Drug Design And Synthesis of Novel Heteroanthracycline Antitumor Drugs.

A Thesis

by

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In memory of Professor B. Belleau

Abstract

Novel heteroanthracycline antitumor drugs were designed based on structure activity relationship studies and known mechanisms of drug action. Their preparation required the development of a general synthetic approach.

After extensive studies, three methodologies were developed for the general synthetic plan. The first method involved photoenolisation of 2,5-dimethoxybenzaldehyde and SO_2 entrapment of the o-quinodimethane to give 4,7-dimethoxy-1-hydroxy-1,3-dihydrobenzo[2,3-c]thiophene-2,2-dioxide. This compound served as a general intermediate towards the synthesis of several heteroanthracyclinones. It could be reduced to the oxathiin-2-oxide derivative which thermally extruded SO_2 to yield the o-quinodimethane. Reentrapment of this latter intermediate with various glyoxalates gave key isochroman derivatives. The second method is an improvement over the first. Isochromandiones with a C-1 hydroxyl functionality were prepared from oxidative demethylation of 1-hydroxyisochromans. These were obtained after acid hydrolysis of the coupling products between epoxides and the cuprate of 2,5-dimethoxy-6-methylbenzaldehydedioxane acetal. The third method involved a sequential cycloaddition routine with two o-quinodimethanes.

By combining newly developed techniques with known methods, a general synthetic plan was developed. Consequently, the total synthesis of six tetracyclic structural hybrids of the naphthoquinone[2,3-c]pyranyl class of antibiotics was accomplished; along with the total synthesis of (R) and (S) 1-(4'-O-p-nitrobenzoyl-N-trifluoroacetyldaunosamine)-1,3-dihydrothioxantho[2,3-c]thiophene-2,2-dioxide, p-nitrobenzyl(5,12-dihydroxy-3,4-

dihydrothioxantho[2,3-c] and [3,2-c]pyran-3-yl)formate, and eight novel heteroanthracyclines with the 5,12-dioxo-2,3,5,12-tetrahydroanthraceno[2,3-c]pyranyl backbone. The diastereomeric mixture of (1'S, 1R, 3S) and (1'S, 1S, 3R) methyl[11-hydroxy-1-(2', 3', 6'-trideoxy-3-trifluoroacetamido-L-lyxohexopyranose)-5,12-dioxo-3,4,5,12-tetrahydroanthraceno[2,3-c]pyran-3-yl]formate was found to possess equipotent antileukemic activity to doxorubicin with no cross resistance.

RESUME

La conception de nouvelles hétéroanthracyclines comme agents antinéoplasiques a été accomplie à partir d'études de mécanismes cytotoxiques et de relations structure - activité d'anthracyclines connues. Leur préparation a nécessité le développement d'un plan de synthèse général.

Deux méthodes ont été développées pour la préparation de dérivés isochromans. La première consiste à piéger avec le SO_2 l'o-quinodiméthane obtenu par photoénolisation de la 2,5-dimethoxybenzaldehyde pour donner le 4,7-dimethoxy-1-hydroxy-1,3-dihydrobenzo [2,3-C] thiophene-2,2-dioxide.

Ce produit a servi comme intermédiaire général dans la préparation de divers hétéroanthracyclinones. Sa réduction a donné le dérivé oxathiin-2-oxide correspondant qui a été utilisé pour générer l'o-quinodiméthane, par extrusion thermique du SO_2. La cycloaddition de ce dernier intermédiane avec divers glyoxylates a permis la synthèse d'isochromans variés. La deuxième méthode est une amélioration de la première. Des isochromandiones ont pu être préparées avec une fonctionnalité hydroxy à la position C-1 par oxidation déméthylante des isochromanes correspondants. Ces dernières ont été synthétisées après l'hydrolyse des produits de couplage obtenus entre certains époxides avec le cuprate de l'acétal dioxane de 2,5-dimethoxy-6-methylbenzaldehyde. La troisième méthode consiste en une séquence successive de cycloadditions avec deux o-quinodimethanes.

En combinant certaine méthodes connues dans la littérature avec notre nouvelle technologie, nous avons réussi le développement d'un plan de synthèse général. En conséquence, la synthèse totale de six analogues tetracycliques d'antibiotiques naphtoquinoniques fut accomplie;

ainsi que la préparation des (R) et (S) 1-(4'-O-p-nitrobenzoyl-N-trifluoarcetyldaunosamine)-1,3-dihydrothiophene-2,2-dioxide des formates [5,12-dihydroxy-3,4-dihydrothioxantho [2,3-C] et [3,2-C] pyran-3-yl] formates du p-nitrobenzyl et de huit nouvelles hétéroanthracyclines incorporant le 5,12-dioxo-2, 3,5,12-tetrahydroanthraceno [2,3-C] pyranyl comme structure de base.

Le mélange diastéréomérique (1'S,1R,3S) et (1'S,1S,3R) du [11-hydroxyl-1-(2',3',6'-trideoxy-3-trifluoroacetamido-L-lyxohexopyranose-5,12-dioxo-3,4,5,12-tétrahydroacenthraceno [2,3-C] pyran-3-yl) formate de méthyl possède une activité égale à la doxorubicine contre la ligné cellulaire leucémique avec absence de resistance croisée.

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List Of Abbreviations And Symbols

A	adenine
Ac	acetyl
AD-32	N-trifluoroacetyladriamycin-14-valerate
AIBN	azobisisobutyronitrile
Ar	aryl
ATP	adenosine triphosphate
bp	boiling point
b	broad
Bu	butyl
t-Bu	tert-butyl
C	cytosine
ca	circa
CAN	ceric ammonium nitrate (ammonium cerium(IV)nitrate)
cat	catalyst, catalytic
cm^{-1}	reciprocal centimeters (wavenumber)
CMR	carbon magnetic resonance
conc.	concentrated
CoQ_{10}	coenzyme Q_{10}
d	doublet
dd	doublet of doublet
ddd	doublet of doublet of doublet
dddd	doublet of doublet of doublet of doublet
DMA	N,N-dimethylacetamide

DMAP	4-dimethylaminopyridine
DME	1,2-dimethoxyethane
DMF	N,N-dimethylformamide
DMSO	dimethyl sulfoxide
DMSO-d_6	deuterated DMSO
DNA	deoxyribonucleic acid
E^+	electrophile
EI	electron impact
EPR	electron paramagnetic resonance
equ.	equivalent
ESR	electron spin resonance
Et	ethyl
$Eu(FOD)_3$	tris(6,6,7,7,8,8,8-heptafluoro-2,2-dimethyl-3,5-octanedionato)europium
eV	electron volt
FMO	frontier molecular orbital
FOD_{Ox}	flavoprotein (oxidized)
FOD_{Red}	flavoprotein (reduced)
FT	Fourier transform
G	guanine
g	gram
gc	gas chromatography
GSH	glutathione reduced
GSSG	glutathione oxidized
hr	hour(s)

HOMO	highest occupied molecular orbital
HPLC	high pressure liquid chromatography
Hz	hertz
IR	infrared
J	coupling constant
k	kilo
kcal	kilocalories
LAH	lithium aluminium hydride
LDA	lithium diisopropylamide
LUMO	lowest unoccupied molecular orbital
M	molar
m	unresolved multiplet
m	meta
Me	methyl
mg	milligram(s)
MHz	megahertz
min	minute(s)
ml	milliliter(s)
mmol	millimole(s)
MP	melting point
MS	mass spectrum
N	Normal
NADH	nicotinamide adenine dinucleotide-reduced
NADP	nicotinamide adenine dinucleotide phosphate-oxidized

NADPH	nicotinamide adenine dinucleotide phosphate-reduced
NBS	N-bromosuccinimide
NMR	nuclear magnetic resonance
Nu	nucleophile(ic)
o	ortho
$O_2^{-\cdot}$	superoxide
Ox	oxidation
p	para
PCC	pyridinium chlorochromate
PDC	pyridinium dichromate
Ph	phenyl
PMR	proton magnetic resonance
PNB	p-nitrobenzyl
PNB_Z	p-nitrobenzoyl
ppm	parts per million
q	quartet
RNA	ribonucleic acid
rt	room temperature
s	singlet
sec	secondary
SOD	superoxide dismutase
T	temperature
T	thymine
t	triplet
TBAF	tetrabutylammonium fluoride

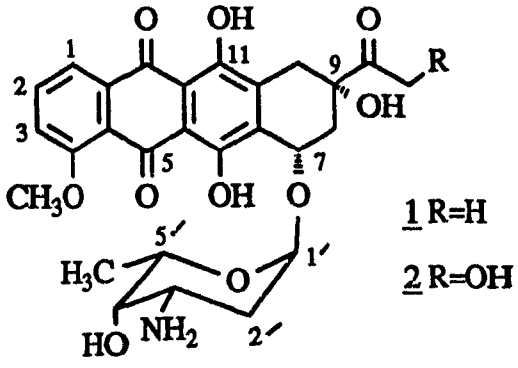
TBDMS	t-butyldimethylsilyl
tert	tertiary
TFA	trifluoroacetic acid
TFAA	trifluoroacetic anhydride
THF	tetrahydrofuran
THP	tetrahydropyran
tlc	thin layer chromatography
TMEDA	N,N,N′, N′-tetramethylethylenediamine
TMS	trimethylsilyl
TMS-Cl	trimethylsilyl chloride
TMS-I	trimethylsilyl iodide
TMSTf	trimethylsilyl trifluoromethanesulfonate
PTSOH	para-toluenesulfonic acid
μ	micron
UV	ultraviolet
v	volume
w	weight

Symbols

	1-electron transfer
	2-electron transfer
Δ	heat
	reaction does not occur
	a series of reactions are involved
[O]	oxidation

INTRODUCTION

Daunorubicin(1) and doxorubicin(2) have been the leading anthracycline antitumor drugs for the past fifteen years and currently account for 20% of all clinically administered antineoplastic agents.[1] While daunorubicin is clinically used mainly against acute childhood and adult leukemias, doxorubicin has the widest spectrum of antitumor activity of all chemotherapeutic agents. It is very useful in adenocarcinomas of the bladder and the breast as well as in thyroidal, bronchogenic and testicular carcinomas. Soft tissue or bone sarcomas, malignant lymphomas, acute leukemia and pediatric solid tumors are among other malignancies where doxorubicin is highly effective.



1 R=H

2 R=OH

The extreme usefulness of both drugs is unfortunately reduced by a great number of undesirable side effects, some of which are very serious and dose limiting. Although doxorubicin and daunorubicin are quite selective for tumor cells in their cytotoxic action,[2-4] several host

cells such as those of the bone marrow, heart, oral mucosa, gastrointestinal epithelium and hair follicles get targetted and consequently damaged.[5,6] Some of the most frequent but clinically manageable toxicities include stomatitis and gastrointestinal disturbances leading to diarrhea, nausea and vomiting. Mutagenicity, carcinogenicity, and immunosuppression are also characteristic of daunorubicin and doxorubicin.[2,3,7-9] The single dose limiting toxicity, myelosuppresion, is quite serious but easily manageable.[1h,1i] By far the most restrictive toxicity which limits the cumulative dosage is irreversible cardiotoxicity manifested by a delayed but progressive destruction of non regenerating cardiac cells. Significant and persistent cardiac dysfunction occurs in 60% of patients at cumulative dosages of 550 mg/m^2 for doxorubicin and 600 mg/m^2 for daunorubicin.[10]

On the onset of cardiotoxicity, chemotherapy must be terminated even though the tumour is still responding. This frustrating setback has incited enormous research efforts aimed towards the elucidation of the mechanisms of action involved in the cytotoxicity and cardiotoxicity of anthracycline antitumor drugs. The preparation of anthracycline analogs with improved therapeutic index has been of utmost importance. Consequently, well over 2000 compounds have been obtained, among which less than a dozen have shown a real improvement in comparison with

daunorubicin or doxorubicin.[10]

The major source of novel derivatives have been from strains of anthracycline producing microorganisms and from the semisynthetic modifications of the naturally occurring compounds. The rational design of totally synthetic compounds has been difficult due to a lack of integrated understanding of the cytotoxic mechanisms of action. However, through retrospective structure-activity analysis of known natural analogs and their derivatives a better understanding of drug induced cellular damage has been achieved.

A. Natural Source of Anthracycline Antitumor Drugs

Daunorubicin was isolated simultaneously but independently at the Farmitalia Research Laboratory of Milan, Italy, from *streptomyces peucetius*[12] and at Rhone-Poulenc in France from *streptomyces coeruleorubidus*.[13] In 1963, DiMarco from Farmitalia observed antitumor activity from his new drug, daunomycin,[14] but in later studies acute and chronic cardiac dysfunction was demonstrated in mammals.[15,16]

DiMarco and Arcamone later obtained from a mutant strain, *streptomyces caesius*, 14-hydroxydaunorubicin (2) and named it adriamycin.[12] Antitumor potency and effectiveness was better than that of daunorubicin and therefore

adriamycin was rapidly introduced into clinical trials. By 1974, approval from the U.S. Food and Drug Administration was obtained and adriamycin became known as doxorubicin.

It soon became evident that doxorubicin and daunorubicin are members of a vast family of biogenetically interrelated anthracyclines.[17-19] Figure 1 shows the multiplicity of anthracyclines that can be produced by various organisms. The biochemical condensation of a propionate starter unit with nine malonate extender units would yield the polyketide(3),[20,21] which can subsequently cyclize to give, after some further adjustments of the oxidation state, the tetrahydronaphtacene ring system of the anthracycline(4).[22] Cyclization of (3) or its reduction product (3a) yield aklavinone(5) which, for example, sequentially gives aclacinomycin A (6) after glycosidation. Oxygenation of aklavinone(5) can occur at C-1 to yield E-pyrromycinone(7), while decarboxylation gives E-rhodomycinone(8). These aglycones can then be glycosidated with mono, di or oligosaccharides to give several anthracycline derivatives(fig 2).

Deoxydaunorubicin(10) can be obtained from E-rhodomycinone(8) after decarboxylation at C-10, 4-O-methylation and glycosidation with daunosamine.[18] Daunorubicin(1) results from oxidation at C-13 in intermediate(10) and C-14 hydroxylation gives doxorubicin(2).[24]

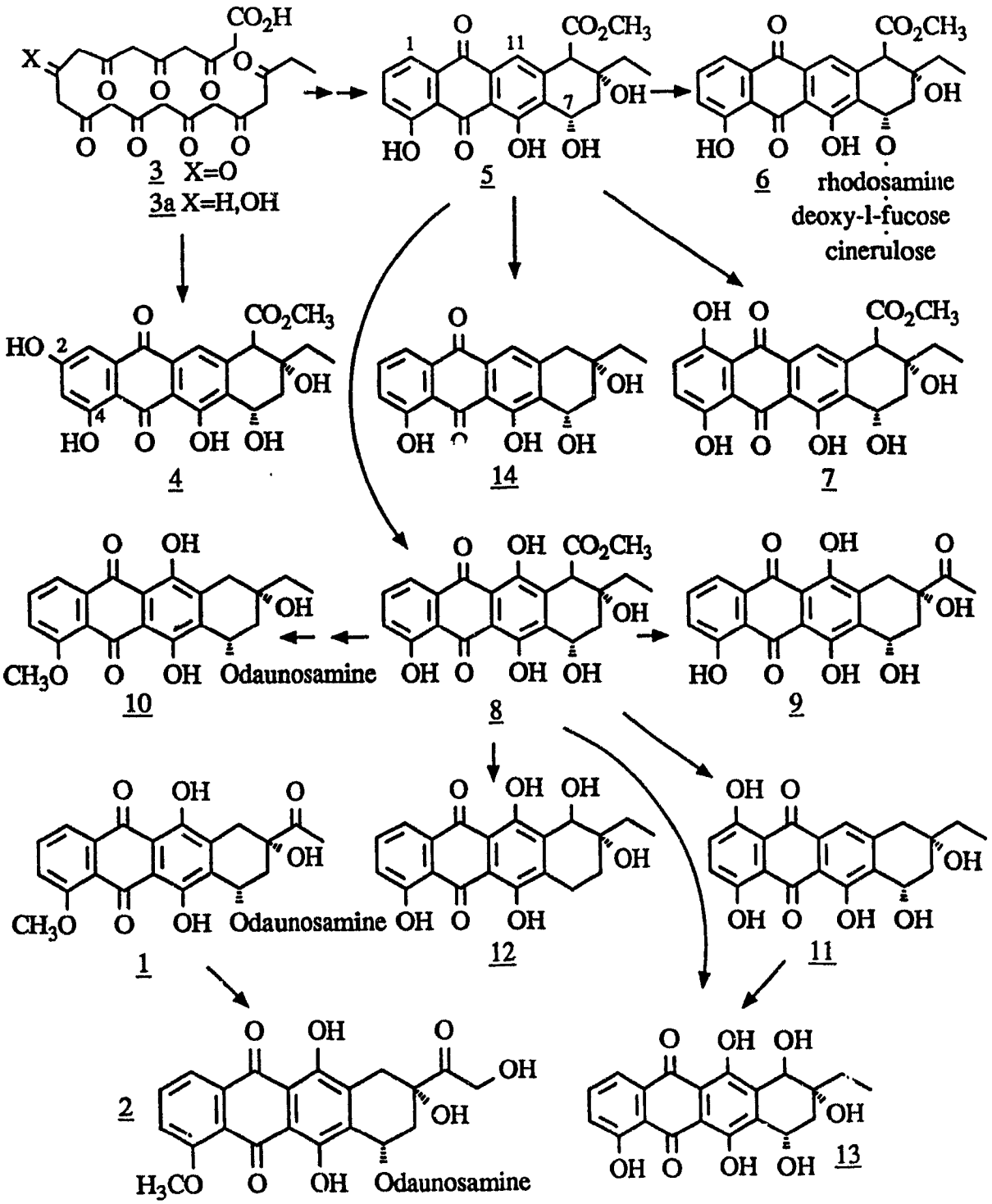

3 X=O
3a X=H,OH
5
6 rhodosamine
deoxy-l-fucose
cinerulose
4
14
7
10 Odaunosamine
8
9
1 Odaunosamine
12
11
2 Odaunosamine
13

Fig 1

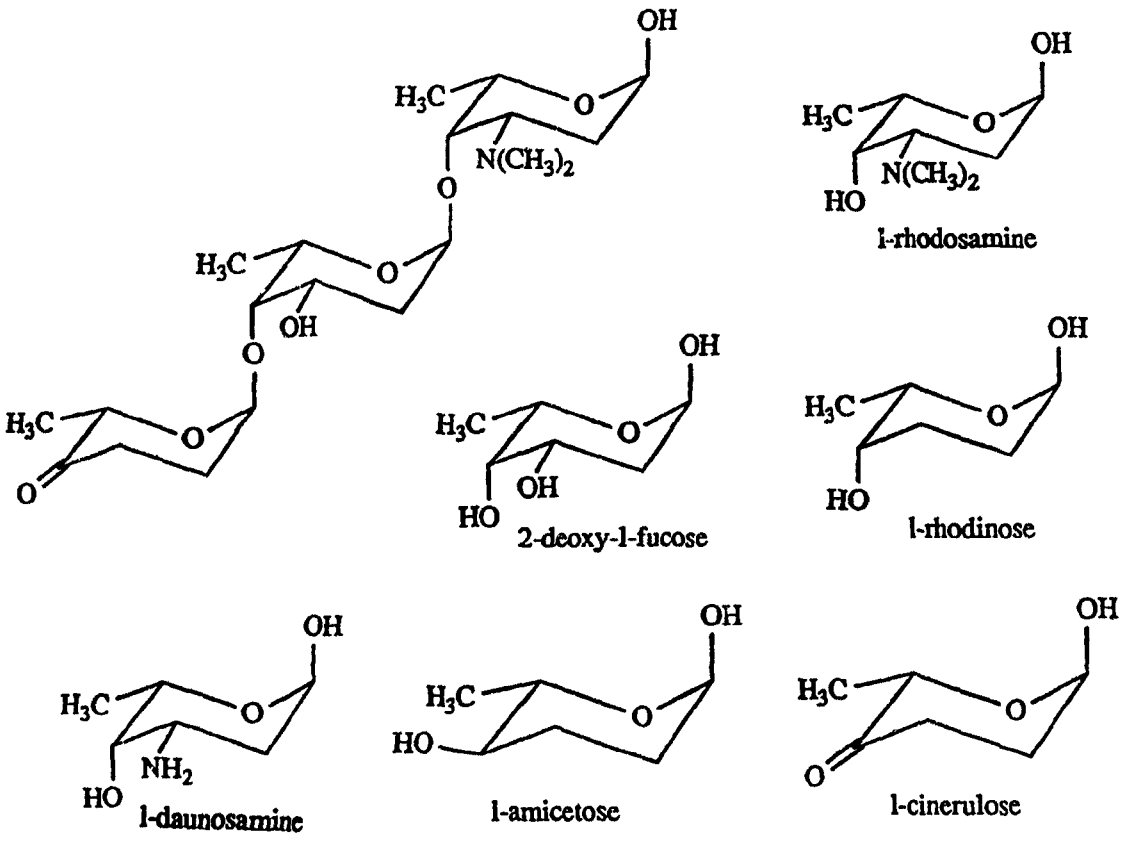


Fig 2 Various sugars found in naturally occuring anthracyclines

Several biosynthetic anthracycline analogs have been obtained through the use of mutant strains of *streptomyces*. However, this approach of preparing derivatives with the hope for a better therapeutic index than that of doxorubicin or daunorubicin has unfortunately not been very productive.

Improved semisynthetic analogs of daunorubicin and doxorubicin have been obtained through various modifications and will be considered in a later section. Rational drug design based on the cytotoxic mechanism of action is desirable, but past endeavors for such a goal have been prevented due to a lack of understanding of drug action and toxicity. However, continuing research in biochemistry and pharmacology as well as in structure-activity relationships should make such a task possible. A review of current cytotoxic and cardiotoxic mechanistic principles will make this apparent.

B. Mechanisms Related to the Cytotoxic Action of Doxorubicin and Daunorubicin.

B-1. Redox Cycling Mechanism

Extensive studies[1k,25] have suggested that the antitumor activity and cardiotoxicity of anthracyclines such as doxorubicin or daunorubicin are related to a redox

cycling mechanism. Cytotoxicity would result from the interaction of key cellular targets with reactive intermediates generated from redox cycling. Both doxorubicin and daunorubicin can undergo reduction by microsomal and nuclear NADPH-cytochome P-450 reductase of various organs. Single electron reduction of the quinone leads to the semiquinone radical anion (15) which can then either react with oxygen to revert back to the quinone or eliminate daunosamine to give the 7-deoxyaglycone (16) (fig 3) after a further reduction.

The exact mechanism of electron transfer from the reductase is speculative and involves two possibilities. The first postulates that electron transfer from NADPH occurs via flavin centered oxido reductases. Alternatively electron transfer would be from superoxide radical anions generated from cytochrome P-450 reductase.[25] This latter proposal seems less likely because under strictly anaerobic conditions, reduction of doxorubicin still takes place with subsequent glycosyl bond cleavage.[26-33]

Besides functioning as an electron shuttle between the NADPH reductases and molecular oxygen the single electron reduction product, the semiquinone, can also undergo deglycosidation to give the carbon centered radical (17) which can subsequently alkylate critical cellular loci.[28,34-38]

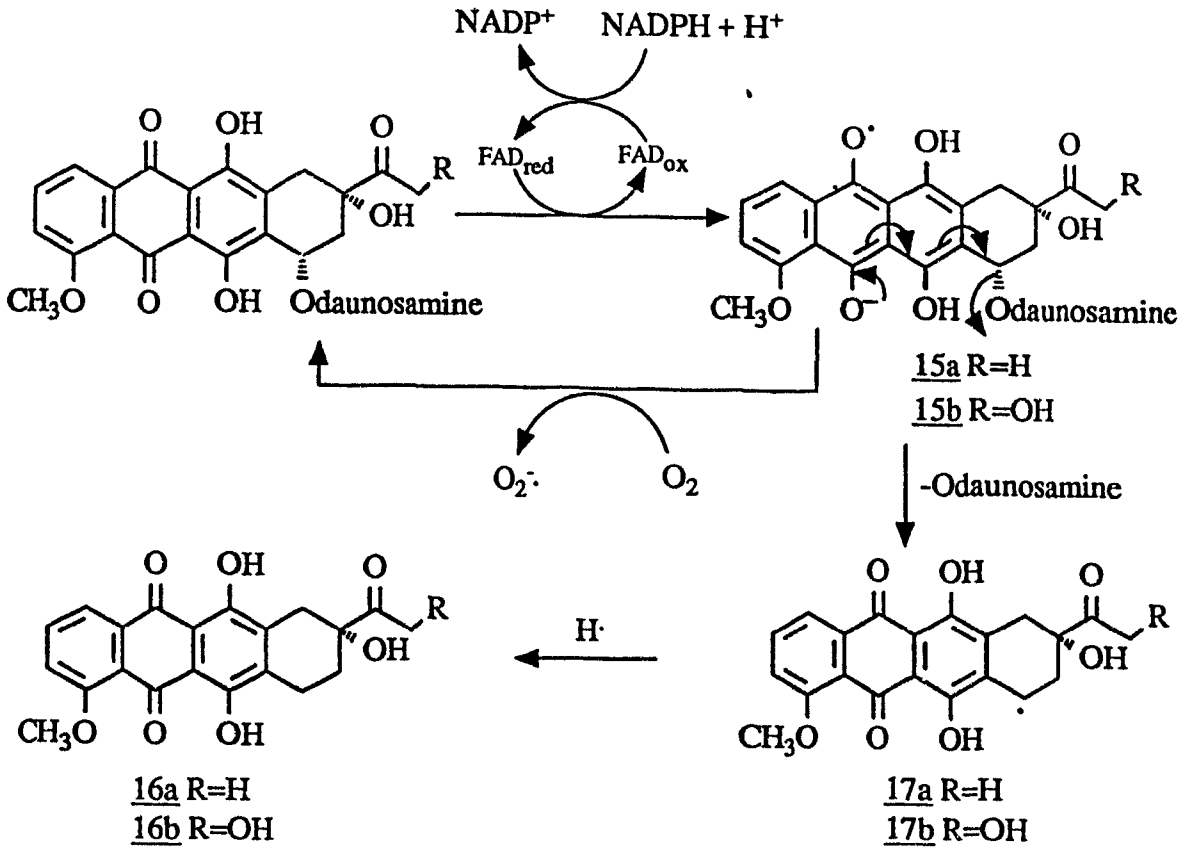


Fig 3

The rationale of bioreductive deglycosidation is not new and is well precedented in the literature.[26-33,37-42] Previously, Keljer and Koch have proposed that reductive elimination of daunosamine occurs from the daunorubicin hydroquinone(18a), generated following a double electron reduction (Fig 4).[43] However, this mechanism is not consistent with experimental evidence[27] which indicates that doxorubicin is a poor substrate for flavoproteins capable of two electron reduction. Furthermore, superoxide anion will

transfer only one electron in aprotic media, and therefore deglycosidation would originate from the semiquinone under these conditions.[44] Dimerization by self association of the quinone radicals has been observed for aclacinomycin A[45], aklavinone[46] and 1-deoxypyrromycin[47], but not for doxorubicin or daunorubicin. The detection of such dimers may be difficult from in vivo interactions because of low intracellular drug concentrations and due to the presence of several cytolytic targets. It is possible that

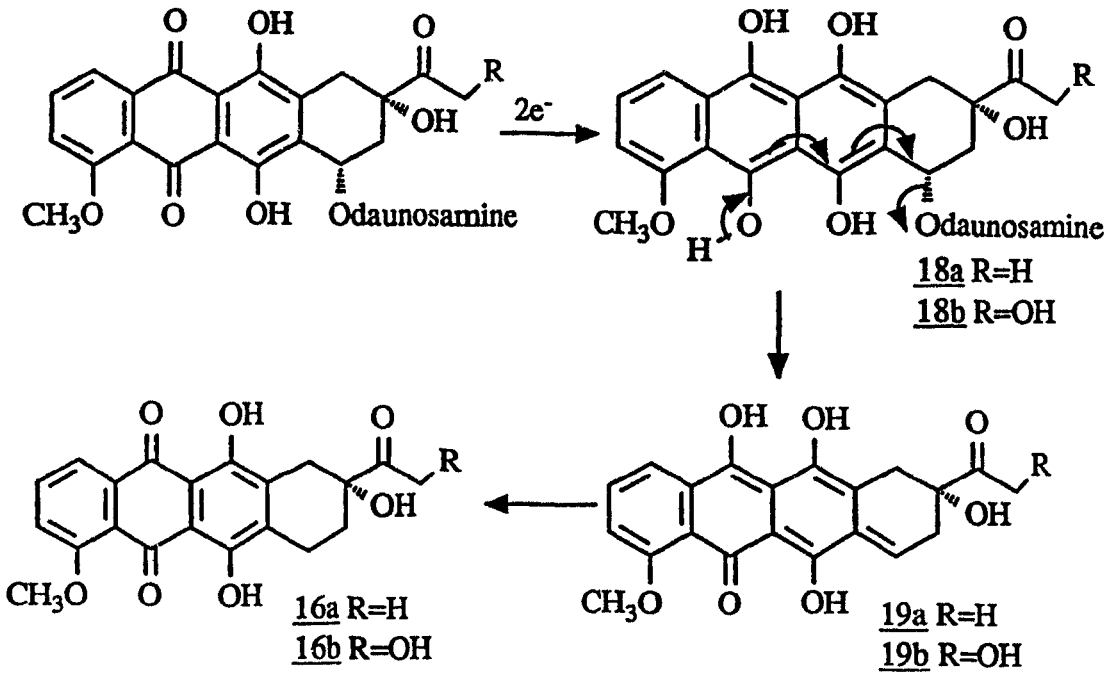


Fig 4

the carbon radical is generated in close proximity to cellular targets with subsequent association. Hydrogen abstraction from cellular lipids would give the 7-deoxyaglycones and the corresponding lipid peroxides.[48]

Indirect evidence for covalent binding of reactive anthracycline intermediates to DNA has been provided by several studies[27,35,36,49-53] and support quinone methide formation over the quinone radical because the free radical scavenger alpha tocopherol does not interfere in covalent binding. The validity of this conclusion is however questionable because the interaction of alpha tocopherol with the quinone radical may be affected by the presence of other radicals which are concurrently generated through redox cycling. The evidence supporting the quinone methide (19) or the radical intermediate (17) can not positively disqualify either reactive intermediate and therefore both possibilities must be considered in cytotoxic events. The possibility also exists that quinone methide results from two consecutive one electron transfers.

The quinone methide, which is also an enol, could act as an electrophile[54] or a nucleophile.[55,56] Nucleophilic attack by the enol on a proton gives the 7-deoxyaglycone but alkylation could take place irreversibly (fig 5) with appropriate intracellular targets. Examples of possible cellular DNA electrophilic sites are the 2 and 4 position of the pyrimidine base and the 2 or 6 position of a purine base. Consequently, covalent binding to DNA via electrophilic substitution is a possibility. Experimentally, the quinone methide has been trapped by benzaldehyde[55,57] but has generally displayed modest nucleophilic character.[55,58]

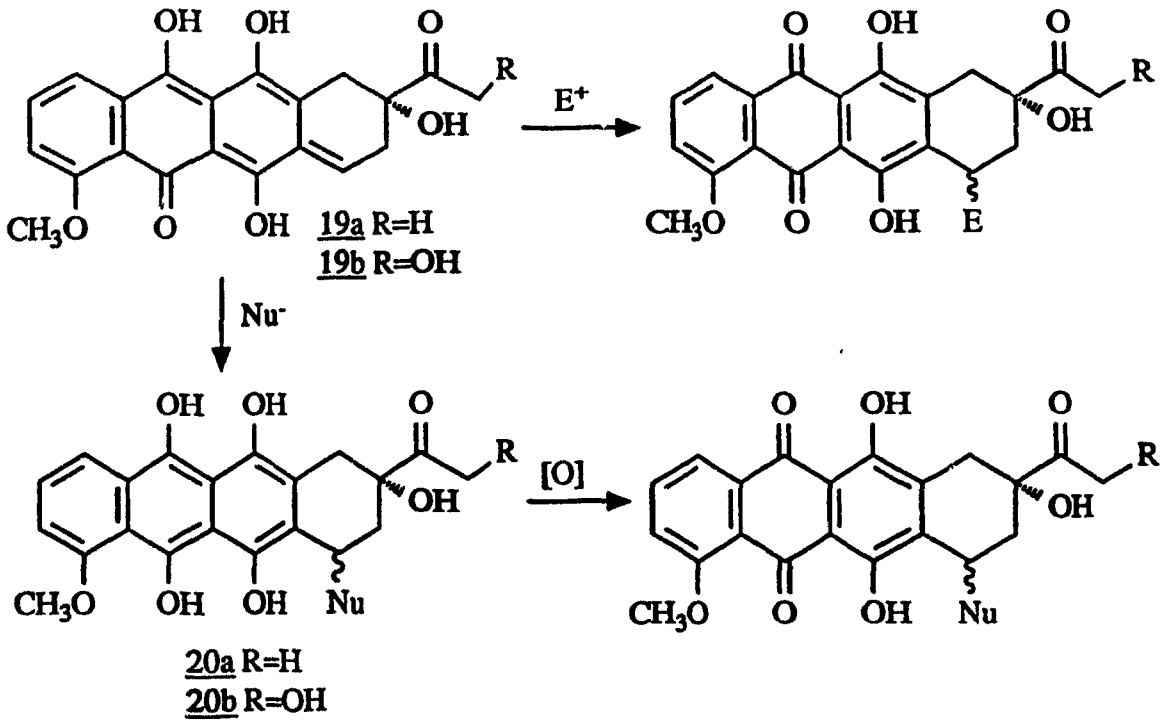


Fig 5

The alternative is that the quinone methide can act as an electrophile. The attack by nucleophiles would yield the hydroquinone adduct (21) (fig 5) which can then undergo oxidation. Earlier attempts of trapping any electrophilic intermediate generated by reductive cleavage of daunorubicin have failed.[35,36,51-55] Recently, however, the quinone methide intermediate obtained under anaerobic conditions from daunomycin reduction was trapped in better than 65% yield by thiolate nucleophiles such as N-acetyl-L-cysteine, N-(tert-butoxycarbonyl)-L-cysteine and 1-thio-D-glucose to give the corresponding C-7 diasteromers.[60]

The low reactivity of the quinone methide may in fact be beneficial for the antitumor activity of the anthracyclines. Too high a reactivity would lead to premature addition whereas a better probability exists that the appropriate targets are reached through lower reactivity. Proximity interactions between drug and active site would increase the effective molar concentration and lead to a positive bimolecular reaction.

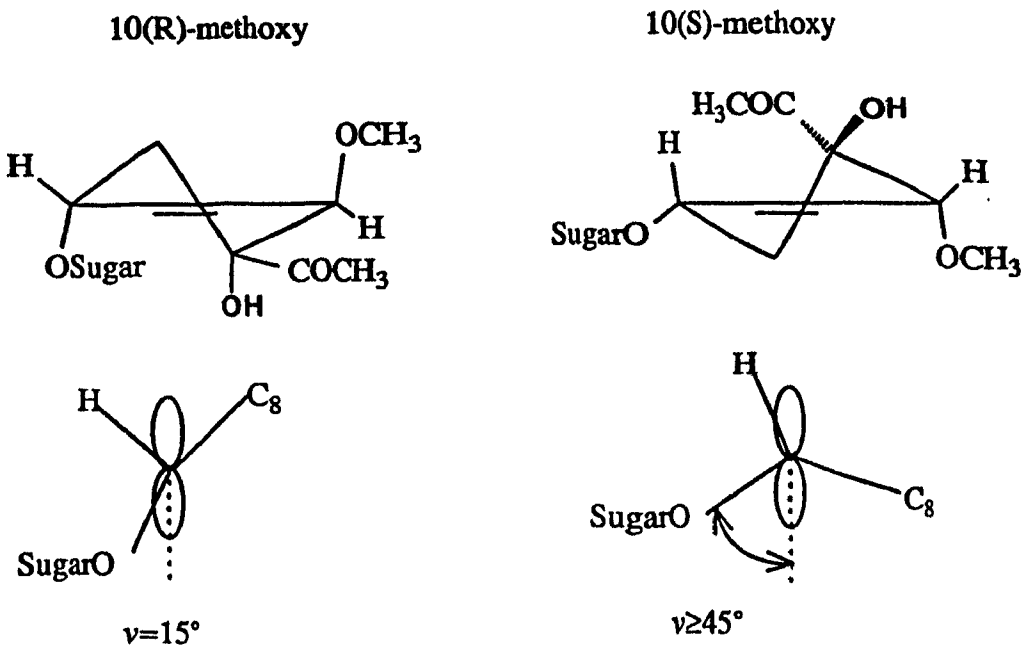


Fig 6

All these reductive deglycosidations are found to be under stereoelectronic control.[61] The stability of the glycosidic bond depends on the conformation of the cyclohexene A ring which in turn is determined by the

substitution pattern of the ring. While daunorubicin, 4-demethoxy-11-deoxydaunorubicin and 10(R)-methoxydaunorubicin easily eliminate the l-daunosamine sugar upon polarographic reduction, the 4-demethoxy-6-deoxydaunorubicin and 10(S)-methoxydaunorubicin do not. This is due to a pronounced stereoelectronic effect where glycosyl bond cleavage is favored when the glycosidic bond has a small dihedral angle with respect to the π-type orbitals of the anthraquinone system.

In aqueous media, the two diastereomers, 10(R)- and 10(S)-methoxydaunorubicin, adopt different half chair conformations[62] (Fig 6). Cleavage of the glycosyl moiety occurs readily for the 10(R)-methoxy derivative because of the small dihedral angle between the glycosidic bond being broken. This is consistent with known stereoelectronic principles which stipulate that heterolytic and/or homolytic fissions are favored when the bond undergoing cleavage can assume near coplanarity with either an adjacent semi occupied p-orbital, an unoccupied non-bonding orbital or an occupied π orbital.[63-67] Thus coplanarity of the C-O5 antibonding orbital (p-σ^*) with the doubly occupied p orbital at C_{6a} is an important requirement for bioreductive deglycosidation and perhaps for antitumor activity.

A second finding worthy of discussion is the inability of 4-demethoxy-6-deoxydaunorubicin to eliminate the sugar. NMR data[68] for the 11-deoxy analog is consistent

with the conformation shown in fig. 7. The dihedral angle for the 6-deoxy analog is too large to favor glycosidic cleavage. The presence of a 6-hydroxy substituent favors the alternative conformation due to periplanar interactions with the C-7 substituted daunosamine and thus the sugar becomes oriented in a pseudoaxial position. Consequently the required dihedral bond is nearly coplanar and bioreductive deglycosidation is allowed.

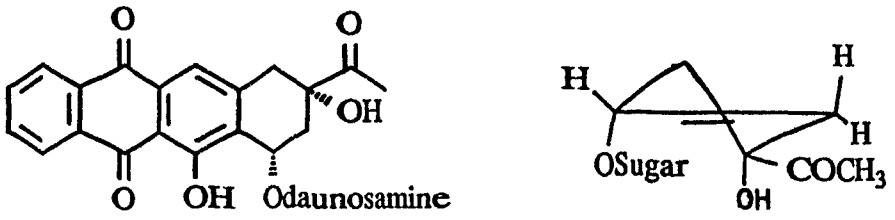


Fig 7

An awareness of the stereoelectronic effects involved in the deglycosidation reaction is important for drug design, because the generation of alkylating tetracyclic intermediates either via single or double electron reduction may constitute an important aspect in cytotoxic processes. Any derivative that has the wrong ring A conformation may have suppressed antitumor activity.

The generation of tetracyclic alkylating agents would take place in competition with the reduction of oxygen to superoxide by the semiquinone radical anion. Reactive

oxygen species generated from bioreduction may contribute to the cytotoxicity and will be considered next.

B-2. Generation of Reactive Oxygen Species

The antitumor effects and cardiotoxicity of anthracyclines like doxorubicin have generally been related to oxygen radical formation occuring during redox cycling in host cells[25,69] (Fig 8). Interaction of the semiquinone radical anion with oxygen will give the superoxide radical which can spontaneously dismutate to hydrogen peroxide and singlet oxygen. Superoxide dismutase can detoxify the host cell by converting superoxide into ground state oxygen and hydrogen peroxide. Catalase detoxifies the host cells by converting hydrogen peroxide into water and oxygen. Glutathione peroxidase removes hydrogen peroxide from the cell to yield water. Hydroxyl radicals can also be formed from hydrogen peroxide in the presence of ferrous ions and would be produced most effectively in host cells with reduced levels of detoxifying enzymes. Cytotoxicity has been related to interactions of hydroxyl radicals with cardiac mitochondrial membranes and DNA.[36,70,71] Single strand breaks in DNA[72] and oxidative modifications of its bases[73] are induced by hydroxyl radicals. Doxorubicin induced oxygen free radical cleavage of DNA has been demonstrated.[51,74.75]

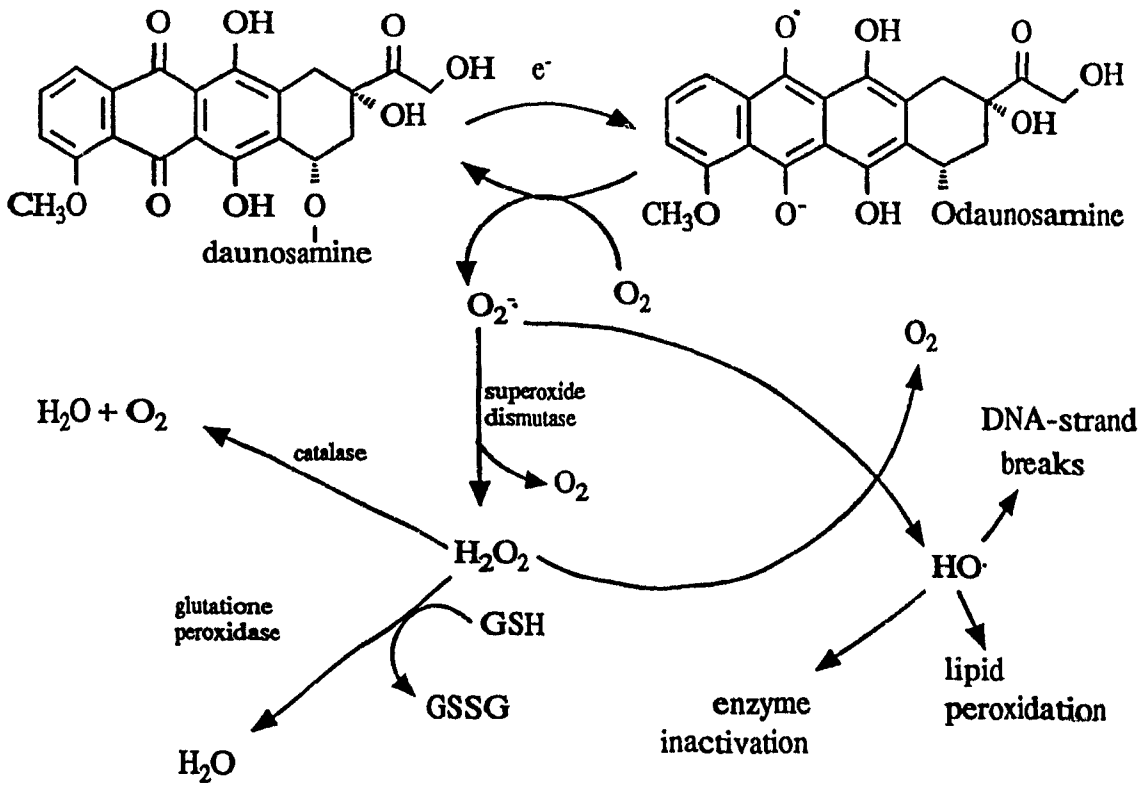


Fig 8 Redox Cycling of Doxorubicin

Several studies support the notion that oxy-radicals are involved in the cytotoxicity. Experimental support for iron requirement in the process include inhibition studies. Doxorubicin cytotoxicity is reduced significantly towards Ehrlich ascites carcinoma cells in the presence of iron chelators such as desferrioxamine, 2,2-bipyridine and diethylenetriaminepentaacetic acid.[76,77] Recently, the production of hydroxyl radicals in microsomal and purified NADPH cytochome P-450 reductase systems in the presence of doxorubicin or daunorubicin was stimulated by Fe-EDTA but

catalase was inhibitory in these processes.[78] These results support the participation of hydrogen peroxide and iron ions in the generation of hydroxyl radicals. Further evidence is obtained from the observations that increased levels of the enzymes catalase and superoxide dismutase as well as the presence of hydroxyl radical scavengers such as dimethyl sulfoxide, diethyl urea and thiourea significantly reduce the cytotoxicity of doxorubicin towards Ehrlich ascites carcinoma.[77]

Anthracycline resistance of tumor cells could result from either a decrease in activity of enzymes responsible for semiquinone formation or from an increase in intracellular concentration of detoxifying enzymes. Enhanced detoxification of drug-induced free radicals has been observed from increases in intracellular glutathione peroxidase concentration.[79] Suppression of glutathione peroxidase enzymatic levels in human ovarian tumor cell lines promotes cytotoxicity, but does not result in the inhibition of cell growth in L1210 mouse or P388 murine leukemia.[79] Evidently, other cytotoxic mechanisms of action predominate in these unresponsive tumor cell lines. Alternatively, in MCF-7 human breast tumor cells, doxorubicin significantly stimulated the formation of hydroxyl radical spin adducts with 5,5-dimethyl-1-pyrroline N-oxide in sensitive cells, but not in the resistant ones. This gives an indication that resistance at least in breast

tumor cells can be related to some interference in the production of hydroxyl radicals from doxorubicin.[80] Interestingly in MCF-7 sensitive cells 60-65% of doxorubicin induced hydroxyl radicals were located extracellularly, and since desferral decreased the concentration by 50%, their production is metal dependent.

The hypothesis that doxorubicin cytotoxicity is mediated by drug semiquinone free radical and oxyradical generation was tested recently on drug sensitive WT human breast tumor cells and MCF-7 doxorubicin resistant cells. The resistant MCF-7 line could tolerate four times more exogenously generated superoxide or hydrogen peroxide than the WT cells. This is explained from a two fold increase in superoxide dismutase activity and a 12 fold augmented selenium dependent glutathione peroxidase activity in MCF-7 resistant cells.[82] Resistance can not be attributed to a diminished ability to activate daunorubicin because the activities of the two enzymes NADPH cytochome P-450 reductase and NADPH dehydrogenase were not significantly different in mitochondria and microsomes isolated from the WT and MCF-7 resistant tumor cells. In a parallel vein, doxorubicin activation increased amounts of reduced oxygen metabolites in Ehrlich ascites tumor cells and drug sensitive MCF-7 human breast tumor cells.[77,82] Exogenously added superoxide dismutase, catalase and known hydroxyl radical scavengers partially protected the MCF-7 sensitive

tumor cells from doxorubicin. It was also found that inhibition of hydroxyl radical production in resistant MCF-7 breast tumor cells did not occur because of impairment in the bioreduction of doxorubicin, but rather from an enhanced activity of cytolytic detoxifying enzyme.[80] Preincubation of the sensitive MCF-7 cell line with superoxide dismutase and catalase was protective against doxorubicin cytotoxicity.[83]

From the evidence presented above, one can conclude that the superoxide cascade contributes significantly to the cytotoxicity. However, in the resistance studies presented here, there was never a direct correlation between the levels of enzymatic activity and the degree of resistance. This is an indication that the bioreductive alkylation mechanism seen previously could also participate in cytotoxic events. The importance and biological impact of each reactive intermediate generated from either mechanism may depend on their interactions with different cellular targets such as the DNA, cellular membranes and cardiac mitochondria. Further consideration of such in vivo interactions is necessary.

B-3. Drug Interactions With DNA

Daunorubicin and doxorubicin are more lethal to cells during the process of replication at the late S

phase[84] and inhibit preferentially the DNA replicative polymerase alpha over the repair polymerase beta enzyme. Numerous studies and reviews are conclusive that synthesis of DNA and RNA are inhibited in vivo and in vitro by both drugs.[85-95] Drug-induced reduction of DNA transcription and replication has been observed as well as an inhibition of the reverse transcriptase activity of RNA tumor viruses.[96-98]

Exposure of tumor cells to doxorubicin or daunorubicin leads to a rapid concentration of drug in the cell nucleus.[99] Mainfait et al have shown from fluorescence emission spectra of doxorubicin at the single cell level that the drug does not interact with other cellular components during its cytoplasmic transit to the nuclei of leukemia cells.[99]

Anthracyclines such as doxorubicin must interact closely with DNA in order to induce the observed interactions. Binding of doxorubicin or daunorubicin to the DNA macromolecule can occur through an attraction of drug to the surface of the double helix[100] or from an insertion of drug between adjacent base pairs, better known as intercalation.[101-103] Physical and chemical properties of the anthracyclines and DNA tend to favor the latter.

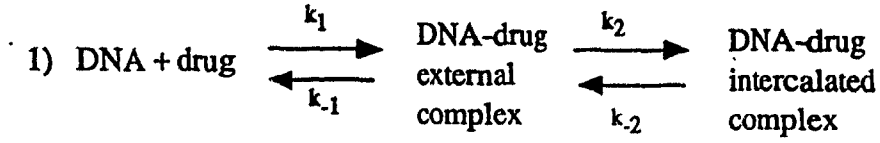
Experimental results supportive of intercalation upon binding of drug on DNA include hypochromic and bathochromic shifts in the visible spectrum,[104] quenching of

the typical semiquinone electron spin resonance signal[106] and absence of the characteristic polarographic wave associated with quinone reduction.[104] These results show that the quinone and hydroquinone functionalities of the tetracycle are no longer available for reductive or ionizing processes. Removal of the anthracycline chromophore from its polar environment to the less polar medium found between adjacent base pairs provides an explanation for these observations. Intercalation also explains the observed effects on DNA upon drug binding such as reduction of the sedimentation coefficient,[100,106,107] lowering of buoyant density,[104] and viscosity increases of DNA solutions.[107] These experimental results as well as the observed uncoiling of supercoiled closed circular DNA by doxorubicin or daunorubicin and drug induced increases of DNA melting temperature are consistent with elongation and stiffening of the double helix.[106,108]

Extensive equilibrium affinity constant determinations of daunorubicin and doxorubicin binding to DNA have been carried out and show a strong reversible binding of ca. 1-7 X 10^{-6} M^{-1} in vitro.[109] The number of binding sites per nucleotide, n, averages 0.17 and corresponds to a maximal binding of one drug molecule per three base pairs. The observed dependence of intercalative binding on ionic strength[110-112] indicate that an electrostatic component exists in intercalative binding.

This can be interpreted in terms of an interaction between the protonated amino group of the daunosamine sugar and the negatively charged phosphate oxygen atoms on the DNA backbone.

There are two possible explanations for this electrostatic contribution in binding. Stabilization of the intercalated anthracycline by drug amino-DNA phosphate attraction would seem to be the most obvious explanation and has been supported in several studies.[107,113,114] This conclusion has however been challenged by very convincing evidence[115], which clearly shows from x-ray analysis of intercalated daunorubicin-nucleic acid oligomer that the amino group of daunosamine does not interact with any functionality of the DNA and protrudes in the solvent region. A more adequate explanation requires that the electrostatic attraction would be necessary in an initial binding of drug (equation 1) on the surface of DNA. This would explain the strong non-intercalative binding, $K_a=1.5\times10^{-5}M^{-1}$, of doxorubicin to heat denatured DNA.[116] Intercalation would occur once the DNA-drug external complex undergoes the required conformational changes.



The best drug DNA intercalation model available today comes from the x-ray crystal structure carried out on a complex of a self-complementary DNA hexamer fragment, d(CGTACG) with daunorubicin.[115] The complex contained two daunorubicin molecules intercalated between CG base pairs with the aglycone chromophore oriented at right angles to the long dimension of the DNA double helix (Fig 9). The D ring protrudes out of the major groove while the B and C rings interact with the top and bottom DNA bases. The cyclohexene A ring and the daunosamine sugar are located in the minor groove of the double helix, consistent with previous NMR studies.[117,118] The O-9 hydroxyl group from ring A hydrogen bonds with the N_3 and N_2 atoms of an adjacent guanine base. No hydrogen bonding is observed between the amino or hydroxyl functionality of the sugar moiety and DNA.

Distortions in the complex from the common conformations of daunorubicin and the double helical DNA are observed. These result from structural adjustments necessary to accomodate the anthracycline in the double helix. The DNA unwinds at the base pair flanking the intercalation site.

Preferential binding to right-handed B-DNA[115,116,119] can be explained from the observed right handedness of the ring A-daunosamine section of daunorubicin, where the amino sugar projects towards the

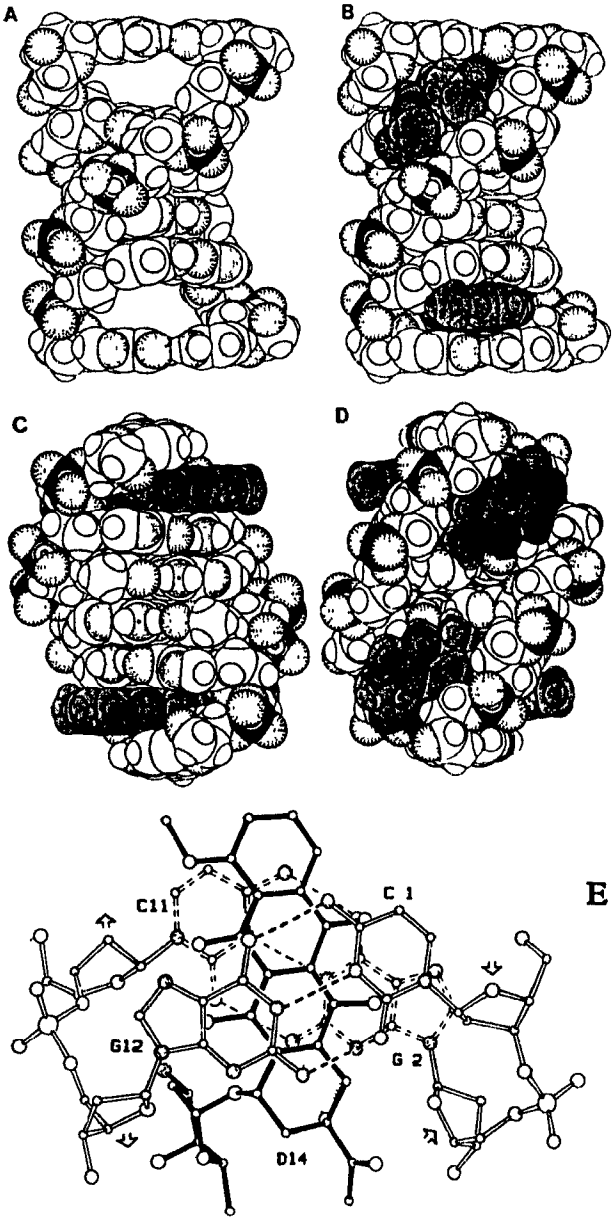


Fig 9 Intercalation model of daunorubicin in a d(CGTACG) DNA hexamer. A is free DNA. B to D are various representations of drug-DNA complex. E is a view from the top.

lower left, while the acetyl group is oriented towards the top right. This is consistent with the experimental observation that daunorubicin shifts the equilibrium of left-handed Z-DNA to the right-handed drug intercalated B-DNA complex in solution.[116]

A similar x-ray crystal structure has been analyzed[115] for the complex of doxorubicin intercalated into an identical d(CGATCG) hexamer of DNA. The complex was found to be virtually isostructural, with the exception that the C-14 hydroxyl proton is hydrogen bonded to a water molecule which in turn is hydrogen bonded to a nearby phosphate group. Differences in the mode of binding to the DNA double helix between daunorubicin and doxorubicin hardly account for the large difference in their biological activity.

Binding of daunorubicin and doxorubicin on DNA may lead to an interaction between polymerases and the exposed amino sequence of the intercalated drug and result in interference in the enzymatic function necessary for DNA replication and repair. Alternatively, DNA damage such as single or double strand breaks may result. This last proposal warrants further consideration.

B-4. DNA Damage

Doxorubicin and daunorubicin are known to generate DNA lesions characterized by single and double strand cleavages.[1h,120-123] Single strand cleavage can be caused by hydroxyl radicals generated from the anthracyclines. The DNA molecule may act as a reservoir of drug where, once intercalative saturation is reached, external binding through electrostatic attraction would then take place. Reduction of these surface bound quinone antibiotics[34] by nuclear NADPH reductases would lead to hydroxyl radical formation in close proximity of DNA and consequently to single strand cleavages.[124-129]

The relation between DNA single strand breaks and cytotoxicity is not convincing. It is current knowledge that drug or radiation induced DNA damage can be divided into two distinct groups where lesions may be repairable or not. Single strand breaks are usually repairable unless two single strand nicks occur very close to each other but on opposite strands. This in fact will result in a double strand break, an unrepairable lesion. With doxorubicin and daunorubicin non-random double strand cleavages were obtained in murine P-388 and human CCRF-CEM leukemia cells four hours after clinical treatment.[130,131] Interestingly, single strand breaks were manifested during the first hour

after drug exposure. Similar results have been obtained in L1210 cells treated with doxorubicin.[132,133] Clinical studies[134,135] showed a good correlation between patient response to doxorubicin therapy and DNA damage produced in vitro from a four hour incubation with doxorubicin of leukemia cells obtained from the patients. It is interesting to note that double strand breaks do not take place immediately as do single strand ones. This delay may reflect the necessary participation in double strand cleavages of endonuclease, the activity of which gradually increases over a period of several hours after treatment. DNA single strand cleavages would occur immediately after drug exposure, possibly from hydroxyl radicals originating from the DNA-drug complex. This would be consistent with the findings that the DNA-doxorubicin-Fe^{3+} complex more efficiently catalyzes the generation of hydroxyl radicals than the doxorubicin-Fe^{3+} complex.[136]

An awareness that significant cytotoxicity could result from drug induced double strand cleavages of DNA with the participation of an enzyme lead to the hypothesis that a topoisomerase was involved in the mechanism of drug action. Topoisomerase I and II are nuclear enzymes which affect several genetic processes and are capable of inducing topological changes of DNA.[137-140] The topological passing of two double-stranded DNA segments is catalyzed by topoisomerase II, possibly by introducing a transient enzyme

linked double strand break in one of the passing strands.[141-144] The intermediate DNA-topoisomerase II cleavable complex would be formed through covalent binding between the phosphate moiety at the 5' terminus of DNA and a tyrosyl residue on the enzyme.[145-148] Doxorubicin or daunorubicin would increase DNA double strand scissions by stimulating the activity of topoisomerase II and by stabilizing the cleavable complex, consequently preventing the rejoining reaction.[138,147,149,150] Topoisomerase II may have a high affinity to the conformationally distorted drug intercalated sites of the DNA double helix. The trapped topoisomerase II after doxorubicin intervention has been detected by filter elution experiments.[149,151,152]

The hypothesis that cytotoxicity may result from double strand cleavages of DNA is supported by drug resistance studies. In P-388 leukemia resistant cells but not in the drug sensitive ones, a lower incidence of drug induced double strand cleavages was observed[153] and has been correlated to a decreased topoisomerase II enzymatic activity.[154] Similarly, in B-16 murine melanoma cells lesser DNA breaks were induced by doxorubicin in the resistant cell line.[155]

The DNA intercalated drug species responsible for topoisomerase II mediated double strand breaks remains to be found. Many possible interactions of the reactive tetracyclic intermediates (15, 17-19) as well as of

inactivated drugs exist.

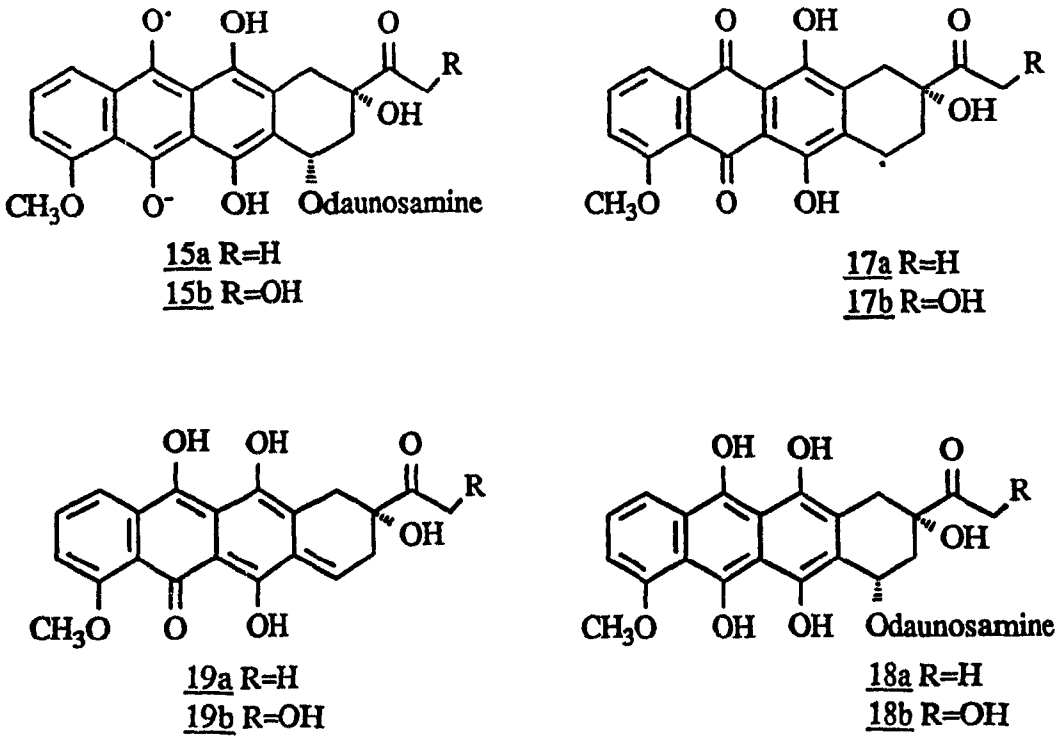


Fig 10

The anthracycline drug itself after intercalation will no longer undergo reduction, but will induce distortions in the DNA double helix. Recognition by topoisomerase is likely but the onset of a dead-end complex is not, because many known intercalators cause DNA distortions but are not cytotoxic.[111,156-158] This indicates that an activation step may be required. Covalent binding of drug has been proven indirectly previously, and may correlate with the following proposal. The possibility exists that the quinone radical or the quinone methide, once generated in the nucleus, may intercalate and subsequently

bind covalently. Alternatively, the semiquinone or hydroquinone could intercalate and subsequently deglycosylate to give the respective alkylating specie in close proximity of possibly reactive DNA functionalities. Covalent binding would result and topoisomerase II, stimulated by the resulting double-helical distortion, will cleave the double strand near the intercalation site.[148] The covalently bound drug would irreversibly damage the DNA macromolecule by preventing the reassembly of the cleaved strands.

