BIOMIMETIC APPROACHES TO THE SYNTHESIS OF MITOMYCIN ANTIBIOTICS

A thesis submitted for the degree of
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I declare that the work described in this thesis is my own, except where due reference has been made. The work was carried out at the Research School of Chemistry, The Australian National University, under the supervision of Professor R. W. Rickards. None of this material has been submitted in support of any other degree.

John Churchill
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for Richard who cast doubt on it all
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Abstract

The chemistry of the mitomycin antibiotics (e.g. 3) has been critically reviewed. Rationalisations are provided for some unexplained observations uncovering some potentially valuable new chemistry; some discrepancies in the literature are pointed out. X-ray crystallographic data has been analysed to discredit a widely accepted belief about intramolecular forces in the mitomycins. The biosynthesis of the mitomycins is reviewed.

\[
\begin{align*}
\text{Mitomycin C (3)}
\end{align*}
\]

Approaches to the synthesis of the mitomycins are summarised. Work on biomimetic cyclisations of ansamycins and mitomycins in the Rickards group is briefly reviewed.

Following the earlier studies of Chai, a Michael cyclisation model for the formation of the C8aC9 bond is investigated. We found the cyclisation of the methoxy and acetylamino substituted β-keto esters 346 and 364 to be facile despite the electronic influence of these substituents. The ramifications of these results for the introduction of 7-substituents in mitomycin biosynthesis is discussed. In the course of the synthetic work leading to 346 and 364, a method for the assignment of phenolic NMR resonances is described. Transannular condensation efficiently generated the mitosene analogues 203 and 367, but elaboration of this biomimetic synthesis to the mitomycins was blocked by facile dehydration and difficulties in converting the 9-carbomethoxy into an hydroxymethyl substituent. The biosynthetic role of a Michael cyclisation is questioned on the basis of new antibiotics whose structures were reported during this work and new biosynthetic models are proposed.
The extension of the synthetic approach to allow incorporation of a chiral aziridine is addressed. The epoxy-β-keto ester 437b has been prepared. This system is found to be unstable isomerising to the furanone 441. Two approaches to the aziridine 455 are described.

Revised biosynthetic models for C8aC9 bond formation and the synthesis of relevant test systems is discussed. A new, concise synthesis of 2-methoxy-3-methylbenzoquinone, an important intermediate in much synthetic work aimed at the mitomycins, is accomplished.
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### CHAPTER ONE THE MITOMYCIN ANTIBIOTICS

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1.1 Introduction

The understanding and treatment of cancers has become the motivation for an incredibly diverse and intensive investigation worldwide. This effort is correlated with an increase in the relative importance of cancers in mortality in the Western world, although from a global perspective, infectious diseases remain a far more compelling problem. Chemists have been involved in this effort to discover the basis of chemical carcinogenesis and because some chemicals can be used to treat cancers. Indeed drug treatment (‘chemotherapy’) is, with surgery and radiation, a major tool in cancer therapy. Among the drugs in clinical use is mitomycin C.

Mitomycin C is active against a wide range of tumours. It is used primarily in the treatment of adenocarcinomas of the stomach, pancreas and colon; it is also used for carcinoma of the breast, lung, malignant melanoma and squamous cell carcinomas. More recently it has been used successfully to treat bladder cancer by direct application. Although in widespread use, clinical application is limited by cumulative, delayed myelosuppression (bone marrow toxicity) and gastrointestinal side effects, and treatment is often not curative. These side affects are due to general inhibition of cellular replication, i.e. a lack of cytoselectivity. An analogue without such side effects would be very valuable and much work has aimed at this (see Section 1.3a).

The name mitomycin was applied to antibiotics isolated from *Streptomyces caespiitosus* in 1956. Hata *et al.* isolated two coloured, crystalline antibiotics, mitomycin A and mitomycin B, and recognised their antitumour properties. Large scale fermentation led Wakaki *et al.* to discover mitomycin C in some alkaline batches where mitomycins A and B were not produced. Mitomycin C was found to be significantly more active as an antitumour agent. Subsequently porfiromycin and the biologically inactive mitiromycin (sometimes written mityromycin) were isolated from other *Streptomyces* species along with the earlier mitomycins. Mitomycins have been obtained from bacteria isolated over a wide geographical range. The mitomycins display strong, broad spectrum antibacterial activity but are too toxic for use as clinical antibiotics. However the conspicuous antitumour properties of mitomycin C led to its
rapid introduction as an anticancer drug in Japan. The term mitomycin is used here to encompass mitomycins A, B and C, porfiromycin and closely related systems discovered more recently (see below).

The clinical use of mitomycin C and the unusual structure have prompted enormous interest in mitomycin chemistry and biology. Any new work is done in the context of this vast literature and an overview is provided here. Reviews dealing with specific aspects are cited in relevant sections. Several more general reviews of varying quality and a book were published contemporaneously \textit{circa} 1980.\textsuperscript{1,9,10,11} A detailed summary of the physical and spectral properties, chromatography and pharmacology of mitomycin C has been published.\textsuperscript{12}

The most recent review of mitomycin chemistry\textsuperscript{50} is not comprehensive and contains some significant errors. Thus we have undertaken a supplementary review of mitomycin chemistry which constitutes the rest of this chapter. X-ray crystallographic data has been used to test some of the issues raised.
1.2 Structure and Nomenclature of the Mitomycins

1.2a Early Mitomycins and Nomenclature

Extensive degradative studies, spectroscopy and interconversions established the structure of the mitomycins, except for their stereochemistry. This work has been reviewed elsewhere\textsuperscript{10} and will not be discussed here. The well established structures for mitomycins A (1), B (2), C (3) and porfiromycin (4) are shown in Figure 1. The most notable feature is the aziridine ring which was previously unknown in natural products and, in interesting contrast to the epoxides, is still extremely rare (see Chapter 4). Three other rings are fused to the aziridine to give a rigid skeleton whose perimeter is heavily functionalised. Variation in natural mitomycins occurs in substitution at C7, methylation of N1a and the O9a and the stereochemistry at C9. The 9a-carbinolamine or corresponding methyl ether functionality is also unusual and important in the mitomycins’ antitumour activity (see Section 1.4).

For continuing reference the more significant structures are reproduced on a fold out sheet inside the back cover.

\[ \text{Mitomycin A (1)} \]
\[ \text{Mitomycin B (2)} \]
\[ \text{Mitomycin C (3)} \]
\[ \text{Porfiromycin (4)} \]

Figure 1. The Early Mitomycin Antibiotics

The extensive mitomycin literature is all written in two related systems of nomenclature based on the original natural product names. The original antibiotics were dubbed mitomycin A, B, and C in order of isolation. Porfiromycin, mitiromycin (sometimes written mityromycin), isomitomycin and albobotomycin are further, inconsistent trivial names. Many synthetic derivatives, \textit{e.g.} N-methylmitomycin A, are
named with respect to these widely recognised parents, using the numbering system indicated. Simultaneously, the original naming system for natural products has been extended, currently up to mitomycin M, with some overlap between the systems e.g. N-methylmitomycin A is mitomycin F (‘mitomycin S’ is merely a mixture of mitomycin C and sodium chloride). A, B and C ring appellations are sometimes used as shown, however, in some earlier work the reverse sequence was used. Occasionally the aziridine ring is called the D ring.

The term ‘apo-mitomycin’ has been used for 2-amino-1-hydroxymitosenes, particularly the cis isomer. Synthetic analogues or derivatives are usually named on the basis of ‘mitosene’ and ‘mitosane’ skeletons shown below. Danishefsky et al. have made several proposals to systematise the usage of the prefixes and suffixes, although these have not all been widely adopted:

- ‘leuco-’ to denote the hydroquinone form of a mitomycin or mitosene;
- ‘-mycin’ only for systems with oxygen at C9a;
- ‘-sane’ for systems bearing hydrogen at C9a;
- ‘aziridino-’ to emphasise the presence of an intact aziridine ring (in synthetic or semi-synthetic products).

In this thesis mitomycins will be named with respect to their natural parents (mitomycin A, B or C, porfiromycin etc.) and the suffix - prefix system above is generally used. The systematic names used in Chemical Abstracts are summarised in Appendix 1, and have a different numbering system.
1.2b X-ray Crystallographic Studies and Absolute Configuration

There is a widespread belief that X-ray crystallography provides an unequivocal structural assignment. This persists despite lucid efforts by crystallographers to explain the pitfalls of the technique.\textsuperscript{15} Tulinsky's crystal structure of N\textsubscript{la}-(4-bromobenzenesulfonyl)-mitomycin A in 1962 confirmed the chemically assigned structure and determined the relative stereochemistry of mitomycin A (1) (and thus of mitomycin C (3) and porfiromycin (4) by known conversions).\textsuperscript{16} Subsequently the absolute stereochemistry was also assigned from anomalous scattering analysis (at an R factor of 0.087).\textsuperscript{17} Mitomycin B (2) had not been converted into, or prepared from other natural mitomycins; its relative and absolute stereochemistry were established by Yahashi and Matsubara's crystal structure analysis in 1976 (R 0.119) in a self-inconsistent paper\textsuperscript{18} which was later corrected.\textsuperscript{19} The 9-substituent in mitomycin B (2) is trans to the aziridine ring, unlike the other natural mitomycins, but the absolute aziridine stereochemistry was found to be the same as that determined for mitomycin A (1) (1R,2R). The structure of mitomycin C (3) was determined three times in 1979\textsuperscript{20,21,22} but the absolute stereochemistry was not addressed, except\textsuperscript{21} "by referring to that of N-(p-bromobenzoyl)-mitomycin C" citing a private communication from "K. Yamaguchi et al." whose work has never been published, but which evidently gave the same (1R,2R) aziridine configuration.

Biosynthetic studies of mitomycin B, however, indicated that D-glucosamine was efficiently incorporated without C-N bond cleavage (see Section 1.6); this implied an unusual inversion during the biosynthesis because S\textsubscript{N}2 cyclisation of D-glucosamine would generate the aziridine epimeric at both C1 and C2 (see Section 1.5). Shirahata and Hirayama determined the structure and absolute configuration of 4-bromobenzoylmitomycin C in 1983 (R 0.0361) and found the relevant absolute stereochemistry was the opposite of that established for mitomycins A (1) and B (2) [and perhaps C (3)], i.e. it was compatible with likely biosynthesis.\textsuperscript{23} This assignment was independently supported by application of the exciton chirality method to the hydrolysed mitomycin C derivative, mitosene 5 (with the assumption that the amino
configuration is retained at C2 during hydrolysis).\textsuperscript{24} There was a faint possibility that the earlier absolute configuration assignments of mitomycin A (1) and B (2) were correct (comparisons of interconverted mitomycins do not appear to have involved chiroptical studies due to the strong UV and visible absorption). This possibility was eliminated by Hirayama and Shirahata’s further X-ray studies on derivatives of both mitomycins A (1) and B (2) (R 0.047 and 0.057).\textsuperscript{25,26} The chirality of mitomycins A (1) and B (2) at C1 and C2 has also been correlated chiroptically. CD spectra of mitomycins are largely determined by C9 stereochemistry (with bathochromic shifts for 7-amino mitomycins).\textsuperscript{27,12} However by removing the C9 and C9a stereogenic centres \textit{via} synthesis of the same aziridinomitosene it was possible to correlate the absolute aziridine configuration of mitomycins A (1) and B (1) (CD comparison).\textsuperscript{28} The possibility of both enantiomers of a natural product occurring must be borne in mind,\textsuperscript{29} but we regard the probability of this in the mitomycin series as vanishingly remote, even though the chiroptical data is sparse and sample origin is not always given, because the source cultures are likely to be related and L-glucosamine is a rare natural carbohydrate. Thus the absolute configuration of the aziridine rings in all of these mitomycins was shown to be the same (1\textit{S},2\textit{S}) and the three earlier X-ray determinations, for mitomycins A, B and C, erroneous. Mitomycin structures published until about 1983 usually show the wrong absolute configurations; all structures in this thesis show the revised absolute configurations.

![Chemical structure](image)

Unfortunately such erroneous assignments in the literature are not very rare; sometimes they arise from simple mistakes in the preparation of structural drawings\textsuperscript{30,31,32} but, as with the mitomycins, the error can also be intrinsic or untraceable.\textsuperscript{29,33,34} Absolute configurations determined for structures with high R values may in fact be justified, but should be assessed with care (in particular due
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allowance must be made for both systematic and random data errors). Furthermore, R values are susceptible to manipulation and the estimated standard deviations should also be considered. Given that most assignments have probably not been reviewed, it seems likely that other literature reports may also be erroneous. Indeed care should be taken even with gross structural formulae 'established' by X-ray. Atoms have been missed or misassigned (which is particularly likely to occur with atoms of similar atomic number such as carbon, nitrogen and oxygen). A recent example is the natural product pyrrolosine (Figure 2) which was misidentified on the basis of X-ray crystallography in the face of compelling synthetic, spectral and biological evidence. The structure was revised using, inter alia, the same crystallographic data (improving the R factor from 0.065 to 0.039). Increasingly structural elucidation by chemical degradation is being displaced by X-ray crystallography. The determination of absolute configuration solely by X-ray crystallography is becoming more common as well (although not "as a matter of course" without ‘heavy atoms’ as has been claimed by one crystallographer). It is to be hoped that critical assessment of X-ray data itself, and using chemical, spectral and biosynthetic evidence will also become standard.

![Structure assigned by X-ray crystallography](image1)

![Revised structure](image2)

**Figure 2. The Structure of Pyrrolosine**

X-ray crystallographic studies of a number of other mitomycins and derivatives that have been published recently are summarised in Appendix 2; the structures are all consistent with earlier assignments but none address absolute stereochemistry. The structure of mitomycin C (3) (from the data of Arora) is represented in Figures 3 and 4. All of the mitomycins are much more concave than is apparent from their two dimensional representation, with the aziridine nitrogen about 2.7 Å from N4 (see Figure 4). Although N4 and C9a are almost coplanar with the quinone ring, C3 is not and thus
N4 is significantly pyramidalised (see Figure 4). This is intriguing because delocalisation of N4 into the quinone is believed to stabilise the 9a-substituent until reductive activation; this is discussed further in Section 1.4. The distance from the aziridine nitrogen to the quinone (N1a to C4a *circa* 3.3 Å) is interesting in light of the discovery of albomitomycin A and isomitomycin A (see below) where N1a and C4a are covalently bonded (1.5 Å for albomitomycin A).

![Figure 3. Mitomycin C (3)](image)

![Figure 4. Mitomycin C (3) (carbamate excised, viewed in the quinone plane)](image)
1.2c Mitiromycin

The structure of mitiromycin (6), an inactive product from *Streptomyces verticillatus*, was elucidated based on spectroscopic evidence; the carbamate is cyclised to C9a giving an unusual oxazinone ring. Franck observed that the oxazinone ring must be cis fused and correlated the relative stereochemistry (C9α) with mitomycin B (2) on the basis of the spectroscopic data. Recently Kono et al. verified this assignment after irreproducibly preparing a synthetic sample by treatment of mitomycin B (2) with sodium methoxide to give mitiromycin in 0.8% yield (rather than the usual decarbamoyl product). Although the melting point, 83 - 88°C, differed from that of the natural monohydrate, mp 124 - 126°C, the product matched natural material spectroscopically and NOE effects and X-ray crystallography confirmed the structure 6. The mechanism of this synthesis is discussed on p20.

\[ \text{MeO} \]

\[ \text{O} \]

\[ \text{O} = \text{O} \]

\[ \text{NMe} \]

\[ \text{NH} \]

\[ \text{NMe} \]

\[ \text{6} \]

1.2d Recently Discovered Mitomycins

After a gap of almost twenty years, a spate of minor fermentation products were isolated. In 1980 Shirahata *et al.* reported the isolation of a large number, significantly expanding the class up to mitomycin M and dramatically increasing the number of C9α mitomycins (Figure 5). The original report was obscure and spectroscopic data was incomplete; further details for some products have been published subsequently. Several of the new mitomycins simply involve variation in methylation and methylamine and anthranilamide were identified as new 7-substituents. More interesting were three mitomycins (G (10), H (11) [isolated by others] and K (14)) with an exocyclic olefin at C9, presumably the result of elimination of carboxylic acid. Indeed mitomycin H (11) has been prepared synthetically from the 9a-hydroxy compound mitomycin B (2) by base catalysed elimination or treatment with alumina, so it is quite possible that mitomycin H (11) is an artefact of isolation. The 9a-methoxy mitomycins are more resistant to such elimination (see Section 1.3a).
Figure 5. Naturally Occurring Mitomycins
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Assessing all of the natural mitomycins, it is apparent that there is variation at four positions:

- The 7-substituent is either a methoxy or amino or substituted amino group.
- The 9a-substituent is a methoxy or hydroxy group (with the exception of mitiromycin).
- The C9 stereochemistry varies; with the β (9S) stereochemistry there is always a 9a-methoxy group. A 9,10-exocyclic double bond is also observed.
- Both the free and methylated aziridines are seen with C9β (9S), but for C9α (9R) mitomycins, only the methylated aziridine is observed.

Two new isomeric “mitomycins”, albomitomycin A (17) and isomitomycin A (18) have been isolated from *Streptomyces caespitosus* and were found to equilibrate with mitomycin A (1) in protic solvents (Scheme 1.1).45 This rearrangement is catalysed by Lewis acids (equilibrium mixture in THF with aluminium isopropoxide is 97:3:trace of 1:17:1846), and appears to involve a Michael addition of the aziridine to the quinone at C4a giving 17 followed by a retro-Michael reaction to 18. The original patent covering isomer 1747 also reported the isolation and preparation of the corresponding mitomycin C (3) analogue of 17 (i.e. 7-amino), but apparently this equilibrium overwhelmingly favours the mitomycin form.48 The reason for the difference in reactivity is not clear. It is conceivable hydrogen bonding by the 7-amino group leads to increased conjugation of N4 with the C8 carbonyl, stabilising the mitomycin form of mitomycin C (3). (In agreement with this, 7-amino substituted mitomycins are slightly less pyramidalised at N4, see Section 1.5).

![Scheme 1.1 Isomerisation of Mitomycin A](image_url)

-Mitomycin A (1)
-Albomitomycin A (17)
-Isomitomycin A (18)
There is a bizarre report of a “zwitterionic form of mitomycin C”, readily separable by HPLC in a ratio affected by γ-cyclodextrin, although “no spectroscopic differences between MMC [mitomycin C] and the zwitterion were found”. We have not been able to obtain further details, but the proposed “zwitterion” structure 19 is simply mesomeric. Physical separation of such a species is unprecedented. A more plausible rationalisation would be the detection of a mitomycin C - albomitomycin C - isomitomycin C rearrangement (the presumably colourless albo intermediate perhaps not being observed by HPLC at the detector wavelength). However, the reported ratio seems inconsistent (“zwitterion” : mitomycin C (3), 2:1 at pH 7). The separation of a 5-hydroxy-7-imino tautomer (20) (compare 31 on p19) would be fascinating if properly established. A possible alternative explanation of stable aziridine invertomers occurred to us during the course of our own work (see Chapter 4).

\[
\begin{align*}
\text{19} & \quad \text{OCONH}_2 \\
\text{20} & \quad \text{OCONH}_2
\end{align*}
\]
CHAPTER ONE

1.3 REACTIONS OF THE MITOMYCINS

The density and variety of the functionalisation of mitomycin skeleton leads to a wide range of chemical reactions; every carbon atom in the skeleton has now been directly affected. The reactivity and sensitivity of the mitomycins have biosynthetic and synthetic ramifications. Thus an overview of mitomycin chemistry will be given here but more attention will be focussed on recent, unusual and unexplained reactions because reactions of the mitomycins up until about 1988 have been reviewed, somewhat cursorily, by Franck and Tomasz50 following two earlier reviews.9,10 Kasai and Kono have published accounts of their group’s extensive research in the area up to 1991.48

1.3a Mitomycin Interconversion and Reactions in Base

The work on structural elucidation by chemical degradation revealed a number of transformations and has allowed the interconversion of many of the now known mitomycins.13,51,52 The aziridine can be methylated, allowing conversion of mitomycin A (1) to F (9) or mitomycin C (3) to porfiromycin (4). The 9a-hydroxyl can also be methylated, allowing conversion of mitomycin B (2) to J (13) or mitomycin H (11) to K (14). With a 7-amino substituent, however, such methylation is less efficient, apparently because of competitive amine methylation.53

The 7-methoxy group is readily displaced by amines or alkoxides. Thus reaction with ammonia converts mitomycin A (1) to mitomycin C (3). More vigorous alcohohlysis cleaves the carbamate generating a C10 alcohol.54 Hydrolysis of the 7-amino or 7-methoxy mitomycins gives the corresponding 7-hydroxy systems which can be alkylated with diazoalkanes or triazenes (ArNNNHR), allowing the two step conversion of mitomycin C (3) to mitomycin A (1), for example.

The reactivity of mitomycins at the aziridine and C7 has led to the preparation of a huge number of derivatives and synthetic analogues substituted at these positions. A number of 10-substituted analogues have been prepared via decarbamoyl intermediates. An analogue more effective than mitomycin C (3), in particular one with
milder side effects would be highly desirable given the significant dose limiting toxicity of mitomycin C (3). While several analogues have reached even clinical trial stage, none is yet in clinical use. Biological activities have been disparately tested and structure-activity relationships have not been very productive. Biological activity will generally not be discussed in this thesis.

Two ‘sets’ of mitomycins are not available either naturally or by the direct semi-synthetic methods described above: mitomycins with both a 9β-substituent and a 9a-hydroxyl and mitomycins with a 9α-substituent and an unsubstituted aziridine ring. In 1989 Kasai et al. reported the first inversion of C9 stereochemistry by indirectly epimerising C9 of mitomycin B (2) (Scheme 1.2), for the first time converting one of the natural C9α mitomycins to a natural C9β mitomycin. The carbamate of mitomycin B (2) was hydrolysed to give the C10 alcohol (minimising later elimination) which was aminated and treated with DBU to give a mixture of epimeric C10 alcohols and as well as some olefin 10. The 1:1 mixture of 22 and 24 was believed to be an equilibrium mixture although conversion of 24 to 22 was not directly established. Attempted epimerisation of the corresponding 7-methoxy system led to decomposition. The carbamate was then reinstated by established procedures to furnish 9-epi-mitomycin D (25) which could be converted to 9-epi-mitomycin B (27) by hydrolysis and methylation. The epimerisation was interpreted as proceeding via equilibration of aminal 22 with the ring opened keto form 23. Danishefsky et al. had reported an alternative reductive approach which converts the 9a-methoxyl of mitomycin F (9) to an hydroxyl; this is discussed in Section 1.3d. The epimerisation of mitomycins A (1) or C (3) to provide the C9α, unsubstituted aziridine series has not been reported. The preparation of 9β, 9a-hydroxy, unsubstituted aziridine systems has very recently been described via a very different approach utilising the mitomycin-albomitomycin rearrangement (see Section 1.3c).
As seen in the preparation of 9-epi-mitomycin B (27) (Scheme 1.2), base catalysed elimination of water can generate a 9-exomethylene group. All three of the natural mitomycins with an exomethylene group (G (10), H (11) and K (14)) have been prepared by synthetic conversion. Urakawa et al. discovered that mitomycin B (2) could be directly converted to mitomycin H (11) through carbamic acid elimination on treatment with base (Scheme 1.3). A similar process appears to occur in the mass spectrometer and on treatment with alumina. Urakawa et al. and Kono et al., who have optimised the process, speculate that the elimination proceeds via the ring open ketone form 23 where H9 is more acidic. Indeed the corresponding 9a-methoxy systems (mitomycins A (1), C (3), F (9) and porfiromycin (4)) where such a ketone is not accessible do not undergo elimination. Gredley has postulated a cyclic mechanism for
elimination of carbamic acid to explain the mass spectrum of mitomycin B (2);\textsuperscript{27} published data does not allow comparison of the MS fragmentation of 9-epi-mitomycin B (27) (this data may be in supplementary material\textsuperscript{56}). Danishefsky \textit{et al.} investigated the elimination reaction for 9-epi-mitomycin B (27) and found that only traces of mitomycin H (11) were generated under conditions that give a 30\% yield from mitomycin B (2) itself (Scheme 1.3).\textsuperscript{56} The cause of this difference in reactivity between 9a-hydroxy mitomycins is not clear; possibly steric hindrance between the aziridine and the carbamate prevents a \textit{trans} elimination in either 27 or its ketone tautomer. The Danishefsky group have also shown that the synthetic 9a\textbeta isomer 11b does not isomerise to mitomycin H (11). Thus the ketone tautomer cannot be accessible from both 11 and 11b if it forms at all.\textsuperscript{57}

![Scheme 1.3 The Preparation of Mitomycin H](image)

An interesting contrast in behaviour of ‘C9 epimers’ unequivocally in the ring open form was observed by Kishi \textit{et al.} with the synthetic ketals 28 and 29. The syn isomer 28 decomposed with loss of carbon dioxide to the ‘C10’ phenyl ether on standing or on mass spectrometry. The anti isomer 29 was stable under these conditions as was the desimino system. This was rationalised by inferring anchimeric assistance by the aziridine which is only possible for the syn isomer 28.\textsuperscript{58}
Net carbamic acid elimination from 9a-methoxy mitomycins has been achieved indirectly by increasing the leaving group ability of the 10-substituent.\textsuperscript{56,59} Hydrolysis of the carbamate, mesylation of the resulting alcohol and treatment with base converted porfiromycin (4) or its C9 epimer into mitomycin G (10). This was extended to give access to unsubstituted aziridine analogues by using acetate protection of the aziridine.

The selective acylation of the 7-amine rather than the aziridine was accomplished by Kraus\textsuperscript{60} via the anion of mitomycin C (3). The same approach for the alkylation of mitomycin C (3) was developed independently by Kaneko \textit{et al.}\textsuperscript{61} who isolated 7-alkylamino mitomycins but also unprecedented triketo byproducts, \textit{e.g.} 30, with undetermined C6 stereochemistry (Scheme 1.4), arising from alkylation of the delocalised anion and imine hydrolysis on workup.

\begin{center}
\includegraphics[width=\textwidth]{Scheme1.4.png}
\end{center}

\textbf{Scheme 1.4 Alkylation of the Anion of Mitomycin C}

The masked $\beta$-diketone nature of C5-C7 is evident in several reactions. 7-Hydroxy mitomycin C can be halogenated at C6 in a manner analogous to enol halogenation.\textsuperscript{48} Sawhney and Kohn have established that hydrazino mitomycins exist as o-azaquinone tautomers such as 31.\textsuperscript{62} Kanda and Kasai have exploited this tendency in order to introduce deuterium at the 6-methyl group. Treating the N1a-acetate of
mitomycin A (32) with ethylene glycol and potassium hydroxide gave acetal 33. After converting this to an unstable unsaturated intermediate 34, reduction with sodium borodeuteride and cleavage of the acetal gave mitomycin C or A derivatives with deuterated methyl groups (35) or (36) (Scheme 1.5).63

![Chemical Structures](image)

**Scheme 1.5 Substitution at the 5-Methyl Group**

A completely unprecedented reaction was discovered by Kasai et al. during attempted decarbamoylation of mitomycin D (7) in sodium isopropoxide. This gave a complex mixture containing two products characterised as 3α-alkoxy derivatives 39 and 40, albeit in low yield (5 and 9%). Their formation was rationalised as involving proton abstraction and alkoxide additions from the less hindered α face (Scheme 1.6). Mitomycin C (3) and porfiromycin (4) (both with 9α-methoxy groups) gave 0% and
0.7% of 3α-alkoxy products respectively. These lower yields were attributed to the lack of stabilisation of the intermediate iminium ions by a ketone tautomer, but note that the C9 stereochemistry also differs. Mitomycin B (2) (the 7-methoxy analogue of mitomycin D (7)) gave a methoxylated product in sodium methoxide in 0.8% yield; the higher yields from mitomycin D (7) were attributed to stronger electron donation at C7 (or contribution of a 7-imine tautomer) promoting conversion of 37 into 38.\textsuperscript{64} It may not in fact be very meaningful to compare yields of methoxylation with isopropoxylation, but under the conditions used the 7-amino group would in fact be fully ionised (pKa 12.4\textsuperscript{65}). Hence the C5 carbonyl may act as an intramolecular kinetic base (O5 to H3a is 2.39 to 2.70 Å in mitomycins where hydrogens are attributed, see Appendix 2).

\begin{center}
\includegraphics[width=0.8\textwidth]{scheme16.png}
\end{center}

\textbf{Scheme 1.6 Formation of 3α-alkoxy Derivates According to Kasai \textit{et al.}}

Kono \textit{et al.} once irreproducibly prepared mitiromycin (6) as the only significant mitomycin product (0.8%) from alcoholysis of mitomycin B\textsuperscript{40} (rather than the usual 49% of decarbamoyl mitomycin B\textsuperscript{66}). As a mechanism they propose intramolecular condensation of intermediate ketone 41 (Scheme 1.7); it is not clear why such a process would be irreproducible. An alternative mechanism (Scheme 1.8) involving reductive activation caused by some unreacted sodium metal would appear to be in accord with reductive sulfonation at C9a described in Section 1.3d (this was done on mitomycin C (3) (\textit{i.e.} C9β), where the aziridine ring may hinder comparable cyclisation from the β
face, or perhaps basic conditions are required to activate the carbamate). Attempts in our laboratory to convert leucomitomycin B or the corresponding 9a-methoxy derivative to mitiromycin under basic conditions (sodium hydride or ammonia) were unsuccessful with quinone being recovered after workup. However the subsequent work of Danishefsky et al. has revealed the distinctive reactivity of semiquinone intermediates (Section 1.3d), which eliminate the 9a-substituent readily. A trace of sodium in an excess of quinone would probably generate some semiquinone anion which on losing hydroxide at C9a could suffer intramolecular attack by a carbamate anion (Scheme 1.8).
1.3b Reactions in Acid

When treated with acids mitomycins decompose with rate limiting elimination of the 9a-substituent followed by aziridine cleavage. This process is apparently triggered by protonation of the 9a oxygen, loss of methanol or water assisted by N4 and then H9 loss leading to a putative aziridinomitosene (Scheme 1.9). More vigorous acidic conditions lead to hydrolysis of the 7-substituent giving a 7-hydroxy mitosene, and then carbamate hydrolysis. Acid treatment in the presence of a range of nucleophiles consistently gives 2-amino derivatives, e.g. 2-amino-1-hydroxymitosenes or “apo­mitomycins” in dilute hydrochloric acid. Although no intermediates have been directly isolated, extensive kinetic studies indicate that the 9a-substituent is lost before the aziridine is opened. An unexpected observation is that predominantly cis-1,2-substituted mitosenes are usually formed, i.e. complete inversion does not occur; the cis : trans ratio depends on the conditions used. Similar results are observed with an aziridinomitosene and a decarbamoyl mitomycin, ruling out neighbouring group participation. While aziridines usually open with inversion, predominant cis products have been observed, e.g. with the opening of 42 by hydrogen fluoride or hydrolysis of indano[1,2-b]aziridine (43) (Scheme 1.9). Unlike simple aziridines, 42 and 43 presumably open in an \( S_N^1 \) process via a benzylic cation. An analogous pseudobenzylic C1 cation in the mitomycin series may be stabilised by N4; this is supported by the complete regioselectivity observed (the 8-hydroxyl may also activate the aziridine in reduced mitosene intermediates, although the ring opening is facile even at the quinone level).
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Intriguingly Rebek et al. have observed cis selectivity from the basic methanolysis of a putative epoxide during the reaction of bromohydrin 44 (Scheme 1.10). The Rebek and Hornemann groups have speculated that these observations of cis selectivity arise from hydrogen bonding of the nucleophile to nitrogen or oxygen before and during opening, resulting in kinetic formation of the cis product. For such a kinetic model to be valid the products must not interconvert under the reaction conditions. Hornemann et al. “established” this for the acidic hydrolysis of mitomycin C by showing that the trans-2-acetamido-1-hydroxymitosene did not equilibrate, which of course proves nothing about the 2-aminomitosenes. However Tomasz et al. report that both cis and trans-2-amino-1-hydroxymitosenes are in fact stable even under strongly acidic conditions. They also note that no deuterium incorporation occurs when D₂O is used, but this is in direct conflict with Iyengar and Remers who report...
45% deuterium incorporation at C1. It is not clear which observation is correct; any deuterium incorporation would presumably occur via 45 (unless mediated by traces of reduced species) which could conceivably also cause cis - trans isomerisation.

Scheme 1.10 Stereoselectivity in the Opening of a Putative Epoxide

Hydrolysis in acetic acid gives unusual results. The Remers’ group found mitomycin C (3) gave, after acetylation of the initial products, cis and trans N,O-diacetates 47 and 48 (X = H₂N, R = H) in a 1 : 4 ratio. Diacetates were noted as only minor products from the reaction of mitomycin C (3) in an earlier report by the Remers’ group;⁷⁶ the major product was a monoacetate which was assigned cis stereochemistry - apparently erroneously given the later results (unless there is epimerisation under acetylation conditions). By contrast mitomycin A (1) which differs from mitomycin C (3) only in C7 substitution gave predominantly the cis product 47 (47 : 48 [X = MeO, R = H], 9 : 1).⁷⁵ The dramatic change in cis : trans ratio with a change in C7 substitution is surprising and was not explained. Aqueous hydrolysis of mitomycins A (1) and C (3) generates isolated products with similar cis : trans ratios, ca 6 : 1,⁷⁶ (the ratio of products from mitomycin C (3) measured directly by HPLC, 3 : 1,⁶⁸ is somewhat different from the isolated product ratio). An early report of significant deuterium incorporation at C1 from treatment of mitomycin C (3) with CH₃CO₂D⁵² could not be repeated, nor could incorporation be detected for other mitomycins.⁷⁵

The corresponding N1a-methyl systems showed the same influence of the C7 substitution on the acetylated product mixture, although to a smaller extent, but the
direct products now included significant amounts of both cis and trans N,O-diacettes 47 and 48 (R = Me). Porfiromycin (4) gave mainly 47 and 48 (X = H₂N, R = Me) and some cis monoacetate 46 (X = H₂N, R = Me). Mitomycin F (9) gave primarily 46 (X = MeO, R = Me), and hence more cis products overall.

The most attractive model for cis selectivity in aqueous hydrolysis is dominant kinetic capture of the developing C1 cation by water bound to the emerging 2-amino group as noted above. The solvation and solvent nucleophilicity in acetic acid will obviously differ and this doubtless leads to a different kinetic product mixture. However we suggest that it is also possible the 1-acetate, unlike the alcohol, is labile and equilibrates after initial kinetic attack. Tests of the stability of the products in acetic acid have not been reported. While cis-2-acetamido-1-hydroxymitosene (46) is easily rationalised as arising from syn acetate attack at C1 followed by acyl migration, the origin of the diacetates is not immediately obvious. Iyengar and Remers speculated that the trans product 48 might arise according to Scheme 1.11. However there seems to be no reason why the opening of oxazole 52 must proceed stereospecifically when that of the original aziridine does not; thus such a mechanism could account for both cis and trans diacetates kinetically. Presumably when R = Me formation of the intermediate cation is more favoured and allows generation of more of the diacetates. The conversion of cis monoacetate to trans diacetate products cannot explain the mitomycin C (3) results where diacetates are minor initial products.

Scheme 1.11 Iyengar and Remers’ Mechanism for the Formation of trans-diacetate
We suggest that the influence of the 7-substituent arises from its competitive effect on the conjugation of N4 with the C5 carbonyl. Thus a 7-amino substituent as in 3 or 4 is strongly conjugated with the C5 carbonyl and thus leads to more stabilisation of a Cl cation by N4 (see 50) compared to the 7-methoxy system. This could influence either the kinetic product mixture or the rate of Cl epimerisation if it occurs, in both cases by stabilising a Cl cation intermediate or transition state. In either case the presumably thermodynamic trans products should be favoured. Such an interaction would also stabilise the oxazole intermediate (52) proposed by Iyengar and Remers and thus lead to more of the diacetate for 7-amino systems, as is seen. An epimerisation process would explain the difference between aqueous and acetic acid hydrolysis, and this possibility is delineated in Scheme 1.12. Note however that if equilibration is the major pathway generating the trans monoacetate from mitomycin C (3), the rate of epimerisation of the cis 1-acetoxy-2-aminomitosene must be faster than transacylation. If Cl epimerisation does proceed at the quinonoid level with some substituents it is possible that DNA adducts (see Section 1.4) might also have mutable stereochemistry.

More definitive studies of stability and the direct products are clearly desirable.

Scheme 1.12 Postulated Mechanism of Mitomycin Hydrolysis in Acetic Acid

-26-
In complete contrast, treatment of mitomycin C (3) with trifluoroacetic acid in acetonitrile gave only the cis-2-trifluoroacetamido-1-hydroxymitosene in 33% yield. If ethyl carbamate (1.1eq) was added the yield improved to 95% and 5% of trans-2-amino-1-hydroxymitosene was also formed. This was interpreted as being a kinetically controlled reaction with the trans product arising from hydrolysis of a 1-trifluoroacetate on workup (and ethyl carbamate probably regenerating 10-carbamate cleaved during reaction). An alternative possibility is that trifluoroacetate, as a better leaving group than acetate, promotes more rapid equilibration in which the cis isomer is trapped by transacylation. Investigation of product stability was again not reported but would only be definitive here if the trans-2-amino-1-trifluoroacetoxymitosene could be studied.
1.3c Chemical Isomerisation of Mitomycin A

Kasai et al. have managed to chemically mimic the spontaneous isomerisation of mitomycin A (1) to albomitomycin A (17) and isomitomycin A (18), allowing more efficient conversion. Reaction with N-bromosuccinimide gives the N-bromoaziridine 53 which cyclises to an epimeric mixture of 8a-bromides 54. These are converted by base to the stable β-bromide (the mechanism is not clear) which is reduced to give albomitomycin A (17) in 76% yield overall (Scheme 1.13). Hydrogenation of albomitomycin A gave selective cleavage of the C4a-N4 bond, apparently for stereoelectronic reasons, to give isomitomycin A (18) after oxidation (90% yield).78

Scheme 1.13 Synthesis of Isomitomycin A and Albomitomycin A

With reasonable quantities of isomitomycin A (18) in hand the same group has developed a neat synthesis of 9β, 9a-hydroxy, unsubstituted aziridine mitomycins, i.e. 55 and 56, unavailable naturally or by earlier synthetic approaches. The key step is the acidic exchange of the ‘9a’-methoxyl in a ring system where subsequent deprotonation at C9 does not ensue (unlike the mitomycins), in accordance with Bredt’s rule. Treatment of isomitomycin A (18) with acid gave low yields of the hydroxyl-substituted and isomerised product 55. By preliminary reduction the exchange reaction and isomerisation could be separated, allowing different pH conditions to be used and giving a higher overall yield of 55 (36%).48 This leaves the C9α, unsubstituted
aziridine mitomycins as the only ‘obvious analogues’ not now available. They may possibly be accessible from 56 via an indirect epimerisation protocol as used to make 9-epi-mitomycin B (27).55

Scheme 1.14 Synthesis of New 9a-Hydroxy Mitomycins
1.3d Chemistry of Reduced Mitomycins

i) In the absence of added nucleophiles

The quinone in mitomycins is readily reduced and its reduction affects the reactivity of the whole mitomycin molecule. This is a crucial element in the selective biological activity of the mitomycins as antitumour drugs (Section 1.4) and is still being actively studied. The biological activity of mitomycins does not correlate well with reduction potentials alone.\(^7\) The reduction potentials of the mitomycins vary, mainly depending on C7 substitution. 7-Methoxy mitomycins are more easily reduced than 7-amino mitomycins; mitomycins are ca 100mV more easily reduced than their corresponding mitosenes.\(^8\) Aerial re-oxidation routinely regenerates the quinone. The ‘leuco’ prefix nomenclature is used here to denote hydroquinone forms as discussed on p5.

Simple catalytic hydrogenation and aerial oxidation of mitomycins B (2) or F (9) in DMF\(^8\) or ethyl acetate\(^8\) over platinum gave the same aziridinomitosene 57 in 40\% yield (Scheme 1.15). Mitomycin A (1), which has a free aziridine, gave a much lower yield of a corresponding mitosene. Apparently attempts to generate a mitosene from mitomycin C (3) were unsuccessful (inferred in reference 95). Porfiromycin (4) could be converted to a 7-amino aziridinomitosene in low yield only when the reduced product was treated with acetic acid. Addition of triethylamine or phenol inhibited the formation of the mitosene from mitomycin B (2), leading to recovery of starting material.\(^8\) Subsequently, mitomycin C (3) has reportedly been converted to a mitosene by hydrogenation in ethyl acetate\(^7\) or controlled potential electrolysis in methanol (30\% yield); the mitosene was not stable to HPLC.\(^7\) Controlled potential electrolysis also converted porfiromycin (4) to a mitosene, in unspecified yield, which was stable to TLC.\(^7\)

Thus reduction of the quinone, probably to the hydroquinone under these conditions, can lead to the elimination of water or methanol across C9,9a but the process appears to be acid-catalysed and elimination does not necessarily ensue. Acid catalysis is also compatible with kinetic studies.\(^8\) The mechanism by which phenol
inhibits elimination is not clear. The variation in reactivity with aziridine and C7 substitution is interesting and has not been explained.

![Chemical structure of Mitomycin F (9) and reaction scheme](image)

**Scheme 1.15 Hydrogenation of Mitomycin F**

The stability of reduced species has only been partially delineated. Kishi reports that hydrogenation of mitomycin B (2) in methanol (Pd-C, 5 min) gave a 50% recovery of mitomycin B (2) after re-oxidation. Danishefsky and Ciufolini found that in pyridine mitomycin B (2) could be cleanly reduced to its hydroquinone and then reoxidised to mitomycin B (2) without elimination. The reaction could be performed in ethanol, though less cleanly, and mitomycin C (3) could be similarly cycled. By contrast Patrick et al. reported observable (UV) conversion of leucomitomycin B to the leucomitosene on standing in DMF (time not stated). Danishefsky’s group adduced evidence that in pyridine it is the semiquinone form of mitomycin F which eliminated methanol. Thus addition of ascorbic acid to mitomycin F (9) in pyridine gave a 90% yield of mitosene 57, whereas leucomitomycin F (58) did not react. Alternatively a 1:1 mixture of the mitomycin F (9) and leucomitomycin F (58) gave, by NMR, a 1:1 mixture of the hydroquinone 58 and the mitosene quinone 51 after evaporation and redissolution in the absence of air. Apparently the mitomycin semiquinone is formed by comproportionation, loses methanol to give a mitosene semiquinone which is selectively oxidised by disproportionation. (The mitosene was not formed by similar treatment of either component alone.) The role of evaporation is not clear, possibly in the absence of any acid methanol must be removed to drive the reaction.

However as Rao et al. pointed out in 1977, semiquinones are much more stable in organic solvents or very alkaline solutions than under conditions that are physiologically relevant. They were able to find no evidence for intermediacy of semiquinones during cyclic voltammetry of mitomycin B (2) and C (3) in water (pH 7).
where reduction led rapidly to aziridinomitosenes then aziridine ring opening. Thus the reactivity of reduced mitomycins depends on the solvent; this is relevant to rationalising the alkylation chemistry of reduced mitomycins (see the next section).

Kinoshita et al. have given two brief accounts of the reaction of mitomycins with hydride reducing agents. Mitomycins A (1) and C (3), with unsubstituted aziridine rings, were relatively unreactive with lithium aluminium hydride in THF giving low yields of decarbamoylmitomycins after reoxidation. Mitomycin F (9) and porfiromycin (4), however, gave products which had both lost the carbamate and 9a-methoxy groups and gained a 9a-hydrogen. This was the first report of C9a substitution. Unfortunately supporting spectral evidence was very limited: the only reference to NMR data was for the product from mitomycin B (2) (see below) “no ... MeO [sic] (3.7 ppm in CDCl3) at C9 [sic] was found” (mitomycin B (2) has an hydroxyl at C9a; the 9a-methoxy of relevant mitomycins resonates at ca 3.3 ppm, the 7-methoxy at ca 4.1 ppm). Treatment of mitomycin B (2) with lithium aluminium hydride gave a colourless solution which was treated with moist ethyl acetate and Fremy’s salt to give, after chromatography, the mitosane (19%), decarbamoyl mitomycin B (60) (23%) and mitomycin B (2) in unspecified yield, see Scheme 1.16. A synthetic diastereomer of 59 has subsequently been reported, with stereochemistry defined by X-ray crystallography; unfortunately the NMR data reported lacks the critical H9H9a coupling for comparison.

\[
\begin{align*}
\text{Mitomycin B (2)} & \xrightarrow{1) \text{LiAlH}_4, 1\text{h}} \text{MeO} \xrightarrow{2) \text{Fremy's salt}} \text{MeO} \\
& \text{59} \\
& \text{59} + 2 \text{MeO} \xrightarrow{59} \text{MeO} \\
& \text{60} \\
\end{align*}
\]

Scheme 1.16 Reduction of Mitomycin B with Lithium Aluminium Hydride

Apparently the quinone is rapidly reduced to a hydroquinonoid anion which slowly suffers reductive cleavage of the carbamate and then reduction at C9a. It seems likely that this proceeds via an iminium ion; note that even under these basic conditions deprotonation of such an ion at C9, which would lead to the mitosene is not reported.
Using sodium borohydride in methanol the reduction of unspecified mitomycins reportedly gave reduction at C9a without decarbamoylation. The stereochemistry of the products of these reactions was not established. Unpublished work in Rickards' laboratory has repeated the reduction of mitomycin B (2) with sodium borohydride on a small scale. This resulted largely in recovery of starting material but also three products, the reduced mitosane 61 and two tentatively identified mitosenes 62 and 63 (Scheme 1.17). NMR spectroscopy indicated that the stereochemistry at C9 of 61 was β, i.e. an unexpected epimerisation had occurred. The mechanism for such an epimerisation is not clear. Presumably the most acidic species is the intermediate iminium ion, however loss of the H9 from this would generate (the mesomer of) a leucomitosene directly and C9,9a reduction of mitosenes has not been reported. It is conceivable that the epimerisation proceeds via the ketone tautomer prior to reduction or even at the quinonoid level during workup.

Scheme 1.17 Reduction of Mitomycin B with Sodium Borohydride

Reduction under acidic conditions in a non-alcoholic solvent (sodium cyanoborohydride, ethyl acetate and 1% acetic acid) was more efficient, giving the same mitosane 61 (27%) together with the aziridine ring opened mitosene 64 (30%) (Scheme 1.18). Thus basic conditions were not required for the epimerisation, although the mechanism may differ of course.

Scheme 1.18 Reduction of Mitomycin B with Sodium Cyanoborohydride
These results were not explained at the time but it seems possible that an unprecedented C9,9a functionalisation of a mitosene is occurring. Elimination of water from leucomitomycin B would give the leucomitosene 67. Protonation of this from the α face due to electrostatic or steric intrusion of the possibly protonated aziridine would give iminium ion 65b. Addition of hydride from the less hindered α face gives the observed mitosane 61 after oxidation, i.e. overall cis hydrogenation of mitosene 67 (Scheme 1.19). Note however that the iminium ion intermediate 65a in the dehydration step is apparently not reduced (i.e. the 9-epi isomer of 61 is not observed). This might be due to steric hindrance of the α face by the 9-substituent, which may also inhibit capture of any mitosene protonated on the β face. The reduction of indoles by sodium cyanoborohydride with acetic acid is a known procedure, occurring via C3 protonation and hydride reduction of the iminium ion, and works for the less basic 3-alkyl indoles also. Thus the proposed mechanism is chemically reasonable.

![Scheme 1.19 Proposed Mechanism of C9a Reduction](image)

It would be interesting to treat isolated aziridinomitosene 57 and the now available 9-epi-mitomycin B 27 with cyanoborohydride under the same conditions. (A high yield of 61 from 57 would rule out conceivable alternative mechanisms involving the ketone tautomer.) If it was possible to intercept the iminium ion 65b with other nucleophiles this may provide novel mitomycin analogues. Alternatively if an ‘interim’ nucleophile (perhaps acetate) could be added under acidic, limited reductive conditions without excessive aziridine cleavage and then displaced or hydrolysed in base this
would be a valuable method of mitomycin interconversion, particularly if compatible with N1a-acetyl protected aziridines. A synthetic route to functionalise mitosenes would be extremely valuable (see Chapter 2) as the only such reaction reported to date is an osmylation\textsuperscript{90} which also adds unwanted C9 functionality.

The mitosene 64 isolated from cyanoborohydride reduction of mitomycin B (2) has subsequently been reported as a trace product from chromatography of the leucomitomycin F (58) along with the aziridino analogue 66.\textsuperscript{56} The mitosene 64 might arise in the hydride reduction reaction \textit{via} two pathways. Tomasz and Lipman first isolated a related 1-unsubstituted mitosene 68a from reduction of mitomycin C (3) with microsomes and NADPH or by hydrogenation in a phosphate buffer. It was rationalised as arising \textit{via} aziridine ring opening at the hydroquinone oxidation level to give 68b which is nucleophilic, allowing protonation at C1 and generating quinone 68a directly.\textsuperscript{91} Kohn \textit{et al.} have shown that in hydrogenation at pH 5 the new C1 proton in 68a comes from the solvent or buffer (not the hydrogen gas), stereoselectively trans to the (protonated) amino group due to electrostatic repulsion.\textsuperscript{92,93} Such a process might also occur with sodium cyanoborohydride and acetic acid. Alternatively the concomitant electrophilic nature of an intermediate like 68b could lead to the hydroquinone of 68a through hydride attack. Such an electrophilic process is believed to lead to alkylation of DNA at C1 (see Section 1.4). Similarly the reduction of C10 may proceed through nucleophilic or electrophilic reaction of intermediate 69 or its ring open analogue where relevant. Hydrogenation of a 2-amino-1-hydroxymitosene in methanol gave the corresponding 9-methyl mitosene with a proton derived from methanol.\textsuperscript{94} Substitution at C10 by nucleophiles under reductive conditions is now well established (see Section 1.4). With sodium cyanoborohydride and acetic acid, either or both pathways may operate to generate 64. With chromatography of the hydroquinone presumably protonation is involved (the formation of 64 would require a disproportionation to reduce an intermediate quinone.).
Another reaction of the C9a position of mitomycins was accomplished by Hornemann et al. Reduction of mitomycin C (3) with dithionite or by hydrogenation in the presence of sulfite in aqueous solution gave the 9a-sulfonate 70 in 32% yield. The sulfonate could be converted back to mitomycin C by hydrogenation in the presence of sodium methoxide in ca 40% yield (Scheme 1.20). The sulfonate reacted further under reducing conditions to give compounds whose structures were not rigorously determined, but which appeared to be decarbamoyl mitosenes. The formation of the sulfonate was rationalised as proceeding via an iminium ion (cf. 65b) arising from methoxide loss from the hydroquinone. Interception of the ion by sulfite leads to 70 whereas deprotonation at C9 would give a mitosene and thus other products. Interestingly, the sulfite addition is reversible allowing regeneration of the iminium ion and interception by methoxide to regenerate mitomycin C. Thus again under basic conditions the iminium ion does not deprotonate at C9 and give a mitosene.

Scheme 1.20 Preparation of a 9a-Sulfonate

The Danishefsky group have converted a 9a-methoxyl to a 9a-hydroxyl under reducing conditions. Hydrogenation of mitomycin F (9) in pyridine and
chromatography of the filtered, evaporated residue gave largely intractable mixtures unless the chromatography was done in the presence of air. Then mitomycin F (9) was recovered (30 - 40%) along with aziridinomitosene 57 (40 - 50%) and 9-epi-mitomycin B (27) (20 - 30%), the latter being confirmed by X-ray crystallography (Scheme 1.21).56 Apparently a semiquinonoid iminium ion 71 is at least partly formed on oxidation, deprotonating to the mitosene or reacting with adsorbed water to give 27. The possibility of hydration of an aziridinomitosene semiquinone was not excluded however. It would be interesting to repeat this reaction using mitomycin J (13), i.e. 9-epi-mitomycin F, to see the effect of hindrance on the α face. Note than in the three known C9a functionalisations the putative nucleophiles (H-, SO32-, OH-) attack from the α face, anti to the aziridine.

