Tetrahedron Vol. 46, No. 3, pp. 935-956, 1990
Printed in Great Britain

0040-4020/90 $3.00+.00

SYNTHESIS OF BIFUNCTIONALISED FLAVINS FOR INCORPORATION INTO WELL DEFINED REDOX SYSTEMS

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(*Received in UK* 18 *October* 1989)

Abstract: The syntheses of isoalloxazines functionalised at C-7 and N-3, suitable for two point parallel face attachment to gold in the construction of flavin-gold electrodes, are described.

The mechanisms of electron transfer in biological redox reactions have been the subjects of intense research effort for many years. Systems which utilise the nicotine [NAD(P)H], flavin and ubiquinone redox coenzymes or that are involved in electron transport have been of foremost interest.[1] More recently, studies have been extended to the construction of a range of biomimetic systems. Currently studies of the systems themselves,[2] studies of model reactions[3] and studies of redox-active proteolytic enzymes[4] are in progress. The last ten years have seen an enormous increase in our understanding of the mechanisms of biological redox reactions, however, the design and development of biomolecular sensors[5], devices which often incorporate a redox enzyme, have made little use of this new knowledge.

Glutathione reductase catalyses the transfer of electrons from NADPH to the peptide disulphide, oxidised glutathione (GS-SG). The X-ray structure of the protein has been determined to 2.6 Å by Schulz and co-workers.[6] The single striking feature of the tertiary protein structure is that the reductant (NADPH) and the electron acceptor (GS-SG) sandwich the flavin, and that the spent species are replaced by NADPH and GS-SG on specific faces of the flavin prosthetic group. In all flavin based electrodes and sensors reported to date, the geometry between the flavin and the conductor has been controlled through a single covalent bonding interaction.[7] There are two major problems associated with this type of flavin-electrode interaction. First, is the low efficiency of electron transfer observed between the flavin and the electrode. Presumably this problem arises because the preferred molecular conformation of a system possessing a single point of attachment does not provide the optimum geometry for efficient electron transfer. Second, is the poor interaction between the apoenzyme and the electrode bound flavin-coenzyme. Clearly, if the flavin

cofactor is attached to a large conductor surface, it is not reasonable to expect the enzyme to bind to the flavin as effectively as it does to the free solution form. Furthermore, it is not reasonable to expect the awkward system which results to be able to efficiently catalyse the normal physiological reaction. Thus, it appears that in order to ensure substrate recognition specificity, either the enzyme should be engineered to work with a conductor-bound flavin, or a free solution form of the flavin should be present in the system.

While the second problem is one of biosensor design and is specific to the type and specificity of the device, the first problem, that of flavin-conductor interaction, is fundamental. Indeed, in order to mimic the natural 'sandwich' geometry of the glutathione reductase system, it would appear logical that the flavin π-system should lie parallel to the conductor. In order to ensure a parallel face geometry it was envisaged that at least two points of attachment for the flavin, preferably at the extremities of the isoalloxazine nucleus, would be required. Since the synthesis of suitably bifunctionalised flavins had not been reported in the literature, the first stage in determining the electron transfer properties of a parallel face flavin-electrode system was to synthesise appropriate flavins.

Here we report on the synthesis of a range of functionalised flavins suitable for the construction of flavin-gold electrodes. The observed electron transfer rates for one of these bifunctionalised flavins, after incorporation into an electrode system, Figure 1, have been previously communicated.[8] The electron transfer rates were determined from cyclic voltammetric data, using the method of Daifuku[9] and the values for the anodic and cathodic rate constants were 5.6×10^2 and 3.2×10^3 sec^{-1} respectively. In comparison with reported rates from similar electrode system these rates are very fast. These observations support the notion that the flavin lies parallel to the surface of the electrode.

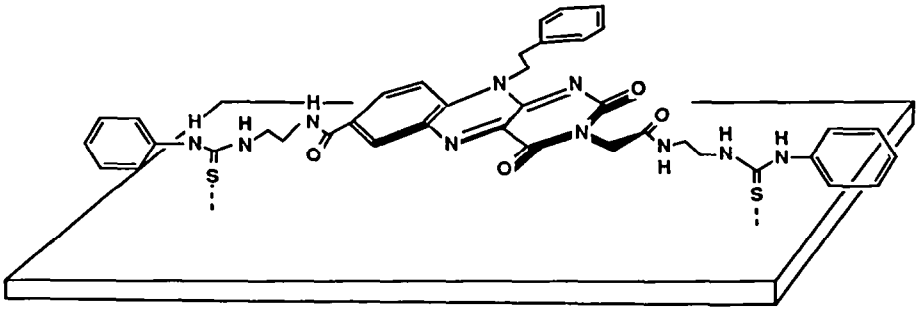
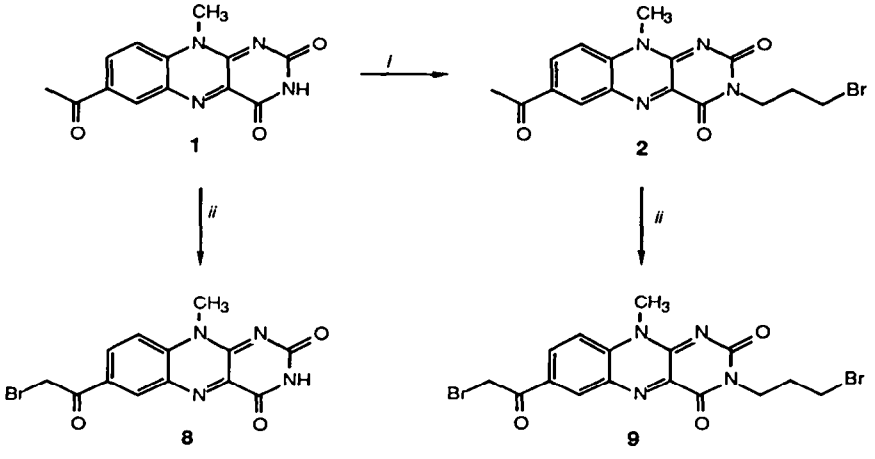


Figure 1

DISCUSSION

A detailed analysis of the glutathione reductase system indicated that the design of a flavin electrode should position the face of the flavin isoalloxazine nucleus parallel to the conductor surface. In order to achieve this geometry it was envisaged that at least two points of attachment for the flavin would be required and, thus, flavins suitably functionalised in two positions would need to be synthesised. To ensure that the correct flavin conformation could be achieved, it was important to place the attachment sites for the flavin as far apart on the isoalloxazine nucleus as possible. A synthetic strategy was necessary which would not disturb the special redox properties of the flavin nucleus, but which would allow flexibility in the type of terminal functionality used ultimately for the attachment of the flavin to the conductor surface. Since gold binds to sulphur ligands very tightly and is otherwise eminently suited as a conductor material, thiol and thiourea groups were chosen to serve as flavin-conductor attachment groups. After some preliminary synthetic work it became evident that positions C-7 and N-3 should be functionalised and used to anchor the flavin molecule to the surface of the electrode. Accordingly, the simultaneous functionalisation of positions C-7 and N-3 of the isoalloxazine nucleus was addressed.

In order to prepare the 7-acetyl isoalloxazine (**1**) the method of Levine and Kaiser was followed.[11] Starting from 4-chloro-acetophenone the sequence involved nitration at C-3 to give the 3-nitroacetophenone, nucleophilic aromatic displacement of chloride with methylamine to give the aromatic amine, reduction to the diamine and finally acid catalysed condensation with alloxan. The synthesis of the flavin (**1**) was achieved with a yield of 44% over the four steps.



(i) 1,3 -dibromopropane, K_2CO_3, DMF, (ii) NBS, TFA.

Scheme 1

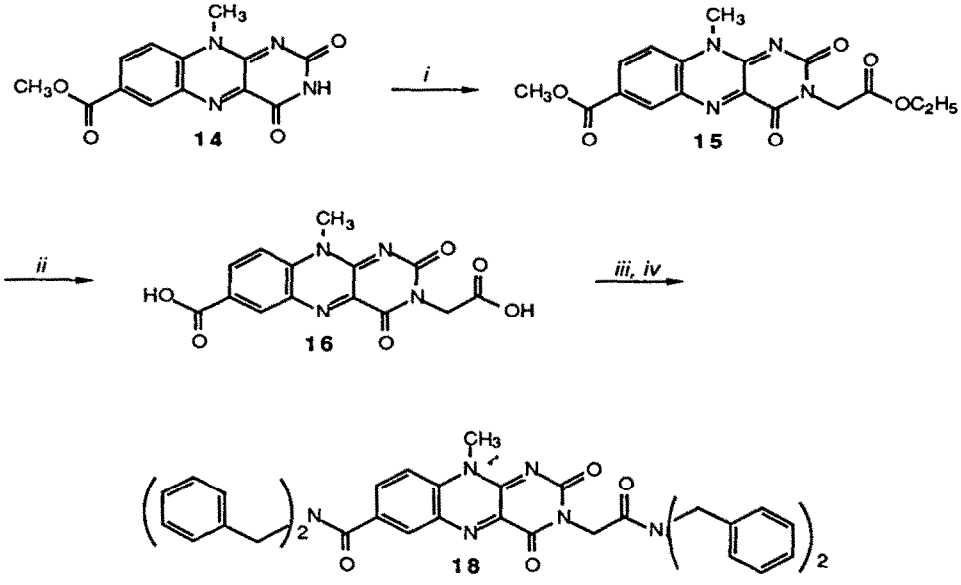
In order to introduce functionality at the N-3 position of the 7-acetyl-10-methylisoalloxazine (**1**) the method of Kraus was used initially.[12] The isoalloxazine was treated with anhydrous potassium carbonate and 1,3-dibromopropane to give the 3-bromopropyl derivative (**2**). Higher yields were obtained with bases other than potassium carbonate. For example, treatment of 7-acetyl-10-methylisoalloxazine with 1.1 equivalents of lithium di-*iso*propylamide at -50°C followed by 10 equivalents of 1,3-dibromopropane gave the desired 3-alkylated product (**2**) in 77% yield. A number of other 3-alkylated 7-acetyl-10-methylisoalloxazines were also synthesised using this method [compounds (**3-7**)]. Yields ranged from 61 to 93%.

