PYRAZOLO[3,4-*d*]PYRIMIDINES AND ADENOSINE RECEPTORS: A STRUCTURE/ACTIVITY STUDY

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ABSTRACT

Pyrazolopyrimidines are a general class of compounds which exhibit A_1 adenosine receptor affinity.

A number of pyrazolo[3,4-*d*]pyrimidine analogues of isoguanosine and 1-methylisoguanosine has been synthesised. All compounds were tested for A_1 adenosine receptor affinity using a [^{3}H] R-PIA competitive binding assay. The N-1 and N-5 positions were substituted with a number of different alkyl and aryl groups. 3-Chlorophenyl substitution of the N-1 position and butyl substitution of the N-5 position greatly enhanced the overall adenosine receptor affinity. Substitution by a methyl group at the N-7 position fixed the C-4 position in the imino tautomeric form. This resulted in a marked reduction in activity. The substitution of the N-2 position with a phenyl group produced an analogue with a similar structure to 1,3-dipropyl-8-(2-amino-4-chlorophenyl)xanthine (PACPX). A 2-phenyl substituent was favourable for interaction with the adenosine receptor.

A number of pyrazolo[3,4-*d*]pyrimidine analogues of 4,6-bis-α-carbamoylethylthio-1-phenylthiopyrazolo[3,4-*d*]pyrimidine (DJB-KK) has also been synthesised and tested for A_1 adenosine receptor affinity. 4,6-Bis-alkylthio-1-phenylpyrazolo[3,4-*d*]pyrimidines with α-carbamoylethyl and α-carbamoylpropyl groups were compared. The additional methylene of the α-carbamoylpropyl group produced increased adenosine receptor affinity. 6-α-Carbamoylethylthio-4-mercapto-1-phenylpyrazolo[3,4-*d*]pyrimidine and 4-α-carbamoylethylthio-1-phenyl-pyrazolo[3,4-*d*]pyrimidine were compared. Substitution of the C-6 position maintained activity, while substitution of the C-4 reduced activity.

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ABBREVIATIONS

Ac	acetyl
ADP	adenosine diphosphate
AMP	adenosine monophosphate
ATP	adenosine triphosphate
cAMP	cyclic adenosine monophosphate
Bu	butyl
Bz	benzoyl
^{13}C NMR	^{13}C nuclear magnetic resonance
CHA	cyclohexyladenosine
CNS	central nervous system
DBN	1,5-diazabicyclo[4.3.0]non-5-ene
DCC	1,3-dicyclohexylcarbodiimide
DMF	dimethylformamide
DMSO	dimethylsulphoxide
DNA	deoxyribonucleic acid
Et	ethyl
GDP	guanosine diphosphate
GTP	guanosine triphosphate
^{1}H NMR	^{1}H nuclear magnetic resonance
HPLC	high pressure liquid chromatography
IC$_{50}$	inhibitory concentration 50 %
IR	infrared
Me	methyl
MS	mass spectrum
NECA	5'-N-ethylcarboxamidoadenosine

Nu	nucleophile
Ph	phenyl
PACPX	1,3-dipropyl-8-(2-amino-4-chlorophenyl)xanthine
PIA	N^6-phenylisopropyladenosine
Pr	propyl
Rb	ribose
RNA	ribonucleic acid
THF	tetrahydrofuran
UV	ultra violet
XAC	xanthine amine congener

CHAPTER ONE

Introduction

[1.0] General Introduction

Adenosine (1) is a nucleoside which consists of a purine base attached to a ribose sugar through a β linkage.

$$NH_2$$

1
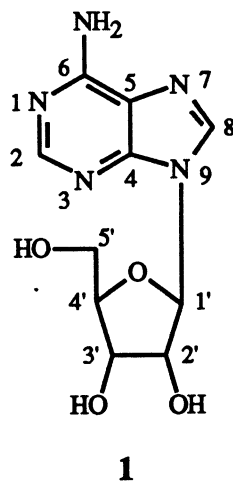
Adenosine possesses a number of unique biochemical and biological properties. It is one of the building blocks of nucleic acids (such as RNA and DNA), which are responsible for encoding genetic information. Adenosine is also a structural element of energy transfer compounds (such as ADP and ATP), which produce chemical energy and secondary messenger compounds (such as cAMP), which couple hormones with enzyme systems.

Adenosine itself was originally shown to produce hypotensive, sedative, anti-spasmodic and vasodilatory effects in 1929.[1] Since then a wide range of other biological effects have been observed. These include coronary vasodilation, reduction of heart rate and force, inhibition of lipolysis, inhibition of platlet aggregation, renal vasoconstriction and behavioural sedation.[2,3]

[1.1] Adenosine Receptors

Adenosine has been shown to activate adenylate cyclase, resulting in stimulation or inhibition of cAMP production depending on the type of cell or tissue. Adenosine increases cAMP levels in brain slices[4] and some cultured cell lines,[5,6] but decreases cAMP levels in fat cells[7] and primary glial cultures.[8] It is believed that the change in intracellular cAMP levels is responsible for the biological response. However, only a limited number of adenosine actions have been proven to involve cAMP systems. Two such examples are adenosine's action on adipocytes and platlets. Adenosine inhibits adipocyte adenylate cyclase, which results in the inhibition of lipolysis in adipocytes.[9] Conversely, adenosine stimulates platlet adenylate cyclase, which results in the inhibition of aggregation of platlets.[2] Adenosine may also regulate other secondary messengers such as calcium.[10]

It was not until the early 1970's that it was realised that adenosine's effect on adenylate cyclase and biological systems was mediated by specific receptors. Burnstock proposed that two classes of purine receptors were present in the membranes of cells.[11] The first type were P_1 receptors, which were sensitive to adenosine and lead to changes in cAMP accumulation. The second type were P_2 receptors, which were sensitive to adenine nucleotides such as AMP, ADP and ATP and lead to prostaglandin production.

In 1977, Londos and Wolff proposed a model to account for the biphasic effects that adenosine produced on adenylate cyclase.[12] Adenosine and its analogues were found to stimulate adenylate cyclase activity in Leydig cells, but inhibit adenylate cyclase activity in liver cells. It was suggested that two adenosine reactive sites were linked to a single adenylate cyclase. The first was the R site, which required the presence of the ribose ring and produced stimulation of cyclase activity. The R site was found to have

the properties of an extracellular receptor. The second was the P site, which required the presence of the purine ring and produced inhibition of cyclase activity. Preliminary evidence suggested that the P site was an intracellular receptor.

In 1978, van Calker, Muller and Hamprecht reported a further study on the extracellular adenosine receptors in cultured glioblasts.[13] Adenosine was found to inhibit increases in the level of cAMP evoked by isoprenaline. Adenosine was already known to stimulate the production of cAMP through interaction with external adenosine receptors.[12,14] This phenomenon was explained by the existence of two kinds of extracellular adenosine receptors: A_1 receptors, which mediated inhibition and A_2 receptors, which mediated stimulation of adenylate cyclase.

In 1980, an independent study by Londos, Cooper and Wolff confirmed these results.[9] Selected adenosine agonists were used to discriminate between two different types of extracellular adenosine receptors. In fat cells the sequence of agonist potency on adenylate cyclase activity was as follows: PIA > adenosine > NECA. In liver and leydig cells the reverse sequence of potency was observed: NECA > adenosine > PIA. From this it was concluded that the inhibition of adenylate cyclase in fat cells and the activation of adenylate cyclase in liver and leydig cells were mediated by different receptor subtypes. An alternative nomenclature was used in which the inhibitory receptors were termed R_i and stimulatory receptors were termed R_a. These receptors were thought to share common properties, but have distinct agonist recognition sites. The R_i receptors were found to be high affinity receptors where adenosine was an effective agonist at 10^{-9} M. The R_a receptors were found to be low affinity receptors where agonists acted at 10^{-6} M.[14] The various subclasses of adenosine receptors are summarised in Table 1.

Table 1   Subclasses of Adenosine Receptors[16,17]

Receptor	Location	Affinity	Effect of Activation
P_1 A_1 (R_i)	Cell Surface	10^{-9} M	Decreased cAMP formation May have effects independent of cAMP.
A_2 (R_a)	Cell Surface	10^{-6} M	Increased cAMP formation May have effects independent of cAMP.
P	Intracellular	10^{-5} M	Decreased cAMP formation
P_2	Cell Surface		Related to increased synthesis of prostaglandins.

[1.2] Mechanism of Action

Extracellular adenosine receptors produce a signal which is transduced across the membrane to the catalytic subunit of adenylate cyclase.[18] Activation of adenylate cyclase results in the conversion of ATP to cAMP, which evokes a biological response. This is believed to occur in the same manner as other receptor-cyclase coupled systems, such as the adrenergic system.[19]

Adenosine receptors do not interact directly with the catalytic subunit of adenylate cyclase, but act via guanine regulatory proteins (G proteins).[18] A large family of these proteins have been found to exist. G_s proteins mediate the activation of adenylate cyclase, G_i proteins inhibit adenylate cyclase, while the function of G_o and G_p proteins remains unknown. All of these coupling proteins are heterotrimers, consisting of α, β and γ subunits. The α subunits differ while the β and γ subunits are identical.

Extracellular adenosine receptors activate the G proteins in the presence of GTP and

Mg^{2+}, which regulate the activity of adenylate cyclase.
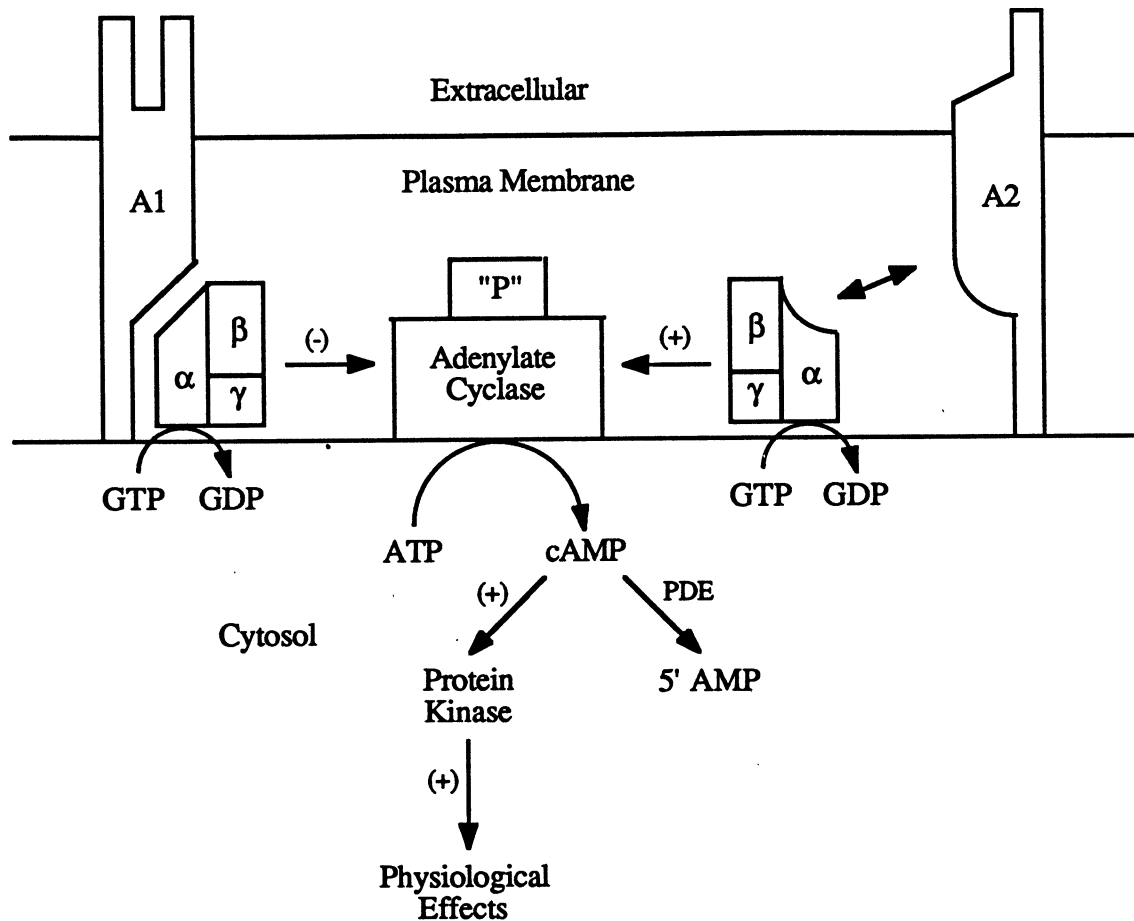
Figure 1 Schematic View of the Adenosine Receptor-Adenylate Cyclase System

Radioligand binding techniques have provided insights into the differences between

agonist and antagonist interactions with the A_1 adenosine receptor.[18] Radiolabelled

agonists, such as [^{3}H] R-PIA, produced saturation binding curves which indicated that

there are both high and low affinity states of the receptor. The presence of guanidine

nucleotides resulted in a decrease in receptor binding as high affinity state receptors were

converted to low affinity state receptors. In contrast, radiolabelled antagonists such as

[^{3}H] XAC produced saturation binding curves that indicated there was only one affinity

state of the receptor. The presence of guanidine nucleotides had no effect on receptor binding as only one affinity state existed. As no fully satisfactory A_2 receptor radioligand has appeared, most studies have been limited to A_1 adenosine receptors.

Agonists interact with the A_1 adenosine receptor and stabilise the high affinity state of the receptor.[18] This is represented by the agonist-receptor-guanidine regulatory protein complex. The formation of this complex then allows GTP to associate with the α subunit of G_i. The agonist-receptor-guanidine regulatory protein complex dissociates and the GTP liganded α subunit of G_i inhibits adenylate cyclase activity. The GTP is then hydrolysed by a GTPase intrinsic to the α subunit of G_i. The coupling mechanism of adenosine receptors with adenylate cyclase is summarised in Figure 1.

[1.3] Structure/Activity Relationships

Structure/activity relationships for adenosine receptors have allowed characterisation of many receptor properties. Many adenosine agonists and antagonists have been studied in a large variety of biological systems with respect to binding of radioactive ligands, cAMP generation and physiological response.[20] A number of commonly used agonists (mainly adenosine analogues) and antagonists (mainly xanthines) are listed in Table 2.

Agonists

The A_1 and A_2 receptors can be thought of in terms of two subdomains; one which binds the ribose moiety and one which binds the purine moiety.[20] The spatial orientation of these domains requires the adenosine analogue to be in the anti-configuration to access both regions and result in activation of the receptor. The purine base of adenosine can change its position relative to the ribose ring by rotation about the glycosidic N-9 to C-1'

bond. By testing several conformationally restricted analogues of adenosine it was possible to demonstrate that adenosine binds to its receptor in the 'anti' conformation. 8-Bromoadenosine, which is quite unstable in the anti-conformation (as a result of interactions between the ribose and bromo groups), has no adenosine receptor activity.

The ribose recognition domain was originally shown to be of prime importance for receptor affinity.[20] The 2', 3' and 5'-hydroxy groups all contribute to the strength of adenosine receptor affinity. Removal or even a change in configuration of these groups has generally resulted in a marked decrease in adenosine receptor affinity. 2'-Deoxyadenosine is inactive, while 3'-deoxyadenosine and 5'-deoxyadenosine are 5 times less potent than adenosine. Arabinofuranoside and xylofuranoside (in which the 2' and 3'-hydroxy groups have the opposite configuration) are both inactive. One modification of the ribose hydroxy groups which resulted in an increase in receptor affinity was the replacement of the 5'-hydroxy group with a carboxamido group. This replacement resulted in an increase in A_2 receptor potency. The most active 5'-carboxamidoadenosine agonists were obtained when the carboxamido group contained an ethyl-sized substituent. N-Ethylcarboxamide and N-cyclopropylcarboxamides are more potent than carboxamide and methylcarboxamide. NECA was found to be a potent A_2 agonist, but was essentially non-selective in its action at A_1 and A_2 receptors. The simplest explanation of the relationship between the ribose moiety of adenosine and its subdomain is that a hydrogen acceptor site must exist. This site must interact with the 2', 3' and 5'-hydroxy groups and the 5'-carboxamido group. N-Ethyl and N-cyclopropyl carboxamides may have enhanced potency through the interaction of the alkyl substituent with an ethyl-sized hydrophobic pocket.

The purine recognition domain also has an influence on receptor affinity.[20] Ring altered adenosines such as 3-deazaadenosine, 7-deazaadenosine, 8-azaadenosine,

7-deaza-8-azaadenosine and 8-aza-9-deazaadenosine have been found to possess minimal activity. This may result from the purine ring binding to its subdomain in a very exact manner. Replacement of the 6-amino group with methyl, hydroxy or mercapto groups has also resulted in a reduction of activity. This suggests the 6-amino group is necessary for interaction with a hydrogen donor located in the purine binding subdomain. N^6-alkyl substitution has produced fluctuations in agonist activity while N^6-cycloalkyl substitution produced a large increase in potency and selectivity for the A_1 adenosine receptor.[21] Recent work has identified a number of optically pure N^6-bicycloalkyladenosines with unusually high potency and A_1 selectivity.[22] A structure/activity analysis of the epimers and diasteriomers of these N^6-norbornyladenosines has led to the construction of a detailed map of the stereochemistry of the N^6 binding region of the A_1 adenosine receptor. Substituents in other positions of the purine ring are tolerated to various degrees by adenosine receptors. The addition of 2-chloro and 2- fluoro groups resulted in a slight decrease in potency, while 2-hydroxy and 2-amino groups produced a marked decrease in adenosine receptor affinity. The 2-phenylamino group produced weak agonist activity, but increased the selectivity for the A_2 receptor.[23] 2-Phenylamino-adenosine was found to be 7 fold less potent and 8-10 fold more selective for A_2 receptors than NECA. Variation of the C-2 substituent and combination with the 5'-N-ethylcarboxamido group produced more potent and selective A_2 agonists. 2-Phenylethylamino-5'-N-ethylcarboxamidoadenosine was found to be more potent and 49 fold selective for the A_2 receptor. Substitution of the phenyl ring led to further increases in potency and selectivity. 2-(4-Carboxyethylphenyl)ethylamino-5'-N-ethylcarboxamidoadenosine was found to be of similar potency and 114 fold selective for the A_2 receptor. It was concluded that bulky aromatic groups in the C-2 position reduce A_1 receptor activity, while carboxamido groups in the C-5' position increase A_2 receptor activity. Substituents in the C-8 position are not tolerated by adenosine receptors as a result of their destabilisation of the anti-configuration of the ribose and the purine ring.

Table 2   Structure of Common Adenosine Agonists and Antagonists
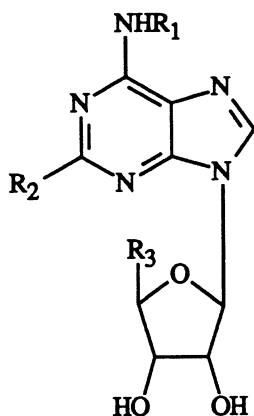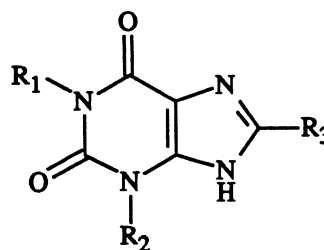
2   3

Agonists (2)	R_1	R_2	R_3
Adenosine	H	H	CH_2OH
5'-N-Ethylcarboxamidoadenosine	H	H	$CONHC_2H_5$
N^6-Cyclopentyladenosine	C_5H_9	H	CH_2OH
N^6-Cyclohexyladenosine	C_6H_{11}	H	CH_2OH
N^6-(2-Phenylisopropyl)adenosine	$CH(CH_3)CH_2C_6H_5$	H	CH_2OH
2-Chloroadenosine	H	Cl	CH_2OH

Antagonists (3)	R_1	R_2	R_3
Xanthine	H	H	H
1,3-Dimethylxanthine	CH_3	CH_3	H
1,3-Dipropylxanthine	C_3H_7	C_3H_7	H
1,3-Dipropyl-8-phenylxanthine	C_3H_7	C_3H_7	C_6H_5
1,3-Dipropyl-8-(2-amino-4-chloro-phenyl)xanthine	C_3H_7	C_3H_7	$C_6H_3NH_2Cl$

Antagonists

Xanthines act as stimulants through their ability to block adenosine receptors.[20] Although xanthine was found to be a relatively weak adenosine antagonist, substitution of the xanthine ring produced a marked increase in antagonist activity. The addition of 1,3-alkyl groups as in 1,3-dimethylxanthine (theophylline) produced an 80 fold increase in adenosine receptor affinity. The increase in the length of the 1,3-dialkyl groups resulted in a further increase in potency and some selectivity for the A_1 adenosine receptor. The addition of an 8-aryl group as in 8-phenylxanthine produced the most dramatic increase in receptor affinity. The variation of the substituents on the 8-phenyl ring resulted in further increases in potency. The combination of 1,3-dipropyl and 8-(2-amino-4-chlorophenyl) substituents in PACPX produced a 4 000 000 fold increase in the receptor affinity (relative to xanthine) and some selectivity for the A_1 receptor. These results suggested that the binding of the heterocyclic ring to the purine site is not as precise for these antagonists as it is for adenosine analogues. As a result of this imprecise fit, the nature of the 1,3-dialkyl groups may affect the positioning of the 8-aryl group, revealing subtle differences in the topography of the A_1 and A_2 receptors around the purine binding domain.

A number of different classes of compounds which contain a planar heterocyclic ring have also been found to possess adenosine antagonist activity. These include benzopteridines[24], pyrazolopyridines[25], pyrazolopyrimidines[26-28], 9-methyladenines[29] and 7-deaza-9-phenyladenines[30]. The pyrazolopyrimidines were the most potent of these classes. Series of 1-substituted 4,6-dialkylthiopyrazolo[3,4-*d*]pyrimidines[26,27] and 5-substituted 1,3-dialkylpyrazolo[4,3-*d*]pyrimidin-7-ones[28] produced antagonists which were much more potent than theophylline at the A_1 adenosine receptor.

[1.4] 1-Methylisoguanosine

In 1980, an interesting adenosine analogue was isolated from the marine sponge, *Tedania digitata*.[31,32] The aqueous ethanolic extract was screened and found to possess muscle relaxant, anti-inflammatory and other pharmocological activity. The active constituent was isolated by ion-exchange chromatography and further purified by recrystallisation. Structural elucidation, predominantly using ^{13}C NMR and mass spectrometry, identified the compound as 1-methylisoguanosine (4). The same compound has since been isolated from the Californian nudibranc, *Anisidoris nobilis* [33] and the Carribean coral, *Madracis mirabilis*.[34]

4
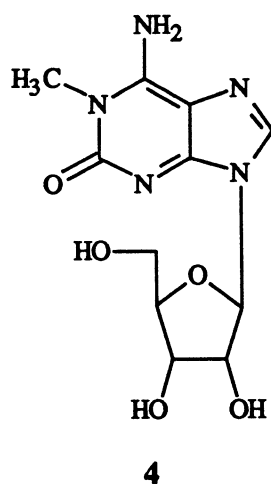
Pure 1-methylisoguanosine produced potent muscle relaxation and hypothermia when given orally or intraperitoneally to mice. It also reduced blood pressure and heart rate in rats and blocked polysynaptic responses at doses which did not affect transmission at the neuromuscular junction in the mouse spinal chord.[35-37] 1-Methylisoguanosine was shown to be resistant to degradation by adenosine deaminase, a poor substrate for the adenosine uptake system and able to interact with extracellular adenosine receptors. These results indicated that this adenosine analogue was metabolically stable and able to remain in the extracellular environment for a sufficient period to activate adenosine

receptors and cause pharmocological effects. Unfortunately, 1-methylisoguanosine was found to exhibit little selectivity for A_1 or A_2 adenosine receptors.

1-Methylisoguanosine has also been shown to interact with benzodiazepine receptors by a [^{3}H] diazepam binding assay.[37] The results of this work have suggested that 1-methylisoguanosine is several hundred fold more potent than inosine or hypoxanthine. These two purines have been suggested as possible endogenous ligands for the stereospecific, saturable benzodiazepine binding sites shown to exist in the mammalian CNS. Although diazepam is a muscle relaxant, 1-methylisoguanosine is not believed to act as a skeletal muscle relaxant through interaction with the benzodiazepine receptor.[38] Benzodiazepine antagonists have been unable to block the skeletal muscle relaxant and hypothermic activity of 1-methylisoguanosine.

A synthesis of 1-methylisoguanosine was developed to obtain enough sample for comprehensive biological testing.[31] 5-Amino-4-carbamoyl-1-β-D-ribofuranosylimidazole (5) was found to be a useful starting material. This compound was obtained from the culture broth of a mutant strain of bacteria (*Bacillus snubtilis*) and was purified by ion-exchange chromatography. The acetylation of the hydroxy groups using acetic anhydride in pyridine produced the protected derivative (6, Scheme 1). The amide was dehydrated using phosphorous oxychloride and triethylamine in chloroform to yield the *ortho*-aminonitrile (7). The addition of this *ortho*-aminonitrile to methyl isocyanate produced an intermediate urea (8). The urea underwent an intramolecular cyclisation with methanolic ammonia in DMF to yield 1-methylisoguanosine (4). The direct methylation of isoguanosine also produced 1-methylisoguanosine (4).
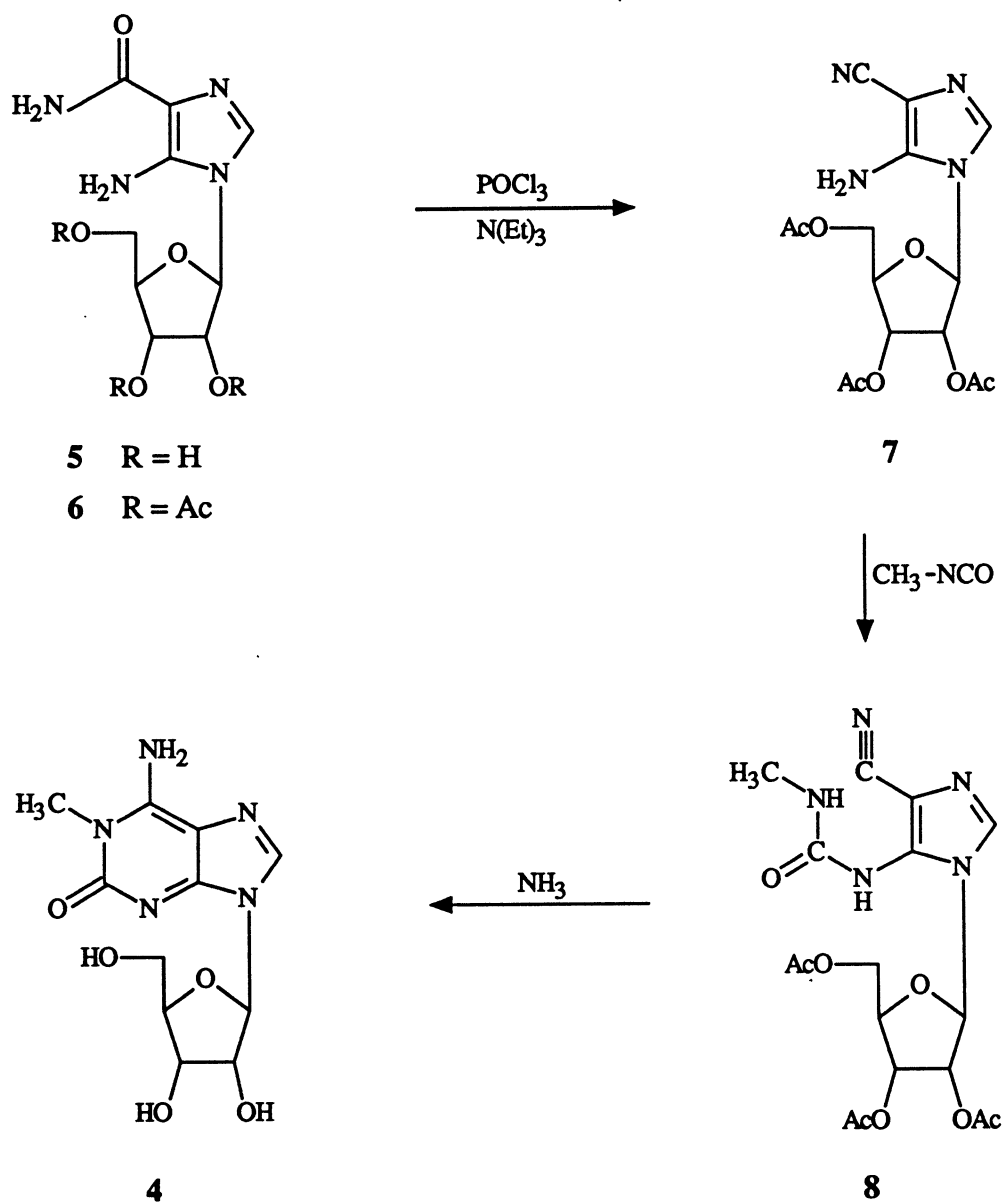
Scheme 1

An improved synthesis of 1-methylisoguanosine was later developed.[39] The addition of 5-amino-4-carbamoyl-1-β-ribofuranosylimidazole (5) to methyl isothiocyanate in DMF produced an intermediate thiourea. The thiourea underwent a novel desulphurisation with DCC to yield the same intermediate urea (8). The cyclisation of this urea with methanolic ammonia produced 1-methylisoguanosine (4). This method required little purification of the intermediates and proceeded in a higher overall yield.

The synthesis of 1-methylisoguanosine analogues may enable more specific A_1 or A_2 receptor interactions. Such compounds would therefore produce more specific pharmacological effects and maybe useful as therapeutic agents. A number of 1-methylisoguanosine analogues have already been prepared. The substituents at N-1 and N-9 were varied, while substituents were added at C-8. The effect of O-2 methylation instead of N-1 methylation and the conversion of N-6 amino to keto analogues were also examined.[40] All of these analogues were tested for their skeletal muscle relaxant, hypothermic, cardiovascular and anti-inflammatory effects following oral administration and for their anti-allergic effects following intravenous administration. None of the analogues exhibited any greater potency or selectivity than 1-methylisoguanosine. A second study involved the preparation of a number of N-7 and N-9 substituted analogues.[34] The compounds were tested in a benzodiazepam binding assay and were found to have similar activity to 1-methylisoguanosine. These analogues had no CNS or anti-inflammatory activity in the same dose range as 1-methylisoguanosine. 1-Ethyl-3-methylisoguanosine was prepared to allow spectroscopic studies of an analogue which was frozen in the 6-imino form.[41]

[1.4] 4,6-Bis-α-carbamoylethylthio-1-phenylpyrazolo[3,4-d]pyrimidine (DJB-KK)

In 1983, a number of heterocycles structurally related to caffeine and theophylline were tested for adenosine antagonist activity.[26,27] Approximately 60 nitrogen and sulphur heterocycles from 11 different classes were examined. The compounds were initially tested for their ability to displace [3H] R-PIA from A_1 adenosine receptors in rat brain membranes. Active compounds were further tested for their ability to antagonise adenosine stimulated cAMP generation and their ability to block the adenosine receptors which mediate presynaptic inhibition of transmitter release from cholinergic nerves. Pyrazolo[3,4-d]pyrimidines were found to be a general class of compounds which acted

as adenosine antagonists. The pyrazolo[3,4-*d*]pyrimidines which were tested, contained different substituents in the N-1, C-4 and C-6 positions (Table 3). The phenyl group was the most potent in the N-1 position, while the α-carbamoylethylthio group was the most potent in the C-4 and C-6 positions. Substitution in the C-4 position alone resulted in a marked reduction in activity. DJB-KK (9, $R_1 = C_6H_5$, R_2 and $R_3 =$ SCH(CH$_3$)CONH$_2$) was found to be the most potent adenosine antagonist in this class. It was 40 times more potent than theophylline. Of the remaining classes only the thiazolo[5,4-*d*]pyrimidines showed significant antagonist activity.

Table 3   Adenosine Receptor Affinity of the Pyrazolo[3,4-*d*]pyrimidines[27]
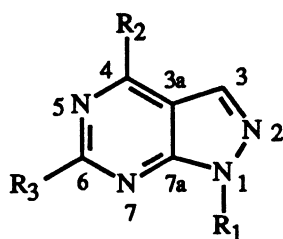
R_1	R_2	R_3	K_i
H	SCH(CH$_3$)CONH$_2$	H	45.6
H	SCH(CH$_3$)CONH$_2$	CH$_3$	19.4
H	SCH(CH$_3$)CONH$_2$	SCH(CH$_3$)CONH$_2$	29.4
CH$_3$	SCH(CH$_3$)CONH$_2$	SCH(CH$_3$)CONH$_2$	14.2
C$_6$H$_5$	SH	H	18.3
C$_6$H$_5$	SCH$_2$CONH$_2$	H	47.7
C$_6$H$_5$	SCH$_2$CONH$_2$	SCH$_2$CONH$_2$	1.7
C$_6$H$_5$	SCH$_2$CONHCH$_3$	H	32.7
C$_6$H$_5$	SCH(CH$_3$)CONH$_2$	SCH(CH$_3$)CONH$_2$	0.37

4,6-Bis-α-carbamoylethylthio-1-phenylpyrazolo[3,4-*d*]pyrimidine (DJB-KK) was originally synthesised as a possible amplifier of the antibiotic and anti-tumour activity of

phleomycin.[42] The reaction of 5-amino-4-cyano-1-phenylpyrazole (**10a**, Scheme 2) and

carbon disulphide in the presence of sodium methoxide produced 1-phenyl-5H,7H-

pyrazolo[3,4-d]pyrimidine-4,6-dithione (**11**). The alkylation of this dithione with

2-bromopropionamide in dilute sodium hydroxide gave DJB-KK (**9**).
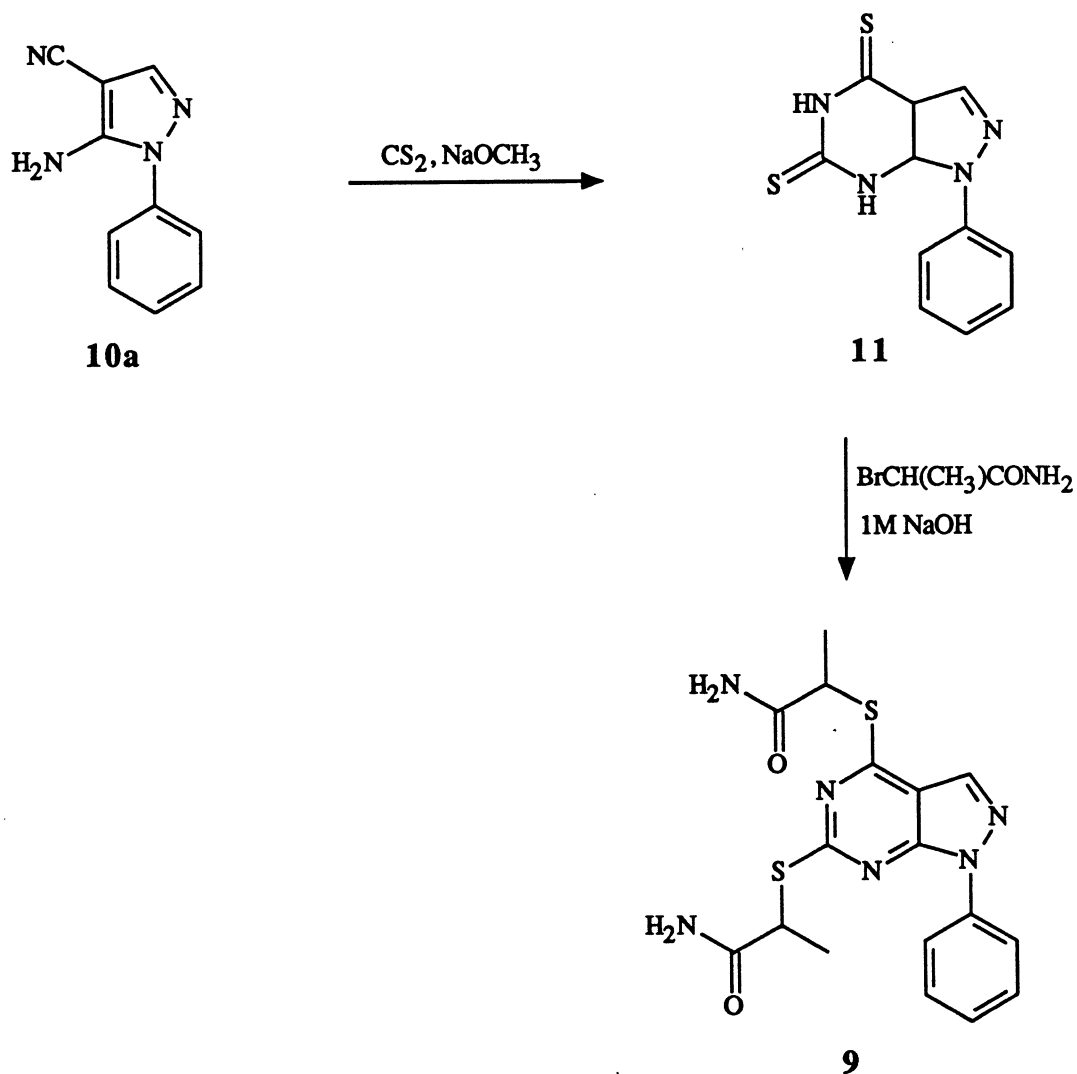
Scheme 2

In 1987, a series of 5-substituted 1,3-dialkylpyrazolo[4,3-d]pyrimidin-7-ones were

synthesised.[28] These compounds were tested for A_1 adenosine receptor affinity by

measuring the inhibition of [^{3}H] CHA binding to rat brain membranes. The most active

compound (**12**) was found to be approximately 15 times more potent than DJB-KK (**9**).

The potency pattern of the 5-phenyl substituents of these 1,3-dialkyl-5-phenylpyrazolo[4,3-*d*]pyrimidin-7-ones was found to be closely related to the 8-phenyl substituents of 1,3-dialkyl-8-phenylxanthines. A quantitative structure/activity relationship was developed between the two series, which accurately predicted the adenosine receptor affinity of additional pyrazolo[4,3-*d*]pyrimidines. On the basis of this strong correlation between the close superposition of the heterocyclic ring and substituents with the adenosine receptor affinity, it was postulated that the two series fit the adenosine receptor in the same fashion.
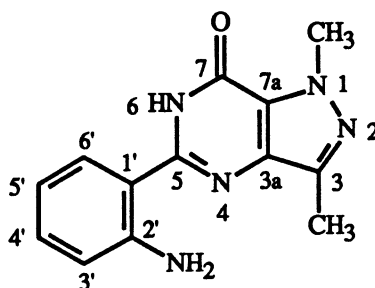
12

[1.6] Drug Development

The compounds described above offer important leads in the development of selective agonists and non-xanthine antagonists active at adenosine receptors. This thesis describes further structure/activity studies which have been undertaken on a number of pyrazolo[3,4-*d*]pyrimidines. Pyrazolo[3,4-*d*]pyrimidine analogues of both 1-methylisoguanosine (**4**) and 4,6-bis-α-carbamoylethylthio-1-phenyl-pyrazolo[3,4-*d*]pyrimidine (**9**) have been investigated. The development of more A_1 or A_2 selective adenosine analogues may provide compounds which are useful as research tools or as therapeutic agents.

A number of pyrazolo[3,4-d]pyrimidine analogues of 1-methylisoguanosine have been prepared. The N-1, N-2, N-5 and C-4 substituents were all varied. The effect of a alkyl and aryl substitution in both the N-1 and N-5 positions was examined. The lack of substitution in the N-5 position produced analogues of isoguanosine. The addition of a methyl substituent to the N-7 position yielded analogues with the C-4 substituent fixed in the imino form. Phenyl substitution in the N-2 position generated analogues which were more like PACPX in structure. A number of analogues of 4,6-bis-α-carbamoylethylthio-1-phenylpyrazolo[3,4-d]pyrimidine were also prepared. The C-4 and C-6 substituents were varied.

The A_1 adenosine receptor affinity of the pyrazolo[3,4-d]pyrimidines was measured using a R-PIA competitive binding assay.[43] R-PIA is a potent adenosine agonist which can be labelled to high specific activity with tritium. R-PIA binding sites appear to be equivalent to the extracellular adenosine receptor sites in rat brain membranes. The ability of the pyrazolo[3,4-d]pyrimidines to compete with [^{3}H] R-PIA binding provides a measure of their A_1 adenosine receptor affinity. All compounds were assayed at 20 μM to obtain the percentage inhibition of [^{3}H] R-PIA binding. The % inhibition values provided an approximation of the A_1 adenosine receptor affinity. Selected compounds were then assayed at various known concentrations to obtain the concentration required to inhibit 50 % of [^{3}H] R-PIA binding. The IC_{50} values provided a more statistically accurate measure of the A_1 adenosine receptor affinity.

CHAPTER TWO

1-Substituted 5-Amino-4-cyanopyrazoles

[2.0] General Introduction

Imidazoles and pyrimidines are common intermediates in the synthesis of purines.[44] The Hoffman degradation of imidazole-4,5-dicarboxamides (13) and the Lossen degradation of the corresponding dihydroxamic acids (14) were among the earliest syntheses of purines. More recently, a number of syntheses have been described which use imidazole compounds such as 15 and 16. These routes are believed to parallel those involved in biological systems. Pyrazoles are common intermediates in the synthesis of pyrazolopyrimidines.[45-47]
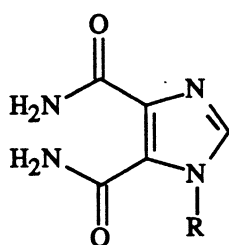
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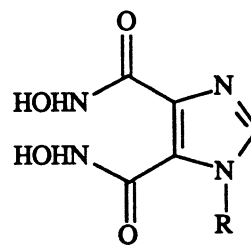
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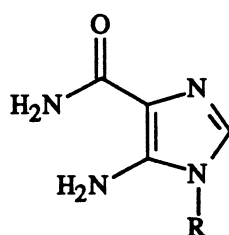
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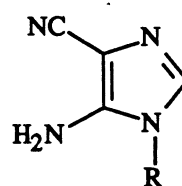
16

The synthesis of 1-substituted 5-amino-4-cyanopyrazoles (10, Scheme 3) involved the condensation of ethoxymethylenemalononitrile (17) with substituted hydrazines (18).[46,47] Ethoxymethylenemalononitrile was prepared by the condensation of malononitrile with triethyl orthoformate, whereas the hydrazines were prepared by the diazotisation and reduction of amines.
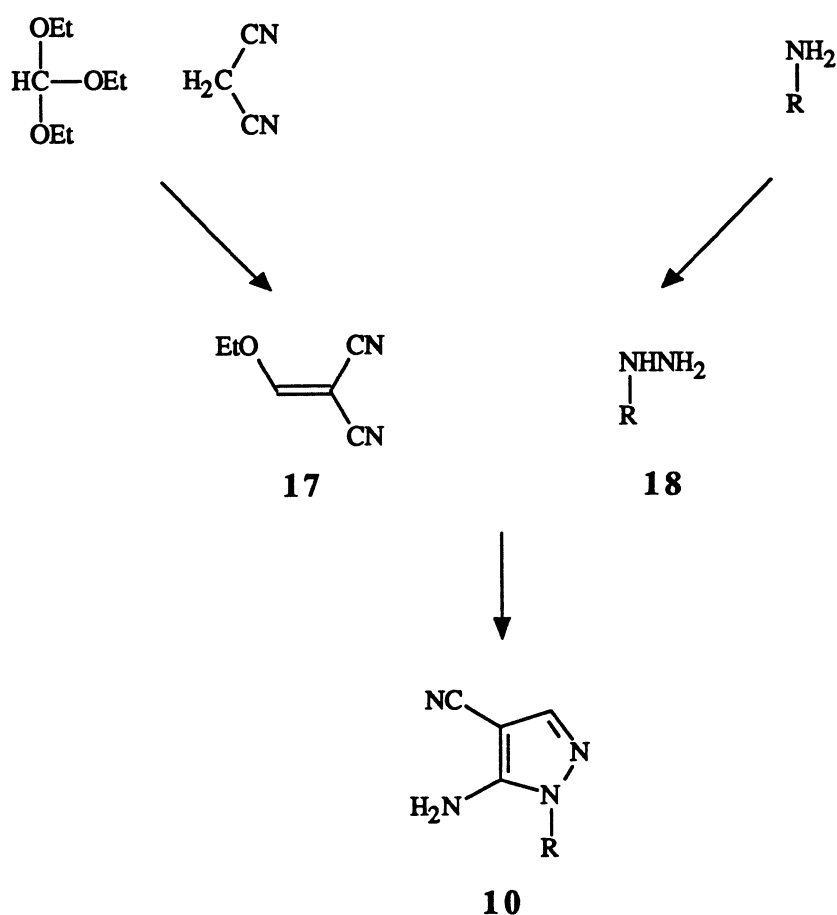
Scheme 3

An alternative synthesis of 1-substituted 5-amino-4-cyanopyrazoles (10) was developed. This method involved a one-pot reaction between malononitrile, triethyl orthoformate and a substituted hydrazine.

[2.1] Synthesis of Ethoxymethylenemalononitrile

The initial starting material necessary for the preparation of 1-substituted 5-amino-4-cyanopyrazoles was ethoxymethylenemalononitrile (17). A number of syntheses of ethoxymethylenemalononitrile have been reported in the literature.[48-50] These methods have involved the condensation of malononitrile with triethyl orthoformate under a variety

of conditions. This reaction has often been accompanied by the formation of undesirable side products.

