Cycloaddition Approaches to Oxygen Heterocycles Applied to Natural Product Synthesis

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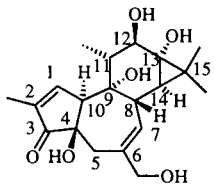
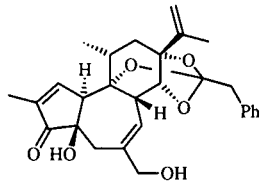
A Synthetic Approach Towards Phorbol *via* the Intramolecular Diels-Alder Reaction of Furan (IMDAF).

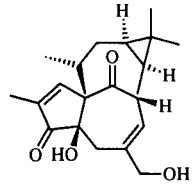
The tiglianes of which the phorbol esters are members and the closely related daphnanes and ingenanes are tri- and tetracarbocyclic diterpenes noted for their cytotoxicity [1]. Phorbol esters are abundant within the genus *Euphorbiaciae* which includes the spurges and the ornamental house plant poinsettia (*E. plucherrima*). The genus owes its name to King Juba the 3rd of Mauritania (25 B.C.), who named the species after his physician Euphorbos [2].



phorbol
(tigliane)



resiniferol
(daphnane)



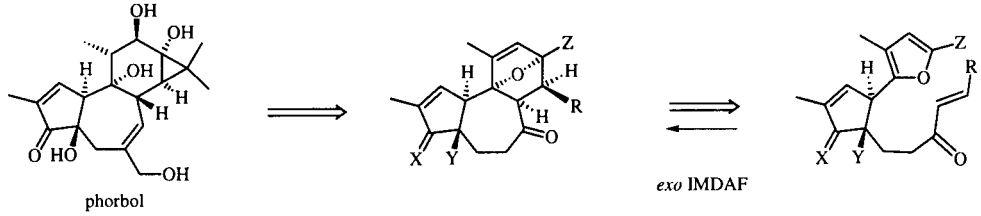
ingenol
(ingenane)

In folkloric medicine the plant extracts have been used to treat tumours, migraine, parasite infections, venereal disease, skin conditions and were also used as purgatives and abortifacients [3]. However, despite their widespread medical use, the toxicological properties of these plants are very severe, inducing intense inflammation [4] and such treatments probably had a rather efficient "kill or cure" result [5]. Indeed, the toxic extract from *E. poisonii* has been used as an arrow tip poison and as a fish poison [6].

Probably the most important physiological property of the phorbol esters is their capacity to act as tumour promoters [7] and tetradodecanoyl phorbol acetate is the most potent tumour promoter known to man, being active at levels of 0.02 μmol [8]. The origin of such activity was identified in 1982, when Castagna [9] showed that tetradecanoyl phorbol acetate bound to the ubiquitous enzyme protein kinase C, substituting for 1-oleyl-2-acetylglycerol which is the natural activator of protein kinase C, a Ca^{2+}-dependent enzyme responsible for regulating serine and threonine phosphorylation [10]. Conversely, several of the ingenanes and daphnanes have been found to show anti-leukaemia activity [11].

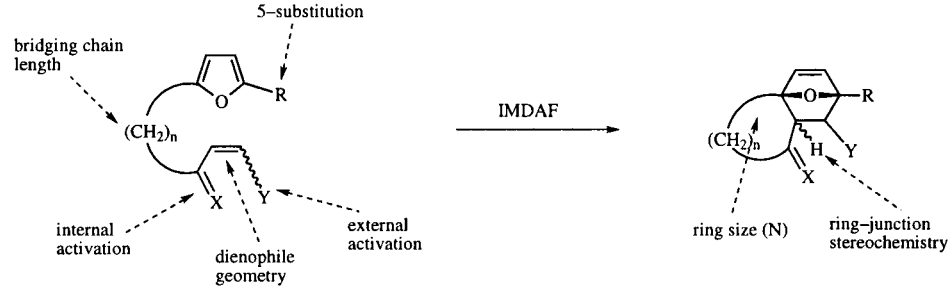
Phorbols are diterpene members of the tigliane family possessing a tetracyclic framework consisting of a *trans*-perhydroazulene (A,B rings) *trans*-fused and a *gem*-dimethylcyclopropyl (D ring) *cis*-fused to a cyclohexane ring (C ring). The complex polycyclic structure of phorbol, together with intense interest in structure-activity relationship studies to map the basis of the tumour promotion activity have fuelled extensive efforts towards establishing efficient synthetic routes to phorbol and its derivatives, culminating in Wender's total synthesis of phorbol [12] which has also been developed in the enantiomerically pure series [13]. Other approaches, yet to lead to a total synthesis, include the work Shibasaki [14], Rigby [15], Bullman-Page [16], Dauben [17], McMills [18], Paquette [19] and Little [20], in addition to our own studies.

At the outset of this work, our original retrosynthetic analysis invoked a novel *exo*-intramolecular Diels-Alder reaction of a furan diene (IMDAF) with an *E*-configured dienophile to construct a highly functionalised advanced intermediate possessing correct stereochemistry at 4 centres of the C-ring. Prediction of *exo*-diastereoselectivity for the IMDAF was based upon the known reversibility of furan Diels-Alder reactions leading to isolation of the thermodynamically more stable *exo*-cycloadducts, but it was impossible to say what control, if any, could be exerted over the stereocentres at the perhydroazulene ring junction.

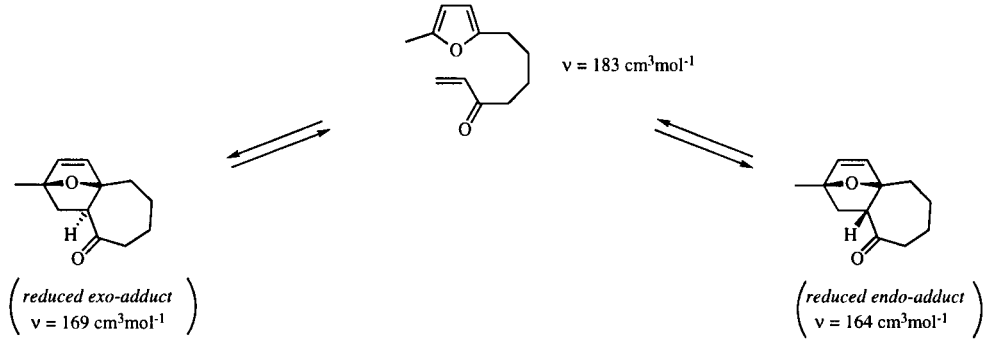


Even with hindsight, it is difficult to conclude whether we were naive or courageous to take on this approach as it had previously been demonstrated that such IMDAF reactions occurred reluctantly at best and not at all for construction of 6,7-fused bicyclic systems [21]. However, our initial model studies mapping out the structure-reactivity profile for this previously unreported process fortunately led us to the discovery that cycloaddition could be induced by the application of ultra-high pressures (up to 20 Kbar) and we were able to formulate a set of ground rules for the cycloaddition as shown below:

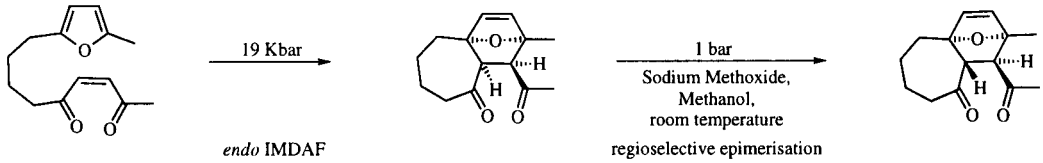
1. High pressures (>10 Kbar) induces IMDAF.
2. Products may cyclorevert at 1 bar.
3. R = H is more reactive than R = Me.
4. Ring size 6 > 7 >>> 5 (for unsubstituted bridging chain).
5. Double activation > internal activation >> external activation.
6. *E*-dienophile > *Z*-dienophile.
7. Under high pressure conditions.
   N = 6; *exo*-adduct kinetically and thermodynamically favoured.
   N = 7: *exo*-adduct kinetically favoured, *endo*-adduct thermodynamically favoured.
8. At 1 bar, *endo*-adduct (N = 7) can be epimerised to the *exo*-stereoisomer.



Thus, our model studies led to the unexpected observation that, for phorbol-like systems generating a 7-membered carbocycle, the kinetic product of the IMDAF is the *exo*-adduct; whereas the thermodynamic product is the smaller, more sterically congested *endo*-adduct [22] *under high pressure conditions*. Molar volume measurements on a model substrate and reduced *exo*- and *endo*-cycloadducts gave values of 183 cm^3mol^{-1}, 169 cm^3mol^{-1} and 164 cm^3mol^{-1} respectively in agreement with this observation.

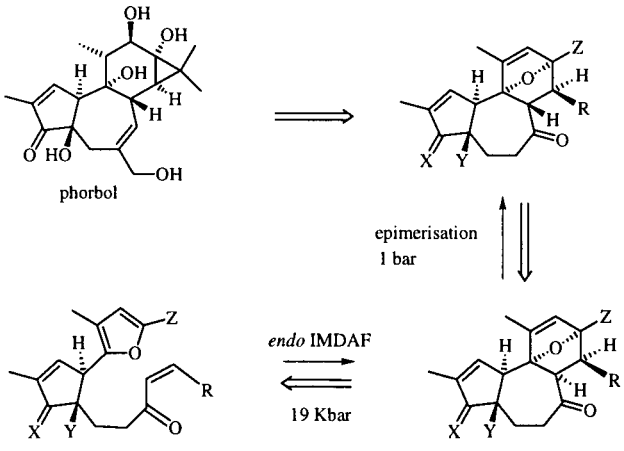


Equally important was the observation that this unexpected thermodynamically favoured *endo*-cycloaddition of a Z-enedione precursor at high pressure is reversed at standard pressure, with epimerisation occurring regioselectively at the ring junction to furnish the desired phorbol relative stereochemistry at all centres of the oxabicyclo-[2.2.1]heptene [23].



