NITROGEN CONTAINING HETEROCYCLES AS POTENTIAL INHIBITORS OF SERINE PROTEINASE.

A thesis submitted for the degree of Doctor of Philosophy

in the faculty of science of the University of London.

by

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"To travel hopefully is a better thing than to arrive,

and the true success is to labour".

- R. L. Stevenson, 13 Nov. 1850 - 3 Dec. 1894.

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To my parents

with love.

Abbreviations

A	absorbance
Ala	alanine
AMC	amino methyl coumarin
Anal.	analysis
Arg	arginine
Asp	aspartic acid
ATP	adenosine triphosphate
aufs	absorbance units for full scale deflection
bp	boiling point
br	broad
Bu	butyl
calcd.	calculated
cAMP	cyclic adenosine monophosphate
CCK-ir	cholecystokinin immunoreactivity
chymotrp	chymotrypsin
cm	centimetre
CNS	central nervous system
conc.	concentrated
cpd	compound
d	doublet
DCC	dicyclohexylcarbodiimide
DCU	dicyclohexylurea
def	deformation
DMAP	dimethylaminopyridine
DMF	dimethylformamide

DMSO	dimethylsulphoxide
EDC	[1-ethyl-3-(dimethylaminopropyl)carbodiimide hydrochloride]
EI	electron impact
eqm	equilibrium
equiv.	equivalent
Et	ethyl
FAB	fast atom bombardment
g	gramme
GABA	γ-aminobutyric acid
GWM	glycine-tryptophan-methionine
His	histidine
HLE	human leukocyte elastase
HPLC	high performance liquid chromatography
hr	hour
Hz	Hertz
IC_{50}	concentration at 50% inhibition
insol	insoluble
^{i}Pr	isopropyl
IR	infra red
J	coupling constant
K_a	acid dissociation constant
K_i	inhibitory constant
Leu	leucine
ln	$\log_{10}$ x 2.303
Log	logarithm
Lys	lysine
m	multiplet (NMR), medium (IR)
M	molarity
max	maximum

m/e	mass to charge ratio
mg	milligramme
MHz	mega Hertz
min	minute
ml	millilitre
mm	millimetre
mM	millimolar
mol	mole
mp	melting point
MP	M. Perera (for identification purposes)
µl	microlitre
µM	micromolar
nM	nanomolar
NMR	nuclear magnetic resonance
obs	observed
oopb	out of plane bending
oopd	out of plane deformation
Ph	phenyl
pNA	*para*-nitroanilide
p-NPGB	*para* -nitrophenyl guanidinobenzoate
PPE	porcine pancreatic elastase
Pr	propyl
prep	preparative
Pro	proline
pyrim.	pyrimidine
q	quartet
RIA	radio immuno assay
rt	room temperature
s	strong (IR), seconds (UV, NMR)

Ser	serine
sh	shoulder (UV)
sm	starting material
sol	soluble
sp. sol	sparingly soluble
str	stretch
Suc	succinic
t	triplet
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TLCK	tosyl-lysyl-chloromethyl-ketone
TMS	tetramethylsilane
TRH	thyrotropin releasing hormone
tryp	trypsin
t.s.	transition state
UCL	University College London (for identification purposes)
UV	ultraviolet
Val	valine
vbr	very broad
w	weak

ABSTRACT

Some nitrogen heterocycles such as pteridines and quinazolines undergo "covalent hydration" - the reversible addition of water across a C=N bond. Alcohols and other nucleophiles can also add across this C=N bond.

Serine proteinases catalyse the hydrolysis of peptide bonds and contain an unusually reactive serine OH residue at the enzyme active site. So far, the most potent serine proteinase inhibitors have been boronic acid derivatives of appropriate peptide substrates, which act as transition-state (t.s.) analogues. Here, the peptide provides enzymic active site recognition and the serine oxygen electron lone pair adds to the boron atom to form a stable tetrahedral intermediate. Aldehydes and electron deficient ketones also form such tetrahedral complexes. It seemed possible that heterocycles which undergo covalent hydration should also act as t.s. analogues to provide a novel approach to designing inhibitors of serine proteinase.

Several 2-substituted pteridines and quinazolines have been synthesised and tested against various serine proteinases such as the CCK-inactivating peptidase, trypsin, chymotrypsin and elastase.

A good synthetic route to 2-N-alkylamidopteridines has also been achieved.

Furthermore, the 2-N-alkylamidopteridines have been investigated by UV and ^{1}H NMR spectroscopy and shown to undergo covalent addition much more readily than either 2-aminopteridine or pteridine itself; the addition follows first order kinetics on approaching equilibrium.

Even though the compounds do not possess specific groups for enzymic active site recognition, some are active in the milli-molar range. It appears that suitably substituted

pteridines and quinazolines may prove to be good inhibitors of serine proteinases but that they require appropriate additional groups to assist binding to the active sites. At this stage, it is concluded that nitrogen containing heterocycles that undergo covalent hydration are potential inhibitors of serine proteinase, where covalent hydration may determine activity, but is not a sufficient condition.

CHAPTER 1

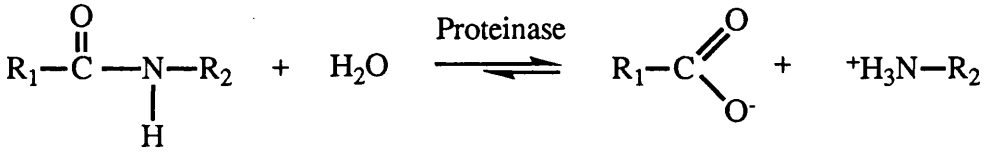
INTRODUCTION

Serine-proteinases: Background and physiological role

Enzymes are specific proteins that catalyse chemical reactions in biological systems. Proteinases are enzymes whose function is to alter or decompose other proteins or polypeptides by splitting them into fragments. Proteinases have been divided into four main groups, namely, serine, cysteine, aspartic and metallo proteinases- on the basis of their catalytic mechanisms [1]. Serine proteinases form the largest group; more than fifty are known in mammals. They are characterised by a reactive serine residue in their catalytic mechanism.

The chemical reactions catalysed by serine proteinases are the hydrolysis of peptide and ester bonds [2]. The hydrolysis of a peptide bond is of physiological significance as it is the fundamental step in the degradation of proteins.

Figure 1.1. Hydrolysis of a peptide bond.



Some of the important physiological processes that involve serine proteinases are coagulation, fibrinolysis, kinin liberation and complement activation as well as fertilization and digestion [3]. They are also involved in the activation of various hormones and in the degradation of endogenous proteins within cells. In all these processes it is important that

proteolytic enzymes are not produced in excess as they could destroy proteins other than the substrate and lead to many pathological states. In such circumstances proteinase inhibitors are potential drugs for the control of physiologically important proteinase-mediated processes [4,5].

Some of the well known serine proteinases are trypsin, chymotrypsin, thrombin, kallikrein, acrosomal serine proteinase, pancreatic elastase, human leukocyte elastase (HLE), cathepsin G and chymase to name but a few [4].

Trypsin, chymotrypsin and elastase in the pancreas are responsible for the digestion of proteins in the small intestine [1-3]. These enzymes act on internal molecular bonds, whilst carboxypeptidase (an aspartic proteinase) acts on the terminal amino acids of proteins. They are initially secreted as inactive pro-enzymes or zymogens in which form they are prevented from exerting their destructive action on the protein components of the tissue in which they originate. During digestion, the enzyme enterokinase activates trypsinogen to trypsin. Trypsin itself further activates trypsinogen and also activates other pro-enzymes.The activated pancreatic proteinases act sequentially at their appropriate sites on the molecule and break down proteins and large polypeptides to oligopeptides and amino acids suitable for absorption. Since trypsin serves as the activator of all other pancreatic zymogens, the inhibition of trypsin has broad consequences in terms of the formation of the endopeptidase and exopeptidase proteinase components of pancreatic juice and is used for the treatment of pancreatitis [4].

The other trypsin-like serine proteinases include:- thrombin, which mediates the coagulation of blood; kallikrein, which is activated in response to tissue injury; complement, which takes part in the immune reaction directed against foreign tissues or organisms; the acrosomal enzyme in the acrosome of the sperm head, which together with a number of other enzymes facilitate the penetration of the ovum, and fibrinolysin, which is involved in the developement of malignancies [3].

The chymotrypsin-like serine proteinases are the elastases, both of pancreatic and human leukocyte origin, cathepsin-G and the chymases [5].

As mentioned earlier, pancreatic elastase is a digestive enzyme and if activated in the pancreas as in acute pancreatitis, it can destroy the structure of the organ, with dire consequences. Most of the studies on pancreatic elastase have been performed with the extensively studied enzyme of porcine pancreas (PP).

HLE has the ability to attack a broad range of natural proteins, particularly those of connective tissue, and the natural substrates include elastin, collagen, fibronectin, basement membrane proteoglycans, fibrinogen, complement components, various plasma protein inhibitors and immunoglobulins. In recent years, HLE, has been the most extensively studied serine proteinase, because of the involvement in a wide variety of pathological states including pulmonary emphysema, rheumatoid arthritis, adult respiratory syndrome, infantile respiratory distress syndrome, glomerulonephritis, pancreatitis, cystic fibrosis, atherosclerosis and psoriasis [5].

Cathepsin-G is present in the white blood cells and can assist elastase in degrading some components of connective tissue, particularly proteoglycan. Chymases are present in human skin and lung mast cells and are released upon stimulation of mast cells under the same conditions that release histamine [5].

The serine proteinases investigated in this thesis, are trypsin, chymotrypsin, PPE, HLE and the CCK-inactivating peptidase.

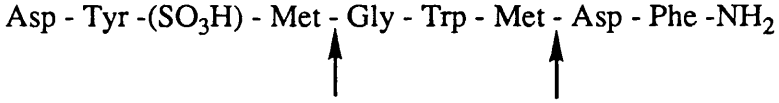
The CCK-inactivating peptidase as a novel serine proteinase

In 1988, Schwartz *et al* [6] identified a major inactivating enzyme for endogenous cholecystokinin (CCK) in brain. They showed that the C-terminal octapeptide of cholecystokinin, CCK_{26-33} or CCK-8 released by depolarisation of slices of rat cerebral

cortex, as measured by it's immunoreactivity (ir) underwent extensive degradation (~85%) before reaching the incubation medium and the recovery of CCK-ir was enhanced upto 3-fold in the presence of serine alkylating agents, with elastase inhibitors being the most potent protecting agents.

Therefore, the enzyme was characterised as a novel elastase-like serine proteinase which cleaves the two peptide bonds of CCK-8 where the carboxyl group is donated by a methionine residue to form CCK-5 and Gly-Trp-Met (GWM) as major metabolites [6].

Figure 1.2. shows the cleavage points of CCK-8 by the CCK-inactivating peptidase.



Further studies showed that the proteinase characterised in rodent brain had a similar role in human brain (unpublished results). The comparison of protective potencies of various peptidase inhibitors in the human and rat models indicated that the serine proteinase activities displayed similar specificities in the two species. In addition, these studies have provided the first direct evidence for CCK8 as a neurotransmitter.

CCK: Background and physiological role

CCK is a hormonal regulator of various gut functions such as gall bladder contraction, pancreatic secretion and gut motility [7]. It was originally discovered by Ivy and Oldberg (1928) [8], who classified it as a hormone. CCK is now known to be present in the nervous system as well and has been classified as a neuropeptide [9-11]. Biochemical studies have shown the existence of two main types of receptors [12]: central receptors -CCKB which are largely distributed in the brain and peripheral receptors-CCKA which are distributed in

peripheral organs such as the gut, pancreas, urinary bladder and peripheral somatic nerves but are also present in some regions of the brain [13].

Peripherally, the highest tissue concentrations of CCK are thought to be in the gut, especially the mucosa and muscle of the upper small intestine [13]. CCK is released in the duodenum by the depolarization of neurons, as well as by the presence of fats or amino acids, and is thought to stimulate the post-prandial secretion of pancreatic enzymes. In the brain, the highest tissue concentrations of CCK are in the cerebral cortex particularly in the neocortical regions of the telencephalon, the pallium which dominates the CNS in man [12]. It is also present in high concentrations in the hippocampus, amygdala and parts of the brain stem. CCK is synthesized presynaptically in the neurons of the brain; stored in vesicles as secretory granules in the cell bodies as well as the nerve endings and is released in a Ca^{2+}-dependent manner on depolarization of neurons [9,11,12,14].

Most neuropeptides are known to exist as precursors which undergo extensive processing to form the biologically active forms. CCK exists as "pro-cholecystokinin", a protein precursor of 115 amino acids (in man), which undergoes tissue-specific processing to form the biologically active forms of CCK-58, CCK-33, CCK-8 and CCK-4 [11]. The intestines produce mainly CCK-33, whilst the brain produces mainly CCK-8 as well as smaller fragments [11].

The existence of CCK in brain was first reported by Vanderhaegen *et al* (1975) [15]. They discovered that the brains of several species of vertebrates, including man, contained substantial quantities of CCK-8. Following this discovery, the role of CCK-8 in the CNS has been extensively investigated [9-14]. CCK-8 is the most abundant neuropeptide in the brain (1-2 mg of CCK-8) compared to other neuropeptides which are present in microgram amounts [13].

As far as CCK-8 is concerned, the greatest and most important dicovery in CCK research came about when Gibbs *et al* (1973) [16] showed evidence for the involvement of CCK-8 in the appetite suppression of rats. CCK-8 appeared to induce satiety in rats and other animals

such as rabbits and monkeys. Following this discovery, several other neuropeptides were investigated as potential regulators of appetite [17,18]. Based on these neuropharmacological experimental data in rats, Morely *et al* (1984) [19], proposed a hypothetical model of appetite regulation which involves a variety of central and peripheral receptor systems [19]. The two major neuropeptide systems thought to be involved are the peripheral satiety system which includes CCK, somatostatin and glucagon; thought to signal the passage of food through the gastrointestinal tract either via or nonvia the vagus and the central satiety system which includes substances such as serotonin, thyrotropin-releasing hormone (TRH) and neurotensin, thought to integrate the multiple signals of the peripheral satiety system.

The central appetite system is thought to be arranged in a cascade with an interaction between opioid peptides and dopamine which is thought to produce part of the feeding drive. This drive is held in check by a variety of neuropeptides such as calcitonin, corticotropin-releasing factor (CRF) and bombesin which in turn are modulated by a γ-aminobutyric acid (GABA) system, and the release of GABA is under the control of both the peripheral and central satiety systems which regulate food intake overall [19]. The model is hypothetical and much more work will be necessary to get a clearer picture of appetite regulation.

However, there is clear evidence to show that CCK does produce a satiety effect as demonstrated by Gibbs and his colleagues. Studies with humans have also shown that CCK decreases food intake in lean and obese men [20]. Therefore, CCK and CCK agonists show great therapeutic potential as appetite suppressants in the treatment of human obesity and bulimia. Similarly, the inhibition of an enzyme that inactivates CCK would be expected to show similar therapeutic potential.

The catalytic mechanism of serine proteinases

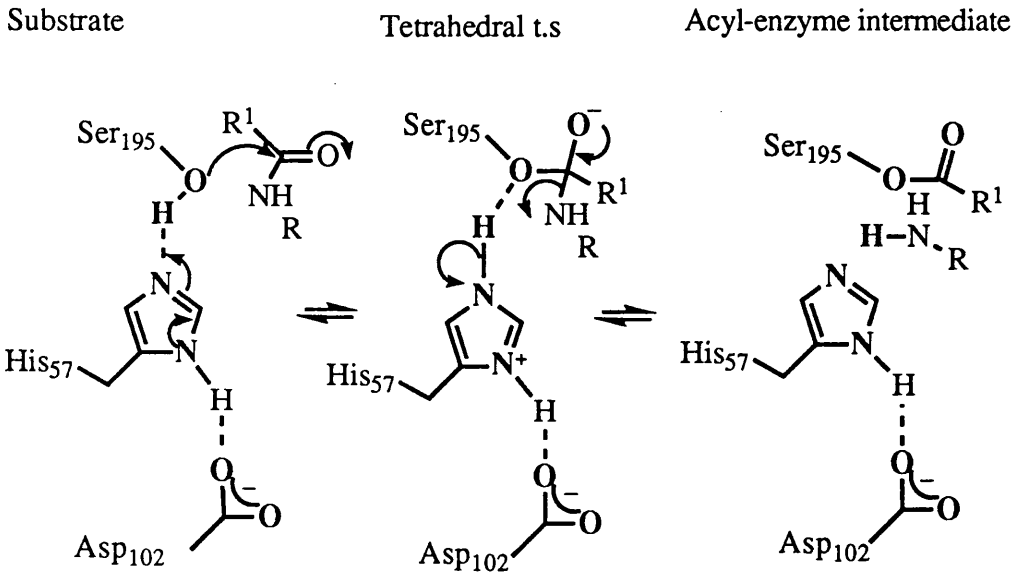
The rational design of synthetic inhibitors requires an understanding both of the catalytic mechanism and the substrate specificity of the chosen enzyme.

Serine proteinases hydrolyse peptide bonds by covalent catalysis with temporary transfer of the carboxylic part of the susceptible peptide bond to the serine CH_2OH residue at the active site [2].

Serine CH_2OH under physiological conditions is quite unreactive. However, at the active site of serine proteinases, serine is assisted by two other specific amino acids Histidine and Aspartate to form a 'catalytic triad' or a 'charge relay system', as was first postulated by Blow *et al* (1969) [21]. This consists of the carboxylate of a buried Asp_{102}, hydrogen bonded to the side chain imidazole (Nπ) [22] of His_{57}, and the imidazole (Nτ) of His_{57} which is within hydrogen bonding distance to the reactive hydroxyl of Ser_{195}, and enables the oxygen atom of the serine to participate in the catalysis as a strong nucleophile.

Hydrolysis begins by the formation of an enzyme-substrate (Michaelis) complex. This is followed by acylation where the electron lone pair on the serine oxygen attacks the C=O carbon atom of the susceptible peptide bond to form a tetrahedral transition state (t.s.) intermediate, which dissociates into a covalent acyl-enzyme complex and an amine component [23,24].

Figure 1.3. Stylized representation of the acylation of the active site of serine proteinases.

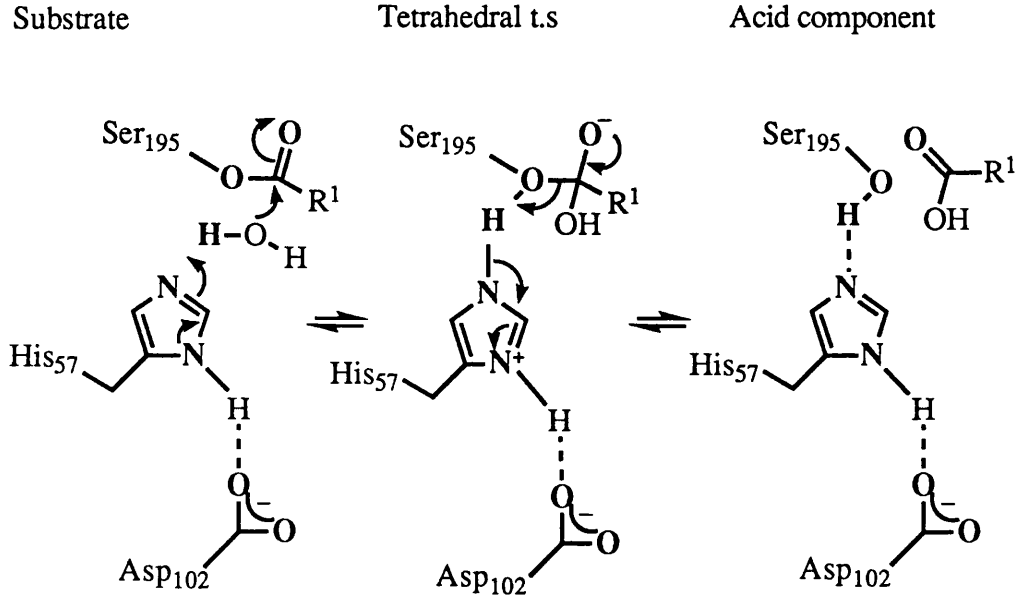


On nucleophilic attack, the carbon-oxygen bond of the C=O group becomes a single bond, and the oxygen atom acquires a net negative charge. The four atoms now bonded to the C=O carbon are arranged as in a tetrahedron. The formation of this transient tetrahedral intermediate from a planar amide group is made possible by hydrogen bonds between the negatively charged carbonyl oxygen atom ("oxyanion binding hole") and two main chain NH groups [23,24].

In the event of the formation of this tetrahedral t.s., a proton is transfered from Ser_{195} to His_{57}. Aspartate precisely orientates the imidazole ring of the His_{57} and partly neutralises the charge that develops on it. The proton held by the protonated form of histidine is then donated to the N atom of the susceptible peptide bond, which is thus cleaved. At this stage, the amine component is hydrogen bonded to the His_{57} whereas the acid component is esterified to Ser_{195} to form the acyl intermediate.

The next step is deacylation which is the reverse of acylation with H_2O substituting for an amine.

Figure 1.4. Stylized representation of the deacylation of the active site of serine proteinases.



First, the catalytic triad draws a proton away from water. The resulting OH^- anion immediately attacks the C=O carbon of the acyl group attached to Ser_{195} to form a transient tetrahedral intermediate. His_{57} then donates a proton to the O atom of Ser_{195}, which then releases the acid component of the substrate. The acid component diffuses away thus regenerating the enzyme [23,24].

There has been considerable discussion about the precise significance of the catalytic triad and the distribution of charge within it. The various hypotheses put forward have been reviewed extensively [25-28]. There has been considerable discussion about the sequence of intermediates in the catalytic pathway of the serine proteinases too. The evidence for the existence of such a pathway has been discussed in detail by Kraut (1977) [29].

Other structural features that are thought to participate in catalysis are the extended polypeptide binding site on the acyl group of the susceptible peptide bond; a number of sites for binding, with greater or lesser specificity, the side chains of a polypeptide substrate; a site for binding the substrate on it's leaving group side andthe oxyanion hole for binding the C=O oxygen atom when the C=O group is in a tetrahedral configuration.

These additional binding sites contribute to the binding of the polypeptide substrate to the enzyme whilst the side chain specificity site provides the structural basis for the specificity of serine proteinase for different substrates. For instance, chymotrypsin, requires an aromatic (such as tyrosine or phenylalanine) or bulky nonpolar side chain on the NH_2 side of the peptide bond to be cleaved, because a nonpolar pocket serves as a niche for aromatic types of compounds. Trypsin requires lysine or arginine residues and elastase is specific towards the smaller uncharged side chains. In trypsin, a serine residue is replaced by aspartate in it's pocket, which can form a strong electrostatic bond with a positively charged lysine or arginine side chain. In elastase, the pocket does not exist, the two glycine residues lining it in chymotrypsin are replaced by the much bulkier valine and threonine [2].

These specificities arise as a result of small structural changes in the binding site, governed by the amino acid sequence in the proteinase; the secondary and tertiary structures;

hydrogen bonding and electrostatic forces. Thus, each of the proteinases provides a specific chemical environment to catalyse the hydrolysis of a peptide bond.

The three most important target areas in serine proteinases that have often been utilized by inhibitor designers are the primary substrate binding sites (S_1) which affect the formation of the E.S complex; the secondary subsite binding sites (S_2, S_3, S_{11} etc.); and the catalytic triad [5].

Enzyme inhibitors as drugs

Two main classes of enzyme inhibitors, irreversible and reversible, could be broadly considered as inhibitors of serine proteinases [30].

Reversible inhibitors are compounds that match the molecular architecture at the active site and posses additional groups for non-covalent binding to adjacent regions, thereby occluding the active site and preventing access to substrate. This non-covalent binding may take place through a suitable combination of van der Waal's, electrostatic, hydrogen bonding, and hydrophobic attractive forces.

Reversible inhibition may be competitive, non-competitive, or of mixed type, depending on their point of entry into the enzyme-substrate reaction scheme. Nearly all reversible inhibitors that have been designed as potential drugs, as well as drugs in current use, are known to be competitive inhibitors-with the exception of the cardiac glycosides, which are non-competitive inhibitors of Na^+, K^+-ATPase. According to Sandler [30], one reason for this may be that competitive inhibitors of the enzyme bear some resemblance to the substrate, since they bind at the same site, and this knowledge has provided a starting point in design, whereas other types of inhibitors bind elsewhere on the enzyme and need not resemble the substrate, so obviously removing some design aspect.

A special type of competitive inhibitor is a transition-state (t.s.) analogue. This is a stable compound thought to resemble in structure the substrate portion(s) of the enzymic transition state for chemical change and forms a stable covalent complex with the enzyme.

Transition states are characterized by incompletely formed bonds and awkward geometries and no real compound is likely to resemble in detail this least stable of structures on the pathway from substrates to products. T.s. analogues are therefore thought more likely to resemble high energy intermediates along the reaction pathway than the t.s. itself. This makes it difficult to tell whether an inhibitor is binding as a poor analogue of a very tightly bound t.s. or as a somewhat better analogue of a less tightly bound high-energy intermediate. However, it has been shown that even an analogue crudely resembling t.s. should bind to the enzyme very strongly, since the affinity of the enzyme for the t.s. is extremely large (10^8-10^{14} fold greater) compared with that for the substrate(s) [31].

Compounds producing irreversible inhibition fall into two groups. Active-site-directed inhibitors possess a reactive function which, after binding of the inhibitor to the enzyme surface, forms a covalent bond with one or more functional groups at or near the active site of the enzyme, thereby removing their critical contribution to the functioning of the active site [30].

Mechanism-based inactivators (suicide inhibitors) do not carry a biologically reactive functional group but, by acting as substrates, are modified by the target enzyme to a moiety containing a reactive function that subsequently forms a covalent bond with a group on the target enzyme. In rare instances, a dead-end complex may be formed with a substrate residue covalently bound to the enzyme or the mechanism-based inactivator may be modified by the target enzyme to give a residue that binds non-covalently but tightly to the enzyme [30].

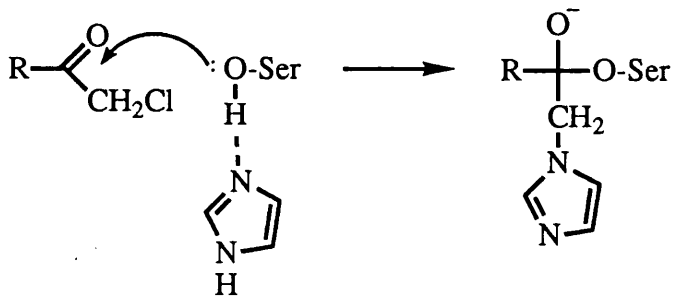
Serine-proteinase inhibitors

Serine proteinase inhibitors could be natural or synthetic. The natural serine proteinase inhibitors are the serpins in human plasma [32]. These are small glycoproteins which are plasma inhibitors, which have evolved specialized roles as regulators of the inflammatory cascades. Each of these cascades involves precisely targeted serine proteinases and is regulated by an appropriately specialized inhibitor. This specificity of control has opened therapeutic possibilities and has prompted efforts to design recombinant serpins which could have potential use as plasma replacements in genetic deficiency and as agents for specific intervention in the inflammatory cascades. Serpins act as irreversible inhibitors.

The number and types of synthetic serine proteinases up to date is so vast that the topic can only be dealt with briefly. Amongst some of the well known synthetic irreversible inhibitors of serine proteinases, have been peptide chloromethyl ketones [33], sulphonyl fluorides [34] and several acylating agents such as the benzoxazin-4-ones [35,36].

Peptide chloromethyl ketones act as active site-directed irreversible inhibitors. The peptide moiety provides enzymic active site recognition, the C=O carbon is attacked by the serine CH_2OH to form a tetrahedral structure and the N atom of the active site histidine imidazole is alkylated.

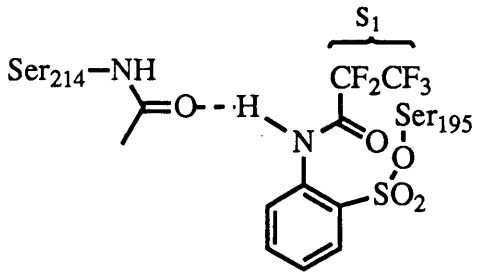
Figure 1.5. shows the structure of a serine proteinase inhibited by a peptide chloromethyl ketone.



One of the most potent chloromethyl ketones has been MeO-Suc-Ala-Ala-Pro-Val-CH_2Cl, specific to HLE with an enzyme inactivation second order rate constant $k_{obs}/[I] = 1560\ M^{-1}\ s^{-1}$. (where [I] = concentration of inhibitor) [33]. Peptides of glycine, leucine and phenylalanine chloromethylketones are specific to cathepsin G, whilst those of lysine and arginine are specific to the trypsin-like enzymes. Selective inactivation of the trypsin-like enzymes has been achieved by using the approach of incorporating part of the sequence of the physiological substrate in the peptide moiety.

Sulphonyl fluorides are active site-directed irreversible inhibitors which inhibit serine proteinases by forming a covalent sulphonyl bond with the serine O atom. It is thought that the fluoroacyl group interacts with the primary substrate recognition site S_1, whilst hydrogen bonding occurs between inhibitor NH and a backbone peptide C=O group.

Figure 1.6 shows the structure of a serine proteinase inhibited by a benzenesulphonyl fluoride.



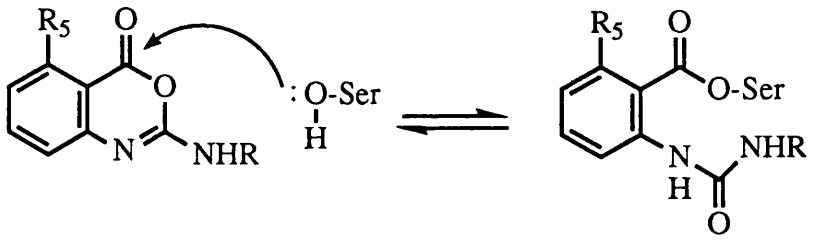
Sulphonyl fluorides have been shown to inhibit most serine proteinases including elastase, chymotrypsin, trypsin and complement, coagulation and fibrinolytic proteinases. Some of the most potent sulphonyl fluorides have been substituted arylsulphonyl fluoride derivatives. Compounds containing a perfluoroacylamino substituent ortho to the sulphonyl fluoride have been shown to be specific for elastases, whereas sulphonyl fluorides with a benzamidine moiety were shown to have the highest inhibitory potency for

trypsin-like enzymes. An example of the former type is 2-pentafluoro propionamido benzene sulphonyl fluoride with $k_{obs}d/[I]$ ($M^{-1}s^{-1}$) = 1700 against HLE [34].

The acylating agents which inhibit serine proteinases are mostly heterocyclic compounds such as benzoxazin-4-ones [35], N-substituted saccharins, 3-alkoxy-4-chloroisocoumarins [5] and ynenol lactones [5]. They acylate the active site serine and give rise to extremely stable enzyme-inhibitor complexes, deacylating with rate constants that are orders of magnitude lower than those of typical substrates.

Krantz *et al* (1987) [35] reported the inhibition of HLE by a series of substituted benzoxazin-4-ones of the general formula shown below, and found that chemically stable potent inhibitors of HLE, with K_is in the nM range could be designed with R^5 alkyl groups to inhibit enzyme-catalysed deacylation, small alkyl substituents linked via heteroatoms to C2 to enhance acylation and limit deacylation rates and strong electron donating groups at C7 to stabilize the oxazinone ring. From this series, the most active compound was 2-isopropylamino-5-ethyl-benzoxazin-4-one (R=^{i}Pr and R_5=Et) with $K_i = 9.4 \times 10^{-9}$ M against HLE [35].

Figure 1.7. shows the inhibition of serine proteinases by benzoxazin-4-ones.

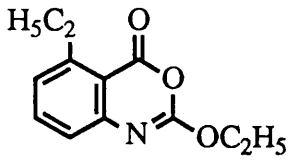


These structures were optimized further and subsequently, Krantz *et al* (1990) [36] reported 2-ethoxy-5-ethyl benzoxazin-4-one with $K_i = 4.2 \times 10^{-12}$M against HLE, making this compound the most potent irreversible inhibitor of a serine proteinase, yet.

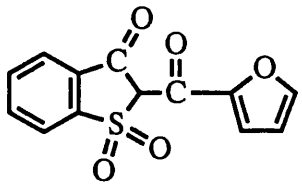
Some other examples of acylating agents are N-furoylsaccharin which inhibits HLE with IC_{50} =0.58mM, PPE with IC_{50} =0.36mM, chymotrypsin with IC_{50} = 0.07 mM and

cathepsin-G with IC_{50} =1.4 mM [37]; 4-chloro-3-ethoxyisocoumarin which inactivates HLE with (k_{obs}/[I]= 43 000 M^{-1} s^{-1} [38]; 2-benzylquinazolin-4-one which inhibits HLE with a K_i value of 8 x 10^{-5}M [39]; and 3-benzyl ynenol tetrahydrofuranone which inhibits HLE with a k_2/K_i value of 28 000 M^{-1} s^{-1} [40]. The latter has been characterized as a mechanism-based inactivator because on acylation of the active site serine, a reactive allene ketone moiety is released which can alkylate any active site nucleophile to give an irreversibly inactivated enzyme. The structures of these compounds are given in Figure 1.8.

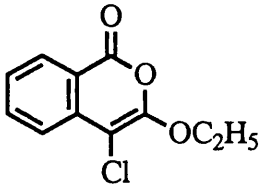
Figure 1.8 shows the structures of some of the acylating agents of serine proteinases.



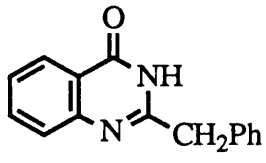
2-Ethoxy-5-ethyl-benzoxazin4-one



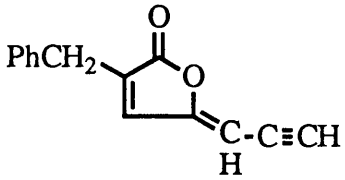
N-furoylsaccharin



4-Chloro-3-ethoxy-isocoumarin



2-Benzyl-quinazolin-4-one



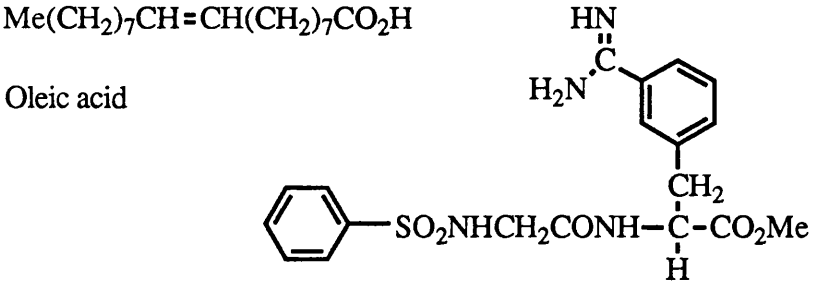
3-Benzyl ynenol tetrahydrofuranone

Some of the well known reversible serine proteinase inhibitors have been trifluoroacetyl peptides [41], cis-unsaturated fatty acids and derivatives [42], phenylguanidine derivatives and benzamidine derivatives [43]. An example of a trifluoroacetyl peptide is CF_3CO-Lys-Leu-NHC_6H_4-4-$CH(CH_3)_2$ which inhibits HLE with K_i=0.3 x 10^{-6} M [41].

An example of a cis -unsaturated fatty acid that inhibits serine proteinases is oleic acid which is specific to HLE with $K_i = 9 \times 10^{-6}$ M, and inactive against other serine proteinases. Oleoyl peptides and peptide aldehydes are also potent inhibitors of HLE. An example is oleoyl-Ala-Ala-Pro-Ala-H with $K_i = 7 \times 10^{-8}$ M.

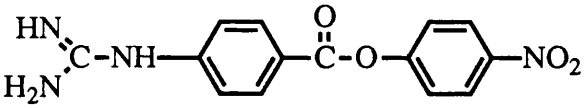
Several benzamidine and phenylguanidine derivatives have been shown to be highly specific for the trypsin-like enzymes. An example of the former type is Nα-tosyl-glycyl-3-amidinophenylalanyl ester which inhibits factor Xa with $K_i = 8.4 \times 10^{-7}$M [43]. An example of the latter type is p-nitrophenyl-p^1-guanidinobenzoate (p-NPGB) which has the highest reactivity and affinity for these types of enzymes. Inhibitors containing benzamidine and phenylguanidine types of derivatives are thought to bind to the specificity pocket of trypsin-like enzymes. Only a few inhibitors are known that inactivate trypsin selectively. An outstanding example is bis-(5-amidino-2-benzimidazolyl)methane, with a K_i value of 1.7 x 10^{-8} M (at pH 8.7, 37°C) [44]. The structures of these compounds are shown in Figure 1.9.

Figure 1.9. shows the structures of some reversible inhibitors of serine proteinases.

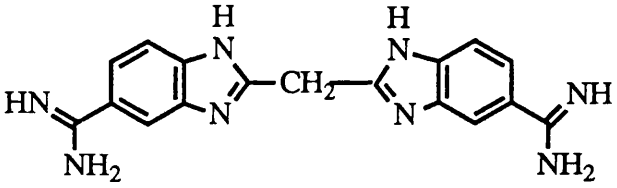


Oleic acid

Nα-tosyl-glycyl-3-amidinophenylalanine methanoate



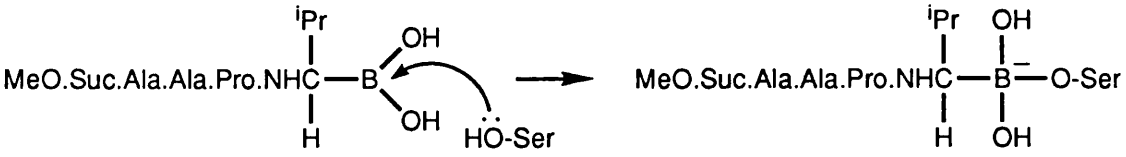
p-NPGB



Bis-(5-amidino-2-benzimidazolyl)methane

So far, the most potent reversible serine proteinase inhibitors reported have been boronic acid derivatives of appropriate peptide substrates. These act as **transition state analogues** [45].

Figure 1.10 Peptidyl boronic acids



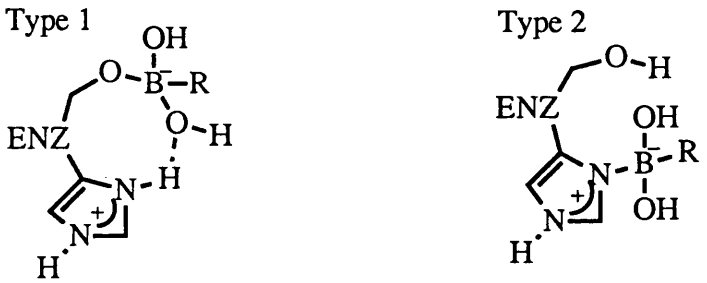
K_i = 1.6 x 10^{-10} M against Chymotrypsin [45].

The peptide provides active site recognition and the boron atom is attacked by the serine OH to form a stable tetrahedral complex.

Kettner *et al* (1988) [46] have shown that there are two types of boronic acid inhibitors.

Type 1 inhibitors are substrate analogues (t.s. inhibitors), such as the compound mentioned above, which form a tetrahedral complex with the serine O atom. These are the most potent inhibitors of a series of boronic acid derivatives and show slow-binding kinetics. Type 2 inhibitors are non-substrate analogues which are believed to form a covalent B-N bond with the active site imidazole. These inhibitors are less effective than substrate analogues but still are effective competitive inhibitors. An example is MeO-Suc-Ala-Ala-Pro-boro-Ala-OH which has a K_i value of 6.7 x 10^{-8} M against α–lytic proteinase at pH 7.5 [46,27].

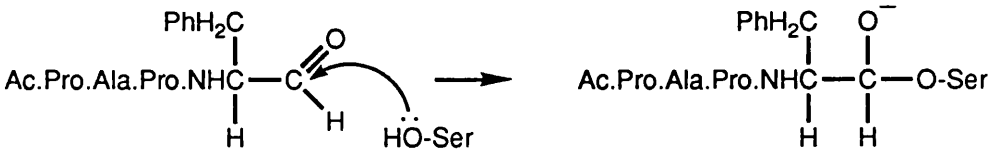
Figure 1.11. The two types of boronic acid inhibitors



Aldehydes and electron deficient ketones also form such tetrahedral complexes.

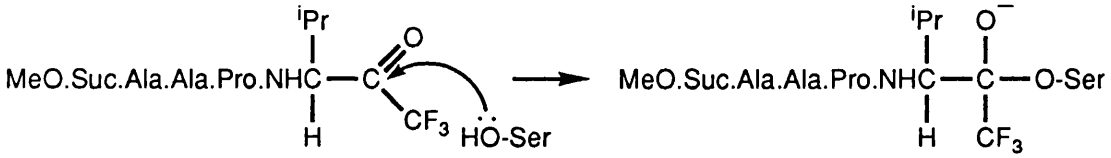
Examples are given below.

Figure 1.12. Peptidyl aldehydes



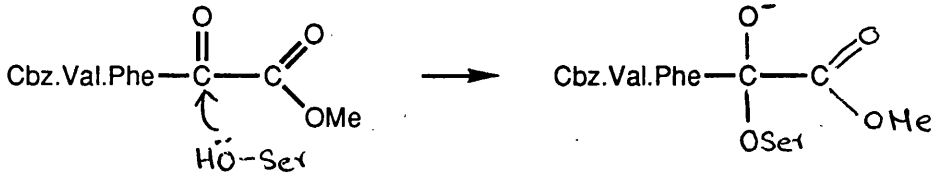
$K_i = 10^{-6}$ to 10^{-7} M(pH dependent) against Streptomyces griseus [47].

Figure 1.13. Peptidyl fluoromethyl ketones



$K_i = 1.4 \times 10^{-8}$ M against HLE [48].

Figure 1.14. α–ketoesters



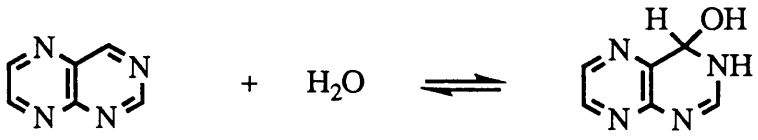
$K_i = 6 \times 10^{-8}$ M against α–chymotrypsin [49].

It seemed possible therefore that **heterocycles that undergo covalent hydration** should also act as transition state analogues, which would be **a novel approach to designing inhibitors of serine proteinase.**

Covalent hydration- a novel approach to enzyme inhibitors?

Albert *et al* (1952) [50] reported that some nitrogen-containing heterocycles undergo covalent hydration, which is - "the reversible addition of water" across the C=N bond of a nitrogen heterocycle. (See figure 1.15.) Much of the earlier work was carried out on heterocycles such as substituted pteridines and quinazolines - which showed anomalous physical properties incompatible with the orthodox formulation of these molecules.

Figure 1.15. Covalent hydration of a nitrogen-containing heterocycle.

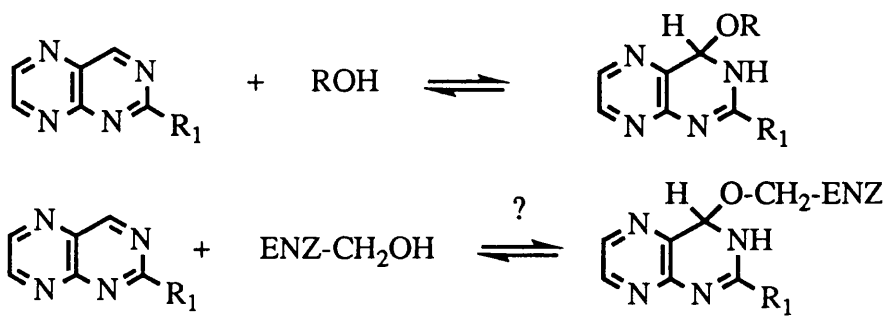


Pteridine

Following Albert's work, several other people investigated this phenomenon in other families of nitrogen-containing heterocycles and discovered that it is very common in several simpler families of polyazanaphthalenes, azapurines, some pyrimidines and biological systems too [51-53]. Some of these compounds such as pteridines also add alcohols and other nucleophiles across the C=N bond, catalysed by either acid or base. In the presence of acid, the heterocyclic cation undergoes covalent hydration. In the presence of base, the alkoxide RO^- ion attacks the C=N bond [54,55].

Therefore it seemed possible that the serine CH_2OH of serine proteinases should also be able to add across the C=N bond if the heterocycle can fit the active site of the appropriate enzyme.

Figure 1.16. Covalent addition of alcohols and possibly serine hydroxyl.



Following this thought, an extensive literature survey was carried out to find out if the idea was novel. The results were very encouraging.When the key words "serine proteinase" or "serine proteinase inhibitors" were programmed into Chemical Abstracts Service on line, 13,137 references were obtained. When combined with "heterocycle", 220 references were obtained. When combined with "heterocycle" and "covalent hydration" - there were no references. Similar results were obtained with Index Medicus on line. Reviews on enzyme inhibitors, serine proteinase inhibitors and covalent hydration up to November 1987, also had no mention of this approach.

The next stage was to decide which nitrogen-containing heterocycles that undergo covalent hydration we should select for investigation. In order to select compounds for investigation, it was necessary to study both the phenomenon of covalent hydration as well as the types of nitrogen-containing heterocycles that were subjected to this phenomenon, in detail.

Much of the early qualitative and quantitative work was done by Albert and Perrin who not only established the phenomenon of covalent hydration but also the techniques used to diagnose and locate covalent hydration and to measure the extent of hydration [51-53]. These techniques and others are still used today and deserve mention.

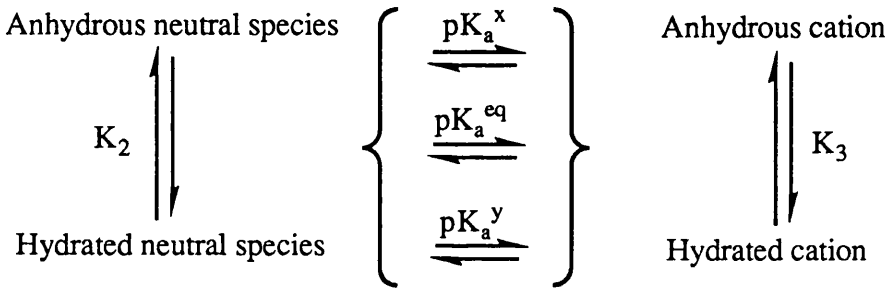
Amongst the methods of qualitatively detecting covalent hydration, anomalous ionisation constants, UV spectra, and ^1H NMR spectra have been the most useful. These same methods have been extended for quantitative measurements too.

Anomalous ionisation constants are detected by measuring and comparing ionisation constants before and after hydration (measured as pK_a values). Very often, the calculated pK_a value is very different from the experimentally determined pK_a value , because of this phenomenon. Progressing along the series of polyazanaphthalenes from di- to tri- to tetra-azanaphthalenes, one would expect a lowering of the pK_a of the nitrogen heterocycle (base-weakening effect). However, with the compounds that do undergo covalent hydration such as 1,3,5-triazanaphthalenes and pteridines, the pK_a values are much higher than expected,

because the measured pK_a value is an equilibrium value involving both hydrated and neutral species.

Bases are usually strengthened and acids weakened; these results would be expected for the deletion of a double bond. The change is marked; the anomalous constant could differ about a 1000 fold from the norm. Sometimes the anomalous value can be detected in a book of pK_a values, otherwise the anomaly is found during pK_a measurements. If the hydration is slow, hysteresis would be seen during a potentiometric titration [51].

Figure 1.17. shows the equilibria encountered during the determination of the ionisation constant of a hydrating heteroaromatic base [51].



Similar equilibria exist for hydrating bases which have an acidic function e.g.the hydroxypteridines. K_a^x and K_a^y are the ionisation equilibrium constants for the anhydrous and the hydrated species, respectively, and can be experimentally determined if measurements could be made much more rapidly than the time required to record significant hydration and dehydration.

K_2 and K_3 are hydration equilibrium constants which include the rates of hydration and dehydration of the neutral species and cation, respectively.

If the equilibria K_2 and K_3 are set up rapidly, then the pK_a value obtained in a potentiometric or spectrometric determination is an overall value (denoted as pK_a^{eq}) which includes K_2, K_3, K_a^x and K_a^y. If however, the equilibria for K_2 and K_3 are attained slowly

and the optical density or pH readings are measured rapidly, either the pK_a^x or pK_a^y value can be obtained directly depending on whether one starts from the predominantly anhydrous neutral species or the predominantly hydrated cation or (anion). However, if the solutions are allowed to come to equilibrium before each reading, only the pK_a^{eq} value can be obtained [51].

The constants pK_a^x, pK_a^{eq}, and pK_a^y are related in the following manner:

$$K_2 = \frac{K_a^y (K_a^x - K_a^{eq})}{K_a^x (K_a^{eq} - K_a^y)} \qquad \text{and} \qquad K_3 = \frac{(K_a^x - K_a^{eq})}{(K_a^{eq} - K_a^y)}$$

where $K_2 = \dfrac{\text{(concentration of hydrated neutral species)}}{\text{(concentration of anhydrous neutral species)}}$

and $K_3 = \dfrac{\text{(concentration of hydrated cation [or anion])}}{\text{(concentration of anhydrous cation [or anion])}}$ at equilibrium.

The above relationships have been used to calculate some of the constants which cannot be obtained by direct measurement, e.g accurate values of K_2 and K_3 which measure the extent of covalent hydration. (See Tables 1.1 and 1.2) [51,56].

Another technique used to diagnose and locate covalent hydration is to measure and compare UV spectra of the hydrated and anhydrous compounds in water, hydrocarbons or dilute aqueous solutions. The electronic absorption spectra of heterocyclic molecules have their origins in the transitions of electrons between different molecular orbitals. In general, the more these orbitals are spread out in space, the closer together are their energy levels and the longer the wavelengths at which absorption maxima occur. Addition of a molecule of water across a C=N bond would be expected to modify the observed spectrum; by reducing the conjugation pathway in the molecule thus producing spectral shifts towards shorter wavelengths. In such systems, a comparable effect on the spectrum is often produced by reduction of the -C=N- bond to -CH-NH- , with spectral shifts of about $\pm$ 5mμ [56].

Sometimes, larger spectral shifts are produced if either the neutral base or the cation has a significant resonance stabilization that is lacking in the other. For example, the neutral molecule of anhydrous quinazoline has λmax = 291mμ (logε = 3.76) [56], whilst the neutral

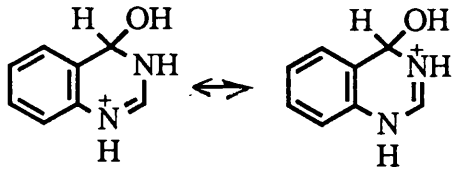
molecule of hydrated quinazoline has $\lambda max = 265m\mu$ ($\log\varepsilon = 3.97$) [56], a difference of $26m\mu$.

Covalent hydration across C=N bonds, takes place readily in nitrogen-containing heterocycles due to electron deficiency in the heterocyclic ring. When a nitrogen atom is doubly bonded, it has the electron-withdrawing force of a nitro group. Several of these in one aromatic ring placed *meta* to each other depletes the π-layer of electrons so strongly that normal aromatic stability is destroyed. As a result, an isolated and highly polarized $^{\delta+}C=N^{\delta-}$ double bond is exposed, which can add H_2O and other nucleophiles [51].

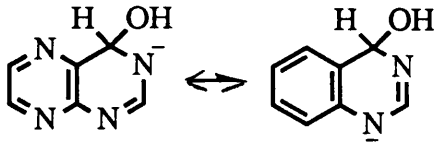
However, H_2O is a weak nucleophile and would not be expected to remain strongly bound unless some further forces were operative. It has been shown that resonance is the principal cause of this extra stabilization. For example, in the hydrated cationic species of quinazoline, an amidine-type of resonance gives this extra stabilization. A similar type of resonance is found with the hydrated neutral species of pteridine. With pteridine, the addition of H_2O produces a system which is capable in alkaline solution, of losing a proton from a nitrogen atom to form an anion-type resonance too. In general, electron-releasing groups such as NH_2 and OH diminish covalent hydration by decreasing the electron deficiency in the nucleus. However, this diminution is ineffective in 2-hydroxypteridine which exists largely as the hydrated form , because the OH facilitates a urea-type resonance. Another example is 2-aminopteridine which is readily hydrated as the cationic species, but not as the neutral species. Albert's explanation [51] for this difference, is that the hydrated cation of 2-aminopteridine is stabilized by a guanidine-type resonance whereas the hydrated neutral species is not. See figure 1.18.

Figure 1.18. shows the different types of resonance systems that stabilize hydrates.

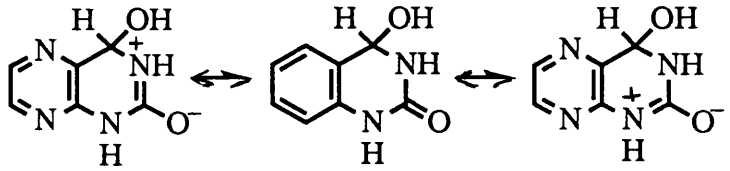
Amidine-type resonance



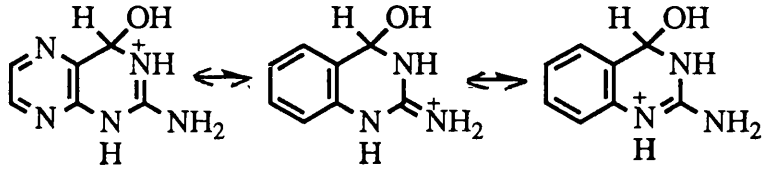
Anion-type resonance



Urea-type resonance



Guanidine-type resonance



Sometimes covalent hydration may produce a bathochromic shift (i.e a shift towards longer wavelengths), as is seen with pteridine which undergoes a bathochromic shift of approximately 20mμ, on forming the hydrate. This is thought to be due to the ease with which the N-3 electrons of the hydrated pteridine can be excited into an orbital in which there is an electron transfer towards N-8 [51].

Generally, NMR spectra of nitrogen-containing heterocycles in D_2O have been difficult to obtain because the neutral species are seldom soluble enough in D_2O. The ^1H NMR spectrum of pteridine in D_2O changes steadily with time until equilibrium is reached. The spectra of the two species is quite distinct and from the measurements of the peak heights, the equilibrium ratio can be calculated [54].The important and distinguishing property is that

the addition reaction converts an unsaturated carbon atom into a saturated one so that the signal for any proton bonded to it undergoes a considerable shift upfield. For further examples, see Chapter 4 on 'the covalent addition reactions of 2-N-alkylamidopteridines'.

Other methods of diagnosing and locating covalent hydration include IR spectroscopy, mass spectroscopy, proton magnetic resonance spectroscopy and the "blocking" effect of a Me group [51-53]. The latter involves examining the homologue which has a Me group attached to that carbon atom which is thought to carry the OH group. If the location is correct, all the above anomalies will disappear. Due to steric hindrance, the proportion of hydrated species will be decreased and both the UV spectra and ionization constants will shift towards normality.

Much of the quantitative work on covalent hydration has been done by Perrin (1965) [56], in determining K_2 values and therefore determining the extent of covalent hydration. Using rapid reaction techniques, Perrin obtained a large number of K_2 values for a whole series of polyazanaphthalenes. These were most useful for the initial selection of compounds for investigation.

Tables 1.1 and 1.2 are on the following page.

Table 1.1. showing K_2 and K_3 ratios for some heterocyclic bases that hydrate reversibly [56].

Base	K_2[1]	K_3[2]
3-Nitro-1,6-naphthyridine	_[3]	appreciable
8-Nitro-1,6-naphthyridine	_	large
Quinazoline	5.5×10^{-5}	100 (estimated)
5-Amino	_	~9
6-Amino	_	large
7-Amino	_	small
8-Amino	_	~9
5-Chloro	_	large
6-Chloro	_	large
7-Chloro	_	large
8-Chloro	_	large
6,8-Dichloro	_	large
2-Hydroxy	0.33	_
5-Hydroxy	_	appreciable
6-Hydroxy	_	appreciable
7-Hydroxy	_	small
8-Hydroxy	_	appreciable
2-Methoxy	_	0.56
5-Methoxy	_	>7
6-Methoxy	_	>6
7-Methoxy	_	>0.28
8-Methoxy	_	large
2-Methyl	0.4	_
4-Methyl	_	>0.23
5-Methyl	_	>9
6-Methyl	_	>12
7-Methyl	_	_
8-Methyl	_	>9
5-Nitro	0.0021	large
6-Nitro	0.0015	large
7-Nitro	0.0080	large
8-Nitro	0.010	large

Base	K_2[1]	K_3[2]
Quinazoline-3-oxide	–[3]	large
7-Chloro	–	large
5-Methoxy	–	appreciable
6-Methoxy	–	appreciable
7-Methyl	–	large
1,3,5-Triazanaphthalene	0.0045	>9
1,3,6-Triazanaphthalene	–	>9
1,3,7-Triazanaphthalene	0.023	>9
1,3,8-Triazanaphthalene	0.0020	>29
1,4,5-Triazanaphthalene	–	16
1,4,6-Triazanaphthalene	0.0001	95
3-Methyl	9×10^{-6}	9
2,3-Dimethyl	very small	very small
Pteridine	0.29	large
2-Amino	–	–
6-Amino	–	–
6-Chloro	–	–
2-Methyl	0.36	large
4-Methyl	0.028	large
7-Methyl	0.040	large
1,4,5,8-Tetraazanaphthalene	–	large
2-Methyl	–	large
2,3-Dimethyl	–	large
2-Amino-8-azapurine	–	–

[1] K_2 = (concentration of hydrated neutral species) / (concentration of anhydrous neutral species) at eqm.

[2] K_3 = (concentration of hydrated cation or anion) / (concentration of anhydrous cation or anion) at eqm.

[3] -- = not determined.

Table 1.2. shows K_1 and K_2 ratios for heterocyclic acids that hydrate reversibly.

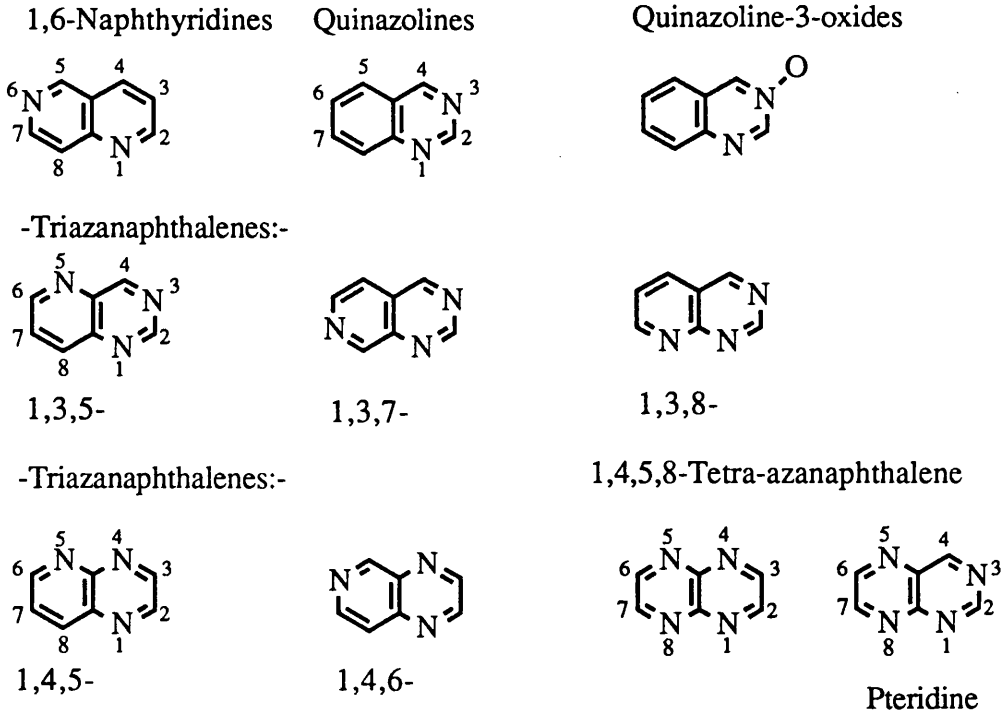
Heterocycle	K_1[4]	K_2
2-Hydroxy-1,3,8-triazanaphthalene	0.063	9
3-Hydroxy-1,4,6-triazanaphthalene	0.00016	0.45
2-Hydroxypteridine	0.14	320
4-Methyl-	0.014	6
6-Methyl-	0.10	110
7-Methyl-	0.058	35
6,7-Diethyl-	0.055	42
6,7-Dimethyl-	0.046	70
4,6,7-Trimethyl-	0.0004	0.42
6-Hydroxypteridine	0.045	125
2-Methyl-	0.028	80
4-Methyl-	0.016	75
7-Methyl-	0.001	1.29
2-Mercaptopteridine	0.24	380
2,6-Dihydroxypteridine	0.57	400
4,6-Dihydroxypteridine	0.06	1.24
2-Amino- (xanthopterin)	0.009	1.01

[4] K_1 = (concentration of hydrated anionic species) / (concentration of anhydrous anionic species) at eqm.

Some nitrogen-containing heterocycles that undergo covalent hydration

Up to 1967, covalent hydration had been demonstrated mainly in the following families of compounds: 1,6-naphthyridines, quinazolines, quinazoline-3-oxides, four families of 1,3,x-triazanaphthalenes, both families of 1,4,x-triazanaphthalenes, pteridines and some other tetra-azanaphthalenes and 8-azapurines [51]. Since then, the phenomenon has been shown to occur in other classes of compounds such as 5-nitropyrimidines, triazines and benzofuranones too [52]. Examples of covalent hydration in nature include xanthopterin, a naturally occuring pteridine which is covalently hydrated; aflatoxin B1, a fungal toxin (liberated by the fungus Aspergillus flavus), which becomes covalently hydrated in the mammalian liver to produce an extremely poisonous toxin which can attack NH_2 groups in key enzymes resulting in severe necrosis and liver cancer; and also certain ergot alkaloids such as ergotamine which becomes physiologically less active on forming the hydrate [53].