Kaiser, in his approach towards synthetic flavopapains,[11] activated 7-acetyl-10-methylisoalloxazine for nucleophilic attack by forming the 7β-bromide (**8**). The derivative was prepared through treatment of 7-acetylisoalloxazine (**1**) with bromine in acetic acid. Using McCormick's bromination method and treating the 7-acetyl isoalloxazine (**1**) with N-bromosuccinimide in trifluoroacetic acid, with a trace of benzoyl peroxide as a catalyst, we were able to increase the yield of the 7β-bromo derivative (**8**) from 67 to 84% and also improve on the purity of the product.[13] Treatment of the isoalloxazine (**2**) in a similar manner gave the bifunctionalised flavin (**9**) which was then suitably activated for reactions with nucleophiles, Scheme 1.

In order to assess the susceptibility of the alkyl bromide side-chain of compound (**9**) to functionalisation by nucleophiles, the mono- and di- bromides [compounds **2**, **8** and **9**] were treated with sodium thiophenolate under a variety of conditions in a series of model reactions. The reaction of the α-bromoketone moiety in compounds (**8** and **9**) was fast and in the case of compound (**9**) could be replaced selectively to give the 7-phenylthioacetyl isoalloxazine (**13**) in low yield after purification. Under similar conditions the alkyl bromide side-chain could be converted to an alkyl thioether side-chain as confirmed by a ^{1}H-nmr spectral shift from 3.50 to 3.02 ppm due to the loss of the bromine atom and the introduction of the thiophenol moiety at C-3'. However, attempts to increase the yield of the reaction by increasing the reaction time or temperature resulted in the formation of several side products.

Thus, the synthesis of a bifunctionalised flavin was complete and, potentially, a range of bifunctionalised flavins suitable for covalent attachment to conductor surfaces were available. For example, through the use of alternative α,ω-dibromoalkanes or alternative types of electrophile the length of the linker at N-3, which ultimately influences the distance between the conductor surface and the flavin, could be varied. Indeed, the reaction of the 7-acetylisoalloxazine (**1**) with ethyl bromoacetate in the presence of potassium carbonate gave the corresponding N-carboxymethyl derivative (**7**). Repetition of the entire synthesis starting from 4-fluorobenzoic acid *via* the

sequence; nitration, nucleophilic displacement of fluorine with methylamine, carboxylic acid esterification, reduction of the nitro group and, finally, condensation with alloxan under acidic conditions gave the isoalloxazine (**14**) in 31% yield.



(i) K_2CO_3, DMF, ethyl bromoacetate, (ii) HCl, (iii) $SOCl_2$, (iv) dibenzylamine, DMF, pyridine.

Scheme 2

Alkylation of the isoalloxazine ester (**14**) with ethyl bromoacetate in the presence of potassium carbonate gave the highly soluble flavin diester (**15**) in 67% yield after recrystallisation from dichloromethane/ diethyl ether.

Since the isoalloxazine nucleus needed to be functionalised for further reactions with nucleophiles, it was necessary to activate the two carboxylic ester groups. Takeda[14] had shown that the acid chloride derivative of various isoalloxazines could be prepared in high yield from the parent acids. Thus, it was first necessary to deprotect the ester functionalities in compound (**15**). In view of the instability of the isoalloxazine nucleus in strongly basic media, the hydrolyses of the esters were performed in hydrochloric acid. The flavin diacid was obtained in 70% yield as bright yellow crystals. Activation of the acid groups through formation of the diacid chloride (**17**) and treatment with dibenzylamine led to the *bis*- N,N-dibenzylamido flavin (**18**) but, in only 12% yield,

Scheme 2. The low yield of the diamide was due, in part, to the instability of the diacid chloride and, in part, to problems in maintaining the flavin in solution. Indeed, many amines were reacted with the diacid chloride and the reaction with dibenzylamine led to the highest isolated yield. Since solubility was proving to be a serious problem in the synthesis of N^{10}-methyl isoalloxazine derivatives, the solubilities of other N^{10}-substituted flavin derivatives were investigated.

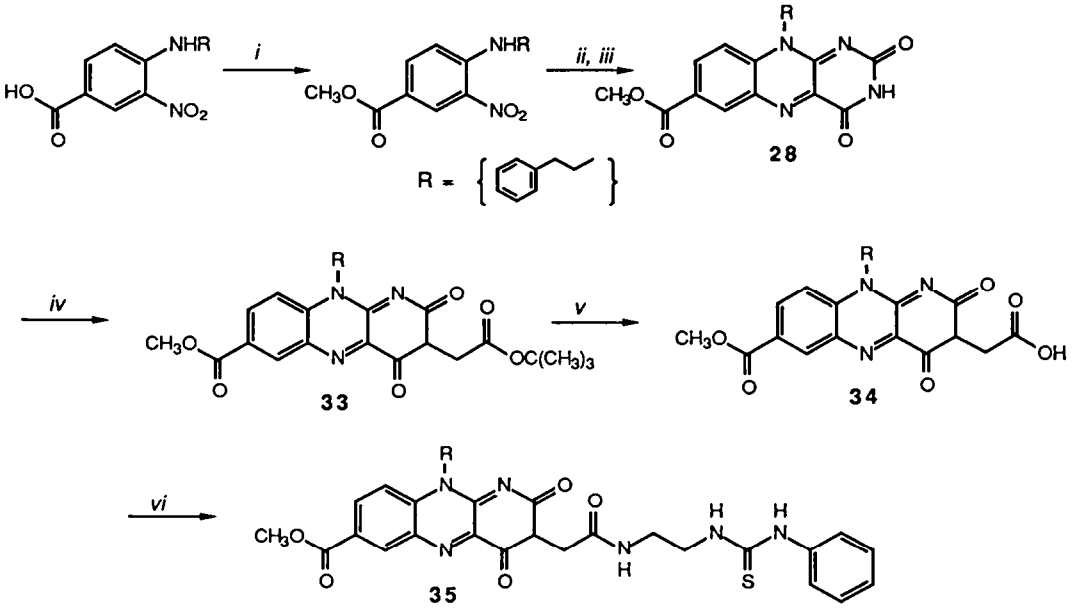
A series of N^{10}-propyl flavins (**22** to **25**), synthesised using similar protocols to those described for the methyl series, proved to have better solubility than their N^{10}- methyl homologues, see experimental section. However, the improvement was only marginal and the entire synthesis was repeated for the N^{10}-phenethyl analogues. These exhibited the required solubility characteristics and were used to reassess the reaction of the activated isoalloxazine diacid with various amine nucleophiles.

The activation of the diacid flavin (**30**), however, could not be achieved through formation of the diacid chloride and alternative methods were examined. Mixed anhydride, dicyclohexylcarbodiimide and N,N'-disuccinimidyl carbonate methodologies also failed. Treatment of the diacid with 1,1'-bis-[6-(trifluoromethyl) benzotriazolyl] oxalate (BTBO) according to the method of Takeda *et al.* [15] gave the *bis*- activated isoalloxazine which reacted smoothly with amines to give the desired amides in good yield. Amide derivatives synthesised using this methodology are detailed in the experimental section, the synthesis of the *bis*-amide (**35**) is shown in Scheme 3. Treatment of the diacid (**30**) with BTBO and DMAP in acetonitrile followed by addition of 1-(2-aminoethyl)-3-phenylthiourea[16] gave the *bis* thiourea (**32**) in 62% yield. The compound showed the expected spectroscopic and analytical properties and was successfully attached to the surface of a gold conductor. The bifunctionalised flavin behaved as a stable adsorbed species and the system displayed very fast electron transfer properties.

The preparation of a mono-thiourea derivative was also required for a comparison of its electrochemical properties with the bifunctionalised system. The synthesis of the monophenylthiourea (**35**) was achieved through formation of the N^3- *t* -butylester flavin (**33**), prepared *via* alkylation with *t*-butyl bromoacetate in 45% yield *vide supra*, followed by the preferential hydrolysis of the butyl ester using trifluoroacetic acid in dichloromethane. The monoacid was obtained in 85% yield.

Treatment of the monoacid with BTBO and DMAP in acetonitrile, followed by addition of the phenylthiourea gave the monothiourea flavin (**35**) in 62% yield. The monothiourea was successfully attached to the surface of a gold electrode and also acted as a stable bound species. However, the rates of electron transfer were ten-fold slower than those observed for the

bifunctionalised flavin electrode. Full details of the electrochemical properties of these and related systems will be reported elsewhere.



(i) $SOCl_2$, MeOH, (ii) H_2/Pd,C, (iii) alloxan, HCl, EtOH, (iv) K_2CO_3, DMF, *t*-butyl bromoacetate, (v) TFA, CH_2Cl_2, (vi) BTBO, CH_3CN, DMAP, 1-(2-aminoethyl) 3-phenyl thiourea.

Scheme 3

EXPERIMENTAL

Melting points were determined using either a Kofler hot-stage or an electrothermal melting point apparatus, and are uncorrected. I.R. spectra were recorded using a Perkin Elmer 298 infrared spectrophotometer. U.v.-visible spectra were obtained using a Pye-Unicam SP8-500 spectrophotometer.^{1}H-nmr spectra were recorded at 90 MHz on a Jeol FX90Q, at 270 MHz on a Jeol JNM-GX270 and at 360 MHz on a Bruker AM360 instrument. ^{13}C-n.m.r. spectra were recorded at 67.9 MHz on a Jeol JNM-GX270 and at 90.56 MHz on a Bruker AM360 instrument. Chemical shift values are given as parts per million downfield shift from TMS. Mass spectra and accurate mass measurements were recorded on either a VG 70 250 SE spectrometer or a VG ZAB E spectrometer. Microanalysis facilities were provided on a service basis by University College London, U.K. All

reactions involving flavins were carried out under subdued lighting and under an atmosphere of argon.

