VINYL AZIDES IN
NATURAL PRODUCT SYNTHESIS

A thesis presented by

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ABSTRACT

The chemistry and biology of the coenzyme PQQ are reviewed.

Several approaches to the synthesis of PQQ are described, culminating in a new total synthesis in which the key step is the formation of the indole ring from a benzaldehyde derived vinyl azide.

The limitations of the more widely used isoquinoline syntheses are briefly discussed and some examples of the use of the intramolecular aza-Wittig reaction in heterocyclic synthesis are described.

A new synthesis of 1,3-disubstituted isoquinolines has been developed in which azidocinnamates containing an ortho-carbonyl group are treated with a phosphorus (III) reagent to give iminophosphoranes. These undergo a spontaneous intramolecular aza-Wittig reaction to form the heterocyclic ring. The scope and limitations of the reaction are discussed with reference to the synthesis of a tricyclic and a tetracyclic isoquinoline.

The thermolyses of a number of azidocinnamates are discussed with regard to the synthesis of 4-acyl indoles.

Approaches to the synthesis of the marine isoquinoline alkaloid amphimedine are described.
I should like to thank Professor C.W. Rees for his guidance throughout the course of this work. His insight, humour and infectious enthusiasm for chemistry were constant sources of inspiration. My thanks are also due to Dr. C.J. Moody for his many ideas, consistently good advice and encouragement.

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A. R. MacKenzie
To Wilma
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<tr>
<td>Bz</td>
<td>Benzyl</td>
</tr>
<tr>
<td>CAN</td>
<td>Cerium (IV) ammonium nitrate</td>
</tr>
<tr>
<td>DIBAL-H</td>
<td>Diisobutylaluminium hydride</td>
</tr>
<tr>
<td>DMF</td>
<td>N,N-Dimethylformamide</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethyl sulphoxide</td>
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<tr>
<td>e.s.r.</td>
<td>Electron spin resonance</td>
</tr>
<tr>
<td>HPLC</td>
<td>High pressure liquid chromatography</td>
</tr>
<tr>
<td>i.r.</td>
<td>Infrared</td>
</tr>
<tr>
<td>LAH</td>
<td>Lithium aluminium hydride</td>
</tr>
<tr>
<td>LDA</td>
<td>Lithium diisopropylamide</td>
</tr>
<tr>
<td>MOM</td>
<td>Methoxymethyl</td>
</tr>
<tr>
<td>NADP</td>
<td>Nicotinamide adenine dinucleotide phosphate</td>
</tr>
<tr>
<td>n.m.r.</td>
<td>Nuclear magnetic resonance</td>
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<tr>
<td>p.l.c.</td>
<td>Preparative layer chromatography</td>
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<tr>
<td>PPA</td>
<td>Polyphosphoric acid</td>
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<tr>
<td>PQQ</td>
<td>Pyrroloquinoline quinone</td>
</tr>
<tr>
<td>Red-Al®</td>
<td>Sodium bis(2-methoxyethoxy) aluminium hydride</td>
</tr>
<tr>
<td>TEP</td>
<td>Triethyl phosphate</td>
</tr>
<tr>
<td>TFA</td>
<td>Trifluoroacetic acid</td>
</tr>
<tr>
<td>TFAA</td>
<td>Trifluoroacetic anhydride</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>t.l.c.</td>
<td>Thin layer chromatography</td>
</tr>
<tr>
<td>TMEDA</td>
<td>(N,N,N^1,N^1)-Tetramethylethylenediamine</td>
</tr>
<tr>
<td>p-TSA</td>
<td>4-Toluenesulphonic acid</td>
</tr>
<tr>
<td>u.v.</td>
<td>Ultraviolet</td>
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PART 1

SYNTHESIS OF COENZYME PQO
1.1 Introduction

The Chemistry and Biology of Coenzyme PQQ

1.1.1 Introduction

The dehydrogenases are a group of enzymes concerned with biological redox processes and as such they belong to the class of enzymes known as the oxidoreductases.¹

For many years it was believed that there were, in general, two types of dehydrogenase, the NAD(P)-dependent and the flavin-containing. Recently, however, it has become increasingly apparent that there is another class, the so-called quinoproteins² in which the coenzyme is 4,5-dihydro-4,5-dioxo-1H-pyrrolo[2,3-f]quinoline-2,7,9-tricarboxylic acid (1).

![Chemical Structure of Coenzyme PQQ](image)

(1)

The aim of this review is to (a) provide an historical background to the isolation and structure elucidation of the novel coenzyme (1), (b) discuss three literature syntheses and one attempted synthesis of (1) and (c) describe the studies, still in progress, aimed at understanding the detailed mechanism of action of quinoprotein dehydrogenases.

1.1.2 Nomenclature

Somewhat confusingly, two trivial names have been proposed for the coenzyme (1). The first, 'methoxatin',³ has tended to be favoured by
chemists while Pyrrolo-Quinoline Quinone (PQQ) is now in general use in the biological literature. The latter name will be used throughout this work because, in the opinion of the author, the increasingly diverse biological role and widespread distribution of (1) have rendered the name 'methoxatin' obsolete and misleading.

1.1.3 Isolation and Structure Elucidation

In 1964 Hauge isolated and purified a glucose dehydrogenase from Acinetobacter calcoaceticus which was found to oxidise glucose and other aldoses to the corresponding lactones. On boiling the enzyme he found that the resulting 'boiled juice' was capable of restoring enzymic activity to an apoenzyme preparation and concluded that the 'boiled juice' contained the liberated coenzyme. From the 'boiled juice' preparations Hauge was able to isolate a few micrograms of the coenzyme which was sufficient to show conclusively, on the basis of its physical and chemical properties, that it was novel. In particular, the u.v. absorption spectrum was characterised by a broad absorption band with a maximum at 330 nm, a shoulder at 280 nm and a second maximum at 248 nm. In addition, the coenzyme was strongly acidic, binding strongly to anion exchangers and containing an uncharged acid group of pK 1.7. The highly polar nature of the new coenzyme was also reflected in its solubility properties, requiring the addition of acid to bring about its dissolution in ethanol.

On the basis of the spectral features described above, Hauge tentatively suggested 'a suitably substituted 1,4-naphthoquinone' as a possible structure for the coenzyme and predicted, accurately, that it 'may well have a wider occurrence'.

Remarkably, Hauge's impressive and pioneering work remained unnoticed, or was ignored, for fifteen years until it was eventually rediscovered by Duine in 1979.
Three years after Hauge's work was published, Anthony and Zatman reported the results of their study of the methanol dehydrogenase from *Pseudomonas* sp. M27, one of a growing number of bacteria, known as methylotrophs, which are capable of growth on methane or methanol as their sole source of cellular carbon and metabolic energy. They were able to show that enzymic activity was independent of nicotinamide nucleotides and that the enzyme did not react directly with oxygen or contain any cytochrome, haem, cobalamin derivatives or significant amounts of metal. Furthermore, convincing spectral and other evidence was presented that the enzyme was not a flavoprotein and the authors were able to conclude that it could not be included in any known class of enzymes.

On boiling the enzyme or, alternatively, on lowering the pH to \( \alpha 3 \) or raising it to \( \alpha 12 \), a 'green fluorescent material' was liberated which was obtained as a red/brown solid after chromatography on cellulose and freeze-drying. It was found that production of the green fluorescent material from the non-fluorescent enzyme was always accompanied by a loss of enzymic activity which led Anthony and Zatman to conclude that they had obtained the free coenzyme, although no attempt to restore enzymic activity in an apoenzyme test was reported.

The isolated coenzyme was found to be freely soluble in water but insoluble in methanol, acetone, ether, light petroleum or chloroform. The u.v. absorption spectrum was very similar to that obtained by Hauge and contained maxima at 330 and 248 nm with a shoulder at 275 nm. Anthony and Zatman placed most emphasis, however, on the fluorescence spectra of the coenzyme. Maximum fluorescence was found to occur at low pH with excitation maxima at 255 and 365 nm and a fluorescence maximum at 460 nm. These values were compared with the published fluorescence spectra of some two hundred pharmaceuticals and compounds.
of biological origin. Of these, only twenty-four showed maximum fluorescence below pH 9 and had excitation maxima between 355 and 385 nm and emission maxima between 450 and 470 nm. Because all of these compounds were derivatives of pteridine, Anthony and Zatman concluded that the new coenzyme was likely to be a pteridine derivative also, and was possibly related to pteroylglutamic acid (2).

![Structure of pteroylglutamic acid (2)](image)

However, the latter suggestion appears to have been based on little more than the qualitative observation that solutions of certain derivatives of (2), on exposure to light and air, produce a red/brown substance of unknown structure which has a green fluorescence.9

Nevertheless, the suggestion that the coenzyme of methanol dehydrogenase was a pteridine derivative was supported by Forrest and coworkers who were investigating whole cell extracts of another methylotrophic bacterium, *Methyloccoccus capsulatus*. From this organism they were able to isolate a new pteridine derivative to which the structure (3) was assigned on the basis of a series of degradations and authentic sample comparisons (Scheme 1). Thus, alkaline permanganate oxidation of (3) gave the known 2-amino-4-hydroxypteridine-6-carboxylic acid (4). Alternatively, acid hydrolysis of (3) gave a blue fluorescent derivative (5) which also underwent oxidative cleavage to give (4). Further hydrolysis of (5), this time using alkaline phosphatase from *E. coli*, gave L-threo-neopterin (6) which was identical to an authentic sample
Scheme 1

(4) \[ \text{NH}_2\text{N} \begin{array}{c} \text{N} \\ \text{N} \end{array} \text{CO}_2\text{H} \] \xrightarrow{\text{KIO}_4} (6) \[ \begin{array}{c} \text{N} \\ \text{N} \end{array} \text{OH} \begin{array}{c} \text{OH} \\ \text{OH} \end{array} \] \xrightarrow{\text{Alkaline phosphatase}} (5) \[ \begin{array}{c} \text{N} \\ \text{N} \end{array} \text{OH} \begin{array}{c} \text{OH} \\ \text{OH} \end{array} \text{PO}_4\text{OH} \]

\[ (7) \begin{array}{c} \text{HO} \\ \text{HO} \\ \text{HO} \\ \text{CHO} \end{array} \] 

\[ (8) \begin{array}{c} \text{NH} \\ \text{NH} \\ \text{NH} \\ \text{NH} \end{array} \] 

\[ (6) \begin{array}{c} \text{OH} \\ \text{OH} \\ \text{OH} \end{array} \]
prepared from L-(-)-xylose (7) and 2,4,5-triamino-6-hydroxypterimidine (8). Since microanalysis had previously shown the ratio of phosphorus to pteridine nucleus in (3) to be 1:1, the authors concluded that (3) had the L-threoneopterin-2',3'-cyclic-phosphate structure shown. The alternative 1',3'-six-membered cyclic phosphate was somewhat unconvincingly ruled out by reference to the ease of hydrolysis of (3) and the ease with which it is prepared from the 3'-phosphate (5) on treatment with trifluoroacetic anhydride.

The spectral characteristics of (3) were found to be similar to those reported earlier by Anthony and Zatman in their study of the isolated coenzyme of methanol dehydrogenase from *Pseudomonas* sp. M27. In particular, the fluorescence spectra contained excitation peaks at 275 and 365 nm and an emission peak at 450 nm while the u.v. absorption spectrum exhibited maxima at 344 and 260 nm. These results led Forrest to suggest not only that the neopterin cyclic-phosphate (3) was the coenzyme of the methanol dehydrogenase from *Methylococcus capsulatus*, but also that (3) might be common to all methanol dehydrogenases present in methylotrophic bacteria. These suggestions were unwarranted however, particularly since Forrest did not present any evidence that (3) was enzyme-derived at all, being simply isolated from a whole-cell extract of the harvested bacteria. As such, the question of its location and biological function, if any, within the cell remains unanswered.

Two years later, in 1973, Forrest and coworkers suggested a different pteridine as the coenzyme. Working now with a purified methanol dehydrogenase from several different methylotrophic bacteria, they isolated a substance, presumed to be the coenzyme, which had u.v. absorption maxima at 346 and 258 nm and produced a fluorescence maximum at 450 nm on excitation at 345 nm. The substance appeared to be
photochemically unstable and on irradiation at 360 nm, a photodegradation product was obtained which was tentatively identified as a uracil derivative on the basis of its spectral characteristics. These findings led the authors to suggest that the coenzyme was a lumazine (9) derivative.

Although at this point three different pteridine derivatives had been proposed as the structure of the new coenzyme, the evidence supporting any of them was at best equivocal, the more so as a result of a study by Patel and coworkers of yet another methylotrophic bacterium, *Methylomonas methanica*. This organism yielded a crystalline methanol dehydrogenase from which it was possible to prepare the apoenzyme by dialysis in acidic buffer solution. However, all attempts to reconstitute an active enzyme using a variety of pteridine derivatives were unsuccessful. The authors suggested that their lack of success might be due to a slight change in the structure of the enzyme on removal of the coenzyme which disturbs the pteridine binding site. Alternatively, they argued that a change in the structure of the coenzyme on release from the enzyme might make reconstitution of the apoenzyme more difficult. The possibility that the coenzyme was other than a pteridine derivative was not mentioned.

The first direct evidence against a pteridine structure was obtained by Duine and coworkers in Holland. Working with a highly
purified methanol dehydrogenase from the methylotrophic bacterium *Hyphomicrobium* X, they observed an e.s.r. signal arising from an enzyme-bound organic free radical.\(^{14}\) Denaturing the enzyme with heat or with high or low pH buffers was found to produce the characteristic green fluorescence of the coenzyme with simultaneous loss of the e.s.r. signal. By considering the line width and \(g_{150}\) value, the Dutch workers concluded that the signal could not be attributed to a pteridine or flavin radical but was typical of the literature spectra of quinone radicals.

Further evidence against a pteridine structure was obtained by the same authors on treatment of the isolated coenzyme with alkaline permanganate. After such treatment, no u.v. absorption spectrum could be obtained, showing that the coenzyme was not the neopterin cyclic-phosphate (3) or a derivative of pteroylglutamic acid (2) since both of these would have been expected to give a characteristic spectrum.\(^{14}\)

The Dutch group were subsequently able to obtain an e.s.r. spectrum of the free coenzyme by releasing it from the enzyme under strictly anaerobic conditions in alkaline salicylate buffer.\(^{15}\) This proved to be particularly informative. Analysis of the hyperfine coupling constants indicated that the unpaired electron was coupled to two nitrogen atoms and three hydrogen atoms. Furthermore, one of the hydrogens was found to be exchangeable when the e.s.r. experiment was conducted in a deuterated solvent.

The final breakthrough came in 1979 when structure (1) was proposed for the coenzyme by Salisbury and Forrest on the basis of an X-ray crystal structure analysis of a derivative (10), obtained on acetone extraction of a cell paste derived from the methylotroph, *Pseudomonas* TPI.\(^{3,16}\)
The adduct (10) was found to be racemic which strongly suggested that the chiral centre was not of biological origin and that (10) was in fact an artefact derived by aldol addition of acetone to C-5 of (1) during the extractive isolation procedure. That (1) was indeed the correct structure of the new dehydrogenase coenzyme was rapidly confirmed by Duine, who reported a procedure for the large-scale extraction of whole cells of methylotrophic bacteria which allowed the isolation of milligram quantities of the purified coenzyme. With these more substantial quantities in hand, Duine was able to record the i.r. and n.m.r. spectra. The former was found to contain strong carbonyl absorptions at 1678 and 1710 cm\(^{-1}\) while the latter consisted only of two broadened singlets at 8.16 and 7.14 ppm. These data, together with the e.s.r. experiments described previously, were found to be fully consistent with the proposed structure (1). Further confirmation was provided by methylation of the coenzyme with dimethyl sulphate and potassium carbonate to give the trimethyl derivative (11).
Based on the systematic name for (1), 4,5-dihydro-4,5-dioxo-1H-pyrrolo[2,3-\(f\)]quinoline-2,7,9-tricarboxylic acid, Duine proposed the trivial name pyrroloquinoline-quinone (PQQ) for the coenzyme and suggested, by analogy with other distinct groups such as the haemoproteins and flavoproteins, that dehydrogenases containing coenzyme PQQ be classified as 'quino-proteins'.

Remarkably, for a molecule with a molecular weight as low as 330, the gap between the first reported isolation of PQQ in 1964 by Hauge and the eventual determination of its molecular structure, was fifteen years. The reasons for the long gap are probably four-fold. Firstly, for many years there was no good method available for obtaining PQQ of high purity. Secondly, and related to the first, only very small quantities (µg) of the coenzyme were available for study. Thirdly, PQQ is a relatively reactive molecule, particularly with nucleophiles, and is unstable in alkaline solution. Finally, there was a tendency by some workers to speculate on the detailed structure of PQQ on the basis of very limited and equivocal evidence. This inevitably led to confusion in the literature and probably slowed progress.

1.1.4 Synthetic Studies

With the elucidation of its molecular structure in 1979, PQQ became an attractive target for synthesis. In addition to the novelty of the
tricyclic orthoquinonoid structure, there was a need for adequate supplies to be made available for use in a variety of mechanistic and biosynthetic studies. At the outset of our own work in this area two syntheses had been reported in the literature, while a third appeared during the course of our studies. These successful syntheses are discussed below together with two unsuccessful approaches which were reported in a thesis.  

The first synthesis of PQQ was reported by Corey and Tramontano in 1981. The route chosen was an efficient one, comprising of a nine step sequence from a commercially available starting material in an overall yield of 19.6% (Scheme 2). Thus, 2-methoxy-5-nitroaniline was converted into the N-formyl derivative (12) which was reduced catalytically to give the mono-protected diamine (13). Diazotisation of (13) followed by treatment with the anion of methyl α-methylacetoacetate gave the hydrazone (14) which underwent Fischer indolisation in formic acid to give the indole (15) with remarkably high regioselectivity. Less than 3% of the isomeric 4-formamido-5-methoxyindole-2-carboxylate could be detected.

Hydrolytic deformylation of (15) under conditions which left the ester function intact, gave rise to the aminoindole (16). Annulation of the third ring was achieved using a two-stage, one-pot reaction in excellent yield. Thus treatment of (16) with dimethyl 2-oxoglutaconate (20), followed by acidification, gave the pyrroloquinoline (17).
Reagents: i, HCO\textsubscript{2}H, Ac\textsubscript{2}O, 25\textdegree{}-50\textdegree{}C; ii, H\textsubscript{2}, PtO\textsubscript{2}, EtOH, 3 atm., 65\textdegree{}C; iii, NaN\textsubscript{2}O, 0.3N HCl, 0-5\textdegree{}C, then MeO\textsubscript{2}CCH(Me)COMe, KOH, 0\textdegree{}C; iv, HCO\textsubscript{2}H, 80\textdegree{}C; v, HCl, acetone-H\textsubscript{2}O (96:4), reflux; vi, MeO\textsubscript{2}CCO-CH=CHCO\textsubscript{2}Me, CH\textsubscript{2}Cl\textsubscript{2}, then H\textsuperscript{+}; vii, Ce(NH\textsubscript{4})\textsubscript{2}(NO\textsubscript{3})\textsubscript{6}, CH\textsubscript{3}CN-H\textsubscript{2}O (4:1), 0\textdegree{}C; viii, HC(O\textsubscript{Me})\textsubscript{3}, p-TSA(cat), MeOH, reflux; ix, aq.K\textsubscript{2}CO\textsubscript{3}, 85\textdegree{}C, then HCl to pH 2.5.
The orthoquinone unit was introduced directly from (17) by oxidation with cerium (IV) ammonium nitrate to give PQQ trimethyl ester (11). The hydrolysis of (11) to give PQQ itself proved troublesome but was finally achieved in a high yielding, two-step procedure in which the orthoquinone unit was first protected by monoketalisation at C-5 to give (18). Hydrolysis of (18) in aqueous potassium carbonate gave, on acidification to pH 2.5, a dark-red precipitate of PQQ.

Shortly after the Corey synthesis was published, a second synthesis was reported by Weinreb and Gainor. Although the broad strategy was similar to that of Corey, whereby the two heterocyclic rings were sequentially fused to a suitably substituted central ring, Weinreb chose to construct the quinoline first. The route chosen to prepare the intermediate quinoline diester (23) involved a Pfitzinger synthesis and is shown in Scheme 3. Lithiation of 2,3-dimethoxytoluene followed by quenching of the resulting anion with CO₂ gave the acid which was converted into the aniline (19) by Curtius rearrangement. A Sandmeyer isatin synthesis was then used to convert (19) into (22). Thus, treatment of the aniline (19) with chloral hydrate, hydroxylamine hydrochloride and sodium sulphate in water gave the oximino compound (21) which underwent acid-catalysed cyclisation to give the isatin (22). Condensation of (22) with pyruvic acid under standard Pfitzinger conditions proved to be straightforward and the resulting quinoline dicarboxylic acid was subsequently esterified to give the required diester (23).
Reagents: i, BuLi, TMEDA, hexane, 20°C, then CO₂, -78°C; ii, ClCO₂Et, Et₃N, acetone, 0°C, then NaN₃, H₂O, 0°C, then toluene, reflux, then 50% aq. KOH; iii, CCl₃CH(OH)₂, H₂NOH·HCl, aq. Na₂SO₄, 70°C; iv, PPA, 100°C; v, 30% aq. KOH, then CH₃COCO₂H, 95°C; vi, MeOH, H⁺, reflux.

Scheme 3

Weinreb's original plan was to annulate the remaining pyrrole ring onto (23) using a classical Reissert indole synthesis. However, although the required nitro-compound (24) was easily prepared, no conditions could be found to convert (24) into the desired Reissert intermediate (25) by condensation with dimethyl oxalate.
This failure was ascribed to steric crowding in (24) which forces the nitro group out of the plane of the aromatic ring thus precluding resonance stabilisation of the benzylic anion. The problem was eventually overcome by employing an 'umpolung' variation of the Reissert synthesis as shown in Scheme 4. Thus benzylic bromination of (23) followed by low-temperature nitration of the resulting bromide (26) gave the nitro-quinoline (27). Although (27) would not alkylate oxalate acyl anion equivalents such as (31) and (32), success was achieved with the sodium enolate of methyl acetoacetate to give the keto-ester (28).

A modified Japp-Klingemann reaction was then used to convert (28) into the Reissert intermediate (29) which underwent catalytic reduction to give the pyrroloquinoline (30) directly. Oxidation of (30) with acidic silver (II) oxide gave PQQ trimethyl ester (11). In contrast to the reported instability of the coenzyme in alkaline solution,\(^3\) Weinreb was able to carry out the final hydrolysis of (11) to give PQQ using lithium hydroxide in aqueous THF. To summarise therefore, the
Reagents: i, NBS, (PhCO₂)₂ (cat.), CCl₄, reflux; ii, cHNO₃, cH₂SO₄, -20°C; iii, MeO₂CCH₂COMe, NaH, THF; iv, PhN₂⁺BF₄⁻, aq.py., then NaBH₄, MeOH; v, H₂, Pd, C, MeOH, 5% HCl (cat.); vi, AgO, 6N HNO₃, THF; vii, LiOH, THF, H₂O (85:15).

Scheme 4
Weinreb synthesis proceeds in 13 steps from 2,3-dimethoxytoluene to give PQQ in an overall yield of 2%.

A third synthesis of PQQ was reported in 1982 by Hendrickson and de Vries. Unlike the previous syntheses, the strategy employed by these workers was convergent and involved coupling of suitably functionalised pyrrole and pyridine subunits followed by oxidative photocyclisation to form the tricyclic ring system (Scheme 5).
Unfortunately the undoubted elegance of the approach was somewhat impaired by the practicalities of the photocyclisation and the tedious manipulations required to convert (36) into the target *(vide infra)*. The pyrrole-aldehyde (33) was prepared from ethyl pyrrole-2-carboxylate by a regioselective Friedel-Crafts reaction using dichloromethyl methyl ether while the Wittig salt (38) was prepared straightforwardly from uvitonic acid (37) as shown in Scheme 6.

![Scheme 6](image)

Reagents: i, MeOH, H⁺, heat; ii, NBS, CCl₄, reflux, hv; iii, PPh₃, benzene, reflux.

Wittig coupling of (33) and (34) proceeded smoothly to give the *trans*-olefin (35). Photocyclisation of (35) to give the pyrroloquinoline (36) was unsuccessful using sulphur or selenium as oxidants but was eventually achieved on prolonged irradiation of a dilute (300 mg/litre) benzene-ether (4:1) solution containing diphenyl diselenide. Unfortunately the irradiation time of one month and the yield of 44% detract somewhat from the usefulness of the reaction. Nevertheless,
the formation of (36) represents the first reported stilbene-type photocyclisation in which one aromatic unit is a pyrrole.\textsuperscript{22}

The conversion of (36) into PQQ was more difficult than originally envisaged due to the lack of phenanthrene-like olefinic character in the C-4 - C-5 bond, thus precluding direct oxidation to introduce the orthoquinone unit. In addition, selective functionalisation at C-5 was not possible due to the high reactivity of C-3 towards electrophiles. Recourse was eventually made to a double nitration at C-3 and C-5. Unfortunately, the unwanted nitro group at C-3 required three steps for its removal and in all, seven steps were required to convert (36) into PQQ (1). The details of the route are shown in Scheme 7. Ultimately then, the Hendrickson synthesis required thirteen steps from uvitonic acid (37) and ethyl pyrrole-2-carboxylate and proceeded in an overall yield of <12%.

Two unsuccessful approaches to the synthesis of PQQ have been reported by Larson in a thesis.\textsuperscript{18} The first approach was similar to that of Hendrickson and involved the attempted photocyclisation of the diaryl ethene (39).

![Diagram](image)

It was hoped that the phenylsulphonyl protecting group would be lost from the intermediate dihydro-pyrroloquinoline (40), either by elimination of phenylsulphinic acid or by photolytic S-N bond scission, thereby removing the need for an external oxidant. Unfortunately no
Reagents: i, fHNO₃, H₂SO₄, 0°C; ii, Na₂S₂·5H₂O, DMF, then CH₂N₂; iii, MnO₂, H₂SO₄, 0°C; iv, H₂, 10% Pd/C, MeOH; v, NaN₃, oHCl, 0°C; vi, 50% H₃PO₄, AcOH, then aq. K₃Fe(CN)₆, KOH; vii, 0.5M LiOH, H₂O-THF (1:1), then H⁺.

Scheme 7
conditions could be found, with or without added oxidants, whereby (39) underwent photocyclisation. The major pathway always appeared to be S-N bond cleavage to give the N-H pyrrole derivative (41) which appeared to be photostable.

![Structure of (41)](image)

The latter conclusion was, however, reached prior to the publication of the Hendrickson synthesis. Presumably (39) would indeed cyclise under the conditions described for the cyclisation of (35) (vide supra).

The second approach to the synthesis of PQQ described by Larson was similar to that of Weinreb and Gainor. Thus the quinoline (43) was prepared from the isatin (42) by Pfitzinger condensation with pyruvic acid and esterification.

![Conversion from (42) to (43)](image)

However, attempts to introduce substituents at C-6 in (43) by sigma-tropic rearrangement of derivatives such as (44) and (45) were unsuccessful. In both cases the substituent migrated to C-8 to give the quinolones (46) and (47).
Similarly an attempted aza-Claisen rearrangement of the lactam (49), prepared from the nitroquinoline (48), did not give any of the desired rearranged product (50) but gave instead, the decarboxylated product (51).
While several other attempts were made to fuse the remaining pyrrole ring onto quinolines derived from (43) and (48), all were ultimately unsuccessful.

Finally, it appears that two other, as yet unsuccessful, approaches to the synthesis of PQQ have been investigated at Oxford and M.I.T.23, 24

1.1.5 Mechanistic Studies

The determination of the structure of PQQ inevitably led to interest in its mechanism of action in co-catalysis. This interest has been enhanced by the increasing commercial importance of methylotrophic bacteria as nutritive single cell protein, and by the discovery that PQQ is also the coenzyme of a number of dehydrogenases from non-methylotrophic bacteria (Table 1).

Table 1

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Organism</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol dehydrogenase</td>
<td>Methylotrophs</td>
<td>3, 7, 17</td>
</tr>
<tr>
<td>Primary amine dehydrogenase</td>
<td><em>Pseudomonas</em> AM1</td>
<td>27</td>
</tr>
<tr>
<td>Alcohol dehydrogenase</td>
<td><em>Acinetobacter calcoaceticus</em></td>
<td>28</td>
</tr>
<tr>
<td>Polyol dehydrogenase</td>
<td><em>Gluconobacter oxydans</em></td>
<td>6</td>
</tr>
<tr>
<td>Long-chain alcohol dehydrogenase</td>
<td>Alkane-grown <em>Ps. aeruginosa</em></td>
<td>6</td>
</tr>
<tr>
<td>Glucose dehydrogenase</td>
<td><em>Acinetobacter calcoaceticus</em></td>
<td>4, 5, 6</td>
</tr>
<tr>
<td>Aldehyde dehydrogenase</td>
<td><em>Gluconobacter suboxydans</em></td>
<td>29</td>
</tr>
<tr>
<td>Lactate dehydrogenase</td>
<td><em>Propionibacterium pentosaceum</em></td>
<td>2</td>
</tr>
</tbody>
</table>

As can be seen from the table, any proposed mechanistic scheme must be able to explain the ability of PQQ to oxidise primary amines and
Scheme 8
aldehydes as well as a wide variety of alcohols.

The first mechanism to be proposed for oxidations involving PQQ was due to Forrest and is shown in Scheme 8. The first step involves the addition of a primary amine (possibly of a lysine residue) or ammonia to C-4 to give (52). Since in solution, nucleophiles are known to add to PQQ at C-5, Forrest is forced to argue that steric constraints within the catalytic site of the enzyme allow attack only at C-4. Subsequent 1,4 elimination of water to give (53) is followed by 1,4 addition of the substrate to give the complex (54). This then undergoes a cyclic rearrangement, driven by aromatisation of the pyrroloquinoline, to give the hydroxy-amine (55) with loss of the product. Reoxidation of the coenzyme then completes the cycle. Forrest proposed the same mechanism for the oxidation of aldehydes since it is known that only those aldehydes which are extensively hydrated in aqueous solution, such as formaldehyde and chloral, are good substrates (Scheme 9).

More recently, Anthony has proposed a similar mechanism for the PQQ-mediated oxidation of methylamine (Scheme 10). Although these mechanisms appear to be chemically feasible, there is now mounting evidence that oxidations involving PQQ are more complicated than was first
thought. In particular, Duine has shown that the methanol dehydrogenase from *Hyphomicrobium X* contains two coenzyme molecules per enzyme molecule and that the second coenzyme is present as 'pyrroloquinoline quinol' (PQQH$_2$). He has proposed that the two coenzyme molecules are able to interact with each other in the enzyme and take part in the series of equilibria shown in Scheme 11. The importance of PQQ semiquinone (PQQH$^\cdot$) in the oxidation mechanism has been investigated by Abeles and coworkers. By comparing the integrated intensity of the e.s.r. signal from the methanol dehydrogenase of *Methylophoras methanica* with that of a standard solution, they claimed to have established that $\alpha 13\%$ of the coenzyme was in the semiquinone form, PQQH$^\cdot$. They also claimed that addition of cyclopropanol, stoichiometrically equivalent to $14\%$ of the enzyme, completely inactivated the enzyme.
From these results Abeles and coworkers proposed that cyclopropanol was oxidised by the enzyme and that subsequent reaction of the enzyme with the oxidation product led to inactivation. They concluded that only the semiquinone form of the coenzyme is catalytically active and that addition of substrate leads to a two-electron reduction of \( \text{PQQH}^+ \) to give a new radical (56).
These conclusions have recently been challenged by Duine who showed that they were based on incorrect interpretation of the e.s.r. spectra. Furthermore, he showed that although both cyclopropanol and cyclopropanone irreversibly inactivate the enzyme, the PQQ adducts (as yet unidentified) extracted after inactivation are spectrally and chromatographically different. He concluded that inactivation by cyclopropanol is not due to initial oxidation to give cyclopropanone followed by an irreversible reaction of the latter with the enzyme. Instead, Duine suggested that PQQ itself reacts with cyclopropanol by an initial single electron transfer followed by ring-opening of the resulting cyclopropanol radical cation (57) to give PQQH\(^-\) and the propionaldehyde radical (58). These then collapse to form the stable PQQ adduct (59).
Further evidence against the three-electron reduced form of PQQ (56) was obtained by Bruice who carried out an electrochemical study of the coenzyme. He showed that while the reduction of PQQ was complex, there was no evidence for an electrochemically reduced state lower than the quinol PQQH$_2$.

![PQQH$_2$](image)

The detailed mechanism of action of quinoproteins remains, therefore, uncertain. In particular, it is still not known whether the oxidation occurs by one-electron steps or by hydride or hydrogen transfer. Neither is it known if the ease of formation of adducts at C-5 of PQQ has any mechanistic significance. Answers to these questions and others related to the mechanism are currently being pursued.