Fig 1.19. shows the major families of polyazanaphthalenes studied by Albert *et al* [51].



Naphthyridines are diazanaphthalenes. The four main groups are the 1,5-, 1,6-, 1,7- and 1,8- naphthyridines. No hydration was demonstrated in either the neutral species or the cations of these compounds [56]. However, the cations of 3-nitro- and 8-nitro-1,6-naphthyridines are largely hydrated whilst the neutral species are predominantly anhydrous.

Quinazolines are diazanaphthalenes too, but differ from naphthyridines in that the two nitrogen atoms are placed meta to each other causing electron deficiency in the pyrimidine ring. Therefore, an appreciable amount of hydration is detected in the neutral species. The neutral species of substituted quinazolines are predominantly anhydrous but the derivatives have somewhat different values depending on the position and nature of the substituent. The two types of substituents that have shown the greatest effect on the hydration of the neutral species have been NO_2 and OH. From the table it can be seen that the NO_2 group affects hydration when substituted in either the 5, 6, 7, or 8-positions, whereas the OH group has the greatest effect on the 2-position [51].

Much of the quantitative results were obtained for the cationic species of substituted quinazolines and from these results it was concluded that electron-withdrawing groups such as NO_2, and Cl in the 5-,6-,7-, or 8- positions greatly promoted hydration at the 3,4 bond by lowering the electron density on C4, whereas electron-donating groups such as MeO, OH, NH_2 and Me decrease hydration [58,59].

Armarego *et al* (1966) [60] investigated the hydration pattern in a series of 2-substituted quinazoline cations and showed that electron-withdrawing groups such as Cl, CHCl and CF_3 in the 2-position of quinazoline cations decrease hydration, whilst electron-donating groups such as Me, Et, and ^{i}Pr and greatly increase hydration. Their explanation was that an electron withdrawing group in the 2-position opposed the polarisation of the 3,4 bond. The cation of 2-hydroxyquinazoline had not been studied to make any comparison. Although, hydrated neutral species of the 2-substituted quinazolines such as 2-amino, 2-chloromethyl and 2-methoxyquinazoline were detected, these were treated as predominantly anhydrous because the adducts were shown to be quite unstable in conditions used for

rapid reaction techniques [60]. For example, the unstable neutral species of 2-aminoquinazoline was shown to have a half-life of 4 s at 200°C and pH 9.6. Therefore, one cannot justifiably make any direct comparisons between cationic and neutral species of substituted quinazolines.

The quinazoline 3-oxides are similar to quinazoline in their mode of hydration. The neutral species of the parent substance and all the derivatives examined are predominantly anhydrous. The cations however are hydrated and the substituted cations show the same hydration pattern as the substituted quinazolines with Cl, Me, and MeO groups substituted in the benzene ring [61].

The triazanaphthalenes fall into three main groups; the 1,2,x-, the 1,3,x- and the 1,4,x-triazanaphthalenes. The 1,2,x-triazanaphthalenes have not been studied due to the unstable nature of the compounds. The neutral species of 1,3,5-, 1,3,7- and 1,3,8-triazanaphthalenes show appreciable hydration and in fact are more hydrated than the diazanaphthalenes [59]. The cations are predominantly hydrated. Of the compounds studied, the neutral species of 2-hydroxy-1,3,8-triazanaphthalene undergoes the greatest amount of hydration K_2= 0.9 [62].

1,4,6-triazanaphthalene hydrates in the 1,2 position and forms a detectable amount of hydrated neutral species at equilibrium [62]. Hydration has also been found in the 3-methyl, 3-hydroxy and 7-amino derivatives but not in the 2- or 8-hydroxy derivatives. No hydration has been detected in 1,4,5-triazanaphthalene. However, the cation of the 2-hydroxy derivative has been shown to hydrate in the 3,4-position with a K_2 value of 16 [63].

The tetra-azanaphthalenes can be divided into two main groups: the 1,4,5,8-tetra-azanaphthalenes (pyrazinopyrazines) and the 1,3,5,8-tetra-azanaphthalenes (the pteridines). 1,4,5,8-tetra-azanaphthalene forms a very stable hydrated neutral species which dehydrates to the anhydrous neutral species over 16 days at pH 8.1. No K_2 values are given. The cations of the parent compound, the 2-Me and 2,3-dimethyl derivatives are > 95% hydrated[64].

If one progresses along the series of azanaphthalenes from di- to tri- to tetra-azanaphthalenes, one can see that the extent of covalent hydration increases with each additional N atom, with pteridines having the largest K_2 values. The high N/C ratio in pteridine ensures the system has a greatly depleted π–electron layer and that the aromaticity is therefore greatly reduced. Moreover, each C atom capable of bearing a substituent is activated by at least one doubly bound ring-nitrogen atom either in the α– or γ– position to it. This results in a semiaromatic system unstable to ring fission and prone to nucleophilic and covalent addition reactions. These reactivities are modified considerably by substitution with electron-donating substituents, which increase stability, or by electron-withdrawing substituents, further exaggerating these tendencies.

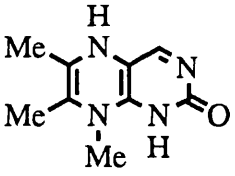
Pteridine undergoes covalent hydration at the 3,4 bond in H_2O, to form 22% of hydrated species at equilibrium [65]. In acidic conditions, pteridine is capable of forming both the 3,4-monohydrated species as well as the 5,6,7,8-dihydrated species at equilibrium [66]. The position and nature of substituent would determine the % and type of hydrated species obtained. Albert *et al* (1966) [66] have investigated the composition of equilibrium mixtures of a large series of substituted pteridine cations. They concluded that the final equilibrium ratios depended upon the relative stabilities of the hydrated species and was related to the efficiency with which the stabilising resonance systems could delocalise the positive charge.

From Tables 1.1 and 1.2, it can be seen that the types of substituents that have the greatest efffect on the hydration of the neutral species of pteridine are Me, OH and SH preferably in the 2-position and Cl in either the 6-, 7- or both 6,7-positions. There are no data on the covalent hydration of 2-chloropteridine. Kinetic studies done by Inoue and Perrin (1963) [67] suggest that a 2-Me group makes only a small difference to the extent of hydration and dehydration, whereas a 7-Me group decreases hydration by the inductive effect on C4. The hydroxypteridines are almost completely hydrated with the equilibrium lying well over that of the hydrated species [68]. It has been suggested that neutral monohydroxypteridines exist predominantly as the amide or lactam form, the formation of which leads to loss of

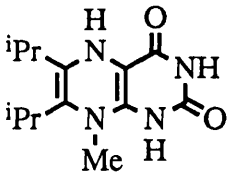
aromaticity in the pyrimidine ring and a more polarised C=N bond highly prone to nucleophilic attack. This effect is further enhanced by cation formation if the proton adds to either N1 or N3, which is perhaps one reason why the cations undergo hydration much more readily than the neutral species. The hydrated form of 2-hydroxypteridine is also stabilized by a urea-type resonance. (See Figure 1.18).

Some of the other pteridines that undergo covalent hydration as the neutral species include 6,7,8-trimethyl-2-pteridinone (an N-methyl-hydroxypteridine) [51], 6,7-diisopropyl-8-alkyllumazine (an 8-alkyllumazine) [53], 1-hydroxy-2,4-dioxo-1,2,3,4-tetrahydropteridine (a pteridine N-oxide) [69] and 4-CO_2Et- [70], 4-$CONH_2$- [71], 4-C=N- [71], and 4-CF_3- substituted derivatives of pteridine [72]. Examples are shown in Figure 1.20.

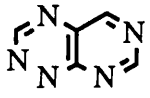
Figure 1.20. shows some examples of other pteridines that undergo covalent hydration.



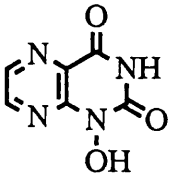
6,7,8-Trimethyl
-pteridin-2-one



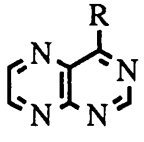
6,7-Diisopropyl
-8-methyllumazine



7-Azapteridine



1-Hydroxy-2,4-dioxo
-1,2,3,4-tetrahydropteridine



4-Substituted-pteridines

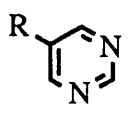
where R = CO_2Et
$CONH_2$
C=N
CF_3

Clark and his colleagues have shown that pteridines with an electron-withdrawing group in the 4-position undergo covalent hydration extremely readily (i.e 100% hydrated) as the neutral species, the position of hydration (i.e 3,4 or 5,6,7,8) depending on the nature of

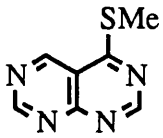
the substituent [71]. Clark and Yates (1971) [73], extended this work to prepare a series of 2-substituted-4-trifluoromethylpteridines to show that the neutral species with substituents such as NH_2 and NMe_2 were only partially hydrated , SMe moderately hydrated (70%) and MeO and Cl completely hydrated. Strong electron-withdrawing groups in the 2-position tended to greatly increase hydration. (cf. with the effect of the 2-substitution pattern on the hydration of quinazolines).

Other heterocyclic ring systems, also known to undergo covalent hydration are cations of 5-substituted pyrimidines [74], 4-methylthiopyrimido[4,5-d]pyrimidine [75], other 2,4-substituted pyrimido[4,5-d]pyrimidine derivatives [75], 1,2,4-triazine, 4,6-dinitrobenzofuroxan [76] and cations of substituted 8-azapurines [77] to name but a few examples. These are shown in figure 1.21.

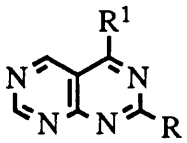
Figure 1.21. Some examples of covalent hydration in other heterocyclic ring systems.



5-Substituted-pyrimidines (R = CN, CO_2H, SO_2Me, NO_2)

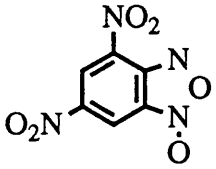


4-Methylthiopyrimido [4,5-d] pyrimidine

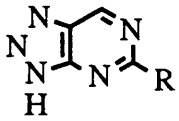


2,4-Disubstituted-pyrimido [4,5-d]pyrimidine (R = R^1= NH_2, OH)

1,2,4-Triazene



4,6-Dinitrobenzofuroxan



2-Substituted-8-azapurines (R= NH_2, SH, OH)

The criteria used for the selection of compounds for investigation will be discussed in Chapter 2.

As can be seen, from all the heterocycles that undergo covalent hydration, pteridines and quinazolines show the greatest extent of hydration and have the greatest scope for substitution. Therefore, in this thesis, I will be concentrating on such nitrogen-containing heterocycles as pteridines and quinazolines, with greater emphasis placed on the former.

My work in this project will be to rationally select and synthesise various substituted pteridines and quinazolines with the idea of investigating the phenomenon of covalent hydration as a potential and novel approach to inhibiting serine proteinases. The compounds will be primarily tested against the CCK-inactivating peptidase and then on trypsin, chymotrypsin, HLE and PPE.

CHAPTER 2

SELECTION OF COMPOUNDS

Compounds were initially selected for testing against the CCK-inactivating peptidase. The compounds that were selected for synthesis are given in Tables 2.1, 2.2 and 2.3.

As was seen in Chapter 1, the extent of covalent hydration as indicated by the ratio of hydrated: anhydrous species is affected by:

- each additional ring N atom in a nitrogen-containing heterocycle; greater resonance stabilisation in the covalent adduct with respect to the anhydrous molecule and appropriate substituents [51].

We require a compound that hydrates as the neutral species and is also not too readily hydrated as otherwise it will immediately react with H_2O when dissolved in the aqueous biophase and be inert towards the enzyme. To start with therefore, we selected for synthesis, compounds such as **2-aminopteridine (1), pteridine (2), quinazolin-2-one (4) and 2-methylpteridine (5)** -each having a ratio of hydrated: anhydrous <1. (See Table 2.1).

Our aim was that:

- compound **(2)**, would probe active site specificity for an unsubstituted heterocyclic ring system known to undergo covalent hydration; **(1)**, the binding affinity of the NH_2 group; **(4)**, the active site specificity of a quinazoline ring system as well as the binding affinity of an OH group and **(5)**, the binding affinity of a Me group. Depending upon the results, we would investigate a range of reactivities and also explore the possibility of achieving additional accessory binding to the enzyme active site by incorporating appropriate substituents. This approach has the potential for increasing potency and selectivity.

Table 2.1 shows the selection of compounds (1) to (12).

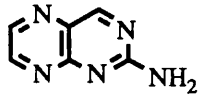
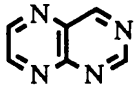
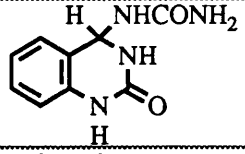
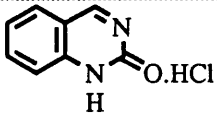
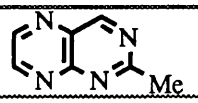
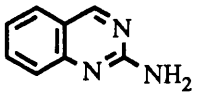
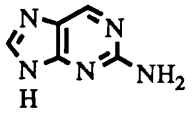
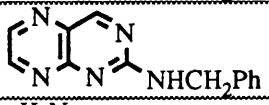
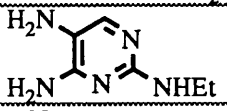
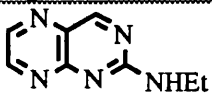
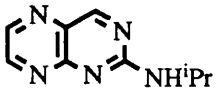
No.	UCL. No.	Ref. No.	COMPOUND	New
(1)	1015	MP61A		
(2)	1016	MP66B		
(3)	1026	MP96C		
(4)	1032	MP164A		
(5)	1041	MP223K		
(6)	1042	MP231D		
(7)	1043	MP225A		
(8)	1059	MP272B		*
(9)	1060	MP274B		*
(10)	1061	MP278B		*
(11)	1062	MP280B		
(12)	1181	MP700A		

Table 2.2 shows the selection of compounds (13) to (23).

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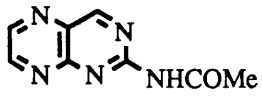
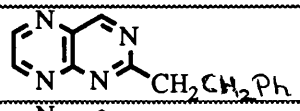
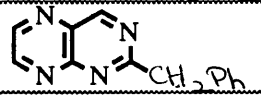
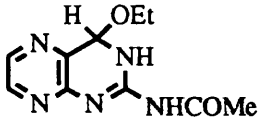
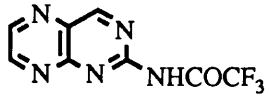
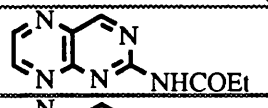
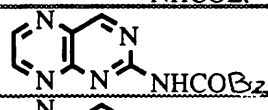
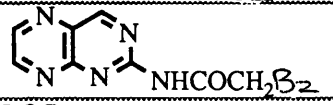
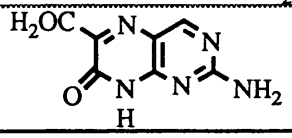
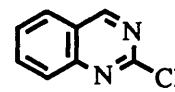
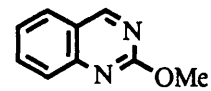
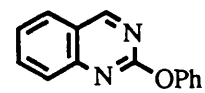
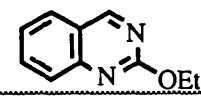
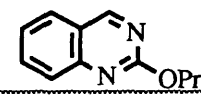
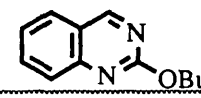
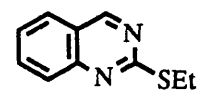
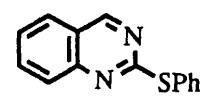
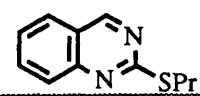
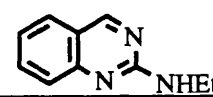
(13)	1085	MP298		
(14)	-----[3]	----		*
(15)	-----	MP818		*
(16)	1096	MP296C		*
(17)	----	MP758		*
(18)	-----	MP804I[1]		*
(19)	-----	MP808B[2]		*
(20)	1179	MP687		*
(21)	1322	MP761C		*
(22)	-----	MP780		*
(23)	1180	MP692C		*

[1] Reference corresponds to that of 2-succinimidopteridine (**18a**), which was obtained instead of (**18**).

[2] Reference corresponds to that of 2-phthalimidopteridine (**19a**), which was obtained instead of (**19**).

[3] ----- means compound was not synthesised.

Table 2.3 shows the selection of compounds (24) to (33)

No.	UCL. No.	Ref. No.	COMPOUND	New
(24)	1207	MP718C		
(25)	1206	MP721A		
(26)	1247	MP756A		*[5]
(27)	1320	MP774D		
(28)	1278	MP772C		*
(29)	NT[4]	MP776E		*
(30)	1279	MP790F		*
(31)	1280	MP798C		*
(32)	1321	MP794F		*
(33)	1281	MP800E		

[4] NT means not tested.

[5] means compound has not been found in the literature or chemical abstracts upto July 1990.

- a compound such as **(1)** should fit the active site since it closely resembles 2-benzyl-quinazolin-4-one and 2-aminobenzoxazin-4-one (see Figure 2.1) which fit the S_1 subsite and are potent inhibitors of HLE. Although predominantly non-hydrated at neutral pH, **(1)** is known to readily react with strong nucleophiles such as acetylacetone, hydrogen sulphite or nitromethane. It should therefore react with serine OH too.

-**(3)**, an intermediate to **(4)**, would be interesting as a compound that lacks a 3,4 C=N bond for covalent addition and would act as a chemical control. (i.e. a compound of similar structure but lacking the particular chemical property whose importance is being tested for).

Two compounds not known to covalently react readily with either strong or weak nucleophiles were next selected. These were **2-aminoquinazoline (6)** and the commercially available **2-aminopurine (7).** These would act as chemical controls too.

We then decided to synthesize substituted pteridines in order to investigate the influence of alkyl and arylalkyl substituents in different positions of the rings, but concentrating initially on the 2-position of both **2-aminopteridine (1)** and unsubstituted **pteridine (2)**.

The substituted aminopteridines thus selected for synthesis were **2-phenylethylaminopteridine (8), 2-benzylaminopteridine (9), 2-ethylaminopteridine (11), 2-isopropylaminopteridine (12)** and **2-N-acetamidopteridine (13).**

The rationale behind this selection was that:

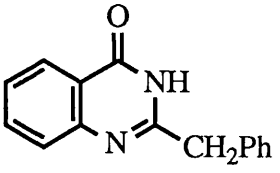
-the 2-alkylaminopteridines closely resemble the structures of both 2-benzylquinazolin-4-one and 2-aminobenzoxazin-4-one and therefore should fit the S_1 subsite at the active site and as with the benzoxazin-4-ones, the various 2-alkyl groups would probe active site specificity at the primary binding site of the CCK-inactivating peptidase.

-**(8)** and **(9)** both, would probe the binding affinity of a benzyl group. (cf. the specificity of the 2-benzyl group in 2-benzylquinazoline-4-one for HLE) [39] and thus, possibly indicate whether there was a hydrophobic pocket near the active site of the CCK-inactivating proteinase too. In the 2-amino-benzoxazin-4-one series [35,36], there is no benzyl analogue to compare.

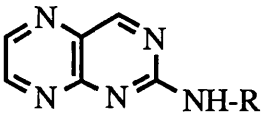
-**(11)** would probe active site specificity for a short straight chain alkyl group, whilst **(12)**, the specificity for a branched chain alkyl group that was not aromatic. **(10)**, an intermediate as well as an open chain analogue of **(11)** would show if the second aromatic ring of pteridine was necessary for activity.

-also, Krantz's work with 2-amino-benzoxazin-4-ones has shown that specificity could be achieved by varying the substituent attached to the NH_2 group [35]. Therefore, we should be able to achieve similar specificity with the alkylaminopteridines if they proved to be active.

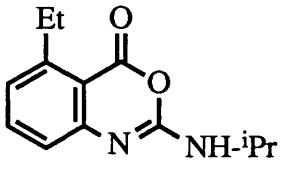
Figure 2.1. shows the structural similarity of 2-alkylaminopteridines, 2-aminobenzoxazin-4-ones and quinazolin-4-ones



2-Benzyl-quinazolin-4-one



R =		
	CH_2CH_2Ph	**(8)**
	CH_2Ph	**(9)**
	Et	**(11)**
	^iPr	**(12)**



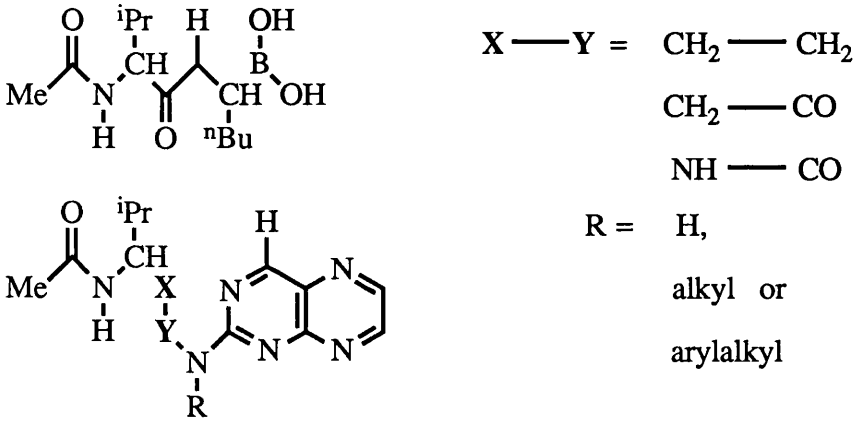
5-Ethyl-2-isopropylamino-benzoxazin-4-one

-depending on the results we could explore the possibility of attaching an appropriate peptide moiety to the most reactive alkylaminopteridine in order to achieve enzyme active site recognition.

(cf. boronic acids which are virtually inactive when unsubstituted, but active in the nM range when substituted with an appropriate peptide group) [45].

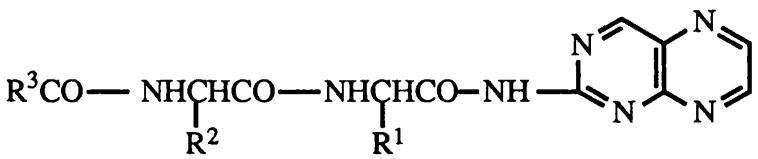
Therefore, a similar approach should be possible with the aminopteridines.

Figure 2.2. Compare substituted boronic acid with a pteridine.



-**2-N-acetamidopteridine (13),** as well as probing active site specificity for an amide group at the 2-position, would indicate if it was possible to incorporate a peptide group at this position, if necessary. (See figure 2.3).

Figure 2.3. Pteridine linked to a peptide.

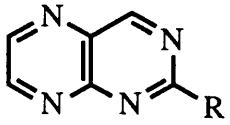


R^3	R^2	R^1	
CH_3	CH_3	CH_3	
Ac —	Ala —	Ala —	NH Pteridine
CH_3	$CHMe_2$	$(CH_2)_3Me$	
Ac —	Val —	Nle —	NH Pteridine

-more importantly, **(13)** with an alkylamido group in the 2-position would be expected to undergo covalent hydration much more readily than either **(1)** or **(2)** because of the electron withdrawing C=O group and therefore should show considerable activity compared to the latter two compounds.

Initially, we decided to investigate several 2-alkyl and 2-aryl-alkyl substituted pteridines such as those shown below.

Figure 2.5. 2-substituted pteridines.



R = Et **(14)**
^{i}Pr
CH_2CH_2Ph
CH_2Ph **(15)**

The rationale was that:

- **2-methylpteridine (5)** undergoes covalent hydration readily, therefore an Et analogue should hydrate in a similar manner and would be interesting to compare the activity of the latter with the former. Similarly, one could compare the extent of covalent addition of the other alkyl analogues too and see whether there is a correlation with biological activity.

- the substituents would probe active site specificity for the various alkyl and alkyl-aryl groups and from the results we would be able to directly compare these activities with those of the analogous substituted aminopteridines which would also indicate if the NH_2 function was necessary for activity.

Unfortunately, due to difficulties associated with obtaining a quick and efficient synthetic route to these compounds, it was decided to concentrate on just two 2-substituted pteridines, namely, **2-phenylethylpteridine (14)** and **2-benzylpteridine (15).**

- compounds **(14)** and **(15)** would probe the active site specificity for phenylethyl and benzyl groups (cf. 2-benzyl-quinazolin-4-one), respectively; are novel and the synthetic scheme would also involve some novel intermediates.

The alcoholate, **4-ethoxy-3,4-dihydro-2-N-acetamidopteridine (16)** was submitted for biological testing too.

The rationale was that:

-covalent adduct formation is a reversible process with both the covalent adduct as well as the anhydrous species present at equilibrium. Therefore, at physiological pH, there will be a sufficient amount of anhydrous species for reaction with serine OH. More importantly, the serine OH will be a much stronger nucleophile than either water or alcohol and therefore should be able to displace the alkoxy group from the adduct too. These properties would make **(16)** an ideal reversible inhibitor, if active. It would be interesting to compare the biological results of **(13)** and **(16).**

The readiness with which **(13)** was seen to undergo covalent adduct formation with alcohols prompted us to investigate other 2-amidopteridine derivatives, also with a view to ultimately making peptide derivatives. To start with, we selected **2-N-trifluoroacetamidopteridine (17).**

The rationale was that:

- a CF_3 group with highly electronegative F atoms, attached to the 2-amino group should withdraw electrons away from the pyrimidine nucleus thus making the 3,4 C=N bond more susceptible to covalent addition reactions, which in practice may not be the case.

- **(17)** would also probe active site specificity for a CF_3 group compared to a CH_3 group.

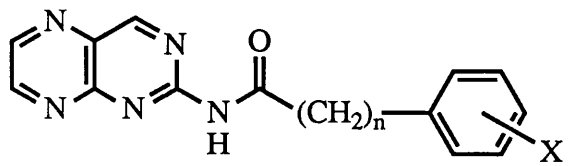
Pteridines are only sparingly soluble in H_2O and other polar solvents, and generally insoluble in most organic solvents. The introduction of substituents such as NH_2, and OH decreases the solubility even further due to strong hydrogen bonding. **(13)** was found to be only sparingly soluble in water. The introduction of large alkyl groups would also tend to decrease solubility in water because of the large hydrophobic content. We anticipated solubility problems to arise with higher alkylamidopteridines. Therefore **2-N-succinamidopteridine (18)** and **2-N-phthalamidopteridine (19)** were next selected for synthesis.

The rationale for the selection of these particular compounds was that:

- **(18)** which is analogous to **2-N-propionamidopteridine (20)** would probe active site specificity for an Et group attached to a C=O function, whereas **(19)**, analogous to **2-N-benzamidopteridine (21)** would probe specificity for a phenyl group adjacent to a C=O function. Both **(18)** and **(19)** would have an advantage over the analogous forms **(20)** and **(21)** in that the CO_2H groups in the former would assist in solubilty and therefore be easier to test for activity.

-depending on the results, we could investigate other alkyl- and alkylaryl-amidopteridines of the general type shown below with appropriate solubilising groups. (See figure 2.6.). The synthesis of these would be preliminary in the investigation of pteridine peptide derivatives.

Figure 2.6. 2-Substituted amidopteridines.



n = 0 X = H
1 CO_2H
2 NO_2
3 Cl

Due to synthetic difficulties anticipated in the acylation of the weakly basic NH_2 group in **(1)** to form these compounds, it was decided to concentrate on a few compounds to start with. These were **2-N-propionamidopteridine (20), 2-N-benzamidopteridine (21)**, where n = 0 and X = H and **2-N-phenylacetamidopteridine (22)** where n = 1 and X = H. If the preparation of these were successful the others would be attempted.

- compounds **(20)** and **(21)** would probe active site specificity for a propionamido group and a benzamido group respectively. **(22)** would be investigating the effect of increasing the chain length, on binding .

- also, as these compounds are novel, it would be interesting to investigate their behaviour towards covalent adduct formation, chemically and biologically. We expect these compounds to undergo covalent adduct formation as readily as **(13)**, too.

Having studied the effect on biological activity of various 2-substituted pteridines, we decided to investigate pteridines substituted in other positions of the ring. To start with we chose **2-amino-7(8H)pteridinone-6-carboxylic acid (23).**

The rationale for the selection of this compound was that:

-substituents in the 7 position have been shown to decrease covalent hydration due to the inductive effect of the latter on C4 (See Chapter 1). Therefore the 6 position was chosen to incorporate substituents.

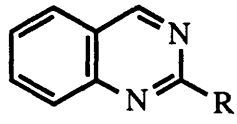
-the C=O group in the 7 position and the CO_2H group attached to the 6 position would decrease aromaticity in the pteridine ring making **(23)** more susceptible to covalent addition reactions. Heterocycles with an acidic function are more prone to covalent addition reactions than those without. (See Table 1.1 of Chapter 1).

-also, depending on the results we could incorporate various other substituents at the 6 position to probe binding affinity of substituents attached to the pyrazine ring of pteridine.

We also decided to synthesize various substituted quinazolines in order to to investigate the influence of alkyl and arylalkyl substituents in different positions of the ring but again concentrating initially on the 2 position. We first selected a series of 2-alkyl and 2-aryl-alkyl oxoquinazolines and subsequently a series of 2-alkyl and 2-aryl-alkyl thioquinazolines and finally, a 2-alkylaminoquinazoline.

The compounds selected were **2-methoxyquinazoline (25), 2-benzoxyquinazoline (26), 2-ethoxyquinazoline (27), 2-propoxyquinazoline (28), 2-butoxyquinazoline (29), 2-ethylthioquinazoline (30), 2-benzylthioquinazoline (31), 2-propylthioquinazoline (32) and 2-ethylaminoquinazoline (33).** The selection of compounds are shown on the following page.

Figure 2.7. 2-substituted quinazolines.



R =	Cl	**(24)**	SEt	**(30)**
	OMe	**(25)**	SCH_2Ph	**(31)**
	OCH_2Ph	**(26)**	SPr	**(32)**
	OEt	**(27)**	NHEt	**(33)**
	OPr	**(28)**		
	OBu	**(29)**		

The two alkoxyquinazolines **(25)** and **(26)** were selected to start with. The rationale was that:

- **(25)** and **(26)** would probe active site specificity for MeO and $PhCH_2O$ groups, respectively. **(26)** is structurally very similar to 2-benzylquinazolin-4-one and should fit the active site in a similar manner as the latter [39], thus orientating the 3,4 C=N bond for covalent reaction with serine OH. Although, **(25)** and **(26)** do not undergo covalent hydration to the same extent as **(4)**, they would react with strong nucleophiles such as serine OH.

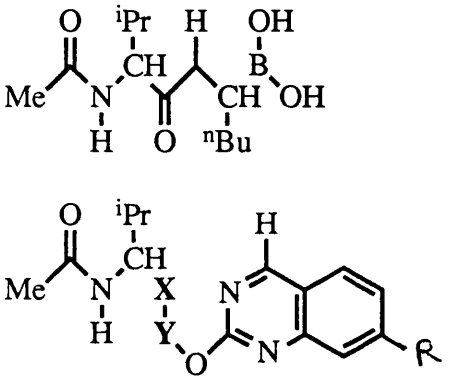
-the alkoxyquinazolines could be compared to 2-ethoxy-5-ethylbenzoxazin-4-one (Chapter 1, figure 1.8), which is the most potent irreversible serine proteinase known [36], yet. HLE seems to prefer alkoxy substituents to alkylamino substituents in the 2 position of the benzoxazin-4-ones. It seemed interesting to see whether similar specificity was seen with the CCK-inactivating peptidase and the other serine proteinases.

Site specificity was explored further by selecting **(27)**, **(28)** and **(29)**.

-these compounds would examine the effect of increasing the chain length of the alkyl substituents, on active site specificity.

-depending on the results, the possibility of attaching an appropriate peptide moiety to the most active alkoxyquinazoline (cf. boronic acids) [45] could be explored.

Figure 2.8. Compare substituted boronic acid with a quinazoline.



X—Y = CH_2—CH_2

CH_2—CO

NH—CO

R = H,

alkyl or

arylalkyl

Oxygen was replaced by other atoms such as S and N in order to examine whether the O atom in the 2 position was critical for activity. i.e. whether activity would be due to specific binding of the alkoxy groups rather than to covalent reaction with serine.
Therefore **(30)** was selected.

-it seemed interesting to see whether an EtS group in the 2 position was specific for the CCK-inactivating peptidase and the other serine proteinases. Such an approach has not been carried out with the benzoxazin-4-ones.
At the same time, **(31)** and **(32)** were also selected.

-together with **(30)**, these two compounds would examine the effect of increasing the chain length of the alkyl substituents, on active site specificity. (cf. the 2-substituted 2-oxoquinazolines).

Finally, **(32)** was selected.

-2-amino quinazolines are known to be predominantly anhydrous and not known to undergo covalent hydration readily. **(33)** is a novel compound and the physical properties have not therefore been studied. However, even if it does not undergo covalent hydration as readily as **(4)**, as before, the arguement was that, if correctly orientated at the active site, the 3,4 C=N bond should be polarised sufficiently for attack by a strong nucleophile such as the serine OH.

The starting material **(24)** to all of the above substituted quinazolines was also submitted for testing. This compound would probe site specific binding for an electron-withdrawing group attached to a quinazoline ring.

Of the 33 compounds selected for investigation, compounds **(1)** to **(6)**, **(8)** to **(13)**, **(16)**, **(20)** to **(28)** and **(30)** to **(33)** were synthesised and submitted for testing. Compound **(7)** was commercially obtained and submitted for testing. Compounds **(18a)** and **(19a)** (see Chapter 3) were obtained instead of **(18)** and **(19)**. The former was submitted for testing too. Compounds **(22)** and **(29)** were synthesised, but purification was problematic and therefore, were not submitted for testing. Of the compounds that were submitted for biological testing, 16 were novel.

The syntheses of these compounds are discussed in the following chapter.

CHAPTER 3

SYNTHESIS

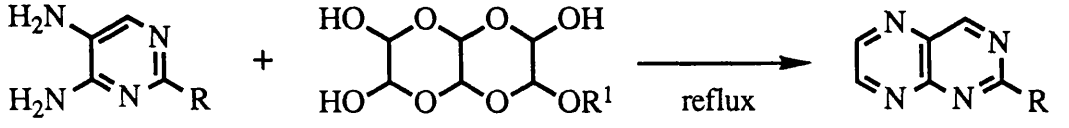
The pteridines and quinazolines were prepared by classical methods. The synthetic routes are shown in the various reaction equations. The literature methods were used to synthesise the known compounds and analogous methods for the synthesis of novel compounds.

All compounds that were submitted for biological testing were characterised by ^{1}H NMR, IR, UV and Mass spectroscopy and Melting points (if solids), TLC, HPLC and Elemental analysis. Intermediates were identified mainly by Melting points (if solids), ^{1}H NMR and Mass spectroscopy.

Pteridines have been prepared from pyrimidines, pyrazines and other heterocyclic systems. Pteridines may be made from the former by three major methods [78]. The first example is the Gabriel and Colman condensation of 4,5-pyrimidinediamines with an α-dicarbonyl reagent such as glyoxal. The other two examples include the Timmis Synthesis which consists of the condensation of a 5-nitroso-4-pyrimidinamine with an α-carbonylmethylene compound, and the Boon Synthesis which comprises of several stages, typically the aminolysis of a 4-chloro-5-nitro-pyrimidine by an α-amino carbonyl compound to give a 4-(substituted amino)-5-nitropyrimidine, the reduction of the latter with subsequent spontaneous cyclization to a 6-substituted-7,8-dihydropteridine, and a final oxidation to the corresponding pteridine [78].

Of these, the Gabriel and Colman synthesis has been utilised the most for the preparation of pteridines. The syntheses of **(1)**, **(2)**, **(5)** and the 2-alkylaminopteridines **(8)**, **(9)**, **(11)** and **(12)** were by this method.

Synthesis of **2-aminopteridine (1) and pteridine (2)** followed the Gabriel and Colman condensation of 4,5-pyrimidinediamines with glyoxal or glyoxal sodium bisulphite according to the general reaction equation shown below.



(**1**-I/1) R = NH_2 (**1**) R = NH_2

(**2**-I/1) H (**2**) H

The reaction conditions used in each case are shown below.

Cpd. No.	R	R^1	Reaction conditions	Exp. yield
(**1**)	NH_2	$NaSO_2$	2.5 hrs reflux, H_2O NaOH	28.7% (crude)
		H	50 min reflux, MeOH	88% (MeOH)
(**2**)	H	H	3 hrs reflux, EtOH	98% (Benzene)

In the literature method, (**1**) was afforded in 20.3% yield (from H_2O) [79], on refluxing 2,4,5-pyrimidinetriamine (**1**-I/1) with 1 mol equiv. of glyoxal sodium bisulphite in H_2O, followed by neutralisation with NaOH, and (**2**) in 63% yield (from benzene) [80] on refluxing 4,5-pyrimidinediamine (**2**-I/1) with 1.1 mol equivs. of polyglyoxal in alcohol.

On following the literature procedure, (**1**) was afforded in 28.7% yield. Using 0.3 mol equiv. of glyoxal trimeric dihydrate instead of polyglyoxal in abs. EtOH afforded (**2**) in 98% yield (from benzene).

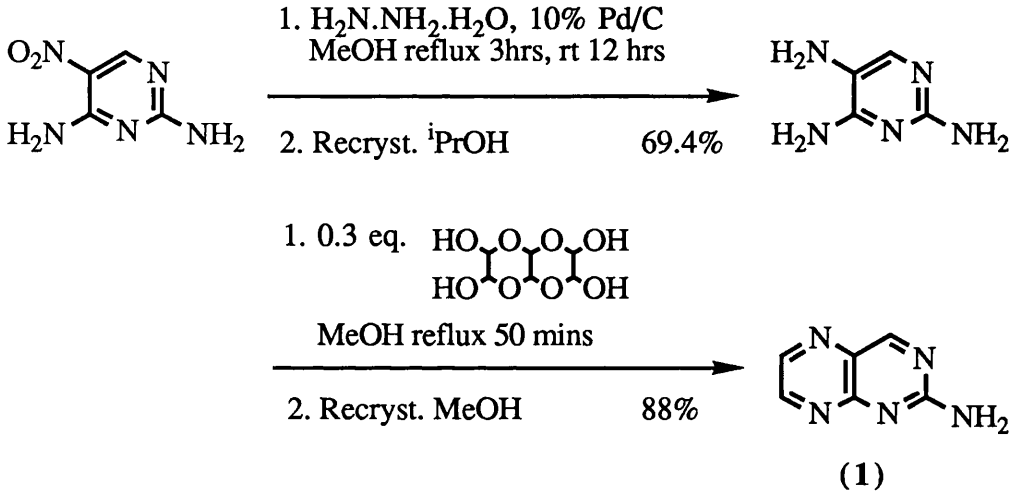
The pteridine nucleus is highly sensitive to strong acids and alkali and will decompose under such conditions [81]. Pteridines are also more prone to undergo covalent addition reactions in acidic or alkali solutions. Albert's method [79] exposed (**1**) to both acidic and alkali conditions. Partial decomposition of the product during synthesis may account for the poor yields. The by-product formed with (**1**), was an orange precipitate which could not be clearly identified at the time. Therefore, at later stages, (**1**), (an intermediate to 2-alkylamidopteridines) was prepared by refluxing 2, 4, 5-pyrimidinetriamine (**1**-I/1) with 0.3 mol equiv. of glyoxal trimeric dihydrate in MeOH and afforded in 80-90% yields. The latter method, preferred to the former, gave higher yields, minimised decomposition and overcame purification problems as the final product crystallised out of the reaction solvent on cooling.

The initial synthesis of the intermediate 2,4,5-pyrimidinetriamine (**1**-I/1) followed the method of Brown (1957) [82] who reported (**1**-I/1) in 79% yield (crude), on hydrogenation of 2,4-diamino-5-nitropyrimidine using Raney Ni catalyst in MeOH at atmospheric pressure. On following the literature method [82], (**1**-I/1) was afforded in 76.9% crude yield. The disadvantages of the above method were that the reaction was extremely slow (~29 hrs for the uptake of 0.04mol H_2) and the hydrogenation apparatus had to be constantly monitored to ensure there were no leakages. The large scale synthesis of (**1**-I/1) at a later stage, called for a more feasible and efficient method. Bavin (1958) [83] describes a method of reducing a NO_2 group to an amine by using hydrazine monohydrate in alcohol, as a reducing agent with Pd/C as a catalyst.

Therefore, at later stages, (**1**-I/1) was synthesised, with slight modifications, according to Bavin's method [83], by refluxing 2,4-diamino-5-nitropyrimidine with hydrazine monohydrate and Pd/C in abs. EtOH for 6 hrs, followed by stirring at rt for 12 hrs. The

reaction was not exothermic and had to be refluxed. Also, the reaction mixture was not added to ice and extracted with an organic solvent as suggested by the literature procedure [83], as pyrimidines are insoluble in most organic solvents and once dissolved in H_2O, could not be extracted out.

The overall scheme utilised for the preparation of **2-aminopteridine (1)** at later stages, is shown below. (Scheme 1).



(1)

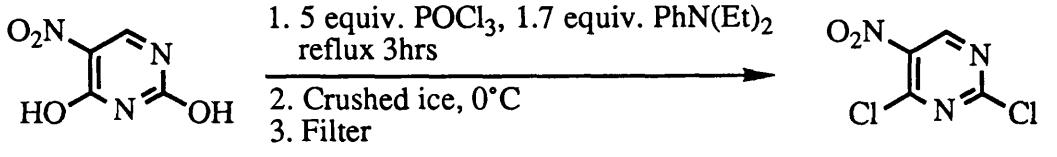
4,5,-pyrimidinediamine (2-I/1), the intermediate to **(2)**, was obtained commercially.

The synthesis of the 2-alkylamino-pteridines: **2-phenylethylaminopteridine (8), 2-benzylaminopteridine (9), 2-ethylaminopteridine** [84] **(11)** and **2-isopropylaminopteridine** [85] **(12)** involved a multi-step synthetic scheme. (See scheme 2 on the following page).

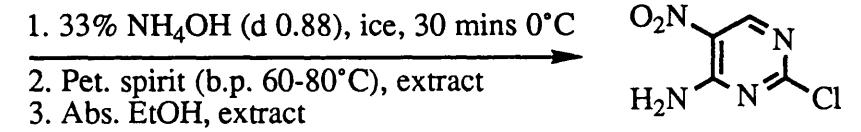
2,4,-dichloro-5-nitropyrimidine (**8**-I/1), was synthesised from 5-nitro-uracil, by refluxing the latter with 5 mol equivs. of $POCl_3$ and 1.7 mol equivs. of diethylaniline for 3 hrs according to the method of Brown (1952) [86]. The product was identified mainly by Mass and ^1H NMR spectroscopy. The crude product was mechanically stirred in crushed ice and

33% NH_4OH solution for 30 mins, filtered off, extracted first with pet. spirit (b.p. 60-80°C), and then with abs. EtOH to obtain 4-amino-2-chloro-5-nitropyrimidine (**8**-I/2).

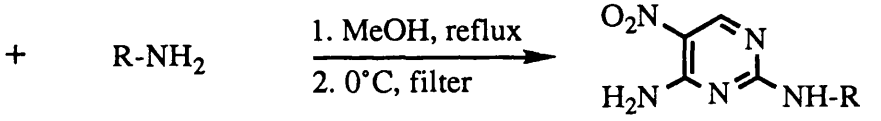
Scheme 2



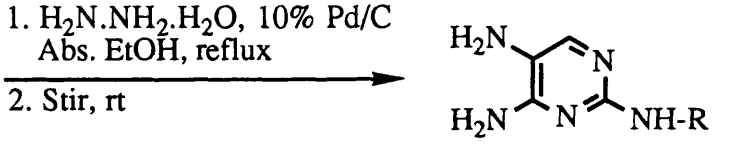
94.5% (crude) (**8**-I/1)



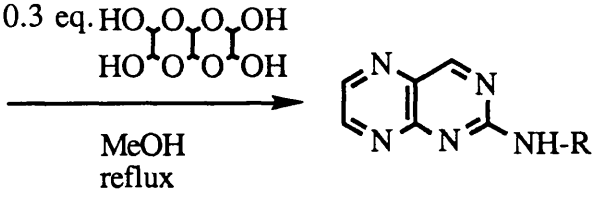
70.2% (EtOH) (**8**-I/2)



38.5% (MeOH) (**8**-I/3) 86.4% (MeOH) (**9**-I/3)
33.1% (MeOH) (**10**/**11**-I/3) 78.5% (MeOH) (**12**-I/3)



89.2% (EtOH) (**8**-I/4) 19.8% (EtOH) (**9**-I/4)
08.4% (^{i}Pr-OH) (**10**) 69.5% (MeOH) (**12**-I/4)



17.2% ($CHCl_3$) (**8**) R = CH_2CH_2Ph. 29.10% ($CHCl_3$) (**9**) CH_2Ph.
29.7% ($CHCl_3$) (**11**) Et. 76.3% ($CHCl_3$) (**12**) ^{i}Pr.

The synthesis of the novel intermediates, 5-nitro-2-phenylethylamino-4-pyrimidinamine (**8**/I-3), 2-benzylamino-5-nitro-4-pyrimidinamine (**9**/I-3), 2-ethylamino-5-nitro-4-pyrimidinamine (**10/11**/I-3) and 2-isopropylamino-5-nitro-4-pyrimidinamine (**12**/I-3) followed the method of Albert *et al* (1951) [79] who prepared an analogous compound, 2-dimethylamino-5-nitro-pyrimidinamine, by refluxing (**8**-I/2) with 50% methanolic dimethylamine (5 mol equivs.) for 1 hr. The particular reaction conditions used in each case are shown below.

Cpd. No.	R	Reaction conditions	Exp. yield
(**8**-I/3)	$Ph(CH_2)_2$	50% methanolic $Ph(CH_2)_2NH_2$ (3 mol equivs.), 2 hrs reflux	38.5% (MeOH)
(**9**-I/3)	$PhCH_2$	50% methanolic $PhCH_2NH_2$ (3 mol equivs.), 2 hrs reflux	86.4% (MeOH)
(**10,11**-I/3)	Et	50% methanolic $EtNH_2$ (7.8 mol equivs.), 2 hrs reflux	33.1% (MeOH)
(**12**-I/3)	^{i}Pr	50% methanolic $^{i}PrNH_2$ (6 mol equivs.), 1 hrs reflux	78.5% (MeOH)

The products crystallised out of solution on cooling to rt and 0°C.

The novel intermediates, 2-phenylethylamino-4,5-pyrimidinediamine (**8**/I-4), 2-benzylylamino-4,5-pyrimidinediamine (**9**/I-4), 2-ethylamino-4,5-pyrimidinediamine (**10/11**/I-4) and 2-isopropylamino-4,5-pyrimidinediamine (**12**/I-4) were prepared according to the method of Bavin (1958) [83] (described earlier for 2,4,5-pyrimidinetriamine (**1**-I/1)). The particular reaction conditions used in each case are shown on the following page.

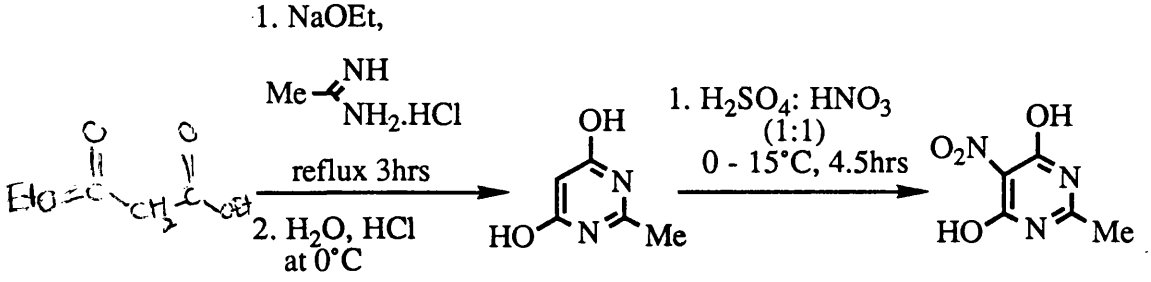
Cpd. No.	R	Reaction conditions	Exp. yield
(**8**-I/4)	$Ph(CH_2)_2$	3 mol equivs. $H_2N.NH_2.H_2O$ in MeOH, 4.5 hrs reflux and 12 hrs at rt	89.2% (EtOH)
(**9**-I/4)	$PhCH_2$	3 mol equivs. $H_2N.NH_2.H_2O$ in MeOH, 3 hrs reflux and 12 hrs at rt	19.8% (EtOH)
(**10**)	Et	3 mol equivs. $H_2N.NH_2.H_2O$ in MeOH, 4 hrs reflux and 12 hrs at rt	08.4% (^{i}PrOH)
(**12**-I/4)	^{i}Pr	6 mol equivs. $H_2N.NH_2.H_2O$ in MeOH, 5 hrs reflux.	69.5% (MeOH)

In retrospect, it seemed unnecessary to stir the reaction mixture at rt for a further 12 hrs, following reflux. The solubilities of these compounds had poor temperature gradients, therefore recrystallisation was problematic. For example, **(10)** was recrystallised from ^{i}PrOH with 14% recovery.

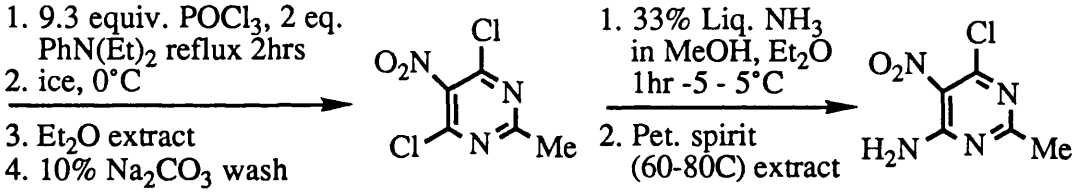
Finally, the 2-alkylaminopteridines were prepared by the general method of condensing the appropriate 4,5,-pyrimidinediamines with 0.3 mol equivs. of glyoxal trimeric dihydrate in MeOH. The compounds were purified by column chromatography (SiO_2; $CHCl_3$:MeOH, 9:1) and crystallised from $CHCl_3$. The reaction conditions were: 3 hrs reflux for **(8)**, 2.5 hrs reflux for **(9)**, 2 hrs reflux for **(11)** and 1 hrs reflux for **(12)**. The products were afforded in 17.2, 29.1, 29.7 and 76.3% yields respectively.

2-Methylpteridine [87] **(5)** was synthesised according to synthetic scheme 3.

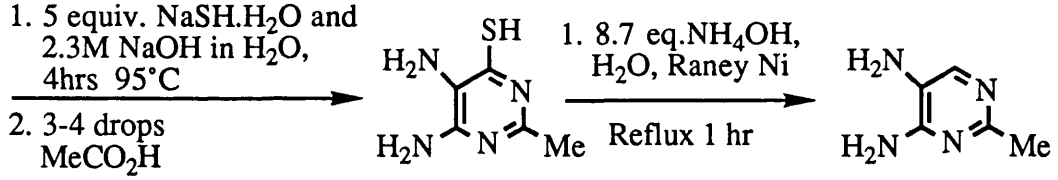
Scheme 3.



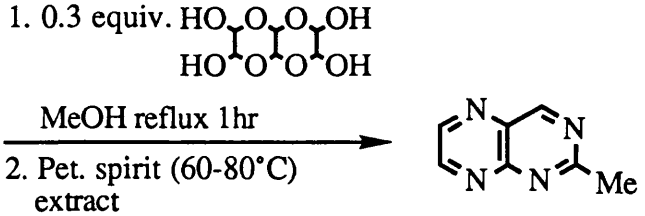
81.5% (crude) (**5**-I/1) 63% (crude) (**5**-I/2)



74.9% (crude) (**5**-I/3) 52.3% (crude) (**5**-I/4)



45.5% (H_2O) (**5**-I/5) 85.5% (EtOH) (**5**-I/6)



22% (crude) (**5**)

4,6,-Dihydroxy-2-methylpyrimidine (**5**/I-1) was prepared by heating acetamidine hydrochloride and diethyl malonate with sodium ethoxide in *situ*, according to the method of Albert *et al* (1954) [87]. Large scale synthesis was necessary in order to obtain sufficient material to see through a 6-step synthetic scheme. The crude product was used for the next stage.

The nitration of (**5**-I/1) to form 4,6,-dihydroxy-2-methyl-5-nitropyrimidine (**5**-I/2) did not proceed according to the reported literature procedure. Albert *et al* (1954) [87] report the synthesis of (**5**-I/2), by reacting (**5**-I/1) with a mixture of HNO_3 (d 1.5) : $MeCO_2H$, in a 1:2 ratio at 15 to 20°C over a period of 40 mins, then pouring the reaction mixture onto crushed ice, to obtain (**5**-I/2) as colourless needles from H_20, (mp not reported) [87]. On repeating the experiment under the same conditions, the starting material was obtained, identified by Mass and ^1H NMR spectroscopy. The latter showed the presence of the peak corresponding to C5-H at δ 4.94.

Other unsuccessful attempts included the use of: glacial $MeCO_2H$:HNO_3 (d 1.4) in a 1:2 ratio at 15 to 20°C for 4 hrs; glacial $MeCO_2H$:HNO_3 (d 1.4) in a 1:2 ratio at 0 to 15°C for 1hr followed by 40 to 50°C for 4 hrs; glacial $MeCO_2H$:fuming HNO_3 (d 1.5) in a 1:2 ratio at 15 to 20°C for 1.5 hrs (which resulted in the reaction mixture turning bright mauve) and finally, $(MeCO)_2O$:HNO_3 (d 1.5) in a 1:2 ratio, refluxed for 2 hrs No exothermic reaction or evolution of H_2 was observed. (**5**-I/1) was recovered in all cases.

Finally, the experiment was performed on a 1g scale using the classic nitrating mixture of 98% H_2SO_4:HNO_3 (d 1.42) in a 1:1 ratio. When maintained at rt, a highly exothermic reaction (with temperatures >50°C) and evolution of H_2 was observed, followed by excessive decomposition of the reaction mixture into an orange gum. (**5**-I/2) was afforded in <50% yield from H_2O. The optimum conditions achieved, by trial and error, were to: add (**5**-I/1) to a well stirred mixture of 98% H_2SO_4:HNO_3 (d 1.42) in a 1:1 ratio over 1 hr below 0°C; stir the reaction mixture at 0 to 15°C until no further rise in temperature was observed; pour onto ice at -5 to 0°C and filter. A series of colour changes, from yellow to

orange to red and back to yellow was observed each time. (**5**-I/2) was obtained as a yellow solid, not white.

(**5**-I/2) was chlorinated to form 4,6,-dichloro-2-methyl-5-nitropyrimidine (**5**-I/3) according to the method of Baddiley and Topham (1944) [88], by refluxing (**5**-I/2) with $POCl_3$ and diethylaniline for 2 hrs. The authors obtained an oil which had to be purified by distillation in order to obtain a solid. (**5**-I/3) was obtained as a viscous liquid which crystallized into a mass of needles on standing at rt.

One of the Cl atoms of (**5**-I/3) was selectively aminated by reaction with 33% liquid NH_3 in MeOH with Et_2O as a solvent at low temperatures (-5 to 5°C) to form 4-amino-2-methyl-6-chloro-5-nitropyrimidine (**5**-I/4) according to the method of Boon *et al* (1951) [89]. The by-products formed were starting material (~20%) and 2-methyl-5-nitro-4,5-pyrimidinediamine (~20%).

The next step involved the nucleophilic substitution of the Cl atom in the 6 position of (**5**-I/4) by an SH group, to form 6-mercapto-2-methyl-4,5-pyrimidinediamine (**5**-I/5) carried out by using commercially available $NaSH.H_2O$ instead of NaSH in *situ* [87]. The product was recrystallized from H_2O before proceeding to the next stage.

The SH group in the 6 position of (**5**-I/5) was reduced by heating with Raney Ni and NH_4OH. The product, 2-methyl-4,5-pyrimidinediamine (**5**-I/6), on recrystallisation from abs. EtOH afforded 11 crops. Analytical HPLC (of the combined crops 1 to 11) indicated 99.7% purity.

The final step involved the condensation of (**5**-I/6) with glyoxal trimeric dihydrate to form **2-methylpteridine (5)** in 22% crude yield. The product was sublimed at 110°C/0.1mmHg, as in the literature procedure [87] and subsequently recrystallised from pet. spirit (b.p. 60-80°C).

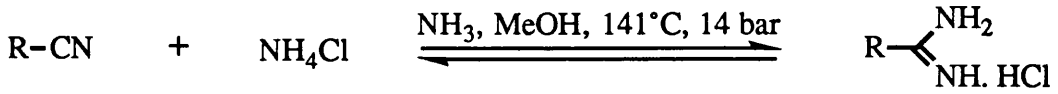
Initially, it was decided to use scheme 3 for the preparation of **2-phenylethylpteridine (14)** and **2-benzylpteridine (15)** too. The reasons for the choice were the familiarity of the synthetic route and the experience gained in handling pyrimidine intermediates in the preparation of **(5)**. Therefore the synthesis of **2-phenylethylpteridine (14)** was initiated *via* scheme 3.

The first step was the synthesis of 2-phenylacetamidine hydrochloride (**14**-I/1). Unsubstituted amidines have been classically obtained by the reaction of imidate hydrochloride salts with alcoholic ammonia or by the reaction of imidate bases with ammonium salts [90]. The alkylimidate salts are generally prepared by the Pinner Synthesis which involves the reaction of a nitrile with an alcohol, generally catalysed by acid [90]. For large scale usage, however, a single step synthesis from the nitrile is more desirable.

Schaefer and Krapcho (1962) [91] report "the simple and efficient synthesis" of a wide variety of unsubstituted amidine salts in high yields from alkyl and aryl nitriles by reaction with NH_4Cl or NH_4Br in the presence of NH_3 under pressure at 125 to 150°C. They performed the reactions in a stainless steel rocking autoclave which was charged with the nitrile, ammonium salt and solvent (if any) and the desired quantity of NH_3 was introduced by way of a transfer bomb.

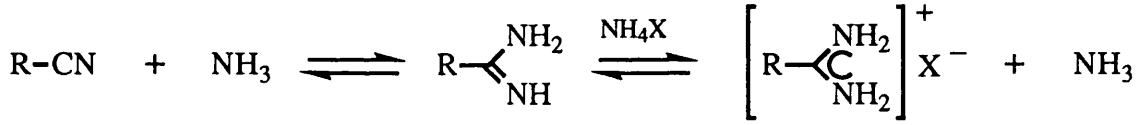
Schaefer and Krapcho (1962) [91] obtained 2-phenylacetamidine hydrochloride (**14**-I/1) in 87% yield by reacting 2-phenylacetonitrile with 4 mol equivs. of NH_4Cl and an excess (22 mol equivs.) of NH_3 at 150°C, (exact pressure not stated) [91]. There is also no mention of the use of a solvent. On repeating the literature method [91], (**14**-I/1) was afforded in 5% crude yield after 14 hrs heating at 141°C/14 Bar. The modifications to the literature method included the use of MeOH as a solvent and the use of a pressure vessel, instead of an autoclave. The experiment was repeated under the same conditions of temperature and pressure, but this time using anhydrous MeOH, to afford (**14**-I/1) in 7.9% crude yield. Dry solvent was used in order to prevent possible hydrolysis of nitrile to amide at high

temperatures. The unchanged nitrile was recovered each time. The reaction equation is shown below.



5% (crude) (**14**-I/1) R = $PhCH_2$

The reaction between nitriles, ammonia and amines has been generally known to cause very little conversion to amidines in the absence of an ammonium salt [92]. This is because the equilibrium itself is unfavourable, particularly at high temperatures and the rate of reaction very slow with nitriles that do not possess electronegative substituents. In the presence of an ammonium salt, the amidine formed, is stabilised as an amidinium salt and higher temperatures can be employed to accelerate the addition reaction. The overall equilibrium reaction is shown below.



X = Cl or Br

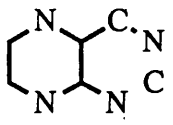
However, from the above equation, it can also be seen that the presence of excess NH_3 will aid the formation of the amidine, but impede the formation of the amidinium salt. In such a situation, the amidine would not be stable at high temperatures and would thermally dissociate back to the nitrile. This phenomenon, as well as the poor solubility of NH_4Cl in MeOH may explain why the reaction failed and starting material was recovered. Another possibility is that the rate of reaction at 141°C, (cf. 150°C) was too small, to achieve

completion of reaction within 14 hrs (literature reaction time for (**14**-I/1a) not stated). Clearly, the experiment should have been carried out with less NH_3 and maintained over a longer period of time. However, the experiment was not repeated as this particular reaction anticipated a lot of synthetic problems, especially, for large scale use.

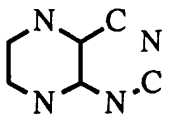
In order to continue with scheme 3, it seemed essential to have a feasible and efficient method of obtaining arylalkylamidines on a large scale. Reviews on amidine chemistry [93-98] did not offer a better alternative at the time. Therefore, scheme 3 was abandoned.

The other alternative synthetic schemes for the preparation of 2-alkylpteridines involved the use of pyrazine intermediates [78].

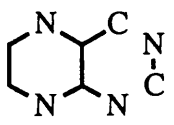
Pteridines may be prepared by building a pyrimidine ring onto a pyrazine intermediate in one of 7 possible ways as shown in the following skeletal formulas (i) - (vii) [78].