N^3-Bromopropyl)-7-acetyl-N^{10}-methylisoalloxazine. (2)

7-Acetyl-N^{10}-methylisoalloxazine (**1**) (50 mg, 0.18 mmol) and potassium carbonate (43 mg, 0.31 mmol) in DMF (5 ml) were heated on an oil bath to 60-70°C. 1,3-dibromopropane (209 µl, 1.8 mmol) was added to the mixture and the temperature maintained at 60-70°C for 3h. Water (2 ml) was added and the mixture extracted with CH_2Cl_2 (3 x 10 ml). The organic layer was washed with water (4 x 10 ml), dried (Na_2SO_4) and the solvent removed *in vacuo*. Yellow crystals were obtained on crystallisation from DMF/diethyl ether (31 mg, 44%), m.p. 210°C dec. (Found: C, 48.9; H, 4.2; Br, 20.0; N, 13.5%; *(M+H)*$^+$, 391.0413. $C_{16}H_{15}BrN_4O_3$ requires C, 49.1; H, 3.9; Br, 20.4; N, 13.3%; *(M+H)*, 391.0408); $\lambda_{max.}$ (DMF) 433 (ε 8 800 $M^{-1}cm^{-1}$) and 289 nm (35 800); δ_H ($CDCl_3$) 2.33 (2H, q, *J* 7 Hz, $C\underline{H}_2CH_2Br$), 2.73 (3H, s, $COCH_3$), 3.48 (2H, t, *J* 6.9 Hz, CH_2Br), 4.15 (3H, s, NCH_3), 4.27 (2H, t, *J* 6.9 Hz, $C\underline{H}_2CH_2CH_2Br$), 7.74 (1H, d, *J* 9 Hz, 9-H), 8.50 (1H, dd, *J* 2.2 & 9 Hz, 8-H) and 8.84 (1H, d, *J* 2 Hz, 6-H); δ_C ($CDCl_3$) 26.71 ($C_{7\beta}$), 30.34 ($\underline{C}H_2CH_2Br$), 31.13 (CH_2Br), 32.50 (NCH_3), 41.35 (NCH_2), 115.89 (C_9), 134.21 (C_8), 134.61 (C_6), 134.78 (C_{5a}), 136.38 (C_{9a}), 149.84 (C_{10a}), 155.17 (C_2), 159.24 (C_4) and 195.57 ($C_{7\alpha}$).

N^3-Methyl-7-acetyl-N^{10}-methylisoalloxazine. (3)

n-Butyl lithium (0.21 ml, 0.21 mmol) was added to a solution of di-*iso* propylamine (0.19 ml, 1.4 mmol) in dry THF (3 ml) at 0°C. After stirring at 0°C for 30 min., the mixture was added to a solution of 7-acetyl-N^{10}-methylisoalloxazine (**1**) (50 mg, 0.18 mmol) in freshly distilled DMF (10 ml) at -50°C. After a further 30 min. at -50°C, methyl iodide (112 µl, 1.8 mmol) was added and then stirred for 1h. at room temperature. The organic solvents were removed *in vacuo* and the residue extracted with CH_2Cl_2 (3 x 10 ml). This was washed with water (3 x 10 ml) and dried (Na_2SO_4). After removal of the CH_2Cl_2 *in vacuo*, the product was crystallised from trifluoroacetic acid/diethyl ether to give yellow crystals (35 mg, 68%), m.p. 240°C dec. (Found: *(M+H)*$^+$, 285.0981. $C_{14}H_{12}N_4O_3$ requires *(M+H)*, 285.0988); δ_H ($CDCl_3$) 2.73 (3H, s, CH_3CO), 3.54 (3H, s, $CONCH_3$), 4.15 (3H, s, NCH_3), 7.73 (1H, d, *J* 8.8 Hz, 9-H), 8.50 (1H, dd, *J* 2 & 9 Hz, 8-H) and 8.88 (1H, d, *J* 2 Hz, 6-H); δ_C ($CDCl_3$) 26.79 ($C_{7\beta}$), 29.16 (N^3CH_3), 32.55 (NCH_3), 115.94 (C_9), 134.30 to 135.04 (aromatics), 136.48 (C_{9a}), 149.82 (C_{10a}), 155.76 (C_2), 159.49 (C_4) and 195.71 ($C_{7\alpha}$).

N^3-Butyl-7-acetyl-N^{10}-methylisoalloxazine. (4)

The procedure was similar to that followed for the N^3-methyl derivative (**3**) where 1-bromobutane (193 µl, 1.8 mmol) was used as the alkylating agent. Purification by suction chromatography (silica gel, CH_2Cl_2/5% MeOH) gave yellow crystals (44 mg, 75%), m.p. 205-207°C (Found: *(M+H)*$^+$, 327.1448. $C_{17}H_{18}N_4O_3$ requires *(M+H)*, 327.1457); δ_H ($CDCl_3$) 0.93 (3H, t, *J* 7.5 Hz, $CH_2\underline{CH_3}$), 1.37 (2H, sextet, *J* 7.5 Hz, $\underline{CH_2}CH_3$), 1.66 (2H, pentet, *J* 7.5 Hz, $\underline{CH_2}CH_2CH_3$), 2.69 (3H, s, CH_3CO), 4.05 (2H, t, *J* 7.5 Hz, $NCH_2\underline{CH_2}$), 4.11 (3H, s, NCH_3), 7.71 (1H, d, *J* 9 Hz, 9-H), 8.44 (1H, dd, *J* 1.7 & 9 Hz, 8-H) and 8.79 (1H, d, *J* 1.7 Hz, 6-H); δ_C ($CDCl_3$) 13.98 ($CH_2\underline{C}H_3$), 20.34 ($\underline{C}H_2CH_3$), 26.69 ($CO\underline{C}H_3$), 29.92 ($NCH_2\underline{C}H_2$), 32.36 (NCH_3), 42.41 ($N\underline{C}H_2CH_2$), 115.80 (C_9), 134.16 to 134.98 (aromatics), 136.41 (C_{9a}), 138.24 (C_7), 149.74 (C_{10a}), 155.33 (C_2), 159.10 (C_4) and 195.63 ($C_{7\alpha}$).

N^3-(2-Bromoethyl)-7-acetyl-N^{10}-methylisoalloxazine. (5)

The procedure was similar to that followed for the N^3-methyl derivative except that 1,2-dibromoethane (155 µl, 1.8 mmol) was used as the alkylating agent. Stirring was continued for a further 2h. at room temperature. The product was purified by suction chromatography (silica gel, CH_2Cl_2/5% MeOH) to give yellow crystals (51 mg, 76%), m.p. 220°C dec. (Found: *(M+H)*$^+$, 377.0256. $C_{15}H_{13}BrN_4O_3$ requires *(M+H)*, 377.0249); δ_H ($CDCl_3$) 2.72 (3H, s, CH_3CO), 3.65 (2H, t, *J* 6.9 Hz, CH_2Br), 4.16 (3H, s, NCH_3), 4.52 (2H, t, *J* 6.9 Hz, NCH_2), 7.74 (1H, d, *J* 9 Hz, 9-H), 8.50 (1H, dd, *J* 1.9 & 9 Hz, 8-H) and 8.84 (1H, d, *J* 1.9 Hz, 6-H); δ_C ($CDCl_3$) 26.69 ($C_{7\beta}$), 27.73 (CH_2Br), 32.57 (NCH_3), 42.91 (NCH_2), 115.96 (C_9), 134.19 to 135.05 (aromatics), 136.36 (C_{9a}), 137.96 (C_7), 149.91 (C_{10a}), 154.82 (C_2), 159.82 (C_4) and 195.54 ($C_{7\alpha}$).

Methyl 7-acetyl-N^{10}-methylisoalloxazine-3-acetate. (6)

The procedure was similar to that followed for the N^3-methyl derivative with methyl bromoacetate (170 µl, 1.8 mmol) as the alkylating agent. The product crystallised from CH_2Cl_2/diethyl ether to give yellow crystals (57 mg, 93%), m.p. 252°C dec. (Found: *(M+H)*$^+$, 343.1057. $C_{16}H_{14}N_4O_5$ requires *(M+H)*, 343.1043); $\lambda_{max.}$ (DMF) 290 (ε 15 230 $M^{-1}cm^{-1}$) and 435 nm (3 550); δ_H ($CDCl_3$) 2.73 (3H, s, $COCH_3$), 3.78 (3H, s, $COOCH_3$), 4.17 (3H, s, NCH_3), 4.87 (2H, s, NCH_2), 7.75 (1H, d, *J* 9 Hz, 9-H), 8.51 (1H, dd, *J* 1.9 & 9 Hz, 8-H) and 8.85 (1H, d, *J* 1.9 Hz, 6-H); δ_C ($CDCl_3$) 26.72 ($C_{7\beta}$), 32.65 (NCH_3), 42.93 (NCH_2), 52.78 ($COO\underline{C}H_3$), 115.99 (C_9), 134.23 to 136.42 (aromatics), 150.13 (C_{10a}), 154.72 (C_2), 158.94 (C_4), 168.17 ($\underline{C}OOCH_3$) and 195.57 ($C_{7\alpha}$).

Ethyl 7-acetyl-N^{10}-methylisoalloxazine-3-acetate. (7)

The procedure was similar to that followed for the N^3-methyl derivative where ethyl bromoacetate (199 µl, 1.8 mmol) was used as the alkylating agent. Yellow crystals were obtained on crystallisation from CH_2Cl_2/diethyl ether (39 mg, 61%), m.p. 228-230°C (Found *(M+H)*$^+$, 357.1207. $C_{17}H_{16}N_4O_5$ requires *(M+H)*, 357.1199); $\lambda_{max.}$ (DMF) 274 (ε 10 600 $M^{-1}cm^{-1}$) and 450 nm (2 970); δ_H ($CDCl_3$) 1.27 (3H, t, *J* 7.1 Hz, $CH_2C\underline{H}_3$), 2.70 (3H, s, CH_3CO), 4.15 (3H, s, NCH_3), 4.20 (2H, quartet, *J* 7.1 Hz, $C\underline{H}_2CH_3$), 4.81 (2H, s, NCH_2), 7.75 (1H, d, *J* 9 Hz, 9-H), 8.48 (1H, dd, *J* 2.1 & 9 Hz, 8-H) and 8.81 (1H, d, *J* 1.9 Hz, 6-H); δ_C ($CDCl_3$) 14.23 ($CH_2\underline{C}H_3$), 26.72 ($C_{7\beta}$), 32.66 (NCH_3), 42.69 (NCH_2), 61.76 ($\underline{C}H_2CH_3$), 116.13 (C_9), 134.07 to 136.42 (aromatics), 137.96 (C_{9a}), 150.07 (C_{10a}), 154.70 (C_2), 158.91 (C_4), 167.68 ($\underline{C}OOC_2H_5$) and 195.61 ($C_{7\alpha}$); *m/z* 356 (*M*$^+$, 30%), 283 (100), 227 (10) and 77 (3).