1.1.6 Miscellaneous Studies

Forrest has recently reported the ultimate proof that PQQ is indeed the coenzyme of glucose dehydrogenase from *Acinetobacter calcoaceticus*. He showed that synthetic PQQ, identical to naturally derived material, was able to restore full enzymic activity to an apoenzyme preparation. He was unable, however, to reactivate the apoenzyme of methanol dehydrogenase and concluded that removing the coenzyme from this enzyme causes irreversible denaturation of the protein, thus making it nonviable in the apoenzyme test.

Ohshiro and coworkers in Japan have reported the results of a study of the nonenzymatic oxidation of amines to the corresponding carbonyl
compounds using PQQ. They found that treatment of cyclohexylamine with PQQ in aqueous solution gave cyclohexanone in 22% yield (based on PQQ). However, on addition of a cationic surfactant such as hexadecyltrimethylammonium bromide (CTAB), the oxidation became catalytic in PQQ. This remarkable micellar catalysis was most efficient at pH 7-8 but was completely suppressed at pH 1.6. Furthermore, neutral or anionic micelles showed no catalytic activity. Although the authors speculated that PQQ semiquinone might be the active species in the oxidation, they were unable to suggest a mechanism for its regeneration. It appears, however, that oxygen plays an important role as benzylamine was only converted into benzaldehyde in low yield when the reaction was carried out under a nitrogen atmosphere.

As an alternative to synthesis as a means of obtaining supplies of PQQ, Ameyama and coworkers have recently reported a microbial method. By growing particular methylotrophic bacteria aerobically in 1% methanol-mineral salt solution they were able to detect more than 10 µg/ml of PQQ in the culture medium after two days. Interestingly, it appears that some acetic acid bacteria can also accumulate PQQ in the culture medium with the result that it is possible to detect PQQ in table vinegars.

A useful and very convenient method for the detection of PQQ has been developed by Duine. The sample containing the coenzyme is first reduced with sodium borohydride to give the dihydrodiol (PQQH₂) and then oxidised with periodate to give a stable, strongly fluorescing compound of unknown structure (presumably a ring-cleavage product) which can be selectively detected by ion-pair HPLC with fluorescence detection.
Using the detection technique just described, Duine has very recently reported the first example of a mammalian enzyme which contains PQQ. The enzyme, bovine serum amine oxidase, is similar to one found in humans and is responsible for the conversion of spermine and spermidine into their corresponding aldehydes. Since these compounds are known to function in the regulation of cell division, it may be possible to design specific inhibitors of PQQ as potential chemotherapeutic agents.

One aspect of PQQ which has not yet been addressed in the literature concerns its mode of biosynthesis. This is of considerable interest as PQQ represents the first naturally occurring example of the pyrrolo[2,3-f]quinoline ring system. While several possible biosynthetic pathways may be envisaged, perhaps the simplest would involve the coupling of phenylalanine and glutamic acid as shown below.
Indeed, crude feeding experiments have shown accelerated growth of methylotrophs when glutamic acid is present in the culture medium.\textsuperscript{18}

1.1.7 Conclusion

Since the discovery of the structure of PQQ in 1979, interest in the novel coenzyme has increased dramatically. It is already clear that the distribution of quinoproteins is much more widespread than was originally thought and that PQQ contributes to a wide range of biological oxidations. Just what the final extent of that contribution will be is impossible to predict at the moment, although the recent discovery of PQQ in a eukaryotic enzyme has led to discussion of the coenzyme's eventual role as a vitamin.\textsuperscript{44}

1.2 Results and Discussion

1.2.1 Introduction

Vinyl azides are versatile intermediates in heterocyclic synthesis and their preparation and reactions have been reviewed.\textsuperscript{47,48,49} Potentially, one of the most useful reactions of vinyl azides is the thermolysis of 3-aryl-2-azido propenoates (60) to give, ultimately, indole-2-carboxylates (64) (Scheme 12). The reaction is thought to proceed by initial cyclisation to give the azirine (61) with concerted loss of nitrogen. The azirine (61) is in thermal, tautomeric equilibrium with the vinyl nitrene (62) which can cyclise onto a free ortho position of the aryl ring. The resulting 7αH-indole (63) undergoes a rapid, aromatising [1,5] hydrogen shift to give the observed product (64). Although the reaction was first reported by Hemetsberger in 1970,\textsuperscript{50} it has since received little attention in the literature.
However, recent studies in this laboratory and elsewhere have shown that the reaction is a general one which, due to the relatively mild and essentially neutral conditions, is able to tolerate a variety of functional groups on the aryl ring. Furthermore, since the vinyl azides (60) are readily prepared by base-catalysed condensation of an alkyl azidoacetate (65) with a benzaldehyde, the two step sequence shown in Scheme 13 now appears to be the method of choice for the
In order to demonstrate and extend the usefulness of the nitrene cyclisation described above, it was decided to employ the reaction as a key step in the synthesis of a natural product. Coenzyme PQQ (1) appeared to be the ideal choice as target molecule since, in addition to containing a recognisable indole-2-carboxylic acid fragment, the overall tricyclic, orthoquinonoid structure is both novel and challenging. Furthermore, in view of the growing biological importance of PQQ and the minute amounts available from natural sources, there was a genuine need for supplies of synthetic material to be made available for study. It was hoped, therefore, that the vinyl azide chemistry described above would allow the construction of a short and efficient synthesis of the coenzyme, devoid of chromatographic separations, which would be amenable to gram-scale preparations.
1.2.2 **Retrosynthetic Analysis**

From the outset, it was intended to follow the same general strategy as Corey and Weinreb, whereby the tricyclic ring system was assembled prior to the introduction of the o-quinone unit in the penultimate step to give PQQ trimethyl ester (11) (Scheme 14).\(^{19,20}\)

![Scheme 14](image.png)

The advantage of such a strategy lies in the late introduction of the o-quinone unit, thereby minimising the difficulties associated with its high reactivity.\(^3,31\)

Having decided to arrive at an intermediate pyrroloquinoline of general type (66), the questions remaining included the method to be used to form the pyridine ring and the order in which the two heterocyclic rings should be formed. These were answered by an examination of the literature, which showed that Corey was able to form the pyridine ring of (17) in one step and >90% yield.\(^{19}\) Thus, treatment of the
6-aminoindole (16) with dimethyl 2-oxoglutarate (20), then acid, gave the pyrroloquinoline (17) directly.

Since this method appeared to offer little scope for improvement, and the aim was to develop as short and efficient a route to PQQ as possible, it was decided to employ a similar reaction to form the pyridine ring in the present synthesis. However, it was recognised that in the absence of a substituent at C-5 in the 6-aminoindole substrate, a question of regioselectivity in the cyclisation, not encountered by Corey, would be created (vide infra).

The considerations described above resulted in the retrosynthetic analysis shown in Scheme 15. Thus disconnection of the pyridine ring in (66) leaves a 6-aminoindole derivative (67) which is in turn derived, by thermolysis and amide cleavage, from a vinyl azide of type (68). Disconnection at the olefinic bond in (68) then reveals a benzaldehyde of type (69).

Based on this retrosynthetic analysis, a series of substituted benzaldehydes (70) - (73) were considered as potential starting materials. Of these, only (72) was ruled out immediately, since it was anticipated that the derived vinyl azide (74), containing two free ortho-positions, would give a mixture of isomeric indoles (75) and (76) on thermolysis.
Scheme 15

R₁, R₂ = H, OR

(66) \[ \text{MeO}_2C\text{NH} \quad \text{CO}_2\text{Me} \]

(67) \[ \text{MeO}_2C\text{NH} \quad \text{CO}_2\text{Me} \]

(68) \[ \text{MeO}_2C\text{N}_3 \]

(69) \[ \text{OHC} \quad \text{NHAc} \]

(70) \[ \text{OHC} \quad \text{NHAc} \]

(71) \[ \text{MeO} \quad \text{NHAc} \]

(72) \[ \text{OMe} \quad \text{NHAc} \]

(73) \[ \text{MeO} \quad \text{OMe} \quad \text{NHAc} \]
Of the remaining aldehydes, 4-acetamido-2,3-dimethoxybenzaldehyde (73) appeared to be the most promising. Not only would the presence of two methoxy groups guarantee regiospecificity in both ring annulations but also the resulting pyrroloquinoline (30) had previously been converted into PQQ by Weinreb. 20

Unfortunately, however, the aldehyde (73) is unknown and, as a 1,2,3,4-tetrasubstituted benzene, the development of a short and efficient method for its large-scale preparation promised to be a less than trivial exercise, and was not attempted. Nevertheless, the attraction of a dimethoxyaldehyde as the starting material remained, and so a modified retrosynthesis of the aminoindole (77) was proposed based on the commercial availability of 2,3-dimethoxybenzaldehyde (Scheme 16).
Thus the intention was to construct the indole (78) prior to the introduction of the amino group either by direct amination or by a nitration/reduction sequence.

1.2.3 Routes from 2,3-Dimethoxybenzaldehyde

As outlined above, the initial objective was to prepare the indole (78). Therefore, commercially available 2,3-dimethoxybenzaldehyde was condensed with methyl azidoacetate under the standard literature conditions \(^{51,53}\) (cf. experimental) at \(-15^\circ C\) to give the vinyl azide (79) in 72% yield after recrystallisation from methanol. It should be noted here that, although (79) is depicted as the Z-isomer, no direct evidence was obtained regarding the stereochemistry about the double-bond. Nevertheless, the Z-isomer is expected to be the thermodynamic product and there is now some indirect evidence (\textit{vide infra}) that this assignment is correct.
correct. For convenience, therefore, all the vinyl azides prepared in this work will be written in the Z-form. Of course, in the indole synthesis, the stereochemistry about the double-bond is irrelevant, since thermolysis of either the \( E \) or \( Z \)-isomer of (79) would give the same product via the derived azirine-vinyl nitrene equilibrium. In the event, thermolysis of (79) in boiling xylene for 4 hours gave the required indole (78) in 98\% yield after sublimation.

With the indole (78) in hand, attention focused on the introduction of an amino group at C-6. It was hoped that this might be achieved in one step by direct amination, since recent interest in the C-amination of organometallic species has led to the introduction of several new reagents (80) - (83)\(^{54-57}\) which act as \( \text{NH}_2^+ \) equivalents.

\[
\begin{align*}
\text{PhSCH}_2\text{N}_3 & \quad \text{(80)} \\
\text{Ph} & \quad \text{N}_3 \quad \text{(81)} \\
\text{H}_2\text{NOP(O)}\text{Ph}_2 & \quad \text{(82)} \\
\text{MeONH}_2/\text{MeLi} & \quad \text{(83)}
\end{align*}
\]
Of these, the most attractive appeared to be α-azidostyrene (81), since Hassner has reported that it reacts with heteroaromatic lithium compounds to give good yields of primary amines on acid hydrolysis of the initially formed triazenes (84) (Scheme 17).55

Furthermore, (81) offered the intriguing possibility of developing a synthesis of PQQ in which both nitrogen atoms were vinyl azide derived.

Less attractive, however, was the prospect of attempting to convert the indole (78) into the required lithium derivative at C-6. In addition to the difficulties created by the electrophilic ester group at C-2, the lithiation of indoles in the carbocyclic ring is very poorly described in the literature.58 Indeed, the only encouraging example found was a report by Sundberg concerning the lithiation of 5-methoxy-1-methylindole (85).59 He found that lithiation of (85) with n-butyllithium in refluxing ether followed by reaction with pyridine-2-carbaldehyde gave rise to a mixture of three isomeric indoles (86)-(88). From the product distribution, he concluded that αα. 60% of the metalation had occurred in the carbocyclic ring.

Scheme 17
An additional problem in the proposed lithiation of (78) concerned the indole N-H. If left unprotected, a dianion must be formed, while prior protection would require at least one extra step. To avoid this problem, it was hoped that protection and deprotection of the indole N-H might be accomplished \textit{in situ} as shown in Scheme 18. Initially, however, a sample of (78) was protected as the $\text{N}$-methyl derivative (89) in order to determine if lithiation could be achieved without interference from the ester group at C-2. Thus treatment of (78) with sodium hydride in DMF, and quenching the resulting anion with methyl iodide gave the protected indole (89) in excellent yield.
Attempts to lithiate (89) were unsuccessful. Using tert-butyl-lithium in THF at room temperature followed by quenching with methyl iodide, a complex mixture of products was obtained whose n.m.r. spectrum contained no signals arising from aromatic methyl groups. Several signals, however, were attributable to tert-butyl groups, indicating
that the lithiating agent had been incorporated, presumably by attack on the ester group. The same result was obtained when the reaction was carried out at \(-78^\circ\text{C}\). Alternatively, using LDA in THF at room temperature as the lithiating agent, merely led to the isolation of starting material after a methyl iodide quench. Presumably, while being sufficiently non-nucleophilic to allow the ester to remain intact, LDA is not a strong enough base to effect deprotonation.

The lack of success in the lithiation of (89), and particularly the high reactivity of the ester group towards a relatively non-nucleophilic base, tert-butyllithium, led to the abandonment of attempts to effect a direct amination of (78).

As an alternative to the failed lithiation/amination approach, attempts were made to introduce a nitrogen function into (78) by nitration. Although, in general, indoles react with electrophiles preferentially at C-3, it is well known that nitration of indoles in sulphuric acid often leads to nitration in the carbocyclic ring. Thus nitration of 2-methylindole (90) gives exclusively 2-methyl-5-nitroindole (92). The effect is ascribed to reaction taking place \textit{via} the conjugate acid (91).

![Chemical reaction diagram]
Unfortunately, however, all attempts to nitrate (78) in sulphuric acid led to decomposition of the ring system to give tars. The lack of stability of (78) in strong acid was disappointing, particularly since it has been reported that indoles containing electron withdrawing groups in the 5-membered ring are stabilised towards oxidation, and that substituents at C-2 tend to inhibit acid-catalysed dimerisation. Presumably, therefore, the origin of the instability is the electron rich carbocyclic ring.

Some measure of the electron-rich nature of (78) was gained when a nitration was attempted using copper(II) nitrate trihydrate in trifluoroacetic anhydride.* Using these reagents a product was obtained in 30% yield which was not a nitro compound but appeared to contain a trifluoroacetyl group. Thus the i.r. spectrum contained an additional carbonyl absorbance at 1650 cm$^{-1}$ while the 250 MHz $^1$H n.m.r. spectrum exhibited a quartet ($J$ 1.6 Hz) at δ7.67 (Figure 1).

![Figure 1](image)

---

*This rather unusual nitration medium is discussed more fully in section 1.2.5.
That the trifluoroacetyl group was situated in the carbocyclic ring was indicated by the presence of a broad singlet at δ10.73 and a doublet \((J = 2.5 \text{ Hz})\) at δ7.47 corresponding to the indole N-H and H-3 respectively. The product was finally identified as the 7-trifluoroacetylindole (93) by nuclear Overhauser difference spectroscopy. The n.o.e. difference spectra are reproduced in Figure 2 together with the normal \(^1\text{H}\) spectrum. Thus, pre-irradiation of the N-H signal gave virtually no enhancement of the quartet at δ7.67, while pre-irradiation of the quartet led to an enhancement of the O-methyl protons at C-5. These results, in conjunction with the other spectral data, are consistent only with structure (93).

The exclusive formation of (93) can be rationalised in terms of the relative stabilities of the intermediates (94) and (95) leading to the 6 and 7-substituted products respectively. Of these, (95) is expected to be of lower energy since it retains an aromatic pyrrole ring.

When the reaction was repeated in the absence of copper(II) nitrate trihydrate there was a dramatic increase in the yield. Thus simply stirring (78) in TFAA at room temperature produced a precipitate of (93) in 97% yield.
Although the reaction with TFAA represented a remarkably facile acylation of the indole nucleus, it was of no value in terms of the synthesis of PQQ. Indeed, the preference for electrophilic substitution at C-7 of (78), coupled with the failure to achieve its lithiation, led to the abandonment of the route from 2,3-dimethoxybenzaldehyde in favour of starting materials which already had the nitrogen function in place.

Of the four such substituted benzaldehydes originally considered as starting materials, only (70) and (71) remained.

It was decided to initiate approaches to PQQ from both of these, although for convenience, the results obtained with 4-acetamidobenzaldehyde (70) are described first.
1.2.4 Routes from 4-Acetamidobenzaldehyde

Commercially available 4-acetamidobenzaldehyde (70) was condensed with methyl azidoacetate to give the vinyl azide (96) in 72% yield after recrystallisation.

\[
\begin{align*}
\text{MeO}_2\text{C} & \quad \text{N}_3 \\
\begin{array}{c}
\text{(70)} \\
\text{NaOMe/MeOH}
\end{array} & \quad \begin{array}{c}
\text{MeO}_2\text{C} \\
\text{NHAc}
\end{array} \\
\text{72%} & \quad \text{xylene/\Delta} & \quad \text{80%}
\end{align*}
\]

Although the azide (96) was found to be insoluble in xylene at room temperature, a stirred suspension rapidly became homogeneous when heated at reflux for 1.5 hours. On cooling, pure methyl 6-acetamido-indole-2-carboxylate (97) crystallised from the solution and was collected by filtration in 80% yield.

Having obtained an indole with a nitrogen function at C-6, the next objective was to cleave the acetamido group and then form the tricyclic ring system of PQQ using a Doebner-von Miller type reaction with dimethyl 2-oxoglutaconate. Of course, as noted previously, the absence of a substituent at C-5 in (97) provides an alternative site for cyclisation. It was anticipated, however, on the basis of 'bond fixation' in indoles, that cyclisation would proceed exclusively to C-7 as required. Thus (98), formed by cyclisation to C-7, can benefit from two other canonical forms (99) and (100) in which the pyrrole ring retains its aromaticity while no such canonical forms can be written for the regioisomer (101).
This 'bond-fixation' effect has recently been noted in the Claisen rearrangement of allyloxyindoles. Thus the 6-allyloxyindole (102) rearranges to give, exclusively, the 7-allyl-6-hydroxyindole (103).

The substrate required for the cyclisation (104), was prepared by heating a solution of the acetamidoindole (97) in dry methanol saturated with hydrogen chloride. Under these conditions, amide cleavage proceeded smoothly and the 6-aminooindole (104) was isolated in 97% yield.
Treatment of (104) with dimethyl 2-oxoglutaconate (20) in dichloromethane at room temperature for 16 hours, followed by the addition of a catalytic amount of dry hydrogen chloride gas gave, after aqueous work-up and trituration with hot methanol, the pyrroloquinoline (105) in 89% yield.

That cyclisation had occurred at C-7 was clear from the n.m.r. spectrum of (105) which contained, *inter alia*, an AB quartet at δ8.05 due to H-4 and H-5, and a broad singlet at δ12.52 due to the N-H. Presumably, the very low-field absorbance of the N-H proton reflects a strong, intramolecular hydrogen-bond to the ester carbonyl at C-9.
Gratifyingly, no trace of the alternative cyclisation product (106) could be detected in the n.m.r. spectrum of the crude reaction product.

Having obtained the pyrroloquinoline (105) in four steps and 50% overall yield from 4-acetamidobenzaldehyde, it remained to introduce the $\sigma$-quinone unit. At the outset it was hoped that this might be achieved by direct oxidation of (105), thus relying on there being some degree of 'phenanthrene-like' double-bond character between C-4 and C-5. However, while the synthesis of (105) was in progress, the Hendrickson and de Vries synthesis of PQQ was published. In their route to the coenzyme, a key intermediate was the pyrroloquinoline (36), which differs from (105) only in the nature of the ester group at C-2.
It was claimed that (36) 'showed no olefinic character towards oxidants' although no further details were given. Furthermore, it had not proved possible to functionalise (36) selectively in the central ring since reactions with electrophiles were dominated by electron release from the 'indole'-like nitrogen to C-3. It was clear, therefore, that the oxidation of (105) to give PQQ trimethyl ester (11) would be more difficult than originally expected. Nevertheless, several approaches were explored.

In spite of Hendrickson's results with (36), an attempt was made to oxidise (105) directly using pyridinium fluorochromate (PFC) since this reagent has recently been claimed to be superior to other oxidants in the oxidation of phenanthrene to phenanthrene-9,10-dione. However, heating a solution of (105) and PFC in glacial acetic acid at reflux led only to the slow decomposition of (105).

While direct oxidation of the pyrroloquinoline (105) was unsuccessful, it seemed likely that the indoline (109) would be more amenable, since it is known that a variety of indolines are oxidised to give the corresponding indole 4,5-quinones on treatment with Fremy's salt (potassium nitrosodisulphonate). For example, the hexahydrocarbazole (107) gives rise to the indole-4,5-quinone (108) in 63% yield.
Unfortunately, all attempts to reduce (105) to give the indoline (109) were unsuccessful. Although a number of reagents, known to reduce indole itself, were tried (Table 2), all merely resulted in varying amounts of decomposition of the starting material.

Table 2. Attempted Reduction of the Pyrroloquinoline (105)

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Reference</th>
<th>Reference</th>
<th>s.m. recovered in 84% yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>H₃B·NMe₃/HCl/Δ</td>
<td>67</td>
<td></td>
<td>s.m. recovered in 84% yield</td>
</tr>
<tr>
<td>H₃B·THF/TFA</td>
<td>68</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>PtO₂/H₂/HBF₄/EtOH</td>
<td>69</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>Zn/H₃PO₄/Δ</td>
<td>70</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>NaBH₃CN/AcOH/Δ</td>
<td>71</td>
<td>&quot;</td>
<td>No isolable products</td>
</tr>
<tr>
<td>Na/liq.NH₃</td>
<td>72</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
</tbody>
</table>

It was concluded that, if any reduction of (105) had occurred, either the product was unstable, or it underwent a spontaneous and rapid oxidation to give the starting material on attempted isolation. In any event, no trace of (109) was detected in the crude mixtures obtained with any of the reagents shown in Table 2.
A successful reduction of (105) was finally achieved using the 'cuprous hydride' reagent described by Semmelhack.\textsuperscript{73} The product was not, however, the indoline (109), but was instead, the 6,9-dihydropyrroloquinoline (110).

The structure assignment of (110) was based on its \textsuperscript{1}H n.m.r. spectrum. Most striking was the >3 ppm upfield shift of the 'indole' N-H proton to δ9.29 from δ12.52 in the starting material, thereby indicating that it was no longer hydrogen-bonded to the ester carbonyl at C-9. In addition, a broad singlet at δ6.63 was present, together with a double doublet (\(\textit{J} 4\) Hz, \(\alpha\). 0.5 Hz) at δ5.76 and a doublet (\(\textit{J} 4\) Hz) at δ5.02. These signals were assigned to H-6, H-8 and H-9 respectively, as shown below.
As expected, decoupling the broad singlet at $\delta 6.63$ caused the signal at $\delta 5.76$ to collapse to a doublet ($\sim 4$ Hz).

The reduction product (110) underwent rapid aerial oxidation, such that a sample could not be obtained totally free from the fully aromatic pyrroloquinoline (105). Indeed, bubbling air through a dichloromethane solution of (110) for 12 hours was sufficient to effect quantitative reversion to the starting material.

Interestingly, no trace of the 6,7-dihydropyrroloquinoline (111) could be detected in the crude reaction mixture.

![Chemical Structure](image)

In retrospect, the formation of a 6,9- rather than a 2,3-dihydropyrroloquinoline in the reduction of (105) is not surprising, since the pyridine ring is the more electron deficient of the two heterocyclic rings.

Another, less direct, approach to the conversion of (105) into PQQ trimethyl ester (11) involved an attempt to alter the intrinsic reactivity of the pyrroloquinoline ring system. First, however, the reactivity of (105) with electrophiles was tested by treatment of a sulphuric acid solution with sodium nitrate (1 equiv.). In confirmation of Hendrickson's results with (36), the product obtained, in 82% yield, was the 3-nitropyroloquinoline (112).
It was hoped that, by preparing the $N$-oxide (113), it would be possible to activate C-4 to electrophilic attack such that a substituent could be introduced selectively into the central ring, thereby providing a handle for subsequent oxidation without the attendant difficulties encountered by Hendrickson, of removing an unwanted substituent at C-3.

Alternatively, it might be possible with acylated derivatives, to transfer functionality from oxygen to C-5.
Although the pyrroloquinoline (105) did not react with \( m \)-chloroperbenzoic acid\(^7^4 \) or pertrifluoroacetic acid,\(^7^5 \) the \( N \)-oxide (113) was prepared in 68% yield by reaction with dichloropermaleic acid.\(^7^6 \)

\[
\text{MeO}_2\text{C} \begin{array}{c} \text{Cl} \\ \text{Cl} \end{array} \text{CO}_2\text{Me} \begin{array}{c} \text{MeO}_2\text{C} \\ \text{CO}_2\text{Me} \end{array} \\
\text{MeO}_2\text{C} \begin{array}{c} \text{Cl} \\ \text{Cl} \end{array} \text{CO}_2\text{Me} \begin{array}{c} \text{MeO}_2\text{C} \\ \text{CO}_2\text{Me} \end{array}
\]

(105) \quad \overset{90\% \text{H}_2\text{O}_2/\text{CH}_2\text{Cl}_2}{\text{68\%}} \quad (113)

Treatment of a sulphuric acid solution of (113) with sodium nitrate (1 equiv.) gave, in 61% yield, the 3-nitro-\( N \)-oxide (114). None of the desired 4-nitro isomer could be detected.

\[
\text{(113)} \begin{array}{c} \text{NaNO}_3 \\ \text{cH}_2\text{SO}_4/0^\circ\text{C} \end{array} \begin{array}{c} \text{MeO}_2\text{C} \\ \text{CO}_2\text{Me} \end{array} \\
\text{MeO}_2\text{C} \begin{array}{c} \text{Cl} \\ \text{Cl} \end{array} \text{CO}_2\text{Me} \begin{array}{c} \text{MeO}_2\text{C} \\ \text{CO}_2\text{Me} \end{array}
\]

(114)

It appears, therefore, that the contribution of canonical form (115) to the \( N \)-oxide resonance hybrid is insufficient to compete with the powerful electron release from the 'indole'-nitrogen to C-3.

Attempts to acylate the \( N \)-oxide (113) were also unsuccessful. No reaction occurred with acetyl or tosyl chloride in chloroform at reflux, while at higher temperatures (113) was prone to thermal deoxygenation. Thus after heating in boiling acetic anhydride or dimethyl-
formamide for 9 hours, the pyrroloquinoline (105) was isolated in 57% and 42% yields respectively.

In each case the balance of the material consisted of tarry, decomposition products.

At this point, with the failure of the $N$-oxide (113) to undergo useful reactions, the results obtained with a different route (vide infra) began to show promise and so no further attempts were made to convert (105) into PQQ trimethyl ester (11).

In conclusion, therefore, the pyrroloquinoline (105) was prepared in 4 steps and 50% overall yield from 4-acetamidobenzaldehyde (70). In terms of directness and efficiency, this represents a considerable improvement on the Hendrickson synthesis, in which the pyrroloquinoline (36) was prepared in 7 steps and less than 23% overall yield.

Unfortunately, however, no short and efficient method was found to introduce the $\sigma$-quinone unit into (105) and so the route developed by Hendrickson, involving a double nitration at C-3 and C-5, remains the only method available to convert (36), and presumably therefore (105) also, into PQQ.

1.2.5 Routes from 4-Aminosalicylic Acid

While the route to PQQ from 4-acetamidobenzaldehyde was under investigation, work started on a similar approach employing 4-acetamido-2-
methoxybenzaldehyde (71) as the starting material.

It was hoped that the added methoxy group would provide a convenient handle with which to introduce the o-quinone unit late in the synthesis.

Specifically, it was anticipated that the pyrroloquinoline (116) would be oxidised to give PQQ trimethyl ester under the conditions described by Corey for the oxidation of (17). \(^\text{19}\)

Unlike the aldehydes used previously, (71) was not available commercially. Furthermore, a literature search revealed that (71) had previously only been prepared in low yield from 2-methoxy-4-nitrotoluene (117) as shown in Scheme 19. \(^\text{77}\) The first objective, therefore, was to develop a synthesis of (71) which was amenable to large-scale preparations.
Scheme 19

The most convenient, commercially available starting material appeared to be 4-aminosalicylic acid (118), particularly since its conversion into the ester (119) was well documented. The eventual route used to convert (118) into the required aldehyde (71) was straightforward, and is shown in Scheme 20.
Thus 4-aminosalicylic acid (118) was first converted, using the literature method, into the ester (119). Initial attempts to reduce (119) to give the required aldehyde (71) directly, with DIBAL-H, led to complex mixtures of products and so recourse was made to a high yielding, two-step procedure. Reduction of the ester (119) with LAH gave the benzyl alcohol (120), which was oxidised cleanly to give the aldehyde (71) with activated manganese (IV) oxide. Thus, as required, (71) was made available in high overall yield (63%) from commercially available material. Furthermore, the sequence was well suited to multi-gram scale since no purification was required prior to recrystallisation of the aldehyde (71).

With the required aldehyde now readily available, attention was focused on its conversion into the pyrroloquinoline (116). The route used was essentially identical to that described previously to prepare the 'unfunctionalised' pyrroloquinoline (105), and is shown in Scheme 21. The two step condensation-thermolysis sequence again proceeded smoothly and in high yield (64%), such that crystals of the indole (122) were collected by simple filtration from the thermolysis solvent. Acid cleavage of the acetamido group in (122) gave rise to the 6-aminoundole (123) which, surprisingly, appeared to be somewhat prone to oxidation. Thus, the crude amine, obtained as a yellow solid, rapidly became dark red on exposure to the air. It was found most convenient, therefore, to use the crude aminoindole (123) immediately, in the next step. Treatment of (123) with dimethyl 2-oxoglutaconate (20) under the conditions described previously gave, after trituration with hot methanol, the 4-methoxypyrroloquinoline (116) in 62% yield from (122). As expected, there was no trace of the alternative cyclisation product (124).
Scheme 21

\[
\text{MeO}_2\text{C} - \text{C} = \text{C} - \text{CO}_2\text{Me} \quad \text{(20)}
\]

then $H^+$

\[
\text{MeO}_2\text{C} - \text{C} = \text{C} - \text{CO}_2\text{Me} \quad \text{(20)}
\]

\[
\begin{align*}
\text{MeO}_2\text{C} - \text{C} = \text{C} - \text{CO}_2\text{Me} \\
\text{MeO} \\
\text{N}
\end{align*}
\]

\[
\text{MeO}_2\text{C} - \text{C} = \text{C} - \text{CO}_2\text{Me} \quad \text{(20)}
\]

\[
\begin{align*}
\text{MeO}_2\text{C} - \text{C} = \text{C} - \text{CO}_2\text{Me} \\
\text{MeO} \\
\text{N}
\end{align*}
\]

\[
\begin{align*}
\text{MeO}_2\text{C} - \text{C} = \text{C} - \text{CO}_2\text{Me} \\
\text{MeO} \\
\text{N}
\end{align*}
\]

\[
\begin{align*}
\text{MeO}_2\text{C} - \text{C} = \text{C} - \text{CO}_2\text{Me} \\
\text{MeO} \\
\text{N}
\end{align*}
\]

\[
\begin{align*}
\text{MeO}_2\text{C} - \text{C} = \text{C} - \text{CO}_2\text{Me} \\
\text{MeO} \\
\text{N}
\end{align*}
\]

\[
\begin{align*}
\text{MeO}_2\text{C} - \text{C} = \text{C} - \text{CO}_2\text{Me} \\
\text{MeO} \\
\text{N}
\end{align*}
\]

\[
\begin{align*}
\text{MeO}_2\text{C} - \text{C} = \text{C} - \text{CO}_2\text{Me} \\
\text{MeO} \\
\text{N}
\end{align*}
\]

\[
\begin{align*}
\text{MeO}_2\text{C} - \text{C} = \text{C} - \text{CO}_2\text{Me} \\
\text{MeO} \\
\text{N}
\end{align*}
\]

\[
\begin{align*}
\text{MeO}_2\text{C} - \text{C} = \text{C} - \text{CO}_2\text{Me} \\
\text{MeO} \\
\text{N}
\end{align*}
\]

\[
\begin{align*}
\text{MeO}_2\text{C} - \text{C} = \text{C} - \text{CO}_2\text{Me} \\
\text{MeO} \\
\text{N}
\end{align*}
\]

\[
\begin{align*}
\text{MeO}_2\text{C} - \text{C} = \text{C} - \text{CO}_2\text{Me} \\
\text{MeO} \\
\text{N}
\end{align*}
\]

\[
\begin{align*}
\text{MeO}_2\text{C} - \text{C} = \text{C} - \text{CO}_2\text{Me} \\
\text{MeO} \\
\text{N}
\end{align*}
\]

\[
\begin{align*}
\text{MeO}_2\text{C} - \text{C} = \text{C} - \text{CO}_2\text{Me} \\
\text{MeO} \\
\text{N}
\end{align*}
\]

\[
\begin{align*}
\text{MeO}_2\text{C} - \text{C} = \text{C} - \text{CO}_2\text{Me} \\
\text{MeO} \\
\text{N}
\end{align*}
\]
As discussed previously, Corey has reported the facile oxidation of (17) to give PQQ trimethyl ester on treatment with cerium (IV) ammonium nitrate (CAN), and it seemed reasonable, therefore, that the same reagent might be employed successfully with the 4-methoxy isomer (116). However, when (116) was subjected to the conditions described by Corey for the oxidation of (17), no reaction took place.

