

The Chemistry of Imidazoles and Pyrimidinones

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To Jenny...

Abstract

The reactions of amino- and formylimidazoles, and amino-pyrimidinones are described. Conditions were found for the successful condensation of 5(4)-aminoimidazole-4(5)-carboxamide with a number of substituted benzaldehydes, to provide a series of novel 5(4)-*N*-benzylidene-aminoimidazole-4(5)-carboxamides. Reaction of a subset of the benzylidene derivatives with sodium hydride furnished novel imidazo[1,5-*a*]quinazoline-3-carbox-amides in good yields, via an intramolecular cyclisation. Attempted mixed acid nitration of imidazo[1,5-*a*]quinazoline-3-carboxamide led only to amide hydrolysis to provide the carboxylic acid. High yielding conditions were sought for the synthesis of a formylimidazole by the selective partial reduction of commercially available 4,5-disubstituted imidazoles. Lithium triethoxy-aluminium hydride reduction of 1-benzyl-4,5-dicyanoimidazole gave a mixture of the isomeric monoaldehydes in a yield of 53%. Methyl 5(4)-formylimidazole-4(5)-carboxylate was formed by acidic hydrolysis of methyl 5(4)-diethoxymethylimidazole-4(5)-carboxylate. Unfortunately, Knoevenagel condensation of the formyl-imidazoles prepared with ethyl nitroacetate could not be achieved. Ethoxycarbonylacetylferrocene and 1,1'-bis(ethoxycarbonylacetyl)ferrocene, prepared from ferrocene in one and two steps respectively via Friedel-Crafts acylations, were heated with guanidine carbonate to provide 1-(2-amino-3,4-dihydro-4-oxopyrimidin-6-yl)ferrocene and 1,1'-bis(2-amino-3,4-dihydro-4-oxopyrimidin-6-yl)ferrocene in low yields, though the latter appeared to decompose on storage. In studies aimed at preparing a pyrimido[5,4-*b*]indole, attempted nitrosation of 2-amino-6-phenylpyrimidin-4-one with nitrosonium tetrafluoroborate gave the unexpected unsymmetrical dimer 2-amino-5-(3,4-dihydro-4-oxo-6-phenylpyrimidin-2-yl)-6-phenylpyrimidin-4-one, in low yield. To overcome the observed poor reactivity of 2-amino-5-halopyrimidin-4-ones towards Suzuki coupling with boronic acids, 2-

amino-5-halo-4-methoxy-6-phenylpyrimidines were prepared and successfully coupled with a range of aryl and heteroaryl boronic acids to provide 5-aryl- and 5-heteroarylpyrimidines in good to excellent yields. As expected, the iodopyrimidine was more reactive to the palladium catalysed coupling than the bromo analogue. Acidic hydrolysis of the 5-arylpyrimidines furnished 2-amino-5-aryl-6-phenylpyrimidin-4-ones in excellent yields. Heck reactions of 2-amino-5-iodo-6-phenylpyrimidin-4-one and 1-hexyne gave only a low yield of 6-amino-2-butyl-4-phenylfuro[2,3-*d*]pyrimidine, involving intramolecular cyclisation. Unlike the trend observed for Suzuki reactions, Heck reaction of 2-amino-5-iodo-4-methoxy-6-phenylpyrimidine with 1-hexyne gave the coupled product, 2-amino-5-(1-hexyn-1-yl)-4-methoxy-6-phenylpyrimidine, but only in a low yield of 27%.

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Abbreviations

ABmFPP	2-amino-5-bromo-6-(3-fluorophenyl)pyrimidin-4-one
ABMP	2-amino-5-bromo-6-methylpyrimidin-4-one
ABPP	2-amino-5-bromo-6-phenylpyrimidin-4-one
Ac	acetyl
ADCC	antibody-dependent cell-mediated cytotoxicity
ADEPT	antibody-directed enzyme prodrug therapy
AIC	5(4)-aminoimidazole-4(5)-carboxamide
AIPP	2-amino-5-iodo-6-phenylpyrimidin-4-one
AP	atmospheric pressure (chemical ionisation)
APP	2-amino-6-phenylpyrimidin-4-one
aq.	aqueous
Ar	aryl / heteroaryl
ATP	adenosine triphosphate
BCG	bacillus calmette-guérin
BOC	<i>tert</i> -butoxycarbonyl
CEA	carcino-embryonic antigen
COSY	correlation spectroscopy
CSF	colony stimulating factor
CTL	cytotoxic T lymphocyte
d.	day
DCCI	<i>N,N'</i> -dicyclohexylcarbodiimide
DEPT	distortionless enhancement by polarisation transfer
DIBAL	diisobutylaluminium hydride
DMAP	4-dimethylaminopyridine
DME	1,2-dimethoxyethane
DMF	<i>N,N</i> -dimethylformamide
DMSO	dimethylsulfoxide

dppf	1,1'-bis(diphenylphosphino)ferrocene
δ	chemical shift (ppm)
EGF-R	epidermal growth factor receptor
EI	electron impact
eq.	equivalents
ES	electrospray
Et	ethyl
FAB	fast atom bombardment
FGF	fibroblast growth factor
G-CSF	granulocyte colony stimulating factor
GI	gastrointestinal
h.	hour
IFN	interferon
IL	interleukin
LHRH	luteinising hormone releasing hormone
LTA	lead tetraacetate
m./min.	minutes
MAB	monoclonal antibody
MDP	muramyl dipeptide
Me	methyl
MHC	major histocompatibility
mol	mole
NCAM	neural cell adhesion molecule
NCI	National Cancer Institute
NK	natural killer
NMR	nuclear magnetic resonance
NOe	nuclear Overhauser effect
PCC	pyridinium chlorochromate
PDGF	platelet derived growth factor

Ph	phenyl
PK	protein kinase
ppm	parts per million
psi	pounds per square inch
PTK	protein tyrosine kinase
rpm	revolutions per minute
TGF	transforming growth factor
THF	tetrahydrofuran
TLC	thin layer chromatography
TNF	tumour necrosis factor
TSA	tumour specific antigen
VDEPT	virally directed enzyme prodrug therapy
VEGF	vascular endothelial growth factor

1. Introduction

1.1 Cancer

Cancer is possibly the most feared disease of mankind - not without good reason, currently accounting for a quarter of all UK deaths.¹ The average age for the diagnosis of cancer is 67, and as life spans increase the incidence of cancer is expected to rise accordingly. However, even when corrected for this factor, mortality rates for a number of common cancers, over several continents, have increased significantly (ref. 2 pp 6-7).

In 1989 (the year for which the most recent complete data are available), 302220 new cases of cancer were registered in the UK, comprising 137690 male and 164530 female cases. The relative incidences of the most common forms for both sexes combined are depicted in Figure 1-1 (Data from ref. 3). Lung cancer was the commonest cancer in men (21%) and breast cancer the commonest in women (19%), with non-melanoma skin cancer the second most common for both sexes (although this has a very high cure rate).

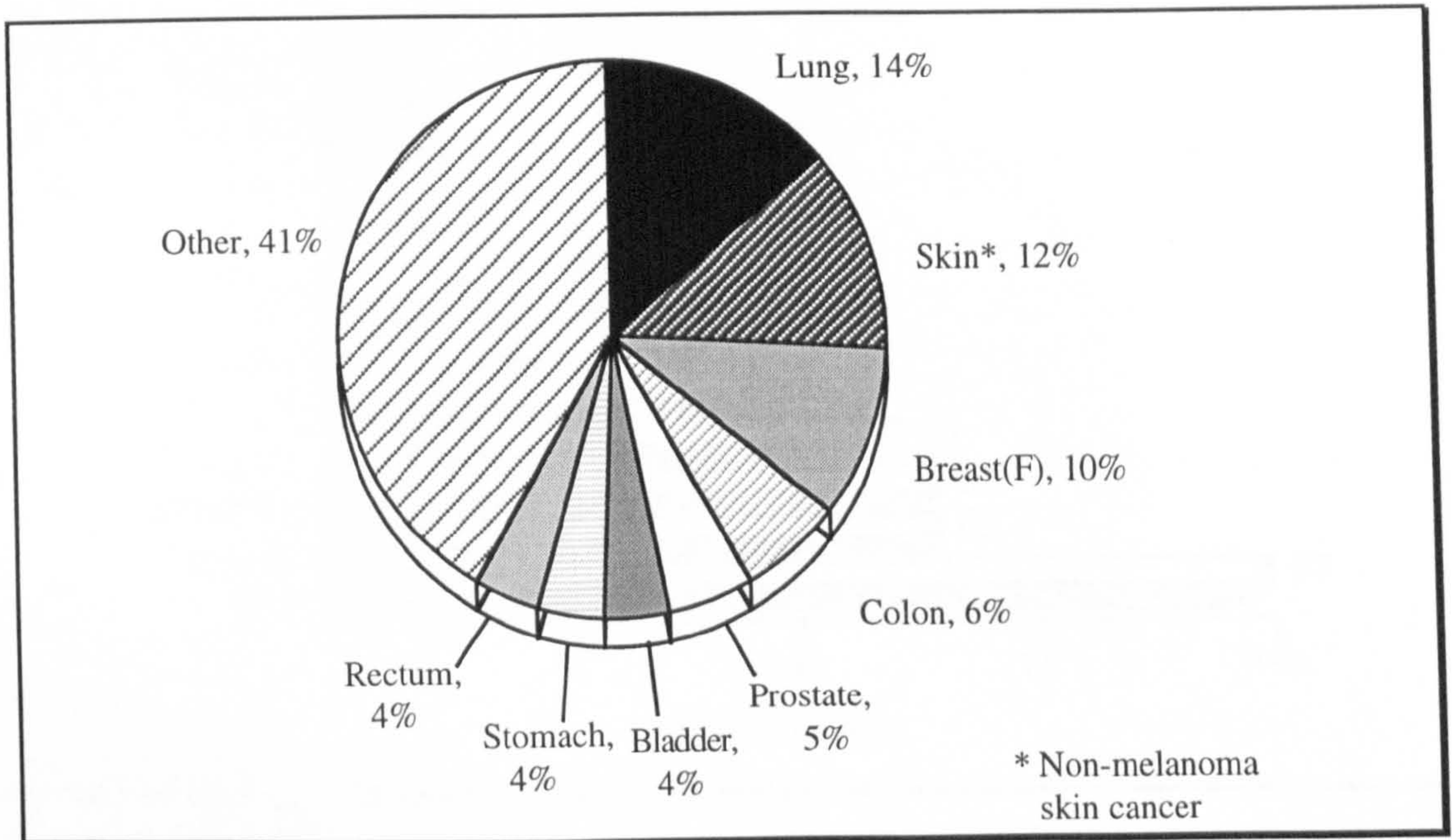


Figure 1-1 Cancer incidence in the UK, 1989.

Cancer is not just one disease, the outcome of treatment being as much a function of cancer type, as stage at presentation. Figure 1-2 shows the 5 year survival rates, for a range of cancers in all stages, registered in England and Wales in 1981, survival rates being very similar between sexes (Data from ref. 3). Certain cancers now have very high cure rates such as testicular cancer, childhood leukaemia, Burkitt's lymphoma and certain ovarian cancers, but these cancers are low in incidence. The most common cancers such as breast, lung, colon and prostate are far less susceptible to chemotherapy, and even with other treatment modalities, such as surgery and radiotherapy, survival rates are still unacceptably low. The earlier the diagnosis the better the prognosis, and screening programmes have been of great benefit in the treatment of certain cancers, such as breast and cervical cancer.

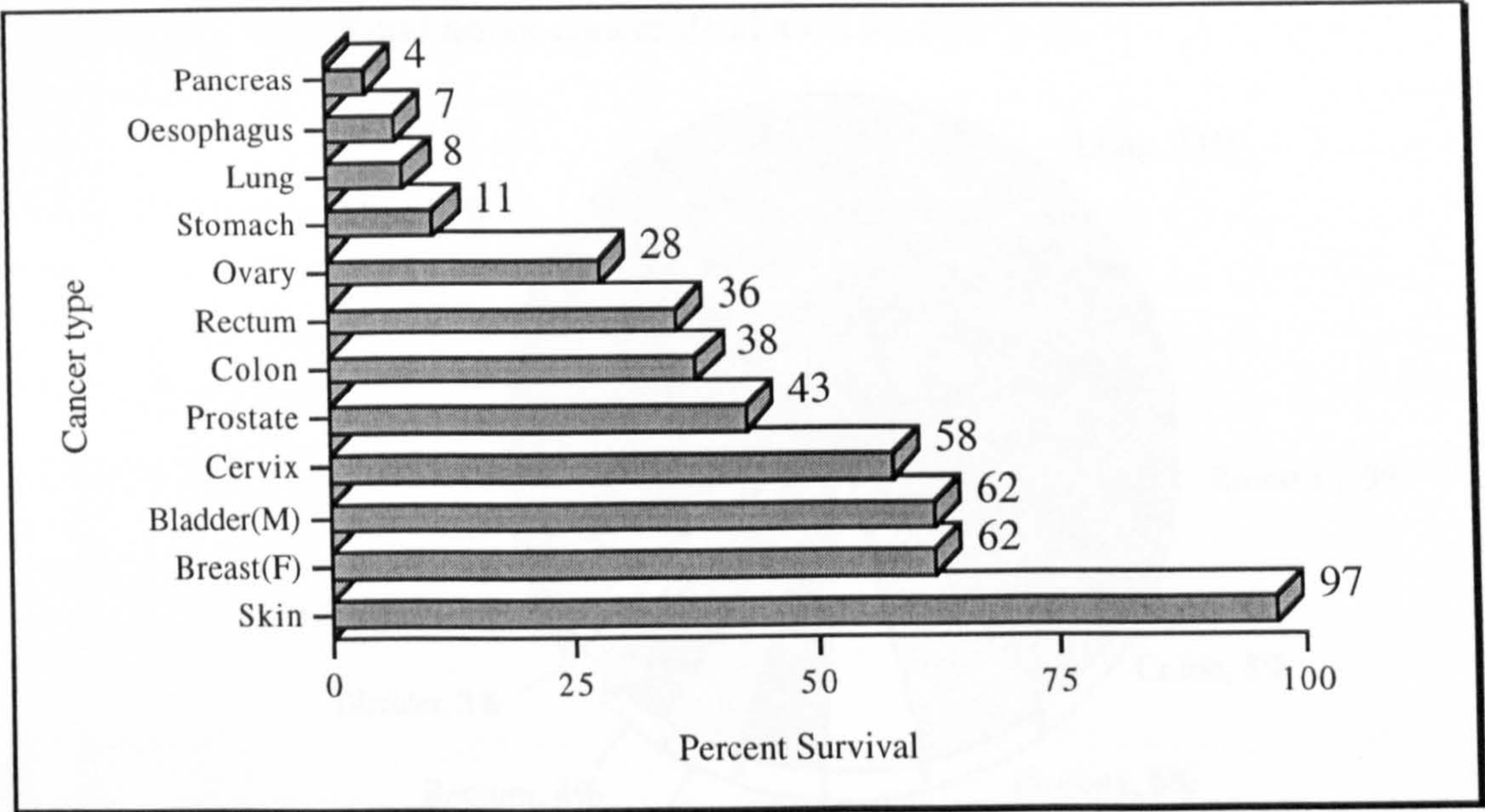


Figure 1-2 Five-year survival rates for cancers registered in England and Wales in 1981.

The most recent mortality statistics for the UK, for 1994, are depicted in Figure 1-3 in descending order for the ten commonest causes of cancer death, both sexes combined (Data from ref. 1). The total figure was 160,680, with 83,342 male and 77,334 female cancer deaths. Lung cancer was the most common cause of male cancer deaths (29%), followed by prostate cancer (12%). For women, breast cancer was the biggest cancer killer (19%), followed by lung cancer (17%). Interestingly, in Scotland, lung cancer overtook breast cancer to become the most common cause of female cancer death, a trend being observed in other industrialised counties, showing a strong correlation with increased smoking among women (ref. 2, p. 6).

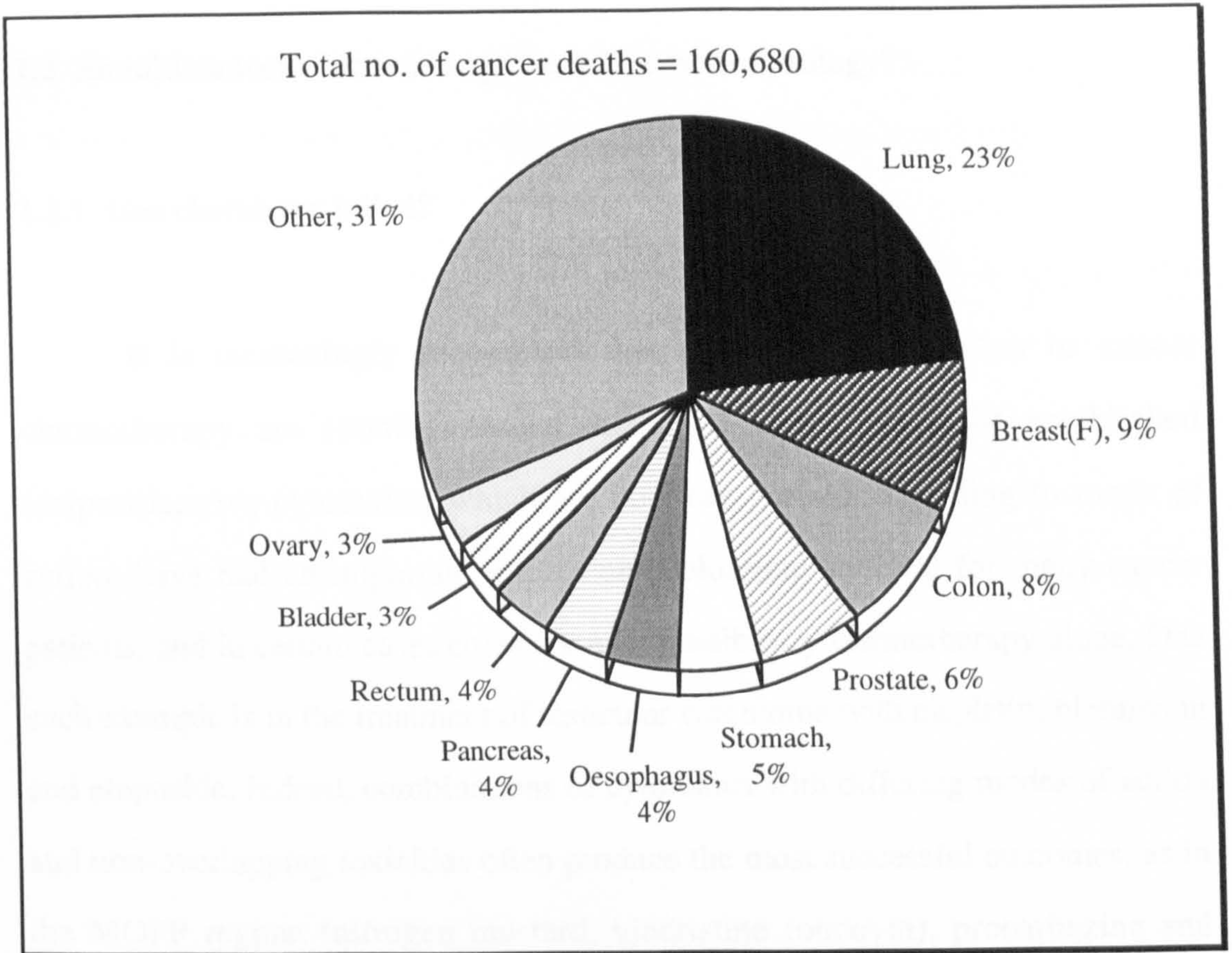


Figure 1-3 Breakdown of cancer mortality, UK, 1994.

Over the past 50 years significant improvements in anticancer therapy have been achieved, with more sophisticated detection, surgery, radiotherapy and the arrival of chemotherapy into the mainstream of treatment all making their mark. As mentioned above, some cancers are now curable by various combinations of these modalities, but the outlook for the most common cancers remains rather bleak. The five year relative survival rates for all malignant neoplasms considered together is about 40% for both sexes.⁴ Major advances are still required in the treatment of this life threatening disease.

1.2 Should cancer chemotherapy be left to biotechnology?

1.2.1 Has chemistry failed?

It is increasingly recognised that traditional approaches to cancer chemotherapy are running out of steam. It is true that the established antiproliferative cytotoxics, which are now categorised according to mode of action, have had an important impact on prolonging survival for many cancer patients, and in certain cases cures are now possible by chemotherapy alone. One such example is in the treatment of testicular carcinoma with cisplatin, bleomycin and etoposide. Indeed, combinations of cytotoxics with differing modes of action and non-overlapping toxicities often produce the most successful outcomes, as in the MOPP regime (nitrogen mustard, vincristine (oncovin), procarbazine and prednisone) introduced in 1963 for the treatment of disseminated Hodgkin's Disease. With improved knowledge of drug pharmacology and tumour growth kinetics, increasing responses continue to be observed, but our current cytotoxics seem fundamentally flawed.

The current cytotoxics all interfere, rather non-specifically, with the biochemistry of cell proliferation, commonly disrupting DNA synthesis. It is perhaps not surprising that apart from their severe toxicities towards rapidly dividing cells of normal tissue, leading commonly to myelosuppression and gastrointestinal (GI) irritation, their main activity is against malignancies of haematopoietic origin such as leukaemias and lymphomas, with their high proliferation rates. Thankfully, fatalities due to childhood cancers such as acute lymphoblastic leukaemia (ALL), Wilm's tumour, Burkitt's lymphoma, other non-Hodgkin's lymphomas and retinoblastoma are becoming a thing of the past, but as

seen in the previous section, the more common, slower growing solid tumours of the breast, lung and GI tract are still not particularly amenable to therapy.

The effect of such growth kinetics is further compounded by the intrinsically higher threshold of certain normal cell types to damage over others before the cell decides, through a biochemical cascade of events, to commit suicide (a process termed apoptosis). Epithelial cells are just such a type, making the more common carcinomas which originate from them inherently more resistant to chemo- and radiotherapy than, for example, tumours whose origins are the more sensitive haematopoietic cells - the leukaemias and lymphomas.⁵ In the malignant phenotype of an aggressive tumour such apoptotic mechanisms may have been subverted by gene mutations or deletions, making them less sensitive still to drug induced damage. This, coupled with the ability of initially responsive tumours to acquire drug-specific/non-specific resistance has been the failing of current chemotherapy.

Variations of existing cytotoxic agents continue to enter clinical trial, and these have been recently reviewed,^{6,7} but in order to achieve the objective, which must remain to cure cancer, radical new approaches are required. The question is - can chemistry deliver?

1.2.2 The problem we face

It is important to remember that cancer is not some sinister enemy with elaborate plans for its every move, rather it is a disease of chance. Consider the scenario of the development of a tumour in epithelial tissue. By chance, a normal cell sustains a genetic mutation - maybe due to a DNA transcription error, or some

mutagenic insult - which is not corrected and which, by chance, increases its propensity to proliferate. It and its descendants still look normal, but they are reproducing too much, giving a *hyperplasia*. After maybe years, one cell in this expanded population sustains another mutation which, by chance, further loosens the controls on cell growth, or maybe affects the cell's ability to correct future transcription errors. The more abnormal cells of this progression lead to the designation *dysplasia*. Again, in time, another mutation in one of the cells alters its and its descendants' behaviour further, creating a progressively more aggressive cancer, less responsive to proper external influences. If the tumour has so far remained contained within its originating tissue it is termed an *in-situ* cancer (in this case a carcinoma). The tumour may remain contained indefinitely, but in the heterogeneous mixture of cells in such a population further mutations in one cell may engender an ability to recruit nearby endothelial cells to vascularise the tumour - angiogenesis - the arrival of nutrients allowing the tumour to expand rapidly. Some cells in the tumour may have, or gain through further mutations, the ability to detach from the primary tumour and travel to distant sites of the body, establishing new tumours (*metastasis*). The tumour is now *malignant*, and it is usually the establishment of secondary tumours in vital organs that leads to fatality in cancer patients.

Given that there are some 30 trillion cells in the average human body, it is perhaps a surprisingly rare event, but that is cold comfort to those afflicted, and more effective therapies are eagerly awaited.

This picture of a tumour's development is not new, but the huge advances in the techniques of molecular biology over the last 20 years, including nucleic acid and protein sequencing and synthesis, mass cell culture, genetic engineering

and recombinant technology have unravelled many of the intricate mechanisms governing normal cell growth and proliferation. It has become clear that the accumulation of mutations in specific classes of genes are required for the malignant transformation of a cell.

Errors in two types of genes are required for uncontrolled cell proliferation - oncogenes and tumour suppressor genes. In a normal cell, protein products of proto-oncogenes are involved in molecular "bucket-brigades", relaying external growth stimulatory signals from neighbouring cells to the nucleus, to initiate the proliferative process. Transformation to oncogenes leads to overproduction of, or a more active form of, the gene product, telling the cell to multiply when it should not. Many defective oncogenes have now been discovered, whose products are involved in various parts of the "signal transduction pathway". For example, the *erb-B2* gene coding for a growth factor receptor is found defective in some breast cancers; members of the *ras* genes coding for cytoplasmic proteins involved in relaying the message are defective in a quarter of cancers (e.g. lung and colon carcinomas), often through a single point mutation; members of the *myc* family of genes coding for transcription factors that activate growth-promoting genes are also defective in a number of tumours.

More than just the generation of oncogenes is required for malignant growth as there are many safeguards wired into all cells, coded by tumour suppressor genes. Some of the tumour suppressor gene proteins are involved in relaying growth inhibitory signals, preventing cell growth. For example, the *NF-1* gene product, in response to such signals, inhibits the *ras* protein in the cytoplasm, preventing the passage of stimulatory signals, and is defective or absent in a number of tumours (e.g. myeloid leukaemia). Similarly, the *p15* gene

protein in the nucleus, in response to transforming growth factor beta (TGF- β), shuts down transcription of growth-promoting genes, and is found discarded in a number of cancers. A number of important tumour suppressor genes act on the cell cycle clock - the molecular apparatus controlling the progress of a cell through its cycle. At points in the cell cycle certain cyclin-dependent kinases (CDKs) require activation by cyclins to allow further progress, their activation bringing about the release of transcription factors required in the activation of growth-promoting genes. Known tumour suppressor genes code for various inhibitory proteins that can restrain forward movement. The p15 protein mentioned above inhibits the cyclin D / CDK4/6 complex at the so called restriction-point when the cell decides whether or not to go into S-phase (DNA synthesis). If not inhibited, this active complex leads to phosphorylation and deactivation of pRB - a protein which when active sequesters an important transcription factor, acting as a 'master-brake' on the system - the released transcription factor generating proteins required in the cell cycle progression. The *pRB* gene is mutated or absent in 40% of cancers, for example paediatric retinoblastoma. Another act of tumour suppressor gene proteins is to ensure that progress through the cell cycle is smooth, checking for mistakes made in DNA replication for example - often known as DNA checkpoint proteins. One ubiquitous example is the p53 protein which, among its many acts in tumour suppression, can halt the cell cycle and induce apoptosis of abnormal cells. As well as evading apoptosis, cells without an effective *p53* gene can more readily accumulate mutations leading to a more malignant phenotype. Unsurprisingly, the *p53* gene is mutated or absent in over half of all cancers. The errors that can occur in the cell cycle clock have been discussed in detail.⁸

Different tumour types commonly have different profiles of oncogenes and defective tumour suppressor genes, though no one tumour fingerprint could be predicted from its type alone. Most familial cancers arise from the inheritance of defective or absent tumour suppressor genes, allowing the time of appearance of tumours to leapfrog many years. Notable examples of inherited defective genes are *pRB* predisposing to retinoblastoma in children, and *BRCA1* and *BRCA2* genes linked to certain breast and ovarian tumours.

Although much less is yet known about the genes involved in later tumour development, including angiogenesis and metastasis, what can be done with the current knowledge of the very real differences between normal and tumour cells?

1.2.3 Does biotechnology have the answer?

Important advances in the techniques of molecular biology are now producing some very exciting approaches to the treatment of cancer.

Perhaps the most exciting come under the gene therapy umbrella, inserting a whole gene into a cell, whose product will ultimately eliminate the tumour. One approach, virally directed enzyme prodrug therapy (VDEPT) aims to introduce selectively into tumour cells genes coding for enzymes able to render cytotoxic an administered prodrug. Another approach involves genes coding for "immunoattraction" such as tumour necrosis factor (TNF), interleukin-2 (IL-2), IL-4, IL-12, interferon-gamma (IFN- γ) and foreign peptides - enhancing immune recognition and tumour cell destruction. For example, inoculation of mice with mammary tumour cells transfected with the IL-10 gene has led to tumour growth inhibition.⁹ Gene replacement therapy has been sold as the "magic bullet" for

cancer - and reversal of tumour phenotype by the insertion of functional tumour suppressor genes has been achieved *in vitro*. For example, the introduction of wild-type *p53* into human gingival carcinoma cells induced differentiation.¹⁰ The various approaches have been discussed in detail.¹¹ At present gene therapy is limited by poor incorporation efficiencies *in vivo*, and despite continued improvements in techniques, progress is slow.

A similar approach is the use of antisense or antigene oligonucleotides, targeted to mRNA or DNA respectively that corresponds to an oncogene, a sequence of about 15 bases required for selectivity. Despite impressive *in vitro* activities, they have yet to show benefit *in vivo*.¹¹ Current problems include metabolic stability and cellular uptake. If successful they would be cytostatic rather than cytotoxic, requiring continued administration.

A number of other approaches are more immunologically based, perhaps the most advanced of which is the use of monoclonal antibodies (MABs). The technique for the production of high affinity antibodies, raised to a specific antigen, in virtually indefinite supply has received widespread clinical use, not least in experimental cancer therapies, since its inception by Köhler and Milstein in 1975. A number of moderately tumour specific antigens (TSAs) are known, including carcino-embryonic antigen (CEA) - present on cells of the foetus but not the adult, CEA is common to many carcinomas, especially of the colon. As well as being able to bring about an immune response (discussed later), MABs have been conjugated to cytotoxic drugs, toxins and radionuclides for tumour targeting - for example, a murine MAB raised against neural cell adhesion molecule (NCAM) conjugated to a ricin toxin has shown *in vitro* activity against a

small cell lung cancer cell line.¹² A number of such approaches are undergoing clinical trial (ref. 2, pp275-276).

MAB approaches are inherently limited by poor tumour penetration of the large macromolecules and the antigenic heterogeneity of cells within a real tumour. An approach able to overcome these problems is known as antibody-directed enzyme prodrug therapy (ADEPT) where an antibody, raised to a TSA, is conjugated to an enzyme that when bound at the tumour site can convert an administered non-toxic prodrug to its cytotoxic form. The final cytotoxic produced is small and easily diffusible, overcoming the poor penetration of the antibody, and the amplification possible from one bound antibody-enzyme converting many prodrugs could overcome antigenic heterogeneity providing the antigen is expressed on enough of the tumour cells, delivering the cytotoxic throughout the tumour. Until recently the major failing of MAB techniques was the immune response raised against such rodent antibodies, limiting treatment to a single administration. The advent of MAB "humanisation" to avoid immune detection, and production of more diffusible antibody fragments is set to revitalise this approach and its future has been discussed.¹³

The commonly observed loss of antigenic presentation by tumour cells has been regarded as evidence of antitumour activity of the immune system, an effective response selective for the emergence of such populations. As a result, immunisation with TSAs (or fragments) has received attention as a potential vaccine. The intention is to create an expanded population of cytotoxic T lymphocytes (CTLs) able to recognise and destroy tumour cells. *In vitro* studies have demonstrated the ability to raise T cells to CEA,¹⁴ and in murine models vaccination has protected against subsequent tumour challenge.¹⁵ However, only

rarely has this approach been effective in the treatment of an established, vascularised tumour. The main difficulties in the treatment of human tumours are their poor antigen presentation and their antigen heterogeneity. The future of vaccine-based immunotherapy has been discussed.¹⁶

With increasing awareness about the molecular messengers involved in regulating the immune response, the administration of such cytokines has received continued interest. Initially with the use of crude cell extracts, and subsequently with high purity products from recombinant technology, the ability of a single agent to trigger a cascade of events ultimately resulting in an effective immune response has stimulated great excitement. However, of the many cytokines investigated to date, few have shown effective single agent activity. The exceptions are IFN- α (approved by the FDA in 1986 for hairy-cell leukaemia and AIDS-associated Kaposi's sarcoma), and IL-2 (with marginal activity against renal cell carcinoma, melanoma and non-Hodgkin's lymphoma). Toxicities are often severe and continue to be a problem, fatalities still being observed with IFN- α for example.¹⁷

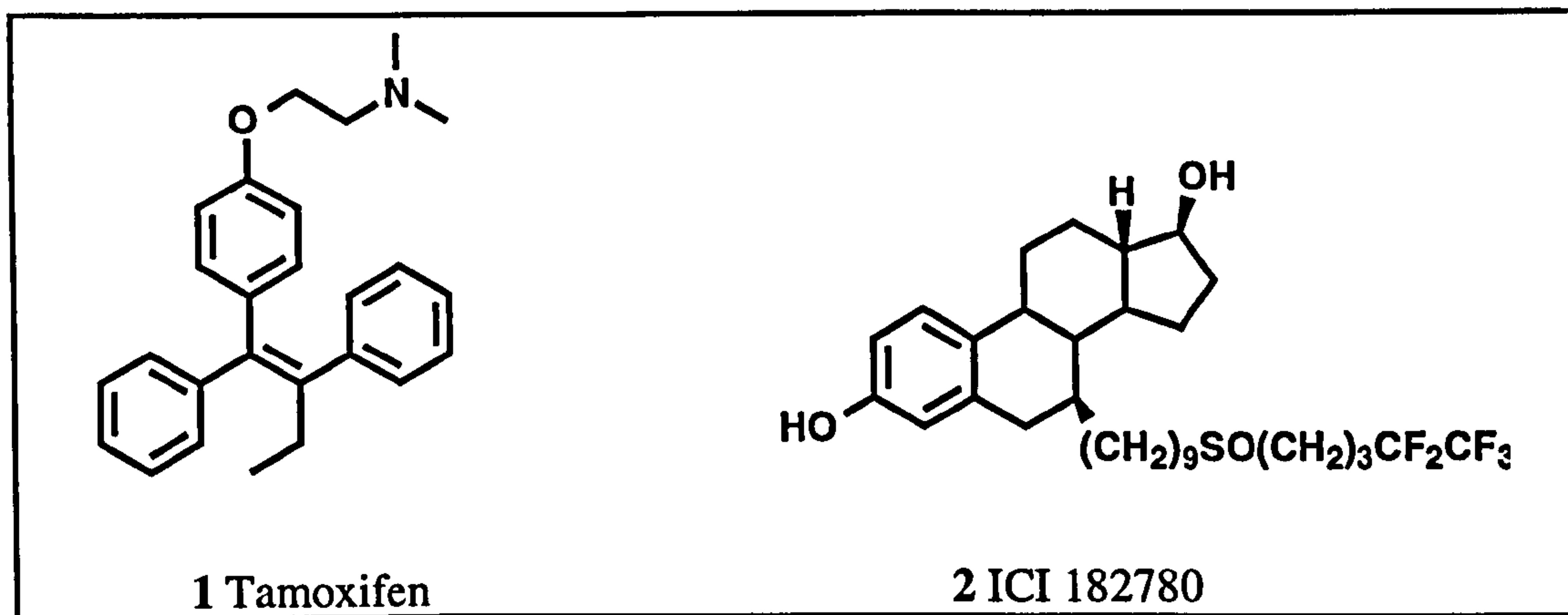
In fairness, the full biological effects of many of these cytokines are still coming to light and their true benefits may await discovery. For example, many are showing potential alongside conventional cytotoxics, combating myelosuppression - such as granulocyte colony stimulating factor (G-CSF) being used in high dose chemotherapy regimes.¹⁸ Another feature of a number of cytokines, including the interferons, is the synergistic activity commonly observed in combination with cytotoxics - for example, shown in a recent phase II trial of dacarbazine or cisplatin in combination with IFN- α and IL-2 in the treatment of metastatic melanoma.¹⁹

Current therapy in the treatment of aggressive, malignant tumours of nearly any sort can be expected to select for the outgrowth of resistant clones that can occur spontaneously within the tumour population. While still offering great promise, biotechnology has so far failed to live up to expectations. With the problems of synthesis, formulation, transport and metabolism of these large fragile molecules, what effect can the small molecule have on the new discoveries of cancer biology?

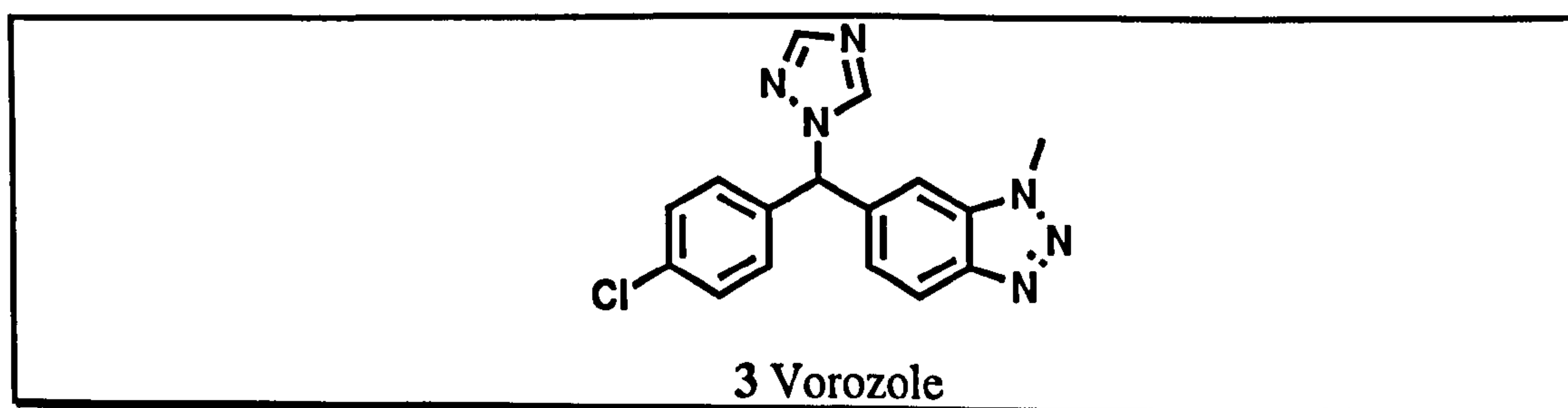
1.2.4 A new age of medicinal chemistry

As well as directing many of the biotechnology approaches, the differences that have been established between normal cells and tumour cells, have provided a wealth of new targets for medicinal chemists. For example, many of the oncogenes involved in tumour growth code for proteins involved in signal transduction pathways where receptors could be antagonised and enzymes inhibited. A great deal of such chemistry has already been done.

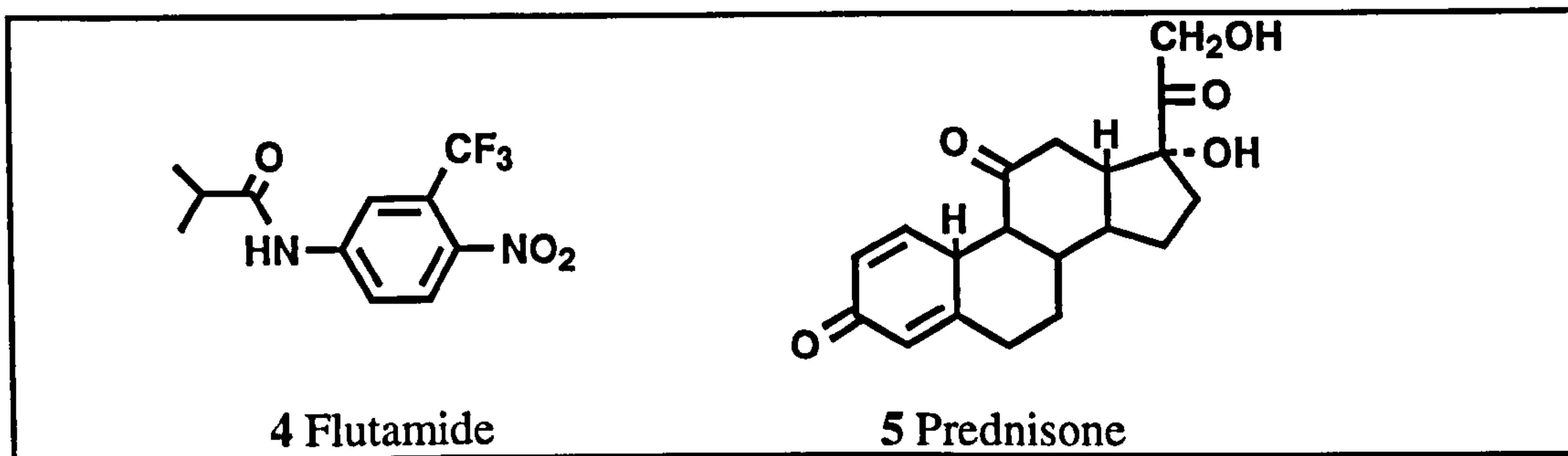
The most studied approach at the receptor approach has been with hormone-dependant tumours of the breast and prostate. The best known drug, tamoxifen (1), is an antioestrogen that competes with oestradiol for the oestrogen receptor. Despite minor toxicities, including increased risk of endometrial cancer,²⁰ it has been the endocrine treatment of choice for breast cancer for nearly a decade. It suffers from residual agonist activity,²¹ and several second generation antioestrogens have been prepared such as ICI 182780 (2) - a pure antioestrogen that has shown *in vivo* activity²² that should help determine the full potential of such growth factor receptor antagonists.



Other approaches to oestrogen blockade have also been established. The aromatase inhibitors block the conversion of androgens to oestrogen, the only source of oestrogen in postmenopausal women. Aminoglutethimide was one of the first aromatase inhibitors used in the treatment of advanced breast carcinoma in postmenopausal women, but it is not very selective and suffers from side effects. Second generation aromatase inhibitors have been discovered such as vorozole (3), that are more selective, exhibiting fewer side effects.²³



Other tumour types are amenable to such steroid therapy. These include ovarian cancers, prostate cancers (commonly treated with luteinising hormone releasing hormone (LHRH) agonists and antiandrogens such as flutamide (4)), and cancers of the lymphocytes - leukaemias and lymphomas - treated with glucocorticoids such as prednisone (5).



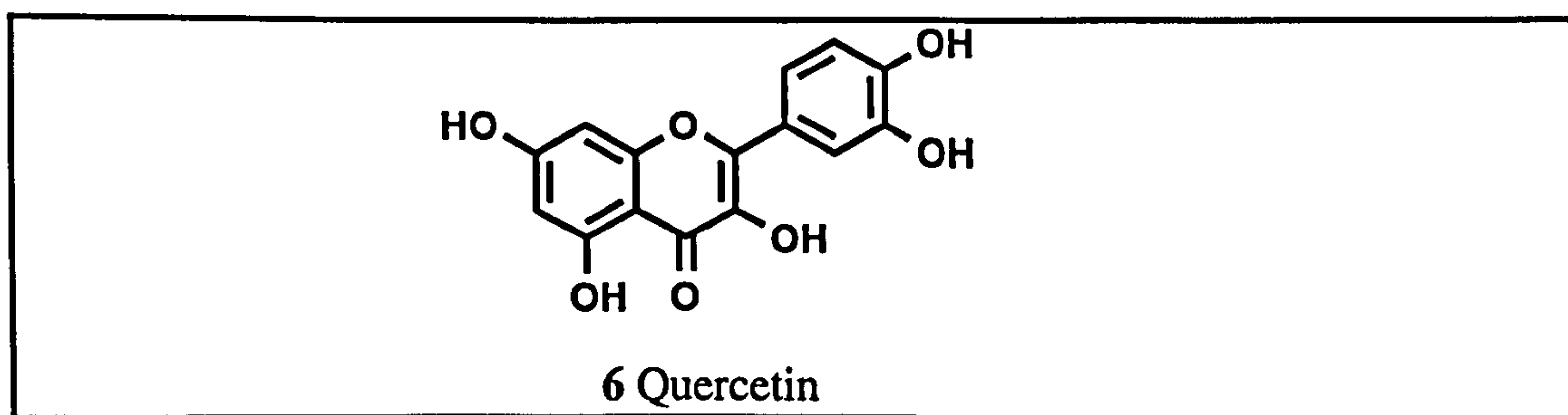
A growing understanding at the molecular level is allowing a more rational approach. However, at present none of these receptor based approaches are curative.

Another group of targets that has already received considerable interest are the protein kinases that are involved in relaying messages along the signal transduction pathway by phosphorylation of tyrosine or serine/threonine residues of the next protein down - thereby activating it to transmit the message. Protein tyrosine kinases (PTKs) are involved in the cytoplasmic region of many receptors that are often over-expressed in tumours such as the epidermal growth factor receptor (EGF-R) in breast and ovarian tumours.²⁴ Other receptors for transforming growth factor α (TGF- α), platelet derived growth factor (PDGF), fibroblast growth factors (FGFs) and the *c-erbB2* receptor whose ligand is unknown are also implicated in a number of tumours. There are also non-receptor PTKs that are cytoplasmic proteins involved in the transduction pathway, including the *src* family of proteins, implicated in colon and breast cancers,²⁵ and the *fes* and *abl* families.

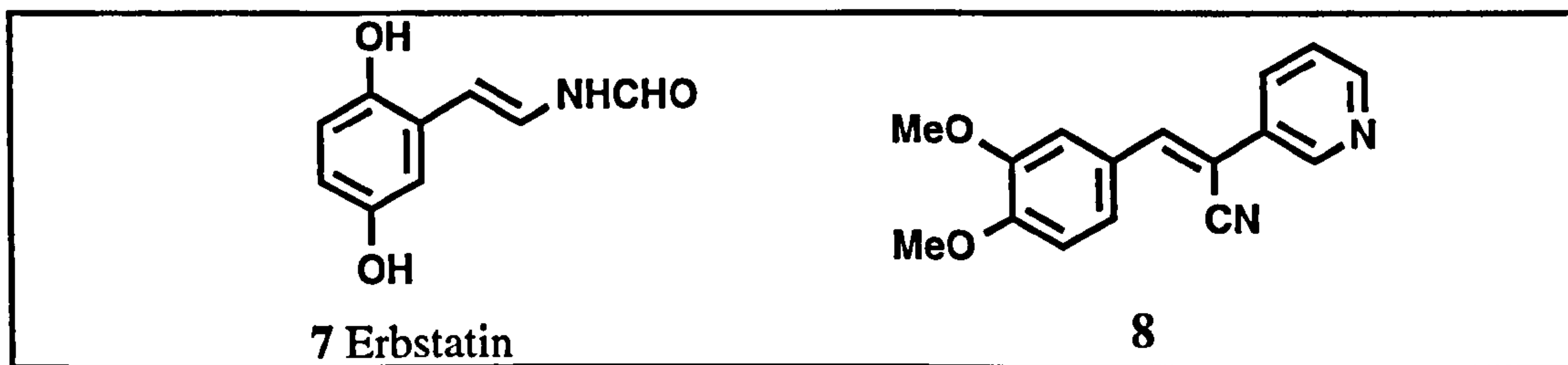
As such, PTK inhibitors have been the subject of considerable recent research. Despite the prediction of up to a thousand different protein kinases (PKs), high throughput screening, coupled with the recent X-ray crystal structures

of serine/threonine cyclic adenosine monophosphate-dependent kinases,^{26,27} and cyclin-dependant kinase 2,²⁸ and a knowledge of the amino acid sequence of the catalytic domain has produced a number of different structures that show a surprising degree of selectivity for individual PKs.

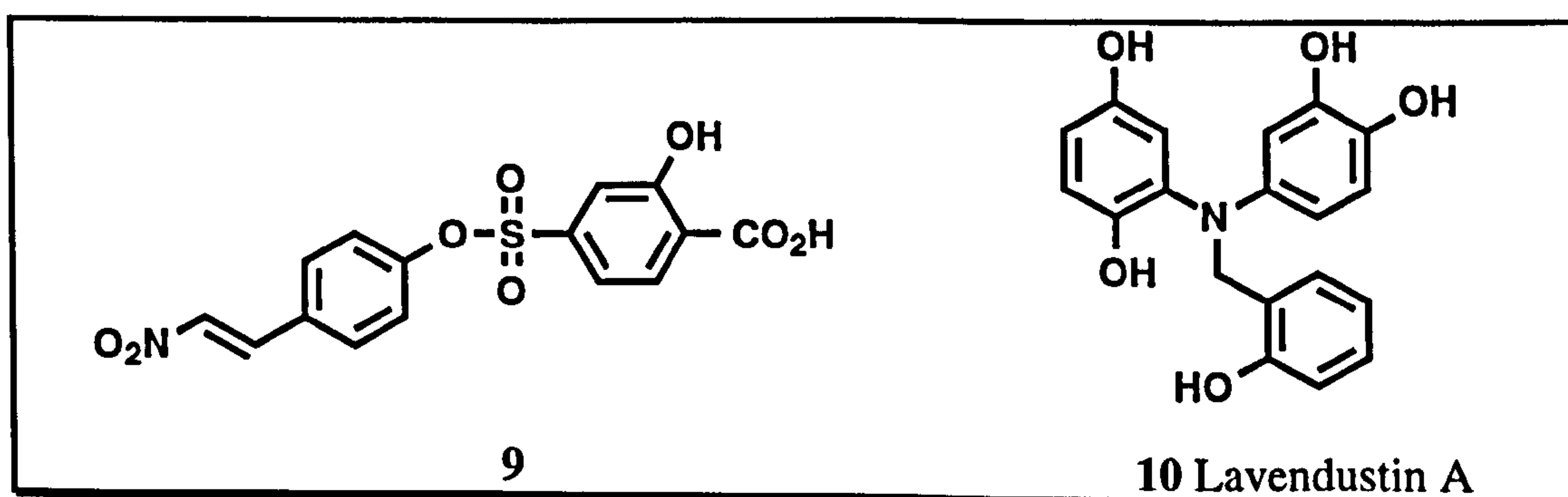
The natural flavone quercetin (6) and iso-flavone genistein show selectivity for PTKs over serine/threonine kinases,²⁹ despite being competitive with adenosine triphosphate (ATP) - the phosphate source - rather than the substrate. However, their antitumour activity is thought to be due, in part, to a cytotoxic effect mediated through interaction with topoisomerases.¹³ Other derivatives have yet to show promise.



The discovery of PTK inhibition and antitumour activity of the styrene containing natural product, erbstatin (7),¹³ prompted the synthesis of hundreds of benzylidene malononitrile analogues now termed tyrphostins.³⁰ They are competitive with substrate, and have shown a great deal of selectivity for the EGF-R PTK, even distinguishing it from the closely related *c-erbB2/neu* PTK.³¹ One such analogue, nitrile 8, has shown potent activity against breast cancer,³² and is thought to block the actions of several growth factors.



Other tyrosine mimics exhibiting PTK inhibitory activity include hydroxycinnamides,³³ thiazolidine-diones,³⁴ aminoalkylacrylophenones,³⁵ quinolines,³⁶ and dianilinophthalimides that are EGF-R selective and have shown *in vivo* activity.¹³ There are also transition state analogues such as the nitrostyryloxysulphonylbenzoic acid 9, again EGF-R selective.³⁷ Other natural products include lavendustin A (10) an EGF-R PTK inhibitor that is competitive with ATP,³⁸ and the benzoquinoid antibiotics herbimycin A and geldanamycin that inhibit the non-receptor src PTK and have shown *in vivo* activity.³⁹



A number of protein kinase inhibitors are in clinical trials and more are expected to follow.

Inhibitors of molecular targets downstream of the receptors carrying out the work of the oncogenes are also under investigation. One such target is the protein-protein interactions involving the so-called SH2 and SH3 homology

domains, important in propagating the 'message'. Phosphorylated peptides have been shown to inhibit these interactions.⁴⁰ That the *ras* gene is over-expressed or mutated in many cancers has made it the target for many approaches. From the initial gene protein three steps are required for the maturation of *ras* to its active form - farnesylation, proteolysis and methylation - occurring at the 'CAAX' box. Farnesyl transferase has been identified and shown to be inhibited by benzodiazepine peptide mimetics.⁴¹ Inhibitors of this enzyme are expected to enter clinical trials soon.

The ether lipids are another class of novel anticancer drugs,⁴² for example miltefosine (hexadecyl phosphocholine) - approved for the topical treatment of skin metastases in breast cancer patients.⁷ They elicit a range of responses including an antitumour immune reaction, but also have numerous effects on signal transduction at the membrane including antagonism of growth factor receptor interactions such as EGF and PDGF, and inhibition of key signalling enzymes.

Suramin is another lead structure that inhibits the binding and mitogenic action of a number of polypeptide growth factors including PDGF, FGF, TGF- α,β and EGF. *In vivo* antitumour activity has been observed in prostate cancer, adrenocortical carcinoma and breast and ovarian cancers.⁴³

There are several potential pitfalls to attacking signal transduction pathways. The first problem is one of selectivity. The target, for example a PTK, may be involved in many signalling pathways. Even if a target is specific for the desired pathway, an inhibitor would inhibit the target in all cells with the risk of toxicity to normal tissue. Fortunately the degeneracy of growth factor signalling

may allow specific pathway inhibition, thereby eliminating the effects of the oncogene, while leaving other paths intact for normal cell function. But, if several pathways were under the controls of several oncogenes, inhibition of all these may not leave enough signalling integrity for normal cell function. Experimentally, the development of regimes based on several drugs, each of which is inactive alone, is not an easy task and would also involve patient selection based on biopsy results to determine the oncogenic profile. As well as these practical problems, the inhibition of signalling pathways is still an antiproliferative approach and may fail on slow growing solid tumours expressing little of this biochemistry, that so desperately need more effective treatments. The results would be cytostatic not cytotoxic, suggesting drugs would be required for extended periods, increasing the chances of drug resistance appearing.

Approaches that address fundamental differences may offer greater potential. The most direct approach, similar to antisense oligonucleotide therapy, is to target drugs to specific DNA sequences of oncogenes that are different by virtue of point mutations or translocations. As stated, this requires the ability to recognise about 15 bases in a sequence. At present we are limited to 5 or 6 base recognition, for example with distamycins. However, some distamycins coupled to nitrogen mustards, have shown the ability to selectively inhibit transcription factors and have entered clinical trial.¹¹ The ability to tailor a molecule for the recognition of any sequence seems a long way off, but more sophisticated DNA binding experiments and molecular modelling packages are hoped to improve our understanding of this challenging area of research.

The search for telomerase inhibitors has also generated considerable excitement. DNA segments at the end of chromosomes called telomeres shorten

every time a cell divides, providing a counting mechanism. Once reduced to a certain length the cell stops dividing, entering senescence. If a cell manages to avoid senescence, further shrinkage of the telomeres will lead to crisis, which means cell death. Any cell escaping crisis will become immortal, in the case of a tumour enhancing the likelihood of acquiring further mutations. If crisis were not avoided, the growth of tumours would be stopped long before they grew large. Mortality is commonly avoided by activation of a gene coding for telomerase, able to replace telomeric DNA. Telomerase is virtually absent in normal cells, but has been found in a large number of tumours. A recent study of head and neck squamous cell carcinoma patients detected telomerase activity in 88% of them.⁴⁴ Telomerase is not a universal marker though, absent in cancer cells still having long telomeres requiring few mutations, including paediatric retinoblastoma,⁴⁵ and other mechanisms have been postulated for telomerase negative cells.⁴⁶ Despite this, a number of groups are seeking telomerase inhibitors.

Initiation of apoptosis is another potential target. Although the precise molecular events are not yet known, a number of genes have been implicated for example, *p53*, *bcl-2* and *fos*. Overexpression of *bcl-2* is common in cancers of haematologic origin. The *bcl-2* protein dimerises with a *bax* protein, preventing the formation of *bax:bax* homodimers which signals apoptosis, making the *bcl-2* protein a target for inhibition.⁴⁷ There appear to be a number of origins of apoptosis, and signal transduction pathways are thought to be involved in some of them, in response to external factors. For example, apoptosis in mammary cells is affected by oestrogen levels. Part of the antitumour effect of tamoxifen is due to activation of apoptosis;⁴⁸ and diethylstilbestrol, leads to apoptosis in hormone-insensitive prostate cancer cells, independent of its oestrogenic activity.⁴⁹ Similarly, testosterone is related to prostatic apoptosis, in the endometrium and

liver TGF- β 1 is important, and glucocorticoids and IL's affect the survival of haematologic cells.

The processes of later stage cancerous development - angiogenesis and metastasis - that ultimately define a cancer's destructive potential, are also the target for medicinal chemists. Not a great deal is understood about metastasis, but protease and collagenase inhibitors are showing promise in preventing its advance.

More is coming to light about the molecules involved in angiogenesis with the discovery of over a dozen promoting factors released by tumour cells (or induced from the surrounding stroma), including FGFs and vascular endothelial growth factor (VEGF) as well as inhibitory factors, including thrombospondin which is usually under the control of the p53 protein.⁵⁰ Various approaches against angiogenesis have been devised including gene therapies, antibodies to growth factors, use of growth factors linked to toxins, but also a number of smaller structures have shown anti-angiogenic activity. This broad group encompasses cytokines such as IFN- α ,⁵¹ small peptides such as angiostatin, to synthetic molecules such as pentosan polysulphate⁶ and TNP-470 (the first anti-angiogenesis agent to enter clinical trial).⁵²

These last two approaches only represent cancer management not elimination, but in an animal model the use of angiostatin reduced established tumours to microscopic dimensions for as long as treatment continued.⁵² As the effects of anti-angiogenesis agents are generally directed away from tumour cells, at slow growing normal cells, the development of resistance is less likely.

While far less advanced, these novel approaches directed at more fundamental differences between tumour and host, are hoped to revolutionise cancer treatment alongside gene therapy and immunotherapy.

The increasing use of cell free enzyme assays etc., and the switch of prescreens from inappropriate animal models that yield active drug candidates, only to perform disappointingly in the clinic, to panels of human tumour cell lines cultured *in vitro* is hoped to yield more realistic results. The American National Cancer Institute (NCI), for example, dropped the use of its P388 leukaemia *in vivo* prescreen in 1989 in favour of a panel of 60 *in vitro* human cell lines, making it now possible to test large numbers of compounds in a short space of time. While *in vitro* tests suffer from obvious limitations including the lack of cell heterogeneity, it is hoped that a more disease orientated approach will be established. Initial results from the NCI panel has shown that mechanistically similar agents of diverse structures often display a common fingerprint of activity across the 60 cell lines. Such fingerprints may also provide clues to the mode of action of agents selected through random screening programs of, for example, natural products.

1.2.5 Non-specific immunotherapy

The immune status of the cancer patient is generally regarded to be very important in determining the outcome of therapy. As stated, the loss of antigen presentation by cancer cells has been considered evidence of an immune system mounting an antitumour response. The fact that tumours are not destroyed before getting established may be due to a number of factors. Firstly, the increase in cancer incidence in old age is thought to be due to a decline in normal immune

function coupled with increasing autoimmunity, allowing aberrant cells to survive.⁵³ Secondly, through the release of as yet unknown factors, the disease itself produces an immunological deficiency. This can be further exacerbated by the immunosuppressive side-effects of many anticancer drugs.

A number of anticancer approaches that activate the immune system exist: from the vaccine and antibody based therapies discussed above, to adoptive immunotherapy, and non-specific immunotherapy. In non-specific immunotherapy the whole immune system is stimulated into activity. A number of anti-tumour agents have been shown to have this property, and can generally be classified as agents of microbial origin, natural biological substances, or synthetic compounds. All these agents are generally pleotropic in their effects on the immune system. Although this pleotropism has hampered efforts to understand the mechanisms of activity, it may be of practical necessity if multiple effector cell activities prove to be important for the successful treatment of cancer.

1.2.5.1 The antitumour immune response

Before we examine the advances in using this sort of approach, we will consider the components of the immune system that are important in the destruction of tumour cells. Figure 1-4 illustrates the major mechanisms involved (reproduced from reference 2, p. 44).

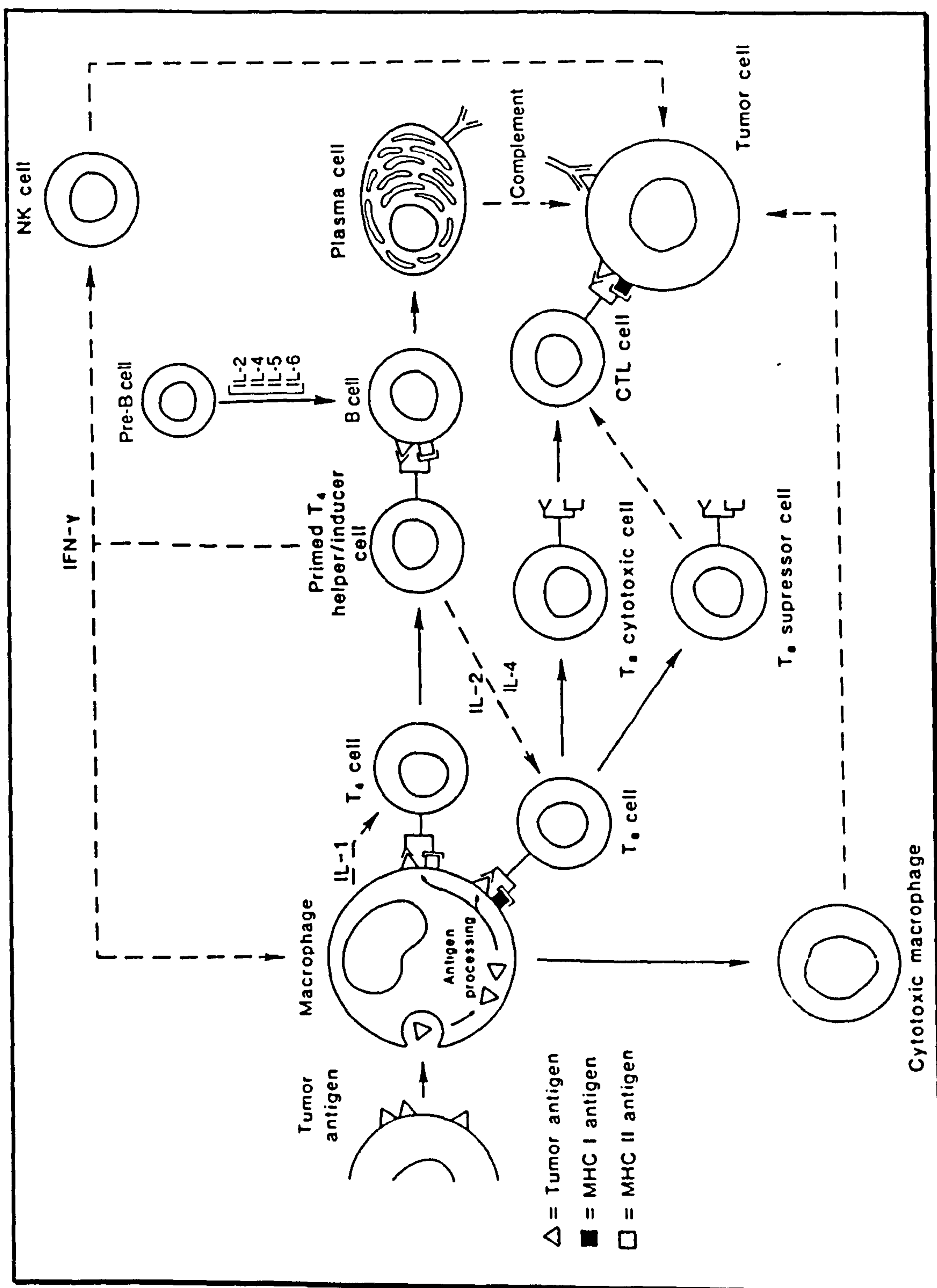


Figure 1-4 Components of the antitumour immune response. Solid lines represent proliferation/conversion of one cell type into another. Dotted lines represent cell-cell interactions.

Macrophages bind to and internalise tumour antigen. Here they are processed by enzymes involving partial degradation. The processed antigenic peptides are then presented at the macrophage cell surface in association with a MHC (major histocompatibility) antigen. The MHC antigens are required in the activation of the T cell response. Helper/inducer-T cells attach to the processed tumour antigen in association with MHC class II antigens, and the cytotoxic/suppressor-T cells in association with MHC class I antigens. Macrophages also secrete IL-1 leading to activation and proliferation of T cells.

The two T cells mentioned differ in their cell surface antigens. The helper/inducer-T cells bear a CD4 antigen, and are also known as T4 cells. The cytotoxic/suppressor-T cells bear a CD8 antigen, hence T8 cells. Once activated the T4 cells secrete a range of cytokines including IL-2, 4, 5 and 6 which stimulate B cells (which also process and present tumour antigens) into proliferation, leading to their differentiation to antibody-producing plasma cells.

Once bound to tumour cell antigen the antibodies lead to tumour cell death in two ways: by antibody-mediated complement-dependent cell lysis; or more importantly by antibody-dependent cell-mediated cytotoxicity (ADCC) employing cells such as macrophages, cytotoxic-T cells (CTLs) and natural killer (NK) cells, which recognise the bound antibodies.

The interleukins produced by the T4 cells also serve to stimulate proliferation and activation of the primed T8 cells. The T8 cells can mature into suppressor-T cells, important in moderating the normal immune response, suppressing the T cell response to antigens. They can also mature into CTLs, able

to kill tumour cells bearing the antigen to which they are activated, in association with MHC class I antigen. This is in addition to their ADCC reactivity.

Another action of primed T4 cells is the production of IFN- γ which activates macrophages and NK cells to become cytotoxic. Unlike the CTLs, NK cells do not require MHC presentation, and although they do attack antibody bound tumour cells this is not necessary for their tumour cell killing either. Thus NK cells have a very broad activity - important when one considers the heterogeneity of cells within a tumour and the low antigenicity inherent to some. Activated NK cells also secrete IFN- γ , producing a positive feedback system.

This picture is something of a simplification of the true story. There are numerous other cytokine messengers involved. As well as those mentioned several other interleukins are known with molecular weights in the range 10-25 kDa, each with a variety of effects on proliferation and activation of effector cells of the immune system.

The interferons are another large group of peptides ranging in size from 16 to 25 kDa and at least 20 are known. They are classified as IFN- α , IFN- β or IFN- γ according to their cellular origins. The interferons exert their effects, as mentioned, by stimulation of NK cells and macrophages and also through a direct antitumour cytotoxic effect.

TNF is another important mediator, with both direct and indirect cytotoxicity. It is postulated that the cell binding of TNF starts a cytolytic cascade, and that there are more TNF receptors on/in tumour cells. TNF also activates macrophages and CTLs.

The colony stimulating growth factors (CSF's) also have a role. Though not involved in direct antitumour effects, as their name suggests these 15-25 kDa peptides are able to promote the proliferation and differentiation of cells - namely granulocytes, monocytes and macrophages.

1.2.5.2 Agents of microbial origin

From the time of "Coley's toxins" in the 1890's, the use of bacterial filtrates to treat cancer was experimented upon sporadically until the 1930's, and was then abandoned. Nonspecific immunotherapy gained prominence once more in the late 1960's when the clinical efficacy of a mycobacterium BCG (*Bacillus Calmette-Guérin*) was reported. A corynebacteria, *C.parvum* has also shown similar results. Their activity is thought to correlate with augmenting the cytotoxic activity of T cells, macrophages and NK cells and there is evidence for their inducing the production of IL-1 and TNF (ref. 2, p285). As well as whole killed or attenuated cells, cell wall constituents have also shown immunomodulation, including MDP (muramyl dipeptide) - identified as the smallest immunomodulating entity of mycobacterial cell wall, and of which numerous analogues have been prepared and investigated. Other active agents include lipopolysaccharides (LPS), an endotoxin from gram-negative bacteria that induces TNF- α , β , and which despite its toxicity is still under investigation having shown moderate activity in colorectal cancer in a recent phase II study;⁵⁴ also yeast polysaccharides; bestatin; and krestin.

To date only BCG has gained approval, and has been in use for the treatment of superficial bladder cancer and carcinoma-in-situ of the bladder since 1990.⁵⁵

1.2.5.3 Natural biological substances

The use of cytokines involved in regulation of the immune system has already been discussed. Despite their exquisite molecular specificities, as mentioned only poor activities have been observed. The natural heterogeneity of each group of cytokines is negated by the use of pure recombinant products, and the relative efficacy of mixtures versus the cloned products remains to be determined. It is possible that the "dirtier" response to BCG is to our benefit. Despite these apparent setbacks a number of recombinant interferons, interleukins, colony stimulating factors and tumour necrosis factor have entered clinical trials (ref. 2, pp275-282).

1.2.5.4 Synthetic drugs

The number of low molecular weight synthetic compounds that have also shown immunomodulatory properties, thought to be responsible for their observed antitumour activities, covers a heterogeneous group of structures. They range from the large double-stranded polyribonucleotides such as poly A:U (polyadenosine-polyuridine) and poly(I,C)-LC (polyinosinic acid-polycytidylic acid complexed with poly-L-lysine); to the carbamoyl aziridine, azimexone (15) (MW=194).