Malononitrile and triethyl orthoformate have been condensed at 140 °C in the absence of solvent.[50] Distillation under reduced pressure gave a 66 % yield of ethoxymethylenemalononitrile. The mechanism of this reaction is believed to be an ionic process (Scheme 4).
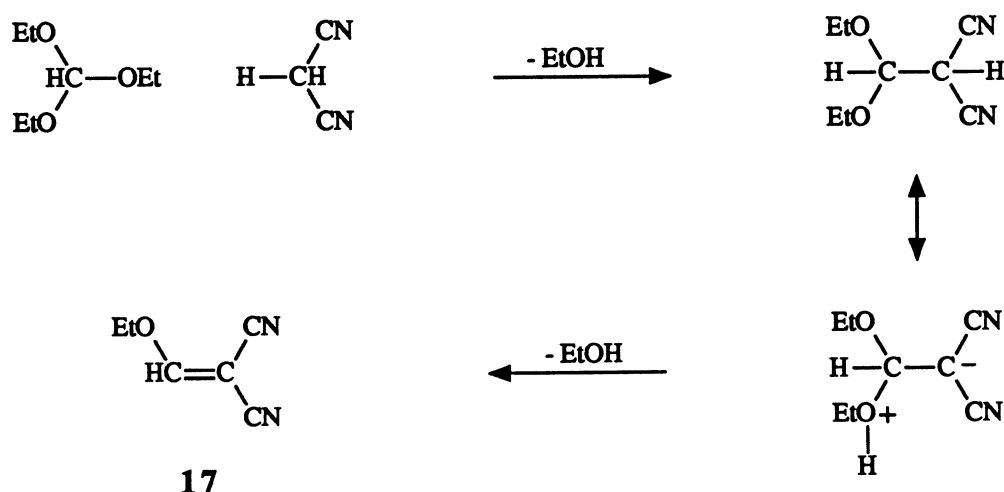
Scheme 4

The ortho ester is capable of becoming polarised, especially under the influence of an activated methylene compound. Nucleophilic attack of the acidic methylene group of malononitrile on the electropositive carbon of triethyl orthoformate, followed by the elimination of ethanol, produced an intermediate acetal. Subsequent elimination of a further molar equivalent of ethanol gave the product (17). As expected for an ionic reaction mechanism, the reaction rate was influenced by the addition of acid or base. It was found that strong base had a slight promoting effect.[50] This reaction was accompanied by the formation of some side products. In addition to ethanol, large quantities of diethyl ether and ethyl formate have been isolated.[50] Malononitrile and more

strongly acidic substances have been reported to catalyse the decomposition of triethyl orthoformate into diethyl ether and ethyl formate.[50-52]

Different conditions have been tested in order to improve the ease of purification and the overall yield of this reaction. Malononitrile and triethyl orthoformate were stirred in toluene at 100 °C with a catalytic amount of boron trifluoride. Distillation under reduced pressure gave a 70 % yield of ethoxymethylenemalononitrile. Identical conditions with zinc chloride gave a 74 % yield of ethoxymethylenemalononitrile. The use of toluene as the solvent allowed the removal of the ethanol via Dean-Stark apparatus. If an equilibrium was established that only allowed the reaction to proceed part of the way, the removal of ethanol would force this equilibrium towards product formation. The presence of a Lewis acid may have assisted the initial attack of malononitrile on triethyl orthoformate through the formation of a malononitrile/Lewis acid adduct or the subsequent loss of ethanol from the intermediate through the formation of an ethanol/Lewis acid adduct.

Malononitrile and triethyl orthoformate were stirred in acetic anhydride at 100 °C and then at 140 °C to remove volatile by-products (ethyl acetate and acetic acid). Distillation under reduced pressure gave a 93 % yield of ethoxymethylenemalononitrile. It has been suggested that the acetic anhydride reacted with ethanol (to form the ethyl acetate and acetic acid) and the removal of ethanol forced the equilibrium towards product formation.[53] It was later found that refluxing ethoxymethylene derivatives with ethanol produced no reaction. This suggested that the reaction was not a reversible, equilibrium process and that acetic anhydride must play a more involved part in the reaction.[50] A mechanism which better explained the role of acetic anhydride has been proposed.[54,55] This mechanism suggested that the triethyl orthoformate initially reacted with acetic anhydride to form diethoxymethyl acetate (Scheme 5). Alkylation by the active methylene

carbon of malononitrile produced an acetal. Elimination of ethanol yielded ethoxymethylenemalononitrile (17).
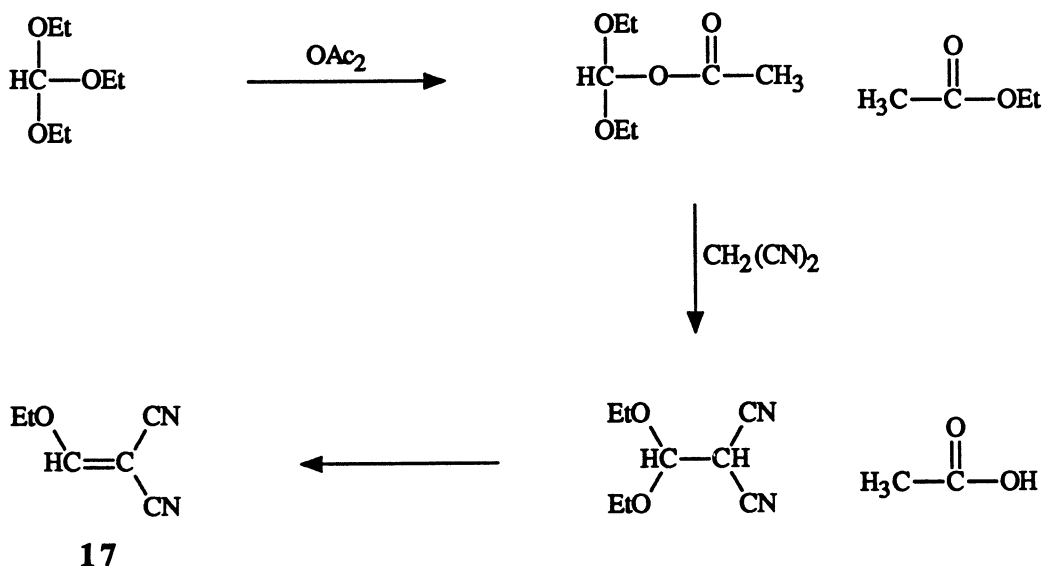
Scheme 5

[2.2] Synthesis of Hydrazines

The other starting material necessary for the preparation of 1-substituted 5-amino-4-cyanopyrazoles was a substituted hydrazine (18). Aryl hydrazines were prepared by the diazotisation and reduction of anilines.[56-59] The appropriate aniline was stirred with sodium nitrite in hydrochloric acid at 0 °C. The diazonium salt which resulted was stirred with sodium sulphite or stannous chloride in hydrochloric acid at room temperature. Sodium sulphite was used to reduce the diazonium salt of aniline, but was found to be unsuitable for the diazonium salts of substituted anilines.[57] Stannous chloride was used to reduce the diazonium salts of substituted anilines.[57-59] The crude hydrazine hydrochlorides were purified by recrystallisation and were neutralised with dilute base to yield free hydrazines. Alkyl hydrazines were prepared by the alkylation of hydrazine.[60] Anhydrous hydrazine was stirred with the appropriate alkyl halide at 0 °C. Although the

reaction was an exothermic process, the temperature was kept below 30 °C to limit bis-alkylation of the hydrazine. The crude hydrazines was purified by fractional distillation.

[2.3] Synthesis of 5-Amino-4-cyanopyrazoles

The condensation of ethoxymethylenemalononitrile (**17**) with substituted hydrazines (**18**) was attempted using the literature procedure.[48,49] The reactants were refluxed in a neutral solution of ethanol. Upon cooling, a solid precipitated and was purified by recrystallisation. A total of 18 pyrazoles with different substituents were prepared using this procedure. The yields for these reactions ranged from 33 % for the 2-nitrophenyl derivative (**10k**) to 82 % for the 2,5-dichlorophenyl derivative (**10r**).

Although no detailed studies have been performed on the mechanism of this reaction a proposed mechanism has been included. Hydrazines are strong nucleophiles which are known to add to α, β-unsaturated carbonyl compounds via a Michael-type addition. In this case, the β nitrogen of the hydrazine attacks the α carbon of the ethoxymethylenemalononitrile to produce a resonance stabilised anion. The oxygen of the ethoxy group could donate electrons producing a carbonyl-type centre at the α-carbon of the double bond (Scheme 6).
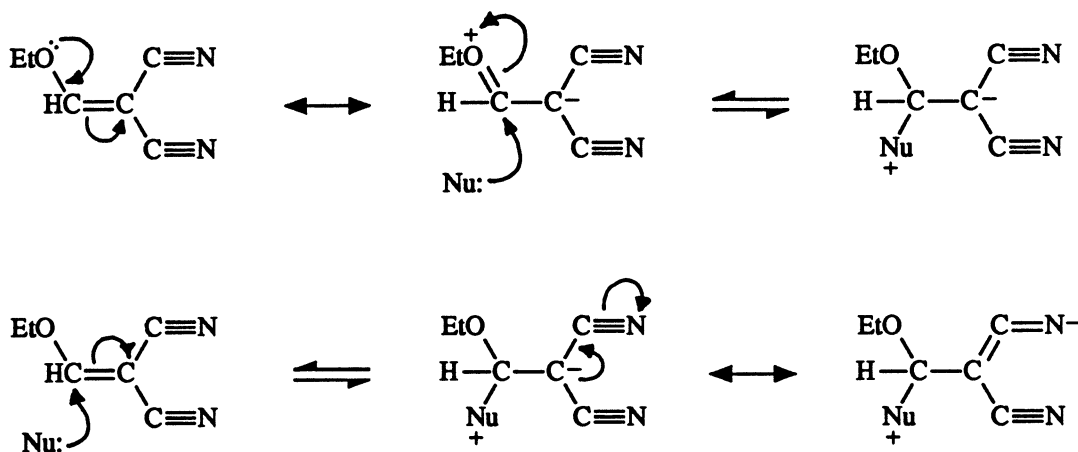
Scheme 6

This would increase the susceptibility of the α carbon to nucleophilic attack. The two nitrile groups possess strong electron withdrawing properties which would activate the α carbon of the double bond and stabilise the intermediate anion produced (Scheme 6). This would also increase the suseptibility of the α-carbon to nucleophilic attack. The resonance stabilised anion loses ethanol to produce a neutral intermediate (19, Scheme 7). This intermediate has not been isolated in this reaction. The α nitrogen of the hydrazine portion attacks a nitrile carbon of this intermediate to effect a facile intramolecular cyclisation. The presence of the second electron withdrawing nitrile group would be expected to favour this cyclisation. Tautomerism of the cyclised intermediate yields the 1-substituted 5-amino-4-cyanopyrazole (10).

The analogous condensation of ethoxymethylenemalonic ester with phenylhydrazine would be expected to proceed by a similar mechanism. In this case, the corresponding neutral intermediate (20) has been isolated and characterised.[61] The greater steric bulk and less electropositive nature of the carboxylic ester groups may hinder cyclisation, thus allowing this intermediate to be isolated.

$$NH—NH—CH{=}C\begin{smallmatrix} COOEt \\ \\ COOEt \end{smallmatrix}$$
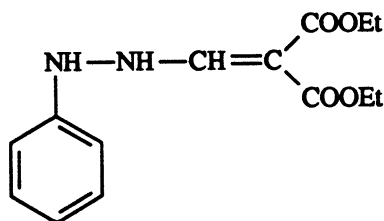
20

The condensation of ethoxymethylenemalononitrile with substituted hydrazines could conceivably produce a 1-substituted 3-amino-4-cyanopyrazoles, rather than 1-substituted 5-amino-4-cyanopyrazoles. For this to occur, the α nitrogen of the hydrazine must attack the α carbon of ethoxymethylenemalononitrile.

19

10
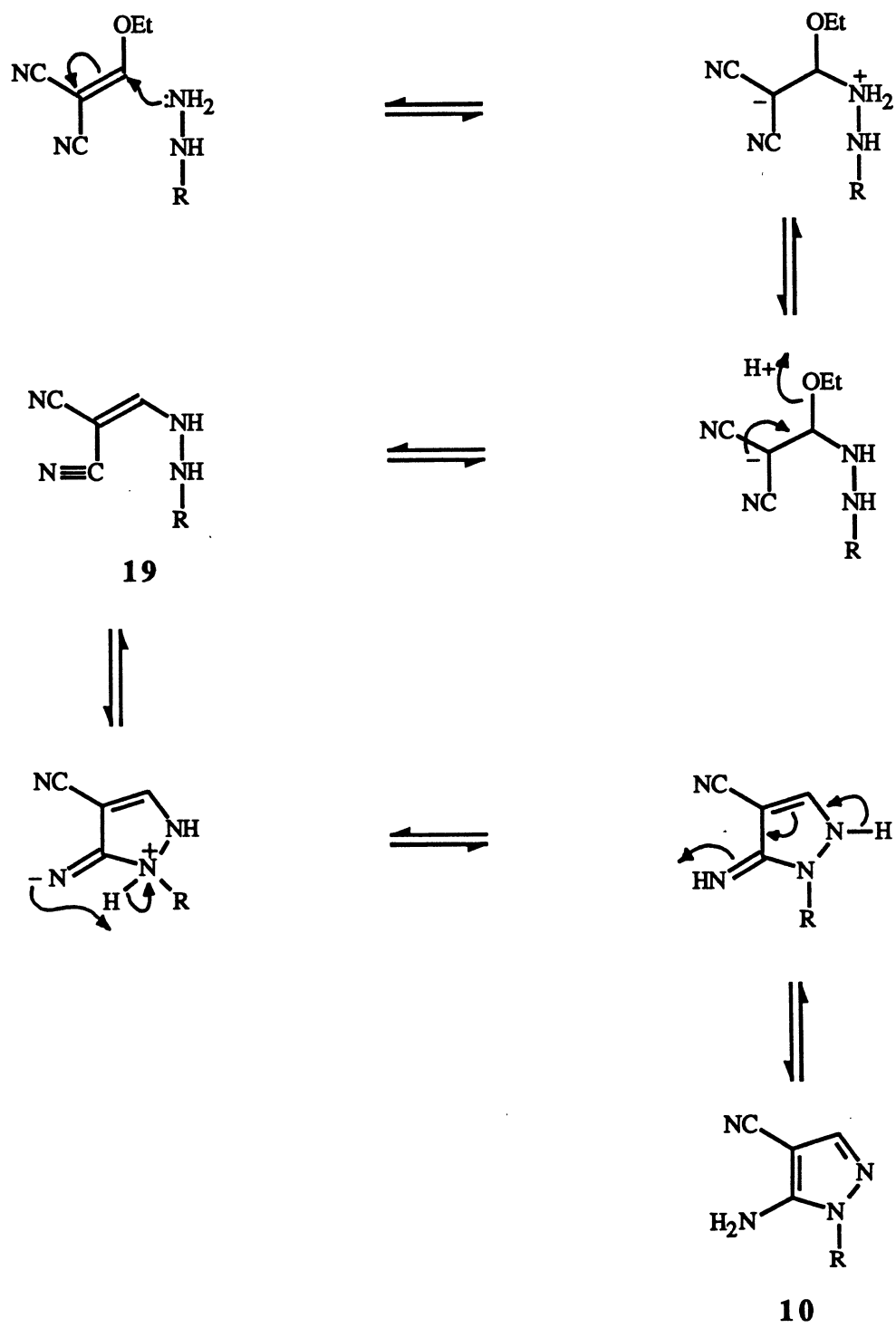
Scheme 7

This would be unlikely as the α nitrogen is less nucleophilic as a result of the electron withdrawing nature of the aryl substituents.[62] This is particularly true in the case of aryl substituents where the lone pair of electrons on the α nitrogen can be delocalised throughout the π system of the aromatic ring. The structure of pyrazoles produced by the reaction of ethoxymethylenemalononitrile and hydrazines has been investigated using an alternative synthesis.[47] This method involved the treatment of ethyl 5-hydroxy-1-phenylpyrazole-4-carboxylate with phosphorous oxychloride and then ammonia to produce 5-amino-1-phenylpyrazole-4-carboxamide. The treatment of 3 or 5-amino-4-cyano-1-phenylpyrazole with sulphuric acid converted the nitrile to a carboxamido group. As the products from both of these reactions were found to be identical, it was concluded that the original pyrazole must have been 5-amino-4-cyano-1-phenylpyrazole.

Pyrazole formation was invariably accompanied by the formation of some brightly coloured material. This intense colouration may be the result of the presence of azo-compounds. Phenylhydrazine has been shown to form azo-benzene by a radical mechanism.[63]

[2.4] Alternative Synthesis of 5-Amino-4-cyanopyrazoles

The condensation of ethoxymethylenemalononitrile and aniline produced anilinomethylenemalononitrile (21).[64] Interestingly, a one-pot reaction between malononitrile, triethyl orthoformate and aniline also yielded anilinomethylenemalononitrile (21).[65] The condensation of ethoxymethylenemalononitrile and phenylhydrazine is believed to produce an analogous nitrile (19a) which readily cyclises to yield 5-amino-4-cyano-1-phenylpyrazole. It was thought that a one-pot reaction between malononitrile, triethyl orthoformate and phenylhydrazine may also produce this nitrile (19a) which would cyclise to yield the 5-amino-4-cyano-1-phenylpyrazole.

NH—NH—CH=C<CN / CN

19a

NH—CH=C<CN / CN
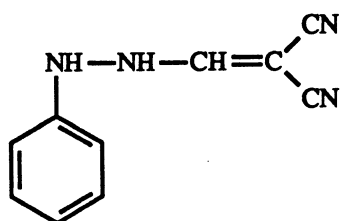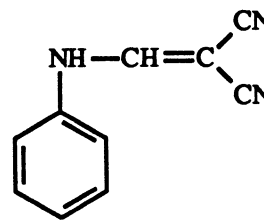
21

Malononitrile, triethyl orthoformate and phenylhydrazines were

refluxed in ethanol. Purification of the crude 5-amino-4-cyano-1-phenylpyrazole (**10a**)

by chromatography gave a 58 % yield. Chromatography was necessary for purification

as a result of the formation of a number of coloured by-products. Similiar problems in

the synthesis of ethoxymethylenemalononilrile were overcome by performing the reaction

in acetic anhydride. In this case, the use of acetic anhydride lowered the yield and

increased the number of by products. An identical one-pot reaction was used for

4-chlorophenylhydrazine and 2,4-dichlorophenylhydrazine. Recrystallisation of the

crude 5-amino-4-cyanopyrazoles (**10d** and **10q**) gave yields of 44 % and 51 %

respectively. The presence of fewer impurities allowed the crude products to be purified

by recrystallisation alone. Malononitrile, triethyl orthoformate and methylhydrazine were

refluxed in ethanol. Purification of the crude 5-amino-4-cyano-1-methylpyrazole (**10n**)

by multiple recrystallisations gave only a 17 % yield.

The mechanism of the one-pot reaction may initially involve the formation of

ethoxymethylenemalononitrile, followed by the formation of the 5-amino-4-

cyanopyrazole. Alternatively, the nucleophilic β nitrogen of the hydrazine may attack the

electron deficient carbon of triethyl orthoformate with the loss of ethanol to generate an

acetal (Scheme 8). The formation of a carbon to nitrogen double bond and the loss of

another equivalent of ethanol would produce an imidate (**22**). Aniline has been reported

to react with triethyl orthoformate to produce ethyl N-phenylformimidate.[66,67] The attack

of the methylene group of malononitrile on the electron deficient carbon with the loss of a further equivalent ethanol would form the intermediate (19). The cyclisation of this intermediate would yield the 5-amino-4-cyanopyrazole (10).

$$
\begin{array}{ccc}
\text{NHNH}_2 & \text{EtO—CH} & \xrightarrow{\text{-EtOH}} & \text{NHNH—CH} \\
| & | & & | \quad | \\
\text{R} & \text{OEt} & & \text{R} \quad \text{OEt}
\end{array}
$$
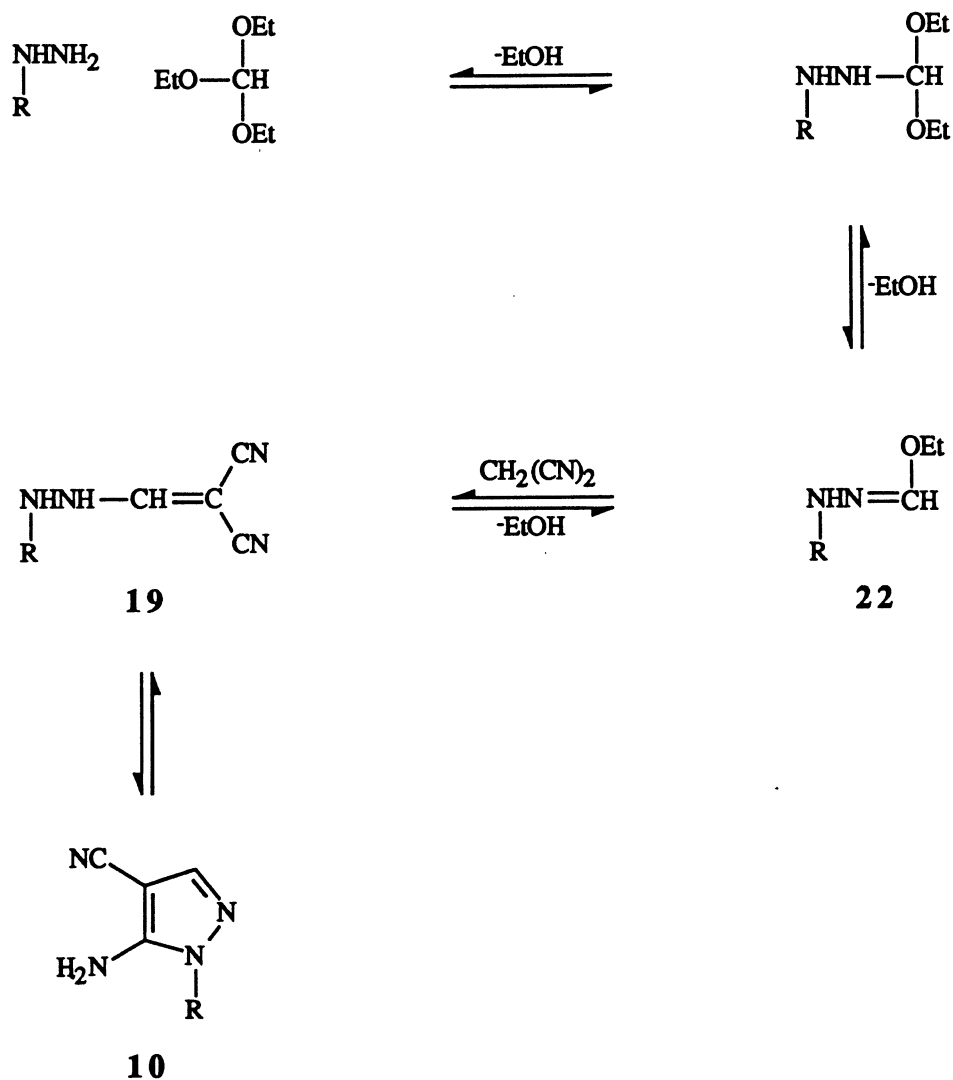
Scheme 8

A side reaction between malononitrile and the substituted hydrazine may have lowered the yield of the one-pot reaction. Malononitrile or malononitrile dimer has been reported to react with hydrazines to give 5-amino-4-cyano-3-cyanomethylpyrazoles.[68,69] Refluxing phenylhydrazine with malononitrile for 8 hours in ethanol gave a 38 % yield of 5-amino-4-cyano-3-cyanomethyl-1-phenylpyrazole. Refluxing methylhydrazine with

malononitrile for 15 minutes gave a 30 % yield of 5-amino-4-cyano-3-cyanomethyl-1-methylpyrazole.

The yields of the new one-pot reaction were compared with those of the established two step synthesis which utilised the best synthesis of ethoxymethylenemalononitrile (Table 4). In general, the reactions with aryl hydrazines produced comparable yields, while the reaction with the alkyl hydrazine produced a lower yield. The lower yield of the reaction with methylhydrazine may have resulted from greater competition from side reactions, such as the formation 5-amino-4-cyano-3-cyanomethyl-1-methylpyrazole.

Table 4   Comparative Yields of the 5-Amino-4-cyanopyrazoles

No.	R	One-Pot (%)	Two Step (%)
10a	C_6H_5	58	66
10d	C_6H_4Cl (4)	44	39
10q	$C_6H_3Cl_2$ (2,4)	51	64
10n	CH_3	17	80

CHAPTER THREE

1,5-Substituted Pyrazolo[3,4-*d*]pyrimidine
Analogues of 1-Methylisoguanosine

[3.0] General Introduction

The addition of *ortho*-aminonitriles to substituted isocyanates has been reported to yield 1-substituted 6-aminopyrimidin-2-ones.[70,71] The addition of the amine of the *ortho*-aminonitrile to the isocyanate produced a urea. The cyclisation of this intermediate urea with sodium methoxide yielded the 1-substituted 6-aminopyrimidin-2-one. The addition of 5-amino-4-cyano-1-β-(2',3',5'-tri-O-acetyl-D-ribofuranosyl)imidazole to methyl isocyanate also produced a urea. The cyclisation of the intermediate urea with methanolic ammonia yielded 1-methylisoguanosine.[31] This synthesis was modified through the use of different substituted isocyanates to obtain N-1 analogues of 1-methylisoguanosine.[40]

The synthesis of pyrazolo[3,4-*d*]pyrimidine analogues of 1-methylisoguanosine was attempted using 1-substituted 5-amino-4-cyanopyrazoles and substituted isocyanates (Scheme 9). The addition of the amine of the 5-amino-4-cyanopyrazole to the isocyanate proved to be the difficult step in this synthesis. This reaction was optimised using the 5-amino-4-cyano-1-phenylpyrazole/methyl isocyanate system. The cyclisation of the intermediate ureas with ammonium hydroxide proceeded smoothly. Two series of analogues were synthesised. The first involved variation of the N-1 substituent using different 1-substituted 5-amino-4-cyanopyrazoles, while the second involved variation of the N-5 substituent using different substituted isocyanates. A number of alkyl and aryl substituents were used.

The A_1 adenosine receptor affinity of the pyrazolo[3,4-*d*]pyrimidine analogues of 1-methylisoguanosine was measured using a [^{3}H] R-PIA competitive binding assay.[43] The most active substituents in the N-1 and N-5 positions were optimised and combined in an attempt to enhance the overall adenosine receptor affinity.
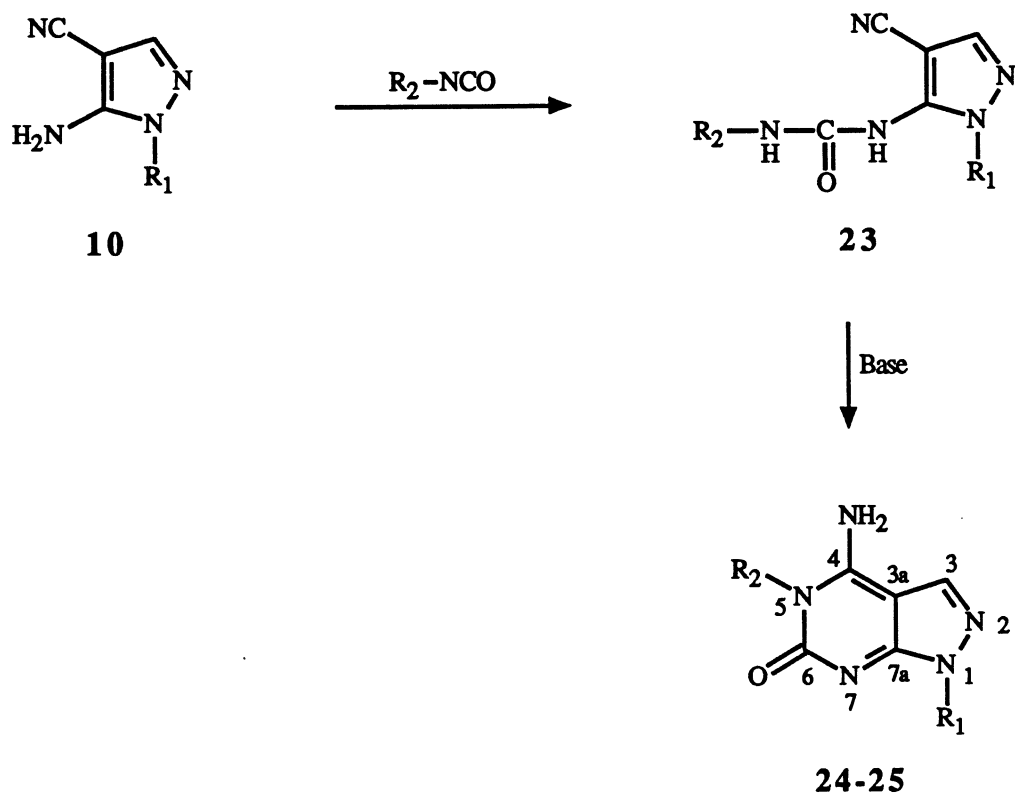
Scheme 9

[3.1] Synthesis of 4-Cyano-5-ureidopyrazoles

The addition of 5-amino-4-cyano-1-phenylpyrazole to methyl isocyanate was initially investigated using pyridine as a solvent. Pyridine has proved to be a useful solvent for similar isocyanate cyclisations involved in the preparation of C-nucleoside analogues of 1-methylisoguanosine.[72] The reactants were stirred at 100 °C in pyridine. A white solid precipitated and was found to be extremely insoluble in organic solvents. A suitable solvent for recrystallisation was not obtained so the solid was triturated in boiling DMSO. It was initially thought that the intermediate urea formed and underwent a facile cyclisation under the basic conditions to yield the required pyrazolo[3,4-*d*]pyrimidine. The IR spectrum showed the disappearance of the nitrile stretch at 2220 cm^{-1}. The NMR spectra were difficult to obtain as a result of the low solubility and the mass spectrum

showed no molecular ion. Further investigation indicated that the solid was actually a dimeric compound. Boiling in d^6-DMSO solubilised enough sample to obtain a ^1H NMR spectrum. The ^1H NMR spectrum contained a broad two proton singlet at δ 6.89 for amine protons. There was also a three proton doublet at δ 2.89 and a broad one proton singlet at δ 10.10 which may have resulted from the NHCH$_3$ protons of a urea. The other NH proton was not detected. The remainder of the spectrum contained two, two proton singlets at δ 8.07 and δ 8.65 for the C-3 protons of two pyrazole rings and a ten proton multiplet from δ 7.39 to 8.04 for the aromatic protons of two phenyl rings. The existence of an amine, a urea, two pyrazole rings and two phenyl rings is consistent with the dimer (28) containing a substituent urea.

A mechanism has been proposed for the dimerisation of *ortho*-aminonitriles.[73] In this case, the amine of one molecule of the 5-amino-4-cyano-1-phenylpyrazole may attack the nitrile of another to form an intermediate amidine (26, Scheme 10). A second intramolecular amine-nitrile addition produces a dimer. This product may contain a six or eight membered ring depending on which of the amines of the intermediate react with the remaining nitrile. As the amidine group is more basic, a six membered ring dimer (27) is generally formed. It has been suggested that an unfavourable equilibrium is involved in the amidine formation which is forced by the formation of a stable, highly aromatic 4-aminopyrimidine system. Experimentation on a number of dimerisations and mixed dimerisations has established that the critical factor in determining the ease of dimerisation is the susceptibility of the nitrile to nucleophilic attack, rather than the nucleophilicity of the amine.[74] A mechanism has also been proposed for the reaction of amines and isocyanates. In this case, an amine of the dimer may attack the carbonyl of methyl isocyanate to form a dimer with a urea substituent. Depending upon which of amines of the dimer react, the urea substituent may be on the C-5 position of the pyrazole fragment (28, R_1 = NH$_2$, R_2 = NHCONHCH$_3$) or the C-4 position of the

pyrazolo[3,4-*d*]pyrimidine fragment (**28**, R_1 = NHCONHCH$_3$, R_2 = NH$_2$). The

sequence of dimer and urea formation may be reversed. The amine of the pyrazole may

attack the carbonyl of methyl isocyanate to form an intermediate urea. The amine of a

second molecule of pyrazole could attack the nitrile of this urea to form a dimer with a

urea substituent on the C-5 position of the pyrazole fragment (**28**, R_1 = NH$_2$,

R_2 = NHCONHCH$_3$).
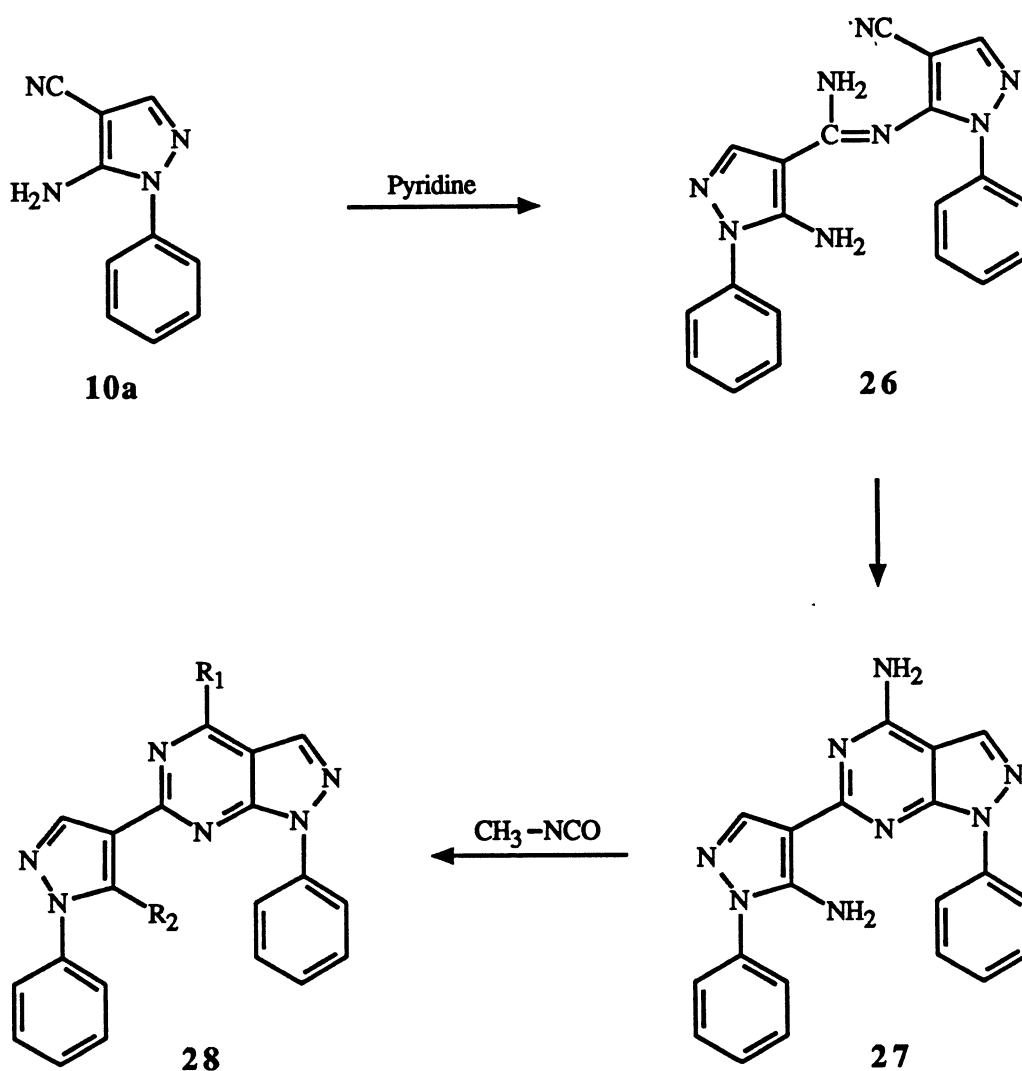
Scheme 10

5-Amino-4-cyano-1-phenylpyrazole has been reported to undergo a base catalysed

dimerisation in the absence of methyl isocyanate.[75] 4-Amino-6-[4-(5-amino-1-phenyl

pyrazolyl)]pyrazolo[3,4-*d*]pyrimidine (**27**) was formed by heating the pyrazole with methanolic ammonia in a bomb. The IR spectrum showed the disappearance of the nitrile stretch at 2220 cm^{-1}, while the UV spectrum contained the characteristic absorbances for a bicyclic ring system at 235 and 322 nm. Elemental analysis was consistent with the structure.

The addition of 5-amino-4-cyano-1-phenylpyrazole to methyl isocyanate was further investigated using modified conditions. The reactants were stirred at 80 °C in DMF. Evaporation of the solvent yielded a white solid which was recrystallised from ethyl acetate and methanol. This solid was found to be the required urea (**23a**). The IR spectrum showed that the nitrile stretch had been retained at 2220 cm^{-1}. The ^{1}H NMR spectrum contained an exchangable one proton singlet at δ 6.53 and a three proton doublet at δ 2.58 for the NHCH$_3$ protons of the urea. There was also an exchangable one proton singlet at δ 8.76 for the NH proton. The remainder of the spectrum contained a one proton singlet at δ 8.17 for the C-3 proton of the pyrazole ring and a five proton multiplet from δ 7.45 to 7.58 for the aromatic protons of the phenyl ring. The ^{13}C NMR spectrum contained a peak at δ 26.4 for the methyl carbon and a peak at δ 154.3 for the carbonyl carbon of the urea. There was also a peak at δ 113.4 for the nitrile carbon. The remainder of the spectrum contained peaks at δ 88.4 (C-4), 142.2 (C-3) and 142.5 (C-5) for the carbons of pyrazole ring and peaks at δ 124.4 (C-2', C-6'), 128.8 (C-4'), 129.4 (C-3', C-5') and 137.5 (C-1') for the carbons of the phenyl ring. These assignments were based on chemical shifts from the ^{13}C NMR spectrum and the multiplicity from the DEPT spectrum.

The mechanism of urea formation involves the nucleophilic attack of the amine nitrogen of the pyrazole on the carbonyl carbon of the isocyanate (Scheme 11). A detailed kinetic study of reactions between amines and isocyanates has suggested that this

initial nucleophilic attack generates a zwitterionic intermediate.[76,77] Proton transfer from

the attacking amine to the nitrogen of the isocyanate then produces the substituted urea.

The slow rate determining step appears to be the first step with strongly basic amines and

the second step with weakly basic amines.

23a
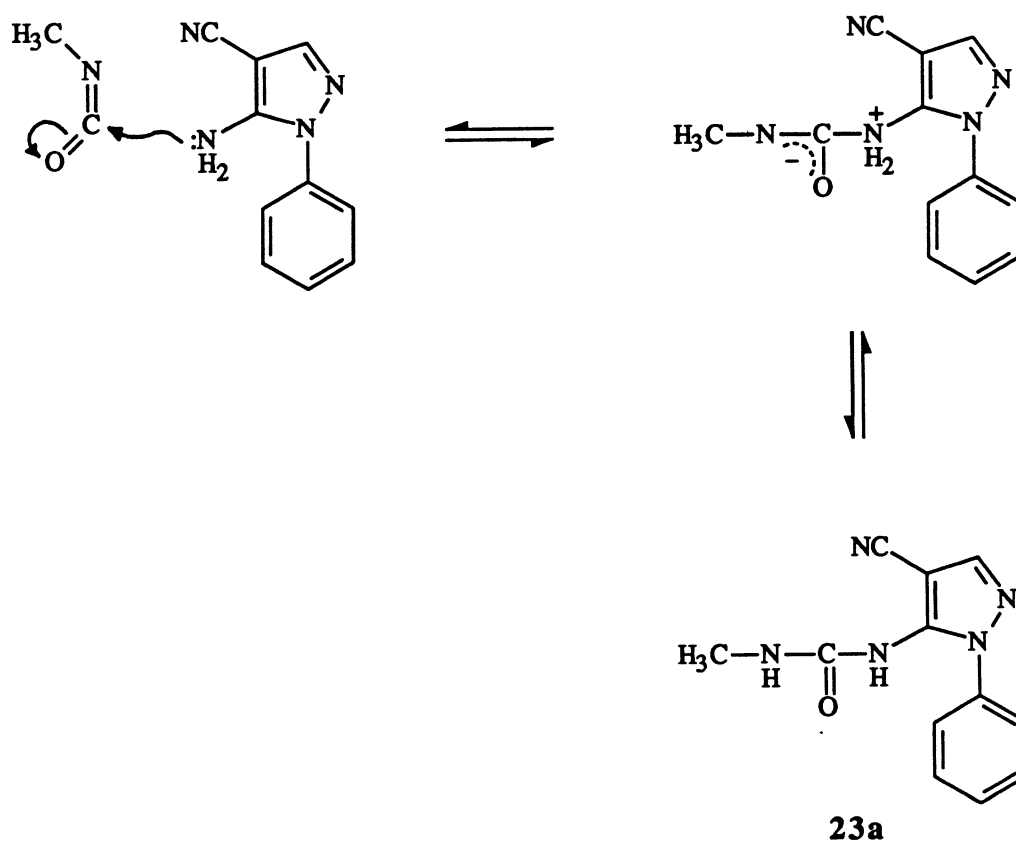
Scheme 11

The reactivity of the pyrazole is primarily governed by the basicity or nucleophilicity of

the amine moiety.[78] Aromatic amines have been found to be generally less nucleophilic

in nature than aliphatic amines. The lone pair of electrons on the nitrogen atom of

aromatic amines is less accessible for bonding as a result of delocalisation about the

aromatic ring.[62] The presence of the electron withdrawing substituents (such as nitriles)

further reduces the electron density around the amine nitrogen, resulting in a greater

decrease in nucleophilicity. Bulky substituents, particularly in the *ortho*-position, also

lower reactivity as a result of steric hindrance.[78] The reactivity of isocyanates can be understood by considering the electronic structure of the isocyanate moiety (Scheme 12).[78]

$$R-N=\overset{+}{C}-\overset{-}{O} \quad \longleftrightarrow \quad R-\overset{-}{N}-\overset{+}{C}=O \quad \longleftrightarrow \quad R-N=C=O$$
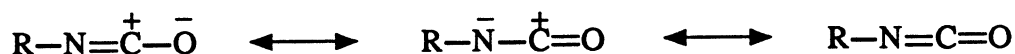
Scheme 12

The electron or charge density is greatest on the oxygen (highest net negative charge) and least on the carbon (highest net positive charge). The nitrogen atom is intermediate with a net negative charge. The nucleophilic attack on the carbonyl carbon of the isocyanate is controlled by these electronic factors. Any electron withdrawing group attached to the isocyanate group will increase the reactivity of the isocyanate to nucleophilic attack. Conversely, electron donating groups reduce the reactivity of the isocyanate moiety. Steric hindrance is also a consideration when bulky substituents are involved. Studies on reactions between amines and isocyanates have indicated that the substituent on the isocyanate functionality hinders the reaction to a greater extent than the same substituent of the amine functionality.[78]

A number of factors may have been responsible for urea formation being favoured over dimerisation under these conditions. The change of solvent from pyridine to DMF would have two main influences on the reaction. Since bases are known to be catalysts of the dimerisation process, the use of pyridine (pK_b = 8.6) may have facilitated the unwanted dimer formation. Since amides (along with carboxylic acids and ureas) are known to be catalysts of reactions between amines and isocyanates, the use of DMF may have facilitated the required urea formation.[79] The change of temperature from 100 °C to 80 °C would also have influenced the reaction. As dimerisation is known to be increased

by high temperatures and long reaction times, the use of a lower temperature would be

expected to favour the required urea formation.[73]

[3.2] Synthesis of 4-Aminopyrazolo[3,4-*d*]pyrimidin-6-ones

The intramolecular cyclisation of 4-cyano-5-(3-methylureido)-1-phenylpyrazole

was attempted using a modified literature procedure.[31] The reactant was stirred at room

temperature in a mixture of DMF and ammonium hydroxide. The DMF was included to

solubilise the urea. A white solid precipitated and was purified by recrystallisation from

DMSO and water. This solid was found to be the required pyrazolo[3,4-*d*]pyrimidine

(24a). The IR spectrum of the product showed the disappearance of the nitrile stretch at

2220 cm^{-1}. The ^{1}H NMR spectrum contained a three proton singlet at δ 3.37 for the

methyl protons and two exchangable one proton singlets at δ 8.26 and 8.94 for the amine

protons. The non-equivalence of the protons on amine nitrogen was caused by hindered

rotation around the C-N bond. Hindered rotation would result from the contribution of

other resonance forms imparting partial double bond character on the C-N bond

(Scheme 13).
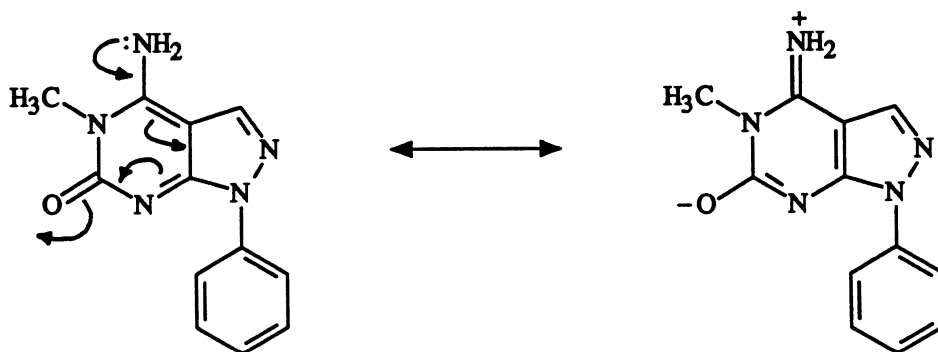
Scheme 13

The spectrum also contained a one proton singlet at δ 8.16 for the C-3 proton of the

pyrazolo[3,4-*d*]pyrimidine ring and a five proton multiplet from δ 7.21 to 8.16 for the

aromatic protons of the phenyl ring. The ^{13}C NMR spectrum contained a peak at δ 29.4

for the methyl carbon. There were also peaks at δ 93.1 (C-3a), 135.3 (C-3), 153.7,

154.7 and 155.6 for the carbons of the pyrazolo[3,4-d]pyrimidine ring. Due to the

similarity of the chemical shifts of the quaternary carbons at δ 153.7, 154.7 and 155.6, a

conclusive assignment of C-4, C-7a and C-6 could not be made. The remainder of the

spectrum contained peaks at δ 119.9 (C-2', C-6'), 125.0 (C-4'), 128.7 (C-3', C-5') and

139.5 (C-1') for the carbons of the phenyl ring.