Using a larger excess of CAN (15 equiv.) and heating the reaction failed to induce (116) to oxidise, even after six hours at reflux. This surprising difference in reactivity between the 4- and 5-methoxy-pyrroloquinolines (116) and (17) towards CAN is difficult to rationalise, particularly since the mechanism of oxidative demethylation by CAN is not known in detail. However, it has been shown by labelling studies on simple 1,4-dimethoxybenzene derivatives, that the reaction proceeds by aryl-oxygen bond cleavage (Scheme 22). Based on these results, a tentative mechanism can be proposed for the oxidation of the Corey intermediate (17), and is shown in Scheme 23. Thus, initial oxidative addition of water to (17) is followed by loss of methanol from the resulting hemiketal (125) to give the \( p \)-quinone imine (126). Addition of water to (126) gives the quinol (127) which is then further oxidised to give the product. If an intermediate such as the \( p \)-quinone imine (126) were to be involved in the oxidation of the Corey compound (17), then by inspecting the equivalent, hypothetical intermediate (128) in
the oxidation of (116), some rationalisation of the reluctance of the latter to undergo oxidation would be possible.

Thus, while (126) is a relatively low energy quinoline \( p \)-quinone imine in which the 'pyridine' ring retains its aromaticity, the extended \( o \)-quinone methide (128) would be expected to be a much higher energy intermediate. While the above is, of course, mere speculation, it is clear that the Corey intermediate (17) was not, after all, a good model for its 4-methoxy isomer (116).

Although CAN is probably the most commonly used reagent to effect oxidative demethylation of methoxyarenes, several other reagent systems are available. However, the 4-methoxypyrroloquinoline (116) did not react with nitric acid, nitrous acid, silver (II) oxide, or manganese (IV) oxide impregnated with nitric acid, and no further attempts at direct oxidation of (116) were made.

The refusal of (116) to undergo direct oxidation was disappointing. However, it was felt that the methoxy group at C-4 in (116) could still be the key factor in developing an alternative, indirect route to PQQ trimethyl ester. In particular, electron release from the methoxy group might allow C-5 to compete successfully with C-3 as the preferred site of electrophilic attack. Therefore, a study of the nitration of (116) was carried out using a range of reagents. The results are
summarised in Table 3.

Table 3. Nitration of the Pyrroloquinoline (116)

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Reference</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaN(_2)O(_3) (1 equiv.)/H(_2)SO(_4)</td>
<td>61</td>
<td>(130), 100%</td>
</tr>
<tr>
<td>fHNO(_3) (excess)/H(_2)SO(_4)</td>
<td>84</td>
<td>(131), 97%</td>
</tr>
<tr>
<td>fHNO(_3)/Ac(_2)O</td>
<td>21</td>
<td>s.m. recovered</td>
</tr>
<tr>
<td>C(NO(_2))(_4)/py</td>
<td>85</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>+ NO(_2)BF(_4)/CH(_2)Cl(_2)</td>
<td>86</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>+ NO(_2)OTf/CH(_2)Cl(_2)</td>
<td>87</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>AgNO(_3)/CH(_3)CN</td>
<td>88</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>N-nitropyrazole/BF(_3)·OEt(_2)</td>
<td>88</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>Cu(NO(_3))(_2)·3H(_2)O/AC(_2)O</td>
<td>89</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>Cu(NO(_3))(_2)·3H(_2)O/TFA</td>
<td>-</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>Cu(NO(_3))(_2)·3H(_2)O/TFAA</td>
<td>90</td>
<td>(129), 25-30% + (130), ca. 32-38% + (131), 8-13% at ca. 84-100% conversion.</td>
</tr>
<tr>
<td>NH(_4)NO(_3)/TFAA</td>
<td>90</td>
<td>(129), 41% + (130), ca. 28% + (131), 22% at ca. 94% conversion.</td>
</tr>
</tbody>
</table>
The initial results were not encouraging. Using an excess of nitrating mixture, a high yield of the 3,5-dinitro derivative (131) was obtained, while one equivalent of sodium nitrate in sulphuric acid gave the 3-nitropyrrroloquinoline (130) quantitatively.* Surprisingly, (116) did not react with the majority of the reagents tried, although in some cases this may simply reflect the low solubility of (116) in the reaction medium. This problem was particularly severe with copper (II) nitrate trihydrate in acetic anhydride, and so an attempt was made to increase the amount of substrate in solution by changing the solvent to the more polar trifluoroacetic anhydride (TFAA). The resulting change was remarkable. Not only did a reaction take place, but for the first time, a mixture of products was obtained which, in addition to some starting material (ca. 16% recovery), contained the three nitropyrrroloquinolines (129)-(131) in a ratio of ca. 3:4:1. The crude mixture could be partially separated by multiple-elution p.l.c. on silica gel to give the pure 5-nitro and 3,5-dinitro derivatives (129) and (131) in 30% and 13% yields respectively. It was not possible, however, to separate the 3-nitro derivative (130) from the unreacted starting material, and so the amount of (130) present could only be gauged approximately, by integration of the singlet arising from H-8 in the n.m.r. spectrum of the mixture.

Attempts to increase the proportion of the desired 5-nitropyrrroloquinoline (129) in the mixture by varying the reaction conditions were largely unsuccessful. Indeed, although the yields varied within the limits shown in the Table, the ratio of the three products remained roughly constant. In addition, the 3-nitro compound (130) was not possible, however, to separate the 3-nitro derivative (130) from the unreacted starting material, and so the amount of (130) present could only be gauged approximately, by integration of the singlet arising from H-8 in the n.m.r. spectrum of the mixture.

*The structure assignments of (129)-(131) were trivial on inspection of their n.m.r. spectra, since in the n.m.r. of (116), H-3 is a doublet ($\nu 1 \text{ Hz}$) while H-5 is a sharp singlet.
was the major product in every case, and there was always approximately equal amounts of the 3,5-dinitro compound (131) and unreacted starting material present in the crude mixtures.

At this point, a literature search revealed that the nitration of aromatics by inorganic nitrate salts in TFAA had recently been studied by Crivello. He found that, although metal nitrate salts could be used, the best reagent was ammonium nitrate due to its greater solubility in the reaction medium. Therefore, the nitration of (116) was repeated using ammonium nitrate to give, as before, a mixture of the nitro compounds (129)- (131) from which the desired 5-nitro derivative (129) was isolated in 41% yield at ca. 94% conversion.

While the increase in the yield of (129) was most welcome, it was hard to rationalise on mechanistic grounds. Crivello has suggested that nitraations in TFAA proceed by initial formation of trifluoroacetyl nitrate (eq. 1). This can undergo heterolytic cleavage of the oxygen-nitrogen bond to generate the nitronium ion (eq. 2) which is assumed to be the actual nitrating species (eq. 3). The overall process, therefore, can be written as in eq. 4.

\[
\begin{align*}
(CF_3CO)_2O + MN_3 & \rightleftharpoons CF_3CO_2NO_2 + CF_3CO_2M \quad (1) \\
CF_3CO_2NO_2 & \rightleftharpoons CF_3CO^- + NO_2^+ \quad (2) \\
CF_3CO^- + NO_2^- + ArH & \rightarrow ArNO_2 + CF_3CO_2H \quad (3)
\end{align*}
\]

\[
(CF_3CO)_2O + MN_3 + ArH \rightarrow ArNO_2 + CF_3CO_2H + CF_3CO_2M \quad (4)
\]

\[M = NH_4, K, Na \text{ etc.}\]

The mechanism is a reasonable one and is analogous to that of nitraations involving acetyl nitrate. It does not, however, provide an explanation
for the increase in the yield of (129) on changing the nitrate counter-ion from copper(II) to ammonium ion. On the contrary, the generation of the nitronium ion is dependent only on a source of nitrate and should, in principle at least, be independent of the inorganic cation present. Furthermore, such a mechanism does not explain the different results obtained with sodium nitrate in sulphuric acid where the effective nitrating agent is also the nitronium ion. It was thought initially, that the formation of (129) in TFAA might be due to prior acylation of the 5-membered ring nitrogen, thereby drastically diminishing electron release to C-3. Subsequent nitration at C-5, followed by cleavage of the reactive N-trifluoroacetyl grouping on alkaline work-up would give (129). However, this was ruled out by treatment of the 'unsubstituted' pyrroloquinoline (105) with copper (II) nitrate trihydrate in TFAA. The only product, in 66% yield, was the 3-nitro derivative (112), and no starting material was recovered.

\[ \text{MeO}_2\text{C} \quad \text{MeO}_2\text{C} \]
\[ \text{NH} \quad \text{NH} \]
\[ \text{CO}_2\text{Me} \quad \text{CO}_2\text{Me} \]
\[ \text{Cu(NO}_3\text{)}_2 \cdot 3\text{H}_2\text{O} \quad \text{Cu(NO}_3\text{)}_2 \cdot 3\text{H}_2\text{O} \]
\[ \text{TFAA} \quad \text{TFAA} \]
\[ 66\% \quad 66\% \]
\[ \text{(105)} \quad \text{(112)} \]

It seems more likely, that the explanation of the different results obtained in TFAA and sulphuric acid lies in the exact nature of the species undergoing attack in these solvents. The yellow pyrroloquinoline (116), in common with all the other pyrroloquinolines prepared in this work, dissolves readily in sulphuric acid to give a dark red solution. Presumably, therefore, in this solvent the species attacked by the nitronium ion is the protonated pyrroloquinoline (132),
to which the canonical forms (132a) and (132b) will be the major contributors.

A much smaller contribution to the resonance hybrid (132) is expected from the canonical form (132c) in which the aromaticity of all three rings is lost. Thus protonation of (116) effectively reduces electron density at C-5 to a greater extent than at C-3, such that nitration takes place at the latter. Alternatively, in the essentially neutral TFAA solution, electron release from the methoxy group causes the reactivity of C-3 and C-5 to be finely balanced, with the result that mixtures of products are formed. Presumably, the formation of the dinitro compound (131) as part of the mixture indicates that the initially formed mono-nitro compounds (129) and (130), undergo further nitration at a comparable rate to the starting pyrroloquinoline (116).

Aside from mechanistic arguments, the formation of the 5-nitropyroloquinoline (129) proved to be the key step in the conversion of (116) to PQQ trimethyl ester. Indeed, the presence of a heteroatom
substituent at C-5 made the task of introducing the o-quinone unit straightforward. Thus, catalytic hydrogenation of the bright yellow 5-nitropyrrroloquinoline (129) gave the dark purple amino compound (133). Oxidation of (133) with manganese (IV) oxide in 35% sulphuric acid at 0°C for 45 minutes proceeded smoothly to give PQQ trimethyl ester (11) as a bright orange solid in 68% yield for the two steps.

In addition to correct microanalytical data and melting point, the n.m.r., i.r., u.v. and mass spectral data obtained for (11) were identical to those reported by Corey, and Weinreb in their syntheses of PQQ, and by Duine for PQQ trimethyl ester derived from natural material.

Although the conversion of (116) into PQQ trimethyl ester (11) formally completed the synthesis of the coenzyme, the overall yield for the 3-step sequence was disappointingly low (28%). In addition, the original objective of a synthesis which required no chromatography, and so was amenable to large-scale preparations had not been achieved. It was decided, therefore, to seek an alternative method of introducing the o-quinone unit into (116).
Although the 4-methoxypyrroloquinoline (116) could not be oxidised directly to give PQQ trimethyl ester (vide supra), it seemed likely that the corresponding phenol (134) would be oxidised more readily.

Attempts were made, therefore, to prepare the phenol (134) by demethylation of (116) (Table 4).

Table 4. Demethylation of the Pyrroloquinoline (116)

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Reference</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>BBr₃(6 equiv.)/CH₂Cl₂/r.t.</td>
<td>92</td>
<td>s.m. recovered</td>
</tr>
<tr>
<td>BBr₃(15 equiv.)/CH₂Cl₂/r.t.</td>
<td>92</td>
<td>(134), 21%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>then MeOH/H⁺/Δ</td>
</tr>
<tr>
<td>TMSI/CHCl₃/Δ</td>
<td>93</td>
<td>s.m. recovered</td>
</tr>
<tr>
<td>SiCl₄/NaI/CH₂Cl₂:CH₃CN, 1:1</td>
<td>94</td>
<td>&quot;</td>
</tr>
<tr>
<td>AlCl₃/xylene/Δ</td>
<td>95</td>
<td>trace of (134)</td>
</tr>
<tr>
<td>LiI/collidine/Δ</td>
<td>96</td>
<td>Decomposition</td>
</tr>
<tr>
<td>HBr/AcOH/Δ</td>
<td>97</td>
<td>&quot;</td>
</tr>
</tbody>
</table>

Frustratingly, most of the reagents tried either did not react with (116) or caused its decomposition. However, after considerable experimentation, it was found that demethylation could be achieved by treatment of (116) with boron tribromide (15 equiv.) in dichloromethane at room temperature for 6 days. Not surprisingly, these relatively fierce conditions also led to a considerable amount of decomposition, as well as ester cleavage, such that the crude reaction mixture had to
be re-esterified prior to isolation of the phenol (134). However, by this method, the optimised yield of (134) was only 21%.

The reason for the reluctance of (116) to undergo ether cleavage is not clear, although it seems likely that, with Lewis acid based reagents at least, the large number of potentially basic sites in (116) plays an important part. Whatever the reason, the yield obtained in the demethylation of (116) was too low to compete with the nitration approach described earlier, and so an alternative route to the phenol (134) was sought.

The most direct approach to the synthesis of (134) would proceed from the hydroxyaldehyde (135).

Unfortunately, however, o-hydroxybenzaldehydes are known not to undergo base-catalysed condensation with methyl azidoacetate. The effect is attributed to deactivation of the aldehyde by formation of the resonance stabilised phenoxide ion (136) as well as the usually poor solubility of (136) in the reaction medium.
What was required, therefore, was a base-stable protecting group which could be removed cleanly and in high yield at the pyrroloquinoline level without affecting the ester groups. The ideal choice appeared to be the benzyl group, particularly since it was already known from the reduction of the 5-nitropyrroloquinoline (129) that the tricyclic ring system was stable under the conditions of catalytic hydrogenolysis.

The required benzyleoxyaldehyde (140) was prepared in three steps from the phenol (137) as shown in Scheme 24.

Thus, alkylation of (137) with benzyl bromide gave the ester (138) which was reduced with LAH to give the benzyl alcohol (139). Oxidation of the alcohol proceeded quantitatively, using barium manganate in chloroform at reflux, to give the aldehyde (140). The overall yield for the three step sequence was 76%.
Initial attempts to condense the aldehyde (140) with methyl azidoacetate under the standard conditions, led to only partial reaction, and the resulting azide/aldehyde mixture proved inseparable by medium-pressure chromatography on silica-gel. Fortunately, however, thermolysis of the crude mixture gave an easily separable mixture of the indole (141) and unchanged aldehyde (140). Using this method, the yield of (141) was 88% at 59% conversion.

Alternatively, and more conveniently, it was found that, by standing the condensation reaction mixture at 2°C overnight, the reaction could be taken to completion. With this modification, the yield of the vinyl azide (142) was 90%.

Thermolysis of the pure azide (142) proceeded cleanly to give, after isolation by filtration and trituration with hot petroleum ether (b.p. 60-80°C), the 4-benzyloxyindole (141) in 82% yield. Cleavage of the
amide group in (141) was straightforward. Thus, heating a solution of (141) in methanolic HCl at reflux for 1-2.5 hours, gave the 6-amino-indole (143) as a tan solid in 58% yield. Interestingly, (143) appeared to be less susceptible to aerial oxidation than its methoxy analogue (123).

Treatment of the amine (143) with dimethyl 2-oxoglutaconate (20) gave, after acid-catalysed dehydration of the initially formed piperidinol and aromatisation, the 4-benzyloxyprroloquinoline (144) in 95% yield. As with the previous cyclisations of this sort, no regioisomeric pyrroloquinoline could be detected in the crude reaction mixture. Gratifyingly, removal of the benzyl protecting group by catalytic hydrogenolysis proved to be straightforward and high yielding. Thus, shaking a suspension of the pyrroloquinoline (144) and 10% palladium on carbon in methanol for 20 hours under 1 atm. of hydrogen, gave the bright yellow phenol (134) in 89% yield. With the phenol (134) available in quantity (36 or 43% from the aldehyde (140)), attention was turned to its oxidation to give PQQ trimethyl ester (11).
As expected, (134) did not react with cerium (IV) ammonium nitrate, a finding which was consistent with the explanation proposed earlier for the lack of reactivity of the 4-methoxypyrrroloquinoline (116), and which emphasised the need for a mechanistically different oxidant.

Fremy's salt (potassium nitrosodisulphonate) has been used extensively in the oxidation of phenols to give $\sigma$- and $\pi$-quinones, and the mechanism of the reaction has been studied in detail (Scheme 25).\textsuperscript{65,100}
Thus, in the formation of o-quinones, initial hydrogen abstraction from the phenol gives a resonance stabilised phenoxy radical which then couples with a second molecule of Fremy's salt. Subsequent loss of the elements of dipotassium imidobisulphate gives the product. This mechanism appeared to be ideally suited to the oxidation of (134) since, in principle, there need be no disruption of the aromaticity of the terminal rings. Indeed, treatment of the phenol (134) with an excess of freshly prepared Fremy's salt in aqueous potassium dihydrogen phosphate buffer solution did give rise to PQQ trimethyl ester (11). However, the yield was a meagre 20% at 63% conversion.

Although the yield of (11) by this method was poor, it seemed likely that this did not reflect a lack of reactivity, but was simply due to mechanical problems associated with the insolubility of the phenol (134) in the reaction medium. To obviate this problem the preparation of an ester of Fremy's salt was considered. However, a literature search revealed that acyl nitroxides are often useful as organic soluble versions of Fremy's salt. In particular, benzoyl tert-butyl nitroxide (145) has been used by Perkins to oxidise a number of monohydradic phenols to give quinones in moderate to good yields.\textsuperscript{101}

\[
\begin{array}{c}
\text{N} \\
\text{O} \\
\text{Ph}
\end{array}
\]

(145)

Therefore, a solution of the phenol (134) in dichloromethane-methanol (9:1) was treated with an excess of the nitroxide (145) at room temperature.\textsuperscript{102} After 16 hours, removal of the solvent and trituration with ether to remove excess of reagent, left pure PQQ trimethyl ester (11) in 93% yield.
Thus (11) was made available from the 4-benzyloxypyrroloquinoline (144) in two steps and 83% yield.

Finally, a sample of PQQ trimethyl ester (11) was converted into PQQ itself using the method described by Corey.\(^\text{19}\)

The resulting synthetic coenzyme had an identical u.v. spectrum to that of an authentic sample.\(^\text{103}\) In addition, a mixture of synthetic and natural PQQ was eluted as a single spot on cellulose t.l.c. (2% aq. ammonium acetate-propanol, 1:1).

1.2.6 Conclusion

The route to indole-2-carboxylates by thermolysis of benzaldehyde-derived vinyl azides, has been successfully applied to the synthesis of coenzyme PQQ. A total of four, substituted benzaldehydes were converted...
into indoles as potential precursors to PQQ and, in every case, the two-step procedure was clean and high-yielding. Although two of the approaches were ultimately unsuccessful, the benzyloxybenzaldehyde (140) was converted into PQQ trimethyl ester (11) in six steps and 40% or 34% overall yield. A summary of the complete synthesis of PQQ trimethyl ester is shown in Scheme 26.

\[
\begin{align*}
\text{HO}_2\text{C} & \overset{5 \text{ steps}}{\longrightarrow} \text{OHC} & \overset{2 \text{ steps}}{\longrightarrow} \\
\text{HO} & \text{NH}_2 & \text{NHAc} & \\
(118) & & (140) & \\
67\% & & 74\% & \\
\end{align*}
\]

\[
\begin{align*}
\text{MeO}_2\text{C} & \overset{2 \text{ steps}}{\longrightarrow} \\
\text{NH} & \text{BzO} & \text{NHAc} & \text{CO}_2\text{Me} & \text{CO}_2\text{Me} \\
\text{BzO} & \text{NHAc} & (141) & (144) & \\
55\% & & 83\% & & \\
\end{align*}
\]

Thus the original objective of developing a short and efficient synthesis has been achieved. In addition, the sequence is amenable to large-scale preparations and is currently being used to prepare PQQ for degradative studies and isotope dilution experiments as part of an
investigation of the biosynthesis of the coenzyme. Finally, the phenol (134) and the 5-amino-4-hydroxypyrroloquinoline (146) (prepared by a modified route involving nitration of the benzyloxypyrroloquinoline (144) and catalytic reduction) are being used in a study of the role of ammonia as an activator in quinoproteins.
PART 2

Synthesis of Isoquinolines by Intramolecular Aza-Wittig Reaction
2.1 Introduction

Interest in the synthesis of isoquinolines dates from the isolation of isoquinoline (147) itself from coal tar in 1885. Although the parent compound is not found free in nature, compounds containing the isoquinoline nucleus are widely distributed in plants. Greater than 1000 isoquinoline alkaloids, including many which are physiologically active, have now been identified. As a result of the historical and continuing interest in alkaloid synthesis and biosynthesis, many methods are now available for the preparation of the isoquinoline ring system and these have recently been the subject of a comprehensive review. The most widely used methods remain, however, the classical Bischler-Napieralski, Pictet-Spengler and Pomeranz-Fritsch reactions. While these have been successful in the preparation of a wide variety of isoquinolines, they all require relatively harsh acid or dehydrating conditions, thereby limiting the number of compatible functional groups. Furthermore, since they all ultimately involve electrophilic attack on a benzene ring, the reaction is greatly facilitated by the presence of electron-donating substituents in the ring. Indeed, in the absence of such activating substituents, ring closure often fails completely or, at best proceeds in low yield under forcing conditions. Thus Table 5 contains some examples of simple systems which do not give any cyclisation products under the classical conditions.
Surprisingly, few attempts to solve the 'activation' problem in isoquinoline synthesis have been reported. Recently, however, the methylthio group has been proposed as an easily removable activating group which allows the preparation of isoquinolines lacking substituents in the carbocyclic ring.\textsuperscript{112} For example, the Schiff base (148) cyclised readily on treatment with trifluoroacetic acid to give the 1,2,3,4-tetrahydroisoquinoline (149) in 75% yield. The methylthio group is then removed by desulphurisation with nickel boride.\textsuperscript{116}

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{HCl/}\Delta$</td>
<td>112,113</td>
</tr>
<tr>
<td>$\text{H}_2\text{SO}_4/\Delta$</td>
<td>112,115</td>
</tr>
<tr>
<td>$\text{HCl}$</td>
<td>112,110</td>
</tr>
</tbody>
</table>

An alternative approach is to employ a ring closure step which does not involve electrophilic attack on a benzene ring. One such
method has been developed independently by Woodward\textsuperscript{117} and Miller\textsuperscript{118} whereby an indene (150) is converted into a homophthalaldehyde derivative (151). Subsequent reaction with ammonia then gives the fully aromatic isoquinoline (152).

\[
\begin{align*}
(150) & \xrightarrow{O_3 \text{ or } OsO_4/NaIO_4} (151) \xrightarrow{NH_2OH} (152)
\end{align*}
\]

The approach is an elegant one which can be used to prepare isoquinolines containing electron-withdrawing groups, but which unfortunately, is somewhat limited by the availability of the indene derivatives (150).

It is clear therefore, that while interest in isoquinoline synthesis is continuing\textsuperscript{119}, there remains considerable scope for improvement. In particular, a method which utilises readily available starting materials and in which the ring closure step is independent of activating substituents, but nevertheless proceeds under mild, ideally neutral conditions would be of considerable value.

2.2 Results and Discussion

2.2.1 Introduction

As part of a study of the chemistry of azidocinnamates, Hickey observed that treatment of azidocinnamates (153) containing an \(\sigma\)-carbonyl group with triethyl phosphite, gave rise to 1,3-disubstituted isoquinolines (155) in high yield (Scheme 27).\textsuperscript{51}
The reaction is thought to proceed by initial Staudinger reaction\textsuperscript{120} to give an iminophosphorane (154) which then undergoes a spontaneous intramolecular aza-Wittig reaction to give the observed product (155) with loss of triethyl phosphate. Although the intramolecular aza-Wittig reaction has been used in the synthesis of a number of heterocyclics (Table 6), it had not previously been used to prepare isoquinolines. The fact that cyclisation proceeded readily, not only with a ketonic carbonyl group, but also with the less reactive carboxylic acid and ester carbonyls, suggested that the ring forming aza-Wittig reaction was particularly favourable in these systems. However, the generality of the reaction, and in particular, its application to the synthesis of 1-alkyl isoquinolines remained to be demonstrated. It was decided, therefore, to investigate the scope and limitations of the new isoquinoline synthesis.

\[
\begin{array}{c}
\text{R}
\end{array}
\]

<table>
<thead>
<tr>
<th>R</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Ph</td>
<td>94</td>
</tr>
<tr>
<td>(b) OEt</td>
<td>90</td>
</tr>
<tr>
<td>(c) OH</td>
<td>74</td>
</tr>
</tbody>
</table>

\textbf{Scheme 27}
Table 6 Examples of the Intramolecular Aza-Wittig Reaction in Heterocyclic Synthesis

1. P(OEt)$_3$
2. hv

Ref. 122

Ref. 123

Ref. 124

Ref. 125

Ref. 126
2.2.2 Synthesis of 1-Alkyl Isoquinolines

In an effort to prepare a simple 1-alkyl isoquinoline, Hickey attempted to condense α-acetylbenzaldehyde (156) with ethyl azidoacetate. Unfortunately, none of the desired vinyl azide (157) was obtained. Instead the aldehyde underwent self-condensation in the basic medium.

The first objective, therefore, was to develop a general route to vinyl azides of type (158) which would allow the preparation of a variety of 1-alkyl isoquinolines.

Rather than investigate alternative methods for introducing the vinyl azide function, it was decided at the outset to protect the ketonic carbonyl group prior to introduction of the azide group by the standard azidoester condensation method. Thus the general sequence envisaged, involved initial protection of an aryl ketone (or aldehyde) followed by the introduction of a formyl group in the ortho position and condensation with methyl azidoacetate. Finally, deprotection of
the carbonyl group would give the vinyl azide (158). In practice, the most convenient starting materials were found to be 2-bromo carbonyl compounds of type (159).

![Chemical Structure](image)

The simplest of these, 2-bromobenzaldehyde (160) was chosen for initial study (Scheme 28). Thus the aldehyde (160) was first protected as its 1,3-dioxolane derivative (161). As expected (161) underwent rapid metal-halogen exchange on treatment with n-butyllithium in THF at -78°C. Quenching the resulting lithio species with dimethyl formamide gave, on aqueous work-up, the mono-protected phthalaldehyde (162). The yield over the two steps was 75%. Condensation of the aldehyde (162) with methyl azidoacetate under the standard conditions, gave the crystalline vinyl azide (163) in 63% yield.

A number of methods are known to cleave 1,3-dioxolanes to give the corresponding carbonyl compounds. Perhaps the mildest of these is the Conia procedure using wet silica gel in dichloromethane. However, (163) was recovered intact even after prolonged exposure (1 week) to this reagent system.

As an alternative to hydrolytic cleavage, an attempt was made to deprotect (163) by acid-catalysed exchange dioxolanation. Thus the azide (163) was dissolved in acetone containing a catalytic amount of p-toluenesulphonic acid (pTSA). After 20.5 hours the azide had been completely converted into a mixture of two compounds which could be
separated by p.l.c. on silica gel. The minor product (19%) was identified as the required deprotected, azido-aldehyde (164) on the basis of its spectral characteristics. The major product, however, was the known isoquinolone (165) which was isolated in 33% yield.\textsuperscript{130} The formation of (165) was unexpected and it seemed likely that it was not a primary product, but was instead derived from the aldehyde (164). Therefore the purified aldehyde (164) was re-subjected to the conditions of its formation. After 2 days it had been converted, almost quantitatively, into the isoquinolone (165).
The formation of the isoquinolone (165) represents a remarkably mild ring closure to form the isoquinoline ring system. As such the mechanism of the reaction is of interest, particularly since a nitrene pathway is clearly not involved. A plausible mechanism is shown in Scheme 29.
Acid-catalysed nucleophilic attack by the azide group on the aldehyde gives an intermediate (166) which loses nitrogen, in a process similar to that involved in the α-ketol rearrangement, to give the product (165). The mechanism is analogous to that proposed for the formation of the indazole derivative (168) in the thermolysis of the 1-acylamino-8-azidonaphthalene (167) (Scheme 30).

Although the aldehyde (164) was available using the acid-catalysed exchange dioxolanation procedure, its ready conversion into the isoquinolone (165) under the reaction conditions inevitably led to low yields, and so an alternative was sought. It was found that deprotection was most conveniently achieved by simply standing a solution of (163) in acidic, aqueous THF at room temperature. Under these conditions, none of the isoquinolone (165) was formed and the required azido-aldehyde (164) could be isolated in 75% yield.
With the aldehyde (164) now available in quantity, it was subjected to the conditions of the aza-Wittig reaction. Thus a solution of (164) in dry benzene was treated with triethyl phosphite (TEP) (1.1 equiv.). As expected, gas evolution was observed and (164) was smoothly converted into methyl isoquinoline-3-carboxylate (169) which was isolated in 91% yield.

Three features of the reaction are worthy of note. Firstly, ring closure takes place readily without the need for activating substituents. Secondly, the reaction occurs under extremely mild and essentially neutral conditions, and finally, a fully aromatic isoquinoline is formed directly.

Having established the viability of the route in the preparation of the 'parent' isoquinoline (169), an identical sequence was followed starting from 2-bromoacetophenone (170) in order to prepare a 1-alkyl
isoquinoline. Thus (170) was converted as before into the protected keto-azide (173) (Scheme 31).

Deprotection of the acetyl group in (173) was achieved using the method described previously for the hydrolysis of (163). When the reaction was carried out on a relatively small scale (50 mg), the only detectable product was the required keto-azide (174) which was obtained in 81% yield. However, on scaling-up the reaction (900 mg), in addition to (174), a second product was obtained in low yield (1.8%) which was shown to be methyl 1-methylisoquinoline-3-carboxylate (175). The formation of (175) was interesting, particularly since none of the corresponding isoquinoline (169) was observed in the deprotection of (163). This may, however, simply reflect the smaller scale (100 mg) of the latter reaction. Mechanistically, (175) could be formed by a
similar process to that proposed in Scheme 29 for the formation of the isoquinolone (165), to give initially, the N-oxide (177) via the oxaziridine (176).

Subsequent, possibly photochemical, deoxygenation would then give the observed product. Alternatively, the isoquinoline (175) might be derived directly from (173) via an intermediate such as (178).

With the required keto-azide (174) in hand, its conversion into the isoquinoline (175) by intramolecular aza-Wittig reaction proved to be straightforward and high yielding. Thus on treatment of (174) with TEP in benzene solution, methyl 1-methylisoquinoline-3-carboxylate (175) was isolated in 93% yield.
In summary, therefore, although the vinyl azide (174) is not available by direct condensation of 2-acetylbenzaldehyde with methyl azidoacetate, it can be prepared indirectly, in 4 steps from 2-bromoacetophenone (170). As expected, (174) cyclised smoothly on treatment with TEP to give the 1-methylisoquinoline (175). In principle the route is a general one which should allow the preparation of a variety of 1-alkyl isoquinolines.
2.2.3 Synthesis of a Tricyclic Isoquinoline

In order to define further the scope of the intramolecular aza-Wittig reaction in isoquinoline synthesis, it was decided to attempt a ring closure in which the carbonyl group was constrained in a ring. The starting material chosen was the known 8-bromotetralone (179) and the route developed in Section 2.2.2 was used to convert it into the vinyl azide (183) (Scheme 32).

Scheme 32
When a solution of the bicyclic vinyl azide (183) in dry benzene was treated with triethyl phosphite (TEP), the initially colourless solution rapidly became bright yellow. After 16 hours t.l.c. indicated that all of the azide had been consumed to give a more polar compound which exhibited the characteristic bright blue fluorescence of isoquinolines, together with a second compound which streaked slightly from the baseline. In an effort to drive the reaction to completion, the mixture was heated at 75°C for one hour. The heating had no effect (t.l.c.) and the reaction was worked-up. However, when magnesium sulphate was added to dry the resulting yellow ether-benzene solution, the yellow colour disappeared instantaneously. Immediate t.l.c. of the now colourless solution showed that the baseline material had disappeared and that the solution now contained a single compound. The solvent was removed to leave a tan solid which was identified as the 1,8-bridged, tricyclic isoquinoline (184) in 93% yield.

\[
\begin{align*}
\text{MeO} & \quad \text{CO}_2\text{Me} \\
\text{N}_3 & \quad \text{P(0Et)}_3, \text{C}_6\text{H}_6 \\
\text{MgSO}_4, \ 93\% & \\
(183) & \quad \rightarrow \\
\text{MeO} & \quad \text{CO}_2\text{Me} \\
\text{N} & \\
(184)
\end{align*}
\]

In order to investigate the effect of magnesium sulphate, TEP was again added to a solution of the azide (183) in dry benzene. After 5 minutes t.l.c. of the yellow solution showed it to contain the isoquinoline (184), some 'baseline streak' and the starting material. To this mixture a large excess of dried magnesium sulphate was added. The solution became colourless instantaneously and t.l.c. immediately after the addition showed that now only the starting material and the
isoquinoline (184) were present. After 16 hours all the azide had been consumed and work-up as before gave the isoquinoline (184), again in 93% yield.

