3H, d, $CHCH_3$), 2.79 (3H, s, NMe), 3.90 (1H, q, CH), 4.31 (2H, q, CH_2), 7.34 (1H, d, 6-H), 8.48 (1H, dd, 5-H), 9.09 (1H, d, 3-H). $J_{CH_2CH_3}$ 7, J_{CHCH_3} 7, $J_{5,6}$ 9, $J_{3,5}$ 3 Hz. The ester was not purified sufficiently for microanalysis.

Reaction of *N*-methyl-*N*-(2,4-dinitrophenyl)-(*RS*)-alanine ethyl ester (219) with potassium carbonate. A solution of *N*-(2,4 dinitrophenyl)-(*RS*)-alanine ethyl ester (0.15 g, 0.5 mmol) in dry ethanol (5 cm^3) containing potassium carbonate (0.08 g, 0.6 mmol) was heated under reflux for 4.5 h, then cooled to room temperature and partitioned between water and ethyl acetate. The aqueous layer was acidified (HCl) and extracted with ethyl acetate, but nothing was isolated. The original ethyl acetate layer was a complex mixture, showing at least 9 spots by tlc.

***N*-(2,4-Dinitrophenyl)-(*S*)-proline ethyl ester (221).** A suspension of (*S*)-proline (2.88 g, 25 mmol) and sodium hydrogen carbonate (6.3 g, 75 mmol) in water (40 cm^3) was added to a stirred solution of fluoro-2,4-dinitrobenzene (3.72 g, 20 mmol) in ethanol (80 cm^3) at room temperature. The mixture was heated under reflux for 6.5 h, concentrated under reduced pressure to 50 cm^3, then extracted with ether and the aqueous layer acidified with HCl. The brown precipitate was recrystallised from ethanol twice (the brown oil crystallised upon stirring for 30 min) to give 5.5 g (98%) of *N*-(2,4-dinitrophenyl)-(*S*)-proline; m.p. 133-6 °C (dec.) (lit.[179] 138 °C). δ_H 1.88-2.11 (2H, m, $CH_2CH_2CH_2$), 2.45-2.52 (2H, m, $CHCH_2$), 3.15-19 (1H, m, NCH_2[a]), 3.38-3.48 (1H, m, NCH_2[b]), 4.68 (1H, t, CH), 7.02 (1H, d, 6-H), 8.25 (1H, dd, 5-H), 8.60 (1H, d, 3-H). $J_{5,6}$ 9.5, $J_{3,5}$ 2.5 Hz.

A solution of *N*-(2,4-dinitrophenyl)-(*S*)-proline (5.30 g, 18.9 mmol) in ethanol (80 cm^3) containing dry HCl (2 g) was heated under reflux for 6 h. The excess ethanol was removed under reduced pressure and the residue taken up in dichloromethane, washed with water, dried over $MgSO_4$ and concentrated under reduced pressure. Crystallisation of the brown oil was attempted from ether/petrol, but the product persisted as an oil (5.49 g, 94%). δ_H ($CDCl_3$) 1.20 (3H, t, CH_3), 1.95-2.02 (2H, m, $CH_2CH_2CH_2$), 2.45 (2H, m, $CHCH_2$), 3.30 (2H, m, NCH_2), 4.14 (2H, q, CH_2CH_3), 4.70 (1H, t, CH), 7.00 (1H, d, 6-H), 8.21 (1H, dd, 5-H), 8.63 (1H, d, 3-H). $J_{CH_2CH_3}$ 9.5, $J_{2,3}$ 9.5, $J_{3,5}$ 2.5 Hz. δ_C 14.2 (CH_3), 24.5 ($CH_2CH_2CH_2$), 30.5 ($CHCH_2$), 52.3 (NCH_2), 61.6 (CH_2CH_3), 62.3 (CH), 117.5 (C-3), 123.5 (C-6), 127.7 (C-5), 135.5 (C-2), 135.7 (C-4), 145.3 (C-1), 171.0 (CO). *m/z* 309 ($M^{+\cdot}$, 5%), 279 (5), 236 (100), 149 (45) *etc*. Microanalytical purity was not obtained, although no impurities could be detected by NMR spectroscopy.

Reaction of *N*-(2,4-dinitrophenyl)-(*S*)-proline ethyl ester (221) with potassium carbonate. A solution of *N*-(2,4-dinitrophenyl)-(*S*)-proline ethyl ester (0.23 g, 0.75 mmol) in dry ethanol (10 cm^3) containing potassium carbonate (0.11 g, 0.8 mmol) was stirred at room temperature for 24 h (no reaction). The mixture

was heated under reflux for 2 h, then cooled to room temperature, concentrated under reduced pressure to dryness and partitioned between water and ethyl acetate. The aqueous layer, upon acidification (HCl) and extraction with ethyl acetate, yielded an intractable brown oil. The original ethyl acetate layer was dried ($MgSO_4$) and concentrated under reduced pressure to an intractable red solid.

Reaction of *N*-(2,4-dinitrophenyl)-(*S*)-proline ethyl ester (221) with sodium ethoxide. A solution of *N*-(2,4-dinitrophenyl)-(*S*)-proline (0.15 g, 0.5 mmol) in dry ethanol (10 cm^3) and *N*,*N*-dimethylformamide (2 cm^3) had added to it sodium ethoxide [from Na (0.012 g, 0.5 mmol) in ethanol (1 cm^3)] at room temperature. The solution was stirred at room temperature for 4 h, then the ethanol removed under reduced pressure and the residue diluted with water. Again, however, no products could be isolated from the reaction mixture, either from the precipitate (after dilution with water) or from the filtrate (which was partitioned between water and ethyl acetate, *etc.*).

***N*-(2,4-Dinitrophenyl)-*trans*-4-hydroxy-(*S*)-proline ethyl ester (222).** A solution of *trans*-4-hydroxy-(*S*)-proline (3.28 g, 25 mmol) in water (40 cm^3) with sodium hydrogen carbonate (6.3 g, 75 mmol) was added to a stirred solution of fluoro-2,4-dinitrobenzene (3.72 g, 20 mmol) in ethanol (80 cm^3) at room temperature. The yellow mixture was heated under reflux for 6.5 h (solution turned red), concentrated under reduced pressure to ~50 cm^3, then extracted with ether and the aqueous layer acidified with HCl. The oil was extracted with ether, dried over $MgSO_4$ and concentrated under reduced pressure; 4.81 g (81%). Attempted recrystallisations from propan-2-ol/water and ether/petrol were both unsuccessful - removal of the solvent under reduced pressure resulted in a brown solid [*N*-(2,4-dinitrophenyl)-*trans*-4-hydroxy-(*S*)-proline]; m.p. 165-168 °C (lit.[180] m.p. 174-5 °C), which was esterified without further purification. δ_H 2.40 (1H, m, pro-3-H), 2.66 (1H, d, pro-3-H), 3.62 (1H, dd, pro-5-H), 4.40 (1H, br s, pro-5-H), 4.73 (1H, dd, pro-2-H), 5.20 (1H, br s, OH; absent

after addition of D_2O), 7.11 (1H, d, 6-H), 8.27 (1H, dd, 5-H), 8.61 (1H, d, 3-H). $J_{5,6}$ 9, $J_{3,5}$ 2.5 Hz.

A solution of *N*-(2,4-dinitrophenyl)-*trans*-4-hydroxy-(*S*)-proline (4.57 g, 15 mmol) in ethanol (100 cm^3) containing dry HCl (2.5 g) was heated under reflux for 7 h. The solution was cooled to room temperature, the solvent removed under reduced pressure and the residue partitioned between water and dichloromethane. The organic layer was dried over $MgSO_4$ and concentrated to yield a brown solid (3.20 g, 66%); m.p. ~90 °C, which could not be recrystallised under a range of different solvent conditions. δ_H ($CDCl_3$) 1.24 (3H, t, CH_2CH_3), 2.00 (1H, br s, OH; exchangeable with D_2O), 2.25 (1H, m, pro-4-H), 2.55 (1H, m, pro-3-H), 2.96 (1H, d, pro-3-H), 3.87 (1H, dd, pro-5-H), 4.20 (2H, q, CH_2CH_3), 4.65 (1H, br s, pro-5-H), 4.79 (1H, dd, pro-2-H), 6.90 (1H, d, 6-H), 8.21 (1H, dd, 5-H), 8.69 (1H, d, 3-H). $J_{5,6}$ 9, $J_{3,5}$ 2.5 Hz. δ_C 14.1 (CH_3), 38.7 (pro-C-3), 60.8 and 60.9 (pro-C-2 and -5), 62.4 (CH_2CH_3), 69.9 (pro-C-4), 116.3 (C-6), 123.9 (C-3), 128.0 (C-5), 136.4 (C-2), 137.2 (C-4), 146.2 (C-1), 171.3 (CO).

Reaction of *N*-(2,4-dinitrophenyl)-*trans*-4-hydroxy-(*S*)-proline ethyl ester (222) with potassium carbonate. A solution of *N*-(2,4-dinitrophenyl)-*trans*-4-hydroxy-(*S*)-proline ethyl ester (0.65 g, 2 mmol) in dry ethanol (15 cm^3) containing potassium carbonate (0.30 g, 2.2 mmol) was heated under reflux for 3.5 h, then cooled to room temperature and filtered (inorganic). The filtrate was concentrated under reduced pressure to an intractable dark red solid.

***N*-(2,4-Dinitrophenyl)-(*S*)-histidine ethyl ester (225).** A suspension of (*S*)-histidine (3.88 g, 25 mmol) and sodium hydrogen carbonate (6.3 g, 75 mmol) in water (40 cm^3) was added to a stirred solution of fluoro-2,4-dinitrobenzene (4.28 g, 20 mmol) in ethanol (80 cm^3) at room temperature. The mixture was heated under reflux for 6 h, concentrated under reduced pressure to dryness, dissolved in water then extracted with ether and the aqueous layer acidified with HCl to yield a yellow precipitate

of *N*-(2,4-dinitrophenyl)-(*S*)-histidine (7.13 g, 97%); m.p. 218-220 °C (lit.[181] 250-280 °C). δ_H 3.20 (2H, d, CH_2), 4.79 (1H, q, CH), 7.00 (1H, s, 5'-H), 7.12 (1H, d, 6-H), 7.88 (1H, s, 2'-H), 8.24 (1H, dd, 5-H), 8.88 (1H, d, 3-H), 9.35 (1H, d, NH). J_{CH_2CH} 7, $J_{3,5}$ 3, $J_{5,6}$ 9 Hz.

A solution of *N*-(2,4-dinitrophenyl)-(*S*)-histidine in ethanol (250 cm^3) containing dry HCl (6 g) was heated under reflux for 6.5 h. The solution was evaporated to dryness under reduced pressure and the residue taken up in dichloromethane, washed with water, dried over $MgSO_4$ and the dichloromethane removed under reduced pressure to yield a yellow solid which was recrystallised from ethanol (2.85 g, 41% from fluoro-2,4-dinitrobenzene); m.p. 170 °C (from ethanol/water). (Found: C, 48.1; H, 4.1; N, 20.2. $C_{14}H_{15}N_5O_6$ requires C, 48.1; H, 4.3; N, 20.05%.) δ_H ($CDCl_3$ + 2 drops DMSO-d_6) 1.28 (3H, t, CH_2CH_3), 3.29 (2H, m, $CHCH_2$), 4.24 (2H, q, CH_2CH_3), 4.72 (1H, m, CH), 6.81 (1H, d, 6-H), 6.83 (1H, s, im-5-H), 7.59 (1H, s, im-2-H), 8.19 (1H, dd, 5-H), 9.09 (1H, s, 3-H), 9.22 (1H, d, exocyclic NH). $J_{CH_2CH_3}$ 9.5, J_{CHCH_2} 7, J_{NHCH} 7, $J_{5,6}$ 9.5, $J_{3,5}$ 2.5 Hz. δ_C 14.2 (CH_2CH_3), 31.0 ($CHCH_2$), 56.4 (CH), 62.2 (CH_2CH_3), 114.7 (C-6), 115.5 (im-C-2), 124.2 (C-3), 130.2 (C-5), 131.1 (im-C-4), 134.0 (C-2), 135.8 (im-C-5), 136.6 (C-4), 147.8 (C-1), 170.7 (CO). *m/z* 349 ($M^{+\cdot}$, 37%), 317 (9), 285 (10), 276 (12), 191 (11), 91 (100) *etc.*

Reaction of *N*-(2,4-dinitrophenyl)-(*S*)-histidine ethyl ester (225) with potassium carbonate. a) Potassium carbonate (1.10 g, 8 mmol) was added to a suspension of *N*-(2,4-dinitrophenyl)-(*S*)-histidine ethyl ester (1.75 g, 5 mmol) in ethanol (50 cm^3) and the mixture heated under reflux for 4 h. The reaction mixture was filtered, the solid dissolved in water and the solution taken to pH 7 (HCl); 5-nitro-1*H*-benzimidazole-2-carboxylic acid 3-oxide (**227**) was filtered off (0.52 g, 47%); m.p. 258-60 °C (dec.). δ_H 7.81 (1H, d, 7-H), 8.05 (1H, dd, 6-H), 8.39 (1H, d, 4-H). $J_{6,7}$ 9, $J_{4,6}$ 2 Hz. δ_C 107.8 (C-4), 117.3 (C-7), 121.7 (C-6), 129.3 (C-3a), 141.7 (C-5), 142.6 (C-7a), 143.1 (C-2), 162.2 (CO) The filtrate was taken to pH 1 (HCl) and 5-nitro-1*H*-benzimidazole 3-oxide (**228**) (0.05 g, 6%) collected; m.p. 265 °C (lit.[182]

274 °C). δ_H 7.86 (1H, d, 7-H), 8.11 (1H, dd, 6-H), 8.38 (1H, d, 4-H), 8.78 (1H, s, 2-H). $J_{6,7}$ 9, $J_{4,6}$ 2 Hz. The original reaction mixture filtrate was concentrated under reduced pressure to dryness, partitioned between water and ethyl acetate, the aqueous phase acidified (HCl) and the precipitate [ethyl 5-nitro-1*H*-benzimidazole-2-carboxylate 3-oxide (**226**)] filtered off and dried; 0.32 g (26%), m.p. 209-10 °C (lit.[88] 209-210 °C). δ_H 1.41 (3H, t, CH_3), 4.49 (2H, q, CH_2), 8.01 (1H, d, 7-H), 8.21 (1H, dd, 6-H), 8.46 (1H, d, 4-H). $J_{CH_2CH_3}$ 7, $J_{6,7}$ 9, $J_{4,6}$ 2 Hz. δ_C 14.2 (Me), 62.4 (CH_2), 107.4 (C-4), 118.9 (C-7), 122.3 (C-6), 132.2 (C-3a), 140.9 (C-5), 142.2 (C-7a), 144.9 (C-2), 157.6 (CO).

b) *N*-2,4-(Dinitrophenyl)-(*S*)-histidine ethyl ester (0.70 g, 2 mmol) and potassium carbonate (0.41 g, 3 mmol) in ethanol (25 cm^3) were heated under reflux for 5 h. The reaction mixture was filtered, the solid taken up in water, acidified and the yellow precipitate [5-nitro-1*H*-benzimidazole-2-carboxylic acid 3-oxide (**227**)] filtered off (0.17 g, 38%). The reaction mixture filtrate was concentrated under reduced pressure to dryness, partitioned between water and ethyl acetate, the aqueous phase acidified (HCl) and ethyl-5-nitrobenzimidazole-2-carboxylate 3-oxide (**226**) (0.15 g, 30%) filtered off (confirmed by m.p.).