Drug-DNA interactions cannot completely account for the observed cytotoxicity. A lack of correlation exists among DNA binding affinity, induction of DNA strand breaks and cytotoxic activity of a series of chromophore-modified anthracyclines which include 11-deoxydaunorubicin, 4-demethoxy-6-O-methyldaunorubicin, 4-demethoxydaunorubicin and 4-demethoxy-11-deoxy-4'-epi-daunorubicin in cultured P-388 leukemia cells.[159] Evidently, other mechanisms and targets are involved. Since the anthracycline drugs pass through several membranes before reaching the cell nucleus, it is conceivable that drug membrane interactions may account for some cytotoxicity. Such a possibility is examined next.

B-5. Drug-Cell Membrane Interactions

In an attempt to distinguish a membranal component of cytotoxicity from one that may be related to drug-DNA interactions, Tritton et Yee[160] examined the effect of immobilized doxorubicin on L1210 leukemic cells. The drug was covalently bound through an N-alkylcarbamate linkage to agarose heads. Both the immobilized and free drug but not the underivatized beads inhibited the growth of the leukemic cells. It was rigourously shown that there was no leakage of drug in leukemic cells exposed to the drug-agarose complex. The effective concentration of agarose-bound doxorubicin was 100 to 1000 times less than free drug. Evidently, the plasma membrane is very sensitive to the cytotoxic effects of doxorubicin.

The cytotoxic action of the doxorubicin-agarose complex may arise from activation to the semiquinone by membranal NADH oxidase or other electron-shuttling enzymes.[161] Aglycones would be released after deglycosidation within the membrane and be trapped due to their high lipophilicity. Redox cycling (fig 10) of the aglycone could then generate enormous quantities of reactive oxygen species, particularly hydroxyl radicals, which would undoubtedly be deleterious to membranal functions. The

potentiation in cytotoxicity observed for the doxorubicin-agarose complex would result because the toxic action of the aglycones could be exerted for long periods of time.

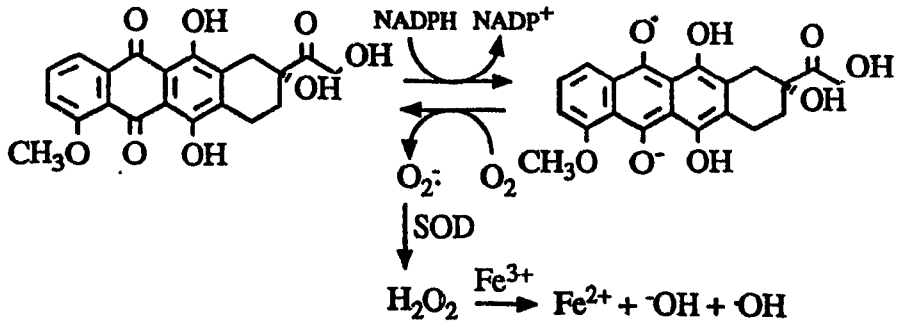


Fig 11

The drug-agarose model unfortunately can not be extrapolated to the free drug system, but does show that membranal interactions are important. Conceivably, a portion of doxorubicin molecules crossing the cell membrane could be bioreduced to the semiquinone, with the consequence that reactive oxygen species are generated from it, or that the aglycones generated from deglycosidation would undergo redox cycling.

The dual hydrophobic and hydrophilic character of doxorubicin and daunorubicin should allow binding of drug in phospholipid bilayers of living membranes. Insertion of the non-polar tetracyclic portion into the non-polar moiety of the membrane and the electrostatic attraction of the ionized

amino sugar to the negatively charged phosphate groups would cause strong binding of drug,[162-164] particularly to negatively charged phospholipids such as cardiolipin. Reasonable concentrations of anthracycline could be achieved due to such interactions in cell membranes.

Daunorubicin binds strongly to the negatively charged phospholipid cardiolipin, $k_a = 1.1 \times 10^6\ M^{-1}$. Interestingly, the strong affinity of cardiolipin for daunorubicin suggests that there could be localisation of drug at the cell membrane in competition with the cell nucleus. Cytotoxic events could then take place independently at both cellular targets. Cardiolipin is a major component of cardiac mitochondria and liver microsomes. It is generally not found in normal cells but is detectable upon malignant transformation.[165] This suggests that some selectivity could be attained between normal and tumor cells from drug cardiolipin mediated cytotoxicity.

An attempt to elucidate the geometry of the drug-cardiolipin complex was made from infrared attenuated total reflexion spectroscopy and conformational analysis.[25] The orientation of the chromophore with respect to the plane of the cardiolipin bilayer was determined and showed that at 36^0C doxorubicin molecules lie parallel to each other but are tilted with respect to the plane of the bilayer (Fig 12). The complex is stabilized from stacking of the

anthraquinone moieties between neighboring anthracycline molecules and by the electrostatic attraction between the positively charged amino sugar and the negatively charged phosphate of cardiolipin.

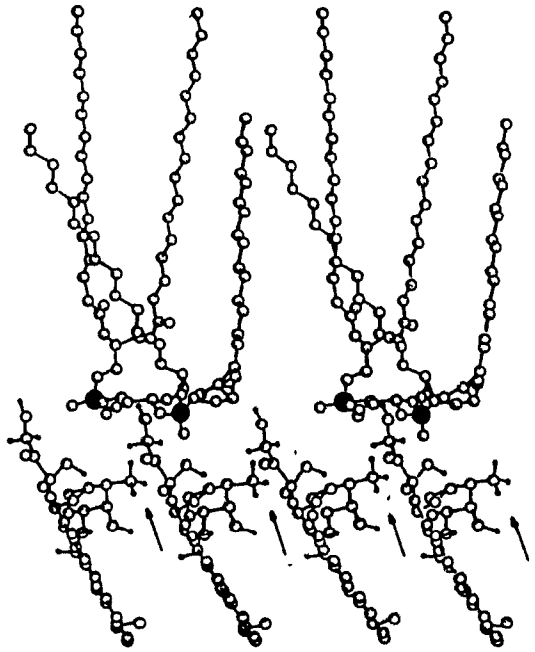


Fig 12

Several drug-induced cell membrane effects have been observed. Tyrosine protein kinases are involved in cell proliferation and transformation.[166] Their inhibition could lead to a reduction in cell growth. Doxorubicin exhibits a remarkable and dose dependent inhibition of tyrosine protein kinases isolated from spleen or produced by the oncogene of Abelson murine leukemia A-MuLV viruses.[167] In Sarcoma 180 ascites cells, doxorubicin changes the surface membrane by increasing the rate of agglutination of cells of concavalin

A[168,169] and by increasing the membrane fluidity.[49,169] Doxorubicin antagonizes cytochome-c oxidase by excluding the enzyme from its essential cardiolipin environment[170], with the consequence that the respiratory chain in mitochondrial membrane is inhibited. Doxorubicin stimulation of NADH oxidase and inhibition of ascorbate oxidase alters the plasma membrane redox function.[161] It also fluidizes pure phosphatidylcholine membranes.[171,172] The presence of anionic phospholipids alters the organization of cell membranal bilayers, consequently changing the affinity of the drug for the membrane at physiological temperature. The presence of small amounts of cardiolipin in cell membranes facilitates the penetration of doxorubicin.[172]

Direct damage to cell membranes by doxorubicin or daunorubicin may result from enhanced lipid peroxidation.[173-176] Under aerobic conditions hydroxyl radicals formed from the redox cycling of the anthracycline or their 7-deoxyaglycones can initiate lipid peroxidation.

In comparison to normal cells, tumor cells are deficient in enzymatic activities of superoxide dismutase,[177] glutathione peroxidase,[178] and catalase.[179] This may enhance the selectivity of doxorubicin or daunorubicin for tumor cells.

Regardless of the actual species involved in cell membrane cytotoxicity, oxy-radicals generated after the activation of anthracycline drugs by the NADPH electron

transport system are expected to participate in the destruction of malignant cells. Unfortunately, as will be seen next, the same deleterious effects to cell membranes may be more significant in cardiac mitochondria and result in cumulative cardiotoxicity.

C. Cardiotoxicity

One hypothesis relates cardiotoxicity to the inhibition of myocardial DNA metabolism such as DNA dependent RNA synthesis[180] and DNA replicative repair processes.[181-183] A lack of protein synthesis would cause myocardial damage and lead to necrosis of the non regenerating cardiac cells. This proposal is however weak because doxorubicin and daunorubicin affect proliferating cells more than the nonproliferating ones. Furthermore, binding for the doxorubicin will be in competition between the DNA and cardiolipin. In contrast to the low concentrations of cardiolipin found in tumor cells, the heart's inner mitochondrial membrane is composed of 24% cardiolipin[184] and therefore the inner leaflet of cardiac mitochondria would probably be the preferred target.

Doxorubicin forms a specific complex with cardiolipin in a 2:1 ratio and causes the collapse of the hexagonal structure of the phospholipid.[185] Structure activity relationship studies show a good correlation

between the drug-cardiolipin association constant of several analogs and cardiotoxicity.[186] The function of cardiolipin-requiring proteins have been impaired by bound drug for cytochome-c oxidase[185], creatine phosphokinase[187], L-glycerol-3-phosphate dehydrogenase[189] and the phosphate carrier.[188] Alterations in mitochondrial membrane functions by doxorubicin localized in cardiac mitochondria may lead to interference of ATP generation and Ca^+ transport[190-194] as well as perturbations of electron transport.[195,196] Recently, 7-hydroxy and 7-deoxyaglycones of doxorubicin were found to trigger Ca^+ release from cardiac mitochondria, consequently causing collapse of the mitochondrial membrane potential, mitochondrial swelling and oxidation of mitochondrial pyridine nucleotides.[194] Mitochondrial membranal lesions have also been observed with a doxorubicin-Fe(III) complex.[197] CoQ dependent enzymes such as NADH dehydrogenase and succinate dehydrogenases are inhibited by doxorubicin.[195,198] All of these results are related to cardiolipin binding.

A recent study[162] showed that some analogs of doxorubicin exhibit the same binding properties as doxorubicin but were less cardiotoxic. The mere binding to cardiolipin therefore does not provoke cardiotoxicity. An activation step may be required after binding. Less cardiotoxicity but equal cardiolipin affinity is seen with 4′-epidoxorubicin. This indicates that the 4′ position on

daunosamine may be important for initiating the biochemical cascade leading to mitochondrial damage.

Heart sarcosomes are poorly active in generating free radicals,[26,199] whereas in the presence of doxorubicin a single electron reduction by NADH dehydrogenase, to give presumably the semiquinone free radical, has been observed in intact mitochondria by electron spin resonance spectroscopy.[200,201] Consequently, electron flow from NADPH to molecular oxygen is enhanced by doxorubicin[26], and thus the formation of oxy-radicals such as the hydroxyl radical could lead to lipid peroxidation.[202]

Cardiac mitochondrial damages from oxy-radicals generated from cardiolipin-bound drug is quite likely, because the heart is particularly deficient in the protective enzymes superoxide dismutase and catalase.[203,204] In comparison, the liver has four times more superoxide activity and ten times greater catalase activity than the heart.[205] Compounding the lack of detoxifying ability is the fact that doxorubicin depresses the activity of glutathione peroxidase in cardiac cells.[206,207] The heart mitochondria basically finds itself unprotected from attack by hydroxyl radicals.

Peroxidized cardiolipin and other phospholipids can cause further damage by reacting covalently with protein.[208,209] Lipid peroxidation in the inner mitochondrial membrane causes a 20 to 30% decrease in

membrane fluidity and inactivates several respiratory reactions.[210]

The exact species, doxorubicin or a metabolite, causing cardiotoxicity, is the subject of current debate. A recent proposal[194] suggests that doxorubicin would accumulate in heart cells through its binding on cardiolipin. Localized depletion of oxygen would occur via redox cycling with subsequent generation of toxic oxygen radicals. Once high hypoxic conditions are reached, bioreductive deglycosidation would yield the 7-deoxyaglycone, which in turn would decrease the ability of cardiac mitochondria to retain Ca^{2+} and possibly participate in the generation of oxy-radicals after cardiac reoxygenation. These combined effects would lead to mitochondrial distress and cell death. This hypothesis would be consistent with the fact that 7-deoxyaglycones are the major physiological metabolites[211-214] which tend to accumulate in heart tissue. As much as 10-15% of a doxorubicin dose has been found as the aglycone 5-30 minutes after drug administration in Syrian golden hamsters hearts.[215,216] The aglycone concentration correlated well with the cardiotoxicity.

The cardiotoxicity hypothesis in question has been very recently challenged by Olson et al, who convincingly showed that cardiotoxicity results from exposure of cardiac tissue to doxorubicinol, a metabolite of doxorubicin

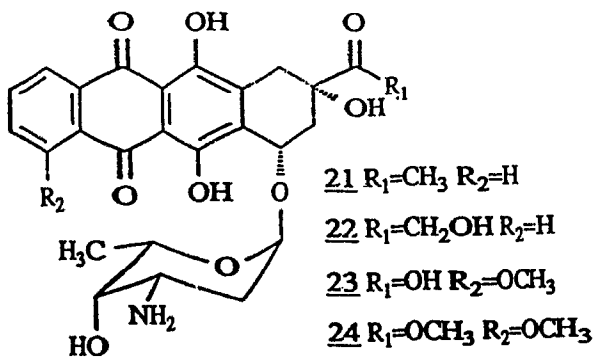
resulting from reduction of the C-13 carbonyl by aldoketo reductases.[10] Doxorubicin at 90 uM extracellular concentration compromised both systolic and diastolic cardiac function and completely inhibited the calcium pump of the sarcoplasmic reticulum, the Na^+/K^+ pump of sarcolemma and the F_oF_1 reversible proton pump of mitochondria. Interestingly, doxorubicin at 700 uM extracellular concentration had little effects on systolic or diastolic function and did not inhibit calcium loading by sarcoplasmic reticulum or the ATPase activities of the three ion pumps. The actual intracellular concentration of doxorubicin was estimated to be 15 ug/g of heart muscle, while 310 ug/g of heart muscle of doxorubicin was measured at 175 uM extracellular concentration. The doxorubicin concentration is too high and is unlikely to be found after therapeutic treatment while concentrations of 15 ug/g of doxorubicinol are more reasonable. The authors also found that the heart muscle metabolizes doxorubicin into doxorubicinol.

While doxorubicinol is nearly thirty times more potent at depressing contractibility, doxorubicin was found to be twenty-five times more potent in the cytotoxicity against two different pancreatic adenocarcinoma cell lines. This encouraging result suggests that the separation of the undesirable cardiotoxic side effect from the beneficial antitumor activity could be achievable.

D. Structure Activity Relationship

Since the study of molecular biological effects of the anthracycline antitumor antibiotics has been lagging behind in vivo testing of new compounds, most of the development of analogs has inevitably been of an empirical nature and has derived mainly from biosynthetic or semisynthetic sources. An awareness of structure – activity relationships coupled with contemporary mechanisms should make rational drug design a more attainable goal. The biological consequence on antitumor activity and cardiotoxicity from pertinent structural modifications of doxorubicin and daunorubicin are reviewed briefly.

The 4-demethoxy-daunorubicin (21), idarubicin, is 4 to 8 times more potent than daunorubicin in L1210 leukemia and Gross leukemia.[217] It is found to accumulate in tumor tissue more than in the heart[218] and is less cardiotoxic, probably because of a lower production of idarubicinol in comparison with daunorubicin.[218] Similarly, the 4-demethoxy-doxorubicin (22) showed lower cardiotoxicity with retention of antitumor activity.[1b,1c] The 11-deoxy analogs are active[219,223] against P-388 leukemic cells[220] as are the 11- and 6-O-methylated homologs of doxorubicin and daunorubicin. Antitumor activity for L1210 was demonstrated



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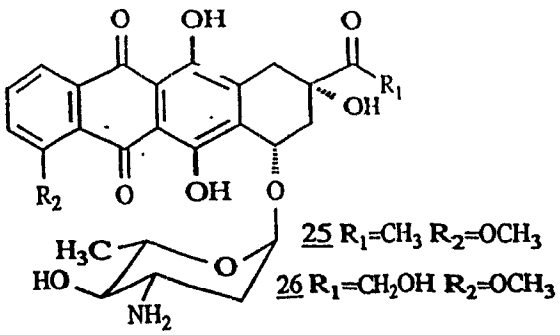
22 R_1=CH_2OH R_2=H

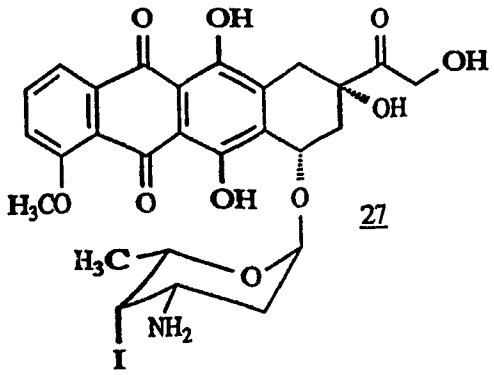
23 R_1=OH R_2=OCH_3

24 R_1=OCH_3 R_2=OCH_3



25 R_1=CH_3 R_2=OCH_3

26 R_1=CH_2OH R_2=OCH_3



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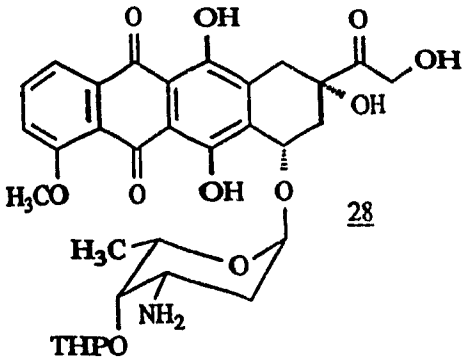

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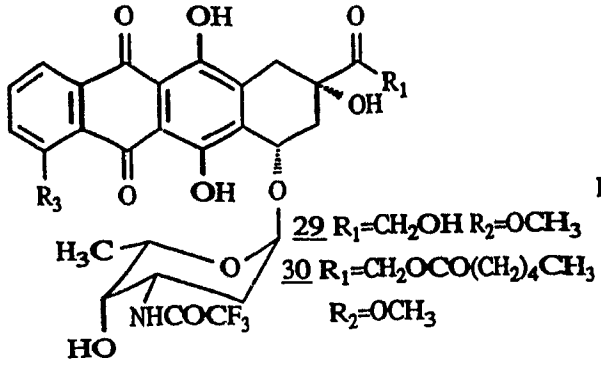
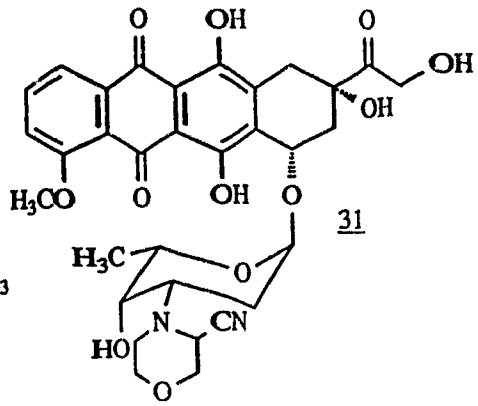


29 R_1=CH_2OH R_2=OCH_3

30 R_1=$CH_2OCO(CH_2)_4CH_3$ R_2=OCH_3



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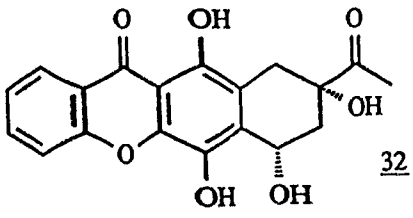

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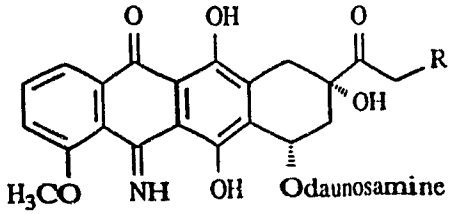


33 R=H

34 R=OH

Fig 13

for 4-demethoxy-11-deoxy modifications of doxorubicin and daunorubicin.[221]

In order to establish the importance of C-9 functionalities, the antitumor activity of compounds with C-9 variations were tested. Removal of the 9-OH gives active compounds[222] which are marginally weaker DNA binders,[223] but 9-O-methylation or replacement of the 9-hydroxyl with a 9-CH_3 inactivates the drug towards P-388 cells.[158,224] Interestingly, the total removal of the 9-acetyl side chain does not result in any loss of antitumor activity.[225] Periodate oxidation of doxorubicin gives the free acid side chain at C-9 (23). Although potency is reduced, both the carboxylic acid derivative (23) and its methyl ester (24) showed equivalent in vivo antileukemic activity with daunorubicin.[1b] It is interesting to note that considerable variations of the C-9 substituents is permissible without great loss in activity.

The C-7 position, however, is more sensitive and inactive compounds are obtained upon epimerization.[226] Analogs with sugar modifications could have important therapeutic promise, because tissue distribution, cellular uptake and intracellular distribution may depend on the structure or stereochemistry of the carbohydrate side chain. Similarly, binding to enzymes and/or enzymatic activation may also be affected. Consequently, structure modification

of the sugar have been exhaustive and therefore a limited but pertinent coverage is possible here.

Epimerization of the C-4′ hydroxyl of doxorubicin to give epirubicin (26) leads to equivalent in vivo activity.[227] The affinities for DNA of 4′-epidaunorubicin (25) or epirubicin are comparable to the parents.[228] Epirubicin has generated great interest because it has an equal antitumor efficacy to doxorubicin with 25% less cardiotoxicity.[11] This therapeutic improvement is reflected in a longer cancer chemotherapy. Clinically, 11 cumulative doses of epirubicin in comparison to 8 for doxorubicin can be administered before there is a high risk of cardiomyopathy.[11] This additional time of treatment is beneficial for patients whose metastatic cancers are responding. The significant reduction of cardiotoxicity is attributed to a faster metabolism and plasma clearance of epirubicin and its metabolites.[229-231] The 4′-deoxy analogs of doxorubicn and daunorubicin are slightly more active than their parents in vivo[227] and cause less cardiotoxicity in mice than epirubicin.[1i] This may result from a more rapid clearance from cardiac tissue and a decreased formation of rubicinol.[232]

Modifications of C-4′ have been seriously considered in view of potential therapeutic improvements. Currently, the 4′-deoxy-4′-iododoxorubicin (27) has entered phase I trials in Italy and France and is promising because of its

greater potency as well as a reduced cross resistance.[233] Enhanced intracellular transport of 4′-iododoxorubicin in K562 human leukemia doxorubicin resistant cells is responsible for the antitumor effectiveness and diminished cross resistance.[234] Another promising candidate with lower cardiotoxicity and cross resistance is pirarubicin, 4′-O-tetrahydropyranyldoxorubicin (28), which has passed phase I trials and is in phase II studies.[235]

Considering these interesting results a great many analogs with modifications at C-3′ have been prepared. Epimerization at C-3′ is not detrimental and leads to a slight loss in potency but equivalent *in vivo* activity.[1b] N-acyl compounds have poor DNA binding properties but are still cytotoxic. Amides, urea ot thiourea derivatives show decreased activity with respect to parent[1b], but N-trifluoroacetyl-doxorubicin (29) is equiactive to doxorubicin in P-388 *in vivo* evaluations.[95] Peptidic derivatives of daunorubicn such as N-glycinyl, N-Leu, N-Leu-Leu and N-Ala-Leu show better *in vivo* L1210 activity.[236] The most interesting N-acyl derivative has been the N-trifluoroacetyldoxorubicin-14-valerate (30), Ad32, which shows greater in vivo activity than the parent though reduced potency.[237] There is less accumulation of this drug in the nucleus, but cellular uptake is faster than the parent[238] and it is not a prodrug of doxorubicin.[239]

Ad32 is less cardiotoxic[240] and causes strand breaks in the DNA double strand with associated DNA-protein cross links as with doxorubicin.[244-246] This is noteworthy, because AD32 is capable of generating DNA damage without intercalating. The possibilty that the lesions are caused by a reactive intermediate of AD32 upon bioreduction is raised. Since the drug is located extranuclearly, it is unlikely that hydroxyl radicals are cleaving DNA. Furthermore, a recent study has shown that AD32 has only 4% of the superoxide generating ability of doxorubicin.[212] Evidently, DNA damage should result from the alternative mechanism in which tetracyclic alkylators are generated. Intercalation to the DNA or surfacial attachement of either the quinone radical or quinomethide could explain the observed DNA strand breakages. The most interesting n-alkylated analogs are the cyclic derivatives at the amino group such as the 3′-deamino-3′-(4-morpholinoyl)daunorubicin.[244-246] The most potent anthracycline antitumor drug known to date is 3′-(3-cyano-4-morpholinyl)-3′-deaminodoxorubicin (31). It is 600 times more potent than doxorubicin against P388 leukemia in mice and has reduced cardiotoxicity.[247]

Modifications dealing with the quinone moiety may prevent the reduction of oxygen. The totally synthetic glycosides of cis-7,9-dihydroxy-9-acetyl-6,7,9,11-tetrahydroxantho[2,3-g] tetralin (32) have exhibited

antileukemic activity but very low superoxide activity[248], thus indicating that the quinone moiety would be necessary for oxygen activation into superoxide. Confirming this requirement are earlier studies carried out with 5-iminodaunorubicin (33) and 5-iminodoxorubicin (34). Both drugs showed important antitumor activity and lower cardiotoxicity than the parents[249] and did not act as prodrugs.[250] A low superoxide generating ability was observed for both imino derivatives.[213] This lack of oxygen reduction provides an explanation for the observed reduction of cardiotoxicity.

The mechanism of action for the iminoanthracycline analogs has been the subject of current debate. The fact that deoxyaglycones are observed in vivo tends to exclude a deglycosidation pathway.[250] This has however been contested by Koch and coworkers[249], who suggested that iminodeoxyaglycones are not detected because they are transformed to compounds (36) and (37). In vitro, 5-iminodaunorubicin was reduced electrochemically under anaerobic conditions. The reaction yielded 5-imino-7-deoxydaunorubicin (35), but continued to ultimately give the overreduction products (36) and (37). These results support the bioreductive deglycosidation of 5-iminoanthracyclines.

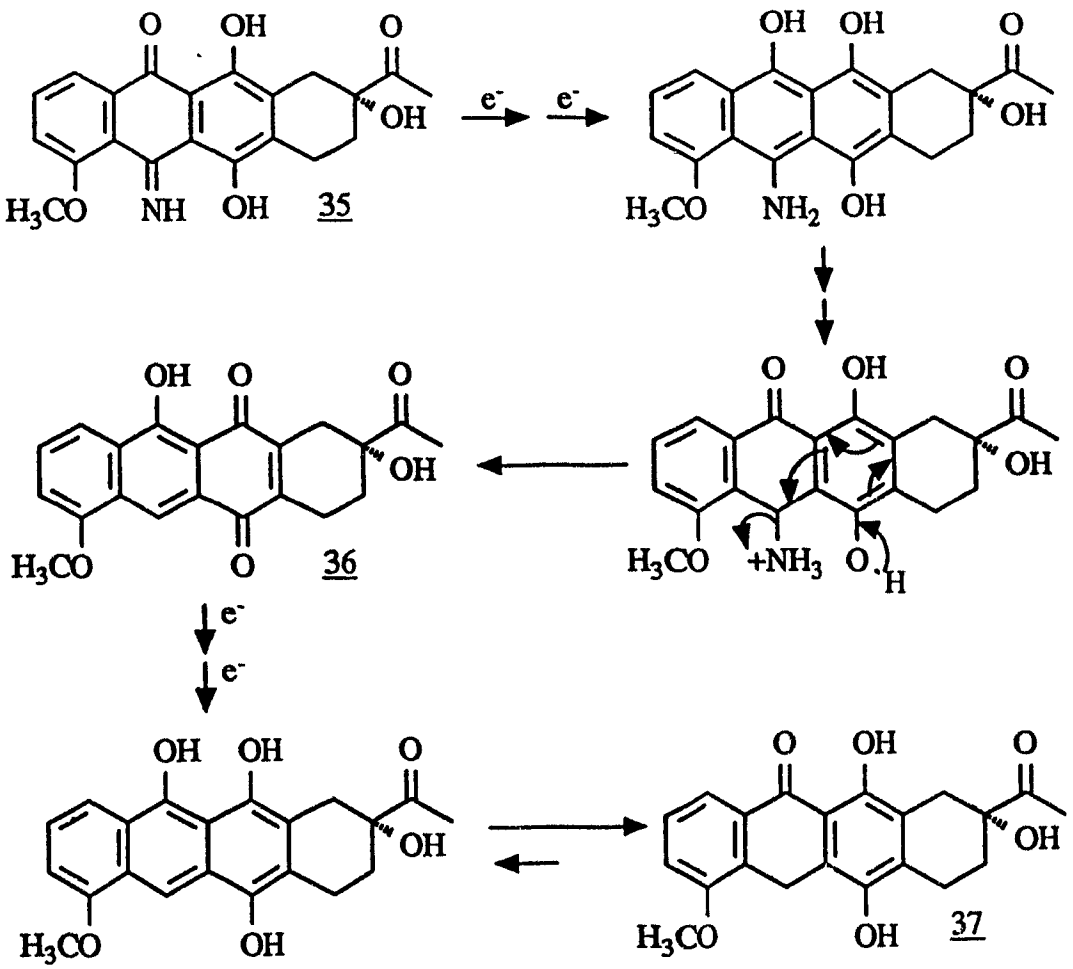


Fig 14

E. Conclusion

The body of data supports a mechanism of drug action in which the anthracycline semiquinone may either reduce oxygen or eliminate the sugar (fig.15). Biochemical consequences can be related to both events. Hydroxyl

radicals produced as a result of superoxide generation would lead to cell membranal damages and DNA single strand nicks.

Deglycosidation would generate reactive intermediates that could covalently bind to DNA and consequently induce topoisomerase II into causing

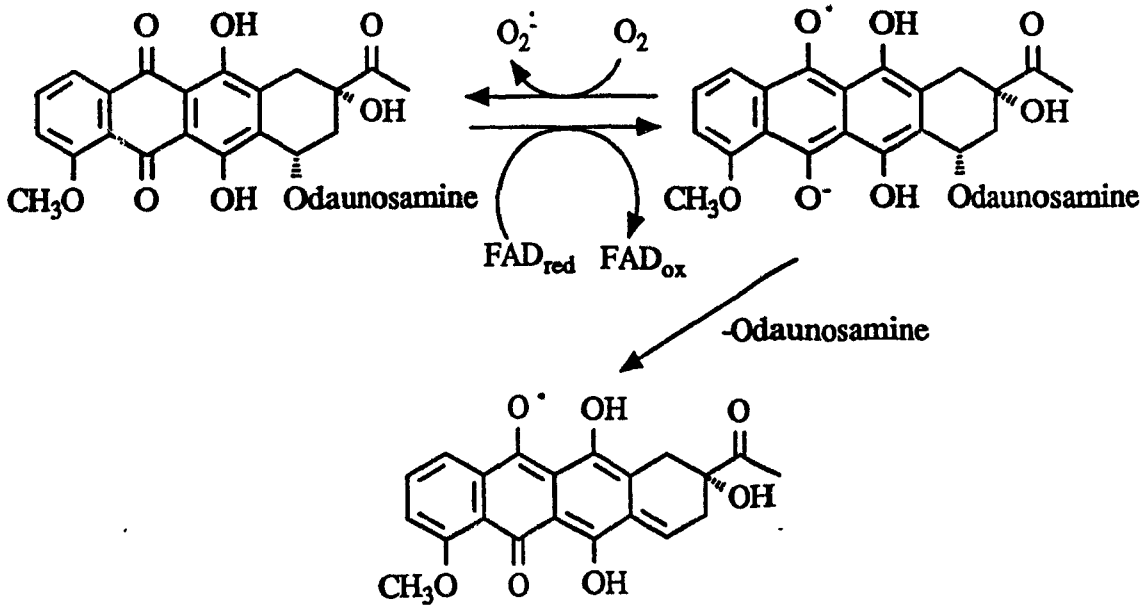


Fig 15

irreversible double strand cleavages. This latter scenario could explain the antitumor activity of quinone modified anthracycline antitumor drugs which have suppressed superoxide activity. Cardiotoxicity would occur, because cardiac cells are poorly protected from attack by free radicals and therefore can be related to the generation of superoxide.

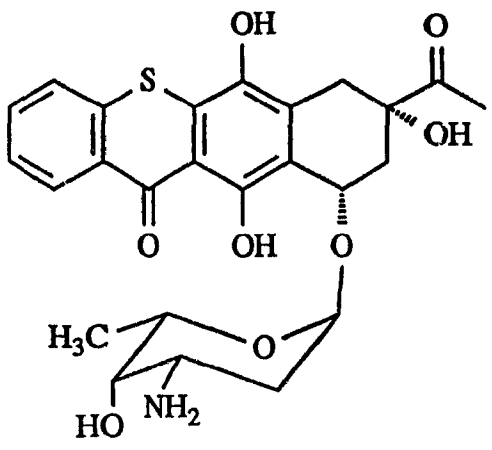
The cardiotoxicity of anthracyclines such as doxorubicin can be separated from the tumor cell toxicity by

preventing the reduction of oxygen. This has been demonstrated with the quinone modified analogs.

The reduction of oxygen and the deglycosidation reaction are in competition with each other. The deglycosidation reaction could be favoured by providing a driving force which is yet unavailable in known anthracyclines. A better leaving group at C-7, or the formation of reactive intermediates with greater stability could satisfy this criteria. The design and synthesis of appropriate structures is required to test such a concept.

F. Goal Of The Project

Based on known mechanistic principles and structure activity relationships, the thioxanthone modification of the quinone moiety should give derivatives which are less prone to participate in a redox cycling mechanism.



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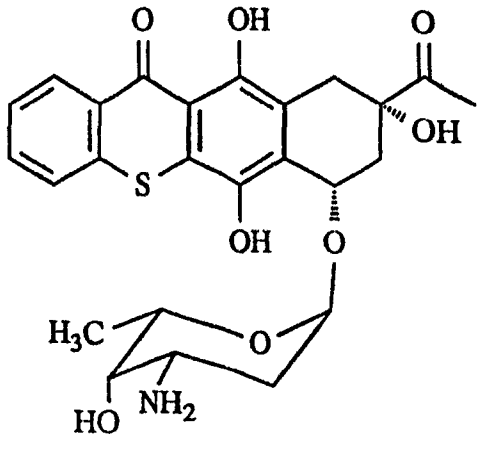


39

Fig 16

Our first goal was an extension of previous work carried out by Honek[251] and involved the total synthesis of compounds (38) and (39) using novel routes of synthesis.

Previously, the major difficulty in the synthesis had been the great driving force towards aromatization, for example:

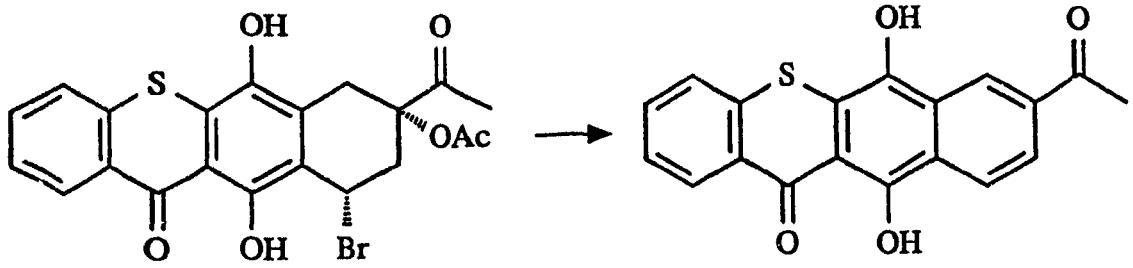


Fig 17

Elimination of the antiperiplanar acetate and bromine leaving groups is facile due to the formation of an aromatic A ring. During the course of the project, the total synthesis of the 7-hydroxy-thioxantone aglycones of (38) and (39) was achieved by Wong et al in 1984[252], but glycosidation of these aglycones has never been reported, possibly due to problems associated with aromatization. Since many known glycosidation reactions involve acidic or basic media[252-256], which will favour aromatization, we proposed to develop a synthetic route in which the last steps could be carried out under neutral conditions. Thus, the total synthesis of the shown thioxanthone intermediate (40) was required as well as the cycloaddition of the thermally generated o-quinodimethide with an appropriate

dienophile.

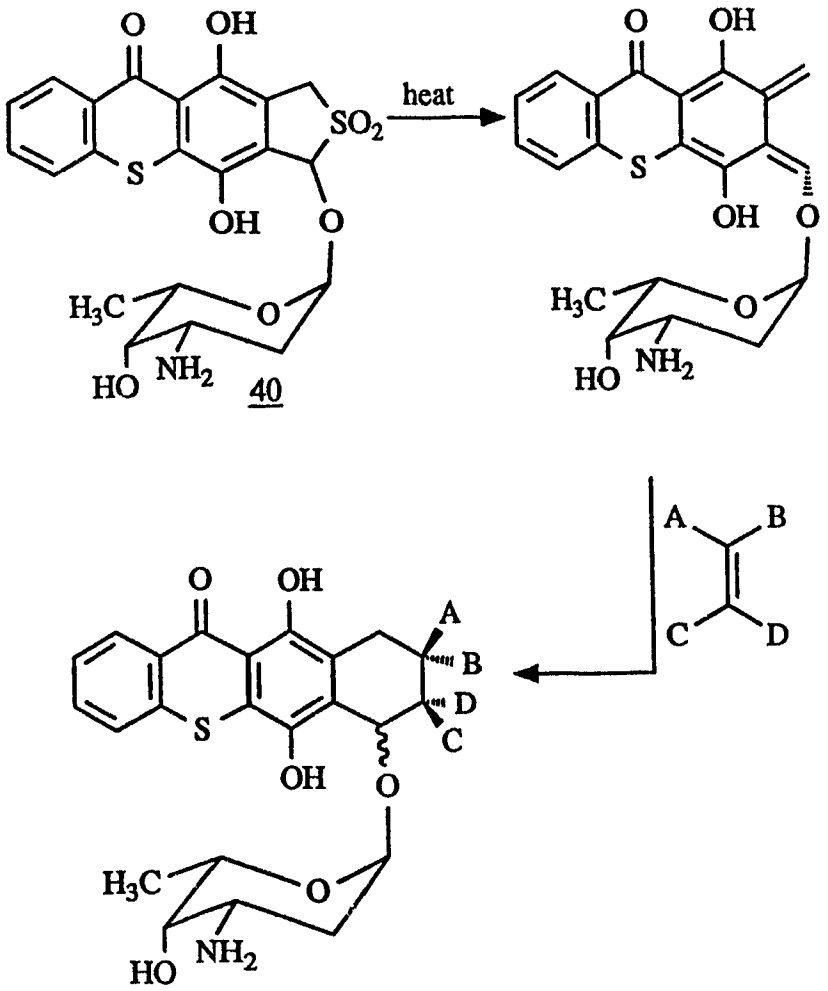


Fig 18

It is interesting to note that (40) in itself constitutes a valid analogue for antitumor evaluation. It fills the requirements for a flat aglycone necessary for intercalation and has the daunosamine sugar which may be necessary for drug transport and distribution purposes in DNA. Reactive intermediates such as (41) or (42) should

have enhanced stability due to the possible conjugative interaction of the sulfone group.

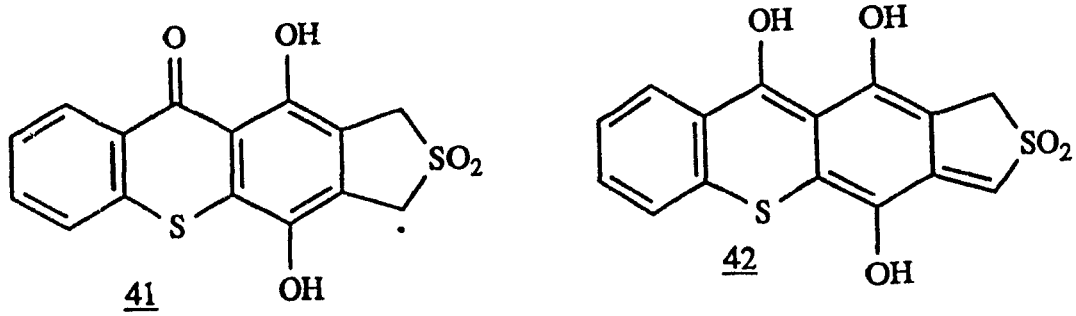


Fig 19

Given the facts that previous synthetic attempts had failed due to aromatization problems, our second goal was to carry out the total synthesis of isochroman structures (43) and (44) where aromatization is prevented due to the presence of the ring A oxygen replacing the C-8 methylene of the natural anthracyclines. Since there are two major modifications in compounds (43) and (44), it became

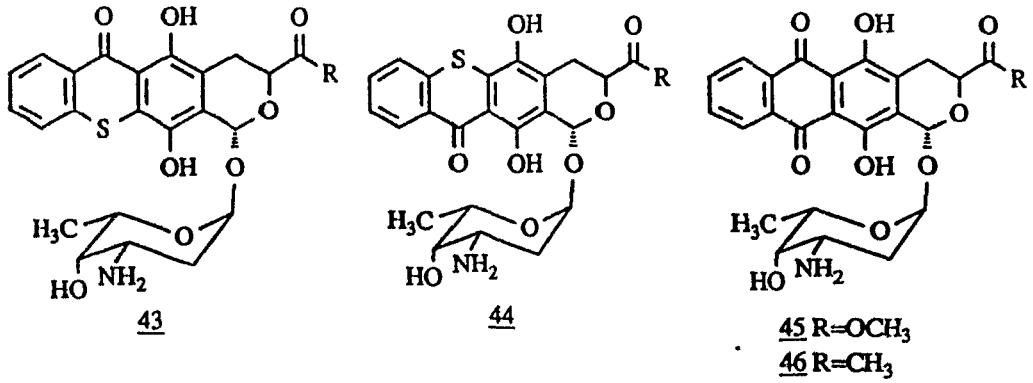


Fig 20

necessary to consider the preparation of analogs having only a ring A modification such as in (45) and (46). These proposed quinones have the possibility of following known mechanisms of antitumor action. Removal of the C-4 methoxy or the C-9 hydroxy of the anthracycline, and an ester C-9 side chain should not be detrimental for antineoplastic activity.

Anthracyclines (45) and (46) should enhance the deglycosidation following reduction, because a significant driving force is provided by the presence of the isochromano-ring oxygen. Captodative[257,258] stabilization due to coupled electron donation from the ring oxygen and

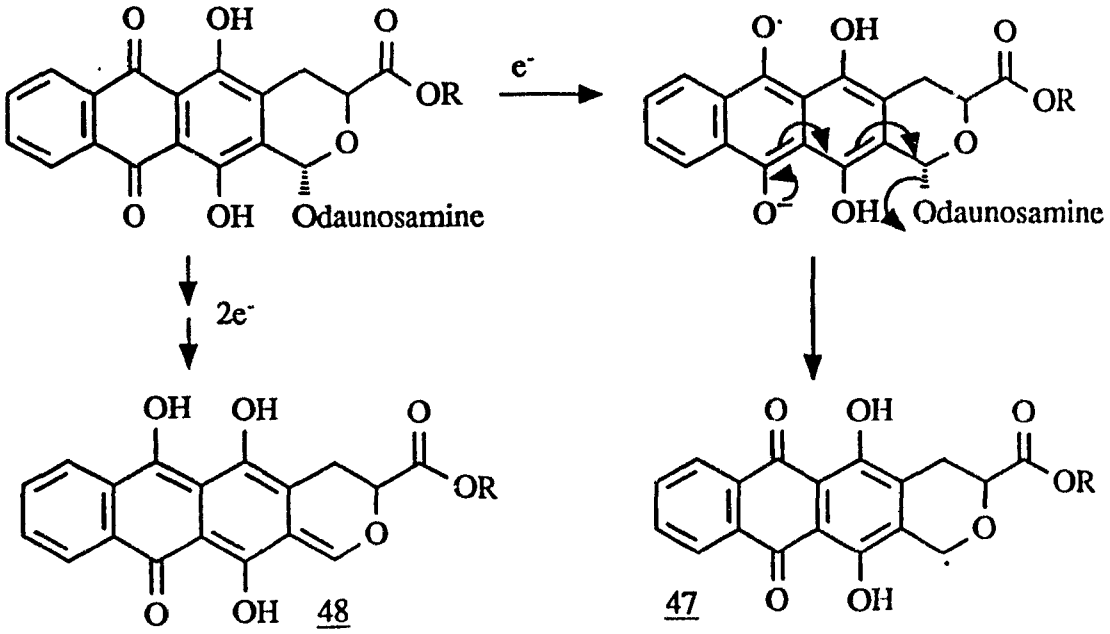
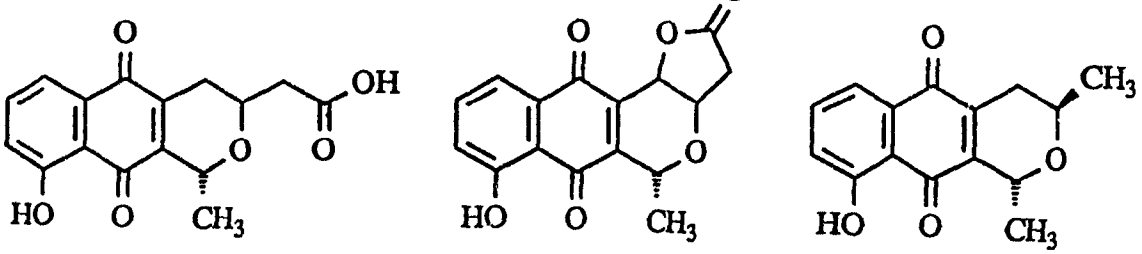


Fig 21

electron withdrawal from the anthraquinone moiety of the quinone radical (47) would greatly favour radical formation

and would extent its half life, thus preventing excessive protonation to yield the aglycone. The captodatively stabilized quinone radical should therefore have more time to reach and alkylate key cellular targets. Alternatively, inductive stabilization of the quinone methide (48) obtained following two electron reduction and deglycosidation should be provided by the presence of the ring oxygen. It was hoped that the added driving force for the bioreductive deglycosidation pathway is sufficient enough to decrease the incidence of oxygen reduction and consequently to decrease cardiotoxicity.

Surprisingly, our literature search for other quinone antibiotics with an isochroman ring led to the



Nanaomycin A Nanaomycin D Eleuterin

Fig 22

discovery of a small family of naphtoquinone antibiotics which possess strong antibiotic and antifungal activities and possibly antitumor activity.[37,38] Some of these compounds are shown in Fig (22). The mechanism of action for these drugs is believed to proceed via bioreductive

alkylation[37,38] in which the lactone moiety is opened (fig 23). Ring opening of the dihydropyran, in which the alcohol

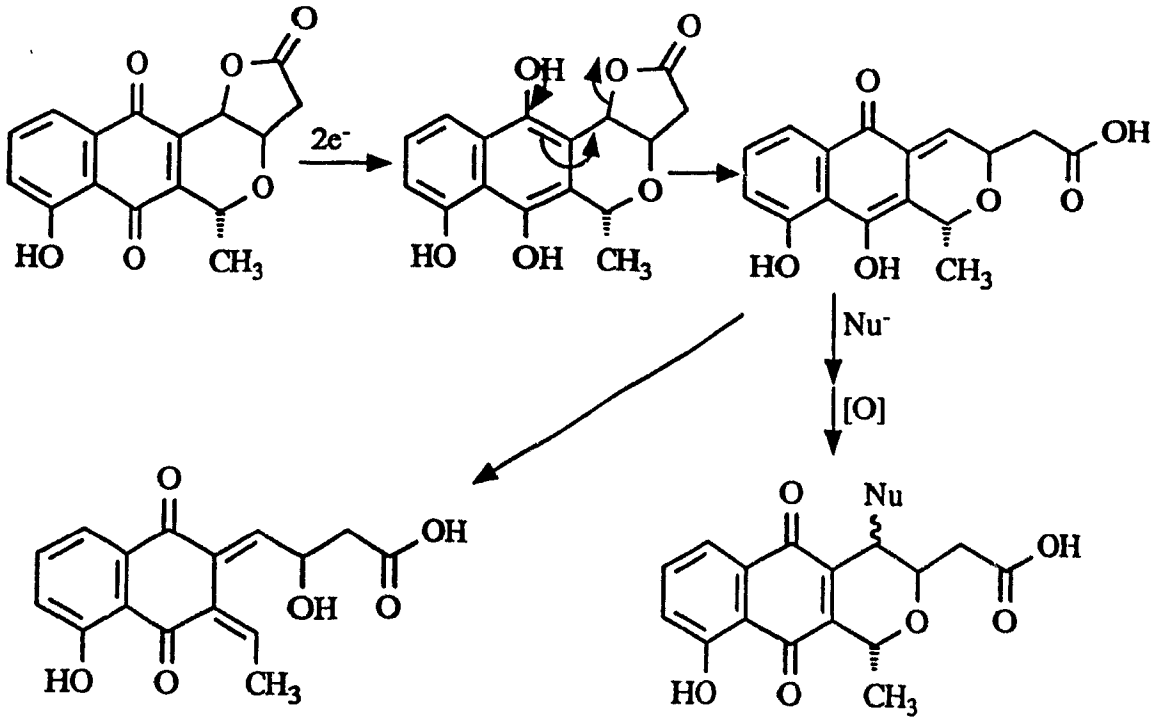
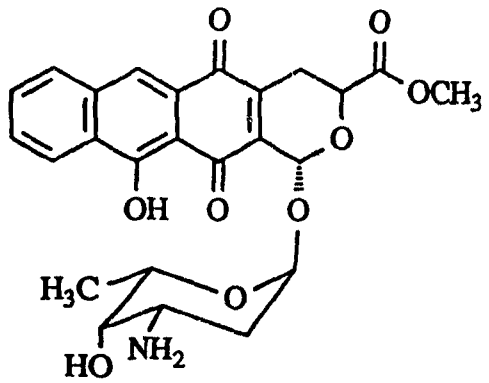


Fig 23

is the leaving group, has been proposed but the stereoelectronics are not ideal for this process.

This literature precedent incited us to broaden our goals and to investigate the possibility of preparing tetracyclic analogs of the naphthoquinone antibiotics of varying ring substitution but with a carboxylic ester side chain and not the homocarboxylate seen in the natural naphthoquinone antibiotics. This would not only allow the synthesis of novel naphthoquinone antibiotic analogs, but also provide synthetic knowledge for the total synthesis of

more complex daunorubicin derivatives, for example (45) and (46).



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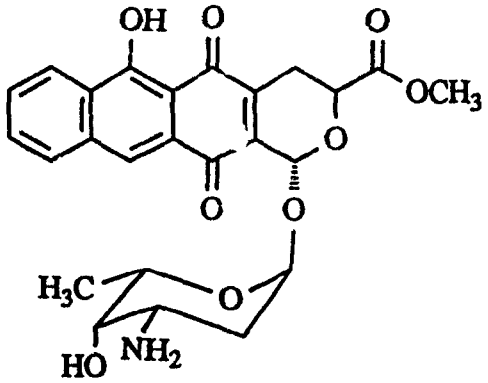
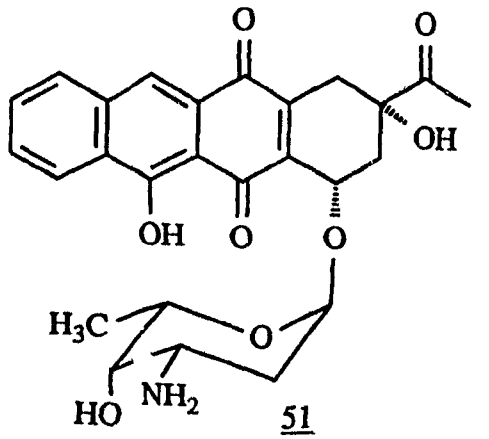


50

Fig 24

An additional goal was the total synthesis of a hybrid between the naphthoquinone antibiotic and 4-demethoxy daunorubicin such as (49) and (50). These can of course enhance the bioreductive deglycosidation pathway for the same reasons as seen previously for analogs (45) and (46).



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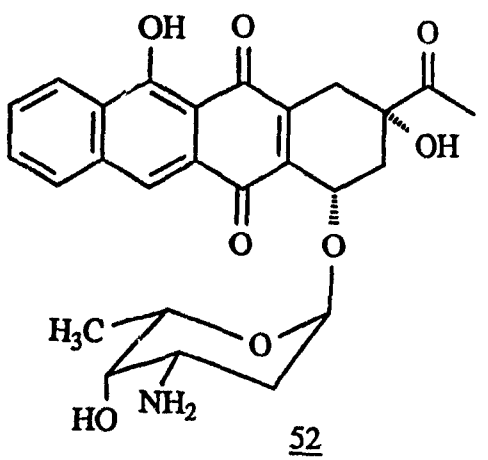


52

Fig 25

Encouragement towards this goal was provided during the course of this project by a 1988 report which indicated that modified anthracyclines such as (51) and (52) were significantly active against leukemia cell lines.[259] The following presents our efforts towards the total synthesis of the proposed anthracycline analogs.

CHAPTER 1

1.1 Studies Towards The Synthesis of Thioxanthone Modified Analogs of 4-Demethoxydaunorubicin.

In our retrosynthetic analysis the major criterion was that the synthetic steps following glycosidation had to be carried out under as neutral conditions as possible. Therefore, the first disconnection in compound (39) was done across ring A as shown in fig. 1.1 to give synthons (63) and (64). The thioxanthone-o-quinodimethide glycosyl intermediate (70) and the mono-enol tautomer of 2,3-butanedione (65) are their equivalents. The latter is not a practical dienophile but can be substituted either by 3-methoxy-3-butene-2-one (66),[260,261] 3-acetoxy-3-butene-2-one (67)[262,263] or 3-[trimethylsilyloxy]-3-butene-2-one (68).[264,265] The preferred dienophile is the latter one, because the silyl protecting group can be removed under mild condition by using fluoride.[266]

Previously, the use of 3-trimethylsilyloxy-3-butene-2-one (68) as a dienophile has been limited because of poor reactivity.[264] The use of $SnCl_4$ as a catalyst has enhanced the reactivity of dienophile (68),[265] but this Lewis acid may cause aromatization in our case. To prevent the elimination of acid-labile groups, tris-(6,6,7,7,8,8,8-heptafluoro-2,2-dimethyl-3,5-octanedionato) europium,

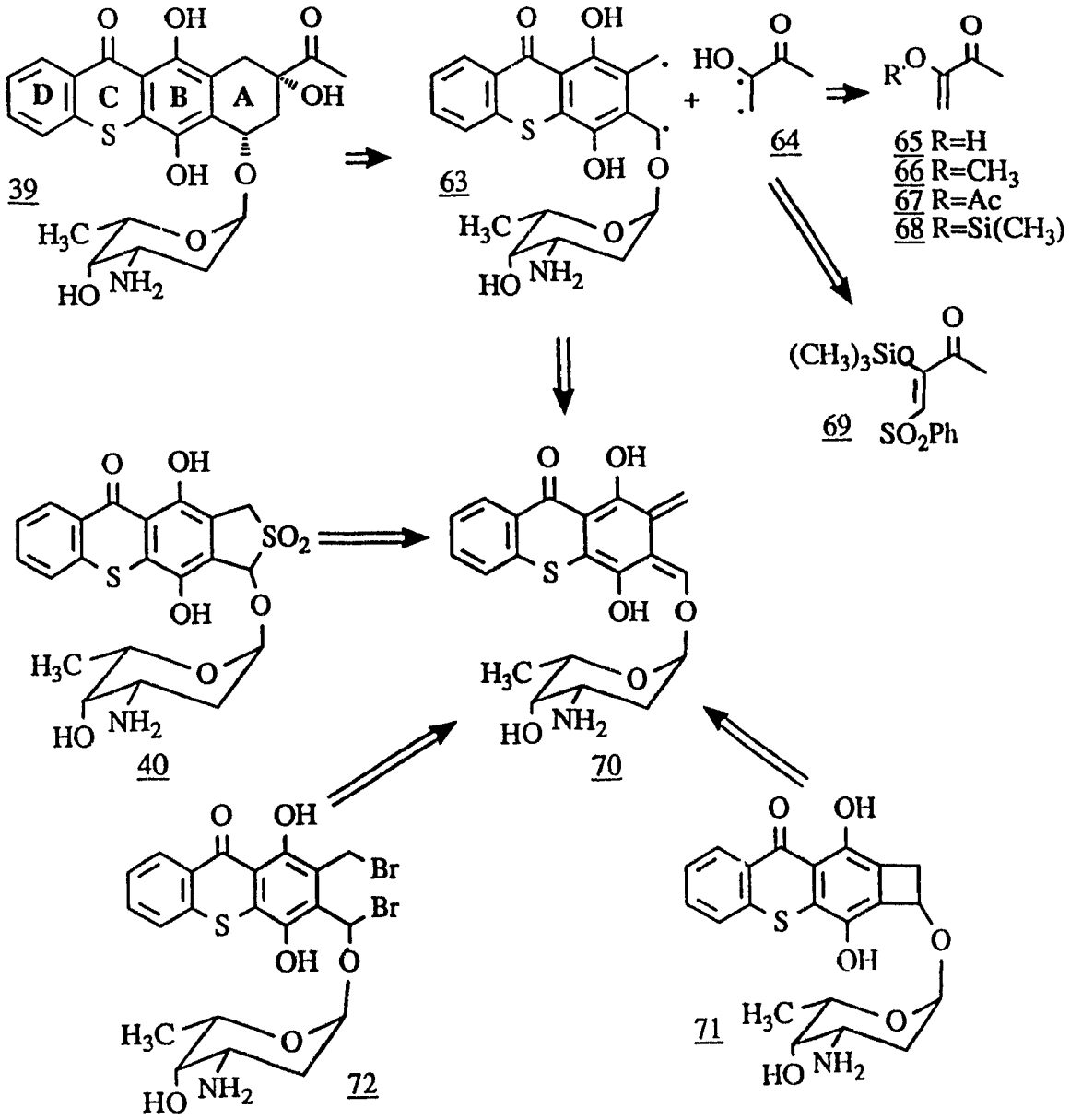


Fig 1.1

$Eu(Fod)_3$, has previously been used as a catalyst in cycloaddition reactions.[267]

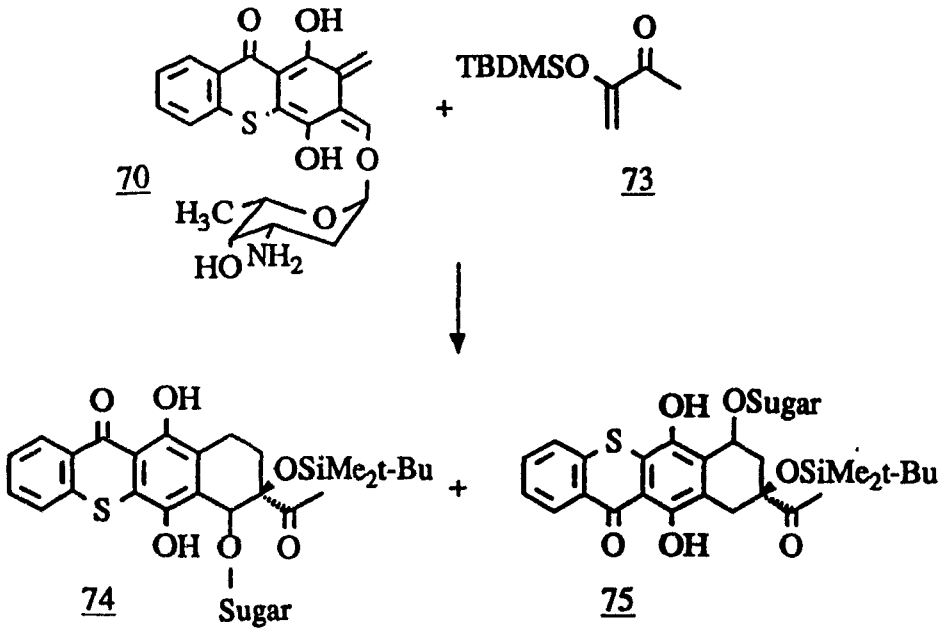


Fig 1.2

A major concern in the use of the silyl enolate (73) as a dienophile was the regio and stereochemical outcome from the cycloaddition (fig. 1.2). A prediction can be attempted based on the frontier molecular orbital (FMO) theory.[268] The most important molecular orbital interactions in the cycloaddition reaction would be between the highest occupied molecular orbital, HOMO, of the diene and the LUMO of the dienophile. The regiochemical outcome depends on the coefficients of the frontier orbitals at the reaction centers. In the quinodimethide (70), the thio and carbonyl fused substituents would not significantly affect

the regiochemistry. This is precedented by a previous experiment,[251] in which a 1:1 regioisomeric mixture of thioxanthyl cycloadducts (77) and (78) was obtained in the trapping of (76) with methyl vinyl ketone (fig. 1.3).