Such an observation is at variance with our results using 4-atom tethers between furan and dienophile where the stereocontrol is the same as observed by others such as Parker [24] and De Clerq [25]. Subsequent to these publications on stereocontrol in the high pressure mediated IMDAF, Keay reported studies which effectively repeated that portion of our work relating to high pressure mediated generation of decalin-derived systems using 4-carbon tethers, apparently unaware of our previous disclosures [26].

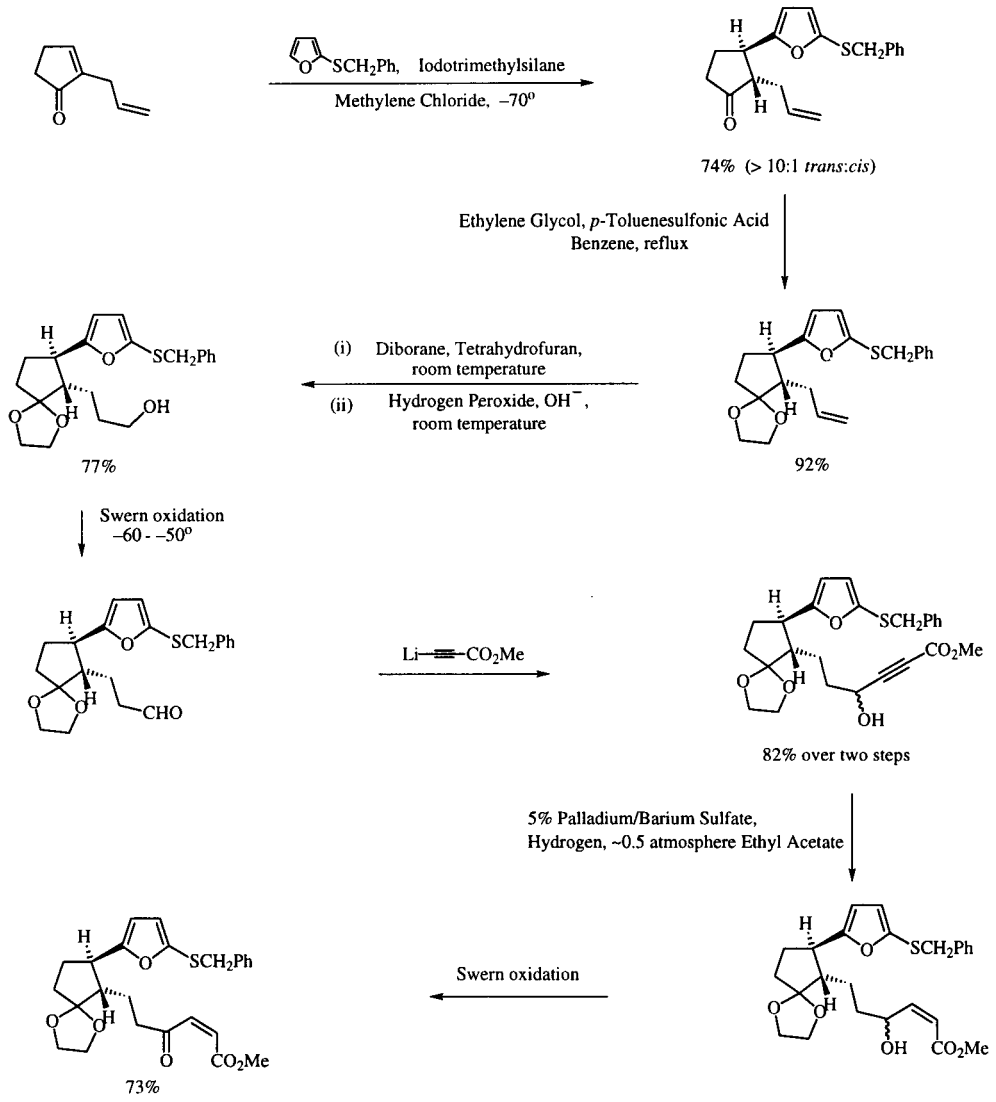
These model studies led to a revision of our synthetic approach to involve high pressure mediated *endo*-IMDAF of a Z-doubly activated dienophile, followed by regioselective epimerisation of the reduced cycloadduct at standard pressure, establishing the required stereochemistry at the B-C ring junction.



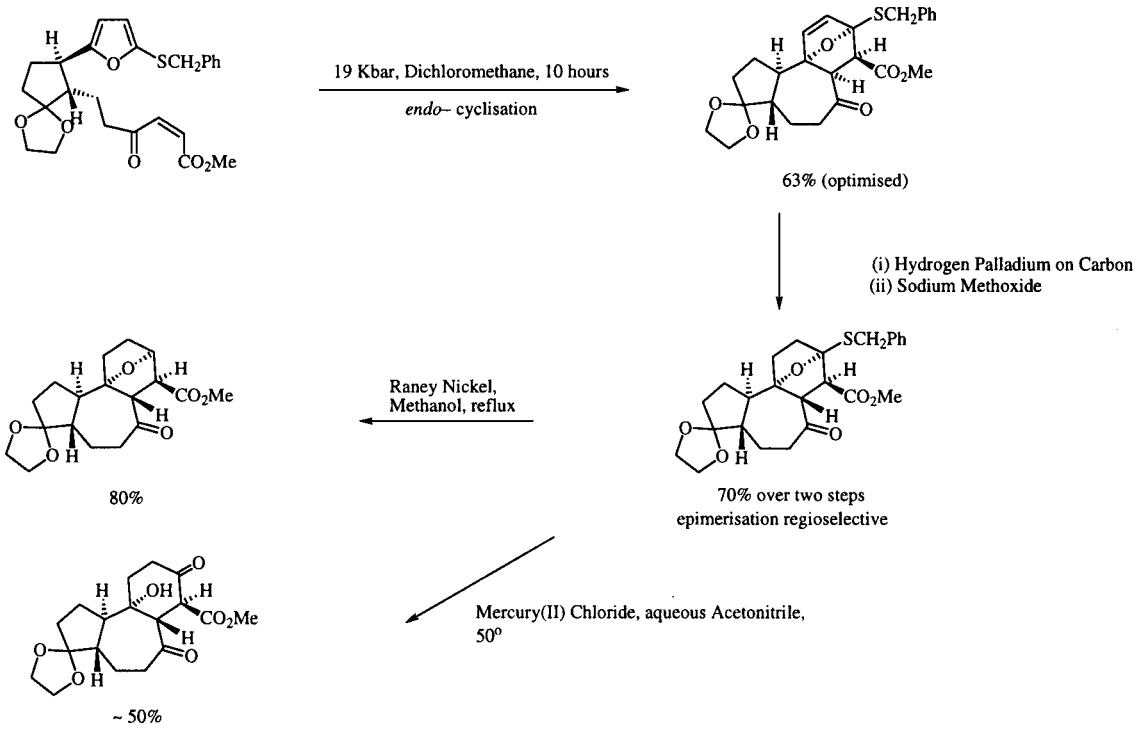
Revised retrosynthetic analysis in light of model studies.

We also had to consider what functionality should be included on the furan to ease cleavage of the oxygen bridge subsequent to IMDAF. Kotsuki *et al.* [27] had previously reported that using 2-methylthiofuran in the high pressure mediated intermolecular Diels-Alder reaction, permitted cleavage of the oxygen bridgehead of the resultant cycloadducts and we decided to adopt this approach for further functionalisation of the C-ring of our cycloadducts.

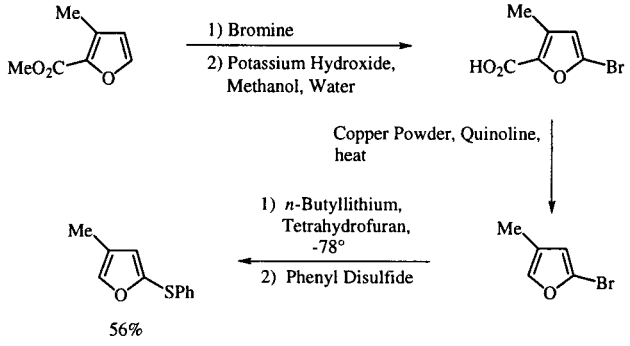
Following the procedure reported by Kraus [28], adding 2-(benzylthio)furan to 2-allylcyclopent-2-enone in the presence of trimethylsilyl iodide at room temperature furnished the 1,4-adduct in a 12:1 ratio in favour of the *trans* isomer in an overall yield of 75% [29]. Treatment of the adduct with ethylene glycol under acid catalysis gave a virtually quantitative yield of the ketal and subsequent hydroboration and oxidation afforded the primary alcohol in a yield of 77%. Swern oxidation gave the unstable aldehyde which was directly treated with methyl lithiopropynoate to give the propynylic alcohol in an overall yield of 80%. Partial hydrogenation was performed in the presence of 5% palladium/barium sulfate to afford the crude α,β-unsaturated ester. Since this compound was prone to lactonisation, it was directly subjected to Swern oxidation conditions to give the IMDAF precursor in 73% yield for both steps.