***N*-(2,4-Dinitrophenyl)-(*S*)-tryptophan ethyl ester.** (With M. Parsons.) A solution of fluoro-2,4-dinitrobenzene (1.87 g, 10 mmol) in ethanol (20 cm^3) was added to a stirred solution of (*S*)-tryptophan (2.15 g, 10.5 mmol) and sodium hydrogen carbonate (1.77 g, 10 mmol) in water (20 cm^3), and the mixture heated under reflux for 12 h. The mixture was concentrated under reduced pressure to dryness, and the residue dissolved in water, extracted with ethyl acetate and the aqueous layer acidified to give *N*-(2,4-dinitrophenyl)-(*S*)-tryptophan which was recrystallised from acetone/ether; 2.41 g (61%); m.p.193-4 °C (lit.[183] 196-8 °C). δ_H 3.37-3.51 (2H, symm. m, CH_2), 4.96-5.10 (1H, symm. m, CH), 6.92 (1H, t, indole 5-H), 7.06 (1H, t, indole 6-H), 7.16 (1H, s, indole 2-H), 7.20 (1H, d, 6-H), 7.29 (1H, d, indole 7-H), 7.36 (1H, d,

indole 4-H), 8.20 (1H, dd, 5-H), 8.83 (1H, d, 3-H), 8.85 (1H, d, NH), 10.95 (1H, s, indole NH). $J_{3,5}$ 3, $J_{5,6}$ 10, J_{NHCH} 7, $J_{indole\ 4,5} \sim J_{indole\ 5,6} \sim J_{indole\ 6,7} \sim 7$ Hz.

A solution of *N*-(2,4-dinitrophenyl)-(*S*)-tryptophan (0.35 g, 0.95 mmol) in ethanol (15 cm^3) containing dry HCl (3.1 g) was heated under reflux for 5 h. The reaction mixture was concentrated under reduced pressure to dryness, and the resultant oil purified by column chromatography on silica gel with dichloromethane/petrol as eluent. Triturating the orange oil in petrol yielded the product as a solid (0.14 g, 24%) which was recrystallised from acetic acid to give orange needles; m.p.113-5 °C. (Found: C, 42.5; H, 4.3; N, 12.7. $C_{19}H_{18}N_4O_6.HCl$ requires C, 42.5; H, 4.4; N, 12.9%.) δ_H ($CDCl_3$) 1.18 (3H, t, CH_2CH_3), 3.39-3.53 (2H, symm. m, $CHCH_2$), 4.12 (2H, q, CH_2CH_3), 4.97-5.11 (1H, symm. m, CH), 6.94 (1H, t, indole 5-H), 7.07 (1H, t, indole 6-H), 7.18 (1H, s, indole 2-H), 7.23 (1H, d, 6-H), 7.30 (1H, d, indole 7-H), 7.34 (1H, d, indole 4-H), 8.21 (1H, dd, 5-H), 8.81 (1H, d, 3-H), 8.83 (1H, d, NH), 10.99 (1H, s, indole NH). $J_{CH_2CH_3}$7, $J_{3,5}$ 3, $J_{5,6}$ 10, J_{NHCH} 7, $J_{indole\ 4,5} \sim J_{indole\ 5,6} \sim J_{indole\ 6,7} \sim 7$ Hz. δ_C 13.9 (CH_3), 27.0 ($CHCH_2$), 55.9 (CH), 61.7 (CH_2CH_3), 107.6 (indole C-3), 111.6 (indole C-7), 116.0 (C-6), 118.0 (indole C-5), 118.7 (indole C-6), 121.3 (indole C-4), 123.6 and 124.6 (C-3 and indole C-2), 127.3 (indole C-7a), 130.1 (C-5), 130.3 (C-2), 135.8 (indole C-3a), 136.2 (C-4), 147.2 (C-1), 170.8 (CO). *m/z* 398 ($M^{+\cdot}$, 7%), 266 (5), 235 (6), 224 (7), 192 (25), 162 (7), 130 (100) *etc.*

***N*-(2,4-Dinitrophenyl)-(*S*)-tryptophan methyl ester (231).** (With M. Parsons.) Fluoro-2,4-dinitrobenzene (1.86 g, 10 mmol), (*S*)-tryptophan methyl ester hydrochloride (2.55 g, 10 mmol) and sodium hydrogen carbonate (1.68 g, 20 mmol) in dry methanol (40 cm^3) were heated together under reflux for 13 h, cooled, poured into ice/water and filtered. The product was recrystallised from acetic acid; yield 3.75 g (98%); m.p. 157-8 °C. (Found: C, 56.0; H, 4.0; N, 14.4. $C_{18}H_{16}N_4O_6$ requires C, 56.0; H, 4.2; N, 14.6%.) δ_H 3.38 (2H, symm. m, CH_2), 3.69 (3H, s, CH_3), 5.06-5.19 (1H, symm. m, CH), 6.95 (1H, t, indole 7-H), 7.04 (1H, t, indole 6-H), 7.17

(1H, s, indole 2-H), 7.21 (1H, d, 6-H), 7.28 (1H, d, indole 7-H), 7.34 (1H, d, indole 4-H), 8.22 (1H, dd, 5-H), 8.79 (1H, d, NH), 8.82 (1H, d, 3-H), 10.99 (1H, s, indole NH). δ_C 27.1 (CH_2), 52.6 (CH_3), 55.7 (CH), 107.5 (indole C-3), 111.5 (indole C-7), 115.8 (C-6), 117.8 (indole C-5), 118.6 (indole C-6), 121.9 (indole C-4), 123.4 and 124.6 (indole C-2 and C-3), 127.0 (indole C-7a), 129.9 (C-5), 130.1 (C-2), 135.6 (indole C-3a), 136.1 (C-4), 147.0 (C-1), 171.0 (CO). *m/z* 384 ($M^{+\cdot}$, 5%), 130 (100), 84 (8), *etc.*

Reaction of *N*-(2,4-Dinitrophenyl)-(*S*)-tryptophan methyl ester (231) with potassium carbonate. (With M. Parsons.) *N*-(2,4-Dinitrophenyl)-(*S*)-tryptophan methyl ester (0.77 g, 2 mmol) and potassium carbonate (0.28 g, 2 mmol) in dry methanol (20 cm^3) were heated together under reflux for 2 h, then cooled to room temperature and filtered. The methanol filtrate was concentrated under reduced pressure to dryness and the residue stirred in water; the insoluble buff-coloured solid [3-(methoxymethyl)indole (**232**)] was filtered off and dried *in vacuo*; 0.22 g (69%); m.p. 94-6 °C [lit.[184,185,186] 96-7 °C, 99-100 °C and 98-99 °C respectively]. δ_H 3.20 (3H, s, CH_3), 4.53 (2H, s, CH_2), 6.98 (1H, t, 5-H), 7.08 (1H, t, 6-H), 7.29 (1H, s, 2-H), 7.35 (1H, d, 7-H), 7.54 (1H, d, 4-H), 10.99 (1H, br s, NH). The aqueous filtrate was acidified (HCl) to precipitate methyl 5-nitro-1*H*-benzimidazole-2-carboxylate 3-oxide (**108**) (0.25 g; 53%); m.p. 194-5 °C (lit.[187] 205-6 °C). δ_H 3.97 (3H, s, CH_3), 7.97 (1H, d, 7-H), 8.18 (1H, dd, 6-H), 8.43 (1H, d, 4-H). $J_{6,7}$ 9, $J_{4,6}$ 2 Hz. The original reaction mixture precipitate was dissolved in water, extracted with ethyl acetate and the aqueous layer acidified (HCl); 80 mg of a buff-coloured solid was isolated as a mixture of methyl 7-nitrobenzimidazole-2-carboxylate 3-oxide and another, unidentified compound.

Diethyl *N*-(2,4-dinitrophenyl)-(*S*)-glutamate (236). (With M. Parsons.) A solution of fluoro-2,4-dinitrobenzene (11.16 g, 60 mmol) in ethanol (120 cm^3) was added to a stirred solution of (*S*)-glutamic acid (11.79 g, 63 mmol) and

sodium hydrogen carbonate (10.59 g, 126 mmol) in water (120 cm^3), and the mixture heated under reflux for 7 h. The mixture was concentrated under reduced pressure to dryness, and the residual oil crystallised to give *N*-(2,4-dinitrophenyl)-(*S*)-glutamic acid, which was a yellow hygroscopic solid (m.p. 115-6 °C; lit.[181] 155-162 °C) from ethyl acetate/petrol (13.33 g; 71%). δ_H 2.10-2.20 (2H, symm. m, $CHCH_2CH_2$), 2.30-2.41 (2H, m, $CHCH_2CH_2$), 4.68-4.76 (1H, symm. m, CH), 7.25 (1H, d, 6-H), 8.26 (1H, dd, 5-H), 8.86 (1H, d, 3-H), 8.93 (1H, d, NH). J_{CHNH} 7, $J_{3,5}$ 3, $J_{5,6}$ 10 Hz.

A solution of *N*-(2,4-dinitrophenyl)-(*S*)-glutamic acid (6.00 g, 19.17 mmol) in dry ethanol (36 cm^3) containing dry HCl (about 9 g) was heated under reflux for 5.5 h, then cooled to room temperature and concentrated under reduced pressure to a yellow-brown oil. This was chromatographed on silica gel with ether-petrol (6:5) as eluent to give the ester as a yellow oil; 4.53 g (75%); b.p. 200 °C/0.5 mmHg. (Found: C, 49.0; H, 5.3; N, 11.0. $C_{15}H_{19}N_3O_8$ requires C, 48.8; H, 5.2; N, 11.4%.) δ_H ($CDCl_3$) 1.28 (3H, t, CH_2CH_3 of α- or γ-ester), 1.34 (3H, t, CH_2CH_3 of α- or γ-ester), 2.24-2.34 (2H, symm. m, $CHCH_2CH_2$), 2.52 (2H, t, $CHCH_2CH_2$), 4.19 (2H, q, CH_2 of α- or γ-ester), 4.31 (2H, q, CH_2 of α- or γ-ester), 4.45-4.53 (1H, symm. m, CH), 6.99 (1H, d, 6-H), 8.33 (1H, dd, 5-H), 8.95 (1H, d, NH), 9.18 (1H, d, 3-H). $J_{CH_2CH_3}$ = $J_{CH_2CH_2}$ = 7, J_{CH_2} 14, J_{CHNH} 7, $J_{3,5}$ 2, $J_{5,6}$ 9.5 Hz. δ_C 13.9 ($CHCH_3$ of both α- and γ-esters), 26.8 ($CHCH_2$), 29.6 (CH_2CO_2Et), 54.7 (CH), 60.7 (CH_2CH_3 of α- or γ-ester), 62.2 (CH_2CH_3 of α- or γ-ester), 114.5 (C-6), 123.8 (C-3), 130.2 (C-5), 130.8 (C-2), 136.4 (C-4), 146.8 (C-1), 170.1 (γ-CO), 172.0 (α-CO). *m/z* 369 ($M^{+\cdot}$, 5%), 324 (55), 296 (82), 262 (16), 250 (100). 222 (42) *etc.*

Reaction of diethyl *N*-(2,4-dinitrophenyl)-(*S*)-glutamate (236) with potassium carbonate. (With M. Parsons.) A solution of diethyl *N*-(2,4-dinitrophenyl)-(*S*)-glutamate (1.52 g, 4.1 mmol) in dry ethanol (35 cm^3) containing potassium carbonate (0.57 g, 4.1 mmol) was heated under reflux for 2 h. The cooled reaction mixture was filtered and the brown precipitate dissolved in water. The aqueous solution yielded (on acidification with HCl) ethyl 3-(5-nitro-3-oxido-1*H*-benzimidazol-

2-yl)propionate (**237**) which was recrystallised from acetone/petrol; 0.41 g (39%); m.p. 79-83 °C. δ_H 1.15 (3H, t, CH_2CH_3), 2.88 (2H, t, CCH_2CH_2), 3.18 (2H, t, CCH_2CH_2), 4.02 (2H, q, CH_2CH_3), 7.70 (1H, d, 4-H), 8.03 (1H, dd, 6-H), 8.24 (1H, d, 7-H). $J_{CH_2CH_3} = J_{CH_2CH_2} = 7$, $J_{4,6}$ 2, $J_{6,7}$ 9 Hz. δ_C 14.0 (Me), 21.1 ($CH_2CH_2CO_2$), 29.8 (CH_2CO_2), 60.1 (CH_2CH_3), 105.2 (C-4), 116.9 (C-7), 119.3 (C-6), 131.8 (C-3a), 142.4 (C-2 and C-5), 155.9 (C-7a), 172.0 (CO). Although (**237**) was not obtained in sufficient purity for microanalysis, the NMR data are consistent with the proposed structure. Extraction with ether of the original reaction mixture filtrate yielded a mixture (0.1 g) of the above ester and the corresponding carboxylic acid (**238**).