24a
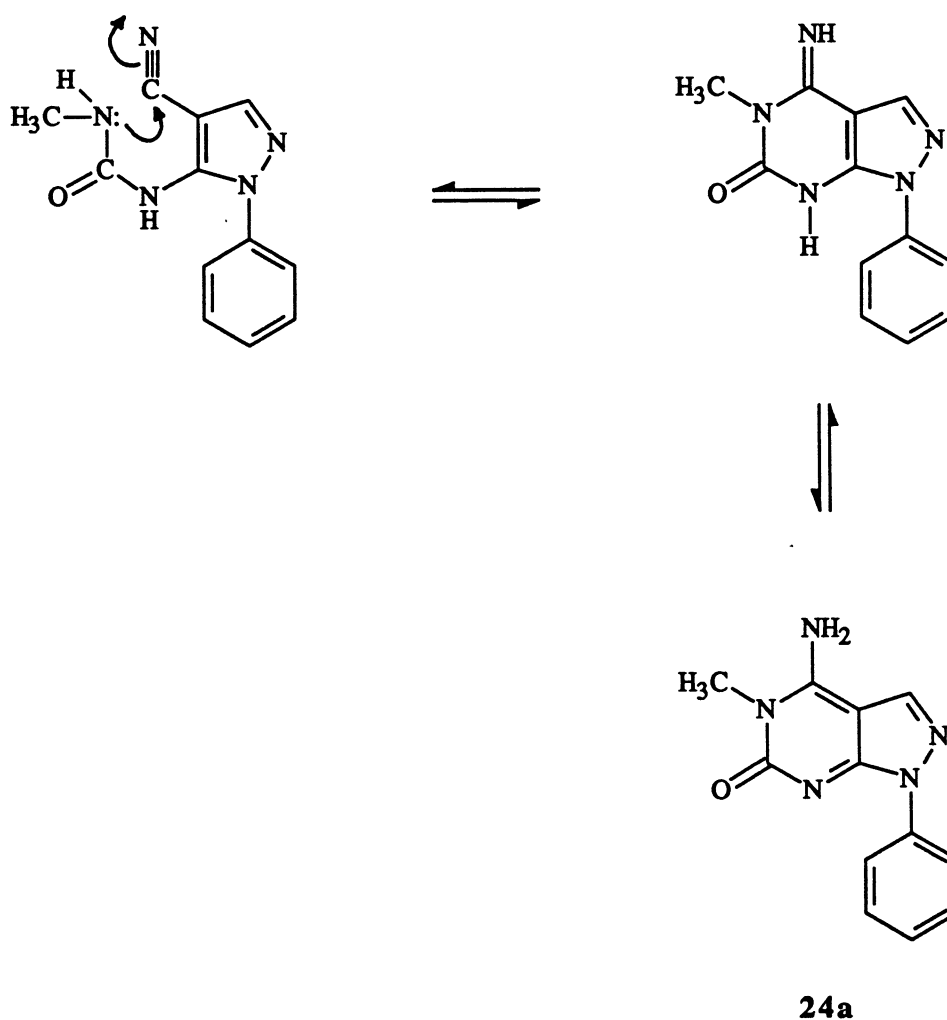
Scheme 14

The mechanism of pyrimidine formation involves the nucleophilic attack of the

amine of the urea on the electron deficient carbon of the nitrile (Scheme 14). This

generates an iminopyrimidine which undergoes a tautomerism to form the stable aminopyrimidine. This intramolecular cyclisation is an example of a Thorpe-Zeigler type reaction. The Thorpe-Zeigler reaction is basically an internal aldol condensation in which a dinitrile is converted to a cyclic enaminonitrile.[80,81] The α carbon of one nitrile is added to the nitrile carbon of another. In our case, the reaction involved the attack of the nucleophilic nitrogen of a urea rather than an α-carbon of a nitrile.

The synthesis of 4-aminopyrazolo[3,4-d]pyrimidin-6-ones was attempted in a one step reaction between 5-amino-4-cyanopyrazole and methyl isocyanate in the presence of base. The reactants were stirred at 60 °C with sodium methoxide in DMF or at -70 °C with n-butyllithium in THF. Neutralisation with dilute hydrochloric acid and evaporation of the solvent gave a white solid which was recrystallised from DMSO and water. This solid was found to be identical to the previous product. This cyclisation has a number of advantages over the two step synthesis:

(1) The one step cyclisation was more convenient to perform as it only required the isolation, purification and characterisation of one product. The two step synthesis required this work to be duplicated.

(2) The one step cyclisation produced a 92 % yield when using sodium ethoxide and a 98 % yield when using n-butyllithium. The two step synthesis produced a 73 % yield for the initial addition of the pyrazole to methyl isocyanate and a 90 % yield for the intramolecular cyclisation of the intermediate urea. The overall yield was only 66 %.

(3) In the one step cyclisation, the intermediate urea underwent an immediate cyclisation which reduced the possibility of undesirable side reactions. The two step synthesis required the urea to be isolated. This increased the probability of the urea reacting with a second equivalent of pyrazole to form a dimer or a second equivalent of methyl isocyanate to form a bis-urea.

The magnitude of these advantages were amplified by the necessity to repeat this reaction many times in order of to prepare a number of different analogues for structure/activity studies.

Once the conditions had been optimised, the reaction was performed using a variety of substituted pyrazoles and isocyanates. The sodium ethoxide/DMF method was used to convert all of the 5-amino-4-cyanopyrazoles to 4-aminopyrazolo[3,4-d]pyrimidin-6-ones. The use of differently substituted pyrazoles allowed variation of the N-1 substituent in the product. Methyl and propyl were the aliphatic groups, while chloro, bromo, fluoro and nitrophenyl were the aromatic groups examined in this position. The reduction of the nitrophenyl groups using palladium under a hydrogen atmosphere [82] gave the corresponding aminophenyl groups. The use of different isocyanates allowed the variation of the N-5 substituent. Methyl, ethyl, propyl, butyl and phenyl were the groups examined in this position. Different groups from the N-1 and N-5 position were combined. This optimisation was guided by the biological activity.

[3.2] Biological Results

The A_1 adenosine receptor affinity was measured using a [^{3}H] R-PIA competitive binding assay. The aromatic groups generally showed greater adenosine affinity than aliphatic groups in the N-1 position (Table 5). Upon examination of the compounds which contained mono-substituted aromatic groups, two main trends emerged. Firstly, substitution of the aromatic group with chlorine produced the greatest adenosine receptor affinity. Substitution with bromine and fluorine produced a moderate response, while substitution with nitro and amine produced little or no response (chlorine > bromine, fluorine >> nitro, amino substitution). Secondly, substitution of the aromatic group in the C-3' position produced the greatest adenosine receptor affinity. Substitution in the

C-4' position produced a moderate response, while substitution in the C-2' position produced little or no response (*meta* > *para* >> *ortho*-substitution). The 3-chlorophenyl group (24c) was the most active N-1 substituent with an IC_{50} of 1.65×10^{-5} M. Similarly, the 3-chlorophenyl group has been reported to be the most active substituent in the corresponding N-9 position in a series of 7-deaza-7,8-dimethyl-9-methyl adenines.[30] This study included chloro, bromo and methoxyphenyl analogues and also measured A_1 adenosine receptor affinity using a [^{3}H] R-PIA competitive binding assay. As the 3-chlorophenyl group showed the greatest activity, a number of di-chlorophenyl substituted compounds were examined. Generally, the addition of a second chlorine reduced the adenosine receptor affinity. The 3,5-dichlorophenyl compound (24t) contained two *meta*-chlorines and was the most active di-chlorophenyl analogue.

The aliphatic groups showed greater adenosine receptor affinity than the aromatic group in the N-5 position (Table 6). Of the aliphatic groups, the ethyl, propyl and butyl substituents were all found to have greater adenosine receptor affinity than the methyl substituent (butyl > propyl, ethyl > methyl substitution). The butyl group was the most active N-5 substituent with an IC_{50} of 0.96×10^{-5} M. A similar trend was apparent for xanthine antagonists.[83] The addition of a methyl substituent to the corresponding N-1 position resulted in an increase in A_1 adenosine receptor affinity. Increases in the length of 1,3-dialkyl substituents from methyl to propyl resulted in a 20 fold increase in receptor affinity. Receptor affinities were measured using a [^{3}H] CHA competitive binding assay. In contrast, the methyl group was reported to be the most active N-1 substituent in a series of isoguanosines.[40] This study included methyl, ethyl, propyl and butyl analogues and analysed skeletal muscle relaxant, hypothermic, cardiovascular, antiinflammatory and antiallergic effects following oral and intravenous administration to mice. This variation in the order of potency may be result of differences in adsorbtion, metabolism and penetration to the target organs in the *in vivo* procedures.[83]

Table 5 Adenosine Receptor Affinity and Hydrophobicity Index of the 1-Substituted 4-Amino-5-methylpyrazolo[3,4-d]pyrimidin-6-ones

No.	R_1	R_2	% I^a	IC_{50}^b	k'^c
24a	C_6H_5	CH_3	30		2.6
24b	C_6H_4Cl (2)	CH_3	7		1.7
24c	C_6H_4Cl (3)	CH_3	53	1.65 ± 0.40	14.2
24d	C_6H_4Cl (4)	CH_3	37		13.6
24e	C_6H_4Br (2)	CH_3	6		1.8
24f	C_6H_4Br (3)	CH_3	40		17.5
24g	C_6H_4Br (4)	CH_3	34		18.9
24h	C_6H_4F (2)	CH_3	8		1.1
24i	C_6H_4F (3)	CH_3	44		5.8
24j	C_6H_4F (4)	CH_3	26		4.1
24k	$C_6H_4NO_2$ (2)	CH_3	5		0.9
24l	$C_6H_4NO_2$ (3)	CH_3	2		>36.5
24m	$C_6H_4NO_2$ (4)	CH_3	5		>36.5
24n	$C_6H_4NH_2$ (2)	CH_3	0		0.8
24p	$C_6H_4NH_2$ (3)	CH_3	0		0.7
24q	$C_6H_4NH_2$ (4)	CH_3	0		0.3
24r	$C_6H_3Cl_2$ (2,4)	CH_3	9		7.1
24s	$C_6H_3Cl_2$ (2,5)	CH_3	1		4.8
24t	$C_6H_3Cl_2$ (3,5)	CH_3	29		>36.5
24u	CH_3	CH_3	6		0.1
24v	C_3H_7	CH_3	5		0.8

[a]% Inhibition of [^{3}H] R-PIA binding to rat brain membranes. [b]Concentration (x 10^{-5} M) required to inhibit 50 % of [^{3}H] R-PIA binding to rat brain membranes. Calculated for all of the compounds which showed greater than 50 % inhibition of [^{3}H] R-PIA binding. [c]Hydrophobicity index measured by reverse phase HPLC.

The most active N-1 and N-5 substituents were combined in an attempt to optimise the overall activity. The combination of the 3-chlorophenyl group in the N-1 position with the butyl group in the N-5 position produced a compound with increased adenosine receptor affinity. The IC_{50} of the optimised compound was reduced by a factor of 2 to 3 relative to compound (24c). The combination of the 3-chlorophenyl group in the N-1 position with ethyl and propyl groups in the N-5 position also produced compounds with increased adenosine receptor affinity.

Table 6 Adenosine Receptor Affinity and Hydrophobicity Index of the
5-Substituted 4-Amino-1-phenylpyrazolo[3,4-d]pyrimidin-6-ones

No.	R_1	R_2	% I^a	$IC_{50}{}^b$	k'^c
24a	C_6H_5	CH_3	30		2.6
25a	C_6H_5	C_2H_5	58	2.01 ± 0.18	4.2
25b	C_6H_5	C_3H_7	52	5.07 ± 0.21	7.5
25c	C_6H_5	C_4H_9	72	0.96 ± 0.33	18.5
25d	C_6H_5	C_6H_5	25		5.1
24c	C_6H_4Cl (3)	CH_3	53	1.65 ± 0.40	14.2
25e	C_6H_4Cl (3)	C_2H_5	70	1.98 ± 0.72	21.9
25f	C_6H_4Cl (3)	C_3H_7	68	1.46 ± 0.50	>36.5
25g	C_6H_4Cl (3)	C_4H_9	88	0.64 ± 0.10	>36.5

a, b, c Refer to Table 5

[3.3] Structure/Activity Relationships

Structure/activity studies attempt to quantify the relationship between chemical structure and biological activity.[84] This approach assigns parameters to the various chemical groups used to modify the chemical structure of a compound. The parameter is a measure of the potential contribution of its group to the biological activity of the parent compound. The types of parameters which are most commonly used are steric and electronic parameters and parameters which relate to solubility. As our series of compounds predominantly contained a number of different phenyl substituents, it was thought the electronic and solubility parameters would impart the greatest influence on biological activity.

Electronic effects are commonly examined using Hammett constants (σ).[84] The Hammett constant was originally defined to quantify the effect of a substituent on the dissociation constant of benzoic acid.

$$\sigma = \log [K_X/K_H]$$

K_X is the dissociation constant of x-substituted benzoic acid and K_H is the dissociation constant of unsubstituted benzoic acid. Both inductive and resonance effects of aromatic substituents are represented by σ values. Electron withdrawing substituents increase dissociation and have a positive σ value. Electron donating groups decrease dissociation and have a negative σ value. *Ortho*-substituents are subject to too many influences and are generally not considered. *Meta* and *para*-substituents have a differing effect on dissociation and have different σ values. The Hammett constant (σ) is widely used to quantify the relationship between the electron density of a substituent with the biological activity of the compound.

In our case, Hammett constants were used to investigate the electronic effects of the different phenyl substituents in the N-1 position of the 4-amino-pyrazolo[3,4-d]pyrimidines on the adenosine receptor affinity (Figure 2). The adenosine receptor affinity increased to a maximum and then decreased in a linear fashion with increasing σ values. The maximum adenosine receptor affinity corresponded to a σ value of 0.37.
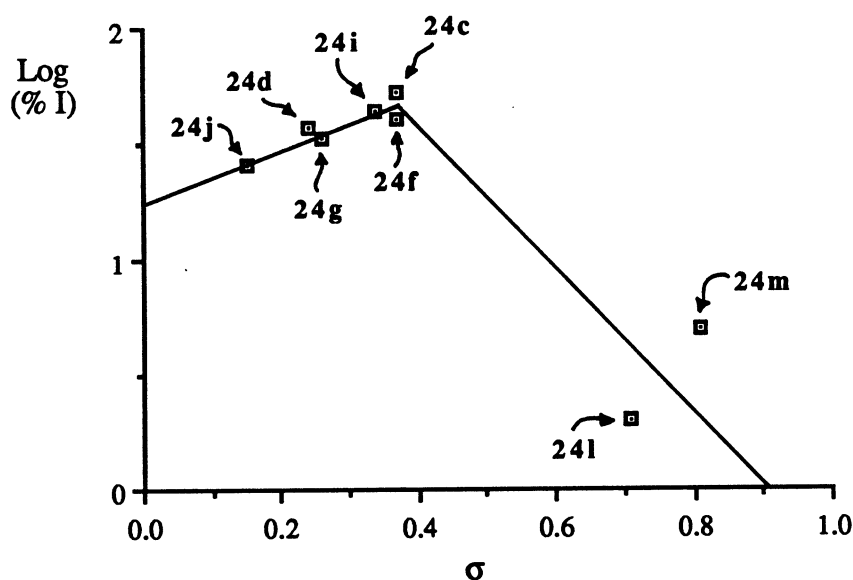
Figure 2   The Relationship between Electronic Effects (Hammett constants) and Adenosine Receptor Affinity (% Inhibition of [^{3}H] R-PIA binding)

Solubility effects are commonly examined using the hydrophobicity index (k'). The hydrophobicity index has been obtained using a HPLC correlation method.[85,86] This method measured the compounds partition between the non-polar octadecylsilyl stationary phase and the polar methanol/aqueous buffer moving phase. The hydrophobicity index of a series of N^6-alkyl adenosines has been determined by this method and used to correlate plasma binding.[87]

No general relationships emerged from the comparison of the hydrophobicity index with adenosine receptor binding for all of the 4-aminopyrazolo[3,4-d]pyrimidin-6-ones. However, when the compounds were broken up into various series, certain trends became apparent. The first series was comprised of the compounds which contained a mono-substituted aromatic group in the N-1 position and a methyl group in the N-5 position. Plotting receptor affinity against hydrophobicity produced a simple curve (Figure 3). As the hydrophobicity index increased, the binding affinity increased to a maximum (k' = 14.2) and then declined. Two of the nitro-substituted compounds were found to have k' values greater than 36.5 and are not shown on the graph. The biological activity of these compounds was low, consistent with the downward trend in activity with high hydrophobicity. The second series was comprised of the compounds which contained a phenyl group in the N-1 position and an aliphatic group in the N-5 position. Plotting receptor affinity against hydrophobicity also produced a simple curve (Figure 4). As the hydrophobicity index increased, the binding affinity increased to a maximum (k' = 18.5). Unfortunately, the unavailability of longer alkyl isocyanates prevented further investigation of this trend.

The hydrophobicity of a compound can influence the biological activity in two ways. A compound may have to cross a series of membranes in order to travel from the point of administration to the site of action. The hydrophobicity of the compound would influence the passage over these hydrophilic and hydrophobic barriers. At the site of action the compound may have to bind to a receptor site. The hydrophobicity of the compound would influence the interactions with certain areas of the receptor. The biological activity of the 4-aminopyrazolo[3,4-d]pyrimidin-6-ones was evaluated using receptor binding techniques. In this case the passage to the site of action would become less important. Relationships between hydrophobicity and receptor binding must result from hydrophobic interactions with the receptor.
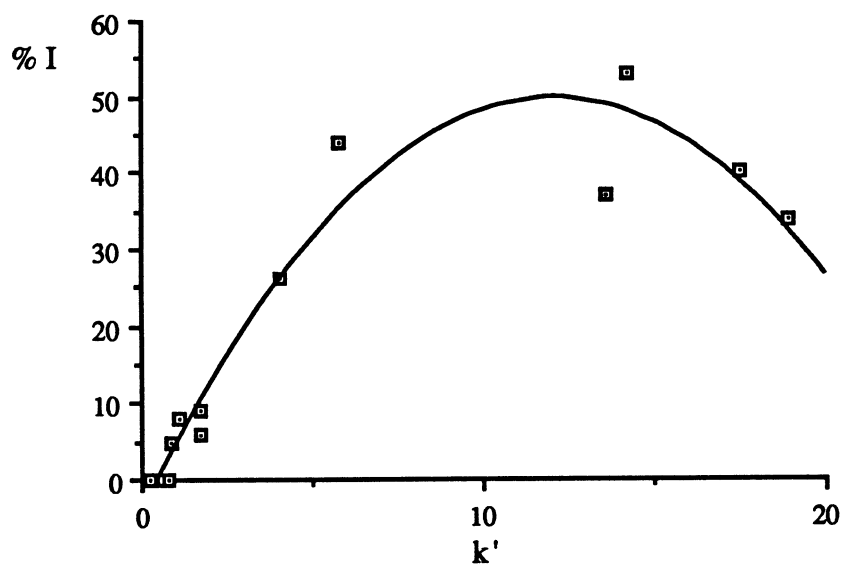
Figure 3 The Relationship between Solubility Effects (Hydrophobicity Index) and Adenosine Receptor Affinity (% Inhibition of [^{3}H] R-PIA binding)
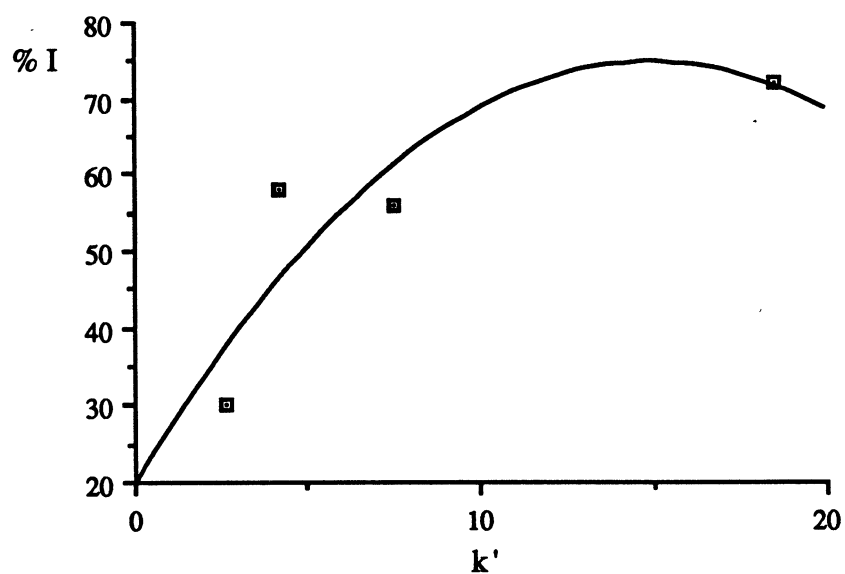
Figure 4 The Relationship between Solubility Effects (Hydrophobicity Index) and Adenosine Receptor Affinity (% Inhibition of [^{3}H] R-PIA binding)

Hydrophobic interactions were examined in the areas occupied by the N-1 and the N-5 substituents. These results relate to the effects of hydrophobicity on these two localised regions of the receptor protein. Combining the groups which produced a maximum receptor affinity in the N-1 and the N-5 positions produced a compound with high hydrophobicity ($k' > 36.5$). Although the hydrophobicity in the N-1 and N-5 regions was optimised the overall hydrophobicity of the molecule was increased.

CHAPTER FOUR

1-Substituted Pyrazolo[3,4-*d*]pyrimidine
Analogues of Isoguanosine

[4.0] General Introduction

4-Aminopyrazolo[3,4-d]pyrimidin-6-ones with no substitution in the N-5 position are pyrazolo[3,4-d]pyrimidine analogues of isoguanosine. Isoguanosine (29) is a naturally occurring nucleoside which was originally isolated from the croton bean (*Croton tiglium L.*).[88] This compound has been reported to possess a number of biological activities. Isoguanosine is incorporated into mammalian, but not bacterial nucleic acids.[89,90] It stimulates the accumulation of cAMP in the brain[91] and acts as an inhibitor of IMP: pyrophosphorylase.[92] Isoguanosine 5'-di and 5'-triphosphates bind strongly and inhibit glutamic acid dehydrogenase.[93]

A number of different syntheses of isoguanosine have been reported.[94-97] The amination of 2-hydroxy-6-methylthio-9-β-(D-ribofuranosyl)purine with ammonia[94] and the deamination of 2,6-diamino-9-β-(D-ribofuranosyl)purine with nitric acid[95] have both been shown to yield isoguanosine. The photolysis of adenosine N'-oxide[96] and 2-iodoadenosine[97] have also produced this compound. These syntheses required a number of steps to prepare the starting material and were subject to low overall yields.

Recently, two new syntheses of isoguanosine have been reported.[98,99] Both of these syntheses involved the addition of 5-amino-4-carbamoyl-1-β-D-ribofuranosylimidazole (5) to benzoyl isothiocyanate to produce an intermediate thiourea (30, Scheme 15). The alkylation of this thiourea with methyl iodide generated an intermediate methylthiourea (31).[98] The cyclisation of this methylthiourea with dilute sodium hydroxide yielded isoguanosine (29). Alternatively, the desulphurisation of the thiourea (30) with DCC generated an intermediate urea (32).[99] The cyclisation of this urea with ethanolic ammonia yielded isoguanosine (29). This synthesis proceeded under milder conditions and gave a higher yield (68 %) of isoguanosine.
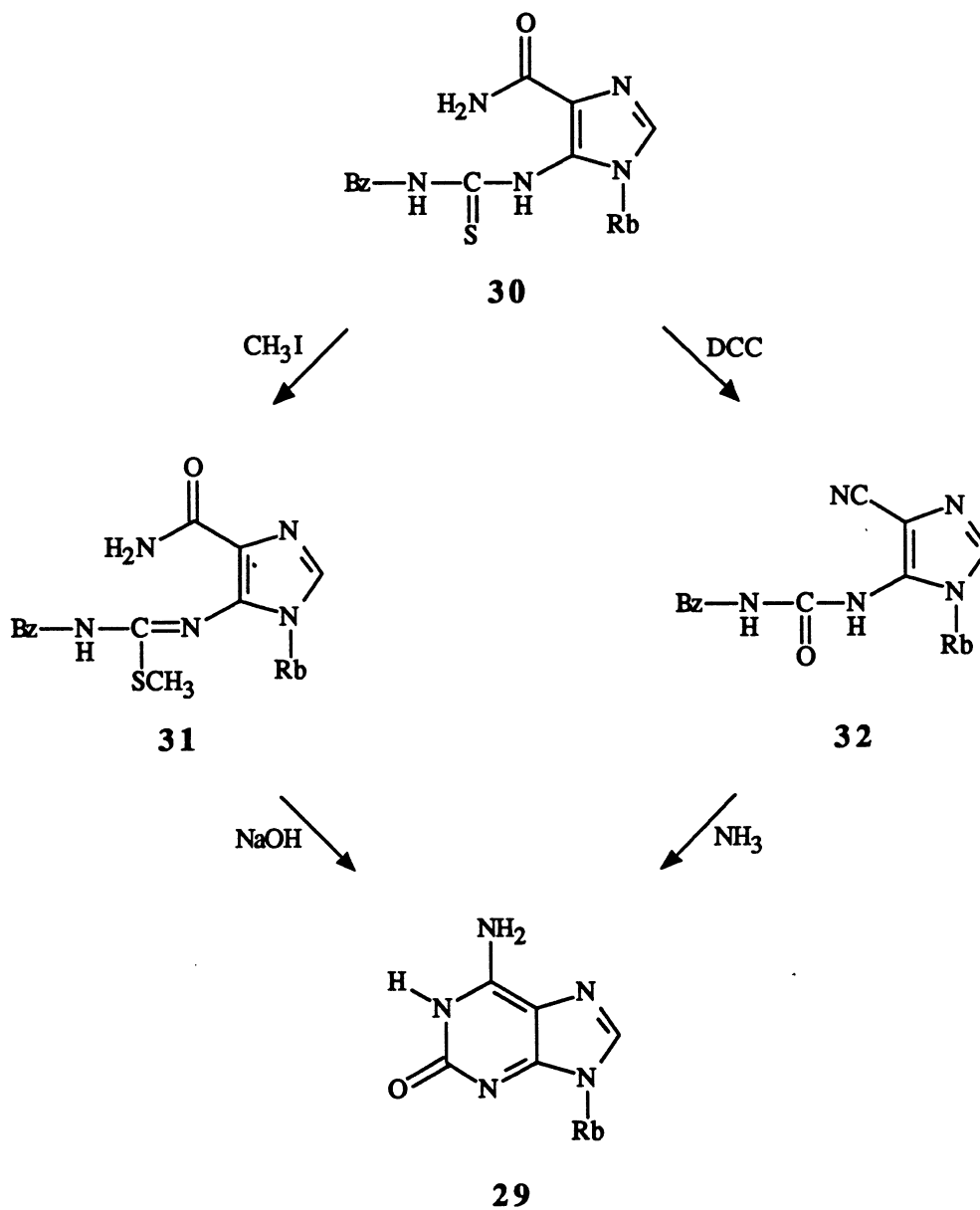
Scheme 15

In this study, pyrazolo[3,4-*d*]pyrimidine analogues of isoguanosine were prepared using this cyclodesulphurative method. Attempts to use a direct pyrazole/isocyanate cyclisation using potassium cyanate or trimethylsilyl isocyanate were unsuccessful.

[4.1] Synthesis of Isoguanosine Analogues using Isocyanic Acid

In order to prepare pyrazolo[3,4-d]pyrimidine analogues of isoguanosine by our pyrazole/isocyanate method it would be necessary to use unsubstituted isocyanate (isocyanic acid, H-N=C=O). Unfortunately, isocyanic acid is not commercially available as it polymerises rapidly when stored as a solution or gas at room temperature.[100] The most common preparation of isocyanic acid involves the depolymerisation of cyanuric acid. Heating cyanuric acid tends to result in sublimation rather than reaction. In order to achieve depolymerisation, heat must be applied to produce a vapour and then the resulting vapour must be heated still further. Cyanuric acid was volatised at 450 °C and then the gas stream was passed through a tube furnace at 700 °C. The crude isocyanic acid was collected in a cold trap at -80 °C. After oxidation with silver oxide (to remove any HCN), drying over phosphorous pentoxide and distillation at -30 °C, the monomeric isocyanic acid was greater than 99.5 % pure. Other workers have used similar procedures, but have collected the crude isocyanic acid in an organic solvent. The preparation of isocyanic acid requires harsh conditions and the use of specialised equipment.

The problem of obtaining isocyanic acid has been overcome by using it as a metal salt. Sodium, potassium and silver salts of isocyanic acid have been commonly used in reactions with ammonia and amines.[101,102] 5-Amino-4-cyano-1-phenylpyrazole and potassium cyanate were stirred at 80 °C in DMF. Evaporation of the solvent produced only starting material. The low solubility of the potassium cyanate in DMF may have inhibited the reaction. The reaction was repeated in water in order to overcome this solubility problem. 5-Amino-4-cyano-1-phenylpyrazole and potassium cyanate were refluxed in water. Upon cooling a white solid crystallised from solution. Column chromatography allowed the separation and purification of 5-amino-1-phenylpyrazole-4-

carboxamide (33, Scheme 16) and unreacted starting material. The IR spectrum showed that the nitrile stretch at 2220 cm^{-1} had been replaced by a carbonyl stretch at 1660 cm^{-1}. The ^{1}H NMR spectrum contained two additional one proton singlets at δ 6.85 and 7.40 for the amide protons. The ^{13}C NMR spectrum showed that the nitrile carbon at δ 114.7 had been replaced by a carbonyl carbon at δ 166.2. This structural assignment was confirmed by comparison of the product with a sample prepared by literature procedures.[47]

KOCN, H$_2$O

10a   **33**
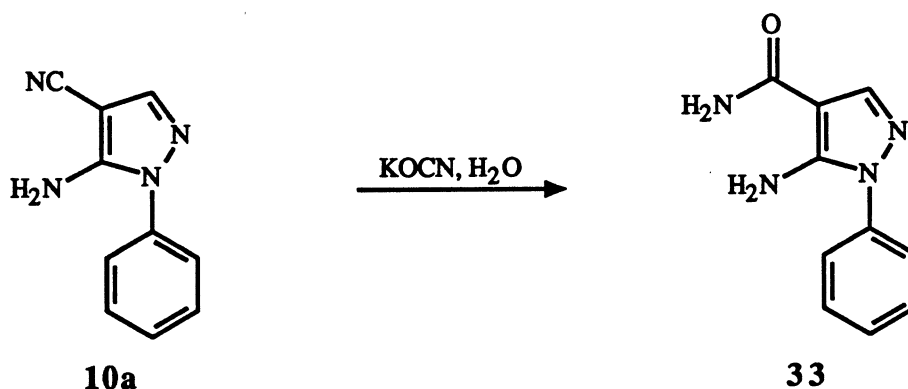
Scheme 16

Under these conditions, hydrolysis of the nitrile was favoured over urea formation at the amine. Nitriles can be readily hydrolysed under acidic or basic conditions to form amides.[103] In this case, the mechanism initially involves the dissociation of the potassium cyanate to potassium and cyanate ions. The basic cyanate ion (conjugate base of cyanic acid, pK$_a$ = 3.7) abstracts a proton from a water molecule to produce a hydroxide ion. Hydroxide catalysed hydrolysis of nitriles is well documented.[103] Nucleophilic attack of the hydroxide ion on the nitrile carbon, followed by protonation of the nitrile nitrogen, generates an intermediate imidate. Tautomerism of this imidate produces the amide (Scheme 17). Hydrolysis of the nitrile may be prevented by maintaining a neutral pH throughout the reaction. This was not attempted due to the

simultaneous success of other methods of producing pyrazolo[3,4-*d*]pyrimidine

analogues of isoguanosine.
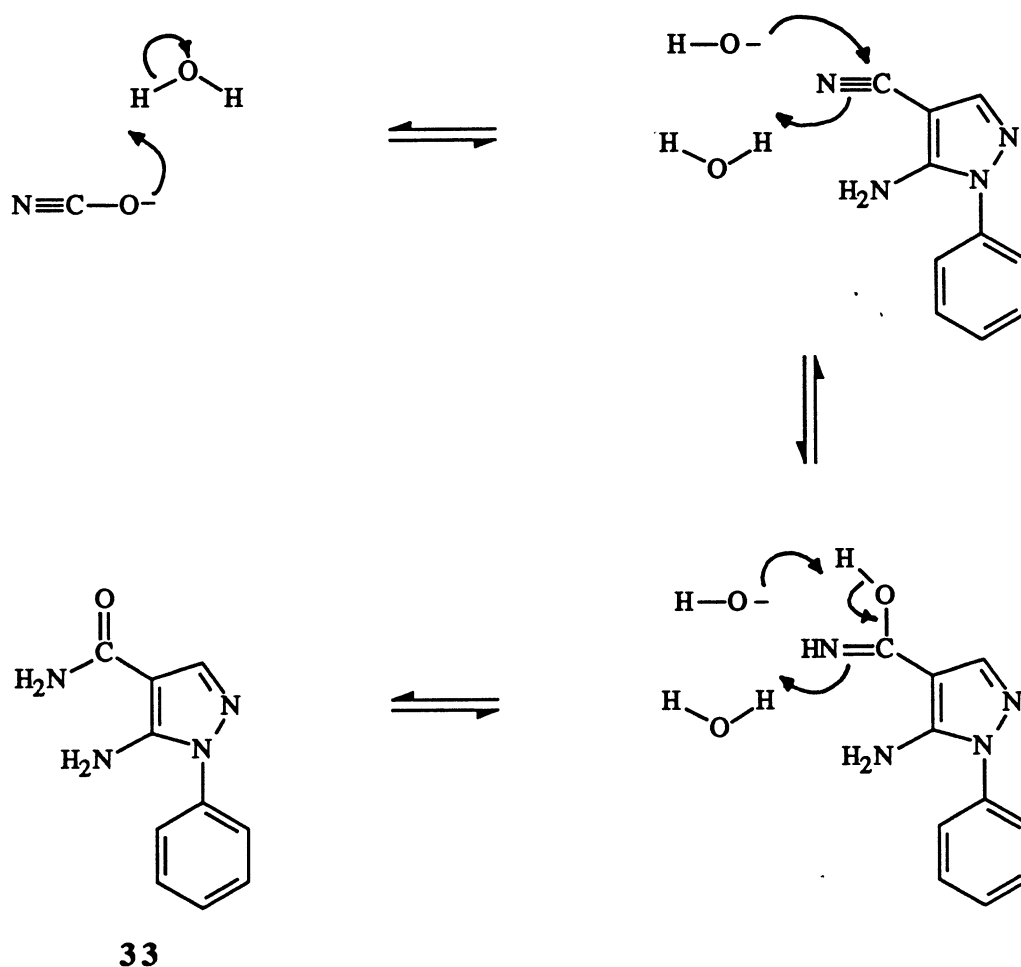
Scheme 17

[4.2] Synthesis of Isoguanosine Analogues using Trimethylsilyl isocyanate

The pyrazole/isocyanate method involving a substituted isocyanate could be used to

obtain isoguanosine analogues, provided the substituent group could be removed after

cyclisation. As the trimethylsilyl group is readily hydrolysed, it was thought that the use

of trimethylsilyl isocyanate may provide a straightforward route to the

pyrazolo[3,4-*d*]pyrimidine analogues of isoguanosine. The addition of 5-amino-4-cyano-

1-phenylpyrazole to trimethylsilyl isocyanate was attempted using the one step synthesis

developed in the previous chapter. 5-Amino-4-cyano-1-phenylpyrazole and sodium

methoxide were dissolved in DMF. The addition of trimethylsilyl isocyanate produced an

exothermic reaction. The reactants were then stirred at 80 °C. Neutralisation with dilute

hydrochloric acid and evaporation of the solvent produced a white solid. This solid was

found to be unreacted 5-amino-4-cyano-1-phenylpyrazole. The nucleophilic base may

have reacted with the silicon of the trimethylsilyl moiety in preference to the pyrazole

amine reacting with the carbon of the isocyanate moiety of the trimethylsilyl isocyanate.

This would result in the cleavage of the trimethylsilyl isocyanate and the pyrazole

remaining unchanged. Trimethylsilyl groups are known to be unstable under nucleophilic

conditions.[104]

Table 7   Conditions for the Reaction of Trimethylsilyl isocyanate with

5-Amino-4-cyano-1-phenylpyrazole.

Base	Solvent	Temperature (°C)	Time (Hrs)
NaOMe	DMF	80	24
DBN	DMF	80	24
DBN	Pyridine	80	24
n-BuLi	THF	-70 to 25	2
-	DMF	80	24
-	DMF	100	24
-	Pyridine	80	24

A non-nucleophilic base was used to prevent any reaction between the base and

trimethylsilyl isocyanate. 5-Amino-4-cyano-1-phenylpyrazole and trimethylsilyl

isocyanate were stirred at 80 °C with DBN in DMF. This reaction was also performed in

pyridine. In each case only unreacted starting material was detected. A stronger non-

nucleophilic base was used to increase the reactivity of the pyrazole. The pyrazole and trimethylsilyl isocyanate were stirred at -70 °C and then at room temperature with n-butyllithium in THF. Again, only unreacted starting material was detected. The two step synthesis was used to allow the formation of the intermediate urea in the absence of base. 5-Amino-4-cyano-1-phenylpyrazole and trimethylsilyl isocyanate were stirred at 80 °C in DMF. This reaction was also performed in pyridine and at higher temperatures. In each case, only unreacted starting material was detected.

The apparent non-reactivity of trimethylsilyl isocyanate after the successful reactions using other substituted isocyanates was quite perplexing. It was initially thought that steric hinderance from the bulky trimethylsilyl group may have limited the reactivity of the trimethylsilyl isocyanate. Steric hinderance has been reported to have a considerable influence on the rate of a number of amine-isocyanate reactions.[78] However, the bulky phenyl group had no such effect on the reactivity of phenyl isocyanate. This indicated that other factors must be involved in the non-reactivity of trimethylsilyl isocyanate. Another possible explanation is that the nucleophilic amine of the pyrazole may have attacked the electropositive silicon, rather than the electropositve carbonyl carbon of the trimethylsilyl isocyanate. This would have resulted in the formation of a trimethylsilyl pyrazole (34) and the loss of an isocyanate ion (Scheme 18). Silicon is markedly more electropositive than nitrogen, resulting in strong polarisation of the silicon-nitrogen bond.[105,106] Polarisation of the silicon-nitrogen bond produces a tendency for nucleophilic attack to occur at the silicon. Nucleophilic attack at the silicon results in heterolysis of the silicon-nitrogen bond. This process is facilitated when the attacking group is a strong nucleophile and the nitrogen containing moiety is a good leaving group. The amine of the pyrazole has proven to be a strong nucleophile in other reactions with substituted isocyanates, while the weakly basic nature of the isocyanate ion would make it a good leaving group. Also, silicon is known to offer a strong stabilising

effect to electropositive carbons and carbonium ions in the β position.[105,106] This is

known as the β-effect and is believed to result from $(\sigma\text{-}p)_\pi$ conjugation

(hyperconjugation). The high degree of polarisation of the silicon-nitrogen σ-bond

produces an electronegative nitrogen which allows stabilisation of an adjacent electron

poor centre by orbital overlap. It has been suggested that hyperconjugation may play a

significant role in the weakening of the silicon-nitrogen σ-bond and thus promote

cleavage processes. β-Ketosilanes and the related esters are readily cleaved by

nucleophiles.[107] The trimethylsilyl pyrazole (34) would be readily hydrolysed back to

the starting material upon contact with water during the reaction workup.

34

10a
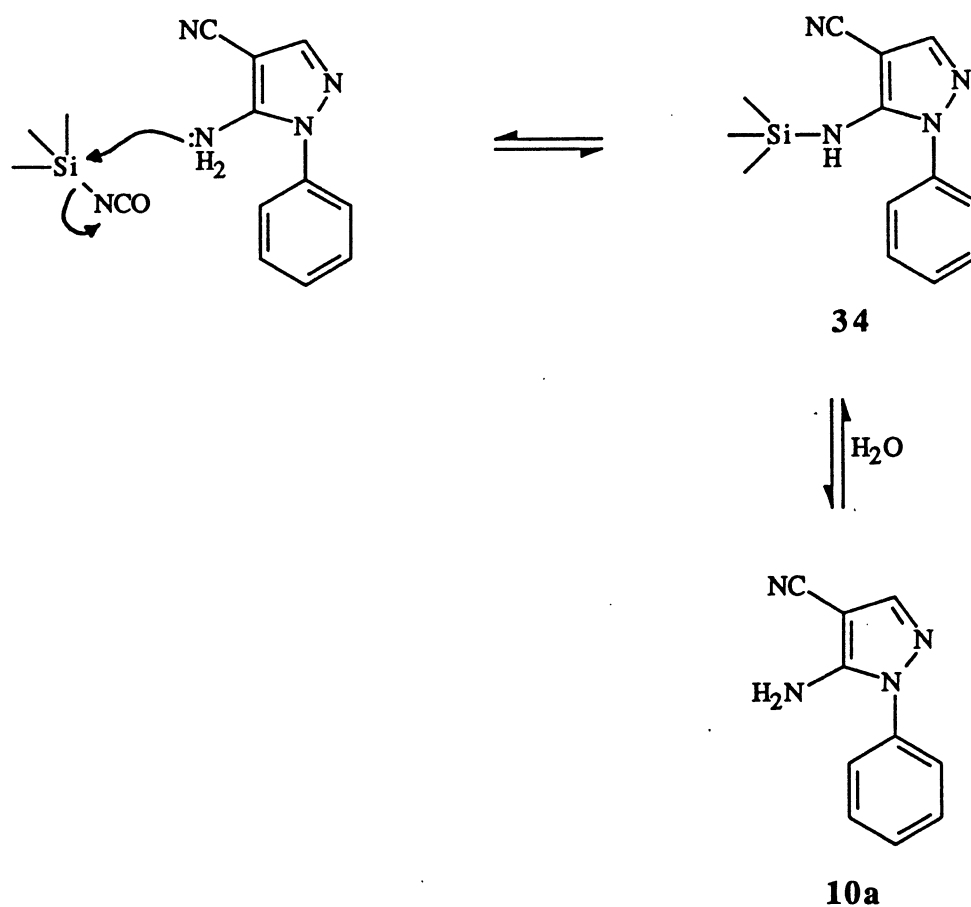
Scheme 18

[4.3] Synthesis of Isoguanosine Analogues using Benzoyl isothiocyanate

The cyclodesulphurative method with pyrazole precursors should allow the preparation of pyrazolo[3,4-*d*]pyrimidine analogues of isoguanosine. In this case, it would be necessary to start with a 5-aminopyrazole-4-carboxamide, rather than 5-amino-4-carbamoyl-1-β-D-ribofuranosylimidazole. 5-Amino-4-cyano-1-phenylpyrazole was refluxed in 2 M sodium hydroxide.[47] Upon cooling a solid precipitated and was recrystallised from water. This product was 5-amino-1-phenylpyrazole-4-carboxamide (33).

5-Amino-1-phenylpyrazole-4-carboxamide and benzoyl isothiocyanate were stirred at room temperature in DMF. A white solid precipitated and was purified by recrystallisation from methanol. Evaporation of the solvent produced an oily solid which was purified by column chromatography. These two products were found to be different compounds (Scheme 19).

(1) The product which was isolated after the solvent was evaporated was found to be the required thiourea (35, 46 %). The IR spectrum showed the carbonyl stretches at 1695 cm⁻¹ and 1700 cm⁻¹ for the benzoyl and amide groups. The ¹H NMR spectrum contained two, one proton singlets at δ 11.81 and δ 12.13 for the two NH protons of the thiourea. There was also a one proton singlet at δ 8.15 for the C-3 proton of the pyrazole ring. The remainder of the spectrum contained a twelve proton multiplet from δ 7.21 to 7.97 for the ten protons of the two phenyl rings and the two protons of the amide. The addition of D₂O resulted in the disappearance of the singlets at δ 11.81 and δ 12.12 and the collapse of the twelve proton multiplet from δ 7.21 to 7.92 to a ten proton multiplet. The ¹³C NMR spectrum contained a peak at δ 167.7 for the carbonyl carbon of the amide. There were also peaks at δ 162.8 and 182.3 for the carbonyl and thiocarbonyl carbons of the thiourea. The remainder of the spectrum contained peaks at δ 113.4 (C-4),

133.4 (C-3) and 138.5 (C-5) for the carbons of the pyrazole ring and eight peaks for the aromatic carbons of the two phenyl rings.

(2) The product which precipitated was found to be 1-phenyl-5*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (**36**, 11 %). The IR spectrum showed the carbonyl stretch at 1725 cm^{-1}. The ^{1}H NMR spectrum contained a one proton doublet at δ 8.19 and a broad one proton singlet at δ 12.47. The addition of D$_2$O resulted in the collapse of the doublet at δ 8.19 to a singlet and the disappearance of the singlet at δ 12.47. This information was consistent for the CHNH protons of the pyrimidine ring. The remainder of the spectrum contained a one proton singlet at δ 8.33 for the C-3 proton of the heterocyclic ring and a five proton multiplet from δ 7.35 to 8.04 for the aromatic protons of the phenyl ring. The ^{13}C NMR spectrum contained peaks at δ 107.6 (C-3a), 136.0 (C-3), 148.8 (C-6), 151.9 (C-7) and 157.3 (C-4) for the carbons of heterocyclic ring. The remainder of the spectrum contained peaks at δ 121.8 (C-2', C-6'), 127.1 (C-4'), 129.2 (C-3', C-5') and 138.3 (C-1') for the carbons of the phenyl ring.

33   **35**   **36**
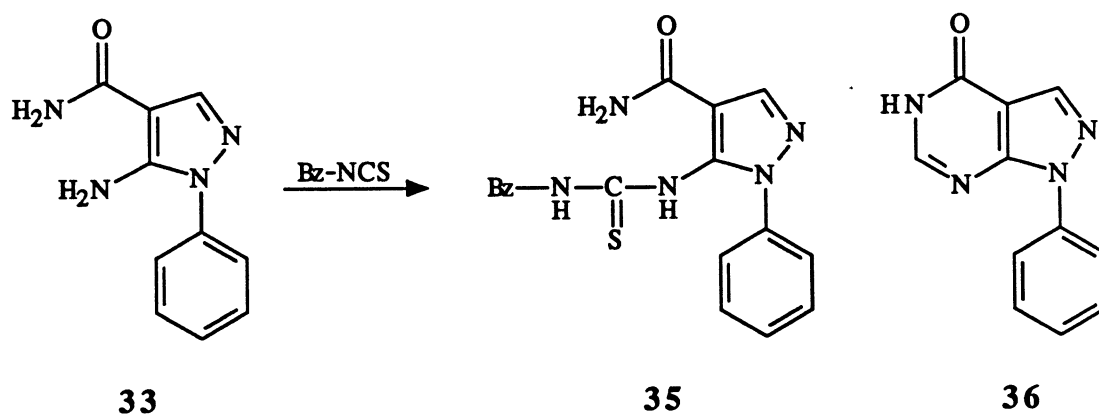
Scheme 19

It was difficult to propose a mechanism for the formation of the inosine analogue (**36**) from the reaction of 5-amino-1-phenylpyrazole-4-carboxamide with benzoyl isothiocyanate. However, it is possible to envisage a mechanism for the formation of this compound from the reaction of the 5-amino-1-phenylpyrazole-4-carboxamide with the

solvent, DMF. This mechanism initially involves the nucleophilic attack of the amine

nitrogen of the pyrazole on the carbonyl carbon of the DMF with the loss of

dimethylamine (Scheme 20). This generates a formylated intermediate. The amide

nitrogen nucleophilically attacks the carbonyl carbon of this intermediate effecting an

intramolecular cyclisation. Aromatisation and the loss of water produces the

pyrazolo[3,4-d]pyrimidine (36). As dimethylamine is a poor leaving group this reaction

would be expected to require harsh conditions. As the reaction proceeded at room

temperature it was suspected that the benzoyl isothiocyanate must have been involved.

The reaction of 5-amino-1-phenylpyrazole-4-carboxamide and DMF was studied in the

absence of benzoyl isothiocyanate. The reactants were stirred at room temperature.