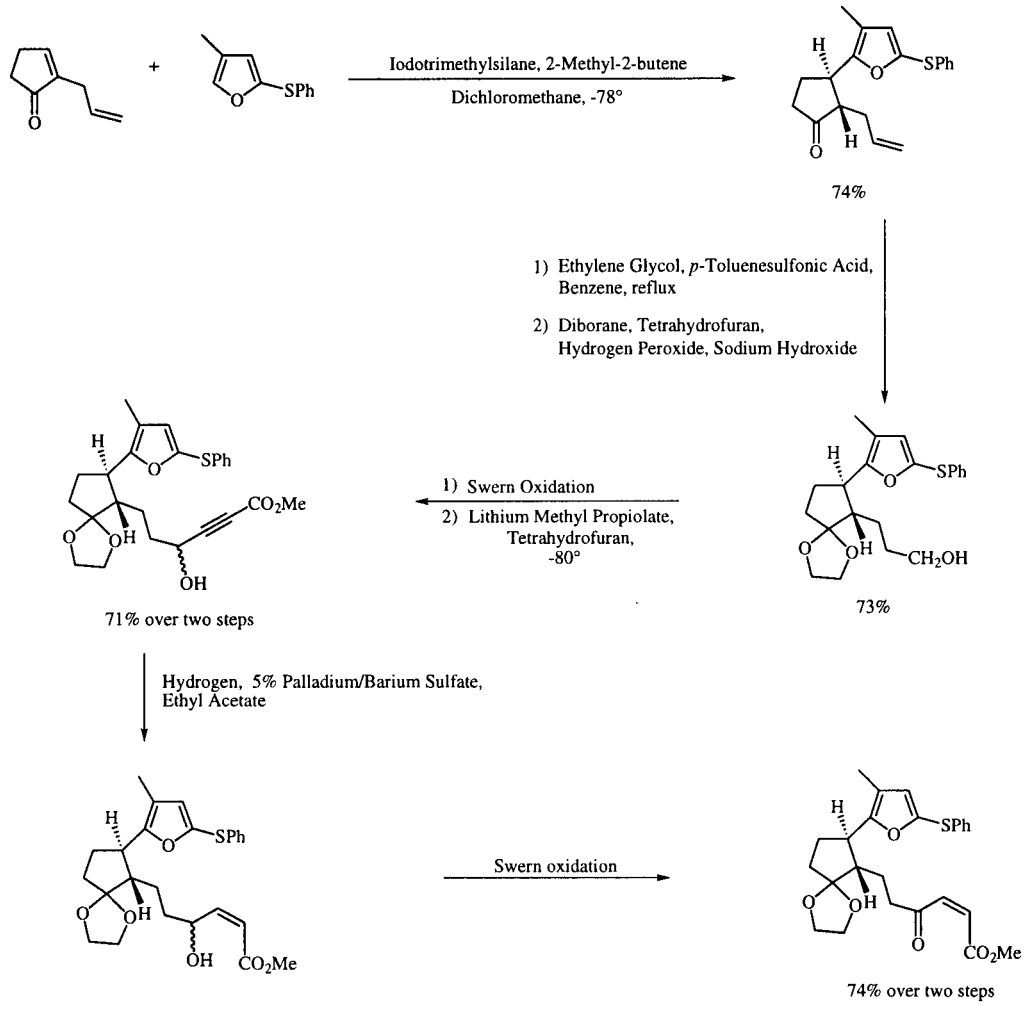
The precursor was subjected to 19 Kbar pressure at room temperature in dichloromethane for 15 hours to afford a single cycloadduct in 65% yield which, after hydrogenation, was shown to be the *endo*-product. Epimerisation furnished the C-8 epimer in 84% yield and this structure was confirmed by X-ray spectroscopic analysis, no epimerisation being observed at C-14. Hydrolytic cleavage of the oxygen bridge using mercuric chloride in aqueous acetonitrile afforded the tricycle in 58% yield. Thus the α'-benzylthiofuran substrate not only permitted oxygen bridgehead cleavage but also appeared to enhance the IMDAF step to yield a cycloadduct which could be readily manipulated to furnish a tricycle with the correct relative stereochemistry at C-4, C-8, C-9, C-10 and C-14.



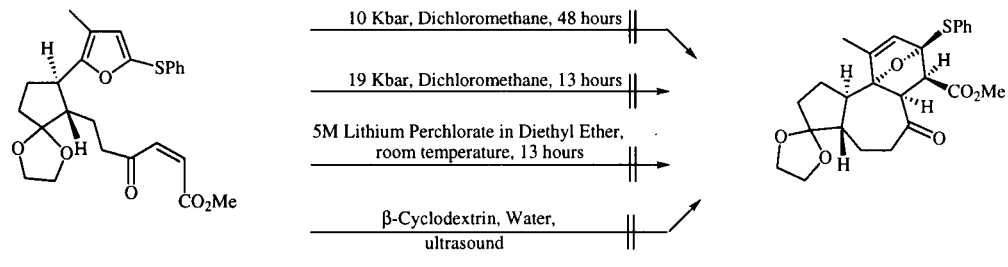
At this stage we wished to synthesise a Diels-Alder precursor in which the furan possessed an additional methyl substituent, in order to generate cycloadducts possessing a methyl group corresponding to that at C-11 on phorbol. Initially we decided to opt for the synthetically more accessible 4-methyl-2-(phenylthio)furan [30].



This was incorporated into the standard synthetic sequence to furnish our desired IMDAF precursor.

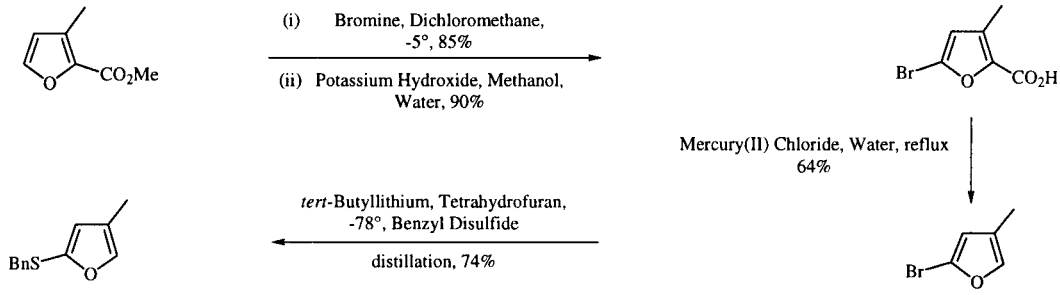


Disconcertingly, it was found that a range of high pressure conditions with this substrate led either to the recovery of starting material or decomposition and no conditions could be established which led to cycloadducts.

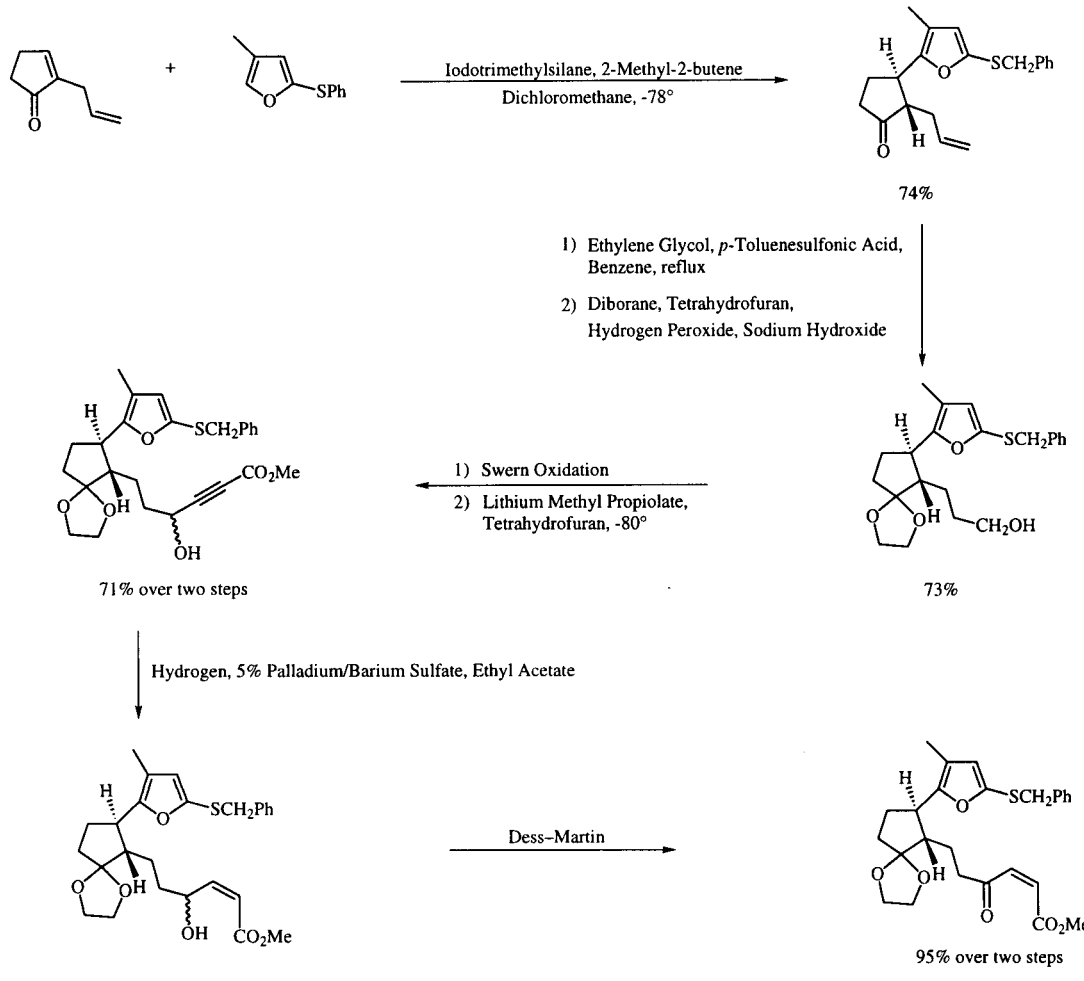


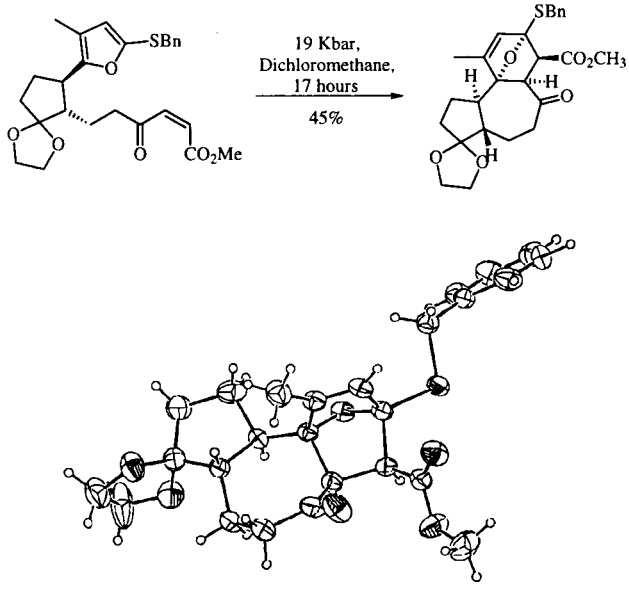
Molecular modeling calculations [31] indicated two possible sources for this reluctance to undergo the key cycloaddition step. Firstly, in the transition state, steric interactions between the methylene protons of the cyclopentane ring and the methyl group of the furan appeared to disfavour cycloaddition (*circa* 1.9 angstroms or less separation at the transition state) and secondly the 2-(phenylthio)-substituent was no longer enhancing electron density of the highest order molecular orbital of the furan, both possible reasons for the lowered propensity for cycloaddition.