Clearly, magnesium sulphate catalyses one (or more) of the steps in the reaction pathway (Scheme 33).

Of the four identifiable steps (A-D) of the reaction, it is possible that magnesium sulphate acts on one (or more) of B-D. No catalysis of step A can be involved since the rate of disappearance of the starting material was unchanged on addition of magnesium sulphate. Step B, in which nitrogen is lost from the initially formed triazene to give an iminophosphorane, might be catalysed by the presence of a solid surface. Such effects have literature precedent. However, it seems more likely that magnesium sulphate acts as a Lewis acid catalyst for step C,
which involves nucleophilic attack on the carbonyl group.

Whatever its mode of action, the addition of magnesium sulphate to the reaction medium had the effect of making the initial triazene formation rate determining. It seemed likely, therefore, that increasing the concentration of the reactants might be beneficial in terms of the reaction time. This was indeed the case such that when the azide (183) was treated with a large excess of TEP and magnesium sulphate, and less solvent was used, the reaction was complete in 3 hours. The isoquinoline (184) was isolated in 91% yield.

Clearly, therefore, the intramolecular aza-Wittig reaction is not limited to the synthesis of simple bicyclic isoquinolines. However, the observation, for the first time, of an intermediate in the formation of (184) suggests that the reaction is sensitive to the constraints imposed by ring closure to cyclic ketones.

2.2.4 Synthesis of an Azafluoranthene as a Model for the Azafluoran­
thene Alkaloids

In her original work, Hickey noted that the vinyl azide (185) was not converted into the azafluoranthene (186) on treatment with TEP. Although the azide was consumed, none of (186) could be detected in the reaction mixture. Despite this failure it seemed worthwhile, for a number of reasons, to investigate this reaction further. Not only would the rigid fluorenone system provide a particularly severe test of the power of the aza-Wittig isoquinoline synthesis, but if successful, the preparation of an azafluoranthene would also be a useful model for the synthesis of the azafluoranthene alkaloids (187). Furthermore, in the light of the results presented in Section 2.2.3, the effect (if any) of added magnesium sulphate on the reaction would be interesting.
The known aldehyde (188) was prepared straightforwardly by ozonolysis of fluoranthene under the literature conditions. Condensation of the aldehyde (188) with methyl azidoacetate gave, in moderate yield, the required vinyl azide (189).
In confirmation of Hickey's results with the azide (185), the azide (189) was rapidly consumed on treatment with triethyl phosphite (TEP) in xylene to give a bright orange compound. This, however, was unaffected by the addition of magnesium sulphate or by heating the solution at 60°C for 8 hours. After aqueous work-up an orange gum was obtained which was identified as the iminophosphorane (190a) on the basis of its n.m.r. spectrum.

\[
\begin{array}{c}
\text{C} & \text{O}_2\text{Me} \\
\text{Ar} & \text{N} - \text{PR}_3 \\
\text{(189)}
\end{array}
\quad \xrightarrow{\text{PR}_3} \quad
\begin{array}{c}
\text{C} & \text{O}_2\text{Me} \\
\text{Ar} & \text{N} - \text{PR}_3 \\
\text{(190)}
\end{array}
\]

\[
\begin{array}{c}
\text{R} & \% \\
\text{(a) OEt} & \text{ca. 100} \\
\text{(b) OMe} & \text{ca. 100} \\
\text{(c) NMe}_2 & -
\end{array}
\]

On standing for several days the iminophosphorane (190a) was slowly converted into an orange solid which was subsequently shown to be the phosphoramide (191).

\[
\begin{array}{c}
\text{Ar} & \text{N} - \text{P(0Et)}_3 \\
\text{(190a)} \\
\end{array}
\quad \xrightarrow{\text{PR}_3} \quad
\begin{array}{c}
\text{Ar} & \text{N} - \text{P(0Et)}_2 \\
\text{O} \\
\text{(191)}
\end{array}
\]

\[\text{Ar} = \text{9-oxofluoren-1-yl}\]

Although it seemed likely that the formation of (191) was simply due to slow hydrolysis of the iminophosphorane (190a), in principle the phosphoramidate (191) could also be derived from (190a) by spontaneous inter- or intramolecular loss of ethylene.
Therefore the azide (189) was treated with trimethyl phosphite to give the iminophosphorane (190b). However this too was converted on standing into the corresponding phosphoramidate (192).

\[
\begin{align*}
\text{CO}_2\text{Me} & \\
\text{Ar} & \\
\text{N} & \text{P(O(OMe))}_2 \\
\text{O} &
\end{align*}
\]

(192)

\(\text{Ar} = 9\text{-oxofluoren-1-yl}\)

In an attempt to increase the reactivity of the iminophosphorane (190), a solution of the azide (189) in xylene was treated with hexamethylyphosphorous triamide. An immediate reaction took place to give, presumably, the iminophosphorane (190c) which was observed as a dark red, highly polar material on t.l.c.. However no change took place at room temperature and on heating the solution at reflux for one hour, the iminophosphorane was converted into a highly complex mixture of products (t.l.c.) which was not investigated further.

A successful cyclisation was finally achieved by subjecting the iminophosphoranes (190a) and (190b) to melt pyrolysis. Thus heating (190a) and (190b) at 250°-300°C for one minute in a sublimation apparatus, followed by p.l.c. of the crude cold finger washings gave
the azafluoranthenes (193) in 10% and 11% yields respectively.

\[
\begin{align*}
&\text{Ar} = 9\text{-oxofluoren-1-yl} \\
&\text{Ar} = 9\text{-oxofluoren-1-yl}
\end{align*}
\]

Although the formation of (193) was encouraging, the low yields obtained limited the preparative usefulness of the reaction. Therefore, in the hope that a bulkier, more stable phosphorus ligand might increase the yield in the cyclisation, the azide (189) was treated with triphenylphosphine to give the iminophosphorane (190d).

\[
\begin{align*}
&\text{Ar} = 9\text{-oxofluoren-1-yl} \\
&\text{Ar} = 9\text{-oxofluoren-1-yl}
\end{align*}
\]

In contrast to the iminophosphoranes discussed previously, (190d) appeared to be stable indefinitely on standing, and was recovered intact after heating in xylene at reflux for 4 hours. In addition it was unchanged after irradiation at 350, 300 and 250 nm in dichloromethane, thereby excluding the possibility that the failure to observe cyclisation was simply a reflection of \(E\)-geometry about the double bond. The iminophosphorane (190d) finally underwent cyclisation using the melt
pyrolysis-sublimation procedure described above, to give the desired azafluoranthene (193). However the isolated yield of 19%, although almost double that obtained with the iminophosphoranes (190a) and (190b), was none the less disappointingly low.

Iminophospholes (194) derived from 1,2,5-triphenylphosphole are known to be more reactive than the corresponding iminophosphoranes derived from triphenylphosphine.\textsuperscript{138}

\[ \text{(194)} \]

\[ \text{(195)} \]

The effect has been rationalised on the basis of the favourable change in configuration at phosphorus on changing from the relatively strained tetracoordinate state of the iminophosphole to the non-strained trigonal bipyramidal intermediate (195) in which the phosphole ring can take up an axial-equatorial position such that the C₁-P-C₅ bond angle is close to ideal (90°).\textsuperscript{138}

Therefore, a solution of the azide (189) and 1,2,5-triphenylphosphole\textsuperscript{139} in toluene was heated at reflux for 1.5 hours. On cooling the dark red, beautifully crystalline iminophosphole (196) was deposited in 71% yield. Initial attempts to thermolyse (196) in boiling 1,2-dichlorobenzene or diphenyl ether solutions gave only traces of the azafluoranthene (193). However melt pyrolysis, under similar conditions (300°C, 2 minutes) to those employed previously with the iminophosphoranes, gave rise to (193) in the improved yield of 34%. 
Clearly, intramolecular attack on the carbonyl group by phosphorus-nitrogen ylids derived from the azide (189) is disfavoured, presumably by the steric constraints imposed by the rigid fluorenone ring system, such that formation of the azafluoranthene (193) requires forcing conditions. In contrast, Hickey has observed that the electronically similar, but more flexible benzophenone derivative (153a) cyclises readily at 35°C.\textsuperscript{51}
2.2.5 Thermolysis of 3-Aryl-2-azidopropenoates as a Route to 4-Acyl Indoles

In order to extend the versatility of 3-aryl-2-azidopropenoates of type \((158)\) in heterocyclic synthesis, a brief study of their thermal reactions was undertaken.

In principle, thermolysis of vinyl azides \((158)\) should allow the preparation of a variety of 4-acyl indoles \((197)\), compounds which are of considerable importance as ergot alkaloid precursors.\(^{140}\) Previous work by Hickey however, had shown that the reaction was not as straightforward as might have been expected.\(^5\) Thus while the ester \((153b)\) gave the indole \((198b)\) in 79% yield, only 10% of the 4-benzoyl indole \((198a)\) was obtained on thermolysis of \((153a)\).
In the present study, thermolysis of the 2-formyl azidocinnamate (164) in boiling xylene gave a mixture of two compounds which were readily separated by p.l.c.. The minor product (28%) proved to be the expected 4-formyl indole (199), while the major product (32%) was identified as the isoquinolone (165).

Although the formation of (165) was unexpected, a similar reaction has been reported by French workers who found that the thieno[2,3-\(\alpha\)]pyridone (201) was formed in high yield on thermolysis of the azide (200).\textsuperscript{141}
Mechanistically, (165) is formally the result of insertion of the nitrene (202) into the aldehydic C-H bond. However, while it is reasonable to assume that the nitrene (202) is an intermediate in the reaction, a number of 'non-insertion' pathways may be envisaged for its conversion into the isoquinolone (165). One such pathway is shown in Scheme 34 whereby an initial [1,6] hydrogen shift in the nitrene gives an intermediate (203) which rapidly aromatises by electrocyclic ring closure to give the product.
Thermolysis of the 2-acetyl azidocinnamate (174) in boiling xylene gave a complex mixture from which the only isolable product, in 22% yield, was the expected 4-acetyl indole (204).

\[
\begin{align*}
\text{O} & \quad \text{CO}_2\text{Me} \\
\text{H} & \quad \text{N}_3 \\
\text{H} & \quad \text{H}
\end{align*}
\]

(174) \rightarrow
\[
\begin{align*}
\text{O} & \quad \text{CO}_2\text{Me} \\
\text{H} & \quad \text{H}
\end{align*}
\]

(204)

\[
\begin{align*}
\text{O} & \quad \text{CO}_2\text{Me} \\
\text{H} & \quad \text{H}
\end{align*}
\]

(189) \rightarrow
\[
\begin{align*}
\text{O} & \quad \text{CO}_2\text{Me} \\
\text{H} & \quad \text{H}
\end{align*}
\]

(205)

In contrast to the results obtained with (174), thermolysis of the vinyl azide (189) was both clean and high yielding such that the tetracyclic indole derivative (205) was isolated in 76% yield.

In contrast to the results obtained with (174), thermolysis of the vinyl azide (189) was both clean and high yielding such that the tetracyclic indole derivative (205) was isolated in 76% yield.

These conflicting results, together with those obtained by Hickey\textsuperscript{51} are difficult to rationalise. In particular, the reason for the low yields of indoles obtained on thermolysis of the vinyl azides (164) and (174) remains obscure and requires further study. However, in terms of the synthesis of 4-acyl indoles, it seemed likely that the problem could be avoided by forming the indole ring prior to deprotection of the carbonyl group. Therefore the protected azido-aldehyde (163) was thermolysed as before in boiling xylene. As with the thermolysis of the unprotected aldehyde (164), two products were formed. The
major product, isolated in 53% yield, was the desired 4-substituted indole (206) while the minor product (44%) was shown to be the isoquinoline (207).

Presumably (207) is formed via the intermediate (208) by a similar mechanism to that which gives the isoquinolone (165) on thermolysis of the unprotected aldehyde (164).

The almost quantitative combined yield in the reaction was encouraging since it was thought unlikely that protected ketones, which lack a reactive benzylic hydrogen, would give rise to any isoquinoline products. That this was indeed the case was shown by thermolysis of the protected 2-acetyl azidocinnamate (173) to give the expected 4-substituted indole (209) as the sole product in quantitative yield.
In summary therefore, although the thermolysis of azidocinnamates containing an ortho-carbonyl group to give 4-acyl indoles can be low yielding, it appears that the problem can be overcome by protecting the carbonyl group during the reaction.

2.2.6 Conclusion

The scope of the intramolecular aza-Wittig reaction of iminophosphoranes derived from azidocinnamates bearing ortho-carbonyl substituents has been extended to include the synthesis of 1-alkyl isoquinolines. In the absence of severe steric strain, the ring closure step occurs under exceptionally mild, neutral conditions and the yields of isoquinolines are excellent.

A convenient route to the required azidocinnamates has been developed starting from 2-bromo aryl-aldehydes and ketones, which should allow the preparation of isoquinolines lacking the activating substituents required by the classical syntheses. Although the method necessarily gives isoquinolines bearing an ester at the 3-position, this limitation is offset by the versatility of the ester group which can, in principle, be modified or removed as required.
In addition to the synthesis of isoquinolines, the route developed to prepare azidocinnamates bearing ortho-carbonyl substituents can also be used in the synthesis of 4-acyl indoles (Scheme 35).

Scheme 35
PART 3

Approaches to the Synthesis of the Marine Alkaloid Amphimedine
3.1 Introduction

Marine organisms continue to provide a rich source of structurally diverse natural products.\textsuperscript{143,144} However the alkaloids, as a structural class, appear to be rare in the marine environment so that relatively few alkaloids have been isolated from marine sources.\textsuperscript{143}

Recently, Schmitz and coworkers reported the isolation of a new pentacyclic aromatic alkaloid from an *Amphimedon* species of sponge collected off the Pacific island of Guam.\textsuperscript{145} By examining natural abundance $^{13}$C-$^{13}$C coupling constants, together with a variety of other n.m.r., i.r. and mass spectral data, they assigned structure (210) to the new alkaloid which they named 'amphimedine'.

![Structure of Amphimedine (210)]

As the first example of a new alkaloid skeleton, amphimedine presents an interesting and challenging target for synthesis. It was decided, therefore, to investigate a vinyl azide based approach to the synthesis of amphimedine in order to demonstrate further the usefulness of vinyl azides in natural product synthesis.
3.2 Retrosynthetic Analysis

The proposed retrosynthetic analysis of amphimedine is shown in Scheme 36.

Thus it was envisaged that the pentacyclic ring system would be formed by sequential fusion of the pyridine and pyridone rings onto an acridine aldehyde of type (211).
3.3 Results and Discussion

The starting material chosen was the known 9-formyl-2-methoxyacridine (212) which was prepared by the literature method\textsuperscript{146} from 2-aminoacetophenone and 4-bromoanisole as shown in Scheme 37.

\begin{equation}
\begin{array}{c}
\text{NH}_2 \\
\text{Br} \\
\text{OMe}
\end{array}
\xrightarrow{1. \text{Cu, Na}_2\text{CO}_3, \text{C}_6\text{H}_5\text{NO}_2, \Delta}
\begin{array}{c}
\text{CHO} \\
\text{OMe}
\end{array}
\xrightarrow{2. \text{AcOH/H}_2\text{SO}_4/\Delta}
\begin{array}{c}
\text{Me} \\
\text{OMe}
\end{array}
\end{equation}

\textbf{Scheme 37}

The condensation of aldehyde (212) with methyl azidoacetate proved troublesome. Although the aldehyde was completely consumed to give one product (by t.l.c.), the desired vinyl azide (213) was isolated from the reaction mixture in only 32% yield. Attempts to increase the yield by varying the reaction conditions were ineffectual. It is possible that the relatively low yield reflects the instability of the azide (213) which, unlike those prepared previously, darkened rapidly on standing and could not be obtained analytically pure.
With the vinyl azide (213) in hand, attention was turned to the key thermolysis reaction. The outcome of the reaction was difficult to predict, since Hemetsberger has reported that the vinyl azide (214), derived from 9-anthraldehyde, gives a complex mixture on thermolysis from which none of the compound derived by cyclisation to the peri position could be isolated.\(^{147}\)

However, in the present case it was hoped that electron release from the methoxy group at C-2 (acridine numbering) would facilitate cyclisation of the vinyl nitrene to C-1. Of course, the other peri-position, C-8, would also benefit, albeit to a lesser extent, from the same effect.

In the event, thermolysis of the vinyl azide (213) gave a mixture of two products in a ratio of ca. 4:1. These were easily separated by
chromatography on silica gel and were assigned the structures (215) and (216) on the basis of their n.m.r. spectra together with a series of n.O.e. difference experiments.

![Chemical Structures](image)

The n.O.e. difference spectra of (215) and (216) are reproduced in Figures 3 and 4 respectively. Thus in the $^1$H n.m.r. spectrum of (215) (Figure 3), pre-irradiation of the singlet due to H-1 results in a strong enhancement of the broadened doublet ($\delta 8.3$ Hz) due to the peri-proton H-11. In addition, pre-irradiation of the singlet (tentatively assigned to the C-4 methoxyl group) at $\delta 3.98$ caused an enhancement of the doublet ($\delta 8.3$ Hz) due to H-5, centered at $\delta 6.98$

Alternatively, in the $^1$H n.m.r. spectrum of (216) (Figure 4), pre-irradiation of the singlet due to H-1 resulted in the enhancement of the doublet ($\delta 2.7$ Hz) at $\delta 7.27$ due to H-11. Finally, as expected for structure (216), pre-irradiation of the methoxyl group singlet at $\delta 3.87$ resulted in the enhancement of both H-9 and H-11 signals.

In summary therefore, the thermolysis of the vinyl azide (213) was both high yielding and regioselective to give the required pyridoacridine
Figure 3
Figure 4
(215) in 78% yield together with 19% of the alternative cyclisation product (216).

With the successful preparation of (215) it remained to add the pyridone ring in order to establish the pentacyclic ring system of amphimedine. First however, it was decided to introduce the required carbonyl group at C-8 (amphimedine numbering). Therefore the methoxy-pyridoacridine (215) was treated with manganese (IV) oxide in 35% sulphuric acid at 0°C. After 45 minutes, work-up and p.l.c. on silica gel gave the pyridoacridinone (217) as a bright yellow solid in 75% yield.

Interestingly, identical treatment of the regioisomeric pyridoacridine (216) caused its complete destruction and no products could be isolated from the reaction. This result was convenient since it removed the need to separate the pyridoacridines (215) and (216). Thus simply oxidising the crude thermolysate gave (217) with no significant diminution of the yield.

It was intended at the outset that the fifth ring of amphimedine would be introduced by a Diels-Alder reaction using a suitably substituted aza-diene. Specifically, the 2-aza-1,3-diene (218) introduced recently by Ghosez and coworkers, appeared to be the ideal candidate.
The diene (218) is known to react with quinones to give fused pyridones in high yield after hydrolysis of the initial adducts. For example, treatment of 1,4-naphthoquinone with the diene (218) in boiling chloroform gave, after hydrolysis, the tricyclic pyridone (219) in 72% yield.\textsuperscript{148}

However, treatment of the enone (217) with the aza-diene (218) (1.1 equiv.) in chloroform gave only starting materials, even after 16 hours at reflux. Using an excess (ca. 20 equiv.) of the diene, the enone was slowly consumed. However, the only isolable product was an unidentified red oil whose n.m.r. spectrum indicated that it was not an adduct of the enone (217).
Attempts to use more forcing conditions (sealed-tube, 160°C or chlorobenzene, 132°C) also resulted in decomposition of the enone. Alternatively, adding catalytic amounts of boron trifluoride etherate or aluminium chloride to the reaction mixture merely resulted in the destruction of the aza-diene.

Finally, an attempt to facilitate the desired Diels-Alder reaction using high pressure was also unsuccessful. Thus the enone (217) was recovered intact after treatment with the aza-diene (218) in chloroform at 40°C and 12 kbar for 40 hours.¹⁴⁹

It seems likely that the failure to form a Diels-Alder adduct is associated with the poor solubility of the enone (217) in most organic solvents. Even in the best solvent, chloroform, a saturated solution at reflux contained only ca. 4-5 mg/ml. It was difficult, therefore, to achieve a high concentration of the reactants, particularly since the aza-diene appeared to be prone to polymerisation.
3.4 Conclusion

A potential precursor (217) to the marine alkaloid amphimedine has been prepared in three steps from the known aldehyde (212) by a route in which the key step involved thermolysis of the vinyl azide (213) to give the pyridoacridine (215) (Scheme 38).

Although attempts to introduce the fifth ring of amphimedine using a Diels-Alder reaction have, as yet, been unsuccessful, further study of the reaction is warranted before recourse is made to alternative, step-wise, strategies.
EXPERIMENTAL
General Experimental Procedures

1. Solvents and Reagents

Petrol refers to the light petroleum fraction boiling in the range 40-60°C which was redistilled through a 36 cm Vigreux column prior to use. Ether refers to diethyl ether which was dried by standing over sodium wire or by distillation from lithium aluminium hydride. THF was dried by distillation from potassium-benzophenone ketyl. Benzene, toluene, xylene, chlorobenzene, 1,2-dichlorobenzene and DMF were dried by distillation from calcium hydride. Chloroform and dichloromethane were purified by passage through a column of Brockmann Grade 1 basic alumina prior to use. Dry methanol and ethanol were prepared by distillation from their respective magnesium alkoxides. All purified and dried solvents were stored under dry nitrogen.

Unless stated otherwise, reagents were obtained from commercial suppliers and were used without further purification.

2. Chromatography

Analytical thin layer chromatography (t.l.c.) was performed on Merck Kieselgel 60 GF$_{254}$ aluminium-backed plates. Plates were visualised under u.v. light at 254 and/or 306 nm.

Preparative layer chromatography (p.l.c.) was performed on glass-backed plates (20 x 20 or 20 x 40 cm) coated to a thickness of ca. 2 mm with Merck Kieselgel 60 GF$_{254}$.

Column chromatography was carried out at medium pressure on Merck Kieselgel 60 H (70-230 mesh) using an aquarium pump or hand-bellows to apply the pressure to the column-head. Samples were applied as a dry, pre-adsorbed mixture with a small amount of adsorbent, or as a solution in the minimum amount of a suitable solvent.
3. Spectra

Infrared (i.r.) spectra were recorded in the range 4000 - 600 cm\(^{-1}\) using a Perkin Elmer 298 spectrophotometer and were calibrated against polystyrene (1603 cm\(^{-1}\)). Spectra of solids were normally recorded as Nujol mulls and those of liquids as thin films between sodium chloride plates or as chloroform solutions.

Ultraviolet (u.v.) spectra were recorded in the range 450 - 200 nm using a Pye Unicam SP 800B spectrophotometer. Points of inflexion are abbreviated (sh).

Proton nuclear magnetic resonance (\(^1\)H n.m.r.) spectra were recorded using Varian EM 360 (60 MHz), Perkin Elmer R 32 (90 MHz), Jeol FX 90Q (90 MHz) or Bruker WM 250 (250 MHz) instruments. All spectra included tetramethylsilane (TMS) as an internal standard; most of those recorded at 90 MHz employed a heteronuclear lock (TMS), while all of those run at 250 MHz employed a homonuclear lock (deuterium of the solvent). Signals are described as singlets (s), doublets (d), triplets (t), multiplets (m), broad (br), double doublets (dd), etc.

Mass spectra (m.s.) were recorded at low resolution using A.E.I. MS 12 and VG Micromass 7070E instruments; the latter was also used for high resolution determinations.

4. Other Data

Melting points were determined on a Reichert Kofler hot stage apparatus and are uncorrected.

Microanalyses were carried out by Mr. K.I. Jones and his staff in the Imperial College Chemistry Department microanalytical laboratory.
EXPERIMENTAL FOR PART ONE
1. **Methyl azidoacetate**

   The procedure is similar to that reported for the preparation of tert-butyl azidoacetate.\(^{150}\)

   In a one litre round-bottom flask equipped with a reflux condenser was placed methyl chloroacetate (50g, 0.461 mol), sodium azide (37.5g, 0.577 mol), acetone (75 ml) and water (50 ml), and the mixture heated under reflux for 16h. On cooling, most of the acetone was removed \textit{in vacuo} and the residue extracted with ether (3 x 50 ml). The combined ethereal extracts were dried (MgSO\(_4\)) and concentrated \textit{in vacuo} to leave a pale yellow mobile oil. The crude material was distilled (CAUTION) (b.p. 45\(^{0}\)C/0.4 mmHg) to give methyl azidoacetate (45.5g, 86\%) as a colourless oil, \(\nu_{\text{max}}\) (film) 2100, 1745, 1290, and 1210 cm\(^{-1}\); \(\delta_H\) (60 MHz; CDCl\(_3\)) 3.74 (3H, s, CO\(_2\)CH\(_3\)), 3.89 (2H, s, CH\(_2\)).

2. **Condensation of 2,3-Dimethoxybenzaldehyde with Methyl azidoacetate**

   Sodium (2.78g, 0.121g atom) was added in portions to methanol (80 ml) and the mixture stirred until all the sodium had dissolved. The solution was cooled to \(-15^{0}\)C whereupon a solution of 2,3-dimethoxybenzaldehyde (5g, 0.03 mol) in methyl azidoacetate (13.86g, 0.121 mol) was added dropwise over 1h. The reaction was stirred for 4h at \(-15^{0}\)C and then allowed to warm to room temperature. When nitrogen evolution had ceased the mixture was poured into saturated aqueous ammonium chloride (200 ml) and extracted with ether (4 x 100 ml). The combined ethereal extracts were washed with water (2 x 150 ml), dried (MgSO\(_4\)) and concentrated \textit{in vacuo} to leave a yellow solid. Recrystallisation from methanol gave methyl 2-azido-3-(2,3-dimethoxyphenyl)propenoate (79) (5.64g, 72\%) as yellow needles, m.p. 90-95\(^{0}\)C (decomp.) (Found: C, 54.9; H, 5.0; N, 15.8. \(C_{12}H_{13}N_3O_4\) requires C, 54.75; H, 4.9; N, 16.0\%); \(\nu_{\text{max}}\) (Nujol) 2130, 1710, 1610, 1570, 1475, 1425, 1283, 1265,
1092, 1070, and 1000 cm\(^{-1}\); \(\delta_H\) (250 MHz; CDCl\(_3\)) 3.84 (3H, s), 3.88 (3H, s), 3.92 (3H, s), 6.92 (1H, dd, \(J\) 7.5 and 1.7 Hz), 7.10 (1H, t, \(J\) 7.5 Hz), 7.33 (1H, s, ArCH), 7.82 (1H, dd, \(J\) 7.5 and 1.7 Hz); \(m/z\) 263 (\(M^+\), 15%), 235 (M-28, 100), 220 (31), 203 (100), 188 (92), 176 (81), 161 (77).

3. Thermolysis of Methyl 2-azido-3-(2,3-dimethoxyphenyl)propenoate (79)

A solution of the vinyl azide (79) (0.5g, 1.90 mmol) in dry xylene (120 ml) was heated under reflux for 4h. On cooling, the solvent was removed \(\text{in vacuo}\) to leave a yellow solid which was sublimed (90°C/0.1 mmHg) to give methyl 4,5-dimethoxyindole-2-carboxylate (78) (0.437g, 98%) as pale yellow prisms, m.p. 125-126°C (from ether-petrol) (Found: C, 61.25; H, 5.5; N, 6.0. \(C_{12}H_{13}NO_7\) requires C, 61.3; H, 5.5; N, 6.0%); \(v_{\text{max}}\) (Nujol) 3310, 1685, 1510, 1330, 1255, 1220, 1090, 980, and 780 cm\(^{-1}\); \(\delta_H\) (250 MHz; CDCl\(_3\)) 3.89 (3H, s), 3.93 (3H, s), 4.09 (3H, s), 7.07 (2H, s, 6-H, 7-H), 7.34 (1H, d, \(J\) 1 Hz, 3-H), 9.75 (1H, br s, NH); \(m/z\) 235 (\(M^+\), 91%), 203 (97), 188 (100), 132 (34), 101 (20).

4. \(N\)-Methylation of Methyl 4,5-dimethoxyindole-2-carboxylate (78)

Sodium hydride (50% suspension in paraffin oil; 77 mg, 3.21 mmol) was de-oiled by washing with petrol and suspended in dry DMF (10 ml). A solution of the indole (78) (0.5g, 2.13 mmol) in DMF (3 ml) was added dropwise at room temperature. When gas evolution had ceased the pale yellow heterogeneous mixture was stirred at room temperature for 30 min whereupon methyl iodide (0.596g, 0.3 ml, 4.20 mmol) was added. After a further 2h stirring, the reaction mixture was diluted with water (25 ml) and extracted with ether (3 x 20 ml). The combined ethereal extracts were washed with water (4 x 20 ml), dried (MgSO\(_4\)) and
concentrated in vacuo to give methyl 4,5-dimethoxy-1-methylindole-2-carboxylate (89) (0.52g, 98%) as a tan solid, m.p. 74-75°C (from petrol) (Found: C, 62.55; H, 6.0; N, 5.5. C₁₃H₁₅NO₄ requires C, 62.6; H, 6.0; N, 5.6%); ν\text{max} (Nujol) 1715, 1510, 1240, 1190, 1070, and 740 cm\(^{-1}\); δ\text{H} (250 MHz; CDCl₃) 3.91 (6H, s), 4.03 (3H, s), 4.09 (3H, s), 7.08 (2H, ABq, J 9 Hz, 6-H, 7-H), 7.39 (1H, s, 3-H); \text{m/z} 249 (M⁺, 100%), 234 (60), 174 (24), 147 (28).

5. Attempted Lithiation of Methyl 4,5-dimethoxy-1-methylindole-2-carboxylate (89)

(a) With tert-Butyllithium at Room Temperature

The indole (89) was treated with tert-butyllithium (2 equiv.) in THF at room temperature for 30 min to give a dark red solution. Methyl iodide (10 equiv.) was added, the red colour was quenched and, after a further 30 min, aqueous work-up gave a brown oil. N.m.r. and t.l.c. analysis of the oil showed it to be a complex mixture which was discarded.

(b) With tert-Butyllithium at -78°C

The indole (89) was treated with tert-butyllithium (1.2 equiv.) in THF at -78°C for 15 min. Methyl iodide (1.2 equiv.) was added and the mixture stirred at -78°C for a further 30 min. Aqueous work-up gave a brown gum whose n.m.r. spectrum was identical to that obtained in 5(a).

(c) With LDA at -78°C

The indole (89) was treated with LDA (1.1 equiv.) at -78°C in THF. After 15 min methyl iodide (1.2 equiv.) was added and the mixture allowed to warm to room temperature. Aqueous work-up gave a pale yellow oil which solidified on standing. The n.m.r. spectrum of the solid showed it to consist solely of the starting material (89).
6. **Attempted Nitration of Methyl 4,5-dimethoxyindole-2-carboxylate**

(a) **With Sodium Nitrate in Sulphuric Acid**

Sodium nitrate (1 equiv.) was added to a solution of the indole (78) in concentrated sulphuric acid at 0°C. After 1h the mixture was poured onto ice and extracted with ether to give a black oil which was shown by n.m.r. and t.l.c. to be a complex mixture and was therefore discarded.

(b) **With Sodium Nitrate in Trifluoroacetic Acid**

The indole (78) was added to a solution of sodium nitrate (1 equiv.) in TFA and the resulting red solution stirred at room temperature for 1.5h. Aqueous work-up gave a small amount of a yellow gum which proved to be a complex mixture (by t.l.c. and n.m.r.) and was not investigated further.

(c) **With Copper(II) Nitrate Trihydrate in Trifluoroacetic Anhydride**

Copper(II) nitrate trihydrate (0.5 equiv.) was added to a solution of the indole (78) in TFAA. After 15 min the reaction mixture was poured onto ice and extracted with chloroform to give a yellow solid. P.l.c. on silica gel gave methyl 4,5-dimethoxy-7-trifluoroacetylindole-2-carboxylate (93) (18 mg, 30%) as a yellow solid, m.p. 116-118°C (from petrol-chloroform) (Found: C, 50.7; H, 3.6; N, 4.2. C_{14}H_{12}F_{3}NO_{5} requires C, 50.8; H, 3.6; N, 4.2%). ν_{max} (CHCl_{3}) 3420, 1715, 1650, 1580, 1495, 1315, 1180, 1140, 1050, 995, and 830 cm^{-1}; δ_{H} (250 MHz; CDCl_{3}) 3.94 (3H, s), 3.98 (3H, s), 4.40 (3H, s), 7.47 (1H, d, J 2.5 Hz, 3-H), 7.67 (1H, q, J 1.6 Hz, 6-H), 10.73 (1H, br s, NH); m/z 331 (M^+, 100%), 316 (11), 299 (50), 284 (32), 262 (11), 230 (68).
7. Reaction of Methyl 4,5-dimethoxyindole-2-carboxylate (78) with Trifluoroacetic Anhydride (TFAA)

The indole (78) (50 mg, 0.21 mmol) was added to freshly distilled TFAA (3 ml) and the resulting heterogeneous mixture stirred at room temperature. After 30 min the indole (78) had dissolved to give a clear, yellow solution. After a further 30 min a yellow precipitate had appeared. The mixture was poured onto ice (10 g) and extracted with chloroform (3 x 10 ml). The combined organic extracts were washed with saturated aqueous sodium hydrogen carbonate (2 x 20 ml), dried (MgSO$_4$) and concentrated in vacuo to give methyl 4,5-dimethoxy-7-trifluoroacetylindole-2-carboxylate (93) (68 mg, 97%) as a yellow solid.