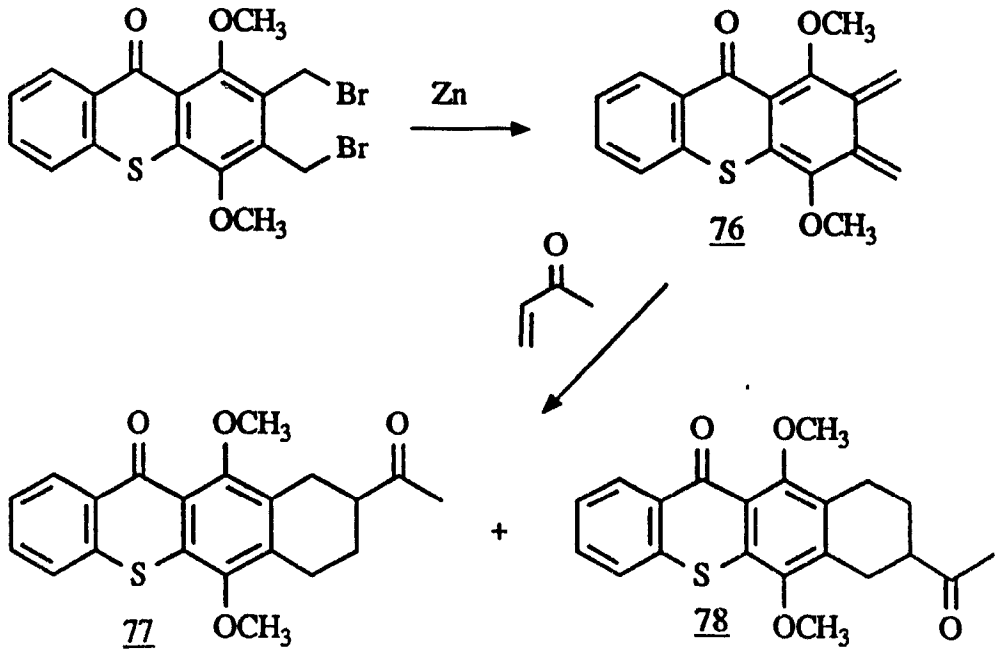


Fig 1.3

The electron donation from the O-daunosamine will increase the electron density at the substituted benzylic carbon and the electron withdrawing effect of the carbonyl, especially if complexed with a metal, will diminish the electron density at the alpha carbon in dienophile (73). These electronic factors will favor isomer (74) over (75) in the cycloaddition reaction.

The dienophile must be modified if experimentally the wrong regioisomer is obtained. Thus, regioselectivity

could be reversed with a phenylsulfone auxiliary as in dienophile (69). The removal of the sulfone moiety should be feasable by using an amalgam under buffered conditions such that neutrality of the medium is maintained.

Stereoselectivity of the cycloaddition reaction in fig. 1.2 can be expected if the Alder rule of maximum accumulation of unsaturation is followed. Cycloaddition reactions of 1-substituted o-quinodimethanes with a variety of dienophiles give the expected products,[269-271] based on endo suprafacial addition of dienophile (fig. 1.4)

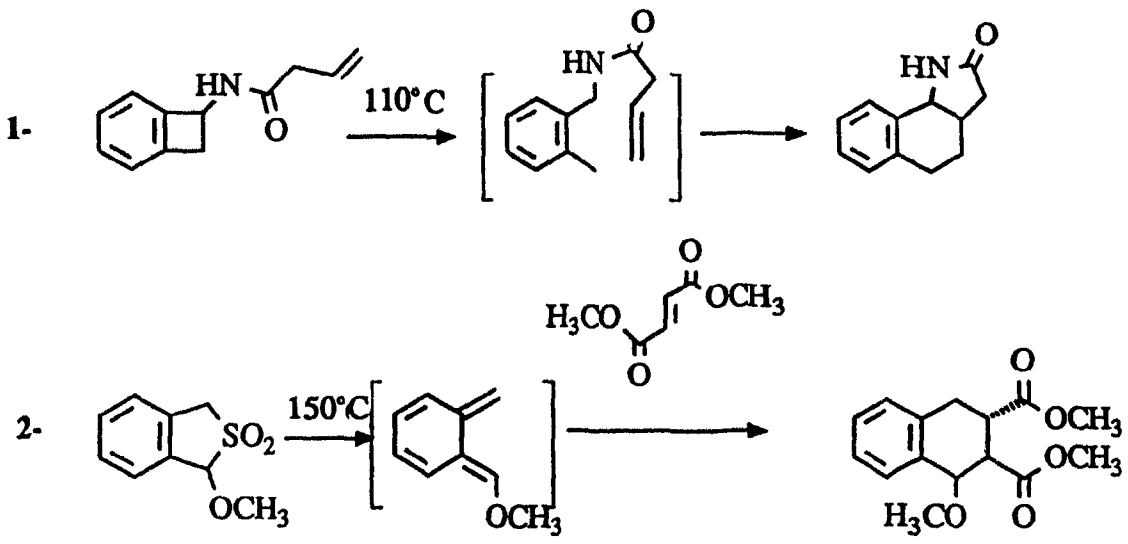


Fig 1.4

Therefore, the cycloaddition reaction with the enolsilyl ether (73) would give preferentially the stereoisomer with the sugar and the acetyl oriented cis to each other as shown in fig. 1.2. The introduction of the

phenylsulfone moiety complicates the prediction for the stereochemical outcome. It was hoped that the attractive interaction of the tetraene system with the phenylsulfone is greater than with the acetyl so that the correct stereoisomer would be obtained.

One of the main goals of our project was the generation of the appropriately functionalized thioxanthobenzyl fused o-quinodimethide (70). Previous studies carried out with the natural anthracycline ring system indicated that the highly reactive intermediary o-quinodimethane (81) could be generated from bis(bromomethyl) anthraquinones (79)[267,272] or thermolytically from the corresponding annelated cyclobutanes (80)[273] (fig. 1.5).

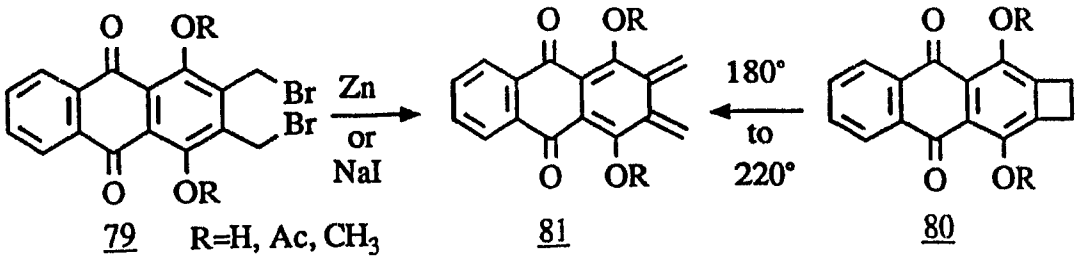
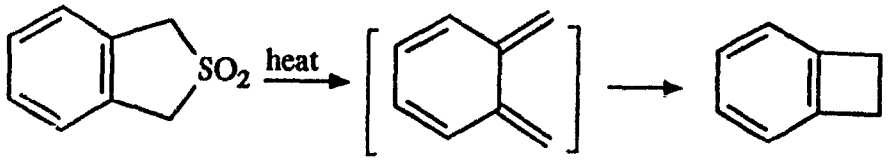


Fig 1.5

In our synthesis, the generation of the o-quinodimethide (70) from a bis-brominated precursor would not be practical because of difficulties associated with the synthesis of an appropriate dibromo derivative. Furthermore, the sugar could act as a leaving group in the presence of chelators, thus complicating the generation of

the o-quinodimethide intermediates. The preferred approach would be to generate compound (70) from the thermolysis of the annelated cyclobutane derivative (71) or through the extrusion of SO_2 from the 1-glycosidated-1,3-dihydrothiophene-2,2-dioxide intermediate (40). This latter reaction, in the absence of dienophiles, could give the cyclobutane derivative (71). In previous studies, thermolysis of 1,3-dihydrobenzo[C]thiophene-2,2-dioxide (82) has given benzocyclobutane (84) from conrotatory ring closure of o-quinodimethane (83) (fig.1.6).[274,275] This provides an example that the o-quinodimethane is generated at a lower temperature from cycloreversion of the cyclic sulfone (82). Following this analogy, the o-quinodimethide intermediate (70) could possibly be generated under milder conditions from the sulfone and therefore we opted to prepare intermediate (40). Furthermore, sulfone (40) in itself is a totally novel analog of 4-demethoxydaunorubicin and therefore its synthesis is well warranted.



82 83 84

Fig 1.6

Continuing the retrosynthetic analysis, deglycosidation of the thioxanthoneglycoside (40) leads to

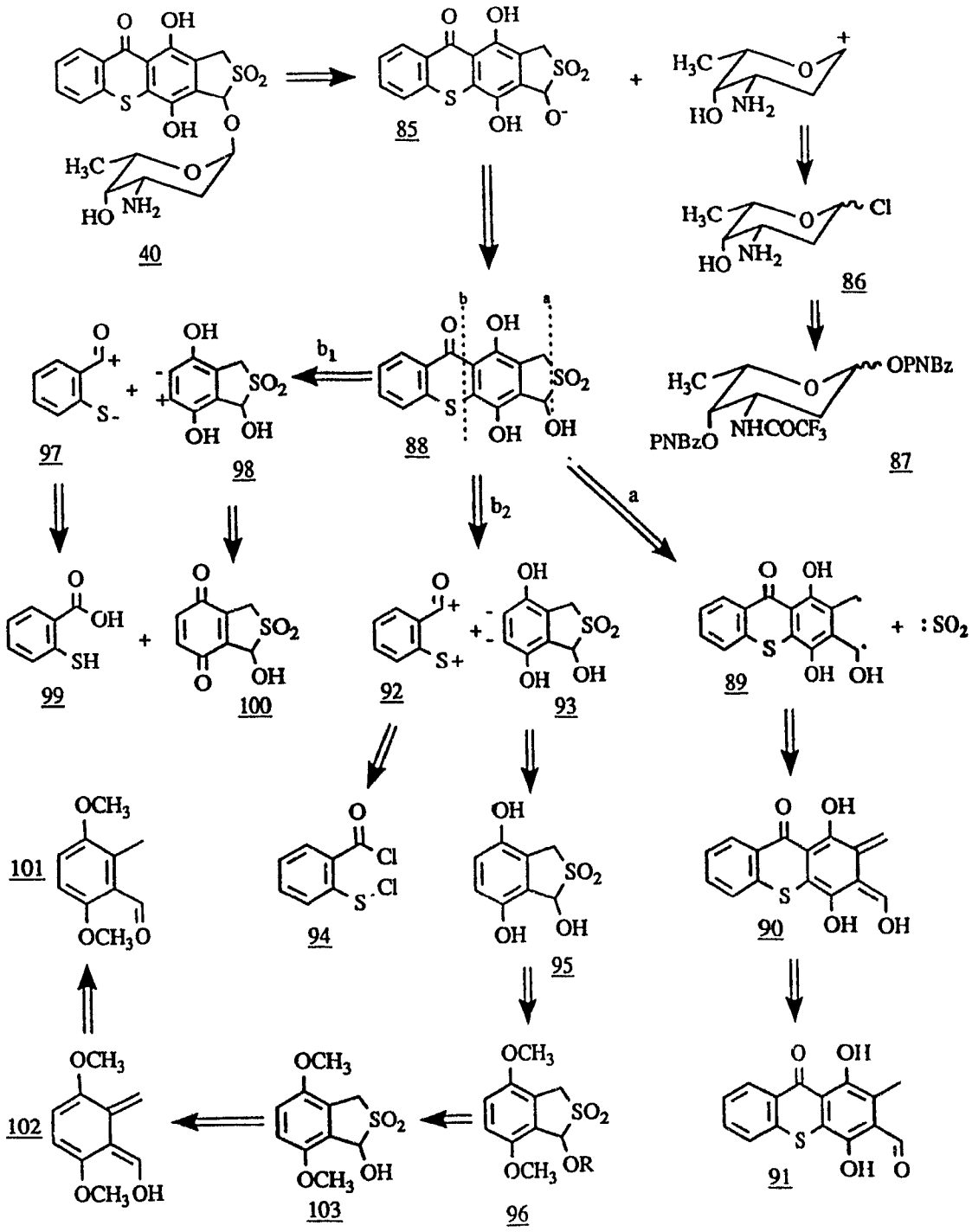


Fig 1.7

the aglycone (88) and the daunosamine sugar (86), which requires protection of the C-3′ amino and C-4′ hydroxyl groups. Thus the previously[254] employed 2,3,6-trideoxy-1-chloro-3-trifluoroacetamido-4-o-p-nitrobenzoyl-L-lyxohexopyranose (87) would be used in the glycosidation reaction. Disconnection A of the tetracyclic intermediate (88) would give the tricyclic o-quinodimethane (90) which could in principle be generated from the photochemical irradiation of the 1,4-dimethoxy-2-methyl-3-formylthioxanthone (91). The photochemical generation of hydroxylated o-quinodimethanes from o-methylbenzaldehydes and their subsequent trapping with sulfur dioxide[271,276] is precedented for simple systems. In the case of compound (91), some exploratory work was required.

The alternative routes B_1 and B_2 take advantage of two types of annulation reactions which have previously been studied on different bicyclic compounds.[251] Thus, the 1,4-Michael addition of o-mercaptobenzoic acid (99) to the bicyclic quinone (100) should yield compound (88) after intramolecular cyclization. Alternatively, the Friedel-Crafts reaction between o-chlorosulfenylbenzoyl chloride (94) and sulfone (96) could yield the tetracyclic hydroxy sulfone (88) after deprotection. It was hoped that the key sulfone (88) could be available from the photochemical irradiation of 2,5-dimethoxy-6-methylbenzaldehyde (101) and

the subsequent photochemical trapping of the 1-hydroxylated o-quinodimethane (102) by sulfur dioxide.

1.1.1 Preliminary Studies

In our synthetic sequence, the feasibility of two key synthetic steps had to be established. These are the photolysis of 2,5-dimethoxy-6-methyl benzaldehyde (101) and the glycosidation of the resulting 1-hydroxy-1,3-dihydro-4,7-dimethoxybenzo[B]thiophene-2,2-dioxide (103) to give glycoside (104) (fig. 1.8).

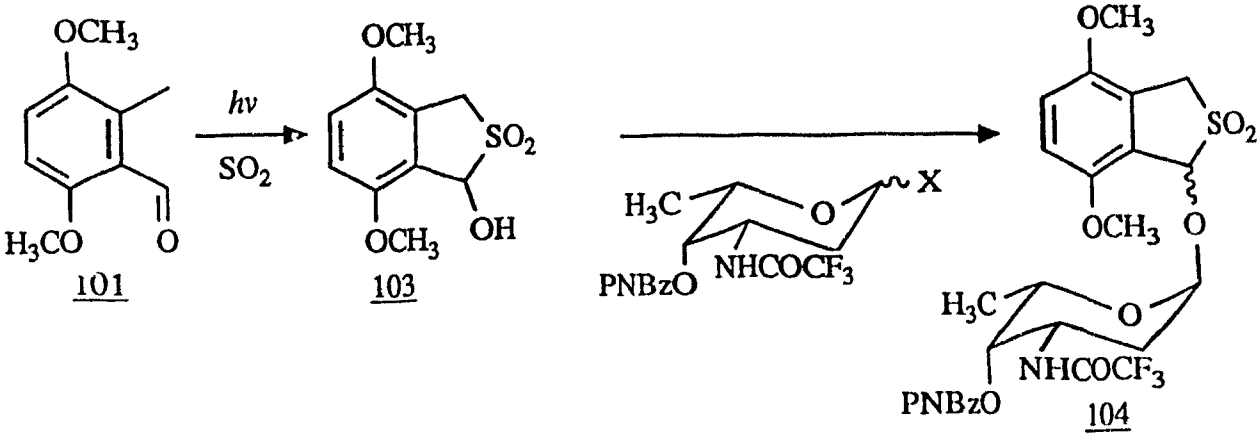
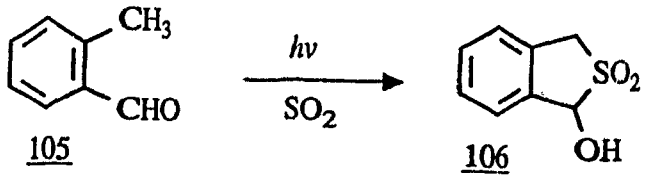


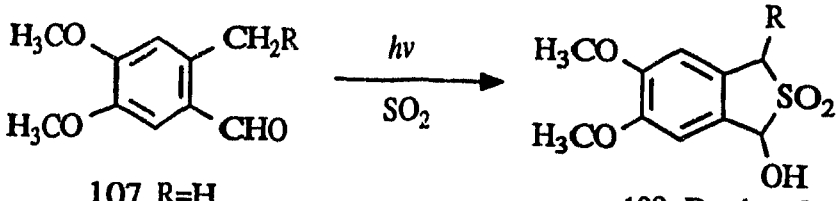
Fig 1.8

While photolysis of 2-methylbenzaldehyde (105) readily yields the corresponding hydroxy sulfone (106) after SO_2 entrapment of the o-quinodimethane,[271] no reaction is reported to take place for the corresponding dimethoxy aldehyde (107)[278] (fig. 1.9). The failure for this reaction is not easily explained, since the corresponding aryl

substituted benzaldehyde (108) readily yields the hydroxysulfone (109) upon photolysis.[278]



105 106



107 R=H

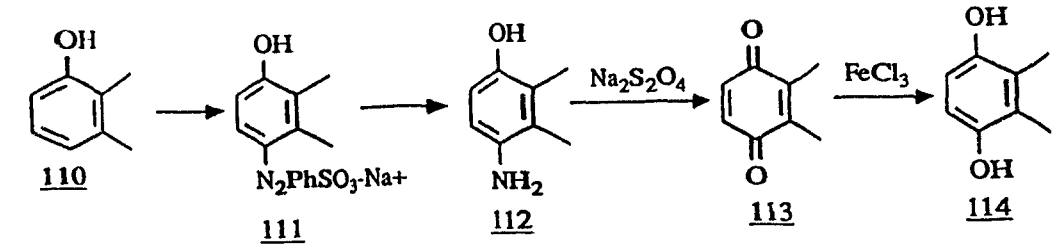
108 R=phenyl

109 R=phenyl

no reaction with 107

Fig 1.9

These results, although disconcerting, cannnot rule out photochemical o-quinodimethane generation from 2,5-dimethoxy-6-methylbenzaldehyde and therefore our synthetic



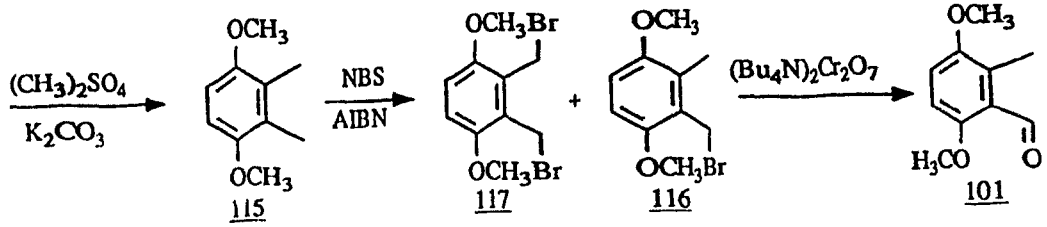


Fig 1.10

efforts were aimed towards the preparation of benzaldehyde (101) to test the likelihood of such a reaction.

Our initial attempt at the synthesis of 2,5-dimethoxy-6-methylbenzaldehyde required oxidation of bromide (116) (fig.1.10). The 2,3-dimethylquinone (113) was prepared in an overall 65% yield from 2,3-dimethylphenol (110) by using the procedure developed in our laboratory by Honek.[251] Its physical and spectral characteristics were identical to the reported ones. Quinone (113) could then be reduced to the hydroquinone (114) in 90% yield by using diethylhydroxylamine.[251] Methylation of the hydroquinone (114) was accomplished in 92% with potassium carbonate and dimethyl sulfate in acetone.[251] Both the dimethylhydroquinone (115) and the hydroquinone (114) have previously been prepared and their reported spectral data (NMR, IR, MS) compared favourably with our synthetic intermediates.

The monobromination of the dimethoxyhydroquinone (115) was accomplished under free radical conditions by using N-bromosuccinimide as the brominating agent and AIBN or irradiation by a sun lamp as a radical initiator. The main side reaction in this process was the generation of 20-30% of the known dibromo[279] derivative (117). After some experimentation, optimum reaction conditions were reached by conducting the reaction at low concentration. In this case, a 78% yield of monobrominated and 10% yield of dibrominated

compound was obtained. The monobrominated derivative was easily characterized by its ^{1}H and ^{13}C NMR, and its mass spectrum.

The direct conversion of bromide (116) into the desired benzaldehyde (101) proceeded in 83% yield when freshly prepared bis-tetrabutylammonium dichromate[280] in refluxing chloroform was used. Other methods[281,282] gave significantly lower yields. The synthetic 2,5-dimethoxy-6-methylbenzaldehyde had the expected spectral features.

The photochemical conversion of compound (101) into hydroxysulfone (103) was undertaken next (fig.1.11).

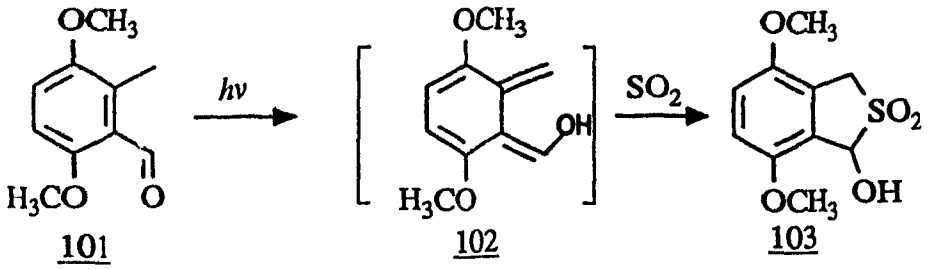


Fig 1.11

Irradiation with a standard General Electric UV sun lamp was unsuccessful. However, irradiation at 350 nm, using a Rayonet reactor, of a solution containing 0.8% of aldehyde (101) and 11% of sulfur dioxide in benzene resulted in the precipitation of the 1-hydroxysulfone (103). In its PMR spectrum, the 1,3-dihydrothiophene ring system could be identified from the doublet of doublets at 4.44ppm corresponding to the methylene protons and from the

downfield shifted methine proton signal at 5.65ppm. At 6.85ppm a doublet of doublet representative of the aromatic protons was found, and at 3.57ppm and 3.60ppm singlets corresponding to the protons from the two methoxy substituents were observed. The hydroxyl group was clearly identified as a broad absorption band between 3300$cm^{-1}$and 3620cm^{-1} in the infrared spectrum. The molecular ion in the CI mass spectrum was found at m/e 245$[M + H]^+$. The signal at m/e 227 was due to loss of H_2O from $[M + H]^+$ and the one at m/e 181 corresponded to the extrusion of SO_2 from $[M + H]^+$.

Having established the feasability of the photolysis reaction, we then tried to generate large quantities of the 1-hydroxysulfone (103) for further elaboration. Scaling up to decagram quantities however gave some serious problems. Although an overall yield of 35% of the benzaldehyde (101) was obtained when a subgram reaction scale was used, yields decreased to 10-15% primarily due to problems in the preparation of quinone (113) and in the oxidation of bromide (116). Problems such as a lenghty synthetic sequence and reduced yields prompted the investigation of a better procedure for the preparation of the required benzaldehyde.

It was reasoned that the o-methylated benzaldehyde (101) could be obtained fairly rapidly from the ortho directed lithiation and subsequent alkylation of an appropriately functionalized intermediate of 2,5-

dimethoxybenzaldehyde. A similar approach was previously exploited in the total synthesis of frustulosin (119).[283] The regio-directed metallation had been achieved by taking

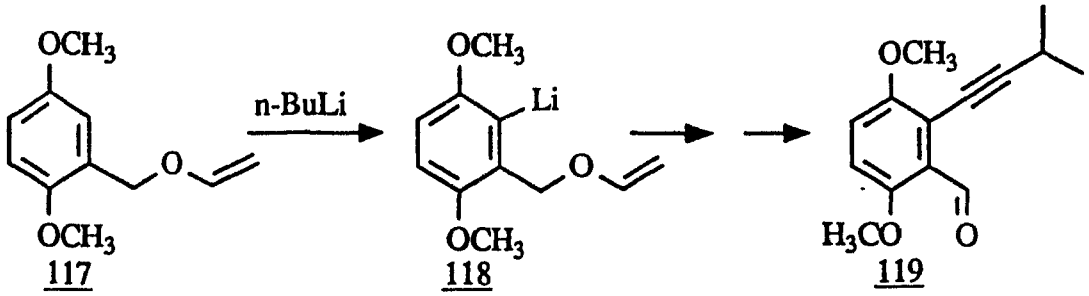


Fig 1.12

advantage of the coordinating ability of the lithium cation with the two proximal oxygens (fig.1.12).

Only the ring metallated product (118) had been obtained from treatment of vinyl ether (117) with n-butyl lithium. The use of a vinyl ether is not appealing because the reduction of benzaldehyde (120) is required. Therefore the possibilty of using the aldehydic moiety, without reducing it, for ortho metalation was investigated. The dioxolane protecting group has previously been used in the ortho directed lithiation of furanic aldehydes[284] and therefore this functional group was considered for our case.

The dioxolane acetal (121) was prepared in 96% yield by treating 2,5-dimethoxybenzaldehyde with 1,2-ethanediol in refluxing benzene overnight and with p-toluenesulfonic acid as catalyst. Its spectral characteristics were consistent with its structure. Subsequent treatment with one

equivalent of n-butyl lithium at room temperature was

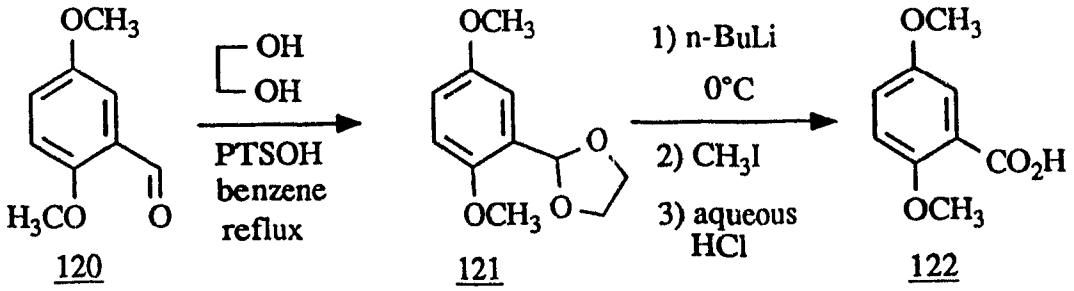


Fig 1.13

troublesome. A slow but gradual evolution of gas was noticed even at lower temperatures (0°C), and 2,5-dimethoxybenzoic acid was isolated in 20% yield from the reaction mixture. Elimination of ethylene had taken place instead of aromatic metallation.

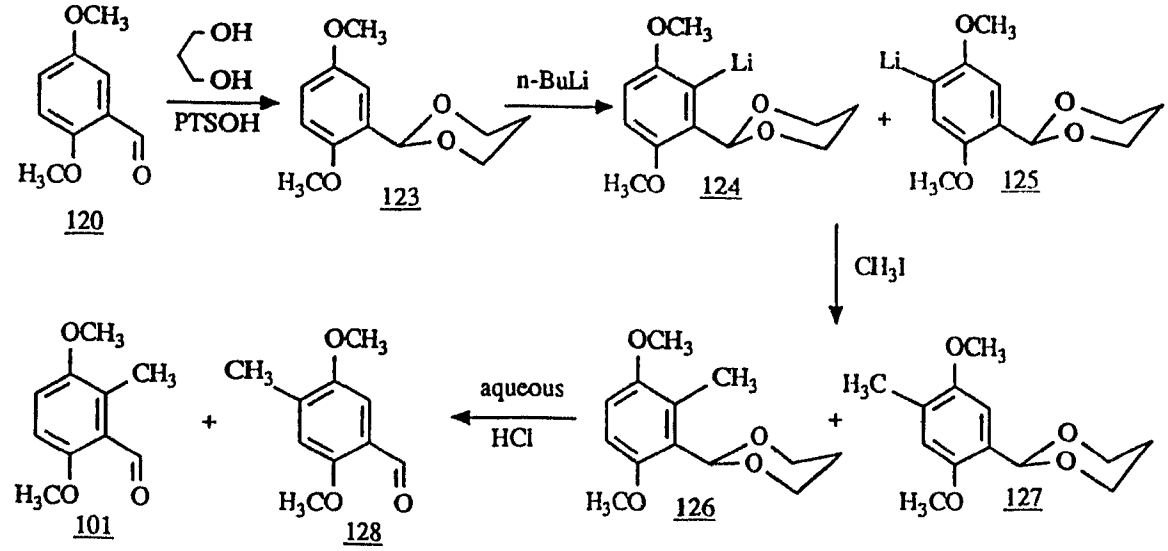


Fig 1.14

This result prompted the use of the alternative dioxane acetal (123), in which an elimination reaction is not possible. Treatment of 2,5-dimethoxybenzaldehyde with 1,3-propanediol under standard conditions gave a quantitative yield of the dioxane acetal (123). All of its spectral characteristics were consistent with its structure. Treatment of benzacetal (123) with one equivalent of n-butyl lithium at 0°C for 20 hours, followed by alkylation of the resulting lithio derivatives with methyl iodide, gave a mixture of methylated benzaldehyde dioxane acetals (126) and (127). These could be separated by flash chromatography, or treated directly as a mixture with 1N aqueous HCl in THF or in a biphasic system of 1N aqueous HCl-diethyl ether to give a 2:1 mixture of methylated (101) and (128) in an overall 40% yield. It was quite difficult to separate these two compounds, but after careful flash chromatography and recrystallization, pure regioisomers were obtained. Compound (101), prepared via the lithiation/methylation route had identical physical and spectral attributes as the previously prepared one. The methoxy benzaldehyde (128) had the expected molecular ion at m/z 180 in the mass spectrum, as well as the correct PMR and CMR spectra. The substitution pattern was determined to be as in (128). This is based on the fact that the two aromatic protons gave apparent singlets in the NMR spectrum. If these two protons would have been meta to each other, a detectable coupling

constant of circa 2Hz should have resulted, whereas the expected coupling constant of circa 0.5Hz between para hydrogens would lead to slightly broadened singlets.[285,286]

The proton at C-4 of the aromatic ring in (123) gets abstracted more easily than the one at C-3 perhaps because the latter position is more sterically hindered due to restricted rotation of the 2-methoxy group caused by a buttressing effect of the dioxane ring. This leads to the interesting observation that although C-6 is the most hindered position, proton abstraction is favoured the most at this location. Evidently the dioxane ring oxygens are reasonably good at coordinating the lithium cation and directing the proton abstraction. To confirm our rationale, the same lithiation/alkylation sequence was carried out in the presence of TMEDA which complexes the lithium cation thus diminishing coordination with the dioxane moiety. A 2:1 ratio in favor of the 4-methylated benzaldehyde was obtained. This indicates that the most kinetically acidic proton is at C-4, but that the dioxane ring oxygens are still able to compete with TMEDA for the lithium cation. In order to improve the lithiation/alkylation sequence, it was reasoned that the complexation pathway should be favoured at lower temperatures. After extensive experimentation, the best reaction condition occured when 1.8 equivalents of n-butyl lithium was added to an appropriately dilute solution of 2,5-dimethoxybenzaldehyde in ether. The temperature was

first maintained at -40°C for 15 hours, and then raised to -15°C for 24 hours before quenching with methyl iodide. This procedure resulted in a reproducible 70% yield of 2,5-dimethoxy-6-methylbenzaldehyde with 20% recovery of unreacted starting material.

The next step which necessitated improvements was the photochemical conversion of benzaldehyde (101) into the 1-hydroxysulfone (103). After some experimentation this reaction was found to depend on the concentration of the benzaldehyde. The best yield (94%) resulted when the photolysis was carried out with solutions containing 1.6% w/w of 2,5-dimethoxybenzaldehyde. The concentration dependence of certain photochemical reactions is well precedented.[287]

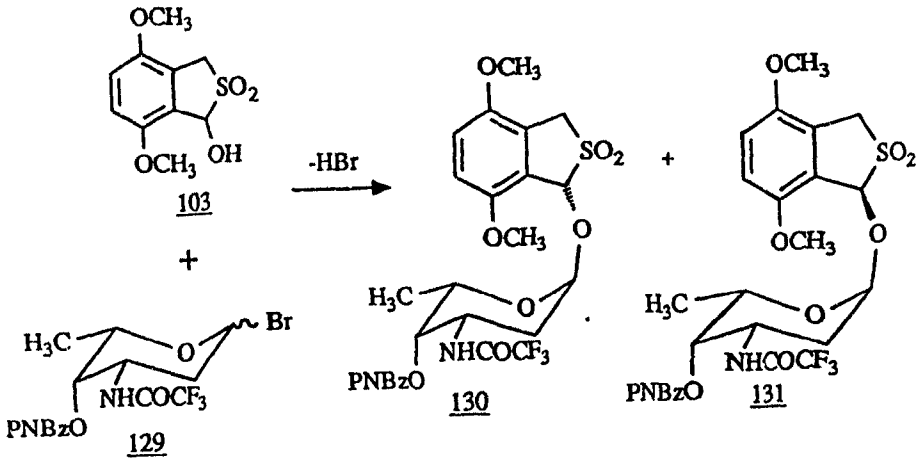
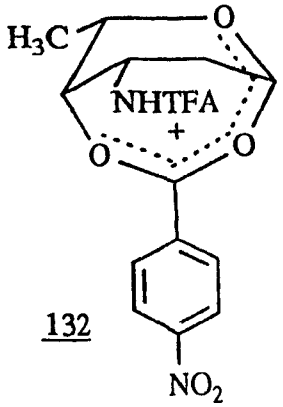


Fig 1.15

The next key step which required some investigation was the glycosidation reaction between the 1-hydroxysulfone (103) and an appropriately functionalized daunosamine moiety (129) (fig.1.15) to give the corresponding alpha glycosides (130) and (131) exclusively.

This glycosidation reaction has been extensively studied in the synthesis of natural anthracycline compounds.[253-256,288] The 4'-O protecting group is capable of sterically controlling the outcome of the glycosidation reaction because of the anchimeric assistance to the halide dispacement by the carbonyl oxygen. It was suggested that with a 4'-O-p-nitrobenzoyl protecting group the coupling reaction would proceed via a 7-membered p-nitrophenyloxonium intermediate (132) and therefore the alpha glycoside would be favoured.[253,254] With the acetyl or trifluoroacetyl group, mixtures of alpha and beta glycosides are obtained from the glycosidation reactions.[255,288] These results convinced us to use the p-nitrobenzoyl group as the protecting group for the C-4' hydroxyl.



132

The required 1,4-di-O-p-nitrobenzoyl derivative (134) of the N-trifluoroacetyldaunosamine (133) was prepared[254] in 88% yield by treating compound (133) with p-nitrobenzoyl chloride and pyridine at 0°C for 18 hours.

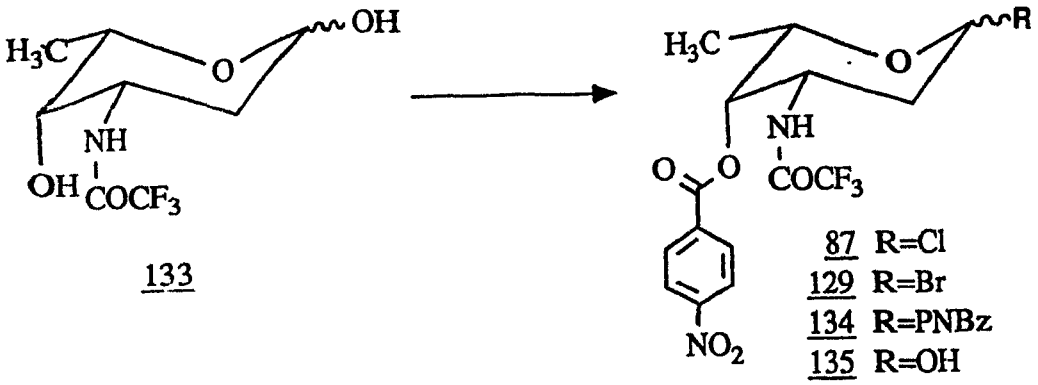


Fig 1.16

The physical and spectral properties of (134) compared well with the reported values. The bromo and chloro sugars (129) and (87) were prepared by a known methodology.[256,289]

Attempts to couple the 1-hydroxysulfone (103) to the protected sugar moiety by using either chlorodaunosamine (87) or bromodaunosamine (129) in the presence of Ag_2CO_3 and $CaSO_4$ or under Koenig-Knorr conditions failed to give the desired glycosides. Instead the protected daunosamine sugar (135) was isolated as judged from ^1H and ^{13}C NMR. Interestingly, the hydroxysulfone was not present in the organic solvent after work up of the reaction mixture. It

was recovered in low yields from the aqueous layer after acidifying and extracting with methylene chloride.

The outcome of these reactions can be explained by the fact that 1-hydroxy-1,3-dihydrothiophene-2,2-dioxides are particularly sensitive to bases.[271] Under basic conditions, they open to give the corresponding sulfinate anion (136) (fig.1.17). Once this occurs, glycosidation can no longer take place.

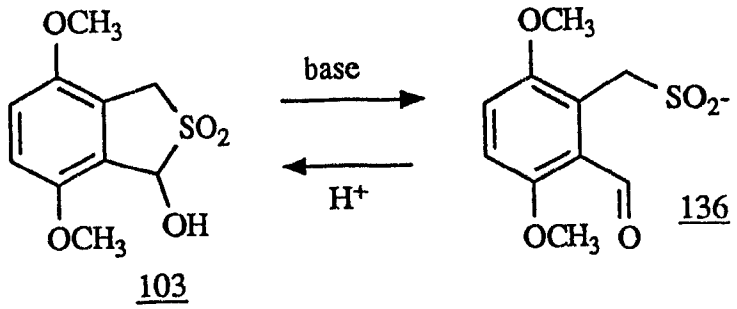


Fig 1.17

It became necessary to find reaction conditions in which the basicity of the medium was low enough to prevent complete conversion to the sulfinate anion but high enough to trap the hydrogen halide produced during the course of the reaction. In order to test various reaction conditions, we chose not to use the precious halodaunosamine derivatives but to employ freshly prepared acetobromoglucose (137)[290] as an inexpensive model.

The coupling reactions were tried at temperatures ranging from 0°C to reflux in CH_2Cl_2, 1,2-dichloroethane or THF as solvent, with $AgClO_4$, $AgBF_4$, $AgCF_3SO_3$ or $HgBr_2$, as

coupling reagent and with or without sodium bicarbonate or imidazole as acid traps. All of these methods failed to give any sugar coupled products such as (138) and invariably gave

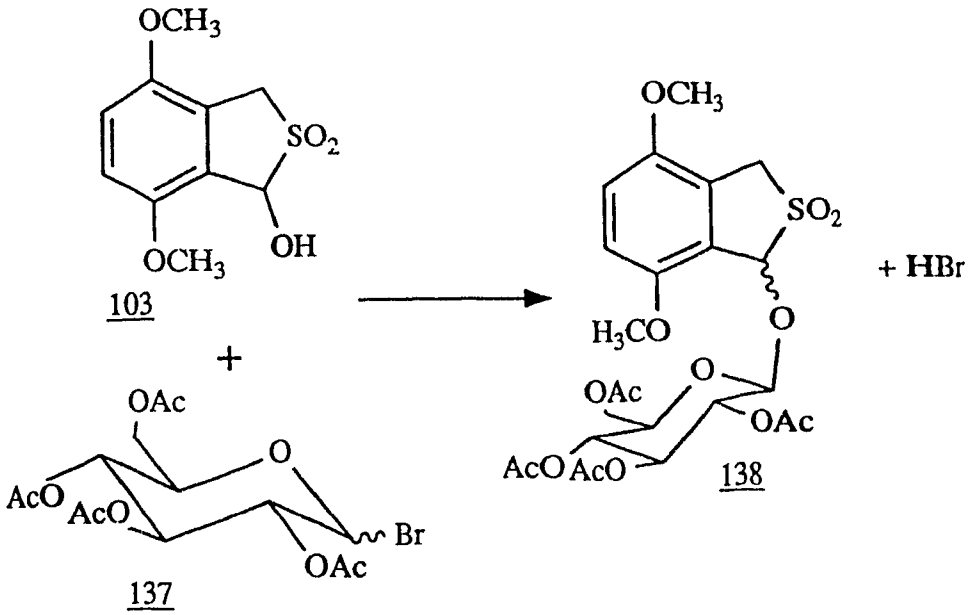


Fig 1.18

back the 1-hydroxysulfone together with several decomposition products. To test the sensitivity of the hydroxyl functionality to bases, a solution of sulfone (103) in CD_2Cl_2 was exposed to imidazole. The PMR spectrum was consistent with the complete disappearance of the sulfone, thus indicating that the hydroxyl proton in sulfone (103) is quite acidic.

Any attempts to acetylate sulfone (103) with acetic anhydride or acetyl chloride in the presence of pyridine or DMAP as catalysts failed to give the corresponding acetate (139). In the absence of these bases, and with trace amount

of sulfuric acid for catalysis, a near quantitative yield of (139) was obtained.

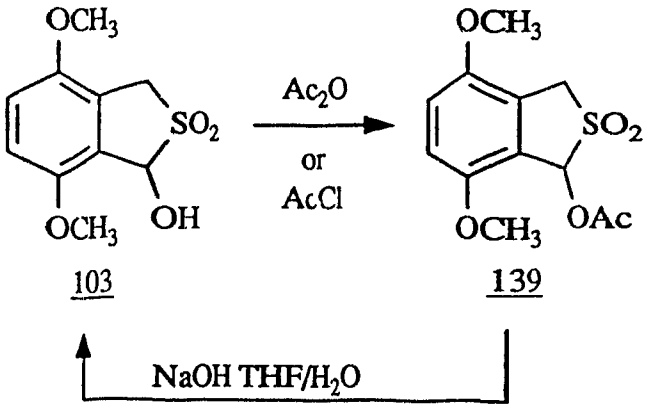
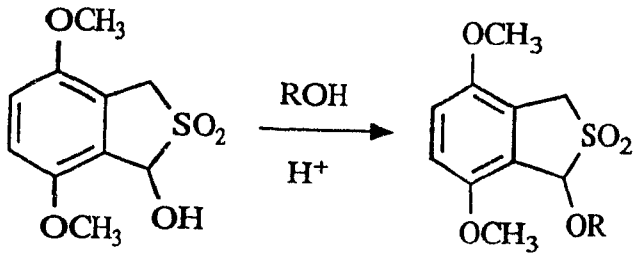


Fig 1.19

The failure to obtain the acetate of other 1-hydroxy-1,3-dihydrothiophene-2,2-dioxides in the presence of bases was later reported by Charlton and Durst.[271] The 1-acetoxysulfone (139) prepared as described above had the expected carbonyl absorption band at 1758cm^{-1} in the infrared spectrum, a singlet at 2.23ppm in the PMR spectrum and the expected ^{13}C signal at 169.09ppm for the carbonyl carbon in the ^{13}C NMR spectrum. The mass spectrum provided further support for the structure (139). A weak molecular ion at m/z 286 could be detected along with major fragmentations at m/z 222 for [$M^{+}\cdot - SO_2$]. The deacetylation of (139) was carried out at room temperature in 10 minutes using 0.1N NaOH or KOH in a 2:1 THF-H_2O solvent system. Reproducible and high yields (90%+) of 1-hydroxysulfone (103) were obtained this way.

During the course of our investigation, we found that the 1-hydroxysulfone (103) could be coupled with various alcohols to give the corresponding 1-alkoxysulfones (140) to (143) by catalyzing the reaction with trace amounts of HCl or p-toluenesulfonic acid. All these derivatives gave the expected ^{1}H, ^{13}C, infrared and mass spectra.



103

140 R=CH_3 90%
141 R=CH_2CH_3 77%
142 R=$CH(CH_3)_2$ 74%
143 R=Menthyl 67%

Fig 1.20

This finding incited us to attempt the coupling reaction between hydroxysulfone (103) and sugars in aprotic media. Unfortunately, all attempts to couple compound (103) with daunosamine derivative (135), and glucose or thioglucose tetraacetate used as model compounds, failed completely. Some trial reaction conditions made use of 1 to 5 equivalents of sugar in the presence of acidic catalysts such as p-toluenesulfonic acid, camphorsulfonic acid, trifluoroacetic acid or trifluoromethanesulfonic acid, in methylene chloride or THF at room temperature or at reflux

with molecular sieves to trap water. In all these attempts only starting materials were recovered even after reaction times as long as 35 days at room temperature.

A then recent approach, which made use of Lewis acid catalysts such as $SnCl_4$[291] or trimethylsilyl trifluoromethanesulfonate[292,293] in the glycosidation reactions between N-trifluoroacetyl-1,4-di-o-p-nitrobenzyl-L-daunosamine (134) and various anthracycline aglycones was found to be successful in our case. Thus, the reaction between the 1-hydroxysulfone (103) and protected daunosamine (134) was done by using a modified procedure of Terashima et al[292] and lead to the desired 1-daunosaminylsulfone

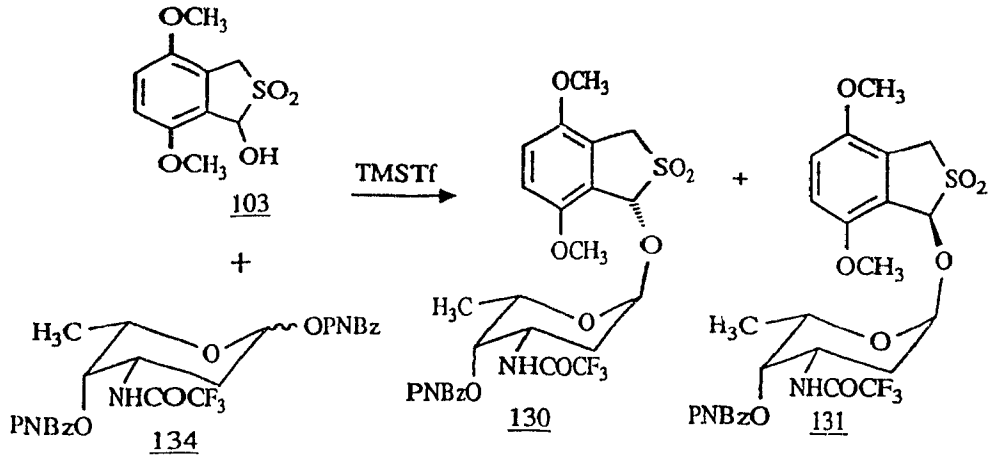


Fig 1.21

glycosides (130) and (131) in an overall 89% yield after separation by flash chromatography of the 1:1 diastereomeric mixture.

Structure (130) was arbitrarily assigned to the less polar compound while (131) was considered the more polar one. Both glycosides gave spectral data as expected. Molecular ions for compounds (130) or (131) could not be detected from the EI, CI, or positive ion FAB mass spectra but the negative molecular ion, ($M^{-}\cdot$), was observed at m/z 618.6 and m/z 618.5, respectively, in the negative ion FAB mass spectra. In the infrared spectrum, the carbonyl stretching frequency of the p-nitrobenzoyl and amide groups could not be distinguished, in either isomer, because of overlap giving a broadened signal at 1736cm^{-1}. Further structural confirmation was obtained from their ^{1}H and ^{13}C NMR spectra. Proof of the presence of the 3'-trifluoroacetamide functionality was obtained from the carbon spectra. The characteristic ^{13}C signal for CF_3 and C=O of the amide occurred at 115.33ppm with J_{CF} = 286.5Hz and at 156.83ppm with J-CCF = 36Hz, respectively, for the glycoside (130). Similar observations were made for compound (131). Thus the ^{13}C signals for the CF_3, and amide carbonyl carbon occurred at 115.44ppm with J_{CF} = 282.8Hz and at 156.79ppm with J_{CF} = 36.3Hz.

The ^{1}H NMR spectra established that the two products obtained from the glycosidation were the desired alpha glycosides. In beta glycosides of anthracycline compounds, conformer C (fig.1.22) is favoured. Therefore the 1' anomeric proton gives rise to a double doublet with J ca 10

and 2Hz due to coupling with $H_{2'a}$ and $H_{2'e}$. A narrow multiplet with a width of ca. 6Hz is obtained for the H1' proton in alpha glycosides due to the absence of a large coupling constant between $H_{1'e}$ and $H_{2'}$ protons in the favoured conformer A in fig.1.22.

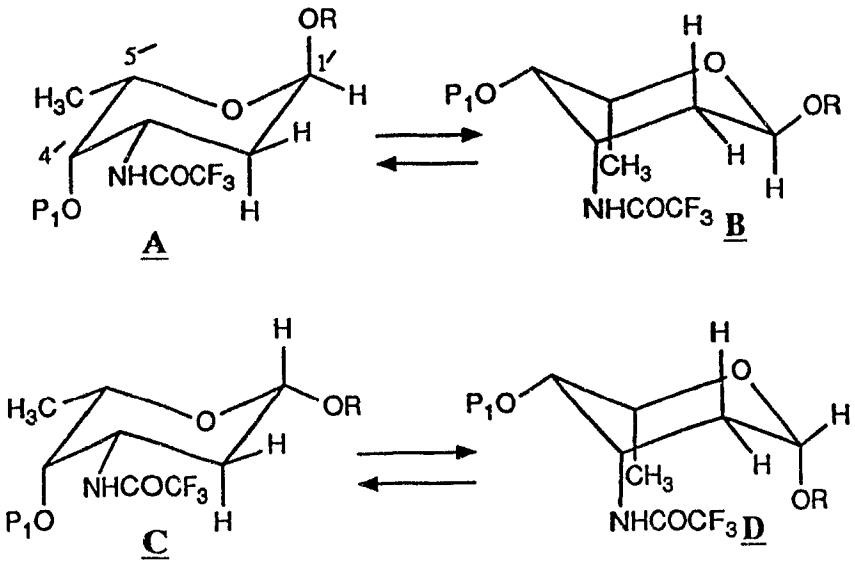
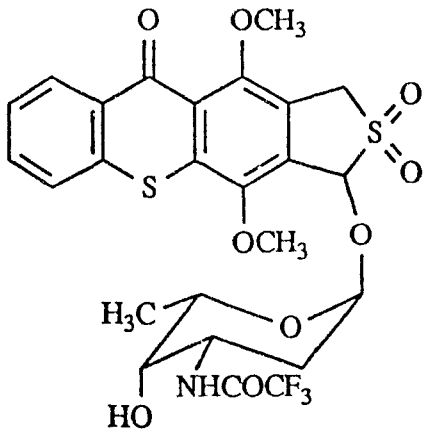


Fig 1.22

The PMR results obtained for glycosides (130) and (131) agree with these observations. Thus for compounds (130) and (131) the $H_{1'}$ anomeric proton signal occurred as a broad singlet at 5.47ppm (W_H = 7Hz) and at 5.55ppm (W_H =7Hz), respectively.

The total synthesis of tetracyclic thioxanthone glycosides like (144) was undertaken next.



144

1.1.2 Studies For The Total Synthesis Of Thioxanthone Analogs Of 4-Demethoxydaunorubicin.

The preparation of derivatives with a thioxanthone moiety was envisaged as previously discussed from three general approaches exemplified in fig.1.23. The first would require the successful trapping by SO_2 of the unprecedented thioxanthyl-o-quinodimethide (146) to give the desired 7-hydroxythioxanthosulfone (147). The second and third procedures would give the required aglycones from the 7-protected intermediates depicted as (150) and (152).

It was anticipated that the thioxanthonaldehyde (145) could be prepared fairly rapidly by taking advantage of the previously developed procedure[251] to make 6,9-dimethoxy-7,8-dimethylthioxanthone (155). Monobromination of (155) and oxidation of thioxanthonebromide (157) by using

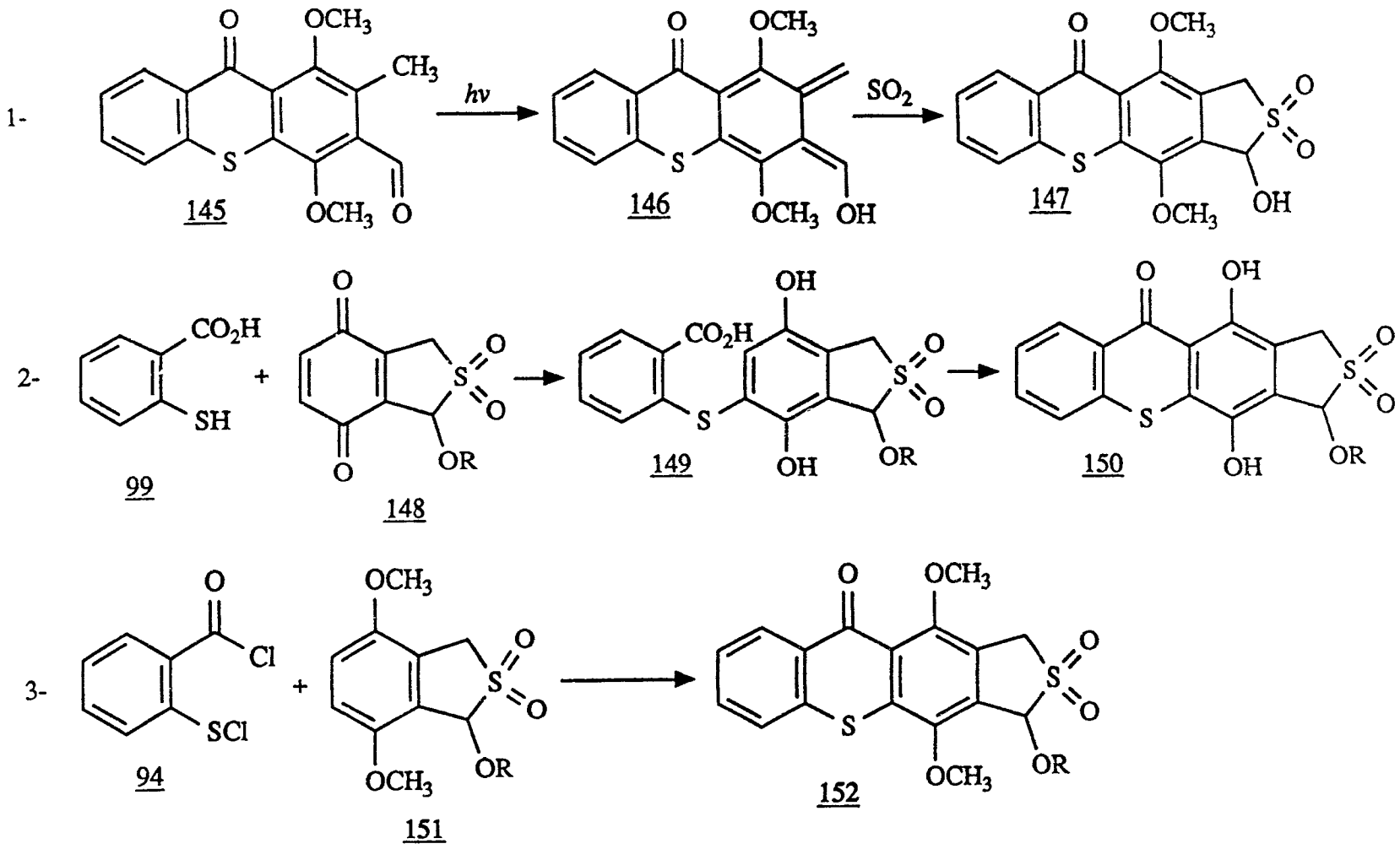

1-
O
OCH3
CH3
S
OCH3
O
145
hv
146
SO2
147
OH
2-
CO2H
SH
99
+
148
OR
149
150
3-
Cl
SCl
94
151
152

Fig 1.23

previously tested methodology should provide the formylthioxanthone (158).

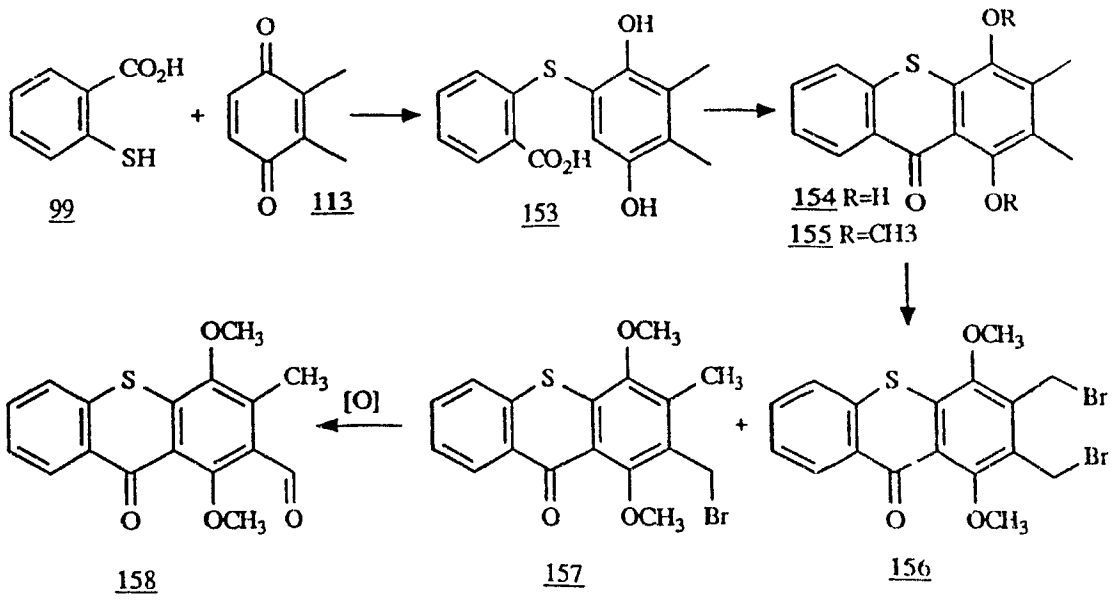


Fig 1.24

Following our analogy, dimethylquinone (113) was reacted with o-mercaptobenzoic acid to give acid (153) which was directly cyclized to compound (154) by using a 2:1 mixture of trifluoroacetic acid and trifluoroacetic anhydride at room temperature for 20 hours. The red thioxanthone (154), obtained in 60% yield from (113), had identical physical and spectral characteristics as reported.[251] Methylation of (154) with CH_3I and NaH gave (155). Monobromination was effected cleanly to give the monobrominated thioxanthone (157) in 60% yield and a small amount (<5%) of the ortho-dibromomethylthioxanthone (156).

Treatment of bromothioxanthone (157) with bis(tetrabutylammonium)dichromate[280] in refluxing chloroform for 24 hours gave in 71% yield formylthioxanthone (158). This compound gave a molecular ion at m/z 314. The two carbonyl absorption bands at 1646 and 1682cm^{-1} were attributed to the thioxanthone and aldehydic moieties, respectively. Both the ^{1}H and ^{13}C NMR spectra showed all the expected resonance signals.

The next step required the photolysis of aldehyde (158) in concentrated (10-13% w/w) benzene solutions of SO_2. In the UV spectrum the n-π^* transition for the aldehydic function occurred at 333nm in ethanol. It is expected that in benzene the prerequisite n-π^* transition would occur quite close to 350nm. Unfortunately, irradiation of solutions containing 0.5 to 2.0% W/W of substrate in a Rayonet photochemical reactor or with the more powerful medium pressure mercury immersion lamp, in the presence or absence of photochemical sensitizers, failed to give the desired 7-hydroxythioxanthonesulfone intermediate (147). The thioxanthone aldehyde (155) remained unchanged even after reaction times as long as 48 hours.

Rapid intramolecular quenching of the aldehydic triplet state by the thioxanthone carbonyl functionality provides an explanation for the observed lack of reactivity.[287] The photochemical reaction could have a better chance of success if the thioxantone carbonyl was

absent. Therefore, we set forth to synthesize the deoxythioxanthenealdehyde (161) in order to test our idea.

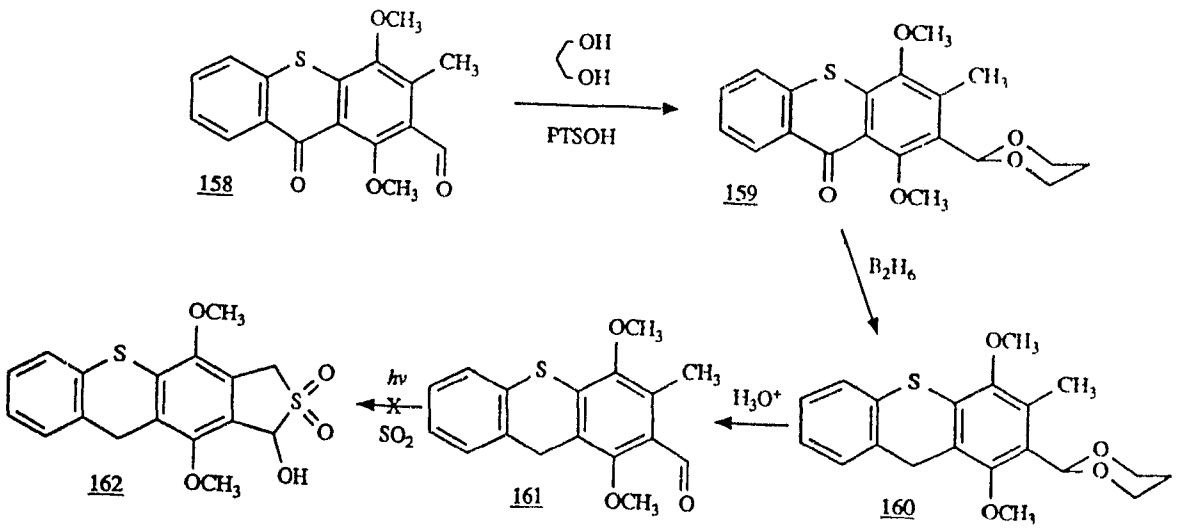


Fig 1.25

The thioxanthonealdehydedioxane acetal was prepared in 98% yield by treating aldehyde (158) with 1,3-propane diol under standard conditions. Acetalation was confirmed from the disappearance of the aldehydic stretching frequency in the infrared spectrum, and the detection of the dioxane methylene groups in the ^{1}H and ^{13}C spectra. An expected molecular ion at m/z 372.44 was also observed.