Scheme 1.21 Synthesis of 9-epi-Mitomycin B

Egbertson and Danishefsky discovered that solutions of the leucoaziridinomitosene 72 slowly formed an unstable quinone, tentatively identified as the pyrrole 73. It was suggested that the pyrrole was formed via a rearrangement and a double elimination as shown in Scheme 1.22.96

Scheme 1.22 Decomposition of a Leucoaziridinomitosene
Zein and Kohn have also isolated a pyrrole under reducing conditions. Treatment of mitomycin C (3) with hydrazine (12 - 50 equivalents) in 2-amino-2-(hydroxymethyl)-1,3-propanediol ('tris') - acetic acid buffered water gave the expected 2-amino-1-hydroxymitosenes, cis-2-acetamido-1-hydroxymitosene and a trace of the pyrrole 74. This became the only product with a vast (1200 eq) excess of hydrazine. Treatment of mitomycin C (3) with 1,1-dimethylhydrazine (440 eq) gave predominantly a mixture of 2-amino-1-hydrazinomitosenes whereas corresponding 1-hydrazinomitosenes were not seen in the hydrazine reaction. An ESR signal matching an aziridinomitosene semiquinone was observable during these reductions. The formation of 74 must involve previously unknown mitomycin chemistry. The 9-methine hydrogen indicates that either the aziridine ring was unprecedentedly reduced without intervention of a mitosene or that such a mitosene was reduced at C9. The 7-hydrazinyl substitution is unusual for 7-aminomitomycins but not unreasonable. While a number of formal mechanisms can be imagined, it is difficult to understand why the hydrazine should cause such unusual reactions. One conceivable mechanism involves the usual reductive aziridine cleavage then nucleophilic attack by hydrazine at C1. Oxidation of the unobserved alkylhydrazine (by quinone or diimide disproportionation) allows an intramolecular reduction of the mitosene which leads to the pyrrole 74 after elimination of ammonia. The later stages of this are shown in Scheme 1.23. Such a mechanism is not possible with the 1,1-dimethylhydrazine-substituted mitosenes which fits the lack of pyrrole products there.
Andrews et al. reported the isolation of a mitosene 75 which had lost C10 after reaction of the hydroquinone dianion of mitomycin C with de-aerated water. The decarbamoyl mitosene 76 was also generated and became the sole product when the dianion was quenched with pH 7.1 phosphate buffer (Scheme 1.24). The authors were unable to explain the formation of 75 and proposed a rather odd mechanism for the formation of 76 (Scheme 1.25).98

The proposed generation of the strained five membered ring in 77 by attack of O8 on C10 (Scheme 1.25) seems unlikely and unnecessary; the mechanism following its cleavage is basically that of Tomasz and Lipman who isolated the corresponding 10-carbamoylmitosene 67 under more normal reductive conditions.89 We suggest that an alternative mode of carbamate cleavage would be inter- or intramolecular transacylation from O10 to O5 or O8 of the hydroquinone dianion, analogous to known
solvolysis of native mitomycins by alkoxide,\textsuperscript{54} the arylcarbamate being hydrolysed on quenching. Whilst transacylation between a phenolate and an alkoxy ester would normally be expected to favour the phenolate, here an electrostatically destabilised hydroquinone dianion is reacting. (This mechanism could be distinguished by observation of isotope incorporation from quenching with H\textsubscript{2}\textsuperscript{18}O where our mechanism predicts no \textsuperscript{18}O incorporation.)

\[ \text{Scheme 1.25 Mechanism Proposed by Andrews et al. for the Formation of Mitosene 76.} \]

We show a formalism for the loss of C10 in Scheme 1.26, where loss of formaldehyde is driven by the iminium ion presumably involved in formation of both mitosenes 75 and 76. The more acidic buffer quench might inhibit such a path by rapidly protonating the 10-alkoxide. Andrews \textit{et al.} very tentatively identified formylated products in the complex mixture of products from quenching the semiquinone radical anion in \textit{both} DMF and DMSO. It would be interesting to check this finding and discover if C10 is a formate source.

Such a sequence is supported by the loss of formaldehyde observed in the well precededent formation of 3,3'-diindolylmethanes from 3-hydroxymethylindoles (\textit{e.g.} Scheme 1.27) and related chemistry. This has been rationalised as proceeding through the iminium ion.\textsuperscript{99}
Scheme 1.26 Postulated Mechanism for C10 Loss

Scheme 1.27 Precedent for the Loss of Formaldehyde
1.3d Chemistry of Reduced Mitomycins

ii) With added nucleophiles

Mimicking the *in vivo* cross linking of DNA by mitomycins has been the target of numerous studies. The results are complex. Many reducing agents or even acidic conditions have been used *in vitro* to simulate the activation of mitomycins in poorly vascularised, hypoxic solid tumours where the mitomycins may be reduced enzymatically. A range of nucleophiles has been used to simulate DNA, and isolated DNA itself has often been utilised. The products depend dramatically on the conditions as well as the nucleophile and a variety of techniques have been used to analyse the process. The usual alkylation site is Cl, giving 1-substituted-2-aminomitosenes. Mitosenes unsubstituted at Cl or ClO are also isolated (see p35). 10-Hydroxy mitosenes are interpreted as arising from nucleophilic attack of water at a ClO cation, although carbamate hydrolysis has not been definitely ruled out. Acetate buffers can give rise to 2-acetamidomitosenes *via* acetate attack at Cl followed by acyl migration. These reactions have been reviewed by Franck and Tomasz\(^5\) and a paper by Li and Kohn provides a bibliography\(^1\). The isolation of 1-hydroxy-2-aminomitosenes is probably largely due to hydrolysis of aziridinomitosenes rather than a reductively activated process\(^2\). Reaction with DNA is discussed more extensively in the next section.

There has been significant controversy about the active reduced species. After Patrick *et al.*'s early observation\(^3\) that the semiquinone of mitomycin B (2) (solvent not stated) was stable, whereas the hydroquinone in DMF gave a leucomitosene, early models formulated the reactive species as the hydroquinone. Tomasz found the alkylation of DNA was optimised by portionwise additions of dithionite or excess mitomycin and attributed this to alkylation by the semiquinone radical form\(^4\). Subsequent chemical work indicated that the semiquinone was sufficiently activated to eliminate the 9a-substituent and alkylate nucleophiles while the hydroquinone was found to be stable. Hoey *et al.* found, however, that the semiquinone of mitomycin C (3) formed by pulse radiolysis in phosphate buffer was stable with respect to methanol elimination, but dismuted rapidly to the hydroquinone which did convert to a mitosene.
(in the concentration range 50-250 µM). Similar results were reported by Machtalere et al. Unfortunately the mitomycin C (3) concentration and the rate of reduction in vivo are not known. This debate has been reviewed by Franck and Tomasz who point out that the reactivity of the semiquinone and hydroquinone species is dependent on the protonation of these species; the protonated semiquinone is probably acidic and this may lead to observed reactivity where the redox reaction generates it rather than the anion.

The current consensus appears to be that the leucoaziridinomitosenes is the primary alkylating agent. The chemistry of the DNA cross-linking process, i.e. alkylating at C10, is less certain as it is more difficult to study or replicate in vitro. At least in vitro the mitosene semiquinone may be more reactive than the hydroquinone at C10. Thus addition of ferric chloride enhances cross-linking by reduced mitomycin F apparently by one electron oxidation of the mono-adduct hydroquinone. No such enhancement, however, was observed with either mitomycin C (3), perhaps due to overoxidation to the quinone, or with the aziridinomitosenes derived from mitomycin F (9), perhaps due to instability of its hydroquinone.

With some reducing agents other species may become important. Dithionite reduction has been widely used in alkylation studies and gives distinctive reactivity e.g. more extensive cross-linking than most reductants. However 9a-sulfonate intermediates (see p36) are almost certainly important, and have very different reactivity from natural mitomycins. Bisulfite addition at C10 has also been observed. Chromium perchlorate also gives distinctive products perhaps via chromium hydroquinone complexes. It is difficult to assess the relevance of the plethora of in vitro studies to the clinical situation where the mitomycin concentration, reducing agent, pH and immediate environment are not well established.
CHAPTER ONE

1.4 MECHANISMS OF ANTITUMOUR ACTION OF THE MITOMYCINS

The mitomycins show a range of biological effects\textsuperscript{108,109} and have been used as reagents in molecular biology to selectively inhibit DNA synthesis. Most work has concentrated on elucidating the carcinostatic mechanism, although an understanding of any cytoselectivity and thereby the origin of side effects would also be important. An understanding of the mode of action of the mitomycins is essential for the rational design of analogues. To function as an anticancer drug, mitomycin C (3) must kill tumour cells or stop them replicating. This requires selective uptake or activation by cancer cells. The useful antitumour activity of the mitomycins seems to arise largely from preferential reduction in solid tumours, which are poorly vascularised and anoxic, but this selectivity is only seen at low doses. The success of radiotherapy is often limited by the refractory nature of such hypoxic cells, which explains the utility of mitomycin C (3) in combination therapy. However the quinone ring of mitomycins may also mediate oxidative damage in oxygenated tissue by redox cycling which generates hydroxyl radicals, superoxide and hydrogen peroxide; this may also serve to increase hypoxia in a cell. The relatively low pH of tumour tissue may also lead to selective activation. The mechanism of action has been reviewed extensively.\textsuperscript{110,111,112,113,114,115,116,117,118,1,50,109}

The lethal effect of the mitomycins in tumour cells is attributed to covalent cross-linking of DNA, thereby inhibiting replication. This crosslinking requires reductive activation, unlike most DNA modifying drugs. Other effects,\textsuperscript{109} however, are not excluded. Monoalkylation of DNA and intrastrand crosslinks are also formed reductively and DNA to protein binding may occur. While covalent crosslinks are much less readily repaired, extensive mono-alkylation may also encumber the DNA. The active alkylating species \textit{in vivo} are short lived and difficult to study even \textit{in vitro} as discussed in the last section.

Much recent work has studied the detail of alkylation of DNA \textit{per se} or of oligonucleotides, mainly \textit{in vitro} but with some model \textit{in vivo} studies. The interaction
with DNA is far from random. Mono-adducts and inter- and intrastrand bis-adducts of mitomycin C (3) with DNA have now been isolated and characterised. The N2 of guanosine from 5'-CG sequences in the minor groove is the dominant alkylation site, giving rise (after hydrolysis) to a mono-adduct 78 through reaction at C1 and then the interstrand adduct 79 (exclusively from 5'-CG sites) or intrastrand adduct 80 (from 5'-GG sites) through reaction at C10 with the N2 of another guanosine. The decarbamoyl adduct 81 has also been isolated, apparently arising from C10 activation and trapping with water. The specificity for guanine is remarkable and models have been proposed to explain the base and sequence selectivity, and why this sequence favours interstrand cross-linking. The acid-catalysed alkylation of oligonucleotides also shows 5'-CG selectivity, which limits the sites likely to be involved in hydrogen bonding during precomplexation of the reduced species. Tomasz has reviewed most of this work, and notes that evolution appears to have selected a drug which forms mono-adducts at a site which favours crosslinking and possibly in a sequence which is common or critical (CG is common in promoter codons) in the natural bacterial target organisms. Which mitomycins are produced by native Streptomyces, their effectiveness against perhaps hypoxic target organisms and mechanisms of self protection by the producing organisms appear to be unstudied. A synopsis of the recent work is given here, and details are available in the reviews.
Under certain conditions the crosslinking is reversible (large excess of sodium dithionite or dithiothreitol, pH 7.4, rt, half-life 4 or 10 h respectively; 1M piperidine, 90°C, 18h). The mitosene products from these reactions were not identified, except for noting the possibility of an “ene-pyrrole” like 73 on the basis of an intense yellow colour. Reversibility of C1 phosphate substitution under reducing conditions has been demonstrated. Whether reversibility is relevant to in vivo alkylation is not clear. Significant reversibility in vivo could make thermodynamics important if re-alkylation by the products is possible.

From the perspective of the mitomycin molecule the cross-linking process is believed to proceed as shown in Scheme 1. Enzymatic reduction of mitomycin C (3), presumably close to DNA, gives the hydroquinone which eliminates methanol via a two step process. Following reduction, the N4 lone pair is no longer deactivated by the C8 carbonyl and aids methoxide loss from C9a (perhaps acid-catalysed), followed by deprotonation at C9 to give the aromatic mitosene 82. This indolic hydroquinone or its aziridine ring open form 83 binds to the DNA, perhaps via hydrogen bonding of the 2'-amino group of guanine of B-form DNA to O10. The ring opening is acid-catalysed and relieves the strain of the three membered ring. Nucleophilic attack by guanine at C1 regenerates a hydroquinone 84 with one covalent link to DNA. Oxidation at this point gives a mitosene quinone bound to DNA which is more difficult to reduce than mitomycin C (3) and which is the source of mono-adduct 78 on hydrolysis. Loss of the carbamate, assisted by N4, gives the iminium ion 85 which suffers nucleophilic attack by a nearby guanine if at a 5'-CG site (or other nucleophile e.g. water) to give a
mitosene hydroquinone which has two covalent links to DNA and oxidises to a corresponding cross-linking quinone 86 (or C10 modified mono-adduct 81).

Scheme 1.28 Alkylation of DNA by Mitomycin C

Thus the mitomycins are masked bis-alkylating, the quinone ring providing a 'safety catch' released by reduction at a (C7 variable) potential which allows methanol (or water) elimination. This in turn allows sequential opening of the strained aziridine ring and carbamate loss, all mediated by N4, giving specific DNA alkylation controlled by the skeletal framework and hydrogen bonding. Overall there is probably no redundant functionality in the molecule.

The reactivity of the C1 and C10 positions in reduced mitomycins was recognised very early.\textsuperscript{114,113} \( \text{Moore} \) subsequently postulated concomitant activation of C1 and C10 to give the diene 87 as the active form, but this is unlikely as nucleophiles must then react initially at C10 whereas only C1 mono-adducts are actually observed.
Leucomitomycin F (58) and leucoaziridinomitosene have been observed in isolation by NMR in pyridine, but no intermediates have been directly identified during reaction with DNA. Most of the evidence comes from kinetic studies and isolation of oxidised products (i.e. quinones) and is summarised in the reviews (p 43).

Almost all of the work has studied the reactivity of mitomycin C (3). Porfiromycin (4) gives apparently related but less rigorously characterised 2-methylamino substituted adducts. McGuinness et al. have recently studied mitomycin A (1) (differing only in C7 substitution) in some detail. Corresponding mono-adducts and an interstrand bis-adduct (7-methoxy analogues of 78, 79 and 81) were isolated, the alkylation efficiency being comparable to mitomycin C. However, while hydrogenation or reductases generated only mono-adduct 78 from mitomycin C (3), these conditions also gave the corresponding decarbamoyl adduct from mitomycin A (1). Also the reaction of mitomycin A (1) was little affected by an aerobic atmosphere, unlike mitomycin C (3). McGuinness et al. propose that another pathway operates to afford the decarbamoyl adduct, namely that the leucomitosene competitively loses carbamate rather than aziridine ring opening, i.e. Path B in Scheme 1.29. This is rationalised as feasible because of decreased "delocalisation of the quinone electrons into the aziridine ring [in the leucomitosene]" or a more acidic 8-hydroxy group compared to the 7-amino compounds. This would be interesting if true, as the only product established with substitution at C10 and an intact aziridine ring is the 9-methyl mitosene 66, a trace product from chromatography of leucomitomycin F (58) which arises from nucleophilic reactivity at C10 (see p35). An alternative explanation was overlooked. Rao et al. have shown that the reduction potential of 1-hydroxy-2-aminomitosene C is significantly lower than mitomycin C (3) (-0.47 vs -0.37 V), thus presumably the mono-adduct hydroquinone 84 is readily oxidised thermodynamically and probably kinetically (e.g. even by another mitomycin C
molecule) which will inhibit subsequent reaction at C10. 7-Methoxy quinones like mitomycin A (1) are much more readily reduced than 7-amino quinones and although the derived mitosenes again have a lower reduction potential (e.g. -0.20 vs -0.32 for mitomycin B (2) and the derived mitosene respectively), the intermediate hydroquinone 88 should be more stable, longer lived and thus more likely to give rise to C10 reactions than the 7-amino analogue 84. Thus unlike the McGuinness proposal, the divergent reactivity may arise at the leucomitosene (88) not the leucoaziridinomitosene (89) level, and no second pathway would be required. This assumes that the proclivity for C10 reaction is not otherwise significantly affected by C7 substitution. Studies of C1 vs C10 reactivity of 89 would be interesting (the related N1a-methyl hydroquinone has been synthesised); the isolation of a 10-hydroxy-aziridinomitosene would support the McGuinness scheme.
Rao et al. also made an unremarked but surprising finding that the reduction potential of the aziridinomitosene C 90 was -0.37 V, i.e. the same as mitomycin C and significantly higher than the apparently more related apo-mitosene 91. If this is true then the aziridine ring has a dramatic effect on the redox properties of the indoloquinone which would have ramifications in the DNA alkylation mechanism. The study involved isolated 90 prepared by catalytic hydrogenation of mitomycin C in ethyl
acetate, the only time this reaction has been reported, whereas Patrick et al. had apparently been unsuccessful in this preparation although able to produce other aziridinomitosenes by hydrogenation in DMF. IR and UV data for compound 90 were reported by Rao et al. and an irrelevant paper was cited for the "general procedure" (Patrick et al. 83 was presumably meant). The preparation of the mitosene 91 by controlled potential electrolysis (-1.0 V) was recently reported by Han and Kohn73 (NMR data only) and intriguingly 90 precipitated directly during the reduction, i.e. as the quinone, which was attributed to "autocatalytic transformation involving mitomycin C, a redox process at the electrode surface, or by reaction with trace amounts of oxygen". Clearly this is not consistent with the measurements of Rao et al. which deserve re-evaluation.

The aziridinomitosene 90 was observed to react readily with nucleophiles (e.g. water or methanol) at the quinone oxidation level.73 Apparently the ring opens primarily in an SN1 fashion via involvement of N4 even without acid catalysis. Acid activation causes mono-functionalisation of DNA by mitomycin C (3), also with 5'-CG selectivity. As acids cause elimination of methanol and aziridine opening, probably in that order, it seems plausible that some mono-adduct arises from reaction of the aziridinomitosene quinone 90 generated either by reduction, elimination and oxidation or by low pH in vivo (solid tumours are usually more acidic than surrounding tissue131). Whether such non-reductive activity is important in limiting the selectivity of mitomycin action is not clear. While 90 could thus lead to mono-alkylation of DNA it is unlikely to generate crosslinks.
CHAPTER ONE

1.5 LONE PAIR EFFECTS IN MITOMYCINS

i) The Case of the Non-basic Aziridine Ring

The aziridine ring *per se* in mitomycins is much less reactive and much less basic than normal aziridines. In their recent review of mitomycin chemistry, Franck and Tomasz state:

"the peculiar lack of reactivity of the aziridine ring of the mitomycins in their native (quinone) form ... [is] ... due to the electron withdrawing effect of the quinone system, transmitted via overlap of the p orbitals of N4 and the aziridine nitrogen"

and cite Tulinsky and van den Hende’s later crystallographic studies in support. The statement is odd as overlap of filled lone pair orbitals is destabilising and Tulinsky and van den Hende indeed attributed pyramidalisation at N4 (away from the aziridine) to deformation to avoid overlap of unshared electrons. Interaction of the aziridine lone pair with a lobe of the C4a-C8a $\pi^*$ orbital could, conceivably, ‘protect’ the aziridine nitrogen and the extreme case of this, covalent bonding N1a to C4a is indeed seen in albomitomycin A. In native mitomycin crystals however, the distance between N1a and C4a is 3.19 to 3.35 Å (see Appendix 2) which suggests that such interaction is at best weak.

The pKa of mitomycin C (3) cannot be accurately determined by titration because of rapid degradation, although a value of 3.2 (in aqueous methanol) has been reported; $p$H - rate profiles suggest that the value is in the range 2.5-2.8. The pKa of mitomycin B (2) was determined by titration in water as 4.3 and by kinetics as 2.80. The pKa values of mitomycin A (1) and porfiromycin (4) were measured kinetically as 2.62 and 2.40 respectively. Apparently the titrimetric values are higher because of partial degradation to the more basic 2-amino-1-hydroxymitosenes. The aziridine is much less basic than cis-2,3-dimethylaziridine ($p$Ka 8.7). The causes of this low basicity are not entirely clear. In part it may be due to fusion with a five membered ring ($p$Ka of trans-6-azabicyclo[3.1.0]hexan-2-ol is 6.1). Presumably there

† Following the normal convention, “pKa” is used here to describe the acidity of the conjugate acid of a given base (the alternative $pK_{\text{HH}^+}$ is sometimes used in the literature).
is steric hindrance to protonation (one proton in the cation must sit inside the concave mitomycin near N4) which will reduce solvation. However, these effects are still present in the hydroquinone yet Hoey et al. suggest the mitomycin C hydroquinone has a pKa of 5.1 on the basis of kinetic studies. Thus some interaction with the quinone ring appears to exist, perhaps an electrostatic one due to polarisation. Iyengar et al. have reported that the aziridinomitosene 57 has a pKa of 5.2, *i.e.* it is much more basic than the mitomycin, consistent with its presumably more planar structure. No information is available regarding the pKa of relevant reduced species.

Tulinsky and van den Hende’s intriguing suggestion that a lone pair interaction with the aziridine caused pyramidalisation at N4 was based on the crystal structure of an N1a-sulfonated mitomycin (where the aziridine lone pair is presumably delocalised into the sulfonate). It is now possible to further assess this postulate using the significant number of native mitomycin X-ray structures now reported. We have examined the structures of mitomycins in the Cambridge Structural Database (Appendix 2). The N1a-N4 distance varies relatively little (2.63 to 2.74 Å) for a range of native and protected mitomycins, whereas the pyramidalisation at N4, measured as an angle sum (341 to 352°) or out of plane distance (0.24 to 0.36 Å) varies significantly and there is no correlation between the N1a-N4 distance and pyramidalisation (see Graphs 1 and 2). The angle sum and out of plane distance are partially dependent on each other and show an almost linear correlation (data not shown). Acylation or sulfonation of the aziridine does not have a significant effect. This suggests that the lone pair interaction is not important in determining N4 geometry. This was confirmed by the unremarked determination of the crystal structure of quinone 94. Despite the absence of an aziridine ring, the nitrogen is significantly pyramidalised (angle sums 352 and 349°, N4 out of plane distances 0.25 and 0.27 Å for two independent molecules) and indeed the geometry matches the mitomycins closely. Thus the pyramidalisation at N4 is unrelated to lone pair interaction and simply arises from requirements of the fused ring system. The pyramidalisation of N4 is discussed further in the next section. It is not possible to properly assess the delocalisation of N1a into the quinone as the N1a-C4a distance varies little.
Tulinsky and van den Hende also state that nuclear quadrupole measurements indicate that the mitomycin ‘free base’ aziridine nitrogen does not invert but no data or reference is given. However, pKα measurements indicate that a proton can fit at the Nla β site (if it is indeed the aziridine that is protonated). Similarly the observation of acylation, alkylation and sulfonylation at Nla suggest that the Nla β invertomer is at least transiently accessible. It might be possible to observe the invertomers by low temperature NMR (see chapter 4) if the energy difference is not too great.
Graphs 1 and 2. Correlation of N4 Pyramidalisation and N1a-N4 Distance for Various Aziridinopyrrolo[1,2a]indoles

(see Appendix 2b; data for KIKSAY (Albomitomycin) and DAKWAN, a hydroquinone, are excluded)
ii) N4 Conjugation

A key point in the accepted model for DNA cross-linking is that loss of the 9a-functionality is slow in the oxidised mitomycin but rapid in some reduced form. The conjugation of N4 with the C4-O8 enone is believed to stabilise the mitomycins by inhibiting the loss of the 9a-substituent. Under acidic conditions equilibrium protonation of the 9a oxygen presumably generates a better leaving group and reductive activation is not required. Two significant, competing factors influence the pyramidalisation of N4. Conjugation with the C4a-O8 enone system favours a coplanar system with N7 or O7 conjugation with the C7-O5 enone doubtless having some subtle effects on the quinone ring geometry and thus on conjugation of N4. Steric requirements of the fused ring system appear to require some pyramidalisation. In his preliminary communication\textsuperscript{16} Tulinsky noted that N4 in N\textsubscript{1a}-(4-bromobenzencesulfonyl)-mitomycin A was “planar within the accuracy of its determination ± 0.03 Å” (at R=0.146). However, this was revised in the full paper (R=0.087)\textsuperscript{17} where it was found to be 0.3 Å out of the C3C4aC9a plane away from the aziridine and “more tetrahedral than trigonal” and that while shortening of the N4-C4a bond (1.36 Å) was seen, C8-O8 (1.23 Å) was not affected. Mitomycin X-ray structures show a significant range of out of plane distances (0.24 to 0.36 Å) as noted above. The non-planar nature of N4 was attributed to deformation to minimise the lone pair overlap with the aziridine nitrogen which we have shown to be unimportant.

Examination of X-ray structures suggest that the mitomycins represent a compromise between the steric influence of the pyrroloindole ring system, which favours a pyramidalised N4, and conjugation, which favours a planar N4. The aromatic pyrroloindoles \textsuperscript{92,93} have effectively fully pyramidalised nitrogen atoms, indicating that the ring system can accommodate this extreme (and that it is probably favoured). The quinone \textsuperscript{94} by contrast is significantly flattened at N4 (see Table 1; for clarity mitomycin like numbering is used). Bond lengths in mitomycins are also consistent with partial conjugation, \textit{e.g.} shortening of N4-C4a, although variations do
not correlate with pyramidalisation of N4, perhaps reflecting random errors in the small range (1.34 to 1.37 Å) for N4-C4a.

<table>
<thead>
<tr>
<th>Angle Sum around N4</th>
<th>332</th>
<th>331</th>
<th>351, 349</th>
</tr>
</thead>
<tbody>
<tr>
<td>Displacement of N4 from C3C4aC9a Plane (Å)</td>
<td>-0.45</td>
<td>-0.47</td>
<td>-0.25, -0.27</td>
</tr>
<tr>
<td>N4-C4a distance (Å)</td>
<td>1.40</td>
<td>1.41</td>
<td>1.35, 1.35</td>
</tr>
</tbody>
</table>

(two independent molecules)

Table 1. Pyramidalisation of N4 in Various Pyrroloindoles

The net result appears to be partial conjugation in native mitomycins that is difficult to quantify but not strong. The limitation of conjugation may be important in the elimination of the 9a-substituent from the quinone under acidic conditions. There is a small difference between 7-amino and 7-methoxy mitomycins. 7-Amino mitomycins have slightly less pyramidalised N4 atoms (angle sum 351°, ‘N4 plane’ 0.25 Å for mitomycin C and 347°, 0.30 Å for mitomycin A). This would be consistent with enhanced delocalisation due to hydrogen bonding of the C8 carbonyl with the 7-amino group.
In order to assess the ‘release’ of the N4 lone pair upon reductive activation it would be desirable to examine structures of reduced mitomycins. There are no X-ray structures of native mitomycin hydroquinones, however the synthetic silylated hydroquinone 95 has been reported. It is not possible to assess the influence of the bulky silyl groups or the 9-hydroxyl, however, the N4 nitrogen is noticeably more pyramidalised than in the mitomycins (angle sum 339° and ‘N4 plane’ 0.39 Å) but not to the extent seen in the 9a-hydro benzenoid systems 92 and 93. Thus it is possible that geometric changes affect the reactivity of the reduced mitomycins, and some increased ‘availability’ of the N4 lone pair is very likely.

![Chemical Structure Image]

...
CHAPTER ONE

1.6 MITOMYCIN BIOSYNTHESIS

The fundamental biogenesis† of mitomycins is now established. The details of the biosynthetic steps, however, are far from clear. Early work, which has been reviewed by Kirsch in 1967 and Hornemann in 1974 and 1981, established that the framework arose from D-glucosamine (96) (see Scheme 1.30) and an unidentified “C7N” source (a meta-C,N substituted hexacycle or precursor). The N- and O-methyl groups derive from methionine and the carbamate from citrulline or arginine.

The origin of a similar C7N unit in ansamycins, which was also known not to be polyketide derived, had attracted significant attention. The Rickards group established that the C7N unit in ansamycin (e.g. actamycin (97), maytansinoid (e.g. maytansine (98), antibiotics arose from incorporation of the same novel amino acid, 3-amino-5-hydroxybenzoic acid (“AHB”, 99), an all-meta C,N and O substituted benzene. Almost simultaneously Ghisalba et al. also identified AHB as the precursor of rifamycin B using blocked mutant studies. The biosynthesis of AHB (99) itself in Nocardia mediterranei from phosphoenolpyruvate, erythrose 4-phosphate and ammonia has recently been established. AHB (99) thus arises from a very early branch point in the shikimic acid pathway. This fits data for incorporation of erythrose and pyruvate into mitomycins.

† “Biogenesis” is used here and in this thesis to mean biosynthetic origin (not process) rather than in its original sense of ‘life arising from life’.
In ansamycin and maytansinoid systems AHB is the starter unit for polyketide chain formation with the chain invariably eventually cyclising at the amino group. Natural ansamycins and maytansinoids contain AHB residues with varied substituents. Chloro, hydroxy, and methylthio substitutions of the nucleus occur and the phenol may be alkylated, acetylated or oxidised to a quinone. However alkylated, chlorinated and fluorinated AHB derivatives were not incorporated into ansamycins and indeed inhibited production. By contrast the addition of AHB itself significantly enhanced antibiotic production. Thus elaboration of the AHB acid nucleus apparently occurs after incorporation.

The Rickards group established that the C7N unit in mitomycin antibiotics also arose from incorporation of the AHB. In the mitomycins the carboxyl of AHB is completely reduced to furnish the 6-methyl group. 3-Amino-5-hydroxybenzylalcohol was not incorporated into porfiromycin and inhibited porfiromycin production, suggesting that it is probably AHB itself which is incorporated with subsequent reduction.

Thus the mitomycins are biosynthesised from AHB, D-glucosamine, methionine and citrulline or arginine (Scheme 1.30). This requires many steps and no details of the sequence are known. Speculations about the mechanisms of formation of the three bonds joining AHB and D-glucosamine are made in the next chapter (Section 2.6) as they led to our own synthetic work. The isolation of some related antibiotics reported during the course of our work also bears on these mechanisms and is discussed at the end of Chapter 3.

Scheme 1.30 Biogenesis of the Mitomycins
The only direct biosynthetic studies relevant to the coupling of AHB (99), D-glucosamine (96) are feeding experiments with selectively radiolabelled D-glucosamines, namely tritium at C2', C4', C5', or C6', and 14C at C1', reported by Hornemann, largely in his later review.\(^{138}\) (For clarity the ultimate mitomycin numbering is generally used in this discussion; other numbering systems, most notably of D-glucosamine (96), are indicated by a prime (') suffix.) Earlier, Hornemann and Cloyd had reported 78% retention of tritium after feeding \([1'-14C,1'-3H]\) labelled D-glucosamine\(^{152}\) but curiously this result was not mentioned in either review. The tritium labels were partially lost during incorporation into mitomycin A (1) and the percentage retentions are shown in Table 2. The locations of the incorporated tritium were not established and full details have not been published so it is not possible to assess the purity of the labelled glucosamines. The loss of \(^3\)H2' and \(^3\)H6' is surprising as C2 and C10 are at the same oxidation state in the derived mitomycins as in glucosamine. The significant retention of \(^3\)H4' is also remarkable as the derived C9a is devoid of hydrogen. The results are not consistent with any simple model of bond formation, perhaps in part due to limited removal and reintroduction of hydrogen atoms during enzyme mediated processes.

<table>
<thead>
<tr>
<th>D-glucosamine (96)</th>
<th>% retention of tritium (based on (^{14})C label)</th>
</tr>
</thead>
<tbody>
<tr>
<td>([1'-14C,6'-3H])</td>
<td>82, (91*)</td>
</tr>
<tr>
<td>([1'-14C,5'-3H])</td>
<td>22</td>
</tr>
<tr>
<td>([1'-14C,4'-3H])</td>
<td>39, (35(\dagger))</td>
</tr>
<tr>
<td>([1'-14C,2'-3H])</td>
<td>44</td>
</tr>
<tr>
<td>([1'-14C,1'-3H])</td>
<td>78*</td>
</tr>
</tbody>
</table>

* Data from Reference 156  
\(\dagger\) Data from Reference 137

Table 2 Retention of Tritium in Mitomycin A Incorporating Labelled D-glucosamine (96) From the Data of Hornemann\(^{138}\)
The results warrant repetition with deuterated glucosamines, if sufficiently low
dilution can be obtained, in order to establish the fate of the deuterons. A comparison of
incorporation into mitomycin B (2) and other mitomycins would be particularly
interesting, given the difference in stereochemistry at C9. It would be worth checking
that the labels are not affected by metabolic cycles involving D-glucosamine (96).

The late stages of mitomycin biosynthesis are also undefined. The conversion of
mitomycin F (9) to porfiromycin (4) (not necessarily occurring intracellurally) is the
only direct feeding experiment reported.146 There is a strange report that cell free
extracts of *Streptomyces caespitosus* convert the 7-hydroxy mitosene 101 into a product
that correlated with mitomycin A (1) by TLC and antibacterial activity. The
decarbamoyl product was not converted.153 This transformation requires methylation of
the 7-hydroxyl, aziridine cyclisation and methanol addition to the mitosene, the last
being completely unprecedented chemically. It is not clear what is occurring but we
doubt that these observations are relevant to biosynthesis, although experiments of this
nature will probably be required to firmly establish the validity of proposed biosynthetic
intermediates. Variation in the relative yields of the mitomycins would be interesting
but is poorly documented, presumably for commercial reasons. Mitomycins A (1) and B
(2) appear to occur at the expense of mitomycin C (3) and *vice versa* in a pH dependent
manner,10,154 but other effects have been noted.138

Claridge *et al.* discovered that supplementation of a mitomycin C (3)
fermentation with many primary amines produced analogues of mitomycin C with
corresponding 7-amine substitution. More interestingly *ca* ten times more of mitomycin
D (7) analogues were also produced, *i.e.* mitomycins with various 7-amine substituents,
C9α stereochemistry, 9a-hydroxy groups and methylated aziridine rings, whereas the
direct fermentation evidently produced no mitomycin B (2) and apparently no
mitomycin D (7). The C9α stereochemistry was assigned on the basis of rather indirect
evidence of aromatic solvent (pyridine) induced shifts by comparison with porfiromycin (4) and the observation that they have a greenish-blue hue on silica which differs from the colour of mitomycin C (3) (although UV spectra were identical). The validity of this comparison is unclear as both C9 stereochemistry and 9a-substitution differ, however the assignment is supported by an alternative NMR method devised by Gredley (with some revisions, see Appendix 3). No unequivocal conclusion can be drawn from this study but the diversion of biosynthesis leading to C9α systems is fascinating.

Hornemann proposed that mitomycin A (1) was the precursor of the other mitomycins. 7-Amination and/or N1α methylation furnishes mitomycins C (3) and F (9) and porfiromycin (4). It was suggested that mitomycin B (2) arose by reduction, methanol elimination and trans addition of water to the intermediate mitosene. This latter proposal has no supporting biosynthetic or chemical evidence. Its only advantage is that it rationalises all of the known mitomycins, including those more recently discovered, in terms of a known product (mitomycin A (1)) as a precursor. It appears to have been postulated because mitomycins A (1) and C (3) (now also M (16)) were the only mitomycins known with an unsubstituted aziridine ring. The requirement for an unprecedented mitosene functionalisation makes it unappealing, however. Note that the putative hydration proceeds to give a different stereochemical outcome from that observed for hydride reduction (see p32) where mitomycin B (2) was converted to a system with mitomycin A (1) stereochemistry (C9β). The conversion of mitomycin F (9) to 9-epi-mitomycin B (27) under reductive conditions (see p37) did not involve C9 epimerisation.

We prefer to postulate that the mitomycins derive from an unknown intermediate 102 (or a 7-hydroxy or unsubstituted analogue) with 9α stereochemistry, a 9α-hydroxyl and an unsubstituted aziridine ring; bear in mind, however, that there is no evidence that the mitomycins all arise from a single biosynthetic path. The elaboration of 102 to the mitomycins would require N1α or O9a methylation, C9 epimerisation and C7 substitution. The only contentious operation is C9 epimerisation. This has been achieved chemically but only indirectly (see p15). Inspection of the known natural
mitomycins (Figure 5, p11) shows that all mitomycins with C9β stereochemistry have a 9a-methoxyl group. This raised the intriguing possibility that the C9β systems might be higher in energy and be trapped by methylation of the 9a-hydroxy, precluding return to a keto tautomer that may be the active species in epimerisation.\textsuperscript{55} However the preparation of 9-\textit{epi}-mitomycin B (27)\textsuperscript{55} and its unsubstituted aziridine analogue \textsuperscript{5548} militate against this. In the light of observations described below, we compared the energies of C9 epimers with 9a hydroxy groups using MACROMODEL v 1.3 (MM2 calculations) and could find no significant difference. We have not yet investigated the relative stability of ‘C9’ epimers and their anions in the ring open keto form. Note that ring opening of a 9a-alkoxide would probably be favoured both entropically and thermodynamically. The pKa of the 7-amino group in mitomycin C (3) is 12.4\textsuperscript{65} so ‘N4’ in the keto form would probably be a more stable anion than the 9a-alkoxide. No direct evidence has been adduced for such ketone tautomerism. Yet even if not thermodynamically driven, C9 epimerisation seems a plausible biosynthetic step.

\begin{center}
\begin{tikzpicture}

\end{center}

\textit{A note on C9 epimerisation}

As our biosynthetic postulate requires C9 epimerisation, any chemical evidence relating to such a process is interesting, even if the biosynthetic step is likely to be enzymatically controlled. In the preparation of 9-\textit{epi}-mitomycin B (27)\textsuperscript{55} epimerisation of the 9-hydroxymethyl substituent of 22 gave a 1:1 mixture of 22 and 24, believed to be an equilibrium mixture, although conversion of 24 to 22 was not directly established.

Beijnen \textit{et al.} have reported CD spectra of mitomycin B (2) and mitomycin A (1) in methanol and the respective derived 7-hydroxy mitomycins in pH 9.5 buffer, wherein they are ionised (Figure 6).\textsuperscript{133} Mitomycins A (1) and B (2) differ in C9
CHAPTER ONE

stereochemistry, 9a-substitution and aziridine substitution. The CD spectra of mitomycins usually reflect C9 stereochemistry with a strong positive extremum at ca 320 nm for 7-methoxy or ca 400 nm for 7-amino C9β (9S) mitomycins and corresponding negative extrema for C9α (9R) systems.27 The CD spectrum “2” reported for mitomycin B (2) agrees qualitatively with data from the Rickards laboratory.27 Both the CD spectra of “3” and “4” are closer to the spectra of porfiromycin (4)27 and mitomycin C (3)12 although with a smaller bathochromic shift. Thus the near identity of spectra “3” and “4” is surprising, given that the C9 stereochemistry should differ and dictate CD effects whatever the effects of ionisation or borate complexation.

Figure 6. CD spectra reported by Beijnen et al.133

Beijnen et al. comment:

"After propionylation of the 7-enolic group the difference in chirality between the compounds becomes obvious in the 300 nm region, analogous with the CD behaviour of their mitosane precursors."133

Unfortunately CD data is not provided for these propionates, nor is spectral data available to assess the site(s) of acylation (e.g. the 9a-hydroxyl for the mitomycin B derivative or aziridine for mitomycin A derivative). Their interpretation requires two unlikely circumstances. Firstly the CD spectra of the derivatives must be virtually identical, despite the normally critical difference in C9 stereochemistry and the usually small effect of N1a and O9a methylation. Secondly it is assumed that acylation of the 7-
hydroxyl abolishes this coincidence. In the absence of further data we tentatively suggest that under the alkaline hydrolysis conditions mitomycin B (2) has actually suffered C9 epimerisation to give the 7-hydroxy mitomycin 103 which has a very similar CD spectrum to the mitomycin A derivative 104, both with bathochromic shifts due to the 7-oxy anion. Propionylation of the aziridine of the mitomycin A derivative would probably affect the quinone ring giving distinct CD effects. The mitomycin B derivative could only be acylated at O7. Note that this requires that mitomycin B (2), or an hydrolysis intermediate, epimerises completely or largely to C9β stereochemistry. Given the facility of carbamate elimination from mitomycin B (2), it is possible some mitomycin H (11) is present but this presumably has a very different CD from 104, alternatively such elimination might be inhibited by a 7-oxy anion. Our proposal could be tested by regenerating “mitomycin B” by methylation of the hydrolysed product with diazomethane and comparison of its CD spectrum (alternatively the hydrolysis products of mitomycin B (2) and synthetic 9-epi-mitomycin B (27) could be compared directly).

\[
\begin{align*}
\text{OH} & \quad \text{NH} \\
\text{O} & \quad \text{NMe}
\end{align*}
\]

\[
\begin{align*}
\text{OH} & \quad \text{O} \\
\text{Me} & \quad \text{NH}
\end{align*}
\]

It would certainly be worth checking this reaction as the known methodology for C9 epimerisation of mitomycin B (2) is indirect (seven steps, see p15). Interestingly this epimerisation methodology\(^5\) involves a similar hydrolysis step for the conversion of 9-epi-mitomycin D (25) to 9-epi-mitomycin B (27) (see Scheme 1.2, p16) evidently without epimerisation of C9β to C9α. Unfortunately mitomycin B (2) is not commercially available and must be prepared by fermentation.
CHAPTER 2 SYNTHETIC APPROACHES TO THE MITOMYCINS

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2.1 INTRODUCTION

The mitomycins are small molecules by comparison to many synthesised today; indeed there are many successful approaches to the fundamental ring system. Nevertheless the density of different functional groups and “chiral centres” that makes the mitomycins potent drugs also provides a significant synthetic challenge. The crucial elements required of any synthesis are the incorporation of an aziridine ring and the control of relative (and preferably absolute) stereochemistry at four adjacent centres including the generation and retention of the 9a-oxy substituent. The seriousness of this challenge is demonstrated by the publication of very many papers describing synthetic approaches but a paucity of total syntheses of mitomycins. An asymmetric synthesis has not yet been accomplished. The few total syntheses afford opportunities to prepare closely related analogues that are unavailable by semisynthesis, but these have not been pursued.

A large number of papers relating the chemistry of indoles identify the mitomycins as their motivation. It would serve little purpose to catalogue all such papers here. A recent review covers synthetic efforts directed at the mitomycins from 1984 up to mid 1989. Three reviews published in 1979 summarised earlier synthetic work. The intervening period is covered in a bibliography a thesis and briefly in a review of quinones. Reviews of pyrrolo[1,2-a]indoles in 1978 and 1986 also cover approaches to the mitomycins. The synopsis here will be selective, focussing especially on synthetic approaches which have come close to the ultimate mitomycin structures or that relate to our own synthetic efforts which are introduced at the end of this chapter.
2.2 Syntheses of Simpler Systems

i) The ABC Ring System

There is a plethora of routes to which generate the ABC ring system of the mitomycins (i.e. a pyrrolo[1,2-a]indole) with various substituents.206 A particularly direct example is a recent mitosene synthesis involving the Nenitzescu style addition of enamino ester 202 to the bromobenzoquinone 201 followed by isomerisation and copper catalysed cyclisation giving indoloquinone 203 in 51% yield.207 This ester 203 has previously been elaborated to carbamate 204 (Scheme 2.1) which has antibacterial activity against Gram-positive species.209 Other approaches to the ABC ring system are documented in the reviews. Much of the synthetic work has involved approaches to mitosenes similar to 204. More interesting and less common, however, are syntheses of systems with the necessary 9a-functionality or the aziridine ring, because the functionalisation of mitosenes such as 204 has been problematic. These problems have necessarily been solved in the total syntheses described later, but it is worth assessing other approaches.

Scheme 2.1 Synthesis of a Pyrrolo[1,2-a]indole
**ii) Introducing 9a-Functionality**

Various methodologies allow introduction of the 9a-oxy functionality or generate it directly in the synthesis of the pyrroloindole skeleton. Kametani *et al.* have functionalised a pyrrolic C ring with singlet oxygen to furnish 9a-oxygenation with simultaneous functionalisation of C3 (Scheme 2.2).\(^{210}\) Danishefsky *et al.* have generated a rather similar system by photolytic cyclisation of a diene and elaborated this to a total synthesis of mitomycin K (14) (see later).

![Scheme 2.2 Functionalisation with Singlet Oxygen](image.png)

Three groups have independently prepared pyrroloindole skeletons with 9a-oxygen functionality and a 3-carbonyl group. Having the N4 lone pair delocalised as an amide presumably increases the stability of the 9a-substituent. Flitsch and Rußkamp were able to generate and preserve a 9a-hydroxyl with an ultrasonically promoted Reformatsky cyclisation of succinimide 205 to form the B ring of amide 206. The 9a-hydroxy group could be methylated.\(^{211}\) The same workers were able to obtain related 9a-methoxy substituted systems by functionalisation of 9,9a-unsaturated amides. Thus treatment of amide 207 with N-bromosuccinimide in methanol gave a bromide 208 which was reduced with tributyl tin hydride to give the *cis* methyl ether 209 in 78% yield (Scheme 2.3). Unfortunately a system with a more functionalised A ring gave a more complex mixture and a much lower yield of the corresponding methyl ether.\(^{212}\)
Scheme 2.3 Syntheses of Pyrrolo[1,2-a]indoles with 9a-Functionality

Vice et al. have used related 9-bromides to synthesise analogues of the olefinic mitomycins G (10), H (11) and K (14). The conditions are not clearly stated but apparently amide 210 reacts with bromine and methanol to give a 9-bromide which eliminates hydrobromic acid when treated with triethylamine in “quantitative” overall yield. The olefin 211 loses methoxide on treatment with acid and regenerates a pyrrole by nucleophilic attack at C10 to give 212 (Scheme 2.4). A related, reductively activated process might occur to give alkylation of the olefinic mitomycins, but their reactions and biological activities are little studied so far.

Scheme 2.4 Synthesis and Reaction of a 9,10-Olefin

Ban et al. have developed an interesting rearrangement that provides the 9a-hydroxy group via transannular cyclisation. Hydrogenolysis of the protected aniline 213 followed by addition of sodium hydride leads to amide 214 via transannular ring opening then reclosure as shown in Scheme 2.5. By delaying the ring closure step in a related system this approach has been elaborated to a synthesis of a mitomycin merely lacking 10-functionality; this is detailed later with total syntheses. The reduction of an amide of this type to the requisite C3 methylene was achieved during the synthesis of mitomycin K (14) by Danishefsky et al. (see later).
Danishefsky et al. have introduced 9a-hydroxy groups by oxidation of mitosenes with osmium tetroxide. Treatment of silylated leucoaziridinomitosene 215 (derived from mitomycin F (9), see p31) with osmium tetroxide gave functionalisation on the less hindered α-face, leading to quinone 216 in unstated yield after deprotection (Scheme 2.6). The concomitant functionalisation of C9 is not desirable for synthesis of mitomycins (although a 9-hydroxy intermediate has been used in the synthesis of mitomycin K (14), see later). The synthetic aldehyde 217 reacted slowly with osmium tetroxide to give, unexpectedly, the ketone 218 whose formation was rationalised as shown in Scheme 2.7. Thus the osmylation had proceeded syn to the aziridine, in contrast to the reaction of 215, perhaps because conjugation of N4 with the aldehyde inhibited coordination of N4 with osmium. The ketone was elaborated to the olefinic quinones 219a and 219b which are diastereomers of mitomycin H (11) and mitomycin K (14) respectively (Scheme 2.7).
Mitomycin F (9)

Scheme 2.6 Osmylation of a Leucoaziridinomitosene

Scheme 2.7 Loss of 9-Formyl Group upon Osmylation
iii) The Aziridine Ring

Early attempts to convert 1,2-disubstituted mitosenes to aziridinomitosenes were unsuccessful. We are aware of three successful cyclisations to generate an aziridine on a mitosene skeleton (Scheme 2.8). Treatment of the 1-amino-2-iodomitosenes with base gave the aziridinomitosenes, in unspecified yield. A putative aniline cyclisation during the annelation shown in Scheme 2.8 gave the isolated phenyl aziridine. Elaboration of the ketomitosenes to the azido mesylate allowed cyclisation to the unstable phosphorylated aziridine in 17% overall yield. This last example is the most surprising as N4 is not conjugated with a carbonyl and the A ring is electron rich. The perception has generally been that cyclisations to give fused aziridines are problematic.

Scheme 2.8 Syntheses of Aziridines Fused to Mitosenes

Cyclisations have also been accomplished on 9a-hydromitosanes where the aziridine product is less activated to nucleophilic attack. Thus osmylation of
olefin 226 from the concave face gave a diol which was converted to the phosphorylated aziridine 227; deprotection by reduction with lithium aluminium hydride gave the free aziridine 228 (Scheme 2.9).

![Scheme 2.9 Synthesis of an Aziridine Fused to a Mitosane](image)

When the double bond in the C ring is conjugated with a carbonyl it is possible to generate the aziridine more directly. Siuta et al. added benzylazide to the amide 229 and photochemically eliminated nitrogen from the triazoline intermediate to give the benzyl aziridine 230, albeit in low yield (Scheme 2.10). A modified version of this process has been used in the total synthesis of mitomycin K (14) (see later).

![Scheme 2.10 Synthesis of an Aziridine via a Triazoline](image)

Unpublished studies in the Rickards laboratories have succeeded in generating an aziridine by direct nitrene addition. Although the quinone 231 itself could not be functionalised, the protected hydroquinone 232 gave aziridinomitosene 233 in 27% yield (Scheme 2.11).
Shaw et al. have described a more convergent approach where the B ring is formed by coupling a fused C ring and aziridine with a quinone (Scheme 2.12). This is, surprisingly, the sole report of asymmetric synthesis aimed at the mitomycins or related systems apart from undeveloped work by Blarer (see Chapter 4). D-methionine was elaborated to the fused aziridine which was coupled with quinone leading ultimately to aziridinomitosene (Scheme 2.12) The benzyl group was not cleaved and the aziridine stereochemistry was selected on the basis of the original, incorrect mitomycin assignment. The options available for introduction of the aziridine ring are discussed further in Chapter 4.
D-methionine (234) 

Scheme 2.12 Synthesis of a Chiral Aziridinomitosene
2.3 Total Syntheses

Three different approaches to the total synthesis of mitomycins have been published and another approach comes very close. The primary target has been mitomycin C (3), but for ease of handling 7-methoxy precursors have usually been utilised to generate mitomycin A (1) and thence mitomycin C (3) as a last step.


The landmark first total syntheses of mitomycins A (1), B (2) and C (3) and porfiromycin (4) by the Kishi group owed relatively little to prior synthetic investigations. The key element in the strategy was to generate the $\theta_a$-methoxy or hydroxy group late in the synthesis and then try to maintain the quinone at that oxidation level, hence N4 conjugated with the 8-carbonyl group, to inhibit elimination of methanol or water from C9,9a. As models the benzazocines 240 and 241 were prepared by Michael cyclisation of the putative quinones 238 and 239 respectively (Schemes 2.13 and 2.14). Although doubtless aided entropically by the ketal, the efficient formation of eight membered rings is notable here and a related cyclisation has been exploited in our own work (the yields of both 240 and 241 were 40-50% from earlier products not shown). The benzazocine 240 was very acid sensitive, decomposing to indole 244. In order to obtain transannular cyclisation without immediate elimination an ingenious route via oxothioketals was devised. Treatment of 240 with methanethiol and boron trifluoride gave the oxothioketal 242 which eliminated methanethiol on treatment with mercuric chloride to give the methyl ether 243. This product was very acid sensitive, losing methanol to form the indole 244, however the requisite transannular cyclisation was seen to be feasible (Scheme 2.13).

The 9-alkylated model system 245 underwent a similar transannular cyclisation. The reaction gave a 1 : 1 mixture of cis : trans isomers 246 and 247 isolable by chromatography on alumina. Treatment with ammonia gave a corresponding mixture of carbamates 248 and 249 (deiminomitomycin A) but alumina now led to the selective conversion of the cis isomer 248 into indole 250 (contrast mitomycin B (2) with a cis
9a-hydroxyl where carbamic acid was eliminated on alumina to give mitomycin H (11), see p16. The isolated trans isomer 249 is reportedly much less stable than mitomycin A (1) with respect to methanol elimination. These reactivity differences have not been explained. Possibly anti elimination of methanol is important in decomposition of 248 despite involvement of N4. The increased flexibility in the deimino systems may increase the reactivity of both on stereoelectronic grounds.

