STRUCTURE-ACTIVITY IN PYRIDINE-CONTAINING

HISTAMINE RECEPTOR ANTAGONISTS

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ABSTRACT

The effect of substitution in some pyridine-containing histamine antagonists on biological activity is described. This work has led to the discovery of potent histamine receptor antagonists of closely related structures which either act at both histamine H_1- and H_2-receptors or show selectivity for one receptor subtype only.

An historical review of the properties of histamine, the classification of histamine receptors and the structural requirements for antagonist activity at histamine receptors is presented.

Substitution at the 3-pyridyl position of N-cyano-N'-methyl-N"-[4-(pyrid-2-yl)butyl]guanidine markedly influenced antagonist activity at the H_2-receptor. This effect was correlated with a steric parameter. The same correlation was apparent when the second methylene of the butyl chain was replaced by sulphur and when the cyanoguanidine group was replaced by a substituted 2-aminopyrimidin-4(1H)-one. The latter series also showed activity at the H_1-receptor which was correlated with the same steric parameter although, for optimal activity, substituents of different size were required.

Comparison of calculated conformations and crystal structures of some analogues indicated that different conformations of the linking butyl chain were required for activity at each receptor. This hypothesis was tested by synthesising a conformationally restricted analogue.

Introduction of a Mannich group at the 6-position reduced potency at the H_2-receptor. Similar substitution at the 4-position gave highly potent and selective H_2-receptor antagonists. These results are compared with other H_2-receptor antagonists containing a Mannich substituent.

Most of the pyridylbutyl analogues were prepared using a malonic ester coupling with a chloronitropyridine. Conditions for hydrolysis and decarboxylation of the malonic esters and elaboration of the nitro groups are discussed. A series of analogues were prepared using free radical chemistry. The presence of a Mannich group was shown to facilitate radical substitution. This has extended the range of pyridyl substituents that can be tolerated when using free radical chemistry for the preparation of substituted pyridines.

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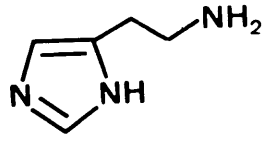
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1.0 REVIEW: HISTAMINE AND HISTAMINE RECEPTOR ANTAGONISTS

1.1. INTRODUCTION

The work described in this thesis is concerned with compounds that are receptor antagonists of the hormone histamine. This chapter contains a brief historical review of the discovery and the properties of histamine and histamine receptor antagonists. The object of the review is to highlight the background knowledge on which the work presented in this thesis is based. This review includes both published literature and data not previously published by Smith Kline and French which is relevant to the work presented in later chapters.

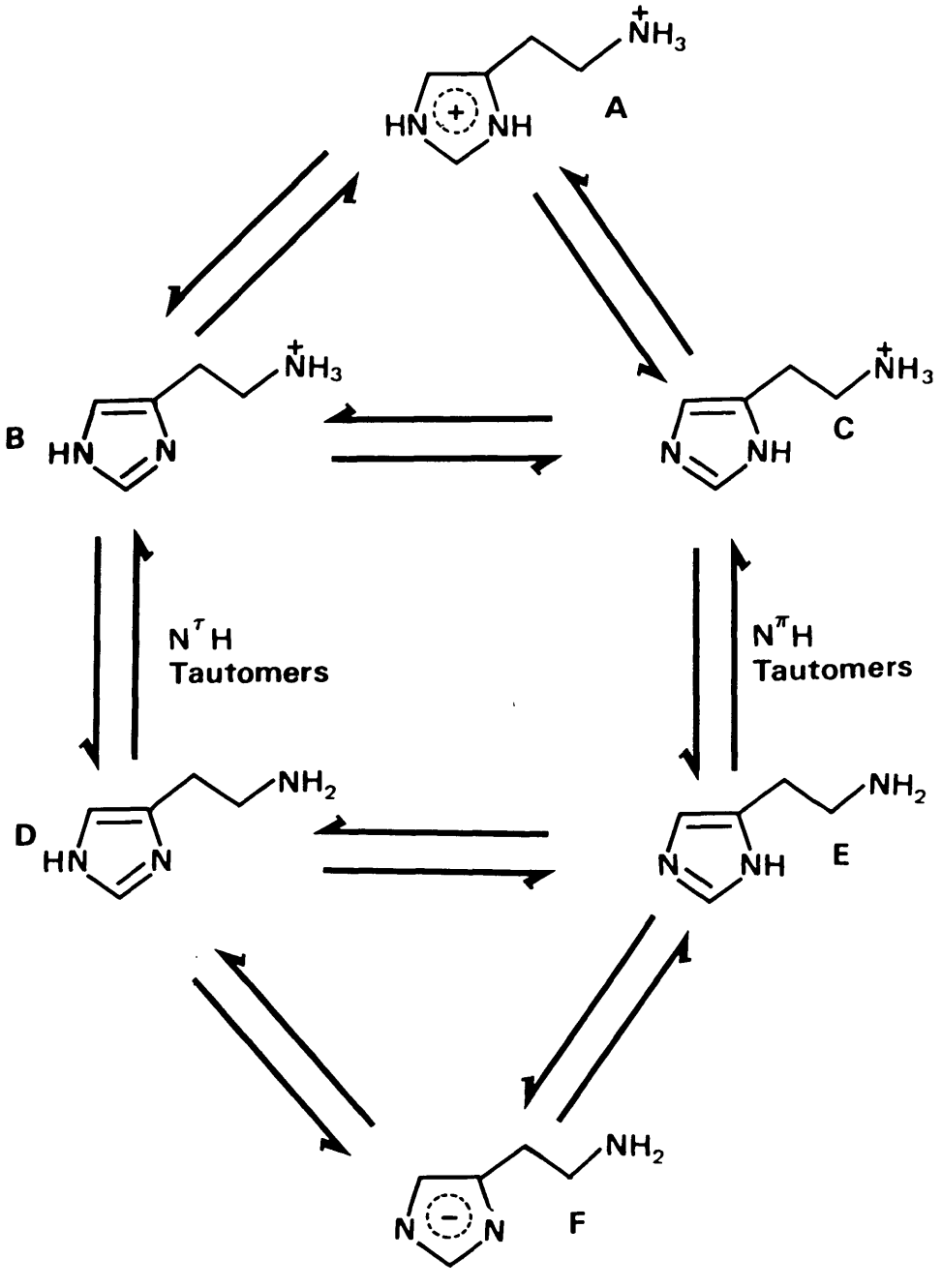
1.2. HISTAMINE

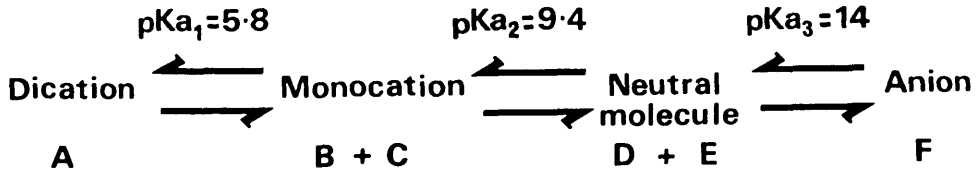


(1)

Histamine (1), 4-(2-aminoethyl)imidazole, was first synthesised in 1907 by Windaus and Vogt[1] as a partial structure of the amino acid histidine. Three years later it was shown to be a naturally occurring compound by its presence in extracts of ergot.[2] In the same year Dale and Laidlaw[3,4] published the first of a series of papers reporting the pharmacological actions of histamine. They noted that histamine was a vasoconstrictor on the larger blood vessels and produced contractions in cardiac muscle. On the capillaries it produced dilation with a resulting fall in the systemic blood pressure. Larger doses of histamine led to loss of plasma through the capillary wall into the extracellular spaces producing oedema, haemoconcentration, increased blood viscosity and a fall in body temperature.[5]

Figure 1.1 Ionic and tautomeric forms of histamine in solution





Histamine also produces constriction in the smooth muscle in organs such as bronchioles and ileum. Popielski[6] reported that histamine is a stimulant of gastric acid secretion in many species, particularly the dog and cat although its response in the rat and mouse is poor.

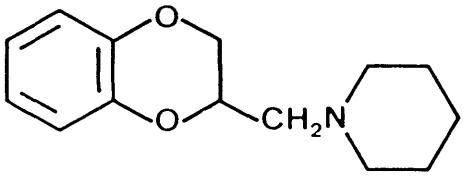
Histamine is found in many types of tissue, both animal and plant. It is formed by the enzymatic decarboxylation of the amino acid L-histidine. It is mainly stored in mast cells and once released is rapidly catabolised by deamination or methylation.[7,8,9]

The overall shape of the histamine molecule is determined by the rotation about the single bonds of the ethylamine side chain. The conformational properties have been studied by NMR,[10,11] X-ray crystallography[12] and theoretical calculations.[10,13,14,15] However the various methods differ in their prediction of the minimum energy conformations. Histamine can exist in a number of ionic and tautomeric forms in solution, shown in Figure 1.1. At physiological condition (pH 7.4; 37°C) the dominant species in solution is the Nτ-H monocation.[16] A series of investigations has suggested that the active form of histamine at both receptors (see later) is likely to be the Nτ-H tautomer of the monocation, although different chemical properties of histamine may be associated with the two receptors.[17]

1.3. HISTAMINE H_1-RECEPTOR ANTAGONISTS

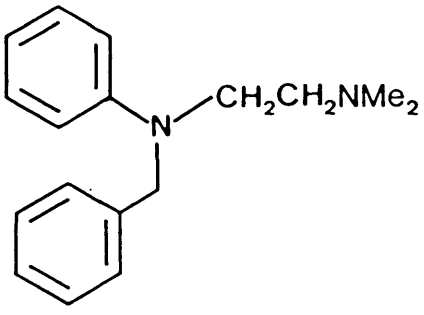
1.3.1. Development of histamine H_1-receptor antagonists

The first histamine receptor antagonist was reported by Fourneau and Bovet.[18] This was the benzodioxane (2) which was only weakly active. Further developments led to a series of aminoalkyl-ethers[19] and ethylenediamine antagonists.[20] The first clinically useful histamine antagonist was Antergan (3).[21] Modification of the aryl rings of Antergan led to the pyridyl aminoethyl analogues.

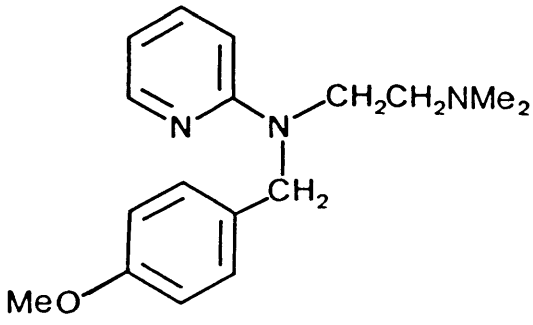


(2)

Mepyramine (4)[22] is an active antihistamine in this class which is still in clinical use today. These antihistamine drugs have been used for the treatment of allergic conditions for over forty years.



(3)



(4)

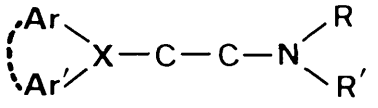
Some of the physiological actions of histamine were found to be unaffected by these drugs. This led Ash and Schild to propose in 1966 that the action of histamine blocked by these antihistaminic drugs characterised only one type of histamine receptor, which they called the histamine H_1-receptor.[23] The histamine H_1-receptor, under the action of histamine, is responsible for the contraction of smooth muscle in various organs[24] such as the guinea-pig ileum and bronchi. Drugs which act at this receptor, blocking the action of histamine, have been defined as histamine H_1-receptor antagonists.

1.3.2. Structure-activity in histamine H_1-receptor antagonists.

The range of structural classes of compounds now known to be antagonists of histamine at the H_1-receptor is extensive and there have been many reviews. The more recent reviews include those of Casy,[25] Nauta and Rekker[26] and Ganellin.[27]

Most of the more active histamine H_1-receptor antagonists can be represented by the general formula (5) which is comprised of an aromatic group linked by a short chain of atoms to a tertiary amino group and encompasses the features shown in Figure 1.2.

Figure 1.2 General structure of histamine H_1-receptor antagonists



(5)

Ar = aryl or hetaryl which may be further substituted.

Ar' = a second aryl ring or an arylmethyl group. The two aryl groups Ar and Ar' may be bridged to form tricyclic derivatives.

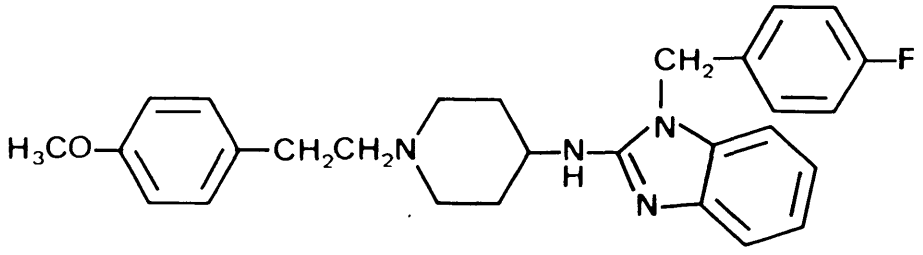
X = a carbon sp_2, carbon sp_3, nitrogen or a carbon sp_3 with an oxygen (ether) link connecting the side chain to the aromatic rings.

C,C = a short chain of carbon atoms which may be saturated, unsaturated, branched or form part of a ring system.

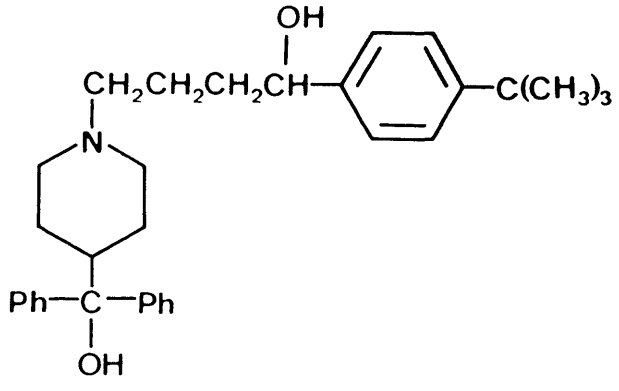
NR,R' = is generally a tertiary basic amino group which may be part of a ring system. The most common groups are dimethylamino and pyrrolidino.

Some of the more recently reported histamine H_1-receptor antagonists include astemizole (6),[28] terfenadine (7)[29] and the cyclic thioamide (8).[30] In the case of terfenadine it has been reported that an active metabolite is responsible for

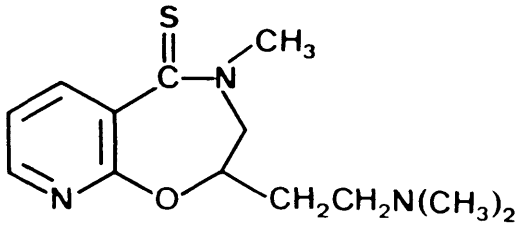
potency.[31] The cyclic thioamide is unusual as it only contains one aromatic ring.



(6)



(7)



(8)

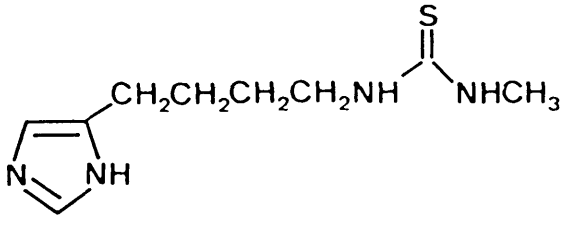
Many attempts have been made to correlate histamine H_1-antagonist activity with the physical properties of the compound and these were reviewed by Nauta and Rekker.[26] The physical properties that have been used include ionisation constants, solubility, surface properties, spectral data (UV, IR and NMR), bond stabilities, charge localization, dipole moment, lipophilicity and 'Hansch' parameters. Two of the most important properties appear to be the lipophilicity of the aryl groups and the basicity of the side chain. Almost all of the reported potent histamine H_1-receptor antagonists are protonated at physiological pH as they contain a tertiary amine side chain with a pKa >7. Some quaternary ammonium derivatives are known to be active[27] which has led to the proposal that the ammonium cation may be the active species. Attempts have been made to correlate activity with various intramolecular distances.[13,32,33] For instance, it was proposed that the most of the active compounds have an intramolecular distance of 5-6Ä between the side chain

ammonium nitrogen and the centroid of one of the aromatic rings,[33] but there are exceptions.[34]

1.4. HISTAMINE H_2-RECEPTOR ANTAGONISTS

1.4.1. Development of histamine H_2-receptor antagonists

In 1964 research was started at Smith, Kline and French Laboratories (England) to try to discover antagonists of histamine that did not act at the histamine H_1-receptor. A systematic modification of the histamine molecule was carried out and in 1972 Black and co-workers[35] at SK&F announced the discovery of antagonists which blocked many of the other actions of histamine. This work led to the definition of the histamine H_2-receptor[35] and the development of the histamine H_2-receptor antagonist, burimamide (9).[36,37]



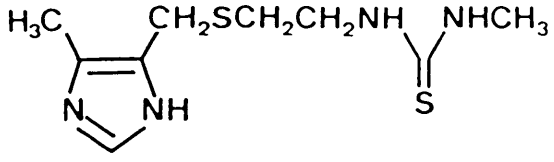
(9)

1.4.2. Development of metiamide and cimetidine

Burimamide although active in man[38] was found not to be sufficiently active orally to be clinically useful. Various ways of increasing activity were investigated. The most successful of these was to modify the microscopic ionisation constants of the imidazole ring and hence the population of the various ionic and tautomeric species in solution.

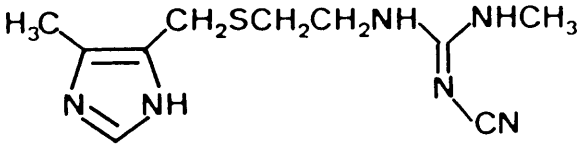
As previously discussed (Section 1.1.) the Nτ-H tautomer is favoured in histamine. It had been proposed that this is due to the electron withdrawing effect of the charged ammonium ethyl side

chain.[39] The tetramethylene side chain present in burimamide is electron donating and this favours the N^{π}-H tautomer.[40] The burimamide structure was therefore modified in order to enhance the stability of the Nτ-H tautomer. The methylene of the side chain was replaced by sulphur and an electron releasing methyl group was introduced into the 5-position of the imidazole ring. This led to metiamide (10) which was 8 to 10 fold more potent <u>in vitro</u>.



(10)

Metiamide was shown to be clinically effective in inhibiting gastric acid secretion in man.[41] However, a low incidence of granulocytopenia[42,43] forced suspension of further trials. These side effects were thought to be associated with the thiourea moiety present in metiamide[44] and prompted the search for a non-thiourea analogue. The cyanoguanidine and thiourea groups have many properties in common and have been regarded as bioisosteres.[45] Replacement of thiourea by cyanoguanidine gave cimetidine (11).

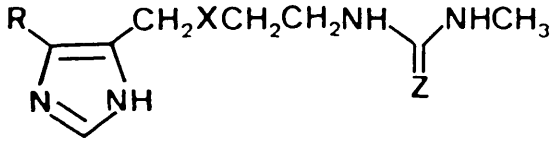


(11)

Cimetidine is slightly more potent than metiamide in inhibiting histamine-stimulated gastric acid secretion in animals[46] and man,[47] and does not show the toxicological effects seen with metiamide at high doses. Towards the end of 1976, cimetidine became

the first histamine H_2-receptor antagonist to be marketed and was called ™Tagamet. Since its introduction, it has proved effective in healing most duodenal ulcers and in reducing the frequency of reulceration, and has become firmly established in the treatment of Zollinger-Ellison Syndrome.[48]

1.4.3. Structure-activity in histamine H_2-receptor antagonists.



(12)

Burimamide (9)	R = H	X = CH_2	Z = S
Metiamide (10)	R = CH_3	X = S	Z = S
Cimetidine (11)	R = CH_3	X = S	Z = NCN

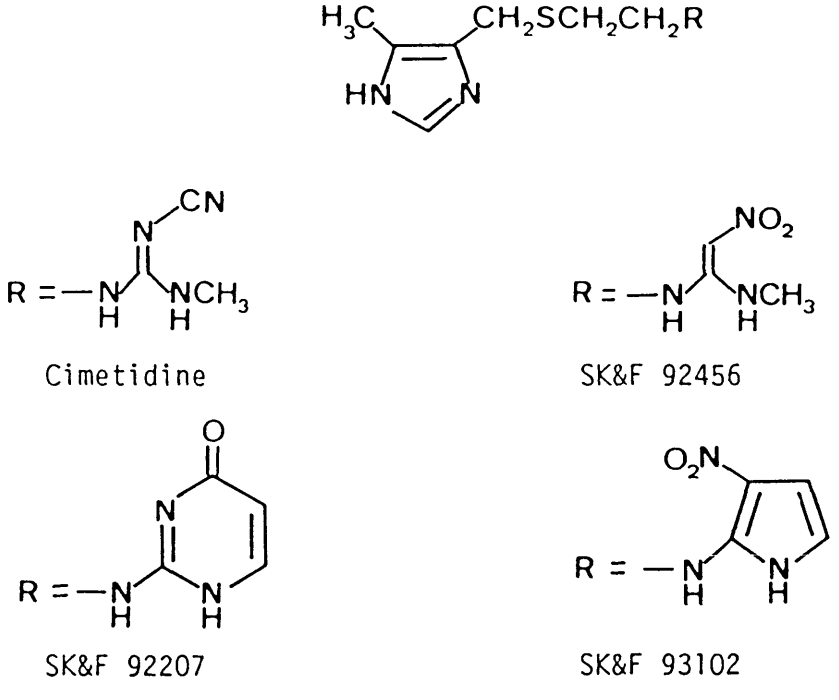
Burimamide, metiamide and cimetidine can be represented by the general structure (12). Since their discovery there has been a lot of effort expended in exploring the structural variations that can be made consistent with maintaining or increasing potency at the histamine H_2-receptor. The compounds represented in structure (12) can be described as an aromatic heterocyclic ring linked by a chain to a dipolar end group. It is convenient to divide the molecule up in this way when reviewing developments. Much of this work has been reviewed elsewhere and it is therefore intended to concentrate only on those developments relevant to the work presented later in this thesis.

The work described in this thesis is only involved with compounds containing the methylenethioethyl and tetramethylene linking chain. Numerous attempts have been made to modify the flexible linking chain and these have been recently reviewed[53] and will not be discussed further.

1.4.4. Modification of the end group

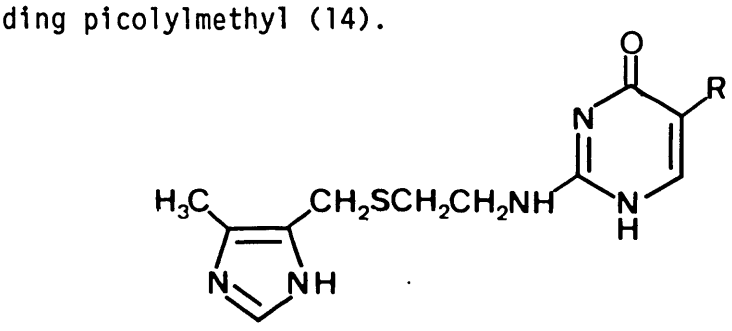
As already discussed the cyanoguanidine was introduced as a bioisostere of the thiourea end group. Some of the other end groups found to be effective are shown in Figure 1.3 and their properties have been reviewed elsewhere.[45] Properties that have been thought to be important in an end group include log P[49,50] and the orientation of the dipole of the end group relative to the side chain.[51]

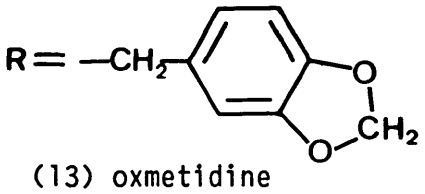
Figure 1.3 Some effective end groups used in histamine H_2-receptor antagonists



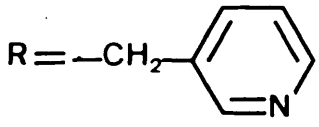
The 2-amino-4-pyrimidone end group, which has the trivial name isocytosine, may be considered as a cyclic acylguanidine. Although this analogue is somewhat less active than the non cyclic analogues this structure did allow further elaboration. Substitution at the

isocytosine 5-position with a 3,4-methylenedioxybenzyl group gave oxmetidine (13)[52] which is a potent selective histamine H_2-receptor antagonist; other 5-substituents were exemplified including picolylmethyl (14).



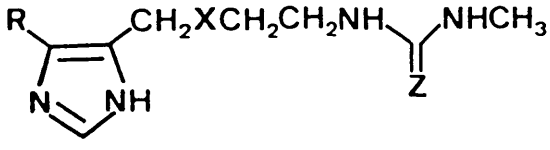


(13) oxmetidine



(14)

1.4.5. Substitution of the imidazole ring

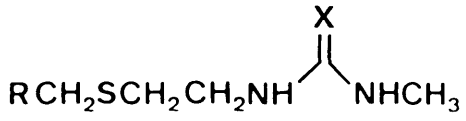


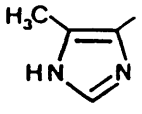
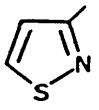
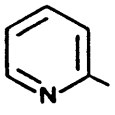
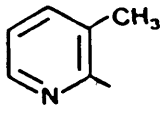
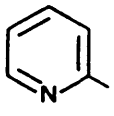
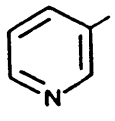
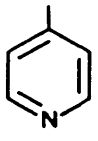
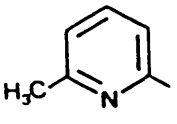
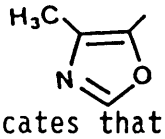
(12)

It has already been shown that the properties of the side chain and the R-substituent in structure (12) modify the ionic and tautomeric properties of the imidazole ring and these effects may be correlated with activity. Modification of the R-substituent in structure (12) has been extensively explored. The introduction of larger alkyl groups reduced histamine H_2-receptor antagonist activity.[54,55,56,57]

Retrospective structure activity analysis in the series of analogues of structure (12) was carried out. If the potency of the compound was corrected for the electronic effect of the R-substituent, then the residual variation in potency could be correlated with a steric

Table 1.1 Comparison of potencies of some histamine H_2-receptor antagonists containing basic heterocyclic rings



R	X	G. pig Atrium pA_2	Rat G.S. ED_{50} µmole/kg
	NCN	6.1	1.4
	NCN	6.15	1.6
	NCN	6.03	9.2
	NCN	6.0	11.0
	S	5.7	13.4
	S	3.5	+(128)*
	S	5.0	+(32)*
	S	<4.07	-
	S	6.26	-

* Indicates that some inhibition was seen at dose given in parenthesis but maximum achieved was less than 50%

parameter.[45,58] It was argued that a favourable balance between the electronic and steric properties of the substituent was required for good potency at the histamine H_2-receptor.

No beneficial effect has been found on substituting the imidazole 2-position in metiamide. Both neutral[59,60] and basic[61] substituents gave substantially reduced activity.

1.4.6. Replacement of the imidazole ring

Replacement of the imidazole ring by other aromatic rings has in many cases yielded potent histamine H_2-receptor antagonists. These can be divided into two classes. The first class contains analogues which have a heterocyclic aromatic ring containing a basic nitrogen. In the second class the aromatic ring is non-basic but includes a basic substituent.

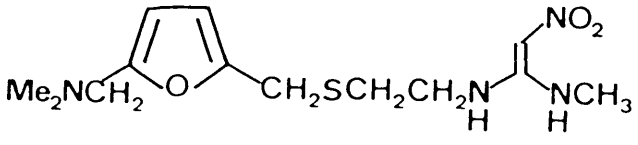
1.4.6.1. With basic heterocyclic systems

A number of potent histamine H_2-receptor antagonists have been reported where the imidazole has been replaced by other basic heterocyclic systems. Some of these are shown in Table 1.1. It had been supposed that a heterocyclic 'pyridine type', that is, with a basic nitrogen, was required 'ortho' to the side chain. This could be concluded from examination of the pyridine analogues; the 2-pyridyl analogue is the most potent.[62] However, one exception is the oxazole. In this example the oxygen atom occupies the position 'ortho' to the side chain.[63] In the 2-pyridyl analogues it can be seen that substitution of a methyl group into the 3-position of the pyridine ring is possible without loss of activity. This may be considered to be comparable to substitution at the 5-position of the imidazole ring (Section 1.4.3.) where substitution was also investigated. Substitution of a methyl into the 6-position of the pyridine ring is detrimental to activity at the histamine H_2-receptor and this may be considered equivalent

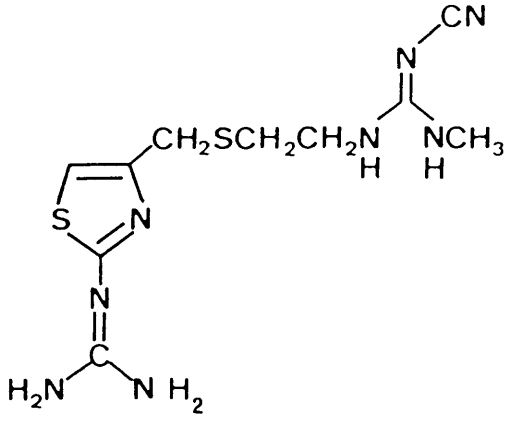
to substitution at the 2-position of the imidazole ring in cimetidine which was also detrimental to activity.

1.4.6.2. With non-basic aromatic rings

In 1979 the structures of two new histamine H_2-receptor antagonists were published, ranitidine (15)[64] and tiotidine (16).[65] Based on these structures many further developments have been made. The developments following the discovery of ranitidine will now be reviewed, while the less relevant developments following the discovery of tiotidine are reviewed elsewhere.[53]

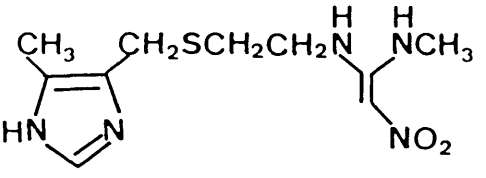


(15)



(16)

1.4.6.3. Analogues of ranitidine



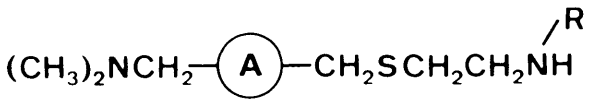
(17)

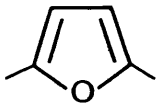
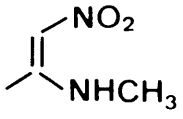
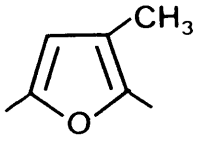
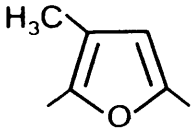
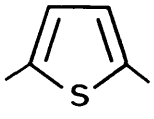
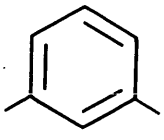
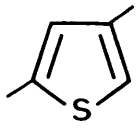
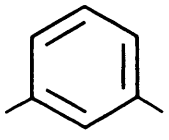
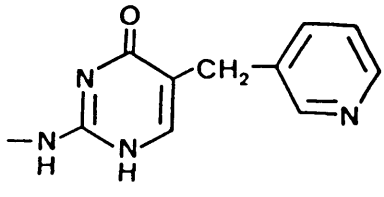
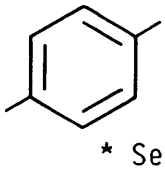
Ranitidine (15) possesses the nitrodiaminoethylene side chain of the cimetidine analogue (17)[50] while the basic imidazole ring is replaced by furan to which is attached a basic dimethylaminomethyl group. Structure activity relationships in this series have been reviewed.[66] Alternative isomeric substitution patterns of the furan ring and the introduction of further substitution into the furan ring were examined. These modifications led to a reduction in potency. Analogues where the furan was replaced by either benzene or thiophene were also reported to be less potent.

Some of these results are shown in Table 1.2. The 2,4-thiophene was reported in a later publication.[67] It may be that in the thiophene series that the 2,4-substitution pattern is optimum for activity. Two benzene analogues prepared at SK&F are also shown. Similar structure activity analysis has been published for the diaminothiadiazole-1-oxide analogues of structure (18).[68,69] Some of the alternative ring systems for "A" are shown in Figure 1.4.

The X-ray crystal structure of the furan example (18a), showed the molecule to be in a folded conformation with the two rings in parallel planes stacked above each other.[69] Attempts were made to correlate the calculated 'stacking energy' for each of the analogues with the histamine H_2-receptor antagonist activity. However, it was concluded that this folded conformation may not be responsible for histamine H_2-receptor antagonist activity. There are three references in the patent literature[70,71,72] which refer to the inclusion of a basic aromatic ring. The examples quoted are pyridyl

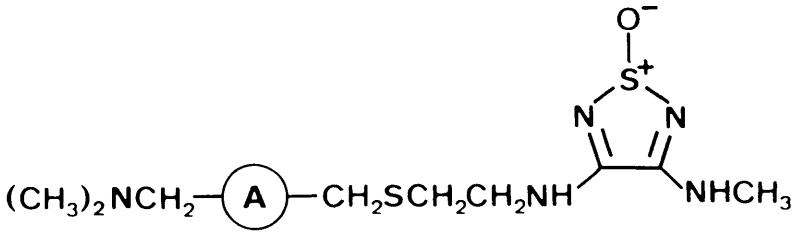
Table 1.2 Comparison of activity of Mannich substituted histamine H_2-receptor antagonists containing various aromatic rings

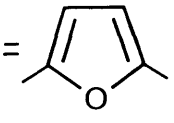


A	R	G. pig Atrium pA_2	Rat G.S. ED_{50} µmole/kg
		-	0.57*
	〃	-	>30*
	〃	-	0.76*
	〃	-	1.52*
	〃	-	2.89*
	〃	-	0.2
		7.29 (1.07)	0.028
	〃	6.56 (0.60)	0.95

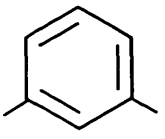
* See reference 66

Figure 1.4 Alternative aromatic ring systems exemplified in the thiadiazole containing histamine H_2-receptor antagonists

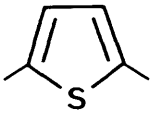


A = 

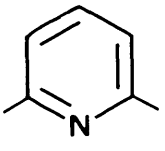
(18a)

A = 

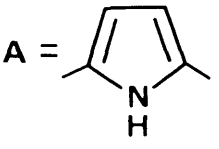
(18b)

A = 

(18c)

A = 

(18d)

A = 

(18e)

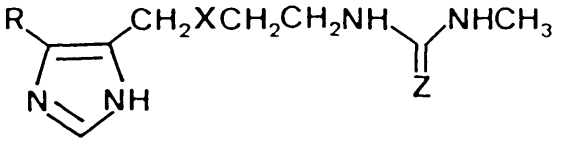
analogues. In the first two examples the substitution pattern is not quoted and in the third example only the 2,6-substituted analogue is exemplified. The thiadiazole analogues (18d) where "A" = 2,6-pyridyl was also quoted to be poorly active.[73] In the crystal this analogue is in a conformation where the two rings are in the same plane and the acidic hydrogens of the diaminothiadiazole form hydrogen bonds with the Mannich group and pyridine nitrogens.

From the above review of the known structure activity data, it may be concluded that either 5 or 6 membered rings may be accommodated and that 1,3-substitution is favoured for optimum activity at the

histamine H_2-receptor. However a 2,6-substituted pyridine system may not be favoured.

Further developments in this area include modification of the aminoalkyl function and the use of various new end groups and linking chains. These have been reviewed by Brown and Young[53] and will not be discussed further.

1.5. AIMS AND OBJECTIVES

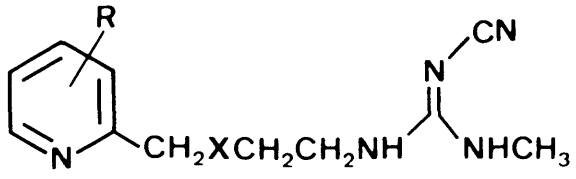


(12)

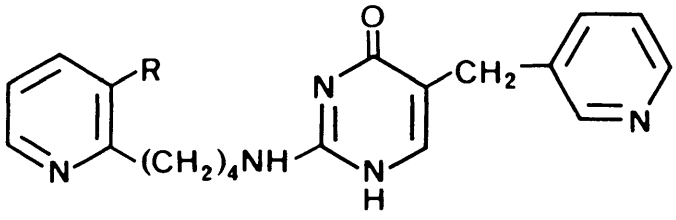
In Section 1.4.3. compounds of structure (12) were discussed. It was shown that the histamine H_2-receptor antagonist potency of the compound was affected by the R-substituent. It had been proposed that the substituent not only altered the ionic and tautomeric properties of the ring, but that steric factors were also important.

The 2-pyridyl analogues of structure (19) were discussed in Section 1.4.6.1; these are non tautomeric examples. It was thought that it may be possible to separate the electronic and steric effects of the R-substituent by preparing analogues substituted at the 3- and 5-positions in a pyridine ring. In pyridine the 3- and 5-positions are electronically equivalent and therefore a substituent in either position should impart the same electronic effect on the ring. If the steric effect operated on the side chain this would occur only in the case of the 3-substituted analogues.

Early in the work the isocytosine analogues of structure (20) were prepared and shown to be potent histamine H_2-receptor antagonists. Interestingly these analogues were also shown to be



(19)



(20)

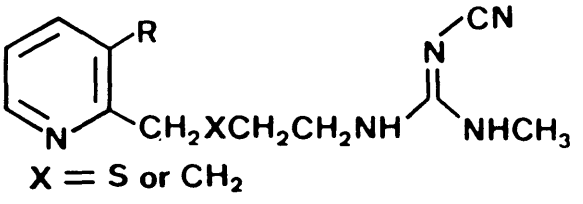
potent histamine H_1-receptor antagonists. Unlike other known histamine H_1-receptor antagonists these compounds would be uncharged at physiological pH.

The work then changed direction to determine if the pyridyl substituent modified the potency of the compound at both the histamine H_1- and H_2-receptors. This has formed the major part of this present work. The objective was then to obtain a compound which was equipotent at both receptors. A second objective was then to prepare compounds of similar structure which were only potent at one of the two receptors. The medicinal chemistry is discussed in Chapter 2 and 3 and the synthesis in Chapter 4.

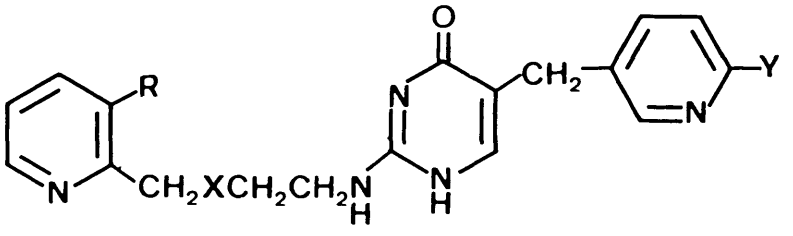
With the publication of the structure of ranitidine the question arose as to whether a dialkylaminoalkyl substituent could be introduced into the pyridine ring in order to increase potency at the histamine H_2-receptor, and, what would be the optimum substitution pattern in the pyridine series? The results of an investigation of various substitution patterns is discussed in Chapter 5 and the synthesis of these compounds is described in Chapter 6.

2.0 Structure-activity correlations in the 3-substituted pyridylbutylcyanoguanidines and isocytosines

2.1. Introduction



(21)



X = S or CH_2 Y = H or CH_3

(22)

The objective of the work presented in this chapter was to examine the effect on antagonist activity at the histamine H_2-receptor of modifying the R-substituent in compounds of structures (21) and (22), and to relate the observed effects to the physical properties of the substituent. The substituent constants used to describe the physical properties of the substituent are reviewed in the first part of this chapter. The effect of introducing a R-substituent into the 3-position of the pyridine ring on antagonist activity at histamine H_2-receptor in the pyridylbutyl and pyridylmethylthioethyl cyanoguanidines of structure (21) will then be discussed, followed by structure-activity analysis in the isocytosines of structure (22). The isocytosine analogues are combined histamine H_1- and H_2-receptor antagonists. The potency of the compounds at both the histamine H_1- and H_2-receptors are shown to correlate separately with the physical properties of the substituents introduced into the 3-position of the pyridine ring.

2.2. THE SUBSTITUENT CONSTANTS

Extensive use is made of 'Hansch analysis' to correlate the biological activity of the compounds with the changes in physical properties of the molecule as a result of modification of substituents. Electronic, lipophilic (hydrophobic) and steric properties of the substituent being introduced will be considered. These parameters have been extensively reviewed[74,75,76] and are discussed briefly below.

2.2.1. The electronic parameters

Introduction of substituents into a pyridine modifies the electronic properties of the ring and this is reflected in the pKa of the pyridine. A number of pKa's were measured, and those not measured were estimated from comparison of literature values[77] of model compounds. Hammett σ constants were also used as a measure of the electronic properties of the substituent.

Equation 1. $$\sigma = \log K_X - \log K_H$$

Equation 1 was proposed by Hammett.[1] K_H is the ionisation constant for benzoic acid in water at 25°C and K_X is the ionisation constant for a 'meta' or 'para' derivative under the same experimental conditions. Separate σ constants are obtained for 'meta' and 'para' substituents. A positive σ constant indicates that the substituent is electron withdrawing while a negative figure indicates the substituent is electron releasing. The use of σ constants has been reviewed by Shorter.[74]

2.2.2. The partitioning parameters

The lipophilic or hydrophobic parameters used are π values which have been well documented by Hansch et al.[75] The importance of the partitioning properties of a molecule was first considered by Meyer[78] and Overtone[79] who showed that the narcotic activity

of many simple organic compounds paralleled their oil/water partition coefficients. An extensive study of the partition coefficients of organic molecules indicated its additive-constitutive nature.[80] The hydrophobic parameter π is derived by application of the Hammett Equation in the form shown in Equation 2.

Equation 2. $$\pi_X = \log P_X - \log P_H$$

In Equation 2, P_X is the partition coefficient of the derivative and P_H is that of the parent compound. This gives an estimate of the effect of a substituent on the partitioning properties of a molecule between aqueous and non-aqueous phases. A positive value for π means that relative to hydrogen the substituent favours the organic phase and the substituent can be considered to be more lipophilic. The values for π can vary depending on the organic phase used. One of the most commonly used systems is octanol/water and it is the data obtained from this system which is used in this work. Most of the data used was obtained from the tables compiled by Hansch and Leo[75] although some log P values were measured by the physical organic department at SK&F. The lipophilic parameter π was also used to estimate the log P values for those analogues not measured.

2.2.3. The steric parameters

One of the simplest measures of steric size is the van der Waals radii of the substituent or its molar volume. Possibly the most widely used quantitative measures of steric size are the E_s values proposed by Taft.[81,82] The Taft E_s parameter is defined in Equation 3.

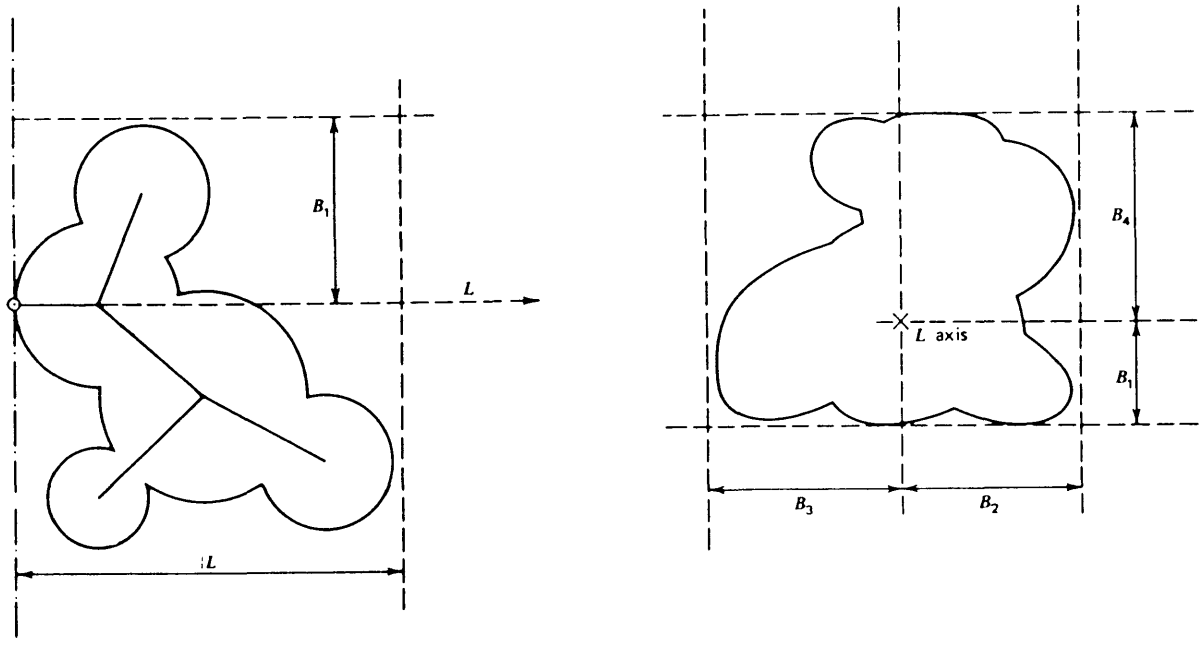
Equation 3. $$E_s = \log [K_X/K_H]$$

K is the acid hydrolysis rate constant of esters of the type $X\text{-}CH_2CO_2R$. Experimental E_s values are available for only a fairly limited range of substituents, although in some cases

they have been calculated from the van der Waals radii.[83,84] In the later reference Kutter and Hansch carried out multiple regression analysis on a series of monoamine oxidase inhibitors and histamine H_1-receptor antagonists. They found a linear regression equation linking E_S and the average van der Waals radii for the measured substituents. They then calculated E_S constants for several of the substituents which could not be determined experimentally.

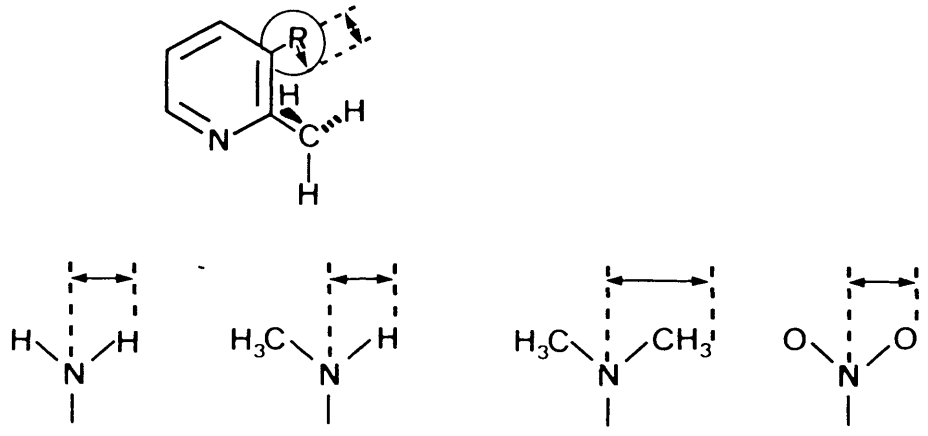
Although E_S constants have been successfully used to rationalise steric effects, they do assume that all substituents are symmetrical. For non symmetrical groups it may be necessary to consider the conformation of the substituent and hence assign different E_S values accordingly. Verloop et al[85] devised a series of five steric parameters which are defined as L, B_1, B_2, B_3, and B_4. They developed a computer programme using van der Waals radii, standard bond angles and lengths to define the space requirement of a substituent. L represents the length of the substituent along the axis of maximum width as shown in Figure 2.1. B_1 is always the smallest and B_4 the largest value.

Figure 2.1 Diagram showing derivation of the Verloop parameters.



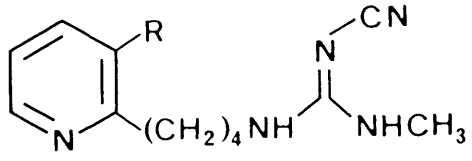
The Verloop parameters are used in order to obtain an estimate of the size of the group in the direction of the side chain. The steric parameter used is defined as the distance by which the substituent extends in the direction of the first methylene of the side chain counting from the pyridine ring as shown in Figure 2.2.

Figure 2.2 Definition of the steric parameter (S_t).



For a symmetrical group such as halogen the radius of the group is used, for an alkoxy or alkylthio group it is the radius of the oxygen or sulphur atom. For example, the methoxy substituent is in a conformation where the methyl group is in the same plane as the pyridine ring and is rotated away from the side chain. There are literature precedents for this being a low energy conformation for a methoxy group in anisole.[86] The use of these distances assumes that the group is in a conformation which minimises the major steric interaction between the side chain and the substituent. For methylamino, dimethylamino and nitro it is the distance as defined in Figure 2.2. It has been assumed that these groups are coplanar with the pyridine ring. However, there are many reported crystal structures containing aromatic rings which have amino and nitro substituents which are not coplanar. The dihedral angle between the nitro substituent and the aromatic ring for example is frequently about 30° which would suggest that the true steric size of these substituents in the direction of the methylene may be over estimated.

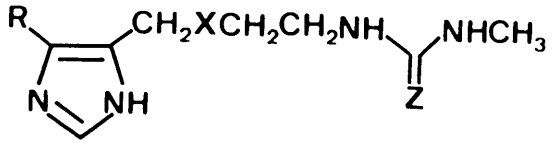
Table 2.1 Comparison of potency at the histamine H_2-receptor and some physical properties for the pyridylbutyl cyanoguanidines



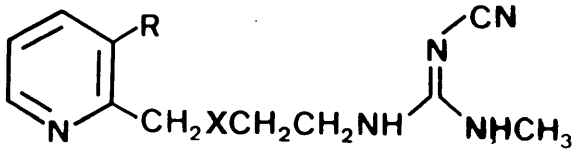
Compound number	R	G.Pig atrium pA_2	log P oct/H_2O	Pyridine ring pKa	Steric parameter (S_t)
24	H	5.64	1.4	5.8	1.00
25	F	6.17	1.4	3.6	1.35
26	OCH_3	6.77	1.5*	5.54-5.58*	1.35
27	OH	5.64	0.6	5.5	1.35
28	OBz**	<3.79	3.7	5.6	1.35
29	SMe	5.56	2.0	5.0	1.70
30	Cl	5.72	2.0	3.5	1.80
31	NH_2	5.01	0.8	6.6	1.84
32	$NHCH_3$	5.42	0.9	7.0	1.84
33	CH_3	5.39	1.9	6.2	1.90
34	Br	5.41	2.1	3.5	1.95
35	NO_2	4.74	1.1	1.9	2.44
36	CF_3	<5.39	2.2	3.0	2.44

* measured (SK&F); other pKa and log P values were calculated
** Bz = benzyl

2.3. THE CYANOGUANIDINES



(12)



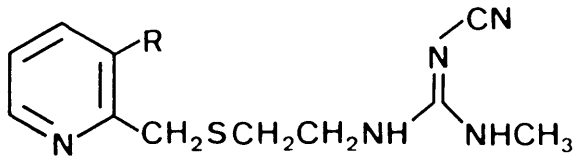
(21)

The effect of modifying the R-substituent in structure (12) which are analogues of cimetidine was discussed in Section 1.4.5. It had been proposed that the R-substituent not only modifies the ionic and tautomeric properties of the imidazole ring but may also influence the conformational properties of the methylthioethyl chain and that a balance between these effects was important for optimum antagonist potency at the histamine H_2-receptor.[45,58]

Similarly, substitution into the 3-position of the pyridine ring in the 2-pyridyl analogues of structure (21) would also modify the ionic properties of the pyridine ring and the conformational properties of the linking chain. As pyridine is a non-tautomeric system any influence of tautomerism on biological activity which complicated structure activity analysis in the imidazole series would be removed in the pyridine series.

A series of substituted pyridylbutyl analogues was prepared and the structure activity relationships in this series were compared with the methylthioethyl analogues prepared by colleagues at SK&F. These two series of compounds are shown in Tables 2.1 and 2.2.

Table 2.2 Comparison of potency at the histamine H_2-receptor and some physical properties for the pyridylmethylthioethyl cyanoguanidines



Compound number	R	G.Pig atrium pA_2	log P oct/H_2O	Pyridine ring pKa	Steric parameter (S_t)
37	H	6.00	0.79*	4.43*	1.00
38	F	6.59	0.8	2.2	1.35
39	OCH_3	6.67	0.97*	4.2*	1.35
40	Cl	6.46	1.4	2.1	1.80
41	NH_2	5.91	0.25	5.3	1.84
42	$NHCH_3$	5.82	0.3	5.6	1.84
43	CH_3	6.0	1.3*	4.8	1.9
44	Br	6.6	1.48*	2.1	1.95
45	I	5.75	1.7	2.2	2.15
46	$N(CH_3)_2$	3.38	0.9	5.0*	2.80

* measured (SK&F); other pKa and log P values were calculated

Analogues were prepared by:- 37,43 - P.D.Miles; 38 - K.Saag; 39 - G.S.Sach; 40 - S.F.Moss; 44,45 - M.E.Footit; 46 - M.A.Wilczynska.

The *in vitro* histamine H_2-receptor antagonist as measured in the guinea-pig atrium assay is compared with the lipophilicity of the molecule and the basicity of the pyridine ring.

Examination of these data indicates that there is no obvious relationship between the *in vitro* histamine H_2-receptor antagonist activity and either the lipophilicity of the molecule or the pKa of the pyridine ring.

The relationship between the steric parameter described in the previous section and the *in vitro* histamine H_2-receptor antagonist activity for the butyl and methylthioethyl analogues are shown in Figures 2.3 and 2.4 respectively.

It is possible to draw a curve through the points with the optimum activity in both series occurring with the methoxy or fluoro analogues which have a steric parameter of 1.35Å. The histamine H_2-receptor antagonist activity decreases with increases in the size of the 3-substituent. Whether there is a true turnover in the curve is debatable as this turnover in each case relies on only one point which is the unsubstituted analogues i.e. when R = H.

The 3-bromo analogue (34) in the methylthioethyl series is an outlier. This analogue was retested on the rat uterus assay and in this assay gave pA_2 in line with the value predicted by the plot.

The benzyloxy analogue (28) has not been included in this analysis as it may be that its low activity was due to the large size of the benzyl group; also in the oxygen series the hydroxy analogue (27) may be anomalous as hydroxypyridines are known to exist in aqueous solution in equilibrium with the corresponding zwitterion. For hydroxypyridine in solution the ratio of hydroxypyridine to zwitter ion is thought to be 1:1.[87] If it is assumed that potency resides in the hydroxypyridine tautomer and the pA_2 is adjusted to account for the presence of the zwitter ion, then the fluoro and hydroxy analogues may be considered to be of comparable *in vitro* potency.

Figure 2.3 Relationship between H_2-antagonism and the steric parameter in the pyridylbutyl cyanoguanidines

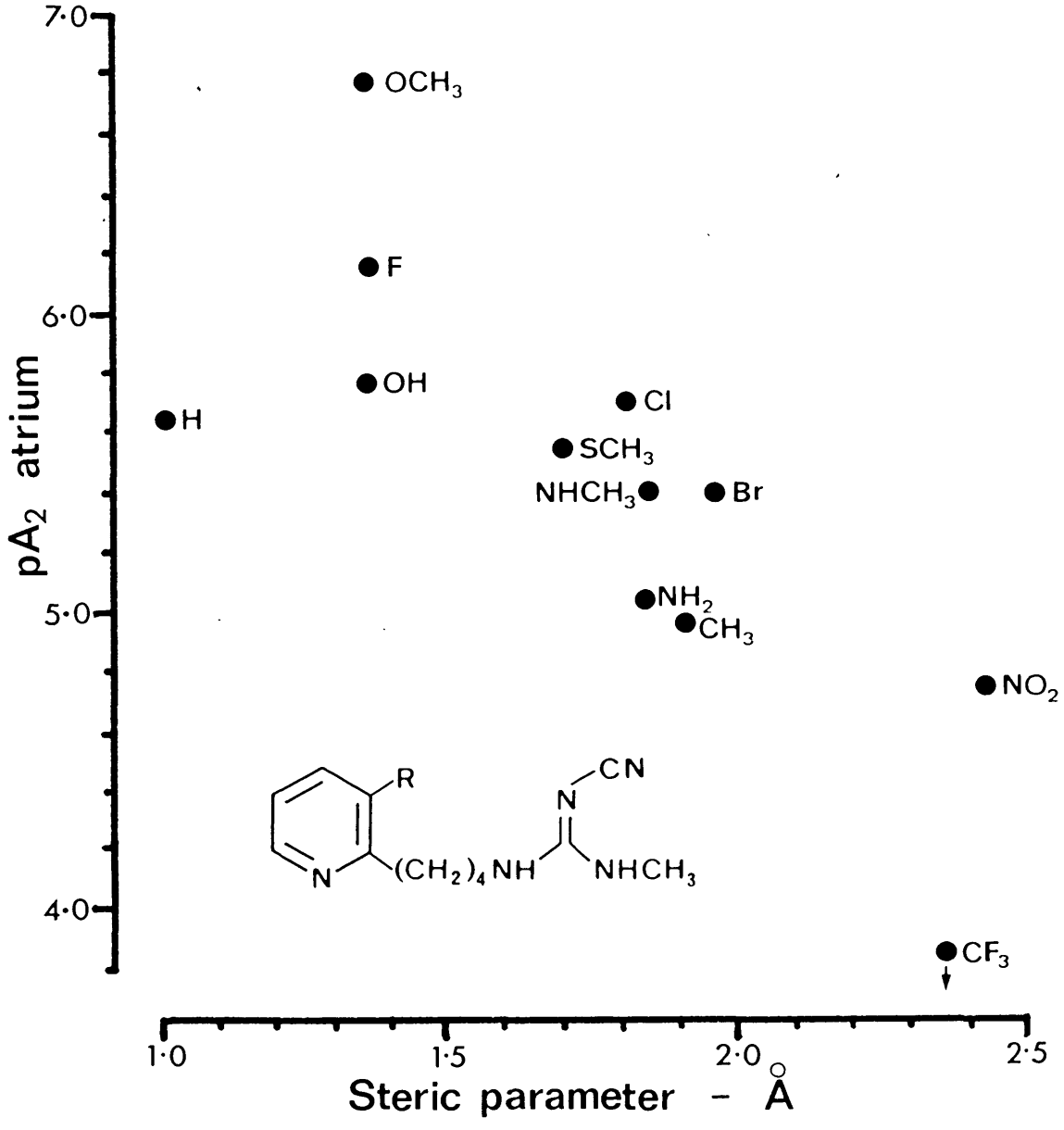
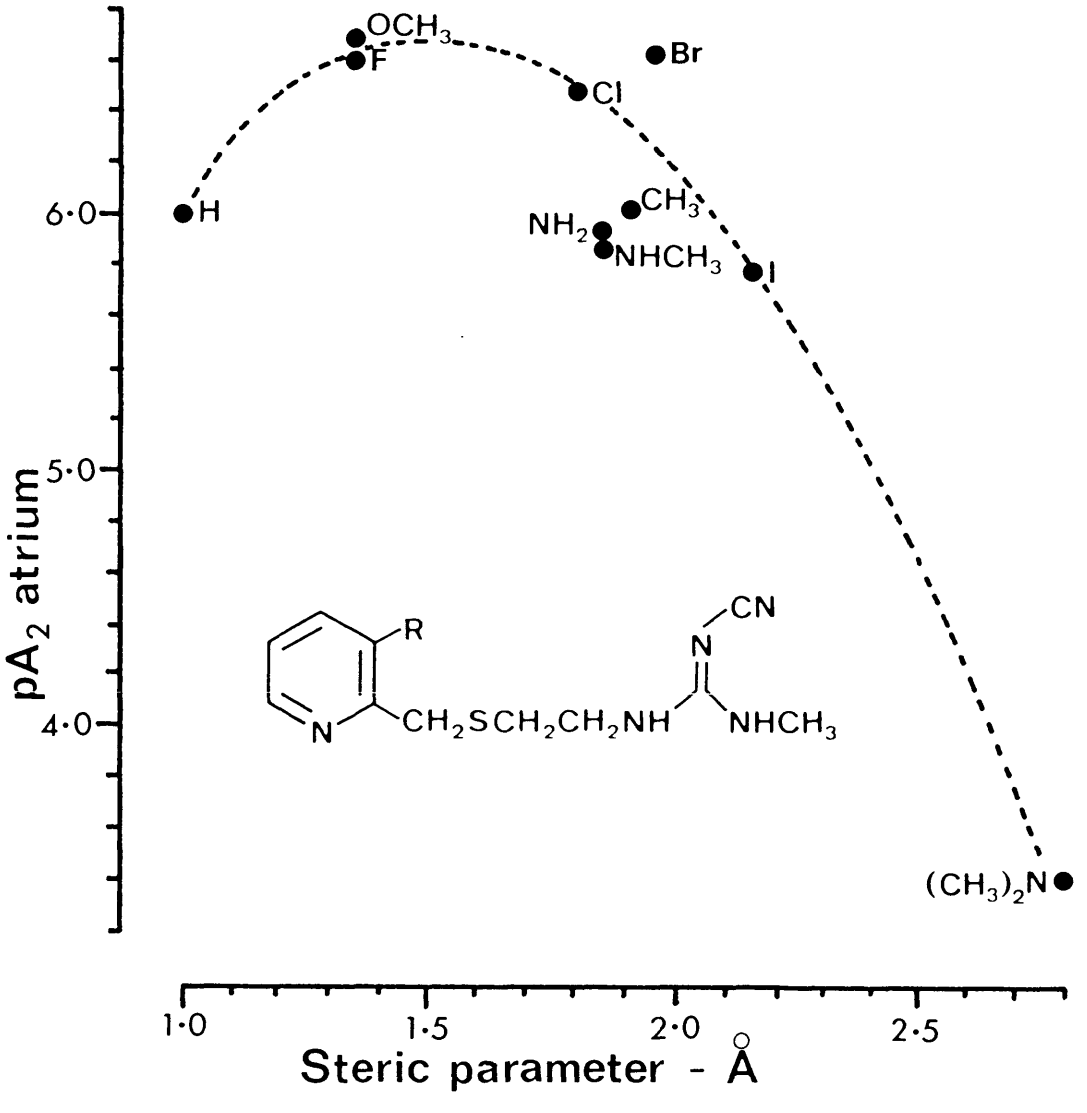
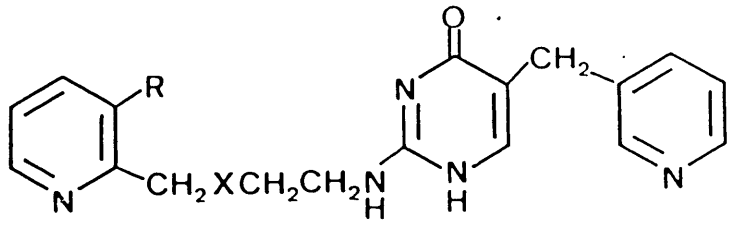


Figure 2.4 Relationship between H_2-antagonism and the steric parameter in the pyridylmethylthioethyl cyanoguanidines



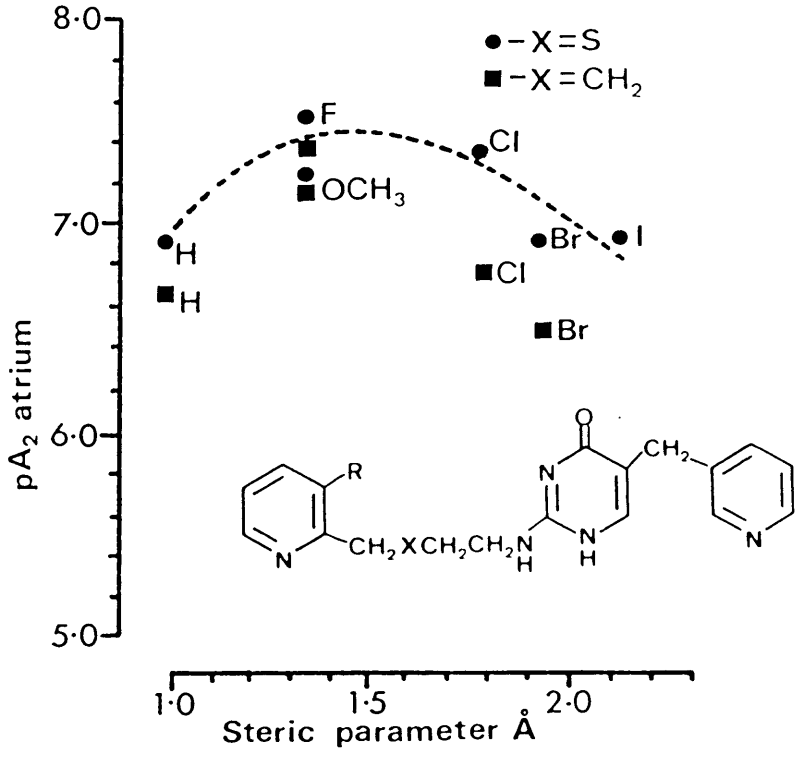
2.4. THE ISOCYTOSINES



X = CH_2 (47) X = S (48)

The isocytosine group had been identified at SK&F[52] as a useful alternative to the cyanoguanidine for histamine H_2-receptor antagonists. A small series of 5-picolyl isocytosine derivatives of structure (47) were prepared. Figure 2.5 shows a plot of the

Figure 2.5 Relationship between H_2-antagonist activity and the steric parameter in the pyridylmethylthioethyl and pyridylbutyl isocytosines



histamine H_2-receptor antagonist activity against the steric parameter in this series of compounds. The corresponding thioether chain analogues (48) prepared by colleagues[88] are also shown. Again a relationship can be seen between the steric parameter and the histamine H_2-receptor antagonist activity. This correlation is similar to that seen in the cyanoguanidine analogues discussed previously.