7β-Bromoacetyl-N^{10}-methylisoalloxazine. (8)

A mixture of 7-acetyl-N^{10}-methylisoalloxazine (**1**) (50 mg, 0.18 mmol), N-bromosuccinimide (36 mg, 0.2 mmol) and a trace of benzoyl peroxide were stirred at 50°C for 2h. in trifluoroacetic acid (15 ml). The waste product, succinimide, was then precipitated by the addition of water (10 ml) and filtered. The filtrate was reduced in volume under vacuum and the residue crystallised from trifluoroacetic acid/diethyl ether to give the titled compound (53 mg, 84%), m.p. 195°C dec. (Found: *(M+H)*$^+$, 348.9928. $C_{13}H_9BrN_4O_3$ requires *(M+H)*, 348.9936); $\upsilon_{max.}$ (nujol) 1725m, 1600m, 1572s and 700 cm^{-1}; δ_H (CF_3CO_2D) 4.67 (3H, s, NCH_3), 4.78 (2H, s, CH_2Br), 8.17 (1H, bs, 9-H), 8.93 (1H, bs, 8-H) and 9.27 (1H, bs, 6-H); δ_C (CF_3CO_2D) 30.25 ($C_{7\beta}$), 38.50 (CH_3), 120.90 (C_9), 135.59 (C_7), 137.48 (C_8), 137.74 (C_{4a}), 141.03 (C_{5a}), 142.14 (C_{9a}), 148.06 (C_{10a}), 153.76 (C_2), 161.61 (C_4) and 195.86 ($C_{7\alpha}$); *m/z* 270 ((*M*-Br)$^+$, 3.5%), 115 (100), 81.9 (81) and 79.9 (83)

7β-Bromoacetyl-N^3-(3-bromopropyl)-N^{10}-methylisoalloxazine. (9)

N-Bromosuccinimide (50 mg, 0.28 mmol) and benzoyl peroxide (catalytic) were added to the N^3-alkylated flavin (**2**) (100 mg, 0.26 mmol) in trifluoroacetic acid (20 ml). The mixture was heated at 60-70°C for 4h. The solvent was removed under vacuum and the residue dissolved in CH_2Cl_2 (20 ml). After washing with water (3 x 5 ml), the yellow organic layer was dried (Na_2SO_4) and vacuum evaporated. Column chromatography (CH_2Cl_2/5% MeOH) of the residual oil yielded the pure product (85 mg, 71%), m.p. 185°C dec. (Found: C, 41.0; H, 3.3; N, 11.6%; *(M+H)*$^+$, 470.9501. $C_{16}H_{14}Br_2N_4O_3$ requires C, 40.9; H, 3.0; N, 11.9%; *(M+H)*, 470.9491); $\upsilon_{max.}$ (nujol) 1725m, 1600m,

1570s and 700s cm^{-1}; δ_H ($CDCl_3$) 2.33 (2H, quintet, *J* 7 Hz, $CH_2C\underline{H}_2CH_2$), 3.48 (2H, t, *J* 7 Hz, $CH_2C\underline{H}_2Br$), 4.15 (3H, s, NCH_3), 4.25 (2H, t, *J* 7 Hz, $C\underline{H}_2CH_2CH_2Br$), 4.49 (2H, s, $BrCH_2CO$), 7.76 (1H, d, *J* 9 Hz, 9-H), 8.52 (1H, dd, *J* 1.95 & 9 Hz, 8-H) and 8.89 (1H, d, *J* 1.95 Hz, 6-H); δ_C (CF_3CO_2D) 30.08 ($\underline{C}H_2CH_2Br$), 30.43 ($C_{7\beta}$), 31.62 ($CH_2\underline{C}H_2Br$), 38.89 (NCH_3), 45.28 (NCH_2), 120 to 145 (aromatics), 151.84 (C_2), 161.06 (C_4) and 195.4 ($C_{7\alpha}$).

Model studies were carried out to determine the susceptibility of the mono- and di-bromo-flavins (**2**, **8** and **9**) to nucleophilic attack, using thiophenol as the nucleophile. In each case reaction occurred to give the thiophenyl ether derivatives as judged from the ^{1}H-nmr spectra of the purified products but, the isolated yields were low. Attempts to improve the yields through the modification of the reaction conditions led to an increase in the number of products formed, as determined by t.l.c. For example, 7β-(Phenylthio)-acetyl-N^{10}-methylisoalloxazine (**10**) was obtained in 7.6% from the action of freshly distilled thiophenol on the brominated flavin (**8**) in DMSO, protected from light and air. Triethylamine was added over 10 min. and the mixture left stirring overnight. After vacuum evaporation of the solvent the product was crystallised from DMF/diethyl ether, δ_H (DMSO-d_6) 3.97 (3H, s, NCH_3), 4.80 (2H, s, SCH_2), 7.30 (5H, m, Ar-H), 8.01 (1H, d, *J* 9 Hz, 9-H), 8.36 (1H, dd, *J* 1.8 & 8.9 Hz, 8-H) and 8.85 (1H, d, *J* 1.9 Hz, 6-H).
Similarly, N^3-(3-Phenylthio)-propyl-7-acetyl-N^{10}-methylisoalloxazine (**11**) was formed by the reaction of freshly prepared sodium thiophenolate on N^3-3-bromopropyl-7-acetyl-N^{10}-methylisoalloxazine (**2**) in dry DMF. Purification by column chromatography (CH_2Cl_2/5% MeOH) gave the 3-thio alkylated product (4.4 mg, 8.2%), δ_H ($CDCl_3$) 2.10 (2H, q, *J* 7.2 Hz, $C\underline{H}_2CH_2S$), 2.72 (3H, s, CH_3O), 3.02 (2H, t, *J* 7.2 Hz, CH_2S), 4.15 (3H, s, NCH_3), 4.25 (2H, t, *J* 7.1 Hz, NCH_2), 7.30 (5H, m, aromatics), 7.74 (1H, d, *J* 8.5 Hz, 9-H), 8.42 (1H, dd, J 2 & 9 Hz, 8-H) and 8.87 (1H, d, *J* 2 Hz, 6-H).

The di-thio flavin N^3-(3-Phenylthio)-propyl-7β-(phenylthio)-acetyl-N^{10}-methylisoalloxazine (**12**) and the mono-thio flavin N^3-(3-Bromopropyl)-7β-(phenylthio)-acetyl-N^{10}-methylisoalloxazine (**13**) were formed by the treatment with sodium thiophenolate in DMF. Purification of the products was achieved by column chromatography, (**12**) in 6% yield δ_H ($CDCl_3$) 2.08 (2H, q, *J* 7 Hz, $C\underline{H}_2CH_2S$), 3.02 (2H, t, *J* 7 Hz, CH_2S), 4.14 (3H, s, NCH_3), 4.27 (2H, t, *J* 7 Hz, NCH_2), 4.31 (2H, s, SCH_2CO), 7.35 (10H, m, C_6H_5S), 7.75 (1H, d, *J* 9 Hz, 9-H), 8.45 (1H, dd, *J* 2 & 9 Hz, 8-H) and 8.87 (1H, d, *J* 2 Hz, 6-H). (**13**) in 6.7% yield δ_H ($CDCl_3$) 2.35 (2H, q, *J* 7 Hz, $C\underline{H}_2CH_2Br$), 3.50 (2H, d, *J* 7

Hz, CH_2Br), 4.15 (3H, s, NCH_3), 4.27 (2H, t, *J* 7 Hz, NCH_2), 4.32 (2H, s, SCH_2CO), 7.34 (5H, m, aromatics), 7.72 (1H, d, *J* 9 Hz, 9-H), 8.46 (1H, dd, *J* 1.95 & 9 Hz, 8-H) and 8.86 (1H, d, *J* 2 Hz, 6-H).

7-Methoxycarbonyl-N^{10}-methylisoalloxazine. (14)

Methyl 3-nitro-N^4-methylaminobenzoate[17] (230 mg, 1.09 mmol) in methanol (25 ml) and water (5 ml) was hydrogenated at atmospheric pressure over palladium on charcoal (5%, 50 mg) for 4h. The product was filtered through celite, washed with methanol and the solvent removed *in vacuo*. To this was added a mixture of alloxan monohydrate (0.17 g, 1.1 mmol) and boric acid (70 mg, 1.1 mmol) in glacial acetic acid (5 ml), which had been thoroughly degassed. The reaction mixture was degassed again and heated to reflux for 10 min. After stirring for 12h. at room temperature a green solid was collected by filtration. This was washed with water and diethyl ether (0.21 g, 67%), m.p. 308-310°C (Found: *(M+H)*$^+$, 287.0770. $C_{13}H_{10}N_4O_4$ requires *(M+H)*, 287.0780); $\upsilon_{max.}$ (nujol) 1750m, 1600m and 1570s cm^{-1}; $\lambda_{max.}$ (EtOH) 223 (ε 16 000 $M^{-1}cm^{-1}$), 275 (17 900), 335 (2 300) and 425 nm (4 600); δ_H (DMSO-d_6) 3.58 (3H, s CH_3), 4.24 (3H, s, $COOCH_3$), 8.23 (1H, d, *J* 9 Hz, 9-H), 8.61 (1H, dd, *J* 2 & 9 Hz, 8-H), 8.75 (1H, d, *J* 2 Hz, 6-H) and 11.82 (1H, s, NH); δ_C (DMSO-d_6) 32.03 (NCH_3), 52.50 ($COO\underline{C}H_3$), 117.10 (C_9), 126.67 (C_8), 130.69 (C_6), 132.40 (C_7), 133.84 (C_{9a}), 136.36 (C_{10a}), 151.25 (C_4) and 165.01 ($C_{7\alpha}$).