Scheme 2.13 Model Cyclisations by Kishi et al.
These model studies were elaborated to a total synthesis of porfiromycin (4) by introducing an aziridine on the uncyclised side chain.\textsuperscript{225} The ketone 251 was synthesised from 1,3-dimethoxy-2-methylbenzene as shown in Scheme 2.15. Ketalisation was problematic because of elimination of acetic acid from C9,10 but was eventually achieved by proceeding through the dithioketal as shown. Indirect dehydrogenation and reduction gave the olefin 252 which slowly osmylated without stereoselectivity, however both diols could be converted to the same epoxide 253. A new method was developed to convert the epoxide into a methyl aziridine (this is discussed in Chapter 4). Michael cyclisation furnished the syn aziridinobenzazocine 254 but neither the alcohol 254 nor the derived phenyl carbonate 28 could be converted to oxothioketals! Hence the crucial transannular cyclisation reaction developed for the model systems could not be used.
Scheme 2.15 Total Synthesis of Porfiromycin
The anti aziridinobenzazocine 255 could be synthesised from diol 256. The diastereomeric alcohols 254 and 255 differed significantly in acid sensitivity. The syn isomer 254 was readily converted to a mitosene by traces of acid whereas the anti isomer 255 was stable under these conditions. Hence mildly acidic conditions were investigated for the cyclisation and tetrafluoroboric acid in dichloromethane was found to successfully convert 254 exclusively to decarbamoylmitomycin F (257) without any methanol elimination. By contrast, under these conditions the deimino system lost methanol. The stereospecific cyclisation and the difference in acid sensitivity of 254 and 255 were rationalised in terms of likely conformers. Established procedures were used to install the 10-carbamate and thus prepare mitomycin F (9), a natural product reported after this synthesis. Porfiromycin (4) was synthesised by substitution of the 7-methoxy group by ammonia (Scheme 2.15).

The synthesis of mitomycins A (1) and C (3) required protection of the aziridine to avoid competitive quinone attack during the Michael cyclisation. After various difficulties were encountered with more conventional protecting groups the 3-acetoxypropyl group was selected, although it required multistep procedures to install and cleave. Otherwise the synthesis proceeded from 258 in accordance with that of porfiromycin (4) via another stereospecific cyclisation to give decarbamoylmitomycin A and thence mitomycins A (1) and C (3) as shown in Scheme 2.16.

The Kishi group has reported the only serious attempt to synthesise mitomycin B (2). This required a different transannular cyclisation in order to generate a 9a-hydroxyl rather than a methoxyl. The ketal 255 had to be hydrolysed but without interception of the intermediate cation by N4 [which would presumably generate the then unreported mitomycin E (8)]. Protection of N4 by carboxybenzylation and dilute acid hydrolysis gave the ketone 259 (Scheme 2.17). Hydrogenolysis and reoxidation gave decarbamoylmitomycin B (260), apparently without excessive dehydration. Thus the stereochemistry of the cyclisation is controlled by the aziridine, not the 9-substituent. The installation of the carbamate was difficult, apparently because of formation of a cyclic carbonate [cf. mitiromycin (6)] by attack of the 9a-hydroxyl. This problem was solved by making a protected carbamate with trichloroacetyl isocyanate
and then cleaving the trichloroacetate group to furnish mitomycin B (2) (3-6% yield from 255).
With impressive conception and the development of several interesting new reactions all of the then known mitomycins had been synthesised. The routes are long and linear and the overall yields low, although there are several steps that could probably be improved.
ii) Mitomycins A and C via Isomitomycin A

The discovery of the remarkable isomerisation of mitomycin A (1) with albomitomycin A (17) and isomitomycin A (18) (see p12) was very quickly exploited by Fukuyama and Yang. Thus before the publication announcing the isomerisation was submitted they had published an approach to a precursor of isomitomycin A (18). The synthesis of mitomycins has almost always been planned according to obvious retrosynthetic analyses (e.g. N4C9a disconnection in the Kishi group strategy). The advantage of targeting isomitomycin A (18) is that the 9a-methoxyl is now insulated from the nitrogen bonded to the quinone (now N1a) until the final isomerisation. The synthesis of isomitomycin is still not trivial of course, but this was rapidly accomplished by Fukuyama and Yang in 29 steps despite the discovery that the isomitomycin system itself was unstable in acid. They have subsequently improved their synthesis and the optimised version is shown in Scheme 2.18. The aziridine is constructed by intramolecular nitrene insertion to give 261 which leads to pyrrolidine 262 containing the ultimate N4. Note that the acid catalysed interchange of the ‘9a’-hydroxyl in 262 for a methoxyl does not lead to a mitosene isomer because such an olefin would be too strained. Deprotection and oxidation gives isomitomycin A (18) which is converted to either mitomycin A (1) by isomerisation, or mitomycin C (3) by 7-substitution then analogous isomerisation. The yield of mitomycin C (3) from 1,3-dimethoxy-2-methylbenzene is 10%.
Scheme 2.18 Total Synthesis of Mitomycins A and C via Isomitomycin A


### iii) Mitomycin K

The Danishefsky group has recently reported the first total synthesis of an olefinic mitomycin, racemic mitomycin K (14) (Scheme 2.19). Photolysis of the diene 263 generated the B and C rings with 3- and 9a-oxygen functionality and 1,2-unsaturation. This interesting reaction probably proceeds via a photochemically mediated dehydration giving a nitroso intermediate, followed by cycloaddition and rearrangement. Osmylation gave a diol with 1β,2β stereochemistry unsuitable for conversion to a β-aziridine. Hence another strategy was adopted to synthesise the aziridine. The 3-alcohol was oxidised to a carbonyl allowing dipolar cycloaddition of phenylthiomethyl azide to give a β-triazoline. After this C3 was completely reduced via an unexpected thiothiooxo intermediate 264. The β-aziridine 265 was formed on photolysis and reduced to the N-methyl aziridine 266. C10 was installed by addition of trimethylsilylmethyl lithium. Oxidation to the quinone in low yield followed by Peterson olefination gave mitomycin K (14).229 This is a very rapid synthesis of the A, B and C rings with 9a-oxygenation in place.
Scheme 2.19 Total Synthesis of Mitomycin K
iv) A Mitomycin Analogue

Ban et al. have extended their earlier work on pyrroloindole synthesis (see p71) to a synthesis of a 9-methyl mitomycin where the 9a-functionality is generated late by transannular cyclisation.230 The benzazocine 272 was available by a rather lengthy and complex sequence (Scheme 2.20).214,231 Unsaturation was introduced to allow creation of the aziridine ring, but functionalisation of the olefin was difficult. This was finally achieved by tethering a nitrogen nucleophile to the 9a-oxygen and intramolecular cyclisation and rearrangement to give the tosyl aziridine 273. Oxidation regenerated the 9a-carbonyl giving the protected hydroquinone 274. The remarkable key step was the detosylation and transannular cyclisation of 274 on treatment with tert-butylidimethylsilyltrifluoromethanesulfonate, giving the 9a-silyl ether 275 in 100% yield. This was “highly stereoselective” and dependent upon benzyl ether protection. The TBDMS protected analogue of 274 gave none of the corresponding pyrroloindole and had a different infrared carbonyl absorption (1730 cm⁻¹ for the silylated hydroquinone, 1700 cm⁻¹ for 274), which was attributed to variation in transannular interactions, presumably due to varying steric interactions. Oxidation and deprotection gave the mitomycin analogue 276, the yield of aziridine detosylation being very low (16%). The stereochemistry of the cyclisation reaction was inferred from an NOE enhancement between the 9-methyl and H1.
Scheme 2.20 Total Synthesis of a Mitomycin Analogue
Contemplation of the known or postulated biosynthesis of a target molecule can suggest fruitful synthetic strategies in vitro. The imitation of key biosynthetic steps in the laboratory has provided impressive biomimetic syntheses of many classes of compounds (although the terminology is not universally liked). Robinson’s short synthesis of tropinone is commonly held as the precocious archetype of this approach and correlates quite well with the established biosynthesis, but the retrosynthesis was explicitly inspired by symmetry, not biosynthetic considerations. Nevertheless biomimetic syntheses of triterpenoids, polyether antibiotics and many others have been reported. A recent example is the synthesis of Daphniphyllum alkaloids by Heathcock et al. where six bonds are formed generating five rings in good yield in the key transformation (Scheme 2.21), a dramatic improvement on a ‘classical’ route. Obviously some enzymatically catalysed biosynthetic reactions will not be readily amenable to in vitro imitation, especially where conformational control is important. For example, the replication in vitro of para-ortho oxidative coupling in morphine biosynthesis has been very difficult, with isomeric products usually dominating. But the value of the approach as one method of retrosynthetic analysis has been established. The biosynthesis is one synthetic path that is, de facto, feasible, if only with enzymatic catalysis. Vice versa, where the biosynthesis is less clearly defined, synthetic success may provide support for the feasibility of a biosynthetic hypothesis, albeit never proof.

![Scheme 2.21 Biomimetic Synthesis of the Daphniphyllum Skeleton](image-url)
2.5 A BIOMIMETIC APPROACH TO ANSAMYCINS

The discovery that naphthoquinoid ansamycin antibiotics, e.g. actamycin (97), are biosynthesised from AHB (99) and various ketide units raises the interesting question of how the naphthoquinone nucleus is formed. Such mechanistic questions are intrinsically more difficult to answer definitively than questions of biogenesis. Two models have been proposed for the coupling of the polyketide chain with the AHB residue; intramolecular Michael addition to an initially formed quinone and radical addition or coupling.240

![Diagram of actamycin (97) and 3-amino-5-hydroxybenzoic acid (99) with Michael cyclisation model]

**i) Michael Cyclisation**

Michael addition of a β-dicarbonyl species (Scheme 2.22) is plausible biosynthetically: the polyketide chain provides the relevant carbonyls and the quinone is evidently generated at some point in the sequence.

![Scheme 2.22 Michael Cyclisation Model]

In contrast to the aldol reaction, Michael additions in biosynthesis appear to be very rare. The addition of activated methylenes to quinones is well precedented chemically,241,242 but intramolecular cyclisation by such a process is almost unknown. Cyclisations *via* Michael addition have been reviewed243 and no examples are given. An example of Michael cyclisation giving an eight membered ring fused to a quinone is discussed later. Ulrich and Rao244 report that oxidation of the hydroquinone 267 with
silver (I) oxide gives the cyclic quinone 268 in 60% yield (Scheme 2.23). The mechanism was not investigated but might well involve Michael addition following oxidation to a benzoquinone as was speculated. Note that a sub-stoichiometric amount of oxidant was used. The corresponding tethered cyclohexanone 269 oxidised to give the quinone 270 without concomitant cyclisation (Scheme 2.24), which was interpreted as due to the lower acidity of this system. Given success in forming eight membered rings by cyclising β-keto esters (albeit under different conditions, see below) it seems that steric factors may be more important. An attempt to cyclise 269 using zinc chloride was unsuccessful; base catalysis was not investigated.

Day and Rickards have explored the chemical feasibility of Michael cyclisation in naphthoquinoid ansamycin biosynthesis using simplified models. Oxidation of the model phenol 277 with Fremy's salt in an aqueous pH 6.8 buffer and methanol gave the naphthoquinone 278 in 40% yield (Scheme 2.25). The moderate yield appears to be due largely to competitive ortho-quinone formation. The observation that all known naphthalenoid ansamycins carry an oxygen function at ‘C1’ and the efficiency of this biomimetic model synthesis support the proposition that the naphthoquinonoid nuclei are formed this way biosynthetically.245
Scheme 2.25 Biomimetic Cyclisation of a Model Actamycin Nucleus

Although Fremy’s salt is known to oxidise 1,3-dicarbonyl moieties,246,247 Day and Rickards have shown that the quinone 279 undergoes spontaneous cyclisation in pH 6.8 buffer alone (Scheme 2.26). Thus the Michael cyclisation presumably proceeds via attack of enol or enolate and radical coupling of the quinone mediated by Fremy’s salt is not involved.

Scheme 2.26 Spontaneous Cyclisation in the Absence of Fremy’s Salt

ii) Radical Cyclisation

The success of these and related model syntheses does not preclude the possibility of a radical coupling of the phenol and the dicarbonyl generating a naphthalene nucleus which is then oxidised to a quinone in vivo. The two most plausible mechanisms would be oxidation to a β-dicarbonyl radical which adds to the aromatic ring (Path A, Scheme 2.27) and oxidation to give a phenoxy radical which adds to the enol (Path B, Scheme 2.27).
Day and Rickards found that oxidation of the phenol 280 with a single electron oxidant, hexacyanoferrate (III), gave a low yield of the \textit{para} coupled product 281, apparently the result of cyclisation and oxidative addition of methanol (Scheme 2.28). It is not possible to distinguish which of the two suggested radical pathways might be operating here but \textit{para} coupling of phenoxy radicals (cf. Path B, Scheme 2.27) has been widely observed.\textsuperscript{248}

\begin{center}
\textbf{Scheme 2.28 Radical Cyclisation of an Ansamycin Model}
\end{center}

Jamie and Rickards have explored these radical cyclisations in greater depth.\textsuperscript{249} One of their more elaborate models was the protected phenol 282 which cyclised to give a diastereomeric mixture of tetralones 283 in high yield when treated with manganese.

-97-
(III) triacetate, another single electron oxidant (Scheme 2.29). Acetoxylation was attributed to oxidative attack on a cyclised intermediate. Other evidence was adduced to support the idea that the cyclisation proceeds via initial oxidation of the dicarbonyl moiety (Path A, Scheme 2.27). The exclusive ‘ortho’ cyclisation was explained in terms of steric hindrance by the acetamido group, although this does not seem entirely compelling; the relative electronic influences of the benzyloxy and acetamido groups are unclear. Hence radical cyclisation is clearly a plausible alternative mechanism for formation of the naphthalenoid nucleus although perhaps less likely than the Michael process for the reasons stated above. [Treatment of the protected phenol 282 with Fremy’s salt led to clean recovery of starting material which further supports the belief that the phenol 277 reacted by initial oxidation to a quinone followed by Michael cyclisation (Scheme 2.25).]

\[\text{NHAc} \]

\[\begin{array}{c}
\text{282} \\
\text{Mn(OAc)}_3
\end{array} \begin{array}{c}
\text{AcOH} \\
\text{93%}
\end{array} \]

\[\begin{array}{c}
\text{283} \\
\text{NHAc}
\end{array} \]

Scheme 2.29 Radical Cyclisation of an Ansamycin Model

2.6. C8aC9 Bond Formation

Mechanisms for C8a-C9 bonding are less clear. Formation of the C8a-C9 bond requires bonding of C8a of the ansamycin 29 to the A7 ring. A plausible mechanism proposed initial C8aC9 bond and subsequent interring rearrangement is possible. An alternative hypothesis is that the initial bond is a rearrangement of the C3 aminoketone that already exists in accordance with Lin's work.

While the bonding of carbonyl carbon to a pyruvate in solution, the overwhelmingly involved bonding at the anomeric center. The mechanism of formation of acyclic diene does not appear to have been established yet although it has
2.6 A BIOMIMETIC APPROACH TO THE MITOMYCINS?

Three bonds link AHB with D-glucosamine to form the mitomycin skeleton during biosynthesis (Scheme 2.30). It is possible to speculate about the likely mechanisms for the formation of each.

2.6a C3N4 Bond Formation

The formation of an imine by condensation of the amine of AHB (99) with the aldehyde of D-glucosamine (96) and then reduction are well precedented steps which would generate the C3N4 bond in mitomycins.

2.6b C8aC9 Bond Formation

Mechanisms for C8a to C9 bonding are less clear. Formation of the C8aC9 bond requires bonding of C5' of D-glucosamine to the AHB residue. It seems reasonable to postulate initial C3N4 bond and subsequent cyclisation generating an eight membered ring. Most plausible C8aC9 bond closures would be disfavoured if the N4C9a bond was also already extant, in accordance with Baldwin’s ‘rules’.250,251,252

While the coupling of carbohydrates and phenols as C-glycosides is common, this overwhelmingly involves bonding at the anomeric carbon. The mechanism of formation of even these does not appear to have been established,253 although it has
been speculated that they form prior to aromatisation of the polyketide precursor to the phenol.\textsuperscript{254} We are not aware of any C-glycosides bonded at C5' of a sugar other than nogalomyacin (100) and the closely related systems decilorubicin, arugomycin and viriplanin A.\textsuperscript{255} Nogalomyacin (100) is biosynthesised from acetate, which gives the ‘aglycone’, and glucose, which probably furnishes both carbohydrate fragments, however details of the crucial coupling have not been elucidated.\textsuperscript{256}

\[
\text{Nogalamycin (100)}
\]

Postulates for C8aC9 bond formation can utilise either the aromatic AHB ring or the ultimate quinone functionality.

\textit{i) Michael Cyclisation}

The facility of the Michael cyclisation in forming a six membered ring fused to a quinone (Section 2.5i) raised the possibility of a related process in mitomycin biosynthesis forming an eight membered ring. Unlike the ansamycins in which the AHB residues are alkylated by the polyketide chain \textit{para} to the amine (if at all), the mitomycins are alkylated \textit{ortho} to the amine. C9a in mitomycins is at the carbonyl oxidation level and AHB is again elaborated to a quinone at some point in their biosynthesis. Thus attack of an enolate on an AHB derived quinone could conceivably form the C8aC9 bond (Scheme 2.31). The pyrroloindole skeleton would then arise by transannular attack by N4 forming the N4C9a bond (see Section 2.6c). Whilst in general cyclisation of eight membered rings is difficult,\textsuperscript{257} the work of Kishi \textit{et al.} (see Section 2.3i) has shown that such Michael cyclisations are quite feasible \textit{in vitro}, at least with an amine as nucleophile.
Unpublished work by Chai and Rickards has investigated this idea in some detail. The ortho-aminophenol 284 was chosen as an AHB equivalent in order to minimise competitive oxidation to ortho-quinones anticipated258 if 3-amino-5-methylphenol was used. The cyclisation of four derived model systems was investigated. The most accessible model was the quinone 285, prepared by DCC coupling of 284 and levulinic acid (286) and oxidation. Treatment of this methyl ketone with bases did not give the desired cyclic product 287 (Scheme 2.32). This is not unexpected as amides are typically more acidic than ketones259 and the amide anion of 285 is further stabilised by the quinone; such ionisation would deactivate the quinone to Michael addition. The protected alkylamino quinone 288 lacks such an acidic proton and did react with LDA or lithium 2,2,6,6-tetramethylpiperidine (LTMP), but only to generate the hydroquinone 289 (Scheme 2.33). The mechanism of reduction by LTMP which lacks β-hydrogens is not clear, possibly a direct electron transfer is involved. Thus no conclusion could be drawn from these two model systems because of side reactions.
Scheme 2.32 Preparation of an Amide Linked Model Ketone

There was, however, precedent for cyclisation of a \( \beta \)-keto ester to give an eight-membered ring. Okamoto and Ohta\textsuperscript{263} reported that the naphthoquinones 290 (R = H or OAc) could be synthesised (the process used for homologation of the acid is discussed in the next chapter) and acetylated with acetic anhydride and boron trifluoride to give, after aqueous workup, the acetates 291. These were cyclised using a base in DMSO to give the benzazocinones 292. Under acidic conditions the acetate was lost and transannular cyclisation gave the pyrroloindole analogues 293 (Scheme 2.34).
Scheme 2.34 Cyclisation of a Naphthoquinonoid β-Keto Ester

The advantage of using a β-keto ester as the nucleophile is that strong bases are not required to generate the enolate. Chai and Rickards prepared amide 294 as shown in Scheme 2.35, but 294 did not cyclise, basic or acidic conditions leading to decomposition or side products. To eliminate problems with ionisation and possibly conformation of the amide, the protected alkylamino phenol 295 was prepared. Oxidation of 295 with Fremy’s salt in acetone and aqueous pH 6.8 buffer led not to the quinone 296 but directly to the desired cyclic system 297 in good yield (Scheme 2.36). Presumably 296 is an intermediate, thus an eight membered ring can be efficiently formed by Michael cyclisation of an enolate.
Scheme 2.35 Preparation of an Amide Linked β-Keto Ester

Scheme 2.36 Successful Michael Cyclisation of a β-Keto Ester

Luly and Rapoport synthesised a related β-keto ester 298 as an intermediate in one approach to the synthesis of 299 (Scheme 2.37). Upon reduction to the hydroquinone, which increases the nucleophilicity of ‘N4’, an intramolecular condensation occurred giving 300. The condensation is reversed by oxidation under acidic conditions, but not cleanly. The pyrrole 300 could be cyclised photochemically only after protection of the hydroquinone. Subsequently the indicated palladium mediated cyclisation of the quinone was developed. Extensive efforts were made investigating cyclisations of 300 and other pyroles linked to quinones, but the cyclisation of β-keto ester 298 itself to generate an eight membered ring was apparently
not contemplated.

![Chemical structure and text]

Scheme 2.37 Synthesis of a Related β-Keto Ester

The stability of the acyclic β-keto esters 290, 291 and 298 under the mildly basic conditions of formation and with dilute acid contrasts with the facile cyclisation of the intermediate quinone 296 (Scheme 2.31), albeit under different conditions. With disparate substrates and conditions it is unwise to draw any conclusions, however the cyclisation is apparently catalysed by base but not acid (which is also used in the workup of 290 and 298). It would be interesting to know if electronic or conformational effects of the amine protection or the halogen affect reactivity.

It has been shown to be chemically feasible to form an eight membered ring by Michael cyclisation of an enolate. However a β-dicarbonyl species is unlikely to be involved in mitomycin biosynthesis given the high retention of label observed when H6'-glucosamine is incorporated (see p60). Incorporation as H10 was not directly
demonstrated but is very likely, so the oxidation state of this carbon probably does not alter during biosynthesis. Nevertheless the Michael cyclisation of an enolised ketone under enzymatic control still seemed plausible as the ionisation site and conformation could in principle be controlled by the enzyme.

The postulated Michael cyclisation mechanism involves conjugate attack on the 5-carbonyl. 7-Substituents are conjugated to this carbonyl and so could conceivably inhibit the cyclisation; the point at which 7-substituents are introduced in mitomycin biosynthesis is unknown. If such substituents were observed to inhibit cyclisation in vitro, it would be reasonable to infer that they are introduced at a later stage in vivo. We have pursued this by preparing the phenols 301 and 302 and studying their oxidation. This work is described in the next chapter.

![Chemical structures](image)

**ii) Radical Cyclisation**

While our work was in progress, Jamie and Rickards investigated the imitation of a possible radical cyclisation mechanism of C8aC9 bond formation. The likely radical progenitor in mitomycin biosynthesis is not clear. During biosynthesis C4' of D-glucosamine is oxidised to a carbonyl or equivalent while C5' is formally reduced. Thus the more obvious possibilities are the enolised methyl ketone 303 or α-hydroxyketone 304 (with the 9-hydroxyl subsequently lost by dehydration in the latter case). The addition of an α-keto radical to an aromatic ring (Scheme 2.38) was modelled by oxidation of a diketone tethered to an electron rich aromatic ring. A diketone was chosen in order to fix the site of radical generation and ensure a sufficient enol content for reasonable reaction.
Chapter Two

Scheme 2.38 Postulated Mechanism for Radical Cyclisation

Treatment of the diketone 305 with manganese triacetate gave a very low yield of the desired fused benzazocine 306 as the only cyclised product (Scheme 2.39). This contrasted with homologous diketones which gave corresponding six and seven membered ring products in 71 and 67% yields respectively. Thus the biomimetic cyclisation was very low yielding, at least for this minimal model system. The model system 305 has a very flexible alkyl chain and entropic factors may inhibit cyclisation whereas in vivo the reaction would presumably be enzyme catalysed and the aziridine ring may be already formed, reducing conformational mobility. Certainly intramolecular radical additions of β-diketones to aromatic rings with certain substituents are feasible. The implications for mitomycin biosynthesis are not entirely clear, but a radical cyclisation is by no means precluded.

Scheme 2.39 Radical Cyclisation of an Eight Membered Ring

During the course of our work the isolation of some antibiotics related to the mitomycins was reported. The structures of these antibiotics have direct implications
for the likely mechanism of formation of the C8aC9 bond which are discussed at the end of Chapter 3.

2.6c N4C9a Bond Formation

Lown and Itoh have suggested that N4C9a bond formation occurs biosynthetically via transannular attack of N4 on a 9a-ketone in a benzazocine intermediate (Scheme 2.40), drawing parallels with senkirkine (307) biosynthesis.\(^{264}\) To model this process they prepared a mixture of three benzazocinones. Deprotection with sodium in liquid ammonia gave a corresponding mixture of pyrroloindoles (Scheme 2.41) which clearly arise from cyclisation and dehydration. Kishi et al. used a transannular cyclisation in their total synthesis of mitomycin B (2), managing to isolate the intermediate alcohol (260, Scheme 2.17), which supports the feasibility of the proposal. Ban et al. have also intercepted an 9a-hydroxyl from transannular cyclisation when N4 was conjugated as an amide (see Scheme 2.5). It seems probable that the 9a-methoxy mitomycins arise from methylation of 9a-hydroxy precursors, which can be accomplished chemically, although direct formation by attack on a ketal cannot be excluded, in principle.

![Scheme 2.40 Postulated Mechanism for N4C9a Bond Formation in Mitomycin Biosynthesis](image_url)
Scheme 2.41 Transannular Cyclisations of Tosylated Benzazocinones

In the model β-keto ester 292, the requisite 9a-carbonyl is present and ‘N4’ is protected with an acetyl group. Okamoto and Ohta found that somewhat unusual acidic conditions caused loss of the acetyl group, transannular cyclisation and dehydration giving the benzomitosene analogue 293 (see Scheme 2.34). Chai and Rickards investigated the deprotection of the related benzazocinone 297. Reduction to the hydroquinone 308 was rapid but decarbamoylation was slow, especially in the presence of added base, and no intermediates were detected between 308 and 310 (Scheme 2.42; the reactions may proceed via semiquinones). Thus it was not possible to trap the presumed intermediate 9a-hydroxy system 309. This is not entirely surprising as the reductive conditions required to cleave the carboxybenzyl protection obviously also reduce the quinone, increasing the nucleophilicity of the released nitrogen. Kishi’s synthesis of mitomycin B (2) (Scheme 2.17) demonstrates that this problem is not insurmountable, but in 309 the corresponding H9 proton is significantly more acidic, presumably enhancing dehydration.
Scheme 2.42 Preparation of a Mitosene Analogue
CHAPTER THREE: MICHAEL CYCLISATIONS WITH DEACTIVATED QUINONES

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3.1 Introduction and Biomimetic Basis

Following the identification of 3-amino-5-hydroxybenzoic acid (AHB, 99) as the in vivo source of the methyl benzoquinone moiety of the mitomycins it was possible to conjecture about mechanisms of its biosynthetic coupling to D-glucosamine (96). These speculations were enunciated in the last chapter. We have further explored the Michael cyclisation model for C8aC9 bond formation in mitomycin biosynthesis developed by Chai and Rickards (see Section 2.6b). In this model D-glucosamine is coupled to AHB (99) by imine formation and reduction, the aziridine is cyclised by intramolecular condensation and the phenol is oxidised to a para-quinone. The crucial C8aC9 coupling then occurs by Michael attack of an enolate on the quinone followed by reoxidation of the hydroquinone intermediate (Scheme 3.1). This leaves the introduction of C7 substituents unaddressed.

The biogenesis of C7 substituents of the mitomycins is not well established. Methionine provides the methyl of the methoxy group in mitomycin A. The origin of the oxygen or nitrogen has not been investigated. There are two plausible mechanisms for the introduction of the C7 oxygen, direct oxidation of a phenolic or hydroquinonoid intermediate and Michael addition of an hydroxide equivalent to a quinone. Initial oxidative hydroxylation could furnish either a ‘7,8’- or a ‘5,8’-hydroquinone (310 or 311) which might both be oxidised to a common trihydroxy benzene 312 and thus to the quinone 313 (Scheme 3.2); the route via 310 could proceed with intermediate methylation of the 7-hydroxy group. Such aryl hydroxylation processes are well established. Alternatively oxidation to the respective quinones 314 and 315 and conjugate addition of an hydroxide equivalent would give the same
trihydroxy benzene intermediate 312. Conjugate addition of hydroxide to unsubstituted quinones has been achieved chemically but the yields are low because of the instability of the products.\textsuperscript{302,303,304}

The two mechanisms could, in principle, be distinguished by comparing incorporation of labelled water and oxygen gas because the direct oxidations would presumably utilise dioxygen while the hydroxide equivalent would arise from water.

![Diagram of pathways for the introduction of oxygen at C7.](image)

Scheme 3.2 Pathways for the Introduction of Oxygen at C7.
The 7-amino mitomycins may well arise by substitution of methoxide by ammonia. Such a process is facile chemically. Feeding N-methyl labelled mitomycin F (9) led to the isolation of labelled porfiromycin (4), although the transformation was not necessarily occurring intracellularly. Transamination of a 7-keto species would also install the nitrogen by displacement of oxygen from a species initially oxidised at C7. Michael addition of ammonia to an unsubstituted quinone and reoxidation could install the 7-amino substituent directly. Such reaction has been achieved chemically only in a few cases and the yields are poor.240,303,305,306

Formation of the C8aC9 bond via Michael cyclisation could occur at a number of points. The most notable variation is between 1,6-conjugate addition to an ortho-quinone like 314 (perhaps activated by an aldehyde at C6) and 1,4-addition to a para-quinone like 315. The precise identity of the reacting species could only be established by extensive blocked mutant or enzyme isolation studies, if at all. Nevertheless one question was relatively amenable to model studies.

The Michael cyclisation in Scheme 2.30 uses the C5 carbonyl as an electron sink. 7-Substituents which reduce the electrophilicity of this carbonyl could conceivably prevent cyclisation. If this was observed in model systems it would be reasonable to infer that the 7-substituents were introduced after the cyclisation in biosynthesis. Such information is important in choosing relevant synthetic substrates to test any isolated enzymes. In a biomimetic total synthesis of the mitomycins it would be preferable to have the 7-substituent installed early for convergence, as long as the cyclisation was still efficient.

Scheme 2.31 Postulated Mechanism for C8aC9 Bond Formation

In unpublished work exploring synthetic approaches to actamycin (97), Rickards and Rukachaisirikul have observed the influence of a methoxy substituent on Michael cyclisation.307 Oxidation of the phenol 316 gave the spiro-enol 317 as the only
characterised product (Scheme 3.3). By contrast the benzyl alcohol 318 gave a 1:1 mixture of the expected cyclisation products 319 and 320 derived from para and ortho-quinone intermediates respectively. The triketo quinone 321 was also isolated. Apparently resonance conjugation of the methoxy group in 316 inhibits cyclisation by attack at ‘C7’, forcing attack at ‘C6’ instead, leading to 317. This effect can be overridden by a benzylic hydroxyl which, presumably by hydrogen bonding to the C5 carbonyl, leads to the desired naphthoquinone 319. This may be due to electronic or conformational effects of the hydrogen bonding or probably both, although a benzylic hydroxyl does not always enhance the yield of such cyclised products. Even a chlorine atom may be sufficiently electron donating to hinder cyclisation, the uncyclised quinone 323 being tentatively identified as a trace product in the oxidation of the ortho-chlorophenol 322 (Scheme 3.3). This is the only instance of an uncyclised 1,3-diketone being isolated in the Rickards laboratories from a Fremy’s salt oxidation.

Scheme 3.3 Michael Cyclisations of Substituted Quinones
In order to study the ramifications of 7-substituents on the proposed Michael cyclisation step in mitomycin biosynthesis we prepared the methoxy and acetamide substituted phenols 301 and 302. The cyclisation of 7-hydroxy and 7-amino systems would also be relevant but such polar quinones have been found to be hard to handle. Inhibition by methoxy or acetamide substituents would suggest stronger effects by hydroxy and amino substituents.

3.2 Will a Methoxy Substituted Quinone Cyclise?

3.2a Synthesis of the Methoxy Substituted Phenol

THE AROMATIC RING

Following from the work of Chai and Rickards (Section 2.6b), it was anticipated that the desired phenol 301 would be accessible from 2-amino-5-methoxy-6-methylphenol (324). The hydrochloride salt of aniline 324 had been reported by Crump et al. who prepared it in five steps from 1,3-dimethoxy-2-methylbenzene using acylation, Beckmann rearrangement and selective demethylation (Scheme 3.4). Rinehart et al. synthesised the aniline 324 by methylation and hydrogenation of the corresponding nitrophenol 325 and protected it immediately by acetylation (Scheme 3.4). We chose to follow the route of Rinehart et al., but no details of the synthesis of the nitrophenol had been given by them. The only reported synthesis of the nitrophenol 325 was an indirect and low yielding rearrangement of the uracil 326 reported by Su et al. (Scheme 3.5). Thus we explored the nitration of 3-hydroxy-2-methylphenol (327) which is readily available commercially, although generally not pure, being contaminated with resorcinol and other material (we found one bottle from Pfaż and Bauer contained pure 2,6-di-t-butyl-4-methylphenol!).
Scheme 3.4 Syntheses of 2-Amino-5-methoxy-6-methylphenol

Scheme 3.5 Synthesis of 3-Hydroxy-2-methyl-4-nitrophenol (325)

Gaude et al. had reported a mild two phase nitration reaction for various phenols using dilute hydrochloric acid, sodium nitrate and ether.\textsuperscript{312} We were interested to try this with 3-hydroxy-2-methylphenol (327), but only recovered starting material under the stated conditions. It was claimed that the method allowed selective nitration of phenols in the presence of anisoles but we found that 1,3-dimethoxy-2-methylbenzene was cleanly demethylated to 327. We were unable to reproduce their nitration of 2-methylphenol.

At our suggestion this reaction was further investigated by Thompson and Zeegers. They were also unable to nitrate 2-methylphenol under the stated conditions.\textsuperscript{313} Subsequently they have determined that there is a significant latency
period which is temperature, phenol and pH dependent but which can be avoided by addition of catalytic nitrite.\textsuperscript{314,315} They commend this modified procedure as clean and high yielding for simple phenols.

We found that 3-hydroxy-2-methylphenol (327) reacted under the more acidic and nitrite catalysed conditions of Thompson and Zeegers, but complete consumption required more than one equivalent of sodium nitrate, giving a complex mixture from which the desired nitrophenol 325 was isolated in 38\% yield from many polar byproducts. In the interim we had prepared 3-hydroxy-2-methyl-4-nitrophenol (325) under conventional conditions (nitric acid, acetic anhydride and acetic acid) in 54\% yield, albeit still with many polar byproducts. The product was isolated by chromatography; steam distillation was ineffective. Comparable yields were obtained by reaction in ether with fuming nitric acid or nitric acid and sodium nitrite. These reactions generated the less polar 3-hydroxy-2-methyl-4,6-dinitrophenol 328 as a minor byproduct.

![Chemical Structures](image)

The NMR spectrum of the nitrophenol 325 in CDCl\textsubscript{3} (in which it is only sparingly soluble) showed two doublets due to the aromatic protons, a methyl singlet and two exchangeable singlets assigned to the hydroxyls at $\delta$ 11.3 and 5.7. The resonance at $\delta$ 11.3 was sharp whereas that at $\delta$ 5.7 was broader and its shift varied between samples. Addition of a drop of DMSO caused a marked downfield movement (to $\delta$ 10.2) of the second hydroxyl resonance but little change in the rest of the spectrum. Su et al.\textsuperscript{310} describe only the signal at $\delta$ 11.3, as a two proton singlet, but this is erroneous as our integration is unequivocal and we assign the signal at $\delta$ 11.3 to the intramolecularly hydrogen bonded C3 hydroxyl and $\delta$ 5.7 to the hydroxyl at C1 (which can be deshielded by intermolecular hydrogen bonding, e.g. with DMSO). This assignment was supported by the NMR spectrum of the symmetrical dinitro byproduct 328 in which both hydroxyls are intramolecularly hydrogen bonded giving a sharp singlet at $\delta$ 11.4.
The mass spectrum of 325 showed an intense molecular ion peak (m/z 169, 99%) and expected\textsuperscript{316} nitric oxide and nitrogen dioxide eliminations. Unexpected however, was a peak m/z 151 (21%) corresponding to loss of water. The dinitro compound 328 gave an even more intense ion corresponding to loss of water (m/z 196, 46%). The losses of oxygen and hydroxyl radicals are well established in various nitroaromatics,\textsuperscript{316} e.g. 2-nitrophenol gives a significant loss of hydroxyl (ca 10%).\textsuperscript{317} The mechanism for loss of water, however, is not clear. We are not aware of any literature on the mass spectroscopy of hydroxynitrophenols except for the spectrum of 3-hydroxy-2-nitrophenol\textsuperscript{317} which also shows notable loss of water (m/z 137 ca 30%). While it is tempting to formulate these ions as the conjugated radical species 329, 330 and 331, drawing analogy to fragmentation of 2-nitroaniline,\textsuperscript{316} the process(es) may be more complicated given that 3-methoxy-2-methyl-6-nitrophenol (333) (see below) also shows such a loss (m/z 165, 20%).

![Diagram](image)

The $^{13}$C NMR spectrum of 325 was unremarkable except for the methyl resonance which was notably shielded ($\delta$ 8.0 cf. $\delta$ 19-22 for normal toluenes\textsuperscript{318}). The shielding effect of one hydroxyl group is apparent in 2-methylphenol ($\delta$ 16.7 cf. $\delta$ 20.6 for 4-methylphenol\textsuperscript{318}). The shielding in 325 is purely due to the influence of the flanking oxygen atoms as the starting material and subsequent methylated, nitrated and other derivatives, even the quinone 203 showed very little variation in the shift of such methyl resonances ($\delta$ 8.0-8.7).

Subsequently Raphael and Ravenscroft have published a preparation of 325 via the nitrosophenol 332 (Scheme 3.6), having been unable to accomplish direct nitration.\textsuperscript{319} Chromatography was still required to isolate pure 325, so this procedure does not offer any obvious improvement over our own. The melting point, mass spectrum and infrared spectrum reported by Raphael and Ravenscroft for 325 matched our own data.
Methylation of 325 following the procedure of Rinehart et al.\textsuperscript{309} gave a monomethyl ether in good yield with traces of what appeared to be the dimethyl ether. The assignment of the structure 333 had been made without comment or any spectral evidence, although it is chemically reasonable. Krause and Hoyer have also synthesised 333 by Wolff-Kishner reduction of the corresponding aldehyde (17\% yield).\textsuperscript{320} This gave a product with the same melting point as ours, but they also report an hydroxyl absorption at 3130 cm\textsuperscript{-1} (solvent and other spectral data not given). In chloroform we observe an absorption at 3195 cm\textsuperscript{-1}. As the structure of their aldehyde was not completely unambiguous we sought confirmation of the structure 333.

The NMR spectrum of 333 contained one sharp exchangeable resonance at $\delta$ 11.1, suggesting the presence of a hydrogen bonded hydroxyl, \textit{i.e.} consistent with methylation of the hydroxyl \textit{para} to the nitro group. Nuclear Overhauser effects (NOEs) can theoretically distinguish between the two monomethyl ether isomers 333 and 333b, with only 3-methoxy-2-methyl-6-nitrophenol (333) expected to show a strong NOE between the ethereal methyl and an aromatic proton. In fact a 16\% enhancement of this methyl was observed when the doublet at $\delta$ 6.5 (H4) was irradiated, and a 9\% reciprocal enhancement was observed, thereby supporting the structural assignment. Neither irradiation resulted in a significant enhancement of the aromatic methyl resonance. Precautions were taken to ensure that the rather long relaxation times observed did not interfere.
THE ALKYL SIDE CHAIN

The sequence developed by Chai and Rickards for the synthesis of β-keto ester 295 was used to prepare the corresponding 7-methoxyphenol 301. Their sequence (Scheme 2.36) involved alkylation of aminophenol 284 giving a γ-substituted butyrate which was protected at nitrogen to give the t-butyl ester. Cleavage of the ester gave the acid which was extended to β-keto ester 295 using the protocol of Brooks et al. This involves formation of an imidazolide and substitution by the magnesium salt of a malonate monoester giving the β-keto ester after decarboxylation (Scheme 3.7); it has the advantage that strongly basic or acidic conditions are avoided. The linear sequence gave 295 in 39% overall yield and avoided protection of the β-keto ester which would be necessary if the entire alkyl chain were to be coupled in one step.

\[ RCO_2H + \text{Im}_2CO \xrightarrow{\text{-CO}_2, \text{-imHf}} \text{R} \overset{\text{N}}{\xrightarrow{\text{Im}} \text{O}} \xrightarrow{1/2 \text{Mg(OMe)}_2 + \text{HO}_2\text{CCH}_2\text{CO}_2\text{Me}} \text{R} \text{C} = \text{O} \overset{\text{Me}}{\text{O}} \]

*Im = imidazole*

**Scheme 3.7 Protocol of Brooks et al. for the Preparation of β-Keto Esters from Acids**

Hydrogenation of the nitrophenol 333 gave the air sensitive aminophenol 334. The amine could be sublimed and NMR and mass spectral data recorded but it was normally used crude. Alkylation with t-butyl 4-bromobutyrate 335 in DMPU gave the air sensitive monoalkylated product 336 and some dialkylated material 337. It was found to be more convenient to protect the amine *in situ* as a benzyl carbamate (Scheme 3.8). Thus the major product was 338, prepared in 60% yield from nitrophenol 333 in one pot, with the dialkylated aniline 337 a minor by-product (9.5%).

-121-
Scheme 3.8 Reduction and Alkylation of Nitrophenol 333

The $^1$H NMR spectrum of 338 showed two doublets due to the aromatic protons and sharp singlets for the methoxy, methyl and $t$-butyl groups; the other resonances were broad. The spectrum was dramatically improved by recording at 50°C; signals due to the methylenes were then sharp and their couplings resolved. Presumably slow rotation in the carbamate leads to broadening at ambient temperature. The $^{13}$C spectrum was similarly enhanced with all aliphatic and most quaternary resonances observed. A signal at $\delta$ 51.3, attributed to the aminomethylene carbon, indicated N rather than O alkylation.

The molecular ion was observed in the mass spectrum but was not strong ($m/z$ 429, 4%). The base peak, as for almost all subsequent carboxybenzyl protected systems, was $m/z$ 91 i.e. $C_7H_7^+$ arising from the benzyl group. Nevertheless, significant fragment ions which were more informative were also observed. Losses of 2-methylpropene, benzyl alcohol and carboxybenzyl are observed directly or in combination as well as subsequent alkyl chain cleavages. The elimination of benzyl alcohol presumably proceeds to give the oxazolone 339, possibly occurring thermally on the probe.
The NMR spectrum of the dialkylated aniline 337 was sharp at ambient temperature. The aminomethylene protons were observed as a triplet at $\delta$ 2.85 indicating that the corresponding protons in the monoalkylated product 338 ($\delta$ 3.66) are deshielded, presumably by the carbonyl of the carboxybenzyl protecting group. The $^{13}$C NMR appeared to be missing one carbon because the resonance of the aminomethylene carbons, $\delta$ 55.48, overlapped with that of the methoxyl carbon $\delta$ 55.83. These were readily separated and assigned using the APT pulse sequence (see experimental).

Klamann has subsequently optimised this and the following steps while repeating the sequence on a large scale. Using dibenzyl dicarbonate rather than benzyl chloroformate he isolated the desired ester 338 (72%), the corresponding benzyl carbonate 340 (2%), the dialkylated aniline 337 (7%) and the corresponding benzyl carbonate 341 (6%) from a 16 mmol scale reaction.

![Chemical structures](image)

Treatment of the t-butyl ester 338 with trifluoroacetic acid gave the butanoic acid 342 as a colourless oil. Attempted low temperature crystallisation was unsuccessful; the product was used crude for subsequent chain extension. The NMR spectrum of 342 was much better resolved than that of the parent ester 338, although the methylene resonances were still distinctly broadened.

Homologation of the crude acid using the protocol of Brooks et al. gave the $\beta$-keto ester 301 in 68% yield (Scheme 3.9). Various minor byproducts were also formed but they varied from reaction to reaction and have not been fully characterised. Klamann scaled up this reaction using dibutyl magnesium to prepare the magnesium salt, thereby avoiding problems with adventitious hydrolysis of magnesium methoxide which had been used previously. On a 4 mmol scale he isolated the $\beta$-keto ester 301 in 51% yield as well as the lactone 344 (12%). The lactone may arise via cyclisation of the imidazolide 345 or from the $\beta$-keto ester 301 by intramolecular hemiketal formation and retroaldol reaction.
Subsequently, Morton et al. have reported that removal of excess carbonyl diimidazole (by reaction with Sephadex) improves the yield and reproducibility of acylation reactions. We have not tried this modification.

In CDCl₃ the β-keto ester 301 was predominantly in the keto form with a trace of the enol present, the latter shown by its characteristic enolic hydroxyl resonance at δ 11.98. The methylene resonances were broad. The mass spectrum, although dominated by the C₇H₇ ion from benzyl cleavage, showed a molecular ion (m/z 429, 15%) and loss of methanol (m/z 397, 6%) inter alia. Interestingly, loss of a methoxy radical was a relatively minor process (m/z 398, 1.6%). Eliminations of benzyl alcohol (m/z 321, 12%) and both benzyl alcohol and methanol (m/z 289, 5%) were apparent. The literature on mass spectrometry of β-keto esters is very limited but losses of both ethoxy radicals and ethanol are usually significant for ethyl β-keto esters. The reasons for variation in the relative importance of the two processes are not clear. The alcohol may be lost sequentially as well as by putative direct elimination from the enol (Figure 7), indeed it is possible that there is no tautomerisation occurring in the

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mass spectrometer. Data in the Wiley/NBS mass spectra collection shows that methoxyl loss is the relatively important process for normal, aliphatic methyl β-keto esters, alcohol loss being somewhat more significant in the corresponding ethyl esters, but still minor compared to ethoxy loss. There is significant variation in alcohol and alkoxy loss from different alkyl acetoacetates, with both processes being weakest for the methyl ester. Why methanol loss is favoured in is not clear.

\[
\text{Figure 7. Direct Elimination of Methanol from the Enol}
\]

3.2b Michael Cyclisation

Oxidation of the phenol 301 with Fremy's salt in a mixture of acetone and pH 6.8 buffer gave an orange-yellow product in 74% yield (Scheme 3.10). The NMR spectrum was very complex; the product was apparently largely enolised (δ 13.00 and 12.96) and present as two conformers, moreover all methylene protons appeared to be non-equivalent. The most dramatic difference in chemical shift was between the methyl ester resonances (δ 3.73 for the minor and δ 3.41 for the major conformer). The extent of enolisation suggested that the product was the cyclised system 348 as the enol content of simple acyclic β-keto esters is minor. The absence of quinonoid singlet resonances expected for the uncyclised quinone 346 supported this assignment. Heating to 50°C gave no improvement in the spectrum.
Scheme 3.10 Oxidation of the Phenol with Fremy’s Salt

The mass spectrum showed a weak ion corresponding to the molecular ion of the cyclised quinone 348. There was some variation between mass spectra of different samples, but a C7H7 ion from benzyl cleavage (m/z 91, 100%) and ions apparently from carboxybenzyl loss (m/z 306, M-CO2CH2Ph, 29%; m/z 274, M-CO2CH2Ph-CH3OH, 26%) were consistently important although variable in intensity. The origin of the other high mass ion, m/z 411 (“M-30”, 4%) was not immediately obvious. The same “loss” was observed by Chai in the mass spectrum of the analogue 297 unsubstituted at C7.203 We attribute these ions to elimination of methanol from the corresponding hydroquinones. “M+2” ions are considered characteristic of quinones, arising to a variable extent by reduction in the inlet chamber, the extent depending on conditions especially the water vapour pressure and the reduction potential of the quinone.327 Although the molecular ion of the parent hydroquinone (m/z 443) was usually negligible, ions were seen at m/z 303 (10%) and 276 (13%) consistent with elimination of methanol and benzyl alcohol, and loss of methanol and a carboxybenzyl radical respectively. These ions (m/z 411, 303 and 276) are the important ions in the mass spectrum of the independently synthesised hydroquinone 347, the molecular ion of which is negligible (the base peak is, as usual, m/z 91, C7H7+).322 The uncyclised quinone 346 has the same molecular weight as the hydroquinone 347 but would not be
expected to eliminate benzyl alcohol. Elimination of methanol from the quinone was a minor process \((m/z \ 409, \ 0.8\%)\) and it seems plausible that the hydroquinone 347 loses methanol via intramolecular lactonisation.

In an effort to obtain more rapid oxidation and escape the problem of almost incompatible solubilities of Fremy’s salt and the phenol 301, we tried a phase transfer catalysed oxidation. Olson et al. had reported that hydrophobic phenols could be efficiently and rapidly oxidised in a benzene - buffer mixture with methyltrioctylammonium chloride as a phase transfer catalyst (“Aliquat”). Unfortunately oxidation of 301 under these conditions was slow and not clean. Other means of circumventing the general problem of mutual solubility and rather low solubility of Fremy’s salt even in water are discussed in Chapter 5. Klamann investigated oxidation using oxygen with a DMF-soluble cobalt catalyst (“salcomine”) but found it was very slow. He optimised the oxidation with Fremy’s salt by varying the solvent mixture and using a large excess of reagent and obtained a 95% yield.

Given the spectral complexity we could not rule out the presence of some uncyclised quinone 346 or traces of a spiro compound analogous to 317, but at least the bulk of the material isolated was the product of oxidation and cyclisation. The structure 348 was confirmed by the next reaction. Thus the presence of a conjugated methoxy group does not noticeably inhibit the Michael cyclisation. Biosynthetically the introduction of a 7-methoxy substituent is clearly plausible either before or after any Michael cyclisation.
CHAPTER THREE

3.2c Transannular Cyclisation

Hydrogenation of the quinone 348 gave a colourless solution which yielded the orange, condensed decarbamoyl product 203 in 82% yield on exposure to air. The indoloquinone 203 had been prepared previously by Gredley and Rickards27 using a Nenitzescu reaction (Scheme 3.12) and this matched our product spectroscopically. More recently Asano et al. (see below) have published 1H NMR data which match ours. The only other data reported was a melting point of 208.5-210°C; we observed a melting point of 210-215°C but the melting point was not well defined.

![Scheme 3.11 Synthesis of a Pyrrolo[1,2a]indole](image)

The NMR spectrum of 203 was well resolved. The mass spectrum showed a molecular ion and extensive fragmentation with losses of methyl radicals, carbon monoxide and methanol (and combinations thereof) responsible for the higher mass peaks. Methanol loss is also seen for the 7-acetylamino analogue 367 (see below) and presumably arises by abstraction of a proton from Cl. Peaks attributable to the hydroquinone 349 were not observed, presumably because the reduction potential of the indoloquinone is lower than that of the benzoquinone precursor 348.

The NMR spectrum of a crude hydrogenated sample indicated that it consisted largely or entirely of the indolic hydroquinone 349. A resonance at δ 10.5 is
characteristic of an 8-hydroxy group in such systems,\textsuperscript{329} apparently due to hydrogen bonding to the ester in a seven membered ring. On exposure to air the phenolic resonances were lost but otherwise spectral changes were minor, leading to the spectrum of the quinone \textbf{203}. This is consistent with the studies of Chai,\textsuperscript{203} who was unable to observe any intermediates in the conversion of \textbf{308} to \textbf{311}. The lack of an aziridine ring and the acidity of H9 compared to the actual mitomycins doubtless facilitates dehydration in these models.

Thus the biomimetic cyclisation and transannular cyclisation both proceed efficiently. The pyrroloindole \textbf{203}\textsuperscript{329,333,207} and its ethyl ester analogue \textbf{299}\textsuperscript{330,331,332,260} have now been synthesised a number of times. These syntheses and the unpublished route of Gredley and Rickards\textsuperscript{27} are summarised in Scheme 3.12. Both esters have been converted to 7-methoxymitosene \textbf{204} (see Scheme 2.1).\textsuperscript{260,330,332,333} As a synthesis of \textbf{203}, our route compares reasonably well to the others, although its primary aim was biomimetic interest rather than synthetic efficiency. Murphy and O'Sullivan's recently published synthesis\textsuperscript{207} is probably the most attractive to date, but note that the preparation of the initial benzoquinone is not detailed. The syntheses of Luly and Rapoport,\textsuperscript{260} Coates and MacManus\textsuperscript{330} and Akiba \textit{et al.}\textsuperscript{331} require related A ring synthons which are not commercially available; the efficient synthesis of suitable benzoquinones is discussed further in Chapter 5.
Scheme 3.12 Literature Syntheses of Pyrrolo[1,2a]indoles 203 and 299 (continues)
Scheme 3.12 Literature Syntheses of Pyrrolo[1,2a]indoles 203 and 299 (continues)
Wender and Cooper 1987

Murphy and O'Sullivan 1992 (see Scheme 2.1)

Scheme 3.12 Literature Syntheses of Pyrrolo[1,2-a]indoles 203 and 299
CHAPTER THREE

3.3 Will an Acetylamino Substituted Quinone Cyclise?

3.3a Synthesis of the Acetylamino Substituted Phenol

THE AROMATIC RING

The acetylaminophenol 302 was also synthesised in order to examine the effect of the protected amino functionality on Michael cyclisation. The difference between an amino group and a methoxy group as a quinone substituent can be significant as seen, for example, in the differences between the chemistry of mitomycin A and C (see Chapter 1). The acetyl protected derivative was investigated to avoid oxidation of intermediates. In order to make the β-keto ester 302 we required 3-acetylamino-6-amino-2-methylphenol (354). This aniline and its obvious precursors had not been reported. We anticipated that 3-acetylamino-2-methyl-6-nitrophenol (352) would be accessible by nitration of 3-acetylamino-2-methylphenol (351), which in turn could be made from 3-amino-2-methylphenol (350). The aniline 350 is commercially available but rather expensive; it has been synthesised by reduction of 2-methyl-3-nitrophenol, but this is also not very cheap, and was not at hand.