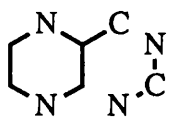
(i)



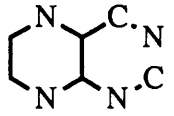
(ii)



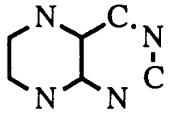
(iii)



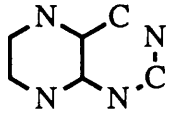
(iv)



(v)



(vi)



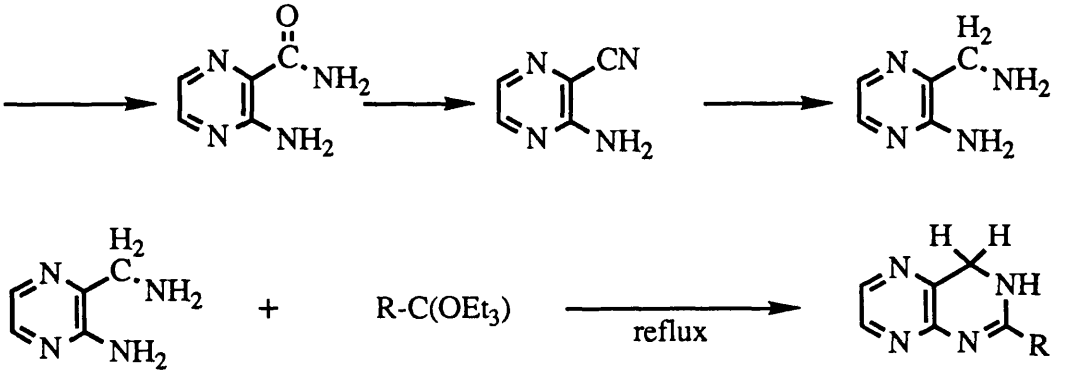
(vii)

For example, the pyrazine intermediate may require the addition of C-2 and the completion of the 1,2 and 2,3 bonds to make a pteridine, as shown in skeletal formula (i); or all the ring atoms may already be attached to the pyrazine, and only require cyclization of the 2,3 bond, as shown in formula (v). Examples for each of these syntheses exists, and are reviewed by Brown (1988) [78]. Of these, the most unambiguous and feasible syntheses for obtaining 4-unsubstituted 2-alkylpteridines have been those that involve the addition of C-2

as in skeletal formula (i) and those that involve the addition of N-3 as in formula (ii). For example, **2-methylpteridine (5)** has been synthesised using both methods. The synthetic schemes which correspond to (i) and (ii) are shown in schemes 4 [99] and 5 [100] respectively.

From the two alternative schemes 4 and 5, the latter was chosen in preference to the former, as the 4th stage in scheme 5, which involves acylation as a means of introducing the various alkyl substituents, seemed more plausible than the 4th stage in scheme 4, which involves the preparation of trialkyl orthoalkylates as a means of introducing the alkyl substituents.

Scheme 4 [99]



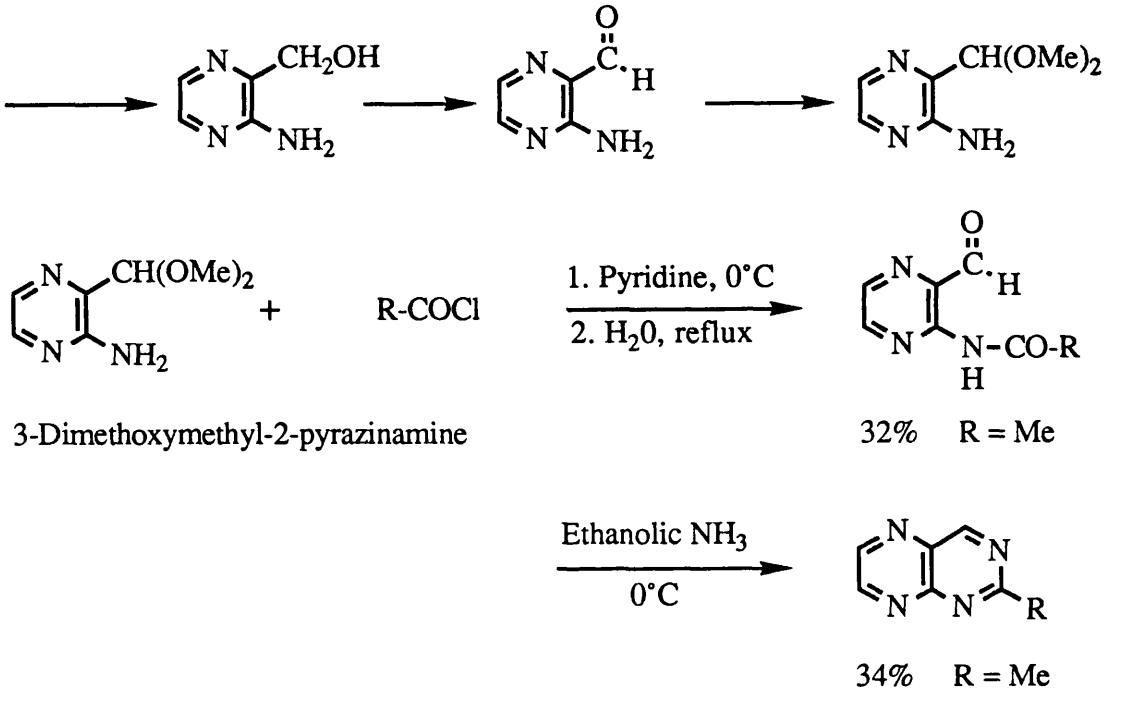
3-Aminomethyl-2-pyrazinamine

70% R = Me

80% R = Me

Scheme 5 is shown on the following page.

Scheme 5 [100]



Synthetic scheme 5 was performed twice, as the desired product (**14**-I/4) could not be obtained on the first attempt (see below, also see Scheme 6).

The first step in the scheme was the reduction of commercially available 2-methoxycarbonyl-3-pyrazinamine to 2-hydroxymethyl-3-pyrazinamine (**14,15** -I/1), performed by refluxing the former with 1.1 mol equivs. of $LiAlH_4$ in THF. The literature method [100] was repeated, using 2 mol equivs. of $LiAlH_4$, to afford (**14,15** -I/1) in 95% crude overall yield.

The next step involved the oxidation of (**14,15** -I/1) to 3-amino-2-pyrazinecarbaldehyde (**14,15** -I/2), achieved by stirring the former (crude) with an excess of MnO_2 in $CHCl_3$ at rt. It was found necessary to stir the reaction mixture for more than 24 hrs, as opposed to 30 mins (cf. the literature method) [100], in order for the reaction to go to completion.

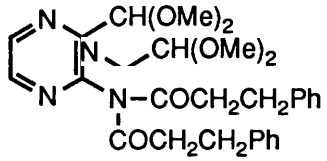
(**14,15** -I/2) was prepared in 3 successive batches to obtain sufficient material for the next stage. The average yield was 58.7% (crude).

Albert and Ohta (1971) [100] found that (**14,15** -I/2) resisted acylation with acid chlorides and acid anhydrides other than acetic methanoic anhydride. The difficulty in acylation was attributed to the strong electron withrawing effect of the aldehyde group and internal hydrogen bonding to the C=O oxygen atom. To overcome these barriers to electrophilic attack, they converted (**14,15** -I/2) into the corresponding methyl acetal, 3-dimethoxymethyl-2-pyrazinamine (**14,15** -I/3) with BF_3-MeOH complex at rt. They did not isolate the product at this stage, but proceeded further in acylating the latter with acetylchloride (~2 mol equivs) in $CHCl_3$ and pyridine. The acylation was performed at 0°C for 30 mins, at the end of which the product was not isolated, but hydrolysed directly to 3-acetamido-2-pyrazinecarbaldehyde. The overall yield was 32% [100].

On following the literature procedure [100], (**14,15** -I/3) was afforded in 57.5% average yield, after purification by column chromatography (SiO_2; $CHCl_3$). The synthesis was repeated twice to gain sufficient material for the next stage.

The acylation of (**14,15** -I/3) by hydrocinnamoyl chloride did not proceed as anticipated. The first attempt involved stirring at 0°C for 30 mins, with 1 mol equiv. of the acid chloride. When no change was observed by tlc, a further 1 mol equiv. of the acid chloride was added and the reaction mixture stirred at 0°C for 1.5 hrs and at rt for 14 hrs. Although, tlc indicated >60% of starting material, the reaction was stopped and worked up, in order to see if the reaction had taken place at all. The mass spectrum of the crude product indicated the correct M^+ ion and the fragmentation corresponding to (**14**-I/4). Therefore, the product was successively purified twice by column chromatography (SiO_2; Pet. spirit 60-80°C:$CHCl_3$:MeOH, 5:5:1) and (SiO_2; EtOAc: Pet. spirit 60-80°C, 1:1). However, (**14**-I/4) could not be identified in any of the 6 fractions collected. The starting materials were recovered.

The second attempt involved stirring at rt for 5 hrs, and at 50°C for 24 hrs with 4 mol equivs. of hydrocinnamoyl chloride. The crude product had 4 major spots on tlc (SiO_2; EtOAc: Pet. spirit 60-80°C, 1:1) of R_f 0.33 (s.m), 0.46 (product), 0.56 (pyridine) and 0.92 (hydrocinnamoyl chloride). On purification by column chromatography, 3-dimethoxymethyl-2-N-N-bis-phenylpropionamidopyrazine (**14**-I/4a) was isolated in 45.2% yield. The stucture was confirmed by ^1H NMR and mass spectroscopy.



(**14**-I/4a)

The final attempt involved stirring at 0 to 5°C for 4 hrs, at 22°C for 14 hrs, at 40°C for 14 hrs and at 50°C for 12 hrs with 1 mol equiv. of hydrocinnamoyl chloride, followed by refluxing for 30 mins with a further 1 mol equiv. of the latter. The required product was not isolated. The starting materials were obtained. The synthesis of 2-alkylpteridines was abandoned for a while (~ 1 year).

The work was resumed once again at the end of the final year. The synthetic scheme was repeated and relatively pure intermediates obtained at each stage. At the end of step 3, 4.2g of (**14**,**15** -I/3) was available for acylation.

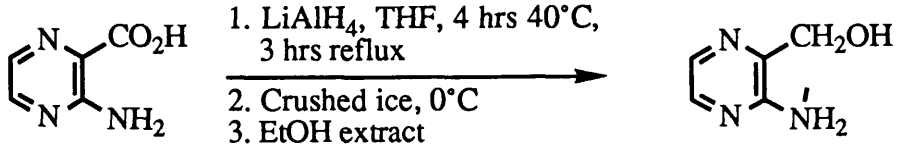
Acylation was now performed on a 0.008 mol scale with phenylacetyl chloride, in an attempt to synthesise (**15** -I/4). (**14**,**15** -I/3) was refluxed with 1.5 mol equivs. of phenylacetyl chloride in pyridine for 3 hrs and the solvent evaporated off to afford a crude product, of which the ^1H NMR spectrum indicated a mixture of starting materials and possible product (masked by pyridine and phenylacetic acid). The experiment was repeated on a large scale and the product obtained purified by column chromatography (SiO_2;

EtOAc: Pet. spirit 60-80°C, 1:1). The ^{1}H NMR spectra of the various fractions were not very clean. All fractions seemed to contain pyridine and either phenylacetyl chloride or phenylacetic acid. Having kept 0.2g aside for further investigation, it was decided to proceed with the rest of the synthetic scheme.

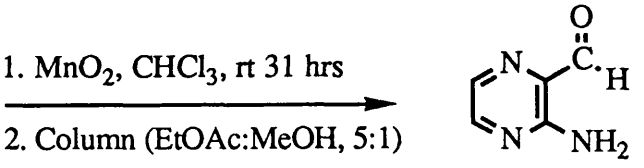
Crude (**15** -I/4) was refluxed with 1.5 mol equivs. of pyridinium hydrochloride in pyridine for 1 hr, at the end of which tlc (SiO_2: EtOAc:Pet. spirit, 1:1) indicated 7 or more products. The ^{1}H NMR spectrum of the crude product showed a mixture of (**15** -I/4) (possibly) and (**15** -I/5) in a ratio of 5:4, based on the ratio of C3-H proton peaks of the two compounds. On two successive purifications by column chromatography (SiO_2; EtOAc: Pet. spirit 60-80°C, 1:1), 3-phenylacetamido-2-pyrazinecarbaldehyde (**15** -I/5) was afforded in 72% purity according to ^{1}H NMR spectroscopy. The other 27% of material was composed of the starting material (**15** -I/4) and other minor impurities. The final fraction afforded pure (**15** -I/4), confirmed by the ^{1}H NMR spectrum, which showed the correct proton peaks corresponding to the required structure.

The final step involved the conversion of (**15** -I/5) by ethanolic NH_3 at rt into **2-benzylpteridine (15)**. (**15** -I/5) was stirred in a mixture of NH_3 (5 ml) in EtOH (20ml) at 0°C for 30 mins according to the analogous literature method [100] and at rt for 2 hrs. The ^{1}H NMR spectrum of the crude product showed a multitude of peaks, amongst which peaks corresponding to neither starting material nor required product could be definitely identified. The experiment was not investigated further due to lack of time and starting material. The overall synthetic scheme is shown on the following page. (See scheme 6). In retrospect, scheme 3 would have been better.

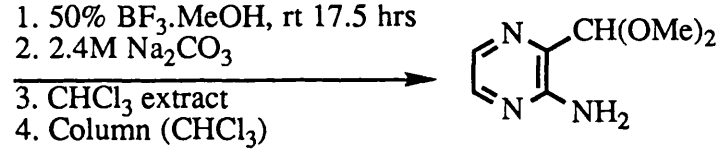
Scheme 6



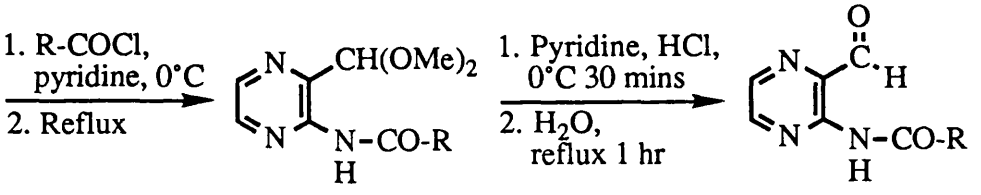
95% (crude) (**14,15** -I/1)



78.6% (crude) (**14,15** -I/2)

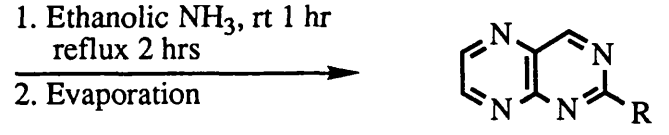


76.6% (crude) (**14,15** -I/3)



not isolated. (**14** - I/4)
52.6% (crude) (**15** - I/4)

not attempted. (**14** - I/5)
23.7% (crude) (**15** - I/5)

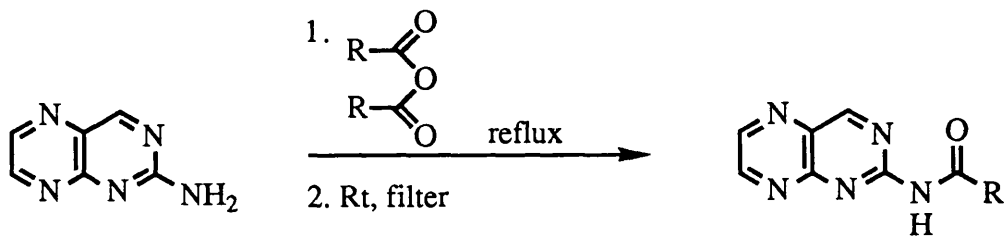


not attempted. (**14**) R = CH_2CH_2Ph.
failed (**15**) CH_2Ph.

The difficulties of acylating heterocyclic amines was encountered in the preparation of 2-N-alkylamidopteridines too.

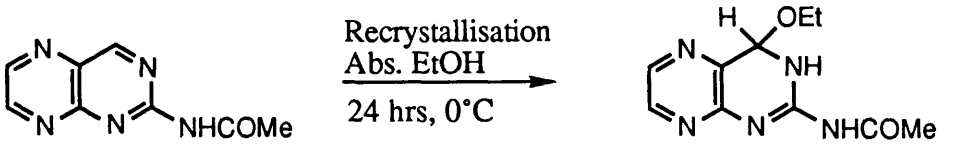
The acylation of primary and secondary aminopteridines have been performed mainly by heating the aminopteridine in the appropriate alkyl anhydride [78] and the majority of these acylation reactions have been with acetic anhydride, either neat [79] or in non-hydroxylic, polar solvents such as DMF [101] or pyridine [102]. Therefore, initially, it seemed interesting to investigate other acylation methods that could be utilised for the preparation of **2-N-acetamidopteridine (13)**. The first of these attempted was acylation of **2-aminopteridine (1)** using acetyl chloride, which proved to be unsuccesful. Acylation was carried out in both DMF (4 hrs, rt followed by 3 hrs reflux) and DMSO (1.5 mol equivs. NaH, 3 hrs reflux). No acylation was observed in either case. The starting material was obtained, confirmed by ^{1}H NMR spectroscopy. On stirring in neat acetyl chloride, **2-aminopteridine (1)** underwent extensive degradation.

The idea of inventing novel acylation routes was therefore abandoned for the time being, and **2-N-acetamidopteridine (13)** was synthesised according to the literature procedure [78], by refluxing **2-aminopteridine (1)** in acetic anhydride (20 mol equivs.) for 20 mins. The product crystallised out of solution on cooling to rt. At a later stage, **2-N-propionamidopteridine (20)** was prepared according to the same method and obtained in 83.7% yield. The reaction time was 20 mins. See reaction equation below.



72.3% (crude) **(13)** R = Me
83.7% (crude) **(20)** R = Et

An attempt to purify **(13)**, by recrystallisation from abs. EtOH, resulted in the formation of the ethanol adduct **4-ethoxy-2-N-acetamido-3,4-dihydropteridine (16)**, as shown below.



(13) **(16)**

The first crop obtained was a mixture of **(13)** and **(16)** as brown and white crystals, respectively, which were separated mechanically. The ^1H NMR spectrum of the latter indicated a mixture of **(16)** and **(13)** in a ratio of 77:22. Although the main purpose, i.e. that of purifying **(13)** was defeated at the time, this discovery proved to be very interesting as it clearly indicated that an amide group at the 2 position was conducive to covalent addition reactions at the 3,4 C=N bond of 2-N-alkylamidopteridines.

Several attempts to prepare **2-N-trifluoroacetamidopteridine (17)**, **2-N-succinamidopteridine (18)** and **2-N-phthalamidopteridine (19)**, proved to be unsuccessful. Details of several examples of attempted synthetic procedures are given in Chapter 5.

Efforts to acylate **2-aminopteridine (1)** with trifluoroacetyl reagents, which did not work included: (i) stirring with neat trifluoroacetic anhydride at rt for 5 hrs; (ii) stirring with methyltrifluoroacetate in DMF at rt for 18 hrs followed by 12 hrs under reflux; (iii) stirring with methyltrifluoroacetate in MeOH at rt for 72 hrs, under reflux for 5 hrs and at rt for another 48 hrs; (iv) stirring with 17.6 mol equivs. of trifluoroacetic anhydride in trifluoroacetic acid (TFA) at rt for 1 hr followed by 30 mins under reflux; (v) stirring with

trifluoroacetic anhydride and a catalytic amount of DMAP at rt for 12 hrs, (the experiment was repeated, taking additional precautions to minimise exposure to UV light and moisture, and stirred at rt for 24 hrs); (vi) stirring with DCC and TFA in CH_2Cl_2 at rt for 5 hrs followed by 1 hrs reflux and finally (vii) 30 mins refluxing with trifluoroacetic anhydride in pyridine.

Carboxylic acid anhydrides, although generally less reactive than acyl halides are useful reagents for the acylation of amines and amides, and as mentioned ealier, have been the most widely used acylating agents for the acylation of **2-aminopteridine (1)** [78]. The general reaction equation for the formation of an amide from an amine and an asymmetric anhydride [103] is shown below.

$$R\text{-}NH_2 \quad + \quad (R^1CO)_2O \longrightarrow R\text{-}NHCOR^1 \quad + \quad R^1CO_2H$$

The mechanism is generally discussed in terms of nucleophilic addition to the C=O carbon, either affording a tetrahedral intermediate or a synchronous displacement process proceeding through a transition state [103]. Both hypotheses lead to the same generalizations concerning the effects of stucture of the reactants on the rates of reaction. An increase in the electronegativity of R^1 in the anhydride will increase the reaction rate by enhancing the electrophilic character of the C=O carbon and by stabilizing the leaving group $R^1CO_2^-$. Therefore, trifluoroacetic anhydride should have been more effective than acetic anhydride in acylating **(1)**.

However, the results did not indicate this. With reactions (ii), (iii) and (iv), **(1)** was recovered. With reactions (i), (v) and (vi), neither the starting material nor the product could be identified by ^1H NMR spectroscopy. The literature [78] also has no mention of any isolated trifluoroacetamidopteridine derivatives. Extensive degradation of the pteridine ring

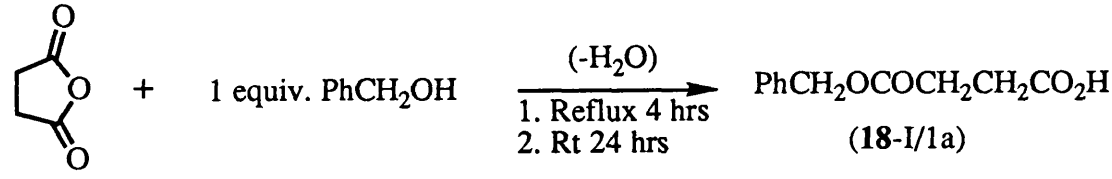
by TFA over a period of time, might be a possible explanation for the failure of reaction. With (vii), the pyridinium trifluoroacetate anion was isolated.

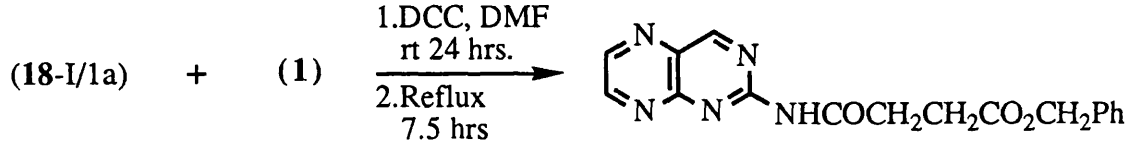
Efforts to acylate **2-aminopteridine (1)** with succinic acid derivatives, which did not work at the time, included: (i) stirring with 1.1 mol equivs. succinic anhydride in pyridine at rt for 1 hr and at 50°C for 2hrs; (ii) refluxing with 29.4 mol equivs. succinic anhydride in DMF for 14 hrs, (repeated with 173.5 mol equivs. of succinic anhydride under reflux for 24 hrs); (iii) refluxing with 29.4 mol equivs. of succinic anhydride in MeCN for 28 hrs, (repeated with 58.8 mol equivs. of succinic anhydride under reflux for 31 hrs and stirred at rt for 48 hrs); (iv) heating with succinic anhydride at 120°C/atmospheric pressure; (v) stirring with 1 mol equivs. monobenzylsuccinate (**18**-I/1a) and 1.1 mol equivs. dicyclohexylcarbodiimide (DCC) in DMF at rt for 24 hrs and under reflux for 7.5 hrs; (vi) stirring with 1 mol equivs. (**18**-I/1a) and 1.1 mol equivs. EDC in H_2O at rt for 2 hrs and under reflux for 2 hrs; (vii) stirring with benzylsuccinimidosuccinate (**18**-I/2a) in DMF at rt for 12 hrs and under reflux for 7.5 hrs; (viii) stirring with (**18**-I/2a) and TEA in DME at 40°C for 14 hrs, (repeated twice, first, stirring at rt for 12 hrs and finally at rt for 24 hrs); (ix) stirring with NaH, and (**18**-I/2a) in DMSO at rt for 17 hrs; and finally (x) refluxing in neat succinyl chloride for 15 mins.

Unlike the previous anhydrides, which were liquids and could be used on their own as reaction solvents for acylation reactions, an appropriate reaction solvent had to be found for acylation with succinic anhydride, a high melting solid. Hydroxylic solvents could not be used due to possible covalent addition reactions between the product and solvent and **(1)** was insoluble in most organic solvents except DMF, DMSO, MeCN and pyridine. Therefore, reactions (i), (ii) and (iii) investigated the best solvent for the acylation reaction.

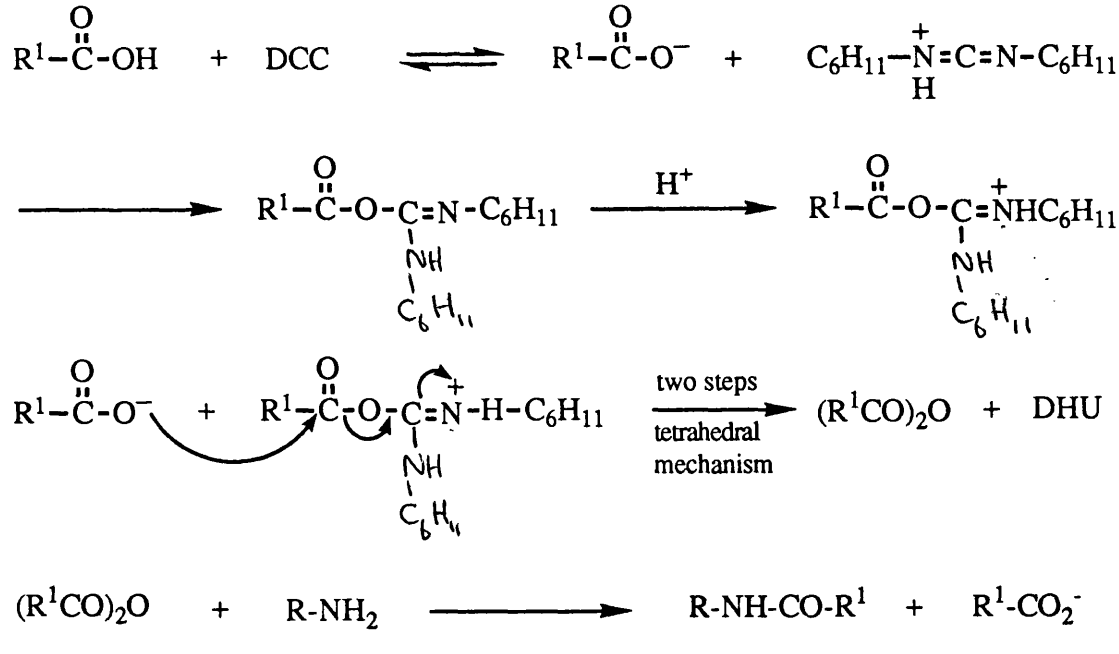
Reaction (iv) investigated the fusion of **(1)** with melted anhydride. The result was the sublimation of the starting materials at their respective melting temperatures.

Reaction (v) investigated acylation of (**1**) with (**18**-I/1a), using DCC, a coupling reagent widely used in peptide synthesis [104]. Synthesis of (**18**-I/1a) was by monoesterification of succinic acid with benzyl alcohol. The overall reaction sequence is shown below.



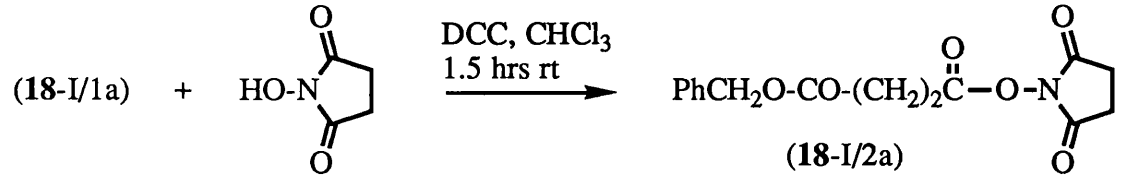


The probable reaction mechanism involved is shown below.



EDC, a water soluble carbodiimide [106] was used as an alternative coupling reagent in reaction (vi).

Reaction (vii) investigated acylation of **(1)** with an activated ester, benzylsuccinimidesuccinate (**18**-I/2a), prepared by adding N-hydroxysuccinimide and DCC to (**18**-I/1a) and stirring at rt for 2 hrs. The reaction equation is shown below



Reaction (ix) investigated the possibility of forming the 2-aminopteridine anion from NaH, with the hope that the anion would be more nucleophilic for acylation with (**18**-I/2a). Although the tautomeric state of primary aminopteridines has not been investigated thoroughly, it is thought that they exist predominantly as such rather than as dihydroiminopteridines [107]. A loss of a proton would therefore be most likely to occur from the NH_2 group. However, the formation of the anion would give rise to other tautomeric states. In such cases, the ring nitrogen atoms would not be sufficiently nucleophilic to attack the C=O group of an activated ester. This may account for the failure of reaction.

Finally, reaction (x) investigated acylation of **(1)** with succinyl chloride. Neither starting material nor product could be identified by ^1H NMR spectroscopy. In this case, the degradation of the pteridine ring by the acid chloride might be a possible explanation for the failure of reaction.

Since, all of the above methods were unsuccesful in the preparation of **(18)**, only a few of those were attempted in the preparation of **(19)**.

Attempts to acylate **2-aminopteridine (1)** with phthalic acid derivatives, which did not work at the time, included: (i) stirring with 1.1 mol equivs. phthalic anhydride in pyridine at rt for 1 hr and at 50°C for 2 hrs; (ii) stirring with NaH and phthalic anhydride in pyridine at rt for 1 hr and finally (iii) heating with phthalic anhydride at 140°C/atmospheric pressure.

Similar results were obtained as with the succinic acid derivatives. The syntheses of **(18)** and **(19)** were abandoned for a while.

Attempts to acylate **2-aminopteridine (1)** with benzoic anhydride to form **2-N-benzamidopteridine (21)**, included: (i) refluxing with 15 mol equivs. of benzoic anhydride in MeCN for 4 hrs and stirring at rt for 12 hrs; (ii) refluxing with 1.8 mol equivs. of benzoic anhydride in pyridine for 30 mins; (iii) refluxing with 2 mol equivs. of benzoic anhydride and 2 drops of $MeCO_2H$ in pyridine for 4.5 hrs and with a further 2.6 mol equivs. of benzoic anhydride for 1hr, followed by stirring at rt for 12 hrs; (repeated as above, but refluxed for 1.5 hrs); and finally, (iv) refluxing with 2 mol equivs. of benzoic anhydride and 2 drops conc. H_2SO_4 in pyridine for 1 hr.

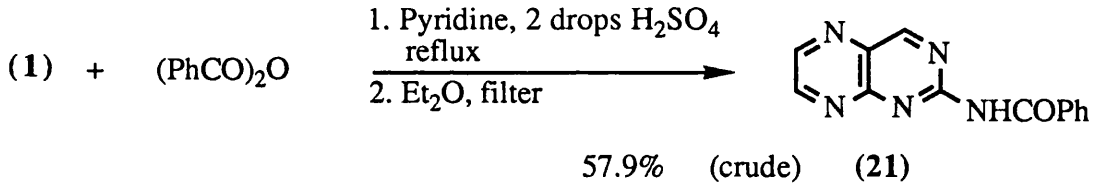
With reaction (i), the starting material was recovered.

With (ii), the ^1H NMR spectrum of the crude product obtained showed the correct proton peaks evident for **(21)**. The mass spectrum confirmed the presence of the M^+ ion, too. However, the material was insufficient for purification , characterisation and submission for biological testing.

Reaction (iii) used $MeCO_2H$ as a catalyst and afforded the product in 28.7% crude yield. An attempt to extract the product with H_2O in the hope of removing benzoic acid, resulted in the formation of the covalently hydrated adduct of **(21)**. Therefore, the experiment had to be repeated. Several unsuccessful purification attempts of the new product included: extraction with toluene, recrystallisation from toluene and recrystallisation from MeCN. By trial and error it was found that purification by column chromatography (SiO_2; MeCN) afforded good separation of impurities.

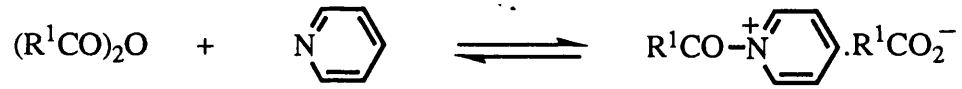
Finally, reaction (iv) was performed by refluxing **(1)** with 2 mol equivs. of benzoic anhydride and 2 drops of conc. H_2SO_4 in pyridine for 1.5 hrs, at the end of which the reaction was complete and had turned a mauve colour. Evaporation of solvent yielded a black tar which was treated with decolourising charcoal in MeCN. Evaporation of the charcoal filtrate, reprecipitation with (Na dried) Et_2O, followed by filtration afforded **(21)** in 57.9% crude yield. After, two successive purifications by column chromatography (SiO_2; MeCN), **(21)** was obtained as a yellow micro-crystalline solid.

The reaction equation is shown below.

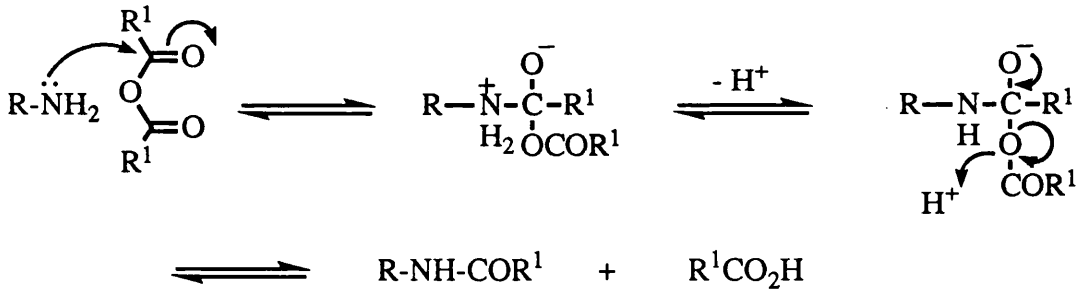


57.9% (crude) **(21)**

The reaction mechanism may involve nucleophilic attack on the N-acylpyridinium ion [108] (as shown below);



or nucleophilic attack directly on the anhydride (as shown below);

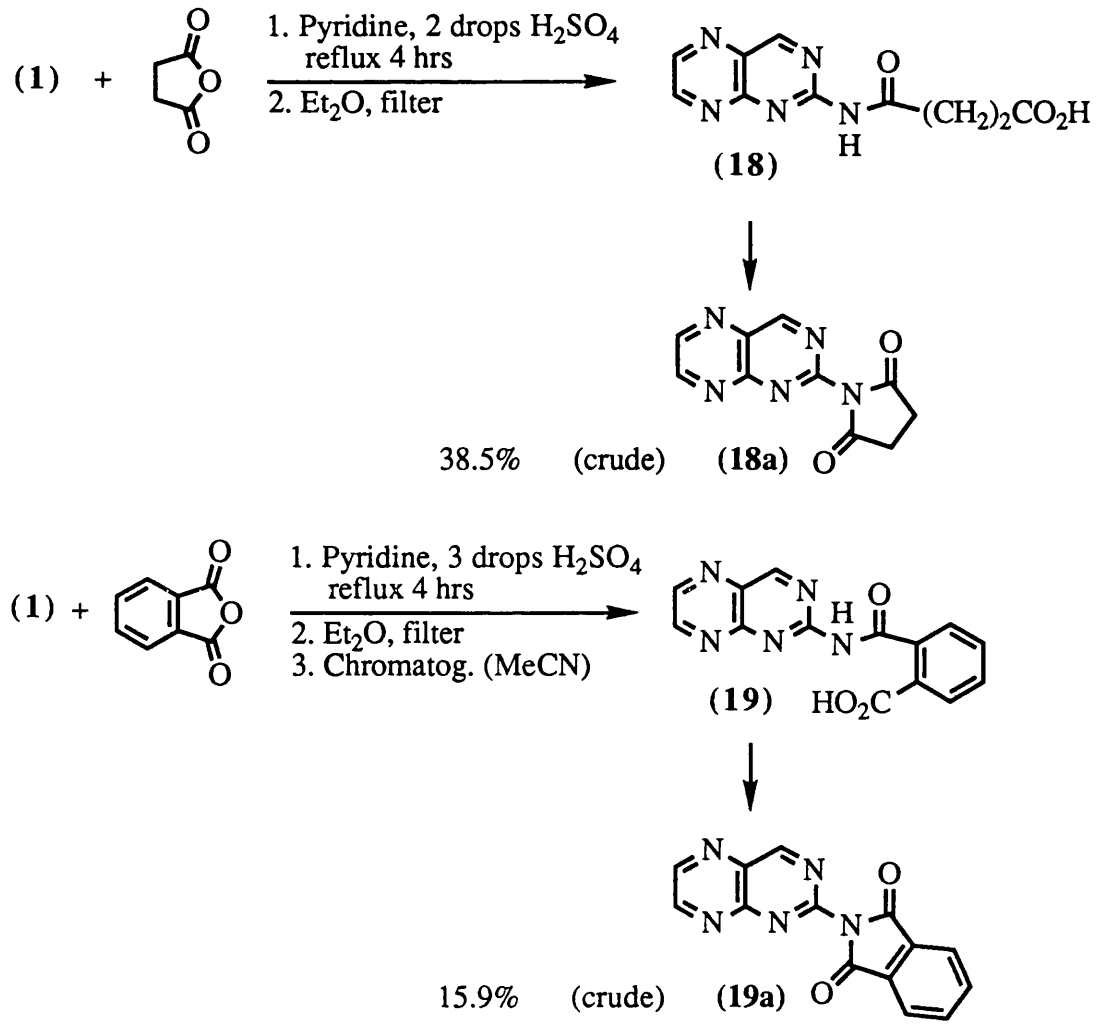


$$\rightleftharpoons \quad R\text{-}NH\text{-}COR^1 \quad + \quad R^1CO_2H$$

or both.

Catalysis by acid would involve protonation of the anhydride, which may undergo direct nucleophilic addition with pyridine or **(1)**, or break down to form RCO^+ ions which could then react with pyridine or **(1)**. The precise mechanism is not evident from these particular experimental results.

The preparation of both **(18)** and **(19)** were then attempted, using the latter method. The experiment, performed with 4.3 mol equivs. of succinic anhydride and **(1)** in pyridine with conc. H_2SO_4 as a catalyst, afforded **2-N-succinimidopteridine (18a)** instead of **2-N-succinamidopteridine (18)**. Similarly, 4.6 mol equivs. of phthalic anhydride with **(1)** under the same conditions, afforded **2-N-phthalimidopteridine (19a)** instead of **2-N-phthalamidopteridine (19)**. The reaction equations are shown on the following page.

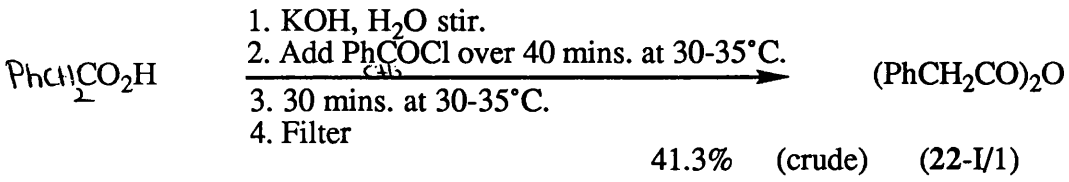


The synthesis **2-phenylacetamidopteridine (22)** proved to be problematic. A major 'stumbling block' was the preparation of 2-phenylacetic anhydride (**22**-I/1) according to the method of Burton and Kaye (1989) [109]. This was the most recently reported method in the literature, for the preparation of (**22**-I/1), at the time.

Burton and Kaye prepared (**22**-I/1) in 56% crude yield by the dehydration of phenylacetic acid in toluene with P_2O_5. Three attempts to synthesise (**22**-I/1) according to this method proved to be consistently unsuccessful. The ester, phenylethyl phenylethanoate (**22**-I/1a) was isolated each time.

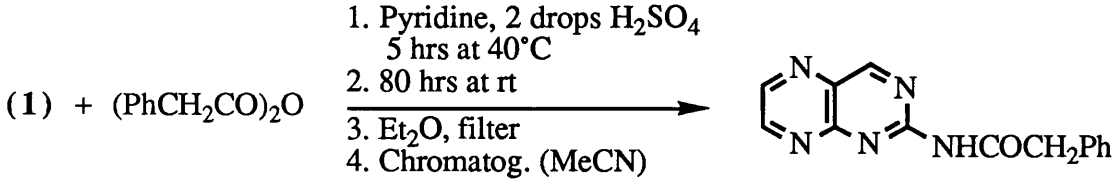
This ester, (22-I/1a) was unreactive towards (1).

Eventually, the synthesis of (22-I/1) followed the method of Cohen and Fager (1965) [110]. Phenylacetic acid was reacted with phenyl acetylchloride and KOH in H_2O to afford (22-I/1) in 41.3% crude yield. The reaction equation is shown below.



41.3% (crude) (22-I/1)

The synthesis of **2-N-phenylethanamidopteridine (22)** was performed by reacting (1) with (22-I/1) and 2 drops of conc. H_2SO_4 in pyridine at 40°C for 5 hrs and at rt for 80 hrs. The product was not evident from the ^1H NMR (200MHz) spectrum of the crude material. Therefore, the latter was purified by column chromatography (SiO_2; MeCN) and the various fractions identified by ^1H NMR (400MHz) spectroscopy. The combined fractions 2 and 3 showed a mixture of (22-I/1) and (22), the majority of product being (22-I/1). The overall crude yield of (22) was 0.6%. The reaction was not repeated further. The reaction equation is shown below.

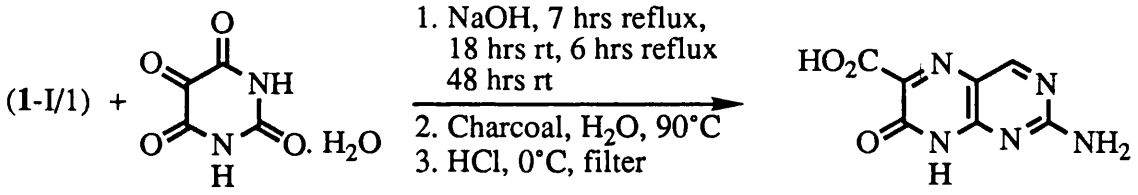


0.6% (crude) (22)

In retrospect, a similar method should have been used for the acylation of the pyrazine intermediates (**14**-I/3) and (**15**-I/3) in synthetic scheme 6.

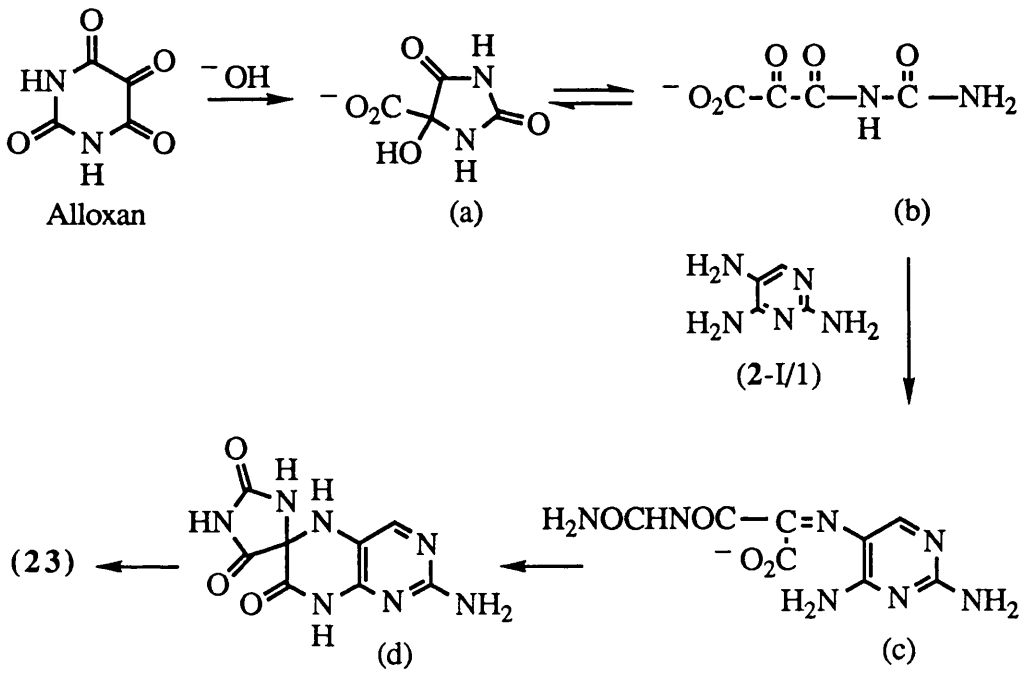
Synthesis of **2-amino-7(8H)-pteridinone-6-carboxylic acid (23)** followed the method of Pleiderer and Taylor (1960) [111] who prepared an analogous compound 2-ethylamino-8-ethyl-7(8H)pteridinone-6-carboxylic acid by refluxing a solution of 2,4-bis-(ethylamino)-5-pyrimidinamine in NaOH with alloxan monohydrate for 17 hrs. In the case of (**23**), a solution of 2,4,5-pyrimidinetriamine (**1**-I/1) in NaOH was refluxed with alloxan monohydrate for 7 hrs, stirred at rt for 18 hrs, refluxed again for 6 hrs and stirred at rt for a further 48 hrs. Treatment with decolourising charcoal in H_2O at 90°C for 1 hr and filtration, followed by precipitation with acid afforded the crude product in 52.3% yield. Further purification with charcoal followed by reprecipitation with acid afforded analytically pure product in 7.1% yield.

The reaction equation is shown below.



52.3% (crude) **(23)**

The probable mechanism is as follows:

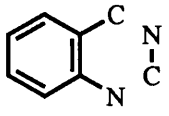


Alloxan undergoes an initial alkaline cleavage to alloxanic acid (a), which exists in equilibrium in alkaline solution with the open chain monoureide of oxomalonic acid (b). Reaction of the keto group of (b) with the 5-NH_2 group of 2,4,5-pyrimidinetriamine would then yield (c), which by cyclization to the spiro compound (d) and finally hydrolysis, leads to the pteridine-6-carboxylic acid (**23**).

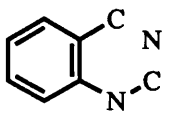
This mechanism is based on that proposed by Taylor and Loux (1959) [112] for the preparation of pteridine-6-carboxylic acids using alloxan and 4,5-pyrimidinediamines in alkaline solution.

Quinazolines may be prepared by both primary and secondary syntheses [113].

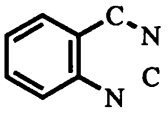
In the primary syntheses, use is made of the intact carbocyclic ring and the quinazoline ring system is built up in many ways; examples (i) - (vii) [113].



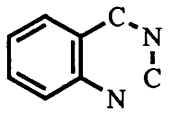
(i)



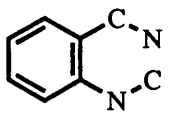
(ii)



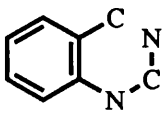
(iii)



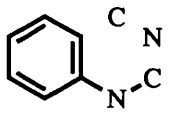
(iv)



(v)



(vi)

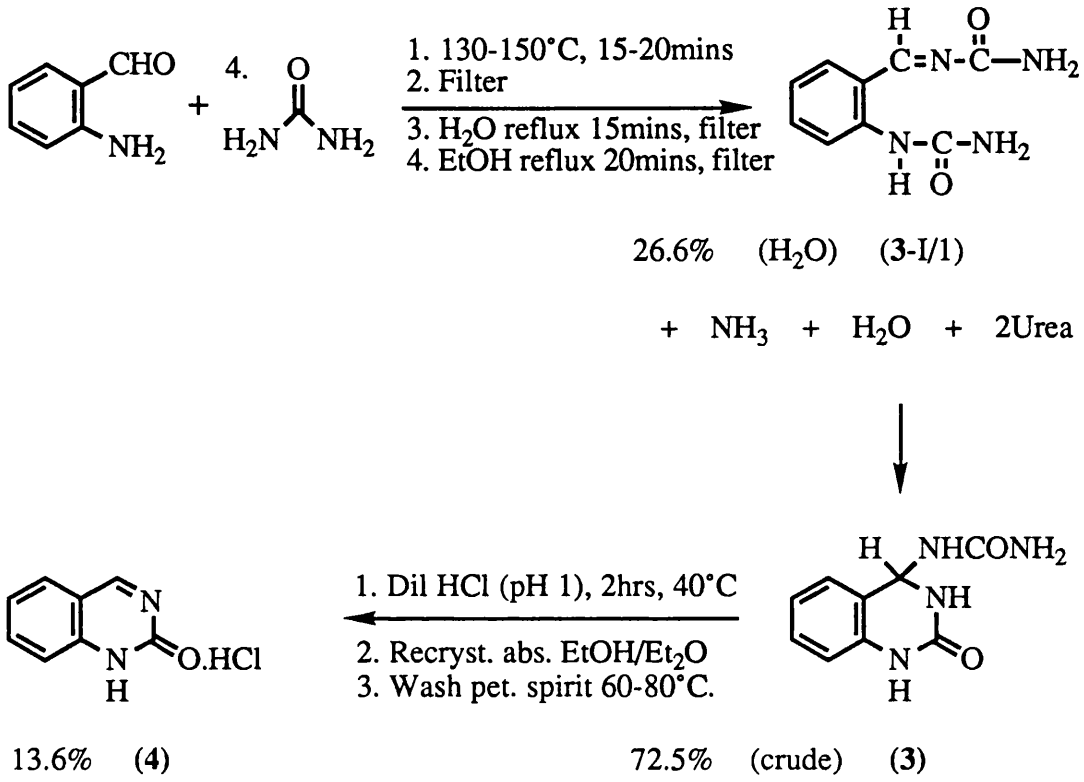


(vii)

In the secondary syntheses, transformations on the intact skeleton are made. Oxidation, reduction, metathesis, addition, and substitution reactions are among the more important examples. The quinazoline derivatives **(3)**, **(4)** and **(6)** were prepared by primary synthetic methods involving a ring closure of type (i). The other quinazoline derivatives used **(4)** as a starting material.

The synthesis of **4-ureido-3,4-dihydroquinazolin-2-one (3)** followed the Gabriel-Posner (1895) [114] method of condensing 2-aminobenzaldehyde with 4 mol equivs. of urea. The intermediate (**3**) was cyclized to **quinazolin-2-one hydrochloride (4)** by HCl acid, as suggested by Postovski *et al* (1975) [115]. The overall synthetic scheme is shown on the following page.

Scheme 7



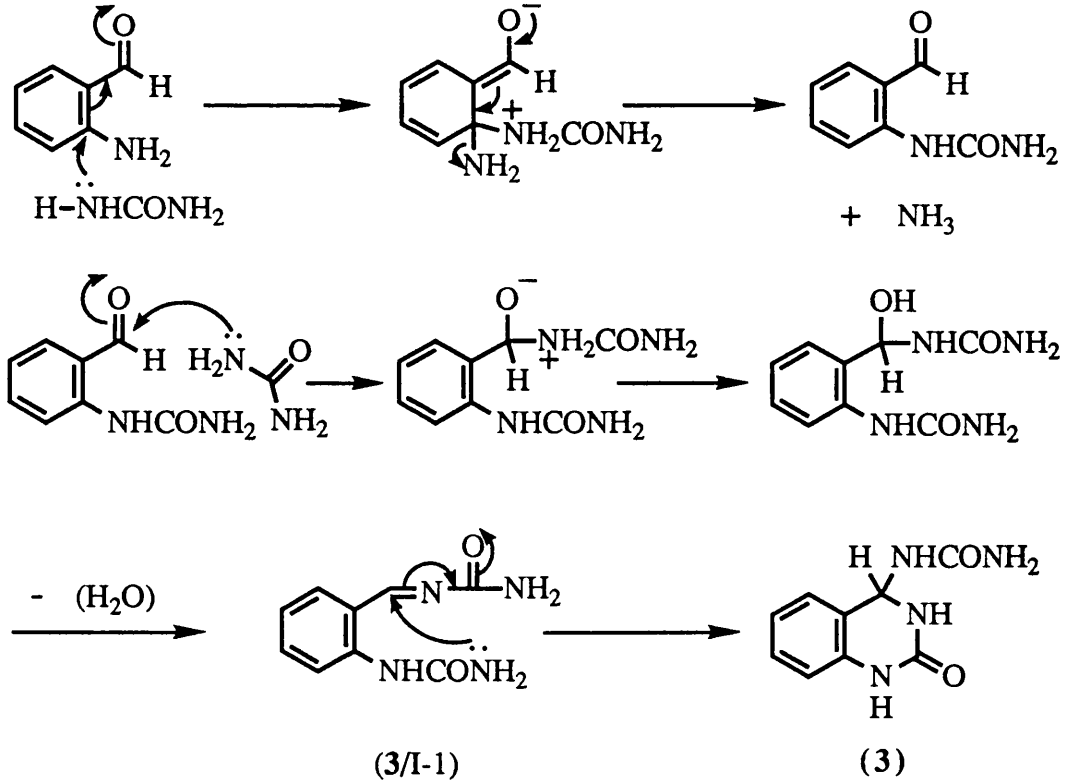
Gabriel and Posner (1895) reported that the product obtained from the condensation of 2-aminobenzaldehyde and 4 mol equivs. of urea, was quinazolin-2-one. Postovski *et al* (1975) on further investigation, found that the above condensation does not form **quinazolin-2-one (4)**, but forms the intermediate o-ureido-benzylidene-urea (**3**-I/1)which could further cycloisomerise to form (**3**). In acidic medium, the intermediate would eliminate urea to form (**4**). The former authors used acid only as a means of purifying their product, and in doing so would have converted (**3**) or (**3**-I/1) to (**4**).

Postovski et al, reported that the structure of the intermediate isolated was more likely to be (**3**-I/1) rather than (**3**), which they thought would be unstable. They based their conclusions on an IR and mass spectrometric investigation. The mass spectrum showed the

absence of the peak corresponding to (M - urea)$^+$ ion which would be extremely characteristic for the latter form.

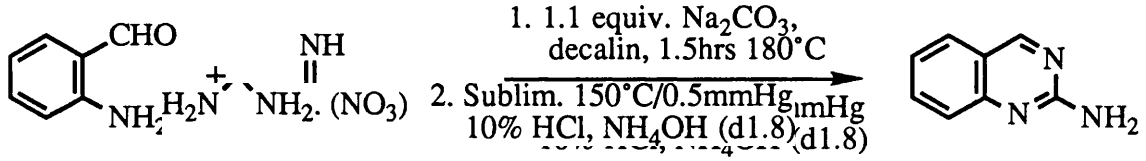
The literature method [114] afforded a product which favoured a structure corresponding to (**3**) rather than (**3**-I/1). The mass spectrum showed an intense peak at 146 corresponding to (M - urea)$^+$. The fragmentation then followed the same pattern as for (**4**). The ^{1}H and ^{13}C NMR spectra were consistent with the structure of (**3**), too. The ^{1}H NMR spectrum of (**3**) shows the characteristic C4-H peak at δ 9.46, the aromatic region and 2 NH peaks (possibly N1-H and N3-H) at δ 7.2 - 6.8, NH peak of urea at δ 5.98 and a single NH_2 peak at δ 5.96. The ^{13}C NMR spectrum shows 2 different types of C atoms at 157.10 ppm and 152.85 ppm corresponding to the C=O carbons of C2 and urea; 6 different types of C atoms of the aromatic ring at 137.02 - 113.85 ppm and the C4 peak at 58.31 ppm. In the ^{13}C spectrum of (**3**-I/1), one would expect the latter peak to be at ~167 ppm, characteristic of a C=N carbon. The C4 peak of (**4**)) is at 167.18 ppm.

One could envisage the overall reaction mechanism to be as follows. (unprecedented).



The first stage involves nucleophilic addition of urea with the elimination of NH_3. In the next stage, a further molecule of urea adds to the C=O bond to eventually eliminate H_2O.

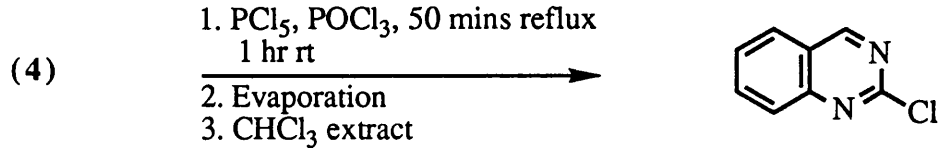
2-Aminoquinazoline (6) was synthesised according to the literature method of Rodda (1956) [116], by refluxing 2-aminobenzaldehyde with 1.5 mol equivs. of guanidine nitrate and 1.1 mol equivs. of Na_2CO_3 in dry decalin for 1.5 hrs, acidifying the mixture using 10% HCl solution and precipitating using NH_4OH. The reaction equation is shown below.



(6)

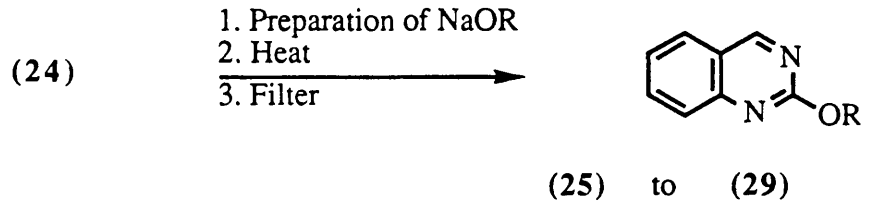
27.3% (crude)

Synthesis of **2-chloroquinazoline (24)** followed the method of Albert and Barlin (1962) [117], by chlorination of **quinazolin-2-one hydrochloride (4)** with a mixture of PCl_5 and $POCl_3$ according to the reaction equation below.



94.1% (crude) **(24)**

Syntheses of the 2-alkoxyquinazolines: **2-methoxyquinazoline** [60] **(25), 2-benzoxyquinazoline (26), 2-ethoxyquinazoline (27), 2-propoxyquinazoline (28)** and **2-butoxyquinazoline (29)** were by nucleophilic substitution of the Cl atom of **(24)** by the appropriate sodium alkoxide. The general reaction equation is shown below.



(25) to **(29)**

The particular reaction conditions used in each case, are shown below.

Cpd. No.	R	Reaction conditions	Exp. yield
(25)	Me	2.3 mol equiv. NaOMe in MeOH, 5 hrs reflux	34.7% (H_2O)
(26)	$PhCH_2$	2.4 mol equiv. $NaOCH_2Ph$ in $PhCH_2OH$, 20 mins at 70°C	13.9% (crude)
(27)	Et	2.7 mol equiv. NaOEt in EtOH, 2 hrs reflux	17.3% (H_2O)
(28)	Pr	2.6 mol equiv. NaOPr in PrOH, 2 hrs reflux	25% (crude)
(29)	Bu	3.4 mol equiv. NaOBu in BuOH, 1.5 hrs at 117°C	26.17% (crude)

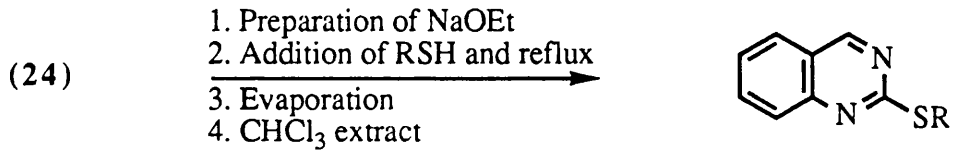
Prep. HPLC on (**26**) afforded an analytically pure product (99.6% by HPLC) in 6.9% overall yield.

Compound (**27**) was further purified by prep. HPLC and afforded in 9.1% overall yield.

Compound (**28**) was 99.5% pure by HPLC as the crude product and needed no further purification, whilst (**29**) was subjected to prep. HPLC twice and afforded in 87.9% purity. The quantity of material available (0.024g) in the latter case, was insufficient for further purification.

Syntheses of the 2-alkythioquinazolines: **2-ethylthioquinazoline (30)**, **2-benzylthioquinazoline (31)** and **2-propylthioquinazoline (31)** followed an analogous method from chemical abstracts [1;8] (full reference was not available) on the preparation of substituted phenyl quinazolinyl sulphides. The reaction involved nucleophilic substitution of the Cl atom in **2-chloroquinazoline (24)** by an appropriate sodium alkylthioxide (prepared in situ by reacting NaOEt with the appropriate alkylthiol in EtOH).

The general reaction equation is shown below.



(30) to **(32)**

The reaction conditions are on the following page.

Cpd. No.	R	Reaction conditions	Exp. yield
(30)	Et	1.1 mol equiv. NaOEt and 1.1 mol equiv. EtSH in EtOH, 1.25 hrs reflux	75.8% (crude)
(31)	$PhCH_2$	1.3 mol equiv. NaOEt and 1.1 mol equiv. $PhCH_2SH$ in EtOH, 3 hrs reflux	38.7% (crude)
(32)	Pr	1.3 mol equiv. NaOEt and 1.1 mol equiv. PrSH in EtOH, 1.85 hrs reflux	64.5% (crude)

Compound (**30**) was purified by column chromatography (SiO_2; $CHCl_3$), to afford an analytically pure product (99.7% by HPLC) in 39.9% overall yield.

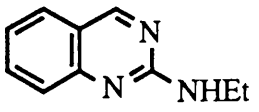
Compound (**31**), initially 82% pure by HPLC, was recrystallised from MeOH to afford the product (97.3% pure by HPLC) in 14.8% overall yield.

Compound (**32**) was purified by column chromatography (SiO_2; $CHCl_3$), to afford a product in 18.7% yield, 90% pure by HPLC. Recrystallisation from abs. EtOH, afforded the pure product (98.2% by HPLC) in 10.6% overall yield.

The synthesis of **2-ethylaminoquinazoline (33)** was by reacting **2-chloroquinazoline (24)** with ethylamine at 0°C for 10 mins, and refluxing for 2.5 hrs The reaction equation is shown below.