Evaporation of the DMF allowed the pyrazole to be isolated quantitatively. This

confirmed that the benzoyl isothiocyanate must facilitate the formation of the inosine

analogue (36).
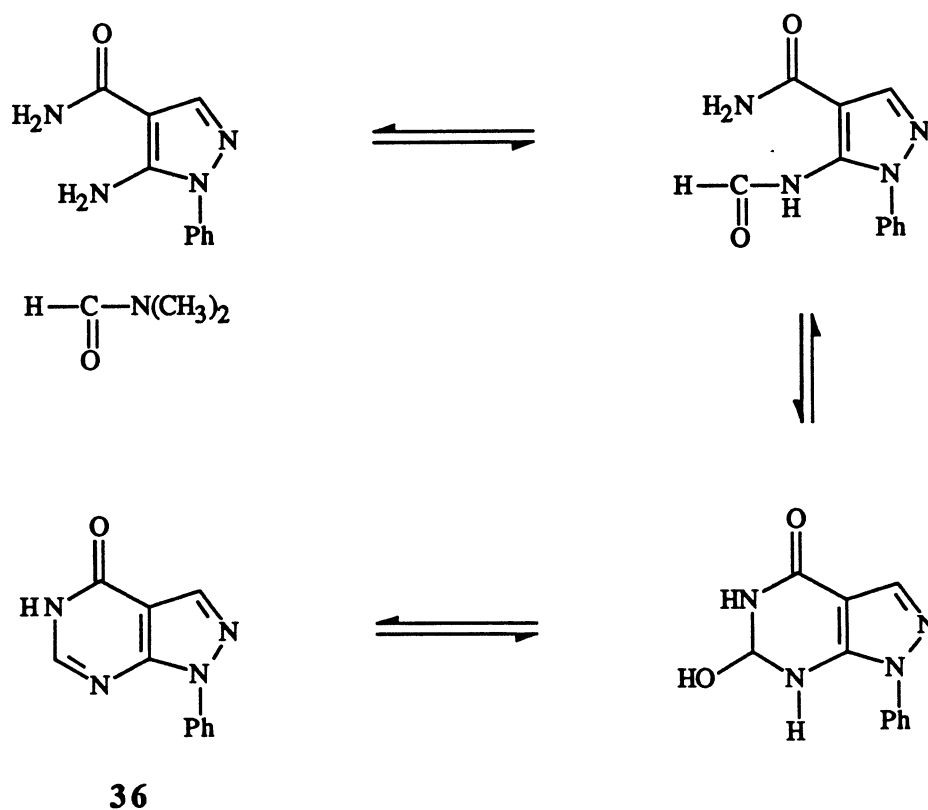
Scheme 20

A number of similar cyclisations have been reported.[47,108] Inosine has been

prepared by refluxing 5-amino-4-carbamoyl-1-β-D-ribofuranosylimidazole and ethyl

formate in ethanolic sodium ethoxide.[108] 1-Phenyl-5H-pyrazolo[3,4-d]pyrimidin-4-one

(36) has been prepared by stirring 5-amino-1-phenylpyrazole-4-carboxamide with

formamide at 200 °C.[47]

The next step in this synthetic method was the DCC mediated cyclodesulphuration.

The thiourea (35) and three molar equivalents of DCC were stirred at room temperature in

DMF. Evaporation of the solvent produced a white solid which was purified by

recrystallisation from ethyl acetate and hexane. This reaction converted the thio group to

a keto group and the amide to a nitrile to produce the urea (37, Scheme 21).

35   DCC   **37**
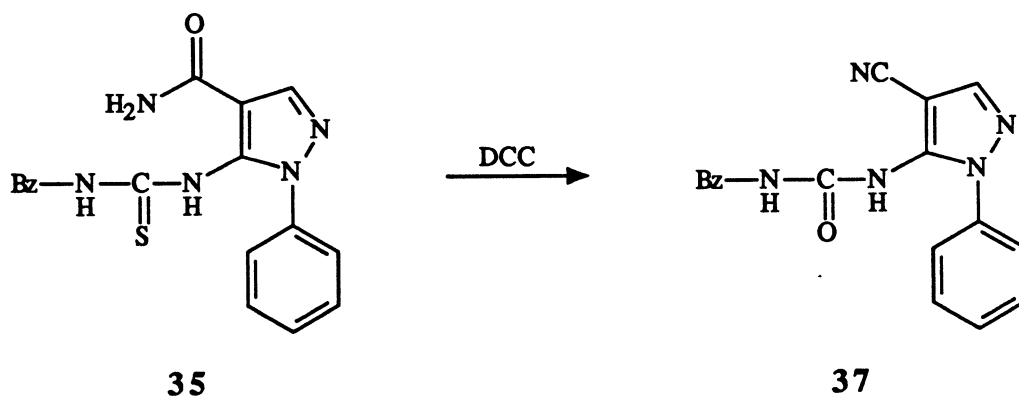
Scheme 21

The IR spectrum showed the appearance of a nitrile stretch at 2230 cm^{-1} and an extra

carbonyl at 1670 cm^{-1}. The benzoyl carbonyl remained at 1700 cm^{-1}. The ^{1}H NMR

contained two, one proton singlets at δ 10.98 and 11.33 for the two NH protons of the

urea. The remainder of the spectrum contained a one proton singlet at δ 8.30 for the C-3

proton of the pyrazole ring and a ten proton multiplet from δ 7.49 to 7.99 for the protons

of the two phenyl rings. The ^{13}C NMR spectrum contained peaks at δ 150.9 and 168.5

for the carbonyl carbons of the benzoyl urea. There was also a peak at δ 112.4 for the nitrile carbon. The remainder of the spectrum contained peaks at δ 89.2 (C-4), 140.4 (C-5) and 142.4 (C-3) for the carbons of the pyrazole ring and eight peaks for the aromatic carbons of the phenyl ring.

A mechanism for similar cyclodesulphurisation reactions involving 3-methoxycarbonyl-1-thioureido substituted compounds has been proposed.[109,110] In this case, attack of the thioenol form of the 3-benzoyl-1-thioureido substituent of 35 on DCC produces the intermediate (38a, Scheme 22). This compound cyclises by one of two possible processes. An intramolecular attack of the 4-carboxamide oxygen on the sp^2 hybridised carbon of the isothiourea moiety of 38a followed by the loss of 1,3-dicyclohexylthiourea generates the [1,3]oxazine intermediate (39), Alternatively, the loss of 1,3-dicyclohexylthiourea produces a carbodiimide intermediate (38b). An intramolecular attack of the 4-carboxamide oxygen on the sp hybridised carbon of the carbodiimide moiety also generates the [1,3]oxazine intermediate (39). A tautomeric ring opening yields the urea (37). There are a number of reasons why the oxygen and not the nitrogen atom of the amide group would act as the nucleophile in the cyclodesulphurisation reaction. The ring closure of 3-methoxycarbonyl-1-thioureido substituents with ortho-amides also proceeded using the mildly acidic DCC-pentafluorophenol complex. Under acidic conditions the excess DCC would not remove a proton from the nitrogen of the amide.[109,110] This indicated that the 'hard end' of the neutral nucleophilic amide group (the oxygen atom) would most likely attack the thiocarbonyl carbon. The ring closure of 3-methoxycarbonyl-1-thioureido substituents with ortho-amides was further investigated by ^{18}O labelling the amide.[109,110] After the reaction with DCC the ^{18}O label was retained, providing indirect evidence for the [1,3]oxazine intermediate and the proposed mechanism. Asparagine and maleamic acids have been reported to react with DCC by a similar mechanism.[111-113]
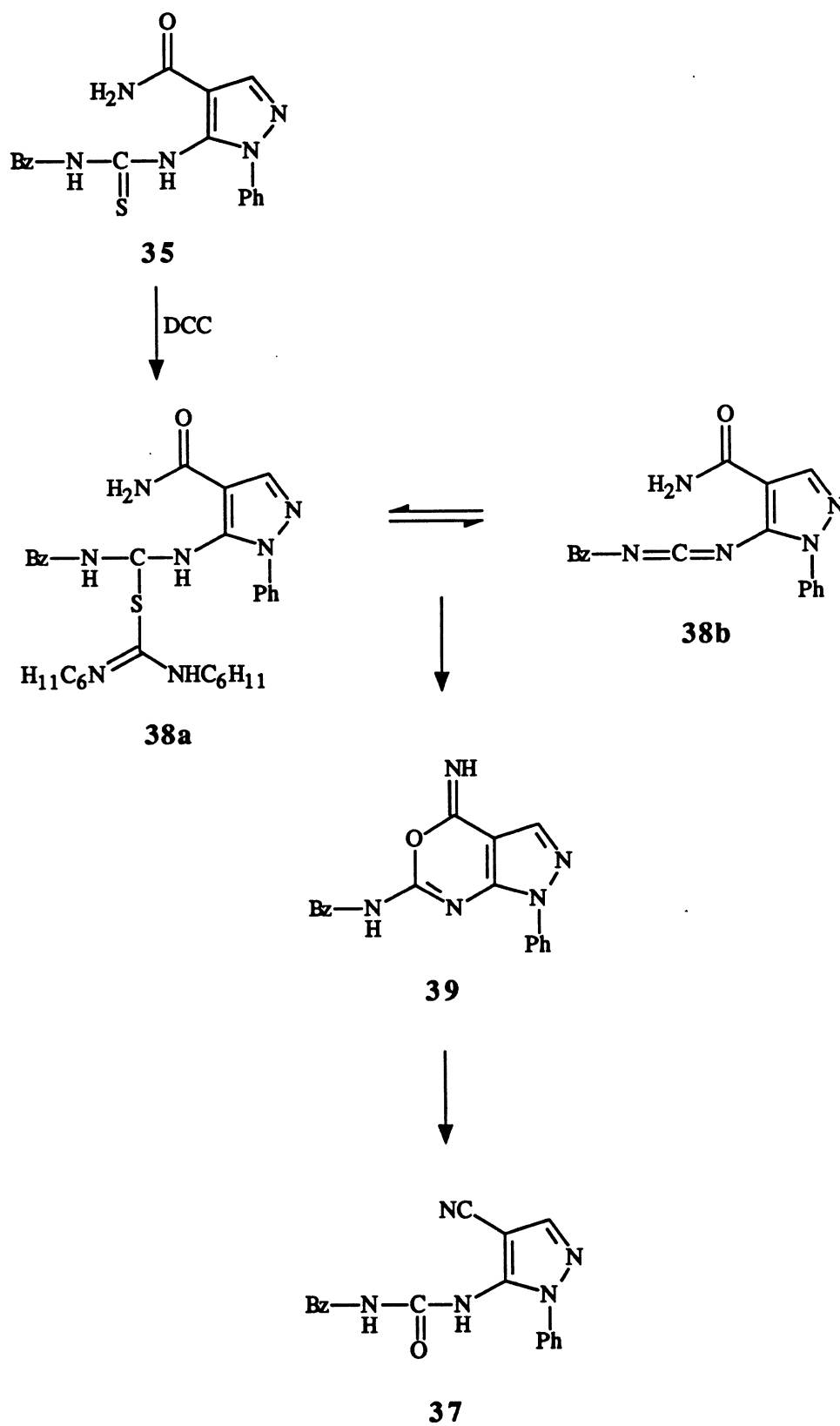
Scheme 22

The dehydration of asparagine was found to proceed via the internal acylation of the amide oxygen, followed by a ring opening of an oxazine intermediate to afford a nitrile product.

The intramolecular cyclisation of the urea (37) was attempted using a modified literature procedure.[99] The reactant was stirred at room temperature in a mixture of DMF and ammonium hydroxide. A white solid precipitated and was purified by recrystallisation from DMSO and water. This solid was pure 4-amino-1-phenyl-5H-pyrazolo[3,4-d]pyrimidin-6-one (40a, Scheme 23). The IR spectrum showed the disappearance of the nitrile at 2230 cm^{-1} and the benzoyl carbonyl at 1700 cm^{-1}. The carbonyl stretch of the C-6 keto group of the product appeared at 1670 cm^{-1}. The ^{1}H NMR spectrum contained three exchangable one proton singlets at δ 7.21, 8.71 and 11.92 for the NH protons. The remainder of the spectrum consisted of a one proton singlet at δ 8.14 for the C-3 proton of the heterocyclic ring and a five proton multiplet from δ 7.21 to 8.13 for the aromatic protons of the phenyl ring. The ^{13}C NMR spectrum contained a peaks at δ 92.3 (C-3a), 135.5 (C-3), 153.9 (C-4), 156.5 (C-7a) and 157.8 (C-6) for the carbons of the pyrazolo[3,4-d]pyrimidine ring. The remainder of the spectrum contained peaks at δ 120.3 (C-2', C-6'), 125.4 (C-4'), 128.8 (C-3', C-5') and 139.3 (C-1') for the carbons of the phenyl ring.

37   NH₃   **40a**
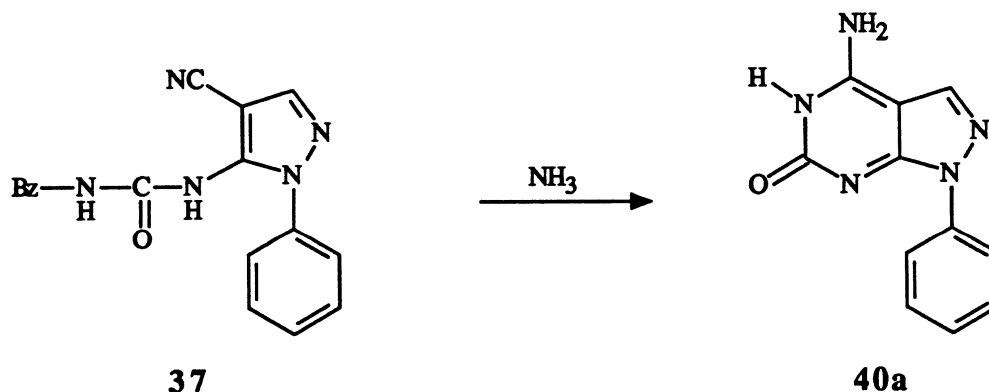
Scheme 23

The mechanism of this reaction involved the cyclisation and debenzoylation of the urea (**37**, Scheme 24). The base abstracts the acidic NH proton of the urea moiety. The nitrogen then undergoes a nucleophilic addition to the electron deficient nitrile carbon to produce a 4-iminopyrimidine. This is another example of the Thorpe-Zeigler type cyclisation which was discussed in the previous chapter. The ammonia then attacks the carbonyl carbon of the benzoyl group. This results in the loss of benzamide and the formation of a stable 4-aminopyrimidine. This 4-aminopyrimidine may exist in a number of different tautomeric forms in solution.

40a
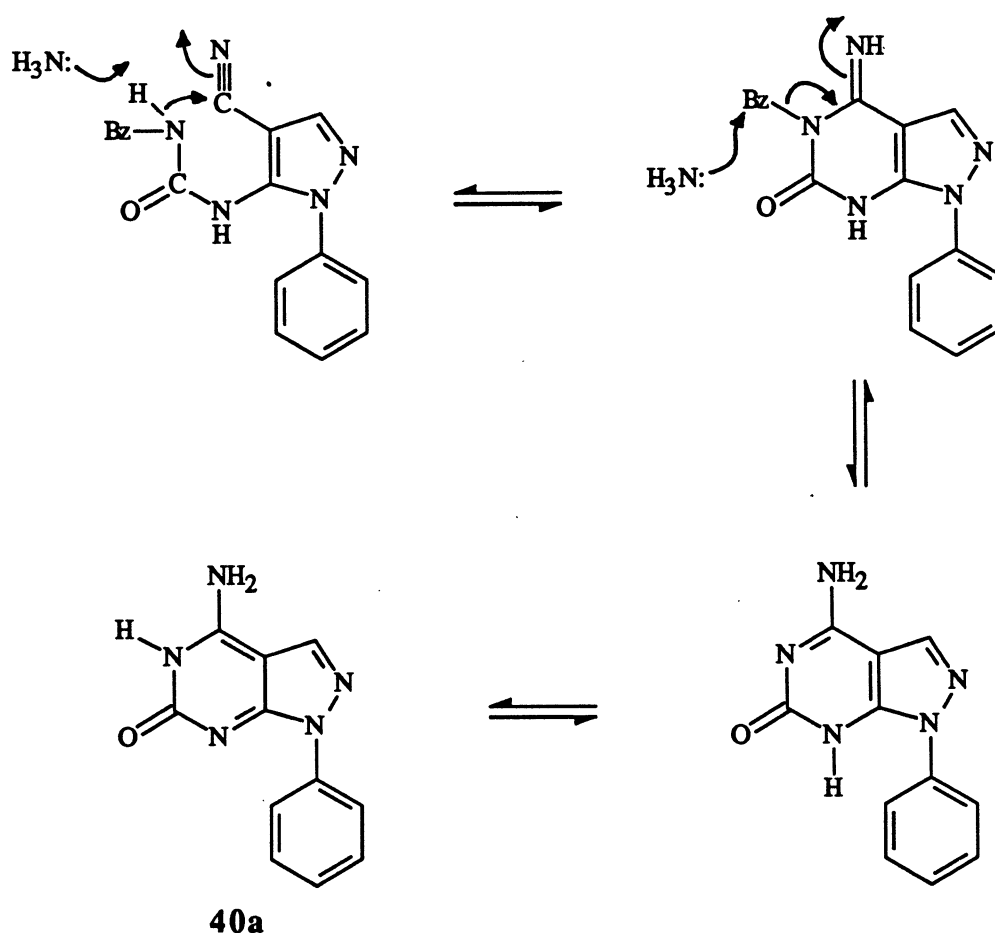
Scheme 24

[4.4] Synthesis of Isoguanosine Analogues using Benzoyl isocyanate

The pyrazole/benzoyl isothiocyanate method required four steps and proceeded in a 28 % yield. The first three steps were required to produce a pyrazole with a nitrile in the C-4 position and a benzoyl urea in the C-5 position. A closer examination of this structure indicated that it may be synthesised directly by the reaction of 5-amino-4-cyano-1-phenylpyrazole with benzoyl isocyanate. The reactants were stirred at 60 °C in DMF. Evaporation of the solvent produced a white solid which was purified by recrystallisation from ethyl acetate and hexane. This solid was found to be the required urea (37, Scheme 25). The intramolecular cyclisation with ammonium hydroxide yielded 4-amino-1-phenyl-5*H*-pyrazolo[3,4-*d*]pyrimidin-6-one (40a). This pyrazole/benzoyl isocyanate method required only two steps and proceeded in a 50 % yield. The problem of side reactions (such as the formation of 36) was eliminated. This method was also used to prepare 4-amino-1-(3-chloro)phenyl-5*H*-pyrazolo[3,4-*d*]pyrimidin-6-one (40b).

10a   37
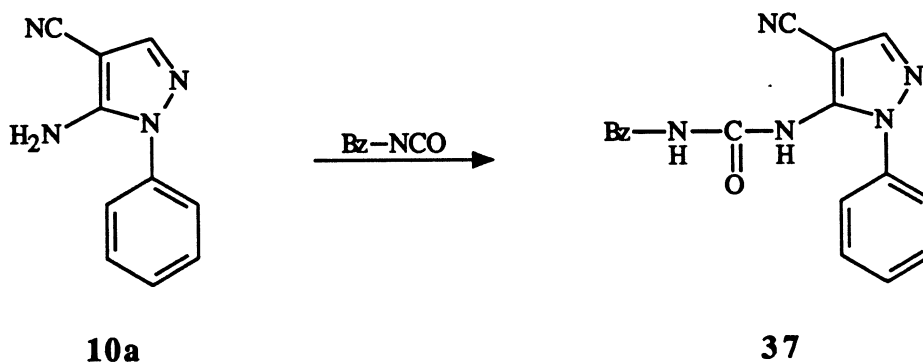
Scheme 25

The reaction between 5-amino-4-cyano-1-phenylpyrazole and benzoyl isocyanate was attempted in one step. The reactants were stirred at 60 °C with sodium methoxide. This produced a number of products. Any base which was strong enough to effect the debenzoylation and cyclisation would presumably react with benzoyl isocyanate prior to the reaction. This difficulty was overcome by adding the base to the reaction mixture

after the urea had formed. The pyrazole and benzoyl isocyanate were stirred at 60 °C for

12 hours. Ammonium hydroxide was added and the reaction was stirred at 60 °C for a

further 12 hours. Evaporation of the solvent gave a white solid which was recrystallised

from DMSO and water. The product of this reaction was found to be identical to the

previous product. The overall yield of the reaction was 68 %.

[4.5] Biological Activity.

The A_1 adenosine receptor affinity of the pyrazolo[3,4-d]pyrimidine analogues of

isoguanosine was measured using a [^{3}H] R-PIA competitive binding assay (Table 8). A

comparison was made with the corresponding pyrazolo[3,4-d]pyrimidine analogues of

1-methylisoguanosine from the previous chapter:

Table 8   Comparison of the Adenosine Receptor Affinity of the

Analogues of Isoguanosine and 1-Methylisoguanosine
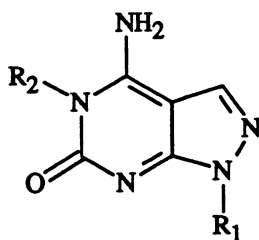
No.	R_1	R_2	% Inhibition
24a	C_6H_5	CH_3	30
24c	C_6H_4Cl (3)	CH_3	53
40a	C_6H_5	H	9
40b	C_6H_4Cl (3)	H	28

(1) The absence of substitution in the N-1 position resulted in an appreciable reduction of adenosine receptor affinity. 4-Amino-1-phenyl-1H-pyrazolo[3,4-d]pyimidin-6-one (40a) was approximately 3 times less potent than 4-amino-5-methyl-1-phenylpyrazolo[3,4-d]pyrimidin-6-one (24a). This result was consistent with the decreases in receptor affinity observed with decreasing chain length of aliphatic groups in the N-5 position. A similar trend exists for the corresponding N-1 substituents of xanthines. Xanthine has been reported to be 40 times less potent that 1-methylxanthine in a [^{3}H] CHA competitive binding assay.[83]

(2) The 3-chlorophenyl group produced greater adenosine receptor affinity than the phenyl group in the N-1 position. This was consistent with the increased receptor affinity observed with both *meta* and chlorine substitution of phenyl groups in the N-1 position.

CHAPTER FIVE

5,7-Substituted Pyrazolo[3,4-*d*]pyrimidine
Analogues of 1-Methylisoguanosine

[5.0] General Introduction

1-Methylisoguanosine has been isolated from three different natural sources.[31-33] The structure was independently reported in the 6-amino (4a)[31,32] and the 6-imino (4b)[33] tautomeric forms. A ^{13}C NMR spin-lattice relaxation study was used to investigate the tautomerism of 1-methylisoguanosine.[114,115] Analysis of the contributions of ^{13}C - ^{1}H dipolar interactions to the ^{13}C NMR relaxation times established that the predominant tautomer in solution was the 6-amino form. A UV study was also used to investigate this tautomerism.[41] Model compounds in which the 6-substituent was frozen in the amino form (N^6, N^6, 9-trimethylisoguanine) and the imino form (1-ethyl-3-methylisoguanosine) were used. However, comparison of the UV spectra of these compounds with 1-methylisoguanosine was inconclusive as all of the compounds produced similar maximum absorbtions in the UV region.
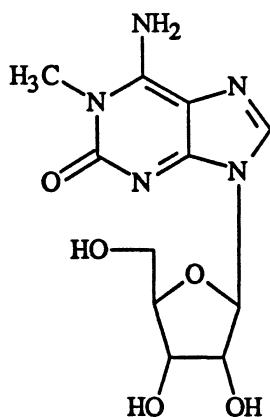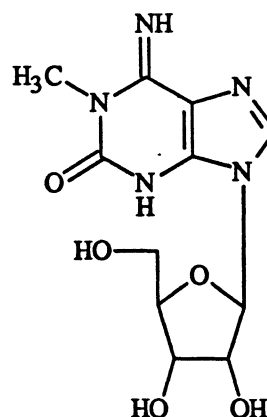
4a   **4b**

1-Methylisoguanosine has been found to be more than twice as potent as 1-methyladenosine in stimulating guinea pig adenylate cyclase.[116] While 1-methylisoguanosine has been found to exist in the 6-amino form, 1-methyladenosine can only exist in the 6-imino form. From this, it has been concluded that an amine was required in this position to obtain maximum activity.

1-Ethyl-3-methylisoguanosine (**42**) contains alkyl substituents in the N-1 and N-3

positions. This effectively prevents any tautomerism to the 6-amino form. 5-Amino-4-

cyano-1-β-D-ribofuranosylimidazole (**7**) was alkylated with formaldehyde and reduced

with sodium borohydride to yield the 5-methylamino derivative (**43**,

Scheme 26).[117,118] Reductive alkylation of the amine allowed the isolation of a mono-

methylated product. Alkylation of the amine with methyl iodide would be expected to

produce a mixture of mono and bis-methylated products. The addition of this

5-methylamino derivative to ethyl isocyanate in DMF produced an intermediate urea (**44**).

The urea underwent an intramolecular cyclisation with methanolic ammonia in DMF to

yield 1-ethyl-3-methylisoguanosine (**42**).
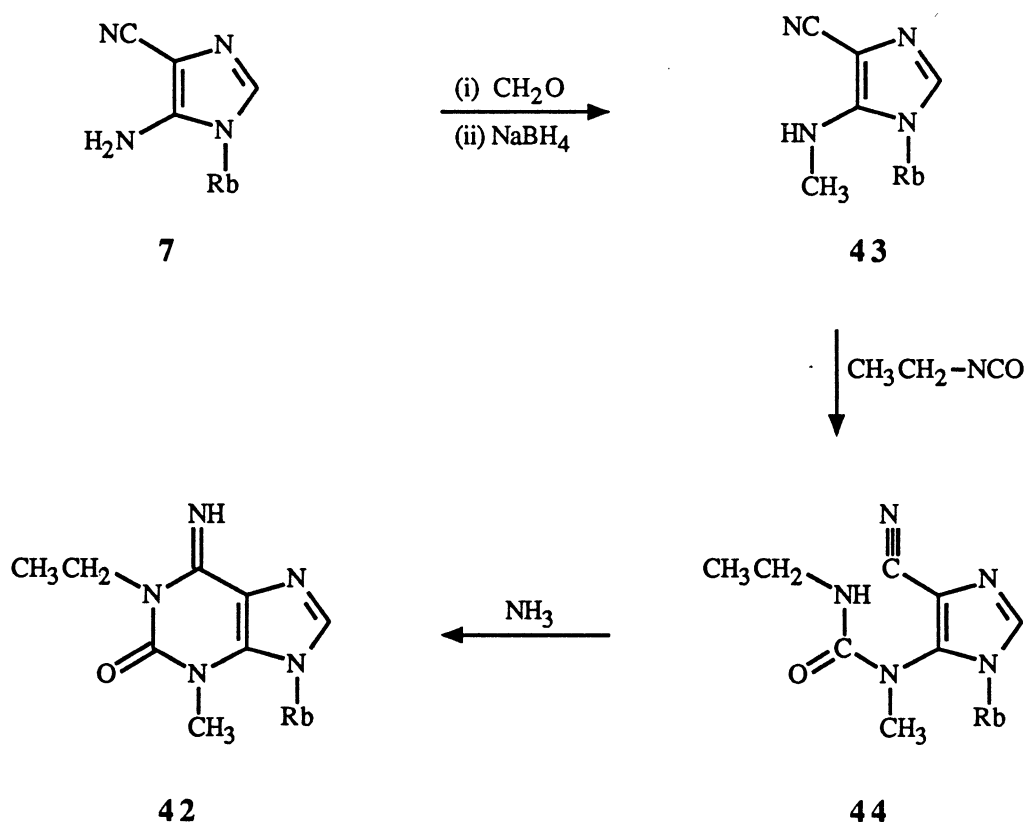
Scheme 26

This method was used to prepare a pyrazolo[3,4-*d*]pyrimidine analogue of 1-methylisoguanosine with the C-4 position frozen in the imino form. A comparison of adenosine receptor affinity was made with the corresponding 4-amino analogue (24a).

[5.1] Synthesis of 4-Cyano-5-methylamino-1-phenylpyrazole

5-Amino-4-cyano-1-phenylpyrazole and paraformaldehyde were refluxed in THF for 24 hours. The reaction was cooled to 0 °C and sodium borohydride in ethanol was added. The reaction was warmed to room temperature and quenched with water. Evaporation of the solvent and a water wash afforded a mixture of two compounds. Column chromatography allowed the separation and purification of 4-cyano-5-methylamino-1-phenylpyrazole (45, 14 %) and unreacted pyrazole (10a, 69 %).
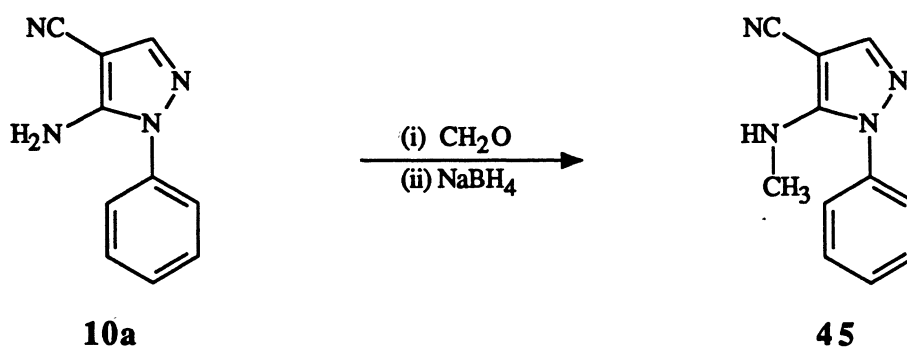
Scheme 27

The IR spectrum of the product showed the NH stretch at 3340 cm^{-1} and the nitrile stretch at 2210 cm^{-1}. The ^{1}H NMR spectrum contained an exchangable one proton singlet at δ 4.53 and a three proton singlet at δ 3.06 for the NHCH$_3$ protons. The methyl signal was not split by the adjacent NH. In cases where an NH exchanges at an intermediate rate, the NH proton is partially decoupled. This results in the observance of a broad NH peak and no splitting of any adjacent CH protons.[119] The ^{1}H NMR spectrum of

N-methyl-4-nitroaniline also contains an exchangable one proton singlet and a three proton singlet for the NHCH₃ functionality. The remainder of the spectrum contained a one proton singlet at δ 7.52 for the C-3 proton of the pyrazole ring and a five proton multiplet from δ 7.35 to 7.50 for the aromatic protons of the phenyl ring. The ^{13}C NMR spectrum contained a peak at δ 31.2 for the methyl carbon and a peak at δ 115.4 for the nitrile carbon. The remainder of the spectrum contained peaks at δ 73.5 (C-4), 142.5 (C-3) and 151.1 (C-5) for the carbons of the pyrazole ring and peaks at δ 124.9 (C-2', C-6'), 128.9 (C-4'), 129.9 (C-3', C-5') and 136.9 (C-1') for the phenyl carbons.

Primary amines are known to react readily with aldehydes and ketones.[120] In this case, the amine of the pyrazole reacted with paraformaldehyde. The reaction mechanism involves the attack of the amine nitrogen on the carbonyl carbon (Scheme 28).
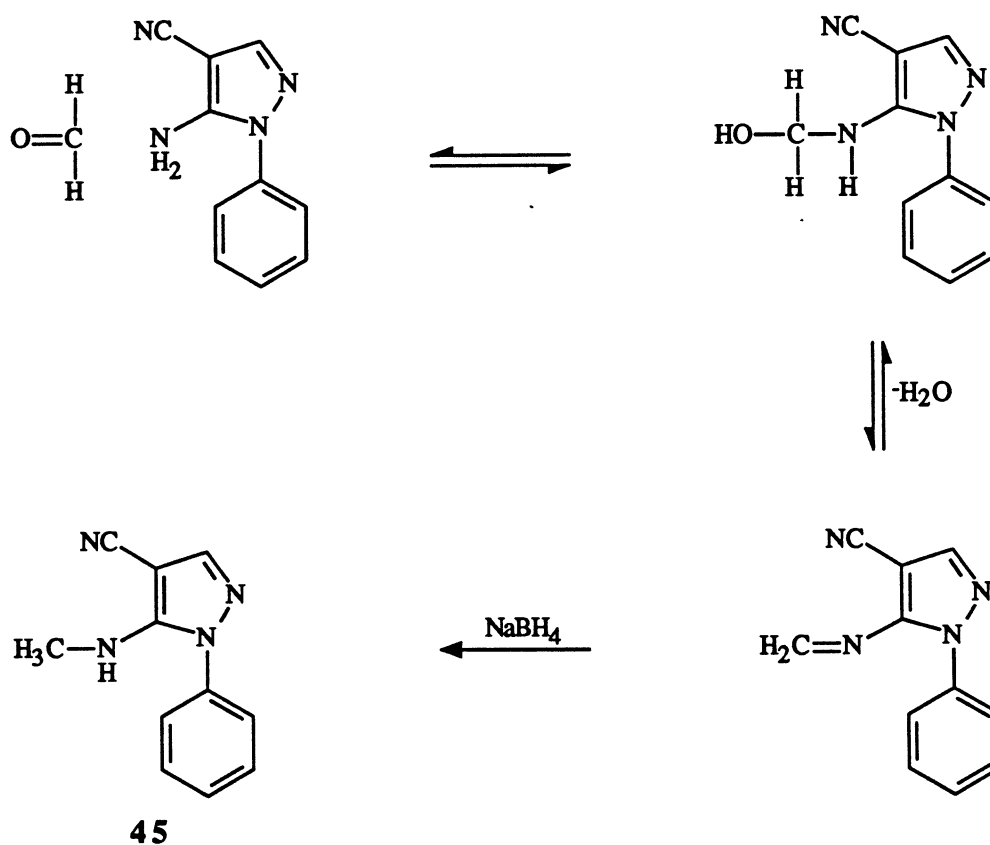
Scheme 28

The resultant carbinolamine dehydrates rapidly to form an intermediate imine. The formation of this imine is an equilibrium process in which the loss of water is a driving force. Reduction of the nitrogen-carbon double bond generates the methyl pyrazole (45). Suitable reducing agents include metal hydrides (such as sodium borohydride, lithium aluminium hydride or sodium cyanoborohydride) and hydrogen with a catalyst.

The isolation of pyrazole (10a) and methyl pyrazole (45) suggested that the limiting step must have been the formation of the intermediate imine. The absence of any imine amongst the reaction products indicated that the reduction step must have proceeded efficiently. Imine formation is an equilibrium process which must have favoured the starting material. A number of different conditions were evaluated in an attempt to shift this equilibrium and improve the yield of methyl pyrazole (45).

Acids are known to catalyse the rate of imine formation by accelerating the dehydration of the intermediate carbinolamine.[120] The pyrazole, paraformaldehyde and a catalytic amount of hydrochloric acid were refluxed in THF for 24 hours. The presence of acid required the use of a different reducing agent, as sodium borohydride is rapidly hydrolysed under acidic conditions. Sodium cyanoborohydride was chosen as a replacement. It is known to rapidly reduce imines and has enhanced stability in acid to pH = 3.[121] The reaction was cooled to 0 °C and sodium cyanoborohydride in ethanol was added. Analysis of the reaction mixture by thin layer chromatography indicated that it contained predominantly unreacted pyrazole (10a) and some methyl pyrazole (45). The presence of acid may also inhibit the rate of imine formation by protonating the more basic amine and retarding the nucleophilic attack on the paraformaldehyde.

The removal of water from the reaction would force the dehydration equilibrium towards the imine. The pyrazole and paraformaldehyde were refluxed in THF in the

presence of 4 Å molecular sieves. As the reaction was only being performed on a small scale, it was thought that the molecular sieves may absorb any water produced during imine formation. The reaction was cooled to 0 °C and sodium borohydride in ethanol was added. Analysis of the reaction mixture by thin layer chromatography indicated that it contained predominantly unreacted pyrazole (10a) and some methyl pyrazole (45).

The presence of sodium borohydride 'in situ' would reduce the imine as soon as it was formed. The removal of the imine would force the dehydration equilibrium in the required direction. This method has the disadvantage that a second equivalent of formaldehyde may react with the 4-cyano-5-methylamino-1-phenylpyrazole to form a bis-methylated product. The pyrazole, formaldehyde and sodium borohydride were stirred at room temperature in THF. Analysis of the reaction mixture by thin layer chromatography indicated that it contained predominantly unreacted pyrazole (10a), some methyl pyrazole (45) and a third compound (suspected to be 4-cyano-5-dimethylamino-1-phenylpyrazole).

The use of higher temperatures may also force the equilibrium in the required direction. The pyrazole and paraformaldehyde were refluxed in DMF for 24 hours. The use of DMF as a solvent allowed the reflux temperature to be raised to 150 °C. The reaction was cooled to 0 °C and sodium borohydride in ethanol was added. The reaction was warmed to room temperature and quenched with water. Evaporation of the solvent and a water wash afforded a mixture of two compounds. Column chromatography allowed the separation and purification of 4-cyano-5-methylamino-1-phenylpyrazole (45, 26 %) and unreacted pyrazole (10a, 58 %).

As the increase in temperature resulted in an increased yield of 4-cyano-5-methylamino-1-phenylpyrazole, further increases in temperature were investigated. The

pyrazole and paraformaldehyde were initially dissolved in THF to ensure thorough mixing. The THF was boiled off and the reactants heated at approximately 200 °C for 24 hours in a fusion-style process. The reaction was cooled to 0 °C and sodium borohydride in ethanol was added. Evaporation of the solvent and a water wash afforded a mixture of two compounds. Column chromatography allowed the separation and purification of 4-cyano-5-methylamino-1-phenylpyrazole (45, 67 %) and a lesser amount of unreacted pyrazole (10a, 32 %).

[5.2] Synthesis of 5,7-Dimethyl-4-imino-1-phenylpyrazolo[3,4-*d*]pyrimidin-6-one

The cyclisation of the 4-cyano-5-methylamino-1-phenylpyrazole and methyl isocyanate was attempted using the pyrazole/isocyanate method developed earlier. The reactants were stirred at 60 °C with sodium methoxide in DMF. As no reaction occurred, the reaction mixture was stirred at 100 °C overnight. During this period the colour darkened to brown. Evaporation of the solvent gave a solid which was found to be unreacted 4-cyano-5-methylamino-1-phenylpyrazole.

The reaction was repeated in the absence of base in order to limit any decomposition at high temperature. The methyl pyrazole and methyl isocyanate were stirred at 60 °C and then at 100 °C in DMF. Evaporation of the solvent yielded an oily solid which was comprised of two compounds. Column chromatography allowed the separation and purification of the urea (46, 9 %) and unreacted methyl pyrazole (45, 86 %). The IR spectrum of the product showed that the nitrile stretch had been retained at 2220 cm^{-1}. The ^{1}H NMR spectrum contained a three proton singlet at δ 2.99 for the NCH$_3$ protons of the urea. There was also an exchangable one proton singlet at δ 4.96 and a three proton doublet at δ 2.66 for the NHCH$_3$ protons. The remainder of the spectrum

contained a one proton singlet at δ 7.87 for the C-3 proton of the pyrazole ring and a five proton multiplet from δ 7.35 to 7.49 for the aromatic protons of the phenyl ring. The ^{13}C NMR spectrum contained peaks at δ 27.5 and 35.7 for the methyl carbons and a peak at δ 155.5 for the carbonyl carbon of the urea. There was also a peak at δ 111.6 for the carbon of the nitrile. The remainder of the spectrum contained peaks at δ 91.1 (C-4), 142.2 (C-3) and 145.5 (C-5) for the pyrazole carbons and peaks at δ 123.4 (C-2', C-6'), 129.3 (C-4'), 129.7 (C-3', C-5') and 137.4 (C-1') for the phenyl carbons. An increase in the reaction temperature to 150 °C also yielded a mixture which contained predominantly starting material and some urea.
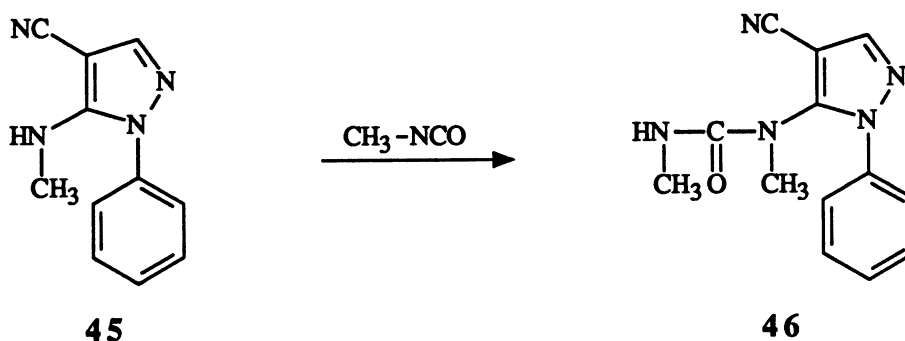
Scheme 29

The intramolecular cyclisation of the urea (**46**) was attempted using a literature procedure.[31] The reactant was stirred at 60 °C in methanolic ammonia. Evaporation of the solvent and recrystallisation from methanol afforded a white solid. This solid was found to be the cyclised pyrazolo[3,4-d]pyrimidine (**47**). The IR spectrum of the product showed the disappearance of the nitrile stretch at 2220 cm^{-1}. The ^{1}H NMR spectrum contained two, three proton singlets at δ 3.00 and 3.50 for the methyl protons and an exchangable one proton singlets at δ 10.65 for the imine proton. The spectrum also contained a one proton singlet at δ 8.93 for the C-3 proton of the

pyrazolo[3,4-*d*]pyrimidine ring and a five proton multiplet from δ 7.21 to 8.16 for the

aromatic protons of the phenyl ring. The ^{13}C NMR spectrum contained peaks at δ 31.9

and 33.1 for the methyl carbons. The remainder of the spectrum contained peaks at

δ 95.1 (C-3a), 137.6 (C-3), 143.9 (C-4), 149.0 (C-7a) and 152.9 (C-6) for the carbons

of the pyrazolo[3,4-*d*]pyrimidine ring and peaks at δ 127.6 (C-2', C-6'), 129.5 (C-4'),

130.4 (C-3', C-5') and 137.7 (C-1') for the carbons of the phenyl ring. This structure

was confirmed by microanalysis and high resolution mass spectroscopy.
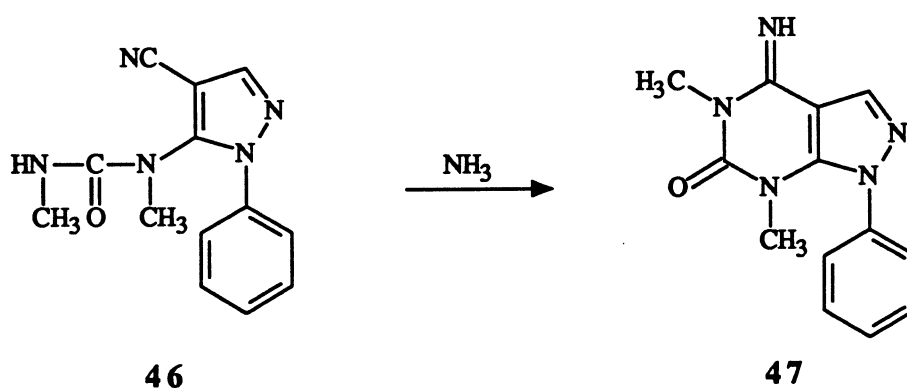
Scheme 30

The cyclisation of the 4-cyano-5-methylamino-1-phenylpyrazole and methyl

isocyanate was attempted using a stronger base in an attempt to improve the yield. The

reactants were stirred at -70 °C and then at room temperature with n-butyllithium in THF.

The reaction was quenched with water and the products were extracted with ethyl acetate.

Evaporation of the solvent yielded an oily solid which was comprised of two compounds.

Analysis of this mixture by thin layer chromatography indicated the it contained

predominantly unreacted methyl pyrazole (45) and some urea (46).

The reaction was repeated using n-butyllithium at a higher temperature. 4-Cyano-5-

methylamino-1-phenylpyrazole and methyl isocyanate were stirred at room temperature

with n-butyllithium in THF. The reaction was followed by thin layer chromatography.

After 4 hours, the reaction still contained some unreacted starting material. A second

portion of methyl isocyanate was added. After a further 30 minutes, all of the starting

material had been consumed. The reaction contained a compound with a similar retention

time to the urea (46) and the pyrazolo[3,4-*d*]pyrimidine (47). The solvent was

evaporated under reduced pressure to yield an oily solid. Ethyl acetate and hexane (1:1)

was added to separate the soluble and insoluble components:

(1) The solid which was insoluble in ethyl acetate was collected and recrystallised

from DMSO and methanol. This product was confirmed to be 5,7-dimethyl-4-imino-1-

phenylpyrazolo[3,4-*d*]pyrimidin-6-one (47, 16 %).

(2) The solid which was soluble in ethyl acetate was purified by chromatography.