Diethyl *N*-(2,4-Dinitrophenyl)-(*S*)-aspartate (239). (With M. Parsons.) A solution of fluoro-2,4-dinitrobenzene (3.72 g, 20 mmol) in ethanol (40 cm^3) was added to a stirred solution of (*S*)-aspartic acid (2.80 g, 21 mmol) and sodium hydrogen carbonate (5.29 g, 63 mmol) in water (40 cm^3), and the mixture heated under reflux for 5 h. The mixture was concentrated under reduced pressure to dryness and the residue dissolved in water and extracted with ether. The aqueous layer was acidified (HCl) and *N*-(2,4-dinitrophenyl)-(*S*)-aspartic acid filtered off and recrystallised from ethyl acetate/petrol to give yellow needles, 5.13 g (86%); m.p. 185-6 °C (lit.[179] 186-7 °C). δ_H 2.70 and 2.91 (each 1H, dd, CH_2), 4.94-5.07 (1H, symm. m, CH), 7.30 (1H, d, 6-H), 8.30 (1H, dd, 5-H), 8.85 (1H, d, 3-H), 9.20 (1H, d, NH). J_{CHNH} 8, J_{CH_2} 16, J_{CHCH_2} 8, $j_{3,5}$ 3, $J_{5,6}$ 10 Hz.

A solution of *N*-(2,4-dinitrophenyl)-(*S*)-aspartic acid (2.50 g, 8.4 mmol) in dry ethanol (35 cm^3) containing dry HCl (5.1 g) was heated under reflux for 7 h, then cooled to room temperature and concentrated under reduced pressure to a yellow-brown oil. Trituration in petrol yielded a yellow solid which was recrystallised from aqueous ethanol; 2.19 g (74%); m.p. 58-9 °C. (Found: C, 47.3; H, 4.8; N, 11.8. $C_{14}H_{17}N_3O_8$ requires C, 47.4; H, 4.8; N, 11.8%). δ_H ($CDCl_3$) 1.28 (3H, t, α- or β- CH_2CH_3), 1.33 (3H, t, α- or β- CH_2CH_3), 2.95 and 3.04 (each 1H, dd, CH_2), 4.18 (2H, q, α- or β- CH_2CH_3), 4.29 (2H, q, α- or β- CH_2CH_3), 4.73 (1H, symm. m, CH), 6.96 (1H, d,

6-H), 8.32 (1H, dd, 5-H), 9.13 (1H, d, 3-H), 9.20 (1H, d, NH). $J_{CH_2CH_3}$ 7, J_{CH_2} 16, J_{CHCH_2} 5, J_{CHNH} 8, $J_{3,5}$ 3, $J_{5,6}$ 9 Hz. δ_C 14.0 (2 x CH_3), 36.6 ($CHCH_2$), 52.4 (CH), 61.7 and 62.7 (CH_2 of α- and β-ester), 113.9 (C-6), 124.2 (C-3), 130.3 (C-5), 131.3 (C-2), 136.8 (C-4), 147.0 (C-1), 169.3 and 169.5 (CO of α- and β-ester). *m/z* 355 ($M^{+\cdot}$, 13%), 282 (100), 268 (10), 236 (55), *etc.*

Reaction of diethyl *N*-(2,4-dinitrophenyl)-(*S*)-aspartate (239) with potassium carbonate. (With M. Parsons.) Potassium carbonate (0.22 g, 1.8 mmol) was added to a solution of diethyl *N*-(2,4-dinitrophenyl)-(*S*)-aspartate (0.6 g, 1.7 mmol) in ethanol (40 cm^3). After stirring for 40 min at room temperature, the solution was filtered, and the brown solid dissolved in water and the solution acidified (HCl). No identifiable product was isolated. The reaction mixture was concentrated under reduced pressure to dryness and the residue dissolved in water. The aqueous solution was acidifed (HCl) and the yellow solid (0.38 g, 63%) filtered off and identified as starting material.

Reaction of diethyl *N*-(2,4-dinitrophenyl)-(*S*)-aspartate (239) with sodium ethoxide. (With M. Parsons.) Sodium ethoxide [1.13 mmol, from Na (0.059 g) in ethanol (3 cm^3)] was added to a stirred solution of diethyl *N*-(2,4-dinitrophenyl)-(*S*)-aspartate (0.40 g, 1.13 mmol) in ethanol (1 cm^3) at 0-5 °C. After stirring for 19 h at room temperature, excess solvent was remove under reduced pressure, and the residual solid dissolved in water. Acidification of this solution (HCl) again failed to yield any identifiable product.

***N*-(4,5-Dichloro-2-nitrophenyl)sarcosine ethyl ester (242).** A suspension of sarcosine (1.07 g, 12 mmol) and sodium hydrogen carbonate (3.02 g, 36 mmol) in water (20 cm^3) was added to a stirred solution of 1,2-dichloro-4-fluoro-5-nitrobenzene (2.10 g, 10 mmol) in ethanol (40 cm^3) at room temperature. The mixture was heated under reflux for 6 h, concentrated under reduced pressure to dryness,

dissolved in water then extracted with ether and the aqueous layer acidified with HCl, giving a yellow precipitate of *N*-(4,5-dichloro-2-nitrophenyl)sarcosine, which was filtered off and recrystallised from ethanol (2.64 g, 95%); m.p. 139-40 °C. δ_H 2.82 (3H, s, CH_3), 4.02 (2H, s, CH_2), 7.33 (1H, s, 6-H), 8.09 (1H, s, 3-H). δ_C 41.2 (CH_3), 54.9 (CH_2), 119.6 (C-4), 120.7 (C-6), 127.6 (C-3), 136.2 (C-2), 137.3 (C-5), 144.2 (C-1), 170.9 (CO). The acid was esterified without further purification for microanalysis.

A solution of *N*-(4,5-dichloro-2-nitrophenyl)sarcosine (2.64 g, 9.46 mmol) in ethanol (30 cm^3) containing dry HCl (1.2 g) was heated under reflux for 5 h. The excess ethanol was removed under reduced pressure, the residue taken up in ether, filtered, and the filtrate concentrated under reduced pressure to leave a yellow solid on cooling which was recrystallised twice from ethanol (1.87 g, 64%); m.p. 66-7 °C. (Found: C, 42.8; H, 4.3; N, 9.0. $C_{11}H_{12}Cl_2N_2O_4$ requires C, 43.0; H, 3.9; N, 9.1%.). δ_H ($CDCl_3$) 1.32 (3H, t, CH_2CH_3), 3.00 (3H, s, NCH_3), 4.21 (2H, s, NCH_2), 4.25 (2H, q, CH_2CH_3), 7.50 (1H, s, 6-H), 8.20 (1H, s, 3-H). $J_{CH_2CH_3}$ 8 Hz. δ_C 14.0 (CH_2CH_3), 40.8 (NCH_3), 54.9 (NCH_2), 60.6 (CH_2CH_3), 120.1 (C-4), 121.0 (C-6), 127.2 (C-3), 136.0 (C-2), 137.7 (C-5), 143.8 (C-1), 169.1 (CO). *m/z* 306 ($M^{+\cdot}$, 7%), 233 (100), 200 (37), 187 (10), 174 (9), 159 (21).

6,7-Dichloro-1-hydroxy-4-methylquinoxaline-2,3-dione (243). A solution of *N*-(4,5-dichloro-2-nitrophenyl)sarcosine ethyl ester (0.92 g, 3 mmol) and potassium carbonate (0.41 g, 3 mmol) in *N,N*-dimethylformamide (13 cm^3) and ethanol (25 cm^3) was stirred at room temperature for 5 h. The solid product was filtered off, dissolved in water and acidified with HCl to furnish 90 mg (11%) of product. The mother liquor was stirred for a further 9 days, then concentrated under reduced pressure and the residue partitioned between water and ether. The aqueous layer was acidified with HCl, but no further precipitation occurred. The crude material melted between 260 °C and 280 °C, with decomposition. δ_H 3.62 (3H, s, CH_3), 7.65 (1H, s, 5-H),

7.75 (1H, s, 8-H). Further purification proved impossible on the small amount of material available.

***N*-(2,4-Dinitrophenyl)iminodiacetic acid.** A solution of iminodiacetic acid disodium salt monohydrate (3.90 g, 20 mmol) in water (30 cm^3) was added to a stirred solution of 2,4-dinitrochlorobenzene (3.65 g, 18 mmol) in ethanol (60 cm^3), and the mixture heated under reflux for 15 h, during which another portion of the disodium salt (0.39 g, 2 mmol) was added. The solution was concentrated under reduced pressure to dryness and the residue partitioned between water and ether. The aqueous layer was acidifed (HCl), extracted with ethyl acetate, and the ethyl acetate dried ($MgSO_4$) and concentrated under reduced pressure to a yellow solid, which was treated with ether to remove traces of starting material to furnish 3.08 g (57%) of product, which was not further purified. δ_H 4.19 (4H, s, 2 x CH_2), 7.14 (1H, d, 6-H), 8.28 (1H, dd, 5-H), 8.61 (1H, d, 3-H). $J_{5,6}$ 9, $J_{3,5}$ 2 Hz.

Attempted esterification of *N*-(2,4-dinitrophenyl)iminodiacetic acid. A solution of *N*-(2,4-dinitrophenyl)iminodiacetic acid (0.58 g. 1.9 mmol) in dry ethanol (30 cm^3) containing dry HCl (2 g) was heated under reflux for 12 h, but only an inseparable mixture (by tlc and ^1H NMR spectroscopy) of products was obtained.

Attempted direct synthesis of diethyl *N*-(2,4-dinitrophenyl)iminodiacetate (244). A solution of diethyl iminodiacetate (2.27 g, 12 mmol) and fluoro-2,4-dinitrobenzene (1.86 g, 10 mmol) in dry ethanol (25 cm^3) with triethylamine (1.74 cm^3, 12.5 mmol) was heated under reflux for 11 h, cooled to room temperature and concentrated under reduced pressure to ~5 cm^3. The yellow crystals were filtered off and washed with a little ethanol to give 2,4-dinitrophenetole (**247**) (0.75 g, 35%); m.p. 83-85 °C (lit.[188] 87 °C). δ_H ($CDCl_3$) 1.55 (3H, t, CH_2CH_3), 4.33 (2H, q, CH_2CH_3), 7.20 (1H, d, 6-H), 8.44 (1H, dd, 5-H), 8.75 (1H, d, 3-H). $J_{3,5}$ 3, $J_{5,6}$ 9 Hz. The ethanol filtrate was concentrated under reduced pressure to a brown oil, which was

distilled (b.p. 160°C/0.5 mmHg) to give the starting material (diethyliminodiacetate; 1.29 g, 57%). The distillation residue yielded a yellow product (b.p. 210 °C/0.5 mmHg; 0.82 g, 23%), tentatively identified as the desired product. Attempted recrystallisation from ethanol, however, led to complete recovery of 2,4-dinitrophenetole (**247**).

Attempted synthesis of diethyl *N*-(2-nitrophenyl)aminomalonate (245). Fluoro-2-nitrobenzene (1.41 g, 10 mmol), diethyl aminomalonate hydrochloride (2.96 g, 14 mmol) and potassium carbonate (4.15 g, 30 mmol) were heated together under reflux in acetonitrile (50 cm^3) for 4 h. Only an intractable mixture was obtained. The same result occurred with the attempted synthesis of diethyl *N*-(2,4-dinitrophenyl)-aminomalonate.

***N*-(2,4-Dinitrophenyl)-2',2',2'-trifluoroethylamine (248).** A solution of fluoro-2,4-dinitrobenzene (1.58 g, 8.5 mmol) and 2,2,2-trifluoroethylamine hydrochloride (1.22 g, 9 mmol) in dry *N*,*N*-dimethylformamide (5 cm^3) with potassium carbonate (2.48 g, 18 mmol) was heated to 100 °C for 5 h with stirring, then cooled and poured into ice/water. The precipitate was filtered off, washed with water, dried and recrystallised from propan-2-ol; 1.37 g (61%), m.p. 116 °C (lit.[189] 115-6 °C). δ_H 4.48 (2H, quintet, CH_2), 7.48 (1H, d, 6-H), 8.34 (1H, dd, 5-H), 8.86 (1H, d, 3-H), 8.94 (1H, t, NH). $J_{CH_2CF} \sim J_{CH_2NH} \sim 7$, $J_{3,5}$ 3, $J_{5,6}$ 9.5 Hz. δ_C 43.3 (CH_2, q, $J_{C,F}$ 33 Hz), 115.7 (C-6), 123.3 (C-3), 124.9 (CF_3, q, $J_{C,F}$ 281 Hz), 130.0 (C-5), 131.1 (C-2), 136.3 (C-4), 147.8 (C-1).