Reduction of compound (159) was carried out with borane-THF[294] at 0°C for 6 hours. The resulting reaction mixture was worked up and the residue was treated directly with 1N. HCl in an aqueous/ether biphasic system. The sensitive thioxanthenealdehyde (161) was obtained in a 40%

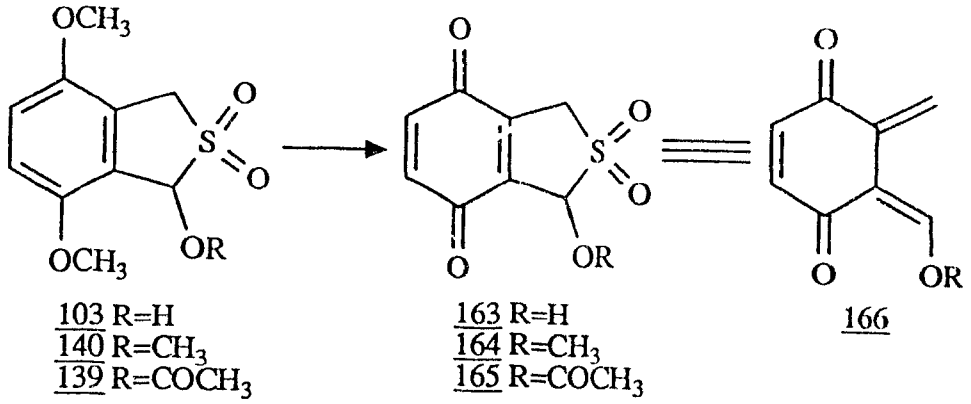
overall yield from the thioxanthonealdehyde (158). Up to 50% of compound (158) could be recovered.

The infrared spectrum of the synthetic thioxanthenealdehyde (161) showed only one carbonyl stretching band at 1682cm^{-1} attributed to the aldehyde. The characteristic carbonyl absorption band at 1645cm^{-1} for thioxanthones was absent. The ^{1}H and ^{13}C NMR spectra confirmed the existence of the new thioxanthene methylene as well as the presence of the other aryl and alkyl functionalities.

Irradiation of the thioxanthenealdehyde (161) at 350nm in benzene this time led to complete reaction within 3 hours, thus support. ng our suggestion that the thioxanthone carbonyl interfered in the photochemical reaction. Unfortunately, this attempt did not lead to the desired 1-hydroxythioxanthenesulfone (162). The PMR spectrum of the resulting polar products showed a large amount of unexplained broad peaks mainly located between 3.3ppm and 5.5ppm, and also between 6.3 and 7.7ppm. These may result from polymerized material. Further investigations of this synthetic approach was abandoned.

An alternative approach, for the thioxanthone tetracyclic aglycones, requires the preparation of quinones having structures such as (163), (164) and (165).

Their synthesis is particularly interesting because these quinones are really representative of synthon (166)



103 R=H
140 R=CH_3
139 R=$COCH_3$

163 R=H
164 R=CH_3
165 R=$COCH_3$

166

Fig 1.26

which is in effect a diene and a dienophile. By masking the diene, the quinone portion could be used as a dienophile at first, while extrusion of SO_2 should ultimately give the diene which may react with a variety of dienophiles.

The preparation of quinonesulfones (163) to (165) was envisaged from the oxidative demethylation of the corresponding dimethyl ethers (103), (140), and (139). Preliminary reactions were carried out on the unprotected 1-hydroxysulfone (103) so as to investigate the necessity of a protecting group. Thus, the reaction of 1-hydroxysulfone with AgO, according to the Rappoport procedure or its modifications,[295-298] with the presence of nitric acid in solvents such as acetone, THF or dioxane at room temperature or at 50°C, led to no reaction whatsoever. The starting material could be recovered in 90 to 95% yield even after a reaction time of 2 hours. When the same reaction conditions were applied to 1,4-dimethoxy-2,3-dimethylbenzene, the

dimethylquinone was obtained in yields ranging from 90 to 95% after a ten minute reaction time.

Evidently there is some considerable resistance towards oxidative demethylation of the 1-hydroxysulfone (103). The use of 2,4,6-pyridinetricarboxylic acid or pyridine-2,6-dicarboxylic acid N-oxide as catalyst, when AgO fails to oxidise the substrate, has been proposed,[299] but this did not improve the oxidative demethylation of 1-hydroxysulfone (103). No reaction was either observed when the reportedly superior oxidant, ceric ammonium nitrate,[298,300,301] was employed or when nitric acid impregnated manganese dioxide[302,303] was used. All of the above methodologies gave high yields of quinone when applied for the oxidative demethylation of 1,4-dimethoxy-2,3-dimethylbenzene.

Attempts to oxidatively demethylate the 1-methoxysulfone (140) and 1-acetoxysulfone (139) by using argentic oxide, ceric ammonium nitrate or nitric acid impregnated manganese dioxide also failed to give the desired products and starting material was invariably recovered. When fifty equivalents of the HNO_3-MnO_2 reagent was used with 1-acetoxysulfone (139) on a 25mg scale, a series of minor products were observed along with the starting material. Any attempt to increase the reaction scale in order to characterize the newly formed compounds resulted in loss of reactivity.

To ascertain that the sulfone moiety was responsible for the lack of reactivity, the unsubstituted benzofused sulfone (168) was prepared from the 1-hydroxysulfone (103) in 78% overall yield.

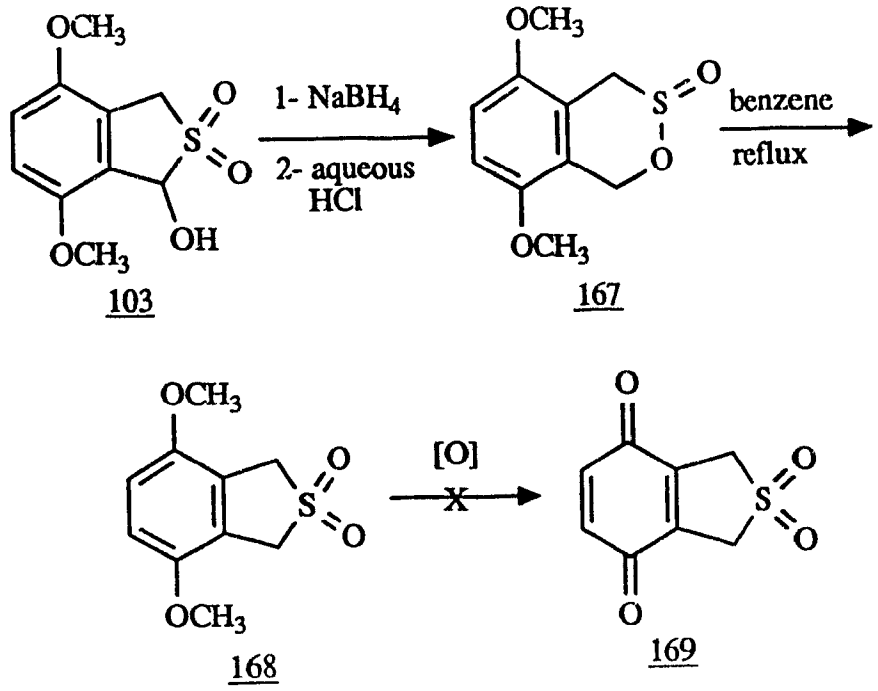


Fig 1.27

Sodium borohydride reduction followed by an acidic workup gave sultine (167) which was characterized by ^{1}H, ^{13}C, mass and infrared spectra. The chemical ionization mass spectrum showed the $[M + {}^{+}NH_4]$ signal at m/z 246 as expected. Loss of SO_2 from the molecular ion adduct was observed at m/z 182 and the major peak at m/z 165 was representative of $[(M + {}^{+}H)-SO_2]$. Proton NMR confirmed the presence of the sulfinate cyclic system. Resonance signals for the methylene protons alpha to the sulfur-oxygen double

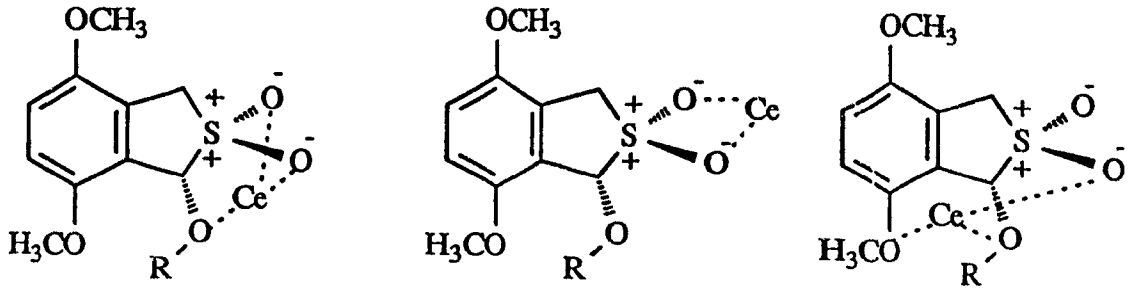
bond were observed at 3.63 and 4.13ppm as doublets while the second methylene protons were found downfield at 5.14ppm as a double doublet.

The sulfone (168) was obtained by refluxing a benzene solution of the sultine (167) for 3 hours. The PMR spectrum clearly showed the symmetry of (168) with resonance signals at 3.81, 4.31 and 6.80ppm corresponding to the protons of the two methoxy, the methylene and aromatic moieties, respectively. The expected molecular ion was detected in the CI mass spectrum at m/z 246 as an adduct with ammonia. The ^{13}C and infrared spectra were also consistent with the structure.

Oxidative demethylation reactions carried out on sulfone (168) with ceric ammonium nitrate failed to give the desired quinosulfone (169). This time in addition to unreacted material, a complex reaction mixture was obtained. Any attempt to separate the products by flash chromatography led to complications due to further decomposition. At least twelve signals in the region of 3.6 to 3.9ppm corresponding to methoxyl protons were observed in the PMR of the crude reaction mixture. This would indicate that the methoxy groups were still resistant towards oxidative demethylation.

Electron withdrawing substituents such as chlorine may prevent oxidative demethylation reactions,[303] but in our case the sulfone is located beta to the aromatic center and therefore its electron withdrawing effect would be non-

conjugative. The reaction should have proceeded to some degree at least in some of the more forcing conditions that were tried. A more profound phenomenon must cause the dimethoxybenzosulfone resistance towards oxidative demethylation. An explanation is somewhat speculative but the likelihood that Ce(IV) or other metallic cations complexes with the 1-oxysulfone may explain the observed resistance.



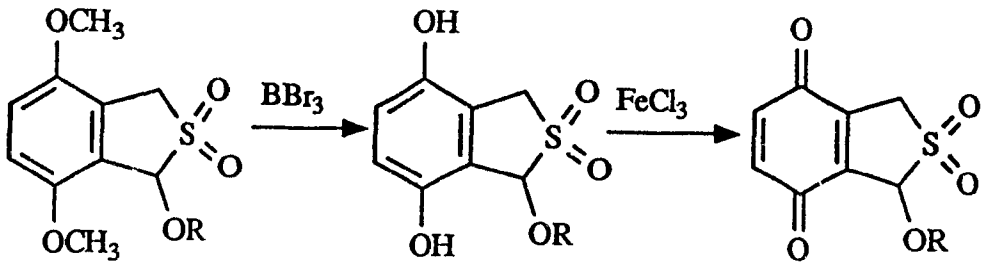
170 171 172

Fig 1.28

Electron withdrawing complexation such as in (170), (171) or (172) could prevent the polarizability of the aromatic electron cloud towards a second cerium cation.

An alternative approach for the preparation of the quinones would be to first remove the methyl protecting groups and then to convert the more easily oxidisable hydroquinone (178) into the quinone (179) (fig.1.29).

Reactions of the 1-hydroxysulfone (103) or the 1-acetoxysulfone (139) could be observed when 5 equivalents of



178 179

Fig 1.29

BBr_3[299] was used at room temperature. Very complex reaction mixtures were obtained and the analysis of every single product was impractical. By following these reactions by NMR it was established that there was demethylation but that the integrity of the dihydrothiophene-2,2-dioxide ring system was lost. It is conceivable that the boron tribromide complexes with the electron-rich oxygen in the sulfur-oxygen double bond.

This polarization could lead to ring opening. An example is shown in fig.1.30. The great number of products formed in this approach would arise because of lack of selectivity of the demethylating agent. As an alternative, BI_3[304] has been proposed when selectivity is a problem but in our case there was no significant improvement from its use. Complex reaction mixtures were also obtained when trimethylsilyl iodide[305] was employed.

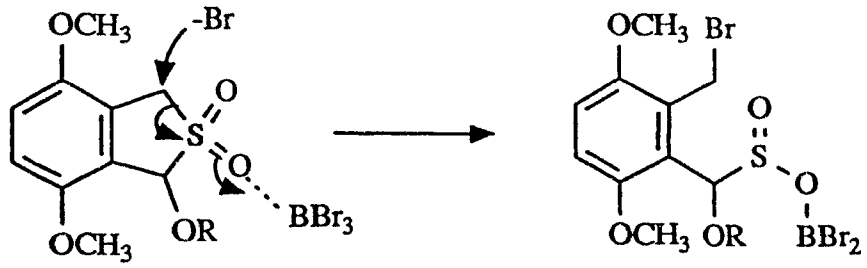
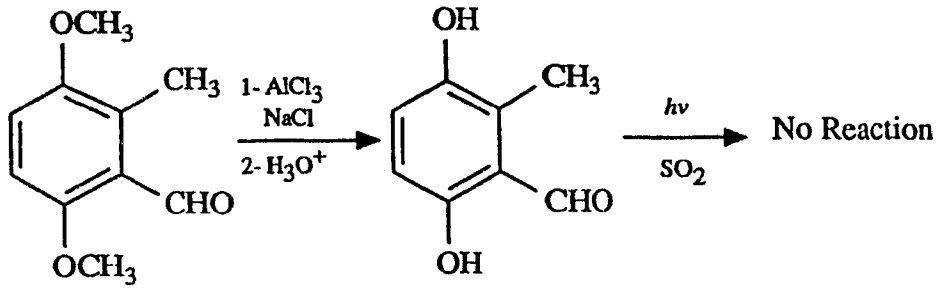


Fig 1.30

It was next decided to regress to the 2,5-dimethoxy-6-methylbenzaldehyde and to remove the methyl groups before



101 180

Fig 1.31

the photochemical reaction. Thus, the hydroquinone (180) was obtained in 82% yield by treating the dimethylether (101) with an $AlCl_3$-NaCl melt at 170°C for five minutes. The ^1H, ^{13}C, infrared, and mass spectra were consistant with structure (180).

Irradiation of a stirred suspension of 2,5-dihydroxy-6-methylbenzaldehyde in a SO_2-benzene solution failed to give any product. This was probably due to insufficient solubility of the aldehyde. The hydroxyl

groups were therefore derivatised into t-butyldimethylsilyl ethers by reacting hydroquinone (180) with t-butyldimethylsilyl chloride in DMF and imidazole as catalyst.[306] Compound (181), obtained in 85% yield, gave the expected spectral results.

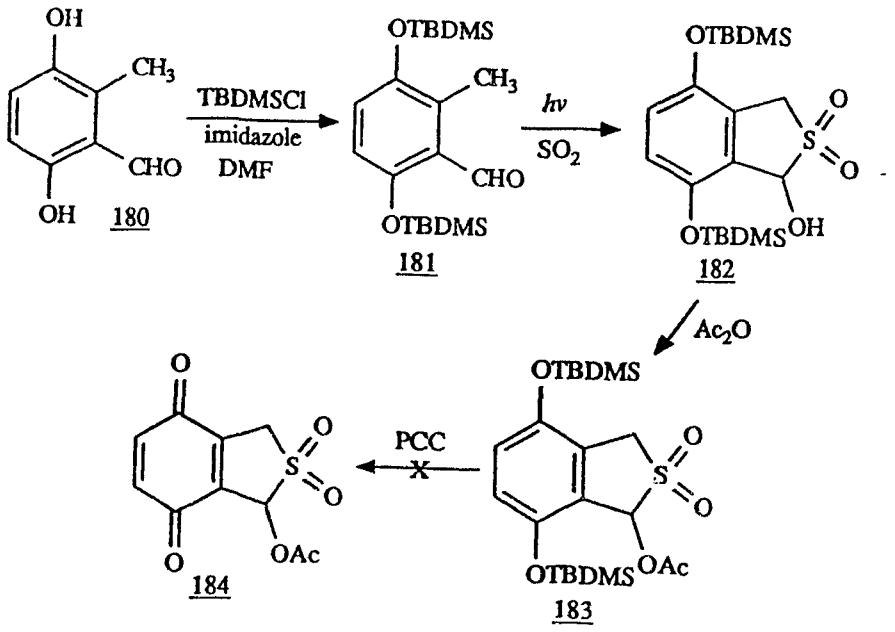
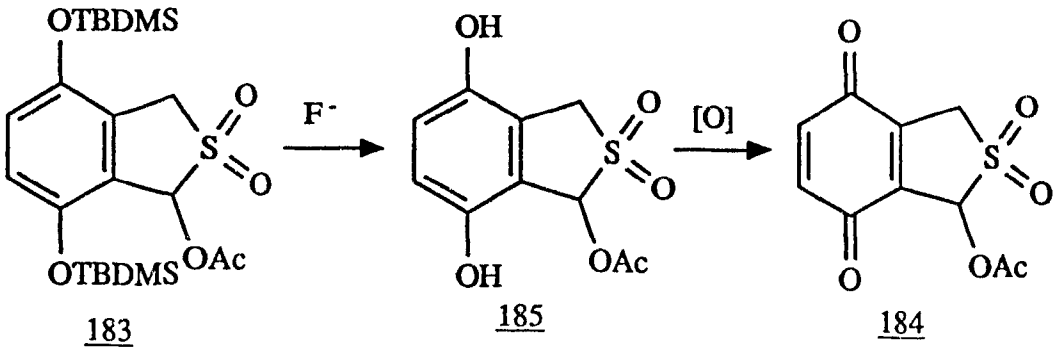


Fig 1.32

Irradiation of the silylated benzaldehyde (181) in a SO_2-benzene solution led to the formation of the unstable 1-hydroxysulfone (182) which had the correct ^1H and ^{13}C NMR spectra. This product was best isolated as the more stable acetoxy derivative (183).

An overall 68% yield of (183) from (181) could be achieved with careful handling of the photochemical product. The 1-acetoxysulfone (183) had the expected PMR and CMR spectra.

The oxidation of hydroquinone silyl ethers has previously been achieved by using pyridinium chlorochromate[307] but failed when electron withdrawing groups were present on the aromatic ring. Attempts to prepare quinone (184) by direct oxidation of (183) with PCC led to no reaction probably because of the electron withdrawing properties of the acetoxysulfone moiety.



183 185 184

Fig 1.33

The sequential preparation of (184) was envisaged next by first removing the t-butyl-dimethylsilyl protecting groups and then conducting the oxidation on the resulting hydroquinone (fig.1.33). The use of tetrabutylammonium fluoride[306] in aprotic media led to decomposition products. Hydrofluoric acid has been proposed as a complementary reagent for the removal of t-butyldimethylsilyl groups.[309-310] After extensive experimental trials it was found that the use of a buffered (pH=4.6) aqueous solution of HF and KF with added CsF resulted in the complete consumption of the

starting hydroquinone silyl ether after a reaction time of two weeks. Among the polar decomposition products one compound could be isolated

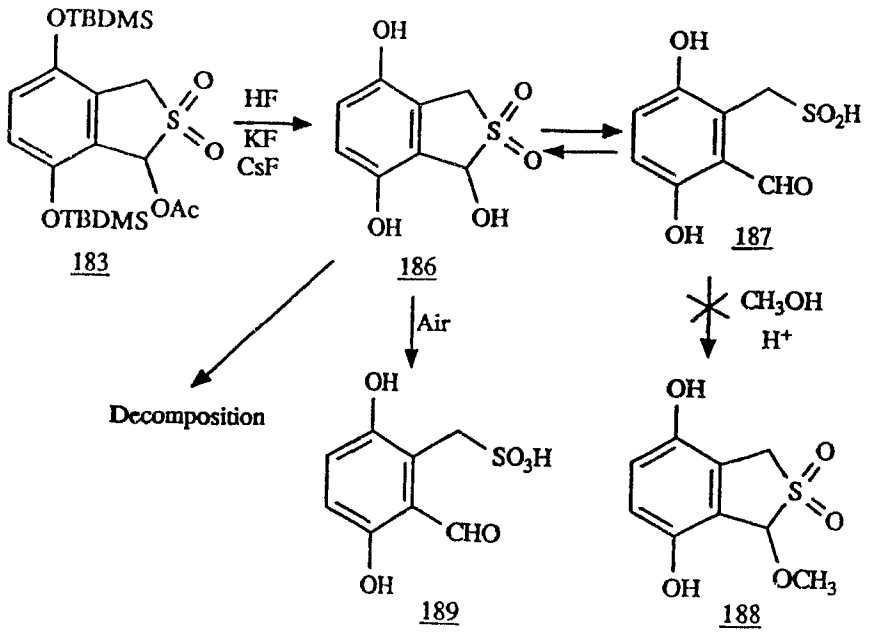


Fig 1.34

(10% yield) in reasonable purity. Its pmr spectrum showed resonances for the phenolic protons at 11.04 and 9.78ppm. The double doublets at 7.03ppm was assignable to the aromatic protons. An aldehydic proton could also be detected at 10.20ppm. The singlet at 5.00ppm corresponded to two protons of a freely rotating methylene. Undoubtedly, desilylation and deacetylation occurred as well as ring opening to give the aldehyde.

The sulfinic acid (187) most likely became oxidized to the sulfonic acid (189). Methylation of the desilylation

reaction products did not yield any 1-methoxysulfone (188) derivative. Sulfonic acid (189) is therefore tentatively assigned as the minor reaction product. Due to the sensitive nature of compound (186), extensive decompostion was inevitable during the course of this long reaction. In consideration of all the difficulties associated with the generation of the quinosulfones of interest, an alternative synthetic sequence for the preparation of the desired thioxanthosulfone glycosides was considered.

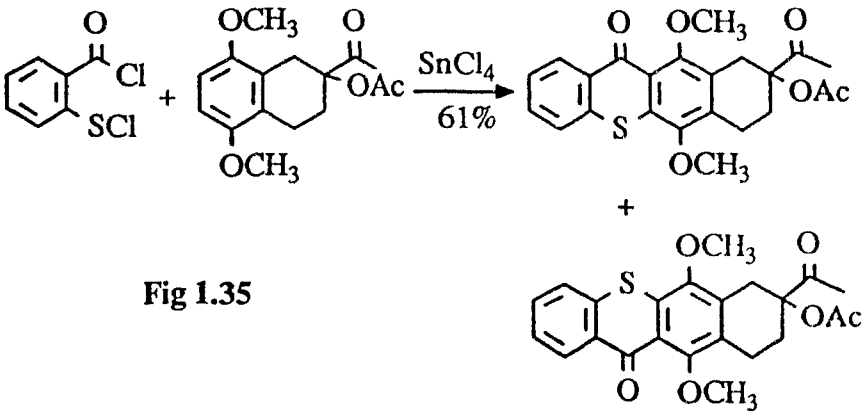


Fig 1.35

Thioxanthones have previously been prepared in our laboratory by carrying out a Friedel-Crafts reaction between o-chlorosulfenylbenzoyl chloride and substituted arenes, such as in fig.1.35.[251] Conceivably, the same reaction could occur with the protected sulfones (fig.1.36) to give the corresponding 1,3-dihydrothioxantho(b)thiophene-2,2-dioxide.

The Friedel-Crafts reaction with 1-isopropoxysulfone (142) and freshly prepared o-chlorosulfenylbenzoyl chloride was not fruitful and led to the formation of the

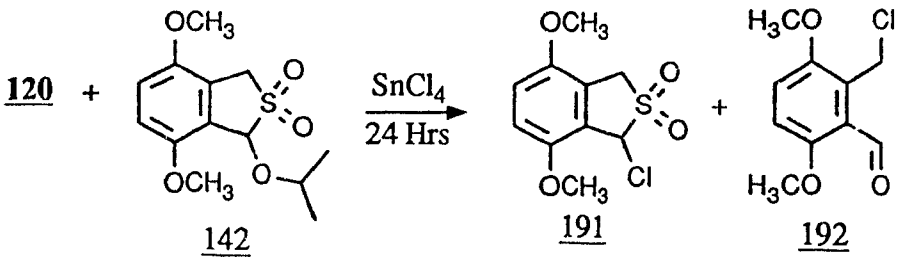


Fig 1.36

chlorosulfone (191) and 2,5-dimethoxy-6-chloromethylbenzaldehyde (192) among other intractable material. Compound (191) gave molecular ions in the mass spectrum at m/z 262 and m/z 264 , with the main fragmentation being loss of SO_2 from the molecular ion. The PMR spectrum was consistent with structure (191) and showed the methine proton at 5.85ppm with the methylene ones at 4.37ppm as a double doublet. Further structural support was provided from the ^{13}C NMR spectrum which showed all the carbon resonances at their expected location. Compound (192) gave a PMR spectrum showing the methylene protons as a sharp singlet at 5.2ppm and the aldehydic proton at 10.68ppm. The carbon NMR spectrum showed the presence of the ten carbons with the aldehydic carbon giving a signal at 191.85ppm, and the chloromethylene carbon at 35.95ppm as expected. These products can be explained if the $SnCl_4$ catalyst coordinates with the dihydrothiophene-2,2-dioxide side of the molecule (fig.1.37). Nucleophilic displacement

with chloride at postion 1 or 3 will ultimately give the observed products.

It was reasoned that a change of protecting group from alkyl to acetoxy may provide a better chance of success. Complexation in this case would occur at the acetoxy carbonyl in competition with the sulfone oxygens (fig.1.38). Since the coordinated site is displaced by one

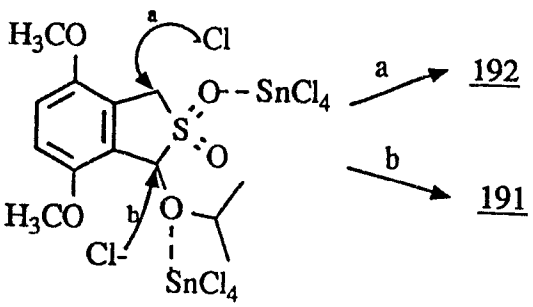


Fig 1.37

atom, it is likely that the aromatic ring is not as deactivated towards electrophilic addition as with the isopropoxy substituent. Chlorination could still take place but the resulting chloride is recyclable to the 1-hydroxysulfone with KOH in THF-H_2O in 87% yield.

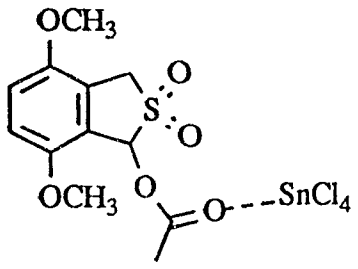


fig 1.38

The reaction between o-chlorosulfenylbenzoyl chloride and the 1-acetoxysulfone (139), carried out with $SnCl_4$ as catalyst in CH_2Cl_2 at room temperature, gave a complex reaction mixture. Some of the products which were isolated and characterized are shown in fig.1.39. Compounds (193) and (194) could not be separated. Separation of (195) from (196) was also impossible. All of compounds (191) to (196) gave satisfactory spectral results. Although low yields were obtained, the Friedel-Crafts reaction gave the desired compound (198). Compound (197) is also useful because it can be converted to the aglycone via alkaline hydrolysis. These two thioxanthones gave satisfactory spectral data. More importantly, the thioxanthone moiety was identified by the infrared spectra from the carbonyl absorption band at 1648 and 1645cm^{-1} for (197) and (198) respectively. The mass spectrum gave the expected molecular ion at m/z 420 for (198) while the thioxanthone chloride (197) was observed at m/z 396 and 398. The pmr spectrum of thioxanthone (198) displayed resonance signals for all the protons. Notably, the four new aromatic protons were located at 7.55 and 7.63ppm as multiplets and 8.48ppm as a broad doublet. The dihydrothiophene-2,2-dioxide could be identified from the doublet of doublets at 4.47ppm for the methylene protons and the singlet at 6.83ppm for the other benzylic proton. The acetyl moiety was apparent from the singlet at 2.30ppm for the methyl group. The ^{13}C spectrum

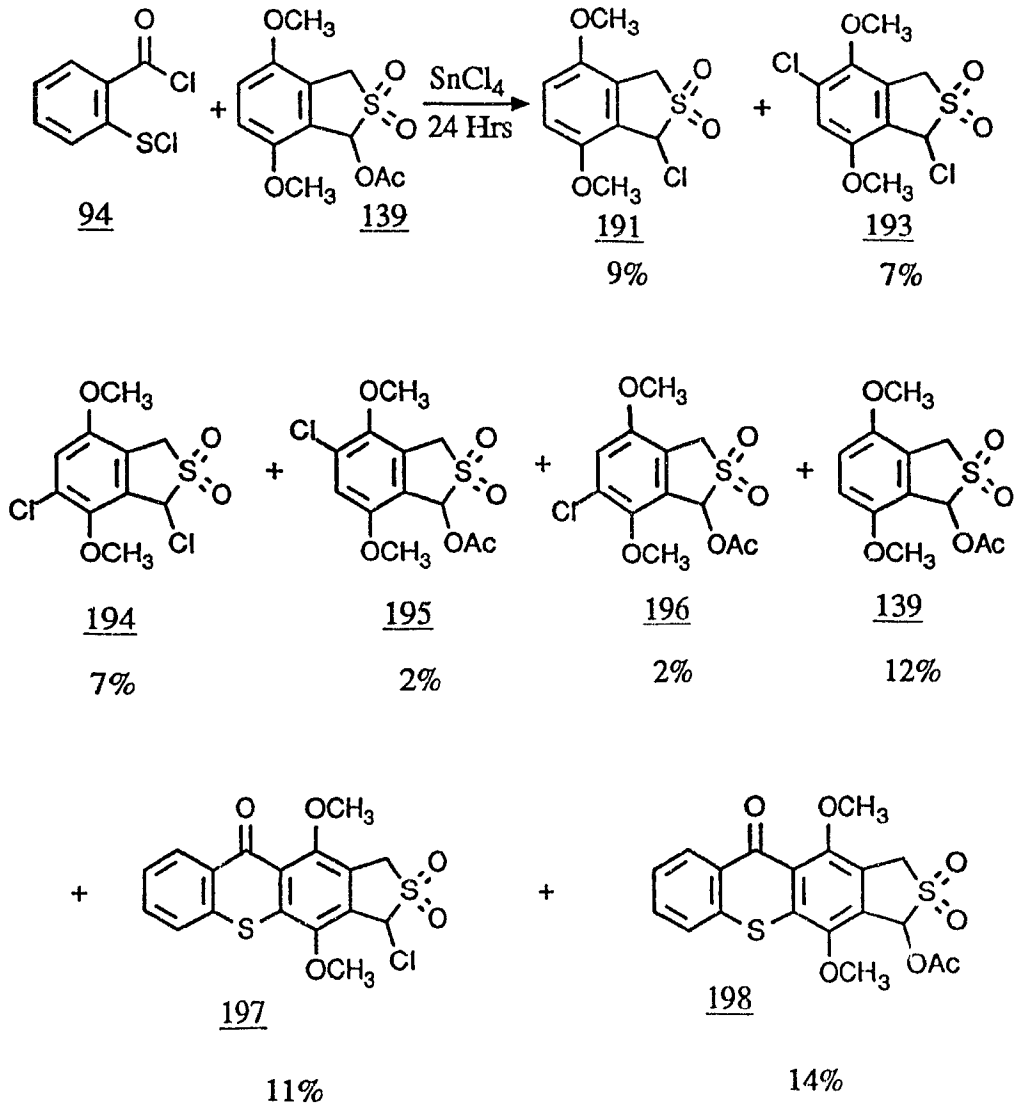

SnCl4
24 Hrs
94
139
191
9%
193
7%
194
7%
195
2%
196
2%
139
12%
197
11%
198
14%

Fig 1.39

showed resonance signals at the correct location for all the nineteen carbons. Similar ^{1}H and ^{13}C spectral attributes was obtained for thioxanthone (197) except that the acetoxyl was absent and the proton at the chloro substituted benzylic position gave a signal at 5.94ppm.

It is interesting to note that only one regioisomer is obtained in the Friedel-Crafts reaction. The regiochemistry was determined based on the hydroxysulfone (147), which was obtained in good yields from alkaline

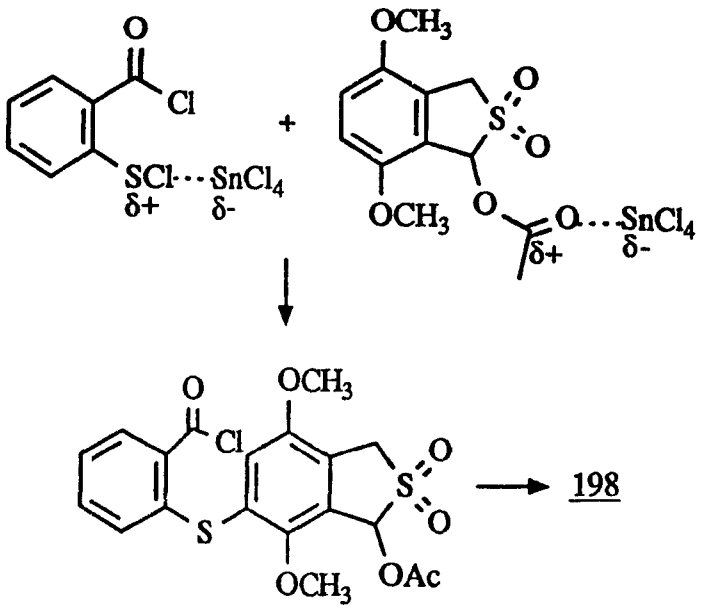


Fig 1.40

treatment of (197) or (198). When (147) was refluxed in xylenes for 24 hours a reasonably good yield (60%) of the aldehyde (145) was obtained.

By comparing the spectral attributes of this new thioxanthone aldehyde with the ones of aldehyde (158) obtained by an alternative route, the regiochemistry of

(145) could be ascertained. Thus, these two thioxanthone aldehydes had different physical and spectral characteristics. The ^{13}C NMR spectra clearly indicated two different carbon structures. Therefore the regiochemistry for (145) is assigned as shown. The sulfenylchloride reacts first with the result that the initial electrophilic addition occurs with the sulfur (fig.1.40). The second electrophilic addition would give regioisomer (198).

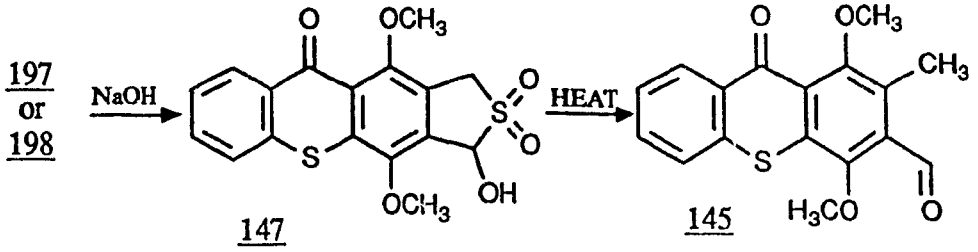
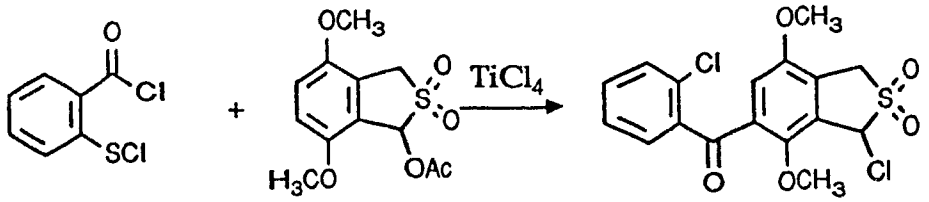


Fig 1.41

Titanium tetrachloride has not previously been employed in this Friedel-Crafts reaction.[251] Since titanium may prefer to complex oxygen more than sulfur, we wondered if its use in the electrophilic reaction would invert the regioselectivity. When $TiCl_4$ was used, the thioxanthones (197) and (198) were no longer obtained. Instead a new compound tentatively assigned as (199) was isolated in 27% yield.

In the PMR spectrum compound (199) gave resonances at 3.84 and 4.04ppm (CH_3O), at 5.14ppm as a doublet of doublets ($ArCH_2$), and at 7.00ppm (ArCH). Five aromatic protons were observed, a singlet at 7.26ppm for the proton



199

Fig 1.42

on the pentasubstituted aromatic ring and two broad triplet at 7.40 and 7.56 ppm with a broad doublet downfield at 8.29ppm for the other four protons. In the ^{13}C NMR spectrum the seventeen carbons gave signals at the expected locations.

As predicted, $TiCl_4$ causes the electrophilic addition of the acylium cation first. Unfortunately the second addition is prevented probably because of strong deactivation by the coordination of titanium to various oxygenated sites. Desulfurization would lead to the observed product.

The glycosidation reaction was carried out next by employing the procedure tested previously. Two glycosides were obtained in 82% yield as a 1:1 diastereomeric mixture, which could be separated by meticulous flash chromatography. Compound (200) was arbitrarily assigned as the less polar glycoside. The ^{1}H and ^{13}C NMR spectra shown in fig.1.44 and 1.45 are consistant with structures (200) and (201). The 2-D heterocorrelated spectrum (fig.1.45) further supported the structural assignment of glycoside (200). Both

diastereomers are assigned as alpha glycosides in accordance

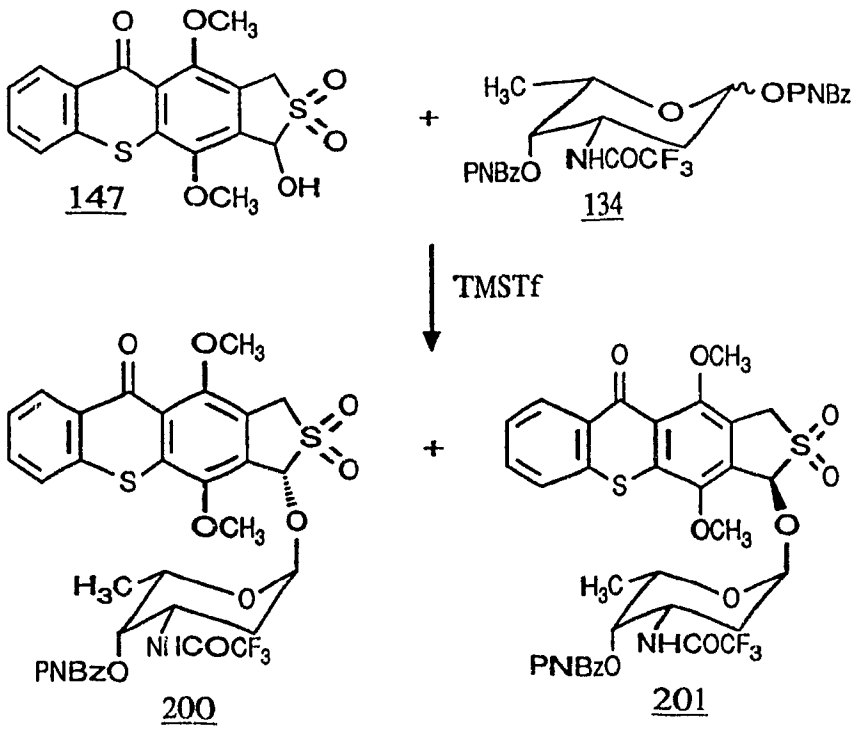


Fig 1.43

with the observed broad singlets at 5.83 (W_H = 6Hz) and 5.63ppm (W_H = 7Hz) for the anomeric proton in (200) and (201), respectively.

1.1.3 Attempted Synthesis of Thioxanthone Glycoside (39).

The next synthetic step for the total synthesis of glycoside (39) was the cycloaddition reaction with glycosides (200) or (201) and an appropriate dienophile.

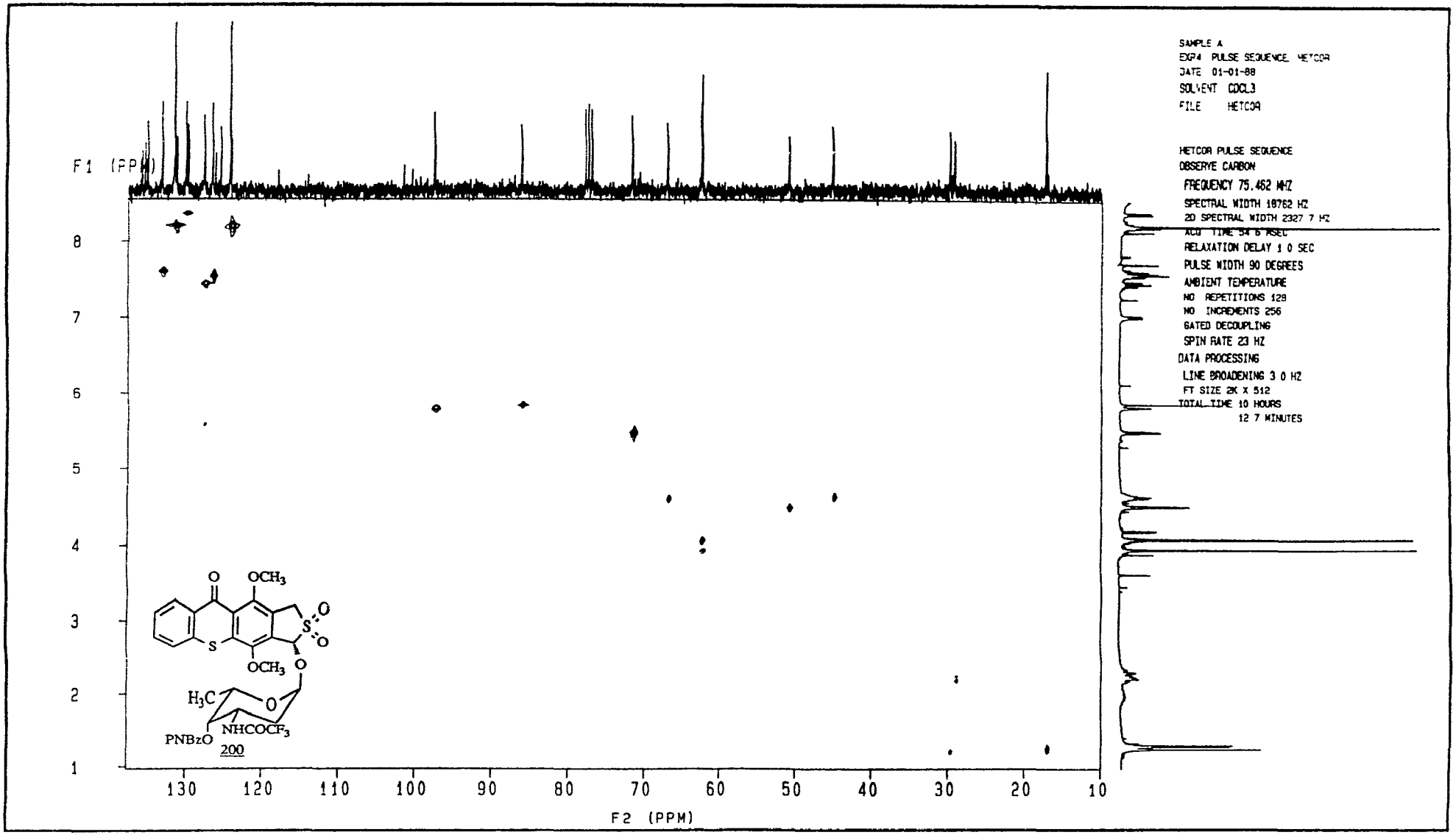
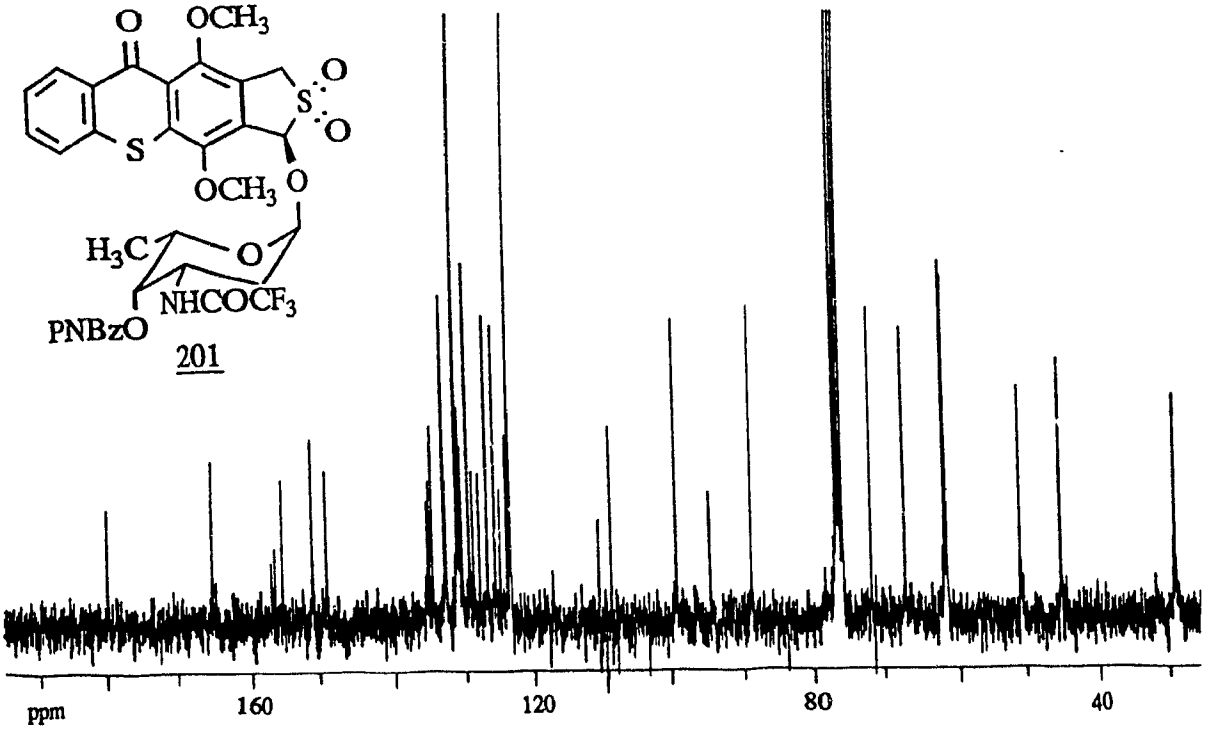

F1 (PPM)
8
7
6
5
4
3
2
1
130
120
110
100
90
80
70
60
50
40
30
20
10
F2 (PPM)
SAMPLE A
EXP4 PULSE SEQUENCE HETCOR
DATE 01-01-88
SOLVENT CDCL3
FILE HETCOR
HETCOR PULSE SEQUENCE
OBSERVE CARBON
FREQUENCY 75.462 MHZ
SPECTRAL WIDTH 18762 HZ
2D SPECTRAL WIDTH 2327 7 HZ
ACQ TIME 54 6 MSEC
RELAXATION DELAY 1 0 SEC
PULSE WIDTH 90 DEGREES
AMBIENT TEMPERATURE
NO REPETITIONS 128
NO INCREMENTS 256
GATED DECOUPLING
SPIN RATE 23 HZ
DATA PROCESSING
LINE BROADENING 3 0 HZ
FT SIZE 2K X 512
TOTAL TIME 10 HOURS
12 7 MINUTES
O
OCH3
S
O
O
S
OCH3
O
H3C
O
PNBzO
NHCOCF3
200

Fig 1.44



201

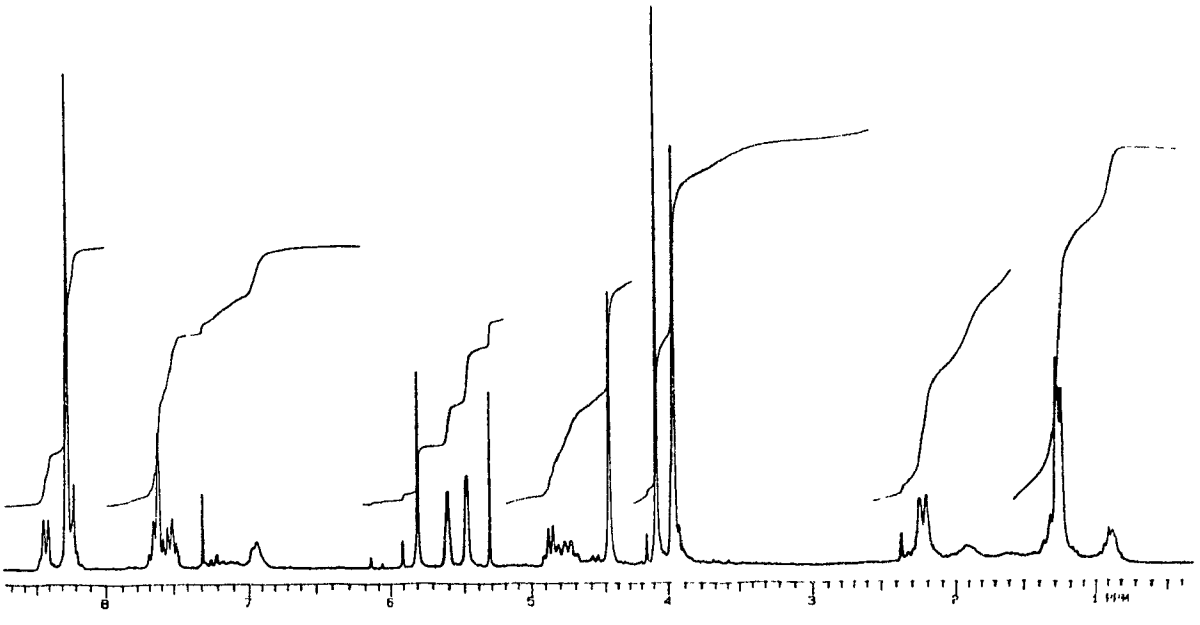


Fig 1.45

Due to the limited supply of thioxanthone glycoside, these reactions were generally carried out on a ten milligram scale. Table 1 is representative of some Diels-Alder attempts. Dimethyl fumarate was used initially because the resulting products may have provided some information on the stereochemical outcome of the reaction. Methyl vinyl ketone was also employed as an alternative, as well as 3-t-butyldimethylsilyloxy-3-butene-2-one. We found that extrusion of SO_2 was particularly difficult from glycosides

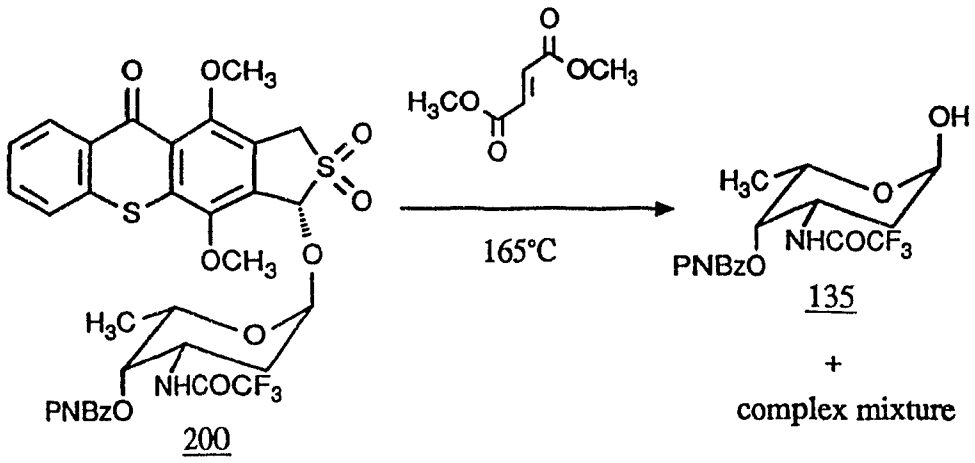


Fig 1.46

(200) and (201). Consequently high temperatures were necessary. The use of oxylophiles has been proposed for the catalysis of cycloaddition reaction[265,271,311] and therefore they were employed extensively in many attempts.

When no catalyst was used in the cyclocondensation, a reflux temperature of 165°C for 24 hours was required to get a reasonable reaction rate. To our great disappointment, this led to complex reaction mixtures

containing a major product (65% yield) identified as being the N-trifluroacetyl-4'-O-p-nitrobenzoyldaunosamine (135). Due to the quantity of side products and the reaction scale, an accurate identification of every single product could not be effected. The use of chlorine containing Lewis acids gave fairly rapidly the sugar with some thioxanthone chloride (197), which surprisingly did not undergo extensive SO_2 extrusion.

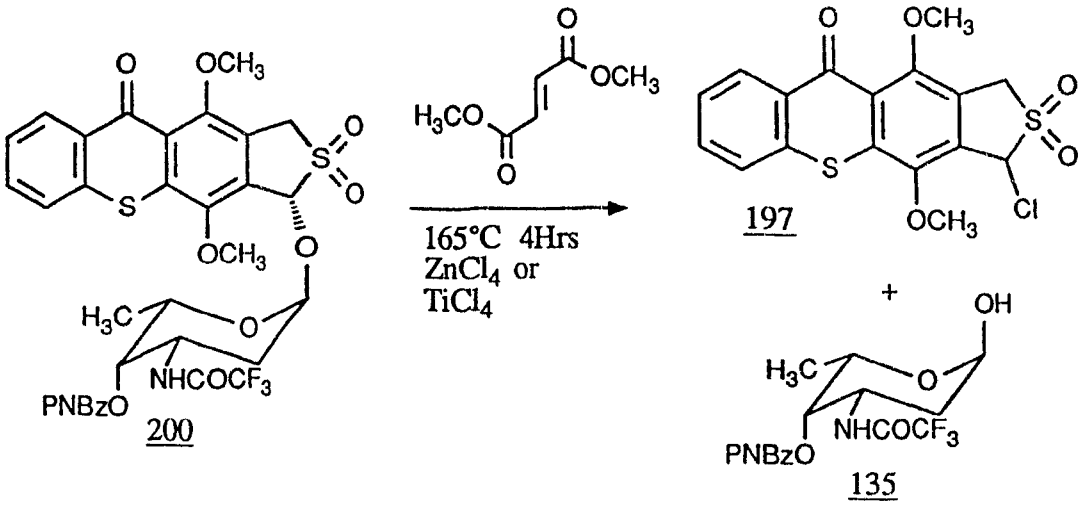


Fig 1.47

A test carried out with 1-chlorosulfone (197) showed that extrusion did not occur even at 200°C. This is probably caused by strong periplanar interactions between the benzylic chlorine and the neighbouring methoxy group, thus inhibiting the formation of an sp^2 benzylic center. The use of other oxylophiles in the cycloaddition reaction mixtures did not improve the outcome and invariably led to the elimination of the daunosamine moiety.

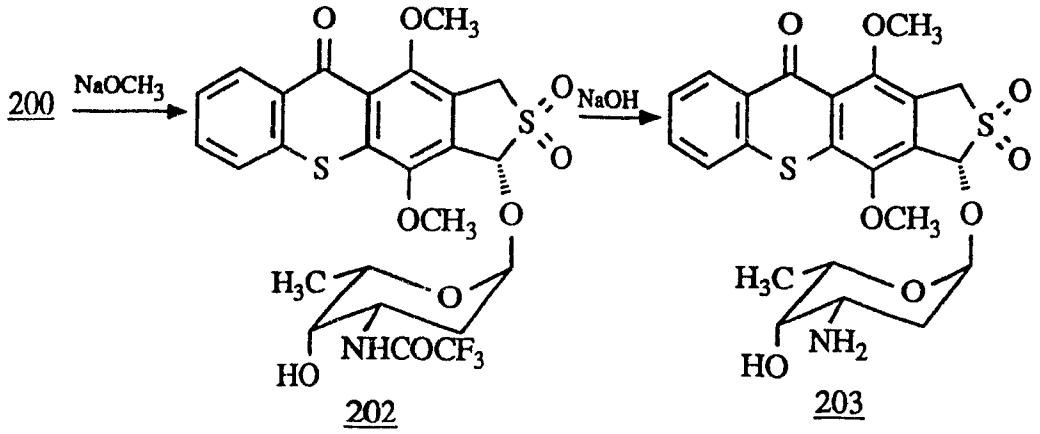


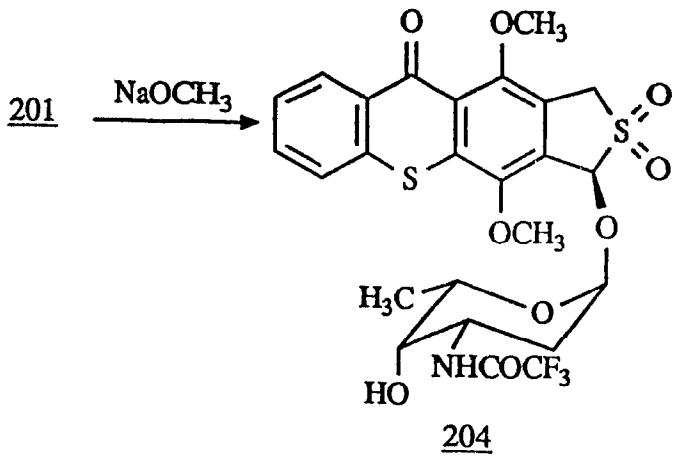
Fig 1.48

In order to test the likelihood that the deprotected thioxanthone glycosides would undergo a cycloaddition reaction, compound (200) was converted in 79% yield to the N-trifluoroacetylglycoside (202) with 0.1N $NaOCH_3$ in methanol.

The absence of the p-nitrobenzoyl group was ascertained from the ^{1}H, ^{13}C and infrared spectra. Treatment of (202) with 0.1N NaOH in aqueous THF gave the deprotected thioxanthoneglycoside (203). The liberated amino group protons were detected as a broad triplet in the pmr spectrum. Similarly, the N-trifluoroacetyl glycoside

(204), obtained in 76% yield from basic methanolysis of glycoside (201), gave satisfactory spectral data.

All cycloaddition reactions in which (202), (203) or (204) were used led to extensive deglycosidation. In view of difficulties in preparing any glycosidated cycloadducts of interest, the synthesis of thioxanthone derivatives containing an heteroatom in ring A was considered next as an alternative. Elimination of the sugar portion should be prevented in this way.



204

Fig 1.49

TABLE 1.2.1

Some Cycloaddition Reactions In Refluxing Mesitylene

Compound	Dienophile	Catalyst	Major Product
(200)	dimethyl fumarate	none	(135)
(200)	dimethyl fumarate	$SnCl_4$	(135) (197)
(200)	dimethyl fumarate	$Eu(FOD)_3$	(135)
(200)	dimethyl fumarate	$ti(o\text{-}iso\text{-}propyl)_2Cl_2$	(135)
(200)	methylvinyl ketone	none	(135)
(201)	dimethyl fumarate	none	(135)
(201)	dimethyl fumarate	$SnCl_4$	(135) (197)
(201)	dimethyl fumarate	$AlEt_2Cl$	(135)
(201)	dimethyl fumarate	$Eu(fod)_3$	(135)
(201)	methylvinyl ketone	none	(135)
(202)	dimethyl fumarate	none	(133)
(202)	methylvinyl ketone	none	(133)
(203)	dimethyl fumarate	none	-----
(203)	dimethyl fumarate	$AlEt_2Cl$	-----
(204)	dimethyl fumarate	none	-----

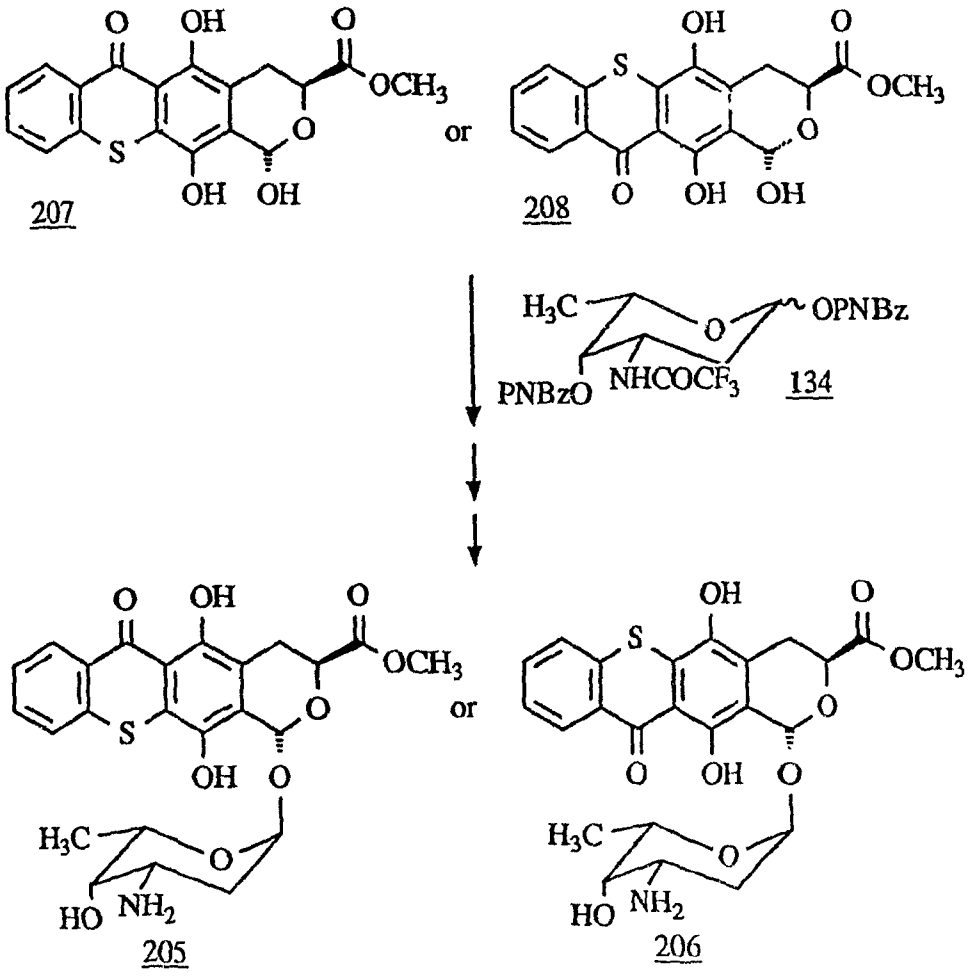
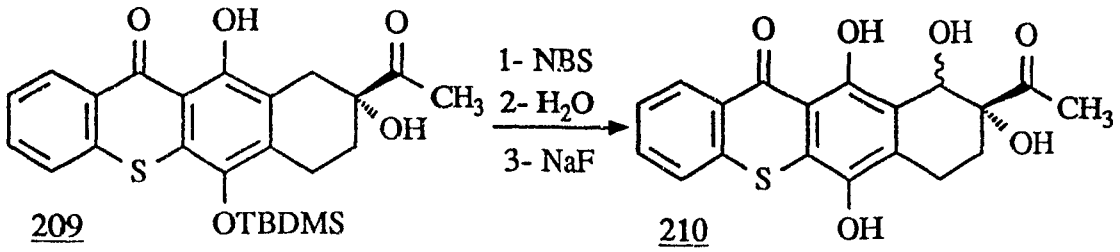

O
OH
O
OCH3
S
O
OH
OH
or
207
OH
O
S
OCH3
O
O
OH
OH
208
H3C
O
OPNBz
PNBzO
NHCOCF3
134
O
OH
O
OCH3
S
O
OH
O
H3C
O
HO
NH2
or
205
OH
O
S
OCH3
O
O
OH
O
H3C
O
HO
NH2
206

Fig 1.50

1.2 Studies Toward The Synthesis of Pyranylthioxanthone Derivatives Of 4-Demethoxydaunorubicin.

The synthesis of derivatives (205) and (206) was considered next. The glycosidation reaction between appropriately protected aglycones such as (207) or (208) and protected daunosamine (134) should provide the desired glycosides. Thus, the preparation of aglycones (207) and (208) was the first goal in this proposed total synthesis. The main criteria in their synthesis would be to functionalize the C-7 position, as the hydroxyl or an appropriate alternative, before constructing the thioxanthone ring system.