8. Condensation of 4-Acetamidobenzaldehyde (70) with Methyl azidoacetate

Sodium (0.282 g, 0.012 g atom) was added in portions to dry methanol (10 ml) and the mixture stirred until all the sodium had dissolved. The solution was cooled to -15°C whereupon a solution of 4-acetamidobenzaldehyde (70) (0.5 g, 3.07 mmol) in methyl azidoacetate (1.41 g, 0.012 mol) and methanol (4 ml) was added dropwise over 30 min. When addition was complete, the reaction was stirred at -15°C for 2 h and then allowed to warm to room temperature. When nitrogen evolution had ceased, the mixture was poured into saturated aqueous ammonium chloride (50 ml) and extracted with ether (4 x 50 ml). The combined ethereal extracts were washed with water (3 x 100 ml), dried (MgSO$_4$) and concentrated in vacuo to leave a yellow solid which was purified by recrystallisation from methanol to give methyl 3-(4-acetamidophenyl)-2-azido-propenoate (96) (0.57 g, 72%), m.p. 118-123°C (decomp.) (from methanol) (Found: C, 55.65; H, 4.65; N, 21.4. C$_{12}$H$_{12}$N$_4$O$_3$ requires C, 55.4; H, 4.6; N, 21.5%); $\nu_{\text{max}}$ (Nujol) 3395, 2110, 1705, 1660, 1600, 1580,
1520, 1430, 1410, 1250, 1180, and 1080 cm\(^{-1}\);  \(\delta_H\) (250 MHz; CDCl\(_3\))
2.19 (3H, s, NHC\(_3\)C\(_2\)F\(_3\)), 3.90 (3H, s, CO\(_2\)C\(_2\)F\(_3\)), 6.87 (1H, s, ArC\(_3\)), 7.55
(2H, d, J 8.3 Hz), 7.67 (1H, br s, NH\(_3\)), 7.78 (2H, d, J 8.3 Hz);  \(m/z\) 232
\((M-28, 67%)\), 190 (35), 158 (100), 130 (49).

9. Thermolysis of Methyl 3-(4-acetamidophenyl)-2-azidopropenoate (96)

A suspension of the vinyl azide (96) (1.5g, 5.77 mmol) in dry xylene
(250 ml) was heated rapidly to 140°C. After 1.5h the solution was
allowed to cool to room temperature, whereupon the resulting pale yellow
prisms were collected by filtration, washed with petrol and dried \textit{in vacuo}
to give methyl 6-acetamidoindole-2-carboxylate (97) (1.07g, 80%),
m.p. 243-245°C (Found: C, 61.8; H, 5.15; N, 11.9. C\(_{12}\)H\(_{12}\)N\(_2\)O\(_3\) requires
C, 62.1; H, 5.2; N, 12.1%); \(\nu_{\text{max}}\) (Nujol) 3380, 3280, 1710, 1655, 1540,
1240, and 1205 cm\(^{-1}\); \(\delta_H\) (250 MHz; d\(_6\)-DMSO) 2.04 (3H, s, NHC\(_3\)C\(_2\)F\(_3\)), 3.84
(3H, s, CO\(_2\)C\(_2\)F\(_3\)), 7.09 (2H, m), 7.54 (1H, d, J 8.4 Hz), 8.02 (1H, s),
9.97 (1H, br s, NH\(_3\)), 11.77 (1H, br s, NH\(_3\)); \(m/z\) 232 (\(M^+\), 70%), 190 (40),
158 (100).

10. Acid Catalysed Methanolysis of Methyl 6-acetamidoindole-2-carboxylate
(97)

To a saturated solution of dry hydrogen chloride in dry methanol
(40 ml) was added the indole (97) (0.44g, 1.90 mmol) and the resulting
pale yellow solution heated under reflux for 6h. On cooling, the solu-
tion was neutralised with saturated aqueous sodium hydrogen carbonate and
extracted with ether (4 x 50 ml). The combined ethereal extracts were
dried (MgSO\(_4\)) and concentrated \textit{in vacuo} to give methyl 6-aminoindole-2-
carboxylate (104) (0.35g, 97%) as a pale yellow solid, m.p. 181-183°C
(Found: C, 63.0; H, 5.25; N, 14.6. C\(_{10}\)H\(_{10}\)N\(_2\)O\(_2\) requires C, 63.2; H, 5.3;
N, 14.7%); \(\nu_{\text{max}}\) (Nujol) 3350, 3320, 1690, 1675, 1630, 1520, 1430, 1275,
11. Reaction of Methyl 6-aminoindole-2-carboxylate (104) with Dimethyl 2-oxoglutaconate (20) 19

To a solution of the aminoindole (104) (0.25g, 1.32 mmol) in dichloromethane (15 ml) was added dimethyl 2-oxoglutaconate (0.339g, 1.97 mmol) and the resulting pale yellow solution stirred at room temperature for 16h. A catalytic amount of dry hydrogen chloride (one drop of a saturated solution in ether) was added and the solution stirred for a further 12h. The reaction mixture was diluted with chloroform (15 ml), washed with saturated aqueous sodium hydrogen carbonate (20 ml), dried (MgSO₄) and concentrated in vacuo to give a yellow/brown solid. Trituration with hot methanol left trimethyl 1H-pyrrolo[2,3-f]quinoline-2,7,9-tricarboxylate (105) (0.4g, 89%) as a bright yellow microcrystalline solid, m.p. 234-235°C (from dichloromethane) (Found: C, 59.7; H, 4.1; N, 8.1. C₁₇H₁₁N₂O₆ requires C, 59.6; H, 4.1; N, 8.2%); νₘₐₓ (Nujol) 3350, 1715, 1700, 1510, 1430, 1300, 1270, 1245, 1225, 1200, 1165, 1010, 1005, 860, 800, and 760 cm⁻¹; δ_H (250 MHz; CDCl₃) 4.02 (3H, s, CO₂CH₃), 4.13 (3H, s, CO₂CH₃), 4.19 (3H, s, CO₂CH₃), 7.39 (1H, d, J 1 Hz, 3-H), 8.05 (2H, ABq, J 3.6 Hz, 4-H, 5-H), 8.92 (1H, s, 8-H), 12.52 (1H, br s, NH); m/z 342 (M⁺, 100%), 284 (81), 252 (75).

12. Attempted Oxidation of Trimethyl 1H-pyrrolo[2,3-f]quinoline-2,7,9-tricarboxylate (105) with Pyridinium Fluorochromate (PFC) 64

A mixture of the pyrroloquinoline (105) and PFC (2.6 equiv.) in glacial acetic acid was heated under reflux for 2h during which time the
reaction mixture slowly became black. T.l.c. analysis showed that the
starting material had been consumed to give a black baseline material
and the solution was discarded.

13. Attempted Reduction of Trimethyl 1H-pyrrolo[2,3-f]quinoline-2,7,9-
tricarboxylate (105) to give the Indoline Derivative (109)

(a) With Borane-Trimethylamine Complex

A suspension of the pyrroloquinoline (105) in dioxan was treated
with borane-trimethylamine complex (4 equiv.) and 10.5N hydrochloric
acid. The resulting solution was heated under reflux for 30 min,
cooled, 6N hydrochloric acid was added, and the solution heated for a
further 15 min under reflux. On cooling, the reaction mixture was made
basic with 6N potassium hydroxide and extracted with chloroform to give
a yellow solid which was identified as the starting material (105) (84%
recovery).

(b) With Borane-THF Complex in Trifluoroacetic Acid (TFA)

A solution of the pyrroloquinoline (105) in TFA at -78°C was
treated with borane-THF complex (2.2 equiv.). After 45 min at -78°C,
aqueous work-up and extraction with dichloromethane gave the starting
material (100% recovery).

(c) Using Catalytic Hydrogenation in Fluoroboric Acid

A suspension of the pyrroloquinoline (105) and platinum oxide
(0.3 equiv.) in 1:1 40% fluoroboric acid-ethanol was shaken under 1 atm.
of hydrogen for 20h. The solution was made basic with 6N potassium
hydroxide, filtered through Celite and extracted with chloroform to give
a yellow solid which was shown by n.m.r. to consist solely of the
starting material (105) (43% recovery).
(d) With Zinc in Phosphoric Acid

A mixture of the pyrroloquinoline (105) and zinc dust (3 equiv.) in 85% phosphoric acid was heated at 80°C for 4h. Aqueous work-up and extraction with chloroform gave a yellow solid which was shown to be the starting material (12% recovery).

(e) With Sodium Cyanoborohydride

Sodium cyanoborohydride (10 equiv.) was added to a solution of the pyrroloquinoline (105) in glacial acetic acid and the mixture stirred at 65°C for 24h. On cooling, the reaction mixture was poured into water, made basic with sodium hydroxide pellets and extracted with chloroform. The resulting brown gum was shown by n.m.r. to be a complex mixture which was discarded.

(f) With Sodium in Liquid Ammonia

Sodium (14 equiv.) was added to a suspension of the pyrroloquinoline (105) in 1:1 THF-liquid ammonia. After 15 min saturated aqueous ammonium chloride was added, the ammonia allowed to evaporate and the residue extracted with chloroform to give a brown gum. N.m.r. analysis showed the gum to be a complex mixture which was discarded.

(g) With 'Cuprous Hydride'

To a suspension of copper(I) bromide (0.419g, 1.46 mmol) in dry THF (5 ml) at 0°C under nitrogen was added Red-A1(R) (0.86 ml of a 3.4M solution in toluene, 2.92 mmol). The resulting black mixture was stirred at 0°C for 30 min and then cooled to -78°C whereupon 2-butanol (0.217g, 0.27 ml, 2.92 mmol) was added followed by a slurry of the pyrroloquinoline (105) (50 mg, 0.146 mmol) in dry THF (10 ml). The reaction mixture was stirred at -78°C for 2h, and then at room temperature for 5h. The black mixture was poured into saturated aqueous
ammonium chloride (50 ml) and filtered through Celite. The solid residue was washed with chloroform (3 x 10 ml) and the filtrates combined. The aqueous layer was separated and extracted with chloroform (2 x 20 ml). The combined organic layers were dried (MgSO₄) and concentrated in vacuo to leave a pale yellow solid. The crude material was chromatographed (p.l.c., CHCl₃, 4 elutions) to give a pale yellow solid (48 mg) which was shown by n.m.r. to be a mixture of the starting material (105) and trimethyl 6,9-dihydro-1H-pyrrolo[2,3-f]quinoline-2,7,9-tricarboxylate (110), δ_H (250 MHz; CDCl₃) 3.72 (3H, s, CO₂CH₃), 3.87 (3H, s, CO₂CH₃), 3.91 (3H, s, CO₂CH₃), 5.02 (1H, d, J 4 Hz, 9-H), 5.76 (1H, dd, J 4 and ca. 0.5 Hz, 8-H), 6.60 (1H, d, J 8.8 Hz, 5-H), 6.63 (1H, br s, 6-H), 7.37 (1H, d, J 2.5 Hz, 3-H), 7.46 (1H, d, J 8.8 Hz, 4-H), 9.29 (1H, br s, 1-H).


(a) With Sodium Nitrate in Sulphuric Acid

Sodium nitrate (13 mg, 0.153 mmol) was added to a solution of the pyrroloquinoline (105) (50 mg, 0.146 mmol) in concentrated sulphuric acid (2 ml) at 0°C. The blood-red solution was stirred at 0°C for 1.5h and then poured onto ice (10g), neutralised with solid sodium hydrogen carbonate and extracted with chloroform (3 x 10 ml). The combined organic extracts were dried (MgSO₄) and concentrated in vacuo to give trimethyl 3-nitro-1H-pyrrolo[2,3-f]quinoline-2,7,9-tricarboxylate (112) (46.3 mg, 82%) as a yellow microcrystalline solid, m.p. 230-232°C (from methanol-nitromethane) (Found: C, 52.6; H, 3.3; N, 10.7. C₁₇H₁₃N₃O₈ requires C, 52.7; H, 3.4; N, 10.9%); ν_max (Nujol) 3180, 1740, 1705, 1515, 1435, 1355, 1290, 1255, 1230, 1200, 1165, 1120, 1075, 1015, 1000, 895, 855, 805, and 775 cm⁻¹; δ_H (90 MHz; CDCl₃) 4.13 (3H, s, CO₂CH₃),
4.17 (3H, s, CO$_2$CH$_3$), 4.23 (3H, s, CO$_2$CH$_3$), 8.19 (1H, d, $J$ 9 Hz, 4-H or 5-H), 8.50 (1H, d, $J$ 9 Hz, 4-H or 5-H), 8.96 (1H, s, 8-H), 13.30 (1H, br s, NH); m/z 387 ($M^+$, 72%), 357 (48), 329 (88), 297 (13), 282 (20), 253 (53), 163 (25), 59 (100).

(b) **With Copper (II) Nitrate Trihydrate in Trifluoroacetic Anhydride (TFAA)**

A suspension of the pyrroloquinoline (105) (50 mg, 0.146 mmol) in TFAA (5 ml) was stirred at room temperature for 4h. The mixture was cooled to 0°C and freshly powdered copper (II) nitrate trihydrate (18 mg, 0.075 mmol) was added. Stirring was continued at 0°C for 2h whereupon the reaction mixture was poured onto ice (20g) and extracted with chloroform (3 x 10 ml). The combined organic extracts were washed with saturated aqueous sodium hydrogen carbonate (3 x 10 ml), dried (MgSO$_4$) and concentrated *in vacuo* to leave a yellow solid (63 mg). The crude material was purified by chromatography (p.l.c., CH$_2$Cl$_2$, 5 elutions) to give trimethyl 3-nitro-1H-pyrrolo[2,3-f]quinoline-2,7,9-tricarboxylate (112) (37 mg, 66%) as bright yellow microcrystals.

15. **$\alpha$-Oxidation of Trimethyl 1H-pyrrolo[2,3-f]quinoline-2,7,9-tricarboxylate (105)**

(a) **With m-Chloroperoxybenzoic Acid (m-CPBA)**

A solution of the pyrroloquinoline (105) and m-CPBA (1.4 equiv.) in dichloromethane was stirred at room temperature for 2 weeks. Only starting material could be detected by t.l.c. and the solution was discarded.

(b) **With Peroxytrifluoroacetic Acid**

30% Hydrogen peroxide (6 equiv.) was added to a solution of the pyrroloquinoline (105) in TFA and the mixture stirred at room temperature
for 3 days. At this time t.l.c. showed that only the starting material (105) was present and the solution was discarded.

(c) With Dichloroperoxymaleic Acid

To a solution of dichloromaleic anhydride (0.305g, 1.83 mmol) in dichloromethane (10 ml) at 0°C was added 90% hydrogen peroxide (56 mg, 1.48 mmol) and the mixture stirred at 0°C for 2h. The pyrroloquinoline (105) (0.25g, 0.731 mmol) was added and the reaction mixture stirred for 15 min at 0°C, and then for 51h at 3°C. The mixture was diluted with chloroform (50 ml) and washed successively with 10% aqueous sodium carbonate (30 ml), 5% aqueous sodium hydrogen sulphite (30 ml) and water (30 ml). The organic phase was dried (MgSO₄) and concentrated in vacuo to give trimethyl 6-oxido-1H-pyrrolo[2,3-f]quinoline-2,7,9-tricarboxylate (113) (0.177g, 68%) as a yellow solid, m.p. 205-207°C (from dichloromethane) (Found: C, 56.9; H, 4.0; N, 7.8). C₁₇H₁₄N₂O₇ requires C, 57.0; H, 3.9; N, 7.8%; λmax (MeOH) 380, 312, 273 (sh), and 210 nm; νmax (Nujol) 3290, 1715, 1695, 1530, 1400, 1315, 1275, 1245, 1010, 980, 795, 760, 750, and 720 cm⁻¹; δH (250 MHz; CDCl₃) 4.02 (3H, s, CO₂CH₃), 4.09 (3H, s, CO₂CH₃), 4.14 (3H, s, CO₂CH₃), 7.39 (1H, d, J 2.1 Hz, 3-H), 8.07 (1H, d, J 9.3 Hz, 4-H or 5-H), 8.58 (1H, s, 8-H), 8.59 (1H, d, J 9.3 Hz, 4-H or 5-H), 13.05 (1H, br s, NH); m/z 342 (M-16, 3%), 324 (16), 308 (47), 277 (20), 250 (84), 218 (100).

16. Nitration of Trimethyl 6-oxido-1H-pyrrolo[2,3-f]quinoline-2,7,9-tricarboxylate (113)

Sodium nitrate (4.7 mg, 0.055 mmol) was added to a solution of the N-oxide (113) (20 mg, 0.056 mmol) in concentrated sulphuric acid (1 ml) at 0°C and the resulting blood-red solution stirred at 0°C for 1.5h. The mixture was poured onto ice (20g) and extracted with chloroform (3 x 20 ml). The combined organic extracts were dried (MgSO₄) and concentrated in vacuo
to give trimethyl 3-nitro-6-oxido-1H-pyrrolo[2,3-f]quinoline-2,7,9-tricarboxylate (114) (13.7 mg, 61%) as a yellow microcrystalline solid, m.p. 213-215°C (decomp.) (from methanol-chloroform) (Found: M⁺, 403.0667. C₁₇H₁₃N₃O₉ requires M, 403.0652); υ_max (CHCl₃) 3180, 1740, 1705, 1595, 1490, 1445, 1395, 1360, 1330, 1260, and 1000 cm⁻¹; δ_H (250 MHz; CDCl₃) 4.09 (3H, s, CO₂CH₃), 4.11 (3H, s, CO₂CH₃), 4.17 (3H, s, CO₂CH₃), 8.52 (1H, d, J 9.3 Hz, 4-H or 5-H), 8.67 (1H, s, 8-H), 8.75 (1H, d, J 9.3 Hz, 4-H or 5-H), 13.98 (1H, br s, NH); m/z 403 (M⁺, 9%), 387 (M⁻16, 68), 342 (11), 329 (69), 297 (11), 282 (23), 267 (12), 253 (41), 239 (10), 207 (6).

17. Thermolysis of Trimethyl 6-oxido-1H-pyrrolo[2,3-f]quinoline-2,7,9-tricarboxylate (113)

A solution of the N-oxide (113) (20 mg, 0.056 mmol) in dry DMF (5 ml) was heated under reflux for 8.5h. On cooling, the reaction mixture was diluted with chloroform (20 ml) and washed with water (5 x 50 ml). The organic layer was dried (MgSO₄) and concentrated to give trimethyl 1H-pyrrolo[2,3-f]quinoline-2,7,9-tricarboxylate (105) (8.1 mg, 42%).

18. Attempted Acylation of Trimethyl 6-oxido-1H-pyrrolo[2,3-f]quinoline-2,7,9-tricarboxylate (113)

(a) With p-Toluenesulphonyl Chloride

A solution of the N-oxide (113) in chloroform was treated with tosyl chloride (1.1 equiv.) and the mixture heated under reflux for 4h. Aqueous work-up gave a yellow solid which was identified as the starting material (113) by n.m.r. (100% recovery).
(b) With Acetic Anhydride

A solution of the N-oxide (113) (50 mg, 0.14 mmol) in freshly distilled acetic anhydride (5 ml) was heated under reflux for 11 h. On cooling, saturated aqueous sodium hydrogen carbonate (20 ml) was added and the mixture stirred for a further 12 h at room temperature. Chloroform (50 ml) was added, the layers were separated and the aqueous layer extracted with chloroform (2 x 20 ml). The combined organic solutions were dried (MgSO₄) and concentrated to leave a brown solid. The crude material was chromatographed (p.l.c., CH₂Cl₂, 3 elutions) to give trimethyl 1H-pyrrolo[2,3-f]quinoline-2,7,9-tricarboxylate (105) (27.2 mg, 57%).

19. Esterification of 4-Aminosalicylic Acid (118)

To a solution of 4-aminosalicylic acid (118) (20 g, 0.131 mol) in methanol (550 ml) was added 96% sulphuric acid (34 ml) and the resulting solution heated under reflux for 20 h. On cooling, the solution was concentrated in vacuo to ca. 150 ml whereupon a mass of colourless crystals were deposited. Water (400 ml) was added and the resulting suspension neutralised with solid sodium hydrogen carbonate. The product was collected by filtration, washed with water (300 ml) and dried in vacuo to give methyl 4-amino-2-hydroxybenzoate (19.2 g, 88%) as colourless needles, m.p. 120-121°C (lit., 152 123-125°C) (Found: C, 57.8; H, 5.4; N, 8.4. Calc. for C₈H₉NŌ₃: C, 57.5; H, 5.4; N, 8.4%); ν_max (Nujol) 3480, 3360, 3250, 1640, 1575, 1515, 1285, 1225, 1200, 1190, 1155, 1110, 1080, 955, and 840 cm⁻¹; δ_H (90 MHz; CDCl₃) 3.90 (3H, s, CO₂CH₃), 4.15 (2H, br s, NH₂), 6.05 - 6.22 (2H, m, 3-H and 5-H), 7.58 (1H, d, J 9 Hz, 6-H), 10.95 (1H, s, OH); m/z 167 (M⁺, 55%), 135 (100), 107 (40), 79 (40).
20. *N*-Acetylation of Methyl 4-amino-2-hydroxybenzoate

To a stirred solution of methyl 4-amino-2-hydroxybenzoate (21.7g, 0.13 mol) in absolute ethanol (300 ml) at 45°C was added, dropwise, acetic anhydride (13.3g, 0.13 mol). When addition was complete the reaction mixture was maintained at 45°C for 3.5h. On cooling, the mixture was poured into water (1 l). The resulting suspension was stirred for 1h and the product collected by filtration and dried *in vacuo* to give methyl 4-acetamido-2-hydroxybenzoate (137) (25.63g, 94%) as colourless needles, m.p. 150-153°C (lit., 152-153°C) (Found: C, 57.5; H, 5.2; N, 6.6. Calc. for C_{10}H_{11}NO_{4}: C, 57.4; H, 5.3; N, 6.7%). \( \nu_{\text{max}} \) (Nujol) 3320, 1675, 1600, 1540, 1495, 1340, 1285, 1270, 1250, 1210, 1170, and 1150 cm\(^{-1}\); \( \delta_{\text{H}} \) (90 MHz; (CD\(_3\))\(_2\)CO) 2.15 (3H, s, NHCO\(_2\)H\(_3\)), 3.94 (3H, s, CO\(_2\)C\(_{13}\)H\(_3\)), 7.09 (1H, dd, J 8 and 2 Hz, 5-H), 7.44 (1H, d, J 2 Hz, 3-H), 7.71 (1H, d, J 8 Hz, 6-H), 9.30 (1H, br s, NH), 10.72 (1H, br s, OH); \( m/z \) 209 (M\(^+\), 38%), 177 (19), 167 (38), 135 (100), 167 (19).

21. *O*-Methylation of Methyl 4-acetamido-2-hydroxybenzoate (137)

To a solution of methyl 4-acetamido-2-hydroxybenzoate (15g, 0.072 mol) in acetone (300 ml) was added powdered, anhydrous potassium carbonate (49.6g, 0.359 mol) and methyl iodide (101.87g, 0.718 mol) and the resulting suspension stirred under reflux for 8h. On cooling, the mixture was filtered through Celite and concentrated *in vacuo* to give methyl 4-acetamido-2-methoxybenzoate (119) (15.5g, 97%) as a colourless solid, m.p. 131-133°C (lit., 127°C) (Found: C, 59.1; H, 5.9; N, 6.25. Calc. for C\(_{11}H_{13}NO_{4}: C, 59.2; H, 5.8; N, 6.3%). \( \nu_{\text{max}} \) (Nujol) 3330, 3210, 3130, 1720, 1690, 1595, 1535, 1500, 1450, 1435, 1405, 1340, 1300, 1250, 1205, 1195, 1160, 1105, 1040, 1015, 865, 840, and 785 cm\(^{-1}\); \( \delta_{\text{H}} \) (250 MHz; CDCl\(_3\)) 2.18 (3H, s, NHCO\(_3\)H\(_3\)), 3.83 (3H, s), 6.91 (1H, dd,
22. Reduction of Methyl 4-acetamido-2-methoxybenzoate (119)

(a) With Diisobutylaluminium Hydride

A solution of the benzoate (119) in dry THF at -100°C was treated with DIBAL-H (2 equiv.). After stirring for 2.5h at -100°C, 10% hydrochloric acid was added and the mixture allowed to warm to room temperature. After 12h at room temperature, work-up gave a green gum which appeared to be a complex mixture which was not investigated further.

(b) With Lithium Aluminium Hydride

To a stirred suspension of lithium aluminium hydride (1.18g, 0.031 mol) in dry THF (40 ml) at 0°C, was added, dropwise, a solution of the benzoate (119) (3.45g, 0.016 mol) in dry THF (20 ml). When addition was complete the mixture was stirred at 0°C for 2h. Saturated aqueous sodium sulphate was added dropwise (CAUTION) to destroy the excess of reagent. The pale yellow solution was decanted from the inorganic salts which were washed with ethyl acetate (3 x 20 ml). The organic solutions were combined, dried (MgSO₄) and concentrated in vacuo to give 4-acetamido-2-methoxybenzyl alcohol (120) (3.0g, 98%) as a pale yellow solid, m.p. 137-139°C (from ethyl acetate) (Found: C, 61.5; H, 6.65; N, 7.1. C₁₀H₁₃N₃O₃ requires C, 61.5; H, 6.7; N, 7.2%); v_max (Nujol) 3480, 3440, 3330, 1660, 1610, 1600, 1530, 1495, 1420, 1320, 1290, 1265, 1160, 1130, 1035, and 1020 cm⁻¹; δ_H (90 MHz; CDCl₃) 2.07 (3H, s, NHCOCH₃), 3.75 (3H, s, ArOCH₃), 4.57 (2H, br s, CH₂OH), 6.98 (1H, dd, J 8 and 2 Hz, 5-H), 7.22 (1H, d, J 8 Hz, 6-H), 7.40 (1H, d, J 2 Hz, 3-H), 8.99 (1H, br s, NH) (OH not assigned); m/z 195 (M⁺, 70%), 153 (100), 138 (37), 136 (49), 124 (27), 110 (17), 93 (17), 92 (17), 65 (17).
23. Oxidation of 4-Acetamido-2-methoxybenzyl alcohol (120)

To a solution of the alcohol (120) (7.17 g, 0.037 mol) in a mixture of chloroform (200 ml) and methanol (5 ml), was added Attenburrow activated manganese (IV) oxide (46 g, 0.561 mol) and the resulting suspension stirred at room temperature for 20 h. The reaction mixture was filtered through Celite and concentrated in vacuo to give a pale brown solid. The crude material was purified by flash chromatography; eluting with ethyl acetate gave 4-acetamido-2-methoxybenzaldehyde (71) (5.64 g, 80%) as a pale yellow solid, m.p. 149-151°C (lit., 141-142°C) (Found: C, 62.3; H, 5.75; N, 7.2. Calc. for C10H11NO3: C, 62.2; H, 5.7; N, 7.25%); \( \nu \text{max} \) (Nujol) 3320, 3270, 3190, 3120, 1700, 1670, 1600, 1540, 1500, 1400, 1315, 1265, 1250, 1200, 1170, 1110, and 1030 cm\(^{-1}\); \( \delta_H \) (90 MHz; CDCl\(_3\)) 2.33 (3H, s, NHC\(_3\)), 4.03 (3H, s, ArOC\(_3\)), 6.87 (1H, dd, \( J \) 8 and 2 Hz, 5-H), 7.77 (1H, d, \( J \) 8 Hz, 6-H), 7.79 (1H, d, \( J \) 2 Hz, 3-H), 7.93 (1H, br s, NH), 10.52 (1H, s, CHO); \( m/z \) 193 (M\(^+\), 100%), 150 (85), 134 (69), 122 (23), 120 (23).

24. Condensation of 4-Acetamido-2-methoxybenzaldehyde (71) with Methyl azidoacetate

Sodium (0.715 g, 0.031 g atom) was added in portions to dry methanol (25 ml) and the mixture stirred until all the sodium had dissolved. The solution was cooled to -15°C whereupon a solution of the aldehyde (71) (1.5 g, 7.77 mmol) in a mixture of methyl azidoacetate (3.58 g, 0.031 mol) and methanol (15 ml) was added dropwise over 45 min. When addition was complete, the solution was stirred at -15°C for 2 h and then allowed to warm to room temperature. When nitrogen evolution had ceased, the mixture was poured into saturated aqueous ammonium chloride (100 ml) and extracted with ethyl acetate (4 x 100 ml). The organic extracts were combined, washed with water (3 x 150 ml), dried (MgSO\(_4\)) and concentrated
in vacuo to give a brown solid. The crude material was tritivated with ice-cold methanol to leave methyl 3-(4-acetamido-2-methoxyphenyl)-2-azido-propenoate (121) (1.65g, 73%) as a yellow solid, m.p. 135-138°C (decomp.) (yellow needles from methanol) (Found: C, 53.5; H, 4.8; N, 19.0. C_{13}H_{14}N_4O_4 requires C, 53.8; H, 4.8; N, 19.3%); \( \nu_{\text{max}} \) (Nujol) 3320, 3260, 3200, 3130, 2130, 1720, 1675, 1600, 1545, 1505, 1440, 1415, 1330, 1280, 1210, 1180, 1130, 1085, 1045, and 850 cm\(^{-1}\); \( \delta_{\text{H}} \) (250 MHz; d\(_6\)-DMSO) 2.03 (3H, s, NHCOC\(_3\)), 3.78 (3H, s), 3.82 (3H, s), 7.14 (1H, br d, \( J \) 9 Hz), 7.21 (1H, s, ArCH\(_2\)), 7.47 (1H, br s), 8.13 (1H, d, \( J \) 9 Hz), 10.18 (1H, br s, NH); \( m/z \) 262 (M\(^+\), 14%), 193 (100), 150 (68), 133 (52), 132 (23), 122 (18), 120 (16), 105 (21), 85 (43), 83 (68).

25. Thermolysis of Methyl 3-(4-acetamido-2-methoxyphenyl)-2-azido-propenoate (121)

A suspension of the vinyl azide (121) (1.26g, 4.35 mmol) in dry xylene (300 ml) was heated rapidly to 140°C. After 4h the solution was allowed to cool to room temperature whereupon the product was collected by filtration, washed with petrol and dried in vacuo to give methyl 6-acetamido-4-methoxyindole-2-carboxylate (122) (0.994g, 87%) as pale yellow prisms, m.p. 241-242°C (Found: C, 59.4; H, 5.4; N, 10.6. C\(_{13}H_{14}N_2O_4\) requires C, 59.5; H, 5.3; N, 10.7%); \( \nu_{\text{max}} \) (Nujol) 3420-3100 (br), 1700 1670, 1630, 1600, 1560, 1530, 1410, 1360, 1280, 1220, 1210, and 1140 cm\(^{-1}\); \( \delta_{\text{H}} \) (90 MHz; d\(_6\)-DMSO) 2.08 (3H, s, NHCOC\(_3\)), 3.85 (3H, s), 3.88 (3H, s), 6.70 (1H, br s, 7-H or 5-H), 7.03 (1H, d, \( J \) 3 Hz, 3-H), 7.62 (1H, br s, 7-H or 5-H), 9.89 (1H, br s, NHCOC\(_3\)), 11.72 (1H, br s, indole NH); \( m/z \) 262 (M\(^+\), 12%), 224 (18), 210 (8), 204 (10), 193 (72), 182 (12), 179 (15), 161 (16), 151 (46), 150 (100), 134 (43), 133 (23).
26. Acid Catalysed Methanolysis of Methyl 6-acetamido-4-methoxyindole-2-carboxylate (122)

To a saturated solution of dry hydrogen chloride in dry methanol (300 ml) was added the indole (122) (0.994 g, 3.79 mmol) and the resulting solution heated under reflux for 6h. On cooling, the solution was concentrated in vacuo to ca. 75 ml and partitioned between saturated aqueous sodium hydrogen carbonate (100 ml) and ethyl acetate (100 ml). The layers were separated and the aqueous layer extracted with ethyl acetate (3 x 50 ml). The combined organic solutions were dried (MgSO₄) and concentrated in vacuo to give methyl 6-amino-4-methoxyindole-2-carboxylate (123) (0.706 g, 85%) as a yellow solid which rapidly became red on standing in the air, δH (90 MHz; (CD₃)₂CO) 3.84 (3H, s), 3.87 (3H, s), 6.08 (1H, d, J 2 Hz, 7-H), 6.35 (1H, d, J 2 Hz, 5-H), 7.15 (1H, d, J 3 Hz, 3-H), 10.90 (1H, br s, indole NH) (NH₂ not assigned).