This product was found to be an inseparable mixture of 1,3,5-trimethyltriazine-2,4,6-

trione (48) and a bis-urea (49).
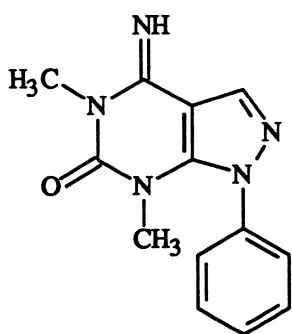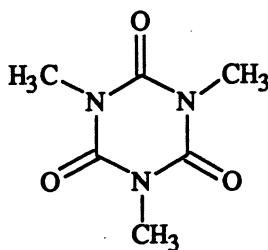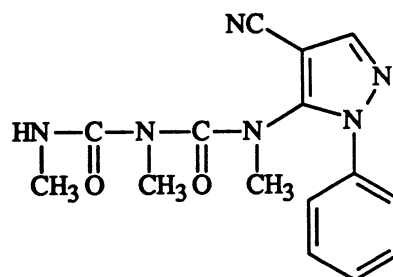
47   **48**   **49**

Analysis of this solid using X-ray crystallography determined the

structure of the isocyanurate (48, Figure 5). The ^{1}H NMR spectrum contained a nine

proton singlet at

δ 3.33 for the three equivalent sets of methyl protons of the isocyanurate. The ^{13}C NMR

spectrum also contained a peak at δ 32.8 for the three equivalent methyl carbons and a

peak at δ 154.5 for the three equivalent carbonyl carbons of the isocyanurate. The

balance of the ^{1}H and ^{13}C NMR spectra determined the structure of the urea.
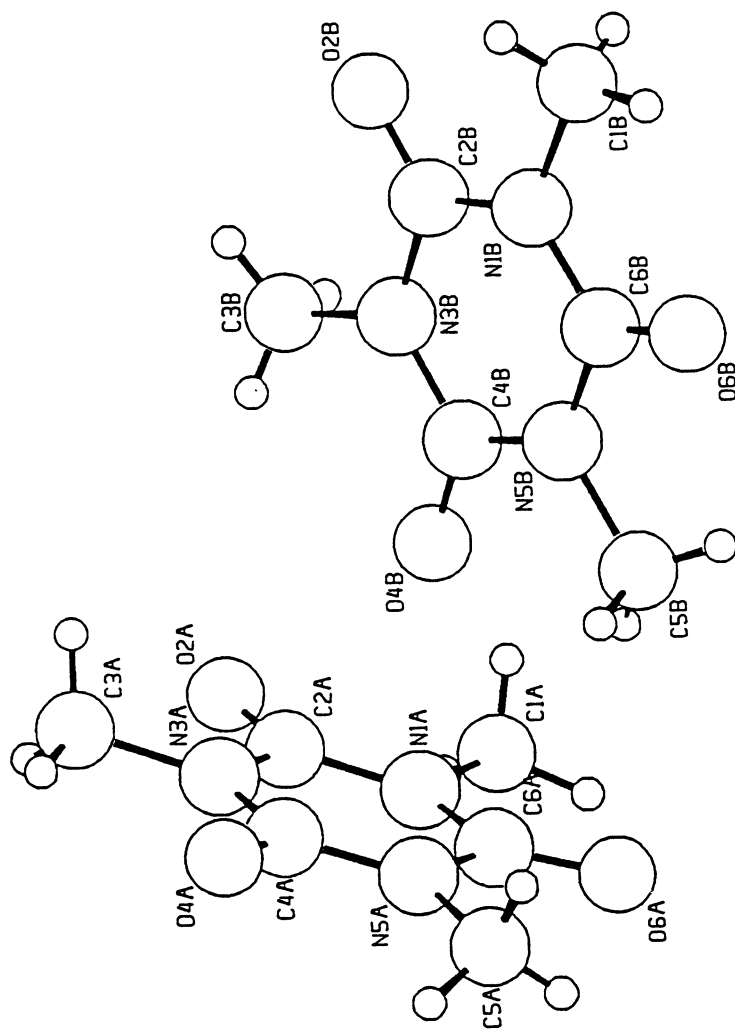
Figure 5 X-Ray Structure of 1,3,5-Trimethyltriazine-2,4,6-trione (48)

The ^{1}H NMR spectrum also contained an exchangable one proton singlet at δ 6.82 and a three proton doublet at δ 2.44 for the terminal NHCH$_3$ protons of the urea. There were two, three proton singlets at δ 2.59 and 3.41 for the other methyl protons of the bis-urea. The remainder of the spectrum contained a one proton singlet at δ 7.93 for the C-3 proton of the pyrazole ring and a five proton multiplet from δ 7.24 to 7.49 for the aromatic protons of the phenyl ring. The ^{13}C NMR spectrum contained peaks at δ 26.7, 29.2, and 38.8 for the methyl carbons and peaks at δ 149.4 and 157.7 for the carbonyl carbons of the urea. There was a peak at δ 111.6 for the nitrile carbon. The remainder of the spectrum contained peaks at δ 89.1 (C-4), 142.4 (C-3) and 145.8 (C-5) for the carbons of the pyrazole rings and peaks at δ 123.6 (C-2', C-6'), 129.4 (C-4'), 129.8 (C-3', C-5') and 142.4 (C-1') for the carbons of the phenyl ring.

The mechanism would initially involve the abstraction of the amine proton of the pyrazole by the base, n-butyllithium (Scheme 31). The negatively charged nitrogen of the amine would attack the electropositive carbonyl carbon of the methyl isocyanate. A urea with a negatively charged terminal nitrogen would result. This anion could attack the nitrile carbon of the pyrazole to effect an intramolecular cyclisation to form the pyrazolo[3,4-*d*]pyrimidine (47). Alternatively, the anion could attack the carbonyl carbon of a second equivalent of methyl isocyanate to effect an intermolecular addition to form the bis-urea (49). Quenching the reaction with water would result in the protonation of any negatively charged species.

The reaction of 5-amino-1-methylpyrazole-4-ethyl carboxylate and two equivalents of methyl isocyanate with sodium hydroxide has also been reported to yield a bis-urea.[122] Alkyl isocyanates are known to trimerise on heating or in the presence of a catalyst to form isocyanurates.[123]
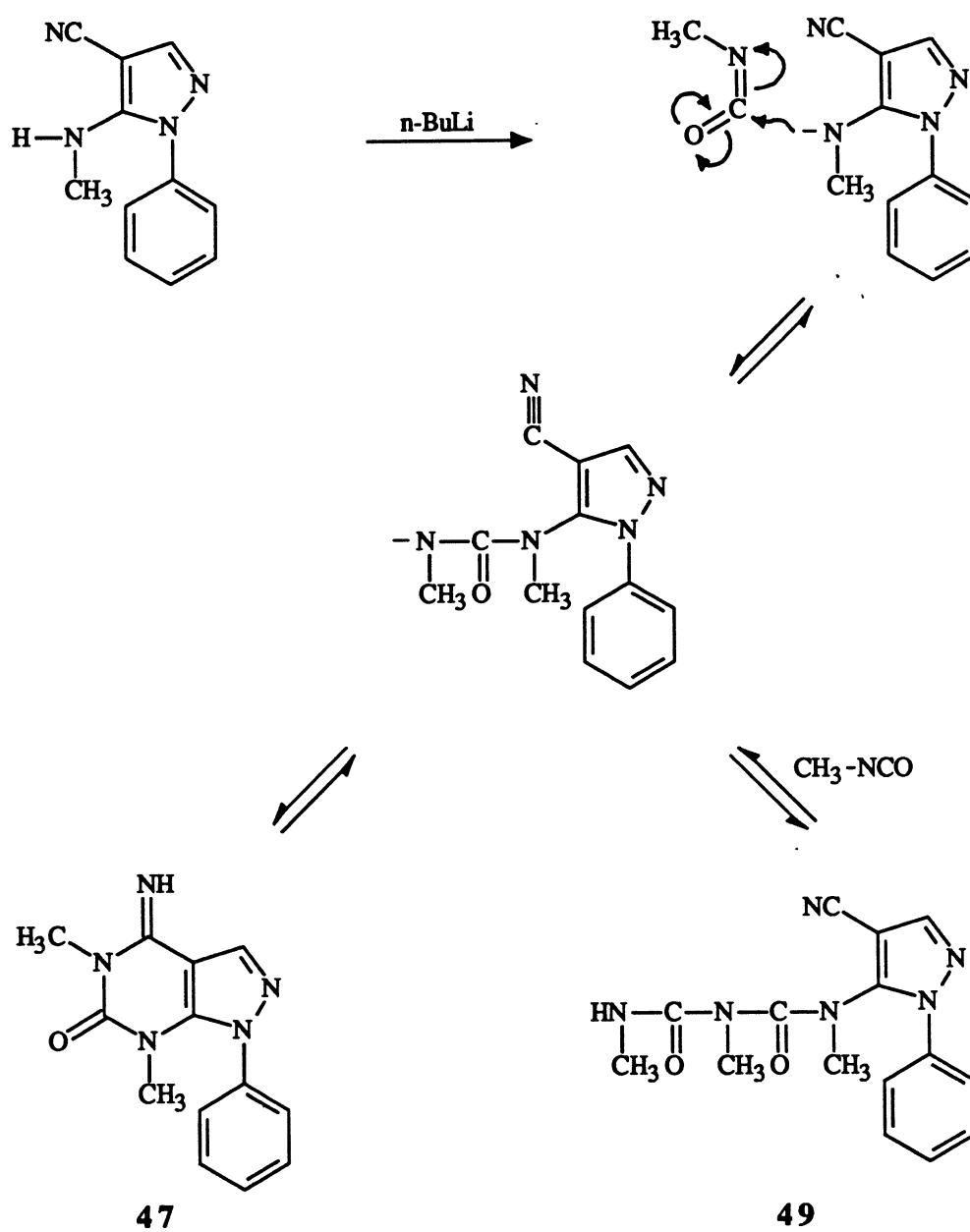
Scheme 31

In order to limit the intramolecular reaction with the second equivalent of methyl

isocyanate, the reaction was performed at a greater dilution. 4-Cyano-5-methylamino-1-

phenylpyrazole and methyl isocyanate were stirred at room temperature in a 5 fold

dilution of THF. After 15 minutes, the reaction consisted of unreacted starting material,

cyclised pyrazolo[3,4-*d*]pyrimidine (**47**) and the mixture of isocyanurate (**48**) and bis-

urea (**49**). A second portion of methyl isocyanate was added to allow the remaining

starting material to react. After a further 15 minutes, all of the starting material had been

consumed. The reaction was quenched with water and the solvent was evaporated under

reduced pressure. The yellow oil produced was triturated with ethyl acetate to afford a

white solid. Recrystallisation from methanol and ethyl acetate yielded the required

product (**47**, 35 %). The conditions used for this reaction are summarised in Table 9.

Table 9   Conditions for the Reaction of Methyl isocyanate with 4-Cyano-
5-methylamino-1-phenylpyrazole

Equivalents of isocyanate	Base	Solvent	Conditions	Product
1.5	NaOMe	DMF	100 °C, 24 hrs	**45**
1.5	-	DMF	100 °C, 24 hrs	**45, 46**
1.5	-	DMF	150 °C, 24 hrs	**45, 46**
1.2	n-BuLi	THF	-70 °C, 1 hr	**45, 46**
2 x 1.2	n-BuLi	THF	25 °C, 1 hr	**47 - 49**
2 x 1.2	n-BuLi	THF	25 °C, 1 hr	**47 - 49**

[5.3] Biological Activity

The A_1 adenosine receptor affinity was measured using a [^{3}H] R-PIA competitive binding assay (Table 10). The 4-imino analogue (47) was found to possess negligible adenosine receptor affinity. The only structural modifications from 4-amino-5-methyl-1-phenylpyrazolo[3,4-d]pyrimidin-6-one (24a) were the imine group in the C-4 position and the additional methyl group in the N-7 position. This information provides further evidence that the 4-amino substituent is necessary for adenosone receptor binding. However, the influence of the additional methyl substituent is uncertain.

Table 10   Adenosine Receptor Affinity of the 4-Imino

Analogue of 1-Methyl isoguanosine

No.	% Inhibition
24a	30
47	0

CHAPTER SIX

2-Substituted Pyrazolo[3,4-*d*]pyrimidine

Analogues of 1-Methylisoguanosine

[6.0] General Introduction

The xanthines, caffeine and theophylline, are classical adenosine antagonists. A structure/activity analysis of a series of xanthine derivatives has allowed the development of more potent adenosine antagonists.[83] The addition of an 8-phenyl substituent produced a dramatic increase in potency. 8-Phenyltheophylline was 1000 times more potent than theophylline in a [^{3}H] CHA binding assay. Combining 2-amino and 4-chloro substituents on the 8-phenyl ring produced further increases in potency. It was thought that similar structural modifications may also produce increased antagonist potency for the 4-aminopyrazolo[3,4-d]pyrimidin-6-ones. Assuming that the heterocyclic rings of these antagonists bind to the receptor in a similar orientation, the N-2 substituent of the pyrazolo[3,4-d]pyrimidine ring and the C-8 substituent of the xanthine ring should access the same area of the receptor.

The synthesis of 2-substituted pyrazolo[3,4-d]pyrimidines requires the use of 1-substituted 3-aminopyrazole precursors. In turn, the synthesis of 1-substituted 3-aminopyrazoles generally involves the condensation of ethoxymethylene derivatives with different types of hydrazines.[46,47]

(1) Substituted Hydrazines: The addition of an electron withdrawing group to the α nitrogen of the hydrazine results in the formation of 1-substituted 5-aminopyrazoles. The reaction of phenyl hydrazine with ethoxymethylenemalononitrile produced 5-amino-4-cyano-1-phenylpyrazole.[47] The electron withdrawing nature of the phenyl group reduces the nucleophilicity of the adjacent α nitrogen of the hydrazine. The more nucleophilic β nitrogen attacks ethoxymethylenemalononitrile generating a neutral intermediate. A facile cyclisation of this intermediate yields 5-amino-4-cyano-1-phenylpyrazole.

(2) 2-Substituted 1-Acetylhydrazines: The addition of a second and stronger electron withdrawing group to the β nitrogen of the hydrazine results in the formation of

1-substituted 3-aminopyrazoles. The reaction of 1-acetyl-2-methylhydrazine (50, Scheme 32) and ethoxymethylenecyanoacetate (51) produced ethyl 3-amino-1-methylpyrazole-4-carboxylate (53).[124-127]
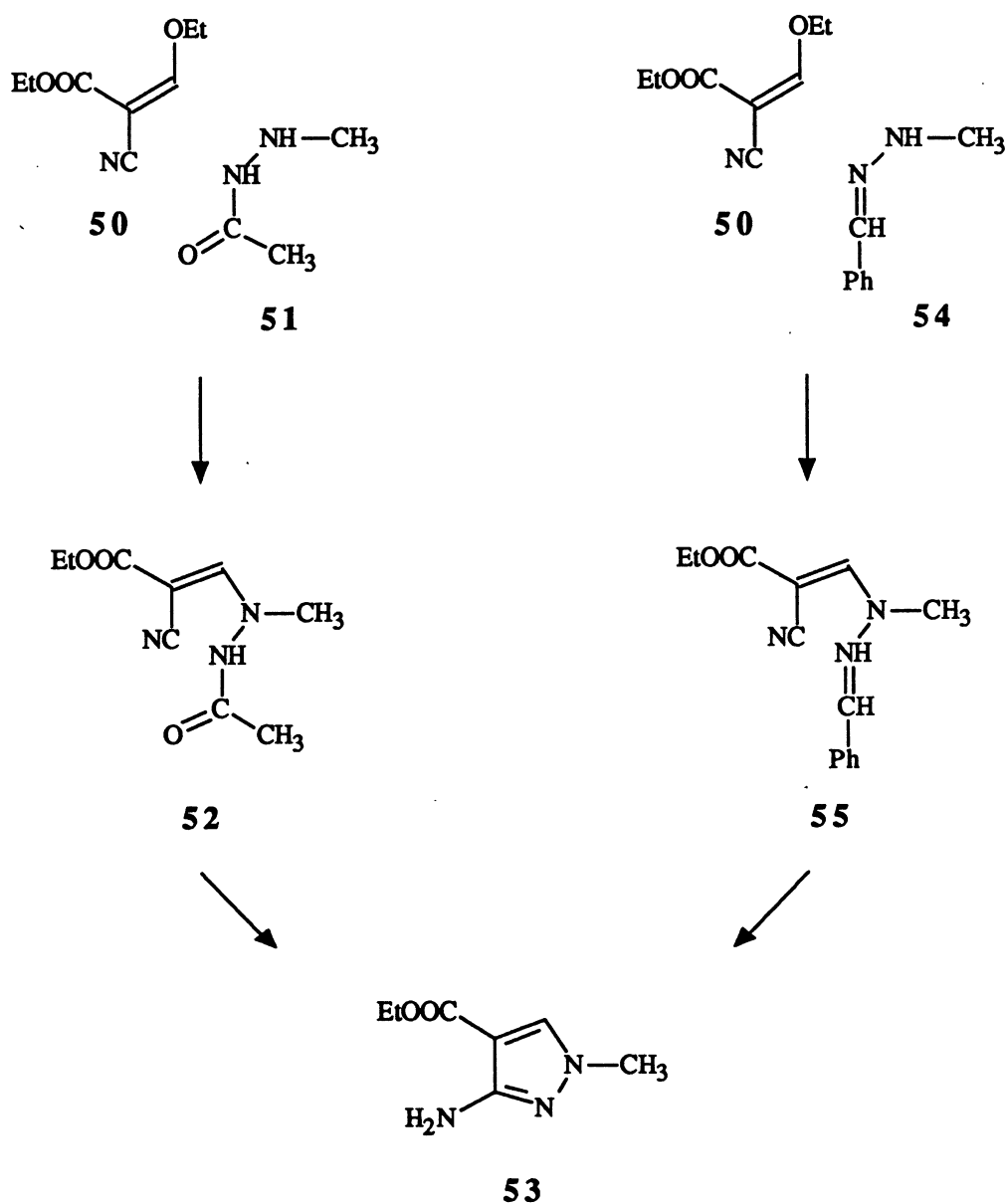
Scheme 32

The greater electron withdrawing nature of the acetyl group reduces the nucleophilicity of the β nitrogen of the hydrazine. The more nucleophilic α nitrogen attacks ethoxymethylenecyanoacetate generating a neutral intermediate (52). Hydrolysis and

cyclisation of this intermediate with hydrochloric acid yields ethyl 3-amino-1-methylpyrazole-4-carboxylate (53). These reactions proceeded in low yield. The addition of the second electron withdrawing substituent would lower the reactivity of the hydrazine. Only one pyrazole with an aromatic substituent has been prepared by this method. The reaction of 1-acetyl-2-phenylhydrazine and ethoxymethylenemalonic ester produced ethyl 3-hydroxy-1-phenylpyrazole-4-carboxylate.[128]

(3) Substituted Benzaldehyde Hydrazones: The use of substituted benzaldehyde hydrazones also results in the formation of 1-substituted 3-aminopyrazoles. The reaction of benzaldehyde methylhydrazone (54, Scheme 32) with ethoxymethylenecyanoacetate (50) produced ethyl 3-amino-1-methylpyrazole-4-carboxylate (53).[124-127] The sp^2 hybridisation of the β nitrogen of the hydrazone allows only the α nitrogen to undergo the reactions of a free amine. The nucleophilic α nitrogen of the hydrazone attacks the ethoxymethylenecyanoacetate generating a neutral intermediate (55). Hydrolysis and cyclisation of this intermediate with hydrochloric acid yields ethyl 3-amino-1-methylpyrazole-4-carboxylate (53).

An alternative synthesis of 1-substituted 3-aminopyrazoles involved the alkylation of an unsubstituted pyrazole ring.[123] The position of substitution was able to be varied by the use of different alkylating agents and reaction conditions. Treatment of ethyl 3-aminopyrazole-4-carboxylate (56, Scheme 33) with diazomethane in ether gave a 2:1 mixture of starting material and ethyl 5-amino-1-methylpyrazole-4-carboxylate (57). Treatment of the same starting material with dimethylsulphate in aqueous alkali gave a 4:1 mixture of ethyl 3-amino-1-methylpyrazole-4-carboxylate (53) and ethyl 5-amino-1-methylpyrazole-4-carboxylate (57). This method required the separation of a mixture of products and generally proceeded in low yield. The type of substituent was limited to groups which could alkylate the pyrazole ring.
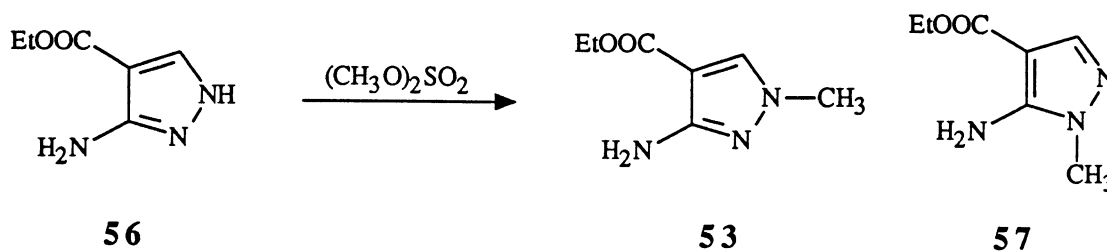
Scheme 33

The synthesis of 2-alkyl pyrazolo[3,4-*d*]pyrimidines has been primarily undertaken using 1-alkyl 3-aminopyrazole-4-carboxylates and 1-alkyl 3-aminopyrazole-4-carboxamides. These pyrazoles have been condensed with urea, thiourea, isocyanates, isothiocyanates, guanidine and formamide to produce a wide range of 2-alkyl pyrazolo[3,4-*d*]pyrimidines.[129] The isocyanate reactions were of particular interest. The addition of ethyl 3-amino-1-methylpyrazole-4-carboxylate to methyl isocyanate generated an intermediate urea. Base catalysed cyclisation of this urea yielded 2,5-dimethyl-7*H*-pyrazolo[3,4-*d*]pyrimidin-4,6-dione (58). This compound represents a 1,8-dimethyl-pyrazolo[3,4-*d*]pyrimidine analogue of xanthine.
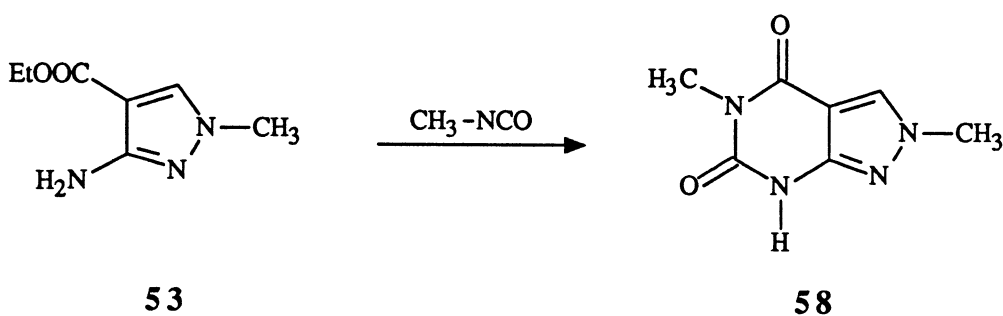
Scheme 34

In this study, 1-acetyl-2-phenylhydrazine and ethoxymethylenemalononitrile were used to prepare 3-amino-4-cyano-1-phenylpyrazole. The addition of this pyrazole to methyl isocyanate yielded a 2-phenylpyrazolo[3,4-*d*]pyrimidine analogue of 1-methylisoguanosine.

[6.1] Synthesis of 3-Amino-4-cyano-1-phenylpyrazole

The reaction of 1-acetyl-2-phenylhydrazine (59) and ethoxymethylenemalononitrile was attempted using a modified literature procedure.[124-127] The reactants were refluxed in ethanol for 12 hours. Analysis of the reaction mixture by thin layer chromatography indicated that no reaction had occurred. Longer reaction times produced no reaction. 1-Acetyl-2-phenylhyrazine was much less reactive than phenylhydrazine in this system. The electron withdrawing acetyl group would reduce the nucleophilicity of the hydrazine and limit the nucleophilic attack on the ethoxymethylenemalononitrile.

1-Acetyl-2-phenylhydrazine and ethoxymethylenemalononitrile were stirred in phosphorous oxychloride at 50 °C for 12 hours. A yellow solid precipitated and was recrystallised from methanol. The spectral information was inconsistent with the expected structure. The amine of the 3-amino-4-cyanopyrazole was substituted with a 2,2-dicyanoethylene group (60). The IR spectrum showed the presence of three nitrile stretches at 2208, 2210 and 2220 cm^{-1}. The ^{1}H NMR spectrum contained a one proton singlet at δ 9.31 for the methine proton and an exchangable one proton singlet at δ 12.19 for the NH proton of 2,2-dicyanoethenylamino group. The remainder of the spectrum contained a one proton singlet at δ 8.45 for the C-3 proton of the pyrazole ring and a five proton multiplet from δ 7.38 to 7.87 for the aromatic protons of the phenyl ring. The ^{13}C NMR spectrum contained peaks at δ 112.1, 113.0 and 115.4 for the nitrile carbon attached to the pyrazole ring and the two nitrile carbons of the 2,2-dicyanoethenylamino group. There were also peaks at δ 55.7 for the quaternary carbon and δ 156.5 for the methine carbon of the 2,2-dicyanoethenylamino group. The remainder of the spectrum contained peaks at δ 84.9 (C-4), 136.1 (C-3) and 149.4 (C-5) for the carbons of the pyrazole ring and δ 119.1 (C-2', C-6'), 128.0 (C-4'), 129.7 (C-3', C-5') and 138.1 (C-1') for the carbons of the phenyl carbons ring.
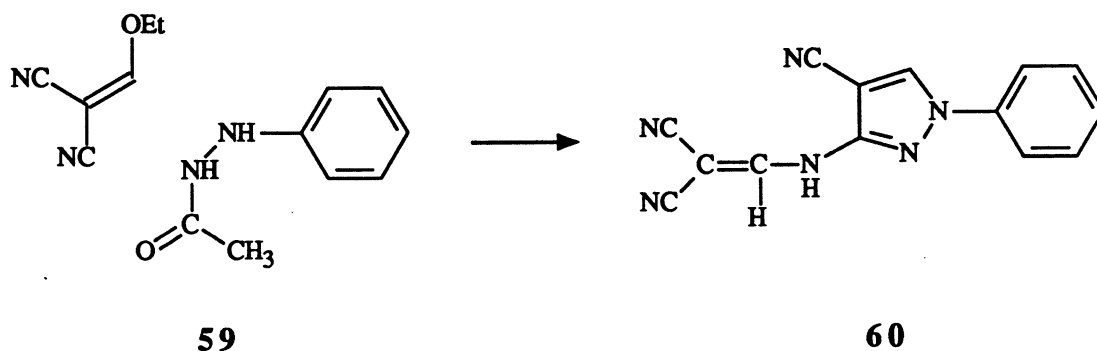
Scheme 35

The quarternary, olefinic carbon of the 2,2-dicyanoethenylamino group had a surprisingly low chemical shift (δ 55.7). In order to confirm this assignment, anilinomethylenemalononitrile (21) was synthesised and the ^{13}C NMR chemical shifts were compared. Aniline and ethoxymethylenemalononitrile were refluxed in ethanol for 2 hours. Upon cooling, a white solid precipitated and was recrystallised from methanol. The IR spectrum showed the three nitrile peaks at 2020, 2025 and 2050 cm^{-1}. The ^1H NMR spectrum contained a one proton singlet at δ 8.49 for the methine proton and a broad singlet at δ 11.12 for the NH proton. The remainder of the spectrum contained a five proton multiplet from δ 7.13 to 7.45 for the protons of the phenyl ring. The ^{13}C NMR contained peaks at δ 114.2 and 116.5 for the nitrile carbons. There were also peaks at δ 51.8 for the quaternary carbon and δ 155.8 for the methine carbon. The remainder of the spectrum contained peaks at δ 118.1 (C-2', C-6'), 125.2 (C-4'), 129.4 (C-3', C-5') and 139.3 (C-1') for the carbons of the phenyl ring. In both compounds, the quarternary carbon of the 2,2-dicyanoethenylamino group had a low chemical shift.

As conclusive proof of the structure of 4-cyano-3-(2,2-dicyanoethenylamino)-1-phenylpyrazole (60), a crystal was grown from methanol and analysed by X-ray crystallography (Figure 6).
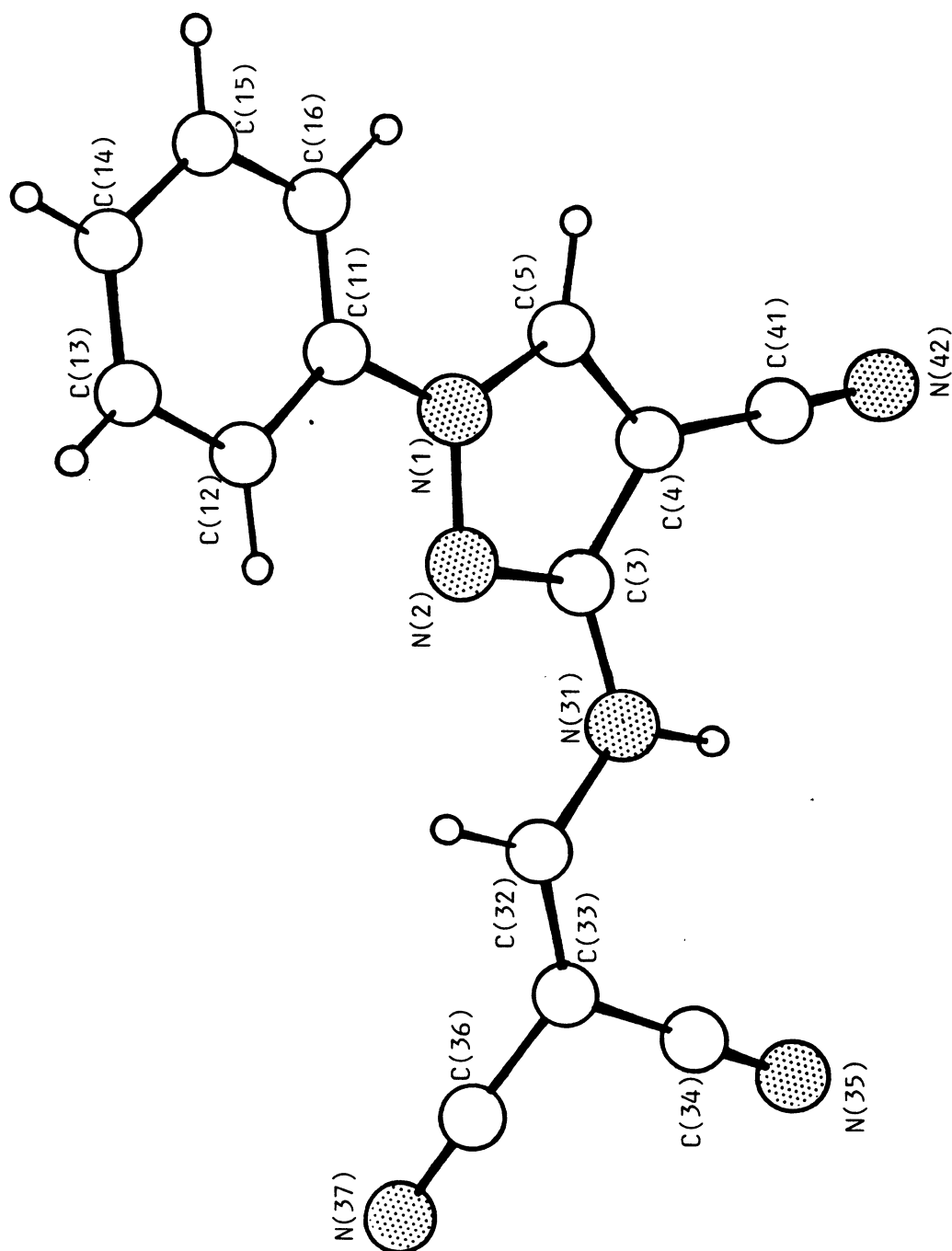
Figure 6   X-Ray Structure of 4-Cyano-3-(2,2-dicyanoethenylamino)-1-phenylpyrazole (60)

An attempt was made to limit the addition of a second equivalent of ethoxymethylenemalononitrile. The reaction was repeated using equimolar amounts of each reagent and milder reaction conditions. This would limit the availability of the ethoxymethylenemalononitrile and lower the reactivity of the system. 1-Acetyl-2-phenylhydrazine, ethoxymethylenemalononitrile and phosphorous oxychloride were stirred at room temperature for 12 hours. A yellow solid precipitated and was found to be 4-cyano-5-(2,2-dicyanoethenylamino)-1-phenylpyrazole (60). Alternatively, the reaction could be repeated in the presence of a proton source. This would allow the reactive amine nitrogen of the pyrazole to be protonated, rather than reacting with a second equivalent of ethoxymethylenemalononitrile. Unfortunately, the use of a protic solvent or the addition of acid would also result in a side reaction with the phosphorous oxychloride.

As this further reaction could not be easily avoided, it was thought that the 2,2-dicyanoethenylamino group may be able to be removed. Enamines (α,β-unsaturated amines) undergo acid catalysed hydrolysis to form amines and carbonyl compounds.[130] 4-Cyano-3-(2,2-dicyanoethenylamino)-1-phenylpyrazole was refluxed in a mixture of dilute hydrochloric acid and DMF. A solid precipitated on cooling and was recrystallised from ethyl acetate. This solid was found to be the required pyrazole (61, Scheme 36).
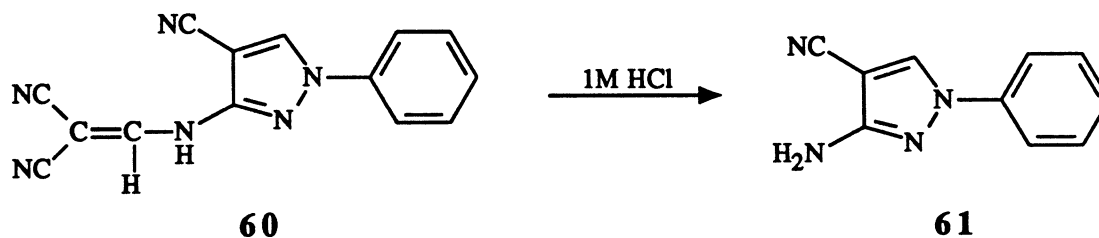
Scheme 36

The IR spectrum showed the presence of only one nitrile peak at 2210 cm^{-1}. The ^{1}H NMR spectrum contained an exchangable two proton singlet at δ 5.99 for the amine protons. The remainder of the spectrum contained a five proton multiplet from δ 7.24 to

7.72 for the aromatic protons of the phenyl ring and a a one proton singlet at δ 8.92 for the C-3 proton of the pyrazole ring. The ^{13}C NMR contained a peak at δ 114.1 for the nitrile carbon. There were also peaks at δ 80.6 (C-4), 133.4 (C-3) and 158.0 (C-5) for the carbons of the pyrazole ring. The remainder the spectrum contained peaks at δ 118.0 (C-2', C-4'), 126.4 (C-4'), 129.5 (C-3', C-5') and 138.7 (C-1') for the carbons of the phenyl ring.

A number of detailed studies have investigated the mechanism of enamine hydrolysis.[131-133] Under acidic conditions, the β carbon of the enamine is protonated and an immonium ion is formed (Scheme 37).

61
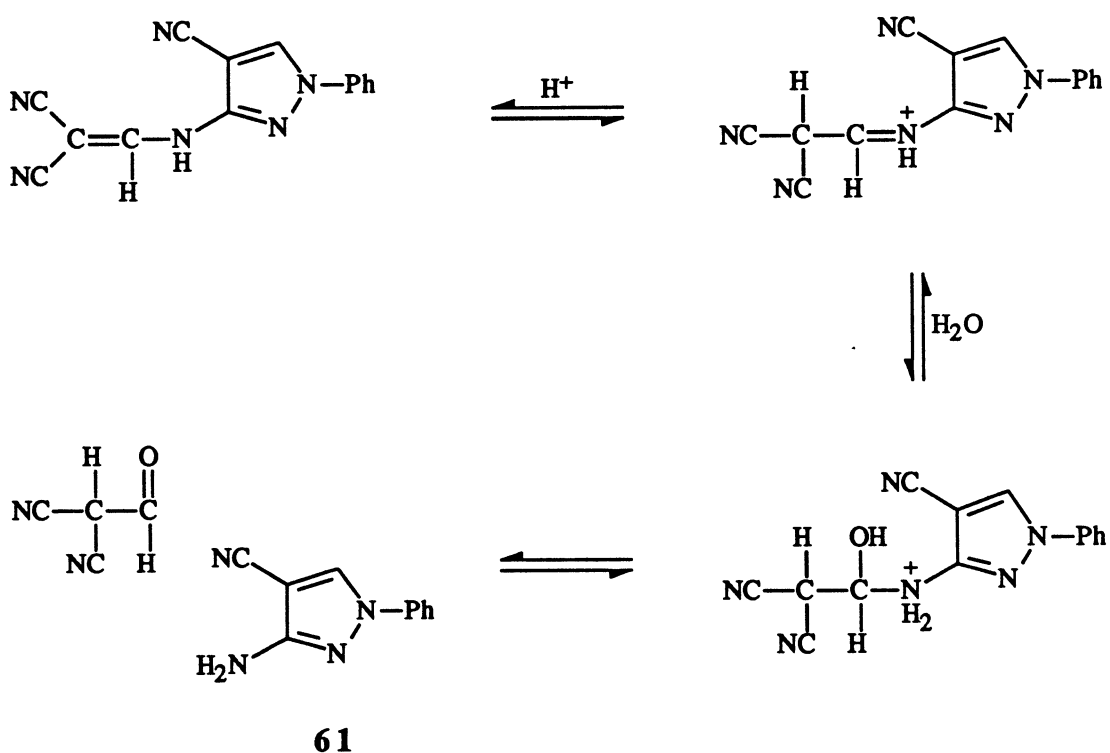
Scheme 37

The attack of a water molecule on the α carbon of the immonium ion generates a carbinolamine. The cleavage of the carbon-nitrogen bond of the carbinolamine results in the formation of the amine (61) and an aldehyde. In weakly acidic solutions, the water

attack on the immonium ion is the rate limiting step. However, in strongly acidic solutions, the decomposition of the carbinolamine is the rate limiting step. This suggests that the N-protonated carbinolamine loses a proton to form a neutral or zwitterionic carbinolamine prior to decomposition into an amine and an aldehyde.

[6.2] Synthesis of 4-Imino-5-methyl-2-phenyl-7H-pyrazolo[3,4-d]pyrimidin-6-one

The cyclisation of 3-amino-4-cyano-1-phenylpyrazole with methyl isocyanate was attempted using the one step synthesis which was developed earlier. The reactants were stirred with sodium methoxide in DMF at 60 °C for 4 hours. Neutralisation with 1 M hydrochloric acid and evaporation of the solvent yielded a crude solid. Recrystallisation from DMSO and water afforded pure pyrazolo[3,4-d]pyrimidine (62, Scheme 38).

61   62
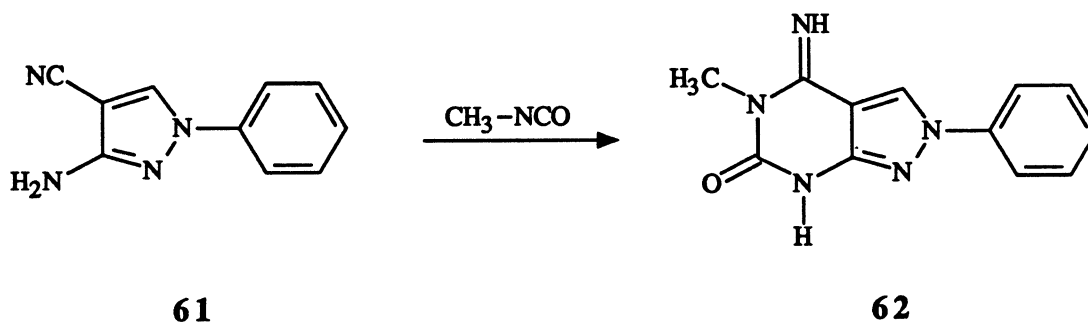
Scheme 38

The IR spectrum showed the disappearance of the nitrile peak at 2210 cm^{-1}. The carbonyl stretch appeared at 1720 cm^{-1} and the NH stretches appeared at 3120 and 3450 cm^{-1}. The ^{1}H NMR spectrum contained a three proton singlet at δ 2.87 for the methyl group and two exchangable one proton singlets at δ 8.24 and 11.27 for the NH protons. The spectrum also contained a one proton singlet at δ 9.02 for the C-3 proton of the pyrazolo[3,4-d]pyrimidine ring and a five proton multiplet from δ 7.31 to 7.73 for the

aromatic protons of the phenyl ring. The ^{13}C NMR spectrum contained a peak at δ 26.8 for the methyl carbon. There were also peaks at δ 102.7 (C-3a), 128.2 (C-3), 150.0 (C-4), 151.5 (C-7a) and 158.8 (C-6) for the carbons of the pyrazolo[3,4-d]pyrimidine ring. The remainder of the spectrum contained peaks at δ 119.0 (C-2', C-6'), 127.4 (C-4'), 129.7 (C-3', C-5') and 138.9 (C-1') for the carbons of the phenyl ring. This structure was confirmed by microanalysis and high resolution mass spectrometry.

[6.3] Biological Activity

The A_1 adenosine receptor affinity was measured using a $[^3H]$ R-PIA competitive binding assay (Table 11).

Table 11   Adenosine Receptor Affinity of the 2-Phenyl

Analogue of 1-Methylisoguanosine

No.	% Inhibition
24a	30
62	41

4-Imino-5-methyl-2-phenyl-7H-pyrazolo[3,4-d]pyrimidin-6-one (62) was found to possess some adenosine receptor affinity. The only structural modifications from 4-amino-5-methyl-1-phenylpyrazolo[3,4-d]pyrimidin-6-one (24a) were the imine substituent in the C-4 position and the phenyl ring in the N-2 position. Studies on 1-methylisoguanosine analogues have suggested that the 6-amino group is necessary for maximum adenosine receptor affinity.[20] Even though this analogue contained an imino group in this position, it still possessed slightly greater adenosine receptor affinity than

24a. This suggests that the positioning of the phenyl substituent in the N-2 position was favourable for interaction with the adenosine receptor.

CHAPTER SEVEN

4,6-Substituted Pyrazolo[3,4-*d*]pyrimidine
Analogues of DJB-KK

[7.0] General Introduction

A number of 4,6-dialkylthiopyrazolo[3,4-d]pyrimidines have been reported to possess A$_1$ adenosine receptor affinity.[26,27] 4,6-Bis-α-carbamoylethylthio-1-phenylthiopyrazolo[3,4-d]pyrimidine (DJB-KK, 9) was found to be approximately 40 times more potent than theophylline as an adenosine antagonist. A related series of 4,6-dialkylthiotriazolo[4,5-d]pyrimidines were examined with different C-4 and C-6 substituents.[134] The effect of methylthio, propylthio, isopropylthio, α-carbamoylethylthio and α-carbamoylpropylthio substituents on A$_1$ adenosine receptor affinity was measured using a [^{3}H] R-PIA binding assay. The α-carbamoylpropylthio group showed the greatest activity.

The reaction of *ortho*-aminonitriles with carbon disulphide in base has provided a convenient synthesis of fused pyrimidine dithiones.[135,136] The reaction of 5-amino-4-cyanopyrazoles with carbon disulphide in sodium methoxide has yielded 1-substituted 5H,7H-pyrazolo[3,4-d]pyrimidin-4,6-dithiones. Alkylation of these dithiones with a variety of alkyl halides has produced a number of novel adenosine antagonists. Thus, the reaction of 1-phenyl-5H,7H-pyrazolo[3,4-d]pyrimidin-4,6-dithione with 2-bromopropionamide in aqueous alkali yielded DJB-KK (9).[42]

The synthesis of mono-alkylated compounds would pin point which α-carbamoylethylthio group of DJB-KK (9) was necessary for adenosine receptor affinity. The reaction of 1-phenyl-5H,7H-pyrazolo[3,4-d]pyrimidin-4,6-dithione with one molar equivalent of 2-bromopropionamide in aqueous alkali yielded 6-α-carbamoylethylthio-1-phenyl-4-mercaptopyrazolo[3,4-d]pyrimidine (63). The site of alkylation was determined by chemical modification and confirmed by NMR experimentation. This compound was used to evaluate the effect of an

α-carbamoylethylthio group in the C-6 position on the A_1 adenosine receptor affinity. Only one similar 1-substituted 6-alkylthio-4-mercaptopyrazolo[3,4-*d*]pyrimidine has been synthesised. 1-Benzyl-6-methylthio-4-mercaptopyrazolo[3,4-*d*]pyrimidine (65) was tested for anti-thrombotic activity.[137]
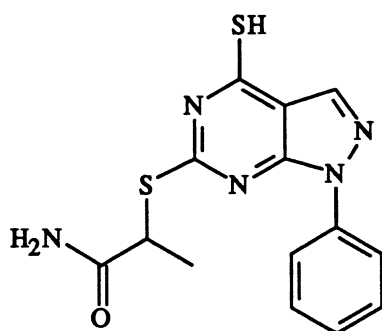
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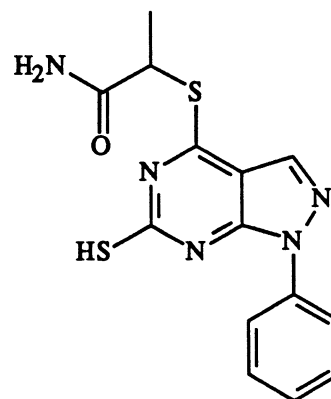
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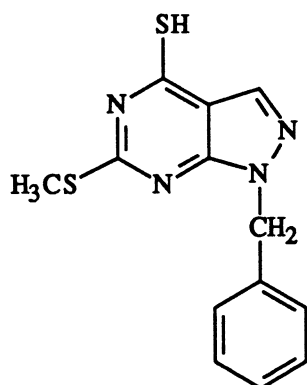
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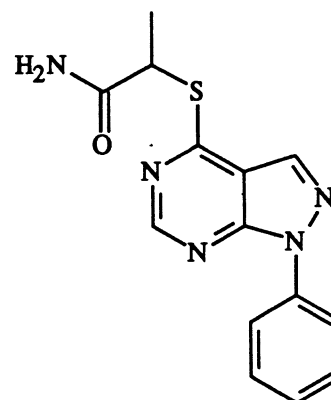
66

The reaction of 1-phenyl-5*H*-pyrazolo[3,4-*d*]pyrimidin-4-thione with 2-bromopropionamide in aqueous alkali yielded 4-α-carbamoylethylthio-1-phenylpyrazolo[3,4-*d*]pyrimidine (66). This compound was used to evaluate the effect of an α-carbamoylethylthio group in the C-4 position on the A_1 adenosine receptor

affinity. A number of similar 1-substituted 4-alkylthiopyrazolo[3,4-*d*]pyrimidines were tested for adenosine antagonistic activity.[27] None of these analogues contained both 1-phenyl and 4-α-carbamoylethylthio substituents.

[7.1] Synthesis of C-4 and C-6 Analogues of DJB-KK

2-Bromopropionyl bromide was converted to 2-bromopropionamide (67a) using a literature procedure.[138] The addition of 2-bromopropionyl bromide to ammonium hydroxide at 0 °C resulted in the rapid precipitation of a white solid. The product was collected and recrystallised from water. The IR spectrum showed the amide NH stretches at 3190 and 3350 cm^{-1} and the amide carbonyl stretch at 1665 cm^{-1}. The ^{1}H NMR spectrum contained a three proton triplet at δ 1.62 and a one proton quartet at δ 4.43 for the protons of the CHCH$_3$ fragment. There were also two, one proton singlets at δ 7.22 and 7.65 for the NH protons of the amide. The ^{13}C NMR spectrum contained peaks at δ 21.6 (CH$_3$), 44.0 (CH) and 170.8 (C=O). 2-Bromobutanamide (67b) was prepared using the same procedure.

The alkylation of 1-phenyl-5*H*,7*H*-pyrazolo[3,4-*d*]pyrimidin-4,6-dithione with two molar equivalents of 2-bromopropionamide produced DJB-KK (9a). The dithione and two equivalents of 2-bromopropionamide were stirred at room temperature in dilute sodium hydroxide. The product precipitated and was recrystallised from DMSO and water. The IR spectrum showed the NH stretch at 3435 cm^{-1} and the carbonyl stretch at 1700 cm^{-1}. The ^{1}H NMR spectrum contained two overlapping three proton doublets at δ 1.56 coupled to two, one proton quartets at δ 4.47 and 4.78 for the CH$_3$CH protons of the propionamide side chains. There were also four exchangable one proton singlets at δ 7.27, 7.35, 7.70 and 7.77 for the NH protons of the propionamide side chains. The remainder of the spectrum contained a one proton singlet at δ 8.50 for the C-3 proton of

the pyrimidine ring and a five proton multiplet at δ 7.38 to 8.16 for the aromatic protons

of the phenyl ring. The ^{13}C·NMR spectrum contained peaks at δ 18.1 (CH_3), 18.8

(CH_3), 42.7 (CH), 44.0 (CH) , 172.3 (C=O) and 172.8 (C=O) for the propionamide

carbons. The remainder of the spectrum contained peaks at δ 110.2 (C-3a), 133.8 (C-3),

151.1 (C-7a), 164.4 (C-4) and 168.1 (C-6) for the carbons of the

pyrazolo[3,4-d]pyrimidine ring and peaks at δ 120.9 (C-2', C-6'), 126.8 (C-4'), 129.4

(C-3', C-5') and 138.2 (C-1') for the carbons of the phenyl ring. The NMR spectra

showed twin peaks for the propionamide signals as a result of the presence of a 1 : 1.7

ratio of diasteriomers. This method was also used to prepare 4,6-bis-α-

carbamoylpropyl-1-phenylpyrazolo[3,4-d]pyrimidine (9b).