2-Trifluoromethyl-5-nitro-1*H*-benzimidazole 3-oxide (249). A suspension of *N*-(2,4-dinitrophenyl)-2',2',2'-trifluoroethylamine (0.53 g, 2 mmol) and potassium carbonate (0.35 g, 2.5 mmol) in dry ethanol (20 cm^3) was heated under reflux for 7 h, cooled to room temperature and filtered. The filtrate was concentrated under reduced pressure to dryness, dissolved in water, extracted with ethyl acetate and the

aqueous phase acidified (HCl). The buff-coloured precipitate was filtered and washed with water to furnish 0.35 g (71%) of product; m.p. 218 °C . (Found: C, 39.1; H, 1.4; N, 16.9. $C_8H_4F_3N_3O_3$ requires C, 38.9; H, 1.6; N, 17.0%.) δ_H 8.04 (1H, d, 7-H), 8.22 (1H, dd, 6-H), 8.51 (1H, d, 4-H). $J_{6,7}$ 9, $J_{4,6}$ 2 Hz. δ_C 107.4 (C-4), 118.1 (CF_3, $J_{C,F}$ 271 Hz), 118.8 (C-7), 122.5 (C-6), 132.0 (C-3a), 140.3 (C-5), 140.3 (C-2, $J_{C,F}$ 32 Hz), 145.2 (C-7a). *m/z* 247 ($M^{+\cdot}$, 37%), 234 (68), 205 (20), 201 (16), 191 (35), 184 (13), 173 (13), 133 (12), 118 (43), 91 (100) *etc.*

***N*-Methyl-2,2,2-trifluoroethylamine hydrochloride (252).** Under nitrogen, a solution of *N*-methyl-2,2,2-trifluoroacetanilide (3.17 g, 25 mmol) in sodium-dried ether (12 cm^3) was added at between -10 to -20 °C over 1 h to a stirred suspension of lithium aluminium hydride (1.90 g, 50 mmol) in ether (25 cm^3). After stirring at this temperature for 1 h, then at room temperature overnight, the reaction mixture was cooled to 0 °C and the excess lithium aluminium hydride decomposed by cautious addition of water (25 cm^3). After stirring at room temperature for 1 h, the volatile portion was distilled off, dried ($MgSO_4$), filtered, and dry HCl bubbled through the solution. The white hydrochloride salt of the product was filtered off and dried *in vacuo*, yielding 1.65 g (44%); m.p. 168-9 °C [lit.[190] >200 °C (dec.)]. δ_H (D_2O) 2.79 (3H, s, NCH_3), 3.90 (2H, q, CH_2). $J_{CH_2CF_3}$ 8.5 Hz. δ_C 36.3 (Me), 51.3 (CH_2, J_{C,CF_3} 35 Hz), 125.1 (CF_3, $J_{C,F}$ 277 Hz).

***N*-(2,4-Dinitrophenyl)-*N*-methyl-2',2',2'-trifluoroethylamine (250).** A solution of fluoro-2,4-dinitrobenzene (0.93 g, 5 mmol) and *N*-methyl-2,2,2-trifluoroethylamine hydrochloride (0.80 g, 5.4 mmol) in dry *N*,*N*-dimethylformamide (3 cm^3) containing potassium carbonate (1.52 g, 11 mmol) was stirred at 120 °C for 66 h, then cooled to room temperature and poured into ice/water with stirring. The brown precipitate was filtered off, washed with water and dried *in vacuo*. The product was distilled (Kugelrohr) as a yellow oil; b.p. 160 °C/0.2 mmHg. δ_H ($CDCl_3$) 3.12 (3H, s, CH_3), 3.94 (2H, q, CH_2), 7.25 (1H, d, 6-H), 8.31 (1H, dd, 5-H), 8.69 (1H, d,

3-H). $J_{CH_2CF_3}$ 8.5, $J_{5,6}$ 8, $J_{3,5}$ 2 Hz. Further attempts at purification were unsuccessful.

Attempted synthesis of *N*-(2,6-dinitrophenyl)-2',2',2'-trifluoroethylamine (253). A solution of chloro-2,6-dinitrobenzene (1.62 g, 8 mmol) and 2,2,2-trifluoroethylamine hydrochloride (1.15 g, 8.5 mmol) in dry *N*,*N*-dimethylformamide (5 cm^3) containing potassium carbonate (2.35 g, 17 mmol) was heated at 110 °C for 6 days, during which time extra portions of the amine (0.41 g, 3 mmol) and potassium carbonate (0.83 g, 6 mmol) were added. The reaction mixture still contained mainly starting material by tlc.

***N*-(3-Nitro-2-pyridyl)-(*S*)-alanine methyl ester (257).** 2-Chloro-3-nitropyridine (1.58 g, 10 mmol) and (*S*)-alanine methyl ester hydrochloride (2.51 g, 17 mmol) in dry methanol (15 cm^3) containing triethylamine (4.6 cm^3, 34 mmol) were heated under reflux for 8.5 h, cooled and concentrated under reduced pressure to dryness. The residue was partitioned between water and ethyl acetate, the organic layer washed with water then saturated brine, dried ($MgSO_4$) and concentrated under reduced pressure to dryness. Yield 1.64 g (73%); b.p. 172 °C/0.9 mmHg [lit.[191] (for the R-enantiomer) m.p. 39-40 °C]. δ_H ($CDCl_3$) 1.59 (3H, d, $CHCH_3$), 3.76 (3H, s, CO_2CH_3), 4.88 (1H, quintet, $CHCH_3$), 6.70 (1H, dd, 5-H), 8.40 (2H, m, 4- and 6-H). J_{CHCH_3} 7, $J_{5,6}$ 4.5, $J_{4,5}$ 8 Hz. δ_C 18.1 ($CHCH_3$), 49.7 (CH), 52.3 (CO_2CH_3), 112.6 (C-5), 123.0 (C-3), 135.2 (C-4), 152.5 (C-2), 155.3 (C-6), 173.7 (CO).

2-Methyl-3*H*-imidazo[4,5-*b*]pyridine 1-oxide (259). A solution of *N*-(3-nitro-2-pyridyl)-(*S*)-alanine methyl ester (0.34 g, 1.5 mmol) in dry methanol (10 cm^3) containing potassium carbonate (0.25 g, 1.8 mmol) was heated under reflux for 2 h, cooled to room temperature and concentrated under reduced pressure to dryness. The residue was partitioned between water and ethyl acetate, and the aqueous layer acidified (HCl) and extracted with ethyl acetate to furnish *N*-(3-nitro-2-pyridyl)-

(*S*)-alanine (0.06 g, 19%). The remaining aqueous layer was taken to pH 8 with sodium hydroxide solution to give a reddish solution, which was concentrated under reduced pressure to dryness and the residue extracted with acetone to give 2-methyl-3*H*-imidazo[4,5-*b*]pyridine 1-oxide (0.04 g, 18%). δ_H 2.28 (3H, s, CH_3), 6.94 (1H, dd, 6-H), 7.45 (1H, dd, 7-H), 8.14 (1H, dd, 5-H). $J_{5,7}$ 1.5, $J_{6,7}$ 8, $J_{5,6}$ 4.5 Hz. The original ethyl acetate extract from the reaction mixture residue was dried ($MgSO_4$) and concentrated under reduced pressure to a brown oil which yielded a very small amount of yellow needles, tentatively identified as ethyl 3*H*-imidazo[4,5-*b*]pyridine-2-carboxylate 1-oxide (**260**); δ_H ($CDCl_3$) 4.12 (CH_3), 7.05 (1H, dd, 6-H), 8.28 (1H, dd, 7-H), 8.41 (1H, dd, 5-H). $J_{5,7}$ 1.5, $J_{6,7}$ 8, $J_{5,6}$ 4.5 Hz.

1,3-Dichloro-4,6-dinitrobenzene. *m*-Dichlorobenzene (29.4 g, 200 mmol) was added to a solution of potassium nitrate (40.4 g, 400 mmol) in concentrated sulphuric acid (150 cm^3) at 50 °C with stirring. The temperature was maintained at 130 °C for 2 h, cooled to room temperature and poured on to crushed ice. The pale yellow product was washed with water, filtered off, dried *in vacuo* and recrystallised from ethanol to give 29.9 g (63%); m.p. 99-101 °C (lit.[192] 103-4 °C). δ_H 7.85 (1H, s, 6-H), 8.58 (1H, s, 3-H).

***N*-(5-Chloro-2,4-dinitrophenyl)glycine ethyl ester (269; R = H).** A solution of glycine (3.0 g, 40 mmol) and sodium hydrogen carbonate (6.72 g, 80 mmol) in water (80 cm^3) was added to a suspension of 1,3-dichloro-4,6-dinitrobenzene (9.48 g, 40 mmol) in ethanol (150 cm^3), and the mixture heated under reflux for 6 h. After cooling to room temperature, the precipitate was filtered off, dissolved in water then *N*-(5-chloro-2,4-dinitrophenyl)glycine reprecipitated by acidification (HCl); the product was filtered and dried *in vacuo*; 9.80 g (89%), m.p. 197-8 °C (lit.[193] 200-1 °C). δ_H 4.33 (2H, d, CH_2), 7.29 (1H, s, 6-H), 8.87 (1H, s, 3-H), 8.93 (1H, t, NH). J_{NHCH_2} 6 Hz.

A suspension of *N*-(5-chloro-2,4-dinitrophenyl)glycine (9.80 g, 35.6 mmol) in ethanol (120 cm^3) had dry HCl (about 7 g) bubbled through it. As soon as the acid dissolved, the ester precipitated, was filtered off, washed with ethanol and dried *in vacuo* to give 7.98 g (74%) of product; m.p. 154 °C (lit.[194] 150-1 °C). δ_H ($CDCl_3$) 1.36 (3H, t, CH_3), 4.15 (2H, d, NCH_2), 4.36 (2H, q, CH_2CH_3), 6.82 (1H, s, 6-H), 8.85 (1H, br s, NH), 9.09 (1H, s, 3-H). $J_{CH_2CH_3}$ 7, J_{NHCH_2} 5 Hz. δ_C 14.0 (CH_3), 44.8 (NCH_2), 62.5 (CH_2CH_3), 116.3 (C-6), 126.8 (C-3), 129.4 (C-2), 129.7 (C-5), 136.0 (C-4), 145.7 (C-1), 167.9 (CO).

6-Chloro-5-nitro-1*H*-benzimidazole 3-oxide (270). a) A solution of *N*-(5-chloro-2,4-dinitrophenyl)glycine ethyl ester (0.61 g, 2 mmol) in boiling ethanol (40 cm^3) had added to it potassium carbonate (0.28 g, 2 mmol). The mixture was heated under reflux for 22 h, then filtered and the precipitate extracted with boiling acetone. The acetone-insoluble residue was dissolved in water, acidified (HCl); the precipitate was washed with water and dried *in vacuo* to give 0.21 g (49%) of the title compound. δ_H 8.05 (1H, s, 4-H), 8.33 (1H, s, 7-H), 8.78 (1H, s, 2-H). δ_C 107.7 (C-4), 117.9 (C-3a), 122.0 (C-7), 129.3 (C-6), 141.3 (C-5), 143.1 (C-7a), 145.2 (C-2).

b) A solution of the ester (7.71 g, 28 mmol) in boiling ethanol (400 cm^3) had added to it potassium carbonate (3.86 g, 28 mmol). The mixture was heated under reflux for 12.5 h, filtered, and the precipitate extracted with boiling acetone, dissolved in water and acidified (HCl). The brown precipitate was filtered off and dried *in vacuo* to give 0.87 g (15%) of the 2-unsubstituted benzimidazole 3-oxide. The reaction mixture filtrate was concentrated under reduced pressure to dryness, dissolved in water, acidified (HCl) and the yellow precipitate filtered off and recrystallised from ethanol to furnish ethyl 6-chloro-5-nitro-1*H*-benzimidazole-2-carboxylate 3-oxide (1.62 g, 20%); m.p. 215-6 °C. δ_H 1.35 (3H, t, CH_3), 4.42 (2H, q, CH_2), 8.20 (1H, s, 4-H), 8.42 (1H, s, 6-H). $J_{CH_2CH_3}$ 7 Hz. δ_C 14.0 (CH_3), 62.3 (CH_2), 108.8 (C-4), 119.1 (C-3a), 123.4 (C-7), 130.7 (C-6), 138.4 (C-5), 142.1 (C-7a), 145.1 (C-2), 157.1 (CO). Further purification for microanalysis was not achieved.

c) Ethyl 6-chloro-5-nitro-1*H*-benzimidazole-2-carboxylate 3-oxide (1.00 g, 3.5 mmol) in concentrated hydrochloric acid (20 cm^3) was heated under reflux for 6 h, the solution cooled to room temperature and concentrated under reduced pressure to dryness. The residue was dissolved in concentrated ammonia (50 cm^3) and concentrated under reduced pressure again to dryness; the residue was stirred in boiling water and the water-insoluble solid filtered off and dried *in vacuo* to give the 2-unsubstituted benzimidazole *N*-oxide (0.45 g, 60%). δ_H as in (a).