Interestingly these compounds also showed activity at the histamine H_1-receptor as measured on the guinea-pig ileum. Variation in the potency of the compound at the histamine H_1-receptor was also seen on modifying the 3-pyridyl substituent. Figure 2.6 shows a plot of histamine H_1-receptor antagonist activity against the same steric parameter for both the butyl and methylthioethyl series of compounds. It can be seen that for both series there is a linear correlation between histamine H_1-receptor antagonist activity and the steric parameter, the antagonist activity increasing with increasing size of the 3-pyridyl substituent. Regression analysis gives Equations 4 and 5 for the butyl and methylthioethyl analogues respectively.

Regression equation for pA_2 against the steric parameter (S_t) for butyl analogues of structure (47).

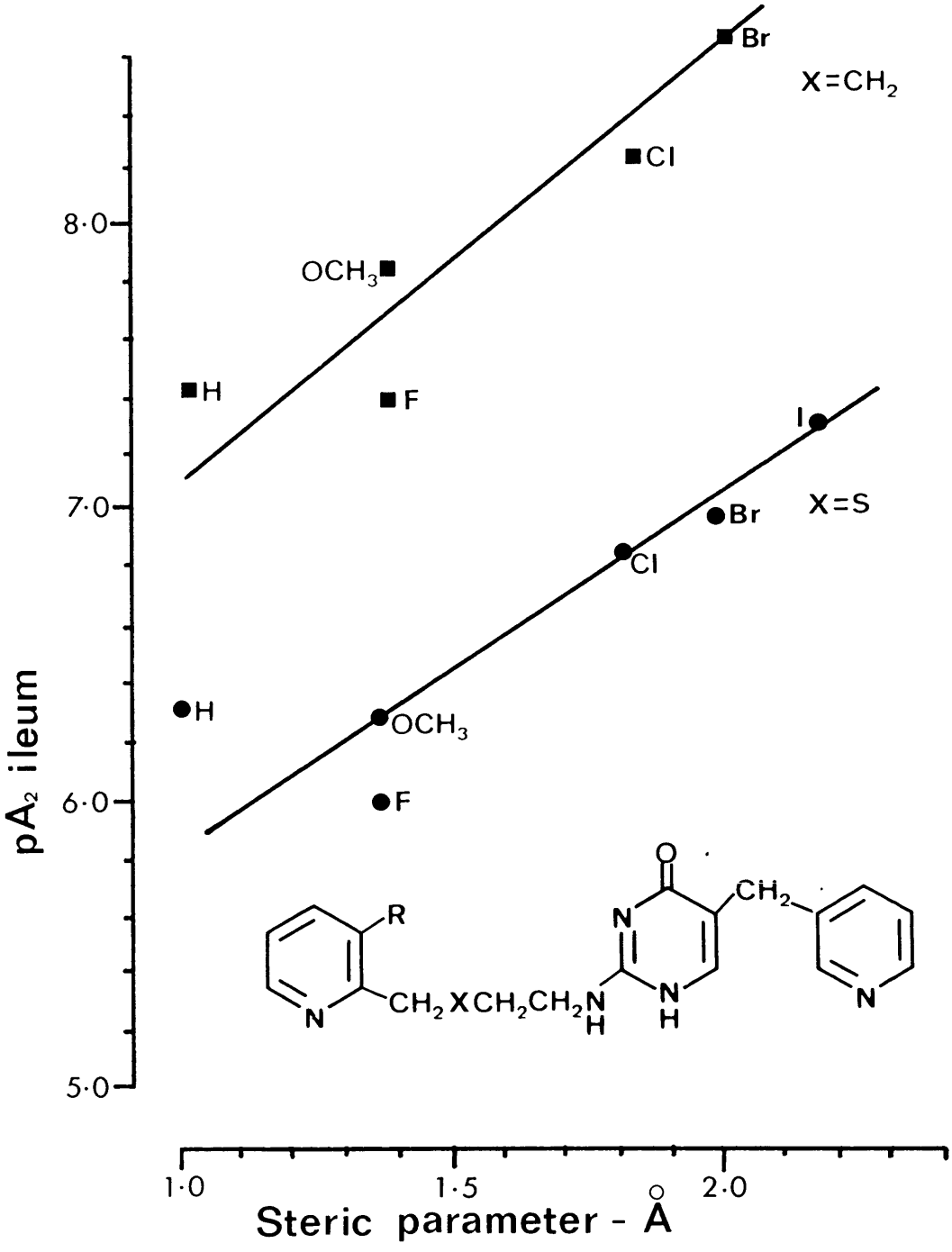
Equation 4. $pA_2 = 5.94 + 1.32S_t \quad r^2 = 0.85$

Regression equation for pA_2 against the steric parameter (S_t) for thioether analogues of structure (48).

Equation 5. $pA_2 = 4.47 + 1.30S_t \quad r^2 = 0.85$

Correlations have been previously reported between histamine H_1-receptor antagonist activity and lipophilicity.[26] For this series of compounds an alternative correlation with the lipophilicity of the substituent could be proposed. Equations 6 and 7 were obtained on regression of the *in vitro* histamine H_1-receptor antagonist activity against the lipophilicity parameter.

Figure 2.6 Effect on histamine H_1-receptor antagonist activity of substituting methylene for sulphur in the pyridyl isocytosines



Regression equation for pA_2 against π for butyl analogues of structure (47).

Equation 6. $pA_2 = 7.51 + 1.17\pi \qquad r^2 = 0.79$

Regression equation for pA_2 against π for thioether analogues of structure (48).

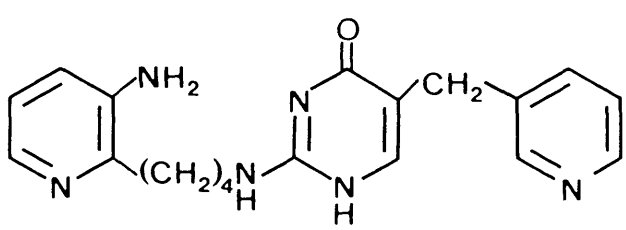
Equation 7. $pA_2 = 6.00 + 1.18\pi \qquad r^2 = 0.88$

The correlation coefficients (r^2) for the equations derived for this series with either the lipophilic or steric parameter are similar. For this series of substituents the lipophilic and the steric parameters are linearly related as can be seen from Equation 8 and therefore if a correlation exists it will be seen with both parameters.

Regression equation for the steric parameter (S_t) against π.

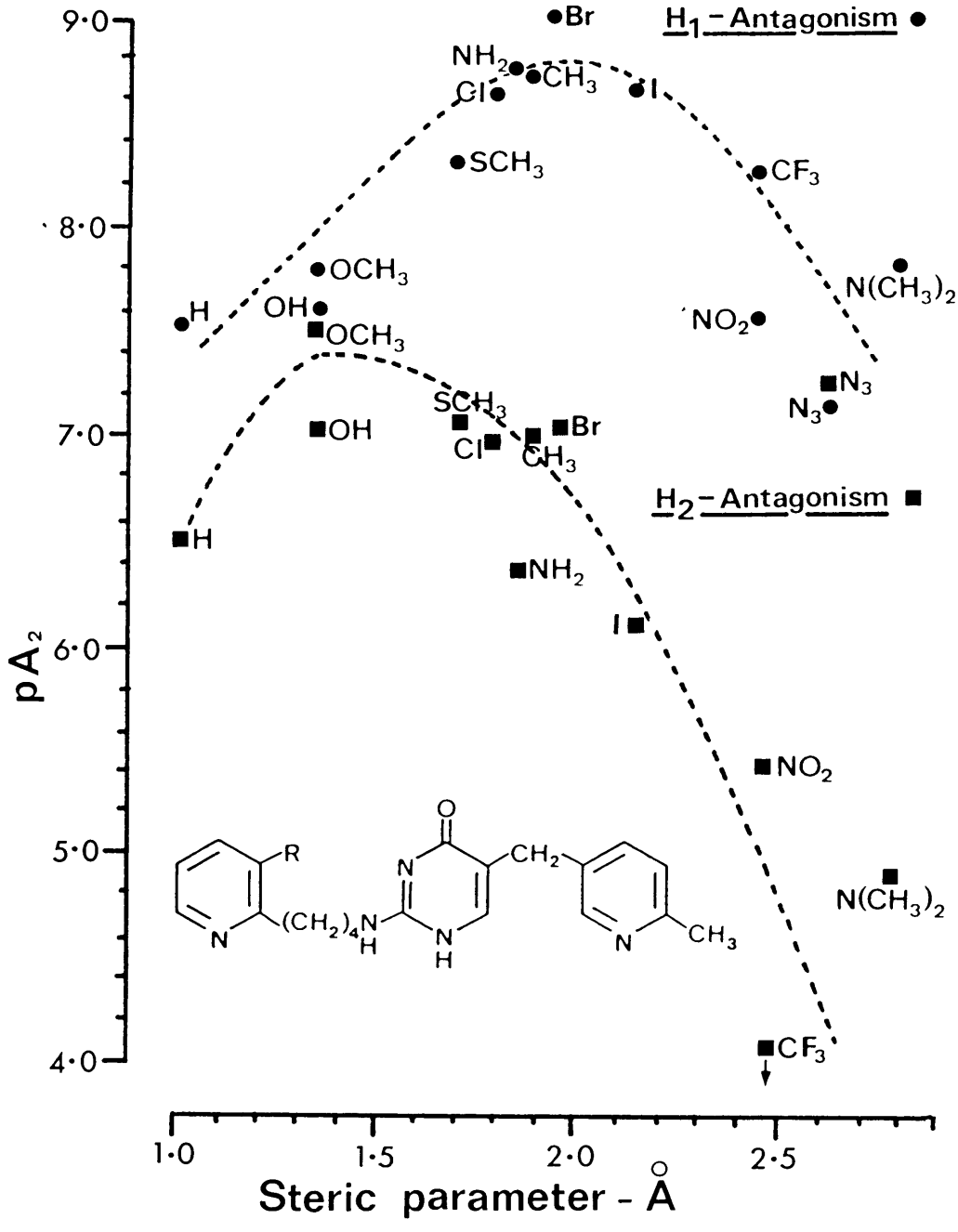
Equation 8. $S_t = 1.20 + 0.86\pi \qquad r^2 = 0.93$

Avoidance of the use of parameters in QSAR which are colinear has been discussed.[89] The subsequent preparation of the 3-amino analogue (49) [pA_2 (ileum) = 8.84] indicated that histamine H_1-receptor antagonist activity may be related to the steric parameter and not lipophilicity. Equation 4 and 6 would predict the 3-amino analogue to have pA_2's of 8.37 and 6.07 respectively.

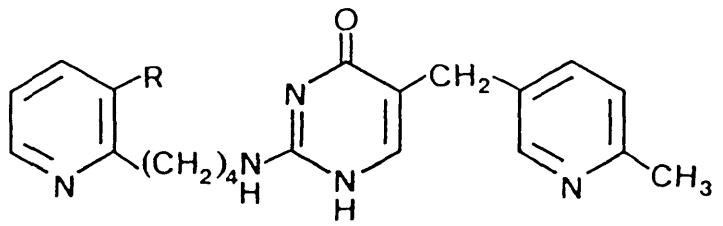


(49)

Figure 2.7 Relationship between the steric parameter and antagonism at both H_1- and H_2-receptors in the pyridylbutyl isocytosines



The use of the steric parameter predicts the high potency of the amino analogue whereas the lipophilic parameter does not. Examination of Figure 2.6 indicates that potency at the histamine H_1-receptor increases with increasing steric parameter of the 3-substituent. The butyl chain analogues are more potent by approximately 1.5 pA_2 units than the corresponding methylthioethyl chain analogues.



(50)

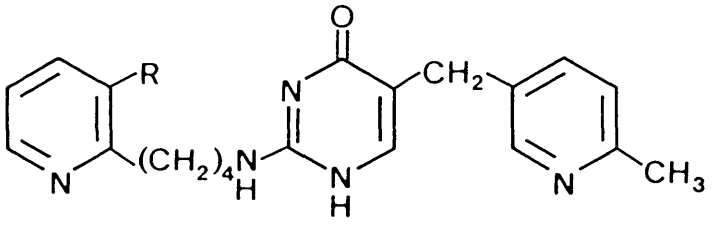
The closely related 3-substituted pyridylbutyl isocytosines with a 6-methyl substituent in the isocytosine 5-picolyl group structure (50) were prepared. Figure 2.7 shows the histamine H_1- and H_2-receptor antagonist activity for this series of compounds plotted against the steric parameter. A relationship is again apparent between the antagonist activity at both histamine receptors and the steric parameter. In this series maximum histamine H_2-receptor antagonist activity (guinea-pig atrial pA_2 7.49) is also found with the methoxy analogue. This analogue shows a similar level of potency at the histamine H_1-receptor (guinea-pig ileum pA_2 7.77).

The choice of parameter for the azido analogue is difficult. Verloop quotes B parameters of 1.5, 2.34, 2.57 and 4.18Å. The three nitrogens of an azido group lie in a straight line and at an angle of 120° to the carbon to which it is attached.[90] If the three nitrogens are in the same plane as the pyridine ring then it may have been expected that the two B parameters which project above or below the ring plane would be equivalent. If a value of about 2.5Å was chosen then potency at the histamine H_1-receptor would be correctly predicted. However, the potency of this analogue at the

histamine H_2-receptor would not be correctly predicted. This analogue is discussed further in Section 5.4.

Increasing the size of the 3-pyridyl substituent from methoxy results in increased histamine H_1-receptor antagonist activity and reduction of histamine H_2-receptor antagonist activity. Maximum histamine H_1-receptor antagonist activity occurs with methyl, amino and bromo.

Table 2.3 Comparison of substituent parameters and histamine H_1-receptor antagonist activity for some pyridylbutyl isocytosines



R	Steric parameter (S_t)	π	σ^m	σ^p	G. pig ileum pA_2	G. pig atrium pA_2
Me	1.90	0.56	-0.07	-0.17	8.68	6.97
Br	1.95	0.86	0.39	0.23	8.92	7.10
NH_2	1.84	-1.23	-0.16	-0.66	8.83	6.34

Some of the substituent parameters for these three analogues are compared in Table 2.3. It is only the steric parameter that has a similar value for the three compounds. This indicates that the major influence of the 3-pyridyl substituents on antagonist potency at either histamine receptor can be related to the size of the substituent.

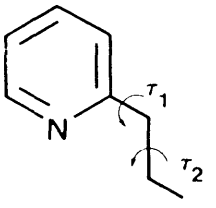
2.5. INFLUENCE OF THE 3-PYRIDYL SUBSTITUENT

In the previous section it has been shown that the 3-pyridyl substituent separately influences activity at both histamine receptors. These effects can be related to the size of the substituent in the 3-position of the pyridine ring. The optimum substitution for histamine H_2-receptor antagonist activity is apparently methoxy or fluoro and for histamine H_1-receptor antagonist activity is that of methyl, bromo or amino. It was postulated that the biological properties of the molecule may be dependent on the conformational properties of the linking chain. It may be that different conformations of the molecule are responsible for potency at the two receptors and it was thought that the 3-pyridyl substituent may modify the potency at both receptors by influencing the conformational properties of the linking chain. Attempts were made to calculate the effect of these substituents on the conformational properties of the linking chain. Also the crystal structures of several of the analogues were determined.

2.5.1. Calculations

The 4-atom linking chain present in these structures contains five rotatable bonds. If an attempt was made to map out the whole conformational space for these molecules it would be necessary to modify systematically one torsion angle at a time while calculating the energy. If each torsion angle was sampled at 30° increments each structure would require 12^5 (248,832) separate calculations which would require an inordinate amount of computing time.

It was assumed that the 3-substituent would exert the greatest effect on the atoms of the chain nearest the pyridine ring. The effect of the 3-substituent on the first two rotatable bonds of the linking chain was investigated. Rotation around these bonds can be defined by the torsion angles τ_1 and τ_2 as defined in structure (52).



(52)

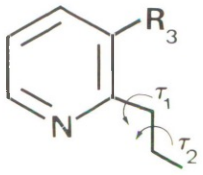
The carbon atom skeleton of the linking chain is in the same plane as the pyridine ring and the bond between the first and second chain atoms is directed away from the 3-substituent. This conformation is defined as $\tau_1 = \tau_2 = 180°$ or the trans configuration.

The calculations were carried out on analogues of structure (52) with various 3-substituents to give the 'Ramachandran' plots shown in Figure 2.8. The plots were calculated using the molecular mechanics programme in 'Cosmic'[91] as described in Section 7.1.1. Similar calculations were carried out by Dr G. Richards at Oxford using 'Extended Huckel Theory' and comparable results were obtained.

The 'Ramachandran' shown in Figure 2.8.a indicates that for the unsubstituted analogue, τ_2 has three minimum energy conformations at $\tau = 60°$, 180° or 300°. These minima are the trans and gauche conformations expected for two methylenes of an alkyl chain. The barrier to rotation around τ_1 is dependant on the conformation of τ_2. When $\tau_2 = 180°$ i.e. the trans form, relatively free rotation is possible around τ_1 with only a low energy barrier passing through $\tau_1 = 0$. Whereas, when $\tau_2 = 60°$ or 300° i.e. the gauche form there is a slightly greater barrier to rotation through the conformation $\tau_1 = 0°$.

The effect of introducing a 3-bromo substituent into the pyridine ring is shown in 'Ramachandran' (Figure 2.8.b). Rotation around τ_2 shows a set of minimum energy conformations similar to that found for the unsubstituted analogue, whereas rotation around τ_1

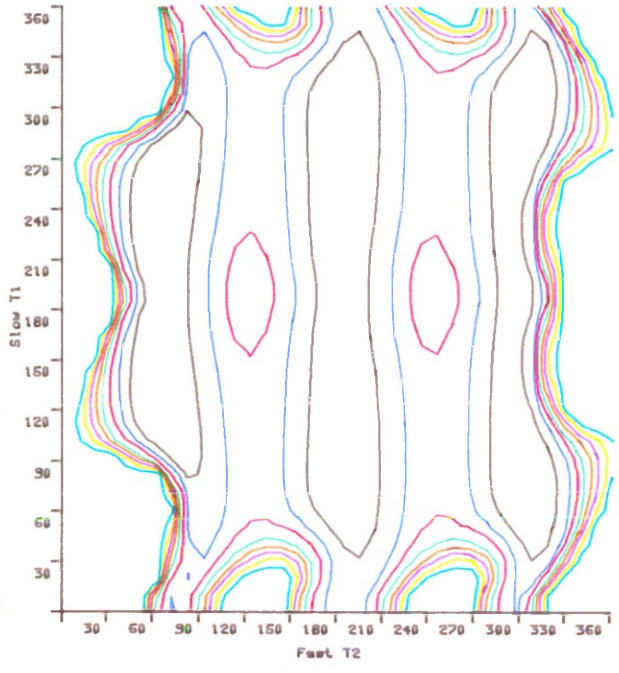
Figure 2.8 Ramachandran plots for 3-substituted 2-propylpyridines



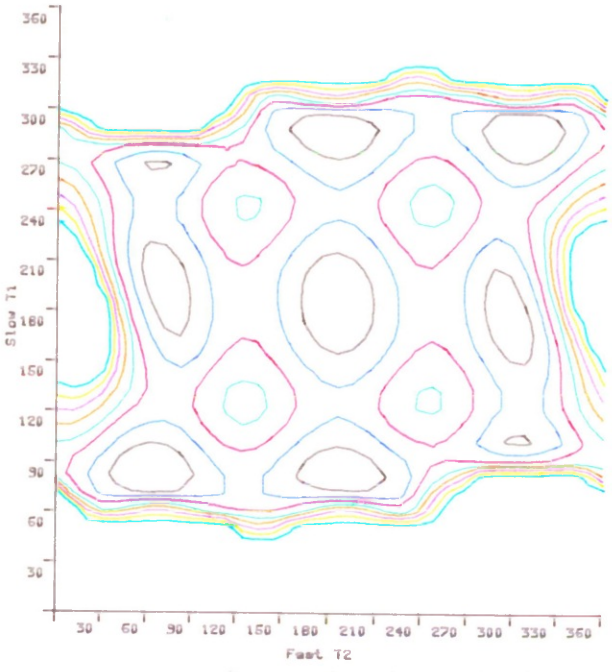
Plots are contoured at 1 kcal intervals using the following colours:

1 kcal — 2 kcal — 3 kcal — 4 kcal —

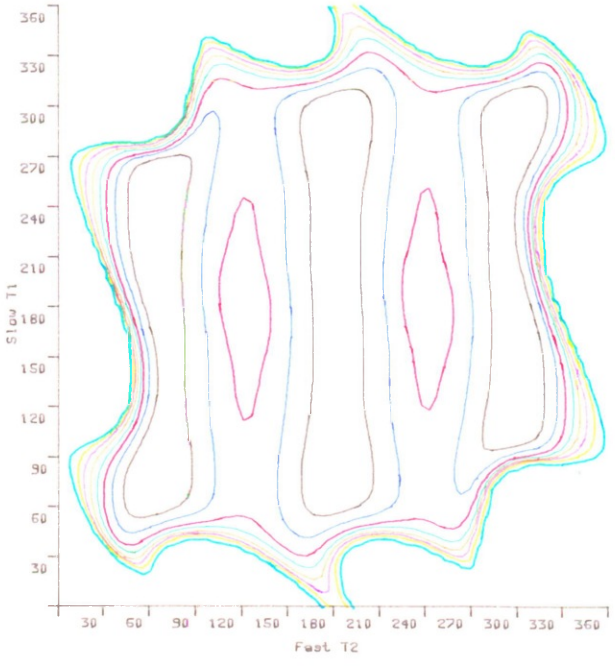
5 kcal — 6 kcal — 7 kcal — 8 kcal —



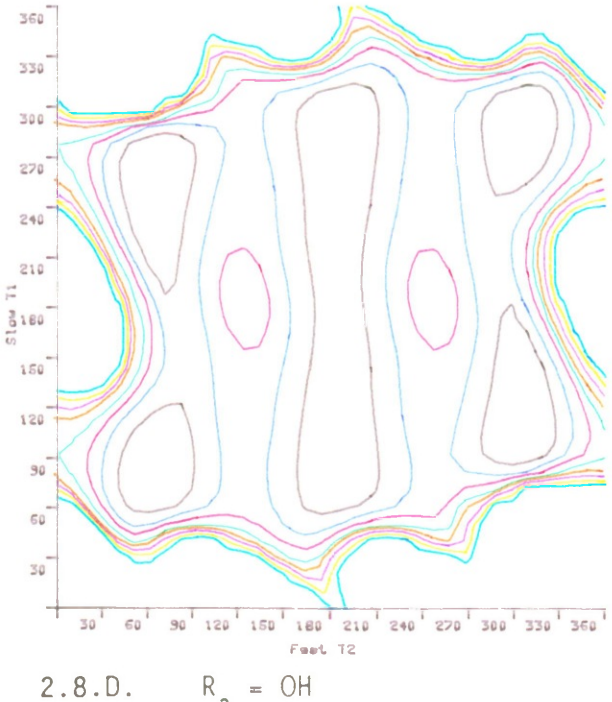
2.8.A. R_3 = H



2.8.B. R_3 = Br

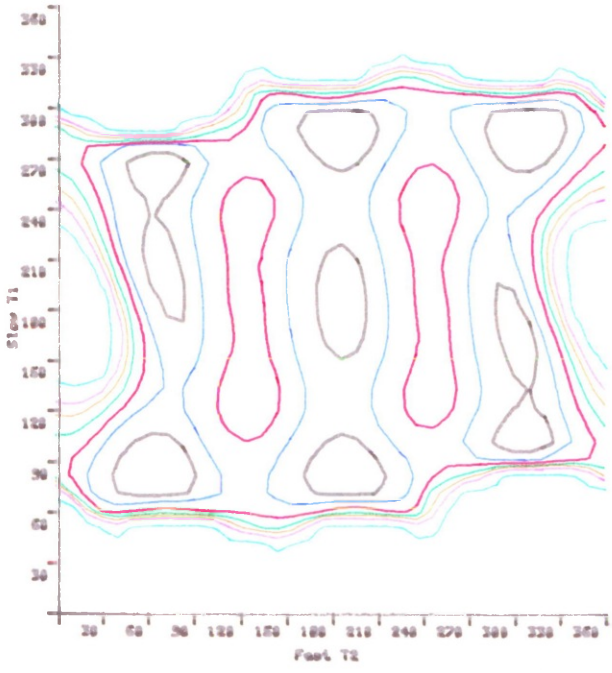


2.8.C. R_3 = F

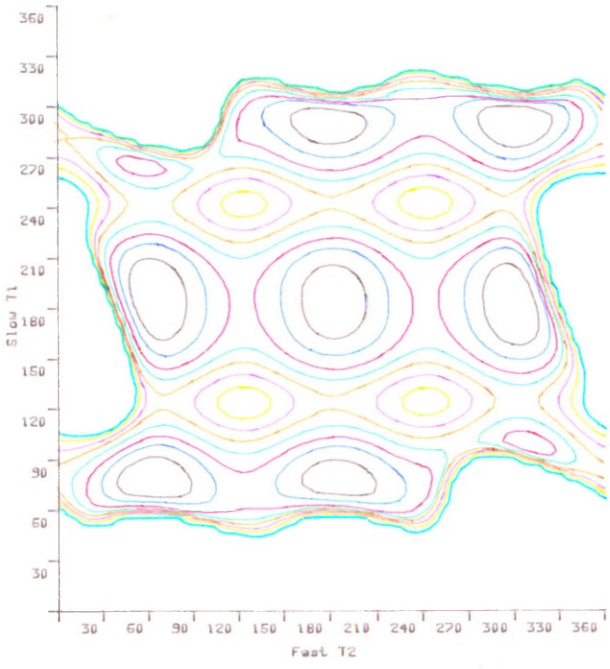


2.8.D. R_3 = OH

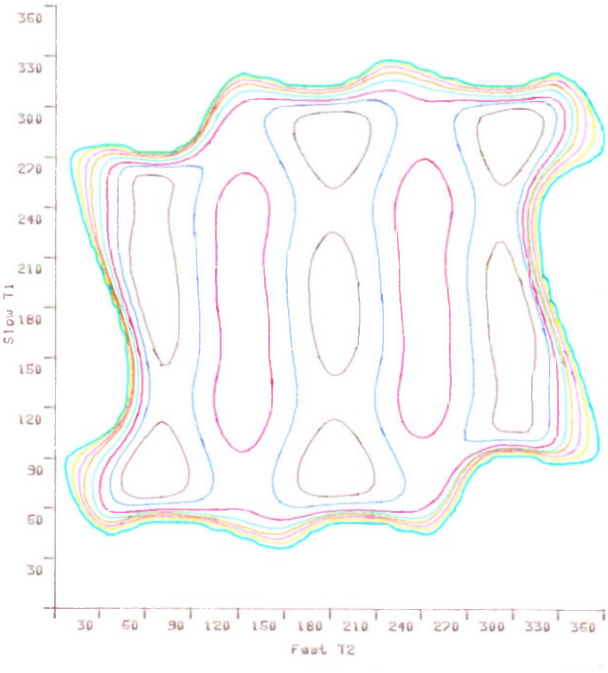
Figure 2.8 (contd)



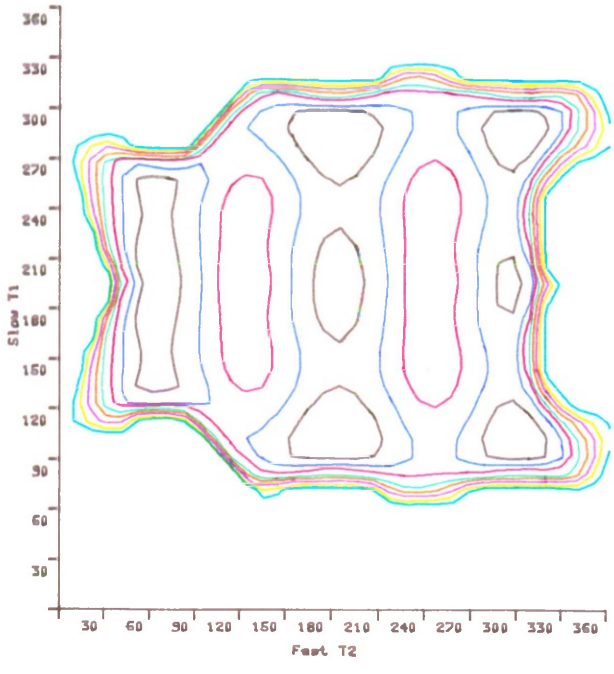
2.8.E. $R_3 = NH_2$



2.8.F. $R_3 = CH_3$



2.8.G. $R_3 = Cl$



2.8.H. $R_3 = I$

is now more restricted. A similar plot was also found for the 3-methyl analogue (Figure 2.8.c). All cases have minima at $\tau_1 = \tau_2 = 180°$.

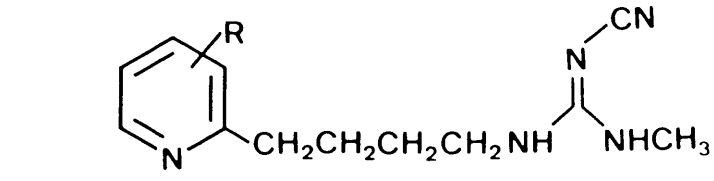
The 3-substituent influences the height of the barrier to rotation around τ_1, the position of the energy minima and the size of the energy well, i.e. the area shown on the 'Ramachandran' which is less than 1 kcal above the absolute minimum.

If the population of molecules in a given minimum energy conformation is taken as being equal to the ratio of the area for that conformational minimum to the total area of all other minimum energy conformations present, then the 'Ramachandrans' indicate that increasing the size of the 3-pyridyl substituent increases the population of molecules in the $\tau_1 = \tau_2 = 180°$ conformation by reducing the number of other low energy conformations available to the molecule. It may be that this is the conformation important for activity at the histamine H_1-receptor as this conformation appears to be more favoured for the methyl and bromo analogues.

If rotation around τ_1 is examined, it can be seen that when R = H there is very little restriction to rotation around τ_1. Introduction of the hydroxy extends this barrier from 25° to about 50°. Increasing the size of the substituent further, to bromo or methyl, pushes the barrier out to about 70°. If the molecule is described by two planes (one being the plane formed by the three carbon atoms of the methylene chain and the other is that of the pyridine ring), then rotation around τ_1 rotates these planes with respect to each other. If torsion angle τ_2 is in the trans conformation ($\tau_2 = 180°$) then these two planes are parallel when $\tau_1 = 0°$ or 180°.

For the unsubstituted analogue free rotation is possible between the two planes with only a small barrier to rotation through $\tau_1 = 0°$. Substitution of fluoro or methoxy into the 3-position of the pyridine ring reduces the population of the $\tau_1 = 0°$ conformation in favour of either the conformation where

Figure 2.9 Comparison of the X-ray crystal structures for several pyridyl cyanoguanidines.



R	
Br	(34)
OCH_3	(26)
F	(25)
H	(24)
CH_3	(33)
OCH_3	(169)

τ_1 = 180° i.e. the two planes are parallel or the conformations where τ_1 is between 60° and 100°. The latter corresponds to either the gauche conformation for τ_1 or a conformation where the two planes are perpendicular to each other. It may be that as a gauche or perpendicular conformation is indicated to be most favoured with the hydroxy or fluoro analogues that one of these conformations is responsible for histamine H_2-receptor antagonist activity.

Further increases in the size of the 3-substituent decreases the population of the conformations, where the planes formed by the chain and the pyridine ring are not parallel, in favour of the τ_1 = 180° conformation. This may correspond to the separate correlations with the size of the 3-substituent found for activity at both the histamine H_1- and H_2-receptor described in Section 2.4.

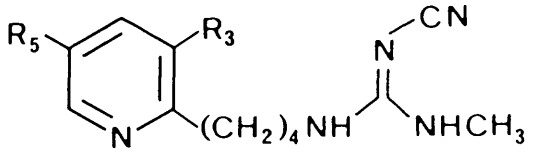
2.5.2 X-ray crystallography

The X-ray crystal structures have been determined for some of the cyanoguanidine analogues.[92] Figure 2.9 shows the different conformations found in the crystal for each of the butyl chain analogues. Although the environmental effects on the molecule either in solution or at the receptor may be very different to those found in the crystal, similarities can be seen between the conformations predicted in the previous section and that found in the crystal.

The torsion angles τ_1 and τ_2 are as defined in structure (52) in the previous section. The torsion angles for the first two bonds are shown in Table 2.4. All the analogues exist in the crystal in a conformation where τ_2 = 180° i.e. in the trans conformation. Various conformations are found for the bond linking the methylene chain to the pyridine ring, as shown by the range of values for τ_1.

The 3-methyl (33) and 3-bromo (34) analogues, which the calculations indicated may favour the conformation where $\tau_1 = \tau_2 = 180°$, are both found to be in this conformation in the crystal. All the other analogues exist in the crystal in conformations where $\tau_1 \neq 0°$. The range of values seen for torsion angle τ_1 is 0° to 100°. As all the values for torsion angle τ_2 are approximately 180° the sign of the torsion angle τ_1 can be disregarded as positive or negative signs give equivalent position on either side of the plane of the pyridine ring.

Table 2.4 Torsion angles found in the crystal for the pyridylbutyl cyanoguanidines.



Compound number	R_3	R_5	τ_1	τ_2
(33)	Me	H	-176.2°	179.2°
(34)	Br	H	179.9°	-177.6°
(26)	OMe	H	-79.3/97.5°	-177.1/-179.3°*
(25)	F	H	-88.8°	179.7°
(24)	H	H	101.3°	172.5°
(169)	H	OMe	4.3°	-176.4°

* This compound exists with 2 separate molecules in the unit cell.

The analogues (24), (25) and (26) exist in the crystal in conformations where τ_1 is approximately 90°. This is a conformation where the plane formed by the first three methylenes of the chain is approximately perpendicular to the plane of the pyridine ring. This was predicted to be a low energy conformation in the previous section. These results do not indicate any difference in conformation for τ_1 on introducing a methoxy or fluoro substituent into the 3-position of the pyridine ring when compared with the unsubstituted analogue.

When comparing the unsubstituted analogue with the fluoro or methoxy analogues, the calculations indicated that for the substituted analogues, a gauche or perpendicular conformation may be favoured over a conformation where $\tau_1 = 0°$. The $\tau_1 = 90°$ conformation was a low energy conformation for the unsubstituted analogue and would therefore be an expected conformation in the crystal.

It is interesting to note that the 5-methoxy analogue (169) exists in the conformation where $\tau_1 = 0°$ i.e. the first three atoms of the linking chain are in the same plane as the pyridine ring with the second atom of the chain directed towards the 3-pyridyl position.

These results support the findings from the molecular mechanics calculations. The X-ray crystal structures correspond to minimum energy conformation predicted by the calculations.

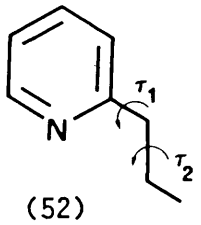
2.5.3. Conclusion

The results presented in Section 2.5.1. and 2.5.2. suggest that 3-substituent modifies the low energy conformations available to the torsion angle τ_1. Increasing the substituent size from H to fluoro or methoxy populates the $\tau_1 = 180°$ and $\tau_1 = 60°$ to 90° conformations. This may be correlated with increases in potency at both the histamine H_1- and H_2-receptor. Further increases in size of the 3-substituent populates the $\tau_1 = 180°$ conformation at the expense of the out of plane conformation.

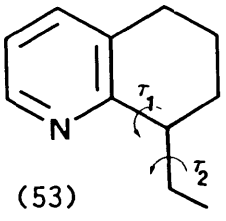
It is possible that an out of plane conformation is therefore responsible for histamine H_2-receptor antagonist activity and the τ_1 = 180° conformation is responsible for histamine H_1-receptor antagonist activity.

2.6. CONFORMATIONALLY RESTRICTED ANALOGUES

In previous sections of this chapter it has been proposed that the 3-substituent influences the antagonist potency at both the histamine H_1- and H_2-receptor by influencing the conformational properties of the torsion angle between the side chain and the pyridine ring. It was proposed that the conformation where $\tau_1 = \tau_2 = 180°$ was important for potency at the histamine H_1-receptor, as shown in structure (52). A similar conformation may also exist in the bicyclic system of structure (53).



(52)



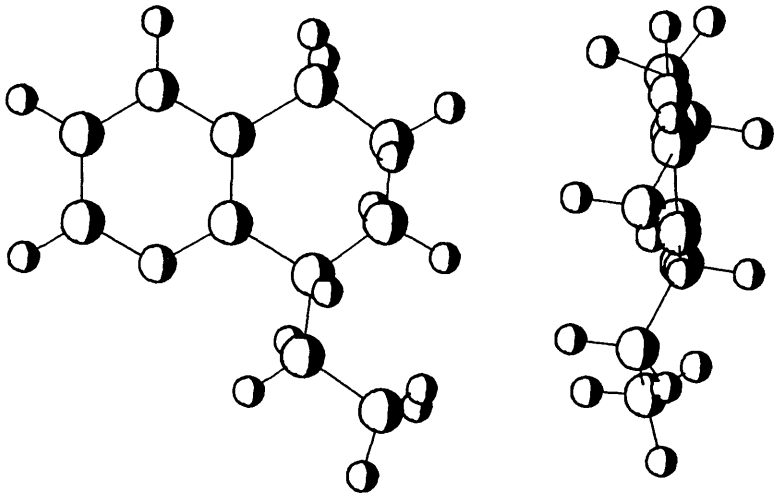
(53)

This analogue may be regarded as a system where the first atom of the chain is embedded into the second ring which is fused onto the 3-position of the pyridine. The presence of the second ring limits the torsion angle τ_1 to certain known positions.

The second ring is a fused cyclohexene and a pseudo chair conformation should be favoured, the ring being flattened due to the double bond.

Figure 2.10 shows orthogonal views for the minimum energy conformation for structure (53) predicted by molecular mechanics calculation. The conformation used for the tetrahydroquinoline ring system is that of tetrahydronaphthalene and was obtained from the

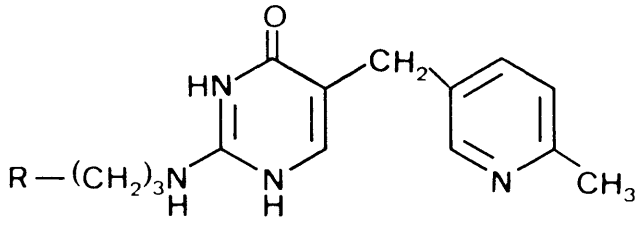
Figure 2.10 Orthogonal views of the 8-substituted tetrahydroquinoline

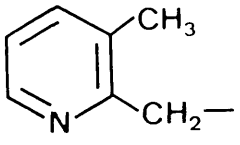
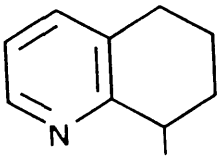


Sybyl data base.[93] This structure was derived from a survey of X-ray crystal and neutron diffraction crystal data bases.[94] The chain was linked in a pseudo equatorial position. The minimum energy conformation for the side chain was found to be as shown in Figure 2.10. In this conformation τ_1 = 148° and τ_2 = 175.9°, when compared with structure (52) corresponds to the side chain being rotated about 30° out of plane from the 180° i.e. trans conformation. Table 2.5 compares the histamine H_1-receptor antagonist activity for a tetrahydroquinoline of structure (55) with the corresponding 3-methylpyridine (54).

Comparison of the potency of analogues (54) and (55) indicates that fusion of the side-chain into a second ring thus freezing out rotation around τ_1, has resulted in a compound of similar or possibly increased potency at the histamine H_1-receptor. However, the variability of the data for the tetrahydroquinoline (55) make this conclusion tentative. This result adds further to the postulate that a conformation near the trans (τ_1 = 180°) conformation is important for potency at the histamine H_1-receptor. The poor activity of the tetrahydroquinoline (55) at the histamine H_2-receptor supports the postulate that an out of plane conformation may be important for potency at this receptor.

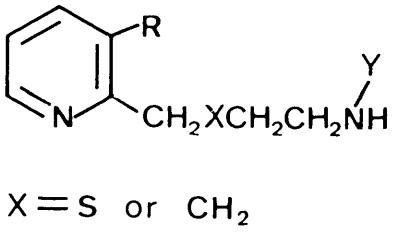
Table 2.5 Comparison of H_1- and H_2-antagonist activity for the 3-methylpyridylbutyl and tetrahydroquinolin-8-ylpropyl isocytosines



Compound number	R	pA_2 Ileum (slope)	pA_2 Atrium (slope)
(54)		8.68 (0.88)	6.97 (0.61)
(55)		8.92 (0.69 ± 0.35)	<4.27

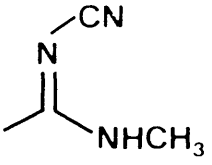
3.0 COMPARISON OF ISOMERIC SUBSTITUTION IN PYRIDINE-CONTAINING HISTAMINE ANTAGONISTS

3.1. INTRODUCTION

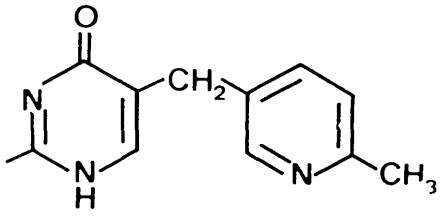


X = S or CH_2

Y =



or



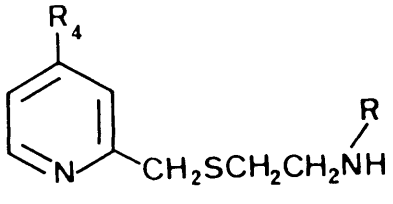
In the previous Chapter the effect of substitution at the 3-pyridyl position on antagonist potency at both histamine receptors was discussed for compounds of the structure shown above. It was shown that the 3-pyridyl substituent separately influenced the antagonist potency at both receptors. The objective of the work presented in this Chapter was to explore the effect of substitution at the remaining pyridyl positions.

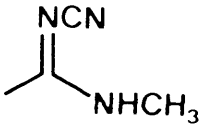
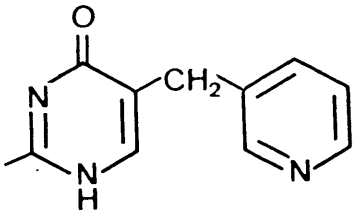
3.2. EFFECT OF SUBSTITUTION INTO THE 4-PYRIDYL POSITION

The effect of introducing a methyl group into the 4-position of the pyridine ring is shown in Table 3.1.

This substitution had little effect on potency at either the histamine H_1- or H_2-receptor in either the isocytosine or cyanoguanidine series. However, there was an apparent increase in potency for the isocytosine analogue at the histamine H_2-receptor as measured in the in vitro assay but the slope of the Schild plot was

Table 3.1. Comparison of effects of substitution at the 4-pyridyl position in cyanoguanidine and isocytosine analogues



R_4	R	pA_2 atrium (slope)	pA_2 ileum (slope)	Rat G.S. ED_{50} µmole/kg
H		6.03	-	9.15
CH_3		6.05	-	11.1
H		6.90 (0.99)	6.04 (0.90)	0.32
CH_3		7.91 (0.43)	6.01 (0.91)	0.31

significantly different from unity for this example making the result inconclusive. The in vivo histamine H_2-receptor potencies for these two analogues were similar. The cyanoguanidine analogues, with or without a 4-methyl substituent, showed very similar potencies at the histamine H_2-receptor both in vivo and in vitro. Therefore, it may be concluded that substitution of a methyl at the 4-position of the pyridine ring has very little effect on potency at either receptor. Further Mannich-4-pyridyl substituents are discussed in Chapter 5.

3.3. EFFECT OF SUBSTITUTION INTO THE 5-PYRIDYL POSITION

Substitution at the 5-pyridyl position was examined for two reasons. The primary objective was, as stated in the introduction to this chapter, to explore the effect of substitution other than at the 3-pyridyl position. However, as the 3- and 5-pyridyl positions are isoelectronic, the effect of introducing a substituent into either position should impart the same electronic effect on the pyridine ring. This would be reflected in similar observed pKa's for the pyridine nitrogen of the isomers and may, therefore, be a way of separating steric and electronic effects.

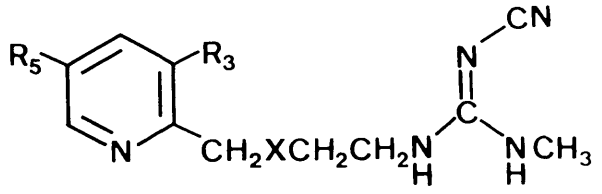
3.3.1. The cyanoguanidine analogues

The effect of substitution into the 5-position of the pyridine ring in the cyanoguanidine series is shown in Table 3.2. The unsubstituted and corresponding 3-substituted analogues are also shown.

All three 5-substituted pyridyl analogues were of similar potency. However, they were much less potent than either the unsubstituted analogue or the corresponding 3-substituted analogues. As all three analogues were of similar potency, it is not possible to define any single property that correlates with activity in the series.

One of the objectives for making the 5-substituted analogues was to separate the electronic and steric effects of the substituent. The measured pKa of the 3- and 5-bromo analogues are shown in Table 3.2. They were very similar being within 0.1 pKa units of each other. This was the postulated result as the 3- and 5-pyridyl position are isoelectronic. This result indicates that as the substituent has different effects on potency depending on whether it is in the 3- or 5-pyridyl position, the activity cannot be simply related to an electronic effect which modifies the pKa of the pyridine ring.

Table 3.2. Effects of substitution into the 5-pyridyl position of the cyanoguanidine analogues.



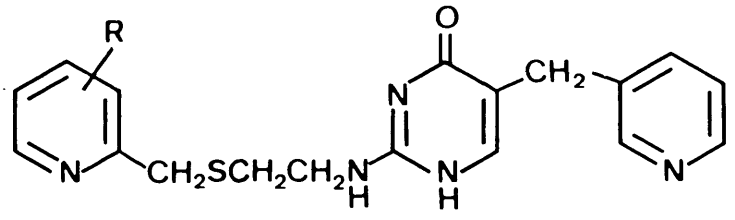
R_3	R_5	X	pA_2 atrium (slope)	Rat G.S. ED_{50} µmole/kg	Pyridine ring pKa
H	H	S	6.03	9.15	4.43*
H	Br	S	4.93	43.0	2.32*
Br	H	S	6.65	2.40	2.23*
H	H	CH_2	5.64	22.0	
H	Cl	CH_2	5.0	39.0	
Cl	H	CH_2	5.72	8.5	
H	OCH_3	CH_2	4.82	29.0	5.58*
OCH_3	H	CH_2	6.75	18.0	

* pKa value measured at 25°C

3.3.2. The isocytosine analogues

The effect of introducing a bromo substituent into the 5-position of the pyridine ring in the methylthioethyl isocytosines is shown in Table 3.3. This substitution reduces potency at the histamine H_2-receptor whereas the corresponding substitution into the 3-position does not affect potency at the histamine H_2-receptor. In

Table 3.3 The effect of introducing a bromo substituent into the pyridine ring of the methylthioethyl isocytosines.



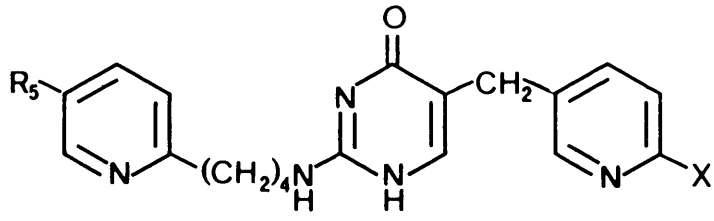
R	pA_2 atrium (slope)	Rat G.S. ED_{50} µmole/kg	pA_2 ileum (slope)
H	6.90 (0.99)	0.32	6.04 (0.90)
5Br	6.18 (0.88)	2.8	7.26 (0.94)
3Br	6.89 (0.84)	0.36	7.03 (0.76)

contrast, substitution of bromo at either the 3- or 5-position increases potency at the histamine H_1-receptor.

A further series of 5-substituted pyridyl analogues were prepared in the tetramethylene series and these analogues are shown in Table 3.4.

Attempts to correlate activity at the histamine H_2-receptor with the physical properties of the substituent do not indicate any single parameter that predicts the potency at this receptor. There was only one pA_2 unit separating the whole range of substituents investigated and therefore any structure activity analysis must be carried out with care due to the inherent inaccuracy in biological data. The slopes of the Schild plots were also different from unity for some of the analogues. The correlation previously seen for the

Table 3.4. The effect of substitution into the 5-position of the pyridine ring of the pyridylbutyl isocytosines.



X	R_5	pA_2 atrium (slope)	pA_2 ileum (slope)	S_t	π	σ^m	π^p
H	H	6.64 (1.14)	7.41 (0.83)	1.00	0.00	0.00	0.00
H	NH_2	6.7 (0.68)	7.6 (0.83)	1.84	-1.23	-0.16	-0.66
H	OMe	6.09 (0.64)	6.62 (0.97)	1.35	-0.02	0.12	-0.27
CH_3	H	6.48 (1.37)	7.28 (0.91)	1.00	0.00	0.00	0.00
CH_3	NO_2	5.52 (0.90)	7.27 (0.55)	2.44	-0.28	0.71	0.78
CH_3	NH_2	-	7.16 (0.88)	1.84	-1.23	-0.16	-0.66
CH_3	F	6.22 (0.85)	7.70 (0.70)	1.35	0.14	0.34	0.06
CH_3	Cl	6.21 (0.82)	8.11 (0.86)	1.80	0.71	0.37	0.23
CH_3	Br	6.29 (0.60)	8.40 (1.15)	1.95	0.86	0.39	0.23
CH_3	I	5.81 (1.0)	8.12 (0.76)	2.15	1.12	0.35	0.18
CH_3	Me	6.5 (0.74)	7.88 (1.28)	1.90	0.56	-0.07	-0.17
CH_3	SMe	-	7.49 (0.96)	1.70	0.61	0.15	0.00
CH_3	NMe_2	5.97 (0.88)	6.26 (0.98)	2.80	0.18	-0.15	-0.83

3-pyridyl analogues with the steric parameter was not apparent in this series.

The 5-pyridyl substituent has a greater influence on activity at the histamine H_1-receptor. A substituent may either have a beneficial influence as with bromo or detrimental influence as with methoxy.

Attempts to correlate the activity at the histamine H_1-receptor with any one single parameter indicate that none of the parameters showed a linear relationship with the potency of the compound at the histamine H_1-receptor (Table 3.4). If the four halogen substituents were considered, a similar relationship to that found for the corresponding 3-substituted analogues was observed with activity increasing through the series F < Cl < Br then falling off to iodo. It is therefore tempting to infer that the same steric parameter used for the 3-substituent holds in this series. This correlation would fit for the methyl analogue which has a similar potency to the chloro analogue. However, the 5-amino analogues showed much lower potencies and these were all of similar potency to the unsubstituted compound.

It is probable, therefore, that a correlation with the steric parameter is not valid. The similar σ values for methyl and amino may also indicate that the σ parameter does not correlate with biological activity. A more reasonable correlation may be seen with the lipophilic parameter. However this would not account for the loss of activity seen with the methoxy or dimethylamino substituents. This may arise as a result of the larger size of these substituents. It may not be possible to accommodate the additional methyl group at the receptor. However, if this is the case one might have expected that the methylthio analogue would also have been less potent. The possibility arises that the methoxy substituent may favour a conformation where the methyl of the methoxy group is in the same plane as the pyridine ring, whereas the methylthio may be able to rotate out of plane and thus avoid a clash at the receptor. The dimethylamino analogue may also avoid a clash in a similar manner although in this case the methyl groups of the out of plane

dimethylamino substituent would project on both sides of the pyridine ring plane and may therefore inhibit binding of the pyridine ring to the receptor.

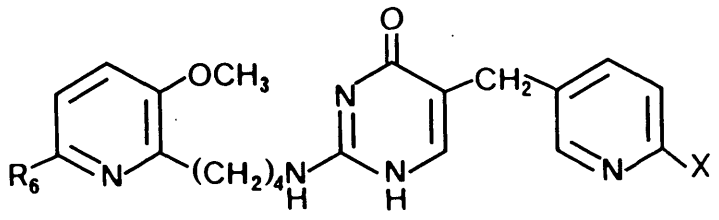
If the above argument explains the activities of the methoxy, dimethylamino and methylthio substituents, it might have been expected that a nitro substituent would also reduce potency. The nitro analogue however, has a similar pA_2 value to the unsubstituted compound although the very low slope to the Schild plot makes any interpretation of this result inconclusive.

The possibility therefore exists that more than one property of the 5-substituent is influencing potency at the histamine H_1-receptor although it is difficult to draw any firm conclusion from a small series of compounds. These results do indicate that the same correlation seen with the steric parameter for the 3-substituent is not apparent for the 5-substituted analogues. It is possible that there is a hydrophopic binding area of limited size which the substituent may bind to. The bromo substituent is most effective for increasing potency at the histamine H_1-receptor.

3.4. EFFECT OF SUBSTITUTION INTO THE 6-PYRIDYL POSITION

Substitution at the 6-position of the pyridine ring had been shown to reduce activity in the thiourea histamine H_2-receptor antagonists (Section 1.4.5.1.). The objective of investigating substitution into this position was to confirm that the detrimental influence seen in the thiourea histamine H_2-antagonists was also found with the isocytosine series. If this was the case, it was of interest to see if a similar reduction in activity was observed at the H_1-receptor. If a reduction in activity was not observed then it may be possible to use such an observation to obtain a selective histamine H_1-receptor antagonist. Only the nonbasic substituents investigated are discussed here; further basic Mannich substituents are discussed in Chapter 5. The effect of introducing a methyl and a hydroxymethyl group into the 6-position in the 3-methoxypyridine series is shown in Table 3.5.

Table 3.5. Influence of 6-methyl and 6-hydroxymethyl substituents on potency at both the histamine H_1- and H_2-receptor in the methoxypyridylbutyl isocytosines



R_6	X	pA_2 atrium (slope)	pA'_2 ileum (slope)	Rat G.S. ED_{50} µmole/kg
H	H	6.64 (1.14)	7.41 (0.83)	0.24
6Me	H	5.63 (0.95)	7.67 (0.94)	4.9
H	Me	6.48 (1.37)	7.28 (0.91)	0.4
$6CH_2OH$	Me	Ppt*	6.34 (0.99)	5.0

*Ppt indicates compound precipitated in the organ bath

Both 6-pyridyl substituents reduced activity at the histamine H_2-receptor. However, for the hydroxy methyl analogue this may be due to insolubility of the compound which did not permit determination of a pA_2 value. This result is consistent with that previously found for the thiourea analogues.

The 6-methyl was only slightly less potent at the histamine H_1-receptor, whereas the hydroxymethyl analogue was an order of magnitude less potent. Again this effect may be due to the insolubility of the hydroxymethyl compound although the slope of the Schild plot for the hydroxymethyl analogue is unity and therefore solubility may not be a problem in this assay. It may be that larger

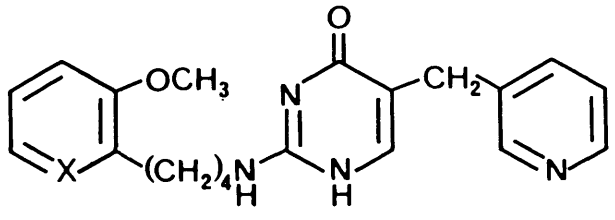
substituents in this position reduce potency at the histamine H_1-receptor. The 6-Mannich-pyridyl analogues discussed in Section 5 also show reduced potency at the histamine H_1-receptor.

These results indicate that it may be possible to separately influence potency at the histamine H_1- and H_2-receptor by substitution at the 6-pyridyl position.

3.5. EFFECT OF SUBSTITUTION OF THE PYRIDINE NITROGEN

From Table 3.6 it can be seen that N-oxidation of the pyridine leads to substantial reduction in potency at both receptors. This indicates that an unsubstituted pyridine nitrogen is important for high potency at both receptors. Either substitution of the pyridine

Table 3.6. The effect of substitution of the pyridine nitrogen in the 3-methoxypyridylbutyl isocytosines.

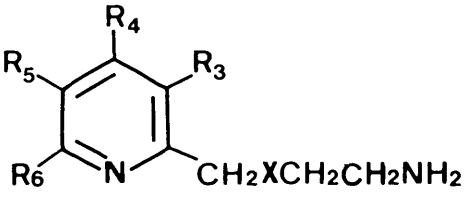


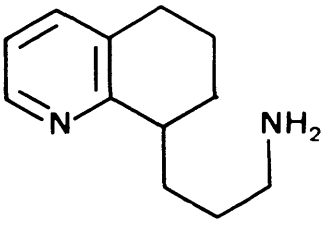
X	pA_2 atrium (slope)	pA_2 ileum (slope)	Rat G.S. ED_{50} µmole/kg
N	7.49 (0.89)	7.77 (1.05)	0.21
NO	4.46 (1.1)	6.35 (0.56)	3.5

nitrogen or introduction of groups into the pyridine ring which would result in steric crowding of this position (see previous section) reduce potency. This indicates that the pyridine nitrogen is important for binding at the receptor and any substituent that inhibits approach to the pyridine nitrogen results in loss of potency.

4.0 SYNTHESIS OF THE SUBSTITUTED PYRIDYL ANALOGUES

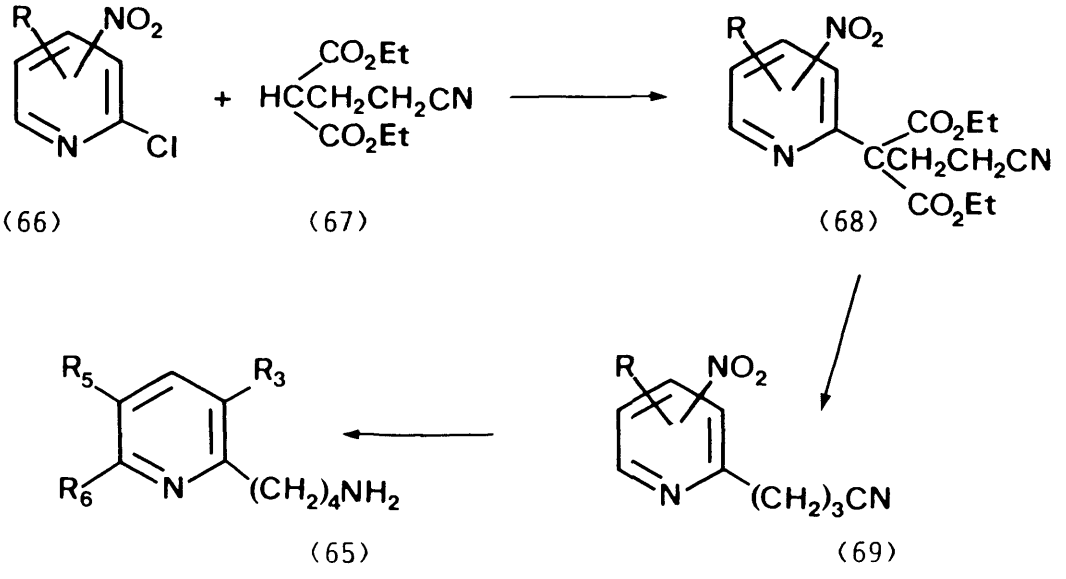
4.1. INTRODUCTION





This chapter describes the preparation of the amines, shown above, which were used to prepare the antagonists discussed in Chapters 2 and 3. Preparation of the antagonist is described in Chapter 6. The major part of this chapter describes the preparation of the substituted pyridyl butylamines, by the route shown in Scheme 4.1. The synthesis of the intermediate nitriles (69) will be discussed first. The methods used for elaboration of those nitriles which give the corresponding substituted pyridylbutylamines structure (65) will then be described. The final part of the Chapter deals with the preparation of the amines not prepared by the malonic ester route.

Scheme 4.1 Preparation of substituted pyridyl analogues

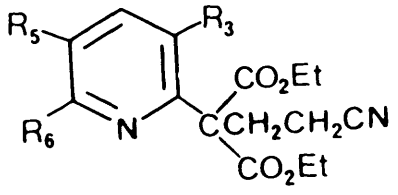


(66) (67) (68) (65) (69)

4.2. PREPARATION OF THE PYRIDYLMALONIC ESTERS

The pyridylmalonic esters synthesised are shown in Table 4.1.

Table 4.1. The pyridylmalonic esters



Compound number	R_3	R_5	R_6
68a	NO_2	H	H
68b	H	NO_2	H
68c	CH_3	NO_2	H
68d	NO_2	H	CH_3

Hurst and Wibberley[95] had shown that the anion of diethyl malonate could be condensed with 2-chloro-3-nitropyridine in refluxing diethyl ether and that hydrolysis and decarboxylation of the product yielded 3-nitro-2-picoline. This reaction was further exemplified[96] for the preparation of 5-nitro-2-picoline from 5-nitro-2-chloropyridine.

At the start of this work it had already been shown that the anion of the substituted malonic ester (67) could be condensed with 2-chloro-3-nitropyridine and the product underwent basic hydrolysis and decarboxylation to give the required pyridylbutyronitrile (69a).[97] However, it had been found that the anion of the cyanoethylmalonate (67) required higher temperatures (103°C). The

anion was first formed using sodium hydride in tetrahydrofuran, 2-chloro-3-nitropyridine was added and the solvent removed by distillation until the internal temperature reached 105°C, the reaction being carried out essentially as a melt; yields of 50-60% of pyridine (68a) could be achieved.[97]

In contrast to the 3-nitro analogue the condensation of 2-chloro-5-nitropyridine with the anion of (67) was found to be exothermic in tetrahydrofuran, the reaction being complete within 1 hour at reflux temperature giving the required malonic ester (68b) in 65% yield.

The anion of (67) underwent condensation with 2-chloro-3-methyl-5-nitropyridine under similar conditions to that required for condensation with 2-chloro-3-nitropyridine.[98]

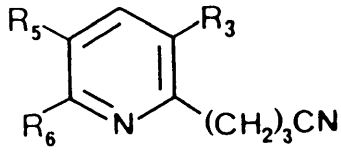
It would appear that the ease of condensation of malonic ester anions with 2-chloropyridines is at least partially governed by the steric crowding at the reaction centre. More vigorous reaction conditions are required with either a substituted malonic ester or when there is a bulky substituent 'ortho' to pyridyl halogen being displaced.

The condensation of the anion of (67) with 2-chloro-6-methyl-3-nitropyridine gave only a very poor yield of malonic ester (68d). The major product of the reaction was a black polymeric material. It may be that the pyridylmethyl group is more acidic than malonic ester anion as it is activated by the ring nitrogen and the nitro group. Proton exchange may therefore occur between the pyridylmethyl group and the malonate anion to give a new pyridylmethyl anion. This anion may then condense with a second pyridine molecule leading to polymerisation of the pyridine starting material.

4.3. PREPARATION OF THE PYRIDYLBUTYRONITRILES

In this section the hydrolysis and decarboxylation of the malonic esters shown in Table 4.2 (68a-d) will be discussed.

Table 4.2 Pyridylbutyronitriles prepared by hydrolysis and decarboxylation of malonic esters.



Compound number	R_3	R_5	R_6
69a	NO_2	H	H
69b	H	NO_2	H
69c	CH_3	NO_2	H
69d	NO_2	H	CH_3

4.3.1. The 3-nitro analogue

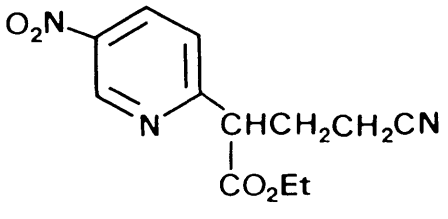
It had been shown[97] that the malonic ester (68a) underwent hydrolysis and decarboxylation under basic conditions in aqueous ethanol to give (69a). Treatment of (68a) with sodium hydroxide (1M ; 4 equivalents) in ethanol for 72 hours was found to be the optimum conditions for the reaction and the product could be isolated in essentially quantitative yield. Subsequent to the different mechanisms for hydrolysis and decarboxylation of (68b) (see later), it was decided to examine the above hydrolysis in a similar manner.

The conversion of the malonic ester (68a) to the butyronitrile (69a) followed a similar course to that of the 3-methyl analogue (68c) in that no monoester intermediates were observed. The reaction was complete in about 72 hours (cf 6 hours for the 3-methyl analogue (68c)). No absorption was seen in the UV visible spectrum of the reaction mixture above 350 nm.

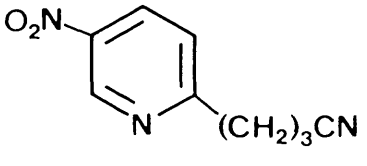
4.3.2. The 5-nitro analogue

The deesterification and decarboxylation of the malonic ester (68b) was first attempted using the method previously described for the 3-nitro analogue (68a). Under these conditions, the nitrile (69b) could only be isolated in low yield from the tarry by-products. It was also observed that on adding the base to the reaction mixture an intense magenta colour developed. The UV spectra of the two malonic esters (68a) and (68b) were compared and it was found that on adding base to the solution of (68b) an intense absorption at 512 nm, $\varepsilon \cong 47{,}000$, developed which was not observed with the malonic ester (68a).