Some examples of well known and tested small molecule immunostimulants are shown in Figure 1-5, and a summary of their main effects on the immune system is collated in Table 1-1. Interestingly, some conventional cytotoxic drugs have also demonstrated immunoenhancing activity, including the alkylating agents uracil mustard (17) and cyclophosphamide (18).⁵⁶

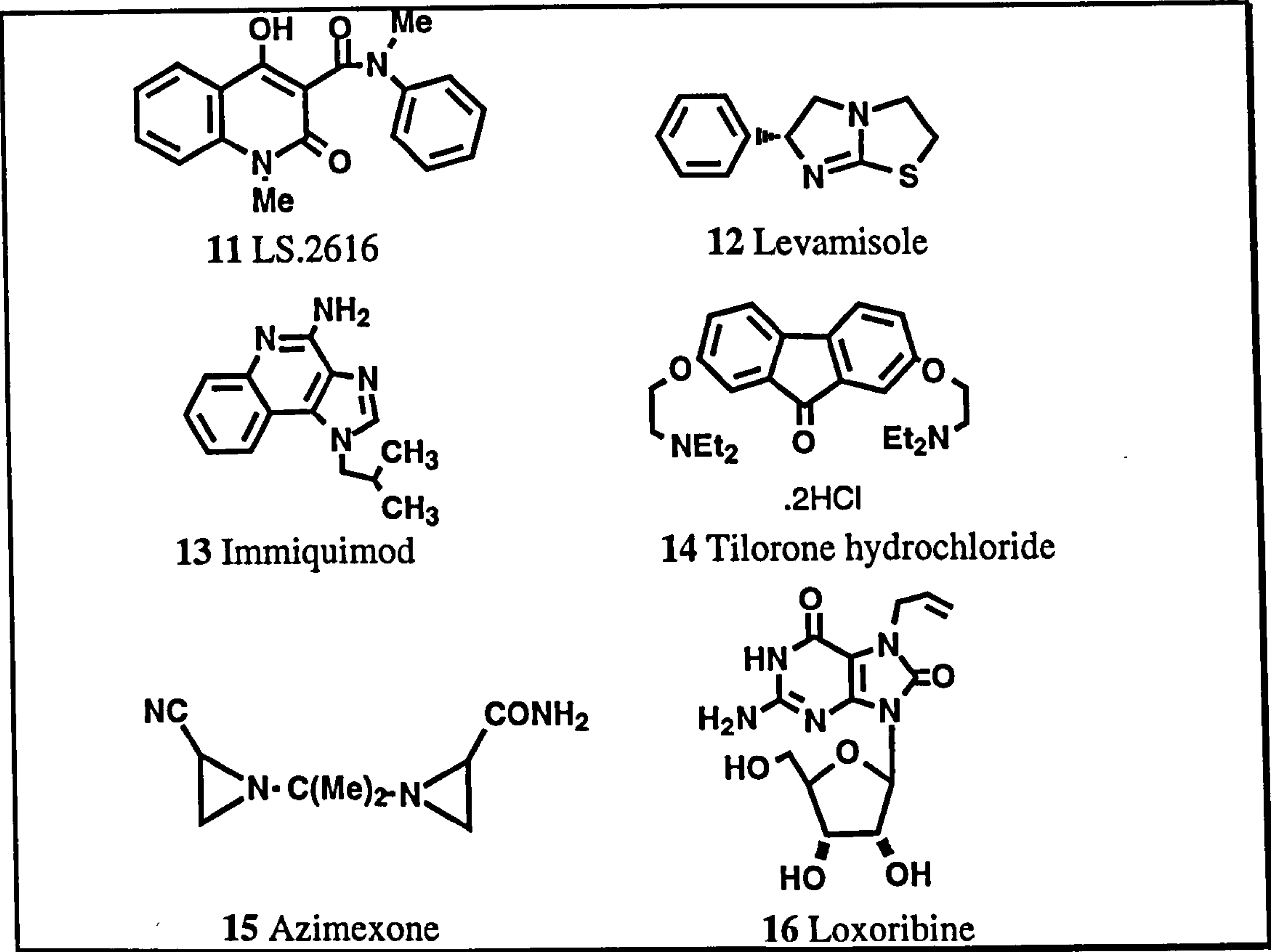
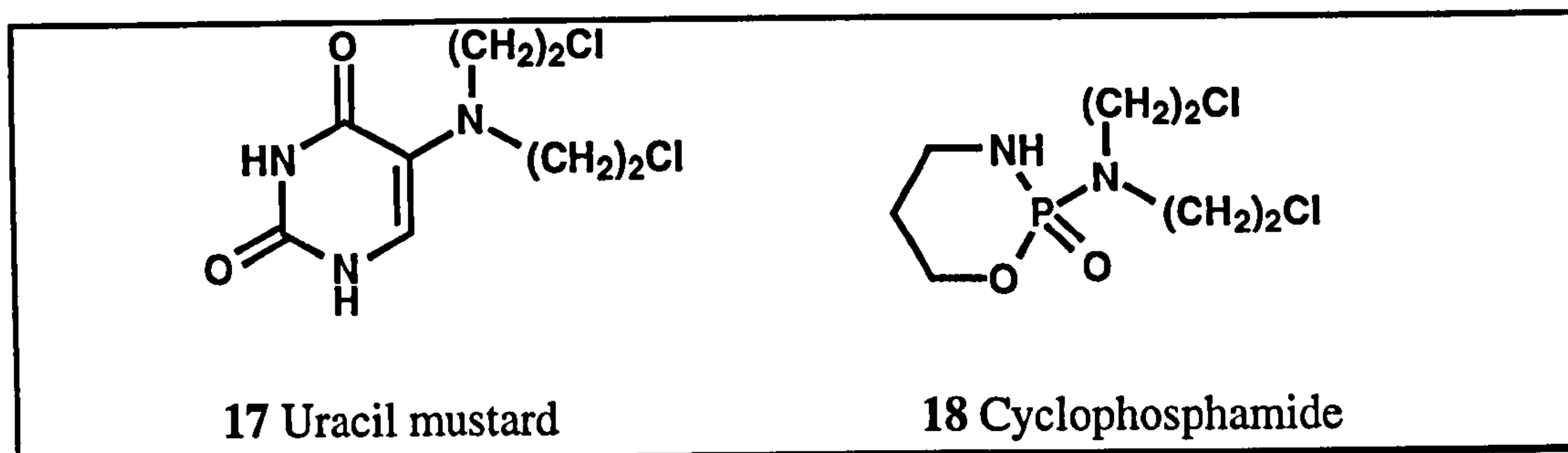


Figure 1-5 Small molecule immunomodulators.

Agent	Immunomodulation	Reference
LS-2616	NK and T cell activation	57
Levamisole	Lymphokine production Antibody formation	58
Tilorone hydrochloride	IFN induction	59
Immiquimod	IFN induction	60
Azimexone	CSF production	2
Loxoribine	NK and B cell activation	61

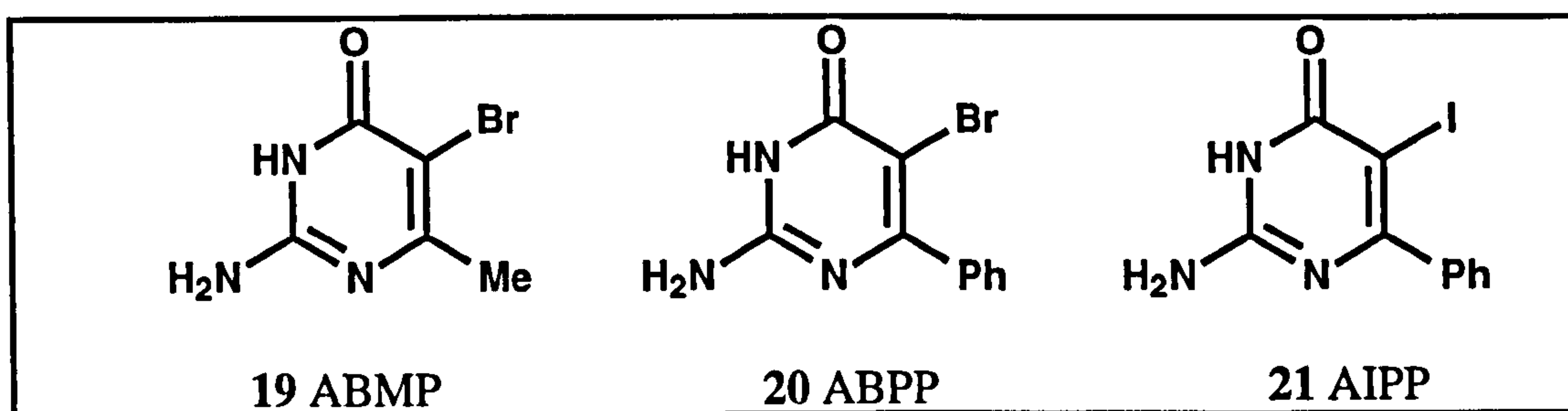
Table 1-1. Major immunomodulatory effects of representative agents.



To date, although the major effects of many of these agents on the immune system have been described, the mechanisms of action are not understood. Of the variety of synthetic structures possessing immunomodulatory activity, only levamisole (12), has demonstrated great clinical utility - as adjuvant to surgical resection in patients with stage B2 or C colon carcinoma, in combination with 5-fluorouracil.⁶²

1.2.5.5 Aminopyrimidinone immunomodulators

As well as immunomodulatory guanosine derivatives such as loxoribine (16), a number of simple 9-alkyl guanines such as 9-hexylguanine have shown antiviral activity thought to be due to immune system potentiation, suggesting the carbohydrate moiety is not required for activity.⁶³ Moreover, previously in 1976 a simple pyrimidinone, 2-amino-5-bromo-6-methylpyrimidin-4(3*H*)-one (19, ABMP) was reported to have antiviral properties and shown to be an interferon inducer in mice.⁶⁴ Unfortunately this compound suffered from unacceptable renal toxicity in certain animal models. The second generation of pyrimidinones to show equal or improved interferon induction and antiviral activity, without the renal toxicity, were the 2-amino-6-phenylpyrimidinones.⁶⁵ The most active were the 5-bromo (20) and 5-iodo (21) derivatives, ABPP (or bropirimine) and AIPP respectively.



ABPP (previously synthesised by Brown and Stevens⁶⁶) was a more potent interferon inducer than ABMP in a range of animals tested. Despite being a weak interferon inducer, AIPP showed equal or better antiviral activity than ABPP, suggesting that the antiviral activity of the 6-phenylpyrimidinones is only partly mediated by the endogenous production of interferon.⁶⁷

In the initial report, several analogues were also prepared, and it was found that the free 2-amino and 4-oxo functions were essential for activity.⁶⁴ Since then other analogue studies have been undertaken varying substitution in the phenyl ring, and replacing the 6-aryl group with heteroaryl groups; other halogens and alkyl groups have been tried in the 5-position.^{65,68,69} Certain constraints for activity have been found such as the 5-substituent must be no smaller than chloro nor larger than propyl, and activity is diminished with para-substituted phenyl groups in the 6-position. No compound showed significantly greater activity than ABPP or AIPP, but some were comparable such as ABmFPP (2-amino-5-bromo-6-(3-fluorophenyl)pyrimidinone). Again there was generally no correlation between interferon induction and antiviral activity.

Further studies with animal tumour models have generally been confined to ABPP, AIPP and ABmFPP - most frequently ABPP with its good oral activity. Broad ranging activity has been noted in several tumour types. For instance,

ABPP as single agent treatment of B16 melanoma in mice; and ABmFPP in combination treatment of P388 Leukaemia in mice with cyclophosphamide.^{70,71} More recently, ABPP has shown activity against murine renal cell carcinoma and rodent prostate cancers.^{72,73} ABPP has also shown activity in human tumours: a recently completed phase II trial in the treatment of carcinoma in situ of the bladder was effective in treating approximately 50% of patients, including those who had previously failed BCG therapy, giving ABPP the potential to replace BCG as frontline therapy because of its ease of administration and low toxicity.⁵⁵ Other indications include upper urinary-tract carcinoma in situ.⁷⁴

The mechanism of action of bropirimine is not known, and its effects on the immune system are wide ranging. Those effects that have been elucidated include the stimulation of NK cells; activation of macrophages; polyclonal B-cell activation; enhancement of antigen-mediated antibody formation; bone marrow proliferation as well as (and possibly involving) the induction of IFN- α , IL-1 (*in vitro*), IL-2 and TNF- α .^{75,76}

A clue to the possible mode of action of bropirimine is provided by its crystal structure, where two molecules are triply hydrogen bonded in the manner of a Watson-Crick cytosine:guanine base pair, with one bropirimine as the 3*H*-tautomer (guanine-like) and the other as the 1*H*-tautomer (cytosine-like) (Fig. 1-6).⁷⁷ This ability to exist as either tautomer could allow bropirimine to recognise guanine or cytosine residues in single-stranded DNA in classical Watson-Crick terms, or to recognise these bases in the major groove of double-stranded DNA by Hoogsteen encounters.

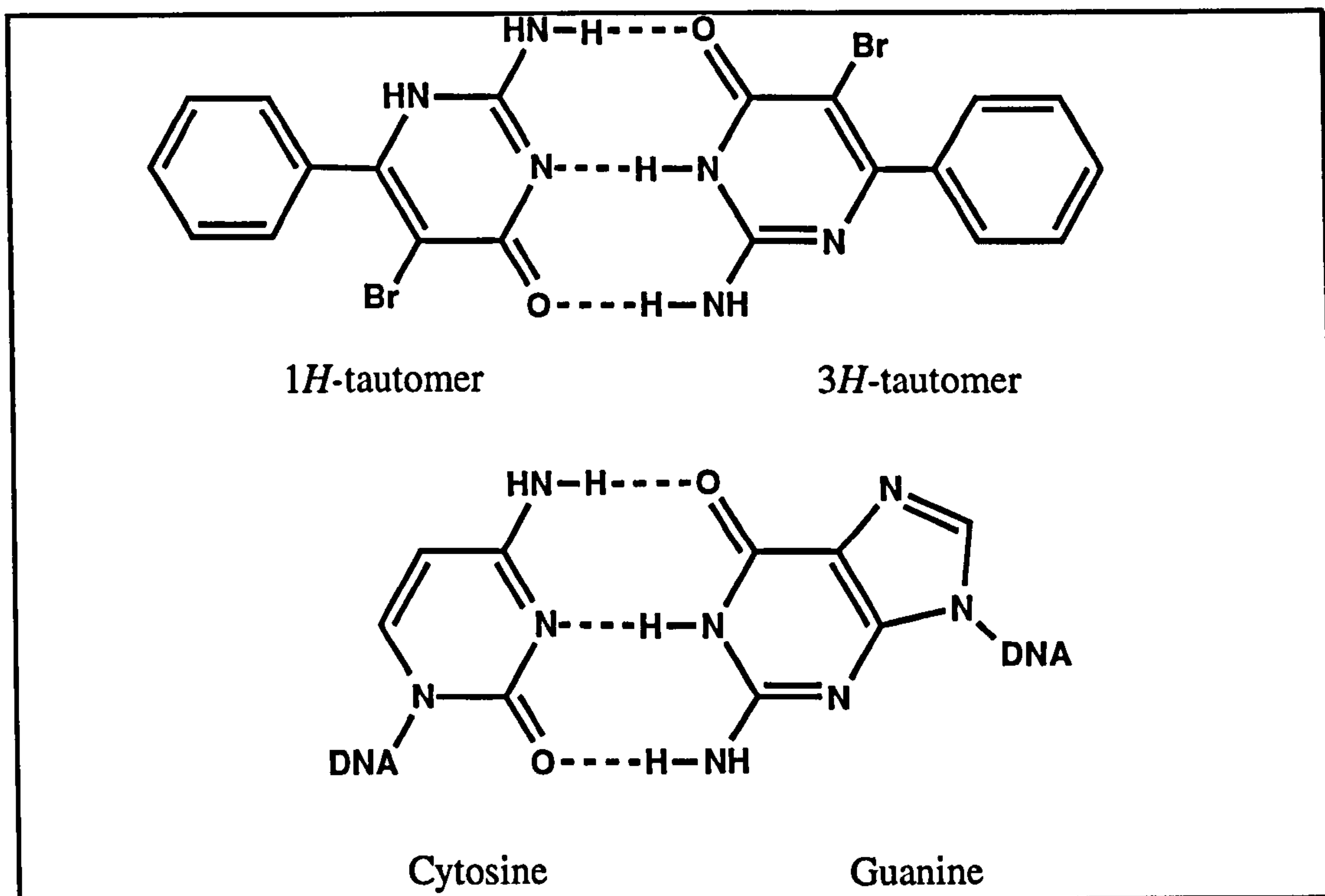


Figure 1-6 "Base-pair" in crystal structure of bropirimine, with a cytosine-guanine Watson-Crick base pair for comparison.

The structural similarity between the bropirimine "base-pair" and the interferon inducer poly(I,C) mentioned above is very striking. However, studies of the structurally similar 9-alkylguanines, and guanosine analogues, with their comparable biological profiles, have implicated so-called G-proteins (cell membrane, guanine-nucleotide binding proteins) as the site of action.⁶³

In answer to the question posed at the beginning of this section, it seems unlikely that any one approach could tackle the collection of diseases that are grouped under the heading "cancer". Far from being dead however, chemistry is becoming increasingly important in probing the mechanisms of this disease at the molecular level. To tackle this apparently complex disease it seems likely that a

combination approach, without prejudice to scientific discipline, will be most effective.

1.3 The role of immunomodulators

In comparison to the impact of cytotoxic drugs, immunotherapy has yet to provide the quantum leap expected in the treatment of cancer, but research in this area is continuing. It is clear that immunomodulators are most effective with low tumour burdens, and given that the smallest currently clinically detectable tumours are of the order of 1 cm^3 , with about 10^9 cells, immunomodulators are unlikely to be effective in eradicating disease alone. The immune status of the host is very important, however, if we are to eliminate every tumour cell to prevent relapse. The optimal setting for immunomodulators is likely to be post-operative, destroying residual tumour cells and perhaps preventing the establishment of metastases, in combination with conventional cytotoxic drugs. At this point it is noteworthy to observe the often synergistic effect observed in the combination of immunomodulators and cytotoxics. For example, the use of levamisole in combination with 5-fluorouracil in the treatment of resected human colon cancer gives a greater than additive effect on survival and remission rates than either agent alone.⁶² Several phase III studies are also looking at the use of 5-fluorouracil in combination with recombinant interferons.(ref. 2, pp. 277-282)

One of the difficulties in the development of small molecule immunomodulators is the lack of knowledge about the molecular targets, negating a rational drug design approach. *In vitro* tests generally fail to demonstrate activity, due to the importance of the host's immune system in bringing about a

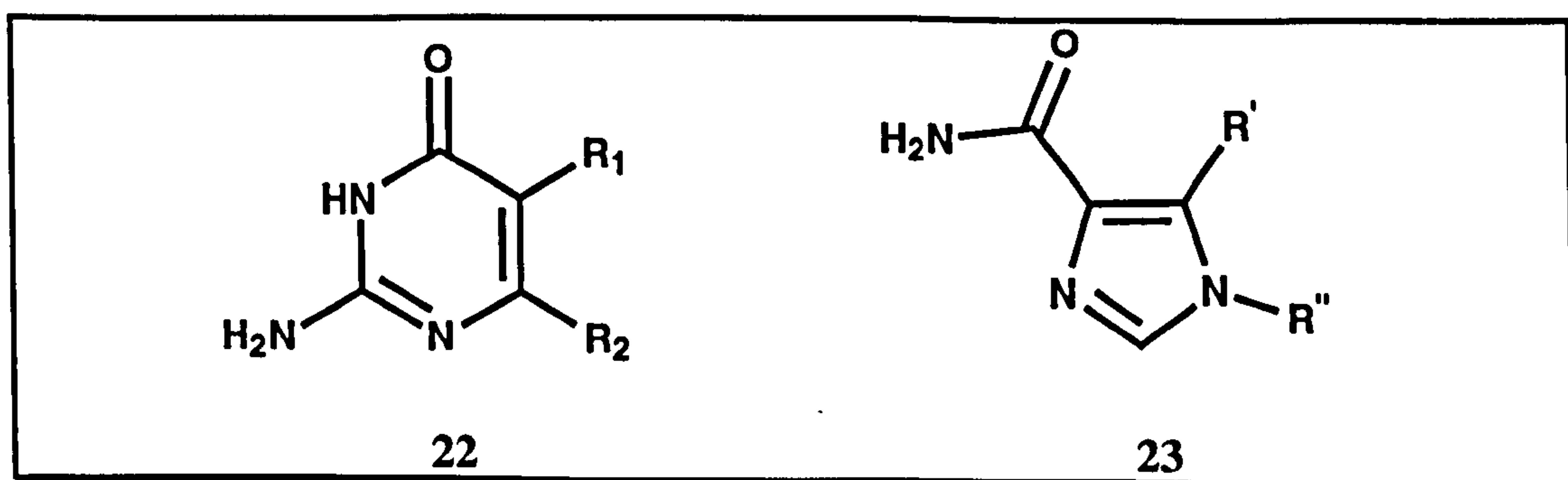
response. Many of the immunomodulators mentioned show *in vivo* activity in the growth and metastasis models of the B16 melanoma in mice (see for example refs. 57 & 70), making this a potential prescreen for new agents. Despite the difficulties, with the potential to harness the power of the body's own defences, such non-specific immunomodulation remains an attractive target for the medicinal chemist.

2. Aims and Objectives

In this chapter the main aims of the experimental work are presented.

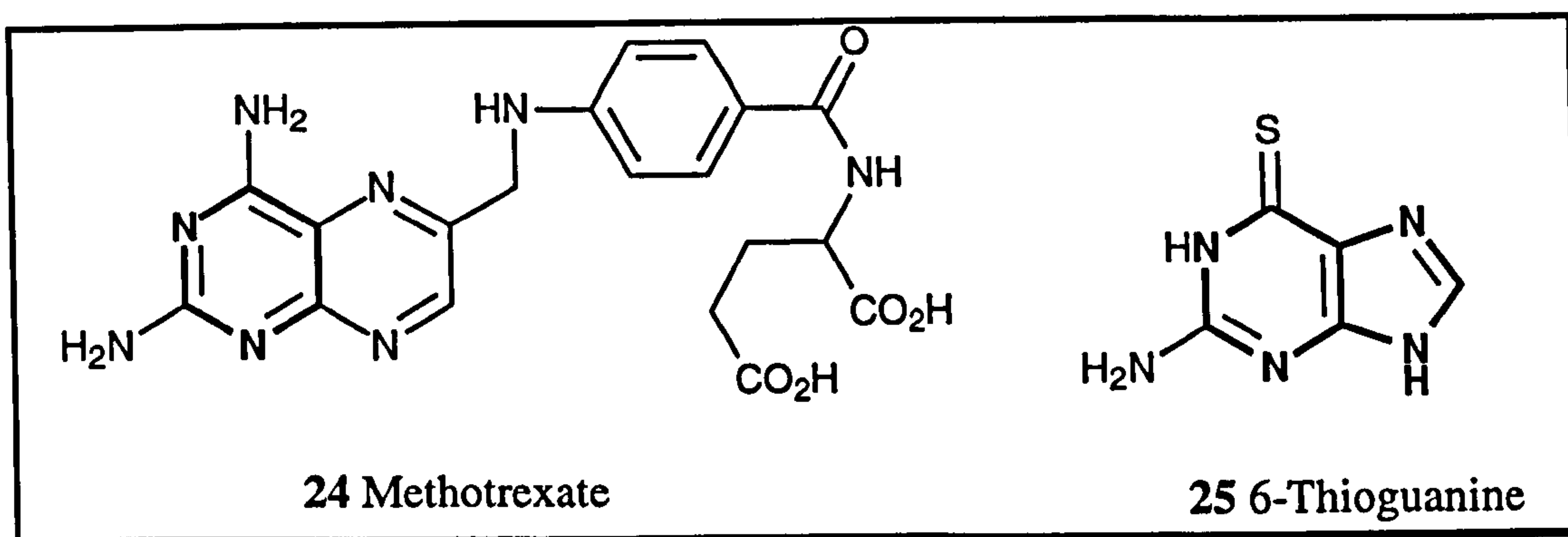
2.1 Pyrimidines and imidazoles

The chemistry of pyrimidines and imidazoles is long established and wide ranging, having been dealt with in a number of texts.⁷⁸⁻⁸² In this work, investigations were restricted to a small class of each type of compound - the aforementioned 2-amino-4-pyrimidinones **22**, and the functionally similar imidazole-4-carboxamides **23**. The pyrimidinones may be regarded as endocyclic-amides, and the imidazolecarboxamides as exocyclic-amides, both with similar intermolecular hydrogen-bonding abilities through the amide groups and two other nitrogen atoms (though not necessarily with the same ratio of hydrogen-bond donors to acceptors).



The imidazole and pyrimidine nuclei, as well as being essential building blocks of living organisms, are important pharmacophores, present in a wide range of biologically active compounds, both natural and synthetic. For example,

both imidazole and pyrimidine subunits feature in the current armoury of anticancer cytotoxics, including methotrexate (24) and numerous DNA base analogues such as 6-thioguanine (25). A number of the small-molecule immunomodulators considered in chapter one also contain one or both subunits (see Figure 1-5, Chapter 1). The aim of the present work was to investigate new chemistry of these systems, with the potential for the synthesis of novel, small molecule immunomodulants.



2.2 Chemistry of 2-aminopyrimidin-4-ones

As mentioned in section 1.2.5.5 it has been found that modifications at positions 1 to 4 of the pyrimidine ring results in loss of interferon induction and antiviral activity, although studies into variants at these positions - including alkylation and oxidation of the ring nitrogens - have yielded compounds with other biological properties including diuretic, hypotensive and anti-inflammatory activity.^{83,84}

More recently, electrophilic and nucleophilic reactions at the carbon atoms of 2-amino-6-arylpyrimidin-4-ones have been described.⁸⁵ The sites of

electrophile and nucleophile attack are depicted in Figure 2-1 upon 2-amino-6-phenylpyrimidin-4(3*H*)-one (26; APP), the inactive precursor of bropirimine (20), the lead compound in this class.

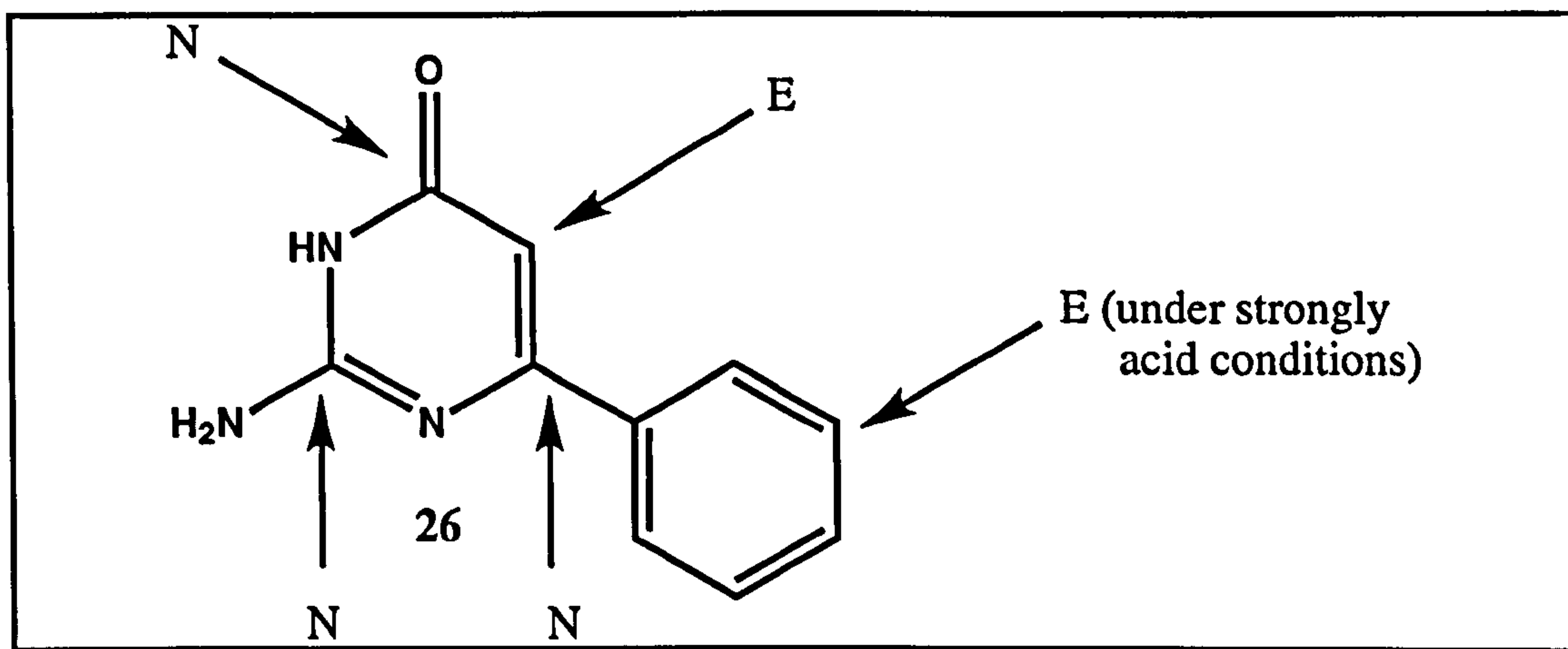


Figure 2-1 Sites of electrophilic and nucleophilic attack on APP, 26.

(E=electrophile; N=nucleophile)

The free pyrimidinones are amphoteric in nature, bropirimine (20) for example ionising to a stabilised anion 20a in alkaline solution, and protonating to a stabilised pyrimidinium ion 20b in acidic solution. The pK_a values for 20b and 20 are 3.18 and 8.53 respectively (Figure 2-2).⁸⁵

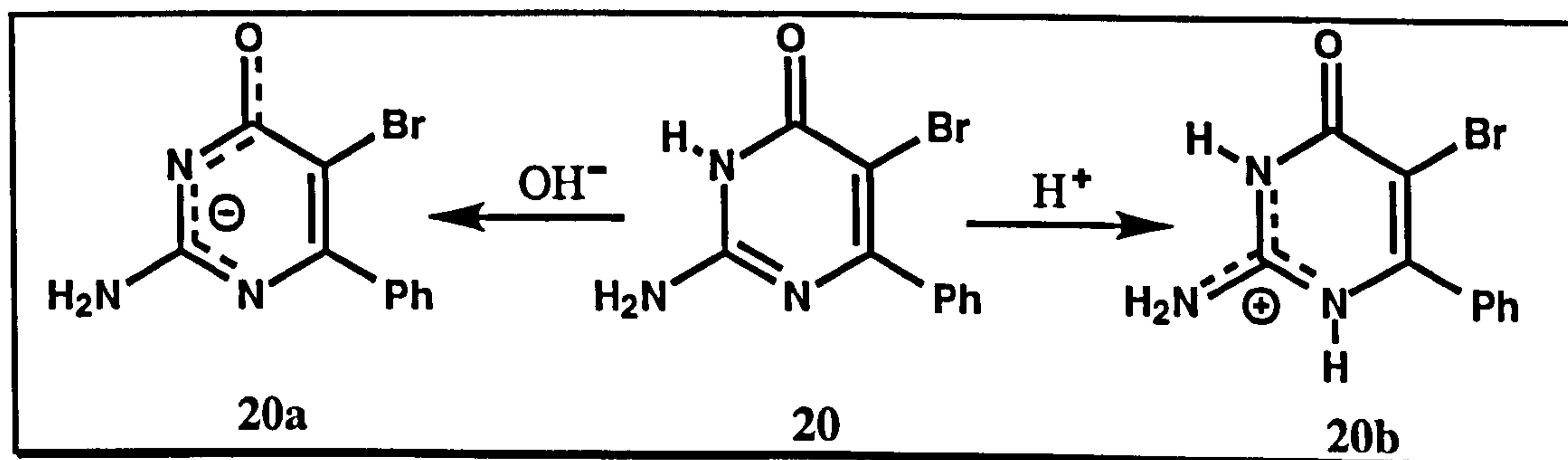


Figure 2-2 The amphoteric nature of bropirimine, 20.

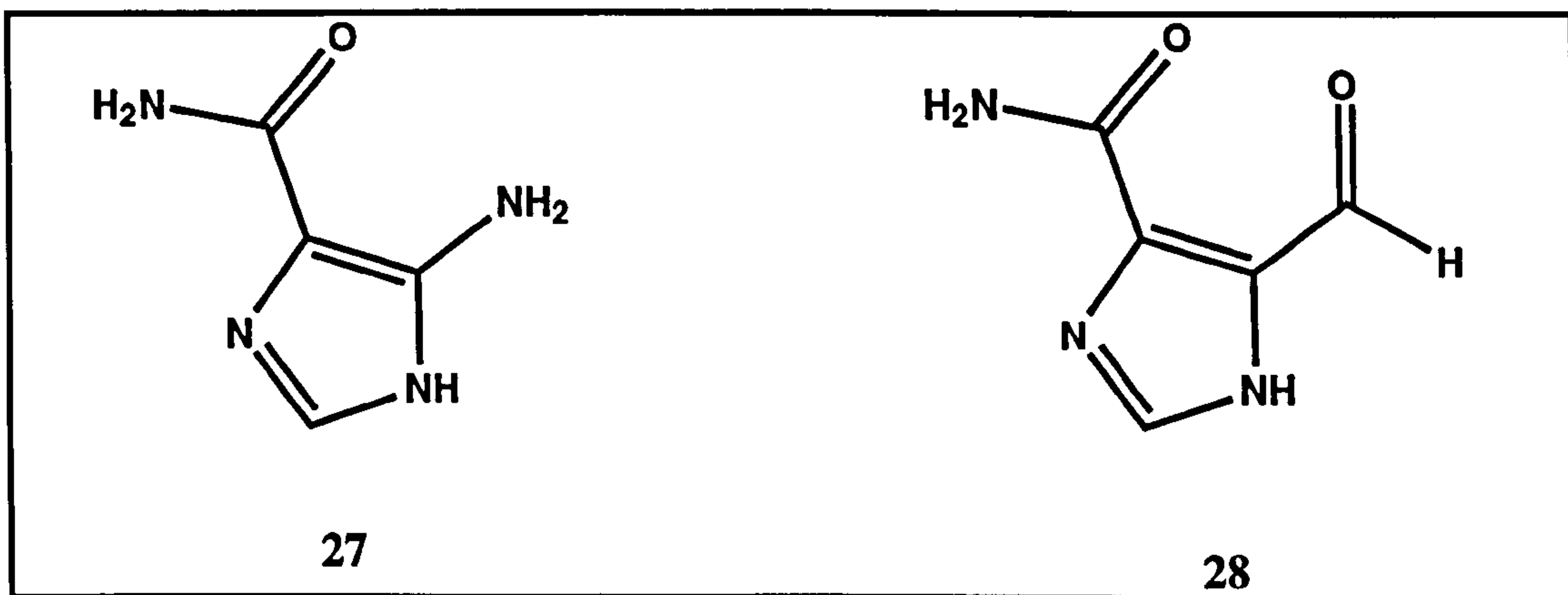
In this work it was decided to retain the basic 2-aminopyrimidin-4-one structure **22**, instead looking at the chemistry of positions 5 and 6 of the pyrimidine ring, to develop novel substitution patterns for R₁ and R₂, including the formation of fused rings with the pyrimidine. An important consideration in the development of pyrimidinone derivatives is their inherent poor aqueous solubility, the formation of Watson-Crick-like pairs in the solid state exposing only hydrophobic groups to an aqueous environment. For example, the aqueous solubility of **20** is less than 40 µg/ml.⁸⁵ The molecular targets of this research will be elaborated in chapter 4.

2.3 Chemistry of imidazole-4-carboxamides

As with studies of the pyrimidinones, the basic structural motif of imidazole **23** was retained, preserving the intermolecular bonding abilities. Studies centered upon varying the substituents R' and R'', again with the intention of developing novel chemistry, including the elaboration to fused ring structures.

Unlike the "π-deficient" aminopyrimidines, the reactions of "π-excessive" imidazoles are characterised by electrophilic attack at carbon and nitrogen - as exemplified by their more basic character, for example the pK_a of protonated imidazole is 6.95.⁸⁶ Free imidazoles are also amphoteric in nature, ionised by strong bases to form a resonance stabilised anion. The pK_a of free imidazole is 14.52⁸⁶ - a weaker acid than bropirimine. The acidity and basicity of imidazoles however, are very sensitive to their ring-substitution pattern. For example, the pK_a's of 5-formylimidazole are 2.90 and 10.66 for the protonated and neutral forms respectively.⁸⁶

In the next chapter will be described the investigations into reactions of 5-aminoimidazole-4-carboxamide (27), studying substitution and condensation reactions; and of 5-formylimidazoles such as 28, studying their synthesis and condensation reactions.

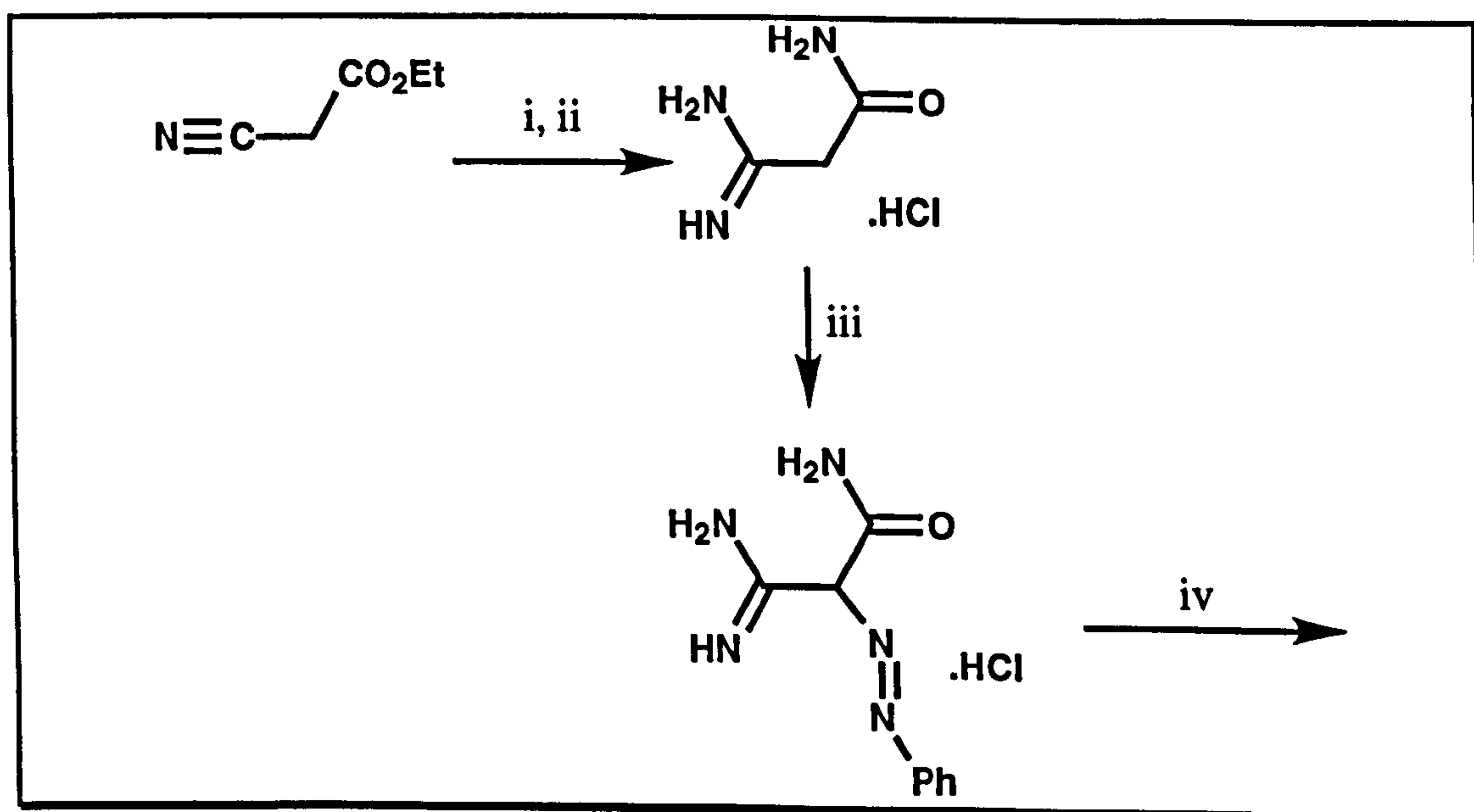


3. Imidazolecarboxamides

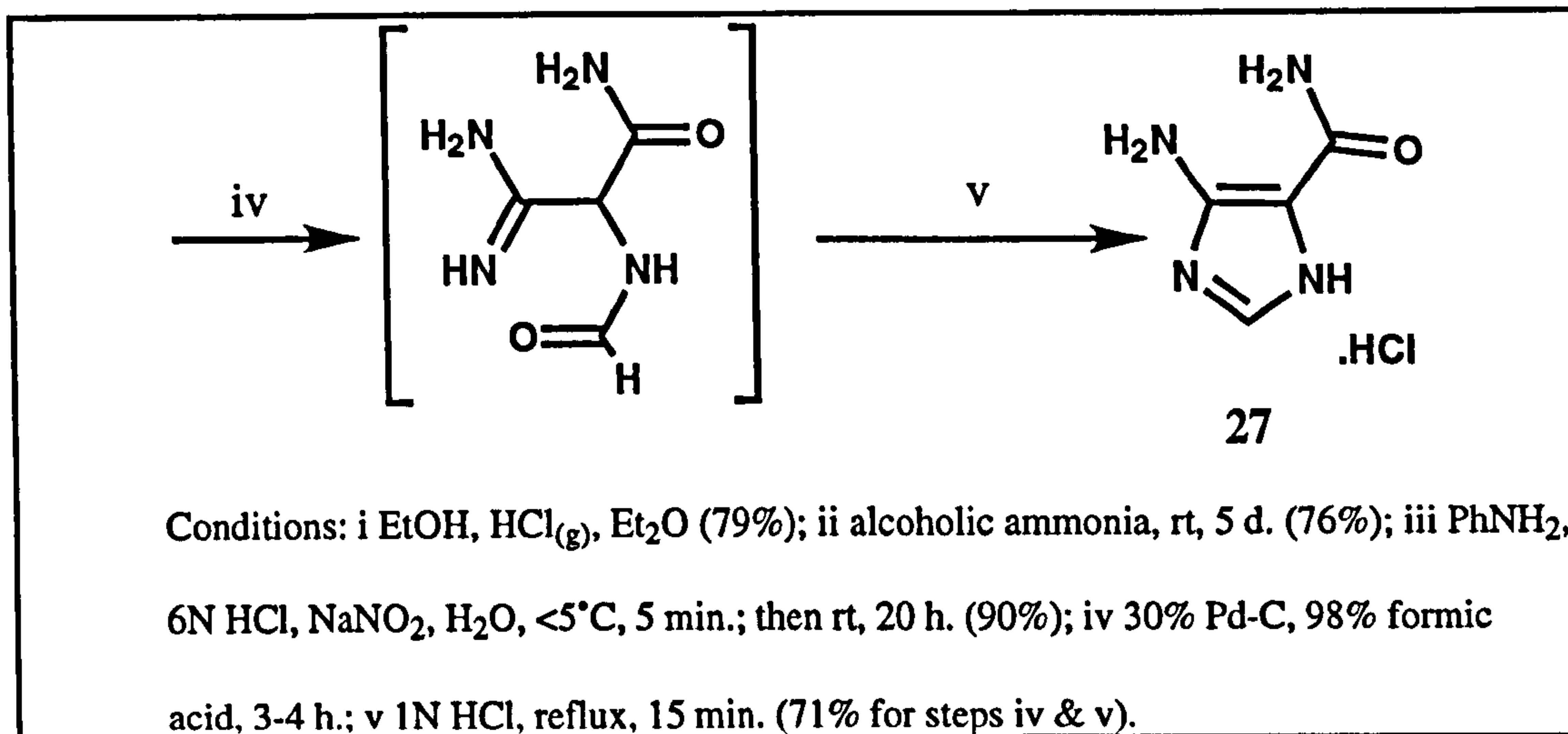
A number of target imidazolecarboxamides were investigated, and each will be considered separately.

3.1 Reactions of 5(4)-aminoimidazole-4(5)-carboxamide

5(4)-Aminoimidazole-4(5)-carboxamide (AIC, 27) was first isolated from a culture of *Escherichia coli* (*E. coli*) during sulfonamide bacteriostasis.⁸⁷ It has been shown to be the precursor of purines in the *de novo* synthesis of nucleic acids.⁸⁸ Now commercially available, AIC has been synthesised by a number of routes, most commonly as the hydrochloride salt (AIC.HCl). An example is the procedure of Shaw and Woolley,⁸⁹ later modified by Montgomery and coworkers⁹⁰ for large scale preparations, starting with ethyl cyanoacetate (Scheme 3-1).

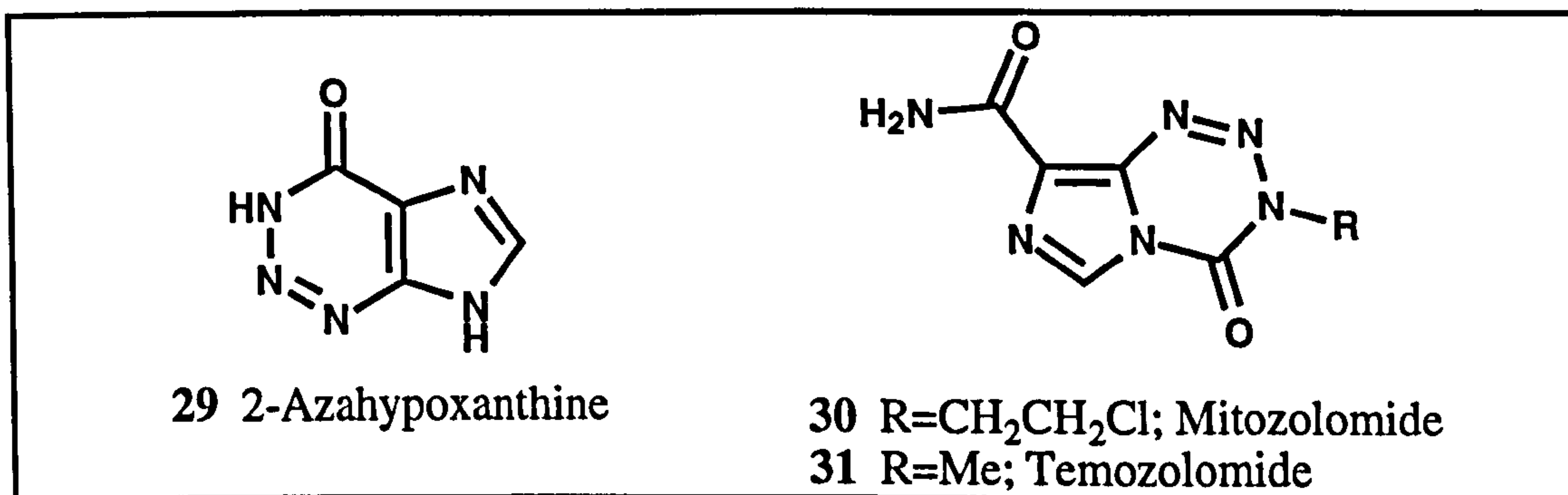


Scheme 3-1 Synthesis of AIC.HCl, 27 (continued overleaf).



Scheme 3-1 Synthesis of AIC.HCl, 27.

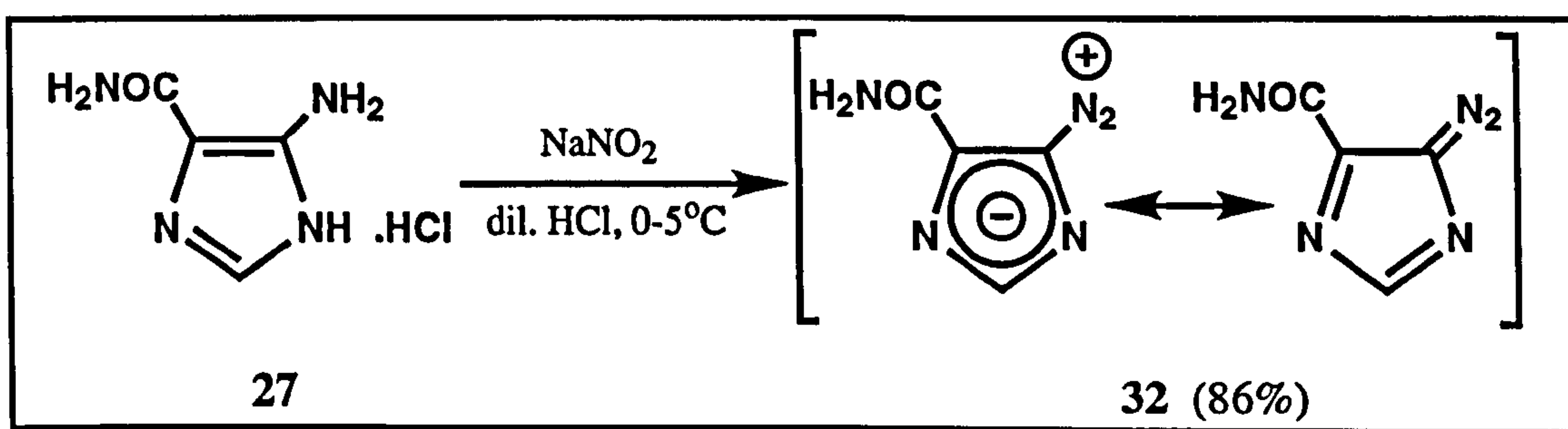
AIC has been used as the starting material for a number of important compounds including purine analogues such as the cytotoxic 2-azahypoxanthine⁹¹ (29) and the antitumour imidazotetrazinones mitozolomide⁹² (30) and temozolomide⁹³ (31).



In this programme, the first reactions of 27 that were investigated involved substitution of the amino group:

3.1.1 Rhodium carbenes

A number of synthetic strategies have employed the internal diazonium salt 5-diazoimidazole-4-carboxamide (**32**) as a common intermediate, by coupling to the terminal nitrogen of the diazo group with a nucleophile. For example, reaction of **32** with the appropriate isocyanates yields the imidazo-tetrazinones **30** and **31**.⁹⁴ In this programme **32** was synthesised on a moderate scale (35g) according to the literature,⁹⁵ in good yield (Scheme 3-2).



Scheme 3-2 Synthesis of 5-diazo-(5*H*)-imidazole-4-carboxamide, **32**.

The transition-metal catalysed carbene reactions of diazo-compounds are well established and have been reviewed.⁹⁶ Employing metals such as rhodium, nickel and copper, reactions such as cyclopropanations, additions to aromatic rings, formation of ylids, atom abstractions and bond insertions can be performed. For example, ethers, thioethers and amines have been formed by reaction of diazo-compounds with alcohols, thiols and amines respectively, in the presence of rhodium(II) acetate.⁹⁷ Procedures are most effective for α -diazocarbonyls,⁹⁶ though sulfonyl and phosphonyl groups are also tolerated α to the diazo group.⁹⁷ The generally accepted mechanism is shown in Figure 3-1 (with a rhodium(II) catalyst), though whether reaction involves a discrete carbene, or a metal-bound carbenoid remains unclear.

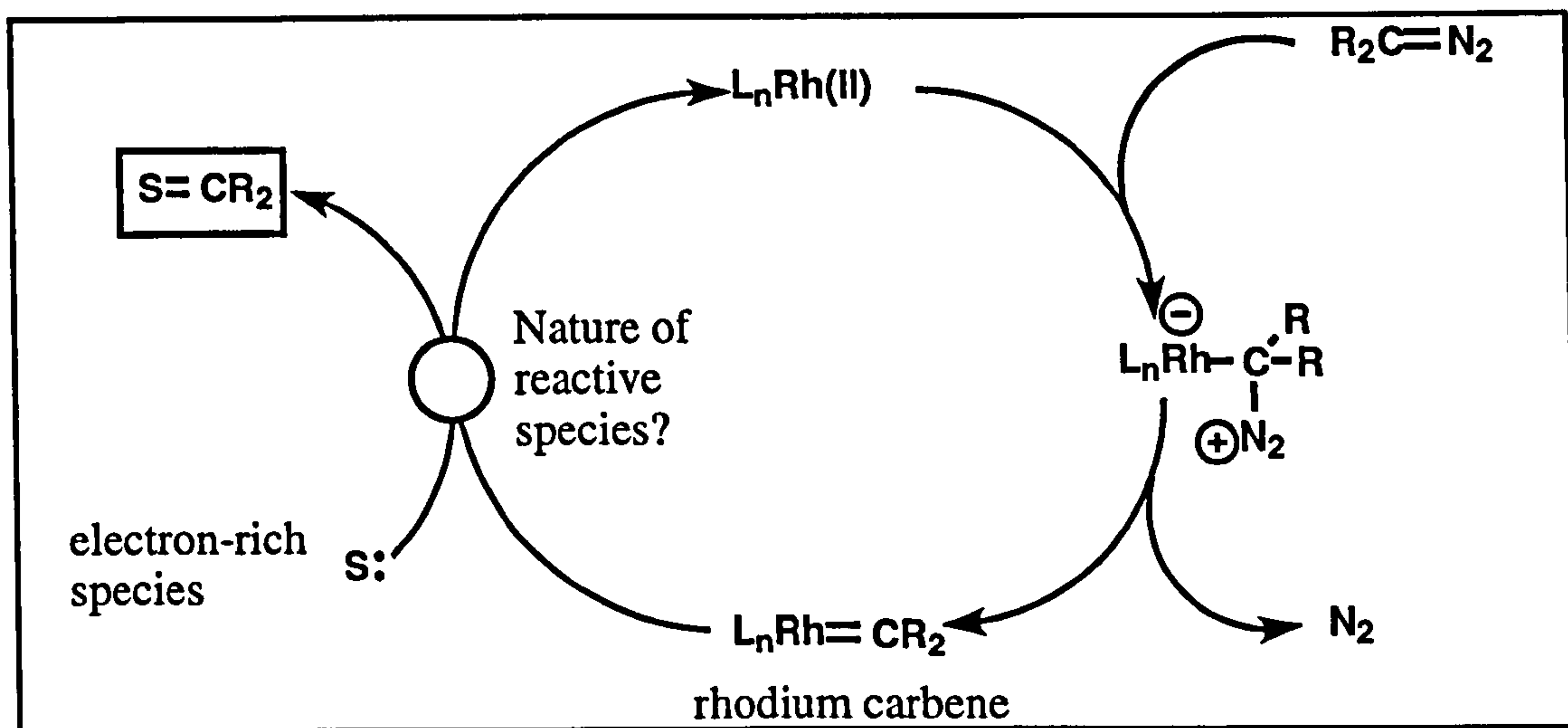
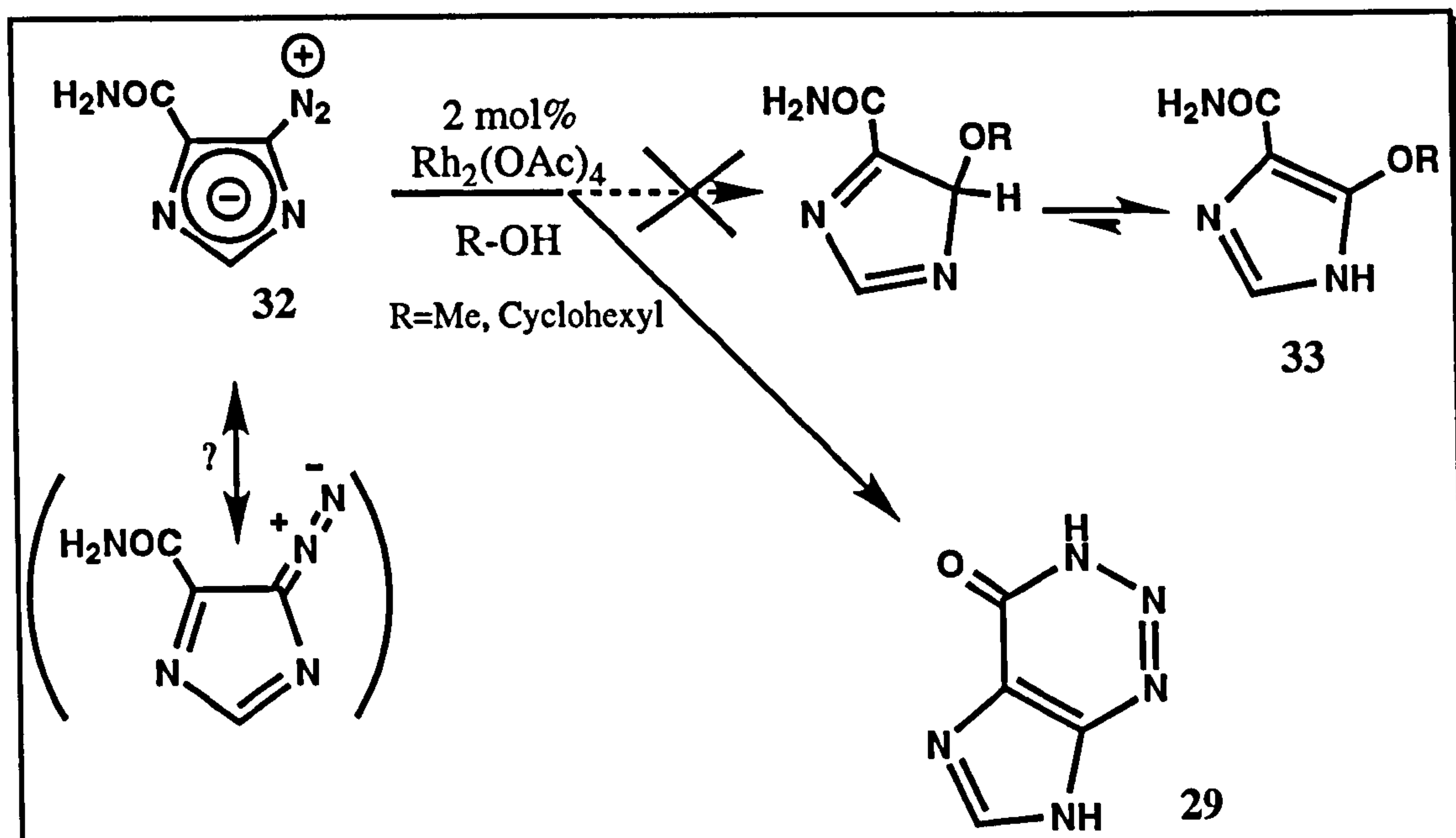


Figure 3-1 Mechanism of metal catalysed carbene formation from diazo-compounds.

It was of interest to determine whether the diazoimidazole **32** could also undergo carbenoid reactions in the presence of a metal catalyst. A number of attempts were made to prepare ethers (**33**) by reaction of diazoimidazole **32** with 2 mol.% rhodium(II) acetate and two equivalents of an alcohol, according to a corresponding literature procedure for transformation of α -diazocarbonyls.⁹⁷ In these studies, instead of using dichloromethane as solvent (as quoted), to counteract the poor solubility of **32**, reaction with methanol was performed in tetrahydrofuran (THF) at room temperature; and reaction with cyclohexanol was performed at room temperature in dimethylsulfoxide (DMSO), and at reflux in THF, employing Soxhlet extraction to introduce **32** slowly to the reaction mixture. Unfortunately, no ether was formed under any of the conditions used and no nitrogen evolution was observed. Instead, the cyclisation product of **32**, 2-azahypoxanthine (**29**) was produced almost quantitatively under all conditions studied, identical with an authentic sample by infrared and ^1H NMR spectroscopy (Scheme 3-3).



Scheme 3-3 Attempted Rh(II) catalysed carbene chemistry.

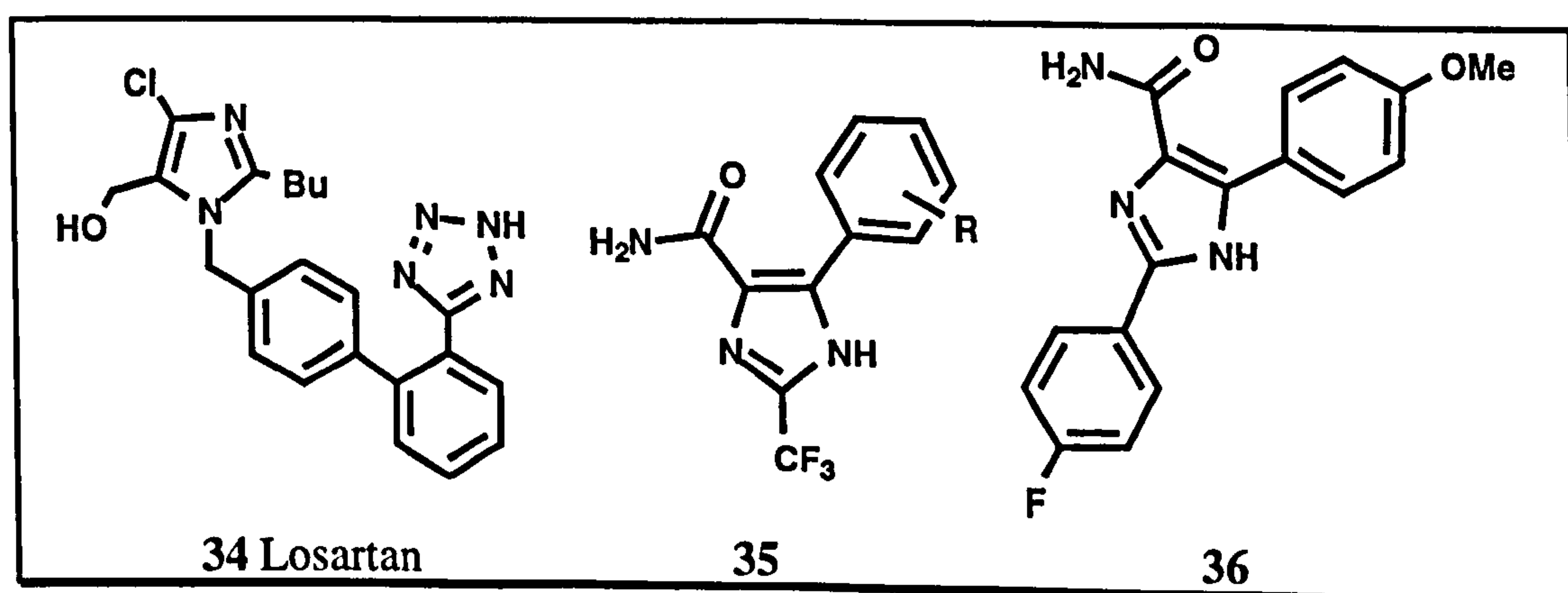
The transformation of diazoimidazole 32 to azahypoxanthine (29) is well known, and the kinetics of the process in aqueous media have been studied spectrophotometrically,⁹⁵ the mechanism presumed to involve nucleophilic attack of the amide nitrogen on the terminal nitrogen of the diazo group. The cyclisation is most rapid at alkaline pH, for example 32 cyclises in 0.1N sodium hydroxide with a half life of less than two minutes,⁹⁵ and it is possible that under the anhydrous conditions employed in our reactions, the alcohols are acting as weak base catalysts for the cyclisation process.

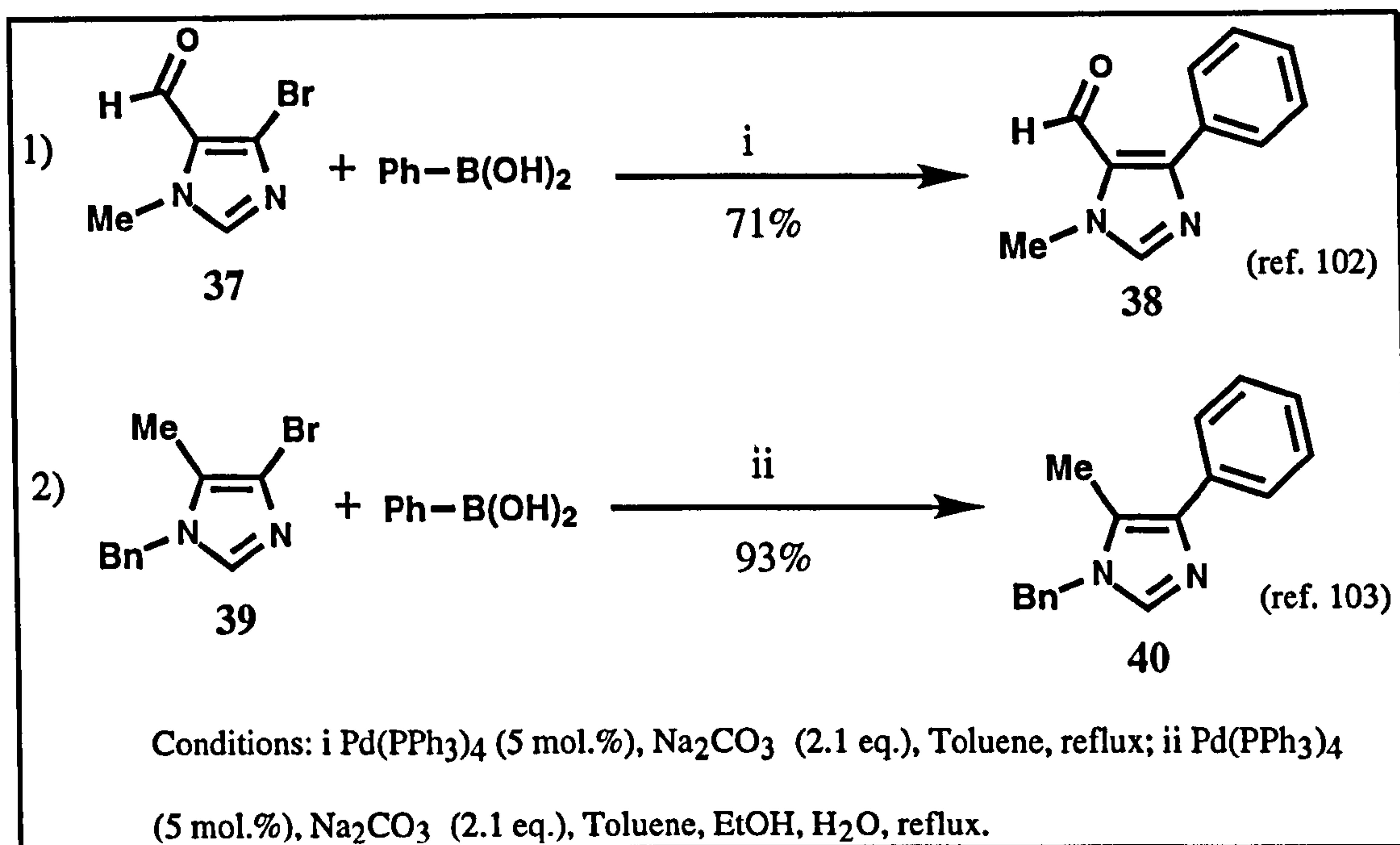
Only rhodium(II) acetate was employed in these studies, but different metals and different ligands, for example trifluoroacetamide, have also been used by researchers in this field - so suitable conditions may exist for this reaction. However, given the intrinsic instability of 32 under likely reaction conditions, this question was not investigated further, the reactivity of 32 more closely mirroring that of aromatic diazonium salts than α -diazocarbonyls.

3.1.2 Suzuki reactions

Since first reported, the palladium-catalysed coupling of a haloarene and an arylboronic acid has become the method of choice for the synthesis of unsymmetrical biaryls.⁹⁸ Moreover, the Suzuki reaction as it has become known, has been extended to a variety of other substrates for C-C bond formation. The scope of the Suzuki reaction will be discussed in more detail in the next Chapter, in the context of pyrimidine couplings.

Biaryl units are important pharmacophores, present in a variety of biologically active compounds. For example, the angiotensin II receptor antagonist losartan (**34**) contains a substituted biphenyl that has been constructed with the Suzuki coupling reaction.⁹⁹ The preparations of a number of biologically active imidazolecarboxamides with aryl substituents have been patented including a range of 5-aryl-2-(trifluoromethyl)imidazole-4-carboxamides (**35**) as pest control drugs,¹⁰⁰ and 2-(4-fluorophenyl)-5-(4-methoxyphenyl)imidazole-4-carboxamide (**36**) as an analgesic antiinflammatory agent.¹⁰¹ However, these aryl-imidazoles were not synthesised using the Suzuki reaction. In fact, there are only a few literature examples of haloimidazoles undergoing Suzuki coupling reactions (Scheme 3-4).