***N*-(5-Chloro-2,4-dinitrophenyl)sarcosine ethyl ester (269; R = Me).** A suspension of 1,3-dichloro-4,6-dinitrobenzene (4.74 g, 20 mmol) in ethanol (80 cm^3) had added to it at room temperature a solution of sarcosine (1.78 g, 20 mmol) and sodium hydrogen carbonate (3.36 g, 40 mmol) in water (40 cm^3). The mixture was heated under reflux for 7 h, concentrated under reduced pressure to dryness and partitioned between water and ether. The aqueous layer was acidified (HCl), and the yellow precipitate filtered, washed with water and dried *in vacuo* ; recrystallisation from propan-2-ol furnished 5.00 g (86%) of *N*-(5-chloro-2,4-dinitrophenyl)sarcosine; m.p. 188 °C. (Found: C, 37.5; H, 2.8; N, 14.5. $C_9H_8ClN_3O_6$ requires C, 37.3; H, 2.8; N, 14.5%.) δ_H 2.90 (3H, s, CH_3), 4.27 (2H, s, CH_2), 7.30 (1H, s, 6-H), 8.62 (1H, s, 3-H). $J_{CH_2CH_3}$ 7 Hz. δ_C 41.7 (CH_2), 54.7 (CH_3), 120.2 (C-6), 126.7 (C-3), 131.2 (C-2), 134.2 (C-5), 134.8 (C-4), 147.2 (C-1), 170.0 (CO). *m/z* 289 ($M^{+\cdot}$, 8%), 244 (100), 241 (26), 211 (52), 198 (37), 181 (19), 165 (20), 152 (34) *etc.*

N-(5-Chloro-2,4-dinitrophenyl)sarcosine (3.76 g, 13 mmol) in ethanol (40 cm^3) containing dry HCl (3 g) was heated under reflux for 7 h, then cooled to room temperature and concentrated under reduced pressure to an oil which solidified upon standing. The solid was recrystallised from propan-2-ol to yield 3.66 g (89%) of product; m.p. 102-3 °C. (Found: C, 41.8; H, 3.9; N, 13.2. $C_{11}H_{12}ClN_3O_6$ requires C, 41.6; H, 3.9; N, 13.3%.) δ_H ($CDCl_3$) 1.33 (3H, t, CH_2CH_3), 3.05 (3H, s, NCH_3), 4.02 (2H, s, NCH_2), 4.29 (2H, q, CH_2CH_3), 7.01 (1H, s, 6-H), 8,62 (1H, s, 3-H). $J_{CH_2CH_3}$ 7 Hz. δ_C 14.0 (CH_2CH_3), 41.5 (NCH_2), 55.3 (NCH_3), 62.1 (CH_2CH_3),

120.7 (C-6), 126.4 (C-3), 133.3 (C-2), 135.4 (C-5), 136.5 (C-4), 147.4 (C-1), 168.2 (CO).

6-Chloro-1-hydroxy-4-methyl-7-nitroquinoxaline-2,3-dione (271). A solution of *N*-(5-chloro-2,4-dintrophenyl)sarcosine ethyl ester (1.27 g, 4 mmol) in ethanol (20 cm^3) and *N*,*N*-dimethylformamide (14 cm^3) with potassium carbonate (0.55 g, 4 mmol) was stirred at room temperature for 72 h, then filtered. The solid was extracted exhaustively with boiling acetone, dissolved in water, acidified (HCl) and the brown precipitate filtered off and dried *in vacuo*; 0.38 g (35%). δ_H 3.54 (3H, s, CH_3), 7.78 (1H, s, 5-H), 8.12 (1H, s, 8-H), 12.15 (1H, br s, OH). δ_C 30.7 (CH_3), 110.2 (C-8), 117.4 (C-5), 120.8 (C-8a), 126.9 (C-6), 129.8 (C-4a), 141.7 (C-7), 150.1 (C-3), 155.2 (C-2).

***N*-(5-Fluoro-2,4-dinitrophenyl)sarcosine ethyl ester (269; R = Me; F for Cl).** A mixture of 1,3-difluoro-4,6-dinitrobenzene (2.04 g, 10 mmol), sarcosine (0.89 g, 10 mmol), sodium hydrogen carbonate (1.68 g, 20 mmol), ethanol (40 cm^3) and water (20 cm^3) was heated under reflux for 5 h, concentrated under reduced pressure to dryness and partitioned between water and ether. The aqueous layer was acidified (HCl) and the yellow *N*-(5-fluoro-2,4-dinitrophenyl)sarcosine filtered and dried *in vacuo*; 1.86 g (68%); m.p. 174 °C (from ethanol). (Found: C, 39.8; H, 2.8; N, 15.2. $C_9H_8FN_3O_6$ requires C, 39.6; H, 2.95; N, 15.4%.) δ_H 2.90 (3H, s, CH_3), 4.25 (2H, s, CH_2), 7.17 (1H, d, 6-H), 8.61 (1H, d, 3-H). $J_{3,F}$ 8, $J_{6,F}$ 15 Hz. δ_C 41.8 (CH_3), 54.9 (CH_2), 105.8 (C-6), 125.5 (C-3), 126.9 (C-4), 132.1 (C-2), 149.5 (C-1), 157.1 (C-5), 169.6 (CO). $J_{5,F}$ 265, $J_{6,F}$ 27, $J_{1,F}$ 13, $J_{3,F}$ 9 Hz.

A solution of *N*-(5-fluoro-2,4-dinitrophenyl)sarcosine (1.65 g, 6 mmol) in ethanol (40 cm^3) containing dry HCl was heated under reflux for 6 h, then cooled and concentrated under reduced pressure to dryness; the residue was stirred in water and the the product filtered and dried *in vacuo*; 1.53 g (85%); m.p. 71-4 °C (from ethanol/water). (Found: C, 43.0; H, 3.7; N, 13.7. $C_{11}H_{12}FN_3O_6.0.25\ H_2O$ requires C, 43.2; H, 4.1;

N, 13.7%.) δ_H ($CDCl_3$) 1.33 (3H, t, CH_2CH_3), 3.05 (3H, s, NCH_3), 4.02 (2H, s, NCH_2), 4.29 (2H, q, CH_2CH_3), 6.68 (1H, d, 6-H), 8.70 (1H, d, 3-H). $J_{CH_2CH_3}$ 7, $J_{3,F}$ 8, $J_{6,F}$ 13 Hz. *m/z* 301 ($M^{+\cdot}$, 6%), 228 (100), 202 (50), 182 (23), 172 (28) *etc.* Further attempts at purification were unsuccessful.

6-Fluoro-1-hydroxy-4-methyl-7-nitroquinoxaline-2,3-dione (271; F for Cl). A solution of *N*-(5-fluoro-2,4-dinitrophenyl)sarcosine ethyl ester (0.30 g, 1 mmol) in dry ethanol (15 cm^3) containing potassium carbonate (0.15 g, 1.1 mmol) was heated under reflux for 6 h, cooled, concentrated under reduced pressure to dryness and the residue dissolved in water. The aqueous solution was acidified (HCl), filtered, and the filtrate extracted with ethyl acetate. The organic extract was dried ($MgSO_4$), concentrated under reduced pressure and recrystallised from water to give 0.14 g (54%) of product. δ_H 3.52 (3H, s, CH_3), 7.73 (1H, d, 5-H), 8.10 (1H, d, 8-H), 12.12 (1H, br s, OH). $J_{5\text{-}H,F}$ 13, $J_{8\text{-}H,F}$ 7 Hz. Attempts at purification were unsuccessful.

***N,N*-(4,6-Dinitro-1,3-phenylene)bis-glycine diethyl ester (266).** A solution of 1,3-dichloro-4,6-dinitrobenzene (2.37 g, 10 mmol) and glycine ethyl ester hydrochloride (2.79 g, 20 mmol) in dimethyl sulphoxide (15 cm^3) containing sodium hydrogen carbonate (3.36 g, 40 mmol) was heated to 90 °C with stirring, until effervescence ceased, then cooled to room temperature and diluted with water; the yellow-brown precipitate was filtered off, washed with water and dried *in vacuo* to give 3.23 g (87%); m.p. 190-2 °C (dec.) (lit.[126] 190-1°C). δ_H 1.23 (6H, t, 2 x CH_3), 4.18 (4H, q, 2 x CH_2CH_3), 4.30 (4H, d, 2 x NCH_2), 5.73 (1H, s, 2-H), 8.68 (2H, t, 2 x NH), 8.98 (1H, s, 5-H). $J_{CH_2CH_3}$ 7, J_{CH_2NH} 7 Hz. δ_C 14.3 (CH_3), 44.9 (NCH_2), 61.5 (CH_2CH_3), 92.7 (C-2), 124.0 (C-4 and -6), 128.6 (C-5), 147.8 (C-1 and -3), 169.4 (CO).

Reaction of *N,N*-(4,6-Dinitro-1,3-phenylene)bis-glycine diethyl ester (266) with potassium carbonate. A mixture of the diester (0.74 g, 2 mmol)

and potassium carbonate (0.55 g, 4 mmol) in dry ethanol (15 cm^3) was heated under reflux for 6 h, cooled to room temperature and the brown solid filtered off. The solid wsa dissolved as much as possible in water; the insoluble product (0.41 g, 55%) was identified as unchanged starting material. The aqueous solution was acidified (HCl), ethyl acetate added, and the mixture stirred until the ethyl acetate had evaporated; the brown solid was filtered off and identified by ^1H NMR spectroscopy as the acid resulting from hydrolysis of the starting material. δ_H 4.22 (4H, d, 2 x CH_2), 5.78 (1H, s, 2-H), 8.69 (2H, t, 2 x NH), 9.00 (1H, s, 5-H).

Sarcosine ethyl ester hydrochloride. A suspension of sarcosine (5.34 g, 60 mmol) in ethanol (40 cm^3) was saturated with dry HCl, then the solution heated under reflux for 2.5 h, cooled to room temperature, concentrated under reduced pressure to dryness and the residue recrystallised from ethanol; 6.02 g (65%), m.p. 127 °C (lit.[195] 127-8 °C). δ_H (D_2O) 1.22 (3H, t, CH_2CH_3), 2.72 (3H, s, NCH_3), 3.92 (2H, s, NCH_2), 4.23 (2H, q, CH_2CH_3). $J_{CH_2CH_3}$ 7 Hz.

***N,N*-(4,6-Dinitro-3,5-phenylene)bis-sarcosine diethyl ester (273).** A solution of 1,3-dichloro-4,6-dinitrobenzene (4.74 g, 20 mmol) and sarcosine ethyl ester hydrochloride (6.45 g, 42 mmol) in dimethyl sulphoxide (20 cm^3) had added to it sodium hydrogen carbonate (7.06 g, 84 mmol), and the mixture heated to 90 °C with stirring, until effervescence ceased. The solution was cooled to room temperature, diluted with water, and the brown solid filtered off and recrystallised from acetic acid; 3.32 g (42%), m.p. 111 °C. (Found: C, 48.25; H, 5.7; N, 14.0. $C_{16}H_{22}N_4O_8$ requires C, 48.2; H, 5.6; N, 14.1%.) δ_H 1.21 (6H, t, 2 x CH_2CH_3), 2.88 (6H, s, 2 x NCH_3), 4.15 (4H, q, 2 x CH_2CH_3), 4.17 (4H, s, 2 x NCH_2), 6.11 (1H, s, 2-H), 8.43 (1H, s, 5-H). $J_{CH_2CH_3}$ 7 Hz. δ_C 14.1 (CH_2CH_3), 40.9 (NCH_3), 54.8 (NCH_2), 60.7 (CH_2CH_3), 104.5 (C-2), 128.6 (C-4,6), 129.3 (C-5), 148.4 (C-1,3), 169.0 (CO). *m/z* 398 ($M^{+\cdot}$, 6%), 381 (5), 325 (100), 291 (7) *etc.*

Reaction of *N*,*N*-(4,6-dinitro-3,5-phenylene)bis-sarcosine diethyl ester (273) with potassium carbonate. A solution of the diester (0.80 g, 2 mmol) in dry ethanol (15 cm^3) containing potassium carbonate (0.62 g, 4.5 mmol) was heated under reflux for 7 h, then cooled to room temperature and the dark red solid filtered off. Exhaustive extraction (Soxhlet) with acetone, and dissolution of the residue in water and acidification (HCl) both failed to yield any identifiable products.

Reaction of *N*,*N*-(4,6-dinitro-3,5-phenylene)bis-sarcosine diethyl ester (273) with sodium ethoxide. A solution of the diester (0.80 g, 2 mmol) in dry ethanol (15 cm^3) and dry *N*,*N*-dimethylformamide (12 cm^3) had added to it sodium ethoxide [from Na (0.10 g, 4.3 mmol) in ethanol (4 cm^3)] dropwise at 0-5 °C with stirring. The solution was warmed to room temperature and stirred for 72 h, then concentrated under reduce pressure to dryness. Again, no identifiable products could be isolated.

5.3 Chapter Three Experimental

Chloro-2,6-dinitrobenzene. Sodium nitrite (4.56 g, 66 mmol) was added over 15 min to concentrated sulphuric acid (50 cm^3) with stirring, then heated to ~60 °C until all the sodium nitrite dissolved. The mixture was cooled in an ice bath, then a solution of 2,6-dinitroaniline (10.98 g, 60 mmol) in hot acetic acid (120 cm^3) was added slowly with stirring, such that the temperature remained below 40 °C. The mixture was then stirred at 40 °C for 30 min. The diazonium salt solution was added to a solution of cuprous chloride (13.2 g, 132 mmol) in concentrated HCl (120 cm^3) over 20 min with stirring and cooling in an ice bath. The reaction mixture was then heated to 80 °C and stirred at that temperature for 20 min, until effervescence ceased. An equal volume of water was added and the mixture cooled in an ice bath. After 1.5 h the yellow crystals (10.03 g, 83%) were collected and dried; m.p. 85-6 °C (lit.[153] 86-7 °C). δ_H ($CDCl_3$) 7.62 (1H, t, 4-H), 8.01 (2H, d, 3- and 5-H). $J_{3,4} = J_{4,5}$ 8 Hz.

***N*-(*n*-Butyl)-2,6-dinitroaniline (274).** A solution of chloro-2,6-dinitrobenzene (0.41 g, 2 mmol) and *n*-butylamine (0.29 g, 4 mmol) in dry *N*,*N*-dimethylformamide (2 cm^3) was left at room temperature for 72 h, then heated to 130 °C for 30 min, cooled to room temperature and poured into ice/water. The yellow precipitate was filtered off, washed with water and ethanol and dried *in vacuo* to give 0.33 g (69%) of product; m.p. 29-30 °C (lit.[196] 39-40 °C). δ_H 0.84 (3H, t, CH_3), 1.27 (2H, sex, CH_2CH_3), 1.56 (2H, quin, $CH_2CH_2CH_2$), 2.89 (2H, dt, NCH_2), 6.88 (1H, t, 4-H), 8.05 (1H, br t, NH), 8.26 (2H, d, 3- and 5-H). $J_{CH_2CH_3} = J_{CH_2CH_2}$ 7, J_{NHCH_2} 5, $J_{3,4} = J_{5,4}$ = 8 Hz.