Treatment of the malonate (68b) in ethanol with 2 mole equivalents of sodium hydroxide (1 M) gave a deep magenta solution. The TLC after 1 hour showed the absence of starting material. Addition of dilute hydrochloric acid to the solution resulted in a steady loss of colour from a deep magenta to pink with a sudden change to yellow at about pH 5; on warming the colour changed to pink (pH 5.5). The solution was warmed and the pH kept at about 4.5 by the dropwise addition of dilute hydrochloric acid. Work up gave an oil which was shown to be a 2:1 mixture of the monoester (70) and the required pyridine (69b).

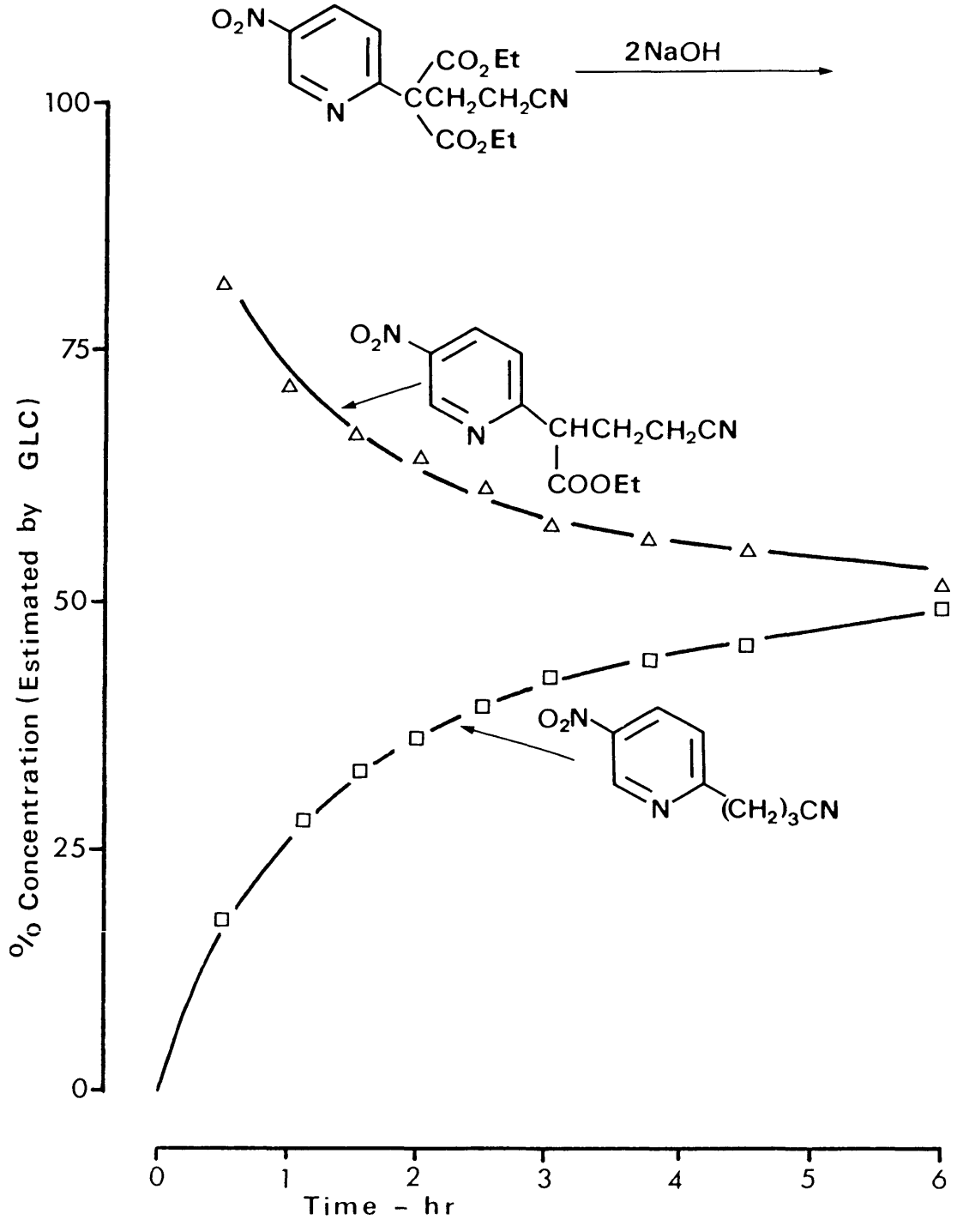


(70)



(69b)

Figure 4.1 Plot showing percentage of monoester and fully hydrolysed product during hydrolysis of diester (68b) using 2 equivalents of base



The above hydrolysis was repeated monitoring the reaction. At time intervals aliquots were withdrawn, acidified, ethanol stripped and extracted with chloroform. The chloroform extracts were examined by GLC and no starting material was detected. Two peaks were seen on GLC which corresponded to monoester (70) and the pyridine (69b) respectively. Figure 4.1 shows the percentage peak area of these two components in the reaction mixture over the course of the reaction.

The above experiment was repeated using 4 equivalents of sodium hydroxide and the results are shown in Figure 4.2. Work up after 6.5 hours gave the pyridine (69b) in greater than 90% yield.

The reaction has been carried out on the 50 g scale using sodium hydroxide (4 equivalents) in ethanol followed by an acid work up to give the pyridine (69b) in 95% yield. The mechanism of the reaction is discussed in Section 4.3.5.

4.3.3. The 3-methyl-5-nitro analogue

The hydrolysis was first carried out using 4 equivalents of sodium hydroxide in ethanol.[97] The reaction was monitored by TLC and GLC in order to compare this analogue with (68a) and (68b). The course of the reaction is shown in Figure 4.3 and the corresponding curve for the formation of the analogue (68b) is shown for comparison. It was found that the formation of the nitrile (69c) followed the same time course as that found for the 5-nitro analogue (69b). However in this case, the loss of starting material (68c) from the reaction mixture paralleled the the formation of product (69c) and none of the intermediate monoester could be detected. The pyridine (69c) was isolated in greater than 90% yield using the conditions described for the malonic ester (68b). However, the mechanism of the hydrolysis appeared similar to that of malonic ester (68a).

Figure 4.2 Plot showing percentage of diester and fully hydrolysed product during hydrolysis of diester (68b) using 4 equivalents of base

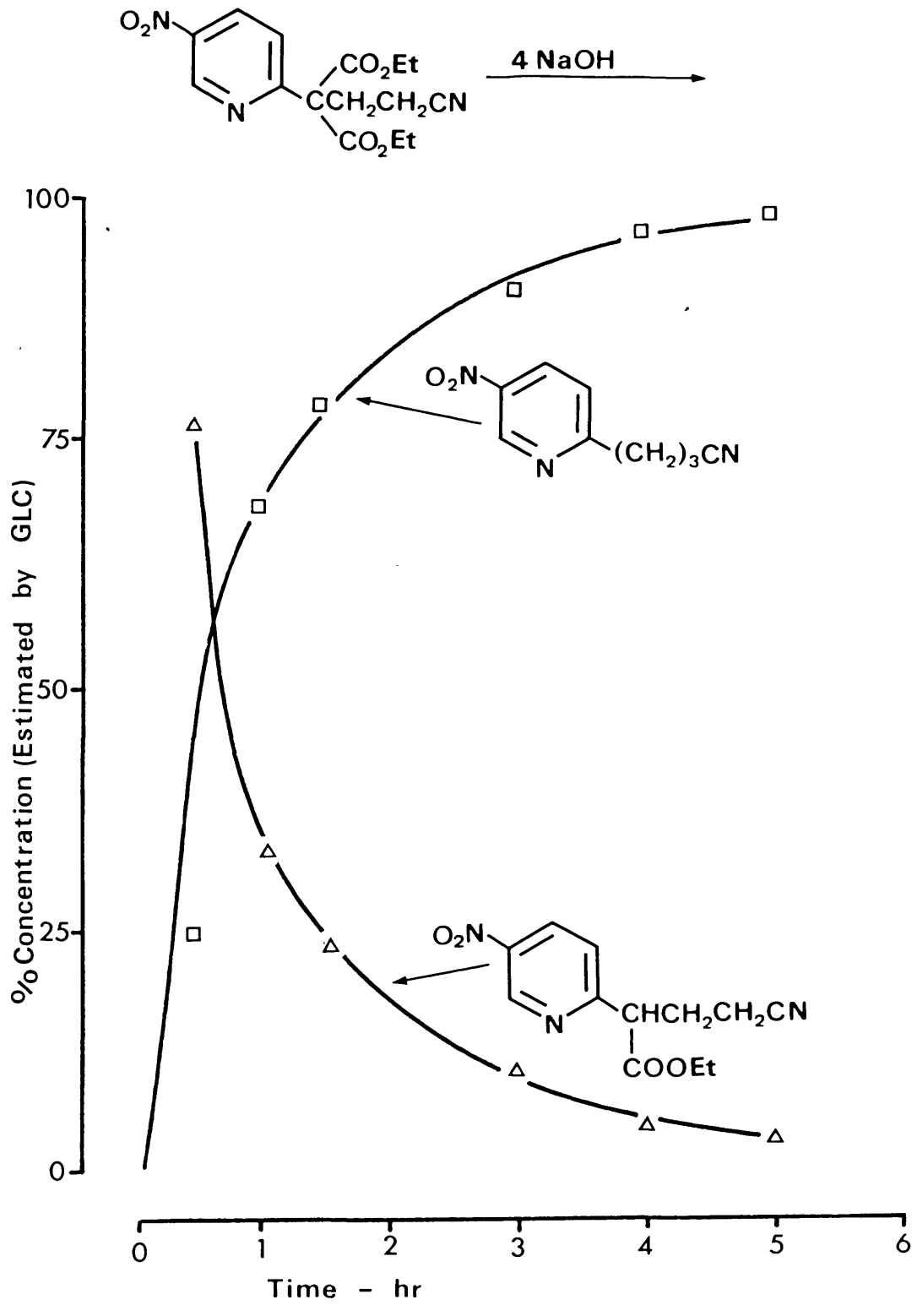
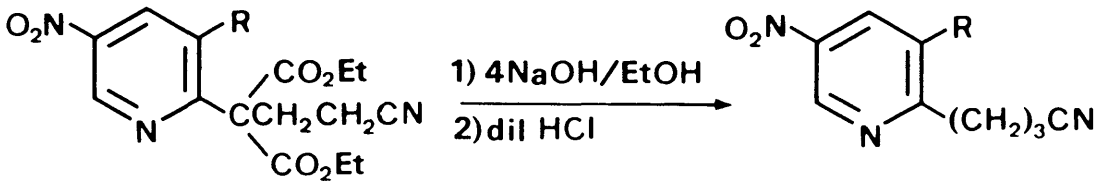
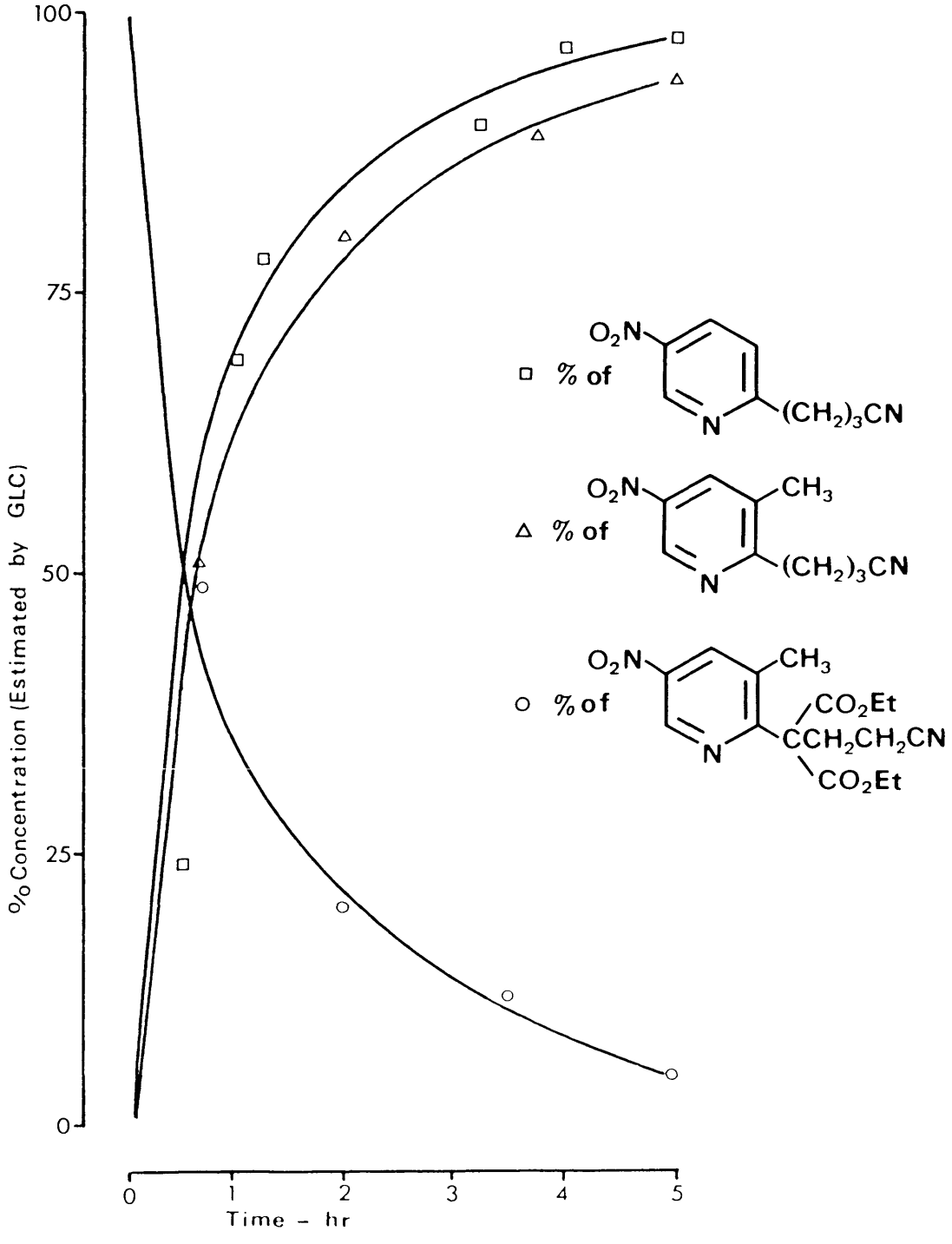


Figure 4.3 Comparison of the hydrolysis of diesters (68b) and (68c)





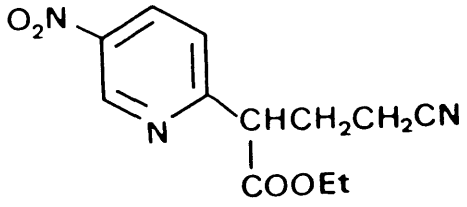
4.3.4. The 6-methyl-3-nitro analogue

The reaction was carried out using the conditions described for the 3-nitro analogue (68a), Section 4.3.1 and the nitrile (69d) was obtained in equally good yield.

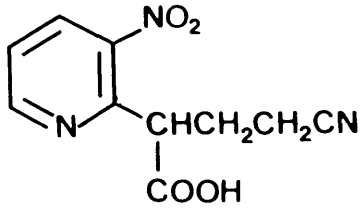
4.3.5. The mechanism of hydrolysis and decarboxylation of pyridylmalonic esters

4.3.5.1. Comparison of the reactions

- o All three reactions require 4 mole equivalents of base.
- o The hydrolysis and decarboxylation of (68a) takes 72 hours whereas both (68b) and (68c) proceed to completion in about 6.5 hours.
- o All the reactions show precipitation of sodium carbonate from the basic reaction mixture.
- o All the reactions give essentially quantitative yield of the fully deesterified and decarboxylated product.
- o On adding base to ethanolic solutions of (68a) and (68c), no colour develops and little absorption is seen above 350 nm in the UV, whereas the diester (68b) under the same conditions gives an intense magenta coloured solution and the the UV visible spectrum showed a large absorption at 512 nm, $\varepsilon \cong 47{,}000$.
- o The two malonic esters (68a) and (68c) are gradually converted into the corresponding fully hydrolysed and decarboxylated products during the reaction whereas the malonic ester (68b) is rapidly converted to the corresponding monoester (70) which is then converted at a much slower rate to the product. The intermediate monoester is not seen in the case of either (68a) or (68c), whereas in the case of (68b) the monoester (70) may be isolated in high yield.



(70)



(71)

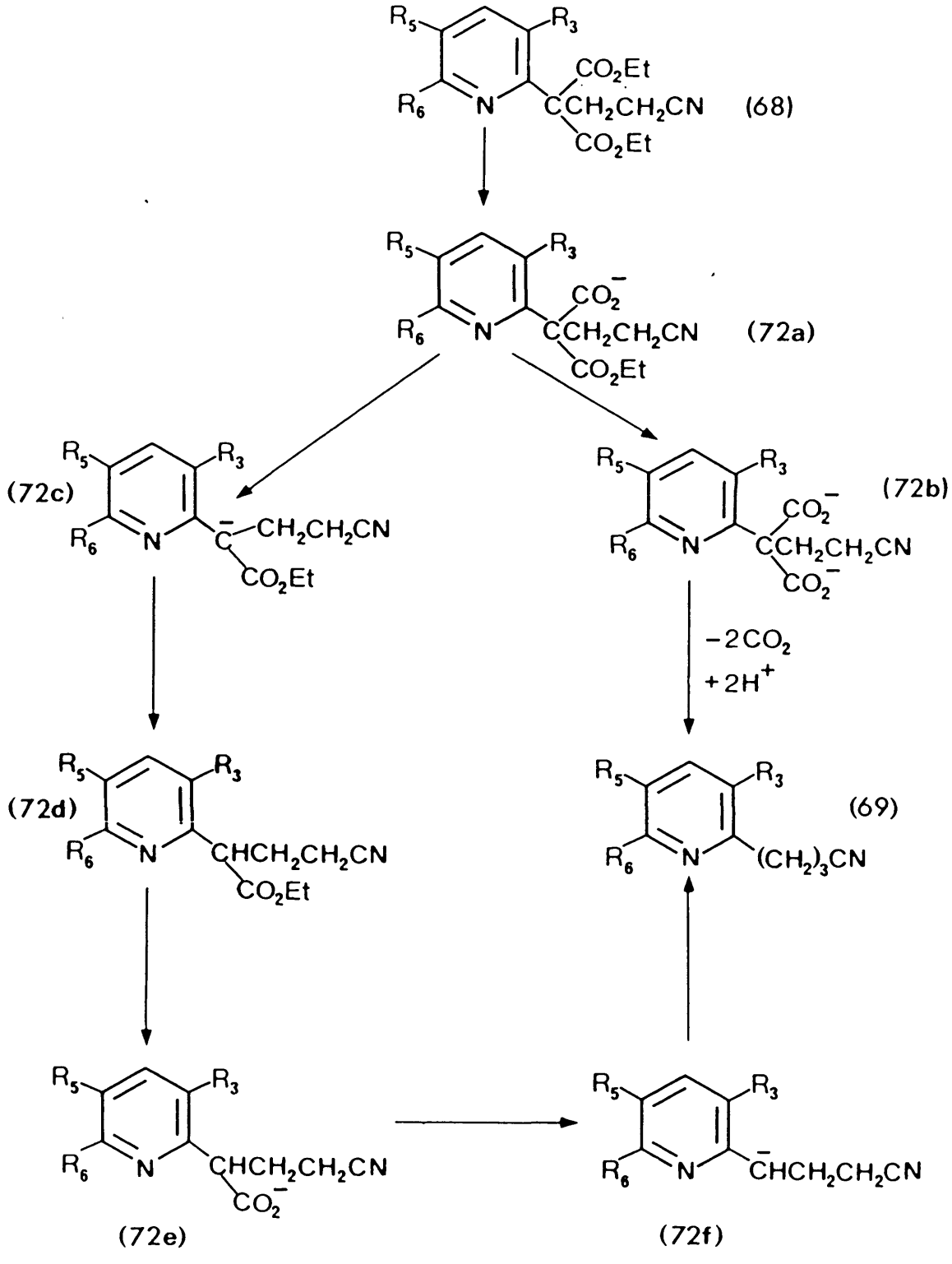
Note: It is not known if the decarboxylation continues on acidification of the reaction mixture or during the removal of the ethanol before assay. It is known that some decarboxylation occurs under basic conditions as carbonate is precipitated from the reaction mixture. The carboxylic acid (71) has been isolated as the sodium salt.[97] However, work up of the chloroform extracts in the GLC assay gave a quantitative recovery of products.

4.3.5.2. Discussion

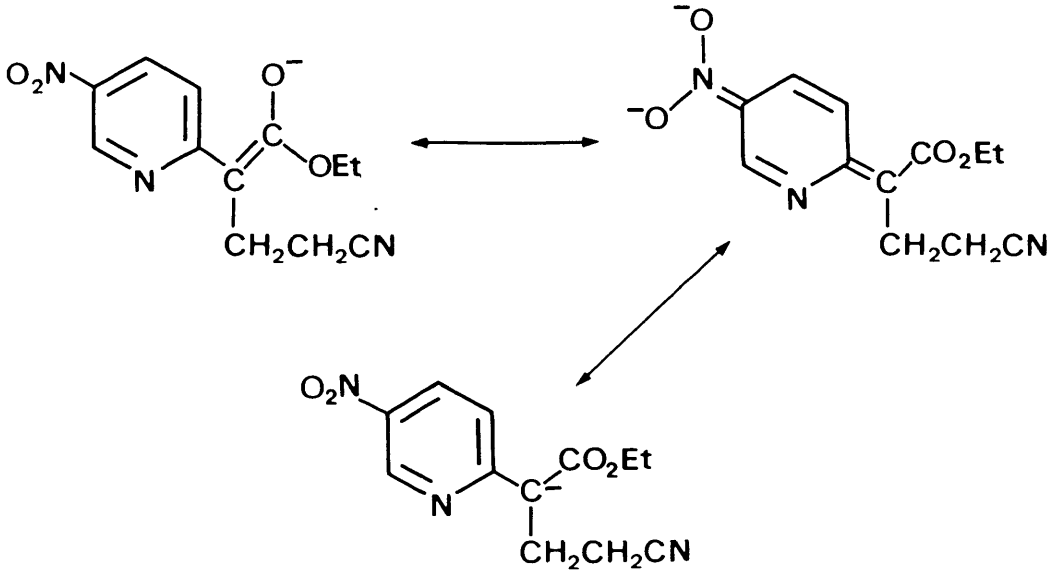
Two possible routes for the reaction are shown in Scheme 4.2. From the data presented above it would appear that in the hydrolysis of either (68a) or (68c) the intermediates are much less stable than the starting material, whereas with (68b) rapid formation of one of the intermediates (72a, 72c, or 72d) must occur and at least one of these intermediates must be stabilized to further hydrolysis, enabling the intermediate (70) to build up in the reaction mixture. The formation of the intense magenta colour (visible band at 512 nm) with (68b) may be attributed to the anion (72c) which could be stabilized by both conjugation with the nitropyridine and the remaining carboethoxy group as shown in Scheme 4.3.

This stabilization will be most favourable if a low energy conformation occurs where the carboethoxy group and the pyridine ring are in the same plane allowing full conjugation of the sp_2 carbanion. This may be the driving force for the rapid loss of the first carboethoxy group in (72a) to give (72c). The malonic ester (68b) does not have a bulky 3-substituent in the pyridine ring

Scheme 4.2 Possible mechanism of hydrolysis and decarboxylation of the pyridyl malonic esters



Scheme 4.3



(72c)

'ortho' to the malonic ester function. This is not the case with the malonic esters (68a) and (68c) where the presence of an 'ortho' substituent on the pyridine ring would hinder formation of an intermediate where the carboethoxy is in the same plane as the pyridine ring. This would disfavour deesterification and decarboxylation in the case of 68a and 68c.

From a comparison of the pKa data shown in Table 4.3 the carbanion intermediate (72c) would be predicted to be acidic, possibly having a pKa <10 and would therefore not protonate under the basic reaction conditions. Further hydrolysis of the unprotonated intermediate would be slow, allowing the concentration of the monocarboethoxy intermediate to build up.

Table 4.3 Comparison of pKa data for various alkanes

	pKa	Reference
$RC\underline{H}_2NO_2$	10	99
(ethane) $RC\underline{H}_3$	42	99
$RO_2C.C\underline{H}_2.R$	24.5	99
$Ar.C\underline{H}_3$	35	99
(2-picoline) $Py.C\underline{H}_3$	29.5	100
(4-nitrotoluene)	28.7	101

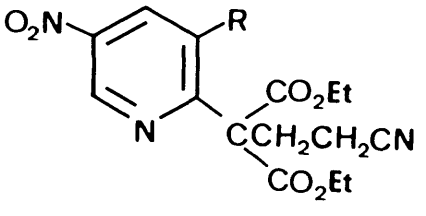
It has already been said that for the anion to be so stable it must be capable of forming a planar intermediate. If the equivalent intermediates were formed during the hydrolysis of either (68a) or (68c) this planar intermediate would be less favourable due to a steric interaction with the 'ortho' substituent present in these examples. These examples would therefore be less stable and would protonate. The second deesterification and decarboxylation may be aided in all three cases as it may be possible for conformations to exist where the carboethoxy group and the pyridine ring are in the same plane.

In summary, hydrolysis and decarboxylation is disfavoured when the carboethoxy group cannot become planar with the pyridine ring which occurs when there is a bulky ortho substituent and two other substituents attached to the carbon from which the carboxy group is lost. When a highly stabilized carbanion is formed, lack of protonation disfavours further reaction of the intermediate.

The differences between the reaction paths can be explained by steric inhibition of resonance which both affects the rate of the initial hydrolysis and the stability of the intermediates. Steric

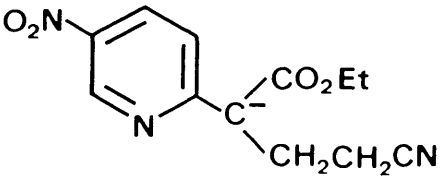
inhibition of resonance by 'ortho' substituents is well documented. This effect has been used to explain pKa changes that do not correlate with the electronic properties of a substituent in benzoic acids,[102] anilines,[103] nitrobenzenes[104] and 4-aminopyridines.[105] For example 'ortho' methyl substitution increases the acidity of benzoic acid. The electron donor properties of the methyl group would be expected to reduce acidity. The 'ortho'-methyl substituent in 'ortho'-toluic acid prevents the carboxy group from being coplanar with the benzene ring and this diminishes the direct resonance interaction between the carboxy group and the aromatic ring.

4.3.5.3. Computer calculations.



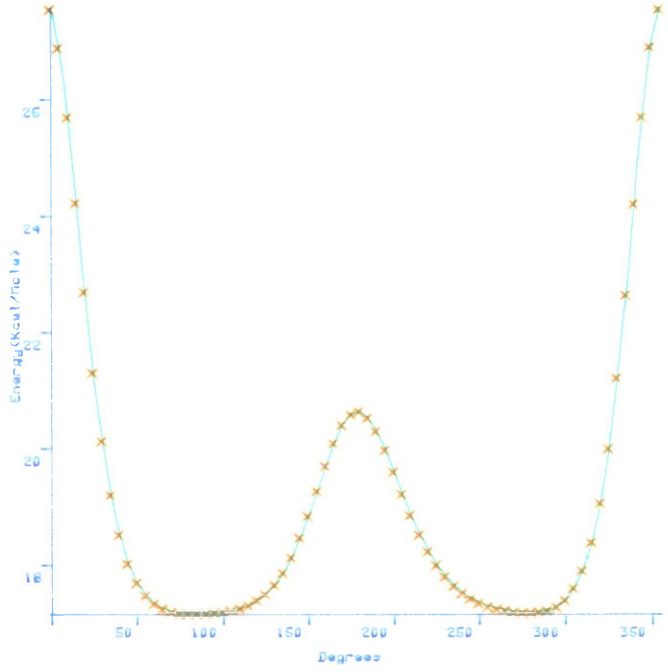
R = H (68a)
R = CH_3 (68c)

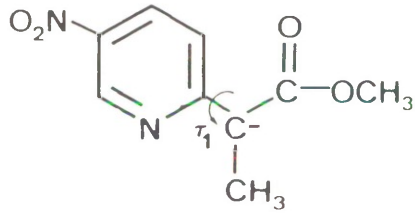
The differences observed on hydrolysis of the two malonic esters (68a) and (68c) has been attributed to the stability of the postulated carbanion intermediate (72c). These anions have been modelled using the SK&F molecular graphics system.[91]



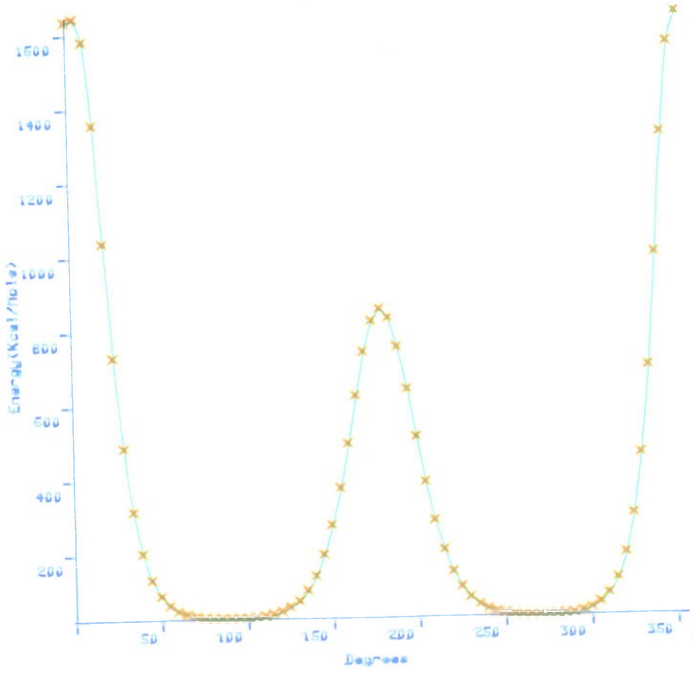
(72c)

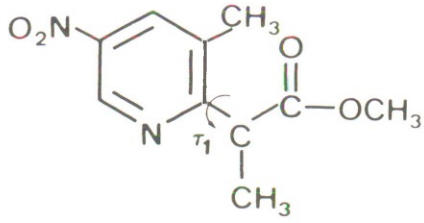
Figure 4.4 Plots for rotation around τ_1 derived by molecular mechanics calculations



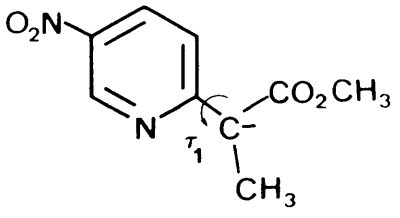


τ_1 = 180° as drawn
3.5 Kcal/mole barrier

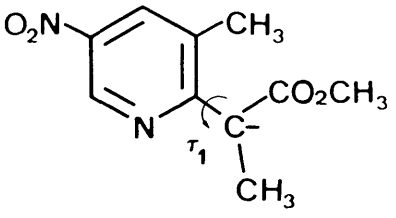




τ_1 = 180° as drawn
800Kcal/mole barrier



(73a)



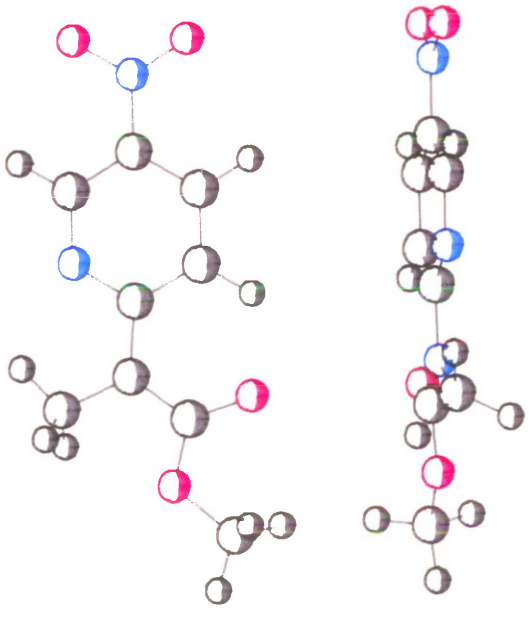
(73c)

The calculation was performed on the model anions (73a) and (73c) in order to reduce the calculation time and simplify the problem. Using a molecular mechanics programme, the energy was calculated for rotation of torsion angle τ_1 as defined in (73a) and (73c) respectively. The deprotonated carbon was parameterised as an isoelectronic sp^2 trigonal planar nitrogen for the molecular mechanics calculation. Figure 4.4 shows the plots of energy against τ_1 for the two anions (73a) and (73c). From the plots it can be seen that there is a high energy barrier (>100Kcal/mol) inhibiting formation of a planar carbanion in the case of the 3-methyl analogue. The barrier is much lower in the case of (73a) (<5Kcal/mol).

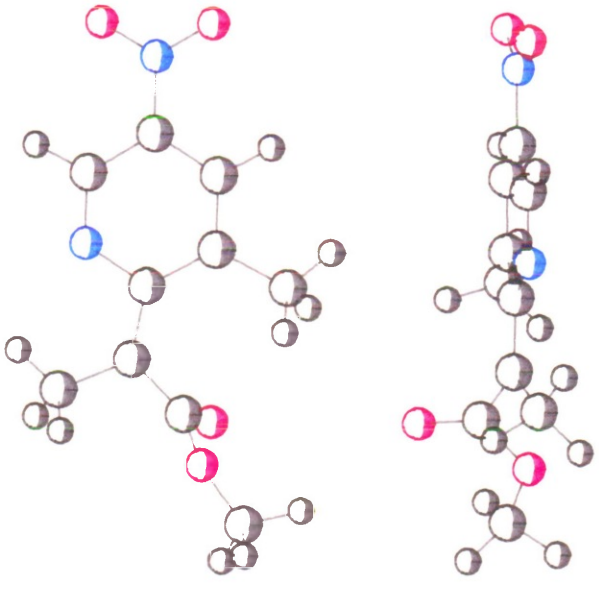
The energy for the rotation around τ_1 in the isoelectronic aminopyridines (74a) and (74c) was calculated by Dr G. Richards at Oxford using 'Extended Huckel Theory' and a similar result was obtained. The two carbanions (73a) and (73c) were geometry-minimized using the MNDO option in the MOPAC molecular orbital package.[106] The carbanion (73a) gave a minimum energy conformation where the carbomethoxy group and the pyridine ring were in plane with the carbanion trigonal planar. The carbanion (73c), however gave a minimum energy conformation with the groups out of plane. The results from these calculations are shown in Figure 4.5.

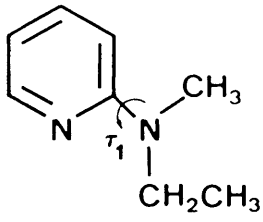
Figure 4.5 Orthogonal views of the minimum energy conformations of pyridylmethyl carbanions obtained using MNDO.

o - Minimum energy conformation for (73a)

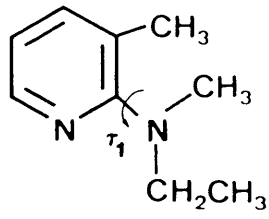


o - Minimum energy conformation for (73c)





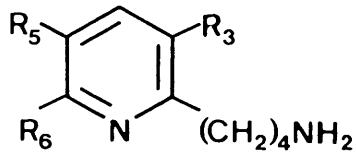
(74a)



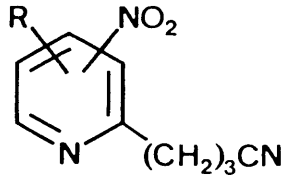
(74c)

These results support the steric inhibition of resonance stabilization by the methyl group in (73c).

4.4. SYNTHESIS OF SUBSTITUTED PYRIDYLBUTYLAMINES



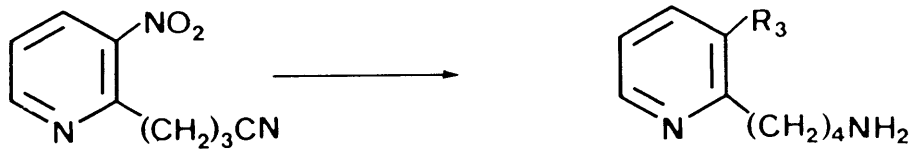
(65)



(69)

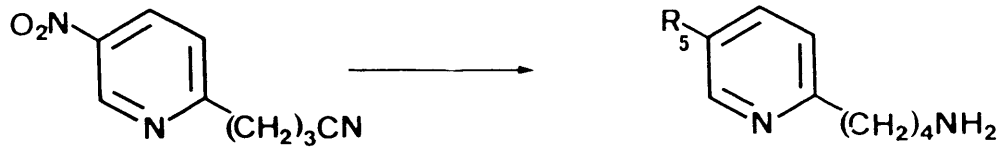
As already discussed the intention was to prepare a range of substituted pyridylbutylamines of structure (65) from the corresponding pyridylbutyronitriles of structure (69). The analogues prepared are shown in Figure 4.6. For each substituent the point at which the side chain nitrile was reduced had to be considered. This depended on the stability to reduction of the substituent being introduced and the stability of the side chain to the conditions required for the introduction of the the new ring substituent. It is proposed to describe the literature precedents for the introduction of each of the chosen substituents into a pyridine ring. The method developed for the 3-substituted-2-(4-aminobutyl)pyridines will be described. A comparison will then be made with methods for the preparation of the 5-substituted-2-(4-aminobutyl)pyridines.

Figure 4.6 Substituted pyridylbutylamines synthesised from the corresponding aminopyridylbutyronitriles



(69a) (65a)

where R_3 = NO_2, NH_2, $NHCH_3$, $N(CH_3)_2$, F, Cl, Br, I, N_3, CF_3, OH, OCH_3, OCH_2Ph, SCH_3.



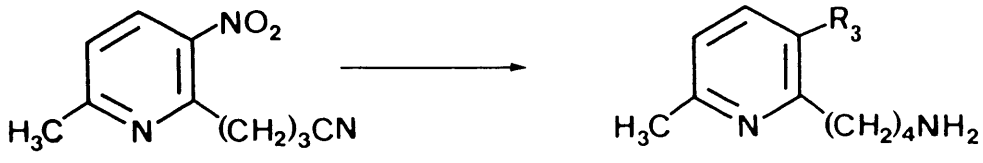
(69b) (65b)

where R_5 = NO_2, NH_2, $N(CH_3)_2$, F, Cl, Br, I, OCH_3.



(69c) (65c)

where R_5 = F, Cl, Br, I.



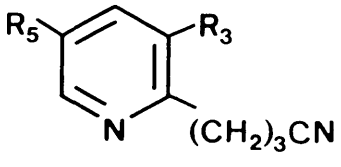
(69d) (65d)

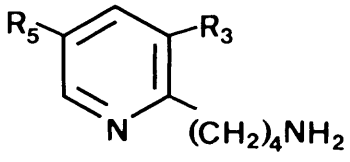
where R_3 = OCH_3.

4.4.1. Reduction of pyridylbutyronitriles

The preparation of aliphatic primary amines from aliphatic nitriles may be accomplished using a variety of reagents. These have been extensively reviewed and include lithium aluminium hydride,[107, 108] diborane[109] and catalytic methods.[110] During this work most of the nitrile reductions were carried out using either lithium aluminium hydride or diborane. A few of the later compounds were reduced with hydrazine and Raney nickel[111] which was a method developed at SK&F for the selective reduction of nitriles in the presence of a halo-pyridine.

4.4.2. Nitro substituted analogues





(69a) $R_3 = NO_2$ $R_5 = H$ (75a) $R_3 = NO_2$ $R_5 = H$

(69b) $R_3 = H$ $R_5 = NO_2$ (75b) $R_3 = H$ $R_5 = NO_2$

Diborane in THF is reported to selectively reduce nitriles in the presence of an aromatic nitro-substituent.[109] The nitropyridine (69a) had been reduced[112] with diborane in THF to give the required amine (75a) in 48% yield. Reduction of the corresponding 5-nitro analogue (69b) gave the aminopyridine (75b) contaminated with about 20% of the product of nitro reduction (77b). No attempt was made to purify the material at this stage. The isocytosine derivative was prepared and the mixture separated by column chromatography.

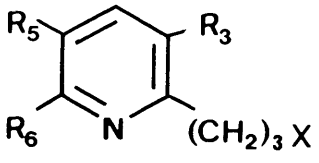
4.4.3. Amino substituted analogues

Reduction of aromatic nitro groups has been extensively reviewed in the literature[113]. Catalytic reduction using palladium on carbon

in hydrogen was found to be the method of choice. The nitropyridines (69a-b) were reduced using 10% palladium on carbon under hydrogen in ethanol at room temperature to give the corresponding aminopyridines (76a-b) shown in Table 4.4. The reductions were exothermic requiring cooling on a scale >2 g and yields were essentially quantitative, providing the solution temperature was not allowed to exceed 25°C during the reduction.

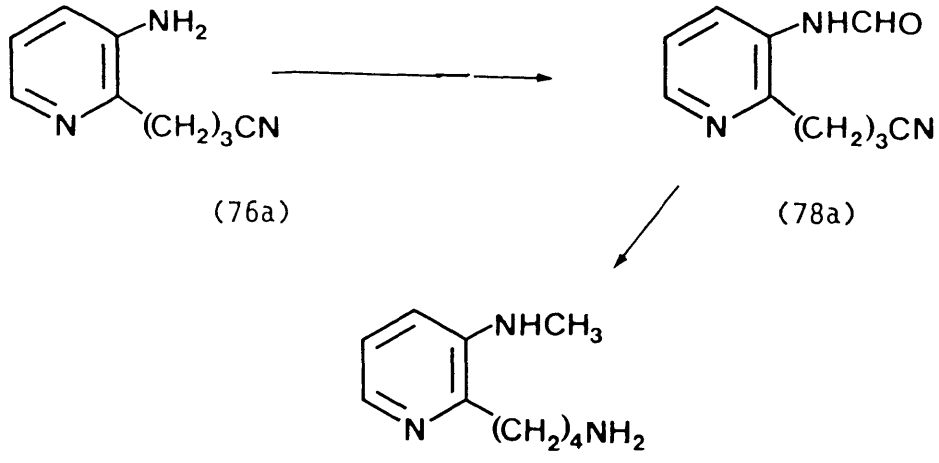
The butylamines (77a-c) were prepared by reduction of the butyronitriles (76a-c) with lithium aluminum hydride and the yields were >90%.

Table 4.4 Preparation of amino substituted pyridylbutylamines



Compound number	X	R_3	R_5	R_6
76a	CN	NH_2	H	H
76b	CN	H	NH_2	H
76c	CN	CH_3	NH_2	H
76d	CN	NH_2	H	CH_3
77a	CH_2NH_2	NH_2	H	H
77b	CH_2NH_2	H	NH_2	H
77c	CH_2NH_2	CH_3	NH_2	H
77d	CH_2NH_2	NH_2	H	CH_3

4.4.4. Alkylamino substituted analogues

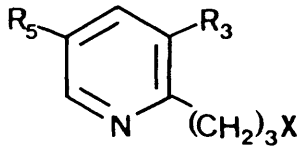


(76a) (78a) (79a)

Scheme 4.4

The methylamino analogue was prepared by treatment of the amine (76a) with ethyl formate to give the formamide (78a), reduction of which with lithium aluminum hydride gave the amine (79a) as shown in Scheme 4.4.

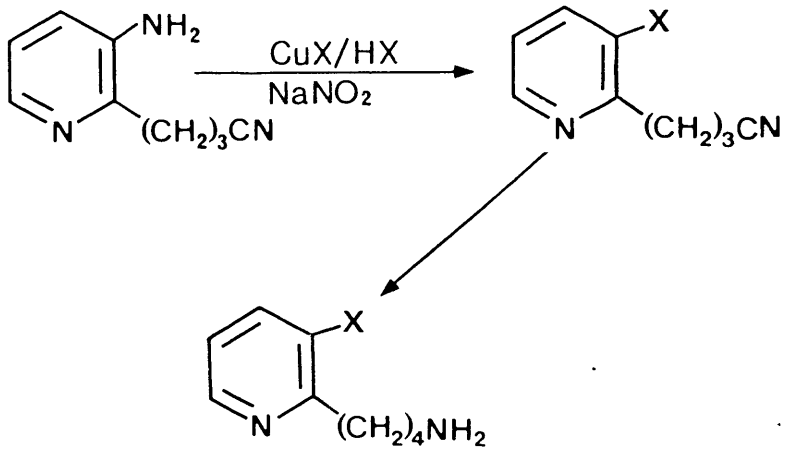
Table 4.5 Dimethylaminopyridylbutylamines



Compound number	X	R_3	R_5
80a	CN	$N(CH_3)_2$	H
80b	CN	H	$N(CH_3)_2$
81a	CH_2NH_2	$N(CH_3)_2$	H
81b	CH_2NH_2	H	$N(CH_3)_2$

N,N-Dimethyl tertiary amines may be prepared from primary amines by reductive alkylation.[114, 115] The two dimethylaminopyridines (80a) and (80b) were prepared by hydrogenating over palladium on carbon, a solution of amines (76a) and (76b) in aqueous formaldehyde. Reduction of (80a) and (80b) with lithium aluminum hydride gave the amines (81a) and (81b) respectively (Table 4.5).

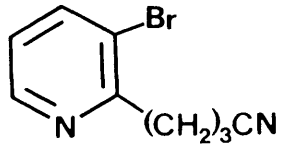
4.4.5. Chloro and bromo substituted analogues



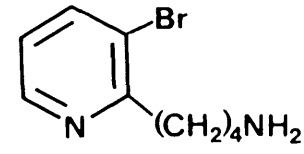
Scheme 4.5

It was initially intended to prepare the chloro and bromo analogues by the route shown in Scheme 4.5. 3-Halopyridines may be prepared from the corresponding 3-aminopyridines under Sandmeyer or Gattermann reaction conditions[116, 117, 118] although yields are usually low. Talik et al[119] have reported an improved method where the diazotisation and halogenation reactions are carried out in one operation. The method involves the treatment of the aminopyridine in the halogen acid and the corresponding copper (I) halide with sodium nitrite. They quote yields of 74-95% for the preparation of 3-chloropyridine, 3-bromopyridine and the corresponding 2,6-lutidines.

Diazotisation of the aminopyridine (76a) using the Talik conditions gave the required bromopyridine (82a) in 40% yield. Unfortunately reduction of (82a) with lithium aluminum hydride resulted in a 3:2 mixture of the required bromopyridine (83a) and the corresponding desbromo analogue (84).

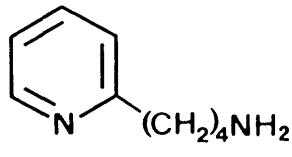


(82a)

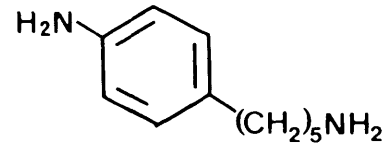


(83a)

One alternative was to try a different reducing agent such as diborane. A second approach would be to reduce the nitrile and protect the aliphatic amine. The selective deamination of an aromatic primary amino in the presence of an aliphatic primary amine has been reported for the substituted aniline (85).[120]



(84)



(85)

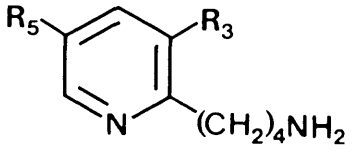
Diazotisation of the benzylamine (85) in hypophosphorous acid leads to selective deamination of the aromatic amino substituent the aliphatic amine being protected as its amine salt. This reaction relies on the large difference in pKa's between the two amines (aniline, pKa 4.6,[121] butylamine, pKa = 10.54,[121] 3-aminopyridine, pKa = -1.5,[122] for protonation of NH_2). It can be seen that from comparison of the pKa's of aniline and 3-aminopyridine with that of butylamine that the pKa separation is even greater in the pyridine case. It was therefore considered that it would be

possible to protect the aliphatic amine as its amine salt during the diazotisation/halogenation of the aminopyridine.

Treatment of the aminopyridine (77a) in hydrobromic acid (8 molar) containing cuprous bromide and copper powder with sodium nitrite gave the bromopyridine (83a) in 90% yield.

The required chloro and bromopyridines were prepared, using this route. The results obtained are shown in Table 4.6. However, some difficulty was found in isolating the products from the reaction mixture. Initially the copper was precipitated as the sulphide, formed by the addition of sodium sulphide to the reaction mixture. This could be removed by filtration and the amine extracted into chloroform from the basified reaction mixture. Filtration was difficult on the larger scale and it was felt that some material was lost in the process. A second method used was to basify the reaction mixture with ammonia; the product was then extracted into chloroform leaving the copper in the aqueous layer as the cuproammonium salt.

Table 4.6 Preparation of chloro- and bromo-pyridylbutylamines



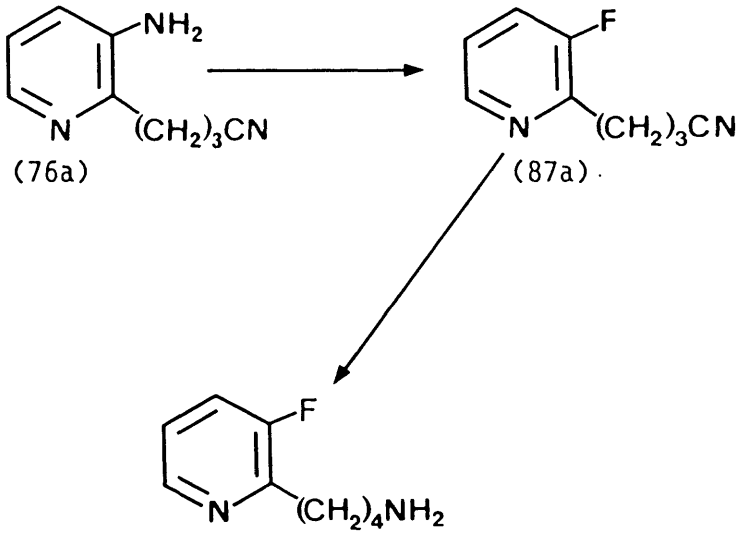
Starting material			Product			Yield
Number	R_3	R_5	Number	R_3	R_5	%
77a	NH_2	H	83a	Br	H	90
77b	H	NH_2	83b	H	Br	72
77a	NH_2	H	86a	Cl	H	42
77b	H	NH_2	86b	H	Cl	75
77c	CH_3	NH_2	83c	CH_3	Br	70

The analogue (83c) was selected for development and the possibility of exclusion of copper from the reaction mixture was investigated as the use of copper presented an effluent problem. However, this led to the production of low yields of an impure product. The product from the reactions where copper had been excluded showed strong absorption in the IR at 2120 cm^{-1}. This will be discussed further in Section 4.4.7.

4.4.6. Fluoro substituted analogues

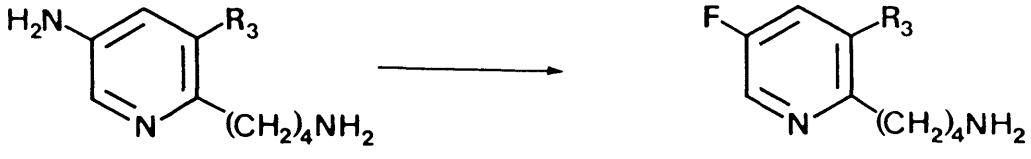
Binz and Rath[123] prepared 3-fluoropyridine in 22% yield by diazotisation of 3-aminopyridine in hydrofluoric acid. Roe and Hawkins[124] reported that 3-fluoropyridine could be prepared in 50% using a modified Schiemann reaction. They treated 3-aminopyridine in ethanol containing fluoroboric acid with ethyl nitrite and obtained the pyridine diazonium fluoroborate salt which was decomposed in petroleum ether to give 3-fluoropyridine. This method was used by Talik and Brekiesz[125] to prepare 3-fluoro-2-picoline from 3-amino-2-picoline in 54% yield. They did not isolate the diazonium salt but allowed it to decompose in the reaction solvent. Belas and Suschitzky[126] prepared 3-fluoro-2,6-lutidine from 3-amino-2,6-lutidine by diazotisation using sodium nitrite in fluoroboric acid to give the diazonium fluoroborate salt; they decomposed this to give the fluorolutidine though they did not quote a yield.

The route shown in Scheme 4.6 was chosen for the preparation of the 3-fluoro analogue (88a). The aminopyridine (76a) was diazotised with ethyl nitrite in fluoroboric acid and ethanol under the conditions quoted by Talik[125]. A steady decomposition of the diazonium salt was observed even at -10°C and no attempt was made to isolate it. The product obtained was shown by GLC and NMR to be a mixture containing the required 3-fluoro analogue (87a), contaminated with the 3-ethoxy analogue and the 3-hydroxy analogue.



Scheme 4.6

The reaction was repeated using fluoroboric acid as solvent and sodium nitrite as the diazotising agent. Again, no attempt was made to isolate the diazonium fluoroborate salt as a steady decomposition was observed even at -5°C. The diazonium salt was allowed to decompose in solution and the required fluoropyridine (87a) was isolated in 59% yield, reduction of which with lithium aluminum hydride gave the butylamine (88a) in 89% yield.

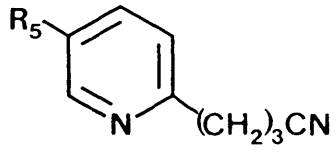


(77b) R_3 = H
(77c) R_3 = CH_3

(88b) R_3 = H
(88c) R_3 = CH_3

The diazotisation of the ring amino group in (77b) and subsequent displacement of the diazonium substituent to give the chloro and bromo analogues leaving the butylamine side chain unaffected has previously been described (Section 4.4.5.). The butylamine chain was protected as its amine salt. This approach should also be possible using fluoroboric acid since this is a strong acid (pKa -4.9).[127] The aminopyridine (77c) was dissolved in fluoroboric acid and treated with sodium nitrite using the conditions described for the preparation of the 3-fluoro analogue (88a). A dark brown viscous oil was obtained which showed a number of impurities on TLC.

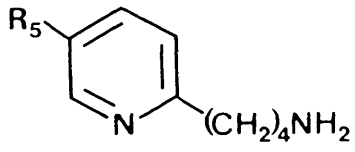
The diazonium substituent in the 5-position does not have a bulky 'ortho' substituent which is present in the 3-substituted analogue. The contrast in reactivity of the two isomeric diazonium substituents had already been seen when attempts were made to displace the diazonium group to give the hydroxy analogues (Section 4.4.9.). The analogue (76b) had already been diazotised to give the diazonium fluoroborate salt (95b) which had then been hydrolysed to give the hydroxypyridine (93b), (Section 4.4.9.).



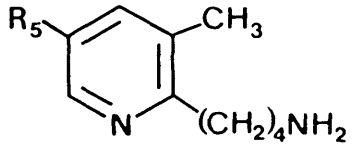
(76b) $R_5 = NH_2$
(87b) $R_5 = F$
(95b) $R_5 = N_2^+BF_4^-$

One possibility was to re-prepare the pyridinediazonium salt (95b) which may then undergo thermal decomposition to give the fluoropyridine (87b). It would then have been necessary to reduce the nitrile to give the required butylamine. The alternative was to isolate the pyridine diazonium salt (98b) which may then undergo thermal decomposition to give the fluoropyridine (88b).

Treatment of the aminopyridines' (77b and 77c) in ethanolic fluoroboric acid with amyl nitrite gave the required diazonium compounds as their fluoroborate salts, which were then decomposed in petroleum ether at 95-100°C to give the fluoropyridine (88b and 88c) in greater than 60% yield.



(77b) $R_5 = NH_2$
(88b) $R_5 = F$
(98b) $R_5 = N_2^+BF_4^-$



(77c) $R_5 = NH_2$
(88c) $R_5 = F$
(98c) $R_5 = N_2^+BF_4^-$

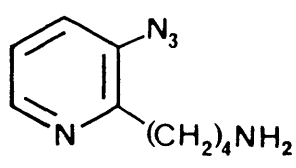
4.4.7. Iodo substituted analogues



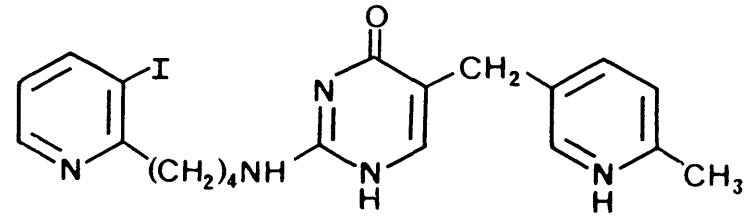
(77a) (89a)

General texts on organic chemistry state that whereas the Sandmeyer reaction for the introduction of Br or Cl requires the presence of the cuprous salt the iodides may be prepared by the treatment of the diazonium salt with iodide ion. The diazotisation and displacement can be carried out as a one or two pot procedure.

Treatment of the amino pyridine (77a) in dilute sulphuric acid containing potassium iodide with sodium nitrite yielded the iodopyridine (89a) which TLC indicated to be one component. However it was contaminated with an impurity which was suspected to be the azido-analogue (90a) because of an IR band 2120 cm^{-1}. Initial preparation of the diazonium salt at -10°C followed by the treatment with potassium iodide gave a similarly contaminated product.



(90a)



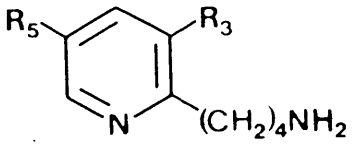
(183)

The isocytosine analogue (183) was prepared and attempts to purify the material by crystallization and chromatography resulted in a product still contaminated with an azide. It was thought that the azido group may arise from reduction of the diazonium intermediate to give a hydrazino compound which could then be converted into the azide by diazotisation. It was observed that iodine was generated during the reaction.

It was noted that although no azido by-product was observed in the preparation of the chloro and bromo analogues, (Section 4.4.5.), the experiments where copper was excluded from the reaction gave products which showed strong absorption at about 2120 cm^{-1}.

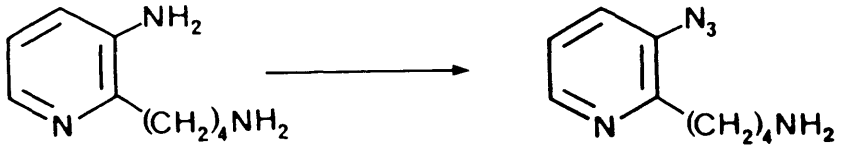
The inclusion of cuprous iodide into the reaction mixture resulted in the formation of the required iodopyridines which were not contaminated with azide impurities. The pyridine diazonium salt was first prepared in dilute sulphuric acid at about -5°C. This solution was then run into a solution of sodium iodide and cuprous iodide, stirred at 10°C. Table 4.7 shows the yields obtained for the iodopyridines prepared.

Table 4.7 Iodopyridylbutylamines



Starting material Number	R_3	R_5	Product Number	R_3	R_5	Yield %
77a	NH_2	H	89a	I	H	54%
77b	H	NH_2	89b	H	I	72%
77c	CH_3	NH_2	89c	CH_3	I	69%

4.4.8. Azido substituted analogues



(77a) (90a)

The azidopyridine (90a) was obtained on diazotisation of (77a) in dilute sulphuric acid and treating the resulting solution with a solution of sodium azide containing sodium acetate. Both the amine (90a) and its isocytosine derivative had the same Rf values on TLC as the corresponding iodo analogues, but they could be resolved using HPLC.

4.4.9. Oxy substituted analogues

4.4.9.1. Alkoxy substituted analogues



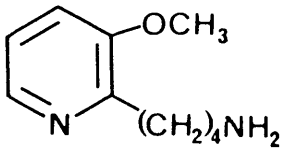
(76a) (91a)

Aromatic amines may be converted into the corresponding alkoxy analogues by diazotisation. The diazotisation is usually carried out using the respective alcohol as a solvent.[128] The methoxypyridine (91a) could be prepared in about 50% yield from the aminopyridine (76a). The product was always found to be contaminated with the deaminated product (92) which could only be removed by lengthy and tedious chromatography. Deamination has been reported to be a common problem with this method.[128]

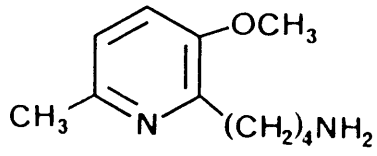


(92) (93a)

The hydroxypyridine (93a) was prepared by diazotisation of the amine (76a) in dilute sulphuric acid. Alkylation of hydroxy pyridines can be a problem as both N- and O-alkylation can occur. Selective alkylation on oxygen has been reported using dimethyl sulphoxide as the solvent.[129] Treatment of the hydroxypyridine (93a) in dimethyl sulphoxide with sodium hydride followed by alkylation with methyl iodide gave the methoxypyridine (91a). The overall yield for the two stages being similar to the previous route.

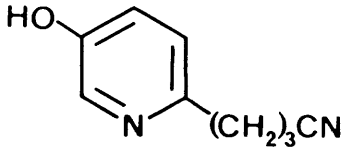


(94a)

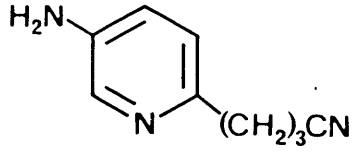


(94d)

Reduction of (91a) with lithium aluminium hydride gave the amine (94a). The methoxypyridine (94d) was prepared in a similar manner from the aminopyridine (76d).



(93b)



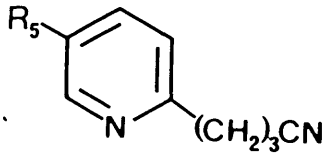
(76b)

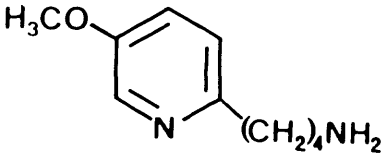
Preparation of the hydroxypyridine (93b) by diazotisation of the aminopyridine (76b) using the method described above for the 3-aminopyridine (76a) failed to give any of the required product. The reaction was carried out at various temperatures and only gave mixtures which showed more than 10 coloured components by TLC.

It is likely that the diazonium intermediate formed from (76a) is less stable due to steric interaction with the 'ortho' methylene chain. The presence of the chain 'ortho' to the diazonium substituent may also disfavour coupling reactions.

The isolation of diazonium compounds as their fluoroborate salts and subsequent oxidation to the corresponding phenol using copper salts has been reported.[130] The diazonium fluoroborate (95b) was prepared by treatment of (76b) in ethanol containing fluoroboric acid with amyl nitrite. Decomposition of (95b) with aqueous copper nitrate gave the hydroxypyridine (93b) but isolation proved very

tedious due to the large quantity of copper present. The reaction conditions quoted required the use of 76g of cuprous nitrate per 1g of starting material. A solution of (95b) in warm water was observed to undergo decomposition and no colour developed. It was found that the diazonium fluoroborate (95b) decomposed in water at 70°C to give the hydroxypyridine (93b) in almost quantitative yield.





(95b) $R_5 = N_2^+BF_4^-$
(93b) $R_5 = OH$

(94b)

The hydroxypyridine (93b) was converted to the methoxypyridine (94b) by the route described for the 3-substituted analogue.

4.4.9.2. Hydroxy substituted analogues

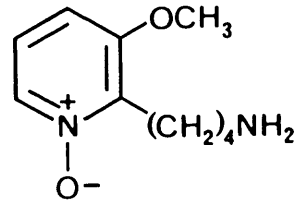


(96a) (97a)

The 3-hydroxy substituent in (93a) was protected by alkylation with benzyl bromide to give the benzyloxy analogue (96a). Selective reduction of the nitrile was accomplished using lithium aluminum hydride to give the amine (97a). This amine was converted into the corresponding cyanoguanidine and isocytosine derivatives.

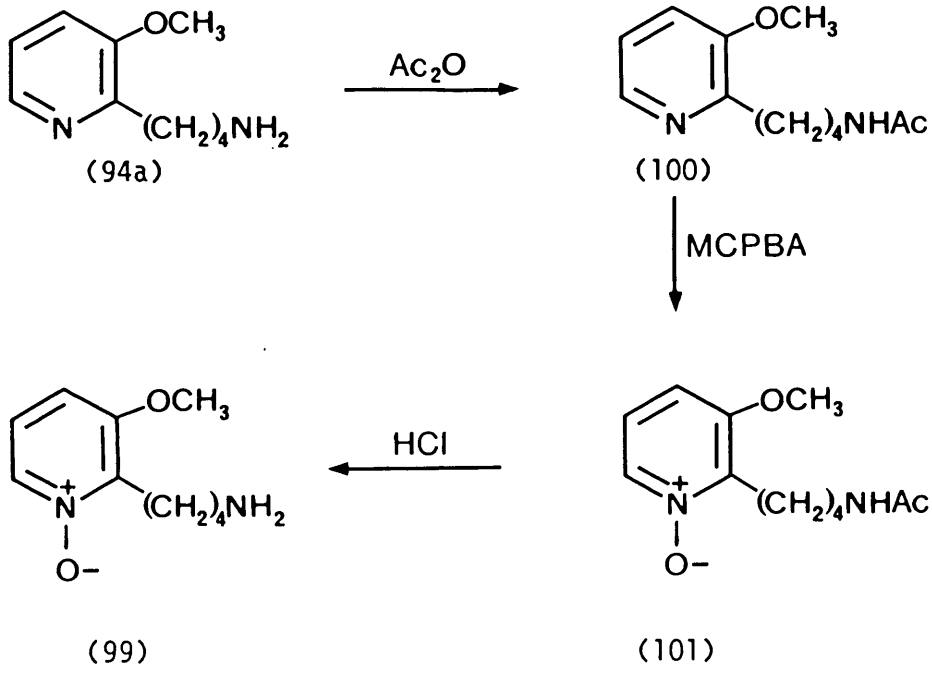
Deprotection was accomplished by hydrogenation over 10% palladium on carbon to give the corresponding hydroxypyridines in essentially quantitative yield.

