SYNTHETIC ROUTES TOWARDS FUNCTIONALISED FLAVINS AS MODELS OF BIOCHEMICAL REDOX SYSTEMS

A thesis submitted by
John Andrew Unikowski
to the
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in application for
the degree of Master of Science

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DECLARATION

I John Andrew Unikowski..... hereby certify that this thesis has been composed by myself, that it is a record of my own work and that it has not been accepted in partial or complete fulfilment of any other degree or professional qualification.

Signed

Date 29/10/93.....

I hereby certify that the candidate has fulfilled the conditions of the Resolution and Regulations appropriate to the degree of M.Sc.

Signature of Supervisor

Date 29/3/94

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ABSTRACT

Recent investigations into biological electron transfer processes have led to the construction of biomimetic systems, a recent example being the biometal electrode. Related work had shown the gold-phenylthiourea flavin electrode to be stable, exhibiting fast electron transfer rates when measured by cyclic voltammetry.

Various N^{10}-phenethylflavins were consequently synthesised, leading to the formation of flavins with terminal amino and thiol functionality. Suitable routes towards these substituted phenylthiourea flavins were investigated. It was found that the mixed anhydride coupling reaction, originally developed for peptide synthesis, proved to be a facile method for the synthesis of functionalised thiourea flavins, especially the bis-phenylthiourea flavins. This method proved to be more successful than the other two methods attempted: the BTBO-mediated reaction of flavins with thioureas and coupling via phenyl esters.

GLOSSARY

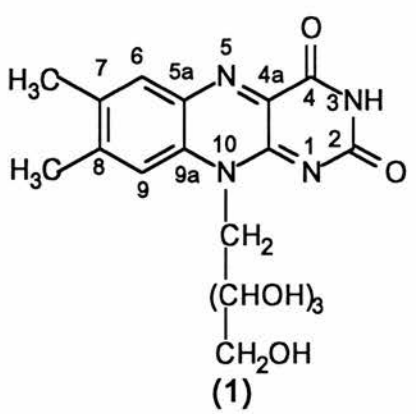
Abbreviation	Meaning
Ac	acetyl
ATP	adenosine-5/-triphosphate
BTBO	1,1/-bis-(6-trifluoromethyl)benzotriazole oxalate
cyt	cytochrome
DMAP	4-N,N-dimethylaminopyridine
DMF	N,N-dimethylformamide
DMSO	dimethyl sulfoxide
ETC	electron transport chain
FAD	flavin adenine dinucleotide
FADP	flavin adenine dinucleotide phosphate
FMN	flavin mononucleotide, a trivial name commonly used, the recommended IUPAC-IUB name is now actually riboflavin-5/-phosphate
NAD	nicotinamide adenine dinucleotide
NADP	nicotinamide adenine dinucleotide phosphate
NMM	N-methyl morpholine
PTSA	*p*-toluenesulfonic acid (*p*-tosylic acid)
py	pyridine
rf.	reflux
sp. gr.	specific gravity
TFA	trifluoroacetic acid
THF	tetrahydrofuran
T.l.c.	thin layer chromatography
Ts	*p*-toluenesulfonyl (*p*-tosyl)

CONTENTS

1.0. INTRODUCTION

1.1. The Biochemical Importance of Flavins as Coenzymes

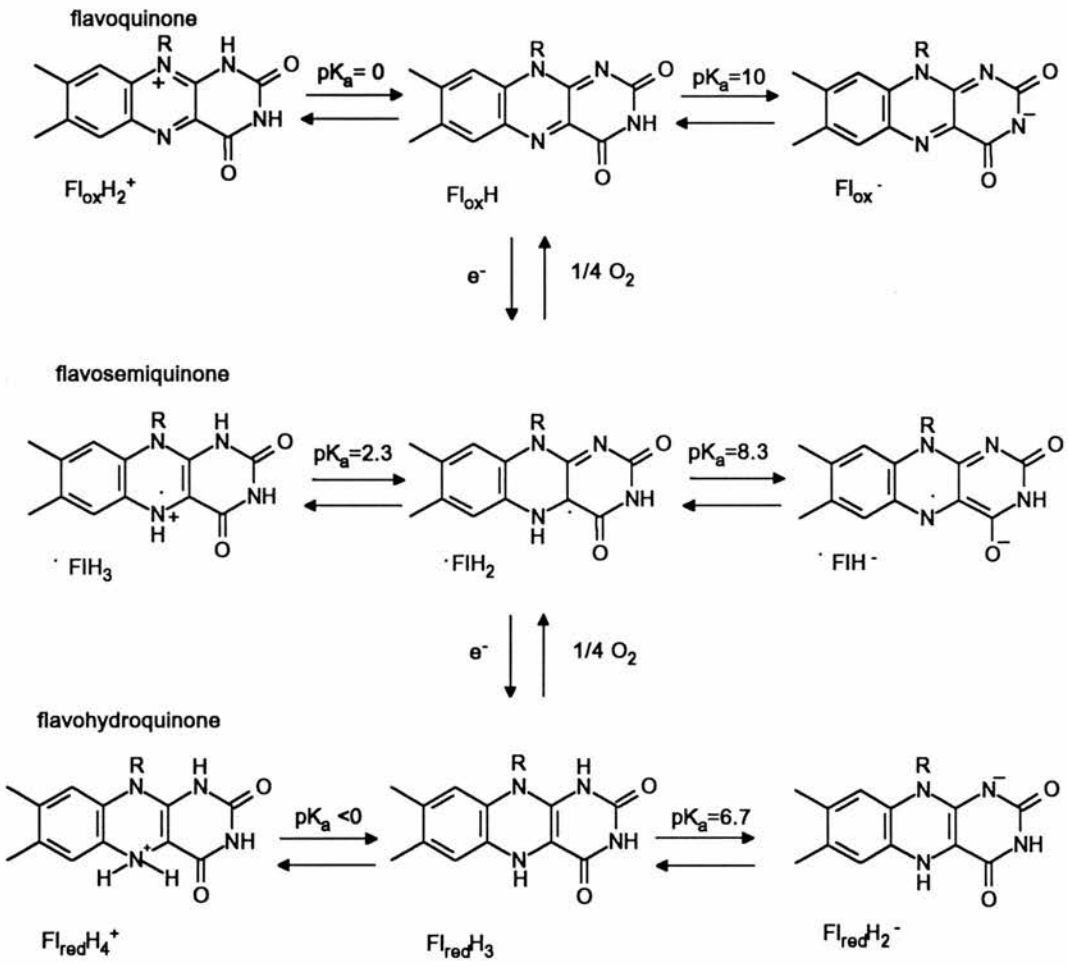
Flavins are vitally important biomolecules, comprising the non-protein portion of flavoenzymes. They possess yellow chromophores, due to their extensive electronic conjugation[1] and serve as prosthetic groups for many different reactions.[2] The flavocoenzymes are the biologically active forms of Vitamin B_2, riboflavin **(1)** (7,8-dimethyl-N^{10}-(1-D/-ribityl) isoalloxazine). The *in vivo* active coenzymes are nucleotide derivatives of riboflavin, namely-flavin mononucleotide (FMN), alternatively called riboflavin-5/-phosphate and flavin adenine dinucleotide (FAD).



(1)

A key feature of flavins is their ability to act as either a two electron or a one electron donor system. The isoalloxazine system is intermediate in character between two electron donors and obligate one electron acceptors, due to the amphoteric nature of flavins. It possesses three species within each redox state *i.e.* neutral, cationic and anionic species, nine in all (Scheme 1). Therefore any flavin reactions would be influenced by the pH of the reaction medium.[3]

No other known coenzyme shows such a diverse variety of reactions. The terms: flavoquinone are used for the most commonly occurring air-stable form, (flavo)semiquinone for the radical form and (flavo)hydroquinone for the completely reduced form.[4,5]



Scheme 1

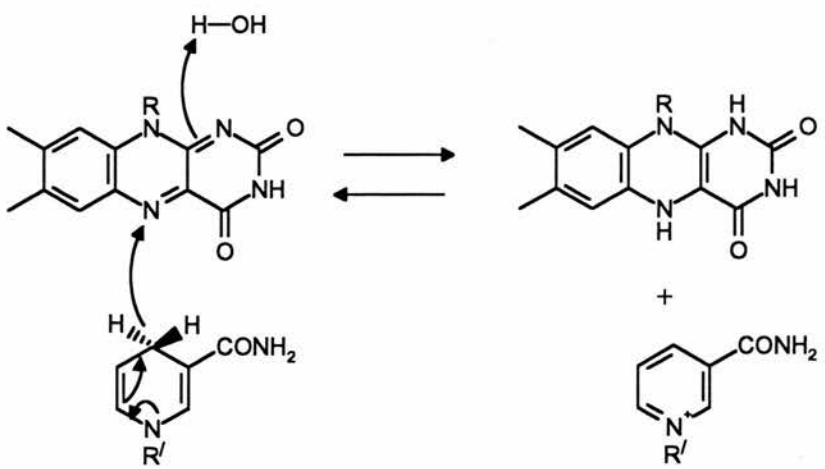
The most common and important reduced form of flavins is the 1,5-dihydroflavin (flavohydroquinone).[6] Thus the reduction or oxidation of flavins is known to occur in two successive equivalent steps. Both these reactions involve the (flavo)semiquinone free radical, which is inherently stable in biological reactions, especially in the electron transport chain (ETC).[7]

The assignment of an absorption band centred around 570 nm to the flavosemiquinone has proved an ideal marker for following the fate of semiquinones in flavoprotein catalysis.[8]

The ETC comprises a particulate complex of redox carriers including: flavoproteins, quinones and cytochromes, located in the mitochondria. This vital metabolic pathway involves the catalytic oxidation of NADH, succinate and a few other reduced substrates, with electrons being passed sequentially from one carrier to the next.[9]

The necessary respiratory enzymes reduce dioxygen to water, producing three moles of ATP for every mole of oxygen consumed. Therefore ancillary sets of enzymes, flavodoxins and iron/sulfur proteins are needed to supply the required electrons.[10]

The oxidation of NADH by flavoenzymes is crucial in the catalytic cycle of many enzymes, especially those that catalyse the reactions in the electron transport chain (Scheme 2). This oxidation reaction involves stereospecific hydride ion transfer from C^4 of the dihydronicotinamide to N^5 of the oxidised flavin and transfer of a solvent proton to N^1 of the flavin.[11]



Scheme 2

Flavoprotein catalysed reactions can conveniently be divided into three major classes.[12]

1) The first class comprises electron transfer reactions with either quinones, metal ions or porphyrin complexes; such as cytochromes or hemes acting as the electron acceptors. An example of an enzyme involved in catalysing such reactions is L-lactate dehydrogenase of yeast, also called cytochrome b_2.[13]
2) The second class constitutes two electron transfer processes, catalysed by oxidases, where either dioxygen or an organic molecule act as the electron donor. A typical example involves xanthine oxidase.[14]
3) The third and final class comprises four electron transfer processes, characteristic of the flavin oxygenases. These enzymes reduce dioxygen to water with the accompanying hydroxylation of an organic substrate, a typical example is L-lactate monooxygenase (L-lactate oxidase).[15]

The fact that flavins could act as the catalytic unit of flavoenzymes was discovered by investigating the photo-oxidation of sarcosine (H_3CNHCH_2COOH) and dimethylglycine ($(H_3C)_2NCH_2COOH$). Using a radiolabel, ^{13}C, it was discovered that both compounds were photo-oxidised to formaldehyde (methanal) in the absence of any protein by riboflavin and riboflavin-5/-phosphate (FMN).[16]

1.2. Bioelectrochemical Significance of Flavins

1.2.1. Relevant Molecules

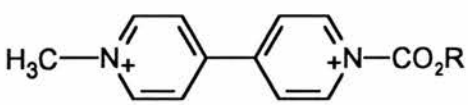
There are three main classes of molecule of interest in bioelectrochemistry. The first type comprises small non-protein redox active molecules, such as: quinones, ascorbate and pyridine nucleotides. A second class comprises redox active proteins, not directly involved in catalysis, such as cytochromes

and flavodoxins. Redox active enzymes comprise the third class, especially oxidoreductases, which include flavoproteins and many metalloenzymes.[17]

1.2.2. Biometal Electrodes

An important area of current research involves the preparation of biometal electrodes to model *in vivo* processes. In an effort to develop electrodes based on immobilised oxidases for constructing amperometric biosensors: colloidal gold was investigated as an enzyme immobilisation matrix. It was shown that two flavoenzymes, xanthine oxidase[18] and glucose oxidase[18,19] could both be adsorbed on to colloidal gold with full and high retention of activity respectively.

An additional example of where gold had been used as such a matrix involved another flavoenzyme, glutathione reductase being covalently attached to a cysteic acid active ester monolayer, which had been chemisorbed to a gold electrode. The resultant electrode-immobilised protein was treated with a bipyridinium derivative **(2)** to form an electron-relay modified enzyme, that exhibited electrical communication with the electrode- for the reduction of oxidised glutathione.



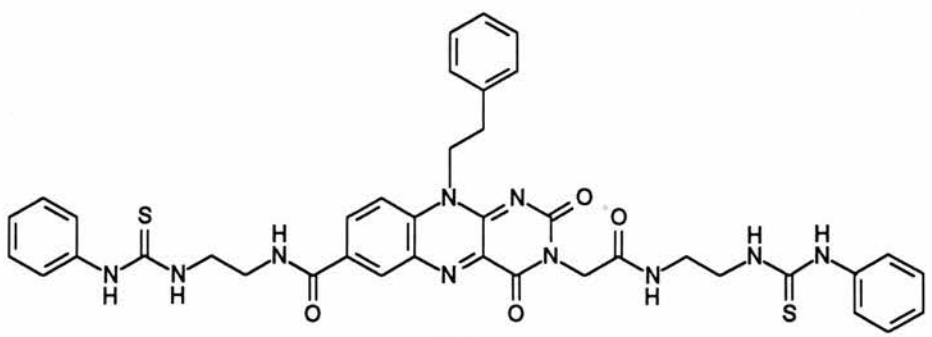
(2)

Increasing the number of relays bound to the enzyme, improved electrical communication between the flavin centres and electrodes. Electron transfer rate constants could thus be correlated with the average distance between the relay site and protein redox centre *i.e.* the geometrical length of the bridging arms anchoring the ET mediator.[20]

In recent years extensive attempts have been made to mimic nature's own coenzymes, employing synthetic flavins in bioelectrode design.[21] This research was an extension of earlier work involving the use of enzymes in bioelectrodes, first described in 1962.[22] Generally such model systems have exhibited slow electron transfer rates between the isoalloxazine and electrode, due to the poor flavin-conductor interactions. In all these systems the geometry between the flavin and conductor was controlled through only one single covalent bonding interaction.[23]

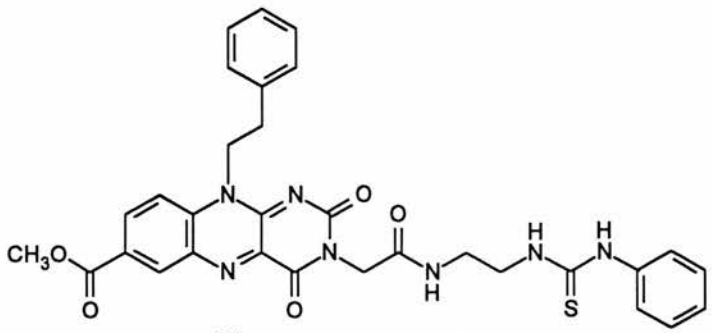
Suitably functionalised isoalloxazines have been synthesised in several sequential steps, then attached to a gold leaf surface to function as a metalloflavin redox system.[24] Thioureas were used to attach the flavin to the gold electrode, since organosulfur derivatives were known to coordinate strongly to gold surfaces. Other thiol derivatives that have been used for this purpose include: alkanethiols and dimethyl-3,3-dithiobispropanimidate.[20]

The electron transfer properties between the bis-functionalised flavin **(85)** and the gold surface were studied by cyclic voltammetry.[25] The anodic and cathodic rate constants measured were 5.6 x 10^2 s^{-1} and 3.2 x 10^2 s^{-1}, the fastest reported so far for such model systems.



(85)

The mono-phenylthiourea flavin **(3)** was also synthesised, comparing its electrochemical properties with those of the bis-functionalised system. This flavin was successfully adsorbed at the gold interface, acting as a stably-bound system.



(3)

The anodic and cathodic rates of electron transfer were 10 times lower than those obtained for the bis-functionalised flavin system. These earlier experiments have indicated that the geometry of the flavins attached to the conductor surface is important, determining the electroactivity of the metalloflavin system.[24,25]

In order to investigate more fully the effects of flavin geometry: isoalloxazines with varying numbers and lengths of phenylthiourea linkers had to be synthesised. Attempts at preparing these flavins are detailed in Sections 4.0 and 5.0.

1.2.3. Biosensors

An application and extension of bioelectrode research led to the use of flavins in biosensors. Such enzyme modified ion selective electrodes were first described as biosensors in 1977.[22] Direct electrical communication between redox enzymes and bare metal electrodes was not an ideal basis for preparing biosensors, due to the electrically insulating protein shell.[27]

One of the most popular uses of biosensors is clinically: in glucose detection, especially in the treatment of *diabetes mellitus*. [28] Although many technical problems need to be tackled, especially in the development stage, biosensors offer a tangible prospect of measuring biomolecules important in biotechnology, medicine and in other areas of technology with convenience, speed and low cost. [29]

2.0. STUDIES ON FLAVIN MODEL SYSTEMS

2.1. Models

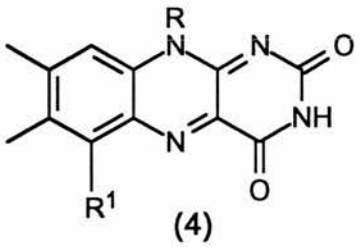
In order to investigate the role and action of flavins in particular in electron transfer kinetics, a number of model systems have already recently been investigated. These have included metalloflavin electrodes designed to mimic nature's own systems (Section 1.2.2.).[24,25]

Flavoenzymes comprise a riboflavin molecule bound noncovalently to a substrate binding site. Hence models of flavoenzymes comprising a flavin molecule acting as a catalyst, attached covalently to a binding site can facilitate the study of their mechanisms of action. These models could be useful catalysts for a variety of chemical transformations in their own right.

Four of the most important and useful classes of model investigated include: i) flavin analogues, ii) flavopolymers, iii) micelles and iv) crown ethers and cyclophanes.

2.2. Flavin Analogues

Recent studies of flavoproteins reconstituted with modified flavins have provided quite useful information about the interactions within the flavin binding site between the coenzyme and the apoenzyme. An example of such a study was where 6-thiocyanato and 6-mercapto FAD **(4)** were substituted for the native flavin of phenol hydroxylase. By examining the relative reactivity of these modified flavins, both free in solution and bound to the active site of the proteins- the accessibility of the isoalloxazine ring positions towards low molecular mass nucleophiles, such as thiolate could be determined.[30]

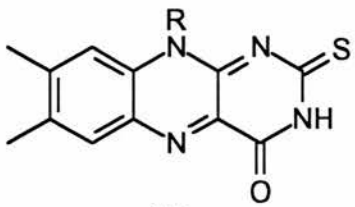


(4)

R= ribityl, $CH_2(CHOH)_3CH_2OH$

R^1=SCN or SH

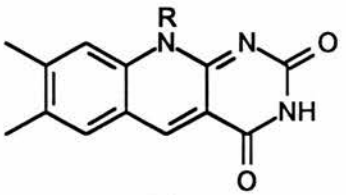
A number of flavoproteins containing the appropriate 2-thioflavin **(5)** (*i.e.* with the carbonyl oxygen at C^2 being replaced by a sulfur atom) were reconstituted. The ionization behaviour of such a modified coenzyme as well as its reactivity towards methyl methanethiosulfonate (MMTS) provided sensitive probes of the flavin environment within these proteins.[31] These derivatives were among analogues used as mechanistic probes for adrenodoxin reductase dependent-electron transfer to cytochrome (Cyt) P-450 of the adrenal cortex.[32]



(5)

The N^5 position is a key locus in the riboflavin molecule and the other end of the diaminoethane redox centre is N^1. The importance of both these positions had been emphasised, synthesising riboflavin derivatives with actual atomic replacement in the redox active isoalloxazine ring. They were the 5-carba-5-deaza analogue, 5-deazariboflavin **(6)** and the 1-carba-1-deaza analogue, 1-deazariboflavin **(7)**. Chemical and coenzymatic properties of both analogues could then be evaluated.