(24) → (33)

1. 50% $EtNH_2$ in MeOH at 0°C
   Stir for 10 mins at 0°C
2. Warm up to rt
3. Reflux for 2.5 hrs
4. Evaporation
5. Chromatog. (EtOAc:pet. spirit 60-80°C, 1:1)



34.6%

As shown above, (**33**) was afforded in 34.6% yield on purification by column chromatography (SiO_2; EtOAc: Pet. spirit 60-80°C, 1:1). Further purification by recrystallisation from propan-2-ol, afforded an analytically pure product (99.9% by HPLC) in 8.6% overall yield.

Finally, **2-Aminopurine (7),** the only compound that was not synthesised, was obtained from Aldrich Chemical Co. The product was pure by ^1H NMR spectroscopy, and tlc (SiO_2: EtOAc:MeOH:NH_4OH). The product was dried at 60°C/0.1mmHg and not further purified.

CHAPTER 4

COVALENT ADDITION REACTIONS OF 2-N-ALKYL AND 2-N-ARYL AMIDOPTERIDINES.

Introduction

Covalent addition reactions of 2-N-alkylamidopteridines have not previously been reported. One reason for this may be the fact that, other than **2-N-acetamidopteridine (13)**, other unsubstituted 2-N-alkylamidopteridines have not been synthesized for a comparative study. However, there is no mention of the tendency of **(13)** to undergo covalent addition reactions readily, either, in the literature [78]. Therefore, the accidental discovery of the adduct, **4-ethoxy-2-N-acetamido-3,4-dihydropteridine (16)** formed on recrystallisation of **(13)** from abs. EtOH, prompted further investigation into the matter of covalent addition reactions of 2-N-alkylamidopteridines, which seemed to provide a novel study. It also seemed interesting to investigate the possibility of a correlation between the adduct formation of 2-N-alkylamido and 2-N-arylamidopteridines and their biological activity, at a later stage.

First of all, it was decided to investigate the behaviour of **(13)** in abs. EtOH by a U.V study, with the hope of carrying out a quantitative investigation, as in the determination of the equilibrium and rate constants for adduct formation in alcohol, at a later stage. A series of covalent adduct forming reactions were then performed with **(13)** in H_2O and EtOH under various conditions of temperature and pH, in order to obtain the optimum conditions for an ^1H NMR spectroscopic kinetic study. The same set of reactions were repeated with **2-aminopteridine (1)** for comparative purposes. A qualitative UV spectroscopic study was performed with **2-N-propionamidopteridine (20)** too. Finally, the covalent

adduct formation in CD_3OD, of **2-N-acetamidopteridine (13)**, **2-N-propionamidopteridine (20)** and **2-N-benzamidopteridine (21)** in DMSO-d_6, were quantitatively investigated by ^1H NMR spectroscopy.

Results and discussion:

6.1 UV spectroscopic study: (The spectra and tables are in Appendix 4.1).

The change in the UV spectrum of (**13**) in abs. EtOH was followed by recording a UV spectrum every 60 mins over a period of 24 hrs. (See Table 4.1a and Spectrum 4.1a). The results were, a decrease in the absorbance of the peaks at λ 232 and λ 255, a slight increase in the absorbance of the peak at λ 330, and the appearance of a new peak at λ 278 which gradually reached an equilibrium value. According to the spectrum, an equilibrium between the hydrated and anhydrous species had been reached by the end of 420 mins. (The equilibrium spectrum is denoted by the thick spectral curve).

The change in the UV spectrum of (**16**) in abs. EtOH was followed by recording a UV spectrum every 60 mins over a period of 24 hrs, as with (**13**). (See Spectrum 4.1b and Table 4.1b). According to the spectrum, an equilibrium between the hydrated and anhydrous species had been reached by the end of 180 mins. (Again, the equilibrium spectrum is denoted by the thick spectral curve). The major changes were a gradual decrease in the peak at λ 278 towards an equilibrium and the appearance of the peak at λ 262. These two peaks of the equilibrium spectrum of (**16**) in abs. EtOH were superimposable on those of (**13**). Both spectra had similar extinction coefficients. ($Log\varepsilon_{eq}$ (**13**) = 5.05, 4.96, 4.90 and 5.01, at λ 232, 255, 278 and 330; whilst $log\varepsilon_{eq}$ (**16**) = 5.03, 4.86, 4.88 and 4.99 at l 231, 262, 278 and 329).

The results obtained from the above UV study did not seem sufficiently valid for a kinetic study at the time, as we did not know exactly what was taking place under these UV

conditions. It did not seem possible to evaporate off 100ml of abs. EtOH and record the UV spectrum of ~ 1mg of product. Therefore, at a later stage, it was decided to mimic these conditions (as far as possible), by reacting 20mg of **(13)** in abs. EtOH (30ml) at rt for 24 hrs, and recording a ^1H NMR spectrum. (See section 4.2a, Table 4.2a). The result showed that under these conditions only 17.4% of adduct was being formed. (The two sets of results could be compared on the assumption that the reaction was first order on approaching equilibrium. In such circumstances, the amount of EtOH present did not matter as long as the solvent was in excess).

The results obtained from section 4.2, caused us to reconsider the choice of solvent for a future kinetic study. The covalent addition reaction in abs. EtOH was too slow to follow by ^1H NMR spectroscopy. Also, **(13)** was insoluble in H_2O. MeOH was the obvious choice.

Therefore, the change in the UV spectrum of **(13)** in MeOH was followed by recording a UV spectrum every 60 mins over a period of 24 hrs. (See Table 4.1c and Spectrum 4.1c). The results showed that the equilibrium spectrum obtained was not the expected equilibrium spectrum. On further investigation, it was found that the final equilibrium spectrum (in spectrum 6.1c) was that of **2-aminopteridine (1)** in MeOH. Also, the initial spectrum (at time 0 to 3 mins) seemed more like the equilibrium spectrum (cf. with the equilibrium spectra of 4.1a and 4.1b).

The hypothetical conclusions were that **(13)** had already undergone covalent adduct formation with MeOH before the experiment had begun and the reaction that was recorded was the hydrolysis of **(13)** to **2-aminopteridine (1)**. The first conclusion was highly possible, as a solution of **(13)** (1.68mg) in MeOH had to be subjected to high heat to aid dissolution, during the preparation of the UV solution, as **(13)** was only partially soluble in MeOH. Therefore, **(13)** could have already reached an equlibrium before the start of the experiment. Possible evidence for this, could be obtained from the tlc (SiO_2; MeCN) of the solution prepared for UV spectroscopy which showed 2 spots, one corresponding to **(13)** and the other to the adduct. The second was possible too, as hydrolysis of **(13)** to **(1)** is seen to occur in both H_2O and alcohol over 24 to 48 hrs. (See results in section 4.2).

The change in the UV spectrum of **(20)** in MeOH was followed by recording a UV spectrum every 60 mins over a period of 24 hrs. (See Table 4.1d and Spectrum 4.1d). The result obtained with **(20)** was similar to that obtained with **(13)**.

Clearly, UV spectroscopy was not the best method to quantitatively investigate the covalent addition reactions of 2-N-alkylamidopteridines.

4.2 Comparison of the covalent addition reactions of (13) in H_2O and alcohol

(Results and examples of spectra are in Appendix 4.2).

Covalent adduct formation is generally accompanied by an upfield shift in the peaks corresponding to protons of C4, C7 and C6. For example, the proton peaks at δ; 9.68 (C4-H), 9.22 (C7-H) and 8.99 (C6-H) of **(13)** are shifted to δ; 5.86 (C4-H), 8.42 (C7-H) and 8.26 (C6-H) on forming the adduct **(16)**. Therefore at any given time, the ratio of adduct: anhydrous species will be the ratio of the C4-H proton peaks of the adduct and the anhydrous species.

A series of reactions in H_2O and abs. EtOH were carried out in order to compare the effect of temperature and the presence or absence of acid on covalent adduct formation of **(13)** in the two solvents. The % of adduct was determined from the ratio of the C4-H proton peak integrals of the adduct and the anhydrous species, in the ^1H NMR spectrum. The general method is described in the experimental section of this chapter. See Table 4.2a for the results. An example of the type of spectrum obtained, is shown in Figure 4.2a.

The results show that: At rt, **(13)** is predominantly anhydrous in neutral solution, however forms ~17.3% of the hydrate in mildly acidic (pH 3.56) solution. In abs. EtOH, the % of adduct formed (~17.3%) is greater than in H_2O and is increased to ~50% in mildly acidic (pH 3.56) conditions. The greater tendency for adduct formation in alcohol compared to

H_2O may be attributed to the greater nucleophilicity of primary alcohols compared to H_2O, or the greater solubility of **(13)** in alcohol compared to H_2O.

At higher temperatures (80°C, 4 hrs), **(13)** is predominantly anhydrous but in mildly acidic conditions, forms a very slight trace of the hydrate (not accurately measurable on a ^1H NMR spectrum recorded at 200 MHz) as well as **2-aminopteridine (1)** (~25%) due to hydrolysis of **(13)**. In abs. EtOH, the % of adduct formed (~47%) is greater than in H_2O and increases to 60% under mildly acidic conditions.

Under more vigorous conditions (i.e reflux 24 hrs), **(13)** is completely hydrolysed to **(1)**. In abs. EtOH, all three species, i.e **(13)** (~33.3%), the adduct (~16.6%) and **(1)** (~57.1%) were present at the end of 3 days of relux.

A similar set of experiments was performed with **(1)** for comparative purposes. The results are in Table 6.2b. The results confirmed the already known finding that 2-aminopteridine **(1)** is fairly resistant to covalent addition reactions either in H_2O or alcohol even in the presence of a weak acid. However, **(1)** has been known to undergo covalent adduct formation with alcohols in the presence of strong acids such as HCl acid.

From this study, the main conclusions were that: **(13)** was more prone to undergo covalent addition reactions with H_2O and alcohol than **(1)** under the same conditions and the former compound formed covalent adducts more readily with alcohol than H_2O.

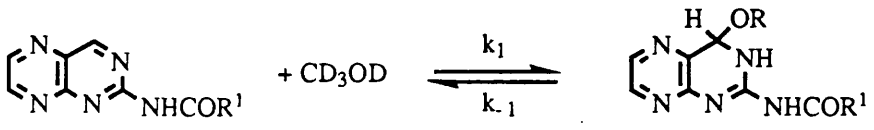
4.3 ^1H NMR spectroscopic study:

The choice of solvent was CD_3OD, rather than CD_3CD_2OD. One of the main reasons for this choice was that covalent adduct formation of **(13)** in MeOH (as indicated by the UV spectroscopy studies) was faster than in abs. EtOH and therefore could be monitored by ^1H NMR spectroscopy over a relatively shorter period of time. The experiment could not be performed in neat CD_3OD as the compound was only partially soluble, and posed a similar problem as with the UV spectroscopic study. Therefore a solution of **(13)** in DMSO-d_6

was used and the experiment performed at 60°C, to prevent reprecipitation of the compound on the addition of CD_3OD, as well as to increase the rate of the reaction.

The ^1H NMR (400 MHz) spectroscopic investigation of the covalent addition reaction of **(13)** with CD_3OD at 60°C, was thus performed by mixing the solution of **(13)** in deuterated DMSO with a solution of CD_3OD (in a NMR tube) at time = 0, and recording a spectrum at ~15 min time intervals. The exact time was recorded using a stopwatch. The rate of reaction was monitored, by following the increase in the C4-H proton peak integral. Spectra were recorded until near equilibrium was reached. Due to limitation of time on the machine, the spectrum at 'infinity' or equilibrium was recorded at a later stage. (~7 days after the kinetic study). The NMR tube (which had been standing at rt) was heated at 60°C for ~3 hrs prior to the recording of the final spectrum, to ensure that equilibrium was reached before the recording. The above procedure was performed twice for **2-N-acetamidopteridine (13)** and once for both **2-N-propionamidopteridine (20)** and **2-N-benzamidopteridine (21)**. The general experimental method is described in the experimental section of this chapter. CD_3OD (12.3M) was in excess (~100 fold), therefore, a first-order reaction was expected.

For a first order reaction approaching equilibrium, the rate of forward and backward reactions are denoted by k_1 and k_{-1} respectively [120]. Assuming the following reaction follows first order kinetics on approaching equilibrium;



A B

The rate of reaction is followed as the rate of formation of the adduct (determined by measurement of the C4-H proton integral I).

At the start the C4-H integral of adduct B = $I_0 = 0$.

The C4-H integral of the adduct at equilibrium = $I_e \alpha [B]$

The C4-H integral of the adduct at time t = $I_t \alpha [B]$

Therefore, $I_e \alpha [A]_0 - [A]_e$ and $I_t \alpha [A]_0 - [A]$

Therefore, $I_e - I_t \alpha [A] - [A]_e$

Now, The rate equation for the above reaction is $-\frac{d[A]}{d[t]} = k_1[A] - k_{-1}[B]$

Integration yields; $\ln\frac{[A]_0 - [A]_e}{[A] - [A]_e} = (k_1 + k_{-1})t$

Substituting for I; $\ln\frac{I_e}{I_e - I_t} = (k_1 + k_{-1})t$

Therefore, on a plot of $\log\frac{I_e}{I_e - I_t}$ against t, the slope = k x 2.303 = $(k_1 + k_{-1})t$ x 2.303.

The slope k is the effective first-order rate constant which is the sum of the rate constants for the forward and reverse directions, on approaching equilibrium [120].

The rate constant k is a pseudo first-order rate constant, because in the above reaction, $[CD_3OD] >> [A]$, therefore $[CD_3OD]$ is constant.

However, for the above reaction, at equilibrium, $k_2[A][CD_3OD] = k_{-1}[B]$

(where $k_2[CD_3OD] = k_1$)

Therefore, $\ln\frac{[B]}{[A][CD_3OD]} = \frac{k_2}{k_{-1}}$

or, the equilibrium constant = K = $\frac{[B]}{[A][CD_3OD]} = \frac{k_2}{k_{-1}} = \frac{k_1}{k_{-1}[CD_3OD]}$

$(k_1 + k_{-1})$ = slope

But, $k_1 = Kk_{-1}[CD_3OD]$,

Therefore, $Kk_{-1}[CD_3OD] + k_{-1}$ = slope

Therefore, $k_{-1} = \frac{slope}{K[CD_3OD] + 1}$

and $k_1 = Kk_{-1}[CD_3OD]$ or $k_2 = Kk_{-1}$

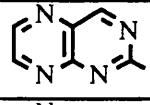
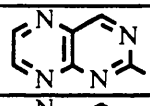
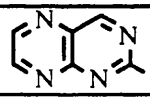
Tables 4.3. a, b, c, and d show the kinetic data obtained for each set of experiments. The stacked spectra 4.3. a, b, c and d (of the C4-H proton peak), the equilibrium spectra 4.3. a, b, c and d, the graphs 4.3. a, b, c and d of the kinetic plots done by a computer analysis and finally fig 4.3e, the overlay plot comparing the 4 graphs are given in Appendix 4.3.

From the graphs, it can be seen that, the best line fitted by the computer is not a straight line for any of the 4 sets of data. Two possible explanations are hypothesised. The first is that the reaction analysed is not following first order kinetics on approaching equilibrium and therefore the above equation is invalid. The second is that the reaction is following first order kinetics at the begining of the reaction (indicated by the greater linearity of the slope at the begining, compared to that at the end), but due to some unknown factor, is not, at the end. However, it seems fair to assume that the second explanation is more feasible and that the gradient at time t = 0 is a fair indication of the rate of reaction. (Some of the CD_3OD could have been evaporating off towards the end). As the rate of reaction of three compounds are being compared, the relative rate is what matters.

The results show that the initial rates of reaction approaching equilibrium (given by the slopes), in the addition of CD_3OD to **2-N-acetamidopteridine (13)** and **2-N-benzamidopteridine (21)** are greater than the initial rate of reaction of the addition of CD_3OD to **2-N-propionamidopteridine (20)**. However, it is not possible to comment on how statistically significant the variations in the values are for each of the above experiments, for the experiments were not repeated, with the exception of **(13)**, and the two sets of data obtained with **(13)** were not identically reproducible too. The major source of error involved in this study has been the measurement of the equilibrium peak I_e. Since I_e was not measured continuously with the other measurements, under the same experimental conditions, the value was not absolute and was subject to error. One also has to consider other factors that may have affected the rate, such as the use of DMSO-d_6

instead of neat solvent and the unavoidable general experimental error involved in the operation of the NMR machine. At 60°C, some CD_3OD could evaporate off. Also, since the equilibrium spectra were run at a later stage, there was ample opportunity for the evaporation of CD_3OD. Also, as mentioned earlier, the reaction may not be a first order reaction.

Table 4.3e compares the K, k_2 and k_{-1} values for the three 2-N-alkylamidopteridines studied, at 60°C.

CPD NO.	SUBSTRATE	K ($mol^{-1}dm^3$)	$k_2 \times 10^{-6}$ ($mol^{-1}s^{-1}dm^3$)	$k_{-1} \times 10^{-4}$ (s^{-1})
(13)	 NHCOMe	0.029[a] 0.031[b]	3.97[a] 5.07[b]	1.38[a] 1.62[b]
(20)	 NHCOEt	0.034	3.06	0.98
(21)	 NHCOPh	0.05	6.35	1.27

[a] and [b] refer to two separate determinations.

From Table 4.3e, it can be seen that the ratio of adduct to anhydrous species at equilibrium, denoted by K, is similar in all three cases, indicating that the nature of the substituent at the 2 position of 2-amidopteridines has liitle or no significant effect on the covalent addition of CD_3OD to the 3,4 C=N bond.

The rate constant for addition reaction, denoted by k_2, is greater for **2-N-benzamidopteridine (21)** than for either **2-N-acetamidopteridine (13)** or **2-N-propionamidopteridine (20)**. Again, it is not possible to comment on how statistically significant the variations in the values are for each of the above experiments, as the experiments were not repeated.

The rate constant for the reverse reaction k_{-1}, is again very similar in all three cases.

The overall conclusion, therefore, is that the rate and extent of covalent addition of CD_3OD to the 3,4 C=N bond to **2-N-acetamidopteridine (13), 2-N-propionamidopteridine (20)** and **2-N-benzamidopteridine (21)** are very much the same. To obtain a more precise conclusion, each set of experiments would have to be repeated at least thrice.

Experimental

UV spectra were recorded on a Perkin Elmer Lambda 5 UV/VIS spectrophotometer using UV cells of 1cm path length. ^1H NMR spectra were recorded on a Varian XL-200 NMR spectrometer at 200 MHz, or a Varian VXR-400 NMR spectrometer at 400 MHz. The kinetic ^1H NMR study was performed using the latter with a variable temperature (VT) probe. All solvents used were dried over 4A molecular sieves. The ^1H NMR spectra of the kinetic study were referenced to DMSO-d_6 at δ; 2.45, whilst the others were referenced to TMS.

UV study:

The experiments were performed according to the following general procedure.

The 2-N-alkyl-amidopteridine (~1mg) was dissolved in the appropriate solvent (100ml) in a volumetric flask. Dissolution was aided by heating with a hair-dryer. The time taken to prepare the solvent was between 1 to 2 mins. The solution was immediately transferred to a UV cell by means of a pipette, placed in the appropriate holder in the spectrometer and the experiment started. The initial spectrum was recorded and the scale adjusted to accomodate the maximum peak height. A spectrum was automatically recorded evry 60 mins for 24 hrs, as programmed.

(Before starting the kinetic study, the apparatus was calibrated to exclude all background radiation. Spectra were recorded at λ 332.8 nm, with a scan speed of 240nm/min; slit width of 2nm; response of 0.2s and a peak threshold of 0.02 A. All spectra were recorded at 25°C).

Study of the covalent addition reactions of (13) and (1) in H_2O and alcohol:

The experiments were performed according to the following general procedure.

The 2-amino- or 2-N-acetamido-pteridine (~20mg) was dissolved in the appropriate solvent (~30ml). At the end of the required time of reaction, the reaction vessel was immediately frozen by means of an acetone/solid CO_2 bath and freeze-dried on a lyophilizer for 24 hrs. Once the solvent was removed, the contents of the vessel were completely dissolved in DMSO (~1-2ml); transferred into a NMR tube and the ^1H NMR spectrum recorded at 200MHz. The ratio of adduct to anhydrous species was determined by the ratio of the C4-H proton peak integrals of the 2 species concerned.

Where acid was required, the pH of the solvent was first acidified with glacial $MeCO_2H$ to 3.56 ± 0.2 (pH meter). A stronger acid was not used for fear of degradation of the pteridine ring. Where higher temperatures (other than reflux) were required, an oil bath with a Kontact thermometer was used to maintain a constant temperature. Constant conditions were maintained (as far as possible) for each set of comparative experiments.

^1H NMR study:

The experiments were performed according to the following general procedure.

The 2-N-alkyl-(or aryl)-amidopteridine (~20mg) (for exact quantity, see tables 4.3 a, b, c and d) and 2,4-dinitrobenzene (reference material) (~1mg) were dissolved in DMSO-d_6

(0.5ml) in a small sample tube and transferred by means of a syringe into a NMR tube. Dissolution of the compounds was aided by heating with a hair-dryer. CD_3OD (0.5ml) was added to the solution (starting the stopwatch at the same time); the tube inverted once to aid mixing of the solvents and the kinetic experiment started. Spectra were recorded at ~15 to 20 min time intervals (for exact times, see tables 4.3 a, b, c and d). The C4-H proton peak integral and the reference peak integral were measured for each spectrum at time t.

(Before starting the kinetic study, a spectrum of the compound and the reference in DMSO-d_6 was run at 60°C; referenced to DMSO-d_6 and the required parameters set up. Spectra were recorded at: 399.95 MHz; at a temperature of 60°C; with a spectral width of 4600Hz; with double precision acquisition and acquisition time of 3.56 s; with a pulse width of 64°; a spin rate of 20Hz and the no: of repetitions = 16. The total time taken for the recording of a single spectrum was 1.1 min.)

Appendix 4.1

Table 4.1a shows the UV spectroscopic data for **2-N-acetamidopteridine (13)** in abs. EtOH at 60 min time intervals.

Time/min = 0 - 3 (Initial spectrum).				
λ/nm	232	255	278	330
A	1.08	0.6	----	0.41
logε	5.19	4.94	----	4.77
Time/min = 60				
λ/nm	232	255	278	330
A	1.24	0.69	----	0.64
Time/min = 120				
λ/nm	232	258	270	330
A	0.98	0.59	0.47	0.69
Time/min = 180				
λ/nm	232	258	278	330
A	0.89	0.56	0.51	0.71
Time/min = 240				
λ/nm	232	258	278	330
A	0.83	0.54	0.53	0.71
Time/min = 300				
λ/nm	232	260	278	330
A	0.81	0.53	0.54	0.71
Time/min = 360				
λ/nm	232	258	278	330
A	0.79	0.53	0.55	0.71
Time/min = (420 - 1440) Equilibrium spectrum				
λ/nm	232	258	278	330
A	0.78	0.63	0.55	0.71
logε	5.05	4.96	4.90	5.01

Spectrum 4.1a. shows the UV spectra of **2-N-acetamidopteridine (13)** in Abs. EtOH, recorded at 60 min time intervals, over a period of 24hrs.

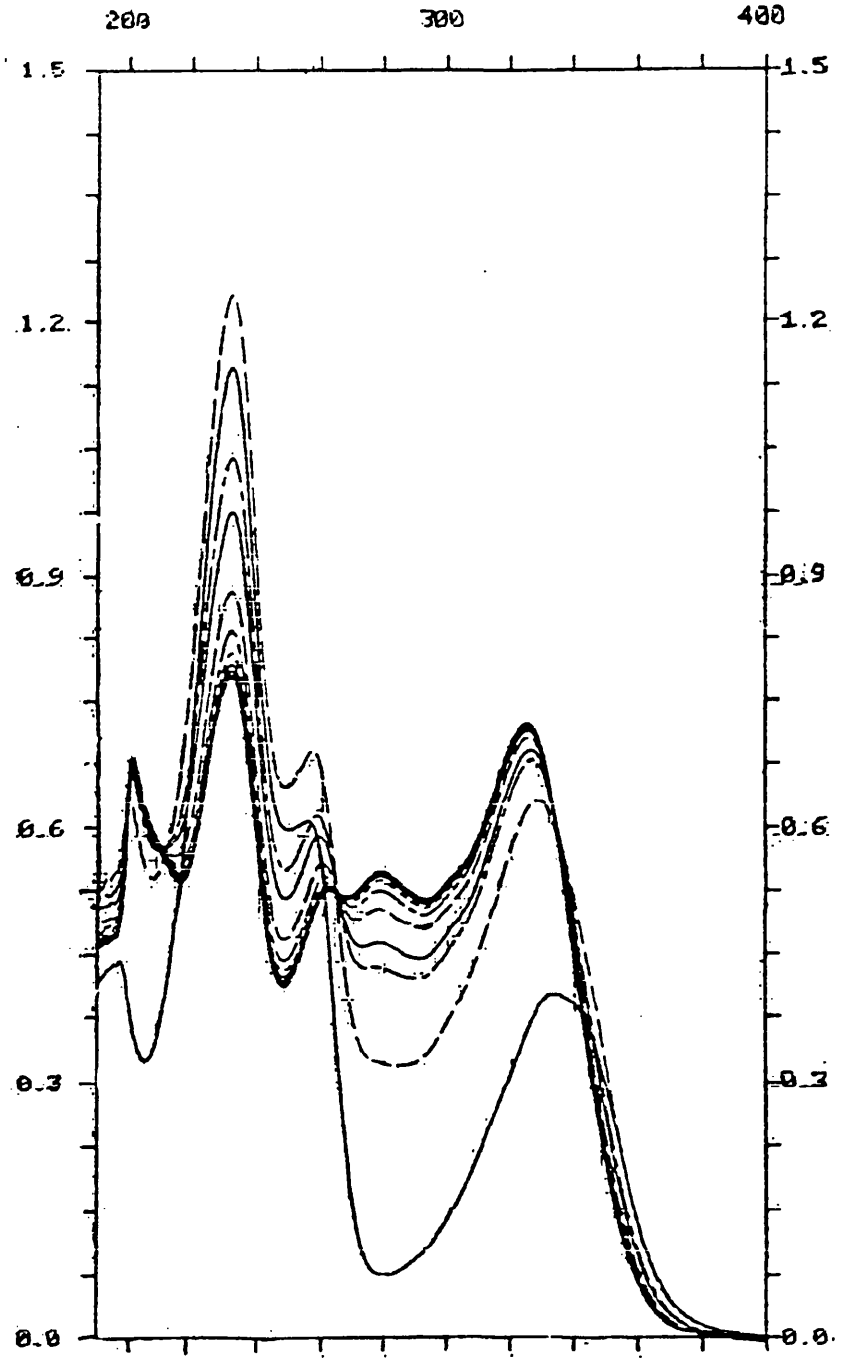


Table 4.1b shows the UV spectroscopic data for (77%) **4-ethoxy-2-N-acetamido-3,4-dihydropteridine (16)** in abs. EtOH at 60 min time intervals.

Time/min = 0 - 3 (Initial spectrum).				
λ/nm	231	262	278	329
A	0.77	----	0.70	0.88
logε	4.97	----	4.93	5.03
Time/min = 60				
λ/nm	231	262	278	328
A	0.88	0.63 (sh)	0.70	0.89
Time/min = 120				
λ/nm	231	262	278	328
A	0.88	0.60	0.64	0.83
Time/min = 180				
λ/nm	231	262	278	328
A	0.88	0.60	0.63	0.82
Time/min = 240. Equilibrium spectrum.				
λ/nm	231	262	278	328
A	0.88	0.60	0.63	0.82
logε	5.03	4.86	4.88	4.99

Table 4.1c shows the UV spectroscopic data for the initial and equilibrium spectra of **2-N-acetamidopteridine (13)** in MeOH.

Time/min = 0 - 3 (Initial spectrum).				
λ/nm	232	261	279	328
A	0.66	0.45	0.49	0.63
logε	4.87	4.70	4.74	4.85
Time/min = 1200 - 1440. (Equilibrium spectrum)				
λ/nm	225	261	279	371
A	1.71	0.60	----	0.45
logε	5.30	4.83	----	4.70

Spectrum 4.1b shows the UV spectra of **4-ethoxy-2-N-acetamido-3,4-dihydropteridine (16)** in Abs. EtOH at 60 min time intervals over 24 hrs.

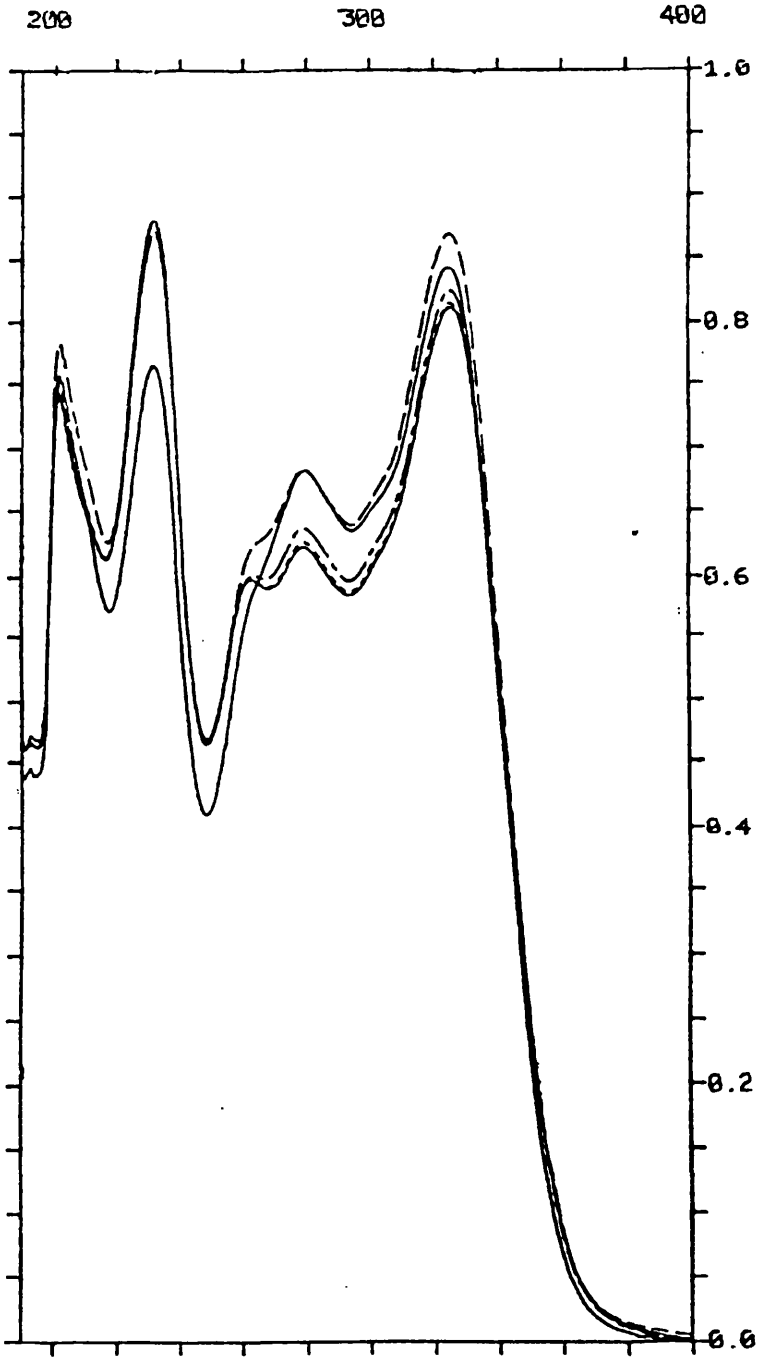


Fig 4.1c shows the UV spectra of **2-N-acetamidopteridine (13)** in MeOH at 60 min time intervals over 24 hrs.

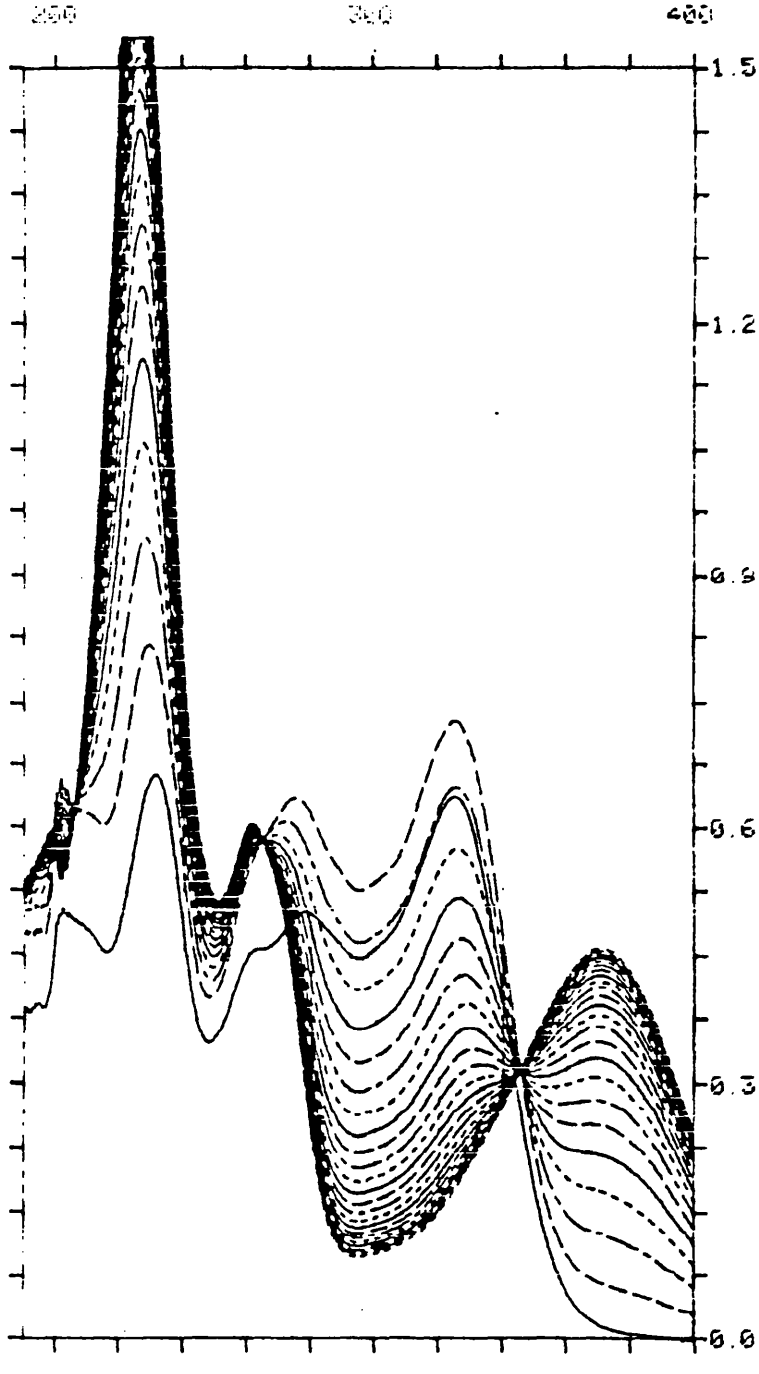
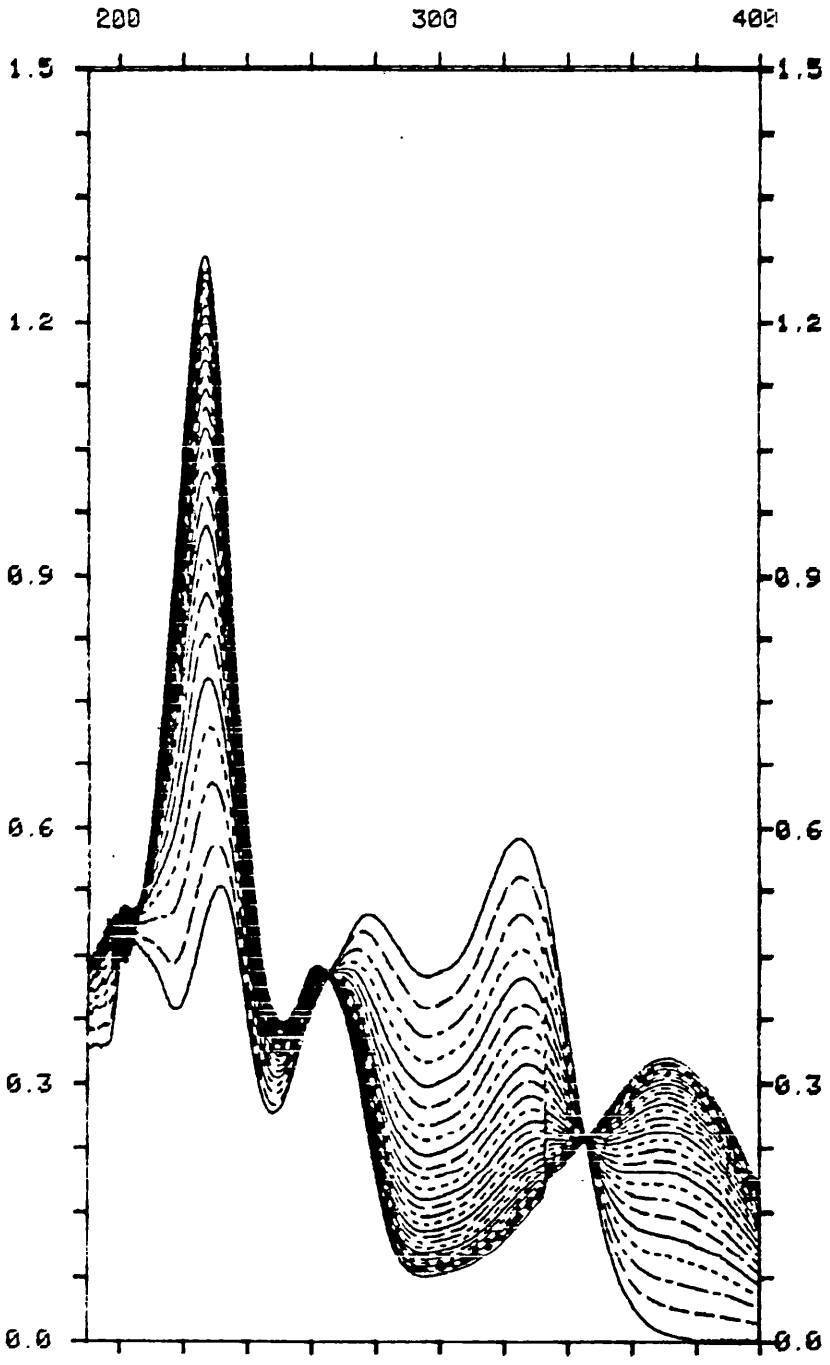


Table 4.1d shows the UV spectroscopic data for the initial and equilibrium spectra of **2-N-propionamidopteridine (20)** in MeOH.

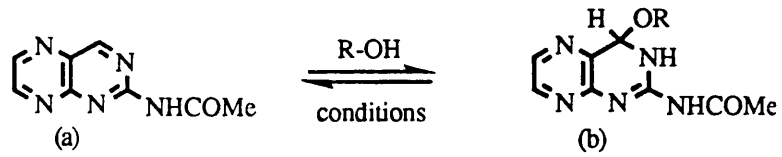
Time/min = 0 - 3 (Initial spectrum).			
λ/nm	231	278	324
A	0.53	0.50	0.59
logε	4.92	4.89	4.97
Time/min = 60			
λ/nm	229	275	324
A	0.58	0.48	0.54
Time/min = 120			
λ/nm	228	273	325
A	0.65	0.46	0.50
Time/min = 180			
λ/nm	227	272	325
A	0.72	0.45	0.46
Time/min = 240			
λ/nm	226	270	326
A	0.78	0.44	0.43
Time/min = 300			
λ/nm	226	269	326
A	0.83	0.43	0.39
Time/min = 360			
λ/nm	231	266	326
A	0.64	0.43	0.37
Time/min = 420 - 1440 (Equilibrium spectrum)			
λ/nm	225	261	370
A	1.23	0.44	0.31
logε	5.29	4.84	4.69

Spectrum 4.1d. shows the UV spectra of **2-N-propionamidopteridine (20)** in MeOH at 60 min time intervals, over 24 hrs.



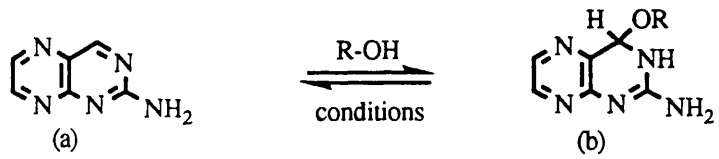
Appendix 4.2

Table 4.2a investigating the effect of solvent, temperature and the presence or absence of acid on the covalent adduct formation of **2-N-acetamidopteridine (13)**



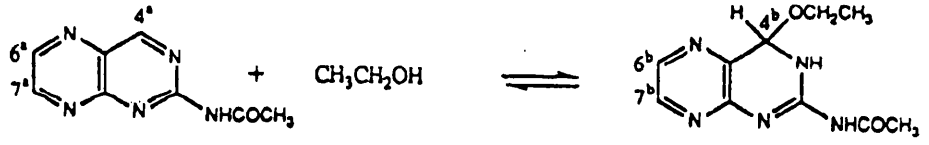
Conditions	R	% Ratio of		
		(a)	(b)	(1)
24 hrs, reflux	H	--	--	100
24 hrs, rt	H	>99	--	trace
24 hrs, rt (pH 3.56)	H	82.7	17.3	--
24 hrs, rt	Et	82.6	17.4	--
24 hrs, rt (pH 3.56)	Et	50	50	--
4 hrs, 80°C	Et	52.4	47.6	--
4 hrs, 80°C (pH 3.56)	Et	40	60	--
4 hrs, 80°C	H	>99	trace	trace
4 hrs, 80°C (pH 3.56)	H	75	trace	25
3 days reflux	Et	33.3	16.6	57.1
NaOEt, 24 hrs, rt	Et	decomposed		

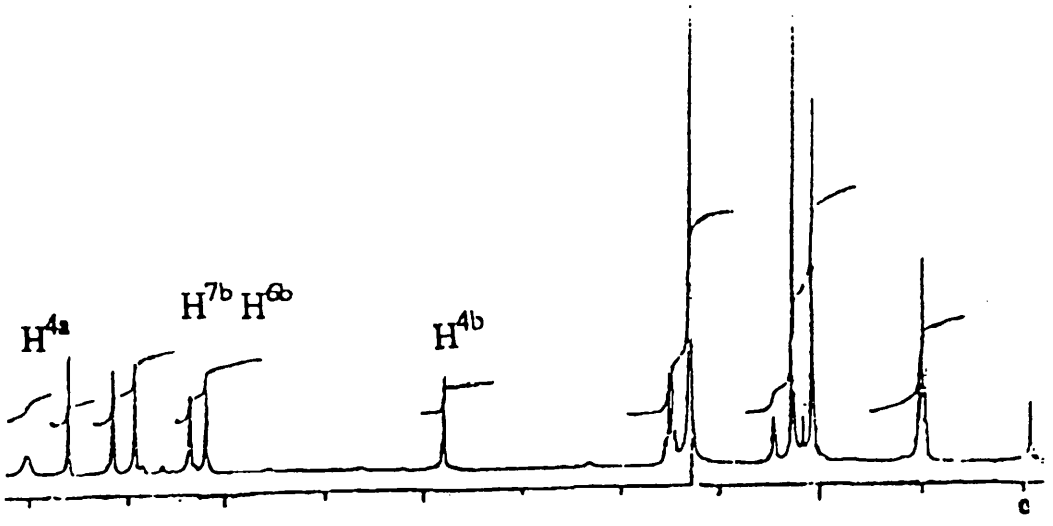
Table 4.2b investigating the effect of solvent, temperature and the presence or absence of acid on the covalent adduct formation of **2-aminopteridine (1)**



Conditions	R	% Ratio of	
		(a)	(b)
24 hrs, rt	H	100	----
24 hrs, rt (pH 3.56)	H	100	----
24 hrs, rt	Et	100	----
24 hrs, rt (pH 3.56)	Et	100	----
4 hrs, 80°C	Et	100	----
4 hrs, 80°C (pH 3.56)	Et	100	----
4 hrs, 80°C	H	100	----
4 hrs, 80°C (pH 3.56)	H	100	----

Fig. 4.2a shows the spectrum of **2-N-acetamidopteridine (13)** in Abs. EtOH at 80°C for 4 hrs. (47% of EtOH adduct is formed).





Appendix 4.3

Table 4.3a shows the ^{1}H NMR kinetic data obtained for the reaction of **2-N-acetamidopteridine (13)** with CD_3OD in DMSO-d_6 at 60°C.

(13)

(20mg, 0.16mmol)

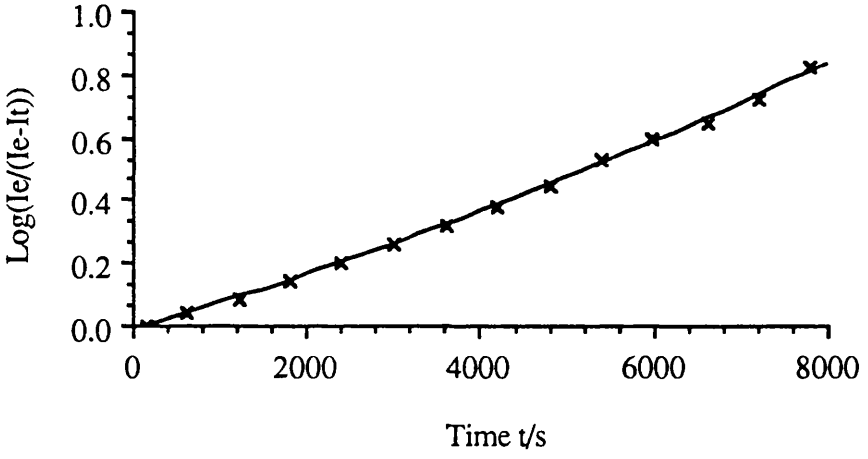
Time t/s x 10^2	I/mm	I_tR/mm	I_t/mm	$Log(I_e/(I_e-I_t)$
1.52	0	149	0	0
6.10	5	140	5.32	0.042
12.30	10	140	10.64	0.088
17.95	15	140	15.96	0.140
24.00	20	139	21.44	0.200
30.00	24.5	137	26.26	0.262
36.25	28	138	30.23	0.320
42.00	31.5	139	33.77	0.379
48.10	34.5	138	37.25	0.446
53.90	38	138	41.03	0.533
59.90	40	138	43.19	0.593
66.20	41.5	137.5	44.97	0.649
72.05	43.5	138	46.97	0.721
77.95	45	136	49.30	0.822
Infinity	58	113	76·40	0

I = C4-H proton peak integral.

$I_t = (I \times 149)/ I_tR$ I_tR = Integral of reference peak at time t.

I_e = I at infinity = 58

Graph 4.3a. shows the kinetic plot of the addition of CD_3OD to **2-N-acetamidopteridine (13)** in DMSO-d_6, followed by ^1H NMR spectroscopy



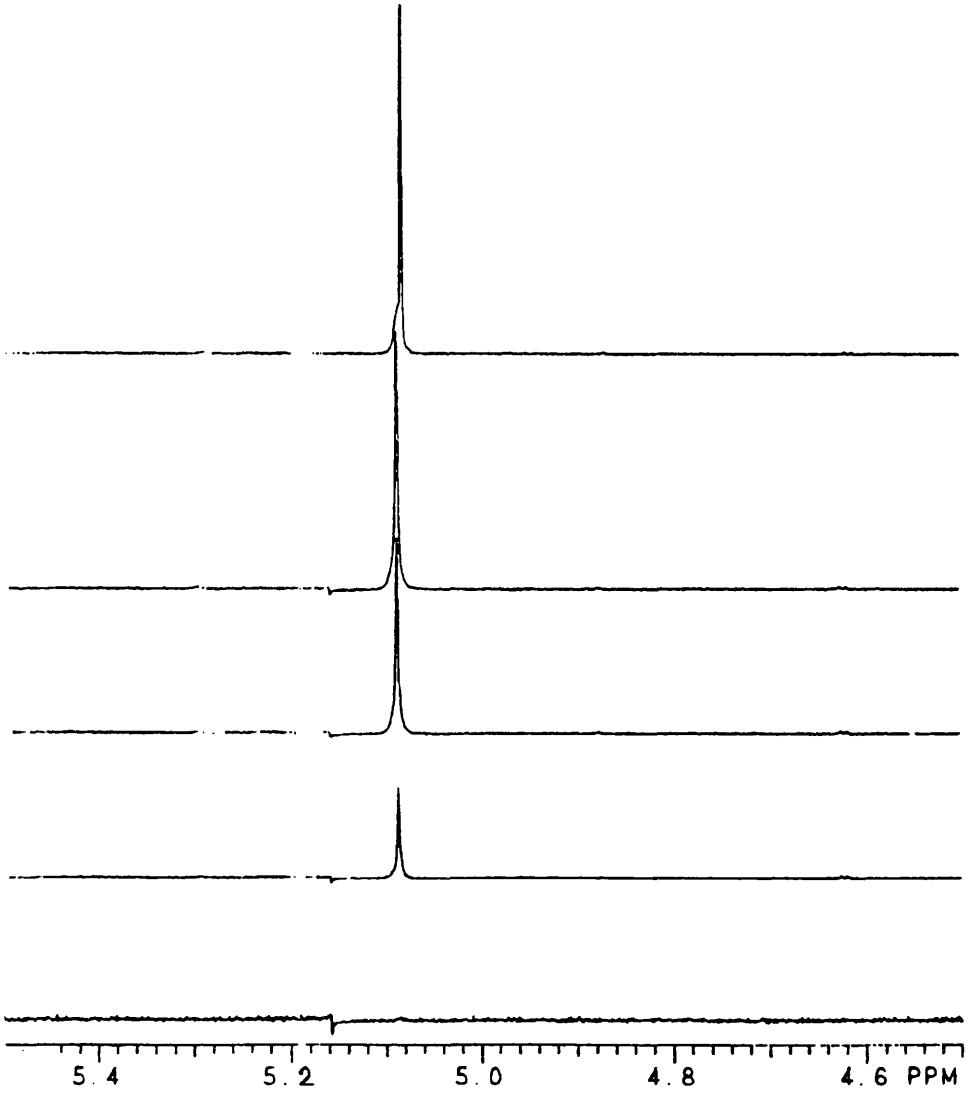
$$y = -1.2566 \times 10^{-2} + 8.0914 \times 10^{-5}x + 3.1665 \times 10^{-9}x^2.$$

Standard deviation = 0.999

At time t = 0, slope = 8.0914×10^{-5} s^{-1}.

$$k = 8.0914 \times 10^{-5} \times 2.303 = 1.86 \times 10^{-4}\ s^{-1}.$$

Stacked spectra 4.3a showing the C4-H peak height of the adduct of **2-N-acetamidopteridine (13)** at 152, 2400, 5390, 7795s and at equilibrium.



Eqm spectrum 4.3a of the addition of CD_3OD to **2-N-acetamidopteridine (13) in** DMSO-d_6.

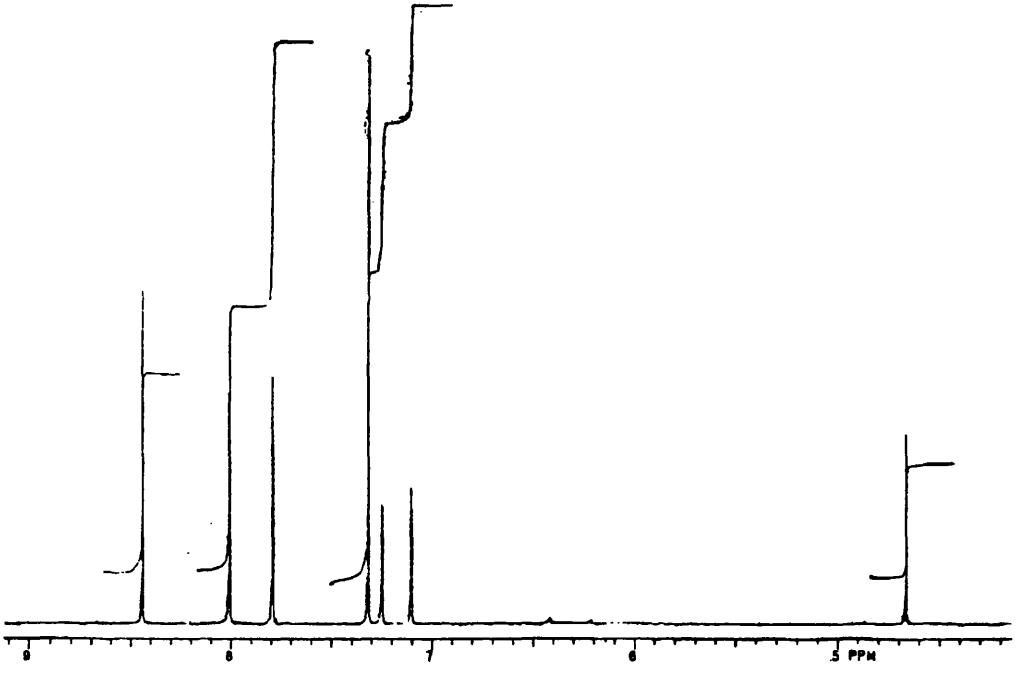


Table 4.3b shows the ^{1}H NMR kinetic data obtained for the reaction of **2-N-acetamidopteridine (13)** with CD_3OD in DMSO-d_6 at 60°C.

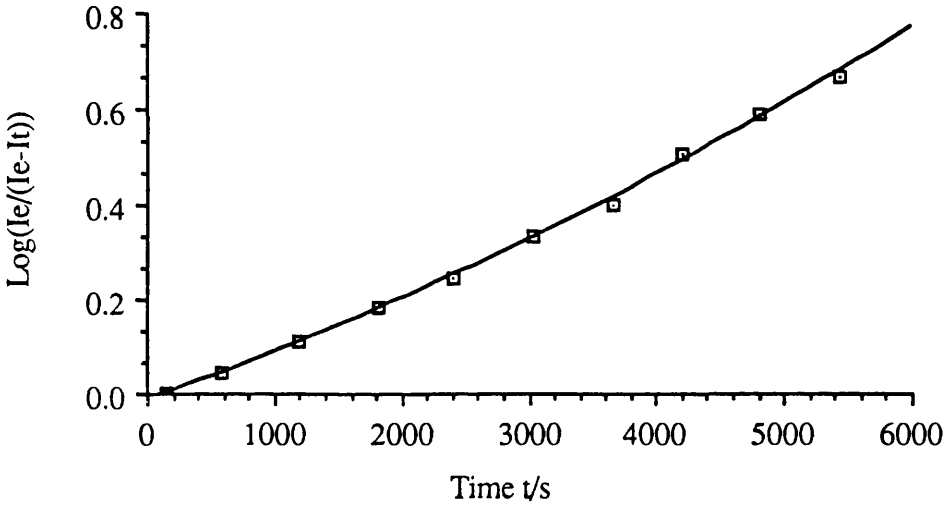
(13)

(20mg, 0.16mmol)

Time t/s x 10^2	I/mm	I_tR/mm	I_t/mm	$Log(I_e/(I_e-I_t))$
1.50	0	182	0	0
5.9	5	172	5.29	0.043
12.00	12	171	12.77	0.111
18.20	18.5	171	19.69	0.186
24.05	23	171	24.47	0.246
30.25	28	169	30.15	0.331
36.65	32	171	34.06	0.401
42.05	36	169	38.77	0.503
48.00	39	169	42	0.591
54.30	41.5	170	44.4	0.669
Infinity	45	145	56.5	0

$I_t = (I \times 182)/ I_tR$ I_e = I at infinity = 56.5

Graph 4.3b shows the kinetic plot of the addition of CD_3OD to **2-N-acetamidopteridine (13)** in DMSO-d_6, followed by ^1H NMR spectroscopy



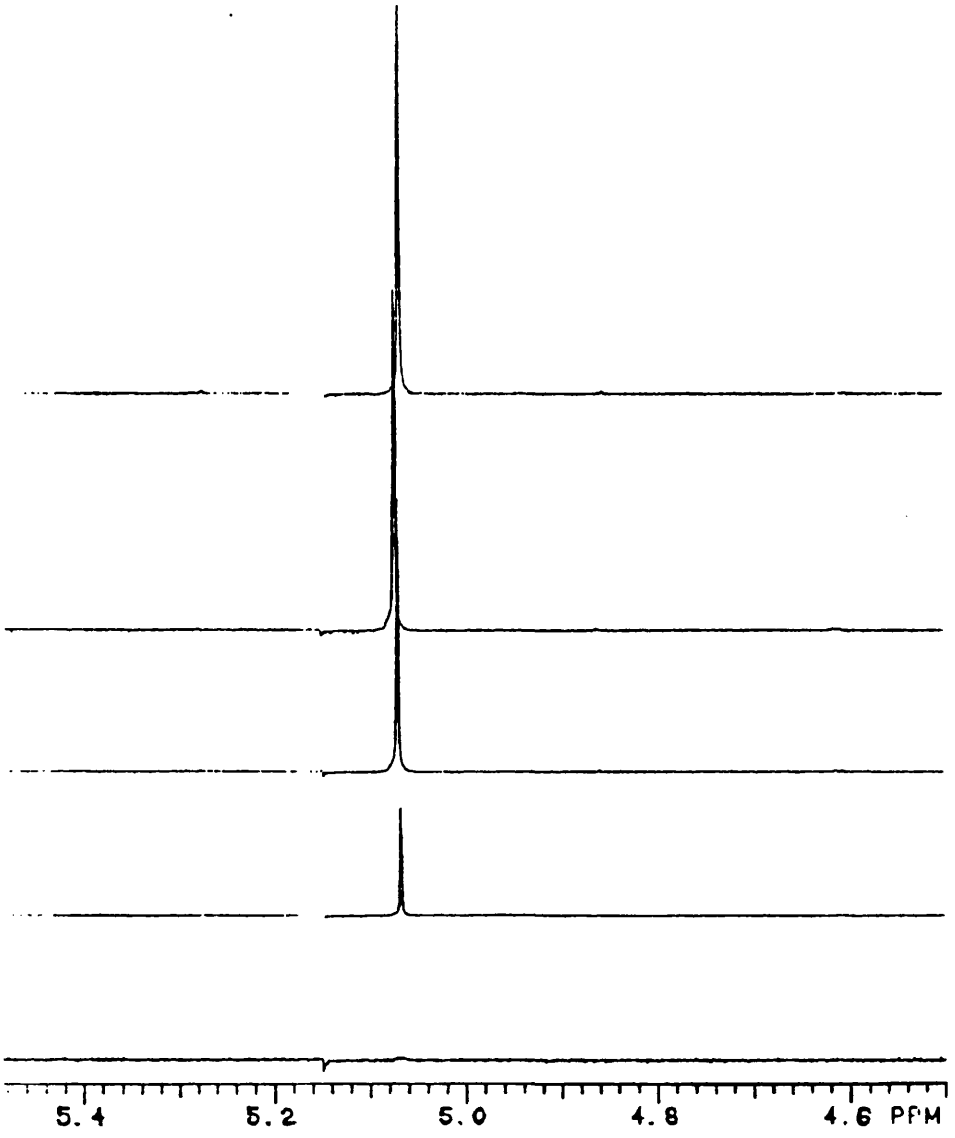
$$y = -1.5020 \times 10^{-2} + 9.7387 \times 10^{-5}x + 5.5441 \times 10^{-9}x^2.$$

Standard deviation = 0.999

At time = t, slope = 9.7387×10^{-5} s^{-1}.

$k = 9.7387 \times 10^{-5}$ 2.303 = 2.243×10^{-4} s^{-1}.

Stacked spectra 4.3b showing the C4-H peak height of the adduct of **2-N-acetamidopteridine (13)** at 152, 1795, 4200, 5990s and at equilibrium.



Eqm spectrum 4.3b of the addition of CD_3OD to **2-N-acetamidopteridine (13) in** DMSO-d_6.

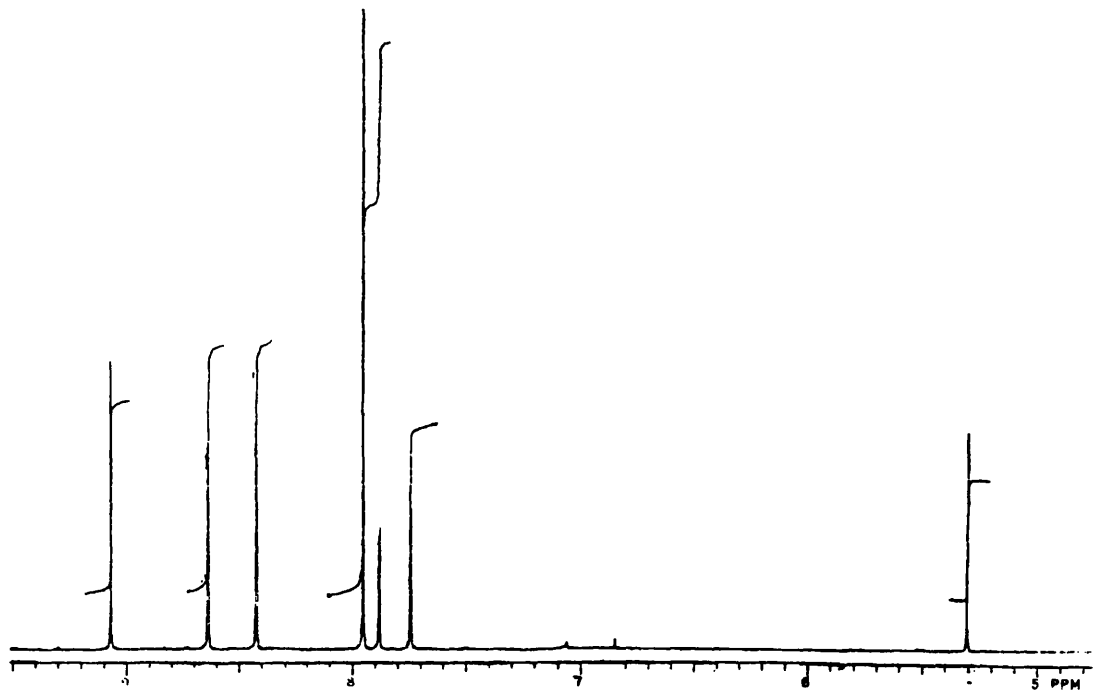
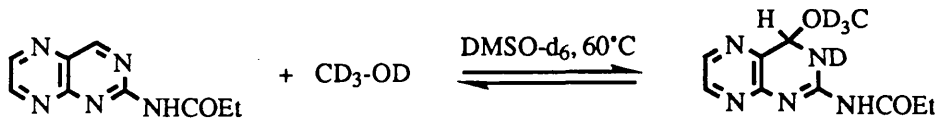


Table 4.3c shows the ^{1}H NMR kinetic data obtained for the reaction of **2-N-propionamidopteridine (20)** with CD_3OD in DMSO-d_6 at 60°C.