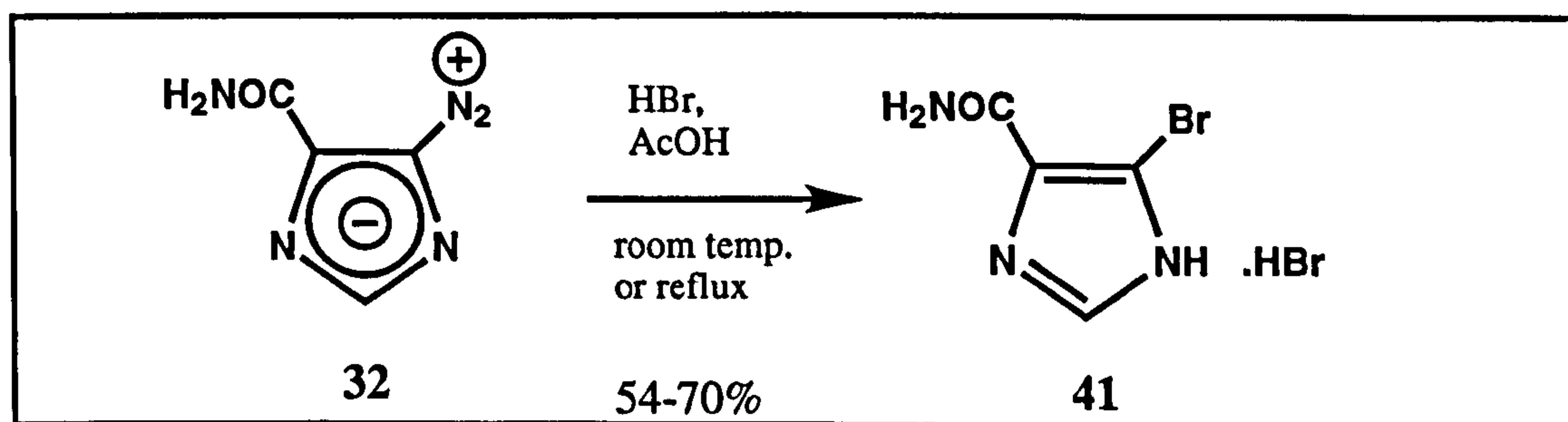
Scheme 3-4 Literature examples of Suzuki reactions upon bromoimidazoles.

To prepare a series of 5-arylimidazole-4-carboxamides first required the synthesis of a bromo- or iodoimidazole.

3.1.2.1 Bromoimidazoles

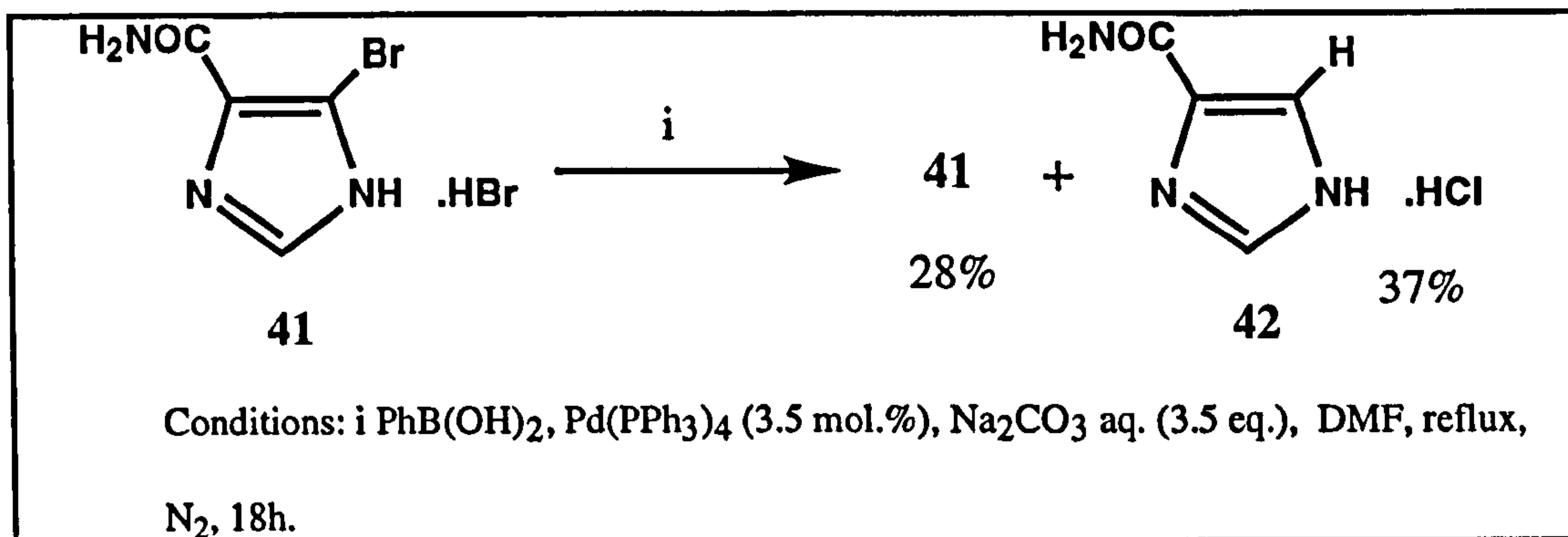
The synthesis of 5(4)-bromoimidazole-4(5)-carboxamide (**41**), as its hydrobromide salt, has been reported by a number of procedures from mitozolomide, **30**, or its diazo precursor, **32**.¹⁰⁴ From **30**, boiling in acetic acid containing sodium bromide or hydrobromic acid gave **41** in 17 and 60% yield respectively, the reaction presumed to proceed via **32** formed *in situ*. Bromoimidazole **41** was also isolated in 60% yield when **32** was stirred with hydrobromic acid in acetic acid at room temperature.¹⁰⁴

In this programme, though reaction of **32** with sodium bromide in acetic acid was unsuccessful, bromoimidazole **41** was prepared in good yield (54 to 70%) by reaction of diazoimidazole **32** with hydrobromic acid in acetic acid, either at room temperature or at reflux, to give a golden crystalline solid with a melting point of 245 - 251°C (lit.,¹⁰⁴ 210°C). Despite the discrepancy in melting points, which may be due to polymorphism, the product thus prepared displayed satisfactory elemental analysis and ¹H and ¹³C NMR spectra consistent with the assigned structure (Scheme 3-5). Comparison with literature NMR data is not possible because only the 2-H signal was quoted, without the identity of the solvent used.¹⁰⁴



Scheme 3-4 Synthesis of 5-bromoimidazole, **41**.

Following a procedure found successful in the Suzuki coupling of an unprotected bromopyrrole,¹⁰⁵ bromoimidazole **41** was reacted with phenylboronic acid in the presence of tetrakis(triphenylphosphine)palladium(0) catalyst and aqueous sodium carbonate as the stoichiometric base (employing more to account for the hydrobromide salt of **41**) in refluxing *N,N*-dimethylformamide (DMF). However, along with unreacted starting material, only a debrominated by-product (**42**) was isolated (both as their hydrochloride salts) - identified by mass spectrometry and ¹H and ¹³C NMR spectra (Scheme 3-6).



Scheme 3-6 Attempted Suzuki reaction on bromoimidazole, **41**.

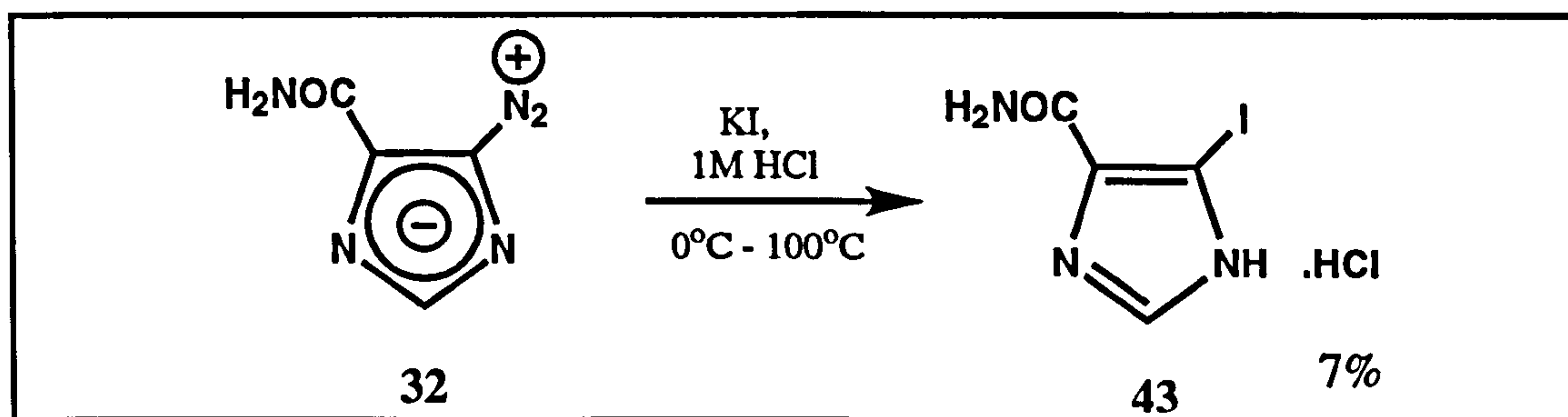
Imidazole **42** has been reported previously,¹⁰⁶ but no physical properties were reported. Imidazoles **41** and **42** proved to be inseparable by crystallisation or chromatography, but in the NMR spectrum, the resonances corresponding to **41** are easily accounted for. The remaining signals are readily assigned to **42**, and in the ¹H NMR spectrum, weak through bond coupling (~1 Hz) is observed between the 2-H and the 5-H protons.

3.1.2.2 Iodoimidazoles

It is widely accepted that in the Suzuki reaction, iodo substrates are more reactive than the corresponding bromo substrates, by virtue of increased reactivity with the catalyst (as will be explained in the next Chapter). In the same communication cited for the bromoimidazole **41** (ref. 104), 5(4)-iodoimidazole-4(5)-carboxamide (**43**) (as its hydroiodide salt) was prepared by refluxing mitozolomide (**30**) with sodium iodide in acetic acid. However no parallel chemistry with diazoimidazole **32** was described.

Efforts were made to prepare **43** by refluxing **32** with sodium iodide in both freshly distilled acetic acid and in dry DMF, but in both cases, as with the

bromide reaction, a dark mixture resulted, little or no nitrogen evolution was observed, and no products were isolated. Iodide **43** was eventually synthesised in low yield by reaction of **32** with potassium iodide in 1M hydrochloric acid (Scheme 3-7) - a method commonly used for the conversion of aromatic diazonium salts to their iodo derivatives.¹⁰⁷



Scheme 3-7 Synthesis of iodoimidazole from **32**.

Given the low yield in the synthesis of **43** and the difficulty in obtaining a pure sample, Suzuki reactions on this compound were not investigated. Also, given the lack of coupling reactivity of bromoimidazole **41**, and its propensity for dehalogenation to **42**, these reactions were not investigated further.

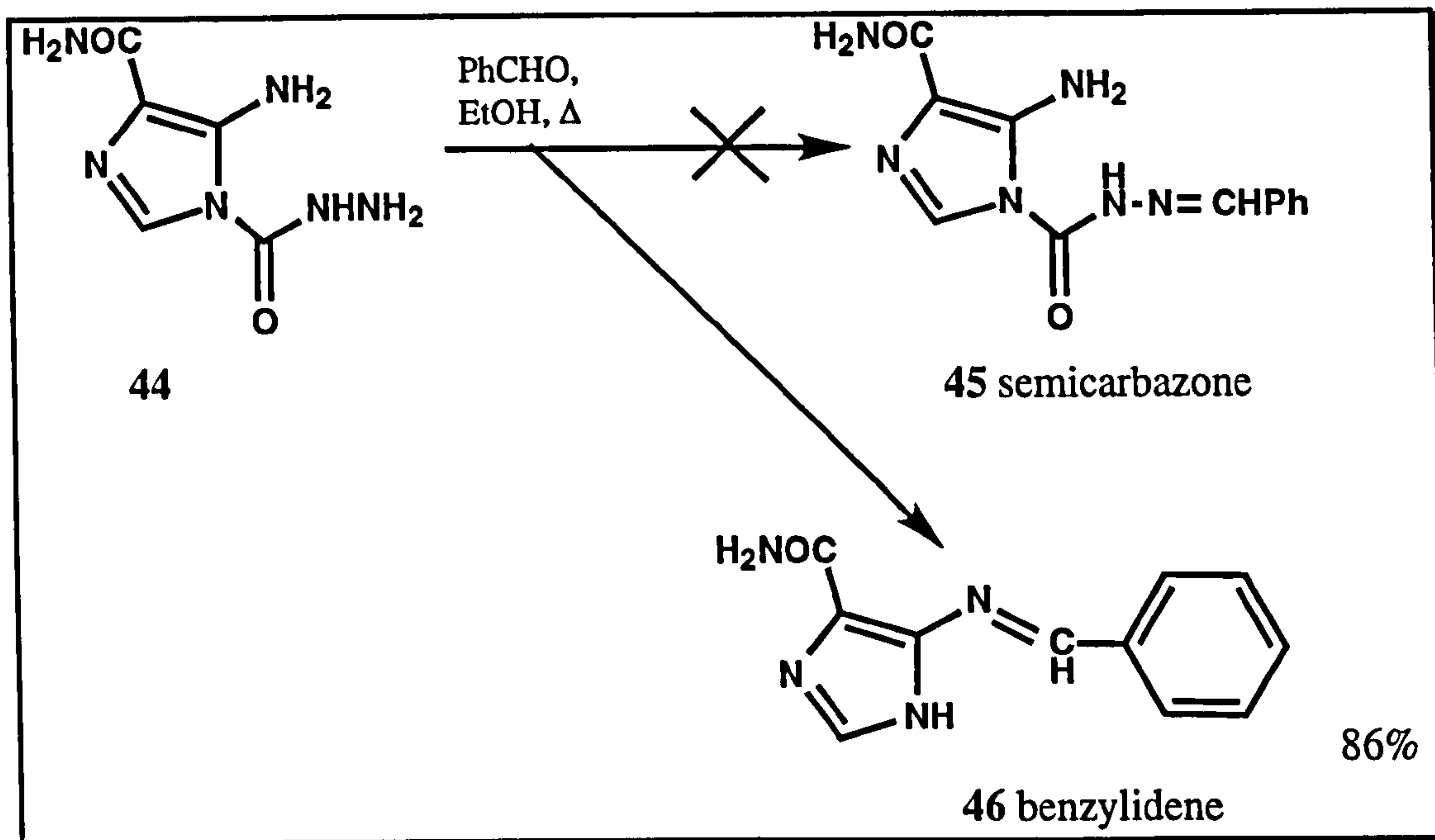
A feature of the Suzuki reaction is that the halide and boronic acid act as the electrophilic and nucleophilic coupling partners respectively. The 5-position of an imidazole is not a particularly electrophilic site, and under the basic conditions of the Suzuki reaction deprotonation of haloimidazoles **41** or **43** is likely to reduce the electrophilicity further still. In the absence of coupling reactivity, other more energetically favoured routes are followed, *i.e.* dehalogenation, a phenomenon noted only rarely in the literature, though an example is in the couplings of bromoimidazoles.¹⁰³ The literature imidazole coupling reactions mentioned above (Scheme 3-4) both involved *N*-protected

imidazoles, and future studies in this area could look at the coupling reactivity of protected derivatives of the bromo- and iodoimidazoles, **41** and **43**.

The second area of interest with AIC **27** concerned the possibilities of benzaldehyde condensations at the amino group:

3.1.3 Benzaldehyde condensations

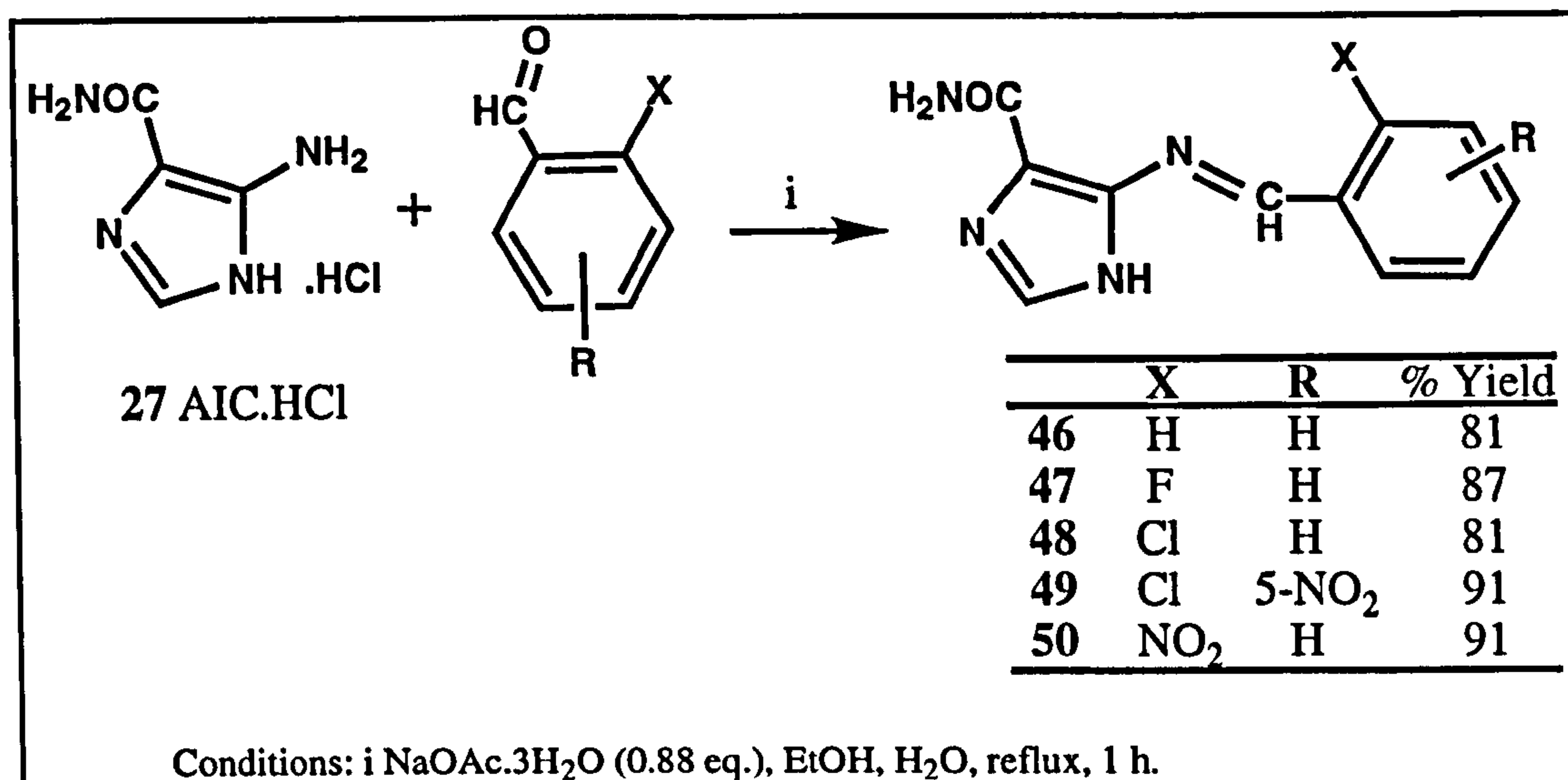
The amino group of **27** resides on an electron rich imidazole ring that makes few demands on the nitrogen lone pair, making it is a strong nucleophile, not only in the diazotisation already mentioned, but in reactions with a range of electrophiles. For example, Baig *et al* discovered that on reacting 5-amino-4-carbamoylimidazole-1-carbohydrazide (**44**) with benzaldehyde in refluxing ethanol, instead of the expected semicarbazone **45** (analogous to the product formed on reaction with acetone) the product formed was identified as the benzylidene derivative (**46**),¹⁰⁴ involving reaction at the 5-amino group (Scheme 3-8). Schiff's base **46** was also prepared in 95% yield in the reaction between **27** (AIC, as the free base) and benzaldehyde in refluxing ethanol.¹⁰⁴



Scheme 3-8 Reaction of aminoimidazole with benzaldehyde.

Instead of a synthesis employing the free base of **27**, one using the commercially available hydrochloride salt (AIC.HCl) was sought. Previous research in this group has shown simple reaction of AIC.HCl with benzaldehydes in refluxing ethanol to be unsuccessful; but that use of a catalytic quantity of sodium acetate, and reaction in aqueous ethanol furnished a range of Schiff bases in good yields.¹⁰⁸

In the present work, a reassessment of these conditions (which employed two equivalents of the benzaldehyde) has shown the use of equimolar quantities of imidazole **27** and benzaldehyde to be equally efficient. As well as the simple benzylidene derivative **46**, the previously unreported derivatives **47** to **50** were prepared in this manner (Scheme 3-9).

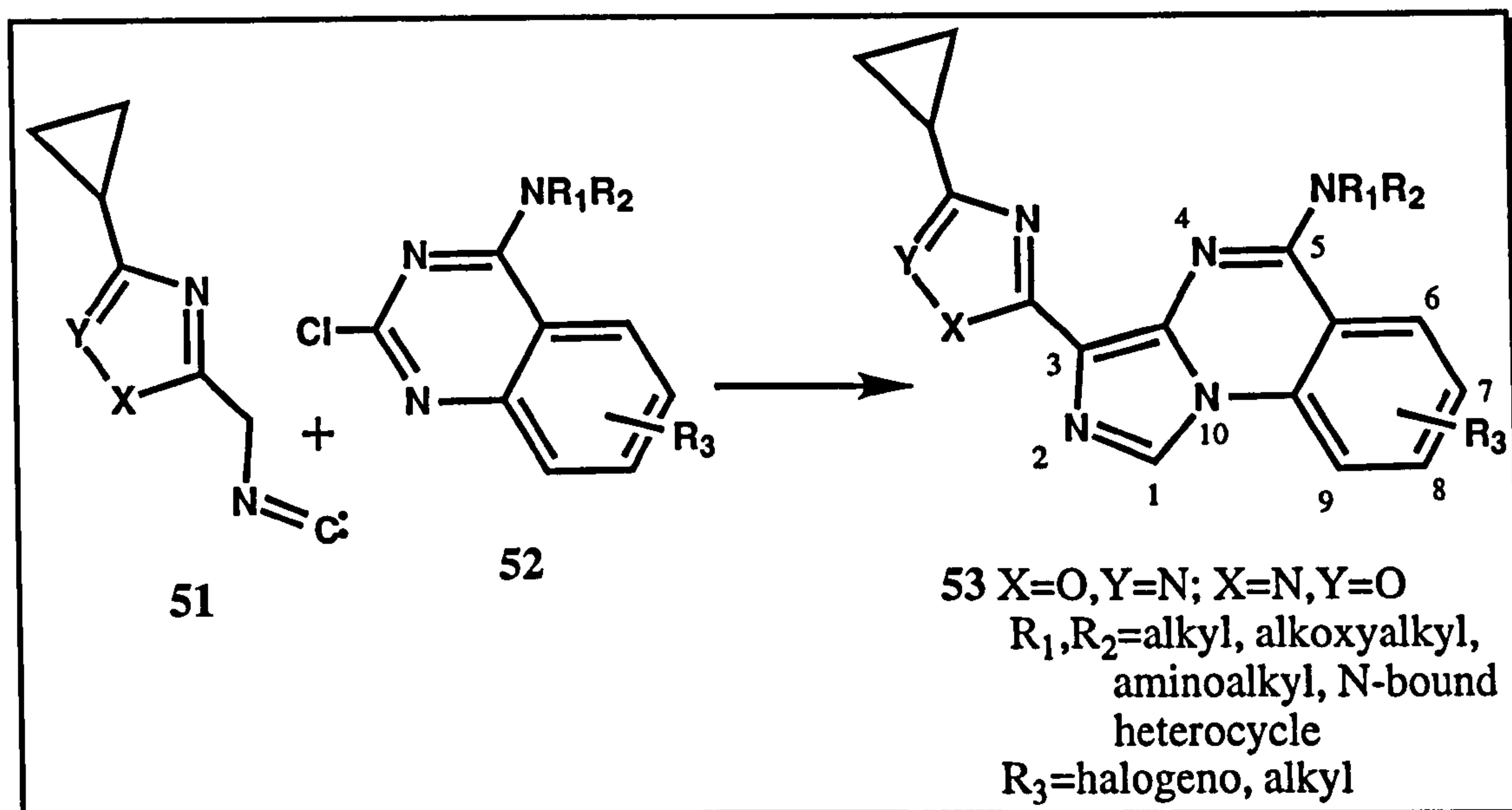


Scheme 3-9 Modified Schiff's base synthesis.

Given the ready availability of substituted benzaldehydes a vast range of Schiff bases could be synthesised. However novel, from a biological standpoint, such compounds would be of little use due to the lability of the imine bond to hydrolysis under weakly acidic or basic conditions, and routes to derivatise these compounds were sought.

3.1.3.1 Imidazo[1,5-*a*]quinazolines

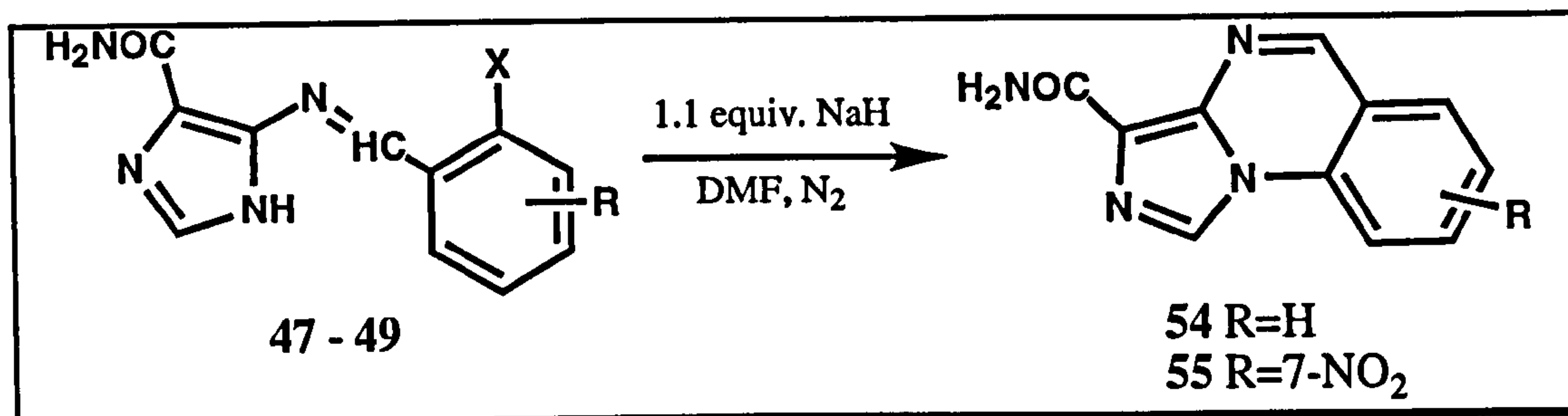
There is relatively little literature regarding the imidazo[1,5-*a*]quinazoline nucleus, although a number of patents do exist on imidazo[1,5-*a*]quinazolines of general structure **53** as central nervous system (CNS) active agents including anticonvulsants, anxiolytics, hypnotics, antipsychotics and antiemetics.^{109,110} The mode of action is thought to involve antagonism at the benzodiazepine receptor. Their synthesis involved reaction of a 4-amino-2-chloroquinazoline (**52**) with a 5/4-cyclopropyl-3/5-isocyanomethyl-1,2,4-oxadiazole (**51**) (Scheme 3-10).



Scheme 3-10 Patented synthesis of CNS active imidazoquinazolines.

This procedure involved formation of the imidazole ring as the last stage. Any likely route to imidazo[1,5-*a*]quinazolines from benzylidene derivatives 46 - 50 would involve formation of the pyrimidine ring last, and leave C-5 in a different oxidation state to compounds of structure 53. Previous studies in this group attempted to achieve cyclisations of 2'-chloro or 2'-nitro derivatives, 48 or 50 respectively, by thermolysis in high-boiling solvents without success.¹⁰⁸

As a different tack the 2'-halogenobenzylidene derivatives, 47 to 49, were treated with sodium hydride in anhydrous DMF, the rationale being that deprotonation of the imidazole ring NH would facilitate intramolecular nucleophilic substitution in the benzene ring, with halides as the leaving groups. In this way the novel imidazoquinazolines 54 and 55 were prepared in good yields (Scheme 3-11 and Table 3-1). Interestingly, the parent compound 54 was fluorescent upon irradiation at 254 nm, but the nitro-substituted 55 was not.



Scheme 3-11 General synthesis of imidazo[1,5-*a*]quinazolines.

	Benzylidene		Conditions		Product	
	X	R	Temp./°C	Time/hours	Yield/%	
47	F	H	25	168	54	0
47	F	H	65	100	"	61
47	F	H	155	3	"	86
48	Cl	H	155	30	"	25
49	Cl	<i>p</i> -NO ₂	65	264	55	77
49	Cl	<i>p</i> -NO ₂	100	3	"	65
49	Cl	<i>p</i> -NO ₂	155	0.5	"	15

Table 3-1 Imidazoquinazoline preparations.

A number of conclusions can be drawn from these results concerning the variables that were changed. Firstly, the effect of temperature: in general cyclisation rates dramatically increased with temperature, as can be seen with the reactions performed with **47** and **49**; also there appeared to be a threshold temperature below which no imidazoquinazoline was formed, as with both **47** and **49** a solution of the anion would persist at temperatures below about 40°C. This threshold temperature could be explained by the need for the imine bond to be *cis* for intramolecular attack of the imidazolyl anion, the heat therefore required to isomerise from the *trans* form produced under the thermodynamic control

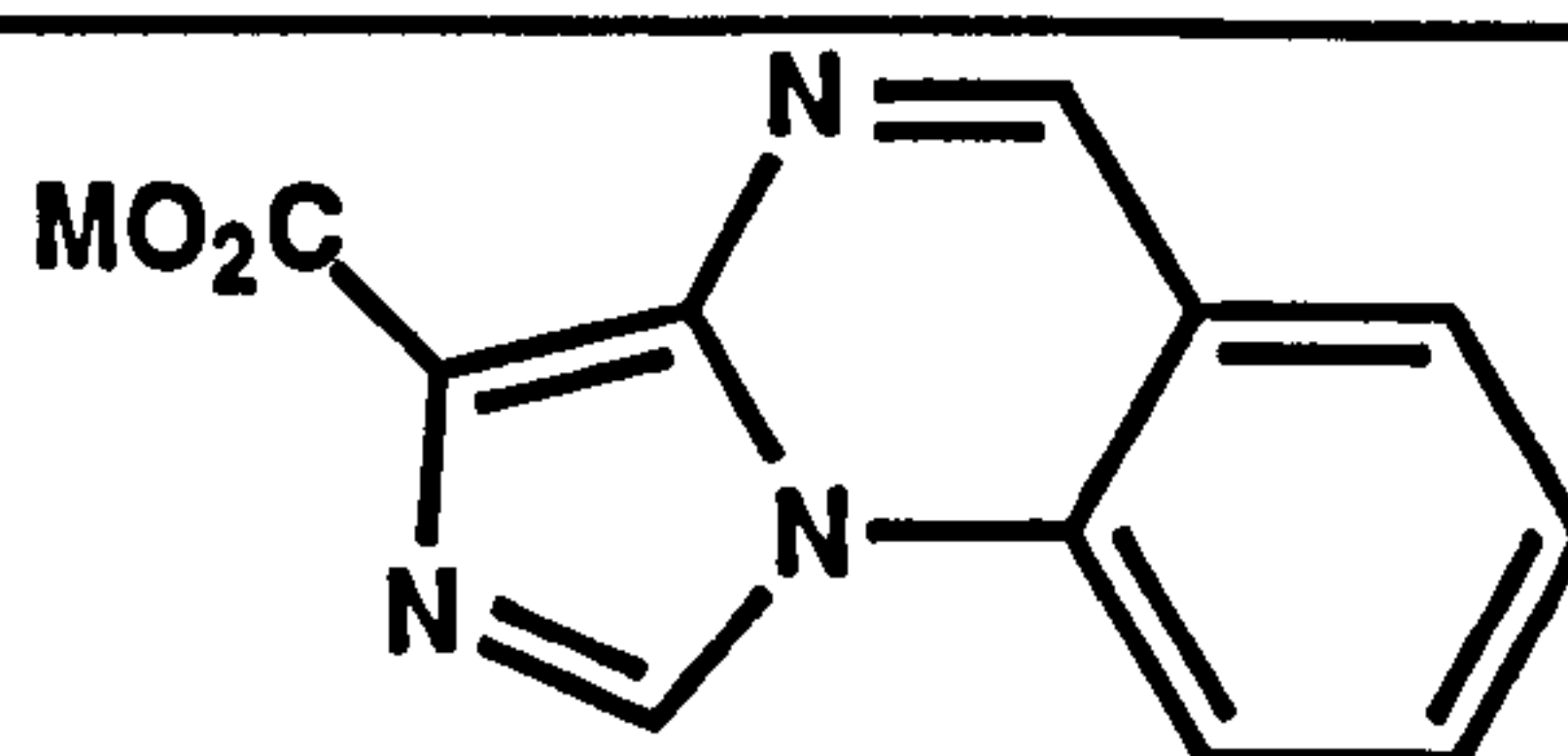
conditions in the Schiff's base synthesis. Secondly, substituent effects: the more electronegative the leaving group the better, the transition from fluoro (47) to chloro (48) leading to a drastic reduction in the cyclisation rate such that after 30 hours at reflux, much of the anion of imidazole 48 still persisted, with the prolonged reaction time leading to by-product formation; the reaction was also very sensitive to the activating ability of R, the introduction of a nitro *para* to the chloro in 49 greatly enhancing reactivity, and not only to reaction along the desired path.

Initially, reaction of 5'-nitro derivative 49 under conditions found optimum in the cyclisation of 47 - 1.1 equivalents of sodium hydride in refluxing DMF - was deemed a failure, with all starting material consumed in 30 minutes leaving a complex mixture of products, none of which were fluorescent and most of which would not elute from the TLC baseline in a solvent system suitable for imidazoquinazoline 54. Alternative conditions were sought in a variety of high-boiling solvents, but again only a complex mixture resulted. Only on lowering the reaction temperature to 65°C was the cyclisation product, 55, slowly formed as a non-fluorescent and highly insoluble solid. For ^{13}C NMR of 55 a sample had to be prepared in deuterio-trifluoroacetic acid (d-TFA), solubility being too low in d_6 -DMSO. With knowledge of the properties of the nitroimidazoquinazoline 55, careful reexamination of products from the initial reflux reaction revealed 55 in 15% yield, though the other components were not identified. The rate of formation of 55 was subsequently greatly improved from the reaction at 65°C, with only a minor reduction in yield, by heating the reaction on a steam bath.

The ^1H NMR spectra of the benzyldienes and imidazoquinazolines are very similar (Figure 3-2). In transition from 47 to 54, the carbocyclic proton

resonances are shifted only slightly downfield, the major shift being that of the imine proton (identified in the Schiff's base **47** by its weak coupling to the fluorine) moving downfield about 1.5 ppm, on effectively becoming attached to an electron deficient pyrimidine ring.

At the time when cyclisation of imidazole **49** was proving difficult, because of the potential of using the nitro group of **55** to introduce other functional groups, alternative routes to this molecule were explored. With reasonable quantities of the mono-substituted imidazoquinazoline **54** in hand direct nitration was an obvious choice. Mixed acid nitration using 70% nitric acid (and 98% sulfuric acid) up to room temperature - sufficient for the nitration of many substrates - left imidazoquinazoline **54** largely intact, only a minute trace of another fluorescent component being observed by TLC. Repeat reaction using 90% nitric acid and heating up to 55°C gave this other fluorescent compound in high yield with a number of minor side-products. The ^1H NMR spectrum of this new compound retained all the CH resonances of **54**, precluding nitration, the only difference being loss of the amide proton resonances. The mystery compound was finally identified as the unknown carboxylic acid of the amide. In the course of the work-up it was isolated as the potassium salt **56a**, recrystallised in 46% yield, and acidification of the filtrate yielded a sample of the free acid **56b** in a further 16% yield.



56a M=K
56b M=H

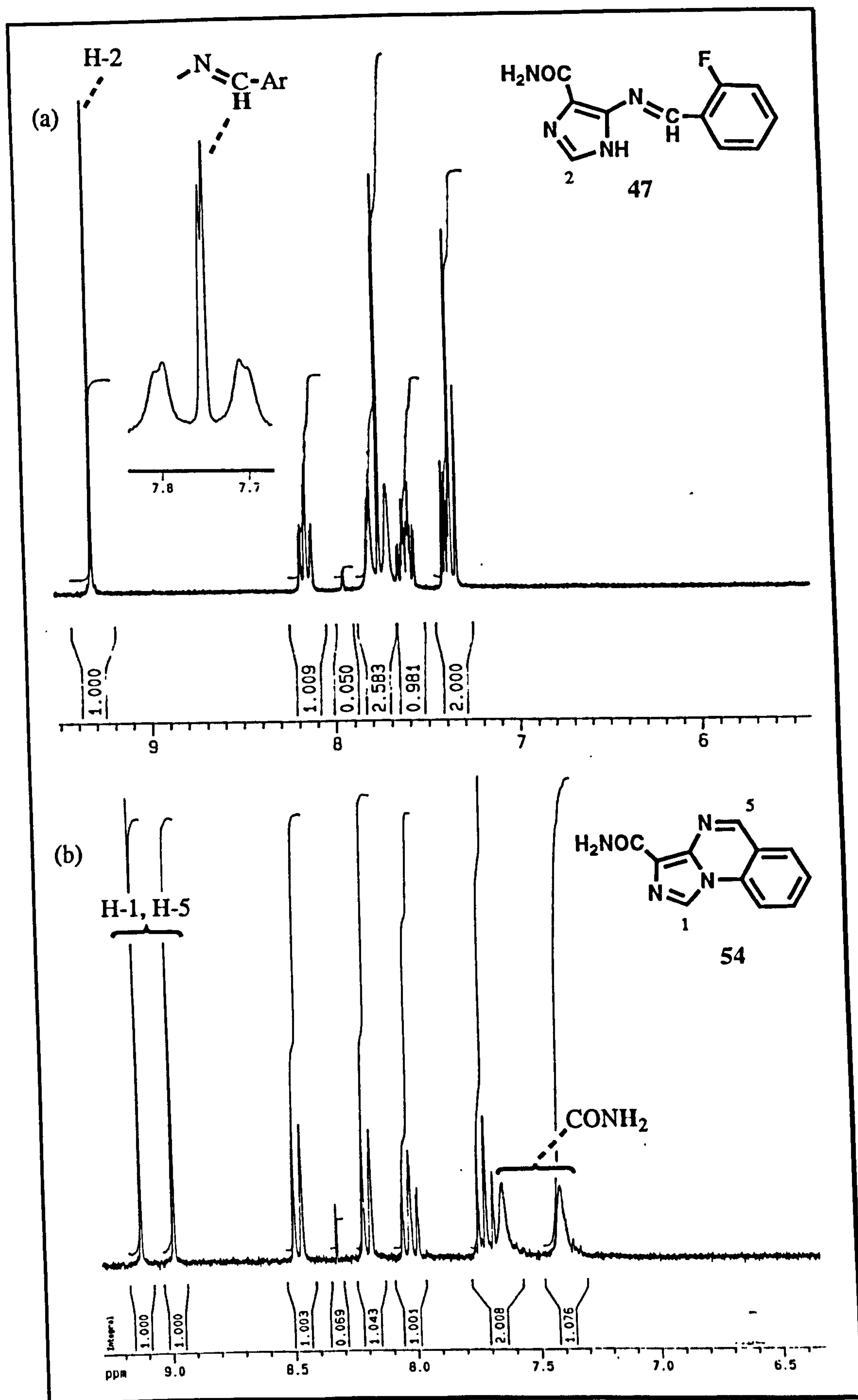
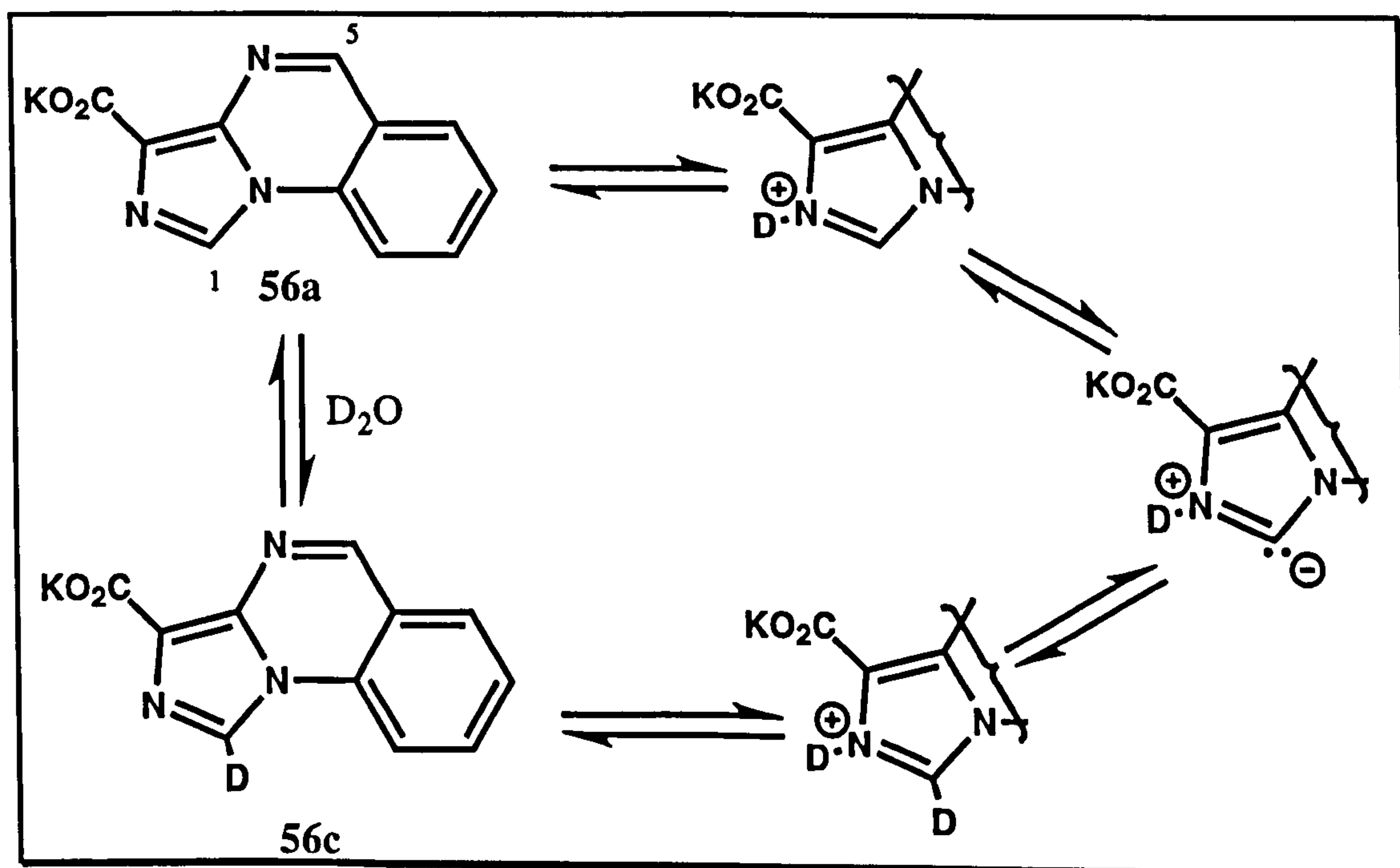


Figure 3-2 ^1H NMR spectra of the Schiff's base 47, and its cyclised derivative 54.

An interesting feature of the ^1H and ^{13}C NMR spectra of the salt **56a**, which had been prepared in deuterium oxide (D_2O), was the slow disappearance of the higher field ($\delta=8.10$ ppm) of the two closely placed singlets in the ^1H spectrum, the pair originally assigned as the 1-H and 5-H resonances. By the time ^{13}C NMR was performed one CH resonance was missing, hampering identification. Consultation of the literature highlighted the ability of imidazoles to undergo deuterium exchange at the 2-position under neutral or basic conditions (see for example ref. 78, p.194), and by analogy the disappearing proton singlet of the potassium salt **56a** was assigned as H-1. Re-examination of the NMR spectrum of a fresh sample in D_2O did indeed show the missing CH signal at $\delta_{\text{C}}=124.8$, the intensity of the singlet at $\delta_{\text{H}}=8.10$ reduced to 13% of original in six days. Such exchange reactions are thought to involve transient ylid formation (Scheme 3-12).



Scheme 3-12 Deuterium exchange at C-1 of imidazoquinazoline **56a**.

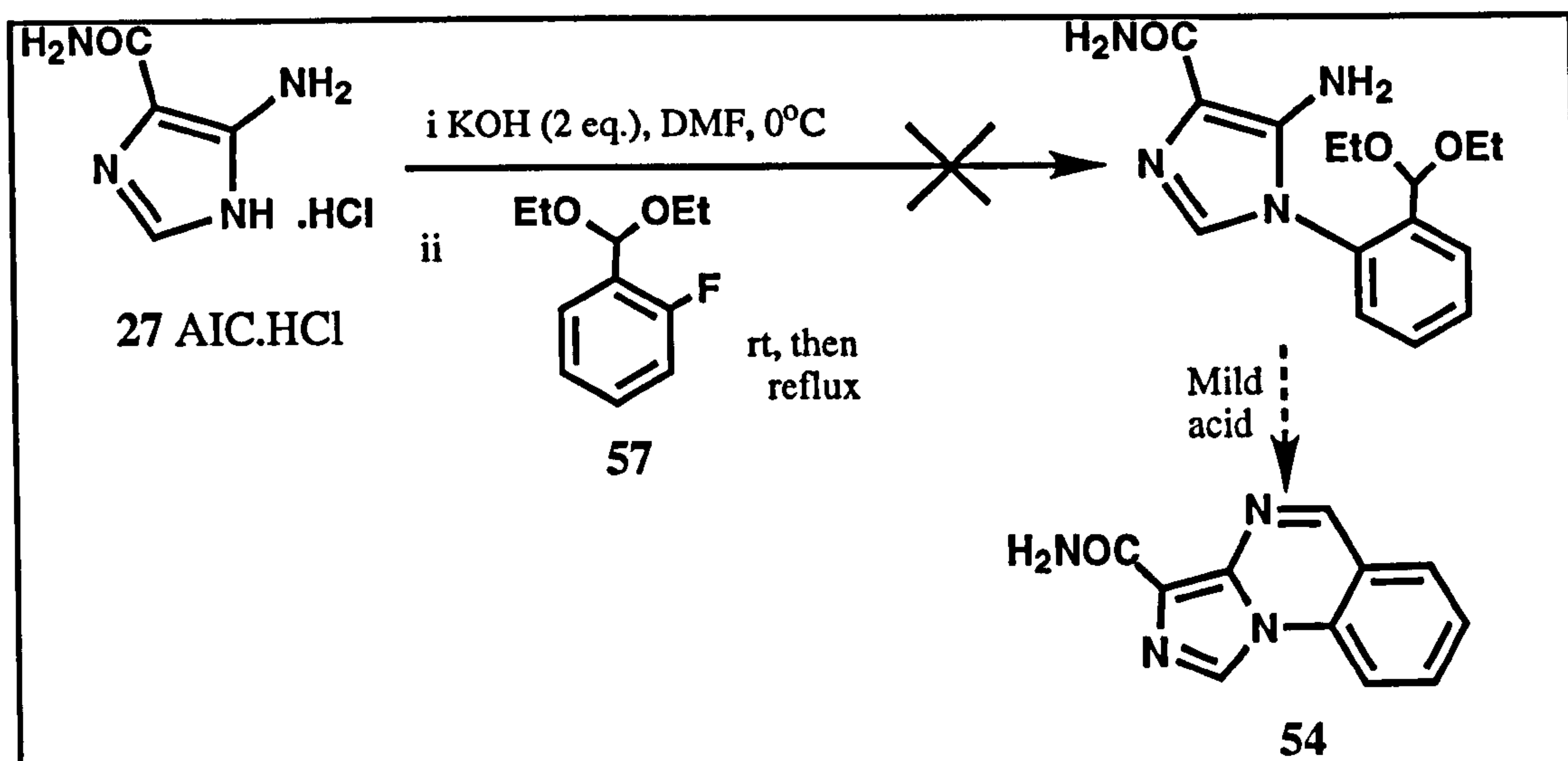
The assignment of H/C-1 and H/C-5 given for the carboxylate salt **56a** cannot be generalised to the other imidazoquinazolines synthesised however, as for the free acid **56b**, the order of the two singlets in the ^1H NMR is reversed such that now the 5-H is the the higher field of the two. This was evidenced by a 2-dimensional ^1H - ^{13}C correlation NMR experiment that linked the lower field proton singlet at $\delta_{\text{H}}=9.22$ to a carbon resonance at $\delta_{\text{C}}=127.5$, the likely region expected for an imidazole C-2,⁷⁹ and the other proton singlet at $\delta_{\text{H}}=9.05$ to a carbon at $\delta_{\text{C}}=154.4$, again in the region expected for a quinazoline C-4.⁷⁹ Complete assignment of all the members synthesised in this class would require a combination of 2-dimensional ^1H - ^{13}C correlation and NOE NMR experiments.

In one final attempt to effect nitration the monosubstituted imidazoquinazoline **54** was heated with 90% nitric acid and concentrated sulfuric acid at 100°C for 24 hours, accepting that amide hydrolysis would occur and hoping that nitration would follow. However, the major product isolated was the acid **56b** as before, obtained in 70% yield. Two other minor products were also isolated in quantities only sufficient for partial identification. Both had imidazoquinazoline-like ^1H NMR spectra, one with a spin system suggesting mono-nitration in the carbocyclic ring; the other, substitution free. Examination of their ^{13}C NMR spectra however suggested decomposition of the imidazole ring. In both cases, 2-dimensional ^1H - ^{13}C correlation NMR linked the two proton singlets at around 9 ppm to two CH carbon signals at chemical shifts greater than 155 ppm, rather than one at around 125 ppm and another at about 155 ppm as observed with the other imidazo[1,5-*a*]quinazolines prepared.

That mixed acid nitration of these imidazoquinazolines is ineffective is perhaps not surprising, as any of the three ring nitrogens could be protonated

under the acidic conditions, deactivating the system to attack by an electrophile. Alternative nitration procedures do exist including the use of acetyl nitrate, or nitronium salts such the tetrafluoroborate salt, but as already mentioned the nitro derivative **55** was finally synthesised in good yield using the original procedure.

An attempt was also made to synthesise the imidazoquinazoline nucleus by an alternative route, arylating the imidazole N-1 nitrogen followed by condensation at the 5-amino group, circumventing the need for high temperatures thought to be required for isomerisation of the imine bond of the benzyldene derivatives. Following a reported general method for the regiospecific 1-alkylation of AIC.HCl (**27**) - employing powdered KOH in DMF, followed by the alkyl halide at or below room temperature¹¹¹ - 2-fluorobenzaldehyde diethyl acetal (**57**), not previously reported, was prepared in 86% yield under standard conditions.¹¹² Attempted arylation of **27** at the imidazole nitrogen with acetal **57**, under the above conditions, gave no reaction, and heating to reflux gave a complex mixture from which no product was isolated - highlighting the difference in reactivity to nucleophiles between alkyl and aryl halides (Scheme 3-13). Perhaps certain aromatic acetals such as a dinitro derivative would be sufficiently activated towards halide displacement, but given the high yield of the established route, this route was abandoned.

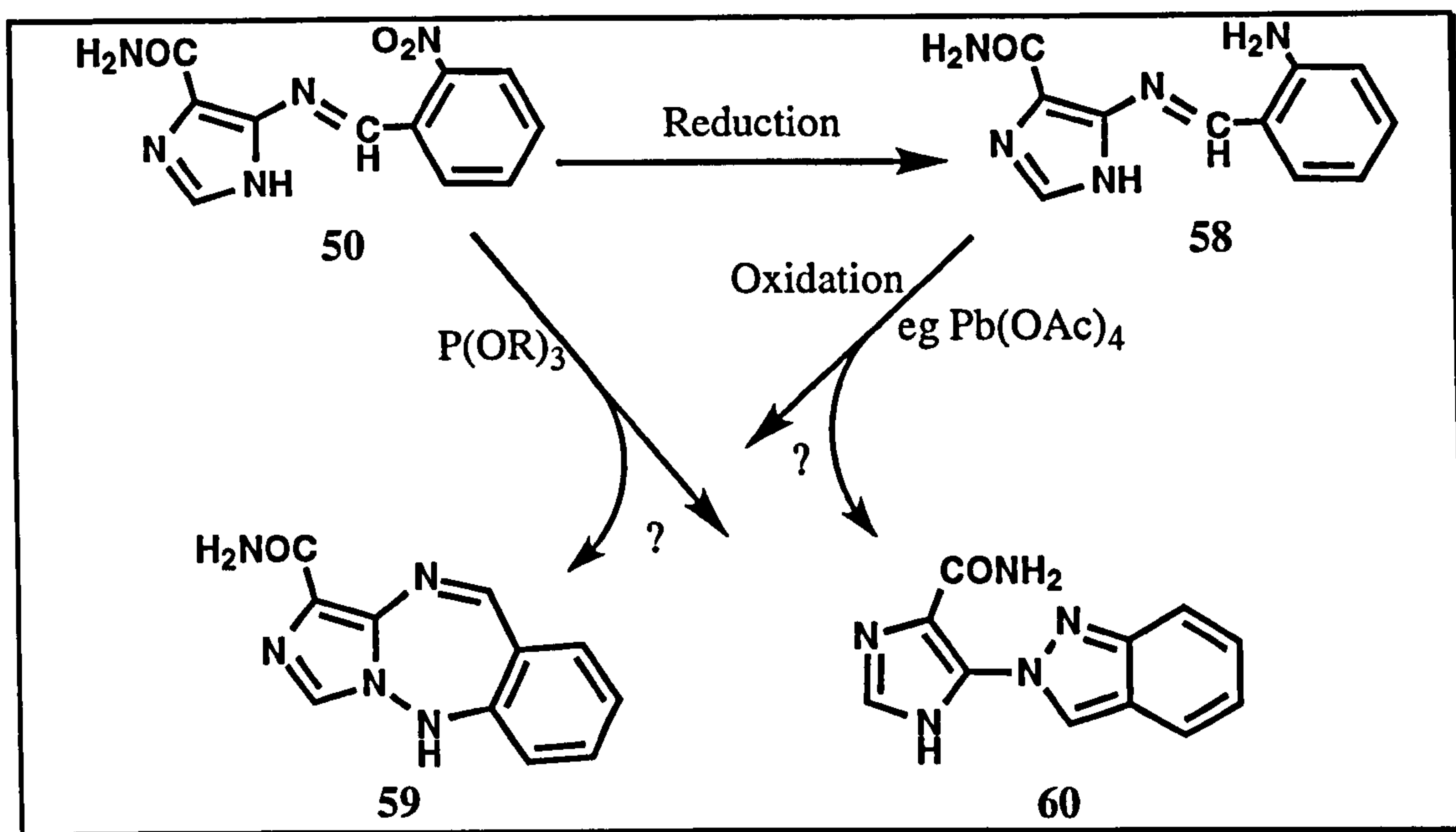


Scheme 3-13 Unsuccessful imidazoquinazoline synthesis.

Given the lack of generality in the cyclisation of benzylidenes to imidazoquinazolines, the preparation of a series of such tricycles for biological evaluation could be limited to particular substitution patterns in the carbocyclic ring, further dependent upon benzaldehyde availability. However, the preparation of derivatives such as 55 opens access to other compounds through functional group interconversion, and other chemistry of these imidazoquinazolines awaits investigation.

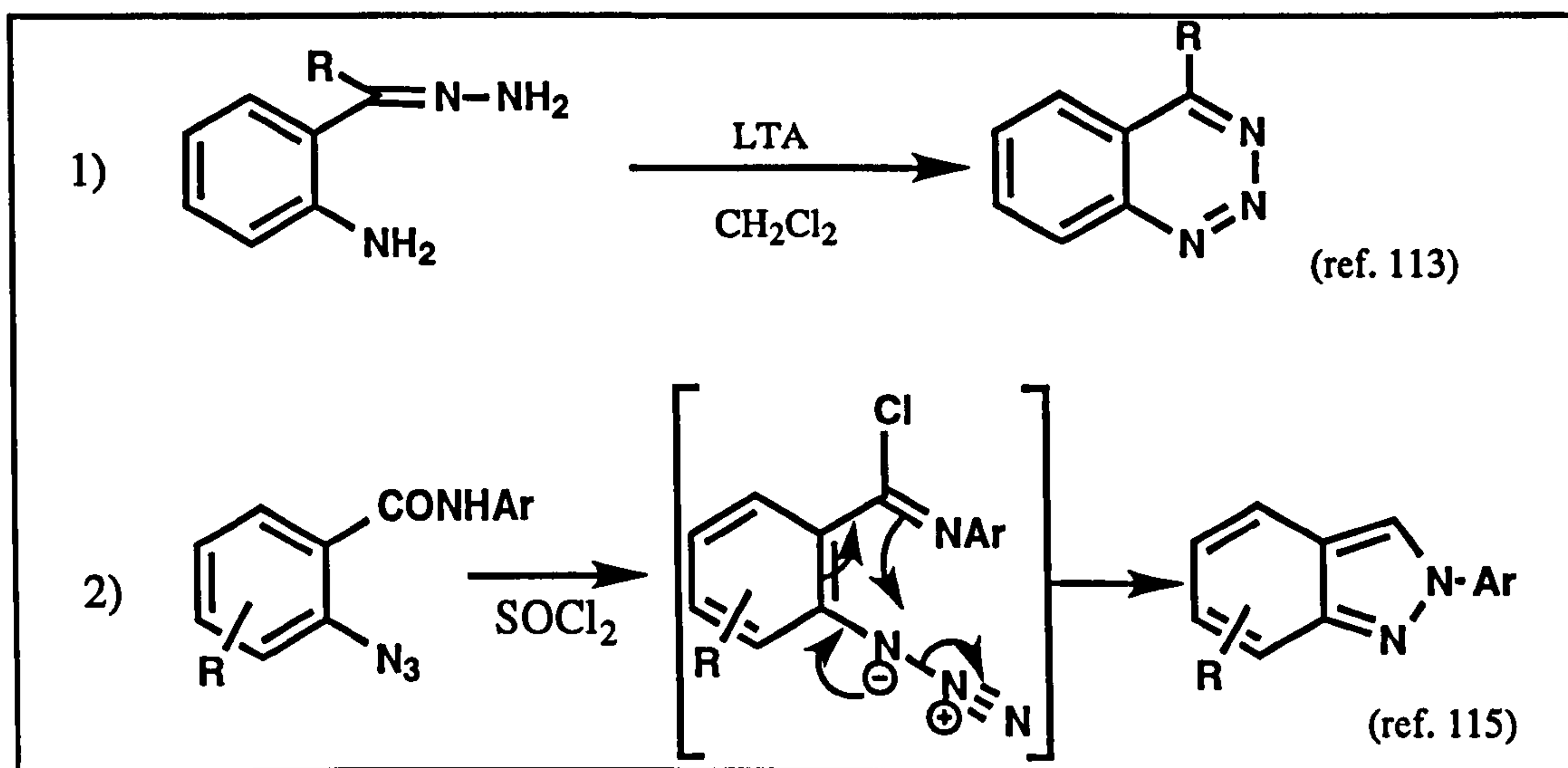
3.1.3.2 Triazepines and indazoles

Investigations were also initiated into the possible derivatisations of a benzylideneaminoimidazole with a nitrogen containing group in the 2'-position of the benzene ring. A number of possibilities, starting from the 2'-nitro derivative 50 were considered, centering on reactions formally involving nitrene intermediates reacting intramolecularly to give the benzoimidazotriazepine 59 or the imidazolyindazole 60 (Scheme 3-14).



Scheme 3-14 Potential cyclisations of a 2-nitrobenzylidene, **50**.

Lead tetraacetate (LTA) is a multi-faceted oxidising agent, able to react by ionic and free radical mechanisms. Of the many and various known reactions of LTA, oxidative formation of nitrogen-nitrogen bonds are fairly common (see for example reaction 1, Scheme 3-15).¹¹³ Trialkyl phosphites have proved effective in the deoxygenative generation of aryl nitrenes from aromatic nitro and nitroso compounds.¹¹⁴ The most widely used source of aryl nitrenes however is the thermolysis or photolysis of aryl azides - for example in the synthesis of a 2*H*-indazole (reaction 2, Scheme 3-15).¹¹⁵ Reaction 2 in Scheme 3-15 might suggest for our series (see Scheme 3-14), the indazole **60** to be more likely than the triazepine **59**, especially starting with a *trans* imine bond.



Scheme 3-15 Intramolecular cyclisations of amino and azido aromatics.

In this area of work, only attempts to form the 2-aminobenzylidene **58** were studied. Firstly, reduction of the 2-nitrobenzylidene **50** was attempted by a number of literature procedures, without success (Table 3-2). The first conclusion to be drawn from these results is that the imine bond is labile under these conditions - either by reduction or simple hydrolysis. Numerous other conditions for the reduction of aromatic nitro groups have been described, but the imine bond is also reduced by a number of common methods including hydrogenation over palladium catalysts, and Raney nickel and zinc metal reductions. Cyclohexene **61** was only partially characterised by ^1H and ^{13}C NMR spectroscopy. Bis(2-amino-benzylidene)hydrazine (**62**) was characterised by its mass spectrum (m/z 239, $M+1$), infrared absorbance and ^1H and ^{13}C NMR spectroscopy and had a mp 249°C (lit.¹¹⁶ $251\text{-}252^\circ\text{C}$). Hydrazine **62** represents the only example where reduction of the nitro group was observed to occur, and though not reinvestigated at the time, a reaction employing careful addition of hydrazine might lead to selective nitro reduction before hydrazine displacement.

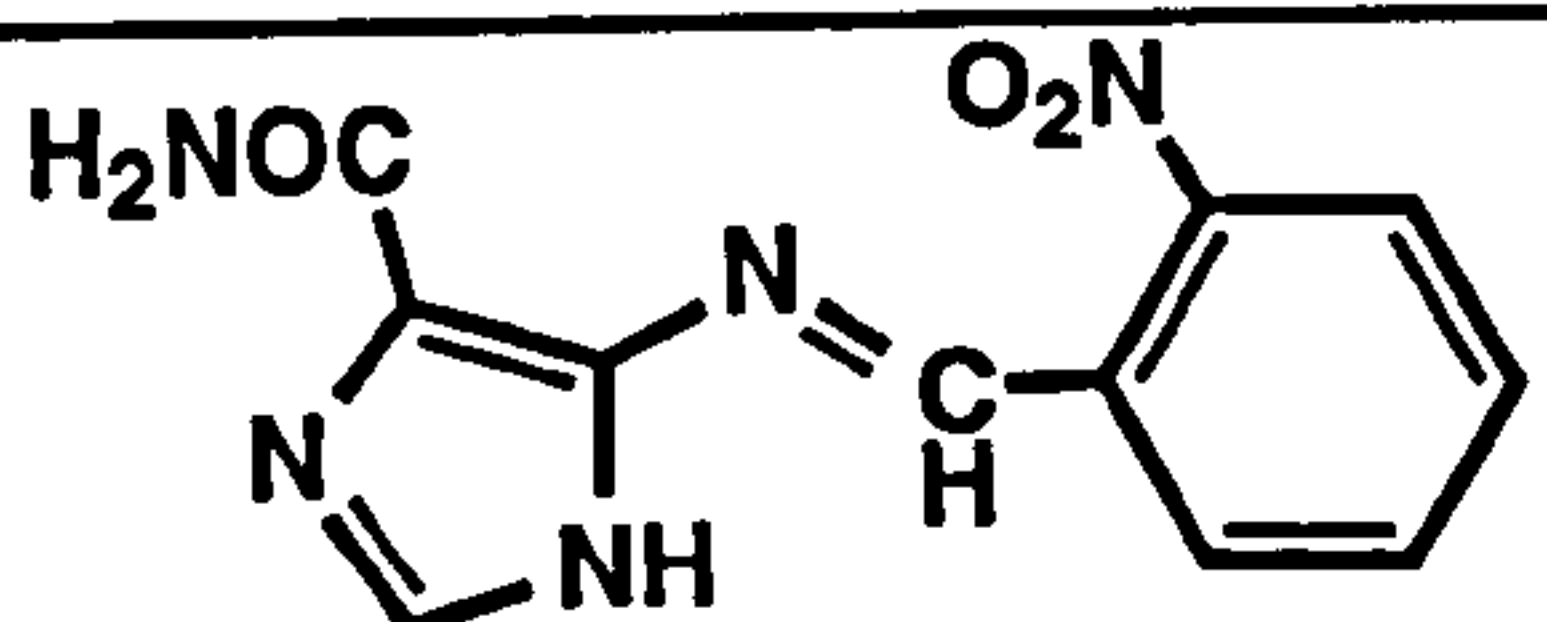
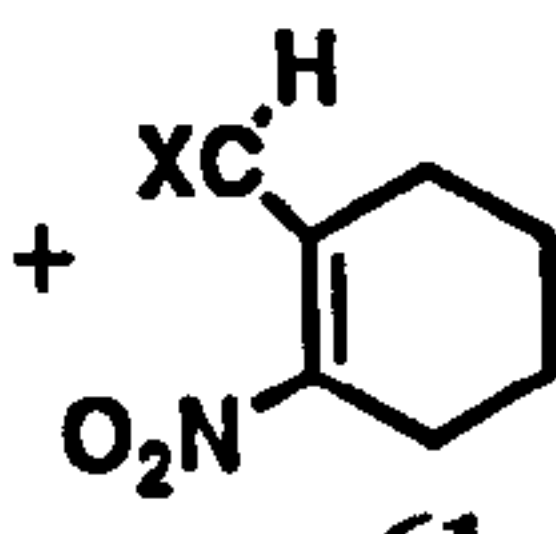
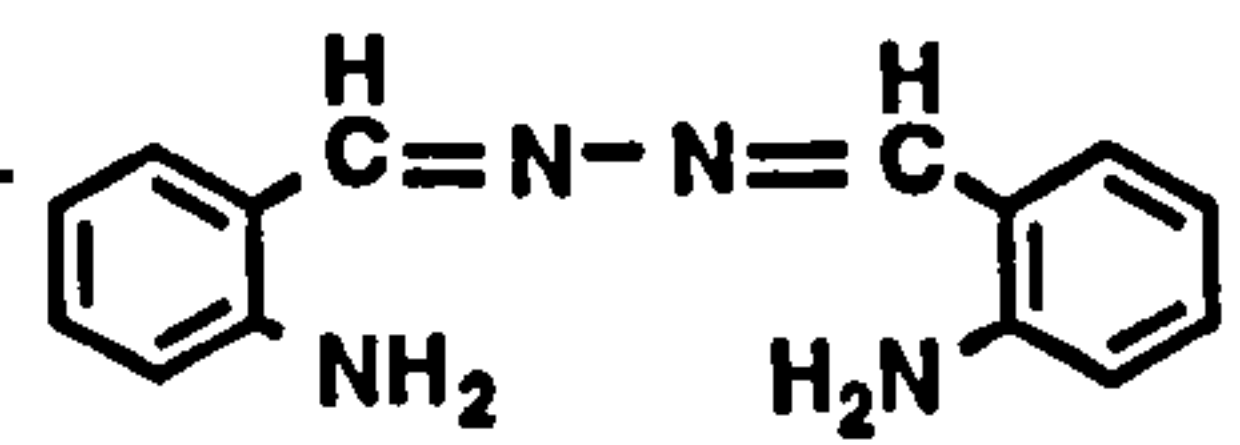
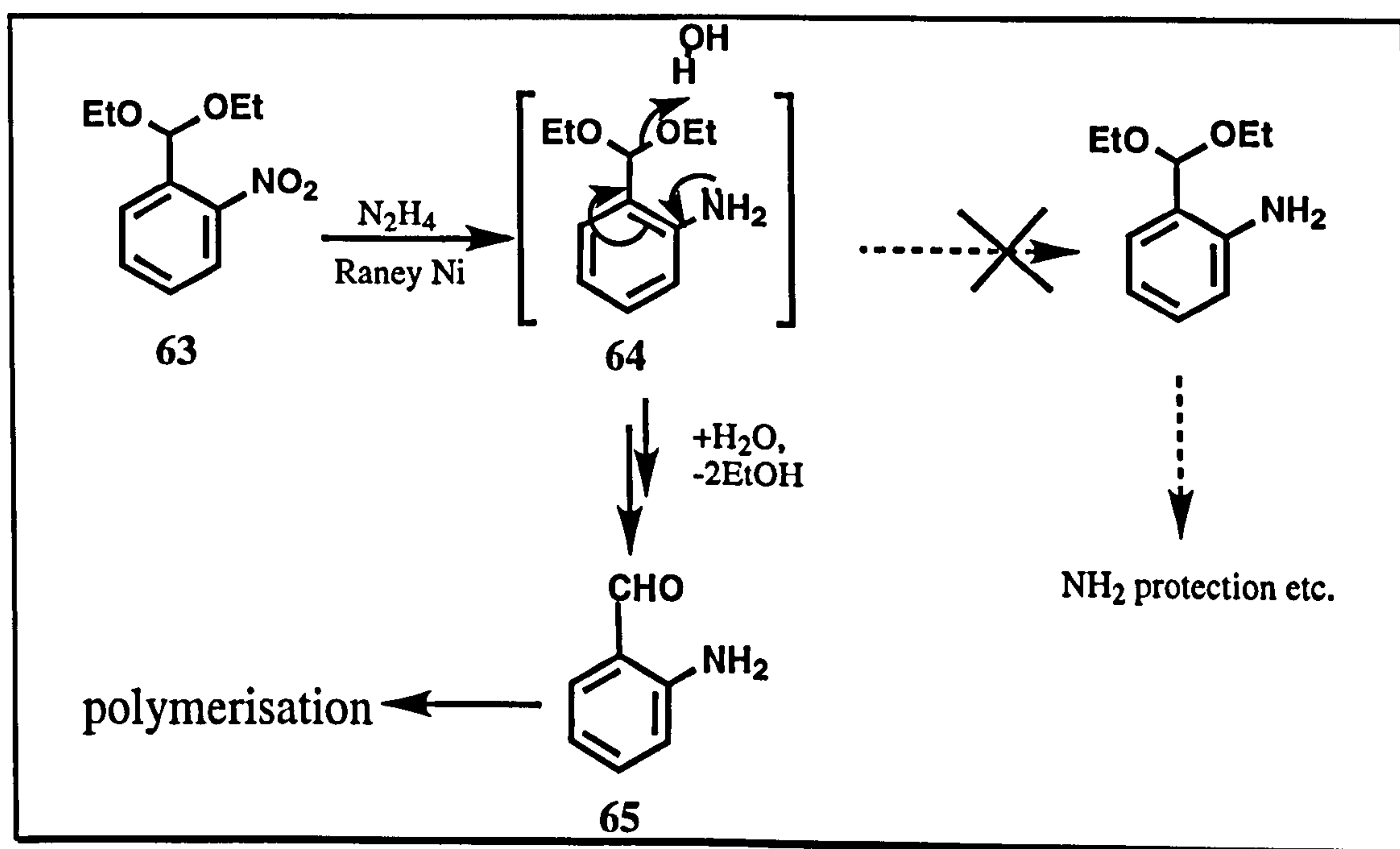
<div style="text-align: center;">  <p>50</p> </div> <div style="text-align: center; margin-top: 10px;"> $\xrightarrow{\text{Reduction}}$ </div>		
Run	Method (Ref.)	Result
1	SnCl ₂ ·2H ₂ O/ EtOH/ reflux (117)	} 27 (AIC) + other unidentified components
2	SnCl ₂ ·2H ₂ O/ EtOAc/ reflux (117)	
3	50 psi H ₂ / 10% Pd-C/ 1M HCl (118)	50 + 27 (AIC) +  (Tentative assignment) 61
4	N ₂ H ₄ / Raney Ni/ EtOH/ HOCH ₂ CH ₂ OH/ 50-60°C (119)	27 (AIC) +  62
5	Zn dust/ 78% aq. EtOH/ reflux (120)	27 + complex mixture

Table 3-2 Attempted nitro reductions of 50.