Ethyl 7-methoxycarbonyl-N^{10}-methylisoalloxazine-3-acetate. (15)

A mixture of the N^{10}-methylisoalloxazine (**14**) (51 mg, 0.18 mmol), potassium carbonate (137 mg, 1.00 mmol) and ethyl bromoacetate (199 µl, 1.8 mmol) were stirred for 4h. in DMF (15 ml) at room temperature. After this time the DMF was removed *in vacuo* and the product crystallised from CH_2Cl_2/diethyl ether (45 mg, 67%), m.p. >250°C (Found: C, 54.1; H, 4.4; N, 14.8. $C_{17}H_{16}N_4O_6.{}^1/_2H_2O$ requires C, 53.6; H, 4.5; N, 14.7%); δ_H ($CDCl_3$) 1.29 (3H, t, *J* 7.1 Hz, $CH_2C\underline{H}_3$), 4.01 (3H, s, CH_3O), 4.16 (3H, s, NCH_3), 4.23 (2H, q, *J* 7.1 Hz, $C\underline{H}_2CH_3$), 4.85 (2H, s, NCH_2), 7.72 (1H, d, *J* 9 Hz, 9-H), 8.52 (1H, dd, *J* 1.9 & 8.9 Hz, 8-H) and 8.96 (1H, d, *J* 1.9 Hz, 6-H); δ_C ($CDCl_3$) 14.36 ($CH_2\underline{C}H_3$), 32.66 (NCH_3), 43.15 (NCH_2), 53.15 ($COO\underline{C}H_3$), 61.97 ($\underline{C}H_2CH_3$), 115.73 (C_9), 135.15 to 136.27 (aromatics), 138.03 (C_{9a}), 150,04 (C_{10a}), 154.78 (C_4), 165.06 ($C_{7\alpha}$) and 167.68 ($C_{3\beta}$).

N^3-Carboxymethyl-7-carboxy-N^{10}-methylisoalloxazine. (16)

The flavin diester (**15**) (63 mg, 0.134 mmol) was dissolved in hydrochloric acid (s.g. 1.18, 0.44 ml) and the resultant mixture stirred at 80-90°C for 45 min. The reaction mixture was cooled, and ice-water (2 ml) added to the solution. Yellow crystals that precipitated were collected by suction filtration and washed with water. Recrystallisation from 2N acetic acid gave fine orange needles (31 mg, 70%), m.p. >300°C (Found: C, 47.1; H, 3.7; N, 15.1%; *(M+H)*$^+$, 331.0666. $C_{14}H_{10}N_4O_6.1^1/_2H_2O$ requires C, 47.1; H, 3.7; N, 15.6%; *(M+H)*, 331.0679); $\upsilon_{max.}$ (nujol) 3420m, 1740m, 1670m and 1600m cm^{-1}; δ_H (CF_3CO_2D) 4.68 (3H, s, NCH_3), 5.20 (2H, s, NCH_2), 8.53 (1H, d, *J* 9 Hz, 9-H), 9.06 (1H, dd, *J* 1.9 & 8.9 Hz, 8-H) and 9.36 (1H, d, *J* 1.9 Hz, 6-H); δ_C (CF_3CO_2D) 38.12 (NCH_3), 44.51 (NCH_2), 119.82 (C_9), 134.20 to 136.40 (aromatics), 137.97 (C_8), 141.75 (C_6), 145.34 (C_{10a}), 151.21 (C_2), 159.90 (C_4), 170.19 ($C_{7\alpha}$) and 173.40 ($C_{3\beta}$); *m/z* 331 (89%).

N^3-Chloroacetyl-7-chloroformyl-N^{10}-methylisoalloxazine. (17)

The diacid flavin (**16**) (50 mg, 0.15 mmol) was added to thionyl chloride (1.0 ml, 13.7 mmol) and the suspension stirred at 45°C for 2h. After this time the solid had dissolved and the remaining thionyl chloride was removed by rotary evaporation at <45°C. The remaining traces of the thionyl chloride were removed by the repeated evaporation of a benzene solution. The crude acid chloride obtained was used without further purification, $\upsilon_{max.}$ (nujol) 1790w, 1760w, 1730w, 1650w and 1590m cm^{-1}; δ_H (DMSO-d_6) 4.03 (3H, s, NCH_3), 4.60 (2H, s, NCH_2), 8.09 (1H, d, *J* 9 Hz, 9-H), 8.40 (1H, dd, *J* 1.9 & 9 Hz, 8-H) and 8.56 (1H, d, *J* 1.9 Hz, 6-H).

N^3-(N,N-Dibenzyl)-acetamido-7-(N,N-dibenzyl)-formamido-N^{10}-methylisoalloxazine. (18)

A solution of dibenzylamine (57 µl, 0.31 mmol) in DMF (2 ml) containing pyridine (96 µl, 1.2 mmol) was added to the flavin diacid chloride (**17**) (55 mg, 0.15 mmol), and stirred under an argon atmosphere at room temperature. After 2h. the reaction mixture was added to water (10 ml) and extracted with CH_2Cl_2 (2 x 20 ml). The organic phase was washed with water (4 x 10 ml), dried (Na_2SO_4) and concentrated by rotary evaporation. The crude product was purified by chromatography on silica gel (CH_2Cl_2/5% MeOH) to give a dark yellow oil which could not be crystallised (12 mg, 12%) (Found: C, 69.8; H, 5.6; N, 11.1%; *(M+H)*$^+$, 689.2924. $C_{42}H_{36}N_6O_4.2H_2O$ requires C, 69.6; H, 5.6; N, 11.5%; *(M+H)*, 689.2876); δ_H ($CDCl_3$) 4.03 (3H, s, NCH_3), 4.20-4.80 (8H,

m, $NC\underline{H}_2Ar$), 4.97 (2H, s, NCH_2), 7.16 to 7.35 (20H, m, Ar-H), 7.60 (1H, d, *J* 9 Hz, 9-H), 7.95 (1H, dd, *J* 1.9 & 8.9 Hz, 8-H) and 8.34 (1H, d, *J* 1.9 Hz, 6-H); δ_C ($CDCl_3$) 32.36 (NCH_3), 43.26 ($C_{3\alpha}$), 48.01 (CH_2), 48.72 (CH_2), 49.40 (CH_2), 50.24 (CH_2), 116.16 (C_9), 126.92 to 129.22 (benzyl aromatics), 134.38 to 136.75 (aromatics), 138.19 (C_{9a}), 149.82 (C_{10a}), 155.81 (C_2), 159.30 (C_4), 166.61 ($C_{7\alpha}$) and 169.58 ($C_{3\beta}$).

N^3-(N-Propyl)-acetamido-7-(N-propyl)-formamido-N^{10}-methylisoalloxazine. (19)

The diacid chloride (**17**) (55 mg, 0.15 mmol) dissolved in DMF (2 ml) was stirred under an atmosphere of argon. Dry pyridine (96 µl, 1.2 mmol) and propylamine (25 µl, 0.31 mmol) were added and the mixture stirred at room temperature for 3h. The reaction was quenched with water (10 ml). The organic phase was separated and washed with water (5 x 10 ml), dried (Na_2SO_4) and vacuum evaporated. The product was purified by column chromatography (CH_2Cl_2/5% MeOH) to yield a yellow solid (9 mg, 15%), m.p. 153-156°C (Found: *(M+H)*$^+$, 413.1927. $C_{20}H_{24}N_6O_4$ requires *(M+H)*, 413.1937); δ_H (DMSO-d_6) 0.85 (3H, t, *J* 7.3 Hz, $CH_2C\underline{H}_3$), 0.95 (3H, t, *J* 7.3 Hz, $CH_2C\underline{H}_3$), 1.42 (2H, m, *J* 7.3 Hz, $C\underline{H}_2CH_3$), 1.62 (2H, m, *J* 7.3 Hz, $C\underline{H}_2CH_3$), 3.05 (2H, q, *J* 7.3 Hz, $C\underline{H}_2CH_2CH_3$), 3.32 (2H, q, *J* 7.3 Hz, $C\underline{H}_2CH_2CH_3$), 3.36 (3H, s, NCH_3), 4.05 (2H, s, NCH_2CO), 8.04 (1H, bt, NH), 8.08 (1H, d, *J* 8.9 Hz, 9-H), 8.40 (1H, dd, *J* 8.9 & 1.9 Hz, 8-H), 8.73 (1H, d, *J* 1.9 Hz, 6-H) and 8.85 (1H, bt, 7β-NH); δ_C (DMSO-d_6) 11.35 (CH_3), 11.52 (CH_3), 22.29 ($\underline{C}H_2CH_3$), 22.34 (CH_2CH_3), 32.06 (NCH_3), 41.26 ($N\underline{C}H_2CH_2$), 43.73 ($N\underline{C}H_2CO$), 116.88 (C_9), 130.01 to 138.50 (aromatics), 149.86 (C_{10a}), 154.50 (C_2), 159.08 (C_4), 164.06 ($C_{7\alpha}$) and 166.38 ($C_{3\beta}$).

3-Nitro-N^4-propylaminobenzoic acid. (20)

Freshly distilled propylamine (1.34 ml, 8.9 mmol) was added to 4-fluoro-3-nitrobenzoic acid (1.5 g, 8.1 mmol) in ethanol (50 ml) and the mixture heated to reflux for 2h. The resultant orange solution was cooled and acidified (HCl, 2N) to give a yellow precipitate. Yellow crystals were obtained after filtration and recrystallisation from methanol (1.51 g, 83%), m.p. 207-209°C (Found: C, 53.6; H, 5.4; N,12.6. $C_{10}H_{12}N_2O_4$ requires C, 53.5; H, 5.4; N, 12.5%); δ_H (DMSO-d_6) 0.95 (3H, t, *J* 7.3 Hz, $CH_2C\underline{H}_3$), 1.65 (2H, m, *J* 7.1 Hz, $C\underline{H}_2CH_3$), 3.65 (2H, m, *J* 7.9 Hz, NCH_2), 7.09 (1H, d, *J* 9.1 Hz, 5-H), 7.94 (1H, dd, *J* 1.9 & 9.1 Hz, 6-H), 8.49 (1H, t, *J* 5.4 Hz, NH) and 8.60 (1H, d, *J* 2.1 Hz, 2-H); δ_C (DMSO-d_6) 11.15 ($CH_2\underline{C}H_3$), 21.49 ($\underline{C}H_2CH_3$), 44.05 (NCH_2), 114.51 to 147.27 (aromatics) and 165.89 (C=O).