2-*n*-Propyl-7-nitro-1*H*-benzimidazole 3-oxide (275). A solution of *N*-(*n*-butyl)-2,4-dinitroaniline (0.20 g, 0.8 mmol) in 60% dioxan/water (50 cm^3) with 0.5 M sodium hydroxide (i.e. 1.6 g NaOH) was heated under reflux for 45 min. The red solution was cooled to room temperature and 1 M aqueous sodium dihydrogen

orthophosphate was added to bring the pH down to 6. The white precipitate was filtered off (inorganic). The solution was concentrated under reduced pressure to dryness and the residue extracted exhaustively (Soxhlet) into acetone, which was removed under reduced pressure to give 0.15 g (83%) of the title compound. δ_H 0.97 (3H, t, CH_3), 1.80 (2H, sex, CH_2CH_3), 2.89 (2H, t, CH_2CH_2), 7.39 (1H, t, 5-H), 7.87 (1H, d, 4-H), 8.00 (1H, d, 6-H). $J_{CH_2CH_2} = J_{CH_2CH_3} = 7$, $J_{4,5} = J_{5,6}$ 8 Hz. δ_C 13.6 (CH_3), 20.0 (C-2'), 27.4 (C-1'), 115.3 (C-4), 118.1 (C-6), 121.1 (C-5), 131.2 (C-7a), 135.0 (C-7), 137.8 (C-3a), 155.1 (CO). NMR data compare favourably with those of de Vargas and Cañas[149], who also further characterised the title compound by high resolution mass spectrometry.

***N*-(2,6-Dinitrophenyl)-γ-aminobutyric acid (279).** A solution of γ-aminobutyric acid (0.54 g, 5.2 mmol) and sodium hydrogen carbonate (0.87 g, 10.4 mmol) in water (10 cm^3) was added to a stirred suspension of chloro-2,6-dinitrobenzene (1.01 g, 5 mmol) in ethanol (20 cm^3) at room temperature. The mixture was heated under reflux for 5 h, concentrated under reduced pressure to dryness, dissolved in water and extracted with ether. The aqueous phase was acidified (HCl) and the yellow precipitate filtered off, washed with water and dried *in vacuo* to give 1.14 g (85%) of the title compound; m.p. 173-4 °C. δ_H 1.80 (2H, quin, $CH_2CH_2CH_2$), 2.24 (2H, t, CH_2CO_2H), 2.91 (2H, q, NCH_2), 6.88 (1H, t, 4-H), 8.02 (1H, t, NH), 8.25 (2H, d, 3- and 5-H), 12.15 (1H, br s, CO). $J_{CH_2CH_2}$ 7, $J_{3,4} = J_{4,5}$ = 8 Hz. δ_C 24.8 ($CH_2CH_2CH_2$), 30.8 (CH_2CO), 45.7 (NCH_2), 114.9 (C-4), 132.3 (C-3 and -5), 137.9 (C-2 and -6), 139.2 (C-1), 173.9 (CO). Although no impurities were detected by NMR spectroscopy, the sample was insufficiently pure for microanalysis.

Reaction of *N*-(2,6-dinitrophenyl)-γ-aminobutyric acid (279) with sodium hydroxide. A solution of *N*-(2,6-dinitrophenyl)-γ-aminobutyric acid (0.22 g, 0.8 mmol) in dioxan (48 cm^3) and water (32 cm^3) containing sodium hydroxide (1.6 g) (*i.e.* 0.5 M NaOH) was heated under reflux for 60 min, cooled to room temperature and

acidified (HCl). The solution was concentrated under reduced pressure to dryness and the residue extracted exhaustively with acetone (Soxhlet); the acetone was removed under reduced pressure to leave a very small amount of impure residue, tentatively identified as 3-(7-nitro-3-oxido-1*H*-benzimidazol-2-yl)propionic acid. δ_H 2.92 (2H, t, CH_2CO_2H), 3.25 (2H, t, CCH_2), *etc.* (see Discussion, pages 105-106). $J_{CH_2CH_2}$ 7 Hz.

***N*-(2,6-Dinitrophenyl)-*N*-methyl-*n*-butylamine (280).** A solution of chloro-2,6-dinitrobenzene (0.61 g, 3 mmol) and *N*-methyl-*n*-butylamine (0.52 g, 6 mmol) in dry *N*,*N*-dimethylformamide (2 cm^3) was stirred at room temperature for 72 h, then poured into ice/water and the orange solid (0.76 g, 100%) filtered off and dried *in vacuo*; m.p. 52-53 °C. δ_H ($CDCl_3$) 0.88 (3H, t, CH_2CH_3), 1.28 (2H, sex, CH_2CH_3), 1.53 (2H, quin, $CH_2CH_2CH_2$), 2.83 (3H, s, NCH_3), 2.91 (2H, t, NCH_2), 7.14 (1H, t, 4-H), 7.81 (2H, d, 3- and 5-H). $J_{CH_2CH_2} = J_{CH_2CH_3} = 7$, $J_{3,4}$ 7 Hz. δ_C 13.6 (CH_3), 19.7 (CH_2CH_3), 29.9 ($CH_2CH_2CH_2$), 40.1 (NCH_3), 54.9 (NCH_2), 121.4 (C-6), 128.8 (C-3 and -5), 139.9 (C-2 and -4), 147.3 (C-1). *m/z* 253 ($M^{+\cdot}$, 9%), 210 (100), 194 (10), 105 (9), 79 (9) *etc.* Further purification for microanalysis was unsuccessful.

Reaction of *N*-(2,6-dinitrophenyl)-*N*-methyl-*n*-butylamine (280) with NaOH. A solution of *N*-(2,6-dinitrophenyl)-*N*-methylbutylamine (0.25 g, 1 mmol) in dioxan (150 cm^3) and water (100 cm^3) containing sodium hydroxide (2.0 g) (*i.e.* 0.2 M NaOH) was heated under reflux for 2 h. The solution was cooled to room temperature, taken to pH 6 with HCl, then concentrated under reduced pressure to dryness and the residue extracted exhaustively with acetone (Soxhlet) to give 100% recovery of starting material.

Reaction of *N*-(2,6-dinitrophenyl)-*N*-methylbutylamine (280) with potassium carbonate. A solution of *N*-(2,6-dinitrophenyl)-*N*-methylbutylamine (0.25 g, 1 mmol) in dry ethanol (10 cm^3) containing potassium carbonate (0.14 g,

1 mmol) was heated under reflux for 18 h, concentrated under reduced pressure to dryness, and the residue partitioned between water and ethyl acetate. The organic layer was dried ($MgSO_4$) and concentrated under reduced pressure to give 0.23 g (92%) of starting material.

***N*-(2,6-Dinitrophenyl)glycine ethyl ester (192).** A solution of glycine (1.35 g, 18 mmol) in water (20 cm^3) with sodium hydrogen carbonate (3.02 g, 36 mmol) was added to a stirred solution of chloro-2,6-dinitrobenzene (3.04 g, 15 mmol) in methanol (30 cm^3) at room temperature. The mixture was heated under reflux for 2 h and concentrated under reduced pressure to dryness. Water was added to the residue, and the insoluble copper-coloured solid was filtered off and treated with 2 M HCl until it turned yellow, whereupon it was filtered off and recrystallised from methanol, giving *N*-(2,6-dinitrophenyl)glycine; 3.05 g (84%), m.p. 173-4 °C (lit.[197] 173 °C). δ_H 3.71 (2H, d, CH_2), 6.86 (1H, t, 4-H), 8.21 (2H, d, 3- and 5-H), 9.06 (1H, br s, NH). $J_{CH_2,NH}$ 4.5, $J_{3,4}$ 8 Hz.

A solution of *N*-(2,6-dinitrophenyl)glycine (2.29 g, 9.5 mmol) in ethanol (60 cm^3) containing concentrated sulphuric acid (1.30 g) was heated under reflux for 4 h, concentrated under reduced pressure to dryness, and the yellow crystals (2.55 g, 100%) were collected and washed with water; m.p. 86 °C (lit.[198] 86 °C). δ_H ($CDCl_3$) 1.31 (3H, t, CH_3), 3.78 (2H, s, NCH_2), 4.28 (2H, q, CH_2CH_3), 6.85 (1H, t, 4-H), 8.22 (2H, d, 3- and 5-H), 9.05 (1H, br s, NH). J_{CH_2,CH_3} 7, $J_{3,4}$ 8 Hz.

1-Hydroxy-5-nitroquinoxaline-2,3-dione (194). A solution of *N*-(2,6-dinitrophenyl)glycine ethyl ester (0.54 g, 2 mmol) in ethanol (20 cm^3) and *N*,*N*-dimethylformamide (2.5 cm^3) with potassium carbonate (0.30 g, 2.2 mmol) was stirred at room temperature for 20 h, filtered, and the solid dissolved in water. The aqueous solution was acidified (HCl) and filtered. The filtrate precipitated on standing for 3 days; the red crystals (0.055 g)were identified as the title compound, and the filtrate was concentrated under reduced pressure to a small volume, extracted into ethyl acetate,

dried ($MgSO_4$) and concentrated under reduced pressure to dryness; 0.11 g (also identified as the title compound) (total yield 37%), m.p. 232 °C (lit.[132] 231-2 °C). δ_H 7.39 (1H, t, 7-H), 7.82 (1H, dd, 6- or 8-H), 7.98 (1H, dd, 6- or 8-H), 11.3-12.0 (2H, 2 x br s, NH and OH). $J_{5,6}$ 9, $J_{6,8}$ 2 Hz. δ_C 119.4 (C-6), 119.8 (C-4a), 121.0 (C-8), 123.6 (C-7), 130.3 (C-8a), 135.3 (C-5), 151.3 (C-3), 154.9 (C-2).

***N*-(2,6-Dinitrophenyl)-(*RS*)-alanine ethyl ester (289).** A solution of (*RS*)-alanine (1.07 g, 12 mmol) in water (20 cm^3) containing sodium hydrogen carbonate (2.94 g, 35 mmol) was added to a stirred solution of chloro-2,6-dinitrobenzene (2.03 g, 10 mmol) in ethanol (30 cm^3), and the mixture heated under reflux for 5.5 h, then concentrated under reduced pressure to dryness. The residue was partitioned between ether and water, the aqueous layer acidified (HCl), extracted with ethyl acetate, dried ($MgSO_4$) and concentrated under reduced pressure to give 1.66 g (65%) of *N*-(2,6-dinitrophenyl)-(*RS*)-alanine m.p. 136-7 °C (lit.[195] 136 °C). δ_H 1.36 (3H, d, CH_3), 3.84 (1H, br quintet, CH), 7.06 (1H, t, 4-H), 8.33 (2H, d, 3- and 5-H). J_{CHCH_3} 6.4, $J_{3,5}$ 8.0. δ_C 18.6 (CH_3), 53.5 (CH), 117.1 (C-4), 132.5 (C-3 and -5), 137.7 (C-2 and -6), 139.5 (C-1), 173.6 (CO).

A solution of *N*-(2,6-dinitrophenyl)-(*RS*)-alanine (1.62 g, 6.4 mmol) in ethanol (50 cm^3) containing dry HCl (2.0 g) was heated under reflux for 4.5 h, then concentrated under reduced pressure to an oil (1.51 g, 83%); b.p. 180 °C/0.05 mmHg. (Found: C, 46.5; H, 4.8; N, 15.1. $C_{11}H_{13}N_3O_6$ requires C, 46.65; H, 4.6; N, 14.8%.) δ_H ($CDCl_3$) 1.25 (3H, t, CH_2CH_3), 1.49 (3H, d, $CHCH_3$), 4.02 (1H, quintet, CH), 4.18 (2H, q, CH_2), 6.90 (1H, t, 4-H), 8.20 (2H, d, 3- and 5-H), 8.58 (1H, d, NH). $J_{CH_2CH_3}$ 7.1, J_{CHCH_3} 6.9, $J_{3,4}$ 8.1. δ_C 14.0 (CH_2CH_3), 19.0 ($CHCH_3$), 53.7 (CH), 62.1 (CH_2), 116.2 (C-4), 132.0 (C-3 and -5), 138.5 (C-2 and -6), 139.6 (C-1), 172.1 (CO).

Reaction of *N*-(2,6-dinitrophenyl)-(*RS*)-alanine ethyl ester (289) with potassium carbonate. A solution of *N*-(2,6-dinitrophenyl)-(*RS*)-alanine ethyl

ester (0.42 g, 1.5 mmol) in dry ethanol (12 cm^3) and *N*,*N*-dimethylformamide (2 cm^3) containing potassium carbonate (0.23 g, 1.7 mmol) was stirred at room temperature for 21 h, then heated under reflux for 4.5 h. The reaction mixture was concentrated under reduced pressure to dryness, dissolved in water and exracted into ethyl acetate. Only an intractable mixture (by tlc and NMR spectroscopy) of products was obtained.