Further reductions of 50 were not carried out. A different approach to amine 58 was planned involving preparation of a benzaldehyde with a protected 2-amino group that could be liberated after formation of the imine bond. The diethyl acetal of 2-nitrobenzaldehyde (63) was prepared in 76% yield (b.p. 146-156°C at 25 mbar, lit.¹²¹ 154-156°C at 18 Torr) by the method used previously¹¹² to prepare the 2-fluoro derivative, 57, and the structure was confirmed by ¹H and ¹³C NMR. Nitroacetal 63 was then reduced according to the method of Ayyangar *et al.* with hydrazine/Raney nickel in a 1:1 mixture of ethanol and 1,2-dichloroethane at 50 to 60°C.¹¹⁹ The reaction appeared a success, but on attempted recrystallisation of the crude white reaction residue from THF/ethanol, a colour change occurred. The orange solid isolated gave a ¹H NMR spectrum

consistent with 2-aminobenzaldehyde (**65**). Reduction of **63** with stannous chloride dihydrate,¹¹⁷ as per run 2 in table 3-2, gave a mixture of products after work-up, none of which were identified.

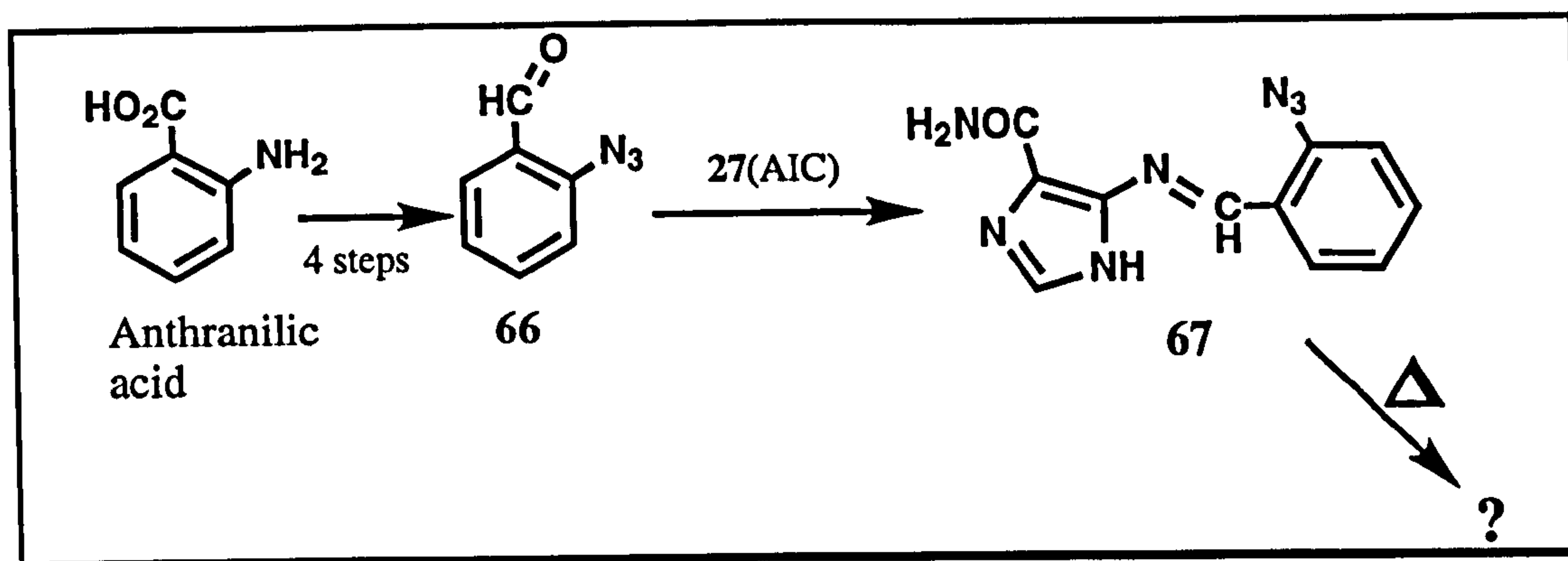
If in the hydrazine reduction of acetal **63**, aniline **64** was formed, acetal hydrolysis in the absence of acid could occur with assistance from the amino group (Scheme 3-16). A high activation energy barrier for this process would explain the apparent reaction during recrystallisation, to give the benzaldehyde **65** as the isolated product. Benzaldehyde **65** is unlikely to be synthetically useful due to a tendency to polymerise, which may also explain the low yield of solid isolated.



Scheme 3-16 Possible degradation in the reduction of **63**.

Future studies in this area could employ alternative aldehyde protecting groups such 1,3-dithianes (not with Raney nickel reductions), but other alternatives should also be tried including trialkyl phosphite reaction with the

nitro derivative **50** as suggested in scheme 3-14. The 2-azidobenzylidene Schiff base **67** also presents possibilities, but would require the preparation of 2-azidobenzaldehyde (**66**),¹¹⁵ adding a further four steps to the procedure (Scheme 3-17).

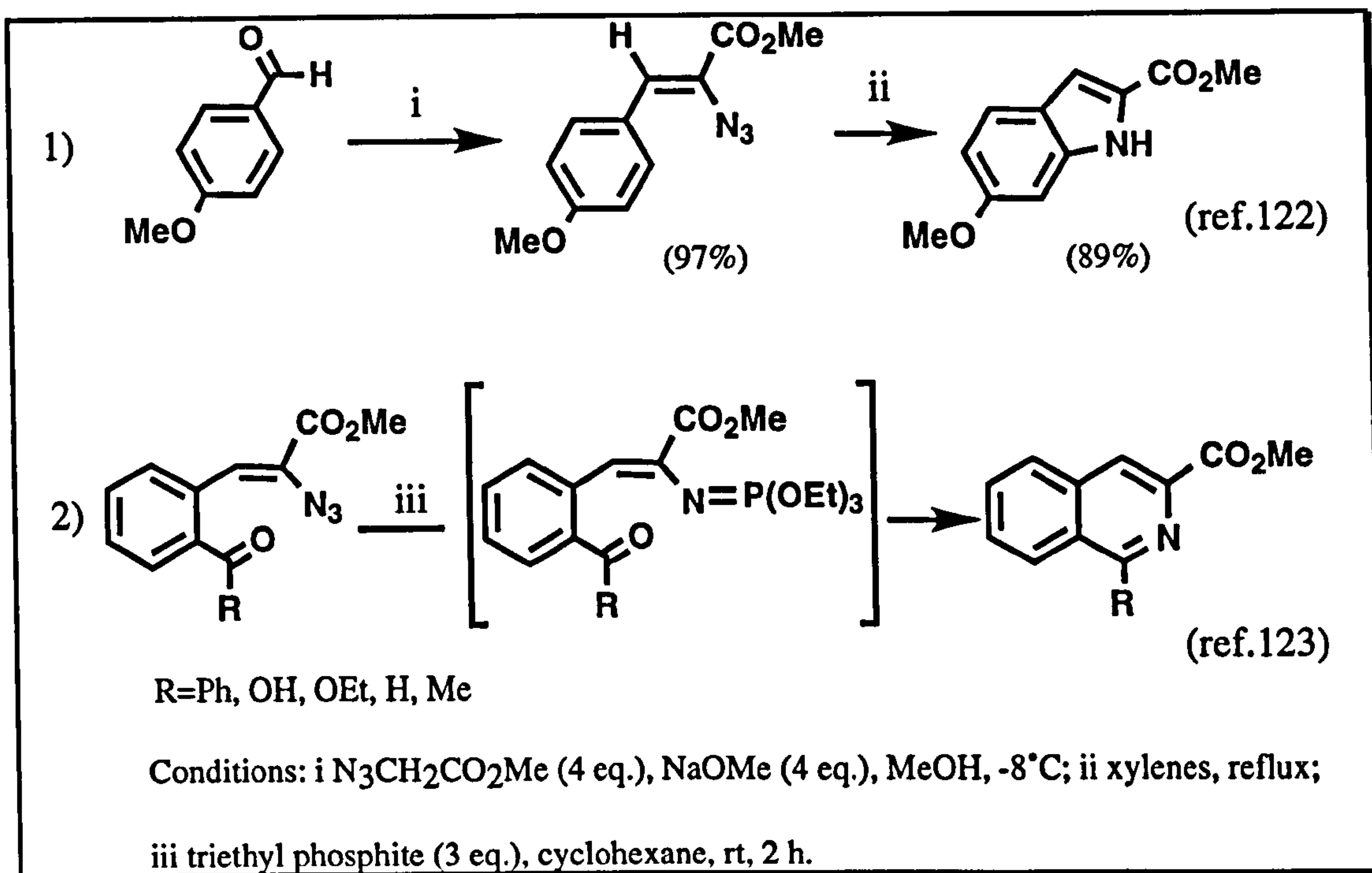


Scheme 3-17 Alternatives to 2-nitrobenzylidene **50**.

3.2 Syntheses and reactions of 5-formylimidazoles

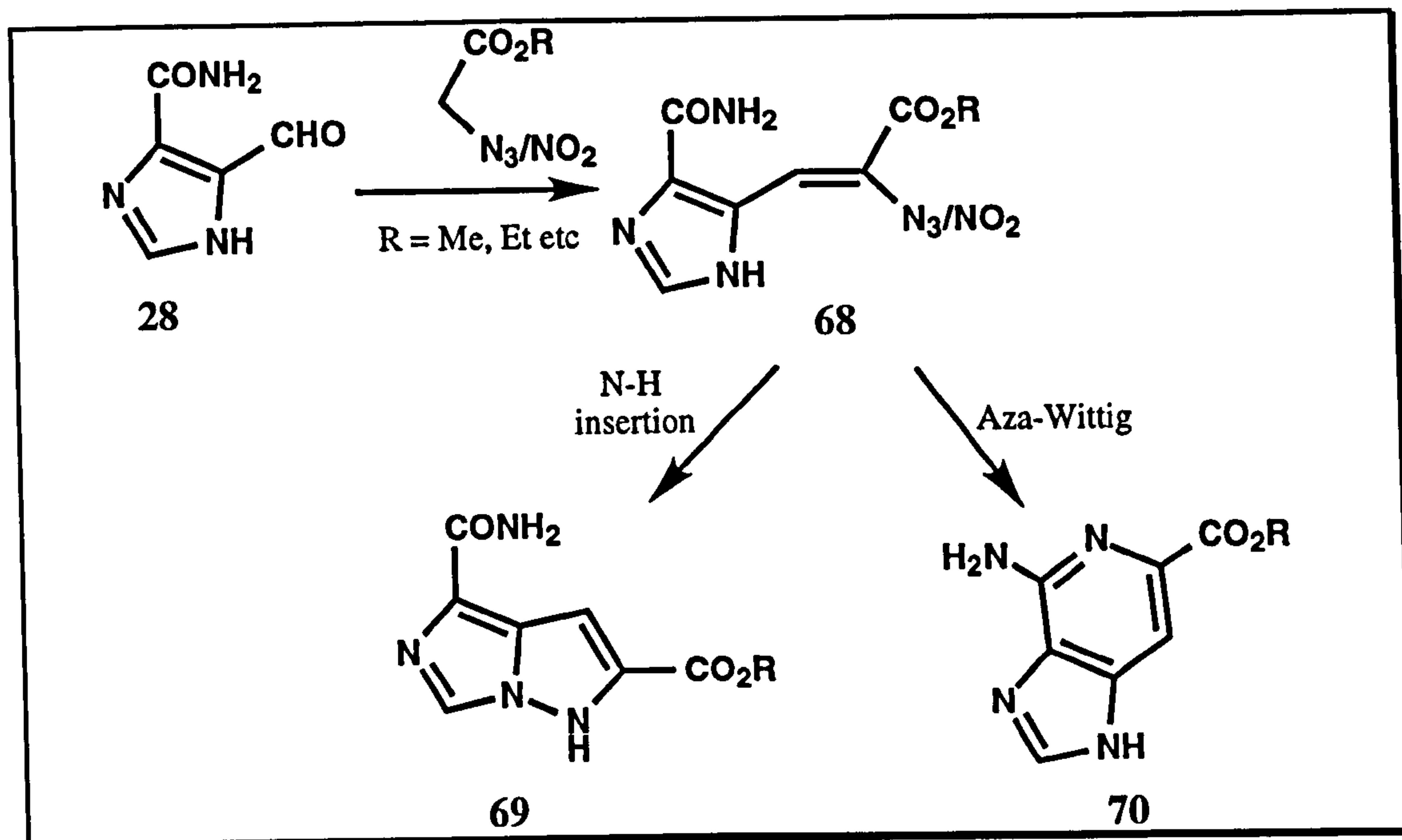
3.2.1 Imidazo[1,5-*b*]pyrazoles

The Knoevenagel condensation of aromatic aldehydes and azido acetates is a well established procedure for the synthesis of aromatic vinyl azides.^{122,123} The main use of such azides formed has been in their thermolyses, generally in boiling non-polar aromatic hydrocarbons, to give indoles in good yields. Aromatic vinyl azides, with suitable ortho substituents in the benzene ring have also been reacted with trialkyl phosphites, the intermediate ylids undergoing aza-Wittig reactions to yield isoquinolines (Scheme 3-18).



Scheme 3-18 Reactions of azido acetates with benzaldehydes.

In this programme the intention was to perform an analogous sequence of reactions upon a formylimidazole, such as **28**, by reaction of azido or nitroacetates to form intermediate azido or nitrovinylimidazoles **68**, with the hope of synthesising novel imidazo[1,5-*b*]pyrazoles **69** in a reaction paralleling the indole formation above. An alternative process, involving an aza-Wittig reaction could yield imidazo[4,5-*c*]pyridines **70** (Scheme 3-19).



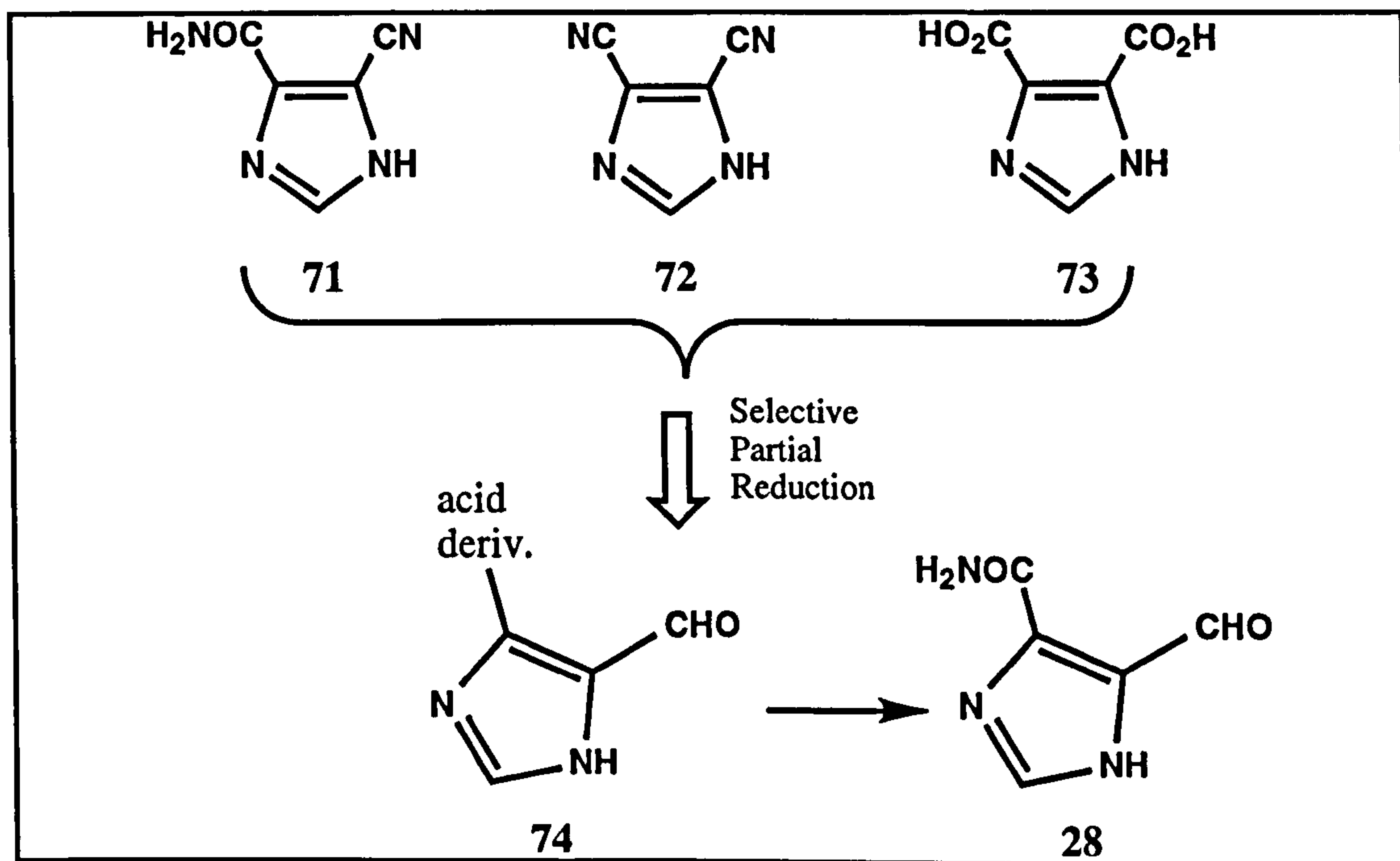
Scheme 3-19 Proposed imidazo[1,5-*b*]pyrazole and imidazo[4,5-*c*]pyridine synthesis.

The first task was to develop an efficient synthesis of 5(4)-formyl-imidazole-4(5)-carboxamide (28) or a synthetic equivalent.

3.2.2 Reduction of imidazoles

In an attempt to restrict the synthesis of formylimidazole 28 to as few steps as possible, transformations of suitably functionalised, commercially available imidazoles were investigated. There is however a relatively small number of such compounds, and initial investigations were restricted to 5(4)-cyanoimidazole-4(5)-carboxamide (71), 4,5-dicyanoimidazole (72) and imidazole-4,5-dicarboxylic acid (73). In all three of these compounds the 4- and 5- substituents are carboxylic acid derivatives. There are numerous literature procedures for the partial reduction of such carboxylic acid derivatives to aldehydes, and using one of these procedures it was hoped to achieve selective

partial reduction of one substituent over the other to give a formylimidazole **74**, with the unaffected group readily converted to an amide, giving **28** (Scheme 3-20).



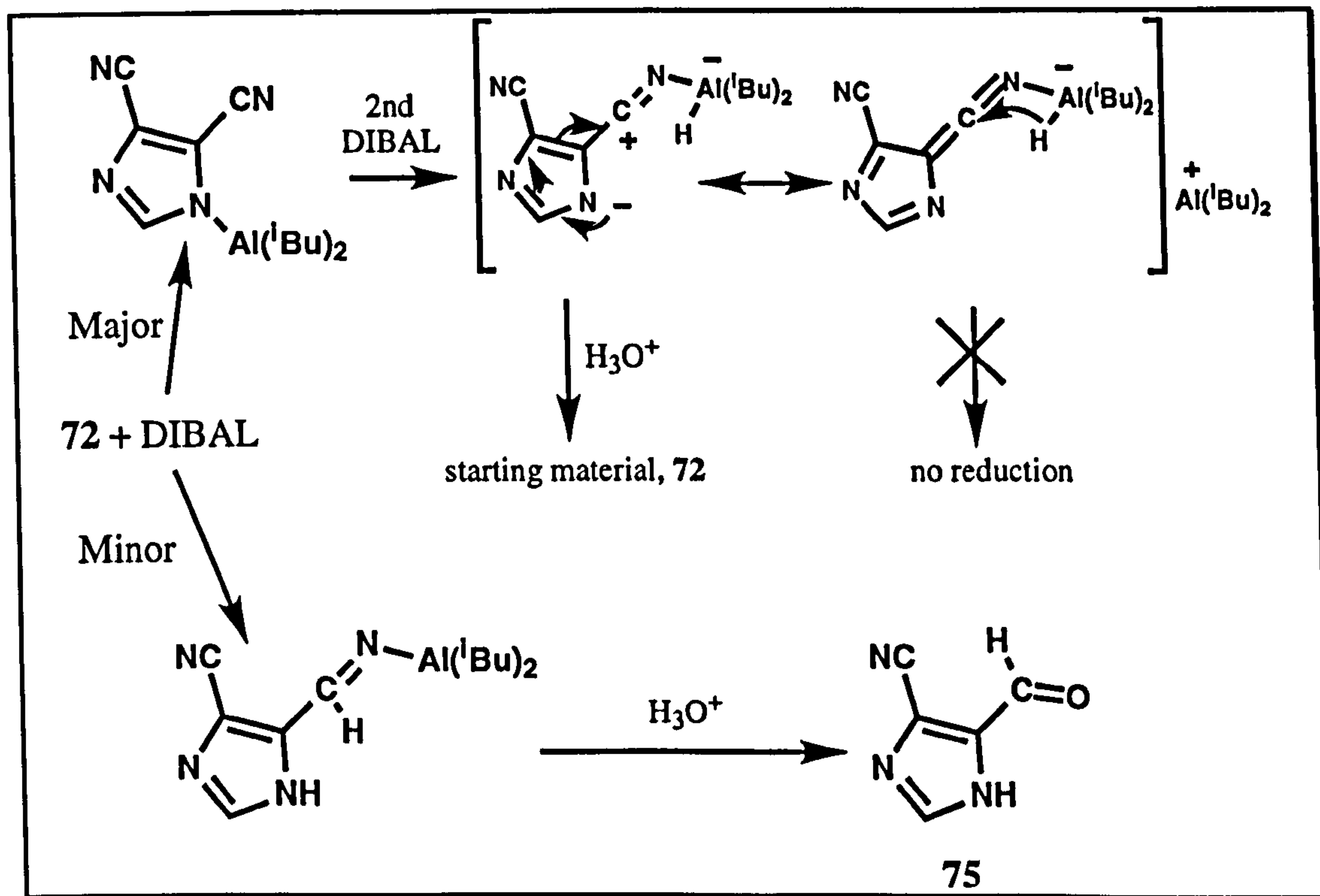
Scheme 3-20 Proposed route to a formylimidazole.

Though perhaps the most popular reagent for the partial reduction of amides, esters and nitriles, diisobutylaluminium hydride (DIBAL)^{124,125} proved to be unsuitable in reductions of imidazoles **71** and **72**. Amide **71** suffered from poor solubility at the low temperatures generally employed. DIBAL reactions of nitrile **72** were more complicated.

It became apparent that rapid deprotonation of **72** occurred in the presence of DIBAL. Employing two equivalents of reductant at 0°C and either basic¹²⁴ or acidic¹²⁶ work-up gave a mixture, from which no products were isolated, though by ¹H NMR trace aldehyde signals were observed. Reaction at -70°C with either one or two equivalents of DIBAL resulted in a mixture of starting material **72** and

the desired monoaldehyde **75** in, at best, a 5:1 ratio. The novel monoaldehyde **75** proved to be inseparable from **72** by chromatography, but was identified in the ^1H NMR spectrum of the mixture, with a characteristic aldehydic signal at $\delta_{\text{H}} = 9.86$, and by its molecular ion in the mass spectrum of the crude mixture (m/z , 121, 118; M^+ (**75**, **72**)).

An explanation for the apparent lack of reactivity of **72** toward DIBAL reduction at low temperatures could be that the anion formed from the initial acid-base reaction, even if it is able to coordinate a second DIBAL molecule, is unreactive to subsequent hydride delivery due to stabilisation of the iminium ion from delocalisation of the negative charge. The small amount of aldehyde observed after work-up could be due to a competitive process acting upon neutral **72** (Scheme 3-21).

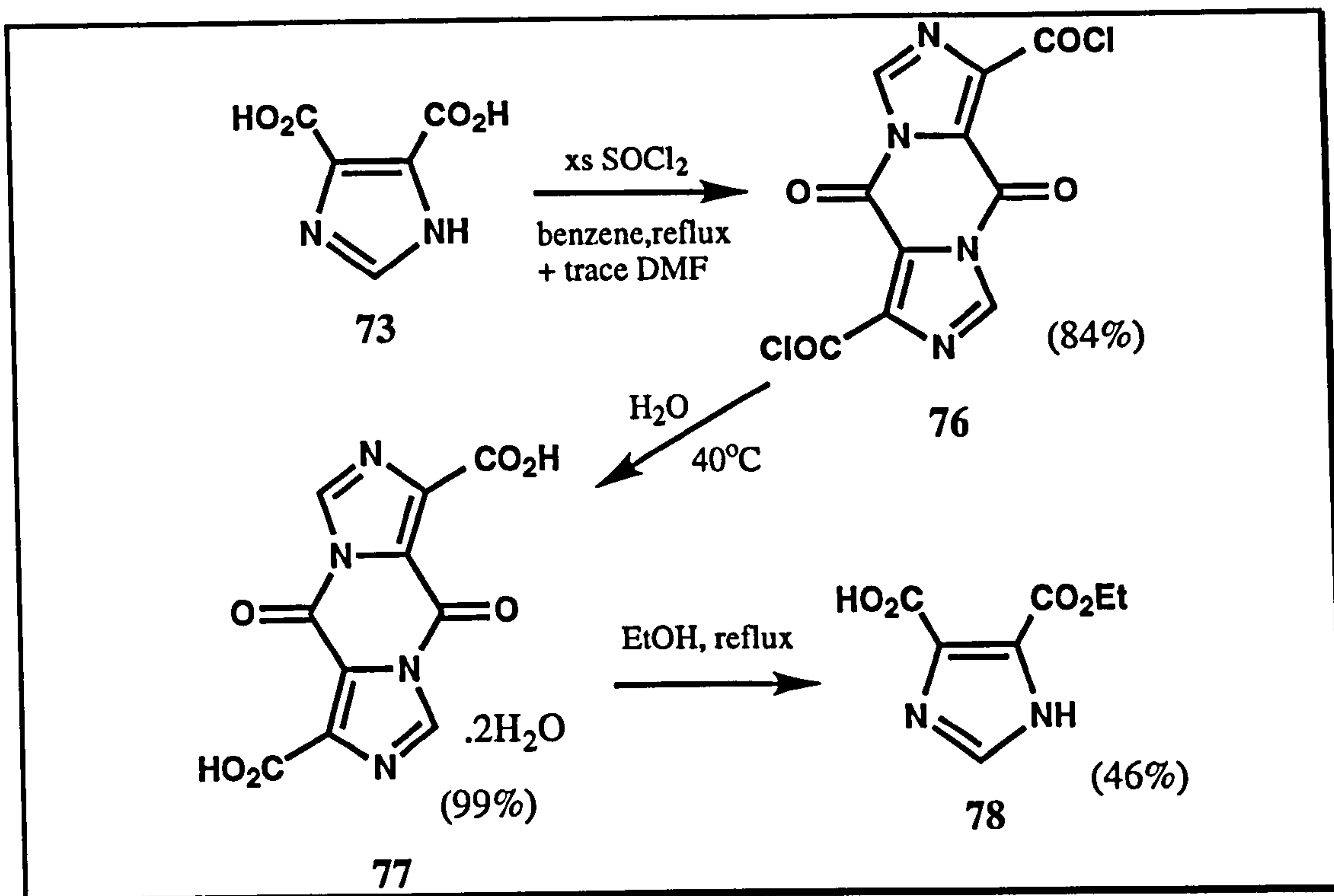


Scheme 3-21 Possible explanation of low reduction yield in low temperature DIBAL reduction of **72**.

Reduction of dicyanoimidazole **72** was then attempted by the method of Fry and Ott,¹²⁷ which involves nitrile *N*-alkylation followed by triethylsilane reduction of the resulting activated *N*-alkylnitrilium ion, and subsequent hydrolysis to yield the aldehyde. The procedure has been successfully applied to the selective reduction of a dicyano compound, reaction upon 1,4-dicyanobenzene giving 4-formylbenzonitrile in good yield.¹²⁷ Employing triethyloxonium tetrafluoroborate as the alkylating agent, reaction of imidazole **72** in both dichloromethane as per the reference, and in THF, more able to dissolve **72**, appeared to return starting material largely intact in both cases, with none of the desired monoaldehyde **75** observed by ¹H NMR.

It would appear that no alkylation of **72** occurred. The procedure could be repeated with alternative alkylating agents, for example dimethylsulfate, but such alternatives were not investigated.

So that more rigorous conditions could be employed, an imidazole with different 4- and 5- substituents was sought, to use chemoselectivity to generate the formyl group, rather than hope for regioselective reduction of identical groups as with dicyanoimidazole **72**. Following a literature procedure,^{128,129} 5(4)-ethoxycarbonylimidazole-4(5)-carboxylic acid (**78**) was synthesised in three steps from the dicarboxylic acid **73**, in 38% overall yield (Scheme 3-22). The low yield of the final step is thought to be due to a competing hydrolysis of tricycle **77** to **73** involving the waters of crystallisation.¹²⁹ Monoester **78**, recrystallised from methanol, had mp 205-207°C (decomp.) (lit.¹³⁰ 215-216°C), ¹H NMR in agreement with the literature,¹²⁹ and ¹³C NMR and mass spectrum analysis consistent with the assigned structure.



Scheme 3-22 Synthesis of monoester, 78 according to the literature.

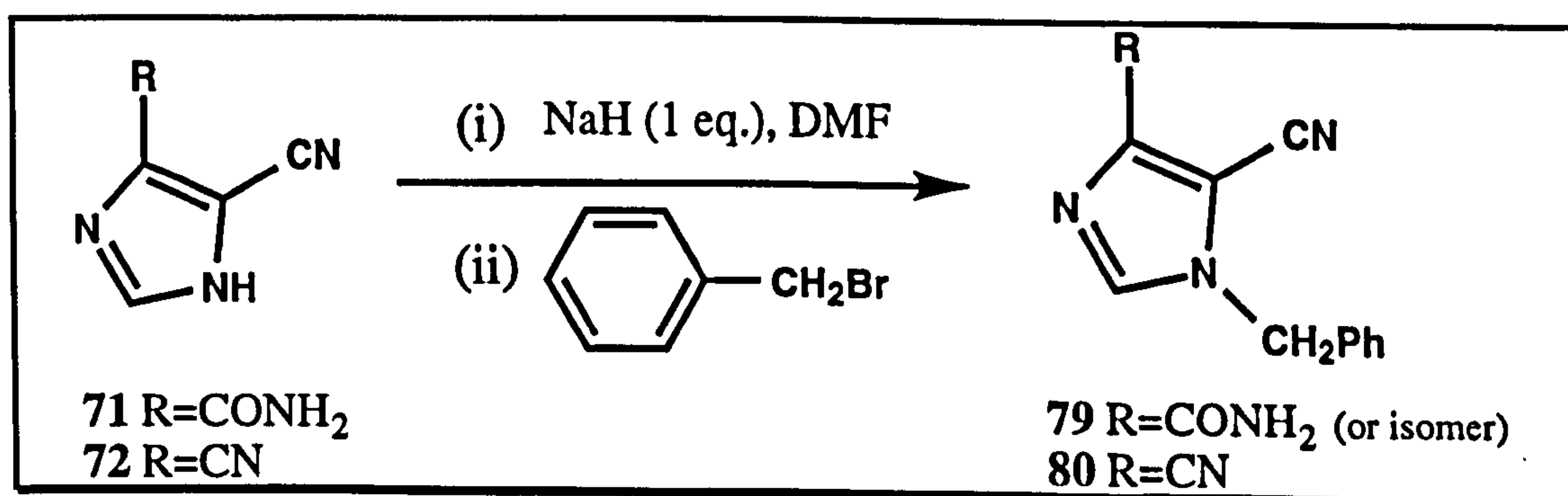
Reduction of monoacid 78 was first attempted with borane-dimethyl sulfide complex, a procedure used to selectively reduce carboxylic acids to alcohols, or from which the intermediate trialkylborane may be directly oxidised to the aldehyde, for example with pyridinium chlorochromate (PCC) (ref.131, p.596). Two borane-dimethyl sulfide reductions of 78 were attempted, one in diethyl ether, and one in THF as solvent, both employing three equivalents of the borane complex as suggested in the presence of amines.¹³² In both cases unreacted acid 78 was returned as the major component, with a mixture of minor components not readily identifiable by ^1H NMR spectroscopy.

The reasoning for the lack of reactivity of 78 towards this borane reagent may mirror the lack of reactivity of the cyano compound 72 to DIBAL reduction, where it was postulated that the intermediate adduct, normally able to activate the

carbon centre to hydride delivery, is counteracted by the electron donating ability of the imidazole ring.

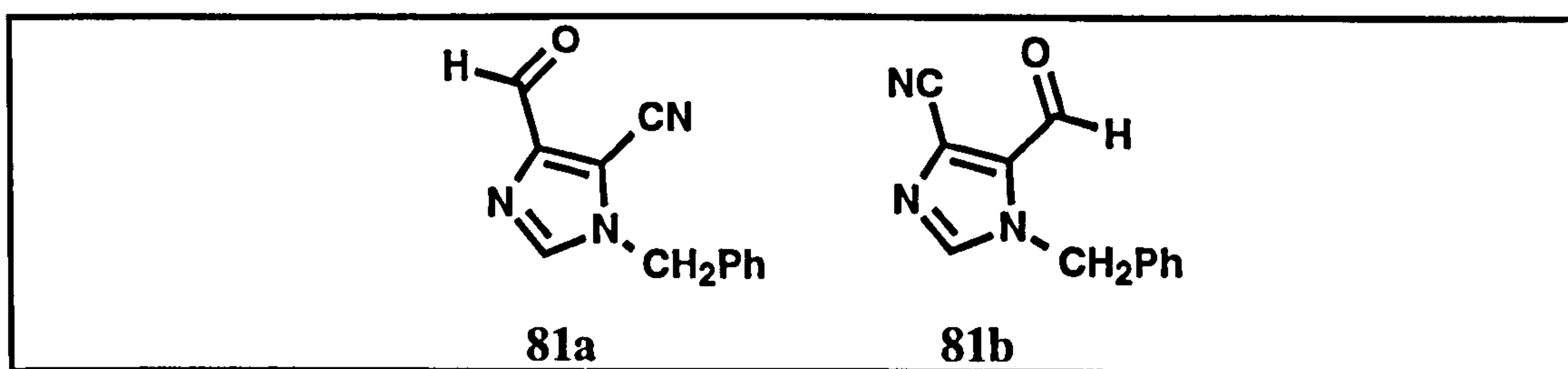
3.2.3 Reduction of *N*-protected imidazoles

Despite the allowance for the free imidazole NH in the borane reactions, as with the DIBAL reductions of **72**, the free imidazoles studied do not seem to be sufficiently reactive under the conditions investigated. Attention then shifted to the reduction of *N*-protected imidazoles. The *N*-benzyl derivative **80** was synthesised in 73% yield from **72** according to the literature method and had mp 128-130°C (lit.¹³³ 124-127°C). The amide **79**, previously reported by a two step procedure from imidazole **80** without experimental details,¹³⁴ was prepared in 75% yield directly from the free imidazole **71** in an analogous manner to the synthesis of **80** (Scheme 3-23). The benzyl group was chosen for its resistance to the reductive and hydrolytic conditions likely to be encountered in these studies, and such *N*-benzyl imidazole derivatives have been cleaved in the presence of other reducible groups by catalytic transfer hydrogenation with 1,4-cyclohexadiene and palladium black catalyst.¹³⁵



Scheme 3-23 *N*-Benzylation of imidazoles **71** and **72**.

With the objective being the selective partial reduction of one of the 4- or 5-substituents of **79** or **80**, amide **79** would be expected to be better with two different substituents, but as with the unprotected precursor **71**, amide **79** suffered from very poor solubility and of the few reductions of **79** attempted this proved to be a severe limitation. Reductions of the dicyanoimidazole **80** were studied in more depth, which if successful would lead to the production of one or both of the possible isomeric monoaldehydes, **81a** and **81b**.



The outcome of DIBAL reduction of **80** was found to be highly solvent dependent. When performed in THF or in toluene, DIBAL reduction of **80** was slow, unreacted reductant leading to hydrogen evolution on acidic work-up. The difference was that in THF, even after warming from -70 to 0°C , the result was starting imidazole **80** (80%) and one of the monoaldehydes **81a** (20%), whereas a low temperature reaction in toluene gave **80** (65%), monoaldehyde **81a** (4%) and the dialdehyde **82** (31%), as determined by analysis of the ^1H NMR spectra.

Curiously, in the 250 MHz ^1H NMR spectrum of the dialdehyde **82**, one of the aldehyde signals appeared as a singlet and the other as a 1 Hz doublet. Long range 2-D ^1H - ^1H COSY NMR showed coupling of this aldehydic doublet to the imidazole 2-H proton. This phenomenon can be rationalised by considering possible canonical forms of dialdehyde **82** (Figure 3-3). In the figure, for the sake of illustration both aldehydes in the starting canonical **82a** are shown in their

ionised forms. Though all the canonicals are inter-convertible, only the positive charge of the 5-CHO group can be shared onto C-2 of the imidazole in a single electron flow (82a-82b-82c). This direct interaction between C-2 and the 5-CHO group leads to enhancement of the through bond coupling between the 2-H and 5-CHO to an observable level. The dialdehyde derivative 82 has been reported previously, but without NMR data, precluding comparison.¹³⁶

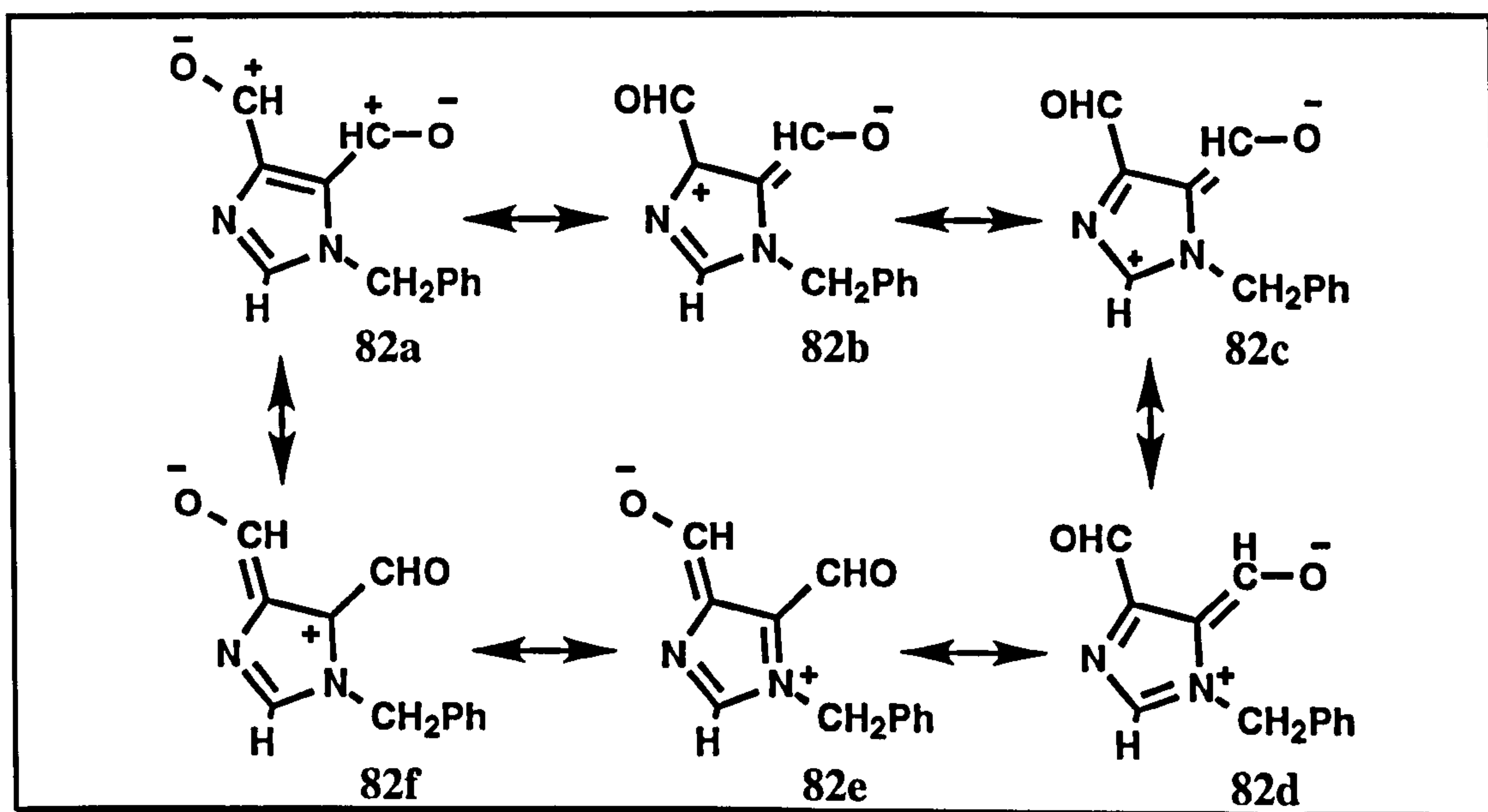


Figure 3-3 Canonical structures of dialdehyde 82.

Stannous chloride reduction of imidazole 80 with ethyl acetate as the solvent was also attempted under the conditions of the Stephen reaction, a method generally reserved for the partial reduction of aromatic nitriles to aldehydes (ref. 131, p. 595). A mixture of products resulted after the hydrolysis step, but with no sign of aldehydes by ^1H NMR. Instead, NMR signals characteristic of an imidazolecarboxamide were present, possibly formed by nitrile hydrolysis under the acidic conditions employed.

Reduction of protected imidazole **80** was then performed according to the method of Brown and Garg, who employed lithium triethoxyaluminium hydride ("LiHAl(OEt)₃") prepared *in situ* as an ethereal suspension, for the partial reduction of nitriles to aldehydes.¹³⁷ Initial LiHAl(OEt)₃ reductions of **80** showed potential, and reactions in a variety of solvent mixtures were investigated with normal or reverse addition of reagents. In all cases studied, mixtures of the two isomeric monoaldehydes (**81a** and **81b**), the dialdehyde (**82**) and starting material resulted. The respective proportions of **80**, **81a**, **81b** and **82** were estimated by analysis of integrations for the benzylic protons in the ¹H NMR spectra of the crude mixtures (see Table 3-3 for optimised results in reactions at 0°C).

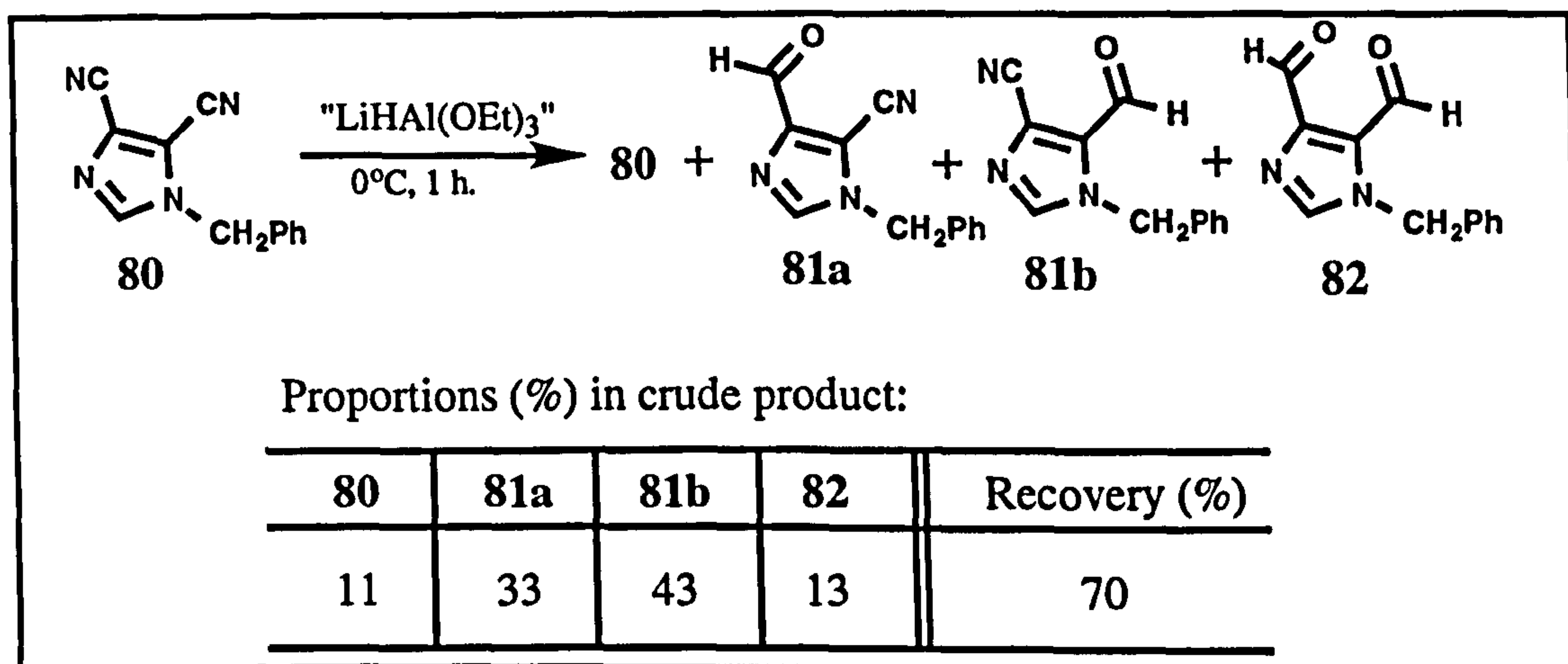


Table 3-3 Optimised results for lithium triethoxyaluminium hydride reduction of **79** at 0°C.

Brown and Garg reported diethyl ether as the most effective general solvent, but in this situation reaction in a mixture of ether (in which the lithium aluminium hydride was supplied) and THF gave consistently more reliable results. Firstly, imidazole **80** was more soluble in THF, and also the LiHAl(OEt)₃ prepared remained in solution, rendering the addition of reagents an easier operation, whichever order of addition was used.

The unknown monoaldehydes **81a** and **81b** were tentatively assigned as the 4- and 5-formylimidazoles respectively on the basis of their deuteriochloroform ^1H NMR spectra, with reasoning analogous to the assignment of the dialdehyde **82** - one of the aldehydic protons of one of the monoaldehydes appeared as a singlet and the other appeared as a 1 Hz doublet (Figure 3-4). Interestingly, as observed in the ^1H NMR of the two aldehyde groups of **82**, the CHO doublet of **81b** was at lower field than the CHO singlet of **81a**.

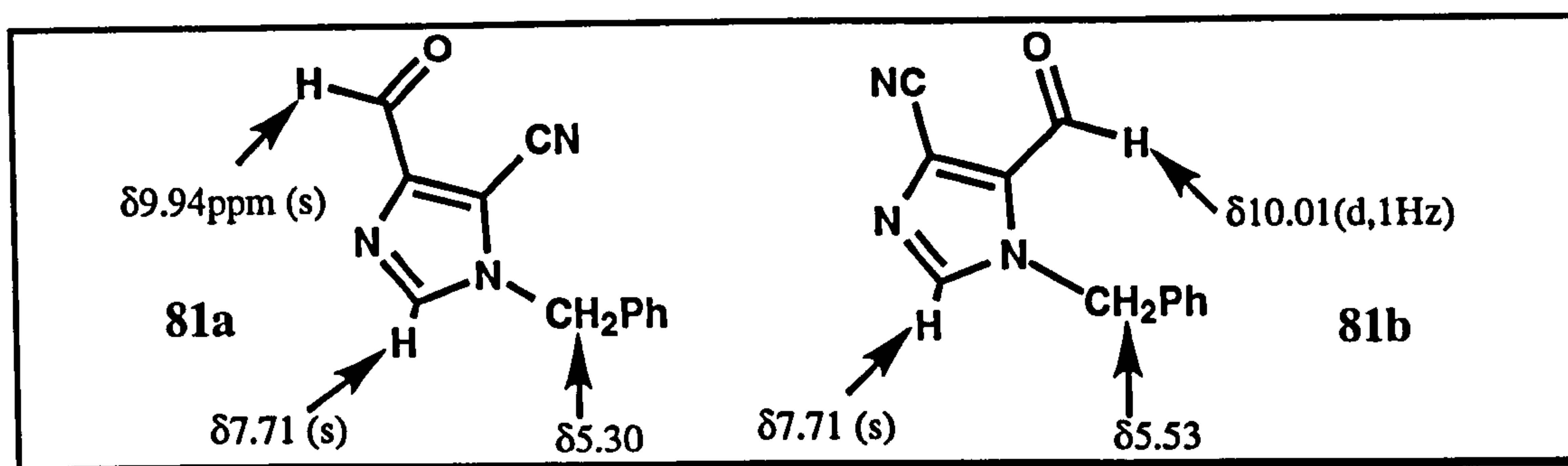
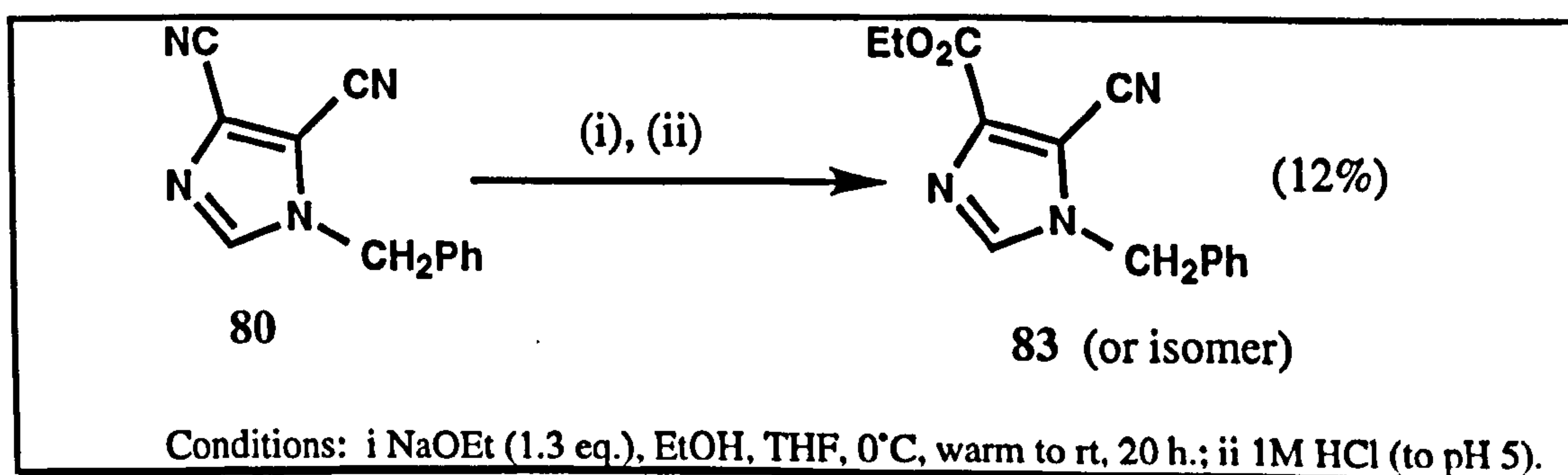


Figure 3-4 Suggested assignment of the ^1H NMR spectra of the isomeric monoaldehydes **81a** and **81b**, in deuteriochloroform.

Although at first glance the yield of monoaldehyde is good, comprising 75% of the crude product, only partial separation of components was possible by silica gel column chromatography such that monoaldehyde **81a** co-eluted with the dialdehyde **82**, and monoaldehyde **81b** co-eluted with starting imidazole **80**, precluding complete characterisation of the formylimidazoles. Debenzylation would reduce the number of components to three, with the desired monoaldehyde **75** comprising 75%, but experience in the DIBAL reductions of the free imidazole **72** also gave problems in the separation of **72** and aldehyde **75**, and debenzylation was not attempted.

The optimum results in the LiAl(OEt)_3 reduction of **80** at 0°C (Table 3-3) suggested the second reduction to be marginally slower than the first. However, LiAl(OEt)_3 reduction of **80** at lower temperatures showed only marginal improvement in selectivity against dialdehyde formation, and the overall much slower reaction created additional problems. For example, slow addition of reductant to **80** over 1.5 hours at -70 to -50°C and allowing to warm to -10°C over 1 hour yielded, after work-up, a crude product consisting of **80** (23%), **81a** (37%), **81b** (32%) and **82** (7%).

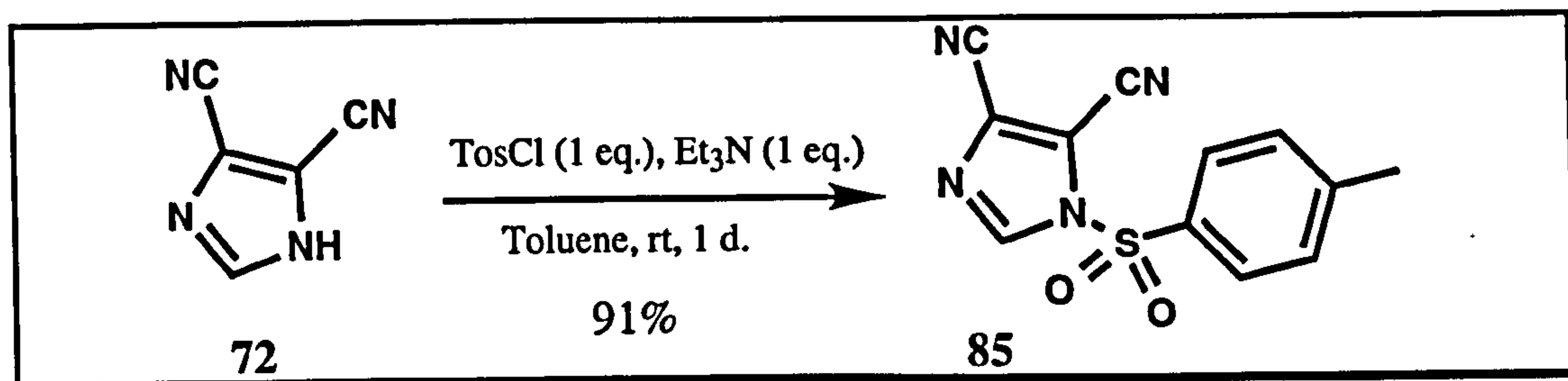
It was of interest to determine whether modification of one of the cyano groups of **80** would improve selectivity for monoreduction by LiAl(OEt)_3 or DIBAL. Bridson and Lambert,¹³⁴ reported the synthesis of ethyl 1-benzyl-5-cyanoimidazole-4-carboxylate (**83**) in good yield, by reaction of the dicyanoimidazole **80** with sodium ethoxide, but without experimental details. An attempt to perform this transformation gave, after column chromatographic purification, a solid with mp 110°C and NMR spectra consistent with the structure **83** (or other isomer), but in only 12% yield (Scheme 3-24). The remaining products were not readily identified, though a number of other ethyl environments were observed by ^1H NMR spectroscopy. Given the poor return of product this approach was not investigated further and no reductions of monoester **83** were carried out.



Scheme 3-24 Monoethanolysis of dicyanoimidazole **80**.

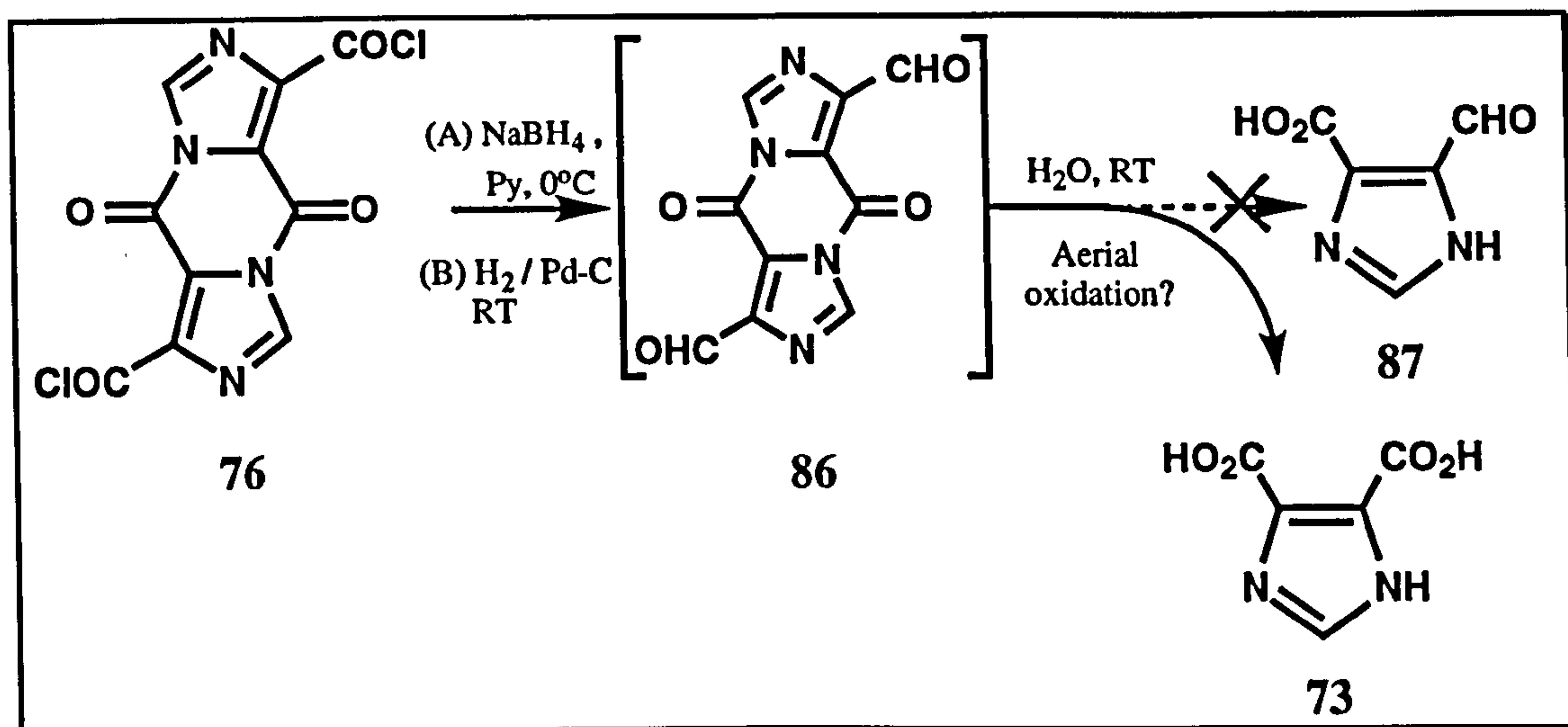
was more readily visible (though the signals of the ethyl carbons were reduced in intensity).

As an alternative to cyano group derivatisation attempted previously, attempts were made to protect the imidazole **72** with more electron-withdrawing groups that could alter the relative reactivities of the two cyano groups, promoting regioselectivity in the DIBAL and $\text{LiAl}(\text{OEt})_3$ reductions for example. Failing that, a mixture of monoaldehydes and dialdehyde might be more readily separated than is the case with a benzyl protecting group. By virtue of the stability of the imidazolyl anion, protected imidazoles are generally cleaved under far milder conditions than required for the cleavage of analogously protected amines.¹³⁸ The previously unreported *N*-tosyl derivative **85** of the dicyano-imidazole **72** was prepared in good yield (91%) by its reaction with tosyl chloride and triethylamine in anhydrous toluene (Scheme 3-26). However, on storage of the solid product, decomposition began to occur within a week, presumably by uptake of moisture to hydrolyse **85** back to **72** and toluene sulphonic acid, and no reductions of this derivative were investigated. An attempt to form the *N*(1)-*tert*-butoxycarbonyl (BOC) derivative of **72** with the anhydride $(\text{BOC})_2\text{O}$ (di-*tert*-butyl dicarbonate) and catalytic 4-dimethylaminopyridine (DMAP), according to the method of Grehn and Raynarsson for the preparation of 1-BOC-pyrroles,¹³⁹ failed completely. This lack of reaction serves to demonstrate the effect of the second nitrogen in the heterocyclic ring, the two electron-withdrawing cyano groups favouring imidazolyl anion formation further.



Scheme 3-26 Synthesis of 1-tosylimidazole, 85.

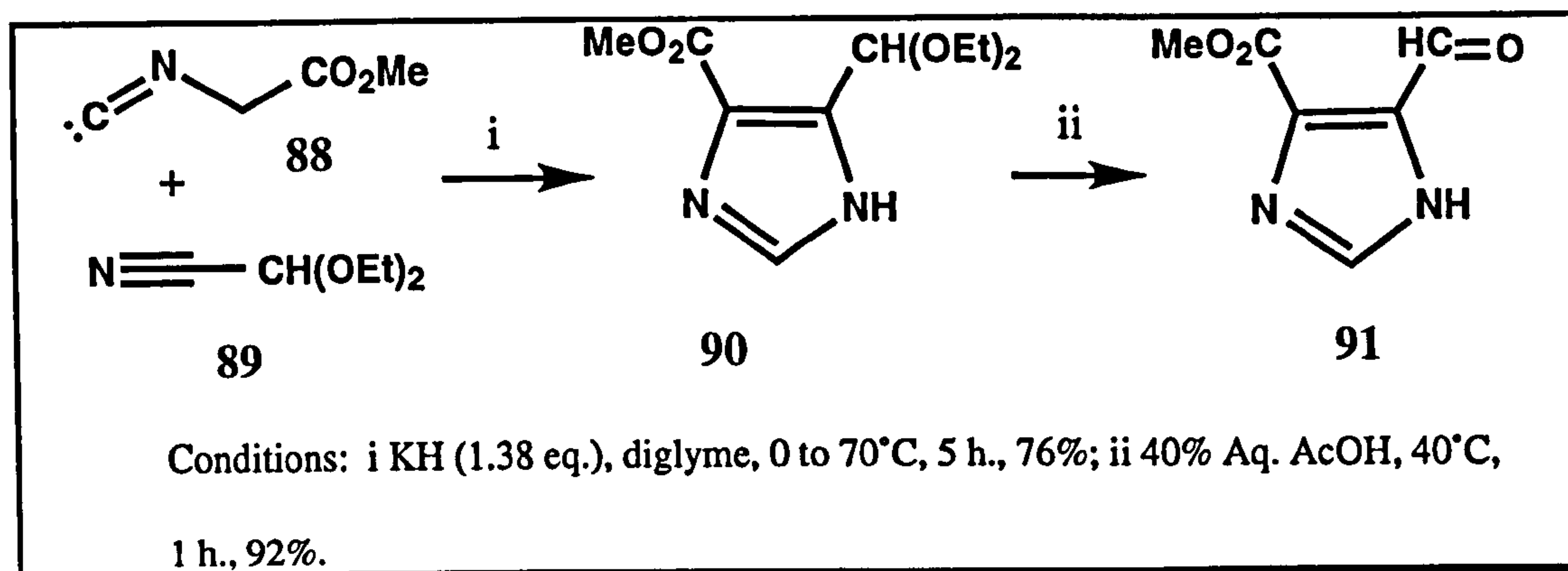
In looking for an alternative reduction approach, reviewing the work already carried out presented another option. In the synthesis of the ester 78, one of the intermediates, the tricycle 76, is effectively a 1-protected-5-carbamoyl-imidazole-4-carboxylic acid chloride. A number of different methods exist for the conversion of acid chlorides to aldehydes. Room temperature, atmospheric pressure hydrogenation with a palladium on carbon catalyst according to a modified Rosenmund procedure of Burgstahler *et al.*;¹⁴⁰ and sodium borohydride reduction at 0°C in THF/ DMF, with pyridine as a borane scavenger to prevent over-reduction, by the procedure of Babler,¹⁴¹ were performed upon 76, the hope being that hydrolysis of an intermediate dicarbalddehyde 86 would yield 5(4)-formylimidazole-4(5)-carboxylic acid (87). Under both sets of conditions a rapid reaction was apparent, but after prolonged aqueous hydrolysis of the crude products from either method, the major component was imidazole-4,5-dicarboxylic acid (73), by comparison with the authentic compound. There are two alternative explanations for this observation: either reductions were not as successful as thought, and hydrolysis of the crude product of these reactions constituted hydrolysis of 76, which would give 73 directly; or the reductions were successful, but at some stage of the hydrolysis of the intermediate dialdehyde 86 aerial oxidation occurred to give the diacid 73 (Scheme 3-27).



Scheme 3-27 Attempted reduction of imidazole acid chloride **76**.

3.2.4 Direct imidazole-ring synthesis

Selective partial reductions of carboxylic acid derivatives linked to imidazoles appeared to present a number of difficulties, and other methods for the introduction of a formyl group onto an imidazole were sought. Reexamination of the literature revealed a one step preparation of methyl 5(4)-diethoxymethyl-imidazole-4(5)-carboxylate (**90**) by reaction of methyl isocyanoacetate (**88**) and diethoxyacetonitrile (**89**).¹⁴² The diethoxymethyl group is a synthetic equivalent of the formyl group, and in this paper anecdotal evidence for the hydrolysis of **90** to the formyl derivative **91** was reported, though without experimental details. In the present work, the imidazole **90** was prepared according to the literature method,¹⁴² and conditions were found for its hydrolysis to the formylimidazole **91** in 92% yield (Scheme 3-28).

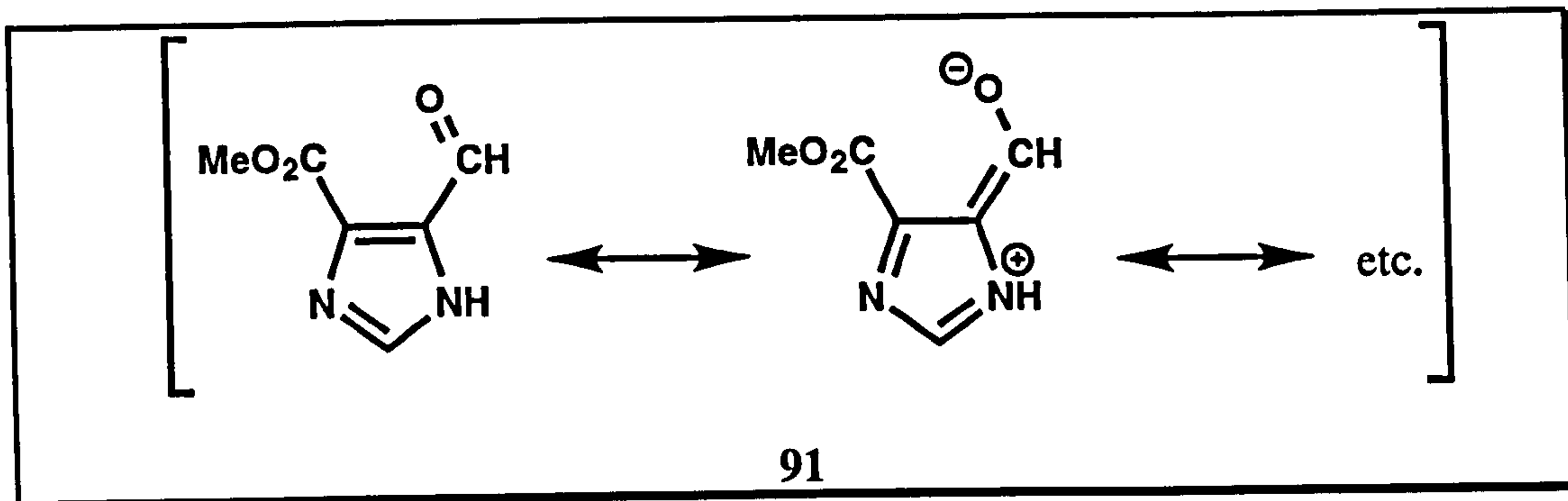


Scheme 3-28 Synthesis of 5-formylimidazole 91.