From these observations it seemed that our synthetic strategy might be fatally flawed if the steric problems of introducing the additional methyl group on the furan were sufficient to tip the balance of a reluctant IMDAF to one which would not even occur under high pressure conditions. It was therefore imperative that we establish the reason for the above failure and we decided to reconsider incorporation of the benzylthioether functionality to see if this would favour cycloaddition. This required a reproducible route for synthesis of large quantities of 2-(benzylthio)-4-methylfuran. We had previously examined the literature procedure [32] and found the copper-quinoline mediated decarboxylation step to be highly capricious, producing only minute quantities of the desired material at the very best (it was this observation which had originally caused us to investigate the 2-(phenylthio)furan series described above). However, after much experimentation it was found that steam distillation of substrate, prepared from ester *via* the known bromoadduct, following the literature procedure, in the presence of mercury(II) chloride [33] led to efficient decarboxylation under mild conditions, permitting the desired, highly volatile 2-bromo-4-methylfuran to be isolated in a reproducible 64% yield. Lithium-halogen exchange by treatment of the bromide with *n*-butyllithium and quenching with dibenzyl disulfide permitted isolation of the target thioether in 74% distilled yield. With access to quantities of 2-(benzylthio)-4-methylfuran established, our previous route was followed to furnish the thiobenzyl IMDAF precursor, the sole modification being that the final oxidation step was carried out using Dess-Martin periodinane.



This precursor was then subjected to the standard ultra-high pressure conditions (19 Kbar, dichloromethane, room temperature, 17 hours) and we were rewarded with the isolation of a single cycloadduct in 45% recrystallised yield which proved more stable than previous cycloadducts, enabling X-ray crystallographic analysis to confirm the predicted structure.





Our most recent result has shown that we can achieve regioselective epimerisation without the need to reduce the double bond - an important advance for completion of the synthetic route.

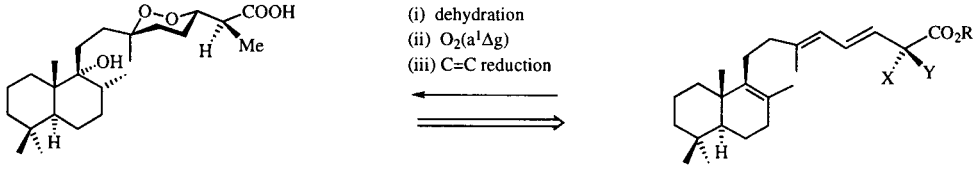
Whatever the steric effect resulting from inclusion of the methyl group on the furan, its presence clearly has a beneficial effect on the stability of the cycloadduct. The effect of the 2-thioether substituent on success or failure of the high pressure mediated IMDAF reaction was an unexpected and disconcerting element in our studies but the fact that cycloaddition is possible with the benzylthiofuran substrate has placed our synthetic approach on a firm footing for future synthetic efforts leading to the total synthesis of phorbol, which we are pursuing actively.

A Singlet Oxygen Photocycloaddition Approach to the Mycaperoxides.

In the second part of this presentation we address the synthetic problems associated with preparing natural products containing an endoperoxide moiety and show how our results shed some light upon the possible biosynthetic pathway to marine secondary metabolites possessing this novel functionality.

The mycaperoxides, typified by mycaperoxide B, make up a family of seven structurally similar norsesterterpene cyclic peroxides isolated from marine sponges of the *Mycale* family, and constitute an important sub-group within the class of marine cyclic peroxides isolated and characterised so far [34]. Isolation of a norsesterterpene triene from an Australian sponge, *Latrunculia brevis* [35] led to the proposal that this, and its side chain stereoisomers, might be biosynthetic precursors to the mycaperoxides, the endoperoxide unit being elaborated by singlet oxygen cycloaddition across the diene, followed by reduction of the unsaturated peroxide intermediate [36]. The co-isolation of related dienic terpenes alongside their cyclic peroxide counterparts, retaining unsaturation in the ring, from the herb *Alpinia chinensis*, has led others to the same biogenetic conclusion [37].

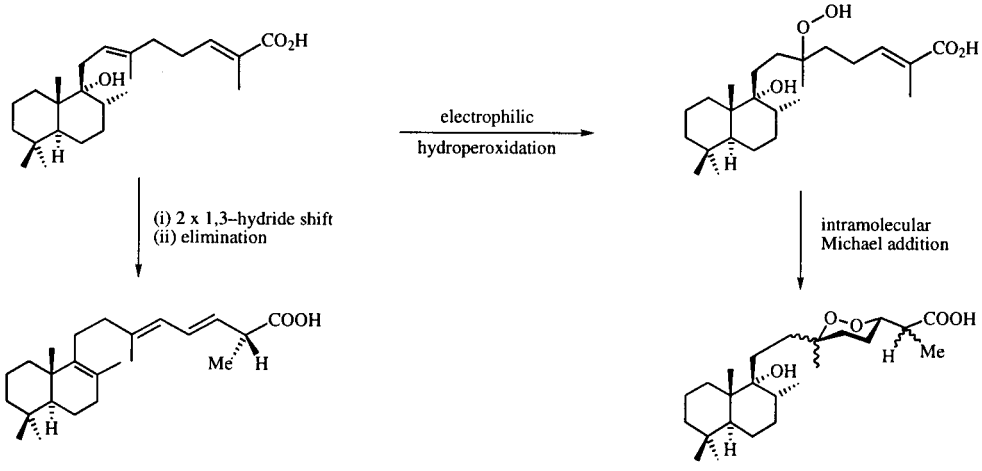
Recently however, isolation of a novel hydroperoxide-containing norsesterterpene endoperoxide from an Australian sponge of the genus *Sigmascreptrella* has led Capon to propose an alternative biosynthetic route [38]. This invokes hydroperoxide formation on the *re* or *si* face of a skipped diene, the newly generated hydroperoxide at C-6 then imposing stereochemical control over intramolecular Michael to afford either *cis* or *trans* configuration of the cyclic peroxide. The different stereochemistries at C-2 would result from protonation of the subsequent enolate from either face. In this way, biosynthesis of all known mycaperoxides can be encompassed and thus the above triene, obtained *via* double bond shifts and dehydration, would be a co-metabolite and not a biosynthetic precursor of the mycaperoxides.



mycaperoxide B

triene isolated from *L. brevis*

Proposed singlet oxygen cycloaddition biosynthetic pathway to mycaperoxides

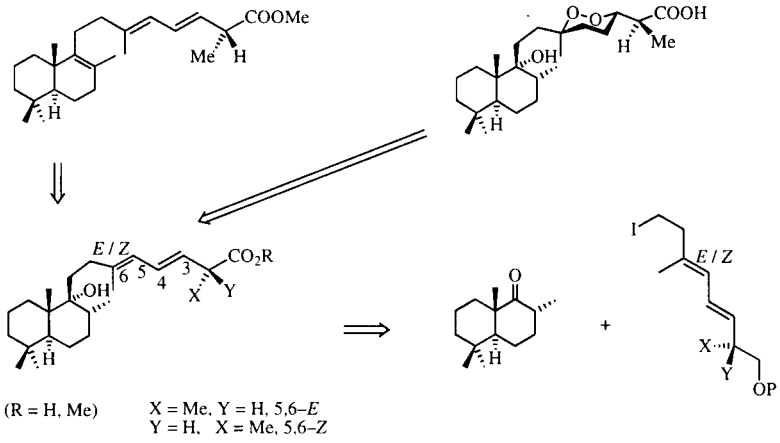


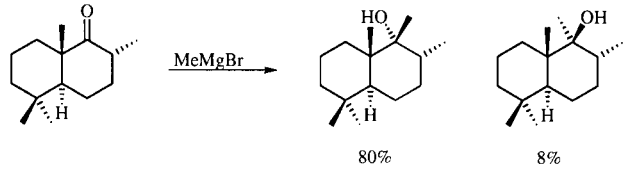
Capon's Michael addition proposal for mycaperoxide biosynthesis

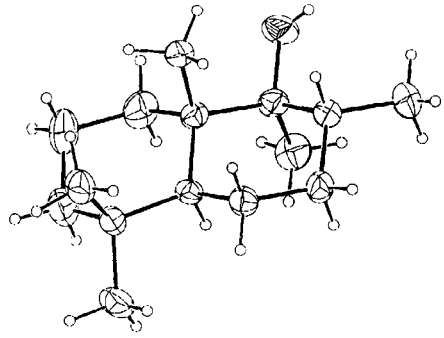
In an attempt to furnish evidence to distinguish between the two pathways we embarked upon a synthetic approach to the mycaperoxides flexible enough to permit laboratory investigation of both modes of cyclic peroxide formation. Our initial efforts focused on the singlet oxygen cycloaddition route and analysis of the mycaperoxide B skeleton indicated that cyclic peroxide formation required facially controlled addition of the singlet oxygen to a 3,4-*E*, 5,6-*Z*-conjugated diene. The synthetic approach was designed to be equally applicable to synthesis of the putative mycaperoxide B biogenetic precursor, as well as providing substrates for singlet oxygen cycloaddition studies.