[7.2] Synthesis of C-6 Analogues of DJB-KK

The alkylation of 1-phenyl-5H,7H-pyrazolo[3,4-d]pyrimidin-4,6-dithione with one

molar equivalent of 2-bromopropionamide produced an analogue of DJB-KK with only

one α-carbamoylethylthio group (63 or 64). The dithione and 2-bromopropionamide

were stirred at room temperature in dilute sodium hydroxide. Neutralisation with dilute

hydrochloric acid resulted in the precipitation of a white solid. The product was

recrystallised from DMSO and water. The IR spectrum showed the NH stretches at

2850, 2980, 3200 and 3395 cm⁻¹ and the carbonyl stretch at 1680 cm⁻¹. The ^1H NMR

spectrum contained a three proton doublet at δ 1.56 coupled to a one proton quartet at

δ 4.43 for the CH_3CH protons and two exchangable one proton singlets at δ 7.34 and

7.79 for the NH protons of the propionamide side chain. There was also a one proton

singlet at δ 11.69 for the thiol proton. The remainder of the spectrum contained a one

proton singlet at δ 8.35 for the C-3 proton of the pyrimidine ring and a five proton

multiplet at δ 7.37 to 8.08 for the aromatic protons of the phenyl ring. The ^{13}C NMR

spectrum contained peaks at δ 18.1 (CH₃), 44.7 (CH) and 172.0 (C=O) for the propionamide carbons. The remainder of the spectrum contained peaks at δ 116.4 (C-3a), 138.2 (C-3), 146.5 (C-7a), 160.2 (C-4) and 180.1 (C-6) for the carbons of the pyrazolo[3,4-*d*]pyrimidine ring and peaks at δ 121.3 (C-2', C-6'), 127.1 (C-4'), 129.4 (C-3', C-5') and 138.0 (C-1') for the carbons of the phenyl ring. This method was also used to prepare the corresponding α-carbamoylpropylthio analogue (63b).

The spectral data showed the presence of one 2-bromopropionamide side chain, but did not allow the assignment of the isomer (63 or 64). In purines, there is a chemical distinction between the C-2 and C-6 positions of the heterocyclic ring. Generally, the C-2 position is inert while the C-6 position is reactive to nucleophilic attack. The methylthio group in the C-2 position of 2-methylthio-6-aminopurine has been shown to be unreactive to displacement by ammonia or amines.[139,140] The methylthio group in the C-6 position of 2,6-bis-methylthiopurine has been selectively displaced by amines.[141] In pyrazolo[3,4-*d*]pyrimidines, there is an analogous chemical distinction between the C-4 and C-6 positions of the heterocyclic ring. The C-4 and C-6 positions of pyrazolo[3,4-*d*]pyrimidines corresponds to the C-6 and C-2 positions of purines due to differences in nomenclature. The methylthio group in the C-4 position of 4,6-bis-methylthio-1-(2,3,5-tri-o-acetyl-β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine has also been selectively displaced by ammonia.[142] In order to determine the structure of the mono-alkylated compound (63 or 64) it was necessary to methylate the remaining mercapto group and test which of the alkylthio groups could be displaced by ammonia.

The mono-alkylated compound (63 or 64) was stirred with methyl iodide in dilute sodium hydroxide at room temperature. A solid precipitated and was recrystallised from DMSO and water. This solid was found to be the required methylthio compound. The IR spectrum showed the NH stretches at 3370 and 3190 cm⁻¹ and the carbonyl stretch at

1658 cm^{-1}. In the ^{1}H NMR spectrum, the one proton singlet at δ 11.69 for the SH proton was replaced by a three proton singlet at δ 2.69 for the SCH$_3$ protons. The ^{13}C NMR spectrum contained an extra peak at δ 11.5 for the SCH$_3$ carbon.

The methylthio compound was heated to 100 °C in ethanolic ammonia in a bomb. Treatment with cold water resulted in the precipitation of a white solid. Recrystallisation from DMSO and water afforded an amino compound. The IR spectrum showed NH stretches at 3470, 3375, 3190 and 3075 cm^{-1} and the carbonyl stretch at 1658 cm^{-1}. In the ^{1}H NMR spectrum, the three proton singlet at δ 2.69 for the SCH$_3$ protons was replaced by two, one proton singlets at δ 7.92 and 8.06 for the amine protons. The three proton doublet at δ 1.56 and the one proton quartet at δ 4.47 for the CHCH$_3$ protons of the propionamide side chain remained. In the ^{13}C NMR spectrum, the peak at δ 11.5 for the SCH$_3$ carbon was no longer present.

The spectral information indicated that the methylthio group had been displaced by ammonia, while the α-carbamoylethylthio group had remained unchanged. By analogy with the literature examples, it was expected that the C-4 position would be the site of displacement by ammonia. Upon combining this information, it was concluded that the methylthio group must have been located in the C-4 position and the α-carbamoylethylthio group must have been located in the C-6 position (68, Scheme 39). Therefore, the α-carbamoylethylthio group of the precursor must have also been in the C-6 position.

SH

CH3I
NaOH

SCH3

63a

68

NH3

NH2

69
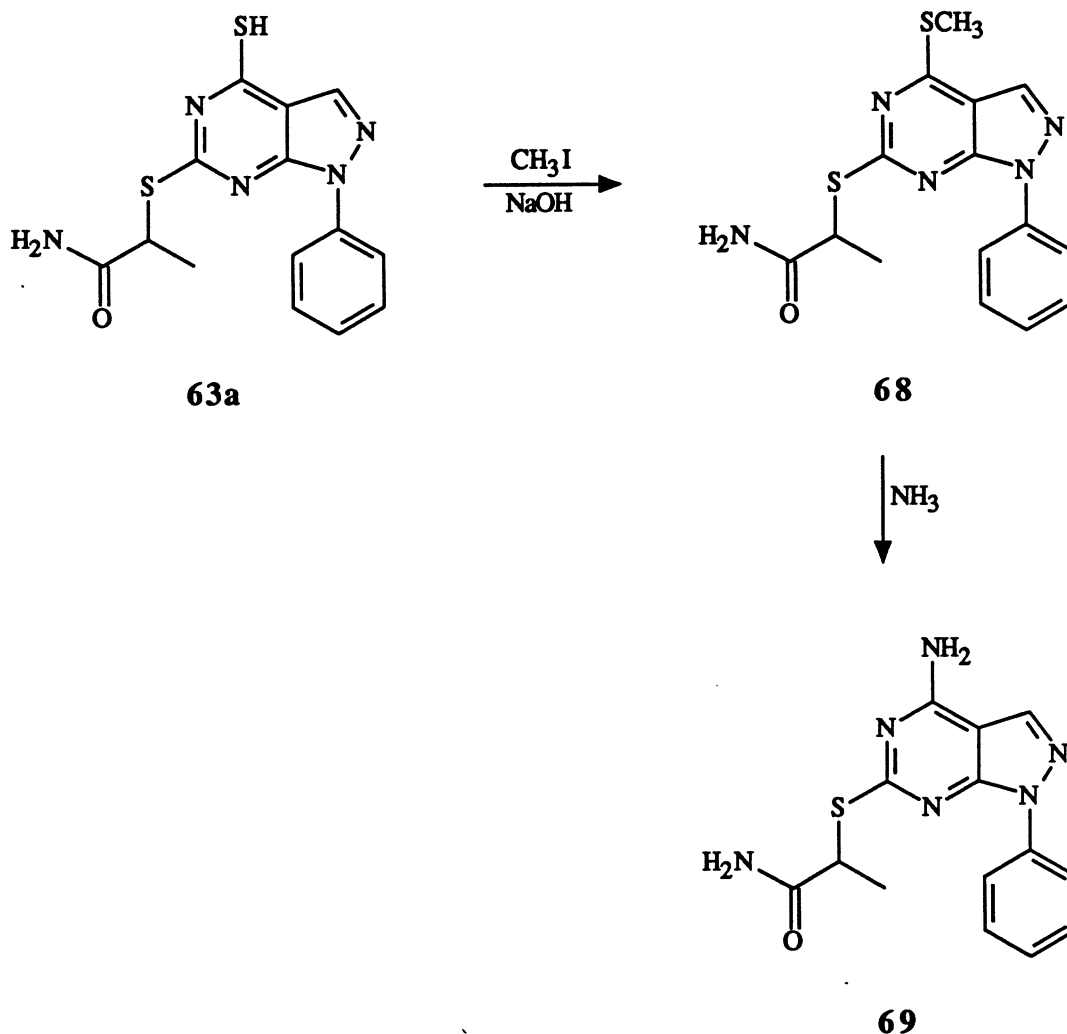
Scheme 39

This is not conclusive proof as some displacements of methylthio groups from the C-2 position of purines have been reported. A number of successful aminations of 2-methylthio-6-hydroxylpurine have been achieved. The nature of group in the C-6 position is believed to have some effect on the ease of displacement of the group in the C-2 position.

The observation of long-range ^1H - ^{13}C couplings may confirm the position of the α-carbamoylethylthio group. In both isomers (**63** and **64**) the olefinic proton (H-3) is

within 3 bonds of C-3a and C-4. In **63** the mercapto proton (SH) is also within 3 bonds

of C-3a and C-4, while in **64** the methine proton of the α-carbamoylethylthio group

(H-α) is also within 3 bonds of C-4. The two isomers could be distinguished from NMR

if all of the possible 2 and 3 bond couplings appeared. If H-3 correlates to the same

carbons as SH then the mercapto group must be in the C-4 position (**63**). Conversely, if

H-3 correlates to the same carbons as H-α then the α-carbamoylethylthio group must be

in the C-4 position (**64**). The SELINEPT[143] experiment was used to observe the long-

range couplings. This pulse sequence uses the refocused INEPT pulse sequence, but

with soft proton pulses to cause polarization transfer from a selected proton and with the

delays optimised for long-range ^{1}H - ^{13}C couplings. SELINEPT spectra were recorded

with selective irradiation at δ 8.35 (H-3), 11.69 (SH) and 4.43 (H-α). The olefinic

proton (H-3) was found to correlate to carbons at δ 116.4 (C-3a) and δ 146.5 (C-7a),

while the methine proton (H-α) correlated to a carbon at δ 160.2. The mercapto proton

(SH) showed no long-range coupling. The absence of further coupling was confirmed

by observation of a proton coupled carbon spectrum in which the peak at δ 180.1 (C-4 or

C-6) appeared as a sharp singlet and the peaks at δ 160.2 and δ 116.4 appeared as sharp

doublets. No distinction between the two isomers was possible from this evidence.

63   **64**
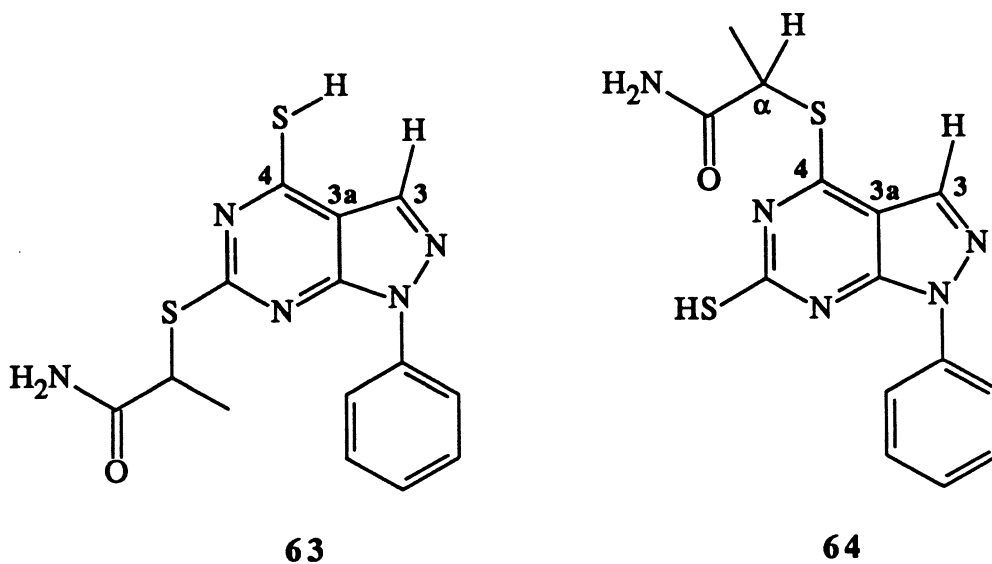
The proton coupled ^{13}C NMR spectrum of the amine compound (produced by methylation and aminolysis) contained a multiplet at δ 99.4 for C-3a. This indicated that this carbon shared a long-range coupling with more than one proton. Attempts to relate the amine protons to C-3a using SELINEPT or COLOC[144] experiments proved unsuccessful, although correlations between H-3 and C-3a and C-7a were observed. Selective decoupling of the ^{13}C NMR spectrum at the frequency of the amine protons resulted in C-3a resonating as a sharp doublet (J = 10 Hz), while selective decoupling at H-3 resulted in C-3a resonating as a triplet (J = 4 Hz). This confirmed the existence of long-range coupling between C-3a and the amine protons and verified that the amine group (and hence the thiol function of the original compound) was in the C-4 position.

[7.3] Synthesis of C-4 Analogues of DJB-KK

The alkylation of 1-phenyl-5H-pyrazolo[3,4-d]pyrimidin-4-thione (70)[145] with 2-bromopropionamide produced an analogue of DJB-KK with an α-carbamoylethylthio group in the C-4 position (66, Scheme 40). The thione and 2-bromopropionamide were stirred at room temperature in dilute sodium hydroxide. The product precipitated and was recrystallised from DMSO and water. The IR spectrum showed the NH stretches at 3470 and 3650 cm^{-1} and the carbonyl stretch at 1800 cm^{-1}. The ^{1}H NMR spectrum contained a three proton doublet at δ 1.58 coupled to a one proton quartet at δ 4.89 for the CH$_3$CH protons of the propionamide side chain. There were also two exchangable one proton singlets at δ 7.30 and 7.80 for the NH protons of the propionamide side chain. The remainder of the spectrum contained a one proton singlet at δ 8.61 for the C-3 proton of the pyrimidine ring and a five proton multiplet at δ 7.37 to 7.61 for the aromatic protons of the phenyl ring. The ^{13}C NMR spectrum contained peaks at δ 18.9 (CH$_3$), 42.4 (CH) and 172.3 (C=O) for the propionamide carbons. The remainder of the spectrum

contained peaks at δ 112.8 (C-3a), 133.4 (C-3), 150.6 (C-7a), 154.7 (C-6) and 164.6

(C-4) for the carbons of the pyrazolo[3,4-*d*]pyrimidine ring and peaks at δ 121.2

(C-2', C-6') and 126.9 (C-4'), 129.3 (C-3', C-5') and 138.0 (C-1') for the carbons of

the phenyl ring.
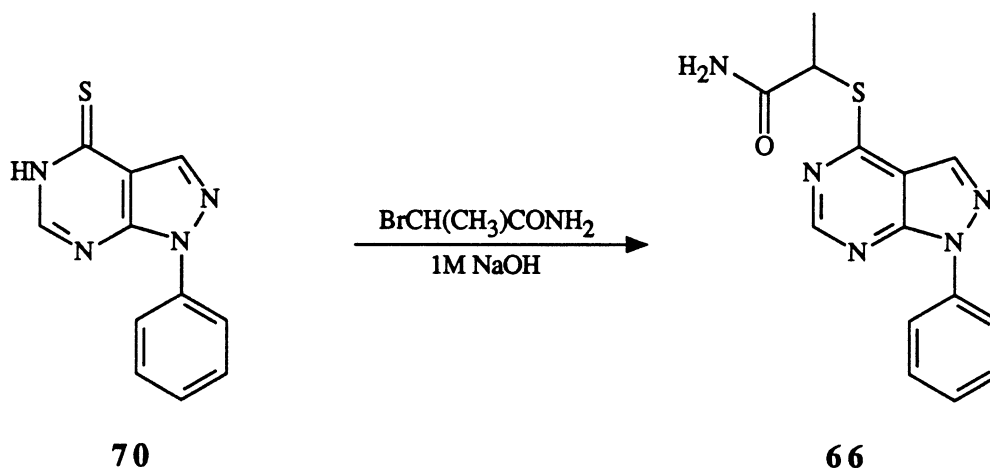
Scheme 40

[7.4] Biological Activity

The A_1 adenosine receptor affinity of all of the DJB-KK analogues was measured

using a [^{3}H] R-PIA competitive binding assay (Table 12). The effect of different

substituents (α-carbamoylethylthio versus α-carbamoylpropylthio) and different

positions of substitution (C-4 versus C-6) was evaluated.

(1) Bis-alkylated dithiones, which contained α-carbamoylethylthio (9a) and

α-carbamoylpropylthio (9b) substituents in the C-4 and C-6 positions, were prepared.

The α-carbamoylpropylthio group produced slightly greater adenosine receptor affinity.

Mono-alkylated dithiones, which contained an α-carbamoylethylthio (63a) and an

α-carbamoylpropylthio (63b) substituent in the C-6 position, were also prepared.

Again, the α-carbamoylpropylthio group produced slightly greater adenosine receptor

affinity. It was concluded that the extra carbon of the α-carbamoylpropylthio group was favourable for interaction with the adenosine receptor.

Table 12 Adenosine Receptor Affinity of the DJB-KK Analogues
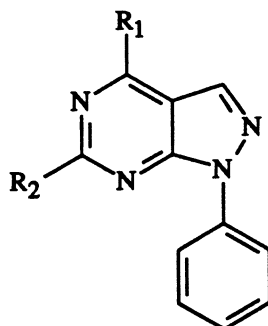
No.	R_1	R_2	% I	IC_{50}
9a	$SCH(Me)CONH_2$	$SCH(Me)CONH_2$	95	$4.41 (\pm 1.51) \times 10^{-7}$
9b	$SCH(Et)CONH_2$	$SCH(Et)CONH_2$	96	$1.08 (\pm 0.01) \times 10^{-7}$
63a	SH	$SCH(Me)CONH_2$	94	$8.14 (\pm 0.86) \times 10^{-7}$
63b	SH	$SCH(Et)CONH_2$	97	$4.92 (\pm 1.06) \times 10^{-7}$
66	$SCH(Me)CONH_2$	H	38	$3.64 (\pm 0.06) \times 10^{-5}$
68	SMe	$SCH(Me)CONH_2$	100	$5.61 (\pm 1.06) \times 10^{-8}$
69	NH_2	$SCH(Me)CONH_2$	100	$3.23 (\pm 0.57) \times 10^{-8}$

(2) The adenosine receptor affinity of the mono-alkylated dithione (63a) was in the same order of magnitude as DJB-KK. An alkylated thione (66), which contained an α-carbamoylethylthio substitutent in the C-4 position, was prepared. The adenosine receptor affinity of this compound was greatly reduced. It was concluded that the 6-α-carbamoylethylthio group was largely responsible for the adenosine receptor affinity. It should be noted that the alkylated thione (66) has no mercapto group in the C-6 position, preventing a direct comparison of 63a and 66.

Interestingly, the methylthio (68) and amino (69) analogues possessed the greatest adenosine receptor affinity.

CHAPTER EIGHT

Experimental

[8.0] General Experimental

Solvents were analytical grade or were distilled through a column of glass helices. DMF was dried by standing with magnesium sulphate and distillation under reduced pressure. Absolute ethanol was dried by reflux and distillation over magnesium ethoxide (prepared from ethanol, magnesium turnings and a catalytic amount of iodine). Pyridine was dried by reflux and distillation over potassium hydroxide and then sodium wire. Toluene was dried by standing with calcium chloride and then distillation over sodium wire. THF was dried by reflux and distillation over lithium aluminium hydride and then sodium sand. Dry DMF, pyridine and THF were stored over 4 Å molecular sieves, while dry ethanol was stored over 3 Å molecular sieves. Dry toluene was stored over sodium wire. Solvents were evaporated under reduced pressure on a Buchi rotary evaporator. The vacuum was provided by water jacket and Javac JDX-120 vacuum pumps. Traces of solvents were evaporated on a high vacuum system.

Thin layer chromatography was performed using glass slides or plates which were coated with Merck Kieselgel 60 HF$_{254}$. Flash chromatography was performed using glass columns, wet packed with Merck Kieselgel 60 (230-400 mesh). Compounds were visualised using UV light, iodine vapour or 5 % vanillin/5 % sulphuric acid sprays. High performance liquid chromatography was performed using an ETP Kortec K35M pump, a Dynamax 60A ODS column and an ETP Kortec K95 variable wavelength UV detector.

Melting points were determined on a Buchi silicon oil bath melting point apparatus or a Gallenkamp electronic melting point apparatus and are uncorrected.

IR spectra were recorded on a Jasco IR-810 spectrophotometer. Smears on NaCl discs were used for liquids, while KBr pellets were used for solids. UV spectra were

recorded on a Varian 634 series spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded at 250.12 and 62.8 MHz on a Bruker WM-250 spectrometer. Off resonance ^{13}C NMR and DEPT experiments were used to confirm the carbon assignment. The following abbreviations describe the type of carbon: s = quaternary, d = methine, t = methylene and q = methyl. SINEPT and COLOC experiment were recorded by Mr David Tucker on a Bruker AC-300 spectrometer at the Chemistry Department, University of New England. Unless otherwise stated, deuterated DMSO was used as a solvent and tetramethylsilane as an internal standard. Low and high resolution mass spectra were recorded by Mr Graham McFarlane on a Kratos MS-25 spectrometer at the Chemistry Department , University of Queensland. Electron impact spectra were recorded at 70 eV and chemical ionisation spectra at 2.5 mA.

Microanalyses were performed by Dr Noel Jacobson at the Chemistry Department, University of Queensland or by the Australian Microanalytical Service.

Crystal structures were performed by Dr Graham Smith and Prof. Colin Kennard at the Chemistry Department, University of Queensland. Data was collected using an Enraf-Nonius CAD-4 four circle diffractometer in the conventional $2\theta/\theta$ scan mode to $2\theta_{max} = 50°$. N independant reflections were obtained and N_0 reflections with I > 2.5 σ (I) were used in structure refinement. Crystal monochromatised MoK_α radiation was used, with no absorption corrections applied to the data. The structure was solved using SHELXS 86[143] and was refined using SHELX 76[144] with neutral atom scattering factors and f', f'' terms for anomalous dispersion.[145] Full-matrix least-squares refinement with anisotropic thermal parameters on all non-hydrogens gave R and R_w. Hydrogens were located by difference methods and their positional and isotropic thermal parameters refined.

[8.1] Chemical Experimental

4,6-Bis-alkylthio-1-phenylpyrazolo[3,4-*d*]pyrimidine (9a,b)

1-Phenyl-5*H*,7*H*-pyrazolo[3,4-*d*]pyrimidin-4,6-dithione (0.50 g, 1.9 mmol) was dissolved in sodium hydroxide (1 M, 10 ml). 2-Bromopropionamide (0.58 g, 3.8 mmol) was added and the reaction was stirred at room temperature for 24 hours. A solid precipitated and was collected by suction filtration. Recrystallisation of the crude product from DMSO and water afforded pure 4,6-bis-α-carbamoylethylthio-1-phenylpyrazolo[3,4-*d*]pyrimidine (9a). The use of 2-bromobutanamide allowed the synthesis of 4,6-bis-α-carbamoylpropylthio-1-phenylpyrazolo[3,4-*d*]pyrimidine (9b).

9a: yield 79 %; mp 213-215 °C; ^{1}H NMR δ 1.56 (overlapping d, 6, CH_3), 4.47 (overlapping q, 1, CH), 4.78 (overlapping q, 1, CH), 7.27 (br s, 1, NH), 7.35 (br s, 1, NH), 7.38-8.16 (m, 5, CH_{arom}), 7.70 (br s, 1, NH), 7.77 (br s, 1, NH), 8.50 (s, 1, H-3); ^{13}C NMR δ 18.1 (q, CH_3), 18.2 (q, CH_3), 18.8 (q, CH_3), 18.9 (q, CH_3), 42.7 (d, CH), 42.8 (d, CH), 44.0 (d, CH), 44.1 (d, CH), 110.2 (s, C-3a), 120.9 (d, C-2', C-6'), 126.8 (d, C-4'), 129.4 (d, C-3', C-5'), 133.8 (d, C-3), 138.2 (s, C-1'), 151.1 (s, C-7a), 164.4 (s, C-4), 168.1 (s, C-6), 172.3 (s, C=O), 172.6 (s, C=O); IR 3435, 1700 cm^{-1}.

9b: yield 71 %; mp 195-198 °C; ^{1}H NMR δ 0.98 (t, 3, J = 7.2 Hz, CH_3), 1.01 (t, 3, J = 7.2 Hz, CH_3), 1.94 (m, 4, 2 x CH_2), 4.34 (q, 1, J = 6.8 Hz, CH), 4.70 (q, 1, J = 6.8Hz, CH), 7.32-8.17 (m, 9, CH_{arom} and 2 x NH_2), 8.50 (s, 1, H-3); ^{13}C NMR δ 11.1 (q, CH_3), 11.6 (q, CH_3), 25.5 (t, CH_2), 25.9 (t, CH_2), 48.5 (d, CH), 50.4 (d, CH), 110.2 (s, C-3a), 120.8 (d, C-2', C-6'), 126.8 (d, C-4'), 129.3 (d, C-3', C-5'), 133.8 (d, C-3), 138.2 (s, C-1'), 151.1 (s, C-7a), 164.5 (s, C-4), 168.2 (s, C-6), 171.5 (s, C=O), 172.0 (s, C=O); IR 3360, 3200, 1660, 1650 cm^{-1}; Anal. Calculated for $C_{19}H_{22}N_6O_2S_2$: C, 53.01; H, 5.15; N, 19.5. Found: C, 53.1; H 4.9; N, 19.5.

1-Substituted 5-Amino-4-cyanopyrazoles (10a-s)

<u>Method A</u>: Ethoxymethylenemalononitrile (8.0 g, 66 mmol) and phenylhydrazine (7.1 g, 66 mmol) were refluxed in dry ethanol for 1 hour. Upon cooling, a solid precipitated and was collected by suction filtration. Recrystallisation of the crude product from ethanol afforded pure 5-amino-4-cyano-1-phenylpyrazole (10a). The use of different hydrazines allowed the synthesis of the 5-amino-4-cyanopyrazoles, 10b-s.

<u>Method B</u>: Malononitrile (27.5 g, 0.42 mol), triethyl orthoformate (61.7 g, 0.42 mol) and phenylhydrazine (30.0 g, 0.28 mol) were refluxed in dry ethanol (100 ml) for 2 hours. During this time the colour darkened to brown. The reaction mixture was cooled and the solvent was evaporated. Any unreacted triethyl orthoformate was evaporated under high vacuum. Flash chromatography of the crude product using methylene chloride as an eluent afforded 5-amino-4-cyano-1-phenylpyrazole (10a). The product was further recrystallised from ethanol. The use of different hydrazines allowed the synthesis of the 5-amino-4-cyanopyrazoles, 10a, d, n and q.

10a: R = C_6H_5; yield 71 % (Method A) and 58 % using (Method B); mp 138-139 °C; ^{1}H NMR δ 6.69 (br s, 2, NH_2), 7.41-7.57 (m, 5, CH_{arom}), 7.80 (s, 1, H-3); ^{13}C NMR δ 73.5, 114.7, 124.1, 127.8, 129.4, 137.4, 141.6, 151.2; IR 3340, 3220, 3050, 2220 cm^{-1}.

10b: R = C_6H_4Cl (2); yield 60 %; mp 139-140 °C; ^{1}H NMR δ 6.69 (br s, 2, NH_2), 7.49-7.69 (m, 4, CH_{arom}), 7.77 (s, 1, H-3); ^{13}C NMR δ 71.7, 115.1, 128.5, 130.6, 131.6, 132.0, 134.5, 142.0, 152.7; IR 3360, 3190, 2220 cm^{-1}.

10c: R = C_6H_4Cl (3); yield 67 %; mp 186-187 °C; ^{1}H NMR δ 6.66 (br s, 2, NH_2), 7.45-7.57 (m, 4, CH_{arom}), 7.81 (s, 1, H-3); ^{13}C NMR 73.7, 114.6, 122.9, 124.1, 127.7, 131.2, 133.7, 138.6, 142.2, 151.7; IR 3460, 3320, 3225, 2240 cm^{-1}.

10d: C_6H_4Cl (4); yield 42 % (Method A) and 44 % (Method B); mp 168.5-170 °C; ^{1}H NMR δ 6.77 (br s, 2, NH$_2$), 7.50-7.60 (m, 4, CH$_{arom}$), 7.80, (s, 1, H-3); ^{13}C NMR δ 73.6, 114.5, 126.0, 129.4, 132.2, 136.4, 142.0, 151.5; IR 3450, 3300, 3190, 2225, cm^{-1}.

10e: R = C_6H_4Br (2); yield 62 %; mp 134-135 °C; ^{1}H NMR δ 6.64 (br s, 2, NH$_2$), 7.44-7.83 (m, 4, CH$_{arom}$), 7.75 (s, 1, H-3); ^{13}C NMR δ 71.9, 114.7, 122.1, 128.9, 130.4, 131.6, 133.4, 136.0, 141.6, 152.4; IR 3360, 3190, 2220 cm^{-1}.

10f: R = C_6H_4Br (3); yield 68 %; mp 191-192 °C; ^{1}H NMR δ 6.86 (br s, 2, NH$_2$), 7.43-7.70 (m, 4, CH$_{arom}$), 7.81 (s, 1, H-3); ^{13}C NMR δ 73.7, 114.5, 121.9, 123.2, 126.6, 130.7, 131.3, 138.9, 142.2, 151.6; IR 3460, 3300, 2240 cm^{-1}.

10g: R = C_6H_4Br (4); yield 67 %; mp 178-180 °C; ^{1}H NMR δ 6.79 (br s, 2, NH$_2$), 7.44-7.73 (m, 4, CH$_{arom}$), 7.80 (s, 1, H-3); ^{13}C NMR δ 73.7, 114.6, 120.7, 126.3, 132.4, 136.8, 142.0, 151.5; IR 3360, 3210, 2220 cm^{-1}.

10h: R = C_6H_4F (2); yield 40 %; mp 149-151 °C; ^{1}H NMR δ 6.77 (br s, 2, NH$_2$), 7.31-7.61 (m, 4, CH$_{arom}$), 7.78 (s, 1, H-3); ^{13}C NMR δ 72.1, 114.8, 117.0, 124.8, 125.3, 129.5, 131.5, 142.3, 152.8, 157.0; IR 3370, 3240, 2245 cm^{-1}.

10i: R = C_6H_4F (3); yield 68 %; mp 163-165 °C; ^{1}H NMR δ 6.85 (br s, 2, NH$_2$), 7.23-7.60 (m, 4, CH$_{arom}$), 7.81 (s, 1, H-3); ^{13}C NMR δ 73.7, 111.5, 114.7, 114.8, 120.1, 131.2, 138.9, 142.1, 151.6, 162.3; IR 3460, 3310, 3220, 2230 cm^{-1}.

10j: R = C_6H_4F (4); yield 56 %; mp 183-185 °C; ^{1}H NMR δ 6.71 (br s, 2, NH$_2$), 7.31-7.55 (m, 4, CH$_{arom}$), 7.77 (s, 1, H-3); ^{13}C NMR δ 73.3, 114.7, 116.3, 126.9, 133.9, 141.7, 151.6, 161.3; IR 3430, 3330, 3240, 2230 cm^{-1}.

10k: R = $C_6H_4NO_2$ (2); yield 33 %; mp 179-180.5 °C; ^{1}H NMR δ 6.93 (br s, 2, NH_2), 7.69-8.16 (m, 4, CH_{arom}), 7.78 (s, 1, H-3); ^{13}C NMR δ 72.7, 114.6, 125.5, 129.6, 130.0, 130.6, 134.5, 142.7, 145.7, 152.9; IR 3350, 3190, 2220, 1522, 1340 cm^{-1}.

10l: R = $C_6H_4NO_2$ (3); yield 68 %; mp 154-156 °C; ^{1}H NMR δ 7.01 (br s, 2, NH_2), 7.77-8.30 (m, 4, CH_{arom}), 7.87 (s, 1, H-3); ^{13}C NMR δ 74.0, 114.4, 118.8, 122.3, 130.3, 131.0, 138.3, 142.6, 148.3, 151.9; IR 3350, 3200, 2220, 1535, 1345 cm^{-1}.

10m: R = $C_6H_4NO_2$ (4); yield 37 %; mp 225-226.5 °C; ^{1}H NMR δ 7.06 (br s, 2, NH_2), 7.80-8.38 (m, 4, CH_{arom}), 7.91 (s, 1, H-3); ^{13}C NMR δ 74.5, 114.2, 124.2, 124.9, 142.8, 143.0, 145.9, 152.0; IR 3450, 3320, 2230, 1518, 1350 cm^{-1}.

10n: R = CH_3; yield 78 % (Method A) and 17 % (Method B); mp 222.5-223.5 °C; ^{1}H NMR δ 3.34 (s, 3, CH_3), 6.54 (br s, 2, NH_2), 7.49 (s, 1, H-3); ^{13}C NMR δ 34.4, 72.2, 115.2, 139.8, 151.6; IR 3375, 3315, 3140, 2200 cm^{-1}.

10p: R = C_3H_7; yield 47 %; mp 163-164.5 °C; ^{1}H NMR δ 0.80 (t, 3, J = 7.4 Hz, CH_3), 1.63 (m, 2, J = 7.1, 7.4 Hz, $CH_3CH_2CH_2$), 3.81 (t, 2, J=7.1 Hz, $CH_2CH_2CH_3$), 6.55 (br s, 2, NH_2), 7.51 (s, 1, H-3); ^{13}C NMR δ 10.6, 21.7, 48.0, 72.0, 115.3, 139.9, 151.3; IR 3400, 3330, 3160, 2200 cm^{-1}.

10q: R = $C_6H_3Cl_2$ (2,4); yield 64 % (Method A) and 51 % (Method B); mp 141-142 °C; ^{1}H NMR δ 6.77 (br s, 2, NH_2), 7.52-7.88 (m, 3, CH_{arom}), 7.78 (s, 1, H-3); ^{13}C NMR δ 71.8, 114.7, 128.6, 130.0, 131.7, 133.2, 133.6, 135.2, 142.1, 152.8; IR 3350, 3200, 2220 cm^{-1}.

10r: R = $C_6H_3Cl_2$ (2,5); yield 82 %; mp 201-204 °C; ^{1}H NMR δ 6.81 (br s, 2, NH_2), 7.62-7.73 (m, 3, CH_{arom}), 7.78 (s, 1, H-3); ^{13}C NMR δ 71.8, 114.8, 130.5, 131.0, 131.5, 131.8, 132.3, 135.6, 142.3, 152.9; IR 3340, 3160, 2230 cm^{-1}.

10s: R = $C_6H_3Cl_2$ (3,5); yield 69 %; mp 166-168 °C; ^{1}H NMR δ 6.99 (br s, 2, NH_2), 7.57-7.68 (m, 3, CHarom), 7.83 (s, 1, H-3); ^{13}C NMR δ 73.9, 114.4, 123.0, 127.4, 134.7, 139.5, 142.6, 151.9; IR 3420, 3340, 3240, 2220 cm^{-1}.

1-Phenyl-5H,7H-pyrazolo[3,4-d]pyrimidin-4,6-dithione (11)

5-Amino-4-cyano-1-phenylpyrazole (10.0 g, 54.3 mmol) and sodium methoxide (5.87 g, 0.12 mol) were dissolved in DMF (100 ml). Carbon disulphide (97.9 ml, 1.63 mol) was added and the reaction was refluxed for 48 hours. The solvent was evaporated under reduced pressure and sodium hydroxide (1 M, 50 ml) was added. The resulting clear solution was filtered and then neutralised with 1 M hydrochloric acid. Upon neutralisation, a solid precipitated and was collected by suction filtration. Recrystallisation of the crude product from DMSO and water afforded pure 1-phenyl-5H,7H-pyrazolo[3,4-d]pyrimidin-4,6-dithione (11): yield 97 %; mp 261-264 °C; ^{1}H NMR δ 7.32-7.64 (m, 5, CH_{arom}), 8.20 (s, 1, H-3), 8.31 (br s, 1, SH), 13.31 (br s, 1, SH)

Ethoxymethylenemalononitrile (17)

Method A: Malononitrile (6.0 g, 91 mmol) and triethyl orthoformate (13.46 g, 91 mmol) were dissolved in toluene (15 ml). A catalytic amount of boron trifluoride etherate (10 μl) was added and the reaction mixture was stirred at 80 °C for 3 hours. Ethanol was removed from the reaction via a Dean-Stark trap. The solvent was evaporated and the crude product was distilled under high vacuum (130-132 °C, 1.5 mmHg) to afford pure ethoxymethylenemalononitrile (17).

<u>Method B</u>: Malononitrile (6.0 g, 91 mmol) and triethyl orthoformate (13.46 g, 91 mmol) were dissolved in toluene (15 ml). A catalytic amount of zinc chloride (10 mg) was added and the reaction mixture was stirred at 80 °C for 4 hours. Ethanol was removed from the reaction via a Dean-Stark trap. Evaporation of the solvent and distillation of the crude product afforded pure **17**.

<u>Method C</u>: Malononitrile (40.0 g, 606 mmol) and triethyl orthoformate (130.0 g, 877 mmol) were stirred at 100 °C in acetic anhydride (187 ml) for 4 hours. The volatile by products (ethyl acetate and acetic acid) were distilled off at 140 °C. Distillation of the crude product afforded pure **17**.

17: yield 70 % (Method A), 74 % (Method B) and 93 % (Method C); mp 66-67 °C; ^{1}H NMR (CDCl$_3$) δ 1.22 (t, 3, J = 6Hz, CH$_3$), 3.69 (q, 2, J = 6 Hz, CH$_2$), 6.41 (s, 1, CH); ^{13}C NMR (CDCl$_3$) δ 14.9, 66.8, 74.9, 109.7, 109.7, 112.1, 174.3; IR 2220, 1610 cm^{-1}.

Phenylhydrazine hydrochloride (18a)

Aniline (20.4 g, 219 mmol) was suspended in concentrated hydrochloric acid (58 ml) and cooled to 0 °C. A solution of sodium nitrite (16.0 g, 231 mmol) in water (30 ml) and then sodium sulphite (123.0 g, mmol) in water (140 ml) were added dropwise. The internal temperature was kept below 5 °C during these additions. The reaction mixture was stirred for 1 hour at 60 °C and acidified slightly with concentrated hydrochloric acid. The reaction mixture was stirred for a further 12 hours at 60 °C before being quenched with concentrated hydrochloric acid (200 ml). A solid precipitated and was collected by suction filtration. Recrystallisation of the crude product from water afforded pure phenylhydrazine hydrochloride (**18a**): yield 78 %; mp 249-253 °C; ^{1}H NMR δ 6.88-7.28 (m, 5, CH$_{arom}$), 8.32 (br s, 1, NH), 10.37 (br s, 3, NH); ^{13}C NMR δ 114.6, 121.4, 128.9, 145.7; IR 3200, 3000, 2690 cm^{-1}.

Substituted Phenylhydrazine hydrochlorides (18b-j)

2-Chloroaniline (10.0 g, 78 mmol) was suspended in concentrated hydrochloric acid (100 ml). The aniline was diazotised with sodium nitrite (5.4 g, 78 mmol) in water (50 ml) and then reduced with stannous chloride (35.4 g, 156 mmol) in concentrated hydrochloric acid (35 ml). The reaction mixture was stirred at 0 °C for 1 hour and then at room temperature for 1 hour. A solid precipitated and was collected by suction filtration. Recrystallisation of the crude product from ethanol afforded pure 2-chlorophenylhydrazine hydrochloride (18b). The use of different anilines allowed the synthesis of the hydrazine hydrochlorides, 18c-m.

18b: R = C_6H_4Cl (2); yield 82 %; mp 200-201 °C dec; ^{1}H NMR δ 6.92-7.41 (m, 4, CH_{arom}), 8.07 (br s, 1, NH), 10.49 (br s, 3, NH); ^{13}C NMR δ 115.0, 119.6, 122.4, 127.7, 129.5, 141.3; IR 2825 cm^{-1}.

18c: R = C_6H_4Cl (3); yield 79 %; mp 240-243 °C dec; ^{1}H NMR δ 6.90-7.38 (m, 4, CH_{arom}), 8.62 (br s, 1, NH), 10.40 (br s, 3, NH); ^{13}C NMR δ 113.1, 114.0, 120.8, 130.6, 133.5, 147.3; IR 3030 cm^{-1}.

18d: R = C_6H_4Cl (4); yield 83 %; mp 225-227 °C; ^{1}H NMR δ 6.97-7.34 (m, 4, CH_{arom}), 8.47 (br s, 1, NH), 10.34 (br s, 3, NH); ^{13}C NMR δ 116.2, 125.1, 128.7, 144.6; IR 2960 cm^{-1}.

18e: R = C_6H_4Br (2); recrystallised from propan-2-ol; yield 49 %; mp 189-201 °C dec; ^{1}H NMR δ 6.86-7.56 (m, 4, CH_{arom}), 7.90 (br s, 1, NH), 10.44 (br s, 3, NH); ^{13}C NMR δ 109.6, 114.9, 123.0, 128.4, 132.7, 142.4; IR 3000 cm^{-1}.

18f: R = C_6H_4Br (3); recrystallised from propan-2-ol; yield 84 %; mp 231-233 °C; ^{1}H NMR δ 6.94-7.25 (m, 4, CH_{arom}), 8.63 (br s, 1, NH), 10.40 (br s, 3, NH); ^{13}C NMR δ 113.5, 116.7, 121.9, 123.8, 130.8, 147.3; IR 3000 cm^{-1}.

18g: R = C_6H_4Br (4); recrystallised from propan-2-ol; yield 63 %; mp 209-211 °C; ^1H NMR δ 6.92-7.44 (m, 4, CH_{arom}), 8.50 (br s, 1, NH), 10.41 (br s, 3, NH); ^{13}C NMR δ 112.8, 116.6, 131.6, 145.1; IR 3000 cm^{-1}.

18h-j: Fluorophenylhydrazines [R = C_6H_4F (2, 3 and 4)] were used directly to prevent decomposition upon standing.

18k: R = $C_6H_4NO_2$ (2); yield 31 %; mp 194-195 °C; ^1H NMR δ 7.03-8.15 (m, 4, CH_{arom}), 9.21 (br s, 1, NH), 10.32 (br s,3, NH); ^{13}C NMR δ 115.7, 120.3, 126.1, 133.9, 136.2, 141.3; IR 2950, 1522, 1330 cm^{-1}.

18l: R = $C_6H_4NO_2$ (3); yield 35 %; mp 210-212 °C; ^1H NMR δ 7.39-7.65 (m, 4, CH_{arom}), 9.04 (br s, 1, NH), 10.66 (br s, 3, NH); ^{13}C NMR δ 107.9, 115.1, 120.3, 129.9, 146.7, 148.0; IR 3000, 1530, 1340 cm^{-1}.

18m: R = $C_6H_4NO_2$ (4); yield 45 %; mp 210-212 °C; ^1H NMR δ 7.03-8.16 (m, 4, CH_{arom}), 9.58 (br s, 1, NH), 10.80 (br s, 3, NH); ^{13}C NMR δ 112.9, 125.3, 140.2, 151.6; IR 2880, 1518, 1322 cm^{-1}.

Propylhydrazine (18p)

Propyl bromide (30.8 g, 251 mmol) was added dropwise to hydrazine hydrate (125.0 g, 251 mmol). The internal temperature was not allowed to exceed 30 °C. The reaction mixture was stirred at room temperature for 2 hours. The hydrazine was extracted with ether (4 x 100 ml) and washed with water (100 ml). Distillation of the crude product afforded pure propylhydrazine (18p): yield 65 %; bp 118-120 °C; ^1H NMR δ 0.38 (t, 3, J = 7.4 Hz, CH_3), 0.96 (m, 2, J = 7.2, 7.4 Hz, $CH_2CH_2CH_3$), 2.17 (t, 2, J = 7.2 Hz, $CH_2CH_2CH_3$), 2.19 (br s, 3, NH); ^{13}C NMR δ 10.4, 19.7, 56.9; IR 3330 cm^{-1}.

Anilinomethylenemalononitrile (21)

Ethoxymethylenemalononitrile (2.0 g, 16.4 mmol) was dissolved in ethanol (40 ml).

Aniline (1.53 g, 16.4 mmol) was added and the reaction was refluxed for 1 hour. Upon

cooling, a solid precipitated and was collected by suction filtration. Recrystallisation of

the crude product from DMSO and methanol afforded pure anilinomethylenemalononitrile

(22): yield 72 %; mp 253-255 °C; ^{1}H NMR δ 7.13-7.45 (m, 5, CH_{arom}), 8.49 (s, 1,

H-3), 11.12 (br s, 1, NH); ^{13}C NMR δ 51.8 (s, C-2), 114.2 (s, CN), 116.5 (s, CN),

118.1 (d, C-2', C-6'), 125.2 (d, C-4'), 129.4 (d, C-3', C-5'), 139.3 (d, C-1'), 155.8

(d, C-1); IR 3500, 2530, 2520 cm^{-1}.

5-(3-Methyl-1-uriedo)-4-cyano-1-phenylpyrazole (23a)

5-Amino-4-cyano-1-phenylpyrazole (1.0 g, 5.4 mmol) was sealed under an atmosphere

of nitrogen. DMF (10 ml) and methyl isocyanate (0.40 ml, 6.5 mmol) were injected by

syringe and the reaction was stirred at 80 °C for 24 hours. The solvent was evaporated

under reduced pressure. The crude product was triturated with ethyl acetate and

recyrstallised from methanol and ethyl acetate to afford pure 5-(3-methyl-1-uriedo)-4-

cyano-1-phenylpyrazole (23a): yield 73 %; mp 255-257 °C; ^{1}H NMR δ 2.58 (d, 3,

J = 4.6 Hz, CH_3), 6.53 (br s, 1, NH), 7.45-7.58 (m, 5, CH_{arom}), 8.17 (s, 1, H-3),

8.80 (br s, 1, NH); ^{13}C NMR δ 26.4 (q, CH_3), 88.4 (s, C-4), 113.4 (s, CN), 124.4 (d,

C-2',C-6'), 128.8 (d, C-4'), 129.4 (d, C-3', C-5'), 137.5 (s, C-1'), 142.3 (d, C-3),

142.5 (s, C-5), 154.4 (s, C=O); IR 3325, 2220, 1640 cm^{-1}; Anal. Calculated for

$C_{12}H_{11}N_5O$: C, 59.74; H, 4.59; N, 29.02. Found: C, 59.8; H, 4.9; N, 28.7.