27. Reaction of Methyl 6-amino-4-methoxyindole-2-carboxylate (123) with Dimethyl 2-oxoglutaconate (20)

To a solution of the indole (123) (0.3 g, 1.36 mmol) in dichloromethane (60 ml) was added dimethyl 2-oxoglutaconate (20) (0.352 g, 2.05 mmol) and the resulting yellow solution stirred at room temperature for 12h. A catalytic amount of dry hydrogen chloride (one drop of a saturated solution in ether) was added and stirring continued for a further 12h. The mixture was diluted with chloroform (20 ml), washed with saturated aqueous sodium hydrogen carbonate (50 ml), dried (MgSO₄) and concentrated in vacuo to leave a brown solid. Trituration with hot methanol left trimethyl 4-methoxy-1H-pyrrolo[2,3-f]quinoline-2,7,9-tricarboxylate (116) (0.371 g, 73%) as bright yellow microcrystals, m.p. 270-274°C (decomp.) (Found: C, 58.0; H, 4.3; N, 7.4. C₁₈H₁₆N₂O₇ requires C, 58.1; H, 4.3; N, 7.5%); νmax (CHCl₃) 3300, 1720, 1605, 1585,
1360, and 1260 cm\(^{-1}\); \(\delta_H\) (250 MHz; CDCl\(_3\)) 4.01 (3H, s), 4.10 (3H, s), 4.12 (3H, s), 4.18 (3H, s), 7.32 (1H, s, 5-H), 7.50 (1H, d, \(j = 1\) Hz, 3-H), 8.78 (1H, s, 8-H), 12.58 (1H, br s, NH); \(m/z\) 372 (M\(^+\), 9%), 314 (6), 279 (23), 265 (34), 247 (9), 205 (6), 167 (45), 149 (100).

28. **Attempted Oxidation of Trimethyl 4-methoxy-1H-pyrrolo[2,3-\(f\)]-quinoline-2,7,9-tricarboxylate (116)**

(a) **With Cerium (IV) Ammonium Nitrate (CAN)\(^\text{19}\)**

CAN (0.41g, 0.748 mmol) was added to a solution of the pyrroloquinoline (116) (50 mg, 0.134 mmol) in 4:1 acetonitrile-water (30 ml) at 0\(^\circ\)C and the resulting orange solution stirred at 0\(^\circ\)C for 2h then heated at reflux for 6h. On cooling, the mixture was diluted with water (20 ml) and extracted with chloroform (3 x 20 ml). The combined extracts were dried (MgSO\(_4\)) and concentrated \textit{in vacuo} to give the starting material, 48 mg, (96% recovery).

(b) **With Silver (II) Oxide\(^\text{82}\)**

Silver (II) oxide\(^\text{153}\) (10 equiv.) and 6N nitric acid were added to a suspension of the pyrroloquinoline (116) in THF. After 1h at room temperature, water was added and the mixture extracted with dichloromethane to give a yellow solid which was shown by n.m.r. to be the starting material (116).

(c) **With Nitric Acid\(^\text{81}\)**

A solution of the pyrroloquinoline (116) in chloroform was treated with the dichloromethane-nitric acid 'reagent' described by Rapoport.\(^\text{81}\) After stirring at room temperature for 22h, the red solution was poured into water and extracted with chloroform to give a yellow solid which proved to be the starting material (116).
(d) **With Nitrous Acid**

A solution of the pyrroloquinoline (116) in chloroform was treated with sodium nitrite (7.3 equiv.) and 3M hydrochloric acid. After stirring at room temperature for 24h t.l.c. showed that only the starting material was present and the reaction mixture was discarded.

(e) **With Manganese (IV) Oxide Impregnated with Nitric Acid**

Excess of manganese (IV) oxide impregnated with nitric acid was added to a solution of the pyrroloquinoline (116) in chloroform. The mixture was stirred at room temperature for 60h and then at 65°C for 3h. T.l.c. analysis showed that only the starting material (116) was present and the mixture was discarded.

29. **Nitration of Trimethyl 4-methoxy-1H-pyrrolo[2,3-f]quinoline-2,7,9-tricarboxylate (116)**

(a) **With Sodium Nitrate in Sulphuric Acid**

Sodium nitrate (12 mg, 0.141 mmol) was added to a solution of the pyrroloquinoline (116) (50 mg, 0.134 mmol) in 96% sulphuric acid (2 ml) at 0°C. The dark red solution was stirred at 0°C for 1.5h then poured onto ice (10g). The resulting suspension was neutralised with solid sodium hydrogen carbonate and extracted with chloroform (3 x 10 ml). The combined extracts were dried (MgSO₄) and concentrated in vacuo to give trimethyl 4-methoxy-3-nitro-1H-pyrrolo[2,3-f]quinoline-2,7,9-tricarboxylate (130) (56 mg, 100%) as yellow microcrystals, m.p. 277-280°C (from methanol-nitromethane) (Found: C, 51.7; H, 3.6; N, 10.1. \( \text{C}_{18}\text{H}_{15}\text{N}_{3}\text{O}_9 \) requires C, 51.8; H, 3.6; N, 10.1%). \( \lambda_{\text{max}} \) (MeOH) 390 (sh), 346 (sh), 289, and 215 nm; \( \nu_{\text{max}} \) (Nujol) 3200, 1740, 1725, 1705, 1610, 1580, 1535, 1360, 1320, 1285, 1255, 1240, 1200, 1175, 1150, and 1010 cm\(^{-1}\); \( \delta_\text{H} \) (250 MHz; CDCl₃) 4.02 (3H, s), 4.06 (3H, s), 4.13 (3H, s),
4.19 (3H, s), 7.41 (1H, s, 5-H), 8.87 (1H, s, 8-H), 13.17 (1H, br s, NH); m/z 417 (M⁺, 7%), 277 (100), 201 (15).

(b) **With Nitrating Mixture**

To a solution of the pyrroloquinoline (116) (20 mg, 0.054 mmol) in 96% sulphuric acid (2 ml) was added an excess of fuming nitric acid (1 drop from a Pasteur pipette). The mixture was stirred at 0°C for 30 min and poured onto ice (10g). The aqueous mixture was extracted with chloroform (3 x 10 ml) and the combined, dried (MgSO₄) organic extracts concentrated in vacuo to give trimethyl 3,5-dinitro-4-methoxy-1H-pyrrolo[2,3-f]quinoline-2,7,9-tricarboxylate (131) (24.1 mg, 97%) as a cream solid, m.p. 253-254.5°C (from methanol-chloroform) (Found: M⁺, 462.0657. C₁₈H₁₄N₄O₁₁ requires M⁺, 462.0659; λmax (MeOH) 406 (sh), 386, 293, 263 (sh), and 212 nm; νmax (Nujol) 3210, 1750, 1710, 1610, 1540, 1280, 1265, 1220, 1215, 1175, and 1025 cm⁻¹; δH (250 MHz; CDCl₃) 4.05 (3H, s), 4.08 (3H, s), 4.19 (3H, s), 4.22 (3H, s), 9.01 (1H, s, 8-H), 13.37 (1H, br s, NH); m/z 462 (M⁺, 50%), 417 (90), 372 (100), 359 (61), 314 (63), 282 (76).

(c) **With Nitric Acid in Acetic Anhydride**

A solution of the pyrroloquinoline (116) in acetic anhydride was treated with excess of fuming nitric acid at 0°C. After 30 min at 0°C the mixture was poured onto ice and extracted with chloroform to give the starting material (116) (97% recovery).

(d) **With Tetranitromethane**

A suspension of the pyrroloquinoline (116) in pyridine was treated with a solution of tetranitromethane (2 equiv.) in ethanol and the mixture stirred at room temperature for 9h. Aqueous work-up and extraction
with chloroform gave a yellow solid which was purified by p.l.c. to give the starting material (60.5% recovery).

(e) With Nitronium Tetrafluoroborate

Nitronium tetrafluoroborate (1 equiv.) was added to a solution of the pyrroloquinoline (116) in dichloromethane. After stirring at room temperature for 6h the mixture was washed with water and concentrated to give the starting material (116) (94% recovery).

(f) With Nitronium Trifluoromethanesulphonate

The pyrroloquinoline (116) was treated with a solution of nitronium triflate-collidine complex (1 equiv.) in dichloromethane at 0°C. The mixture was stirred at room temperature for 1h and then heated under reflux for 7h. Aqueous work-up and extraction with chloroform gave a quantitative return of the starting material (116).

(g) With Silver Nitrate

Boron trifluoride etherate (excess) was added to a suspension of the pyrroloquinoline (116) and silver nitrate (1 equiv.) in dry acetonitrile. The red solution was stirred at room temperature for 2h whereupon aqueous work-up and extraction with chloroform gave the starting material (100% recovery).

(h) With N-Nitropyrazole

A solution of the pyrroloquinoline (116) dichloromethane was treated with N-nitropyrazole (4 equiv.) and boron trifluoride etherate (excess). After stirring at room temperature for 4h the mixture was poured into water and extracted with chloroform to give a yellow solid. P.l.c. of the crude material gave the starting material (97% recovery).
(i) With Copper (II) Nitrate Trihydrate in Acetic Anhydride

A suspension of the pyrroloquinoline (116) in acetic anhydride was treated with copper (II) nitrate trihydrate (1 equiv.) at room temperature. After 1.5h the mixture was poured into saturated aqueous sodium hydrogen carbonate and extracted with chloroform to give the starting material (116) (98% recovery).

(j) With Copper (II) Nitrate Trihydrate in Trifluoroacetic Acid

Copper (II) nitrate trihydrate (1 equiv.) was added to a solution of the pyrroloquinoline (116) in trifluoroacetic acid. After stirring at room temperature for 1.5h, the blood-red solution was poured onto saturated aqueous sodium hydrogen carbonate and extracted with chloroform to give a yellow solid. N.m.r. analysis of the solid showed it to be >98% starting material (116).

(k) With Copper (II) Nitrate Trihydrate in Trifluoroacetic Anhydride (TFAA)

A suspension of the pyrroloquinoline (116) (0.1g, 0.269 mmol) in TFAA (10 ml) was stirred at room temperature for 4h. The mixture was cooled to 0°C and copper (II) nitrate trihydrate (33 mg, 0.137 mmol) was added. Stirring was continued at 0°C for 2h whereupon the reaction mixture was poured onto ice (20g) and extracted with chloroform (3 x 20 ml). The combined organic extracts were washed with saturated aqueous sodium hydrogen carbonate (3 x 20 ml), dried (MgSO\(_4\)) and concentrated \textit{in vacuo} to give an orange solid (123 mg). The crude material was chromatographed (p.l.c., CH\(_2\)Cl\(_2\), 8 elutions) to give: (i) the starting material (116) \((ca. 16 \text{ mg, ca. 16% recovery})\), (ii) trimethyl 4-methoxy-3-nitro-1H-pyrrolo[2,3-f]quinoline-2,7,9-tricarboxylate (130) \((ca. 36 \text{ mg,}\

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ca. 38% based on 84% conversion), (iii) trimethyl 3,5-dinitro-4-methoxy-1H-pyrrolo[2,3-f]quinoline-2,7,9-tricarboxylate (131) (8.3 mg, 13% based on Cu(NO₃)₂·3H₂O), (iv) trimethyl 4-methoxy-5-nitro-1H-pyrrolo[2,3-f]quinoline-2,7,9-tricarboxylate (129) (27.7 mg, 30% based on 84% conversion) as a yellow solid, m.p. 263-265°C (Found: M⁺, 417.0824; C₁₈H₁₅N₃O₉ requires M⁺, 417.0808); \( \lambda_{\text{max}} \) (MeOH) 376, 298, 273, and 215 nm; \( \nu_{\text{max}} \) (CHCl₃) 3280, 1720, 1610, 1535, 1435, 1360, 1260, and 1000 cm⁻¹; \( \delta_{\text{H}} \) (250 MHz; CDCl₃) 4.05 (6H, s), 4.20 (3H, s), 4.36 (3H, s), 7.67 (1H, d, \( J = 2.8 \) Hz, 3-H, collapses to singlet on irradiation of signal at \( \delta 12.99 \)), 8.90 (1H, s, 8-H), 12.99 (1H, br s, NH); m/z 417 (M⁺, 100%), 387 (24), 359 (81), 327 (38).

(1) With Ammonium Nitrate in TFAA

A suspension of the pyrroloquinoline (116) (20 mg, 0.054 mmol) in TFAA (3 ml) was stirred at room temperature for 2h. Ammonium nitrate (4.3 mg, 0.054 mmol) was added and the mixture stirred at room temperature for a further 3.75h then poured onto ice (10g) and extracted with chloroform (3 x 10 ml). The combined organic extracts were washed with saturated aqueous sodium hydrogen carbonate (3 x 15 ml), dried (MgSO₄) and concentrated \textit{in vacuo} to give a yellow solid (29.1 mg). The crude material was chromatographed to give: (i) the starting material (116) (ca. 1.3 mg, ca. 6% recovery), (ii) trimethyl 4-methoxy-3-nitro-1H-pyrrolo[2,3-f]quinoline-2,7,9-tricarboxylate (130) (ca. 5.8 mg, ca. 28% based on 94% conversion), (iii) trimethyl 3,5-dinitro-4-methoxy-1H-pyrrolo[2,3-f]quinoline-2,7,9-tricarboxylate (131) (5.2 mg, 22%), (iv) trimethyl 4-methoxy-5-nitro-1H-pyrrolo[2,3-f]quinoline-2,7,9-tricarboxylate (129) (8.7 mg, 41% based on 94% conversion).
30. **Catalytic Reduction of Trimethyl 4-methoxy-5-nitro-1H-pyrrolo-[2,3-f]quinoline-2,7,9-tricarboxylate (129)**

A suspension of the nitropyrroloquinoline (129) (60 mg, 0.144 mmol) and 10% palladium on carbon (30 mg) in methanol (120 ml) was shaken at room temperature under 1 atm of hydrogen for 20 h. Chloroform (10 ml) was added and the mixture filtered through Celite. The filtrate was concentrated *in vacuo* to give a purple/black solid. The crude material was chromatographed (p.l.c., 5% methanol-dichloromethane, 1 elution) to give trimethyl 5-amino-4-methoxy-1H-pyrrolo[2,3-f]quinoline-2,7,9-tricarboxylate (133) (52 mg, 93%) as a purple solid, m.p. 202-204°C (Found: C, 55.9; H, 4.5; N, 10.9. C₁₉H₁₇N₃O₇ requires C, 55.8; H, 4.4; N, 10.85%); \( \lambda_{\text{max}} \) (MeOH) 388 (sh), 336, 309 (sh), 295 (sh) and 220 nm; \( \nu_{\text{max}} \) (CHCl₃) 3340, 1720, 1625, 1440, 1325, 1220, 1155, and 1000 cm⁻¹; \( \delta_H \) (250 MHz; CDCl₃) 4.00 (3H, s), 4.07 (3H, s), 4.09 (3H, s), 4.16 (3H, s), 4.92 (2H, br s, NH₂), 7.36 (1H, d, \( J \approx 2 \) Hz, collapses to singlet on irradiation of signal at \( \delta 12.24, 3-H \)), 8.82 (1H, s, 8-H), 12.24 (1H, br s, NH); \( m/z \) 387 (M⁺, 60%), 372 (27), 355 (29), 340 (100), 280 (49), 220 (20), 209 (16), 177 (20), 147 (24).

31. **Oxidation of Trimethyl 5-amino-4-methoxy-1H-pyrrolo[2,3-f]quinoline-2,7,9-tricarboxylate (133)**

Manganese (IV) oxide (44 mg, 0.537 mmol) was added to a solution of the aminopyrroloquinoline (133) (52 mg, 0.134 mmol) in 35% sulphuric acid (10 ml) at 0°C. The mixture was stirred at 0°C for 40 min then filtered through Celite. The filtrate was neutralised with solid sodium hydrogen carbonate and extracted with chloroform (3 x 10 ml). The combined extracts were dried (MgSO₄) and concentrated *in vacuo* to give trimethyl 4,5-dihydro-4,5-dioxo-1H-pyrrolo[2,3-f]quinoline-2,7,9-tricarboxylate (11) (36.5 mg, 73%) as a bright orange solid, m.p.
265-269°C (decomp.) (lit.,¹⁹ 260-263°C, decomp.; lit.,²⁰ 220°C, decomp.)

(Found: C, 54.6; H, 3.2; N, 7.45. Calc. for C₁₇H₁₂N₂O₈: C, 54.8; H, 3.2; N, 7.5%); λ_max (MeOH) 376, 306, and 251 nm; ν_max (CHCl₃) 1720, 1680, 1270, 1200, and 910 cm⁻¹; δ_H (250 MHz; CDCl₃) 3.99 (3H, s, CO₂CH₃), 4.08 (3H, s, CO₂CH₃), 4.19 (3H, s, CO₂CH₃), 7.48 (1H, d, J 1 Hz, 3-H), 8.90 (1H, s, 8-H), 12.98 (1H, br s, NH); m/z 372 (M⁺, 40%), 341 (75), 314 (45), 286 (75), 282 (50), 254 (100).

32. Demethylation of Trimethyl 4-methoxy-lH-pyrrolo[2,3-f]quinoline-2,7,9-tricarboxylate (116)

(a) With Boron Tribromide ⁹²

(i) A suspension of the pyrroloquinoline (116) in dichloromethane at -78°C was treated with boron tribromide (6 equiv.). The resulting clear, red solution was stirred at -78°C for 2h and then at room temperature for 12h. The mixture was poured into water and extracted with chloroform to give the starting material (96% recovery).

(ii) Boron tribromide (2 ml of a 1M solution in dichloromethane, 2 mmol) was added to the pyrroloquinoline (116) (50 mg, 0.134 mmol) and the resulting blood-red solution stirred at room temperature for 14h. Methanol (20 ml) was added (CAUTION) followed by 96% sulphuric acid (1 ml) and the mixture heated under reflux for 48h. On cooling the mixture was poured onto saturated aqueous sodium hydrogen carbonate (30 ml) and extracted with chloroform (3 x 30 ml). The combined extracts were dried (MgSO₄) and concentrated in vacuo. The residue was chromatographed (p.l.c., 5% methanol-chloroform, 2 elutions) to give, in addition to the starting material (116) (12 mg, 24% recovery), trimethyl 4-hydroxy-lH-pyrrolo[2,3-f]quinoline-2,7,9-tricarboxylate (134) (9.9 mg, 21% or 27% at 76% conversion) as yellow microcrystals,
m.p. 279-281°C (Found: C, 56.8; H, 4.1; N, 7.5. \(\text{C}_{17}\text{H}_{14}\text{N}_{2}\text{O}_{7}\) requires C, 57.0; H, 3.9; N, 7.8%); \(\lambda_{\text{max}}\) (MeOH) 368, and 300 nm; \(\nu_{\text{max}}\) (Nujol) 3290, 1700, 1590, 1260, and 1200 cm\(^{-1}\); \(\delta_{\text{H}}\) (250 MHz, d\(_6\)-DMSO) 4.00 (3H, s, CO\(_2\)CH\(_3\)), 4.07 (3H, s, CO\(_2\)CH\(_3\)), 4.18 (3H, s, CO\(_2\)CH\(_3\)), 7.32 (1H, s, 5-H), 7.54 (1H, d, \(J\) 2 Hz, 3-H), 8.67 (1H, s, 8-H), 12.48 (1H, br s, NH) (OH not assigned); \(m/z\) 358 (M\(^+\), 100%), 300 (61), 268 (53).

(b) With Trimethylsilyl Iodide

Trimethylsilyl iodide (6 equiv.) was added to a solution of the pyrroloquinoline (116) in deuteriochloroform in an n.m.r. tube. The tube was sealed and heated at 60°C. The n.m.r. spectrum of the mixture was recorded after 5h, 12h and 24h. No demethylation was observed and the mixture was discarded.

(c) With Trichlorosilyl Iodide

Silicon tetrachloride (5.5 equiv.) was added to a suspension of the pyrroloquinoline (116) and sodium iodide (5.5 equiv.) in 1:1 dichloromethane-acetonitrile at room temperature. The resulting red solution was heated under reflux for 14h. On cooling, the mixture was poured into methanol and concentrated \(\text{in vacuo}\). The residue was chromatographed (p.l.c., 5% methanol-chloroform) to give the starting material (116) (12.7 mg, 64% recovery) as the only isolable product.

(d) With Aluminium Chloride

Aluminium chloride (6 equiv.) was added to a suspension of the pyrroloquinoline (116) in xylene and the resulting red solution heated under reflux for 7h. On cooling, the mixture was poured onto ice and extracted with chloroform to give a dark yellow gum. T.l.c. showed the gum to consist of the starting material (116) together with a trace of the phenol (134).
(e) **With Lithium Iodide**

Lithium iodide (14 equiv.) was added to a suspension of the pyrroloquinoline (116) in 2,4,6-collidine and the mixture heated under reflux for 4h. On cooling, the mixture was poured into water, made acidic with 6N hydrochloric acid and extracted with chloroform. The residue was shown to consist entirely of collidine hydrochloride. No pyrroloquinoline products could be detected.

(f) **With Hydrobromic Acid**

A solution of the pyrroloquinoline (116) in 1:1 46% hydrobromic acid-acetic acid was heated under reflux for 5h. On cooling, the mixture was poured onto ice to give an aqueous mixture from which nothing could be extracted with chloroform. On standing, an orange solid separated from the aqueous phase but this appeared to be inorganic in nature (i.r.).

33. **Benzylation of Methyl 4-acetamido-2-hydroxybenzoate (137)**

To a solution of the benzoate (137) (20.75g, 0.099 mol) in acetone (400 ml) was added powdered anhydrous potassium carbonate (68.6g, 0.496 mol) and benzyl bromide (30.2g, 21 ml, 0.177 mol) and the resulting suspension stirred under reflux for 24h. On cooling, the mixture was filtered through Celite and concentrated *in vacuo*. The residual oil was triturated with petrol and the resulting solid filtered off and dried *in vacuo* to give methyl 4-acetamido-2-benzyloxybenzoate (138) (27.34g, 92%) as colourless prisms, m.p. 113-115°C (from ethyl acetate) (Found: C, 68.3; H, 5.8; N, 4.6. C_{17}H_{17}NO_{4} requires C, 68.2; H, 5.7; N, 4.7%); $\nu_{\text{max}}$ (Nujol) 3360, 1710, 1680, 1620, 1415, 1315, 1290, 1150, and 840 cm$^{-1}$; $\delta_{H}$ (90 MHz; CDCl$_3$) 2.14 (3H, s, NHC0CH$_3$), 3.90 (3H, s,
34. Reduction of Methyl 4-acetamido-2-benzyloxybenzoate (138)

To a suspension of lithium aluminium hydride (6.35g, 0.168 mol) in dry THF (300 ml) at 0°C under nitrogen, was added dropwise over 30 min, a solution of the benzoate (138) (25g, 0.084 mol) in THF (150 ml). The reaction mixture was stirred at 0°C for 3h whereupon 10% sodium hydroxide solution was added dropwise (CAUTION) to destroy excess of hydride. Magnesium sulphate (20g) was added and the mixture stirred at room temperature for 10 min then filtered through Celite. The filtrate was concentrated in vacuo to leave a pale yellow gum which crystallised from ethyl acetate to give 4-acetamido-2-benzyloxybenzyl alcohol (139) (18.86g, 83%) as colourless rods, m.p. 115-116°C (Found: C, 70.7; H, 6.3; N, 5.2. C16H17N03 requires C, 70.85; H, 6.3; N, 5.2%); νmax (Nujol) 3280 (br), 1655, 1610, 1540, 1510, 1420, 1280, 1260, 1170, 1130, 1045, 1030, 860, and 735 cm⁻¹; δH (90 MHz; (CD3)2C0) 2.15 (3H, s, NHC0CH3), 3.35 (1H, t, J 6 Hz, OH), 4.70 (2H, d, J 6 Hz, CH2OH), 5.10 (2H, s, CH2Ph), 6.97 (1H, dd, J 8 and 2 Hz, 5-H), 7.20 - 7.60 (7H, m), 8.82 (1H, br s, NH); m/z 271 (M⁺, 29%), 163 (13), 91 (100).

35. Oxidation of 4-Acetamido-2-benzyloxybenzyl alcohol (139)

Barium manganate¹⁵⁵ (50g, 0.195 mol) was added to a solution of the alcohol (139) (5g, 0.019 mol) in chloroform (250 ml) and the resulting suspension stirred under reflux for 1h. On cooling, the mixture was filtered through Celite and the filtrate concentrated in vacuo to
leave a tan solid. The crude material was recrystallised from methanol to give 4-acetamido-2-benzyloxybenzaldehyde (140) (5.03g, 100%) as a colourless solid, m.p. 156-158°C (sublimed at 140°C/0.04 mmHg) (Found: C, 71.1; H, 5.7; N, 5.25. C16H15NO3 requires C, 71.4; H, 5.6; N, 5.2%); \( \nu_{\text{max}} \) (Nujol) 3270, 1700, 1670, 1585, 1530, 1420, 1250, 1175, 1115, 1025, 730, and 695 cm\(^{-1}\); \( \delta_H \) (250 MHz; CDCl\(_3\)) 2.21 (3H, s, NHCOC\(_3\)), 5.18 (2H, s, CH\(_2\)Ph), 6.77 (1H, dd, \( J \) 8 and 2 Hz, 5-H), 7.30 - 7.48 (5H, m), 7.73 (1H, br s, NH), 7.79 (1H, d, \( J \) 8 Hz, 6-H), 7.90 (1H, d, \( J \) 2 Hz, 3-H), 10.42 (1H, s, CO); \( m/z \) 269 (M\(^+\), 39%), 240 (10), 227 (1), 199 (2), 178 (10), 136 (6), 91 (100).

36. Condensation of 4-Acetamido-2-benzyloxybenzaldehyde (140) with Methyl azidoacetate

Sodium (2.75g, 0.12g atom) was added in portions to dry methanol (80ml) and the mixture stirred under nitrogen until all of the sodium had dissolved. The solution was cooled to -10°C whereupon a solution of the aldehyde (140) (5.0g, 0.019 mol) and methyl azidoacetate (13.8g, 0.12 mol) in dry THF (50 ml) was added dropwise at such a rate that the temperature did not rise above -5°C. When addition was complete, the reaction mixture was stirred at -10°C for 5h and then at 2°C for 16h. The mixture was poured into saturated aqueous ammonium chloride (500 ml) and extracted with ethyl acetate (4 x 500 ml). The organic extracts were combined, washed with water (3 x 500 ml), dried (MgSO\(_4\)) and concentrated in vacuo to give methyl 3-(4-acetamido-2-benzyloxyphenyl)-2-azidopropenoate (142) (6.13g, 90%) as a pale yellow solid, m.p. 101-104°C (decomp.) (Found: C, 62.2; H, 4.85; N, 15.05. C\(_{19}\)H\(_{18}\)N\(_4\)O\(_4\) requires C, 62.3; H, 4.9; N, 15.3%); \( \nu_{\text{max}} \) (Nujol) 3320, 2120, 1700, 1665, 1605, 1585, 1520, 1420, 1300, 1280, 1180, 1125, 1085, 1045, 850, 815, and 740 cm\(^{-1}\); \( \delta_H \) (90 MHz; CDCl\(_3\)) 2.10 (3H, s, NHCOC\(_3\)), 3.82 (3H,
s, CO$_2$CH$_3$), 5.04 (2H, s, CH$_2$Ph), 6.75 (1H, dd, $\text{J}$ 8 and 2.5 Hz), 7.16 - 7.68 (8H, m), 8.09 (1H, d, $\text{J}$ 8 Hz); $m/z$ 338 ($M^+-28$, 9%), 269 (13), 91 (100).

37. **Thermolysis of Methyl 3-(4-acetamido-2-benzyloxyphenyl)-2-azidopropenoate (142)**

A suspension of the vinyl azide (142) (3.0g, 8.20 mmol) in dry xylene (350 ml) was rapidly heated to 140°C. After 1.5h, the solution was allowed to cool to room temperature whereupon the solid was filtered off and triturated with hot light petroleum (b.p. 60-80°C) to give methyl 6-acetamido-4-benzyloxyindole-2-carboxylate (141) (2.26g, 82%) as a tan solid, m.p. 263-265°C (from ethyl acetate) (Found: C, 67.5; H, 5.4; N, 8.3. C$_{19}$H$_{18}$N$_2$O$_4$ requires C, 67.5; H, 5.3; N, 8.3%); $\nu_{\text{max}}$ (Nujol) 3320, 1720, 1600, 1555, 1535, 1350, 1275, 1210, and 1135 cm$^{-1}$; $\delta_H$ (250 MHz, d$_6$-DMSO) 2.04 (3H, s, NHC0C$_3$), 3.82 (3H, s, CO$_2$CH$_3$), 5.19 (2H, s, CH$_2$Ph), 6.73 - 7.72 (8H, m), 9.98 (1H, br s, NH), 11.85 (1H, br s, NH); $m/z$ 338 ($M^+$, 24%), 297 (10), 248 (11), 216 (18), 91 (100).

38. **Alternative Small Scale Condensation - Thermolysis Procedure for the Preparation of Methyl 6-acetamido-4-benzyloxyindole-2-carboxylate (141)**

Sodium (0.171g, 0.007g atom) was dissolved in dry methanol (5 ml) and the solution cooled to -10°C. A solution of the aldehyde (140) (0.31g, 1.15 mmol) in dry THF (10 ml) was added dropwise, taking care that the temperature did not rise above -5°C. When addition was complete the mixture was stirred at -10°C for 4h and then allowed to warm to room temperature. When nitrogen evolution had ceased, the mixture was poured into saturated aqueous ammonium chloride (50 ml) and extracted with ethyl acetate (3 x 50 ml). The combined extracts were washed with
water (3 x 50 ml), dried (MgSO₄) and concentrated in vacuo to leave a bright yellow oil (0.667g). The oil was dissolved in methanol (5 ml) and added, dropwise, to boiling xylene (175 ml). When addition was complete the solution was heated under reflux for 1.5h. On cooling, the xylene was removed in vacuo to leave a brown solid. The crude mixture was column chromatographed to give, (i), on elution with 80% ether-petrol, 4-acetamido-2-benzylxybenzaldehyde (140) (0.128g, 41% recovery), (ii), on elution with ether, methyl 6-acetamido-4-benzylxy-indole-2-carboxylate (141) (0.202g, 52% or 88% at 59% conversion) which gave spectral data identical to that reported in Experiment 37.

39. Acid Catalysed Methanolysis of Methyl 6-acetamido-4-benzylxy-indole-2-carboxylate (141)

The indole (141) (1.85g, 5.47 mmol) was added to a saturated solution of dry hydrogen chloride in methanol (300 ml) and the solution heated under reflux for 2.5h. On cooling the solution was neutralised with saturated aqueous sodium hydrogen carbonate. Most of the methanol was removed in vacuo and the residue extracted with ethyl acetate (4 x 150 ml). The combined extracts were dried (MgSO₄) and concentrated in vacuo to leave a brown solid. The crude material was flash chromatographed (1:1 ether-ethyl acetate) to give methyl 6-amino-$\beta$-benzylxy-indole-2-carboxylate (143) (0.944g, 58%) as a tan solid, m.p. 217-220°C (decomp.) (Found: C, 69.05; H, 5.5; N, 9.55. C₁₇H₁₆N₂O₃ requires C, 68.9; H, 5.4; N, 9.5%); νₘₐₓ (Nujol) 3430, 3370, 1680, 1630, 1580, 1515, 1440, 1280, 1125, and 1080 cm⁻¹; δᵢ (250 MHz; CDC1₃) 3.88 (3H, s, CO₂CH₃), 5.15 (2H, s, CH₂Ph), 6.04 (1H, br s, 7-H), 6.26 (1H, br s, 5-H), 7.27 - 7.57 (5H, m), 8.52 (1H, br s, NH) (NH₂ not assigned); m/z 296 (M⁺, 74%), 265 (18), 205 (75), 177 (30), 173 (69), 145 (34), 117 (33), 91 (100).
40. Reaction of Methyl 6-amino-4-benzyloxyindole-2-carboxylate (143) with Dimethyl 2-oxoglutaconate (20)

Dimethyl 2-oxoglutaconate (20) (6.8 mg, 0.04 mmol) was added to a solution of the indole (143) (7.8 mg, 0.026 mmol) in dry dichloromethane (2 ml), and the resulting yellow solution stirred at room temperature for 12h. Dry hydrogen chloride (1 drop from a Pasteur pipette of a saturated solution in ether) was added and stirring continued for a further 12h. The mixture was diluted with chloroform (5 ml) and washed with saturated aqueous sodium hydrogen carbonate (5 ml). The organic solution was dried (MgSO$_4$) and concentrated in vacuo to leave a yellow-brown solid which was chromatographed (p.l.c., CH$_2$Cl$_2$, 2 elutions) to give trimethyl 4-benzyloxy-1H-pyrrolo[2,3-f]quinoline-2,7,9-tricarboxylate (144) (11.2 mg, 95%) as a bright yellow solid, m.p. 215-217°C (from methanol-chloroform) (Found: C, 64.65; H, 4.6; N, 6.3. C$_{24}$H$_{20}$N$_2$O$_7$ requires C, 64.3; H, 4.5; N, 6.25%); $\nu_{\max}$ (CHC1$_3$) 3310, 1720, 1600, 1580, 1360, 1290, 1260, and 1000 cm$^{-1}$; $\delta_H$ (250 MHz; CDCl$_3$) 4.00 (3H, s, CO$_2$C$_3$), 4.09 (3H, s, CO$_2$C$_3$), 4.14 (3H, s, CO$_2$C$_3$), 5.35 (2H, s, C$_6$H$_5$Ph), 7.36 - 7.56 (7H, m), 8.76 (1H, s, 8-H), 12.29 (1H, br s, NH); m/z 448 (M$^+$, 100%), 416 (14), 406 (14), 388 (18), 360 (11), 343 (4), 329 (7), 297 (18).