4.4.9.3. N-Oxypyridylbutylamines



(99)

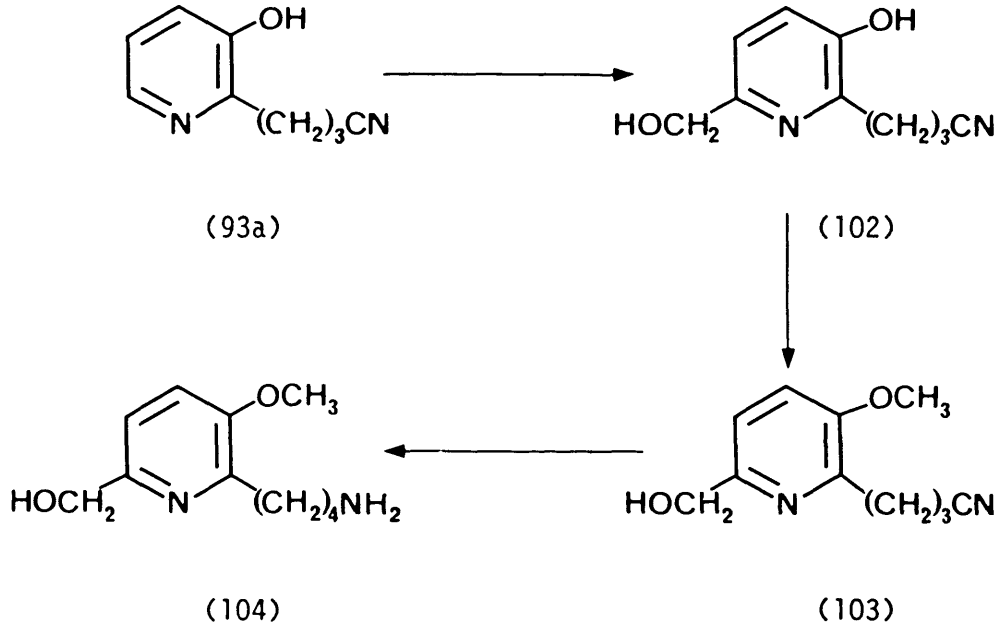
The N-oxypyridylbutylamine (99) was prepared by the route shown in Scheme 4.7. The butylamine (94a) was first protected by treatment with acetic anhydride. Oxidation with m-chloroperbenzoic acid followed by deprotection under acidic conditions gave the required amine in good yield.



Scheme 4.7

4.4.9.4. 6-Hydroxymethyl substituted analogues

3-Hydroxypyridines are reported to undergo Mannich reactions at the 2-position of the pyridine ring.[131] If the 2-position is blocked by an alkyl group, substitution occurs at the 6-position of the pyridine ring.[132] The reaction may also be accomplished with formaldehyde using sodium hydroxide as the base when a hydroxymethyl substituent is introduced into the pyridine ring.[133] The 6-hydroxymethyl analogue (102) was prepared as shown in Scheme 4.8. Alkylation of (102) under the conditions used in Section 4.4.9.1. and reduction with lithium aluminum hydride gave the amine (104).



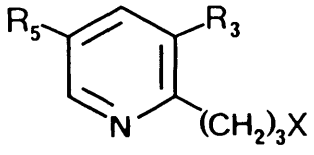
Scheme 4.8

4.4.10. Methylthio substituted analogues.

3-Methylthiopyridine is obtained on treatment of 3-aminopyridine with a mixture of amyl nitrite and dimethyl disulphide at 80° - 90°.[134] Under these conditions the methylthiopyridines (105a) and (105b) were obtained from the corresponding aminopyridines (76a) and (76b) in

yields of 32% and 70% respectively. Reduction of (105a) and (105b) gave the required amines (106a) and (106b). These structures are shown in Table 4.8.

Table 4.8 Methylthio substituted analogues

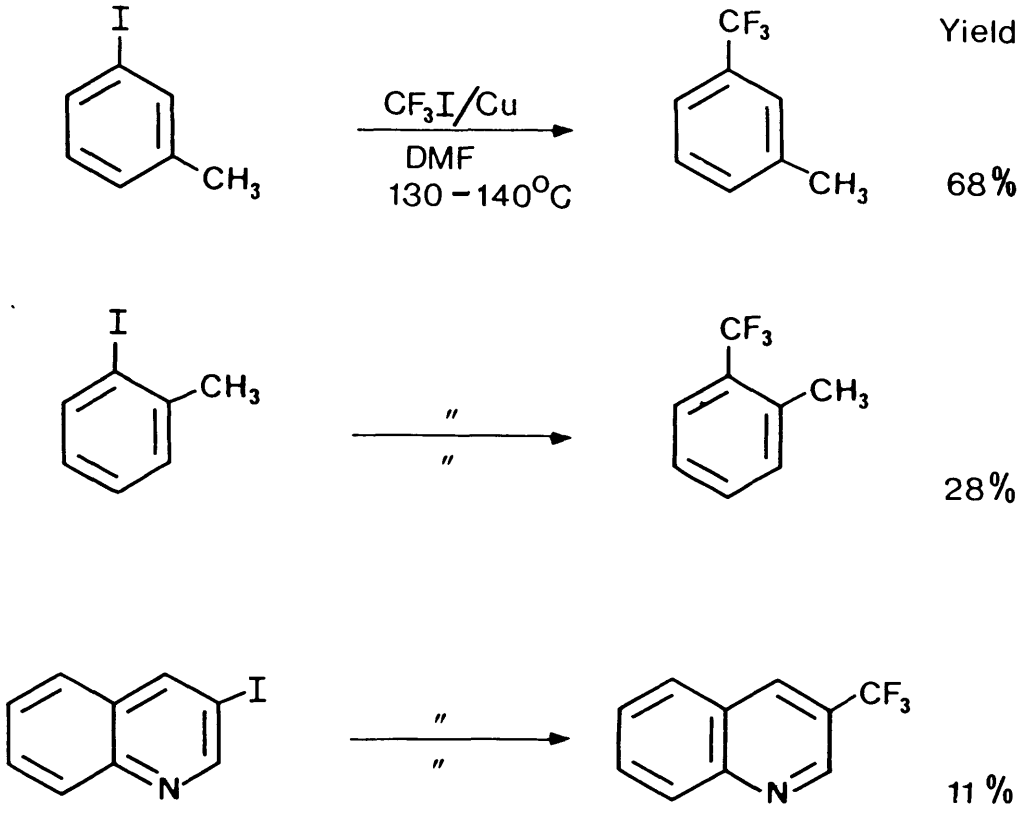


Compound number	X	R_3	R_5
105a	CN	SCH_3	H
105b	CN	H	SCH_3
106a	CH_2NH_2	SCH_3	H
106b	CH_2NH_2	H	SCH_3

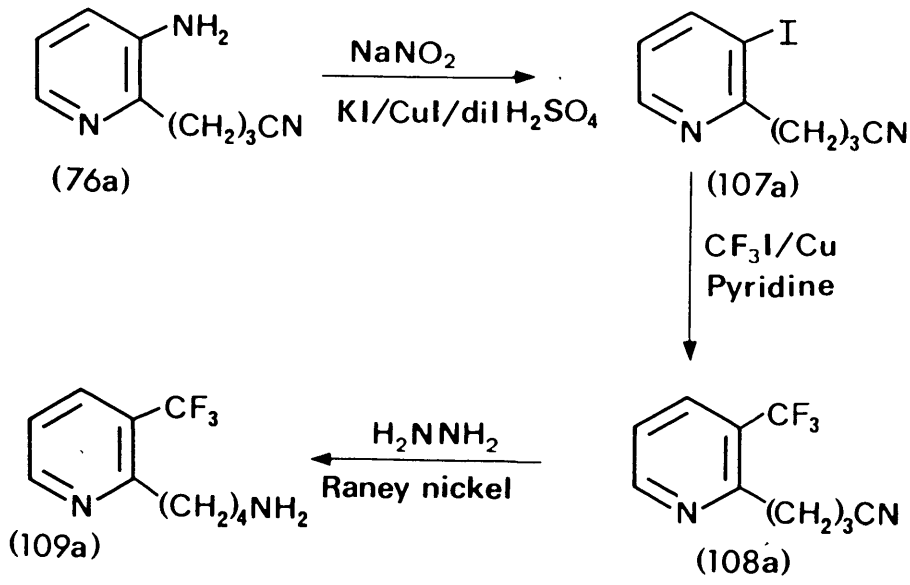
4.4.11. Trifluoromethyl substituted analogues

Aromatic iodides and bromides react with trifluoromethyl iodide at 120° - 130°C in the presence of copper as a catalyst to give the corresponding trifluoromethyl aromatic.[135] The choice of solvent is reported to be important and the following order of ease of reaction was observed: pyridine > HMPT >> DMF > acetonitrile.[136,137] The examples shown in Table 4.9 are quoted. Only poor yields are quoted for 3-iodoquinolines and o-iodotoluene. A colleague had attempted to prepare 3-trifluoromethyl-2-picoline from 3-iodo-2-picoline and had failed to obtain any of the required product.[138]

Table 4.9 Published yields for preparation of trifluoromethyl aromatics



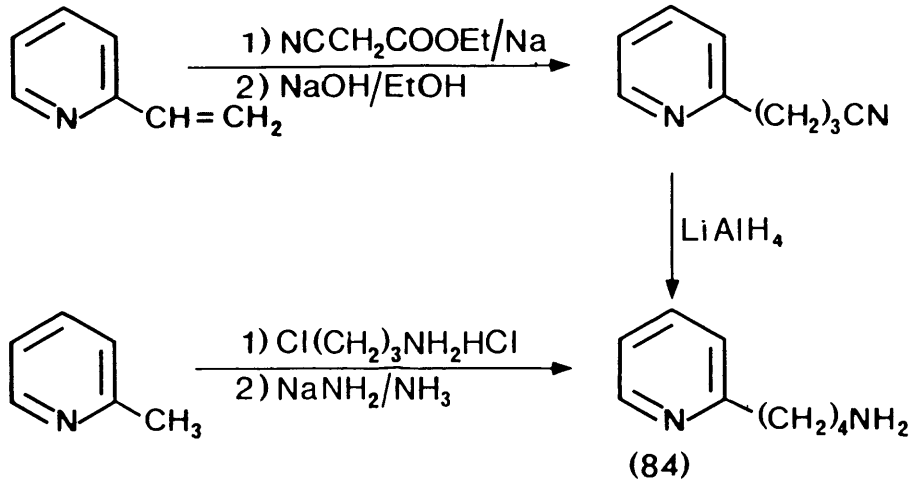
The trifluoromethyl analogue (109a) was prepared by the route shown in Scheme 4.9 The iodopyridine (107a) was first prepared from the aminopyridine (76a) by the method described in Section 4.4.7. The trifluoromethylpryidine (108a) was obtained in 66% yield on heating the iodopyridine (107a) at 140°C with trifluoromethyl iodide in the presence of copper as catalyst using pyridine as the solvent. The use of either DMF or acetonitrile failed to give any reaction. The nitrile (108a) was reduced using Raney nickel/hydrazine to give the butylamine (109a).



Scheme 4.9

4.5. ANALOGUES PREPARED BY ALKYLATION OF 2-PICOLINE

4.5.1 4-(Pyrid-2-yl)butylamine (84)

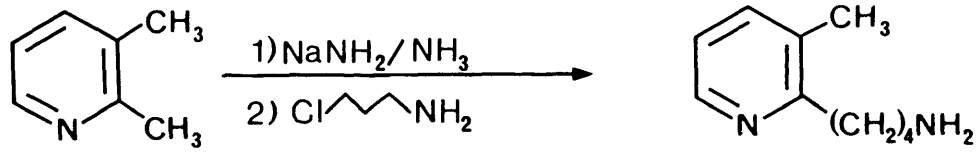


Scheme 4.10

The butylamine (84a) had previously been prepared[138] from 2-vinylpyridine as shown in Scheme 4.10. However, the sample used in this work was prepared by alkylation of 2-picoline, also shown in

Scheme 4.10. This method was developed by colleagues at SK&F.[140] The anion formed by deprotonation of the α methyl substituent using sodamide in liquid ammonia is alkylated with chloropropylamine generated *in situ* from its hydrochloride salt.

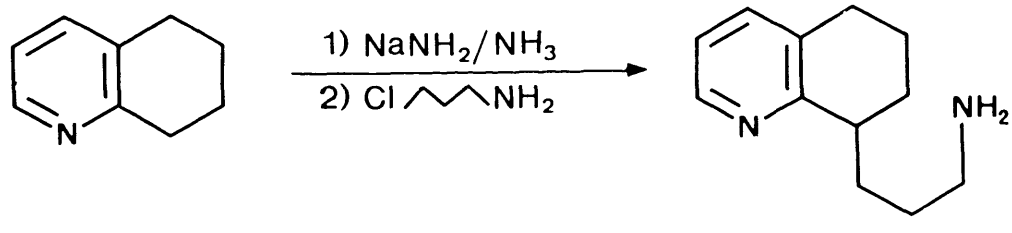
4.5.2. *3-Methyl substituted analogue*



(110) (111)

The 3-methyl analogue (111) was prepared as shown above.

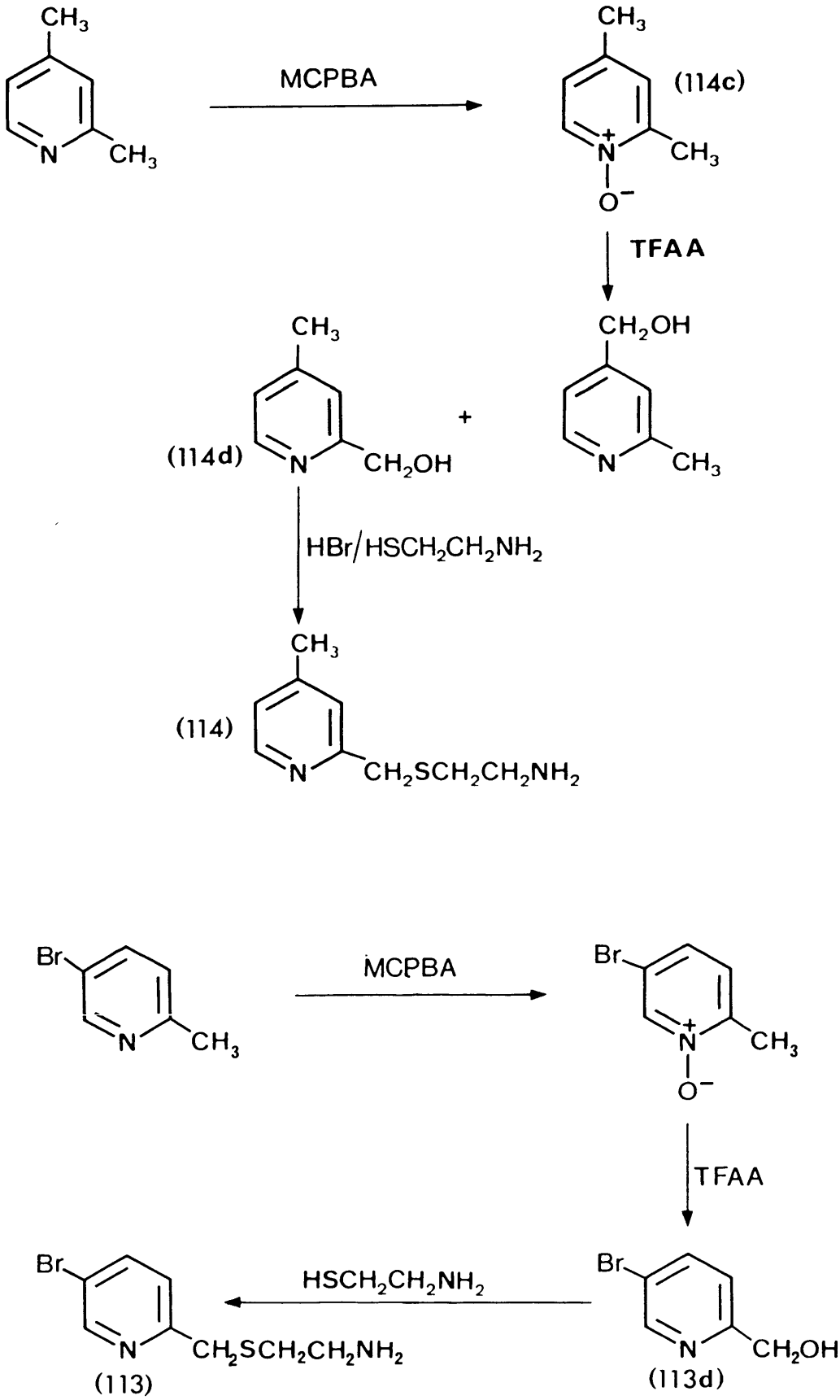
4.5.3. *Tetrahydroquinoline analogues*



(112)

The 5,6,7,8-tetrahydroquinoline (112) was prepared by the analogous route shown above. Tetrahydroquinoline is reported to undergo deprotonation in the presence of alkali metals[141] at the 8-position.

Scheme 4.11


CH3
MCPBA
(114c)
CH3
N
CH3
O−
TFAA
CH2OH
CH3
(114d)
N
CH2OH
+
N
CH3
HBr/HSCH2CH2NH2
CH3
(114)
N
CH2SCH2CH2NH2
Br
MCPBA
Br
N
CH3
N
CH3
O−
TFAA
Br
HSCH2CH2NH2
Br
N
CH2SCH2CH2NH2
(113)
N
CH2OH
(113d)

4.6. MISCELLANEOUS AMINES

4.6.1. Pyrid-2-yl-methylthioethylamines

The 5-bromo and 4-methyl analogues were prepared as shown in Scheme 4.11. 2-Picoline-N-oxides are reported to rearrange to give the isomeric hydroxypyridines with either acetic anhydride[142] or trifluoroacetic anhydride.[143]

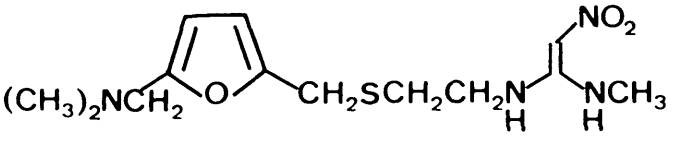
5-Bromolutidine was converted into 5-bromo-2-hydroxymethylpyridine but either reagent gave an isomeric mixture of the 2- and 4-hydroxymethyl compounds with 2,4-lutidine. These isomers were separated by column chromatography. However, the 2-hydroxymethyl compound could be prepared by the alternative radical hydroxymethylation discussed in Section 6. Reaction of cysteamine hydrochloride in hydrobromic acid with the hydroxymethyl pyridines gave the corresponding amines (113) and (114).

5.0 MANNICH PYRIDYL HISTAMINE H_2-RECEPTOR ANTAGONISTS

5.1. INTRODUCTION

In this chapter the effect of introduction of a dialkylaminoalkyl substituent into 2-pyridyl histamine H_2-receptor antagonists will be discussed.

The dialkylaminoalkyl furan and benzene histamine H_2-receptor antagonists were reviewed in Chapter 1. Ranitidine (15) was the first published Mannich furan histamine H_2-receptor antagonist.



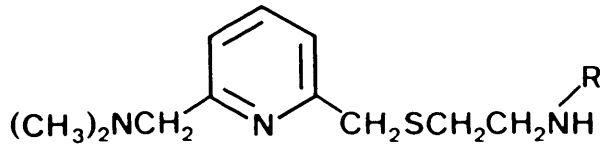
(15)

All the possible isomeric substitutions of the furan ring had been examined. It had been shown that a 2,5-disubstituted furan gave optimum potency at the histamine H_2-receptor.[66] The furan ring may also be replaced by benzene and a 1,3-substitution gave optimum potency in these analogues.

The pyridyl histamine H_2-receptor antagonists were discussed in Chapter 2. We wished to explore the possibility of introducing a dimethylaminomethyl substituent into the pyridine ring in order to increase antagonist potency at the histamine H_2-receptor.

5.2. SUBSTITUTION AT THE 6-POSITION OF THE PYRIDINE RING

Comparison of the furan and benzene analogues may suggest that the analogous substitution in the pyridine series would be that shown in



(115)

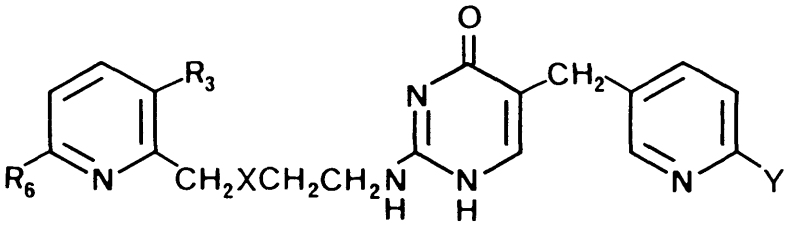
structure (115). This structure combines the 1,3 arrangement found to be optimum for the benzene and furan analogues, also both the Mannich group and the side chain occupy positions 'ortho' to the ring hetero atom as found in ranitidine. This substitution pattern had been described in the patent literature.[68,69,70] However, the influence of substitution at the 6-position of the pyridine ring had been shown to reduce activity at the histamine H_2-receptor, although only non-basic substituents had been investigated, (Section 3.4.). Table 5.1 shows a small series of 6-Mannich-pyridyl analogues which were prepared in order to examine which of the following possibilities held:-

- The 6-Mannich pyridyl substituent behaved as previous 6-pyridyl substituents, lowering histamine H_2-receptor antagonist activity, as described in Section 3.4.

- The 6-Mannich pyridyl substituent behaved in the same way as a Mannich substituent introduced into the furan ring giving increased potency at the histamine H_2-receptor.

The isocytosine derivatives containing the 6-Mannich pyridyl side chain are compared in Table 5.1. Also shown are the corresponding des-Mannich analogues. The 3-methoxypyridyl analogues (116 & 117) were the most potent histamine H_2-receptor antagonist in this series. The 6-methyl analogue (118) had already been prepared and shown to reduce antagonist potency at the histamine H_2-receptor. The same reduction in potency was seen with the 6-Mannich analogue (119). Activity at the histamine H_1-receptor was also reduced by substitution of a Mannich group into the 6-pyridyl positions.

Table 5.1 Effect of introducing a Mannich group at the 6 position of the pyridine ring on the potency at the H_1- and H_2-receptor in the isocytosines



No.	R_3	R_6	X	Y	pA_2 atrium (slope)	Rat G.S. ED_{50} µmole/kg	pA_2 ileum (slope)
116	OMe	H	CH_2	H	7.37 (0.87)	0.23	7.84 (1.05)
118	OMe	Me	CH_2	H	5.63 (0.95)	4.9	7.67 (0.94)
117	OMe	H	CH_2	Me	7.49 (0.89)	0.21	7.77 (1.05)
119	OMe	CH_2NMe_2	CH_2	Me	5.88 (0.72)	9.3	5.1 (1.14)
120	H	H	S	Me	7.08 (0.93)	0.22	5.81 (1.22)
121	H	CH_2NMe_2	S	Me	7.28 (1.0)	0.25	- -

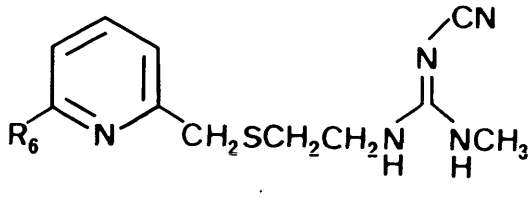
The reduction in potency at the histamine H_2-receptor was the postulated result. However, the possibility exists that the beneficial effect of a 3-methoxy substituent and a 6-dimethylamino-methyl substituent could not be demonstrated in the same molecule. It may be that these two substituents somehow act in a non-cooperative

manner. The 6-Mannich analogue (121) without a 3-methoxy group was prepared and this is of similar potency to the parent (120). In this series substitution of the Mannich group into the 6-position of the pyridine ring has no effect on histamine H_2-receptor antagonist potency.

The same comparison was carried out in the cyanoguanidine series, and these are compared in Table 5.2. In this case the introduction of the Mannich group reduces <u>in vitro</u> potency, although it should be noted that slopes of the Schild plot for these analogues, although similar, are significantly different from unity. The <u>in vivo</u> potency for the two compounds are similar.

The results from both the cyanoguanidine and isocytosine series indicate that substitution of a Mannich group into the 6-pyridyl position in the pyridyl histamine H_2-receptor antagonists either reduces or has no effect on potency at the histamine H_2-receptor.

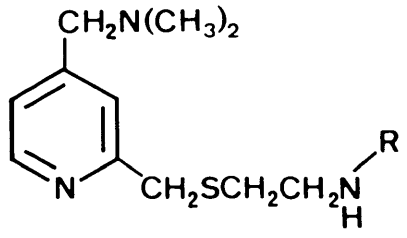
Table 5.2 <u>Effect of substituting a Mannich group into the 6-position of the pyridine ring in the pyridylcyanoguanidines</u>



Compound number	R_6	pA_2 atrium (slope)	Rat G.S. ED_{50} µmole/kg
37	H	6.03 (0.63)	9.15
122	CH_2NMe_2	5.0 (0.58)	10.2

5.3. SUBSTITUTION AT THE 4-POSITION OF THE PYRIDINE RING

Structure (123) shows an alternative 1,3-disubstituted pyridyl system. The side chain is 'ortho' to the hetero atom. This position of substitution for the side chain gave the most potent pyridyl histamine H_2-receptor antagonists, (Section 1.4.6.1.).

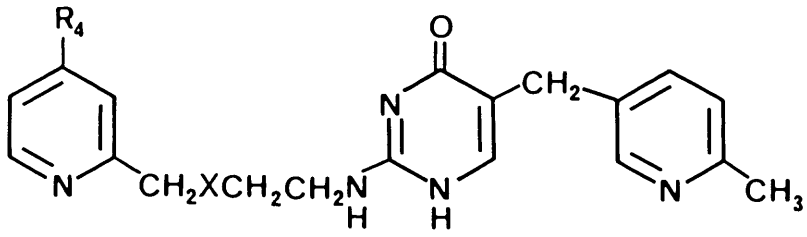


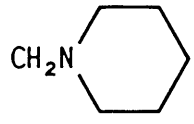
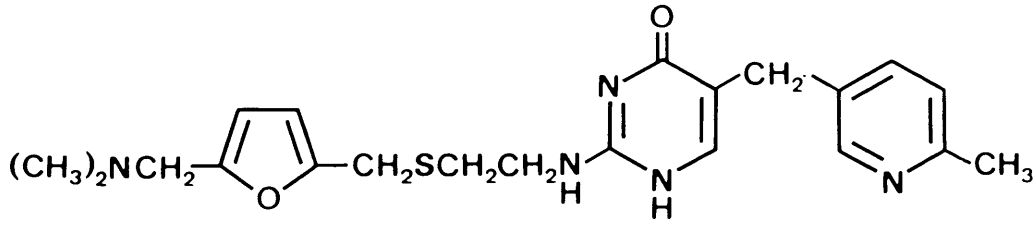
(123)

Introduction of a methyl group into the 4-position of the pyridine ring had been investigated, (Section 3.2.) and was shown to have very little effect on potency at the histamine H_2-receptor. This was in contrast to substitution of a methyl group into the 6-pyridyl position which reduced potency at the histamine H_2-receptor. This result indicated that substitution at the 4-position of the pyridine ring may be tolerated. Introduction of a Mannich substituent into the 4-pyridyl position was therefore investigated. The isocytosine derivatives with this substitution are shown in Table 5.3.

Both the tetramethylene and methylthioethyl chain analogues were prepared. In this series introduction of the Mannich group at the 4-position of the pyridine ring results in increased potency at the histamine H_2-receptor when compared with the corresponding unsubstituted analogue. The 4-Mannich pyridyl analogues have similar *in vitro* potency to the furan (129). The piperidinomethyl substituent had been found to be an alternative Mannich substituent and was therefore investigated. This substituent was also found to have a beneficial influence on potency at the histamine H_2-receptor when introduced into the 4-position of the pyridine ring.

Table 5.3 Comparison of the 4-Mannich pyridyl and Mannich furyl isocytosines

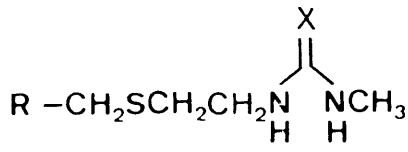


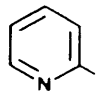
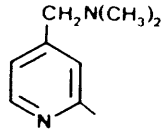
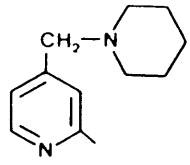
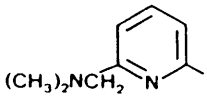
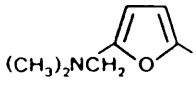
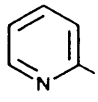
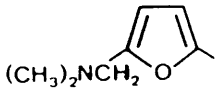
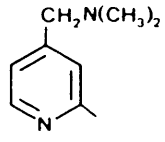
Compound number	R_4	X	pA_2 atrium (slope)	Rat G.S. ED_{50} µmole/kg	pA_2 ileum (slope)
120	H	S	7.08 (0.93)	0.22	5.81 (1.22)
125	CH_2NMe_2	S	7.92 (0.63)	0.064	wide scatter no regression
126	H	CH_2	6.48 (1.37)	0.40	7.28 (0.91)
127	CH_2NMe_2	CH_2	7.76 (0.95)	0.045	5.32 (1.28)
128		S	7.25 (1.16)	0.172	-
129			7.78 (0.88)	0.14	4.24 (1.50)

Although increases in potency can be seen for the 4-Mannich pyridyl analogues, this effect is not great due to the high intrinsic potency of the parent compound (120). A further comparison was therefore carried out in the cyanoguanidine series and these analogues are compared in Table 5.4. Again the beneficial effect of introducing a Mannich group into the 4-position of the pyridine ring is seen. However, in this series the difference in potency between the two isomeric Mannich pyridyl analogues is striking. The 4-substituted analogue (130) is two orders of magnitude more potent than the 6-substituted analogue (122). There is no advantage gained on introducing a dimethylaminomethyl substituent into the 6-position of the pyridine ring. In contrast, a definite advantage can be seen on introducing a dimethylaminomethyl substituent into the 4-position of the pyridine ring. The resulting 4-dimethylaminomethyl analogue is slightly more potent than the furan compound (133).

The histamine H_2-receptor antagonist ranitidine (15) has the diaminonitroethylene end group. The increase in potency on replacing furan by pyridine in the cyanoguanidine series prompted further comparison to be made in the diaminonitroethylene series. The results are also shown in Table 5.4. Here too, a beneficial effect of introducing a 4-Mannich group into the pyridine ring is seen. Furthermore, comparison with ranitidine (15) indicates that the 4-Mannich pyridyl analogue (136) is a more potent compound <u>in vivo.</u>

Table 5.4 Comparison of the Mannich pyridyl and furyl cyanoguanidines



Compound number	R	X	pA_2 atrium (slope)	Rat G.S. ED_{50} µmole/kg
37		NCN	6.03 (0.63)	9.15
130		NCN	7.79 (0.80)	0.081
131		NCN	7.55 (0.74)	0.75
122		NCN	5.00 (0.58)	10.2
133		NCN	6.81 (0.50)	1.77
134		$CHNO_2$	5.65 (0.62)	3.12
15		$CHNO_2$	7.05 (0.70)	0.57
136		$CHNO_2$	7.22 (0.95)	0.044

5.4. STRUCTURE ACTIVITY RELATIONSHIPS IN THE MANNICH DERIVATIVES

In a recent publication all the possible isomers of the 'Mannich' furans have been compared.[66] The authors quoted the 2,5-isomer as being the most potent by a factor of four. The thiophene analogues have also been described and in this case the authors quote the 2,4-isomer as being 9 times more active than the 2,5-isomer. This was reviewed in Section 1.4.6.3.

A comparison of the geometries of the Mannich pyridine and Mannich furan rings are shown in Figure 5.1. The ring bond angles and lengths are taken from the molecular structure literature.[144] The bond angle to the side chain and Mannich group are taken as those quoted for hydrogen on the ring. The dimensions for the Mannich group are those found in trimethylamine. The same dimensions for the Mannich group are used in each structure. Figure 5.1 shows the heavy atom skeleton for the 2,5-furan and the 2,4-substituted pyridine analogues The structures have been drawn with the bond joining the ring to the side chain superimposed. The Mannich group has been drawn in a conformation where the nitrogen and the linking carbon are in the same plane as the aromatic ring and the 2-methyl groups are above and below the plane of the ring respectively. It is interesting to note that the nitrogen of the Mannich groups are almost superimposed. Also shown are the approximate positions that the nitrogen of the Mannich group would occupy for the alternative isomers and ring systems, as detailed in the Figure 5.1.

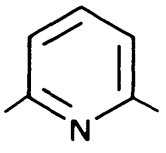
The position of the nitrogens can be defined by an arc. If this arc is measured from the vector of the bond between the ring and the side chain, then all the nitrogen positions are in an arc of about 110° - 150° from this bond.

The 2,6-pyridyl isomer gives the most acute angle while the 2,4-furan and the 2,5-thiophene give the most obtuse angles. The Mannich nitrogen of the most active isomer for each system occupies a very similar position in space.

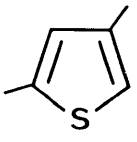
Figure 5.1 Comparison of geometries of some Mannich heterocycles

N_A - N_B indicate the positions the exocyclic nitrogen would occupy for the Mannich substituted heterocycles A - D.

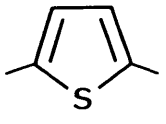
A =



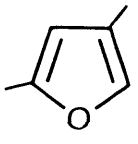
B =

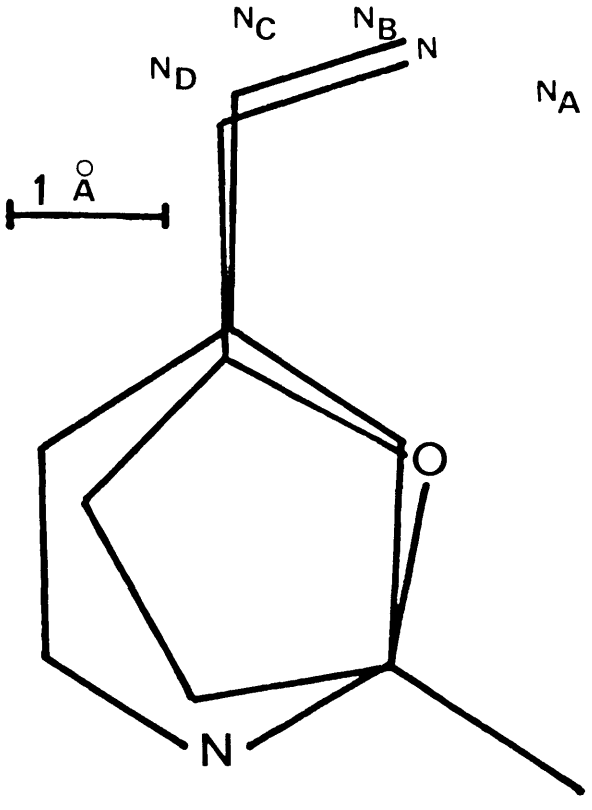


C =

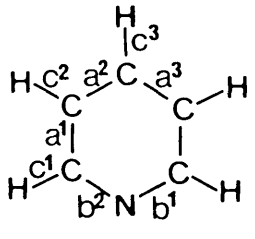


D =



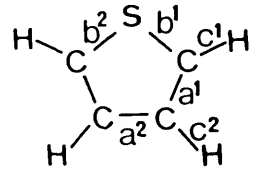


Bond lengths (Ä) and bond angles taken from reference 144



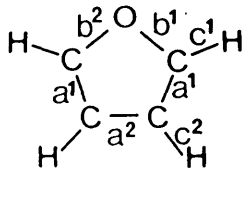
Pyridine

a^1 = 1.39	$\angle a^1a^2$ = 119°
a^2 = 1.39	$\angle a^2a^2$ = 118°
b = 1.34	$\angle bb$ = 117°
c^1 = 1.08	$\angle ba^1$ = 124°
c^2 = 1.08	$\angle bc^1$ = 116°
c^3 = 1.08	$\angle c^2a^2$ = 121°



Thiophen

a^1 = 1.35	$\angle b^1b^2$ = 91°
a^2 = 1.45	$\angle b^1a^1$ = 113°
b = 1.72	$\angle a^1a^2$ = 112°
c^1 = 1.08	$\angle b^1c^1$ = 119°
c^2 = 1.07	$\angle a^2c^2$ = 123°



Furan

c = 1.07	$\angle a^1a^2$ = 106°
a^2 = 1.44	$\angle bb$ = 106°
a^1 = 1.35	$\angle bc^1$ = 115°
b = 1.37	$\angle a^2c^2$ = 128°

It may therefore be that one of the requirements of the aromatic ring is to provide the correct relative disposition of the Mannich group with respect to the side chain. If this is the case, it might be expected that the two possible isomers with a 2,4-substituted pyridine ring i.e. the isomers of structure (137) and (127), as shown in Table 5.5, would be of similar potency. Although it is difficult to draw conclusions in the isocytosine case due to the high affinity of the isocytosine end group, it can be noted that the two Mannich pyridyl analogues (127 & 137) are of similar potency and are an order of magnitude more potent than the unsubstituted pyridyl analogue (126).

During the development of ranitidine it had been postulated that a basic heterocyclic nitrogen was not required providing it was replaced by a non basic ring bearing a basic substituent.[66] This implies that either of the basic centres may be fulfilling the same role at the receptor.

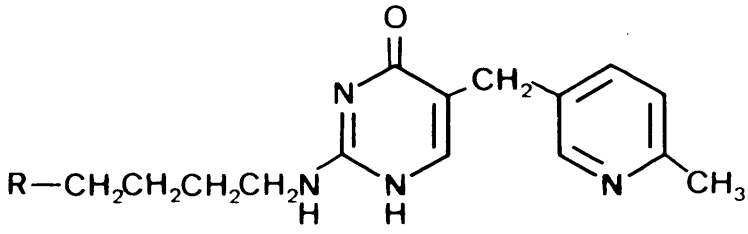
However, the 4-Mannich pyridyl analogues presented in this chapter have tended to show increased potency at the histamine H_2-receptor when compared with the corresponding furan. It is therefore possible that the ring nitrogen and the exocyclic nitrogen bind to separate points on the receptor. Thus the 4-Mannich pyridyl analogues have increased affinity due to both the binding of the Mannich group and the pyridine nitrogen. Also as the geometry of the ring would appear to be critical in order for the Mannich group to achieve optimum binding, it is unlikely that the pyridine nitrogen could bind to the same site.

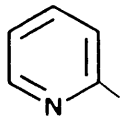
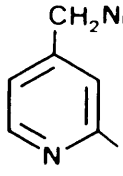
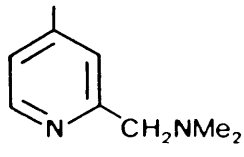
The introduction of further substituents into the furan ring was discussed in Section 1.4.6.3. The implication of the overlay of the 4-Mannich pyridine with the 2,5-furan, shown in Figure 5.1, implies that the 3-position of the furan ring may be equivalent to the 6-position or nitrogen of the pyridine ring. Substitution of a methyl into the 3-position of the furan caused a reduction in potency at the histamine H_2-receptor.[66] However, if the furan oxygen

and the pyridine nitrogen were equivalent, the 3-position on the furan would then be equivalent to the 3-position in pyridine.

In the pyridine series substitution at the 3-position was tolerated, whereas in furan it is not, thus indicating that these positions are not equivalent.

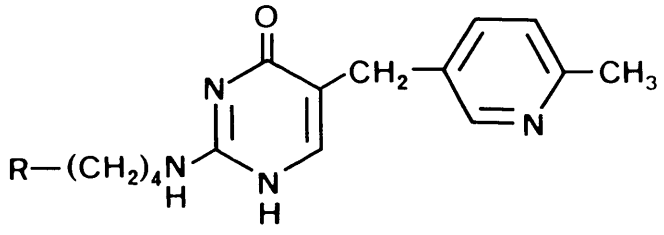
Table 5.5 Comparison of isomeric Mannich pyridyl analogues



Compound number	R	pA_2 atrium (slope)	Rat G.S. ED_{50} µmole/kg	pA_2 ileum (slope)
126		6.48 (1.37)	0.4	7.28 (0.91)
127		7.76 (0.95)	0.045	5.32 (1.28)
137		7.88 (1.01)	0.034	4.19 (1.50)

The azido analogue (138) was discussed in Section 2.4. and showed high potency at the histamine H_2-receptor. Comparison of this analogue with the 4-Mannich analogue (127), shown in Table 5.6, indicates that these two analogues show similar high potency at the histamine H_2-receptors. Figure 5.2 compares the overlay for the Mannich analogue and the azido analogue. It is possible that the azido and the 4-Mannich group may be able to bind to the same point on the receptor. This analogue may therefore also fit this model for the binding of the Mannich aryl substituents to the histamine H_2-receptor.

Table 5.6 Comparison of the 3-azidopyridine with the 4-Mannich pyridine



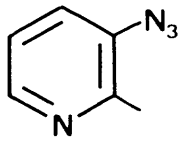
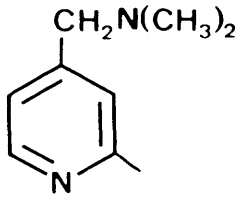
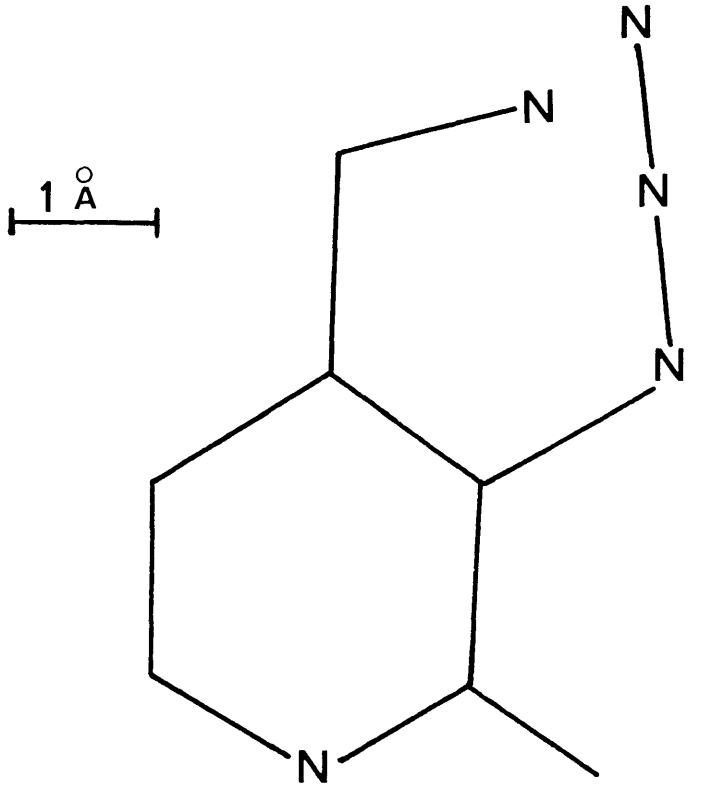
Compound number	R	pA_2 atrium (slope)	Rat G.S. ED_{50} µmole/kg
138		7.04 (0.79)	0.032
127		7.76 (0.95)	0.045

Figure 5.2 Comparison of geometries for a 4-Mannich and a 3-azidopyridyl substituent



5.5. CONCLUSION

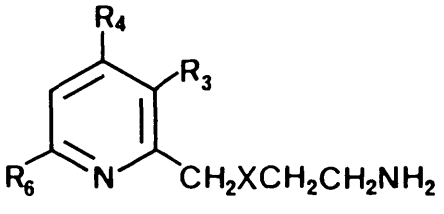
In this chapter the 4-Mannich pyridine has been shown to give rise to high potency at the histamine H_2-receptor. However, this was not the case with the corresponding 6-Mannich pyridyl analogues. The 4-Mannich subsitution also leads to a reduction in potency at the histamine H_1-receptor and therefore these analogues may be considered as potent selective histamine H_2-receptor antagonists. A model for the binding of the Mannich aryl histamine H_2-receptor antagonists has been presented which includes the published structure activity data for the furan and thiophene analogues along with the new data for the pyridine analogues.

6.0 SYNTHESIS OF THE MANNICH SUBSTITUTED PYRIDINES

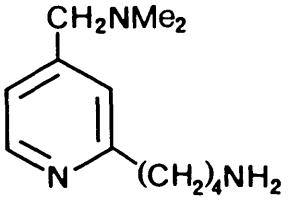
6.1. INTRODUCTION

In this chapter the preparation of the dialkylaminoalkyl substituted pyridines (141) to (145) (Mannich pyridines, Table 6.1) is described. The compounds were prepared in order to investigate the effect on activity at the histamine H_2-receptor of introduction of a Mannich substituent into the pyridine ring of known pyridyl histamine H_2-receptor antagonists. For analogues (142) to (145) only the preparation of the amines of structure (140), where Y = H is discussed. The coupling of the amines with the end-group to give

Table 6.1 Mannich substituted pyridines



(140)



(150)

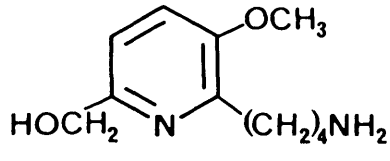
Number	R_4	R_6	R_3	X
146	H	CH_2OH	OMe	CH_2
141	H	CH_2NMe_2	OMe	CH_2
142	H	CH_2NMe_2	H	S
143	CH_2NMe_2	H	H	S
144	CH_2NMe_2	H	H	CH_2
145	CH_2N⬡	H	H	S

the antagonists is discussed in Chapter 7. The analogue (142) was prepared from the corresponding 6-hydroxymethylpyridyl isocytosine. A route to the amine (150) is also described.

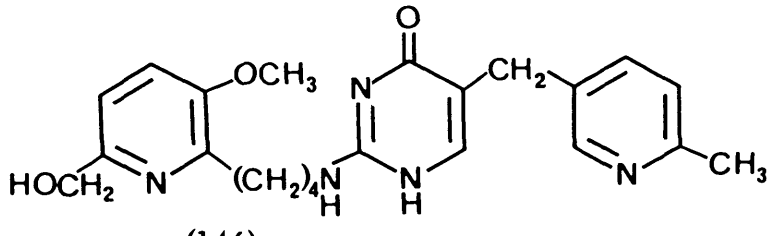
6.2. THE 2,6-SUBSTITUTED ANALOGUES

The preparation of the 6-hydroxymethyl pyridine (104) was described in Section 4.4.9.4. The isocytosine derivative (146) had been prepared and was converted using standard conditions into the 6-dimethylaminomethyl analogue (119), as shown in Scheme 6.1.

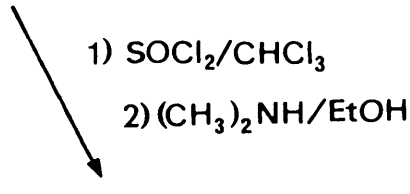
Scheme 6.1

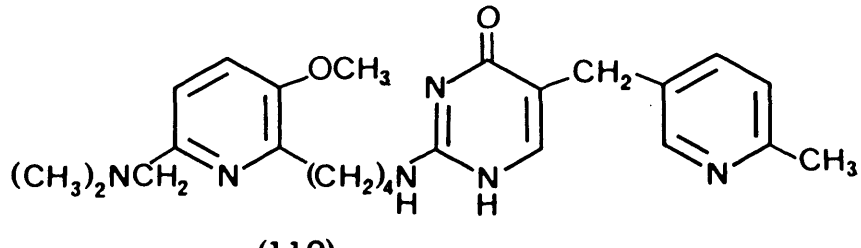


(104)



(146)

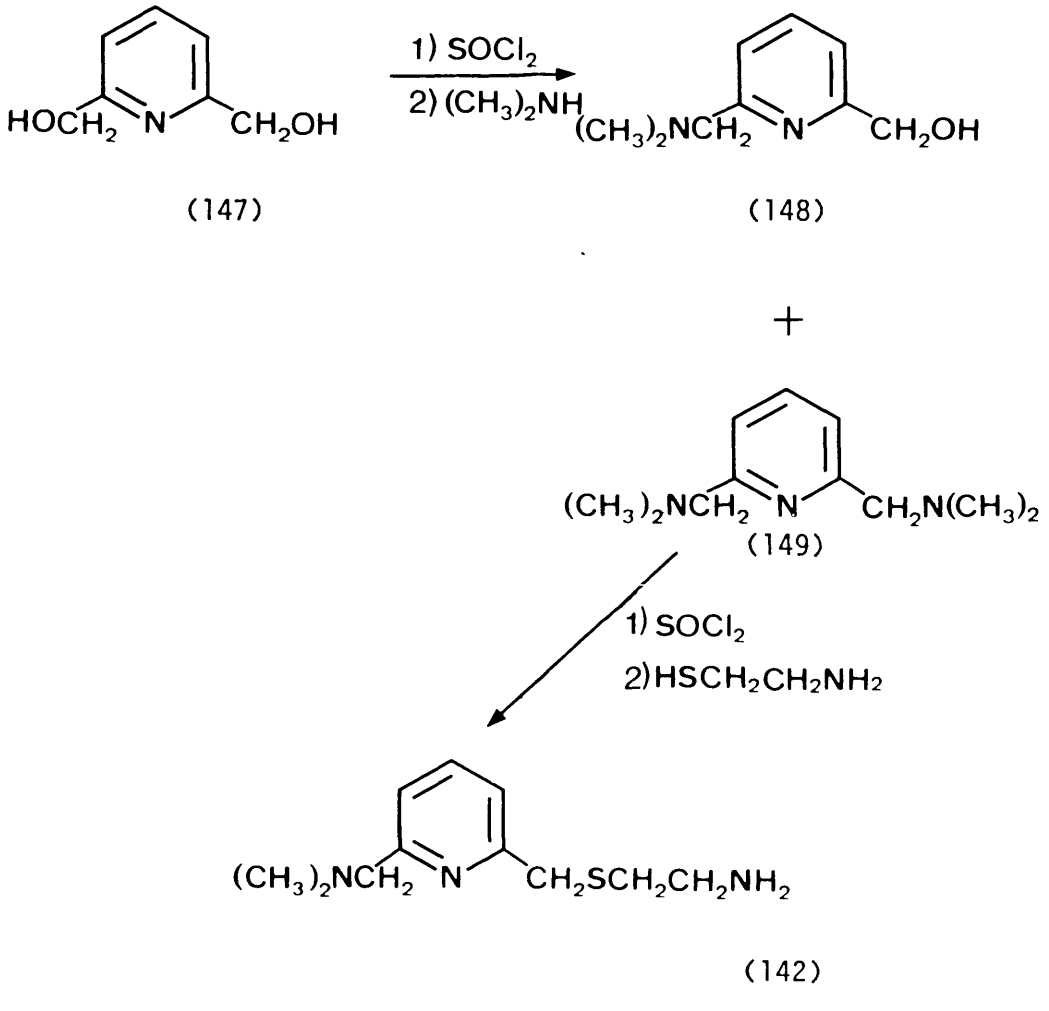


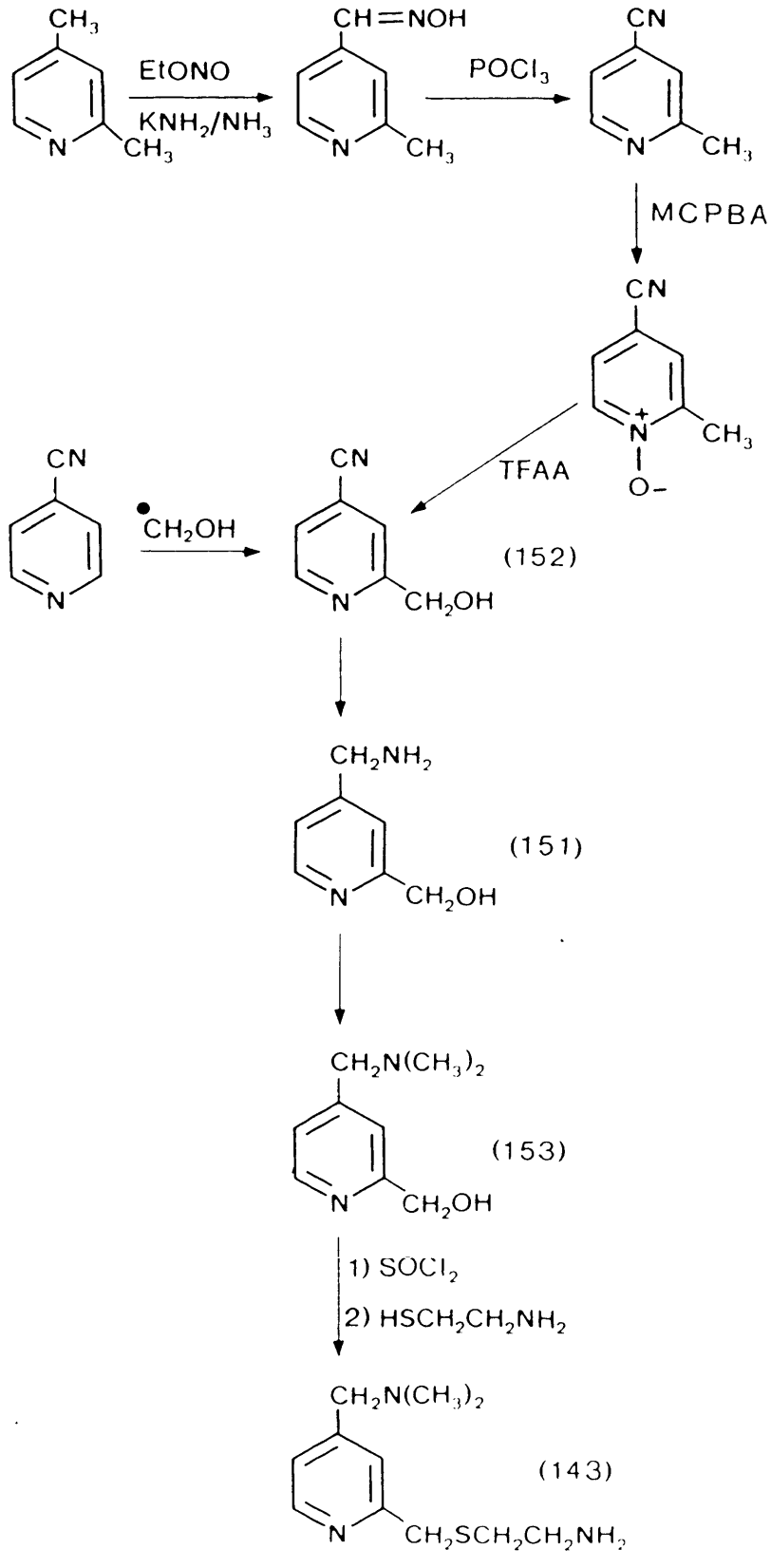


(119)

The 2,6 analogue (142) was prepared by standard methods as shown in Scheme 6.2. Reaction of (147) with thionyl chloride in chloroform led to a mixture of starting material and the bis-product (149) due to the low solubility of the starting material in chloroform. However using pyridine as solvent gave the required intermediate and reaction with dimethylamine gave a mixture of mono- and bis-dimethylaminomethyl pyridines (148) and (149) respectively. The mixture was not separated as only (148) would react further, and separation could be more easily accomplished after introduction of the cysteamine side chain. Reaction of the mixture with thionyl chloride followed by cysteamine gave the amine (142).

Scheme 6.2



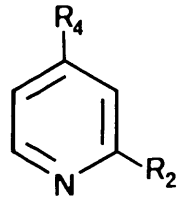


Scheme 6.3 Synthesis of (143)

6.3. THE 2,4-SUBSTITUTED ANALOGUES

In this section the synthesis of the amines shown in Table 6.2 is described.

Table 6.2 2,4-substituted Mannich pyridines

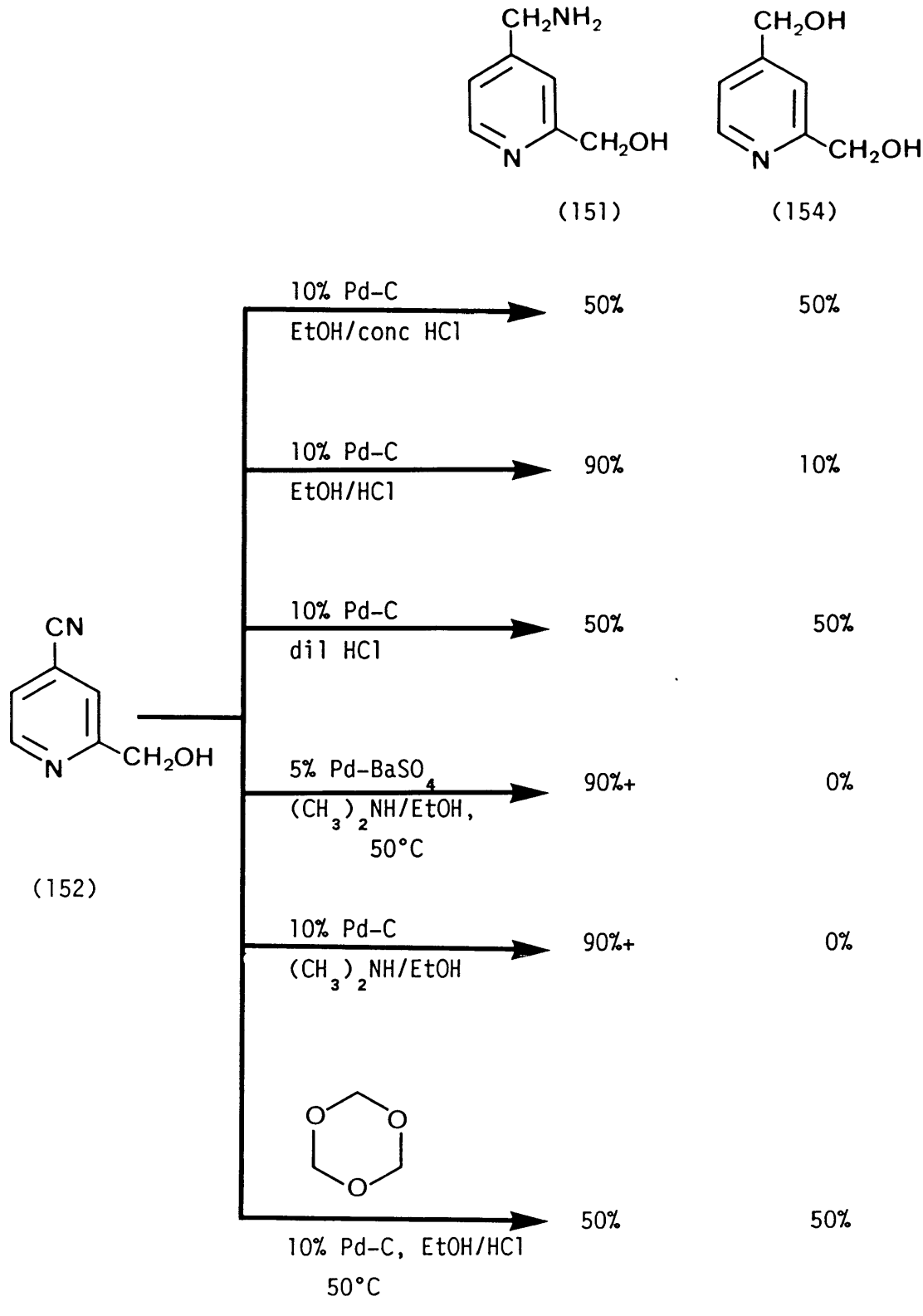


Compound number	R_4	R_2
143	CH_2NMe_2	$CH_2SCH_2CH_2NH_2$
145	CH_2N⟨⟩	$CH_2SCH_2CH_2NH_2$
144	CH_2NMe_2	$CH_2CH_2CH_2CH_2NH_2$
150	$CH_2CH_2CH_2CH_2NH_2$	CH_2NMe_2

The synthesis of the above amines requires either the regio-selective functionalisation of carbon substituents already in the pyridine ring or the regio-selective introduction of a carbon unit into the pyridine ring. Examples of these two alternative approaches are shown in Scheme 6.3.

Selective functionalisation of 2,4-lutidine has been reported[145] via the aldoxime. However, this stage is low yielding. Also difficulty had been found carrying out a selective rearrangement of an N-oxy function, (Section 4.6.1.) This alternative was therefore not p rsued.

Table 6.3 Catalytic hydrogenation of 4-cyano-2-hydroxymethyl pyridine



All hydrogenations were carried out at 50 psi of hydrogen and 25°C except where stated.

The alternative route starting from 4-cyanopyridine involves a regio selective radical substitution at the 2-position of the pyridine ring; pyridine is reported to undergo 'nucleophilic radical substitution'.[146] In acidic media substitution occurs at the 2- and 4-position of the pyridine ring. Electron withdrawing substituents are reported to facilitate the reaction.[147] The ease of reaction was correlated with the NMR shift of the α protons in the protonated 4-substituted pyridines. If the 4-position of the pyridine ring is blocked then substitution must occur at one of the two equivalent 2-positions. The carbon centred radicals required may be obtained by several routes which include persulphate oxidation of alcohols[148] and oxidative decarboxylation of carboxylic acids.[149]

Radical hydroxymethylation of 4-cyanopyridine using the 'Minisci' conditions[148] gave a 1:2:1 mixture of starting material, mono- and bis-hydroxymethylated products which were separated by chromatography. Reduction with lithium aluminum hydride gave only a poor yield of the required product (151); at least part of the problem was due to the insolubility of the product making isolation difficult. The results of catalytic reduction of (151) under various conditions are shown in Table 6.3.

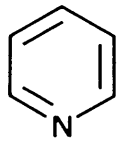
Reduction under acidic conditions always led to contamination of the required product with the 2,4-bishydroxymethylpyridine (154). It was presumed that (154) arose from hydrolysis of an enamine intermediate.

Benzyl cyanides are reported to undergo reductive aminolysis in ethanolic dimethylamine to give dimethylaminoethylbenzenes.[150] It was hoped that catalytic reduction of (152) in ethanolic dimethylamine would give the required dimethylaminomethylpyridine directly. However, the use of either palladium on carbon or palladium on barium sulphate as catalyst gave the aminomethyl pyridine (151) in essentially quantitative yield. Reductive alkylation using the conditions described in Section 3.4.5.2. followed by treatment of the product with thionyl chloride and cysteamine gave the required amine (143). Although, the above route

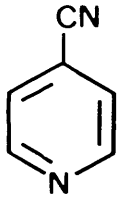
gave the required amine, it was however both long and difficult to scale up due to chromatography being required at the first stage.

The possibility of preparation of the pyridine intermediate (153) by direct hydroxymethylation of 4-dimethylaminomethylpyridine was investigated. Under the conditions used for the hydroxymethylation the dimethylaminomethyl group will be protonated and as such is a powerful electron withdrawing group. The pKa's of various 4-substituted pyridines are compared in Table 6.4.