(20)

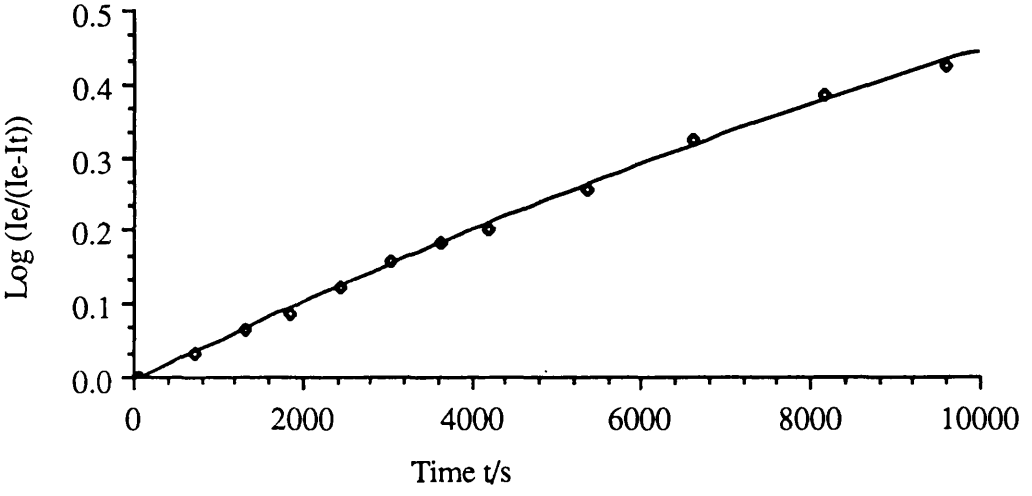
(20mg, 0.098mmol)

Time t/s x 10^2	I/mm	I_tR/mm	I_t/mm	$\log(I_e/(I_e-I_t))$
0.75	0	71	0	0
7.23	3.5	68	3.5	0.033
13.16	6.5	67	6.6	0.064
18.41	8.5	66	8.8	0.088
24.32	13	75	11.8	0.123
30.28	16	75	14.5	0.157
36.28	18.5	76	16.6	0.185
41.91	20	77	17.7	0.200
53.66	24	77	21.2	0.254
66.25	28	76	25.1	0.323
81.60	31	75	28.1	0.385
96.05	43	98	29.8	0.425
Infinity	71	101	47.8	0

I = Measured C4-H proton peak integral.

$I_t = (I \times 68)/ I_tR$ $I_e = 47.8$

Graph 4.3c. shows the kinetic plot of the addition of CD_3OD to **2-N-propionamidopteridine (20)** in DMSO-d_6, followed by ^1H NMR spectroscopy



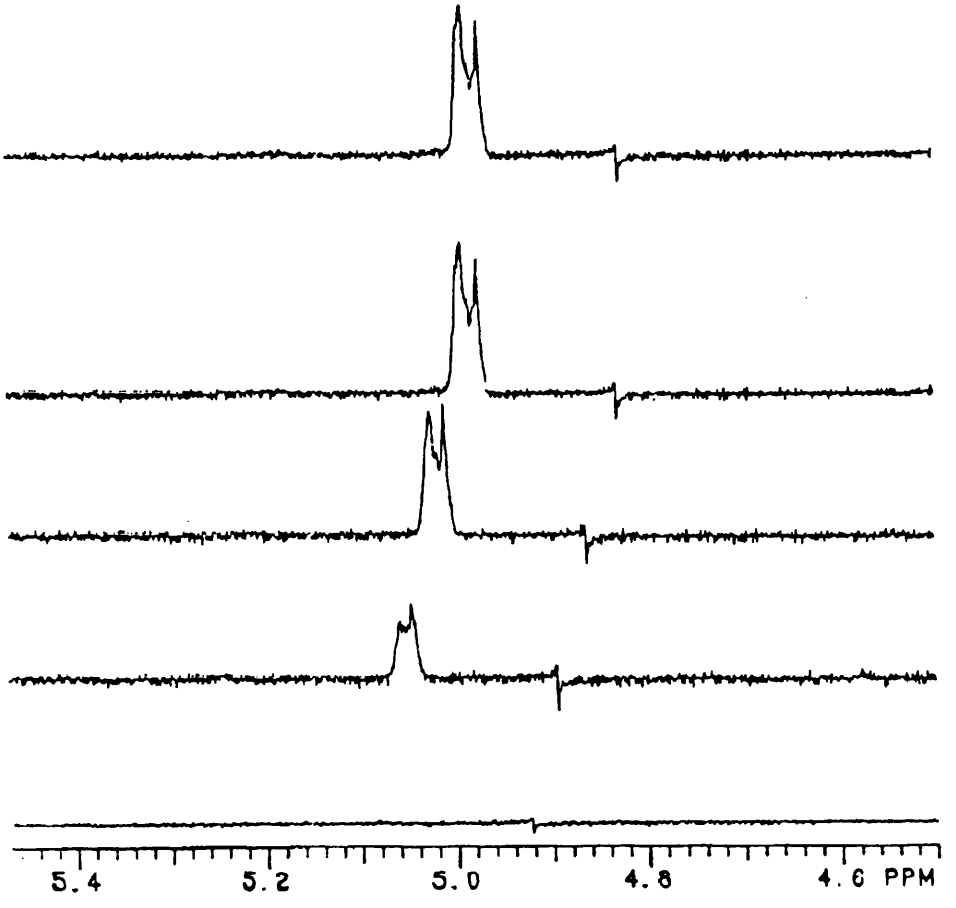
$y = -6.4990 \times 10^{-3} + 5.5536 \times 10^{-5}x - 1.0399 \times 10^{-9}x^2$.

Standard deviation = 0.998

At time t = 0, slope = 5.5536×10^{-5} s^{-1}.

$k = 5.5536 \times 10^{-5} \times 2.303 = 1.28 \times 10^{-4}$ s^{-1}.

Stacked spectra 4.3c showing the C4-H peak height of the adduct of **2-N-propionamidopteridine (20)** at 75, 1841, 4191, 8160s and at equilibrium.



Eqm spectrum 4.3c of the addition of CD_3OD to **2-N-propionamidopteridine (20)** in DMSO-d_6

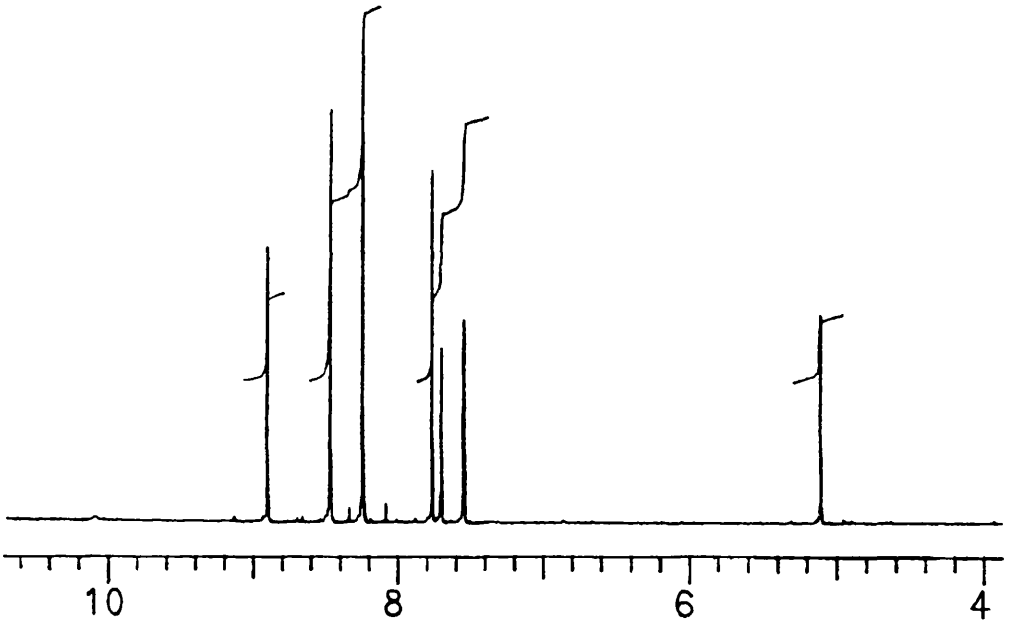
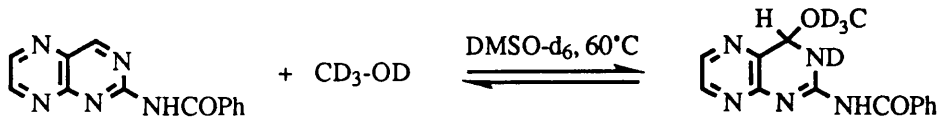


Table 4.3d shows the ^{1}H NMR kinetic data obtained for the reaction of **2-N-benzamidopteridine (21)** with CD_3OD in DMSO-d_6 at 60°C.



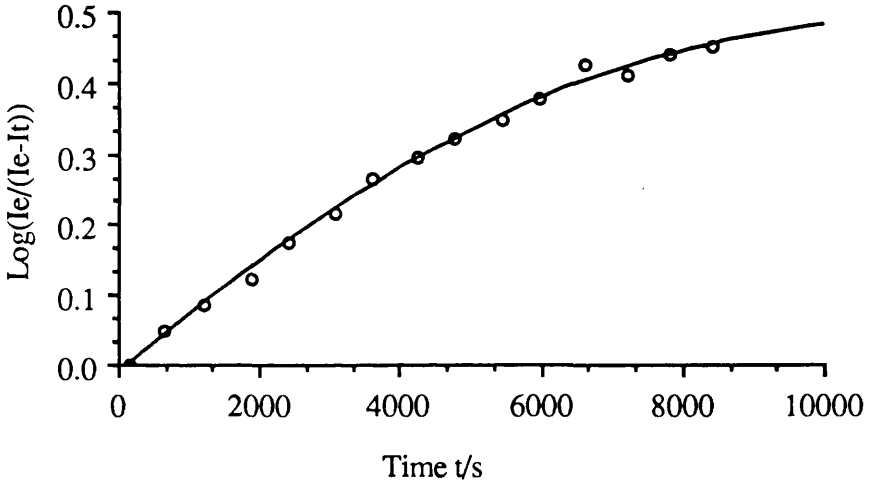
(21)

(20mg, 0.079mmol)

Time t/s x 10^2	I/mm	I_tR/mm	I_t/mm	$Log(I_e/(I_e-I_t))$
1.35	0	47	0	0
6.23	2	43	2.19	0.049
12.15	3.5	44	3.74	0.087
18.90	5.0	46	5.11	0.124
24.15	7.0	48	6.85	0.176
30.91	8.5	49.5	8.07	0.216
36.25	10	50	9.40	0.265
42.45	11	51	10.14	0.295
47.92	12	52.5	10.74	0.321
54.60	13	54	11.32	0.347
59.92	14	55	11.96	0.378
66.32	15	55	12.82	0.424
72.08	15	56	12.58	0.411
78.08	17	61	13.09	0.440
84.10	17.5	62	13.26	0.450
Infinity	21	48	20.56	0

$I_t = (I \times 47)/ I_tR$ $I_e = 20.56$

Graph 4.3d. shows the kinetic plot of the addition of CD_3OD to **2-N-benzamidopteridine (21)** in DMSO-d_6, followed by ^1H NMR spectroscopy.



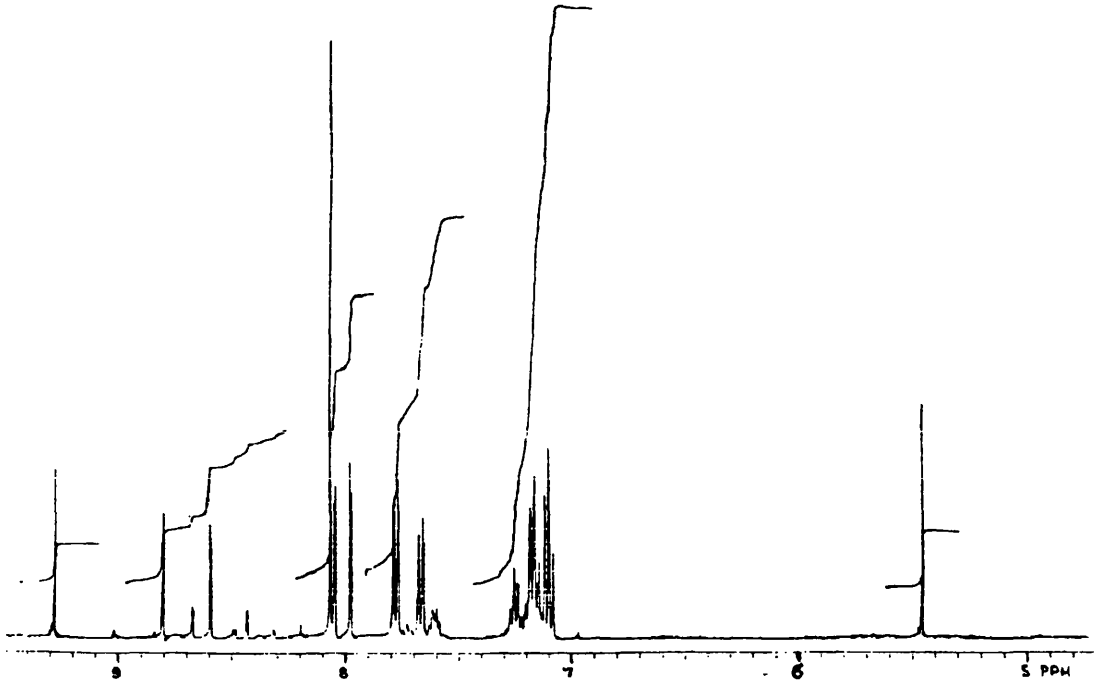
$$y = -1.4078 \times 10^{-2} + 8.8880 \times 10^{-5}x - 3.9408 \times 10^{-9}x^2.$$

Standard deviation = 0.996

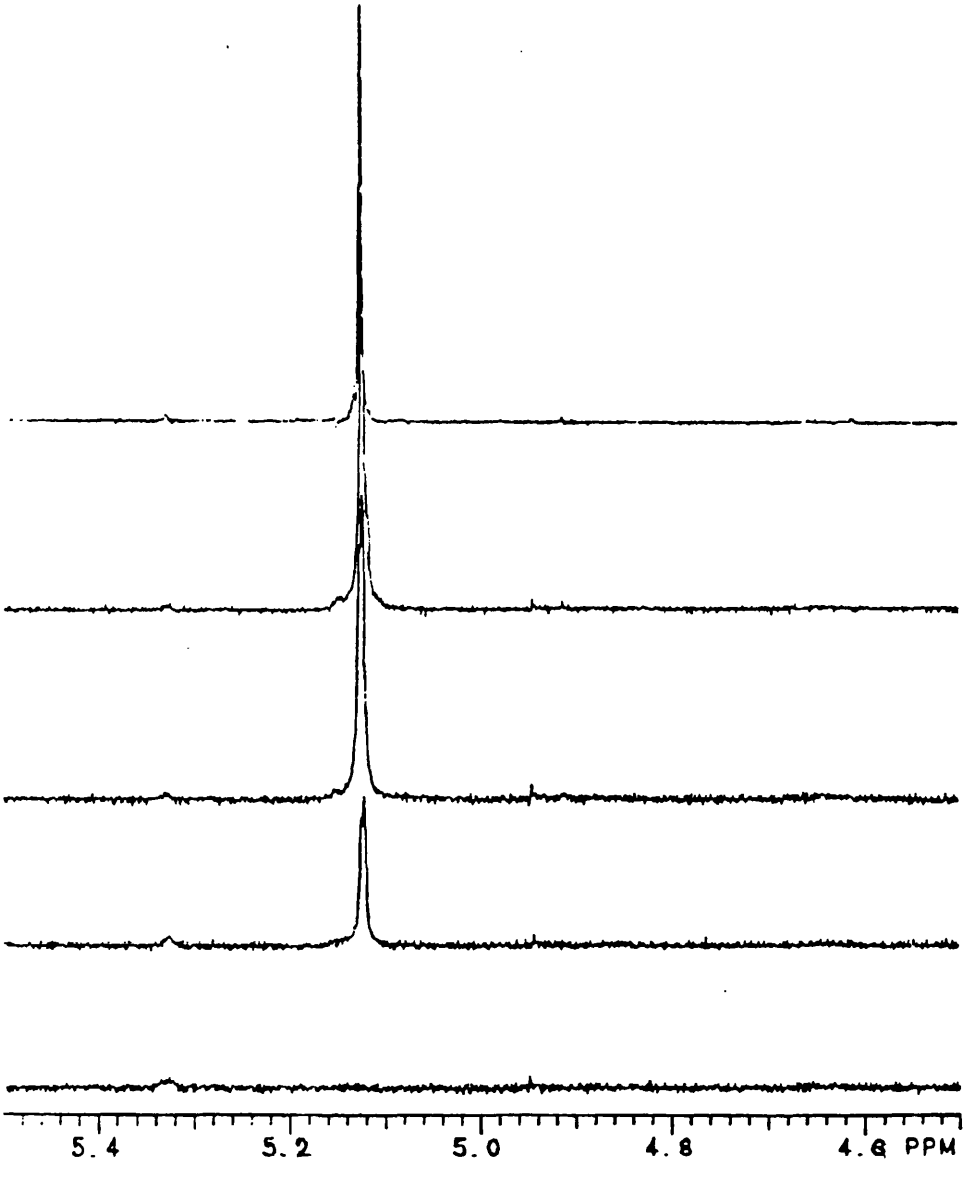
At time t = 0, slope = 8.8880×10^{-5} s^{-1}.

$k = 8.8880 \times 10^{-5} \times 2.303 = 2.047 \times 10^{-4}$ s^{-1}.

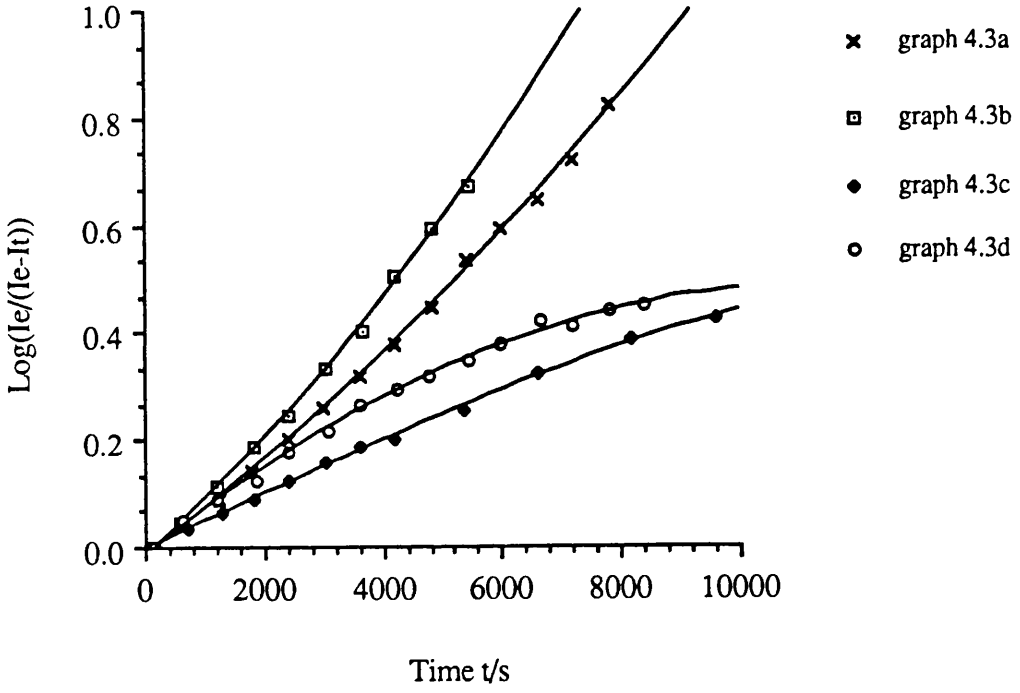
Eqm spectrum 4.3d of the addition of CD_3OD to **2-N-benzamidopteridine (21)** in DMSO-d_6



Stacked spectra 4.3c showing the C4-H peak height of the adduct of **2-N-benzamidopteridine (21)** at 135, 2415, 5460, 7808s and at equilibrium.



Overlay plot 4.3e of graphs 4.3 a, b, c and d



CHAPTER 5

STRUCTURE ACTIVITY

The idea of "heterocycles which undergo covalent hydration acting as transition stae analogues, to provide a novel approach to designing inhibitors of serine proteinase" has been tested by synthesising various 2-substituted pteridines and quinazolines and testing them against the CCK-inactivating peptidase, trypsin, chymotrypsin, porcine pancreatic elastase (PPE) and human leukocyte elastase (HLE).

The effect of the structural modifications of the pteridines and quinazolines on the biological activity of the various serine proteinases is discussed in a qualitative manner.

1.The CCK8-inactivating peptidase:

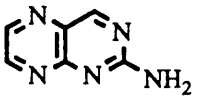
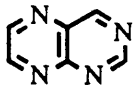
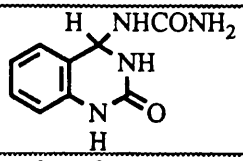
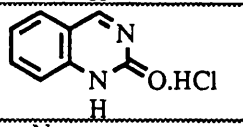
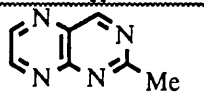
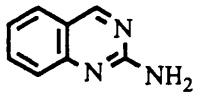
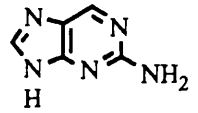
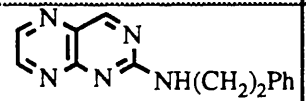
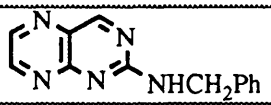
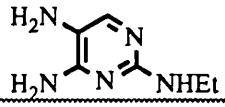
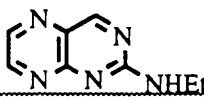
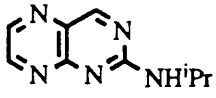
The % inhibition *in vitro*, at a given concentration, of the hydrolysis of the artificial substrates:

- Suc-AAPM-pNA (for Enzyme 1) in assay 7.2, by compounds **(1)** to **(13)**, **(16)**, **(20)**, **(24)** and **(25)**,

- AAF-pNA (for Enzyme 2 - the endogenous CCK8-inactivating peptidase) in assay 7.3, by compounds **(1)** to **(12)**, **(20)**, **(24)** and **(25)**, as well as compounds **(23)**, **(26)**, **(28)**, **(30)**, **(31)** and **(33)**, and

- AAF-AMC (the latest substrate for Enzyme 2) in assay 7.4, by compounds **(18a)**, **(21)**, **(27)** and **(32)** which were submitted for testing at a later stage, as well as some of the

Table 5.1 shows the % inhibition in vitro of the CCK-inactivating peptidase, by compounds (1) to (12).

COMPOUND No.	UCL.	Ref.	STRUCTURE	% INHIBITION A. (7.2)[1]	A. (7.3)[2]	A. (7.4)[3]
(1)	1015	MP61A		25% 9mM	IC_{50} >3mM	NT[4]
(2)	1016	MP66B		20% 12mM	IC_{50} >3mM	NT
(3)	1026	MP96C		IC_{50} 3mM	IC_{50} 3mM	NT
(4)	1032	MP164A		IC_{50} 4mM	IC_{50} 3mM	NT
(5)	1041	MP223K		26% 3mM	NT	NT
(6)	1042	MP231D		0 3mM	IC_{50} >3mM	NT
(7)	1043	MP225A		0 3mM	IC_{50} >3mM	NT
(8)	1059	MP272B		11% 3mM	IC_{50} >3mM	NT
(9)	1060	MP274B		12% 3mM	IC_{50} >3mM	NT
(10)	1061	MP278B		12% 3mM	IC_{50} >3mM	NT
(11)	1062	MP280B		0 3mM	IC_{50} >3mM	NT
(12)	1181	MP700A		6% 3mM	IC_{50} >3mM	IC_{50} 0.4mM

[1] Assay using Suc-AAPM-pNA as a substrate.

[2] Assay using AAF-pNA as a substrate.

[3] Assay using AAF-AMC as a substrate.

[4] Not tested.

Table 5.2 shows the % inhibition in vitro of the CCK-inactivating peptidase, by compounds (13) to (23).

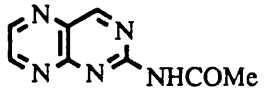
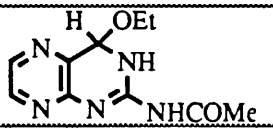
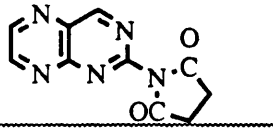
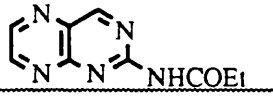
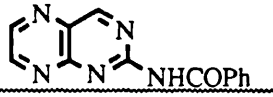
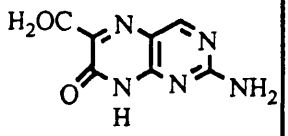
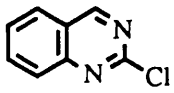
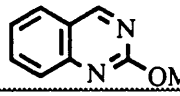
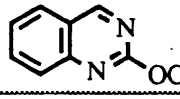
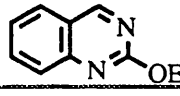
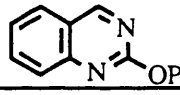
COMPOUND No.	UCL.	Ref.	STRUCTURE	% INHIBITION A. (7.2)[1]	A. (7.3)[2]	A. (7.4)[3]
(13)	1085	MP298		IC_{50} 3mM	NT	IC_{50} >5mM
(16)	1096	MP296C		IC_{50} 4mM	NT	IC_{50} 1.5mM
(18a)	1323	MP804I		NT	NT	IC_{50} 3mM
(20)	1179	MP687		33% 3mM	IC_{50} >3mM	IC_{50} 5mM
(21)	1322	MP761C		NT	NT	IC_{50} 1mM
(23)	1180	MP692C		NT	IC_{50} >1mM	NT (interf)

Table 5.3 shows the % inhibition in vitro of the CCK-inactivating peptidase, by compounds (24) to (33)

COMPOUND No.	UCL.	Ref.	STRUCTURE	% INHIBITION A. (7.2)[1]	A. (7.3)[2]	A. (7.4)[3]
(24)	1207	MP718C		32% 3mM	IC_{50} 5mM	IC_{50} >5mM
(25)	1206	MP721A		25% 3mM	IC_{50} 5mM	IC_{50} >5mM
(26)	1247	MP756A		NT	IC_{50} 6mM	NT
(27)	1320	MP774D		NT	NT	IC_{50} 20mM
(28)	1278	MP772C		NT	IC_{50} >10mM	NT

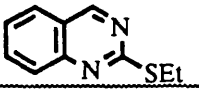
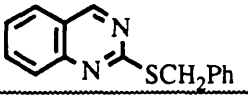
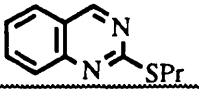
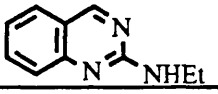
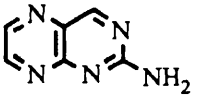
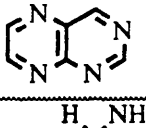
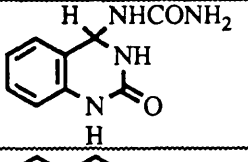
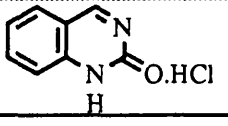
COMPOUND No.	UCL.	Ref.	STRUCTURE	% INHIBITION A. (7.2)[1]	A. (7.3)[2]	A. (7.4)[3]
(30)	1279	MP790F		NT	IC_{50} 5mM	NT
(31)	1280	MP798C		NT	IC_{50} >10mM	NT
(32)	1321	MP794F		NT	NT	IC_{50} >2mM
(33)	1281	MP800E		NT	IC_{50} >10mM	NT

Table 5.4 shows the IC_{50} values *in vitro* for the CCK-inactivating peptidase by compounds (1) to (4) using the artificial substrate Suc-AAPM-AMC.

COMPOUND No.	UCL.	Ref.	STRUCTURE	IC_{50} A [7.1][5]
(1)	1015	MP61A		0.5mM
(2)	1016	MP66B		>3mM
(3)	1026	MP96C		1mM
(4)	1032	MP164A		1mM

[5] Assay using Suc-AAPM-AMC as a substrate.

earlier compounds **(12)**, **(13)**, **(16)**, **(20)** and **(23)** to **(25)**, are given in Tables 5.1, 5.2 and 5.3.

The % inhibition *in vitro* by compounds **(1)** to **(4)**, of the hydrolysis of Suc-AAPM-AMC, the first artificial substrate used to measure the activity of the CCK-inactivating peptidase, is given in Table 5.4.

Details of the classification of Enzyme 1 and 2 and the different bioassay systems are given in Chapter 7. The structures of AMC and pNA are given in Chapter 7 too.

As mentioned in Chapter 2, the first compounds selected for synthesis and biological testing were **(1)**, **(2)**, **(4)** and **(5)**. These compounds were selected on the basis that each had a ratio of hydrated : anhydrous <1.

Initially, **(2)** was found to be active against Enzyme 1 (thought to be the CCK8-inactivating peptidase, at the time) with an IC_{50} value in the 10^{-4} M range. See Table 5.4. This prompted us to investigate whether it was the binding affinity of the NH_2 group rather than covalent addition by the enzyme which was contributing to the activity, since **(2)** was inactive. Therefore, two compounds not known to covalently react readily with either strong or weak nucleophiles, **(6)** and **(7)**, were selected. Subsequent evaluation of the test procedure led to a revised result (as shown in Table 5.1) which was much less interesting (25% inhibition at 9mM). It still appeared that activity might be specific to the pteridine structure since the non-pteridine analogues such as **(6)** and **(7)** were found to be inactive. Therefore, the 2-alkylaminopteridines **(8)**, **(9)**, **(11)** and **(12)** and the 2-N-alkylamidopteridine **(13)** were synthesised.

The initial activity of quinazolines **(3)** and **(4)** against Enzyme 1, prompted us to investigate whether activity was due to a preference in binding of the 2-oxoquinazoline ring system compared to the pteridine ring system or the greater ability of **(4)** to undergo covalent adduct formation than the rest of the pteridines with the enzyme. Therefore, to

start with, **(25)**, a quinazoline not kown to undergo covalent adduct formation as readily as **(4)**, was synthesised. The s.m. **(24)** was submitted for testing too.

The results (Tables 5.1 and 5.2) show that with the exception of the quinazolines **(3)** and **(4)** and the 2-N-alkylamidopteridines **(13)**, **(16)** and **(20)**, the compounds are only very weakly active on Enzyme 1.

Amongst the pteridines, the 2-N-alkylamidopteridines were much more active than either the unsubstituted pteridine or the pteridines with the electron donating substituents, such as 2-aminopteridine, 2-methylpteridine and the 2-alkylaminopteridines. From the results, it was not possible to deduce whether the differences in activity were due to the differences in binding affinity or the differences in the extent to which each compound underwent covalent adduct formation with the enzyme.

Further studies carried out (see Chapter 4) showed that **(13)** underwent covalent adduct formation much more readily than **(1)** in both EtOH and H_2O, either at 25°C or at higher temperatures under neutral conditions.

Of the other two compounds known to undergo covalent hydration readily, **(3)** and **(5)**, the former was active whilst the latter was not as active.

Also, both **(24)** and **(25)** are not known to undergo covalent hydration readily as the neutral species and yet the activity was comparable to that of the alkylamidopteridine **(20)**.

These results together with the covalent adduct formation studies of **(13)** suggested that the extent of covalent addition may determine activity but that it is not a sufficient condition.

At this stage, pharmacological studies (unpublished results) indicated that Enzyme 1 was not the CCK8-inactivating neuropeptidase we were interested in. (See Chapter 7). This led to the retesting of some the above compounds on a new assay system which measured the activity of Enzyme 2 - the endogenous CCK8-inactivating neuropeptidase. Several other compounds submitted for testing at this stage were also tested on the new assay.

The results (Tables 5.1, 5.2 and 5.3) show that with the exception of the quinazolines **(3)**, **(4)**, **(24)**, **(25)**, **(26)** and **(30)** which show specific activity, the other compounds tested are relatively inactive on the CCK8-inactivating peptidase.

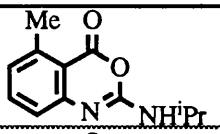
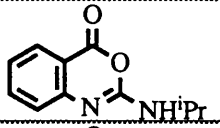
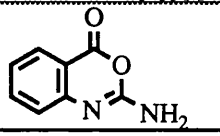
Neither the unsubstituted pteridine nor the pteridines with electron donating substituents were found to be active.

The result for **(12)** was surprising, as this compound structurally resembles 5-ethyl-2-isopropylamino-benzoxazin-4-one, which was active against HLE with $K_i = 9.4nM$ [35]. (See Chapter 1 and Chapter 2, Fig. 2.1 for the structure). Therefore, one would have expected the CCK8-inactivating peptidase to have shown similar active site specificity for the NH-^{i}Pr group.

Krantz's study with the 2-aminobenzoxazin-4-ones [35,36] has shown that the activity of these compounds are highly sensitive to their having appropriate alkyl substituents at positions 2 and 5. In order to investigate whether 2-aminobenzoxazin-4-ones showed similar specificity for the CCK8-inactivating peptidase too, 5-methyl-2-isopropylaminobenzoxazin-4-one (UCL 1139), the methyl analogue of Krantz's potent inhibitor, was synthesised by Dr. R. Bambal (a colleague working on the same project with Prof. C. R. Ganellin) and submitted for testing. This compound would probe active site specificity for the 5-Me and 2-^{i}Pr-NH groups.

The *in vitro* IC_{50} values for the hydrolysis of the CCK8-inactivating peptidase (CIP), PPE, bovine trypsin and HLE by UCL 1139, and the hydrolysis of the former by two other 2-aminobenzoxazin-4-ones, are given in Table 5.5.

Table 5.5. The *in vitro* IC_{50} values for CIP, PPE, trypsin and HLE.

COMPOUND UCL.	STRUCTURE	IC_{50} CIP.	PPE	Tryp.	HLE.
1139		>3mM	1μM	0.4μM	1.5nM
1197		>3mM	NT	NT	NT
1204		>3mM	NT	NT	NT

The results showed UCL 1139 to be inactive against the CCK8-inactivating peptidase (IC_{50} > 3mM), but active against PPE, trypsin and HLE with IC_{50}s of 1mM, 0.4mM and 0.0015mM respectively.

It was surprising to find that UCL 1139 was not as active on the CCK8-inactivating peptidase, but the results show that the different serine proteinases are remarkably sensitive in their binding site specificities.

Two analogues (UCL 1197 and 1204), in which the 5-Me or 2-^{i}Pr groups were absent were examined to see whether these groups act sterically to inhibit binding to the CCK8-inactivating peptidase, however none of these compounds was active.

Therefore, it was concluded that the complete lack of activity of the benzoxazin-4-ones as inhibitors of the CCK8-inactivating peptidase might serve as an analogy to account for the poor showing of pteridine derivatives as inhibitors.

Amongst the quinazolines that are active, (**4**) is the only compound known to undergo covalent hydration readily as the neutral species and is is also the most active. (Under the

testing conditions, which uses HCl, **(3)** would most definitely ring-close to form **(4)**. Therefore, it is the activity of **(4)** that is being determined in either case).

Compounds **(26)** and **(30)** are novel and covalent adduct formation has not been studied, however, one would expect the covalent adduct formation of **(26)** to be similar to that of **(25)** and the covalent adduct formation of **(30)** to be similar to that of 2-methylthioquinazoline. The latter has also been reported to undergo covalent adduct formation readily as the cationic species, but to remain anhydrous as the neutral species [60]. Although the hydrated neutral species of all three compounds **(24)**, **(25)** and 2-methylthioquinazoline have been detected by Armarego and Smith (1966) [60], these have been treated as predominantly anhydrous because the adducts were shown to be quite unstable in conditions used for rapid reaction techniques. Therefore, it is still possible that a strong nucleophile such as the enzymic serine OH might covalently react with these compounds.

Except for **(20)**, there are no results (on this assay) for the other compounds that do undergo covalent hydration readily, such as the 2-N-alkylamidopteridines **(13)** and **(16)** and the 2-alkylpteridine **(5)**, to see whether it is the extent of covalent adduct formation that determines activity.

Compound **(20)** undergoes covalent adduct formation readily but is not as active as the quinazolines. A similar result was observed with Enzyme 1. Therefore, the conclusion was that the extent of covalent addition may determine activity but it is not a sufficient condition.

The introduction of a more sensitive assay at this stage enabled potential inhibitors to be tested at much lower concentrations than was previously possible. Although it was beyond the time limit of the project, Prof. Schwartz and his team were kind enough to retest a few of the above compounds using the new assay, for a comparative study. The results are given in Tables 5.1, 5.2 and 5.3.

The biological results based on the new assay show that the pteridines are more active than the quinazolines on the CCK8-inactivating peptidase. The most active compound is **2-**

isopropylaminopteridine (12) with an IC_{50} value of 0.4mM. The enzyme was clearly showing active site specificity for the ^{i}Pr group. Unfortunately, the active site specificity for the other groups such as Et, CH_2Ph and CH_2CH_2Ph in the 2 position of aminopteridines cannot be compared, due to the results not being available.

The other active compounds are the 2-N-alkylamidopteridines **(16)**, **(18a)**, **(20)** and **(21)**. It is surprising that compound **(13)** is not active whilst the adduct **(16)** is active with an IC_{50} value of 1.5mM. It seems as if the OEt group in **(16)** is in some way or another contributing to binding at the active site. On forming the adduct, the planarity of the pteridine molecule is lost. The conformational change may affect the way in which the molecule binds at the active site.

The active site is a three-dimensional entity made up of groups that come from different parts of the linear amino acid sequence, and residues far apart in the linear seqence may interact more strongly than adjacent residues in the amino acid sequence by folding of the polypeptide chains. The clefts and crevices formed in the process create a microenvironment in which certain polar residues (i.e. the catalytic groups such as serine OH) acquire special properties essential for their catalytic role. The substrate molecule is bound to a cleft or crevice from which water is usually excluded. The non-polar character of the cleft enhances the binding of the substrate, the specificity of binding depending on the precisely defined arrangement atoms at these binding sites.

The shape of the active site is modified by the binding of the substrate. However, some enzymes preferentially bind a strained form of the substrate corresponding to the transition state (i.e. the t.s. analogues). Compound **(16)** may be acting as a true t.s. analogue, which may account for the greater activity of **(16)** compared to that of **(13)**.

Extending the chain from Me to Et causes an increase in activity, giving **(20)** an IC_{50} value of 5mM compared to **(13)** which has an IC_{50} value >5mM. The substitution of an alkyl chain by an aromatic ring increases activity even further as shown by **(21)** which has an IC_{50} value of 1mM. Compound **(18a)** which has a bulky succinimide group in the 2

position is also active with an IC_{50} value of 3mM. The considerable activity of compounds **(12)**, **(18a)** and **(21)** having ^{i}Pr, succinimido and Ph groups respectively, show that the enzyme clearly has a hydrophobic cavity at the active site which can accomodate and bind these bulky substituents.

The study carried out to determine the binding properties of the Enzyme 2 active site towards dipeptide derivatives (unpublished results) showed that in dipeptides of the following type: $H.P_2.P_1R$, acylation at P_1 (corresponding to the S_1 subsite of the enzyme) were not tolerated, and amides and esters at P_2 were affinity enhancing but a free NH_2 group was preferred.

Amides at the 2 position of pteridines are tolerated and are affinity enhancing. Therefore, the pteridines may be binding at the S_2 subsite.

Of the tested quinazolines, only **(27)** showed definite activity. The activity of this particular quinazoline may be due to the greater binding affinity of the OEt group compared to the other groups, by the enzyme active site. Unfortunately, the results for the other compounds which have Et groups in the 2 position, such as **(11)**, **(30)** and **(33)** are unavailable for comparison.

2. Trypsin, chymotrypsin, PPE and HLE:

The *in vitro* IC_{50} values for the hydrolysis of the artificial substrates:

- Z-Arg-AMC (for trypsin) in assay 7.5,

- AAF-AMC (for α-chymotrypsin) in assay 7.6 and

- Suc-AAA-AMC (for PPE) in assay 7.7, by compounds **(1)** to **(9)**, **(11)** to **(13)**, **(16)**, **(18a)**, **(20)**, **(21)** and **(23)** to **(33)**, are given in Tables 5.6 and 5.7.

Only a few compounds were tested on HLE using the artificial substrate Suc-AAV-pNA and these were all found to be inactive at a concentration of 10^{-4}M. It is hoped that all the compounds discussed so far, will be tested at a much higher concentration against HLE at a later stage, for a comparative study. The compounds that were tested, were **(1)**, **(3)**, **(4)**, **(5)**, **(9)**, **(13)**, **(16)**, **(20)**, **(24)** and **(25)**. The biological results are given in Tables 5.6 and 5.7 too.

Details of the different bioassay systems are given in Chapter 7.

Table 5.6 shows the biological activities against Trp, chymotrp, PPE, HLE and the CCK-inactivating peptidase (CIP).

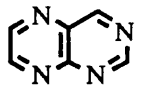
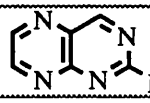
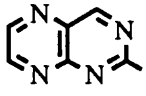
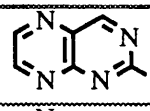
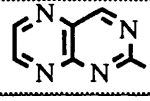
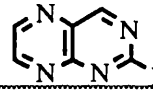
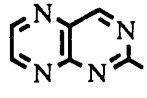
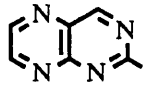
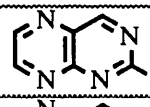
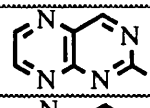
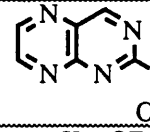
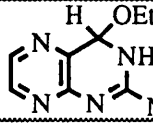
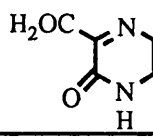
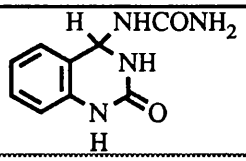
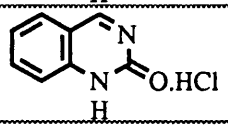
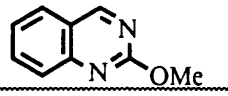
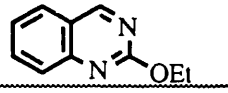
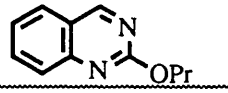
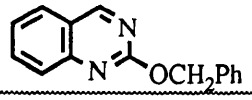
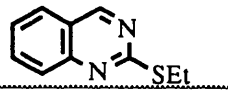
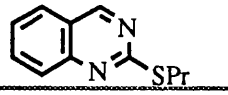
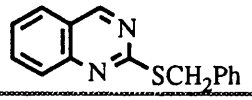
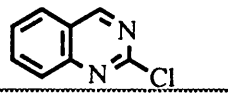
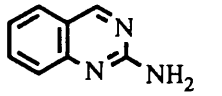
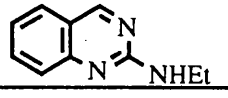
COMPOUND No.	UCL.	STRUCTURE	IC_{50}/mM Trp.	Chym.	PPE.	HLE.	CIP.
(2)	1016		>>3[a]	>>3[a]	>>3[a]	NT	NT
(5)	1041		>>3[a]	>>3[a]	>>3[a]	>>0.1	NT
(1)	1015		>>3[a]	>>3[a]	>>3[a]	>>0.1	NT
(8)	1059		>>3[a]	>>3[a]	>>3[a]	NT	NT
(9)	1060		>>3[a]	>>3[a]	>>3[a]	>>0.1	NT
(10)	1061		>>3[a]	>>3[a]	>>3[a]	NT	NT
(12)	1181		1.5	0.17	0.17	NT	0.4
(13)	1085		1.7	1.5 >0.1[a]	1.7 >0.1[a]	>>0.1	>5.0
(20)	1179		2.0	1.5	1.0	>>0.1	5.0
(21)	1322		1.0	1.0	1.0	NT	1.0
(18a)	1323		2.5	2.0	1.0	NT	3.0
(16)	1096		1.0	0.8	0.5 >0.1[a]	>>0.1	1.5
(23)	1180		1.2	0.6	0.5	NT	Int.

Table 5.7 shows the biological activities of quinazoline derivatives against Trp, chymotrp, PPE, HLE and CIP.

COMPOUND No.	COMPOUND UCL.	STRUCTURE	IC_{50}/mM Trp.	Chym.	PPE.	HLE.	CIP.
(3)	1026		>3[a]	>3[a]	>3[a]	>>0.1	NT
(4)	1032		>3[a]	>3[a]	>3[a]	>>0.1	NT
(25)	1206		5.0	2.0	5.0	>>0.1	>5.0
(27)	1320		12.0	12.0	12.0	NT	20.0
(28)	1278		>10.0	6.0	>10.0	NT	NT
(26)	1247		>10.0	5.00	12.0	NT	NT
(30)	1279		3.00	8.00	4.00	NT	NT
(32)	1321		3.00	1.5	1.0	NT	NT
(31)	1280		>10.0	>10.0	>10.0	NT	NT
(24)	1207		5.00	1.6	5.0	>>0.1	>5.0
(6)	1042		>>3[a]	>>3[a]	>>3[a]	NT	NT
(33)	1281		>10.0	>10.0	>10.0	NT	NT

[a] Refers to test results from a previous occasion.

The first six compounds, **(1)**, **(2)**, **(5)**, **(8)**, **(9)** and **(11)** in Table 5.6, which include unsubstituted pteridine, and pteridine with electron donating groups, are inactive against trypsin, chymotrypsin and PPE. Compound **(12)**, however, shows considerable activity. The former compounds were tested on a previous occasion using a different substrate, which could account for the discrepancy. They would have to be retested in order to make a fair comparison of the activities between the various 2-alkylaminopteridines.

Compound **(12)** is consistently active against trypsin, chymotrypsin, PPE and the CCK8-inactivating peptidase, showing that the ^{i}Pr group in 2-alkylaminopteridines is accommodated very well at the active site of all four serine proteinases. The greatest activity of **(12)** is shown against chymotrypsin and PPE with an IC_{50} value of 0.17mM for both enzymes, then, against the CCK8-inactivating peptidase with an IC_{50} value of 0.4mM and finally against trypsin with an IC_{50} value of 1.5mM. Chymotrypsin and PPE both have similar binding specificities. Both enzymes have hydrophobic binding regions which can accommodate branched chain alkyl groups and aromatic rings. PPE is also known to have high affinity for amino acids such as alanine and valine which have Me and iPr groups respectively, which again shows the binding specificity for the ^{i}Pr group. The CCK-inactivating peptidase can accommodate bulky groups too. However, trypsin is specific to straight chain alkyl amino groups as those in lysine and arginine. The differences in activities could thus be attributed to the differences in the binding specificities.

The 2-N-alkylamidopteridines are not as active as **(12)**, but nevertheless show considerable activity against trypsin, chymotrypsin, PPE and the CCK8-inactivating peptidase. Covalent addition studies with the 2-N-alkylamidopteridines (see Chapter 4) have shown that a substituent at the 2-position of 2-amidopteridines has little or no effect on addition at the 3,4 C=N bond. Therefore, the differences in activity amongst the latter compounds must reflect the differences in the binding affinities at the enzyme active site.

The Me analogue **(13)** is active against the three former enzymes, but not against the latter enzyme, clearly showing that the Me group in 2-N-alkylamidopteridines is tolerated at the active sites of the former three, but not at that of the latter.

The covalent adduct **(16)** is more active than the anhydrous species **(13)** and is also the most active 2-N-alkylamidopteridine against all four serine proteinases. The activity of **(16)** might be due to the compound being bound more favourably as the t.s analogue, however the inhibitory activity does not by itself provide an indication of mechanism.

Extending the chain length by a methylene group to form the Et analogue **(20)**, does not significantly change activity against trypsin and chymotrypsin, however, increases activity considerably against PPE, showing that the Et group in 2-N-alkylamidopteridines is accommodated at the active sites of all four enzymes, but to a greater extent at that of PPE.

Replacing alkyl groups with an benzene ring to form **(21)** results only in a slight increase in activity against trypsin and chymotrypsin but a marked increase in activity against the CCK8-inactivating peptidase. PPE does not seem to discriminate between either an Et group or a Ph group in the 2 position of amidopteridine. Activity remains at an IC_{50} value of 1mM. All four serine proteases show an IC_{50} value of 1mM for **(21)**, clearly showing that the Ph group in 2-N-alkylamidopteridines is well tolerated at the respective active sites.

One of the major problems associated with the testing of the pteridines and quinazolines, was the low solubility in H_2O and other organic solvents. Most of the compounds had to be dissolved in DMSO. Compound **(13)** was only sparingly soluble in H_2O. Therefore, it was decided to incorporate a CO_2H group, as in compound **(18)** (see Chapter 2, Table 2.2), to assist solubility. It seemed interesting to investigate the extent to which increased solubilty affected the activity. Unfortunately, **(18a)** was obtained instead of **(18)**, and in terms of solubility, the former is not very different to **(13)**. However, in terms of activity, **(18a)** is more active against both PPE and the CCK8-inactivating peptidase, than the former. The succinimido group seems to be accommodated well at the active sites of both enzymes, though not as well as the Ph group. Amongst the 2-N-alkylamidopteridines,

(18a) is the least active against both trypsin and chymotrypsin, again showing that the succinimido group is accommodated, but not as readily as a Ph group. A possible explanation is steric hindrance by the two C=O groups in the succinimido moiety.

Amongst the pteridines, **(23)** is the second most active compound against chymotrypsin and PPE with IC_{50} values of 0.6mM and 0.5mM, respectively. This compound was also active against trypsin with an IC_{50} value of 1.2mM. A result for the CCK8-inactivating peptidase for this compound could not be obtained due to a problem associated with the bioassay. Both the free NH_2 group in the 2 position and the CO_2H group in the 6 position of pteridin-7-one, seem to be accommodated very well at the active sites of both chymotrypsin and PPE. It is difficult to ascertain which of the two groups, CO_2H or NH_2 contributes most to the activity. The C=O group in the 7 position may contribute to activity too. The activity against trypsin may be as a result of the interaction between the free NH_2 group in **(23)** with the aspartic acid residue at the enzymic active site. Another explanation for the considerable potency of **(23)** against the three serine proteinases is esterification of the serine OH by the CO_2H group of the compound. One cannot also rule out the possibility of covalent adduct formation with the enzyme.

Amongst the quinazolines, compounds **(3)**, **(4)** and **(6)** were tested on a previous occasion, on a different substrate, and therefore the results of these cannot be compared with the others.

The 2-alkoxyqinazolines show activity against trypsin, chymotrypsin and PPE, but not against the CCK-inactivating peptidase. The Me analogue **(25)** is the most active 2-alkoxyquinazoline against the former three enzymes. It is however, inactive against the latter. This result may be compared with that of the Me analogue **(13)** of the 2-N-alkylamidopteridines, which was also active against the former three serine proteinases, but not as active against the latter. These results clearly confirm that a Me group at the active site of the CCK8-inactivating peptidase is not tolerated as well as it is at the active sites of the other three serine proteinases.

Increasing the chain length by a CH_2 group as in the Et analogue **(27)**, results in a decrease in activity against trypsin, chymotrypsin and PPE, but an increase in activity against the CCK8-inactivating peptidase. The Pr analogue **(28)**, restores activity against chymotrypsin, however, decreases activity in both trypsin and PPE. The benzyl analogue **(26)** restores activity against chymotrypsin and PPE but not against trypsin. Of the 2-alkoxyquinazolines, **(26)** is the second most active compound against chymotrypsin. Again, the results show that aromatic and bulky groups are well accommodated at the active sites of PPE and chymotrypsin but not at that of trypsin. Neither **(28)** nor **(26)** have been tested against the CCK8-inactivating peptidase (new assay) for comparison.

The 2-alkylthioquinazolines show activity against trypsin, chymotrypsin and PPE. The compounds have not been tested on the CCK-inactivating peptidase (new assay) yet. The Et analogue **(30)** is most active against trypsin and PPE. Increasing the chain length to Pr **(32)** causes a significant increase in the activity against chymotrypsin and PPE, to make **(32)** the most potent quinazoline compound against the latter two enzymes. The activity of trypsin remains unaltered. Trypsin does not seem to differentiate between either an Et or Pr analogue. Generally, activity seems to increase with increasing chain length, amongst the 2-alkylthioquinazolines. The Me and Bu analogues, if present, would have shed further light on the subject.

The benzyl analogue **(31)** diminishes activity against all three serine proteinases tested. The results for trypsin and PPE are as expected, however, the result for chymotrypsin is somewhat surprising, as the hydrophobic pocket of the latter should have high binding affinity for the $PhCH_2$ group of **(31)**. Also, the corresponding 2-oxo analogue **(26)** showed considerable activity against the latter enzyme. Unfortunately, the result for the CCK8-inactivating peptidase is not available for comparison.

An electron withdrawing group such as Cl in the 2 position of quinazoline is similar in activity to a MeO group in the 2 position, against all four serine proteinases tested. Compound **(24)** is most active against chymotrypsin with an IC_{50} value of 1.6mM. The Cl

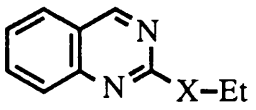
atom seems to be accommodated at the active sites of trypsin, chymotrypsin and PPE, but not at that of the CCK8-inactivating peptidase. The compound was inactive against the latter.

The two examples of quinazolines with electron donating groups are **(6)** and **(33)**. As mentioned earlier, compound **(6)** was tested on a previous occasion, on a different substrate, and therefore the result of **(6)** cannot be compared with the others. Compound **(33)** is inactive against trypsin, chymotrypsin and PPE. The compound has not been tested against the CCK8-inactivating peptidase, yet. The biological results clearly show that an Et-NH group at the two position of quinazoline is not tolerated at either of the enzymic active sites.

On comparing the activities of the 2-alkoxyquinazolines, 2-alkylthioquinazolines and 2-alkylaminoquinazolines, it can be seen that the heteroatom in the 2-position of quinazolines significantly affects the activities of these compounds against the serine proteinases tested.

Activity decreases in the order: 2-alkylthioquinazolines > 2-alkoxyquinazolines > 2-alkylaminoquinazolines.

This trend in activity is exemplified by the Et analogues. Activity decreases in the order: 2-ethylthioquinazoline > 2-ethoxyquinazoline > 2-ethylaminoquinazoline. (See Table 5.7 for IC_{50} values).



X = S > O > NH.

COMPOUND No.	UCL.	X	IC_{50}/mM Trp.	Chym.	PPE.
(30)	1279	S	3.00	8.00	4.00
(27)	1320	O	12.00	12.00	12.00
(33)	1281	NH	>10.00	>10.00	>10.00

The S atom is in some way or another contributing to binding affinity, much more than either an O atom or an N atom. The precise mechanism is not known.

To summarize:

1. The complete lack of activity of the benzoxazin-4-ones as inhibitors of the CCK-inactivating peptidase might serve as an analogy to account for the poor showing of pteridines and quinazolines as inhibitors. However, generally, the pteridines are more active than quinazolines against the CCK8-inactivating peptidase, trypsin, chymotrypsin and PPE.

2. Compound **(12)** is the most active pteridine tested yet, against the CCK8-inactivating peptidase, chymotrypsin and PPE with IC_{50} values of 0.4, 0.17 and 0.17mM. The IC_{50} value for trypsin was 1.5mM. All four serine proteinases show active site specificity for an ^{i}Pr-NH group.

3. The alkylamidopteridines are the next most active compounds. Of these, the CCK-inactivating peptidase is specific for those with Ph and succinimido groups; trypsin for those with Ph and Me groups and chymotrypsin for those with a Ph group. PPE binds Et, Ph and succinimido groups with equal affinity.

4. All four serine proteinases bind the covalent adduct **(16)** more favourably than either the anhydrous compound **(13)** or the majority of the other alkylamidopteridines, probably because the former is in a strained conformation resembling the transition state.

5. The pteridin-7-one-6-carboxylic acid (**23**) is active against trypsin, chymotrypsin and PPE with IC_{50} values of 1.2, 0.6 and 0.5mM. It is not possible to say which particular functional group(s) is/are contributing to activity.

6. Of the quinazolines, the 2-alkylthioquinazolines are more active than either the 2-alkoxyquinazolines or 2-alkylaminoquinazolines against trypsin, chymotrypsin and PPE. Only a few quinazolines were tested on the CCK-inactivating peptidase using the new assay.

Of the 2-alkoxyquinazolines, a Me-O group is tolerated best by all three serine proteinases. The Et-S and Pr-S groups are accommodated better than the analogous Et-O and and Pr-O groups by all three enzymes. The Pr-O group is accommodated best by chymotrypsin. A $PhCH_2$-O group is accommodated better than a $PhCH_2$-S group by chymotrypsin and PPE. Trypsin does not tolerate either $PhCH_2$-O or $PhCH_2$-S.

7. The Cl group in (**24**) has the greatest effect on chymotrypsin with an IC_{50} value of 1.6mM. The Cl group is also well accommodated at the active sites of trypsin and PPE, but not at that of the CCK8-inactivating peptidase.

8. An Et-NH group is not tolerated by any of the serine proteinases.

9. All compounds tested on HLE were inactive at a concentration of 10^{-4}M.

Conclusion:

Even though the pteridine and quinazoline derivatives do not possess specific groups for binding, they are active in the 10^{-3} to 10^{-4} M range, against the CCK-inactivating peptidase, trypsin, chymotrypsin and HLE. From the results, it is clear that the compounds are inhibiting the various serine proteinases, thus proving that nitrogen-containing heterocycles are acting as inhibitors of serine proteinases, as was hypothesised. What is not clear however, is whether the compounds are acting as t.s.analogues.

The activities of the compounds are comparable to those of N-furoylsaccharin which is known to inhibit HLE, PPE and chymotrypsin with IC_{50} values of 0.58, 0.07 and 1.4mM, respectively [37]. (See Chapter 1, Figure 1.8). The mechanism of inhibition in this case, is irreversible acylation of serine OH. If the pteridines and quinazolines are accommodated at the active sites of the serine proteinases (which they must be, in order to show such a degree of activity), they too must be in close proximity to serine for covalent addition to serine OH.

Some of these compounds such as **2-isopropylaminopteridine (12), 2-N-benzamidopteridine (21)** and **2-N-succinimidopteridine (18a)** are sufficiently active to serve as leads for incorporation into suitable peptide analogues (cf. peptidyl boronic acids), which was the original aim in synthesising these 2-N-alkylamidopteridines. A good synthetic route to 2-N-alkylamidopteridines has also been achieved, therefore higher analogues and peptide derivatives could be synthesised if required.

Compounds which have a greater tendency to undergo covalent hydration show greater activity than those which do not. Exceptions are **2-isopropylaminopteridine (12)** and **2-propylthioquinazoline (32)** which are not known to undergo covalent hydration as readily as the 2-N-alkylamidopteridines but show comparable activity. The activity of the former may be attributed to increased binding affinity.

All pteridines and quinazolines are capable of undergoing covalent hydration under suitable conditions, if the compounds are soluble in H_2O. Even those that are least likely to undergo covalent adduct formation, would do so extremely readily, with a strong nucleophile. Therefore, all the compounds that have been tested so far, are potentially capable of covalently reacting with serine OH, when precisely orientated at the active site. Precise orientation should be possible with suitable peptidyl binding groups. Therefore, at this stage, one cannot definitely say whether the compounds are acting according to the proposed mechanism.

The conclusion that can be safely derived at this stage is that, nitrogen-containing heterocycles that undergo covalent hydration are potential inhibitors of serine proteinase, where covalent hydration may determine activity but is not a sufficient condition.

At a later stage, when greater binding affinity has been achieved by exploring other substituents and also incorporating into a suitable substrate analogue, the precise mechanism of inhibition of serine proteinases by heterocycles that under covalent hydration, could be explored in detail.

Some suggestions for further work include:

1. Synthesising 2-isopropoxy-, 2-isopropylthio- and 2-isopropylamino quinazolines to investigate active site specificity of the ^{i}Pr- group attached to O, S and NH atoms at the 2-position of quinazolines.

2. Synthesising an analogous series of 2-alkoxy and 2-alkylthio pteridines for comparison.

3. Preparing 2-N-isopropionamidopteridine to investigate active site specificity for an ^{i}Pr-NHCO group as opposed to ^{i}Pr-NH in **(12)**, and also to complete the series of the 2-N-alkylamidopteridines.

4. Preparing 2-isopropylamino-7(8H)pteridinone-6-carboxylic acid to investigate if the ^{i}Pr in the 2 position causes a significant change in binding affinity.

5. Preparing pteridine-2-carboxylic acid to investigate the active site specificity for a CO_2H group in the 2-position.

6. Synthesising pteridines with substituents in positions other than the 2-position, and

7. Preparing a pteridine-peptide derivative to follow up the 2-N-alkylamidopteridines.

CHAPTER 6

EXPERIMENTAL

General methods:

Melting points were determined on an Electrothermal (open capillary) melting point apparatus and are uncorrected.