This approach is preferred because the introduction of the C-7 hydroxyl was proven to be very difficult in related tetracyclic thioxanthones[251] (fig.1.51). In one of the most successful attempts compound (210) was obtained from (209) in 21% yield. The sulfur directs the bromination to the para benzylic position.



209 210

Fig 1.51

In our case the ring A oxygen may direct the bromination reaction to C-7 but the extent of bromination at that position is uncertain when a sulfur atom is located at the 5 position.

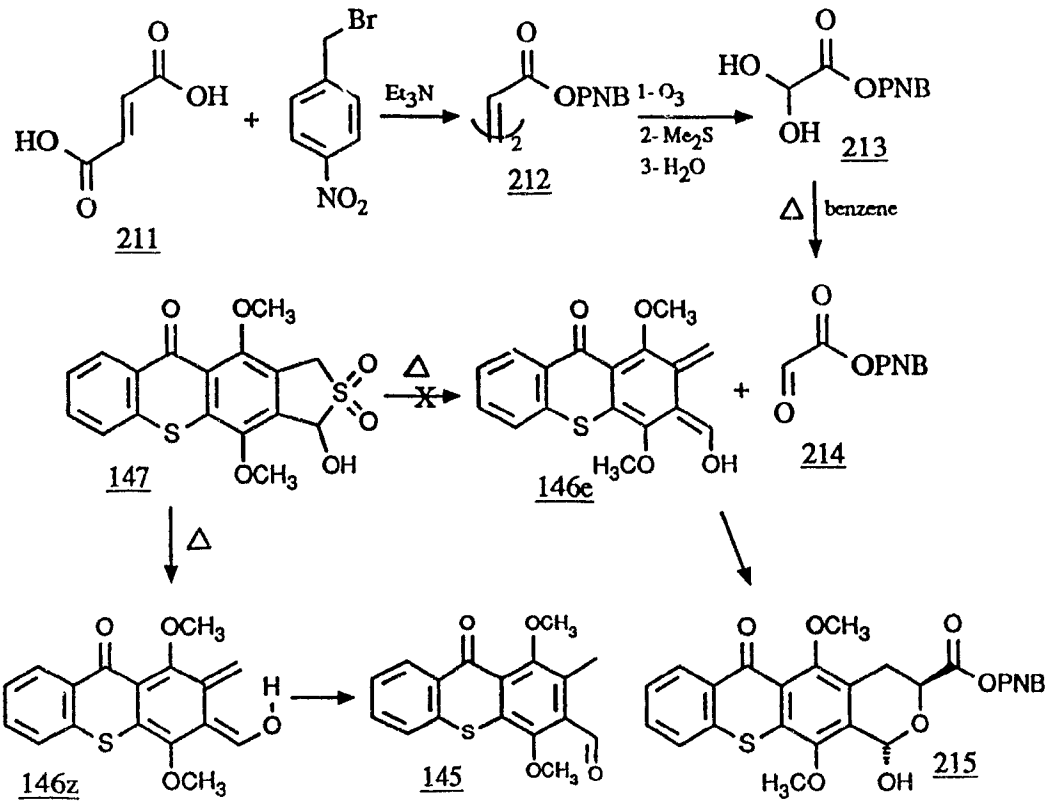


Fig 1.52

Rapid access to one of the required aglycones (215) was envisaged from the cycloaddition reaction between the thermally generated o-quinodimethide (146e) and an activated aldehyde such as the p-nitrobenzylglyoxylate (214).

The use of glyoxalates in Diels-Alder reactions has previously been reported.[313,313] Glyoxalate (214) was chosen over the methyl or ethyl glyoxalates because the p-

nitrobenzyl group may enhance the reactivity of the aldehyde through its electron withdrawing ability. An eventual transesterification could be accomplished with catalytic sodium methoxide in methanol.

The p-nitrobenzylglyoxylate hydrate (213) was prepared by using a known procedure.[309] The resulting glyoxylate (214) was reacted with 7-hydroxysulfone (147) at reflux. In all of our attempts, the thioxanthone aldehyde (145) was formed in good yields (60-75%) and none of the desired cycloadduct was isolated.

In an alternative strategy, the synthesis of pyranylthioxanthones (218) and (219) was envisaged from the coupling reaction between quinone (217) and o-thiobenzoic acid (99). Unfortunately, the preparation of (216) via the

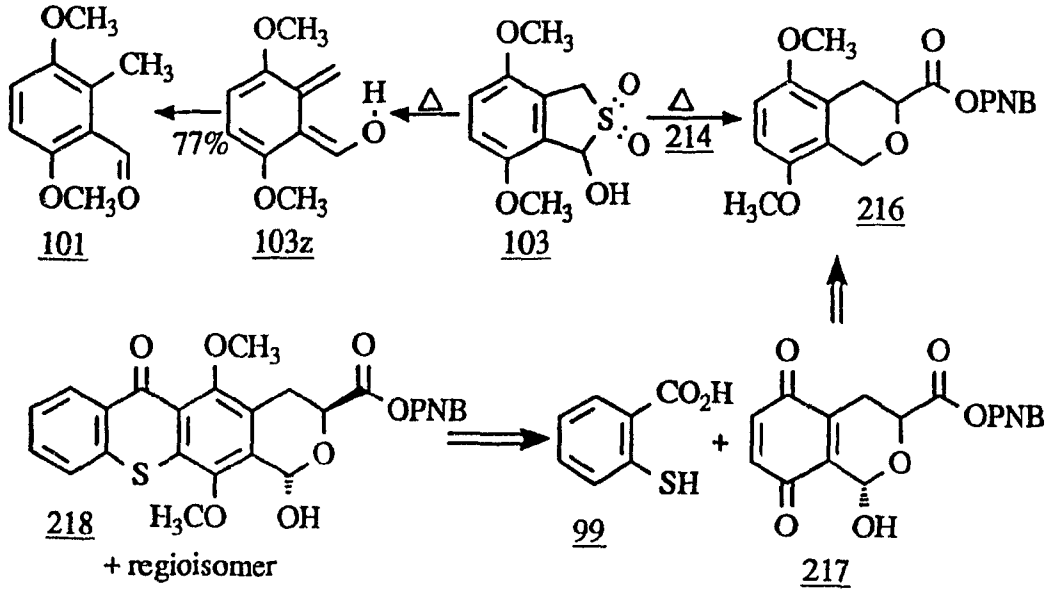
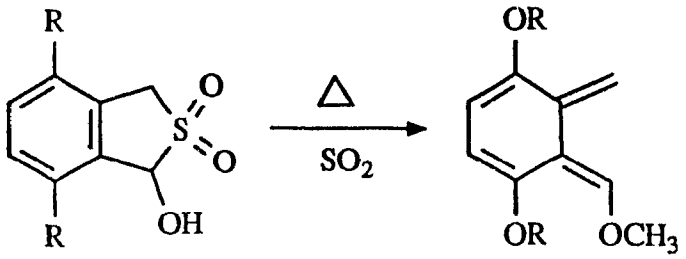


Fig 1.53

extrusion of SO_2 from the 1-hydroxysulfone (103) and trapping of the o-quinodimethane with glyoxalate (214) failed. The major product was 2,5-dimethoxy-6-methylbenzaldehyde.

In all likelihood the extrusion of sulfur dioxide from hydroxysulfones having a bulky periplanar aromatic substituent will lead to the formation of the Z-dienols such as [146_z, Fig 1.52] and [103_z, Fig 1.53]. This is contrary to literature precedents[315] which show that the E-dienol is formed preferentially, or faster than the Z-isomer. The fact that the Z-dienol is formed in the ortho substituted hydroxysulfones explains the requirement for high SO_2 extrusion temperatures. As a comparison our dimethoxysubstituted 1-methoxysulfone (140) extrudes SO_2 at ca 260°C whereas the analogous 1-methoxysulfone (219)



219 R=H T=150°C
140 R=OCH_3 T=260°C

Fig 1.54

undergoes thermolytic loss of SO_2 at 150°C.[271] Periplanar interactions between the benzylic substituent and the ortho aromatic substituent prevents the formation of the E-dienol when bulk is introduced.

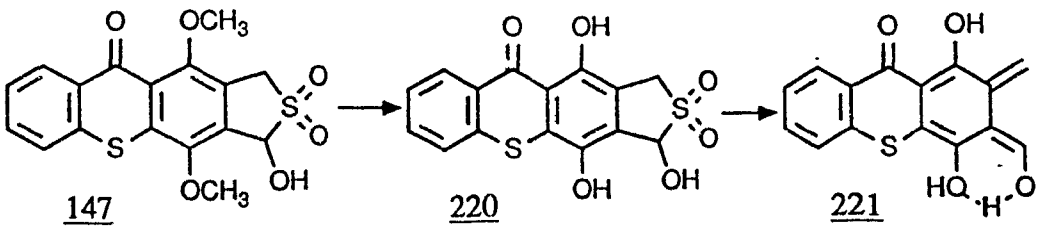


Fig 1.55

The idea of reducing steric repulsion and increasing the likelihood of E-dienol formation by introducing a hydrogen bond as in (221) has value (fig.1.55).

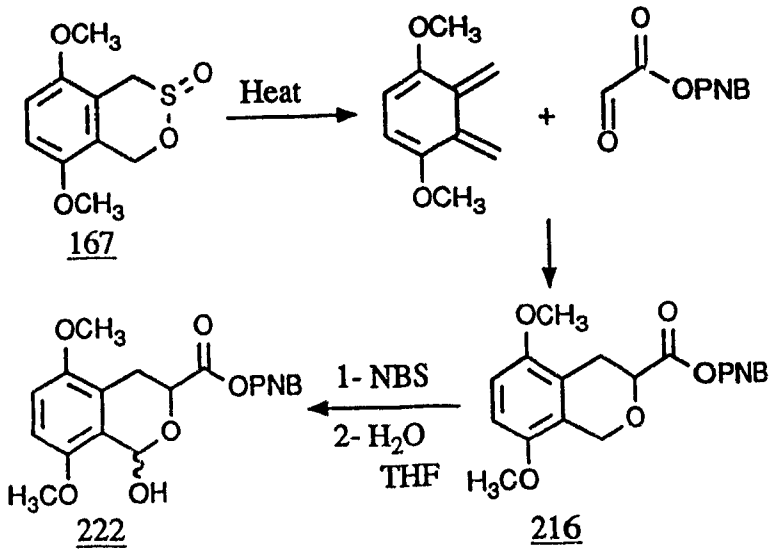


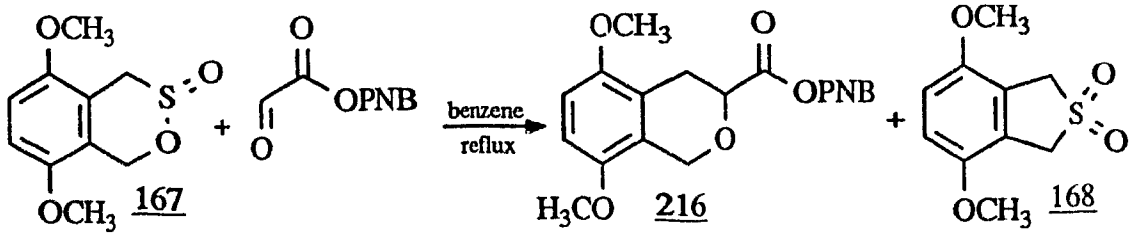
Fig 1.56

Unfortunately,the demethylation step proved to be difficult and any attempt to react thioxanthone (147) with BBr_3 or BI_3 led to complex reaction mixtures, from which none of the desired thioxanthone (220) could be isolated. In

consideration of these difficulties, the synthetic strategy was modified as shown in fig.1.56.

Generation of the o-quinodimethane from previously prepared sultine (167) followed by cycloaddition with the glyoxalate should provide the isochroman cycloadduct. Free radical bromination followed by solvolysis could provide compound (222).

Sultines extrude SO_2 at lower temperatures than dihydrothiophene-2,2-dioxides.[276,316,317] Dimethoxysultine (167) underwent SO_2 extrusion at 80°C. The product distribution of the cycloaddition reaction depended on the number of glyoxalate equivalents and rate of addition of the sultine (fig. 1.57). The highest yield of compound (216) was obtained when the sultine was added over two hours to twenty equivalents of glyoxalate in refluxing benzene.



3 equivalents	18.8%	52%
20 equivalents	30.6%	37%
20 equivalents slow addition	42.3%	45.5%

Fig 1.57

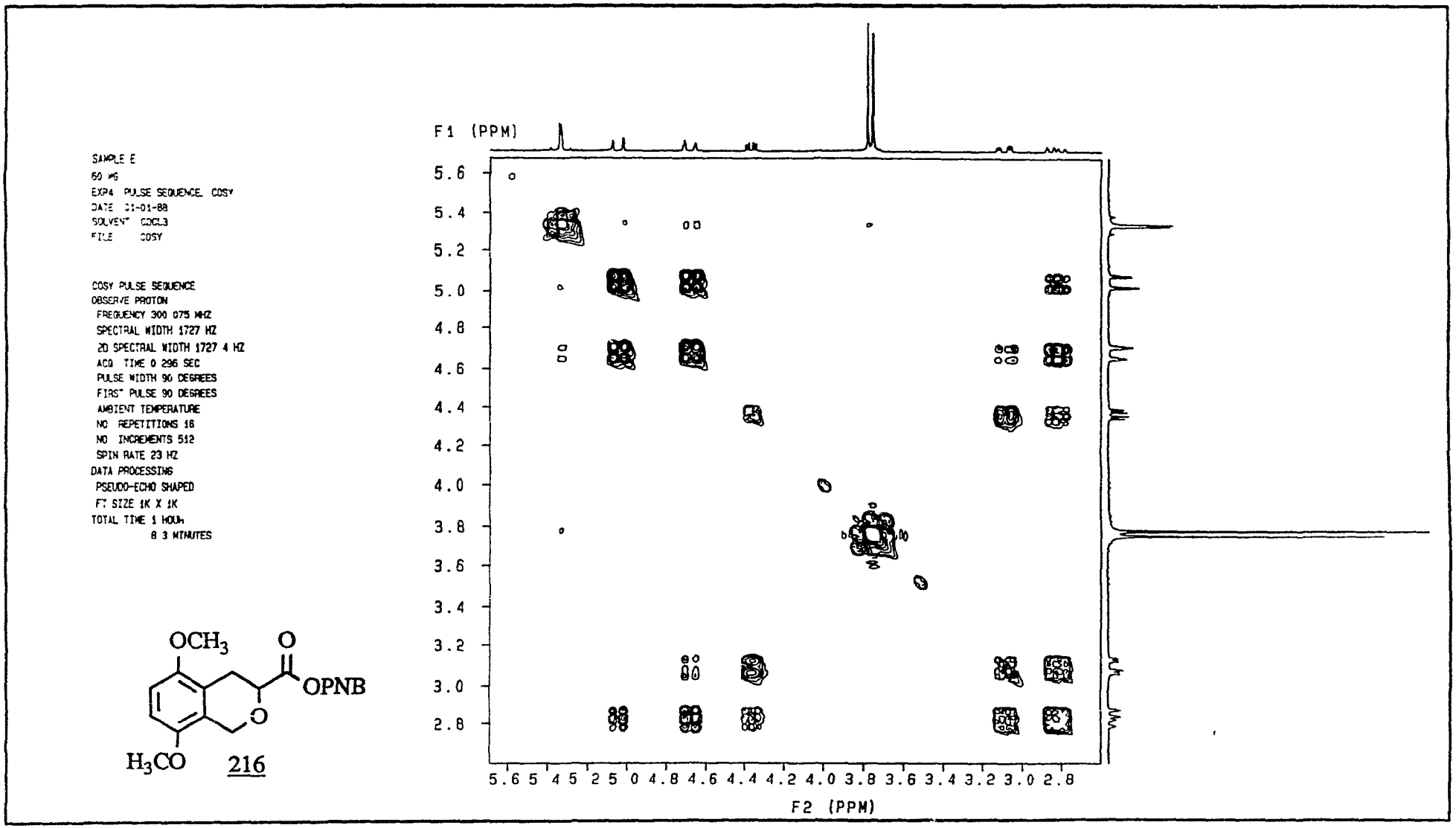
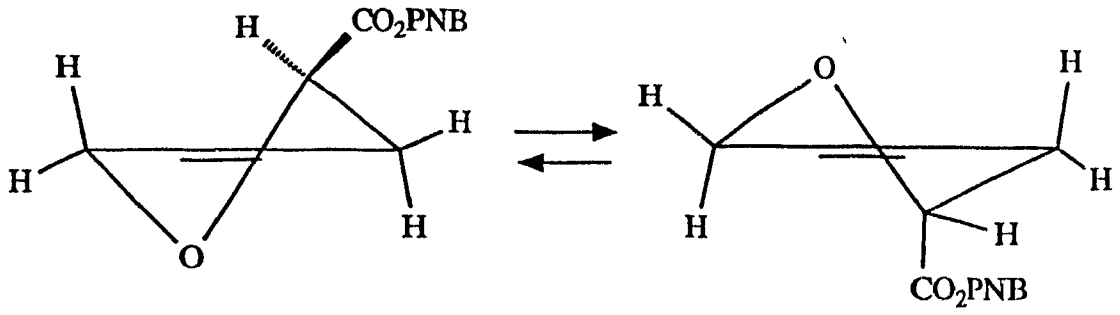

SAMPLE E
60 MG
EXP4 PULSE SEQUENCE COSY
DATE 01-01-88
SOLVENT CDCL3
FILE COSY
COSY PULSE SEQUENCE
OBSERVE PROTON
FREQUENCY 300 075 MHZ
SPECTRAL WIDTH 1727 HZ
2D SPECTRAL WIDTH 1727 4 HZ
ACQ TIME 0 296 SEC
PULSE WIDTH 90 DEGREES
FIRST PULSE 90 DEGREES
AMBIENT TEMPERATURE
NO REPETITIONS 16
NO INCREMENTS 512
SPIN RATE 23 HZ
DATA PROCESSING
PSEUDO-ECHO SHAPED
FT SIZE 1K X 1K
TOTAL TIME 1 HOUR
8 3 MINUTES
OCH3
O
OPNB
O
H3CO
216
F1 (PPM)
5.6
5.4
5.2
5.0
4.8
4.6
4.4
4.2
4.0
3.8
3.6
3.4
3.2
3.0
2.8
5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8
F2 (PPM)

Fig 1.58

Isochroman (216) gave a molecular ion at m/z 391 as the (M + $^{+}NH_4$) adduct in the CI mass spectrum. The presence of the p-nitrobenzyl functionality was confirmed from the infrared spectrum from the carbonyl absorption band at 1756cm^{-1}, and the nitro absorption bands at 1350cm^{-1} and 1526cm^{-1}. The CMR and PMR spectra contained all the required resonance signals for the alkyl and aryl functionalities. The COSY 2D spectrum is shown in Fig 1.58. Most interestingly, the pyran ring conformation could be assigned as the pseudochair conformation with the ester in a pseudoequatorial orientation (fig.1.59, 216b).



216a 216b

Fig 1.59

This is based on the observed coupling constants of 4.0 and 10.3Hz assigned to the coupling of C-3 Ha with C-4 He, and C-3 Ha with C-4 Ha, respectively. Long range coupling occurred between the C-1 and the C-4 protons. The magnitude and incidence of long range coupling between protons at the two benzylic position has been highly diagnostic for assigning the relative stereochemistry when

both carbons, linked to the pyran oxygen, are monosubstituted.[318-322] This will be discussed in a later section.

The bromination of isochroman (216) led to a ring opened compound in 40% yield the structure of which could not be unambigously assigned. Structure (223) is possible, based on the ^1H spectrum. The presence of the aldehyde was confirmed from its proton signal at 10.11ppm. Resonances for the pyran ring protons were absent, but two new doublets were observed at 4.22 and 5.15ppm with a coupling constant of 7Hz and were reluctantly assigned to the protons at the epoxide moiety.

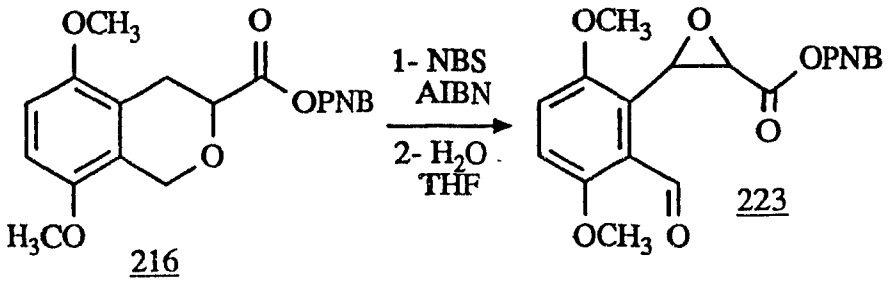


Fig 1.60

Ring opening would occur due to a lack of specificity in the bromination of isochroman (216) , and should be prevented by providing more stabilization to the benzylic radical. Since radicals are particularly strongly stabilized when both an electron withdrawing and an electron donating substituent are present on the radical carbon, then

the presence of electron attracting substituents in the aromatic part should provide this enhancement.

Capto-dative stabilization is shown for the thioxanthone derivative in fig.1.61. It was hoped that the combined stabilizing effect of the thioxanthone carbonyl and pyran ring oxygen would be sufficient to counterbalance the

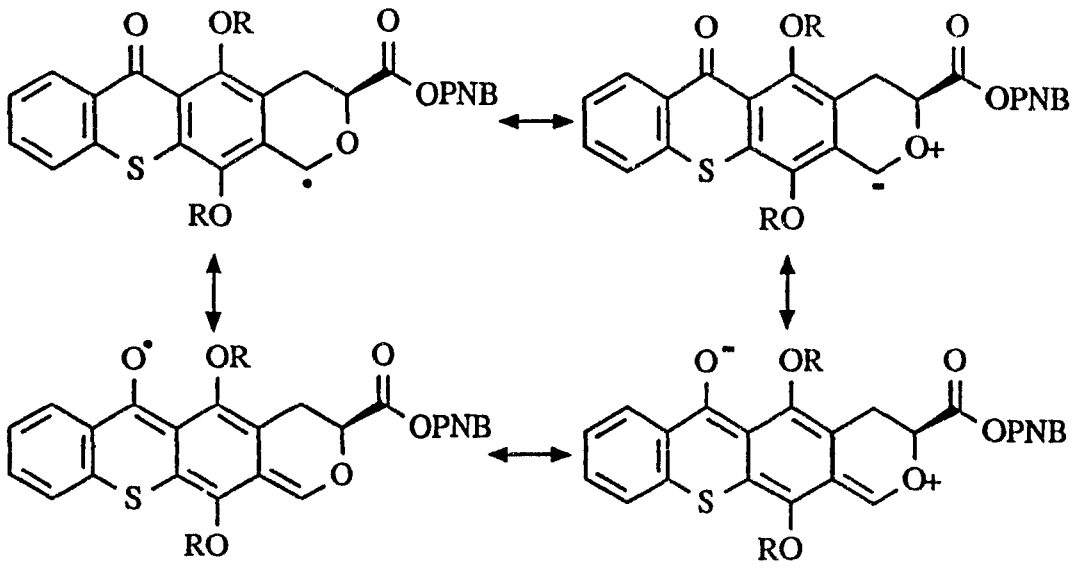


Fig 1.61

directing ability of the sulfur in the free radical reactions. Therefore the synthesis of the 7-deoxythioxanthone derivatives was initiated.

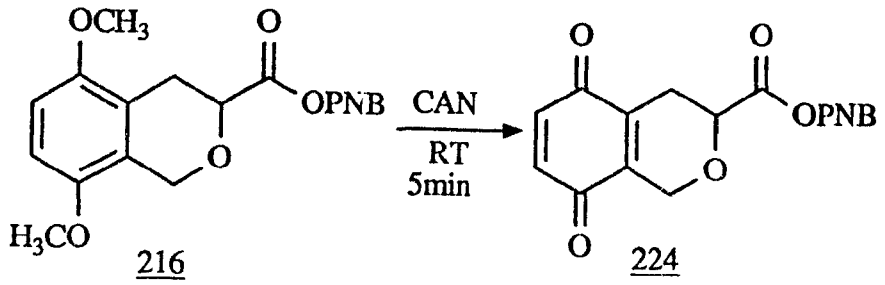


Fig 1.62

Treatment of dimethyl ether (216) with ceric ammonium nitrate, as described for similar isochromans,[320,323] gave a 92% yield of pyranoquinone (224). The infrared spectrum clearly showed the presence of the newly formed quinone as a strong carbonyl absorption band at 1658cm^{-1}. The ^{1}H, ^{13}C, and mass spectra further supported the proposed structure.

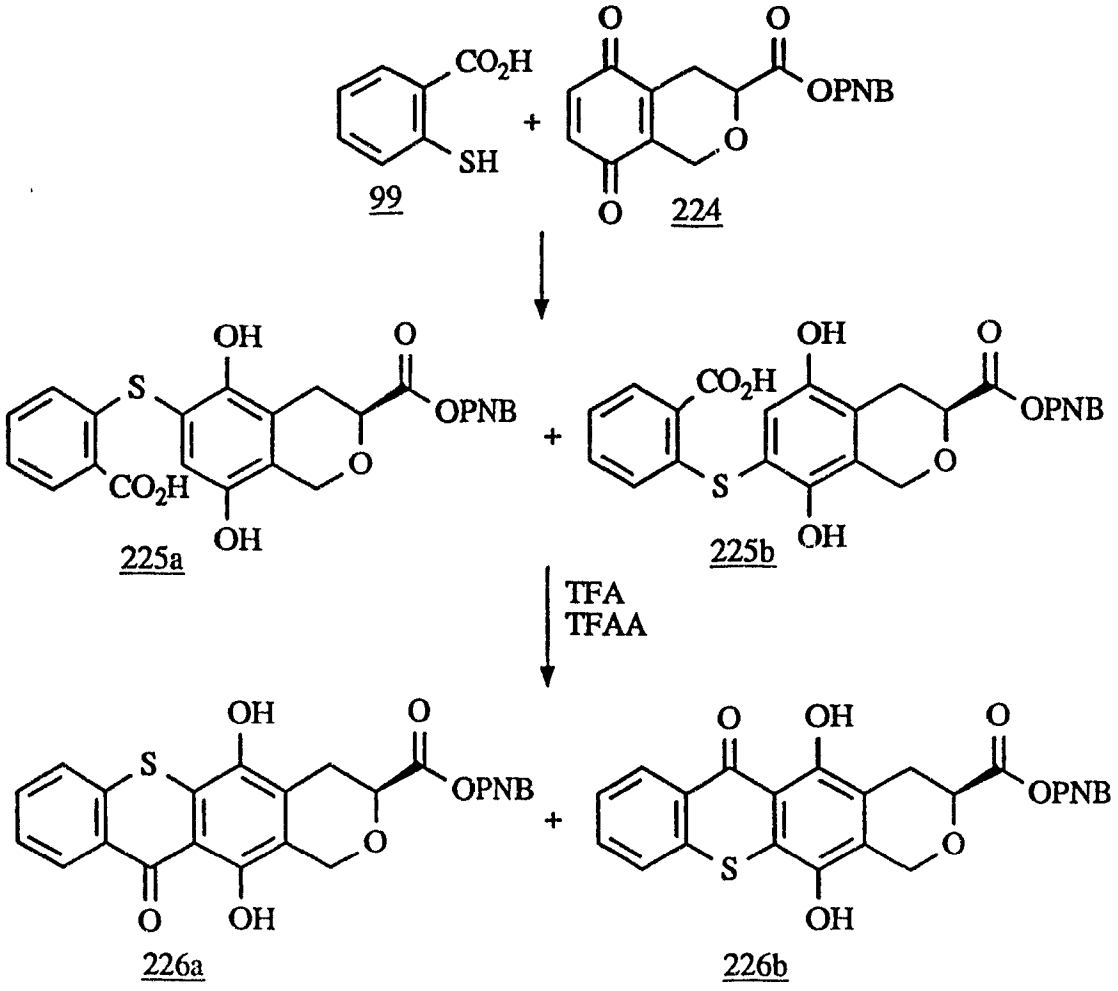
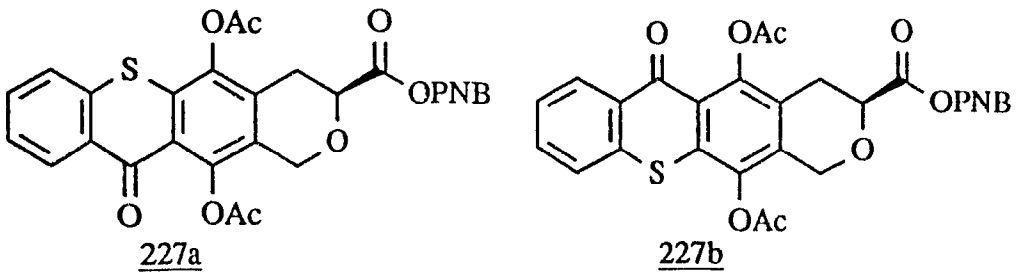


Fig 1.63

The Michael addition[251] of the o-mercaptobenzoic acid (99) to quinone (224) gave acids (225a) and (225b), which were directly cyclized with TFA-TFAA to give a 1:1 regioisomeric mixture of pyrano thioxanthones (226a) and (226b) in 72% yield. The separation of these regioisomers could be done by flash chromatography. Structure (226a) was arbitrarily assigned to the less polar fraction and (226b) to the more polar one.

The infrared spectrum of (226a) showed the thioxanthone hydrogen bonded carbonyl at 1610cm^{-1} and a broad band at 3420cm^{-1} for the hydroxyls. Resonances for the four aromatic protons of the thioxanthone system occured at 8.26, 7.82 and 7.58 ppm. The two hydroxyl hydrogens gave signals at 9.56 and 13.85ppm for the hydrogen bonded one. The ^{13}C NMR spectrum provided further support for the structure. Compound (226b) showed very similar spectral attributes as (226a).

Acetylation of either thioxanthone (226a) or (226b) gave the diacetates (227a) and (227b) in good yields.



227a 227b

fig 1.64

Subsequent bromination of either tetracyclics with NBS or Br_2 with AIBN, UV light or t-butylhydroperoxide as initiator and in refluxing solvents such as CCl_4 or CH_2Cl_2 failed to give any reaction after three to five hours. The use of longer reaction times (24-48hrs) with NBS and UV light in CCl_4 also gave back the starting compound with some additional minor products. Any attempts to isolate these compounds failed because of their extremely low yields.

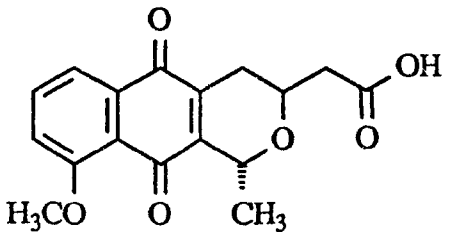
These results are not unexpected. As previously discussed the bromination of similar thioxanthones is not a very good process. Coupled to this problem is the fact that all of the pyranothioxanthones are highly insoluble in the required solvents. It was hoped that as the bromination proceeded, more substrate would dissolve and consequently undergo reaction. This however failed, since longer reaction times, with periodic addition of NBS, gave only a very small yield of a complex mixture. Evidently introduction of the benzylic hydroxyl prior to the synthesis of the thioxanthone ring is the preferred route.

Synthetic efforts for the thioxanthone derivatives were ended at this point and our efforts were concentrated on the synthesis of ring A pyran modified anthracycline ring systems.

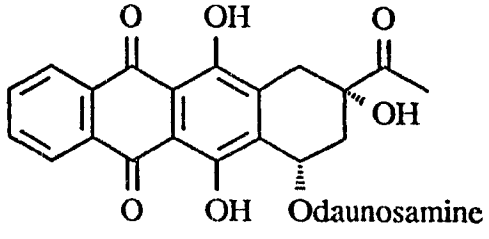
CHAPTER 2

2.1 Total Synthesis Of Tetrahydroanthraceno[2,3-c]pyranyl Analogs Of 4-Demethoxydaunorubicin.

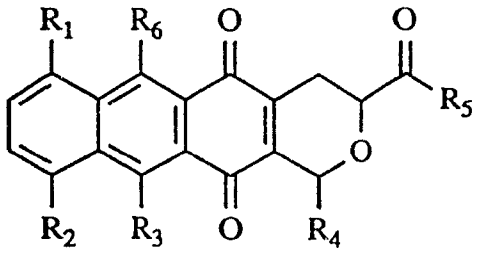
In the second part of the project, synthetic efforts were directed towards the preparation of various dioxotetrahydroanthracenopyranyl derivatives of general formula (228). Compounds specifically having a methyl R_4 substituent can be classified as nanaomycin analogs, whereas novel heteroanthracycline derivatives are obtained when a daunosaminyl sugar is located at that position.



nanaomycin



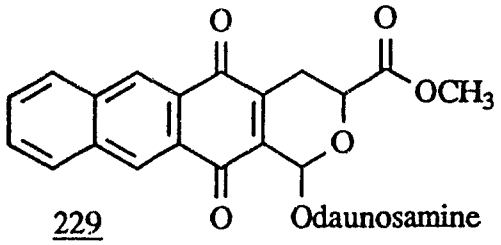
1



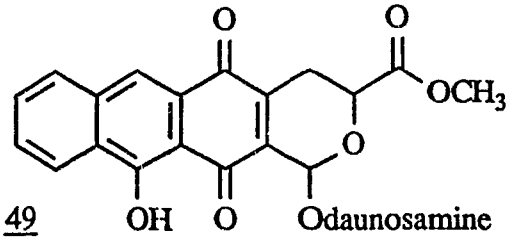
228

Our efforts were directed towards the preparation of hybrid structures containing structural features of nanaomycin and 4-demethoxydaunorubicin. More specifically,

compounds (229), (45), (49), and (50), were selected as synthetic targets. Attempts were made to develop a generalized synthetic approach from which several totally synthetic pyranoanthracycline derivatives could be accessed. Known synthetic methods as well as new ones were combined to create novel routes of synthesis.



229



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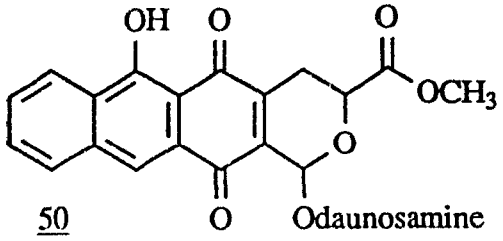
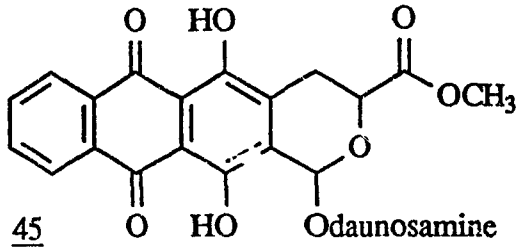


50



45

2.1.1 Studies Toward The Total Synthesis Of 3,4,5,12-Tetrahydroanthraceno[2,3-c]pyran-3-yl Derivatives of Nanaomycin Related Antibiotics.

Retrosynthetic analysis indicates that structures such as (228) could be assembled from the combination of synthons (230) and (231) with aldehyde (232). This assembly can be carried out sequentially by first combining the aldehyde

(232) with an appropriate o-quinodimethide derivative such as (235) to give isochroman (234).

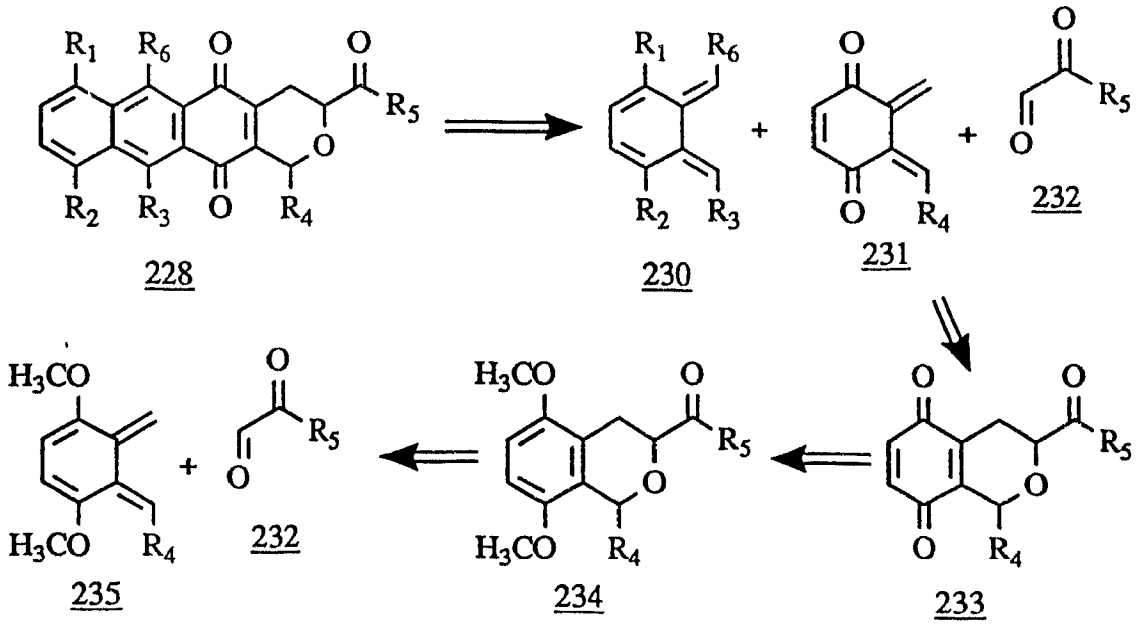


Fig 2.1

Quinone (233) should then be liberated through oxidative demethylation of (234) and trapped with a second o-quinodimethane such as (230) to ultimately give compound (228) after adjustment of the oxidation state.

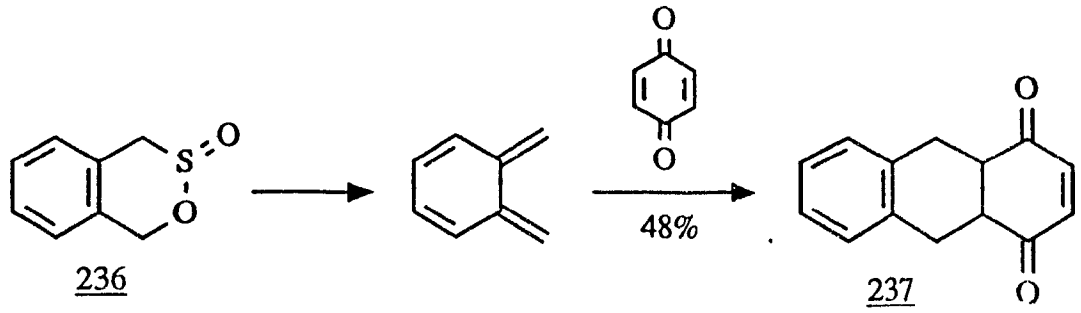


Fig 2.2

The trapping of an o-quinodimethane generated via extrusion of SO_2 from 3,6-dihydro-1,2-oxathiin-2-oxide (236) with benzoquinone to give the tetracyclic compound (237) has been described by Scheffer and coworkers.[324]

Application of this approach for the preparation of tetracyclic derivatives (238) to (241) was considered with more complex quinones such as (233). In our preliminary

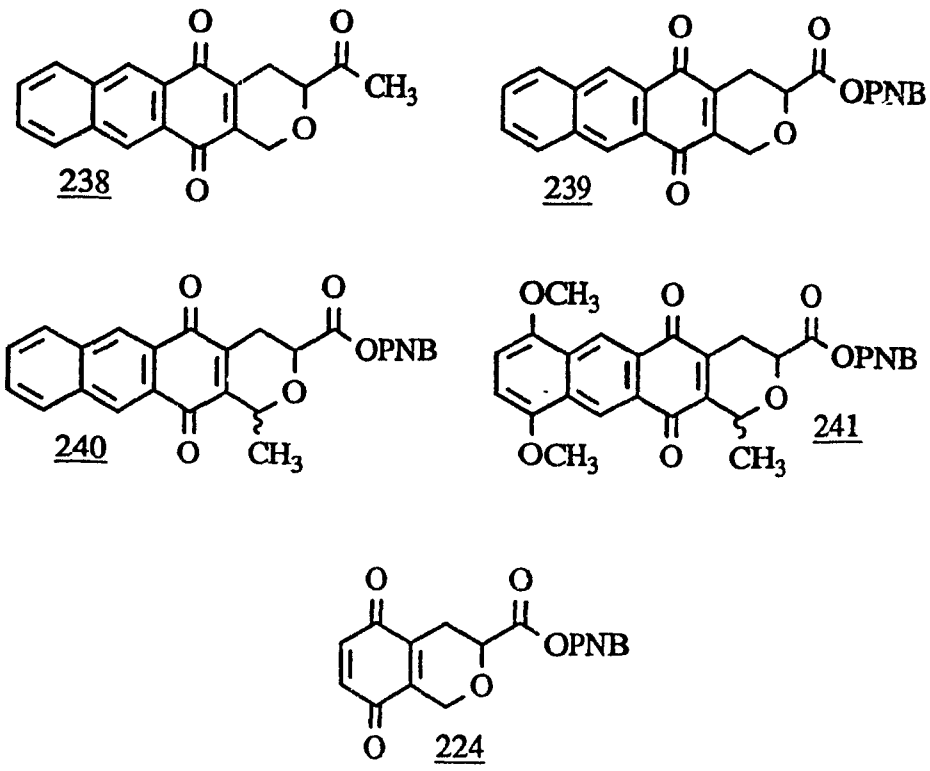
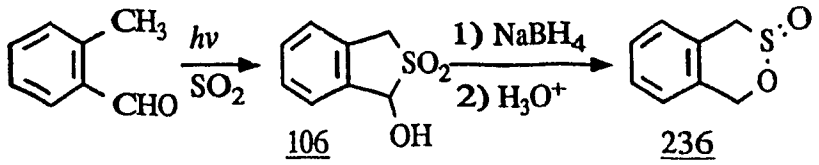


Fig 2.3

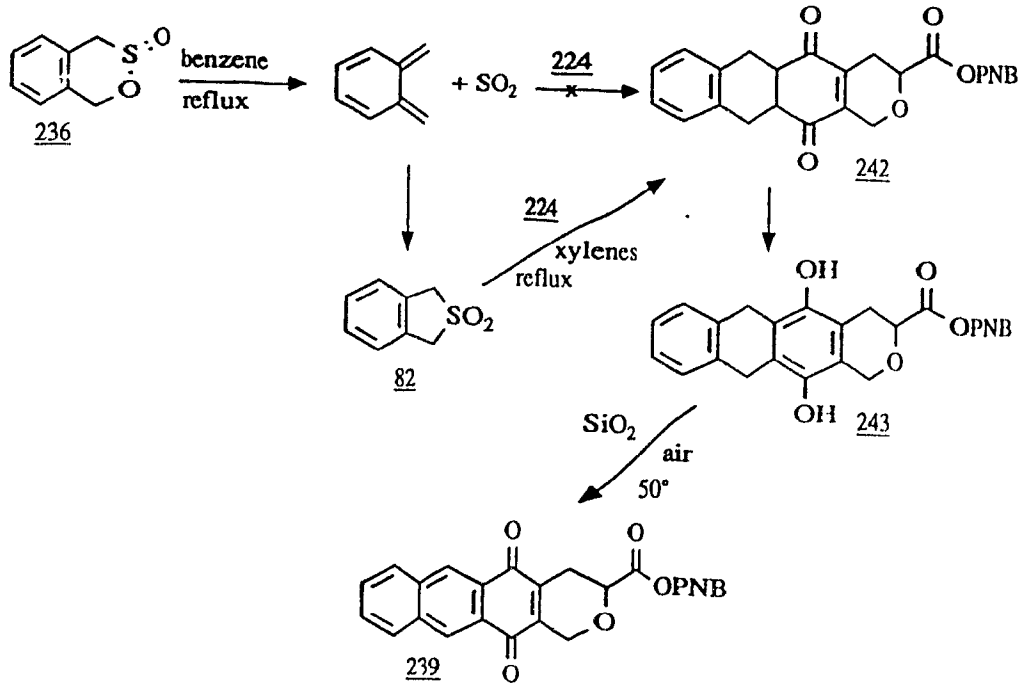
work, the previously prepared pyranoquinone (224) was used to test the synthetic route.



106 236

Fig 2.4

The known sultine (236)[276] was prepared in 77% yield by sodium borohydride reduction of 1-hydroxysulfone (106). Extrusion of SO_2 from sultine (236) in the presence of quinone (224) in refluxing benzene lead to problems because



236 242 82 243 239

Fig 2.5

of reentrapment of the thermally generated o-quinodimethane by SO_2. Fortunately, in refluxing xylenes, the resulting dihydrothiophene-2,2-dioxide (82) underwent extrusion and the o-quinodimethane was trapped with (224) to give a polar compound, presumably cycloadduct (243). Flash chromatography of the reaction mixture led to problems because of the appearance of a yellow compound mixed with the polar one. After some experimentation, the best work up procedure was to adsorb the products from the cycloaddition on silica gel and to warm the mixture at 50°C for 8 hours under air. A 60% yield of the oxidized tetracyclic pyranoquinone (239) was obtained after flash chromatography.

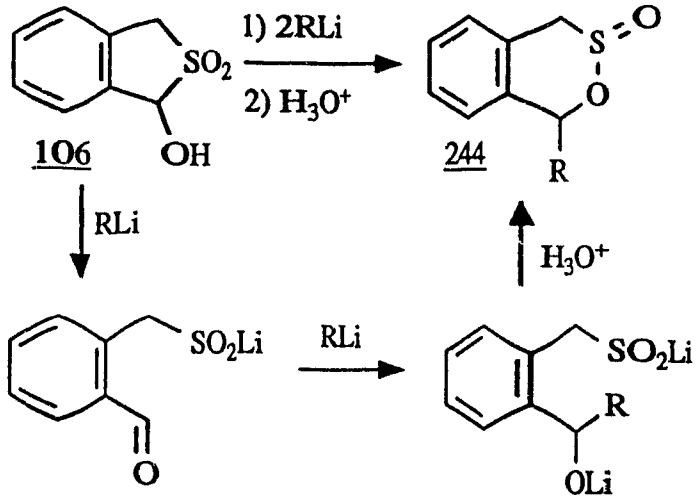


Fig 2.6

The preparation of the prerequisite quinones was carried out next. Durst and Charlton have reported an efficient preparation of 1-alkyl sultines such as (244) from 1-hydroxysulfone (106).[276] Extension of this methodology to our aryl substituted sulfone (103) led to the formation of a

1:10 mixture of 1-methylsulfone (245) and 1-methylsultine (246) which could be separated by flash chromatography.

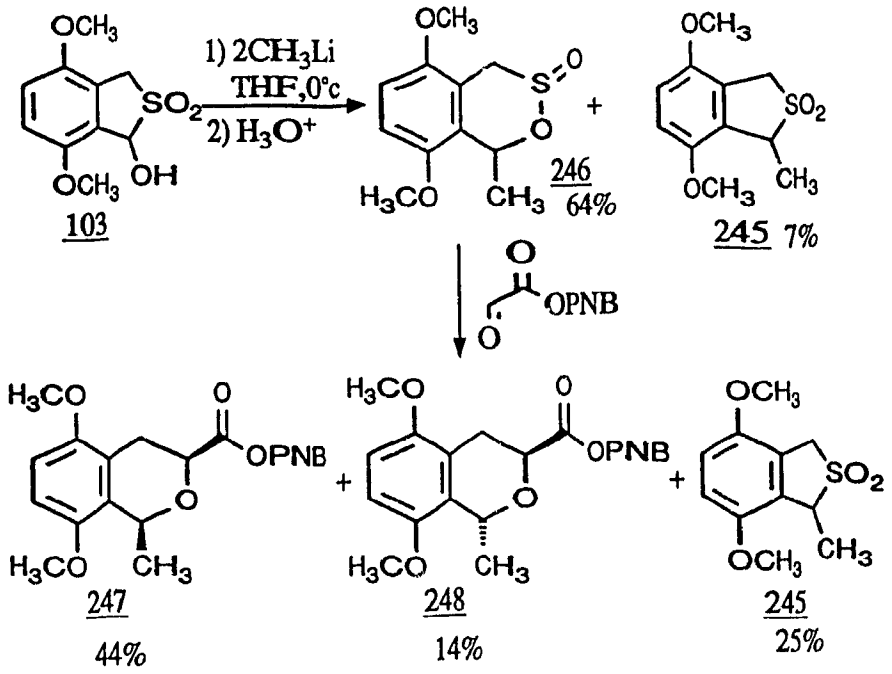
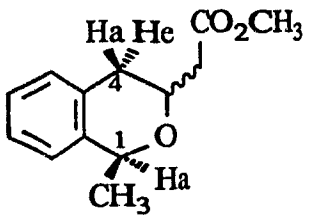
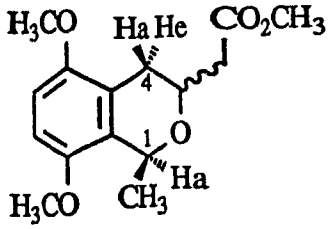


Fig 2.7

The 1-methylsultine (246) was then reacted with excess p-nitrobenzylglyoxalate in refluxing toluene for 10 hours. In addition to a 25% yield of the 1-methylsulfone (245), a 3:1 mixture of isochromans (247) and (248) was obtained. The stereochemistry of compound (247) was assigned by analogy with closely related isochroman derivatives[318-322] such as (249) and (250). The pyran ring prefers a pseudochair conformation with the C-3 substituent oriented

pseudoequatorially. The benzylic methine hydrogen (H_a at C-1) is pseudoaxial and shows significant long-range, homobenzylic coupling constants in the cis isomer but not in the trans isomer (Fig 2.8).



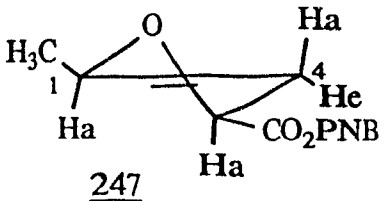


249a cis: $J_{H1a-H4a}$= 1.5Hz
$J_{H1a-H4e}$= 1.3Hz
249b trans: J_{1-4}< 0.5Hz

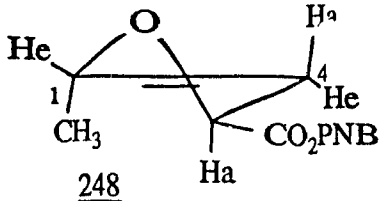
250a cis: $J_{H1a-H4a}$= 1.5Hz
$J_{H1a-H4e}$= 1.5Hz
250b trans: J_{1-4}< 1Hz

Fig 2.8

Thus isochroman (247) has significant homobenzylic coupling constants whereas compound (248) does not (Fig 2.9). Therefore (248) is assigned the trans conformation.



247



248

$J_{H1a-H4a}$= 1.5Hz
$J_{H1a-H4c}$= 1.2Hz
$J_{H3a-H4a}$= 11.4Hz
$J_{H3a-H4c}$= 3.0Hz

J_{1-4}<0.7Hz
$J_{H3a-H4a}$= 9.0Hz
$J_{H3a-H4c}$= 4.4Hz

Fig 2.9

Oxidative demethylation was carried out with ceric ammonium nitrate (CAN) as reported for other isochromans.[320,323] Thus, both quinones (251) and (252) were obtained in good yield by treating the isochroman precursors with CAN for 5 minutes at room temperature.

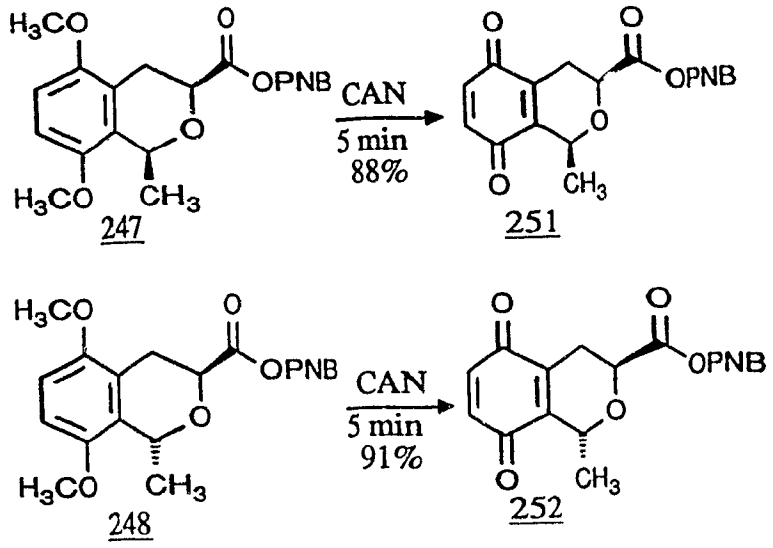


Fig 2.10

Cycloaddition between these quinones and two equivalents of o-quinodimethane, generated from sultines (167) and (236), gave the tetracyclic derivatives (253) to (256) in reasonable yields. All these tetracyclic analogs of nanaomycin gave the expected ^{1}H, ^{13}C, infrared and mass spectra.

Having established the feasibility of building tetracyclic pyrano structures with a carboxylate C-9 side

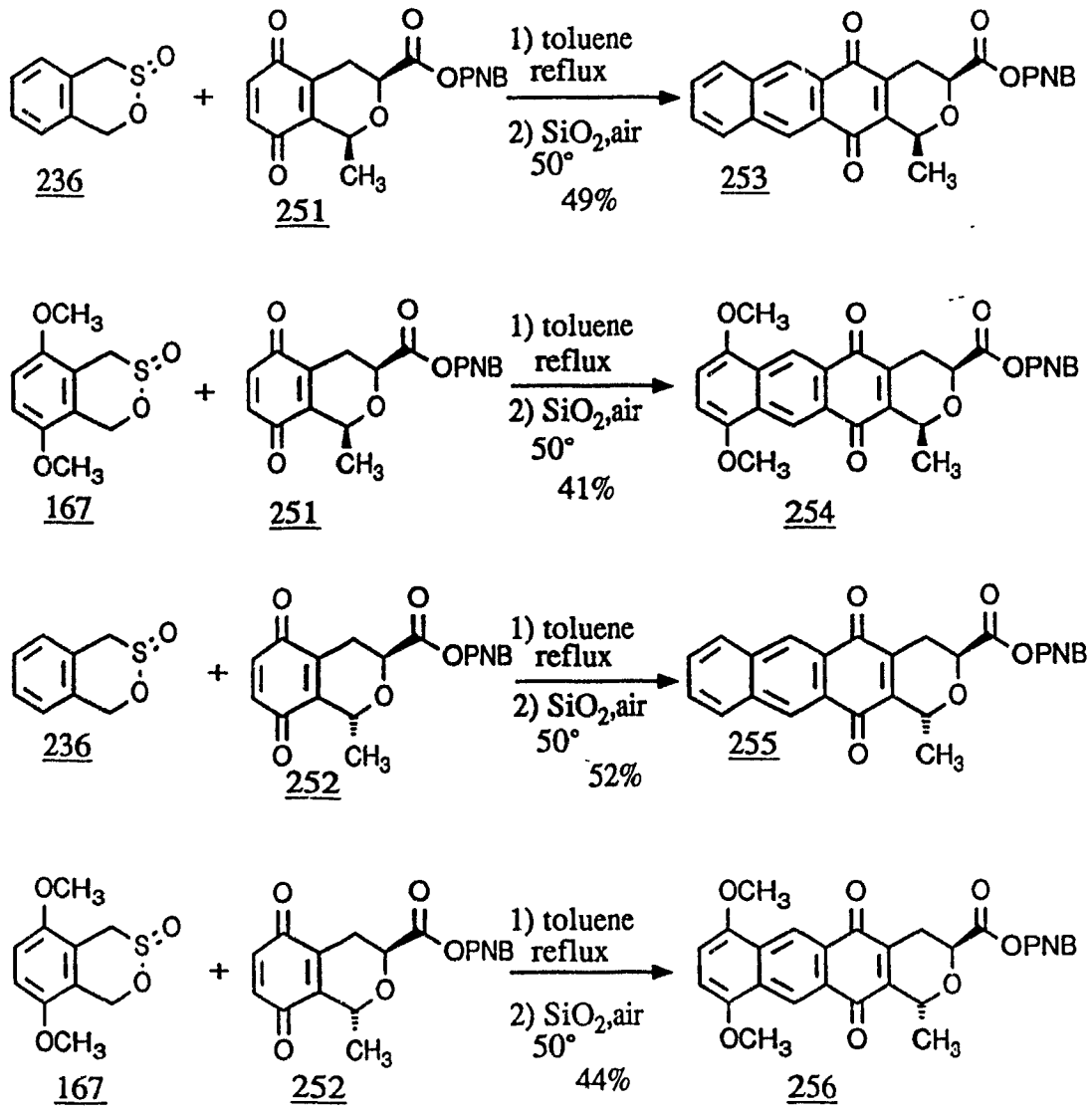

236
251
1) toluene reflux
2) SiO2,air 50°
49%
253
167
251
1) toluene reflux
2) SiO2,air 50°
41%
254
236
252
1) toluene reflux
2) SiO2,air 50°
52%
255
167
252
1) toluene reflux
2) SiO2,air 50°
44%
256

Fig 2.11

the preparation of one tetracyclic methyl ketone cycloadduct was considered next.

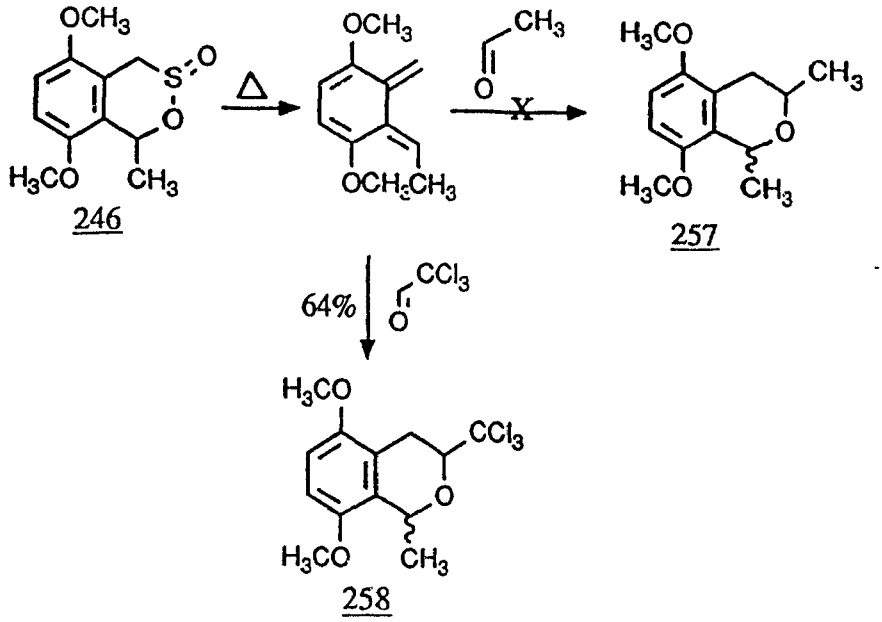


Fig 2.12

The possibility of trapping the o-quinodimethane with methylglyoxal, acetaldehyde, and chloral was investigated. Attempts with acetaldehyde failed to give cycloadduct (257) and invariably gave the dihydrothiophene-2,2-dioxide. This is attributed to the low dienophilic activity of the carbonyl. When chloral was used, a reasonable yield of isochroman (258) was obtained, consistent with the fact that electron withdrawing substituents are required to activate the aldehyde for cycloaddition.