Methyl 3-nitro-N^4-propylaminobenzoate. (21)

A solution of 3-nitro-N^4-propylaminobenzoic acid (**20**) (1g, 4.46 mmol) in dry MeOH (50 ml) was cooled to -5°C, and whilst maintaining this temperature, thionyl chloride (1.63 ml, 22 mmol) was added over 15 min. After heating to reflux (1.5h.) the excess thionyl chloride and methanol were removed *in vacuo*. Bright yellow crystals were obtained on recrystallisation from methanol (707 mg, 66%), m.p. 62-64°C (Found: C, 55.4; H, 5.8; N, 11.7. $C_{11}H_{14}N_2O_4$ requires C, 55.5; H, 5.9; N, 11.8%); δ_H ($CDCl_3$) 1.08 (3H, t, *J* 7.4 Hz, $CH_2\underline{CH_3}$), 1.79 (2H, m, *J* 6.9 Hz, $\underline{CH_2}CH_3$), 3.34 (2H, m, *J* 7.6 MHz, NCH_2), 3.90 (3H, s, CH_3O), 6.87 (1H, d, *J* 9.1 Hz, 5-H), 8.04 (1H, dd, *J* 9.1 & 2.2 Hz, 6-H), 8.37 (1H, bs, NH) and 8.88 (1H, d, *J* 2.1 Hz, 2-H); δ_C ($CDCl_3$) 11.74 ($CH_2\underline{C}H_3$), 22.40 ($\underline{C}H_2CH_3$), 45.21 (NCH_2), 52.09 (CH_3O), 113.75 to 148.07 (aromatics) and 165.91 (C=O).

7-Methoxycarbonyl-N^{10}-propylisoalloxazine. (22)

Methyl 3-nitro-N^4-propylaminobenzoate (**21**) (5.94 g, 24.9 mmol) in methanol (250 ml) was hydrogenated over palladium on charcoal (10%, 500 mg) at room temperature. The resultant mixture was filtered through a celite plug, washed with methanol and evaporated *in vacuo*. The diamine thus obtained was dissolved in absolute ethanol (500 ml). Alloxan monohydrate (4.04 g, 30 mmol), dissolved in hot concentrated hydrochloric acid (47 ml), was added to the diamine and the solution refluxed in the dark for 15 min. The product which crystallised at 4°C was collected and washed with diethyl ether. Recrystallisation from acetic acid (2N) gave lime-coloured crystals (5.32 g, 68%), m.p. >300°C (Found: C, 55.7; H, 4.2; N, 17.6. $C_{15}H_{14}N_4O_4.{}^1/_2H_2O$ requires C, 55.7; H, 4.6; N, 17.3%); $\upsilon_{max.}$(nujol) 1730m, 1605m and 1570m cm^{-1}; δ_H (DMSO-d_6) 1.08 (3H, t, *J* 7.3 Hz, $CH_2\underline{CH_3}$), 1.79 (2H, m, *J* 7.1 Hz, $\underline{CH_2}CH_3$), 3.97 (3H, s, CH_3O), 4.55 (2H, t, *J* 7.8 Hz, NCH_2), 8.12 (1H, d, *J* 9 Hz, 9-H), 8.33 (1H, dd, *J* 1.9 & 9.1 Hz, 8-H) and 8.53 (1H, d, *J* 1.9 Hz, 6-H); δ_C (DMSO-d_6) 10.92 ($CH_2\underline{C}H_3$), 19.70 ($\underline{C}H_2CH_3$), 45.78 (NCH_2), 52.62 (CH_3O), 117.05 (C_9), 126.45 (C_{4a}), 132.54 (C_6), 133.77 (C_{5a}), 134.00 (C_{9a}), 135.52 (C_7), 150.78 (C_{10a}), 155.55 (C_2), 159.35 (C_4) and 164.81 ($C_{7\alpha}$); *m/z* (FAB+) ($(M+H)^+$, 100%), 273 (60), 232 (20), 137 (14) and 79 (35).

Ethyl 7-methoxycarbonyl-N^{10}-propylisoalloxazine-3-acetate. (23)

The N^{10}-propylisoalloxazine (**22**) (1 g, 3.2 mmol) and anhydrous potassium carbonate (2.48 g, 17.8 mmol) were suspended in dry DMF (100 ml). To this suspension was added freshly distilled ethyl bromoacetate (1.34 ml, 12 mmol) and the mixture stirred at room temperature. After 5h. the reaction mixture was filtered and the DMF solvent removed by rotary evaporation. Recrystallisation

from methanol afforded fine yellow crystals (1.12 g, 88%), m.p. 205-207°C (Found: C, 56.9; H, 5.0; N, 13.9. $C_{19}H_{20}N_4O_6$ requires C, 57.0; H, 5.0; N, 14.0%); $\upsilon_{max.}$ (nujol) 1720m, 1670m, 1600m and 1570 cm^{-1}; $\lambda_{max.}$ 432 (ε 1 150 $M^{-1}cm^{-1}$) and 285 nm (6 700); δ_H ($CDCl_3$) 1.06 (3H, t, *J* 7.34 Hz, $CH_2CH_2C\underline{H}_3$), 1.21 (3H, t, *J* 7.15 Hz, CH_3), 1.85 (2H, m, *J* 7.9 Hz, $CH_2C\underline{H}_2CH_3$), 3.91 (3H, s, CH_3O), 4.13 (2H, q, *J* 7.15 Hz, $C\underline{H}_2CH_3$), 4.60 (2H, m, *J* 7.5 Hz, $C\underline{H}_2CH_2CH_3$), 4.74 (2H, s, CH_2), 7.66 (1H, d, *J* 9.08 Hz, 9-H), 8.40 (1H, dd, *J* 1.93 & 9.08 Hz, 8-H) and 8.80 (1H, d, *J* 1.74 Hz, 6-H); δ_C ($CDCl_3$) 11.17 ($CH_2CH_2\underline{C}H_3$), 14.09 ($\underline{C}H_3CH_2O$), 20.43 ($CH_2\underline{C}H_2CH_3$), 42.85 ($CH_3\underline{C}H_2O$), 46.66 ($N\underline{C}H_2CH_2$), 52.82 (CH_3O), 61.62 ($N\underline{C}H_2CO$), 115.70 (C_9), 128.08 (C_{4a}), 134.82 (C_6), 135.07 to 135.67 (aromatics), 137.76 (C_7), 149.34 (C_{10a}), 154.69 (C_2), 158.78 (C_4), 164.84 ($C_{7\alpha}$) and 167.58 ($C_{3\beta}$); *m/z* (FAB^+) 401 (*(M+H)*$^+$, 100%), 327 (30), 285 (15) and 228 (17).

N^3-Carboxymethyl-7-carboxy-N^{10}-propylisoalloxazine. (24)

The diester (**23**) (567 mg, 1.42 mmol) was dissolved in hydrochloric acid (s.g. 1.18, 4.65 ml) and the resultant mixture stirred at 80-90°C for 45 min. The reaction mixture was cooled and ice-water (14 ml) added to the solution. Yellow crystals that precipitated were collected by suction filtration and washed with water. Yellow needles were obtained on recrystallisation from 2N acetic acid (361 mg, 71%), m.p. 250°C dec. (Found: C, 50.2; H, 4.4; N, 14.2. $C_{16}H_{14}N_4O_6.1^1/_2H_2O$ requires C, 49.9; H, 4.5; N, 14.4%); $\upsilon_{max.}$ (nujol) 3425w, 1750m, 1668m and 1600m cm^{-1}; δ_H (CF_3CO_2D) 1.37 (3H, t, *J* 7.0 Hz, CH_3), 2.23 (2H, bm, $C\underline{H}_2CH_3$), 5.04 (2H, bm, CH_2), 5.20 (2H, s, NCH_2), 8.48 (1H, d, *J* 9.27 Hz, 9-H), 9.08 (1H, dd, *J* 1.7 & 9.3 Hz, 8-H) and 9.35 (1H, d, *J* 1.7 Hz, 6-H); δ_C (CF_3CO_2D) 10.89 (CH_3), 22.90 ($\underline{C}H_2CH_3$), 44.82 ($N\underline{C}H_2CH_2$), 53.58 ($N\underline{C}H_2CO$), 120.00 (C_9), 134.53 (C_{4a}), 136.01 (C_{5a}), 138.40 (C_6), 141.55 (C_8), 142.06 (C_7), 144.59 (C_{10a}), 152.16 (C_2), 160.11 (C_4), 170.36 ($C_{7\alpha}$) and 173.45 ($C_{3\beta}$); *m/z* (FAB^+) 359 (*(M+H)*$^+$, 100%), 307 (22), 232 (32), 176 (33), 137 (25) and 79 (26).

N^3-(N-Propyl)-acetamido-7-(N-propyl)-formamido-N^{10}-propylisoalloxazine. (25)

Triethylamine (85.6 µl, 0.61 mmol) and *iso* butyl chloroformate (40 µl, 0.61 mmol) were added to a degassed solution of the diacid flavin (**24**) (50 mg, 0.28 mmol) in dry DMF (5 ml) at room temperature. After 30 min., propylamine (115 µl, 1.4 mmol) was added and the mixture left stirring for 3h. Excess reactants and DMF were removed *in vacuo* and the residue extracted with CH_2Cl_2 (2 x 10 ml). The organic phase was washed with water (3 x 5 ml), dried (Na_2SO_4) and vacuum evaporated. Purification of the product was achieved by column chromatography (silica gel,

CH_2Cl_2/7.5% MeOH), (31 mg, 25%), m.p. 172-175°C dec. (Found: M^+, 440.2143. $C_{22}H_{28}N_6O_4$ requires M, 440.2172); $\lambda_{max.}$ (DMF) 436 (ε 4 100 $M^{-1}cm^{-1}$), 332 (2 950) and 282 nm (16 500); δ_H (DMSO-d_6) 0.85 (3H, t, J 7.4 Hz, 3-$CH_2C\underline{H}_3$), 0.94 (3H, t, J 7.3 Hz, 7-$CH_2C\underline{H}_3$), 1.07 (3H, t, J 7.3 Hz, N^{10}-$CH_2C\underline{H}_3$), 1.43 (2H, sextet, J 7.1 Hz, 3-$C\underline{H}_2CH_3$), 1.60 (2H, sextet, J 7.1 Hz, 7-$C\underline{H}_2CH_3$), 1.81 (2H, sextet, J 7.1 Hz, 10-$C\underline{H}_2CH_3$), 3.05 (2H, q, J 5.4 Hz, 3-$NHC\underline{H}_2$), 3.28 (2H, q, J 5.4 Hz, 7-$NHC\underline{H}_2$), 4.48 (2H, s, NCH_2), 4.65 (2H, q, J 5.4 Hz, 10-NCH_2), 8.01 (1H, t, J 5.4 Hz, 3-NH), 8.14 (1H, d, J 9.1 Hz, 9-H), 8.38 (1H, dd, J 1.7 & 9.1 Hz, 8-H), 8.74 (1H, d, J 1.9 Hz, 6-H) and 8.82 (1H, t, J 5.4 Hz, 7-NH); δ_C (MeOH-d_4) 12.09 (CH_3), 12.48 (CH_3), 12.60 (CH_3), 22.39 ($CH_3\underline{C}H_2$), 24.40 ($CH_3\underline{C}H_2$), 24.50 ($CH_3\underline{C}H_2$), 43.13 ($NHCH_2$), 43.85 ($NHCH_2$), 45.81 (NCH_2), 118.74 (C_9), 121.99 to 137.31 (aromatics), 151.90 (C_4), 168.27 ($C_{7\alpha}$) and 170.59 ($C_{3\beta}$); *m/z* (EI) 440 ($M^{+\cdot}$ 37%), 398 (58), 382 (36), 355 (100), 313 (62), 255 (92) and 104 (23).