It was envisaged that a series of potential diene substrates would be derived by equatorial attack of organometallic species upon (2*R*,9*S*,10*S*)-2,5,5,10-tetramethyloctahydronapthalen-1-one, available in 33% yield over 4 steps from (–)-carvone [39].

A literature survey of nucleophilic additions to decalones indicated that the methyl groups flanking the carbonyl have a profound effect on the stereochemical outcome, making it difficult to predict whether axial or equatorial attack would predominate [40]. In a model study to examine alkylation of our substrate, treatment with methyl magnesium bromide gave a 10:1 mixture of isomeric tertiary alcohols in almost 90% yield. Separation of the isomers by column chromatography afforded the minor isomer as a crystalline solid. X-ray crystallographic analysis showed the hydroxyl group to be in an equatorial configuration and hence demonstrating that the major isomer was derived from equatorial attack of the Grignard reagent, in accordance with our requirements.

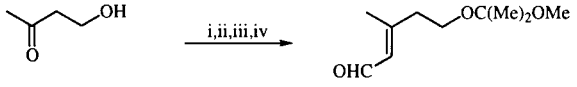






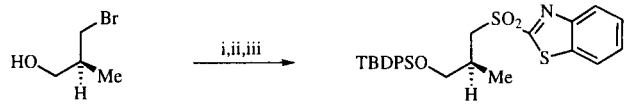
X-ray structure of minor adduct

Encouraged by this finding we embarked upon the synthesis of *E,E*-diene using a Julia coupling protocol. The aldehyde substrate for Julia coupling was prepared from 4-hydroxy-2-butanone in 30% overall yield by protection of the primary alcohol, Wadsworth-Emmons reaction and two-step conversion of the ester to the aldehyde [41]. The nmr analysis of the material obtained from the Wadsworth Emmons reaction showed a mixture of isomers (*E*:*Z* 4:1) but these proved inseparable at this stage.



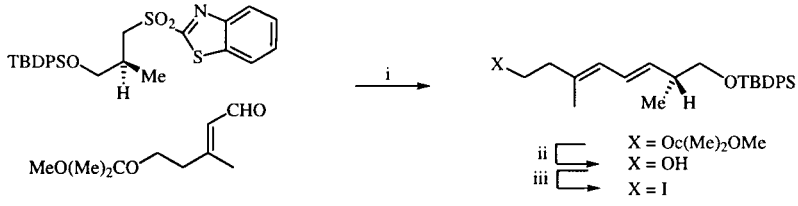
i. 2-Methoxypropene, Phosphorus Oxychloride, 93%; ii. Triethyl Phosphonoacetate, Sodium Hydride, Tetrahydrofuran, 25°, 47%; iii. Diisobutylaluminum Hydride, Diethyl Ether, -78°, 88%; iv. Tetrapropylammonium Perruthenate, 4-Methylmorpholine *N*-Oxide, 4-(Dicyanomethylene)-2-methyl-6-(4-dimethylaminostyryl)-4*H*-pyran, 25°, 78%.

The synthesis of 2-[(3*S*)-6-*t*-butyldiphenylsilyloxy)-3-methylpropylsulfonyl]benzothiazole was achieved in three steps from (*S*)-3-bromo-2-methylpropan-1-ol in an overall yield of 83%.



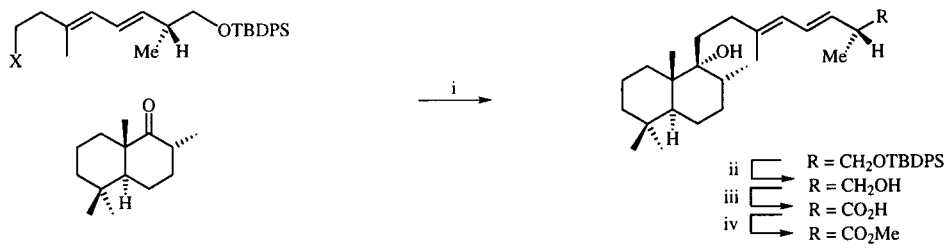
i. Imidazole, *tert*-Butylchlorodiphenylsilane, *N,N*-Dimethylformamide, 25°, 97%; ii. Mercaptobenzothiazole, Sodium Hydride, *N,N*-Dimethylformamide, 25°, 92%; iii. *m*-Chloroperoxybenzoic Acid, *N,N*-Dimethylformamide, 25°, 93%.

The stereochemical outcome of the one-pot olefination procedure developed by S. Julia [42] is often substrate dependent [43] but, when lithium bis(trimethylsilyl)amide (2 equivalents) was added to a mixture of the two substrates at -78°, coupling was achieved in high yield and stereoselectivity (95%, no *Z*-isomer detectable). Selective cleavage of the ketal permitted separation of the 4:1 mixture of alkene isomers resulting from the earlier Wadsworth Emmons reaction, furnishing pure *E,E*-alcohol in 52% yield which was converted to the iodide in 95% yield using triphenylphosphine/imidazole/I_2 [44].



i. Lithium bis(Trimethylsilyl)amide, 2 equivalents, Tetrahydrofuran, -78°, 87%, (>95/5 *E*:*Z*); ii: *p*-Toluenesulfonic Acid, Ethanol, 25°, 52%; iii: Triphenylphosphine, Imidazole, I_2, 4-(Dicyanomethylene)-2-methyl-6-(4-dimethylaminostyryl)-4*H*-pyran.

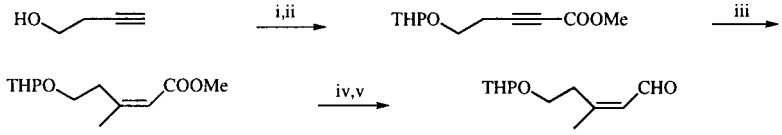
The coupling was achieved by direct treatment of a mixture of the two substrates, at -90° with two equivalents of *tert*-butyllithium to furnish a 7:1 mixture of diastereoisomers. Column chromatography furnished the major isomer in 56% yield and desilylation with *tert*-butylammonium fluoride furnished the primary alcohol in 95% yield. Finally, two step oxidation to the carboxylic acid and esterification with TMS-diazomethane without purification of intermediates gave methyl ester in 65% overall yield.



i. *tert*-Butyllithium (2 equivalents), Diethyl Ether, -90°, 56%; ii. Tetrabutylammonium Fluoride, Tetrahydrofuran, 25°, 95%; iii. a) Dess-Martin Periodinane, 4-(Dicyanomethylene)-2-methyl-6-(4-dimethylaminostyryl)-4*H*-pyran, 25°; b) Sodium Chlorite, 2-Methyl-2-butene, Sodium Hydrogen Phosphate, 25°; iv. Tetramethylsilane(cyanamide), Toluene, Methanol, 25°, 65%.

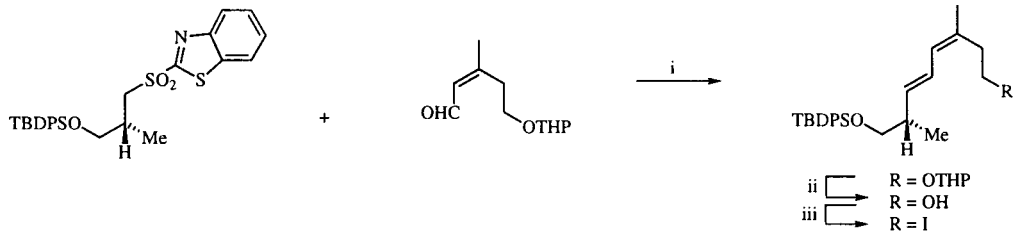
The final dehydration of ester was accomplished with 50% sulfuric acid to afford the natural product in 65% yield. The ^{1}H and ^{13}C nmr data were identical with those reported for the natural product and the specific rotation $[\alpha]_D^{22}$ = +5.5 (c = 2.4, chloroform) was in accord with the reported value for the isolated material, $[\alpha]_D$ = +13.3 (c = 2.55, chloroform), supporting the absolute configuration assigned to the triene isolated from *L brevis*.

The proposed biogenetic precursor to mycaperoxide B differs in possessing the (*R*)-configuration at the side chain stereocentre and the *Z*-geometry of the 5,6-double bond. The *Z*-2-methyl-5-hydroxypent-2-enal precursor for side chain construction was efficiently prepared as its tetrahydropyran ether in five steps from 3-butyn-1-ol in an overall yield of 75%.



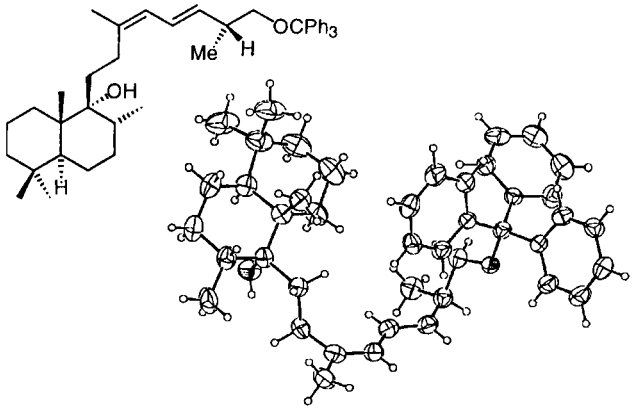
i. 3,4-Dihydro-2*H*-pyran, Pyridinium *p*-Toluenesulfonate, 4-(Dicyanomethylene)-2-methyl-6-(4-dimethylaminostyryl)-4*H*-pyran, 25°, 96%; ii. *n*-Butyllithium, Methyl Chloroformate, Tetrahydrofuran, -60°, 95%; iii. Copper(I) Iodide, Iodomethane, -78°, 91%; iv. Diisobutylaluminum Hydride, Diethyl Ether, -78°, 97%; v. Tetrapropylammonium Perruthenate, 4-Methylmorpholine *N*-Oxide, 4-(Dicyanomethylene)-2-methyl-6-(4-dimethylaminostyryl)-4*H*-pyran, 93%.