3.2.5 Knoevenagel condensation reactions of 5-formylimidazoles

With a formylimidazole **91** in hand, at last, Knoevenagel condensation reactions were attempted with ethyl nitroacetate. Reaction in the presence of titanium(IV) chloride and pyridine, according to the method used by Végh and coworkers¹⁴³ in condensations between furancarbaldehydes and nitroacetates, was unsuccessful, returning **91** and ethyl nitroacetate essentially intact with only minor by-products.

This lack of reactivity towards a carbon nucleophile parallels the difficulty found reducing cyanoimidazoles in the previous sections, by all but the most reactive reductants. Electron donation from the heterocyclic ring decreases the electrophilic character of the formyl carbon, deactivating it to nucleophilic attack, even in the presence of the Lewis acid titanium tetrachloride (Figure 3-5). Imidazole deprotonation in the presence of pyridine is likely to further favour the deactivation of the formyl group.



Scheme 3-28 Potential deactivation of the 5-formyl group of 91 to nucleophilic attack.

In the hope of establishing the importance of the NH group in precluding reaction, it was of interest to attempt Knoevenagel condensation upon the *N*-benzyl formylimidazoles prepared in the $\text{LiAl}(\text{OEt})_3$ reductions of 80. As mentioned previously, monoaldehydes 81a and 81b were not isolated pure, but a sample of 81b was isolated by silica gel column chromatography with the dicyanoimidazole 80 as the only contaminant (35%). Imidazole 80 should not interfere with any condensation reaction, which was performed on this mixture and ethyl nitroacetate under a number of conditions.

A repeat of the titanium tetrachloride catalysed reaction of this impure 81b returned only starting materials. Reaction in pyridine with a catalytic amount of piperidine, used for the condensation of aromatic aldehydes and malonic acid,¹⁴⁴ again returned the starting materials. Condensation was also tried according to the conditions reported satisfactory for the condensation of aromatic aldehydes and azido acetates,¹²² which involved adding a mixture of alkyl azidoacetate and the corresponding alkoxide ion to the aldehyde at about 0°C. Reacting the mixture of 81b and 80 with ethyl nitroacetate (pretreated with sodium ethoxide) at low temperatures did not promote a reaction, and after refluxing in ethanol a mixture of products resulted. Silica gel column chromatography of the mixture was unable

to completely separate the components, and none could be adequately characterised.

It would appear that the benzyl group, though masking a potentially acidic proton, is unable to prevent aldehyde deactivation by the delocalisation route suggested above with **91**, and derivatives of aldehyde **91** with more deactivating imidazole protecting groups were sought. However, mirroring the difficulties observed in the preparation of 1-tosyl and 1-BOC derivatives of dicyanoimidazole **72**, all attempts to protect the imidazole NH of **91** failed. Reaction of **91** with tosyl chloride (successful in the protection of imidazole **72**) returned only starting material; no 1-BOC derivative was formed by reaction of **91** with "BOC-ON" (2-*tert*-butoxycarbonyloxyimino-2-phenylacetonitrile; and attempted protection of **91** as the 1-formamide by reaction with formic acid according to a number of literature procedures,¹⁴⁵⁻¹⁴⁷ also returned only starting material, on all occasions.

While affording some valuable insights into the chemistry of these substituted imidazoles, given the difficulties encountered by all the synthetic approaches to formylimidazoles investigated, it was of some disappointment to find the formylimidazoles finally prepared unreactive toward Knoevenagel condensation with ethyl nitroacetate.

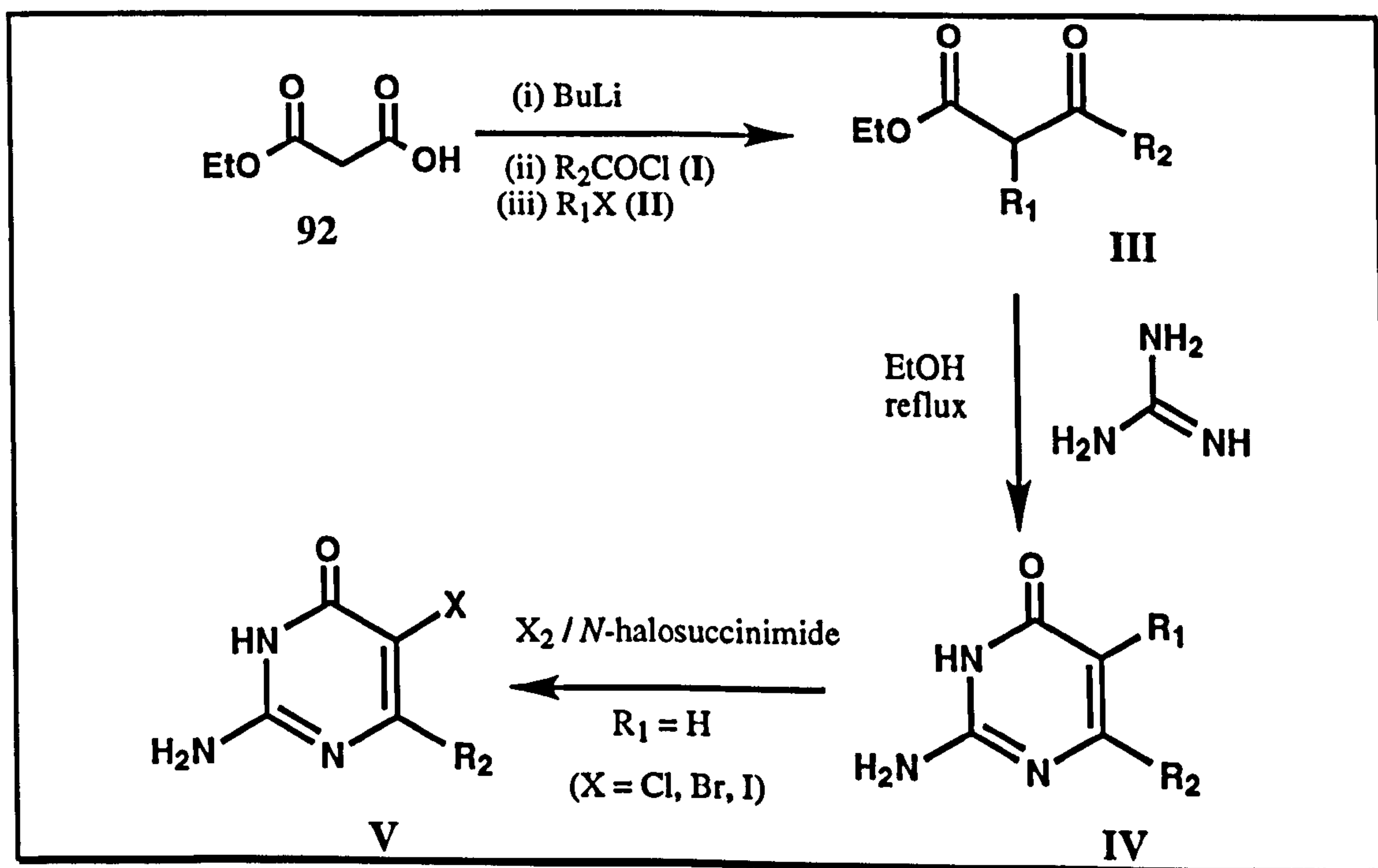
Future studies in this area might look at more stable imidazole protecting groups, that still have sufficient electron-withdrawing ability to reduce the formyl-destabilising activity of the N-1 nitrogen lone pair. A 2,4-dinitrophenyl derivative for example, though still reactive to nucleophilic attack - being generally cleaved with mercaptoethanol (ref. 138, p. 390) - is strongly mesomerically electron-withdrawing, and expected to be more resistant to hydrolytic cleavage than acyl,

carbonate and sulfonate derivatives. Also, the intermediate imidazole **90** might prove a better subject for imidazole NH protection, without the influence of a formyl group helping to stabilise a deprotonated species and prevent protection.

4. Aminopyrimidinones

4.1 General 2-aminopyrimidin-4(3*H*)-one synthesis

At the time of the initial pyrimidinone analogue studies mentioned in the introduction, aimed at finding a successor to the interferon inducer **19** (ABMP), a general synthetic route for pyrimidinone preparation was devised (Scheme 4-1).⁶⁸ Requisite β -keto esters (**III**) were prepared from monoethyl malonate (**92**) and appropriate acid chlorides (**I**), and the intermediate anion formed could be alkylated with alkyl halides (**II**).¹⁴⁸ The β -keto esters (**III**) were then refluxed with guanidine, or guanidine salts such as the carbonate, to give the pyrimidinones (**IV**) directly. In the case of $R_1 = H$, halogenation readily occurred at the 5-position to give 5-halogenopyrimidinones (**V**).



Scheme 4-1 2-Aminopyrimidinone synthesis.

In this programme, the pyrimidinones **19** to **21**, **26** and **93** to **97** were prepared in good yields, starting from commercially available β -keto esters (Table 4-1, yields are from β -keto esters). The initial 5-H-pyrimidinones **26**, **93** and **94** were prepared according to the method of Kulkarni *et al.*,¹⁴⁹ by refluxing the β -keto ester with guanidine carbonate in ethanol. The pyrimidinones thus formed were then either brominated according to the method of Stevens *et al.*,⁸⁵ heating with bromine in acetic acid; or they were iodinated with iodine in a mixture of chloroform and aqueous sodium hydroxide, as reported by Skulnick *et al.*⁶⁹ All the pyrimidinones formed were characterised by ^1H NMR, and melting points were in agreement with literature values.

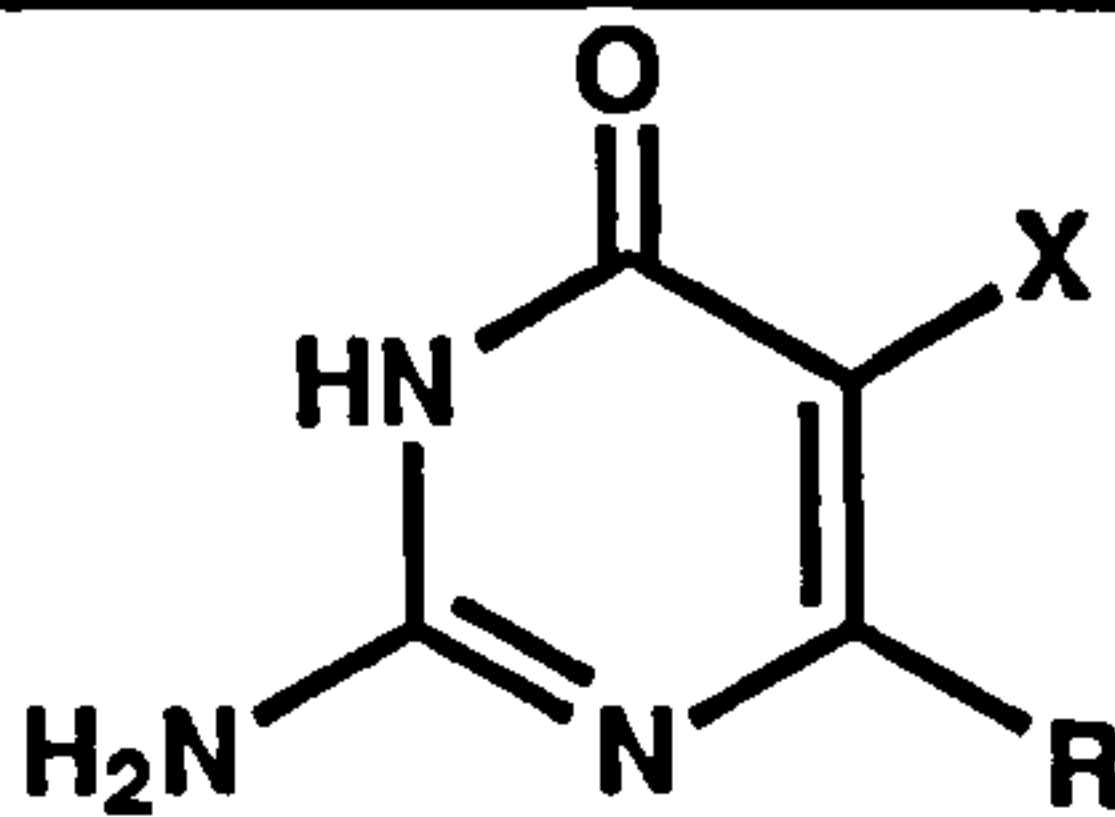
							
	R	X	Yield/ %		R	X	Yield/ %
19	Me	Br	55	94	Et	H	86
20	Ph	Br	48	95	Et	Br	48
21	Ph	I	62	96	Me	I	71
26	Ph	H	69	97	Et	I	72
93	Me	H	83				

Table 4-1 Synthesised pyrimidinones.

As previously mentioned, a wide range of substituted 2-aminopyrimidin-4(3*H*)-one derivatives has been prepared according to the procedure of Scheme 4-1, and their chemistry has been studied at length.⁸³⁻⁸⁵ In the present work a number of discrete novel derivatives were sought as targets, but also the

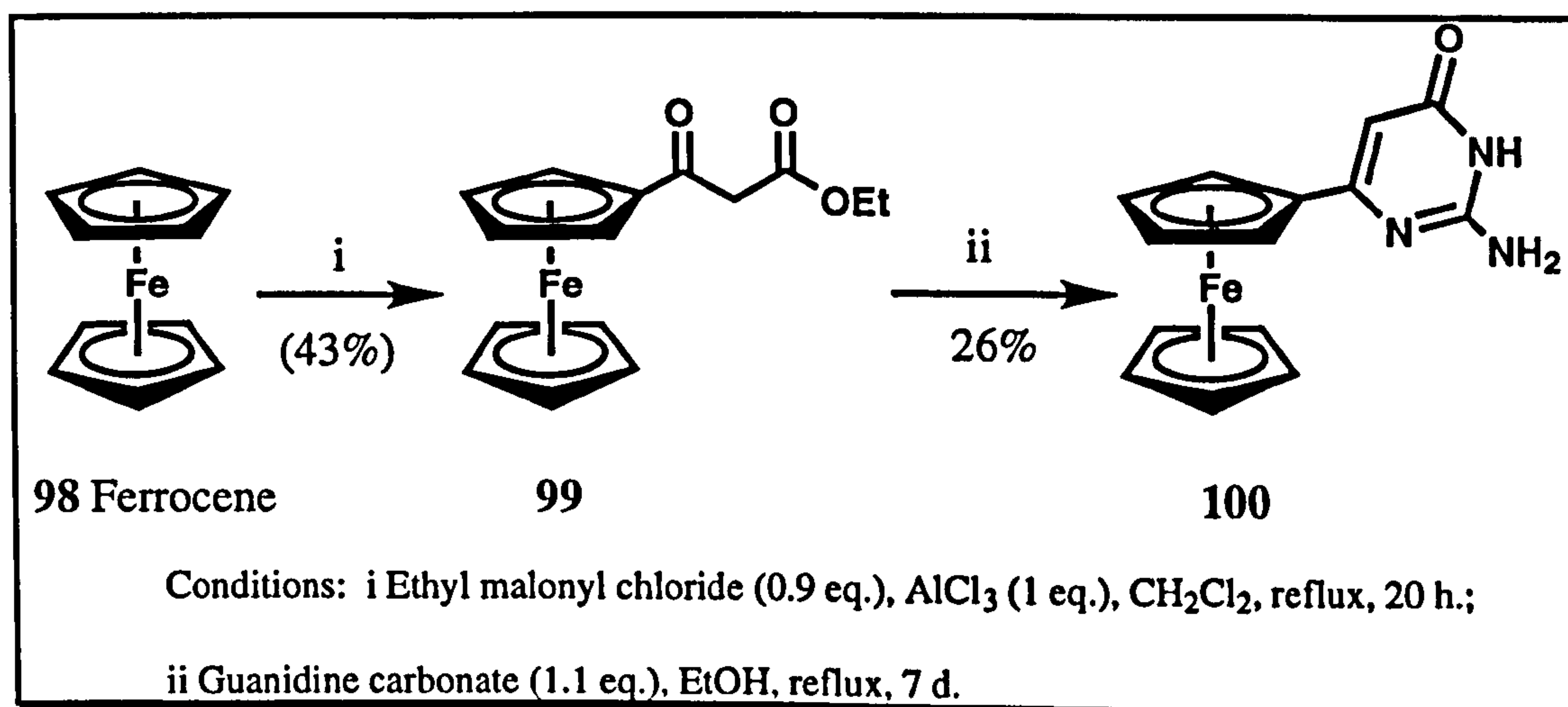
development of new chemistry applicable to the synthesis of a range of new derivatives was investigated. The results of these studies are described in the following sections.

4.2 Ferrocenyl derivatives

Given the previous discovery of the novel crystal structure of broprimine (20),⁷⁷ it was of interest to prepare pyrimidinone derivatives with different aromatic substituents in the 6-position. It was hoped that studies of crystal structures of other derivatives might shed light on the influence of the 6-substituent on the structure adopted, as well as investigating π -stacking interactions in the solid state. The cyclopentadienyl rings of ferrocene presented a unique opportunity to link the pyrimidine ring to a novel group, and routes were sought to prepare two derivatives - with ferrocene singularly attached to the 6-position of a pyrimidine ring (100), and with each of the cyclopentadienyl rings similarly bonded to pyrimidines (103).

A further interest in such derivatives, as well as their structural novelty, stems from the putative mode of action of the immunomodulatory broprimine (20), thought to involve interaction with guanine or cytosine bases of DNA. If derivatives 100 and 103 were able to bind in this fashion, then they may be able to deliver iron, known to cleave DNA (for example the chemistry of the involvement of iron in the DNA cleaving action of the antitumour agent bleomycin is well known, and has been reviewed¹⁵⁰), thereby bringing about a potentially cytotoxic event.

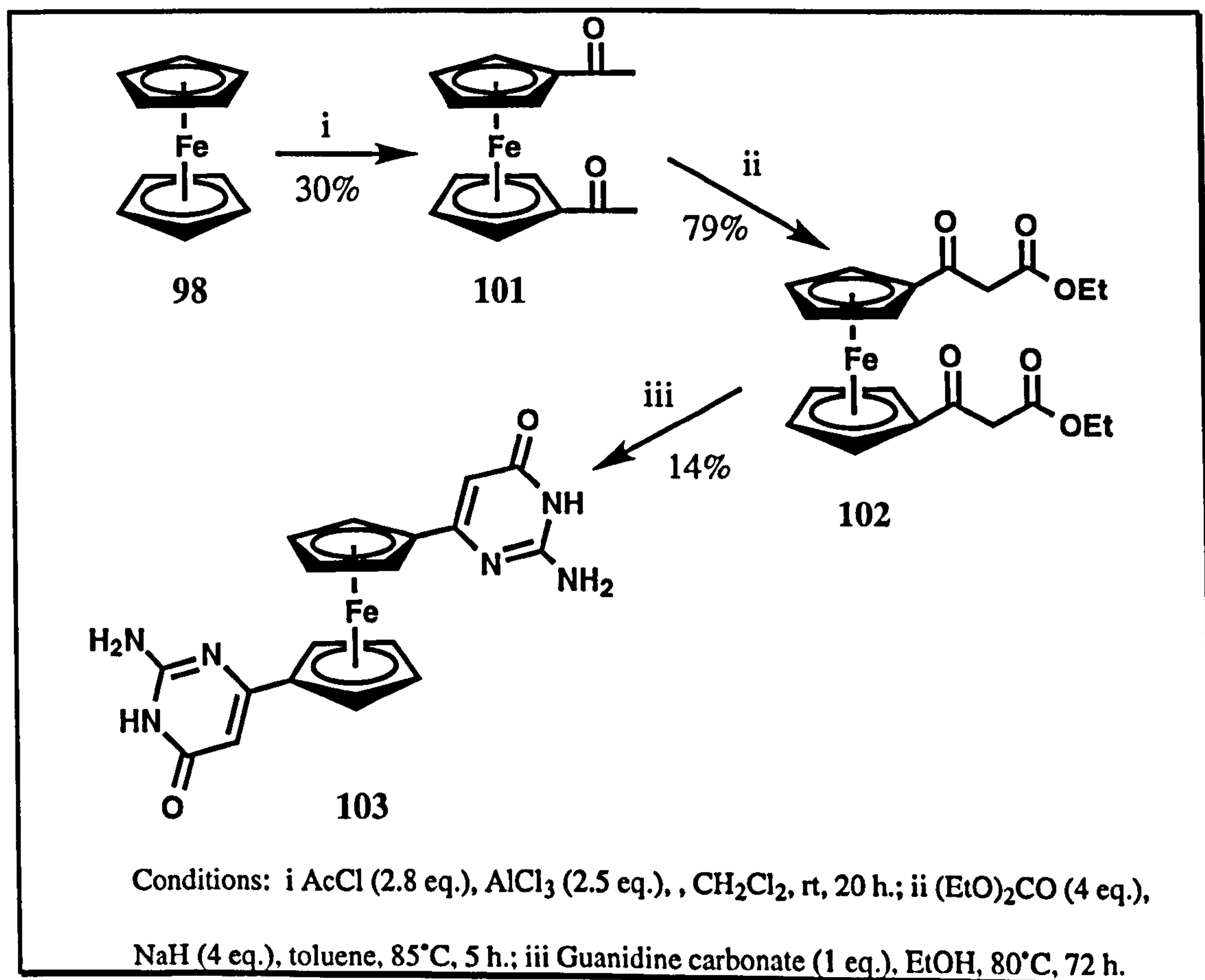
The mono-substituted ferrocene derivative **100** was successfully prepared in two steps, in low yield (11%) from ferrocene (**98**) (Scheme 4-2). The known β -keto ester **99** was prepared by Friedel-Crafts acylation of ferrocene (**98**) with ethyl malonyl chloride, according to the procedure of Dormond.¹⁵¹ Purified by silica-gel chromatography, **99** was then reacted with guanidine carbonate under the standard conditions to furnish the pyrimidinone **100**, which readily crystallised from ethanol as purple prisms. The reaction with guanidine carbonate was considerably slower than the usual pyrimidinone formation reactions, traces of **99** persisting after a week at reflux, despite periodic additions of guanidine carbonate.



Scheme 4-2 Synthesis of ferrocenylpyrimidinone **100**.

An attempt to adapt the above synthesis of **99** to give the bis- β -keto ester **102**, employing two equivalents of ethyl malonyl chloride, yielded a mixture of the orange monoester **99** and the desired red diester **102**. The mono and bis derivatives **99** and **102** have very similar R_f values, proving to be difficult to separate by silica gel column chromatography. Reaction of a fraction partially

purified by chromatography, (thought to be richest in ester **102**), with guanidine carbonate, gave only the pyrimidinone **100**, seen previously. The required β -keto ester **102** was finally prepared in two steps from ferrocene (**98**), according to the literature. Firstly, Friedel-Crafts acylation of ferrocene with acetyl chloride gave bisacetylferrocene **101**,¹⁵² which after recrystallisation was reacted with diethyl carbonate in the presence of sodium hydride¹⁵³ to furnish the β -keto ester **102** in moderate overall yield. Reaction of **102** with guanidine carbonate then gave the desired pyrimidinone **103** as an almost black solid, in low yield, as its DMF solvate (14%), and a significant amount of material that showed no resonances by ^1H NMR (Scheme 4-3).



Scheme 4-3 Synthesis of bispyrimidinylferrocene **103**.

A curious feature of the mass spectrum of **100** was that in two independent EI⁺ (electron impact) experiments, one showed the molecular ion (m/z 295) as the parent peak, but under different conditions the highest mass observed corresponded to the uptake of water (m/z 313).

Pyrimidinone **103** had rather unique physical properties: not melting with heating to 360°C, producing no EI⁺ mass spectrum with heating of the sample probe to 500°C, and due to its very poor solubility in all solvents encountered was only identified by ¹H NMR. Such properties are explained by the potential of **103**, if it forms hydrogen bonds in the solid equivalent to those of bropirimine (**20**), to form an extended hydrogen bonded polymer by self assembly.

The ¹H NMR spectra of both ferrocenylpyrimidinones **100** and **103** are shown in Figure 4-1. In both examples, as well as resonances entirely characteristic of the NH, NH₂ and H-5 protons of pyrimidinones, the resonances of the substituted cyclopentadienyl rings appear as two pseudo-triplets (expanded regions), and the unsubstituted cyclopentadienyl ring of **100** appears as a singlet, indicating unrestricted independent rotation of the ferrocene rings. Unfortunately, pyrimidinone **103** seemed to decompose on storage, it proving impossible to prepare fresh NMR samples from the original solid, precluding more complete analysis. The β-keto ester **102** also appeared to degrade on storage, and no more pyrimidinone **103** could be readily prepared.

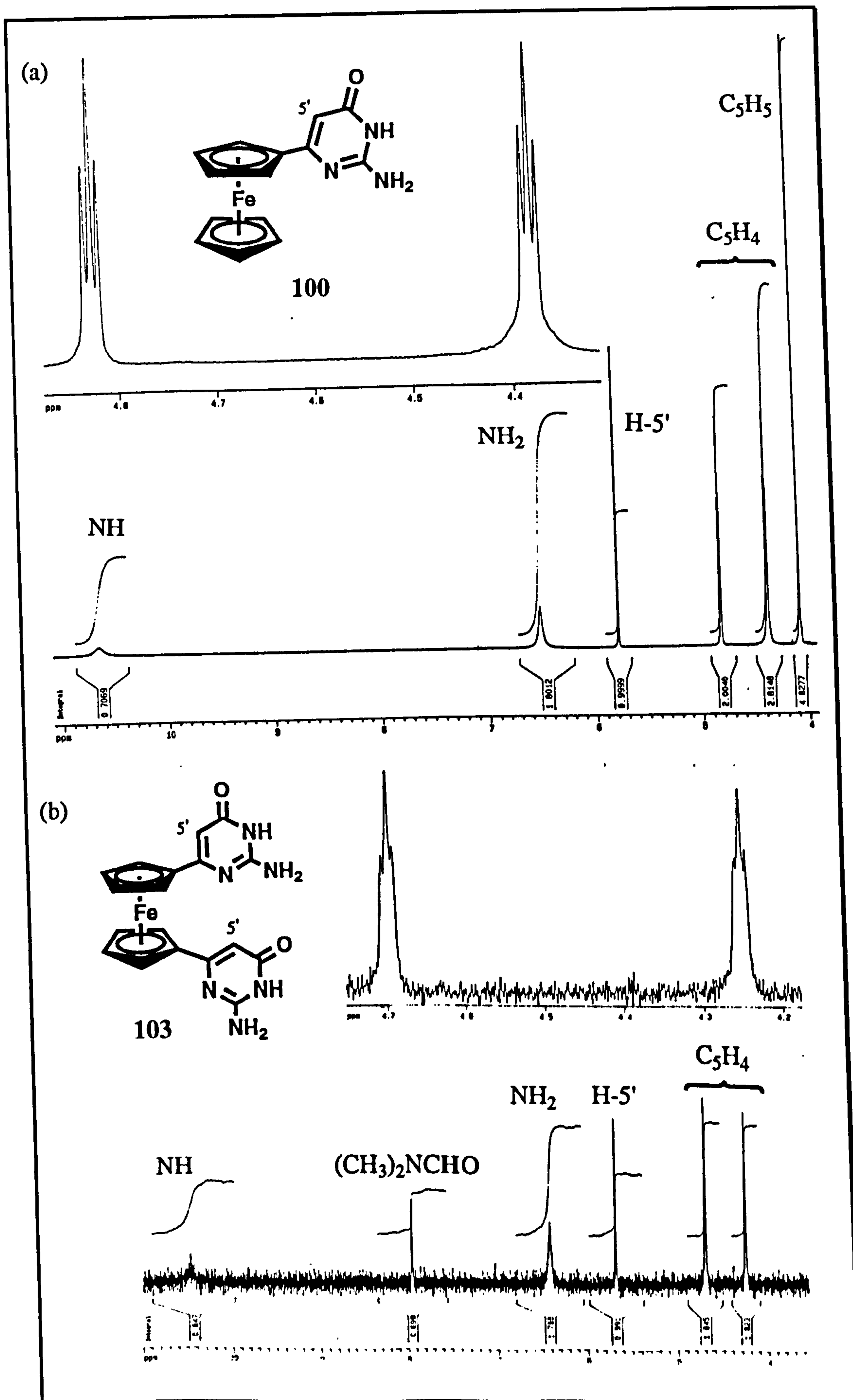
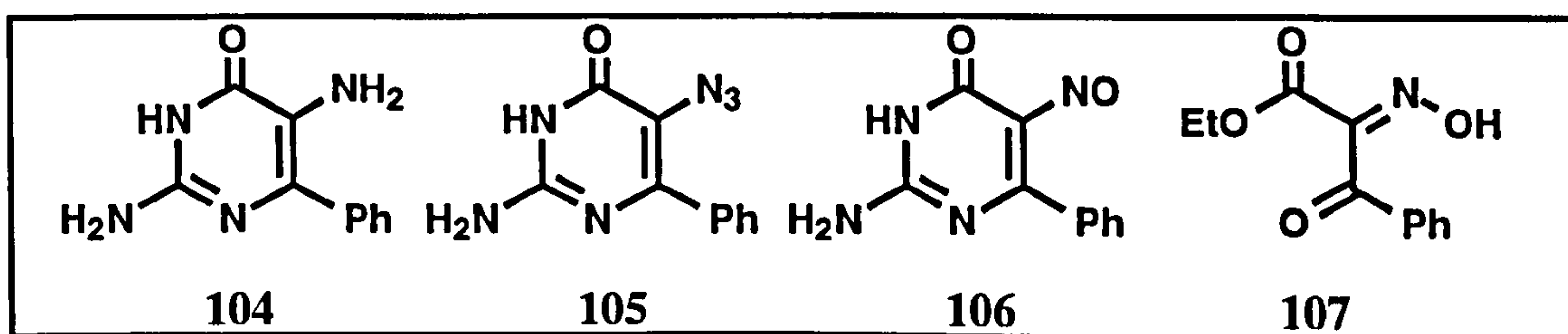


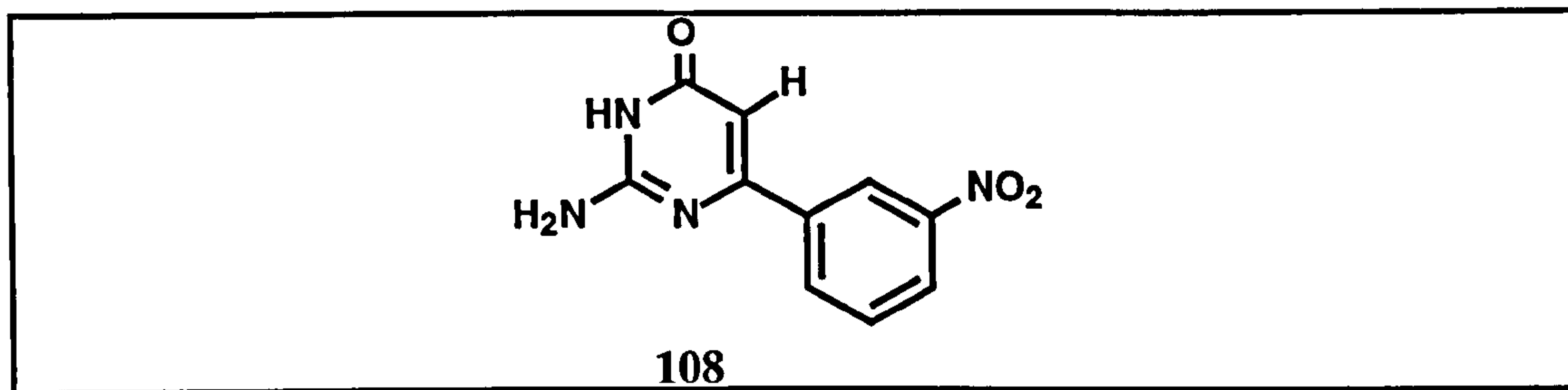
Figure 4-1 ¹H NMR spectra of pyrimidinylferrocenes 100 and 103, acquired in (CD₃)₂SO.

4.3 Attempted introduction of nitrogen containing groups at the pyrimidine 5-position

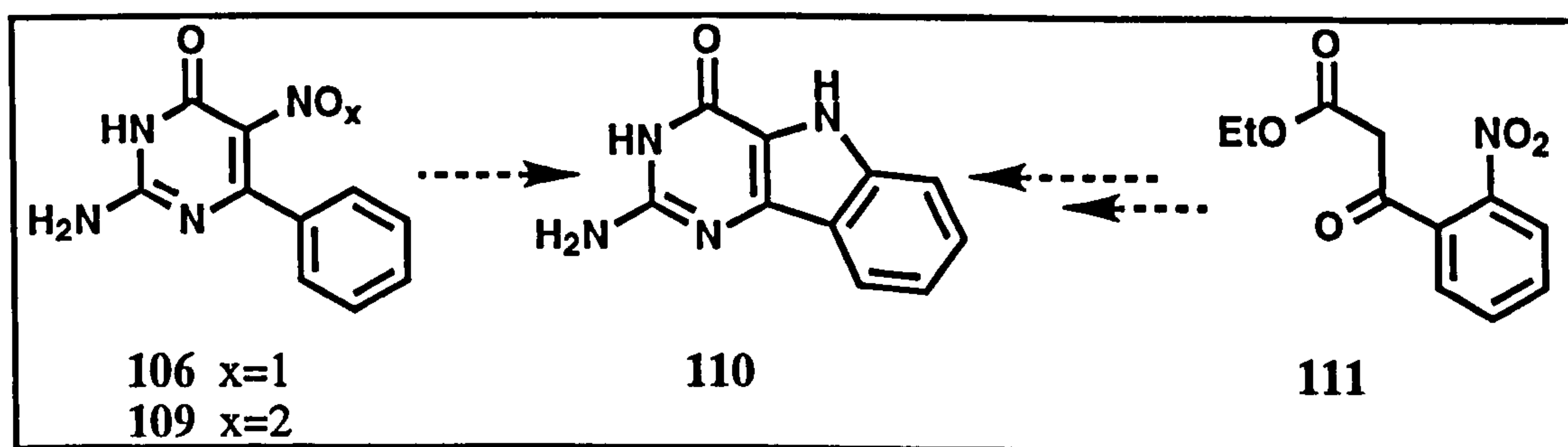
In a recent paper already cited,⁸⁵ the diaminopyrimidinone **104** was prepared in low yield by reduction of the azo compound formed by the reaction of **26** (APP) and 4-chlorophenyl diazonium chloride (7% from **26**). A crude azide **105** was also reported, formed by diazotisation of **104** and subsequent azide displacement, but purification attempts led to decomposition. The same article also observed that no 5-nitrosopyrimidinone **106** was formed by reaction of **26** with inorganic and organic nitrites, or by the reaction of guanidine and the oxime **107**.



Under relatively mild conditions, electrophilic substitution occurs at the 5-position of these aminopyrimidinones, in preference to many aromatic substituents such as the phenyl group of **26**, aided by the electron donating 2-amino group. The halogenations mentioned previously, and the azo coupling reaction above are two relevant examples. However, conventional mixed acid nitration of **26** leads to substitution in the phenyl ring to give **108**, where presumably protonation of the pyrimidine ring nitrogens deactivates the pyrimidine 5-position to electrophilic attack.⁸⁵



It was of interest to study alternative reactions capable of introducing nitrogen containing groups at the 5-position. One application of, for example, a 5-nitro (109) or 5-nitroso (106) derivative would be in reductive cyclisations with trialkylphosphites in an attempt to form the novel pyrimido[5,4-*b*]indole 110. Alternatively, the nitrogen could be initially attached to the phenyl ring, for example via the β -keto ester 111 (Scheme 4-4).



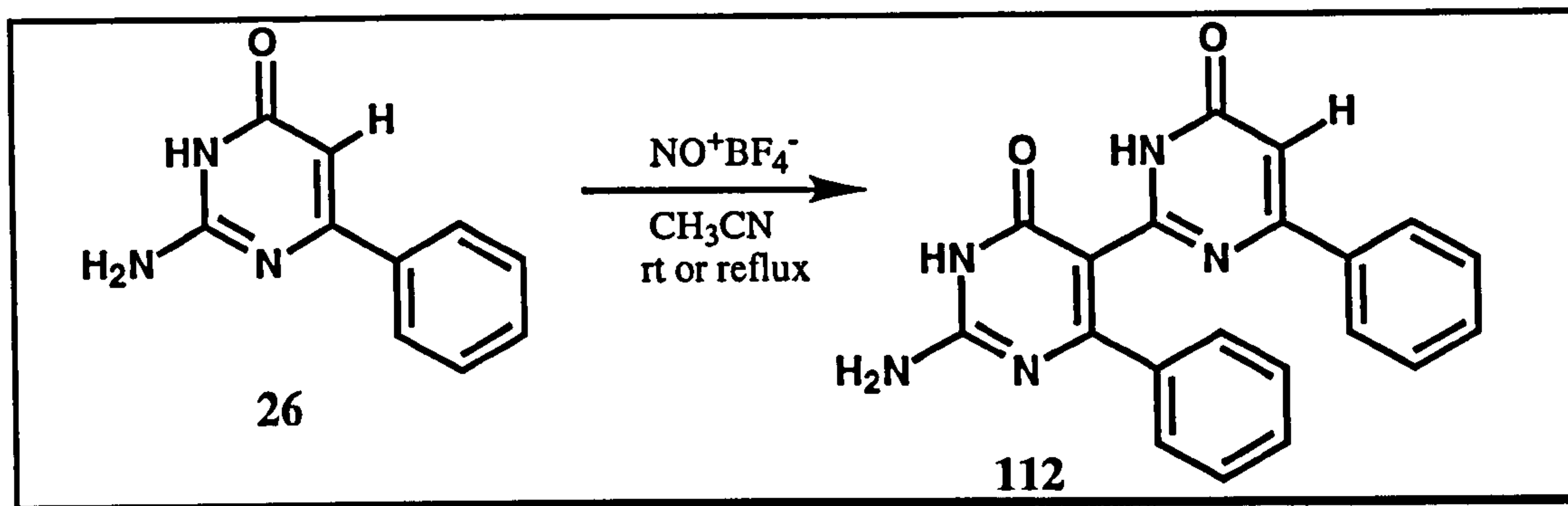
Scheme 4-4 Potential routes to pyrimido[5,4-*b*]indole 110.

4.3.1 Nitration / nitrosation reactions

Endeavouring to follow the left-hand route of Scheme 4-4 to the pyrimidoindole 110, nitration and nitrosation of 26 (APP) was attempted with the nitronium and nitrosonium tetrafluoroborate salts respectively.

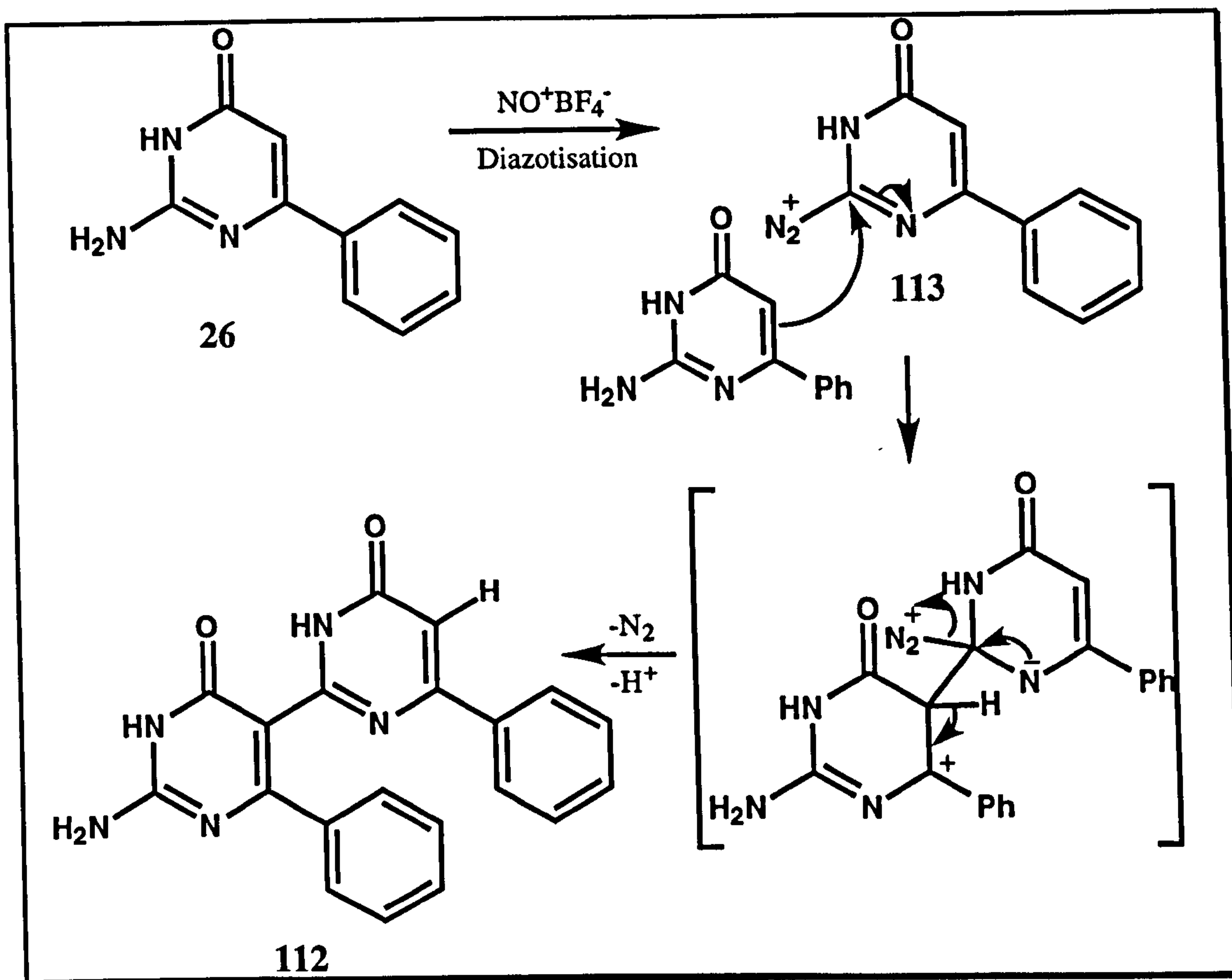
Nitronium tetrafluoroborate (NO_2BF_4) has previously been used very effectively for the nitration of substituted benzenes, including deactivated substrates such as nitrobenzene, in nonaqueous, acid-free systems.¹⁵⁴ It was hoped that such non-acidic nitration conditions would lead to preferential substitution of **26** in the pyrimidine ring. However, ambient temperature reactions of **26** with NO_2BF_4 in both anhydrous acetic acid and in dry acetonitrile returned only starting material, identified by ^1H and ^{13}C NMR spectra and mass spectrometry. The distinctive sharp singlet from the C-5 proton in the ^1H NMR spectrum at around 6 ppm readily confirms the lack of substitution at this position.

In an analogous manner to the use of NO_2BF_4 , it was of interest to determine whether nitrosonium tetrafluoroborate (NOBF_4) could effect nitrosation of **26**, the desired site of attack being the 5-position of the pyrimidine ring. Reactions were performed with three equivalents of NOBF_4 in dry acetonitrile, both at ambient temperature and at reflux (82°C). In both cases a mixture of several components resulted as monitored by TLC, including unreacted **26**. In either case, the most abundant component, variously isolated by column chromatography or crystallisations, was identified as the novel unsymmetrical dimer **112** (for example, 13% from the reaction at reflux), rather than the desired 5-nitroso derivative **106** (Scheme 4-5). On the small scale of reaction employed it proved impractical to isolate **112** as a pure compound, but samples were obtained in sufficient purity for analysis by ^1H and ^{13}C NMR and high resolution mass spectrometry, all in agreement with the structure **112**.



Scheme 4-5 Production of unsymmetrical dimer **112**.

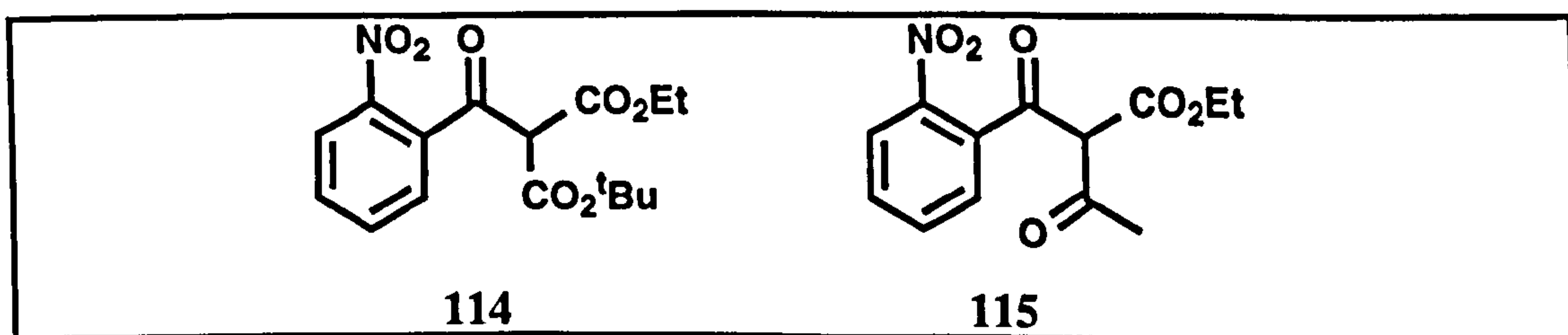
The formation of **112** could be explained by an ionic mechanism involving initial nitrosation of the primary amine of **26**, leading to the production of a diazonium species **113**. Electrophilic substitution at the 5-position of another molecule of **26** by **113** would yield the dimer **112** directly (Scheme 4-6). Alternatively, a radical mediated pathway operates from the diazonium species **113** as in the Gomberg reaction,¹⁵⁵ requiring the presence of hydroxide ions, potentially generated from the molecule of water produced in diazonium ion **113** formation. Through either mechanism, the mixture of other products observed in the reaction mixtures by TLC could be higher oligomers of this dimeric structure.



Scheme 4-6 Mechanistic explanation for formation of dimer 112.

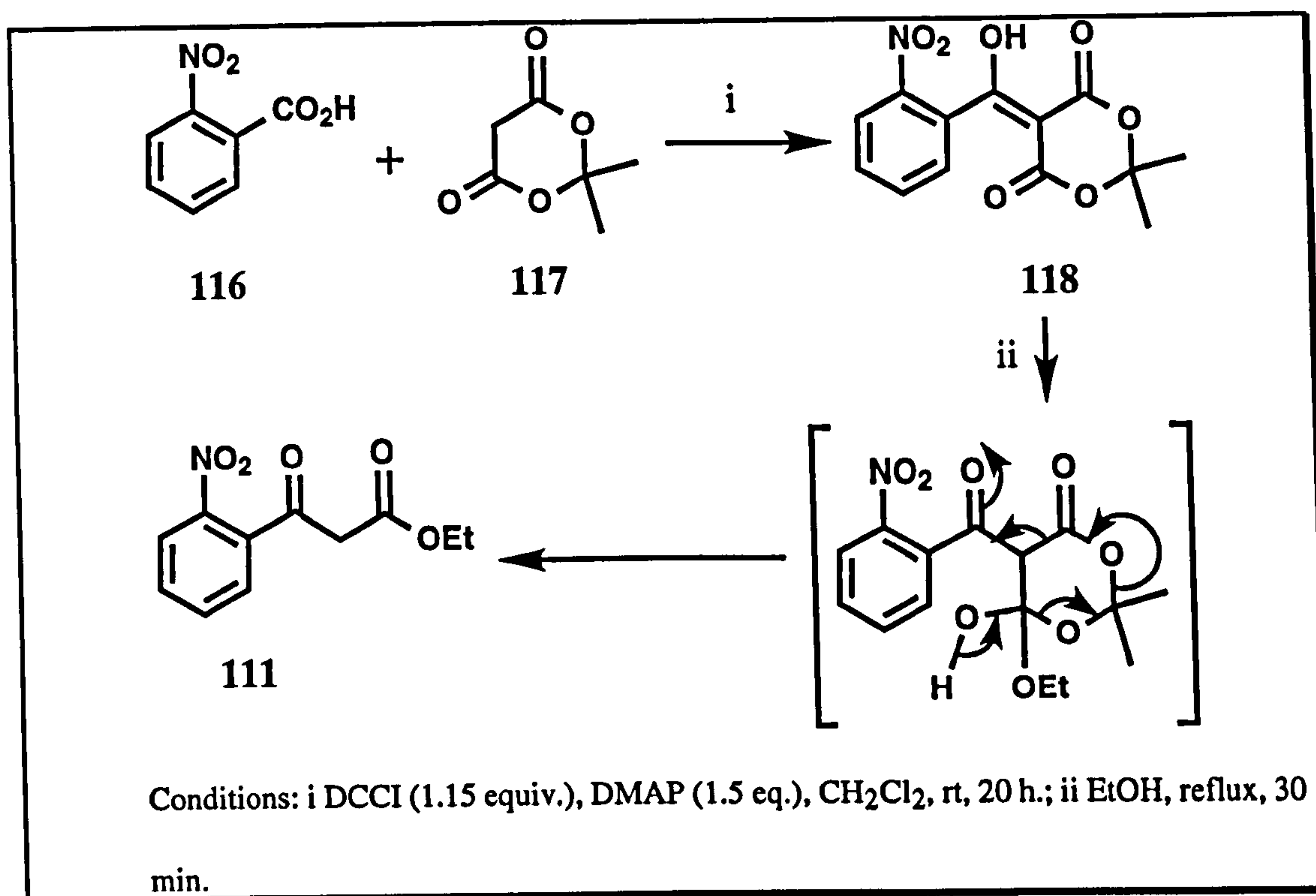
4.3.2 Synthesis and reaction of a 2-nitrophenyl- β -keto ester

Given the difficulties encountered in achieving nitration or nitrosation at the 5-position of 26, the alternative route suggested in Scheme 4-4 was investigated. The required ethyl 3-(2-nitrophenyl)-3-oxopropanoate (111) has been previously prepared by decarboxylation of a malonate 114,¹⁵⁶ and by deacylation of an acetoacetate 115.¹⁵⁷



In the present work it was of interest to prepare the β -keto ester **111** by a novel route. Holmquist and Roskamp reported the preparation of β -keto esters by reaction of aldehydes with ethyl diazoacetate, catalysed by tin(II) chloride.¹⁵⁸ Aromatic and tertiary aldehydes were observed to react the slowest, but under the conditions employed - room temperature in dichloromethane - nitro groups were unaffected, an important consideration in this case. An attempt to react 2-nitrobenzaldehyde under these conditions however was unsuccessful, yielding a mixture of products that were not readily separable. A number of ethyl ester resonances were observed in a ^1H NMR spectrum of the crude mixture, as well as several different aldehyde signals.

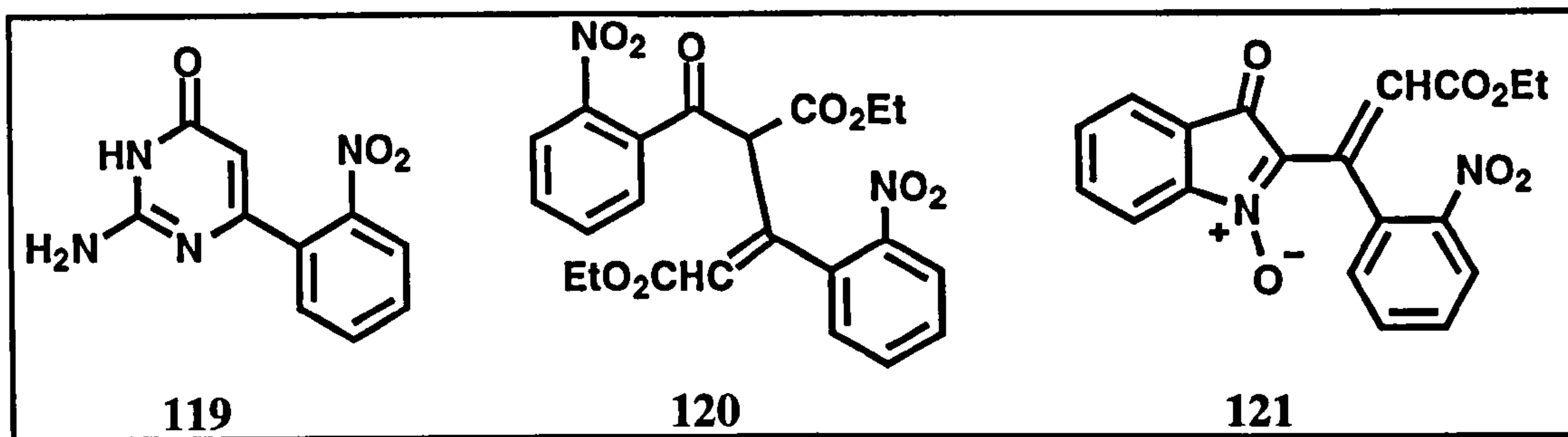
Through a modification of the procedure of Oikawa *et al.*,¹⁵⁹ the β -keto ester **111** was finally prepared in low yield (21%). Reaction of 2-nitrobenzoic acid (**116**) with Meldrum's acid, 2,2-dimethyl-1,3-dioxane-4,6-dione (**117**), in the presence of dicyclohexylcarbodiimide (DCCI) as coupling reagent, afforded the acyl derivative **118** (Oikawa *et al.*¹⁵⁹ employed acid chlorides). Ethanolysis of the crude **118** then furnished the required β -keto ester **111**, which was purified by silica gel chromatography (Scheme 4-5). ^1H NMR of the product ester isolated was in agreement with literature values,¹⁶⁰ showing evidence of keto-enol tautomerism in the chloroform solution.



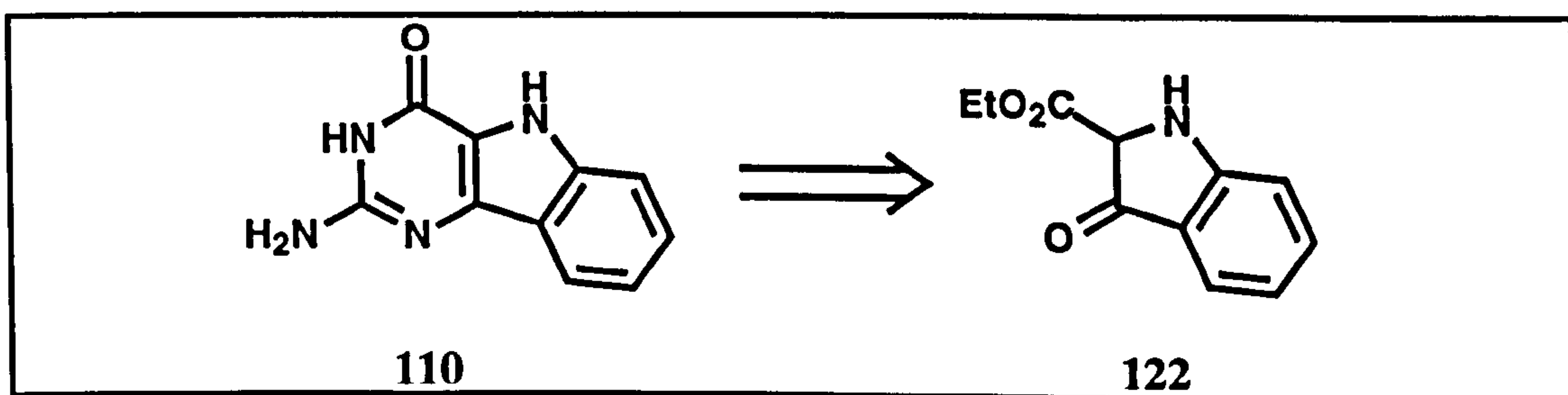
Scheme 4-5 Synthesis of β -keto ester **111**, employing Meldrum's acid.

With ester **111** prepared, formation of pyrimidinone **119** was attempted by reaction with guanidine carbonate in refluxing ethanol. Ester **111** appeared to be only slowly consumed, and after 48 hours at reflux TLC analysis showed a complex mixture including highly coloured blue, orange, purple and brown spots. No pyrimidinone was isolated from the reaction.

Consultation of the literature revealed a propensity of ethyl and methyl *o*-nitrophenyl- β -keto esters to decompose under basic conditions.¹⁶¹ Standing in aqueous sodium bicarbonate solution, ester **111** has been observed to degrade (through several proposed intermediates) to a number of highly coloured products including the orange diester **120** and the purple isatogen **121**. Given the equivalent colours formed in the reaction of **111** with guanidine carbonate, and the basic conditions present, similar degradation processes may have occurred.



Though not investigated in this project, reaction of ester **111** with guanidine as the free base, or as a less basic salt such as the hydrochloride, is a potential alternative route to pyrimidinone **119**. The known indoxylic acid derivative **122**¹⁶² might also lead to the pyrimidoindole **110** directly, if it reacted as an ordinary β -keto ester. It was felt however, that this constituted a further step away from the original objective of this particular project, which was to attempt to broaden the known chemistry of the 5-position of these pyrimidinones.

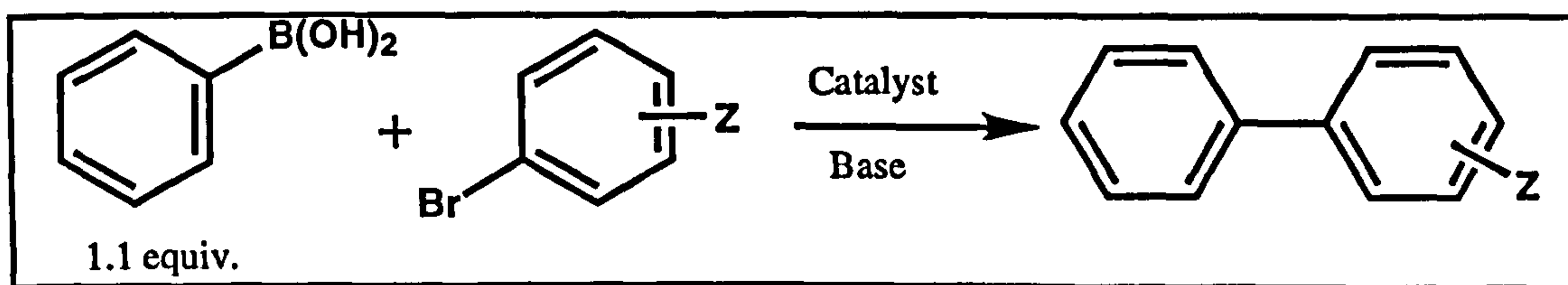


4.4 Palladium catalysed coupling reactions

As well as the specific targets of sections 4.2 and 4.3, it was of interest to develop novel 2-aminopyrimidinone chemistry applicable to the generation of a series of new compounds. It was envisaged that palladium-catalysed chemistry of the 5-halopyrimidinones, specifically Suzuki and Heck coupling reactions, would provide such an opportunity.

4.4.1 Suzuki coupling reactions

The regiospecific palladium-catalysed cross-coupling of phenylboronic acid with haloarenes, in the presence of bases, was first reported in 1981,¹⁶³ and has since become known as the Suzuki reaction (Scheme 4-6). The reaction was shown to be highly dependent upon the base employed, weak bases such as sodium carbonate producing optimum yields, and was only applicable to the coupling of bromides and iodides. Tetrakis(triphenylphosphine)palladium(0), $\text{Pd}(\text{PPh}_3)_4$ was found to be an effective catalyst, even at just 3 mole %, and the reaction was usually conducted in refluxing benzene or toluene with two equivalents of the aqueous base. Generally complete in under 6 hours, yields in excess of 90% were reported.¹⁶³



Scheme 4-6 General Suzuki reaction.

Biaryl units are important pharmacophores, present in a wide variety of biologically active compounds, and the use of boronic acids in Suzuki coupling reactions has presented significant advantages over other transition-metal catalysed C-C bond forming reactions of organometallics and organic halides in use at the time of its publication. For example, the reaction is tolerant of a wide variety of functional groups on either reactant, unlike Grignard reagents or organolithiums; and boronic acids are easily prepared, generally air and moisture stable solids; and they don't suffer from the high toxicities of organotin reagents employed in Stille couplings.¹⁶⁴

Given the advantages, it is not surprising that the Suzuki reaction has been the subject of a great deal of research, and is arguably a favoured method in the formation of biaryls. A variety of different conditions have been devised involving changing the nature of the palladium catalyst, for example $\text{Pd}(\text{dppb})\text{Cl}_2$ ([1,4-bis(diphenylphosphino)butane]palladium(II) dichloride) has broadened the process to the coupling of certain heteroaromatic chlorides.¹⁶⁵ Changes of base and solvent have also been effective for the optimisations of particular couplings. The scope of the reaction has further widened beyond aryl halide and aryl boronic acid to include vinyl halides,¹⁶⁶ vinyl boronic acids,¹⁶⁷ alkyl boranes,¹⁶⁸ boronic esters,¹⁶⁸ as well as aryl and vinyl triflates.^{169,170}

A widely accepted catalytic cycle has been proposed by Martin and Yang,¹⁷¹ for the simple biaryl coupling with sodium hydroxide as base (Figure 4-2). Oxidative addition of the aryl halide to a palladium(0) species forms an organopalladium halide (Ar-Pd-X) which undergoes metathetical displacement to form an organopalladium hydroxide (Ar-Pd-OH). The electrophilicity of the palladium is increased in this step by virtue of the increased electronegativity of the oxygen atom over bromine or iodine, but at 2.0 the electronegativity of boron is very similar to that of carbon at 2.5,¹⁷¹ making the aryl group of the boronic acid insufficiently reactive to perform the transmetallation reaction. The boronic acid is activated by boronate formation with the second equivalent of base, a process shown to produce a 10^6 fold increase in reactivity with electrophiles.¹⁷² Boronate and Ar-Pd-OH then transmetallate to form the diorganopalladium (Ar-Pd-Ar'), from which the biaryl product readily forms by reductive elimination, regenerating the catalytic palladium. In effect, the aryl halide and boronic acid behave as electrophilic and nucleophilic coupling partners respectively, and

substituents that enhance these characteristics are generally beneficial to the process.

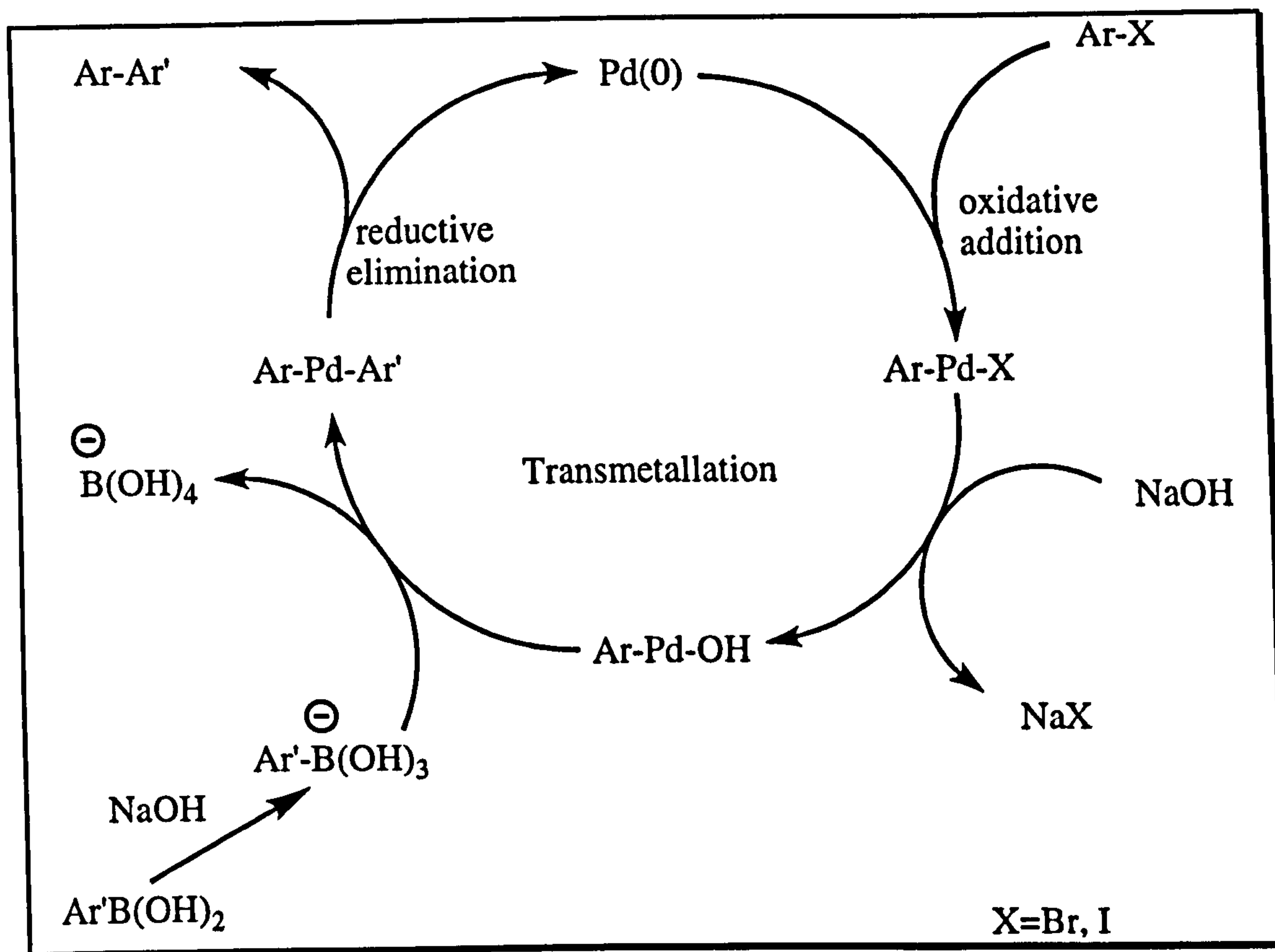
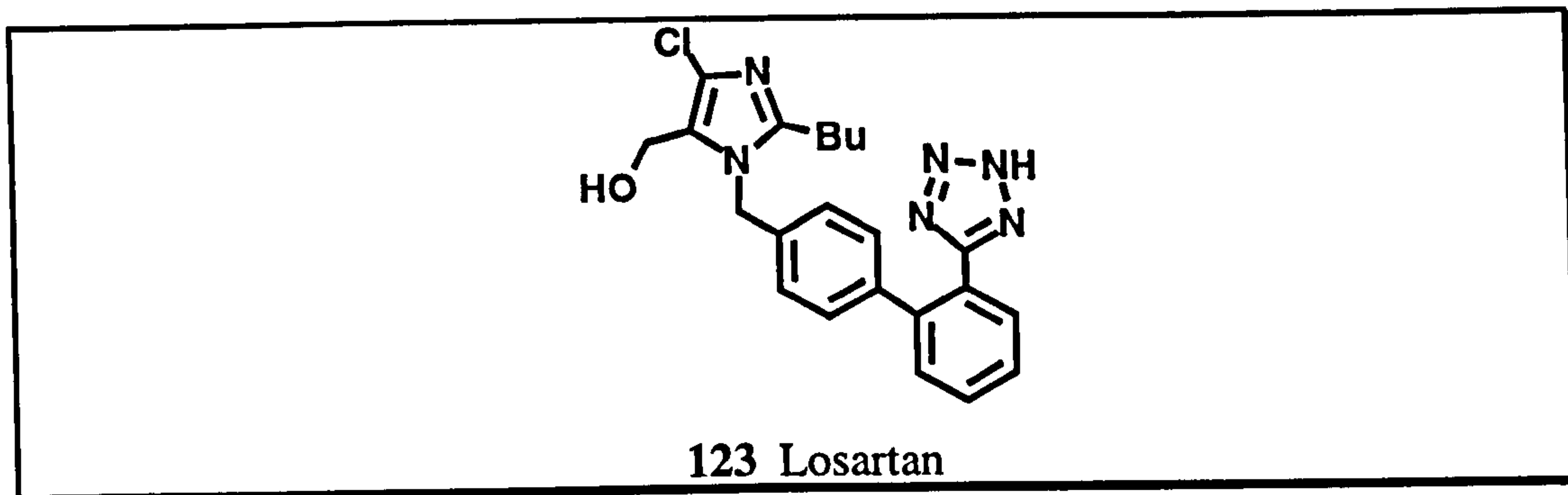


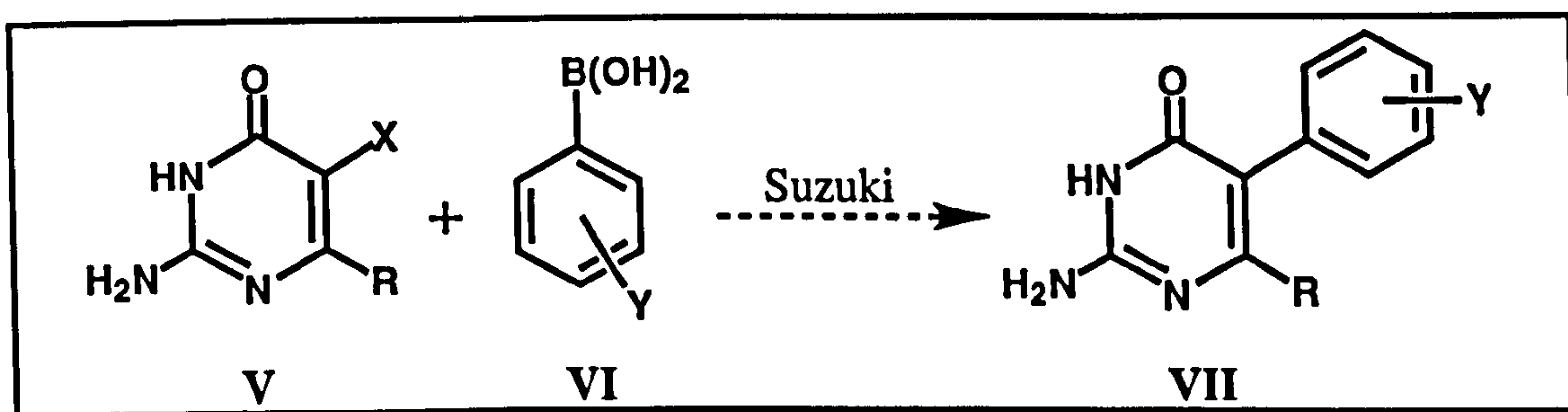
Figure 4-2 Mechanism of the Suzuki coupling reaction.