We prepared 350 by heating 3-hydroxy-2-methylphenol (327) with ammonia and ammonium chloride at 225°C for 38 h in a pressure vessel. Addition of sodium bisulfite, i.e. the use of Bucherer conditions was unnecessary. Brief attempts to catalyse the reaction with cobalt chloride as described by Enomoto et al. were unsuccessful. The desired aminophenol 350 was separated from starting material and 3-amino-2-methylaniline by base then acid extraction in 70% yield. Selective acetylation with acetic anhydride in ethanol gave the unknown acetanilide 351 in quantitative yield (Scheme 3.13). The aryl methyl group of 351 was significantly shielded in the 13C NMR (δ 10.8 in CD3OD) presumably in a manner analogous to the 3-hydroxy-2-methylphenol derivatives discussed above.
Nitration of 351 with nitric acid in acetic anhydride gave two new mononitrophenols (Scheme 3.14). These were readily separable because the more polar, minor isomer (mp 202-203°C, 37%) was effectively insoluble in chloroform but could be extracted with ethyl acetate. The less polar, major isomer mp 184-185°C was isolated in 52% yield. The aromatic protons were seen as ortho coupled doublets (J ~ 9 Hz) in both products, indicating that the expected ortho- and para-nitrophenols (352 and 353) had been formed. The assignment of these structures to the products was less obvious.

Scheme 3.14 Nitration of 3-Acetylamino-2-methylphenol

There are clear and related differences between the physical properties of ortho- and para-nitroacetanilides and nitrophenols arising from intramolecular hydrogen bonding in the ortho isomers. Unfortunately, 352 is an ortho-nitrophenol and 353 is an ortho-nitroacetanilide thus differences in polarity, melting point, solubility etc. were not diagnostic.

The two isomers exhibit quite distinct 1H NMR spectra. The major product has aromatic protons which resonate at δ 8.00 and 7.86 in CDCl₃ or δ 7.90 and 7.40 in DMSO. The minor product has protons at δ 7.71 and 6.83 in DMSO. Calculation of spectra using substituent additivity with various substituted benzenes failed to predict the observed spectra; this was not surprising given the polycsubstitution, polarisation and solvent effects. Nevertheless a tentative assignment was possible based on OH and NH resonances.
We had observed deshielded and sharp resonances of the hydrogen bonded hydroxyl protons in 3-hydroxy-2-methyl-4-nitrophenol (325) and its methyl ether 333. For comparison we measured the NMR spectra of ortho- and para-nitrophenol and nitroacetanilide in chloroform, acetone and dimethyl sulfoxide (Table 3). While there was a dramatic solvent effect on linewidths, in all cases the intramolecularly hydrogen bonded amide or hydroxyl resonances were broader than the corresponding resonances of the para isomers in the same solvent. More importantly the amide resonances were consistently broader than the hydroxyl resonances in each solvent; this effect was dramatic in CDCl₃. Presumably quadrupole interactions and viscosity affect the broadening. We were unable to perform experiments with nitrogen decoupling. Drying of the CDCl₃ solutions (molecular sieve) did not affect the shifts or linewidths. The reproducibility of the linewidth measurements was only about ±2 Hz. We measured the linewidths of some other acetanilides and phenols as shown in Figure 8; clearly the observation of a narrow resonance in CDCl₃ is indicative of a phenolic resonance whether or not intramolecular hydrogen bonding is present.

Table 3. Linewidths of Amide and Hydroxyl Proton Resonances in ortho- and para- Nitroacetanilides and Nitrophenols

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Acetanilides</th>
<th>Phenols</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AcNH-NO₂</td>
<td>AcNH-NO₂</td>
</tr>
<tr>
<td></td>
<td>δ 10.34</td>
<td>δ 7.4</td>
</tr>
<tr>
<td></td>
<td>15.9 Hz</td>
<td>13.4 Hz</td>
</tr>
<tr>
<td>CD₃COCD₃</td>
<td>δ 10.34</td>
<td>δ 9.74</td>
</tr>
<tr>
<td></td>
<td>24.9 Hz</td>
<td>21.1 Hz</td>
</tr>
<tr>
<td>CD₃SOCD₃</td>
<td>δ 10.28</td>
<td>δ 10.56</td>
</tr>
<tr>
<td></td>
<td>4.9 Hz</td>
<td>4.8 Hz</td>
</tr>
</tbody>
</table>

(† these linewidths are comparable to the digital resolution and not precise)
The spectrum of the major, less polar nitration product in CDCl₃ showed a sharp, exchangeable resonance at δ 11.25 (linewidth 0.6 Hz) and a broad exchangeable resonance at δ 7.19 (linewidth 12 Hz) consistent with the hydroxyl of an ortho-nitrophenol and the amide of a para-nitroacetanilide respectively. Thus the major product appeared to be the desired isomer 352. Unfortunately, the minor product 353 was not sufficiently soluble in chloroform to enable an NMR spectrum to be recorded.

Attempts to confirm the structural assignment by NOE studies were complicated by the effective overlap of the two methyl groups in both isomers in a range of solvents and the similar shifts of the aromatic and amide protons in the major product. With the major product in dichloromethane, irradiation of the broad resonance at δ 7.25 resulted in an enhancement of both methyl resonances (23% combined) and a 6% negative NOE of the sharp resonance at δ 11.2. Irradiation of the aromatic protons gave no significant enhancement of other resonances. This is consistent with δ 7.25 being the resonance of the amide of 352 in the conformation shown (Figure 9), irradiation leading to enhancement of the adjacent methyl groups and a relayed effect to the intramolecularly hydrogen bonded hydroxyl (δ 11.2). Amides, including 2-methylacetanilides, are known to prefer a Z conformation. The NOE result is not compatible with the isomeric structure 353 as relay effects from the hydroxyl (putatively δ 7.25, since it is not hydrogen bonded) to the acetamide methyl would not be expected (relay effects through more than one nucleus are almost always negligible).
The minor product was sparingly soluble in acetone, with one exchangeable resonance observed at δ 9.1. On cooling to -46°C to minimise exchange with water in the solvent, a broad resonance at δ 10.15 appeared. This was, however, still exchanging with water as irradiation either at δ 10.15 or of the water peak gave identical NOE effects (a 6% enhancement of the doublet at δ 6.9 and negative effects at the water peak or δ 10.15). This result cannot be unambiguously interpreted on its own. NOE experiments in dimethyl sulfoxide were similarly inconclusive. Given the structure 353, it is apparent that an NOE between the hydroxyl and the ortho aromatic proton is being observed. Subsequently we have discovered that addition of a 3Å molecular sieve to the NMR solution removed water and led to observation of the two exchangeable resonances at ambient temperature but we have not repeated the NOE experiment. (Note that molecular sieves can cause decomposition of acetone after initial drying.)

$^{13}$C NMR spectroscopy also seemed consistent with the structural assignments. Unfortunately substituent additivity calculations involve parameters for chloroform solution and the solvent effect of acetone is not clear. The calculated spectra of the two isomers differ most significantly in predictions of COH and CNH resonances. Thus for the desired isomer 352 COH shifts of δ 156, 149 and 153 were calculated (starting with shifts for 2-nitrophenol, 2-methylphenol and 4-nitroacetanilide respectively); the major product had a resonance at δ 154.3 in CDCl$_3$. Similarly CNH shifts of δ 148, 147 and 148 were calculated and a resonance at δ 144.2 observed. For the 4-nitrophenol isomer 353 COH shifts of δ 162, 160 and 163 and CNH shifts of δ 136, 136 and 136 were calculated from appropriate disubstituted benzenes; the minor product in acetone showed resonances at δ 161.1 and 140.0 (in dimethylsulfoxide δ 160.2 and 138.4).
These correlations seem reasonable given the deviations from additivity known to arise with steric crowding, intramolecular hydrogen bonding and polarisation.\textsuperscript{344}

The mass spectra of the two isomers were remarkably different. The major product 352 showed a strong molecular ion (m/z 210, 47\%) and a base peak corresponding to the loss of ketene (m/z 168). The minor product 353 gave a weak molecular ion (m/z 210, 2\%), strong peaks from water and ketene loss (m/z 192, 39\% and m/z 168, 78\%) and a base peak due to loss of nitrogen dioxide (m/z 164). Ions due to water and nitrogen dioxide loss in the mass spectrum of the major product 352 were negligible (0.3 and 0.6\% respectively). By contrast the lower mass ranges were very similar.

Loss of the nitro substituent is a general feature of the mass spectra of nitroaromatics,\textsuperscript{316} but it is not clear why it is not significant for the \textit{para}-nitroacetanilide 352. Conceivably the acetyl group can stabilise an \textit{ortho}-cation allowing more favourable nitrogen dioxide loss in the \textit{ortho}-isomer 353. The mass spectra of nitroacetanilides have only been cursorily discussed in the literature\textsuperscript{345,317} thus we measured the mass spectra of \textit{ortho}- and \textit{para}-nitroacetanilide. Loss of ketene dominates both spectra but nitrogen dioxide loss is more intense for the \textit{ortho} isomer (m/z 134 7\% cf. 0.4\% for \textit{para}) matching the correlation seen with our more substituted products. We also recorded the mass spectra of \textit{ortho}- and \textit{para}-nitrophenol and observed similar and more significant nitrogen dioxide loss for both isomers (m/z 93, 19 and 24\% respectively).

The loss of water in the mass spectrum of the minor product 353 was reminiscent of the mass spectra of hydroxynitrophenols discussed earlier in this chapter. Furthermore both products showed ions at m/z 150 and 151, presumably due to loss of water and a hydroxyl radical respectively from the aminonitrophenols generated by ketene loss. This loss of water appears to be a general process for 1-amino/hydroxy-3-hydroxy/amino-2-methyl-4-nitro substituted benzenes, perhaps dominated by competitive ketene loss in the major product 352.
Hydrogenation of the nitrophenol gave the air sensitive aminophenol 354 which was directly alkylated and protected to give the ester 355 in 43% yield (Scheme 3.15). The NMR spectrum contained sharp methyl and t-butyl singlets but other resonances were broad. Heating to 50°C sharpened all resonances except one methyl singlet which was significantly broadened; coupling in the alkyl chain was now resolved. Presumably carbamate rotation at the higher temperature is fast enough for coalescence while rotation of the acetamide becomes fast enough to broaden that methyl resonance (the amide and phenol resonances were not resolved at low temperature, at high temperature the amide resonance appears to be a very broad signal at δ 7.07).

\[
\begin{align*}
\text{AcNH} & \rightarrow \text{AcNH} \\
\text{OH} & \rightarrow \text{OH} \\
\text{Br(CH}_3)_2\text{CO}_2\text{-Bu} & \rightarrow \text{Br(CH}_3)_2\text{CO}_2\text{-Bu} \\
\text{NaHCO}_3 & \rightarrow \text{NaHCO}_3 \\
\text{DMPU} & \rightarrow \text{DMPU} \\
\text{43%} & \rightarrow \text{43%} \\
\end{align*}
\]

\[
\begin{align*}
\text{AcNH} & \rightarrow \text{AcNH} \\
\text{OH} & \rightarrow \text{OH} \\
\text{CO}_2\text{Bn} & \rightarrow \text{CO}_2\text{Bn} \\
\text{O}^\text{Bu} & \rightarrow \text{O}^\text{Bu} \\
\end{align*}
\]

Scheme 3.15 Reduction and Alkylation of Nitrophenol 352

Herlt has repeated the reaction on a larger scale in these laboratories and isolated the unalkylated, protected aniline 356 as well as traces of what appeared to be an N,N-dialkylated byproduct. The unalkylated aniline 356 was still a major product even when 1.6 equivalents (rather than 1.05) of the alkyl bromide were used (yield of 355 47%, yield of 356 43%). The NMR spectrum of 356 was not sharp, apparently due to slow conformational changes of the carbamate. Chai\textsuperscript{203} has shown that ortho-(carboxybenzylamino)-phenols are relatively unstable, heat or base causing elimination of benzyl alcohol. Sublimation of the ester 355 cleanly gave the oxazolone 357. The
NMR spectrum of the oxazolone was sharp with coupling in the alkyl chain well resolved. This oxazolone was not detected as a byproduct in the preparation of \textbf{355}.

![Structures](image1.png)

Treatment of the ester \textbf{355} with trifluoroacetic acid gave the corresponding butanoic acid \textbf{358}, which we were unable to recrystallise. NMR spectra of \textbf{358} were consistently broader than those of the corresponding methoxy analogue \textbf{342}. Heating to 51°C improved the resolution markedly except for the acetamide methyl group which was broadened, paralleling the behaviour of the ester \textbf{355}. Unlike most compounds in this and the methoxy series, the base peak in the mass spectrum of \textbf{358} was not \textit{m/z} 91 (C\textsubscript{7}H\textsubscript{7}\textsuperscript{+} from benzyl cleavage) but \textit{m/z} 79. This ion and \textit{m/z} 108, 107 and 77 were consistently intense for a number of different samples run at different times, and they correspond to benzyl alcohol (molecular weight 108) and its fragments,\textsuperscript{317} clearly formed by elimination which forms the oxazolone \textbf{359} (\textit{m/z} 292, M-PhCH\textsubscript{2}OH \textit{i.e.} the molecular ion of this oxazolone is also observed, 47%). The oxazolone \textbf{359} could be prepared by base hydrolysis or heating but was not detectable (NMR) in the crude acid \textbf{358}.

![Structures](image2.png)

Extension of the acid \textbf{358} (Scheme 3.16) was problematic. The reaction was performed a number of times and in several cases none of the \(\beta\)-keto ester \textbf{302} was isolated. The products varied quite widely and have not all been characterised; the most common undesired product was the lactone \textbf{361} which was identified largely by mass spectrometry because its NMR spectra were very complex due to overlap and the presence of two conformers. The oxazolone \textbf{362} was also isolated. At best the desired...
β-keto ester was isolated in 44% yield. Why this reaction was lower yielding and more variable than that of the methoxy analogue is not clear.

\[ \text{Scheme 3.16. Extension of the Acid to a β-Keto Ester} \]

The NMR spectrum of the oxazolone 362 was well resolved, typical of systems without a carboxybenzyl group. The molecular ion (\(m/z\) 348) was the base peak in the mass spectrum, and loss of ketene and methanol were also observed with direct methoxyl loss being less significant (M-\(\text{CH}_3\text{OH}\), 31%; M-\(\text{CH}_3\text{O}\), 12%). Ions corresponding to various McClafferty rearrangements (\(m/z\) 232 and 206) and a cleavage (\(m/z\) 219), and then further ketene loss (\(m/z\) 190, 177, 164) were also observed with \(m/z\) 164 being intense (90%).

The NMR spectrum of the β-keto ester 302 was not well resolved (we have not tried high temperature NMR for this system); a trace of the enol form was betrayed by a small singlet at \(\delta\) 11.97. The mass spectrum showed a modest molecular ion peak (\(m/z\) 456, 12%) and losses of methanol and methoxyl (\(m/z\) 424 and 425, 4% and 1%), benzyl alcohol (presumably giving the oxazolone 362, \(m/z\) 348, 17%) and the (net) loss of a carboxybenzyl radical (\(m/z\) 321, 17%). The only intense ion, other than the ubiquitous base peak \(m/z\) 91, was \(m/z\) 191 (44%). Mass measurement of this latter ion revealed a composition \(\text{C}_{10}\text{H}_{11}\text{N}_2\text{O}_2\). The analogous 7-methoxy and 7-unsubstituted203 systems 301 and 295 gave rise to corresponding intense ions (\(m/z\) 164, 45% and \(m/z\) 134, 48% respectively) suggesting that the species formed is 363 (i.e. rather than possible isomers arising via ketene loss). The mechanism of formation is not
clear. The oxazolone 362 does not give the same fragment, indicating \( \alpha \) cleavage and decarbonylation of 362 are not involved. Routes via carboxybenzyl loss and a cleavage require further hydrogen radical loss.

3.3b Michael Cyclisation

Oxidation of 302 with Fremy’s salt in pH 6.8 buffer gave an orange product in 75% yield (Scheme 3.17). The spectra of the product were complex; the NMR spectrum was similar to that of the methoxy substituted quinone 348, and apparently the product was enolised (\( \delta \) 13.03 major and \( \delta \) 13.07 minor) and present as a mixture of rotamers. Again quinonoid singlet resonances were not observed. The mass spectrum showed very weak ions at \( m/z \) 468 and 470 (0.03 and 0.03%) matching the molecular ions of the desired cyclic quinone 366 and its hydroquinone. Weak fragments arising from methanol, ketene and carboxybenzyl (or combination) loss from both parents were observed, with loss of benzyl alcohol from the hydroquinone 365 as well. The hydroquinone could arise from \textit{in situ} reduction as discussed for the methoxy analogue 348; again the loss of benzyl alcohol (benzyl alcohol, \( m/z \) 108, 15%, was also observed) is only expected for the hydroquinone, not the isobaric uncyclised quinone 364. Similarly direct loss of methanol was a significant process only for the hydroquinone (\( m/z \) 438, 1% \textit{cf.} \( m/z \) 436, 0.04%) consistent with an intramolecular lactonisation.

Thus the presence of a conjugated acetamide group does not noticeably inhibit the Michael cyclisation. Studying the cyclisation of a 7-amino (or hydroxy) substituted system would be interesting, as any inhibition should be more marked, but such a system was not readily accessible.
Scheme 3.17 Oxidation of the Phenol with Fremy’s Salt

3.3c Transannular Cyclisation

Hydrogenation of the quinone and reoxidation led to the decarbamoyl, condensed product 367 in 47% yield on a small scale (Scheme 3.18). The \(^1\)H NMR spectrum of 367 was well resolved and matched that of the methoxy analogue 203 very closely (± 0.02 ppm, except of course for the acetamide/methoxy resonances). The mass spectrum showed a molecular ion, loss of methanol and ketene and a base peak from loss of both. Hydroquinone derived ions were not observed.

Scheme 3.18 Synthesis of a Pyrrolo[1,2a]indole

We hoped to correlate the methoxy and acetamide substituted pyrroloindoles 203 and 367 by chemical conversion. Substitution of a 7-methoxy group with ammonia is standard mitomycin chemistry. Acetyl cleavage or acetamide displacement was
expected to give the same aminoquinone 368 from the acetamide substituted pyrroloindole (Scheme 3.18).

Scheme 3.19 Intended Correlation of Pyrroloindoles

Treatment of the methoxy substituted pyrroloindole 203 with ammonia gave a very sluggish reaction. This may be in part due to the dichloromethane required for solution, since the pyrroloindole 203 is not very soluble in neat methanol (less than 1 mg per ml). Such displacement reactions with mitomycins are known to be very solvent dependent, proceeding best in polar, protic solvents. It also seems possible that substitution of pyrroloindoles is intrinsically more difficult in line with their lower reduction potential (compare mitomycins and mitosenes, see Chapter 1). With little of the benzoquinone precursors available we have not pursued the correlation of the two series by 7-substitution at that stage.
3.4 Hence the Mitomycins?

With a reasonably efficient route to the carbocyclic skeleton of the mitomycins available, the obvious extension was to elaborate the process by incorporating an aziridine ring and convert the ester group to an alcohol. These changes were expected to lead to the retention of the 9a-hydroxyl group upon transannular cyclisation. Efforts initiated to incorporate the aziridine ring are outlined in the next chapter. While that work was in progress, independent studies were undertaken by Klamann to study the conversion of the ester 348 to alcohol 369.322

Reduction of ClO₂ or an alkylation-decarboxylation protocol, was expected to give the 3-hydroxy ketone 369, which is similar to Kishi et al.'s intermediate 259. The amine protection would then be removed reductively (or perhaps hydrolytically). Transannular cyclisation of the amino ketone should then proceed with retention of the 9a-hydroxy group, at least for an aziridine fused system, by analogy to the synthesis of mitomycin B (see Chapter 2). The ester 348 was found to form a stable anion which could not be alkylated, nor could the hydroquinone. Removal of the ester substituent using hot aqueous dimethylsulfoxide was accomplished in reasonable yield (Scheme 3.20). The hydroxylated byproduct 371 is apparently formed by attack on oxygen by the enolate produced on decarboxylation. Attempts to trap the anion in situ with formaldehyde or diiodomethane were unsuccessful. The anion of the ketone 370 was found to be unstable and the enol-trimethylsilyl ether gave a complex mixture when treated with titanium tetrachloride and trioxane. Reduction of ester 348 was also explored. Attempts to protect the ketone as a ketal or enol ether were unsuccessful or low yielding. Reduction with various hydride reducing agents gave, at best, very low yields of products with ester reduction. Thus the quinone functionality appeared to preclude useful elaboration of the ester.
Scheme 3.20 Demethoxycarbonylation of Ester 348

With the failure of this transformation of the ester 348 to the alcohol 369, a synthesis of mitomycins becomes problematic. A C10 ester makes the 9-methine proton appreciably acidic and it seemed very unlikely that dehydration to the mitosene could be avoided upon cleavage of the N4 protecting group and transannular cyclisation. Thus the most likely product of our extended synthesis was the aziridinomitosene ester 372 or its 7-N-acetylamino analogue. While the stability of such an ester might be of some interest, given the marked reactivity of the mitomycin C derived aziridinomitosene 90,73 it is unlikely that such a system could be readily elaborated to a mitomycin with requisite 9a- and 10-functionality. Obviously the need to reintroduce 9a-functionality was distasteful even if possible. Furthermore, the achiral aziridinomitosene ester 373 had already been synthesised in the Rickards laboratory by a different route347 and Shaw et al.219 had synthesised the chiral analogue 237 (see Chapter 2).

Clearly the β-ketoester functionality we required to obtain C8a-C9 bond formation complicates subsequent elaboration. It was possible that this problem could be circumvented by synthesising an α-hydroxymethyl-β-keto ester (374) where
appropriate ester protection would allow mild decarboxylation following cyclisation. The stability of such systems to elimination and the effect of steric hindrance on cyclisation are not clear. But there was now also doubt about the biosynthetic relevance of our Michael cyclisations.

3.5 The Isolation of FR-900482 and FR-66979

The biosynthetic postulates on which our Michael cyclisation model studies were based had been seriously challenged during the course of the work. Uchida et al. reported the isolation of FR-900482 (375), a potent antibiotic with antitumour activity from S. sandensis. The structure was determined by X-ray crystallography of an acetate derivative. FR-900482 exists as a mixture of two stereoisomers 375 and 376 which interconvert, presumably via ketone 377; the dominant isomer is 375 which is furtherfavoured in the presence of acid. The aziridine ring, carbamate and carbocyclic skeleton bespeak an obvious relationship to the mitomycins.

Subsequently the reduced derivative FR-66979 (378) was isolated with an hydroxymethyl rather than an aldehyde at ‘C6’; FR-66979 also exists as a stereoisomeric mixture. Interestingly FR-66979 crosslinks DNA without activation whereas FR-900482 needed one equivalent of thiol to crosslink significantly. Both FR-900482 and FR-66979 are weak antibacterial agents. The triacetate of FR-900482
has attracted interest as an antitumour agent and forms DNA crosslinks and DNA-protein linking in cells. Unlike mitomycins and other quinonoid antibiotics these phenolic systems cannot directly mediate oxidative damage.\textsuperscript{351,352}

\[
\text{FR-66979}
\]

Two models for crosslink formation for these FR-antibiotics have been speculatively proposed on the basis of similarity with the mitomycins. Goto and Fukuyama suggested that two electron reduction cleaving the N-O bond would give a ketone \textsuperscript{379} that could dehydrate giving the aziridinoindole \textsuperscript{380}. This is competent to alkylate DNA at C1 and C10 in a manner completely analogous to leucoaziridinomitosenes (see Section 1.4).\textsuperscript{353} McClure and Danishefsky have proposed that nucleophilic aromatic substitution at ‘C5’ with consequent N-O cleavage would also lead to a mitosene analogue \textsuperscript{381} (Scheme 3.21).\textsuperscript{354} Note that intermediates \textsuperscript{380} and \textsuperscript{381} would be likely to crosslink with high efficiency (or at least suffer both C1 and C10 substitution) as deactivation of N4 by oxidation to a quinone is not quickly accessible.

These conjectures remain to be tested \textit{e.g.}, most directly, by isolation of the crosslinked adduct and determination of the structure of the FR-900482 residue. Studies by Williams and Rajski\textsuperscript{355} found that thiol activation is not required for FR-66979 to crosslink DNA, suggesting that, at least for FR-66979, nucleophilic activation is unlikely and that if reductive activation occurs it presumably must proceed by intramolecular redox reaction. In contradiction, however, Woo \textit{et al.} have recently reported that reductive conditions (dithionite) are required for significant crosslinking by either FR-900482 or FR-66979 and that the sequence selectivity is similar to that of mitomycin.\textsuperscript{356} Thus the crosslinking mechanism remains unclear.
The isolation of FR-900482 and FR-66979 has implications for mitomycin biosynthesis. The antibiotics appear to be structurally related to the mitomycins, but with oxidation at nitrogen rather than C5 and, crucially, cyclisation of the glucosamine moiety onto a benzenoid ring. Subsequent biosynthetic studies on FR-900482 demonstrated the efficient incorporation of both 3-amino-5-hydroxybenzoic acid and D-glucosamine and enhanced antibiotic production (indicating a biogenetic origin identical with the mitomycins).\textsuperscript{357} This suggests FR-900482 is a divergent product from the mitomycin biosynthetic pathway. It is even conceivable that FR-900482 is an intermediate, \textit{e.g.} by subsequent Bamberger rearrangement\textsuperscript{358} isomerising the hydroxylamine to oxidise either ‘C5’ or ‘C7’. It is extremely unlikely that mitomycin-like quinones are precursors of FR-900482; although phenolic deoxygenation is known to occur in biosynthesis (\textit{i.e.} beyond the polyketide precursor level),\textsuperscript{359} we are unaware of any examples of a benzenoid hydroquinone suffering such a transformation.

In the absence of knowledge about the point of divergence between the biosyntheses of mitomycins and FR-900482, it is dangerous to draw inferences about mitomycin biosynthesis. Nevertheless, it is rather tempting to view FR-900482 as related to early intermediates in the mitomycin biosynthesis sequence. We note that aziridine cyclisation and carbamoylation may well occur early in the sequence and,
more tentatively, that our postulation of initial C9α stereochemistry is consistent with FR-900482 stereochemistry. The absence of a substituent at C7 is noteworthy and leaves open both direct oxidative and Michael addition mechanisms for its introduction in mitomycin biosynthesis. The most significant conclusion is that coupling of C5' of D-glucosamine (96) to form the ultimate C8aC9 bond is almost certainly feasible with the AHB residue at the benzenoid oxidation level. Possible mechanisms for this bond formation are discussed in Chapter 5. The existence of another mode of AHB to glucosamine coupling (perhaps a radical process, see Chapter 5) in another pathway does not preclude our postulated Michael cyclisation mechanism for mitomycin biosynthesis, but it seems reasonable to invoke Occam's razor and postulate a common mechanism. The Michael cyclisation is facile chemically, and synthetically effective despite the medium ring which it generates, but it is probably not mimicking the biosynthetic process. (We view a 1,6 Michael addition to the 6-aldehyde as extremely unlikely).

Dmitrienko et al. have very recently speculated that the acid 382 serves as a biosynthetic intermediate for both mitomycins, by A ring oxidation, and FR-900482, perhaps by N4 oxidation and rearrangement. They provide evidence that the latter process is chemically feasible. This speculation seems sensible, although there is no evidence for the oxidation state of the 6-substituent of early intermediates, except that it is unlikely to be a methyl group.

Thus we were forced to devise a more flexible synthetic approach which would also allow exploration of other models of C8aC9 bond formation; this is described in Chapter 5.
CHAPTER FOUR: INCORPORATION OF A CHIRAL AZIRIDINE

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4.1 Introduction

The aziridine ring is the more reactive of the two alkylating moieties in the mitomycins and provides two of the four chiral centres. A crucial element in the elaboration of our model studies to an enantioselective total synthesis of the mitomycins was the enantioselective incorporation of an aziridine in the aliphatic portion. The aziridine ring is also the obvious source of induced asymmetry at C9 and C9a. All of the known natural mitomycin antibiotics have the same aziridine ring configuration (1S,2S) and the 9a-substituent is always trans to the aziridine (9aR). Kishi's synthetic work on transannular cyclisations (Section 2.3i) suggested that the aziridine chirality would induce the desired 9a-stereochemistry if dehydration could be prevented. Control of stereochemistry at C9 was less certain.

There were several points in our synthesis where an aziridine ring might be formed from an olefin, epoxide or other suitable precursor in the aliphatic segment. These are summarised in Scheme 4.1.

Scheme 4.1 Strategies for Introducing the Aziridine Ring
Adapting Kishi's strategy (Section 2.3i), the aziridine could be introduced after coupling to the quinone synthon but before cyclisation (Path A). Without conformational constraints, all the methods of aziridine synthesis should be available, avoiding problems that can occur with preparations of fused aziridines. The major disadvantage is the lack of convergence which tends to result in lower overall yields.

Similarly, an aziridine ring could be formed after the first cyclisation, along the lines of Ban et al.'s route to decarbamoyloxymitomycin derivatives (see Section 2.3iv); again the route is not convergent (Path B). Establishing C9 chirality before the aziridine ring is formed may cause problems if stereoselectivity is sought.

Aziridine ring formation after the transannular cyclisation (Path C) was the least attractive proposition since the sensitive 9a-functionality would be present and aziridine formation on model fused systems has been unsuccessful\(^{219,400}\) or low yielding\(^{401}\). Indeed the 9a-functionality appears to be more sensitive in the absence of the aziridine ring. (see Section 2.3i).

The most convergent and flexible approach is to couple an aliphatic segment with an extant aziridine to the quinone synthon (Path D). This strategy, using the aziridine and quinone rings as the starting points, has not been utilised before except in modified form (Path E) in a mitosene synthesis by Shaw et al. (Scheme 2.12, p77)\(^{219}\). There the aziridine was fused to a pyrrolidine (N4C9a bond formation) before coupling (N4C4a bond formation).

The extension of our model system using this approach (Path D) required a six carbon synthon equivalent to aziridine 400. The aziridine 400 itself is liable to cyclise intramolecularly to give a furan\(^{402}\) so the β-keto ester would presumably require protection during the coupling step.
Thus it was hoped that 400 might be elaborated to provide the first asymmetric synthesis of the mitomycins. The chirality of 400 was chosen on this basis. In retrospect we would argue that the enantiomer of 400 should have been chosen. The enantiomers of natural products are equally challenging synthetic targets, usually as informative in structural confirmation, but potentially far more valuable in their own right than the natural products because of their own possible activity. Thus we would argue that the antipode of a natural product with biological activity is always the preferable synthetic target, except when biological testing is required to confirm the target structure. Unfortunately, even where a natural product is available in large quantities this approach is very seldom taken [for a case where the synthetic enantiomer proved to be more biologically active, see Fleet and Smith403].

It would be attractive in a biomimetic synthesis to modify D-glucosamine (96) itself to yield the required intermediate 400. The utilisation of carbohydrates as starting materials in organic synthesis is now well developed.404 To use D-glucosamine requires reduction of C5' (perhaps via a furanose derivative) and oxidation of C4' to a ketone, operations which are not particularly well preceded. More importantly, any ultimate synthesis would be relatively linear and inflexible.

The ‘chiral pool’ of commercially available chiral natural products is frequently exploited. It suffers from the disadvantage that often only one enantiomer of the starting material is readily available, limiting the synthesis of enantiomers. The cost of starting materials obviously varies widely. Blarer and Rickards developed an approach to a D-glucosamine equivalent from S-serine for application in mitomycin synthesis.405 The known serine derivative 401 was alkylated with various lithio dithianes to give ketones such as 402, which was diastereoselectively reduced to the masked β-amino-α-hydroxyketone 403, (Scheme 4.2). This work, however, has not been further pursued.
The asymmetric synthesis of the mitomycins was one of the major prospective targets of this work. While this is obviously a dramatic and indeed still unaccomplished challenge, in the light of the two established synthetic routes success would not in itself be especially important. However the synthesis of new classes of analogues would certainly be worthwhile. The analogues investigated up to now have almost entirely been C7, carbamate or aziridine substituent variations prepared from mitomycins, or synthetic mitosenes or mitosanes. Analogues with more fundamental changes at the key alkylating sites are almost unstudied, indeed even the activity of the racemic mitomycins is apparently unknown, so just the enantiomers would be of interest. Major variations such as the preparation of epoxide analogues or variation in ring sizes would be intriguing (the only such analogue prepared so far is a mitosene with a cyclopropane ring in place of the aziridine\footnote{This is particularly true as the detail of mode of action and DNA base selectivity is discovered, laying the way for rational analogue design.}). After some consideration we decided against using carbohydrates as the source of our chiral aliphatic segment.

Many possible routes to 400 come to mind. The synthesis of enantiomerically pure products has become a major goal in organic chemistry and now offers a sensible alternative to resolution.\footnote{Resolution of simple aziridines has been reported by Mori and Toda.\footnote{The key element is the synthesis of the aziridine ring.}} Resolution of simple aziridines has been reported by Mori and Toda.\footnote{The key element is the synthesis of the aziridine ring.}
4.2 Natural and Synthetic Aziridines

4.2a Natural Aziridines

The aziridine ring has very rarely been identified in natural products. The mitomycins themselves, with the related FR-900482 (375) and FR-66979 (378), provide the most notable examples and by far the largest class. (2S,3S)-(+)-aziridine-2,3-dicarboxylic acid 404 has been isolated from a *Streptomyces* strain.409 The azinomycins, e.g. 405 have been identified as containing a fused aziridine;410 intriguingly these compounds are antitumour antibiotics which cross-link sequence specific guanine residues in DNA (note however that the structure of carzinophilin, which was recently established as identical with azinomycin B, has been controversial and frequently revised).411 The mechanism of cross-linking has not been elucidated. Thus there are no intact aziridines available in the ‘chiral pool’.

4.2b Synthetic Aziridines

Synthetic aziridines, by contrast, are far better precedent. The literature of aziridine synthesis has been reviewed up to *ca* 1982 by Deyrup.412 There is a large number of methods for the synthesis of aziridines. We confined our attention to methods compatible with asymmetric synthesis.
AZIRIDINES via NITRENES

A common methodology for the synthesis of aziridines is the addition of nitrenes to olefins. Only hetero- and carbonyl substituted nitrenes are useful synthetically in general. Carbalkoxynitrenes furnish aziridine carbamates which can potentially be deprotected. In the Rickards laboratories the racemic aziridinomitosene 233 has been prepared from olefin 232 in 27% yield using this methodology (Scheme 2.11, p76) providing rapid access to the aziridine in comparable yield to multistep procedures.

Chai and Rickards have investigated the synthesis of aziridines for an approach to the racemic mitomycins. They tried a variety of methods to aziridinate the double bonds in three furanoid derivatives 406, 407 and 408. All of the procedures investigated were unsuccessful except for the low yielding conversion of 408 to aziridines 409 and 410 (Scheme 4.3). The lack of reactivity observed was attributed to deactivation by the allylic oxygen, steric hindrance or the electron withdrawing functionality.

\[
\begin{align*}
\text{Scheme 4.3 Preparation of Aziridines by Nitrene Addition}
\end{align*}
\]

Asymmetric reactions of free nitrenes have not been reported. Aziridination using metal complexed nitrenes was known to proceed in varying yields but without any stereospecificity (for aryl substituted olefins) in interesting contrast to epoxidation using analogous metal-oxo complexes. Subsequently the preparation of cis- and trans-alkylaziridines with good stereospecificity has been reported. Recently the first case of asymmetric aziridination using copper (I) catalysis (Scheme 4.4) was reported. Note that tosyl aziridines are not easily deprotected.
When we were planning our synthesis however, the only reported asymmetric aziridination reactions were those of Atkinson’s group.\textsuperscript{418} The oxidation of various N-amino heterocycles with lead tetraacetate in the presence of olefins gave good yields of aziridines (e.g. Scheme 4.5). The reactions had been assumed to proceed via nitrenes however it was subsequently established that N-acetoxyamines were the reacting species.\textsuperscript{419} In fact, Atkinson has not reported the preparation of enantiomerically pure aziridines apparently due to difficulties in obtaining the N-amino heterocycles enantiomerically pure, so the “chiral aziridination” refers to the preparation of only or predominantly one diastereomer using racemic nitrenes. In the context of our synthesis, the crucial problem was cleavage of the nitrogen-nitrogen bond, which had not been accomplished. Subsequently, one cleavage via 1,2-elimination from a silylated aziridine has been reported to provide an azirine.\textsuperscript{420} This could conceivably provide a tenuous route to chiral unsubstituted aziridines if a diastereoselective reduction is possible. The synthesis of useful chiral aziridines via direct aziridination of olefins did not look promising.

\begin{equation}
\text{Ph} \quad + \quad \text{PhI-NSO}_2\text{PhCH}_3 \quad \xrightarrow{\text{CuSO}_2\text{CF}_3} \quad \text{Ph} \quad \text{NSO}_2\text{PhCH}_3 \quad + \quad \text{PhI}
\end{equation}

\textbf{Scheme 4.4 Asymmetric Aziridination}

When we were planning our synthesis however, the only reported asymmetric aziridination reactions were those of Atkinson’s group.\textsuperscript{418} The oxidation of various N-amino heterocycles with lead tetraacetate in the presence of olefins gave good yields of aziridines (e.g. Scheme 4.5). The reactions had been assumed to proceed via nitrenes however it was subsequently established that N-acetoxyamines were the reacting species.\textsuperscript{419} In fact, Atkinson has not reported the preparation of enantiomerically pure aziridines apparently due to difficulties in obtaining the N-amino heterocycles enantiomerically pure, so the “chiral aziridination” refers to the preparation of only or predominantly one diastereomer using racemic nitrenes. In the context of our synthesis, the crucial problem was cleavage of the nitrogen-nitrogen bond, which had not been accomplished. Subsequently, one cleavage via 1,2-elimination from a silylated aziridine has been reported to provide an azirine.\textsuperscript{420} This could conceivably provide a tenuous route to chiral unsubstituted aziridines if a diastereoselective reduction is possible. The synthesis of useful chiral aziridines via direct aziridination of olefins did not look promising.
AZIRIDINES via EPOXIDES

There are a number of methodologies for the elaboration of epoxides to aziridines. For instance Kishi et al. converted the epoxide to an azidomesylate and then to a phosphorylated aziridine by treatment with trimethylphosphite and base. Reduction released the free aziridine (see Scheme 2.15, pp81-82). Chai and Rickards attempted to prepare a fused, furanoid aziridine from the corresponding epoxide. 2(5H)-Furanone was epoxidised and treated with trimethylsilyl azide giving predominantly the unexpected and undesired cis-azidoalcohol (Scheme 4.6), apparently caused by the high acidity of the α-proton. Conversion of the minor trans isomer into an aziridine was not pursued.

Blum et al. have reported an efficient method for converting 1,2-azidoalcohols into aziridines with the opposite configuration, but same relative geometry as the parent epoxide by heating with triphenylphosphine. This method is now a standard method for synthesising aziridines. Apparently a Staudinger adduct is formed which eliminates nitrogen to give an hydroxy-iminophosphorane in equilibrium with an oxazaphospholidine. Elimination of triphenylphosphine oxide apparently gives the desired aziridine and triphenylphosphine oxide (Scheme 4.7).
4.3 Proposed Aziridine Synthesis

Given the accessibility of chiral epoxides, their conversion to aziridines was an attractive route. We anticipated that the cis-aziridine 419 might be prepared from the known epoxide 418 (Scheme 4.8). Distinct substitution of the hydroxyls must be maintained for the chirality to be preserved. It was hoped that the route from this epoxide would be relatively malleable, perhaps also leading to the very interesting but unknown epoxy analogues of the mitomycins. Oxidation of the intact aziridino alcohol 419 was expected to avoid the problem of epimerisation. Extension of the acid 420, as in the model sequence, should give the β-keto ester 421. Aziridine protection might be required.

\[
\text{Scheme 4.8 Proposed Aziridine Synthesis}
\]

We anticipated preparing the epoxide 418 by enantioselective epoxidation (see below). During the course of our work, two groups have reported the preparation of related chiral systems using enzymatic reactions to exploit the meso character of some butanediols. Grandjean et al. prepared the acylated epoxide 422 in 90% yield and 95% ee using an enzymatic hydrolysis, providing a good alternative to Sharpless epoxidation. Fuji et al. prepared the aziridine 423 by enzymatic transesterification in 68% yield and 98% ee. These are synthetically equivalent to 418 and 419 respectively. With suitable protecting group manipulation, both enantiomers should also be accessible by these routes.
4.4 Preparation of the Epoxy Alcohol

The desired epoxy alcohol 418 had been synthesised by Hungerbühler and Seebach in many steps starting from L-(+) -diethyl tartrate 426 and by Katsuki et al. by asymmetric epoxidation of the Z-allylic alcohol 426 using L-(+)-diethyl tartrate as the chiral ligand. 427 Subsequently many other groups have synthesised both enantiomers using the epoxidation procedure. Fluka Ltd. has started selling both enantiomers as p-nitrobenzoate esters but the price is high. In both preparations the product is not enantiomerically pure. The enantioselectivity of the 'Sharpless' epoxidation reaction is known to be worse for 4-substituted cis-allylic alcohols than for most other substrates. 428 Lowering the temperature increases the selectivity but slows the reaction. Weishan et al. have reported a significant acceleration for the epoxidation of this and other substrates through the addition of catalytic calcium hydride and silica gel. 429 This protocol has not been widely adopted and is not clear whether the effect is due to removal of adventitious water 430 or some more complex interaction (both of the additives are reportedly necessary to obtain an increased rate with sustained enantioselection).

The acetal 425 was prepared by condensation of benzaldehyde and (Z)-but-2-ene-1,4-diol (424) in 79% yield. 431 Cleavage with lithium aluminium hydride and aluminium chloride 432 gave the monoprotected diol 426 433 in 85% yield (Scheme 4.9). With hindsight, direct protection of the diol (see below) is probably a more attractive preparation.
We epoxidised 426 using catalytic conditions (0.5 eq TiO\textsubscript{4}Pr\textsubscript{4}) with added powdered molecular sieves.\textsuperscript{434} The reaction was slow at low temperatures, some uncharacterised by-products were formed (one apparently due to epoxide opening by t-butylic alcohol) and there were technical problems with maintaining low temperatures for the long periods required (greater than a week.) With almost stoichiometric ‘catalyst’ loading at -26°C only a 53% yield of product ([\alpha]_D -23°, c 0.59, CHCl\textsubscript{3}) was obtained after seven days with significant residual starting material. However by allowing the reaction mixture to warm overnight, a good yield of product (85%) was isolated with little effect on optical purity ([\alpha]_D -22°, c 0.51, CHCl\textsubscript{3}). This reaction was not further developed because it was subsequently decided to utilise bromobenzyl protection. The yield we obtained is comparable to those reported in the literature (78-87%). Subsequently, the Sharpless group detailed the importance of ageing of the titanium - tartrate catalyst; they found allylic alcohol 426 to behave atypically, reacting extremely slowly but noting that other workers had succeeded with (unpublished) modified conditions.\textsuperscript{436}

Hungerbühler and Seebach\textsuperscript{426} had obtained enantiomerically pure alcohol 418 by recrystallisation of the p-nitrobenzoate ester and hydrolysis but with only 50% recovery; Mori and Seu\textsuperscript{437} have recently purified the epoxide as its 3,5-dinitrobenzoate with 72% recovery. The optical rotation of our product suggests 79% enantiomeric excess based on the optical rotation of the pure enantiomer [\alpha]_D -28°, c 0.93, CHCl\textsubscript{3}) reported by Hungerbühler and Seebach\textsuperscript{426} and Mori and Seu.\textsuperscript{437} The optical rotations (when reported) are not always compatible with the enantiomeric purity established by NMR. This may be due to solvent and concentration effects on the optical rotation and several authors quote the optical rotation of Hungerbühler and Seebach's impure...
product ([α]D -27°), but the precision of the optical rotation method appears to be poor.

At this point the moderate enantiomeric purity and its precise quantification was not a concern. The enantiomeric purity of derived aziridine becomes important somewhat later in our planned synthesis when cyclisation creates another chiral centre. Thus with enantiomerically pure epoxide potentially available we investigated the conversion of 418 into an aziridine. While this work was in progress Chong and Wong reported the preparation of the 4-bromobenzyl epoxide 428 from the corresponding allylic alcohol 427, which itself was prepared by direct benzylation of (Z)-but-2-ene-1,4-diol (424). This epoxide is a solid at room temperature and could be directly recrystallised to enantiomeric purity. As this also offered the possibility that more of our intermediates would be crystalline we adopted this epoxide for further work. Recently it was reported that the simple benzyl epoxide 418 could itself be enriched to greater than 97% ee by recrystallisation at low temperature.439

For exploratory work we used racemic epoxide 428. The vanadium catalysed epoxidation noted by Chong and Wong and recently detailed by Page et al.440 seemed unnecessarily complicated and we simply epoxidised the allylic alcohol 427 with m-chloroperbenzoic acid in chloroform to give 428 in 96% yield (Scheme 4.10).

Scheme 4.10 Preparation of the Racemic Epoxy Alcohol 428
CHAPTER FOUR

4.5 Oxidation to an Epoxy Acid

The incorporation of the four carbon aziridine 419 into our mitomycin synthesis required oxidation, a two carbon extension to a (protected) six carbon aziridino β-keto ester 421 (Scheme 4.8) and then coupling to a suitable aniline. Oxidation of the aziridino alcohol 419 should furnish an acid which can be extended as in the model sequence (Chapter 3). Coupling of this unit to the anilines used in the model sequence would require hydrogenolysis to release the free hydroxyl and suitable activation. Thus we required a method for the oxidation of alcohol 419 to aziridino acid 420. As a model for the aziridine and recognising the interest in epoxy analogues, the oxidation of the epoxy alcohol 418 to epoxy acid 429 was investigated.

CONVENTIONAL OXIDANTS

The preparation of the acid 429 by ‘ruthenium tetroxide’ (i.e. sodium periodate with catalytic ruthenium trichloride) oxidation had been perfunctorily noted by Scolastico et al. (Scheme 4.11).441

\[
\begin{align*}
\text{OH} & \quad \text{RuO}_4^+ \\
\text{OBn} & \quad \text{OBn} \\
418 & \quad 429
\end{align*}
\]

\[
\begin{align*}
\text{H} & \quad \text{OCOPh} \\
\text{OBn} & \quad \text{OBn} \\
430 & \quad 431
\end{align*}
\]

Scheme 4.11 Preparation of the Epoxy Acid 429

Scolastico kindly forwarded us experimental details reporting contamination by the intermediate aldehyde 430 and a 50% yield of unstable acid 429. Ruthenium tetroxide has been the favoured reagent for the oxidation of epoxy alcohols to acids (and a number of other oxidations) since Sharpless et al. reported significant improvements using acetonitrile as an added cosolvent.442 A further improvement in the general procedure involving the use of periodic acid rather than sodium periodate, was
subsequently reported. Using this modified procedure to oxidise the alcohol (Scheme 4.11), we obtained a crude product mixture in 88% yield. This consisted of the desired acid (ca 70%), intermediate aldehyde (ca 16%), benzoate acid (ca 4%) and traces of benzaldehyde and another aldehyde (presumably the benzoate) by NMR. Evidently there is competitive oxidation of the benzylic methylene and the alcohol, with another minor process leading to cleavage of the benzyl group; the results are consistent with complete consumption of oxidant (2.5 equivalents of periodate).

When the bromobenzyl epoxide was oxidised under the same conditions a similar mixture was obtained (Scheme 4.12), containing somewhat more 4-bromobenzaldehyde and noticeably more benzoate. Approximately 60% of the product was the desired acid and 25% was the benzoate by NMR. Simultaneously changing the ruthenium source to ruthenium dioxide and cooling to 0°C reduced the amount of benzoate only marginally (to ca 23%). While the neutral aldehydes could easily be removed, the two acids were not readily separable.

The oxidation of benzyl ethers with ruthenium tetroxide is preceded, although reportedly slower than the oxidation of epoxy alcohols. It should be possible to optimise the reaction by adjusting the amount of oxidant, temperature and the time and perhaps recycling the aldehyde. First, however, we investigated other oxidants reported to be compatible with acid sensitive substrates, in an effort to avoid benzylic oxidation. Bonini and Fabio have subsequently recommended the addition of sodium hydrogen carbonate in the preparation of a silyl protected analogue of 432, but we have not tried this. There is a plethora of methods for the oxidation of alcohols to acids in one step, although ruthenium tetroxide is the favoured reagent for epoxy.
Pyridinium dichromate has been reported to oxidise primary alcohols to aldehydes in dichloromethane solution, but to acids in dimethylformamide. The epoxy alcohol 428 reacted extremely slowly, a problem that has been observed by others with this oxidant. Reaction in the presence of powdered molecular sieves was faster, and gave relatively little benzylic oxidation, but the isolated yield of acid 432 was only 40%. Addition of anhydrous acetic acid as well has been recommended for the preparation of ketones with pyridinium dichromate in dichloromethane, but we found that oxidation of the epoxy alcohol 428 in dimethylformamide with added acetic acid gave only aldehyde 433 (21%).

Jones reagent in acetone at low temperature has been used to oxidise a primary alcohol in the presence of a tetrahydropyranyl (or TBDMS) group in high yield. We were aware of a potential problem with Jones oxidation giving epimerisation (leading to the trans-epoxy acid) in the reaction of another epoxy alcohol. Reaction of epoxide 428 at -20°C was reasonably clean although fairly slow, with no epimerisation or significant ring opening evident, but with significant benzylic oxidation. Subsequently Williams et al. have reported the surprisingly selective oxidation of a triol with Jones’s reagent in THF at 0°C, apparently without affecting a benzyl group.

Oxidation over Platinum Black

While this work was in progress, the oxidation of the trans analogue of 428 was reported to proceed in “82% yield” using platinum and oxygen. Rich kindly furnished us with full experimental conditions, slightly different from the reported synopsis (67% crude yield). Nevertheless we found treatment of the cis-bromobenzyllic alcohol 428 under these conditions gave pure epoxy acid 432 in 96% yield and residual starting material (3%) with no detectable epoxy aldehyde 433 and only traces of benzylic oxidation (Scheme 4.13). Thus other plausible oxidants such as alkaline permanganate were not pursued.

The doublet coupling of 4.6 Hz for H2 indicated cis stereochemistry had been
Electron impact mass spectra for most compounds in this series were uninformative being dominated by ions from benzyl cleavage (m/z 169/171), but the acid 432 showed a significant molecular ion.

A clean and efficient route to the epoxy acid 432 was available, if slow (the reaction takes one week). Attempts to increase the rate of reaction by heating or using ultrasound were not successful, perhaps due to reduced gas solubility. The epoxy acid 432 was smoothly converted to the methyl ester 435 in 95% yield on treatment with diazomethane (Scheme 4.13).

Scheme 4.13 Preparation of the Epoxy Ester 435

4.6 Attempted Extension of the Epoxy Acid

With an efficient synthesis of the epoxy acid 432 we attempted to transform it into β-keto ester 437 using the methodology of Brooks et al. that was successfully applied in the last chapter (see Scheme 3.7, p121). However, this generally gave products with broadened NMR spectra which were not consistent with 437a. Mass spectra were also uninformative being dominated by ions from benzyl cleavage (m/z 169/171). Treatment of the methyl ester 435 with the anion of methyl acetate gave a similar outcome. Oikawa et al. have reported that acid chlorides could be condensed with Meldrum’s acid (436) and the products converted to β-keto esters by heating in alcohol (Scheme 4.14).458

Scheme 4.14 Preparation of β-Keto Esters using Meldrum’s Acid
Coupling of the epoxy acid 432 with Meldrums acid (436) using carbonyl diimidazole activation did not give a clean product.459 Similarly, diethyl phosphorocyanidate and triethylamine460 failed to cleanly couple the epoxy acid 432 and Meldrum’s acid (436). Refluxing the crude product in methanol did not appear to give any β-keto ester 437a by NMR.