Following the previously established conditions for Julia olefination, reaction of 2-[(3*R*)-6-*t*-butyldiphenylsilyloxy)-3-methylpropylsulfonyl]benzothiazole with 2 equivalents of lithium bis(trimethylsilyl)amide gave the *E,Z*-diene in excellent yield and stereoselectivity. Removal of the tetrahydropyran ether and conversion of the alcohol to the iodide using previous conditions occurred in greater than 80% overall yield.

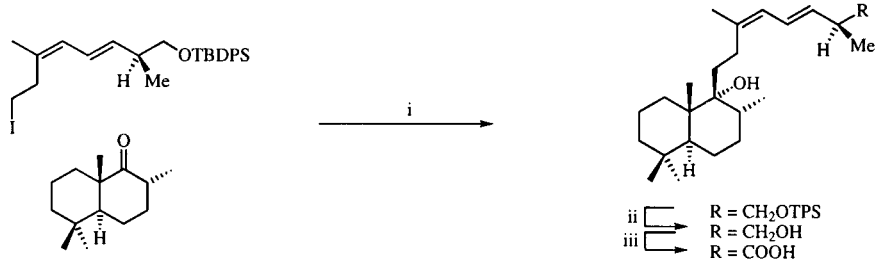


i. Lithium bis(Trimethylsilyl)amide, 2 equivalents, Tetrahydrofuran, -78°, 95% (>95/5 *E:Z*); ii. Pyridinium *p*-Toluenesulfonate, Ethanol, 55°, 85%; iii. Triphenylphosphine, Imidazole, I_2, 4-(Dicyanomethylene)-2-methyl-6-(4-dimethylaminostyryl)-4*H*-pyran, 25°, 95%.

The coupling of decalone and iodide was achieved in 70% yield applying previously optimized conditions. Attempts to confirm the stereochemistry of the alkylative coupling step were thwarted by our inability to obtain crystals of sufficient quality for X-ray crystallographic analysis. However, the trityl ether of the side-chain epimer did provide crystals which permitted X-ray crystallographic analysis to be carried out, confirming equatorial alkylation in this instance.



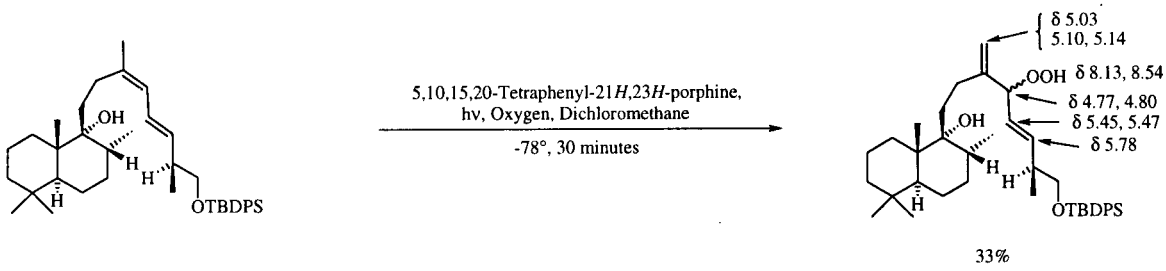
Desilylation with *tert*-butylammonium fluoride was achieved in 94% yield and two-step oxidation of the primary alcohol as before furnished the target carboxylic acid in 80% yield.



i. *tert*-Butyllithium (2 equivalents), Diethyl Ether, -85°, 70%; ii. Tetrabutylammonium Fluoride, Tetrahydrofuran, 25°, 94%; iii. a) Dess-Martin Periodinane, 4-(Dicyanomethylene)-2-methyl-6-(4-dimethylaminostyryl)-4*H*-pyran, 25°; b) Sodium Chlorite, 2-Methyl-2-butene, Sodium Dihydrogen Orthophosphate, 25°, 80%.

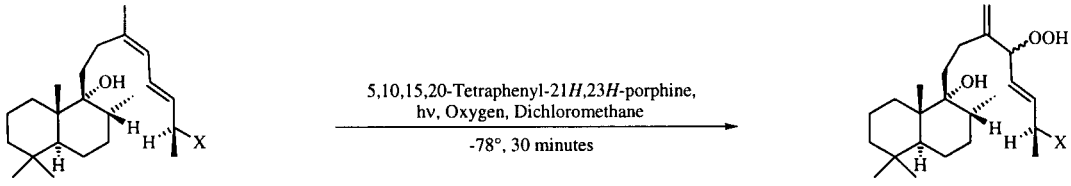
With a series of substrates available, we were now able to turn our attention to generation of the cyclic peroxides *via* singlet oxygen $4\pi + 2\pi$ photocycloaddition across the side chain diene. A solution of silyl ether in dichloromethane containing 10 mole % tetraphenylporphyrin as sensitiser was cooled to -78° and irradiated using a high pressure mercury lamp (125 W). The reaction mixture was agitated by passage of a stream of dry oxygen during 30 minutes when analysis by tlc indicated appearance of a more polar material. Removal of solvent and column chromatography furnished a single product along with unreacted starting material (40% recovery). Analysis by ^1H and ^{13}C nmr spectroscopy indicated the new material to consist of a 1:1 mixture of diastereoisomers. Particularly noteworthy was the appearance in the ^1H nmr spectrum of two deuterium oxide removable singlets at δ 8.13 and 8.54, indicative of hydroperoxide protons. In addition, two doublets were observed at δ 4.77 (0.5H, J = 8.0 Hz) and δ 4.80 (0.5H, J = 7.5 Hz) coupled to two double double doublets at δ 5.45 (0.5H, J = 1.0, J' = 8, J" = 16 Hz) and δ 5.47 (0.5H, J = 1.0, J' = 7.5, J" = 16 Hz) and these in turn were coupled to a broadened double doublet at δ 5.78

(1H, J = 7.5, J' = 16 Hz). The 1 Hz coupling of the absorptions centred on δ 4.77 and δ 4.80 and the 7.5 Hz coupling of the absorption centred on δ 5.78 could be correlated with the broadened apparent septet at δ 2.45 corresponding to the methine proton at C-2. Finally, the appearance of two broadened singlets at δ 5.10 and δ 5.14 (0.5H apiece) and a broad singlet centred on δ 5.03 (1H) led us to conclude that desired cycloaddition had not occurred but that an ene reaction had led to the formation of two diastereoisomeric hydroperoxides in 33% isolated yield.



33%

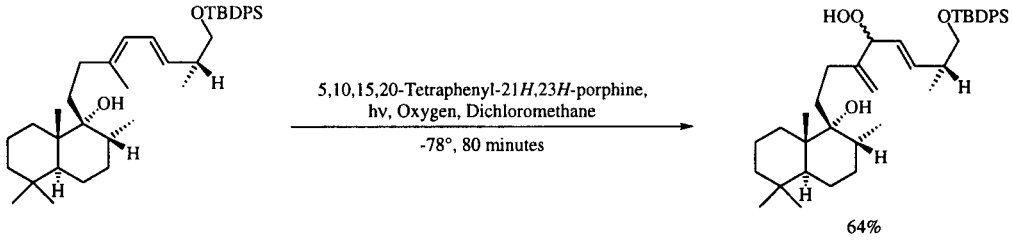
Repeating the reaction with unprotected alcohol likewise gave a mixture of diastereoisomeric ene products isolated in 66% yield. The ^{1}H nmr spectrum was directly comparable to before, showing *inter alia* two pairs of broadened singlets at δ 5.03, 5.11 and δ 5.04, 5.15, corresponding to the *geminal* vinylic protons, in a ratio of 2:1. Finally, the acid was also subjected to the same conditions and gave rise to a 35% purified yield of a 1:1 mixture of diastereomeric hydroperoxides with no starting material



X = CH_2OH, CO_2H

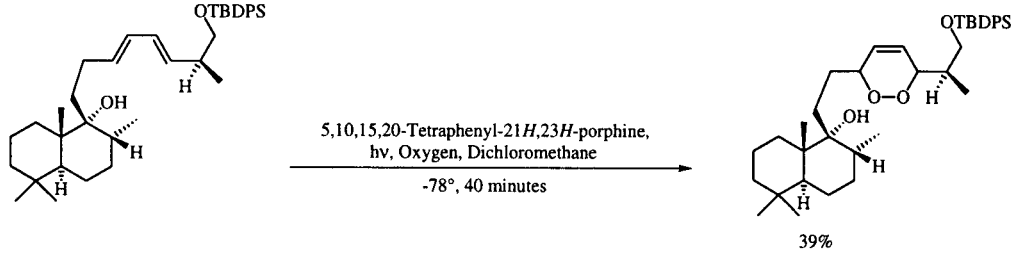
X = CH_2OH, 66%
X = CO_2H, 35%

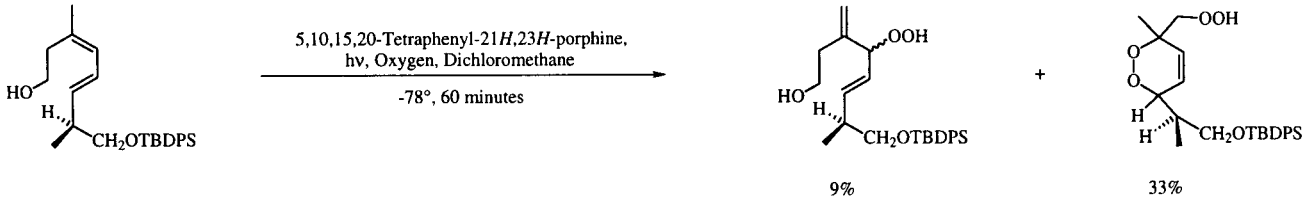
being recovered. Judging that reluctance of the *E,Z*-diene moiety to adopt the required *S-cis* conformation could explain absence of 4π + 2π cycloaddition [45], we subjected the epimeric *E,E*-diene-silyl ether to the same conditions. After 80 minutes only a single material was visible by tlc analysis. Once again, the ^{1}H nmr spectrum indicated signals consistent with a pair of diastereoisomers arising from an ene reaction, isolated in 64% yield.