The redox potentials involving two electron changes for either analogue were considerably more negative than those for riboflavin. Carbon substitution at either former nitrogen locus had made reduction less thermodynamically favourable.[33]



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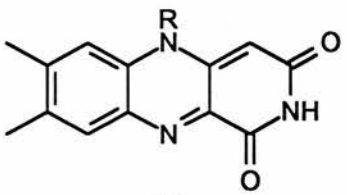
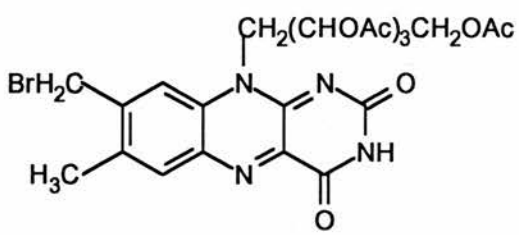


(7)

The actual chemistry of the flavins and their deaza analogues was fundamentally different. The reactions and properties of the deaza-flavins resembled those of the nicotinamides, which are pyridine nucleotides.[34] In another study 5-deazaflavins were used as models to test the validity of the Marcus Theory of Electron Transfer to hydride ion flow in NAD^+ analogues.[35]

2.3. Flavopolymers

Another model system investigated was a flavopolymer formed by combining 8-bromo-N^{10}-tetraacetyl riboflavin **(8)** with a matrix of a dodecylated polyaziridine derivative, having deacylated the ribityl side chain.



(8)

It was found that the rate of oxidation of NADH by such a flavopolyaziridine was more than 100 times faster than that for riboflavin.[36]

Flavocyclodextrins would appear to have the best promise as chemically useful models of flavoenzymes among flavopolymers, since cyclodextrins have been shown to possess enzyme-like binding capacity for small organic molecules.[37]

To demonstrate the usefulness of flavocyclodextrins: the rates of oxidation of substituted benzyl alcohols to their aldehydes, catalysed by flavodextrins and riboflavin were compared. The highest acceleration factor of 6500 exhibited by the cyclodextrin derivative, relative to riboflavin could be attributed to effective flavosubstrate geometry within the enzyme-substrate complex.

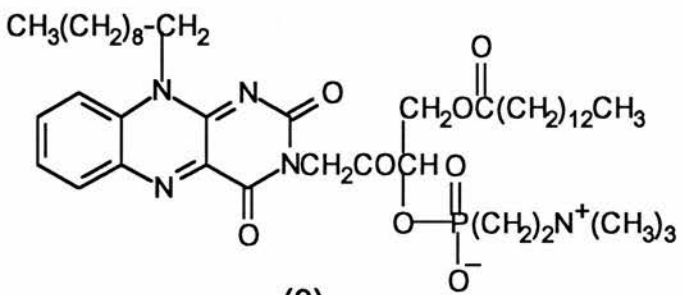
These flavopolymers show two advantages that such redox systems could confer to a reaction; converting a slow process, incompletely catalysed by the flavin to an efficient reaction. Also the system could benefit from conditions in the study not normally used by real enzymes, such as photochemical stimulation.[38]

2.4. Micelles

Since electrostatic and hydrophobic reactions are major driving forces for coenzyme binding to enzymes, causing activation of adsorbed substrates, the use of micelles, which frequently act as efficient catalysts for many organic reactions would prove to be a promising method to employ for simulating these interactions in model enzyme systems.[39] Micelles are organised molecular aggregates with the hydrophobic groups inside and the charged hydrophilic groups in contact with the aqueous medium.[40]

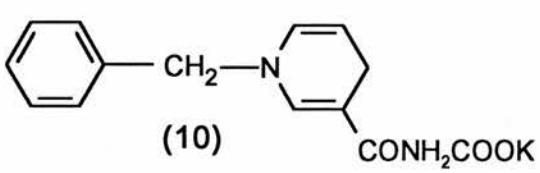
In one study the flavin oxidation of both thiols and carbanions was markedly accelerated by flavin binding in the micellar hydrophobic region. It is believed that such rate enhancement was due to: the increased local concentration of anionic substrates in the flavin-bound catalytic sites, enhanced nucleophilicity of bound anions in the hydrophobic environment and the shift of the flavin redox potential.[39]

In another study to simulate the action of membrane bound flavins; a synthetic isoalloxazine was attached to the polar head group of a natural lipid, phosphatidyl choline, forming an artificial flavolipid **(9)**.



(9)

A hydrophilic NADH analogue, potassium N-(carboxymethyl)-1-benzyl-1,4-dihydronicotinamide (BzNAHCOOK) **(10)** was used as a model electron donor.



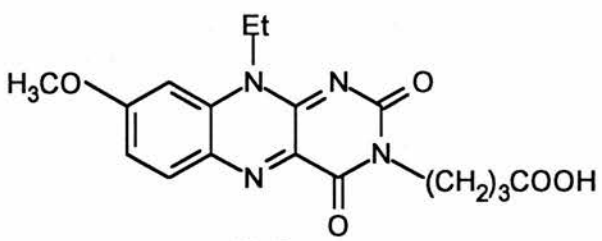
(10)

An efficient transmembrane electron transfer was observed, so the flavolipid-BzNAHCOOK micelle system provided a simplified model of the NADH-flavoprotein interactions occurring in the electron transport chain of the mitochondrial inner membrane.[41]

2.5. Crown Ethers and Cyclophanes

Flavo-crown ethers and flavinophanes are two additional important models recently reported in the literature.[42] Crown ethers can serve as useful mimics of enzyme action in that they simulate enzymatic binding pockets.[43] However flavo-crown ethers have limited use, since they can only bind positively charged substrates.[42]

In one investigation a novel water-soluble flavinophane was prepared as a potential redox mediator host along with the corresponding non-macrocyclic molecule **(11)** as a comparison compound. Previous work had indicated large selective acceleration of arene transport in aqueous solutions, mediated by complexation to cyclophanes.[44]

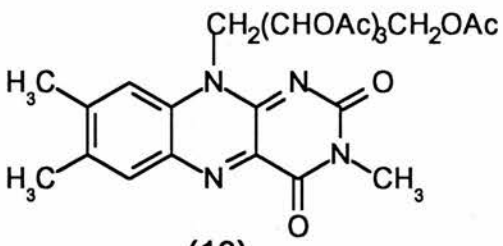


(11)

The research addressed the activation of the coenzymes by the specific microenvironment of the cyclophane binding site. Hence the association (aggregation) constants between the flavin and cyclophane were evaluated in the presence of naphthalene guest compounds by an NMR spectroscopy method previously developed for micellization studies of surfactants.[45] The values of the association constants varied from 145-934 l mol^{-1}, depending on the naphthalene.

The influence on substrate binding of the flavins in their different oxidation states could be determined. It was found that the flavinophane could alternate between cavity and non-cavity binding in a redox process. [44]

In another study a model reaction involving the one-pot synthesis of aromatic methyl esters by electrochemical oxidation of aldehydes, mediated by the flavin **(12)** was carried out. When the supramolecular catalyst was used, the cyclophane significantly increased both the rates and yield of aromatic ester formation. The increased rates were probably due to entropically favourable orientation, proximity and microenvironmental effects within the apolar cyclophane cavity.[46]

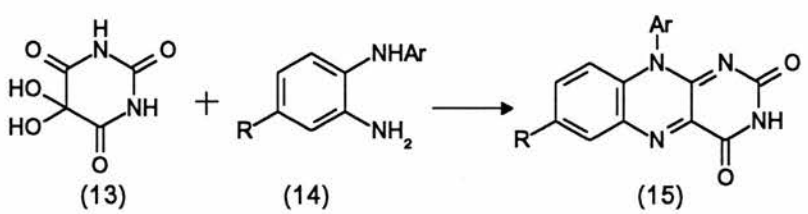


(12)

3.0. SYNTHETIC APPROACHES TO ISOALLOXAZINES

Riboflavin, Vitamin B_2, a naturally occurring isoalloxazine was first isolated from animal and vegetable tissue, characterized and synthesised in the early to mid 1930s.[47] Recent work has shown that the postulated precursors for the *in vivo* synthesis of riboflavin are ribulose-1,5-bisphosphate and guanosine triphosphate (GTP) respectively. [48]

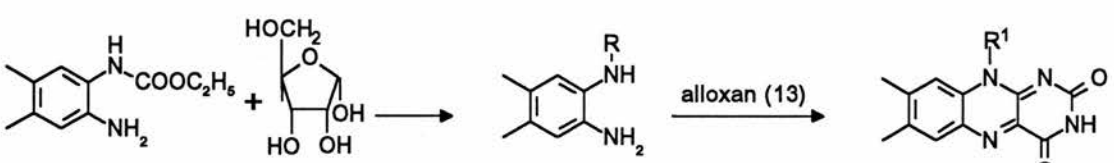
The term, isoalloxazine stems from the use of alloxan in the early syntheses of these molecules, involving the condensation of alloxan monohydrate **(13)** with 1,2-diaminobenzene derivatives (Scheme 3).[3,4]



(13) (14) (15)

Scheme 3

This method was used in a confirmatory synthesis of riboflavin (Scheme 4).



(16) (17) (18) (1)

R= $CH_2(CHOH)_3CHO$ R^1= $CH_2(CHOH)_3CH_2OH$

Scheme 4

The diaminoxylene derivative, 4-amino-5-ethoxycarbonylamino-1,2-dimethylbenzene **(16)** underwent a reductive condensation reaction with D-ribose **(17)**, forming 4-amino-1,2-dimethyl-5-D-ribitylaminobenzene **(18)**.

Condensation of this derivative with alloxan monohydrate **(13)** formed riboflavin **(1)** (Scheme 4).[49] Suitable reaction media for these condensations would have been glacial acetic (ethanoic) acid in the presence of boric acid or 4 M hydrochloric acid in methanol.[3]

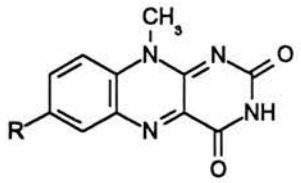
The first syntheses of riboflavin and lumiflavin were carried out in this way, by Kuhn and Weygand, using aromatic *o*-diamines and alloxan.[50] However their method had limited application, not allowing much variation in the pyrimidine subnucleus of the flavin, due to the lability of even the simplest alloxan derivatives. There was another disadvantage, alloxan and its derivatives behaved as oxidants towards diaminobenzenes. The yields were often unsatisfactory.[3,4]

Despite its limitations this method could be and was used successfully. Flavocyclodextrins were synthesised to act as potential flavoenzyme models. [38,42] There were two strategies for the synthesis of such flavin models. The first possibility was the coupling of functionalised flavins on to a binding site *e.g.* flavopapain. The alternative approach was to construct a flavin moiety on a binding site *e.g.* a flavo-crown ether.

The first strategy would have been potentially more attractive, since the syntheses of flavin derivatives as electrophiles and cyclodextrins as nucleophiles were well-developed. It was discovered that preparing flavocyclodextrins by attaching fully constructed flavins on to cyclodextrins was not a facile strategy.

The alternative approach involved synthesising a 1,2-diamino-benzene derivative of cyclodextrin, then condensing it with alloxan **(13)**, giving the flavocyclodextrin **(19)**. The condensation reaction had to be carried out at high temperatures in acidic conditions.[51] Cyclodextrins were known to be slightly hydrolysed by acids, even in dilute solution. To minimise

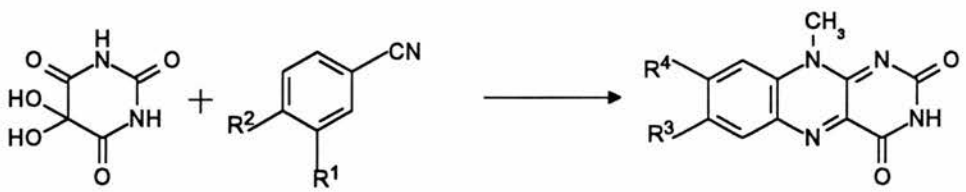
decomposition of the dextrin ring the conditions used were mild enough with the condensation reaction being carried out in 1 M HCl.[38,42]



(19)

R=β-cyclodextrin ring

The classic method was repeated by Bruice *et al* [52] to synthesise electron-deficient flavins, 7- and 8-cyanoflavins to act as analogues to elucidate the mechanisms of various flavoenzyme-catalysed reactions. The diaminonitrile, 4-methylamino-3-aminobenzonitrile **(20)** was formed from *p*-aminobenzonitrile in five steps. Condensation of the *o*-diamine with alloxan **(13)** in a mixture of acetic and boric acids, formed the flavin, 7-cyano-N^{10}-methylisoalloxazine **(22)** in good yield, 75% (Scheme 5).

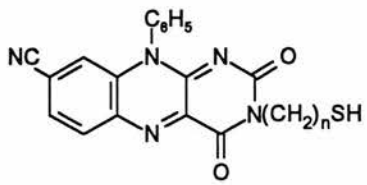


(13) **(20): R^1= NH_2, R^2= $NHCH_3$**
(21): R^1= $NHCH_3$, R^2= NH_2
(22): R^3= CN, R^4= H
(23): R^3= H, R^4= CN

Scheme 5

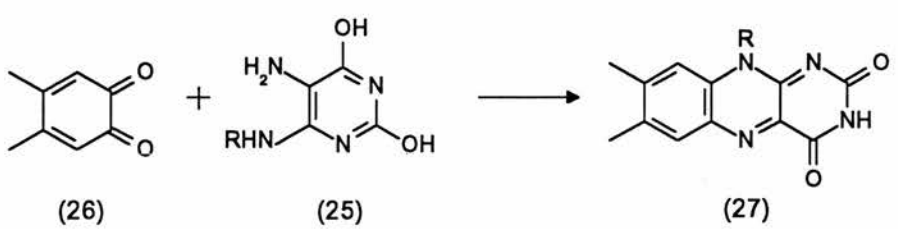
The 8-substituted flavin, 8-cyano-N^{10}-methylisoalloxazine **(23)** was prepared in the same way, using a *o*-diamino precursor, 4-amino-3-methylaminobenzonitrile **(21)**. Condensation of this diaminonitrile with alloxan **(13)** in a mixture of acetic and boric acids led to the 8-cyanoflavin **(23)** in 73% yield (Scheme 5).

The method of Bruice *et al* [52] was successfully repeated by Cashman during the preparation of similar electron-deficient flavins, 8-cyano-N^3-mercaptoalkyl-N^{10}-phenyl isoalloxazines **(24)**. [53] These 8-cyanoflavins were prepared by condensation of an appropriate diamino derivative with alloxan **(13)**. The mercaptoalkyl derivatives were then obtained in a five reaction sequence to act as model systems for disulfide prodrug formation during the metabolism of thiol-containing drugs. [53]



(24)

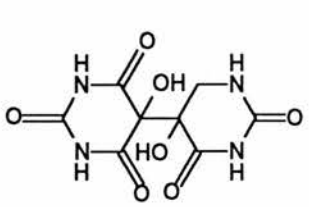
In the synthesis, using alloxan, the N^5 and N^{10} loci of the flavin nucleus are introduced by the homoaromatic component. The reverse path, which is the natural scheme, where both nitrogen loci are derived from a heterocyclic precursor, guanosine triphosphate (GTP) has been followed. During a study to investigate and mimic possible, potential pathways in the biosynthesis of riboflavin: Creswell *et al* [54] prepared 4-alkylamino-5-amino-pyrimidines **(25)** and treated them with 3,4-dimethyl-benzoquinones (*o*-xylo-quinone) **(26)** in order to obtain riboflavin analogues (Scheme 6).



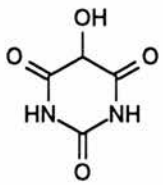
(26) (25) (27)

Scheme 6

The 5-aminopyrimidines were prepared by reduction of the corresponding 5-nitro compounds with sodium dithionite. The nitropyrimidines had been obtained by the reaction of the appropriate halonitrouracils and amines. Such a synthetic method suffered from the sensitivity of both starting components and their tendency towards autocondensation. [4,54] These difficulties were avoided by the condensation of 5-nitrosopyrimidones with aromatic *m*-diamines.[4] Diaminobenzenes were also condensed with alloxantin **(28)** and 5-hydroxybarbituric acid (dialuric acid) **(29)**.[51]

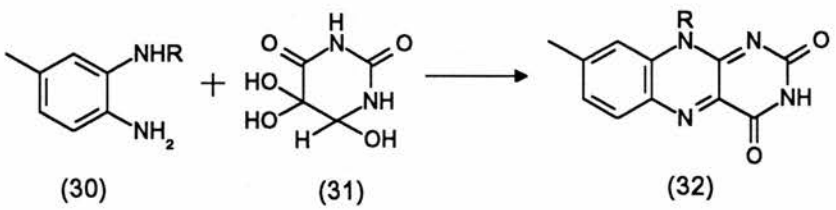


(28)



(29)

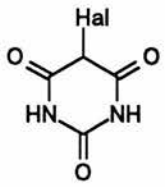
The isomer of 5-hydroxybarbituric acid, isodialuric acid **(31)** was condensed with *o*-diamines. Desoxo flavins were the anticipated products, *i.e.* isoalloxazines with a hydrogen atom or an alkyl group at position C^4 next to N^3 in the ring. Instead 2,4-dioxo products, normal flavins were obtained (Scheme 7).[4]



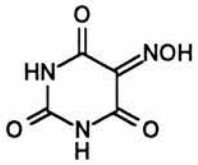
(30) (31) (32)

Scheme 7

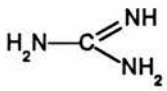
Halobarbituric acids, **(33)** [55] and violuric acid [56] **(34)** have also been condensed with diaminobenzenes.