With $\text{Pd(PPh}_3)_4$ as the catalyst, studies have shown $\text{Pd(PPh}_3)_2$ to be the likely catalytic species.¹⁷³ Mechanistic studies of the Suzuki reaction in the synthesis of the biaryl unit of Losartan **123**, an angiotensin II receptor antagonist, demonstrated that the rate determining step under conventional Suzuki conditions depends upon the nature of the halide.¹⁷⁴ With the bromide, oxidative addition was rate determining, requiring heat to proceed; whereas with the iodide, oxidative addition was rapid and transmetallation became rate determining, a common observation in oxidative additions of organic halides to palladium.¹⁷⁵



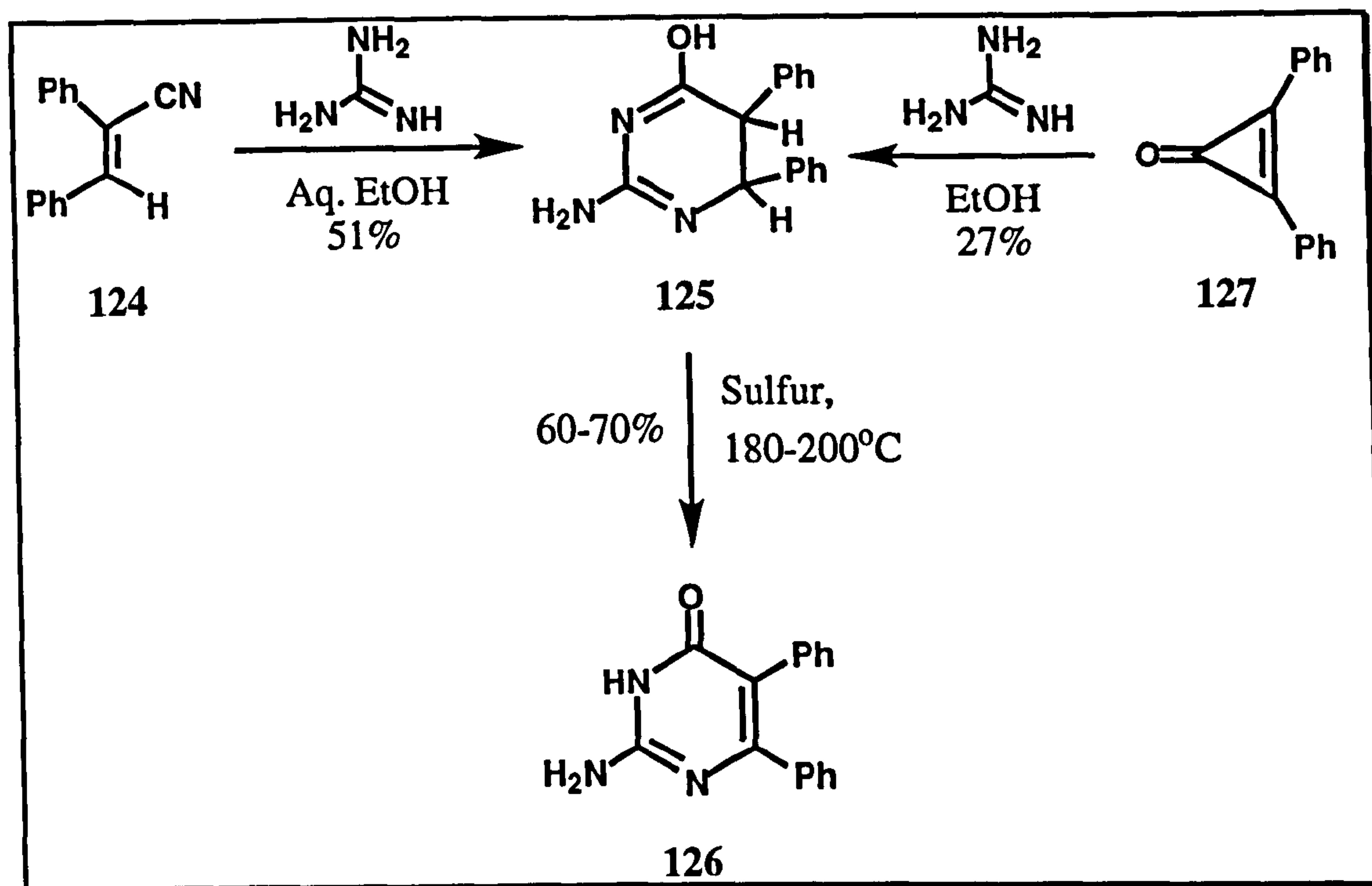
4.4.1.1 Reaction of 5-halopyrimidinones

In the present work it was hoped that Suzuki coupling reactions of the readily prepared 5-halopyrimidinones (V) with aryl / heteroaryl boronic acids (VI) would provide access to a series of novel 5-aryl / heteroaryl pyrimidinones (VII) (Scheme 4-7).



Scheme 4-7 Proposed route to 5-aryl / heteroaryl pyrimidinones.

The simple diphenyl derivative 126 has been prepared previously by two similar routes, involving dehydrogenation of an intermediate dihydropyrimidine 125 (Scheme 4-8). The intermediate 125 was prepared either from the acrylonitrile 124,¹⁷⁶ or the cyclopropanone 127,¹⁷⁷ by reaction with guanidine carbonate.

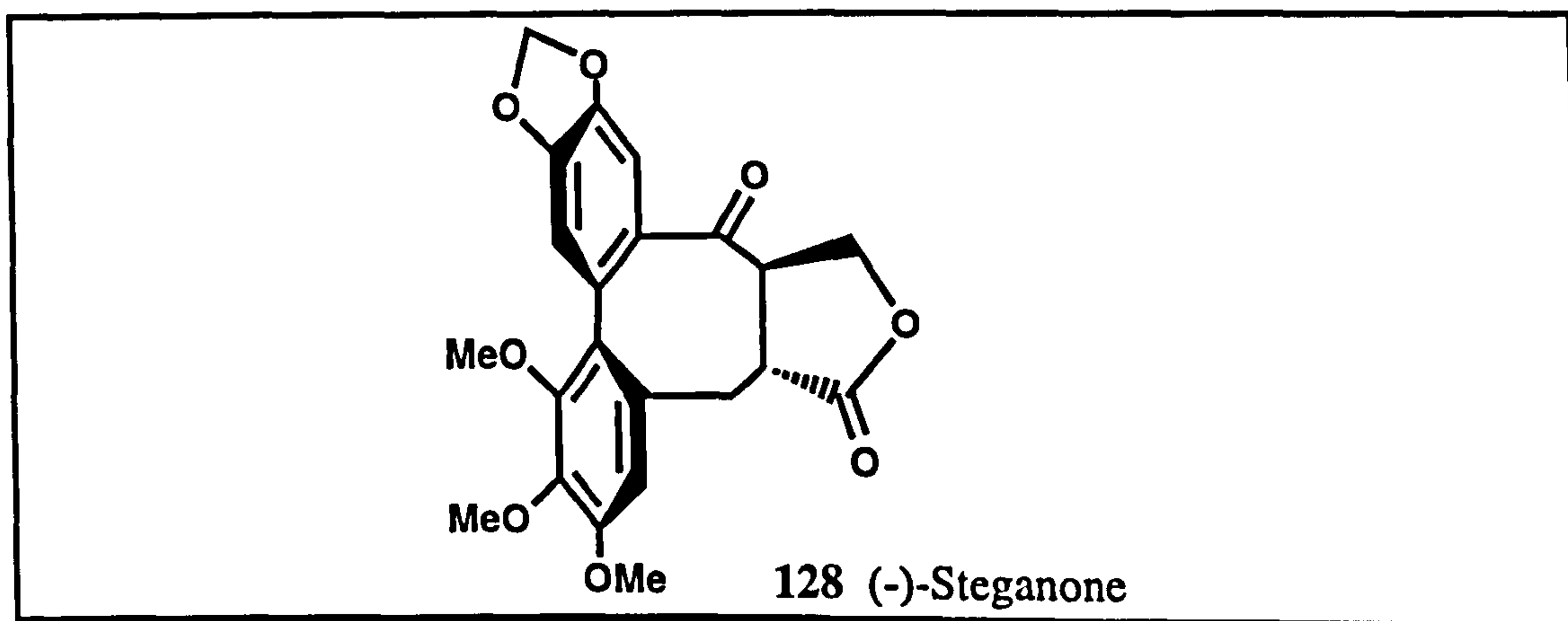


Scheme 4-8 Literature preparations of diphenylpyrimidinone 126.

Suzuki reactions have not been reported with 2-amino-5-halopyrimidinones, though a limited number of couplings have been successfully performed upon 5-halouracils, 5-iodouridine derivatives for example have been coupled with aryl boronic acids under standard conditions.¹⁷⁸ As well as further probing the chemistry of the immunomodulatory 5-halopyrimidinones, if successful, the advantage of this proposed new route to 5-arylpyrimidinones (VII) is that novelty is introduced in the final step. Targets VII (Scheme 4-7) would be formed in one step by reaction upon a common intermediate (V), rather than diverging at the beginning with the synthesis of different acrylonitriles or cyclopropenones, as would be the case if one of the previous routes were employed.

An added facet to compounds of structure VII would be the potential to form atropisomers, by coupling with suitable *ortho*-substituted aryl boronic acids,

such that rotation about the biaryl bond is prevented by steric clashes with the *ortho*-substituent and the oxygen at the 4-position, or the R group at the 6-position. Such atropisomers might be expected to show the same differences in biological activities as a pair of enantiomers, and if the barrier to rotation were high enough then separation of atropisomers by chiral chromatographic techniques might be possible. Alternatively, atropisomer selective Suzuki coupling reactions could be investigated. The use of a chromium tricarbonyl complexed aryl halide, for example, has been reported in an atropisomer selective Suzuki coupling in a stereospecific route to the antileukaemic agent (-)-Steganone (128).¹⁷⁹



Continuing in our interest of reactions of 6-phenylpyrimidinones, Suzuki coupling reactions were first attempted with broprimine (20) and phenylboronic acid. As well as employing fairly standard reaction conditions - utilising the method of Wang and Haseltine,¹⁸⁰ with 3.5 mole % $\text{Pd}(\text{PPh}_3)_4$, in a refluxing mixture of toluene, ethanol and aqueous sodium carbonate - coupling was also attempted under the same conditions used for the bromoimidazole 41 in section 3.1.2.1, at reflux in DMF (Table 4-2).

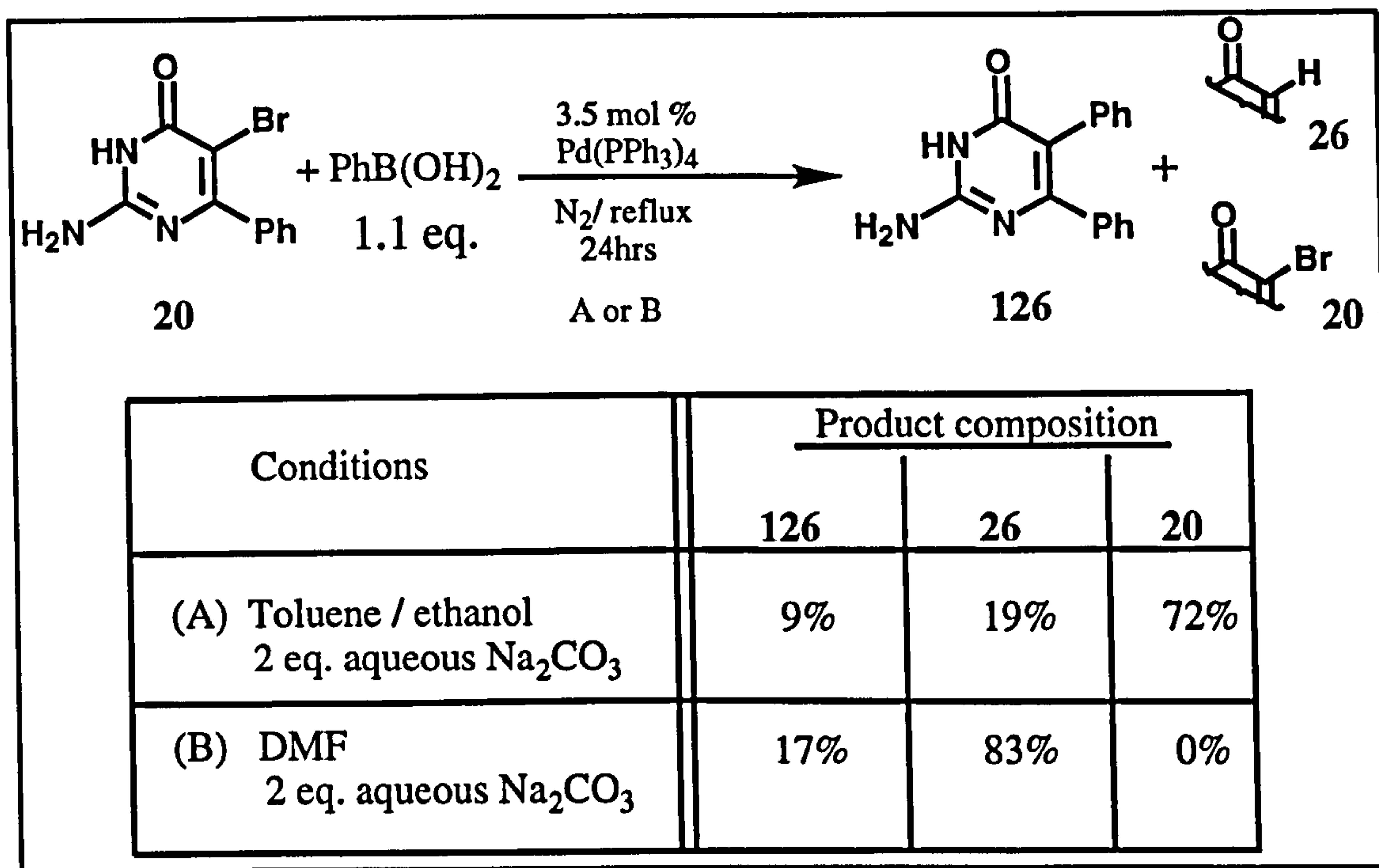


Table 4-2 Initial Suzuki reactions of bropirimine 20 and phenylboronic acid.

A number of conclusions can be drawn from these initial results. The major observation was the difficulty with which the components of the product mixture (126, 26 and 20) were separated - a portion of 126 serendipitously crystallising from the ethyl acetate phase during conventional work up of the toluene reaction, greatly facilitating its identification, was confirmed by ¹H NMR, mass spectroscopy and elemental analysis, with mp 312-314°C (lit.,¹⁷⁶ 319°C). The remaining mixtures were not separable by silica gel chromatography, instead the values given above were determined by analysis of ¹H NMR spectra of the mixtures. Also, although in refluxing DMF all the bropirimine was consumed, the selectivity for coupled product (126) versus debrominated side product (26) was considerably poorer than resulted from the toluene reaction.

On the grounds that successful coupling of the 6-phenyl derivative might be disfavoured due to steric clashes, couplings were also attempted with the 6-

methylpyrimidinone, ABMP (19), again under the same two sets of conditions (Table 4-3). However, in the refluxing toluene / ethanol reaction, pyrimidinone 19 proved even less reactive than 20, with only traces of coupled product 129 by ^1H NMR. From the refluxing DMF reaction the selectivity for coupling versus debromination was even worse than with 20. However, a sample of the novel pyrimidinone 129 was isolated by silica gel chromatography, and its structure confirmed by ^1H and ^{13}C NMR and its mass spectrum. Unfortunately, elemental analysis of a recrystallised sample of 129 was inconclusive, suggesting co-crystallisation with an inorganic substance.

Conditions	Product composition		
	129	93	19
(A) Toluene / ethanol 2 eq. aqueous Na_2CO_3	Trace	Trace	>90%
(B) DMF 2 eq. aqueous Na_2CO_3	10%	90%	0%

Table 4-3 Initial Suzuki reactions of ABMP (19) and phenylboronic acid.

The presence of the phenyl group actually appeared to increase both reactivity and selectivity for the coupling process, and attentions reverted to improving the Suzuki reactions of 20. A repeat reaction in toluene / ethanol as before, over 76 hours, with periodic additions of more boronic acid, base and

catalysts did consume more starting material, but the ratio of coupled to debrominated product remained poor at approximately 1:2 (the isolated yields were **126** (21%), **26** (46%) and **20** (20%)). A series of reactions were then performed, varying the nature of the halide, base, solvent and different supplies of catalyst, as well as reaction with *in situ* formation of the presumed catalytic species $\text{Pd}(\text{PPh}_3)_2$,¹⁷⁴ by reaction of $\text{Pd}(\text{OAc})_2$ and PPh_3 (Table 4-4).

20 X=Br
 21 X=I

$\text{A = Toluene, ethanol, water}$
 B = DMF, water

Isolated yield */ %

X	Base	Catalyst	Conditions	126	26	SM	Total
I	Na_2CO_3	$\text{Pd}(\text{PPh}_3)_4$ batch 1	A / 24 h.	1	30	38	70
Br	Et_3N	$\text{Pd}(\text{PPh}_3)_4$ batch 1	B / 24 h.	0	29	48	77
Br	NaHCO_3	$\text{Pd}(\text{PPh}_3)_4$ batch 1	A / 24 h.	4	18	56	78
Br	Na_2CO_3	$\text{Pd}(\text{PPh}_3)_4$ batch 2	A / 24 h.	15	31	34	80
Br	Na_2CO_3	$\text{Pd}(\text{PPh}_3)_4$ batch 3	A / 24 h.	16	25	37	77
Br	Na_2CO_3	$\text{Pd}(\text{PPh}_3)_2$	A / 18 h.	10	23	31	64

* determined by ^1H NMR of crude product mixture

Table 4-4 Further Suzuki coupling reactions of pyrimidinones **20** and **21**.

A number of conclusions can be drawn from these results. Firstly, contrary to the literature precedent, the iodopyrimidinone **21** gave a lower yield of coupled product **126** than the corresponding bromo analogue **20**, but with comparable

dehalogenation to **26**, under identical conditions-suggesting a difference in reactivity between Ar-Pd-Br and Ar-Pd-I (with reference to Fig. 4-2). Preventing deprotonation of **20** with weaker bases, namely triethylamine (Et₃N) and sodium bicarbonate (NaHCO₃), also resulted in poorer coupling yields though debromination still occurred. It is generally held that Pd(PPh₃)₄ is best prepared as required, and as the age of 'batch 1' was unknown, reaction was repeated with two new batches bought-in. However, in all such cases, and with Pd(PPh₃)₂ prepared *in situ*, reactions were slow and the ratio of coupled product **126** to debrominated side product **26** failed to improve above 1:2.

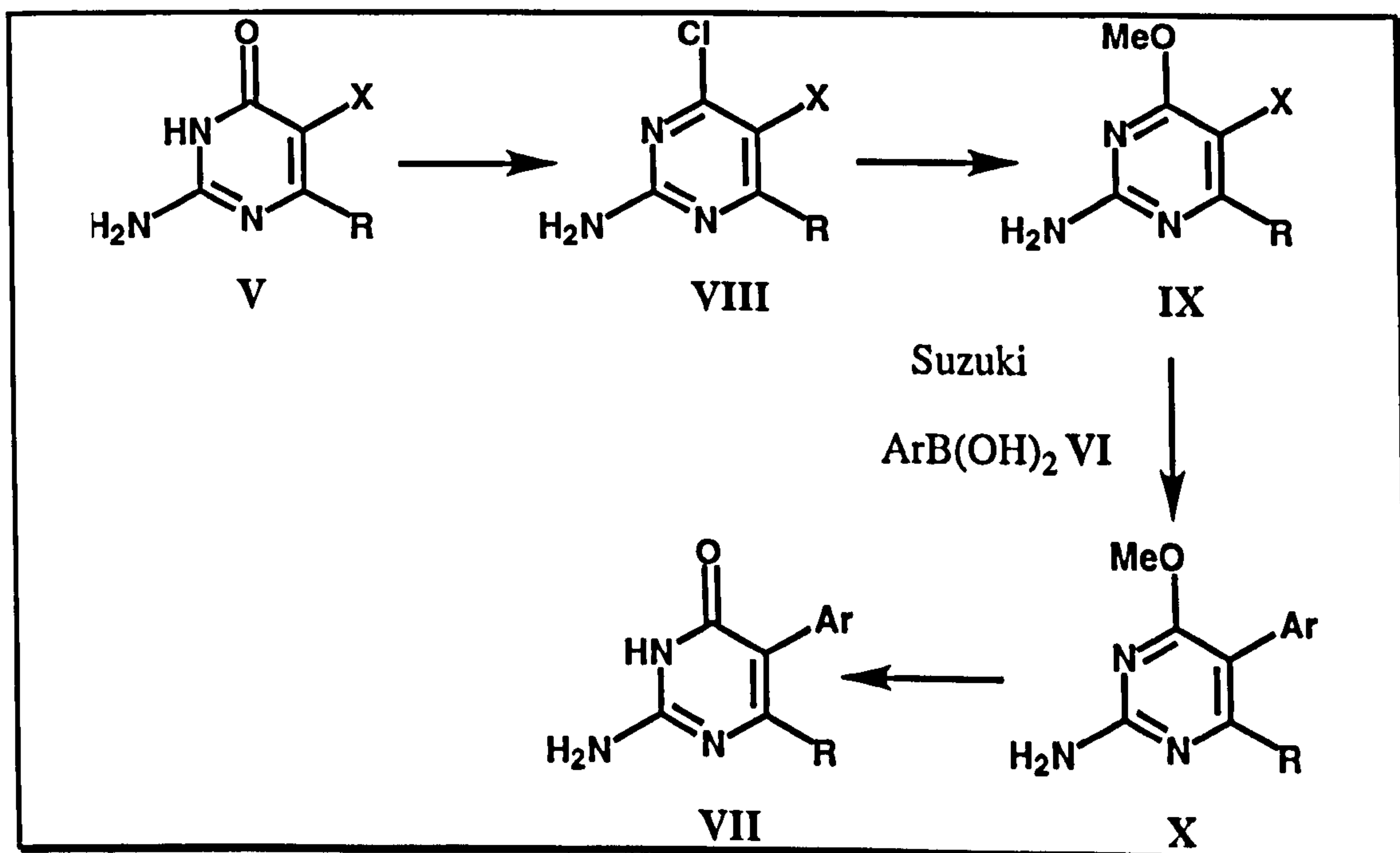
Bropirimine **20** was found stable to Na₂CO₃ at reflux in aqueous acetone, and has been reported stable to 0.1 N sodium hydroxide at 37°C for 96 hours,¹⁸¹ suggesting involvement of palladium in the debrominations observed, though as mentioned in the previous chapter such occurrences are not widely reported in the literature. It would appear that oxidative addition of halide to palladium is occurring, but that the transmetallation with boronic acid is so slow that an alternative reaction pathway competes, leading to dehalogenation. Bromopyrimidinone **20** is substituted at both positions *ortho* to the site of reaction, hence steric crowding could be a factor affecting rates of reaction.

A requirement of the Suzuki reaction is that the halide is electrophilic, which is entirely opposite to the nature of the C-5 carbon of these 2-amino-pyrimidinones, a fact likely to be exaggerated by NH deprotonation under the basic conditions of the Suzuki reaction. Though couplings have been achieved in these studies, a more reliable high yielding approach was desired, especially given the difficulties encountered in the purifications of the mixtures formed. At this stage, rather than continue coupling reactions of these pyrimidinones employing

alternative reported conditions, approaches that could improve the electrophilicity of the C-5 carbon and/or eliminate the acidic proton of the pyrimidinones were sought.

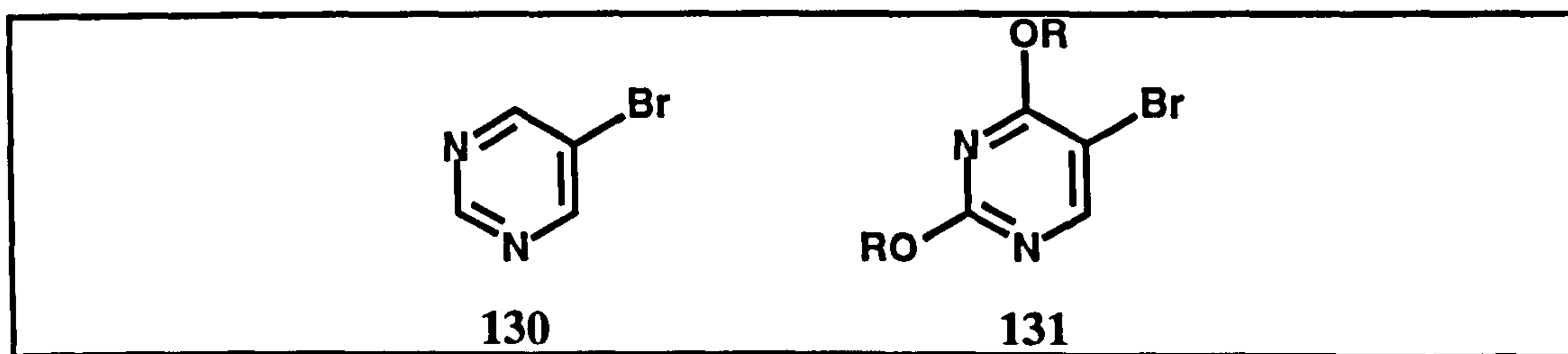
4.4.1.2 Reaction of 5-halopyrimidines

A stable 4-pyrimidinone derivative from which the pyrimidinone can be readily regenerated is a 4-alkoxypyrimidine. Employing such an intermediate, a potential alternative route to 5-arylpyrimidinones (VII) involves transformation of halopyrimidinones (V), which have already been prepared, to corresponding 4-alkoxypyrimidines (IX) via the 4-chloro derivatives (VIII). After successful Suzuki coupling, the target pyrimidinones (VII) would be obtained by hydrolysis of pyrimidines (X) (Scheme 4-9).



Scheme 4-9 Proposed route to 5-arylpyrimidinones via pyrimidines.

A limited number of groups have reported successful Suzuki coupling reactions with 5-bromopyrimidines. Reactions were confined to 5-bromopyrimidine (130),^{182,183} or 5-bromo-2,4-dialkoxypyrimidines 131.^{182,184} Suzuki reactions have also been reported with 2,4-dichloro and 2,4-dibromopyrimidines, undergoing cross-coupling preferentially at the 4-position,¹⁸² raising interesting possibilities for reactions upon 4-chloropyrimidines (VIII). However, no Suzuki reactions have been reported with a 2-amino-5-bromopyrimidine, or a 5-bromopyrimidine with a 6-substituent, either of which may have led to the observed reduced Suzuki reactivity of the pyrimidinones.



Conversion of pyrimidinones and uracils to their 2- or 4-chloro derivatives is a well known reaction, performed with a number of reagents,^{81,82} for example thionyl chloride and DMF, phosphorous oxychloride (POCl_3), and phosphorus pentachloride, the formation of 2,4-dichloropyrimidines from uracils generally requiring the presence of an added amine base. The 4-chloro-5-bromopyrimidine 132 has been previously prepared in low yield (41%) by refluxing bropirimine (20) in POCl_3 .¹⁸⁵ This reaction was briefly investigated in the conversion of bromo and iodopyrimidinones 20 and 21, to their 4-chloro derivatives 132 and 133 respectively, with and without an added amine base, *N,N*-dimethylaniline (Table 4-4).

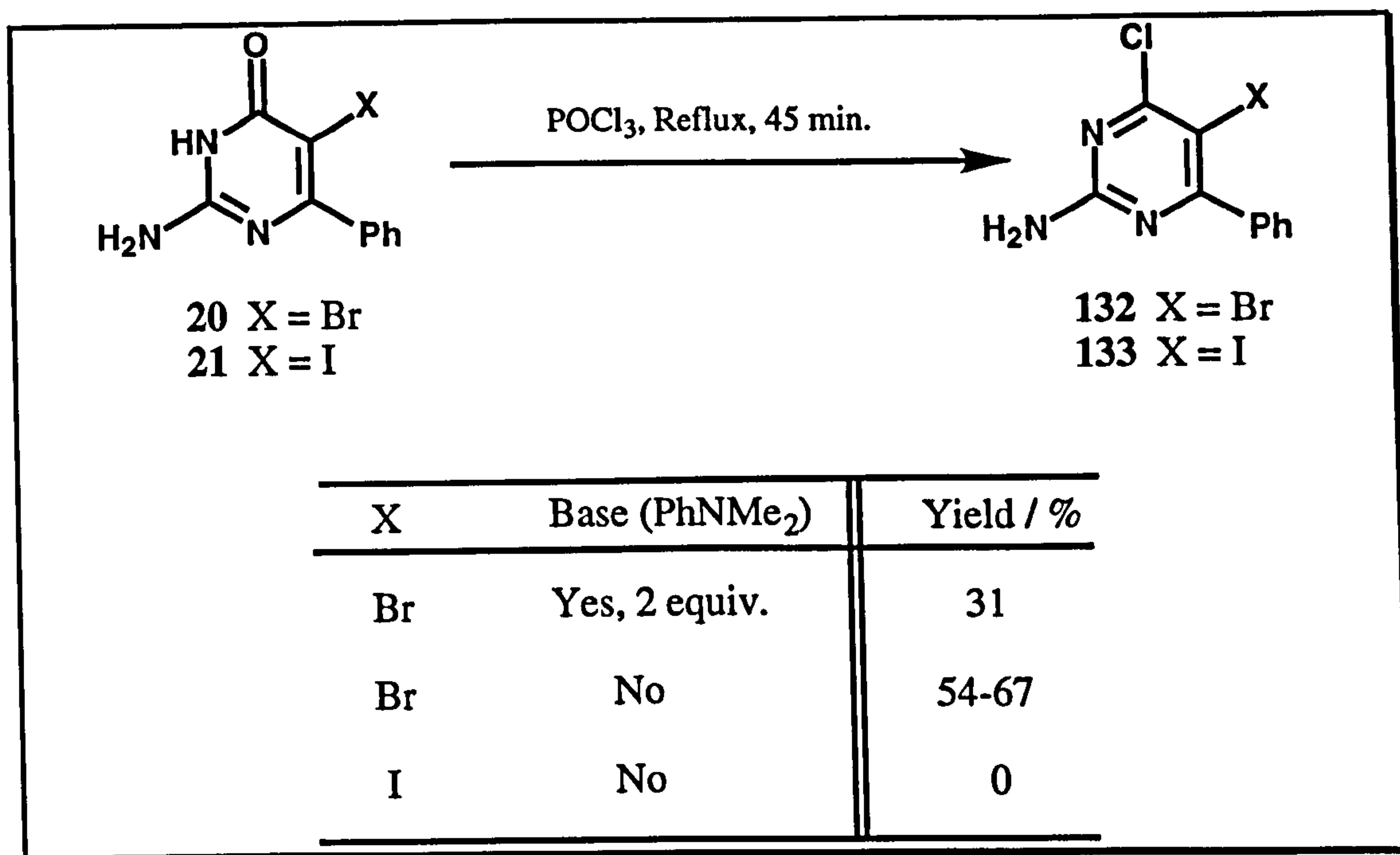
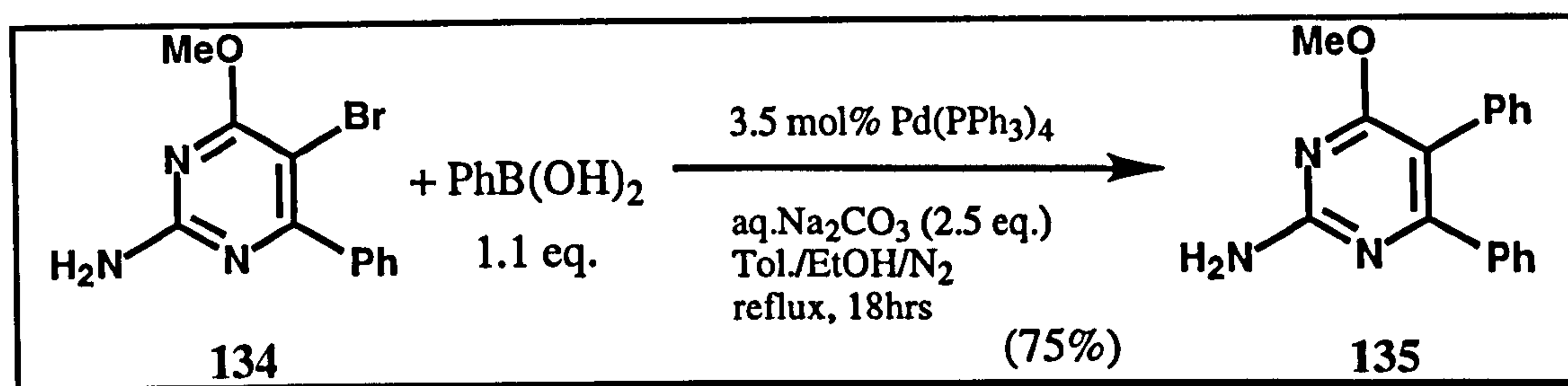


Table 4-4 Phosphorus oxychloride chlorination of pyrimidinones **20** and **21**.

The reaction of **20** with POCl_3 was somewhat capricious, though yields of **132** isolated appeared greater in the absence of base. The identity of pyrimidine **132** was confirmed by ^1H and ^{13}C NMR and mass spectroscopy, and had mp 135°C (lit.⁸⁵ $136\text{-}138^\circ\text{C}$) after silica gel column purification. An acetone insoluble by-product in the synthesis of **132** could not be identified by ^1H NMR, but on treatment with 1 M hydrochloric acid overnight, a sample of reasonably pure **20** was returned. The insoluble solid may have resulted from intermolecular condensation of **132** between 2-amino and 4-chloro groups, with the potential to form oligomers. Acid hydrolysis would then cleave the connections and return pyrimidinone **20**. Unfortunately, heating AIPP (**21**) with POCl_3 led to decomposition from which no chloropyrimidine **133** was isolated.

Chloropyrimidine **132** was then converted in excellent yields (82-93%) to the novel 4-methoxy derivative **134**, according to the method of Prystas and Sorm

for the conversion of a 2,4-dichloropyrimidine to its 2,4-dimethoxy derivative,¹⁸⁶ by treating **132** with sodium methoxide in refluxing methanol. Suzuki reaction of **134** and phenylboronic acid, according to the conditions used previously (3.5 mol% Pd(PPh₃)₄, toluene, ethanol, aq. Na₂CO₃), resulted in essentially complete conversion to the novel diphenylpyrimidine **135**, which was isolated in 75% yield after recrystallisation (Scheme 4-10).



Scheme 4-10 Successful Suzuki reaction of 5-bromopyrimidine **134**.

Unfortunately, these conditions did not appear general. Reaction of **134** with other boronic acids was slow, a possible result of which was a resurgence of competing debromination, though to a lesser extent than observed with pyrimidinones (Table 4-5). The novel 2,4-dichlorophenyl-coupled pyrimidine **136** presents the potential to form separable atropisomers, but, produced in only 24% yield, it proved difficult to separate from **134** and **138**, and was not isolated pure. Readily identifiable by its fluorescence at 254 nm on TLC plates, a sample 89% pure in **136** (with **134** the only contaminant by ¹H NMR) was obtained by three sequential silica gel columns followed by a recrystallisation, and had ¹H and ¹³C NMR spectra and mass spectrum in agreement with the structure. Reaction of **134** with 3-thiopheneboronic acid failed, only a trace of the thienylpyrimidine **137** being observed in the residue by ¹H NMR after recovery of 89% of starting material **134**.

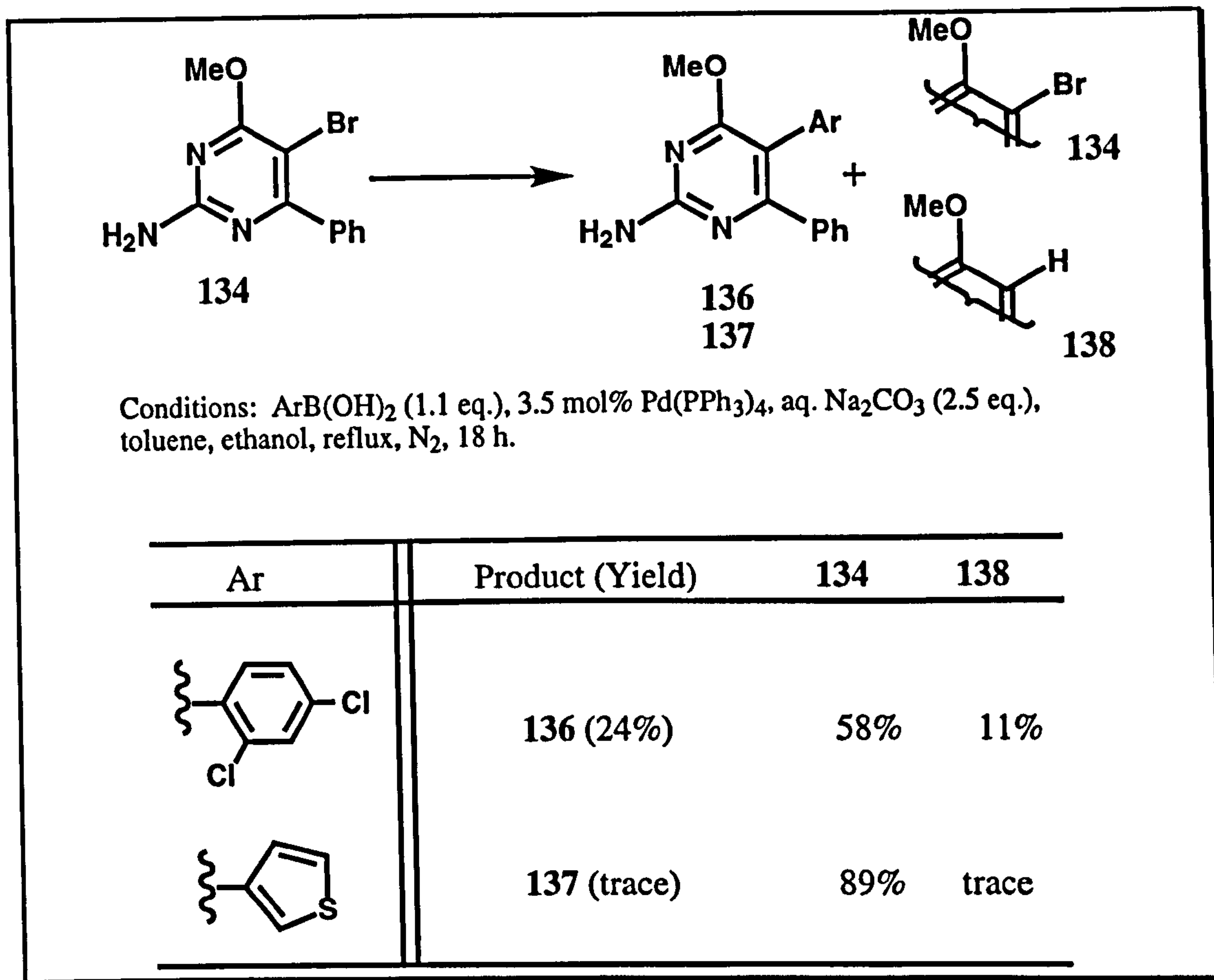
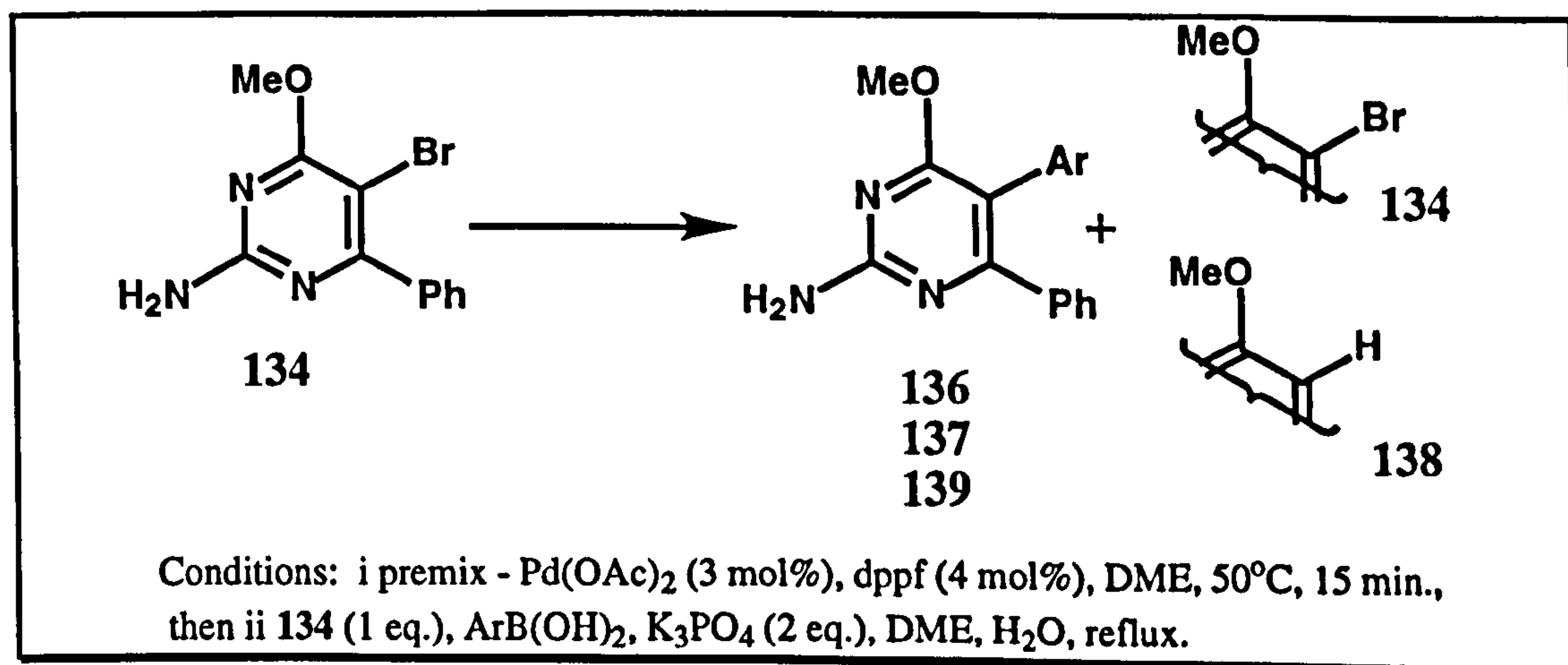


Table 4-5 Further Suzuki couplings of 134 with $\text{Pd(PPh}_3)_4$ as catalyst.

Scrutiny of the literature suggested a number of alternative conditions. Gronowitz *et al.*¹⁸⁷⁻¹⁸⁹ have shown that the use of 1,2-dimethoxyethane (DME) in place of benzene or toluene, reduces the deboronation of boronic acids - deboronated by-products have been observed in the couplings performed. The use of alternative bases, including barium and thallium(I) hydroxides, and potassium phosphate (K_3PO_4), have been reported to produce dramatic rate enhancements in Suzuki reactions, especially in the synthesis of hindered biaryls,^{190,191} presumably through acceleration of transmetallation. Thompson *et al.*¹⁹² described the use of 1,1'-bis(diphenylphosphino)ferrocenepalladium(II) acetate (Pd(dppf)(OAc)_2), prepared *in situ*, for cross-couplings of moderately hindered halopyrazines where $\text{Pd(PPh}_3)_4$ had failed. It was believed that the longer Pd-P bonds, forced by the

rigid ferrocene backbone, decreased steric crowding at the palladium and also forced the coupling partners *cis* for the reductive elimination step.¹⁹²

A combination of these variants, employing K_3PO_4 as base and $Pd(dppf)(OAc)_2$ as catalyst in a mixture of water and DME, were then applied to the coupling of **134** and 2,4-dichlorobenzene, 3-thienyl and 4-methoxybenzene boronic acids. Reactions were partially successful, though the outcome still appeared highly dependent upon boronic acid involved (Scheme 4-11 and Table 4-6). Coupling was successfully achieved with 3-thiophene and 4-methoxybenzeneboronic acids, forming 5-arylpyrimidines **137** and **139** respectively in moderate yields, though reaction was obviously slow, accompanied by increased amounts of debromination. Interestingly, no **136** was formed under these conditions.



Scheme 4-11 Scheme for Table 4-6.

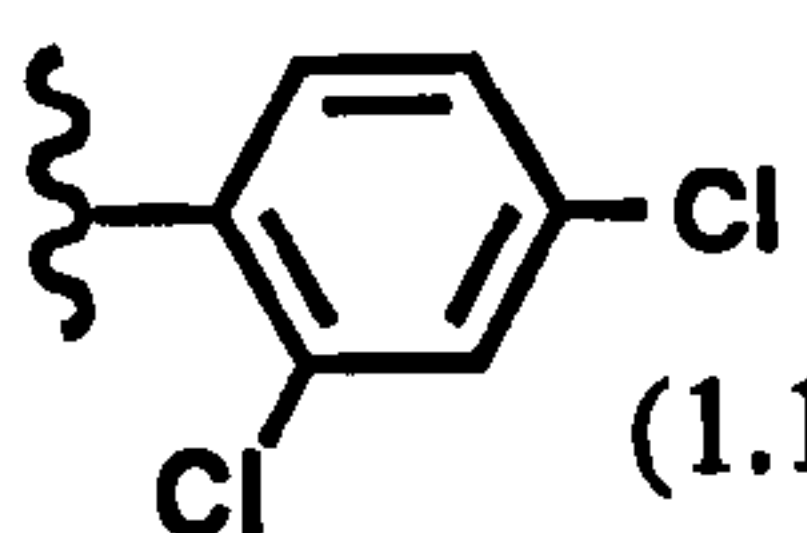
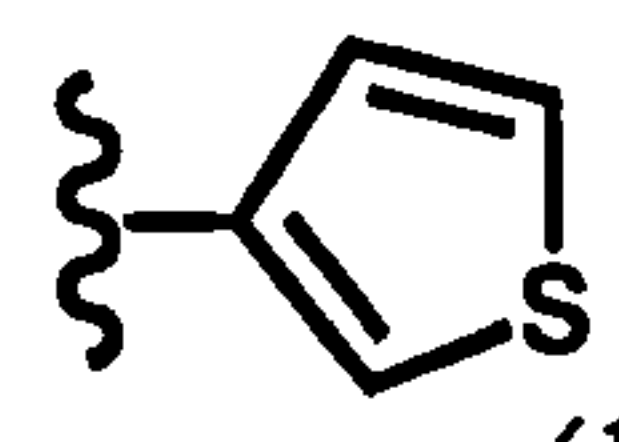
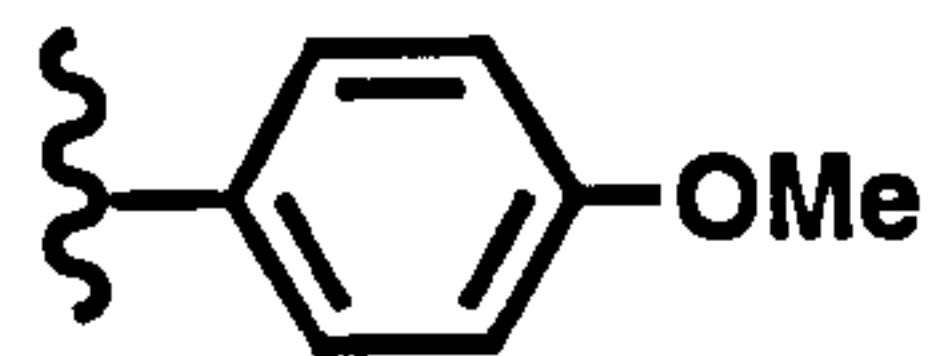
Ar	Reaction time / h.	Product composition		
		Coupled product	134	138
 (1.1 equiv.)	24	136 (0%)	71%	24%
 (1.1 equiv.)	24	137 (50%)	24%	16%
 (1.7 equiv.)	90	139 (55%)	20%	18%

Table 4-6 Suzuki couplings of 134 with Pd(dppf)(OAc)₂ as catalyst.

Suzuki reaction of the 5-bromo-4-chloropyrimidine **132** with phenylboronic acid, under these modified conditions with Pd(dppf)(OAc)₂ as catalyst, was also briefly investigated. A mixture of products resulted, the composition of which, by TLC, seemed independent of whether one or two equivalents of phenylboronic acid had been employed. The products were not readily separable by silica gel chromatography, but seemed likely to comprise mixtures of mono and bis-coupled products, as well as oligomers via intermolecular condensation between amino and chloro groups.

Given the interest in forming the coupled pyrimidine **136**, with its potential for atropisomerism, alternative high yielding coupling conditions were sought for its synthesis. Employing Pd(dppf)(OAc)₂ as catalyst, reaction of **134** was repeated in refluxing DMF in the hope of forcing reaction; reaction was also performed in toluene / ethanol which had given a low yield of **136** with Pd(PPh₃)₄

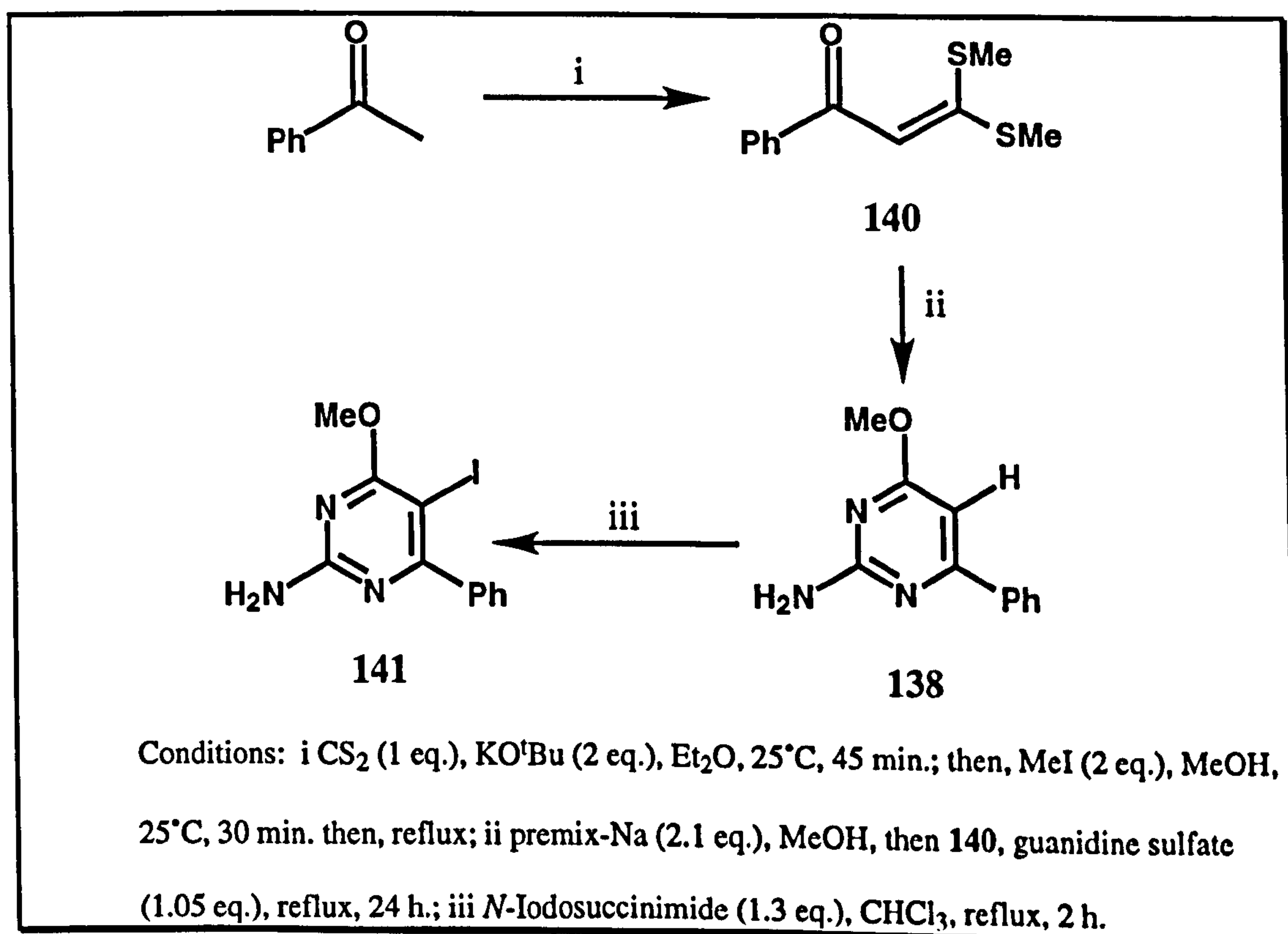
as catalyst, but employing K_3PO_4 as base. Campi *et al.*¹⁹³ reported the use of catalytic $Pd(OAc)_2$, in degassed 95% ethanol, with Na_2CO_3 as the base, for the coupling of an *ortho*-substituted benzenboronic acid at ambient temperature, and these conditions were also applied to the synthesis of 136 (Table 4-7). Once more however, only varying amounts of debromination of 134 to 138 were observed.

<p> <chem>COc1nc(N)c(Br)c1-c2ccccc2</chem> + <chem>Clc1cc(B(O)O)cc(Cl)c1</chem> (1.1 equiv.) \rightarrow <chem>COc1nc(N)c(c1-c2ccccc2)-c3cc(Cl)cc(Cl)c3</chem> + 134 + 138 </p>			
Conditions	Product composition		
	136	134	138
3 mol% $Pd(dppf)(OAc)_2$, Na_2CO_3 (2 eq.) DMF, N_2 , reflux, 24 h.	0%	28%	4%
3 mol% $Pd(dppf)(OAc)_2$, K_3PO_4 (2 eq.) Toluene, Ethanol, N_2 reflux, 6 h.	0%	67%	29%
5 mol% $Pd(OAc)_2$, Na_2CO_3 (1.5 eq.) 95% ethanol, N_2 , reflux, 14 h.	0%	77%	13%

Table 4-7 Further attempts to form bisarylpyrimidine 136.

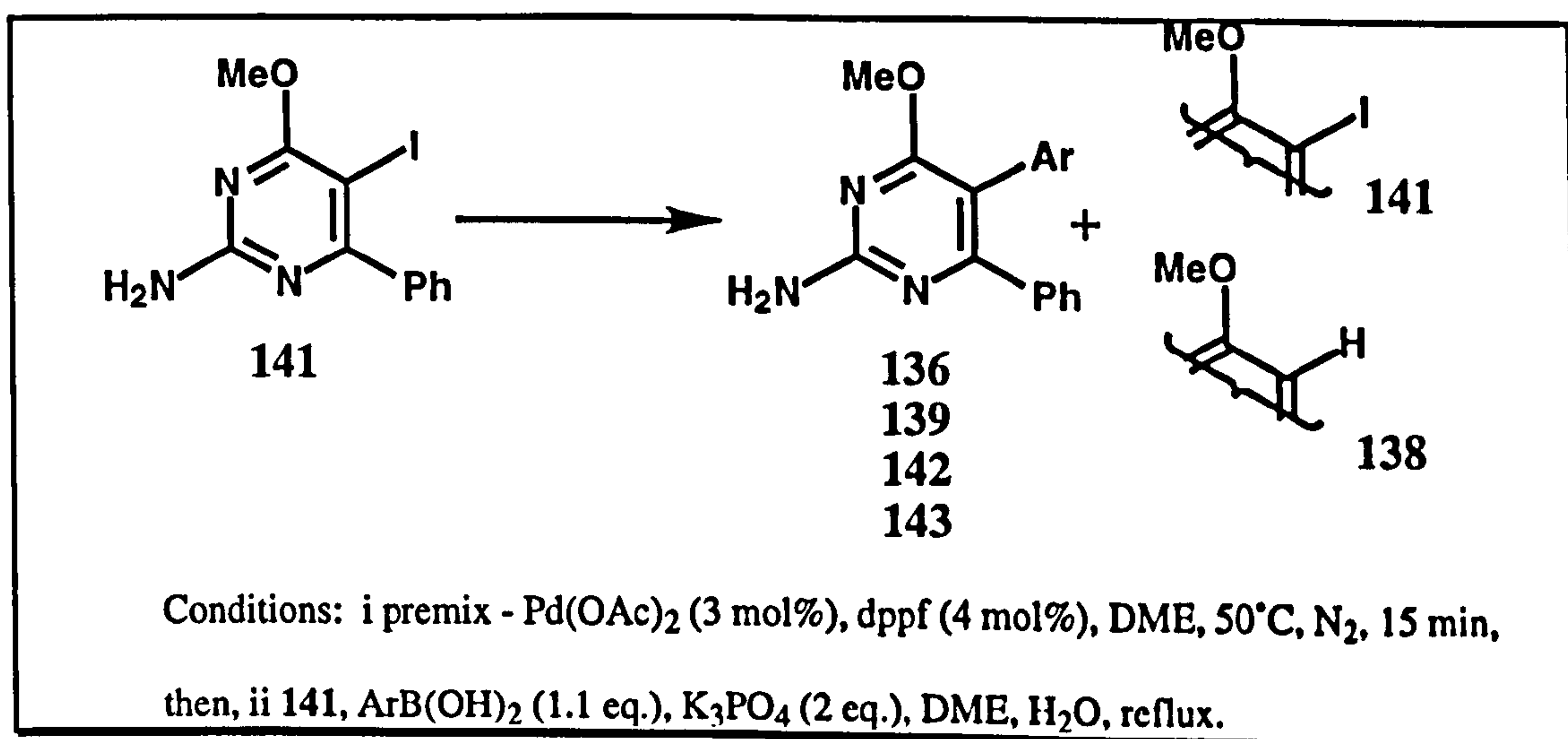
Despite the previous disappointing results on changing from bromo to iodopyrimidinones in Suzuki reactions, a route to a 5-iodopyrimidine was sought, in the hope that if more reactive than bromide 134, Suzuki reaction could be achieved with a wider range of boronic acids, and with improved yields.

The novel 5-iodopyrimidine **141** was successfully prepared in three steps (Scheme 4-12). The α -ketoketene *S,S*-diacetal **140** has been previously prepared in two steps by the condensation of acetophenone with carbon disulfide to yield a dithioacid,¹⁹⁴ which has been alkylated with methyl iodide to provide **140**.¹⁹⁵ In these studies, the dithioacid dianion initially formed was alkylated directly to provide a one-pot synthesis of **140** in 62 % yield, which was then reacted with guanidine sulfate according to Chauhan and Junjappa,¹⁹⁶ to furnish the 4-methoxypyrimidine **138** in 51% yield. Pyrimidine **138** thus prepared had mp 151-152°C (lit.,¹⁹⁶ 152°C), and was identical by ¹H NMR to the by-product observed in previous couplings, assigned as **138**. Iodination with *N*-iodosuccinimide, based upon the method of Nishiwaki,¹⁹⁷ provided clean conversion to iodopyrimidine **141** in almost quantitative yield.



Scheme 4-12 Synthesis of a 5-iodopyrimidine, **141**.

Suzuki reaction of iodopyrimidine **141** was then attempted with a series of four boronic acids, employing $\text{Pd}(\text{dppf})(\text{OAc})_2$ prepared *in situ*, in refluxing aqueous DME, with K_3PO_4 as base (Scheme 4-13 and Table 4-8). Reaction with all but 2,4-dichlorobenzeneboronic acid completely consumed starting material within 24 hours, with almost complete conversion to Suzuki coupled products **139**, **142** and **143**, and only traces of deiodinated **138**. The yields in Table 4-8 of coupled products are after recrystallisation, but for **138** figures refer to proportions in the original crude products by ^1H NMR. Interestingly, not only was reaction of **141** and 4-methoxybenzeneboronic acid more rapid than that of the bromo analogue **134** (see Table 4-6), but also the selectivity for coupled **139** versus dehalogenated **138** product was considerably greater. With reference to the catalytic cycle (Figure 4-2), this would suggest a difference in reactivity between not only Ar-Br and Ar-I (as is generally accepted), but also between Ar-Pd-Br and Ar-Pd-I, if dehalogenation does indeed involve palladium.



Scheme 4-13 Scheme for Table 4-8.

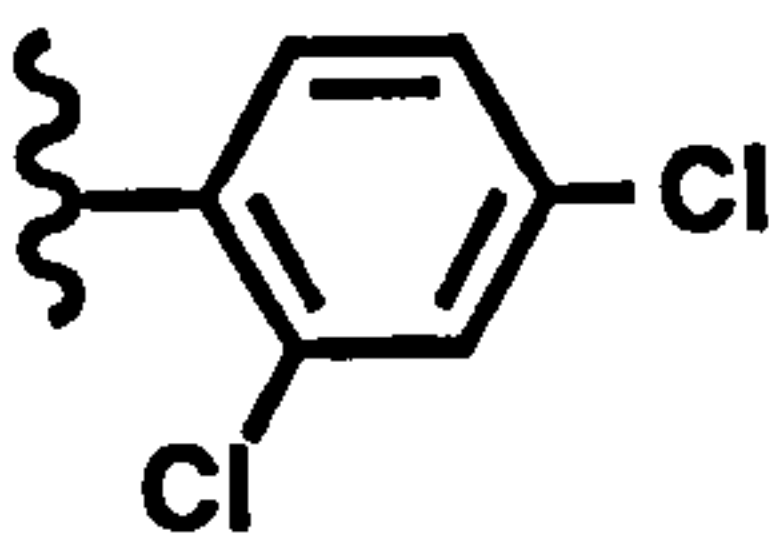
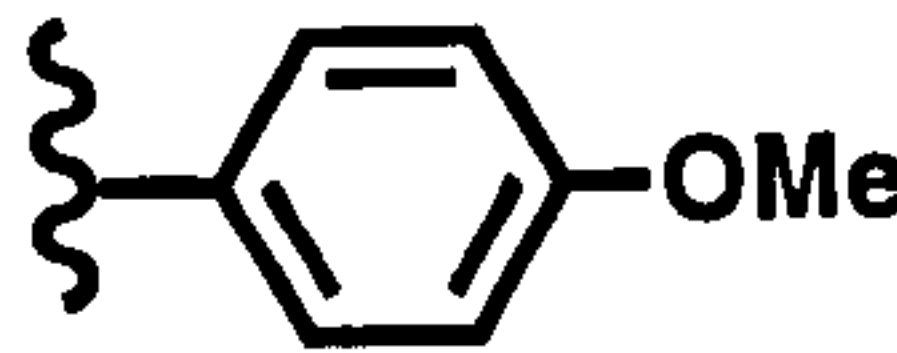

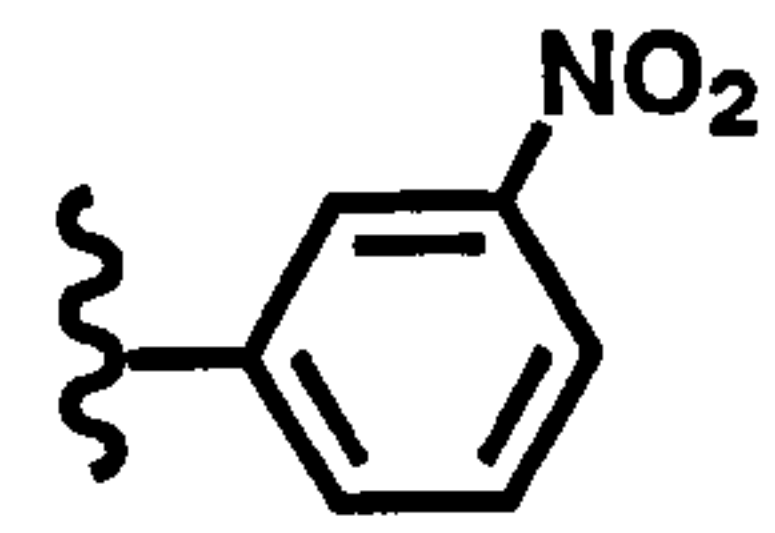
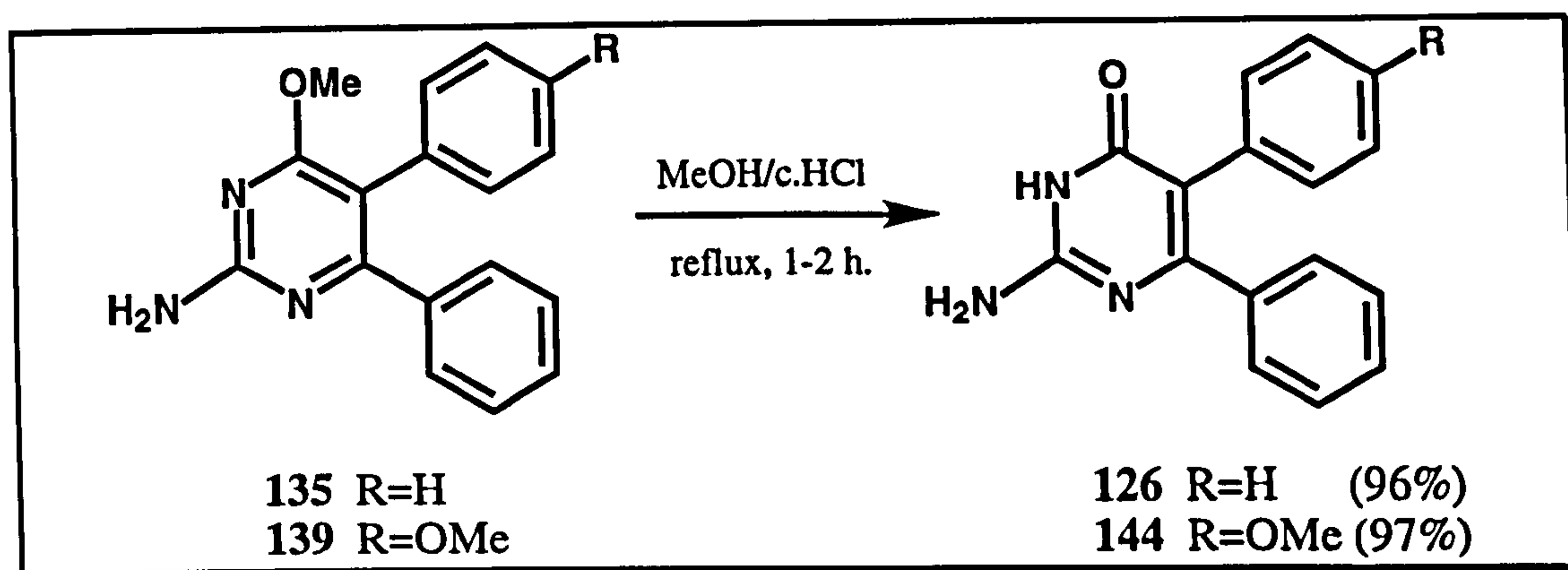
Ar	Reaction time / h.	Yield		
		Coupled product	141	138
	5	136 (0%)	67%	22%
	24	139 (72%)	0%	6%
	16	142 (78%)	0%	4%
	16	143 (75%)	0%	3%

Table 4-8 Suzuki reactions of iodopyrimidine, 141, with Pd(dppf)(OAc)₂ catalyst.

With a protocol for the Suzuki coupling of 5-halopyrimidines with a range of substituted arylboronic acids successfully developed, except in the reaction with more hindered boronic acids, the next stage in this project was to hydrolyse the 4-methoxypyrimidines (X) to the target 5-arylpyrimidin-4-ones (VII). To determine suitable conditions for this transformation, the hydrolyses of the bisphenylpyrimidine 135 and the 5-(4-methoxybenzene) derivative 139 were investigated.