As it was becoming evident that the C-6 methyl group was the conduit for ene reaction, we constructed the silyl ether possessing a 1,4-disubstituted *E,E*-diene and subjected this to the standard singlet oxygen conditions, giving a new material by tlc analysis. Removal of solvent and chromatographic purification led to the recovery of starting material (35%) and a substance which, although clearly a 1:1 mixture of diastereoisomers by ^{1}H nmr analysis, gave different spectroscopic data to the hydroperoxides previously isolated from such reaction mixtures. In the ^{1}H nmr spectrum, the key resonances were a 2H multiplet at δ 5.94, two broadened doublets at δ 4.61 (0.5H, J = 7.3 Hz) and δ 4.64 (0.5H, J = 5.5 Hz) and a 1H multiplet at δ 4.35-4.45. These absorptions correlate well with those for the ring protons of the endoperoxide derived from reaction of singlet oxygen with *E,E*-hexa-2,5-diene [46] which appear at δ 5.95 and δ 4.65 respectively. The ^{13}C spectrum of the new material showed pairs of peaks at δ 65.2, 65.6 and δ 78.9, 79.0 as well as a group at δ 126.1, 126.9, 127.6 and δ 128.5, 129.6. These correspond with ^{13}C spectrum for the simpler endoperoxide which shows resonances at δ 129.0 and δ 74.7 for the ring carbons. On the basis of these data, the new product could be assigned a cyclic peroxide structure with an isolated yield of 39%.

As the absorptions due to protons of the decalin system were complicating the spectroscopic analysis of the products, we decided to study singlet oxygen reactions on side chain fragments in order to better verify our initial conclusions. Accordingly, the *E,Z*-alcohol coupling precursor was treated under standard conditions for 1 hour to furnish a reaction mixture containing two new components and residual starting material, isolated in 12% yield after chromatography. The most polar component, isolated in 19% yield, was readily identified as a mixture of diastereoisomers resulting from ene reaction showing a pair of singlets at δ 9.79 and δ 9.85 integrating to one proton as well as characteristic multiplets corresponding to four alkene protons and a proton adjacent to the hydroperoxide. The third, least polar component showed signals at δ 8.37 and δ 8.53, implying the presence of a pair of diastereoisomeric hydroperoxide containing compounds, but also signals at δ 5.65, δ 6.45 and δ 4.53, characteristic of an endoperoxide. The ^{13}C nmr spectrum exhibited nine pairs of signals, excluding those corresponding to the *tert*-butyldiphenylsil group and a distortionless enhancement by polarization transfer experiment indicated only two methylene groups to be present showing that the product possessed one less carbon than the starting material. These data, in addition to consideration of couplings by 2D-analysis, led to assignment of a hydroperoxy-endoperoxide structure to this new product in which a methylene group has been lost. Spectrometric analysis provided further evidence for this unexpected result, showing a highest mass peak of 377, corresponding to M^+-O_2/O_2H.

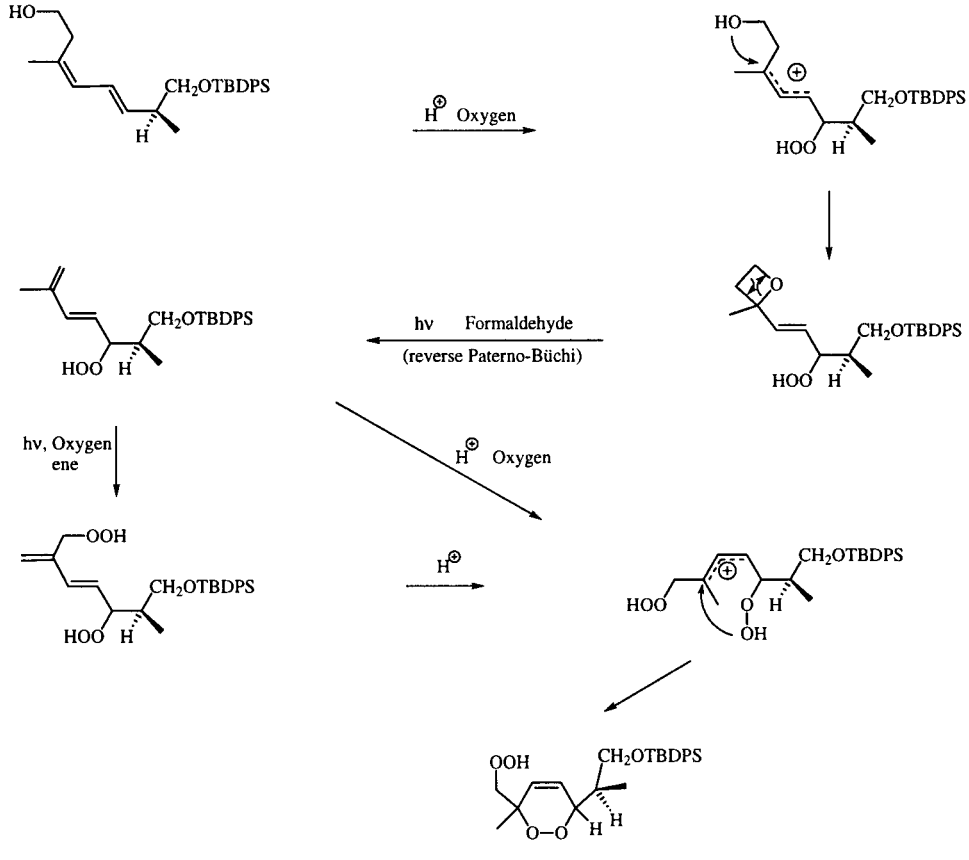




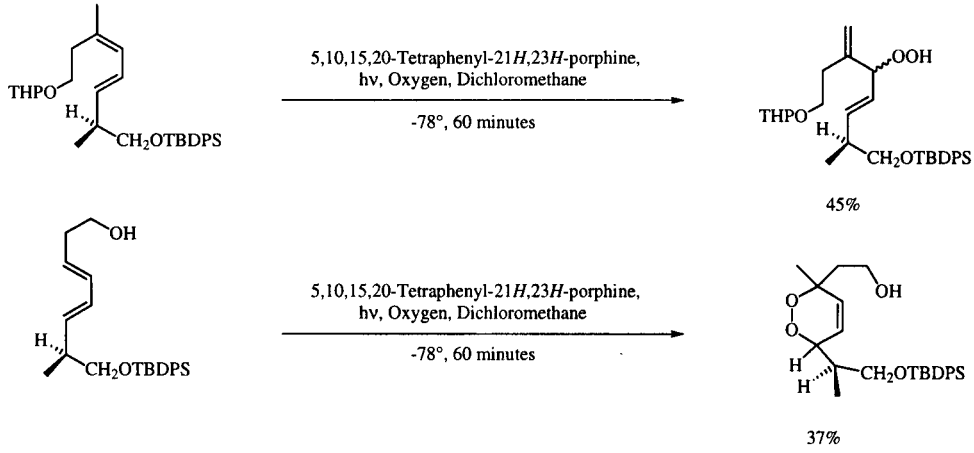
This totally unexpected result can be rationalised by the mechanistic sequence outlined below which takes into account the known reluctance of these trisubstituted *E,Z*-diene systems to undergo $4\pi + 2\pi$ singlet oxygen cycloaddition. Acid catalyzed addition of oxygen [47] to the least substituted double bond of the substrate in an *S-trans* conformation would generate a stabilised tertiary allylic carbocation which can be trapped by the hydroxyl to generate an oxetane intermediate.

Photochemically allowed reverse Paterno-Büchi extrusion of formaldehyde would then form a 1,3-disubstituted diene. This could then undergo either an ene reaction with more singlet oxygen followed by acid catalysed closure of the secondary hydroperoxide group or a second acid catalysed addition of oxygen to generate a tertiary carbocation which is quenched by the secondary hydroperoxide.

Support for this mechanism comes from the fact that the tetrahydropyran ether derivative furnished only the



normal ene product in 45% isolated yield under the same conditions; whereas the *E,E*-disubstituted analogue with an unprotected hydroxyl group gave recovered starting material in 42% yield and the expected cyclic endoperoxide in 37% yield.



Taken as a whole, these results challenge the viewpoint that the mycaperoxides - or any other 3,3,6-trisubstituted-1,2-dioxanes are constructed *in vivo* by singlet oxygen cycloaddition. However, they do not invalidate such a biosynthetic pathway for 3,6-disubstituted-1,2-dioxanes. Indeed, a survey of naturally occurring cyclic peroxides gives circumstantial support for the operation of both pathways. Most trisubstituted endoperoxides, such as the mycaperoxides have saturated rings and possess a 3-(2-propanoic acid) or related substituent, as would be expected from a hydroperoxide Michael type cyclisation pathway; whereas the disubstituted systems commonly possess the 4,5-double bond that would be formed in the singlet oxygen cycloaddition to a 1,4-disubstituted diene.

The implications regarding the biosynthesis of the mycaperoxides notwithstanding, the consequences of the intervention of ene-type chemistry in the reaction of singlet oxygen with *E,Z*-trisubstituted dienes means that the singlet oxygen approach is unlikely to lead to successful synthetic access to the mycaperoxides, although it remains a valid approach to disubstituted unsaturated endoperoxides.

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REFERENCES AND NOTES

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[1] Naturally Occurring Phorbol Esters, F. J. Evans, ed, C. R. C. Press, Boca Raton, 1986.

[2] L. Seghal and G. S. Paliwal, *Bot. J. Linn. Soc.*, **68**, 173 (1974).

[3] J. L. Hartwell, *Lloydia*, **32**, 153, (1969); J. M. Watt and M. G. Breyer-Brandwijk, The Medicinal and Poisonous Plants of Southern and Eastern Africa, 2nd Ed, E. & S. Livingstone, Edinburgh, 1962, p 395; Th. J. C. Up Hof, Dictionary of Economic Plants, H. R. Engelmann, London, 1959, p 151; W. Dymock, C. J. H. Warden and D. Hooper, Pharmacographica Indica, Vol **1**(iii), Hambard National Fondation, Karachi, Pakistan, 1890, p 247 (reprinted 1972); J. R. Ainslie, A List of Plants in Native Medicine in Nigeria, Inst. pap. no. 7, Oxford University, Oxford, 1937, p 27.