(33)



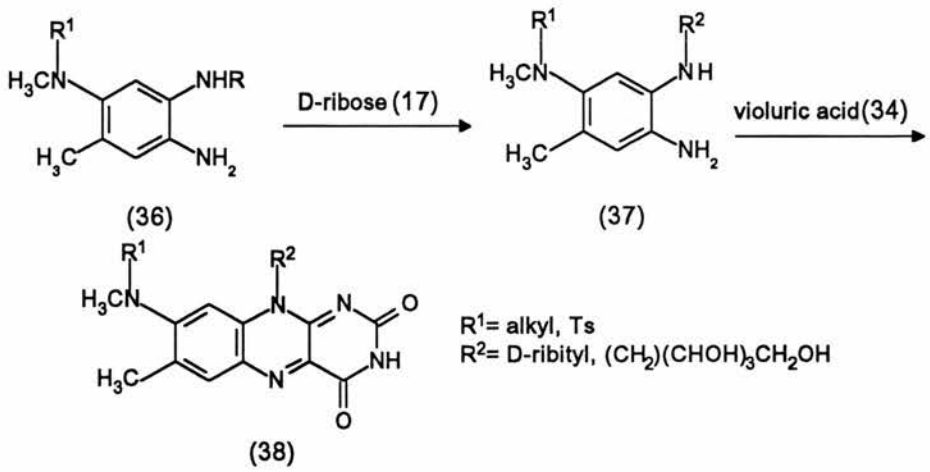
(34)



(35)

The use of halobarbituric acids **(33)** by Tishler, [55] instead of alloxan **(13)** allowed variation in the pyrimidine moiety of flavins. This procedure involved the condensation of the *o*-diamine with 5-mono or dihalobarbituric acids in pyridine. Harsher conditions were used for this method than for the corresponding synthesis with alloxan. However the advantage was that the starting pyrimidine components were more accessible than any alloxan derivatives. Using the appropriate diamines and 5-chlorobarbituric acids, riboflavin **(1)**, tetraacetyl riboflavin, 1-araboflavin, 6,7-dimethyl-9-benzyl- and 6,7-dimethyl-9-methylisoalloxazines were ultimately prepared.[55]

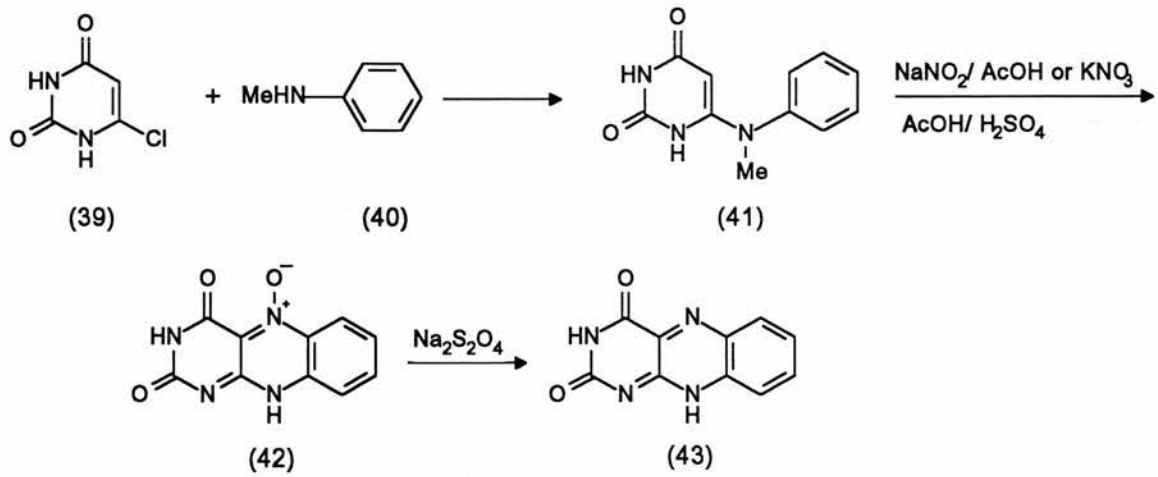
In an investigation into antimicrobial (bacteriostatic) agents a number of riboflavin analogues **(38)** were prepared and tested (Scheme 8).These isoalloxazines were obtained by reacting the corresponding N-polyhydroxy-alkyl substituted 1,3-diaminobenzenes **(37)** with violuric acid **(34)**.
The *m*-diamine derivatives **(37)** were obtained, reacting the appropriate 1,3-diaminobenzene **(36)** with D-ribose **(17)**. No actual yields of the riboflavin analogues were quoted, since the study was more concerned with the biochemical and microbiological effects of the riboflavin derivatives.[56]



Scheme 8

A more recent synthetic method for flavins has involved cyclisation of alkylphenyl aminouracils, followed by reduction of the isoalloxazine-5-oxides formed (Scheme 9).[57] The precursors were formed by the fusion of 6-chlorouracil **(39)** with the appropriate N-alkylaniline by the methods of Goldner. [58] Using N-methylaniline **(40)** formed 6-N-(methylanilino)uracil **(41)** in high yield. Nitrosation of the uracil **(41)**, using excess sodium nitrite, led to the exclusive formation of the 5-oxide derivative **(42)** in good yield (> 80%) by a nitrosative cyclisation reaction.

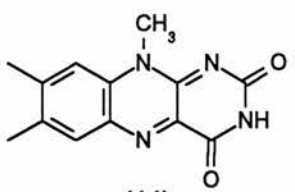
Alternatively the 6-N-alkylanilinouracil **(41)** was heated with potassium nitrate in a mixture of acetic and sulfuric acids to give the isoalloxazine 5-oxide **(42)** by nitrative cyclisation. The yields obtained were slightly lower than by nitrosative cyclisation, but were still 75-80%. Reduction of the 5-oxide **(42)**, using sodium dithionite, produced the isoalloxazines **(43)** in high yield (> 80%).[57]



(39) (40) (41) (42) (43)

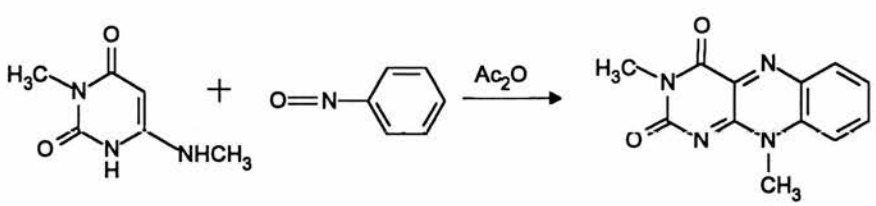
Scheme 9

This method was repeated by Takeda *et al* during the synthesis of flavin-linked porphyrins to act as models for enzymatic electron transfer mechanisms. The relevant flavin **(44)** was synthesised from its 5-oxide and coupled with the porphyrins.[59]



(44)

Condensation reactions of 6-alkylaminouracils with nitrosobenzenes have also been used to prepare flavins (Scheme 10).[57]



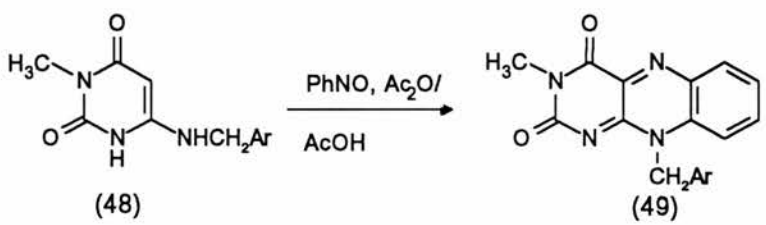
(45) (46) (47)

Scheme 10

Refluxing 3-methyl-6-methylaminouracil **(45)** with a three fold excess of nitrosobenzene **(46)** in acetic (ethanoic) anhydride, followed by dilution, precipitated the flavin, 3,10-dimethylisoalloxazine **(47)**.

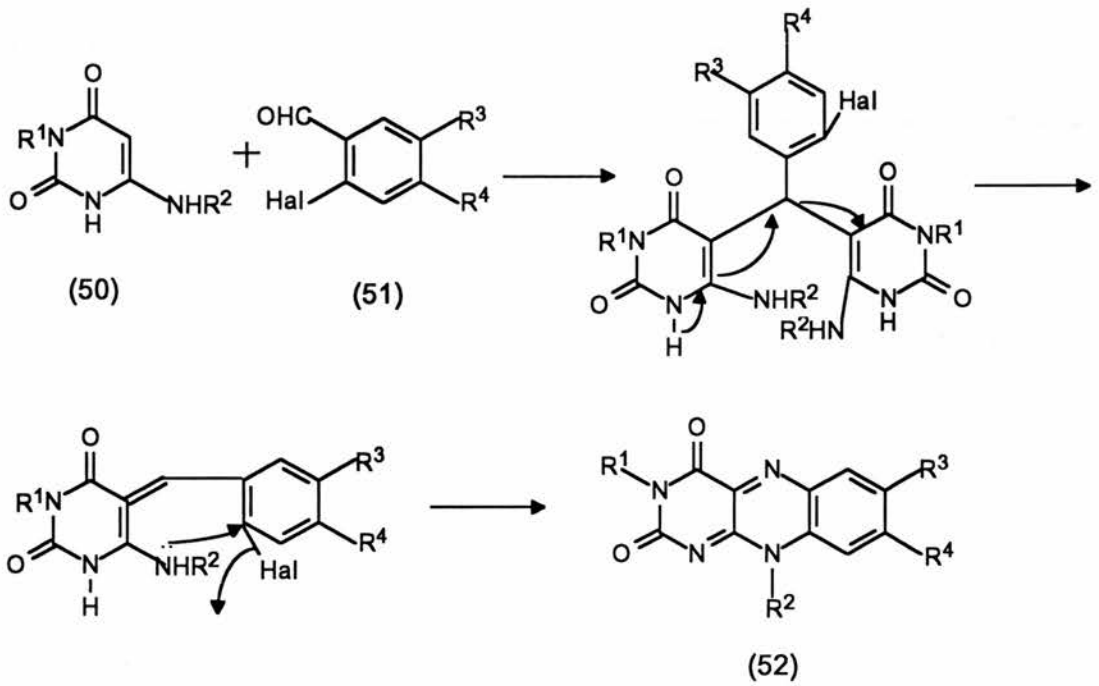
This method was successfully repeated to obtain 3-methyl-N^{10}-phenylflavins **(49)** during a study of potential antimalarial agents. The 6-anilino-3-methyluracils **(48)** were obtained, heating the appropriate aniline (phenylamine) with 6-chloro-3-methyluracil.

To determine the effect on antimalarial activity of the N^{10} aryl substituent different anilines were used. The phenylflavins **(49)** were readily obtained by means of the nitrosative cyclisation reaction on the uracils **(48)** (Scheme 11).[60]



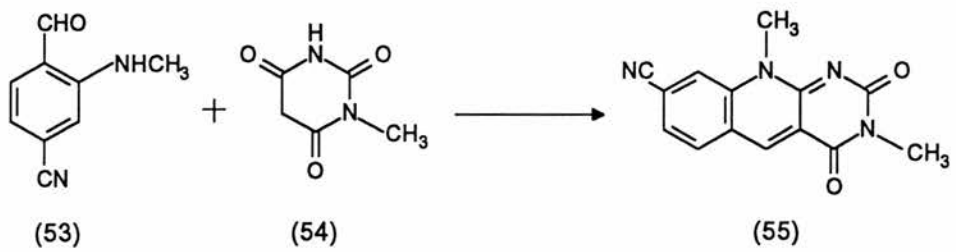
Scheme 11

Many 5-deazaflavins **(52)** have been prepared in good yields (> 75%) by condensation reactions of 6-substituted aminouracils **(50)** with *o*-halobenzaldehydes **(51)** in refluxing DMF (where R^1, R^2=H, alkyl, aryl; R^3, R^4=H, Cl, NO_2 & OH). The method has had wide applicability, being used to prepare 48 different analogues. The reaction was believed to have proceeded as shown by the postulated mechanism in Scheme 12. [61,62]



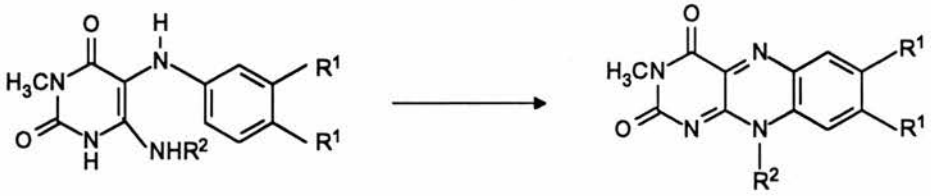
Scheme 12

The important analogues, 5-deazaflavins **(55)** were also synthesised by Chan and Bruice in a multistep sequence. The aminobenzaldehyde **(53)** was synthesised in a number of steps from 4-tolunitrile. Then this araldehyde **(53)** was condensed with N-methylbarbituric acid **(54)**, which had been formed from diethyl malonate and N-methylurea in up to 60% yield. The electron-deficient analogue, 8-cyano-3,10-dimethylisoalloxazine **(55)** was obtained in 75% yield (Scheme 13).[52]



Scheme 13

The one-pot conversion of pyrimidinediones **(56)** to isoalloxazines **(57)** has been reported to proceed in good yield (>85%) by oxidative cyclisation in DMF at 120 °C in an oxygen atmosphere (where R^1=R^2=Me, R^1=Me, R^2=Ph, R^1=H, R^2=Me, Et, Ph and 2,6-Me_2-C_6H_3) (Scheme 14).[61,63]



(56) (57)

Scheme 14

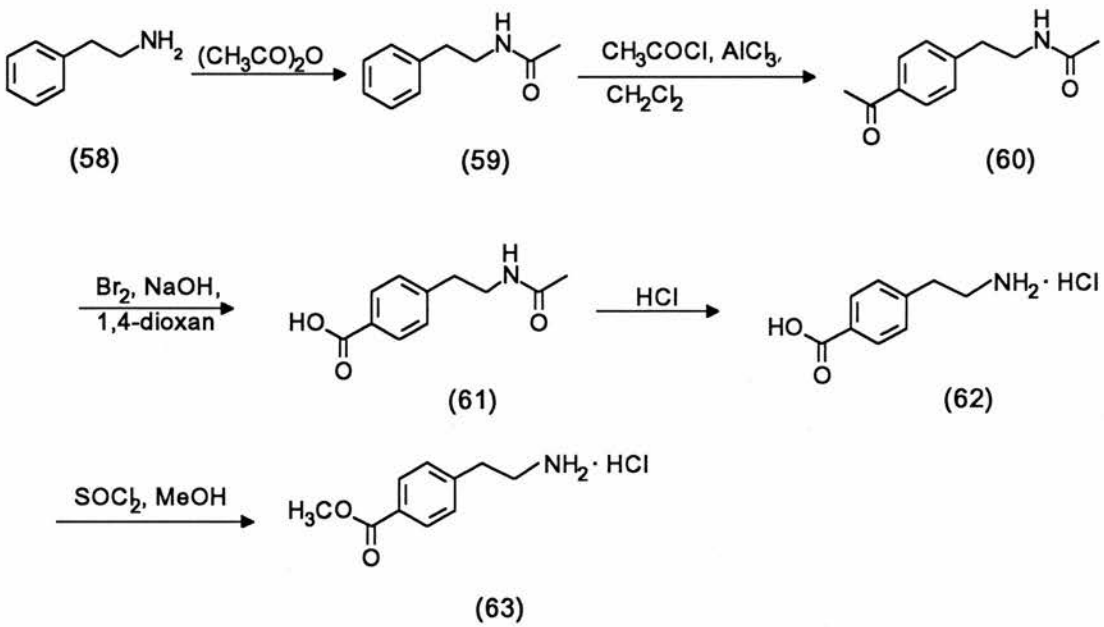
Additional routes to isoalloxazine systems include the condensation of: 1) 2-arylazoanilines with barbituric acid, 2) butanedione with diamino-uracils and 3) quinoxalines with guanidine (iminocarbamide) **(35)**.[51]

4.0. DISCUSSION

4.1. Reactions leading to the formation of the flavin triacid, 7-carboxy-N^3-carboxymethyl-N^{10}-(4-carboxyphenethyl)isoalloxazine

4.1.1. Formation of the amino acid methyl ester

Similar methods to those used by previous workers [64] were used to synthesise the aminomethyl ester intermediate **(63)** (Scheme 15).



Scheme 15

The initial reaction involved protecting the amine **(58)** in the form of an acetamide, a commonly used group. [65] A modification of the method of Bischler and Napieralski [66] was followed to prepare the acetamide (acylated amine), N-acetyl-2-phenethylamine **(59)** (Scheme 15).

Purification of the product **(59)** initially proved to be a problem. Toluene was added to the crude compound to form an azeotropic mixture with any unreacted acetic (ethanoic) anhydride and acid, which could be removed *in vacuo*. Recrystallisation of the product **(59)** was attempted,

using a variety of common solvents: acetone (propanone), ethyl acetate (ethyl ethanoate), ether, (light) petroleum and toluene. None of these solvents proved to be suitable. So fractional distillation was tried and proved to be a good method for purifying the compound. The product was formed in good yield, 78%.

The next step, the Friedel Crafts reaction, using the method of Blicke and Lillenfeld [67] to prepare the phenone, *p*-β-acetylaminoethyl acetophenone **(60)** proceeded smoothly (Scheme 15). The product was formed in good yield, 64%. The classical Friedel Crafts method, the Elbs procedure, where the catalyst was added to the acid chloride/ arene mixture was the method used.[68] Characteristically the Friedel Crafts ac(et)ylation led to the exclusive formation of the *para* substituted acetophenone **(60)**. [68,69]

The next reaction, the bromoform variant of the haloform (trihalo-methane) reaction, using this acylated phenone **(60)** to prepare the carboxylic acid, *p*-(β-acetylaminoethyl) benzoic acid **(61)** (Scheme 15)- at first proved to be difficult to carry out. Initial attempts, following the method of Blicke and Lillenfeld [67] were failures, with the benzoic acid **(61)** being formed in poor yield.

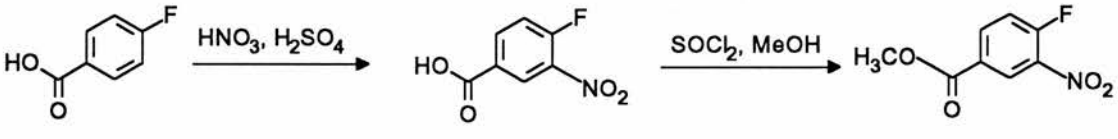
However a slight modification was then made, referring to a more recent literature method[70], where the haloform reaction was used to prepare a carboxylic (alkanoic) acid from an aliphatic methyl ketone. In this method after addition of the methyl ketone to the cooled hypobromite solution, the mixture was reacted at 0 °C for only one hour, then allowed to warm to room temperature and stirred for two, not three hours at 0 °C- as originally suggested. This modified method proved to be more successful, using it, the benzoic acid **(61)** was formed in a much higher yield, 64%.

The subsequent conversion of the benzoic acid **(61)** to the amine hydrochloride salt, 2-(4-carboxyphenyl)ethylamine hydrochloride **(62)**, proceeded comparatively more smoothly. The acetamido function was hydrolysed easily to the amine by the common method of refluxing with concentrated HCl [71], producing the hydrochloride salt **(62)** in high yield, 93% (Scheme 15).

The method of esterification devised by Brenner and Boissonnas was used to form the methyl ester **(63)** from the acid **(62)** (Scheme 15).[72] The reaction proceeded readily with methanol reacting as both the nucleophile and solvent. The ester **(63)** was formed in very high yield, 93%.

4.1.2. Synthesis of the fluoromethyl ester

Following the methods used by previous workers [64], the preparation of the methyl ester **(66)** proceeded well (Scheme 16).



(64) (65) (66)

Scheme 16

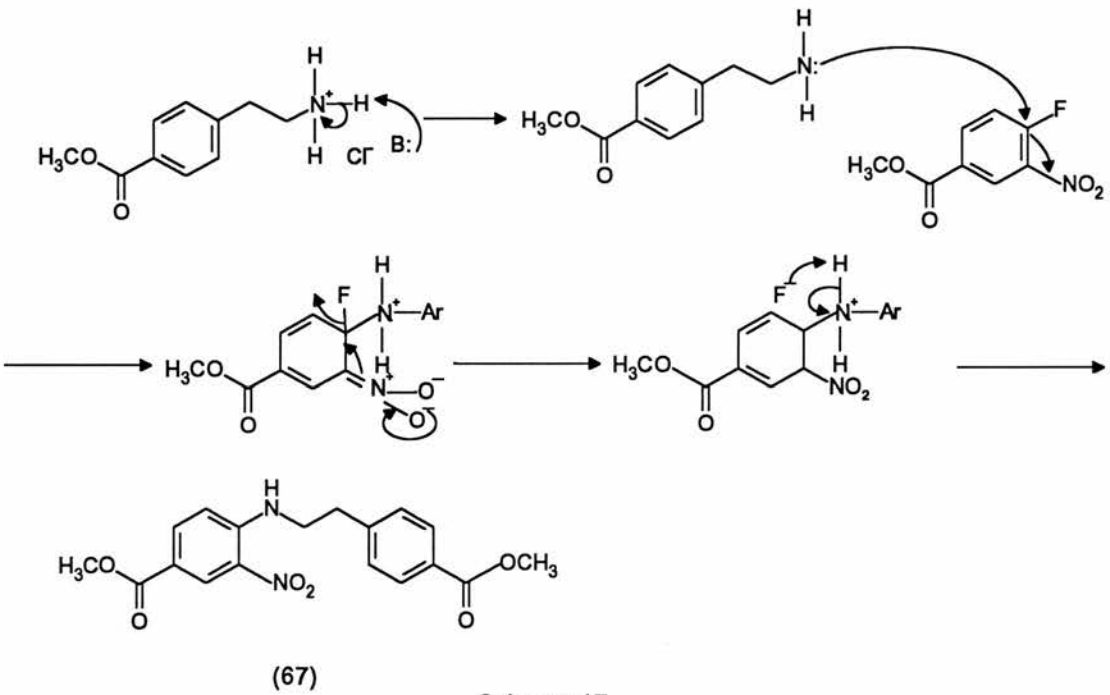
The disubstituted acid, 4-fluoro-3-nitrobenzoic acid **(65)** was formed in good yield, 77% from 4-fluorobenzoic acid **(64)** under standard nitration conditions, using the method of Rouche.[73] The presence of the weakly deactivating *m*-directing carboxyl group and the mesomeric influence of the *o,p*-directing inductively deactivating fluoro group caused nitration to occur *ortho* to the fluoro group and *meta* to the acid function.[74]

The ester, methyl 4-fluoro-3-nitrobenzoate **(66)** was prepared readily, reacting the benzoic acid **(65)** with thionyl chloride in methanol, following the

same methods used to prepare the amino acid methyl ester **(63)**. The quantities of thionyl chloride needed were less than half those used by previous workers.[64] The product **(63)** was still formed in good yield, 72%.