TLC and column chromatography were performed on Merck Kieselgel 60 F254 (for tlc) and Merck Kieselgel 60 (for column chromatography), respectively.

HPLC (analytical and preparative) was performed by Mr. S. Corker (or myself), on a Gilson Binary Gradient System combined with Gilson 714 software and a Hewlett Packard Integrator System for data analysis.

UV spectra were recorded on a Perkin Elmer 554 UV-VIS spectrophotometer using UV cells of 1cm path length.

IR spectra were recorded on a Perkin Elmer 983 IR spectrophotometer using NaCl plates or KBr discs.

^{1}H NMR spectra were recorded on Jeol PMX60SI, Varian XL-200 and Varian VXR-400 NMR spectrometers at 60, 200 and 400 MHz, respectively. ^{13}C NMR spectra were recorded on Varian XL-200 and Varian VXR-400 NMR spectrometers at 50 MHz. The (400 MHz) ^{1}H and ^{13}C spectra were recorded by Ms. J Maxwell.

Mass spectra (EI) were recorded by Dr. M. Mruzek, on a VG 7070H double focussing mass spectrometer attached to a Finnigan Incos data system.

Elemental analysis was performed by Mr. A. Stones on a Perkin Elmer 2400 analyser.

2,4,5-Pyrimidinetriamine [82]

(**1**-I/1) (MP57A, MP33A)

Commercially obtained 5-nitro-2,4-pyrimidinediamine (1.81g, 0.012mol) suspended in MeOH (60ml), was hydrogenated at atmospheric pressure over activated Raney Ni catalyst (5 spatulas full) at rt with continuous magnetic stirring according to the literature method [82]. Uptake of H_2 (~800ml, 0.04mol) was complete in ~29 hrs. On addition of the catalyst, a colour change of yellow to colourless was observed. At the end of the reaction, the reaction mixture was a pink/brown colour which gradually darkened on exposure to the atmosphere. Filtration, first under suction and then under gravity to remove further particles of catalyst, followed by evaporation under reduced pressure afforded MP57A (1.12g, 76.9% overall crude yield) as a pink powder; mp 169-172°C (from the reaction solvent MeOH); reported 171-173°C [82].

TLC R_f 0.64 (silica; EtOAc: MeOH:NH_4OH, 5:1:1 UV+KIP sensitive).

^1H NMR (200MHz, DMSO) δ 7.42 (s, 1H, C-6-H), 5.8 (s, (br), 2H, C2-NH_2), 5.18 (s, (br), 2H, C4-NH_2), 3.7 (s, (br), 2H, C5-NH_2).

IR (Nujol mull) 3477, 3390, 3323 (m, anti and symmetrical N-H str, primary NH_2), 1647, 1627, 1590, 1554, 1376, 1305 (m, aromatic C=N str, N-H def, aromatic C-C str).

Mass spectrum: (EI) m/e; 125 $(M)^+$, 98 $(M - HCN)^+$, 109 $(M - NH_2)^+$, 93 $(M - 2NH_2)^+$, 77 $(M - 3NH_2$ or pyrimidine$)^+$.

The same experiment performed on a 0.013mol scale afforded MP33A in 78% overall crude yield.

Note. At a later stage, (**1**-I/1) was synthesised using the following method.

(1-I/1) (MP406A, MP292B, MP680A, MP688A)

To 5-nitro-2,4-pyrimidinediamine (20g, 0.129mol) and Pd/C (2g), MeOH (400ml) was added and the contents stirred mechanically at room temperature (rt) under N_2. $H_2N.NH_2.H_2O$ (37.5ml, 38.7g, 0.77mol) was added dropwise over 30 mins and the reaction mixture warmed at 30-40°C for 20 mins. Exothermic reaction refluxed on it's own. The reaction was left to stand 12 hrs at rt, followed by 3 hrs reflux, ensured completion of reaction. The Pd/C was filtered off; the filtrate concentrated down to ~100 ml and refridgerated overnight. Evaporation to dryness followed by recrystallization from ^{i}PrOH gave white crystals of MP406A (11.17g, 69.4%).

Note. Previously, the same experiment performed on a 0.032mol scale afforded MP292B in 61.5% crude yield. At a later stage, the experiment was repeated on 0.13 and 0.118mol scales to afford MP680A and MP688A in 68.7 and 94.2% yields respectively. In the latter case, the reaction mixture was refluxed for 6 hrs and maintained at rt for 14 hrs before work up.

2-Aminopteridine [79]

(1) (MP61A/UCL1015)

A suspension of sodium glyoxal bisulphite addition compound (2.87g, 0.0092mol) in warm H_2O (19ml) was added to 2,4,5-pyrimidinetriamine MP57A (1.15g, 0.0092mol) in H_2O (7ml) and refluxed according to the literature method [79]. The reaction was complete at the end of 2.5 hrs and the reaction mixture had turned a bright yellow. The yellow solution was filtered whilst warm and the filtrate refridgerated overnight. (Precautions were taken at all times to minimise exposure to UV light in fear of decomposition of the product). The brown sulphonic acid precipitate MP61a (0.97g) was filtered off; washed with EtOH abs (20ml) and dried at 80°C. Treatment with aqueous NaOH (1M) for 35 mins with continuous stirring at 20°C resulted in the formation of a green/mustard residue which on

filtration afforded MP61A (0.38g, 28.7% crude yield overall).

HPLC (MP61A) (see conditions below) confirmed 94% purity. TLC showed two spots corresponding to R_f 0.67 (sλ active) and R_f 0.60 (long wave active) (SiO_2; EtOAc:MeOH:NH_4OH, 5:1:1).

Attempts to recrystallize MP61A were unsuccessful due to lack of a suitable solvent. MP61A was insoluble in H_2O, alcohol, $CHCl_3$ and most other organic solvents. MP61A (0.3g, 0.002mol) was dissolved in warm H_2O (~30ml) and the undissolved material MP61B (0.29g, 98.6%) recovered. HPLC confirmed 96% purity. The long wave active spot of R_f 0.6 was absent by tlc too.

MP61B was obtained as a micro-crystalline greenish/yellow solid: mp 263-267°C, reported 268°C from H_2O [79].

Solubility, sol (H_2O, DMSO), sp. sol (MeOH, EtOAc), insol (Et_2O).

TLC R_f 0.43 (SiO_2; EtOAc:MeOH:NH_4OH, 25:1:1), R_f 0.6 and 0.15 trace (SiO_2; EtOAc:MeOH:NH_4OH, 5:1:1).

HPLC Major peak 96.4% at 3.28min and minor peaks 1.45%, 1.34%, and 0.85% at 0.65min, 1.16min and 4.57min respectively. (100 x 4.6mm Spherisorb ods 2 5µm + guard, 1.0ml/min, UV 254nm. 0.1 aufs, A/B (80/20) where A is H_2O with 0.01% triethyl amine (TEA) and B is MeOH with 0.01% TEA).

UV (H_2O) λmax; 223, 258, 370nm (logε 3.94, 3.39, 3.37).

IR (nujol mull); 3250 (br, aromatic N-H str, amine), 2931 (s, Nujol, C-H str), 1662, 1602 (s, C=N def, aromatic C=C str), 949, 863, 821 (m, aromatic =C-H def, oopb) cm_{-1}.

^1H NMR (200MHz, DMSO) δ; 9.26 (s, 1H, C4-H), 8.92 (d, 1H, $^3J_{H-H}$ = 1.9Hz, H-C7-C6-H), 8.61 (d, 1H, $^3J_{H-H}$ = 1.9Hz, H-C7-C6-H), 7.62 (s, (vbr), 2H, NH_2).

Mass spectrum (EI) m/e; 147 $(M)^+$, 131 $(M - NH_2)^+$, 120 $(M - HCN)^+$, 105 $(M - HCN - NH)^+$, 93 $(M - 2HCN)^+$, 79 (pyrazine), 66 $(M - 3HCN)^+$, 52 (pyrazine - $HCN)^+$, 43 $(NH\text{-}CH_2{=}N)^+$, 26 $(H\text{-}C{=}C\text{-}H)^+$.

Anal. Calcd. for $C_6H_5N_5$: C, 48.96%; H, 3.43%; N, 47.59%

Found: C, 48.32%; H, 3.51%; N, 47.14%.

Three previous attempts to synthesise (**1**) using the method above failed due to excessive decomposition of the product during the aqueous workup.

At later stages, the synthesis of (**1**) was according to the following method.

(**1**) (MP407A, MP294B, MP748A)

A suspension of glyoxal trimeric dihydrate (5.6g, 0.027mol) was added to a warmed suspension of 2,4,5-pyrimidinetriamine MP406A (10g, 0.08mol) in MeOH (150ml). An instant colour change from colourless to yellow was observed. At the end of 50 mins of reflux, the reaction was complete. Cooling to rt over 2 hrs resulted in the formation of yellow crystals which on filtration and drying overnight under vacuum afforded MP407A (10.3g, 88%), mp 282-289°C, reported 268°C from H_2O [79].

HPLC (MP407A) major peak 97.9% at 11.27 min, and minor peaks 1.18% and 0.85% at 3.52 and 1.45 mins respectively (4.00 x 250mm + Lichrosorb RP select B 7µm, 1.0ml/min, UV 254nm. 0.1 aufs, A/B gradient of (95/5), (95/5) and (50/50) at 0.00 mins, 10 mins, and 30 mins where A is H_2O with 0.01% triethyl amine (TEA) and B is MeOH with 0.01% TEA).

^1H NMR was identical with that of previous sample.

Anal. Calcd. for $C_6H_5N_5$: C, 48.96%; H, 3.43%; N, 47.59%

Found: C, 48.48%; H, 3.20%; N, 46.84%.

The same experiment performed on a 0.012mol scale previously, afforded MP294B

(1.22g, 69.3%) yield. The experiment repeated on a 0.09mol scale, at a later stage afforded MP748A (9.4g, 83.6%) yield. Here, the reaction mixture was refluxed for 1.5 hrs and the product crystallised out of solution on standing.

HPLC (MP748A) 100% at 11.27 min (4.00 x 4.00mm + Lichrosorb RP select B 7μm, 1.0ml/min, UV 254nm. 0.1 aufs, A/B (95/5) where A is H_2O with 0.01% triethyl amine (TEA) and B is MeOH with 0.01% TEA).

Pteridine [80]

(2) (MP66B/UCL1016)

A suspension of commercially obtained 4,5-pyrimidinediamine (**2**-I/1) (1g, 0.009 mol) in EtOH abs. (6.3ml) was added to a boiling suspension of glyoxal trimeric dihydrate (0.636g, 0.003 mol) in EtOH abs. (24ml), and refluxed for 3 hrs according to the general method [80]. The reaction mixture brownish orange to start with, turned deep orange at the end of 1 hr and bright yellow at the end of 3 hrs. When the reaction was complete, the solvent was removed under reduced pressure below 30°C to afford yellow fluffy crystals of MP64A (1.4g, 116% crude overall yield due to retention of solvent); mp 110°C.

MP64A (1.39g, 0.011mol) was recrystallized from a mixture of benzene/pet. spirit (40-60°C) in the ratio of 10:1 to give an undissolved brown solid MP65A (0.474g) which was recovered by filtration, and three successive crops of MP65B (0.244g, 20%), MP66A (0.421g, 35%) and MP66B (0.217g, 18%) with mp values of 135-137°C; reported 140°C (benzene) [80]. MP65A, on further recrystallization afforded MP66α (0.184, 15.4%) and MP66β (0.130g, 10.9%) with mp values of 135°C. Combined yield was 98%.

MP66B was afforded as a mustard yellow micro-crystalline solid and was the most pure by tlc.

Solubility, sol (MeOH, Benzene, Cyclohexane), sp. sol (H_2O).

TLC (MP66B) R_f 0.3 (SiO_2; EtOAc:MeOH:NH_4OH, 5:1:1), 0.6 (SiO_2; MeOH).

HPLC (MP66B) 100.00% (7.54 mins). (50mm + 100mm Lichrosorb 5μm silica, 1.0ml/min, UV 254 nm, 0.2 aufs, A/B = 80/20 where A is CH_3CN:CH_2Cl_2 80:20 and B is pet. spirit:CH_2Cl_2, 50:50).

UV (0.015g/l) (H_2O) λmax; 204, 230 (sh), 294, 308nm (logε 3.93, 3.59, 3.87, 3.84).

IR (Nujol mull); 2970, 2700 (s, nujol, C-H str), 1600, 1599, 1550 (m, aromatic C=C str, C=N def), 950, 880, 820 (m, aromatic =C-H def, oopb) cm^{-1}.

^1H NMR (200MHz, $CDCl_3$) δ; 9.77 (s, 1H, H-C2), 9.63 (s, 1H, H-C4), 9.27 (d, 1H, $^3J_{H-H}$ = 1.6Hz, H-C7-C6-H), 9.08 (d, 1H, $^3J_{H-H}$ = 1.6Hz, H-C7-C6-H).

Mass spectrum (E/I) m/e; 132 $(M)^+$, 119 $(M - CH)^+$, 105 $(M - HCN)^+$, 78 (M - 2.HCN or pyrazine)$^+$, 51 (pyrazine - HCN)$^+$, 26 $(H-C{=}C-H)^+$.

Anal. Calcd. for $C_6H_4N_4$: C, 54.54%; H, 3.05%; N, 42.34%.

Found: C, 54.40%; H, 3.05%; N, 42.40

4-Ureido-3,4-dihydroquinazolin-2-one

(3) (MP96C/UCL1026) (also MP158A, MP706C, MP714A)

2-Aminobenzaldehyde (2g, 0.0165mol) and urea (4g, 0.0067mol) were mechanically stirred at rt. for 5 mins and gradually heated to ~180°C according to the general method. In ~7 mins the urea melted to form a clear yellow solution which started to solidify above 140°C with the evolution of NH_3 gas and H_2O to form NH_4OH. The reaction was maintained between 130-150°C for 15 mins After cooling to ~60°C, H_2O (50ml) was

added; the solid broken down into smaller particles via a spatula and the mixture refluxed for 15 mins to dissolve excess urea. The solid was filtered under suction and refluxed in EtOH abs (50ml) for 30 mins On filtration, a creamy white residue of MP68B (2.56g, 72.5%) was obtained. Mp 230-237°C, reported mp for the isomeric form (**3**-I/1) is 250°C (reprecipitation from DMSO with H_2O) [115].

MP68B (0.5g, 0.0034mol) was recrystallized from H_2O (20ml) to give undissolved MP96A. MP96A on further recrystallization from H_2O (20ml) gave undissolved MP96B (0.2g). The combined filtrates of MP96 A and B, on etching and refridgeration yielded pure white crystals which were filtered off and dried at 100°C/reduced pressure (760mm Hg) for 3 hrs to give MP96C (0.133g, 26.6%), mp 228-230°C from H_2O, reported 250°C (reprecipitation from DMSO with H_2O).

At later stages, the same experiment repeated on 0.017, 0.165 and 0.264mol scales gave MP158A, MP706C and MP714A in 17.6%, 60% and 68.8% yields respectively.

Solubility, sp. sol (DMSO, MeCN, EtOH abs., H_2O), insol (EtOAc, Et_2O, benzene).

TLC R_f 0.3 (SiO_2; EtOAc:MeOH:NH_4OH, 5:1:1), 0.00 (SiO_2; $CHCl_3$:MeOH, 9:1).

HPLC 100% at 4.80min (250 x 4mm Lichrosorb RP select B 5µm, 1.00ml/min, UV 254nm 0.05 aufs, A/B (60/40) where A is H_2O with 0.01% TEA and B is MeOH with 0.01% TEA).

UV (MeOH) λmax; 226, 236, 274, 338nm (logε 3.96, 4.03, 3.27, 2.61).

IR (KBr); 3425, 3268, 3134, 3057 (m, N-H str, primary and secondary amide, aromatic C-H str), 1649, 1551 (m, aromatic C-C str, C=N str, amide I and II N-H def and C=O str), 1467, 1413 (s, amide I and II C=O str), 939, 924, 856 (w, aromatic =C-H oopb) cm^{-1}.

^1H NMR (400MHz, DMSO) δ; 9.46 (s, 1H, C4-H), 7.28 (s, 1H, N1-H), 7.18-6.81 (m, 6H, C5-H, C6-H, C7-H, C8-H, N3-H, N1-H), 5.98, 5.96 (d, 1H, $^3J_{H-H}$ = 6Hz, C2-NH-

CO-NH_2), 5.5 (s, (vbr), C2-NH-CO-N$\underline{H}_2$).

^{13}C NMR (100MHz, DMSO) ppm; 157.10, 152.85 ($\underline{C}$2, C4-NH-$\underline{C}$O-NH_2), 137.02, 128.64, 126.65, 121.24, 119.89, 113.85 (aromatic region), 58.3 ($\underline{C}$4).

Mass spectrum (EI) m/e; 206 $(M)^+$, 146 $(M - NHCONH_2)^+$, 118 $(M - HCN)^+$, 91 $(M - HCN - CO)^+$, 77 $(M - HCN - CONH)^+$.

Anal. Calcd. for $C_9H_{10}N_4O_2$: C, 52.39%; H, 4.89%; N, 27.16%

Found: C, 52.35%; H, 4.96%; N, 26.97%.

Quinazolin-2-one hydrochloride [115]

(4) (MP164A/UCL1032) (also MP74G, MP716E)

MP96C (1g, 0.0048mol) dissolved in dilute HCl (pH 1) (50ml) was warmed at 40°C for 2 hrs. A colour change from colourless to yellow was observed. The solvent was removed under reduced pressure at 30°C. A hygroscopic yellow/orange residue was obtained. The crude product was recrystallized from abs. EtOH (~50ml) and Et_2O. Refridgeration overnight yielded the first crop of mustard yellow micro-crystals which on filtration followed by washing with pet.spirit (40-60°C) afforded MP164A (0.12g, 13.6%) overall yield, mp 245-250°C (EtOH); reported 280-282°C [115]. Concentration of the filtrate under reduced pressure caused decomposition.

Solubility, sol (H_2O, EtOH abs, MeOH), sp.sol ($CHCl_3$), insol (Et_2O).

TLC R_f 0.4 (SiO_2; EtOAc:MeOH:NH_4OH, 5:1:1), 0.31 (SiO_2; $CHCl_3$:MeOH, 9:1).

HPLC Major peak 95.05% at 19.7min and minor peaks 0.34%, 1.56% and 1.56% at 8.53, 20.87 and 23.12mins (250 x 4mm Lichrosorb RP select B 5µm, 1.00ml/min, UV 254nm 0.05 aufs, A/B gradient run: 10-90% B in 35 min, B = 90% for 15 min, where A is H_2O

with 0.01% TEA and 0.5% $MeCO_2H$ and B is MeOH with 0.01% TEA and 0.5% $MeCO_2H$.

UV (MeOH) λmax; 201, 222, 270, 338nm (logε 3.84, 4.011, 3.27, 2.95).

IR (KBr); 3412, 2938 (m, N-H str, primary and secondary amide, aromatic C-H str), 1730, 1628, 1598 (m, aromatic C-C str, C=N str, amide I and II N-H def and C=O str), 1467 (s, amide I and II C=O str), 977, 937, 870 (w, aromatic =C-H oopb) cm^{-1}.

^1H NMR (400MHz, DMSO) δ; 9.5 (s, (vbr), C4-H), 8.022-7.34 (m, 4H, C5-H, C6-H, C7-H, and C8-H), 5.9-5.2 (s, (v.vbr), N1-H and HDO).

^{13}C NMR (100MHz, DMSO) ppm; 167.18 (br. C4), 151.36 (C2), 138.29, 130.44, 123.53, 115.43 (aromatic region).

Mass spectrum (EI) m/e; 146 $(M)^+$, 118 $(M - HCN)^+$, 91 $(M - HCN - CO)^+$, 77 $(M - HCN - CONH)^+$.

Anal. Calcd. for $C_8H_6N_2O.HCl$: C, 52.62%; H, 3.86%; N, 15.34%; Cl, 19.42%

Found: C, 52.74%; H, 4.12%; N, 15.21%; Cl, 19.04%.

The same experiment repeated on a 0.003mol scale previously afforded MP74G in 42.2% crude yield overall. When repeated on a 0.04mol scale at a later stage, MP716E was afforded in 70.8% crude yield overall.

Multi-step synthesis of (5).

4,6,Dihydroxy-2-methylpyrimidine [87]

(5-I/1) (MP84A, MP80A)

5a was synthesized in 2 batches in order to have sufficient starting material to enable a 6 step synthetic scheme to be carried out.

Na metal (26g, 1.13mol) was reacted with abs. EtOH (500ml) at rt under N_2. At the end of 60 mins, diethyl malonate (60g, 0.37mol) and acetamidine hydrochloride (35.5g, 0.37mol) were added and the mixture refluxed according to the general method [87]. The reaction was complete at the end of 3 hrs with the formation of a creamy white precipitate. Cooling to rt followed by vacuum filtration afforded a white solid MP84A which was left to dry in air for ~12 hrs To a solution of MP84A in H_2O (222ml), HCl acid (10M, 75ml) was added in 1ml portions, cooling over ice. The solution was left to stand for 15 mins at rt, filtered and the precipitate collected. MP84A (38.32g, 81.5% crude yield overall) was collected as a white solid: mp 325-350°C (dec), mp not reported [87].

^{1}H NMR (200MHz, DMSO) δ; 4.94 (s, 1H, C-5-H), 4.0 (s, (vbr), OH), 2.17 (s, 3H, CH_3).

IR (Nujol mull); 3580, 3082 (w/m and br, O-H str), 1685, 1634, 1571, 1457 (s, aromatic C=N str, aromatic C-C str), 1377, 1350, 1328, 1298, 1273, 1214 (s/m, C-O str or O-H def. coupled), 734, 684, 644, 622 (m, aromatic oop C-H def) cm^{-1}.

Mass spectrum (E/I) m/e; 126 $(M)^+$, 98 $(M - H\text{-}C{=}N\text{-}H)^+$, 85 $(M - H\text{-}C{=}N\text{-}H - C\text{-}H)^+$, 69 $(M - H\text{-}C{=}N\text{-}H - C\text{-}OH)^+$, 57 $(M - H\text{-}C{=}N\text{-}H - 2.C\text{-}OH)^+$.

The previous batch MP80A was obtained in 59.5% crude overall yield.

At a later stage, it was necessary to synthesize 2 further batches of 5a, as the first 2 were all

used up in the subsequent nitration reaction which posed problems. On repeating the experiment on the same scale as above, MP114A and MP118Awere afforded in 22% and 35.5% yields respectively. These were identified by ^1H NMR and mass spectroscopy.

4,6-Dihydroxy-2-methyl-5-nitropyrimidine [87]

(5-I/2) (MP130A, MP104, MP126, MP134, MP136, MP142A).

To a mixture of HNO_3 acid (d 1.42, 11ml) and 98% conc. H_2SO_4 acid (11ml) (mechanically stirred at 0°C for 1 hr), 4,6-dihydroxy-2-methylpyrimidine MP118A (5g, 0.079mol) was added portionwise over 1 hr. The temperature was maintained below 15°C throughout the reaction by the use of an acetone/solid CO_2 bath. The colour changes observed during the course of the reaction were yellow to orange to red. At the end of 2.5 hrs, the reaction mixture was a deep orange with a yellow precipitate. The reaction was continuously stirred for a further 2 hrs until there was no further rise in temperature. The mixture was added portionwise to crushed ice (200ml) at -5° to 0°C. Stirring with a glass rod hurried the formation of a thick creamy yellow precipitate in an orange solution. Filtration afforded MP130A (3.17g, 63% crude yield overall) as yellow crystals, mp 280°-char, dec.

^1H NMR (200MHz, DMSO) δ; 7.2 (s, (br), 2H, O<u>H</u>), 2.31 (s, 3H, C<u>H</u>$_3$).

IR (Nujol mull); 3580, 3402, 3083 (w/m and br, O-H str), 1692, 1642, (s, aromatic C=N str, aromatic C-C str), 1572(s, aromatic C=N str, or aromatic anti N-O str), 1454, 1377, 1350, 1330, 1299,1274, 1217 (m, 1377, 1350, 1328, 1298, 1273, 1214 (s/m, C-O str or O-H def. coupled, aromatic sym. N-O str), 815, 723 (m, oopb C-H def) cm^{-1}.

Mass spectrum (E/I) m/e; 171 $(M)^+$, 141 $(M - NO_2)^+$, 111 $(M - NO_2 - H\text{-}CO\text{-}H)^+$, 95 $(M - NO_2 - H_2CO2)^+$, 70 $(M - NO_2 - H_2CO_2 - C_2H_2)^+$.

Note. When the experiment was initially performed on the same scale as above, MP104

(1.6g) was afforded in 23.63% yield. Subsequently, repeating the experiment 4 times on a 0.04mol scale afforded MP126 (4.57g, 67.4%), MP134 (2.9g, 57.5%) and MP136 (3.18g, 46.9%) crude yields overall. Repeating the experiment on a 0.008mol scale afforded MP142A (9.20g, 67.7% crude yield overall) . The products were identified by ^1H NMR and mass spectroscopy. The fractions were combined to give MP142B (24.5g).

4,6-Dichloro-2-methyl-5-nitropyrimidine [88]

(5-I/3) (MP146A, MP150A, MP154A)

$POCl_3$ (d 1.675ml, 0.055mol) was added to 4,6-dihydroxy-2-methylpyrimidine MP142B (1g, 0.0059mol) as in the literature method [88], and mechanically stirred for 5 mins. The dropwise addition of fuming aniline (d 0.93, 1.8ml, 0.012mol) resulted in a colour change from colourless to yellow, along with the evolution of HCl fumes. Reflux for 2 hrs with continuous stirring resulted in the reaction mixture turning a green/black colour. The cooled mixture when poured onto crushed ice (~300ml) in a beaker at 0°C, cooled by an acetone/solid CO_2 bath, formed a brown oil in a brown aqueous solution. The ice mixture was extracted with Et_2O (4 x 150ml) and washed with 10% $NaHCO_3$ solution (500ml). The ether extract was dried ($MgSO_4$) overnight. Filtration followed by evaporation of solvent under reduced pressure at rt afforded a brown oil which crystallized on standing. MP146A (1.22g, 97.2% crude yield overall) was obtained as brown/yellow needles: mp 48-52°C; reported 54-55°C [88].

^1H NMR (200MHz, DMSO), δ; 2.75 (s, 3H, $C\underline{H}_3$).

Mass spectrum (E/I) m/e; 208 $(M)^+$, 162 $(M - NO_2)^+$, 108 $(M - N{=}CN{-}CH_3)^+$, 91 $(M - NO_2 - 2Cl)^+$.

The experiment repeated on 0.058mol and 0.072mol scales afforded MP150A (9.1g, 74.9% crude overall yield) and MP154A (19.6g, crude). The fractions were combined to afford MP154B(29.92g).

4-Amino-6-chloro-2-methyl-5-nitropyrimidine [89]

(5-I/4) (MP169C, MP173C, MP177C)

A solution of 4,6-dichloro-2-methyl-5-nitropyrimidine MP154B (4g, 0.0192mol) was cooled to) -5°C, using an acetone/solid CO_2 bath. A solution of liquid NH_3 (1ml, 0.039mol) in MeOH (3ml) was added dropwise over 20 mins via a pre-cooled dropping funnel. (The methanolic NH_3 was obtained by condensing NH_3 gas into a pre-cooled measuring cylinder filled with MeOH). The reaction mixture was stirred for 2 hrs, maintaining the temperature between -5°C to 5°C. A yellow solid had formed in a brown solution. On filtration an off-white solid 4,6,-pyrimidinediamine MP169A (0.78g) mp >250°C, dec, was isolated. The filtrate was left aside. MP169A was extracted with Et_2O (3 x 50ml) and EtOAc (3 x 50ml). The combined extracts and the filtrate were first filtered under gravity (to remove any traces of MP169A) and evaporated off under reduced pressure to afford MP169B as a brown/white solid (crude). The solid was extracted with hot pet. spirit (60-80°C) (4 x 100ml) and evaporated off to afford the starting material (1.09g). The remaining residue MP169C (1.90g, 52.3% crude yield overall) was obtained as a brown solid, mp 150-155°C, reported 155-156°C [89].

^1H NMR (200MHz, $CDCl_3$) δ; 6.8 (s, (v.br. hump), 2H, $N\underline{H}_2$), 2.54 (s, 3H, $C\underline{H}_3$).

Mass spectrum (E/I) m/e; 188 $(M)^+$, 158 $(M - NH_2)^+$, 142 $(M - NO_2)^+$, 137$(M - Cl - NH_2)^+$, 130 $(M - NH_2 - HCN)^+$, 103 $(M - NH_2.HCN - C_2H_2)^+$, 89 $(M - NH_2.HCN - C_2H_2 - N)^+$.

Note. The experiment repeated on 0.048 and 0.067mol scales afforded MP173C (3.99g, 43.9% crude yield overall) and MP177C (5.75g, 46.5% crude yield overall) respectively, as yellow solids. The fractions were combined to afford MP177D (11.63g).

6-Mercapto-2-methyl-4,5-pyrimidinediamine [87]

(5-I/5) (MP190B, MP187A)

$NaSH.H_2O$ (19.7g, 0.266mol) dissolved in H_2O (50ml) was added to 4-amino-6-chloro-2-methyl-5-nitropyrimidine MP177D (10g, 0.053mol) in NaOH solution (2.3M, 150ml). The reaction was maintained at 95°C for 4 hrs by which time the reaction was complete. The addition of glacial $MeCO_2H$ acid (3-4 drops) via a pasteur pipette resulted in a solution of pH 5. The solution on refridgeration overnight afforded a yellow precipitate. Filtration afforded MP190A (13.15g, 157% crude yield due to retention of H_2O), which on drying overnight in a vacuum oven afforded MP190B (11.1g).

Note. On a previous attempt, the same experiment carried out on a 0.0027mol scale afforded MP187A (0.93g, crude).

Solubility: sp. sol. in H_2O, MeOH, acetone, and insol in EtOH, Et_2O, pet. spirit (60-80°C), $CHCl_3$, CH_2Cl_2, dioxan, THF and MeCN.

Recrystallization of the crude product MP187A (1g) from water (300ml) afforded undissolved material MP193A (0.264g) and four successive crops of MP193B (0.367g), MP193C (0.027g), MP193D (0.040g) and MP193E (0.212g). Fractions were combined to afford MP193F (0.64g, 64.6% recovery).

Recrystallization of the crude product MP190B (11g) from H_2O (2.5l) afforded undissolved material MP197A (7g wet) and 3 crops MP197B (2.39g), MP197C (0.616g) and MP197D (0.606g). Further recrystallization of MP197A afforded MP197E (0.9g) and MP197F (0.5g). The fractions that had identical melting points and R_f values were combined to afford MP197G (5.01g, 45.5% overall yield) as pale yellow crystals: mp>280°C- dec; reported 246-248°C [87].

^1H NMR (60MHz, DMSO) δ; 6.4 (s, (br), 2H, $N\underline{H}_2$), 5.25 (s, (vbr), 2H, $N\underline{H}_2$), 3.4 (s, (vbr), 1H, S<u>H</u>), 2.18 (s, 3H, $C\underline{H}_3$).

IR (Nujol mull); 3312, 3151 (m, anti and sym. N-H str, primary amine), 2333 (m, S-H str), 1641, 1583, 1517, 1464, 1431 (m, aromatic C=N and C-C str, N-H def), 1382, 1373, 1332 (m, aromatic N-H str) cm^{-1}.

Mass spectrum (E/I) m/e; 156 $(M)^+$, 128 $(M - C_2H_4)^+$, 115 $(M - HN{=}CN)^+$, 98 $(M - HS{-}C{=}N{-}H)^+$, 73 $(M - HS{-}C{=}N - C{-}CH_3)^+$.

2-Methyl-4,5-pyrimidinediamine [87]

(5-I/6) (MP207A, MP202B)

NH_4OH solution (d 1.8, 5ml) was added to 6-mercapto-2-methyl-4,5-pyrimidinediamine MP197G (4.9g, 0.0314mol) in H_2O (125ml) and heated. Activated Raney Ni catalyst (~5 spatulas full) was added to the hot solution and the mixture refluxed vigorously for 1 hr according to the literature method [87]. At the end of a further 1 hrs reflux, a yellow precipitate had formed. On filtration, MP207A (3.89g, 100% crude yield) was afforded as a yellow solid, mp 238-242°C, reported mp 246-248°C (from alcohol) [87].

Note. On a previous attempt, the same experiment performed on a 0.0032mol scale afforded MP202A (0.39g, 98% crude yield overall). Recrystallisation from abs. EtOH afforded MP202B (0.36g, 92% recovery) and MP202C (0.022g) on evaporation of the mother licqour.

MP207A (3.8g) was recrystallised from abs. EtOH to yield 11 crops of 0.06, 0.50, 0.509, 0.27, 0.189, 0.260, 0.299, 0.125, 0.052, 0.247, 0.563, 0.062, 0.509, 0.098 and 0.010g (on evaporation of the mother licqour) which were combined to give MP209P (3.3g, 85.5% overall yield).

HPLC (MP209P) 99.7% at 10.10 min. (4.00 x 250mm Lichrosorb RP select B 5μm, 1.00ml/min, UV 254nm 0.05 aufs., gradient 10-25% B in 25 min where A is H_2O and B is MeOH, mobile phase contained o-phosphoric acid, also 5ml of hexane sulphonic acid

was added to every 100ml of both solvents).

^{1}H NMR (60MHz, DMSO) δ; 7.48 (s, 1H, C6-H), 6.1 (s, (vbr. hump), 2H, C4-NH_2), 4.4 (s, (vbr. hump), 2H, C5-NH_2), 2.18 (s, 3H, CH_3).

Mass spectrum (EI) m/e; 123 $(M)^+$, 107 $(M - NH_2)^+$, 91 $(M - 2NH_2)^+$, 77 (pyrimidine)$^+$.

2-methylpteridine [87]

(5) (MP223K/UCL1041) (MP213C, MP217E)

2-Methyl-4,5-pyrimidinediamine MP209P (1.4g, 0.013mol) in MeOH (50ml) was added to glyoxal trimeric dihydrate (2.75g, 0.013mol) in MeOH (20ml) as in the general method[87]. Further MeOH (100ml) was added to dissolve more of the solids and the mixture refluxed for 1 hr, by which time the reaction was complete. The solvent was evaporated off under reduced pressure and the brown oil MP221A (4.68g) was stored under pet. spirit (60-80°C) overnight to solidify. Some of the solid had dissolved in the solvent as indicated by tlc. Therefore, the solid was extracted with pet. spirit (60-80°C, 2l) and the solvent evaporated off to give MP221B (0.42g, 22% crude yield) as yellow leaflets, mp 135-138°C. The residual brown solid MP221C on sublimation at 110°C/0.1mmHg for 3 hrs afforded MP221D (0.040g), mp135-136°C and a black residue, mp > 280°C.

Note. The same experiment carried out previously on a 0.0008mol scale afforded MP213C (0.088g, 74.7% crude overall yield). Sublimation at 110°C/0.1mmHg for 3 hrs afforded MP213D (0.011g, 12.5% recovery). When repeated on a 0.0096mol scale, MP217E (0.570g) was afforded in 40.34% crude overall yield.

0.054g of MP221B sublimed whilst being dried in a drying piston at 60°C/0.05mmHg. Therefore, MP221B (0.36g) was sublimed at 110°C/0.1mmHg for 3.5 hrs to afford yellow

leaflets of MP223H (0.233g, 25.5%), mp 141-142°C. The remaining residue (0.011g), mp >280°C was discarded.

MP223H (0.203g) on recrystalization from pet. spirit (60-80°C) (~75ml) afforded MP223J (0.088g) as a fine yellow powder. On drying in a drying piston at 60°C/0.1mmHg for 3 hrs, MP223J had sublimed as before to afford yellow needles of MP223K (0.051g), mp 138-140°C; reported 141-142°C [87].

Solubility, sol (MeOH, EtOH abs, H_2O, Et_2O, pet. spirit 60-80°C).

TLC R_f 0.4 (SiO_2; EtOAc:MeOH:NH_4OH, 5:1:1), 0.4 (SiO_2; $CHCl_3$:MeOH, 9:1).

HPLC (was not carried out as the product tended to undergo covalent addition reactions with the solvents used).

UV (MeOH) λmax; 208, 268, 303, 313 (loge 3.82, sh, 3.62, s, 3.85, sh, 3.82, s).

IR (KBr disc); 1644, 1566, 1549, 1442 (m/s, C=N def, aromatic C=C str), 1197, 1151, 1038 (s, C$\underline{H}_3$ def, C$\underline{H}_3$ str), 949, 824, 802 (s, aromatic =C-H str, oopb) cm^{-1}.

^1H NMR (200MHz, $CDCl_3$) δ; 9.67 (s, 1H, C4-$\underline{H}$), 9.21 (d, 1H, $^3J_{H-H}$ = 1.6Hz, $\underline{H}$-C7-C6-H), 9.00 (d, 1H, $^3J_{H-H}$ = 1.7Hz, H-C7-C6-$\underline{H}$), 3.04 (s, 3H, C$\underline{H}_3$).

Mass spec (EI) m/e; 146 $(M)^+$, 131 $(M - CH_3)^+$, 119 (M - HCN or M - $C_2H_3)^+$, 104 (M - CH_3 - HCN)$^+$, 92 (M - 2.HCN or M - C_2H_3 - HCN)$^+$, 78 (M - C_2H_3 - HCN or pyrazine)$^+$.

Anal. Calcd. for $C_7H_6N_4$: C, 57.53%; H, 4.13%; N, 38.34%

Found: C, 56.25%; H, 3.91%; N, 36.24%.

2-Aminoquinazoline [116]

(6) (MP231D/UCL1042)

2-Aminobenzaldehyde (1g, 0.0083mol) was thoroughly ground with guanidine nitrate (1.5g, 0.012mol) and Na_2CO_3 (1g, 0.009mol); suspended in dry decalin (20ml) and stirred under reflux according to the general method, for 1.5 hrs. The reaction mixture was filtered whilst hot to afford a residual solid which was further refluxed in decalin and filtered. Evaporation of the first filtrate afforded a yellow solid MP229α (0.335g), mp 170-190°C and evaporation of the second filtrate, MP229β (0.129g) mp 180-190°C. The residual solid MP229A was refluxed in decalin (~300ml) and filtered whilst hot to give MP229B as a mustard solid (0.201g, 16.7% crude yield overall), mp 300-310°C and a filtrate which was evaporated off to afford MP229γ (0.040g).

Sublimation of MP229α (0.1g, 0.00068mol) at 150°C/0.5mm Hg afforded MP229C (0.045g, 45%) mp 195-198°C. Sublimation of the combined fractions MP229 β, γ and B (0.372g, 0.0025mol) under the above conditions afforded MP229F (0.145g, 38.9%). The combined fractions MP229C and F, treated with 10% HCl, followed by reprecipitation with NH_4OH solution and filtration afforded MP231D (0.159g, 13.2% overall yield), mp 203-207°C; reported 203-204°C [116].

Solubility, sol (MeOH, EtOH abs, H_2O), insol (Et_2O, pet. spirit 60-80°C).

TLC R_f 0.5 (SiO_2; pet. spirit 80-100°C:Et_2O, 50:50), 0.43 (SiO_2; $CHCl_3$).

HPLC Major peak 92.0% at 2.12min, and minor peaks 0.9% and 7.0% at 7.3 and 8.1 mins (Lichrosorb RP select B 7μm, 1.00ml/min, UV 254nm, A/B (30/70) where A is H_20 with 0.1% TFA and B is MeOH with 0.1% TFA).

UV (MeOH) λmax; 230, 257, 349nm (logε 4.37, s, 3.66, sh, 3.35, br).

IR (Nujol mull); 3308, 3137 (s, br, N-H str, amine), 1653, 1617, 1589, 1571, 1479 (s, C=N def, aromatic C=C str), 971, 919, 856 (m, =C-H def, oopb) cm^{-1}.

^{1}H NMR (200MHz, $CDCl_3$) δ; 9.05 (s, 1H, C4-H), 7.77-7.56 (m, 3H, C5-H, C8-H and C6-H or C7-H), 7.35-7.27 (m, 1H, C6-H or C7-H), 5.4-5.3 (s (vbr), 2H, C2-NH_2).

Mass spectrum (EI) m/e; 146 $(M)^+$, 129 $(M - NH_2)^+$, 118 $(M - HCN)^+$, 103 $(M - HCN - NH_2)^+$, 91 $(M - HCN - NH_2 - C)^+$, 76 $(C_6H_5)^+$.

Anal. Calcd. for $C_8H_7N_3$: C, 66.18%; H, 4.86%; N, 28.95%

Found: C, 66.05%; H, 4.81%; N, 28.91%.

2-Aminopurine [119]

(7) (MP225A)

2-Aminopurine was obtained commercially from Aldrich Chemical Co. Tlc indicated a single spot in 2 solvent systems. (see below). ^{1}H NMR confirmed the product was pure. Therefore, the product was dried at 60°C/0.1mmHg, for 6 hrs and submitted for analysis. Mp 267-270°C, reported 277-278°C [119].

Solubility, sol (H_20), sp. sol (MeOH, EtOH abs.), insol (Et_2O, pet. spirit 60-80°C).

TLC R_f 0.3 (SiO_2; EtOAc:MeOH:NH_4OH, 5:1:1), 0.28 (SiO_2; $CHCl_3$:MeOH, 9:1).

HPLC Major peak 96.1% at 2.09min, and minor peaks 1.5% and 2.3% at 2.6 and 8.2 mins (Lichrosorb RP select B 7μm, 1.00ml/min, UV 254nm, A/B (30/70) where A is H_20 with 0.1% TFA and B is MeOH with 0.1% TFA.

UV (MeOH) λmax; 215, 235, 298nm (logε 4.13, s, 3.70, shoulder, 3.76, br).

IR (KBr disc); 1550, 1400 (m, aromatic C=N str), 1299, 1279, 1245, 1152 (m, aromatic C-C def, aromatic N-H def, primary and secondary NH_2), 920, 892, 845 (w, aromatic =C-H def, oopb), 780, 735, 705, 690, 680, 663, 650 (s, aromatic =C-H def, oopb) cm^{-1}.

^{1}H NMR (200MHz, DMSO) δ; 8.59 (s, 1H, C6-H), 8.09 (s, 1H, C4-H), 6.38 (s, (br), 2H, C2-NH_2), 4-3 (s (vbr), 1H, N-H).

Mass spectrum (EI) m/e; 135 $(M)^+$, 119 $(M - NH_2)^+$, 108 $(M - 2HCN)^+$, 65 $(pyrimidine)^+$.

Anal. Calcd for $C_5H_5N_5$: C, 44.44%; H, 3.72%; N, 51.83%

Found: C, 42.02%; H, 4.03%; N, 49.38%.

Multi-step synthesis of compounds (8), (9), (10), (11) and (12).

2,4,-Dichloro-5-nitropyrimidine [86]

(**8**-I/1) (MP248A)

$POCl_3$ (142ml,) was added to commercially obtained 5-nitrouracil (30g, 0.191mol) and stirred for 15 mins at rt. Diethylaniline (50ml) was added dropwise over 20 mins and the mixture refluxed for 3 hrs according to the literature method [86]. Excess $POCl_3$ was evaporated off under reduced pressure at 45-50°C. The oily residue, was poured in portions onto crushed ice (~600ml) at 0°C (maintained by using an acetone/solid CO_2 bath), which resulted in the formation of a green/black tar. On filtration, a grey solid MP248A (35.4g, 94.5% crude yield due to retention of water) was obtained. The solid was dried overnight in a vacuum oven to afforded MP248B (34g).

It was decided not to purify all of the solid at this stage, since the product was pure by tlc (SiO_2; MeOH:$CHCl_3$, 9:1). Recrystallisation of MP248B (1g) from pet. spirit 60-80°C afforded MP248C (0.92g) as shiny light brown crystals, mp 300°C, dec, mp not reported in the literature [86].

^1H NMR (60MHz, DMSO) δ; 8.90 (s, 1H, C6-H).

Mass spectrum (EI) m/e; 193 $(M)^+$, 158 $(M - Cl)^+$, 147 $(M - NO_2)^+$, 123 $(M - 2Cl)^+$, 77 $(pyrimidine)^+$.

4-Amino-2-chloro-5-nitropyrimidine [86]

(**8**-I/2) (MP248I)

2,4,Dichloro-5-nitropyrimidine MP248B (34g, 0.189mol) was added portionwise over 10

mins to a vigorously stirred mixture of NH_4OH (d 0.88) (200ml) and crushed ice (~600ml) according to the literature method [86]. The grey suspension on stirring at 0°C for 30 mins, turned yellow/brown. The precipitate was filtered and washed with ice H_2O (200ml) followed by cold EtOH (200ml). The brown residue was successively boiled in pet.spirit (60-80°C) (200ml) with continuous stirring and filtered. The residue obtained on filtration was dried under vacuum at 120°C/760mmHg for 3 hrs to obtain MP248E (31g, 93.9% crude overall yield).

Attempted recrystallization of MP248E (10g) from toluene was unsuccesful. MP24E (0.5g) was recrystallised from EtOH abs to obtain MP248F (0.20g) as shiny pale yellow crystals, mp 205-210°C, reported 215-217°C [86].

MP248E (~30g) was extracted with EtOH abs. over 48 hrs (using a soxhlet extractor) to obtain a combined yield of MP248I (23.3g, 70.2% overall yield).

(MP248F): ^1H NMR (60MHz, DMSO) δ; 8.90 (s, 1H, C6-H), 8.6-8.2 (s, (vbr), 2H, C4-$N\underline{H}_2$).

Mass spectrum (EI) m/e; 174 $(M)^+$, 139 $(M - Cl)^+$, 128 $(M - NO_2)^+$, 112 (M - Cl - HCN or M - NO_2 -$NH_2)^+$, 77 (pyrimidine)$^+$.

2-Phenethylamino-5-nitro-4-pyrimidinamine

(**8**-I/3) (MP226A)

A mixture of 4-amino-5-nitro-2-chloropyrimidine 248I (5.03g, 0.028mol) and phenethylamine (10ml, 0.08mol) in MeOH (10ml) was refluxed in MeOH (20ml) for 2 hrsusing an oil bath. An brown/orange solution was obtained. The reaction was first cooled to rt and then to 0°C by immersing the reaction vessel in an acetone/solid CO_2 bath. Etching with a glass rod resulted in the formation of crystals. On filtration, yellow leaflets of MP226A (2.76g, 38.5%) were obtained; mp 105-110°C (from MeOH).

^{1}H NMR (400MHz, DMSO) δ; 8.82 (s, 1H, C6-H), 8.25-8.22 (s, (vbr), 2H, NH_2), 8.06 (s, (vbr), 1H, C2-NH), 7.95-7.17 (m, 5H, C_6H_5), 3.56-3.46 (m, 2H, NH-CH_2-CH_2), 2.85-2.82 (t, 2H, $^3J_{H-H}$ = 6.4Hz, C2-NH-CH_2-CH_2-C_6H_5).

Mass spectrum (EI) m/e; 259 $(M)^+$, 168 $(M - CH_2-C_6H_5)^+$, 155 $(M - CH_2-CH_2-C_6H_5)^+$, 213 $(M - NO_2)^+$, 242 $(M - NH_2)^+$, 105 $(CH_2-CH_2-C_6H_5)^+$, 91 $(CH_2-C_6H_5)^+$, 77 $(C_6H_5)^+$.

2-Phenethylamino-4,5-pyrimidinediamine

(8-I/4) (MP268A)

$H_2N.NH_2.H_2O$ (1.89ml, 0.039mol) and active 10% Pd/C (0.1g) was added to 2-phenethylamino-5-nitro-pyrimidinamine MP266A (3.5g, 0.013mol) in EtOH (50ml) under N_2, refluxed for 4.5 hrs and left at rt for 12 hrs. The reaction mixture was filtered off first under suction and then under gravity, the filtrate evaporated to dryness under reduced pressure and azeotroped with ^{i}PrOH (3 x 100ml). The orange solid MP268A (1.57g, 89.2% crude overall yield) obtained was dried on a vacuum line (rt/1mmHg), mp 229-276°C, dec.

^{1}H NMR (400MHz, DMSO) δ; 7.31-7.14 (m, 5H, C_6H_5) 6.020 (s, (vbr), 2H, C4-NH_2), 5.59 (t, $^3J_{H-H}$ = 6.4Hz, C2-NH-CH_2-CH_2), 3.77-3.72 (s, (vbr), 2H, C5-NH_2, HDO), 3.33 (q, 2H, $^3J_{H-H}$ = 6.4Hz, C2-NH-CH2-CH2-C6H5), 2.75 (t, 2H, $^3J_{H-H}$ = 6.4Hz, C2-NH-CH_2-CH_2-C_6H_5).

Mass spectrum (EI) m/e; 229 $(M)^+$, 213 $(M - NH_2)^+$, 197 $(M - 2.NH_2)^+$, 150 $(M - C_6H_5)^+$, 138 $(M - CH_2-C_6H_5)^+$, 124 $(M - CH_2-CH_2-C_6H_5)^+$, 109 $(M - NH-CH_2-CH_2-C_6H_5)^+$, 105 $(CH_2-CH_2-C_6H_5)^+$, 91 $(CH_2-C_6H_5)^+$, 77 $(C_6H_5)^+$.

2-Phenethylaminopteridine

(8) (MP272B/UCL 1059)

2-Phenethylamino-4,5-pyrimidinediamine MP268A (0.31g, 0.0013mol) was dissolved in MeOH (10ml). To this, glyoxal trimeric dihydrate (0.1g, 0.00047mol) was added and refluxed for 3 hrs at the end of which the reaction was complete. The reaction was stopped and the mixture allowed to cooled to rt. The solvent was evaporated off under reduced pressure to afford MP272A (0.38g, 115% crude overall yield due to retention of solvent). MP272A (0.3g) was adsorbed onto silica and purified by column chromatography, (SiO_2; $CHCl_3$:MeOH, 9:1). MP272B (0.055g, 17.2%) was obtained as shiny bright yellow micro-crystals from $CHCl_3$, mp 140-145°C.

Solubility, sol (MeOH, $CHCl_3$, Et_2O), sp. sol (H_2O, EtOAc).

TLC R_f 0.87 (SiO_2; EtOAc:MeOH:NH_4OH, 5:1:1) 0.82 (SiO_2; $CHCl_3$:MeOH, 9:1).

HPLC Major peak 99.37% at 9.30 min and minor peaks 0.5 and 0.1% at 3.72 and 4.45 mins (4.00 + 250mm Lichrosorb RP select B 5 μm, 1.00ml/min, UV 254nm 0.05 aufs., A/B is 40/60 where A is H_2O with 0.1% TEA and B is MeOH with 0.1% TEA).

UV (MeOH) λmax; 225, 266, 325nm (logε 3.31, br, 3.22, br, 2.76).

IR (Nujol mull); 3234 (m, N-H str, secondary NH_2), 1623, 1528, 1459, 1376 (s, C=N def, aromatic C=C def, C-C str, Nujol), 965, 945, 921, 844, 822 (m, aromatic =C-H- def, oopb), 784, 751, 722, 700, 636, 611 (s, aromatic C_6H_5 str) cm^{-1}.

^1H NMR (400MHz, $CDCl_3$) δ; 9.14 (s, 1H, C4-H), 8.85 (d, 1H, $^3J_{H-H}$ = 1.7Hz, C7-H), 8.53 (d, 1H, $^3J_{H-H}$ = 1.7Hz, C6-H), 7.31-7.19 (m, 5H, CH_2-CH_2-C_6H_5 and $CDCl_3$), 5.8 (s, (vbr), 1H, -NH-), 3.93-3.88 (m, 2H, $^3J_{H-H}$ = 7Hz, $^4J_{H-H}$ = 5.8Hz, NH-CH_2-CH_2-C_6H_5), 3.02-2.95 (m, 2H, CH_2-C_6H_5).

13C NMR (100MHz, DMSO) ppm;164.66 (C4), 161.30 (C2), 155.97, 151.79 (C7 and C9), 141.51 (C6), 138.75 (C10), 131.08, 128.85, 128.65, 126.55 (C_6H_5), 42.87, 35.13 (CH_2CH_2).

Mass spectrum (EI) m/e; 251 $(M)^+$, 174 $(M - C_6H_5)^+$, 160 $(M - CH_2\text{-}C_6H_5)^+$, 147 (2-am. pter.)$^+$, 133 (2-am.pter. - NH)$^+$, 120 (2-am.pter. - HCN)$^+$, 105 $(CH_2\text{-}CH_2\text{-}C_6H_5)^+$, 79 (pyrazine)$^+$, 52 (pyrazine - HCN)$^+$.

Anal. Calcd. for $C_{14}H_{13}N_5$: C, 66.91%; H, 5.22%; N, 27.87%.

Found: C, 66.78%; H, 5.41%; N, 27.66%.

2-Benzylamino-5-nitro-4-pyrimidinamine

(9-I/3) (MP250A)

A mixture of 4-amino-2-chloro-5-nitropyrimidine MP248I (4.9g, 0.028mol) and benzylamine (10ml, 0.092mol) in MeOH (10ml) was refluxed in MeOH (20ml) for 2 hrs using an oil bath. An orange solution was obtained. The reaction was first cooled to rt and then to 0°C by immersing the reaction vessel in an acetone/solid CO_2 bath. Etching with a glass rod resulted in the formation of crystals. On filtration, shiny yellow crystals of MP250A (6.062g, 86.4%) were obtained; mp 190-193°C.

^{1}H NMR (60MHz, DMSO) δ; 8.8 (s, 1H, C6-H), 7.3 (m, 5H, C_6H_5), 4.4 (s, (br), 2H, NH-CH_2), 4.0 (s, (vbr), NH masked by DHO).

Mass spectrum (EI) m/e; 245 $(M)^+$, 228 $(M - NH_2)^+$, 198 $(M - NO_2)^+$, 168 $(M\text{-}CH_2\text{-}C_6H_5)^+$, 106 $(M - N\text{-}CH_2\text{-}C_6H_5)^+$, 91 $(CH_2\text{-}C_6H_5)^+$.

2-Benzylamino-4,5-pyrimidinediamine

(9-I/4) (MP258A)

$H_2N.NH_2.H_2O$ (0.35ml, 0.0072mol) and active 10% Pd/C (0.1g) was added to 2-benzylamino-5-nitro-4-pyrimidinamine MP250A (0.5g, 0.002mol) in EtOH (50ml) under N_2. After 20 hrs of continuous stirring, tlc (eluant; $CHCl_3$:MeOH:Pet.spirit 60-80°C, 10:10:2) showed no evidence of a new product. Refluxing for 3 hrs ensured completion of reaction. The catalyst was filtered off and the filtrate added to iced water (250ml). The organic layer was extracted with toluene (2 x 100ml) and EtOAc (3 x 100ml) and dried ($MgSO_4$) overnight. Filtration followed by evaporation to dryness under reduced pressure resulted in yellow oil which solidified on azeotroping with ^iPrOH (3 x 100ml), mp 90-96°C, red oil.

The same experiment repeated on a 0.02mol scale, afforded MP254A in 78% crude overall yield.

^1H NMR (400MHz, DMSO) δ; 7.26-7.22 (m, 5H, $C_6\underline{H}_5$), 6.17 (t, $^3J_{H\text{-}H}$ = 6.4Hz, C2-N$\underline{H}$-CH_2), 6.01 (s, (vbr), 2H, C4-N$\underline{H}_2$), 4.36 (d, 2H, $^3J_{H\text{-}H}$ = 6.4Hz, C2-NH-C$\underline{H}_2$), 3.7 (s, (vbr), 2H, C5-N$\underline{H}_2$, HDO).

Mass spectrum (EI) m/e; 215 $(M)^+$, 197 $(M - NH_2)^+$, 181 $(M - 2NH_2)$+, 155 $(M - 2NH_2$ - HCN or M - N-$CH_2C_6H_5$)+, 138 (M - N-CH_2-C_6H_5 - $NH_2)^+$, 110 (2-NH_2-pyrimidine - $HCN)^+$, 91 $(CH_2\text{-}C_6H_5)^+$, 83 (2-NH_2-pyrimidine - $2HCN)^+$.

2-Benzylamino pteridine

(9) (MP274B/UCL 1060)

2-Benzylamino-4,5-pyrimidinediamine MP258A (0.51g, 0.0023mol) was dissolved in MeOH (10ml). To this, glyoxal trimeric dihydrate (0.16g, 0.0008mol) was added and

refluxed for 2.5 hrs at the end of which the reaction was complete. The reaction was stopped and cooled to rt. The crystallised solid MP274A (0.46g, 85.1% crude overall yield) was collected on filtration and washed with cold MeOH. MP274A (0.4g) was adsorbed onto silica and purified by column chromatography (SiO_2; $CHCl_3$:MeOH, 9:1). MP274B (0.16g, 29%) was afforded as yellow micro-crystals from $CHCl_3$, mp 165-168°C.

Solubility, sol (MeOH, $CHCl_3$, EtOAc), sp. sol (H_2O, Et_2O).

TLC R_f 0.87 (SiO_2; EtOAc:MeOH:NH_4OH, 5:1:1), 0.78 (SiO_2; $CHCl_3$:MeOH, 9:1).

HPLC 100% at 6.92 mins (4 + 250mm Lichrosorb RP select B 5μm, 1.00ml/min, UV 254nm 0.05 aufs., A/B is 40/60 where A is H_2O with TEA and B is MeOH with TEA).

UV (MeOH) λmax; 229, 268, 380nm (logε 4.48, br, 4.18, s, 3.94, s).

IR (Nujol mull); 3219 (m, N-H str, secondary NH_2), 1611, 1585, 1527 (s, C=N str, aromatic C-C def), 945, 909, 865, 823 (s, aromatic =C-H str), 787, 743, 701, 656, 631, 620, 610 (s, aromatic =C-H def, oopb) cm^{-1}.

^1H NMR (400MHz, $CDCl_3$) δ; 9.15 (s, 1H, C4-H), 8.85 (d, 1H, $^3J_{H-H}$ = 1.7Hz, C7-H), 8.55 (d, 1H, $^3J_{H-H}$ = 1.7Hz, C6-H), 7.42-7.26 (m, 5H, NH-CH_2-C_6H_5), 6.12 (s, (vbr), 1H, -NH-), 4.837, 4.823 (d, 2H, $^4J_{H-H}$ = 5.8Hz, NH-CH_2-C_6H_5).

Mass spectrum (EI) m/e; 237$(M)^+$, 160 $(M - C_6H_5)^+$, 146 (M - CH_2-C_6H_5 or 2-am. pter.)$^+$, 132 (2-am. pter. - NH)$^+$, 120 (2-am. pter - HCN)$^+$, 105 (2-am. pter - HCN-NH)$^+$, 91 $(CH_2-C_6H_5)^+$, 79 (pyrazine)$^+$, 52 (pyrazine - HCN)$^+$.

Anal. Calcd. for $C_{13}H_{11}N_5.0.6H_2O$: C, 62.94%; H, 4.46%; N, 28.23%

Found: C, 63.33%; H, 4.73%; N, 27.88%.

2-Ethylamino-5-nitro-4-pyrimidinamine

(**10**-I/3) (MP270A)

A mixture of 4-amino-2-chloro-5-nitropyrimidine MP248I (2.53g, 0.028mol) and ethylamine (10ml, 0.22mol) in MeOH (10ml) was refluxed in MeOH (20ml) for 2 hrs using an oil bath. A yellow solution was obtained. The reaction was cooled to rt and then to 0°C by immersing the reaction vessel in an acetone/solid CO_2 bath. Etching with a glass rod resulted in the formation of yellow crystals. On filtration, yellow crystals of MP270A (1.99g, 33.1%) were obtained; mp 159-163°C.

^{1}H NMR (400MHz, DMSO) δ; 8.82 (s, 1H, C6-H), 8.21-8.12 (d, (vbr), 2H, NH_2), 7.92-7.86 (m, (vbr), 1H, NH), 3.36-3.27 (m, 2H, NH-CH_2-CH_3), 1.09 (t, 3H, $^3J_{H-H}$ = 7.1Hz, CH_2CH_3).

Mass spectrum (EI) m/e; 183 $(M)^+$, 167 $(M - NH_2)^+$, 154 $(M - CH_2\text{-}CH_3)^+$, 140 $(M - N\text{-}CH_2\text{-}CH_3)^+$, 137 $(M - NO_2)^+$, 92 $(pyrimidine)^+$.

2-Ethylamino-4,5-pyrimidinediamine

(10) (MP278B/UCL 1061)

$H_2N.NH_2.H_2O$ (1ml, 1.03g, 0.02mol) and active 10% Pd/C (0.1g) was added to 2-ethylamino-5-nitro-4-pyrimidinamine MP270A (1.10g, 0.006mol) in EtOH (20ml) under N_2, refluxed for 4 hrs and left at rt for 12 hrs. The reaction mixture was filtered off first under suction and then under gravity, the filtrate evaporated to dryness under reduced pressure and azeotroped with ^{i}PrOH (3 x 100ml). A reddish brown solid MP278A (0.56g, 60.9% crude yield) was obtained. MP278B (0.076g, 8.4%) was afforded as the first crop on recrystallisation from ^{i}PrOH, mp 160-165°C.

Solubility, sol (MeOH, EtOAc, H_2O, Et_2O), sp. sol (^{i}PrOH).

TLC R_f 0.62 (SiO_2; EtOAc:MeOH:NH_4OH, 5:1:1), 0.1 (SiO_2; $CHCl_3$:MeOH, 9:1).

HPLC Major peak 99.34% at 6.02 min and a minor peak 0.66% at 2.97 min (4 + 250mm Lichrosorb RP select B 5μm, 1.00ml/min, UV 254nm 0.05 aufs., A/B is 80/20 where A is H_2O with TEA and B is MeOH with TEA).

UV (MeOH) λmax; 208, 232, 307nm (logε 3.77, shoulder, 4.05, br, 3.59, br).