In a separate experiment on a larger scale (0.7g), involving trituration of the crude product with hot methanol as a means of purification, the yield was 68%.

41. Catalytic Hydrogenolysis of Trimethyl 4-benzyloxy-1H-pyrrolo[2,3-f]quinoline-2,7,9-tricarboxylate (144)

A suspension of the benzyloxyquinoquine line (144) (0.677g, 1.51 mmol) and 10% palladium on carbon (0.25g) in methanol (250 ml) was shaken at room temperature under 1 atm of hydrogen for 20h. The
mixture was diluted with chloroform (300 ml) and filtered through Celite. The filtrate was concentrated \textit{in vacuo} to give trimethyl 4-hydroxy-1H-pyrrolo[2,3-\textit{f}]quinoline-2,7,9-tricarboxylate (134) (0.484 g, 89\%) as a bright yellow powder whose spectral and other data were identical to that obtained for (134) prepared by demethylation of the methoxypyrroloquinoline (116).

42. Oxidation of Trimethyl 4-hydroxy-1H-pyrrolo[2,3-\textit{f}]quinoline-2,7,9-tricarboxylate (134)

(a) With Cerium (IV) Ammonium Nitrate (CAN)

A solution of the hydroxypyrroloquinoline (134) and cerium (IV) ammonium nitrate (5.5 equiv.) in 4:1 acetonitrile-water was stirred at room temperature for 20 h. A further 6.5 equiv. of CAN was added and the mixture heated at 60°C for 12 h. At this time no trace of PQQ trimethyl ester (11) could be detected by t.l.c. and the solution was discarded.

(b) With Fremy's Salt 65

Fremy's salt (24 mg, 0.089 mmol) was added to a suspension of the hydroxypyrroloquinoline (134) (13 mg, 0.036 mmol) in 1:1 methanol-water (6 ml) containing potassium dihydrogen phosphate (7 mg, 0.051 mmol), and the resulting mixture stirred at room temperature for 14 h. More Fremy's Salt (50 mg, 0.186 mmol) and potassium dihydrogen phosphate (10 mg, 0.073 mmol) were added and the mixture heated at 65°C for 10 h. On cooling the reaction mixture was diluted with water (15 ml) and extracted with chloroform (3 x 10 ml). The combined chloroform extracts were washed with saturated aqueous sodium hydrogen carbonate (10 ml), dried (MgSO$_4$) and concentrated \textit{in vacuo}. The solid residue was chromatographed (p.l.c., 2\% methanol-chloroform) to give (i), the
starting material (134) (4.8 mg, 37% recovery); (ii), trimethyl 4,5-
dihydro-4,5-dioxo-1H-pyrrolo[2,3-f]quinoline-2,7,9-tricarboxylate (11)
(1.7 mg, 13% or 20% at 63% conversion), identical (by n.m.r. and t.l.c.)
with PQQ trimethyl ester prepared in Experiment 31.

(c) With Benzoyl tert-Butyl Nitroxide

Benzoyl tert-butyl nitroxide (0.536g, 2.79 mmol, prepared (93%) by
potassium ferricyanide oxidation of tert-butylbenzhydroxamic acid
was added to a solution of the hydroxypyrroloquinoline (134) (0.1g, 0.279
mmol) in 9:1 dichloromethane-methanol (300 ml). The resulting dark
green solution was stirred at room temperature for 16h. The solvent
was removed *in vacuo* and the residue triturated with ether to leave
trimethyl 4,5-dihydro-4,5-dioxo-1H-pyrrolo[2,3-f]quinoline-2,7,9-tricar-
boxylate (11) (96.4 mg, 93%) as a bright orange solid whose spectral
characteristics and m.p. were identical to those of PQQ trimethyl ester
prepared in Experiment 31 and in the literature. 19,20

43. Hydrolysis of Trimethyl 4,5-dihydro-4,5-dioxo-1H-pyrrolo[2,3-f]-
quinoline-2,7,9-tricarboxylate, PQQ Trimethyl Ester (11) 19

A solution of PQQ trimethyl ester (11) (10 mg, 0.027 mmol) and
methyl orthoformate (28 mg, 29 μl, 0.264 mmol) in dry methanol (25 ml)
containing a trace of p-toluenesulphonic acid was heated under reflux
for 3h. On cooling, the solvent was removed *in vacuo* to leave a yellow
solid. 0.5M Aqueous potassium carbonate (15 ml) was added and the
resulting suspension heated at 85°C for 4h. On cooling the, now
homogeneous, yellow solution was acidified to pH 2.5 with 6N hydrochloric
acid, whereupon a red solid precipitated. The solid was collected by
centrifugation and dried *in vacuo* to give 4,5-dihydro-4,5-dioxo-1H-
pyrrolo[2,3-f]quinoline-2,7,9-tricarboxylic acid, coenzyme PQQ (1) (7 mg,
79%) as a dark red solid, identical to a sample of the natural coenzyme (kindly supplied by Dr. J.A. Duine of Delft University of Technology), t.l.c.; $R_f$ 0.38 (cellulose, 2% aqueous ammonium acetate-propanol, 1:1); $\lambda_{max}$ (H$_2$O, pH 7) 248, 269 (sh), and 332 nm, (H$_2$O, pH 1.5) 251 and 349 nm.
EXPERIMENTAL FOR PART TWO
1. Preparation of the Aryl Bromides (161), (171), (179) and (180)

(i) 2-(2-Bromophenyl)-1,3-dioxolane (161)

The title compound was prepared (87%) from 2-bromobenzaldehyde by the literature method, \(^{156}\) b.p. 95°C/0.35 mmHg (Kugelrohr) (lit., \(^{156}\) 126-127°C/5 mmHg).

(ii) 2-(2-Bromophenyl)-2-methyl-1,3-dioxolane (171)

The title compound was prepared (86%) from 2-bromoacetophenone by the literature method, \(^{157}\) b.p. 85°C/0.3 mmHg (Kugelrohr) (lit., \(^{157}\) 141-142°C/15 mmHg).

(iii) 8-Bromo-7-methoxy-1,2,3,4-tetrahydronaphthalen-1-one (179)

7-Methoxy-1-tetralone was brominated by the literature method\(^{134}\) to give the title compound (87%), m.p. 96-99°C (lit., \(^{134}\) 99°C).

(iv) Ketalisation of 8-Bromo-7-methoxy-1,2,3,4-tetrahydronaphthalen-1-one (179)

To a solution of the tetralone (179) (2.63 g, 0.01 mol) in dry benzene (40 ml) was added ethylene glycol (5.6 g, 5 ml, 0.09 mol) and p-toluenesulphonic acid (50 mg, 0.263 mmol) and the mixture heated under reflux for 24 h. On cooling the mixture was diluted with ether (40 ml) and washed with 10% aqueous sodium hydroxide (2 x 40 ml) and water (2 x 30 ml). The organic solution was dried (K₂CO₃) and concentrated \textit{in vacuo} to give the ethylene ketal (180) of 8-Bromo-7-methoxy-1,2,3,4-tetrahydronaphthalen-1-one (2.93 g, 95%) as a pale yellow oil which crystallised on standing, m.p. 90-91°C (from cyclohexane) (Found: C, 52.5; H, 5.0. C₁₃H₁₅BrO₃ requires C, 52.2; H, 5.0%); \(\nu_{\text{max}}\) (film) 1600, 1560, 1475, 1440, 1400, 1340, 1290, 1220, and 1140 cm\(^{-1}\); \(\delta_H\) (60 MHz; CDCl₃) 1.7 - 2.1 (4H, m), 2.7 (2H, br t, \(J\) 6 Hz), 3.75 (3H, s),
4.2 (4H, m), 6.85 (2H, ABq, $\delta$ 8.5 Hz); $m/z$ 298-300 (1:1, $M^+$, 52%), 270-272 (1:1, 35), 254-256 (1:1, 31), 226-228 (1:1, 18), 213-215 (1:1, 15), 198-200 (1:1, 18), 99 (100).

2. Preparation of the Aldehydes (162), (172), (181) and (188)

(i) 2-(1,3-Dioxolan-2-yl)-benzaldehyde (162)

$n$-Butyllithium (2.05 ml of a 1.6M solution in hexane, 3.28 mmol) was added dropwise to a solution of the bromide (161) (0.5g, 2.18 mmol) in dry THF (10 ml) at -100°C under nitrogen. When addition was complete, the solution was stirred at -100°C for 15 min whereupon dry DMF (0.48g, 0.51 ml, 6.6 mmol) was added. The mixture was allowed to warm to room temperature over 2h then poured into 0.05M hydrochloric acid (30 ml) and extracted with ether (3 x 20 ml). The combined ethereal extracts were dried ($K_2CO_3$) and concentrated in vacuo to leave a pale yellow oil (0.41g) which was distilled to give 2-(1,3-dioxolan-2-yl)-benzaldehyde (162) (0.34g, 86%) as a colourless oil, b.p. 95°C/0.35 mmHg (lit., 81°C/0.1 mmHg) (Found: C, 67.6; H, 6.0. Calc. for C$_{10}$H$_{10}$O$_3$: C, 67.4; H, 5.6%); $\nu_{\max }$ (film) 1690, 1600, 1405, 1385, 1270, 1215, 1075, 945, 820, and 760 cm$^{-1}$; $\delta_H$ (90 MHz; CDCl$_3$) 4.13 (4H, m), 6.41 (1H, s, ArCH), 7.30 - 7.80 (4H, m, ArH), 10.37 (1H, s, CHO); $m/z$ 178 ($M^+$, 24%), 149 (33), 133 (91), 122 (18), 118 (33), 105 (100), 89 (36), 77 (73).

(ii) 2-(2-Methyl-1,3-dioxolan-2-yl)-benzaldehyde (172)

$n$-Butyllithium (1.87 ml of a 1.65M solution in hexane, 3.09 mmol) was added dropwise to a solution of the bromide (171) (0.5g, 2.06 mmol) in dry THF (10 ml) at -78°C under nitrogen. When addition was complete, the pale orange suspension was stirred at -78°C for 5 min whereupon dry DMF (0.451g, 0.48 ml, 6.18 mmol) was added. The resulting clear solution was allowed to warm to room temperature over 2h then poured into
0.05M hydrochloric acid (25 ml) and extracted with ether (3 x 20 ml). The combined ethereal extracts were washed with water (3 x 15 ml), dried (K₂CO₃) and concentrated \textit{in vacuo} to leave a pale yellow oil. The crude material was distilled to give 2-(2-methyl-1,3-dioxolan-2-yl)-benzaldehyde (172) (0.38 g, 96%) as a colourless oil, b.p. 50°C/0.25 mmHg (Kugelrohr) (Found: C, 68.95; H, 6.5. C₁₁H₁₂O₃ requires C, 68.75; H, 6.25%); ν \text{max} (film) 3000, 2900, 1690, 1600, 1375, 1335, 1190, 1035, 950, 870, 825, and 765 cm⁻¹; δ \text{H} (60 MHz; CDCl₃) 1.77 (3H, s, C₃tf 3), 3.50 - 4.19 (4H, m, -0C₂tf 2C tf 20-), 7.13 - 7.98 (4H, m, ArH), 10.67 (1H, s, CtfO); m/z 193 (M⁺, 90%), 177 (7), 149 (66), 133 (14), 105 (19), 87 (63), 77 (20), 65 (11).

(iii) Formylation of the Tetralone Ketal (180)

n-Butyllithium (8.82 ml of a 1.65M solution in hexane, 0.015 mol) was added dropwise over 10 min to a stirred solution of the bromide (180) (2.9 g, 9.70 mmol) in dry THF (60 ml) at -78°C under nitrogen. The resulting red-brown solution was stirred at -78°C for 20 min whereupon dry DMF (2.12 g, 2.25 ml, 0.029 mol) was added dropwise. The reaction mixture was allowed to warm to room temperature over 2 h during which time the mixture became yellow and a precipitate appeared. The reaction mixture was poured into 0.05M sulphuric acid (100 ml) and extracted with ether (3 x 50 ml). The combined ethereal extracts were washed with water (3 x 50 ml), dried (K₂CO₃) and concentrated \textit{in vacuo} to leave a yellow oil which was purified by column chromatography. Eluting with 40% ether-petrol gave the aldehyde (181) (1.52 g, 63%) as a colourless oil, b.p. 135°C/0.35 mmHg (Kugelrohr) (Found: C, 67.9; H, 6.4. C₁₆H₁₆O₄ requires C, 67.7; H, 6.45%); ν \text{max} (film) 1710, 1615, 1600, 1585, 1480, 1350, 1290, 1265, 1145, 1120, 1055, 950, 935, and 860 cm⁻¹; δ \text{H} (60 MHz; CDCl₃) 1.70 - 2.00 (4H, m), 2.45 - 3.85 (2H, m, ArCH₂), 3.70 (3H, s, ArOCH₃), 4.00 (4H, s, -OCH₂CH₂O-), 6.90 (2H, ABq,
J 8.5 Hz, ArH), 10.25 (1H, s, CH0); m/z 248 (M^+, 30%), 220 (29), 203 (100), 115 (41), 99 (36), 91 (38), 77 (51), 54 (56).

(iv) 9-Oxofluorene-1-carbaldehyde (188)

The title compound was prepared (66%) by ozonolysis of fluoranthene under the conditions described by Callaghan and coworkers, m.p. 184-186°C (lit., 188-191°C).

3. Preparation of Vinyl Azides

(i) Condensation of 2-(1,3-Dioxolan-2-yl)-benzaldehyde (162) with Methyl azidoacetate

Sodium (0.26 g, 0.011 g atom) was added to dry methanol (10 ml) and the mixture stirred until all the sodium had dissolved. The solution was cooled to -15°C whereupon a solution of the aldehyde (162) (0.5 g, 2.83 mmol) in methyl azidoacetate (1.23 g, 0.011 mol) was added dropwise over 5 min. The reaction mixture was stirred at -15°C for 3 h and then allowed to warm to room temperature. When nitrogen evolution had ceased the mixture was poured into saturated aqueous ammonium chloride (20 ml) and extracted with ether (3 x 20 ml). The combined ethereal extracts were washed with water (3 x 10 ml), dried (MgSO\textsubscript{4}) and concentrated \textit{in vacuo} to leave a yellow oil which was purified by column chromatography. Eluting with 5% ether-petrol gave methyl 2-azido-3-[2-(1,3-dioxolan-2-yl)phenyl]propenoate (163) (0.491 g, 63%) as a pale yellow oil which solidified on standing at 2°C, m.p. 73-74°C (from ether) (Found: C, 56.6; H, 4.8; N, 15.2. C\textsubscript{13}H\textsubscript{13}N\textsubscript{3}O\textsubscript{4} requires C, 56.7; H, 4.7; N, 15.3%); ν\textsubscript{max} (film) 2140, 1720, 1620, 1440, 1380, 1310, 1260, 1115, 1080, 970, 950, 770, 760, and 660 cm\textsuperscript{-1}; δ\textsubscript{H} (250 MHz; CDCl\textsubscript{3}) 3.91 (3H, s, CO\textsubscript{2}CH\textsubscript{3}), 4.02 - 4.17 (4H, m, -OCH\textsubscript{2}CH\textsubscript{2}O-), 5.96 (1H, s, ArCH), 7.38 (2H, m, ArH), 7.39 (1H, s, -CH=C(N\textsubscript{3})CO\textsubscript{2}CH\textsubscript{3}), 7.58 (1H, dd, 6.88 (1H, d, J 8.8 Hz, ArH), 8.12 (1H, d, J 8.8 Hz, ArH).
J 8 and 2 Hz, ArH), 7.98 (1H, m, ArH); m/z 247 (M–28, 16%), 203 (63), 188 (16), 175 (14), 162 (27), 143 (48), 116 (92), 105 (63), 103 (19), 89 (61), 73 (80), 59 (91), 45 (56).

(ii) Cleavage of the Dioxolane Ring in Methyl 2-azido-3-[2-(1,3-dioxolan-2-yl)phenyl]-propenoate (163)

(a) With Wet Silica Gel

Wet silica gel (excess) was added to a solution of the vinyl azide (163) and the resulting suspension stirred at room temperature for one week. T.l.c. showed that no reaction had taken place and the mixture was discarded.

(b) By Acid Catalysed Exchange Dioxolanation

p-Toluenesulphonic acid (10 mg, 0.053 mmol) was added to a solution of the vinyl azide (163) (0.1g, 0.364 mmol) in acetone (5 ml) and the mixture stirred at room temperature for 20.5h. Ether (20 ml) was added and the solution washed with saturated aqueous sodium hydrogen carbonate (10 ml). The organic solution was dried (K₂CO₃) and concentrated in vacuo to give a pale yellow solid. The crude mixture was separated by chromatography (p.l.c., ether, 1 elution) to give (i) methyl 2-azido-3-(2-formylphenyl)propenoate (164) (15.9 mg, 19%), m.p. 95-97°C (Found: C, 56.9; H, 3.9; N, 18.1. C₁₁H₉N₃O₃ requires C, 57.1; H, 3.9; N, 18.2%); νₓ max (Nujol) 2140, 1710, 1695, 1600, 1420, 1260, 1075, and 760 cm⁻¹; δₓ (250 MHz; CDCl₃) 3.94 (3H, s, CO₂CH₃), 7.52’ (1H, dt, J 8 and 1.5 Hz), 7.63 (1H, dt, J 8 and 1.5 Hz), 7.71 (1H, s, ArCH₂C=N₃)CO₂CH₃), 7.87 (1H, dd, J 8 and 1.5 Hz), 7.94 (1H, d, J 8 Hz), 10.15 (1H, s, CHO); m/z 203 (M–28, 37%), 187 (6), 171 (8), 143 (21), 129 (34), 118 (M–113, 100), 89 (58), 59 (39), 51 (10); (ii) methyl isoquinol-1-one-3-carboxylate (165) (24.2 mg, 33%), m.p. 162-163°C (lit., 130 159-160°C) (Found: C, 65.0;
H, 4.3; N, 6.9. Calc. for C_{11}H_{9}NO_{3}: C, 65.0; H, 4.4; N, 6.9%; v_{\text{max}} (Nujol) 1725, 1660, 1305, 1215, 1150, 1000, 860, and 765 cm^{-1}; \delta_{\text{H}} (250 MHz; CDCl_{3}) 4.00 (3H, s, CO_{2}C_{3}), 7.38 (1H, s, 4-H), 7.58 - 7.78 (3H, m), 8.46 (1H, br d, J 8 Hz), 9.37 (1H, br s, NH); m/z 203 (M^{+}, 100%), 172 (6), 143 (69), 115 (28), 89 (33), 63 (15).

In a separate experiment, a solution of the azido-aldehyde (164) (0.1g, 0.433 mmol) in acetone (5 ml) containing p-toluenesulphonic acid (20 mg, 0.105 mmol) was stirred at room temperature for 2 days. The solvent was removed \textit{in vacuo} and the residual brown solid purified by flash chromatography (1:1 ether-petrol) to give methyl isoquinol-1-one-3-carboxylate (165) (87 mg, 99%).

(c) By Acid Catalysed Hydrolysis

6N Hydrochloric acid (6 drops) was added to a solution of the vinyl azide (163) (0.5g, 1.82 mmol) in 1:1 THF-water (100 ml) and the mixture stirred at room temperature for 22h. The reaction mixture was poured into saturated aqueous sodium hydrogen carbonate (50 ml) and extracted with ether (3 x 50 ml). The combined ethereal extracts were dried (MgSO_{4}) and concentrated \textit{in vacuo} to leave a pale yellow solid. The crude material was purified by column chromatography to give methyl 2-azido-3-(2-formylphenyl)propenoate (164) (0.316g, 75%) as colourless crystals whose spectral data were identical to those reported in Experiment 3(ii)(b).

(iii) Condensation of 2-(2-Methyl-1,3-dioxolan-2-yl)-benzaldehyde (172) with Methyl azidoacetate

Sodium (0.958g, 0.042g atom) was added in portions to dry methanol (30 ml) and the mixture stirred until all the sodium had dissolved. The solution was cooled to -15°C whereupon a solution of the aldehyde (172) (2g, 0.01 mol) in methyl azidoacetate (4.79g, 0.042 mol) was added.
dropwise over 10 min. When addition was complete the mixture was stirred for 5 h at -15°C and then allowed to warm to room temperature and poured into water (50 ml). The resulting precipitate was filtered off to give methyl 2-azido-3-[2-(2-methyl-1,3-dioxolan-2-yl)phenyl]propenoate (173) (1.08 g, 36%) as colourless crystals, m.p. 67-68°C (Found: C, 58.4; H, 5.2; N, 14.4. C₁₄H₁₅N₃O₄ requires C, 58.1; H, 5.2; N, 14.5%); ν<sub>max</sub> (Nujol) 2120, 1715, 1610, 1425, 1360, 1255, 1180, 1080, 1030, 945, 865, and 750 cm<sup>-1</sup>; δ <sub>H</sub> (60 MHz; CDCl₃) 1.8 (3H, s, CH₃), 3.9 (3H, s, CO₂CH₃), 3.5 - 4.2 (4H, m, -OCH₂CH₂O-), 7.1 - 7.6 (4H, m, ArH), 7.7 (1H, s, ArCH); m/z 261 (M-28, 26%), 246 (100), 214 (39), 202 (18), 176 (17), 158 (27), 105 (38), 87 (45).

(iv) Acid Catalysed Hydrolysis of Methyl 2-azido-3-[2-(2-methyl-1,3-dioxolan-2-yl)phenyl]propenoate (173)

6N Hydrochloric acid (6 drops) was added to a solution of the vinyl azide (173) (0.9 g, 3.11 mmol) in 1:1 THF-water (180 ml) and the mixture stirred at room temperature for 1 week. The reaction mixture was poured into saturated aqueous sodium hydrogen carbonate (150 ml) and extracted with ether (3 x 100 ml). The combined ethereal extracts were dried (MgSO₄) and concentrated in vacuo to leave a yellow solid. The crude mixture was column chromatographed to give (i) methyl 3-(2-acetyl-phenyl)-2-azidopropenoate (174) (0.47 g, 62%) as colourless crystals, m.p. 111-115°C (decomp.) (Found: C, 59.0; H, 4.5; N, 17.15. C₁₂H₁₁N₃O₃ requires C, 58.8; H, 4.5; N, 17.1%); ν<sub>max</sub> (Nujol) 2110, 1710, 1670, 1440, 1270, 1085, and 780 cm<sup>-1</sup>; δ <sub>H</sub> (60 MHz; CDCl₃) 2.6 (3H, s, COCH₃), 3.9 (3H, s, CO₂CH₃), 7.35 (1H, s, ArCH), 7.2 - 7.9 (4H, m, ArH); m/z 217 (M-28, 5.5%), 201 (8), 158 (12), 143 (60), 132 (100), 103 (62), 77 (37), 59 (39), 43 (60); (ii) methyl 1-methylisoquinoline-3-carboxylate (175) (11.4 mg, 1.8%) as a colourless oil which solidified on standing, m.p. 104-105°C (from cyclohexane) (Found: C, 71.8; H, 5.4; N, 6.95.
C_{12}H_{11}NO_2 requires C, 71.6; H, 5.5; N, 7.0%; ν\text{max} (Nujol) 1730, 1285, 1235, 1210, 1150 and 795 cm\(^{-1}\); δ\text{H} (90 MHz; CDCl\(_3\)) 3.05 (3H, s, CH\(_3\)), 4.08 (3H, s, CO\(_2\)CH\(_3\)), 7.65 - 8.20 (4H, m), 8.40 (1H, s, 4-H); m/z 201 (M\(^+\), 14%), 171 (8), 143 (100), 115 (24).

(v) Condensation of the Aldehyde (181) with Methyl azidoacetate

Sodium (0.482g, 0.021g atom) was added in portions to dry methanol (15 ml) and the mixture stirred until all the sodium had dissolved. The solution was cooled to -15°C whereupon a solution of the aldehyde (181) (1.3g, 5.24 mmol) in methyl azidoacetate (2.41g, 0.021 mol) was added dropwise over 15 min. When addition was complete the mixture was stirred at -15°C for 2h and then at 2°C for 14h. The reaction mixture was poured into water (50 ml) and extracted with ether (3 x 30 ml). The combined ethereal extracts were dried (K\(_2\)CO\(_3\)) and concentrated \textit{in vacuo} to leave a gum (1.08g). The crude material was column chromatographed to give, (i) the starting material (181) (0.568g, 44% recovery); (ii) the azidopropenoate (182) (0.407g, 40% at 56% conversion) as a pale yellow gum, ν\text{max} (film) 2130, 1720, 1635, 1480, 1440, 1350, 1265, 1140, 1060, and 950 cm\(^{-1}\); δ\text{H} (60 MHz; CDCl\(_3\)) 1.7 - 2.0 (4H, m), 2.6 - 2.9 (2H, m, ArCH\(_2\)), 3.75 (3H, s), 3.85 (3H, s), 4.0 (4H, s, -OCH\(_2\)CH\(_2\)O-), 6.95 (2H, ABq, J 8.5 Hz, ArH), 7.30 (1H, s, ArCH=CH(N\(_3\))CO\(_2\)CH\(_3\)); m/z 317 (M-28, 5%), 287 (5), 257 (55), 232 (19), 199 (100), 176 (15), 154 (16), 119 (30). No satisfactory microanalysis was obtained for compound (182).

(vi) Acid Catalysed Hydrolysis of the Azidopropenoate (182)

6N Hydrochloric acid (6 drops) was added to a solution of the vinyl azide (182) (0.4g, 1.16 mmol) in 1:1 THF-water (70 ml) and the mixture stirred at room temperature for 4h. The reaction mixture was poured into saturated aqueous sodium hydrogen carbonate (100 ml) and extracted
with ether (3 x 50 ml). The combined ethereal extracts were dried
(MgSO₄) and concentrated in vacuo to give a green solid which was tri-
turated with ether to leave methyl 2-azido-3-(7-methoxy-1,2,3,4-tetra-
hydronaphthalen-1-on-8-yl)propenoate (183) (0.255 g, 73%) as a colour-
less solid, m.p. 100°C (decomp.) (from dichloromethane-cyclohexane)
(Found: C, 60.0; H, 5.0; N, 14.0. C₁₅H₁₅N₃O₄ requires C, 59.8; H, 5.0;
N, 13.95%); \( \nu_{\text{max}} \) (Nujol) 2120, 1720, 1710, 1630, and 1110 cm⁻¹; \( \delta \) \( \text{H} \)
(60 MHz; CDCl₃) 1.9 - 2.4 (2H, m), 2.5 - 3.1 (4H, m), 3.9 (3H, s), 7.1
(2H, ABq, \( j \) 8.5 Hz, ArH), 7.35 (1H, s, ArCH=C(N₃)CO₂CH₃); \( m/z \) 188 (M-
113, 100%), 173 (38), 145 (8), 129 (10), 115 (25), 84 (13), 75 (7),
54 (67).

(vii) Condensation of 9-Oxofluorene-1-carbaldehyde (188) with Methyl
azidoacetate

Sodium (1.33 g, 0.058 g atom) was added in portions to dry methanol
(80 ml) and the mixture stirred until all the sodium had dissolved. The
solution was cooled to -15°C whereupon a solution of the aldehyde (188)
(3 g, 0.014 mol) in a mixture of methyl azidoacetate (6.64 g, 0.058 mol)
and dry methanol (20 ml) was added dropwise over 30 min. When addition
was complete, the mixture was stirred at -15°C for 2 h and then at 2°C
for 14 h. The reaction mixture was poured into saturated aqueous
ammonium chloride (150 ml) and extracted with ethyl acetate (4 x 50 ml).
The combined organic extracts were washed with water (3 x 50 ml), dried
(MgSO₄) and concentrated in vacuo. The residue was triturated with
ice-cold methanol to leave methyl 2-azido-3-(fluoren-9-on-1-yl)propenoate
(189) (2.21 g, 50%) as bright yellow needles, m.p. 125°C
(decomp.) (from dichloromethane) (Found: C, 66.7; H, 3.6; N, 13.5.
C₁₇H₁₁N₃O₃ requires C, 66.9; H, 3.6; N, 13.8%); \( \nu_{\text{max}} \) (Nujol) 2140, 2120,
1710, 1700 (sh), 1610, 1430, 1300, 1255, 930, 805, 765, 755, and 670
cm⁻¹; \( \delta \) \( \text{H} \) (250 MHz; CDCl₃) 3.98 (3H, s, CO₂CH₃), 7.27 - 7.34 (1H, m),
7.44 - 7.54 (4H, m), 7.63 (1H, dt, J 8 and 1 Hz), 8.17 (1H, m), 8.21 (1H, s, ArCH=C(N3)CO2CH3); m/z 305 (M⁺, 1%), 291 (5), 277 (M-28, 57), 245 (100), 217 (11), 203 (37), 189 (39), 163 (12), 123 (12), 109 (18), 101 (6), 95 (13), 87 (8), 82 (20).

4. Reactions of Vinyl Azides with Phosphorus (III) Reagents

(i) Reaction of Methyl 2-azido-3-(2-formylphenyl)propenoate (164) with Triethyl Phosphite (TEP)

To a solution of the vinyl azide (164) (0.1g, 0.433 mmol) in dry benzene (10 ml) was added triethyl phosphite (79.1 mg, 0.082 ml, 0.476 mmol) and the resulting colourless solution stirred at room temperature for 21h. The mixture was diluted with ether (50 ml), washed with water (2 x 30 ml), dried (MgSO₄) and concentrated in vacuo to give methyl isoquinoline-3-carboxylate (169) (74.1 mg, 91%) as a pale yellow solid, m.p. 88-89°C (colourless rods from cyclohexane) (lit., 86-88°C) (Found: C, 70.6; H, 4.8; N, 7.4. Calc. for C₁₁H₉N₂O₂: C, 70.6; H, 4.8; N, 7.5%); ν max (Nujol) 1730, 1430, 1330, 1290, 1225, 1180, 1140, 1095, 960, 915, 800, 790, 770, 755, and 640 cm⁻¹; δH (60 MHz; CDCl₃) 4.03 (3H, s, CO₂CH₃), 7.6 - 8.1 (4H, m), 8.52 (1H, s, 4-H), 9.27 (1H, s, 1-H); m/z 187 (M⁺, 38%), 157 (30), 129 (100), 101 (25), 77 (25), 64 (7), 51 (16).

(ii) Reaction of Methyl 3-(2-acetylphenyl)-2-azidopropenoate (174) with Triethyl Phosphite (TEP)

To a solution of the vinyl azide (174) (0.1g, 0.408 mmol) in dry benzene (7 ml) was added triethyl phosphite (0.102g, 0.614 mmol) and the colourless solution stirred at room temperature for 4.5h. The solvent, and excess of TEP, were removed in vacuo to leave a tan crystalline solid which was purified by flash chromatography. Eluting with ethyl acetate gave methyl 1-methylisoquinoline-3-carboxylate (175) (76.6 mg,
as colourless plates whose spectral data were identical with those reported in Experiment 3(iv).