4.1.3. Synthesis of the diarylmethyl ester

The aromatic nucleophilic substitution reaction of the amino methyl ester **(63)** and the fluoro methyl ester **(66)** initially proceeded smoothly, forming the diaryl amine diester **(67)** in high yield, 93% (Scheme 17).

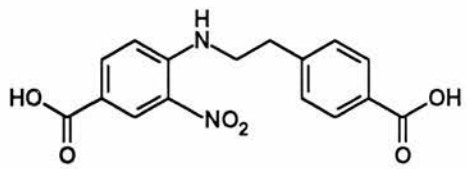


(67)

Scheme 17

In this activated aromatic nucleophilic substitution (S_NAr) reaction: the amine nitrogen displaced the fluorine atom on the arene, which was a good leaving group, facilitated by the strongly electron-withdrawing nitro group.[75] However on subsequent occasions there were complications. When using potassium carbonate as the base, significant hydrolysis to the diaryl diacid

(68) took place. Also some transesterification began to occur, prompting a change in the reaction conditions.



(68)

To minimise ester hydrolysis and transesterification: the solvent, ethanol was replaced by anhydrous methanol and then the base previously used, potassium carbonate was replaced by sodium hydrogencarbonate. The reaction then proceeded in almost equally high yield, 94%. The diaryl amine ester **(67)** was not formed by methylation of the corresponding diaryl diacid **(68)**, due to this acid's instability and formation in low yield.

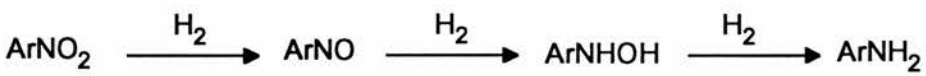
An indication of the unsuitabilty of such an approach was provided by the subsequent attempts at re-methylating this diaryl diacid **(68)**, that had been formed on hydrolysis of the ester **(67)**. The standard method of thionyl chloride in anhydrous methanol was initially tried [72], which did not succeed.

The Fischer method of refluxing the diacid **(68)** in anhydrous methanol with a trace of concentrated HCl, as employed by Tabushi *et al* [76]; likewise did not succeed.

A third method was attempted, using diazomethane as a methylating agent, following a similar protocol to that used by both Kosak [77] and Lee[78]. Again insufficient remethylation occurred.

4.1.4. Synthesis of the flavin diester

The formation of the isoalloxazine, flavin diester **(69)** involved two subsequent reactions. Initially the nitro function on the diaryl methyl ester **(67)** was reduced to an amine by catalytic hydrogenation. It is believed that the reduction would have proceeded by the simplified pathway as shown in Scheme 18.[79]

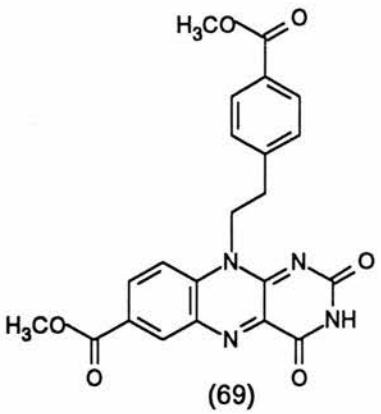


Scheme 18

It was found that, increasing the quantity of the catalyst from 10% to 20% by weight relative to the substrate, allowed the reaction to proceed more readily. Anhydrous methanol was initially tried as a solvent, however the diester **(67)** was sparingly soluble in the cold alcohol, but it did dissolve readily in ethyl acetate at room temperature, a definite advantage, since the hydrogenation had to be carried out at that temperature.

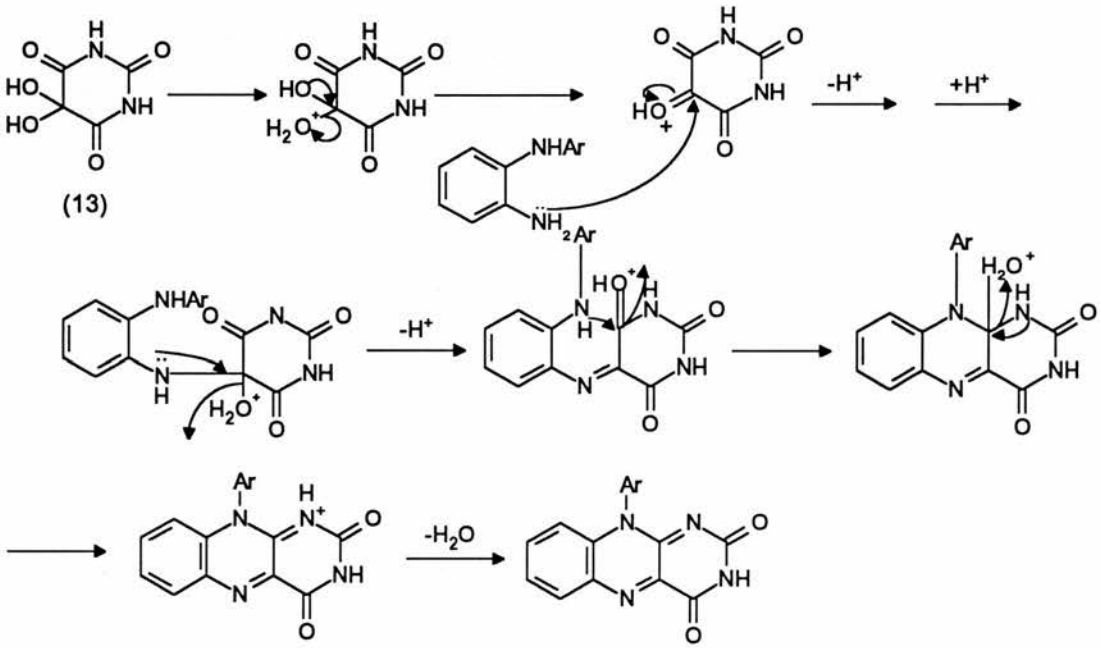
T.l.c. and spectral evidence, both ^{13}C and ^{1}H-NMR confirmed reduction had occurred. Replacing the electron-withdrawing nitro group by the electron-donating amino group, caused a shift upfield in both the δ_H and δ_C values for the arene nucleus.

The nitro group should have been readily reduced, especially in the case of simpler aliphatic and aromatic nitro compounds.[80,81] Other factors had an effect in this case. The reducible substrate **(67)** was a multifunctional compound. The resultant amine was not isolated, but used *in-situ* and coupled with alloxan **(13)**. The subsequent acid-mediated condensation and cyclisation reaction gave the flavin **(69).**



(69)

The isoalloxazine nucleus was formed as shown in Scheme 19.



Scheme 19

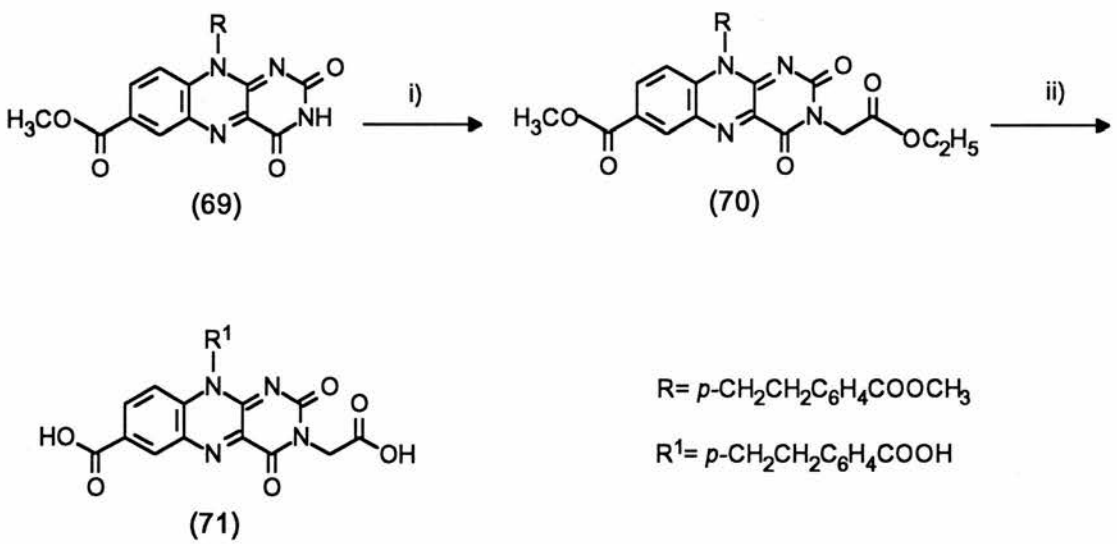
4.1.5. Formation of the flavin triacid

The flavin diester **(69)** had been synthesised in a total yield of 9.6% in 10 reactions. The actual yield of 67% from the 2-nitro compound **(67)** was reasonable, compared with previous related work. In an investigation into potential anti-malarial agents: Cowden *et al* synthesised similar N^{10}-aryl

substituted flavins, condensing 2-aminophenylamines with alloxan **(13)**. The yields varied from 15 to 76%, with an average of 43%, depending on the substituent attached to the N^{10} locus. [60]

Alkylation of the diester **(69)** at the N^3 position, with ethyl bromoacetate, using the method of Kraus [82] then formed the triester **(70)** in reasonably good yield, 58% (Scheme 20). The hydrolysis of triester **(70)** was done in acid solution, following previous worker's methods [64], due to the instability of the isoalloxazine nucleus in basic media.[83]

The triacid **(71)** was then recrystallised from acetic (ethanoic) acid, a suitable solvent for flavins.[84] The acid **(71)** was prepared from the diester flavin **(69)** with an actual yield of 56% and in twelve steps overall with a yield of 5.3%.

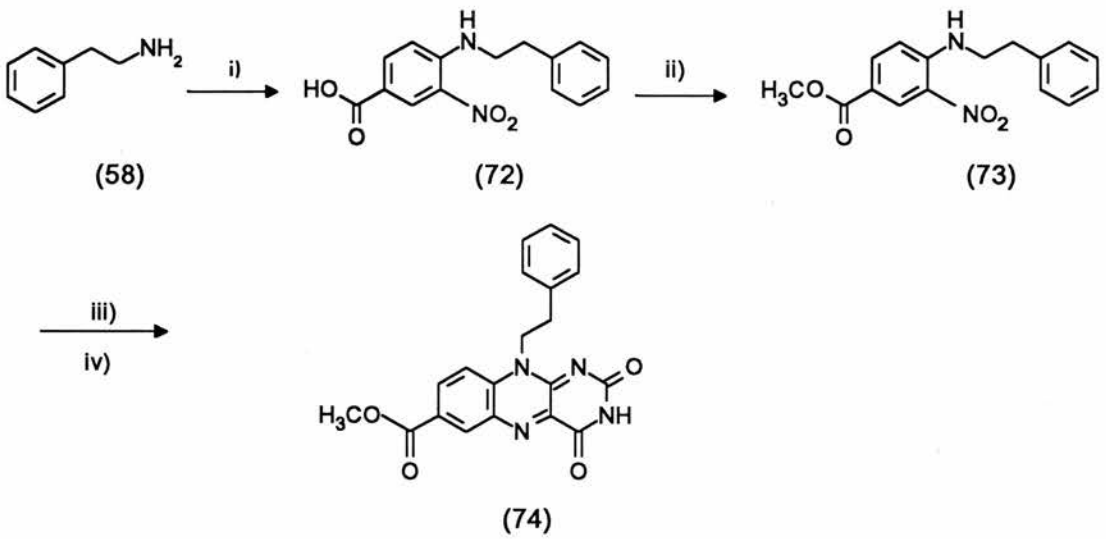


i) DMF, K_2CO_3, ethyl bromoacetate and ii) concentrated HCl

Scheme 20

4.2. Formation of the flavin diacid, 7-carboxy-N^3-carboxymethyl-N^{10}-phenethylisoalloxazine

The corresponding monoester **(74)** was synthesised in a similar way to the diester **(69)**, using the methods of Edwards and Gani (Scheme 21).[24,25]



(58) (72) (73) (74)

i) 3-nitro-4-fluorobenzoic acid (65), EtOH, HCl, ii)$SOCl_2$, CH_3OH, iii) H_2, Pd/C
iv) alloxan, EtOH and HCl

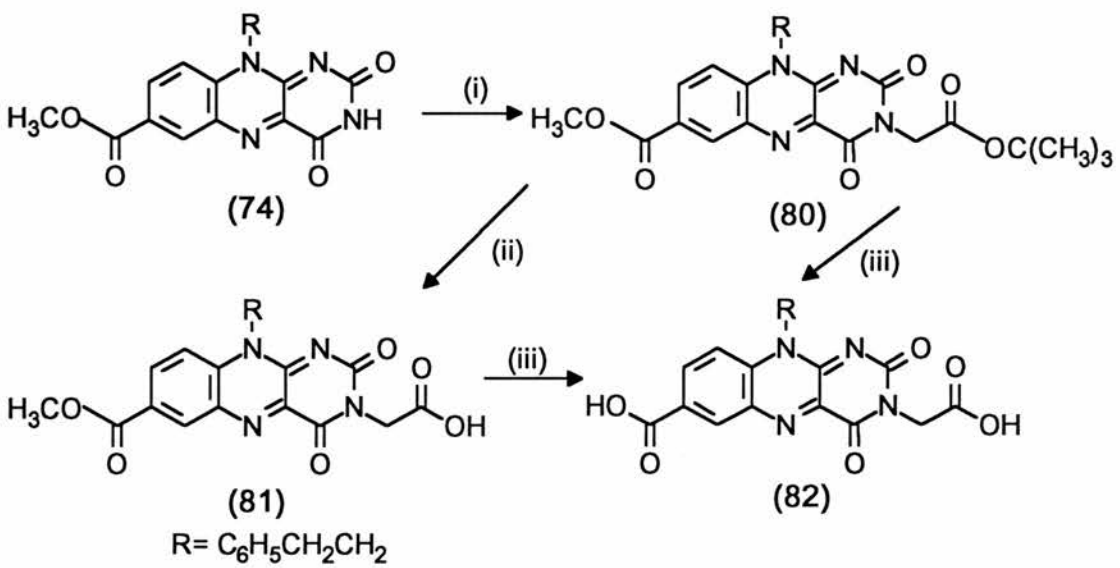
Scheme 21

The condensation reaction of 2-phenethylamine **(58)** with the nitrobenzoic acid **(65)**, analogous to Scheme 17, formed the phenyl benzoic acid **(72)** in good yield, 70%. Subsequent methylation of the benzoic acid **(72)** led to the diaryl methyl ester **(73)**.

Initial yields of the methyl ester **(73)** were low (< 50%). An attempt to couple 2-phenethylamine **(58)** directly with methyl 4-fluoro-3-nitrobenzoate **(66)** did not lead to any significant increases in yield. However increasing the refluxing time for the nitrobenzoic acid **(73)** and thionyl chloride mixture from 1.5 to 2 hours promoted a considerable gain in product yield to 95%. Reduction of the methyl ester **(73)**, followed by condensation of the

resulting amine with alloxan monohydrate **(13)** in acid (*cf.* Scheme 19) led to the formation of the flavin monoester **(74)**.

Attempts were made to form the diester **(80)** from the monoester **(74)** (Scheme 22). The method employed to form the triester **(70)** from the corresponding diester **(69)** by alkylation at the N^3 position, using ethyl bromoacetate was tried. However attempts at this reaction were unsuccessful. Instead t-butyl bromoacetate was used as the alkylating agent, again with potassium carbonate used to deprotonate at the N^3 position, following the methods of Kraus. [82]



i) K_2CO_3, DMF, t-butyl bromoacetate, ii) TFA, CH_2Cl_2, iii) concentrated HCl

Scheme 22

The resultant diester **(80)** could be purified by column chromatography and was formed in reasonable yield, 57%. The diester **(80)** could be hydrolysed to either the monoacid monoester flavin **(81)** or the required diacid **(82)**, depending on the conditions selected (Scheme 22). The t-butyl esters being stable under basic conditions, had to be

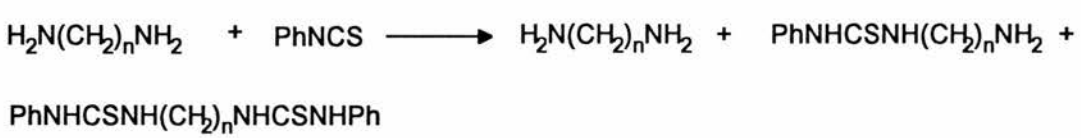
hydrolysed using acid.[85] Reacting the diester **(80)** in a vast excess of TFA led to the bisfunctional flavin **(81).**

More vigorous conditions were required to cleave the methyl ester. An initial attempt, heating the diester at 80 °C with concentrated hydrochloric acid in a water bath for 45 minutes readily cleaved the t-butyl function, but however failed to satisfactorily hydrolyse the methyl ester. Then refluxing the diester in 6 M HCl for *ca.* 2 hours removed the t-butyl group, but not the methyl ester. Refluxing the diester in concentrated hydrochloric acid finally hydrolysed this group, leading to the diacid **(82)** (Scheme 22).

4.3. Coupling of the flavins with the thioureas

4.3.1. Formation of the thioureas

The phenylthioureas **(75)** and **(76)** were synthesised, using the method of Lee *et al*, [86] an adaptation of the method of Stoutland *et al.* [87] The maximum possible yield obtained of the required monophenylthiourea, when using a two fold excess of the diamine would have been 50%, due to by-product formation (Scheme 23).[88]



(75): n=2, (76): n=3

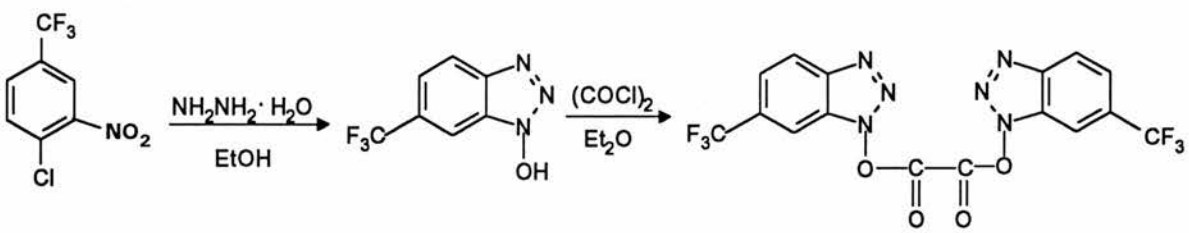
Scheme 23

Using a vast (>10-fold) excess of 1,2-diaminoethane, to prepare thiourea **(75)**, failed to increase the yield of the reaction.

4.3.2. Coupling via BTBO activated esters

The first coupling agent tried was an activator for carboxylic acid groups, BTBO **(79)**. Previous workers had managed to couple both the flavin monoacid **(81)** and diacid **(82)** with 1-(2-aminoethyl)-3-phenylthiourea **(75)**, using BTBO **(79)** in the presence of DMAP. Mono- and bis-functionalised thiourea flavins had been formed.[24,25]

BTBO **(79)** was synthesised in two steps (Scheme 24). The method of Takeda *et al*, [89] an adaptation of the method of Itoh *et al* [90] was used to form the triazole **(78)**. The S_NAr reaction of the arene **(77)** with hydrazine was enhanced by the presence of a good leaving group, a chlorine atom *ortho* to a strongly electron-withdrawing substituent, a nitro group.[91] The crude triazole **(78)** was dark brown, however use of decolorising charcoal and recrystallising from ether/ light petroleum, yielded a lighter, cream-coloured compound. BTBO **(79)** was formed from this triazole **(78)**, reacting with oxalyl chloride, though in a low yield as obtained in the literature.[89]



(77) (78) (79)

Scheme 24

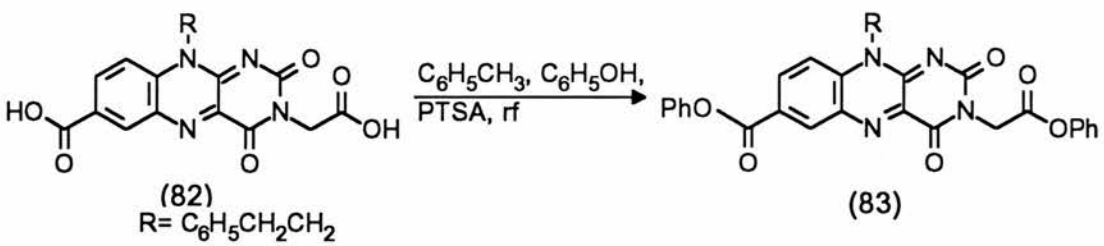
The coupling reaction using BTBO **(79)** was initially tried with the flavin monoacid **(81)** in an attempt to prepare the mono-phenylthiourea flavin **(3)** that had been previously made. [24] The reaction did not succeed.