Gronowitz *et al.*¹⁸² reported the use of a 1:1 mixture of methanol and 5 M hydrochloric acid for the room temperature hydrolysis of 2,4-dialkoxypyrimidines to the corresponding uracils. However pyrimidine 135 was unaffected under these conditions. Pyrimidine 135 also proved to be stable to warming with 1 M sodium

hydroxide, and to warm 5 M hydrochloric acid. Pyrimidines **135** and **139** were finally hydrolysed to their pyrimidinone derivatives **126** and **144** respectively, by heating to reflux in a 1:1 mixture of methanol and 12 M hydrochloric acid (Scheme 4-14). Pyrimidinone **126** thus prepared had mp 314-316°C, in close agreement to that previously isolated in minor quantities from the Suzuki reactions upon bropirimine **20** (312-314°C), and had identical ^1H NMR spectra.



Scheme 4-14 Acid hydrolysis of pyrimidines to pyrimidinones.

A possible future alternative route to 5-arylpyrimidines/pyrimidinones, that could facilitate the coupling of more hindered groups such as the 2,4-dichlorophenyl, stems from the work of Gronowitz *et al.* who successfully prepared and cross-coupled 5-pyrimidine boronic acids and heteroaromatic halides.¹⁸² Though adaptation of this route to the aminopyrimidinone substrates would require NH protection during boronic acid formation, the general nucleophilicity at pyrimidine C-5 positions might prove more in keeping with the boronic acid partner in Suzuki reactions, and overcome the difficulties encountered in coupling 5-halopyrimidines/pyrimidinones.

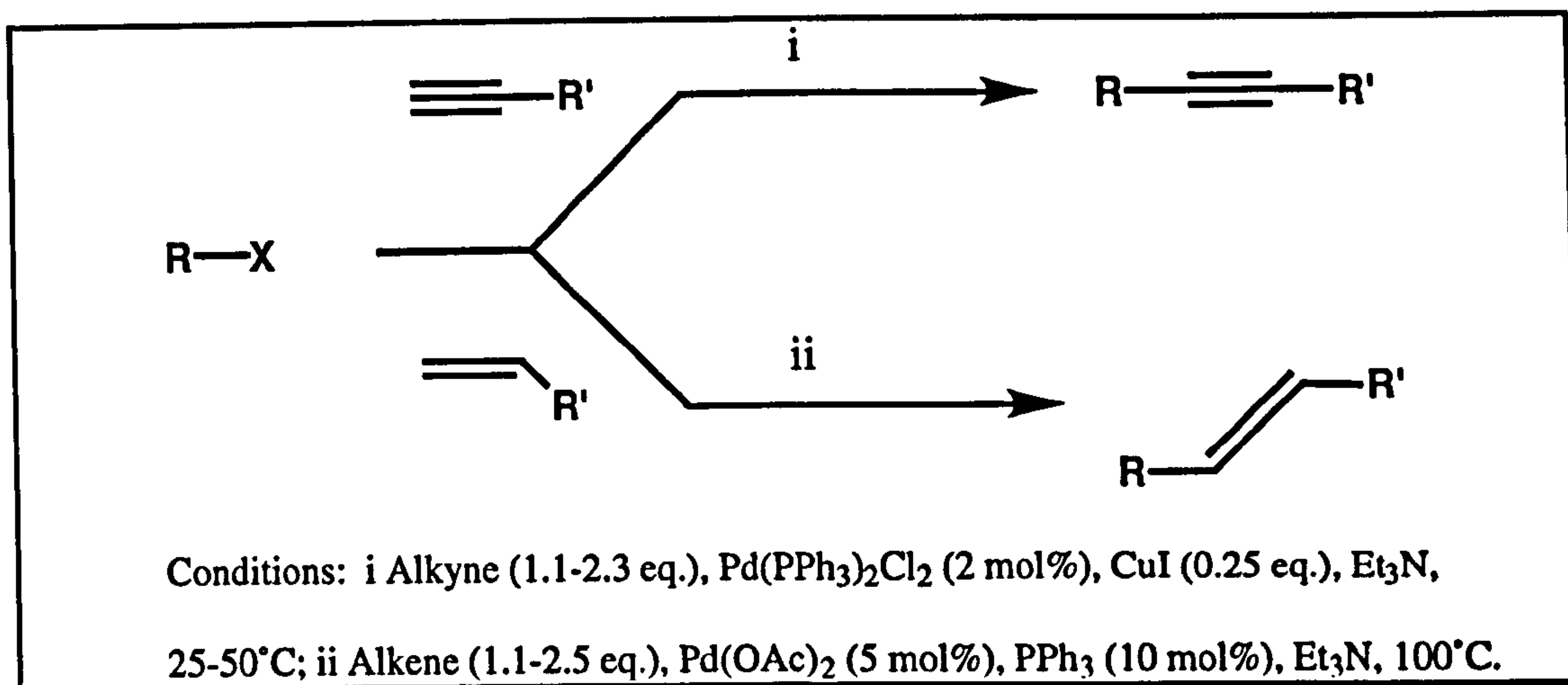
4.4.2 Heck coupling reactions

The pioneering work of Heck in 1968 originally involved stereospecific arylation of olefins.¹⁹⁸ The procedure involved reaction of organometallics such as arylmercuric chlorides (ArHgCl) with olefins in the presence of palladium(II) salts, for example Li_2PdCl_4 , and an amine base such as triethylamine. In the initial reactions, a stoichiometric quantity of palladium was required, as reaction produced $\text{Pd}(0)$. However, a reaction catalytic in Pd was soon realised, employing cupric chloride (CuCl_2) to reoxidise the palladium.

It was only later discovered that aryl halides could be used in the presence of Pd metal,^{199,200} or Pd-triarylphosphine complexes,²⁰¹ removing the need for stoichiometric quantities of mercury, lead or tin salts, and making the reaction of more general utility. The olefination of benzylic and vinylic halides, and heterocyclic bromides has also been described.²⁰²

The Heck reaction however, is perhaps more commonly thought of as the coupling of aromatic / heteroaromatic halides and terminal alkynes (Scheme 4-15). This process was first reported independently by Cassar²⁰³ and Heck²⁰⁴ in 1975, though the high yielding conditions in general use today, involving a bis-(triphenylphosphine)palladium(II) dichloride - cuprous iodide mixed catalyst system, were first reported by Sonogashira *et al.*²⁰⁵ for the formation of symmetrical disubstituted acetylenes from acetylene gas and aryl / vinyl bromides / iodides. A more recent report by Heck and coworkers²⁰⁶ on the alkenylation and alkynylation of polyhaloarenes, conveniently describes general conditions (see Scheme 4-15). Alkynylation of vinyl chlorides,²⁰⁷ vinyl triflates²⁰⁸ and imidoylchlorides²⁰⁹ have also been described, and, as with the Suzuki reaction, a

wide range of functionality is tolerated in either coupling partner in Heck reactions.



Scheme 4-15 General Heck reactions of (hetero)aryl / vinyl halides.

Sonogashira *et al.*²⁰⁵ also proposed a mechanism for their reaction (Figure 4-3). The first steps involve generation of the catalytic palladium species, $\text{Pd}(\text{PPh}_3)_2$, by reduction with some of the acetylene in the presence of CuI . The catalytic cycle then follows the general pattern of oxidative addition of $\text{Pd}(0)$ to the halide, followed by copper catalysed alkynylation of the Palladium. Reductive elimination of coupled product then regenerates the catalytic $\text{Pd}(0)$ species.

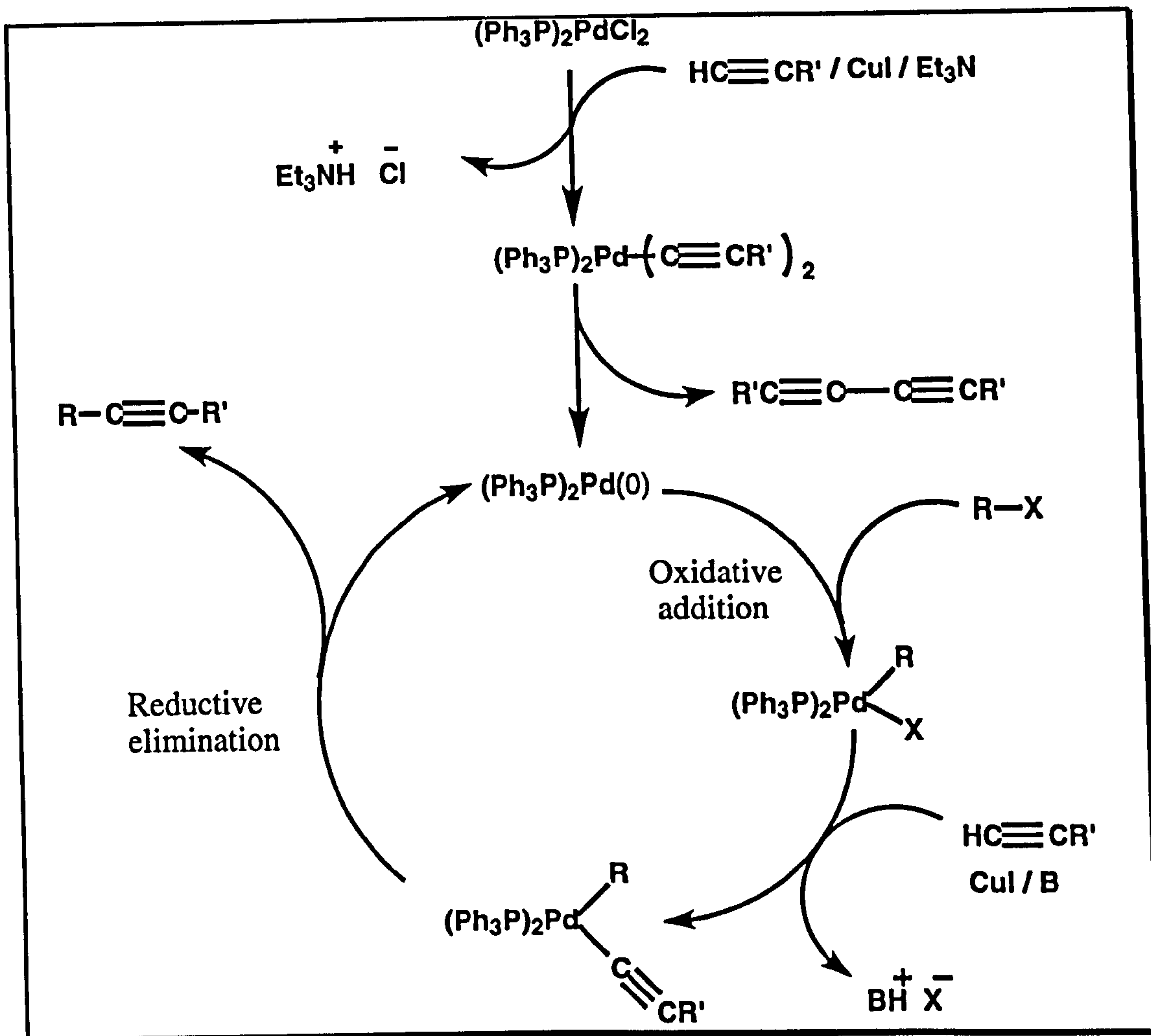
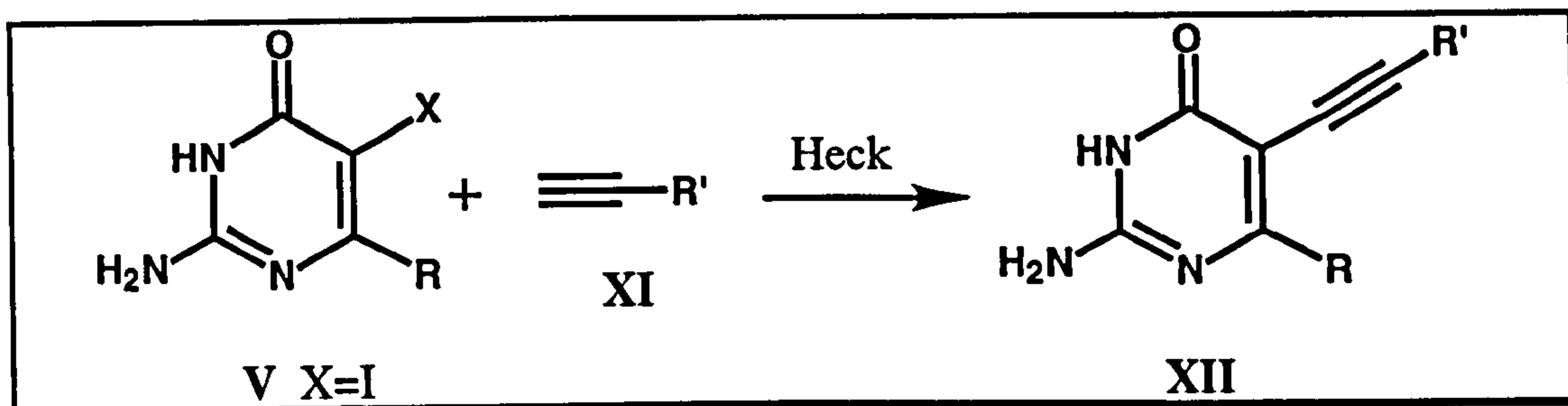


Figure 4-3 Proposed mechanism of Heck alkynylation of organic halides.

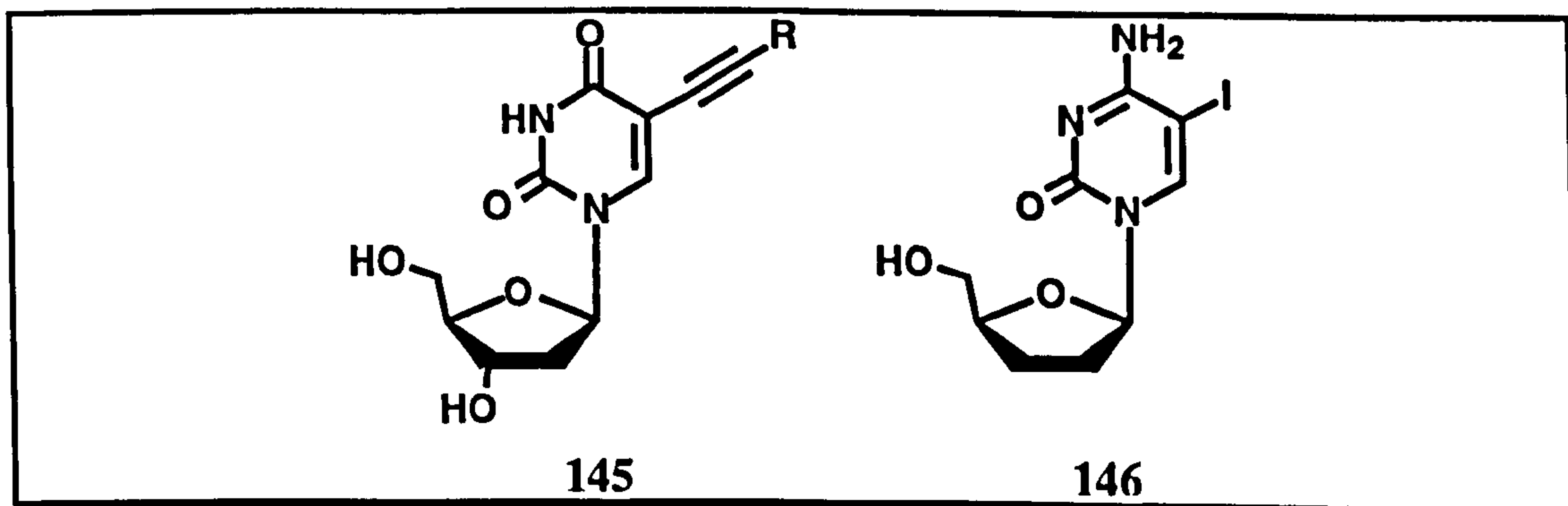
4.4.2.1 Reaction of 5-iodopyrimidinones

Alkynylations are generally performed under milder conditions than alkenylations and, as with Suzuki reactions, iodides have in general proved more reactive than corresponding bromides. By application of the Heck reaction conditions to 5-iodopyrimidinones (V) in the presence of terminal alkynes (XI), it was hoped to synthesise a range of novel 5-alkynylpyrimidinones (XII) (Scheme 4-16).



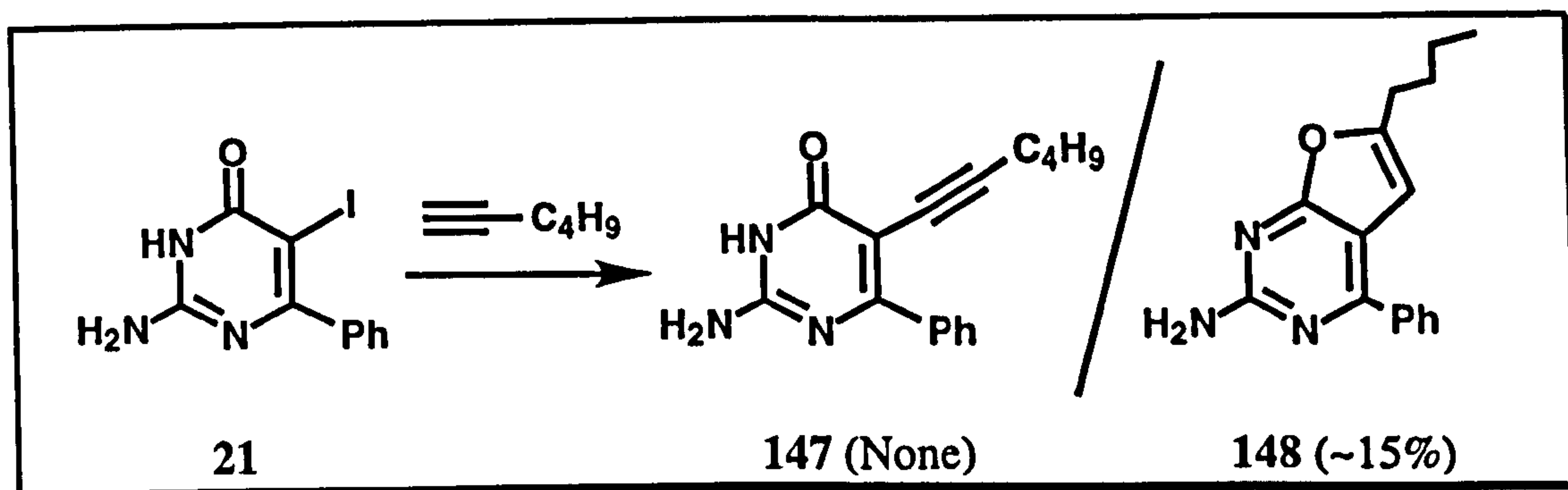
Scheme 4-16 Proposed route to 5-alkynylpyrimidinones, XII.

Literature searches revealed no record of compounds of structure XII, or the same without a 6-substituent. However, Heck alkynylations of iodopyrimidines have been widely reported (e.g. ref. 210). More closely related, the pronounced cytotoxicity and antiviral activity of 5-ethynyl-2'-deoxyuridine (145)^{211,212} has prompted the successful alkynylations of 5-iodouracils,²¹³⁻²¹⁵ 5-iodouracil nucleosides,²¹³⁻²¹⁵ and related 2,4-dialkoxy-5-iodopyrimidines²¹⁵ under conventional Heck conditions, in the search for active analogues. Less widely reported, high yielding alkynylations have also been performed upon even more structurally similar aminopyrimidinones such as the cytosine analogue 146,²¹⁶ suggesting the coupling proposed in scheme 4-16 to be feasible.



In the present work, continuing the interest in 6-phenylpyrimidinones, Heck reaction was initially attempted between AIPP (21) and 1-hexyne, according to the general procedure.²⁰⁶ Employing a mixture of triethylamine and DMSO as

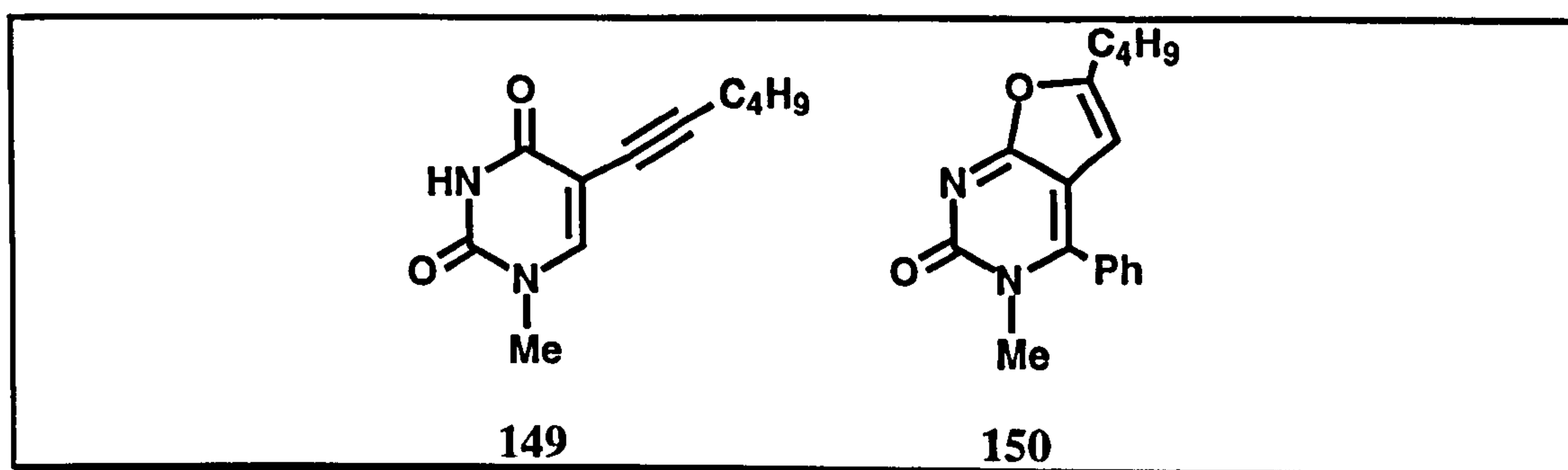
solvent, with a $\text{Pd}(\text{PPh}_3)_2\text{Cl}_2$ - CuI mixed catalyst system, the components were maintained at 50°C overnight. TLC of the mixture appeared to show one major component, highly fluorescent at 254 nm irradiation, and considerably less polar than **21**. During work up, the fluorescent compound was not extracted into 1 M sodium hydroxide, suggesting it not to be the desired 5-alkynylpyrimidinone **147**. An impure sample of the fluorescent compound finally obtained was tentatively assigned as the novel furo[2,3-*d*]pyrimidine **148**, though despite its suggested abundance by TLC only accounting for approximately 15% of starting material (Scheme 4-17).



Scheme 4-17 Heck reaction of AIPP (**20**) and 1-hexyne.

Similar cyclisations have been reported in the literature, for example in attempted Heck alkynylations of 5-iodouracils,^{213,214} as well as with other substrates including 3-iodo-2-pyridones and 4-pyridones.²¹⁷ In all these cases, iodine was only flanked by the oxo function. With iodide **21** a phenyl group is also adjacent, and steric effects favouring cyclisation cannot be ruled out. In many of the literature examples above, the bicyclic products were just minor ones, but in certain cases the major, simple alkynylated product was cyclised under coupling conditions but at elevated temperatures. For example, uracil **149** cyclised to **150** by treatment with CuI in refluxing triethylamine / methanol, in 92% yield.²¹⁴ In a repeat attempt to prepare **147**, reaction of **21** and hexyne was rerun

at room temperature, the only change being that DMSO was replaced with DMF to circumvent previous isolation difficulties. After 40 hours, TLC indicated a mixture, which included starting material, and possible deiodinated **26** as well as **148**, still forming at the lower temperature. At this stage the desire for **147** was abandoned, and the mixture was heated to 50°C for 4 hours to try and produce more **148**. Despite its apparent intensity by TLC, **148** was isolated in only 4% yield, compared with 64% returned **21** and 11% of deiodination by-product **26**.



Though not obtained sufficiently pure for elemental analysis, the assigned structure **148** was supported by ¹H and ¹³C NMR spectra and further corroborated by a series of ¹H NMR NOe measurements (Figure 4-4). Importantly, they show enhancement between the single 'vinylic' proton and the nearest methylene units of the butyl chain, as well as one of the phenyl group multiplets - indicative of their spacial proximity.

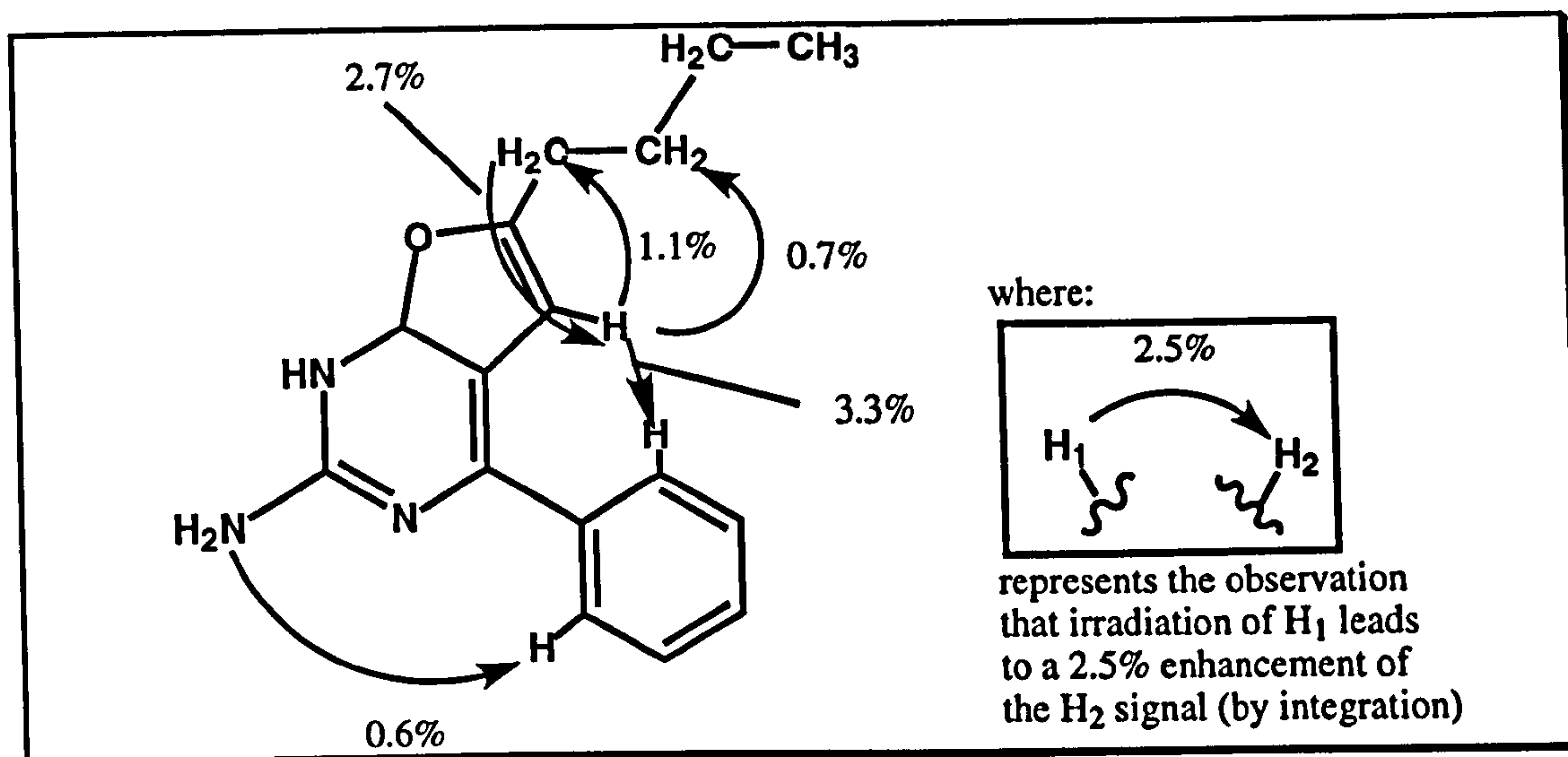


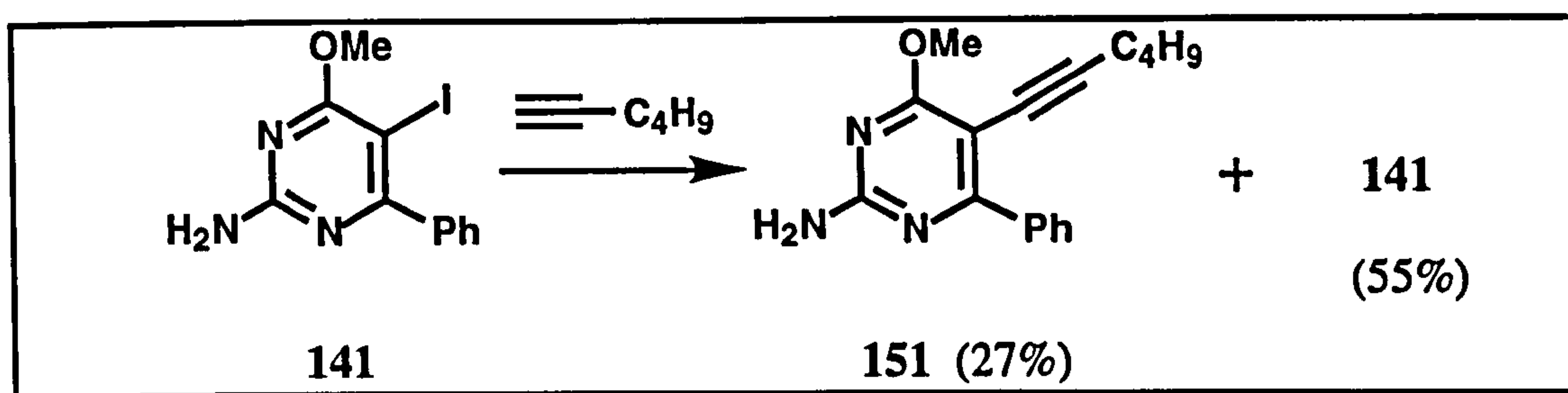
Figure 4-4 ^1H NMR NOe enhancements within furopyrimidine 148.

As observed in many of the Suzuki reactions of halopyrimidinones and halopyrimidines, competitive dehalogenation was a problem in the Heck reaction of **21**. The milder conditions employed in this Heck reaction further support the previous assertion that palladium is involved in this process. Unlike the literature of the Suzuki reaction, dehalogenations of substrates in Heck alkenylations and alkynylations have been reported on a number of occasions, though without rationalisation.^{201,206}

4.4.2.2 Reaction of 5-iodopyrimidines

Though an exact parallel cannot be drawn between the conditions of Suzuki and Heck reactions, given the previous experiences in the Suzuki reactions of 5-halopyrimidines versus pyrimidinones, and the difficulties encountered in Heck reactions upon pyrimidinone substrates, it seemed reasonable to attempt Heck reactions upon halopyrimidines. If successful, the 5-alkynylpyrimidines could then be converted to the target pyrimidinones **XII** by hydrolysis.

To this end, just one exploratory Heck reaction was performed. Iodopyrimidine **141**, previously synthesised for Suzuki coupling, and 1-hexyne, were reacted under identical conditions to those used previously, with the exception that triethylamine was the sole solvent (there being no solubility problems with **141**). After reaction work up and silica gel chromatography, a mixture of starting material (**141**) and novel coupled product **151** was obtained, which proved inseparable by a number of techniques (Scheme 4-18).



Scheme 4-18 Heck reaction of 5-iodopyrimidine **141** and hexyne.

The likely explanation for the low yield stems from the presence of two strongly electron donating substituents on the halide. Other researchers have noted lower reactivity towards alkynylation of such electron rich halides.²¹⁰

On the positive side, though the yield of alkynylated product was low, clean conversion was observed, without deiodination. With further studies, the potential to drive such coupling reactions to completion exists - for example, by attenuating the electron donating ability of the 2-amino group by carbamate formation. As with the pyrimidine Suzuki products, the alkynylpyrimidines thus generated would be ripe for conversion to the targets, a series of novel 5-alkynyl-pyrimidinones (**XII**), or potentially to related furo[2,3-*d*]pyrimidines.

5. Experimental

All mps were recorded on a Gallenkamp melting point apparatus, and are uncorrected. IR spectra were taken on a Mattson 2020 GALAXY Series FT-IR spectrometer as KBr plate or CHCl_3 films. ^1H , ^{13}C and ^{19}F NMR spectra were recorded on a Bruker ARX250 spectrometer at 250.1 MHz, 62.9 MHz and 235.3 MHz respectively in solvents as specified, with tetramethylsilane or residual protic solvents as internal standard, or with CFCl_3 as external standard with ^{19}F NMR, J values being in Hz. Low resolution mass spectra were recorded on a Micromass Platform (AP^+ , ES^+ and GC-EI), a VG70E (EI) or a VG Autospec (FAB^+). High resolution mass spectra (HRMS) were performed by the EPSRC Mass Spectrometry Service Centre, Swansea. Silica gel TLC was performed on 60F-254 pre-coated sheets (E. Merck) and column chromatography was done on silica gel C60 (60-120 mesh). Elemental analyses were kindly performed by Knoll Pharmaceuticals, Nottingham.

5.1 Imidazole chemistry

5-Diazoimidazole-4-carboxamide 32.⁹⁵ 5(4)-Aminoimidazole-4(5)-carboxamide hydrochloride (**27**) (48.77 g, 0.30 mmol) was dissolved in cold 1M hydrochloric acid (400 ml), decolourised with activated carbon, and added dropwise to a stirred solution of sodium nitrite (22.11 g, 0.33 mmol) in water (600 ml), maintained at 0-5°C, whereupon a precipitate began to form. Before the addition of **27** was complete, the mixture began to assume a slight pink colour, and the addition was discontinued. The precipitate was isolated by filtration, and washed with water (2 x 50 ml). The filtrate was then charged with more sodium nitrite (2 g), and the addition of **27** resumed. Once the

addition was complete a further crop of solid was isolated by filtration, and washed with water. The combined solids were then dried in a vacuum desiccator over phosphorus pentoxide to constant weight, to furnish the *title compound 32* as a yellow solid (35.38 g, 86%). The melting point was not determined, as explosions have been reported,⁹⁵ but a sample of the product gave the following data: $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$ 3282, 3151, 2193, 1710, 1449, 1369, 1249 and 1147 (lit.,⁹⁵ 2190 (diazo) and 1380 cm^{-1}); $\delta_{\text{H}}([\text{2H}_6]\text{DMSO})$ 7.62 (1H, s, 2-H) and 7.81 and 8.00 (2H, 2 x brs, CONH₂).

Attempted carbenoid reaction of 5-diazoimidazole-4-carboxamide 32: Procedure A. Methanol (0.18 ml, 4.5 mmol) was added to a stirred suspension of 5-diazoimidazole-4-carboxamide (**32**) (0.310 g, 2.26 mmol) in anhydrous THF (30 ml), followed by rhodium(II) acetate (0.010 g, 0.023 mmol). The reaction mixture was then stirred at room temperature, under nitrogen, for 76 h. Analysis of an aliquot by IR showed almost complete disappearance of the diazo band at 2188 cm^{-1} . A small amount of solid was removed by filtration, and the filtrate was concentrated *in vacuo* to leave a pale pink solid (0.49 g). ¹H NMR analysis of the pink solid revealed it to be a mixture of starting imidazole **32** (10%) and 2-azahypoxanthine (**29**) (90%). By-product **29** gave the following spectroscopic data: $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$ 3428, 3115, 2804, 1702 (lit.,⁹⁵ 1690), 1093, 921, 782 and 615; $\delta_{\text{H}}([\text{2H}_6]\text{DMSO})$ 8.52 (1H, s, 2-H) and 13-15 and 14.8-15.2 (2H, 2 x brs, 2xNH). These spectroscopic data were in exact agreement with those of an authentic sample of **29** available within the group.

Procedure B. Rhodium(II) acetate (0.010 g, 0.023 mmol) and 5-diazoimidazole-4-carboxamide (**32**) (0.310 g, 2.26 mmol) were dissolved in anhydrous DMSO (20 ml), under nitrogen. After 10 min at room temperature, cyclohexanol (0.47 ml, 4.5 mmol) was added via syringe, and the mixture

stirred at room temperature for 20h. The resultant red solution was diluted with diethyl ether (100 ml), and a pale pink solid isolated by filtration (0.160 g). IR analysis of the solid formed was in close agreement with an IR spectrum of an authentic sample of 2-azahypoxanthine (29). TLC analysis of the filtrate also correlated with an authentic sample of 29.

Procedure C. A dry flask was charged with rhodium(II) acetate (0.010 g, 0.023 mmol), anhydrous THF (100 ml) and cyclohexanol (0.47 ml, 4.5 mmol), and arranged for Soxhlet extraction with 5-diazoimidazole-4-carboxamide (32) (0.310 g, 2.26 mmol) in the extractor thimble. Held under nitrogen, the contents were heated to reflux for 20 h. After cooling, a small amount of solid was removed from the reaction mixture by filtration. TLC and IR analysis of the filtrate were consistent with the formation of 2-azahypoxanthine (29) in high yield, with reference to an authentic sample.

5-Bromoimidazole-4-carboxamide hydrobromide 41. A suspension of 5-diazoimidazole-4-carboxamide (32) (0.411 g, 3.0 mmol) in glacial acetic acid (25 ml) was treated with 30% wt hydrobromic acid in acetic acid (9 ml), and the mixture heated to reflux for 2.5 h. After slow cooling, a pale brown precipitate was removed by filtration, washed with acetic acid, followed by acetone, and dried in a vacuum desiccator to provide the *title compound* 41 (0.44 g, 54%) as pale brown crystals, and had mp 245-251°C (lit.,¹⁰⁴ 210°C) (Found: C, 17.8; H, 1.8; N, 15.8; Br, 59.2%. $C_4H_4BrN_3O \cdot HBr$ requires C, 17.7; H, 1.9; N, 15.5; Br, 59.0%); $\nu_{max}(KBr)/cm^{-1}$ 2980, 2806, 1701, 1609, 1473, 1395, 832 and 623; $\delta_H([^2H_6]DMSO)$ 7.21 and 7.71 (2H, 2xbrs, CONH₂), 8.13 (1H, s, 2-H) and 11.57 (2H, brs, 2xNH); $\delta_C([^2H_6]DMSO)$ 111.8, 124.9, 137.3 (CH) and 159.6.

Attempted Suzuki reaction of 5-bromo-1*H*-imidazole-4-carboxamide hydrobromide 41. Phenylboronic acid (0.275 g, 2.25 mmol) was added to a mixture of 5-bromo-1*H*-imidazole-4-carboxamide hydrobromide (**41**)¹⁰⁴ (0.550 g, 2.03 mmol) and tetrakis(triphenylphosphine)-palladium(0) (0.080 g, 0.07 mmol) in degassed DMF (15 ml), followed by a degassed solution of sodium carbonate (0.765 g, 7.22 mmol) in water (4 ml), and the mixture heated to reflux under nitrogen, for 18 h. After cooling, a white solid was removed by filtration. The filtrate was evaporated to give a gummy purple solid which was extracted with boiling ethyl acetate (200 ml), leaving a grey solid. Hydrogen chloride gas was bubbled through the ethyl acetate solution, and a pale pink solid collected by filtration (0.239 g). ¹H NMR analysis of the pink solid revealed it to be a mixture of starting imidazole **41** (43%) and 1*H*-imidazole-4-carboxamide (**42**) (57%), both as their hydrochloride salts, accounting for 65% of starting imidazole. Crystallisation of the mixture from acetic acid gave a product 71% pure in imidazole **42** (with imidazole **41** the contaminant), where the NMR signals assigned to **42** were as follows: $\delta_{\text{H}}([\text{}^2\text{H}_6]\text{DMSO})$ 7.93 (1H, br s, CONH₂), 8.35 (1H, d, *J* 1, 5-H), 8.56 (1H, br s, CONH₂), 8.22 (1H, d, *J* 1, 2-H) and 14.90 (1-2H, br s, [-NHCHNH-]⁺); $\delta_{\text{C}}([\text{}^2\text{H}_6]\text{DMSO})$ 120.9 (C-5), 128.0 (C-4), 135.8 (C-2) and 158.6 (CONH₂).

5-Iodoimidazole-4-carboxamide hydrochloride 43. 5-Diazoimidazole-4-carboxamide (**32**) (0.411 g, 3.0 mmol) was added in one portion to an ice-cold solution of potassium iodide (4.98 g, 30 mmol) in 1 M hydrochloric acid. Protected from light, the flask was connected to a bubbler to observe nitrogen evolution, and stirred in the ice-bath. Once effervescence had subsided (after about 10 min), the flask was allowed to warm to room temperature, whereupon effervescence appeared to resume. After 45 min at room temperature, the mixture was then heated to 50°C for 10 min, followed

by heating on a steam bath for a further 10 min. After prolonged cooling, a metallic looking solid was removed by filtration (iodine), and the solid washed with water. The combined dark red filtrate and washings were washed with ethyl acetate (4 x 30 ml). The remaining pale yellow aqueous phase was then basified with solid sodium hydroxide to pH 9-10 and extracted with ethyl acetate (18 x 20 ml). The combined extracts were dried (MgSO_4), and concentrated *in vacuo* to 50 ml. Hydrogen chloride gas was passed through the remaining solution, and after cooling in ice an impure sample of the *title compound 43* (0.061 g, 7%) was obtained on filtering the suspension, which though initially white, appeared to discolour with the formation of blue and orange tinges on drying at the filter. A sample of **43** gave the following data: $\delta_{\text{H}}([\text{}^2\text{H}_6]\text{DMSO})$ 7.71 (2H, brs, CONH_2), 8.72 (1H, s, 2-H) and 9-10 (2H, brs, 2xNH). Residual resonances attributable to imidazole **42** were also present in the ^1H NMR spectrum of the crude product at $\delta_{\text{H}}=8.26$ (1H, d, J 1, 2 or 5-H) and $\delta_{\text{H}}=9.19$ (1H, d, J 1, 2 or 5-H).

5(4)-N-(Benzylidene)amino-1H-imidazole-4(5)-carboxamide 46.

Benzaldehyde (0.51 ml, 5.0 mmol) was added to a stirred solution of 5-amino-1H-imidazole-4-carboxamide hydrochloride (**27**) (0.81 g, 5.0 mmol) and sodium acetate trihydrate (0.60 g, 4.4 mmol) in ethanol (10 ml) and water (10 ml). The mixture was heated to reflux for 1 h. After cooling in ice, the mixture was filtered and the precipitate washed with ethanol, then diethyl ether and dried *in vacuo* to furnish the *title compound 46* (0.86 g, 80%) as a white solid, mp 252°C (decomp.) (from DMF) (lit.,¹⁰⁴ 242-245°C for the crude solid); $\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$ 3363, 3118, 1656, 1589, 1421, 1091, 828 and 685 (lit.,¹⁰⁴ 3400, 3160 and 1645); $\delta_{\text{H}}([\text{}^2\text{H}_6]\text{DMSO})$ 7.49-7.59 (3H, m, ArH), 7.68 and 7.84 (2x1H, 2xbrs, CONH_2), 7.74 (1H, s, $\text{ArCH}=\text{N}$), 7.95-8.02 (2H, m, ArH), 9.18 (1H, s, 2-H) and 13.06 (1H, brs, NH) (in agreement with the literature¹⁰⁴); $\delta_{\text{C}}([\text{}^2\text{H}_6]\text{DMSO})$ 118.7 (C), 128.8 (CH, 2xArC), 129.0 (CH,

2xArC), 131.8 (CH, ArC), 135.5 (C, ArC), 136.0 (CH, C-2), 146.8 (C), 158.3 (CH, ArCH=N) and 160.8 (C, CONH₂); m/z (EI), 214, C₁₁H₁₀N₄O requires M , 214.

5(4)-N-(2-Fluorobenzylidene)amino-1H-imidazole-4(5)-

carboxamide 47. Prepared according to the method for the preparation of 46, with 5-amino-1H-imidazole-4-carboxamide hydrochloride (27) (1.626 g, 10.0 mmol), 2-fluorobenzaldehyde (1.407 g, 11.0 mmol) and sodium acetate trihydrate (1.2 g, 8.8 mmol), to provide the *title compound* 47 (2.068 g, 89%) as a white solid, mp 278°C (with decomp.) (from DMF) (Found: C, 56.7; H, 4.1; N, 24.2%; m/z (FAB⁺), 233. C₁₁H₉FN₄O requires C, 56.9; H, 3.9; N, 24.1%; $M+1$, 233); ν_{\max} (KBr)/cm⁻¹ 3368, 3124, 1668, 1594, 1457, 1421, 1098 and 582; δ_{H} ([²H₆]DMSO) 7.34-7.42 (2H, m, 2xArH), 7.58-7.63 (1H, m, ArH) 7.71 and 7.80 (2x1H, 2xbr s, CONH₂) 7.76 (1H, d, J 0.7, N=CHAr), 8.15-8.19 (1H, m, ArH), 9.32 (1H, s, 2-H) and 13.0 (1H, br s, ring NH); δ_{C} ([²H₆]DMSO, ¹⁹F coupled) 116.5 (d, J 21, C-3'), 119.4 (C-5), 123.2 (d, J 9, C-1'), 125.3 (d, J 3.3, C-6' or 4'), 128.7 (d, J 2.5, C-5'), 133.9 (d, J 9, C-4' or 6'), 136.5 (C-2), 146.9 (C-4), 151.3 (d, J 4, N=CHAr), 160.8 (CONH₂) and 162.3 (d, J 253, C-2'); δ_{F} ([²H₆]DMSO) -119.79 to -119.90 (m).

5(4)-N-(2-Chlorobenzylidene)amino-1H-imidazole-4(5)-

carboxamide 48. Prepared according to the method for the preparation of 46, with 5-amino-1H-imidazole-4-carboxamide hydrochloride (27) (1.626 g, 10.0 mmol), 2-chlorobenzaldehyde (1.24 ml, 11.0 mmol) and sodium acetate trihydrate (1.2 g, 8.8 mmol), to yield the *title compound* 48 (2.301 g, 93%) as a white solid, mp 275°C (decomp.) (from DMF) (Found: C, 53.1; H, 3.8; N, 22.6%; m/z (ES⁺), 249, 251. C₁₁H₉ClN₄O requires C, 53.1; H, 3.6; N, 22.5%; $M+1$, 249, 251); ν_{\max} (KBr)/cm⁻¹ 3389, 3150, 1655, 1584, 1422, 1157, 837 and 679; δ_{H} ([²H₆]DMSO) 7.48-7.63 (3H, m, 3xArH), 7.73 (2H, br s, CONH₂),

7.76 (1H, s, N=CHAr), 8.25 (1H, dd, J 8 and 2, ArH), 9.52 (1H, s, 2-H) and 13.0 (1H, br s, ring NH); $\delta_{\text{C}}([^2\text{H}_6]\text{DMSO})$ 119.7 (C), 128.0 (CH), 128.6 (CH), 130.5 (CH), 132.4 (C), 133.3 (CH), 135.3 (C), 136.5 (CH), 146.7 (C), 154.1 (N=CHAr) and 160.8 (CONH₂).

5(4)-*N*-(2-Chloro-5-nitrobenzylidene)amino-1*H*-imidazole-4(5)-carboxamide 49. Prepared according to the method for the preparation of 46, with 5-amino-1*H*-imidazole-4-carboxamide hydrochloride (27) (1.626 g, 10.0 mmol), 2-chloro-5-nitrobenzaldehyde (2.041 g, 11.0 mmol) and sodium acetate trihydrate (1.2 g, 8.8 mmol), to provide the *title compound* 49 (2.767 g, 94%) as a yellow solid, that would not crystallise from common solvents tried, and had mp 298°C (with decomp.) (Found: C, 45.4; H, 3.1; N, 23.8%; m/z (ES⁺), 294, 296. C₁₁H₈ClN₅O₃ requires C, 44.8; H, 2.7; N, 23.8%; $M+1$, 294, 296); $\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$ 3407, 3181, 1678, 1586, 1530, 1350, 739 and 523; $\delta_{\text{H}}([^2\text{H}_6]\text{DMSO})$ 7.67 and 7.84 (2x1H, 2xbr s, CONH₂), 7.79 (1H, s, N=CHAr), 7.92 (1H, d, J 9, 3'-H), 8.34 (1H, dd, J 9 and 3, 4'-H), 8.87 (1H, d, J 3, 6'-H), 9.50 (1H, s, 2-H) and 12.9 (1H, br s, ring NH); insufficiently soluble for ¹³C NMR; m/z (HRMS-ES⁺) 294.0394 ($M+(^{35}\text{Cl})+1$. C₁₁H₈ClN₅O₃+H requires 294.0394).

5(4)-*N*-(2-Nitrobenzylidene)amino-1*H*-imidazole-4(5)-carboxamide 50. Prepared according to the method for the preparation of 46, with 5-amino-1*H*-imidazole-4-carboxamide hydrochloride (27) (1.626 g, 10.0 mmol), 2-nitrobenzaldehyde (1.696 g, 11.0 mmol) and sodium acetate trihydrate (1.2 g, 8.8 mmol), to furnish the *title compound* 50 (2.357 g, 91%) as a yellow solid and had mp 256°C (decomp.) (from DMF) (Found: C, 51.0; H, 3.8; N, 27.1%; m/z (EI), 259. C₁₁H₉N₅O₃ requires C, 51.0; H, 3.5; N, 27.0%; M , 259); $\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$ 3399, 3150, 1654, 1559, 1541, 1522, 1091 and 782; $\delta_{\text{H}}([^2\text{H}_6]\text{DMSO})$ 7.53 (1H, br s, CONH₂), 7.74-7.81 (3H, m, CONH₂, ArH

and N=CHAr), 7.87 (1H, t, J 7.5, ArH), 8.09 (1H, t, J 7.5, ArH), 8.27 (1H, d, J 7.5, ArH), 9.40 (1H, s, 2-H) and 13.1 (1H, br s, ring NH); $\delta_{\text{C}}([^2\text{H}_6]\text{DMSO})$ 120.0 (C-1'), 124.6 (CH), 129.3 (C-2'), 130.2 (CH), 132.2 (CH), 133.6 (CH), 136.6 (CH), 146.3 (C), 149.4 (C), 154.1 (C-2) and 160.6 (CONH₂).

Imidazo[1,5-*a*]quinazoline-3-carboxamide 54. Sodium hydride as a 60% dispersion in mineral oil (0.379 g, 9.5 mmol NaH) was placed in a flask and triturated via syringe with freshly distilled petroleum ether (bp 40-60°C) (3x10 ml), and then suspended in anhydrous DMF (60 ml). Solid 5(4)-*N*-(2-fluorobenzylidene)amino-1*H*-imidazole-4(5)-carboxamide (**47**) (2.00 g, 8.6 mmol) was added in a single portion to the stirred DMF suspension, sealed under a nitrogen atmosphere connected to a bubbler, and stirred at room temperature. Once a clear solution was obtained, the mixture was heated to reflux for 3 h. The dark mixture was allowed to cool, water (10 ml) was added and the pH adjusted to neutral to litmus with 1 M hydrochloric acid. After further cooling at 4°C, a solid was filtered from solution, washed with water, then ethanol, and dried at 60°C/4x10⁻² mbar to yield the *title compound* **54** (1.444 g, 79%) directly, as a dark yellow crystalline solid. A further crop of **54** (0.119 g, 6.5%) was obtained by evaporation of the mother liquor, followed by crystallisation of the residue from DMF. A sample recrystallised from DMF had mp 294-294.5°C (with decomp.) (Found: C, 61.55; H, 4.1; N, 26.2%; m/z (EI), 212. C₁₁H₈N₄O.1/6 C₃H₇NO requires C, 61.6; H, 4.1; N, 26.0%; M , 212 (for C₁₁H₈N₄O)); $\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$ 3436, 3154, 1665, 1612, 1548, 1424, 1228 and 695; $\delta_{\text{H}}([^2\text{H}_6]\text{DMSO})$ 7.41 and 7.64 (2x1H, 2xbr s, CONH₂), 7.71 (1H, t, J 8, 7 or 8-H), 8.03 (1H, dt, J 8 and 1, 8 or 7-H), 8.20 (1H, dd, J 8 and 1, 6 or 9-H), 8.48 (1H, d, J 8, 9 or 6-H), 8.99 (1H, s, 1 or 5-H) and 9.12 (1H, s, 5 or 1-H); $\delta_{\text{C}}([^2\text{H}_6]\text{DMSO})$ 116.3 (CH), 118.9 (C), 125.7 (C), 127.6 (CH), 127.7 (CH), 130.4 (CH), 132.9 (C), 135.5 (CH), 136.6 (C),

153.2 (C-5) and 163.9 (CONH₂); *m/z* (HRMS-EI) 212.0698 (M⁺. C₁₁H₈N₄O requires 212.0698).

7-Nitroimidazo[1,5-*a*]quinazoline-3-carboxamide 55. Reactants were mixed as in the synthesis of **54**, employing sodium hydride (60% dispersion in oil) (0.177 g, 4.4 mmol), DMF (60 ml) and 5(4)-*N*-(2-Chloro-5-nitrobenzylidene)-amino-1*H*-imidazole-4(5)-carboxamide (**49**) (1.179 g, 4.0 mmol). Once a clear solution was obtained, the reaction was heated in an oil bath maintained at 55-65°C for 11 days, until reaction was determined complete by TLC. The mixture was allowed to cool, diluted with water (10 ml) and neutralised with 1 M hydrochloric acid. After cooling in ice, a solid was isolated by filtration, washed with water then ethanol, and dried at 60°C/6.5x10⁻² mbar to furnish the *title compound 55* (0.797 g, 77%) in acceptable purity. Evaporation of the mother liquor and crystallisation from aqueous DMF provided a second crop of **55** (0.061 g, 6%). A sample crystallised from 50% aqueous DMF had mp >360°C (Found: C, 51.2; H, 3.1; N, 27.0%; *m/z* (ES⁺), 358. C₁₁H₇N₅O₃ requires C, 51.4; H, 2.7; N, 27.2%; *M*+1, 358); *v*_{max}(KBr)/cm⁻¹ 3358, 3181, 1686, 1595, 1570, 1528, 1350 and 698; δ_{H} (CF₃CO₂D) 9.44 (1H, d, *J* 10, 9-H), 9.70 (1H, dd, *J* 10 and 3, 8-H), 9.90 (1H, d, *J* 3, 6-H), 10.07 (1H, s, 1/5-H) and 10.83 (1H, s, 5/1-H); δ_{C} (CF₃CO₂D) 116.1 (C), 118.1 (CH), 119.8 (C), 126.1 (CH), 126.5 (CH), 130.6 (CH), 134.0 (C), 135.3 (C), 147.7 (C), 158.0 (CH) and 159.4 (CONH₂).

Hydrolysis of imidazo[1,5-*a*]quinazoline-3-carboxamide 54 to imidazo[1,5-*a*]quinazoline-3-carboxylic acid, potassium salt 56a, and imidazo[1,5-*a*]quinazoline-3-carboxylic acid 56b. Concentrated (90%) nitric acid (0.494 g, 7.1 mmol), concentrated (98%) sulfuric acid (5 ml) and amide **54** (1.00 g, 4.7 mmol) were heated on a water bath to 57°C (internal temperature) for 45 min. The yellow solution formed was allowed to cool,

poured into water (20 ml), further cooled, and a yellow solid isolated by filtration which was washed with small portions of water and ethanol. After air drying, the solid was dissolved in 1 M potassium hydroxide (5 ml), washed with chloroform (30 ml), carefully neutralised with dilute sulfuric acid and evaporated to give a pale yellow solid. The crude yellow solid was crystallised from aqueous ethanol to yield imidazo[1,5-*a*]quinazoline-3-carboxylic acid, potassium salt (**56a**) (0.605 g, 46%) as its hydrate, and had mp 311-313°C (Found: C, 46.7; H, 3.3; N, 14.6. $C_{11}H_6KN_3O_2 \cdot 1.75H_2O$ requires C, 46.7; H, 3.4; N, 14.9%); $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$ 3432, 3108, 1609, 1551, 1427, 1370, 1231 and 754; $\delta_{\text{H}}(\text{D}_2\text{O})$ 7.12 (1H, t, *J* 7, 7 or 8-H), 7.28-7.40 (3H, m, 3xArH), 8.10 (1H, s, slow exchange, 1-H) and 8.21 (1H, s, 5-H); $\delta_{\text{C}}(\text{D}_2\text{O})$ 113.1 (CH), 116.6 (C), 124.8 (slow exchange, C-1), 126.4 (CH), 126.8 (C), 128.2 (CH), 130.2 (C), 133.9 (CH), 134.9 (C), 151.7 (CH) and 169.8 (CO_2^-). The residue after evaporation of the aqueous ethanol filtrate was dissolved in the minimum volume of water (2 ml) and acidified with concentrated hydrochloric acid. The precipitate produced was filtered, washed with a little water and dried in *vacuo* to provide imidazo[1,5-*a*]quinazoline-3-carboxylic acid (**56b**) (0.124 g, 16%) as its hydrate, as a yellow solid, and had mp 194°C (decomp. with effervescence) (Found: C, 60.7; H, 3.7; N, 19.3; H_2O , 1.3%; *m/z* (ES^+), 214. $C_{11}H_7N_3O_2 \cdot 0.2 \text{H}_2\text{O}$ requires C, 60.9; H, 3.4; N, 19.4; H_2O , 1.7%; *M*+1, 214); $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$ 3401, 3160, 1705, 1603, 1549, 1317, 1175 and 772; $\delta_{\text{H}}([\text{}^2\text{H}_6]\text{DMSO})$ 7.73 (1H, t, *J* 8, 7 or 8-H), 8.03 (1H, t, *J* 8, 8 or 7-H), 8.21 (1H, d, *J* 8, 6 or 9-H), 8.50 (1H, d, *J* 8, 9 or 6-H), 9.05 (1H, s, 5-H) and 9.22 (1H, s, 1-H); $\delta_{\text{C}}([\text{}^2\text{H}_6]\text{DMSO})$ 115.9 (CH), 118.5 (C), 120.9 (C), 127.5 (CH), 127.6 (CH), 129.7 (CH), 132.0 (C), 135.0 (C), 137.7 (C), 154.4 (CH) and 162.6 (CO_2H); *m/z* (HRMS- Cl^+) 214.0617 ($\text{M}^+ C_{11}H_7N_3O_2 + \text{H}$ requires 214.0616).

2-Fluorobenzaldehyde diethyl acetal 57. 2-Fluorobenzaldehyde (3.838 g, 30 mmol) and *p*-toluenesulfonic acid monohydrate (0.145 g, 0.8 mmol) were heated to reflux in a mixture of triethyl orthoformate (25 ml) and anhydrous ethanol (25 ml) for 2 h, under nitrogen. After cooling, the solution was diluted with diethyl ether (250 ml), washed with a 1:1 mixture of 5% sodium hydroxide and saturated brine (40 ml), then water (20 ml) followed by saturated brine (20 ml). The ethereal solution was then dried (MgSO₄) and reduced to 10 ml. Fractional distillation of the residue under reduced pressure provided the *title compound 57* (5.151 g, 86%) as a colourless oil, and had bp 99-101°C/22 mbar (Found: C, 66.5; H, 7.5%; *m/z* (GC-EI), 198. C₁₁H₁₅FO₂ requires C, 66.6; H, 7.6%; *M*, 198); $\nu_{\max}(\text{CHCl}_3)/\text{cm}^{-1}$ 2978, 2882, 1489, 1458, 1229, 1115, 1061 and 760; $\delta_{\text{H}}([\text{}^2\text{H}_6]\text{DMSO})$ 1.16 (6H, t, *J* 7, 2xMe), 3.44-3.65 (4H, m, 2xCH₂), 5.67 (1H, s, acetal CH), 7.15-7.24 (2H, m, ArH), 7.35-7.45 (1H, m, ArH) and 7.47 (1H, m, ArH); $\delta_{\text{C}}([\text{}^2\text{H}_6]\text{DMSO}, \text{}^{19}\text{F coupled})$ 15.3 (2xCH₃), 61.7 (2xCH₂), 96.5 (d, *J* 3, acetal C), 115.5 (d, *J* 21, C-3), 124.3 (d, *J* 3, C-5), 126.4 (d, *J* 13, C-1), 128.0 (d, *J* 4, C-4 or 6), 130.6 (d, *J* 8, C-6 or 4) and 159.9 (d, *J* 247, C-2).

Attempted reduction of 5(4)-*N*-(2-nitrobenzylidene)amino-1*H*-imidazole-4(5)-carboxamide (50), to give 5(4)-*N*-(2-aminobenzylidene)-amino-1*H*-imidazole-4(5)-carboxamide 58: Procedure A. According to the literature method,¹¹⁷ 5(4)-*N*-(2-nitrobenzylidene)amino-1*H*-imidazole-4(5)-carboxamide (50) (0.130 g, 0.50 mmol) and stannous chloride dihydrate (0.564 g, 2.50 mmol) were heated to reflux in absolute ethanol (20 ml), under an argon atmosphere, for 1 h, whereupon TLC analysis indicated complete disappearance of imidazole 50. The resultant yellow solution was poured into ice-water (50 ml), basified with 5% sodium bicarbonate solution to pH 7-8 to decompose tin salts, and extracted with 5% ethanol/ethyl acetate (50 ml). TLC analysis of the organic extract showed a complex mixture of many

components, including one with an identical R_f to AIC (27), and the reaction was not progressed further.

Procedure B. According to the literature method,¹¹⁷ 5(4)-*N*-(2-nitrobenzylidene)amino-1*H*-imidazole-4(5)-carboxamide (50) (0.130 g, 0.50 mmol) and stannous chloride dihydrate (0.564 g, 2.50 mmol) were heated to reflux in ethyl acetate (10 ml), under argon, for 20 h. The solvent was evaporated *in vacuo*, and the residue suspended in water (10 ml), basified with 5% sodium bicarbonate solution to pH 7-8, and extracted with 5% ethanol/ethyl acetate (50 ml). As previously observed, TLC analysis of the organic extract showed a mixture of components, including AIC (27). TLC analysis of the remaining aqueous phase also demonstrated the presence of imidazole 27. The reaction was not progressed further.

Procedure C. According to the literature method,¹¹⁸ 5(4)-*N*-(2-nitrobenzylidene)amino-1*H*-imidazole-4(5)-carboxamide (50) (0.644 g, 2.50 mmol) was suspended in water (5 ml). 12 M Hydrochloric acid (0.5 ml) was added, followed by 10% palladium on carbon (approximately 25 mg), and the flask connected to a Parr hydrogenation apparatus, which was charged with hydrogen (50 psi). After shaking at room temperature for 5 h, the mixture was filtered, and the solids washed with water, then acetone. TLC analysis identified the pale green crystalline solid filtered off as unreacted 50, contaminated with the palladium catalyst, which was later confirmed by ¹H NMR, on a portion recrystallised from DMF. The combined filtrates above were extracted with ethyl acetate (2 x 30 ml). TLC analysis of the aqueous and organic phases showed the aqueous phase to contain AIC (27), later confirmed by ¹H NMR after removal of the water *in vacuo*; and the organic phase to be a mixture of three components, with one in far greater abundance than the other two. The combined ethyl acetate extracts were combined, and reduced *in*

vacuo to leave a green oil which gave NMR data consistent with the cyclohexene structure **61**: $\delta_{\text{H}}(\text{CDCl}_3)$ 1.69-1.87 (4H, m, 2xCH₂), 2.58 (2H, t, *J* 6, CH₂), 2.78 (2H, t, *J* 6, CH₂) and 8.09 (1H, s, CHX); DEPT- $\delta_{\text{C}}(\text{CDCl}_3)$ 18.6 (CH₂), 21.4 (CH₂), 22.5 (CH₂), 22.6 (CH₂), 152.5 (CHX).

Procedure D. According to the literature method,¹¹⁹ a suspension of 5(4)-*N*-(2-nitrobenzylidene)amino-1*H*-imidazole-4(5)-carboxamide (**50**) (0.500 g, 1.93 mmol) in 1:1 v/v ethanol/ethylene glycol (10 ml) was treated with hydrazine hydrate (0.87 ml, 15 mmol). After stirring for 10 min at room temperature, a 50% slurry of Raney nickel in water (2 drops) was added and the mixture stirred for a further 30 min. The mixture was then heated in an oil bath at 50-60°C for 2 h, cooled, and the catalyst filtered from the solution and washed with ethanol. The combined filtrates were diluted with water (50 ml) and extracted with diethyl ether (2 x 30 ml). TLC analysis of the remaining aqueous phase showed one major component, which appeared identical to an authentic sample of AIC (**27**). The organic extracts were concentrated to dryness, and the residue purified by silica gel column chromatography, with diethyl ether as eluent, to provide a yellow solid that was identified as the hydrazine derivative **62** (0.21 g, 91% of theoretical yield) and had mp 249°C (lit.,¹¹⁶ 251-252°C) (Found *m/z* (ES⁺), 239. C₁₄H₁₄N₄ requires *M*+1, 239); $\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$ 3486 (asym. NH str.), 3304 (sym. NH str.), 3011 (ArH), 1711 (asym. C=N str.), 1624 (sym. C=N str.), 1595, 1364 and 1159; $\delta_{\text{H}}(\text{CDCl}_3)$ 6.35 (4H, brs, 2xNH₂), 6.71-6.77 (4H, m, ArH), 7.18-7.29 (4H, m, ArH) and 8.74 (2H, s, 2xCH=N).

Procedure E. Based on the literature method,¹²⁰ a suspension of 5(4)-*N*-(2-nitrobenzylidene)amino-1*H*-imidazole-4(5)-carboxamide (**50**) (0.500 g, 1.93 mmol) in 78% ethanol/water (15 ml) was treated with calcium chloride (0.138 g, 1.24 mmol), as a solution in water (0.3 ml), followed by zinc powder (0.51

g, 7.7 mmol), and the mixture heated to reflux. After 1 h, TLC analysis showed only starting material, but after a further 20 h at reflux, TLC indicated complete consumption of starting material **50** to give a multicomponent mixture, including one component identical by TLC with an authentic sample of AIC (**27**). No attempt was made to isolate any of the products, and the reaction was abandoned.

2-Nitrobenzaldehyde diethyl acetal 63. 2-Nitrobenzaldehyde (4.53 g, 30.0 mmol) and *p*-toluenesulfonic acid monohydrate (0.15 g, 0.8 mmol) were heated to reflux, under nitrogen, in a mixture of triethyl orthoformate (25 ml) and ethanol (25 ml). After 2 h, the yellow solution was cooled, diluted with diethyl ether (250 ml), and washed with a 1:1 mixture of 5% NaOH and saturated brine (40 ml), water (20 ml), saturated brine (20 ml) and dried (MgSO₄). After removal of solvents *in vacuo*, the residue was purified by distillation under reduced pressure, collecting the fraction boiling at 146-156°C/25 mbar, to provide the *title compound 63* (5.17 g, 76%) (lit.,¹²¹ bp 154-156°C/18 Torr); $\delta_{\text{H}}(\text{CDCl}_3)$ 1.24 (6H, t, *J* 7, 2xCH₃), 3.54-3.78 (4H, m, 2xCH₂), 6.03 (1H, s, acetal-CH), 7.43-7.50 (1H, m, ArH), 7.57-7.64 (1H, m, ArH) and 7.80-7.85 (2H, m, ArH); $\delta_{\text{C}}(\text{CDCl}_3)$ 15.1 (2xCH₃), 63.4 (2xCH₂), 98.3 (acetal-C), 124.1 (CH, ArC), 128.0 (CH, ArC), 129.1 (CH, ArC), 132.5 (CH, ArC), 133.7 (ArC) and 149.1 (ArC).

Attempted synthesis of 4(5)-cyano-5(4)-formylimidazole, 75, via DIBAL reduction of 4,5-dicyanoimidazole (72). A solution of 4,5-dicyanoimidazole (**72**) (0.591 g, 5.0 mmol) in anhydrous THF (10 ml), cooled to -78°C and held under a nitrogen atmosphere, was treated with a 1.0 M solution of DIBAL in THF (5.0 ml, 5.0 mmol), dropwise over 10 min. After a further 20 min at -78°C, the yellow solution formed was poured into ice-water (50 g). The mixture was then acidified with 1 M hydrochloric acid, a white

solid removed by filtration and washed with ethanol, followed by diethyl ether, and the combined filtrates evaporated under vacuum to leave the crude product, an orange solid (0.499 g). The mass spectrum (EI) of the crude mixture contained two peaks corresponding to molecular ions of **72** and **75**, at m/z 118 (100%) and 121 (34) respectively; and in the ^1H NMR spectrum of the mixture, after consideration of the signals attributable to starting imidazole **72**, the remaining signals readily assign to formylimidazole **75** $\delta_{\text{H}}([\text{DMSO}-d_6])$ 8.27 (1H, s, 2-H) and 9.86 (1H, s, CHO).