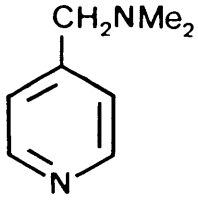
Table 6.4 Comparison of pKa values for several pyridines



pKa = 5.12 (Ref 127)



pKa = 1.86 (Ref 127)

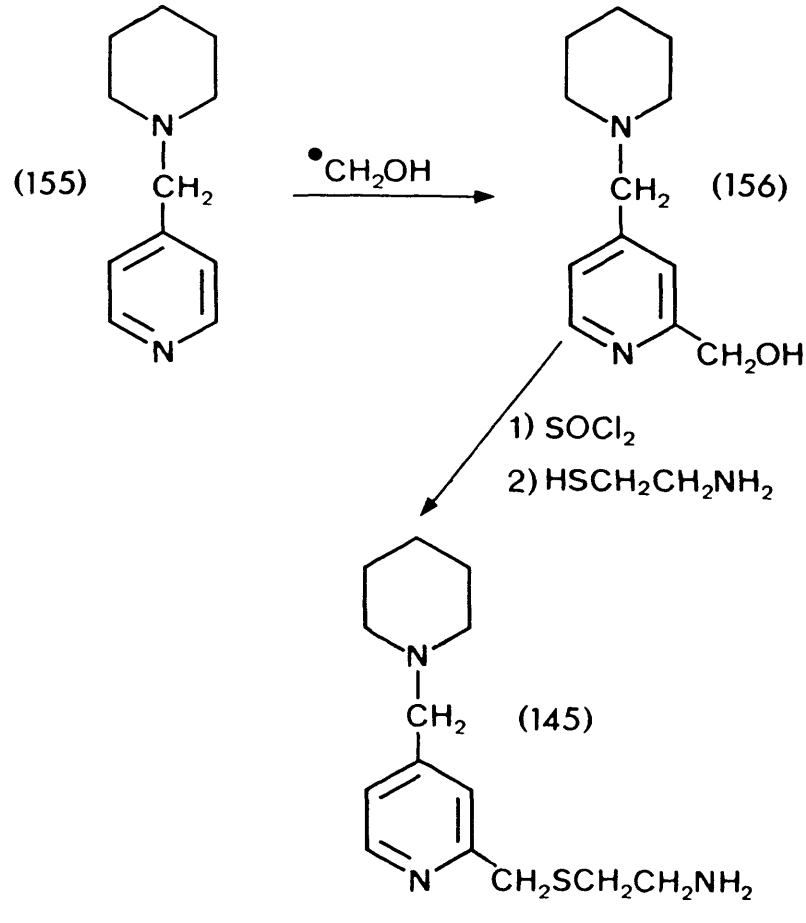


pKa_1 = 7.70 exocyclic nitrogen[151]
pKa_2 = 3.45 ring nitrogen

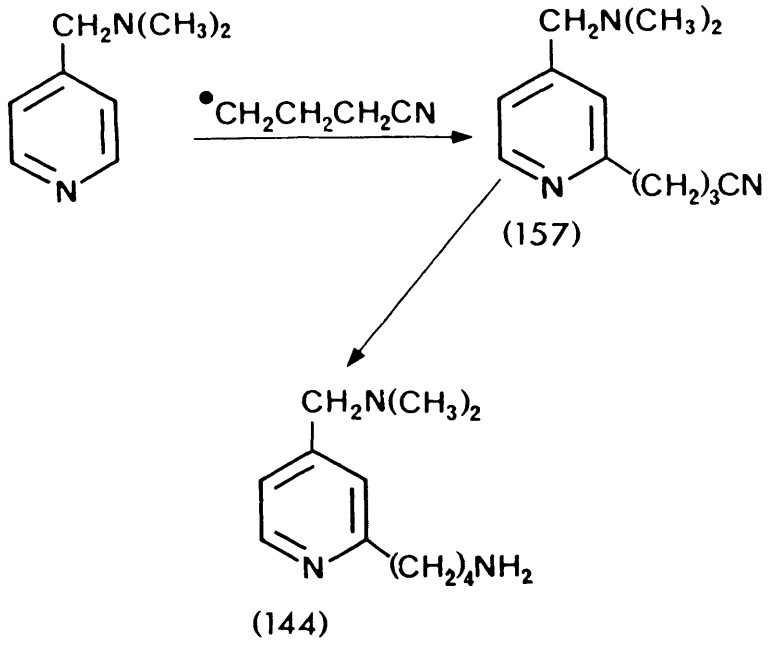
In 4-dimethylaminomethylpyridine protonation will occur first at the exocyclic nitrogen. The presence of a protonated centre lowers the basicity of the pyridine ring.

Radical hydroxymethylation of 4-dimethylaminomethyl pyridine gave a 1:2:1 mixture of starting material, mono- and bis-hydroxymethylated products. These were easily separated by fractional distillation to give the required hydroxymethyl pyridine in about 35% yield. This was then converted, using the route shown in Scheme 6.3 into the amine (143).

The piperidinomethyl analogue (145) was prepared by the analogous route shown in Scheme 6.4. Hydroxymethylation of (155) again yielded a mixture of mono- and bis-hydroxymethylated products. These were separated by distillation and converted into the amine (145) by the route shown in Scheme 6.4.



Scheme 6.4 The piperidinomethyl analogue (145)



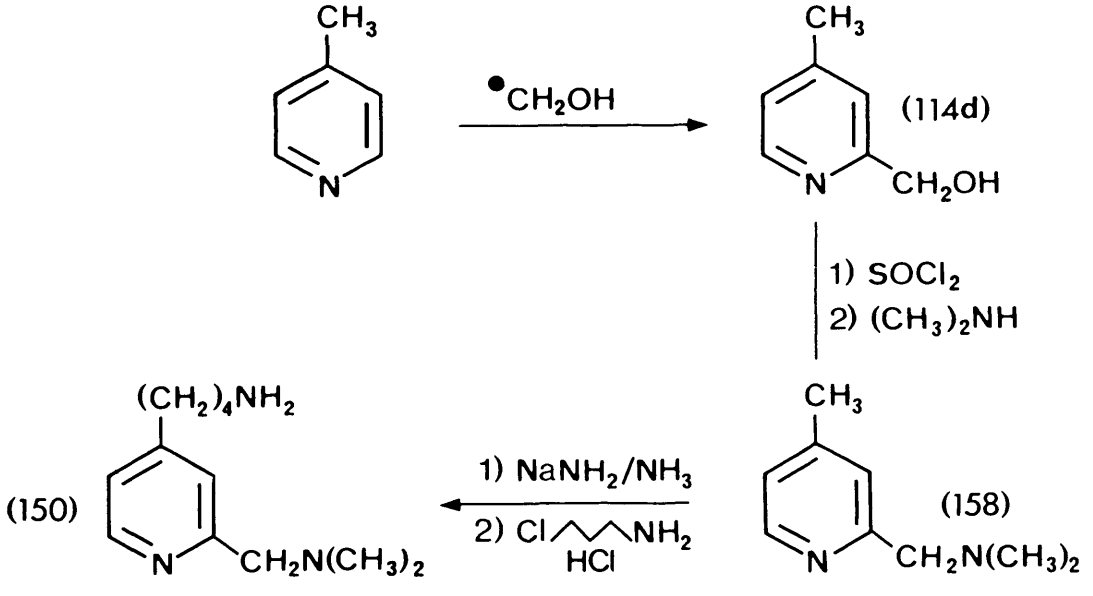
Scheme 6.5 Preparation of the butyl chain analogue (144)

The tetramethylene analogue (144) was initially prepared by the route shown in Scheme 6.5. Alkylation of 4-dimethylaminomethyl pyridine with cyanopropyl radicals generated by silver catalysed decarboxylation of 4-cyanobutyric acid gave a mixture of the cyanopropylpyridine (157), starting material and the bis-product in a 2:1:1 ratio. These were easily separated by distillation to give the pyridine (157) in 26% overall yield. Reduction of (157) with lithium aluminum hydride gave the amine (144) in quantitative yield.

The possibility of direct introduction of the aminobutyl chain by radical alkylation of 4-dimethylaminomethylpyridine with the radicals generated from oxidative decarboxylation of 5-amino-valeric acid was investigated. This reaction gave the required amine contaminated with the bis-product which was again easily separated by distillation. This method allowed the preparation of the amine (144) in one step from 4-dimethylaminomethylpyridine.

The decarboxylation of 6-aminocaproic acid which is the next higher homologue of 5-aminovaleric acid has been reported to give 5-aminopentyl radicals which were reported to add to 4-cyanopyridine.[152]

The amine (150) was prepared by the route shown in Scheme 6.6. In this case the substituent introduced by the radical alkylation is elaborated to give the Mannich group. The hydroxymethylpyridine (114d) had previously been prepared in Section 4.6.1. by rearrangement of 2,4-lutidine-N-oxide. However, this method also gave the alternative isomer. The hydroxymethylpyridine (114d) was therefore prepared by the method of 'Minisci'[148] and converted using standard methods to the Mannich pyridine (158). It has been shown[140] that the anion formed from picoline on treatment with sodamide in liquid ammonia can be alkylated with chloropropylamine to give a butylpicoline. Alkylation of (158) under similar conditions gave the amine (150).



Scheme 6.6 Synthesis of the alternative isomer (150)

6.4. CONCLUSION.

In this section it has been shown that 'Minisci' type nucleophilic radical substitution can be carried out in the presence of a protonated dimethylaminomethyl substituent. The use of radical chemistry has led to the preparation of the required Mannich pyridyl compounds by relatively short routes.

7.0 COMPUTATIONAL AND BIOLOGICAL METHODS

7.1 COMPUTATIONAL METHODS

7.1.1 Introduction

The calculations described in the previous chapter were performed using the S&KF molecular modelling system (COSMIC)[91] running on a Digital VAX 11/780 computer.

COSMIC is a graphic's package which allows the loading of molecular structures into the computer. These molecules may then be displayed and manipulated on the graphic's screen. COSMIC also provides a simple means of submitting structures to computational programmes. These programmes include both molecular mechanics and molecular orbital packages for calculation of energy. Substructure searches may also be carried out using the Cambridge crystal data base.

The results from these calculations and searches may then be displayed graphically or plotted as hard copy.

7.1.2 Molecular Mechanics

Molecular mechanics methods assume that the energy of a molecule can be described by the sum of the energies of its various mechanical and electrical contributions.[153,154] Thus, a bond will come to rest at a predefined length. Any stretch or compression which changes that length will result in an increase in energy and that energy change will follow some simple mechanical formula. For example, bond stretchings are assumed to follow Hooke's Law. There are at least five contributions to the total energy (E) which are included in the molecular mechanics algorithms currently in use, as shown overleaf,[155] so that:-

$$E = E_b + E_a + E_t + E_w + E_c.$$

- Energy increases due to deviations from equilibrium bond lengths are given by Equation 7.1 and are around 300 kcal $mole^{-1}Å^{-2}$.

  Equation 7.1 $E_b = k/2\ (\Delta l)^2$ for each bond;
  k = stretch force constant,
  Δl = difference between actual and equilibrium bond length

- Energy increases due to deviations from equilibrium bond angles are given by Equation 7.2. These are around 0.1 kcal $mole^{-1}deg^{-2}$.

  Equation 7.2 $E_a = k/2\ (\Delta\Theta)^2$ for each angle,
  k = bending force constant,
  $\Delta\Theta$ = difference between actual and equilibrium bond angle

- Periodic energy changes with rotation of torsional angles are given by Equation 7.3. Increases of about 2 kcal $mole^{-1}$ would be expected on going from staggered to eclipsed forms of a tetrahedral carbon-carbon bond.

  Equation 7.3 $E_t = V/2\ (1+\cos\#w)$ for each torsional angle;
  V = barrier to rotation,
  w = torsional angle

- The interaction of two approaching atoms separated by 3 or more bonds. These are the van der Waals interactions and are a combination of attractive and repulsive terms. As two atoms close within touching distance of their experimentally determined surfaces, the repulsive term predominates and the resulting energy may reach hundreds of kcal $mole^{-1}$. The formula for the van der Waals interactions can take one of several forms or be a mixture of these forms. The three Equations 7.4-7.6 are examples of those used. The molecular mechanics programmes in COSMIC use Equation 7.6.

Equation 7.4 $E_w = a/r\ 12 - b/r\ 6$

Equation 7.5 $E_w = ce\ d/r - d/r\ 6$

Equation 7.6 $E_w = f[-g/r^6 + he^{ir^1}]$ for all 1-4 non bonded pairs

a to d and f to i are constants

r = distance between atoms

r^1 = r/(sum of van der Waals radii)

o The non-bonding interactions of two charged atoms separated by 3 or more bonds are given by Equation 7.7. This is the coulombic energy and is simply the product of the two charges divided by the distance between them and the dielectric constant of the surrounding medium. Energies can reach 300 kcal $mole^{-1}$ at close quarters.

Equation 7.7 $E_c = q^1.q^2/Dr$ for each 1-4 non-bonded pair.

q = partial change

r = distance between charged atoms

D = dielectric constant (COSMIC takes a default value of one for the dielectric constant).

Each of the formulae associated with the five interactions is programmed into the computer. All of the individual atoms of the molecule are assigned atom types (Csp^3, Nsp^3, etc). The necessary coefficients for each atom are held in a database called the force field. The partial charges required for the coulombic energy calculations are added using a molecular orbital programme CNDO[156] or MOPAC[106].

The programmes can then be used to either calculate the energy of the molecule in a given conformation or the system can be used to minimise the energy of the molecule using an iterative process.

7.1.3 Method of calculating the change in energy with variation of the torsion angle of a bond

The molecule was drawn into the computer and minimised using the molecular mechanics methods. Charges were then added to the atoms using the molecular orbital package CNDO.[156] The molecule was then re-minimised and the CNDO calculation was repeated. This procedure was repeated until an energy minimum was found.

Variation of the energy of the molecule on rotating around a torsion angle of a bond could be then be investigated. The torsion angle was altered with 5° increments, the energy being calculated at each step. The results are then plotted in a graphical form using the programme "Simple plot".[157] Alternatively, rotation around 2 separate bonds can be investigated at the same time the results being plotted as a 2-dimensional contour plot (a Ramachandron plot). The angle of rotation for each torsion angle is plotted on the abscissa and ordinate, then a contour map is used to show the energy of the system for any given combination of the two torsion angles.

7.2 BIOLOGICAL METHODS

7.2.1 Determination of potency at the histamine H_1-receptor.

Histamine causes contraction of certain smooth muscle preparations from tissues such as bronchioles and ileum.[24] The most commonly used preparation for the assay of histamine H_1-receptor activity is the guinea pig ileum.[23] This tissue was used in this work and the methods used are outlined below.

A portion of guinea pig terminal ileum was isolated and mounted on a force transducer in a 10 ml tissue bath filled with magnesium free Tyrode solution equilibrated with $95\%O_2/5\%CO_2$ maintained at a temperature of 30°C. The tissue was loaded with 0.5 g tension and the force of contraction was detected by a force transducer. The signal was amplified and displayed on a potentiometric recorder.

Cumulative dose-response curves were obtained by the addition of measured amounts of histamine until the response reached a maximum.

The tissue bath was washed out and filled with fresh bathing medium containing the compound under test (antagonist). The solution was left in contact with the tissue for 8 minutes and a second cumulative dose-response curve was obtained by addition of measured amounts of histamine until a maximum response was observed. Further dose-response curves were obtained with increasing concentrations of antagonist. The doses of histamine giving 50% of the maximum response in the presence of different concentrations of antagonist were noted. A dose ratio (DR) was calculated by comparing the concentrations of histamine required to produce 50% of the maximum response in the absence and in the presence of each dose of the antagonist.

The relationship between the dose of antagonist and the ratio of equi-effective doses of agonist (Equation 7.8) can be used to assess if an antagonist is competitive over the dose-range investigated and to determine an estimate of antagonist potency.[158]

<u>Equation 7.8</u> $x - 1 = [D]_x / K_A$

or $\log (x - 1) = \log [D]_x - \log K_A$

where x is the ratio of equi-effective doses of agonist (DR), $[D]_x$ is the concentration of antagonist giving a dose-ratio, x, and K_A is the antagonist dissociation constant.

The negative log of the molar concentration of antagonist with which the ratio of equi-effective concentrations of agonist in the presence and absence of antagonist is two (i.e. $x = 2$) has been designated by Schild as the pA_2 value[23] i.e. $pA_2 = -\log [D]_2 = \log K_A$.
A plot (Schild plot) of log DR-1 against log [D] was made and the point of intersection where log (DR-1) = 0 gives an estimate of the pA_2 value (i.e. activity of the antagonist). Equation 7.8 also predicts that, for a competitive antagonist, the slope of the Schild plot should be not significantly different from unity. If values

significantly different from 1 are obtained experimentally, some caution should be used in interpreting the data.

7.2.2 Measurement of potency at the histamine H_2-receptor.

Histamine H_2-receptors are also widely distributed.[24] In this case, two assays have been used to determine antagonist activity. An *in vitro* test was selected in order to determine pA_2 values but in order to assess inhibitory effects on gastric secretion, an *in vivo* test was also required.

o *In vitro* test - Guinea pig right atrium

In the guinea pig heart, histamine has the effect of increasing atrial rate; this provided the basis for the guinea pig atrium assay which was carried out in the following way.

A spontaneously beating portion of the guinea pig right atrium was isolated and mounted on a force transducer in a 15 ml tissue bath filled with McEwens solution equilibrated with $95\%O_2/5\%CO_2$ at a temperature of 37°C. The rate of beating was determined by feeding the amplified signal from the force transducer into a rate meter and displaying the output on a potentiometric recorder.

Following completion of the first cumulative histamine dose-response curve, the bathing solution was replaced with fresh solution containing the antagonist. The solution was left in contact with the tissue for 60 minutes before repeating the cumulative dose-response curve. Cumulative dose-response curves were constructed in the presence of increasing concentrations of antagonist. The pA_2 values were calculated as described under the previous section (Section 7.2.1).

o *In vivo* test - Rat gastric secretion

Histamine stimulated gastric acid secretion was measured in the following way, using a modification of the method of Ghosh and Schild.[159]

Female Sprague-Dawley rats (160-200 g) were starved overnight and anaesthetised by intraperitoneal injection of urethane, 200 mg. The trachea and jugular veins were both cannulated and a mid-line incision was made in the abdomen exposing the stomach which was cleared of connective tissue. A small incision was made in the rumen of the stomach and the stomach washed with 5% w/v glucose solution. The oesophagus was cannulated with polythene tubing and the oesophagus and vagi are then cut above the cannula. An incision was made in the antrum and a cannula passed into the stomach via the ruminal incision and through into the antrum so that the head of the cannula lay in the body of the stomach. A funnel-shaped cannula was inserted in the ruminal incision and tied into position so that the line between the rumen and the body was coincident with the edge of the funnel. The antral cannula was tied into place to reduce the possibility that antrally released gastrin would affect gastric acid secretion. Two stab wounds were made in the abdominal wall, and the stomach cannulae passed through. The stomach was perfused through the oesophageal and stomach cannulae with 5.4% w/v glucose solution at 37°C at 1-2 ml min^{-1}. The effluent was passed over a micro-flow pH electrode and recorded by a pH meter fed to an anti-log unit and potentiometric recorder. The basal output of acid secretion from the stomach was monitored by measurement of the pH of the perfusion effluent.

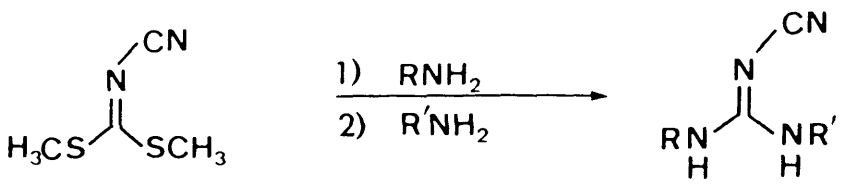
A sub-maximal dose of histamine was continuously infused into the jugular vein in order to produce a stable plateau of acid secretion and the pH of the perfusion effluent was determined when this condition was obtained. Infusion of histamine at a rate of 0.25 μmol $kg^{-1}min^{-1}$ produced 70% of maximum histamine stimulated gastric acid secretion. The test compound was then administered intravenously into the second jugular vein and washed in

with glucose solution (0.2 ml, 5.4% w/v). The difference in acid secretion between basal output and the histamine stimulated plateau level and the reduction of acid secretion caused by the test compound was calculated from the difference in pH of the perfusion effluent. ED_{50} values (for inhibition sub-maximal acid secretion by 50%) were determined by administering one dose of test compound to one rat and repeating this in at least four rats for each of three or more dose levels. The results obtained were then used to calculate the ED_{50} value by the standard method of least squares.

8.0 PREPARATION OF ANTAGONISTS AND TABLES

8.1. THE CYANOGUANIDINES

8.1.1. Preparation



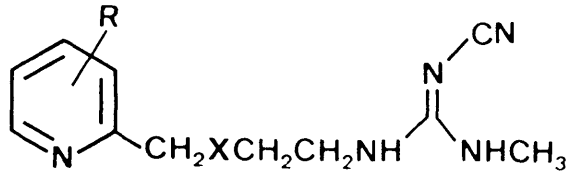
(165) (160)

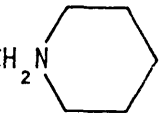
The N-cyano-N'-methyl-N"substituted guanidines (the cyanoguanidines) of structure (160) were prepared as shown above. This method involves the sequential displacement of the methylthio groups of (165) first with the substituted amine, then with methylamine as described by Durant et al.[44]

8.2.2. Table of analogues

The following table lists the compounds prepared and their biological activities, and, is also intended as an index to the experimental section.

Table 8.1 The pyridylbutyl cyanoguanidines



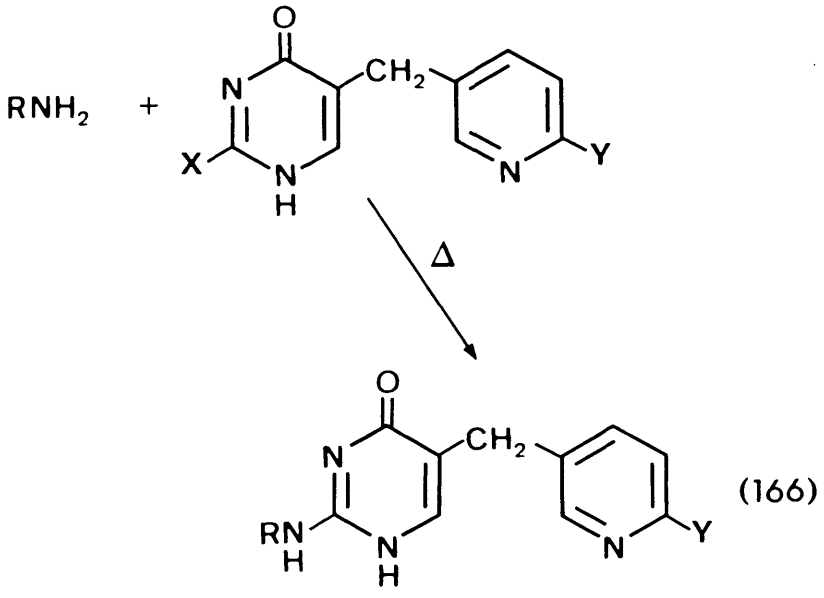
Compound number	R	X	G.Pig atrium pA_2	Rat G.S. μmoles/kg	Yield %	Page number
24	3H	CH_2	5.64	22	30	224
25	3F	CH_2	6.17	-	46	225
26	3OMe	CH_2	6.75	18	61	225
27	3OH	CH_2	5.64	-	68*	226
28	3OBz†	CH_2	<3.79	-	50	226
29	3SMe	CH_2	5.56	-	48	227
30	3Cl	CH_2	5.72	8.5	61	228
31	$3NH_2$	CH_2	5.01	-	63	228
32	3NHMe	CH_2	5.42	-	30	229
33	3Me	CH_2	5.39	-	74	230
34	3Br	CH_2	5.41	-	33	230
35	$3NO_2$	CH_2	4.74	-	42	231
36	$3CF_3$	CH_2	<3.97	-	41	232
37	H	S	6.03	9.15	-	233
122	$6CH_2NMe_2$	S	5.0	10.2	73	235
130	$4CH_2NMe_2$	S	7.79	0.081	34	236
131		S	7.55	0.75	52	237
166	4Me	S	6.05	11.1	61	230
167	5Br	S	4.93	43	34	233
168	5Cl	CH_2	5.0	39	52	234
169	5OMe	CH_2	4.82	29	53	234

* This compound was prepared by hydrogenation of (28).

† - Bz = benzyl

8.2 THE ISOCYTOSINES

8.2.1. Preparation



(166)

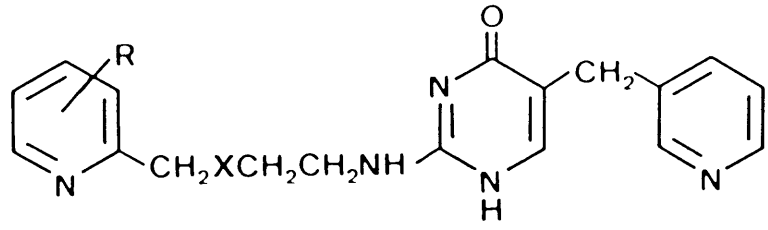
where:- X	Y	Compound no.
H_3CS	H	161
H_3CS	CH_3	163
O_2NNH	H	162
O_2NNH	CH_3	164

The 2-amino-1(H)-pyrimidine-4-ones structure (166), (the isocytosines), were prepared as shown above. The reactions were either carried out either as a fusion or in pyridine at reflux. The starting materials of structure (161-164) were prepared by colleagues at SK&F using the methods described in the patent literature.[160]

8.2.2. Tables of analogues

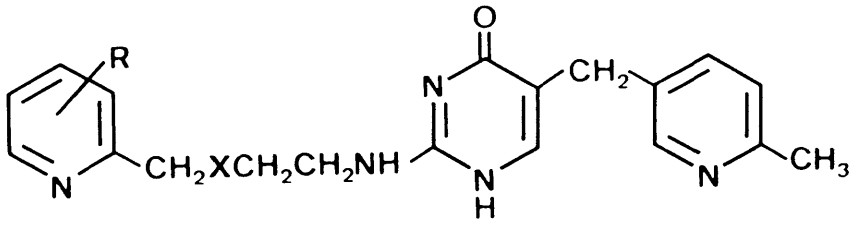
The following tables list the compounds prepared and their biological activities, and, are also intended as an index to the experimental section.

Table 8.2 The 5-picolyl isocytosines



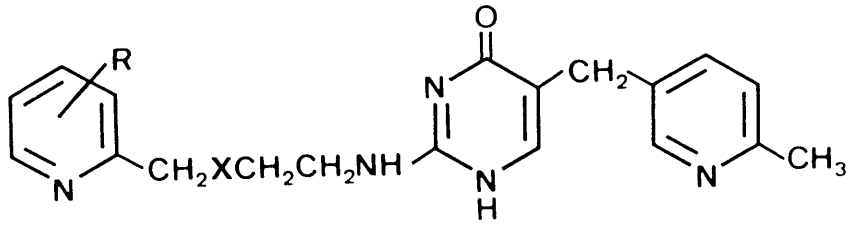
Compound number	R	X	G.Pig atrium pA_2 (slope)	G. Pig ileum pA_2 (slope)	Rat G.S. µmoles/kg	Yield %	Page number
49	$3NH_2$	CH_2	6.64 (0.76)	8.84 (0.80)	0.42	44	240
116	3OMe	CH_2	7.37 (0.87)	7.84 (1.05)	0.23	73	239
118	3OMe,6Me	CH_2	5.63 (0.95)	7.67 (0.94)	4.90	60	242
170	H	CH_2	6.64 (1.14)	7.41 (0.83)	0.24	-	237
171	3F	CH_2	7.21 (0.90)	7.40 (0.67)	0.20	73	238
172	3Cl	CH_2	6.78 (0.94)	8.23 (0.96)	0.56	65	238
173	3Br	CH_2	6.50 (0.92)	8.67 (0.99)	0.40	34	239
174	4Me	S	7.91 (0.43)	6.01 (0.91)	0.31	57	240
175	5Br	S	6.18 (0.88)	7.26 (0.94)	2.80	54	241
176	$5NH_2$	CH_2	6.70 (0.68)	7.60 (0.83)	0.70	37	241
177	5OMe	CH_2	6.09 (0.64)	6.62 (0.97)	1.36	77	242

Table 8.3 The 5-(6-methylpicolyl) isocytosines



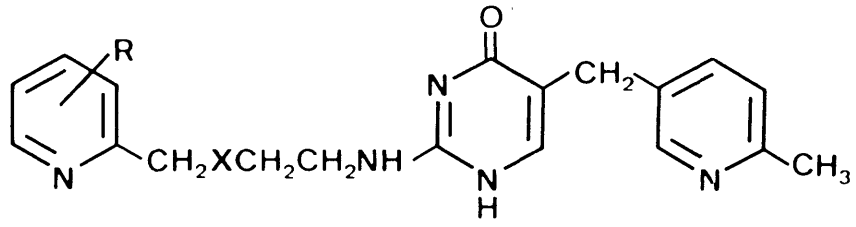
Compound number	R	X	G.Pig atrium pA_2 (slope)	G Pig ileum pA_2 (slope)	Rat G.S. μmoles/kg	Yield %	Page number
117	3OMe	CH_2	7.49 (0.89)	7.77 (1.05)	0.21	62	247
126	H	CH_2	6.48 (1.37)	7.28 (0.91)	0.40	92	243
138	$3N_3$	CH_2	7.04 (0.79)	6.86 (1.13)	0.032	33	246
178	$3NO_2$	CH_2	5.37 (0.59)	7.53 (0.83)	1.13	67	243
179	$3NH_2$	CH_2	6.34 (0.94)	8.83 (1.06)	0.56	65	244
180	$3NMe_2$	CH_2	4.90 (0.97)	7.78 (0.88)	1.54	55	244
181	3Br	CH_2	7.10 (0.62)	8.92 (0.75)	0.58	65	245
182	3Cl	CH_2	6.94 (0.65)	8.60 (1.12)	0.73	75	245
183	3I	CH_2	6.06* (0.98)	6.82 (1.43)	-	50	246
184	3OBz†	CH_2	**	7.16 (0.99)	+ve	47	247
185***	3OH	CH_2	6.81 (0.69)	7.44 (0.85)	0.3	53	248
186	3SMe	CH_2	7.03 (0.80)	8.26 (1.09)	2.19	83	248

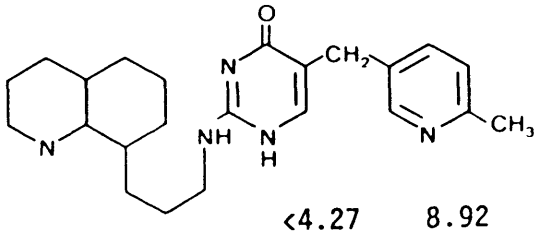
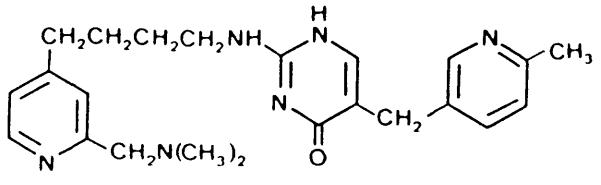
Table 8.3 (contd)



Compound number	R	X	G.Pig atrium pA_2 (slope)	G. Pig ileum pA_2 (slope)	Rat G.S. μmoles/kg	Yield %	Page number
54	3Me	CH_2	6.97 (0.61)	8.68 (0.88)	1.12	28	250
146	3OMe, $6CH_2OH$	CH_2	**	6.34 (0.99)	5.00	30	255
187	$3CF_3$	CH_2	<4.27	8.25 (0.69)	-	71	249
188	$5NO_2$	CH_2	5.52 (0.90)	7.27 (0.55)	7.59	33	251
189	$5NH_2$	CH_2	-	7.16 (0.88)	-	82	251
190	$5NMe_2$	CH_2	5.97 (0.88)	6.26 (0.98)	5.31	54	252
191	5Br	CH_2	6.29 (0.60)	8.40 (1.15)	4.75	53	252
192	5Cl	CH_2	6.21 (0.82)	8.11 (0.86)	-	76	253
193	5F	CH_2	6.22 0.85)	7.70 (0.70)	1.90	71	253
194	5I	CH_2	5.81 (1.00)	8.12 (0.76)	22.5	93	254
195	5SMe	CH_2	-	7.49 (0.96)	-	70	254
196	3OMe, N-O	CH_2	≃4.46 (1.10)	6.35 (0.56)	3.50	69	255

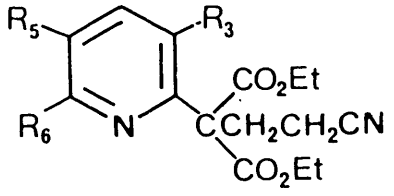
Table 8.3 (contd)



Comp. number	R	X	G.Pig atrium pA_2 (slope)	G.Pig ileum pA_2 (slope)	Rat G.S. μmoles/kg	Yield %	Page number
119	3OMe, $6CH_2NMe_2$	CH_2	5.88 (0.72)	5.10 (1.14)	9.30	50	256
121	$6CH_2NMe_2$	S	7.28 (1.00)	-	0.25	45	256
125	$4CH_2NMe_2$	S	7.92 (0.63)	**	0.064	62	257
127	$4CH_2NMe_2$	CH_2	7.76 (0.95)	5.32 (1.28)	0.045	62	258
128	$4CH_2N$	S	7.25 (1.16)	-	0.172	75	257
55			<4.27	8.92 (0.69)	-	26	250
137			7.88 (1.01)	4.19 (1.50)	0.034	42	258

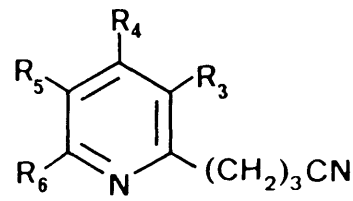
* - Some precipitation observed in organ bath.
** - No regression, compound precipitated in organ bath.
*** - This analogue was prepared by hydrogenation of (185).
† - Bz = benzyl

8.3 TABLE OF STRUCTURES INCLUDED IN EXPERIMENTAL



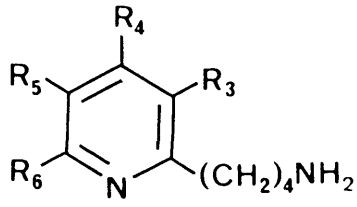
(68)

Compound number	R_3	R_5	R_6	Page
68a	NO_2	H	H	173
68b	H	NO_2	H	173
68c	Me	NO_2	H	174
68d	NO_2	H	Me	175



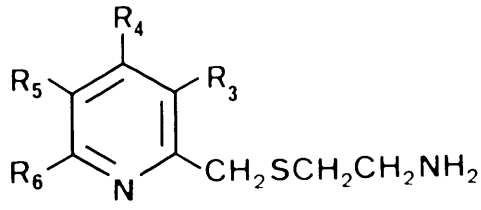
Compound number	R_3	R_4	R_5	R_6	Page
69a	NO_2	H	H	H	176
69b	H	H	NO_2	H	177
69c	Me	H	NO_2	H	178
69d	NO_2	H	H	Me	178
76a	NH_2	H	H	H	180
76b	H	H	NH_2	H	181
76c	Me	H	NH_2	H	181
76d	NH_2	H	H	Me	182
78a	NHCHO	H	H	H	183
80a	NMe_2	H	H	H	184
80b	H	H	NMe_2	H	185
82a	Br	H	H	H	187
87a	F	H	H	H	191/2
91a	OMe	H	H	H	198/9
91b	H	H	OMe	H	203
91d	OMe	H	H	Me	201
93a	OH	H	H	H	198
93b	H	H	OH	H	202
93d	OH	H	H	Me	200
95b	H	H	N_2^+	H	202
96a	OBz†	H	H	H	204
102	OH	H	H	CH_2OH	206
103	OMe	H	H	CH_2OH	207
105a	SMe	H	H	H	208
105b	H	H	SMe	H	208
107a	I	H	H	H	110
108a	CF_3	H	H	H	110
157	H	CH_2NMe_2	H	H	221

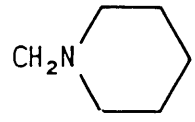
† - Bz = benzyl

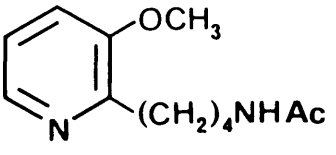
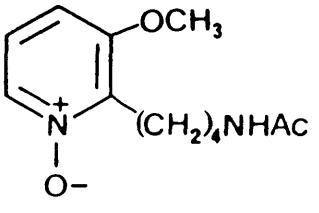
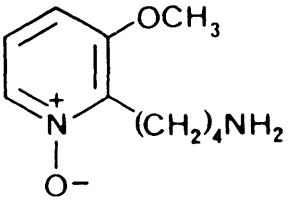
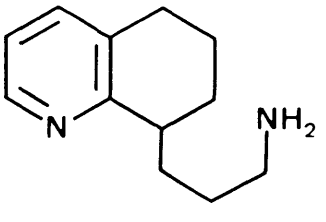


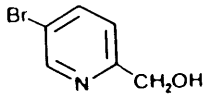
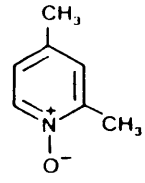
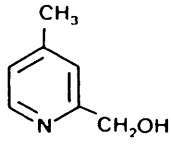
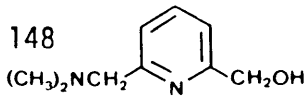
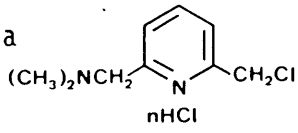
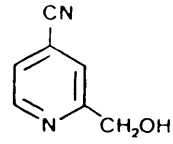
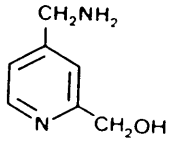
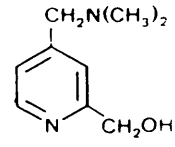
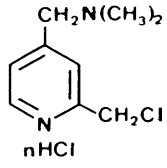
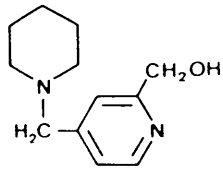
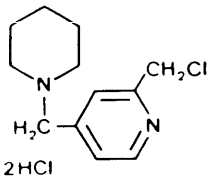
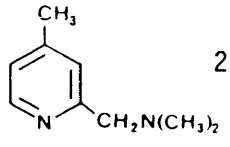
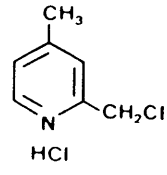
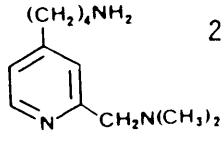
Compound number	R_3	R_4	R_5	R_6	Page
75a	NO_2	H	H	H	179
75b	H	H	NO_2	H	179
77a	NH_2	H	H	H	182
77b	H	H	NH_2	H	182
77c	Me	H	NH_2	H	183
79a	NHMe	H	H	H	184
81a	NMe_2	H	H	H	186
81b	H	H	NMe_2	H	186
83a	Br	H	H	H	187/8
83b	H	H	Br	H	188
83c	Me	H	Br	H	190
86a	Cl	H	H	H	190
86b	H	H	Cl	H	191
88a	F	H	H	H	192
88b	H	H	F	H	193
88c	Me	H	F	H	194
89a	I	H	H	H	195
89b	H	H	I	H	196
89c	Me	H	I	H	197
98b	H	H	N_2^+	H	193
98c	Me	H	N_2^+	H	194
90a	N_3	H	H	H	197
94a	OMe	H	H	H	199
94b	H	H	OMe	H	203
94d	OMe	H	H	Me	201
97a	OBz†	H	H	H	204
104	OMe	H	H	CH_2OH	207
106a	SMe	H	H	H	209
106b	H	H	SMe	H	209
109a	CF_3	H	H	H	211
111	Me	H	H	H	211
144	H	CH_2NMe_2	H	H	222

† - Bz = benzyl



Compound number	R_3	R_4	R_5	R_6	Page
113	H	H	Br	H	213
114	H	Me	H	H	214
142	H	H	H	CH_2NMe_2	216
143	H	CH_2NMe_2	H	H	219
145	H		H	H	220

Compound number		Page
100		205
101		205
99		206
112		212

Compound number		Page	Compound number		Page
113d		213	114c		213
114d		214	148		215
149a		215	152		216
151		217	153		217
153a		219	156		220
156a		220	158		223
159		222	150		223

9.0 EXPERIMENTAL

GENERAL

Starting materials were prepared according to literature procedures as indicated and, if no reference is quoted, are available commercially.

Solvents

In the text petrol refers to petroleum ether b.p. 60-80°C. Ether and xylene were dried over sodium wire. Tetrahydrofuran (THF) was dried over sodium wire and distilled from lithium aluminium hydride immediately prior to use. Dimethylsulphoxide (DMSO) was dried over 4Å molecular sieve.

Melting points

Melting points (m.p.) were carried out on a Thomas Hover capillary melting point apparatus and are uncorrected.

Chromatography

Column chromatography was carried out on Merck silica gel type H using either gravity elution or elution at about 50 PSI on a medium pressure chromatography system comprising of a Gilson Model 302 pump and Altex glass columns.

Thin layer chromatography (TLC) was carried out on Merck Kieselgel 60 F254 plates. The plates were visualised at 254 nm and 350 nm and then with either gaseous iodine or potassium iodoplatinate spray reagent.

Gas liquid chromatography (GLC) was carried out on a Pye Unicam G.C.D. gas chromatograph fitted with 1.5 mm x 2 m glass columns packed as stated in the experimental. A flame ionisation detector and Hewlett Packard 3390A integrator was used.

elemental analysis was performed on a Control Equipment Corporation model 240 C,H,N analyser or a Perkin-Elmer 240C C,H,N analyser. Sulphur and halogen determinations were performed using the Schoniger flask technique.

Differential scanning calorimetry was performed on a Perkin-Elmer DSC 2C instrument and thermogravimetric analysis was performed on a Perkin-Elmer TGS2 instrument.

Spectroscopy

Infra-red (IR) spectra were recorded on one of the following instruments:- Perkin-Elmer model 157, 298 or 580 spectrometers, and spectra were calibrated against polystyrene. Spectra were recorded in one of the following ways:- thin film between sodium chloride plates (LF), Nujol mulls (NM), 1% w/w KBr discs (KBr disc) or in bromoform solution ($CHBr_3$).

Proton NMR spectra were recorded on one of the following instruments:- Bruker AM 360 (360 MHz), a Jeol PFT 100P (100 MHz) or a Jeol FX60Q (60 MHz). Tetramethylsilane (TMS) was used as internal standard. Signals are indicated to be singlets (s), doublets (d), doublet of doublets (dd), triplets (t), quartets (q), multiplets (m) and values of coupling constants (J) are quoted in Hz. Broad signals are listed as (br) and those which underwent exchange on treatment with D_2O as (exch. D_2O).

Carbon (^{13}C) spectra were recorded on an Jeol PFT 100P NMR spectrometer operating at 25.14 MHz.

UV/visible spectra were determined on a Sp 800 spectrophotometer. Cells of 1 cm path length were used and the solvent was ethanol. Extinction coefficients (ε) are quoted in parenthesis.

Mass spectra were recorded on a V.G. analytical 7070F instrument fitted with a DS 2050 Data System using direct insertion probes. Spectra were recorded at 70 or 12 eV using one of the following

techniques:- electron impact (EI) (this technique was used unless stated otherwise), chemical ionisation (CI), field desorption (FD) or fast atom bombardment (FAB).

Diethyl 2-(2-cyanoethyl)-2-(3-nitropyrid-2-yl)malonate (68a)

This procedure is a modification of that of Sach.[97] Sodium hydride in oil (29.6 g, 0.66 mole) was washed by decantation with THF (2 x 100 ml) and finally suspended in THF (245 ml) under an atmosphere of nitrogen. A solution of diethyl 2-(2-cyanoethyl)-malonate (156.9 g, 0.73 mole) in THF (100 ml) was added over 40 min keeping the internal temperature at 20°C ± 2°C (ice/water cooling). The resulting suspension cleared over 1 hr when 2-chloro-3-nitro-pyridine (88.3 g, 0.55 mole) was added and the solution was heated to reflux. The solvent was allowed to distil off and distillation was continued until the internal temperature reached 110°C which took 3 hr. The mixture was then heated at 110°C for 1 hr, cooled to room temperature and the black oil was partitioned between water (300 ml) and chloroform (400 ml) and the pH taken to ~ 6 with concentrated hydrochloric acid. The chloroform layer was run off and the aqueous layer was further extracted with chloroform (2 x 200 ml). The chloroform extracts were combined, treated with magnesium sulphate (20 g) and charcoal (10 g), filtered and the solvent removed to give a black oil. Ethanol (100 ml) was added and the solution allowed to crystallise. The solid was collected by filtration and recrystallised from ethanol to give malonate (68a) (93.32 g, yield 50%), m.p. 93.5-94.5°C. (Found: C, 53.6; H, 5.05; N, 12.3%. $C_{15}H_{17}N_3O_6$ requires C, 53.75; H, 5.1; N, 12.55%); NMR ($CDCl_3$, 100 MHz); δ 1.22 (6H, t); 2.60 (2H, m); 3.00 (2H, m); 4.24 (4H, q); 7.57 (1H, dd); 8.42 (1H, dd); 8.72 (1H, dd); IR υ_{max} (NM) 2250, 1760, 1740, 1600, 1580 and 1520 cm^{-1}.

Diethyl 2-(2-cyanoethyl)-2-(5-nitropyrid-2-yl)malonate (68b)

Sodium hydride (53% dispersion in oil) (30.71 g, 0.66 mole) was washed by decantation with xylene (2 x 150 ml), ether (150 ml),

THF (150 ml) and finally suspended in THF (245 ml). Diethyl 2-(2-cyanoethyl)malonate (156.9 g, 0.73 mole) in THF (80 ml) was added dropwise over 1 hr keeping the internal temperature at 18°C to 22°C (with ice bath cooling). The resulting suspension cleared over 15 min when 2-chloro-5-nitropyridine (88.3 g, 0.55 mole) was added to give a deep magenta solution. The solution was heated in an oil bath at ~ 85°C when an exotherm was observed, the internal temperature rose (22-74°C in <10 min) and the THF distilled off. The oil bath was removed and distillation continued for ~ 10 min. The resulting solution was refluxed for 1 hr and the solvent was removed on the rotary evaporator. The resulting oil was partitioned between water (500 ml) and chloroform (800 ml), pH adjusted to ~ 7 (concentrated hydrochloric acid), and the chloroform was run off. The aqueous layer was extracted with a further (2 x 250 ml) chloroform, the extracts were combined, dried over magnesium sulphate and the solvent was removed to give an amber oil (~ 245g). Ether (150 ml) was added and the solution was allowed to crystallise to give the malonic ester (68a) (121.98 g, 65%), m.p. 59.5-61°C. (Found: C, 53.7; H, 5.05; N, 12.35%. $C_{15}H_{17}N_3O_6$ requires C, 53.75; H, 5.1; N, 12.55%); NMR ($CDCl_3$, 60 MHz); δ 1.37 (6H, t); 2.65 (4H, m); 4.26 (4H, q); 7.84 (1H, dd, J 2,10); 8.49 (1H, dd, J 4,10); 9.32 (1H, dd, J 4,10); IR υ_{max} (NM) 2220, 1750, 1730, 1600, 1580 and 1520 cm^{-1}.

Diethyl 2-(2-cyanoethyl)-2-(5-methyl-3-nitropyrid-2-yl)malonate (68c)

This compound was first prepared by Mr M.L. Meeson. Sodium hydride (53% dispersion in oil) (28.86 g, 0.64 mole) was washed by decantation with hexane (2 x 150 ml), ether (150 ml), THF (150 ml) and finally suspended in THF (250 ml). Diethyl 2-(2-cyanoethyl)-malonate (148.6 g, 0.70 mole) in THF (70 ml) was added over 1 hr keeping the internal temperature at 18-22°C. The resulting suspension cleared over 15 min when 2-chloro-3-methyl-5-nitro-pyridine (100 g, 0.58 mole) was added. The solution was heated to 100°C (some of the THF distilled off) and maintained at 100°C for 13 hr. The resulting dark brown oil was partitioned between water

(800 ml) and chloroform (800 ml). The chloroform layer was run off and dried over magnesium sulphate. The solution was treated with charcoal, filtered through a bed of silica gel and then evaporated. The residue was recrystallised from ethanol to give the malonic ester (68c) (87.5 g, 44%), m.p. 64.5-65.5°C. (Found: C, 55.05; H, 5.75;N, 12.0 %. $C_{16}H_{19}N_3O_6$ requires C, 55.0; H, 5.5;N, 12.05 %); NMR ($CDCl_3$, 60 MHz); δ 1.29 (6H, t); 2.41 + 2.70 (7H, s + m); 4.28 (4H, q); 8.27 (1H, d); 9.12 (1H, d); IR υ_{max} (NM) 2240, 1590, 1580, 1510, and 1410 cm^{-1}.

Diethyl 2-(2-cyanoethyl)-2-(6-methyl-3-nitropyrid-2-yl)malonate (68d)

Sodium hydride in oil (6.40 g, 0.14 mole) was washed by decantation with xylene (2 x 50 ml) and finally suspended in THF (50 ml) under an atmosphere of nitrogen. A solution of diethyl 2-(2-cyanoethyl)-malonate (31.2 g, 0.15 mole) in THF (25 ml) was added over 40 min keeping the internal temperature at 20°C ± 3°C (ice/water cooling). The resulting suspension cleared over 1 hr when 2-chloro-6-methyl-3-nitropyridine (18.0 g, 0.10 mole) was added and the resulting dark green solution was heated to reflux. The solvent was allowed to distil off until the internal temperature reached 100°C which took 1 hr. The mixture was then heated at 100°C for 3.5 hr, cooled, and the black oil was partitioned between water (300 ml) and chloroform (400 ml) and the pH taken to ~ 6 with concentrated hydrochloric acid. The chloroform layer was run off and the aqueous layer was further extracted with chloroform (2 x 200 ml). The chloroform extracts were combined, treated with magnesium sulphate (10 g) and charcoal (5 g) and then filtered through a bed of silica gel. The filtrate was evaporated and the residue was allowed to crystallise under ether to give the malonic ester (68d) (3.75 g, 10%), m.p. 66-67°C. (Found: C, 55.1; H, 5.5; N, 11.8 %. $C_{16}H_{19}N_3O_6$ requires C, 55.0; H, 5.5; N, 12.0 %); NMR ($CDCl_3$, 100 MHz); δ 1.27 (6H, t); 2.60 + 2.64 (5H, t + s); 3.00 (2H, t); 4.24 (4H, q); 7.31 (1H, d); 8.34 (1H, d); IR υ_{max} (NM) 2230, 1760, 1740, 1600 and 1580 cm^{-1}.

General procedure for monitoring the malonic ester hydrolyses

A stirred solution of the pyridyl malonic ester (68) (0.01 mole) in ethanol (150 ml) was treated with sodium hydroxide solution (1 M; 40 ml, 0.04 mole). Aliquots (10 ml) were withdrawn and acidified (pH 3) with dilute hydrochloric acid. The ethanol was removed on the rotary evaporator and the residual aqueous layer was extracted with chloroform (10 ml). The chloroform extract was then assayed by GLC and TLC. GLC assays were carried out on an OV1 column at 225°C. At the end of the experiment the remaining reaction mixture was worked up in the same way as for a single aliquot. Evaporation of the chloroform extract gave an essentially quantitative recovery of organic material.

2-(3-Cyanopropyl)-3-nitropyridine (69a)

This compound was first prepared by Dr G.S. Sach and this is a modification of that method. Diethyl 2-(2-cyanoethyl)-2-(3-nitropyrid-2-yl)malonate (68a) (84.25 g, 0.251 mole) was dissolved in ethanol (3.5 l) and sodium hydroxide solution (1 M; 1.005 l, 1.005 mole) and stirred at ambient temperature for 100 hr. The solution was heated to reflux and stirred at reflux for 1 hr then cooled and filtered. The filtrate was concentrated under reduced pressure until an oil separated. The cooled solution was extracted with chloroform (3 x 500 ml). The combined chloroform extracts were dried over magnesium sulphate, the solvent was removed to give the pyridine (69a) (47 g, 97%) as a straw coloured oil.
NMR ($CDCl_3$, 100 MHz); δ 2.26 (2H, m); 2.50 (2H, m); 3.28 (2H, m); 7.42 (1H, dd); 8.26 (1H, dd); 8.77 (1H, dd); IR υ_{max} (LF) 3050, 2940, 2850, 2230, 1600, 1560, 1520 and 1340 cm^{-1}.

A sample was converted to the hydrochloride salt by treatment with ethanol saturated with hydrogen chloride gas and recrystallisation from ethanol, m.p. 143-145.5°C. (Found: C, 47.65; H, 4.45; N, 18.6%.
$C_9H_9N_3O_2.HCl$ requires C, 47.5; H 4.45; N, 18.45%); NMR (DMSO d_6,

100 MHz); δ 2.08 (2H, m); 2.63 (2H, m); 3.16 (2H, m); 7.61 (1H, dd); 8.43 (1H, dd); 8.86 (1H, dd).

Hydrolysis of diethyl 2-(2-cyanoethyl)-2-(5-nitropyrid-2-yl)-malonate (68b)

1) With 2 equivalents of base

Diethyl 2-(2-cyanoethyl)-2-(5-nitropyrid-2-yl)malonate (68b) (4.36 g, 0.013 mole) was dissolved in ethanol (180 ml) and sodium hydroxide solution (1 M; 26 ml, 0.026 mole) and the reaction was monitored by GLC. The reaction appeared to give two components by GLC and had stopped after 24 hr. The red solution was acidified with dilute hydrochloric acid to give a solution of pH 3. The solution was concentrated on the rotary evaporator to about 30 ml before being cooled and extracted with chloroform (3 x 20 ml). The combined chloroform extracts were dried over magnesium sulphate, the solvent was removed to give a oil which GC/MS and NMR indicated to be a mixture of the monoester (70) and the fully hydrolysed product (69b) in a ratio of 2 : 1.

2) With 4 equivalents of base: Preparation of 2-(3-cyanopropyl)-5-nitropyridine (69b)

Diethyl 2-(2-cyanoethyl)-2-(5-nitropyrid-2-yl)malonate (68b) (43.6 g, 0.13 mole) was dissolved in ethanol (2.0 l) and sodium hydroxide solution (1 M; 520 ml, 0.52 mole) and the deep magenta solution was stirred at ambient temperature for 6.5 hr. The now brown-red solution was acidified with dilute hydrochloric acid to give a solution of pH 3. The solution was concentrated on the rotary evaporator to about (700 ml) before being cooled and extracted with chloroform (3 x 200 ml). The combined chloroform extracts were dried over magnesium sulphate, the solvent was removed and the residue was recrystallised from ether to give the pyridine (69b) (23 g, 93%) as a white solid, m.p. 55-55.5°C. (Found: C, 56.65; H, 4.75; N, 21.8%. $C_9H_9N_3O_2$ requires C, 56.55; H, 4.75; N, 22.0%); NMR ($CDCl_3$, 100 MHz); δ 2.18 (2H, m); 2.44 (2H, m);

3.08 (2H, m); 7.48 (1H, d, J 10); 8.42, (1H, dd, J 10,3); 9.35 (1H, d, J 3); IR υ_{max} (NM) 2230, 1600, 1585 and 1520 cm^{-1}.

2-(3-Cyanopropyl)-3-methyl-5-nitropyridine (69c)

This compound was first prepared by Mr M.L. Meeson. A suspension of diethyl 2-(2-cyanoethyl)-2-(5-methyl-3-nitropyrid-2-yl)malonate (68c) (99 g, 2.83 mole) in ethanol 1.2 l and sodium hydroxide solution (1 M; 1.13 l, 1.13 mole) was stirred at room temperature for 5.5 hr. The solution was acidified (pH = 3) and its volume was reduced on the rotary evaporator to about (1.25 l) and was extracted with chloroform (2 x 400 ml). The chloroform extracts were combined and dried over magnesium sulphate. The solvent was removed to give a fawn solid which was dissolved in water (480 ml) and concentrated hydrochloric acid (74 ml). The solution was extracted with chloroform (3 x 200 ml). The combined chloroform extracts were dried over magnesium sulphate and the solvent removed to give a white solid which was recrystallised from ether to give the pyridine (69c) as a white solid (49.54 g, 85%), m.p. 51.5-53°C. (Found: C, 58.25; H, 5.6; N, 20.4%. $C_{10}H_{11}N_3O_2$ requires C, 58.55; H, 5.4; N, 20.5%); NMR ($CDCl_3$, 100 MHz); δ 2.25 (2H, m); 2.46 + 2.52 (5H, s + m); 3.02 (2H, m); 8.22 (1H, d); 9.18 (1H, d); m/e 205 (M^+) 204, 190, 177, 165, 152, 106 and 79; IR υ_{max} (NM) 2240, 1590, 1520 and 1350 cm^{-1}.

2-(3-Cyanopropyl)-6-methyl-3-nitropyridine (69d)

A solution of diethyl 2-(2-cyanoethyl)-2-(6-methyl-3-nitropyrid-2-yl)malonate (68d) (3.5 g, 0.01 mole) in ethanol (175 ml) and sodium hydroxide solution (1 M; 40 ml, 0.04 mole) was allowed to stand at room temperature for 4 days. The solution was refluxed for 1 hr, cooled and filtered. The filtrate was concentrated to about 50 ml and extracted with chloroform (3 x 30 ml). The combined chloroform extracts were dried over magnesium sulphate and the solvent was removed to give the pyridine (69d) as a straw coloured

oil (1.91 g, 99%). NMR ($CDCl_3$, 100 MHz); δ 2.26 (2H, m); 2.51 (2H, m); 2.64 (3H, s); 3.25 (2H, m); 7.23 (1H, d); 8.20 (1H, m); IR υ_{max} (LF) 2920, 2230, 1590, 1520, 1460 and 1350 cm^{-1}.

2-(4-Aminobutyl)-3-nitropyridine (75a)

This amine was first prepared by Dr G.S. Sach and this is a minor modification of that method. Boron trifluoride diethyletherate (30 ml) was added over 10 min to a solution of 2-(3-cyanopropyl)-3-nitropyridine (69a) (10 g, 52 mmole) in THF (100 ml), stirred under nitrogen. To this solution was added borane in THF (1 M; 235 ml, 0.235 mole) over 40 min and the resulting solution was stirred for 2.5 hr. Ethanol (500 ml) was added over 20 min and the solution was stirred for 40 min. Hydrochloric acid (1 M; 500 ml, 0.5 mole) was added over 30 min maintaining the temperature below 20°C and the solution was stirred for a further hour. The mixture was concentrated to 600 ml on the rotary evaporator. The solution was adjusted to pH 12 and extracted with chloroform (4 x 300 ml). The chloroform extracts were combined, dried over magnesium sulphate and the solvent removed to give the nitropyridine (75a) as an oil (4.95 g, 48%). NMR ($CDCl_3$, 100 MHz); δ 1.42 - 1.95 (6H, m); 2.72 (2H, t); 3.10 (2H, t); 7.35 (1H, dd, J 7,5); 8.18 (1H, dd, J 7,2); 8.73 (1H, dd, J 2,5); IR υ_{max} (LF) 3380, 2950, 2880, 1600, 1585, 1420 and 1340 cm^{-1}.

2-(4-Aminobutyl)-5-nitropyridine (75b)

Diborane solution (1 M; 45 ml, 45 mmole) was added dropwise over 20 min to a solution of 2-(3-cyanopropyl)-5-nitropyridine (69b) (1.91 g, 10 mmole) and boron trifluoride diethyletherate (5.7 ml) in THF (20 ml). The solution was stirred for 2 hr when ethanol (100 ml) was added over 20 min. Hydrochloric acid (1 M; 100 ml, 0.1 mole) was added and the solution was concentrated on the rotary evaporator to about 90 ml. The solution was extracted with chloroform (2 x 100 ml) and the chloroform extracts were discarded. The

solution was adjusted to pH 12 and extracted with chloroform (4 x 100ml). The chloroform extracts were combined, dried over magnesium sulphate and the solvent was removed to give the nitropyridine (75b) (1.44 g, 73%) contaminated with approximately 20% of the corresponding aminopyridine (77b). NMR ($CDCl_3$, 60 MHz); δ 1.42-1.95 (6H, m); 2.51-3.10 (4H, m); 6.90 (0.4H, m); 7.31 (0.8H, d, J 8); 7.97 (0.2H, m); 8.35 (0.8H, dd, J 2,8); 9.3 (0.8H, d, J 2) the peaks at 6.9 and 7.32 corresponding to those for the 5-aminopyridine (77b); IR υ_{max} (LF) 3360, 3280, 2950, 2800, 1600, 1585, 1420 and 1350 cm^{-1}.

3-Amino-2-(3-cyanopropyl)pyridine (76a)

The hydrogenation was carried out on a Parr rocking hydrogenator fitted with a 1.7 l stainless steel vessel with integral ice water cooling jacket. The vessel was charged with 2-(3-cyanopropyl)-3-nitropyridine (69a) (30.2 g, 0.158 mole), 10% palladium on carbon (3.0 g), ethanol (650 ml) and pressurised with hydrogen to 20 PSI. The vessel was agitated and the internal temperature was maintained at 10°C and hydrogen uptake was complete within 50 min. Agitation was continued for 1 hr at 20°C. The catalyst was filtered off and washed with ethanol. The filtrates were evaporated leaving an oil (25.6 g), which was dissolved in ether (60 ml), and allowed to crystallise to give the aminopyridine (76a) (21.5 g, 85%), m.p. 59.5-63.5°C.

A further crop (2.25 g) was obtained from the mother liquors. Total yield (23.75 g, 94%). (Found: C, 67.1; H, 6.8; N, 26.3%. $C_9H_{11}N_3$ requires: C, 67.05; H, 6.9; N, 26.1%); NMR ($CDCl_3$, 100 MHz); δ 2.22 (2H, m); 2.50 (2H, m); 2.80 (2H, m); 3.68 (2H, br); 6.9 (2H, m); 7.97 (1H, dd); IR υ_{max} (NM) 3420, 3340, 2230, 1630 and 1580 cm^{-1}.

5-Amino-2-(3-cyanopropyl)pyridine (76b)

A 250 ml Parr hydrogenator was charged with a mixture of 2-(3-cyanopropyl)-5-nitropyridine (69b) (3 g, 15.6 mmole), 10% palladium on carbon (0.3 g) in ethanol (80 ml). This was shaken for 1 hr under an atmosphere of hydrogen at 50 PSI, when uptake was complete. The solution was filtered and the filtrate was evaporated to give the aminopyridine (76b) as a straw coloured oil (2.5 g, 100%). NMR ($CDCl_3$, 60 MHz); δ 2.15 (2H, m); 2.33 (2H, m); 2.80 (2H, m); 6.90 (2H, m); 7.98, (1H, m); IR υ_{max} (LF) 3500-2800 (series of bands), 2200, 1620, 1600, 1585, 1490 and 1420 cm^{-1}.

A sample was converted to the hydrochloride salt by treatment with ethanol saturated with hydrogen chloride gas and recrystallised from isopropanol to give the salt as prisms, m.p. 149.5-151°C. (Found: C, 54.85; H, 6.15; N, 21.2; Cl, 17.75%. $C_9H_{15}N_3.HCl$ requires C, 54.7; H, 6.1; N, 21.25; Cl, 17.95%). NMR (D_2O, 100 MHz); δ 2.14 (2H, m); 2.60 (2H, m); 3.04 (2H, m); 7.70 (1H, m); 7.80, (1H, m); 7.98 (1H, m).

5-Amino-2-(3-cyanopropyl)-3-methylpyridine (76c)

A 250 ml Parr hydrogenator was charged with a mixture of 2-(3-cyanopropyl)-3-methyl-5-nitropyridine (69c) (10 g, 0.048 mole), 10% palladium on carbon (1.0 g) in ethanol (100 ml). This was shaken under an atmosphere of hydrogen at 50 PSI, maintaining the temperature of the solution at 15°C. Uptake of hydrogen was complete within 1 hr and the mixture was shaken for a further 1 hr. The solution was filtered, the filtrate evaporated to dryness and the residue recrystallised from ether to give the aminopyridine (76c) as a cream coloured solid (7.79 g., 91%), m.p. 101.5-104.5°C. (Found: C, 68.75; H, 7.75; N, 23.95%. $C_{10}H_{13}N_3$ requires C, 68.55; H, 7.5; N, 24.0%). NMR ($CDCl_3$, 100 MHz); δ 1.9 - 2.6 + 2.13 (7H, m + s); 2.80 (2H, m); 3.48 (2H, br); 6.77 (1H, m); 7.85 (1H, m); IR υ_{max} (NM) 3420, 3380, 3320, 3280, 2880, 2240, 1650, 1600 and 1480 cm^{-1}.

3-Amino-2-(3-cyanopropyl)-6-methylpyridine (76d)

A 250 ml Parr hydrogenator was charged with a mixture of 2-(3-cyanopropyl)-6-methyl-3-nitropyridine (69d) (1.8 g, 8.7 mmole), 10% palladium on carbon (0.27 g) in ethanol (50 ml). This was shaken under an atmosphere of hydrogen at 50 PSI. Uptake of hydrogen was complete within 30 min and the mixture was shaken for a further 30 min. The solution was filtered, the filtrate evaporated to dryness and the residue recrystallised from ether to give the aminopyridine (76d) as a cream coloured solid (1.51 g, 98%), m.p. 80.5-83.5°C. NMR (DMSO d_6, 100 MHz); δ 1.95 (2H, m); 2.27 (3H, s); 2.55 (4H, m); 4.85 (2H, m); 6.82 (2H, m); IR υ_{max} (NM) 3420, 3320, 3200, 2240, 1630 and 1570cm^{-1}.

3-Amino-2-(4-aminobutyl)pyridine (77a)

3-Amino-2-(3-cyanopropyl)pyridine (76a) (7.22 g, 0.045 mole) in dry THF (220 ml) was added over 1 hr to a stirred suspension of lithium aluminium hydride (5.0 g, 0.13 mole) in ether (150 ml) and THF (150 ml). The suspension was stirred at room temperature for 4 hr and then the complex was decomposed by the addition of water (5 ml) in THF (150 ml), 16% w/v sodium hydroxide solution (5 ml) and water (15 ml). The suspension was filtered and the solid washed with THF (50 ml) and ether (2 x 50 ml). The filtrates were combined and the solvent removed to give the butylamine (77a) as a low melting hygroscopic solid (5.98 g, 80%), m.p. 35°C. NMR ($CDCl_3$, 100 MHz); δ 1.4-1.9 (6H, m); 2.80 (2H, m); 3.65 (2H, br); 6.88 (2H, m); 7.95 (1H, m); IR υ_{max} (LF) 3320, 3220, 2980, 2860, 1620, 1580 and 1450 cm^{-1}.