***N*-(2,6-Dinitrophenyl)-(*RS*)-norvaline methyl ester (290).** A solution of chloro-2,6-dinitrobenzene (0.81 g, 4 mmol) and (*RS*)-norvaline (0.49 g, 4.2 mmol) in ethanol (15 cm^3) with sodium hydrogen carbonate (1.06 g, 12.6 mmol) in water (10 cm^3) was heated under reflux for 5 h, concentrated under reduced pressure to dryness, dissolved in water, extracted with ether and the aqueous phase acidified (HCl) to give a yellow precipitate of *N*-(2,6-dinitrophenyl)-(*RS*)-norvaline (0.96 g, 85%), which was filtered off and dried *in vacuo*; m.p. 156-7 °C. δ_H 0.81 (3H, t, CH_3), 1.24 (2H, sex, CH_2CH_3), 1.68 (2H, m, $CHCH_2$), 3.76 (1H, m, CH), 7.04 (1H, t, 4-H), 8.14 (1H, d, NH), 8.30 (2H, d, 3- and 5-H). $J_{CH_2CH_2} = J_{CH_2CH_3} = 7$, J_{CHCH_2} 7, J_{NHCH_2} 8, $J_{3,4} = J_{4,5} = 8$ Hz. δ_C 13.4 (CH_3), 17.8 (CH_2CH_3), 34.1 ($CHCH_2$), 57.3 (CH), 117.2 (C-4), 132.2 (C-3 and -5), 137.5 (C-2 and -6), 139.3 (C-1), 172.6 (CO). The acid was esterified without further purification.

A solution of *N*-(2,6-dinitrophenyl)-(*RS*)-norvaline (0.85 g, 3 mmol) in dry methanol (20 cm^3) containing dry HCl was heated under reflux for 3 h, then cooled to room temperature, concentrated under reduced pressure to ~10 cm^3 and the yellow crystals filtered off and dried *in vacuo*. Yield 0.58 g (65%); m.p. 76 °C (from methanol). (Found: C, 48.7; H, 4.95; N, 14.1. $C_{12}H_{15}N_3O_6$ requires C, 48.5; H, 5.1; N, 14.1%.) δ_H 0.92 (3H, t, CH_3), 1.39 (2H, sex, CH_2CH_3), 1.80 (2H, m, $CHCH_2$), 3.73 (3H, s, $COCH_3$), 3.97 (1H, m, CH), 6.89 (1H, t, 4-H), 8.18 (2H, d, 3- and 5-H), 8.47 (1H, d, NH). $J_{CH_2CH_3} = J_{NHCH} = 7$, $J_{3,4}$ 8 Hz. δ_C 13.6 (CH_3), 18.4 (CH_2CH_3), 35.2 ($CHCH_2$), 52.7 ($COCH_3$), 58.1 (CH), 116.2 (C-4), 132.0 (C-3 and -5), 138.7 (C-2 and -6), 139.4 (C-1), 172.0 (CO).

Reaction of *N*-(2,6-dinitrophenyl)-(*RS*)-norvaline methyl ester (290) with potassium carbonate. A solution of *N*-(2,6-dinitrophenyl)-(*RS*)-norvaline methyl ester (0.30 g, 1 mmol) in dry methanol (10 cm^3) and *N*,*N*-dimethylformamide (1 cm^3) containing potassium carbonate (0.15 g, 1.1 mmol) was stirred at room temperature for 26 h. No product could be identified from the reaction mixture, which was (by tlc and NMR spectroscopy) a complex mixture.

5.4 Chapter Four Experimental

3-Iodopropan-1-ol. 3-Bromopropan-1-ol (10.43 g, 75 mmol) and sodium iodide (22.8 g, 150 mmol) in anhydrous acetone (75 cm^3) were heated under reflux for 6 h. The sodium bromide precipitate was filtered off and most of the acetone removed under reduced pressure. The residue was diluted with water, and extracted with ether. The ether layer was dried over $MgSO_4$ and concentrated under reduced pressure to an oil; 12.89 g (92%); b.p. 68-70 °C/30 mmHg (lit.[167] 105 °C/25 mmHg). δ_H 2.05 (2H, quin, $CH_2CH_2CH_2$), 3.31 (2H, t, CH_2I), 3.78 (2H, t, CH_2OH). $J_{CH_2CH_2}$ 7, $J_{CH_2CH_2}$ 6 Hz. δ_C 3.01 (CH_2I), 35.5 ($CH_2CH_2CH_2$), 62.3 (CH_2OH).

***N*-(2,4-Dinitrophenyl)sarcosine ethyl ester (186; X= 4-NO_2).** A solution of sarcosine (4.45 g, 50 mmol) in water (150 cm^3) with sodium hydrogen carbonate (12.6 g, 150 mmol) was added to a stirred solution of fluoro-2,4-dinitrobenzene (7.44 g, 40 mmol) in ethanol (160 cm^3) at room temperature. The mixture was heated under reflux for 6 h, concentrated under reduced pressure to about 100 cm^3, then extracted with ether and the aqueous layer acidified with HCl. The brown precipitate was filtered off, washed with water and recrystallised from ethanol to give 10.8 g (85%) of *N*-(2,4-dinitrophenyl)sarcosine; m.p. 175 °C (lit.[179] 176 °C). δ_H 2.92 (3H, s, CH_3), 4.33 (2H, s, CH_2), 7.19 (1H, d, 6-H), 8.25 (1H, dd, 5-H), 8.60 (1H, d, 3-H). $J_{5,6}$ 8, $J_{3,5}$ 2.5 Hz.

A solution of *N*-(2,4-dinitrophenyl)sarcosine (11.71 g, 46 mmol) in ethanol (150 cm^3) containing dry HCl (7.3 g) was heated under reflux for 6 h. The solution was then cooled to room temperature and the yellow-green precipitate filtered off, affording 12.71 g (98%) of product; m.p. 103-4 °C (lit.[130] 103 °C). δ_H ($CDCl_3$) 1.25 (3H, t, CH_2CH_3), 2.95 (3H, s, NCH_3), 4.20 (2H, q, CH_2CH_3), 4.38 (2H, s, NCH_2), 7.20 (1H, d, 6-H), 8.25 (1H, dd, 5-H), 8.60 (1H, d, 3-H); $J_{CH_2CH_3}$ 7.5, $J_{3,5}$ 2.5, $J_{5,6}$ 10 Hz. δ_C 14.3 (CH_2CH_3), 41.8 (NCH_3), 54.9 (CH_2CO_2), 61.3 (CH_2CH_3),

118.7 (C-3), 123.7 (C-6), 127.8 (C-5), 135.7 (C-2), 135.6 (C-4), 148.6 (C-1), 168.8 (CO).

1-Hydroxy-4-methyl-7-nitroquinoxaline-2,3-dione (187; X = 7-NO_2). A solution of *N*-(2,4-dinitrophenyl)sarcosine ethyl ester (12.45 g, 44 mmol) and potassium carbonate (6.23 g, 45 mmol) in ethanol (250 cm^3) was stirred at room temperature for 24 h. The solid product was filtered off and extracted exhaustively with boiling acetone to give an acetone-insoluble solid which was dissolved in water - this was acidified with HCl to give a precipitate which was recrystallised from acetic acid to give 4.28 g (41%) of yellow-green crystals; m.p. 245 °C (lit.[130] 243 °C). δ_H 3.60 (3H, s, NCH_3), 7.67 (1H, d, 5-H), 8.15 (1H, dd, 6-H), 8.25 (1H, d, 8-H); $J_{5,6}$ 9, $J_{6,8}$ 3 Hz. δ_C 30.8 (CH_3), 107.4 (C-8), 116.0 (C-5), 119.5 (C-6), 128.2 (C-8a), 131.1 (C-4a), 142.9 (C-7), 150.4 (C-3), 155.4 (C-2).

1-Ethoxy-4-methyl-7-nitroquinoxaline-2,3-dione (312). Ethyl iodide (0.34 g, 2.2 mmol) and 1-hydroxy-4-methyl-quinoxaline-2,3-dione (0.47 g, 2 mmol) were dissolved in dry *N*,*N*-dimethylformamide, and triethylamine (0.22 g, 2.2 mmol) was added with stirring at room temperature. After 48 h at room temperature, the reaction mixture was filtered, the filtrate diluted with water and the buff-coloured precipitate filtered off, washed with water and recrystallised from water; 0.22 g (42%), m.p. 210-11 °C. (Found: C, 49.5; H, 4.0; N, 15.6. $C_{11}H_{11}N_3O_5$ requires C, 49.8; H, 4.2; N, 15.8%.) δ_H 1.41 (3H, t, CH_2CH_3), 3.58 (3H, s, NCH_3), 4.30 (2H, q, CH_2), 7.69 (1H, d, 5-H), 8.12 (1H, d, 8-H), 8.18 (1H, dd, 6-H). $J_{CH_2CH_3}$ 7, $J_{5,6}$ 9, $J_{6,8}$ 2 Hz. δ_C 13.2 (CH_2CH_3), 30.6 (NCH_3), 71.3 (CH_2), 107.2 (C-8), 116.2 (C-5), 119.7 (C-6), 127.1 (C-8a), 131.2 (C-4a), 142.9 (C-7), 150.0 (C-3), 155.5 (C-2). *m/z* 265 ($M^{+\cdot}$, 52%), 237 (12), 221 (10), 209 (65), 192 (100), 160 (14), 146 (35) *etc.*

1-(3-Hydroxypropoxy)-4-methyl-7-nitroquinoxaline-2,3-dione (314). A solution of 1-hydroxy-4-methyl-7-nitroquinoxaline-2,3-dione (0.43 g, 1.8 mmol) in

dry *N*,*N*-dimethylformamide had added to it triethylamine (0.20 g, 2 mmol). The resultant precipitate dissolved when 3-iodopropanol (0.37 g, 2 mmol) was added at 120 °C. The red solution was stirred at room temperature for 24 h, then concentrated under reduced pressure (Kugelrohr) to dryness. The residue was stirred in boiling water and filtered to give a brown solid which was recrystallised from water; 0.23 g (43%), m.p. 101-3 °C. The filtrate yielded a further crop of crystals; 0.21 g (40%). Total yield 83%. (Found: C, 48.4; H, 4.3; N, 14.0. $C_{12}H_{13}N_3O_6$ requires C, 48.8; H, 4.4; N, 14.2%). δ_H 1.93 (2H, quin, $CH_2CH_2CH_2$), 3.55 (3H, s, NCH_3), 3.82 (2H, q, CH_2OH), 4.28 (2H, t, OCH_2), 4.67 (1H, t, OH), 7.66 (1H, d, 5-H), 8.15 (1H, dd, 6-H), 8.17 (1H, d, 8-H). $J_{5,6}$ 8, $J_{6,8}$ 2, $J_{CH_2CH_2}$ 6.5, J_{CH_2OH} 4.5 Hz. δ_C 31.0 and 31.3 (CH_3 and $CH_2CH_2CH_2$), 57.8 (CH_2OH), 73.4 (OCH_2), 107.7 (C-8), 116.6 (C-5), 120.1 (C-6), 127.5 (C-8a), 131.7 (C-4a), 143.4 (C-7), 150.3 (C-3), 156.0 (C-2). *m/z* 295 ($M^{+\cdot}$, 38%), 237 (58), 222 (35), 221 (36), 209 (72), 192 (100), *etc.*

***N*-(2-Nitrophenyl)sarcosine ethyl ester (186; X = H).** A solution of sarcosine (4.45 g, 50 mmol) in water (80 cm^3) containing sodium hydrogen carbonate (12.60 g, 150 mmol) was added to a stirred solution of fluoro-2-nitrobenzene (6.35 g, 45 mmol) in ethanol (140 cm^3) at room temperature. The mixture was heated under reflux for 6.5 h, concentrated under reduced pressure to dryness and dissolved in water. This was extracted with ether, the aqueous layer acidified (HCl), extracted with ethyl acetate and the organic phase dried ($MgSO_4$) and concentrated under reduced pressure to a red oil [*N*-(*o*-nitrophenyl)sarcosine] which crystallised on standing. Yield 7.66 g (81%); m.p. 93-5 °C (lit.[122,131] 88-92 °C and 75 °C respectively). δ_H 2.84 (3H, s, CH_3), 3.99 (2H, s, CH_2), 6.93 (1H, t, 4-H), 7.08 (1H, d, 6-H), 7.51 (1H, t, 5-H), 7.76 (1H, d, 3-H). $J_{3,4}$ 6.5 Hz.

A solution of *N*-(*o*-nitrophenyl)sarcosine (6.20 g, 30 mmol) in ethanol (125 cm^3) conatining dry HCl (5.8 g) was heated under reflux for 6 h. The solution was then concentrated under reduced pressure to 5.00 g (70%) of an oil, b.p. 160 °C/0.1 mmHg (lit.[131] 170 °C/0.2 mmHg). δ_H ($CDCl_3$) 1.29 (3H, t, CH_2CH_3), 2.99 (3H, s, NCH_3),

3.91 (2H, s, NCH_2), 4.23 (2H, q, CH_2CH_3), 6.93 (1H, t, 4-H), 7.06 (1H, d, 6-H), 7.43 (1H, t, 5-H), 7.78 (1H, d, 3-H). $J_{3,4} = J_{4,5} = J_{5,6} = 8$ Hz. δ_C 14.0 (CH_2CH_3), 40.7 (NCH_3), 55.9 (NCH_2), 61.0 (CH_2CH_3), 119.8 and 120.0 (C-4 and -6), 126.5 (C-3), 133.4 (C-5), 136.3 (C-2), 145.0 (C-1), 170.1 (CO).

1-Hydroxy-4-methylquinoxaline-2,3-dione (187; X = H). Sodium ethoxide [11 mmol; from sodium (0.25 g) in ethanol (12 cm^3)] was added to a stirred solution of *N*-(2-nitrophenyl)sarcosine ethyl ester (2.38 g, 10 mmol) in ethanol (12 cm^3) at 0-5 °C. Stirring was continued at room temperature for 3 days, then the brown precipitate filtered off and recrystallised from acetic acid. Yield 0.59 g (31%); m.p. 239-240 °C (lit.[199]. 253 °C). δ_H 3.58 (3H, s, CH_3), 7.29-7.34 (2H, m, 6- and 7-H), 7.44-7.50 (1H, m, 5-H), 7.55-7.60 (1H, m, 8-H). (These assignments are provisional.)