The reaction between the dimethoxy-o-quinodimethane and pyruvaldehyde gave a low yield of isochroman (259), probably due to the poor reactivity of the dienophile.

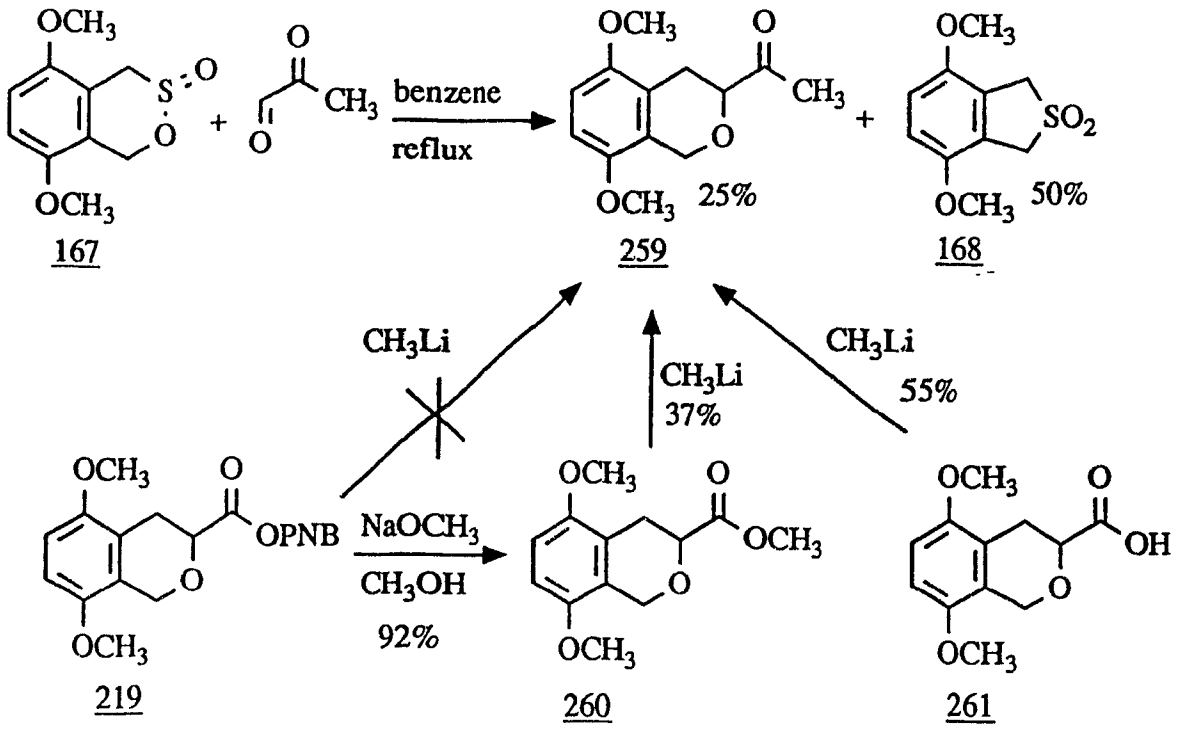


Fig 2.13

Compound (259) could also be obtained from (260) or (261) by carefully treating them with methyl lithium. With (260) poor results were obtained due to the rapid addition of a second methyl anion giving a 40% yield of the tertiary alcohol. Interestingly the p-nitrobenzyl ester is resistant to attack by CH_3Li even at room temperature. Proton abstraction at the p-nitrobenzyl position must occur. Quenching of a mixture of methyl lithium and (219) with

methanol gave the methyl ester (260) in good yield. An explanation for this is illustrated in fig 2.14.

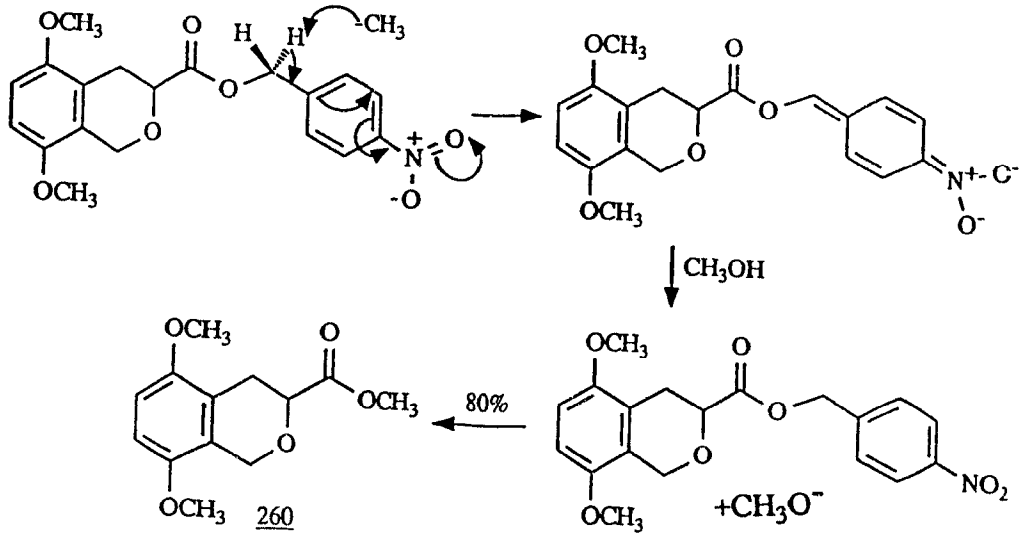


Fig 2.14

The oxidative demethylation of isochroman (259) with ceric ammonium nitrate went well and gave the pyranoquinone (262) in 92% yield.

The cycloaddition reaction between the o-quinodimethane generated from the sultine and quinone (262) gave the desired pyranotetracyclic cycloadduct (238), but its separation from the benzyl fused dihydrothiophene-2,2-dioxide was very difficult. After extensive experimentation analytical amounts of compound (238) could be obtained in 10% yield after repeated fractional crystallizations, followed by preparative TLC. Further elaboration for the

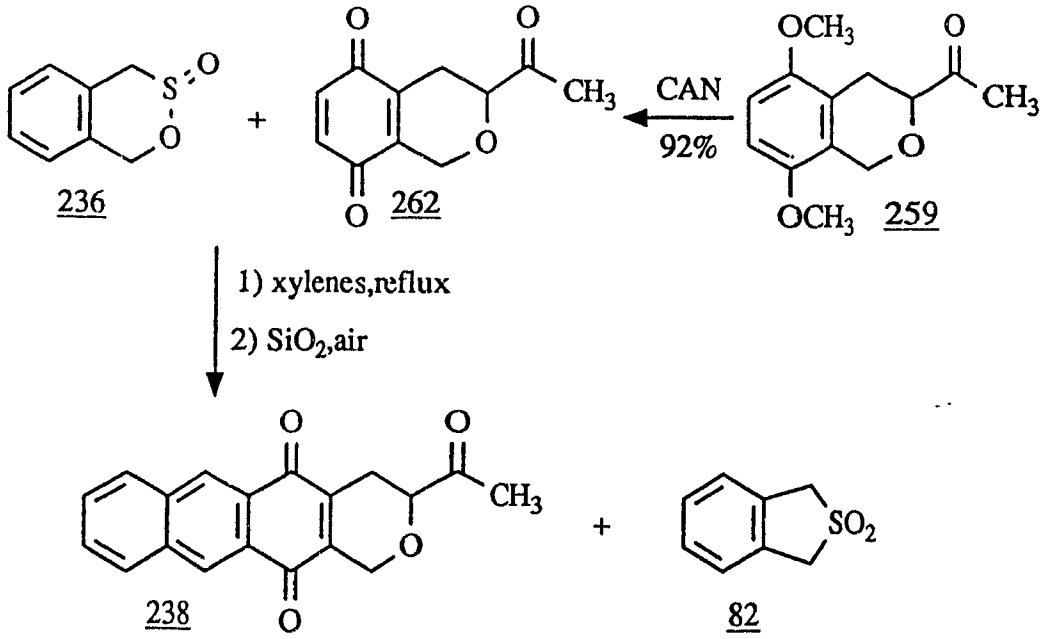


Fig 2.15

preparation of derivatives with an acetyl side chain was discontinued. The examples shown here indicate that the sequential addition of two o-quinodimethane and an aldehyde is a viable route for the preparation of anthraceno[2,3-C]pyranyl tetracycles.

2.1.2 Total Synthesis of 5,12-Dioxo-3,4,5,12-tetrahydroanthraceno[2,3-c]pyran-3-yl Glycosides

The functionalization of the benzylic position to get the tetracyclic aglycone (263) was considered next.

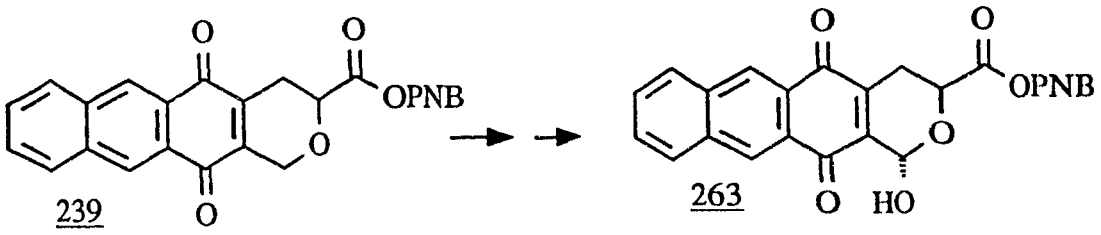


Fig 2.16

Bromination of (264) followed by displacement of the bromide with trifluoroacetate and methanolysis (Fig 2.17) generally gives a poor yield of daunomycinone (266).[325] Aromatized material and epidaunomycinone (265) are obtained in significant amounts. Hassal [326] has obtained a higher yield of a correctly functionalized daunomycinone, but this required the bromination of the fully protected 7-deoxydaunomycinone substrate (267), thus extending the synthesis by four additional steps.

The problems observed with the natural deoxyaglycones should not be significant with the pyranotetracyclic derivative (239). The pyran ring oxygen should efficiently direct the formation of the benzylic radical, which would be captodatively stabilized. To prevent the formation of the wrong cis isomer, the hydroxylation should be carried out

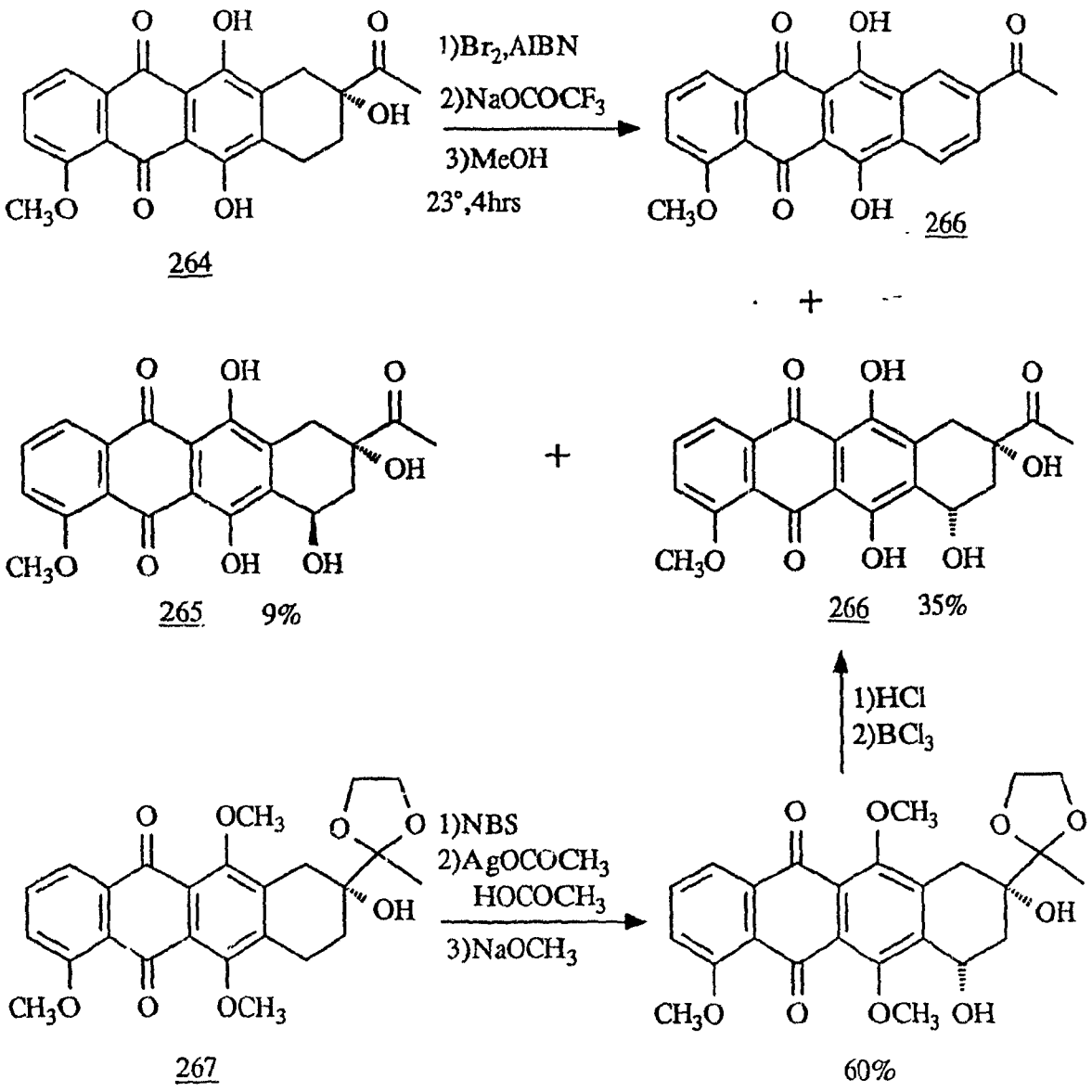

1)Br2,AIBN
2)NaOCOCF3
3)MeOH
23°,4hrs
264
266
265 9%
266 35%
1)HCl
2)BCl3
1)NBS
2)AgOCOCH3
HOCOCH3
3)NaOCH3
267
60%

Fig 2.17

under Sn^1 condition. The ester side chain would direct the addition of water towards the less hindered face (fig 2.18). Furthermore, the flat carbonium ion may attract the carbonyl oxygen towards itself and consequently only the trans isomer would be formed.

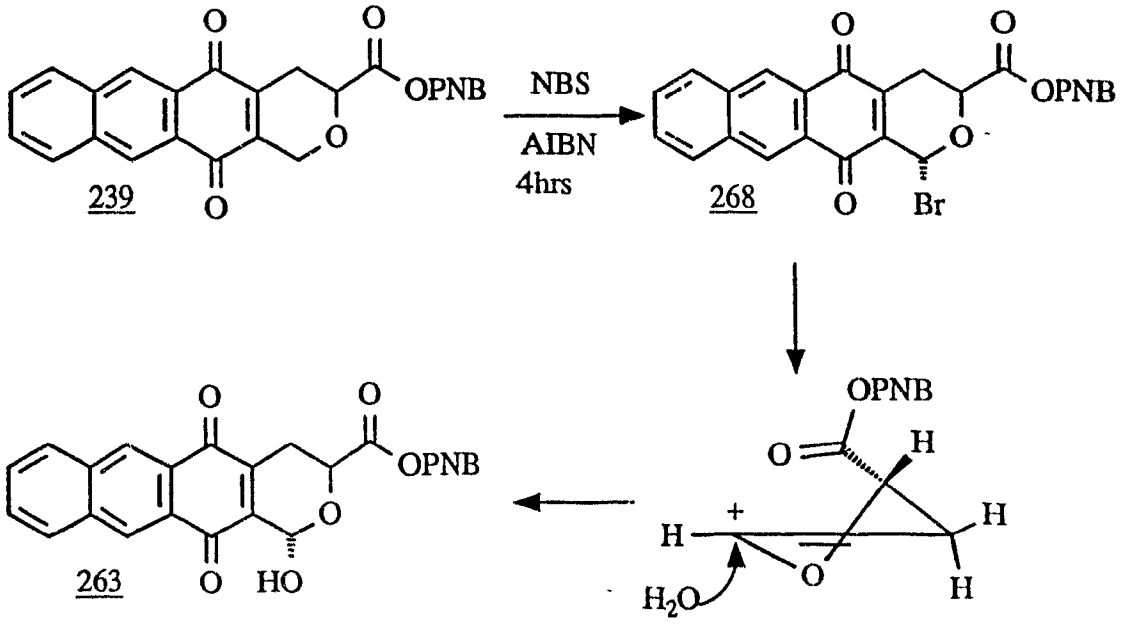
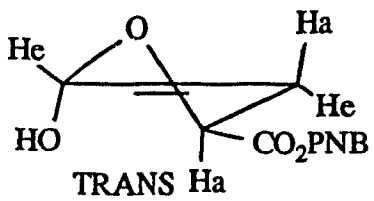


fig 2.18

Our rationale was confirmed experimentally. Bromination was effected with N-bromosuccinimide in the presence of AIBN and the resulting reaction mixture was stirred in THF-water for 1 hour. Following flash chromatography, a 78% yield (2 steps) of aglycone (263) was obtained. No other product was isolated with the exception of 9% of starting material.

Compound (263) gave the expected IR, PMR and CMR spectra. The PMR spectral analysis of the coupling constants of the protons in the pyran ring of (263) was helpful for a stereochemical assignment. From the couplings between H-3 and H-4, a pseudochair conformational assignment is made in which the p-nitrobenzyl ester side chain is oriented pseudoequatorially.



$J_{H3a-H4a} = 11.4Hz$

$J_{H3a-H4e} = 4.3Hz$

$J_{1-4} < 0.5Hz$

Fig 2.19

No homobenzylic long range coupling was observed from the PMR spectrum obtained with a 300 MHz spectrometer. At best a width of 0.5 HZ could be measured from the peaks corresponding to the C-4 protons. A cis arrangement of the hydroxyl and ester groups would have given a conformation with the C-1 proton oriented pseudoaxially and, by analogy with previously encountered isochromans,[318-322] significant long range coupling constants would have been observed. Consequently, a trans stereochemical arrangement of the hydroxyl and the ester groups is assigned.

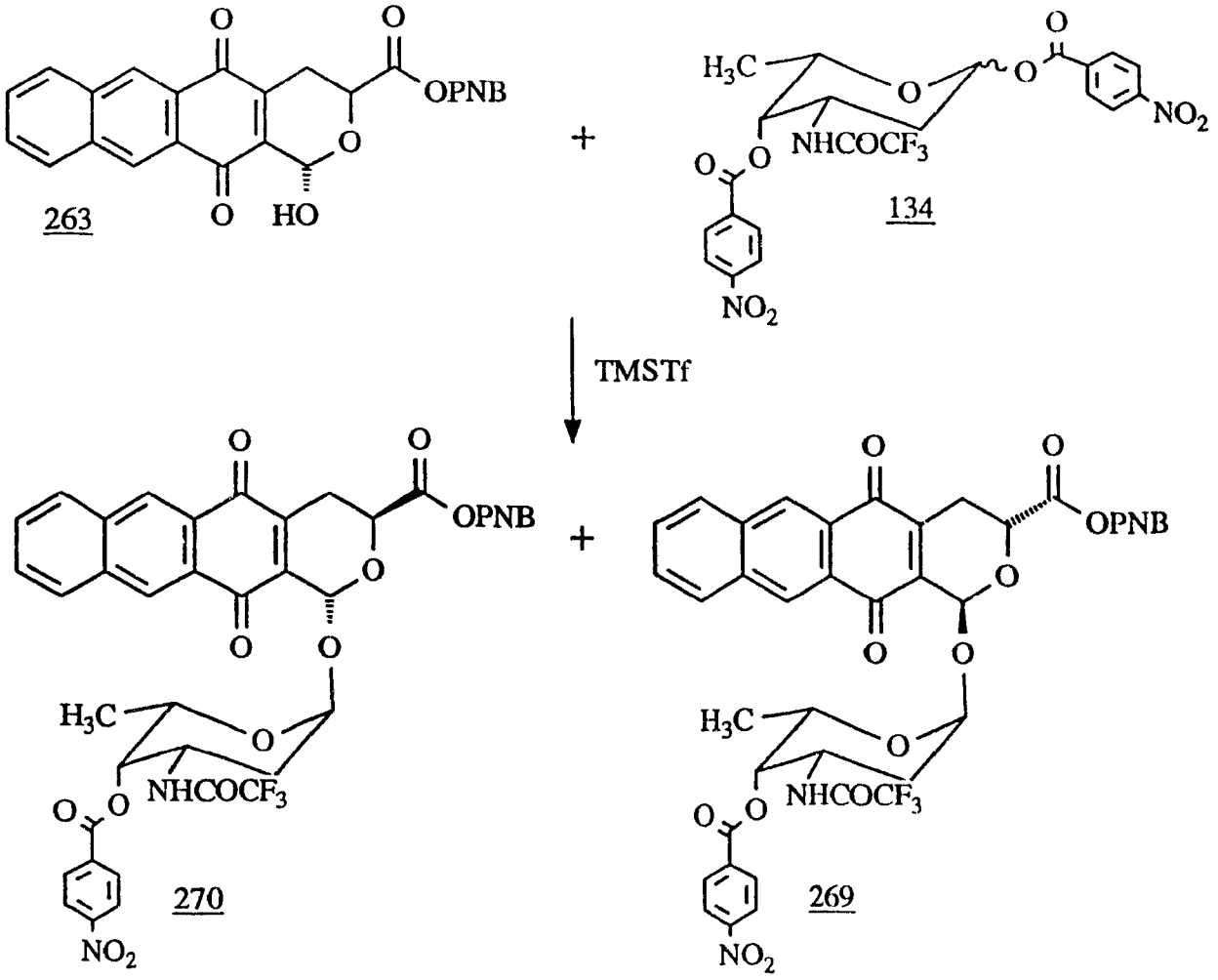
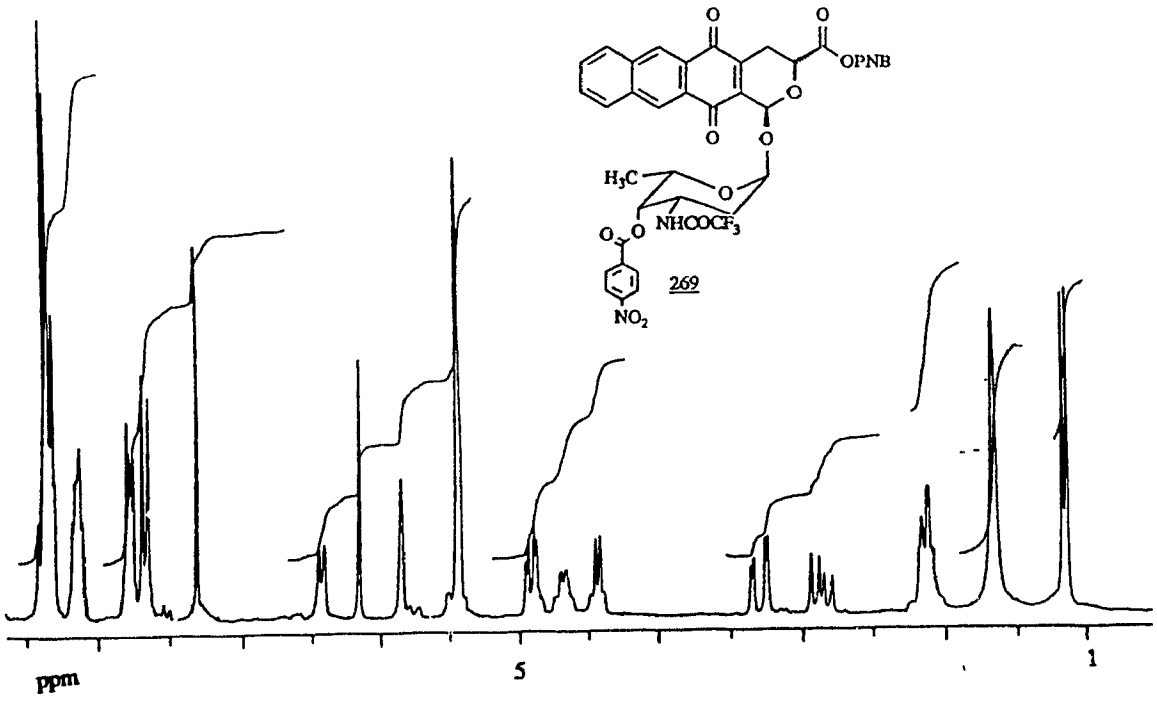


Fig 2.20

The glycosidation reaction was carried out with aglycone (263) and the protected daunosamine sugar (134) using a modification of the previously described reaction conditions (chapter 1). Botn glycosides (269) and (270) were obtained in a combined 75% yield after repeated flash chromatography. The less polar isomer was tentatively assigned structure (269), while compound (270) was considered as the more polar one. The proton NMR spectrum



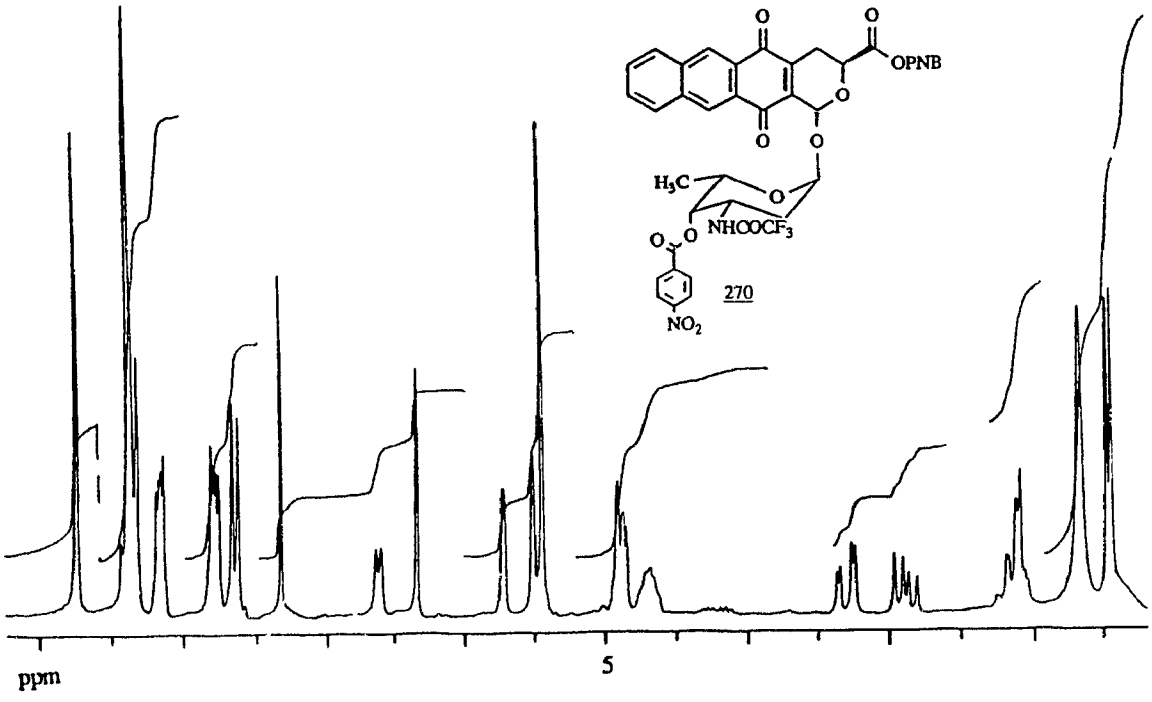


Fig 2.21

of both compounds is shown in fig.2.21. The infrared spectrum showed a broad ester and carbonyl absorption band centered at 1733 cm^{-1} for glycoside (270) and 1738 cm^{-1} for (269). The quinone carbonyl absorption band occurred at 1669 cm^{-1} for both compounds. The ^{13}C NMR spectra showed reasonances for all the forty carbons in the structure. The proton NMR spectra was consistent with structures (270) and (269), and was very informative of the stereochemistry. In the case of ring A, coupling constants were determined as $J_{H3a-H4a}$=11.7Hz and $J_{H3a-H4e}$=3.7Hz for compound (269) and $J_{H3a-H4a}$=11.3Hz and $J_{H3a-H4e}$=4.3Hz for (270), consistent with a pseudochair conformation (fig.2.22). Since the

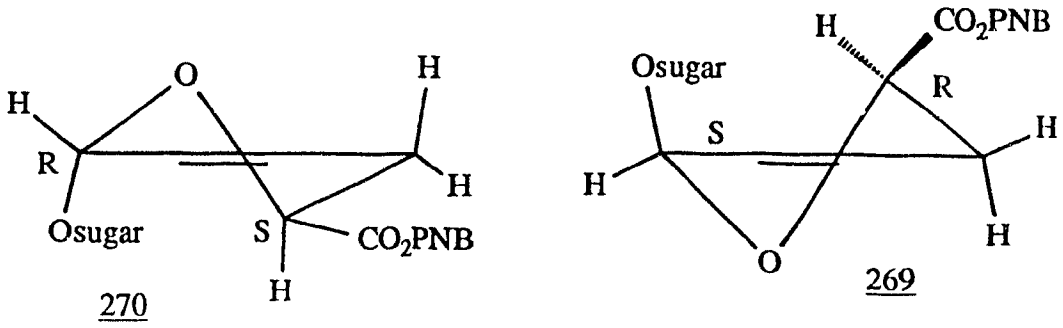


Fig 2.22

long range coupling constants, J_{1-4}, was less than 0.7Hz for both glycosides, the C-1 and C-3 substituents are placed trans to each other. The sugar conformation is assignable as shown in fig.2.23 and both anthracycline derivatives are alpha glycosides. The H-1′ proton gave a broad singlet at 5.82 (W_H 6hz) and 5.69 ppm (W_H 7Hz) for compounds (269) and (270), respectively. By analogy to the natural system and

as previously discussed, this would occur with the alpha glycoside and the sugar conformation can be assigned as shown in fig.2.23. Once the conformation of ring A and the daunosamine was established, an assignment of the R and S stereochemistry at C-1 was attempted.

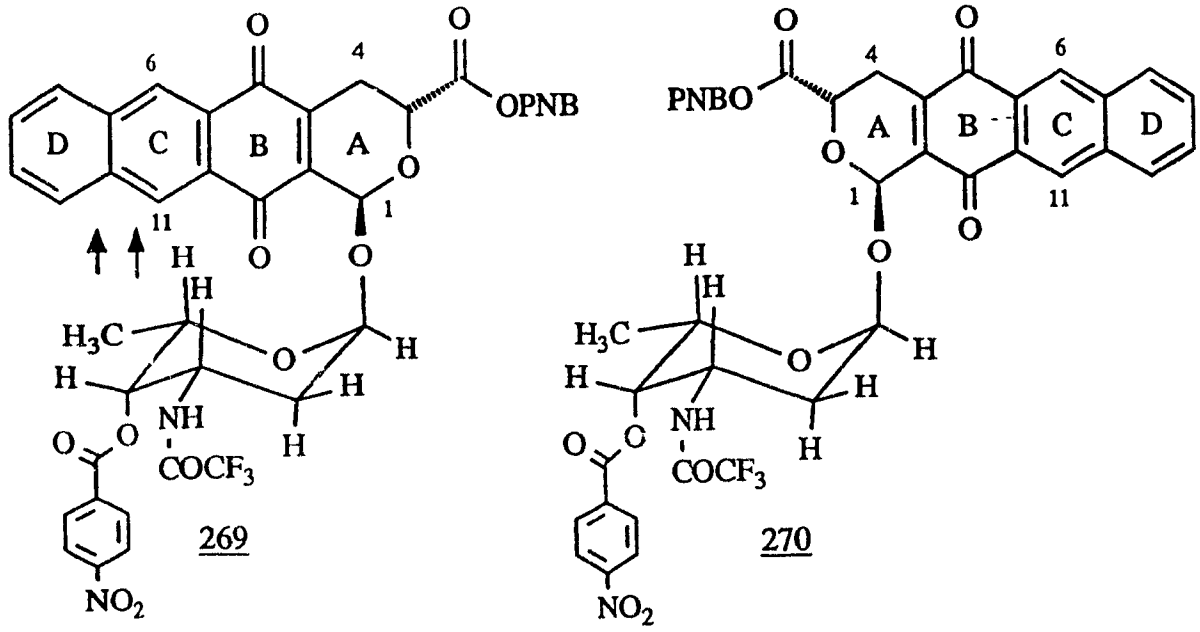


Fig 2.23

In the PMR spectrum of compound (270) resonances occured at 8.69 (s, 2H, ring-C), 5.47 (broad s, H-4′), 4.87 (m, H-5′), and 1.42 ppm (d, CH_3). These signals were shifted upfield in compound (269) and occured at 8.69 (s, 1H, ring-C), 8.64 (s, 1H, ring-C), 5.42 (broad s, H-4′), 4.40 (m, H-5′), and 1.12 ppm (d, CH_3). This can be explained if the sugar ring interacts with the aromatic portion of the tetracycle in glycoside (269). After

detailed analysis of CPK molecular models, it became apparent that in structure (269) the C-4′ to C-6′ portion of the sugar interacts with ring C of the tetracycle. The methyl group , the C-4′ and C-5′ protons can lie very closely on top of ring C, thus experiencing the shielding effect of the aromatic ring. This would be reflected on the PMR spectrum as an upfield shift. Two signals are observed for the ring C aromatic protons because the environment close to H-11 is much more different to H-6. In compound (270), these two protons experience a similar environment, because the daunosamine moiety cannot easily approach the tetracycle, and therefore are not easily distinguished.

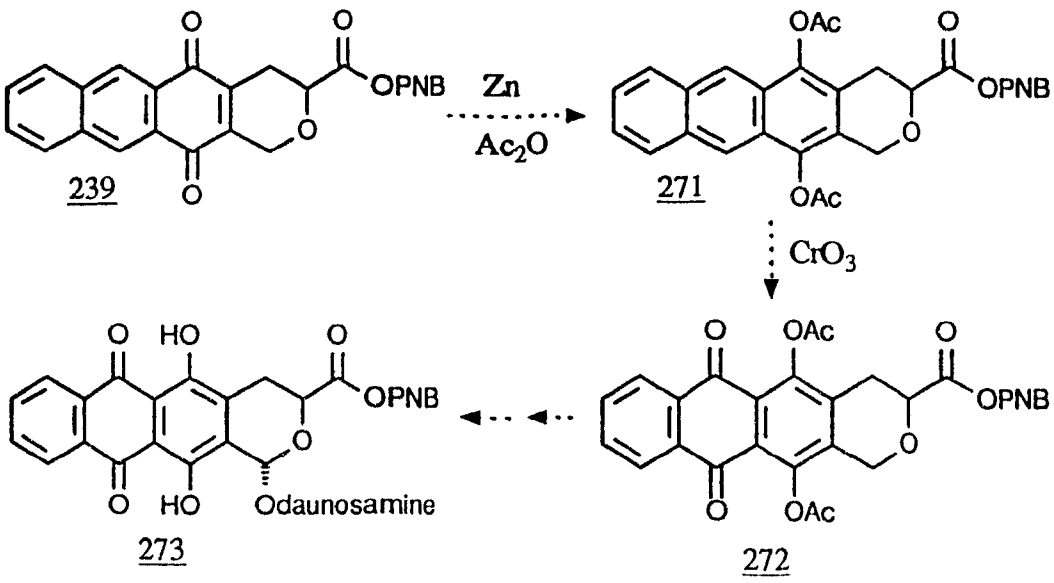


Fig 2.24

The synthesis of quinizarin functionalized pyranotetracyclic glycosides such as (273) was undertaken

next. It was anticipated that quinone tetracycle (239) should be converted to the required deoxyaglycone (272) by employing a reductive acylation-oxidation sequence as previously used in the synthesis of the natural 7-deoxydaunomycinones.[327,330] Our attempted reductive acylation of tetracycle (239) however gave very complex reaction mixtures, from which pure (271) could not be isolated. Activated Zn dust in acetic anhydride or acetyl chloride at 110°C for 14 hours was used. Any attempts to oxidize the reaction mixture from the reductive acetylation with CrO_3 in acetic acid-water at room temperature resulted in worse reaction mixtures. After extensive experimentation it became apparent that the desired deoxyaglycone could not be obtained from the pyranyltetracycle (239).

An alternative strategy which used the known bis(bromomethyl)quinizarin (274) was considered. The corresponding o-quinodimethane (275) has been generated and trapped with a variety of dienophiles[263,272,273,331] to give the 7-deoxyaglycone (276). In principle, the cycloaddition of p-nitrobenzylglyoxalate to (275) should rapidly provide the desired deoxyaglycone (277).

The required bis(bromomethyl)quinizarin was synthesized in 55% yield in two steps from the reported condensation of phthalic anhydride with 2,3-dimethylhydroquinone in a melt of $AlCl_3$/NaCl at 185°C followed by bromination with bromine or NBS in carbon tetrachloride.[272] Compound (274) had

physical and spectral data in agreement with the reported ones.

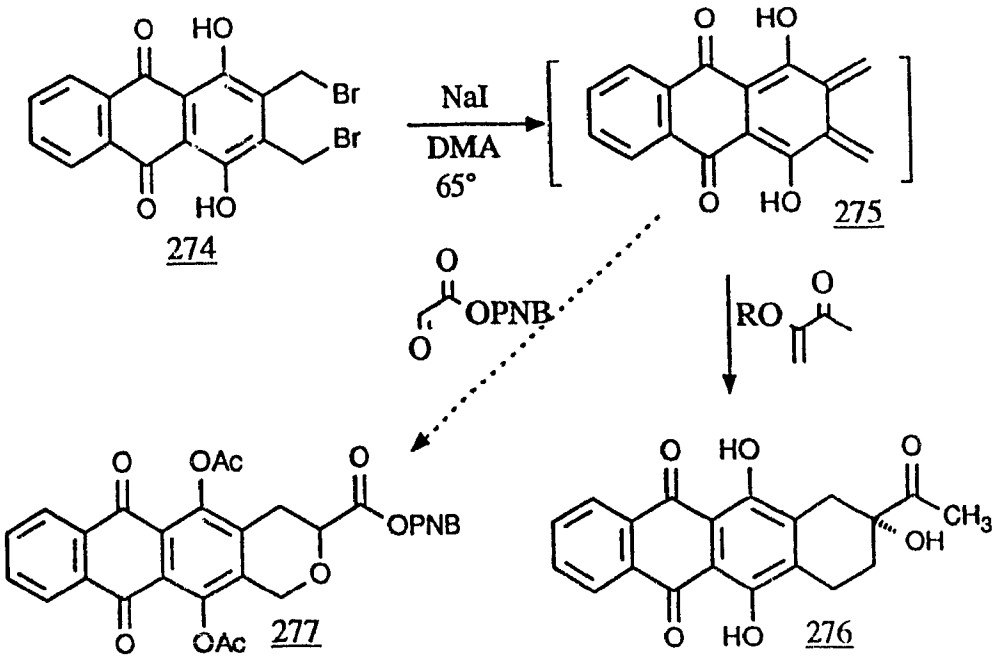


Fig 2.25

Bis(bromomethyl)quinizarin (274) proved to be synthetically useless in the reaction with p-nitrobenzylglyoxalate. The only product obtained was the highly insoluble dimer (278), even when 25 equivalents of the glyoxalate was used as dienophile. Compound (278) gave comparable physical and spectral characteristics as reported.[272]

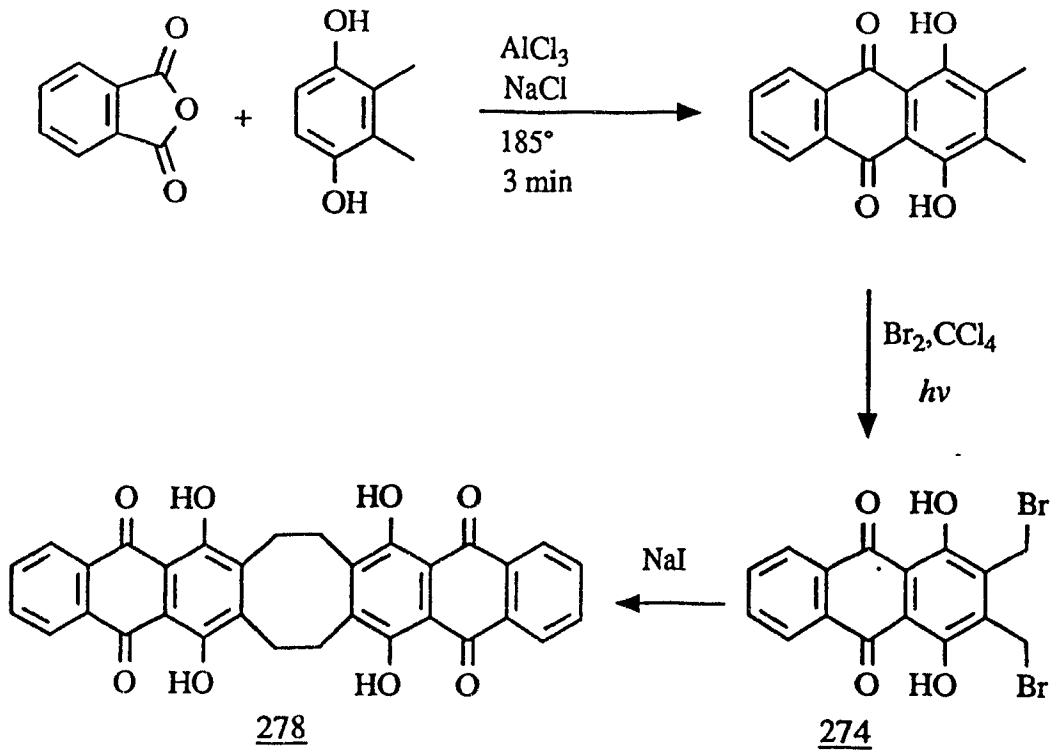


Fig 2.26

As seen previously, the trapping of an o-quinodimethane with a glyoxalate was successful when a sultine was used as precursor. Therefore, the possibility of generating the

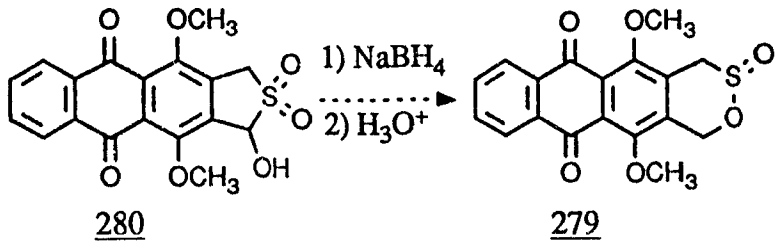


Fig 2.27

anthraquinodimethane from a sultine such as (279) was considered next. The synthesis of quinizarin fused sultine (279) was envisaged from the 7-hydroxyquinizarinesulfone (280).

The methylation of dimethylquinizarin (274) was effected in 87% yield with methyl tosylate and potassium carbonate.[263,272] Monobromination with NBS in CCl_4 gave the monobromide (282) in 80% yield along with 5% of the bis(bromomethyl)quinizarin derivative.

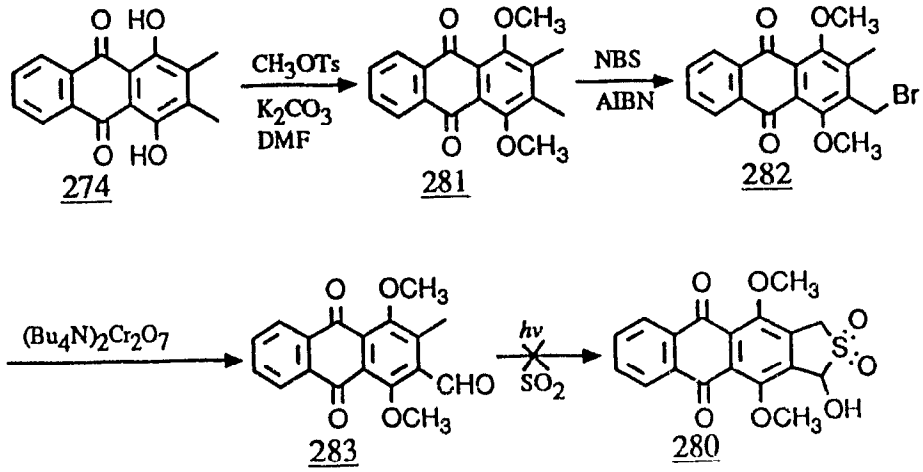


Fig 2.28

Oxidation of (282) gave a 50% yield of 1,4-dimethoxy-2-formyl-3-methylanthraquinone (283). Subsequent photochemical irradiation of (283) in the presence or absence of sensitisers did not yield any of the anthraquinone fused hydroxydihydrothiophene-2,2-dioxide

(280). Starting material was invariably recovered. Intramolecular quenching of the aldehydic triplet state by the quinone provides an explanation for this result. Triplet state quenching should be prevented if the irradiation were carried out on the hydroquinone (284). Its preparation was attempted from anthraquinone (283).

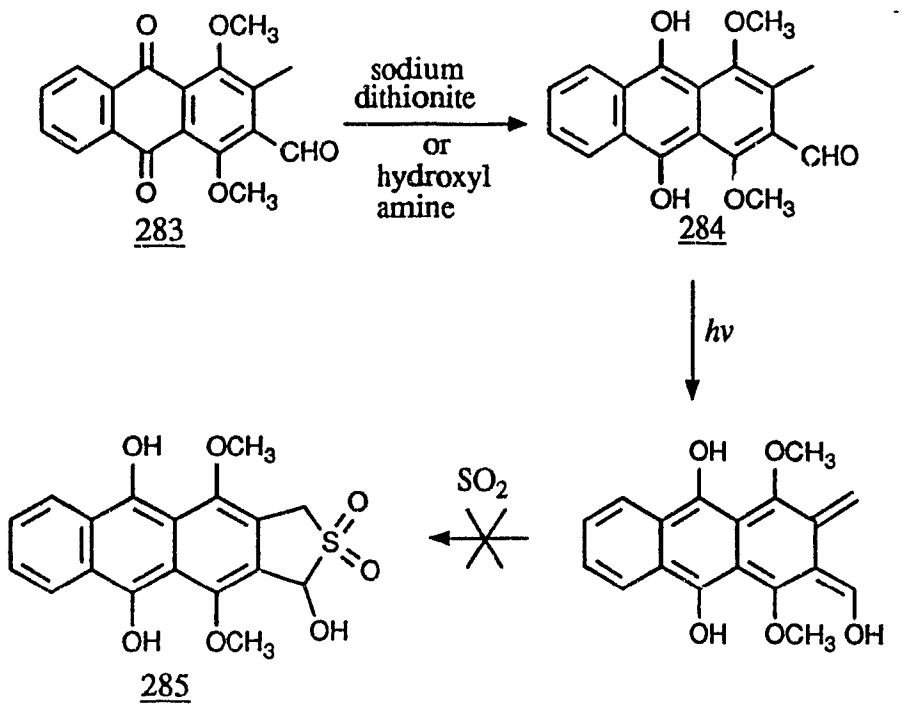


Fig 2.29

Thus, treatment of quinonealdehyde (283) with hydroxylamine or sodium dithionite[251] rapidly led to the consumption of starting material to give a polar air sensitive compound, presumably (284). Any attempts to isolate this product by flash chromatography or

crystallization led to the recovery of starting quinone. Since the infrared spectrum did show the disappearance of the quinone absorption band and the appearance of phenolic bands when the anthraquinone was treated with the reducing agents, the reaction mixture resulting from reduction of (283) was irradiated at 300 and 350nm in an SO_2-benzene solution. This however did not lead to any dihydrothiophene-2,2-dioxide (285) but only to recovery of quinonealdehyde (283). Failure to generate the anthracenic o-quinodimethane is possibly due to the quenching of any aldehydic triplet of anthracene (284) by unreduced quinone aldehyde (283). Further photochemical reactions were abandoned.

In an alternative approach, the preparation of the anthraquinonehydroxysulfone (280) was envisaged from the Friedel-Crafts reaction between phthalic anhydride or

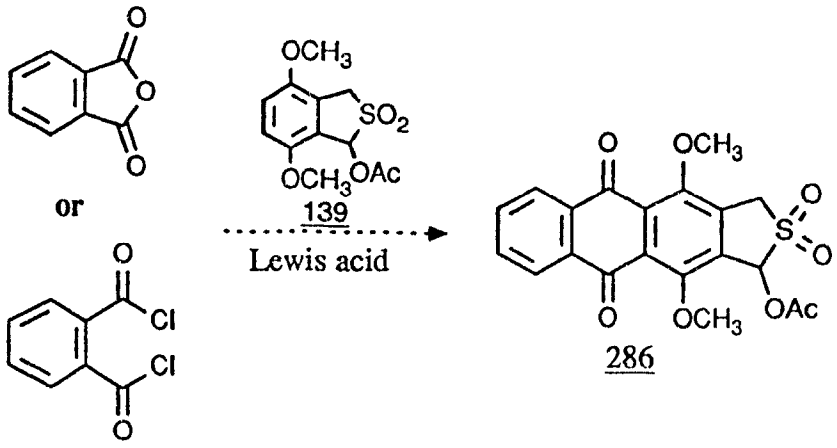


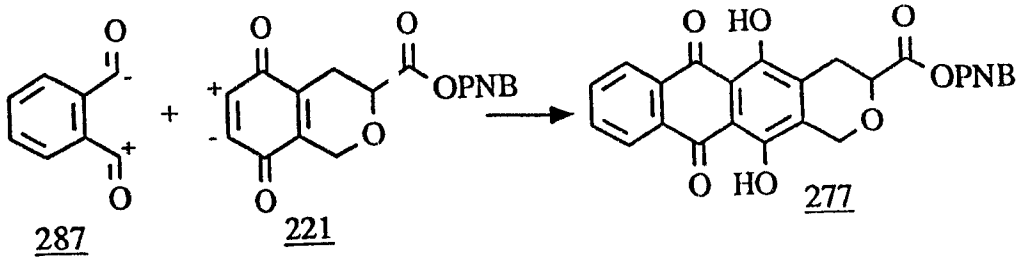
Fig 2.30

phthaloyl dichloride and the 1-acetoxysulfone (139) followed by alkaline hydrolysis of tetracycle (286).

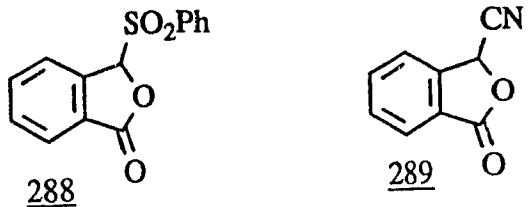
Application of Ishizumi et al procedure[332] for the electrophilic addition of phthalic anhydride to the sulfone catalyzed by an $AlCl_3$/NaCl melt under a variety of conditions unfortunately led to complex reaction mixtures of intractable materials. Only the previoulsy encountered 1-chlorosulfone (191) could be isolated in low yields. This is not surprising considering the drastic conditions employed in the reaction. The alternative Friedel-Crafts reaction which made use of phthaloyl dichloride and $TiCl_4$ under milder conditions also led to complex mixtures in which the chlorosulfone (191) predominated. Treatment of these reaction mixtures with TFA/TFAA for reaction times as long as four days at room temperature or at reflux in CH_2Cl_2 or dichloroethane failed to give any of the desired tetracyclic compound (286). Examination of the crude reaction mixtures by PMR spectroscopy pointed out the absence of proton reasonances for the methylene near 4.0 ppm. The signal associated with the acetate was no longer present. New proton signals were observed between 9.5 and 11.0 ppm possibly due to aldehydic prctons. These results indicate that the integrity of the sulfone ring was lost. The extension of the $TiCl_4$ catalyzed or the $AlCl_3$-NaCl melt Friedel-Crafts reactions to the sulfones containing an alkoxy alpha substituent such as methoxy, ethoxy or

isopropoxy, did not give cleaner reaction mixtures. The preparation of the quinonoid tetracycle (280) was abandoned.

In our next strategy, the synthesis of the pyranoquinone tetracycle (277) was considered from a benzannelation reaction of quinone (221). This approach has been extensively used in the preparation of anthracyclinones. The 1,4-dipole synthon (287) can be replaced by 3-(phenylsulfonyl)-1(3H)-isobenzofuranone (288)[333-340] or 3-cyano-1(3H)-isobenzofuranone (289).[341-347]



287 221 277



288 289

Fig 2.31

Benzannelation reactions with cyanoisobenzofuranone (289) generally gives better yields[248] and therefore its use was considered first.

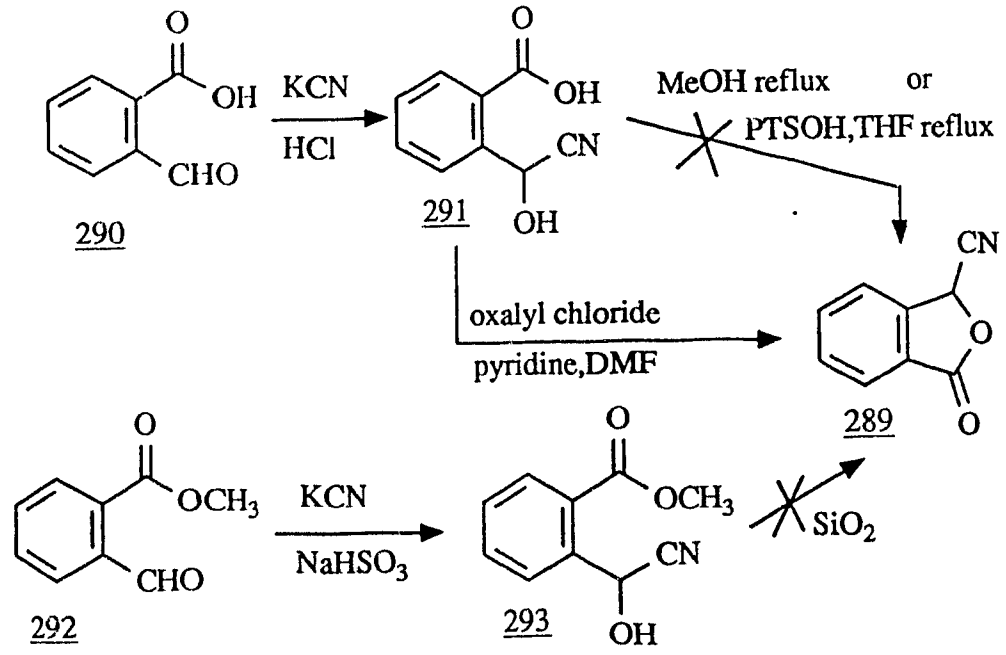


Fig 2.32

Surprisingly the attempted preparation of compound (289) via several methods reported in the literature failed in our hands. Thus, cyclization of cyanohydrin (291), obtained in 85% yield by treating opranic acid (290) with HCN, in refluxing methanol or THF with or without added acid catalysts was unsuccessful.[349] An alternate known route which uses cyanohydrin (293)[350] was also unsuccessful (Fig.2.32). After searching extensively the literature, we discovered that Swenton[351] had previously experienced the same difficulties in the preparation of cyanolactone (289). A new approach was reported in which the cyanohydrin (291) is cyclized with oxalyl chloride. Application of this technique gave a 90% yield of the desired compound (289).

The coupling of the cyanoisobenzofuranone with the p-nitrobenzyl ester derivative of pyranylquinone (221) or the methyl ester (294), obtained in 90% yield from the ceric ammonium nitrate oxidation of (264), failed to give (277) or (295) under numerous reaction conditions. Only complex mixtures of intractable polar products resulted.

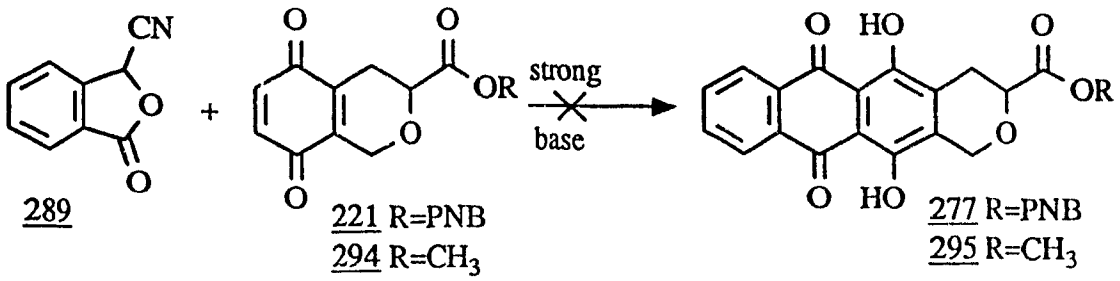


Fig 2.33

The fact that very polar products were obtained from the attempted benzannelation reactions with cyanoisobenzofuran suggested the formation of carboxylic acids such as (298) and (299) from the monoadducts (296) and (297).

In an attempt to separate some compounds, the above resulting reaction mixtures were treated with t-butyldimethylsilyl chloride in DMF with imidazole as catalyst or alternatively with t-butyldimethylsilyl trifluoromethane sulfonate. This however did not provide any clean products and isolation of pure compounds for

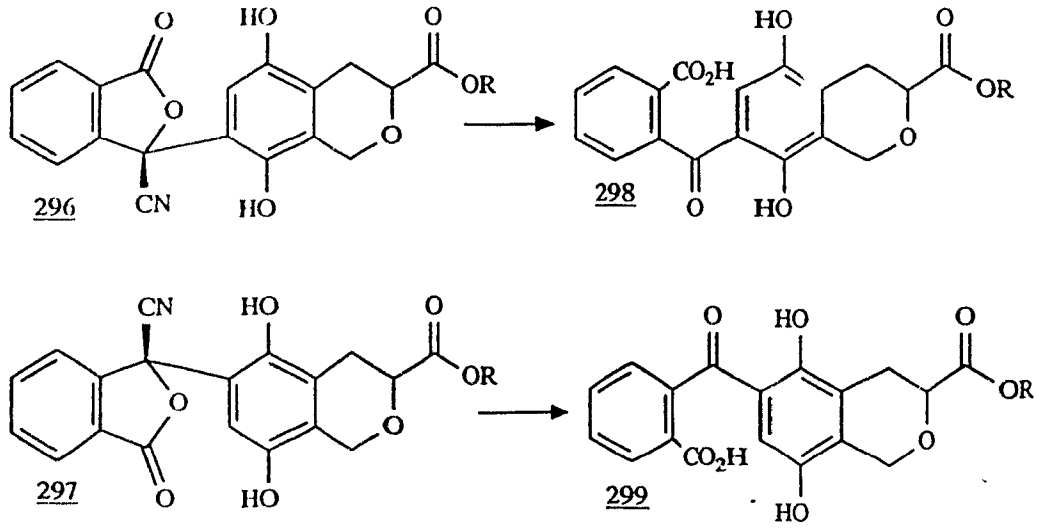


Fig 2.34

identificat:on purpose was not possible. In consideration of the difficulties associated with the cyanolactone benzannelation approach, further trials were discontinued.

Our attention was next turned to a process developed by Tamura in which a cycloaddition reaction between an enol (300) or an enolate (302) obtained from homophthalic anhydride with naphtoquinone can give the tetracyclic naphtacenedione (301).[352-355] This approach is of interest

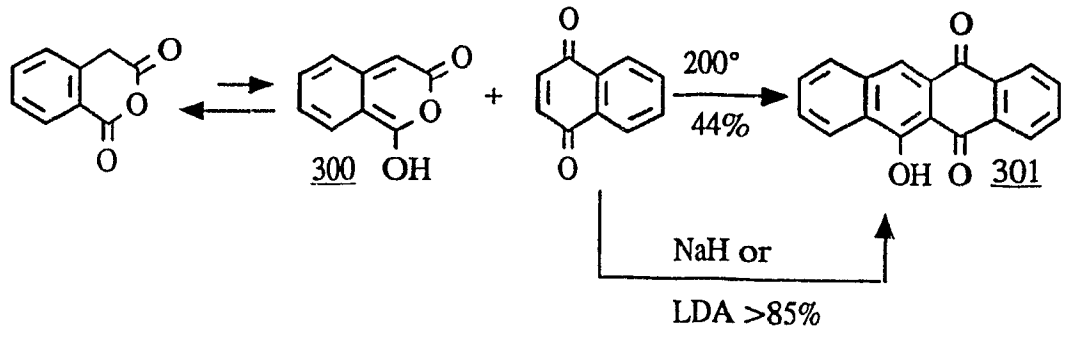


Fig 2.35

because analogs which contain the quinone at ring B can be prepared. A second hydroxyl moiety on ring C would be introduced oxidatively later on.

Our first attempt to prepare the pyranylanthraceno derivative (303) with the p-nitrobenzyl protected isochromandione was unsuccessful. This is not surprising because of the incompatibility of the protecting group with strong base.

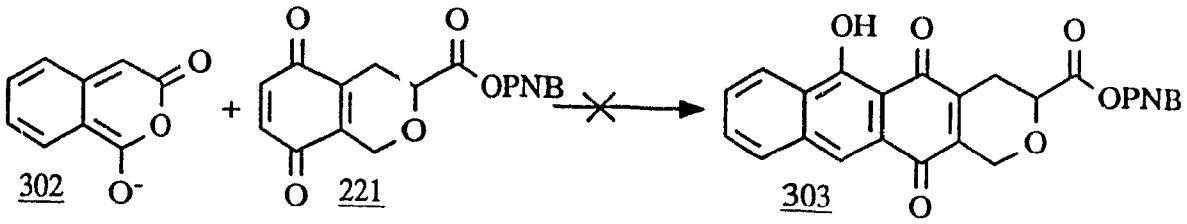


Fig 2.36

After numerous attempts the preparation of anthraceno[2,3-C]pyranyl tetracycles (308) and (309) could be effected in an 18% overall yield when lithium diisopropylamide was used as the base with isochromandione (305), obtained in 94% yield from (304). When sodium hydride was used a lower yield resulted. The major problem in this reaction was the resiliency of the tetracyclic carboxylic acids (306) and (307) to decarboxylate. Partial decarboxylation took place when these compounds were adsorbed on silica gel and allowed to stand under air for several days. An extra 12% yield of pyranyl tetracycles (308) and (309) could be obtained with this method.