5-Amino-2-(4-aminobutyl)pyridine (77b)

5-Amino-2-(3-cyanopropyl)pyridine (76b) (2 g, 12.4 mmole) in dry THF (50 ml) was added over 40 min to a stirred suspension of lithium aluminium hydride (1.4 g, 37 mmole) in THF (90 ml) and ether

(40 ml). The complex was stirred for 3 hr and then decomposed by the addition of water (1.4 ml) in THF (15 ml), 16% w/v sodium hydroxide (1.4 ml) and water (4.2 ml). The white suspension was filtered and the residue was washed with ether (3 x 50 ml). The filtrates were combined, the solvent was removed and the oil was distilled in a Kugelrohr apparatus (oven temp. 150°C at 0.05 mmHg) to give the butylamine (77b) as a low melting solid (1.76g, 86%).
NMR ($CDCl_3$, 100 MHz); δ 1.35-1.85 (6H, m); 2.55-2.8 (4H, m); 3.6 (2H, br); 6.90 (2H, m); 8.0 (1H, m); m/e 165 (M^+), 148, 135, 122, 121, 108, 107, 95, 80, 53, 30; IR υ_{max} (LF) 3900-2800 (series of bands), 1620, 1600, 1585 and 1490 cm^{-1}.

5-Amino-2-(4-aminobutyl)-3-methylpyridine (77c)

This compound was first prepared by Mr M.L. Meeson. A solution of 5-amino-2-(3-cyanopropyl)-3-methylpyridine (76c) (23.0 g, 0.12 mole) in dry THF (250 ml) was added over 20 min to a suspension of lithium aluminium hydride (12.4 g, 0.32 mmole) in dry ether (750 ml) and THF (500 ml). The suspension was stirred for 3.5 hr and then decomposed by the addition of water (12.5 ml) in THF (100 ml) over 20 min, 16% w/v sodium hydroxide (12.5 ml) over 20 min and water (36 ml) over 20 min. The inorganic solid was filtered off and the solvent was removed to give the pyridine (77c) as an oil (20.8 g, 88%). (Found: C, 66.7; H, 9.65; N, 23.0%. $C_{10}H_{17}N_3$ requires C, 67.0; H, 9.6; N, 23.4%). NMR ($CDCl_3$, 100 MHz); δ 1.3-1.9 (6H, m + s); 2.22 (3H, s); 2.7 (4H, m); 3.5 (2H, br); 6.77 (1H, d); 7.88 (1H, d); IR υ_{max} (LF) 3500-2800 (series of bands), 1620, 1600 and 1480 cm^{-1}.

2-(3-Cyanopropyl)-3-formylaminopyridine (78a)

A 90 ml stainless steel pressure vessel was charged with a solution of 3-amino-2-(3-cyanopropyl)pyridine (76a) (2.5 g, 15.5 mmole) in ethylformate (50 ml), sealed and heated at 115°C for 4 hr. The solvent was removed to yield the pyridine (78a) (3 g, 100%) as an

oil. NMR ($CDCl_3$, 60 MHz); δ 1.9-2.6 (4H, m); 3.06 (2H, t); 7.16(1H, dd); 7.62 (1.5H, br); 7.88(1H, d); 8.35 (1H, dd); IR υ_{max} (LF) 2350, 1690, 1580, 1520 and 1460 cm^{-1}.

2-(4-Aminobutyl)-3-methylaminopyridine (79a)

A solution of 2-(3-cyanopropyl)-3-formylaminopyridine (78a) (1.5 g, 18 mmole) in dry THF (10 ml) and ether (30 ml) was added over 20 min to a suspension of lithium aluminium hydride (1.3 g, 34 mmole) in dry ether (120 ml). The suspension was stirred for 3.5 hr, then decomposed by the addition of water (1.5 ml) in THF (20 ml) for 20 min, 40% w/v sodium hydroxide (1.5 ml) over 20 min and water (3.6 ml) over 20 min. The inorganic solid was filtered off and washed with ether (150 ml) to give a viscous oil (0.36 g). The inorganic residue was washed twice with chloroform. The chloroform extracts were combined and the solvent removed to give pyridine (79a) as a straw coloured oil (1.04 g, 84%). NMR (DMSO d_6, 100 MHz); δ 1.6 (4H, m); 2.6 + 2.7 + 2.9 (8H, m); 5.5 (1H, br); 6.9 + 7.1 (2H, m + dd); 7.72(1H, m); IR υ_{max} (LF) 3330, 2920, 2850, 1580, 1410 and 1360 cm^{-1}.

2-(3-Cyanopropyl)-3-dimethylaminopyridine (80a)

A Parr rocking hydrogenator was charged with a mixture of 3-amino-2-(3-cyanopropyl)pyridine (76a) (8.05 g, 50 mmole), 10% palladium on carbon (2.0 g), 40% w/w formaldehyde solution (30 ml) and water (20 ml). The suspension was shaken at 30°C under an atmosphere of hydrogen at 50 PSI for 6 hr. TLC indicated the reaction was not complete. 10% palladium on carbon (2 g) in 40% w/w formaldehyde solution (10 ml) was added and the suspension was shaken at 30°C under an atmosphere of hydrogen at 50 PSI for 5 hr. The suspension was filtered and the catalyst was washed with water (2 x 50 ml). The filtrates were combined and extracted with chloroform (3 x 50 ml). The chloroform extracts were washed consecutively with water (2 x 50 ml) in a counter current manner. The chloroform

extracts were combined, dried over magnesium sulphate and the solvent removed to give an oil (9.75 g) which was dissolved in ethanol (50 ml) and treated with ethanol saturated with hydrogen chloride gas (50 ml). A solid crystallised which was recrystallised from ethanol to give the pyridine (80a) as the dihydrochloride (12.05 g, 92%),m.p. 150-152°C as a hygroscopic solid. (Found: C, 49.35; H, 6.5; N, 15.5; Cl, 26.1% $C_{11}H_{15}N_3.2HCl.0.4H_2O$ requires C, 49.05; H, 6.6; N, 15.6; Cl, 26.3%); DSC/TGA weight loss of 2.5% w/w 60-100°C ($0.4H_2O$ = 2.6% w/w); NMR (D_2O, 60 MHz); δ 2.15 (2H, m); 2.65 (2H, m); 2.9 (6H, s); 3.25 (2H, m); 7.86, (1H, m); 8.25 (1H, m).

The hydrochloride of (80a) (11.0 g) was dissolved in dilute sodium hydroxide (40 ml) and extracted with chloroform (3 x 40 ml). The chloroform extracts were dried over magnesium sulphate, filtered and the solvent removed to give pyridine (80a) (7.65 g, 96%) as a straw coloured oil. NMR ($CDCl_3$, 100 MHz); δ 2.25 (2H, m); 2.44 (2H, m); 2.72 (6H, m); 3.05 (2H, m); 7.14 (1H, m); 7.38 (1H, m); 8.24 (1H, m); IR υ_{max} (LF) 3000-2500 (series of bands), 2240, 1590 and 1440 cm^{-1}.

2-(3-Cyanopropyl)-5-dimethylaminopyridine (80b)

A Parr rocking hydrogenator was charged with a mixture of 5-amino-2-(3-cyanopropyl)pyridine (76b) (3.22 g, 20 mmole), 40% w/w formaldehyde solution (12 ml), 10% palladium on carbon (2 g) and water (20 ml) and shaken at 35°C for 18 hr under an atmosphere of hydrogen at 50 PSI The suspension was filtered through Hyflow and the catalyst was washed by filtration with water (2 x 30 ml). The filtrates were combined and extracted with chloroform (3 x 60 ml). The chloroform extracts were washed consecutively with water (2 x 50 ml) in a counter current manner. The chloroform extracts were dried over magnesium sulphate, filtered and the solvent was removed to give an oil which was dissolved in ethanol (20 ml) and treated with ethanol saturated with hydrogen chloride gas (20 ml). The product was collected by filtration and attempts to recrystallise

the solid resulted in a very hygroscopic product (~ 4 g) which was dissolved in dilute sodium hydroxide solution (50 ml) and extracted with chloroform (3 x 50 ml). The chloroform extracts were combined, dried over magnesium sulphate, filtered and the solvent was removed to give pyridine (80b) as a straw coloured oil (2.24 g, 59%). NMR ($CDCl_3$, 60 MHz); δ 2.15 (2H, m); 2.32 (2H, m); 2.85 (2H, m); 2.95 (6H, s); 6.98 (2H, m); 8.08 (2H, m); IR υ_{max} (LF) 3000-2600 (series of bands), 2230 and 1600 cm^{-1}.

2-(4-Aminobutyl)-3-dimethylaminopyridine (81a)

A solution of 2-(3-cyanopropyl)-3-dimethylaminopyridine (80a) (1 g, 5.3 mmole) in ether (50 ml) was added dropwise over 30 min to a stirred suspension of lithium aluminium hydride (0.5 g, 13 mmole) in ether (50 ml). The mixture was stirred for 4 hr when the complex was decomposed by the consecutive addition of water (0.5 ml) in THF (10 ml), 16% w/v sodium hydroxide solution (0.5 ml) and water (1.5 ml). The suspension was filtered and the inorganic solid was washed with ether (2 x 50 ml). The filtrates were combined and the solvent removed to yield the butylamine (81a) (1 g, 98%) as a straw coloured oil. NMR ($CDCl_3$, 100 MHz); δ 1.32 (2H, s); 1.4-1.9 (4H, m); 2.68 (6H, s); 2.7-2.95 (4H, m); 7.04 (1H, dd, J 8,4); 7.33 (1H, dd, J 8,2); 8.22 (1H, dd, J 4,2); IR υ_{max} (LF) 3380, 3280, 2920, 2850, 1600, 1560 and 1500 cm^{-1}; m/e 193 (M^+), 163, 149, 135 and 30.

2-(4-Aminobutyl)-5-dimethylaminopyridine (81b)

A solution of 2-(3-cyanopropyl)-5-dimethylaminopyridine (80b) (2.0 g, 10.5 mmole) in ether (30 ml) was added dropwise to a stirred suspension of lithium aluminium hydride (1 g, 26 mmole) in ether (160 ml). The suspension was stirred for 5 hr and then decomposed by the addition of water (5 ml). The suspension was filtered and the residue washed with ether (3 x 100 ml). The filtrates were combined and the solvent was removed to give the amine (81b)

(1.81 g, 89%) as a straw coloured oil. NMR ($CDCl_3$, 60 MHz); δ 1.4 (2H, s); 1.77 (4H, m); 2.72 (4H, m); 2.92 (6H, s); 6.96 (2H, m); 8.04 (1H, m); IR υ_{max} (LF) 3360, 3280, 2920, 2850, 1600, 1585 and 1500 cm^{-1}.

3-Bromo-2-(3-cyanopropyl)pyridine (82a)

Sodium nitrite (0.94 g, 13.6 mmole) in water (3 ml) was added over 50 min to a rapidly stirred solution of 3-amino-2-(3-cyanopropyl)-pyridine (76a) (2 g, 12.4 mmole), cuprous bromide (2.13 g, 14.8 mmole), 48% w/v hydrobromic acid and water (2.2 ml). The solution was maintained at 5°C during the addition and for a further 1 hr after which time evolution of nitrogen had ceased. The solution was treated with hydrogen sulphide gas and 40% w/v sodium hydroxide (14 ml) to give a black suspension. The suspension was filtered through Hyflow and the black solid washed by filtration with water (2 x 20 ml). The filtrates were combined, adjusted to pH 8 and extracted with chloroform (3 x 70 ml). The chloroform extracts were combined, dried over magnesium sulphate, filtered and the solvent removed to give the bromopyridine (82a) (1.87 g, 67%) as an oil. NMR ($CDCl_3$, 60 MHz); δ 1.90-2.60 (4H, m); 3.05 (2H, t); 6.92 (1H, dd); 7.86 (1H, dd); 8.40 (1H, dd); IR υ_{max} (LF) 3020, 2940, 2220, 1590, 1540 cm^{-1}.

2-(4-Aminobutyl)-3-bromopyridine (83a)

A solution of 3-bromo-2-(3-cyanopropyl)pyridine (82a) (1.16 g, 5.15 mmole) in ether (15 ml) was added to a suspension of lithium aluminium hydride (0.57 g, 15 mmole) in ether (50 ml) and the solution was stirred for 2.5 hr. The complex was decomposed by the consecutive addition of water (0.6 ml) in ether (20 ml), 16% w/v sodium hydroxide solution (0.6 ml), and water (1.5 ml). The suspension was filtered and the residue washed with ether (3 x 50 ml). The combined filtrates were evaporated to dryness to give an oil which NMR and GLC indicated to be a mixture of the

butylamine (83a) and 2-(4-aminobutyl)pyridine (84) in a ratio of 6 : 4.

2-(4-Aminobutyl)-3-bromopyridine (83a)

Sodium nitrite (2.08 g, 24 mmole) in water (10 ml) was added dropwise to a rapidly stirred solution of 3-amino-2-(4-aminobutyl)-pyridine (77a) (4 g, 24 mmole), cuprous bromide (4.3 g, 30 mmole), copper bronze (0.16 g), 48% w/w hydrobromic acid (40 ml) and water (4 ml) maintained at 5-8°C. After the addition was complete the solution was stirred at 5-8°C for 1 hr, then at room temperature for 1 hr. The solution was poured onto crushed ice (60 g) containing 40% w/v sodium hydroxide solution (10 ml) and treated with hydrogen sulphide gas. The black suspension was filtered and the filtrate adjusted to pH 4 and retreated with hydrogen sulphide gas and filtered. The filtrates were combined, adjusted to pH 12 and extracted with chloroform (3 x 120 ml). The chloroform extracts were dried over magnesium sulphate, the solvent was removed and the oil was distilled in a Kugelrohr apparatus (oven temp. 105°C at 0.05 mmHg) to give the bromopyridine (83a) (4.08g, 74%) as a straw coloured oil. NMR ($CDCl_3$, 60 MHz); δ 1.4-1.9 (4H, m); 1.6 (2H, s); 2.65-3.15 (4H, m); 6.98(1H, dd, J 8,5); 7.81 (1H, dd, J 8,2); 8.46 (1H, dd, J 5,2); m/e 229/231 $(M + H)^+$; 230/228, 213/211, 212/210, 200/198, 187/185, 184/186, 173/171, 160/158, 149, 132, 91, 64, 30; IR υ_{max} (LF) 3380, 3280, 2920, 2860, 1570 and 1460 cm^{-1}.

2-(4-Aminobutyl)-5-bromopyridine (83b)

Sodium nitrite (1.04 g, 15 mmole) in water (10 ml) was added dropwise over 45 min to a rapidly stirred solution of 5-amino-2-(4-aminobutyl)pyridine (77b) (2 g, 12 mmole). Cuprous bromide (4.3 g, 30 mmole), copper bronze (0.07 g), 48% w/w hydrobromic acid (20 ml) and water (2 ml) at 5-8°C. The resulting solution was then stirred at 5-8°C for 1 hr, at room temperature for a further 1 hr,

and then poured onto crushed ice (~ 50 g) containing 40% w/v sodium hydroxide solution (5 ml). The solution was treated with hydrogen sulphide gas, filtered and the filtrate adjusted to pH 11. The filtrate was extracted with chloroform (3 x 75 ml). The chloroform extracts were combined, dried over magnesium sulphate and the solvent removed to give the bromopyridine (83b) (2.0 g, 72%) as a straw coloured oil. NMR ($CDCl_3$, 60 MHz); δ 1.35-2.05 (4H, m); 1.7 (2H, s); 1.6-1.95 (4H, m); 7.05 (1H, d, J 8); 7.72 (1H, dd, J 8,2); 8.57(1H, d, J 2); m/e 230/ 228 (M^+) 213/211, 200/198, 187/185, 186/184, 173/171, 160/158, 92, 65, 30; IR υ_{max} (LF) 3340, 3260, 2910, 2860, 1580 and 1460 cm^{-1}.

Examination of the Sandmeyer reaction of 5-amino-2-(4-aminobutyl)-3-methylpyridine under various conditions

5-Amino-2-(4-aminobutyl)-3-methylpyridine (77c) (2 g, 11 mmole) was dissolved in 48% w/w hydrobromic acid (20 ml) and water (2 ml). The solution was cooled to -5°C and treated with a solution of sodium nitrite (0.86 g, 0.12 mmole) over 20 min. The solution was cooled to -10°C and 5 ml aliquots were treated as below:-

1) Added to 20% hydrobromic acid at 10°C over 2 min.
2) Added to 20% hydrobromic acid at 10°C and copper powder (15 mg).
3) Added to 20% hydrobromic acid at 10°C and cuprous bromide (0.1 g).
4) Added to 20% hydrobromic acid at 10°C and cupric bromide (0.1 g).
5) Cuprous bromide (150 mg) was added to the remaining solution which was then allowed to warm to room temperature.

All the above solutions were allowed to warm to room temperature over 30 min. Comparison of the solutions by TLC indicated that solutions 3 to 5 contained mainly the 2-(4-aminobutyl)-5-bromo-3-methylpyridine whereas solutions 1 and 2 contained much less of this product and were more highly contaminated by coloured by-products. Solution 1 was basified and extracted with chloroform (2 x 10 ml). The chloroform extracts were dried over magnesium sulphate and the solvent removed to give a black oil (0.3 g). TLC indicated this product was multi-component mixture and the IR showed a strong absorption band at 2120 cm^{-1}.

2-(4-Aminobutyl)-5-bromo-3-methylpyridine (83c)

This amine was first prepared by Mr M.L. Meeson. Sodium nitrite (17.51 g, 0.25 mole) in water (100 ml) was added dropwise to a rapidly stirred solution of 5-amino-2-(4-aminobutyl)-3-methylpyridine (77c) (36.4 g, 0.203 mole), cuprous bromide (35.5 g, 0.25 mole), copper bronze (1.3 g) 48% w/w hydrobromic acid (335 ml) and water (36 ml) maintained at 5-8°C. The addition took 1 hr and the solution was stirred at 5-8°C for 1 hr, then at room temperature for 1 hr. The solution was poured onto crushed ice (500 g) containing 40% w/v sodium hydroxide solution (80 ml) and was treated with sodium sulphide (60 g). The suspension was filtered and the filtrate adjusted to pH 4 and retreated with sodium sulphide and filtered. The filtrates were combined, adjusted to pH 12 and extracted with chloroform (3 x 700 ml). The chloroform extracts were dried over magnesium sulphate and the solvent was removed to give a straw coloured low melting solid which was vacuum distilled to give the bromopyridine (83c) (34.55 g, 70%) as a hygroscopic solid, b.p. 118-120°C at 0.05 mm Hg. (Found: C, 49.1; H, 6.2; N, 11.2; Br, 32.4%. $C_{10}H_{15}BrN_3$ requires C, 49.4; H, 6.2; N, 11.5; Br, 32.8%). NMR ($CDCl_3$, 100 MHz); δ 1.4-1.9 (6H, m); 2.3 (2H, s); 2.7-2.85 (4H, m); 7.53 (1H, d); 8.41 (1H, d); IR υ_{max} (LF) 3360, 3260, 3290, 2850, 1580 and 1460 cm^{-1}.

2-(4-Aminobutyl)-3-chloropyridine (86a)

Sodium nitrite (2.74 g, 40 mmole) in water (10 ml) was added over 1 hr to a stirred solution of 3-amino-2-(4-aminobutyl)pyridine (77a) (5.25 g, 32 mmole), cuprous chloride (3.88 g, 39 mmole), copper powder (0.2g) in hydrochloric acid (8 M; 65 ml, 0.52 mole) maintained at 6°C ± 1°C. The solution was stirred for 3 hr when TLC indicated the presence of the starting material. Sodium nitrite (1 g, 15 mmole) in water (5 ml) was added over 1 hr and the solution stirred at 6°C ± 1°C for 2 hr and then allowed to warm to room temperature. The solution was saturated with hydrogen sulphide gas and the black precipitate was filtered and washed with water (50 ml).

The combined filtrates were adjusted to pH 5, saturated with hydrogen sulphide and filtered. The solid was washed with water (50 ml). The filtrates were combined, adjusted to pH 13 and extracted with chloroform (3 x 200 ml). The chloroform extracts were combined, dried over magnesium sulphate, and the solvent removed to give pyridine (86a) (3.1 g, 53%) as a straw coloured oil.
NMR ($CDCl_3$, 60 MHz); δ 1.4-2.0 (6H, m); 2.55-3.05 (4H, m); 7.08 (1H, dd, J 5,8); 7.63 (1H, dd, J 8,1.5); 8.45 (1H, dd, J 5,1.5); IR υ_{max} (LF) 3360, 3290, 2920, 2850, 1585, 1460 and 1380 cm^{-1}.

2-(4-Aminobutyl)-5-chloropyridine (86b)

Sodium nitrite (2.09 g, 30 mmole) in water (10 ml) was added dropwise over 30 min to a rapidly stirred solution of 5-amino-2-(4-aminobutyl)pyridine (77b) (4 g, 24 mmole), cuprous chloride (2.95 g, 30 mmole), copper bronze (0.16 g), concentrated hydrochloric acid (36 ml) and water (13.5 ml) at 10°C. The solution was stirred at 10°C for 30 min then allowed to warm to room temperature. The solution was poured into water (200 ml) containing saturated sodium sulphide solution (20 ml) and a black precipitate was filtered and washed with water (20 ml). The combined filtrates was adjusted to pH 10 with 40% w/v sodium hydroxide solution and extracted with chloroform (3 x 200 ml). The combined chloroform extracts were dried over magnesium sulphate and solvent removed to give an oil which was distilled in a Kugelrohr apparatus (oven temp.110°C at 0.02 mm Hg) to give the chloropyridine (86b) as a clear oil (3.36 g, 75%).
NMR ($CDCl_3$, 100 MHz); δ 1.38 (2H, s); 1.3-1.9 (4H, m); 2.15-2.9 (4H, m); 7.10 (1H, d, J 8); 7.56 (1H, dd, J8,2); 8.48 (1H, d, J 2); m/e 184/6, 167/9, 154/6, 140/2, 127/9, 92; IR υ_{max} (LF) 3360, 3280, 2920, 2860, 1600, 1480 and 1370 cm^{-1}.

Attempted preparation of 2-(3-cyanopropyl)-3-fluoropyridine (87a)

3-Amino-2-(3-cyanopropyl)pyridine (76a) (1 g, 62 mmole) was dissolved in ethanol (10 ml) and 40% w/v fluoroboric acid (2.3 ml) and cooled

to -5°C which resulted in precipitation of a white solid. The mixture was treated with ethyl nitrite[161] over 1 hr to give a clear solution which was stirred at 0°C for 1 hr then warmed to 60°C for 20 min, cooled and diluted with water (50 ml). The solvent was partially removed on the rotary evaporator to give a solution volume of ~ 30 ml which was basified with sodium hydroxide and extracted with dichloromethane (4 x 30 ml). The combined dichloromethane extracts were dried over magnesium sulphate and the solvent was removed to give an oil (1 g) which NMR and GLC indicated to be a mixture of 2-(3-cyanopropyl)-3-fluoropyridine (87a) (60%), 2-(3-cyanopropyl)-3-ethoxypyridine (21) (26%) and 2-(3-cyanopropyl)-3-hydroxypyridine (4) (4%).

2-(3-Cyanopropyl)-3-fluoropyridine (87a)

Sodium nitrite (1.73 g, 25 mmole) was added portionwise over 1 hr to a solution of 3-amino-2-(3-cyanopropyl)pyridine (76a) (3.22 g, 20 mmole) in 40% w/v fluoroboric acid (18 ml) stirred at -5°C which resulted in a steady evolution of nitrogen. The solution was stirred at -5°C for 2 hr when evolution of nitrogen had ceased. The solution was allowed to warm to room temperature and was then stirred at 60°C for 20 min, cooled, basified with sodium hydroxide solution and extracted with chloroform (3 x 75 ml). The chloroform extracts were washed consecutively with dilute sodium hydroxide (50 ml), combined, dried over magnesium sulphate and the solvent removed to give the fluoropyridine (87a) as an oil (1.90g, 59%).
NMR ($CDCl_3$, 100 MHz); δ 2.05-2.35 (2H, m); 2.40-2.60 (2H, m); 2.85-3.15 (2H, m); 7.1-7.5 (2H, m); 8.3-8.45 (1H, m);
IR υ_{max} (LF) 3020, 2920, 2220, 1600, 1585 and 1460 cm^{-1}.

2-(4-Aminobutyl)-3-fluoropyridine (88a)

A solution of 2-(3-cyanopropyl)-3-fluoropyridine (87a) (1.8g, 11 mmole) in ether (40ml) was added over 30 min to a suspension of lithium aluminium hydride (1.15g, 30 mmole) in ether (200ml). The

suspension was stirred for 4 hr and then the complex was decomposed by the addition of water (1 ml) in THF (20ml), 16% w/v sodium hydroxide solution (1.2 ml) and water (3.6 ml). The suspension was allowed to stand at room temperature overnight, filtered and the inorganic solid was then washed with ether (200 ml). The combined filtrates were evaporated to dryness to give the fluoropyridine (88a) (1.65g, 89%) as an oil. NMR ($CDCl_3$, 100 MHz); δ 1.35-1.85 + 1.47 (6H, m + s); 2.72-3.00 (4H, m); 7.05-7.45 (2H, m); 8.3-8.5 (1H, m); m/e 168 (M^+), 151, 138, 132, 124, 111; GLC (OV1 at 160°C) retention time 5.8 min. IR υ_{max} (LF) 3360, 3280, 2920, 2860, 1600, 1585 and 1460 cm^{-1}.

2-(4-Aminobutyl)-5-pyridinediazonium tris-tetrafluoroborate (98b)

Amyl nitrite (9 ml) was added dropwise over 15 min to a solution of 5-amino-2-(4-aminobutyl)pyridine (77b) (3 g, 18 mmole), 40% w/v fluoroboric acid (15 ml) in ethanol (90 ml) stirred at 0°C to -5°C. The solution was stirred for 20 min at -3°C when a product had crystallised. Ether (200 ml) at -5°C was added and the solid was collected by filtration, washed with (2 x 20 ml) cold ether and transferred to a vacuum desiccator. The pale straw coloured needles (7.29 g) darkened in colour on storage at 5°C for 1.5 hr, m.p. 54-57°C dec; IR υ_{max} (NM) 2940, 2280, 1570, 1460 and 1050 cm^{-1}.

2-(4-Aminobutyl)-5-fluoropyridine (88b)

The diazonium salt (98b) (7.2g) was added in small portions over 30 min to petroleum ether, b.p. 100-120°C, stirred at 95-100°C. The mixture was stirred at 100°C for 15 min, cooled and the petrol decanted to leave a brown oil which was dissolved in dilute sodium hydroxide (125 ml) and extracted with dichloromethane (3 x 125 ml). The dichloromethane extracts were combined and dried over magnesium sulphate. The solvent was removed and the product was distilled in a Kugelrohr apparatus (oven temp. 90°C at 0.05 mm Hg) to give the fluoropyridine (88b) as a straw coloured oil, (1.91 g, 62% from 5-aminopyridine (77b)). NMR ($CDCl_3$, 100 MHz); δ 1.32 (2H, s);

1.4-1.95 (4H, m); 1.65-1.9 (4H, m); 7.05-7.45 (2H, m); 8.35-8.4 (1H, m); m/e 168 (M^+), 151, 138, 124, 111, 30; IR υ_{max} (LF) 3360, 3280, 2920, 2850, 1585, 1460 and 1380 cm^{-1}.

Attempted preparation of 2-(4-aminobutyl)-5-fluoro-3-methylpyridine (88c)

Sodium nitrite (1 g, 14.4 mmole) was added over 20 min to a stirred solution of 5-amino-2-(4-aminobutyl)-3-methylpyridine (77c) (2.17 g, 12.1 mmole) in 40% w/v fluoroboric acid (20 ml) at -6°C. The solution was stirred at -5°C for 1 hr, then at room temperature for 1 hr and then at 60°C for 30 min. The solution was poured onto ice, basified with sodium hydroxide and extracted into chloroform (3 x 100 ml). The chloroform extracts were combined, dried over magnesium sulphate and solvent removed to give a dark brown oil (1.1 g). TLC indicated one major component and many minor components. The product was not isolated.

2-(4-Aminobutyl)-3-methyl-5-pyridinediazonium tris-tetrafluoroborate (98c)

Amyl nitrite (3 ml) was added over 15 min to 5-amino-2-(4-aminobutyl)-3-methylpyridine (77c) (0.86 g, 4.8 mmole) in 40% w/v fluoroboric acid (5 ml) and ethanol (30 ml) stirred at 0°C to -5°C. The suspension was stirred at -5°C for 20 min. Ice cold ether (30 ml) was then added and the product collected by filtration and washed with ice cold ether (30 ml) to give a cream coloured solid (1.88 g), m.p. darkens from 80°C, melts ~ 120°C. IR υ_{max} (NM) 3280, 2280, 1610, 1585 and 1050 cm^{-1}.

2-(4-Aminobutyl)-5-fluoro-3-methylpyridine (88c)

The pyridinediazonium salt (98c) (1.78 g) was added in small portions over 10 min to petroleum ether, b.p. 100-120°C stirred at

95-100°C. The mixture was stirred at 100°C for 15 min, cooled and the petrol was decanted. The orange solid was dissolved in dilute sodium hydroxide solution (50 ml) and extracted with chloroform (3 x 50 ml). The chloroform extracts were combined, dried over magnesium sulphate and solvent removed to give the fluoropyridine (88c) (0.69 g, 78%) as an oil (yield quoted from the amino pyridine (77c)). NMR ($CDCl_3$, 100 MHz); δ 1.39 + 1.4-1.9 (6H, s + m); 2.30 (3H, s); 2.6-2.8 (4H, m); 7.14 (1H, dd, J 9,2); 8.19 (1H, d, J 2); IR υ_{max} (LF) 3360, 3280, 2950, 2860, 1600 and 1460 cm^{-1}.

Attempted preparation of 2-(4-aminobutyl)-3-iodopyridine (89a)

Sodium nitrite (2.08 g, 30 mmole) in water (20 ml) was added over 10 min to a rapidly stirred solution of 3-amino-2-(4-aminobutyl)-pyridine (77a) (4 g, 24 mmole) in concentrated sulphuric acid (8 ml) and water (40 ml) maintained at 6°C. The solution was stirred at 6°C for 1 hr then allowed to warm to room temperature over 1 hr after which time evolution of gas had ceased. The resulting black suspension was basified with ammonia (d 0.88) and extracted with chloroform (3 x 200 ml). The combined chloroform extracts were dried over magnesium sulphate, filtered and the solvent removed to give a black oil (1.95 g). This oil showed a strong absorption at 2120 cm^{-1}. NMR ($CDCl_3$, 100 MHz); δ 1.6 (2H, s); 1.55-1.95 (4H, m); 2.76 (2H, t); 3.0 (2H, t); 6.84 (1H, dd, J 8,5); 8.06 (1H, dd, J 8,2); 8.48 (1H, J 5,2). All attempts to purify this product failed.

2-(4-Aminobutyl)-3-iodopyridine (89a)

Sodium nitrite (5.65 g, 0.082 mole) in water (20 ml) was added over 10 min to a rapidly stirred solution of 3-amino-2-(4-aminobutyl)-pyridine (77a) (10.84 g, 0.065 mole) in concentrated sulphuric acid (25 ml) and water (125 ml) maintained at -5°C to -10°C. The solution was stirred at -6°C for 10 min then run into a rapidly stirred suspension of potassium iodide (21.68 g, 0.13 mole) and cuprous iodide (2.71 g, 0.014 mole) in water (400 ml) maintained at 8°C. The

resulting black suspension was allowed to warm to room temperature over 1 hr, basified with ammonia (d 0.88) and extracted with chloroform (3 x 200 ml). The combined chloroform extracts were dried over magnesium sulphate, filtered and the solvent removed to give a black oil (14.22 g) which was distilled in a Kugelrohr apparatus (oven temp. 130°C at 0.02 mm Hg) to give the pyridine (89a) (9.72 g, 54%) as a straw coloured oil. NMR ($CDCl_3$, 100 MHz); δ 1.6 (2H, s); 1.55-1.95 (4H, m); 2.76 (2H, t); 3.0 (2H, t); 6.84 (1H, dd, J 8,5); 8.06 (1H, dd, J 8,2); 8.48 (1H, J 5,2); m/e 276 (M^+), 259, 246, 233, 219, 206, 119, 105, 92, 65; IR υ_{max} (LF) 3360, 3280, 2920, 2850, 1600, 1540 and 1460 cm^{-1}.

2-(4-Aminobutyl)-5-iodopyridine (89b)

Sodium nitrite (2.15 g, 31 mmole) in water (10 ml) was added dropwise over 10 min to a rapidly stirred solution of 5-amino-2-(4-aminobutyl)pyridine (77b) (4.13 g, 25 mmole) in concentrated sulphuric acid (12 ml) and water (60 ml) at -8°C. The resulting solution was maintained at -8°C for 15 min then run into a rapidly stirred suspension of cuprous iodide (2 g, 10 mmole) and potassium iodide (10 g, 60 mmole) in water at 10°C. The resulting black suspension was stirred at room temperature for 1 hr, basified with ammonia (d 0.88) and extracted with chloroform (3 x 100 ml). The combined chloroform extracts were treated with magnesium sulphate. The solvent was removed to give an amber oil which was distilled in a Kugelrohr apparatus (oven temp. 150°C at 0.05 mmHg) to give the iodopyridine (89b) as a straw coloured oil (5.01g, 72%). NMR ($CDCl_3$, 100 MHz); δ 1.44 (2H, s), 1.4-1.85 (4H, m); 2.6-2.84 (4H, m); 6.95 (1H, d, J 8); 7.87 (1H, dd, J 8,2); 8.72 (1H, d, J 2); m/e 276 (M^+), 259, 246, 233, 219, 206, 119, 105, 92, 65, 30; IR υ_{max} (LF) 3360, 3280, 2920, 2860, 1570, 1460, 1360 and 1000 cm^{-1}.

2-(4-Aminobutyl)-5-iodo-3-methylpyridine (89c)

A solution of sodium nitrite (1 g, 14.5 mmole) in water (5 ml) was added over 20 min to a solution of 5-amino-2-(4-aminobutyl)-3-methylpyridine (77c) (2.17 g, 12.1 mmole) in concentrated sulphuric acid (10 ml) and water (5 ml) stirred at -9°C. The solution was stirred for 15 min at -9°C then poured into a rapidly stirred suspension of cuprous iodide (0.5 g, 2.6 mmole) and potassium iodide (0.4 g, 2.4 mmole) in water (65 ml) maintained at 10°C. The resulting suspension was allowed to warm to room temperature, adjusted to pH 12 and extracted with chloroform (4 x 100 ml). The chloroform extracts were washed consecutively with dilute sodium hydroxide solution. The combined chloroform extracts were dried over magnesium sulphate and the solvent was removed to give the iodopyridine (89c) (2.45 g, 69%) as an oil. NMR ($CDCl_3$, 100 MHz); δ 1.45-1.95, + 1.69 (6H, m + s); 2.30 + 2.60 (5H, t + s); 2.75 (2H, m); 7.74 (1H, m); 8.64 (1H, m); IR υ_{max} (LF) 3360, 3280, 2920, 2850, 1600, 1540 and 1460 cm^{-1}.

2-(4-Aminobutyl)-3-azidopyridine (90a)

Sodium nitrite (2 g, 29 mmole) in water (5 ml) was added dropwise to a rapidly stirred solution of 3-amino-2-(4-aminobutyl)pyridine (77a) (4 g, 24 mmole), concentrated sulphuric acid (10 ml) and water (50 ml) maintained at -7°C ± 2°C. The addition took 15 min and the solution was stirred for a further 15 min before being poured into a stirred solution of sodium azide (1.96 g, 30 mmole) and sodium acetate (30 g) in water (125 ml) at 8-9°C. The solution was stirred at room temperature for 1 hr and then extracted with chloroform (150 ml). The chloroform layer was discarded and the aqueous layer was adjusted to pH 12 and extracted with chloroform (3 x 150 ml). The chloroform extracts were combined, dried over magnesium sulphate and solvent removed to give the azidopyridine (90a) as an oil (2.34 g, 51%). NMR ($CDCl_3$, 100 MHz); δ 1.35-1.9 (4H, m); 1.45 (2H, s); 2.6-2.9 (4H, m); 7.16 (1H, dd, J 5,8); 7.38 (1H, dd, J 8,2); 8.28 (1H, dd, J 5,2); IR υ_{max} (LF) 3420, 3320, 2940, 2880, 2120, 1595 and 1450 cm^{-1}.

2-(3-Cyanopropyl)-3-methoxypyridine (91a)

A solution of 3-amino-2-(3-cyanopropyl)pyridine (76a) (9.05 g, 0.056 mole) was dissolved in methanol (70 ml) and concentrated sulphuric acid (6.5 ml) and stirred at 5°C during the slow addition of finely ground sodium nitrite (8 g, 0.12 mole) over 1 hr. The solution was stirred at 5-9°C for 3 hr then allowed to warm to room temperature. Water (150 ml) was added, the solution adjusted to pH 13 by the addition of sodium hydroxide solution and extracted with chloroform (1 x 200 ml, 2 x 100 ml). The chloroform extracts were combined and dried over magnesium sulphate. The solvent was removed and the residue was chromatographed on silica gel eluted with 10% methanol in chloroform to give the methoxypyridine (91a) (5.2 g, 52%) as a red oil. NMR ($CDCl_3$, 100 MHz); δ 2.15 (2H, m); 2.42 (2H, m); 2.96 (2H, m); 3.83 (2H, s); 7.15 (2H, m); 8.12 (1H, m). Impurity peaks at δ 7.75 and 8.55 integrating for ~ 10%. GLC (OV 17 at 175°C) peak 1 at 3.5 min ~ 5% by peak area, 2 at 7.25 min ~ 95% by peak area: peak 1 corresponds to 2-(3-cyanopropyl)pyridine. IR υ_{max} (LF) 3100-2600 (series of bands), 2240, 1595, 1585, 1450 and 1420 cm^{-1}.

2-(3-Cyanopropyl)-3-hydroxypyridine (93a)

3-Amino-2-(3-cyanopropyl)pyridine (76a) (8.41 g, 0.052 mole) was dissolved in a mixture of concentrated sulphuric (4.7 ml) and water (40 ml) at 8°C. Sodium nitrite (4.57 g, 0.066 mole) in water (14 ml) was added over 35 min while maintaining the temperature at 9°C. The solution was stirred at 8°C for 2 hr when evolution of nitrogen had ceased. The solution was allowed to warm to room temperature. Water (50 ml) was then added and the solution adjusted to pH 12 with 40% w/v sodium hydroxide solution and extracted with chloroform (2 x 100 ml). The chloroform extracts were discarded. The aqueous layer was adjusted to pH 6.5 and extracted with chloroform (3 x 100 ml). The chloroform extracts were combined, dried over magnesium sulphate, evaporated to dryness and the residue was recrystallised from chloroform/petrol to give

the pyridine (93a) (6.94 g, 81%), m.p. 112-112.5°C. (Found: C, 66.65; H, 6.2; N, 17.4%. $C_9H_{10}N_2O$ requires C, 66.65; H, 6.2; N, 17.3%). NMR (DMSO d_6, 100 MHz); δ 1.93 (2H, m); 2.53 (2H, m); 2.80 (2H, m); 7.1 (2H, m); 7.96 (1H, m); IR υ_{max} (NM) 2580, 2460, 2240, 1810 and 1585 cm^{-1}.

2-(3-Cyanopropyl)-3-methoxypyridine (91a)

A solution of 2-(3-cyanopropyl)-2-hydroxypyridine (93a) (6.9 g, 0.042 mole) in DMSO (50 ml) was stirred at 17-21°C during the addition of sodium hydride (1.02 g, 0.042 mole) over 30 min. The solution was stirred at 18°C for a further 10 min. Methyl iodide (5.61 g, 0.042 mole) in DMSO (10 ml) was added dropwise over 30 min and then the solution was stirred at room temperature for 2 hr. The resulting solution was evaporated to dryness (70°C at 1 mmHg) to give a waxy solid which was partitioned between water (50 ml) and chloroform (60 ml). The chloroform layer was run off and the aqueous layer was further extracted with chloroform (2 x 60 ml). The chloroform extracts were washed with sodium hydroxide solution (1 M; 60 ml, 0.06 mole), dried over magnesium sulphate, filtered and the filtrate evaporated to dryness to give the methoxypyridine (91a) (5.62 g, 74%) as an oil. NMR ($CDCl_3$, 100 MHz); δ 2.15 (2H, m); 2.42 (2H, m); 2.96 (2H, m); 3.83 (2H, s); 7.15 (2H, m); 8.12 (1H, m); IR υ_{max} (LF) 3100-2700 (series of bands), 2230, 1595, 1580, 1450 and 1420 cm^{-1}.

2-(4-Aminobutyl)-3-methoxypyridine (94a)

A solution of 2-(3-cyanopropyl)-3-methoxypyridine (91a) (5.2 g, 0.029 mole) in ether (80 ml) was added over 1 hr to a stirred suspension of lithium aluminium hydride (3.08 g, 0.081 mole) in ether (280 ml). The suspension was stirred for 3.5 hr when the complex was decomposed by the consecutive addition of water (3 ml) in THF (30 ml), 16% w/v sodium hydroxide solution (3.1 ml) and water (9.2 ml). The inorganic solid was filtered off and washed by

filtration with ether (2 x 100 ml). The combined filtrates were evaporated to dryness to yield a amber oil (5.1 g) which was partitioned between water (60 ml) and chloroform (60 ml). The solution was adjusted to pH 6 and the chloroform layer was run off. The aqueous layer was extracted with chloroform (2 x 60 ml). The chloroform extracts were discarded. The aqueous layer was adjusted to pH 12 and extracted with chloroform (3 x 60 ml). The combined chloroform extracts were dried over magnesium sulphate, filtered and the filtrate concentrated to give the methoxypyridine (94a) (4.15 g, 81%) as a straw coloured oil. NMR ($CDCl_3$, 100 MHz); δ 1.27 (2H, s); 1.35-1.8 (4H, m); 2.65-2.95 (4H, m); 3.8 (3H, s); 7.08 (2H, m); 8.1 (1H, m); IR υ_{max} (LF) 3380, 3260, 3100-2800 (series of bands), 1590, 1570, 1450, 1430 and 1280 cm^{-1}; m/e 180 (M^+), 164, 150, 137, 136, 123, 122, 110 and 30.

2-(3-Cyanopropyl)-3-hydroxy-6-methylpyridine (93d)

3-Amino-2-(3-cyanopropyl)-6-methylpyridine (76d) (1.45 g, 8.3 mmole) was dissolved in a mixture of concentrated sulphuric acid (1.1 ml) and water (14 ml) at 7°C. Sodium nitrite (0.85 g, 12.3 mmole) in water (4.4 ml) was added over 30 min while maintaining the temperature at 7°C. The solution was stirred at 7°C for 2 hr when evolution of nitrogen had ceased. The solution was allowed to warm to room temperature, adjusted to pH 6 with 10% w/v sodium hydroxide solution and extracted with chloroform (2 x 30 ml). The chloroform extracts were combined, dried over magnesium sulphate, filtered and the filtrate concentrated to give the pyridine (93d) (0.875 g, 60%), m.p. 152.5-154°C. NMR (DMSO d_6, 60 MHz); δ 1.85 (2H, m); 2.30 (3H, s); 3.3-3.8 (4H, m); 6.92 (2H, m); 9.45 (1H, exch.); IR υ_{max} (LF) 3400-2800 (series of bands), 2130, 1800, 1640, 1580 and 1460 cm^{-1}.

2-(3-Cyanopropyl)-6-methyl-3-methoxypyridine (91d)

A solution of 2-(3-cyanopropyl)-2-hydroxy-6-methylpyridine (93d) (0.8 g, 4.62 mmole) in DMSO (8 ml) was stirred at 17-21°C during the addition of sodium hydride (in oil) (0.2 g, 4.7 mmole) over 10 min. The solution was stirred at 19°C for a further 30 min. Methyl iodide (0.66 g, 4.6 mmole) in DMSO (2 ml) was added over 10 min and then the solution was stirred at room temperature for 16 hr. The resulting solution was evaporated to dryness (70°C, 1 mmHg) to give a waxy solid which was partitioned between water (20 ml) and chloroform (20 ml). The chloroform layer was run off and the aqueous layer was further extracted with chloroform (2 x 20 ml). The chloroform extracts were washed with sodium hydroxide solution (1 M; 20 ml, 0.02 mole), dried over magnesium sulphate, filtered and the filtrate evaporated to dryness to give methoxypyridine (91d) as a straw coloured oil (0.525 g, 55%). NMR ($CDCl_3$, 100 MHz); δ 2.04 (2H, m); 2.42 + 2.48 (5H, m + s); 2.92 (2H, m); 3.80 (3H, s); 7.00 (2H, m); IR υ_{max} (LF) 3500-2800 (series of bands), 2130, 1600, 1580 and 1460 cm^{-1}.

2-(4-Aminobutyl)-3-methoxy-6-methylpyridine (94d)

A solution of 2-(3-cyanopropyl)-3-methoxy-6-methylpyridine (91d) (0.49 g, 2.3 mmole) in ether (25 ml) was added over 30 min to a stirred suspension of lithium aluminium hydride (0.27 g, 7.1 mmole) in ether (25 ml). The suspension was stirred for 3.5 hr when the complex was decomposed by the addition of water (0.3 ml) in THF (10 ml) over 15 min, 16% w/v sodium hydroxide solution (0.3 ml) and water (0.8 ml). The inorganic solid was filtered off and washed by filtration with ether (2 x 40 ml). The combined filtrates were evaporated to dryness to yield the butylamine (94d) as a straw coloured oil (0.48 g, 98%). NMR ($CDCl_3$, 100 MHz); δ 1.55 (4H, m); 1.82 (2H, s); 2.43 (3H, s); 3.86 (3H, s); 6.95 (2H, m); IR υ_{max} (LF) 3400-2800 (series of bands), 1610, 1590 and 1460 cm^{-1}.

Attempted preparation of 2-(3-cyanopropyl)-5-hydroxypyridine (93b)

5-Amino-2-(3-cyanopropyl)pyridine (76b) (0.84 g, 5.2 mmole) was dissolved in a mixture of concentrated sulphuric acid (1.2 ml) and water (10 ml) at 7°C. Sodium nitrite (0.46 g, 6.6 mmole) in water (4 ml) was added over 20 min while maintaining the temperature at 7°C. The solution was stirred at 7°C for 2.5 hr to give a dark coloured solution which was then allowed to warm to room temperature, adjusted to pH 6 with 10% w/v sodium hydroxide solution and extracted with chloroform (2 x 30 ml). The chloroform extracts were combined, dried over magnesium sulphate, filtered and the filtrate concentrated to give a black tar which on TLC showed many coloured components.

2-(3-Cyanopropyl)-5-diazoniumpyridine di-tetrafluoroborate (95b)

A solution of 5-amino-2-(3-cyanopropyl)pyridine (76b) (4.2 g, 2.6 mmole), 40% w/v fluoroboric acid (17 ml, 0.1 mole) and ethanol (75 ml) was added dropwise to a stirred solution of amyl nitrite (17 ml, 0.12 mole) in ethanol (75 ml) stirred at 0°C over 1 hr. The resulting solution was stirred at 0°C for 1 hr and poured into cold ether (200 ml) causing precipitation of the diazonium salt (95b) (5.9 g, 62%), m.p. 56-67°C; IR υ_{max} (NM) 2280, 2220, 1570 and 1050 cm^{-1}.

2-(3-Cyanopropyl)-5-hydroxypyridine (93b)

A solution of 2-(3-cyanopropyl)-5-diazoniumpyridine di-tetrafluoroborate (95b) (5.9 g, 0.016 mole) in water (150 ml) was heated at 60°C for 40 min when a steady evolution of a gas was observed. The resulting solution was cooled, adjusted to ~ pH 6.5 and extracted with chloroform (3 x 100 ml). The chloroform extracts were combined, dried over magnesium sulphate and the solvent removed to give a yellow solid, (2.7 g), m.p. 100-104°C. The residue was dissolved in ether, treated with charcoal, filtered through Hyflow

and crystallised to give the pyridine (93b), (1.96 g, 72%), m.p. 105.5-107°C. (Found: C, 66.8; H, 6.2; N, 17.25%. $C_9H_{10}N_2O$ requires C, 66.65; H, 6.2; N, 17.3%). NMR (DMSO d_6, 100 MHz); δ 1.93 (2H, m); 2.48 (2H, m); 2.72 (2H, m); 7.08 (2H, m); 8.05 (1H, m); 10.00 (1H, exch.); IR υ_{max} (NM) 2560, 2460, 2230 and 1585 cm^{-1}.

2-(3-Cyanopropyl)-5-methoxypyridine (91b)

Sodium hydride (53% dispersion in oil; 0.37 g, 8 mmole) was added to a stirred solution of 2-(3-cyanopropyl)-5-hydroxypyridine (93b) (1.3 g, 8 mmole) in DMSO (50 ml) maintained at 17-21°C. The solution was stirred at 21°C for 30 min to give a clear solution, when methyl iodide (1.14 g, 8 mmole) in DMSO (5 ml) was added. The solution was stirred at room temperature for 1 hr. The DMSO was removed on the rotary evaporator (0.1 mmHg) to give a brown slurry which was partitioned between chloroform (20 ml) and dilute hydrochloric acid (20 ml). The chloroform layer was run off and the aqueous layer was re-extracted with chloroform (20 ml), the chloroform extracts being discarded. The aqueous layer was basified (pH 12) and extracted with chloroform (3 x 20 ml). The chloroform extracts were combined, washed with sodium hydroxide (1M; 30 ml) and then dried over magnesium sulphate. The solvent was removed to give the methoxypyridine (91b) (1.12 g, 80%) as a straw coloured oil. NMR (100 MHz, $CDCl_3$); δ 2-2.5 (4H, m); 2.8-3.0 (2H, m); 3.83 (3H, s); 7.1 (2H, m); 8.20 (1H, m); m/e 176 (M^+) 136, 123, 108, 93, 80, 78 and 65; IR υ_{max} (LF) 2950, 2860, 2220, 1600, 1585 and 1490 cm^{-1}.

2-(4-Aminobutyl)-5-methoxypyridine (94b)

2-(3-Cyanopropyl)-5-methoxypyridine (91b) (1.48 g, 8.3 mmole) in ether (50 ml) was added dropwise to a stirred suspension of lithium aluminium hydride (0.86 g, 22 mmole) in ether (60 ml). The addition took 40 min and the resulting grey suspension was stirred for a

further 3 hr. The complex was decomposed by the consecutive addition of water (0.8 ml) in THF over 20 min, 16% w/v sodium hydroxide (0.9 ml) over 20 min and water (2.5 ml) over 20 min. The inorganic solid was filtered off and washed with ether (2 x 30 ml). The filtrates were combined and the solvent removed to give the methoxypyridine (94b) (1.41 g, 93%) as a straw coloured oil. NMR ($CDCl_3$, 100 MHz); δ 1.4-1.9 (6H, m); 2.6-2.85 (4H, m); 2.83 (3H, s); 7.1 (2H, m); 8.2 (1H, m); IR υ_{max} (LF) 3320, 3280, 2980, 2880, 1585 and 1420 cm^{-1}; m/e 180 (M^+), 164, 150, 150, 137, 336, 123, 122, 110 and 30.

3-Benzyloxy-2-(3-cyanopropyl)pyridine (96a)

Sodium hydride (0.89 g, 37 mmole) was added slowly to a solution of 2-(3-cyanopropyl)-3-hydroxypyridine (93a) (6 g, 37 mmole) in DMSO (45 ml) stirred at 19°C. The resulting solution was stirred for 45 min when a solution of benzyl bromide (6.33 g, 37 mmole) in DMSO (10 ml) was added and the solution was stirred for 2 hr. The solvent was removed in vacuo (0.12 mm Hg at 65°C) to give a brown slurry which was dissolved in water (75 ml) and extracted with chloroform (3 x 75 ml). The chloroform extracts were combined, dried over magnesium sulphate and charcoal, filtered and the solvent was removed to give the pyridine (96a) as an amber coloured oil (9.15 g, 98%). NMR ($CDCl_3$, 100 MHz); δ 2.2 (2H, m); 2.5 (2H, m); 3.48 (2H, m); 5.1 (2H, s); 7.15 (2H, m); 7.37 (5H, m); 8.1 (1H, m); IR υ_{max} (NM) 2220, 1580 and 1460 cm^{-1}.

2-(4-Aminobutyl)-3-benzyloxypyridine (97a)

A solution of 3-benzyloxy-2-(3-cyanopropyl)pyridine (96a) (9 g, 35.7 mmole) in THF (100 ml) was added over 1 hr to a stirred suspension of lithium aluminium hydride (3.75 g, 98 mmole) in THF (600 ml). The suspension was stirred for 2 hr before being decomposed by the dropwise addition of water (3.75 ml) in THF (40 ml), 16% w/v sodium hydroxide solution (3.75 ml) and water

(11 ml). The suspension was filtered and the residue washed with THF (3 x 50 ml). The filtrates were combined and the solvent removed to give a brown oil which was dissolved in dilute hydrochloric acid (100 ml) and extracted with chloroform (100 ml). The aqueous layer was extracted with chloroform at pH 3, 5, 7 and 8 and the chloroform extracts were discarded. The aqueous layer was then extracted with chloroform (4 x 100 ml) at pH 12. The chloroform extracts were dried over magnesium sulphate and the solvent removed to give the butylamine (97a) as an oil (5.56 g, 60%). NMR ($CDCl_3$, 100 MHz); δ 1.75 (4H, m); 2.85 (4H, m); 5.05 (2H, s); 5.95 (2H, br); 7.05 (2H, m); 7.45 (5H, m); 8.05 (1H, m); IR υ_{max} (LF), 3360, 3280, 3050, 2920, 2860, 1590 and 1440 cm^{-1}.

2-(4-Acetylaminobutyl)-3-methoxypyridine (100)

Acetic anhydride (5.7 g) in dichloromethane (20 ml) was added over 15 min to a stirred solution of 2-(4-aminobutyl)-3-methoxypyridine (94a) (5 g, 28 mmole) in dichloromethane. The solution was stirred for 1 hr. The solvent was removed and the resulting oil was dissolved in water (50 ml), adjusted to pH 8 and extracted with chloroform (3 x 50 ml). The chloroform extracts were dried over magnesium sulphate and the solvent was removed to give the methoxypyridine (100) as a clear oil (5.5 g, 90%). NMR ($CDCl_3$, 60 MHz); δ 1.65 (2H, m); 1.97 (3H, s); 2.83 (2H, m); 3.25 (2H, m); 3.80 (3H, s); 7.06 (2H, m); 8.03 (1H, m); IR υ_{max} (LF) 3280, 3040, 2920, 1660, 1560, 1450, 1430, 1320, 1270 cm^{-1}.

2-(4-Acetylaminobutyl)-3-methoxypyridine-N-oxide (101)

m-Chloroperbenzoic acid (4.85 g, 0.028 mole) was added to a stirred solution of 2-(4-acetylaminobutyl)-3-methoxypyridine (100) (5.0 g, 0.022 mole) in chloroform (100 ml) over 40 min. The solution was allowed to stand at room temperature overnight before being saturated with ammonia gas and the precipitate collected by filtration. The filtrate was saturated with ammonia until no further precipitation

occurred. The solvent was removed to give the methoxypyridine (101) as a clear oil (5.25 g, 96%). NMR ($CDCl_3$, 60 MHz); δ 1.65 (2H, m); 1.98 (3H, s); 3.00 (2H, m); 3.25 (2H, m); 3.77 (3H, s); 6.84 (1H, d); 6.97 (1H, d); 7.86 (1H, dd); IR υ_{max} (NM) 3380, 3220, 1650, 1560 cm^{-1}.

The sample crystallised on trituration with ether and was recrystallised from ethanol/ether to give the methoxypyridine (101) as the monohydrate. m.p. 91-92°C (Found: C, 56.35; H, 7.8; N, 11.0%. $C_{12}H_{18}N_2O_3.H_2O$ requires C, 56.25; H, 7.6; N, 11.75%).

2-(4-Aminobutyl)-3-methoxypyridine-N-oxide (99)

A solution of 2-(4-acetylamino)-3-methoxypyridine-N-oxide (101) (3.5 g, 14.6 mmole) was refluxed in dilute hydrochloric acid (35 ml) for 20 hr. The solution was basified and extracted with chloroform (3 x 100 ml). The chloroform extracts were dried over magnesium sulphate and the solvent removed to give the pyridine-N-oxide (99) as a clear oil (2.68 g, 92%). NMR ($CDCl_3$, 60 MHz); δ 1.48 + 1.70 (6H, s + m); 2.76 (2H, m); 3.00 (2H, m); 3.87 (3H, s); 6.87 (2H, m); 7.78 (1H, dd); IR υ_{max} (LF) 3380, 3280, 2950, 2860, 1600, 1540, 1480, 1430 and 1220 cm^{-1}.

2-(3-Cyanopropyl)-3-hydroxy-6-hydroxymethylpyridine (102)

A solution of 2-(3-cyanopropyl)-3-hydroxypyridine (93a) (4.87 g, 0.03 mole), sodium hydroxide (1.20 g, 0.03 mole) and 40% w/v aqueous formaldehyde (6.6 ml, 0.03 mole) in water (15 ml) was refluxed for 1.5 hr. The solvent was removed on the rotary evaporator to give an amber oil (~ 9 g) which was chromatographed on silica gel eluted with ethyl acetate/ethanol/ammonia (d 0.88) in the ratio 15 : 10 : 2 to give the pyridine (102) (4.9 g, 84%) as an oil. m/e (FD) 143 (M + H) and m/e 286 (2(M + H)); NMR (DMSO d_6, 60 MHz); δ 1.95 (2H, m); 2.64 (2H, m); 2.85 (2H, m); 4.40 (2H, s); 7.08 (2H, m);

IR υ_{max} (LF) 3400-2800 (series of bands), 2130, 1640, 1580 and 1460 cm^{-1}.

A second component (0.4 g) was isolated and identified by NMR as 2-(3-cyanopropyl)-4,6-dihydroxymethyl-3-hydroxypyridine. NMR (DMSO d_6, 60 MHz); δ 2.0 (2H, m); 2.54 (2H, m); 2.90 (2H, m); 4.40 (2H, s); 4.65 (2H, s); 7.20 (1H, m).

2-(3-Cyanopropyl)-6-hydroxymethyl-3-methoxypyridine (103)

Sodium metal (0.5 g, 22 mmole) was dissolved in ethanol (40 ml). The resulting solution was added dropwise to a stirred solution of 2-(3-cyanopropyl)-3-hydroxy-6-hydroxymethylpyridine (102) (4.2 g, 22 mmole) in ethanol (20 ml). The solvent was removed on the rotary evaporator to give a pale orange solid which was dried in vacuo, (0.5 mmHg, 50°C, 2 hr) (4.65 g, 98%). The solid was dissolved in DMSO (40 ml) and treated with methyl iodide (3.1 g, 22 mmole) in DMSO (10 ml). The solution was stirred for 1 hr, the solvent was removed on the rotary evaporator (0.1 mmHg) to give an orange slurry which was extracted with hot ether (2 x 250 ml). The ether extracts were combined, the solvent was removed and the residue was chromatographed on silica eluted with 10% v/v methanol in chloroform to give the pyridine (103) (3.93 g, 87%) as a straw coloured oil. NMR ($CDCl_3$, 60 MHz); δ 1.9-2.6 (4H, m); 2.8-3.1 (2H, m); 3.46 (1H, s); 3.83 (3H, s); 4.64 (2H, s); 7.09 (2H, s); IR υ_{max} (LF) 3400, 2950, 2230, 1590, 1460 and 1420 cm^{-1}.

2-(4-Aminobutyl)-6-hydroxymethyl-3-methoxypyridine (104)

A solution of 2-(3-cyanopropyl)-6-hydroxymethyl-3-methoxypyridine (103) (5 g, 0.015 mole) in ether (75 ml) was added dropwise to a stirred suspension of lithium aluminium hydride (1.73 g, 0.043 mole) in ether (200 ml) over 40 min. The resulting pink-grey suspension was stirred at room temperature for a further 2 hr. The complex was decomposed by the addition of water (1.7 ml) in THF (20 ml) over

50 min, 16% w/v sodium hydroxide solution (1.7 ml) over 5 min and water (2.5 ml) over 20 min. The suspension was filtered and the residue was washed with ether (2 x 30 ml). The combined filtrates were evaporated to give the methoxypyridine (104) (2.24 g, 72%) as a straw coloured oil. NMR ($CDCl_3$, 60 MHz); δ 1.6 (4H, m); 2.5 + 2.7 + 2.8 (7H, s + m + m); 3.80 (3H, s); 4.62 (2H, s); 7.03 (2H, s); IR υ_{max} (LF) 3400-2500 (series of bands), 1610, 1590, 1460 and 1420 cm^{-1}.

2-(3-Cyanopropyl)-3-methylthiopyridine (105a)

3-Amino-2-(3-cyanopropyl)pyridine (76a) (10 g, 0.062 mole) was added slowly to a rapidly stirred solution of dimethyl disulphide (65 ml) and amyl nitrite (12.5 ml) at 80-90°C. The rate of addition was controlled to keep frothing to a minimum and was completed in 40 min. The solution was stirred at 80-90°C for 1 hr when the solvent was removed under reduced pressure (water pump) to yield a black oil which was chromatographed on silica gel eluted with chloroform and recrystallised from ether/petrol to give the methylthiopyridine (105a) (3.79 g, 32%) as prisms, m.p. 54.5-56.5°C. (Found: C, 62.5; H, 6.4; N, 14.55; S, 16.45%. $C_{10}H_{12}N_2S$ requires C, 62.45; H, 6.3; N, 14.6; S, 16.65%). NMR ($CDCl_3$, 100 MHz); δ 2.0-2.35 (2H, m); 2.45 (2H, t); 2.48 (3H, s); 3.0 (2H, t); 7.16 (1H, dd, J 8,5); 7.47 (1H, dd, J 8,2); 8.32 (1H, dd, J 5,2); IR υ_{max} (NM) 2220, 1580 and 1410 cm^{-1}.

2-(3-Cyanopropyl)-5-methylthiopyridine (105b)

A solution of 5-amino-2-(3-cyanopropyl)pyridine (76b) (5 g, 31 mmole) in dimethyl disulphide (10 ml) was added dropwise over 20 min to a solution of amyl nitrite (12.5 ml) and dimethyl disulphide (30 ml) stirred at 80-90°C. The solution was maintained at 80-90°C for 1 hr. The solvent was removed under reduced pressure and the residue was chromatographed on silica gel eluted with chloroform followed by distillation in a Kugelrohr apparatus (oven

temp. 120°C at 0.05 mmHg) to give the methylthiopyridine (105b) (4.23 g, 70%) as a straw coloured oil. NMR ($CDCl_3$, 100 MHz); δ 1.98-2.25 (4H, m); 2.5 (3H, s); 2.82-3.0 (2H, t); 7.1 (1H, d, J 8); 7.54 (1H, dd, J 8,2); 8.45 (1H, d, J 8,2); m/e 192 (M^+), 152, 139, 124, 111, 96 and 65; IR υ_{max} (LF) 2880, 2220, 1580, 1540, 1470 and 1420 cm^{-1}.

2-(4-Aminobutyl)-3-methylthiopyridine (106a)

A solution of 2-(3-cyanopropyl)-3-methylthiopyridine (105a) (3.5 g, 18 mmole) in ether (100 ml) was added over 30 min to a stirred suspension of lithium aluminium hydride (2.42 g, 63 mmole) in ether (250 ml). The suspension was stirred for 2 hr then the complex was decomposed by the consecutive addition of water (2.5 ml) in THF (10 ml), 16% w/v sodium hydroxide solution (2.5 ml) and water (7.5 ml). The suspension was filtered and the inorganic solid washed with ether (5 x 50 ml). The filtrates were combined and the solvent removed to give the butylamine (106a) (3.59 g, 98%) as a straw coloured oil. NMR ($CDCl_3$, 100 MHz); δ 1.45 (2H, s); 1.4-1.9 (4H, m); 2.45 (3H, s); 2.65-2.95 (4H, m); 7.1 (1H, dd, J 7,5); 7.45 (1H, dd, J 7,2); 8.31 (1H, dd, J 5,2); m/e 196 (M^+), 180, 166, 164, 152, 149, 139, 138, 132, 120, 106, 93 and 30; IR υ_{max} (LF) 3380, 3330, 2980, 2880, 1585 and 1420 cm^{-1}.

2-(4-Aminobutyl)-5-methylthiopyridine (106b)

A solution of 2-(3-cyanopropyl)-5-methylthiopyridine (105b) (3.8 g, 19.6 mmole) in ether (200 ml) was added over 40 min to a stirred suspension of lithium aluminium hydride (2.5 g, 66 mmole) in ether (300 ml). The suspension was stirred for 3 hr then treated with water (2.5 ml) in THF (10 ml), 16% w/v sodium hydroxide solution (2.5 ml) and water (7.5 ml). The suspension was filtered and the residue was washed with ether (2 x 250 ml). The combined filtrates were evaporated to dryness and the residue was distilled in a Kugelrohr apparatus (oven temp. 135°C at 0.01 mmHg) to give the

butylamine (106b) (3.23 g, 83%) as a straw coloured oil. NMR ($CDCl_3$, 100 MHz); δ 1.4-1.9 (6H, m); 2.48 (3H, s); 2.62-2.85 (4H, m); 7.07 (1H, d, J 8); 7.52 (1H, dd, J 8,2); 8.45 (1H, d, J 2). m/e 196 (M^+), 179, 166, 153, 152, 139, 126, 124, 111, 96, 80, 65 and 30; IR υ_{max} (LF) 3360, 3300, 2920, 2880, 1585, 1540 and 1450 cm^{-1}.