Similarly the corresponding reaction with the flavin diacid **(82)** to prepare the bis-phenylethylthiourea flavin **(85)** failed to work satisfactorily. The synthesis of the tris-phenylethylthiourea flavin **(88)** was attempted from the triacid **(71)**, using BTBO **(79)**, following previous worker's methods.[64] Again the reaction did not work.

The main reasons for the failures of these attempts to synthesise these substituted flavins would have been the purity and efficacy of the BTBO. Any deteriation and impurities in the BTBO would have led to poor activation of the carboxylic acid functions. The coupling agent had a limited lifespan, even stored frozen within a desiccator.

4.3.3. Coupling via phenyl esters

After the lack of success in using BTBO to activate the flavin carboxylic acid functions for coupling with the thiourea, a second approach involving phenyl esters as intermediates was tried. Initially the acid-catalysed synthesis of the diphenyl ester flavin **(83)** was attempted (Scheme 25).



Scheme 25

Since the reaction between the flavin carboxylic acid **(82)** and phenol was reversible, equilibrium would have normally been attained under vigorous conditions, such as refluxing for several days. A convenient way of displacing the equilibrium towards the product, diphenyl ester **(83)** was to remove the water formed in the reaction with a Dean-Stark trap.[92]

A modification of the methods of Cope *et al* [93] and the more recent one of Furniss *et al* [94] was used, replacing the solvent originally used, benzene by toluene. However the ultimate yield of diphenyl ester **(83)** was low, 34%. There were problems in purification. Initially column chromatography was used to try and purify the diester, using 92.5% CH_2Cl_2/7.5% MeOH as eluent. The ^{1}H-NMR spectra of the collected fractions indicated that the ester functions had been cleaved. Also phenol typically reacted to such a small extent in the esterification.[92]

The diphenyl ester **(83)** was then reacted with a 6-fold excess of a primary amine, isobutylamine to show, if such a method could be viable for activating carboxyl groups towards nucleophilic substitution with thioureas. The ester without further purification- was reacted with the excess amine in dichloromethane for 3 days. However the reaction failed to work satisfactorily as shown by the ^{1}H-NMR spectrum of the product. The methyl peaks of the iso-butyl group at *ca.* 1.0 p.p.m. were too small- as indicated by their relative integration- for sufficient amination to have occurred.

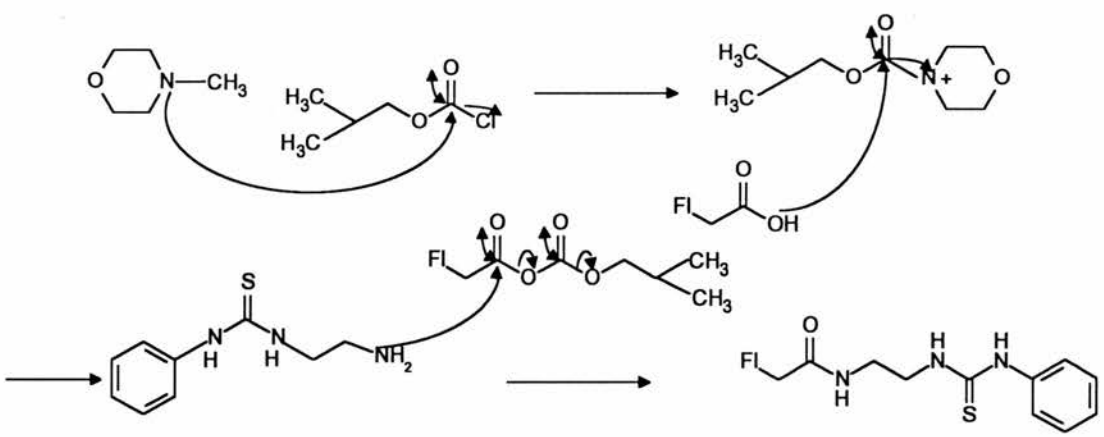
Due to the low yield of the phenol esterification accompanied by the subsequent lability of the synthesised esters, the method of preparing the thiourea flavins via the phenyl ester functions was not a viable one. Consequently the corresponding phenol esterification reaction with the flavin triacid **(71)** was not attempted.

4.3.4. Mixed anhydride coupling of flavins and thioureas

The mixed anhydride method has been widely used in amino acid and peptide chemistry. It was developed independently by Vaughan and Boissonnas in 1951 as a way of activating the carboxyl group for peptide (amide) bond formation.[95]

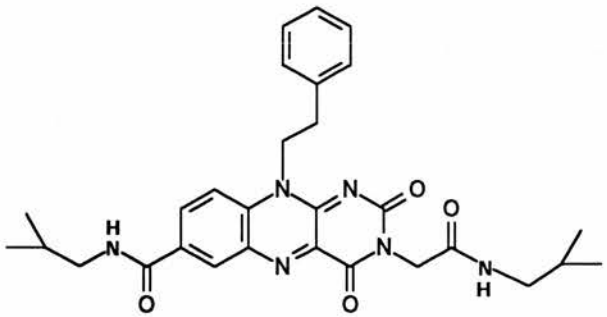
The advantage of this reaction was that the mixed anhydride did not have to be isolated. During work-up only carbonic half-esters were formed, which decomposed to an alcohol and carbon dioxide. The subsequent aminolysis of the carbonic-carboxylic anhydride could have occurred with any amino or amide derivative with a free primary amino group acting as the nucleophile (Scheme 26).[96]

During the amide bond formation the phenylthiourea behaved as the nucleophile. The acylating species, the flavin carboxylic-carbonic anhydride acted as the electrophile as shown in Scheme 26. The acetic (ethanoic) acid end of the flavin diacids and triacids reacted as shown. The other carboxylic groups present in both the di- and triacids would have reacted likewise. Isobutyl chloroformate and NMM were used to activate the flavin carboxylic acid groups, with THF chosen as the solvent, since such a combination of reagents had been shown to lead to a faster formation of the mixed anhydride.[97,98]



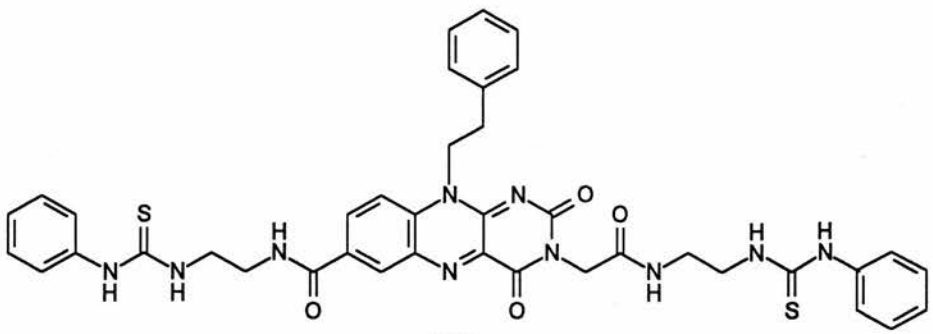
Scheme 26

To prove the viabilty of this method the mixed anhydride reaction of the flavin diacid **(82)** was initially tried with a primary amine, isobutylamine, leading to the bis-amido flavin **(84)** in good yield, 79%.

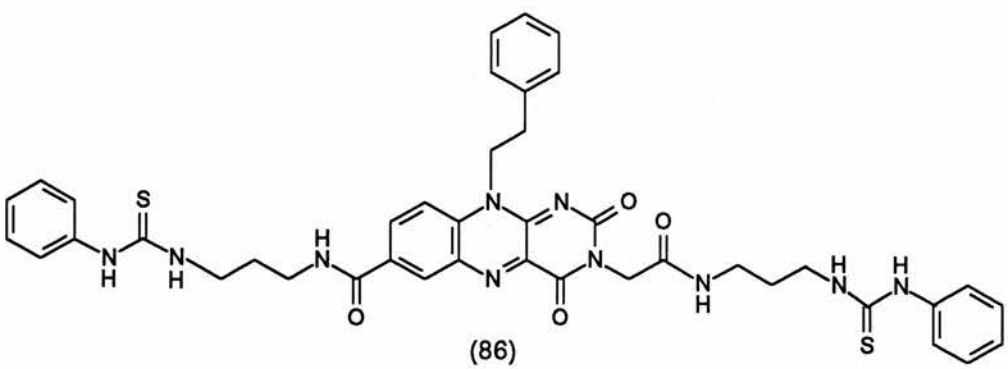


(84)

So the reaction was repeated with 1-(2-aminoethyl)-3-phenylthiourea **(75)** and 1-(3-aminopropyl)-3-phenylthiourea **(76)**, giving both bis-phenyl-thiourea flavins **(85, 86)** in moderate yield, 48% and 53% respectively.



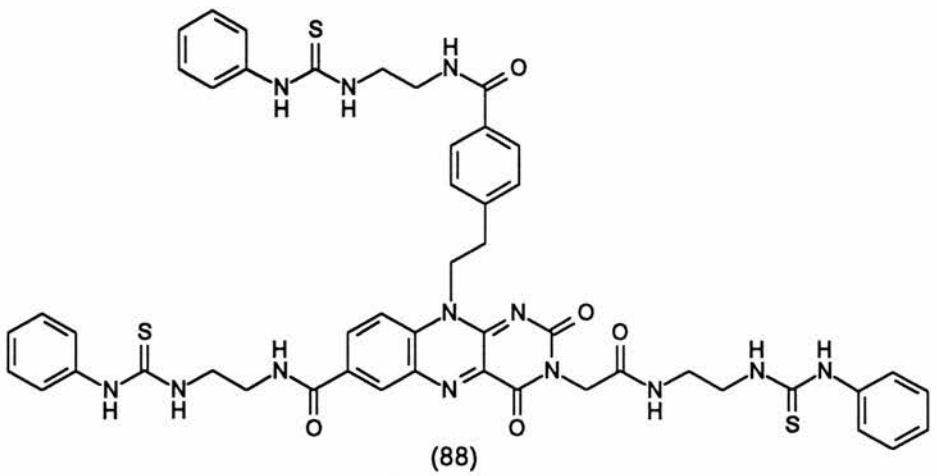
(85)



(86)

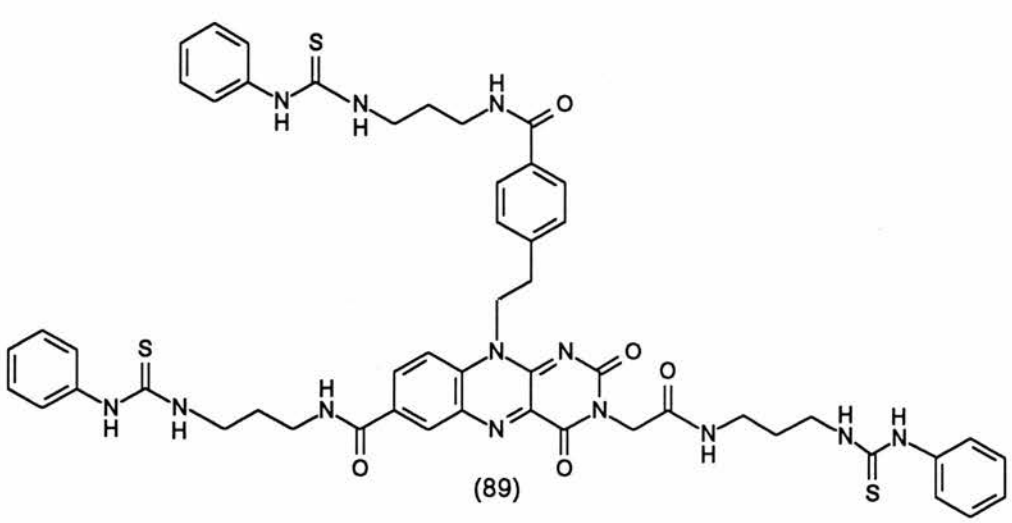
Similarly the mixed anhydride reaction was attempted with the flavin triacid **(71)**, initially with the primary amine, isobutylamine (2-methyl-1-propylamine). On that occasion the yield was lower, 34%. The reaction was

repeated, using both thioureas **(75, 76)**, forming the tris-phenylthiourea flavins **(88, 89)**.



(88)

The flavin triacid **(71)** was sparingly soluble in THF, which was a definite disadvantage. Changing the solvent to 1,4-dioxan for the final reaction to form the tris-phenylthiourea flavin **(89)** meant that only approximately half the volume of solvent was required. To further overcome solubility problems this reaction was carried out at room temperature.



(89)

The tris-phenylthiourea flavins **(88, 89)** had been made in a crude form as indicated by their ^{1}H-NMR spectra. Column chromatography, using a mixture of 90% CH_2Cl_2/10% CH_3OH and 92.5% CH_2Cl_2/7.5% CH_3OH respectively as eluents - did not lead to a successful purification of these compounds as shown by their ^{1}H-NMR spectra.

Despite the failure of the chromatographic separations for the tris-phenylthiourea flavins, the mixed anhydride method has the potential for formation of functionalised thiourea flavins. Manipulation of the reaction conditions and purification procedures, namely chromatography should lead to higher yields of these flavins with adequate purity.

5.0. EXPERIMENTAL

All solvents and where necessary commercial grade reagents were purified according to established literature procedures.[99] Light petroleum comprised the alkane portions boiling between 40-60 °C and 60-80 °C. All reactions involving flavins were carried out in subdued light and in an inert atmosphere of either argon or nitrogen at s.t.p.- unless otherwise stated.

Melting points were determined, using an Electrothermal melting point apparatus and are uncorrected. N.M.R. spectra were recorded on a Bruker AM-300 (300 MHz ^{1}H & 74.76 MHz ^{13}C N.M.R.) and a Varian Gemini 200 (200 MHz, 50.3 MHz, ^{13}C N.M.R.) spectrometers. ^{1}H N.M.R. spectra are described in parts per million (p.p.m.) downfield shift from TMS and are reported consecutively in terms of position (δ_H), relative integral, multiplicity (s-singlet, d-doublet, t-triplet, q-quartet, qn- quintet, m-multiplet, d.d.-double doublet, app- apparent and br-broad), coupling constant and assignment (numbering according to the IUPAC nomenclature for that particular compound).[100]

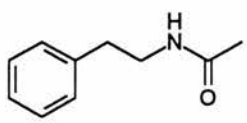
I.R. spectra were obtained on a Perkin Elmer 298 infrared spectrophotometer or mostly on a Perkin Elmer 1710 Infrared Fourier Transform Spectrometer. Samples were prepared as Nujol mulls between sodium chloride discs. Absorption maxima are stated in wavenumbers (cm^{-1}), relative to a polystyrene standard. The intensity of the signal was specified (v.s. - very strong, s-strong, m-medium and w-weak).

U.V. spectra were recorded on a Pye Unicam SP8-100 ultraviolet spectrophotometer, using a matched pair of 1 cm path length quartz cells for the samples. Electron impact (EI) mass spectra were obtained on an AE1 MS50 mass spectrometer. FAB mass spectra and accurate mass

measurements were recorded, using 3-nitrobenzyl alcohol (NOBA) as a matrix and polyethene glycol (PEG) as a reference mass on a VG AutoSpec model at the SERC Mass Spectrometry Service Centre, University College, Swansea.

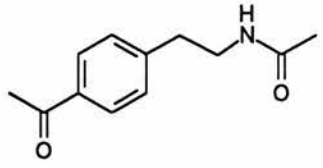
Analytical thin layer chromatography was carried out, using precoated 0.25 mm thick silica gel plates (Macherey Nagel SILG/ UV_{254} or Whatman PE SILG/ UV). Visualisation methods of compounds included UV fluorescence, iodine treatment and occasionally permanganate staining. Column chromatography was carried out, using Prolabo Sorbsil 40-60 μm mesh silica gel.

N-Acetyl-2-phenethylamine (59)



Excess distilled acetic (ethanoic) anhydride (50 ml, 530 mmol) was added dropwise to 2-phenethylamine **(58)** (21 ml, 167 mmol) at 0 °C with constant stirring. The mixture was allowed to reach room temperature, while stirring for *ca.* 1 hour. Excess acetic acid and anhydride were removed *in vacuo.* The product was then distilled under vacuum and the wax dried *in vacuo* over either phosphorus pentoxide or sodium hydroxide to give a white solid (21.31 g, 78%), m.p. 50 °C (Lit [101] 51 °C). ν_{max} (nujol)/cm^{-1} 3270 m., 3050-3080 w. (Ar CH), 1660,1540 s. (CONH) & 1500 m. (Ar C=C); δ_H (300 MHz, C^2HCl_3): 1.97 (3H, s., CH_3CO), 2.85 (2H, t., *J* 6.7 Hz, $C_6H_5CH_2$), 3.40 (2H, q., *J* 6.7 Hz, $NHCH_2$), 6.70 (1H, s., NH) and 7.30 (4H, m., ArH); δ_C (75 MHz, C^2HCl_3): 23.2 ($\underline{C}H_3CO$), 35.6 ($C_6H_5\underline{C}H_2$), 40.7 ($NH\underline{C}H_2$), 126.5 (C_2), 129.5 (C_4), 130.0 (C_3), 140.0 (C_1) and 170.2 ($CH_3\underline{C}O$).

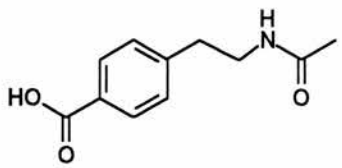
p-(β-acetylaminoethyl) acetophenone (60)



Acetyl chloride (14 ml, 197 mmol) was added to a solution of N-acetyl-2-phenethylamine **(59)** (10 g, 61 mmol) in dichloromethane (24 ml) at 0 °C. Granular aluminium chloride (27 g) was added in small quantities, keeping the temperature relatively constant. The mixture was warmed gradually to room temperature, then refluxed for 30 minutes. The resultant viscous liquid was then poured on to crushed ice (400 ml).

The organic layer was extracted with 3 volumes of dichloromethane and dried over magnesium (or sodium) sulfate. Diethyl ether was added to induce crystallisation. Excess ether was removed from the product *in vacuo*, which was then recrystallised from xylene (dimethylbenzene) and washed with petroleum to give white crystals (8.01 g, 64%), m.p. 100-102 °C (Lit [67] 99-101 °C). ν_{max} (nujol)/cm^{-1} 3300 m. (2°NH), 3080-3050 m. (Ar CH), 2850 w. (CH_3CO), 1680 m. (Ar C=O) & 1490 m. (Ar C=C); δ_H (300 MHz, C^2HCl_3): 2.10 (3H, s., CH_3CONH), 2.40 (2H, t., *J* 6 Hz, $ArCH_2$), 2.50 (3H, s., CH_3COAr), 2.85 (1H, t., *J* 6 Hz, NH), 3.40 (2H, q., *J* 6 Hz, $NHCH_2$), 7.30 (2H, d., *J* 6.6 Hz, ArH) and 7.90 (2H, d., *J* 6.6 Hz, ArH); δ_C (75 MHz, C^2HCl_3): 24.0 ($\underline{C}H_3CO$), 35.7 ($Ar\underline{C}H_2$), 40.4 ($NH\underline{C}H_2$), 128.7 (C_3), 129.4 (C_2), 130.3 (C_1), 144.8 (C_4), 168.8 ($CH_3\underline{C}ONH$) and 197.8 ($CH_3\underline{C}OAr$).