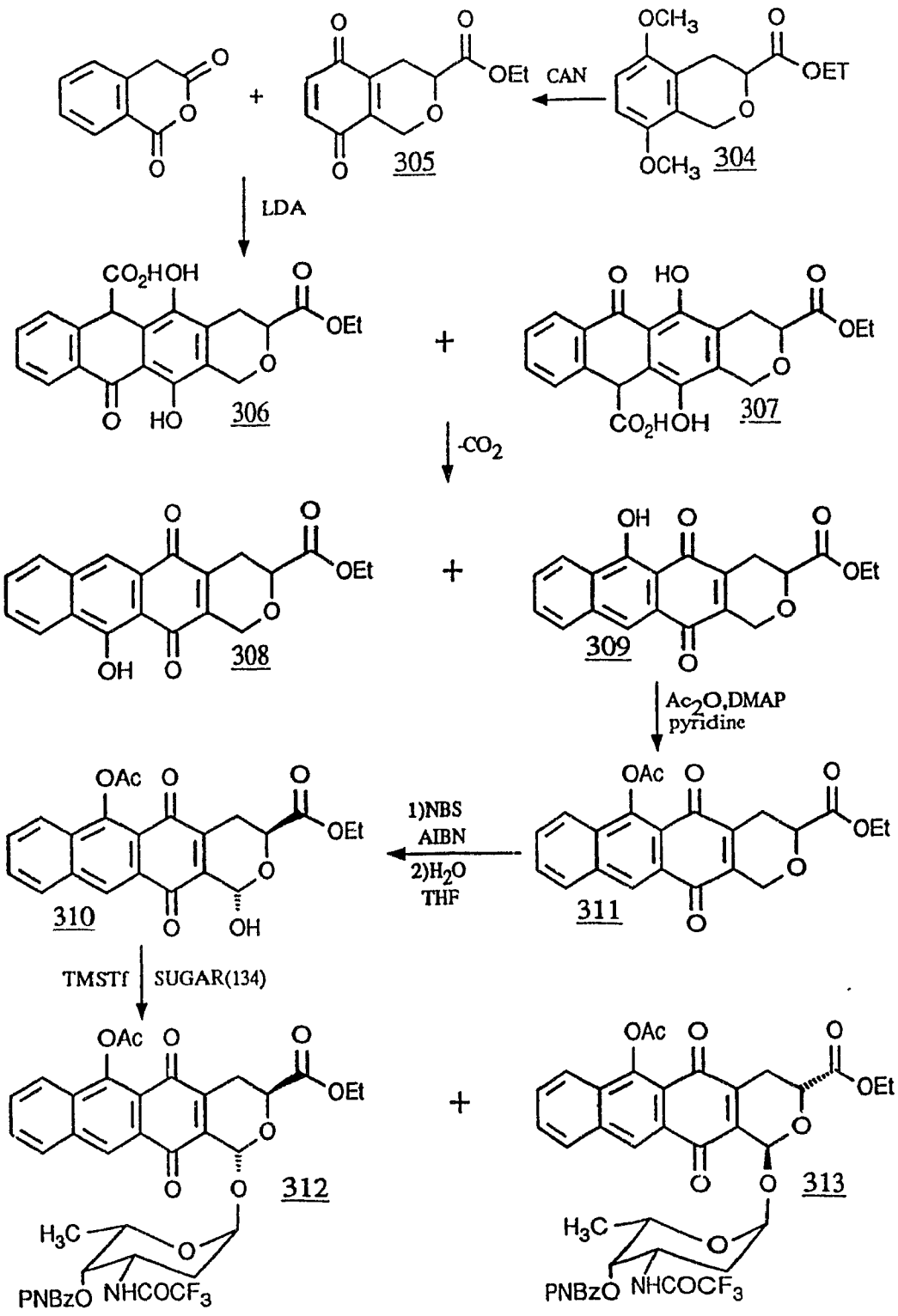
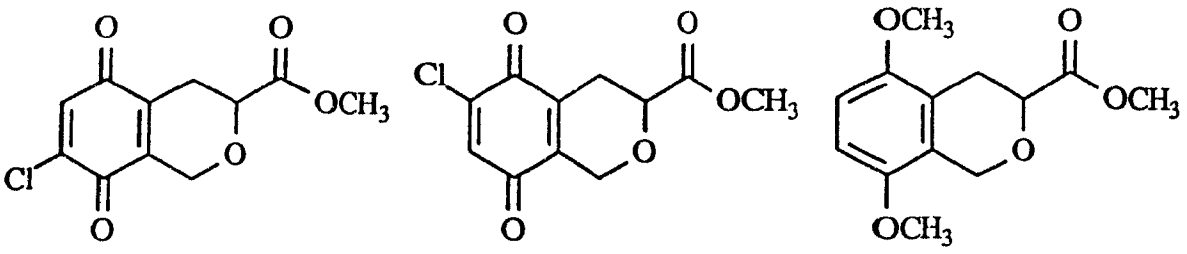

CAN
305
304
LDA
306
307
-CO2
308
309
Ac2O,DMAP
pyridine
1)NBS
AIBN
2)H2O
THF
310
311
TMSTf
SUGAR(134)
312
313
PNBzO
NHCOCF3

Fig 2.37

Separation of the two regioisomers was difficult and resulted in great losses of compound. Nevertheless, pure pyranotetracycle (309) was obtained and the synthetic sequence for glycosides (312) and (313) was carried out without any major difficulties. Thus, acetylation of (309) was best effected with acetic anhydride or acetyl chloride with one equivalent of dimethylaminopyridine to give the acetate (311) in 85% yield. Subsequent bromination and solvolysis of the bromide gave a 60% yield of the pyranotetracyclic aglycone (310), which was then glycosidated with the appropriate daunosaminyl derivative in an overall yield of 49% with 20% recovery of aglycone. The resulting diastereomeric glycosides (312) and (313) could not be separated by flash chromatography. Spectral analysis of these novel compounds leads to the structural assignment shown in figure 2.37.

In consideration of the problems associated with the separation of regioisomeric tetracycles, a regiospecific synthesis was considered next. Tamura[355] has reported that regiospecificity in condensations with homophthalic anhydride enolates can be achieved by using monochlorinated quinones. Furthermore, yields are generally improved when such quinones are used. Consequently, the preparation of chloropyranoquinones such as (314) and (315) was envisaged from the corresponding dimethoxyisochroman derivative (260).



314 315 260

Fig 2.38

Since the need for a good supply of intermediate (260) arose, a direct approach for its preparation was considered.

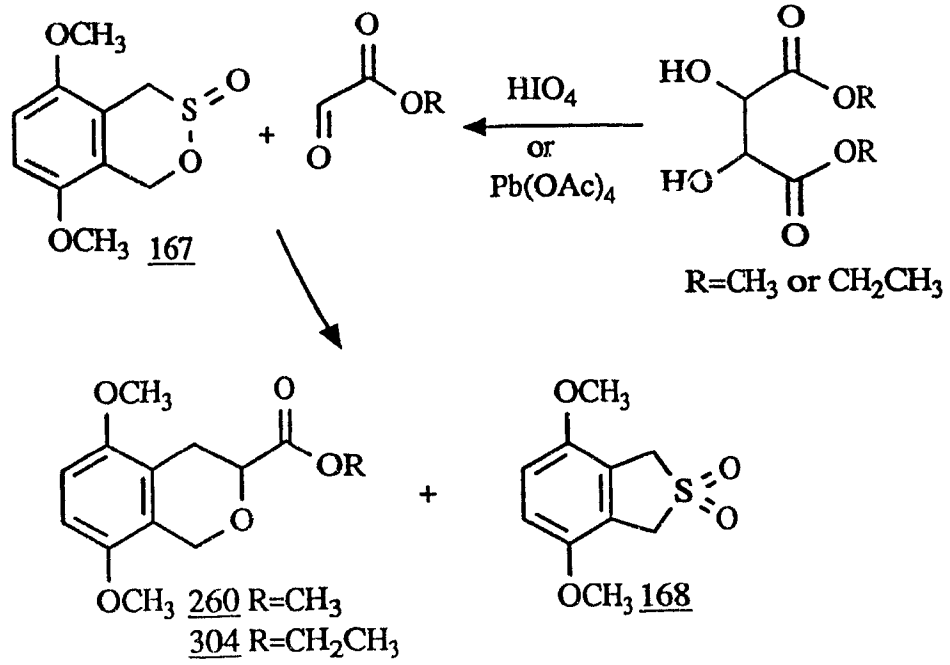


Fig 2.39

The cyclocondensation reaction between the o-quinodimethane, generated thermally from dimethoxybenzosultine (167) and methyl glyoxalate, conveniently prepared from the oxidative cleavage with periodic acid of dimethyl tartrate,[356] resulted in a low yield (10-15%) of the desired isochroman (260) under several reaction conditions. In contrast, the dimethoxybenzosulfone

(168) was always formed in moderate yields (40-60%). When ethyl glyoxalate was used for the cycloaddition in refluxing benzene, a surprisingly higher yield of 61% of isochroman (304) was obtained along with only 20% of sulfone (168). This may be accounted by the fact that both methyl and ethyl glyoxylates exist in oligomeric forms, and that the free aldehyde is generated thermolytically.[356] It is possible that, in the case of ethyl glyoxalate, there is a higher concentration of free aldehyde in refuxing benzene.

Chlorination of isochroman (304) with n-chlorosuccinimide[357] under ionizing conditions did not yield the desired chloroisochromans (316) and (317). The use of t-butyl hypochlorite and silicic acid has been suggested when aromatic chlorination is sluggish,[357] but this led to overchlorination in our case, with the result that the

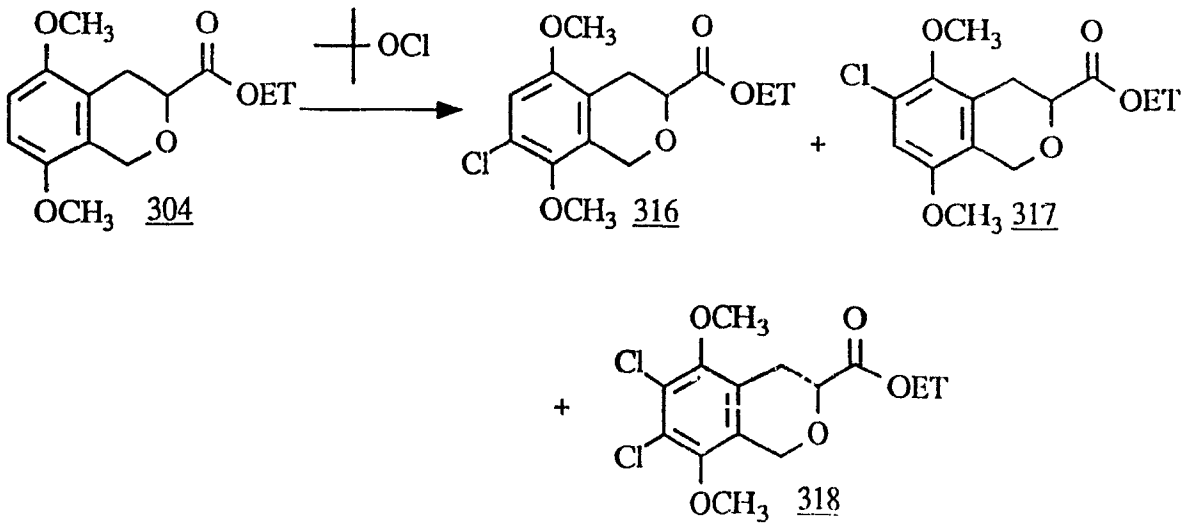


Fig 2.40

dichloroisochroman (317) was formed preferentially. Finally, after modulating carefully the reaction conditions, monochlorination was carried out in high yield (95%) with t-butyl hypochloride in the dark without silicic acid.

Separation of the two chlorides was very difficult and was carried out only on a small scale. The next step involved oxidative demethylation of (316) and (317) as a mixture, using ceric ammonium nitrate in acetonitrile-water. This reaction was quite sluggish and took 8 hours.

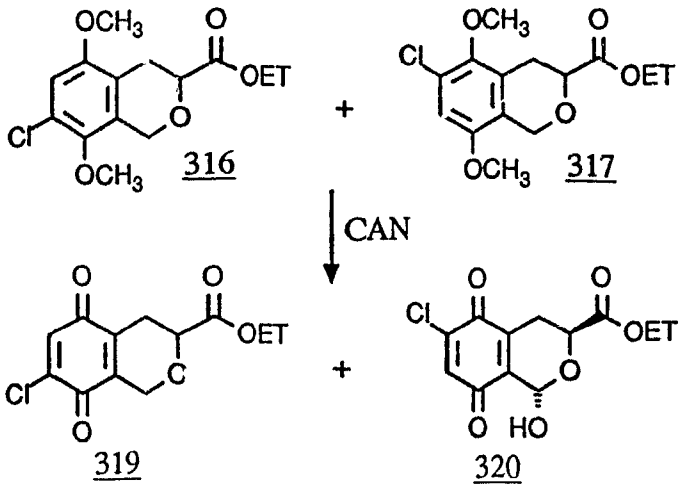


Fig 2.41

Most interestingly, the 1-hydroxylated pyranochloroquinone (320) was obtained in 25% yield while a 40% yield of (319) could be achieved. The same experiment carried out on the separated chloroisochromans (316) and (317) indicated that only one of the chloroisochromans was oxidized at the C-1 benzylic position. The combined effects of the p-chloro atom with the pyrano oxygen facilitates the

oxidation. Although quinone (320) could represent a strategic synthetic intermediate, it proved to be useless in further synthetic application with the homophtalic anhydride cyclocondensation.

Intermediate (319) was more useful and the reaction between the enolate of homophtalic anhydride and (319) gave exclusively pyranotetracycle (308) in 40% yield. After some experimentation, acetoxylation of (308) could be effected by using a modification of a known procedure.[355] Thus, treatment of (308) with excess lead tetraacetate in acetic acid for 3 days gave a modest yield (38%) of pyranotetracycle (321). Bromination of compound (321) followed by solvolysis proved to be troublesome. However, with intermediate (322), obtained in 87% yield from acetylation of pyranotetracycle (321), the bromination-solvolysis sequence gave the fully functionalised pyranoanthracene aglycone (323) in 70% yield. Subsequent glycosidation, as described previously, gave glycosides (324) and (325) in 61% overall yield. These novel heteroanthracycline derivatives were separated by flash chromatography and their structures assigned based on their PMR and CMR spectra.

Pyrano tetracycle (308) was also converted to glycosides (328) and (329). Thus, acetylation of (308) with acetyl chloride and dimethylaminopyridine for catalysis led to an 87% yield of compound (326), which was then subjected

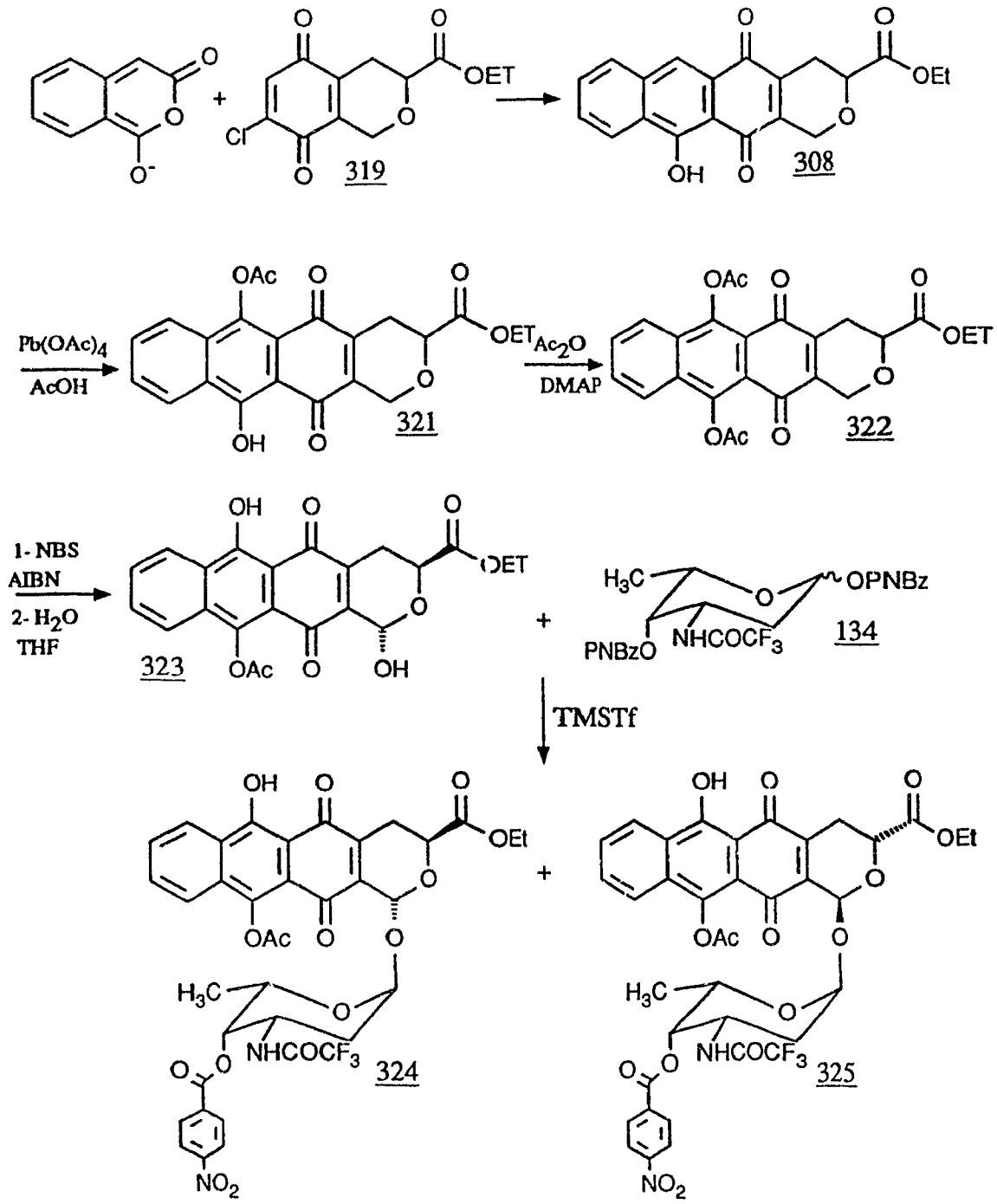

OEt
319
308
OAc
OH
Pb(OAc)4
AcOH
321
Ac2O
DMAP
322
1- NBS
AIBN
2- H2O
THF
323
H3C
OPNBz
PNBzO
NHCOCF3
134
TMSTf
324
325
NO2

Fig 2.42

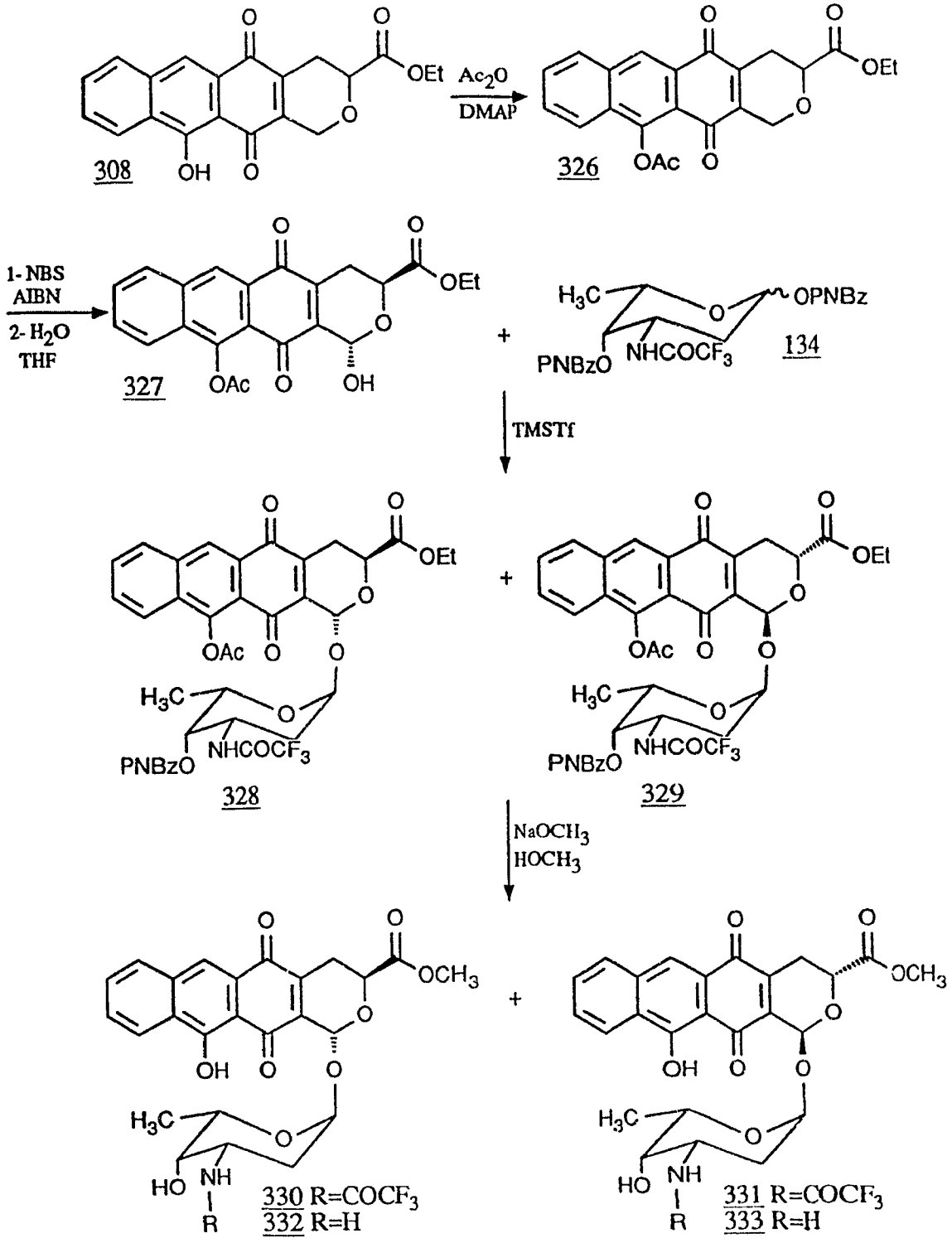

308
OH
OEt
Ac2O
DMAP
326
OAc
1- NBS
AIBN
2- H2O
THF
327
OAc
OH
H3C
OPNBz
PNBzO
NHCOCF3
134
TMSTf
328
329
NaOCH3
HOCH3
OCH3
HO
NH
R
330 R=COCF3
332 R=H
331 R=COCF3
333 R=H

Fig 2.43

to bromination with N-bromosuccinimide and AIBN. Solvolysis of the resulting bromide in THF-water gave in 67% yield the 1-hydroxyanthraceno[2,3-C]pyranyl derivative (327). Glycosidation of (327) as described before resulted in a 65% yield of a 1:1 mixture containing glycosides (328) and (329) which could not be separated by flash chromatography. All the ester based protecting groups were next removed in one step with catalytic sodium methoxide in methanol to give the 1-(trifluoroacetyldaunosaminyl)pyrano tetracycles (330) and (331) as a 1:1 mixture. All attempts to remove the trifluoroacetyl protecting group with 1 to 50 equivalents of sodium methoxide failed to give the 1-daunosaminylpyrano tetracycles (332) and (333). Further deprotection trials were stopped because of the limited supply of compounds (330) and (331).

Biological screening carried out at the National Cancer Institute, at Bethesda, Maryland (USA), showed that the 1:1 diasteriomeric mixture of (330) and (331) possesed equivalent in vitro anticancer activity as doxorubicin towards p388 mouse leukemia. The novel heteroanthracyclines had no cross resistance whatsoever in a 160 fold doxorubicin resistant p388 cell line. This is noteworthy since other ring A heteroanthracycline derivatives (334-337) reported during the course of our studies did not have any significant antitumor activity.[358-360]

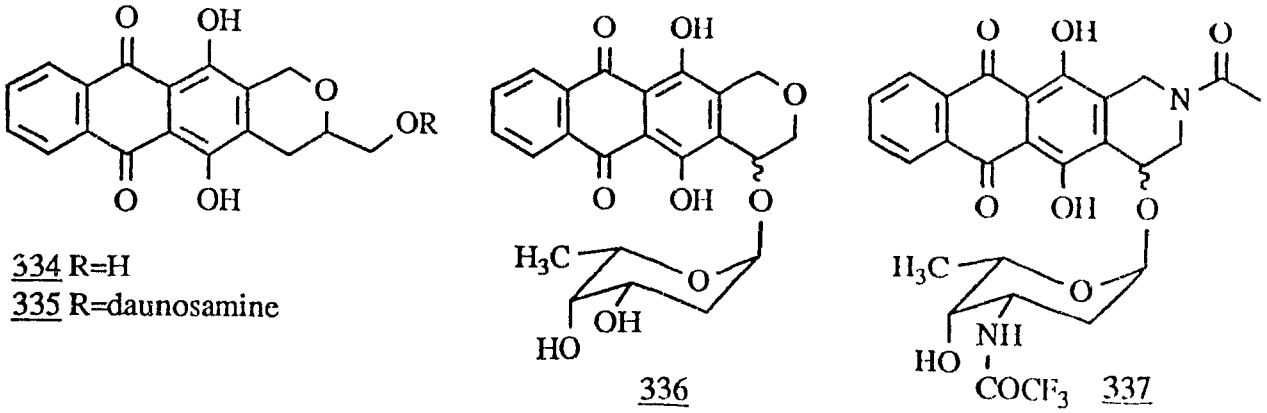


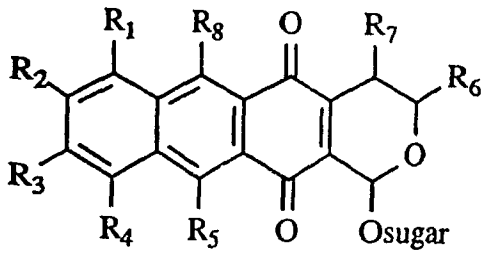
Fig 2.44

Evidently, the position of the A-ring oxygen is important. An oxygen atom at C-8 is not detrimental for the cytotoxicity and may in fact be beneficial. The finding that p388/dox do not resist the combined cytotoxic action of (330) and (331) indicates that these novel drugs operate via a mechanism not available to doxorubicin. A mechanistic rational would be premature at this point, but a limited proposal can be developped. It is known that p388 cells expressing resistance towards doxorubicin contain a depressed topoisomerase II activity. Consequently, even if doxorubicin or any of its reactive intermediates bind to the DNA, lethal double strand cleavage would decrease with the result that cytotoxicity is diminished. With the dihydropyranyl derivatives, any reactive intermediates that would result following bioreduction could be longer lived because of the additional stabilization provided by the ring

oxygen, and consequently there could be an increase of DNA binding of reactive species. This enhanced binding could counteract the lower levels of topoisomerase II found in doxorubicin resistant P388 cells and consequently no cross resistance would be observed.

2.1.3 Preliminary Experiments For Further Studies.

The fact that anticancer activity was observed with heteroanthracyclines (330) and (331) warrants further studies. The biological evaluation of heteroanthracyclines possessing a general structure such as (338) is of interest.



338

This presents a major difficulty, because a lengthy and tedious synthetic route is required for their preparation. Consequently, a shorter synthetic route should be developped.

Close retrosynthetic consideration of structure (338) leads to the disconnections shown in fig.2.45. Synthetic

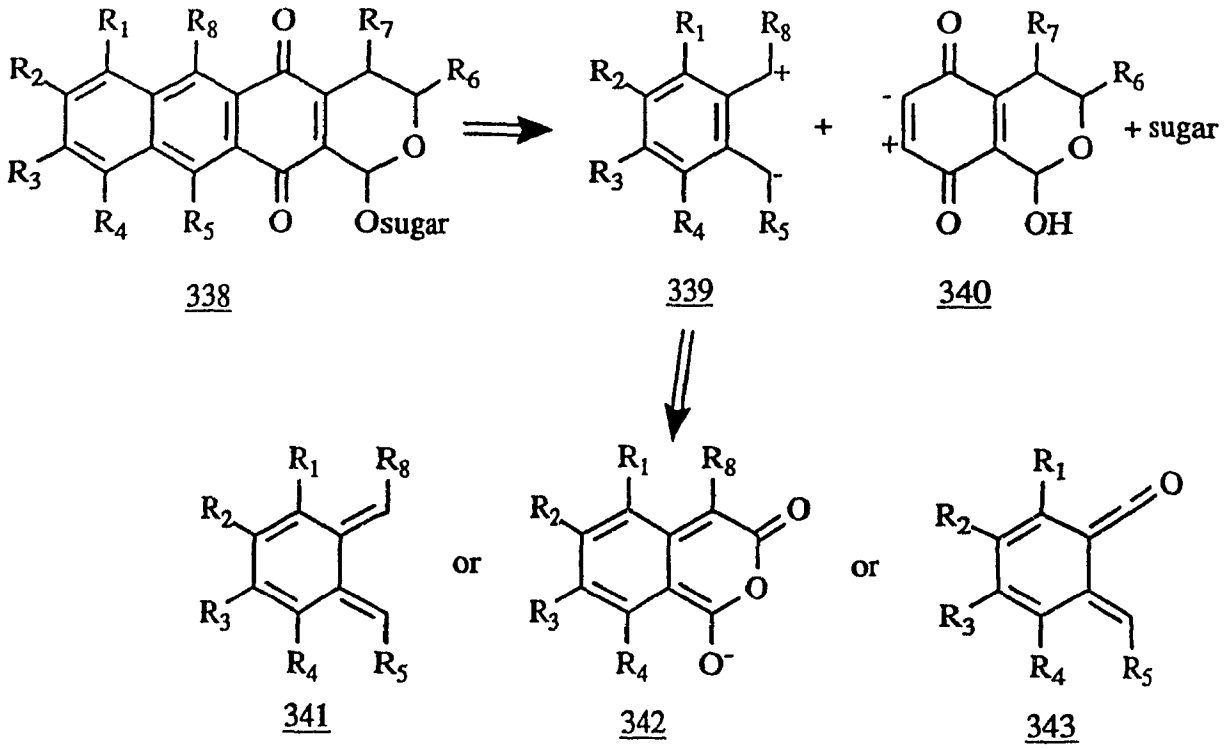
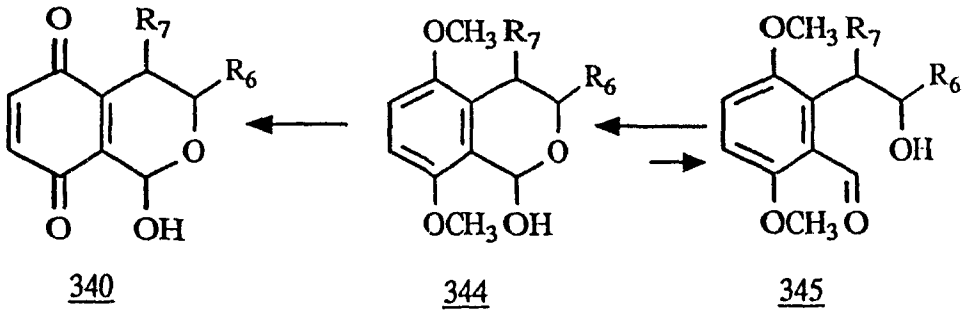


Fig 2.45

equivalents for (339) have been extensively considered in anthracyclinone research. Intermediates (341), (342) and (343) are three possibilites.[361-363] The highly convergent approach proposed in fig.2.45. would be succesful if a practical method for the preparation of hydroxypyranyl quinones of formula (340) existed. The preparation of such quinones can be rationalized from the fact that structure (340) is really a quinone fused to a sugar. Furthermore, (340) should be obtained easily from an isochroman such as (344) after oxidative demethylation. Thus a simple synthesis of compounds such as (345) would be important.



340 344 345

Fig 2.46

The preparation of examples of (345) was first considered from the electrophilic addition of epoxides such as ethylene and propylene oxides to the previously encountered lithio salt of 2,5-dimethoxybenzaldehyde dioxane acetal. All attempts however failed, possibly due to the poor reactivity of these epoxides as electrophiles. Starting material was invariably recovered after work up. However, when the lithio derivative was converted into the cuprate,[364,365] prior to the addition of the epoxide, a yield of circa 45% of homobenzylic alcohol (346) or (347) was achieved. Either compounds could be cyclized to give good yields (>80%) of isochromans (348) and (349). These were then oxidatively demethylated with ceric ammonium nitrate to give in 85-90% yield 1-hydroxypyranyl quinones (350) and (351).

Interestingly, the 1-methoxylated isochromans (352) and (353) were obtained when the cyclization was carried out in methanol with acid catalysis in 80-85% yield.

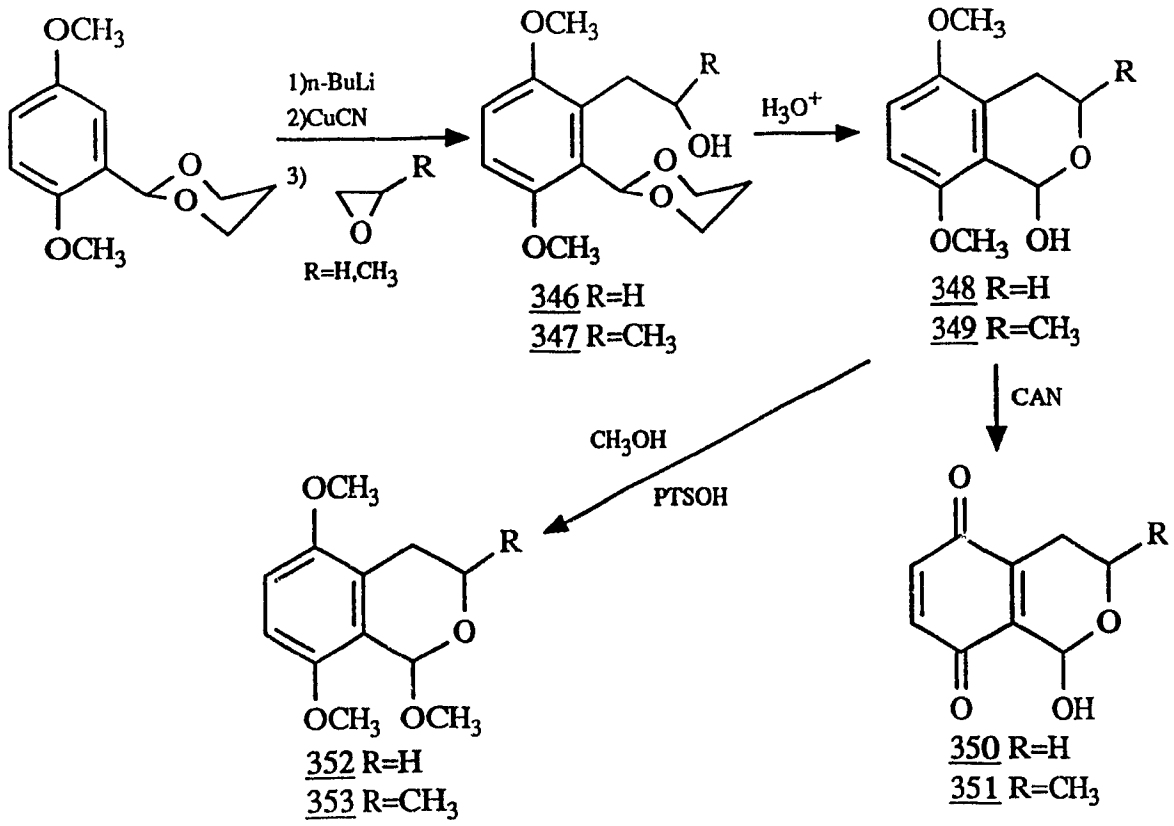
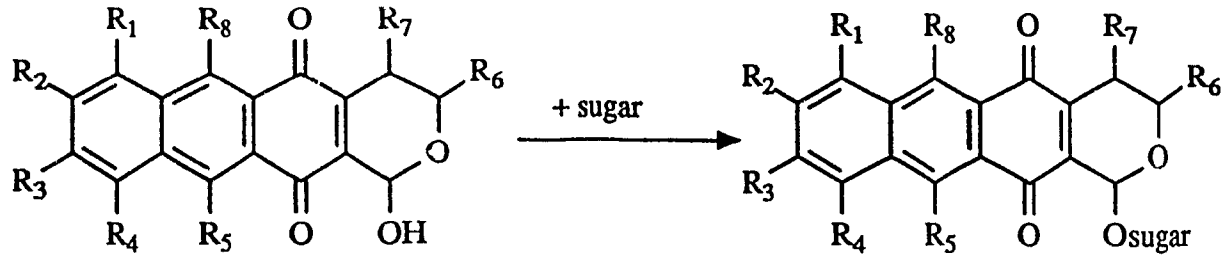


Fig 2.47

Since the preparation of quinones (350) and (351) could be effected in three steps from the dioxane acetal of 2,5-dimethoxybenzaldehyde, the synthesis of heteroanthracyclinones of formula (354) could conceptually



354 338

Fig 2.48

be achieved in four or five synthetic steps. Such an approach should permit the total synthesis of various glycosides of general formula (338) in less than eight steps.

The application and further developement of the proposed approach requires much research and consequently our efforts were ended at this stage.

Summary

Novel heteroanthracyclines were designed based on structure activity relationships of known anthracyclines and contemporary drug action mechanisms. The preparation of these glycosides required the development of a general synthetic route. Fig.A summarizes the synthetic map which resulted from our studies.

The thioxanthosulfonyl glycosides (202) and (204) could be prepared in seven steps in 4.8% and 4.6% overal yields by glycosidating, with a daunosaminyl derivative, the thioxanthone aglycone (147), itself obtained , after deacetylation of (198), from the double Friedel-Crafts reaction between o-chlorosulfenylbenzoyl chloride and 1-acetoxy-4,7-dimethoxy-1,3-dihydrobenzo[2,3-c]thiophene-2,2-dioxide (139). This latter compound was obtained after acetylating the hydroxysulfone derivative (103), a key intermediate diverging towards other synthetic targets. Its reduction with $NaBH_4$ gives the sultine (167) after acidic treatment. Thermal extrusion of SO_2 from the sultine gives the o-quinodimethane which can be trapped with p-nitrobenzylglyoxalate or ethyl glyoxalate to give isochromans (216) and (304). Oxidative demethylation of (216) gives the isochromandione (224) which can be used in a Michael addition with thiobenzoic acid. The resulting adduct can be cyclized under electrophilic conditions to

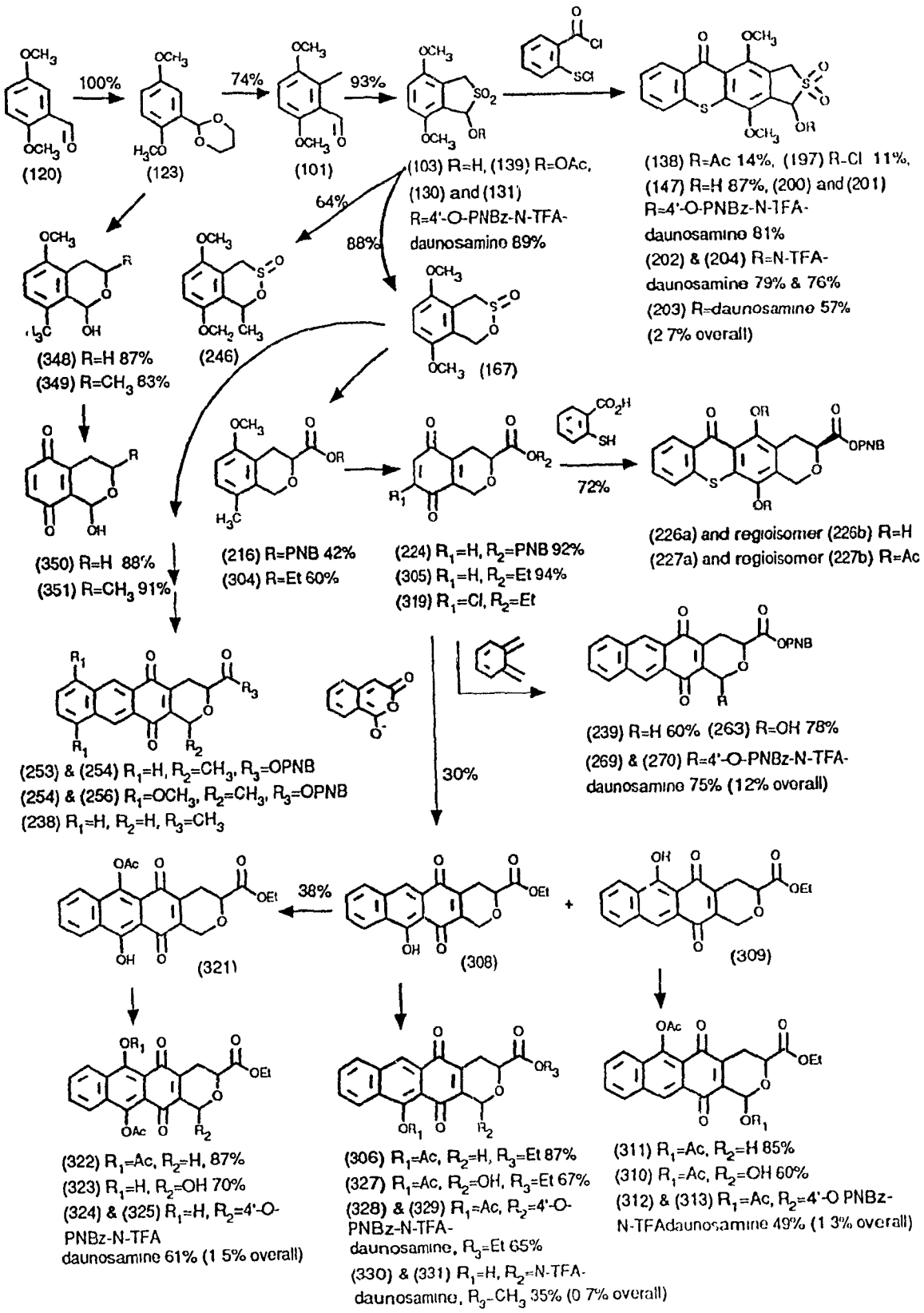

100%
74%
93%
(120)
(123)
(101)
(103) R=H, (139) R=OAc,
(130) and (131)
R=4'-O-PNBz-N-TFA-
daunosamine 89%
(138) R=Ac 14%, (197) R=Cl 11%,
(147) R=H 87%, (200) and (201)
R=4'-O-PNBz-N-TFA-
daunosamine 81%
(202) & (204) R=N-TFA-
daunosamine 79% & 76%
(203) R=daunosamine 57%
(2 7% overall)
64%
88%
(348) R=H 87%
(349) R=CH3 63%
(246)
(167)
(350) R=H 88%
(351) R=CH3 91%
(216) R=PNB 42%
(304) R=Et 60%
(224) R1=H, R2=PNB 92%
(305) R1=H, R2=Et 94%
(319) R1=Cl, R2=Et
72%
(226a) and regioisomer (226b) R=H
(227a) and regioisomer (227b) R=Ac
(253) & (254) R1=H, R2=CH3, R3=OPNB
(254) & (256) R1=OCH3, R2=CH3, R3=OPNB
(238) R1=H, R2=H, R3=CH3
30%
(239) R=H 60% (263) R=OH 78%
(269) & (270) R=4'-O-PNBz-N-TFA-
daunosamine 75% (12% overall)
38%
(321)
(308)
(309)
(322) R1=Ac, R2=H, 87%
(323) R1=H, R2=OH 70%
(324) & (325) R1=H, R2=4'-O-
PNBz-N-TFA
daunosamine 61% (1 5% overall)
(306) R1=Ac, R2=H, R3=Et 87%
(327) R1=Ac, R2=OH, R3=Et 67%
(328) & (329) R1=Ac, R2=4'-O-
PNBz-N-TFA-
daunosamine, R3=Et 65%
(330) & (331) R1=H, R2=N-TFA-
daunosamine, R3=CH3 35% (0 7% overall)
(311) R1=Ac, R2=H 85%
(310) R1=Ac, R2=OH 60%
(312) & (313) R1=Ac, R2=4'-O PNBz-
N-TFAdaunosamine 49% (1 3% overall)

Fig A

give the thioxanthonepyranyl derivatives 226a and 226b in a 1:1 ratio; each obtained in 12% overall yield from 2,5-dimethoxybenzaldehyde. Benzylic bromination of these deoxyaglycones, or of the acetates (227a) and (227b) proved to be difficult. Consequently, the thioxanthopyranyl daunosamine glycosides could not be prepared.

The preparation of anthraceno[2,3-c]pyranyl analogs of anthracyclinones was more successful. In our first attempts six tetracyclic analogs (238,239,253,254,255,256)of the naphthoquinone class of antibiotics were prepared from a tandem cycloaddition route involving two o-quinodimethanes, generated thermally from appropriately functionalized oxathiin-2-oxide precursors. An example is the preparation of p-nitrobenzyl (5,12-dioxo-3,4,5,12-tetrahydro[2,3-c]pyran-3-yl)formate (239) which involved the cycloaddition between an o-quinodimethane and the isochromandione (224); 14% overall yield from (120). Subsequent monobromination followed by solvolysis gave the aglycone (239) which was then coupled with 1,2-di-O-p-nitrobenzoyl-N-trifluoroacetyldaunosamine to give the first pair of anthraceno[2,3-c]pyranyl glycosides (269) and (270) in 37.5% yield each (12% overall, 9 steps from 2,5-dimethoxybenzaldehyde). However, the semisynthetic preparation of the aromatic substituted glycosides (324,325,328,329,312 and 313) was not feasible from (239). Therefore, the synthetic route was modified.

The homophthalic anhydride enolate cyclocondensation with pyranylquinone (305) gave the key tetracyclic intermediates (308) and (309). Pyranoanthracenedione (308) could also be prepared regiospecifically by condensing the homophthalic anhydride enolate with chloroisochromandione (314).

Either tetracyclic derivative (308) or (309), could be used to prepare the pyranoanthracenedione diacetate (322). All of intermediates (326), (311) or (322) could be brominated and then solvolysed with water to give the desired aglycones (327), (310) and (323) in good yield. The protected anthraceno[2,3-c]pyranyldaunosamine glycosides (324), (325), (329), (328), (312) and (313) could be prepared in respectable yields. In one case, deprotection gave the diastereomeric mixture of N-TFAdaunosamine anthraceno[2,3-c]pyranyl glycosides (330) and (331) which was found to have equipotent antileukemic activity to doxorubicin without any cross-resistance.

Some preliminary work for further studies was started in an attempt to shorten the synthesis. Thus the preparation of isochromandiones such as (350) and (351) was done from the oxidative demethylation of isochromans (348) and (349) obtained after acid hydrolysis of the products resulting from the addition of an epoxide to the cuprate of 2,5-dimethoxybenzaldehydedioxane acetal (123). The two hydroxylated isochromandiones (350) and (351) were prepared

in three steps from commercial 2,5-dimethoxybenzaldehyde and in an overall yield of circa 75%. In comparison, six steps were required to prepare quinone (305) in 34% overall yield from compound (120). This improved methodology should help in shortening future synthesis of pyranyl based heteroanthracyclines.

Contributions To Knowledge

1- Novel heteroanthracycline antitumor antibiotics were designed and a general synthetic map was developed.

2- Two methods for the synthesis of isochromans were developed.

A) The first involves photoenolisation of 2,5-dimethoxybenzaldehyde and SO_2 entrapment of the o-quinodimethane to give the dimethoxybenzo[c]dihydrothiophene-2,2-dioxide. Reduction to the sultine, followed by SO_2 thermal extrusion yields the o-quinodimethane, which can be cycloadded to a glyoxylate.

B) The second method is an improvement and involves the addition of an epoxide to the cuprate of 2,5-dimethoxybenzaldehydedioxane acetal followed by acidic hydrolysis.

3- The total synthesis of (R) and (S) 1-(N-trifluoroacetyldaunosamine)-1,3-dihydrothioxantho[2,3-c]thiophene-2,2-dioxide was carried out.

4- Six tetracyclic structural hybrids of the naphthoquinone[2,3-c]pyranyl class of antibiotics were synthesized by folowing a sequential cycloaddition routine requiring two o-quinodimethanes.

5- The total synthesis of p-nitrobenzyl(5,12-dihydroxy-3,4-dihydrothioxantho[2,3-c] and [3,2-c]pyran-3-yl) formates was carried out.

6- Eight novel heteroanthracyclines with the 5,12-dioxo-3,4,5,12-tetrahydroanthraceno[2,3-c]pyranyl backbone were synthesized.

7- The diastereomeric mixture of (1′S,1R,3S) and (1′S,1S,3R) methyl[11-hydroxy-1-(2′,3′,6′-trideoxy-3-trifluoroacetamido-L-lyxohexopyranose)-5,12-dioxo-3,4,5,12-tetrahydroanthraceno[2,3-c]pyran-3-yl]formate was found to possess equipotent antileukemic activity to doxorubicin with no cross resistance. This may indicate that a new family of antineoplastic agents was created by rational drug design.

General Experimental

Reagents were purchased from Aldrich Chemical Co and used without further purification except for homophthalic anhydride, which was treated with acetyl chloride in dry acetone for six hours, under argon, at room temperature. After evaporation of solvent and drying, the product was used as such in coupling reactions. N-trifluoroacetyldaunosamine was donated by Bristol-Myers Co, USA.

Organic solvents used in reactions and work up procedures were of ACS grade. Further purification of solvents was achieved as follows. Thiophene free benzene was obtained by stirring over concentrated sulfuric acid, decanting over dry KOH pellets and distilling under argon. Dry THF was prepared by distilling under argon over sodium-benzophenone. Dichloromethane was distilled over phosphorous pentoxide or calcium hydride. Dichloroethane was dried over calcium hydride and distilled. Dry diisopropylamine, pyridine or triethylamine was prepared by distilling over calcium hydride. Anhydrous ether was obtained from Mallinckrodt Chemicals, Montreal. Carbon tetrachloride was deoxygenated by bubling argon for one hour prior to use.

Organic solutions were dried over anhydrous $MgSO_4$ or $NaSO_4$. Solvents were removed under reduced pressure with a Buchi rota-evaporator with gentle heating when removing toluene (40°C). Residues were dried under high vacuum.

Reactions were followed by analytical thin layer chromatography from 0.2mm thick aluminum backed Kieselgel $60F_{254}$ precoated sheets (British Drug Houses,BDH). Preparative thin layer chromatography was carried out on 0.5-1.0mm thick glass backed Kieselgel $60F_{254}$ precoated plates (BDH). Flash chromatography was carried out as described by Still and co-workers[366] on 400 to 230 mesh silica gel (BDH), with the exception that crude reaction mixtures, adsorbed on three weight equivalents of silica gel, were transferred dry to the packed column.

Photochemical reactions were carried out with a Rayonet Photochemical setup (Photron Co,London,Ontario) or with a medium pressure Hg immersion lamp (Hanovia Co., New Jersey,USA). Ozonolysis was done at Bristol-Myers, Candiac, using a custom made ozone generator.

Melting points (mp) were determined on a Buchi SMP-20 apparatus in closed capillary tubes and are uncorrected. Ultraviolet spectra, (UV), were obtained with a Carry 1 spectrophotometer, data is reported as wavelength in nm. Infrared spectra (IR) were obtained either on an Analect or a Nicolet Fourier-transform spectrophotometer, and absorptions are reported in wavenumbers (cm^{-1}). Mass

spectra, ms, were recorded at the Biomedical Mass Spectrometry Center (McGill) on a LKB model 9000 or HP Model 5984 mass spectrometers. Mass peak intensities are reported as % relative intensity. High resolution mass measurements were obtained from the University of Montreal mass spectrometry regional center. PMR were recorded either on a Varian XL-200 or XL-300 spectrometers. Chemical shifts are reported in units, parts per million (PPM) relative to tetramethylsilane (δ =0.0ppm). In the PMR spectra, coupling constants are reported in hertz (Hz). Simple multiplets are described as doublets (d), doublet of doublet (dd), doublet of doublet of doublet (ddd), doublet of doublet of doublet of doublet (dddd), doublet of triplets (dt), triplets (t) or quartets (q). The notation broad (b) may be assigned to signals which are unresolved (eg. bdd); m refers to an unresolved or complex multiplet. CMR spectra were recorded on a Varian XL-300 spectrometer. Chemical shifts are reported in δ units.

The purity of new compounds was ascertained from single component TLC analysis, sharp melting points, and frequent HPLC analysis on various Waters HPLC instrumentation. Other criteria for the purity and structural integrity of important intermediates were clean PMR and CMR spectra as well as accurate elemental composition obtained by high resolution mass spectra.

EXPERIMENTAL

1,4-Dimethoxy-2-bromomethyltoluene (116)

A mixture containing 15.0g (90mmol) of 1,4-dimethoxy-2,3-dimethylbenzene, 14.39g (81mmol) of N-bromosuccinimide, and .05g of AIBN in 2l of deoxygenated CCl_4 was refluxed under argon for 0.5 hour.[251] An additional 1.61g (9mmol) of NBS and 0.01g of AIBN was then added and reflux was continued for an extra hour. After cooling, the mixture was filtered and the solvent was evaporated. Flash chromatography of the residue (7% ethyl acetate in hexanes) gave 17.3g (78% yield) of 1,4-dimethoxy-2-bromomethyltoluene (116). MP: 130-131°C ^{1}H NMR (200 MHz, $CDCl_3$) δ : 2.17 (s, 3H, CH_3), 3.69 (s, 3H, OCH_3), 3.75 (s, 3H, OCH_3), 4.78 (s, 2H, CH_2Br), 6.65 (dd, 2H, J=9.3 Hz, ArH). CMR (75.44 MHz, $CDCl_3$) δ : 11.1, CH_3; 26.2, CH_2Br; 55.86, 55.93, $PhOCH_3$; 108.2, 111.2, aryl CH; 125.5, 127.5, 151.5, 151.7, aryl C. IR (FT,$CDCl_3$) ν_{MAX}: 1258, C-O. MS (EI, 70eV, 40°C) m/e; 244 (37, $M^{+\cdot}$), 165 (100, $M^{+\cdot}$-Br).

The 1,4-dimethoxy-2,3-dibomomethylbenzene was obtained in 19% yield. MP:148-150°C (lit[251] MP:149-151°C). ^{1}H NMR (200 MHz, $CDCl_3$) δ: 3.63 (s, 6H, OCH_3), 4.74 (s, 4H, CH_2Br), 6.82 (s, 2H, ArH). CMR (75.44 MHz, $CDCl_3$) δ: 23.9, CH_2Br; 56.2, OCH_3; 112.1, aryl CH; 126.3, 151.7, aryl C. MS (EI, 70eV, 80°C) m/e; 322 (32, $M^{+\cdot}$), 243 (53, $M^{+\cdot}$-Br), 164 (100, $M^{+\cdot}$-2Br), 149 (54, $M^{+\cdot}$-2Br-CH_3).

2,5-Dimethoxybenzaldehydedioxane acetal (123)

A solution containing 200g (1.2 mole) of 2,5 dimethoxybenzaldehyde, 150g (2.0 mole) of 1,3-propanediol, and 1.0g of p-toluenesulfonic acid in 1.0 l of benzene was refluxed until no more water could be isolated in the Dean-Stark water separator (6 hours). The reaction mixture was then cooled and washed with 400 ml of saturated aqueous sodium bicarbonate, 200 ml of water and 200 ml of saturated aqueous sodium chloride. The organic layer was then dried over $MgSO_4$ and the solvent was removed in vacuo. Distillation of the residue under reduced pressure (B.P 167° at 1 mmHg) gave 263.7g (98% yield) of a slightly yellow oil characterised as 2,5-dimethoxybenzaldehydedioxane acetal. ^1H NMR (200 MHz, $CDCl_3$) δ: 1.40 (d m, 1H, HC<u>Ha</u>), 2.24 (m, 1H, HC<u>He</u>), 3.77 (s,3H, OCH_3), 3.78 (s, 3H, OCH_3), 4.00 (dt, 2H, O-HC<u>Ha</u>), 4.24 (dd, 2H, O-CH-O), 6.82 (dd, 2H, ArH), 7.19 (d, 1H, ArH). CMR (75.44 MHz, $CDCl_3$) δ; 25.3 CH_2; 55.1 OCH_3; 55.7 OCH_3; 67.0 2X OCH_2; 96.2, 111.6, 115.0, aromatic CH; 127.3, 150.1, 153.2 aromatic C. MS (EI, 70eV, 40°C) m/e: 224 (100, $M^{+}\cdot$), 193 (32, $M^{+}\cdot-OCH_3$), 166 (37, $M^{+}\cdot-C_3H_6O$), 138 (36, $M^{+}\cdot-C_4H_6O_2$). IR ($CDCl_3$) ν_{MAX} 3050 (ArH), 2960, 2936, 2838 (alkyl H), 1147, 1096, 1092, 1047 (O-C).

2,5-Dimethoxybenzaldehydedioxolane acetal (121)

This compound was prepared in 98% yield by employing the procedure described for (101) but by using ethylene glycol instead of propane-1,3-diol. The crude oily product, after work up, was found to be sufficiently pure for further use. ^1H NMR (200 MHz, $CDCl_3$) δ : 3.70 (s, 3H, OCH_3), 3.74 (s, 3H, OCH_3), 3.98 (m, 4H, $2XOCH_2$), 6.08 (s, 1H, O-CH-O), 7.09 (bs, 2H, ArH), 7.10 (bs, 1H, ArH). CMR (75.44 MHZ, $CDCl_3$) δ : 55.1, 55.7, OCH_3; 64.7, $2XOCH_2$; 98.5, O-CH-O; 111.6, 111.9, 114.5, aryl CH; 126.3, 151.4, 153.0, aryl C.

2,5-Dimethoxy-6-methylbenzaldehyde (101)

Procedure A:

An homogeneous solution containing 1.72g (7 mmol) of 1,4-dimethoxy-2-bromomethyltoluene (116) and 17.2g (2.5mmol) of freshly prepared bis-tetrabutylammonium dichromate[280] in 30 ml of chloroform was refluxed under argon for 11 hours. After cooling, the reaction mixture was filtered through 40g of silica gel. The gel was then washed with 300 ml of ether. Recrystallization from hexanes of the residue obtained after removal of solvent from the combined filtrates gave 1.1g (87% yield) of 2,5-dimethoxy-6-

methylbenzaldehyde (101).

Procedure B:

To a cooled (-40°C) solution containing 84.0g (0.37 mole) of 2,5-dimethoxybenzaldehydedioxane acetal in 2.0 l of dry diethylether was added with stirring and under argon 240 ml of a 2.5M n-butyl lithium solution in hexanes. The mixture was stirred for four hours at -25°C and then 24 hours at -10°C. Then to the cooled (-25°C) stirred reaction mixture under argon was added 90.0g of methyl iodide and stirred overnight at room temperature. The solution was then washed twice with 300 ml of water, once with 300 ml of saturated sodium chloride and dried over $MgSO_4$. The organic solvent was evaporated and the residue was dissolved in 500 ml of ether and stirred for 1-1/2 hours with 500 ml of 1N aqueous HCl. The organic layer was separated and washed twice with 200 ml water, once with 200 ml of brine and dried over $MgSO_4$. Evaporation of the solvent gave a yellow oil which was flash chromatographed with 2.5% ethyl acetate in toluene. A 67% yield (45g) of 2,5-dimethoxy-6-methylbenzaldehyde was obtained (MP: 61-61.5°C). ^1H NMR (200 MHz, $CDCl_3$) δ: 2.46 (s, 3H, CH_3), 3.78 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 6.90 (dd, 2H, ArH), 10.58 (s, 1H, CHO). CMR (75.44 MHz, $CDCl_3$) δ: 12.0, CH_3; 55.9, 56.3, $ArOCH_3$; 108.7, 116.6, aryl CH; 124.2, 130.0, 151.8, 156.8, aryl C;

192.7, CHO. UV (MeOH) λ_{Max}: 223, 260, 348. MS (EI, 70eV, 45°C) m/e: 180 (100, $M^{+}\cdot$), 165 (70, $M^{=}\cdot-CH_3$), 150 (34, $M^{+}\cdot-CH_2O$). HRMS calculated for $C_{10}H_{12}O_4S$: [$M^{+}\cdot$] 180.0787 found 180.0776.

2,5-Dimethoxy-4-methylbenzaldehyde was obtained in 12% yield. MP:82-83°C (lit.[294] 80.5-81.5°C). ^1H NMR (200 MHz, $CDCl_3$) δ: 2.31 (s, 3H, CH_3), 3.84 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 6.85 (s, 1H, ArH), 7.27 (s, 1H, ArH), 10.42 (s, 1H, CHO). CMR (75.44 MHz, $CDCl_3$) δ: 17.3, CH_3; 55.7, 56.1, OCH_3; 107.6, 114.6, aryl CH; 122.8, 136.5, 151.9, 156.6, aryl C; 189.2, CHO. MS (EI, 70eV, 45°C) m/e: 180 (100, $M^{+}\cdot$), 165 (43, $M^{+}\cdot-CH_3$), 151 (12, $M^{+}\cdot-CHO$).

4,7-dimethoxy-1-hydroxy-1,3-dihydrobenzo[2,3-c]thiophen-2,2-dioxide (103)

Procedure 1:

Under an argon atmosphere, a deoxygenated solution containing 1.60g (8.9 mmole) of 2,5-dimethoxy-6-methylbenzaldehyde, 11.0g of SO_2 in 100 ml of thiophene free benzene was irradiated at 350 nm for 36 hours. The precipitated crystals (2.01g, 93% yield) were filtered and found sufficiently pure for further use.