3-Nitro-N^4-phenethylaminobenzoic acid. (26)

4-Fluoro-3-nitrobenzoic acid (1.0 g, 5.4 mmol) and phenethylamine (1.36 ml, 10.8 mmol) were dissolved in ethanol (50 ml) and the orange mixture heated to reflux for 2h. The mixture was allowed to cool and acidified with 2N HCl. The resultant yellow precipitate was filtered and recrystallised from methanol (1.14 g, 74%), m.p. 200-202°C (Found: C, 62.8; H, 4.9; N, 9.7; $C_{15}H_{14}N_2O_4$ requires C, 62.9; H, 4.9; N, 9.8%; $\upsilon_{max.}$(nujol) 3400s, 1690s, 1630s and 1590w cm^{-1}; δ_H (DMSO-d_6) 2.98 (2H, t, J 7 Hz, $C_6H_5C\underline{H}_2$), 3.30 (1H, bs, NH), 3.68 (2H, q, J 7 Hz, $NHC\underline{H}_2$), 7.01 (1H, d, J 9 Hz, 5-H), 7.31 (5H, m, C_6H_5), 7.95 (1H, dd, J 2 & 8.9 Hz, 6-H) and 8.60 (1H, d, J 2 Hz, 2-H).

Methyl 3-nitro-N^4-phenethylaminobenzoate. (27)

To a suspension of the benzoic acid (**26**) (2 g, 7 mmol) in dry methanol (100 ml), at 0 to -5°C, was added thionyl chloride (2.6 ml, 35 mmol) dropwise over 15 min. After heating to reflux for 1.5h., the excess solvent was removed *in vacuo* and the product recrystallised from methanol (1.81 g, 86%), m.p. 97 - 99°C (Found: C, 64.1; H, 5.3; N, 9.4. $C_{16}H_{16}N_2O_4$ requires C, 64.0; H, 5.4; N, 9.3%); $\upsilon_{max.}$ (nujol) 3490w, 1730m, 1640m, 1580w and 1530w cm^{-1}; δ_H ($CDCl_3$) 3.05 (2H, t, J 7.15 Hz, $C_6H_5C\underline{H}_2$), 3.63 (2H, q, J 7.15 Hz, $NHC\underline{H}_2$), 3.90 (3H, s, CH_3O), 6.87 (1H, d, J 9.07 Hz, 5-H), 7.30 (5H, m, C_6H_5), 8.04 (1H, dd, J 1.9 & 9 Hz, 6-H) and 8.88 (1H, d, J 1.94 Hz, 2-H); δ_C ($CDCl_3$) 35.30 ($C_6H_5\underline{C}H_2$), 44.80 (NCH_2), 52.28 (CH_3O), 113.64 to 147.69 (aromatics) and 165.81 (C=O).

7-Methoxycarbonyl-N^{10}-phenethylisoalloxazine. (28)

The methyl ester (**27**) (1.75 g, 5.8 mmol) in methanol (150 ml) was hydrogenated over palladium on charcoal (10%, 170 mg) for 24 h. The catalyst was removed by filtration through a celite pad and the solvent evaporated to give a dark residue. This residue was dissolved in absolute ethanol (227 ml) and treated with alloxan monohydrate (1.03 g, 6.38 mmol) in hot HCl (s.g. 1.18, 11 ml). The mixture was heated to reflux for 15 min, and then left to cool at 4°C overnight. Yellow crystals were filtered, washed with diethyl ether and recrystallised from methanol (1.50 g, 64%), m.p. >300°C (Found: C, 58.8; H, 4.2; N, 13.7; M^+, 376.1145. $C_{20}H_{16}N_4O_4$ requires C, 58.4; H, 4.6; N, 13.6%; *M*, 376.1172); $\upsilon_{max.}$ (nujol) 1725m, 1600m, and 1574m cm^{-1}; $\lambda_{max.}$ (DMF) 430 (ε 2,400 M^{-1}cm^{-1}), and 286 nm (10,500); δ_H (DMSO-d_6) 3.03 (2H, t, *J* 7.5 Hz, $C_6H_5C\underline{H}_2$), 3.94 (3H, s, CH_3O), 4.81 (2H, t, *J* 7.4 Hz, NCH_2), 7.25 to 7.41 (5H, m, C_6H_5), 7.99 (1H, d, *J* 9.08 Hz, 9-H), 8.26 (1H, dd, *J* 1.9 & 9.0 Hz, 8-H), and 8.52 (1H, d, *J* 1.9 Hz, 6-H); δ_C (DMSO-d_6) 31.83 ($C_6H_5\underline{C}H_2$), 45.47 (NCH_2), 52.67 (CH_3O), 117.09 (C_9), 126.42 to 137.60 (aromatics), 140.10 (C_7), 150.70 (C_{10a}), 155.59 (C_2), 159.44 (C_4) and 164.84 ($C_{7\alpha}$); *m/z* 376 (M^+, 2%), 272 (5), 241 (15) and 104 (100).

Ethyl 7-methoxycarbonyl-N^{10}-phenethylisoalloxazine-3-acetate. (29)

A mixture of the phenethylisoalloxazine (**28**) (100 mg, 0.27 mmol), potassium carbonate (206 mg, 1.49 mmol) and ethyl bromoacetate (111 µl, 1.01 mmol) were stirred in DMF (17 ml) for 4h. The DMF was removed *in vacuo* and the product crystallised from methanol (8.8 mg, 72%), m.p. 195-197°C (Found: C, 60.2; H, 4.8; N, 11.5; M^+, 462.1590. $C_{24}H_{22}N_4O_6$ requires C, 60.0; N, 5.0; N, 11.7%; *M*, 462.1540); $\upsilon_{max.}$(nujol) 1750m, 1720m, 1680m, 1640m, 1605m, 1570m, 1540m and 1430w cm^{-1}; $\lambda_{max.}$ (DMF) 430 (ε 1,302 M^{-1}cm^{-1}), and 285 nm (5,100); δ_H ($CDCl_3$) 1.20 (3H, t, *J* 7.1 Hz, $CH_2C\underline{H}_3$), 3.11 (2H, t, *J* 7.5 Hz, $C_6H_5C\underline{H}_2$), 3.91 (3H, s CH_3O), 4.18 (2H, q, *J* 7.1 Hz, $C\underline{H}_2CH_3$), 4.77 (2H, s, NCH_2CO), 4.84 (2H, t, *J* 7.5 Hz, $NC\underline{H}_2CH_2$), 7.19 (5H, m, C_6H_5), 7.46 (1H, d, *J* 9.8 Hz, 9-H), 8.30 (1H, dd, *J* 1.9 & 9.8 Hz, 8-H), and 8.84 (1H, d, *J* 1.9 Hz, 6-H); δ_C ($CDCl_3$) 14.29 ($CH_2\underline{C}H_3$), 33.08 ($C_6H_5\underline{C}H_2$), 43.08 ($CH_3\underline{C}H_2O$), 46.65 ($N\underline{C}H_2CH_2$), 53.03 (CH_3O), 61.88 ($N\underline{C}H_2CO$), 115.51 (C_9), 127.58 to 136.52 (aromatics), 149.39 (C_{10a}), 154.75 (C_2), 158.95 (C_4), 165.03 ($C_{7\alpha}$) and 167.74 ($C_{3\beta}$); *m/z* 462 (M^+, 3%), 358 (5), 285 (10), 228 (10) and 104 (100).

N^3-Carboxymethyl-7-carboxy-N^{10}-phenethylisoalloxazine. (30)

The diester (**29**) (1 g, 2.16 mmol) was dissolved in hydrochloric acid (s.g. 1.18, 7.1 ml) and the resultant mixture stirred at 80 to 90°C for 45 min. The reaction mixture was cooled and ice-water

(14 ml) added to the solution. Yellow crystals that precipitated were collected by suction filtration and washed with water. Yellow needles were obtained on recrystallisation from 2N acetic acid (772 mg, 85%), m.p. 183-185°C (Found: C, 58.6; H, 4.2; N, 12.8. $C_{21}H_{16}N_4O_6.{}^1/_2H_2O$ requires C, 58.7; H, 4.0; N, 13.0%); $\upsilon_{max.}$ (nujol) 3425w, 1730m, 1672m, 1600 and 1560 cm^{-1}; *m/z* 418 (M^+, 21%), 404 (30), 272 (37), 214 (40) and 170 (45).