IR (Nujol mull); 3322, 3109 (s, N-H str, primary NH_2), 1650, 1595, 1505 (s, C=N def, aromatic C-C def), 1450, 1372, 1337 (s, Nujol, C-H def, CH_2CH_3), 889, 780 (s, aromatic =C-H def, oopb) cm^{-1}.

^1H NMR (400MHz, DMSO) δ; 7.26 (s, 1H, C6-H), 5.92 (s, 2H, C4-NH_2), 5.48 (t, 1H, $^3J_{H-H}$ = 5.8Hz, -NH-CH_2), 3.692 (s, 2H, C5-NH_2), 3.14-3.08 (m, 2H, NH-CH_2-CH_3), 1.017 (t, 3H, $^3J_{H-H}$ = 7.1Hz, CH_2CH_3).

Mass spectrum (EI) m/e; 153 $(M)^+$, 137 $(M - NH_2)^+$, 124 $(M - CH_2\text{-}CH_3)^+$, 121 $(M - 2NH_2)^+$, 110 $(M - NH\text{-}CH_2CH_3)^+$, 77 $(pyrimidine)^+$.

Anal. Calcd. for $C_6H_{11}N_5$: C, 47.04%; H, 7.24%, N, 45.72%

Found: C, 47.25%; H, 7.31%; N, 44.68%.

2-Ethylaminopteridine [84]

(11) (MP280B/UCL 1062)

To 2-ethylamino-4,5-pyrimidinediamine (0.22g, 0.0014mol) in MeOH (10ml), glyoxal trimeric dihydrate (0.1g, 0.00048mol) was added and refluxed for 2 hrs, at the end of which the reaction was complete. The reaction was stopped; cooled to rt and the solvent evaporated off under reduced pressure to afford MP280A (~0.3g, wet) which was adsorbed onto silica and purified by column chromatography (SiO_2; $CHCl_3$:MeOH, 9:1). MP280B (0.075g, 29.7%) was obtained as a bright yellow micro-crystalline solid from

$CHCl_3$, mp 122-125°C, reported 137-138°C [84].

Solubility, sol (MeOH, H_2O, $CHCl_3$, EtOAc), sp. sol (Et_2O).

TLC R_f 0.85 (SiO_2; EtOAc:MeOH:NH_4OH, 5:1:1), 0.74 (SiO_2; $CHCl_3$:MeOH, 9:1).

HPLC Major peak 98.3% at 5.5 min, and minor peaks 0.20%, 0.23% and 1.28% at 2.4, 3.6 and 3.6mins respectively, (Lichrosorb RP select B 7 μm, 1.00ml/min, UV 254nm, A/B (40/60) where A is H_2O with 0.1% TFA and B is MeOH with 0.1% TFA).

UV (MeOH) λmax; 232, 272, 387nm (logε 4.32, s, 4.00, s, 3.78, br).

IR (Nujol mull); 3271 (s, N-H str, secondary NH_2), 1607, 1568, 1540 (s, C=N str, aromatic C-C def), 1404, 1376, 1337 (m/s, Nujol, C-C str, -CH_2CH_3 def), 947, 864, 823 (s, aromatic =C-H def, oopb) cm^{-1}.

^1H NMR (200MHz, DMSO) δ; 9.13 (s, 1H, C4-H), 8.87 (d, 1H, $^3J_{H-H}$ = 1.7Hz, C7-H), 8.56 (d, 1H, $^3J_{H-H}$ = 1.7Hz, C6-H), 8.2-8.05 (m, (br), 1H, -NH-CH_2-CH_3), 3.41 (q, 2H, $^3J_{H-H}$ = 7.1Hz, -NH-CH_2-CH_3), 1.20 (t, 3H, $^3J_{H-H}$ = 7.1Hz, -NH-CH_2-CH_3).

Mass spectrum (EI) m/e; 175 $(M)^+$, 146 $(M - CH_2CH_3)^+$, 133 (2-am. pter. - NH)$^+$, 120 (2-am. pter. - HCN)$^+$, 79 (pyrazine)$^+$, 52 (pyrazine - HCN)$^+$.

Anal. Calcd. for $C_8H_9N_5$: C, 54.84%; H, 5.17%; N, 39.97%

Found: C, 54.74%; H, 5.10%; N, 39.79%.

2-Isopropylamino-5-nitro-4-pyrimidinamine

(**12**-I/3) (MP698C)

A 50% mixture of isopropylamine (5ml) in MeOH (5ml) was added to 4-amino-2-chloro-5-nitropyrimidine MP248I (1.5g, 0.0086mol). A further portion of MeOH (10ml) was added whilst cooling the reaction mixture over ice. (Exothermic reaction with fizzing was

observed). The mixture was refluxed for 1 hr, cooled to rt and filtered. Buff coloured crystals of MP698A (0.312g, 18.4%) were obtained. The filtrate was reduced to half the volume and left overnight in the freezer. Buff coloured crystals of MP698B (0.406g, 24.02%), mp 15$\overset{\wedge}{1}$-152°C were obtained on filtration. The filtrate was concentrated down and left in the freezer. MP698C (0.61g, 36.04%) was collected on filtration. Overall yield was 78.5%.

Mass spectrum (EI) m/e; 197 $(M)^+$, 181 $(M - NH_2)^+$, 155 $(M - {}^iPr)^+$, 151 $(M - NO_2)^+$, 141 $(M - N\text{-}{}^iPr)^+$, 56 $(NH\text{-}{}^iPr)^+$, 43 $({}^iPr\text{-}H)^+$.

2-Isopropylamino-4,5-pyrimidinediamine

(**12**-I/4) (MP699B)

$H_2N.NH_2.H_2O$ (1.7ml, 0.035mol) was added dropwise over 20 mins to active 10% Pd/C (0.2g) and 2-isopropylamino-5-nitro-4-pyrimidinamine MP698D (1.1g, 0.0056) in MeOH (50ml) under N_2. MeOH (50ml) was added and the mixture refluxed for 5 hrs. The reaction mixture was filtered off first under suction and then under gravity, the filtrate evaporated off under reduced pressure and azeotroped with ${}^iPrOH$ (3 x 100ml). On drying in a vacuum oven (rt/760mmHg), MP699A (0.951g, 101%)was obtained as sticky light pink solid. Repeatedly stirring in Et_2O (2 x 50ml) followed by filtration afforded MP699B (0.65g, 69.5%) as a yellow solid, mp 110-114°C.

^1H NMR (400MHz, DMSO) δ; 8.82 (s, 1H, C6-H), 8.19-7.88 (m, (vbr), 4H, C4-NH_2, C5-NH_2), 4.09-4.05 (m, 1H, NH-$CH(CH_3)_2$), 1.13 (d, 6H, $^3J_{H\text{-}H}$ = 7.1Hz, $-CH_2-(CH_3)_2$).

Mass spectrum (EI) m/e; 167 $(M)^+$, 152 $(M - CH_3$ or $M - NH_2)^+$, 135 $(M - 2.NH_2$ or $M - C_2H_4)^+$, 125 $(M - {}^iPr)^+$, 110 $(M - NH\text{-}{}^iPr)^+$, 94 $(NH_2\text{-pyrim.})^+$, 76 $(pyrim.)^+$.

2-Isopropylaminopteridine [85]

(12) (MP700A/UCL1181)

Glyoxal trimeric dihydrate (0.21g, 0.0099mol) in MeOH (5ml) was added to 2-isopropylamino-4,5-pyrimidinediamine MP699B (0.5g, 0.003mol) in MeOH (5ml). The undissolved glyoxal was washed with further MeOH (10ml), added to the reaction mixture and refluxed for 1 hr. The crude reaction product was adsorbed to silica and columned twice (SiO_2; $CHCl_3$:MeOH, 9:1). Fractions 1-9 were collected and evaporated off under reduced pressure to afford MP700A (0.43g, 76.3%) as a yellow micro-crystalline solid: mp 156-160°C (from $CHCl_3$), reported 136°C [85].

Solubility, sol (H_2O, MeOH, EtOH, Et_2O), sp. sol (pet. spirit 60-80°C).

TLC R_f 0.87 (SiO_2; EtOAc:MeOH:NH_4OH, 5:1:1), 0.76 (SiO_2; $CHCl_3$:MeOH, 9:1).

HPLC Major peak 97.7% at 6.13 min, and minor peaks 0.37%, 0.55% and 1.38% at 3.17, 3.36 and 5.3mins respectively, (Lichrosorb RP select B 7 μm, 1.00ml/min, UV 254nm, A/B (40/60) where A is H_2O with 0.1% TFA and B is MeOH with 0.1% TFA).

UV (MeOH) λmax; 231, 271, 385 (logε 4.32, s, 3.97, 3.79, br).

IR (Nujol mull); 3292 (s, N-H str, secondary amine), 1600, 1558, 1528 (m, C=N str, C-C str), 1458, 1407, 1376, 1364, 1340, 1320 (s, ^{i}Pr, C-H def, str), 941, 862, 793 (m, aryl =C-H def, oopb) cm^{-1}.

^1H NMR (400MHz, $CDCl_3$) δ; 9.14 (s, 1H, C4-H), 8.82 (d, 1H, $^3J_{H-H}$ = 1.7Hz, C7-H), 8.50 (d, 1H, $^3J_{H-H}$ = 1.7Hz, C6-H), 5.68-5.66 (s/d, (vbr), 2H, C2-NH-CH), 4.39 (m, 1H, $^3J_{H-H}$ = 6Hz, NH-CH-$(CH_3)_2$), 1.27 (d, 6H, $^3J_{H-H}$ = 7.1Hz, -CH_2-$(CH_3)_2$).

Mass spec (EI) m/e; 189 $(M)^+$, 146 (M - iPr or 2-am. pter)$^+$, 130 (M - iPr-NH)$^+$, 120 (2-am. pter. - HCN)$^+$, 79 (pyrazine)$^+$, 52 (pyrazine - HCN)$^+$, 43 (iPr-H)$^+$.

Anal. Calcd. for C9H11N5. 3% inorganic: C, 55.54%; H, 5.68%; N, 35.90%

Found: C, 55.23%; H, 5.58%; N, 34.42%.

2-N-acetamidopteridine [78]

(13) (MP298/UCL 1085) (also MP296A, MP408)

2-aminopteridine MP294B (0.204g, 0.0014mol) was dissolved in acetic anhydride (3.5ml) with heating and refluxed for 20 mins. A gradual colour change from yellow to orange to brown was observed during the course of the reaction. The brown solution was left for 16 hrs at rt. The crystals were filtered, washed with cold acetic anhydride, H_2O and pet. spirit (60-80°C) and dried at 60°C/0.1mmHg for 3 hrs. MP298 (0.18g, 72.3%) was afforded as shiny pale brown crystals, mp 230-235°C, reported 225-229°C (from H_2O) [78].

Solubility, sol (DMSO, MeCN), sp. sol (MeOH, EtOH abs., H_2O, $CHCl_3$), insol (Et_2O, pet. spirit 60-80°C).

TLC R_f 0.3 (MeCN), 0.54 and 0.29 trace of covalent adduct, ($CHCl_3$:MeOH, 9:1).

HPLC 98.8% at 6.57min and minor peaks 0.8% and 0.3% at 4.13 and 8.3 mins (250 x 4mm Lichrosorb RP select B 5μm, 1.00ml/min, UV 254nm 0.05 aufs., A/B is (95/5) where A is H_2O with 0.1% TEA and B is MeCN + 5% H_2O with 0.1% TEA).

UV λmax; 198, 232, 257, 329nm (logε 4.9, 5.23, 4.98, 4.92).

IR (Nujol mull); 3357, 3214, 3147 (m, secondary amide N-H str), 1794, 1777, 1674, 1599, 1553 (m/s, secondary amide C=O str and N-H def, C=N str and aromatic C-C str), 1522, 1459, 1407, 1375 (m, C-H def, Nujol, CH_3-), 807, 766, 752, 722, 635 (s, aryl oop =C-H def,) cm^{-1}.

^1H NMR (200MHz, DMSO) δ; 11.4-11.2 (s (vbr), possibly C2-N<u>H</u>-$COCH_3$), 9.68 (s, 1H, C4-<u>H</u>), 9.22 (d, 1H, $^3J_{H-H}$ = 1.7Hz, C7-<u>H</u>), 8.99 (d, 1H, $^3J_{H-H}$ = 1.7Hz, C6-<u>H</u>), 2.33 (s, 3H, $COC\underline{H}_3$).

Mass spectrum (EI) m/e; 189 $(M)^+$, 161 $(M - HCN)^+$, 147 ($M - NHCOCH_3$ or 2-ampter.)$^+$, 120 $(147 - HCN)^+$, 105 $(147 - NH)^+$, 79 $(pyrazine)^+$, 52 $(pyrazine - HCN)^+$.

Anal. Calcd. for $C_8H_7N_5O$: C, 50.78%; H, 3.73%; N, 37.03%

Found: C, 50.41%; H, 3.62%; N, 36.63%.

Previously, the experiment repeated on a 0.007mol scale afforded MP296A in 69.6% yield. At a later stage, the same experiment performed on a 0.014mol scale afforded MP408 in 91% yield.

4-Ethoxy-2-N-acetamido-3,4-dihydropteridine

(16) (MP296C/UCL 1096)

2-N-acetamidopteridine MP296A (0.73g, 0.0038mol) was recrystallised from abs. EtOH (50ml). The undissolved brown solid MP296B (0.29g) was recovered on filtration. The EtOH filtrate was refridgerated for 24 hrs. White crystals and yellow crystal had formed. The crystals were filtered, washed with pet. spirit 60-80°C and dried under suction. The White crystals were separated out using a spatula and dried in a vacuum oven at 50°C/0.1mmHg. MP296C (0.093g, which consists of ~77% of 4-Ethoxy-2-N-acetamido-3,4-dihydropteridine and 22% of 2-N-acetamidopteridine, according to the ^1H NMR (200MHz, DMSO) spectrum, was obtained as white crystals of mp 230-235°C.

Solubility sol (DMSO, MeCN), sp. sol (H_2O, MeOH, EtOH abs., $CHCl_3$), insol (pet. spirit 60-80°C, Et_2O).

TLC R_f 0.3 ($CHCl_3$:MeOH, 9:1).

HPLC 97.8% at 6.66min. and minor peaks 0.43%, 0.083%, 1.59%, 0.07% and 0.01% at 4.15, 6.01, 8.4, 11.3 and 12.4mins respectively (250 x 4mm Lichrosorb RP select B 5μm, 1.00ml/min, UV 254nm 0.05 aufs., A/B is (95/5) where A is H_2O with 0.1% TEA

and B is MeCN + 5% H2O with 0.1% TEA).

UV (MeOH) λmax; 207, 227, 274, 319nm (logε 3.85, 3.93, 3.97, 4.04).

IR (Nujol mull); 3237 (m, secondary amide N-H str), 1701, 1617, 1602, 1573 (m/s, secondary amide C=O str and N-H def, C=N str and aromatic C-C str), 2850, 2722, 2355, 2321 (m, C-H str, Nujol, CH_3CH_2-), 1523, 1458, 1404, 1375 (m, C-H def, Nujol, CH_3-), 1284, 1239, 1193, 1153, 1114 (s, C-O str, ether), 849, 820, 778, 722, 660, 621 (s, aryl oop =C-H def,) cm^{-1}.

(MP296C) ^1H NMR (200MHz, DMSO) ~77.8% of 4-Ethoxy-2-N-acetamido-3,4-dihydropteridine [δ; 8.42 (d, 1H, $^3J_{H-H}$ = 1.7Hz, C7-H), 8.26 (d, 1H, $^3J_{H-H}$ = 2Hz, C6-H), 5.86 (s, 1H, C4-H), 3.56 (q, 2H, $^3J_{H-H}$ = 7Hz, O-CH_2-CH_3), 2.13 (s, 3H, COCH_3), 1.06 (t, 3H, $^3J_{H-H}$ = 7Hz, O-CH_2-CH_3)], and

~22% of 2-N-acetamidopteridine [δ; 9.68 (s, 1H, C4-H), 9.22 (d, 1H, $^3J_{H-H}$ = 1.7Hz, C7-H), 8.99 (d, 1H, $^3J_{H-H}$ = 1.7Hz, C6-H), 2.33 (s, 3H, COCH_3)] based on the ratio of proton peak integrals.

Mass spectrum (EI) m/e; trace of 235 $(M)^+$, 189 (M - EtO or 2-acetamidopter.)$^+$, 161 (189 - HCN)$^+$, 147 (2-ampter.)$^+$, 120 (147 - HCN)$^+$, 105 (147 - NH)$^+$, 93 (147 - 2.HCN)$^+$, 79 (pyrazine)$^+$, 52 (pyr. - HCN)$^+$, 45 $(EtOH)^+$.

Anal.Calcd. for 98% $C_{10}H_{13}N_5O_2$ + 2% $C_8H_7N_5O$: C, 50.25%; H, 5.48%; N, 29.30%

Found: C, 50.29%; H, 5.44%; N, 27.67%.

2-N-Propionamidopteridine

(20) (MP687/UCL1179)

2-aminopteridine MP407 (0.20g, 0.0014mol) was dissolved in propionic anhydride (5ml)

with heating. The mixture was left to reflux. A gradual colour change from yellow to orange to brown was observed during the course of the reaction. The reaction was complete in 20 mins. The brown solution was left standing at rt for 3 hrs. The crystals were filtered, washed with cold propionic anhydride, H_2O and pet. spirit (60-80°C) and dried at 60°C/0.1mmHg for 3 hrs. MP687 (0.231g, 83.7%) was obtained as shiny pale brown crystals, mp 179-180°C.

Solubility, sol (DMSO, MeCN), sp. sol (H_2O, MeOH, EtOH,), insol (Et_2O, pet. spirit 60-80°C).

TLC R_f 0.4 (MeCN); 0.63 and 0.33 trace of covalent adduct, ($CHCl_3$:MeOH, 9:1).

HPLC 98.8% at 8.01 min and 1.05% at 4.38 min (250 x 4.6mm Ultratechsphere 5μm, 1.0ml/min, UV 254nm 0.1 aufs, isocratic conditions: A/B (90/10) where A is H_2O and B is MeCN with 0.01% TFA.

UV (MeOH) λmax; 228, 274, 322nm (logε 3.98, s, 3.97, 4.0).

IR (nujol mull); 3218, 3149 (m, secondary amide N-H str), 1789, 1684, 1598 (m/s, secondary amide C=O str and N-H def, C=N str, aromatic C-C str), 790, 751, 722, 664, 618 (s, aryl =C-H def, oopb) cm^{-1}.

^1H NMR (400MHz, DMSO) δ; 9.65 (s, 1H, C4-H), 9.19 (d, 1H, $^3J_{H-H}$ = 1.7Hz, C7-H), 8.96 (d, 1H, $^3J_{H-H}$ = 1.7Hz, C6-H), 2.63 (q, 2H, $^3J_{H-H}$ = 1Hz, NH-CO-CH_2-CH_3), 1.09 (t, 3H, $^3J_{H-H}$ = 7Hz, NH-CO-CH_2-CH_3), NH masked due to DHO.

Mass spectrum (EI) m/e; 203 $(M)^+$, 175 $(M - Et)^+$, 146 $(2\text{-ampter.})^+$, 120 $(147 - HCN)^+$, 105 $(147 - NH)^+$, 93 $(147 - 2.HCN)^+$, 79 $(pyrazine)^+$, 57 $(EtCONH2)^+$, 52 $(pyr. - HCN)^+$.

Anal. Calcd. for $C_9H_9N_5O$: C, 53.19%; H, 4.46%; N, 34.46%

Found: C, 52.90%; H, 4.46%; N, 34.25%.

Attempted syntheses of compounds (14) and (15).

Phenylacetamidine hydrochloride [91]

(**14**-I/1a) (MP244C, MP244D)

NH_3 gas (~9ml) was condensed into cold MeOH in a test tube at -40°C and transferred immediately to a pressure vessel cylinder pre-cooled to -20°C. Phenyl acetonitrile (2g, 0.017mol) and NH_4Cl (3.6g, 0.068mol) were added, the vessel concealed and the pressure vessel set up. The reaction mixture was initially heated at 155°C. In 1hr, the internal temperature was 125°C at 14 Bars. The reaction mixture was then heated at 165°C for 14 hrs with an internal temperature of 141°C at 11.14 Bars. The reaction was stopped and cooled to rt. On filtration, NH_4Cl was recovered. The p.t.f.e cylinder was washed with MeOH. The MeOH washings and filtrate were evaporated off under reduced pressure and extracted with Et_2O. Phenyl acetonitrile (1.7g) was recovered. The remaining residue was extracted with hot abs. EtOH. On evaporation of EtOH, phenylacetamidine hydrochloride MP244C (0.15g, 5% crude yield) was obtained as a white solid, mp 142-145°C, reported 145-147°C [91].

^1H NMR (60MHz,DMSO) δ; 7.23 (s, br, 5H, $C_6\underline{H}_5$), 3.51 (s, 2H, Ph-$C\underline{H}_2$). c.f. phenylacetonitrile ^1H NMR (60MHz,DMSO) d 7.23 (s, br, 5H, $C_6\underline{H}_5$), 3.86 (s, 2H, Ph-$C\underline{H}_2$).

Mass spectrum (EI) m/e; 133 $(M)^+$, 104 $(Ph\text{-}CH_2\text{-}CH_2\text{-})^+$, 91 $(Ph\text{-}CH_2)^+$, 77 $(C_6H_5)^+$.

Note. The experiment was repeated on the same scale as above to obtain MP244D (0.23g, 7.9% crude yield).

2-Hydroxymethyl-3-pyrazinamine [100]

(**14,15**-I/1) (MP637B, MP302A)

A reaction vessel, fitted with mechanical stirrer, condenser and N_2 inlet was flushed continuously with N_2. 2-Methylcarboxylate-3-pyrazinamine (20g, 0.13mol) and THF (600ml) (distilled over Na wire and benzophenone) were placed in the vessel and stirred at rt for 30 mins A suspension of $LiAlH_4$ (10g, 0.26mol) in dry THF (250ml) was added portionwise over 1 hr. Efferverscence, with slight evolution of heat was observed. At the end of 2 hrs stirring at ~40°C, tlc (eluant: EtOAc:MeOH: NH_4OH, 5:1:1) indicated only slight presence of product R_f 0.47. Therefore, the reaction mixture was refluxed for a further 2 hrs, at the end of which the reaction was complete.The reaction was stopped and cooled to rt. To the cooled mixture, crushed ice (~100ml) was added portionwise over 30 mins, in order to deactivate $LiAlH_4$, maintaining the temperature at -5 to 0°C, by the use of a MeOH/solid CO_2 bath. A colour change of brown to orange with the formation of a white precipitate of $LiAl(OH)_4$ was observed. The reaction mixture was filtered and the residue washed with more THF to obtain a filtrate, which when evaporated under reduced pressure afforded MP636A.

The $LiAl(OH)_4$ was extracted with EtOAc (5 x 100ml; the combined extracts dried (Na_2SO_4) for ~ 12 hrs, filtered and the solvent evaporated off. MP636A was further extracted with EtOAc (4 x 250ml), the combined extracts dried (Na_2SO_4) for ~ 12 hrs, filtered and the solvent evaporated off under reduced pressure to afford MP637A (11.16g, 68.4% wet, crude overall yield) as a white solid. On freeze-drying, MP637B (9.7g, 59.5% crude overall yield) was afforded as a yellow solid, mp 105-110°C crude, reported 110-114°C (sublimation) [100].

^1H NMR (200MHz, DMSO) δ; 7.91 (d, 1H, $^3J_{H-H}$ = 2 Hz, C6-H or C5-H), 7.71 (d, 1H, $^3J_{H-H}$ = 2 Hz, C6-H or C5-H), 6.3 (s, br, 2H, NH_2), 5.6-5.3 (s, (vbr), 1H, OH), 4.58 (s, 2H, CH_2-OH).

Mass spectrum (EI) m/e; 125 $(M)^+$, 109 $(M - NH_2)^+$, 107 $(M - H_20)^+$, 77 $(pyrazine)^+$.

Note. Previously, the same experiment repeated on a 0.065mol scale afforded MP302 in 95% crude overall yield. MP302 (2.00g) sublimed at 110°C/0.1mmHg over 3 hrs afforded MP302A (0.56g), mp 109-114°C.

Anal. Calcd. for $C_5H_7N_3O$: C, 47.99%; H, 5.64%; N, 33.50%

Found: C, 48.37%; H, 5.75%; N, 32.40%.

3-Amino-2-pyrazinecarbaldehyde [100]

(**14**,**15**-I/2) (MP639A, MP305, MP306, MP307)

A suspension of 2-hydroxymethyl-2-pyrazinamine MP637B (9.1g, 0.073mol) in $CHCl_3$ (300ml) (distilled over CaH_2 and dried over 4Å molecular sieves) was stirred for 30 mins according to the literature method [100]. Further $CHCl_3$ (200ml) was added to aid dissolution. MnO_2 (50.5g, 0.93mol) was added portionwise over 15 mins and left stirring at rt. The reaction progress was monitored by tlc (SiO_2: EtOAc:MeOH, 5:1). At the end of 29 hrs, the reaction was still incomplete. Further MnO_2 (3.8g, 0.07mol) was added and the reaction mixture stirred for a further 2 hrs. The reaction was stopped, the MnO_2 filtered off, by suction and then under gravity and the solvent evaporated off under reduced pressure to afford MP638A (8.5g, 96.5% crude, overall yield) as a brown solid.

MP638A was purified by column chromatography (SiO_2: EtOAc:MeOH, 95:5) in two successive batches to give MP639A (4.5g, 51%, combined yield) as a yellow solid, mp 105-108°C, reported 117°C (on sublimation) [100].

Solubility, sol (EtOAc, Et_2O, $CHCl_3$), sp. sol (pet. spirit 60-80°C).

^1H NMR (200MHz, $CDCl_3$) δ; 10.08 (s, 1H, CO<u>H</u>), 8.23 (d, 1H, C5-<u>H</u> or C6-<u>H</u>), 8.08 (d, 1H, C5-<u>H</u> or C6-<u>H</u>), 7 - 6 (s, (vbr), N<u>H</u>$_2$).

Mass spectrum (EI) m/e; 123 $(M)^+$, 95 $(M - COH)^+$, 79 $(M - COH - NH_2)^+$, 68 $(M - COH - HCN)^+$.

Anal. Calcd. for $C_5H_5N_3O$: C, 48.78%; H, 4.09%; N, 34.12%

Found: C, 49.10%; H, 3.97%; N, 34.00%.

Note. Previously, the same experiment repeated on 0.004, 0.04, and 0.072mol scales afforded MP305 (78.6%), MP306 (72.7%) and MP307 (25%) respectively.

3-Dimethoxymethyl-2-pyrazinamine [100]

(**14**,**15**-I/3) (MP641A, MP400, MP402B)

3-Amino-2-pyrazinecarbaldehyde MP639A (0.1g, 0.00081mol) was placed in a flame-dried apparatus. MeOH (10ml), (distilled over CaH_2) was added and stirred for 10 mins. 50% BF_3.MeOH complex (1ml, 0.009mol) was added, cooling the reaction mixture over a acetone/solid CO_2 bath. The reaction mixture was stirred at rt for ~17.5 hrs, monitoring the reaction progress by tlc (SiO_2: EtOAc:MeOH, 95:5 and $CHCl_3$:pet. spirit 60-80°C:MeOH, 10:10:2). The MeOH was evaporated off under reduced pressure to afford an orange sticky solid. Na_2CO_3 solution (2.4M, 5ml) was added to the solid, cooling over ice. The resulting yellow solution was extracted with $CHCl_3$ (5 x 15ml), the combined extracts dried (Na_2SO_4) for 4 hrs, filtered and the solvent evaporated off under reduced pressure to afford MP641A (0.23g, crude, wet).

MP641A was purified by column chromatography (SiO_2; $CHCl_3$) to afford MP641B (0.11g, 76.6%) as a brown oil.

^1H NMR (200MHz, $CDCl_3$) δ; 7.97 (d, 1H, $^3J_{H-H}$ =2 Hz, C5-H or C6-H), 7.84 (d, 1H, $^3J_{H-H}$ =2 Hz, C5-H or C6-H), 5.61 (s, (vbr), 2H, NH_2), 5.24 (s, 1H, $CH(OMe)_2$), 3.45 (s, 6H, $O(CH_3)_2$).

Mass spectrum (EI) m/e; 169 $(M)^+$, 153 $(M - NH_2)^+$, 138 $(M - OMe)^+$, 124 (M - OMe - Me)$^+$, 109 $(M - (OMe)_2)^+$, 95 ($M - CH(OMe)_2)^+$.

Note. The experiment was repeated thrice on a 0.016mol scale to obtain MP646B (4.139g, 34.5% combined yield) after freeze drying for 24 hrs.Previously, the same experiment, repeated on 0.0008 and 0.0045 mol scales afforded MP400 (0.075g, 60.4%) and MP402B (1.3g, 17.3% overall yield) after purification by column chromatography (SiO_2; EtOAc:MeOH, 95:5). MP402B was obtained as a brown oil.

3-Dimethoxymethyl-2-N-Phenylpropionamidopyrazine

(**14**-I/4)

3-Dimethoxymethyl-2-pyrazinamine MP402B (0.5g, 0.0029mol) was dissolved in $CHCl_3$ (15ml) and pyridine (1ml) according to the analogous method of Albert and Ohta [100]. The mixture was stirred at rt under N_2 for 30 mins. Hydrocinnamoyl chloride (0.5ml, 0.0033mol) was added dropwise over 10 mins at 0°C, cooling the reaction vessel by means of an acetone/solid CO_2 bath. The reaction mixture was stirred at 0°C for 30 mins. Tlc (SiO_2: EtOAc:MeOH:, 95:5) indicated the presence of s.m. Further hydrocinnamoyl chloride (0.5ml, 0.0033mol) was added dropwise and the mixture stirred at 0°C for 1.5 hrs and at rt for 14 hrs. Tlc indicated the slight presence of a product. Further hydrocinnamoyl chloride (0.5ml, 0.0033mol) and pyridine (0.5ml) were added over 5 mins and the reaction mixture stirred for a further 1.5 hrs at rt and 5hrs at 50°C. Tlc indicated > 60% s.m. The reaction was stopped and the solvents evaporated off under reduced pressure to yield MP603A (2.3g, with residual pyridine) as a brown oil.

The mass spectrum indicated the presence of the M^+ ion at 300 and the correct fragmentation pattern with peaks at m/e; 270 $(M - OCH_3)^+$, 239 $(M - OCH_3 - OCH_3)^+$, 208 $(M - CH_2$-Ph)$^+$. ^1H NMR ($CDCl_3$) showed a mixture.

The first purification of MP603A by column chromatography (SiO_2: pet. spirit 60-80°C:$CHCl_3$:MeOH, 5:5:1) was unsuccesful due to the uneven mixing of solvents. The column was washed with MeOH, the material recovered on evaporation of solvent and re-eluted on a column (SiO_2; EtOAc:pet.spirit 60-80°C, 1:1). The product could not be identified in either of the 6 fractions collected. The ^1H NMR ($CDCl_3$) spectrum indicated pyridine and starting materials.

3-Dimethoxymethyl-2-N-N-bis-phenylpropionamidopyrazine

(MP612E)

The above experiment was repeated on a 0.0024mol scale with 4 mol equivs. of hydrocinnamoyl chloride (1.39ml, 0.0094mol). The mixture was stirred at rt for 5 hrs and heated at 50°C for 24 hrs. The crude material MP609 (1.6g, crude, with residual pyridine) was purified by column chromatography (SiO_2: EtOAc:pet.spirit 60-80°C, 1:1). The crude product had 4 spots corresponding to R_f 0.33 (s.m), 0.46, 0.56 (pyridine) and 0.92 (hydrocinnamoyl chloride). Fractions 1-18 (R_f 0.92) afforded hydrocinnamoyl chloride (0.37g, after freeze-drying for 3 hrs). Fraction 19-25 (R_f 0.56) afforded pyridinium hydrochloride (0.27g, after freeze-drying for 24 hrs). Fractions 26-40 (R_f 0.46) were pooled, the solvent evaporated off under reduced pressure and freeze-dried to afford 3-Dimethoxymethyl-2-N-N-bis-phenylpropionamidopyrazine MP612E (0.47g) as a yellow oil.

^1H NMR (200MHz, $CDCl_3$) δ; 8.58 (d, 1H, $^3J_{H-H}$ = 2 Hz, C5-H or C6-H), 8.53 (d, 1H, $^3J_{H-H}$ = 2 Hz, C5-H or C6-H), 7.29 -7.13 (m, 10H, $NHCO(CH_2\text{-}CH_2\text{-}C_6H_5)$), 5.11 (s, 1H, $CH(OCH_3)_2$), 3.27 (s, 6H, $(OCH_3)_2$), 2.94 - 2.78 (m, 8H, $2.CH_2\text{-}CH_2\text{-}Ph$).

Mass spectrum (EI) m/e; 433 $(M)^+$, 401 $(M - MeOH)^+$, 270 (M - MeOH - $NCOCH_2CH_2Ph)+$, 239 (pyrazine-$NCOCH_2CH_2Ph)+$, 179 (239 - CH_2-Ph)$^+$, 165 (239 - $CH_2CH_2Ph)^+$, 105 $(CH_2CH_2Ph)^+$, 91 $(Ph)^+$, 75 $(pyrazine)^+$.

Note. The experiment was repeated on the same scale as above, with 1 mol equiv. of hydrocinnamoyl chloride. The latter (0.35ml, 0.0024mol) in dry $CHCl_3$ (0.5ml) was added dropwise over 10 mins to 3-dimethoxymethyl-2-pyrazinamine (0.4g, 0.0024mol) in dry $CHCl_3$ (40ml) and dry pyridine (2ml, 0.025mol) at 0°C. The reaction mixture was stirred at 0-5°C for 4 hrs, at 22°C for 14 hrs, at 40°C for 14 hrs and 50°c for 12 hrs. Tlc (SiO_2: EtOAc:pet. spirit 60-80°C) showed s.m. The reaction mixture was then refluxed for 4 hrs. Tlc indicated no change. A further 0.5 mol equiv. of hydrocinnamoyl chloride (0.17ml, 0.0012mol) was added dropwise and the reaction mixture refluxed. At the end of 30 mins, tlc indicated the appearance of a spot of R_f 0.5 (possibly the bis-adduct). The reaction was stopped, cooled to rt and freeze-dried for 24 hrs. MP626A (0.99g) was obtained as a brown oil. The mass spectum did not show the presence of either the required product or the bis-compound. The ^1H NMR spectrum showed a mixture of starting materials and pyridine. The reaction was abandoned.

3-Dimethoxymethyl-2-N-phenylacetamidopyrazine

(**15**-I/4) (MP810, MP812)

Phenylacetyl chloride (1ml, 0.0076mol) was added dropwise over 10 mins, at 0°C, to a solution of 3-dimethoxymethyl-2-pyrazinamine (0.9g, 0.0053mol) in pyridine (10ml). On addition of the acid chloride, the evolution of white fumes of HCl and a colour change of orange to dark brown was observed. The reaction mixture was warmed up to rt and heated under reflux for 1hr. Tlc (SiO_2; EtOAc:pet.spirit 60-80°C, 1:1) showed 6 different spots. At the end of 2 hrs, the reaction was stopped and the pyridine evaporated off under reduced pressure to yield MP810A (3.7g). ^1H NMR and the mass spectra indicated the presence of the product.

The experiment was repeated on a 0.017mol scale to obtain MP812A (7.41g, crude due to residual pyridine). MP812A was purified by column chromatography (SiO_2:

EtOAc:pet.spirit 60-80°C, 1:1) to afford MP812B (2.57g, 52.6%). In the ^1H NMR spectrum, the proton peaks of the required product were masked by residual pyridine, phenylacetic acid and phenylacetyl chloride.

2-N-Phenylacetamido-3-pyrazinecarbaldehyde

(**15**-I/5) (MP816)

HCl (d 1.88) (0.2ml) was added to pyridine (20ml) at 0°C, cooling the reaction vessel over an acetone/solid CO_2 bath and stirred for 30 mins. 3-Dimethoxymethyl-2-N-phenylacetamidopyrazine (2g, 0.0068mol) was added and refluxed for 1 hr, the reaction progress being monitored by tlc (SiO_2: EtOAc:pet.spirit 60-80°C, 1:1). The reaction was stopped, cooled to rt and the solvent evaporated off under reduced pressure to yield MP816A (1.1g, 67.1% crude).

The ^1H NMR (60MHz) spectrum indicated a mixture of (**15**-I/4) and (**15**-I/5) in a ratio of 5:4 based on the ratio of proton peak integrals. Other impurities were present too. The first purification by column chromatography (SiO_2: EtOAc:MeOH, 95:5) was unsuccessful. MP816B (0.8g) was rechromatographed on silica with the same eluant as above, to afford MP816C (0.14g), MP816D (0.25g), with a combined yield of (0.39g, 23.7%). The ^1H NMR (60MHz) spectrum indicated a mixture of (**15**-I/4) and (**15**-I/5) in a ratio of 72:27 as well as other impurities. The final fraction MP816E (0.27g) was s.m. (**15**-I/4).

^1H NMR (60MHz, $CDCl_3$) δ; 8.22 (s, 1H, C5-H or C6-H), 8.05 (s, 1H, C5-H or C6-H), 7.23 (s, 5H, C_6H_5), 5.01 (s, 1H, $CH(OMe)_2$), 3.8 (s, 2H, CH_2-Ph), 3.18 (s, 6H, $O(CH_3)_2$).

2-Benzylpteridine

(15)

NH_3 gas (5ml) was condensed in abs. EtOH (20ml) at -10 to -5°C. The ethanolic NH_3 was added to crude 2-phenylacetamido-3-pyrazinecarbaldehyde (0.3g, 0.0012mol) at -5°C and stirred at 0°C for 30 mins according to the literature method [100]. Tlc (SiO_2: EtOAc:MeOH, 95:5) indicated s.m. The reaction mixture was stirred at rt for 2 hrs. The colour was a bright mauve. The solvent was evaporated off under reduced pressure to afford MP818 (0.27g).The ^1H NMR, 60MHz spectrum of the crude product showed a multitude of peaks. No pteridine peaks were observed. Purification was abandoned due to lack of time.

Some attempted syntheses of (17).

Reaction with trifluoroacetic anhydride (neat) (MP629)

Trifluoroacetic anhydride (15ml, 0.106 mol, 31.2 equiv.) was added to 2-aminopteridine (0.5g, 0.0034mol) and stirred at rt (~23°C). A colour change from yellow to orange to brown was observed within 10 mins. The reaction was stirred at rt for a further 4 hrs and refidgerated for 12 hrs. Tlc (SiO_2: Et2O:MeOH, 1:1) indicated the presence of a new spot (~40%). On removal of excess anhydride under reduced pressure a black solid MP629A was obtained. MP629A was dissolved in DMF (30ml) and toluene (20ml) and decolourising charcoal (2 spatulas) for 30 mins, filtered by suction followed by gravity and the solvent evaporated off on the freeze-dryer to avoid decomposition by heating. The black tar obtained MP629B was extracted with $CDCl_3$. The ^1H NMR, DMSO spectrum showed DMF peaks. The tar was dissolved in DMSO and the ^1H NMR spectrum recorded. Peaks corresponding to either s.m or product were absent.

Reaction with methyl trifluoroacetate ester in DMF (MP630)

Methyl trifluoroacetate (3.4ml, 0.034mol) was added to a solution 2-aminopteridine (0.5g, 0.0034mol) in DMF (30ml) and stirred at rt. for 18 hrs. Tlc (SiO_2: Et_2O:MeOH, 1:1) indicated no reaction. The reaction was refluxed for 12 hrs, at the end of which the reaction mixture had turned black. The solvent was removed under reduced pressure and the ^1H NMR, DMSO spectrum recorded. The s.m. was obtained.

Reaction with methyl trifluoroacetate ester in MeOH (MP631)

2-Aminopteridine (0.050g, 0.00034mol) was partially dissolved in MeOH (50ml). Methyl

trifluoroacetate (10ml, 0.09mol) was added and stirred at rt for 72 hrs. Tlc indicated no reaction. The reaction mixture was refluxed for 5 hrs and stirred at rt for 2 days. The MeOH was evaporated off and the ^1H NMR spectrum recorded. The s.m. was obtained.

Reaction with trifluoroacetic anhydride in trifuoroacetic acid (TFA) (MP632)

2-Aminopteridine (0.050g, 0.00034mol) was reacted with trifluoroacetic anhydride (1ml, 0.006mol) in TFA (5ml, 0.06mol) at rt for 1 hr. The reaction had turned black. At the end of 30 mins reflux, the solvents were evaporated off under reduced pressure to afford a black solid. The mass spectrum confirmed the product to be s.m. and TFA. The ^1H NMR spectrum showed peaks due to s.m.

Reaction with trifluoroacetic anhydride and 4-dimethylaminopyridine (DMAP) as a catalyst. (MP633)

2-Aminopteridine (0.050g, 0.00034mol) was reacted with trifluoroacetic anhydride (7ml, 0.056mol) and DMAP (0.010g, 0.000082mol) at rt. for 1 hr. Tlc (SiO_2: EtOAc:MeOH, 1:1) indicated the gradual appearance of a yellow long wave active spot. The reaction was stirred for 12 hrs at rt. The reaction mixture had turned a black colour. The solvent was evaporated under reduced pressure and the ^1H NMR spectrum recorded in DMSO. There were no peaks recorded. The product had decomposed.

The experiment was repeated on the same scale as above. Additionally, the apparatus was flame-dried, the reaction was performed under N_2, the reaction vessel was covered with silver foil to prevent exposure to UV and visible light and a drying tube attached to the condenser. The reaction mixture was brown at the end of 3 hrs and black at the end of 24 hrs. The ^1H NMR spectrum indicated decomposition of the product.

Reaction with TFA and DCC in dichloromethane (MP682)

To DCC (0.56g, 0.003mol) dissolved in CH_2Cl_2 (10ml), TFA (1.15ml, 0.014mol) was added dropwise via a syringe over 10 mins cooling over ice. A suspension of 2-aminopteridine (0.2g, 0.0014mol) in CH_2Cl_2 (10ml) was added and stirred at rt for 5 hrs, and refluxed for 1 hr. Reaction mixture had turned black. Evaporation of the solvent under reduced pressure gave a black sticky solid. The ^1H NMR spectrum showed no evidence of the required product.

Reaction with trifluoroacetic anhydride in pyridine (MP758)

To a solution of 2-aminopteridine (1g, 0.007mol) in pyridine (20ml), trifluoroacetic anhydride (4ml, 0.027mol, 4 equivs.) was added at rt. An exothermic reaction with evolution of white fumes, and a colour change from orange to brown was observed. At the end of 30mins, tlc (SiO_2; MeCN) showed the absence of s.m. Pyridine was evaporated off under reduced pressure to yield a black tar which was azeotroped with Et_2O (4 x 100ml). The sticky solid was suspended in Et_2O (100ml) and refridgerated for 48 hrs. The black solid (slightly less sticky) was broken down into smaller particles. On filtration, MP758A (3.7g) was isolated as the first crop and MP758B (1.2g) as a second crop. The ^1H NMR spectrum identified both products as pyridine trifluoroacetate. The filtrate was evaporated under reduced presure to afford MP758C (0.065g) as a black gum. The ^1H NMR, 200MHz, DMSO spectrum indicated MP758C to consist mainly of pyridine trifluoroacetate. However, a trace of possible product could be identified. These were peaks at δ 9.7 (s, 1H, C4–H), 9.36 (s, 1H, C7–H) and 9.19 (s, 1H, C6-H). The molecular ion $(M)^+$ was not evident from the mass spectrum.

Some attempted syntheses of (18).

Reaction of 2-aminopteridine with succinic anhydride in pyridine

To a suspension of 2-aminopteridine (0.1g, 0.00068mol) in pyridine (10ml), a solution of succinic anhydride (0.078g, 0.0075mol, 1.1 mol. equiv.) in pyridine (5ml) was added and stirred at rt. for 1hr and heated at 50°C for 2 hrs. Further heating for 2hrs, resulted in a colour change from yellow to orange. The mixture was refluxed for 12 hrs. Tlc (SiO_2; $CHCl_3$:pet. spirit 60-80°C:MeOH, 5:5:1) indicated s.m. The solvent was evaporated off under reduced pressure. The ^1H NMR spectrum indicated starting materials and solvent.

Reaction of 2-aminopteridine with succinic anhydride in DMF (MP653, MP655)

The experiment was repeated on a 0.00034mol scale with 29.4 equivs. of succinic anhydride in DMF (5ml). The reaction mixture was refluxed for 14 hrs. Tlc (SiO_2; $CHCl_3$:pet. spirit 60-80°C:MeOH, 5:5:1) indicated that the reaction had not taken place. The solvent was evaporated off under reduced pressure. The ^1H NMR spectrum indicated starting materials and solvent.

The experiment was repeated on the same scale as above with an excess of succinic anhydride (173.5 equivs). The reaction mixture was refluxed for 24 hrs and stirred at rt. for 3 days. Tlc (SiO_2; EtOAc:MeOH:NH_4OH, 5:1:1) indicated no s.m. Filtration, followed by evaporation gave a sticky brown solid which was azeotroped with Et_2O. Succinic acid was obtained. The ^1H NMR spectrum showed no evidence of product.

Reaction of 2-aminopteridine with succinic anhydride in MeCN (MP654, MP656)

The experiment was repeated on a 0.00034mol scale with a suspension of 29.4 equivs. of

succinic anhydride in MeCN (5ml). The reaction mixture was refluxed for 28 hrs. Tlc (SiO_2; EtOAc:MeOH:NH_4OH) indicated that the reaction had not taken place. A black charred product was obtained. The product was treated with decolourising charcoal (3 spatulas full) for 1hr, filtered, the solvent evaporated off, and dried on a vacuum line (1mmHg/rt.) for 1 hr. The solid obtained was succinic anhydride. The ^1H NMR spectrum indicated neither s.m nor product.

The experiment was repeated on the same scale as above, with an excess of succinic anhydride (58.8 equivs) in MeCN (15ml). The reaction mixture was refluxed for 31 hrs and stirred at rt. for 3 days. Tlc (SiO_2; EtOAc:MeOH:NH_4OH) indicated no s.m. A brownish yellow solution with crystallised anhydride was obtained. Filtration followed by evaporation gave succinic acid and s.m. The ^1H NMR spectrum showed no product.

Reaction of 2-aminopteridine with succinic anhydride at 120°C (MP661)

2-Aminopteridine (0.1g, 0.00068mol) and 15.4 equivs. of succinic anhydride (1.05g, 0.011mol) were heated at 140°C/atmospheric pressure. At 120°C, charring, followed by sublimation of succinic anhydride, was observed. The reaction failed.

Preparation of monobenzylsuccinate

(**18**-I/1a) (MP653, MP665)

A Dean Stark apparatus was assembled. A solution of benzyl alcohol (2.3g, 0.021mol, 1.1 equiv) in benzene (10ml) was added to succinic anhydride (2g, 0.019mol) in benzene (30ml). At the end of 4 hrs reflux, further succinic anhydride (0.54g, 0.0015mol) was added and the mixture stirred at rt for 24 hrs Evaporation of the solvent under reduced pressure, followed by drying on a vacuum line at 1.00mmHg/rt, afforded MP653 (4.22g, 101% crude).

^1H NMR (200MHz) δ; 7.45-7.24 (m, 5H, $C_6\underline{H}_5$), 4.68 (s, 2H, C_6H_5-$C\underline{H}_2$), 2.9 (s, 2H, $C\underline{H}_2$-$C\underline{H}_2$).

Mass spectrum (EI) m/e; 208 $(M)^+$, 117 $(M - CH_2\text{-}Ph)^+$, 91 $(Ph\text{-}CH_2)^+$.

Note. The same experiment repeated on a 0.039mol scale afforded MP665 (8.54g) in 102% crude yield.

Reaction of 2-aminopteridine with monobenzylsuccinate and DCC in DMF (MP669)

A solution of 2-aminopteridine (0.050g, 0.00034mol) in DMF (5ml) was added to monobenzylsuccinate MP653 (0.071g, 0.0034mol) in DMF (5ml) and stirred. To this, DCC (0.077g, 0.00037mol) was added and stirred at rt for 24 hrs and refluxed for 7.5 hrs. Tlc (SiO_2; MeOH:$CHCl_3$, 6:4) indicated no reaction. No peaks due to the desired product could be identified by the ^1H NMR spectrum.

Reaction of 2-aminopteridine with monobenzylsuccinate and EDC in H_2O (MP667)

An emulsion of monobenzylsuccinate (0.1g, 0.00068mol) in H_2O (10ml) was added to a partially dissolved suspension of 2-aminopteridine (0.1g, 0.00068mol) in H_2O (30ml). To this, EDC (0.13g, 0.00075mol) in H_2O (5ml) was added and stirred for 2 hrs. An instantaneous colour change from orange to pale yellow was observed. At the end of 2 hrs reflux, tlc showed s.m.. The solvent was evaporated off on the freeze-dryer. The ^1H NMR spectrum showed no product.

Preparation of benzyl-succinimido-succinate

(**18**-I/2a) (MP663, MP672)

To a suspension of monobenzylsuccinate (3.7g, 0.018mol) in CH_2Cl_2 (35ml), N-hydroxysuccinamide (2.24g, 0.019mol) and melted DCC (3.664g, 0.018mol) were separately added. Instantaneous fizzing and the formation of DCU was observed. Reaction was complete at the end of 2 hrs. Filtration, followed by evaporation under reduced pressure afforded MP663 (5.67g, 100.7%, due to retention of solvent).

^1H NMR (200MH, $CDCl_3$) δ; 7.36 (s, 5H, $C_6\underline{H}_5$), 5.13 (s, 2H, $C\underline{H}_2$-Ph), 3.01-2.9 (m, 2H, BOC-$C\underline{H}_2$-$C\underline{H}_2$), 2.84 (s, 2H, N-CO-$C\underline{H}_2$-$C\underline{H}_2$-CO-).

At a later stage, the same experiment afforded MP672 (6.1g) in 100.3% crude yield.

Reaction of 2-aminopteridine with benzyl-succinimido-succinate in DMF (MP668)

A solution of 2-aminopteridine (0.050g, 0.00034mol) in DMF (10ml) was added to a solution of benzyl-succinimido-succinate (0.11g, 0.00034mol) in DMF and stirred at rt for 12 hrs and refluxed for 7.5 hrs. Tlc indicated no reaction. The solvent was evaporated off to give a black tar. The ^1H NMR spectrum showed no product.

Reaction of 2-aminopteridine with benzyl-succinimido-succinate in DME

(MP673, MP674)

The experiment was repeated as above, on a 0.037mol scale using DME as a solvent. TEA (3ml) was added too. The reaction mixture was stirred at 40°C. At the end of 14 hrs, the reaction mixture had turned a violet colour. The ^1H NMR spectrum did not indicate the desired product.

The experiment was repeated as before, but without heating. At the end of 12 hrs stirring at rt, tlc indicated that the reaction had not taken place. Therefore, the reaction was abandoned.

The experiment was repeated on a 0.00095mol scale. Here, the reaction was maintained at rt for 24 hrs. The solvents were evaporated off under reduced pressure and the crude product suspended in $CHCl_3$. On filtration 2-aminopteridine (0.065g) was isolated. The filtrate was evaporated off and purified by column chromatography (SiO_2; $CHCl_3$:MeOH:$MeCO_2H$, 10:1:0.2). The ^1H NMR spectrum confirmed the recovery of starting materials.

Reaction of 2-aminopteridine with NaH and benzyl-succinimido-succinate in DMSO (MP676)

A solution of NaH (0.025g, 0.00063mol) in anhydrous DMSO (5ml) (previously distilled over CaH_2) was stirred at 0°C (cooling over ice) for 10 mins. 2-Aminopteridine (0.1g, 0.00068mol) in DMSO (5ml) was added dropwise from a pressure equalising funnel, over 20 mins, cooling the reaction vessel over ice. The reaction mixture turned red instantaneously. The reaction mixture was stirred for 2 hrs, maintaining the temperature at 0 to 20°C. Benzyl-succinimido-succinate (0.22g, 0.00069mol) in DMSO (5ml) was added dropwise at 0°C. The reaction mixture turned brown. At the end of 17 hrs stirring at rt, tlc indicated s.m. The solvents were evaporated off under reduced pressure for 12 hrs and a ^1H NMR spectrum recorded. 2-aminopteridine was recovered.

Reaction of 2-aminopteridine with succinyl chloride (MP675)

2-Aminopteridine (0.1g, 0.00068mol) was refluxed in neat succinyl chloride (10ml). At the end of 15 mins, the reaction mixture was dark brown in colour. Tlc (SiO_2; MeOH:$CHCl_3$,

3:2) indicated the absence of s.m. The precipitate was filtered off and washed with Et_2O. The Et_2O started precipitating out more product from the filtrate which was recovered on filtration. Combined product was a greenish brown solid (~4g, wet). The filtrate was evaporated off to give succinic acid. The ^1H NMR spectrum of the residue did not show the required product.

2-N-Succinimido pteridine

(18) (MP804I/UCL1323) (MP754A)

A solution of succinic anhydride (1.36g, 0.014mol) in pyridine (10ml) was added in one step to a solution of 2-aminopteridine (0.5g, 0.003mol) in pyridine (10ml) at rt. To this, 2 drops of concentrated H_2SO_4 was added via a pipette, which instantaneously resulted in a series of colour changes from yellow to orange to brown and finally to a deep purple/brown. The mixture was refluxed for 1hr at the the end of which a further 1 mol equiv. of succinic anhydride (0.34g, 0.003mol) was added. At the end of 3.5hrs, the reaction was complete. Tlc (SiO_2; MeCN) showed a dark brown spot on the baseline, a pink spot (tail) at R_f 0.13 and an intense orange spot of R_f 0.53 corresponding to the product. The solvent was removed under vacuum and azeotroped with Et_2O (3 x 200ml) to give a black tar MP802A which solidified on standing in Et_2O (200ml), for 17 hrs at rt.. A brown solid had formed. Attempts to filter the solid were unsuccesful due to the hygroscopic nature. On filtration, the residue started to turn black and decompose. The residue was washed into the Et_2O filtrate with $CHCl_3$.The filtrate was added to a mixture of $CHCl_3$ (50ml) and MeCN (100ml) and stirred with decolourising charcoal (3 spatulas full) at 50°C for 30 mins. The charcoal was filtered, the solvents removed under vacuum and the residue azeotroped with Et_2O (2x100ml) to give a brown solid MP804A (0.43g). ^1H NMR indicated the correct peaks corresponding to a 2-alkylamidopteridine as well as impurity peaks due to pyridine and succinic acid. MP804A was washed with Et_2O (5x100ml) to remove excess pyridine and succinic acid to yield (0.3g, 38.5% crude yield)

of product which was chromatographed (SiO_2: MeCN). Pooling and evaporation of the first 3 fractions gave MP804C (0.084g, 10.77%) as a mustard solid. The black residue remained on the top of the column.

CHN analysis indicated the product contained some silica. Therefore, MP804C was extracted with MeCN, filtered and the solvent evaporated off under reduced pressure, dried at 60°C/ 0.1mmHg for 4 hrs and then for 24 hrs to obtain MP804I (0.065g, 8.3%) as a brown micro-crystalline solid, mp 215-220°C.

Solubility, sol (DMSO, MeOH, MeCN, EtOH abs), sp. sol (H_2O), insol (Et_2O).

TLC R_f 0.7, 0.66 (SiO_2; MeCN, $CHCl_3$:MeOH, 9:1).

HPLC Major peak 95.4% at 6.43 mins and minor peaks 2.6 and 2.1% at 3.34 and 9.9 mins, (250 x 4.6mm Phenomenex Ultracarb ODS 20 5μm, 1.00 ml/min, UV 254 nm 0.01 aufs., A/B is (90/10) where A is H_2O with 0.01% TEA, 0.1% TFA and hexane sulphonic acid (0.005M) and B is MeOH with 0.01% TEA).

UV (MeOH) λmax; 214, 305nm (logε 3.96, s, 3.75, br).

IR (Nujol mull); 3065 (m, aromatic C-H str), 1781, 1708 (s and br, C=O str and N-H def, coupled, tertiary amide, aromatic C-C str), 1583, 1552, 1453, 1375, 1352 (s, Nujol, aromatic C-C and C=N str, C-H def of $-CH_2-CH_2$) cm^{-1}.

^1H NMR (400MHz, DMSO) δ; 10.01 (s, 1H, C4-H), 9.42 (d, 1H, $^3J_{H-H}$ = 1.7Hz, C7-H), 9.29 (d, 1H, $^3J_{H-H}$ = 1.7Hz, C6-H), 3.30 (s, 4H, CO-CH_2-CH_2-CO).

^{13}C NMR (100MHz, DMSO) ppm; 175.62 (2.C=O), 166.04 (C4), 154.21 (C7 and C9), 152.42 (C2), 149.69 (C6), 133.43 (C10), 28.93 (2.CH_2).

Mass spectrum (EI) m/e; 229 $(M)^+$, 202 $(M - HCN)^+$, 174 $(M - COEt)^+$, 146 $(M - N(COEt))^+$, 131 $(146 - NH)^+$, 119 $(146 - HCN)^+$, 91 $(146 - 2.HCN)^+$, 105 $(146 - HCN - NH_2)^+$, 55 (N-succinamide)$^+$.

Anal. Calcd. for $C_{10}H_7N_5O_2$ + 4% silica gel: C, 50.30%; H, 2.95%; N, 29.33%

Found: C, 50.86%; H, 3.02%; N, 29.33%.

Note. Previously, the experiment performed on a 0.0068mol scale afforded MP754A (2.1g, 76.9% crude yield). Having tried several methods of purification, only 0.1g of crude product remained. On purifying 0.1g by column chromatography (SiO_2: MeCN) as a last resort, 0.027g of MP755C was obtained (insufficient for characterisation and testing).

Some attempted syntheses of (19).

Reaction of 2-aminopteridine with phthallic anhydride in pyridine (MP652)

To a suspension of 2-aminopteridine (0.1g, 0.00068mol) in pyridine (10ml), a suspension of phthallic anhydride (0.11g, 0.00075mol, 1.1 mol equivs.) in pyridine (5ml) was added and stirred at rt for 1hr and heated at 50°C for 2 hrs. The mixture was further refluxed for 12 hrs. The starting materials had dissolved and the reaction mixture was brown. Tlc (SiO_2: $CHCl_3$:pet. spirit 60-80°C:MeOH, 5:5:1) indicated s.m. The solvent was evaporated off under reduced pressure. The ^1H NMR spectrum indicated starting materials and solvent.

Reaction of 2-aminopteridine with NaH and phthallic anhydride in pyridine (MP652)

A suspension of NaH (0.017g, 0.00071mol) in pyridine (20ml) (previously distilled over KOH) was stirred in a flame-dried apparatus for 5 mins under N_2. 2-aminopteridine (0.1g, 0.00068mol) in pyridine (20ml) was added portionwise over 10 mins. An instantaneous colour change from orange to red was observed. The mixture was stirred at rt for 15 mins. Phthallic anhydride (0.11g, 0.00074mol) in pyridine (10ml) was added dropwise over 10 mins. A colour change from red to orange was observed. At the end of 1 hr stirring at rt, the s.m. was recovered.

Reaction of 2-aminopteridine with phthallic anhydride at 120°C (MP662)

2-aminopteridine (0.1g, 0.00068mol) and 15 equivs. of phthallic anhydride (1.56g, 0.011mol) were heated at 140°C/atmospheric pressure. At 120°C, charring followed by sublimation of phthallic anhydride was observed. White crystals of phthallic anhydride had

sublimed on the neck of the reaction vessel. The reaction failed.

2-N- Phthallimido pteridine

(19) (MP808B)

A solution of phthallic anhydride (2.0g, 0.013mol) in pyridine (10ml) was added in one step to a solution of 2-aminopteridine (0.5g, 0.003mol) in pyridine (10ml) at rt. To this, 3 drops of conc. H_2SO_4 acid was added via a pipette, which instantaneously resulted in a series of colour changes from orange to brown to purple and finally to a deep purple/black. The mixture was refluxed for 2.5hrs at the the end of which tlc (SiO_2; MeCN) indicated completion of reaction. The solvent was removed under vacuum and azeotroped with Et_2O (3x100ml) to afford a black tar which would not solidify on standing in Et_2O. The residue was dissolved in a mixture of $CHCl_3$ (50ml) and MeCN (100ml) and stirred with decolourising charcoal (3 spatulas full) at 50ºC for 30 mins. The charcoal was filtered, the solvents removed under vacuum and the residue azeotroped with Et_2O (2x100ml) to afford MP806A as a brown oil. (The ^1H NMR (60MHz, DMSO) spectrum indicated the correct proton peaks evident for the required structure). MP806A was extracted with Et_2O (4x100ml) to remove excess pyridine and phthallic acid. The residue MP806B (1.86g) of was chromatographed (SiO_2: MeCN). Pooling and evaporation of the first 4 fractions (R_f 0.8) afforded MP808A (0.15g, 15.93%) as a brown solid. MP808A was chromatographed a second time (SiO_2; MeCN) to afford MP808B (0.029g) in 3.5% overall yield.

^1H NMR (400MHz, DMSO) δ; 10.01 (s, 1H, C4-H), 9.42 (d, 1H, $^3J_{H-H}$ = 1.7Hz, C7-H), 9.28 (d, 1H, $^3J_{H-H}$ = 1.7Hz, C6-H), 8.09-7.98 (m, 4H, C_6H_4).

Mass spectrum (EI) m/e; 277 $(M)^+$, 249 $(M - HCN)^+$, 222 $(M - 2.HCN)^+$, 147 (2-am.pter.)$^+$, 104 $(147 - HCN)^+$, 76 $(C_6H_4)^+$.