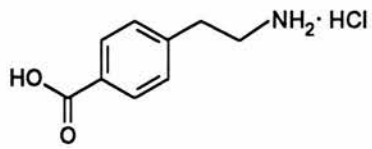
p-(β-acetylaminoethyl) benzoic acid (61)



Sodium hydroxide (8.7 g, 218 mmol) was dissolved in water (65 ml) and cooled to 0 °C. Bromine (4 ml, 156 mmol) was added dropwise. The *p*-(β-acetylaminoethyl) acetophenone **(60)** (5 g, 24 mmol), dissolved in the minimum volume of 1,4-dioxan was added. The solution was stirred at 0 °C for 1 hour, then slowly warmed to room temperature and reacted for a further 2 hours.

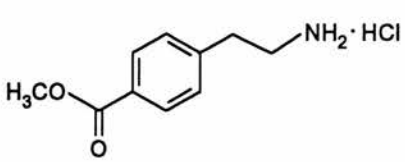
The mixture was neutralized with c. HCl to induce separation of the bromoform (tribromomethane) layer, which was extracted with dichloromethane. Diethyl ether was then added to efficiently extract the aqueous layer, which was then acidified to pH 4.5 and cooled to form crystals of the acid. Recrystallisation from water gave a white crystalline solid (3.19 g, 64%), m.p. 171-173 °C (Lit [67] 173-175 °C). δ_H (300 MHz, DMSO-d_6): 1.90 (3H, s., CH_3CO), 2.78 (2H, t., *J* 6 Hz, $ArCH_2$), 3.30 (2H, q., *J* 6 Hz, $NHCH_2$), 7.30 (2H, d., *J* 7.7 Hz, ArH), 7.85 (2H, d., *J* 7.7 Hz, ArH) and 7.95 (1H, br.t., NH); δ_C (75 MHz, DMSO-d_6): 23.3 ($\underline{C}H_3CO$), 35.8 ($Ar\underline{C}H_2$), 38.9 ($NH\underline{C}H_2$), 129.3-145.6 (4 ArC), 164.5 ($CH_3\underline{C}O$) and 166.0 (COOH); m/z (EI) 207 (M^+, 23%), 131 (11, $[M-C_6H_4]^+$), 118 (14) and 30 (100, $CH_2=NH_2^+$).

2-(4-Carboxyphenyl)ethylamine hydrochloride (62)



Initially *p*-(β-acetylaminoethyl) benzoic acid **(61)** (1.0 g, 4.8 mmol) was refluxed in c. HCl (sp. gr. 1.18) (2.5 ml) for 5 hours. Ethanol was added to induce crystallisation and the mixture was then cooled overnight. The crystals were collected by vacuum filtration. (0.90 g, 93%), m.p. > 300 °C (Lit [67] m.p. > 300° C). δ_H (200 MHz, ^2H_2O): 2.90 (2H, t., *J* 6 Hz, $ArCH_2$), 3.10 (2H, t., *J* 6Hz, NH_2CH_2), 7.20 (2H, d., *J* 7.5 Hz, ArH) and 7.70 (2H, d., *J* 7.5 Hz, ArH); δ_C (50 MHz, ^2H_2O): 35.69 ($Ar\underline{C}H_2$), 43.06 ($NH_2\underline{C}H_2$), 131.39-145.48 (4 ArC) and 173.26 (COOH); m/z (EI) 165 (45, $[M\text{-}HCl]^+$), 136 (59), 118 (62), 90 (75), 77 (55), 51 (53) and 30 (100).

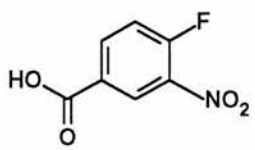
2-(4-methoxycarbonylphenyl)ethylamine hydrochloride (63)



2-(4-carboxyphenyl)ethylamine hydrochloride **(62)** (0.60 g, 2.97 mmol) was dissolved in methanol (45 ml) and stirred vigorously, cooling to 0 °C. Previously cooled thionyl chloride (2.25 ml, 30.9 mmol) was added dropwise, minimising any temperature rises. The mixture was allowed to rise to room temperature and refluxed for 1 hour. On cooling the methanol was removed *in vacuo*. Recrystallisation from ethanol/diethyl ether (1:1)

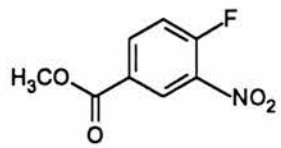
led to a white crystalline solid (0.93 g, 93%), m.p. 224-225 °C (Lit [64] 222-224 °C). ν_{max} (nujol)/cm^{-1} 2723 w. (NH_2^+), 1723 v.s. ($CH_3\underline{C}OOAr$), 1683 m., 1107 s. (Ar) and 856 v.s. (*p*-substituted arene); δ_H (300 MHz, ^2H_2O): 2.95 (2H, t., *J* 6.3 Hz, $ArCH_2$), 3.20 (2H, t., *J* 6.3 Hz, $NHCH_2$), 3.80 (3H, s., CH_3COO), 7.35 (2H, d., *J* 7.5 Hz, ArH) and 7.90 (2H, d., *J* 7.5 Hz, ArH); δ_C (75 MHz, ^2H_2O): 30.8 ($Ar\underline{C}H_2$), 38.2 ($NH\underline{C}H_2$), 50.7 ($\underline{C}H_3COO$), 126.4-140.7 (4 ArC) and 167.2 ($CH_3\underline{C}OO$); m/z (EI) 179 (40, $[M\text{-}HCl]^+$), 148 (40, $[M\text{-}HCl\text{-}OCH_3]^+$), 135 (78, $C_6H_4CO_2CH_3^+$), 150 (88, $[M\text{-}HCl\text{-}CH_2NH]^+$), 119 (70), 91 (77, $C_6H_5CH_2^+$), 90 (72) and 30 (100, $CH_2NH_2^+$).

4-fluoro-3-nitrobenzoic acid (65)



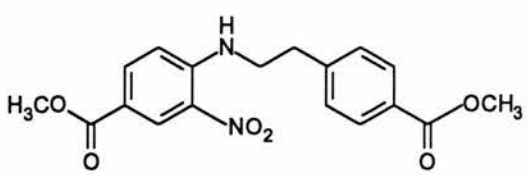
Concentrated sulfuric acid (sp. gr. 1.84) (11 ml) was added to c. nitric acid (sp. gr. 1.42) (7.5 ml) at 0 °C. Then 4-fluorobenzoic acid **(64)** (6 g, 43 mmol) was slowly added. After allowing the mixture to slowly warm to room temperature, it was then refluxed for 1 hour and poured on to crushed ice (400 ml), forming crystals that were collected by vacuum filtration. The acid was then recrystallised from water. (7.96 g, 77%), m.p. 120-122 °C (Lit [73] 122 °C). δ_H (300 MHz, DMSO-d_6): 7.80 (1H, d.d., *J* 2.1 & 8.2 Hz, 5-H), 8.45 (1H, m., 6-H) and 8.75 (1H, d., *J* 2.54 Hz, 2-H); δ_C (75 MHz, DMSO-d_6): 118.9 (C-5, $J_{C\text{-}F}$ 260 Hz), 119.2 (C-1), 127.1 (C-2), 127.9 (C-6), 136.7 (C-3, $J_{C\text{-}F}$ ~ 21 Hz), 155.3 (C-4) and 164.6 (COOH).

Methyl 4-fluoro-3-nitrobenzoate (66)



At 0 °C 4-fluoro-3-nitrobenzoic acid **(65)** (1.0 g, 5.4 mmol), dissolved in methanol (30 ml) was stirred vigorously. Thionyl chloride (2.2 ml, 30.2 mmol) was added dropwise. After complete addition, the reaction vessel was allowed to rise to room temperature, then refluxed for 1 hour. The solvent was removed *in vacuo* and the product was recrystallised from petroleum (or heptane), giving a white solid (0.77 g, 72%), m.p. 52-54 °C (Lit [64] 54-55 °C). δ_H (300 MHz, C^2HCl_3): 3.9 (3H, s., CH_3COO), 7.35 (1H, d., *J* 7.7 Hz, 5-H), 8.40 (1H, m., 6-H) and 8.75 (1H, d., *J* 2.1 Hz, 2-H); δ_C (75 MHz, C^2HCl_3): 54.7 ($\underline{C}H_3COO$), 118.7 (C-5, J_{C-F} ~260 Hz), 119.0 (C-1), 127.3 (C-2), 127.9 (C-6), 136.5 (C-3, J_{C-F} ~21 Hz), 160 (C-4) and 164.1 ($CH_3\underline{C}OO$); m/z (EI) 199 (M^+, 37%), 168 (100, $[M\text{-}OCH_3]^+$) and 122 (42).

methyl N^4-(4-methoxycarbonylphenethylamino)-3-nitrobenzoate (67)



Method 1

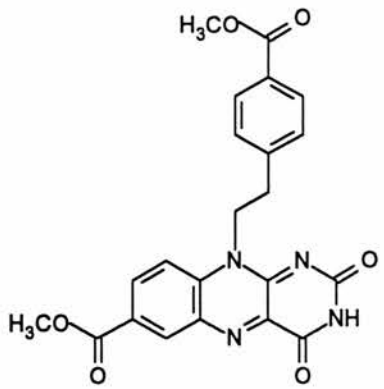
A solution of the amine methyl ester **(63)** (0.7 g, 3.25 mmol), dissolved in absolute ethanol (25 ml) was adjusted to pH 8 with 10% (w/w) aqueous potassium carbonate solution, then added to methyl 4-fluoro-3-nitro-benzoate **(66)** (0.6 g, 3.01 mmol), dissolved in absolute ethanol (20 ml).

The mixture was refluxed for 3 hours and filtered hot to remove any impurities. Washing with aqueous ethanol (*ca.* 50% v/v) and recrystallising from methanol gave a yellow product, that was identical to that produced by Method 2 (1.00 g, 93%), m.p. 142-143 °C.

Method 2

The ester **(63)** (1.65 g, 7.65 mmol) was dissolved in anhydrous methanol (100 ml), the solution was basified to pH 8, adding sodium hydrogen carbonate (1.30 g, 15.47 mmol). Then methyl 4-fluoro-3-nitrobenzoate **(66)** (1.38 g, 6.93 mmol) was added and the mixture was refluxed for 3 hours, monitoring to ensure that the pH remained *ca.* pH 8. The compound was initially purified, washing with aqueous methanol. Recrystallisation from methanol gave rise to yellow crystals (2.32 g, 94%), m.p. 143-45 °C. m/z (Found $[M]^+$ 358.1165, $C_{18}H_{18}N_2O_6$ requires 358.1165); ν_{max} (nujol)/cm^{-1} 3358 m. (2° NH), 1728, 1713 v.s. ($CH_3\underline{C}OOAr$), 1568 w. (2° NH), 1338 and 1318 w. ($ArNO_2$), 854, 843 and 833 s. (Ar); λ_{max} (CH_2Cl_2)/nm 288 (ε/8082 $dm^{-3}mol^{-1}cm^{-1}$) and 416 (3836); δ_H (300 MHz, C^2HCl_3): 3.10 (2H, t., *J* 7.5 Hz, $ArCH_2$), 3.65 (2H, t., *J* 7.5 Hz, $NHCH_2$), 3.88 (3H, s., CH_3COO), 3.90 (3H, s., CH_3COO), 6.88 (1H, d., *J* 8.5 Hz, 6-H), 7.35 (2H, d., $J_{1,2}$ 8.5 Hz, ArH), 8.05 (2H, d., *J* 8.5 Hz, ArH), 8.10 (1H, br.d., 5-H), 8.40 (1H, br.s., NH) and 8.90 (1H, s., 3-H); δ_C(75 MHz, C^2HCl_3): 35.14 ($Ar\underline{C}H_2$), 44.17 ($NH\underline{C}H_2$), 52.10 ($\underline{C}H_3COO$), 113.40-147.36 (10 ArC), 165.64 ($CH_3\underline{C}OO$) and 166.77 ($CH_3\underline{C}OO$); m/z (EI) 358 (M^+, 8%), 209 (100), 179 (10) and 150 (12).

7-methoxycarbonyl-N^{10}-(4-methoxycarbonylphenethyl)isoalloxazine (69)

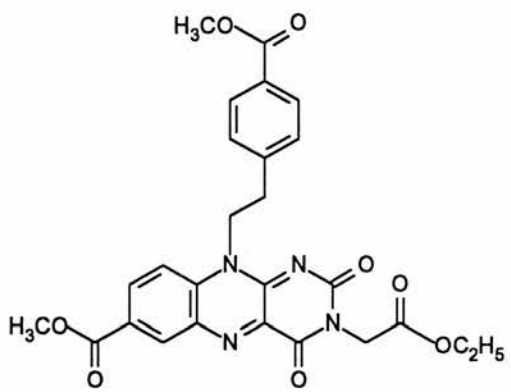


The ester **(67)** (1.00 g, 2.79 mmol) was dissolved in ethyl acetate (110 ml) and active palladium on carbon (10% w/w) (200 mg) was added. The mixture was then hydrogenated for *ca.* 24 hours. The solvent was then removed *in vacuo*. The catalyst was removed by suction filtration through Celite. The resulting amino ester was then dissolved in absolute ethanol (100 ml) and thoroughly degassed with argon.

Alloxan monohydrate **(13)** (0.49 g, 3.06 mmol), dissolved in hot concentrated hydrochloric acid (sp. gr. 1.18) (15 ml) was added to the degassed solution of the amino ester. The mixture was refluxed for 1 hour. Cooling yielded a yellow residue, which was recrystallised from methanol, forming yellow crystals (0.81 g, 67%), m.p. > 300 °C (Lit [64] m.p. > 300 °C). m/z (Found $[M+H]^+$, 435.1304. $C_{22}H_{19}N_4O_6$ requires 435.1304); ν_{max} (nujol)/cm^{-1} 1729 v.s. (ester CO), 1705 m. (CO), 1668 m. (CONH), 1590 and 1558 m. (Heterocyclic Ar), 866, 843 and 833 w. (Ar); λ_{max} (CH_3CN)/nm 284 (ε/305 $dm^3mol^{-1}cm^{-1}$) and 428 (119); δ_H (300 MHz, DMSO-d_6): 3.25 (2H, t., *J* 7.5 Hz, $ArCH_2$), 3.97 (3H, s., CH_3COO), 4.07 (3H, s., CH_3COO), 4.95 (2H, t., *J* 7.5 Hz, $N^{10}CH_2$), 7.66 (2H, d., *J* 8.4 Hz, ArH), 8.02 (2H, d., *J* 8.4 Hz,

ArH), 8.14 (1H, d., *J* 9 Hz, ArH), 8.39 (1H, d., *J* 9 Hz, ArH) and 8.64 (1H, s., ArH); δ_C (75 MHz, DMSO-d_6): 35.72 (Ar<u>C</u>H_2), 41.52 (N^{10}<u>C</u>H_2), 55.95 (<u>C</u>H_3COO), 56.54(<u>C</u>H_3COO), 120.91-139.40 (9 ArC), 143.00 (C-4a), 147.27 (C-10a), 154.44 (C-2), 159.36 (C-4), 163.28 (CH_3<u>C</u>OO) and 170.01 (CH_3<u>C</u>OO); m/z (EI) 434 (M^+, 2%), 241 (16), 162 (100), 131 (75), 103 (11) and 31 (75, OCH_3^+).

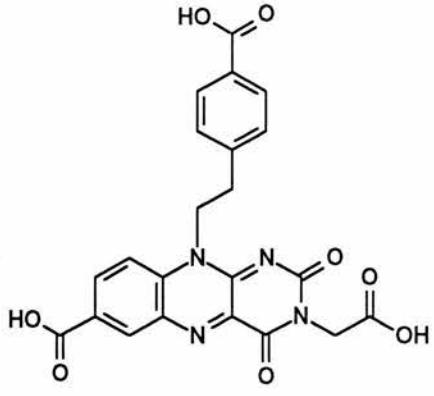
N^3-ethoxycarbonylmethyl-7-methoxycarbonyl-N^{10}-(4-methoxycarbonyl-phenethyl)isoalloxazine (70)



The diester **(69)** (100 mg, 0.23 mmol) was dissolved in DMF (10 ml) and potassium carbonate (200 mg, 1.45 mmol) was added. The mixture was stirred for 5 minutes. Then ethyl bromoacetate (100 μl, 0.90 mmol) was added and the mixture stirred for 48 hours. The resultant dark red mixture was poured into water (10 ml), then extracted with dichloromethane (20 ml) and washed with water (10 ml). The organic fraction was dried with anhydrous magnesium sulfate and residual DMF was removed *in vacuo.* The residue was added to anhydrous methanol, cooling below 0 °C to form a yellow precipitate. Recrystallisation from methanol gave a yellow crystalline solid (70 mg, 58%), m.p. 204-06 °C. m/z (Found $[M+H]^+$

521.1705. $C_{26}H_{25}N_4O_8$ requires 521.1672); ν_{max} (nujol)/cm^{-1} 2853 m. (CH_2), 1742 and 1724 s. (ester CO), 1705 and 1665 s. (NCO), 1590 and 1558 m. (Heterocyclic Ar), 866, 843 and 833 w. (Ar); $\lambda_{max}(CH_2Cl_2)$/nm 236 (ε/9900 $dm^3mol^{-1}cm^{-1}$), 272 (10818), 332 (3663) and 434 (5544); δ_H (200 MHz, C^2HCl_3): 1.30 (3H, q., *J* 7.5 Hz, CH_3CH_2), 3.25 (2H, t., *J* 7.5 Hz, $ArCH_2$), 3.90 (3H, s., CH_3COO), 4.00 (3H, s., CH_3COO), 4.25 (2H, q., *J* 6.7 Hz, CH_2COO), 4.85 (2H, s., NCH_2CO), 4.95 (2H, t., *J* 7.5 Hz, $N^{10}CH_2$), 7.40 (2H, d., *J* 8.8 Hz, ArH), 7.55 (1H, d., *J* 8.8 Hz, ArH), 8.00 (2H, d., *J* 8.8 Hz, ArH), 8.40 (1H, d., *J* 8.8 Hz, ArH) and 8.98 (1H, br.d., ArH); m/z (FAB) 521 ($[M+H]^+$, 100%), 135 (67) and 163 (15).

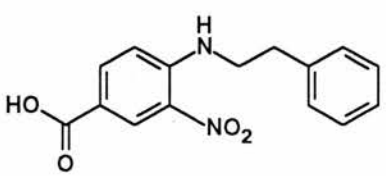
7-carboxy-N^3-carboxymethyl-N^{10}-(4-carboxyphenethyl)isoalloxazine (71)



The triester **(70)** (50 mg, 0.096 mmol) was heated with concentrated hydrochloric acid (sp. gr. 1.18) (1 ml) at 80 °C for 30 minutes. Crushed ice (20 ml) was added and the residue was further cooled, causing a yellow solid to precipitate, that was collected by vacuum filtration. Recrystallisation from 2 M acetic (ethanoic) acid gave rise to yellow crystals (43 mg, 96%), m.p. > 300 °C. m/z (Found: $[M+H]^+$, 465.1046. $C_{22}H_{17}N_4O_8$ requires 465.1046); ν_{max} (nujol)/cm^{-1} 2854 s. (CO<u>OH</u>), 1739 m. (CH_2<u>CO</u>OH),

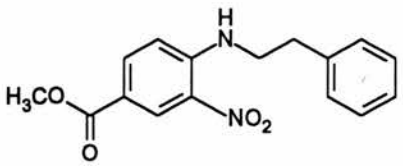
1640 w. (CO-N-CO), 1587 and 1556 m. (Heterocyclic Ar); λ_{max} (THF)/nm 246 (ε/3460 $dm^3mol^{-1}cm^{-1}$), 294 (5190) & 428 (2076); δ_H (200 MHz, DMSO-d_6): 3.95 (2H, br.t., $ArCH_2$), 4.50 (2H, s., NCH_2CO), 4.90 (2H, br.t., $N^{10}CH_2$), 7.55 (2H, d., *J* 7.5 Hz, ArH), 7.90 (2H, d., *J* 7.5 Hz, ArH), 8.30 (2H, br.d., ArH) and 8.50 (1H, br.d., ArH); δ_C (75 MHz, DMSO-d_6): 35.54 ($Ar\underline{C}H_2$), 42.64 ($N^{10}\underline{C}H_2$), 45.12 ($N\underline{C}H_2CO$), 117.16 (C-9), 129.09-138.19 (8 ArC), 142.65 (C-4a), 149.38 (C-10a), 154.24 (C-2), 159.32 (C-4), 167.04 (C-7α), 170.79 (C-3β) & 173.28 (*p*-COOH); m/z (FAB) 465 ($[M+H]^+$, 100%), 447 (20, $[M-H_2O]^+$), 389 (17, $[M-H_2O-CH_2COOH]^+$) and 273 (15).