1-Ethoxy-4-methylquinoxaline-2,3-dione (311). Ethyl iodide (0.09 g, 0.6 mmol) and 1-hydroxy-4-methylquinoxaline-2,3-dione (0.10 g, 0.5 mmol) in dry *N*,*N*-dimethylformamide (2 cm^3) were heated to 75 °C, then triethylamine (0.06 g, 0.6 mmol) was added with stirring. The temperature was maintained at 80 °C for 5 h, then cooled to room temperature, diluted with water and the very small amount of yellow precipitate filtered off and discarded . The filtrate was concentrated under reduced pressure (Kugelrohr) to dryness, and the residue recrystallised from water to give the title compound (50 mg, 45%; m.p. 172-3 °C). (Found: C, 60.3; H, 5.4; N, 12.9. $C_{11}H_{12}N_2O_3$ requires C, 60.0; H, 5.5; N, 12.7%.) δ_H 1.36 (3H, t, CH_2CH_3), 3.52 (3H, s, NCH_3), 4.20 (2H, q, CH_2), 7.32 (2H, m, 6- and 7-H), 7.47 (2H, m, 5- and 8-H). (These assignments are provisional.) $J_{CH_2CH_3}$ 7. δ_C 13.1 (CH_2CH_3), 30.0 (NCH_3), 70.8 (CH_2), 112.4 (C-8), 115.5 (C-5), 124.2 (C-7), 124.7 (C-6), 125.7 (C-4a), 126.5 (C-8a), 145.9 (C-3), 155.5 (C-2). *m/z* 220 ($M^{+\cdot}$, 80%), 192 (8), 176 (13), 164 (24), 147 (100), 119 (68) *etc.*

1-(3-Hydroxypropoxy)-4-methylquinoxaline-2,3-dione (313). A solution of 1-hydroxy-4-methylquinoxaline-2,3-dione (0.10 g, 0.5 mmol) and 3-iodopropanol (0.11 g, 0.6 mmol) in dry *N*,*N*-dimethylformamide (2 cm^3) containing triethylamine (0.06 g, 0.6 mmol) was stirred at 95 °C for 15 h, then cooled to room temperature, diluted with water and concentrated under reduced pressure (Kugelrohr) to dryness. The residue was recrystallised from water to give 0.07 g (58%) of product; m.p. 187-8 °C. (Found: C, 54.0; H, 6.2; N, 10.65. $C_{12}H_{14}N_2O_4.H_2O$ requires C, 53.7; H, 6.0; N, 10.4%.) δ_H 1.92 (2H, quin, $CH_2CH_2CH_2$), 3.52 (3H, s, CH_3), 3.60 (2H, q, CH_2OH), 4.22 (2H, t, OCH_2), 4.65 [1H, t, OH (exchangeable with D_2O)], 7.32 (2H, m, 6- and 7-H), 7.48 (2H, m, 5- and 8-H). (These assignments are provisional.) δ_C 30.2 and 30.9 (CH_3 and $CH_2CH_2CH_2$), 57.4 (CH_2OH), 72.7 (OCH_2), 112.4 (C-8), 115.4 (C-5), 124.2 (C-7), 124.8 (C-6), 125.7 (C-4a), 126.3 (C-8a), 150.0 (C-3), 155.3 (C-2). *m/z* 250 ($M^{+\cdot}$, 57%), 192 (29), 176 (33), 164 (50), 147 (100), 119 (64) *etc.*

1-Ethoxy-6-nitro-2-trifluoromethylbenzimidazole (315). A solution of 5-nitro-2-trifluoromethylbenzimidazole 3-oxide (0.12 g, 0.5 mmol) and triethylamine (6 drops, 0.6 mmol) in dry *N*,*N*-dimethylformamide (1.5 cm^3) had added to it ethyl iodide (0.09 g, 0.6 mmol) at room temperature. After 24 hours, the solution was diluted with water and the buff-coloured product filtered off and dried *in vacuo*; yield 0.10 g (71%); m.p. 117 °C. (Found: C, 43.7; H, 2.7; N, 15.2. $C_{10}H_8F_3N_3H_3$ requires C, 43.65; H, 2.9; N, 15.3%.) δ_H 1.44 (3H, t, CH_3). 4.60 (2H, q, CH_2), 8.10 (1H, d, 4-H), 8.27 (1H, dd, 5-H), 8.82 (1H, d, 7-H). $J_{CH_2CH_3}$ 7, $J_{4,5}$ 9, $J_{5,7}$ 2 Hz. δ_C 13.1 (CH_3), 77.5 (CH_2), 107.8 (C-7), 117.7 (CF_3, q, $J_{C,F}$ 271 Hz), 119.2 (C-4), 122.6 (C-5), 130.5 (C-7a), 140.0 (C-2, q, $J_{C,F}$ 32 Hz), 140.1 (C-6), 145.6 (C-3a). *m/z* 275 ($M^{+\cdot}$, 75%), 256 (8), 247 (100), 227 (9), 201 (14), 184 (19), *etc.*

1-(3-Hydroxypropoxy)-6-nitro-2-trifluoromethylbenzimidazole (316). A solution of 5-nitro-2-trifluoromethylbenzimidazole 3-oxide (0.12 g,

0.5 mmol) and triethylamine (6 drops, 0.6 mmol) in dry *N*,*N*-dimethylformamide (1.5 cm^3) had added to it 3-iodopropanol (0.11 g, 0.6 mmol) and the solution heated to 120 °C for 6 h, then left at room temperature for 72 h, diluted with water and extracted with dichloromethane. The dichloromethane extract was washed with 0.1 M aqueous sodium hydroxide, then water, dried ($MgSO_4$) and concentrated to dryness (excess *N*,*N*-dimethylformamide was removed on a Kugelrohr) to furnish 0.13 g (87%) of product; m.p. 68-69 °C (from water). δ_H ($CDCl_3$) 2.00 (2H, quintet, $CH_2CH_2CH_2$), 3.64 (2H, q, CH_2OH), 4.61 (2H, t, OCH_2), 4.78 (1H, t, OH), 8.10 (1H, d, 4-H), 8.27 (1H, dd, 5-H), 8.82 (1H, d, 7-H). $J_{CH_2CH_2}$ 7, $J_{4,5}$ 9, $J_{5,7}$ 2 Hz. δ_C 31.0 ($CH_2CH_2CH_2$), 57.0 (CH_2OH), 79.1 (OCH_2), 108.1 (C-4), 118.1 (CF_3, q, $J_{C,F}$ 264 Hz), 119.8 (C-7), 123.1 (C-6), 130.8 (C-3a), 140.3 (C-2, q, $J_{C,F}$ 35 Hz), 140.6 (C-5), 146.0 (C-7a). *m/z* 305 ($M^{+\cdot}$, 41%), 247 (96), 231 (14), 201 (15), 69 (29), 59 (100), *etc*. The sample was not purified sufficiently for microanalysis.

***N*-(3-Nitro-2-pyridyl)sarcosine ethyl ester (190).** A suspension of sarcosine (2.67 g, 30 mmol) and sodium hydrogen carbonate (10.08 g, 120 mmol) in water (60 cm^3) was added to a stirred solution of 2-chloro-3-nitropyridine (4.78 g, 30 mmol) in ethanol (150 cm^3) at room temperature. The solution was heated under reflux for 4 h, then concentrated under reduced pressure to 70 cm^3, extracted with ether and the aqueous layer acidified with HCl. The precipitate was filtered off and recrystallised from propan-2-ol/water to give 4.51 g (71%) of *N*-(3-nitro-2-pyridyl)sarcosine; m.p. 121-4 °C (dec.). The mother liquor afforded a further crop of product (0.63 g, 10%); m.p. 121-4 °C (dec.) (lit.[131] 126-8 °C). Overall yield: 81%. δ_H 3.02 (3H, s, CH_3), 4.32 (2H, s, CH_2), 6.90 (1H, dd, 5-H), 8.25 (1H, dd, 4-H), 8.30 (1H, dd, 6-H). $J_{4,5}$ 8, $J_{5,6}$ 4.6, $J_{4,6}$ 1.6 Hz. δ_C 40.1 (CH_3), 52.2 (CH_2), 112.7 (C-5), 132.0 (C-3), 135.9 (C-4), 150.8 (C-6), 150.9 (C-2), 170.5 (CO).

N-(3-Nitro-2-pyridyl)sarcosine (4.99 g, 23.6 mmol) was dissolved in ethanol (100 cm^3) containing dry HCl (2.4 g) and heated under reflux for 5 h. The solution was concentrated under reduced pressure to 50 cm^3, then poured into vigorously stirred

ice/water (200 cm^3). The precipitate was filtered off and recrystallisation was attempted from ethanol/water, but an oil persisted, so this was concentrated under reduced pressure to give the title compound (4.13 g, 73%); m.p. 49-50 °C (lit.[131] 52 °C). δ_H ($CDCl_3$) 1.25 (3H, t, CH_2CH_3), 2.95 (3H, s, NCH_3), 4.20 (2H, q, CH_2CH_3), 4.32 (2H, s, NCH_2), 6.75 (1H, m, 5-H), 8.10 (1H, dd, 4-H), 8.25 (1H, dd, 6-H). $J_{4,5}$ 8, $J_{5,6}$ 4.6, $J_{4,6}$ 1.7 Hz.

1-Hydroxy-4-methylpyrido[2,3-*b*]pyrazine-2,3-dione (191). *N*-(3-Nitro-2-pyridyl)sarcosine ethyl ester (3.59 g, 15 mmol) and potassium carbonate (2.07 g, 15 mmol) were stirred in ethanol (90 cm^3) at room temperature for 48 h. The precipitate was filtered off, dissolved in water and acidified (HCl), to give 0.46 g (16%) of the title compound; m.p. 244-6 °C (lit.[131] 242-4 °C). δ_H 3.49 (3H, s, CH_3), 7.35 (1H, dd, 7-H), 7.85 (1H, d, 8-H), 8.29 (1H, d, 6-H). $J_{6,7}$ 5.0, $J_{7,8}$ 8.0, $J_{6,8}$ 1.6 Hz. δ_C 28.7 (CH_3), 119.6 (C-8), 120.7 (C-7), 124.4 (C-8a), 137.6 (C-4a), 142.6 (C-6), 150.0 (C-3), 155.8 (C-2).

1-Ethoxy-4-methylpyrido[2,3-*b*]pyrazine-2,3-dione (317). A solution of 1-hydroxy-4-methylpyrido[2,3-*b*]pyrazine-2,3-dione (0.19 g, 1 mmol), triethylamine (0.11 g, 1.1 mmol) and ethyl iodide (0.17 g, 1.1 mmol) in dry *N*,*N*-dimethylformamide (2 cm^3) was stirred at room temperature for 24 h, then diluted with water and the buff-coloured product filtered off and dried *in vacuo*; 70 mg (32%); m.p. 164 °C (from water). (Found: C, 53.2; H, 4.8; N, 18.7. $C_{10}H_{11}N_3O_3.0.25\ H_2O$ requires C, 53.2; H, 5.1; N, 18.6%.) δ_H 1.35 (3H, t, CH_2CH_3), 3.55 (3H, s, NCH_3), 4.20 (2H, q, CH_2), 7.34 (1H, dd, 7-H), 7.82 (1H, dd, 8-H), 8.28 (1H, dd, 6-H). $J_{CH_2CH_3}$ 7, $J_{6,7}$ 4.6, $J_{7,8}$ 8, $J_{6,8}$ 1.5 Hz. δ_C 13.0 (CH_2CH_3), 28.3 (NCH_3), 71.0 (OCH_2), 119.4 (C-8), 120.0 (C-7), 123.2 (C-8a), 137.7 (C-4a), 142.5 (C-6), 149.7 (C-3), 156.0 (C-2). *m/z* 221 ($M^{+\cdot}$, 66%), 193 (9), 177 (13), 165 (29), 148 (100), 120 (71), *etc.*

1-(3-Hydroxypropoxy)-4-methylpyrido[2,3-*b*]pyrazine-2,3-dione (318). A solution of 1-hydroxy-4-methylpyrido[2,3-*b*]pyrazine-2,3-dione (0.11 g, 1 mmol), triethylamine (0.11 g, 1.1 mmol) and 3-iodopropanol (0.20 g, 1.1 mmol) in dry *N*,*N*-dimethylformamide (2 cm^3) was stirred at room termperature for 24 h, heated to 110 °C for 2 h, then cooled to room temperature, diluted with water and extracted with dichloromethane. The organic extract was dried ($MgSO_4$) and concentrated to dryness (Kugelrohr) to yield 0.15 g (60%) of the title compound; m.p. 130-132 °C (from water). (Found: C, 50.9; H, 4.9; N, 16.1. $C_{11}H_{13}N_3O_4.1/3\ H_2O$ requires C, 51.4; H, 5.2; N, 16.3%.) δ_H 1.91 (2H, quintet, $CH_2CH_2CH_2$), 3.55 (3H, s, NCH_3), 3.60 (2H, q, CH_2OH), 4.23 (2H, t, CH_2O), 4.64 (1H, t, OH), 7.34 (1H, dd, 7-H), 7.83 (1H, dd, 8-H), 8.28 (1H, dd, 6-H). $J_{6,7}$ 5, $J_{7,8}$ 8, $J_{6,8}$ 1.4 Hz. δ_C 28.5 (NCH_3), 30.7 ($CH_2CH_2CH_2$), 57.2 (CH_2OH), 72.6 (CH_2ON), 119.5 (C-8), 120.0 (C-7), 123.2 (C-8a), 137.8 (C-4a), 142.7 (C-6), 149.8 (C-3), 156.2 (C-2). *m/z* 251 ($M^{+\cdot}$, 55%), 193 (45), 177 (49), 165 (53), 148 (100), 120 (66), *etc.*

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