MP808B was pure by ^1H NMR spectroscopy, but not by CHN analysis. Extraction with

^{i}Pr-OH in order to remove possible silica gel did not improve the analysis data. Insufficient material was available for further purification.

Some attempted syntheses of (21)

Reaction of 2-aminopteridine with benzoic anhydride in MeCN (MP689)

2-aminopteridine (0.1g, 0.00068mol) was refluxed with benzoic anhydride (2.3g, 0.0102mol) in MeCN (20ml) for 4 hrs and stirred at rt for 12 hrs. Tlc (SiO_2; pet. spirit 60-80°C:$CHCl_3$:MeOH, 5:5:1) indicated s.m.. The solvent was evaporated off under reduced pressure to afford a black tar. ^1H NMR showed peaks corresponding to the starting materials.

Reaction of 2-aminopteridine with benzoic anhydride in pyridine (MP695B)

2-Aminopteridine (0.1g, 0.00068mol) was refluxed with benzoic anhydride (0.3g, 0.0014mol) in pyridine (5ml) for 30 mins. On cooling to rt, a solid precipitated out. On filtration, 2-aminopteridine MP695A (0.054g) was afforded, mp 280-285°C. The filtrate was evaporated off under reduced pressure to yield MP695B (0.068g, 39.8%) as a brown solid. The ^1H NMR and mass spectrum confirmed the presence of the product.

Reaction of 2-aminopteridine with benzoic anhydride and acetic acid (as a catalyst) in pyridine (MP738B, MP751C)

2-Aminopteridine (1g, 0.0068mol) was refluxed with benzoic anhydride (3g, 0.014mol) and 2 drops of Me_2CO_2H acid in pyridine (20ml). At the end of 4.5 hrs reflux, tlc indicated the presence of s.m. Benzoic anhydride (4g, 0.018mol) was added, refluxed for 1 hr and stirred at rt for 12 hrs. On filtration, 2-aminopteridine was recovered. The filtrate was treated with decolourising charcoal for 30 mins, filtered, the solvent evaporated off under reduced pressure and the brown tar azeotroped with Et_2O. On stirring in Et_2O (50ml), a

floculant solid precipitated out. On filtration MP738B (0.49g, 28.7% crude yield) was obtained as a pink solid, mp 140-150°C. The filtrate was extracted with H_2O. Benzoic acid (3.7g) was recovered. MP738B on further extraction with Et_2O gave MP739C (0.14g). An attempt to purify MP739 by column chromatography (SiO_2; Et_2O:MeOH, 9:1) was unsuccesful. The product had covalently added MeOH across the 3,4 C=N bond to form the adduct. (identified by ^1H NMR spectroscopy).

The experiment was repeated on the same scale as above. The reaction was stopped at the end of 1.5 hrs reflux. MP750A (0.88g) and MP750B (0.58g) were isolatd. Combined yield was 1.46g, 85.5% (crude). Unsuccessful purification attempts included: extraction with toluene; recrystallisation from toluene, and recrystallisation from MeCN. Finally, the product was treated with charcoal in MeCN. On evaporation of the solvent MP751C (0.046g) was obtained. The product, identified by ^1H NMR and mass spectroscopy was impure by CHN analysis.

2-Benzamidopteridine

(21) (MP761C/UCL 1322)

2-aminopteridine MP748A (1g, 0.0068mol) was added to a stirred solution of benzoic anhydride (3.1g, 0.014mol) in pyridine (10ml) and refluxed for 1 hr, at the end of which the reaction mixture had turned a black/mauve colour. As tlc (SiO_2; MeCN) indicated the presence of s.m., the reaction was left stirring for 12 hrs at rt. At the end of a further 1 hrs reflux, the reaction was complete. The solvent was removed at ~95°C/0.1mmHg, using a vacuum pump attached to the rotary evaporator. The black tar MP760A obtained, was dissolved in MeCN (100ml). Decolourising charcoal (~ 3 spatulas full) was added and stirred for 45 mins at ~60°C.The charcoal was removed by filtration. The pink/brown solution obtained was evaporated off under reduced pressure to give MP760B as a brown oil. The oil was dried on a vacuum line rt/1mmHg for ~8 hrs MP760B was then azeotroped with Et_2O (4 x 50ml), suspended in Et_2O (50ml) and refridgerated for ~12 hrs. The pink

solid was filtered off and repeatedly washed with warm Et_2O (4 x 50ml). MP761A (0.99g, 57.9% crude overall yield) was obtained as a pink powder. MP761A (0.2g) was chromatographed (SiO_2; MeCN) to afford MP761B (0.16g) as a beige powder, mp 160-165°C pure by ^1H NMR spectroscopy, but not by CHN analysis.

MP716B (0.1g) was chromatographed a second time (SiO_2; MeCN) to afford a yellow oil which was azeotroped with Et_2O (3 x 20ml) and dried in a vacuum oven at 60°C/0.1mmHg, for 24 hrs MP716C was obtained as a yellow micro-crystalline solid, mp 170-174°C.

Solubility, sol (DMSO, MeCN), sp. sol (MeOH, EtOH abs., $CHCl_3$), insol (pet. spirit 60-80°C, Et_2O).

TLC R_f 0.5 (MeCN); 0.66 and 0.5 trace of adduct ($CHCl_3$:MeOH, 9:1).

HPLC Major peak 97.12% at 7.9 min and minor peaks 0.1 and 2.8% at 3.1 and 4.8 mins respectively, (250 x 4.6mm Kromasil C18 5μm, 1.00 ml/min, UV 254 nm 0.05 aufs., A/B is (55/45) where A is H_2O with 0.01% TEA and B is MeOH with 0.01% TEA).

UV (MeOH) λmax; 234, 254, 323nm (logε 4.06, s, 4.03, sh, 3.83, br).

IR (Nujol mull); 3437, 3371 (m, secondary amide N-H str), 1700, 1633, 1596, 1558 (m/s, secondary amide C=O str and N-H def, C=N str and aromatic C-C str), 979, 826, 774, 721, 675 (s, aryl oop =C-H def,) cm^{-1}.

^1H NMR (400MHz, DMSO) δ; 9.74 (s, 1H, C4-H), 9.24 (d, 1H, $^3J_{H-H}$ = 1.7Hz, C7-H), 9.03 (d, 1H, $^3J_{H-H}$ = 1.7Hz, C6-H), 8.032, 8.014 (d, 2H, $^3J_{H-H}$ = 7Hz, possibly C1-H and C5-H of C_6H_5), 7.65-7.52 (m, 3H, C2-H, C3-H and C4-H of C_6H_5).

Mass spectrum (EI) m/e; 251 $(M)^+$, 147 $(M - NHCOC_6H_5)^+$, 120 (2-ampter. - HCN)$^+$, 105 (2-ampter. - NH and PhCO)$^+$, 93 (2ampter. - 2.HCN)$^+$, 79 (pyrazine)$^+$, 52 (pyrazine - HCN)$^+$.

Anal. Calcd. for $C_{13}H_9N_5O$ + 3% silica gel: C, 60.27%; H, 3.50%; N, 27.03%

Found: C, 60.77%; H, 3.42%; N, 26.67%.

Attempted preparation of phenylacetic anhydride using the method of Burton and Kaye [109]

(22-I/1) (MP742, MP744 and MP768)

P_2O_5 (15.6g, 0.11mol) was added to a stirred solution of phenylacetic acid (15g, 0.1mol) in toluene (100ml) (dried over CaH_2) under Ar. The reaction mixture was mechanically stirred at 100°C for 1.5 hrs according to the literature method [109]. A series of colour changes of white to yellow to orange to brown was observed. The reaction was stopped and filtered whilst hot. The brown residue was extracted with Et_2O (3 x 200ml). The combined extracts were treated with decolourising charcoal, and K_2CO_3 (4g) under N_2, for 1 hr, filtered and the solvent evaporated off under reduced pressure. MP742 (15.3g, 54.7%) was afforded as a white solid, mp 110-120°C, reported 77-78°C [109].

^1H NMR (200MHz,$CDCl_3$) δ; 7.32-7.17 (m, 10H, 2.$C_6\underline{H}_5$), 3.69 (s, 4H, 2.Ph-$C\underline{H}_2$-CO).

cf. phenylacetic acid ^1H NMR (60MHz, $CDCl_3$) δ; 7.3 (s, 5H, $C_6\underline{H}_5$), 3.46 (s, 2H, Ph-$C\underline{H}_2$-CO).

IR (Nujol mull); 1812 (w, C=O str, possibly anhydride), 1760, 1710, 1679, 1603 (m, C=O str, esters, ketones), 849, 814 (m, =C-H def, oopb) cm^{-1}.

cf. phenylacetic acid IR (Nujol mull); 3936, 3731 (m, (br), O-H str, free CO_2H), 1709, 1601, 1548, 1496 (s, C=O str, CO_2H), 839, 750, 699, 673, 603 (m, =C-H def, oopb) cm^{-1}.

Mass spectrum (EI) m/e; 226 (M^+ ion corresponding to Ph-CH_2-CO-O-CH_2-Ph), 210 (Ph-CH_2-CO-CH_2-Ph)$^+$, 178 (Ph-CH_2-CO-CH_3)$^+$, 165 (Ph-CH_2-CO-O-CH(OH))$^+$, 136 (Ph-CH_2-CO_2H)$^+$.

Note. The reaction repeated on the same scale as above afforded MP744 (16.07g, 57.5% crude yield) as a white solid, mp 115-120°C. The reaction repeated on a 0.07mol scale gave MP768 (7.9g, 42.3% crude yield). The ^1H NMR and mass spectra were identical in all three cases.

Reaction of 2-aminopteridine with MP742

2-Aminopteridine (2g, 0.01mol) was added to MP742 (15g, 0.06mol) in pyridine (20ml) (dried over KOH) refluxed for 1.5 hrs and stirred at rt for 14 hrs. Tlc (MeCN) showed the presence of s.m. as well as 6 other spots. The solvent was evaporated off under reduced pressure. Addition of Et_2O (100ml) to the residue resulted in the precipitation of a floculant mustard solid MP743A (2.19g) which was isolated on filtration. The filtrate was evaporated off to obtain an oil (14.7g with residual pyridine). The mass spectrum of MP743A indicated the presence of the (265) M^+ ion, although the expected fragmentation pattern was not seen. The ^1H NMR spectrum did not show the required product. Attempts to recrystallise MP743A from MeCN and toluene were unsuccesful. MP743A was treated with decolourising charcoal in MeCN, filtered and the solvent evaporated off to afford MP743B (0.14g), mp 110-115°C as the first crop and MP743C (0.25g), mp 110-115°C. (similar to that of MP742).

The experiment was repeated with MP744 and MP768 on the same scale as above. The required product was not obtained in either case.

Preparation of phenylacetic anhydride using the method of Cohen and Fager [110]

(22-I/1) (MP778)

A solution of KOH pellets (7.4g, 0.13mol) in H_2O (25ml) was added at rt to a slurry of phenylacetic acid (20g, 0.15mol) in H_2O (21ml) according to the literature procedure [110].

The slurry immediately dissolved to form a clear solution. Phenylacetyl chloride (5ml, 0.037mol) was added dropwise via a 5ml syringe, over 40 mins, maintaining the temperature at 30 to 35°C using an ice bath. The gradual formation of a white precipitate ceased at the end of 30 mins. MP778A (15.4g, 41.3%) was obtained as a white solid (mp 65-70°C, reported 77-78°C [110]) on filtration and washing with cold H_2O. MP778A was refridgerated for 12 hrs and dried on a vacuum line at rt/1mmHg, for 12 hrs to obtain MP778B.

^1H NMR (200MHz) δ; 7.29 (s, 10H, 2.C_6H_5), 4.21 (s, 4H, 2.Ph-CH_2-CO) and a trace of the acid.

IR (Nujol mull); 1815 (s, C=O str, anhydride), 1763, 1737, 1709, 1678, 1603 (m, C=O str, esters, ketones), 814, 787, 776, 751, 728, 696 (m, =C-H def, oopb) cm^{-1}.

Mass spectrum (EI) m/e; 255 $(M)^+$, 227 $(M - CO)^+$, 181 $(M - CO - CO_2H)^+$, 136 $(M - CO-CH_2-Ph)^+$, 119 $(Ph-CH_2-CO-)^+$, 91 $(Ph-CH_2)^+$.

Reaction of 2-Aminopteridine with phenylacetic anhydride

2-Aminopteridine (0.5g, 0.0034mol) was added to phenylacetic anhydride MP778B (2g, 0.0068mol) in pyridine (10ml). Conc. H_2SO_4 acid (2 drops) were added and stirred at 40°C for 5 hrs at the end of which tlc (SiO_2; EtOAc:MeOH:NH_4OH, 5:1:1) indicated a new spot. The reaction mixture was stirred at rt for 80 hrs. The reaction was not complete. However, the reaction mixture was not refluxed in fear of decomposition. The pyridine was evaporated off under reduced pressure at 40°C/0.1mmHg to obtain a dark brown residue MP780A. On addition of Et_2O, a brown floculant solid precipitated out. On filtration, MP780B (0.99g) was obtained. The appropriate pteridine peaks could not be detected by ^1H NMR (200MHz, DMSO) spectroscopy. The mass spectrum showed the presence of 265 $(M)^+$ ion.

MP780B (0.4g) was purified by column chromatography (SiO_2; MeCN). Fractions (1-3)

MP780C (0.005g, 0.6%), (4 and 5) MP780D (0.016g) and (8-10) MP780E (0.029g) were collected and analysed by ^{1}H NMR spectroscopy. The brown residue remains on top of the column. MP780D and MP780E consisted largely of s.m. and (**22**-I/1).

MP780C: ^{1}H NMR (400MHz, DMSO) δ; 9.66 (s, 1H, C4-H), 9.19 (d, 1H, $^3J_{H-H}$ = 0.97Hz, possibly C7-H), 8.97 (d, 1H, possibly C6-H), 7.43-7.19 (m, 23H, possibly C_6H_5 of (**22**) as well as (**22**-I/1)), 3.92 (s, 2H), 3.88 (s, 2H), 3.75 (s, 2H), 3.58 (s, 2H).

Synthesis of (23).

2-Amino-7(8H)pteridinone-6-carboxylic acid

(23) (MP692C/UCL1180)

Alloxan monohydrate (2.83g, 0.018mol) in NaOH (25ml), was added to a solution of 2,4,5-pyrimidinetriamine MP686 (2g, 0.016mol) in aqueous NaOH (25ml) according to the analogous method [111] and refluxed for 7 hrs and stirred at rt for a further 18 hrs. Further alloxan monohydrate (1.2g, 0.0081mol) was added, stirred at rt for 22 hrs and refluxed for 6 hrs. The reaction had not gone to completion. At the end of a further 48 hrs at rt, a brown precipitate had formed. MP691A (2.5g) was recovered on filtration. The brown filtrate, treated with decolourising charcoal (~3 spatulas full) at 90°C for 1 hr followed by filtration under gravity gave a clear yellow solution of pH 6. Conc. HCl acid was added dropwise to the solution at 0°C, swirling and cooling the reaction vessel over ice, until the solution was pH 1. Standing at 0°C for 1 hr resulted in the formation of a yellow precipitate. MP692A (1.42g) was obtained on filtration and MP692B (0.3g) on reprecipitation to afford a combined yield of (1.72g, 52.3% crude yield overall).

Products, MP692 A and B were dissolved in boiling H_2O, treated with decolourising charcoal (1 spatula full) for 20 mins, filtered, acidified to pH1 with conc. HCl and the precipitate collected on filtration. MP692C (0.23g, 7.11%) was afforded as a yellow micro-crystalline solid, mp >280°C. decomposes.

Solubility, sol (DMSO), sp. sol (H_2O, MeOH, EtOH), insol (Et_2O, pet. spirit 60-80°C).

TLC R_f 0.5 (SiO_2; EtOAc:MeOH:NH_4OH, 5:1:1).

HPLC could not be performed as the compound was insoluble in most HPLC solvents.

UV (MeOH) λmax; 211, 230, 284, 342nm (log ε 3.68, s, 3.46, sh, 3.50, sh, 3.58, s).

IR (nujol mull); 3924, 3368, 3190 (m, carboxyl O-H str, primary amine N-H str), 1906 (m, aryl carboxyl C=O def), 1627, 1552 (m, secondary amide C=O str, C=N str), 854, 816 (m, aryl =C-H, def, oopb) cm^{-1}.

^1H NMR (400MHz, DMSO) δ; 12.8-12.2 (s, (vbr), 1H, C6-$CO_2\underline{H}$), 8.72 (s, 1H, C4-$\underline{H}$), 7.62 (s, (vbr), 3H, C2-$N\underline{H}_2$ and C7-$N\underline{H}$-).

^{13}C NMR (100MH, DMSO) ppm; 164.18 ($\underline{C}O2H$), 163.37, 160.54 ($\underline{C}ONH$, $\underline{C}4$), 156.47 ($\underline{C}1$-NH2), 150.72, 142.51, 118.82 ($\underline{C}5$, $\underline{C}9$, $\underline{C}10$).

Mass spectrum (EI) m/e; 207 $(M)^+$, 179 $(M - HCN)^+$, 163 $(M - NH_2\text{-}CH_2\text{=}N$ or $M - CO_2H)^+$, 135 $(M - HCN - NH_2\text{-}CH_2\text{=}N$ or $M - HCN - CO_2H)^+$, 119 $(M - NH_2\text{-}CH_2\text{=}N - CO_2H)^+$, 108 $(135 - HCN)^+$, 81 $(135 - 2.HCN)^+$, 44 (CO_2H and/or $NH_2\text{-}CH_2\text{=}N)^+$.

Anal. calcd. for $C_7H_5N_5O_3.1.5H_2O$: C, 35.9%; H, 2.79%; N, 29.90%

Found: C, 36.41%; H, 2.75%; N, 29.48%.

2-Chloroquinazoline [117]

(24) (MP718C/UCL1207)

PCl_5 (5.9g, 0.028mol) was added to a stirred suspension of quinazolin-2-one hydrochloride MP716E (5g, 0.027mol) in $POCl_3$ (150ml, 1.61mol) and refluxed for 50 mins according to the general method [117]. A colour change of yellow to orange was observed. Stirring for a a further hour at rt ensured completion of reaction. The reaction was stopped, cooled to 25°C and the $POCl_3$ evaporated off at 70°C/760mm Hg. The brown/yellow residue was dissolved in $CHCl_3$ (~200ml) and washed with 10% Na_2CO_3 solution (4 x 200ml) until the washings were pH 10-11 and the $CHCl_3$ extracts pH 6. The combined $CHCl_3$ extracts were dried (Na_2SO_4) for 16 hrs, filtered and the solvent evaporated off under reduced pressure at 40°C. MP718A (4.24g, 94.1% crude overall yield) was afforded as a yellow solid.

MP718A (2.5g) recrystallized from pet.spirit (60-80°C) (200ml) gave undissolved MP718B (0.506g) and three successive crops MP718C (0.542g, 12%) mp 99-102°C, MP718D (0.57g, 12.6%) mp 99-102°C and MP718E (0.55g, 12.2%) mp 90-92°C (from pet. spirit 60-80°C), reported 107-108°C [117]. MP718C was selected for characterisation.

Solubility, sol (MeOH, EtOAc, Et_2O), sp. sol (H_2O), insol (pet. spirit 60-80°C).

TLC R_f 0.87 (SiO_2; $CHCl_3$:MeOH, 9:1), 0.9 (SiO_2; EtOAc:MeOH:NH_4OH, 5:1:1), 0.47 (SiO_2; pet. spirit 60-80°C:Et_2O, 1:1).

HPLC 100% at 8.52 mins (250 x 4 Lichrosorb RP select B 7μm, 1ml/min, UV 254nm 0.1 aufs, A/B (50/50) where A is H_2O + 0.1% TFA and B is MeOH + 0.1% TFA).

UV (MeOH) λmax; 230, 266, 311 nm (log ε 4.33, 3.42, br, 3.39, br).

IR (Nujol mull); 1735, 1685, 1652, (s, C=N def), 791, 859, 974 (s, aromatic =C-H def, oopb), 657, 690 (s, aromatic C-Cl str) cm^{-1}.

^1H NMR (200MHz, $CDCl_3$) δ; 9.32 (s, 1H, C4-H), 7.99-7.96 (m, 3H, C5-H , C8-H and C7-H or C6-H), 7.75-7.67 (m, 1H, C6-H or C7-H).

Mass spectrum (EI) m/e; 166 $(M)^+$:164 $(M)^+$ (Cl^{35}:Cl^{37}), 129 $(M - Cl)^+$, 102 $(M - Cl - HCN)^+$, 76 $(M - Cl - 2.HCN)^+$.

Anal. Calcd. for $C_8H_5N_2Cl$: C, 58.37%; H, 3.06%; N, 17.02%; Cl, 21.54%

Found: C, 58.39%; H, 2.84%; N, 16.95%; Cl, 21.49%.

Note. The same experiment, repeated at a later stage on a 0.029mol. scale, afforded MP782A in 72.7% crude overall yield.

2-Methoxyquinazoline [60]

(25) (MP721A/UCL1206)

Na metal (0.164g, 0.0071mol) was reacted with MeOH (8ml) (distilled over Mg metal and dried over 4Å molecular sieves), under N_2 at rt. 2-Chloroquinazoline MP718A (0.51g, 0.0031mol) in MeOH (2ml) was added and refluxed. A colour change of orange to yellow with the formation of a white precipitate was observed within 20 mins of reaction. At the end of 5 hrs, the reaction was cooled to rt, the NaCl filtered off and the solvent evaporated off under reduced pressure. The residue was azeotroped with Et_2O (3 x 100ml) to give a sticky solid of two components, (mp 53-56°C and mp > 250°C). Heating in H_2O (~30ml) resulted in the formation of a yellow/orange oil which on refridgeration at 0°C for 16 hrs followed by standing at rt for 5 hrs afforded white needles. The needles were filtered off and dried in a drying piston at 30°C/0.1mm Hg, for 18 hrs to afford MP721A (0.169g,

34.7%) mp 45-50°C, reported 50-55°C [60].

Solubility, sol (MeOH, EtOH, EtOAc), sp. sol (H_2O), insol (pet. spirit 60-80°C).

TLC R_f 0.8 (SiO_2; $CHCl_3$:MeOH, 9:1).

HPLC 99.95% at 9.77 min (250 x 4 Lichrosorb RP select B 7μm, 1ml/min, UV 254nm 0.1 aufs, A/B (50/50) where A is H_2O + 0.1% TFA and B is MeOH + 0.1% TFA).

UV (MeOH) λmax; 220, 256, 322 (loge 4.32, 3.43, sh, 3.47, br).

IR (Nujol mull); 1621, 1590, 1572 (s, C=N str), 1459 (s, CH3 def), 1376, 1045 (s, C-O str), 799, 762, 722 (aryl, =C-H def, oopb) cm^{-1}.

^1H NMR (200MHz, $CDCl_3$) δ; 9.24 (s, 1H, C4-H), 7.88-7.783 (m, 3H, C5-H , C8-H and C7-H or C6-H), 7.48-7.45 (m, 1H, C5-H or C6-H), 4.15 (s, 3H, O-CH_3).

Mass spectrum (EI) m/e; 160 $(M)^+$, 145 $(M - Me)^+$, 133 $(M- HCN)^+$, 129 $(M - OMe)^+$, 102 $(129 - HCN)^+$, 77 $(Ph)^+$, 31$(OMe)^+$.

Anal. Cald. for $C_9H_8N_2O$: C, 67.48%; H, 5.03%; N, 17.49%

Found: C, 67.69%; H, 5.03%; N, 17.35%.

2-Benzoxyquinazoline

(26) (MP756A/UCL1247)

Na metal (0.17g, 0.0074mol) was reacted with $PhCH_2OH$ (8ml) (dried overnight over activated 4Å molecular sieves), under N_2 at rt for 20 mins. 2-Chloroquinazoline MP718A (0.51g, 0.0031mol) in $PhCH_2OH$ (10ml) was added and heated at ~70°C. An instantaneous colour change of orange to yellow with the formation of a white precipitate was observed. At the end of 20 mins, when the reaction was complete, $PhCH_2OH$ was

evaporated off at 90°C/0.1mmHg to give a yellow gum MP724A (8.3g, wet) which would not solidify on refridgeration (12 hrs). A mass spectrum confirmed the presence of the product. Azeotroping with Et_2O (3 x 200ml) and further drying resulted in the formation of a solid MP724B (4.78g).

Attempted recrystallisation from H_2O was unsuccessful. MP724B (4.78g) was suspended in Et_2O (100ml), stirred and filtered. The filtrate was evaporated off to afford MP725A (0.1g) as an oil which would not recrystallise from H_2O. Purification by prep. HPLC (see conditions below) afforded MP725B (0.050g, 7%), mp 60-65°C, as the first crop on crystallisation from the HPLC (H_2O:MeOH, 30:70) mixture. Elemental analysis suggested 1.5% inorganic impurity. MP725B (0.050g) was dissolved in propan-2-ol (30ml) filtered and the solvent evaporated off under reduced pressure over 6 hrs Drying at rt/0.1mmHg for 12 hrs, followed by 30°C/0.1mmHg for 6 hrs, afforded MP756A (0.040g, 5.6%) as a white solid, mp 70-73°C.

Solubility, sol. (MeOH, Et_2O, $CHCl_3$), sp. sol. (pet. spirit 60-80°C), insol. (H_2O).

TLC R_f 0.5 (SiO_2; pet. spirit 80-100°C: Et_2O, 50:50), 0.43 (SiO_2; $CHCl_3$)

HPLC Major peak 99.6% at 7.6min and minor peak 0.41% at 2.3min (Lichrosorb RP select B 7μm, 1.00ml/min, UV 254nm, A/B is (30/70) where A is H_2O with 0.1% TFA and B is MeOH with 0.1% TFA).

UV (MeOH) λmax; 221, 256, 317nm (logε 4.54, s, 4.38, br, 3.43, br).

IR (Nujol mull); 1590, 1571, 1558, 1459, 1411 (m, aromatic C=N def), 1212, 1156, 1143, 1078 (m, C-O def), 909, 873 (s, aromatic =C-H def, oopb) cm^{-1}.

^1H NMR (200MHz, $CDCl_3$) δ; 9.51 (s, 1H, C4-H), 8.13-7.38 (m, 9H, C5-H , C8-H , C7-H , C6-H) and C_6H_5), 5.53 (s, 2H, O-CH_2).

Mass spectrum (EI) m/e; 236 $(M)^+$, 207 $(M - HCN)^+$, 147 $(M - CH_2Ph)^+$, 130 $(M - OCH_2Ph)^+$, 108 $(OCH_2Ph)^+$, 91 $(CH_2Ph)^+$, 77$(Ph)^+$.

Anal. Calcd. for $C_{15}H_{12}N_2O$. $0.25H_2O$: C, 74.80%; H, 5.02%; N, 11.63%
Found: C, 75.14%; H, 5.02%; N, 11.58%.

2-Ethoxyquinazoline

(27) (MP774D/UCL1320)

Na metal (0.193g, 0.0084mol) was reacted with abs. EtOH (8ml) (distilled over Mg metal and dried over 4Å molecular sieves for 2 days), under N_2 at rt for 10 mins. 2-Chloroquinazoline MP718D (0.51g, 0.0031mol) in abs. EtOH (2ml) was added and refluxed. A colour change of orange to bright yellow with the formation of a white precipitate was observed within 3 mins. At the end of 2 hrs, the reaction was cooled to rt, the NaCl filtered off and the solvent evaporated off under reduced pressure and azeotroped with Et_2O (3 x 100ml) to give a brown/orange solid MP774A. Recrystallization from H_2O, gave undissolved MP774B (0.137g), and MP774C (0.092g, 17.3%) as a brown solid mp 55-60°C. Further purification by prep. HPLC afforded MP774D (0.048g, 8.9%) mp 55-60°C.

Solubility, sol (MeOH, Et_2O, dil. HCl, dil. Na_2CO_3), sp. sol (H_2O).

TLC R_f 0.64 (SiO_2; pet. spirit 80-100°C: Et_2O, 50:50), 0.68 (SiO_2; $CHCl_3$).

HPLC Major peak 97.5% at 4.9 min and minor peaks 0.29% and 2.12% at 2.32 and 4.2 mins (250 x 4 Lichrosorb RP select B 7µm, 1.00ml/min, UV 254nm, A/B is (30/70) where A is H_2O with 0.1% TFA and B is MeOH with 0.1% TFA).

IR (KBr disc); 1575, 1558, 1482, 1412, 1383 (m/s C=N def, aryl C-C str), 1217, 1147, 1131, 1114 (s, C-O def, $C\underline{H}_3$ str), 800, 787, 763, 743, 721 (s, aryl =C-H def, oopb) cm^{-1}.

UV (MeOH) λmax; 225, 254, 324nm (logε 4.306, br, 3.48, sh, 3.39, s).

^{1}H NMR (400MHz, $CDCl_3$) δ; 9.21 (s, 1H, C4-H), 7.84-7.77 (m, 3H, C5-H , C8-H and C7-H or C6-H), 7.45-7.41 (m, 1H, C5-H or C6-H), 4.54 (q, 2H, $^3J_{H-H}$ = 7Hz, 0-CH_2CH_3), 1.48 (t, 3H, $^3J_{H-H}$ = 7Hz, 0-CH_2CH_3).

Mass spectrum (EI) m/e; 174 $(M)^+$, 145 (M -Et or M -HCN)$^+$, 129 (M -OEt)$^+$, 77 $(Ph)^+$, 45 $(EtOH)^+$.

Anal. Calcd. for $C_{10}H_{10}N_2O$. 6% inorganic: C, 64.81%; H, 5.44%; N, 15.11%

Found: C, 64.75%; H, 5.79%; N, 15.00%.

2-Propoxyquinazoline

(28) (MP772C/UCL1278)

Na metal (0.191g, 0.0083mol) was reacted with propan-1-ol (8ml) (distilled over Mg metal and dried over 4Å molecular sieves for 2 days), under N_2 at rt for 10 mins. 2-Chloroquinazoline MP718C (0.52g, 0.0032mol) in propan-1-ol (2ml) was added and refluxed. An instantaneous colour change from orange to yellow with the formation of a precipitate was observed. At the end of 2 hrs, $CHCl_3$ (5ml) was added to dissolve any precipitated product, the reaction mixture filtered, the solvent evaporated off under reduced pressure and azeotroped with Et_2O to afford MP772A (1.23g) as a yellow oil. Attempted crystallization from H_2O was unsuccessful. The product was extracted with Et_2O (3 x 100ml), the Et_2O removed under reduced pressure and the residual oil dried at 70°C/0.1mmHg on the rotory evaporator for 4 hrs. Analytical HPLC indicated 99.5% purity. Freeze drying overnight afforded MP772C (0.150g, 25%) as a yellow solid, mp 30-32°C.

Solubility, sol (MeOH, dil. HCl, dil. Na_2CO_3, Et_2O), sp. sol. (H_2O).

TLC R_f 0.47 (SiO_2; pet. spirit 60-80°C:Et_2O, 1:1), 0.6 (SiO_2; $CHCl_3$).

HPLC 99.47% at 5.71 min and 0.5% at 4.59 min (250 x 4 Lichrosorb RP select B 7μm, 1ml/min, UV 254nm 0.1 aufs, A/B (30/70) where A is H_2O + 0.1% TFA and B is MeOH + 0.1% TFA).

UV (MeOH) λmax; 226, 255, 324nm (logε 4.37, v. s, 3.55, sh, 3.50, br).

IR (KBr disc); 1558 (m, C=N def), 1212, 1168, 1140, 1127 (s, C-O def), 800, 786, 760, 722 (aryl =C-H def, oopb) cm^{-1}.

^1H NMR (400MHz, $CDCl_3$) δ; 9.22 (s, 1H, C4-H), 7.852-7.804 (m, 3H, C5-H , C8-H and C7-H or C6-H), 7.47-7.43 (m, 1H, C5-H or C6-H), 4.46 (t, 2H, $^3J_{H-H}$ = 7Hz, 0-CH_2-CH_2-CH_3), 1.94-1.87 (m, 2H, 0-CH_2CH_2-CH_3), 1.09 (t, 3H, $^3J_{H-H}$ = 7Hz, 0-CH_2-CH_2CH_3).

Mass spectrum (EI) m/e; 189 $(M)^+$, 147 $(M - Pr)^+$, 131 (M - $OPr)^+$, 118 $(147 - HCN)^+$, 77 $(Ph)^+$, 43 $(Pr)^+$.

Anal. Calcd. for $C_{11}H_{12}N_2O$: C, 70.19%; H, 6.43%; N, 14.88%

Found: C, 70.19%; H, 6.65%; N, 14.68%.

2-Butoxyquinazoline

(29) (MP776E)

Na metal (0.260g, 0.0113mol) was reacted with butan-1-ol (8ml) (distilled over Mg metal and dried over 4Å molecular sieves for 1 day), under N_2 at 30°C for 20 mins. 2-Chloroquinazoline MP718E (0.52g, 0.0032mol) in butan-1-ol (5ml) was added and heated

at 117°C. An instantaneous colour change from orange to yellow with the formation of a precipitate was observed. At the end of 1.5 hrs, the reaction mixture was cooled to rt, filtered and the solvent evaporated off to afford an oil (0.176g, 26.17% crude yield overall). Analytical HPLC (same conditions as below) indicated 61% purity. Purification by prep. HPLC afforded 3 fractions MP776B (0.03g), MP776C (0.063g) and MP776D (0.085g). The required product was in fraction MP776C. Further purification by prep. HPLC afforded MP776E (0.024g, 37%) as an oil.

HPLC 87.9% at 6.15 mins and 5 other impurity peaks (See conditions below.)

Solubility, sol. (MeOH, EtOH, $CHCl_3$), insol. (H_2O).

TLC R_f 0.75 trace, 0.61 major spot, 0.46 trace (SiO_2; pet. spirit 60-80°C : Et_2O, 1:1), 0.9 trace, 0.8 major spot, 0.5 trace (SiO_2; $CHCl_3$).

HPLC Major peak 85.41% at 7.4 min (250 x 4 Lichrosorb RP select B 7µm, 1.00ml/min, UV 254nm, A/B is (30/70) where A is H2O with 0.1% TFA and B is MeOH with 0.1% TFA).

UV (MeOH) λmax; 222, 254, 323nm (logε 4.29, v. s, 3.35, sh, 3.29, br).

^1H NMR (200MHz, $CDCl_3$) δ; 9.18 (s, 1H, C4-H), 7.78-7.82 (s, 3H, C5-H , C8-H and C7-H or C6-H), 7.46-7.40 (m, 1H, C5-H or C6-H), 4.46 (m, 2H, 0-$C\underline{H}_2$-$(CH_2)_2CH_3$), 1.79 (t, 2H, 0-$CH_2C\underline{H}_2$-CH_2CH_3), 1.48 (t, 2H, 0-CH_2CH_2-$C\underline{H}_2CH_3$), 0.97 (t, 3H, 0-CH_2-CH_2-$CH_2C\underline{H}_3$).

Mass spectrum (EI) m/e; 203 $(M)^+$, 161 $(M - Pr)^+$, 147 $(M - Bu)^+$, 133 $(M - OBu)^+$, 73 $(M - BuOH)^+$, 59 $(Bu)^+$, + impurity peaks at 225, 239, 251, 265, 279, and 291.

Anal. Calcd. for $C_{12}H_{14}N_20$: C, 71.2%; H, 6.97%; N, 13.85%.

Found: C, 53.74%; H, 4.93%; N, 9.05%.

2-Ethylthioquinazoline

(30) (MP790F/UCL1279)

Na metal (0.085g, 0.0037mol) was reacted with abs. EtOH (10ml) (distilled over magnesium and dried over 4Å molecular sieves), under N_2 at rt for 15 mins. Ethanethiol (0.3ml, 0.0041mol) was added via a syringe in one step which resulted in a colour change from colourless to pale yellow. 2-Chloroquinazoline MP782B (0.5g 0.00304mol) in EtOH (15ml) was added and refluxed for 1.25 hrs. The reaction was stopped, cooled to ~40°C and N_2 flushed through the solution for 1.45 hrs to remove excess ethanethiol. The solvent was removed under reduced pressure and azeotroped with Et_2O (2 x 50ml) to afford MP790A (1.814g) as a yellow solid. A suspension of MP790 (1.8g) in H_2O (30ml) was extracted with $CHCl_3$ (4 x 200ml), the combined extracts dried (Na_2SO_4) filtered and evaporated to dryness to afford MP790B (0.44g, 75.8% crude overall yield) as a yellow oil. Purification by column chromatography (SiO_2; $CHCl_3$) afforded MP790C (0.231g, 39.9%) as a yellow oil.

Solubility, sol (MeOH, dil. HCl, dil. Na_2CO_3, Et_2O), insol (H_2O).

TLC R_f 0.6 (SiO_2; pet. spirit 60-80°C:Et_2O, 1:1), 0.6 (SiO_2; $CHCl_3$).

HPLC 99.7% at 6.09 min and 0.28% at 3.15 min (250 x 4 Lichrosorb RP select B 7μm, 1ml/min, UV 254nm 0.1 aufs, A/B (30/70) where A is H_2O + 0.1% TFA and B is MeOH + 0.1% TFA).

UV (MeOH) λmax; 209, 245, 256, 336nm (logε 4.13, v. s, 4.34, 4.34, 3.44, br).

IR (Nujol mull); 2955, 2919, 2865, 2733, 2539, 2395 (s, Nujol, aliphatic C-H str, CH_3CH_2 str), 1546, 1558, 1581 (m, C=N def), 865, 791, 781, 755 (m, aryl =C-H def, oopb), 692, 681, 658, 600 (m, C-S str) cm^{-1}.

^1H NMR (400MHz, $CDCl_3$) δ; 9.03 (s, 1H, C4-H), 7.72-7.67 (m, 3H, C5-H , C8-H and C7-H or C6-H), 7.39-7.36 (m, 1H, C5-H or C6-H), 3.18 (q, 2H, $^3J_{H-H}$ = 7Hz, 0-CH_2-

CH_3), 1.94-1.87 (m, 2H, 0-$CH_2\underline{CH_2}$-CH_3), 1.36 (t, 3H, $^3J_{H-H}$ = 7Hz, 0-CH_2-$\underline{CH_3}$).

Mass spectrum (EI) m/e; 190 $(M)^+$, 163 $(M - HCN)^+$, 161 $(M - Et)^+$, 129 $(M - SEt)^+$, 102 $(129 - HCN)^+$, 77 $(Ph)^+$, 61 $(SEt)^+$.

Anal. Calcd. for $C_{10}H_{10}N_2S$: C, 63.13%; H, 5.29%; N, 14.72%

Found: C, 62.99%; H, 5.61%; N, 14.80%.

2-Benzylthioquinazoline

(31) (MP798C/UCL1280)

Na metal (0.090g, 0.0039mol) was reacted with abs. EtOH (10ml) (distilled over Mg metal and dried over 4Å molecular sieves), under N_2 at rt for 20 mins. Phenylmethyl thiol (0.4ml, 0.0034mol) was added via a syringe in one step and stirred for 2 mins. 2-Chloroquinazoline (0.5g 0.003mol) in abs. EtOH (20ml) was added and refluxed for 3hrs. The reaction mixture a clear orange to start with had turned yellow with a precipitate. Flushing the apparatus with Ar gas over 24 hrs removed excess alcohol and thiol to leave behind a mustard residue. A suspension of this residue in H_2O (35ml) was extracted with $CHCl_3$ (3x200ml), the combined extracts dried (Na_2SO_4) for 24 hrs, filtered, evaporated off at 50°C under reduced pressure and azeotroped with Et_2O (3 x 100ml) to afford MP798A (0.283g, 38.7%% crude overall yield) as a yellow solid. Analytical HPLC (same conditions as below) indicated the major product in 82.2% at 9.4 mins and other impurity peaks in 1.2, 3.1, 13.2, and 0.37% at 2.52, 3.1, 6.0 and 7.9 mins.

Recrystallization of MP798A from MeOH afforded pale yellow needles of both MP798B (0.025g) mp 82-84°C, and MP798C (0.079g) mp 82-84°C. Combined yield was 14.8%. MP798C was characterised fully .

Solubility, sol (MeOH, dil. Na_2CO_3, Et_2O), insol (H_2O, dil. HCl).

TLC R_f 0.6 (SiO_2; pet. spirit 60-80°C:Et_2O, 1:1), 0.7 (SiO_2; $CHCl_3$).

HPLC 97.3% at 9.43 min and 2.47% at 2.69 min (250 x 4 Lichrosorb RP select B 7μm, 1ml/min, UV 254nm 0.1 aufs, A/B 30/70 where A is H_2O + 0.1% TFA and B is MeOH + 0.1% TFA).

UV (MeOH) λmax; 210, 245, 258, 336nm (logε 4.19 s, 4.41, 4.41, 3.46 br).

IR (Nujol mull); 1610, 1590, 1579, 1554, 1546 (s/m, C=N def), 800, 790, 779, 758, 722, 703 (m, aromatic, =C-H def, oopb), 690, 658, 600, 566, 526 (m, C-S str, def) cm^{-1}.

^1H NMR (400MHz, $CDCl_3$) δ; 9.16 (s, 1H, C4-H), 7.89-7.22 (m, 9H, C5-H, C8-H, C7-H, C6-H, and CH_2-C_6H_5), 4.56 (s, 2H, -CH_2-C_6H_5).

Mass spectrum (EI) m/e; 240 $(M)^+$, 213 $(M - HCN)^+$, 149 $(M - CH_2Ph)^+$, 123 $(SCH_2Ph)^+$, 117 $(M - SCH_2Ph)^+$, 91 $(CH_2Ph)^+$.

Anal. Calcd. for $C_{15}H_{12}N_2S$: C, 71.41%; H, 4.79%; N, 11.11%; S, 12.68%

Found: C, 71.10%; H, 4.90%; N, 11.09%; S, 12.73%.

2-Propylthioquinazoline

(32) (MP794F/UCL1321)

Na metal (O. 091g, 0.0039mol) was reacted with abs. EtOH (10ml) (distilled over Mg and dried over 4Å molecular sieves), under N_2 at rt for 15 mins. Propanethiol (0.3ml, 0.0033mol) was added via a syringe in one step and stirred for 2 mins which resulted in a colour change from colourless to pale yellow. 2-Chloroquinazoline (0.5g 0.00304mol) in abs. EtOH (15ml) was added and refluxed. A clear orange solution with a white precipitate had formed at the end of 35 mins. The mixture was refluxed for a further 1.5 hrs. Flushing

the apparatus with N_2 over 1hr at ~50°C removed excess alcohol and thiol to leave behind a sticky mustard residue. The residue was dissolved in EtOH, transferred to a vessel and evaporated to dryness. A suspension of this residue in H_2O (50ml) was extracted with $CHCl_3$ (4 x 200ml), the combined extracts dried (Na_2SO_4) for 24 hrs, filtered, evaporated off at 50°C under reduced pressure and azeotroped with Et_2O (2 x 100ml) to afford MP794A (0.4g, 64.5% crude overall yield) as a yellow oil.

MP794A was purified by column chromatography (SiO_2; $CHCl_3$). Pooling and evaporation of fractions 10-13 afforded MP794D (0.16g, 18.7%) as a brown oil. Analytical HPLC (same conditions as below) indicated 90% purity.

Recrystallization of MP794D from EtOH abs afforded MP794F (0.066g, 10.6%) as a yellow oil.

Solubility, sol (MeOH, Et_2O), insol (H_2O).

TLC R_f 0.6 (SiO_2; pet. spirit 80-100°C:Et_2O, 1:1), 0.6 (SiO_2; $CHCl_3$).

HPLC 98.24% at 8.17 min and 0.22%, 0.27%, 0.303% and 0.93% at 2.3, 2.8 and 5.9mins (250 x 4 Lichrosorb RP select B 7µm, 1ml/min, UV 254nm, A/B 30/70 where A is H_2O + 0.1% TFA and B is MeOH + 0.1% TFA).

UV (MeOH) λmax; 210, 246, 258, 340nm (logε 3.90 s, 3.85, 3.84, 2.91 br).

IR (Nujol mull); 2851, 2722, 2673 (s, Nujol, propyl C-C str, 1558, 1582 (s, C=N def, aryl C-C str), 864, 790, 754, 721 (m, aryl =C-H def, oopb), 692 (s, C-S def) cm^{-1}.

^1H NMR (400MHz, $CDCl_3$) δ; 9.11 (s, 1H, C4-H), 7.79-7.76 (m, 3H, C5-H , C8-H and C7-H or C6-H), 7.48-7.44 (m, 1H, C5-H or C6-H), 3.24 (t, 2H, $^3J_{H-H}$ = 7Hz, -S-CH_2-CH_2-CH_3), 1.839-1.79 (t/q, 2H, $^3J_{H-H}$ = 7Hz, -S-CH_2-CH_2-CH_3), 1.07 (t, 3H, $^3J_{H-H}$ = 7Hz, -S-CH_2-CH_2-CH_3).

Mass spectrum (EI) m/e; 204 $(M)^+$, 189 $(M - CH_3)^+$, 176 $(M - Et)^+$, 162 $(M - Pr)^+$, 130 $(M - SPr)^+$, 103 $(130 - HCN)^+$, 76 $(SPr)^+$.

Anal. Calcd. for $C_{11}H_{12}N_2S.0.3H_2O$: C, 63.01%; H, 6.06%; N, 13.36%; S, 15.29%

Found: C, 62.99%; H, 5.99%; N, 13.28%; S, 15.56%.

2-Ethylaminoquinazoline

(33) (MP800E/UCL1281)

A 50% methanolic solution (40ml) of $EtNH_2$ (20ml, 0.3mol), pre-cooled at 0°C, was added in one step to a solution of 2-chloroquinazoline (0.5g, 0.0039mol) in MeOH (10ml) at -10°C and stirred magnetically at 0°C for 10 mins. The reaction was maintained at 0°C by immersing the reaction vessel in an acetone/solid CO_2 bath. The escape of $EtNH_2$ was minimized by having an acetone/solid CO_2/NaCl/H_2O mixture passing through the condenser. The mixture was allowed to warm up to rt and then refluxed for 2.5 hrs. The solvent was removed under reduced pressure and azeotroped with Et_2O. MP784A (0.849g) was obtained as a yellow residue consisting of two components (mp 45-50°C and mp 210-230°). Analytical HPLC indicated a purity of 70%.

MP784A was column chromatographed (SiO_2; EtOAc:pet. spirit (60-80°C), 1:1) to give MP800C (0.182g, 34.6%), which on recrystallization from propan-2-ol afforded MP800E (0.045g, 6.7%) as yellow crystals, mp 87-90°C.

Solubility, sol (MeOH, Et_2O, dil. HCl, dil. Na_2CO_3), insol (H_2O).

TLC R_f 0.30 (SiO_2; pet. spirit 60-80°C:EtOAc, 1:1), 0.9 (SiO_2; MeCN).

HPLC 99.9% at 2.5 min (250 x 4 Lichrosorb RP select B 7μm, 1ml/min, UV 254nm 0.1 aufs, A/B (30/70) where A is H_2O + 0.1% TFA and B is MeOH + 0.1% TFA).

UV (MeOH) λmax; 205, 241, 363 (logε 3.79, s, 4.49, sh, 4.42, br).

IR (KBr disc); 3262 (s, N-H str), 2965, 2925 (m, CH_2CH_3 str), 1597, 1560 (m, C=N def), 795, 759, 718 (m,aryl, =C-H def, oopb) cm^{-1}.

^1H NMR (400MHz, $CDCl_3$) δ; 8.9 (s, 1H, C4-H), 7.69-7.58 (m, 3H, C5-H , C8-H and C7-H or C6-H), 7.27-7.20 (m, 1H, C5-H or C6-H), 5.271-5.264 (s, (vbr), 1H, -NH-CH_2-CH_3), 3.62-3.55 (m, 2H, $^3J_{H-H}$ = 7Hz, -NH-CH_2-CH_3), 1.30 (t, 3H, $^3J_{H-H}$ = 7Hz, -S-CH_2-CH_2-CH_3).

Mass spectrum (EI) m/e; 204 $(M)^+$, 189 $(M - CH_3)^+$, 176 $(M - Et)^+$, 162 $(M - Pr)^+$, 130 $(M - SPr)^+$, 103 $(130 - HCN)^+$, 76 $(SPr)^+$.

Anal. Cald. $C_{10}H_{11}N_3$: C, 68.78%; H, 6.48%; N, 23.66%

Found: C, 68.95%; H, 6.62%; N, 23.54%.

CHAPTER 7

BIOLOGICAL TESTING

The biological tests were carried out at Centre Paul Broca de l'I.N.S.E.R.M., Paris, by Ms. C. Rose, Mr. P. Bourgeat and Dr. F. Vargas.

The general *in vitro* bioassay systems utilised for the testing of compounds against the CCK-inactivating peptidase, trypsin, chymotrypsin, PPE and HLE will be discussed briefly.

First, it is necessary to give a brief summary of the characterisation and development of a suitable bioassay system of the CCK-inactivating peptidase, as the testing of compounds was initially targeted at this enzyme.

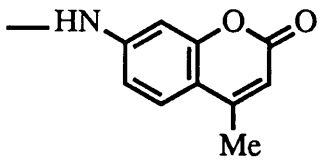
The bioassay systems of the CCK-inactivating peptidase:

As mentioned earlier, the CCK-inactivating peptidase was identified as a physiologically relevant serine endopeptidase cleaving endogenous CCK8 in brain at the level of the Met-Gly and Met-Asp bonds, using depolarised rat brain slices [6,122]. The enzyme was characterised as a membrane-bound elastase-like enzyme by the inhibitory effects of a series of serine reagents and serine proteinase inhibitors [122,123].

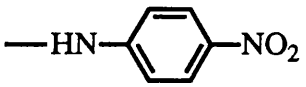
Tests based upon the measurement of endogenous or exogenous CCK hydrolysis were too cumbersome to assay the enzyme activity in purification or extensive pharmacological studies. Therefore a reliable and simple test to measure the activity and test novel compounds was developed by Prof. Schwartz and his team, using chromogenic or flourescent substrates. These first artificial substrates chosen were Suc-Ala-Ala-Pro-Met-

aminomethylcoumarin (Suc-AAPM-AMC) and Suc-Ala-Ala-Pro-Met-p-nitroanilide (Suc-AAPM-pNA).

The structures of AMC and pNA are shown below.



7-Amido-4-methylcoumarin



para -nitroanilide

The former proved to be much more sensitive but less selective and the assay required the co-presence of TLCK in order to avoid the contribution of an interfering activity. The former was therefore abandoned. The latter proved to be more selective and the sensitivity of the assay was enhanced using a colourimetric assay based upon a final diazo reaction. Using this model substrate, the following studies were performed: correlation of activities measured in membranes and brain slices; determination of kinetic parameters of the enzyme activity; determination of subcellular and regional distributions; determination of optimum pH; initial purification studies; subsite characterisation and testing of novel compounds (unpublished results).

Therefore, for approximately two and a half years, the testing of compounds and purification of the enzyme relied upon enzyme activity measurements performed using this substrate which showed excellent correlation between activities measured in membranes and brain slices, until two potent inhibitors were synthesised. MeO-Suc-AAP-boroV and CBz-Val-boro-Nleu (UCL 1158, prepared by Dr. J. Law) showed activity against the purified enzyme (with IC_{50} values of 10 and 91μM respectively) yet were almost inactive against protection of endogenous CCK. The latter was inactive at 0.5mM on protection of endogenous CCK. At this stage, it was concluded that this artifical substrate did not measure the activity of the relevant peptidase, and that the purified enzyme, although a

CCK-hydrolysing peptidase, was presumably not involved in the primary inactivation of the endogenous neuropeptide. (This enzyme was identified as Enzyme 1.)

The foregoing result led to the investigation of several alternative tests to monitor purification and testing of compounds, which were: the protection of endogenous CCK-8 released from depolarised brain slices using an improved radioimmunoassay (RIA) procedure and incubation of brain slices; protection of exogenous CCK-8 from brain slices or membranes using a RIA procedure which measures the formation of Gly-Trp-Met (GWM) and the use of a novel artificial substrate Ala-Ala-Phe-pNA (AAFpNA).

With the first of these, MeOSuc-AAPboroV was effective in protecting endogenous CCK-8 release with an IC_{50} value of 0.2mM whereas UCL 1158 was ineffective at 0.5mM. However, with increasing concentrations, UCL1158 caused a shift in the dose-response curve of MeOSuc-AAPboroV (at 30mM) towards the right, by 2 log units. It seemed that UCL 1158 might be acting on an allosteric site of the relevant CCK-inactivating peptidase to diminish the affinity of inhibitors. Whatever the underlying molecular mechanism, this observation proved to be useful in identifying and purifying the relevant CCK-inactivating peptidase.

The second test involved measuring the formation of Gl-Trp-Met (GWM) by RIA using exogenous CCK-8 in low concentrations as a substrate. Again, UCL 1158 was found to be ineffective as an inhibitor on its own, but in its presence (30mM), the inhibition curve by MeOSuc-AAPboroV was shifted towards the right by two log units. Similar results were obtained when CCK-8 in higher concentrations or when cortical membranes, instead of slices were used.

The artificial substrate was chosen on the basis of its ability to be inhibited by MeOSuc-AAPboroV (3μM), which is known to enhance endogenous CCK recovery maximally. The results of a vast screening programme showed that only a few substrates were almost completely inhibited by the above compound. Amongst these, preference was given to a

substrate with a high rate of hydrolysis and with a pNA group which is more easily used in a large series of assays. Hence, AAFpNA.

In the presence of 500μM of AAFpNA , UCL 1158 was completely ineffective as an inhibitor (IC_{50} >1000μM), whereas the IC_{50} values of the other potent inhibitors, MeOSuc-AAPboroV and AAF-CH_2Cl were in the low μM range (IC_{50} = 0.3 and 10.00μM respectively) . Similar results were obtained when brain slices were used instead of membranes. UCL 1158 had an IC_{50} value >300mM, whereas MeOSuc-AAPboroV and AAF-CH_2Cl had IC_{50} values of 0.16 and 5.4μM respectively. These results indicated the enzyme activity to be that of an ectopeptidase. On cerebral membranes, the apparent KM of AAF-pNA regarding enzyme activity was ~125mM. Comparison of potencies of other inhibitors on CCK-ir released from rat brain slices and on enzyme activity from cortical membranes showed good correlation. Furthermore, the presence of UCL 1158 caused a similar shift in the dose response curve of MeOSuc-AAPboroV, as that seen on exogenous CCK-8 hydrolysis and endogenous CCK-8 recovery. These data gave strength to the idea that the assay based on AAFpNA measures the activity associated with endogenous CCK hydrolysis. Purification of the relevant enzyme (Enzyme 2) was then pursued using this model substrate.

At a later stage, the bioassay system for the testing of compounds against Enzyme 2, was altered again, by using an artificial flourescent substrate, AAF-AMC. The main reason for this choice was that the sensitivity of this substrate was much higher than that of the previous one, which meant that both enzyme and substrate could be used in much smaller concentrations than before. This is especially advantageous for testing sparingly soluble compounds, such as the pteridines, quinazolines and certain dipeptides.

These different assay systems used for monitoring the serine proteinase activity from rat cortical membranes, are described below.

The IC_{50} values were determined from dose-response curves.

Membrane preparation:

Rats were killed by decapitation and the cerebral cortex dissected and cleaned out of meninges and white matter. Tissue (rat brain cortex) was homogeneized in 15 mls of cold buffered 0.32M sucrose (5mM, Tris-Cl pH 7.4) using a Potter-Elvehjem homogeniser. Homogenates were centrifuged at 1,000g for 5 mins. and the supernatant centrifuged at 20,000g for 15 mins and the pellet resuspended in 50mM Tris-Cl, pH 7.4 (5ml/g tissue) with sonication. This suspension was diluted to 40 mg/g tissue and centrifuged at 20, 000g for 15 mins. The pellet was washed twice more with the same buffer in order to eliminate any insoluble enzyme which could have bound onto the membrane. The final pellet was resuspended at 10mg/ml of membrane protein in 50mM Tris-Cl buffer, pH 7.4 (approximately 2ml / cerebral cortex). Membranes were used fresh or frozen within 2 days.

7.1 Fluorometric assay of the CCK-inactivating peptidase activity using Suc-AAPM-AMC as a substrate.

The assay system contained: 100µl of membrane fraction (200µg of protein), 20µl of bestatin (2mM), 20µl of a solution of the inhibitor compound to be tested (ten fold the final desired concentration), 20µl of Me-Suc-Ala-Ala-Pro-Met-AMC (5mM) and 20µl of Tris HCl (50mM, pH 7.4). Separate assay mixtures were prepared as before and 10µl of DFP (40mM) was added. Mixtures were preincubated in the absence of peptide and substrate (Me-Suc-Ala-Ala-Pro-Met-AMC) for 20-45 mins. at 37°C and the proteinase reaction started with the addition of the peptide substrate. Incubation was performed for 60 mins. at 37°C. Reaction was stopped by heating for 10 mins at 95°C. Released AMC was determined at λex = 370nm, λem = 460nm as described by Castillo *et al* (1979) [124].

7.2 Assay of the CCK-inactivating peptidase activity using Suc-AAPM-pNA as a substrate.

The membrane fraction was prepared as described earlier.

The assay system contained: 100µl of membrane fraction (1mg of protein), 40 µl of bestatin (2mM), 40 µl of a solution of the inhibitor compound to be tested (ten fold the final desired concentration), 40 µl of Suc-Ala-Ala-Pro-Met-pNA (5mM) and 180 µl of 50mM Tris-Cl, pH 7.4. Separate assay mixtures were prepared as before and 10 µl of DFP (80 mM) was added.

Mixtures were preincubated in the absence of peptide substrate (Suc-AAPM-pNA) for 20-45 mins. at 37°C and proteinase reaction started with the addition of the peptide substrate. Incubation was performed for 60 mins at 37°C. The reaction was stopped by the addition of 10µl DFP (80mM) followed by heating for 10 mins at 95°C. Assay mixtures were cooled and 20µl of 3M HCl was added. Samples were centrifuged for 15 mins at 13,000 rpm. using an Eppendorf centrifuge.

Serine proteinase activity was determined by the release of p-nitroanilide (pNA) from the peptide substrate. The released pNA was either read at 405nM or coupled with N-naphthyl-N-N-diethylpropylenediamine to produce the diazo derivation according to the method of Bieth and Wermuth (1973) [125] given below.

To 300µl of the acidified and deproteinised sample, 25µl of $NaNO_2$ (12mg/ml) is added and left to stand for 3 mins. To this, 50µl of freshly prepared ammonium sulfamate (30mg/ml) is added and left to stand for 5 mins and finally 25µl of N-naphthyl-N-N-diethylpropylenediamine (12mg/ml) is added.This results in the formation of a rose colour, the intensity of colour depending on the concentration of released pNA.

Samples were then read at 550nm (on a Dynatech Micro Fluor Reader) within 2 hrs of preparation. Using this apparatus, a microplate of 100 wells can be read in 3 seconds.

Thus, serine proteinase activity with different inhibitor concentrations can be assayed over a relatively short period of time.

7.3 Assay of the CCK-inactivating peptidase activity using AAF-pNA as a substrate.

The membrane fraction was prepared as before.

The assay system was the same as that for using Suc-AAPM-pNA as a substrate. The only modification to the procedure was the addition of 10µl of 3M HCl instead of 20µl at the end of the reaction.

7.4 Assay of the CCK-inactivating peptidase activity using Ala-Ala-Phe-AMC as a substrate.

The membrane fraction was prepared as before.

The assay system was the same as that for using MeSuc-AAPM-AMC as a substrate. (i.e. the fluorometric assay.)

The inhibitor compounds were preincubated for 10 mins at 37°C in the incubation media containing the serine proteinase and the reaction started by the addition of the peptide substrate (AAF-AMC). The incubation was continued for 15 mins and the hydrolysed substrate measured by fluorescence on a Dynatech Micro Fluor Reader.

The incubation medium consisted of: 0.1M Potasium phosphate buffer pH 7.4; 0.1% Brij 35; 0.1mM Bestatin; 25mg of membrane protein; 2.5mM NaN_3 and 0.05mM AAF-AMC.

The bioassay systems of trypsin, chymotrypsin, PPE and HLE:

Serine proteinase activity with commercially available enzymes were assayed according to the same method as described above. However, the incubation medium differed for each type of enzyme used.

7.5 Trypsin assay using Z-Arg-AMC as a substrate.

The incubation medium consisted of : 0.1M Potassium phosphate buffer pH 7.4; 0.5M NaCl; 0.1% Brij 35; 0.1% BSA; 50ng Trypsin; 2.5mM NaN_3 and 0.05mM Z-Arg-AMC.

7.6 α-Chymotrypsin assay using AAF-AMC as a substrate.

The incubation medium consisted of : 0.1M Potassium phosphate buffer pH 7.4; 0.5M NaCl; 0.1% BSA (Bovine serum albumin); 0.1% Brij 35; 25ng α-Chymotrypsin; 2.5mM NaN_3 and 0.5mM AAF-AMC.

7.7 PPE assay using Suc-AAA-AMC as a substrate.

The incubation medium consisted of : 0.1M Potassium phosphate buffer pH 7.4; 0.5M NaCl; 0.1% BSA; 0.1% Brij 35; 250ng PPE; 2.5mM NaN_3 and 0.5mM Suc-Ala-Ala-Ala-AMC.

7.8 HLE assay using Suc-AAV-pNA as a substrate.

The assay system was the same as that for using Suc-AAPM-pNA as a substrate for the CCK-inactivating peptidase. The modifications to the procedure included the use of commercially available HLE; the use of Suc-AAV-pNA as a substrate and a different incubation medium.

The incubation medium consisted of 55µl of (PBS + 0.1% BSA); 25ml of HLE solution (5mg of HLE was dissolved in 750ml of H_2O + 0.01% Triton X 100); 10µl of inhibitor compound and 0.5mM of Suc-AAV-pNA in a 1:1 mixture of H_2O: DMSO.

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