1-Substituted 4-Amino-5-methylpyrazolo[3,4-d]pyrimidin-6-ones (24a-v)

Method A: 5-(3-Methyl-1-uriedo)-4-cyano-1-phenylpyrazole (0.2 g, 0.8 mmol) was

dissolved in DMF (5 ml). Ammonium hydroxide (30 %, 3 ml) was added and the

reaction mixture was stirred at room temperature for 24 hours. The reaction was

neutralised with 1 M hydrochloric acid and the solvent was evaporated under reduced pressure. Recrystallisation of the crude product from methanol and ethyl acetate afforded pure 4-amino-5-methyl-1-phenylpyrazolo[3,4-d]pyrimidin-6-one (**24a**).

Method B: 5-Amino-4-cyano-1-phenylpyrazole (1.0 g, 5.4 mmol) and sodium methoxide (0.59 g, 10.8 mmol) were sealed under an atmosphere of nitrogen. DMF (10 ml) and methyl isocyanate (0.40 ml, 6.5 mmol) were injected by syringe and the reaction mixture was stirred at 60 °C for 4 hours. The reaction was neutralised with 1 M hydrochloric acid and the solvent was evaporated under reduced pressure. The crude product was triturated with ethyl acetate (to remove any organic impurities) and then with water (to remove any inorganic impurities). Recrystallisation from methanol and ethyl acetate afforded pure **24a**. The use of different 5-amino-4-cyanopyrazoles allowed the synthesis of the 4-amino-5-methylpyrazolo[3,4-d]pyrimidin-6-ones, **24b-s**.

Method C: 5-Amino-4-cyano-1-phenylpyrazole (0.50 g, 2.7 mmol) was sealed under an atmosphere of nitrogen. THF (15 ml) was injected by syringe and the flask was cooled to -70 °C on a methanol/dry ice bath. n-Butyllithium (2.0 ml, 1.6 M, 6.5 mmol) and methyl isocyanate (0.40 ml, 6.5 mmol) were injected and the reaction was stirred at -70 °C for 1 hour and then at room temperature for 1 hour. Water (5 ml) was added to quench the reaction and the solvent was evaporated under reduced pressure. The crude product was triturated with ethyl acetate and then with water. Recrystallisation from methanol and ethyl acetate afforded pure **24a**.

24a: R = C$_6$H$_5$; yield 90 % (Method A), 92 % (Method B) and 98 % (Method C); mp 308-310 °C; ^{1}H NMR δ 3.37 (s, 3, CH$_3$), 7.21-8.16 (m, 5, CH$_{arom}$), 8.16 (s, 1, H-3), 8.26 (br s, 1, NH), 8.94 (br s, 1, NH); ^{13}C NMR δ 29.4 (q, CH$_3$), 93.1 (s, C-3a), 119.9 (d, C-2', C-6'), 125.0 (d, C-4'), 128.7 (d, C-3', C-5'), 135.3 (d, C-3), 139.5 (s, C-1'), 153.7 (s, C-4), 154.7 (s, C-7a), 155.6 (s, C-6); IR 3300, 1640 cm^{-1};

Anal. Calculated for $C_{12}H_{11}N_5O$: C, 59.74; H, 4.59; N, 29.02. Found: C, 59.4; H, 4.5; N, 28.7.

24b: R = C_6H_4Cl (2); recrystallised from DMSO and water; yield 81 %; mp 261-262 °C; ^1H NMR δ 3.35 (s, 3, CH_3), 7.46-7.66 (m, 4, CH_{arom}), 8.15 (s, 1, H-3), 8.26 (br s, 1, NH), 8.99 (br s, 1, NH); ^{13}C NMR δ 29.6 (q, CH_3), 91.2 (s, C-3a), 127.8 (d, C-6'), 130.1 (d, C-4'), 130.3 (d, C-5'), 130.5 (d, C-3'), 131.5 (s, C-2'), 135.4 (d, C-3), 135.6 (s, C-1'), 153.4 (s, C-4), 154.4 (s, C-7a), 156.7 (s, C-6); IR 3410, 3050, 1690 cm^{-1}; Anal. Calculated for $C_{12}H_{10}N_5OCl$: C, 52.27; H, 3.65; N, 29.40. Found: C, 52.7; H, 3.2; N, 25.3.

24c: R = C_6H_4Cl (3); recrystallised from DMSO and water; yield 67 %; mp 296-299 °C; ^1H NMR δ 3.38 (s, 3, CH_3), 7.26-8.36 (m, 4, CH_{arom}), 8.19 (s, 1, H-3), 8.29 (br s, 1, NH), 9.00 (br s, 1, NH); ^{13}C NMR δ 29.7 (q, CH_3), 92.6 (s, C-3a), 117.9 (d, C-6'), 119.0 (d, C-2'), 124.8 (d, C-4'), 130.6 (d, C-5'), 133.2 (s, C-3'), 135.7 (d, C-3), 140.6 (s, C-1'), 153.3 (s, C-4), 154.2 (s, C-7a), 156.1 (s, C-6); IR 3340, 2960, 1690 cm^{-1}; Anal. Calculated for $C_{12}H_{10}N_5OCl$: C, 52.27; H, 3.65; N, 25.40. Found: C, 52.5; H, 3.5; N, 25.8.

24d: R = C_6H_4Cl (4); recrystallised from DMSO and water; yield 69 %; mp 298-301 °C; ^1H NMR δ 3.37 (s, 3, CH_3), 7.51-8.24 (m, 4, CH_{arom}), 8.18 (s, 1, H-3), 8.24 (br s, 1, NH), 8.98 (br s, 1 NH); ^{13}C NMR δ 29.7 (q, CH_3), 92.6 (s, C-3a), 121.2 (d, C-2', C-6'), 128.8 (d, C-3', C-5'), 129.1 (s, C-4'), 135.7 (d, C-3), 138.3 (s, C-1'), 153.3 (s, C-4), 154.2 (s, C-7a), 155.8 (s, C-6); IR 3465, 3090, 1685 cm^{-1}; Anal. Calculated for $C_{12}H_{10}N_5Cl$: C, 52.27; H, 3.65; N, 25.40. Found: C, 52.2; H, 3.4; N, 25.4.

24e: R = C_6H_4Br (2); recrystallised from DMSO and methanol; yield 47 %; mp 362-364 °C; ^1H NMR δ 3.33 (s, 3, CH_3), 7.40-7.82 (m, 4, CH_{arom}), 8.12 (s, 1, H-3),

8.23 (br s, 1, NH), 8.90 (br s, 1, NH); ^{13}C NMR δ 29.5 (q, CH$_3$), 91.1 (s, C-3a), 121.8 (s, C-2'), 128.4 (d, C-6'), 130.5 (d, C-4'), 130.8 (d, C-5'), 133.2 (d, C-3'), 135.1 (d, C-3), 137.3 (s, C-1'), 153.4 (s, C-4), 154.3 (s, C-7a), 156.6 (s, C-6); IR 3400, 3050, 1685 cm^{-1}; Anal. Calculated for C$_{12}$H$_{10}$N$_5$OBr: C, 45.02; H, 3.14; N, 21.87. Found: C, 45.4; H, 3.1; N, 22.0.

24f: R = C$_6$H$_4$Br (3); recrystallised from DMSO and methanol; yield 44 %; mp 318-320 °C; ^{1}H NMR δ 3.37 (s, 3, CH$_3$), 7.42-8.49 (m, 4, CH$_{arom}$), 8.19 (s, 1, H-3), 8.64 (br s, 2, NH$_2$); ^{13}C NMR δ 29.6 (q, CH$_3$), 92.7 (s, C-3a), 118.3 (d, C-6'), 121.6 (s, C-3'), 121.8 (d, C-2'), 127.7 (d, C-4'), 130.9 (d, C-5'), 135.9 (d, C-3), 140.7 (s, C-1'), 153.4 (s, C-4), 154.2 (s, C-7a), 156.0 (s, C-6); IR 3325, 2940, 1690 cm^{-1}; Anal. Caculated for C$_{12}$H$_{10}$N$_5$OBr: C, 45.02; H, 3.14; N, 21.87. Found: C, 44.8; H, 3.1; N, 21.7.

24g: R = C$_6$H$_4$Br (4); recrystallised from DMSO and methanol; yield 48 %; mp 350-355 °C; ^{1}H NMR δ 3.37 (s, 3, CH$_3$), 7.63-8.20 (m, 4, CH$_{arom}$), 8.18 (s, 1 H-3), 8.26 (br s, 1, NH), 8.97 (br s, 1, NH); ^{13}C NMR δ 29.6 (q, CH$_3$), 92.6 (s, C-3a), 117.3 (s, C-4'), 121.5 (d, C-2', C-6'), 131.7 (d, C-3', C-5'), 135.7 (d, C-3), 138.7 (s, C-1'), 153.3 (s, C-4), 154.1 (s, C-7a), 155.9 (s, C-6); IR 3450, 3070, 1680; Anal. Calculated for C$_{12}$H$_{10}$N$_5$OBr: C, 45.02; H, 3.14; N, 21.87. Found: C, 45.0; H, 3.1; N, 21.7.

24h: R = C$_6$H$_4$F (2); recrystallised from methanol; yield 64 %; mp 334-337 °C; ^{1}H NMR δ 3.38 (s, 3, CH$_3$), 7.32-7.59 (m, 4, CH$_{arom}$), 8.36 (s, 1, H-3), 8.76 (br s, 1, NH), 9.63 (br s, 1, NH); ^{13}C NMR δ 29.9 (q, CH$_3$), 92.1 (s, C-3a), 116.7 (d, C-3'), 124.8 (d, C-6'), 125.1 (s, C-1'), 128.8 (d, C-5'), 130.7 (d, C-4'), 136.5 (d, C-3), 152.4 (s, C-4), 152.7 (s, C-7a), 153.5 (s, C-6), 156.5 (s, C-2'); IR 3320, 3145, 1735 cm^{-1}; Anal. Calculated for C$_{12}$H$_{10}$N$_5$OF: C, 55.59; H, 3.88; N, 27.01. Found: C, 56.0; H, 3.6; N, 27.2.

24i: R = C$_6$H$_4$F (3); recrystallised from methanol; yield 61 %; mp 306-307.5 °C; ^{1}H NMR δ 3.38 (s, 3, CH$_3$), 7.03-8.15 (m, 4, CH$_{arom}$), 8.19 (s, 1, H-3), 8.29 (br s, 1, NH), 9.01 (br s, 1, NH); ^{13}C NMR δ 29.7 (q, CH$_3$), 92.7 (s, C-3a), 106.5 (d, C-2'), 111.6 (d, C-4'), 115.3 (d, C-6'), 130.7 (d, C-5'), 135.8 (d, C-3), 140.8 (s, C-1'), 153.3 (s, C-4), 154.2 (s, C-7a), 156.1 (s, C-6), 162.2 (s, C-3'); IR 3485, 2920, 1695 cm^{-1}; Anal. Calculated for C$_{12}$H$_{10}$N$_5$OF: C, 55.59; H, 3.88; N, 27.01. Found: C, 55.7; H, 3.9; N, 27.1.

24j: R = C$_6$H$_4$F (4); recrystallised from methanol; yield 59 %; mp 291-292 °C; ^{1}H NMR δ 3.37 (s, 3, CH$_3$), 7.27-8.20 (m, 4, CH$_{arom}$), 8.16 (s, 1, H-3), 8.29 (br s, 1, NH), 8.93 (br s, 1, NH); ^{13}C NMR δ 29.6 (q, CH$_3$), 92.4 (s, C-3a), 115.5 (d, C-3', C-5'), 121.8 (d, C-2', C-6'), 135.3 (d, C-3), 135.8 (s, C-1'), 153.3 (s, C-4), 154.2 (s, C-7a), 155.5 (s, C-6), 159.5 (s, C-4'); IR 3475, 2920, 1680 cm^{-1}; Anal. Calculated for C$_{12}$H$_{10}$N$_5$OF: C, 55.59; H, 3.88; N, 27.01. Found: C, 55.4; H, 3.8; N, 27.1.

24k: R = C$_6$H$_4$NO$_2$ (2); recrystallised from DMSO and water; yield 46 %; mp 344-346 °C; ^{1}H NMR δ 3.34 (s, 3, CH$_3$), 7.60-8.07 (m, 4, CH$_{arom}$), 8.16 (s, 1, H-3), 8.35 (br s, 1, NH), 9.02 (br s, 1, NH); ^{13}C NMR δ 29.6 (q, CH$_3$), 91.5 (s, C-3a), 124.9 (d, C-6'), 127.7 (d, C-3'), 128.4 (d, C-4'), 130.5 (s, C-1'), 133.5 (d, C-5'), 136.4 (d, C-3), 144.2 (s, C-2'), 153.4 (s, C-4), 154.1 (s, C-7a), 156.2 (s, C-6); IR 3455, 3060, 1700, 1540, 1370 cm^{-1}; Anal. Calculated for C$_{12}$H$_{10}$N$_6$O$_3$: C, 50.35; H, 3.52; N, 29.35. Found: C, 50.1; H, 3.7; N, 29.1.

24l: R = C$_6$H$_4$NO$_2$ (3); recrystallised from DMSO; yield 40 %; mp 396-398 °C; ^{1}H NMR δ 3.38 (s, 3, CH$_3$), 7.75-9.12 (m, 4, CH$_{arom}$), 8.25 (s, 1, H-3), 8.35 (br s, 1, NH), 9.08 (br s, 1, NH); ^{13}C NMR δ 29.7 (q, CH$_3$), 92.7 (s, C-3a), 113.6 (d, C-2'), 119.4 (d, C-4'), 125.2 (d, C-6'), 130.5 (d, C-5'), 136.5 (d, C-3), 140.2 (s, C-1'), 146.2 (s, C-3'), 153.4 (s, C-4), 154.2 (s, C-7a), 156.5 (s, C-6); IR 3440, 3100, 1700,

1560, 1325 cm^{-1}; Anal. Calculated for $C_{12}H_{10}N_6O_3$: C, 50.35; H, 3.52; N, 29.35. Found: C, 50.1; H, 3.4; N, 29.1.

24m: R = $C_6H_4NO_2$ (4); recrystallised from DMSO; yield 49 %; mp 340-366 °C; ^{1}H NMR δ 3.37 (s, 3, CH$_3$), 8.27 (s, 1, H-3), 8.36-8.58 (m, 4, CH$_{arom}$), 8.36 (br s, 1, NH), 8.96 (br s, 1, NH); IR 3445, 3100, 1685, 1560, 1350; Anal. Calculated for $C_{12}H_{10}N_6O_3$: C, 50.35; H, 3.52; N, 29.35. Found: C, 50.0; H, 3.7; N, 29.3.

24n: R = CH$_3$; recrystallised from water; yield 72 %; mp 326-328 °C; ^{1}H NMR δ 3.32 (s, 3, CH$_3$), 3.57 (s, 3, CH$_3$), 7.87 (s, 1, H-3), 8.10 (br s, 1, NH), 8.76 (br s, 1, NH); ^{13}C NMR δ 29.4 (q, CH$_3$), 32.4 (q, CH$_3$), 91.1 (s, C-3a), 133.1 (d, C-3), 153.2 (s, C-4), 154.0 (s, C-7a), 155.1 (s, C-6); IR 3410, 3100, 1700 cm^{-1}; Anal. Calculated for $C_7H_9N_5O$: C, 46.92; H, 5.06; N, 39.08. Found: C, 46.6; H, 5.2; N, 39.1.

24p: R = C_3H_7; recrystallised from methanol; yield 74 %; mp 253-256 °C; ^{1}H NMR δ 0.78 (t, 3, J = 7.4 Hz, CH$_3$), 1.69 (m, 2, J = 7.0, 7.4 Hz, CH$_2$*CH$_2$*CH$_3$), 3.32 (s, 3, CH$_3$), 3.90 (t, 2, J = 7.0 Hz, *CH$_2$*CH$_2$CH$_3$), 7.97 (s, 1, H-3), 8.41 (br s, 2, NH$_2$); ^{13}C NMR δ 10.8 (q, CH$_3$), 22.0 (t, CH$_2$*CH$_2$*CH$_3$), 29.5 (q, CH$_3$), 46.6 (t, *CH$_2$*CH$_2$CH$_3$), 91.1 (s, C-3a), 133.4 (d, C-3), 153.2 (s, C-4), 154.2 (s, C-7a), 155.0 (s, C-6); IR 3410, 2970, 1640 cm^{-1}; Anal. Calculated for $C_9H_{13}N_5O$: C, 52.16; H, 6.32; N, 33.79. Found: C, 52.0; H, 6.6; N, 33.4.

24q: R = $C_6H_3Cl_2$ (2,4); yield 73 %; mp 266.5-267.5 °C; ^{1}H NMR δ 3.34 (s, 3, CH$_3$), 7.50-7.86 (m, 3, CH$_{arom}$), 8.15 (s, 1, H-3), 8.37 (br s, 1, NH), 8.87 (br s, 1, NH); ^{13}C NMR δ 29.6 (q, CH$_3$), 91.1 (s, C-3a), 128.0 (d, C-6'), 129.7 (d, C-5'), 131.3 (d, C-3'), 132.5 (s, C-2'), 134.0 (s, C-4'), 134.7 (s, C-1'), 136.0 (d, C-3), 153.4 (s, C-4), 154.3 (s, C-7a), 156.8 (s, C-6); IR 3310, 2940, 1700 cm^{-1}; Anal.

Calculated for $C_{12}H_9N_5OCl_2$: C, 46.47; H, 2.92; N, 22.58. Found: C, 46.4; H, 2.9; N, 22.4.

24r: R = $C_6H_3Cl_2$ (2,4); yield 77 %; mp 373-375 °C; ^{1}H NMR δ 3.34 (s, 3, CH$_3$), 7.59-7.73 (m, 3, CH$_{arom}$), 8.15 (s, 1, H-3), 8.38 (br s, 1, NH), 8.85 (br s, 1, NH); ^{13}C NMR δ 29.5 (q, CH$_3$), 91.0 (s, C-3a), 129.8 (d, C-6'), 130.2 (s, C-2'), 130.2 (d, C-4'), 131.6 (d, C-3'), 131.7 (s, C-5'), 135.7 (d, C-3), 136.6 (s, C-1'), 153.4 (s, C-4), 154.2 (s, C-7a), 156.9 (s, C-6); IR 3250, 2960, 1700 cm^{-1}; Anal. Calculated for $C_{12}H_9N_5OCl_2$: C, 46.47; H, 2.92; N, 22.58. Found: C, 46.6; H, 2.9; N, 22.6.

24s: R = $C_6H_3Cl_2$ (3,5); recrystallised from DMSO and methanol; yield 65 %; mp 354-356 °C; ^{1}H NMR δ 3.34 (s, 3, CH$_3$), 7.48 (t, 1, J = 1.9 Hz, H-4'), 8.21 (s, 1, H-3), 8.32 (d, 2, J = 1.9 Hz, H-2', H-6'), 8.43 (br s, 1, NH), 9.02 (br s, 1, NH); ^{13}C NMR δ 29.7 (s, CH$_3$), 92.7 (s, C-3a), 117.3 (d, C-2', C-6'), 124.2 (d, C-4'), 134.4 (s, C-3', C-5'), 136.5 (d, C-3), 141.2 (s, C-1'), 153.3 (s, C-4), 154.1 (s, C-7a), 156.5 (s, C-6); IR 3350, 2960, 1685 cm^{-1}; Anal. Calculated for $C_{12}H_9N_5OCl_2$: C, 46.47; H, 2.92; N, 22.58. Found: C, 46.3; H, 3.2; N, 22.2.

4-Amino-1-(aminophenyl)-5-methylpyrazolo[3,4-*d*]pyrimidin-6-ones (24t-v)

4-Amino-5-methyl-1-(2-nitrophenyl) pyrazolo[3,4-*d*]pyrimidin-6-one (1.0 g, 3.5 mmol) was finely ground and suspended in acetic acid (20 ml). Palladium on carbon (0.4 g) was added and the reaction was placed under a 50 kPa atmosphere of hydrogen for 24 hours. The reaction mixture was filtered and the acetic acid was evaporated under reduced pressure. Recrystallisation from DMSO and water afforded pure 4-amino-1-(2-aminophenyl)-5-methylpyrazolo[3,4-*d*]pyrimidin-6-one (24t). The use of different 4-

amino-5-methyl-1-(nitrophenyl)pyrazolo[3,4-*d*]pyrimidin-6-ones allowed the synthesis of the 4-amino-1-(aminophenyl)-5-methylpyrazolo[3,4-*d*]pyrimidin-6-ones, **24u** and **v**.

24t: R = $C_6H_4NH_2$ (2); yield 89 %; mp 283-290 °C; ^{1}H NMR δ 3.36 (s, 3, CH_3), 5.16 (br s, 2, NH_2), 6.61-7.19 (m, 4, CH_{arom}), 8.15 (s, 1, H-3), 8.21 (br s, 1, NH), 8.94 (br s, 1 NH); ^{13}C NMR δ 29.5 (q, CH_3), 91.5 (s, C-3a), 116.2 (d, C-3'), 116.7 (d, C-5'), 124.1 (s, C-1'), 127.0 (d, C-6'), 128.4 (d, C-4'), 135.1 (d, C-3), 143.3 (s, C-2'), 153.4 (s, C-4), 154.1 (s, C-7a), 155.5 (s, C-6); IR 3325, 2945, 1705; Anal. Calculated for $C_{12}H_{12}N_6O$: C, 56.24; H, 4.71; N, 32.79. Found: C, 56.4; H, 4.7; N, 32.5.

24u: R = $C_6H_4NH_2$ (3); recrystallised from methanol; yield 66 %; mp 233-235 °C; ^{1}H NMR δ 3.37 (s, 3, CH_3), 5.31 (br s, 2, NH_2), 6.41-7.34 (m, 4, CH_{arom}), 8.13 (s, 1, H-3), 8.24 (br s, 1, NH), 8.90 (br s, 1, NH); ^{13}C NMR δ 29.7 (q, CH_3), 92.5 (s, C-3a), 105.9 (d, C-2'), 108.2 (d, C-6'), 111.3 (d, C-4'), 129.0 (d, C-5'), 134.7 (d, C-3), 140.1 (s, C-1'), 149.2 (s, C-3'), 153.3 (s, C-4), 154.3 (s, C-7a), 155.4 (s, C-6); IR 3440, 3330, 3100, 1680; Anal. Calculated for $C_{12}H_{12}N_6O$: C, 56.24; H, 4.71; N, 32.79. Found: C, 56.3; H, 4.8; N, 32.9.

24v: R = $C_6H_4NH_2$ (4); recrystallised from DMSO and methanol; yield 75 %; mp 290-294 °C; ^{1}H NMR δ 3.38 (s, 3, CH_3), 5.16 (br s, 2, NH_2), 6.59-7.63 (m, 4, CH_{arom}), 8.06 (s, 1, H-3), 8.19 (br s, 1, NH), 8.81 (br s, 1 NH); ^{13}C NMR δ 29.6 (q, CH_3), 92.1 (s, C-3a), 113.5 (d, C-3', C-5'), 122.2 (d, C-2', C-6'), 128.6 (s, C-1'), 134.0 (d, C-3), 146.8 (s, C-4'), 153.3 (s, C-4), 154.2 (s, C-7a), 154.5 (s, C-6); IR 3340, 3100, 1665 cm^{-1}; Anal. Calculated for $C_{12}H_{12}N_6O$: C, 56.24; H, 4.71; N, 32.79. Found: C, 56.3; H, 4.8; N, 33.0.

5-Substituted 4-Amino-1-phenylpyrazolo[3,4-*d*]pyrimidin-6-ones (25a-d)

5-Amino-4-cyano-1-phenylpyrazole (1.0 g, 5.4 mmol) and sodium methoxide (0.59 g, 10.8 mmol) were sealed under an atmosphere of nitrogen. DMF (10 ml) and ethyl isocyanate (0.51 ml, 6.5 mmol) were injected by syringe and the reaction mixture was stirred at 60 °C for 12 hours. The reaction was neutralised with 1 M hydrochoric acid and the solvent was evaporated under reduced pressure. This crude product was triturated with ethyl acetate and then with water. Recrystallisation from methanol and ethyl acetate afforded pure 4-amino-5-ethyl-1-phenylpyrazolo[3,4-*d*]pyrimidin-6-one (25a). The use of different isocyanates allowed the synthesis of the 4-amino-1-phenylpyrazolo[3,4-*d*]pyrimidin-6-ones, 25b-d.

25a: R = C_2H_5; yield 65 %; mp 306-308 °C; ^1H NMR δ 1.14 (t, 3, J = 7.0 Hz, CH_3), 4.00 (q, 2, J = 7.0 Hz, CH_2), 7.19-8.16 (m, 5, CH_{arom}), 8.19 (s, 1, H-3), 8.38 (br s, 1, NH), 8.87 (br s, 1, NH); ^{13}C NMR δ 12.3 (q, CH_3), 37.1 (t, CH_2), 92.6 (s, C-3a), 120.0 (d, C-2', C-6'), 125.2 (d, C-4'), 128.8 (d, C-3', C-5'), 135.4 (d, C-3), 139.3 (s, C-1'), 152.6 (s, C-4), 153.9 (s, C-7a), 155.7 (s, C-6); IR 3400, 2930, 1680 cm^{-1}; Anal. Calculated for $C_{13}H_{13}N_5O$: C, 61.16; H, 5.13; N, 27.43. Found: C, 61.0; H, 5.2; N, 27.1.

25b: R = C_3H_7; recrystallised from DMSO and methanol; yield 80 %; mp 232-234 °C; ^1H NMR δ 0.90 (t, 3, J = 7.3 Hz, CH_3), 1.56 (m, 2, J = 7.3, 7.7 Hz, $CH_2CH_2CH_3$), 3.88 (t, 2, J = 7.7 Hz, $CH_2CH_2CH_3$), 7.20-8.15 (m, 5, CH_{arom}), 8.19 (s, 1, H-3), 8.29 (br s, 1, NH), 8.85 (br s, 1, NH); ^{13}C NMR δ 10.7 (q, CH_3), 19.9 (t, $CH_2CH_2CH_3$), 43.3 (t, $CH_2CH_2CH_3$), 92.6 (s, C-3a), 120.0 (d, C-2', C-6'), 125.2 (d, C-4'), 128.8 (d, C-3', C-5'), 135.4 (d, C-3), 139.3 (s, C-1'), 152.7 (s, C-4), 154.0 (s, C-7a), 155.7 (s, C-6); IR 3610, 3345, 3025, 1700 cm^{-1}; Anal. Calculated for $C_{14}H_{15}N_5O$: C, 62.44; H, 5.61; N, 26.00. Found: C, 62.0; H, 5.5; N, 25.7.

25c: R = C₄H₉; recrystallised from DMSO and methanol; yield 68 %, mp 224-226 °C, ¹H NMR δ 0.89 (t, 3, J = 7.2 Hz, CH₃), 1.33 (m, 2, J = 7.0, 7.2 Hz, CH₂CH₂CH_2CH₃), 1.50 (m, 2, J = 7.0, 7.3 Hz, CH₂CH_2CH₂CH₃), 3.93 (t, 2, J = 7.3 Hz, CH_2CH₂CH₂CH₃), 7.20-8.15 (m, 5, CH$_{arom}$), 8.19 (s, 1, H-3), 8.29 (br s, 1, NH), 8.53 (br s, 1, NH); ¹³C NMR δ 13.6 (q, CH₃), 19.3 (t, CH₂CH₂CH_2CH₃), 28.8 (t, CH₂CH_2CH₂CH₃), 41.8 (t, CH_2CH₂CH₂CH₃), 92.6 (s, C-3a), 120.0 (d, C-2', C-6'), 125.2 (d, C-4'), 128.8 (d, C-3', C-5'), 135.5 (d, C-3), 139.3 (s, C-1'), 152.7 (s, C-4), 154.1 (s, C-7a), 155.7 (s, C-6); IR 3320, 3080, 1700 cm⁻¹; Anal. Calculated for C₁₅H₁₇N₅O: C, 63.58; H, 6.04; N, 24.71. Found: C, 63.9; H, 5.9; N, 24.5.

25d: R = C₆H₅; recrystallised from DMSO and methanol; yield 55 %; mp 249-250 °C; ¹H NMR δ 7.12 (br s, 1, NH), 7.26-8.20 (m, 10, CH$_{arom}$), 8.29 (s, 1, H-3), 9.04 (br s, 1, NH); ¹³C NMR δ 93.8 (s, C-3a), 120.1 (d, C-2', C-6'), 125.1 (d, C-2',C-6'), 128.4 (d, C-4'), 128.8 (d, C-4'), 129.1 (d, C-3', C-5'), 129.8 (d. C-3', C-5'), 136.0 (d, C-3), 137.0 (s, C-1'), 139.7 (s, C-1'), 153.9 (s, C-4), 154.9 (s, C-7a), 156.3 (s, C-6); IR 3460, 3090, 1695 cm⁻¹; Anal. Calculated for C₁₇H₁₃N₅O: C, 67.31; H, 4.31; N, 23.08. Found: C, 67.7; H, 4.0; N, 23.5.

5-Substituted 4-Amino-1-(3-chlorophenyl)pyrazolo[3,4-*d*]pyrimidin-6-ones (25e-g)

5-Amino-1-(3-chlorophenyl)-4-cyanopyrazole (1.0 g, 5.4 mmol) and sodium methoxide (0.59 g, 10.8 mmol) were sealed under an atmosphere of nitrogen. DMF (10 ml) and ethyl isocyanate (0.51 ml, 6.5 mmol) were injected by syringe and the reaction mixture was stirred at 60 °C for 12 hours. The reaction was neutralised with 1 M hydrochoric acid and the solvent was evaporated under reduced pressure. The crude product was triturated with ethyl acetate and then with water. Recrystallisation from methanol and ethyl acetate afforded pure 4-amino-1-(3-chlorophenyl)-5-ethylpyrazolo[3,4-*d*]pyrimidin-

6-one (25e). The use of different isocyanates allowed the synthesis of the 4-amino-1-(3-chlorophenyl)pyrazolo[3,4-d]pyrimidin-6-ones, 25f and g.

25e: R = C_2H_5; recrystallised from DMSO and water; yield 71 %; mp 278-280 °C; ^{1}H NMR δ 1.14 (t, 3, J = 7.0 Hz, CH_3), 3.98 (q, 2, J = 7.0 Hz, CH_2), 7.27-8.36 (m, 4, CH_{arom}), 8.20 (s, 1, H-3), 8.37 (br s, 1, NH), 8.94 (br s, 1, NH); ^{13}C NMR δ 12.2 (q, CH_3), 37.2 (t, CH_2), 92.7 (s, C-3a), 117.9 (d, C-6'), 119.0 (d, C-2'), 124.8 (d, C-4'), 130.6 (d, C-5'), 133.3 (s, C-3'), 136.0 (d, C-3), 140.6 (d, C-1'), 152.6 (s, C-4), 153.9 (s, C-7a), 156.1 (s, C-6); IR 3350, 2945, 1680 cm^{-1}; Anal. Calculated for $C_{13}H_{12}N_5OCl$: C, 53.89; H, 4.17; N, 24.17. Found: C, 54.0; H, 4.3; N, 24.1.

25f: R = C_3H_7; recrystallised from DMSO and methanol; yield 62 %; mp 233-235 °C; ^{1}H NMR δ 0.90 (t, 3, J = 7.3 Hz, CH_3), 1.56 (m, 2, J = 7.3, 7.7 Hz, $CH_2CH_2CH_3$), 3.88 (t, 2, J = 7.7 Hz, $CH_2CH_2CH_3$), 7.26-8.36 (m, 4, CH_{arom}), 8.20 (s, 1, H-3), 8.41 (br s, 1, NH), 8.88 (br s, 1, NH); ^{13}C NMR δ 10.7 (q, CH_3), 19.8 (t, $CH_2CH_2CH_3$), 43.4 (t, $CH_2CH_2CH_3$), 92.7 (s, C-3a), 117.9 (d, C-6'), 119.0 (d, C-2'), 124.8 (d, C-4'), 130.6 (d, C-5'), 133.2 (s, C-3'), 136.0 (d, C-3), 140.5 (s, C-1'), 152.8 (s, C-4), 154.0 (s, C-7a), 156.1 (s, C-6); IR 3650, 3310, 3030, 1700 cm^{-1}; Anal. Calculated for $C_{14}H_{14}N_5OCl$: C, 55.35; H, 4.64; N, 23.05. Found: C, 55.1; H, 5.0; N, 22.6.

25g: R = C_4H_9; recrystallised from DMSO and water; yield 72 %; mp 230-232 °C; ^{1}H NMR δ 0.85 (t, 3, J=7.2 Hz,CH_3), 1.32 (m, 2, J = 7.2, 7.2 Hz, $CH_2CH_2CH_2CH_3$), 1.51 (m, 2, J = 7.2, 7.4 Hz, $CH_2CH_2CH_2CH_3$), 3.92 (t, 2, J = 7.4 Hz, $CH_2CH_2CH_2CH_3$), 7.26-8.36 (m, 4, CH_{arom}), 8.20 (s, 1, H-3), 8.35 (br s, 1, NH), 8.91 (br s, 1, NH); ^{13}C NMR δ 13.6 (q, CH_3), 19.3 (t, $CH_2CH_2CH_2CH_3$), 28.8 (t, $CH_2CH_2CH_2CH_3$), 41.8 (t, $CH_2CH_2CH_2CH_3$), 92.7 (s, C-3a), 117.9 (d, C-6'), 119.0 (d, C-2'), 124.8 (d, C-4'), 130.6 (d, C-5'), 133.3 (s, C-3'), 136.1 (d,

C-3), 140.6, (s, C-1'), 152.7 (s, C-4), 154.0 (s, C-7a), 156.1 (s, C-6); IR 3350, 3100,

2950, 1700 cm-1; Anal. Calculated for $C_{15}H_{16}N_5OCl$: C, 56.69; H, 5.07; N, 22.03.

Found: C,57.0; H, 5.3; N, 22.2.

Dimerisation of 5-Amino-4-cyano-1-phenylpyrazole

5-Amino-4-cyano-1-phenylpyrazole (1.0 g, 5.4 mmol) was sealed under an atmosphere

of nitrogen. Pyridine (10 ml) and methyl isocyanate (0.40 ml, 6.5 mmol) were injected

by syringe and the reaction was stirred at 100 °C for 24 hours. A solid precipitated and

was collected by suction filtration. The crude product was extremely insoluble and was

unable to be recrystallised using common organic solvents. Trituration with DMSO

afforded a compound which was suspected to be a dimer of 5-amino-4-cyano-1-

phenylpyrazole (28): yield 52 %; mp 318-321 °C; ^{1}H NMR δ 2.89 (d, 3, CH_3), 6.89

(br s, 2, NH_2), 7.39-8.65 (s, 10, CH_{arom}), 8.07 (s, 1, H-3), 8.65 (s, 1, H-3), 10.10 (s,

1, NH).

Reaction of 5-Amino-4-cyano-1-phenylpyrazole with Potassium cyanate

Method A: 5-Amino-4-cyano-1-phenylpyrazole (1.0 g, 5.4 mmol) and potassium cyanate

(0.88 g, 10.8 mmol) were stirred at 80 °C in DMF (10 ml) for 24 hours. A large portion

of the potassium cyanate remained undissolved. The solvent was evaporated under

reduced pressure. Only starting material (10a) was isolated.

Method B: 5-Amino-4-cyano-1-phenylpyrazole (1.0 g, 5.4 mmol) and potassium cyanate

(0.88 g, 10.8 mmol) were refluxed in water (50 ml) for 6 hours. Upon cooling, a white

solid precipitated and was collected by suction filtration. Flash chromatography using

ethyl acetate as an eluent afforded a mixture of starting material (10a, 63 %) and

5-amino-1-phenylpyrazole-4-carboxamide (33).

33: yield 26 %; mp 170-172 °C; ^{1}H NMR δ 6.36 (br s, 2, NH$_2$), 6.85 (br s, 1,

NH), 7.40 (br s, 1, NH), 7.34-7.58 (m, 5, CH$_{arom}$), 7.89 (s, 1, H-3); ^{13}C NMR δ

97.5 (s, C-4), 123.0 (d, C-2', C-6'), 127.0 (d, C-4'), 129.3 (d, C-3', C-5'), 138.3 (s,

C-1'), 139.0 (d, C-3), 149.3 (s, C-5), 166.2 (s, C=O); IR 3380, 3270, 3175,

1660 cm^{-1}.

Reaction of 5-Amino-4-cyano-1-phenylpyrazole with Trimethylsilyl isocyanate

Method A: 5-Amino-4-cyano-1-phenylpyrazole (0.5 g, 2.7 mmol) and sodium

methoxide (0.29 g, 5.4 mmol) were sealed under an atmosphere of nitrogen. DMF

(5 ml) and trimethylsilyl isocyanate (0.37 g, 3.2 mmol) were injected by syringe and the

reaction mixture was stirred at 80 °C for 1 hour. The reaction was neutralised with

1 M hydrochloric acid and the solvent was evaporated under reduced pressure. Only

starting material (**10a**) was isolated.

Method B: 5-Amino-4-cyano-1-phenylpyrazole (0.5 g, 2.7 mmol) was sealed under an

atmosphere of nitrogen. DMF (5 ml), DBN (0.67 ml, 5.4 mmol) and trimethylsilyl

isocyanate (0.37 g, 3.2 mmol) were injected by syringe and the reaction mixture was

stirred at 80 °C for 24 hours. Thin layer chromatography revealed only starting material

(**10a**).

Method C: 5-Amino-4-cyano-1-phenylpyrazole (0.5 g, 2.7 mmol) was sealed under an

atmosphere of nitrogen and cooled to -70 °C on a methanol/dry ice bath. THF (5 ml) and

n-butyllithium (0.20 ml, 6.5 mmol) were injected by syringe and the reaction mixture was

stirred for 5 minutes. Trimethylsilyl isocyanate (0.37 g, 3.2 mmol) was injected and the

reaction mixture was stirred at -70 °C for 1 hour and at room temperature for 1 hour.

Only starting material (**10a**) was isolated.

<u>Method D</u>: 5-Amino-4-cyano-1-phenylpyrazole (0.5 g, 2.7 mmol) was sealed under an atmosphere of nitrogen. DMF (5 ml) and trimethylsilyl isocyanate (0.37 g, 3.2 mmol) were injected by syringe and the reaction mixture was stirred at 80 °C for 1 hour. Only starting material (10a) was isolated. Increasing the temperature to 100 °C and changing the solvent to pyridine produced the same result.

5-Amino-1-phenylpyrazole-4-carboxamide (33)

5-Amino-4-cyano-1-phenylpyrazole (2.0 g, 10.8 mmol) was refluxed in 2 M NaOH (100 ml) for 30 minutes. Upon cooling, a solid precipitated and was collected by suction filtration. Recrystallisation from water afforded pure 5-amino-1-phenylpyrazole-4-carboxamide (33): yield 66 %; mp 170-172 °C; ^{1}H NMR δ 6.36 (br s, 2, NH$_2$), 6.85 (br s, 1, NH), 7.40 (br s, 1, NH), 7.34-7.58 (m, 5, CH$_{arom}$), 7.89 (s, 1, H-3); ^{13}C NMR δ 97.5 (s, C-4), 123.0 (d, C-2', C-6'), 127.0 (d, C-4'), 129.3 (d, C-3', C-5'), 138.3 (s, C-1'), 139.0 (d, C-3), 149.3 (s, C-5), 166.2 (s, C=O); IR 3380, 3270, 3175, 1660 cm^{-1}.

Reaction of 5-Amino-1-phenylpyrazole-4-carboxamide and Benzoyl isothiocyanate

5-Amino-1-phenylpyrazole-4-carboxamide (0.5 g, 2.5 mmol) was sealed under an atmosphere of nitrogen. DMF (10 ml) and benzoyl isothiocyanate (0.48 g, 3.0 mmol) were injected by syringe and the reaction mixture was stirred at room temperature for 12 hours. During this period, a small amount of solid precipitated and was collected by suction filtration. Recrystallisation from methanol afforded pure 1-phenyl-5H-pyrazolo[3,4-d]pyrimidine (36). The remainder of the reaction mixture was evaporated under reduced pressure. Flash chromatography using a 1:1 mixture of ethyl acetate and hexane as an eluent afforded pure 5-(3-benzoylthioureido)-1-phenylpyrazole-4-carboxamide (35).

35: yield 46 %; mp 178-180 °C; ^{1}H NMR δ 7.21 (br s, 1, NH), 7.35-7.97 (m,

11, CH$_{arom}$ and NH), 8.15 (s, 1, H-3), 11.81 (br s, 1, NH), 12.13 (br s, 1, NH);

^{13}C NMR δ 113.4 (s, C-4), 123.8 (d), 128.0 (d), 128.5 (d), 128.8 (d), 129.0 (d), 131.7

(s), 133.4 (d), 137.1 (s, C-5), 138.5 (s), 139.8 (d, C-3), 162.8 (s, C=O), 167.7 (s,

C=O), 182.3 (s, C=S); IR 3500, 3250,1700, 1695 cm^{-1}.

36: yield 11 %; mp 301-303 °C; ^{1}H NMR δ 7.35-8.04 (m, 5, CH$_{arom}$), 8.19 (d,

1, H-6), 8.33 (s, 1, H-3), 12.47 (br s, 1, NH); ^{13}C NMR δ 107.6 (s, C-3a), 121.8 (d,

C-2', C-6'), 127.1 (d, C-4'), 129.2 (d, C-3', C-5'), 136.0 (d, C-3), 138.3 (s, C-1'),

148.8 (d, C-6), 151.9 (s, C-7a), 157.3 (s, C-4); IR 3100, 1725 cm^{-1}.

1-Substituted 5-(3-benzoylureido)-4-cyanopyrazoles (37a,b)

Method A: 5-(3-Benzoylthioureido)-1-phenylpyrazole-4-carboxamide (0.4 g, 1.1 mmol)

was sealed under an atmosphere of nitrogen. DMF (5 ml) and DCC (0.68 g, 3.3 mmol)

were injected by syringe and the reaction was stirred at room temperature for 48 hours.

The solvent was evaporated under reduced pressure to produce an oily solid. The crude

product was triturated with hexane to remove some of the impurities. Recrystallisation

from ethyl acetate and hexane afforded pure 5-(3-benzoylureido)-4-cyano-1-

phenylpyrazole (37a).

Method B: 5-Amino-4-cyano-1-phenylpyrazole (0.5 g, 2.7 mmol) was sealed under an

atmosphere of nitrogen. DMF (10 ml) and benzoyl isocyanate (0.60 g, 4.1 mmol) were

injected by syringe and the reaction was stirred at 60 °C for 12 hours. The solvent was

evaporated under reduced pressure to produce an oily solid. The crude product was

triturated with a 1:1 mixture of ethyl acetate and hexane to remove some of the impurities.

Recrystallisation from ethyl acetate and hexane afforded pure **37a**. The use of 5-amino-

1-(3-chlorophenyl)-4-cyanopyrazole allowed the synthesis of 5-(3-benzoylureido)-1-(3-

chlorophenyl)-4-cyanopyrazole (**37b**).

37a: yield 85 % (Method A) and 63 % (Method B); mp 194-195.5 °C; ^{1}H NMR

δ 7.49-7.99 (m, 10, CH$_{arom}$), 8.30 (s, 1, H-3), 10.98 (br s, 1, NH), 11.33 (br s, 1,

NH); ^{13}C NMR δ 89.2 (s, C-4), 112.9 (s, CN), 124.4 (d), 128.4 (d), 128.6 (d), 129.1

(d), 129.5 (d), 131.7 (s), 133.4 (d), 137.2 (s), 140.4 (s, C-5), 142.4 (d, C-3), 150.9 (s,

C=O), 168.5 (s, C=O); IR 3250, 3200, 1700, 1670 cm^{-1}.

37b: yield 91 % (Method B); mp 146-149 °C; ^{1}H NMR δ 7.50-8.00 (m, 4,

CH$_{arom}$), 8.32 (s, 1, H-3), 10.96 (br s, 1, NH), 11.40 (br s, 1, NH); ^{13}C NMR δ 89.6

(s, C-4), 112.7 (s, CN), 122.9 (d), 124.2 (d), 128.4 (d) 128.6 (d), 128.9 (d), 131.2

(d), 131.8 (s), 133.4 (d), 138.4 (s), 140.9 (s, C-5), 142.8 (d, C-3), 150.9 (s, C=O),

168.5 (s, C=O); IR 3450, 2220, 1720, 1660 cm^{-1}.

1-Substituted 4-Amino-5H-pyrazolo[3,4-d]pyrimidin-6-ones (40a,b)

Method A: 5-(3-benzoylthioureido)-4-cyano-1-phenylpyrazole (0.2 g, 0.6 mmol) was

dissolved on DMF (5 ml). Ammonium hydroxide (28 %, 10 ml) was added and the

reaction mixture was stirred at room temperature for 48 hours. The solvent was

evaporated under reduced pressure. Recrystallisation of the crude product from DMSO

and water to afforded pure 4-amino-1-phenyl-5H-pyrazolo[3,4-d]pyrimidin-6-one (40a).

The use of 5-(3-benzoylureido)-1-(3-chlorophenyl)-4-cyanopyrazole allowed the

synthesis of 4-amino-1-(3-chlorophenyl)-5H-pyrazolo[3,4-d]pyrimidin-6-one (40b).

Method B: 5-Amino-4-cyano-1-phenylpyrazole (0.5 g, 2.7 mmol) was sealed under an

atmosphere of nitrogen. DMF (10ml) and benzoyl isocyanate (0.60 g, 4.1 mmol) were

injected by syringe and the reaction mixture was stirred at 60 °C for 12 hours.

Ammonium hydroxide (28 %, 20 ml) was added and the reation was stirred at 60 °C for a

further 24 hours. The solvent was evaporated under reduced pressure. Recrystallisation

of the crude product from DMSO and water to afforded pure 40a.

40a: yield 80 % (Method A) and 68 % (Method B); mp 345-348 °C; ^{1}H NMR δ

7.21-8.13 (m, 5, CH arom), 7.27 (br s, 1, NH), 8.14 (s,1, H-3), 8.71 (br s, 1, NH),

11.92 (br s, 1, NH); ^{13}C NMR δ 92.7 (s, C-3a), 120.3 (d, C-2', C-6'), 125.4 (d,

C-4'), 128.8 (d, C-3', C-5'), 135.5 (d, C-3), 139.3 (s, C-1'), 153.9 (s,C-4), 156.5 (s,

C-7a), 157.8 (s, C-6); IR 3350, 3180, 1670 cm^{-1}; Anal. Calculated for $C_{11}H_9N_5O$: C,

58.14; H, 3.99; N, 30.82. Found: C, 57.9; H, 4.1; N, 30.5.