3-Nitro-N^4-phenethylaminobenzoic acid (72)



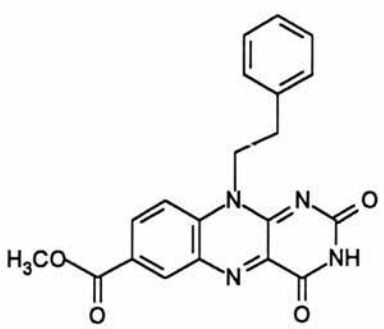
A mixture of both 2-phenethylamine **(58)** (1.36 ml, 10.8 mmol) and 4-fluoro-3-nitrobenzoic acid **(65)** (1 g, 5.4 mmol) was refluxed in absolute ethanol (50 ml) for 2 hours. The solution was concentrated *in vacuo* to *ca.* 20 ml and then acidified with 2 M hydrochloric acid, cooling to promote precipitation. Recrystallisation from methanol gave a yellow crystalline product (1.09 g, 70%), m.p. 197-199 °C (Lit [24] 200-202 °C). δ_H (300 MHz, DMSO-d_6): 3.14 (2H, t., *J* 7.5 Hz, $C_6H_5CH_2$), 3.30 (1H, br.s., NH), 3.83 (2H, q., *J* 7.5 Hz, $NHCH_2$), 7.43 (5H, m., C_6H_5), 8.13 (1H, d.d., *J* 2 and 8.6 Hz, 6-H), 8.60 (1H, d., *J* 8.5 Hz, 5-H), 8.69 (1H, d., *J* 2 Hz, 2-H); δ_C (75 MHz, DMSO-d_6): 34.2 ($C_6H_5\underline{C}H_2$), 43.8 ($NH\underline{C}H_2$), 114.5 (C-5), 117.5 (C-4), 126.3-128.7 (4 Ar/C), 130.3 (C-6), 136.0 (C-2), 138.5 (C-1), 146.9 (C-3) and 165.9 (COOH); m/z (EI) 286 (M^+, 16%), 195 (100), 91(20) and 44 (21).

Methyl 3-nitro-N^4-phenethylaminobenzoate (73)



Thionyl chloride (1.3 ml, 17.5 mmol) was added to a suspension of the nitrobenzoic acid **(72)** (1 g, 3.5 mmol) in methanol (50 ml) at 0-5 °C. The mixture was then refluxed for 2 hours. The compound was recrystallised from methanol, forming yellow-orange crystals (0.89 g, 95%), m.p. 97-99 °C (Lit [24] 97-99 °C). λ_{max} (CH_2Cl_2)/nm 266.5 (ε/35285 $dm^3mol^{-1}cm^{-1}$), 291.5 (36486) and 416 (9760); δ_H (300 MHz, C^2HCl_3): 3.05 (2H, t., *J* 7.5 Hz, $C_6H_5CH_2$), 3.60 (2H, q., *J* 7.5 Hz, $NHCH_2$), 3.90 (3H, s.,CH_3COO), 6.85 (1H, d., *J* 8.6 Hz, 5-H), 7.30 (5H, m., C_6H_5), 8.03 (1H, d.d., *J* 2.1 and 8.6 Hz, 6-H), 8.35 (1H, br.s., ArNH) and 8.86 (1H, d., *J* 2 Hz, 2-H); δ_C(75 MHz, C^2HCl_3): 35.1 ($C_6H_5\underline{C}H_2$), 44.60 ($NH\underline{C}H_2$), 113.4 (C-5), 117.3 (C-4), 127.0-129.5 (4 Ar/C), 131.3 (C-6), 136.3 (C-2), 137.8 (C-1), 147.5 (C-3) and 165.6 ($CH_3\underline{C}OO$); m/z (EI) 300 (M^+, 23%), 209 (100), 103 (10) and 77 (10).

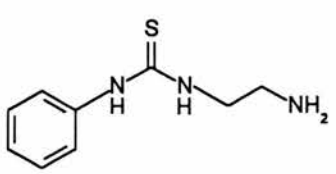
7-methoxycarbonyl-N^{10}-phenethylisoalloxazine (74)



The ester **(73)** (1.65 g, 5.49 mmol) was dissolved in ethyl acetate (90 ml). Activated palladium on carbon (10% w/w) (330 mg) was added and the mixture was hydrogenated for *ca.* 24 hours. The catalyst was removed by suction filtration through Celite. The resulting amino ester was then dissolved in absolute ethanol (80 ml) and degassed thoroughly with argon.

Then alloxan monohydrate **(13)** (0.98 g, 6.12 mmol), dissolved in hot concentrated hydrochloric acid (sp. gr. 1.18) (20 ml) was added and the mixture was refluxed for 15 minutes. The product was recrystallised from methanol, giving yellow crystals (0.98 g, 47%), m.p. > 300 °C (Lit [24] m.p > 300 °C). m/z (Found $[M+H]^+$ 377.1250. $C_{20}H_{17}N_4O_4$ requires 377.1250). λ_{max} (EtOH)/nm 272 (ε/25990 $dm^3mol^{-1}cm^{-1}$) and 428 (6188); δ_H (200 MHz, DMSO-d_6): 3.06 (2H, br.t., $C_6H_5CH_2$), 3.96 (3H, s., CH_3COO), 4.82 (2H, br.t., $N^{10}CH_2$), 7.34 (5H, m., C_6H_5), 8.01 (1H, d., *J* 7.5 Hz, 9-H), 8.30 (1H, d.d., *J* 2.5 and 7.5 Hz, 8-H) and 8.55 (1H, br.d., 6-H); δ_C (75 MHz, DMSO-d_6): 35.95 ($C_6H_5\underline{C}H_2$), 44.86 ($N^{10}\underline{C}H_2$), 56.77 ($\underline{C}H_3COO$), 121.10-139.50 (8 ArC), 141.64 (C-7), 142.14 (C-4a), 149.00 (C-10a), 154.76 (C-2), 159.71 (C-4) and 164.22 ($CH_3\underline{C}OO$); m/z (FAB) 376 (M^+, 75%), 253 (25), 197 (100), 135 (88) and 104 (52).

1-(2-Aminoethyl)-3-phenylthiourea (75)

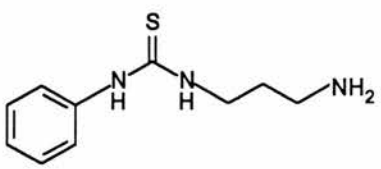


Initially 1,2-diaminoethane (1.15 ml, 17.2 mmol) was dissolved in 2-propanol (13 ml). Phenyl isothiocyanate (1 ml, 8.36 mmol), dissolved in diethyl ether (2 ml) was then added dropwise at 0 °C. A white precipitate resulted.

On complete addition the mixture was diluted with water to *ca.* 50 ml. The flask was left stirring overnight. Then the reaction mixture was acidified with concentrated hydrochloric acid (sp. gr. 1.18) to pH 2.6 and heated to 70 °C for 30 minutes.

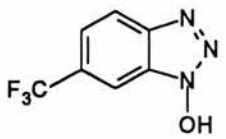
The remaining white precipitate was filtered and discarded. The filtrate was evaporated *in vacuo*, then basified with saturated sodium hydroxide solution to pH 8.5, producing white crystals, which were recrystallised from water (0.78 g, 48%), m.p. 133-35 °C (Lit [86] 135-136 °C). δ_H (200 MHz, DMSO-d_6): 2.85 (2H, t., *J* 6.4 Hz, NH_2CH_2), 3.45 (2H, t., *J* 6.4 Hz, $CSNHCH_2$), 3.70 (2H, br.s., NH_2), 7.40 (5H, m., C_6H_5) and 7.80 (1H, br.s., ArNH); δ_C (75 MHz, DMSO-d_6): 44.86 ($NH_2\underline{C}H_2$), 49.74 ($NH\underline{C}H_2$), 123.02-139.40 (4 ArC) and 180.65 (C=S); m/z (EI) 195 (M^+, 2%), 135 (100), 93 (84) and 77 (90).

1-(3-Aminopropyl)-3-phenylthiourea (76)



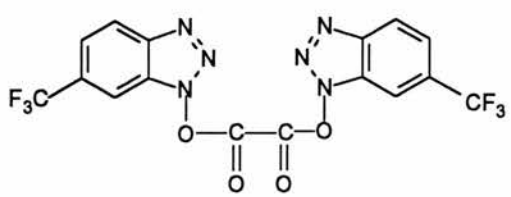
The procedure for **(75)** was repeated in an analogous manner, using the same relative quantities of reagents, replacing 1,2-diaminoethane by 1,3-diaminopropane (1.27 g, 48%), m.p. 105-06 °C (Lit [86] 106-07 °C). δ_H (300 MHz, C^2HCl_3): 1.60 (2H, qn., *J* 6.4 Hz, $NH_2CH_2C\underline{H}_2$), 1.85 (2H, t., *J* 6.4 Hz, NH_2CH_2), 3.25 (2H, t., *J* 6.4 Hz, $CSNHCH_2$), 3.70 (2H, br.s., NH_2), 7.27 (5H, m., C_6H_5) and 7.60 (1H, br.s., ArNH); δ_C (75 MHz, C^2HCl_3): 30.77 ($NHCH_2\underline{C}H_2$), 40.36 ($NH_2\underline{C}H_2$), 45.21 ($NH\underline{C}H_2$), 124.82-136.45 (4 ArC) and 180.32 (C=S); m/z (EI) 209 (M^+, 2%), 174 (51), 93 (79) and 77 (27).

1-Hydroxy-6-(trifluoromethyl) benzotriazole (78)



Initially a mixture of 4-chloro-3-nitro-ααα-trifluorotoluene (4-chloro-3-nitrobenzotrifluoride) **(77)** (6.6 ml, 44 mmol) and hydrazine hydrate (6.4 ml, 132 mmol) was refluxed in absolute ethanol (20 ml) for 24 hours. The residue was dissolved in 10% (w/w) sodium carbonate solution. The aqueous layer was extracted with three volumes of diethyl ether and acidified with concentrated hydrochloric acid (sp. gr. 1.18), cooling to promote crystal formation. Recrystallisation from diethyl ether/ petroleum and decolorising with charcoal gave an off-white solid (7.54 g, 84%), m.p. 141-44 °C (Lit.[89] 143-47 °C). δ_H (300 MHz, $C^2H_3COC^2H_3$): 7.81(1H, d.d., *J* 2.2 and 8 Hz, ArH) and 8.29 (2H, m., ArH); δ_C (75 MHz, $C^2H_3COC^2H_3$): 120.62 (CF_3, q., *J* 255 Hz), 122.17-127.34 (ArC); m/z (EI) 203 (M^+, 5%), 187 (88), 159 (35), 140 (39) and 63 (40).

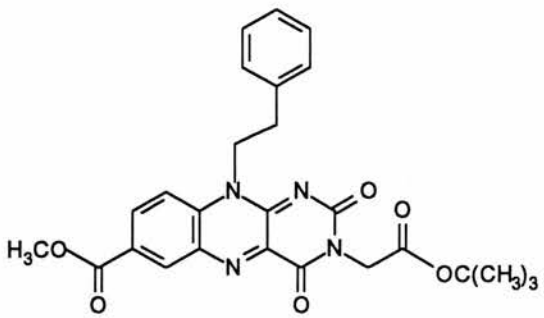
1,1/-Bis [6-(trifluoromethyl)benzotriazolyl] oxalate (BTBO) (79)



Initially the benzotriazole **(78)** (0.5 g, 2.46 mmol) was dissolved in diethyl ether (13 ml). Then oxalyl chloride (0.45 ml, 5.16 mmol) was slowly added. The mixture was stirred for 3 hours. The resulting white precipitate was

collected by suction filtration (0.39 g, 35%), m.p. 143-144 °C (Lit [89] 141-145 °C). δ_H (300 MHz, $C^2H_3COC^2H_3$): 7.80 (2H, d., *J* 7.8 Hz, ArH), 8.25 (2H, br.s., ArH) and 8.33 (2H, d., *J* 7.8 Hz, ArH); m/z (EI) 460 (M^+, 2%), 258 (52), 214 (75), 202 (17) and 187 (88).

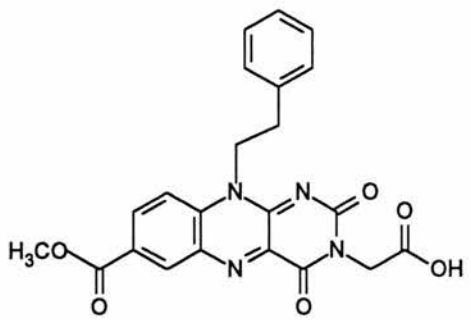
t-Butyl 7-methoxycarbonyl-N^{10}-phenethylisoalloxazine-3-acetate (80)



The monoester **(74)** (1.00 g, 2.66 mmol) was dissolved in DMF (70 ml) and potassium carbonate (2.04 g, 14.76 mmol) added. The mixture was then stirred for *ca.* 5 minutes after which freshly distilled t-butyl bromoacetate (2.63 ml, 16.30 mmol) was added. The mixture was then stirred for 14 hours. After completion of the reaction the residual potassium carbonate was removed by gravity filtration. Column chromatography, using 99% CH_2Cl_2/1% MeOH as eluent, followed by recrystallisation from methanol gave rise to yellow crystals (0.74 g, 57%), m.p. 212-214 °C (Lit.[24] 213-215 °C). m/z (Found $[M+H]^+$ 491.1913. $C_{26}H_{27}N_4O_6$ requires 491.1931); ν_{max} (nujol)/cm^{-1} 1729 s. (ester CO), 1675 m. (NCO), 1590 w. (Heterocyclic Ar) & 866 w. (Ar); λ_{max} (EtOH)/nm 428 (ε/3776 $dm^{-3}mol^{-1}$ cm^{-1}) and 260 (23776); δ_H (200 MHz, C^2HCl_3): 1.45 (9H, s., $(CH_3)_3$), 3.15 (2H, t., *J* 7.5 Hz, $C_6H_5CH_2$), 3.94 (3H, s., CH_3O), 4.72 (2H, s., NCH_2CO), 4.85 (2H, t., *J* 7.5 Hz, $N^{10}CH_2$), 7.25 (5H, m., C_6H_5), 7.50 (1H, d., *J* 10 Hz, 9-H), 8.30 (1H,

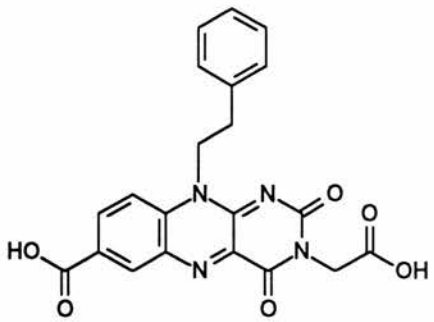
d.d., *J* 2.5 and 10 Hz, 8-H) and 8.88 (1H, d., *J* 2.5 Hz, 6-H); δ_C (50 MHz, C^2HCl_3): 28.53 (CH_3), 33.40 ($C_6H_5\underline{C}H_2$), 44.09 ($N^{10}\underline{C}H_2$), 46.96 ($N\underline{C}H_2CO$), 53.96 (CH_3O), 83.00 ($\underline{C}(CH_3)_3$), 115.86 (C-9), 127.94-135.97 (8 ArC), 149.75 (C-10a), 155.00 (C-2), 159.50 (C-4), 165.50 (C-7α) and 167.00 (C-3β); m/z (FAB) 491 ([M+H]$^+$, 39%), 435 (100) and 389 (38).

N^3-carboxymethyl-7-methoxycarbonyl-N^{10}-phenethylisoalloxazine (81)



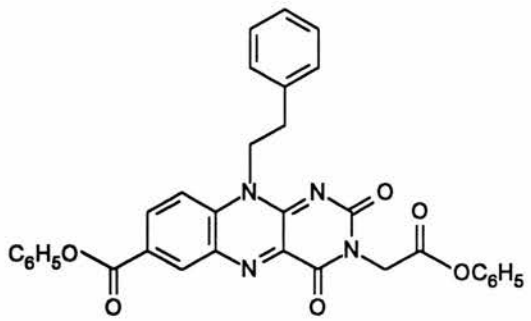
The diester **(80)** (120 mg, 0.245 mmol) was dissolved in dichloromethane (12 ml) and TFA (6 ml, 278 mmol) added. The mixture was stirred for 6 hours. The compound was recrystallised from methanol (83 mg, 78%), m.p. 288 °C dec (Lit.[24] 285 °C dec). (Found [M+H]$^+$ 435.1305. $C_{22}H_{19}N_4O_6$ requires 435.1305); ν_{max} (nujol)/cm^{-1} 2853 s. (CO$\underline{OH}$), 1721 m. (Ar $\underline{CO}$), 1668 s. ($\underline{CO}$NH), 1594, 1558 and 830 w. (ArC); λ_{max} (EtOH)/nm 432 (ε/1406 dm^{-3}mol^{-1}cm^{-1}) and 280 (7348); δ_H (200 MHz, DMSO-d_6): 3.10 (2H, t., $C_6H_5CH_2$, *J* 7.5 Hz), 3.95 (3H, s., CH_3O), 4.50 (2H, s., NCH_2CO), 4.85 (2H, t., $N^{10}CH_2$, *J* 7.5 Hz), 7.35 (5H, m., C_6H_5), 8.05 (1H, d., *J* 10 Hz, 9-H), 8.30 (1H, d.d., *J* 2.5 and 10 Hz, 8-H) and 8.60 (1H, d., *J* 2.5 Hz, 6-H); δ_C (50 MHz, DMSO-d_6): 31.91 ($C_6H_5\underline{C}H_2$), 42.54 ($N^{10}\underline{C}H_2$), 45.60 ($N\underline{C}H_2CO$), 52.57 ($\underline{C}H_3O$), 117.16 (C-9), 126.69-138.59 (8 ArC), 149.52 (C-10a), 154.23 (C-2), 158.61 (C-4), 164.74 (C-3β) and 168.94 (C-7α); m/z (FAB) 435 ([M+H]$^+$,100), 301 (88) and 125 (48).

7-carboxy-N^3-Carboxymethyl-N^{10}-phenethylisoalloxazine (82)



The t-butyl ester **(80)** (0.85 g, 1.73 mmol) was added to c. HCl (sp. gr. 1.18) (35 ml) and the mixture refluxed for 1 hour. It was then allowed to cool to room temperature. Ice water (25 ml) was added, precipitating the product. Yellow crystals were obtained, recrystallising from methanol (0.61 g, 84%) m.p. 187-88 °C (Lit. [24] 183-85 °C). m/z (Found $[M+H]^+$ 421.1150. $C_{21}H_{17}N_4O_6$ requires 421.1148); ν_{max} (nujol)/cm^{-1} 2855 v.s. (CO<u>OH</u>), 1713 s. (CH_2<u>CO</u>OH), 1662 s. (N<u>CO</u>), 1590 and 1558 w. (Heterocyclic ArC); λ_{max} (EtOH)/nm 214 (ε/10333 $dm^3mol^{-1}cm^{-1}$), 270 (16988), 340 (3503) and 436 (4904); δ_H (200 MHz, DMSO-d_6): 3.10 (2H, t., *J* 7.5 Hz, $C_6H_5CH_2$), 4.60 (2H, s., NCH_2CO), 4.90 (2H, t., *J* 7.5 Hz, $N^{10}CH_2$), 7.37 (5H, m., C_6H_5), 8.05 (1H, d., *J* 10 Hz, 9-H), 8.35 (1H, d.d., *J* 2.5 and 10 Hz, 8-H) and 8.60 (1H, br.d., 6-H); δ_C (50 MHz, DMSO-d_6): 32.23 (C_6H_5<u>C</u>H_2), 41.50 (N^{10}<u>C</u>H_2), 42.90 (N<u>C</u>H_2CO), 127.07-139.99 (9 ArC), 149.57 (C-10a), 153.51 (C-2), 159.25 (C-4), 169.00 (C-7α) and 169.90 (C-3β); m/z (FAB) 421 ($[M+H]^+$, 35%), 307 (23), 289 (23), 155 (46), 137 (100), 103 (24) and 89 (26).