2-(3-Cyanopropyl)-3-iodopyridine (107a)

This reaction was carried out by Miss A. Paul-Clark. Sodium nitrite (5.00 g, 0.072 mole) in water (25 ml) was added over 10 min to a rapidly stirred solution of 3-amino-2-(3-cyanopropyl)pyridine (76a) (10.0 g, 0.062 mole) in concentrated sulphuric acid (25 ml) and water (125 ml) maintained at -10°C. The solution was stirred at -6°C for 10 min then run into a rapidly stirred suspension of potassium iodide (20.0 g, 0.12 mole) and cuprous iodide (2.50 g, 0.013 mole) in water (300 ml) maintained at 8°C. The resulting black suspension was allowed to warm to room temperature over 1 hr, basified with ammonia (d 0.88) and extracted with chloroform (3 x 200 ml). The combined chloroform extracts were dried over magnesium sulphate, filtered and the solvent removed to give a brown oil which was distilled in a Kugelrohr apparatus (oven temp. 130°C at 0.02 mmHg) to give the iodopyridine (107a) (10.07 g, 60%) as a straw coloured oil. NMR ($CDCl_3$, 60 MHz); δ 1.95-2.60 (4H, m); 3.06 (2H, t); 6.92 (1H, dd); 8.03 (1H, dd); 8.23 (1H, dd); m/e 272 (M^+), 232, 219, 205, 145, 105, 92, 78, 65 and 51; IR υ_{max} (LF) 2940, 2220, 1585, 1540, 1470, 1420 cm^{-1}.

2-(3-Cyanopropyl)-3-trifluoromethylpyridine (108a)

A 250 ml Parr stainless steel pressure vessel was charged with 2-(3-cyanopropyl)-3-iodopyridine (107a) (6.5 g, 24 mmole), copper powder (15 g) and pyridine (50 ml). The vessel was cooled to about -30°C when trifluoromethyl iodide (40 g) was condensed into the reactor and the vessel was sealed and heated at 145°C for 70 hr

(internal pressure 190 PSI). The reactor was cooled and the black slurry was suspended in ether (700 ml), filtered and the solid washed with ether (250 ml). The filtrates were combined, the solvent removed and the residue was distilled in a Kugelrohr apparatus (oven temp. 120°C at ~ 0.4 mmHg) to give the pyridine (108a) (3.38 g, 66%) as a yellow oil. NMR ($CDCl_3$, 60 MHz); δ 1.95-2.55 (4H, m); 3.06 (2H, t); 7.12 (1H, m); 7.84 (1H, dd); 8.62 (1H, dd); IR υ_{max} (LF) 2940, 2220, 1590, 1540, 1480, 1340 and 1140 cm^{-1}.

2-(4-Aminobutyl)-3-trifluoromethylpyridine (109a)

Hydrazine hydrate (10 ml) in ethanol (15 ml) was added over 30 min to a suspension of 2-(3-cyanopropyl)-3-trifluoromethylpyridine (108a) (2.61 g, 12.2 mmole), Raney nickel (2 g) in ethanol (100 ml) and stirred under nitrogen. The mixture was stirred for 90 min when TLC indicated the presence of the starting material. Hydrazine hydrate (10 ml) in ethanol (15 ml) was added over 30 min and the suspension was stirred for 90 min. The suspension was filtered through Hyflow and the catalyst was washed with ethanol (2 x 50 ml). The combined filtrates were evaporated to dryness and dissolved in dilute ammonium hydroxide solution (50 ml) and extracted with chloroform (3 x 50 ml). The combined chloroform extracts were dried over magnesium sulphate, filtered and the solvent removed to give an oil which was distilled in a Kugelrohr apparatus (oven temp. 95°C at 0.02 mmHg) to give the butylamine (109a) (1.79g, 67%) as a water white oil. NMR ($CDCl_3$, 100 MHz); δ 1.3-1.9 (4H, m); 1.4 (2H, s); 2.65-3.1 (4H, m); 7.2 (1H, m); 7.9 (1H, dd, J 8,2); 8.7 (1H, dd, J 4,2); IR υ_{max} (LF) 3360, 2280, 2920, 2860, 1590, 1570, 1440, 1320 and 1140 cm^{-1}.

2-(4-Aminobutyl)-3-methylpyridine (111)

This amine was first prepared[98] by deamination of 5-amino-2-(4-aminobutyl)-3-methylpyridine (77c). The later batch was prepared as below by colleagues at Bridge Chemicals[140].

3-Chloropropylamine hydrochloride (522.6 g, 4.02 mole) was added to liquid ammonia (5 l) contained in a 10 l flange flask fitted with a mechanical stirrer and dry ice/n-propanol condenser. Sodamide (274.4 g, 7.04 mole) was carefully added with stirring followed by 2,3-lutidine (358.5 g, 3.35 mole). The reaction was followed by HPLC and seven portions of sodamide (each 16.3 g, 0.42 mole) were added over 4 hr. The reaction was quenched after 5 hr and the ammonia was evaporated. The residue obtained after the standard work up was distilled (90-102°C 0.4 mmHg) to give the desired aminopicoline (111) (316 g, 58%). (Found: C, 73.15; H, 9.85; N, 17.05%. $C_{10}H_{16}N_2$ requires C, 73.15; H, 9.85; N, 17.05%. NMR ($CDCl_3$) δ 1.26 (2H, s); 1.65 (4H, m); 2.29 (3H, s); 2.78 (4H, m); 7.01 (1H, m); 7.40 (1H, m); 8.37 (1H, m); IR υ_{max} (LF) 3650, 3590, 3375, 3310, 1585 and 1575 cm^{-1}.

3-(5,6,7,8-Tetrahydroquinol-8-yl)propylamine (112)

This amine was prepared by Miss A. Paul-Clark. 5,6,7,8,Tetrahydroquinoline (20 g, 0.138 mole) was added quickly to sodamide (17.6 g, 0.44 mole) in liquid ammonia (250 ml) to give a dark red coloured solution. 3-Chloropropylamine hydrochloride (28.9 g, 0.22 mole) was added in portions over 4 hr when loss of colour was permanent. The reaction was then stirred for a further 2 hr befre being quenched with ammonium chloride (20 g). The liquid ammonia was allowed to evaporate and the residues were partitioned between chloroform and water. The pH was lowered to 6 and the chloroform layer was discarded. The aqueous layer was basified (pH 12), and extracted with chloroform. The chloroform extracts were dried, combined, evaporated and the residue was vacuum distilled to give the pyridine (112) (7.58 g, 63%), b.p. 92-94°C at 0.1 mm Hg. NMR ($CDCl_3$, 250 MHz); δ 1.43-2.15 (6H, m); 2.75 (3H, m); 2.88 (1H, m); 7.01 (1H, m); 7.32 (1H, m); 8.39 (1H, m); IR υ_{max} (LF) 3360, 3280, 2920, 2850, 1570, 1440, 1420, 1120, 790 cm^{-1}.

5-Bromo-2-hydroxymethylpyridine (113d)

5-Bromo-2-hydroxymethylpyridine hydrochloride was prepared as described in the literature[162], m.p. 210-212°C, lit. m.p. 211-212°C. NMR (DMSO d_6, 100 MHz); δ 4.58 (3H, s); 7.55 (1H, d); 8.14 (1H, dd); 8.67 (1H, d).

2-(5-Bromopyrid-2-ylmethylthio)ethylamine (113)

A mixture of 5-bromo-2-hydroxymethylpyridine hydrochloride (10 g, 0.044 mole) and cysteamine hydrochloride (5.06 g, 0.44 mole) was refluxed in 48% w/v hydrobromic acid (120 ml) for 7.5 hr. The solvent was removed on the rotary evaporator and the residue was azeotroped with ethanol (4 x 100 ml) and recrystallised from isopropyl alcohol to give the dihydrobromide salt of the amine (113) (9 g, 49%) as a hygroscopic solid, m.p. 216.5-217.5°C. NMR (D_2O, 100 MHz); δ 2.87 (2H, t); 3.27 (2H, t); 4.06 (2H, s); 7.94 (1H, d); 8.64 (1H, dd); 8.92 (1H, d).

2,4-Lutidine-N-oxide (114c)

m-Chloroperbenzoic acid (34.5 g, 0.24 mole) was added in portions over 1 hr to a solution of 2,4-lutidine (21.43 g, 0.24 mole) in chloroform (200 ml). The solution was stirred for 2 hr, saturated with ammonia gas and filtered. The filtrate was saturated with ammonia gas, filtered and the solvent was removed to give 2,4-lutidine-N-oxide (114c) as a straw coloured oil (24 g, 97%). NMR ($CDCl_3$, 100 MHz); δ 2.30 (3H, s); 2.48 (3H, s); 6.96 (1H, m); 7.08 (1H, m); 8.14 (1H, m); IR υ_{max} (LF) 3100-2850 (series of bands), 1470, 1420, 1370 and 1240 cm^{-1}.

2-Hydroxymethyl-4-methylpyridine (114d)

Method 1

Trifluoroacetic anhydride (50 ml) was added to a solution of 2,4-lutidine-N-oxide (19.5 g, 0.158 mole) in dichloromethane (200 ml) which had been dried over alumina. The solution was left for 4 days. Methanol (150 ml) was added and the solution was refluxed for 1 hr. The solvent was removed and the residue was dissolved in water (100 ml), adjusted to pH 6 and extracted with chloroform (4 x 100 ml). The chloroform extracts were combined, dried over magnesium sulphate and the solvent was removed. The residue was chromatographed on silica eluted with 10% v/v methanol in chloroform to give the methylpyridine (114d) (7 g, 35%) as a straw coloured oil. NMR ($CDCl_3$, 100 MHz); δ 2.36 (3H, s); 4.72 (2H, s); 7.05 (1H, m); 7.16 (1H, m); 8.37 (1H, m); IR υ_{max} (LF) 3400-2300 (series of bands), 1605, 1520 and 1480 cm^{-1}.

Method 2

Ammonium persulphate (136.8 g, 0.60 mole) in water (250 ml) was added dropwise over 1 hr to a refluxing solution of 4-methylpyridine (93.13 g, 1 mole), methanol (450 ml), concentrated sulphuric acid (45 ml) and water (100 ml). The solution was refluxed for 3 hr and then the methanol was removed on the rotary evaporator. The cooled solution was basified and extracted with chloroform (3 x 300 ml). The chloroform extracts were combined, dried over magnesium sulphate and the solvent was removed. The residue was double distilled to give the pyridine (114d) (18.66 g, 15%) as a clear oil, b.p. 92-94°C at 5 mmHg which was indistinguishable from the product from Method 1.

2-(4-Methylpyrid-2-ylmethylthio)ethylamine (114)

A mixture of 2-hydroxymethyl-4-methylpyridine (3.75 g, 0.03 mole) and cysteamine hydrochloride (3.5 g, 0.03 mole) was refluxed in 48% w/v hydrobromic acid (60 ml) for 7.5 hr. The solvent was

removed on the rotary evaporator and the residue was azeotroped with ethanol (2 x 70 ml) and recrystallised from ethanol to give the amine (114) as the di-hydrobromide salt (5.87 g, 56%), m.p. 173-174°C. NMR (D_2O, 100 MHz); δ 2.66 (3H, s); 2.88 (2H, t); 3.28 (2H, t); 4.17 (2H, s); 7.88 (1H, m); 7.90 (1H, m); 8.56 (1H, m); IR υ_{max} (NM) 2900-2500 (series of bands), 1630, 1620, 1495 and 1370 cm^{-1}.

2-Dimethylaminomethyl-6-hydroxymethylpyridine (148)

Thionyl chloride (5.21 ml, 0.07 mole) in pyridine (20 ml) was added dropwise to a rapidly stirred solution of 2,6-pyridinedimethanol (0.072 mole) in pyridine (80 ml) over 40 min. The resulting white suspension was stirred for 1.5 hr, cooled to 5°C and anhydrous dimethylamine (40 ml) was added over 30 min, to give a straw coloured suspension. The solvent was removed in vacuo and the resulting straw coloured slurry was dissolved in water (150 ml) and extracted with chloroform (3 x 100 ml). The aqueous layer was adjusted to pH 12 with 40% w/v sodium hydroxide and extracted with chloroform. The chloroform extracts were dried over magnesium sulphate, and concentrated to give the pyridine (148) (4.01 g, 34%) as an oil. GLC (OV1 at 150°C) peak 1, 7.2 min (6% by area) corresponds to 2,6-bis-dimethylaminomethylpyridine; peak 2, 9.2 min (94% by area). NMR ($CDCl_3$, 100 MHz); δ 2.28 (6H, m); 3.60 (2H, m); 4.15 (1H, br); 4.76 (2H, s); 7.24 (2H, m); 7.61 (1H, dd); IR υ_{max} (LF) 3400, 3000-2500 (series of bands), 1600, 1590, 1460 and 1360 cm^{-1}.

2-Chloromethyl-6-dimethylaminomethylpyridine (149a)

Thionyl chloride (8.2 ml) in chloroform (30 ml) was added over 30 min to a stirred solution of 2-dimethylaminomethyl-6-hydroxymethyl-pyridine (3.8 g) in chloroform (150 ml) at 10°C and the resulting solution was stirred at room temperature for 1 hr. The solvent was removed in vacuo to yield crude 2-chloromethyl-6-dimethylaminomethyl-

pyridine (149a) as the hydrochloride salt (6.0 g, 96%, assume dihydrochloride) which was used without further purification.

2-(6-Dimethylaminomethylpyrid-2-ylmethylthio)ethylamine (142)

Sodium (2.06 g, 0.09 mole) was dissolved in ethanol (150 ml) and the resulting solution cooled to 5°C when cysteamine hydrochloride (2.75 g, 0.024 mole) was added. The resulting white suspension was stirred for 5 min when a solution of crude 2-chloromethyl-6-dimethylaminomethylpyridine hydrochloride (149a) (5.8 g, 0.022 mole) in ethanol (50 ml) was added and the resulting brown suspension was stirred at room temperature for 1 hr. The solvent was removed *in vacuo* and the residue was dissolved in water (80 ml). The solution was adjusted to pH 10 and extracted with chloroform. The chloroform extracts were dried over magnesium sulphate and concentrated *in vacuo* to give an oil which was chromatographed on a silica gel column eluted with ethyl acetate/ethanol/ammonia (d 0.88) to give the amine (142) (1.98 g, 42%) as an oil. NMR ($CDCl_3$, 100 MHz); δ 1.95 (2H, s); 2.27 (6H, s); 2.60 (2H, m); 2.80 (2H, m); 3.56 (2H, s); 3.81 (2H, s); 7.24 (2H, m); 7.61 (1H, dd); IR υ_{max} (LF) 3360, 3280, 2980-2760 (series of bands), 1595, 1570, 1450 and 1350 cm^{-1}.

4-Cyano-2-hydroxymethylpyridine (152)

A mixture of 4-cyanaopyridine (31.2 g, 0.3 mole) ammonium persulphate (136.8 g, 0.6 mole), concentrated sulphuric acid (16.2 ml) and water (210 ml) was refluxed under nitrogen for 24 hr after which time the methanol was removed by distillation under reduced pressure. The resulting solution was poured onto crushed ice (~ 400 g) made basic by addition of sodium hydroxide (10 M) and extracted with chloroform (3 x 700 ml). The combined chloroform extracts were dried over magnesium sulphate, filtered and the solvent was removed to give a pink crystalline solid (~ 42 g), m.p. 70-78°C. This solid was chromatographed on silica eluted with

7.5% v/v methanol in chloroform to give 4-cyano-2-hydroxymethylpyridine (18.59 g, 46%) and 4-cyanopyridine (10 g, 32%). The pyridien (152) was recrystallised from chloroform/hexane, m.p. 92-94°C. (Found: C, 62.7%; H, 4.6%; N, 20.8% calc for $C_7H_6N_2O$: C, 62.7%; H, 4.5% N, 20.9%. NMR ($CDCl_3$, 100 MHz); δ 3.55 (1H, broad s); 4.79 (2H, s); 7.46 (1H, dd); 7.61 (1H, d); 8.74 (1H, d); IR υ_{max} (NM) 3400, 2220, 1600, 1550, 1460 cm^{-1}.

4-Aminomethyl-2-hydroxymethylpyridine (151)

4-Cyano-2-hydroxymethylpyridine (152) (0.5 g, 37 mmole) in THF (25 ml) was added dropwise to a stirred suspension of lithium aluminium hydride (0.35 g, 93 mmole) in THF. The addition took 20 min and the resulting grey suspension was stirred for a further 2.5 hr. The complex was decomposed by the addition of water (0.3 ml) in THF (15 ml) over 20 min, 16% w/v sodium hydroxide solution (0.35 ml) over 2 min and water (1 ml) over 20 min. The suspension was filtered and the residue was washed with THF (3 x 20 ml). The combined orange filtrates were evaporated in vacuo to give the pyridine (151) (0.28 g, 54%) as an orange oil. NMR ($CDCl_3$, 100 MHz); δ 3.87 (2H, s); 4.61 (2H, s); 7.11 (1H, dd); 7.30 (1H, d); 8.40 (1H, d); IR υ_{max} (LF) 3400-2600 (series of bands), 1610, 1560, 1410 cm^{-1}.

4-Dimethylaminomethyl-2-hydroxymethylpyridine (153)

Method 1

A mixture of 4-aminomethyl-2-hydroxymethylpyridine (151) (1.38 g, 0.01 mole), 10% palladium on carbon (1 g) and aqueous formaldehyde (20 ml, 24%) was shaken under hydrogen at a pressure of 50 PSI for 23 hr, the temperature being maintained at 30°C. The catalyst was removed by filtration and washed with water (3 x 50 ml). The combined filtrates were extracted with chloroform (3 x 50 ml). The chloroform extracts were combined, dried over magnesium sulphate and

the solvent removed on the rotary evaporator to give an amber oil which was partitioned between chloroform (15 ml) and dilute hydrochloric acid (15 ml). The chloroform layer was run off and the aqueous layer was further extracted with chloroform at pH 9. This extract was dried over magnesium sulphate and the solvent removed in vacuo to give the pyridine (153) (0.47 g, 28%) as a clear oil. NMR ($CDCl_3$, 100 MHz); δ 2.26 (6H, s); 3.42 (2H, s); 3.75 (1.3H, broad resonance); 4.78 (2H, s); 7.19 (1H, dd); 7.28 (1H, d); 8.49 (1H, d); m/e 166, (M^+) 121, 123, 108, 94 and 58. IR υ_{max} (LF) 3450-2740 (series of bands), 1605, 1560, 1450, 1410 and 1360 cm^{-1}.

Method 2

Ammonium persulphate (102.7 g, 0.45 mole) in water (200 ml) was added over 40 min to a refluxing solution of 4-dimethylaminomethyl-pyridine (40.86 g, 0.30 mole), methanol (450 ml), water (210 ml) and concentrated sulphuric acid (30 ml). The resulting solution was refluxed for 2 hr, water (300 ml) was added and the methanol was distilled off. The cooled solution was basified and extracted with chloroform (3 x 200 ml). The combined chloroform extracts were dried over magnesium sulphate, the solvent was removed and the residue was vacuum distilled. The fraction boiling at 92-120°C at 0.06 mmHg was collected (19.96 g, 40%). This was found to be identical to the product (153) of the previous reaction by GLC, TLC, NMR and IR. NMR ($CDCl_3$, 100 MHz); δ 2.26 (6H, s); 3.42 (2H, s); 3.75 (1.3H, broad resonance); 4.78 (2H, s); 7.19 (1H, dd); 7.28 (1H, d); 8.49 (1H, d). GLC (OV 17 at 150-200°C at 8°C/minute) retention time 5.7 min <99% by peak area.

A second fraction was collected (b.p. 160-170°C) at 0.05 mmHg (3.42 g, 7%) which was shown to be 4-dimethylaminomethyl-2,6-bis-hydroxymethylpyridine. NMR ($CDCl_3$, 100 MHz); δ 2.16 (6H, s); 3.40 (2H, s); 4.54 (4H, s); 2.4 (2H, br); 7.26 (2H, s); IR υ_{max} (LF) 3500-2700 (series of bands), 1610, 1530, 1450, 1410 and 1360 cm^{-1}.

2-Chloromethyl-4-dimethylaminomethylpyridine dihydrochloride (153a)

4-Dimethylaminomethyl-2-hydroxymethylpyridine (153) (13.45 g, 0.081 mole) in dichloromethane (200 ml) was added dropwise to a stirred solution of thionyl chloride (30 ml, 0.41 mole) in dichloromethane (150 ml). The resulting red mixture was stirred for 1 hr then concentrated in vacuo. The residue was treated with ether (300 ml) to give a solid which was recrystallised from methanol/ether (1 : 3) to give the pyridine (153a) as an orange solid, (20.04 g, 96%) m.p. 202-204°C. (Found: C, 42.15; H, 5.95; Cl, 41.1; N, 10.85% $C_9H_{15}ClN_2.HCl$ requires: C, 41.95; H, 5.9; Cl, 41.3; N, 10.85%). NMR (D_2O, 100 MHz); δ 2.96 (6H, s); 4.78 (2H, s); 5.03 (2H, s); 8.12 (1H, m); 8.23 (1H, m); 8.90 (1H, m); IR υ_{max} (NM) 2700-1980 (series of bands), 1640, 1620 and 1530 cm^{-1}.

2-(4-Dimethylaminomethylpyrid-2-ylmethylthio)ethylamine (143)

Cysteamine hydrochloride (3.75 g, 0.033 mole) was added to a solution of sodium (2.83 g, 0.123 mole) in etharol (200 ml). The resulting white suspension was cooled to ~ 10°C when 2-chloromethyl-4-dimethylaminomethylpyridine dihydrochloride (153a) (7.72 g, 0.03 mole) was added. The resulting red suspension was stirred for 20 min after which time the suspension was filtered and the inorganic solid washed with ethanol (2 x 50 ml). The filtrates were combined and the solvent removed in vacuo. The resulting slurry was partitioned between dilute hydrochloric acid (200 ml) and chloroform (200 ml). The chloroform layer was run off and the aqueous layer further extracted with chloroform (3 x 200 ml) at pH 10. The pH 10 chloroform extracts were combined, dried over magnesium sulphate and the solvent removed on the rotary evaporator to give the amine (143) (4.70 g, 69%) as a straw coloured oil. NMR ($CDCl_3$, 100 MHz); δ 1.51 (2H, s); 2.28 (6H, s); 2.65 (2H, m); 2.85 (2H, m); 3.46 (2H, s); 3.86 (2H, s); 7.17 (1H, dd) 7.36 (1H, d); 8.49 (1H, d); m/e 226 $(M + H)^+$ 225, 208, 182, 150, 107, 58 and 30.
IR υ_{max} (LF) 3360, 3280, 3000-2750 (series of bands), 1605, 1560, 1450 and 1410 cm^{-1}.

2-Hydroxymethyl-4-piperidinomethylpyridine (156)

Ammonium persulphate (68.52 g, 0.30 mole) in water (130 ml) was added over 30 min to a refluxing solution of 4-piperidinomethylpyridine (155) (35.20 g, 0.20 mole), methanol (250 ml), water (80 ml) and concentrated sulphuric acid (20 ml). The reflux condenser was inverted and the methanol was distilled off over 1.25 hr. The cooled solution was basified and extracted with chloroform (3 x 200 ml). The combined chloroform extracts were dried over magnesium sulphate, the solvent was removed and the residue was vacuum distilled. The fraction boiling at 110-130°C at 0.1 mmHg was collected (18.12 g) and was redistilled to give the pyridine (156) (3.71 g, 33%) as a colourless oil. NMR ($CDCl_3$, 100 MHz); δ 1.50 (6H, m); 2.35 (4H, m); 3.44 (2H, s); 4.10 (1H, br); 4.72 (2H, s); 7.17 + 7.26 (2H, dd + d); 8.43 (1H, d).
IR υ_{max} (LF) 3500-2400 (series of bands), 1610, 1560, 1440 and 1410 cm^{-1}.

2-Chloromethyl-4-piperidinomethylpyridine dihydrochloride (156a)

2-Hydroxymethyl-4-piperidinomethylpyridine (156) (13.00 g, 0.063 mole) in chloroform (50 ml) was added dropwise to a stirred solution of thionyl chloride (104 g) in chloroform (200 ml). The resulting mixture was stirred for 2 hr then concentrated *in vacuo*. The residue was treated with ethanol saturated with hydrogen chloride gas (100 ml) and evaporated to dryness to give the pyridine (156a) as a solid foam (18 g, 96%). NMR (D_2O, 100 MHz); δ 1.90 (6H, m); 3.30 (4H, m); 4.59 (2H, s); 5.02 (2H, s); 8.09 + 8.21 (2H, dd + s); 8.88 (1H, d) IR υ_{max} (NM) 2900-1980 (series of bands), 1640, 1610 and 1540 cm^{-1}.

2-(4-Piperidinomethylpyrid-2-ylmethylthio)ethylamine (145)

Cysteamine hydrochloride (7.87 g, 0.069 mole) was added to a solution of sodium (6.37 g, 0.027 mole) in ethanol (300 ml). The

resulting white suspension was cooled to ~ 10°C when 2-chloromethyl-4-piperidinomethylpyridine dihydrochloride (156a) (18 g, 0.063 mole assumed quantative yield for the previous reaction) was added. The resulting suspension was stirred for 18 hr after which time the suspension was filtered and the inorganic solid washed with ethanol (3 x 75 ml). The filtrates were combined and the solvent removed *in vacuo*. The resulting slurry was partitioned between dilute hydrochloric acid (200 ml) and chloroform (200 ml). The chloroform layer was run off and the aqueous layer further extracted with chloroform (3 x 200 ml) at pH 10. The pH 10 chloroform extracts were combined, dried over magnesium sulphate and solvent removed on the rotary evaporator to give the amine (145) (10.96 g, 65%) as a colourless oil. NMR ($CDCl_3$, 60 MHz); δ 1.55 (8H, m); 2.30 + 2.60 + 2.80 (8H, m); 3.42 (2H, s); 3.81 (2H, s); 7.12 + 7.31 (2H, dd + d); 8.40 (1H, d). IR υ_{max} (LF) 3360, 3280, 3100-2500 (series of bands), 1610, 1560 and 1410 cm^{-1}.

2-(3-Cyanopropyl)-4-dimethylaminomethylpyridine (157)

Ammonium persulphate (54.8 g, 0.24 mole) in water (150 ml) and 4-cyanobutyric acid (68 g, 0.6 mole) in water (300 ml) were added separately and simultaneously over 30 min to a mixture of 4-dimethylaminomethylpyridine (16.32 g, 0.12 mole), silver nitrate (4 g, 0.024 mole), water (200 ml) and concentrated sulphuric acid (25 ml) stirred at 80°C. The reaction mixture was stirred at 80°C for 1.5 hr, cooled and poured onto crushed ice (400 g) containing 28% w/w aqueous ammonia (200 ml). The solution was extracted with chloroform (900 ml) and the chloroform extract was washed with dilute sodium hydroxide, dried over magnesium sulphate, concentrated *in vacuo* and distilled to give the pyridine (157) (9 g, 37%) as a clear oil, b.p. 110-112°C at 0.1 mmHg. NMR ($CDCl_3$, 100 MHz); δ 2.15 + 2.23 + 2.40 (10H, m + s + m); 2.92 (2H, t); 3.41 (2H, s); 7.10 (2H, m); 8.46 (1H, d); IR υ_{max} (LF) 3360, 3280, 3000-2700 (series of bands), 2240, 1600, 1560, 1450 and 1410 cm^{-1}.

4-(4-Dimethylaminomethylpyrid-2-yl)butylamine (144)

Method 1

2-(3-Cyanopropyl)-4-dimethylaminomethylpyridine (157) (1 g, 5 mmole) in ether (15 ml) was added dropwise to a rapidly stirred suspension of lithium aluminium hydride (0.76 g, 20 mmole) in ether (45 ml). The solution was stirred for 2.5 hr. Water (1 ml) in THF (10 ml), followed by 16% w/v sodium hydroxide (1 ml) and then water (2 ml) was added and the mixture was filtered. The filtrate was evaporated to give the pyridine (144) (1.02 g, 100%) as a clear oil. NMR ($CDCl_3$, 100 MHz); δ 1.64 + 1.70 (6H, s + m); 2.23 (6H, s); 2.75 (4H, m); 3.39 (2H, m); 7.10 (2H, m); 8.46 (1H, d); m/e 208 $(M + H)^+$ 207, 164, 147, 58 and 30; IR υ_{max} (LF) 3360, 3280, 3000-2700 (series of bands), 1600, 1560, 1450 and 1420 cm^{-1}.

Method 2

Ammonium persulphate (27.4 g, 0.12 mole) in water (50 ml) and 5-aminovaleric acid (17.07 g, 0.30 mole) in water (50 ml) were added separately and simultaneously to a solution of 4-dimethylamino-methylpyridine (4.10 g, 0.03 mole), silver nitrate (1 g, 5.8 mmole), concentrated sulphuric acid (30 ml) and water (80 ml) stirred at 80-90°C. The addition took 20 min and the reaction was stirred at 90°C for a further 20 min. The cooled reaction mixture was basified with ammonium hydroxide solution and extracted with chloroform (3 x 150 ml). The combined chloroform extracts were evaporated and distilled in a Kugelrohr apparatus (oven temp. 125°C at 0.07 mmHg) to give the pyridine (144) as a clear oil. This product was identical by TLC, GLC, NMR and IR to the product from Method 1.

2-Chloromethyl-4-methylpyridine hydrochloride (159)

2-Hydroxymethyl-4-methylpyridine (114d) (17.50 g, 0.14 mole) in chloroform (50 ml) was added dropwise to a stirred solution of thionyl chloride (52 ml, 0.71 mole) in chloroform (200 ml). The resulting mixture was stirred for 3 hr then concentrated in vacuo to give a straw coloured solid. This was recrystallised from ethanol

to give the pyridine (159) (23.97 g, 94%) as a cream coloured very hygroscopic solid, m.p. 161-163°C.

2-Dimethylaminomethyl-4-methylpyridine (158)

2-Chloromethyl-4-methylpyridine hydrochloride (158) (23 g, 0.13 mole) was added over 15 min to a stirred solution of anhydrous dimethylamine (100 ml) in ether (300 ml). The solution was left at room temperature for 18 hr when water (200 ml) was added. The ether layer was separated and the aqueous layer was extracted with ether (3 x 200 ml). The combined ether extracts were dried over magnesium sulphate, the solvent was removed and the residue was distilled to give the pyridine (158) (17.78 g, 92%) as a clear oil, b.p. 62°C at 3 mmHg. NMR ($CDCl_3$, 100 MHz); δ 3.30 (6H, s); 2.36 (2H, s); 3.55 (2H, s); 6.98 (1H, m); 7.22 (1H, m); 8.38 (1H, m); IR υ_{max} (LF) 3200-2500 (series of bands), 1605, 1560, 1450 and 1360 cm^{-1}.

4-(2-Dimethylaminomethylpyrid-4-yl)butylamine (150)

Ammonia (250 ml) was condensed into a 1 l flask. To this was added sodamide (12.7 g, 0.327 mole) followed by 2-dimethylaminomethyl-4-methylpyridine (158) (16 g, 0.107 mole) over 10 min. The solution was stirred for 1 hr when the green/brown mixture was treated with chloropropylamine hydrochloride (14.30 g, 0.11 mole). The mixture was stirred for 5 hr and then treated with ammonium chloride (20 g) and the solvent was allowed to evaporate. The residue was dissolved in water (150 ml) and extracted with chloroform (3 x 200 ml). The combined extracts were dried over magnesium sulphate, the solvent was removed and the residue was distilled in a Kugelrohr apparatus twice (oven temp. 150°C at 0.05 mmHg) to give the pyridine (150) (6.79 g, 31%) as a clear oil. Found; C, 69.25; H, 10.55; N, 19.9%). $C_{12}H_{21}N_3$ requires C, 69.5; H, 10.2; N, 20.25%. m/e 207 (M^+), 206, 192, 190, 177, 164, 147, 134, 120, 106, 58 and 30; NMR ($CDCl_3$, 100 MHz); δ 1.60 (4H, m); 1.82 (2H, s); 2.30 (6H, s); 2.65 (4H, m);

3.58 (2H, m); 7.0 (1H, m); 7.24 (1H, m); 8.44 (1H, d). IR υ_{max} (LF) 3360, 3280, 3000-2600 (series of bands), 1590, 1550, 1450 and 1365 cm^{-1}.

N-Cyano-N'[4-(pyrid-2-yl)-butyl]-S-methylthiourea (24a)

A solution of 2-(4-aminobutyl)pyridine (84) (1.50 g, 10 mmole) in ethanol (10 ml) was added over 30 min to a stirred solution of dimethylcyanodithioimidocarbonate (1.46 g, 10 mmole) in ethanol (10 ml). The suspension was left at room temperature for 15 hr when the product was collected by filtration and washed with ether to give the thiourea (24a) as a white solid (1.1 g, 44%), m.p. 120-121°C. (Found: C, 57.95; H, 6.5; N, 22.75; S 13.2%. $C_{12}H_{16}N_4S$ requires C, 58.0; H, 6.5; N, 22.55; S, 12.9%)

N-Cyano-N'-methyl-N"-[4-(pyrid-2-yl)butyl]guanidine (24)

A solution of the thiourea (24a) (0.6 g, 2.4 mmole) in 16% w/v methylamine in ethanol (15 ml) was allowed to stand at room temperature for 18 hr. The solvent was removed and the residue was chromatographed on silica gel eluted with 10% v/v methanol in chloroform and recrystallised from ethanol/ether to give the guanidine (24) (0.375 g, 67%) as white prisms (hygroscopic), m.p. 84-85.5°C. (Found: C, 62.2; H, 7.45; N, 30.25%. $C_{12}H_{17}N_5$ requires C, 62.3; H, 7.4; N, 30.3%). NMR (DMSO d_6, 100 MHz); δ 1.6 (4H, m); 2.68 + 2.73 (5H, d + t); 3.1 (2H, q); 6.79 (2H, br); 7.20 (2H, m); 7.68 (1H, m); 8.49 (1H, m). ^{13}C NMR; δ 26.45 (t); 28.39 (q); 28.78 (t); 37.18 (t); 41.01 (t); 41.30 (t); 118.38 (s); 121.15 (d of t); 122.70 (d); 136.35 (dd); 148.91 (dd); 160.03 (s); 161.44 (s); IR υ_{max} (NM) 3260, 2150 and 1580 cm^{-1}.

N-Cyano-N'[4-(3-fluoropyrid-2-yl)-butyl]-S-methylthiourea (25a)

A solution of 2-(4-aminobutyl)-3-fluoropyridine (88a)(0.85 g, 5.05 mmole) in ethanol (8 ml) was added over 30 min to a stirred

solution of dimethylcyanodithioimidocarbonate (0.815 g, 5.5 mmole) in ethanol (8 ml). The solution was left for 15 hr when the residue was collected by filtration and washed with ether to give the thiourea (25a) as a pale orange solid (1.09 g, 80%.), m.p. 157-158.5°C.

N-Cyano-N'-[4-(3-fluoropyrid-2-yl)butyl]-N"-methylguanidine (25)

A solution of N-cyano-N'-[4-(3-fluoropyrid-2-yl)butyl]-S-methylthiourea (25a) (1g, 3.7 mmole) in 16% w/v methylamine in ethanol (24 ml) was allowed to stand for 72 hr. The solvent was removed and the residue was chromatographed on silica gel eluted with 10% v/v methanol in chloroform and recrystallised from ethanol/ether to give the guanidine (25) (0.67 g, 57%) as white prisms, m.p. 89-90°C. (Found: C, 57.55; H, 6.4; N, 27.8%. $C_{12}H_{16}FN_5$ requires C, 57.8; H, 6.45; N, 28.1%). NMR (DMSO d_6, 100 MHz); δ 1.6 (4H, m); 2.67 + 2.80 (5H, d + m); 3.1 (2H, m); 6.9 (2H, br); 7.34 + 7.66 (2H, m); 8.38 (1H, m); IR υ_{max} (NM) 3270, 2180, 1615 and 1580 cm^{-1}.

N-Cyano-N'-[4-(3-methoxypyrid-2-yl)butyl]-S-methylthiourea (26a)

A solution of 2-(4-aminobutyl)-3-methoxypyridine (94a) (2.4 g, 14 mmole) in ethanol (20 ml) was added dropwise to a stirred solution of dimethylcyanodithioimidocarbonate (2.03 g, 14 mmole) in ethanol (25 ml) over 20 min. The solution was stirred for 1 hr when the residue was collected by filtration to give the thiourea (26a) (3.25 g, 83%), m.p. 136-137.5°C. (Found: C, 56.2; H, 6.6; N, 19.95%. $C_{13}H_{18}N_4OS$ requires C, 56.1; H, 6.5; N, 20.1%)

N-Cyano-N'-[4-(3-methoxypyrid-2-yl)butyl]-N"-methylguanidine (26)

The thiourea (26a) (3 g, 10.7 mmole) was stirred in 16% w/v methylamine in ethanol (50 ml) over 18 hr. The solvent was removed and the residue was recrystallised from ethanol to give the guanidine (26) (2.06 g, 73%) as white prisms, m.p. 118.5-119.5°C. (Found: C,

59.7; H, 7.4; N, 27.1%. $C_{13}H_{19}N_5O$ requires C, 59.75; H, 7.35; N, 26.8%); NMR (DMSO d_6, 100 MHz); δ 1.58 (4H, m); 2.69 + 2.73 (5H, d + m); 3.13 (2H, m); 3.81 (3H, s); 6.78 (2H, m); 7.19 (1H, m); 7.34 (1H, m); 8.07 (1H, m); ^{13}C NMR; δ 24.80 (t); 28.35 (q); 28.93 (t); 31.36 (t); 41.06 (t); 55.33 (q); 117.17 (d); 118.33 (s); 121.98 (d); 139.93 (d); 150.66 (s); 153.09 (s); 159.98 (s); IR υ_{max} (NM) 3260, 2170 and 1580 cm^{-1}.

N-[4-(3-Benzyloxypyrid-2-yl)butyl]-N-cyano-S-methylthiourea (28a)

A solution of 2-(4-aminobutyl)-3-benzyloxypyridine (97a) (2 g, 7.8 mmole) in ethanol (10 ml) was added over 10 min to a stirred solution of dimethyldithiocyanoimidocarbonate (1.26 g, 8.6 mmole) in ethanol (20 ml). The solution was stirred for 18 hr, the solvent was removed and the residue was chromatographed on silica eluted with ethyl acetate and crystallised under ether to give the thiourea (28a) (1.87 g, 67%) as a white solid, m.p. 98-101°C.

N-[4-(3-Benzyloxypyrid-2-yl)butyl]-N-cyano-N-methylguanidine(28)

The thiourea (28a) (1.75 g, 4.9 mmole) was treated with 16% w/v methylamine in ethanol (30 ml) for 72 hr. The solvent was removed and the residue was recrystallised from ethanol to give the guanidine (28) (1.23 g, 74%) as white prisms, m.p. 124-126°C. (Found: C, 67.75; H, 6.85; N, 20.55%. $C_{19}H_{23}N_5O$ requires C, 67.55; H, 6.9; N, 20.75%); NMR ($CDCl_3$, 60 MHz); δ 1.70 (4H, m); 2.85 + 2.92 + 3.23 (7H, d + m + m); 5.06 (2H, s); 5.8 (2H, m); 7.1 + 7.33 (7H, m + m); 8.02 (1H, m); IR υ_{max} (NM) 3270, 3140, 2165 and 1590 cm^{-1}.

N-Cyano-N-[4-(3-hydroxypyrid-2-yl)butyl]-N-methylguanidine (27)

A mixture of N-[4-(3-benzyloxypryid-2-yl)butyl]-N'-cyano-N"-methyl-guanidine (28) (0.90 g, 0.26 mmole) and 10% palladium on carbon

(0.25 g) in ethanol (60 ml) was shaken under an atmosphere of hydrogen at 50 PSI on a Parr hydrogenator for 2 hr. The solution was filtered and the catalyst was washed with ethanol (25 ml). The filtrates were combined, the solvent removed and the residue twice recrystallised from ethanol to give the guanidine (27) (0.44 g, 68%) as white prisms, m.p. 151-153°C. (Found: C, 58.1; H, 6.95; N, 28.2%. $C_{12}H_{17}N_5O$ requires C, 58.3; H, 6.95; N, 28.3%); NMR (DMSO d_6, 60 MHz); δ 1.57 (4H, m); 2.66 + 2.70 + 3.12 (7H, d + m + m); 6.53 + 7.0 (7H, m + m); 7.88 (1H, dd); 9.2 (1H, br); IR υ_{max} (NM) 3410, 3260, 3170, 2500, 2170 and 1585 cm^{-1}.

N-Cyano-N'-[4-(3-methylthiopyrid-2-yl)butyl]-S-methylthiourea (29a)

A solution of 2-(4-aminobutyl)-3-methylthiopyridine (106a) (1.0 g, 5.1 mmole) in ethanol (5 ml) was added dropwise to a stirred solution of dimethylcyanodithioimidocarbonate (0.82 g, 5.5 mmole) in ethanol (10 ml) over 20 min. The solution was stirred for 1 hr during which a solid crystallised from the solution. Ether (30 ml) was added and the residue was collected by filtration and washed with ether to give the thiourea (29a) (0.885 g, 56%), m.p. 106.5-108.5°C.

N-Cyano-N'-[4-(3-methylthiopyrid-2-yl)butyl]-N"-methylguanidine (29)

Thiourea (29a) (0.79 g, 2.6 mmole) in 20% w/v methylamine in ethanol (40 ml) was stirred for 72 hr. The solvent was removed and the residue was recrystallised from ethanol to give the guanidine (29) (0.64 g, 86%) as white prisms, m.p. 137-139°C. (Found: C, 56.05; H, 6.95; N, 25.2; S, 11.4%. $C_{13}H_{19}N_5S$ requires C, 56.3; H, 6.9; N, 25.25; S, 11.55%); NMR ($CDCl_3$, 100 MHz); δ 1.70 (4H, m); 2.46 (3H, s); 2.88 + 2.92 (5H, d + t); 3.30 (2H, m); 5.90 (2H, br); 7.16 (1H, dd); 7.47 (1H, dd); 8.28 (1H, dd); IR υ_{max} (NM) 3290, 3180, 2160, 1605 and 1575 cm^{-1}.

N-[4-(3-Chloropyrid-2-yl)butyl-N'-cyano-N"-methylguanidine (30)

A solution of 2-(4-aminobutyl)-3-chloropyridine (86a) (0.43 g, 2.5 mmole) in ethanol (4 ml) was added, dropwise over 10 min, to a stirred solution of dimethylcyanodithioimidocarbonate (0.37 g, 2.5 mmole) in ethanol (5 ml). The solution was stirred for 5 hr when the residue was collected by filtration and washed with ethanol (5 ml) and ether (2 x 10 ml) to give a white solid which was suspended in 16.5% w/v methylamine in ethanol (30 ml). The solution was stirred for 18 hr, the solvent removed and the residue chromatographed on silica gel eluted with 10% v/v methanol in chloroform followed by crystallisation under ether to give the guanidine (30) (0.365g, 61%), m.p. 66.5-62.5°C. (Found: C, 54.05; H, 6.05; Cl, 13.5, N, 26.35%. $C_{12}H_{16}ClN_5$ requires C, 54.25; H, 6.05; Cl, 13.35; N, 26.35%); NMR (DMSO $d_6$6, 100 MHz) δ 1.61 (4H, m); 2.68 (3H ,d); 2.89 + 3.15 + 3.28 (6H, m + m + s); 6.81 (2H, m); 7.28 (1H, dd); 7.60 (1H, dd); 8.45 (1H, dd); IR υ_{max} (NM) 3270, 2160, 1620, 1575 and 792 cm^{-1}.

N-[4-(3-Aminopyrid-2-yl)butyl]-N'-cyano-S-methylthiourea (31a)

3-Amino-2-(4-aminobutyl)pyridine (83a) (1g, 6 mmole) in ethanol (8 ml) was added dropwise to a stirred solution of dimethylcyanodithio-imidocarbonate (0.89 g, 6.6 mmole) in ethanol (8 ml). The resulting solution was stirred for 4 hr, the solvent removed and the residue chromatographed on silica, eluted with 5% v/v methanol in chloroform followed by recrystallisation from chloroform to give the thiourea (31a) (1.11g, 70%) as a white plates, m.p. 129-130°C.
(Found: C, 54.55; H, 6.6; N, 26.25, S, 11.95%. $C_{12}H_{17}N_4S$ requires C, 54.75; H, 6.5; N, 26.6; S, 12.2%).

N-[4-(3-Aminopyrid-2-yl)butyl]-N'-cyano-N"-methylguanidine (31)

A solution of the thiourea (31a) (1.0 g, 3.8 mmole) in 33% w/v methylamine in ethanol (25 ml) was stirred at room temperature for

18 hr. The solvent was removed and the residue recrystallised from ethanol to give the guanidine (31) (0.84 g, 90%) as white micro crystals, m.p. 154-155°C. (Found: C, 58.55; H, 7.5; N, 34.1%. $C_{12}H_{18}N_6$ requires C, 58.5; H, 7.4; N, 34.1%); NMR (DMSO d6, 100 MHz) δ 1.55 (4H, m); 2.65 + 2.67 (obs., m + d); 3.11 (2H, m); 4.99 (2H, br); 6.90 (4H, m); 7.70 (1H, dd); IR υ_{max} (NM) 3460, 3330, 3300, 3220, 2160, 1630, 1605 and 1570 cm^{-1}.

N-Cyano-N'-methyl-N"-[4-(3-methylaminopyrid-2-yl)-butyl]-guanidine (32)

A solution of 2-(4-aminobutyl)-3-methylaminopyridine (79a) (1.00 g, 5.5 mmole) in ethanol (9 ml) was added, dropwise over 10 min, to a stirred solution of dimethylcyanodithioimidocarbonate (0.81 g, 5.5 mmole) in ethanol (10 ml). The solution was stirred for 18 hr when the solvent was removed in vacuo to give a clear oil which was dissolved in 16.5% w/w methylamine in ethanol (30 ml). The solution was stirred for 18 hr, when the solvent was removed and the residue chromatographed on silica eluted with 10% v/v methanol in chloroform and recrystallised from ethanol/ether to give the guanidine (32) (0.44 mg, 30%), m.p. 124.5-127.0°C. (Found: C, 60.35; H, 7.8; N, 32.65%. $C_{13}H_{20}N_6$ requires C, 60.0; H, 7.75; N, 32.3%); NMR (DMSO d6, 100 MHz) δ 1.60 (4H, m); 2.62 + 2.68 + 2.72 (8H, m + d + d); 3.14 (2H, m); 5.26 (1H, m); 6.8 + 6.8 + 7.01 (4H, br + m + dd); 7.72 (1H, m); IR υ_{max} (NM) 3410, 3260, 3140, 2160 and 1590 cm^{-1}.

N'-Cyano-N-[4-(3-methylpyrid-2-yl)butyl]-S-methylthiourea (33a)

A solution of 2-(4-aminobutyl)-3-methylpyridine (111) (1.64 g, 0.01 mole) in ethanol (20 ml) was added, dropwise over 30 min, to a stirred solution of dimethylcyanodithioimidocarbonate (3 g, 0.02 mole) in ethanol (50 ml). The solution was stirred for 2 hr when a white solid had crystallised which was collected by filtration to give the thiourea (33a) (2.22 g, 84%), m.p. 130-132°C.

N-Cyano-N'-methyl-N"-[4-(3-methylpyrid-2-yl)butyl]guanidine (33)

The thiourea (33a) (2 g, 7.6 mmole) was suspended in 33% w/w methylamine in ethanol (50 ml). The solution was stirred for 72 hr, the solvent removed and the residue was recrystallised from acetonitrile to give the cyanoguanidine (33) (1.65 g, 88%), m.p. 102-104°C. (Found: C, 60.6; H, 8.15; N, 27.2%. $C_{13}H_{19}N_5.0.8H_2O$ requires C, 60.0; H, 8.0; N, 26.9%); DSC/TGA sharp symmetric endotherm T = 103°C associated with a weight loss of 5.7% w/w ($0.8H_2O$ = 5.6% w/w); NMR ($CDCl_3$, 100 MHz) δ 1.70 (4H, m); 2.30 (3H, s); 2.86 + 2.91 + 3.30 (7H, m + d + m); 5.90 + 6.21 (2H, br + m); 7.08 (1H, m); 7.47 (1H, m); 8.32 (1H, m); IR υ_{max} (NM) 3520, 3260, 3180, 2155, 1645, 1608 and 1590 cm^{-1}.

N-[4-(3-Bromopyrid-2-yl)butyl]-N'-cyano-S-methylthiourea (34a)

A solution of 2-(4-aminobutyl)-3-bromopyridine (83a) (0.75 g, 3.2 mmole) in ethanol (5 ml) was added, dropwise over 10 min, to a stirred solution of dimethylcyanodithioimidocarbonate (0.750 g, 5.1 mmole) in ethanol (5 ml). The solution was stirred for 2 hr, concentrated in vacuo and the residue chromatographed on silica eluted with 5% v/v methanol in chloroform to give the thiourea (34a) (0.42 g, 40%), m.p. 133.5-134.5°C.

N-[4-(3-Bromopyrid-2-yl)-butyl]-N'-cyano-N"-methylguanidine (34)

The thiourea (34a) (0.4 g, 1.2 mmole) was suspended in 16% w/w methylamine in ethanol (20 ml). The solution was stirred for 18 hr, the solvent removed and the residue was chromatographed on silica eluted with 10% v/v methanol in chloroform and recrystallised from isopropyl alcohol to give the cyanoguanidine (34) (0.375 g, 82%), m.p. 77-77.5°C. (Found: C, 46.55; H, 5.1; Br, 25.8; N, 22.4%. $C_{12}H_{16}BrN_5$ requires C, 46.45; H, 5.2; Br, 25.75; N, 22.6%); NMR (DMSO d_6, 100 MHz) δ 1.60 (4H, m); 2.68 (3H, d); 2.90 (2H, m); 3.10 (2H, m); 6.80 (2H, br); 7.18 (1H, dd); 7.99 (1H, dd);

8.51 (1H, dd); ^{13}C NMR 25.50 (t); 28.39 (q); 28.78 (t); 36.40 (t); 40.97 (t); 118.33 (s); 120.71 (s); 123.19 (dd); 140.23 (dd); 148.00 (d of q); 159.20 (s); 159.98 (s); IR υ_{max} (NM) 3420, 3380, 3260, 2150, 1605 and 1590 cm^{-1}.

N-Cyano-N"-[4-(3-nitropyrid-2-yl)butyl]-S-methylthiourea (35a)

This analogue was prepared by Dr G.S. Sach from 2-(4-aminobutyl)-3-nitropyridine (75a) and dimethylcyanodithioimidocarbonate in ethanol to give the thiourea (35a) (69%), m.p. 115-117°C.

N-Cyano-N'-methyl-N"-[4-(3-nitropyrid-2-yl)butyl]-guanidine (35)

A solution of the thiourea (35a) in ethanol was treated with methylamine in ethanol. The solvent was removed and the residue recrystallised from ethanol to give the guanidine (35) (62%), m.p. 134-135°C. (Found: C, 52.35; H, 5.7; N, 30.45%. $C_{12}H_{16}N_6O_2$ requires C, 52.15; H, 5.85; N, 30.4%); NMR (DMSO d_6, 100 MHz); δ 1.4-1.9 (4H, m); 2.67 (3H, d); 3.04 (4H, m); 6.90 (2H, br); 7.55 (1H, dd); 8.38 (1H, dd); 8.83 (1H, dd); IR υ_{max} (KBr disc) 3370, 3300, 2160, 1580, 1560, 1515 and 1345 cm^{-1}.

N-Cyano-N"-[4-(3-trifluoromethylpyrid-2-yl)butyl]-S-methyl-thiourea (36a)

A solution of 2-(4-aminobutyl)-3-trifluoromethylpyridine (109a) (0.5 g, 2.3 mmole) in ethanol (5 ml) was added dropwise to a stirred solution of dimethylcyanodithioimidocarbonate (0.84 g, 5.7 mmole) in ethanol (25 ml). The solution was stirred for 2 hr, the solvent was removed and the residue was chromatographed on silica gel eluted with 5% v/v methanol in chloroform to give the thiourea (36a) (0.48 g, 66%), m.p. 115-117°C.

N-Cyano-N'-methyl-N"-[4-(3-trifluoromethylpyrid-2-yl)butyl]-guanidine (36)

The thiourea (36a) (0.46 g, 1.45 mmole) was treated with 16% w/w methylamine in ethanol (40 ml) for 18 hr. The solvent was removed and the residue was recrystallised from chloroform/petrol to give the guanidine (36) as white needles (0.27 g, 62%), m.p. 131-133°C. (Found: C, 52.3; H, 5.3; N, 23.3%. $C_{13}H_{16}FN_5$ requires: C, 52.15; H, 5.4; N, 23.4%); NMR ($CDCl_3$, 100 MHz); δ 1.77 (4H, m); 2.88 + 3.00 (5H, d + s); 3.29 (2H, m); 5.62 (2H, m); 7.28 (1.3H, m + $CHCl_3$); 7.93 (1H, m); 8.71 (1H, m); IR υ_{max} (NM) 3290, 3170, 2160 and 1590 cm^{-1}.

N-Cyano-N'-[2-(4-methylpyrid-2-ylmethylthio)ethyl]-S-methyl-thiourea (166a)

This analogue was prepared by Miss M.A. Wilczynska. A solution of 2-(4-methylpyrid-2-ylmethylthio)ethylamine (114) (0.83g, 4.5 mmole) in ethanol (5 ml) was added dropwise over 20 min, to a stirred solution of dimethylcyanodithioimidocarbonate (0.67 g, 4.4 mmole) in ethanol (10 ml). The solution was stirred for 18 hr to give the thiourea (116a) as a white solid (0.93 g, 73%), m.p. 103-104°C.

N-Cyano-N'-methyl-N"-[2-(4-methylpyrid-2-ylmethylthio)ethyl]-guanidine (166)

The thiourea (116a) (0.9 g, 3.2 mmole) was suspended in 33% w/w methylamine in ethanol (20 ml). The solution was stirred for 18 hr, the solvent removed and the residue was recrystallised from ethanol to give the cyanoguanidine (166) (0.70 g, 83%) as a white solid, m.p. 145-146°C. (Found: C, 54.95; H, 6.4; N, 26.4; S, 11.9%. $C_{12}H_{17}N_5S$ requires C, 54.7; H, 6.5; N, 26.4; S, 12.15%); NMR (DMSO d_6, 100 MHz) δ 2.31 (3H, s); 2.60 + 2.69 (5H, m + s); 3.3 (obscured by HOD, m); 3.80 (2H, s); 7.0 + 7.10 + 7.25 (4H, br + m + m); 8.34 (1H, d); IR υ_{max} (NM) 3260, 3160, 2170, 1610 and 1590 cm^{-1}.

N-Cyano-N'-methyl-N"-[2-(pyrid-2-ylmethylthio)ethyl]guanidine (37)

This analogue was prepared by Mr P.D. Miles from 2-(pyridylmethylthio)ethylamine and dimethylcyanodithioimidocarbonate in ethanol and subsequently treating the intermediate with methylamine in ethanol. Recryallisation from water gave the guanidine (37) as a white solid, m.p. 123-125°C. (Found: C, 53.05; H, 6.1; N, 28.15; S, 12.6%. $C_{11}H_{15}N_5S$ requires C, 53.0; H, 6.05; N, 28.1; S, 12.85%); NMR (DMSO d_6, 100 MHz) δ 2.59 + 2.67 (5H, t + d); 3.26 (2H, t); 3.82 (2H, t); 6.92 + 7.13 + 7.36 + 7.71 (5H, m); 8.42 (1H, m).

N-[2-(5-Bromopyrid-2-ylmethylthio)ethyl]-N'-cyano-S-methylthiourea (167a)

A solution of 2-(5-bromopyrid-2-ylmethylthio)ethylamine (113) (1.2 g, 4.8 mmole) in ethanol (10 ml) was added, dropwise over 20 min, to a stirred solution of dimethylcyanodithioimidocarbonate (0.8 g, 5.4 mmole) in ethanol (20 ml). The solution was stirred for 3 hr when a white solid had crystallised. This was collected by filtration and recrystallised from isopropyl alcohol to give the thiourea (167a) (0.8 g, 42%), m.p. 117-118°C.

N-[2-(5-Bromopyrid-2-ylmethylthio)ethyl]-N'-cyano-N"-methylguanidine (167)

N-[2-(5-Bromopyrid-2-ylmethylthio)ethyl]-N'-cyano-S-methylthiourea (167a) (0.8 g, 2.3 mmole) was suspended in 16% w/w methylamine in ethanol (20 ml). The solution was stirred for 18 hr, the solvent removed and the residue was chromatographed on silica eluted with 10% v/v methanol in chloroform to give the cyanoguanidine (167) (0.62 g, 81%) as a clear oil. (Found: C, 40.25; H, 4.5; Br, 24.15; N, 21.2; S, 9.5%. $C_{11}H_{14}BrN_5S$ requires C, 40.25; H, 4.3; Br, 24.35; N, 21.35; S, 9.75%); NMR ($CDCl_3$, 100 MHz) δ 2.68 + 2.69 (5H, m + d); 3.47 (2H, m); 3.80 (2H, s); 6.07 (2H, m); 7.29 (1H, d); 7.85 (1H, dd); 8.56 (1H, d); IR υ_{max} (LF) 3280, 3160, 2160 and 1590 cm^{-1}.

N-[4-(5-Chloropyrid-2-yl)-butyl]-N'-cyano-N"-methylguanidine (168)

A solution of 2-(4-aminobutyl)-5-chloropyridine (86b) (0.8 g, 4.3 mmole) in ethanol (5 ml) was added dropwise over 10 min to a stirred solution of dimethylcyanodithioimidocarbonate (1.5 g, 10 mmole) in ethanol (25 ml). The solution was allowed to stand at room temperature for 18 hr, the solvent was removed and ether (100 ml) was added to give a white solid (0.98 g), m.p. 97-99°C. This was suspended in 33% w/w methylamine in ethanol (20 ml) and allowed to stand at room temperature for 18 hr. The solvent was removed and the residue was recrystallised from acetonitrile to give a white solid (0.79 g), m.p. 85-90°C. Recrystallisation from ethanol gave the cyanoguanidine (168) (0.63 g, 52%) as prisms, m.p. 85-94°C. (Found: C, 50.8; H, 6.35; Cl, 12.55; N, 26.35%. $C_{12}H_{16}N_5Cl.H_2O$ requires C, 50.8; H, 6.4; Cl, 12.5; N, 24.7%). DSC/TGA endothermic weight loss 4.4% over range 42-97°C, weight loss 2.0% over range 97-183°C ($1H_2O$ = 6.35% w/w); NMR ($CDCl_3$, 100 MHz), δ 1.7 (4H, m), 2.81 + 2.86 (5H, t + d), 3.25 (2H, m), ~5.80 (2H, m), 7.13 (1H, d), 7.59 (1H, dd), 8.47 (1H, d); IR υ_{max} (NM) 3490, 3310, 3240, 3170, 2155, 1604 and 1585 cm^{-1}.

N-Cyano-N'-[4-(5-methoxypryid-2-yl)butyl]-S-methylthiourea (169a)

A solution of 2-(4-aminobutyl)-5-methoxypyridine (96b) (0.65 g, 3.6 mmole) in ethanol (5 ml) was added dropwise to a stirred solution of dimethyldithiocyanoimidocarbonate (0.54 g, 3.7 mmole) in ethanol (10 ml). The solution was stirred for 18 hr, the solvent was removed and the residue chromatographed on silica eluted with 10% v/v methanol in chloroform followed by recrystallisation from ethanol/ether to give the thiourea (169a) (0.7 g, 70%) as white needles, m.p. 99-100°C.

N-Cyano-N'-[4-(5-methoxypyrid-2-yl)butyl]-N"-methylguanidine (169)

A solution of the thiourea (169a) (0.65 g) in 16% w/w methylamine in ethanol (20 ml) was stirred at room temperature for 36 hr. The

solvent was removed and the product recrystallised from ethanol/ether to give the guanidine (169) (0.5 g, 76%) as a cream coloured solid, m.p. 73-75°C. (Found: C, 55.9; H, 7.55; N, 24.95%. $C_{13}H_{19}N_5O.H_2O$ requires C, 55.9; H, 7.6; N, 25.05%); NMR (DMSO d_6, 60 MHz), δ 1.53 (4H, m), 2.5 + 2.66 (5H, m + d), 3.09 (2H, m), 3.78 (3H, s), 6.9 + 7.2 (4H, br + m), 8.15 (1H, m); IR υ_{max} (NM) 3580, 3280, 3180, 3130, 2170, 1600 and 1578 cm^{-1}.

N-Cyano-N'-[2-(6-dimethylaminomethylpyrid-2-ylmethylthio)ethyl]-S-methylthiourea (122a)

A solution of 2-(6-dimethylaminomethylpyrid-2-ylmethylthio)-ethylamine (142) (0.6 g, 2.6 mmole) in ethanol (10 ml) was added, over 20 min, to a stirred solution of dimethylcyanodithioimido-carbonate (0.43 g, 2.9 mmole) in ethanol (15 ml). The solution was stirred for 1.5 hr when the solvent removed and the residue was chromatographed on silica eluted with 20% v/v methanol in chloroform to give the thiourea (122a) (0.76 g, 90%) as a clear oil.

N-Cyano-N'-[2-(6-dimethylaminomethylpyrid-2-ylmethylthio)ethyl]-N"-methylguanidine (122)

The thiourea (122a) (0.71 g, 2.1 mmole) was suspended in 16% w/w methylamine in ethanol (30 ml). The solution was stirred for 18 hr, the solvent removed and the residue was chromatographed on silica eluted with 20% v/v methanol in chloroform to give the cyanoguanidine (122) (0.48 g, 81%) as a clear oil. (Found: C, 54.0; H, 7.1; N, 27.31; S, 10.0%. $C_{14}H_{22}N_6S$ requires C, 54.85; H, 7.25; N, 27.4; S, 10.45%); NMR ($CDCl_3$, 100 MHz) δ 2.30 (6H, s); 2.71 + 2.91 (5H, m + d); 3.56 + 3.60 (4H, m + s); 6.14 (1H, br); 6.60 (1H, br); 7.25 (2H, m); 7.69 (1H, dd); IR υ_{max} (NM) 3670, 3590, 3450, 3410, 3270, 2170 and 1590 cm^{-1}.

N-Cyano-N'-[2-(4-dimethylaminomethylpyrid-2-ylmethylthio)ethyl]-S-methylthiourea (130a)

A solution of 2-(4-dimethylaminomethylpyrid-2-ylmethylthio)-ethylamine (143) (1.0 g, 4.82 mmole) in ethanol (20 ml) was added, dropwise over 20 min, to a stirred solution of dimethylcyanodi-thioimidocarbonate (0.77 g, 5.3 mmole) in ethanol (15 ml). The solution was stirred for 1.5 hr when the solvent removed and the residue was chromatographed on silica eluted with 20% v/v methanol in chloroform to give the thiourea (130a) (0.95 g, 61%) as a clear oil.

N-Cyano-N'-[2-(4-dimethylaminomethylpyrid-2-ylmethylthio)ethyl]-N"-methylguanidine (130)

The thiourea (130a) (0.92 g, 2.8 mmole) was suspended in 16% w/w methylamine in ethanol (40 ml). The solution was stirred for 18 hr, the solvent removed and the residue was chromatographed on silica eluted with 20% v/v methanol in chloroform and recrystallised from ethanol/ether to give the cyanoguanidine (130) (0.48 g, 56%) as white prisms m.p. 114-115.5°C. (Found: C, 54.85; H, 7.2; N, 27.3; S, 10.85%. $C_{14}H_{22}N_6S$ requires C, 54.85; H, 7.25; N, 27.4; S, 10.45%); NMR ($CDCl_3$, 100 MHz) δ 2.27 (6H, s); 2.68 (2H, t); 2.92 (3H, d); 3.45 + 3.49 (4H, s + t); 3.82 (2H, s); 6.45 (2H, br); 7.20 + 7.31 (2H, dd + d); 8.39 (1H, d); IR υ_{max} (NM) 3630, 3400, 3260, 3140, 2170 and 1585 cm^{-1}.

N-Cyano-N'-[2-(4-piperidinomethylpyrid-2-ylmethylthio)ethyl]-S-methyl-thiourea (131a)

A solution of 2-(4-piperidinomethylpyrid-2-ylmethylthio)ethylamine (145) (2.65 g, 10 mmole) in ethanol (25 ml) was added, dropwise over 30 min, to a stirred solution of dimethylcyanodithioimidocarbonate (3 g, 20 mmole) in ethanol (40 ml). The solution was stirred for 2 hr when the solvent was removed and the residue was

chromatographed on silica eluted with 15% w/w methanol in chloroform to give the thiourea (131a) (3.12 g, 86%) as a clear oil.