40b: yield 71 % (Method A); mp 355-358 °C; ^{1}H NMR δ 7.28-8.36 (m, 4,

CH_{arom}), 7.51 (br s, 1, NH), 8.17 (s, 1, H-3), 8.79 (br s, 1, NH), 10.99 (br s, 1, NH);

^{13}C NMR δ 92.6 (s, C-3a), 118.1 (d, C-6'), 119.2 (d, C-2'), 124.9 (d, C-4'),130.6 (d,

C-5'), 133.2 (s, C-3'), 136.1 (d, C-3), 140.5 (s, C-1'), 153.6 (s, C-4), 156.2 (s, C-7a),

158.5 (s, C-6); IR 3450, 3100, 1675 cm^{-1}; Anal. Calculated for $C_{11}H_8N_5OCl$: C,

50.49; H, 3.08; N, 26.76. Found: C, 50.6; H, 3.0; N, 26.9.

4-Cyano-5-methylamino-1-phenylpyrazole (45)

Method A: 5-Amino-4-cyano-1-phenylpyrazole (4.0 g, 21.7 mmol) and

paraformaldehyde (0.78 g, 26.1 mmol) were refluxed in THF for 24 hours. After

cooling to 0 °C, sodium borohydride (1.64 g, 43.4 mmol) in ethanol was added. The

reaction mixture was stirred at room temperature for 1 hour. The solvent was evaporated

under reduced pressure. The crude solid was dissolved in ethyl acetate (100 ml) and

washed with water (100 ml). The water fraction was re-extracted with ethyl acetate (3 x

100 ml). The organic extracts were combined, dried over anhydrous magnesium sulphate

and evaporated. Flash chromatography using methylene chloride as a eluent afforded

starting material (**10a**, 69 %) and 4-cyano-5-methylamino-1-phenylpyrazole (**45**, 14 %).

Method B: 5-Amino-4-cyano-1-phenylpyrazole (1.84 g, 10.0 mmol) and

paraformaldehyde (0.36 g, 12.0 mmol) were dissolved in THF. Dilute hydrochloric acid

was added dropwise until the pH was approximately 4. The reaction mixture was

refluxed for 24 hours. After cooling to 0 °C, sodium cyanoborohydride (1.64 g,

43.4 mmol) in ethanol was added. The reaction mixture was stirred at room temperature

for 1 hour. Thin layer chromatography identified a mixture of starting material (10a) and

methyl pyrazole (45).

Method C: 5-Amino-4-cyano-1-phenylpyrazole (1.84 g, 10.0 mmol) and

paraformaldehyde (0.36 g, 12.0 mmol) were dissolved in THF. Molecular sieves (4 Å,

10 g) were added and the reaction mixture was refluxed for 24 hours. After cooling to

0 °C, sodium borohydride (1.64 g, 43.4 mmol) in ethanol was added. The reaction

mixture was stirred at room temperature for 1 hour. Thin layer chromatography identified

a mixture of starting material (10a) and methyl pyrazole (45).

Method D: 5-Amino-4-cyano-1-phenylpyrazole (0.5 g, 2.7 mmol), formaldehyde (36 %

in methanol) and sodium borohydride (0.10 g, 2.7 mmol) were refluxed in THF for

12 hours. Thin layer chromatography identified a mixture of starting material (10a),

methyl pyrazole (45) and another product.

Method E: 5-Amino-4-cyano-1-phenylpyrazole (0.5 g, 2.7 mmol) and paraformaldehyde

(0.1 g, 3.2 mmol) were dissolved in DMF. The reaction mixture was refluxed for

24 hours. Sodium borohydride reduction and reaction work up (as per Method A)

afforded starting material (10a, 55 %) and methyl pyrazole (45, 26 %).

Method F: 5-Amino-4-cyano-1-phenylpyrazole (9.0 g, 48.9 mmol) and

paraformaldehyde (2.93 g, 97.8 mmol) were dissolved in THF. After thorough mixing,

the solvent was boiled off. The reactants were heated to approximately 200 °C in a

fusion-style process. Sodium borohydride reduction and reaction work up (as per

Method A) afforded starting material (10a, 32 %) and methyl pyrazole (45, 67 %).

45: mp 115-116 °C; ^{1}H NMR (CDCl$_3$) δ 3.06 (s, 3, CH$_3$), 4.53 (br s, 1, NH),

7.35-7.50 (m, 5, CH$_{arom}$), 7.52 (s, 1, H-3); ^{13}C NMR (CDCl$_3$) δ 31.3 (q, CH$_3$), 73.5

(s, C-4), 115.4 (s, CN), 124.9 (d, C-2', C-6'), 128.9 (d, C-4'), 129.9 (d, C-3', C-5'),

136.9 (s, C-1'), 142.5 (d, C-3), 151.1 (s, C-5); IR 3340, 2210 cm^{-1}.

4-Cyano-5-(1,3-dimethylureido)-1-phenylpyrazole (46)

<u>Method A</u>: 4-Cyano-5-methylamino-1-phenylpyrazole (0.5 g, 2.52 mmol) was sealed

under an atmosphere of nitrogen. DMF (5 ml) and methyl isocyanate (0.19 ml,

3.02 mmol) were injected by syringe and the reaction mixture was stirred at 60 °C for

4 hours and then at 100 °C for 24 hours. The solvent was evaporated under reduced

pressure. Flash chromatography using a 1:1 mixture of ethyl acetate and hexane as an

eluent afforded starting material (**45**, 86 %) and 4-cyano-5-(1,3-dimethylureido)-1-

phenylpyrazole (**46**, 9 %).

<u>Method B</u>: 4-Cyano-5-methylamino-1-phenylpyrazole (0.5 g, 2.52 mmol) was sealed

under an atmosphere of nitrogen. DMF (5 ml) and methyl isocyanate (0.19 ml,

3.02 mmol) were injected by syringe and the reaction mixture was stirred at 150 °C for

24 hours. Thin layer chromatography identified a mixture of starting material (**45**) and

urea (**46**).

46: mp 228-230 °C; ^{1}H NMR (CDCl$_3$) δ 2.66 (d, 3, CH$_3$), 2.99 (s, 3, CH$_3$),

4.96 (br s, 1, NH), 7.35-7.49 (m, s, CH$_{arom}$), 7.87 (s, 1, H-3); ^{13}C NMR (CDCl$_3$) δ

27.5 (q, CH$_3$), 35.7 (q, CH$_3$), 91.1 (s, C-4), 111.6 (s, CN), 123.4 (d, C-2', C-6'),

129.3 (d, C-4'), 129.7 (d, C-3', C-5'), 137.4 (s, C-1'), 142.2 (d, C-3), 145.5 (s, C-5),

155.5 (s, C=O); IR 3350, 2210, 1660 cm^{-1}.

5,7-Dimethyl-4-imino-1-phenylpyrazolo[3,4-d]pyrimidin-6-one (47)

Method A: 4-Cyano-5-(1,3-dimethylureido)-1-phenylpyrazole (0.5 g, 2.2 mmol) was dissolved in methanol (10 ml). Ammonium hydroxide (28 %, 20 ml) was added and the reaction was stirred at 60 °C for 12 hours. The solvent was evaporated under reduced pressure. The crude product was recrystallised from methanol to afford pure 5,7-dimethyl-4-imino-1-phenylpyrazolo[3,4-d]pyrimidin-6-one (47, 69 %).

Method B: 4-Cyano-5-methylamino-1-phenylpyrazole (0.5 g, 2.52 mmol) and sodium methoxide (0.27 g, 5.0 mmol) were sealed under an atmosphere of nitrogen. DMF (5 ml) and methyl isocyanate (0.17 g, 3.0 mmol) were injected by syringe and the reaction was stirred at 60 °C for 4 hours and then at 100 °C for 24 hours. During this period the colour darkened to brown. The solvent was evaporated under reduced pressure. Trituration of the crude product with ethyl acetate afforded only starting material (45).

Method C: 4-Cyano-5-methylamino-1-phenylpyrazole (0.5 g, 2.52 mmol) was sealed under an atmosphere of nitrogen. THF (20 ml) was injected by syringe and the reaction mixture was cooled to -70 °C in a dry ice/methanol bath. n-Butyllithium (1.89 ml, 1.6 M, 3.0 mmol) was injected and the reaction was stirred for 5 minutes. Methyl isocyanate (0.17 g, 3.0 mmol) was injected and the reaction was stirred at -70 °C for 1 hour and then at room temperature for 1 hour. Water (5 ml) was added to quench the reaction and the solvent was reduced in volume by evaporation under reduced pressure. The remaining reaction mixture was extracted with ethyl acetate (3 x 50 ml) to produce an oily solid. Flash chromatography using ethyl acetate as an eluent afforded only starting material (45, 80 %) and urea (46, 14 %).

Method D: 4-Cyano-5-methylamino-1-phenylpyrazole (1.0 g, 5.0 mmol) was sealed under an atmosphere of nitrogen. THF (10 ml) was injected by syringe and the reaction mixture was cooled to -70 °C on a methanol/dry ice bath. n-Butyllithium (3.56 ml,

1.6 M, 5.8 mmol) was injected and the reaction was warmed to room temperature. Methyl isocyanate (0.86 g, 15.1 mmol) was injected and the reaction was stirred for 15 minutes. Thin layer chromatography identified a mixture of starting material (45), a compound with similar retention time to the urea (46) and 4-imino-5,7-dimethyl-1-phenylpyrazolo[3,4-d]pyrimidin-6-one (47). A second portion of methyl isocyanate (0.86 g, 15.1 mmol) was injected and the reaction was stirred for a further 15 minutes. Thin layer chromatography indicated that all of the starting material (45) had been consumed. The reaction was quenched with water (5 ml) and the solvent was evaporated under reduced pressure. A 1:1 mixture of ethyl acetate and hexane (10 ml) was added to separate the soluble and insoluble components. The solid which was insoluble was recrystallised from DMSO and methanol to afford 5,7-dimethyl-4-imino-1-phenylpyrazolo[3,4-d]pyrimidin-6-one (47, 16 %). The solid which was soluble was purified by column chromatography using ethyl acetate and hexane (1:1) as an eluent. An inseparable mixture of 1,3,5-trimethyltriazine-2,4,6-trione (48) and a bis-urea (49) was obtained.

Method E: 4-Cyano-5-methylamino-1-phenylpyrazole (1.0 g, 5.0 mmol) was sealed under an atmosphere of nitrogen. THF (50 ml) was injected by syringe and the reaction mixture was cooled to -70 °C on a methanol/dry ice bath. n-Butyllithium (3.56 ml, 1.6 M, 5.8 mmol) was injected and the reaction mixture was warmed to room temperature. Methyl isocyanate (0.86 g, 15.1 mmol) was injected and the reaction was stirred for 15 minutes. A second portion of methyl isocyanate (0.86 g, 15.1 mmol) was injected and the reaction was stirred for another 15 minutes. The reaction was quenched with water and the solvent was evaporated under reduced pressure. A 1:1 mixture of ethyl acetate and hexane (10 ml) was added and the insoluble portion was collected by suction filtration. Recrystallisation from DMSO and methanol afforded 47 (35 %). The other products were not purified.

47: mp 268-270 °C; ^{1}H NMR δ 3.00 (s, 3, CH$_3$), 3.50 (s, 3, CH$_3$), 7.56-7.67

(m, 5, CH$_{arom}$), 8.93 (s, 1, H-3), 10.65 (br s, 1, NH); ^{13}C NMR δ 31.9 (q, CH$_3$),

33.1 (q, CH$_3$), 95.1 (s, C-3a), 127.6 (d, C-2',C-6'), 129.5 (d, C-4'), 130.4 (d, C-3',

C-5'), 137.6 (d, C-3), 137.7 (s, C-1'), 143.9 (s, C-4), 149.0 (s, C-7a), 152.9 (s, C-6);

IR 3440, 3140, 3000, 1670 cm^{-1}; Anal. Calculated for C$_{13}$H$_{13}$N$_5$O: C, 61.17; H, 5.13;

N, 27.43. Found: C, 61.0; H, 5.3; N, 27.4; HRMS Calculated for C$_{13}$H$_{13}$N$_5$O:

255.1120. Found: 255.1120.

48/49: mp 137-138 °C; ^{1}H NMR δ 2.39 (d, 3, CH$_3$), 2.41 (s, 3, CH$_3$), 3.15 (s,

3, CH$_3$), 3.21 (s, 3, CH$_3$), 7.40 (br q, 1, NH), 7.44-7.59 (m, 5, CH$_{arom}$), 8.26 (s, 1,

H-3); ^{13}C NMR δ 26.7 (q, CH$_3$), 28.8 (q, CH$_3$), 32.7 (q, CH$_3$), 37.5 (q, CH$_3$), 88.2

(s, C-4), 112.4 (s, CN), 123.8 (d, C-2', C-6'), 129.0 (d, C-4'), 129.4 (d, C-3', C-5'),

137.2 (s, C-1'), 142.3 (d, C-3), 146.2 (s, C-5), 149.7 (s, C=O), 154.8 (s, C=O), 156.1

(s, C=O); ^{1}H NMR (CDCl$_3$) δ 2.44 (d, 3, CH$_3$), 2.59 (s, 3, CH$_3$), 3.33 (s, 3, CH$_3$),

3.41 (s, 3, CH$_3$), 6.82 (br s, 1, NH), 7.24-7.49 (m, 5, CH$_{arom}$), 7.93 (s, 1, H-3);

^{13}C NMR (CDCl$_3$) δ 26.7 (q, CH$_3$), 29.2 (q, CH$_3$), 32.8 (q, CH$_3$), 38.8 (q, CH$_3$),

89.1 (s, C-4), 111.6 (s, CN), 123.6 (d, C-2', C-6'), 129.4 (d, C-4'), 129.8 (d, C-3',

C-5'), 136.6 (s, C-1'), 142.4 (d, C-3), 145.8 (s, C-5), 149.4 (s, C=O), 154.5 (s,

C=O), 157.7 (s, C=O); IR 3310, 2240, 1690, 1680, 1670 cm^{-1}; Crystal Data for **48**

C$_6$H$_9$N$_3$O$_3$, M = 171.2, Monoclinic, space group P2$_1$/a, a = 8.132 (16), b = 13.386 (2),

c = 14.812 (3) Å, β = 100.896 (9)°, v = 1583.4 (5) Å^3, D$_c$ (Z = 4) = 1.549 gcm^{-3},

F(000) = 776, μ(MoK$_α$) = 0.71 cm^{-1}, specimen: 0.25 x 0.27 x 0.38, N = 2784,

N$_0$ = 1696, R = 0.059, R$_w$ = 0.065, T = 296 K. Final atomic coordinates are listed in

Table 13, while bond distances and angles are listed in Tables 14 and 15.

Table 13   Atomic Coordinates of 1,3,5-Trimethyltriazine-2,4,6-trione

(48)

Atom	x/a	y/b	z/c
N-1A	0.0605 (4)	0.4029 (2)	0.0918 (2)
C-1A	0.0335 (6)	0.2965 (3)	0.0680 (3)
C-2A	0.2057 (5)	0.4458 (3)	0.0762 (3)
O-2A	0.3038 (4)	0.4012 (3)	0.0384 (2)
N-3A	0.2313 (4)	0.5431 (3)	0.1042 (2)
C-3A	0.3887 (7)	0.5892 (4)	0.0921 (4)
C-4A	0.1255 (6)	0.5958 (3)	0.1483 (3)
O-4A	0.1547 (4)	0.6810 (2)	0.1747 (2)
N-5A	-0.0160 (4)	0.5472 (3)	0.1604 (2)
C-5A	-0.1355 (6)	0.6018 (4)	0.2036 (4)
C-6A	-0.0499 (5)	0.4487 (3)	0.1376 (3)
O-6A	-0.1718 (4)	0.4065 (3)	0.1548 (2)
N-1B	0.5046 (5)	0.3617 (3)	0.5355 (2)
C-1B	0.5840 (8)	0.3372 (5)	0.6300 (3)
C-2B	0.5952 (6)	0.3472 (3)	0.4670 (3)
O-2B	0.7402 (4)	0.3198 (3)	0.4835 (3)
N-3B	0.5117 (4)	0.3653 (3)	0.3798 (2)
C-3B	0.6055 (7)	0.3570 (5)	0.3053 (4)
C-4B	0.3473 (6)	0.3937 (3)	0.3579 (3)
O-4B	0.2769 (4)	0.4070 (3)	0.2792 (2)
N-5B	0.2677 (4)	0.4078 (3)	0.4301 (2)
C-5B	0.0887 (6)	0.4339 (5)	0.4093 (4)
C-6B	0.3401 (6)	0.3926 (3)	0.5206 (3)
O-6B	0.2647 (5)	0.4071 (3)	0.5823 (2)

Table 14   Bond Distances of 1,3,5-Trimethyltriazine-
2,4,6-trione (**48**)

Atoms	Distance (Å)
C-1A to N-1A	1.474 (5)
C-6A to N-1A	1.367 (5)
N-3A to C-2A	1.371 (6)
C-4A to N-3A	1.370 (6)
N-5A to C-4A	1.363 (6)
C-6A to N-5A	1.377 (5)
C-1B to N-1B	1.464 (6)
C-6B to N-1B	1.378 (6)
N-3B to C-2B	1.363 (5)
C-4B to N-3B	1.368 (5)
N-5B to C-4B	1.364 (6)
C-6B to N-5B	1.374 (6)
C-2A to N-1A	1.372 (5)
O-2A to C-2A	1.213 (6)
C-3A to N-3A	1.462 (7)
O-4A to C-4A	1.214 (5)
C-5A to N-5A	1.457 (6)
O-6A to C-6A	1.209 (6)
C-2B to N-1B	1.374 (6)
O-2B to C-2B	1.215 (6)
C-3B to N-3B	1.459 (7)
O-4B to C-4B	1.211 (5)
C-5B to N-5B	1.472 (6)
O-6B to C-6B	1.209 (7)

Table 15   Bond Angles of 1,3,5-Trimethyltriazine-2,4,6-

trione (48)

Atoms	Angle (°)
C-2A to N-1A to C-1A	117.2 (4)
C-6A to N-1A to C-2A	124.3 (3)
N-3A to C-2A to N-1A	115.6 (4)
C-3A to N-3A to C-2A	116.7 (4)
C-4A to N-3A to C-3A	119.0 (4)
N-5A to C-4A to N-3A	116.0 (4)
C-5A to N-5A to C-4A	117.9 (4)
C-6A to N-5A to C-5A	118.0 (4)
O-6A to C-6A to N-1A	122.5 (4)
C-2B to N-1B to C-1B	118.2 (4)
C-6B to M-1B to C-2B	124.3 (4)
N-3B to C-2B to N-1B	115.7 (4)
C-3B to N-3B to C-2B	117.7 (4)
C-4B to N-3B to C-3B	117.9 (4)
N-5B to C-4B to N-3B	116.1 (3)
C-5B to N-5B to C-4B	117.8 (4)
C-6B to N-5B to C-5B	117.7 (4)
O-6B to C-6B to N-1B	122.9 (4)
C-6A to N-1A to C-1A	118.0 (4)
O-2A to C-2A to N-1A	122.3 (4)
N-3A to C-2A to O-2A	122.1 (4)
C-4A to N-3A to C-2A	124.1 (4)
O-4A to C-4A to N-3A	122.3 (4)
N-5A to C-4A to O-4A	121.7 (4)
C-6A to N-5A to C-4A	124.1 (4)
N-5A to C-6A to N-1A	115.5 (4)
O-6A to C-6A to N-5A	121.9 (4)
C-6B to N-1B to C-1B	117.4 (4)
O-2B to C-2B to N-1B	122.0 (4)
N-3B to C-2B to O-2B	122.3 (5)
C-4B to N-3B to C-2B	124.3 (4)
O-4B to C-4B to N-3B	122.2 (4)
N-5B to C-4B to O-4B	121.7 (4)
C-6B to N-5B to C-4B	124.4 (4)
N-5B to C-6B to N-1B	115.2 (4)
O-6B to C-6B to N-5B	121.9 (4)

Reaction of 1-Acetyl-2-phenyl hydrazine with Ethoxymethylene-malononitile

Method A: 1-Acetyl-2-phenylhydrazine (0.5 g, 3.3 mmol) and ethoxymethylene-malonontrile (0.41 g, 3.3 mmol) were refluxed in dry ethanol for 12 hours. The solvent was evaporated to produce a crude solid. The ^{1}H NMR spectrum identified this solid as starting material.

Method B: 1-Acetyl-2-phenylhydrazine (0.5 g, 3.3 mmol) and ethoxymethylene-malononitrile (0.41 g, 3.3 mmol) were sealed under an atmosphere of nitrogen. Phosphorous oxychloride (0.56 g, 3.7 mmol) was injected and the reaction was stirred at 100 °C for 1 hour. Any remaining phosphorous oxychloride was evaporated under vacuum and the reaction was quenched with water (5 ml). Evaporation of the solvent and recrystallisation from methanol afforded pure 4-cyano-3-(2,2-dicyanoethenylamino)-1-phenylpyrazole (60).

Method C: 1-Acetyl-2-phenylhydrazine (0.5 g, 3.3 mmol) and ethoxymethylene-malononitrile (0.41 g, 3.3 mmol) were stirred at room temperature in phosphorous oxychloride (0.56 g, 3.7 mmol) for 12 hours. An identical work up afforded only 4-cyano-3-(2,2-dicyanoethenylamino)-1-phenylpyrazole (60).

Method D: 1-Acetyl-2-phenylhydrazine (5.0 g, 33.3 mmol) and ethoxymethylene-malononitrile (8.13 g, 66.6 mmol) were stirred at 100 °C in phosphorous oxychloride (10.21 g, 66.6 mmol) for 12 hours. An identical work up afforded only 4-cyano-3-(2,2-dicyanoethenylamino)-1-phenylpyrazole (60, 49 %).

60: mp 225-228 °C; ^{1}H NMR δ 7.38-7.87 (m, 5, CH_{arom}), 8.45 (s, 1, CH), 9.31 (s, 1, H-3), 12.19 (br s, 1, NH); ^{13}C NMR δ 55.7 (s, C-1"), 84.9 (s, C-4), 112.1 (s, CN), 113.0 (s, CN), 115.4 (s, CN), 119.1 (d, C-2', C-6'), 128.0 (d, C-4), 129.7 (d, C-3', C-5'), 136.1 (d, C-3), 138.1 (s, C-1'), 149.4 (s, C-5), 156.5 (d, C-2"); IR

3210, 3150, 2220, 2210, 2208 cm^{-1}; Crystal Data $C_{14}H_8N_6$, M = 260.3, Monoclinic, space group $P2_1/n$, a = 9.732 (2), b = 5.566 (1), c = 23.659 (6) Å, β = 97.39 (1)°, v = 1271.0 (5) Å^3, D_c (Z = 4) = 1.359 gcm^{-3}, F(000) = 824, $\mu(MoK_\alpha)$ = 0.96 cm^{-1}, specimen: 0.35 x 0.14 x 0.13, N = 1909, N_O = 1158, R = 0.036, R_w = 0.038, T = 296 K. Final atomic coordinates are listed in Table 16, while bond distances and angles are listed in Tables 17 and 18.

3-Amino-4-cyano-1-phenylpyrazole (61)

4-Cyano-3-(2,2-dicyanoethenylamino)-1-phenylpyrazole (0.5 g, 1.9 mmol) was refluxed in hydrochloric acid (1 M, 20 ml) for 3 hours. Upon cooling, a white solid precipitated and was collected by suction filtration. Recrystallisation from ethyl acetate and hexane afforded pure 3-amino-4-cyano-1-phenylpyrazole (61): yield 92 %; mp 155-156 °C; ^{1}H NMR δ 5.99 (br s, 2, NH$_2$), 7.24-7.72 (m, 5, CH$_{arom}$), 8.92 (s, 1, H-3); ^{13}C NMR δ 80.6 (s, C-4), 114.1 (s, CN), 118.0 (d, C-2', C-6'), 126.4 (d, C-4'), 129.5 (d, C-3', C-5'), 133.4 (d, C-3), 138.7 (s, C-1'), 158.0 (s, C-5); IR 3360, 3220, 2210 cm^{-1}.

4-Imino-5-methyl-2-phenyl-7H-pyrazolo[3,4-d]pyrimidin-6-one (62)

3-Amino-4-cyano-1-phenylpyrazole (1.0 g, 5.4 mmol) and sodium methoxide (0.59 g, 10.8 mmol) were sealed under an atmosphere of nitrogen. DMF (20 ml) and methyl isocyanate (0.46 g, 8.1 mmol) were injected by syringe and the reaction mixture was stirred at 60 °C for 12 hours. The reaction was neutralised with 1 M hydrochoric acid and the solvent was evaporated under reduced pressure. Recrystallisation of the crude product from DMF afforded pure 4-imino-5-methyl-2-phenyl-7H-pyrazolo[3,4-d]pyrimidin-6-one (62): yield 66 %; mp 242-244 °C; ^{1}H NMR δ 2.87 (s, 3, CH$_3$), 7.31-7.73 (m, 5, CH$_{arom}$), 8.24 (br s, 1, NH), 9.02 (s, 1, H-3), 11.27 (br s, 1, NH); ^{13}C NMR δ 26.8 (q, CH$_3$), 102.7 (s, C-3a), 119.0 (d, C-2', C-6'), 127.4 (d, C-4'), 129.7 (d, C-3), 129.9 (d, C-3', C-5'), 138.9 (s, C-1'), 150.0 (s, C-7a),

Table 16   Atomic Coordinates of 4-Cyano-3-(2,2-dicyanoethenyl

amino)-1-phenylpyrazole (60)

Atom	x/a	y/b	z/c
N-1	0.6877 (2)	0.2813 (4)	0.6459 (1)
N-2	0.6764 (2)	0.1238 (4)	0.6011 (1)
C-3	0.7637 (2)	0.2072 (5)	0.5675 (1)
C-4	0.8324 (2)	0.4162 (5)	0.5900 (1)
C-5	0.7793 (3)	0.4567 (6)	0.4600 (1)
C-11	0.6001 (3)	0.2516 (5)	0.6894 (1)
C-12	0.5173 (3)	0.0509 (6)	0.6886 (1)
C-13	0.4329 (3)	0.0258 (8)	0.7315 (2)
C-14	0.4319 (3)	0.1950 (8)	0.7737 (1)
C-15	0.5160 (4)	0.3937 (7)	0.7739 (1)
C-16	0.6003 (4)	0.4212 (4)	0.7319 (1)
N-31	0.7755 (2)	0.0903 (4)	0.5162 (1)
C-32	0.6966 (3)	-0.1006 (5)	0.4993 (1)
C-33	0.6991 (3)	-0.2262 (5)	0.4505 (1)
C-34	0.7882 (3)	-0.1690 (5)	0.4093 (1)
N-35	0.8603 (3)	-0.1251 (6)	0.3765 (1)
C-36	0.6115 (3)	-0.4314 (6)	0.4392 (1)
N-37	0.5425 (3)	-0.5966 (5)	0.4302 (1)
C-41	0.9361 (3)	0.5571 (5)	0.5683 (1)

Table 17   Bond Distances of 4-cyano-3-(2,2-dicyano

ethenylamino)-1-phenylpyrazole (60)

Atoms	Distance (Å)
N-1 to N-2	1.368 (3)
N-1 to C-5	1.341 (3)
N-1 to C-11	1.427 (3)
N-2 to C-3	1.321 (3)
C-3 to C-4	1.412 (4)
C-3 to N-31	1.395 (3)
C-4 to C-5	1.369 (4)
C-4 to C-41	1.424 (4)
C-41 to N-41	1.142 (3)
N-31 to C-32	1.341 (3)
C-32 to C-33	1.352 (4)
C-33 to C-34	1.420 (4)
C-34 to N-35	1.138 (3)
C-33 to C-36	1.429 (4)
C-36 to N-37	1.142 (3)
C-11 to C-12	1.376 (4)
C-11 to C-16	1.379 (4)
C-12 to C-13	1.391 (4)
C-13 to C-14	1.374 (5)
C-14 to C-15	1.375 (5)
C-15 to C-16	1.377 (4)

Table 18   Bond Angles of 4-cyano-3-(2,2-dicyano

ethenylamino)-1-phenylpyrazole (60)

Atoms	Angle (º)
N-2 to N-1 to C-5	112.0 (2)
N-2 to N-1 to C-11	119.2 (2)
C-5 to N-1 to C-11	128.7 (3)
N-1 to N-2 to C-3	104.4 (2)
N-2 to C-3 to N-31	119.5 (2)
N-2 to C-3 to C-4	111.8 (2)
C-4 to C-3 to N-31	128.7 (2)
C-3 to C-4 to C-5	104.4 (2)
C-3 to C-4 to C-41	129.8 (3)
C-5 to C-4 to C-41	125.7 (3)
C-4 to C-41 to N-42	179.5 (3)
C-4 to C-5 to N-1	107.4 (3)
N-1 to C-11 to C-12	119.2 (3)
N-1 to C-11 to C-16	120.1 (3)
C-12 to C-11 to C-16	120.8 (3)
C-11 to C-12 to C-13	118.1 (3)
C-12 to C-13 to C-14	121.5 (3)
C-13 to C-14 to C-15	119.6 (3)
C-14 to C-15 to C-16	119.7 (4)
C-15 to C-16 to C-11	120.4 (3)
C-3 to N-31 to C-32	121.4 (2)
N-31 to C-32 to C-33	126.2 (2)
C-32 to C-33 to C-34	119.7 (2)
C-32 to C-33 to C-36	123.3 (3)
C-34 to C-33 to C-36	116.9 (2)
C-33 to C-34 to N-35	179.3 (3)
C-33 to C-36 to N-37	179.4 (3)

151.5 (s, C-4), 158.8 (s, C-6); IR 3450, 3120, 1720 cm^{-1}; Anal. Calculated for

$C_{12}H_{11}N_5O$: C, 59.74; H, 4.60; N, 29.03. Found: C, 59.8; H, 4.5; N, 28.9; HRMS

Calculated for $C_{12}H_{11}N_5O$: 241.0964. Found: 241.0965.

6-Alkylthio-4-mercapto-1-phenylpyrazolo[3,4-d]pyrimidines (63a,b)

1-Phenyl-5H,7H-pyrazolo[3,4-d]pyrimidin-4,6-dithione (0.50 g, 1.92 mmol) was

dissolved in 1 M sodium hydroxide (10 ml). 2-Bromopropionamide (0.35 g,

2.30 mmol) was added and the reaction was stirred at room temperature for 24 hours.

Upon neutralisation with 1 M hydrochloric acid, a solid precipitated and was collected by

suction filtration. Recrystallisation from DMSO and water to afforded pure 6-α-

carbamoylethylthio-4-mercapto-1-phenylpyrazolo[3,4-d]pyrimidine (63a). The use of

2-bromobutanamide allowed the synthesis of 6-α-carbamoylpropylthio-4-mercapto-1-

phenylpyrazolo[3,4-d]pyrimidine (63b).

63a: yield 72 %; mp 285-286 °C; ^{1}H NMR δ 1.56 (d, 3, J = 7.1 Hz, CH$_3$),

4.43 (q, 1, J = 7.1 Hz, CH), 7.34 (br s, 1, NH), 7.42 (t, 1, H-4'), 7.57 (t, 2, H-3',

H-5'), 8.07 (d, 2, H-2', H-6'), 7.79 (br s, 1, NH), 8.35 (s, 1, H-3), 11.69 (br s, 1,

SH); ^{13}C NMR δ 18.1 (q, CH$_3$), 44.7 (d, CH), 116.4 (s, C-3a), 121.3 (d, C-3', C-5'),

127.1 (d, C-4'), 129.4 (d, C-2', C-6'), 138.0 (s, C-1'), 138.2 (d, C-3), 146.5 (s,

C-7a), 160.2 (s, C-6), 172.0 (s, C=O), 180.1 (s, C-4); IR 3395, 3200, 2980, 2850,

1680 cm^{-1}; Anal. Calculated for $C_{14}H_{13}N_5OS_2$: C, 50.7; H, 3.9; N, 21.1. Found: C,

50.4; H, 3.5; N, 21.1.

63b: yield 67 %; mp 227-237 °C; ^{1}H NMR δ 0.99 (t, 3, J = 7.2 Hz, CH$_3$), 1.98

(m, 2, J = 6.7, 7.2 Hz, CH$_2$), 4.35 (t, 1, J = 6.7 Hz, CH), 7.40-8.55 (m, 8, CH$_{arom}$,

NH and SH), 8.36 (s, 1, H-3); ^{13}C NMR δ 11.4 (q, CH$_3$), 25.6 (t, CH$_2$), 50.8 (d,

CH), 116.4 (s, C-3a), 121.3 (d, C-2', C-6'), 127.1 (d, C-4'), 129.3 (d, C-3', C-5'),

138.0 (s, C-1'), 138.1 (d, C-3), 146.4 (s, C-7a), 160.3, (s, C-6), 171.0 (s, C=O), 180.1 (s, C-4); IR 3375, 2960, 2850, 1660 cm^{-1}.

4-α-Carbamoylethylthio-1-phenylpyrazolo[3,4-d]pyrimidine (66)

1-Phenyl-5H-pyrazolo[3,4-d]pyrimidin-4-thione (2.0 g, 8.8 mmol) was dissolved in sodium hydroxide (1 M, 20 ml). 2-Bromopropionamide (1.60 g, 10.5 mmol) was added and the reaction was stirred at room temperature for 24 hours. A solid precipitated and was collected by suction filtration. Recrystallisation of the crude product from DMSO and water to afforded 4-α-carbamoylethylthio-1-phenylpyrazolo[3,4-d]pyrimidine (66): yield 78 %; mp 190-195 °C; ^{1}H NMR δ 1.58 (d, 3, J = 7.0 Hz, CH$_3$), 4.89 (q, 1, J = 7.0 Hz, CH), 7.30 (br s, 1 NH), 7.37-7.61 (m, 5, CH$_{arom}$), 7.80 (br s, 1, NH), 8.61 (s, 1, H-3), 8.87 (s, 1, H-6); ^{13}C NMR δ 18.9 (q, CH$_3$) 42.4 (d, CH), 112.8 (s, C-3a), 121.2 (d, C-2', C-6'), 126.9 (d, C-4'), 129.3 (d, C-3', C-5'), 133.4 (d, C-3), 138.2 (s, C-1'), 150.6 (s, C-7a), 154.7 (s, C-6), 164.6 (s, C-4), 172.0 (s, C=O); IR 3650, 3470, 1800 cm^{-1}; Anal. Calculated for C$_{14}$H$_{13}$N$_5$OS: C, 56.2; H, 4.4; N, 23.4. Found: C, 56.3; H, 4.4; N, 23.4.

2-Bromo alkylcarboxamides (67a,b)

Ammonium hydroxide (15 ml, 33 %) was cooled to 0 °C on an ice bath. 2-Bromopropionyl bromide (10.7 g, 49.8 mmol) was added at a rate which did not raise the temperature above 10 °C. The reaction mixture was stirred at room temperature for 30 minutes. A solid precipitated from solution and was collected by suction filtration. Recrystallisation from water afforded 2-bromopropionamide (67a). The use of 2-bromobutyryl bromide allowed the synthesis of 2-bromobutanamide (67b).

67a: yield 77 %; mp 123-124.5 °C; ^{1}H NMR δ 1.62 (d, 3, J = 6.8 Hz, CH$_3$), 4.43 (q, 1, J = 6.8 Hz, CH$_3$), 7.22 (br s, 1, NH), 7.65 (br s, 1, NH); ^{13}C NMR δ 21.6, 44.0, 170.8; IR 3350, 3175, 1670 cm^{-1}.

67b: yield 58%; mp 108-110 °C; ^{1}H NMR δ 0.88 (t, 3, J = 7.3 Hz, CH$_3$), 1.88

(m, 2, J = 7.3, 7.3 Hz), 4.21 (t, 1, J = 7.3 Hz, CH), 7.26 (br s, 1, NH), 7.66 (br s, 1,

NH); ^{13}C NMR δ 11.6, 28.0, 51.2, 170.2; IR 3360, 3180, 1670 cm^{-1}.

6-α-Carbamoylethylthio-4-methylthio-1-phenylpyrazolo[3,4-*d*]pyrimidine

(68)

6-α-carbamoylethylthio-4-mercapto-1-phenylpyrazolo[3,4-*d*]pyrimdine (2.0 g,

6.03 mmol) was dissolved in 1 M NaOH (20 ml). Methyl iodide (0.38 ml, 6.03 mmol)

was added and the reaction was stirred at room temperature for 1 hour. A solid

precipitated from solution and was collected by suction filtration. Recrystallisation from

DMSO and water to afforded pure 6-α-carbamoylethylthio-4-methylthio-1-

phenylpyrazolo[3,4-*d*]pyrimidine (68): yield 70 %; mp 230-232 °C; ^{1}H NMR δ 1.56

(d, 3, J = 7.1 Hz, CH$_3$), 2.69 (s, 3, SCH$_3$), 4.47 (q, J = 7.1 Hz, CH), 7.23 (br s, 1,

NH), 7.37 (t, 1, H-4'), 7.57 (t, 2, H-3', H-5'), 7.71 (br s, 1, NH), 8.14 (d, 2, H-2',

H-6'), 8.48 (s, 1, H-3); ^{13}C NMR δ 11.5 (q, SCH$_3$), 18.1 (q, CH$_3$), 44.1 (d, CH),

110.4 (s, C-3a), 120.8 (d, C-3', C-5'), 126.7 (d, C-4'), 129.4 (d, C-2', C-6'), 133.7

(d, C-3), 138.2 (s, C-1'), 151.0 (s, C-7a), 165.7 (s, C-6), 168.0 (s, C-4), 172.7 (s,

C=O); IR 3370, 3190, 1658 cm^{-1}; Anal. Calculated for C$_{15}$H$_{15}$N$_5$OS$_2$: C, 52.2; H, 4.4;

N, 20.3. Found: C, 52.2; H, 4.1; N, 20.4.

4-Amino-6-α-Carbamoylethylthio-1-phenylpyrazolo[3,4-*d*]pyrimidine (69)

6-α-carbamoylethylthio-4-methylthio-1-phenylpyrazolo[3,4-*d*]pyrimidine (0.5 g,

1.45 mmol) was dissolved in DMSO (10 ml). Ethanolic ammonia (20 ml) was added and

the solution was heated at 100 °C for 24 hours in a bomb. Ice cold water (40 ml) was

added and a solid precipitated from solution. This crude product was collected by suction

filtration and recrystallised from DMSO and water to afford pure 4-amino-6-α-

carbamoylethylthio-1-phenylpyrazolo[3,4-*d*]pyrimidine (69): yield 79 %; mp 284-

286 °C; ^{1}H NMR δ 1.50 (d, 3, J = 7.1 Hz, CH$_3$), 4.37 (q, 1, J = 7.1 Hz, CH), 7.14

(br s, 1, NH), 7.31 (t, 1, H-4'), 7.53 (t, 2, H-3', H-5'), 7.92 (br s, 1, NH), 8.06 (br s,

1, NH), 8.18 (d, 2, H-2', H-6'), 8.26 (s, 1, H-3); ^{13}C NMR δ 18.0 (q, CH$_3$), 42.9 (d,

CH), 99.4 (s, C-3a), 120.4 (d, C-3', C-5'), 126.0 (d, C-4'), 129.2 (d, C-2', C-6'),

134.3 (d, C-3), 139.0 (s, C-1'), 153.6 (s, C-7a), 157.5 (s, C-4), 68.8 (s, C-6), 173.3

(s, C=O); IR 3470, 3375, 3190, 3075, 1658 cm^{-1}; Anal. Calculated for C$_{14}$H$_{14}$N$_6$OS:

C, 53.5; H, 4.5; N, 26.7. Found: C, 53.6; H, 4.4; N, 27.0.

1-Phenyl-5H-pyrazolo[3,4-d]pyrimidin-4-thione (70)

5-Amino-4-cyano-1-phenylpyrazole (6.0 g, 32.6 mmol) and triethyl orthoformate (60 ml)

were refluxed in acetic anhydride (60 ml) for 3 hours. Evaporation of the solvent yielded

a brown oily solid. Sodium hydrosulphide (1.5 M, 150 ml) was added and the reaction

was refluxed for a further 12 hours. The solvent was evaporated under reduced pressure

and the residue was taken up in hot water. Acidification with glacial acetic acid resulted

in the precipitation of a solid. This crude product was collected by suction filtration and

dissolved in 1 M sodium hydroxide. Neutralisation with 1M hydrochloric acid afforded

pure 1-phenyl-5H-pyrazolo[3,4-d]pyrimidin-4-thione (70): yield 91%; mp 279-282 °C;

^{1}H NMR δ 7.37-8.05 (m, 5, CH$_{arom}$), 8.29 (s, 1, H-3), 8.42 (s, 1, H-6); ^{13}C NMR δ

118.9 (s, C-3a), 121.8 (d, C-2', C-6'), 127.3 (d, C-4'), 129.3 (d, C-2', C-6'), 137.9

(s, C-1'), 138.0 (d, C-3), 147.0 (s, C-7a), 147.8 (s, C-6), 179.4 (s, C-4).

*Due to the similarity of the chemical shifts of the C-4, C-6 and C-7a carbons of most

pyrazolo[3,4-d]pyrimidines, a definative assignment of these carbons could not be made.

[8.2] Biological Experimental

[^{3}H] R-PIA Competitive Binding Assay

The binding assay involved the displacement of [^{3}H] R-PIA (Amersham TRK.783: 15,

36, 45 Ci / mM) binding to rat brain membranes. Assays were performed in triplicate and

included internal controls. All compounds were assayed at 20 μM to obtain the

% Inhibition values and then selected compounds were assayed at various known

concentrations to obtain IC$_{50}$ values. Washed synaptosomal membranes were obtained

from male Wistar rats and were incubated with adenosine deaminase for 30 minutes at

37 °C to remove any endogenous adenosine. Total binding was determined by

incubating the compound and 5 nM [^{3}H] R-PIA in buffer (1 ml, 50 mM TRIS-HCl,

1 mM MgCl$_2$, pH = 7.4) at 37 °C for 40 minutes. Non-specific binding was determined

using a large excess of unlabelled [^{3}H] R-PIA under the same conditions. Separation of

bound and free ligand was achieved using a vacuum filtration technique. The reaction

was terminated by the addition of 4 ml of ice cold incubation buffer and then filtered

through Whatman GF/B glass fiber filters using millipore apparatus. Non-specific

binding of free radioligand to the filter was reduced by rapid washing of the filter with

two 4 ml aliquots of ice cold incubation buffer. The filtration process, from application

of the sample to the filter to removal of the filter, did not exceed 30 seconds. The filter

was placed in 10 ml of aqueous scintillation fluid and stored for a minimum of 6 hours.

Samples were then counted in a Liquid Scintillation counter for 5 minutes. Specific

binding was calculated by subtracting non-specific binding from total binding. IC$_{50}$

values were calculated using EBDA.[146]

Hydrophobicity Index

An index of the hydrophobicity was provided by the retention time of a number of the

pyrazolo[3,4-d]pyrimidines on a reverse phase HPLC column. An ETP Kortec K35M

pump and an ETP Kortec K95 variable wavelength UV detector fitted with a Dynamax

60A ODS silica column (0.45 x 20 cm) was used. This system was isocratically eluted

with 6:4 mixture of NaH_2PO_4/Na_2HPO_4 (0.05 M, pH = 7.4):CH_3OH which had been

adjusted to pH = 7.4 using 1 M phosphoric acid. The hydrophobicity index (k') was

calculated for each compound using the formula:

$$k' = (t - t_0)/t_0$$

where t is the retention time of the compound and t_0 is the retention time of the solvent

front.

Table 19   Adenosine Receptor Affinity and Hydrophobicity

No.	% I	IC_{50}	k'
9a	95	$4.41 \pm 1.51 \times 10^{-7}$	
9b	96	$1.08 \pm 0.01 \times 10^{-7}$	
24a	30		2.6
24b	7		1.7
24c	53	$1.65 \pm 0.40 \times 10^{-5}$	14.2
24d	37		13.6
24e	6		1.8
24f	40		17.5
24g	34		18.9
24h	8		1.1
24i	44		5.8
24j	26		4.1
24k	5		0.9
24l	2		>36.5
24m	5		>36.5
24n	0		0.8
24p	0		0.7
24q	0		0.3
24r	9		7.1
24s	1		4.8
24t	29		>36.5
24u	6		0.1
24v	5		0.8
25a	58	$2.01 \pm 0.18 \times 10^{-5}$	4.2
25b	52	$5.07 \pm 0.21 \times 10^{-5}$	7.5
25c	72	$0.96 \pm 0.33 \times 10^{-5}$	18.5
25d	25		5.1
25e	70	$1.98 \pm 0.72 \times 10^{-5}$	21.9
25f	68	$1.46 \pm 0.50 \times 10^{-5}$	>36.5
25g	88	$0.64 \pm 0.10 \times 10^{-5}$	>36.5
40a	9		
40b	28		
47	0		
62	41		
63a	94	$8.14 \pm 0.86 \times 10^{-7}$	
63b	97	$4.92 \pm 1.06 \times 10^{-7}$	
66	38	$3.64 \pm 0.06 \times 10^{-5}$	
68	100	$5.61 \pm 1.06 \times 10^{-8}$	
69	100	$3.23 \pm 0.57 \times 10^{-8}$	

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LIST OF PUBLICATIONS

(1) Synthesis of 5-Aminopyrazole-4-carbonitriles, Peter J. Scammells, Michael J. Dooley and Ronald J. Quinn, *Aust. J. Chem.*, 1989, **42**, 747.

(2) Synthesis and Adenosine Receptor Affinity of a Series of Pyrazolo[3,4-*d*] pyrimidine Analogues of 1-Methylisoguanosine, Peter J. Scammells, Fiona A. Harden and Ronald J. Quinn, accepted for publication by *J. Med. Chem.*

(3) Mono-(α-carbamoylethylthio)-substituted Pyrazolo[3,4-*d*]pyrimidines: The Position of Substitution, Peter J. Scammells, Ronald J. Quinn and David J. Tucker, accepted for publication by *Aust. J. Chem.*

(4) Synthesis of 2-Substituted Pyrazolo[3,4-*d*]pyrimidines, Peter J. Scammells and Ronald J. Quinn, submitted to *Aust. J. Chem.*

(5) 4-Amino-1-phenyl-5*H*-pyrazolo[3,4-*d*]pyrimidin-6-one, an Isoguanosine Analogue, Peter J. Scammells and Ronald J. Quinn, submitted to *Aust. J. Chem.*