5(4)-Ethoxycarbonylimidazole-4(5)-carboxylic acid 78. According to the literature procedure,¹²⁸ thionyl chloride (30 ml, 413 mmol) was added to a suspension of 4,5-imidazoledicarboxylic acid (**73**) (7.80 g, 50 mmol) in dry benzene (100 ml) containing DMF (4 ml), and the mixture was heated to reflux for 6 h, sealed with a calcium chloride guard tube. The resultant bright yellow solution was allowed to cool, and evaporated to dryness. More anhydrous benzene (50 ml) was added to the residue, and the mixture concentrated to dryness once more. A second portion of dry benzene (50 ml) was added, and after stirring for 30 min, the precipitate was isolated to provide 5,10-dioxo-5*H*,10*H*-diimidazo[1,5-*a*:1',5'-*d*]pyrazine-1,6-dicarbonyl dichloride (**76**) (6.56 g, 84%) as a bright yellow solid. The crude acid chloride **76** (6.56 g, 21.0 mmol) was then suspended in water (80 ml) and heated to 40°C for 6 h. After allowing to cool, the solids were removed by filtration, washed with water (5 x 10 ml), followed by acetone (5 x 10 ml), then dried under vacuum to provide 5,10-dioxo-5*H*,10*H*-diimidazo[1,5-*a*:1',5'-*d*]pyrazine-1,6-dicarboxylic acid dihydrate (**77**) (6.45 g, 99%) as an off-white solid, and had mp 275-276°C (decomp.) (lit.,¹²⁸ 284°C).

According to the literature method,¹²⁹ the crude diacid **77** (6.4 g, 20.0 mmol) was then heated to reflux in anhydrous ethanol for 17 h, with the

addition of molecular sieves (type 4Å), sealed with a calcium chloride guard tube. After cooling, the crude product was isolated by filtration, and washed with ethanol. Recrystallisation from methanol, employing hot filtering to remove molecular sieves and diacid **73**, furnished the *title compound 78* (3.42 g, 46%) as a white crystalline solid, and had mp 205-207°C (decomp.) (lit.,¹³⁰ 215-216°C) (Found: m/z (EI), 184. $C_7H_8N_2O_4$ requires M , 184); $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$ 3423, 2924, 1735, 1637, 1459, 1328, 1069 and 955; $\delta_{\text{H}}([\text{}^2\text{H}_6]\text{DMSO})$ 1.29 (3H, t, J 7, CH_3), 4.31 (2H, q, J 7, CH_2), 7.90 (1H, s, 2-H) and, 13.43 and 13.58 (2H, 2 overlapping brs, CO_2H and NH); DEPT- $\delta_{\text{C}}([\text{}^2\text{H}_6]\text{DMSO})$ 13.9 (CH_3), 61.2 (CH_2), 138.0 (C-2).

1-Benzyl-5/4-cyano-1H-imidazole-4/5-carboxamide 79. (The 5-cyano-4-carboxamide isomer of this compound was previously reported by a different route, without experimental details.¹³⁴ 5(4)-Cyano-1H-imidazole-4(5)-carboxamide (**71**) (2.31g, 17 mmol.) was added to a vigorously stirred suspension of 95% sodium hydride (0.408g, 17 mmol.) in anhydrous DMF (30 ml), and stirred at room temperature for 30 min giving a pale yellow solution. Benzyl bromide (2ml, 17 mmol.) was added and the mixture stirred for a further 2 h, whereupon a precipitate slowly formed. The mixture was then poured into water (200 ml) to ensure all product had precipitated and the solid collected by filtration, washed with water, dried and recrystallised from 2-ethoxyethanol to give the *title compound 79* (2.88 g, 75%) as a flaky white crystalline solid, and had mp 259°C (with decomp.); $\delta_{\text{H}}([\text{}^2\text{H}_6]\text{DMSO})$ 5.41 (2H, s, CH_2), 7.27-7.46 (5H, m, Ph), 7.59 and 7.74 (2x1H, 2xbr s, CONH_2) and 8.30 (1H, s, 2-H); $\delta_{\text{C}}([\text{}^2\text{H}_6]\text{DMSO})$ 50.6 (CH_2), 106.2 (C), 111.5 (C), 128.4 (2xCH, ArC), 129.3 (CH, ArC), 129.8 (2xCH, ArC), 136.2 (C), 142.2 (C-2), 146.1 (C) and 162.3 (C).

General procedure for lithium triethoxyaluminium hydride reduction of 1-benzyl-4,5-dicyano-1*H*-imidazole 80. Anhydrous ethyl acetate (0.73 ml, 7.5 mmol.) was added dropwise over a period of 15-30 min to a stirred solution of lithium aluminium hydride as a 1.0 M solution in diethyl ether (5 ml, 5 mmol.) diluted with anhydrous tetrahydrofuran (15-20 ml), cooled in ice bath, and held under a nitrogen atmosphere. After a further 15 min stirring at 0°C the substrate (e.g. 80¹³³) was added to this reductant, or the solution of the reductant was added to the substrate as desired. The mixture was stirred at 0°C for a further 1 h followed by the addition of wet diethyl ether (10-30 ml) to decompose any unreacted reductant, and 5 N sulfuric acid (5 ml) followed by a further 10 ml of water to decompose the intermediate aluminium adducts. The layers were separated and the aqueous phase extracted with diethyl ether (3 x 25 ml). The combined ethereal layers were washed with saturated sodium bicarbonate solution (50 ml), water (40 ml), dried over magnesium sulphate and solvents removed in *vacuo* to leave the products of the reaction. Silica gel column chromatography of the product mixture with ethyl acetate as eluent separated the mixture into two fractions. The faster running components, with an *R_f* of 0.75-0.81, were identified as unreacted 80 and 1-benzyl-4-cyano-5-formylimidazole (81b). The slower running components, with an *R_f* of 0.54-0.63, were identified as 1-benzyl-5-cyano-4-formylimidazole (81a) and 1-benzyl-4,5-diformylimidazole (82).

1-Benzyl-5-cyano-4-formyl-1*H*-imidazole 81a. $\delta_{\text{H}}(\text{CDCl}_3)$ 5.30 (2H, s, CH₂), 7.27-7.32 (2H, m, ArH), 7.40-7.46 (3H, m, ArH), 7.71 (1H, s, 2-H) and 9.94 (1H, s, CHO).

1-Benzyl-4-cyano-5-formyl-1*H*-imidazole 81b. $\delta_{\text{H}}(\text{CDCl}_3)$ 5.33 (2H, s, CH₂), 7.20-7.25 (2H, m, ArH), 7.36-7.42 (3H, m, ArH), 7.71 (1H, s, 2-H) and 10.01 (1H, d, *J* 1, CHO).

1-Benzyl-4,5-diformyl-1*H*-imidazole 82. $\delta_{\text{H}}(\text{CDCl}_3)$ 5.56 (2H, s, CH_2), 7.19-7.25 (2H, m, ArH), 7.33-7.38 (3H, m, ArH), 7.71 (1H, s, 2-H), 10.08 (1H, s, C(4)CHO) and 10.42 (1H, d, J 1, C(5)CHO).

Attempted Knoevenagel condensation of 1-benzyl-4-cyano-5-formylimidazole (81b) and ethyl nitroacetate: Procedure A. According to the literature method,¹⁴³ titanium tetrachloride (0.44 ml, 4.0 mmol), in carbon tetrachloride (2 ml, previously dried over type 4Å sieves and distilled from phosphorus pentoxide), was added to vigorously stirred anhydrous THF (10 ml) cooled to -5°C , and the resultant yellow precipitate stirred for a further 30 min under argon. 1-Benzyl-4-cyano-5-formylimidazole (81b) (0.653 g (65% pure), equivalent to 2.0 mmol) was then added, as a solution in dry THF (2 ml), followed by ethyl nitroacetate (0.23 ml, 2.0 mmol). After a further 30 min at -5°C , pyridine (0.65 ml, 8.1 mmol) was added dropwise via syringe, as a solution in dry THF (2 ml), over 30 min. The reaction mixture was then stirred at -5°C for 24 h, treated with water (2 ml) and extracted with diethyl ether (3 x 10 ml). TLC analysis of the two resultant phases indicated only starting materials, residing in the organic phase, which was confirmed by ^1H NMR after drying the organic phase (MgSO_4) and concentration *in vacuo*.

Procedure B. According to the literature method,¹⁴⁴ 1-benzyl-4-cyano-5-formylimidazole (81b) (0.653 g (65% pure), equivalent to 2.0 mmol) was dissolved in pyridine (2 ml) and treated with ethyl nitroacetate (0.215 ml, 1.95 mmol), followed by piperidine (1 drop), and the mixture stirred at room temperature. After a total of 2 d at room temperature, TLC analysis showed only starting materials. The mixture was then heated to reflux for 1 h, but TLC analysis indicated no reaction. Samples of starting imidazole 81b were indeed recovered, characterised by ^1H NMR, after dilution of the reaction mixture

with 5% hydrochloric acid and extraction into chloroform, and the reaction was abandoned.

Procedure C. According to the literature method,¹²² ethyl nitroacetate (0.74 ml, 6.5 mmol), as a solution in ethanol (1-2 ml), was added dropwise to a solution of sodium (0.15 g, 6.5 mmol) in ethanol (20 ml), cooled in an ice-water bath, and held under a nitrogen atmosphere. The resultant fine white suspension was then added to a solution of 1-benzyl-4-cyano-5-formyl-imidazole (**81b**) (1.31 g (65% pure), equivalent to 4.0 mmol) cooled in ice. After stirring for 2 h, TLC analysis indicated only starting materials remaining. The mixture was then heated to reflux under nitrogen for 20 h, whereupon TLC analysis showed a mixture of several components, including highly polar products that would not elute on the TLC plate. Dilution of the reaction mixture with water, and adjustment of the pH to 5-6 with dilute hydrochloric acid yielded a viscous red precipitate that by ¹H NMR and TLC analysis consisted of many products. Attempts to purify the components of the crude solid by silica gel column chromatography were not successful, and the reaction was abandoned.

Ethyl 1-benzyl-5/4-cyano-1*H*-imidazole-4/5-carboxylate 83.¹³⁴ A stirred solution of 1-benzyl-4,5-dicyano-1*H*-imidazole (**80**)¹³³ (1.04 g, 5.0 mmol) in a mixture of dry ethanol (20 ml) and dry THF (10 ml), cooled in an ice-water bath, was treated with a solution of sodium metal (0.15 g, 6.5 mmol) in anhydrous ethanol (5 ml), and the mixture allowed to warm to room temperature over 18 h. The reaction was quenched by the addition of 1 M hydrochloric acid (6.5 ml), and the pH of the mixture adjusted to 5 with more of the acid. The mixture was reduced in *vacuo* to leave the aqueous phase, which was diluted with more water (30 ml) and extracted with chloroform (2x40 ml). The combined extracts were washed with water (30 ml), dried

(MgSO₄) and reduced in *vacuo* to leave a pale yellow solid (1.28 g). Silica gel chromatography of the yellow solid, with ethyl acetate as eluent, furnished the *title compound 83* (0.153 g, 12%) as a single regioisomer. A sample crystallised from petroleum ether (bp 40-60°C) / acetone had mp 110°C and gave the following ¹H NMR spectrum: δ_H(CDCl₃) 1.41 (3H, t, *J* 7, CH₂CH₃), 4.43 (2H, q, *J* 7, CH₂CH₃), 5.28 (2H, s, CH₂Ph), 7.25-7.31 (2H, m, ArH), 7.36-7.45 (3H, m, ArH) and 7.68 (1H, s, 2-H).

1-Benzyl-4,5-dicyano-3-ethylimidazolium tetrafluoroborate 84. 1-Benzyl-4,5-dicyano-1*H*-imidazole (80)¹³³ (1.25 g, 6.0 mmol) and triethyl-oxonium tetrafluoroborate (2.35 g, 12.4 mmol) were heated to gentle reflux in anhydrous dichloromethane for 4 days. According to the procedure to achieve nitrile reduction,¹²⁷ freshly distilled triethylsilane (0.82 g, 7.1 mmol) was then added to the mixture, and reflux was continued for 18 h. Water (10 ml) was added and the reaction mixture refluxed for a further 4 h. The resultant suspension was filtered and the solid washed with water and dried on the filter. Crystallisation from ethanol gave the *title compound 84* (1.49 g, 76%) as white fluffy needles, and had mp 167-168.5°C (Found: C, 51.9; H, 3.95; N, 17.2%; *m/z* (ES⁺), 237. C₁₄H₁₃BF₄N₄ requires C, 51.9; H, 4.0; N, 17.3%; *M*-BF₄, 237); ν_{max}(KBr)/cm⁻¹ 3151, 1451, 1450, 1341, 1184, 1071, 762 and 713; δ_H([²H₆]acetone) 1.70 (3H, t, *J* 7, CH₃), 4.68 (2H, q, *J* 7, CH₂), 5.85 (2H, s, CH₂Ph;), 7.47-7.52 (3H, m, ArH), 7.58-7.62 (2H, m, ArH) and 9.74 (1H, s, D₂O exchange, 2-H); δ_C([²H₆]DMSO) 13.8 (CH₃), 46.7 (CH₂), 53.6 (CH₂), 105.8 (C), 106.0 (C), 114.2 (C), 116.1 (C), 128.9 (2xCH, ArC), 129.1 (2xCH, ArC), 129.5 (CH, ArC), 131.7 (C, ArC) and 141.8 (C-2); δ_F([²H₆]DMSO) 152.01 (82%, ¹¹BF₄) and 151.96 (18%, ¹⁰BF₄).

4,5-Dicyano-1-(*p*-toluenesulfonyl)imidazole 85. Triethylamine (0.42 ml, 3.0 mmol) was added slowly via syringe to a stirred suspension of 4,5-

dicyanoimidazole (**72**) (0.354 g, 3.0 mmol) and *p*-toluenesulfonyl chloride (0.572 g, 3.0 mmol) in anhydrous toluene (15 ml) held at room temperature under nitrogen, and the mixture stirred at this temperature for 24 h. More toluene (10 ml) was added to aid stirring. The resultant suspension was filtered and the solid washed with toluene. The remaining solid was partitioned between water (50 ml) and diethyl ether (60 ml). The ethereal layer was washed with saturated brine, dried (MgSO₄) and evaporated to leave a white solid. Evaporation of the initial toluene filtrate also yielded a white solid. Crystallisation of the combined white solids from toluene gave the *title compound* **85** (0.745 g, 91%) as a crystalline solid $\delta_{\text{H}}(\text{CDCl}_3)$ 2.52 (3H, s, Me), 7.48-7.54 (2H, m, ArH), 8.00-8.05 (2H, m, ArH) and 8.25 (1H, s, 2-H); $\delta_{\text{C}}(\text{CDCl}_3)$ 22.0 (Me), 106.5 (C), 110.2 (C), 110.3 (C), 126.1 (C), 128.9 (2xCH, ArC), 131.2 (2xCH, ArC), 131.5 (C), 140.4 (C-2) and 149.3 (C); note-decomposed before complete characterisation carried out.

Attempted synthesis of 5(4)-formylimidazole-4(5)-carboxylic acid, **87, via reduction of 5,10-dioxo-5*H*, 10*H*-diimidazo[1,5-*a*:1',5'-*d*]pyrazine-1,6-dicarbonyl dichloride (**76**): Procedure A.** Based upon the literature procedure for the sodium borohydride reduction of acid chlorides,¹⁴¹ the acid chloride **76**¹²⁸ (see **78** for synthesis of **76**) (0.626 g, 2.0 mmol), as a solution in anhydrous DMF (15 ml), was added to a solution of sodium borohydride (0.129 g, 3.4 mmol) and pyridine (2.0 ml) in a mixture of dry THF (2 ml) and dry DMF (5 ml), under argon, cooled in an ice-bath. After stirring for 1 min, water (0.5 ml) was added. After a further 1 min stirring, diethyl ether (50 ml) was added, and a brown solid of presumably the dialdehyde **86** was isolated by filtration, and washed with further portions of diethyl ether. The crude brown solid was then suspended in water (30 ml) and heated to 40°C for 3 d, to leave an orange solution over a pale brown solid. TLC and ¹H NMR

analysis of the crude product suggested it to be the diacid **73**, and the reaction was not progressed further.

Procedure B. Based upon the literature procedure for the hydrogenation of acid chlorides,¹⁴⁰ a flask was charged with freshly distilled 2,6-lutidine (0.47 ml, 4.0 mmol), 10% palladium on carbon catalyst (10-15 mg) and anhydrous THF (10 ml), and held under an atmosphere of hydrogen. Then, from a pressure-equalising addition funnel was added the acid chloride **76** (0.789 g, 2.52 mmol) as a solution in dry THF (50 ml), over a period of 4 min, forming a vivid red suspension. Stirring was continued at room temperature for 20 h, after which the red solid, presumed to be the tricyclic dialdehyde **86**, was filtered from the solution. The crude intermediate was then suspended in water (40 ml), and stirred at room temperature for 3 d, to leave a pale orange solution over an orange solid. TLC analysis of the solid and supernatant liquid indicated the diacid **73** as the major product, and the reaction was not progressed further.

Methyl 5(4)-diethoxymethylimidazole-4(5)-carboxylate 90.¹⁴²

According to the literature method,¹⁴² potassium hydride, as a 35% wt. dispersion in mineral oil (4.79 g, 41.8 mmol KH), was placed in a flask and, under argon, the oil removed by washing with dry hexane (2 x 20 ml). The remaining solids were then suspended in freshly distilled diglyme (20 ml), cooled in an ice-water bath, and treated with a solution of methyl isocyanoacetate (**88**) (3.00 g, 30.3 mmol) and diethoxyacetonitrile (**89**) (3.91 g, 30.3 mmol) in diglyme (20 ml), dropwise over a period of 20 min. Once the addition was complete, the mixture was heated to 70-80°C for 5 h. The dark reaction mixture was then cooled, quenched by the cautious addition of saturated ammonium chloride solution (80 ml), and extracted with chloroform (3 x 50 ml) and ethyl acetate (50 ml). The combined organic extracts were

then dried (MgSO_4) and reduced *in vacuo* to leave the *title compound* **90** (5.26 g, 76%). A sample recrystallised from hexane/ethanol had mp 147°C (decomp. with effervescence) (lit.,¹⁴² $148\text{-}150^\circ\text{C}$); $\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$ 3434, 2979, 1718, 1509, 1447, 1323, 1195, 1147, 1106, 1061 and 1006 (lit.,¹⁴² 3420, 2500-3100, 1715, 1510, 1440, 1320, 1190, 1145, 1100, 1060 and 1000); $\delta_{\text{H}}(\text{CDCl}_3)$ 1.23 (6H, t, J 7, $2\times\text{OCH}_2\text{CH}_3$), 3.58-3.81 (4H, m, $2\times\text{OCH}_2\text{CH}_3$), 3.92 (3H, s, CO_2CH_3), 6.12 (1H, s, $\text{CH}(\text{OEt})_2$), 7.62 (1H, s, 2-H) and 10.02 (1H, brs, NH), in good agreement with the literature.¹⁴²

Methyl 5(4)-formylimidazole-4(5)-carboxylate 91.¹⁴² Methyl 5(4)-diethoxymethylimidazole-4(5)-carboxylate (**90**) (0.736 g, 3.22 mmol) was suspended in 40% acetic acid/water (25 ml), and heated to $40\text{-}50^\circ\text{C}$ for 3 h. TLC analysis showed complete consumption of **90**, and the mixture was evaporated to dryness under vacuum and then triturated with chloroform (10 ml) to provide the *title compound* **91** (0.455 g, 92%), as a golden yellow solid, and had mp 222.5°C (decomp.) (Found: m/z (EI), 154. $\text{C}_6\text{H}_6\text{N}_2\text{O}_3$ requires M , 154); $\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$ 3432, 2962, 1726, 1673, 1493, 1322, 1261, 1069 and 962; $\delta_{\text{H}}([\text{D}_6]\text{DMSO})$ 3.88 (3H, s, CH_3), 8.08 (1H, d, J 0.6, 2-H), 10.22 (1H, d, J 0.6, CHO) and 13.81 (1H, brs, NH); $\delta_{\text{C}}(\text{CF}_3\text{CO}_2\text{D})$ 56.6 (Me), 130.6 (C), 134.1 (C), 140.0 (CH, C-2), 159.6 (C, CO_2CH_3) and 182.8 (CH, CHO).

Attempted Knoevenagel condensation of methyl 5(4)-formylimidazole-4(5)-carboxylate (91) and ethyl nitroacetate. According to the literature method,¹⁴³ titanium tetrachloride (0.89 ml, 8.1 mmol), in carbon tetrachloride (5 ml, previously dried over type 4Å sieves and distilled from phosphorus pentoxide), was added to vigorously stirred anhydrous THF (25 ml) cooled to -5°C , and the resultant yellow precipitate stirred for a further 20 min under argon. Methyl 5(4)-formylimidazole-4(5)-carboxylate (**91**) (0.624 g, 4.05 mmol) was then added, as a solution in dry DMF (10 ml),

followed by ethyl nitroacetate (0.45 ml, 4.05 mmol). After stirring at -5°C for a further hour, pyridine (1.31 ml, 16.2 mmol), as a solution in dry THF (3 ml), was added dropwise to the reaction mixture, over a period of 30 min. The reaction was then stirred at -5 to 5°C for 24 h, after which water (3 ml) and diethyl ether (5ml) were added, and the mixture allowed to warm to room temperature. The resultant layers were separated, and the aqueous phase extracted with THF (4 x 25 ml). TLC analysis of the aqueous and organic phases suggested only the presence of starting materials. As confirmation, after drying the combined organic extracts over MgSO₄, followed by concentration *in vacuo*, a pale pink solid crystallised from the residual oil that was identical by ¹H NMR to an authentic sample of imidazole 91; concentration of the remaining mother liquor gave an oil rich in ethyl nitroacetate, as determined by ¹H NMR. A further crop of starting imidazole 91 was obtained after neutralisation of the remaining aqueous phase, and further analysis was abandoned.

5.2 Pyrimidinone chemistry

2-Amino-5-bromo-6-methylpyrimidin-4(3*H*)-one 19. According to the literature method,⁸⁵ a solution of bromine (6.71 g, 42 mmol) in glacial acetic acid (15 ml) was added dropwise to a solution of 2-amino-6-methylpyrimidin-4(3*H*)-one (93) (4.97 g, 40 mmol) in glacial acetic acid (40 ml), heated to 70°C. As the product precipitated, more acetic acid (25 ml) was added to aid stirring. After all the bromine had been added, the reaction mixture was stirred at 70°C for a further hour, then allowed to cool slowly. The suspension was then filtered and the solid residue washed with ethanol (3 x 20 ml), saturated sodium bicarbonate solution (until effervescence in the filter subsided), water (3 x 20 ml), acetone (20 ml) then dried in a vacuum

desiccator to give a white solid (6.77 g). The crude white solid was recrystallised from DMF to provide the *title compound* 19 (5.42 g, 66%), and had mp 234-234.5°C (with decomp.) (lit.,²¹⁸ 249°C (from water)) (Found: C, 29.9; H, 3.0; N, 20.4; Br, 39.2%. $C_6H_6BrN_3O$ requires C, 29.4; H, 3.0; N, 20.6; Br, 39.2%); $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$ 3314, 3160, 1644, 1604, 1560, 1392, 1220 and 765; $\delta_{\text{H}}([\text{}^2\text{H}_6]\text{DMSO})$ 2.20 (3H, s, Me), 6.62 (2H, brs, NH_2) and 11.18 (1H, brs, NH).

2-Amino-5-bromo-6-phenylpyrimidin-4(3*H*)-one 20. Prepared according to the method for the preparation of 19, with 2-amino-6-phenylpyrimidin-4(3*H*)-one (26) (28.08 g, 0.15 mol) and bromine (25.25 g, 0.158 mol), to provide a crude white solid (29.82 g) which was recrystallised from 2-ethoxyethanol (800 ml), yielding the *title compound* 20 (27.85 g, 70%) as a white solid, and had mp 274-277°C (with decomp.) (lit.,⁶⁹ 268-270°C (from aqueous DMF)) (Found: C, 45.4; H, 3.0; N, 15.6; Br, 30.4%. $C_{10}H_8BrN_3O$ requires C, 45.1; H, 3.0; N, 15.8; Br, 30.0%); $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$ 3431, 3312, 1676, 1583, 1454, 1338, 1226 and 1013; $\delta_{\text{H}}([\text{}^2\text{H}_6]\text{DMSO})$ 6.76 (2H, brs, NH_2), 7.42 (3H, m, ArH), 7.54 (2H, m, ArH) and 11.42 (1H, brs, NH).

2-Amino-5-iodo-6-phenylpyrimidin-4(3*H*)-one 21. According to the literature method,⁶⁹ to a solution of 2-amino-6-phenylpyrimidin-4(3*H*)-one (26) (28.08 g, 0.15 mol) in 1 M sodium hydroxide (450 ml) was added chloroform (500 ml) and iodine (41.88 g, 0.165 mol), and the mixture was heated to gentle reflux on a steam bath for 4 h. The biphasic mixture was then cooled and filtered, removing a crop of the *title compound* 21 (8.21 g) as a pale yellow solid. After separation of the phases, the aqueous phase was layered with ethyl acetate (250 ml), and the pH of the aqueous phase lowered to 9-10 with 12 M hydrochloric acid, to provide a second crop of 21 as a pale yellow solid, that was isolated by filtration, washed with water, and dried to

constant weight *in vacuo*. The combined solids (42.21 g, 90%) were pure by ^1H NMR. Attempts to recrystallise the crude product from high boiling solvents, including DMF, and 2-ethoxyethanol, appeared to lead to decomposition to give 26, but a sample recrystallised from ethanol had mp 270-274°C (Found: C, 38.4; H, 2.5; N, 13.4; I, 40.2%. $\text{C}_{10}\text{H}_8\text{IN}_3\text{O}$ requires C, 38.4; H, 2.6; N, 13.4; I, 40.5%); $\delta_{\text{H}}([\text{}^2\text{H}_6]\text{DMSO})$ 6.76 (2H, brs, NH_2), 7.35-7.55 (5H, m, Ph) and 11.32 (1H, brs, NH).

2-Amino-6-phenylpyrimidin-4(3*H*)-one 26. According to the literature method,¹⁴⁹ ethyl benzoylacetate (95.14 g, 0.495 mol) and guanidine carbonate (40.54 g, 0.450 mol) were heated to reflux in absolute ethanol (450 ml) for 24 h. After cooling, the reaction mixture was cooled and filtered, and the residue washed with ethanol (2 x 30 ml), water (2 x 30 ml), acetone (20 ml), and dried *in vacuo* to provide the *title compound* 26 (58.28 g, 69%), as a white solid, in acceptable purity, and had mp 304-306°C (with decomp.) (lit.,¹⁴⁹ 304°C); $\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$ 3351, 3091, 2951, 1657, 1503, 1475, 1380 and 763; $\delta_{\text{H}}([\text{}^2\text{H}_6]\text{DMSO})$ 6.10 (1H, s, 5-H), 6.61 (2H, brs, NH_2), 7.44 (3H, m, ArH), 7.94 (2H, m, ArH) and 10.82 (1H, brs, NH).

2-Amino-6-methylpyrimidin-4(3*H*)-one 93. Prepared according to the method for the preparation of 26, with ethyl acetoacetate (13.4 ml, 0.105 mol) and guanidine carbonate (9.01 g, 0.100 mol), to provide a crude solid (11.97 g), which was recrystallised from DMF (250 ml) to yield the *title compound* 93 (10.35 g, 83%) as a pale brown crystalline solid, and had mp 311-314°C (with decomp.) (lit.,²¹⁹ 297-299°C (from water)) (Found: C, 47.7; H, 5.7; N, 33.2%. $\text{C}_5\text{H}_7\text{N}_3\text{O}$ requires C, 48.0; H, 5.6; N, 33.6%); $\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$ 3335, 3082, 2945, 1662, 1499, 1390, 1184 and 832; $\delta_{\text{H}}([\text{}^2\text{H}_6]\text{DMSO})$ 1.98 (3H, s, Me), 5.39 (1H, s, 5-H), 6.47 (2H, brs, NH_2) and 10.68 (1H, brs, NH).

2-Amino-6-ethylpyrimidin-4(3*H*)-one 94. Prepared according to the method for the preparation of 26, with ethyl propionylacetate (15.0 ml, 0.105 mol) and guanidine carbonate (9.01 g, 0.100 mol), to provide a crude solid (12.21 g), which was recrystallised from DMF (40 ml) to yield the *title compound* 94 (11.80 g, 86%) as a white solid, and had mp 262-264°C (with decomp.); $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$ 3338, 3077, 2949, 1663, 1501, 1385, 1320 and 841; $\delta_{\text{H}}([\text{}^2\text{H}_6]\text{DMSO})$ 1.08 (3H, t, *J* 7.5, CH_2CH_3), 2.26 (2H, q, *J* 7.5, CH_2CH_3), 5.38 (1H, s, 5-H), 6.46 (2H, brs, NH_2) and 10.64 (1H, brs, NH) (94 has been previously reported,²²⁰ but without supporting physical data).

2-Amino-5-bromo-6-ethylpyrimidin-4(3*H*)-one 95. Prepared according to the method for the preparation of 20, with 2-amino-6-ethylpyrimidin-4(3*H*)-one (94) (3.48 g, 25 mmol) and bromine (4.20 g, 26 mmol), to yield a crude product (4.02 g) that was recrystallised from DMF/2-ethoxyethanol to provide the *title compound* 95 (3.03 g, 55%) as a white solid. Drying the solid under high vacuum failed to remove the final traces of 2-ethoxyethanol, as determined by ^1H NMR, and the solid had mp 232-234°C (with decomp.) (lit.,²²⁰ 225-225.5°C); $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$ 3375, 3107, 1671, 1591, 1464, 1350, 1219 and 1007; $\delta_{\text{H}}([\text{}^2\text{H}_6]\text{DMSO})$ 1.10 (3H, t, *J* 7.5, CH_2CH_3), 2.47-2.56 (multiplet coincident with DMSO signal, CH_2CH_3), 6.64 (2H, brs, NH_2) and 11.18 (1H, brs, NH).

2-Amino-5-iodo-6-methylpyrimidin-4(3*H*)-one 96. Prepared according to the method for the preparation of 21, with 2-amino-6-methylpyrimidin-4(3*H*)-one (93) (4.97 g, 40 mmol) and iodine (10.66 g, 42 mmol), and a reaction time of 28 h, to give the *title compound* 96 (8.63 g, 86%) directly, as a pale yellow solid, in acceptable purity, and had mp 216-217°C (with decomp.) (lit.,²²¹ 211.5-212.5°C); $\delta_{\text{H}}([\text{}^2\text{H}_6]\text{DMSO})$ 2.28 (3H, s, Me), 6.64 (2H, brs, NH_2) and 11.12 (1H, brs, NH).

2-Amino-6-ethyl-5-iodopyrimidin-4(3H)-one 97. Prepared according to the method for the preparation of **21**, with 2-amino-6-ethylpyrimidin-4(3H)-one (**94**) (6.96 g, 50 mmol) and iodine (13.33 g, 52.5 mmol), to provide the *title compound* **97** (11.11 g, 84%) directly, as a pale yellow solid, in reasonable purity. A sample was purified by dissolving in 2 M sodium hydroxide, decolourising the solution with charcoal, before acidifying with acetic acid to precipitate **97**. After filtering off the solid, washing with water and drying *in vacuo*, the resultant white solid had mp 203-206°C (with decomp.) (lit.,²²¹ 199-200°C (from ethanol)); $\delta_{\text{H}}([^2\text{H}_6]\text{DMSO})$ 1.08 (3H, t, J 7.5, CH_2CH_3), 2.56 (2H, t, J 7.5, CH_2CH_3), 6.64 (2H, brs, NH_2) and 11.08 (1H, brs, NH).

1-(2-Amino-3,4-dihydro-4-oxopyrimidin-6-yl)ferrocene 100. Guanidine carbonate (0.45 g, 2.5 mmol) and ethoxycarbonylacetylferrocene (**99**)⁹⁰ (1.54 g, 5.0 mmol) were heated to reflux in absolute ethanol (20 ml), and the reaction periodically monitored by TLC. After 4 days another portion of guanidine carbonate (0.045 g, 0.5 mmol) was added and the reaction returned to reflux. When ester **99** was judged to have been consumed by TLC (7 days) the mixture was cooled, reduced in *vacuo* to *ca.* 2 ml and filtered to give a brown solid (0.62 g). Crystallisation of the crude brown solid from ethanol furnished the *title compound* **100** (first crop-0.212 g, 14%; second crop-0.176 g, 12%) as the hemihydrate, a purple crystalline solid, and had mp 220-230°C (onset of decomp.) (Found: C, 55.0; H, 4.5; N, 13.7%; m/z (EI), 295. $\text{C}_{14}\text{H}_{13}\text{FeN}_3\text{O} \cdot 0.5\text{H}_2\text{O}$ requires C, 55.3; H, 4.6; N, 13.7%; M , 295); $\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$ 3397, 3088, 1655, 1640, 1603, 1460, 1254 and 814; $\delta_{\text{H}}([^2\text{H}_6]\text{DMSO})$ 4.08 (5H, s, C_5H_5), 4.38 (2H, t, J 2, C_5H_4), 4.83 (2H, t, J 2, C_5H_4), 5.79 (1H, s, pyrimidine 5-H), 6.58 (2H, br s, NH_2) and 11.01 (1H, br s, NH); $\delta_{\text{C}}([^2\text{H}_6]\text{DMSO})$ 67.8 (2xCH, C_5H_4), 69.7 (5xCH, C_5H_5), 70.1 (2xCH, C_5H_4), 81.9 (C, C_5H_4), 96.4 (CH, pyrimidine C-5), 155.7, 163.5 and 166.1

(3xC, pyrimidine C-2,4 and 6); m/z (HRMS-EI) 295.0408 (M^+ , $C_{14}H_{13}FeN_3O$ requires 295.0407).

1,1'-Bis(2-amino-3,4-dihydro-4-oxopyrimidin-6-yl)ferrocene 103.

1,1'-Bis(ethoxycarbonylacetyl)ferrocene (102) (2.72 g, 6.57 mmol) (prepared according to the literature method,^{91,92} and used crude) and guanidine carbonate (1.18 g, 6.57 mmol) were heated to 80°C in ethanol (150 ml). After 76 h the dark red mixture was cooled, concentrated in *vacuo* to approximately 50 ml, re-cooled in ice and a solid was filtered from the solution and washed with ethanol. The solid was then triturated with boiling DMF (3x100 ml), washed with diethyl ether and dried to provide the *title compound* 103 (0.43 g, 14%) as the 1:2 DMF solvate, an almost black powder, and had mp >360°C (no elemental analysis obtained, EI mass spectroscopy failed); $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$ 3390, 1651, 1463, 1393, 1250, 1080, 821 and 595; $\delta_{\text{H}}([\text{}^2\text{H}_6]\text{DMSO})$ 2.73 (6H, s, 2xDMF-Me), 2.90 (6H, s, 2xDMF-Me), 4.25 (4H, m, 2xC₅H₄), 4.70 (4H, m, 2xC₅H₄), 5.69 (2H, s, 2xpyrimidine-5-H), 6.41 (4H, br s, 2xNH₂), 7.95 (2H, s, 2xDMF-CHO) and 10.50 (2H, br s, 2xNH); note insufficiently soluble for ¹³C NMR.

Ethyl 2-nitrobenzoylacetate 111. To a stirred suspension of 2-nitrobenzoic acid (116) (0.836 g, 5.0 mmol) in dichloromethane (25 ml, freshly distilled from calcium hydride) was added dicyclohexylcarbodiimide (DCCI) (1.186 g, 5.75 mmol), followed by 2,2-dimethyl-1,3-dioxane-4,6-dione (117) (Meldrum's acid) (0.749 g, 5.2 mmol) and 4-dimethylamino-pyridine (DMAP) (0.916 g, 7.5 mmol), and the mixture stirred at room temperature for 18 h. A white solid was removed by filtration, which was washed with dichloromethane until washings were colourless. The combined filtrates were then washed with 1 M hydrochloric acid (20 ml), water (20 ml), dried (MgSO₄) and concentrated in *vacuo* to leave an orange oil (0.94 g). The

crude oil, taken to be 2,2-dimethyl-5-(2-nitrobenzoyl)-1,3-dioxane-4,6-dione (**118**), but used without analysis, was heated to reflux in ethanol (50 ml) for 30 min, until complete disappearance of intermediate **118** noted by TLC. After cooling, the orange solution was concentrated to give an oily orange solid which was purified by silica gel column chromatography, with ethyl acetate / hexane (3:7) as eluent, to provide the *title compound* **111** (0.249 g, 21%) as a yellow oil; $\delta_{\text{H}}(\text{CDCl}_3)$ 1.24 (2.57H, t, J 7, CH_2CH_3 (keto)), 1.34 (0.43H, t, J 7, CH_2CH_3 (enol)), 3.88 (1.71H, s, CH_2 (keto)), 4.16 (1.71H, q, J 7, CH_2CH_3 (keto)), 4.28 (0.29H, q, J 7, CH_2CH_3 (enol)), 5.42 (0.14H, s, CH (enol)) and 7.51-8.20 (4H, m, ArH) (in agreement with literature values¹⁶⁰).

Reaction of Ethyl 2-nitrobenzoylacetate (111**) with guanidine carbonate.** Guanidine carbonate (0.094 g, 1.05 mmol) and ethyl 2-nitrobenzoylacetate (**111**) (0.249 g, 1.05 mmol) were heated to reflux in ethanol (10 ml). After 4 h, though the colour of solution had changed from pale yellow to dark red, TLC analysis indicated **111** remaining, with residual, highly coloured, polar components not eluting from the baseline of the chromatogram. After a further 24 h at reflux, TLC analysis indicated the presence of several coloured components with varying R_f values, but also remaining **111**. More guanidine carbonate (0.047 g, 0.53 mmol) was added, and reflux continued until all **111** was consumed (24 h). After cooling, a small amount of a brown solid was filtered from the reaction mixture, but ^1H NMR analysis showed no sign of the intended product, pyrimidinone **119**, only an overlapping set of weak signals between 7 and 8 ppm. The filtrate was reduced to leave a red oil, but which again, by ^1H NMR, showed a complex pattern between 7 and 8 ppm, as well as several ethyl environments, but no sign of the pyrimidinone **119** - for which would be expected a sharp singlet at about 6 ppm, for the 5-H.

2-(2-Amino-3,4-dihydro-4-oxo-6-phenylpyrimidin-5-yl)-6-phenylpyrimidin-4(3H)-one 112 (example preparation). Nitrosonium tetrafluoroborate (0.175 g, 1.5 mmol) was added, as a solution in acetonitrile (1 ml, dried by azeotropic distillation with dichloromethane), to a stirred solution of 2-amino-6-phenylpyrimidin-4(3H)-one (26)¹⁴⁹ (0.094 g, 0.5 mmol) in acetonitrile (20 ml), cooled in an ice-water bath. The reaction was allowed to warm slowly to room temperature. After stirring at room temperature for 2 days, the reaction mixture was heated to reflux for a further 2 days. After cooling, water (3 ml) was added and the mixture neutralised with 1 M sodium hydroxide. The mixture was cooled to 4°C and a solid removed by filtration, which was discarded. Dilution of the filtrate with more water and evaporation of the acetonitrile in *vacuo* gave a second precipitate which was filtered from the solution and washed with small portions of water. This second precipitate was then dissolved in 2 M sodium hydroxide (1-2 ml) with heating, decolourised with activated carbon, and the clear orange solution acidified with acetic acid to pH 5 forming a brown precipitate. Direct crystallisation of the mixture by the addition of the minimum quantity of DMF yielded the *title compound* **112** (6.5 mg, 7 %) as a brown solid. A further crop of **112** (5.6 mg, 6%) was obtained on dilution of the crystallisation mother liquor with water, and had mp 332°C (with decomp.); $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$ 3424, 2920, 2851, 1655, 1638, 1561, 1544 and 698; $\delta_{\text{H}}([\text{}^2\text{H}_6]\text{DMSO})$ 6.62 (1H, s, 5-H), 7.24-7.46 (12H, m, ArH and NH₂), 11.67 (1H, br s, NH) and 12.52 (1H, br s, NH); $\delta_{\text{C}}([\text{}^2\text{H}_6]\text{DMSO})$ 105.8 (pyrimidine C-5'), 106.5 (C-5), 127.0 (2xCH, ArC), 128.1 (4xCH, ArC), 128.6 (2xCH, ArC), 129.0 (CH, ArC), 130.4 (CH, ArC), 136.5 (C), 140.1 (C), 155.6 (C), 155.9 (C), 160.7 (C), 162.8 (C) and 163.0 (broad signal, coincidence of 2xC?); m/z (HRMS-FAB⁺) 358.1228 (MH⁺, C₂₀H₁₆N₅O₂ requires 358.1304).

2-Amino-5,6-diphenylpyrimidin-4(3*H*)-one 126. 12 M Hydrochloric acid (5 ml) was added to a solution of 2-amino-5,6-diphenyl-4-methoxypyrimidine (135) (0.044 g, 0.16 mmol) in methanol (5 ml), and the mixture was heated to reflux for 1 h. After cooling, the solution was basified with solid sodium hydroxide (2.27 g) and then re-acidified with acetic acid to pH 6. After cooling in ice, the precipitate was collected by filtration, washed with water and dried in *vacuo* to furnish the *title compound* 126 (0.040 g, 96%) as a fine white solid. A sample crystallised from ethanol had mp 314-316°C (lit.,¹⁷⁶ 319°C). The following data have not been reported previously: $\delta_{\text{H}}([^2\text{H}_6]\text{DMSO})$ 6.64 (2H, br s, NH_2), 6.86-7.02 (2H, m, ArH), 7.06-7.22 (8H, m, ArH) and 11.12 (1H, br s, NH); $\delta_{\text{C}}([^2\text{H}_6]\text{DMSO})$ 113.2 (C-5), 126.1 (CH, ArC), 127.5 (2xCH, ArC), 127.6 (2xCH, ArC), 128.2 (CH, ArC), 129.4 (2xCH, ArC), 131.6 (2xCH, ArC), 135.7 (C, ArC), 139.7 (C, ArC), 154.3 (C), 162.1 (C) and 162.7 (C). Also gave m/z (HRMS-EI), 263.1038. (M^+ . $\text{C}_{16}\text{H}_{13}\text{N}_3\text{O}$ requires M , 263.1058).

2-Amino-6-methyl-5-phenylpyrimidin-4(3*H*)-one 129. 2-Amino-5-bromo-6-methylpyrimidin-4(3*H*)-one (19)²¹⁸ (0.414 g, 2.03 mmol) and tetrakis(triphenylphosphine)palladium(0) (0.080 g, 0.07 mmol) were stirred with DMF (5 ml) for 5 min. Phenylboronic acid (0.275 g, 2.25 mmol) and more DMF (10 ml) were added, followed by sodium carbonate (0.550 g, 5.19 mmol) dissolved in water (2 ml) and the mixture was heated to reflux for 24 h, under a gentle flow of nitrogen. After cooling, the reaction was filtered, and the filtrate evaporated in *vacuo*. The residue was purified by silica gel column chromatography, with ethyl acetate / methanol / acetic acid (16:4:1) as eluent to yield the *title compound* 129 (0.040 g, 10%) as a white solid with R_f 0.61. Crystallisation of 129 from ethanol or from 50% aqueous DMF yielded solids that failed to give satisfactory elemental analyses, with broad melting ranges, but a sample crystallised from ethanol had $\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$ 3368, 3134, 2925,

1655, 1613, 1508, 1048 and 704; $\delta_{\text{H}}([^2\text{H}_6]\text{DMSO})$ 1.98 (3H, s, Me), 6.96 (2H, br s, NH_2), 7.18-7.40 (5H, m, Ph) and 10.5-12.5 (1H, br s, NH); $\delta_{\text{C}}([^2\text{H}_6]\text{DMSO})$ 20.8 (Me), 114.1 (C-5), 126.8 (CH, ArC), 127.8 (2xCH, ArC), 130.6 (2xCH, ArC), 134.4 (C, ArC), 153.1 (C), 157.0 (C) and 161.9 (C); m/z (HRMS-EI), 201.0899. (M^+ . $\text{C}_{11}\text{H}_{11}\text{N}_3\text{O}$ requires M , 201.0902).

2-Amino-5-bromo-4-chloro-6-phenylpyrimidine 132. 2-Amino-5-bromo-6-phenylpyrimidin-4(3*H*)-one (20) (3.725 g, 14.0 mmol) was heated to reflux in phosphorus oxychloride (12 ml) for 45 min, the vessel fitted with a calcium chloride guard tube. The solution was concentrated *in vacuo* to leave an orange oil, which was treated with ice-water (50 ml), and the oil broken up by the action of a glass rod. The mixture was then neutralised by the dropwise addition of aqueous ammonia to pH 7. After then standing overnight at 4°C, more ammonia was added to restore the pH to 7. The solid was then removed by centrifugation of the suspension formed, at 4000 rpm, washing with water, and drying the solid to constant weight. On standing, the supernatant precipitated more white solid, which was combined with that isolated by centrifugation. The combined crude solids were then warmed with dichloromethane, filtered to remove an insoluble by-product (2.09 g, see text), and reduced *in vacuo* to ca. 40 ml. Precipitation was then induced by the addition of petroleum ether (bp 40-60°C), to provide the *title compound* 132 (2.681 g, 67%) as a yellow crystalline solid, and had mp 135°C (lit.,⁸⁵ 136-138°C); $\delta_{\text{H}}([^2\text{H}_6]\text{DMSO})$ 7.36 (2H, brs, NH_2), 7.45-7.50 (3H, m, ArH) and 7.57-7.61 (2H, m, ArH); $\delta_{\text{C}}([^2\text{H}_6]\text{DMSO})$ 102.0 (C-5), 128.0 (CH, 2xArC), 128.8 (CH, 2xArC), 129.7 (CH, ArC), 138.3 (C, ArC), 160.4 (C), 161.4 (C) and 168.0 (C); m/z (ES^+), 284, 286, 288; $\text{C}_{10}\text{H}_7\text{BrClN}_3$ requires $M+1$, 284, 286, 288).

2-Amino-5-bromo-4-methoxy-6-phenylpyrimidine 134. Sodium methoxide, 25%wt solution in methanol (2 ml, ~8.8 mmol) was added via syringe to a stirred suspension of 2-amino-5-bromo-4-chloro-6-phenylpyrimidine (132)¹⁸⁵ (0.647 g, 2.27 mmol) in anhydrous methanol (25 ml), and the mixture heated to reflux for 30 min, under nitrogen. After slowly cooling overnight, excess sodium methoxide was decomposed by the addition of solid carbon dioxide, followed by water (200 ml). The suspension was extracted with chloroform (3x50 ml) and the combined organic extracts were washed with water (25 ml), dried (MgSO₄) and reduced to give a white solid. The solid was crystallised from hexane to furnish the *title compound* 134 (0.588 g, 93%) and had mp 174°C (Found: C, 47.1; H, 3.5; N, 14.8%; *m/z* (ES⁺), 280, 282. C₁₁H₁₀BrN₃O requires C, 47.2; H, 3.6; N, 15.0%; *M*+1, 280, 282); $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$ 3486, 3291, 3154, 1630, 1543, 1375, 1200 and 698; $\delta_{\text{H}}(\text{CDCl}_3)$ 4.01 (3H, s, Me), 5.11 (2H, br s, NH₂), 7.40-7.47 (3H, m, ArH) and 7.61-7.65 (2H, m, ArH); $\delta_{\text{C}}(\text{CDCl}_3)$ 54.8 (Me), 92.5 (C-5), 127.9 (2xCH, ArC), 128.8 (2xCH, ArC), 129.2 (CH, ArC), 138.1 (C, ArC), 160.9 (C), 166.0 (C) and 166.8 (C).

2-Amino-5,6-diphenyl-4-methoxypyrimidine 135. Sodium carbonate (0.276 g, 2.61 mmol), as a solution in water (2 ml), was added to a mixture of 2-amino-5-bromo-4-methoxy-6-phenylpyrimidine (134) (0.287 g, 1.02 mmol), phenylboronic acid (0.138 g, 1.13 mmol) and tetrakis(triphenylphosphine)palladium(0) (0.040 g, 0.035 mmol) in toluene (2 ml) and ethanol (2 ml), and the mixture heated to reflux for 18 h, under nitrogen. After cooling, the mixture was extracted with chloroform (3x25 ml), and the combined extracts washed with water (10 ml), 0.5 M sodium hydroxide (2x5ml), water (10 ml) and saturated brine (10 ml). The organic solution was then dried (MgSO₄) and reduced to give a grey solid which was crystallised from 10% ethyl acetate / hexane to provide the *title compound* 135 (0.212 g,

75%), as a crystalline white solid, and had mp 185-185.5°C (Found: C, 73.5; H, 5.4; N, 15.0%; m/z (ES⁺), 278. C₁₇H₁₅N₃O requires C, 73.6; H, 5.45; N, 15.15%; $M+1$, 278); ν_{\max} (KBr)/cm⁻¹ 3379, 3171, 1638, 1568, 1543, 1371, 1198 and 696; δ_{H} (CDCl₃) 3.88 (3H, s, Me), 5.14 (2H, br s, NH₂) and 7.08-7.28 (10H, m, 2xPh); δ_{C} (CDCl₃) 53.9 (Me), 110.9 (C-5), 126.7 (CH, ArC), 127.7 (2xCH, ArC), 127.8 (2xCH, ArC), 128.3 (CH, ArC), 129.4 (2xCH, ArC), 131.2 (2xCH, ArC), 134.4 (C, ArC), 138.6 (C, ArC), 161.3 (C), 165.2 (C) and 168.5 (C).

2-Amino-5-(2,4-dichlorophenyl)-4-methoxy-6-phenylpyrimidine

136. Prepared according to the method for the preparation of **135**, with 2-amino-5-bromo-4-methoxy-6-phenylpyrimidine (**134**) (0.210 g, 0.75 mmol), 2,4-dichloro-benzeneboronic acid (0.158 g, 0.83 mmol), tetrakis(triphenylphosphine)palladium(0) (0.030 g, 0.026 mmol) and sodium carbonate (0.203 g, 1.92 mmol) in toluene (2 ml), ethanol (2 ml) and water (1 ml), and the mixture heated to reflux for 24 h. Three sequential silica gel column purifications of the residue with chloroform / diethyl ether (85:15) as eluent gave a white solid (0.093 g) that by ¹H NMR contained the *title compound* **136** and starting pyrimidine **134** in approximately equal amounts. Crystallisation of this mixture from acetone (1 ml) gave a sample 90% pure in **136** (0.021 g) which gave the following data: (m/z (ES⁺), 346, 348, 350. C₁₇H₁₃Cl₂N₃O requires $M+1$, 346, 348, 350); ν_{\max} (KBr)/cm⁻¹ 3408, 3183, 1645, 1568, 1368, 1051, 814 and 698; δ_{H} (CDCl₃) 3.89 (3H, s, Me), 5.23 (2H, br s, NH₂), 6.92 (1H, d, J 8, 6'-H (5-Ar)), 7.09 (1H, dd, J 8 and 2, 5'-H (5-Ar)), 7.17-7.31 (5H, m, Ph) and 7.40 (1H, d, J 2, 3'-H (5-Ar)); δ_{C} (CDCl₃) 54.0 (Me), 107.5 (C-5), 126.9 (CH, ArC), 127.9 (2xCH, ArC), 128.6 (2xCH, ArC), 128.7 (CH, ArC), 129.1 (CH, ArC), 132.8 (C, ArC), 133.6 (CH, ArC), 133.7 (C, ArC), 136.1 (C, ArC), 138.1 (C, ArC), 162.1 (C), 165.7 (C) and 168.4 (C).

2-Amino-4-methoxy-6-phenyl-5-(3-thienyl)pyrimidine 137.

Palladium acetate (5.1 mg, 0.023 mmol) and 1,1'-(diphenylphosphino)-ferrocene (dppf) (16.6 mg, 0.03 mmol) were heated in degassed 1,2-dimethoxyethane (DME) (3 ml) for 15 min, under nitrogen. After cooling of this mixture 2-amino-5-bromo-4-methoxy-6-phenylpyrimidine (134) (0.210 g, 0.75 mmol), thiophene-3-boronic acid (0.106 g, 0.83 mmol) and potassium phosphate tribasic (0.318 g, 1.5 mmol) were added, along with DME (3 ml) and water (1 ml), and the mixture was heated under gentle reflux for 22 h, under nitrogen. The reaction mixture was then cooled, diluted with water (10 ml) and extracted with diethyl ether (3x25 ml). The combined ethereal extracts were washed with water (10 ml), saturated brine (10 ml), dried (MgSO₄) and reduced in *vacuo* to leave a pale brown solid. Successive crystallisations of the brown solid from hexane / ethyl acetate (5:1), then acetone yielded the *title compound* 137 (0.055 g, 26%) as a crystalline white solid, and had mp 206-207°C (Found: C, 63.6; H, 4.55; N, 14.6%; *m/z* (ES⁺), 284. C₁₅H₁₃N₃OS requires C, 63.6; H, 4.6; N, 14.8%; *M*+1, 284); ν_{\max} (KBr)/cm⁻¹ 3461, 3144, 1630, 1549, 1479, 1452, 1344 and 1198; δ_{H} (CDCl₃) 3.93 (3H, s, Me), 4.99 (2H, br s, NH₂), 6.82 (1H, dd, *J* 1 and 5, 4'-H (thienyl)), 6.96 (1H, dd, *J* 1 and 3, 2'-H (thienyl)), 7.18 (1H, dd, *J* 3 and 5, 5'-H (thienyl)) and 7.23-7.32 (5H, m, Ph); δ_{C} (CDCl₃) 53.9 (Me), 115.4 (C-5), 124.7 (CH, thienyl), 125.2 (CH, thienyl), 128.2 (2xCH, ArC), 129.0 (CH), 130.2 (2xCH, ArC), 131.2 (CH), 135.3 (C), 140.4 (C), 162.9 (C), 166.2 (C) and 169.3 (C).

2-Amino-4-methoxy-6-phenylpyrimidine 138.¹⁹⁶ According to the literature method,¹⁹⁶ guanidine sulfate was added to a solution of sodium (0.699 g, 30.4 mmol) in anhydrous methanol (50 ml), immediately followed by 3,3-bis(methylthio)-1-phenyl-2-propen-1-one (140) (3.252 g, 14.5 mmol), and the mixture heated to reflux, protected from moisture with a calcium chloride guard tube, for 24 h. The reaction mixture was evaporated under

vacuum, and the residue triturated with water, to leave a pale orange solid. The crude solid was then recrystallised from methanol (30 ml) to provide a crop of the *title compound* **138** (1.13 g), as a white, crystalline solid. From the filtrate, a further crop of **138** (0.37 g) was isolated by recrystallisation from hexane (75 ml) (total yield of 1.50 g, 51%). The product obtained had mp 151-152°C (lit.,¹⁹⁶ 152°C) ; $\delta_{\text{H}}(\text{CDCl}_3)$ 3.94 (3H, s, Me), 5.02 (2H, brs, NH_2), 6.51 (1H, s, 5-H), 7.42-7.48 (3H, m, ArH) and 7.92-7.96 (2H, m, ArH).

2-Amino-4-methoxy-5-(4-methoxyphenyl)-6-phenylpyrimidine

139. Prepared according to the method for the preparation of **137**, with palladium acetate (5.1 mg, 0.023 mmol), dppf (16.6 mg, 0.03 mmol) 2-amino-5-iodo-4-methoxy-6-phenylpyrimidine (**141**) (see below) (0.245 g, 0.75 mmol), 4-methoxybenzeneboronic acid (0.126 g, 0.83 mmol) and potassium phosphate tribasic (0.318 g, 1.5 mmol), in DME (6 ml) and water (1 ml), with a reaction time of 24 h. The crude residue of the organic extract, after work up as before, was purified by silica gel chromatography, with chloroform / diethyl ether (4:1) as eluent, to provide impure **139** (0.20 g). Crystallisation from acetone yielded the *title compound* **139** (0.165 g, 72%) as a white crystalline mass, and had mp 174°C (Found: C, 70.4; H, 5.5; N, 13.55%; m/z (ES^+), 308. $\text{C}_{18}\text{H}_{17}\text{N}_3\text{O}_2$ requires C, 70.3; H, 5.6; N, 13.7%; $M+1$, 308); $\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$ 3484, 3140, 1545, 1371, 1246, 1042, 831 and 696; $\delta_{\text{H}}(\text{CDCl}_3)$ 3.78 (3H, s, Me), 3.90 (3H, s, Me), 5.05 (2H, br s, NH_2), 6.76-6.80 (2H, m, ArH), 7.00-7.04 (2H, m, ArH) and 7.19-7.31 (5H, m, Ph); $\delta_{\text{C}}(\text{CDCl}_3)$ 54.0 (Me), 55.1 (Me), 110.5 (C-5), 113.4 (2xCH, ArC), 126.4 (C, ArC), 127.8 (2xCH, ArC), 128.2 (CH, ArC), 129.4 (2xCH, ArC), 132.2 (2xCH, ArC), 138.8 (C, ArC), 158.3 (C), 161.1 (C), 165.1 (C) and 168.7 (C).

3,3-Bis(methylthio)-1-phenyl-2-propen-1-one 140.¹⁹⁵ Acetophenone (2.821 g, 23.5 mmol) and carbon disulfide (1.787 g, 23.5 mmol) were added dropwise under argon, to a stirred suspension of potassium *tert*-butoxide (5.546 g, 46.9 mmol) in anhydrous diethyl ether (35 ml) in a cold water bath, forming an orange precipitate. After 45 min stirring, iodomethane (2.9 ml, 47 mmol) was added via syringe and the mixture stirred for a further 45 min at room temperature. Anhydrous methanol (100 ml) was added, and the diethyl ether was distilled from the reaction. The resultant red solution was poured into ice-cold water (200 ml), and the red precipitate formed was collected, washed with water and dried in *vacuo*. Crystallisation of the solid from ethyl acetate yielded the *title compound* 140 (2.809 g, 53%) as a crystalline orange solid. To recover more 140, the aqueous filtrate was extracted with chloroform (3x25 ml) and the combined extracts dried (MgSO₄) and concentrated in *vacuo* to give a red oil. The ethyl acetate mother liquor from the first crystallisation was added to the oil, concentrated to an oil once more and triturated with cold toluene to provide a second crop of 140 (0.455 g, 9%). The sample crystallised from ethyl acetate had mp 92-93°C (lit.,¹⁹⁵ 93°C) and the following ¹H NMR spectrum: $\delta_{\text{H}}(\text{CDCl}_3)$ 2.54 (3H, s, Me), 2.63 (3H, s, Me), 6.78 (1H, s, 2-H), 7.41-7.51 (3H, m, ArH) and 7.91-7.95 (2H, m, ArH).

2-Amino-5-iodo-4-methoxy-6-phenylpyrimidine 141. 2-Amino-4-methoxy-6-phenylpyrimidine (138)¹⁹⁶ (1.29 g, 6.41 mmol) was heated to reflux with *N*-iodosuccinimide (1.88 g, 8.33 mmol) in anhydrous chloroform (20 ml) for 2 h, under nitrogen. After cooling, the red solution was diluted to 50 ml with more chloroform and washed with water (2x25 ml), 5% sodium metabisulfite solution (15 ml), water (10 ml), then dried (MgSO₄) and concentrated to dryness in *vacuo* to provide the *title compound* 141 (2.07 g, 99%) directly in good purity, as a pale yellow solid. A portion crystallised from hexane had mp 179-180°C (Found: C, 40.7; H, 3.0; N, 12.9%; *m/z* (AP⁺),

328. $C_{11}H_{10}IN_3O$ requires C, 40.3; H, 3.0; N, 12.8%; $M+1$, 328); $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$ 3476, 3150, 1628, 1545, 1362, 1198, 1005 and 696; $\delta_{\text{H}}(\text{CDCl}_3)$ 3.96 (3H, s, Me), 5.46 (2H, br s, NH_2) and 7.42-7.54 (5H, m, Ph); $\delta_{\text{C}}(\text{CDCl}_3)$ 54.9 (Me), 64.9 (C-5), 127.9 (2xCH, ArC), 128.6 (2xCH, ArC), 129.0 (CH, ArC), 140.5 (C, ArC), 162.2 (C), 168.7 (C) and 170.2 (C).

2-Amino-5-(4-chlorophenyl)-4-methoxy-6-phenylpyrimidine 142.

Prepared according to the method for the preparation of 137, with palladium acetate (5.1 mg, 0.023 mmol), dppf (16.6 mg, 0.03 mmol) 2-amino-5-iodo-4-methoxy-6-phenylpyrimidine (141) (0.245 g, 0.75 mmol), 4-chlorobenzeneboronic acid (0.130 g, 0.83 mmol) and potassium phosphate tribasic (0.318 g, 1.5 mmol), in DME (6 ml) and water (1 ml), with a reaction time of 16 h. The crude reaction product was crystallised from hexane / acetone (approx.2:1) to provide the *title compound* 142 (0.182 g, 78%) as pale brown crystals, and had mp 196-198°C (Found: C, 65.6; H, 4.75; N, 13.4%; m/z (ES^+), 311, 313. $C_{17}H_{14}ClN_3O$ requires C, 65.5; H, 4.5; N, 13.5%; $M+1$, 311, 313); $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$ 3493, 3140, 1634, 1566, 1543, 1370, 833 and 694; $\delta_{\text{H}}(\text{CDCl}_3)$ 3.90 (3H, s, Me), 5.16 (2H, br s, NH_2), 7.01-7.06 (2H, m, 2xArH) and 7.17-7.30 (7H, m, ArH); $\delta_{\text{C}}(\text{CDCl}_3)$ 53.7 (Me), 107.5 (C-5), 127.7 (2xCH, ArC), 127.9 (2xCH, ArC), 128.4 (CH, ArC), 129.4 (2xCH, ArC), 131.2 (C, ArC), 133.1 (2xCH, ArC), 134.0 (C, ArC), 138.8 (C, ArC), 162.2 (C), 164.9 (C) and 167.8 (C).

2-Amino-4-methoxy-5-(3-nitrophenyl)-6-phenylpyrimidine 143.

Prepared according to the method for the preparation of 137, with palladium acetate (5.1 mg, 0.023 mmol), dppf (16.6 mg, 0.03 mmol) 2-amino-5-iodo-4-methoxy-6-phenylpyrimidine (141) (0.245 g, 0.75 mmol), 3-nitrobenzeneboronic acid (0.139 g, 0.83 mmol) and potassium phosphate tribasic (0.318 g, 1.5 mmol), in DME (6 ml) and water (1 ml), with a reaction time of 16 h.

Crystallisation of the crude reaction product from hexane / acetone (approx. 5:2) yielded the *title compound* **143** (0.197 g, 75%) as its 2:1 solvate with acetone, and had mp 184-185°C (Found: C, 63.0; H, 4.8; N, 16.0%; m/z (ES⁺), 323. C₁₇H₁₄N₄O₃ · 0.5 C₃H₆O requires C, 63.2; H, 4.9; N, 15.9%; $M+1$, 323 (for free molecule)); ν_{\max} (KBr)/cm⁻¹ 3322, 3198, 1640, 1545, 1346, 1200, 1065 and 775; δ_{H} (CDCl₃) 2.18 (3H, s, Me-acetone), 3.91 (3H, s, Me), 5.24 (2H, br s, NH₂), 7.16-7.31 (5H, m, Ph), 7.32-7.39 (2H, m, ArH) and 8.00-8.09 (2H, m, ArH); δ_{C} (CDCl₃) 30.9 (Me-acetone), 54.1 (Me), 108.6 (C-5), 121.6 (CH, ArC), 126.2 (CH, ArC), 128.2 (2xCH, ArC), 128.6 (CH, ArC), 128.8 (CH, ArC), 129.4 (2xCH, ArC), 136.5 (C, ArC), 137.5 (CH, ArC), 137.8 (C, ArC), 147.9 (C, ArC), 161.7 (C), 165.9 (C), 168.2 (C) and 207 (C-acetone).

2-Amino-5-(4-methoxyphenyl)-6-phenylpyrimidin-4(3H)-one 144.
 2-Amino-4-methoxy-5-(4-methoxyphenyl)-6-phenylpyrimidine (**139**) (0.17 g, 0.55 mmol) was heated to reflux in a mixture of methanol (5 ml) and 12 M hydrochloric acid (5 ml) for 2 h. After cooling the reaction mixture was diluted with water (10 ml), basified with solid sodium hydroxide (2.24 g) and re-acidified with acetic acid to pH 6. The suspension formed was stirred in ice, filtered, and washed with water followed by a small portion of acetone, to furnish the *title compound* **144** (0.16 g, 97%) as a white solid. Crystallisation from ethanol / ethyl acetate yielded fine white crystals, and had mp 322-326°C (Found: C, 69.1; H, 5.4; N, 14.1; H₂O, 0.6%; m/z (AP⁺), 294. C₁₇H₁₅N₃O₂ · 0.1 H₂O requires C, 69.2; H, 5.2; N, 14.2; H₂O, 0.6%; $M+1$, 294); ν_{\max} (KBr)/cm⁻¹ 3383, 3108, 1651, 1582, 1518, 1242, 993 and 704; δ_{H} ([²H₆]DMSO) 3.68 (3H, s, Me), 6.61 (2H, br s, NH₂), 6.68-6.75 (2H, m, ArH), 6.89-6.95 (2H, m, ArH), 7.16-7.23 (5H, m, Ph) and 11.10 (1H, br s, NH); δ_{C} ([²H₆]DMSO) 55.1 (Me), 112.9 (C-5), 113.1 (2xCH, ArC), 127.5 (2xCH, ArC), 127.7 (C, ArC), 128.1 (CH, ArC), 129.4 (2xCH, ArC), 132.5 (2xCH, ArC), 139.9 (C, ArC), 154.1 (C), 157.6 (C), 161.7 (C) and 162.9 (C).

6-Amino-2-butyl-4-phenylfuro[2,3-*d*]pyrimidine 148.

Triethylamine (8 ml) was added to a stirred suspension of 2-amino-5-iodo-6-phenylpyrimidin-4(3*H*)-one (**21**)⁶⁹ (0.626 g, 2.00 mmol), bis(triphenylphosphine)palladium dichloride (0.028 g, 0.04 mmol), cuprous iodide (0.100 g, 0.52 mmol) in DMF (8 ml), held under nitrogen, forming a deep green solution. 1-Hexyne (0.329 g, 4.00 mmol) was added and the mixture stirred at room temperature for 40 h. The reaction mixture was then heated to 50°C (internal temperature) for 4 h. Concentration of the reaction mixture in *vacuo* gave a brown solid, which was suspended in diethyl ether (50 ml) and washed with 1 M sodium hydroxide (2x10 ml), water (10 ml), 1 M hydrochloric acid (2x10 ml), water (10 ml) and finally saturated brine (10 ml). The remaining ethereal solution was dried (MgSO₄) and reduced in *vacuo* to give a brown oil, which was purified by silica gel column chromatography with 10% diethyl ether / dichloromethane, to give a sample of the *title compound* **148** (0.021 g, 4%). The fluorescent **148** thus obtained was not totally pure and was not subjected to elemental analysis, but yielded the following spectroscopic data: ν_{max} (CDCl₃ film)/cm⁻¹ 3530, 3422, 2961, 2934, 1616, 1460, 1371 and 698; δ_{H} (CDCl₃) 0.95 (3H, t, *J* 7, Me), 1.43 (2H, quintet, *J* 7, CH₂CH₂CH₂CH₃), 1.68-1.79 (2H, m, CH₂CH₂CH₂CH₃), 2.74 (2H, td, *J* 7 and 1, CH₂CH₂CH₂CH₃), 5.20 (2H, br s, NH₂), 6.52 (1H, t, *J* 1, 3-H), 7.47-7.56 (3H, m, ArH) and 7.95-8.01 (2H, m, ArH); δ_{C} (CDCl₃) 13.7 (Me), 22.1 (CH₂CH₂CH₂CH₃), 27.9 (CH₂CH₂CH₂CH₃), 29.3 (CH₂CH₂CH₂CH₃), 100.1 (C-3), 108.2 (C-3a), 128.3 (2xCH, ArC), 128.7 (2xCH, ArC), 130.1 (CH, ArC), 137.5 (C, ArC), 156.7 (C), 158.6 (C), 159.9 (C) and 169.4 (C).

2-Amino-5-(1-hexyn-1-yl)-4-methoxy-6-phenylpyrimidine 151. 1-

Hexyne (0.14 ml, 1.2 mmol) was added to a mixture of 2-amino-5-iodo-4-methoxy-6-phenylpyrimidine (**141**) (0.327 g, 1.0 mmol), bis(triphenylphosphine)palladium dichloride (0.014 g, 0.02 mmol) and cuprous iodide

(0.050 g, 0.26 mmol) in degassed (argon) triethylamine (10 ml), and the reaction mixture was then heated to 50-60°C for 16 h. The reaction mixture was then concentrated to dryness in *vacuo*, suspended in water (50 ml), and the mixture extracted with dichloromethane (3x25 ml). The combined organic extracts were washed with water (10 ml), dried (MgSO₄), and evaporated to leave an oily brown solid. Silica gel column purification of the crude product, with 10% diethyl ether / chloroform as eluent, was only able to isolate a mixture (*R_f* 0.36) of starting pyrimidine **141** and the *title compound* **151** (67/33%), the total return (0.255 g) accounting for 82% of starting material. The ¹H NMR spectrum of the weakly fluorescent **151** (at 254 nm) was determined: δ_H(CDCl₃) 1.22 (3H, t, *J* 7, CH₂CH₂CH₂CH₃), 1.26-1.56 (4H, m, CH₂CH₂CH₂CH₃), 2.38 (2H, t, CH₂CH₂CH₂CH₃), 3.97 (3H, s, OMe), 5.57 (2H, br s, NH₂), 7.39-7.45 (3H, m, ArH) and 7.88-7.92 (2H, m, ArH).

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