It appeared that the epoxy β-keto esters 437 might be unstable under the conditions of formation. We have not been able to obtain an online search for the relevant substructure but it appears that γ,δ-epoxy β-keto esters have never been reported. Jamie and Rickards have subsequently made the epoxy β-keto ester 438 in these laboratories by coupling a bromoacetoacetate with a benzaldehyde (Scheme 4.15).249 The yield is low (11% optimised) and the methodology is not suitable for the preparation of chiral epoxides. The epoxide 438 decomposed on preparative thin layer chromatography.

An obvious alternative option is to chain extend the epoxy aldehyde 433 rather than the epoxy acid 432. Attack on the aldehyde by a suitable two carbon anion would give an alcohol which could in principle be oxidised to the β-keto ester 437. We thus turned our attention to the isolated bromobenzyl protected aldehyde 433. Indeed
Escudier et al. have since reported the preparation of the β-hydroxy ester 439 from the aldehyde 430 (Scheme 4.16).}

\[
\begin{align*}
\text{H} & \quad \text{O} \\
\text{OBn} & \quad \text{O} \\
\text{430} & \quad \text{O} \\
\end{align*}
\]

\[
\begin{align*}
\text{O} & \quad \text{O} \\
\text{OBn} & \quad \text{O} \\
\text{439} & \quad \text{O} \\
\end{align*}
\]

Scheme 4.16 Synthesis of an Epoxy β-Hydroxy Ester

4.7 Preparation of the Epoxy Aldehyde

The aldehyde 433 was also of interest for use in a reductive coupling with anilines and as a model for two step oxidations to carboxylic acids (see below). The oxidation of epoxy alcohols to epoxy aldehydes is well explored, many methods have been adopted and some problems noted. The preparation of aziridino aldehydes, by contrast, is almost unprecedented. The most commonly used procedures for epoxides are the “Swern” oxidation, pyridinium dichromate in dichloromethane and the related “Collins” oxidation (chromium trioxide and pyridine). The usual Swern conditions (i.e. oxalyl chloride, DMSO and triethylamine) can cause problems with epoxy alcohol oxidations;\(^{462}\) tetra-n-propylammonium per-ruthenate has recently been advocated as a significantly better oxidant for the preparation of several epoxy aldehydes.\(^{463}\) The Collins oxidation has also been used in preference\(^{464}\) but requires a large excess of reagent.

The benzyl protected epoxy aldehyde 430 has been utilised in literature syntheses a number of times but details of its preparation have not been reported. Behrens and Sharpless prepared 430 with pyridinium chlorochromate in 71% yield and had difficulty converting it to the methyl acetal under a range of conditions (maximum 42% yield),\(^{465}\) whereas Furukawa et al. later reported a sequential Swern oxidation and ethyl acetalisation (Amberlyst and triethyl formate) as proceeding in 76% overall yield.
(no further details). Other reports noted preparation of 430 by Swern oxidation (no details), pyridinium dichromate in dichloromethane ("reagent of choice", no details), sulfur trioxide-pyridine-DMSO-triethylamine ("reagent of choice", no details), or do not comment on the preparation. The bromobenzyl protected aldehyde 433 has not been reported. Nicolaou et al. have reported full details of the preparation of the benzyl protected trans-epoxy aldehyde by Swern oxidation (98% crude).

The reaction of the bromobenzyl protected epoxy alcohol 428 with pyridinium dichromate in dichloromethane and molecular sieves was rapid and operationally simple (Scheme 4.17). The crude product contained the desired aldehyde 433, significant amounts of the bis-epoxy ester 440, traces of 4-bromobenzaldehyde and other minor impurities. It was difficult to quantify proportions by NMR due to the overlap of comparable resonances of the aldehyde and ester. Short path distillation did not significantly affect the composition but pure, somewhat unstable aldehyde could be obtained in 60% yield by careful silica chromatography, although the ester tended to co-elute. Reaction at 0°C gave a similar mixture and yield.

### Scheme 4.17 Preparation of the Epoxy Aldehyde 433

Recently the NMR spectra of the benzyl aldehyde 430, prepared in "high yield" by sulfur trioxide-pyridine oxidation were reported. The non-aromatic resonances (1H and 13C) match those we observed for the bromobenzyl aldehyde 433. The diastereomeric mixture of esters 440 (8%) was isolated with no apparent separation.
The infrared and $^{13}$C absorptions (1752 cm$^{-1}$ and 167.4 ppm) clearly indicated an ester. The $^1$H NMR was very complex, although grossly consistent with the assignment. The $^{13}$C NMR was more informative, resonances of the two diastereomers being distinguished for several carbons. These $^{13}$C intensities suggested that an approximately equal mixture of diastereomers was generated.

Oxidation of the epoxy alcohol 428 with sulfur trioxide-pyridine complex in DMSO was problematic. A crude product (89%) was prepared which contained no 4-bromobenzaldehyde and little ester 440 but a significant proportion of an unidentified byproduct. We found that the order of addition of reagents is important. In a brief investigation we observed oxidation only when a DMSO solution of sulfur trioxide-pyridine was added to a solution of the alcohol and triethylamine, the converse of Baker and Castro’s 473 observation that solid sulfur trioxide-pyridine must be added last in the preparation of another aldehyde. Variations on our procedure gave what appeared to be the sulfate of the alcohol or no reaction. We had obtained sufficient aldehyde 433 for use in various reactions and have not pursued further improvements in its preparation.

4.8 Oxidation of the Epoxy Aldehyde to an Acid

The further oxidation of epoxy aldehydes to epoxy acids is poorly preceded. With the efficient one step process using platinum black available, such a step was not directly useful, especially given the moderate yield of aldehyde obtained. However, as a model study for the preparation of the corresponding aziridino acid (see below) the oxidation of the epoxy aldehyde 433 was investigated. Oxidation with sodium chlorite proceeded efficiently. This mild oxidation procedure, reported by Lindgren and Nilsson, 474 is believed to occur by chlorite attack on protonated aldehyde; the hypochlorous acid that is generated causes side reactions unless it is removed. 475 Oxidation of 433 in buffered aqueous THF with resorcinol as a hypochlorous acid scavenger gave an almost quantitative yield of epoxy acid 432, contaminated with a small amount of phenolic byproducts after extractive workup. There was no evidence
of benzylic oxidation. Alternatively oxidation of crude aldehyde with bromine in methanol\(^{476,477}\) directly gave the methyl ester \(435\) in 63\% yield.

### 4.9 Extension of the Epoxy Aldehyde

With the aldehyde \(433\) in hand we considered a two step, addition and oxidation protocol to generate the epoxy \(\beta\)-keto ester but were attracted by a different reaction. Holmquist and Roskamp had reported a direct method for the conversion of aldehydes to \(\beta\)-keto esters. Treatment of various aldehydes with ethyl diazoacetate and catalytic stannous chloride gave ethyl \(\beta\)-keto esters in good yields.\(^{478,479}\) This utilises a higher oxidation level in the two carbon fragment to provide oxidation of the aldehyde carbon by \textit{in situ} rearrangement (Scheme 4.18), perhaps \textit{via} a carbene. The conditions are mild and simple so we investigated their application to the epoxy aldehyde \(433\).

![Scheme 4.18 Conversion of an Aldehyde to a \(\beta\)-Keto Ester](image)

The NMR spectrum of the crude, filtered reaction product was fairly clean, apparently consisting largely of \(437b\) and its enol. Attempted chromatography led to partial decomposition but we were able to obtain a pure sample by extraction with sodium hydroxide and reacidification, albeit with low recovery. The NMR spectrum showed a significant enol content (\textit{ca} 16\%), apparent from the enolic hydroxyl (\(\delta\) 12.1) and the benzylic protons which were split as an AB quartet for the enol (\(\delta\) 4.51) but which were isochronous in the major ketone (\(\delta\) 4.45). Unfortunately, the more interesting aliphatic resonances all overlap in the range \(\delta\) 3.8 - 3.4. Electron impact mass spectrometry was not informative but NICIMS showed a strong M-H cluster. The sample slowly decomposed on storage.

In an attempt to prepare \(437b\) on a larger scale we observed that the bulk of
material exposed to base was not base soluble and resembled the impurity formed on chromatography. The crude decomposition product consisted of two components which could be separated by chromatography. Both components were very similar spectroscopically (UV, IR, NMR, MS) and the UV absorption at \( \lambda \) 253 nm together with the very strong IR absorption at 1576 cm\(^{-1}\) observed for both suggested that they were 3(2H)-furanone isomers of the desired \( \beta \)-keto ester 441 (Scheme 4.19).\textsuperscript{480,481,482,483,484,485} Observation of NMR resonances at \( \delta \) 4.82 and 4.80 was consistent with H4 in such a structure.\textsuperscript{484} The cyclisation of \( \beta \)-keto esters with \( \gamma \)-leaving groups has been used to prepare furanones.\textsuperscript{481,482,486} We assume the two products are diastereomers arising from epimerisation at C2, but we have not assigned relative stereochemistry.

![Scheme 4.19 Decomposition of the \( \beta \)-Keto Ester 437b.](image)

Thus the epoxy \( \beta \)-keto ester 437b is unstable and isomerises. The crude products from the attempted extensions of the epoxy acid do not all show the presence of these furanones, suggesting other modes of decomposition occur as well. The epoxy \( \beta \)-keto ester 437b is clearly unsuitable for use as a synthetic intermediate. However, corresponding aziridino \( \beta \)-keto esters are apparently more stable; Shaw \textit{et al.} have prepared the aziridino \( \beta \)-keto ester 442 by nucleophilic attack on an ester without difficulty.\textsuperscript{219}
4.10 Synthesis of the Aziridine

It was possible to open the epoxide 428 with sodium azide and ammonium chloride in aqueous 2-methoxyethanol.\(^\text{487}\) This gave azidodiols with little if any regioselectivity, as shown by integration of the secondary hydroxyl NMR resonances. Reaction of the silylated epoxide 443 with sodium azide and magnesium perchlorate in methanol gave a similar mixture of azidoalcohols 444 in 82% yield with some unidentified byproducts, possibly the diol, but without significant desilylation (sodium azide and ammonium chloride in aqueous 2-methoxyethanol\(^\text{487}\) gave less reliable results). This route was preferable as it avoided the need for selective silylation. While it may be possible to alter the regioselectivity of the epoxide opening, it is not necessary here as both isomers should lead to the same aziridine.

Conversion of the hydroxy azides into aziridines was problematic. After one successful, small scale reaction of 444 which gave the desired aziridine 445 in 81% yield (Scheme 4.20), numerous further attempts gave typically low (6-36%), variable yields of 445 together with the amino alcohols 449. Chromatographic removal of triphenyl phosphine oxide from the aziridine was tedious and crystallisation did not remove it efficiently. The usual byproduct was a regioisomeric mixture of amino alcohols. Varying the solvent and the phosphine reagent did not solve the problem. Similar difficulties have been reported by others\(^\text{488}\) and Tanner informs us that he obtains lower yields for cis systems.\(^\text{489}\)

Subsequently we discovered that use of polymer bound triphenylphosphine gave a reproducible yield of 87% in the reaction of the azido alcohols. This reagent has the further significant advantage that the derived phosphine oxide can be simply filtered off. The reasons for the difference in reactivity are not obvious.

![Scheme 4.20 Preparation of the Aziridine](image)
The NMR spectrum of 445 was not particularly informative due to pseudosymmetry in the molecule, but the aziridine protons (overlapping at δ 2.3) and carbons (δ 33 and 35) were in the expected range. Cleavage of the silyl ether gave the polar bromobenzyl aziridine 446 (see Section 4.12), the benzyl analogue of which, 419, had been isolated by Cardillo et al. as a dominating byproduct in the preparation of dihydro-oxazole 447 (Scheme 4.21). The chemical shift ranges reported for the non-aromatic protons of 419 were consistent with those we observed for 446. More critically, the non-aromatic 13C resonances also matched (± 0.5 ppm, benzylic -0.8 ppm).

Scheme 4.21 Literature Precedent for the Aziridine

The EIMS of 445 was consistent with the structure, with a weak M+1 cluster at m/z 386/8 arising from self protonation (essentially self-Cl). The EIMS of these bromobenzyl derivatives was almost always uninformative with m/z 169/71 (C7H6Br) the highest mass ions with significant intensity. The noteworthy ions above m/z 169/71 were m/z 328/30, corresponding to the loss of a C4H9 radical at silicon, and m/z 284/6 (M-101). This second cluster cannot arise from any simple cleavage and its identity was unclear. The same loss was observed with the simple (unbrominated) benzyl aziridine (m/z 206, M-101), but not with the corresponding epoxide. HRMS of 445 confirmed the expected composition of the protonated molecular ion, C17H29NO279BrSi (M+H ±1 ppm), and gave a composition for m/z 284 of C11H15NO79BrSi (±3 ppm), identifying a net loss of C6H13O from the molecular ion. If an initial loss of C4H9 is postulated then the subsequent loss of C2H4O must take place by rearrangement. Interestingly the same fragment ions m/z 284/6 are seen in the EIMS of the derived t-butyl carbamate protected aziridine 448 (which may well decompose to 445 by loss of CO2 and C4H8 in
the spectrometer) and HRMS showed the same composition, \( \text{C}_{11}\text{H}_{15}\text{NO}^{79}\text{BrSi} (\pm 2 \text{ ppm}) \). The mechanism for the rearrangement is not clear.

The positive chemical ionisation (NH\(_3\)) mass spectrum of 445 showed a strong M+1 cluster as well as the \( m/z \) 284/6 ions. The CIMS base peak (above \( m/z \) 100) was, however, \( m/z \) 556 corresponding to M+C\(_7\)H\(_6\)Br; thus an intermolecular ‘benzyl’ transfer had occurred in the spectrometer, perhaps thermally. Another process, debromination, was suggested by \( m/z \) 306 (M-Br, 1%) and 308 (M-Br+H+H, 10%) (high resolution CIMS was not available); the CIMS of aryl bromides is little studied, but these are different from the fragmentation observed (methane CI) for 1-bromo-4-methylbenzene\(^{492}\) or 4-bromobenzenamine\(^{493}\). In the negative ion chemical ionisation (NH\(_3\)) spectrum the base peak (above \( m/z \) 200) was \( m/z \) 466, compatible (with the associated isotope peaks) with addition of bromine to the molecular ion; the only other significant peaks were a three-bromine cluster \( m/z \) 544/6/8/550 presumably M+Br+HBr and an unassigned cluster \( m/z \) 510/12/14. This sample derived bromide flux in the CIMS source is interesting and was generally observed with the series of bromobenzyl protected compounds. Although complex, mass spectrometry supported the assigned aziridine structure 445.

The choice of protecting group for the aziridine was not obvious, indeed this has been a significant problem in mitomycin synthesis.\(^{58}\) For convenience in exploring further chemistry we used the \( t \)-butyl carbamate (BOC) group, recognising that its ultimate removal might not be possible without opening the aziridine ring (but note that acidic detritylation of aziridines is preceded\(^{494}\)). It was readily installed by reaction of 445 with di-\( t \)-butyl dicarbonate in 85% yield (Scheme 4.22), and provided a stable protecting group in most of the subsequent reactions without noticeably activating the aziridine to nucleophilic opening. No molecular ion was observed in the EIMS of 448 but the fragmentation was sensible, the only intense ion above \( m/z \) 169/171 being \( m/z \) 284/6 (M-201). The origin of this cluster was discussed above in relation to the MS of 445. HRMS of 448 confirmed that here they had the same composition, arising by net loss of \( \text{C}_{11}\text{H}_{21}\text{O}_3 \) (not by loss of \( \text{C}_4\text{H}_8 \) and \( \text{CH}_2\text{OSiMe}_2\text{Bu} \}). This presumably involves generation of the free aziridine 445 by loss of \( \text{C}_4\text{H}_8 \) and \( \text{CO}_2 \) (M-100) then
rearrangement as before. HRMS also showed that the very small peaks at m/z 384/6 (M-101) were not formed directly by such a rearrangement of the protected aziridine itself, but by loss of CO$_2$C$_4$H$_9$. NICIMS of 445 showed bromide addition again (m/z 564/6/8, M+Br) and the subsequent loss of 2-methylpropene giving the base peak m/z 508/10/12, M+Br-C$_4$H$_8$.

![Scheme 4.22 Protection of the Aziridine 445](image)

### 4.11 An Alternative Aziridine Cyclisation

When problems were initially experienced with the azide route, other methods for the conversion of epoxides to aziridines were considered. The ‘Mitsunobu-like’ cyclisation of amino alcohols$^{495}$ or acylated amino alcohols was attractive because the starting material was available from the epoxide in one step. Heating the epoxide 443 with ammonia in methanol yielded the desired amino alcohol mixture 449 in 77% yield (Scheme 4.23). Treatment of this with triphenylphosphine and diethylazodicarboxylate (DEAD) gave a mixture which appeared to contain the desired aziridine 445 by NMR, but separation of the polar aziridine from hydrazine and phosphine oxide byproducts was difficult. The corresponding mixture of t-butyl carbamates 450 was prepared from the amino alcohols 449 by treatment with di-t-butyl dicarbonate in methanol. Treatment of this regioisomeric mixture with DEAD and triphenylphosphine in dilute THF solution gave 68% of the protected aziridine 448 (Scheme 4.23).
From a practical standpoint, other methods for the preparation of the aziridine on a large scale were considered preferable because of the difficulty of separating triphenylphosphine oxide, hydrazine and unreacted reagents from the product. The aminoalcohol cyclisation was not pursued after the discovery of polymer bound triphenylphosphine for the azidoalcohol reaction (see above).

4.12 Oxidation of the Aziridine

With the aziridine formed we investigated oxidising the silyl-protected alcohol. Cleavage of the silyl ether with tetra-n-butyl ammonium fluoride proceeded smoothly and gave the alcohol in 88% yield. A number of attempts to oxidise the protected aziridino alcohol to the corresponding acid, under the same conditions used for the epoxide (O₂, Pt, NaHCO₃, ethyl acetate, water, see Scheme 4.13), resulted in very little reaction; starting material was recovered efficiently with traces of aldehyde in the neutral fraction.

The cause of this difference in reactivity was not clear. There are examples of primary hydroxyl groups in amino-sugars being selectively oxidised under such
catalytic oxidation conditions both with and without amino protection.\textsuperscript{496} We are not aware of any aziridines having been subjected to these conditions. It is not known in which phase the oxidation occurs but we speculated that the difference in water solubility between 428 and 451 might be the cause. We found that the oxidation of the epoxide 428 proceeded in neat ethyl acetate, giving approximately 50\% acid 4.32 and 10\% aldehyde 4.33 (by NMR) after eight days. It is possible that the acid inhibits further oxidation as is known to occur in neutral aqueous solution.\textsuperscript{496} Reaction in basic homogeneous solution (NaHCO\textsubscript{3}, THF, acetone and water) gave, however, a complex mixture. Reaction of the aziridino alcohol 451 in neat ethyl acetate for ten days gave little reaction with only traces of the aldehyde 452 formed. A concurrent reaction in THF-heptane gave a more complex mixture but little of the desired acid 453 by NMR.

Cleavage of the silyl group in the unprotected aziridine 445 with tetra-n-butylammonium fluoride gave the polar alcohol 446. Chromatography gave a product contaminated with tetrabutylammonium salts. Fortunately treatment with aqueous sodium perchlorate\textsuperscript{497} precipitated these and the alcohol was isolated in 71\% yield. Oxidation of this aziridine 446 for seven days under similar conditions to those used with the epoxide (except that the alcohol concentration was approximately one tenth) gave a neutral fraction containing starting material (72\%) with no aldehyde apparent by NMR. To isolate the amino acid the aqueous part was treated with methanol and di-t-butyl dicarbonate and acidified. Extraction with dichloromethane gave the protected aziridine acid 453 contaminated with acetic acid (from ethyl acetate hydrolysis). This acid was identical by NMR with that produced by oxidation of the aldehyde (see below). Unfortunately, the sample decomposed significantly during the running of a \textsuperscript{13}C NMR spectrum. Some reasonably pure material was recovered by extraction but the mass spectrum was dominated by protecting group cleavage with ions
of m/z 57 (C₄H₉) and 169/71 (C₇H₆Br) and no significant ions of high mass. Thus aqueous solubility does seem to enhance oxidation but not to useful levels here.

The instability of acid 453 was not entirely surprising as the t-butyl carbonate and aziridine ring are acid sensitive. The compound appears to be stable as the sodium salt. It is interesting that the putative intermediate aldehyde⁴⁹⁶ survives long enough to be selectively oxidised. This oxidation has not been further investigated, but the aqueous solubility of the substrate appears to be important in determining the success of catalytic oxidation over platinum.

Thus we were forced to investigate alternative routes for oxidation of the aziridine. Because of the difficulties experienced with clean preparation of the epoxy aldehyde 433, we were interested in reports of oxidation under very mild conditions using 12-I-5 triacetoxyperiodinane.⁴⁹⁸ Indeed this reagent oxidised the aziridino alcohol 451 very simply and smoothly to the aldehyde 452 in 84% yield (Scheme 4.24). Oxidation of the aldehyde with sodium chlorite gave what appeared to be the acid 453 by NMR but which again decomposed rapidly.

![Scheme 4.24 Preparation of an Aziridine Acid via the Aldehyde](image)

**Scheme 4.24 Preparation of an Aziridine Acid via the Aldehyde**

### 4.13 An Alternative Approach to Aziridino Esters

During the course of our work Zwanenburg et al. reported the transformation of *trans*-epoxy esters into aziridines.⁴⁹⁹ We had originally avoided such a route to the *cis*-aziridine because of concern about the compatibility of the ester in such aziridine synthesis and the risk of epimerisation of the azide intermediate (notably the 2-azido isomer if formed). Subsequently, significant epimerisation has been independently
observed under mildly acidic conditions with 2-azido-3-hydroxysuccinates prepared from bromides\textsuperscript{500} and epoxy succinates\textsuperscript{501} but has not been observed in a range of monoesters.\textsuperscript{499,502}

Nevertheless, with an efficient synthesis only of the epoxy acid 432, this path seemed worth trying. The advantage of such a route in our synthesis is that only monoprotection of the butane diol moiety is required (the ester serving as ‘protection’ for the aziridine formation step) allowing more flexibility in protection choice.

Treatment of the epoxy ester 435 with sodium azide and ammonium chloride in methanol gave a mixture of products, predominantly two azido alcohols presumed to be regioisomers 454\textsuperscript{a} and 454\textsuperscript{b} in 92\% total yield (Scheme 4.25). Starting material was also isolated and was still present after prolonged reaction suggesting that the epoxide opening may be reversible. Little regioselection was evident (the isomers are clearly distinguishable by NMR, most notably the hydroxyl doublets are well separated: $\delta$ 2.5 and 3.0). The coupling of the hydroxyl protons led to their assignment as 2- and 3-azido isomers respectively 454\textsuperscript{a} and 454\textsuperscript{b}. A third, minor component was apparent from a doublet at $\delta$ 2.7.

Treatment of the mixture of azido alcohols 454 with polymer bound triphenylphosphine in refluxing toluene gave a low yield of the desired aziridino ester 455 (see below), 4-bromobenzylalcohol, and other products. The mechanism of formation of benzyl alcohol is not clear, conceivably a competitive Grob fragmentation is occurring.\textsuperscript{503}
However, reaction with triphenylphosphine in acetonitrile or DMF at 90°C (the conditions of Zwanenburg et al.\textsuperscript{499}) cleanly gave the aziridino ester \textit{455} in 79% yield. The NMR spectrum was broadened with resonances at δ 2.8 and 2.6, consistent with an aziridine moiety. \textsuperscript{13}C resonances were observed at δ 36.6 and 33.2 which is in the range expected for aziridine carbons. The sharpness of the peaks in the \textsuperscript{1}H NMR varied between samples. At best the methine resonances were observed as a broad doublet (δ 2.8, J 6 Hz) and a broad pseudo-quartet (δ 2.6, J 6 Hz). A 6 Hz coupling is consistent with a \textit{cis} stereochemistry.\textsuperscript{504}

However, in some samples of \textit{455}, apparently identical and pure by TLC, NMR showed three broad resonances in this region (δ 2.9, 2.7 and 2.55) and more distinctive resonances in the range δ 1.6 to 0.8 (otherwise very broad). Filtration or mild warming (50°C) did not significantly affect these spectra. The addition of 3Å sieves, however, resulted in a dramatic change over 30 hours. Well resolved multiplets were now seen at δ 2.9, 2.7 and 2.55 and two triplets were apparent at high field in a ratio of about 1:2. The \textsuperscript{13}C NMR showed duplication of all resonances (except carbonyl resonances which were too weak to observe) with shifts on either side of those previously observed, most different for the aziridine carbons. Thus the addition of sieves had slowed an isomerisation or exchange process and frozen out two forms. The variation in spectra observed earlier presumably arose from differences in the interconversion rate in those
samples. Although molecular sieves are well known to remove water, this did not seem to be the cause of the change because variation between earlier spectra did not correlate with water content (δ ca 1.5). Thus we speculate that variable acid content was causing variable nitrogen inversion rates. Cooling to -50°C resulted in even better resolution. Han and Kohn have recently established the existence of very stable NH invertomers in the aziridino mitosene 90 (p51), but did not observe them with other aziridines examined.73 Martino et al. have observed slow nitrogen inversion with some aziridines in acid free chloroform.505 It is not clear what structural factors affect the inversion barrier.

The azido alcohol mixture 454 could be partially separated and when the more polar part, containing the minor component 456, was treated under the same conditions a less polar byproduct was formed as well. The amount of byproduct was proportional to the amount of the minor component. The byproduct was identified as the trans-aziridine 457. NMR, IR and MS data were very similar to those of the major product. When the NMR solution was percolated through potassium carbonate the amine proton was resolved as a triplet (J 7Hz) and H2 as a doublet of doublets (J 7 and 2.5 Hz) consistent with trans stereochemistry,504 but no exchange process was observed. Formation of the trans-aziridine is consistent with the minor azido alcohol 456 arising by epimerisation.
4.14 A Note on the Mass Spectra of the Aziridines and Epoxides

The base peak in the MS of both aziridino esters 455 and 457 was m/z 56; this was reproducibly the dominant ion in the spectra (above the low mass cut-off of m/z 50). It was not immediately clear how such an ion arose. The composition was determined by HRMS for the trans-aziridine 457 as C$_3$H$_6$N (± 6 ppm), suggesting loss of carbomethoxy and benzyloxy groups and hydrogen transfer. An intense m/z 56 ion was also observed in the spectra of the aziridines 446 and 445 suggesting that the fragmentation process is general and insensitive to the functionality at C1 which must be lost. HRMS of the aziridino alcohol 446 also indicated a composition of C$_3$H$_6$N (± 11 ppm; other CHNO compositions differ by more than 200 ppm). The BOC protected aziridines 451 and 448 gave only weak m/z 56 ions, variable but weak m/z 156 ions (cf. C$_3$H$_5$NCO$_2$Bu) and intense m/z 57 ions, presumably C$_4$H$_9$ derived from the BOC group (and perhaps the TBDMS for 448). Selected data is summarised in Table 4.1.

<table>
<thead>
<tr>
<th>m/z</th>
<th>OH</th>
<th>OTBDMS</th>
<th>OMe</th>
<th>OTBDMS</th>
<th>OMe</th>
<th>OH</th>
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<tr>
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<td>1.3*</td>
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</tbody>
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BnBr = \(-\text{CH}_2\text{\raisebox{-1.5pt}{\(\cond\)}}\text{-Br}\) \quad Bn = \(-\text{CH}_2\text{\raisebox{-1.5pt}{\(\cond\)}}\)

* M-ArCHO = m/z 187

Table 4.1. Intensities (% of base peak) of Selected Fragment Ions in EIMS of Various Aziridines

-184-
Intriguingly, the mass spectra of some analogous epoxides showed strong m/z 57 ions (see Table 4.2). HRMS indicated a composition of C₃H₅O (± 13 ppm), i.e. the oxygen analogue of C₃H₆N, for m/z 57 from the epoxy alcohol 428. This suggests that a parallel fragmentation process can occur in epoxides, although other pathways may again dominate of course (e.g. for the anilide 459). Two samples of the epoxy ester 440 gave very different intensity ratios (see Table 4.2), for reasons that are not clear. The intensity of m/z 57 in MS of the epoxy aldehyde 433 also varied between samples, perhaps due to instability; by GCMS the intensity was 7% (data not shown).

<table>
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<tr>
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<th>435</th>
<th>440</th>
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</tr>
</tbody>
</table>

Table 4.2. Intensities (% of base peak) of Selected Fragment Ions in EIMS of Various Epoxides

The MS of aziridines is relatively little studied. For both aziridines and epoxides α-cleavage is usually a minor process in contrast to acyclic amine and ether
analogues, perhaps because it leads to greater ring strain. We discussed our observations with Porter\textsuperscript{507} who suggested that an “inside” McLafferty rearrangement\textsuperscript{508} may be involved. Thus transfer of a benzylic hydrogen leads to elimination of 4-bromobenzaldehyde with cleavage of the ring (shown for aziridine \textbf{455} in Scheme 4.25). Subsequent $\alpha$-cleavage would afford the C\textsubscript{3}H\textsubscript{6}N or C\textsubscript{3}H\textsubscript{5}0 ion.

Hydrogen abstraction by “inside” McLafferty rearrangement of alkyl epoxide and aziridine radical ions has been proposed by Djerassi \textit{et al.}\textsuperscript{509} and Porter and Spear\textsuperscript{562} respectively. The alternative sequence cannot be excluded, but note that it would start with unfavoured $\alpha$-cleavages of various moieties and involve the rearrangement of a non-radical azirine ion or corresponding oxy-system.

![Scheme 4.26 Postulated Mechanism for Generation of m/z 56](image)

Ions corresponding to the possible intermediates for either sequence were very weak for compounds in the aziridine series. For the epoxy ester \textbf{435}, however, $m/z$ 116 (M-BrPhCHO?) which would be produced by initial aldehyde elimination is significant (32 or 24% with different samples). The corresponding ion $m/z$ 102 (M-BrPhCHO?) is also significant (28%) in the MS of the epoxy acid \textbf{432} although $m/z$ 57 is negligible. Ions associated with 4-bromobenzaldehyde ($m/z$ 186/185/184/183, M and M-1, and 157/155, M-CHO), although ubiquitous in MS of bromobenzylxoy compounds, were much more intense in the MS of these two epoxides. (The ions $m/z$ 187/185 (BrC\textsubscript{6}H\textsubscript{4}CH\textsubscript{2}O) overlap partially but are clearly independent.) Metastable ion information was not available from the digitised spectral data. If the mechanism postulated is generally valid this suggests that $\alpha$-cleavage from the aziridine derived amine radical is more facile than from the corresponding alcohol radical.
Comparison with the few available unsubstituted benzyl analogues is also interesting; \( m/z \) 56 and 57 ions are much less intense (even after allowing for isotope effects in base peaks of the bromobenzyl series) and the base peak is consistently the ‘benzyl’ (probably tropylium) ion, \( m/z \) 91. The corresponding ions \( m/z \) 171/169 are also always present in the bromobenzyl series, and are clearly more intense for the epoxides than for the aziridines. If the postulated mechanism for formation of \( m/z \) 56 and 57 is correct, it evidently competes with simple fission of the ‘benzyl’ ion. Simplistically these processes involve ionisation at different sites. Bromine substitution will affect the energetics of both benzyl fission and benzylic hydrogen transfer, as well as initial ionisation. The difference between the aziridines and epoxides may reflect either their ionisation energies or their propensity to abstract hydrogen via ring cleavage.

Confirmation of the proposed mechanism and teasing out the influence of these factors would require a more detailed mass spectral (e.g. daughter ion) study and examination of labelled and differently substituted benzyl derivatives.

### 4.15 Coupling of the Aziridine and Benzyl Cleavage

We have efficient access to the aziridino ester 455. For the reasons discussed at the end of Chapter 3, extension to an aziridino \( \beta \)-keto ester was now less relevant. In order to increase the flexibility in cyclisation reactions (see next chapter) it seemed sensible to couple the aziridine as a four carbon fragment and then extend it. However it seemed unlikely that we could remove the benzyl ether from the aziridino ester 455 without lactonisation. The amination of such a butyrolactone at C4 was not expected to be compatible with the aziridine ring. Thus coupling of the aziridino alcohol 460 and then oxidation was the desired route.

In fact our trepidation regarding the lactonisation was not entirely warranted as revealed by Jones and Rapoport’s recent utilisation of an aziridine substituted 4-hydroxy ester in an intermolecular Mitsunobu reaction (see below).

One obvious and probably biomimetic coupling is to oxidise the released alcohol to an aldehyde, couple by imine formation and then reduce. We have modelled
this by coupling the epoxy aldehyde 433 to aniline. Condensation in the presence of molecular sieves and reduction with sodium cyanoborohydride gave the alkylaniline 459 in 55% yield (Scheme 4.27).

\[
\begin{align*}
\text{NH}_2 + \text{H} & \xrightarrow{1) \text{3Å sieves}} \xrightarrow{2) \text{NaCNBH}_3} 55\% \\
& \begin{array}{c}
\text{O}\text{BnBr} \\
433 \\
\end{array} \\
& \begin{array}{c}
\text{OH} \\
459 \\
\end{array}
\end{align*}
\]

Scheme 4.27 Reductive Coupling of the Epoxy Aldehyde

From a synthetic perspective this requires an oxidation and a reduction which it would be desirable to avoid. The conventional methodology would be to convert the alcohol to a leaving group and then alkylate the aniline, probably generating some dialkylated product unless an excess of the aniline is used. We considered direct coupling of the alcohol via a Mitsunobu reaction. These reactions are believed to require a nucleophile which is acidic (pK_a < 14), but intramolecular cyclisations of diols and aminoalcohols suggested that this is not a strict requirement.512,514 Nevertheless we were unable to alkylate the aniline 334 with 428, aniline with 424 or acetanilide with 2-phenylethanol. Simultaneously it was becoming clear from the literature that only acidic amines would be synthetically useful for intermolecular reaction.515,516,517 Koppel et al. have specifically correlated the yields of such aminations with the acidity of the amine.518 High yields are only obtained with sulfonamides; unfortunately the cleavage of sulfonamides generally requires forcing conditions. Jones and Rapoport used a Mitsunobu coupling in an approach to the synthesis of FR-900482 (375) (Scheme 4.28); the cleavage of the tosyl group was not addressed.519 Note that Ban et al. have achieved concomitant detosylation and cyclisation in their approach to the mitomycins (Section 2.3iv). It is possible that 2-(trimethylsilyl)ethanesulfonate would be a useful protecting group.
Coupling of an aniline to the aziridine fragment requires cleavage of the benzyl group. The relative reductive lability of the aziridine and benzyl group were not clear. An attempt to hydrogenate the silylated epoxide in ethanol gave only very polar products. Hydrogenation of the protected aziridine over palladium on charcoal in methanol gave a complex mixture. The NMR spectrum of the crude product suggested desilylation, ring opening (from the absence of signals in the range δ 2 to 3) and the continued presence of benzyl and bromobenzyl residues. The removal of silyl ethers by hydrogenation in methanol has recently been advocated as a useful deprotection method.

Hydrogenation of the unprotected aziridine in ethyl acetate in the presence of sodium hydrogen carbonate did not give observable desilylation but ring opening was again apparent; the benzyl group was merely debrominated. Similar results were obtained on catalytic transfer hydrogenation of using palladium black, triethylamine and formic acid or ammonium formate. Reduction over platinum reduced the benzene ring without cleavage. Aziridines have reportedly been cleaved by sodium in ammonia, but only aryl-substituted systems were studied. We found reduction of with lithium in ammonia gave the polar, debenzylated alcohol in 45% yield (Scheme 4.29). The aziridine ring appeared to be intact on the basis of a two proton multiplet in the NMR spectrum at δ 2.44. Acetylation appeared to give the acetylaziridine (multiplet at δ 2.80) but this was unstable to chromatography.
Before attempting to optimise the preparation of 460 we wanted to have a suitable aziridine available for coupling. The choice of an aniline (AHB equivalent) to couple with the aziridine affects the subsequent cyclisation reaction. Options suitable for exploration of revised biomimetic cyclisations and synthetic goals are discussed in the next chapter.
CHAPTER FIVE: NEW MODELS FOR C8aC9 BOND FORMATION

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5.2 Biomimetic Models for C8aC9 Bond Formation ......................... 193
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5.3 Conclusion .................................................................................... 202

Scheme 5.1. Biosynthesis of the Mitomycins

The biosynthesis of the mitomycins by a C8/C9 bond is achieved
by Scheme 5.1, which involves a C8-C9 bond. This bond is
formed by the isomerization of a C9-C11 bond. The isomerization
of a C9-C11 bond is achieved by the isomerization of a C8-C9 bond.
Scheme 5.1 is followed by a C8-C9 bond isomerization. This bond is
formed by the isomerization of a C8-C9 bond. The isomerization
of a C8-C9 bond is achieved by the isomerization of a C8-C9 bond.
Scheme 5.1 is followed by a C8-C9 bond isomerization. This bond is
formed by the isomerization of a C8-C9 bond. The isomerization
of a C8-C9 bond is achieved by the isomerization of a C8-C9 bond.
Scheme 5.1 is followed by a C8-C9 bond isomerization. This bond is
formed by the isomerization of a C8-C9 bond. The isomerization
of a C8-C9 bond is achieved by the isomerization of a C8-C9 bond.
Scheme 5.1 is followed by a C8-C9 bond isomerization. This bond is
formed by the isomerization of a C8-C9 bond. The isomerization
of a C8-C9 bond is achieved by the isomerization of a C8-C9 bond.
Scheme 5.1 is followed by a C8-C9 bond isomerization. This bond is
formed by the isomerization of a C8-C9 bond. The isomerization
of a C8-C9 bond is achieved by the isomerization of a C8-C9 bond.
5.1 The Biosynthetic Ramifications of FR-900482 and FR-66979

The isolation of FR-900482 and FR-66979 (see Chapter 3) forced us to re-evaluate mechanisms for the coupling of AHB (99) and D-glucosamine (96) in mitomycin biosynthesis (Scheme 5.1). The formation of the N4C3 bond by imine reduction (Section 2.6a) remains plausible as does transannular N4C9a bond formation by attack on a ketone (Section 2.6c). The crucial C8aC9 coupling, however, was unlikely to involve a Michael cyclisation given the absence of a quinone (see Section 3.5). The formation of the bond requires a formal dehydration and it seems likely that the formal oxidation of C4' is associated in some way. Two general processes seem plausible, aryl alkylation of the aminophenol or a radical process.

![Scheme 5.1 Biogenesis of the Mitomycins](image)

Electrophilic substitution of the aminophenol by a C5' electrophile, probably tethered by N4C4a, would generate the C8aC9 bond and an eight membered ring. Phosphorylation of the 5'-hydroxyl could readily activate such a process. This would be somewhat analogous to the electrophilic alkylation of orsellinic acid by pyrophosphates, for example.\(^5\) Similarly, a '4,5'- or '5,6'-epoxide could be opened by attack of the AHB residue (involvement of a 5,6-epoxide would preclude early introduction of the carbamate). We are not aware of any biosynthetic epoxy-arene reactions but epoxy-alkene cyclisations have been established and imitated,\(^5\) and cyclisations of epoxides tethered to benzenes are synthetically useful.\(^5\) Even seven membered rings can be formed with an unactivated benzene (e.g. see Scheme 5.2).\(^5,6\) A ketone can be sufficiently electrophilic to allow cyclisation to form a seven membered ring with a more activated ring (Scheme 5.3).\(^5\)
Biosynthetically such processes could also proceed with anionic activation, and the anionic cyclisation of substituted phenols is an established synthetic methodology. We are not aware of any eight-membered rings being formed by any of the above reactions - a fused aziridine ring would reduce the entropic problem in the biosynthetic process which may well be enzymatically controlled as well. The successful biomimetic imitation of these processes might well lead to a mixture of regioisomers (i.e. alkylation ortho and para to the phenol) although the electronic influences are not clear as they depend on the substituents on the aromatic ring as well as the process involved. Again, the biosynthetic reaction is probably enzyme controlled.

A particularly economical version of an electrophilic cyclisation would start with oxidation of the 5′-hydroxyl to a ketone 501. Cyclisation would give a benzyl alcohol 502 which could dehydrate and directly generate the 9α-ketone 503 required for transannular cyclisation (Scheme 5.4). Note that [5′-3H]-D-glucosamine suffers the greatest loss (78%) of label upon incorporation into mitomycin A (1) of the various glucosamines studied by Homemann et al. (see Section 1.6); this is perhaps compatible with oxidation of C5′ although the results are complex. Note that [4′-3H]-D-glucosamine also loses most (56%) of its label during incorporation into mitomycin A; for any retention of this label in Scheme 5.4 there must be reincorporation from the water lost on dehydration.
The other obvious possibility for C8aC9 bond formation is a radical coupling. Oxidation of C4' or C5' and enolisation would give an ene-diol which could alkylate a phenolic radical cation or lead to a semidione radical which could undergo radical coupling. Jamie and Rickards have investigated the coupling of 1,3-dicarbonyl derived radicals with aromatic systems; this was discussed in Section 2.5ii.

5.2 Biomimetic Models for C8aC9 Bond Formation

It occurred to us that a very flexible substrate for the investigation of various biomimetic couplings was the tethered nitroalkane 504, with either an alcohol or ketone at C4'. The nitro group potentially gives access to C5' radicals, either as intermediates in reductive denitration\(^{557,558}\) or through oxidation with manganese (III)\(^ {559}\). Alternatively hydrolysis should give the 5'-ketone; this is a “Nef reaction” and can be performed under mild conditions\(^ {560}\). Reduction and diazotisation should generate a C5' electrophile. We anticipated that 504 would be accessible by coupling a 3-amino-5-substituted-phenol with a four carbon aziridine (as sought in the last Chapter) and 2-nitroethanol. Various AHB analogues are available \textit{via} the Bucherer reaction from substituted 3-hydroxyphenols\(^ {561}\). For example we have synthesised 3-amino-5-
methylphenol from 3-hydroxy-5-methylphenol according to the procedure of Wessely et al.\textsuperscript{562} However, with limited time we decided to pursue a different target.

![Chemical structure](image)

The protected hydroquinone 505 has activation of ‘C8a’ that may be similar to that in acyclic precursors of FR-900482 and FR-66979. The hydroquinone 505 thus offered the chance to study different cyclisation methodologies and generate a probable mitomycin precursor if successful. We hoped that a tosyl protecting group on the aniline would allow a Mitsunobu coupling of the aziridine fragment and then efficient concomitant deprotection and transannular cyclisation, following from the work of Ban et al. (see Section 2.3iv). To this end our initial target was the aniline 506.

![Chemical structures](image)

5.2a The Aromatic Ring

With 3-methoxy-2-methyl-6-nitrophenol (333) in hand we hoped to obtain rapid access to the nitroquinone 507 by oxidation. Nitroquinones have occasionally been reported and are, not surprisingly, unstable\textsuperscript{563,564} but we thought that 507 might be useable as a transient intermediate if rapidly reduced. Indeed Parker and Sworin have synthesised this nitroquinone, albeit in low yield.\textsuperscript{565} They prepared the nitrohydroquinone 509 from vanillin (508) in low yield, then obtained the nitroquinone 507 by oxidation, trapping it by intermolecular Michael addition (Scheme 5.5).
Scheme 5.5 Preparation of a Nitroquinone

In the event the nitrophenol 333 was completely inert to oxidation by Fremy’s salt. (The same batch of Fremy’s salt was successfully used to prepare 2-hydroxy-3-methyl-1,4-benzoquinone 522, see Scheme 5.11). Oxidations of phenols with Fremy’s salt are known to be sensitive to electron withdrawing groups, with both electron withdrawing and electron donating substituents the outcome varies.566 Rather than pursue more vigorous oxidants we reduced the nitro group to give, after tosylation, the aminophenol 510. Oxidation with Fremy’s salt was smooth giving the aminoquinone 511 in 86% overall yield (Scheme 5.6).

Scheme 5.6 Preparation of a Protected Aminoquinone
The mass spectrum of 511 contained a weak molecular ion (4%), an intense C\textsubscript{7}H\textsubscript{7} ion (base peak) and ions from N-S cleavage (\textit{m/z} 166 and 155, 17 and 13%). More surprising was an ion at \textit{m/z} 257 (3%) suggesting elision of sulfur dioxide (supported by the weakness of the isotope peak at \textit{m/z} 259). An analogous loss has been observed for sulfonyl ureas;\textsuperscript{567} two mechanisms were found to be operating, migration of the toluene moiety to either nitrogen or oxygen. For the quinone 511 it is hard to envisage a mechanism for transfer to the \textit{ortho} carbonyl oxygen but a direct extrusion seems reasonable.

Reduction of the quinone and benzylation gave two products (Scheme 5.7). The more polar product (mp 144-145°C, 33%) was the N,O-dibenzyl derivative 513. The \textsuperscript{1}H NMR spectrum contained an exchangeable, one proton resonance at δ 6.05 with a line width of 1.1 Hz, clearly a phenolic resonance (see section 3.3c). The benzylic proton resonances were all broad, presumably due to restricted rotation of the benzyl and sulfonamide groups. The \textsuperscript{13}C NMR spectrum was consistent with the assignment with benzylic resonances at δ 71.6 (OCH\textsubscript{2}) and 55.8 (NCH\textsubscript{2}). Assignment as the 4-benzyloxy-2-sulfonamidophenol isomer was made on steric grounds and very tentative support for this came from observation of an ion \textit{m/z} 256 (M-C\textsubscript{7}H\textsubscript{7}-HSO\textsubscript{2}PhCH\textsubscript{3} ?, 6%). This presumably arose via elimination of toluenesulfonic acid and a benzyl radical (Scheme 5.8), which requires an \textit{ortho} hydroxy group; a weak ion at \textit{m/z} 156 (CH\textsubscript{3}PhSO\textsubscript{2}H ?, 1%) was observed. Also isolated was the tribenzyl derivative 514 (37% based on the quinone) as an oil. The \textsuperscript{13}C NMR spectrum of 514 showed benzylic resonances at δ 74.8, 70.8 (2 x OCH\textsubscript{2}) and 54.8 (NCH\textsubscript{2}). None of the desired O,O-dibenzyl product appeared to be present in the crude product by NMR.

\[
\begin{align*}
\text{MeO} & \quad 1) \text{H}_2, \text{Pd-C} \\
\text{O} & \quad \text{MeO} \\
\text{NH} & \quad \text{OH} \\
\text{SO}_2\text{PhCH}_3 & \quad \text{MeO} \\
\text{511} & \quad \text{512} \\
& \quad 2) \text{BnBr, K}_2\text{CO}_3, \text{acetone} \\
& \quad \text{513 (33%)} \\
& \quad \text{514 (37%)}
\end{align*}
\]

\textbf{Scheme 5.7 Benzylation of the Hydroquinone}
Thus the tosyl protecting group which would have enabled subsequent Mitsunobu coupling (see Section 4.15) leads to facile, unwanted N-benzylation. (Without protection, oxidation of the aminophenol 334 would generate an ortho-quinone.) It might be possible to selectively O-benzylate compound 512 by formation of a polyanion, thereby ‘protecting’ the nitrogen, or avoid the problem by alkylation prior to benzylation. However, we turned to more direct syntheses of a suitable A ring precursor.

When making the aminoquinone 511 we were reminded of the difficulties associated with scaling up Fremy’s salt reactions. Fremy’s salt itself is erratically available commercially and expensive. Furthermore the solubility of Fremy’s salt in water is relatively low and thus very large volumes of solution are required when working on a moderate scale. The aqueous solution prevents the oxidation of hydrophobic phenols unless phase transfer catalysis is used (see Section 3.2b). This has led to the development of nitroxides (R₂NO) which are soluble in organic solvents,₅₆₈,₅₆₉,₅₇₀ but these are not readily available. Given such problems we preferred to avoid Fremy’s salt as a reagent in the preparation of early intermediates.

We conceived two strategies for the possible, large scale preparation of the protected aminohydroquinone 506. Starting with 2-methoxy-3-methyl-1,4-benzoquinone (515), we anticipated that reduction to the hydroquinone, benzylation and selective nitration para to the methoxy group would give the desired 506 (Scheme 5.9). If nitration was impractical, regioselective amination of the quinone with ammonia or a synthetic equivalent may provide an alternative route. The second envisaged strategy begins with the dinitro compound 328. Reduction and reoxidation should, after controlled hydrolysis, give the aminohydroxyquinone 516. If it is feasible
to selectively methylate the hydroxy group, this gives the aminoquinone 517 which, on reduction and benzylation would furnish 506 (Scheme 5.9).

Scheme 5.9 Proposed Synthesis of Protected Aminohydroquinone

3-Methoxy-2-methyl-1,4-benzoquinone (515) is the sort of starting material one would prefer to have commercially available. It has been used quite often in synthetic work aimed at mitomycins. The benzoquinone 515 has been synthesised by oxidation of various phenols, an aniline, anisoles and a benzyl alcohol as summarised in Table 5. Most of the precursors themselves require several step syntheses. The most obvious synthesis involves oxidation of 3-methoxy-2-methylphenol with Fremy's salt (Entry 2). However, we preferred to avoid Fremy's salt for the reasons outlined above and 3-methoxy-2-methylphenol is not efficiently accessible, as has been discussed by Luly and Rapoport. They developed an alternative route from 1,3-dimethoxy-2-methylbenzene (518) involving formylation, Baeyer-Villiger oxidation, hydrolysis and oxidative demethylation (Entry 5). A similar route from 3-hydroxy-2-methylphenol (327) was recently reported by van der Steeg et al. (Entry 7). Both routes are multistep although the overall yields are acceptable (77 and 50% respectively).