N^3-(N-propyl)-acetamido-7-(N-propyl)-formamido-N^{10}-phenethylisoalloxazine. (31)
Suspended 1,1-Bis [6-(trifluoromethyl) benzotriazolyl] oxalate (BTBO) (120 mg, 0.26 mmol) in acetonitrile (5 ml) was added to a solution of the diacid flavin (**30**) (50 mg, 0.12 mmol) and pyridine (21 µl, 0.26 mmol) in acetonitrile (10 ml). Afer evolution of gas had ceased, the clear mixture was stirred for 1h. at room temperature. Propylamine (49 µl, 0.60 mmol) was added, without isolation of the active ester, and the resultant orange reaction mixture stirred for 2h. The solvent was removed *in vacuo* and the product purified by crystallisation from MeOH/diethyl ether (29.8 mg, 51%), m.p. 172°C dec. (Found: M^+, 502.2126. $C_{27}H_{30}N_6O_4$ requires *M*, 502.2329); $\lambda_{max.}$ (DMF) 434 (ε 3 300 $M^{-1}cm^{-1}$) and 284 nm (17 100); δ_H (MeOH-d_4) 0.95 (3H, t, *J* 7.5 Hz, CH_3), 0.97 (3H, t, *J* 7.5 Hz, CH_3), 1.55 (2H, sextet, *J* 7.3 Hz, $C\underline{H}_2CH_3$), 1.67 (2H, sextet, *J* 7.3 Hz, $C\underline{H}_2CH_3$), 3.20 (4H, q, *J* 6.9 Hz, $NHC\underline{H}_2CH_2$), 3.30 (2H, m, $C_6H_5C\underline{H}_2$), 4.72 (2H, s, NCH_2CO), 4.95 (2H, t, *J* 7.5 Hz, NCH_2), 7.35 (5H, m, C_6H_5), 7.88 (1H, d, *J* 9.27 Hz, 9-H), 8.27 (1H, dd, *J* 2.13 & 9.08 Hz, 8-H) and 8.55 (1H, d, *J* 1.93 Hz, 6-H); δ_C (MeOH-d_4) 12.51 (CH_3), 12.61 (CH_3), 24.43 ($\underline{C}H_2CH_3$), 24.50 ($\underline{C}H_2CH_3$), 34.64 ($C_6H_5\underline{C}H_2$), 43.12 ($\underline{C}H_2CH_2CH_3$), 43.85 ($\underline{C}H_2CH_2CH_3$), 45.83 (NCH_2), 118.68 (C_9), 128.89 to 141.26 (aromatics), 169.64 ($C_{7\alpha}$) and 170.60 ($C_{3\beta}$); *m/z* (EI) 502 (M^+, 8%), 445 (13), 398 (22), 313 (31), 255 (16) and 104 (100).

[7-(2-Formamido-ethyl)-3-(2-acetamido-ethyl)]-3,3'-bis-phenyl-thiourea-N^{10}-phenethylisoalloxazine. (32)
BTBO (120 mg, 0.26 mmol) was added to a suspension of the diacid flavin (**30**) (50 mg, 0.19 mmol) and DMAP (32 mg, 0.26 mmol) in acetonitrile (10 ml). The solution was left stirring for 1h. after which time the solution became clear. 1-(2-Aminoethyl)-3-phenylthiourea (117 mg, 0.6 mmol) was added to the resultant red solution at room temperature and the mixture stirred for a further 3h. Excess solvent was removed *in vacuo* and the product purified by column chromatography (silica gel, CH_2Cl_2/7.5% MeOH) (57 mg, 62%), m.p. 155°C dec. $\upsilon_{max.}$ 1730w, 1670w, 1605m and 1570w cm^{-1}; $\lambda_{max.}$(DMF) 436 (ε 16 100 $M^{-1}cm^{-1}$), 332 (12 900) and 280 nm (106 400); δ_H (MeOH-d_4/$CDCl_3$)

3.15 (2H, t, *J* 7.98 Hz, $N^{10}CH_2C\underline{H}_2$), 3.45 (2H, bt, CH_2), 3.67 (2H, t, *J* 2.76 Hz, CH_2), 3.77 (2H, t, *J* 4.94 Hz, CH_2), 4.76 (2H, s, NCH_2CO), 4.88 (2H, t, *J* 7.90 Hz, $N^{10}C\underline{H}_2CH_2$), 7.10 to 7.35 (aromatics), 7.70 (1H, d, *J* 9.22 Hz, 9-H), 8.30 (1H, dd, *J* 2.05 & 9.06 Hz, 8-H) and 8.57 (1H, d, *J* 2.08 Hz, 6-H); δ_C (MeOH-d_4/$CDCl_3$) 34.22 ($C_6H_5\underline{C}H_2CH_2$), 41.74 ($N\underline{C}H_2CH_2C_6H_5$), 44.62 ($CH_2$), 45.42 ($CH_2$), 45.77 ($CH_2$), 45.77 ($CH_2$), 48.07 ($CH_2$), 117.66 ($C_9$), 126.03 to 139.27 (aromatics), 150.86 (C10a), 157.31 (C_2), 161.34 (C_4), 167.62 ($C_{7\alpha}$), 170.23 ($C_{3\beta}$) and 182.61 (C=S); *m/z* (FAB) 776 ($(M+H)^+$, 33%), 500 (25), 139 (27), 77 (100) and 63 (41).

t-Butyl 7-methoxycarbonyl-N^{10}-phenethylisoalloxazine-3-acetate. (33)

A mixture of the phenethylisoalloxazine (**28**) (100 mg, 0.27 mmol), potassium carbonate (206 mg, 1.49 mmol) and *t*-butylbromo acetate (163 µl, 1.01 mmol) were stirred for 14h. in DMF (17 ml). After this time the DMF was removed *in vacuo* and the product purified by column chromatography (CH_2Cl_2/1% MeOH) (58 mg, 45%), m.p. 213 - 215°C δ_H ($CDCl_3$) 1.50 (9H, s, $(CH_3)_3$), 3.19 (2H, t, *J* 7.5 Hz, $C_6H_5C\underline{H}_2$), 4.00 (3H, s, CH_3O), 4.77 (2H, s, NH_2CO), 4.91 (2H, t, *J* 7.5 Hz, $NC\underline{H}_2CH_2$), 7.26 (5H, m, C_6H_5), 7.50 (1H, d, *J* 9.8 Hz, 9-H), 8.37 (1H, dd, *J* 1.9 & 9.8 Hz, 8-H) and 8.92 (1H, d, *J* 1.9 Hz, 6-H); δ_C ($CDCl_3$) 28.16 (CH_3), 33.07 ($NCH_2\underline{C}H_2$), 43.74 ($N\underline{C}H_2CH_2$), 46.57 ($N\underline{C}H_2CO$), 52.97 (CH_3O), 82.60 ($\underline{C}(CH_3)_3$), 115.37 (C_9), 127.54 to 138.00 (aromatics), 149.34 (C_{10a}), 154.76 (C_2), 158.89 (C_4), 165.00 ($C_{7\alpha}$) and 166.71 ($C_{3\beta}$); *m/z* 490 (M^+, 12%), 417 (10), 389 (7), 286 (10), 104 (100).

N^3-Carboxymethyl-7-methoxycarbonyl-N^{10}-phenethylisoalloxazine. (34)

To a solution of the flavin (**33**) (50 mg, 0.1 mmol) in CH_2Cl_2 (5 ml) was added trifluoroacetic acid (1.25 ml, 16 mmol) and the mixture stirred at room temperature for 2h. The organic solvents were removed *in vacuo* and the titled compound recrystallised from MeOH (36.9 mg, 85%), m.p. 290°C dec. (Found: C, 60.3; H, 4.0; N, 12.5. $C_{22}H_{18}N_4O_6.{}^1/_2\,H_2O$ requires C, 60.2; H, 4.2; N,12.8%); δ_H (DMSO-d_6) 3.15 (2H, t, *J* 7.5 Hz, $C_6H_5C\underline{H}_2$), 3.98 (3H, s, CH_3O), 4.65 (2H, s, NCH_2CO), 4.90 (2H, t, *J* 7.5 Hz, $NC\underline{H}_2CH_2$), 7.40 (5H, m, C_6H_5), 8.10 (1H, d, *J* 9.8 Hz, 9-H), 8.35 (1H, dd, *J* 1.9 & 9 Hz, 8-H) and 8.65 (1H, d, *J* 2 Hz, 6-H).

7-Methoxycarbonyl-3-(2-acetamido-ethyl)-3-phenylthiourea-N^{10}-phenethylisoalloxazine. (35)

BTBO (120 mg, 0.26 mmol) was added to a suspension of the monoacid flavin (**34**) (50 mg, 0.19

mmol) and DMAP (32 mg, 0.26 mmol) in acetonitrile (10 ml). The solution was left stirring for 1h. after which time the solution became clear. 1-(2-Aminoethyl)-3-phenylthiourea (117 mg, 0.6 mmol) was added to the resultant red solution at room temperature and stirring continued for a further 3h. Excess solvent was removed *in vacuo* and the product purified by column chromatography (silica gel, CH_2Cl_2/7.5% MeOH) (41 mg, 62%), m.p. 225°C dec. (Found: C, 60.2; H, 4.7; N, 15.8; S, 5.5%; *(M+H)*$^+$, 612.2037. $C_{31}H_{29}N_7O_5S$ requires C, 60.8; H, 4.8; N, 16.0; S, 5.2%; *(M+H)*, 612.2029); δ_H ($CDCl_3$) 3.20 (2H, t, *J* 7.6 Hz, $N^{10}CH_2C\underline{H}_2$), 3.48 (2H, m, CH_2), 3.90 (2H, m, CH_2), 3.99 (3H, s, CH_3O), 4.80 (2H, s, NCH_2), 4.89 (2H, t, *J* $N^{10}C\underline{H}_2CH_2$), 6.35 (1H, t, *J* 6.06 Hz, NH), 7.25 to 7.40 (aromatics), 7.47 (1H, d, *J* 9.15 Hz, 9-H), 8.36 (1H, dd, *J* 9.15 & 2.1 Hz, 8-H) and 8.85 (1H, d, *J* 1.84 Hz, 6-H); δ_C ($CDCl_3$) 33.14 ($NCH_2\underline{C}H_2C_6H_5$), 41.58 ($NCH_2\underline{C}H_2C_6H_5$), 43.08 ($NCH_2CO$), 45.40 ($CH_2$), 46.67 ($CH_2$), 52.96 ($OCH_3$), 115.61 ($C_9$), 125.87 to 138.82 (aromatics), 149.72 (C_{10a}), 155.17 (C_2), 159.33 (C_4), 165.11 ($C_{7\alpha}$), 166.22 ($C_{3\beta}$) and 181.97 (C=S).

We thank the S.E.R.C. for an IT studentship to T.R.G.E. and for financial support and the Royal Society for a University Fellowship to D.G.

Contribution from the Institute of Biomolecular Science, Southampton University.

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