[4] S. von Reis Altschul, Drugs and Foods from Little Known Plants Notes in Harvard University Herbaria, Harvard University Press, Boston, 1973, p 143; R. N. Chopra, I. C. Chopra, K. L. Handa and L. D. Kapur, Indigenous Drugs of India, 2nd Ed, H. N. Dhur & Sons, Calcutta, 1958; F. J. Evans, The British Herbal Pharmacopoeia, British Herbal Medical Association, London, 1976, p 81; F. J. Evans, A. D. Kinghorn and R. J. Schmidt, *Acta Pharmacol. Toxicol.*, **37**, 250 (1975); K. Lampe and Fagerström, Plant Toxicity and Dermatitis, Williams & Wilkins, Baltimore, 1968; J. Kingsbury, Poisonous Plants of the United States and Canada, Prentice Hall, Eaglewood Cliffs, NJ, 1964.

[5] British Pharmacopoeia, The Pharmaceutical Press, London, 1949.

[6] R. Castagnou, R. Baudriment and J. Gauthier, *Compt. Rend.*, **260**, 4109 (1965).

[7] E. Hecker and R. Schmidt, *Fortschr. Chem. Organ. Naturst.*, 377 (1974).

[8] J. G. Kidd and P. Rous, *J. Exp. Med.*, **68**, 529 (1938).

[9] M. Castagna, Y. Takai, K. Kaibuchi, S. Kimihiko, U. Kikkawa and Y. Nishizuka, *J. Biol. Chem.*, **13**, 7847 (1982).

[10] M. Castagna, Y. Takai, K. Kaibuchi, K. Sano, U. Kikkawa and Y. Nishizuka, *J. Biol. Chem.*, **257**, 7847 (1982); J. F. Kuo, R. G. G. Anderson, B. C. Wise, L. Mackerlova, I. Salmonsson, N. L. Brackett, N. Kato, M. Shoji and R. W. Wrenn, *Proc. Natl. Acad. Sci. U. S.A.*, **77**, 7039 (1980).

[11] S. M. Kupchan, I. Vchida, A. R. Branfman, R. G. Dailey and B. Yu Fei, *Science*, **191**, 571 (1976).

[12] P. A. Wender, R. M. Keenan and H. Y. Lee, *J. Am. Chem. Soc.*, **109**, 4390 (1987); P. A. Wender, H. Kogen, H. Y. Lee, J. D. Junger, R. S. Wilheim and P. D. Williams, *J. Am. Chem. Soc.*, **111**, 8954 (1989); P. A. Wender, H. Kogen, H. Y. Lee, J. D. Junger, R. S. Wilheim and P. D. Williams, *J. Am. Chem. Soc.*, **111**, 8957 (1989).

[13] P. A. Wender, K. D. Rice and M. E. Schnute, *J. Am. Chem. Soc.*, **33**, 7897 (1997).

[14] K. Shigeno, K. Ohne, T. Yamaguchi, H. Sasai and M. Shibasaki, *Heterocycles*, **33**, 161 (1992); K. T. Shigeno, H. Sasai and M. Shibasaki, *Tetrahedron Letters*, **33**, 4937 (1992); K. Sugita, K. Shigeno, C. F. Neville, H. Sasai and M. Shibasaki, *Synlett*, 325 (1994); R. Tokunoh, H. Tomiyama, M. Sodeska and M. Shibaski, *Tetrahedron Letters*, **37**, 2449 (1996).

[15] J. H. Rigby and P. Ch. Kierkus, *J. Am. Chem. Soc.*, **111**, 4125 (1989); J. H. Rigby, P. Ch. Kierkus and D. Head, *Tetrahedron Letters*, **30**, 5073 (1989).

[16] P. C. B. Page, D. C. Jennens, R. A. Porter and A. N. Baldock, *Synlett*, 472 (1991); P. C. B. Page and D. C. Jennens, *J. Chem. Soc., Perkin Trans. 1*, 2587 (1992); P. C. B. Page, D. C. Jennens and H. Mc Farland, *Tetrahedron Letters*, 5395 (1997).

[17] W. G. Dauben, J. Dinges and T. C. Smith, *J. Org. Chem.*, **58**, 7635 (1993).

[18] M. C. McMills, L. Zhuang, D. L. Wright and W. Watt, *Tetrahedron Letters*, 8311 (1994).

[19] L. A. Paquette, D. R. Sauer, S. D. Edmondson and D. Friedrich, *Tetrahedron*, **50**, 4071 (1994).

[20] J. I. Mc Loughlin, R. Brahma, O. Campopiano and R. D. Little, *Tetrahedron Letters*, 1377 (1990).

[21] K. A. Parker and M. R. Adamchuk, *Tetrahedron Letters*, 1689 (1978).

[22] L. M. Harwood, S. A. Leeming, N. S. Isaacs, G. Jones, J. Pickard, R. M. Thomas and D. Watkin, *Tetrahedron Letters*, 5017 (1988); D. Dolata and L. M. Harwood, *J. Am. Chem. Soc.*, **114**, 10738 (1992).

[23] L. M. Harwood, G. Jones, J. Pickard, R. M. Thomas and D. Watkin, *Tetrahedron Letters*, 5825 (1988).

[24] K. A. Parker and M. R. Adamchuk, *Tetrahedron Letters*, 1685 (1978).

[25] L. A. Van Royen and P. J. De Clercq, *Synth. Commun.*, **9**, 771 (1979).

[26] B. A. Keay and P. W. Dibble, *Tetrahedron Letters*, 1045 (1989).

[27] H. Kotsuki, Y. Mori, T. Ohtsuka, H. Nishizawa, M. Ochi and K. Matsuoka, *Heterocycles*, **26**, 2347 (1987).

[28] G. A. Kraus and P. Gottschalk, *Tetrahedron Letters*, 2727 (1983).

[29] L. M. Harwood, T. Ishikawa, H. Phillips and D. Watkin, *J. Chem. Soc., Chem. Commun.*, 527 (1991).

[30] E. Niwa, H. Aski, H. Tanaka, K. Munakata and M. Namiki, *Chem. Ber.*, **99**, 3215 (1966).

[31] L. M. Harwood, P. Marais and S. West, unpublished results.

[32] D. W. Knight and D. C. Rustidge, *J. Chem. Soc., Perkin Trans I*, 679 (1981).

[33] H. Gilman and G. F. Wright, *J. Am J. Soc.*, **55**, 3302 (1933).

[34] S. Sperry, F. A. Valeriote, T. H. Corbett and P. Crews, *J. Nat. Prod.*, **61**, 241 (1998).

[35] R. J. Capon and M. S. Butler, *Aust J. Chem.*, **44**, 77 (1991).

[36] R. J. Capon and J. K. Mcleod, *Tetrahedron*, **41**, 3391 (1985); L-K. Sy and G. D. Brown, *J. Nat. Prod.*, **60**, 904 (1997); R. S. Compagnone, I. C. Pina, H. R. Rangel, F. Dagger, A. I. Suárez, M. V. R. Reddy and J. D. Faulkner, *Tetrahedron*, **54**, 3057 (1998).

[37] L-K. Sy and G. D. Brown, *J. Nat. Prod.*, **60**, 904 (1997).

[38] S. P. Ovenden and R. J. Capon, *J. Nat. Prod.*, **62**, 214 (1999).

[39] J-P. Gesson, J-C. Jacquesy and B. Renoux, *Tetrahedron*, **45**, 5853 (1989).

[40] For examples see: J. D. Ballantyne and P. J. Sykes, *J. Chem. Soc. (C)*, 731 (1970); S. C. Welch, A. S. C. P. Rao, J. T. Lyon and J-M. Assercq, *J. Am. Chem. Soc.*, **105**, 252 (1983); W. M. Daniewski, E. Kubak and J. Jurczak, *J. Org. Chem.*, **50**, 3963 (1985); K. H Schulte-Elte, W. Giersch, B. Winter, H. Pamingle and G. Ohloff, *Helv. Chim. Acta*, **68**, 1961 (1985); H. Hagiwara and H. Uda, *J. Chem. Soc., Chem. Commun.*, 815 (1988).

[41] For related synthetic approaches see R. Esmond, B. Fraser Reid and B. B. Jarvis, *J. Org. Chem.*, **47**, 3358 (1982); A. Kanazawa, P. Declair, M. Pourashraf and A. E. Greene, *J. Chem. Soc., Perkin Trans 1*, 1911 (1997).

[42] J. B. Baudin, G. Hareau, S. A. Julia and O. Ruel, *Tetrahedron Letters*, 1175 (1991); J. B. Baudin, G. Hareau, S. A. Julia, R. Lorne and O. Ruel, *Bull. Soc. Chim. France*, **130**, 336 (1993).

[43] R. Bellingham, K. Jarowicki, P. Kocienski and V. Martin, *Synthesis*, 285 (1996).

[44] G. L. Lange and C. Gottardo, *Synth. Commun.*, **20**, 1473 (1990).

[45] E. L. Clennan, *Tetrahedron*, **47**, 1343 (1991); M. Matsumoto, S. Dobashi, K. Kuroda and K. Kondo, *Tetrahedron*, **41**, 2147 (1985).

[46] K. E. O'Shea and C. S. Foote, *J. Am. Chem. Soc.*, **110**, 7167 (1988).

[47] Zwitterionic or pre-epoxide species have been proposed as intermediates in singlet oxygen cycloadditions: T. Linker and L. Frölich, *Angew. Chem., Int. Ed. Engl.*, **33**, 1971 (1994); T. Linker and L. Frölich, *J. Am. Chem. Soc.*, **117**, 2694 (1995); T. Linker, F. Rebein and G. Tóth, *J. Chem. Soc., Chem Commun.*, 2585 (1996).