N-Cyano-N'-methyl-[2-(4-piperidinomethylpyrid-2-ylmethylthio)ethyl]-N"-guanidine (131)

The thiourea (131a) (1.01 g, 2.7 mmole) was suspended in 33% w/w methylamine in ethanol (25 ml). The solution was stirred for 16 hr, the solvent removed and the residue was chromatographed on silica eluted with 10% v/v methanol in chloroform and recrystallised from chloroform/pentane to give the cyanoguanidine (131) (0.59 g, 61%) as a white solid, m.p. 78-80°C. (Found: C, 58.95; H, 7.55; N, 24.3; S, 9.25%. $C_{17}H_{26}N_6S$ requires C, 58.95; H, 7.55; N, 24.25; S, 9.25%); NMR ($CDCl_3$, 100 MHz) δ 1.50 (6H, m); 2.40 (4H, m); 2.68 (2H, t); 2.91 (3H, d); 3.45 + 3.48 (4H, m + s); 3.81 (2H, s); 6.50 (2H, br); 7.30 (2H, m); 8.37 (1H, d); IR υ_{max} (NM) 3260, 3160, 3100-2500 (series of bands), 2170, 1605 and 1570 cm^{-1}.

2-[4-(Pyrid-2-yl)butylamino]-5-[(pyrid-3-yl)methyl]pyrimidin-4(1H)-one (170)

This analogue was prepared by Dr R. J. Ife from 2-(4-aminobutyl)-pyridine and 2-methylthio-5-[(pyrid-3-yl)methyl]-pyrimidin-4(1H)-one (161) and recrystallised from ethanol/ether to give the isocytosine (170), m.p. 144-145°C. (Found: C, 68.05; H, 6.5; N, 20.65%. $C_{19}H_{21}N_5O$ requires C, 68.0; H, 6.3; N, 20.9%); NMR (DMSO d_6, 100 MHz); δ 1.60 (4H, m); 2.75 (2H, m); 3.30 + 3.54 (obscured by HOD, m + s); 6.45 (1H, br); ~7.25 (3H, m); ~7.6 (3H, m); ~8.45 (3H, m); 10.78 (1H, br); IR υ_{max} (NM) 3300, 2500, 1655, 1615 and 1590 cm^{-1}.

2-[4-(3-Fluoropyrid-2-yl)butylamino]-5-[(pyrid-3-yl)methyl]pyrimidin-4(1H)-one (171)

A mixture of 2-(4-aminobutyl)-3-fluoropyridine (88a) (0.7 g. 4.2 mmole) and 2-methylthio-5-[(pyrid-3-yl)methyl]pyrimidin-4(1H)-one (161) (0.875 g, 3.75 mmole) was fused under nitrogen at 170-175°C for 4.5 hr. The resulting glass was chromatographed on silica eluted with 7.5% v/v methanol in chloroform and recrystallised from ethanol to give the isocytosine (171) (0.95 g, 73%), m.p. 149-150.5°C. (Found: C, 64.25; H, 5.65; N, 19.6%. $C_{19}H_{20}FN_5O$ requires C, 64.55; H, 5.7; N, 19.8%); NMR (DMSO d6, 100 MHz); δ 1.64 (4H, m); 2.83 (2H, m); 3.28 + 3.55 (5.8H, m + s + HOD resonance); 7.32 (1H, br); ~7.3 (2H, m); ~7.6 (3H, m); ~8.4 (3H, m); 10.79 (1H, br); IR υ_{max} (NM) 3230, 3170, 1660, 1620 and 1595 cm^{-1}.

2-[4-(3-Chloropyrid-2-yl)butylamino]-5-[(pyrid-3-yl)methyl]pyrimidin-4(1H)-one (172)

A mixture of 2-(4-aminobutyl)-3-chloropyridine (86a) (0.9 g, 5.3 mmole) and 2-nitroamino-5-[(pyrid-3-yl)methyl]pyrimidin-4(1H)-one (162) in ethanol (15 ml) was refluxed for 15 hr. TLC indicated that there was very little product formed. The solvent was allowed to distil off and the oil was fused at 120°C for 5 hr. The resulting glass was chromatographed on silica eluted with 10% v/v methanol in chloroform and recrystallised from ethanol/ether to give the isocytosine (172) (1.14 g, 65%) as prisms, m.p. 146-147.5°C. (Found: C, 61.65; H, 5.6; N, 19.0; Cl, 9.7%. $C_{19}H_{20}N_5ClO$ requires C, 61.7; H, 5.45; N, 18.95; Cl, 9.6%); NMR (DMSO d6, 100 MHz,) δ 1.6 (4H, m), 2.95 (2H, m), 3.30 (obscured by HOD, m), 3.54 (2H, s), 6.4 (1H, br), 7.3 (2H, m), 7.61 + 7.65 (2H, s + m), 7.89 (1H, dd), 8.45 (3H, m), 10.8 (1H, br); IR υ_{max} (NM) 3220, 2720, 1660, 1605 and 795 cm^{-1}.

2-[4-(3-Bromopyrid-2-yl)butylamino]-5-[(3-pyrid-3-yl)methyl]pyrimidin-4(1H)-one (173)

An intimate mixture of 2-(4-aminobutyl)-3-bromopyridine (83a) (0.55 g, 2.6 mmole) and 2-methylthio-5-[(pyrid-3-yl)methyl]pyrimidin-4(1H)-one (161) (0.6 g, 2.3 mmole) was fused at 150°C for 6 hr under nitrogen. The residue was dissolved in hot isopropyl alcohol and treated with charcoal, filtered and twice recrystallised from isopropanol to give the isocytosine (173) (0.33g, 34%) as prisms, m.p. 155.5-157°C. (Found: C, 55.0; H, 4.95; N, 16.7; Br, 18.9%. $C_{19}H_{20}BrN_5O$ requires C, 55.1; H, 4.9; N, 16.9; Br, 19.2%); NMR (DMSO d6, 100 MHz); δ 1.6 (4H, m); 2.91 (2H, m); ~3.3 + 3.5 (obscured by HOD resonance, m + m); 6.43 (1H, m); 7.19 + 7.28 (2H, m); 7.59 + ~7.65 (2H, s + m); 8.02 (1H, dd); ~8.4 (3H, m); IR υ_{max} (NM) 3330, 3250, 1660, 1600 and 1560 cm^{-1}.

2-[4-(3-Methoxypyrid-2-yl)butylamino]-5-[(pyrid-3-yl)methyl] pyrimidin-4(1H)-one (116)

A mixture of 2-(4-aminobutyl)-3-methoxypyridine (94a) (3.75 g, 20.8 mmole) and 2-methylthio-5-[(pyrid-3-yl)methyl]-pyrimidin-4(1H)-one (161) (4.37 g, 18.7 mmole) was fused under nitrogen for 6 hr at 152°C. The residue was chromatographed on silica gel eluted with 10% v/v methanol in chloroform and recrystallised from ethanol/ether to give the isocytosine (116) (5.1\g, 73Δ), m.p. 116.5-118°C. (Found: C, 65.7; H, 6.4; N, 19.2Δ. $C_{20}H_{23}N_5O_2$ requires C, 65.75; H, 6.35; N, 19.15%); NMR (100 MHz, DMSO d_6); δ 1.6 (4H, m); 2.75 (2H, m); 3.4 (obscured by HOD resonance, m); 3.55 (2H, s); 3.79 (3H, s); 6.3 (1H, br); ~ 7.25 (3H, m); 7.56 + 7.62 (2H, s, m); 8.06 (1H, dd); 8.38 + 8.49 (2H, m); 10.54 (1H, br); IR υ_{max} (NM) 3230, 2720, 1670 and 1605 cm^{-1}.

2-[4-(3-Aminopyrid-2-yl)butylamino]-5-[(pyrid-3-yl)methyl]-pyrimidin-4(1H)-one (49)

A mixture of 3-amino-2-[4-aminobutyl)pyridine (77a) (1.81 g, 11 mmole) and 2-nitroamino-5-[(pyrid-3-yl)methyl]pyrimidin-4(1H)-one (162) (2.47 g, 10 mmole) was refluxed in ethanol (12 ml) for 24 hr. The solvent was removed and the resulting glass was partitioned between water (35 ml) and chloroform (35 ml) when a white solid precipitated. The mixture was filtered and the residue washed with water (30 ml) and recrystallised from ethanol to give the isocytosine (49) (1.53 g, 44%), m.p. 130-131.5°C. (Found: C, 62.9; H, 6.35; N, 23.0%. $C_{19}H_{22}N_6O.0.7H_2O$ requires C, 62.85; H, 6.45; N, 23.15%). DSC/TGA broad asymmetric endotherm T = 60-90°C associated with a weight loss of 3.34% ($0.7H_2O$ = 3.5%); NMR (DMSO d6, 100 MHz); δ 1.6 (4H, m); 2.60 (obscured by DMSO d_5 resonance, t); 3.3 + 3.52 (obscured by HOD resonance, m + s); 5.00 (2H, br); 6.46 (1H, br); 6.88 (2H, m); 7.27 (1H, dd); 7.58 (1H, s); 7.70 (1H, m); 8.36 + 8.47 (2H, dd + d); 11.00 (1H, br); IR υ_{max} ($CHBr_3$) 3660, 3620, 3450, 3330, 3190, 2720, 1680, 1660 and 1620 cm^{-1}.

2-[2-(4-Methylpyrid-2-ylmethylthio)ethylamino]-5-[(pyrid-3-yl)methyl]-pyrimidin-4(1H)-one (174)

A mixture of 2-[4-methylprid-2-ylmethylthio)ethylamine (114) (0.8 g, 4.5 mmole) and 2-nitroamino-5-[(pyrid-3-yl)methyl]pyrimidin-4(1H)-one (162) (1 g, 4 mmole) was refluxed in ethanol (5 ml) for 18 hr. The solvent was removed and the residue was chromatographed on silica eluted with 10% methanol in chloroform and recrystallised from ethanol/ether to yield the isocytosine (174) (0.95 g, 57%), m.p. 128-129°C. (Found: C, 61.75; H, 5.75; N, 19.0; S, 8.9%. $C_{19}H_{21}N_5OS$ requires C, 62.1; H, 5.75; N, 19.05; S, 8.75%); NMR (DMSO d6, 60 MHz); δ 2.26 (3H, s); 2.67 (obscured by DMSO d_5 resonance, m); 3.44 + 3.54 (4H, m + s); 6.9-7.4 (3H, m); 7.49 + 7.58 (2H, s + m); 8.2-8.5 (3H, m); IR υ_{max} ($CHBr_3$) 3420, 3250, 1660, 1615 and 1600 cm^{-1}.

2-[2-(5-Bromopyrid-2-ylmethylthio)ethylamino]-5-[(pyrid-3-yl)methyl]-pyrimidin-4(1H)-one (175)

A mixture of 2-(5-bromoprid-2-ylmethylthio)ethylamine (113) (1.15 g, 4.6 mmole) and 2-nitroamino-5-[(pyrid-3-yl)methyl]pyrimidin-4(1H)-one (162) (0.975 g, 4.2 mmole) was fused at 170-180°C for 5 hr. The residue was chromatographed on silica eluted with 10% v/v methanol in chloroform and recrystallised from ethanol to yield the isocytosine (175) (1.06 g, 54%), m.p. 159-164.5°C. (Found: C, 49.75; H, 4.05; Br, 18.4; N, 16.15; S, 7.4%. $C_{18}H_{18}BrN_5OS$ requires C, 50.0; H, 4.2; Br, 18.5; N, 16.2; S, 7.4%); NMR (DMSO d6, 100 MHz); δ 3.54 + 3.5 (obscured by HOD resonance s + m); 2.63 (obscured by DMSO d5 resonance, m); 3.84 (2H, s); 6.50 (1H, br); 7.27 + 7.44 + 7.60 + 7.64 (4H, dd + d + s + m); 8.00 + 8.39 + 8.48 + 8.60 (4H, dd + m + m + d); IR υ_{max} (NM) 3300-2500 (series of bands), 1660, 1610 and 1600 cm^{-1}.

2-[4-(5-Aminopyrid-2-yl)butylamino]-5-[(pyrid-3-yl)methyl]-pyrimidin-4(1H)-one (176)

A mixture of 5-amino-2-(4-aminobutyl)pyridine (77b) (0.55 g, 3.3 mmole) and 2-nitroamino-5-[(pyrid-3-yl)methyl]pyrimidin-4(1H)-one (162) (0.74 g, 3 mmole) was refluxed in pyridine (4 ml) for 24 hr. The solvent was removed and the residue chromatographed on silica eluted with ethyl acetate/ethanol/ammonia (d 0.88) in the ratios 15 : 10 : 2, followed by recrystallisation from ethanol to give the isocytosine (176) (0.39g, 37%), m.p. 184-186°C. (Found: C, 64.65; H, 6.5; N, 23.6%. $C_{19}H_{22}N_6O$ requires C, 65.1; H, 6.35; N, 24.0%). DSC/TGA small endotherm T = 156°C associated with a weight loss of 0.87% followed by sharp endotherms T = 166°C and T = 183°C; NMR (DMSO d6, 100 MHz); δ 1.56 (4H, m); 2.55 (obscured by DMSO d_5 resonance, m); 3.24 + 3.54 (obscured by HOD resonance, m + s); 5.53 (2H, br); 6.42 (1H, br); 6.86 (2H, m); 7.27 (1H, m); 7.58 + 7.62 (2H, s + m); 7.86 (1H, m); 8.32 + 8.48 (2H, m + m); IR υ_{max} (NM) 3320, 3190, 1670 and 1595 cm^{-1}.

2-[4-(5-Methoxypyrid-2-yl)butylamino]-5-[(pyrid-3-yl)methyl]-pyrimidin-4(1H)-one (177)

A solution of 2-(4-aminobutyl)-5-methoxypyridine (94b) (0.55 g, 3.05 mmole) and 2-methylthio-5-[(pyrid-3-yl)methyl]pyrimidin-4(1H)-one (161) (0.74 g, 3 mmole) in pyridine (3 ml) was refluxed for 5 hr. The solvent was removed and the residue was chromatographed on silica eluted with 10% v/v methanol in chloroform and recrystallised from ethanol/ether to give the isocytosine (177) (0.84 g, 77%) as white prisms, m.p. 159-160°C. (Found: C, 65.95; H, 6.4; N, 19.15%. $C_{20}H_{23}N_5O_2$ requires C, 65.75; H, 6.35; N, 19.15%); NMR (DMSO d_6, 100 MHz); δ 1.56 (4H, m); 2.68 (2H, m); 3.24 + 3.53 (integral obscured by HOD resonance, m + s); 3.78 (3H, s); 6.45 (1H, br); 7.1-7.4 (3H, m); 7.58 + 7.63 (2H, s + m); 8.18 (1H, m); 8.37 + 8.47 (2H, m + m); 10.77 (1H, br); IR υ_{max} (NM) 3230, 2720, 1670 and 1605 cm^{-1}.

2-[4-(3-Methoxy-6-methylpyrid-2-yl)butylamino]-5-[(pyrid-3-yl)methyl]-pyrimidin-4(1H)-one (118)

A solution of 2-(4-aminobutyl)-5-methoxy-6-methylpyridine (94d) (0.43 g, 2.2 mmole) and 2-methylthio-5-[(pyrid-3-yl)methyl]-pyrimidin-4(1H)-one (161) (0.52 g, 2.2 mmole) were fused for 6 hr. The residue was chromatographed on silica eluted with 10% v/v methanol in chloroform and recrystallised from ethanol/ether to give the isocytosine (118) (0.5 g, 60%) as a white solid; m.p. 95-96.5°C. (Found: C, 60.5; H, 6.75; N, 16.8%. $C_{21}H_{25}N_5O_2.2H_2O$ requires C, 60.7; H, 7.05; N, 16.85%); NMR (DMSO d_6, 100 MHz); δ 1.55 (4H, m); 2.33 (2H, s); 2.70 (2H, m); 3.20 + 3.52 (integral obscured by HOD resonance, m + s); 3.73 (3H, s); 6.40 (1H, br); 7.00 + 7.22 + 7.25 (3H, d + d + m); 7.58 + 7.60 (2H, s + m); 8.38 + 8.47 (2H, dd + d); 10.80 (1H, br); IR υ_{max} (NM) 3430, 3220, 2720, 1670 and 1620 cm^{-1}.

2-[4-(Pyrid-2-yl)butylamino]-5-[(6-methylpyrid-3-yl)methyl]pyrimidin-4(1H)-one (126)

A mixture of 2-(4-aminobutyl)pyridine (84) (0.66 g, 4.4 mmole) and 2-methylthio-5-[(6-methylpyrid-3-yl)methyl]pyrimidin-4(1H)-one (163) (1 g, 4 mmole) was fused at 160°C for 4 hr under nitrogen. The product was chromatographed on silica eluted with 5% v/v methanol in chloroform and recrystallised from ethanol to give the isocytosine (126) (1.3 g, 92%), m.p. 156.5-157.5°C. (Found: C, 68.9; H, 6.75; N, 20.0%. $C_{20}H_{23}N_5O$ requires C, 68.75; H, 6.65; N, 20.05%); NMR (DMSO d_6, 60 MHz); δ 1.62 (4H, m); 2.41 (3H, s); 2.77 (2H, m); 3.27 (integral obscured by HOD, s); 3.50 (2H, s); 6.2 (1H, br); 7.2 (2H, m); 7.49 + 7.5 + 7.65 (3H, m + m + m); 8.30 + 8.44 (2H, m); 10.48 (1H, br); IR υ_{max} (NM) 3230, 3160, 3090, 1660, 1610 and 1595 cm^{-1}.

2-[4-(3-Nitropyrid-2-yl)butylamino]-5-[(6-methylpyrid-3-yl)methyl]-pyrimidin-4(1H)-one (178)

A solution of 2-(4-aminobutyl)-3-nitropyridine (75a) (1 g, 5.1 mmole) and 5-[(6-methylpyrid-3-yl)methyl]-2-nitroaminopyrimidin-4(1H)-one (164) (1.22 g, 4.6 mmole) in pyridine (5 ml) was refluxed under nitrogen for 5.5 hr. The solvent was removed and the residue chromatographed on silica eluted with 5% v/v methanol in chloroform followed by recrystallisation from acetone to give the isocytosine (178) (1.22 g, 67%), m.p. 134.5-135.5°C. (Found: C, 61.0; H, 5.7; N, 21.25%. $C_{20}H_{22}N_6O_3$ requires C, 60.9; H, 5.6; N, 21.3%); NMR (DMSO d_6, 60 MHz, δ 1.6 (4H, m), 2.39 (obscured by DMSO d_5, s), 3.01 + 3.25 + 3.48 (7H, t + t + s), 6.2 (0.6H, br), 7.03 (1H, d), 7.42 + 7.4 + 7.4 (3H, s + m + m), 8.22 + 8.28 (2H, m + m), 8.72 (1H, q); IR υ_{max} (NM) 3240, 3180, 1655, 1610, 1590, 1525 and 1340 cm^{-1}.

2-[4-(3-Aminopyrid-2-yl)butylamino]-5-[(6-methylpyrid-3-yl)methyl-pyrimidin-4(1H)-one (179)

A mixture of 3-amino-2-(4-aminobutyl)pyridine (77a) (0.52 g, 3.45 mmole) and 5-[(6-methylpyrid-3-yl)methyl]-2-nitroaminopyrimidin-4(1H)-one (164) (0.9 g, 3.5 mmole) was refluxed in pyridine (3 ml) for 6 hr. The solvent was removed and the residue chromatographed on silica eluted with ethylacetate/ethanol/ammonia (d 0.88) in the ratios 20 : 5 : 2, followed by recrystallisation from ethanol/ether to give the isocytosine (179) as a white solid (0.84 g, 65%), m.p. 103-105°C. (Found: C, 59.2 H, 7.3; N, 20.65%. $C_{20}H_{24}N_6O.2.3H_2O$ requires C, 59.15; H, 7.1; N, 20.7%). DSC/TGA complex endotherm T = 73°C associated with a weight loss of 10.2% (10.2% = $2.3H_2O$); NMR (DMSO d_6, 60 MHz, δ 1.6 (4H, m), 2.39 + 2.60 (obscured by DMSO d_5, s + m), 3.35 + 3.48 (obscured by HOD resonance, m + s); 4.98 (0.9H, br), 6.85 + 7.06 (3H, m + d), 7.44 + 7.50 + 7.66 (3H, dd + s + dd), 8.27 (1H, d); IR υ_{max} (NM) 3460, 3400-2500 (series of bands), 1685, 1620 and 1600 cm^{-1}.

2-[4-(3-Dimethylaminopyrid-2-yl)butylamino]-5-[(6-methylpyrid-3-yl-methyl]pyrimid-4(1H)-one (180)

A mixture of 2-(4-aminobutyl)-3-dimethylaminopyridine (81a) (0.77 g, 4 mmole) and 5-[(6-methylpyrid-3-yl)methyl]-2-nitroaminopyrimidin-4(1H)-one (164) (0.95 g, 3.6 mmole) was refluxed in pyridine (3 ml) for 5 hr. The mixture was chromatographed on silica gel eluted with 20% v/v methanol in chloroform and the residue was recrystallised from acetonitrile to give the isocytosine (180) as the trihydrate (0.77 g, 55%), m.p. 60-70°C. (Found: C, 59.05; H, 7.5; N, 18.65%. $C_{22}H_{28}N_6O.3H_2O$ requires: C, 59.15; H, 7.6; N, 18.8%). DSC/TGA complex endotherm T = 50-90°C associated with a weight loss of 12.3% w/w ($3.0H_2O$ = 12.1% w/w); NMR ($CDCl_3$, 100 MHz,); δ 1.7 (4H, m); 2.49 (3H, s); 2.65 + 2.88 (8H, s + m); 3.33 + 3.59 (4H, m + s); 6.9-7.5 (6H, m); 8.14 (1H, m); 8.36 (1H, m); IR\ $_{max}$(NM) 3360, 3300-2100 (sieries of bands), 1675 and 1620 cm^{-1}.

2-[4-(3-Bromopyrid-2-yl)butylamino]-5-[(6-methylpyrid-3-yl)methyl]-pyrimidine-4(1H)-one (181)

A solution of 2-(4-aminobutyl)-3-bromopyridine (83a) (1.0 g, 4.36 mmole) and 5-[(6-methylpyrid-3-yl)methyl]-2-nitroaminopyrimidin-4(1H)-one (164) (1.14 g, 4.36 mmole) in pyridine (5 ml) was refluxed for 5 hr. The mixture was chromatographed on silica eluted with 10% v/v methanol in chloroform and the residue was recrystallised from 10% v/v water in acetonitrile to give the isocytosine (181) (1.36 g, 65%) as white needle clusters, m.p. 88-92°C. (Found: C, 49.8; H, 5.55; N, 14.6; Br, 16.35%. $C_{20}H_{22}BrN_5O.3H_2O$ requires: C, 49.8; H, 5.85; N, 14.5; Br, 16.55%). DSG/TGA endothermic weight loss 11% w/w over a temperature range of 80-140°C ($3H_2O$ = 11.2% w/w); NMR (DMSO d6, 100 MHz); δ 1.65 (4H, m), 2.42 (3H, s); 2.92 (2H, m); 3.28 + 3.50 (Integral obscured by HOD resonance, m + s); ~6.4 (1H, br); ~7.2 (2H, m); 7.52 + 7.57 (2H, dd + m); 8.04 (1H, dd); 8.33 (1H, m); 8.51 (1H, dd); IR υ_{max} (KBr disc) 3200, 3300-2100 (series of bands), 1675 and 1620 cm^{-1}.

2-[4-(3-Chloropyrid-2-yl)butylamino]-5-[(6-methylpyrid-3-yl)methyl-pyrimidin-4(1H)-one (182)

A mixture of 2-(4-aminobutyl)-3-chloropyridine (86b) (2.0 g, 12 mmole) and 5-[(6-methylpyrid-3-yl)methyl]-2-methylthiopyrimidin-4(1H)-one (163) was fused at 160°C under nitrogen for 4 hr. The residue was chromatographed on silica eluted with 5% v/v methanol in chloroform followed by recrystallisation from ethanol/ether to give the isocytosine (182) (3.1 g, 75%), m.p. 132.5-133.5°C. (Found: C, 62.5; H, 5.75; Cl, 9.05; N, 18.0%. $C_{20}H_{22}ClN_5O$ requires C, 62.55; H, 5.85; Cl, 9.25; N, 18.25%); NMR (DMSO, 60 MHz) δ 1.62 (4H, m), 2.40 (obscured by DMSO d5, s), 2.89(2H, m), 3.3 + 3.48 (4H, m + s), 6.40(1H, m), 7.07 + 7.25 + 7.47 + 7.52 (4H, m + m + m + m), 7.84 (1H, dd), 8.30 + 8.43 (2H, m + dd); IR υ_{max} (KBr disc) 3350-2300 (series of bands), 1665, 1605 and 1570 cm^{-1}.

2-[4-(3-Iodopyrid-2-yl)butylamino]-5-[(6-methylpyrid-3-yl)methyl]-pyrimidine-4(1H)-one (183)

A solution of 2-(4-aminobutyl)-3-iodopyridine (89a) (0.6 g, 2.1 mmole) and 5-[(6-methylpyrid-3-yl)methyl]-2-nitroaminopyrimidin-4(1H)-one (164) (0.68 g, 2.6 mmole) in pyridine (2 ml) was refluxed for 5 hr. The solvent was removed and the residue chromatographed on silica eluted with 10% v/v methanol in chloroform followed by recrystallisation from ethanol/ether to give the isocytosine (183) as the trihydrate (0.55 g, 50%), m.p. 98-106°C. (Found: C, 45.4; H, 5.25, N, 13.45; I, 24.15%. $C_{20}H_{22}IN_5O.3H_2O$ requires C, 45.35; H, 5.3; N, 13.2; I, 23.8%). DSC/TGA endothermic weight loss of 10.2%, T = 60-100°C, ($3H_2O$ = 10.2% w/w H_2O); NMR ($CDCl_3$, 100 MHz); δ 1.7 (4H, m); 2.48 (3H, s); 2.95 (2H, t); 3.35 (2H, m); 3.59, (2H, s); 6.80 + 6.85 + 7.02 (3H, dd + br + d); 7.45 (2H, m); 8.03 (1H, dd); 8.40 (2H,m); IR υ_{max} ($CHBr_3$) 3670, 3590, 3420, 3300, 1650 and 1600 cm^{-1}.

2-[4-(3-Azidopyrid-2-yl)butylamino]-5-[(6-methylpyrid-3-yl)methyl]-pyrimidin-4(1H)-one (138)

A solution of 2-(4-aminobutyl)-3-azidopyridine (90a) (0.6 g, 3.1 mmole) and 5-[(6-methylpyrid-3-yl)methyl]-2-nitroaminopyrimidin-4(1H)-one (164) (1.23 g, 4.7 mmole) in pyridine (5 ml) was refluxed for 3.5 hr. The solvent was removed and the residue chromatographed on silica eluted with 10% v/v methanol in chloroform followed by recrystallisation from acetonitrile/ethanol to give the isocytosine (138) (0.39 g, 33%), m.p. 79-85°C. (Found: C, 57.6; H, 5.85. N, 26.7%. $C_{20}H_{22}N_8O$ + 6% w/w H_2O requires C, 57.8; H, 6.0; N, 26.9%). DSC/TGA; 40-80°C (3.3%) endothermic weight loss, 80-140°C (2.7%) weight loss associated with minor thermal effects, 140-160°C (6.6%) weight loss with a large endotherm (loss of N_2 = 6.7% w/w); NMR ($CDCl_3$, 100 MHz); δ 1.66 (4H, m); 2.48 (3H, s); 2.76 (2H, m); 3.30 + 3.58 (4H, m + s); 6.9-7.5 (5H, m); 7.0-7.5 (2H, br); 7.42 (1H, s); 8.24 + 8.36 (2H, m + m); IR υ_{max} (KBr disc) 3420, 3260, 2120, 1650, 1610, on heating the disc in vacuo to 150°C the

band at 2120 completely disappeared and changes in intensity were seen in bands at 1485 and 1295 cm^{-1}.

2-[4-(3-Methoxypyrid-2-yl)butylamino]-5-[(6-methylpyrid-3-yl)-methyl]pyrimidin-4(1H)-one (117)

A mixture of 2-(4-aminobutyl)-3-methoxypyridine (94a) (1.84 g, 10 mmole) and 5-[(6-methylpyrid-3-yl)methyl]-2-methylthiopyrimidin-4(1H)-one (163) (2.30 g, 9.3 mmole) was fused at 160°C under nitrogen for 4 hr. The residue was triturated with water (100 ml) to give a white solid which was collected by filtration and washed with water (2 x 50 ml). The solid collapsed to a glass on drying and was dissolved in ethanol (50 ml) and treated with ethanol saturated with hydrogen chloride gas (10 ml) to give a white residue which was recrystallised from ethanol to yield the isocytosine (117) as the trihydrochloride salt. (2.82 g, 62%), m.p. 209.5-210°C. (Found: C, 50.95; H, 5.9, N, 14.1; Cl 21.15%. $C_{21}H_{25}N_5O_2.3HCl.0.24H_2O$ requires C, 51.15; H, 5.85; N, 14.2; Cl, 21.6%). DSC/TGA endothermic weight loss 0.88% w/w, t = 60-100°C; ($0.24H_2O$ = 0.8% w/w H_2O); NMR (D_2O, 100 MHz); δ 1.85 (4H, m); 2.81 (3H, s); 3.16 (2H, m); 3.52 (2H, m); 3.94, (2H, s); 4.11 (3H, s); 7.79 + 7.95 + 8.20 + 8.27 + 8.41 + 8.61 (7H, s + m + dd + dd + dd + d); IR υ_{max} (KBr disc) 3400, 3300-1800 (series of bands) and 1660 cm^{-1}.

2-[4-(3-Benzyloxypyrid-2-yl)butylamino]-5-[(6-methylpyrid-3-yl)-methyl]pyrimidin-4(1H)-one (184)

A mixture of 2-(4-aminobutyl)-3-benzyloxypyridine (97a) (2.77 g, 11 mmole) and 5-[(6-methylpyrid-3-yl)methyl]-2-nitroaminopyrimidin-4(1H)-one (164) (2.61 g, 10 mmole) was refluxed in pyridine (15 ml) for 18 hr. The solvent was removed and the residue chromatographed on silica eluted with 10% v/v ethanol in chloroform and recrystallised from ethanol/ether to give the isocytosine (184) (2.14 g, 47%) as a white solid, m.p. 88-94°C. (Found: C, 63.3; H,

6.9; N, 13.6%. $C_{27}H_{29}N_5O_2.3H_2O$ requires C, 63.35; H, 6.9; N, 13.7%). DSC/TGA endothermic weight loss 10.5% w/w, T = 60-90°C; ($3H_2O$ = 10.6% w/w H_2O); NMR ($CDCl_3$, 100 MHz); δ 1.70 (4H, m); 2.44 (3H, s); 2.89 (2H, t); 3.30 (2H, m); 3.55, (2H, s); 5.102 (2H, s); 6.9-7.5 (12H, m); 8.01 (1H, m); 8.34 (1H, d); IR υ_{max} (KBr disc) 3340, 3300-2300 (series of bands), 1668 and 1607 cm^{-1}; m/e (EI) 455 (M^+) 364, 348, 270, 257, 243, 239, 229, 226, 212, 199, 108, 106, 91 and 65.

2-[4-(3-Hydroxypyrid-2-yl)butylamino]-5-[(6-methylpyrid-3-yl)methyl]-pyrimidin-4(1H)-one (185)

A mixture of 2-[4-(3-benzyloxypyrid-2-yl)butylamino]-5-[(6-methyl-pyrid-3-yl)methyl]pyrimidin-4-one (184) (1.25 g, 2.4 mmole), 10% palladium on carbon (0.5 g) and triethylamine (1 ml) in ethanol (50 ml) was shaken under an atmosphere of hydrogen at 50 PSI for 22 hr in a Parr hydrogenation apparatus. The solution was filtered and the catalyst washed with ethanol (25 ml). The combined filtrates were evaporated to dryness and the residue recrystallised from ethanol to give the isocytosine (185) (0.47 g, 53%), m.p. 192-194°C. (Found: C, 64.95; H, 6.55; N, 18.8%. $C_{20}H_{23}N_5O_2.0.25H_2O$ requires C, 64.9; H, 6.35; N, 18.9%). DSC/TGA sharp slightly asymmetric endotherm T = 195°C associated with a weight loss of 1.1% w/w; ($0.25H_2O$ = 1.2% w/w H_2O); NMR (DMSO d_6, 60 MHz); δ 1.6 (4H, m); 2.38 + 2.72 (5H, s + m); 3.23 + 3.47 (4H, m + s); ~ 7.0 (3H, m); 7.42 + 7.45 (2H, dd + s); 7.88 (1H, dd); 8.25 (1H, d); IR υ_{max} (NM) 3260, 3180, 2620, 1660, 1610 and 1595 cm^{-1}.

2-[4-(3-Methylthiopryid-2-yl)butylamino]-5-[(6-methylpyrid-3-yl)-methyl]-pyrimidin-4(1H)-one (186)

A solution of 2-(4-aminobutyl)-3-methylthiopyridine (106a) (0.8 g, 4.1 mmole) and 5-[(6-methylpyrid-3-yl)methyl]-2-nitroaminopyrimidin-4(1H)-one (164) (1.06 g, 4.5 mmole) in pyridine (3 ml) was

refluxed for 3 hr. The solvent was removed and the residue chromatographed on silica eluted with 10% v/v methanol in chloroform followed by recrystallisation from acetonitrile/water/ether in the ratios of approximately 10 : 1 : 10 by volume, to give a white solid (5.16 g) which collapsed to a glass on drying and was recrystallised from ethanol/ether (1 : 3) to give the isocytosine (186) as the dihydrate (1.57 g, 83%), m.p. 83-90°C. (Found: C, 58.3; H, 6.65; N, 16.05; S, 7.45%. $C_{21}H_{25}N_5OS$ requires C, 58.45; H, 6.75; N, 16.25; S, 7.4%). DSC/TGA; a broad complex endotherm is observed T = 40-100°C associated with a weight loss of 8.5% w/w from 40-170°C. ($C_{21}H_{25}N_5OS.2H_2O$ = 8.4% w/w H_2O); NMR ($CDCl_3$, 100 MHz); δ ~ 1.75 (4H, m); 2.41 + 2.49 (6H, s + s); 2.88 (2H, t); ~ 3.35 (2H, m); 3.61 (2H, s); 7.04 + 7.1 + 7.11 (3H, d + dd + br); 7.45 (3H, m); 8.24 + 8.39 (2H, dd + d); IR υ_{max} (NM), 3450, 3400-2800 (series of bands), 1674 and 1615 cm^{-1}.

2-[4-(3-Trifluoromethylpyrid-2-yl)butylamino]-5-[(6-methylpyrid-3-yl)-methyl]pyrimidin-4(1H)-one (187)

A solution of 2-(4-aminobutyl)-3-trifluoromethylpyridine (109a) (0.5 g, 2.3 mmole) and 5-[(6-methylpyrid-3-yl)methyl]-2-nitroamino-pyrimidin-4(1H)-one (164) (0.6 g, 2.3 mmole) in pyridine (3 ml) was refluxed for 18 hr, cooled and chromatographed on silica gel eluted first with with chloroform and then 10% v/v methanol in chloroform. The residue was recrystallised from 5% v/v water in acetonitrile to give the isocytosine (187) (0.68 g, 71%) as needles, m.p. 90-95°C. (Found: C, 60.4; H, 5.3; N, 16.6%. $C_{21}H_{22}F_3N_5O$ requires C, 60.4; H, 5.3; N, 16.8%); NMR ($CDCl_3$, 100 MHz); δ 1.7 (4H, m); 2.48 (3H, s); 2.9 + 3.3 + 3.6 (integral obscured by HOD resonance, m + m + s); 6.42 (1H, br); 7.02 (1H, d); 7.22 (obscured by $CHCl_3$ resonance, m); 7.44 + 7.57 (2H, m + br); 7.88 (1H, m); 8.37 (1H, m); 8.66 (1H, m); IR υ_{max} (KBr disc) 3350-2200 (series of bands), 1671, 1610, 1578, 1320 and 1125 cm^{-1}.

2-[4-(3-Methylpyrid-2-yl)butylamino]-5-[(6-methylpyrid-3-yl)-methyl]pyrimidin-4(1H)-one (54)

A solution of 2-(4-aminobutyl)-3-methylpyridine (111) (0.5 g, 3 mmole) and 5-[(6-methylpyrid-3-yl)methyl]-2-nitroaminopyrimidin-4(1H)-one (164) (0.7 g, 2.7 mmole) in pyridine (7 ml) was refluxed for 4 hr, cooled and chromatographed on silica gel eluted with chloroform followed by 16% v/v methanol in chloroform. The residue was recrystallised from ethanol/ether to give the isocytosine (54) (0.25 g, 28%), m.p. 80-81°C. (Found: C, 69.3; H, 5.95; N, 19.05%. $C_{21}H_{25}N_5O$ requires C, 69.4; H, 6.95; N, 19.3%); NMR (DMSO d_6, 100 MHz); δ 1.6 (4H, m); 2.27 + 2.4 + 2.73 (obscured by DMSO d_5, m + s + s); 3.3 + 3.47 (obscured by HOD resonance, m + s); 6.36 (1H, br); 7.1 (2H, m); 7.5 (3H, m); 8.3 (2H, s + br); IR υ_{max} (KBr disc) 3340-2500 (series of bands), 1675 and 1620 cm^{-1}.

2-[3-(5,6,7,8,-Tetrahydroquinol-8-yl)propylamino]-5-(6-methylpyrid-3-ylmethyl)pyrimidin-4-(1H)-one (55)

A solution of 3-(5,6,7,8,-tetrahydroquinol-8-yl)propylamine (112) (0.75 g, 3.8 mmole) and 5-[(6-methylpyrid-3-yl)methyl]-2-nitroamino-pyrimidin-4(1H)-one (164) (0.99 g, 3.7 mmole) in pyridine (1 ml) was refluxed for 8 hr, cooled and chromatographed on silica gel eluted with chloroform and recrystallised from chloroform/petrol to give the isocytosine (55) (0.38 g, 26%) as white solid, m.p. 75-80°C. (Found: C, 69.75; H, 7.1; N, 17.3%. $C_{23}H_{27}N_5O.0.4H_2O$ requires: C, 69.45; H, 7.05; N, 17.6%); NMR (CDCl3, 100 MHz); δ 1.51-2.1 (8H, m); 2.51 (3H, s); 2.75 (2H, m); 2.90 (1H, m); 3.37 (2H, m); 3.62 (2H, s); ~ 7.0 (2H, m); 7.35 (1H, m); 7.46 + 7.5 (2H, m + br); 8.30 (1H, m); 8.39 (1H, m); IR υ_{max} (NM) 3450, 3400-2000 (series of bands), 1668 and 1605 cm^{-1}.

2-[4-(5-Nitropyrid-2-yl)butylamino]-5-[(6-methylpyrid-3-yl)methyl]-pyrimidin-4(1H)-one (188)

A solution of 2-(4-aminobutyl)-5-nitropyridine (75b) (0.5 g, 2.5 mmole) and 5-[(6-methylpyrid-3-yl)methyl]-2-nitroaminopyrimidin-4(1H)-one (164) (0.75 g, 2.9 mmole) and pyridine (3 ml) was refluxed under nitrogen for 5 hr. The solvent was removed and the residue chromatographed on silica eluted with 10% v/v methanol in chloroform followed by recrystallisation from isopropanol to give the isocytosine (188) (0.32 g, 33%), m.p. 193-195°C. (Found: C, 60.1; H, 5.65; N, 20.75%. $C_{20}H_{22}N_6O_3$ requires C, 60.9; H, 5.6; N, 21.3%. $C_{20}H_{22}N_6O_3$ + 1% C_3H_8O + 0.5% H_2O requires C, 60.35; H, 5.7; N, 20.9%). DSC/TGA endothermic weight loss of 1.36% over range of 130-140°C followed by asymmetric endotherm at 186°C; NMR (DMSO d_6, 60 MHz); δ 1.6 (4H, m); 2.40 (3H, s); 2.90 + 3.24 + 3.47 (6H, m + m + s); 6.42 (1H, m); 7.06 (1H, d); 7.44 + 7.50 + 7.54 (3H, m + s + d); 8.27 + 8.45 (2H, m + dd); 9.23 (1H, m); IR υ_{max} (NM) 3230, 3170, 3090, 1660, 1611, 1595 and 1515 cm^{-1}.

2-[4-(5-Aminopyrid-2-yl)butylamino]-5-[(6-methylpyrid-3-yl)methyl]-pyrimidin-4(1H)-one (189)

A mixture of 5-amino-2-(4-aminobutyl)pyridine (77b) (1 g, 6.05 mmole) and 5-[(6-methylpyrid-3-yl)methyl]-2-nitroaminopyrimidin-4(1H)-one (164) (1.42 g, 5.4 mmole) was refluxed in pyridine (5 ml) for 6 hr. The solution was poured into ether (200 ml) and the white solid was collected by filtration and recrystallised from acetonitrile to give a white solid, m.p. 65-70°C which was recrystallised from ethanol/ether to give the isocytosine (189) as a white solid (1.62 g, 82%). (Found: C, 65.25 H, 6.65; N, 22.95%. $C_{20}H_{24}N_6O$.0.8% w/w H_2O ,requires C, 65.55; H, 6.65; N, 22.9%). DSC/TGA asymmetric endotherm T = 108°C, endothermic weight loss of 0.8% over range of 93-140°C; NMR ($CDCl_3$, 100 MHz); δ 1.65 (4H, m); 2.46 + 2.65 (5H, s + t); 3.24 (2H, m); 3.38 + 3.70 (4H, s + br); 6.90 + 7.02 (3H, m + d); 7.40 (2H, m); 7.90 (1H, m); 8.37 (1H, m); IR υ_{max} (NM) 3500-2500 (series of bands), 1668, 1635, 1598 and 1570 cm^{-1}.

2-[4-(5-Dimethylaminopyrid-2-yl)butylamino)-5-[(6-methylpyrid-3-yl-methyl]pyrimidin-4(1H)-one (190)

A mixture of 5-[(6-methylpyrid-3-yl)methyl]-2-nitroaminopyrimidin-4(1H)-one (164) (0.95 g, 3.6 mmole) and 2-(4-aminobutyl)-5-dimethyl-aminopyridine (81b) (0.85 g, 4.4 mmole) was refluxed in pyridine (3 ml) for 4 hr. The mixture was cooled and chromatographed on silica gel eluted with 15% v/v methanol in chloroform and recrystallised from methanol/acetonitrile to give the isocytosine (190) (0.76 g, 54%) as cream coloured prisms, m.p. 181.5-183°C. (Found: C, 67.3; H, 7.2; N, 21.4%. $C_{22}H_{28}N_6O$ requires C, 67.3; H, 7.2; N, 21.4%); NMR ($CDCl_3$, 100 MHz); δ 1.75 (4H, m); 2.46 (3H, s); 2.69 + 2.88 (8H, m + s); 3.30 (2H, m); 3.57 (2H, s); ~ 7.0 (3H, m); 7.38 + 7.44 (2H, br + dd); 7.93 (1H, d); 8.35 (1H, d); IR υ_{max} (NM) 3240-2500 (series of bands), 1658, 1620 and 1595 cm^{-1}.

2-[4-(5-Bromopyrid-2-yl)butylamino]-5-[(6-methylprid-3-yl)methyl]-primidin-4(1H)-one (191)

A mixture of 2-(4-aminobutyl)-5-bromopyridine (83b) (0.7 g, 3.05 mmole) and 5-[(6-methylpyrid-3-yl)methyl]-2-nitroamino-pyrimidin-4(1H)-one (164) (0.8 g, 3.06 mmole) was refluxed in pyridine (3 ml) for 5 hr. The residue was chromatographed on silica eluted with 20% v/v methanol in chloroform and recrystallised from ethanol to give the isocytosine (191) (0.7 g, 53%), as white microcrystals, m.p. 194-195.5°C. (Found: C, 56.1; H, 5.2; N, 16.3; Br, 18.4%. $C_{20}H_{22}BrN_5O$ requires C, 56.1; H, 5.2; N, 16.35; Br 18.65%); NMR (DMSO d6); δ 1.60 (4H, m); 2.41 (3H, s); 2.71 (2H, m); 3.25 + 3.48 (integral obscured by HOD, t + s); 6.40 (1H, br); 7.12 + 7.23 (2H, d + dd); 7.49 + 7.53 (2H, dd + s); 7.92 (1H, dd); 8.31 (1H, d); 8.59 (1H, d); IR υ_{max} (NM) 3230, 3170, 1660, 1610 and 1595 cm^{-1}.

2-[4-(5-Chloropyrid-2-yl)butylamino]-5-[(6-methylpyrid-3-yl)methyl]-pyrimidin-4(1H)-one (192)

A solution of 2-(4-aminobutyl)-3-chloropyridine (86b) (0.8 g, 4.3 mmole) and 5-[(6-methylpyrid-3-yl)methyl]-2-nitroaminopyrimidin-4(1H)-one (164) (1.04 g, 4 mmole) in pyridine (3 ml) was refluxed for 5 hr and then poured into ether (200 ml). The product was filtered and recrystallised from methanol to give the isocytosine (192) as white needles, (1.17 g, 76%), m.p. 191-192°C. (Found: C, 62.25; H, 5.6; Cl, 9.1; N, 18.05%. $C_{20}H_{22}ClN_5O$ requires C, 62.55; H, 5.8; Cl, 9.2%); NMR (DMSO d6, 100 MHz); δ 1.6 (4H, m), 2.4 (2.40, s), 2.73 (2H, m), approx 3.2 (obscured by HOD, m), 3.48 (2H, s), approx 6.3 (1H, br), 7.09 (1H, d), 7.28 (1H, d), 7.47 + 7.50 (2H, dd + s), 7.77 (1H, dd), 8.30 (1H, d), 8.49 (1H, d), approx 10.6 (1H, br); IR υ_{max} (NM) 3360-2500 (series of bands), 1660, 1615, 1600 and 1470 cm^{-1}.

2-[4-(5-Fluoropyrid-2-yl)butylamino]-5-[(6-methylpyrid-3-yl)methyl]-pyrimidin-4(1H)-one (193)

A solution of 2-(4-aminobutyl)-3-fluoropyridine (88b) (0.77 g, 4.5 mmole) and 5-[(6-methylpyrid-3-yl)methyl]-2-nitroaminopyrimidin-4(1H)-one (164) (1 g, 3.8 mmole) in pyridine (5 ml) was refluxed for 4 hr, cooled and poured into ether. The residue was filtered and recrystallised from methanol to give (193) (0.99g, 71%), m.p. 175-177°C. (Found: C, 65.35; H, 5.95; N, 19.1%. $C_{20}H_{22}FN_5O$ requires C, 65.4; H, 6.05; N, 19.05%; NMR ($CDCl_3$, 100 MHz); δ 1,70 (4H, m); 2.46 (3H, s); 2.77 (2H, t); 3.30 (2H, m); 3.57 (2H, s); ~7.2 (7H, m); 8.35 (2H, m); IR υ_{max} (NM) 3400-2300 (series of bands), 1660, 1615 and 1597 cm^{-1}.

2-[4-(5-Iodopyrid-2-yl)butylamino]-5-[(6-methylpyrid-3-yl)methyl]-pyrimid-4(1H)-one (194)

A solution of 2-(4-aminobutyl)-5-iodopyridine (89b) (1 g, 3.6 mmole) and 5-[(6-methylpyrid-3-yl)methyl]-2-nitroaminopyrimidin-4(1H)-one (164) (0.78 g, 3 mmole) in pyridine (5 ml) was refluxed for 5 hr, cooled and poured into ether (200 ml). The residue was filtered and recrystallised from methanol to give the isocytosine (194) (1.38 g, 93%), m.p. 196-198°C. (Found: C, 50.5; H, 4.5; N, 14.45; I, 26.45%. $C_{20}H_{22}IN_5O$ requires C, 50.5; H, 4.65; N, 14.75; I, 26.7%); NMR (DMSO d6, 100MHz); δ 1.60 (4H, m); 2.42 (3H, s); 2.71 (2H, t); 3.3 + 3.48 (integral obscured by HOD resonance, m + s); 6.30 (1H, br); 7.15 (2H, m); 7.45 (2H, m); 8.04 (1H, dd); 8.32 (1H, d); 8.69 (1H, d); IR υ_{max} (NM) 3300-2500 (series of bands), 1658, 1612 and 1593 cm^{-1}.

2-[4-(5-Methylthiopyrid-2-yl)butylamino]-5-[(6-methylpyrid-3-yl)-methyl]pyrimidin-4(1H)-one (195)

A mixture of 2-(4-aminobutyl)-5-methylthiopyridine (106b) (0.75 g, 3.8 mmole) and 5-[(6-methylpyrid-3-yl)methyl]-2-nitroamino-pyrimidin-4(1H)-one (164) (0.9 g, 3.4 mmole) was refluxed in pyridine (3 ml) for 5 hr. The solution was poured into ether (250 ml) and the white residue was collected by filtration and recrystallised from ethanol to give a white solid (1.45 g), m.p. 168.5-170°C. This was dissolved in 10% v/v methanol in chloroform (100 ml) and filtered through a bed of silica gel. The silica gel was washed by filtration with solvent (500 ml). The filtrates were combined, the solvent was removed and the residue was recrystallised from ethanol to give the isocytosine (195) (1.05 g, 70%), m.p. 170-171.5°C. (Found: C, 63.8; H, 6.25; N, 17.55; S, 8.2%. $C_{21}H_{25}N_5OS$ requires C, 63.75; H, 6.35; N, 17.7; S, 18.1%); NMR ($CDCl_3$, 100MHz); δ 1.66 (4H, m); 2.44 + 2.66 (6H, s + s); 2.72 (2H, m); 3.30 + 3.58 (4H, m + s); 6.8 + 7.01 (3H, br + m); 7.46 + 7.55 (3H, m + br); 8.37 (1H, m); IR υ_{max} (NM) 3400-2500 (series of bands), 1660, 1612 and 1597 cm^{-1}.

2-[4-(6-Hydroxymethyl-3-methoxypyrid-2-yl)butylamino]-5-[(6-methylprid-3-yl)methyl]pyrimidin-4(1H)-one (146)

A mixture of 2-(4-aminobutyl)-6-hydroxymethyl-3-methoxypyridine (104) (2.31 g, 0.011 mole), 5-[(6-methylpyrid-3-yl)methyl]-2-nitro-aminopyrimidin-4(1H)-one (164) (2.61 g, 0.01 mole) and pyridine (15 ml) was refluxed for 20 hr under nitrogen. The solvent was removed on the rotary evaporator to give a dark amber viscous oil. The residue was chromatographed on silica eluted with 10% v/v methanol in chloroform followed by recrystallisation from ethanol/ether (3:1) to give the isocytosine (146) as white needles (1.27 g, ~ 30%), m.p. 55-100°C, on drying (at 55°C, 0.005 mm Hg, for 6 hr) the melting point fell to ~ 60-70°C. (Found: C, 61.8; H, 6.5; N, 16.45%. $C_{22}H_{27}N_5O_3.H_2O$ requires C, 61.8; H, 6.85; N, 16.4%). DSC/TGA complex endotherms T = 40-90°C associated with a weight loss of 4.4% w/w ($1H_2O$ = 4.2% w/w); NMR (DMSO d_6, 100MHz); δ 1.57 (4H, m); 2.40 (3H, s); 2.72 (2H, m); 3.23 + 3.48 (obscured by HOD resonance, m + s); 3.77 (3H, s); 4.47 (2H, d); 5.27 (1H, br); 6.40 (1H, m); 7.1-7.5 (6H, m); 8.31 (1H, m); IR υ_{max} (NM) 3400, 3300-2500 (series of bands), 1685 and 1610 cm^{-1}.

2-[4-(3-Methoxy-N-oxopyrid-2-yl)butylamino]-5-[(6-methylpyrid-3-yl)methyl]pyrimidine-4(1H)-one (196)

A solution of 2-(4-aminobutyl)-3-methoxypyridine-N-oxide (99) (0.75 g, 3.83 mmole) and 5-[(6-methylpyrid-3-yl)methyl]-2-nitroamino-pyrimidin-4(1H)-one (164) (1.0 g, 3.72 mmole) in pyridine (5 ml) was refluxed for 6 hr. The mixture was chromatographed on silica eluted with 15% v/v methanol in chloroform and the residue was recrystallised from ethanol/ether (1 : 1) to give the isocytosine (196) (1.01g, 69%) as white micro crystals, m.p. 200-201.5°C. (Found: C, 63.75; H, 6.45; N, 17.65%. $C_{21}H_{25}N_5O_3$ requires C, 63.8; H, 6.4; N, 17.7%); NMR (DMSO d_6, 60MHz); δ 1.55 (4H, m); 2.40 (3H, s); 2.90 + 3.25 (obscured by HOD resonance, m + m); 3.48 (2H, s); 3.82 (3H, s); 6.10 (1H, br); 7.05 (3H, m); 7.42 + 7.44 (2H, dd + s); 7.81 (1H, dd); 8.26 (1H, d). IR υ_{max} (NM) 3380, 1651, 1625, 1599 and 1570 cm^{-1}.

2-[4-(6-Dimethylaminomethyl-3-methoxypryid-2-yl)butylamino]-5-[(6-methylpyrid-3-yl)methyl]pyrimidin-4(1H)-one (119)

Thionyl chloride (2.8 ml) in chloroform (15 ml) was added dropwise to a stirred solution of 2-[4-(6-hydroxymethyl-3-methoxypyrid-2-yl)butylamino]-5-[(6-methylpyrid-3-yl)methyl]pyrimidin-4(1H)-one (146) (0.8 g, 0.002 mole) in chloroform (80 ml). The addition was controlled maintaining the solution temperature below 22°C. The resulting solution was stirred at room temperature for 3 hr when the solvent was removed to give a white solid which was dissolved in ethanol (40 ml) and treated with 16% w/v dimethylamine in ethanol (20 ml) and the resulting solution was allowed to stand at room temperature for 16 hr. The solvent was removed give an amber oil (~ 1.5 g) which was partitioned between chloroform (10 ml) and water (10 ml). The chloroform layer was run off and the aqueous layer re-extracted with chloroform (10 ml). The chloroform extracts were washed with water (10 ml), dried over magnesium sulphate and the solvent removed on the rotary evaporator to give a viscous straw oil (0.7 g). This was chromatographed on silica gel eluted with 20% v/v methanol in chloroform to give the isocytosine (119) as a glass (0.4 g, ~ 50%). (Found: C, 64.7; H, 7.3; N, 18.7%. $C_{24}H_{32}N_6O_2.0.5H_2O$ requires C, 64.7; H, 7.45; N, 18.85%). DSC/TGA weight loss of 1.9% w/w over the range 80-200°C associated with minor thermal effects ($0.5H_2O$ = 2% w/w); NMR ($CDCl_3$, 100MHz); δ 1.75 (4H, m); 2.33 (6H, s); 2.48 (3H, s); 2.95 (2H, m); 3.40 + 3.56 + 3.61 (6H, m + s + s); 3.82 (3H, s); 7.05 (3H, m); 7.50 + 7.52 (2H, dd + s); 8.41 (1H, d); 9.15 (1H, br); IR υ_{max} (KBr disc) 3410, 3250, 1670 and 1610 cm^{-1}.

2-[2-(6-Dimethylaminomethylpyrid-2-yl)methylthio)ethylamino-5-[(6-methylpyrid-3-yl)methyl]pyrimidin-4(1H)-one (121)

A mixture of 2-(6-dimethylaminomethylprid-2-ylmethylthio)ethylamine (142) (0.5 g, 2.2 mmole) and 5-[(6-methylpyrid-3-yl)methyl]-2-nitro-aminopyrimidin-4(1H)-one (164) (0.7 g, 2.6 mmole) was refluxed in pyridine (3 ml) for 6 hr. The solvent was removed and the residue

was chromatographed on silica eluted with ethyl acetate/ethanol/ ammonium hydroxide in the ratios 15 : 10 : 2, and recrystallised from acetonitrile to give the isocytosine (121) (0.42 g, 45%), m.p. 114-115.5°C. (Found: C, 62.1; H, 6.65; N, 19.6; S, 7.45%. $C_{22}H_{28}N_6OS$ requires C, 62.25; H, 6.65; N, 19.8; S, 7.55%); NMR ($CDCl_3$, 100 MHz); δ 2.33 + 2.48 (9H, s + s); 2.82 (2H, t); 3.59 + 3.62 + 3.75 + 3.82 (8H, s + s + m + s); 7.05 (3H, m); 7.47 + 7.51 + 7.60 (3H, s + dd + dd); 8.39 (1H, d); 9.35 (1H, br); IR υ_{max} (NM) 3240, 3170, 1660, 1615 and 1600 cm^{-1}.

2-[2-(4-Dimethylaminomethylpyrid-2-yl)methylthio]ethylamino-5-[(6-methylpyrid-3-yl)methyl]pyrimidin-4(1H)-one (125)

A mixture of 2-(4-dimethylaminomethylprid-2-ylmethylthio)ethylamine (143) (0.5 g, 2.2 mmole) and 5-[(6-methylpyrid-3-yl)methyl]-2-nitro-aminopyrimidin-4(1H)-one (164) (0.7 g, 2.6 mmole) was refluxed in pyridine (3 ml) for 2.5 hr. The solvent was removed and the residue was chromatographed on silica eluted with ethyl acetate/ethanol/ ammonium hydroxide in the ratios 15 : 10 : 2, and recrystallised from acetonitrile to give the isocytosine (125) (0.58 g, 62%), m.p. 128-125.5°C. (Found: C, 62.35; H, 6.5; N, 19.9; S, 7.6%. $C_{22}H_{28}N_6OS$ requires C, 62.25; H, 6.65; N, 19.8; S, 7.55%); NMR ($CDCl_3$, 100 MHz); δ 2.23 (6H, s); 2.51 (3H, s); 3.72 (2H, t); 3.4 + 3.5 + 3.63 + 3.86 (8H, s + m + s + s); 7.05 + 7.13 + 7.32 + 7.45 + 7.5 (5H, d + m + s + m + br); 8.4 (2H, m); IR υ_{max} (NM) 3240, 3170, 2767, 1660, 1616 and 1600 cm^{-1}.

2-[2-(4-Piperidinomethylpyrid-2-yl)methylthio]ethylamino-5-[(6-methyl-pyrid-3-yl)methyl]pyrimidin-4(1H)-one (128)

A mixture of 2-(4-piperidinomethylprid-2-ylmethylthio)ethylamine (145) (1.1 g, 4.1 mmole) and 5-[(6-methylpyrid-3-yl)methyl]-2-nitro-aminopyrimidin-4(1H)-one (164) (0.98 g, 3.7 mmole) was refluxed in pyridine (3 ml) for 5 hr. The solvent was removed and the residue was chromatographed on silica eluted with 20% v/v methanol in

chloroform and recrystallised from acetonitrile to give the isocytosine (128) (1.29 g, 75%), m.p. 123.5-125°C. (Found: C, 64.4; H, 6.9; N, 18.0; S, 6.75%. $C_{25}H_{32}N_6OS$ requires C, 64.6; H, 6.95; N, 18.1; S, 6.9%); NMR ($CDCl_3$, 100 MHz); δ 1.55 (6H, m); 2.35 + 2.48 + 2.72 (9H, m + s + t); 3.42 + 3.50 + 3.61 + 3.84 (8H, s + m + s + s); ~ 7.30 (6H, m); 8.35 (2H, m); IR υ_{max} (NM) 3200 (complex series of bands), 1668, 1615 and 1600 cm^{-1}.

2-[4-(4-Dimethylaminomethylpyrid-2-yl)butylamino]-5-[(6-methylpyrid-3-yl)methyl]pyrimidin-4(1H)-one (127)

A mixture of 4-(4-dimethylaminomethylprid-2-yl)butylamine (144) (1 g, 4.8 mmole) and 5-[(6-methylpyrid-3-yl)methyl]-2-nitro-aminopyrimidin-4(1H)-one (164) (1.26 g, 4.8 mmole) was refluxed in pyridine (4.5 ml) for 5 hr. The solvent was removed and the residue was chromatographed on silica eluted with ethyl acetate/ethanol/ammonium hydroxide in the ratios 15 : 10 : 2, and recrystallised from ethanol ether to give the isocytosine (127) (1.21 g, 62%), m.p. 135-137°C. (Found: C, 67.75; H, 7.35; N, 20.5%. $C_{23}H_{30}N_6O$ requires C, 67.95; H, 7.45; N, 20.8%); NMR ($CDCl_3$, 100 MHz); δ 1.7 (4H, m); 2.22 (6H, s); 2.49 (3H, s); 2.80 (2H, m); 3.3 + 3.39 (4H, m + s); 3.60 (2H, s); 6.9-7.2 (3H, m); 7.4-7.6 (2H, m); 8.4 (2H, m); IR υ_{max} (KBr disc) 3240, 3170, 3100, 1660, 1612 and 1598 cm^{-1}.

2-[4-(2-Dimethylaminomethylpyrid-4-yl)butylamino]-5-[(6-methylpyrid-3-yl)methyl]pyrimidin-4(1H)-one (137)

A mixture of 4-(2-dimethylaminomethylprid-4-yl)butylamine (150) (0.75 g, 3.6 mmole) and 5-[(6-methylpyrid-3-yl)methyl]-2-nitroamino-pyrimidin-4(1H)-one (164) (0.9 g, 3.4 mmole) was refluxed in pyridine (3 ml) for 5 hr. The solvent was removed and the residue was chromatographed on silica eluted with 20% v/v methanol in chloroform. The resulting oil was dissolved in isopropyl alcohol and treated with ethanol saturated with hydrogen chloride gas. The resulting white solid was recrystallised from methanol/ethanol to

give the isocytosine (137) as the hydrochloride salt (0.83 g, 42%), m.p. 223-225°C. (Found: C, 49.8; H, 6.25; N, 15.15; Cl, 24.9%. $C_{23}H_{30}N_6O.3.9HCl.0.3H_2O$ requires C, 49.8; H, 6.3; N, 15.15; Cl, 25.0%). DSC/TGA weight loss of 1.1% w/w over the range 30-90°C (1.1% w/w = $0.3H_2O$); NMR (D_2O, 100 MHz); δ 1.85 (4H, m); 2.78 (3H, s); 3.03 + 3.03 (8H, s + m); 3.50 (2H, m); 3.92 (2H, s); 4.73 (obscured by HOD, s); 7.75 + 7.84 + 7.98 + 8.07 (4H, s + d + m + m); 8.39 (1H, dd); 8.57 (1H, d); 8.78 (1H, d); IR υ_{max} (KBr disc) 3420, 3300-2200 (series of bands), 1675 and 1640 cm^{-1}.

1-Nitro-2-methylamino-2-[2-(pyrid-2-ylmethylthio)ethylamino]ethylene (134)

This analogue was prepared by Mr D. W. Hills from 2-(pyrid-2-yl-methyl-thio)ethylamine and 1-nitro-2,2-bis(methylthio)ethenemono-sulphoxide[163], treating the intermediate with methylamine and recrystallising the residue from ethanol/ether to give the nitroethylene (134) m.p. 112-113°C. (Found: C, 49.45; H, 5.95; N, 20.9; S, 11.75%. $C_{11}H_{16}N_4O_2S$ requires C, 49.4; H, 5.65; N, 20.95; S, 12.0%); NMR ($CDCl_3$, 100 MHz); δ 2.72 + 2.94 (5H, t + d); 3.40 (2H, m); 3.85 (2H, s); 6.75 (1H, s); 7.10 + 7.20 + 7.38 + 7.67 (4H, m + m + m + m); 8.43 (1H, m).

2-[2-(4-Dimethylaminomethylpyrid-2-ylmethylthio)ethylamino]-2-methyl-amino-1-nitroethylene (136)

A solution of 2-(4-dimethylaminomethylprid-2-ylmethylthio)ethyl-amine (143) (0.75 g, 3.3 mmole) in methanol (10 ml) was added over 10 min to a solution of 1-nitro-2,2-bis(methylthio)ethene monosulphoxide[163] (0.66 g, 3.6 mmole) stirred at 30°C. The solution was stirred for 1.5 hr, evaporated to dryness and chromatographed on silica gel eluted with 10% v/v methanol in chloroform. The resulting clear oil (0.85 g) was dissolved in 16% v/v methylamine in ethanol (20 ml) and allowed to stand for 24 hr. The solvent was removed and the residue was recrystallised from

acetonitrile to give the nitroethylene (136) (0.32 g, 30%) as a creamed coloured solid, m.p. 113-115°C. (Found: C, 51.45; H, 6.95; N, 21.3; S, 9.95%. $C_{14}H_{23}N_5O_2S$ requires C, 51.65; H, 7.1; N, 21.5; S, 9.85%); NMR (DMSO d_6, 100 MHz); δ 2.12 (6H, s); 2.75 (5H, m); 3.40 (obscured by HOD, m); 3.87 (2H, s); 6.50 (1H, br); 7.30 (3H, m); 8.42 (1H, d); IR υ_{max} (NM) 3300-2700 (series of bands), 1625, 1607, 1575 and 1565 cm^{-1}.

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