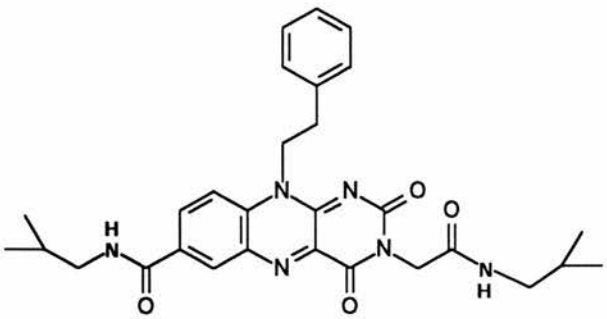
7-phenoxycarbonyl-N^3-phenoxycarbonylmethyl-N^{10}-phenethyl-isoalloxazine (83)



The diacid **(82)** (50 mg, 0.119 mmol) was added to toluene (10 ml), followed by phenol (45 mg, 0.48 mmol). A trace amount of PTSA was added. The mixture was refluxed for *ca.* 5 hours, using a Dean-Stark condenser.

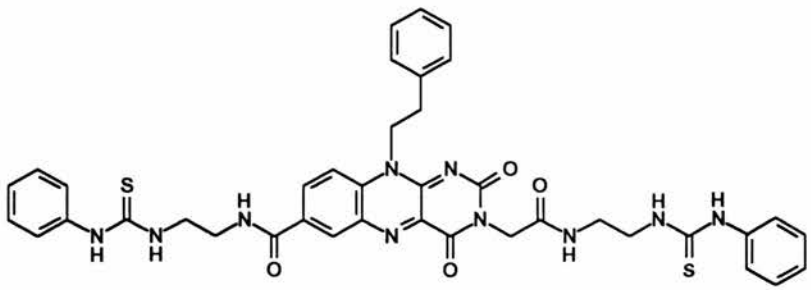
The residue was then taken up in dichloromethane and then washed with 10% (w/w) $NaHCO_3$. The organic layer was taken and the solvent, dichloromethane removed *in vacuo.* Recrystallisation with methanol/ether gave yellow crystals 0.23 g (34%), m.p. dec. 300 °C. m/z (Found $[M+2H]^+$ 574.1830, $C_{33}H_{26}N_4O_6$ requires 574.1852); ν_{max} (nujol)/cm^{-1} 2853 m. (CH_2), 1743 m. (ArCOOPh), 1705 w. and 1683 m. (C=O), 1634 s. (CO-N-CO), 1594 and 1558 s. (Heterocyclic ArC); δ_H (200 MHz, C^2HCl_3): 3.10 (2H, t., *J* 7.5 Hz, $C_6H_5CH_2$), 4.85 (2H, s., NCH_2CO), 5.05 (2H, br.t., $N^{10}CH_2$), 6.90-7.40 (15H, 2m., 3 x C_6H_5), 7.65 (1H, d., *J* 7.5 Hz, 9-H), 8.35 (1H, br.d., 8-H) and 8.60 (1H, d., *J* 2.5 Hz, 6-H); m/z (FAB) 574 ($[M+2H]^+$, 61%), 460 (50), 421 (100), 391 (92), 385 (43) and 355 (38).

7-(2-methylpropanamido)-3-[(2-methylpropanamido)methyl]-N^{10}-phenethylisoalloxazine (84)



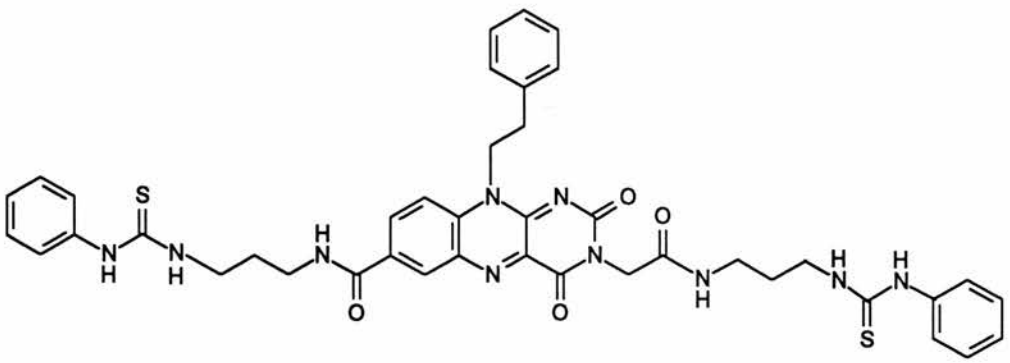
The flavin diacid **(82)** (50 mg, 0.119 mmol) was dissolved in THF (10 ml) and cooled to -15 °C, using a mixture of Cardice and acetone or ethanol. Then N-Methylmorpholine (NMM) (26 μl, 0.238 mmol) was added, followed by isobutylchloroformate (IBCF) (32 μl, 0.238 mmol). Then isobutylamine (35 μl, 0.35 mmol) was added, the mixture was stirred for *ca.* 10 minutes at -15 °C and allowed to rise to room temperature, reacting for a further 4 hours. On completion of the reaction the solvents and excess reagents were removed *in vacuo*. Water was then added to the residue to precipitate the product, which was recrystallised from a mixture of methanol/ether (0.05 g, 79%), m.p. 218-20 °C. m/z (Found $[M+H]^+$ 531.2702, $C_{29}H_{35}N_6O_4$ requires 531.2720); δ_H (200 MHz, C^2HCl_3/d_4-MeOH): 0.98 (12H, app.m., 4x CH_3), 1.7 (1H, m., CH), 1.9 (1H, m., CH), 3.10 (2H, t., *J* 7.5 Hz, $C_6H_5CH_2$), 3.18 (2H, d., *J* 6.8 Hz, NCH_2CH), 3.28 (2H, d., *J* 6.8 Hz, NCH_2CH), 4.53 (2H, s., NCH_2CO), 4.90 (2H, t., *J* 7.5 Hz, $N^{10}CH_2$), 7.23-7.38 (5H, m., C_6H_5), 8.06 (1H, d., *J* 10 Hz, 9-H), 8.32 (1H, d., *J* 10 Hz, 8-H), 8.65 (1H, br.d., 6-H) and 8.85 (1H, br.t., NH); m/z (FAB) 531 ($[M+H]^+$, 61%), 458 (20) and 111 (12).

[3-(2-acetamido-ethyl)-7-(2-formamido-ethyl)]-3,3′-bisphenylthiourea-N^{10}-phenethylisoalloxazine (85)



The procedure was repeated in an analogous manner to that for the bis-isobutylamino flavin **(84)**, using 1-(2-aminoethyl)-3-phenylthiourea **(75)** (117 mg, 0.6 mmol). The mixture was stirred for 4 hours. The compound was purified by column chromatography, using 92.5% CH_2Cl_2/7.5% CH_3OH as eluent (44 mg, 48%), m.p. 157-158 °C dec (Lit [25] 155 °C dec). m/z (Found $[M+H]^+$ 775.2594, $C_{39}H_{39}N_{10}O_4S_2$ requires 775.2597); ν_{max} (nujol)/cm^{-1} 2250 m., 1663 m. (2° CONH), 1594 and 1558 s. (Heterocyclic ArC), 1319 and 1249 w. (NCSN); δ_H (200 MHz, C^2HCl_3/d_4-MeOH): 3.15 (2H, t., *J* 7.5 Hz, $C_6H_5CH_2$), 3.30 (2H, br.t., CH_2), 3.45 (2H, br.t., CH_2), 3.70 (2H, br.t., *J* 2.5 Hz, CH_2), 3.92 (2H, br.t., CH_2), 4.75 (2H, s., NCH_2CO), 4.88 (2H, t., *J* 7.5 Hz, $N^{10}CH_2$), 7.15-7.40 (5H, m., C_6H_5), 7.70 (1H, d., *J* 7.5 Hz, 9-H), 8.30 (1H, d.d., *J* 2.5 and 7.5 Hz, 8-H) and 8.60 (1H, br.d., 6-H); δ_C (50 MHz, C^2HCl_3/d_4-MeOH): 32.97 ($C_6H_5\underline{C}H_2$), 43.61 ($N^{10}\underline{C}H_2$), 44.09 ($N\underline{C}H_2CO$), 44.82 ($CSNH\underline{C}H_2$), 44.92 ($CSNH\underline{C}H_2$), 46.56 ($\underline{C}H_2NHCO$), 116.02 (C_9), 124.12-138.50 (16 ArC), 149.40 (C-10a), 155.40 (C-2), 159.75 (C-4), 165.50 (C-3β), 168.20 (C-7α), 181.33 (C=S) and 181.40 (C=S); m/z (FAB) 776 ($[M+H]^+$, 21%), 391 (53), 296 (100), 149 (64) and 111 (42).

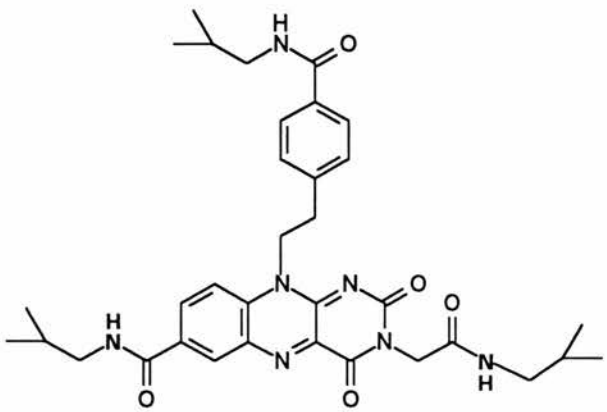
[3-(3-acetamido-propyl)-7-(3-formamido-propyl)]-3,3/-bisphenylthiourea-N^{10}-phenethylisoalloxazine (86)



The procedure was repeated in an analogous manner to that for the bis-isobutylamino flavin **(84)**, using 1-(2-aminopropyl)-3-phenylthiourea **(76)** (126 mg, 0.6 mmol). The mixture was reacted at least 4 hours. On completion of the reaction, excess solvents and reagents were removed *in vacuo*. The compound was purified by column chromatography, using 90% CH_2Cl_2/10% CH_3OH as eluent, dry loading the mixture (51 mg, 53%), m.p. dec. 178-180 °C. m/z (Found $[M+H]^+$ 803.2908, $C_{41}H_{43}N_{10}O_4S_2$ requires 803.2910); ν_{max} (nujol)/cm^{-1} 1733 m. (NCO-NCO), 1654 m., 1596 & 1538 s. (Heterocyclic ArC), 1461 (CH_2), 1377 (ArCH), 1290 and 1201 w. (NCSN); $\lambda_{max}(CH_2Cl_2)$/nm 280 (ε/5191 $dm^3mol^{-1}cm^{-1}$) and 436 (95); δ_H (200 MHz, C^2HCl_3/d_4-MeOH): 3.00 (2H, t., *J* 7.5 Hz, $C_6H_5CH_2$), 3.15 (2H, br.t., CH_2), 3.30 (2H, t., *J* 6.2 Hz, CH_2), 3.55 (2H, br.t., CH_2), 3.65 (2H, t., *J* 6.2 Hz, CH_2), 3.75 (2H, br.t., CH_2), 4.75 (2H, s., NCH_2CO), 4.90 (2H, br.t., $N^{10}CH_2$), 7.15-7.40 (5H, m., C_6H_5), 7.66 (1H, d., *J* 7.5 Hz, 9-H), 8.30 (1H, d.d., *J* 2.5 and 7.5 Hz, 8-H) and 8.65 (1H, br.d., 6-H); δ_C (50 MHz, C^2HCl_3/d_4-MeOH): 34.54 ($C_6H_5\underline{C}H_2$), 42.30 ($N^{10}\underline{C}H_2$), 43.18 ($N\underline{C}H_2CO$), 43.24 ($CSNH\underline{C}H_2$), 43.41 ($CSNH\underline{C}H_2$), 48.25 ($NHCH_2\underline{C}H_2$), 48.36 ($NHCH_2\underline{C}H_2$), 50.41 ($\underline{C}H_2NHCO$), 50.84 ($\underline{C}H_2NHCO$), 117.09 (C-9), 125.66-138.29 (16 ArC),

150.79 (C-10a), 155.31 (C-2), 159.81 (C-4), 167.06 (C-3β), 169.81 (C-7α), 182.77 (C=S) and 182.81 (C=S); m/z (FAB) 803 ([M+H]$^+$, 27%), 391 (54), 149 (100), 131 (16) and 111 (61).

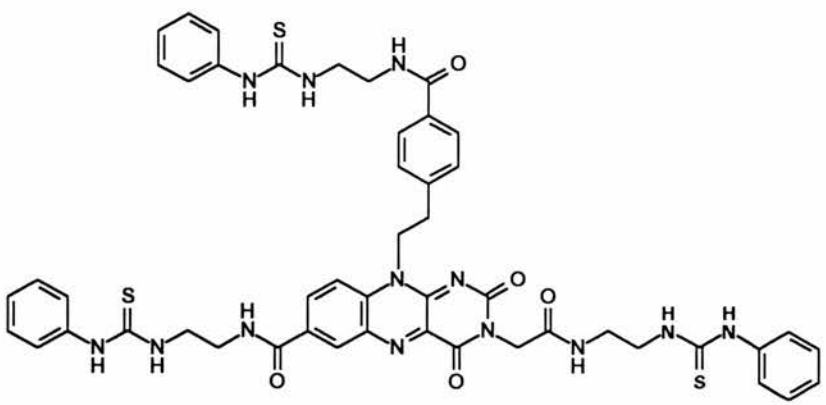
7-(2-methylpropanamido)-3-[(2-methylpropanamido)methyl]-N^{10}-4-[(2-methylpropanamido)phenethyl]isoalloxazine (87)



The flavin triacid **(71)** (50 mg, 0.108 mmol) was dissolved in THF (30 ml) and cooled to -15 °C, using a mixture of Cardice and acetone or ethanol. Then N-Methylmorpholine (NMM) (39 μl, 0.36 mmol) was added, followed by isobutylchloroformate (IBCF) (49 μl, 0.36 mmol), maintaining the temperature at -15 °C. Isobutylamine (64 μl, 108 mmol) was then added, the mixture was stirred for *ca.* 10 minutes at -15 °C and allowed to rise to room temperature, stirring for a further 4 hours. On completion of the reaction, excess solvents and reagents were removed *in vacuo.* Column chromatography (90% CH_2Cl_2/10% CH_3OH) gave a yellow solid (23 mg, 34%), m.p. dec. > 300 °C. m/z (Found [M+H]$^+$ 630.3447, $C_{34}H_{44}N_7O_5$ requires 630.3404); λ_{max} (CH_2Cl_2)/nm 282 (ε/2480 $dm^3mol^{-1}cm^{-1}$), 438 (512) and 560 (315); δ_H (200 MHz, C^2HCl_3/d_4-MeOH): 0.98 (18H, app.m.,

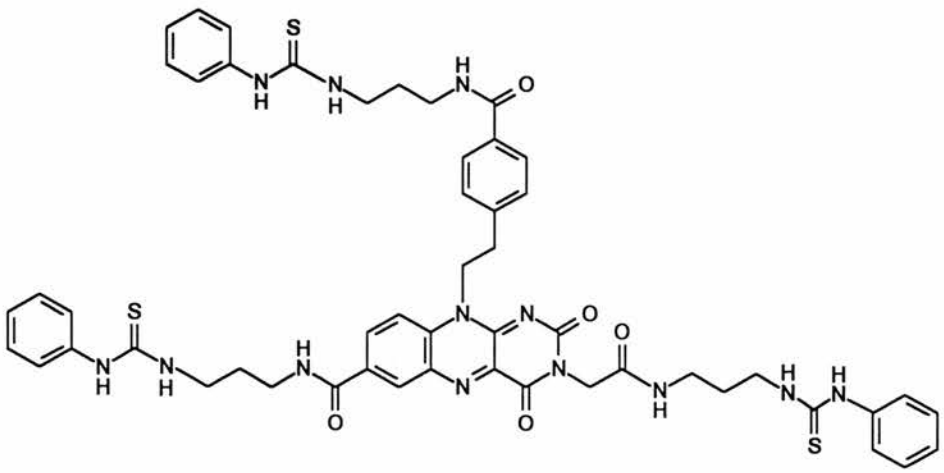
6 x CH_3), 1.66 (3H, app.m., 3 x CH), 3.00 (2H, t., *J* 7.5 Hz, $ArCH_2$), 3.08-3.32 (6H, 2 q., *J* 6.8 Hz, 3 x NCH_2CH), 4.64 (2H, s., NCH_2CO), 4.94 (2H, br.t., $N^{10}CH_2$), 7.38 (2H, d., *J* 7.5 Hz, ArH), 7.63 (1H, d., *J* 7.5 Hz, ArH), 7.96 (1H, d., *J* 10 Hz, ArH), 8.40 (1H, d., *J* 10 Hz, ArH), 8.65 (1H, s., ArH) and 8.85 (1H, br.t., NH); m/z (FAB) 630 ($[M+H]^+$, 74%), 516 (16), 589 (100), 198 (16), 149 (33), 131 (54) and 111 (62).

Reaction leading to fomation of : [3-(2-acetamido-ethyl)-7-(2-formamido-ethyl)]-3,3/-bisphenylthiourea-N^{10}-[4-((2-formamido-ethyl)phenylthiourea) phenethyl]isoalloxazine (88)



The procedure was similar to that for the tris-isobutylamino flavin **(87)**, dissolving the triacid in THF (50 ml) and using 1-(2-aminoethyl)-3-phenyl-thiourea **(75)** (127 mg, 0.648 mmol), dissolved in DMF (4 ml). The mixture was reacted for 36 hours. δ_H (200 MHz, C^2HCl_3/d_4-MeOH): 3.05 (2H, br.t., $ArCH_2$), 3.20 (2H, t., *J* 6.7 Hz, CH_2), 3.30 (2H, br.t., CH_2), 3.45 (2H, br.t., CH_2), 3.55 (2H, t., *J* 6.7 Hz, CH_2), 3.64 (2H, br.t., CH_2), 3.80 (2H, br.t., CH_2), 4.63 (2H, s., NCH_2CO), 4.75 (2H, br.t., $N^{10}CH_2$), 6.98-7.36 (15H, m., 3 x C_6H_5), 7.46 (2H, d., *J* 7.5 Hz, ArH), 7.53 (1H, br.d., ArH), 8.08 (1H, br.d., ArH), 8.28 (1H, d., *J* 10 Hz, ArH) and 8.68 (1H, s., ArH).

Reaction leading to: [3-(3-acetamido-propyl)-7-(3-formamido-propyl)]-3,3/-bisphenylthiourea-N^{10}-[4-((3-formamido-propyl)phenylthiourea) phenethyl]isoalloxazine (89)



The procedure was repeated in an analogous manner to that for the tris-isobutylamino flavin **(87)**, dissolving the triacid in 1,4-dioxan (30 ml) and using 1-(3-aminopropyl)-3-phenylthiourea **(76)** (136 mg, 0.648 mmol). The mixture was reacted for 36 hours. On completion of the reaction, excess solvents and reagents were removed *in vacuo*. δ_H (200 MHz, C^2HCl_3/ d_4-MeOH): 2.93 (2H, t., *J* 7.5 Hz, $ArCH_2$), 3.10 (2H, t., *J* 6.7 Hz, CH_2), 3.16-3.25 (10 H, app.m., 5 x CH_2), 3.44 (2H, br.t., CH_2), 3.78 (2H, br.t., CH_2), 3.85 (2H, t., *J* 6.7 Hz, CH_2), 4.69 (2H, s., NCH_2CO), 4.84 (2H, br.t., $N^{10}CH_2$), 7.10-7.40 (15H, m., 3 x C_6H_5), 7.68 (2H, br.d., ArH), 7.89 (1H, d., *J* 10 Hz, ArH), 8.30 (1H, d., *J* 10 Hz, ArH) and 8.61 (1H, s., ArH).

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