Examination of this literature and consideration of the availability of precursors directed our attention to the oxidative demethylation of 1,3-dimethoxy-
2-methylbenzene (518) (Entries 6a and 6b). This substrate is commercially available in high purity at a reasonable price. If a better yield for the transformation was achievable, the one step reaction would become the preparation of choice. Matsumoto et al. have reported an efficient synthesis of 2,6-dimethoxy-1,4-benzoquinone by oxidation of 1,3,5-trimethoxybenzene with hydrogen peroxide and iron catalysis (Scheme 5.10).\textsuperscript{572} It occurred to us that such a process potentially offered an efficient method for preparing 515. Matsumoto et al. observed significantly better reaction in aqueous acetone than in acetic acid, suggesting that the existing procedures (Entries 6a and 6b) could be improved.

\begin{center}
\textbf{Scheme 5.10 Synthesis of 2,6-dimethoxy-1,4-benzoquinone}
\end{center}

\begin{center}
\includegraphics[width=0.5\textwidth]{scheme_5.10.png}
\end{center}
<table>
<thead>
<tr>
<th>Entry</th>
<th>Precursor</th>
<th>Reagents</th>
<th>Yield (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><img src="image1" alt="Entry 1" /></td>
<td>Na$_2$Cr$_2$O$_7$, H$_2$SO$_4$</td>
<td>33</td>
<td>Majima and Okazaki$^{573}$ Winzor$^{574}$ Anslow et al.$^{575}$ Hanger et al.$^{576}$</td>
</tr>
<tr>
<td>2</td>
<td><img src="image2" alt="Entry 2" /></td>
<td>a) Fremy’s salt</td>
<td>71 (crude)</td>
<td>Rashid and Read$^{577}$ Kirkemo and White$^{578}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>b) Fremy’s salt</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>c) NaI$_2$O$_4$</td>
<td>11</td>
<td>Andersson$^{579}$</td>
</tr>
<tr>
<td>3</td>
<td><img src="image3" alt="Entry 3" /></td>
<td>Fremy’s salt</td>
<td>89</td>
<td>Saa et al.$^{580}$</td>
</tr>
<tr>
<td>4</td>
<td><img src="image4" alt="Entry 4" /></td>
<td>HCO$_2$H</td>
<td>90</td>
<td>Parker et al.$^{581}$</td>
</tr>
<tr>
<td>5</td>
<td><img src="image5" alt="Entry 5" /></td>
<td>i. POCl$_3$, DMF ii. mCPBA iii. KOH iv. CAN</td>
<td>77 (overall)</td>
<td>Luly and Rapoport$^{587}$</td>
</tr>
<tr>
<td>6</td>
<td><img src="image6" alt="Entry 6" /></td>
<td>a) Na$_2$Cr$_2$O$_7$, AcOH</td>
<td>18</td>
<td>Mandell and Roberts$^{582}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>b) H$_2$O$_2$, HCO$_2$H</td>
<td>12</td>
<td>Orita et al.$^{583}$</td>
</tr>
<tr>
<td>7</td>
<td><img src="image7" alt="Entry 7" /></td>
<td>not detailed: apparently methylation then similar to Entry 5</td>
<td>50 (overall)</td>
<td>van der Steeg et al.$^{521}$</td>
</tr>
</tbody>
</table>

Table 5 Syntheses of 3-Methoxy-2-methyl-1,4-benzoquinone (515)
Treatment of 1,3-dimethoxy-2-methylbenzene with hydrogen peroxide and potassium ferricyanide did indeed generate the desired methoxybenzoquinone 515 (Scheme 5.11). We have not optimised the reaction and there appear to be significant losses on workup (e.g. noticeable loss on evaporation of a dichloromethane solution of 515 with nitrogen gas) but we isolated 515 in 47% on a 7 mmol scale reaction. It would be worth optimising this procedure given its suitability for scaling up and the utility of the product.

\[
\begin{align*}
\text{MeO} & \quad \text{H}_2\text{O}_2, \text{K}_3\text{Fe(CN)}_6 \\
\text{OMe} & \quad \text{acetone, water} \\
\rightarrow & \quad \text{MeO} \\
\text{MeO} & \quad \text{Me}_2\text{SO}_4, \text{K}_2\text{CO}_3 \quad \text{acetone} \\
\text{HO} & \quad \text{518} \\
\text{522} & \quad \text{515}
\end{align*}
\]

Scheme 5.11 Synthesis of 2-Methoxy-3-methyl-1,4-benzoquinone

A synthesis of 506 from 328 (Scheme 5.9) requires methylation of the hydroxyquinone 516. We prepared 2-hydroxy-3-methyl-1,4-benzoquinone 522 by oxidation of 3-hydroxy-2-methylphenol according to the procedure of Rashid and Read.527 The methylation of this hydroxyquinone to form 515 has not been reported. Treatment of the sample with dimethyl sulphate and potassium carbonate in acetone gave no detectable trace of 515, only decomposition. This may be a reflection of the instability of hydroxyquinones.302,303,304 We have not pursued alternatives such as reaction with diazomethane, and note that such methylations are not necessarily clean, possibly because of competitive addition reactions.584 Whether the 2-amino-5-hydroxybenzoquinone 516 could be efficiently O-methylated remains unexplored.

We have not attempted to convert 515 to the aminohydroquinone 506. There is some precedent for this process in the efficient conversion of hydroquinone itself to 2,5-dibenzylxoyaniline,585 although the nitration there requires no regioselectivity.
5.2b Application to Mitomycin Synthesis

From a purely synthetic perspective the quinone 520 has struck us as a worthwhile target. The nitro group makes H5' appreciably acidic and it seemed possible that a quinone such as 520 would undergo Michael cyclisation to give a benzazocine 521 with the desired hydroxymethyl substituent in place. If reductive removal of the nitro group was possible this would circumvent the critical problem of converting a carboxymethyl to an hydroxymethyl group (see 3.4). The corresponding system with a 4'-alcohol might allow denitration by simple elimination of nitrous acid.586 Thus the nitro group might offer a synthetic improvement on our earlier Michael cyclisation work (Chapter 3), albeit probably not biomimetic. The most obvious precursor to the quinone 520 was the phenol 519, by extension of our earlier work. The protected hydroquinone 505 was less attractive as debenzylation in the presence of an aliphatic nitro group would be difficult.

5.3 Conclusion

The revised biomimetic models have not been taken beyond these very preliminary investigations. We believe however that the strategies described in this chapter would be worth pursuing both as models to study the biomimetic cyclisation and for synthetic ends.
Par une belle matinee du mois de mai, une elegante amazone parcourait,
sur une superbe jumente alezane, les allees fleuries du Bois de Boulogne

Albert Camus, *La Peste* p 84 (Gallimard: Paris 1947)
Caution! The mitomycins and some simple aziridines have been shown to be very potent mutagens. Appropriate care should be taken in handling these compounds.

General Procedures

Melting points (mp) were determined on a Reichert hot-stage apparatus and are uncorrected.

Microanalyses were carried out by the Australian National University Microanalytical Service.

Ultra-violet (UV) and visible spectra were recorded on either a Varian DMS 90 or a Hewlet-Packard 8450A spectrophotometer using solutions in spectroscopic grade methanol, ethanol or chloroform. "sh" indicates shoulder.

Infra-red spectra were recorded on a Perkin-Elmer 683 spectrophotometer. Fourier transform infra-red spectra (FTIR) were recorded on a Perkin-Elmer 1800 (FT) with appropriate solvent subtraction. Strong peaks and some others deemed pertinent are recorded.

$^1$H NMR spectra were recorded on a Varian Gemini-300 or a Varian VXR-300 spectrometer. Spectra were recorded in deuteriochloroform (CDCl$_3$) unless otherwise indicated. Tetramethylsilane is used as an internal reference and resonances are quoted in ppm. Multiplicities and peak shapes are abbreviated: d (doublet), dd (doublet of doublets), t (triplet), q (quartet), m (multiplet), br (broad) and v br (very broad).

$^{13}$C NMR spectra were recorded on a Varian Gemini-300 spectrometer at 75.5 MHz or a Varian VXR-300 at 75.4 MHz. Spectra are referenced to the middle peak of deuteriochloroform as 77.00 ppm. The Attached Proton Test (APT) pulse sequence$^{600}$ was frequently used. Assignment of $^{13}$C NMR resonances is based on literature comparison, most notably using the compilations of Kalinowski et al.$^{601}$ Bremser et al.$^{602}$ and Breitmaier and Voelter$^{603}$ and on information from the APT, when run. Assignments of resonances marked ‘*’ may be interchanged. ‘Bn’ indicates a carbon in the benzyl group.

Mass spectra were recorded on AEI 902 and V.G.-Micromass 7070F double-focussing mass spectrometers. Peaks with intensity greater than 20% and others...
considered pertinent have been recorded. All mass spectra were run using electron
impact ionisation (ca 70 eV) unless otherwise indicated.

High resolution mass measurements were made on the AEI 902 spectrometer
using perfluorokerosene as a reference.

Positive (CI) and negative (NICI) chemical ionisation mass spectrometry was
performed using ammonia as the reagent gas in all cases; CI were recorded down to
approximately m/z 100 and NICI to approximately m/z 200.

Optical Rotations were measured on a Perkin Elmer Model 241 polarimeter
using a 1 dm cell at ambient temperature (ca 20°); concentrations are noted in grams per
100 ml.

Flash column chromatography was carried out using 230-400 mesh silica gel.
For thin layer chromatography (TLC), 0.25 mm Merck silica gel plates (60 F254) were
used for analytical purposes and 2 mm plates for preparative work. Radial
chromatography (Chromatatron®) was routinely used in preference in later work.

Buffered aqueous solutions at pH 6.8 referred to in Fremy's salt oxidations were
prepared by dissolving disodium hydrogen phosphate (355 mg) and potassium
dihydrogen orthophosphate (340 mg) in distilled water (100 ml).

Where necessary, solvents and reagents were purified and dried according to the
procedures described in Perrin et al.604 All organic solvents were distilled before use.
Petroleum ether refers to the light petroleum fraction bp 40-60°.

All air sensitive reactions were carried out under a positive pressure of dried
argon and the glassware was dried with a flame before use.
3-hydroxy-2-methyl-4-nitrophenol (325)*

Concentrated nitric acid (70%, 0.786 g) was added cautiously to ice cooled acetic anhydride (1.34 g, 13.1 mmol) and the cold mixture added to a cooled solution of 3-hydroxy-2-methylphenol (commercial and not pure; 1.01 g, 8.16 mmol) in acetic acid (4.1 g). After stirring for one hour the mixture was heated for ten minutes on a steam bath (care! fumes), cooled and diluted with water (15 ml). Extraction with ether (4 × 15 ml), drying over magnesium sulfate and evaporation with azeotropic removal of acetic acid using cyclohexane gave a crude product which was chromatographed over silica (ethyl acetate: toluene, 1:9) (analytical chromatography with dichloromethane) to give 3-hydroxy-2-methyl-4-nitrophenol (325) as a pale yellow solid (745 mg, 54%). The product could not be usefully steam distilled. Recrystallisation from benzene gave brown needles mp 128-130° (lit.605 and 606 124-125° and 128-129°).

IR: \(\nu_{\text{max}}\) (KBr) 3390, 1621, 1599, 1544, 1462, 1427, 1276, 1157, 1075, 815, 762 cm\(^{-1}\).

\(^1\)H NMR: The NMR spectrum was in accord with that of Su et al.\(^{310}\), except that the resonance at \(\delta\) 11.33 is due to only one hydroxyl, another is observed at \(\delta\) 5.68:

\[
\begin{align*}
\delta & 11.33 (s, 1H, C3-OH), 7.93 (d, J 9.3 \text{ Hz}, H5), 6.45 (d, J 9.3 \text{ Hz}, H6), 5.68 (s, 1H, C1-OH), 2.19 (s, \text{CH}_3).
\end{align*}
\]

\(^{13}\)C NMR: \(\delta\) (CDCl\(_3\) + CD\(_3\)SOCD\(_3\)) 164.1 (C1), 156.1 (C3), 126.9 (C4), 123.9 (C5), 112.6 (C2), 108.9 (C6), 8.0 (CH\(_3\)).

EIMS: 169 (M, 99%), 151 (M-H\(_2\)O, 21), 139 (M-NO, 17), 123 (M-NO\(_2\), 36), 111 (30), 95 (20), 94 (25), 80 (73), 77 (25), 67 (58), 66 (53), 65 (73), 63 (22), 55 (87), 54 (21), 53 (100), 52 (43), 51 (59).

* This nomenclature is used instead of the benzenediol stem to allow facile comparison of assigned spectra.
3-hydroxy-2-methyl-4,6-dinitrophenol (328)

Fuming nitric acid (10 ml) was added dropwise to a stirred solution of 3-hydroxy-2-methylphenol (commercial and not pure; 20 g, 0.16 mol) in ether (1000 ml) cooled in ice-salt. After 3 h, more fuming nitric (5 ml) was added and the mixture stirred for a further 1 h. The mixture was washed with brine, dried with magnesium sulfate and evaporated. Chromatography (2: 8, ethyl acetate : toluene) gave 3-hydroxy-2-methyl-4, 6-dinitrophenol (328) (417 mg, 1.2%) as a red microcrystalline solid. (Found: M, 214.0225; C7H6N2O6 requires 214.0226).

$^1$H NMR: $\delta$ 11.39 (s, 2H, OH), 9.00 (s, 1H, H5), 2.28 (s, 3H, CH$_3$).

$^{13}$C NMR: $\delta$ 158.6 (COH), 127.2 (CNO$_2$), 122.1 (CH), 117.3 (CCH$_3$), 8.5 (CH$_3$).

EIMS: $m/z$ 214 (M, 94%), 196 (M-H$_2$O, 46), 99 (86), 93 (28), 83 (74), 69 (21), 68 (26), 67 (48), 55 (69), 54 (29), 53 (100), 52 (27), 51 (41).

Further elution gave 3-hydroxy-2-methyl-4-nitrophenol (325) (13.6 g, 50%).
3-methoxy-2-methyl-6-nitrophenol (333)

3-Methoxy-2-methyl-6-nitrophenol (333) was prepared from 3-hydroxy-2-methyl-4-nitrophenol (325) according to the literature procedure<sup>607</sup> giving yellow prisms from hexane, mp 71.5-72.5° (lit.<sup>310</sup> and <sup>319</sup> 68-69° and 70-71.5°).

IR: $v_{\text{max}}$ (KBr) 3440 br w, 1615, 1475, 1283, 1247, 1110, 761 cm$^{-1}$.

FTIR: $v_{\text{max}}$ (CHCl$_3$) 3195, 1617, 1536, 1478, 1436, 1291, 1251, 1112 cm$^{-1}$.

$^1$H NMR: δ 11.13 (s, Cl-OH), 8.02 (d, J 9.6 Hz, H5), 6.54 (d, J 9.6 Hz, H4), 3.94 (s, CH$_3$O), 2.15 (s, CH$_3$).

$^{13}$C NMR: δ 164.4 (C3), 154.8 (COH), 128.2 (CNO$_2$), 124.4 (C5), 114.9 (CCH$_3$), 103.1 (C4), 56.2 (CH$_3$O), 8.10 (CH$_3$).

EIMS: $m/z$ 183 (M, 100%), 165 (M-H$_2$O, 20), 153 (M-NO, 14), 125 (23), 107 (81), 93 (27), 92 (24), 91 (20), 79 (29), 78 (31), 77 (47), 67 (26), 66 (50), 65 (81), 64 (23), 63 (31), 59 (24), 55 (35), 53 (90), 52 (52), 51 (67).
EXPERIMENTAL

2-amino-5-methoxy-6-methylphenol (334)

Platinum oxide (1 mg) was added to a solution of 3-methoxy-2-methyl-6-nitrophenol (333) (50 mg, 0.27 mmol) in ethanol (3 ml) and the mixture was stirred for 3h under an atmosphere of hydrogen. Evaporation of the colourless solution gave the air-sensitive crude product. Sublimation (90°, 0.1 mm) gave 2-amino-5-methoxy-6-methylphenol (334) as white crystals (mp 123-6°) which rapidly became coloured. Crude material was used for further reactions.

1H NMR: δ 6.70 (d, 1H, J 8.6 Hz), 6.32 (d, 1H, J 8.6 Hz), 3.90 (br s, 3H, NH₂ and OH), 3.77 (s, 3H, CH₃O), 2.13 (s, 3H, CH₃).

13C NMR: δ 153.4 (CH₃OC), 146.5 (COH), 126.0 (CNH₂), 117.0 (C3), 112.8 (CCH₃), 102.6 (C4), 56.0 (CH₃O), 8.5 (CH₃).

EIMS: m/z 153 (M), 138 (M-CH₃), 65.

2-[tert-butoxycarbonylpropanamino]-5-methoxy-6-methylphenol (336)

Crude 2-amino-5-methoxy-6-methylphenol (334) [from reduction of 21 mg, 0.12 mmol of 3-methoxy-2-methyl-6-nitrophenol (333)] was dissolved in DMPU (0.5 ml) and sodium hydrogen carbonate (12 mg, 0.14 mmol) and tert-butyl 4-bromobutanoate (29.2 mg, 0.13 mmol) added. After stirring for 24 hours the mixture was chromatographed to give 2-[3-tert-butoxycarbonylpropanamino]-5-methoxy-6-methylphenol (336) as an air sensitive solid (15.7 mg, 38%). (Found: M, 295.1784; C₁₆H₂₅N₀₄ requires 295.1785).

EIMS: m/z 295 (M, 24%), 239 (M-C₄H₈, 57), 224 (M-C₄H₈-CH₃, 17), 222 (M-C₄H₉O, 38), 167 (11), 166 (M-CH₂CH₂CO₂tBu, 100), 57 (C₄H₉, 15), 41 (12).
2-\([\text{N-(benzyloxycarbonyl)-3-tert-butoxycarbonylpropanamino]-5-methoxy-6-methylphenol (338)}\)

To crude 2-amino-5-methoxy-6-methylphenol (334) (from hydrogenation of 161 mg, 0.88 mmol of 3-methoxy-2-methyl-6-nitrophenol (333)) and sodium hydrogen carbonate (90 mg) under argon was added tert-butyl 4-bromobutanoate (335)\(^{203}\) (204 mg, 0.91 mmol) \textit{via} DMPU (1.5 ml). After stirring at rt for 85 h the mixture was diluted with THF (2 ml), cooled to 0° and pyridine (85 µL, 1.1 mmol) and benzylcholoroformate (160 µL, 1.1 mmol) added. After stirring at 0° for another 2 h the mixture was added to aqueous sodium dihydrogen phosphate (5%, 3 ml) and extracted with ethyl acetate (3 x 2 ml). The combined extracts were washed with water, dried with sodium sulfate and evaporated. Repeated chromatography (ethyl acetate : hexane, 1 : 4) removed residual DMPU giving 2-\([\text{N-(benzyloxycarbonyl)-3-tert-butoxycarbonylpropanamino]-5-methoxy-6-methylphenol (338)}\) (227 mg, 60%) as an oil which slowly solidified on standing. Recrystallisation from benzene-hexane gave white crystals, mp 106°. Also isolated was the less polar 2-\([\text{N,N-bis-3-tert-butoxycarbonylpropanamino]-5-methoxy-6-methyl phenol (337)}\) (37 mg, 9.5%) as a colourless oil.

2-\([\text{N-(benzyloxycarbonyl)-3-tert-butoxycarbonylpropanamino]-5-methoxy-6-methylphenol (338):}\)

\begin{align*}
\text{Found C: 67.1; H 7.6, N 3.2%. C24H31NO6 requires C, 67.1; H, 7.3; N, 3.3%.} \\
\text{FTIR: } \nu_{\text{max}} (\text{CHCl}_3) 3549 \text{ (weak)}, 3308, 1710, 1497, 1302, 1156, 1110 \text{ cm}^{-1}. \\
\text{\textsuperscript{1}H NMR: } \delta 7.4-7.2 \text{ (br m), 6.87 (d, 1H, J 8.8 Hz), 6.42 (d, 1H, J 8.8 Hz), 5.12 (br s),} \\
\text{3.81 (s), 3.8-3.4 (vbr m), 2.38 (br m), 2.16 (s), 1.9-1.6 (br m), 1.41 (s), OH not resolved.} \\
\text{at 50°: } \delta 7.35-7.20 \text{ (m, 5H, Ph), 6.86 (d, 1H, J 8.9 Hz), 6.54 (br s, 1H, OH), 6.42 (d, 1H, J 8.9 Hz), 5.13 (s, 2H, CH}_2\text{Ph), 3.81 (s, 3H, CH}_3\text{O), 3.66 (br t, 2H, J 6.7 Hz, NCH}_2\text{), 2.30 (br m, 2H, J 6.9 Hz, COCH}_2\text{), 2.15 (s, 3H, CH}_3\text{), 1.81 (quintet, 2H, J 6.7 Hz, CH}_2\text{), 1.41 (s, 9H, tBu).} \\
\text{\textsuperscript{13}C NMR at 50°: } \delta 173.0 \text{(CO}_2\text{), 157.9* (NCO}_2\text{), 156.5* (MeOC), 151.4* (HOC),} \\
136.7 \text{(Bn quaternary), 128.5* (Bn or CN), 127.9* (Bn or CN), 127.5* (Bn or CN).}
\end{align*}
CN), 124.6 (C3), 102.9 (C4), 80.7 (CMe3), 67.5 (Bn methylene), 55.7 (CH3O), 51.0 (NCH2), 33.3 (CH2CO2), 28.1 (Me3), 23.7 (CH2), 8.6 (CH3C). CH3C and either CN or a Bn resonance not seen.

EIMS: m/z 429 (M, 4%), 373 (M-C4H8, 14), 321 (M-PhCH2OH, 27), 265 (M-C4H8-PhCH2OH, 50), 248 (M-PhCH2OH-OC4H9, 37), 238 (M-C4H8-CO2CH2Ph, 66), 192 (M-PhCH2OH-CH2CH2CO2C4H9, 16), 179 (M-PhCH2OH-CH2CHCH2CO2tBu, 52), 164 (179-CH3, 27), 108 (PhCH2OH, 20), 107 (C7H7O, 17), 91 (C7H7, 100), 79 (22), 57 (C4H9, 21).

2-[N,N-bis-3-tert-butoxycarbonylpropanamino]-5-methoxy-6-methylphenol (337)

Found: C, 66.1; H, 9.2; N, 3.2%. C24H39N06 requires C, 65.9; H, 9.0; N, 3.2%.

FTIR: $\nu_{\text{max}}$ (CHCl3) 3315, 2981, 1720, 1496, 1369, 1155, 1109 cm$^{-1}$.

$^1$H NMR: $\delta$ 7.43 (br s, <1H, OH), 6.92 (d, 1H, J 8.7 Hz), 6.37 (d, 1H, J 8.7 Hz), 3.80 (s, 3H, CH3O), 2.85 (t, 4H, J 7.5 Hz, NCH2 x2), 2.17 (t, 4H, J 7.3 Hz, CH2CO x2), 2.12 (s, 3H, CH3), 1.65 (quintet, 4H, J 7.4 Hz, CH2 x2), 1.42 (s, 18H, tBu x2).

$^{13}$C NMR: $\delta$ 172.6 (CO2), 156.2 (MeOC), 152.3 (HOC), 129.6 (CN), 119.8 (C3), 111.3 (CH3C), 101.3 (C4), 80.3 (CMe3), 55.53 (CH3O), 55.48 (NCH2), 33.1 (CH2CO2), 28.0 (Me3), 22.9 (CH2), 8.7 (CH3C).

EIMS: m/z 437 (M, 13%; found 437.2776; C24H39NO6 requires 437.2777), 381 (M-C4H8, 2), 364 (M-OC4H9, 2), 325 (M-C4H8-C4H8, 8), 308 (M-CH2CH2CO2tBu or M-C4H8-OC4H9, 22), 252 (M-CH2CH2CO2tBu-C4H8, 59), 166 (M-(CH2)2CO2tBu-CH2CHCH2CO2tBu, 21), 87 (CH2CH2CH2CO2H, 38), 57 (C4H9, 100).
2-[N-(benzyloxycarbonyl)-3-carboxypropanamino]-5-methoxy-6-methylphenol (342)

2-[N-(Benzyloxycarbonyl)-3-tert-butoxycarbonylpropanamino]-5-methoxy-6-methylphenol (338) (85.6 mg, 0.2 mmol) was dissolved in 40% trifluoroacetic acid in dichloromethane (v/v, 3 ml) and stirred at rt for 1 h. Evaporation and azeotropic distillation of residual trifluoroacetic acid with toluene gave crude 2-[N-(benzyloxycarbonyl)-3-carboxypropanamino]-5-methoxy-6-methylphenol (342) (74 mg, 99%). (Found: M, 373.1526; C20H23N06 requires 373.1525).

1H NMR: δ 8.25 (br s, 2H, CO2H and OH), 7.4-7.1 (br m, 5H, Ph), 6.87 (d, 1H, J 8.8 Hz), 6.43 (d, 1H, J 8.8 Hz), 5.12 (br s, 2H, CH2Ph), 3.81 (s, 3H, CH3O), 3.65 (br t, 2H, J 7 Hz, NCH2), 2.38 (br t, 2H, J 7 Hz, CH2CO), 2.13 (s, 3H, CH3), 1.83 (br quintet, 2H, J 7 Hz, CH2).

13C NMR: δ 178.4 (CO2H), 157.8* (NCO2), 156.9* (MeOC), 150.9* (HOC), 136.1 (Bn quaternary), 128.4* (Bn or CN), 128.3* (Bn or CN), 127.9* (Bn or CN), 127.5* (Bn or CN), 124.8 (C3), 102.6 (C4), 67.8 (Bn methylene), 55.6 (CH3O), 50.0 (NCH2), 31.4 (CH2CO2), 23.1 (CH2), 8.7 (CH3C). CH3C not seen.

EIMS: m/z 373 (M, 2%), 265 (M-PhCH2OH, 18), 238 (M-CO2CH2Ph, 6), 192 (M-PhCH2OH-CH2CH2CO2H, 5), 179 (265-CH2CHCH2CO2H, 23), 164 (179-CH3, 22), 108 (PhCH2OH, 33), 107 (26), 91 (C7H7, 100), 87 (CH2CH2CH2CO2H, 35), 79 (63), 77 (40), 65 (31), 53 (23), 52 (25), 51 (39).

Propanedioic acid monomethyl ester

Propanedioic acid monomethyl ester was prepared according to the procedure of Grakauskas and Guest as a colourless liquid bp 97°, 0.55 mm (lit 90°, 0.5 mm).

1H NMR: δ 9.7 (shift variable; BR s, 1H, CO2H), 3.79 (s, 3H, OCH3), 3.46 (s, 2H, CH2).

13C NMR: δ 171.8 and 166.9 (CO2H and CO2), 52.7 (OCH3), 40.7 (CH2).

EIMS: m/z 101 (M-OH, 5%), 100 (M-H2O, 3), 87 (M-OCH3, 48), 74 (M-CO2, 23), 59 (29), 45 (26), 44 (20), 43 (100).
2-[N-(benzyloxycarbonyl)-5-methoxycarbonyl-4-oxo-pentanamino]-5-methoxy-6-methylphenol (301)

To a solution of crude 2-[N-(benzyloxycarbonyl)-3-carboxypropanamino]-5-methoxy-6-methylphenol (342) (33 mg, 0.08 mmol) in THF (0.4 ml) was added 1,1'-carbonyl-diimidazole (18 mg, 0.11 mmol) and the mixture was stirred at rt for 5 h.

Magnesium methoxide (7.5 mg, 0.09 mmol) and propanedioic acid monomethyl ester (19 mg, 0.16 mmol) were stirred in THF (0.4 ml) for 1 h, the solvent was evaporated and the solution of imidazolide added to the crude magnesium salt. After stirring for 23 h the mixture was partially evaporated, diluted with water and extracted with ethyl acetate. Drying and evaporation gave a crude product which was purified by chromatography (ethyl acetate: hexane: dichloromethane, 4:7:4) to give 2-[N-(benzyloxycarbonyl)-5-methoxycarbonyl-4-oxopentanamino]-5-methoxy-6-methylphenol (301) (25 mg, 68%) white crystals mp 62°. (Found: C, 64.2; H, 6.6; N, 3.5%. C23H27N07 requires C, 64.3; H, 6.3; N, 3.3%).

FTIR: $\nu_{\text{max}}$ (CHCl3) 3552, 3359, 1747, 1714, 1496, 1304, 1110 cm$^{-1}$.

$^1$H NMR: 11.98 (s, trace, enol OH), 7.27 (br s, 5H, Ph), 6.85 (d, 1H, J 8.8 Hz), 6.43 (d, 1H, J 8.8 Hz), 5.08 (br s, 2H, CH2Ph), 3.81 (s, 3H, CH3O), 3.69 (s, 3H, CO2CH3), 3.67 (v br m 2H, NCH2), 3.36 (br s, 2H, CH2CO2), 2.65 (m, 2H, CH2CO), 2.15 (s, 3H, CH3), 1.80 (br m, 2H, CH2); OH not resolved.

EIMS: m/z 429 (M, 15%; found 429.1789; C23H27NO7 requires 429.1788), 397 (M-CH3OH, 6), 321 (M-PhCH2OH, 12), 294 (M-CO2CH2Ph, 13), 289 (321-CH3OH, 5%), 262 (M-CO2CH2Ph-CH3OH, 12), 164 (see discussion of the 7-acetylamino analogue in text, 45), 91 (C7H7, 100).
1-(benzyloxycarbonyl)-8-methoxy-9-methyl-5,7,10-trioxo-1,2,3,4,5,6,7,10-octahydro-1-benzazocine-6-carboxylic acid methyl ester (348)

A solution of 2-[N-(benzyloxycarbonyl)-5-methoxycarbonyl-4-oxo-pentanamino]-5-methoxy-6-methyl-phenol (301) (40 mg, 0.093 mmol) in acetone (2 ml) was added to a solution of Fremy's salt (40 mg, 0.15 mmol) in pH 6.8 buffer (1.8 ml). The solution was stirred for 4 days with daily addition of Fremy's salt (30 mg, 0.11 mmol) and acetone and buffer to maintain the solution. The reaction mixture was quenched with saturated NH₄Cl solution (5 ml) and extracted with chloroform (3 x 5 ml). The organic phase was washed with brine, dried (Na₂SO₄) and the solvent evaporated under reduced pressure to yield the crude product. Chromatography (ethyl acetate: hexane: dichloromethane, 1: 2: 1) gave 1-(benzyloxycarbonyl)-8-methoxy-9-methyl-5,7,10-trioxo-1,2,3,4,5,6,7,10-octahydro-1-benzazocine-6-carboxylic acid methyl ester (348) (30 mg, 74%) as an orange oil which solidified on standing to yellow crystals, mp 75-78°. (Found: C, 62.8; H, 5.4%. C₂₃H₂₃NO₈ requires C, 62.6; H, 5.3%).

UV: λₘₐₓ (CHCl₃) 261, 332 nm.

IR: νₘₐₓ (CCl₄): 2950, 2930, 2855, 1720, 1650, 1610, 1445, 1340, 1320, 1245 cm⁻¹.

FTIR: νₘₐₓ (CHCl₃) 2957, 2931, 2872, 1712, 1669, 1650, 1610, 1448, 1341, 1321, 1297, 1244, 1178, 1124, 1052 cm⁻¹.

¹H NMR: the NMR spectrum is complicated and has not been fully assigned: in CDCl₃ the product appears to be largely in the enol form and present as a major and minor conformer in a ratio of about 2: 1; for the major conformer:

δ 12.96 (s, 1H, enol OH), 7.40-7.10 (m, 5H, Ph), 5.13 (d, 1H, J 13 Hz, CHPh), 4.93 (d, 1H, J 13 Hz, CHPh), 4.49 (br d, 1H, J 15 Hz, NCH), 4.01, (s, 3H, CH₃O), 3.41 (s, 3H, CO₂CH₃), 2.7-2.4 (m, 2H, NCH and CHCO), 1.99 (s, 3H, CH₃), 2.1-1.7, (m, 3H, CHCO and CH₂).

In addition some resonances attributed to the minor conformer were distinguishable:

δ 13.03 (br s, enol OH), 5.22 (d, 1H, J 12 Hz, CHPh), 5.03 (d, 1H, J 12 Hz, CHPh), 4.29 (br d, 1H, J 16 Hz, NCH), 3.73 (s, 3H, CO₂CH₃).

EIMS: m/z 441 (M, 2%; found 441.1426; C₂₃H₂₃NO₈ requires 441.1424), 411 (M+2-
CH$_3$OH, 4), 306 (M-CO$_2$CH$_2$Ph, 29), 303 (M+2-PhCH$_2$OH-CH$_3$OH, 10), 276
(M+2-CH$_3$OH-CO$_2$CH$_2$Ph, 13), 274 (M-CO$_2$CH$_2$Ph-CH$_3$OH, 26), 91 (C$_7$H$_7$,
100).

7-methoxy-6-methyl-5,8-dioxo-2,3,5,8-tetrahydro-1H-pyrolo[1,2-a]indole-9-
carboxylic acid methyl ester (203)

1-(benzyloxy carbonyl)-8-methoxy-9-methyl-5,7,10-trioxo-1,2,3,4,5,6,7,10-
octahydro-1-benzazocine-6-carboxylic acid methyl ester (348) (132 mg, 0.30 mmol)
was dissolved in methanol (20 ml) and 10% palladium on charcoal (8 mg) added. The
mixture was stirred under an atmosphere of hydrogen for 21 h (colourless), then the
apparatus was flushed with nitrogen. Methanol was added to dissolve a small amount of
white precipitate. The solution was stirred in air for 24 h to give a tan precipitate. The
mixture was filtered (celite) and the residue washed with dichloromethane; evaporation
of the combined filtrates gave a crude yellow-orange product. Chromatography (ethyl
acetate : hexane : dichloromethane, 70: 90: 130) gave 7-methoxy-6-methyl-5,8-dioxo-
2,3,5,8-tetrahydro-1H-pyrolo[1,2-a]indole-9-carboxylic acid methyl ester (203) (71 mg,
82%). Recrystallisation from dichloromethane-hexane gave very fine orange needles
which appear to be light sensitive; the mp was not sharp, the bulk melted in the range
210-215°. (Asano et al.).

UV: $\lambda_{max}$ (CHCl$_3$) 292, 330 (sh), 432 nm.

FTIR: $\nu_{max}$ (CHCl$_3$) 1725, 1673, 1645, 1507, 1439, 1288, 1261, 1125, 1094 cm$^{-1}$.

$^1$H NMR: NMR data was in accordance with that reported by Gredley and Asano et
al.

$\delta$ 4.30 (t, 2H, J 7.4 Hz, NCH$_2$), 4.06 (s, 3H, CH$_3$O), 3.87 (s, 3H, CO$_2$ CH$_3$), 3.11
(t, 2H, J 7.6 Hz, H1,1'), 2.59 (quintet, 2H, J 7.5 Hz, NCH$_2$CH$_2$), 1.95 (s, 3H,
CH$_3$).

$^{13}$C NMR: $\delta$ 179.3 and 177.0 (quinone CO), 163.4 (CO$_2$), 157.7 (CH$_3$OC), 150.7
(C4a), 127.9*, 126.0*, 124.9*, (C8a, C9 or C9a), 107.0 (CH$_3$C), 61.3 (CH$_3$O),
51.6 (CO$_2$CH$_3$), 47.8 (NCH$_2$), 26.4*, 25.5*, (C1 or C2), 8.2 (CH$_3$C).

-E 13-
EIMS: $m/z$ 289 (M, 40%), 274 (M-CH$_3$, 9), 258 (26), 257 (M-CH$_3$OH, 73), 246 (M-CH$_3$-CO, 28), 230 (20), 229 (257-CO, 53), 228 (31), 227 (40), 218 (26), 214 (24), 201 (229-CO, 29), 199 (29), 186 (24), 171 (33), 143 (20), 133 (30), 132 (24), 131 (28), 130 (39), 116 (20), 115 (40), 105 (27), 104 (45), 103 (54), 102 (21), 91 (32), 90 (20), 89 (27), 83 (55), 79 (34), 78 (58), 77 (100), 76 (44), 67 (40), 66 (28), 65 (44), 63 (31), 59 (45), 55 (75), 53 (36), 52 (25), 51 (54).
3-amino-2-methylphenol (350)

Ground 3-hydroxy-2-methylphenol (327) (commercial and not pure; 609 1.0 g, 8 mmol) and ammonium chloride (1.0 g) were dissolved in aqueous ammonia (28%, 19 ml) and heated in a pressure vessel at 225° for 38h. After cooling, sodium hydrogen sulfite (70 mg) was added and the solution partially evaporated to remove ammonia. The solution was extracted with ethyl acetate (3 x 15 ml) and the combined organic extracts extracted with sodium hydroxide (2M, 3 x 10 ml). The aqueous extracts were acidified with concentrated hydrochloric acid to pH 1 with cooling, extracted with ethyl acetate then neutralised with saturated sodium carbonate solution; extraction with ethyl acetate (4 x 10 ml), washing with brine and drying over sodium sulfate gave, on evaporation, 3-amino-2-methylphenol (350) (6.93g, 70%). Recrystallisation from water gave pale pink crystals mp 127-8° (lit. 610 129°).

IR: IR data was in accord with the literature: 611

$\nu_{\text{max}}$ (KBr) 3355, 3290, 1592, 1468, 1371, 1301, 1284, 1078, 901, 767, 708, 683 cm$^{-1}$.

$^1$H NMR: $\delta$ 6.87 (t, 1H, J 7.9 Hz, H5), 6.31 (d, 1H, J 7.9 Hz) and 6.23 (d, 1H, J 7.9 Hz, H4 and H6), 4.68 (br s, 1H, OH), 3.65 (br s, 2H, NH$_2$), 2.06 (s, 3H, CH$_3$). The phenol is only sparingly soluble in CDCl$_3$.

EIMS: $m/z$ 124 (11%), 123 (M, 100), 122 (49), 106 (M-OH, 16), 104 (11), 94 (34), 93 (11), 78 (16), 77 (17).
3-(acetylamino)-2-methylphenol (351)

3-amino-2-methylphenol (350) (142 mg, 1.15 mmol) was dissolved in ethanol (2.5 ml) and acetic anhydride (120 µl, 1.27 mmol) added to the stirred solution. After ten minutes the solution was evaporated to give crude 3-(acetylamino)-2-methylphenol (351) in quantitative yield suitable for further reaction. Recrystallisation from acetone-hexane gave white needles, mp 163.5-164.5°; sublimation gave analytically pure material (found C, 65.7; H, 7.0; N, 8.6%. C₉H₁₁NΟ₂ requires C, 65.4; H, 6.7; N, 8.5%). UV: λ_{max} (EtOH) 278 (ε 1.4x10³), 217 nm (1.0x10⁴).

FTIR: ν_{max} (KBr) 3293, 2925, 1732 (weak), 1627, 1600, 1542, 1471, 1281, 782, 755, 708 cm⁻¹.

¹H NMR: δ (CD₃OD) 6.97 (t, 1H, J 8 Hz, H₅), 6.77 (d, 1H, J 8 Hz), 6.66 (d, 1H, J 8 Hz), 2.13 (s, 3H, CH₃), 2.06 (s, 3H, CH₃).

δ (CD₃COCD₃) 8.5 (br s 1H), 8.32 (s 1H), 7.13 (d, 1H, J 8 Hz), 6.94 (t, 1H, J 8 Hz, H₅), 6.66 (d, 1H, J 8 Hz), 2.10 (s 6H, CH₃ and CH₃).

¹³C NMR: δ (CD₃OD), 172.2 (CO), 157.1 (COH), 137.7 (CN), 126.9 (C₅), 121.6 (C₂), 118.4*, 113.8* (C₄ or C₆), 22.9 (CH₃CO), 10.8 (CH₃).

EIMS: m/z 165 (M, 45%), 123 (M-CH₂CO, 100), 122 (34), 106 (12), 94 (21), 77 (17).
EXPERIMENTAL

3-(acetylamino)-2-methyl-6-nitrophenol (352) and 3-(acetylamino)-2-methyl-4-nitrophenol (353)

A cooled (ice-salt) mixture of nitric acid (70%, 0.10 ml, 1.6 mmol) in acetic anhydride (15 ml) (care, exotherm!) was added with stirring to cold 3-(acetylamino)-2-methylphenol (351) (251 mg, 1.52 mmol). An orange yellow solution slowly formed and after 30 minutes, when TLC (ethyl acetate) indicated complete consumption of starting material, the solution was poured onto ice (30 g). On standing a yellow precipitate of pure 3-(acetylamino)-2-methyl-6-nitrophenol (352) formed in variable yield, this was filtered and washed with ice water to give typically 128 mg, 40% of the nitrophenol (352). The filtrate was extracted with chloroform (3x20 ml) and the combined extracts washed with brine and dried over sodium sulfate to give more of the nitrophenol (352) (total yield 166 mg, 52%). Recrystallisation from ethyl acetate gave 3-(acetylamino)-2-methyl-6-nitrophenol (352) as fine yellow needles, mp 184-185°.

(Found: C, 51.5; H, 4.7; N 13.2%. C9H10N2O4 requires C, 51.4; H, 4.8; N 13.3%).

UV: $\lambda_{\text{max}}$ (EtOH) 328, 209 nm.

+ 1 drop 10% NaOH: 432, 308, 229 (sh), 205 nm.

FTIR: $\nu_{\text{max}}$ (CHCl3) 3449, 1705, 1611, 1545, 1473, 1431, 1287, 1255, 1168, 1090 cm$^{-1}$.

FTIR: $\nu_{\text{max}}$ (KBr) 3235, 3103, 1678, 1661, 1606, 1544, 1471, 1419, 1355, 1256, 1211, 1167, 1091, 724 cm$^{-1}$.

$^1$H NMR: $\delta$ 11.25 (s, 1H, OH), 8.00 (d, 1H, J 9.5 Hz), 7.86 (d, 1H, J 9.5 Hz), 7.19 (br s, 1H, NH), 2.28 (s, 3H, CH$_3$), 2.23 (s, 3H, CH$_3$).

$\delta$ (CD$_3$SOCD$_3$) 10.78 (br s, 1H), 9.61 (s 1H), 7.90 (d, 1H, J 9.3 Hz), 7.40 (d, 1H, J 9.3 Hz), 2.147 (s 3H, CH$_3$), 2.139 (s, 3H, CH$_3$).

$^{13}$C NMR: $\delta$ 168.5 (CO), 154.3 (C1), 144.2 (C3), 130.0 (C6), 123.7 (C5), 115.5 (C2), 112.9 (C4), 25.0 (CH$_3$CO), 9.4 (CH$_3$).

EIMS: $m/z$ 210 (M, 47%), 192 (M-H$_2$O, 0.3), 168 (M-CH$_2$CO, 100), 164 (M-NO$_2$, 0.6), 151 (16), 150 (35), 138 (22), 122 (22), 110 (21), 105 (16), 94 (13), 93 (17), 92 (13), 80 (11), 78 (12), 77 (14), 67 (20), 66 (22), 65 (43), 64 (11), 63 (13), 55 (10), 54 (14), 53 (28), 52 (26), 51 (22).
Further extraction with ethyl acetate (3×30 ml), washing of the combined extracts with brine and drying over sodium sulfate gave 3-(acetylamino)-2-methyl-4-nitrophenol (353) (119 mg, 37%). Recrystallisation from acetone gave off white plates, mp 202-203°. (Found: C, 51.2; H, 5.0; N, 13.0%. C₉H₁₀N₂O₄ requires C, 51.4; H, 4.8; N, 13.3%.)

UV: λₒ (EtOH) 317, 247 (sh), 206 nm.

+ 1 drop 10% NaOH: 418, 271, 211 nm.

FTIR: ν (KBr) 3376, 2925, 1742 (weak), 1668, 1604, 1585, 1486, 1452, 1300, 1263, 1092 cm⁻¹.

¹H NMR: δ (CD₃COCD₃) 9.65 (v br s, 1H, OH), 9.07 (br s, 1H, NH), 7.76 (d, 1H, J 9 Hz, H5), 6.90 (d, 1H, J 9 Hz, H6), 2.15 (s, 3H, CH₃), 2.14 (s, 3H, CH₃).

δ (CD₃SOCD₃) 10.84 (v br s, 1H), 9.77 (s, 1H), 7.71 (d, 1H, J 9 Hz), 6.83 (d, 1H, J 9 Hz), 2.028 (s, 3H, CH₃), 2.023 (s, 3H, CH₃).

¹³C NMR: δ (CD₃COCD₃) 169.1 (CO), 161.1 (C1), 140.0 (C3), 133.1 (C4), 124.8 (C5), 113.0 (C6), 23.2 (CH₃CO), 11.6 (CH₃); C2 not observed.

δ (CD₃SOCD₃) 168.2, 160.2, 138.4, 131.4, 123.7, (C5), 122.7 (C2), 111.7, 22.6, 10.9.

EIMS: m/z 210 (M, 2%), 192 (M-H₂O, 39), 169 (11), 168 (M-CH₂CO, 78), 165 (11), 164 (M(NO₂, 100), 151 (26), 150 (25), 138 (39), 133 (23), 122 (30), 121 (28), 120 (17), 100 (16), 94 (26), 93 (13), 80 (22), 79 (10), 77 (20), 67 (22), 66 (19), 65 (35), 64 (10), 63 (11), 55 (15), 54 (19), 53 (25), 52 (30), 51 (21).
EXPERIMENTAL

3-(acetylamino)-6-[N-(benzyloxycarbonyl)-3-(tert-butoxycarbonyl)propanamino]-2-methylphenol (355)

To a solution of the nitrophenol (352) (16 mg, 0.08 mmol) in ethanol (1.5 ml) was added platinum oxide (3 mg). The mixture was stirred under a hydrogen atmosphere for 16 h and the colourless solution filtered and evaporated under argon to give the air sensitive, crude 3-(acetylamino)-6-amino-2-methylphenol (354).

EIMS m/z 180 (M, 86%), 138 (M-CH2CO, 100), 137 (44), 109 (80), 43 (29).

To the crude solid under argon was added sodium hydrogen carbonate (7 mg, 0.08 mmol) and the tert-butyl 4-bromobutanoate (335) (17.5 mg, 0.08 mmol) via DMPU (0.5 ml). The mixture was stirred for 48 h, diluted with THF (1 ml) and cooled to -20°. Pyridine (7 µl, 0.09 mmol) and benzyl chloroformate (11 µl, 0.08 mmol) were added and the mixture was allowed to stir for 2 h then stirred for 4 h at 0°. The solution was diluted with water (2 ml), extracted with ethyl acetate (3 x 5 ml); the extracts were washed with water (3 x 5 ml), dried with sodium sulfate and evaporated.

Chromatography with neat ethyl acetate gave 3-(acetylamino)-6-[N-(benzyloxycarbonyl)-3-(tert-butoxycarbonyl)-propanamino]-2-methylphenol (355) (15 mg, 43%) as a colourless glass. Low temperature recrystallisation from dichloromethane - hexane gave white crystals mp 120-121.5°. We have been able to obtain satisfactory high resolution mass spectral data but not microanalytical data (found: C, 66.3; H, 7.2; N, 6.1%; C25H32N2O6 requires C, 65.8; H, 7.1; N, 6.1%; found: M 456.2259; C25H32N2O6 requires 456.2260).

IR: \( \nu_{\text{max}} \) (CCl4) 3460 (weak), 3320, 2930, 1743, 1714, 1600 cm\(^{-1}\).

\(^1\)H NMR: \( \delta \) (CDCl3, 50°) 7.3 (m, 6H, PhH and H4 or 5), 7.07 (v br s, 1H, NH), 6.97 (br s, 1H, OH), 6.90 (d, 1H, J 8.5 Hz, H4 or 5), 5.14 (s, 2H, CH2Ph), 3.68 (br, 2H, NCH2), 2.32 (t, 2H, J 6.8 Hz, CH2CO), 2.17 (s, 3H, CH3), 2.15 (br s, 3H, CH3), 1.80 (quintet, 2H, J 6.9 Hz, NCH2 CH2), 1.41 (s, 9H, tBu).

\(^13\)C NMR: \( \delta \) (CDCl3, 25°) 173.8 br (CO2tBu), 168.6 (CH3CON), 156.3 (NCO2), 151.4 br, 136.4 br, 135.9, 128.5, 128.1, 127.6, 126.1 br, 125.1 br, 115.8 br, 81.0 (CMe 3), 67.6 br (CH2Ph), 51.3 br (NCH2), 33.8 br (CH2CO2), 28.0 (Me3), 24.3 (CH3CON), 23.6 (CH2), 10.6 (CH3C); one aromatic resonance not observed.
Mr A.J. Herlt has repeated this preparation on a larger scale in these laboratories with proportionately more of the bromobutanoate (335) [525 mg of 352; 16 ml DMPU, 420 mg NaHCO3, 892 mg of 335, 22 h; 33 ml THF, 0.244 ml pyridine, 0.43 ml benzyl chloroformate, 50 ml H2O, 3 x 50 ml ethyl acetate, 5 x 50 ml H2O] to obtain 355 (541 mg, 47%). Also isolated was:

3-(acetylamino)-6-(benzyloxycarbonylamino)-2-methylphenol (356) (341 mg, 43%). The solubility of 356 was low in all solvents investigated. Recrystallisation from methanol-water gave white crystals, mp 170-172°. (Found: C, 65.1; H, 6.0; N, 8.8%. C17H18N2O4 requires C, 65.0; H, 5.8; N, 8.9%).

FTIR: \( v_{\text{max}} \) (KBr) 3301, 3033, 1685, 1653, 1521, 1281, 1256, 1089, 1070, 789, 747, 696 cm\(^{-1}\).

\(^1\)H NMR: \( \delta \) (CD\(_3\)COCD\(_3\)) 8.56 (br s, 1H), 8.42 (br s, 2H), 7.42 (m, 5H, Ph), 7.21 (d, 1H, J 8.7 Hz, H4 or 5), 7.10 (d, 1H, J 8.7 Hz, H5 or 4), 5.21 (s, CH\(_2\)), 2.14 (s, CH\(_3\)), 2.09 (s, CH\(_3\)).

\(^{13}\)C NMR: \( \delta \) (CD\(_3\)COCD\(_3\)) 169.0 (CH\(_3\)CO), 156.3 (NCO\(_2\)), 147.4, 137.6, 135.1, 129.4, 129.1, 124.3, 121.8, 119.8, 117.6, 67.8 (CH\(_2\)), 23.6 (CH\(_3\)CO), 11.3 (CH\(_3\)); one aromatic resonance was not observed.

EIMS: \( m/z \) 314 (M, 3%; found 314.1268; C17H18N2O4 requires 314.1267), 206 (M-PhCH\(_2\)OH, 7), 179 (M-CO\(_2\)CH\(_2\)Ph, 8), 164 (M-PhCH\(_2\)OH-CH\(_2\)CO, 20), 137 (M-CO\(_2\)CH\(_2\)Ph-CH\(_2\)CO, 13), 108 (PhCH\(_2\)OH, 20), 91 (C\(_7\)H\(_7\), 100), 79 (19).
6-(acetylamino)-3-(3-tert-butoxycarbonylpropyl)-7-methyl-2-benzoxazalone
tert-butyl ester (357)

3-(acetylamino)-6-[N-(benzyloxycarbonyl)-3-(tert-butoxycarbonyl)-
propanamino]-2-methylphenol (355) (4 mg) was sublimed at 174°, 0.05 mm to give 6-
(acetylamino)-3-(3-tert-butoxycarbonylpropyl)-7-methyl-2-benzoxazalone tert-butyl ester (357) as a white solid in quantitative yield (found: M, 348.1686; C18H24N2O5 requires 348.1685).

$^1$H NMR: $\delta$ 7.39 (d, 1H, J 8.3 Hz, H4 or 5), 6.93 (s, 1H, NH), 6.88 (d, 1H, J 8.3 Hz, H4 or 5), 3.86 (t, 2H, J 7.1 Hz, NCH$_2$), 2.31 (t, 2H, J 7.2 Hz, CH$_2$CO), 2.28 (s, 3H, CH$_3$), 2.26 (s, 3H, CH$_3$), 2.03 (quintet, 2H, J 7 Hz, CH$_2$), 1.45 (s, 9H, C$_4$H$_9$).

EIMS: $m/z$ 348 (M, 24%), 292 (M-C$_4$H$_8$, 22), 275 (M-OC$_8$H$_9$, 29), 250 (292-CH$_2$CO, 36) 206 (M-CH$_2$CHCH$_2$CO$_2$C$_4$H$_9$, 11), 164 (206-CH$_2$CO, 54), 87 (28), 57 (100).
EXPERIMENTAL

3-(acetylamino)-6-[N-(benzyloxycarbonyl)-3-carboxypropanamino]-2-methylphenol (358)

3-(Acetylamino)-6-[N-(benzyloxycarbonyl)-3-(tert-butoxycarbonyl)propanamino]-2-methylphenol (355) (20 mg, 0.04 mmol) was dissolved in 40% trifluoroacetic acid in dichloromethane (v/v, 1 ml) and stirred for 2 h. Cautious evaporation gave crude 3-(acetylamino)-6-[N-(benzyloxycarbonyl)-3-carboxypropanamino]-2-methylphenol (358) as a glassy solid in quantitative yield. We have not been able to satisfactorily recrystallise the acid. HRMS found: 400.1636; C21H24N2O6 requires 400.1634.

FTIR: \( \nu_{\text{max}} \) (KBr) 3265, 2931, 1728, 1685, 1624, 1389, 1317, 1293, 1087, 746, 698 cm\(^{-1}\).

IR: (CCl4): \( \nu_{\text{max}} \) 1695, 1600 cm\(^{-1}\).

\(^1\)H NMR: \( \delta \) (CDCl\(_3\), 51°) 7.30 (br M, 6H, Ph and H4 or 5), 6.92 (d, 1H, J 8 Hz, H4 or 5), 5.14 (s, 2H, CH\(_2\)Ph), 3.72 (t, 2H, J 7 Hz NCH\(_2\)), 2.39 (t, 2H, J 7 Hz), 2.16 (s, 3H, CH\(_3\)), 2.12 (br s, 3H CH\(_3\)CO), 1.83 (quintet, 2H, J 7 Hz, CH\(_2\)); exchangeable protons were not resolved. The solubility in CDCl\(_3\) varies depending on the sample history.

\(^13\)C NMR: \( \delta \) (CDCl\(_3\) + CD\(_3\)SOCD\(_3\)) 175.6 (CO\(_2\)H), 168.7 (CONH), 155.8 (NCO\(_2\)), 151.3, 135.7, 128.8, 128.1, 128.0, 127.5, 127.2, 125.0, 116.1, 67.0 (CH\(_2\)Ph), 50.0 (NCH\(_2\)), 31.8 (CH\(_2\)CO), 23.7*, 23.3* (CH\(_3\)CON or CH\(_2\)), 10.8 (CH\(_3\)).