(iii) Reaction of Methyl 2-azido-3-(7-methoxy-1,2,3,4-tetrahydro-1H-naphthalen-1-on-8-yl)propenoate (183) with Triethyl Phosphite (TEP)

Method A

To a solution of the vinyl azide (183) (0.1g, 0.332 mmol) in dry benzene (12 ml) was added TEP (82.8 mg, 85 µl, 0.498 mmol) and the mixture stirred at room temperature for 16h. T.l.c. analysis of the yellow solution indicated that the starting material had been consumed to give two compounds, one of which exhibited the characteristic bright blue fluorescence of isoquinolines. The solution was heated at 75°C for 1h whereupon t.l.c. analysis showed no change in the composition of the mixture. On cooling, the mixture was diluted with ether (50 ml) and washed with water (3 x 20 ml). Magnesium sulphate was added to the yellow ether-benzene solution whereupon the yellow colour disappeared instantaneously. T.l.c. analysis of the colourless solution showed it to contain only a single component. The solution was concentrated in vacuo to give methyl 5-methoxy-cyclohex[1j]isoquinoline-3-carboxylate (184) (79.6 mg, 93%) as colourless plates, m.p. 122-123°C (from cyclohexane) (Found: C, 70.1; H, 5.9; N, 5.4. C_{15}H_{15}NO_3 requires C, 70.0; H, 5.8; N, 5.45%); ν_{max} (Nujol) 1715, 1615, 1495, 1470, 1270, 1240, 1215, 1195, 1050, 1030, and 815 cm\(^{-1}\); δ_{H} (60 MHz; CDCl_{3}) 1.95 - 2.3 (2H, m, CH\_2CH\_2CH\_2), 2.8 - 3.4 (4H, m, CH\_2CH\_2CH\_2), 3.95 (3H, s), 4.0 (3H, s), 7.05 (2H, ABq, J 8.5 Hz), 8.7 (1H, s); m/z 257 (M\(^+\), 47%), 199 (100), 182 (5), 154 (8), 127 (8).
Method B

To a solution of the vinyl azide (183) (10 mg, 0.033 mmol) in dry benzene (0.5 ml) was added TEP (40 mg, 0.241 mmol) and magnesium sulphate (0.1 g, 0.831 mmol). The mixture was stirred at room temperature for 3 h, diluted with ether (5 ml), and washed with water (3 x 2 ml). The organic solution was dried (MgSO_4) and concentrated in vacuo to give methyl 5-methoxy-cyclohex[\(\alpha\)]isoquinoline-3-carboxylate (184) (7.8 mg, 91%).

(iv) Reaction of Methyl 2-azido-3-(fluoren-9-on-1-yl)propenoate (189) with Triethyl Phosphite (TEP)

To a suspension of the vinyl azide (189) (0.1 g, 0.328 mmol) in dry xylene (10 ml) was added TEP (81.7 mg, 0.492 mmol) and the mixture stirred at room temperature for 18 h. T.l.c. analysis of the resulting orange solution showed that the vinyl azide (189) had been completely converted into an orange compound. Magnesium sulphate (excess) was added but had no effect (as judged by t.l.c.) and the mixture was heated at 60°C for 8 h. On cooling, the mixture was filtered, washed with water (2 x 10 ml), dried (MgSO_4) and concentrated in vacuo to give methyl 3-(fluoren-9-on-1-yl)-2-iminotriethoxyphosphoranylpropenoate (190a) (0.147 g, ca. 100%) as an orange gum, \(\delta_H\) (60 MHz; CDCl_3) 1.3 (9H, dt, \(J = 8\) and 1 Hz, -OCH_2CH_3), 3.8 (3H, s, CO_2CH_3), 4.1 (6H, m, -OCH_2CH_3), 6.9-7.7 (6H, m, ArH), 8.0 (1H, d, \(J = 9\) Hz), 8.9 (1H, dd, \(J = 6\) and 3 Hz). On standing in the air for several days, the iminophosphorane (190a) was converted into the corresponding phosphoramidate (191), m.p. 166-168°C (from chloroform-cyclohexane) (Found: C, 60.8; H, 5.4; N, 3.3. \(C_{21}H_{22}NO_6P\) requires C, 60.7; H, 5.3; N, 3.4%); \(\nu_{\text{max}}\) (Nujol) 3090, 1720, 1700, 1630, 1610, 1435, 1300, 1280, 1240, 1190, 1130, 1035, 970, and 750 cm\(^{-1}\); \(\delta_H\) (250 MHz; CDCl_3) 1.28 (6H, dt, \(J = 7.6\) and 0.3 Hz, -OCH_2CH_3),
3.91 (3H, s, CO₂CH₃), 3.98 (4H, dq, J 7.6 and 3.8 Hz, -OC₂H₃), 4.98 (1H, br s, exch. D₂O, NH), 7.31 (1H, dt, J 7 and 0.3 Hz), 7.45 - 7.56 (4H, m, ArH), 7.64 (1H, br d, J 7 Hz), 7.76 (1H, m), 7.89 (1H, d, J 1.7 Hz); m/z 415 (M⁺, 68%), 356 (70), 328 (8), 262 (7), 246 (6), 220 (100), 203 (16), 193 (59), 164 (25), 137 (12), 109 (62).

(v) Reaction of Methyl 2-azido-3-(fluoren-9-on-l-yl)propenoate (189) with Trimethyl Phosphite (TMP)

To a solution of the vinyl azide (189) (0.1g, 0.328 mmol) in dry benzene (10 ml) was added trimethyl phosphite (61 mg, 0.492 mmol) and the mixture stirred at room temperature for 24h. The solvent and excess of TMP were removed in vacuo to give methyl 3-(fluoren-9-on-yl)-2-iminotrimethoxyphosphoranylpropenoate (190b) (0.13g, ca. 100%) as an orange gum, λ_max (EtOH) 327, 293, and 256 nm; ν_max (CHCl₃) 1710, 1610, 1595, 1450, 1260, 1050, 910, and 840 cm⁻¹; δ_H (90 MHz; CDCl₃) 3.82 (9H, d, J 12 Hz, -P(0)(0C₆H₅)₃), 3.93 (3H, s, CO₂CH₃), 6.90 - 7.65 (6H, m, ArH), 8.04 (1H, d, J 8 Hz), 8.83 (1H, dd, J 7 and 2 Hz). On standing in the air, the iminophosphorane (190b) was slowly converted into the corresponding phosphoramidate (192), m.p. 184-187°C (from chloroform-petrol) (Found: C, 58.7; H, 4.7; N, 3.6. C₁₉H₁₈N₂O₆P requires C, 58.9; H, 4.65; N, 3.6%); ν_max (Nujol) 3130, 1725, 1710, 1630, 1605, 1430, 1275, 1245, 1185, 1130, 1040, 830, 790, and 755 cm⁻¹; δ_H (250 MHz; CDCl₃) 3.60 (6H, d, J 11.4 Hz, P(0)(0C₆H₅)₂), 3.92 (3H, s, CO₂CH₃), 5.03 (1H, br s, NH), 7.27 - 7.35 (1H, dt, J 11 and 2 Hz, ArH), 7.45 - 7.54 (4H, m, ArH), 7.61 - 7.67 (2H, m, ArH), 7.86 (1H, d, J 1.3 Hz); m/z 387 (M⁺, 20%), 328 (46), 219 (5), 203 (7), 193 (17), 163 (8), 109 (100).
(vi) Reaction of Methyl 2-azido-3-(fluoren-9-on-1-yl)propenoate (189) with Hexamethyl Phosphorous Triamide

A solution of the vinyl azide (189) in xylene was treated with hexamethyl phosphorous triamide (1.1 equiv.). The solution immediately turned red and t.l.c. analysis showed that the azide had been completely consumed to give a red, baseline material. The red solution was stirred at room temperature for 4h whereupon t.l.c. analysis showed no change and the mixture was heated under reflux for 1h. On cooling, the solvent was removed in vacuo to leave a black gum which appeared to be a highly complex mixture by t.l.c. and was discarded.

(vii) Reaction of Methyl 2-azido-3-(fluoren-9-on-1-yl)propenoate (189) with Triphenylphosphine

A solution of the vinyl azide (189) (0.5g, 1.64 mmol) and triphenylphosphine (0.473g, 1.81 mmol) in dry xylene (50 ml) was stirred at room temperature for 16h. The solvent was removed in vacuo and the residue purified by flash chromatography to give methyl 3-(fluoren-9-on-1-yl)-2-iminotriphenylphosphoranylpropenoate (190d) (0.871g, 99%) as a bright orange solid, m.p. 209-211°C (from petrol-chloroform) (Found: C, 77.7; H, 4.8; N, 2.5. C_{35}H_{26}NO_{3}P requires C, 77.9; H, 4.8; N, 2.6%); \( \lambda_{\text{max}} \) (EtOH) 358, 290 (sh), and 255 nm; \( \nu_{\text{max}} \) (Nujol) 1700, 1610, 1585, 1430, 1400, 1240, 1225, 1190, 1110, 760, 715, and 700 cm\(^{-1}\); \( \delta_{\text{H}} \) (90 MHz, CDCl\(_3\)) 3.55 (3H, s, CO\(_2\)CH\(_3\)), 7.14 - 7.89 (21H, m, ArH), 8.03 (1H, d, \( J \) 8 Hz), 9.10 (1H, dd, \( J \) 6 and 3 Hz); \( m/z \) 539 (M\(^+\), 2%), 497 (5), 480 (2), 469 (3), 395 (2), 354 (3), 304 (9), 277 (100), 262 (12), 216 (13), 201 (51), 183 (22), 152 (20), 77 (44).
(viii) Reaction of Methyl 2-azido-3-(fluoren-9-on-1-yl)propenoate (189) with 1,2,5-Triphenylphosphole

A solution of the vinyl azide (189) (1g, 3.28 mmol) and 1,2,5-triphenylphosphole (1.53g, 4.90 mmol) in dry toluene (50 ml) was heated under reflux for 1.5h. On cooling to room temperature the product was collected by filtration and dried in vacuo to give methyl 3-(fluoren-9-on-1-yl)-2-{imino-1,2,5-triphenylphosphoryl}propenoate (196) (1.37g, 71%) as a dark red microcrystalline solid, m.p. 249-251°C (from petrol-chloroform) (Found: C, 79.3; H, 4.7; N, 2.3. C_{39}H_{28}NO_{3}P requires C, 79.5; H, 4.75; N, 2.4%). λ_{max} (EtOH) 370, 300 (sh), 265, and 261 nm; ν_{max} (Nujol) 1700, 1690, 1610, 1580, 1430, 1400, 1420, 890, 860, and 760 cm\(^{-1}\); δ_{H} (90 MHz; CDCl\(_{3}\)) 3.69 (3H, s, CO\(_2\)C\(_2\)), 7.05 - 8.20 (23H, m, Artt), 8.15 (1H, d, J 8 Hz), 9.20 (1H, dd, J 7 and 3 Hz); m/z 589 (M\(^{+}\), 40%), 530 (5), 407 (11), 354 (12), 344 (27), 328 (82), 312 (9), 297 (23), 277 (39), 261 (20), 251 (22), 245 (65), 203 (100), 187 (32), 157 (25), 145 (33), 131 (65), 105 (78).

5. Preparation of Methyl 1-azafluoranthene-2-carboxylate (193)

(i) By Melt Pyrolysis of the Iminophosphorane (190a)

The iminophosphorane (190a) (0.2g, 0.451 mmol) was placed in a sublimation apparatus and lowered into a pre-heated Woods-metal bath at 300°C. After 1 min the heating bath was removed and the cold-finger washed with dichloromethane. The cold-finger washings were concentrated in vacuo to leave a yellow gum which was chromatographed (p.l.c., 1:1 ether-petrol, 2 elutions) to give methyl 1-azafluoranthene-2-carboxylate (193) (12.3 mg, 10%) as a pale yellow solid, m.p. 115-117°C (Found: M\(^{+}\), 261.0797. C\(_{17}\)H\(_{11}\)NO\(_{2}\) requires M, 261.0789); ν_{max} (Nujol) 1710, 1625, 1360, 1285, 1230, 1210, 1100, 785, and 745 cm\(^{-1}\); δ_{H} (90 MHz;
(ii) By Melt Pyrolysis of the Iminophosphorane (190b)

The iminophosphorane (190b) (0.19g, 0.474 mmol) was subjected to the conditions described in 5(i) above. The crude material was chromatographed (p.l.c., 1:1 ether-petrol, 3 elutions) to give methyl 1-azafluoranthene-2-carboxylate (193) (14 mg, 11%).

(iii) By Melt Pyrolysis of the Iminophosphorane (190d)

The iminophosphorane (190d) (50 mg, 0.093 mmol) was pyrolysed under the conditions described above (5(i)). The crude mixture was chromatographed (p.l.c., 60% ether-petrol, 2 elutions) to give methyl 1-azafluoranthene-2-carboxylate (193) (4.7 mg, 19%).

(iv) By Melt Pyrolysis of the Iminophosphole (196)

The iminophosphole (196) (0.1g, 0.17 mmol) was placed in a 50 ml round-bottom flask equipped with a cold-finger. The flask was lowered into a pre-heated Woods-metal bath at 300°C. After 2 min, the heating bath was removed and the cold-finger washed with dichloromethane. The dichloromethane washings were concentrated in vacuo to leave a yellow gum which was chromatographed (p.l.c., 1:1 ether-petrol, 3 elutions) to give methyl 1-azafluoranthene-2-carboxylate (193) (15.1 mg, 34%).

(v) By Solution Thermolysis of the Iminophosphole (196)

(a) In 1,2-Dichlorobenzene

A solution of the iminophosphole (196) in 1,2-dichlorobenzene was heated under reflux for 2h. On cooling, t.l.c. showed that none of the starting material remained. The solvent was removed in vacuo to leave a black gum. The n.m.r. spectrum of the gum showed that a trace
of the required azafluoranthene (193) was present but purification was not attempted.

(b) In Diphenyl Ether

A solution of the iminophosphole (196) in diphenyl ether was heated under reflux for 20 min. On cooling the solution was diluted with petrol and extracted with 6N hydrochloric acid. The aqueous extracts were basified with solid sodium hydrogen carbonate and extracted with dichloromethane to give a red gum. T.l.c. and n.m.r. analysis of the gum showed that some of the desired azafluoranthene (193) was present but the mixture was not investigated further.

(vi) Attempted Photolysis of the Iminophosphorane (190d)

A solution of the iminophosphorane (190d) in dichloromethane was irradiated at 350 nm in a Rayonet photochemical reactor for 3h whereupon t.l.c. analysis showed that no reaction had taken place. Subsequent irradiation at 300 and 254 nm produced the same result and the solution was discarded.

(vii) Attempted Solution Thermolysis of the Iminophosphorane (190d)

A solution of the iminophosphorane (190d) in dry xylene was heated under reflux for 4h. On cooling, t.l.c. analysis showed that only the starting material was present and the solution was discarded.

6. Thermolysis of 3-Aryl-2-azidopropenoates

(i) Thermolysis of Methyl 2-azido-3-(2-formylphenyl)propenoate (164)

A solution of the azido-aldehyde (164) (15 mg, 0.065 mmol) in dry xylene (10 ml) was heated under reflux for 1h. On cooling, the solvent was removed in vacuo and the residual solid chromatographed (p.l.c.,
1:1 ether-petrol, 1 elution) to give, (i) methyl 4-formylindole-2-carboxylate (199) (3.7 mg, 28%) as a tan solid, m.p. 187-189°C (Found: \(M^+\), 203.0591. \(C_{11}H_{10}NO_3\) requires \(M\), 203.0582); \(\nu_{\text{max}}\) (Nujol) 3180, 1705, 1670, 1440, 1350, 1295, 1260, 1210, 1050, 1000, 770, and 715 cm\(^{-1}\); \(\delta_H\) (90 MHz; CDCl\(_3\)) 3.99 (3H, s, CO\(_2\)CF\(_3\)), 7.38 - 7.80 (3H, m, 5-H, 6-H, 7-H), 8.00 (1H, d, \(J\) 2.6 Hz, 3-H), 9.26 (1H, br s, NH), 10.26 (1H, s, CHO); \(m/\alpha\) 203 (\(M^+\), 90%), 171 (100), 143 (17), 115 (19), 89 (14), 63 (10); (ii) methyl isoquinol-1-one-3-carboxylate (165) (4.2 mg, 32%) whose spectral data were identical to those reported for (165) in Experiment 3(ii)(b).

(ii) Thermolysis of Methyl 3-(2-acetylphenyl)-2-azidopropenoate (174)

A solution of the vinyl azide (174) (0.1g, 0.408 mmol) in dry xylene (100 ml) was heated under reflux for 1.5h. On cooling the solvent was removed in vacuo to leave a yellow gum. The crude material was chromatographed (t.l.c., ether, 1 elution) to give methyl 4-acetyl-indole-2-carboxylate (204) (19 mg, 22%) as a colourless solid, m.p. 178.5 - 179.5°C (from cyclohexane-chloroform) (Found: C, 66.6; H, 5.1; N, 6.4. \(C_{12}H_{11}NO_3\) requires C, 66.4; H, 5.1; N, 6.45%); \(\nu_{\text{max}}\) (Nujol) 3240, 1720, 1655, 1570, 1500, 1295, 1275, 1240, 1205, 1140, 1005, 920, 830, and 770 cm\(^{-1}\); \(\delta_H\) (250 MHz; CDCl\(_3\)) 2.72 (3H, s, CO\(_2\)CF\(_3\)), 7.40 (1H, m, 6-H), 7.65 (1H, d (with additional fine splitting), \(J\) 8.5 Hz), 7.78 (1H, d (with additional fine splitting), \(J\) 8.5 Hz), 8.00 (1H, d, \(J\) 2 Hz, 3-H), 9.40 (1H, br s, NH); \(m/\alpha\) 217 (\(M^+\), 83%), 202 (84), 185 (21), 170 (100), 142 (26), 114 (32).

(iii) Thermolysis of Methyl 2-azido-3-(fluoren-9-on-1-yl)propenoate (189)

A solution of the vinyl azide (189) (0.2g, 0.656 mmol) in dry xylene (175 ml) was heated under reflux for 4h. On cooling to room temperature, the product was collected by filtration and dried in vacuo to give methyl
pyrrolo[2,3-a]fluoren-10-one-2-carboxylate (205) (0.138g, 76%) as a red solid, m.p. 280-282°C (Found: C, 73.55; H, 3.9; N, 5.1. C_{17}H_{11}NO_3 requires C, 76.65; H, 4.0; N, 5.05%); \( \nu_{\text{max}} \) (Nujol) 3320, 1705, 1700, 1610, 1530, 1315, 1265, 1220, 1175, 1140, 810, and 760 cm\(^{-1}\);
\( \delta_{\text{H}} \) (250 MHz; d_6-DMSO) 3.90 (3H, s, CO_2CH_3), 7.18 - 7.30 (2H, m), 7.43 - 7.52 (2H, m), 7.60 - 7.64 (2H, m), 7.65 (1H, s, 3-H), 11.30 (1H, br s, NH); \( m/z \) 277 (M\(^+\), 30%), 245 (55), 233 (13), 219 (62), 189 (35), 180 (20), 167 (52), 163 (28), 152 (18), 131 (28), 119 (34), 115 (28), 109 (30), 105 (48).

(iv) Thermolysis of Methyl 2-azido-3-[2-(1,3-dioxolan-2-yl)phenyl]-propenoate (163)

A solution of the vinyl azide (163) (0.1g, 0.364 mmol) in dry xylene (100 ml) was heated under reflux for 1.5h. On cooling, the solvent was removed \textit{in vacuo} to leave a gum. The crude mixture was chromatographed to give, (i) methyl 4-(1,3-dioxolan-2-yl)indole-2-carboxylate (206) (47.6 mg, 53%) as a colourless solid, m.p. 145-146°C (from ether-petrol) (Found: C, 62.9; H, 5.3; N, 5.6. C_{13}H_{13}NO_4 requires C, 63.2; H, 5.3; N, 5.7%); \( \nu_{\text{max}} \) (Nujol) 3330, 1695, 1525, 1445, 1350, 1300, 1260, 1210, 1160, 1100, 1040, 1000, 950, 800, 775, and 770 cm\(^{-1}\); \( \delta_{\text{H}} \) (250 MHz; CDCl_3) 3.94 (3H, s, CO_2CH_3), 4.15 (4H, m, -OCH_2CH_2O-), 6.15 (1H, s, ArCH), 7.23 - 7.43 (4H, m, ArH), 9.35 (1H, br s, NH); \( m/z \) 247 (M\(^+\), 100%), 214 (35), 201 (6), 188 (17), 175 (54), 156 (8), 143 (41), 127 (8), 114 (6), 73 (28); (ii) methyl 1-(2-hydroxyethoxy)isoquinoline-3-carboxylate (207) (39.7 mg, 44%) as a colourless gum which solidified on standing, m.p. 101-102°C (from ether-petrol) (Found: C, 63.0; H, 5.3; N, 5.7. C_{13}H_{13}NO_4 requires C, 63.2; H, 5.3; N, 5.7%); \( \nu_{\text{max}} \) (Nujol) 3500, 1715, 1590, 1570, 1500, 1455, 1410, 1350, 1330, 1295, 1250, 1210, 1100, 1000, 935, 895, 800, 785, and 675 cm\(^{-1}\);
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δ_H (250 MHz; CDCl_3) 4.00 (3H, s, CO_2CH_3), 4.06 (2H, br s, CH_2OH), 4.77 (2H, m, ArOCH_2), 5.57 (1H, br s, OH), 7.64 - 7.90 (3H, m, ArH), 8.17 (1H, s, 4-H), 8.30 (1H, m, ArH); m/z 247 (M^+, 8%), 228 (6), 216 (11), 203 (100), 172 (8), 143 (47), 127 (12), 115 (21), 89 (30).

(v) Thermolysis of Methyl 2-azido-3-[2-(2-methyl-1,3-dioxolan-2-yl)-phenyl]propenoate (173)

A solution of the vinyl azide (173) (0.1g, 0.346 mmol) in dry xylene (100 ml) was heated under reflux for 1.5h. On cooling, the solution was concentrated _in vacuo_ to leave a cream solid. The crude material was flash chromatographed (20% ether-petrol) to give methyl 4-(2-methyl-1,3-dioxolan-2-yl)indole-2-carboxylate (209) (90 mg, 100%) as colourless rods, m.p. 179-180°C (Found: C, 64.5; H, 5.8; N, 5.3. C_{14}H_{15}NO_4 requires C, 64.4; H, 5.75; N, 5.4%); ν_max (Nujol) 3310, 1690, 1520, 1340, 1290, 1265, 1240, 1210, 1040, 1000, 865, 830, and 765 cm⁻¹; δ_H (60 MHz; (CD_3)_2CO) 1.7 (3H, s, CH_3), 3.9 (3H, s, CO_2CH_3), 3.7 - 4.1 (4H, m, -OCH_2CH_2O-), 7.2 (1H, d, J 2 Hz, 3-H), 7.3 - 7.6 (3H, m, 5-H, 6-H, 7-H), 10.9 (1H, br s, NH); m/z 261 (M^+, 19%), 246 (100), 214 (48), 202 (9), 170 (22), 142 (6), 114 (18), 87 (21).
EXPERIMENTAL FOR PART THREE
1. 9-Formyl-2-methoxyacridine (212)

The title compound was prepared by the literature method, \(^\text{146}\) m.p. 145-148.5°C (lit., \(^\text{146}\) 146.5 - 148°C); \(\delta_H\) (90 MHz; CDCl\(_3\)) 4.00 (3H, s, ArOC\(_3\)), 7.35 - 7.80 (3H, m), 8.02 - 8.25 (3H, m), 8.52 - 8.68 (1H, m), 11.37 (1H, s, C=O).

2. Condensation of 9-Formyl-2-methoxyacridine (212) with Methyl azidoacetate

Sodium (0.175g, 0.008g atom) was added to dry methanol (10 ml) and the mixture stirred until all the sodium had dissolved. The solution was cooled to -15°C whereupon a solution of the aldehyde (212) (0.45g, 1.90 mmol) in methyl azidoacetate (0.873g, 7.59 mmol) and dry methanol (5 ml) was added dropwise over 15 min. The reaction mixture was stirred at -15°C for 4h and then at 0°C for 24h. On warming to room temperature, the mixture was poured into saturated aqueous ammonium chloride (50 ml) and extracted with ethyl acetate (3 x 20 ml). The combined organic extracts were washed with water (3 x 20 ml), dried (MgSO\(_4\)) and concentrated \textit{in vacuo} to give a black gum which was chromatographed. Eluting with 10% ether-petrol gave methyl 2-azido-3-(2-methoxyacridin-9-yl)propenoate (213) (0.201g, 32%) as a red foam, m.p. 116-118°C (decomp.) (from methanol); \(\nu_{\text{max}}\) (CHCl\(_3\)) 2120, 1720, 1630, 1560, 1550, 1430, 1360, 1270, 1140, 1080, 955, 905, and 825 cm\(^{-1}\); \(\delta_H\) (90 MHz; CDCl\(_3\)) 3.91 (3H, s), 4.05 (3H, s), 6.90 (1H, d, \(J = 2\) Hz), 7.32 - 7.85 (5H, m), 7.96 - 8.25 (2H, m); \(m/z\) 334 (M\(^+\)), 308 (M-28, 53), 291 (12), 267 (100), 247 (43), 237 (48), 223 (87), 208 (38), 180 (53), 166 (33), 84 (44). Despite several attempts, no satisfactory microanalysis could be obtained for compound (213). Crystals grown in methanol rapidly became dark and opaque when exposed to air.
3. **Thermolysis of Methyl 2-azido-3-(2-methoxyacridin-9-yl)propenoate (213)**

A solution of the vinyl azide (213) (0.285g, 0.853 mmol) in dry xylene (150 ml) was heated under reflux for 1.5h. On cooling, the solvent was removed *in vacuo* to leave a dark purple solid. The crude mixture was separated by column chromatography to give, (i) (90% ether-petrol) *methyl 10-methoxy-3H-pyrido[4,3,2-ntt]acridine-2-carboxylate* (216) (49.3 mg, 19%) as a purple solid, m.p. 210-212.5°C (Found: $M^+$, 306.1011. $C_{18}H_{14}N_2O_3$ requires $M$, 306.1004); $\nu_{\text{max}}$ (CHCl$_3$) 3440, 1720, 1620, 1600, 1580, 1345, 1275, 1145, and 1000 cm$^{-1}$; $\delta_H$ (250 MHz; CDCl$_3$) 3.87 (3H, s, ArOC$_3$), 4.04 (3H, s, CO$_2$CH$_3$), 6.58 (1H, dd, $J$ 6.8 and 2.3 Hz, 4-H), 6.82 (1H, d, $J$ 8.3 Hz, 8-H), 6.98 (1H, dd, $J$ 8.3 and 2.7 Hz, 9-H), 7.27 (1H, d, $J$ 2.7 Hz, 11-H), 7.43 (2H, m, 5-H, 6-H), 7.85 (1H, s, 1-H) (NMR not observed); $m/z$ 306 ($M^+$, 100%), 291 (27), 248 (51), 233 (15), 205 (19), 177 (8), 149 (9), 137 (10), 124 (23), 111 (21); (ii) (10% methanol-ether) *methyl 4-methoxy-3H-pyrido[4,3,2-ntt]acridine-2-carboxylate* (215) (0.204g, 78%) as a purple solid, m.p. 225-227°C (Found: $M^+$, 306.1011. $C_{18}H_{14}N_2O_3$ requires $M$, 306.1004); $\nu_{\text{max}}$ (Nujol) 3300 (br), 1720, 1630, 1315, 1280, 1240, 1140, 1015, and 750 cm$^{-1}$; $\delta_H$ (250 MHz; CDCl$_3$) 3.98 (3H, s, ArOCH$_3$), 4.01 (3H, s, CO$_2$CH$_3$), 6.57 (1H, d, $J$ 8.3 Hz, 6-H), 6.78 (1H, br d, $J$ 8.3 Hz, 10-H), 6.98 (1H, d, $J$ 8.3 Hz, 5-H), 7.25 - 7.35 (2H, m, 8-H, 9-H), 7.92 (1H, br d, $J$ 8.3 Hz, 11-H), 8.07 (1H, s, 1-H) (NMR not observed); $m/z$ 306 ($M^+$, 100%), 291 (95), 245 (21), 233 (11), 217 (32), 205 (8), 105 (47).


To a stirred solution of the pyridoacridine (215) (0.25g, 0.817 mmol) in 35% sulphuric acid (35 ml) at 0°C was added Attenburrow
activated manganese (IV) oxide (0.268g, 3.27 mmol) and the resulting suspension stirred at 0°C for 40 min. The mixture was diluted with chloroform (100 ml), neutralised with solid sodium hydrogen carbonate and filtered through Celite. The filtrate was placed in a separatory funnel and the layers separated. The aqueous layer was extracted with chloroform (1 x 30 ml) and the combined organic layers dried (MgSO₄). The solvent was removed in vacuo to give methyl 4-oxo-4H-pyrido[4,3,2-mn]acridine-2-carboxylate (217) (0.179g, 75%) as a bright yellow solid, m.p. 288-291°C (decomp.) (from chloroform-methanol) (Found: M⁺, 290.0695. C₁₇H₁₀N₂O₃ requires M, 290.0691); ν max (Nujol) 1720, 1670, 1585, 1400, 1260, 1200, 1130, 840, and 760 cm⁻¹; δ H (90 MHz; CDCl₃) 4.21 (3H, s, CO₂CH₃), 7.15 (1H, d, J 11 Hz, 5-H), 7.91 (1H, d, J 11 Hz, 6-H), 7.80 - 8.10 (2H, m), 8.26 - 8.40 (1H, m), 8.65 - 8.75 (1H, m), 9.42 (1H, s); m/z 292 (M+2, 14%), 290 (M⁺, 3), 232 (100), 204 (25), 176 (10).


A solution of the pyridoacridine (216) in 35% sulphuric acid at 0°C was treated with Attenburrow activated manganese (IV) oxide (4 equiv.) and the suspension stirred at 0°C for 40 min. The mixture was diluted with chloroform, neutralised with solid sodium hydrogen carbonate and filtered through Celite. The filtrate was extracted with chloroform to give a small amount of a brown gum. T.l.c. analysis of the gum showed it to be a complex mixture which was discarded.
6. Attempted Diels-Alder Reaction of Methyl 4-oxo-4H-pyrido[4,3,2-mn]-acridine-2-carboxylate (217) with the Aza-Diene (218)

(a) Using 1.1 Equivalent of the Diene

A mixture of the pyridoacridine (217) (20 mg, 0.069 mmol) and the aza-diene (218) (24 mg, 0.076 mmol, prepared by the method of Sainte\textsuperscript{148b}) in dry, acid-free chloroform (2 ml) was heated under reflux for 16h. T.l.c. analysis showed that the starting material (217) was unchanged. The chloroform was removed \textit{in vacuo} and the residue triturated with ether to leave (217) as a bright yellow solid.

(b) Using 20 Equivalents of the Diene

A solution of the pyridoacridine (217) in chloroform was treated with the aza-diene (218) (20 equiv.). The mixture was heated under reflux until all of the starting material had been consumed (20h), whereupon the solvent was removed \textit{in vacuo} to leave a dark-red oil. The crude material was chromatographed to give a red oil whose n.m.r. spectrum contained no signals in the aromatic region and was, therefore, not investigated further.

(c) In Chloroform at 160°C

A mixture of the pyridoacridine (217) and the aza-diene (218) (2 equiv.) in chloroform was heated at 160°C in a sealed tube for 14h. The solvent was removed \textit{in vacuo} to leave a black tar which was discarded.

(d) In Boiling Chlorobenzene

A solution of the pyridoacridine (217) and the aza-diene (218) (excess) in chlorobenzene was heated under reflux for 5h. On cooling, the solvent and excess of the diene were removed \textit{in vacuo} to leave a
black gum. T.l.c. analysis of the gum showed it to be a complex mixture which was discarded.

(e) Using Boron Trifluoride Etherate as Catalyst

A mixture of the pyridoacridine (217) and the aza-diene (218) (2 equiv.) in chloroform was treated with a catalytic amount of boron trifluoride etherate. The mixture was stirred at room temperature for 1h and then heated under reflux for 24h. At this time, t.l.c. analysis of the mixture showed only the presence of the pyridoacridine (217) and some baseline material. The chloroform was removed \textit{in vacuo} to leave a gum. Trituration of the gum with ether gave none of the aza-diene (218) and the gum was discarded.

(f) Using Aluminium Chloride as Catalyst

A mixture of the pyridoacridine (217) and the aza-diene (218) (2 equiv.) in chloroform was treated with a catalytic amount of freshly sublimed aluminium chloride and the mixture stirred for 1h at room temperature. T.l.c. analysis of the black solution showed that the pyridoacridine (217) was unchanged but that the aza-diene (218) had been consumed to give a black baseline material. The mixture was discarded.

(g) Using High Pressure

A mixture of the pyridoacridine (217) (20 mg, 0.069 mmol) and the aza-diene (218) (43.4 mg, 0.138 mmol) in chloroform was held at 40°C and 12 kbar for 40h. The solvent was removed \textit{in vacuo} and the residue trituated with ether to leave the starting material (217) as a bright yellow solid.
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38. J.A. Duine, personal communication.


52. continued


98. D.M.B. Hickey, unpublished work.

99. I thank Miss E.M. Naylor for carrying out this reaction.


102. I thank Professor M.J. Perkins for a gift of t-butylbenzhydroxamic acid.

103. R.J. Parry, personal communication.

104. E.M. Naylor, unpublished work.

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139. I thank Dr. I. Gosney for a generous gift of 1,2,5-triphenyl-phosphole.


144. (a) D.J. Faulkner, Tetrahedron, 1977, 33, 1421.
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149. I thank Dr. N. Isaacs of the University of Reading for carrying out this experiment.