EIMS: \( m/z \) 400 (m, 1.5%), 292 (M-PhCH\(_2\)OH, 47), 250 (292-CH\(_2\)CO, 40), 164 (250-CH\(_2\)CHCH\(_2\)CO\(_2\)H, 78), 108 (PhCH\(_2\)OH, 85), 107 (PhCH\(_2\)O, 59), 91 (C\(_7\)H\(_7\), 35), 79 (100), 77 (53).
EXPERIMENTAL

3-(acetylamino)-6-[N-(benzyloxycarbonyl)-5-methoxycarbonyl-4-oxopentanamino]-2-methylphenol (302)

To a solution of crude 3-(acetylamino)-6-[N-(benzyloxycarbonyl)-3-carboxypropanamino]-2-methylphenol (358) (from 20 mg, 0.044 mmol of ester 355) in THF (0.4 ml) was added 1,1-carbonyl diimidazole (11 mg, 0.067 mmol) and the solution was stirred for 6 h.

Propanedioic acid monomethyl ester (13 mg, 0.11 mmol) was added to magnesium methoxide (4.5 mg, 0.05 mmol) in THF (0.3 ml) and the mixture stirred for 4.5 h then evaporated and vacuum dried.

The solution of imidazolide was added to the dried magnesium salt (canula) with a further 0.6 ml of THF used to rinse. The suspension was stirred under argon for 80 h then partially evaporated and diluted with water (2 ml). Extraction with chloroform, drying over sodium sulfate and evaporation gave a crude product which was chromatographed with neat ethyl acetate to give 3-(acetylamino)-6-[N-(benzyloxycarbonyl)-5-methoxycarbonyl-4-oxopentanamino]-2-methylphenol (302) (9 mg, 44%) as a colorless oil (found: M, 456.1894; C24H28N2O7 requires 456.1897).

Also isolated was the more polar 6-(acetylamino)-3-(5-methoxycarbonyl-4-oxopentyl)-7-methyl-2-benzoxazalone (362) (3 mg, 20%) (Found: M, 348.1322; C17H20N2O6 requires 348.1321).

3-(acetylamino)-6-[N-(benzyloxycarbonyl)-5-methoxycarbonyl-4-oxopentanamino]-2-methylphenol (302):

$^{1}$H NMR: δ 11.98 (s, << 1H, enol OH), 7.45-7.20 (br m, 6H, Ph and H4 or 5), 7.08 (s, 1H, OH or NH), 6.90 (d, 1H, H4 or 5), 5.10 (br s, 2H, CH$_2$Ph), 3.9-3.5 (v br s, 2H, NCH$_2$), 3.70 (s, 3H, OCH$_3$), 3.38 (br s, 2H, CH$_2$CO$_2$), 2.65 (br s, CH$_2$CO), 2.20 (s, 3H, CH$_3$), 2.17 (s, 3H, CH$_3$), 1.75 (br s, 2H, CH$_2$); NH or OH not observed.

$^{13}$C NMR: δ 168.5* (CONH), 167.5* (CO$_2$), 156.4 (NCO$_2$), 135.8, 128.47, 128.44, 128.43, 67.7 (CH$_2$Ph), 52.3 (CO$_2$CH$_3$), 48.8 (CH$_2$CO$_2$), 24.3 (CH$_3$CON), 22.1 (CH$_2$), 10.5 (CH$_3$); ketone and six aromatic resonances not observed, two
methylene not observed possibly because of broadening.

EIMS: m/z 456 (M, 12%), 425 (M-OCH3, 1), 424 (M-CH3OH, 4), 348 (M-PhCH2OH, 17), 321 (M-CO2CH2Ph, 17), 191 (see text; found 191.0831, C10H11N2O2 requires 19.0820, 44), 108 (PhCH2OH, 26), 91 (C7H7, 100); the low mass cutoff was m/z 90.

6-(acetylamino)-3-(5-methoxycarbonyl-4-oxopentyl)-7-methyl-2-benzoxazalone (362):

1H NMR: δ 7.37 (d, 1H, J 8.3 Hz, H4 or 5), 6.98 (br s, 1H, NH), 6.89 (d, 1H, J 8.3 Hz, H4 or 5), 3.83 (t, 2H, J 7 Hz, NCH2), 3.74 (s, 3H, CO2CH3), 3.47 (s, 2H, CH2CO2), 2.66 (t, J 6.7 Hz, CH2CO), 2.28 (s, 3H, CH3), 2.22 (s, 3H, CH3), 2.06 (quintet, J 6.8 Hz, CH2).

EIMS: m/z 349 (20%), 348 (M, 100), 317 (M-CH3O, 12), 316 (M-CH3OH, 31), 306 (M-CH2CO, 20), 275 (M-CH2CO2CH3, 17), 232 (M-CH2COHCH2CO2CH3, 22), 219 (M-CH2CH2CO2CH3, 5), 206 (M-CH2CH2CH2CO2CH3, 10), 190 (232-CH2CO, 32), 177 (219-CH2CO, 6), 164 (206-CH2CO, 90), 143 ((CH2)3COCH2CO2CH3, 62), 133 (20), 111 (89), 101 (COCH2CO2CH3, 76); the low mass cutoff was m/z 90.

The major product from some reactions was 9-(acetylamino)-6-(benzyloxy carbonyl)-10-methyl-3,4,5,6-tetrahydro-1,6-benzoxacin-2(2H)-one (361). (Found 382.1529, C21H22N2O5 requires 382.1529.)

1H NMR: The NMR spectrum is complex apparently because of the presence of two conformers in a ratio of about 1.1:1 (major : minor).

δ 8.00 (br s, 1H major), 7.74 (br d, J 8.1 Hz, 1H minor), 7.49 (br d, J 8.5 Hz) with 7.45-7.15 (m) and 7.10 (d, J 8.5 Hz) and 7.04 (d, J 8.5 Hz, 7H combined), 5.20 (s, 2H major, CH2Ph), 5.07 (s, 2H minor, CH2Ph), 3.7 (v br s, 2H, NCH2), 2.4-1.9 (br m, 10H) including 2.34 (br s), 2.216 (s), 2.18 (s), 2.11 (s), 1.94 (s)

EIMS: m/z 382 (M, 45%), 247 (M-CO2CH2Ph, 79), 91 (C7H7, 100).
EXPERIMENTAL

8-(acetylamino)-1-(benzyloxycarbonyl)-6-methoxycarbonyl-9-methyl-
1,2,3,4,5,6-hexahydro-1-benzazocine-5,7,10-trione (366)

3-(acetylamino)-6-[N-(benzyloxycarbonyl)-5-methoxycarbonyl-4-
oxopentanamino]-2-methylphenol (302) (10.8 mg, 0.023 mmol) was dissolved in
acetone and a solution of Fremy’s salt (65 mg, 0.24 mmol) in pH 6.8 buffer (3.5 ml)
added. The mixture was stirred for 28 h, diluted with saturated ammonium chloride
solution (3.5 ml) and extracted with dichloromethane (4 x 2 ml). The extracts were
dried over sodium sulfate, evaporated and the crude product was chromatographed with
methanol - dichloromethane (4: 96) to give 8-(acetylamino)-1-(benzyloxycarbonyl)-6-
methoxycarbonyl-9-methyl-1,2,3,4,5,6-hexahydro-1-benzazocine-5,7,10-trione (366)
(8.3 mg, 75%) as an orange solid (note the product streaks on chromatography).

1H NMR: the NMR spectrum is complicated and has not been fully assigned: in CDCl3
the product appears to be largely in the enol form and present as a major and
minor conformer in a ratio of about 3: 1; for the major conformer:
δ 13.03 (s, 1H, enol OH), 7.56 (s, 1H, NH), 7.40-7.10 (m, 5H, Ph), 5.15 (d, 1H, J
12.7 Hz, CHPh), 4.90 (d, 1H, J 12.7 Hz, CHPh), 4.49 (ddd, 1H, J 14.5, 4.0 and 2.2
Hz, NCH), 3.37 (s, 3H, CO2CH3), 2.7-2.45 (m, 2H, NCH and CHCO), 2.3-2.2 (m,
1H, CHCO), 2.25 (s, 3H, CH3CONH), 2.01 (s, 3H, CH3), 2.1-1.7 (m, 2H, CH2).

In addition some resonances attributed to the minor conformer were
distinguishable:
δ 13.07 (br s, enol OH), 7.52 (s, 1H, NH), 5.19 (d, 1H, J 12.3 Hz, CHPh), 5.03 (d,
1H, J 12.3 Hz, CHPh), 4.28 (ddd, 1H, J 15.2, 4.3, 1.9 Hz, NCH), 3.72 (s, 3H,
CO2CH3).

13C NMR: δ 183.0 and 182.7 (C7, C10), 178.0 (C5 major), 177.3 (C5 minor), 171.0
(CO2CH3), 167.6 (CONH), 153.7 (NCO2), 141.6*, 137.8*, 136.3*, 132.7*, (C6a,
C8, C9, C10a or benzyl quaternary), 128.5, 128.2 and 127.9 (3 x benzyl CH
minor), 128.4, 127.8 and 127.2 (3 x benzyl CH major), 94.2 (C6), 67.7 (CH2Ph
minor), 67.4 (CH2Ph maj), 51.8 (CO2CH3), 47.7 (NCH2), 33.0 (CH2CO), 24.2
(CH3CO), 24.1 (C3), 14.2 (CH3).

EIMS: m/z 470 (M+2, 0.03%), 468 (M, 0.03), 438 (M+2-CH3OH, 1), 436 (M-CH3OH,
EXPERIMENTAL

0.04), 362 (M+2-PhCH₂OH, 0.3), 333 (M-CO₂CH₂Ph, 6), 330 (438-PhCH₂OH, 4), 320 (M+2-CH₂CO-PhCH₂OH, 0.3), 303 (438-CO₂CH₂Ph, 4), 291 (M-CH₂CO-CO₂CH₂Ph, 4), 288 (438-CH₂CO-PhCH₂OH, 15), 261 (303-CH₂CO, 3), 259 (291-CH₃OH, 4), 108 (PhCH₂OH, 15), 107 (PhCH₂O, 13), 91 (C₇H₇, 100), 79 (22). One resonance (C₆a, C₈, C₉, C₁₀a or benzyl quaternary) of major conformer not observed.

7-(acetylamino)-6-methyl-5,8-dioxo-2,3,5,8-tetrahydro-1H-pyrrolo[1,2-a]indole-9-carboxylic acid methyl ester (367)

8-(acetylamino)-1-(benzyloxycarbonyl)-6-methoxycarbonyl-9-methyl-1,2,3,4,5,6-hexahydro-1-benzazocine-5,7,10-trione (366) (6.6 mg, 0.014 mmol) was dissolved in methanol (1 ml) and 10% palladium on charcoal (4 mg) added. The mixture was stirred under an atmosphere of hydrogen for 2.5 h, flushed with argon and opened to the air. After stirring for 1 h the mixture was filtered through celite, washing with methanol and dichloromethane. Chromatography with methanol: dichloromethane (3: 97) gave 7-(acetylamino)-6-methyl-5,8-dioxo-2,3,5,8-tetrahydro-1H-pyrrolo[1,2-a]indole-9-carboxylic acid methyl ester (367) (2.1 mg, 47%) as an orange solid (found: M, 316.1059; C₁₆H₁₆N₂O₅ requires 316.1059). (Note that the starting material and product have similar Rfs, starting material turns purple when the TLC plate is exposed to ammonia vapour.)

¹H NMR: δ 7.82 (s, 1H, NH), 4.32 (t, 2H, J 7.4 Hz, NCH₂), 3.87 (s, 3H, OCH₃), 3.13 (t, 2H, J 7.7 Hz, CH₂CO), 2.61 (quintet, 2H, J 7.4 Hz, CH₂), 2.22 (s, 3H, CH₃CON), 1.95 (s, 3H, CH₃).

¹³C NMR: δ 178.6 and 178.0 (quinone CO), 167.5 (CONH), 163.2 (CO₂), 151.1*, 137.0*, 129.9* (some of C₄a, C₆, C₇, C₈, C₉ and C₉a), 51.7 (CO₂CH₃), 48.0 (NCH₂), 26.5*, 25.5* (C₁ or C₂), 24.2 (CH₃CON), 13.3 (CH₃C).

EIMS: m/z 316, (M, 19%), 285 (5), 284 (M-CH₃OH, 15), 274 (M-CH₂CO, 15), 243 (17), 242 (M-CH₃OH-CH₂CO, 100), 241 (20), 214 (214-CO, 52), 104 (24), 103 (23), 79 (20), 78 (35), 77 (56), 76 (32), 65 (26), 54 (24), 53 (26), 52 (32), 51 (35).

-E 26-
2-Phenyl-4,7-dihydro-1,3-dioxepin (425)

2-Phenyl-4,7-dihydro-1,3-dioxepin (425) was prepared in 79% yield by condensation of benzaldehyde and (Z)-but-2-ene-1,4-diol (424); bp 128°, 11.5 torr (lit.612 128-30°, 15 torr). The NMR was in accord with the literature220.

4-Benzylxy-but-2-en-1-ol (426)

4-Benzylxy-but-2-en-1-ol (426) was prepared in 85% yield by reaction of 425 with lithium aluminium hydride and aluminium chloride according to Eliel et al.;613 bp 115°, 2 mm (short path) (lit.614 114-120°, 0.4 mm). The 1H NMR and 13C NMR were in accord with the literature.615

(±)-4-(4-bromobenzyloxy)-cis-2,3-epoxybutan-1-ol (428)

To a solution of 4-(4-bromobenzyloxy)-but-2-en-1-ol (427)438 (1.00 g, 3.89 mmol) in chloroform (10 ml) at 0°C was added a solution of m-chloroperbenzoic acid (0.988 g, 5.73 mmol) in chloroform (10 ml). The solution was allowed to warm to room temperature overnight. Washing with 10% aqueous sodium hydrogen sulfite, 5% aqueous sodium carbonate and brine and extraction of the combined aqueous washes with chloroform, gave a combined organic solution which was dried over magnesium sulfate. Evaporation gave a crude white solid which was chromatographed (ethyl acetate: hexane: dichloromethane, 6: 4: 4) to afford 4-(4-bromobenzyloxy)-cis-2,3-epoxybutan-1-ol (428) (1.02 g, 96%). The 1H and 13C NMR were identical with those of chiral material prepared according to Chong and Wong.438

EIMS: m/z 274/273/272/271 (M and M-H, 0.1/0.1/0.1/0.1%), 187/185 (78/93), 171/169 (C7H6Br, 93/100), 90 (56), 89 (39), 77 (28), 57 (C3H5O, 56; found: 57.0347; C3H5O requires 57.0340).
(2S,3R)-4-benzyloxy-2,3-epoxybutan-1-ol (418)

To a stirred mixture of dichloromethane (1.1 ml) and molecular sieves (6, 4Å) was added titanium tetra-isopropoxide (84 µl, 0.28 mmol). After cooling in acetone-dry ice (+)-diethyl tartrate (57 µl, 0.33 mmol) and 4-benzyloxybut-2-en-1-ol (426) (69 mg, 0.39 mmol) were added followed by more dichloromethane (0.5 ml) and a cold solution of t-butylhydroperoxide in toluene616 (0.29 ml, 2.65 M, 0.77 mmol). After 4.5 hours the reaction flask was transferred to a carbon tetrachloride-dry ice bath (ca -230°C) and allowed to warm overnight. Ether (1 ml) and saturated sodium sulfate solution (0.5 ml) were added and the mixture stirred for 2 hours then filtered (celite) washing with sodium hydroxide in brine. Flash chromatography (ethylacetate: hexane: dichloromethane, 6:3:4) gave (2S,3R)-4-benzyloxy-2,3-epoxybutan-1-ol (418) (64 mg, 85%), [α]D -22° (c 0.51, CHCl3) otherwise identical with that reported by Hungerbühler and Seebach.426
EXPERIMENTAL

(2R,3R)-4-(4-bromobenzyloxy)-2,3-epoxy-butanoic acid (432)

(2S,3R)-4-(4-Bromobenzyloxy)-2,3-epoxybutan-1-ol (428) (500 mg, 1.83 mmol) and sodium hydrogen carbonate (3.71 mg, 4.41 mmol) were dissolved in a mixture of ethyl acetate (5 ml) and water (5 ml). Platinum black (Aldrich Chemical Co., 34 mg) was added and oxygen was bubbled through the stirred mixture. Portions of platinum black (9 mg) were added at 48 hour intervals and solvent lost by evaporation was replaced. The reaction is not easily monitored by TLC. After 190 hours the platinum was removed by centrifugation and washed with aqueous sodium hydrogen carbonate and ethyl acetate. Separation of the supernatant mixture gave an organic phase which was dried (MgSO₄) and evaporated to yield starting material (14 mg, 2.7%). The aqueous layer was acidified with acetic acid and extracted five times with chloroform to give, after drying (MgSO₄) and azeotropic evaporation of several aliquots of cyclohexane, essentially pure (2R,3R)-4-(4-bromobenzyloxy)-2,3-epoxy-butanoic acid (432) (506 mg, 96%) as an oil which solidified on standing. Recrystallisation from dichloromethane-hexane gave poor white crystals, mp 97-98° (Found: C, 46.2; H, 3.8; Br, 28.0%. C₁₁H₁₁O₄Br requires C, 46.0, H, 3.9; Br, 27.8%).

IR: \( \nu_{\text{max}} \) (KBr) 3425 (br), 3104 (br), 2925, 1743, 1215, 1120, 1106, 926, 840, 798 cm\(^{-1}\).

\(^1\)H NMR: \( \delta \) 10.3 (brs, 1H, CO₂H), 7.46, 7.19 (AA'BB', 4H, J \(_{\text{AB}}\) 8.1 Hz, PhBr), 4.50 (ABq, 2H, \( \Delta \delta 0.08, J_{\text{AB}} 12.0 \)), 3.81-3.68 (m, 2H, OCH₂), 3.59 (d, 1H, J 4.6 Hz, H2), 3.48 (dt=q, 1H, J 4.8 and 4.8 Hz, H3).

\(^13\)C NMR: \( \delta \) 172.9, 136.3, 131.6, 129.4, 121.9, 72.7, 66.9, 56.0, 51.1.

EIMS: \( m/z \) 288/286 (M, 25/25%), 188/187/186/185/184/183 (C\(_7\)H\(_6\)BrO etc., 24/84/57/100/49/49), 172/171/170/169 (C\(_7\)H\(_6\)Br, 27/98/29/98), 157 (22), 155 (11), 119 (23), 107 (37), 102 (28), 91 (31), 90 (70), 89 (63), 85 (23), 79 (25), 78 (33), 77 (55), 76 (20), 75 (30), 74 (14), 63 (22), 62 (44), 61 (17), 53 (68).
Methyl (2R,3R)-4-(4-bromobenzyl)-2,3-epoxybutanoate (435)

Freshly prepared ethereal diazomethane was added dropwise to a stirred, cooled suspension of (2R,3R)-4-(4-bromobenzyl)-2,3-epoxy-butanoic acid (432) (137.1 mg, 0.50 mmol) in ether (10 ml) until a yellow colour persisted. This colour was discharged by the addition of acetic acid. Evaporation from several aliquots of cyclohexane gave the crude ester. Chromatography (ethyl acetate : hexane : dichloromethane, 1:5:1) gave methyl (2R,3R)-4-(4-bromobenzyl)-2,3-epoxybutanoate (435) (136.8 mg, 95%) as an oil which solidified on standing (mp of solid 35-37°), [α]D -11° (c 2.3, CHCl3). (Found: C, 47.8; H, 4.2; Br, 26.4%. C12H13O4Br requires C, 47.9; H, 4.4; Br, 26.5%.)

IR: (KBr) νmax 2952, 1753, 1214, 1096, 812 cm⁻¹.

1H NMR: δ 7.48, 7.21 (AA'BB', 4H, JAB 8.3 Hz, PhBr), 4.51 (ABq, 2H, Δδ 0.086, JAB 12.1 Hz, CH₂Ph), 3.75 (s, 3H, OCH₃), 3.72 (d, 2H, J 5.2 Hz, OCH₂), 3.58 (d, 1H, J 4.6 Hz, CHCO₂), 3.42 (dt=q, 1H, J 4.9 and 4.9 Hz, OCH₂CH).

13C NMR: δ 168.0 (CO), 136.6 (CCH₂), 131.5 (BnC’), 129.3 (BnC’), 121.6 (CBr), 72.4 (CH₂Ph), 67.0 (OCH₂), 55.5 (C3), 52.3 (CO₂CH₃), 51.1 (C2).

EIMS: m/z 302/300 (M, 0.2/0.2%), 187/185 (C₇H₆BrO, 41/49), 186 (11), 171/169 (C₇H₆Br, 94/100), 116 (32), 115 (19), 113 (34), 99 (23), 91 (15), 90 (70), 89 (59), 87 (17), 84 (19) 77 (23), 69 (21), 59 (14), 57 (19).

NICI: m/z 383/381/379 (M+Br, 51/100/51%), 382 (14), 369/367/365 (M-CH₃+HBr?, 8/15/8), 301/299 (M-H, 16/18).
4-(4-bromobenzyloxy)-2,3-cis-epoxybutanal (433)

a) Using Pyridinium Dichromate

Crushed molecular sieves (4Å, 0.5 g) were heated under vacuum overnight at 200°C. After cooling, a stirrer, pyridinium dichromate (272 mg, 0.72 mmol) and dry dichloromethane (2.5 ml) were added and the mixture was cooled in ice. 4-(4-bromobenzyloxy)-cis-2,3-epoxybutan-1-ol (428) (126 mg, 0.46 mmol) was added neat and the mixture stirred at 0°C for 2 hours. More pyridinium dichromate (83 mg, 0.22 mmol) was added and after twenty minutes the mixture was diluted with ether (4 ml) and filtered on celite and magnesium sulfate to give a colourless crude oil on evaporation. Chromatography with neat dichloromethane gave pure 4-(4-bromobenzyloxy)-2,3-cis-epoxybutanal (433) (75 mg, 60%) as a colourless oil. An analytical sample was obtained by gas chromatography (dimethyl silicone, SGE 12QC3BP1 at 230°C) (found: C, 49.0, H, 4.1; Br, 29.8%. C11H11O3Br requires C, 48.7, H, 4.1, Br, 29.5%). Other data as below. Also isolated by chromatography was the more polar 4-(bromobenzyloxy)-cis-2,3-epoxybutyl 4-(4-bromobenzyloxy)-cis-2,3-epoxybutanoate (440) (10 mg, 8 mol% of starting material).

4-(bromobenzyloxy)-cis-2,3-epoxybutyl 4-(4-bromobenzyloxy)-cis-2,3-epoxybutanoate (440):

An analytical sample was prepared by short path distillation (150°, 0.1 mm) (found C, 48.7; H, 4.2; Br, 29.5%. C22H22O6Br2 requires C, 48.7; H, 4.1; Br, 29.5%).

IR: \( \nu_{\text{max}} \) (neat) 2860, 1752, 1592, 1486, 1195, 1092, 1012, 802 cm\(^{-1}\).

\(^1\)H NMR: \( \delta \) 7.50-7.45 (m, 4H, PhBr), 7.23-7.18 (m, 4H, PhBr), 4.57-4.36 (m, 5H, includes \( \text{CH}_2\text{PhX}_2 \)), 4.15-4.06 (m, 1H), 3.8-3.15 (m, 8H).

\(^13\)C NMR: \( \delta \) 167.4 (CO\(_2\)), 136.5/136.4 (Bn), 131.6/131.5 (Bn), 129.3 (Bn), 121.8/121.7 (Bn), 72.59/72.55, 67.74, 66.99/66.96, 63.65/63.62, 55.74/55.69 (CH), 54.55 (CH), 52.62/52.56 (CH), 51.08 (CH).

NICI: \( m/z \) 625/623/621/619 (M+Br, 19/71/69/24%), 543/541/539 (M-H, 4/7/4), 343/341 (M-\text{ArCH}_2\text{OCH}_2, 100/100), 287/285 (carboxylate, 32/32), 201/199 (\text{ArCH}_2\text{OCH}_2, 48/49).
b) Using Sulfur Trioxide-Pyridine

To an ice cooled solution of 4-(4-bromobenzyloxy)-2,3-epoxybutan-1-ol (428) (88 mg, 0.32 mmol) in dichloromethane (1 ml) was added triethylamine (0.5 ml, 11 mmol) and sulfur trioxide-pyridine complex (155 mg, 0.97 mmol) dissolved in dimethylsulfoxide (2 ml). After 2 hours more sulfur trioxide-pyridine (50 mg, 0.31 mmol) was added directly. After stirring for a further 2.5 hours the solution was diluted with ether (5 ml) and aqueous ammonium chloride (5 ml, 5.8 M). The mixture was separated and the aqueous phase extracted with ether; the combined ether extracts were dried over magnesium sulfate and evaporated to give, after vacuum drying, crude 4-(4-bromobenzyloxy)-2,3-cis-epoxybutanal (433) (78 mg, 89%). This material was used crude in further oxidation with bromine (see below).

4-(4-bromobenzyloxy)-2,3-cis-epoxybutanal (433):

$^1$H NMR: δ 9.44 (d, 1H, J 4.7 Hz, CHO), 7.48, 7.18 (AA'BB', 4H, J$_{AB}$ 8.3 Hz, PhBr), 4.50 (s, 2H, CH$_2$Ar), 3.86-3.72 (AB(X), 2H, J$_{AB}$ 11.7, J$_{AX}$ 3.0, J$_{BX}$ 4.6 Hz, OCH$_2$), 3.52-3.48 (m, 1H, OCH$_2$CH), 3.43 (dd=t, 1H, J 4.7, 4.7 Hz, CHCHO).

$^{13}$C NMR: δ 197.6 (CO), 136.1 (CCH$_2$), 131.6 (Bn), 129.4 (Bn), 121.9 (CBr), 72.7 (CH$_2$Ar), 66.3 (OCH$_2$), 57.8, 57.2 (C2 and C3).

EIMS: m/z 271/269 (M-H, 0.05/0.03%), 229/227 (M-CH$_2$CHO, 3/3), 185 (10), 171/169 (C$_7$H$_6$Br, 63/65), 119 (28), 91 (21), 90 (63), 89 (40), 86 (37), 85 (17), 78 (12), 77 (30), 75 (11), 71 (59), 70 (17), 69 (25), 63 (21), 59 (14), 58 (22), 57 (39), 51 (22), 50 (19), 46 (26), 45 (100).
EXPERIMENTAL

(±)4-(4-bromobenzyloxy)-cis-2,3-epoxy-butanoic acid (432) from the aldehyde

4-(4-Bromobenzyloxy)-2,3-cis-epoxybutanal (433) (51 mg, 0.19 mmol) and 3-hydroxyphenol (55 mg, 0.5 mmol) in THF (2 ml) were treated with a solution of sodium chlorite (technical, 43 mg ca 0.5 mmol) in pH 3.7 buffer (1 ml). The solution was stirred for 25 h, evaporated and partitioned between ether and aqueous sodium hydrogen carbonate (5%, 7.5 ml). After further extraction with ether (3x) the aqueous part was acidified with saturated oxalic acid solution (5.5 ml) and extracted with dichloromethane. Drying over magnesium sulfate and evaporation gave crude 4-(4-bromobenzyloxy)-cis-2,3-epoxy-butanoic acid (432) in quantitative yield. Data as before.

Methyl (2R,3R)-4-(4-bromobenzyloxy)-2,3-epoxybutanoate (435) from the aldehyde

Crude 4-(4-bromobenzyloxy)-2,3-cis-epoxybutanal (433) (see above, 73 mg, 0.29 mmol) in methanol (3.2 ml) and water (0.25 ml) was added to a mixture of bromine (0.29 g, 1.8 mmol), methanol (1.2 ml), water (0.13 ml) and sodium hydrogen carbonate (0.49 g, 6 mmol). The mixture was stirred for 5h then quenched with sodium thiosulfate (colourless), diluted with dichloromethane and filtered through magnesium sulfate. Chromatography (dichloromethane : hexane : methanol, 75:75:1) gave traces of impure starting material and the less polar methyl (2R,3R)-4-(4-bromobenzyloxy)-2,3-epoxybutanoate (435) (51 mg, 63%). Data as before.
Ethyl 6-(4-bromobenzyloxy)-cis-4,5-epoxy-3-oxohexanoic acid (437b)

4-(4-Bromobenzyloxy)-2,3-cis-epoxybutanal (433) (41 mg, 0.15 mmol) was dissolved in dichloromethane (0.3 ml) and stannous chloride (4 mg, 0.02 mmol) then ethyldiazoacetate (17 µl, 0.16 mmol) added. Evolution of gas was evident. After stirring for 30 minutes the suspension was filtered and evaporated to give crude, unstable product, fairly pure by NMR. Part of the crude product from this reaction (ca 10 mg) was dissolved in ether (5 ml) and extracted once with aqueous sodium bicarbonate (5%, 5 ml) then with sodium hydroxide solution (8%, 5 ml). The sodium hydroxide solution was acidified with sulfuric acid (3 M) and extracted with ether (1x5 ml) to give, on evaporation, ethyl 6-(4-bromobenzyloxy)-cis-4,5-epoxy-3-oxohexanoic acid (437b) (1.6 mg) which was pure by NMR.

\[ \delta \text{ H NMR: } 12.06 (s, <1H, enol OH), 7.48 (d, 2H, } J 8.4 \text{ Hz, PhBr), 7.17 (d, 2H, } J 8.4 \text{, PhBr), 4.52 (ABq, <1H, } J 12 \text{ Hz, enol CH}_2\text{PhBr), 4.45 (s, 2H, CH}_2\text{Ph), 4.19 (q, 2H, } J 7.1 \text{ Hz, CH}_2\text{CH}_3\text{), 3.75-3.4 (m, 6H, CH}_2\text{CHCHCOCH}_2\text{), 1.21 (t, 3H, } J 7.1 \text{ Hz, CH}_2\text{CH}_3\text{).} \]

\[ \delta \text{ C NMR: } 198.0 (C3), 136.1, 131.6, 129.5, 121.9, 90.6 (enol CH), 72.7 (OCH}_2\text{Ph), 66.3 (C6), 61.6 (CH}_2\text{CH}_3\text{), 57.3 and 57.1 (C4 and 5), 47.0 (CH}_2\text{CO}_2\text{), 14.1 (CH}_2\text{CH}_3\text{); other enol form and CO}_2\text{ resonances not observed.} \]

Some peaks in the MS may be due to impurities notably the furanones described below.

EIMS: \[ m/z \text{ 187/185 (14/15%), 171/169 (56/59), 128 (60), 100 (45), 90 (47), 89 (33), 70 (23), 69 (100).} \]

NICI \[ m/z \text{ 439/437/435 (M+Br, 11/21/11%), 358/357/356/355 (M and M-H, 30/100/31/98), 329/327 (27/28), 172 (26), 170 (19), 169 (21).} \]
**EXPERIMENTAL**

**2-[2-(4-bromobenzyloxy)-1-hydroxyethyl]-5-ethoxy-3 (2H)-furanone (441)**

The remaining crude ethyl 6-(4-bromobenzyloxy)-cis-4,5-epoxy-3-oxohexanoic acid (437b) (ca 44 mg) was dissolved in ether (5 ml) and dichloromethane (1 ml) and extracted with sodium hydroxide solution (2 M, 5 ml). The organic solution was washed with brine, dried over magnesium sulfate and evaporated to give a white solid (23 mg) mp 85-90°. Chromatography with 4% ethanol in dichloromethane gave a less polar fraction (3 mg) and a more polar fraction (16 mg); these are diastereomers of 2-[2-(4-bromobenzyloxy)-1-hydroxyethyl]-5-ethoxy-3 (2H)-furanone (441), the stereochemistry of the products has not been assigned.

The minor, less polar product was recrystallised from dichloromethane - hexane as fine needles mp 109-110° (found: C; 50.6; H, 4.7; Br, 22.7%. C15H17O5Br requires C, 50.4; H, 4.8; Br, 22.4%).

**UV:** \( \lambda_{\text{max}} \) (EtOH) 253 (\( e \ 1.5 \times 10^4 \)), 228 (sh), 222 (1.1 x 10^4).

**FTIR:** \( \nu_{\text{max}} \) (CHCl_3) 1689, 1576 (strong), 1388, 1358, 1011 cm\(^{-1}\).

**\( ^1H \text{NMR} \):** \( \delta \) 7.48 (d, 2H, \( J \ 8.4 \text{ Hz} \), PhBr), 7.21 (d, 2H, \( J \ 8.4 \text{ Hz} \), PhBr), 4.80 (s, 1H, H4), 4.70 (d, 1H, \( J \ 6.8 \text{ Hz} \), H2), 4.56 (ABq, 2H, \( \Delta \delta \ 0.05 \)), \( J_{\text{AB}} \ 12.1 \text{ Hz} \), CH\(_2\)Ph), 4.26 (q, 2H, \( J \ 7.1 \text{ Hz} \), CH\(_2\)CH\(_3\)), 4.09 (m, 1H, CHOH), 3.70 (m, 2H, OCH\(_2\)), 3.60 (br d, 1H, \( J \ 3 \text{ Hz} \), OH), 1.46 (t, 2H, \( J \ 7.1 \text{ Hz} \), CH\(_2\)CH\(_3\)).

**\( ^13C \text{NMR} \):** \( \delta \) 198.9, 185.1, 136.8, 131.5, 129.4, 121.6, 83.2, 80.5, 72.9, 70.4, 69.8, 68.3, 14.2.

**EIMS:** \( m/z \) 171/169 (C\(_7\)H\(_6\)Br, 24/27%), 157 (M-ArOCH\(_2\), 15), 128 (McLafferty rearrangement, 100), 100 (62), 90 (23), 69 (74).

**NICI:** \( m/z \) 320/327 (M-CH\(_2\)CH\(_3\), 96/100%), 311/309 (327-H\(_2\)O, 60/62), 248 (327-Br, 83); 169 (M-C\(_7\)H\(_6\)Br-H\(_2\)O, 70).
The dominant, more polar product was recrystallised from dichloromethane - ether as fine clear needles mp 111-114° (found: C, 50.3; H, 4.9%. C15H17O5Br requires C, 50.4; H, 4.8%).

UV: $\lambda_{\text{max}}$ (ETOH) 222, 252 nm.

FTIR: $\nu_{\text{max}}$ (CHCl3) 1694, 1576 (strong), 1474, 1425, 1388, 1358, 1013 cm$^{-1}$.

$^1$H NMR: $\delta$ 7.48 (d, 2H, $J$ 8.4 Hz, PhBr), 7.21 (d, 2H, $J$ 8.4 Hz, PhBr), 4.82 (s, 1H, H4), 4.68 (d, 1H, $J$ 3 Hz, H2), 4.54 (s, 2H, CH$_2$Ph), 4.30 (m, 1H, CHO), 4.26 (q, 2H, $J$ 7.1 Hz, CH$_2$CH$_3$), 3.69 (m, 2H, OCH$_2$), 3.00 (varies, br d, 1H, $J$ 5.5 Hz, OH), 1.46 (t, 3H, $J$ 7.1 Hz, CH$_2$CH$_3$).

$^{13}$C NMR: $\delta$ 197.4, 185.4, 136.7, 131.6, 129.3, 121.7, 85.5, 81.1, 72.7, 70.2, 68.8, 68.2, 14.2.

EIMS: $m/z$ 171/169 (C$_7$H$_6$Br, 19/20%), 157 (M-ArO$\text{CH}_2$, 13), 128 (McClafferty rearrangement, 100), 100 (69), 90 (24), 69 (72).

NICI: $m/z$ 439/437/435 (M+Br, 2/4/2%), 358/357/356/355 (M and M-H, 3/2/3/2), 329/327 (M-CH$_2$CH$_3$, 97/100), 311/309 (95/96), 248 (327-Br, 74), 169 (83).
EXPERIMENTAL

1-(4-bromobenzyloxy)-4-(tert-butyldimethylsilyloxy)-cis-2,3-epoxybutane (443)

To 4-(4-bromobenzyloxy)-cis-2,3-epoxybutan-1-ol (428) (5.23 g, 19.1 mmol) dissolved in DMF (20 ml) was added imidazole (2.87 g, 42.1 mmol) and tert-butyldimethylsilyl chloride (3.32 g, 22.0 mmol). After stirring overnight under Ar the reaction was quenched with water (100 ml) and extracted with ether (2 x50 ml). The extracts were washed with water and brine and dried over magnesium sulfate. Evaporation gave the crude product which was chromatographed (ethyl acetate: hexane, 1: 9) to give 1-(4-bromobenzyloxy)-4-(tert-butyldimethylsilyloxy)-cis-2,3-epoxybutane (443) (6.53 g, 88%) as a free flowing oil. Alternatively short path distillation (210°, 0.03 mm) gave a slightly higher yield of product, pure by NMR but with minor contaminants indicated by TLC. A small sample was rechromatographed: found: C, 52.6; H, 7.0; Br, 21.0%; C17H27O3BrSi requires C, 52.7; H, 7.0; Br, 20.6%.

A small sample was prepared from (2S,3R)-4-benzyloxy-2,3-epoxy-1-butanol (438) ([α]D -16.6°, c 1.5, CHCl3 i.e. 96% ee) to give (2R,3S)-1-(4-bromobenzyloxy)-4-(tert-butyldimethylsilyloxy)-2,3-epoxybutane (443) ([α]D -0.975°; c 0.14 CHCl3).

1H NMR: δ 7.48, 7.24 (AA'BB', 4H, JAB 8.3 Hz, PhBr), 4.53 (ABq, 2H, JAB ~0.09, CH2Ph), 3.80-3.67 (m, 3H), 3.57-3.49 (m, 1H), 3.28-3.22 (m, 1H), 0.89 (s, 9H, SiC4H9), 0.08 (s, 3H, SiCH3), 0.06 (s, 3H, SiCH3).

13C NMR: δ 136.8, 131.5, 129.4, 121.6, 72.3, 68.3, 61.5, 56.1, 55.0, 25.8, 18.3; (SiCH3 signals (ca -6ppm) were lost by foldback.

EIMS: mlz 301/299 (0.2, 0.2%), 171/169 (C7H6Br, 95/100), 117 (18), 101 (14), 90 (51), 89 (34), 77 (12), 75 (57), 73 (55), 59 (44), 58 (13), 57 (64), 55 (11), 45 (12), 43 (16).

CIMS: mlz 389/387 (M+H, 0.3/0.4%), 171/169 (C7H6Br, 100/100), 132 (14), 129 (12), 117 (13), 106 (13).

NICI: mlz 219 (18%), 218 (100), 217 (M-C7H6Br, 67).
(±)-(2R,3S)-3-azido-4-(4-bromobenzyloxy)-1-(tert-butydimethylsilyloxy)-2-butanol and
(±)-(2R,3S)-3-azido-1-(4-bromobenzyloxy)-4-(tert-butydimethylsilyloxy)-2-butanol (444)

1-(4-Bromobenzyloxy)-4-(tert-butydimethylsilyloxy)-cis-2,3-epoxybutane (443) (108 mg, 0.28 mmol) was dissolved in methanol and treated with sodium azide (73 mg, 1.1 mmol) and magnesium perchlorate in water (0.25 ml). The mixture was heated at 70°C for 34 hours. Filtration and evaporation gave a crude product which was chromatographed (ethyl acetate: hexane, 1:9) to give starting material (5 mg, 5%) and a partially separated mixture of (±)-(2R,3S)-3-azido-4-(4-bromobenzyloxy)-1-(tert-butydimethylsilyloxy)-2-butanol and (±)-(2R,3S)-3-azido-1-(4-bromobenzyloxy)-4-(tert-butydimethylsilyloxy)-2-butanol (444) (total 98 mg, 82%).

The less polar isomer:

FTIR: \( \nu_{\text{max}} \) (CHCl\(_3\)) 2105 cm\(^{-1}\).

\( ^1\text{H} \) NMR: \( \delta \) 7.49 (d, 2H, \( J \) 8.4 Hz, PhBr), 7.23 (d, 2H, \( J \) 8.4 Hz, PhBr), 4.54 (s, 2H, \( \text{CH}_2\text{Ph} \)), 3.8-3.6 (m, 3H), 2.42 (d, 1H, \( J \) 5.1 Hz, OH), 0.89 (s, 9H, \( \text{C}_4\text{H}_9 \)), 0.074 (s, 3H, \( \text{SiCH}_3 \)), 0.068 (s, 3H, \( \text{SiCH}_3 \)).

\( ^{13}\text{C} \) NMR: \( \delta \) 136.5, 131.5, 129.2, 121.7, 72.6, 71.5, 70.7, 63.6, 61.8, 25.7, 18.1, -5.6.

NICI: \( m/z \) 512/510/508 (M+Br, 32/60/31%), 431/430/429/428 (M and M-H, 46/100/43/94), 260 (M-C\(_7\)H\(_6\)Br, 86).

The more polar isomer:

FTIR: \( \nu_{\text{max}} \) (CHCl\(_3\)) 2104 cm\(^{-1}\).

\( ^1\text{H} \) NMR: \( \delta \) 7.48 (d, 2H, \( J \) 8.4 Hz, PhBr), 7.20 (d, 2H, \( J \) 8.4 Hz, PhBr), 4.50 (s, 2H, \( \text{CH}_2\text{Ph} \)), 4.0-3.8 (m, 3H), 3.50 (m, 3H), 2.67 (d, 1H, \( J \) 5.0 Hz, OH), 0.90 (s, 9H, \( \text{C}_4\text{H}_9 \)), 0.09 (s, 6H, \( \text{Si(CH}_3)_2 \)).

\( ^{13}\text{C} \) NMR: \( \delta \) 136.9, 131.8, 129.6, 121.9, 72.7, 71.2, 70.3, 64.2, 63.4, 25.5, 17.9, -6.0.

NICI: \( m/z \) 512/510/508 (M+Br, 53/100/48%).
1-(4-bromobenzyloxy)-4-(tert-butyldimethylsilyloxy)-cis-2,3-iminobutane (445)

To a mixture of (±)-(2R,3S)-3-azido-4-(4-bromobenzyloxy)-1-(tert-butyldimethylsilyloxy)-2-butanol and (±)-(2R,3S)-3-azido-1-(4-bromobenzyloxy)-4-(tert-butyldimethylsilyloxy)-2-butanol (444) (97 mg, 0.22 mmol) in toluene (2 ml) was added polymer bound triphenyl phosphine (25.7 mg, Aldrich, 3.06 mmolP/g polymer, 1.5 eq). The mixture was refluxed under Ar for 9 hours, cooled and filtered. Evaporation and chromatography (chloroform: methanol, 97: 3) gave 1-(4-bromobenzyloxy)-4-(tert-butyldimethylsilyloxy)-cis-2,3-iminobutane (445) (75 mg, 87%) as a colourless oil. (Found: C, 53.1; H, 7.6; N, 3.9; Br, 20.8%. C17H28N02SiBr requires C, 52.8; H, 7.3; N, 3.6; Br, 20.7%).

FTIR: \( \nu_{\text{max}} \) (CHCl\(_3\)) 3328, 2956, 2931, 2859, 1594, 1488, 1472, 1405, 1362, 1259, 1090, 1012 cm\(^{-1}\).

\(^1\)H NMR: \( \delta \) 7.47 (d, 2H, J 8.4 Hz, PhBr), 7.23 (d, 2H, J 8.4 Hz, PhBr), 4.51 (ABq, 2H, \( \Delta \delta \) 0.06, \( J_A B \) 12.1 Hz, CH\(_2\)Ph), 3.73-3.68 (m, 1H), 3.61-3.55 (m, 2H), 3.50-3.44 (m, 1H), 2.42-2.28 (m, 2H, H2 and H3), 0.89 (s, 9H, C\(_4\)H\(_9\)), 0.063 and 0.050 (s and s, 6H, Si(CH\(_3\))\(_2\)). NH not resolved.

\(^13\)C NMR: \( \delta \) 137.3 (Bn), 131.6 (Bn), 129.5 (Bn), 121.6 (Bn), 72.4, 69.9, 62.9, 35.1 and 33.3 (C2 and C3), 25.9 (Me\(_3\)), 18.4 (CMe\(_3\)), -5.2 and -5.3 (Si(CH\(_3\))\(_2\)).

EIMS: \( m/z \) 388/386 (M+H, 0.1/0.1%; found 386.1151; C17H29NO2SiBr requires 386.11517), 330/328 (M-C\(_4\)H\(_9\), 0.9/1.0), 286/284 (M-C\(_6\)H\(_{13}\)O, 4/4; found 284.0098; C11H15NO79BrSi requires 284.01065), 171/169 (C\(_7\)H\(_6\)Br, 47/49), 116 (28), 90 (31), 89 (19), 75 (39), 73 (39), 69 (100), 68 (27), 59 (26), 57 (21), 56 (70), 54 (17), 45 (14), 43 (24), 42 (10), 41 (28).

CIMS: \( m/z \) 559/558/557/556/555/554 (M+BrH\(_6\)Br, 17/62/33/100/17/48%), 389/388/387/386 (M+1, 17/66/17/69), 330/328 (M-C\(_4\)H\(_9\), 11/11), 308 (10), 306 (1), 286/284 (M-C\(_6\)H\(_{13}\)O, 21/22), 200 (10), 186 (13), 171/169 (C\(_7\)H\(_6\)Br, 17/18), 144 (10), 142 (18), 128 (15), 116 (13).

NICI: \( m/z \) 550/548/546/544 (M+Br+HBr, 6/16/15/6%), 514/512/510 (6/11/7), 469/468/467/466/465/464 (M+Br, 13/50/26/100/13/49), 386/384 (M-1, 1/1).
A sample of 1-benzyloxy-4-tert-butylmethylsilyloxy-cis-2,3-iminobutane was prepared in a similar manner from (2S,3R)-4-benzyloxy-2,3-epoxy-1-butanol (418).

EIMS: \textit{m/z} 308 (M+H, 0.1%), 250 (M-C_4H_9, 3), 206 (M-C_6H_13O, 8), 116 (12), 91 (C_7H_7, 100), 73 (13), 69 (13), 68 (11).

CIMS: \textit{m/z} 398 (M+C_7H_7, 1%), 309/308 (M+H, 24/100), 250 (M-C_4H_9, 3), 206 (M-C_6H_13O, 1).
N-(tert-butoxycarbonyl)-1-(4-bromobenzyloxy)-4-(tert-butyldimethylsilyloxy)-cis-2,3-iminobutane (448) by protection of the aziridine

To a solution of 1-(4-bromobenzyloxy)-4-(tert-butyldimethylsilyloxy)-cis-2,3-iminobutane (445) (839 mg, 2.17 mmol) in methanol was added ditert-butyl dicarbonate (550 mg, 2.52 mmol). After stirring for 1 hour more dicarbonate (100 mg, 0.46 mmol) was added and the solution stirred for a further 30 minutes. Ammonia saturated methanol (5 ml) was added and after 15 minutes the solution was evaporated. Chromatography (ethylacetate: hexane, 1: 9) gave N-(tert-butoxycarbonyl)-1-(4-bromobenzyloxy)-4-(tert-butyldimethylsilyloxy)-cis-2,3-iminobutane (448) (893 mg, 85%) as a colourless oil (found C, 54.3, H, 7.8; N, 3.0; Br, 16.5%. C22H36N04BrSi requires C, 54.3; H, 7.5; N, 2.9; Br, 16.4%).

FTIR: \( \nu_{\text{max}} \) (CHCl\(_3\)) 2956, 2931, 2859, 1717, 1595 (weak), 1488, 1472, 1369, 1154, 1093, 1012 cm\(^{-1}\).

\(^1\)H NMR: \( \delta \) 7.46 (d, 2H, J 8.3 Hz, PhBr), 7.24 (d, 2H, J 8.3 Hz, PhBr), 4.59 (ABq, 2H, \( \Delta \delta \) 0.14, \( J_{AB} \) 12.1 Hz, CH\(_2\)Ph), 3.81 (dd, 1H, J 5.6, 11.4), 3.70-3.50 (m, 3H), 2.76-2.62 (m, 2H, H2 and H3), 1.45 (s, 9H, OC\(_4\)H\(_9\)), 0.89 (s, 9H, C\(_4\)H\(_9\)), 0.07 and 0.06 (s and s, 6H, Si(CH\(_3\))\(_2\)).

\(^13\)C NMR: \( \delta \) 162.1 (CO\(_2\)), 137.3 (Bn), 131.6 (Bn), 129.5 (Bn), 121.6 (Bn), 81.5 (OCMe\(_3\)), 71.8, 67.9, 61.4, 41.8 and 40·4 (C2 and C3), 27·9 (OC(CH\(_3\))\(_3\)), 25·9 (Si(CH\(_3\))\(_3\)), 18·3 (CMe\(_3\)), -5.3 and -5.4 (Si(CH\(_3\))\(_2\)).

EIMS: \( m/z \) 386/384 (M-CO\(_2\)C\(_4\)H\(_9\), 0.02/0.04%; found 386.0976 and 384.0993; C17H27NO2BrSi requires 386.0974 and 384.0994), 374/372 (M-C\(_4\)H\(_9\)-C\(_4\)H\(_8\), 1/1; found 374.0245 and 372.0266; C14H19NO4BrSi requires 374.0246 and 372-0267), 330/328 (M-C\(_4\)H\(_8\)-CO\(_2\)-C\(_4\)H\(_9\), 0·6/0·6; found 328-0367; C13H19NO279BrSi requires 328-0368), 286/284 (M-C\(_11\)H\(_21\)O\(_3\), 3·5/3·4; found 284-0101; C11H15NO79BrSi requires 284-0106), 171/169 (C\(_7\)H\(_6\)Br, 46/50), 90 (20), 89 (15), 75 (25), 73 (24), 69 (15), 68 (10), 59 (13), 57 (C\(_4\)H\(_9\), 100), 56 (13), 41 (20).

NICI: \( m/z \) 568/566/564 (M+Br, 19/34/16%), 513/512/511/510/509/508 (M+Br-C\(_4\)H\(_8\), 14/58/28/100/13/50), 432/431/430/429/428 (8/13/13/10/6).
EXPERIMENTAL

N-(tert-butoxycarbonyl)-1-(4-bromobenzoyloxy)-4-(tert-butyldimethylsilyloxy)-cis-2,3-iminobutane (448) by Mitsunobu cyclisation

(±)-(2R,3S)-3-amino-4-(4-bromobenzoyloxy)-1-(tert-butyldimethylsilyloxy)-2-butanol and (±)-(2R,3S)-3-amino-1-(4-bromobenzoyloxy)-4-(tert-butyldimethylsilyloxy)-2-butanol (449)

1-(4-bromobenzoyloxy)-4-(tert-butyldimethylsilyloxy)-cis-2,3-epoxybutane (443) (891 mg, 2.30 mmol) was heated in a pressure vessel with ammonia saturated methanol (11 ml) at 100°C for 36 hours. After evaporation of the solvent, the residue was chromatographed with chloroform: methanol (24:1) to give a mixture of (±)-(2R,3S)-3-amino-4-(4-bromobenzoyloxy)-1-(tert-butyldimethylsilyloxy)-2-butanol (714 mg, 77%).

1H NMR: δ 7.49-7.45 (m, 2H, ArH), 7.22-7.19 (m, 2H, ArH), 4.55-4.41 (m, 2H, CH2Ph), 3.81-3.32 (m, 5H), 3.22-3.10 and 3.00-2.92 (m, 1H), 2.22 (brs, 3H, NH2 and OH), 0.890 and 0.887 (s, s, 9H, SiC4H9), 0.10-0.05 (m, 6H, Si(CH3)2).

EIMS: m/z 406/404 (M+H, 0.5/0.5%), 260/258 (M4-TBDMSOCH2, 8/8), 230/228 (M1-TBDMSOCH2CHOH, 9/8), 204 (M1-BrBnOCH2, 31), 174 (M4-BrBnOCH2CHOH, 39), 171/169 (C7H6Br, 94/100), 101 (13), 90 (30), 89 (28), 75 (34), 74 (20), 73 (53), 59 (13), 43 (16); where M4 and M1 refer to the 4- and 1-silyloxy isomers respectively.
EXPERIMENTAL

(±)-(2R,3S)-4-(4-bromobenzyloxy)-3-(tert-butoxycarbonylamino)-1-(tert-butyldimethylsilyloxy)-2-butanol and (±)-(2R,3S)-1-(4-bromobenzyloxy)-3-(tert-butoxycarbonylamino)-4-(tert-butyldimethylsilyloxy)-2-butanol (450)

To a mixture of (±)-(2R,3S)-3-amino-4-(4-bromobenzyloxy)-1-(tert-butyldimethylsilyloxy)-2-butanol and (±)-(2R,3S)-3-amino-1-(4-bromobenzyloxy)-4-(tert-butyldimethylsilyloxy)-2-butanol (449) (191 mg, 0.47 mmol) in ethanol (10 ml) was added ditert-butyl dicarbonate (117 mg, 0.54 mmol). The mixture was stirred for 4 hours then evaporated. Chromatography (chloroform : methanol, 75:1) gave (±)-(2R,3S)-4-(4-bromobenzyloxy)-3-(tert-butoxycarbonylamino)-1-(tert-butyldimethylsilyloxy)-2-butanol and (±)-(2R,3S)-1-(4-bromobenzyloxy)-3-(tert-butoxycarbonylamino)-4-(tert-butyldimethylsilyloxy)-2-butanol (450) (total 203 mg, 85%) as an unseparated mixture. (In retrospect the ammonia workup developed for the preparation of 448 by protection would be advantageous to remove residual ditert-butyl dicarbonate.)

$^1$H NMR: $\delta$ 7.46 (d, 2H, J 8.4 Hz, PhBr), 7.21 and 7.19 (d and d, 2H combined, J 8.1, 8.1 Hz, PhBr), 5.09 (br m, 1H, NH), 4.49 (br s, 2H, CH$_2$Ph), 4.14 (m) and 4.0-3.4 (m, 6H combined), 3.20 (br s) and 2.91 (br s, 1H combined, OH), 1.432 and 1.429 (s and s, 9H combined, OC$_4$H$_9$), 0.88 (s, 9H, SiC$_4$H$_9$), 0.06 and 0.05 (s and s, 3H, Si(CH$_3$)$_2$).

$^{13}$C NMR: $\delta$ 155.8 (NCO$_2$), 137.0 and 136.8 (Bn), 131.5 (Bn), 129.4 and 129.3 (Bn), 121.63 and 121.57 (Bn), 79.4 (OCMe$_3$), 72.7 and 72.5, 71.6 and 71.4, 71.1, 65.1 and 64.1, 51.7 and 50.2, 28.3 (OC(CH$_3$)$_3$), 25.8 (SiC(CH$_3$)$_3$), 18.2 and 18.1 (CMe$_3$), -5.47, -5.50 and -5.59 (Si(CH$_3$)$_2$).

EI MS: $m/z$ 506/504 (M+H, 0.04/0.04%), 392/390 (M-C$_4$H$_8$C$_4$H$_9$, 19/21), 348/346 (392/390-CO$_2$, 24/23), 204 (16), 174 (15), 171/169 (C$_7$H$_6$Br, 73/73), 90 (28), 89 (26), 75 (30), 73 (35), 56 (41), 53 (100).

-E 43-
N-({\textit{tert}}-butoxycarbonyl)-1-(4-bromobenzyloxy)-4-({\textit{tert}}-butyldimethylsilyloxy)-{\textit{cis}}-2,3-iminobutane (448)

To a mixture of (±)-(2R,3S)-4-(4-bromobenzyloxy)-3-({\textit{tert}}-butoxycarbonylamino)-1-({\textit{tert}}-butyldimethylsilyloxy)-2-butanol and (±)-(2R,3S)-1-(4-bromobenzyloxy)-3-({\textit{tert}}-butoxycarbonylamino)-4-({\textit{tert}}-butyldimethylsilyloxy)-2-butanol (450) (47.5 mg, 0.094 mmol) was added triphenyl phosphine (27.5 mg, 0.10 mmol), tetrahydrofuran (2 ml) and diethyl azodicarboxylate (17.5 µl, 0.11 mmol). After stirring for 2 hours the solution was evaporated and treated with ether-hexane (1: 1, 5.5 ml). The supernatant was removed and evaporated to an orange oil which was chromatographed (neat chloroform) to give N-({\textit{tert}}-butoxycarbonyl)-1-(4-bromobenzyloxy)-4-(4-butyldimethylsilyloxy)-{\textit{cis}}-2,3-iminobutane (448) (31.5 mg, 69%). Data as above.
N-(tert-butoxycarbonyl)-4-(4-bromobenzyloxy)-cis-2,3-imino-1-butanol (451)

N-(tert-butoxycarbonyl)-1-(4-bromobenzyloxy)-4-(tert-butyldimethylsilyloxy)-cis-2,3-iminobutane (448) (32 mg, 0.06 mmol) was dissolved in THF (2 ml), cooled in ice and treated with tetrabutylammonium fluoride (0.1 ml, 1 M, 0.1 mmol). After stirring for 1 hour the reaction was quenched with aqueous sodium hydrogen carbonate (2 ml), extracted with ether (x3) and the extracts dried over magnesium sulfate. Chromatography (ether: hexane 1: 1) gave N-(tert-butoxycarbonyl)-4-(4-bromobenzyloxy)-cis-2,3-imino-1-butanol (451) (22 mg, 92%).

$^1$H NMR: $\delta$ d 7.49 (d, 2H, J 8.5 Hz, PhBr), 7.22 (d, 2H, J 8.5 Hz, PhBr), 4.54 (ABq, 2H, $\Delta \delta$ 0.1, $J_{AB}$ 12.0 Hz, CH$_2$Ph), 3.86-3.78 (m, 2H), 3.61-3.46 (m, 2H), 2.85-2.72 (m, 2H, H2 and H3), 2.22 (dd, 1H, J 5.1, 8.1 Hz, OH), 1.45 (s, 9H, C$_4$H$_9$).

$^{13}$C NMR: $\delta$ 162.2 (CO$_2$), 136.9 (Bn), 132.2 (Bn), 130.0 (Bn), 122.4 (Bn), 82.1 (CMe$_3$), 72.7, 68.4, 60.9, 41.7 and 39.7 (C2 and C3), 28.0 (Me$_3$); referenced to tetramethylsilane as 0.00 (CDCl$_3$ is 77.3).

EIMS: $m/z$ 316/314 (M-C$_4$H$_9$, 0.4/0.4), 272/270 (M-CO$_2$C$_4$H$_9$, 0.4/0.4), 171/169 (C$_7$H$_6$Br, 17/17), 100 (31), 86 (27), 57 (100), 56 (71), 41(44).

NICI: 454/452/450 (M+Br, 52/100/47), 398/396/394 (M+Br-C$_4$H$_9$, 48/86/48).
4-(4-bromobenzyloxy)-cis-2,3-imino-1-butanol (446)

N-(tert-butoxycarbonyl)-1-(4-bromobenzyloxy)-4-(tert-butyldimethylsilyloxy)-
cis-2,3-iminobutane (448) (69 mg, 0.18 mmol) was dissolved in THF (2 ml), cooled in
ice and treated with tetrabutylammonium fluoride (0.18 ml, 1 M, 0.28 mmol). After
stirring for 2 hours the reaction was quenched with ammonium chloride (14 mg) in
water (2 ml), extracted with dichloromethane after the addition of brine (10 drops) and
the extracts dried over magnesium sulfate. After chromatography (7% methanol in
dichloromethane) the product appeared to be contaminated by tetrabutylammonium
salts (NMR). The chromatographed product was dissolved in sodium bicarbonate
solution (5%, 4 ml) and THF (1 ml) and sodium perchlorate (50 mg) added. Extraction
with dichloromethane, drying over magnesium sulfate and chromatography (ether:
methanol: triethylamine, 120: 15: 1.4) gave 4-(4-bromobenzyloxy)-cis-2,3-imino-1-
butanol (446) (34 mg, 71%) as fine needles. Recrystallisation from dichloromethane-
hexane gave crystals mp 79-84°. (Found 272.0287; C11H14NO279Br+H requires
272.0286.)

UV: $\lambda_{\text{max}}$ (EtOH) 222 nm (e 1.1x10⁴).

FTIR: $\nu_{\text{max}}$ (CHCl₃) 3467, 3330, 2872, 1594, 1489, 1405, 1085, 1073, 1013 cm⁻¹

1H NMR: $\delta$ 7.48 (d, 2H, $J$ 8.3 Hz, PhBr), 7.22 (d, 2H, $J$ 8.3 Hz, PhBr), 4.50 (ABq, 2H,
$\Delta \delta$ 0.06, $J_{AB}$ 12.0 Hz, CH₂Ph), 3.77-3.69 (m, 2H), 3.55-3.49 (m, 1H), 3.44-3.38
(m, 1H), 2.49-2.46 (m, 2H, H2 and H3), 1.95 (brs, 2H, NH and OH).

$^{13}$C NMR: $\delta$ 136.6, 131.6, 129.5, 121.8, 72.5 (CH₂Ph), 69.9 (CH₂), 61.4 (CH₂), 34.8
and 32.9 (C2 and C3).

EIMS: $m/z$ 274/272 (M+H, 2/2%), 242/240 (M-CH₂OH, 0.06/0.06; found 240.0024,
C10H11NO79Br requires 240.0024), 171/169 (C₇H₆Br, 14/15), 85 (22), 56
(C₃H₆N, 100; found 56.0500, C₃H₆N requires 56.0500), 54 (57).

NICI: $m/z$ (354/352/350 (M+Br, 51/100/52%), 273/272/271/270 (M and M-H,
17/13/18/12).
N-(tert-butoxycarbonyl)-4-(4-bromobenzyloxymethyl)-cis-2,3-iminobutanoic acid (453)

a) via N-(tert-butoxycarbonyl)-4-(4-bromobenzyloxymethyl)-cis-2,3-iminobutanal (452)

To a solution of 12-1-5 triacetoxyperiodinane498 (106 mg, 0.25 mmol) in dichloromethane (1.5 ml) at 0°C was added N-(tert-butoxycarbonyl)-4-(4-bromobenzyloxy)-cis-2,3-imino-1-butanol (451) (59 mg, 0.16 mmol) in dichloromethane (2 ml). After stirring for 2 hours TLC indicated residual starting material was present so further oxidant (150 mg) was added. After 2 hours the reaction was quenched with ether (5 ml), sodium thiosulphate (500 mg) and aqueous sodium hydrogen carbonate (5%, 5 ml). The ether layer was washed with saturated bicarbonate, dried (MgSO4) and chromatographed (ether: hexane, 1: 1) to give N-(tert-butoxycarbonyl)-4-(4-bromobenzyloxymethyl)-cis-2,3-iminobutanal (452) (50 mg, 85%).

1H NMR: δ 9.32 (d, 1H, J 4.4 Hz, CHO), 7.47 (d, 2H, J 8.1 Hz, PhBr), 7.17 (d, 2H, J 8.1 Hz, PhBr), 4.5 (ABq, 2H, Δδ 0.04, JAB 12.1 Hz, CH2Ph), 3.80-3.64 (m, 2H, CH2), 3.10-2.98 (m, 2H, H2 and H3), 1.47 (s, 9H, C4H9).

The aldehyde was all dissolved in THF (2 ml) and treated with 3-hydroxyphenol (59 mg) and sodium chlorite (technical, 42 mg, 0.47 mmol) in pH 3.7 buffer (1 ml). The mixture was stirred for 22 hours, partially evaporated and partitioned between ether (4 ml) and sodium bicarbonate (5%, 7 ml). The basic solution was acidified with saturated oxalic acid solution (4 ml) and extracted with dichloromethane. Drying over MgSO4 and evaporation gave crude N-(tert-butoxycarbonyl)-4-(4-bromobenzyloxymethyl)-cis-2,3-iminobutanoic acid (453) (48 mg, 94% if pure). The acid matched that prepared as described below (NMR) and quickly decomposed.
b) by oxidation over platinum

4-(4-bromobenzyloxy)-cis-2,3-imino-1-butanol (446) (17 mg, 0.06 mmol) was stirred in a mixture of ethyl acetate (2 ml), water (2 ml), sodium hydrogen carbonate (164 mg) and platinum black (2 mg) with continuous bubbling of oxygen gas. Evaporated solvent was replaced and more platinum added every 48 hours. After 144 hours the mixture was centrifuged. The ethyl acetate layer was dried (MgSO$_4$) and evaporated to give starting material (12 mg, 72%). The aqueous part was diluted to 5 ml and treated with ditert-butyl dicarbonate (50 mg, 0.23 mmol) and methanol (2 ml) for 24 hours. The solution was extracted twice with dichloromethane, acidified with saturated oxalic acid solution and extracted with dichloromethane (x3). The latter extracts were dried (Na$_2$SO$_4$) and evaporated to give crude N-(tert-butoxycarbonyl)-4-(4-bromobenzyloxymethyl)-cis-2,3-iminobutanoic acid (453) contaminated with tert-butanol and acetic acid.

1H NMR: δ 9.5 (v br s, shift varies, 1H, CO$_2$H), 7.46 (d, 2H, J 8.3 Hz, PhBr), 7.20 (d, 2H, J 8.3 Hz), 4.61 (d, 1H, J 12.1 Hz, CHPh), 4.46 (d, 1H, J 12.1 Hz, CHPh), 3.80 (dd, 1H, J 11.1 and 5.7 Hz, OCH), 3.64 (dd, 1H, J 11.1 and 5.9 Hz, OCH), 3.20 (d, 1H, J 6.7 Hz, H2), 3.01 (q, 1H, J 6 Hz, H3), 1.46 (s, 9H, C$_4$H$_9$).

The sample decomposed significantly during an attempt to acquire a $^{13}$C NMR spectrum.

EIMS: m/z 171/169 (C$_7$H$_6$Br, 66/69%), 130 (37), 107 (48), 91 (27), 90 (100), 89 (76), 79 (84), 77 (93), 57 (99) [selected peaks].
methyl (2S,3S)-2-azido-4-(4-bromobenzyloxy)-3-hydroxybutanoate (454a),
methyl (2R,3S)-3-azido-4-(4-bromobenzyloxy)-2-hydroxybutanoate (454b)
and methyl (2R,3S)-2-azido-4-(4-bromobenzyloxy)-3-hydroxybutanoate (456)

To a solution of (+) methyl (2R,3R)-4-(4-bromobenzyloxy)-2,3-epoxybutanoate (435) (125 mg, 0.416 mmol) in methanol (5 ml) was added sodium azide (90 mg, 1.38 mmol) and ammonium chloride (50 mg, 0.94 mmol). After refluxing for 20 hours the methanol was removed by evaporation and the residue dissolved in a mixture of ether (5 ml) and water (5 ml). The aqueous layer was separated and further extracted with ether (3x5 ml) and the combined extracts were dried over magnesium sulfate and evaporated to give the crude product as a colourless oil. Chromatography with neat dichloromethane gave starting material (4.8 mg, 3.8%) and then a mixture of two azido alcohols (132 mg, 92%, less polar : more polar ca 1.0: 1.1). Further chromatography with neat dichloromethane and ethanol : dichloromethane (1: 99) allowed separation of these isomers.

The less polar isomer was methyl (2R,3S)-3-azido-4-(4-bromobenzyloxy)-2-hydroxybutanoate (454b) which was isolated as a colourless oil. (Found: C, 42.2; H, 4.0; N, 12.0%. C12H14N3O4Br requires C, 41.9; H, 4.1; N, 12.2%).

IR: \(\nu_{\text{max}}\) (neat) 3490, 2955, 2862, 2110, 1745, 1487, 1270, 1125, 1013, 795 cm\(^{-1}\).

\(^1\)H NMR: \(\delta\) 7.49 (d, 2H, \(J\ 8.3\ Hz,\ \text{PhBr})\), 7.23 (d, 2H, \(J\ 8.3\ Hz,\ \text{PhBr})\), 4.55 (s, 2H, \(\text{CH}_2\text{Ph})\), 4.33 (dd, 1H, \(J\ 2.1,\ 6.2\ Hz,\ H2)\), 3.91 (m, 1H, H3), 3.85 (s, 3H, \(\text{CH}_3\text{O})\), 3.81 (s, 1H, H4), 3.78 (pseudo doublet, 1H, H4'), 3.00 (d, 1H, \(J\ 6.2\ Hz,\ \text{OH})\). Spin simulation indicates that the H4,4' pattern arises from near overlap of H4 and H4' in an ABC system (H4H4'H3); simulated couplings: \(J_{4,4'} \approx 10\ Hz,\ J_{3,4} \approx 5.7\) and 6.0 Hz.

\(^13\)C NMR: \(\delta\) 172.7, 136.5, 131.7, 129.3, 121.9, 72.8, 70.4, 69.2, 62.1, 53.2.

EIMS: \(m/z\) 316/314 (0.5/0.4%), 228/226 (18/19), 171/169 (C\(_7\)\text{H}_6\text{Br}, 98/100), 90 (76), 89 (49), 63 (24).
The more polar isomer was methyl (2S,3S)-2-azido-4-(4-bromobenzyloxy)-3-hydroxybutanoate (454a) which was isolated as a colourless oil.

$^1$H NMR: δ 7.49 (d, 2H, $J$ 8.4 Hz, PhBr), 7.20 (d, 2H, $J$ 8.4 Hz, PhBr), 4.51 (s, 2H, CH$_2$Ph), 4.32-4.25 (m, 1H, H3), 4.07 (d, 1H, $J$ 3.3 Hz, H2), 3.81 (s, 3H, CH$_3$O), 3.63-3.53 (m, 2H, H4,4'), 2.47 (d, 1H, $J$ 6.5 Hz, OH).

$^{13}$C NMR: δ 169.376, 136.406, 131.533, 129.387, 121.762, 72.698, 70.724, 70.333, 62.991, 52.489.

Prolonged reaction (45h) under the same conditions generated noticeable amounts of a third, minor isomer believed to be methyl (2R,3S)-2-azido-4-(4-bromobenzyloxy)-3-hydroxybutanoate (456) which coeluted with methyl (2S,3S)-2-azido-4-(4-bromobenzyloxy)-3-hydroxybutanoate (454a) in a ratio of about 1:4. (Found for the mixture of these isomers: C, 42.2; H, 4.2; N, 12.0%. C$_{12}$H$_{14}$N$_3$O$_4$Br requires C, 41.9; H, 4.1; N, 12.2%).

IR: $\nu_{\text{max}}$ (neat) 3460, 2955, 2918, 2865, 2110, 1745, 1488, 1435, 1270, 1205, 1070, 1013, 795 cm$^{-1}$.

EIMS: m/z 316/314 (0.6/0.5%), 258/256 (1/1), 171/169 (94/100), 91 (24), 90 (70), 89 (48), 88 (49), 77 (23), 72 (47), 63 (25), 59 (35), 51 (21).

Some spectral information for the minor isomer (456) itself could be distinguished:

$^1$H NMR: δ 4.12 (m, 2H), 3.78 (s, 3H, CH$_3$O), 3.64-3.63 (m, 2H), 2.68 (d, 1H, $J$ 6.3 Hz, OH).

$^{13}$C NMR: δ 169.113, 136.380, 131.505, 129.412, 72.725, 70.636, 69.938, 63.169, 52.728; one resonance not observed.
methyl 4-(4-bromobenzyloxy)-cis-2,3-iminobutanoate (455) and methyl 4-(4-bromobenzyloxy)-trans-2,3-iminobutanoate (457)

A mixture of the more polar azidoalcohols (454a) and (456) (ca 4: 1, 25 mg, 0.07 mmol) was dissolved in DMF (1 ml) and treated with triphenylphosphine (22 mg, 0.08 mmol). The solution was heated at 90° for 8 h under Ar then evaporated. Chromatography with 2% methanol in dichloromethane gave two products:
The less polar methyl 4-(4-bromobenzyloxy)-trans-2,3-iminobutanoate (457) (4 mg, 18%).

FTIR: \( \nu_{\text{max}} (\text{CHCl}_3) \) 3295, 3019, 2956, 2862, 1730, 1488, 1447, 1360, 1092, 1013 cm\(^{-1}\).

\( ^1\text{H} \) NMR: \( \delta \) 7.48, 7.22 (AA’BB’, 4H, \( J_{AB} \) 8.3 Hz, PhBr), 4.51 (ABq, 2H, \( \Delta \delta \) 0.03, \( J_{AB} \) 12.1 Hz, \( \text{CH}_2\text{Ph} \)), 3.77 (s, 3H, OCH\(_3\)), 3.48 and 3.46 (pseudo d, 2H, H\(_4\) and H\(_4'\) ), 2.54 (m, 1H, H3), 2.43 (dd, 1H, \( J \) 2.5 and 7.5 Hz, H2), 1.44 (br t, 1H, \( J \) 8 Hz, NH). In some samples the methine (H2 and H3) and amino resonances were broadened so that coupling was not apparent.

\( ^1\text{C} \) NMR: \( \delta \) 172.6 (Cl), 136.9 (C\(_{CH_2}\)), 131.5 (BnC3), 129.3 (BnC2), 121.7 (CBr), 72.4 (\( \text{CH}_2\text{Ar} \)), 71.1 (CH\(_2\text{O}\)), 52.6 (OCH\(_3\)), 37.8 and 33.1 (C2 and C3).

EIMS: \( m/z \) 302/300 (M+H, 0.1/0.1%), 270/268 (M-OCH\(_3\), 0.3/0.3), 242/240 (M-CO\(_2\text{CH}_3\), 0.3/0.3), 186/185/184/183 (1/2/1/2), 171/169 (C\(_7\text{H}_6\)Br, 29/30), 130 (M-C\(_7\text{H}_6\)Br, 3), 113 (8), 90 (25), 89 (19), 70 (22), 56 (C\(_3\text{H}_6\)N, 100; found 56.0503; C\(_3\text{H}_6\)N requires 56.0500), 54 (36).

CIMS: \( m/z \) 302/300 (M+H, 95/100%), 222 (M-Br+2H, 42).

Also isolated was the more polar methyl 4-(4-bromobenzyloxy)-cis-2,3-iminobutanoate (455) (13 mg, 61%). Short path distillation gave a clear oil which solidified in the freezer (mp of solid 38-41°). (Found C, 47.9; H, 4.7; N, 4.8%.

\( \text{C}_{12}\text{H}_{14}\text{N}_0\text{Br} \) requires C, 48.0; H, 4.7; N, 4.7%).

FTIR: \( \nu_{\text{max}} (\text{CHCl}_3) \) 3329, 3273, 3009, 2956, 2865, 1730, 1489, 1443, 1089 cm\(^{-1}\).

\( ^1\text{H} \) NMR: \( \delta \) 7.47, 7.20 (AA’BB’, 4H, \( J_{AB} \) 8.3 Hz, PhBr), 4.46 (ABq, 2H, \( \Delta \delta \) 0.05, \( J_{AB} \) 12.0 Hz, \( \text{CH}_2\text{Ph} \)), 3.74 (s, 3H, OCH\(_3\)), 3.59 (dd, 1H, \( J \) 6.1, 10.6 Hz, H4), 2.77 (br s or br d, 1H, \( J \) 6 Hz, H2), 2.57 (br s or q, 1H, \( J \) 6 Hz, H3), 1.4-0.8 (vbr, 1H, NH).
In some samples the methine (H2 and H3) resonances were observed as three broad signals $\delta$ 2.9, 2.7 and 2.55 (1:3:2), the H4 resonances were broadened and broad NH signals were observed at $\delta$ 1.35 and 1.05 (1:2). A 3Å molecular sieve was added to the NMR solution which was left at rt for 30h whence resonances for two components were well resolved (ratio of components ca 2:1); we interpret this as being due to slow nitrogen inversion.

$^1$H NMR: with sieve, distinctive resonances for major component:

$\delta$ 3.75 (s, 3H, OCH$_3$), 2.69 (dd, 1H, $J$ 6.0, 7.9 Hz, H2), 2.51 (m, 1H, H3), 1.03 (pseudo t, 1H, $J$ 7.8 and 8.4 Hz, NH).

distinctive resonances for minor component:

$\delta$ 3.72 (s, 3H, OCH$_3$), 2.91 (dd, 1H, $J$ 6.7, 8.8 Hz, H2), 2.69 (buried m, 1H, H3), 1.34 (pseudo t, 1H, $J$ 8.3 and 9.6 Hz, NH).

$^{13}$C NMR: $\delta$ 170.8 (Cl), 136.8 (CCH$_2$), 131.5 (BnC3), 129.2 (BnC2), 121.6 (CBr), 72.3 (CH$_2$Ar), 68.2 (CH$_2$O), 52.4 (OCH$_3$), 36.6br and 33.2br (C2 and C3).

with sieve: major comp. $\delta$ 136.8, 131.53, 129.23, 121.66, 72.3, 68.1, 52.6, 37.3 and 33.7.

with sieve: minor comp. $\delta$ 137.0, 131.48, 129.34, 121.55, 72.5, 68.7, 52.2, 35.6 and 32.5.

EIMS: $m/z$ 603/601/599 (2M+H, 0.4/0.7/0.3%), 472/470/468 (M+C$_7$H$_6$Br, 1/2/1), 302/300 (M+H, 11/12), 242/240 (M-CO$_2$CH$_3$, 0.4/0.4), 186/185/184/183 (0.4/0.6/0.3/0.5), 171/169 (C$_7$H$_6$Br, 25/26), 130 (M-C$_7$H$_6$Br, 6), 115 (M-ArCHO?, 4), 114 (10), 113 (22), 90 (15), 89 (17), 70 (33), 56 (C$_3$H$_6$N, 100), 54 (53), 51 (24).

Repeated chromatography of a mixture of the more polar azidoalcohols (454a) and (456) gave a sample with somewhat more of the minor isomer (454a:456 ca 1.3:1). This sample (10 mg, 0.03 mmol) was dissolved in acetonitrile (1 ml) and treated with triphenylphosphine (10 mg, 0.04 mmol). The solution was heated at reflux for 7 h under Ar then evaporated. Chromatography gave methyl 4-(4-bromobenzyloxy)-trans-2,3-iminobutanoate (457) (3.1 mg, 36%) and methyl 4-(4-bromobenzyloxy)-cis-2,3-iminobutanoate (455) (3.5 mg, 41%).

Treatment of the less polar azidoalcohol (454b) under these conditions gave only the cis product, methyl 4-(4-bromobenzyloxy)-cis-2,3-iminobutanoate (455).
N-(phenyl)-4-(4-bromobenzylxoy)-cis-2,3-epoxybutanamine (459)

Freshly chromatographed 4-(4-bromobenzylxoy)-cis-2,3-epoxybutanal (433) (53 mg, 0.20 mmol) was dissolved in dichloromethane (2 ml) and added to activated sieves (3Å). Aniline (25 µl, 0.27 mmol) was added and after 3 hours sodium cyanoborohydride (31 mg, 0.24 mmol), methanol (1 ml) and acetic acid (3 drops) were added and the mixture stirred for 5 hours, with a further 12 mg of borohydride added after 3 hours. Sodium hydrogen carbonate was added and the mixture was filtered and evaporated. Chromatography of the crude product (ethylacetate: hexane, 1: 1) gave crude material which was rechromatographed (neat dichloromethane) to give N-(phenyl)-4-(4-bromobenzylxoy)-cis-2,3-epoxybutanamine (459) (37.6 mg, 55%). Recrystallisation from dichloromethane-hexane gave white crystals mp 88-89°. (Found: C, 58.5; H, 5.0; N, 4.0; Br, 23.4%. Cl7H18N02Br requires C, 58.6; H, 5.2; N, 4.0; Br, 23.9%).

IR: $\nu_{\text{max}}$ (CHCl3) 3414, 2866, 1604, 1507, 1489, 1092, 1071, 1013 cm$^{-1}$.

$^1$H NMR: $\delta$ 7.47 (d, 2H, J 8.4 Hz, BrPhH3'), 7.26-7.16 (m, 4H, BrPhH2' andNPhH3), 6.75 (tm, 1H, J 7.4 Hz, NPhH4), 6.63 (dm, 2H, J 8.4 Hz, NPhH2), 4.54 (ABq, 2H, $\Delta$$\delta$ 0.10, $J_{AB}$ 12 Hz, CH2Ph), 3.90 (br s, 1H, NH), 3.74-3.62 (m, 2H, CHCH), 3.40-3.20 (m, 4H, OCH2 and NCH2).

$^{13}$C NMR: $\delta$ 147.6, 136.6, 131.6, 129.4, 121.8, 118.1, 113.1, 72.6, 68.1, 54.70, 54.67, 42.6.

EIMS: $m/z$ 349/347 (M, 1.5/1.4%; found 347.0518; Cl7H18NO2Br requires 347.0521), 171/169 (C7H6Br, 13/14), 107 (16), 106 (C6H5NHCH2, 100), 93 (C6H5NH2, 19), 91 (11), 90 (14), 77 (22), 51 (11).
4-(tert-butyldimethylsilyloxy)-cis-2,3-imino-1-butanol (460)

To a solution of lithium (9 mg, 1.3 mmol) in ammonia (distilled from sodium and ferric chloride, ca 4 ml) at -78° was added a solution of 1-(4-bromobenzyloxy)-4-(tert-butyldimethylsilyloxy)-cis-2,3-iminobutane (445) (86.5 mg, 0.22 mmol) in tert-butyl alcohol (0.17 ml) and THF (1.6 ml). More lithium was added until the solution turned blue. After 5 min isoprene (0.3 ml) was added, then excess ammonium acetate. After evaporation under Ar the residue was diluted with brine (2 ml) and pH 7 buffer (2 ml). Extraction with dichloromethane (x3), drying over magnesium sulfate and evaporation gave the crude product (41 mg). Chromatography (2% methanol in dichloromethane) gave the polar 4-(tert-butyldimethylsilyloxy)-cis-2,3-imino-1-butanol (460) (22 mg, 45%).

$^1$H NMR: $\delta$ 4.03 (dd, 1H, J 11.4, 5.5 Hz), 3.83 (dd, 1H, J 11.7, 5.7 Hz), 3.48 (m, 2H), 2.44 (m, 2H, H2 and H3), 1.7 (v br s, NH and OH), 0.91 (s, 9H, SiC4H9), 0.11 and 0.10 (s and s, 6H, Si(CH3)2).

EIMS: m/z 186 (M-CH2OH, 0.6%), 158 (2), 142 (3), 116 (100), 85 (26), 75 (79), 73 (31), 59 (26), 56 (42), 54 (41).

4-(tert-Butyldimethylsilyloxy)-cis-2,3-imino-1-butanol (460) (20 mg, 0.09 mmol) was dissolved in ethanol (1 ml) at 0° and treated with acetic anhydride (14 µl, 0.14 mmol) for 30 min. The solution was evaporated to give what appeared to be fairly pure N-acetyl-4-(tert-butyldimethylsilyloxy)-cis-2,3-imino-1-butanol by NMR, but which partially decomposed on attempted chromatography.

$^1$H NMR: $\delta$ 4.03 (dd, 1H, J 11.5, 5.5 Hz), 3.91 (dd, 1H, J 12.0, 5.5 Hz), 3.63 (m, 2H), 2.80 (m, 2H, H2 and H3), 2.15 (s, 3H, COCH3), 0.91 (s, 9H, SiC4H9), 0.114 and 0.107 (s and s, 6H, Si(CH3)2); OH not resolved.

-E 54-
**3-amino-5-methylphenol**

This was prepared from 3-hydroxy-5-methylphenol according to the procedure of Wessely *et al.*

\[ \delta (\text{CDCl}_3 + \text{CD}_3\text{SOCD}_3) \]

1\(^{H}\) NMR: 8.15 (s, 1H, OH), 6.09 (s, 1H) and 6.03 (s, 2H, H2, H4 and H6), 3.63 (br s, 2H, NH2), 2.18 (s, 3H, CH3).

1\(^{3}\)C NMR: 8 (\text{CDCl}_3 + \text{CD}_3\text{SOCD}_3) 158.1, 147.8, 140.2, 107.7, 106.8, 99.7, 21.5.

EIMS: \textit{m/z} 123 (M, 100%), 122 (39), 106 (5), 94 (12).
3-methoxy-2-methyl-6-(4-methylbenzenesulfonamido)-phenol (510)

A mixture of 3-methoxy-2-methyl-6-nitrophenol (333) (200 mg, 1.1 mmol) and palladium on charcoal (10%, 3 mg), in ethanol (5 ml) was stirred under an atmosphere of hydrogen for 12 h. 4-Methylbenzenesulfonyl chloride (221 mg, 1.2 mmol) and sodium hydrogen carbonate (102 mg, 1.2 mmol) were added and the mixture was stirred for 3 h when more of both reagents (77 and 35 mg) was added. After stirring for a further 2 h the mixture was diluted with a pH 4 buffer (6 ml) and extracted with dichloromethane. Drying over magnesium sulfate and evaporation gave a crude product which was chromatographed with methanol in dichloromethane (1.5%) to give 3-methoxy-2-methyl-6-(4-methylbenzenesulfonamido)-phenol (510) (302 mg, 90%) as a white solid. Recrystallisation from dichloromethane-hexane gave white needles mp 134-136° (found: C, 58.5; H, 5.9; N, 4.5; S, 10.1%. C15H17NO4S requires C, 58.6; H, 5.6; N, 4.6; S, 10.4%).

FTIR: $\nu_{\text{max}}$ (CHCl$_3$) 3515 (OH), 3343 (NH), 1615, 1598, 1496, 1470, 1317, 1161, 1109 cm$^{-1}$.

$^1$H NMR: $\delta$ 7.60 (d, 2H, $J$ 8.2 Hz, SO$_2$ArH), 7.25 (d, 2H, $J$ 8.2 Hz, SO$_2$ArH), 6.58, (s, 1H, OH), 6.32 (d, 1H, $J$ 8.8 Hz, H4 or 5), 6.19 (d, 1H, $J$ 8.8 Hz, H4 or 5), 6.14 (br s, 1H, NH), 3.75 (s, 3H, CH$_3$O), 2.42 (s, 3H, SO$_2$ArCH$_3$), 2.11 (s, 3H, CH$_3$).

$^{13}$C NMR: $\delta$ 158.1, 151.6, 144.2, 134.7, 129.6 (CH), 127.7 (CH), 124.7 (CH), 115.0, 114.1, 102.2 (CH), 55.6 (CH$_3$O), 21.6 (SO$_2$ArCH$_3$), 8.6 (CH$_3$).

EIMS: $m/z$ 307 (M, 5%), 152 (M-SO$_2$Ar, 100), 124 (31).
3-methoxy-2-methyl-6-(4-methylbenzenesulfonamido)-1,4-benzoquinone (511)

To a solution of Fremy's salt (286 mg, 1.1 mmol) and potassium dihydrogen phosphate (4 mg) in water (10 ml) was added 3-methoxy-2-methyl-6-(4-methylbenzenesulfonamido)-phenol (510) (51 mg, 0.18 mmol) in methanol (3 ml). Water (8 ml) and acetone (1 ml) were added to give a solution. After stirring for 2 h an orange precipitate was filtered. This was washed with water, dissolved in dichloromethane, dried over magnesium sulfate and chromatographed (1% methanol in dichloromethane) to give 3-methoxy-2-methyl-6-(4-methylbenzenesulfonamido)-1,4-benzoquinone (511) as an orange solid (51 mg, 96%). Recrystallisation from benzene-hexane gave orange needles mp 155-157° which contain benzene. An analytical sample was prepared by sublimation (found: C, 56.3; H, 4.8; N, 4.3; S, 9.6%. C15H15N05S requires C, 56.1; H, 4.7; N, 4.4; S, 10.0%).

UV: \( \lambda_{\text{max}} \) (ETOH) 414 (\( \epsilon \) 1.1 x 10^3), 301 (2.2 x 10^4), 222 cm\(^{-1}\) (2.2 x 10^4).

+ NaOH: 500 (\( \epsilon \) 3.7 x 10^3), 316 (1.8 x 10^4), ca 220 cm\(^{-1}\) (> 2 x 10^4).

\(^1\)H NMR: \( \delta \) 7.83 (br s, 1H, NH), 7.81 (d, 2H, J 8.5 Hz, SO\(_2\)ArH), 7.34 (d, 2H, J 8.5 Hz, SO\(_2\)ArH), 6.29 (s, 1H, H5), 4.07 (s, 3H, CH\(_3\)O), 2.43 (s, 3H, SO\(_2\)ArCH\(_3\)), 1.88 (s, 3H, CH\(_3\)).

\(^{13}\)C NMR: \( \delta \) 182.4 and 182.2 (CO), 156.3 (C3), 145.4, 137.8, 134.9, 130.1, 127.6, 124.3, 108.5, 61.4 (CH\(_3\)O), 21.7 (SOArCH\(_3\)), 8.5 (CH\(_3\)).

EIMS: \( m/z \) 321 (M, 4%), 257 (M-SO\(_2\), 3), 166 (M-SO\(_2\)Ar, 17), 155 (SO\(_2\)Ar, 13), 91 (C\(_7\)H\(_7\), 100).
EXPERIMENTAL

2-[N-benzyl,N-4-methylbenzenesulfonamido]-4-benzyl-oxy-5-methoxy-6-methylphenol (513)

To 3-methoxy-2-methyl-6-(4-methylbenzenesulfonamido)-1,4-benzoquinone (511) (38 mg, 0.12 mmol) was added palladium on charcoal (10%, 0.5 mg) and acetone (2 ml). The mixture was stirred under an atmosphere of hydrogen gas causing a rapid loss of colour. The mixture was filtered through celite under Ar onto potassium carbonate (65 mg, 0.47 mmol) with more acetone (2 ml). Benzyl bromide (30 µl, 0.25 mmol) was added and the mixture heated at reflux for 17 h. After dilution with sodium dihydrogen phosphate (20%), extraction with dichloromethane (x3), washing with brine and drying over magnesium sulfate, a crude semisolid product was obtained. Chromatography with neat dichloromethane gave two products. The more polar product was 2-[N-benzyl,N-4-methylbenzenesulfonamido]-4-benzyl-oxy-5-methoxy-6-methylphenol (513) (19 mg, 33%) a white solid mp 144-145° from ethanol (found: C, 69.1; H, 6.1; N, 2.7%. C29H29N05S requires C, 69.2; H, 5.8; N, 2.8%).

$^1$H NMR: $\delta$ 7.60 (d, 2H, J 8.3 Hz, SO$_2$ArH), 7.40-7.17 (M, 10H), and 7.03 (M, 2H, SO$_2$ArH and Ph x 2), 6.05 (s, 1H, OH), 5.75 (s, 1H, H3), 5.05 (br s, 1H, CHPh), 4.67 (br s, 1H, CHPh), 4.59 (br s, 1H, CHPh), 3.95 (br s, 1H, CHPh), 3.78 (s, 3H, CH$_3$O), 2.47 (s, 3H, SO$_2$ArCH$_3$), 2.07 (s, 3H, CH$_3$).

$^{13}$C NMR: $\delta$ 149.2, 148.4, 144.7, 144.2, 137.1, 135.2 and 134.1 (some of C1, C2, C4, C5, C6, benzyl and benzenesulfonamide quaternaries), 129.6, 128.7, 128.55, 128.49, 128.3, 128.0, 127.9 and 127.1 (benzyl and benzenesulfonamide CH), 120.6 and 119.4 (remaining quaternaries), 111.3 (C3), 71.6 (OCH$_2$), 60.5 (CH$_3$O), 55.8 (NCH$_2$), 21.6 (SO$_2$ArCH$_3$), 9.4 (CH$_3$)

EIMS: m/z 348 (M-SO$_2$Ar, 7), 256 (M-HSO$_2$Ar-C$_7$H$_7$, 6), 91 (C$_7$H$_7$, 100).
The less polar product was N-benzyl,N-(4-methylbenzenesulfonyl)-2,5-dibenzyl oxy-4-methoxy-3-methylbenzenamine (514) (26 mg, 37%) isolated as an oil (found M: 593.2236; C36H35N05S requires 593.2236).

$^1$H NMR: $\delta$ 7.63 (d, 2H, $J$ 8.4 Hz, $\text{SO}_2\text{ArH}$), 7.40-7.05 (m, 13H, PhH), 7.20 (d, 2H, $J$ 8.4 Hz, $\text{SO}_2\text{ArH}$), 6.94 (M, 2H, PhH), 6.29 (s, 1H, H6), 4.79 (br s, 2H, CH$_2$Ph), 4.72 (s, 1H, CHPh), 4.61 (br s, 3H, CHPh), 3.82 (s, 3H, CH$_3$O), 2.41 (s, 3H, $\text{SO}_2\text{ArCH}_3$), 2.15 (s, 3H, CH$_3$).

$^{13}$C NMR: $\delta$ 149.7, 148.3, 147.4, 143.3, 137.7, 137.6, 136.9 and 136.0 (some of C1, C2, C3, C4, C5, benzyl and benzenesulfonamide quaternaries), 129.4, 129.2, 128.5, 128.3, 128.1, 127.93, 127.87, 127.7, 127.64, 127.57, 127.1 (benzyl and benzenesulfonamide CH), 127.4 and 127.0 (remaining quaternaries), 113.6 (C6), 74.8 and 70.8 (OCH$_2$), 60.3 (CH$_3$O), 54.8 (NCH$_2$), 21.5 (SO$_2$ArCH$_3$), 10.3 (CH$_3$).

EIMS: $m/z$ 593 (M, 0.6%), 502 (M-C$_7$H$_7$, 1), 91 (C$_7$H$_7$, 100).
**EXPERIMENTAL**

2-methoxy-3-methyl-1,4-benzoquinone (515)

To a suspension of potassium fericyanide (142 mg, 0.43 mmol) and 1,3-dimethoxy-2-methylbenzene (1.05 g, 6.9 mmol) in water (0.7 ml) and acetone (8 ml) was added hydrogen peroxide (30%, 2.4 ml) and the mixture was stirred for 80 h. After dilution with water (5 ml) and extraction with dichloromethane (x3), drying over sodium sulfate and evaporation gave a crude orange product which was sublimed (0.2 torr) to give 490 mg (47%) of a product largely consisting of 2-methoxy-3-methyl-1,4-benzoquinone (515). This procedure has not been optimised and the work up appears to lead to significant losses.

1H NMR: The NMR was in accord with the literature.\textsuperscript{580b}

\[ \delta 6.69 (d, 1H, J 10 Hz, H5 or 6), 6.60 (d, 1H, J 10 Hz, H5 or 6), 4.03 (s, 3H, CH$_3$O), 1.96 (s, 3H, CH$_3$). \]

2-hydroxy-3-methyl-1,4-benzoquinone (522)

2-hydroxy-3-methyl-1,4-benzoquinone (522) was prepared according to the method of Rashid and Read.\textsuperscript{527}

UV: UV data match the spectrum reported by Flaig et al.\textsuperscript{618}

\[ \lambda_{\text{max}} \text{(CHCl$_3$) 397 (}\epsilon 1.2 \times 10^3\text{), 255 nm (}\epsilon 9.3 \times 10^3\text{).} \]

1H NMR: NMR data fits that reported by Rashid and Read.\textsuperscript{527}

\[ \delta 6.86 (s, 1H, OH), 6.74 (s, 2H, H5 and H6), 1.97 (s, 3H, CH$_3$). \]

13C NMR: \( \delta 187.7 \text{ and } 182.8 \text{ (CO), } 151.2 \text{ (C2), } 139.0 \text{ (CH), } 131.7 \text{ (CH), } 117.9 \text{ (C3), } 8.0 \text{ (CH$_3$).} \)

EIMS: \textit{m/z} 138 (M, 79%), 110 (M-CO, 18), 83 (28), 82 (110-CO, 79), 81 (31), 56 (42), 55 (100), 54 (92), 53 (63).
Par une matinée de mai, une amazone, montée sur une jumente, parcourait, au milieu des fleurs, les allées du Bois de Boulogne

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REFERENCES


Appendix 1 Chemical Abstracts Service Names of the Mitomycins

Chemical Abstracts Service names and registry numbers for representative mitomycins are shown (as indexed).

Azirino[2',3':3,4]pyrrolo[1,2-a]indole

**Mitomycin A**
Azirino[2',3':3,4]pyrrolo[1,2-a]indole-4,7-dione, 8-[[aminocarbonyl]oxy]methyl]-1,1α,2,8α,8b-hexahydro-6,8a-dimethoxy-5-methyl-, [1αS-(1αα,8β,8αα,8βα)]- [4055-39-4]

**Mitomycin B**
Azirino[2',3':3,4]pyrrolo[1,2-a]indole-8-[[aminocarbonyl]oxy]methyl]-1,1α,2,8α,8b-hexahydro-8a-hydroxy-6-methoxy-1,5-dimethyl-, [1αS-(1αα,8β,8αα,8βα)]- [4055-40-7]

**Mitomycin C**
Azirino[2',3':3,4]pyrrolo[1,2-a]indole-4,7-dione, 6-amino-8-[[aminocarbonyl]oxy]methyl]-1,1α,2,8α,8b-hexahydro-8a-methoxy-5-methyl-, [1αS-(1αα,8β,8αα,8βα)]- [50-07-7]

**Porfiromycin**
Azirino[2',3':3,4]pyrrolo[1,2-a]indole-4,7-dione, 6-amino-8-[[aminocarbonyl]oxy]methyl]-1,1α,2,8α,8b-hexahydro-8a-methoxy-1,5-dimethyl-, [1αS-(1αα,8β,8αα,8βα)]- [801-52-5]

**Mitromycin (or Mityromycin)**
Azirino[2',3':3,4]oxazino[4,5-b]indole-2,5,8(1H)-trione,4,4α,10,10a,11,11α-hexahydro-6-methoxy-7,11-dimethyl-, [4αS-(4αα,10αβ,11αβ,11βR*)] [27164-43-8]

**Mitomycin H**
Azirino[2',3':3,4]pyrrolo[1,2-a]indole-4,7-dione,1,1α,2,8α,8b-hexahydro-8a-hydroxy-6-methoxy-1,5-dimethyl-8-methylene, [1αS-(1αα,8αα,8βα)] [74148-44-0]
Appendix 2a. Selected Statistics for All Natural and Some Very Similar Synthetic Mitomycins (no aziridine substituted derivatives) from the Cambridge Structural Database

Data were extracted using GSTAT and Chem3D and converted to the accepted absolute configuration (1S,2S) where necessary (to correlate sense).

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<td>140.6</td>
<td>138.9</td>
<td>139.7</td>
<td>144.7</td>
<td>146.9</td>
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<tr>
<td>N1a-C4a</td>
<td>2.67</td>
<td>2.65</td>
<td>2.67</td>
<td>2.68</td>
<td>2.68</td>
<td>2.74</td>
<td>2.66</td>
<td>2.68</td>
<td>2.67</td>
<td>2.74</td>
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<td>N4 plane</td>
<td>0.257</td>
<td>0.239</td>
<td>0.267</td>
<td>0.293</td>
<td>0.273</td>
<td>0.275</td>
<td>0.362</td>
<td>0.313</td>
<td>0.287</td>
<td>0.293</td>
</tr>
</tbody>
</table>

Note some structures have two independent molecules; data for both are given.

Bond Lengths (Å): A = O8-C8 B = C8-C8a C = C8a-C4a D = C4a-N4 E = O5-C5 F = C5-C6 G = C5-C7 H = C7-N4

Bond Angles: <1 = C4a,N4,C9a; <2 = C4a,N4,C3; <3 = C3,N4,C9a; Angle sum = <1 + <2 + <3

Atomic Separations N1a-N4 and N1a-C4a in Å.

N4 Plane = perpendicular distance (Å) between C3-C4a-C9a plane and N4.
Appendix 2b. Crystallographic Data for All Mitomycin Related Systems
# Appendix 2c X-ray Structures of Mitomycins

Reference codes (Cambridge Structural Database) and/or references for X-ray crystal structures of natural and synthetic mitomycins and closely related compounds are given.

<table>
<thead>
<tr>
<th>REFCODE</th>
<th>Structure</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>* BRBSMA</td>
<td>1-N-(4-Bromophenylsulphonyl)-mitomycin A, benzene solvate</td>
<td>1</td>
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<tr>
<td>* CEGNIL</td>
<td>1-N-(4-Bromobenzoyl)-mitomycin C</td>
<td>2</td>
</tr>
<tr>
<td>*</td>
<td>1-N-(4-Bromobenzoyl)-mitomycin A</td>
<td>3</td>
</tr>
<tr>
<td>* COPCAL</td>
<td>1-N-(4-Bromobenzoyl)-mitomycin A</td>
<td>4</td>
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<tr>
<td>*</td>
<td>“7-(4-Bromoanilino)-mitomycin B” monohydrate</td>
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</tr>
<tr>
<td>* COPCIT</td>
<td>“7-(4-Bromoanilino)-mitomycin B” monohydrate</td>
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<td>ψ KIKSAY</td>
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<tr>
<td>ψ MITCDH</td>
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<td>Mitomycin A hemihydrate</td>
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<td>ψ JIGFUA</td>
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<td>GESJET</td>
<td>9α-Hydroxy-mitomycin B</td>
<td>15</td>
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<tr>
<td>KABKIH</td>
<td>t-Butyl[1αS-(1αα,8β,8αα,8βα)]-8-[[aminocarbonyloxy]methyl]-1,1α,2,8a,8b-hexahydro-4-hydroxy-8a-methoxy-5-methyl-7-oxoazirino[2'3':3,4]pyrrolo[1,2-a]indol-6[7H]-ylidene]hydrazinecarboxylate monohydrate</td>
<td>17</td>
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<tr>
<td>DAKWAN</td>
<td>[1αR*,8αR*,8αB*,8B<em>S</em>]-7-hydroxy-8-Hydroxyethyl-1,1α,2,8a,8b-hexahydro-4,6-dimethoxy-1,5-dimethylazirino[2'3':3,4]pyrrolo[1,2-a]indole acetone solvate</td>
<td>18</td>
</tr>
</tbody>
</table>

* = absolute configuration determined  ψ = naturally occurring
APPENDIX 3  A NOTE ON THE DETERMINATION OF C9 STEREOCHEMISTRY

Franck assigned the relative stereochemistry of mitiromycin (6) on the basis of C ring shifts and the observation that the carbamate must be cis fused.9 The key point was that the resonance assigned as H3β in mitomycins is not coupled to H2 and is deshielded by the C5 carbonyl (‘A’ in Figure 7). The same pattern is observed in mitiromycin and does not fit the postulated coupling pattern for the other, unknown cis fused diastereomer (‘B’), where the deshielded H3 proton should be coupled to H2. Whatever the validity of the assumptions, the conclusion was correct.40,41 However such analysis does not assign C9 stereochemistry in systems where C9 and C9a are not fused and necessarily cis related.

Figure 7. NMR Coupling in Mitiromycin Diastereomers

The assignment of C9α stereochemistry to the mitomycins isolated by Claridge et al. was based on rather indirect evidence of aromatic solvent (pyridine) induced shifts by comparison with porfiromycin (4) and the observation that they have a greenish-blue hue on silica which differs from the colour of mitomycin C (3) (although UV spectra were identical). The validity of this comparison is unclear as both C9 stereochemistry and 9a-substitution differ. Gredley has proposed a method of assigning the C9 configuration of mitomycins based on the shifts of H9, H3 and H3′.27 In the light of data now available for 9-epi-mitomycin D (25) obtained in C5D5N, extension of the method to that common solvent requires revision of the model (Gredley used CD3OD
and CDCl₃). Data for 9-epi-mitomycin B (27) are also given but we do not have data for mitomycin B (2) in the same solvent available; relevant data may be in supplementary material for another paper. Gredley proposed that the chemical shift of H₉ and H₃α were smaller for 9S-mitomycins than 9R, while the shift of H₃β (or endo) was greater.

However H₃α (or exo) in the mitomycin D (7) system in C₅D₅N does not fit the pattern (δ 3.69 for 9-epi-mitomycin D (25) (9S), δ 3.68 ppm for mitomycin D (7) (9R); for the decarbamoyl compounds δ 3.67 and 3.60). The shifts for H₉ and H₃β do fit (Δδ₉,₀⁻₀.₀₉ and +₀.₁₁; -₀.₀₇ and +₀.₁₅ ppm respectively). Thus in C₅D₅N the assignment can only be based on the shifts of H₉ and H₃β. Claridge et al. only report average shifts in C₅D₅N for five new mitomycins (varying in 7-amino substitution) but assuming they are very similar, the revised model agrees with the C₉α assignment when comparison is made with 9-epi-mitomycin D (25) although the difference in H₉ shift is very small (Δδ₉,₀⁻₀.₀₄ for H₉ and +₀.₁₂ for H₃β).

The magnitudes of the shift differences are obviously rather small for comparisons of spectra recorded independently and corroborating CD or X-ray evidence is clearly desirable in general. It seems likely that ¹³C NMR would allow an alternative method of assignment, however there is presently insufficient data for epimeric mitomycins to analyse. Chemical correlations should be treated with some caution as the propensity for epimerisation at C9 is not always clear (e.g. see p32). In some instances NOE effects may be indicative, for example there is an 11% NOE between the 9a-methoxy and H₉ of porfiromycin (4) but generally the technique has not been useful.
The Important Mitomycins and Related Systems