Procedure 2:

For larger scale, under argon, a deoxygenated solution containing 10.0g (55.5 mmole) of 2,5-dimethoxy-6-methylbenzaldehyde, 50g of SO_2 in 600 ml of thiophene free benzene was irradiated with a medium pressure mercury immersion lamp with pyrex filtration for four days. The resulting sludge was extracted three times with 400 ml of 1N NaOH and the combined aqueous layer was washed twice with 200 ml of methylene chloride. The aqueous layer was then neutralised with concentrated aqueous HCl and the resulting mixture was then extracted three times with 500 ml of methylene chloride. The combined organic layer was then washed once with 200 ml water, 200 ml of saturated aqueous sodium bicarbonate, 200 ml of water, 200 ml of brine, and then dried over $MgSO_4$. Following evaporation of solvent, 11.2g (83%) of pure 5,8-dimethoxy-1-hydroxy-1,3-dihydrobenzo[2,3-c]thiophene-2,2-dioxide was obtained (MP: 140°C decomposes). ^1H NMR (200 MHz, $CDCl_3$) δ: 3.57 (s, 3H, OCH_3), 3.60 (s, 3H, OCH_3), 4.44 (dd, 2H, CH_2), 5.65 (s, 1H, CH), 6.85 (dd, 2H, ArH). CMR (75.44 MHz, $CDCl_3$) δ: 49.8, CH_2; 55.9, 56.0, OCH_3; 85.1, CH; 111.4, 113.0, aryl CH; 121.7, 124.1, 149.2, 151.0, aryl C. IR (KBr) ν_{MAX}: 3300-3600, OH; 1290, SO_2. MS (CI, i-but, 58°C) m/e: 245 (18, M + H), 227 (14, M + H-H_2O), 181 (100, M + H-SO_2). HRMS calculated for $C_{10}H_{12}O_5S$: [$M^{+\cdot}$] 244.0406 found 244.0385.

4,7-Dimethoxy-1-acetoxy-1,3-dihydrobenzo[2,3-c]thiophene-2,2-dioxide (139).

To a stirred suspension containing 2.928g (12mmol) of hydroxysulfone (103) in 200 ml of acetic anhydride was added one drop of concentrated sulfuric acid. Stirring was continued for four hours under argon, the precipitate was filtered, then washed with water and air dried. Flash chromatography from 10% ethyl acetate in toluene of the precipitate gave the acetoxysulfone in 87% yield (2.98g). MP: 227.5-228.5°C (decomposes). ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.23 (s, 3H, $COCH_3$), 3.82 (s, 6H, $2XOCH_3$), 4.27 (dd, 2H, J=16.5 Hz,CH_2), 6.72 (s, 1H, CH), 6.9 (dd, 2H, J=9.7 Hz, ArH). CMR (75.44 MHz, $CDCl_3$) δ: 20.4, CH_3; 51.0, CH_2; 52.2, 55.9, $ArOCH_3$; 103.8, CH; 111.0, 113.2, aryl CH; 121.9, 122.6, 151.4, 151.6, aryl C; 169.1, C=O. IR (KBr) ν_{MAX}: 1758, ester C=O; 1323, 1190, SO_2. MS (EI, 70eV, 60°C) m/e: 286 (2.3, $M^{+}\cdot$), 255 (3, $M^{+}\cdot-OCH_3$), 222 (16, $M^{+}\cdot-SO_2$), 194 (24, $M^{+}\cdot-SO_2-C_2H_4O$). HRMS calculated for $C_{10}H_{14}O_6S$: [$M^{+}\cdot$] 286.0511 found 286.0507.

1,4,7-Trimethoxy-1,3-dihydrobenzo[2,3-c]thiophene-2,2-dioxide (140).

A solution containing 350mg (14.3mmol) of hydroxysulfone (103) and 5mg p-toluenesulfonic acid in 50 ml of methanol was stirred overnight at room temperature. The solvent was then removed and the residue was dissolved in 75 ml of dichloromethane. Washing of the organic solution was then successively done with 25 ml aliquots of saturated aqueous sodium bicarbonate, water and brine. After drying and removal of solvent, flash chromatography of the residue with 30% ethyl acetate in hexanes gave 3.32g (90% yield) of 1-methoxysulfone (140). MP: 147-148°C. ^{1}H NMR (200 MHz, DMSO-d_6) δ: 3.34 (s, 3H, OCH_3), 3.80 (s, 3H, $ArOCH_3$), 3.85 (s, 3H, $ArOCH_3$), 4.52 (bs, 2H, CH-2), 6.40 (s, 1H, CH),7.13 (dd, 2H, J=9.3 Hz, ArH). CMR (75.44 MHz, DMSO-d-6) δ: 51.3, CH_2; 55.8, 56.0, $ArOCH_3$; 59.7, OCH_3; 93.0, CH; 110.9, 112.8, aryl CH; 121.9, 122.2, 149.6, 151.5, aryl C. IR (Ft, $CDCl_3$) ν_{MAX}: 1126, 1313, sulfone. MS (EI, 70 eV, 70°C) m/e: 258 (1.4, $M^{+}\cdot$), 194 (73, $M^{+}\cdot-SO_2$), 179 (100, $M^{+}\cdot-SO_2-CH_3$), 164 (52, $M^{+}\cdot-SO_2-CH_3-CH_3$).

4,7-Dimethoxy-1-ethoxy-1,3-dihydrobenzo[2,3-c]thiophene-2,2-dioxide (141).

Application of the procedure described for sulfone (140) gave 3.00g (77% yield) of ethoxysulfone (141). After flash chromatography (30% ethyl acetate in hexanes), from ethanolysis of 350mg (14.3mmol) of hydroxysulfone (103). MP: 235°C decomposes. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.25 (t, 3H, J=7.0 Hz, CH_3), 3.71 (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3), 3.87 (q, 2H, J=7.0 Hz, OCH_2), 4.14 (bs, 2H, CH_2), 5.32 (s, 1H, O-CH-S), 6.78 (dd, 2H, J=9.0 Hz, ArH). CMR (75.44 MHz, $CDCl_3$) δ: 14.9, CH_3; 51.2, CH_2; 55.8, 56.0, OCH_3; 68.1, OCH_2; 91.7, OCHS; 111.0, 112.6, aryl CH; 121.8, 122.6, 149.6, 151.4, aryl C. MS (ei, 70eV, 65°C) m/e: 272 (0.7, $M^{+}\cdot$), 208 (100, $M^{+}\cdot-SO_2$), 193 ($M^{+}\cdot-SO_2-CH_3$), 179 (96, $M^{+}\cdot-SO_2-C_2H_5$). HRMS calculated for $C_{12}H_{16}O_3$: [$M^{+}\cdot-SO_2$] 208.1067 found 208.1099.

4,7-Dimethoxy-1-isopropoxy-1,3-dihydrobenzo[2,3-c]thiophene-2,2-dioxide (141)

Application of the same procedure described for sulfone (140) gave 3.00g (74%) of isopropoxysulfone (142), after flash chromatography (30% ethylacetate in hexanes), from 350 mg (1.4 mmol) of hydroxysulfone (103). MP: 245°C decomposes. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.39 (d, 3P, J=7.1

Hz, CH_3), 1.42 (d, 3H, J=7.1 Hz, CH_3), 3.83 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 4.27 (bs, 2H, CH_2SO_2), 4.36 (m, 1H, $C\underline{H}(CH_3)_2$), 5.56 (s, 1H, OCH), 6.89 (dd, 2H, J=8.9 Hz, ArH). CMR (75.44 MHz, $CDCl_3$) δ: 20.5, $2XCH_3$; 52.2, CH_2; 55.9, 56.0, OCH_3; 76.4, OCH; 82.7, $OCHSO_2$; 110.9, 113.2, aryl CH; 119.9, 122.6, 149.6, 151.5, aryl C. MS (EI, 70eV, 160°C) m/e 222 (0.2, $M^{+}\cdot-SO_2$), 180 (100, $M^{+}\cdot-SO_2-C_3H_6$), 165 (37, $M^{+}\cdot-SO_2-C_3H_6-CH_3$), 150 ($M^{+}\cdot-SO_2-C_3H_6-2CH_3$).

4,7-Dimethoxy-1-menthyl-1,3-dihydrobenzo[2,3-c]thiophene-2,2-dioxide (143)

A solution containing 1g (4.1mmol) of hydroxysulfone (103), 5g (20.5mmol) of menthol and 10mg of p-toluenesulfonic acid in 150 ml of CH_2Cl_2 was stirred for 24 hours at room temperature. The reaction mixture was then washed with successively with 50 ml aliquots of saturated aqueous sodium bicarbonate, water, brine and dried over $MgSO_4$. After removal of solvent, flash chromatography of the residue from 10% ethyl acetate in toluene gave the menthylsulfone (143) as a 1:1 diastereomeric mixture in 67% yield. MP: 145-146°C. ^1H NMR of the 1:1 diastereomeric mixture (200 MHz, $CDCl_3$) δ: 0.95 (overlapped d, 9H, CH_3), 1.14-1.81 and 1.41 (overlapped m, 9H, menthyl protons), 3.74 and 3.94 (m, 1H, OCH), 3.84 and 3.86 (s, 6H, OCH_3), 4.23 (bs, 2H, CH_2SO_2), 5.56 and 5.70 (bs, 1H, $OCHSO_2$), 6.89

(overlapped dd, 2H, ArH). CMR of the 1:1 diastereomeric mixture (75.44 MHz, $CDCl_3$) δ : 15.8, 16.2, 21.1, 21.5, 22.2, 22.4, CH_3; 23.0, CH_2; 24.6, 24.8, 31.3, 31.7, 48.3, 48.6, CH; 34.2, 34.3, 39.1, 41.6, 50.7, 50.9, CH_2; 55.5, 55.7, 55.8, OCH_3; 79.7, 82.7, OCH; 87.9, 91.3, $OCHSO_2$; 110.3, 110.9, 112.2, 112.4, aryl CH; 121.8, 122.2, 123.4, 123.6, 149.6, 149.7, 151.17, 151.22, aryl C.

4,7-Dimethoxy-1-(2',3',6'-trideoxy-3'-trifluoroacetamido-4'-o-p-nitrobenzoyl-L-lyxohexopyranose)-1,3-dihydrobenzo[2,3-c]thiophene-2,2-dioxides (130) and (131).

Following a modification of Terashima procedure,[292] to a stirred and cooled (-25°C) mixture containing 2.24g (4mmole) of 1,4-di-O-p-nitrobenzoyl-N-trifluoroacyldaunosamine (134), 0.5g of activated 4a molecular sieves in 400 ml of a 1:3 ether/dichloromethane solvent system was added dropwise and under argon, 2.0 ml of trimethylsilyltrifluoromethane sulfonate. The mixture was then stirred 1 hour at -5°C and then a solution containing 1.15g (4.7mmol) of hydroxysulfone (103) in 225 ml of dichloromethane was added at -10°C. After stirring for three hours, the mixture was added to a solvent system composed of 250 ml of saturated aqueous sodium bicarbonate and 250 ml of ethyl acetate. The two phases were separated and the aqueous layer was extracted twice with 150 ml of

ethyl acetate. The combined organic phase was then washed consecutively with 100 ml aliquots of saturated aqueous sodium bicarbonate, water, saturated aqueous NaCl, and then dried over anhydrous $MgSO_4$. After evaporation of solvent, flash chromatography of the residue with 30% ethyl acetate in hexanes yielded the two diastereomers in pure form (44.5% of each diastereomer). The less polar glycoside, (130), had: MP: 176-177°C decomposes. ^1H NMR (200 MHz, $CDCl_3$) δ: 1.27 (d, 3H, J=6.7 Hz, CH_3), 2.15 (m, 2H, $C'H_2$), 3.82 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 4.24 (bs, 2H, CH_2), 4.59 (overlapped m, CHNH and $OCHCH_3$), 5.47 (bs, 1H, OCH), 5.79 (bs, 1H, O-CH-O), 5.86 (s, 1H, $OCHSO_2$), 6.38 (bd, 1H, J=7.3 Hz, NH), 6.91 (dd, 2H, J=9.2 Hz, ArH), 8.31 (dd, 4H, J=9.1 Hz, ArH). CMR (75.44 MHz, $CDCl_3$) δ: 16.6, CH_3; 28.7, CH_2; 44.9, CH; 50.9, CH_2; 55.6, 55.7, OCH_3; 65.9, 70.9, 85.9, OCH; 96.1, O-CH-O; 110.9, 113.1, 123.4, 130.2, aryl CH; 115.3, quartet, J=286.5 Hz, CF_3; 121.0, 121.9, 134.5, 149.6, 150.4, 150.8, aryl C; 156.8, quartet, J=36.0 Hz, $COCF_3$; 164.3, ester C=O. IR ($CDCl_3$) ν_{MAX}: 1736, ester C=O; 1531, amide. MS (Negative Ion Fab, diethanolamine, RT) m/e: 618.67, M-.

The more polar glycoside, (131), had: MP: 203-204°C. ^1H NMR (200 MHz, $CDCl_3$) δ: 1.23 (d, 3H, J=6.5 Hz, CH_3), 2.12 (m, 2H, $C'H_2$), 3.82 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 4.24 (dd, 2H, J=16.0 Hz, CH_2), 4.68 (m, 1H, CH, NH), 4.87 (bq, 1H, J=6.5 Hz, $CHCH_3$), 5.44 (bs, 1H, OC'H), 5.56 (bs, 1H, O-CH-

o), 5.65 (s, 1H, $OCHSO_2$), 6.48 (bd, 1H, J=6.9 Hz, NH), 6.88 (dd, 2H, J=9.0Hz, ArH), 8.30 (dd, 4H, J=9.4 Hz, ArH). CMR (75.44 MHz, $CDCl_3$) δ: 16.6, CH_3; 29.1, CH_2; 45.3, CH; 51.4, CH_2; 55.8, $2XOCH_3$; 66.4, 71.8, 89.6, 99.0, CH;, 110.8, 112.6, 123.6, 130.8, aryl CH; 115.4, quartet, J=282.8 Hz, CF_3; 122.5, 134.5, 149.6, 150.4, 151.2, aryl C; 156.8, quartet, J=36.3 Hz, $COCF_3$; 164.4, ester C=O. IR (FT, $CDCl_3$) ν_{MAX}: 1736, ester C=O; 1531, amide. MS (Negative Ion FAB, diethanolamine, RT) m/e: 618.53 (55, M^-, calculated for $C_{25}H_{25}F_3N_2O_{11}S$: 618.54), 554 (13, $M^- - SO_2$), 468 (24, M^- - PNBz).

1,4-Dimethoxy-2-bromomethyl-3-methylthioxanthone (157).

Following Honek procedure,[251] a solution containing 2.10g (.9 mmol) of thioxanthone (156), 1.12g of (.7 mmol) of N-bromosuccinimide and 10mg of AIBN in 160 ml of deoxygenated CCl_4 was refluxed for one hour under argon. An additional 0.126g (.08 mmol) of NBS with 10mg of AIBN was added and reflux was continued for two more hours. The mixture was then cooled, filtered and evaporated in vacuo. Flash chromatography of the residue with dichloromethane gave 1.58g of monobrominated thioxanthone (157). MP: 162-164°C (lit[251] MP: 163-166°C). ^1H NMR (200 MHz, $CDCl_3$) δ: 2.48 (s, 3H, CH_3), 3.91 (s, 3H, OCH_3), 4.03 (s, 3H, OCH_3), 4.74 (bs, 2H, CH_2Br), 8.44 (m, 1H, ArH), 8.58 (m, 2H, ArH),

8.49 (d, 1H, J=8.0 Hz, ArH). CMR (75.44 MHz, $CDCl_3$) : 12.4, CH_3; 25.5, CH_2Br; 60.5, 62.5, $ArOCH_3$; 125.9, 126.4, 129.4, 131.8, aryl CH; 121.7, 130.7, 179.7, 135.0, 135.4, 135.8, 148.8, 157.3, aryl C; 224.1, CO. IR ($CDCl_3$) ν_{MAX} : 1646, C=O. MS (EI, 70 eV, 155°C) m/e: 378 (7, $M^{+}\cdot$), 299 (67, $M^{+}\cdot$-Br), 284 (68, $M^{+}\cdot$-Br-CH_3).

1,4-Dimethoxy-2-formyl-3-methylthioxanthone (158).

Following the same procedure as described for (101), treatment of 2.50g (8.0 mmmol) of bromomethylthioxanthone (157) with 8.59g (12.0 mmol) of bis-tetrabutylammonium dichromate gave 1.47g (71% yield) of thioxanthyl aldehyde (158) after flash chromatography with 10% ethyl acetate in toluene. MP: 196-197°C. ^1H NMR (200 MHz, $CDCl_3$) δ: 2.61 (s, 3H, CH_3), 3.80 (s, 3H, OCH_3), 4.00 (s, 3H, OCH_3), 7.4 (m, 3H, ArH), 8.45 (d, 1H, J=8.9 Hz, ArH), 10.62 (s, 1H, ArH). CMR (75.44 MHz, $CDCl_3$) δ: 13.6, CH_3; 60.5, 64.6, OCH_3; 126.1, 126.9, 129.5, 132.2, aryl CH; 121.6, 126.8, 130.6, 134.9, 136.7, 140.6, 149.1, 163.7, aryl C; 179.4, CHO; 192.1, thioxanthyl C=O. IR (FT, $CDCl_3$) ν_{MAX}: 1682, CHO; 1648, thioxanthone C=O. UV (ethanol) λ_{Max}: 375, 333, 267, 249nm. MS (EI, 70eV, 180°C) m/e: 314 (100, $M^{+}\cdot$), 299 (58, $M^{+}\cdot$-CH_3), 286 (24, $M^{+}\cdot$-CO), 269 (42, $M^{+}\cdot$-CH_3-CH_2O). HRMS calculated for $C_{17}H_{14}O_4S$: [$M^{+}\cdot$] 314.0613 found 314.0613.

1,4-Dimethoxy-2-formyl-3-methylthioxanthone dioxane acetal.

In a triple necked round bottom flask equipped with a Dean-Stark water separartor was refluxed for 7.5 hours a mixture containing 0.50g (1.6 mmol) of thioxanthone aldehyde (158), 0.15g (2 mmol) of 1,3-propanediol and 10mg of PTSOH in 50 ml of benzene. The mixture was cooled washed successively with 25 ml aliquots of saturated sodium bicarbonate, water, brine, and then dried over $MgSO_4$. Flash chromatography (15% ethyl acetate in toluene) of the residue after evaporation of solvent gave 0.62g (100% yield) of the formylthioxanthone dioxane acetal (159). MP: 169-170°C. ^1H NMR (200 MHz, $CDCl_3$) δ: 1.47 (bd, 1H, J=12.0 Hz, HCHa), 2.28 (m,1H, HCHe), 2.70 (s, 3H, CH_3), 3.88 (s, 3H, OCH_3), 3.92 (s, 3H, CH_3), 4.02 (m, 2H, O-HCHa), 4.27 (m, 2H, O-HCHe), 5.24 (s, 1H, CH), 7.46 (m, 1H, ArH), 7.58 (m, 2H, ArH), 8.49 (d, 1H, J=8.0 Hz, ArH). CMR (75.44 MHz, $CDCl_3$) δ: 13.5, CH_3; 25.8, CH_2; 60.1, 63.4, $ArOCH_3$; 67.7, OCH_2; 98.3, OCH; 125.9, 126.3, 129.4, 131.6, aryl CH; 121.4, 130.8, 132.2, 135.0, 135.6, 137.0, 156.4, 179.9, aryl C; 192.2, C=O. IR ($CDCl_3$) ν_{MAX}: 1646, C=O. MS (EI, 70eV, 175°C) m/e: 372 (17, $M^+\cdot$), 357 (37, $M^+\cdot$-15), 314 (69, $M^+\cdot$-C_2H_6O), 299 (53, $M^+\cdot$-C_2H_6O-CH_3).

1,4-Dimethoxy-2-formyl-3-methylthioxanthene (161)

Following a similar procedure as described by Mancini,[294] to a solution containing 0.500g (1.3 mmol) of formylthioxanthonedioxane acetal (159) in 15 ml of CH_2Cl_2 was added slowly, under argon at 0°C, 7 ml of a 1M borane-THF solution. The mixture was stirred for 5.5 hours at room temperature, then quenched carefully with 5 ml methanol and diluted with 50 ml of CH_2Cl_2. The solution was then washed with 20 ml of water, 20 ml of brine, and dried. After evaporation of solvent the residue was dissolved in 20 ml of ether, and the resulting solution was stirred with 20 ml of 1N aqueous HCl for one hour at room temperature. The organic layer was separated, washed successively with 10 ml aliquots of water, saturated sodium bicarbonate, water, brine, and dried over $MgSO_4$. Flash chromatography (CH_2Cl_2) of the residue obtained after evaporation of solvent, gave g (50% yield) of thioxanthone aldehyde (161). MP: 125°C decomposes. ^1H NMR (200 MHz, $CDCl_3$) δ: 2.48 (s, 3H, CH_3), 3.81 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 3.90 (s, 2H, CH_2), 7.21 (m, 2H, ArH), 7.33 (m, 1H, ArH), 7.43 (m, 1H, ArH), 10.46 (s, 1H, CHO). CMR (75.44 MHz, $CDCl_3$) δ: 12.9, CH_3; 30.9, CH_2; 60.5, 64.2, $ArOCH_3$; 127.0, 127.2, 128.3, aryl CH; 126.1, 127.1, 131.6, 132.3, 134.3, 137.0, 151.0, 157.8, aryl C; 192.0, CHO. IR ($CDCl_3$) ν_{MAX}: 1682, aldehyde. HRMS calculated for $C_{17}H_{16}O_3S$: [$M^{+\cdot}$] 300.0820 found 300.0830.

4,7-Dimethoxy-3,8-dihydrobenzo[b]-1,2-oxathiin-2-oxide (167).

Following a slightly modified procedure,[276] to a stirred and cooled (0°C) solution of 7.30g (30 mmol) of the 1-hydroxysulfone (103) in 275 ml of methanol was added in portions over fifteen minutes 5.65g of sodium borohydride. The mixture was stirred for one hour and then warmed at 50°C for five minutes. The reaction mixture was then evaporated to dryness and to the residue was added 200 ml of concentrated aqueous HCl. After warming at 50°C for five minutes, 300 ml of water was addded and the aqueous mixture was extracted three times with 300 ml of CH_2Cl_2. the combined organic layer was washed twice with 200 ml of water, once with 200 ml of brine and dried over $MgSO_4$. After evaporation of the solvent, the residue was found to be sufficiently pure to be used in the next step (MP: 90.0-91.0°C). ^1H NMR (200 MHZ, DMSO-d_6) δ: 3.63 (d, 1H, J=16Hz, CH_2SO), 3.80 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 4.13 (d, 1H, J=16Hz, CH_2SO), 5.14 (dd, 2H, CH_2O-), 6.78 (s, 2H, aryl-H). CMR (75.44 MHz, $CDCl_3$) δ: 55.6 2XOCH_3; 55.7 CH-2SO; 56.6 CH_2O; 108.9, 109.1 ArCH; 110.2, 114.4, 149.1, 151.1 ArC. IR ($CDCl_3$) ν_{MAX}: 1081 (C-O-S), 1121 (S=O), 1266 (O-C). MS (CI, NH_3, 140°C) m/e: 246 (100, M+NH_4), 182 (38, M+NH_4-SO_2),

165 (65, M+H-SO_2). HRMS calculated for $C_{10}H_{12}O_4S$: [$M^{+\cdot}$] 224.0457 found 228.0434.

4,7-Dimethoxy-1,3-dihydrobenzo[2,3-c]thiophene-2,2-dioxide (168).

A solution containing 1.75g of sultine (167) in 10 ml of benzene was refluxed for one hour under argon. After evaporation of solvent, flash chromatography of the residue, using 10% ethyl acetate in toluene, gave 1.47g (84% yield) of sulfone (168). MP: 139.0-140.0°C decomposes. ^1H NMR (300 MHz, $CDCl_3$) δ: 3.81 (s, 6H, 2XOCH_3), 4.31 (s, 4H, 2XCH_2), 6.80 (s, 2H, ArH). CMR (75.44MHz, $CDCl_3$) δ: 55.7, 55.8, OCH_3; 110.2, aryl CH_2; 120.8, 149.8, aryl C. IR (FT,$CDCl_3$) ν_{MAX}: 1319, 1129, SO_2;1076, 1265, C=C-O-C. MS (CI, NH_3, 143°C) m/e: 246 (100, M+NH_4). HRMS calculated for $C_{10}H_{12}O_4S$: [$M^{+\cdot}$] 228.0457 found 228.0455.

2,5-Dihydroxy-6-methylbenzaldehyde (180).

A mixture containing 2.5g (13.9mmol) of 2,5-dimethoxy-6-methylbenzaldehyde, 12.0g of $AlCl_3$ and 3.0g of NaCl was heated at 170°C for eight minutes, then cooled, and quenched with 50 ml of saturated aqueous oxalic acid solution. The resulting mixture was extracted three times with 50 ml of ethylacetate, and the combined organic phase was washed

successively with 50 ml aliquots of water, saturated sodium bicarbonate, water, and brine. After drying over $MgSO_4$ and evaporation of solvents, flash chromatography of the residue (30% ethyl acetate in hexanes) gave 1.95g (92% yield) of 2,5-dihydroxy-6-methylbenzaldehyde. MP: 290-291°C. ^1H MNR (200 MHz, DMSO-d_6) δ: 2.40 (s, 3H, CH_3), 6.88 (dd, 2H, J=8.5 Hz), 9.11 (s, 1H, exchangeable, OH), 10.39 (s, 1H, CHO), 10.84 (s, 1H exchangeable, OH). CMR (75.44 MHz, DMSO-d_6) δ: 10.8, CH_3; 114.5, 124.4, aryl CH; 119.7, 126.1, 147.7, 155.30, aryl C; 195.8, CHO. MS (EI, 70eV, 70°C) m/e: 152 (100, $M^{+}\cdot$), 123 (22, $M^{+}\cdot$-CHO). HRMS calculated for $C_8H_8O_3$: [$M^{+}\cdot$] 152.0473 found 152.0468.

2,5-Bis(t-butyldimethylsilyloxy)-6-methylbenzaldehyde (181).

A solution containing 1.7g (11.2 mmol) of 2,5-dihydroxy-6-methylbenzaldehyde 4.22g (28 mmol) of t-butyldimethylsilylchloride,[306] 1.91g (28 mmol) of imidazole in 50ml DMF was stirred overnight at room temperature and under argon. The mixture was diluted with 100 ml of dichloromethane and washed once with 25 ml aliquots of 0.1N aqueous HCl, water, and saturated aqueous NaCl. After drying and removal of solvent, recrystallization of the residue from petroleum ether gave g (85% yield) of silylated benzaldehyde (181). MP: 65-66°C. ^1H NMR (200 MHz, $CDCl_3$) δ: 0.19 and 0.25 (s, 6H, $SiCH_3$), 1.01 and 1.02

(s, 12H, t-butyl), 2.4 (s, 3H, CH_3), 6.61 (d, 1H, J=8.5 Hz, ArH), 6.90 (d, 1H, J=8.5 Hz, ArH), 10.59 (s, 1H, CHO). CMR (75.44 MHz, $CDCl_3$) δ: -4.49, $SiCH_3$; 13.2, CH_3; 18.1, SiC; 25.6, CH_3(t-butyl); 117.0, 124.8, aryl CH; 126.4, 131.3, 148.0, 153.9, aryl C; 192.7, CHO.

4,7-Bis(t-butyldimethylsilyloxy)-1-acetoxy-1,3-dihydrobenzo[2,3-c]thiophene-2,2-dioxide (183).

A solution containing 4.0g (10.5 mmol) of 2,5-bis-t-butyldimethylsilyloxy-6-methylbenzaldehyde and 30g of SO_2 in 250 ml of thiophene free benzene was irradiated at 350nm for three days under argon and at room temperature. Removal in vacuo of the benzene layer gave a sensitive brown product. Flash chromatography (ethyl acetate) gave the silylated hydroxysulfone (182) (3.1g, 66% yield). MP: 138-140°C decomposes. ^1H NMR (200 MHz, $CDCl_3$) δ: 0.20, 0.24, 0.26 and 0.27 (s, 3H, $SiCH_3$), 0.98 and 1.04 (s, 12H, t-butyl H), 3.66 (d, 1H, J=4.0 Hz, exchangeable OH), 4.27 (dd, 2H, J=18 Hz, CH_2), 5.63 (d, 1H, J=4.0 Hz,CHOC), 6.75 (dd, 2H, J=9.0 Hz, ArH). CMR (75.44 MHz, $CDCl_3$) δ: -4.44, -4.5, $SiCH_3$; 17.83,17.93, SiC; 25.5, 6XCH_3; 51.3, CH_2; 85.0, CHOH; 119.1, 120.7, aryl CH; 123.9, 125.9, 145.7, 148.1, aryl C. The unstable hydroxysulfone (182) was immediately added to 50 ml of acetic anhydride. One drop of sulfuric acid was added and the mixture was stirred for two hours under argon at

room temperature. The mixture was then added to 100g of ice and extracted three times with 100 ml of dichloromethane. After washing the combined organic layer with 100 ml of water and drying over $MgSO_4$, evaporation of solvent in vacuo gave a beige solid. Recrystallization from hexane gave 3.0g (91% yield) of benzosulfone (183). MP: >250°C. ^1H NMR (200 MHz, $CDCl_3$) δ: 0.23, 0.25, 0.254, and 0.27 (s, 3H, $SiCH_3$), 0.974 and 1.00 (s, 9H, t-butyl CH_3), 2.22 (s, 3H, $COCH_3$), 4.26 (dd, 2H, J=18.0Hz, CH_2), 6.66 (s, 1H, OCH), 6.79 (dd, 2H,J=8.9Hz, ArH).

4,11-Dimethoxy-1-hydroxy-1,3-dihydrothioxantho[2,3-c]thiophene-2,2-dioxide (220).

Procedure A:

A solution containing 425mg (1.0 mmol) of 1-acetoxythioxanthosulfone (198) in 50 ml of tetrahydrofuran was stirred at room temperature with 15 ml of 1M aqueous NaOH for five minutes. The reaction mixture became cloudy at first but gradually turned yellow. The mixture was then diluted with 150 ml of water, extracted twice with dichloromethane to remove organic impurities, and the organic layers were discarded. The aqueous layer was then neutralized with 12M HCl and reextracted three times with 100 ml of dichloromethane. The combined organic layers were washed successively once with 50 ml of water, 50 ml of

brine, and then dried over $MgSO_4$. Evaporation of solvent gave 375mg (99% yield) of 1-hydroxythioxantosulfone (220). MP: 148-150°C decomposes. ^1H NMR (200 MHz, $CDCl_3$) δ : 3.36 (broad s, 1H exchangeable, OH), 3.82 (s, 3H, OCH_3), 4.03 (s, 3H, OCH_3), 4.54 (bs, 2H, CH_2), 5.83 (bs, 1H, CH), 7.57 (bt, 1H, ArH), 7.80 (m, 2H, ArH), 8.32 (bd, 1H, J=8.1 Hz). IR (FT,$CDCL_3$) ν_{MAX}: 3350-3600, bs, OH; 1676, thioxanthone C=O; 1289, 1183, SO_2.

Procedure B:

The same procedure as described above but carried out with 147mg (3.7 mmol) of 1-chlorothioxanthosulfone (197) gave 118mg (76% yield) of compound (220).

Chlorosulfone (191) and Chloromethylbenzaldehyde (192)

Application of the procedure used to prepare the thioxanthosulfonyl derivative (198) but with 1.1g (3.8 mmol) of isopropoxysulfone (142) gave the chlorosulfone (191) (448mg, 45% yield) and 2,5-dimethoxy-6-chloromethylbenzaldehyde (192) (146mg, 18% yield). Chloromethylbenzaldehyde (192): MP: 101-102°C. ^1H NMR (200 MHz, $CDCl_3$) δ: 3.98 (s, 6H, OCH_3), 5.20 (s, 2H, CH_2Cl), 7.17 (dd, 2H, J=6.4 Hz, ArH), 10.68 (s, 1H, CHO). CMR (75.44 MHz, $CDCl_3$) δ: 35.9, CH_2Cl; 56.2, 56.6, $ArOCH_3$; 112.8, 117.9, aryl CH; 122.7, 127.1, 151.6, 156.9, aryl C; 191.9,

CHO. IR ($CDCl_3$) ν_{MAX}: 1689, C=O. HRMS calculated for $C_{10}H_{11}ClO_3$: [$M^{+}\cdot$] 214.0397 found 214.0383.

Chlorosulfone (191): MP: 244-245°C. ^1H NMR (200 MHz, $CDCl_3$) δ: 3.90 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 4.37 (dd, 2H, J=16.6 Hz, CH_2), 5.85 (s, 1H, CH), 6.92 (dd, 2H, J=9.3 Hz, ArCH). CMR (75.44 MHz, $CDCl_3$) δ: 50.9, CH_2; 56.0, 56.2, OCH_3; 68.9, CHCl; 111.5, 113.2, aryl CH; 121.0, 124.0, 149.9, 150.9, aryl C. MS (EI, 70 eV, 145°C) m/e: 262 (41, $M^{+}\cdot$), 198(100, $M^{+}\cdot-SO_2$), 183 (58, $M^{+}\cdot-SO_2-CH_3$), 163 (40, $M^{+}\cdot-SO_2-Cl$). HRMS calculated for $C_{10}H_{11}ClO_4S$: [$M^{+}\cdot$] 262.0067 found 262.0105.

4-11-Dimethoxy-1-acetoxy-1,3-dihydrothioxantho[2,3-c]thiophene-2,2-dioxide (198)

Following a modification of Honek' procedure,[251] 1.37g (4.8mmol) of benzosulfone acetate (139) was added under argon to a solution containing 1.91g (9.4mmol) of o-chlorosulfenylbenzoylchloride and 0.5 ml of $SnCl_4$ in 25 ml of dichloromethane. After stirring at room temperature for 24 hours, the mixture was thrown in 50g of ice, diluted with 100 ml of dichloromethane., and the separated organic phase was washed consecutively with 50 ml aliquots of water, saturated sodium bicarbonate, water, brine, and then dried over $MgSO_4$. After evaporation of solvent, flash chromatography of the residue (ethyl acetate-toluene) gave

the chlorosulfone (191) (113mg, 9% yield), the starting material (165mg, 12% yield), as well as the following compounds:

Thioxanthone (198) (282mg, 14% yield) MP:190-195°C decomposes. ^{1}H NMR (200 MHz, $CDCl_3$) δ : 2.29 (s, 3H, CH_3), 3.97 (s, 3H, OCH_3), 4.01 (s, 3H, OCH_3), 4.47 (dd, 2H, J=16.0 Hz, CH_2), 6.83 (s, 1H, CH), 7.60 (m, 3H, ArH), 8.47 (d, 1H, J=6.0 Hz, ArH). CMR (75.44 MHz, $CDCl_3$) δ : 20.4, CH_3; 51.9, CH_2; 62.1, 62.2, OCH_3; 82.1, CH; 126.0, 127.0, 129.6, 132.6, aryl CH; 124.9, 125.6, 127.6, 130.7, 134.9, 135.1, 149.3, 155.2, aryl C; 168.5, ester C=O; 179.9, thioxanthone C=O. IR (ft, $CDCl_3$) ν_{MAX}: 1766, ester C=O; 1645, thioxanthyl C=O. MS (EI, 70eV, 225°C) m/e: 420 (41, $M^+\cdot$), 356 (62, $M^+\cdot-SO_2$), 313 (56, $M^+\cdot-SO_2-C_2H_3O$. .

Thioxanthone (197) (218mg, 11% yield) MP: 242-244°C decomposes. ^{1}H NMR (200 MHz, $CDCl_3$) δ : 4.00 (s, 3H, OCH_3), 4.15 (s, 3H, OCH_3), 4.56 (dd, 2H, J=15.5 Hz, CH_2), 5.94 (s, 1H, CHCl), 7.63 (m, 3H, ArH), 8.49 (dd, 1H, J=1.6,7.9 Hz, ArH). IR (KBr) ν_{MAX}: 1650, thioxanthone C=O; 1323, 1190, SO_2; 810, C-Cl. MS (EI, 70eV, 230°C) m/e 396 (49, $M^+\cdot$), 332 (59, $M^+\cdot-SO_2$).

A 1:1 mixture of sulfones (195) and (196) in 4% yield (61mg). ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.28 (s, 3H, $COCH_3$), 3.86 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 4.28 (dd, 2H, J=16.0 Hz, CH_2), 6.70 (s, 1H, CH), 7.02 (s, 1H, ArH). ^{1}H NMR of the other isomer (200 MHz, $CDCl_3$) δ: 2.24 (s, 3H, $COCH_3$), 3.80

(s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 4.50 (bs, 2H, CH_2), 6.60 (s, 1H, CH), 7.40 (s, 1H, ArH). MS (EI, 70eV, 110°C) m/e: 320 (18, $M^{+}\cdot$).

A 1:1 mixture of (193) and (194): (199mg, 14% yield) MP:> 250°C. ^1H NMR of the mixture of 1,5 and 1,6-dichloro-4,7-dimethoxy-1,3-dihydrobenzo[2,3-c]thiophene-2,2-dioxide δ: 3.90, 3.91, 3.97 and 4.04 (s, 6H, OCH_3), 4.40 (overlapped dd, 2H, CH_2), 5.86 and 5.89 (s, 1H, CHCl), 7.02 and 7.06 (s, 1H, ArH). MS (EI, 70eV, 120°C) m/e: 296 (23, $M^{+}\cdot$), 261 (24, $M^{+}\cdot-Cl$), 232 (76, $M^{+}\cdot-SO_2$), 217 (66, $M^{+}\cdot-SO_2-CH_3$), 197 (23, $M^{+}\cdot-SO_2-Cl$).

Chlorosulfone (199)

Repetition of the procedure described for thioxanthone (198) with $TiCl_4$ as Lewis acid gave the chlorosulfone (199) (27% yield) instead of the desired tetracycle (198) or its regioisomer. MP: 178-180°C. ^1H NMR (200 MHz, $CDCl_3$) δ: 3.85 (s, 3H, OCH_3), 5.14 (dd, 2H, J=11.1 Hz, CH_2SO_2), 7.01 (s, 1H, ArH), 7.30-7.53 (m, 3H, ArH), 8.29 (d, 1H, J=8.0 Hz, ArH). CMR (75.44 MHz, $CDCl_3$) δ: 37.5, CH_2; 56.5, 62.1, OCH_3; 76.8, CHCl; 113.8, 126.8, 127.5, 133.0, 133.7, aryl CH; 120.5, 123.8, 130.7, 134.5, 139.7, 150.0, 159.9, aryl C; 164.0, C=O. MS (CI, NH_3, 137°C) m/e: (CI, NH_3, 154°C) m/e: 385 (100, $M^{+}+H-CH_3$), 387 (71, $M^{+}+H-CH_3$)

(5) and (R) 1-(4'-O-p-nitrobenzoyl-N-trifluoroacyldaunosamine)-113-dihydrothioxantho[2,3-C]thiophene-2,2-dioxide (200) and (201)

Following a modification of Kimura procedure,[292] 0.70 ml (2.6 mmol) of trimethylsilyl trifluoromethane sulfonate was added dropwise, at -25°C and under argon, to a mixture containing 0.70g (1.3 mmol) of protected daunosamine (134), 1g of 4A molecular sieves in 100 ml of a 1:3 ether/ dichloromethane solvent system. After stirring at -5°C for one hour, the mixture was cooled to -10°C, and a solution containing 400 mg (1.1 mmol) of thioxanthone (147) in 50 ml of dichloromethane was transfered under argon over one minute. The resulting mixture was stirred overnight and then added to 100 ml of a 1:1 saturated aqueous sodium bicarbonate-ethyl acetate solvent system. The organic phase was separated and the aqueous layer was extracted twice with 50 ml of dichloromethane. The combined organic layer was washed successively with 100 ml of water, 100 ml of brine and then dried over $MgSO_4$. Evaporation of solvents yielded a yellow residue which was flash chromatographed with 10% ethyl acetate in toluene. The first collected fraction (39% yield) is arbitrarily assigned as compound (200). (MP: 140-144°C decomposes). ^1H NMR (200 MHz, $CDCl_3$) δ: 1.22 (d,3H, J=6.5Hz, CH_3), 2.16 (m,2H,2'-CH_2), 3.87 (s,3H,OCH_3), 3.99,(s,3H, OCH_3),

4.36 (bs, 2H,CH_2), 4.48 (overlapped m, 2H, C<u>H</u>NH and C<u>H</u>CH_3), 5.38 (bs,1H,OCH), 5.71 (bs,1H,O-CH-O), 5.76 (s, 1H, OC<u>H</u>SO_2), 6.96 (bd,1H, J=7.0Hz,NH), 7.40-7.65 (m,3H, ArH), 8.14 (bs,4H, ArH), 8.32 (d,1H, J=6.8Hz,ArH). CMR (75.44 MHz, $CDCl_3$) δ: 17.0, 6'-CH_3; 29.0, 2'-CH_2; 44.8, 3'-CHNH; 50.6, CH_2SO_2; 62.1, 62.2, ArOCH_3; 66.6, O<u>C</u>HCH_3; 71.3, 4'-OCH; 85.7 $OCHSO_2$; 97.0, O-CH-O; 115.4, quartet, J=287.8 Hz, CF_3; 123.6, 130.9, p-nitrobenzoyl CH; 125.9, 127.0, 129.4, 132.5, thioxanthone, CH; 124.9, 125.5, 128.1, 128.9, 134.4, 134.7, 135.1, 148.9, 150.6, 155.4, aryl C; 157.0, quartet, J=37.7 Hz, <u>C</u>OCF_3; 164.3, ester C=0; 179.8, thioxanthone C=0.

The more polar epimer (38% yield) assigned as (201), had: (MP: 147-148°C - decomposes). ^{1}H NMR (200 MHz, $CDCl_3$) δ:1.18 (d,3H, J=6.4Hz, CH_3), 2.15 (m, 2H, 2'- CH_2), 3.87 (s, 3H, OCH_3), 3.98 (s,3H, OCH_3), 4.37 (bs, 2H, CH_2SO_2), 4.69 (m,1H,C<u>H</u>NH), 4.77 (bq, 1H, J=6.6 Hz, C<u>H</u>CH_3), 5.38 (bs, 1H, OCH), 5.50 (bs, 1H,O-CH-O), 5.69 (s,1H, $OCHSO_2$), 6.74 (d, 1H,J=7.3Hz, NH), 7.40-7.64 (m, 3H, ArH), 8.17 (s,4H, ArH), 8.33 (d,1H, J=8,0Hz, ArH). CMR (75.44MHz, $CDCl_3$) δ: 16.8, 6'- CH_3; 29.5, 2'-CH_2; 45.6, 3'-CHNH; 51.1, CH_2SO_2; 61.9, 62.2, ArOCH_3; 67.2, O<u>C</u>HCH_3; 72.1, 4'-OCH; 89.0, $OCHSO_2$; 99.3, O-CH-O; 117.3, quartet, J=287.7Hz, CF_3; 123.8, 131.0, p-nitrobenzoyl CH; 126.0, 127.1, 129.5, 132.6, thioxanthone CH; 124.2, 125.2, 128.2, 130.0, 132.6, 134.3, 134.7, 148.9,

150.8, 155.5 aryl C; 157.8, quartet, J=37.5Hz, $\underline{C}OCF_3$; 164.8, ester C=O, 179.9 thioxanthone C=O.

(1S)1-(N-trifluoroacyldaunosamine)-4,11-dimethoxy-1,3-dihydrothioxantho[2,3-C]thiophene-2,2-dioxide (202).

To a solution containing 88 mg (0.12 mmol) of glycoside (200) in 5 ml of methanol was added under argon at 0°C, 0.01 ml of a 1M methanolic sodium methoxide solution. After stirring for 0.5 hr, three drops of saturated aqueous ammonium chloride were added, and the reaction mixture was evaporated to dryness. The residue was dissolved in dichloromethane and the undissolved white solid was filtered off. The filtrate was evaporated to give a yellow residue to which was added 0.5 ml of CH_2Cl_2 for dissolution, followed by 30 ml of pentane. Filtration of the suspension gave a yellow precipitate found to be almost pure (202). Flash chromatography using 20% ethyl acetate in toluene gave 55 mg (78% yield) of thioxantyl glycoside (202). (MP: 150-155°C - decomposes). ^1H NMR (300 MHz, $CDCl_3$) δ: 1.40 (d,3H,J=6.5Hz, CH_3), 1.96 (dt,1H,J= 3.8,13.4 Hz,2'-HC$\underline{H}$a), 2.14 (dd,1H,J= 5.0, 13.5 Hz,2'-HC$\underline{H}$e), 3.66 (bs,1H,4'-OCH), 4.01 (s,3H, OCH_3), 4.07 (s,3H, OCH_3), 4.37 (overlaped m, 2H, 3'-CH and 5'-OCH),

4.46 (d,2H,CH_2SO_2), 5.71 (bd,1H,J<3.1Hz,O-CH-O), 5.88 (s, 1H,O-$CHSO_2$), 6.80 (bd,1H,J=8.7Hz,NH), 7.50 - 7.70 (m, 3H, ArH), 8.50 (dd,1H,J=0.8,7.5Hz,ArH). CMR (75.44 MHz, $CDCl_3$) δ: 16.7, 6'-CH_3; 28.5, 2'- CH_2; 45.4, 3'-CH; 50.4, CH_2SO_2; 62.2, 2XArOCH_3; 67.3, 5'-CH; 68.7, 4'- CH; 85.3, $OCHSO_2$; 96.8, O-CH-O; 117.7, quartet, J=285.9Hz, CF_3; 125.4, 129.2, 129.8, 132.7, aryl CH; 126.1, 127.2, 128.4, 129.7, 130.9, 134.9, 149.2, 155.8, aryl C; 156.5, quartet, J=37.5Hz, $\underline{C}OCH_3$; 180.3, C=O.

(1S)1-daunosamine-4-11-dimethoxy-1,3-dihydrothioxantho [2,3-C] thiophene-2,2-dioxide (203).

A solution containing 30 mg (0.05 mmol) of glycoside (202) in 1 ml of THF was stirred for 0.5 hr at room temperature with 10 ml of a 0.1M aqueous NaOH solution. The reaction mixture was then neutralized with 1M aqueous HCl and evaporated to dryness. The residue was then stirred with 5 ml of dichloromethane, then filtered and the precipitate was triturated three times with ethylacetate. The combined organic filtrate was evaporated to dryness. Recrystallization of the residue from dichloromethane pentane gave 20.5 mg (81% yield) of thioxanthylglycoside (203). (MP: 139-141°C decomposes.)

^{1}H NMR (300 MHz,$CDCl_3$) δ: 1.21 (bd,3H,J= 6.4H_3, 6'- CH_3), 2.12 (m,2H,2'-CH_2), 3.62 (bs,1H,4'- OCH), 3.83 (s, 3H,OCH_3), 3.93 (s,3H,OCH_3), 4.11 (m,1H, 3'-CH), 4.27 (m,5'-OCH), 4.34 (bs,2H,CH_2SO_2), 5.58 (bs, 1H,O-CH-O), 5.77 (s,1H,O-CH-SO_2), 7.35 - 7.55 (m,3H,ArH), 7.90 (bs,2H, NH_2), 8.33 (d,1H,J=7.7Hz,ArH).

(1R)1-(N-trifluoroacyldaunosamine)-4,11-dimethoxy-1,3-dihydrothioxanto[2,3-C]thiophene-2,2-dioxide (204).

Following the procedure described for compound (202), 15 mg (74% yield) of thioxanthyl glycoside (204) was obtained from the catalytic methanolysis of 22 mg (0.04 mmol) of (201). (MP: 144-148°C - decomposes).

^{1}H NMR (300 MHz,$CDCl_3$) δ: 1.32 (d,3H,J=6.6Hz, 6'- CH_3), 1.96 (dt,1H,J=3.9,13.5Hz,2'-HC<u>Ha</u>), 2.10 (dd,1H, J=5.4 13.6Hz,2'-HC<u>He</u>), 3.72 (bs,1H,4'-OCH), 3.98 (s,3H, OCH_3), 4.03 (s,3H,OCH_3), 4.44 (s,2H,CH_2SO_2), 4.47 (m,1H,3'- CH), 4.66 (bq,1H,J=6.6Hz,5'-CH), 5.44 (bd,1H, J<3.5Hz,O-CH- O), 5.73 (s,1H,$OCHSO_2$), 6.79 (bd,1H,J= 8.5Hz,NH), 7.5 - 7.75 (m,3H,ArH), 8.48 (dd,1H,J=1.3,8.0Hz, ArH).

Attempted Synthesis of Thioxanthonetetralin Glycosides

In all of our cycloaddition attempts significant deglycosidation resulted. The daunosamine counterpart could be isolated in varying yields. For example, daunosamine (135) ^1H NMR (200 MHz, $CDCl_3$)δ: 1.23 (d, 3H, J=6.3 Hz, CH_3), 2.10 (m, 2H, CH_2), 4.23 (bq, 1H, J=6.1 Hz, $CHCH_3$), 4.73 (m, 1H, CHNH), 5.43 (bs, 1H, OCH), 5.48 (bs, 1H, O-CH-O), 6.33 (bd, 1H, J=6.7 Hz, NH), 8.32 (dd, 4H, J=6.4 Hz, ArH). CMR (75.44 MHz, $CDCl_3$) δ: 16.9, CH_3; 29.2, CH_2; 45.5, HC-NH; 65.8, HC-O; 71.2, HC-O; 91.8, O-CH-OH; 123.7, 130.9, aryl CH; 115.4, quartet, J=288 Hz, CF_3; 134.5, 150.6, aryl C; 157.1, quartet, J=38.0 Hz, $COCF_3$.

1,4-dimethoxy-2-formyl-3-methylthioxanthone (145)

A solution containing 50 mg (0.13 mmol) of 1-hydroxythioxanthosulfone (147) in 10 ml of xylenes was refluxed for 5 hours under argon. After removal of solvent, flash chromatography of the residue (ethyl acetate/hexanes) gave 18.5 mg (45% yield) of thioxanthone aldehyde (145). (MP: 179-180°C). ^{1}H NMR (200 MHz,$CDCl_3$) δ: 2.61 (s,3H,CH_3), 3.92 (s, 3H,OCH_3), 4.05 (s,3H,OCH_3), 7.5-7.8 (m,3H,ArH), 8.24 (d, 1H,J=8.9 Hz,ArH), 10.61 (s, 1H,CHO). CMR (75.44 MHz, $CDCl_3$) δ: 12.2, CH_3; 61.9, 64.2, OCH_3; 126.0, 126.7, 127.8, 129.6, aryl CH; 123.7, 127.0, 127.8, 131.3, 131.5, 132.2, 135.3, 144.4, aryl C; 159.9, CHO; 191.3, thioxantyl C=0. HRMS calculated for $C_{17}H_{14}O_4S$: [M+·] 314.0613 found 314.0613.

p-Nitrobenzyl(5,8-dimethoxyisochroman-3-yl) formate (216)

The same methodology as employed for compound (304) was used but with 40g (.18mmol) of p-nitrobenzylglyoxalate hydrate.[309] A 58% yield (4.1g) of the titled compound was obtained after flash chromatography (MP: 140-141°C). ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.84 (dd,1H,J=16.3, 10.3 Hz, HC<u>Ha</u>CHC=O), 3.09 (dd,1H,J=16.2, 4.0Hz, HC<u>He</u>CHC=0), 3.77 (s,3H, OCH_3), 3.80

(s,3H,OCH_3), 4.39 (dd,1H,J=10.3, 4.0Hz, OCHC=O), 4.70 (d, 1H,J=16, OH_3, HC<u>Ha</u>O), 5.07 (d,1H, J=15.9 Hz, HC<u>He</u>O), 5.36 (broad,s,2H,CH_2), 6.67 (broad,s,2H,ArH), 7.54 (d,2H, ArH), 8.24 (d,2H,ArH). CMR (75.44 MHz,$CDCl_3$) δ: 25.4, CH_2; 55.2, 55.4, $ArOCH_3$; 64.3, 65.0, OCH_2; 72.4, OCH; 107.2, 107.6, 123.6, 128.3, aryl CH; 121.4, 123.3, 142.6, 147.5, 149.1, 150.6, aryl C; 170.7, ester CO, IR ($CDCl_3$) νmax: 1756, ester CO; 1526, 1350, nitro. MS (CI,NH_3, 160°C) m/e: 391 (100,$M+NH_4$).

p-nitrobenzyl(5,8-dioxo-5,8-dihydroisochroman-3-yl) formate (224)

p-Nitrobenzyl (5,8-dimethoxyisochroman-3-yl) formate was oxidised as described for (262). The titled compound was obtained in 92% yield. (MP:133°C decomposes) ^{1}H NMR (200 MHz,$CDCl_3$) δ: 2.70 (ddt,1H,J=18.7,9.0,3.0Hz, HC<u>Ha</u>CHC= O), 2.95 (d multiplet,1H,J=19.0Hz,HC<u>He</u>CHC=O), 4.38 (dd,1H,J= 8.9, 4.3 Hz,OCHC=O), 4.56 (dt,1H,J=17.1,3.0Hz, HC<u>Ha</u>-O) 4.88 (ddd,1H, J=17.3,2.8,1.7 Hz,HC<u>He</u>-O), 5.36 (broad s,2H, CH_2, ArH), 6.79 (dd,2H,ArH), 7.57 (d,2H,ArH), 8.29 (d,2H, ArH). CMR (75.44 MHz,$CDCl_3$) δ: 24.1, CH_2; 62.3, 65.6, OCH_2; 71.4, OCH; 123.8, 128.6, aryl CH; 136.1, 136.3, CH; 137.6, 139.5, quaternary C; 142.1, 147.8, aryl C; 169.5, ester CO; 185.0, 185.1, quinone CO. IR ($CDCl_3$) νmax: 1756, ester CO; 1658, quinone CO; 1526, 1350, nitro. MS (CI, NH_3, 161°C) m/e: 361 (47,$M+NH_4$).

p-Nitrobenzyl (5,12-dihydroxy-2,3-dihydrothioxantho [3,2-C] and [2,3-c] pyran-3-yl) formate (226a and 226b).

Following a slight modification of Honek procedure,[251] a solution containing 610 mg (1.8 mmol) of isochromandione (224) in 10 ml of dry acetonitrile was added dropwise over one hour to a warmed (55°C) mixture containing 525 mg (3.4 mmol) of O-mercaptobenzoic acid in 15 ml of anhydrous methanol. After stirring overnight, solvents were removed in vacuo and to the residue was added 30 ml of a 2:1 solution of TFA/TFAA at room temperature. Stirring was continued overnight and then mixture was added slowly to 100 ml of cold saturated sodium bicarbonate. The mixture was extracted four times with 50 ml of dichloromethane, and the combined organic layer was washed once with 100 ml of water, 100 ml of brine and dried over $MgSO_4$ solvents were then removed in vacuo and the dark red residue was flash chromatographed. Two components were obtained. The first is assigned to 226a. (250mg, 29% yield) had: (MP: 213.5-214°C). ^1H NMR (200 MHz, DMSO-D_6) δ: 2.98 (dd,1H,J= 8.8, 17.5 Hz, HCHa), 3.20 (dd,1H,J=4.13, 17.5 Hz,HCHe), 4.68 (overlapped dd,1H,OCH), 4.74 (d,1H,J=15.9 Hz,HCHa), 4.99 (d, 1H,J=15.9 Hz,HCHe), 5.40 (bs,2H,OCH_2), 7.6-8.0 (m,3H, ArH) 7.68 (d,2H,J=8.9Hz,ArH), 8.26 (d,2H,J=8.9Hz, ArH), 8.49 (dd, 1H,J=0.9,8.0 Hz,ArH), 9.56 (s,1H, exchangeable OH), 13.85(s, 1H, exchangeable, chelated OH). CMR (75.44MHz,DMSO-D_6)

δ: 26.5, CH_2, 63.1, 65.4, OCH_2; 71.7, OCH; 124.0, 129.0, aryl PNBCH; 127.1, 127.3, 128.9, 134.0, aryl CH; 111.4, 118.5, 129.6, 138.., 140.5, 143.8, 147.5, 153.8, aryl CH; 170.6, ester C=O; 185.5, thioxanthone C=O. The more polar component (284 mg, 33% yield) assigned as 226b had (MP: 218-220°C). ^1H NMR (200 MHz, DMSO-d_6) δ: 2.86 (dd,1H,J=9.2, 16.7 Hz,HCHa), 3.07 (dd,1H,J=4.2, 16.8 Hz, HCHe), 4.68 (dd,1H,J= 4,5,9.2 Hz,OCH), 4.82 (d,1H,J= 16.7Hz, OHCHa), 5.09 (d,1H,J= 16.5 Hz,OHCHe), 7.5-8.1 (m,3H, ArH), 7.66 (d,2H,J= 8.9 Hz,ArH), 8.9 (d,2H,J=8.9Hz,ArH), 8.49 (dd, 1H,J=1.0, 8.0 Hz,ArH), 9.35 (s,1H, exchangeable,OH), 13.64 (s,1H, exchangeable, chelated OH). IR (FT,$CDCl_3$) νmax: 3420, broad, OH; 1753, ester C=O; 1682, thioxanthone C=O.

Pyranylthioxanthonediacetate (227b)

A mixture containing 150 mg (3.1 mmol) of thioxantho [2,3-c] pyran derivative (226b), 10 ml of acetic anhydride, 10 ml of pyridine, 10 mg of dimethylaminopyridine in 25 ml of dichloromethane was stirred for three hours at room temperature under argon atmosphere.[251] The mixture was then added to 50 g of ice and extracted three times with 75 ml of dichloromethane. The combined organic layer was then washed twice with 100 ml of 1N aqueous HCl, once with 100 ml of water, once with 100 ml of brine, and then dried over $MgSO_4$. Solvent was removed <u>in vacuo</u> and the crude

diacetate was found to be pure enough for further use. (MP: 254-255°C). ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.48 (s,3H,$COCH_3$), 2.53 (s,3H,$COCH_3$), 3.1 (bm,2H,CH_2), 4.55 (bm,1H,CH), 4.70 (bd,1H,J=16.2 Hz,HCha), 5.03 (bd,1H,J=16.2 Hz,HChe), 5.35 (dd,2H,J=13.8 Hz,OCH_2) 7.55 (overlapped m,5H,ArH), 8.40 (d, 2H,J=8.9 Hz,ArH), 8.46 (d,1H,J=7.7 Hz, ArH). CMR (75.44MHz, $CDCl_3$) δ:20.25, 21.17, CH_3; 25.0, CH_2; 63.6, 65.6, OCH_2; 72.4, OCH; 123.9, 128.6, PNB aryl CH; 125.9, 126.9, 129.7, 132.5, aryl CH; 124.5, 130.0, 131.2, 132.2, 134.3, 138.6, 142.2, 147.8, aryl C; 167.5, 16°.3, acyl C=0, 169.8, PNBOC=0; 178.8, thioxanthone C=0. IR (FT,$CDCl_3$) νmax: 1757, 1744, 1733, ester C=O; 1645, thioxanthone C=O.

Pyranylthioxanthonediacetate (227a)

A quantitative yield (148 mg from 125 mg of 226a) of this compound was obtained by following the procedure described for (227b). (MP: 253-254°C). ^{1}H NMR (200 MHz, DMSO-d_6, 100°C) δ: 2.40 (s,3H, $COCH_3$), 2.50 (s,3H,$COCH_3$), 2.90 (m,2H,CH_2), 4.71 (dd, 1H,J=5.4,7.8 Hz, OCH), 4.69 (d,1H,J= 16.0Hz, OHCHa), 4.95 (d,1H,J=16.0 Hz, OHCHe), 6.9-7.6 (m,3H, ArH), 7.62 (d,2H,J=8.6Hz, ArH), 8.18 (d,2H,J=8.7Hz,ArH) 8.33 (d,1H,J=8.7 Hz,ArH). IR (FT,$CDCl_3$) νmax: 1756, 1746, 1733, ester C=O; 1646, thioxanthone C=O.

p-Nitrobenzyl [5,12-dioxo-3,4,5,12-tetrahydroanthraceno [2,3-c] pyran-3-yl] formate (239)

A solution containing 669 mg (1.9 mmol) of pyranoquinone (221), 485 mg (2.9 mmol) of 3,6-dihydrobenzo [b]-1,2-oxathiin-2-oxide[276] in 50 ml of xylenes was refluxed overnight. The solvent was then removed in vacuo. The residue was flash chromatographed with 10% ethyl acetate in toluene and gave 536 mg (62%) of the titled pyranoanthraquinone. (MP: 214-215°C).

^{1}H NMR (200 MHz, $CDCl_3$) δ:2.84 (ddt,1H, J=18.9, 9.0, 2.7 Hz, HCHaCHC=0), 3.12 (broad d,1H,J=19.0 Hz, HCHeCHC=0), 4.40 (dd,1H,J=9.1, 4.4 Hz,OCHC=0), 4.67 (dt, 1H,J=19.0, 3.0 Hz, HCHa-0), 5.04 (broad d,1H, J=18.9 Hz, HCHe-0), 5.33 (broad s,2H,$ArCH_2$), 7.53 (d,2H,ArH), 7.67 (m,2H,ArH), 8.04 (m,2H,ArH), 8.21 (d,2H,ArH), 8.58 (s,1H, ArH), 8.62 (s,1H, ArH). CMR (75.44 MHz, DMSO-d_6) δ: 24.5, CH_2; 62.2, 65.1, OCH_2; 70.9, OCH; 123.6, 128.0, 128.9, 129.8, 130.2, aryl CH; 128.3, 134.3, 141.3, 142.7, 143.4, 147.2, aryl C; 169.7, ester CO; 182.0, 182.2, quinone CO. IR($CDCl_3$) νmax: 1746, ester CO; 1661, quinone CO. MS (CI,NH_3, 160°C). m/e: 461 (100, M + NH_4). HRMS calculated for $C_{25}H_{17}NO_7$: [M+·] 443.1005 found 443.1003.

5,8-Dimethoxy-1-methylbenzo[C]1,2-oxathiin-2-oxide (246)

Following a slight modification of Durst and Charlton procedure[277], 800 ml of a 2.5 M solution of methyl lithium in hexanes was added, at room temperature and under argon, to a solution containing 14.38 g (59 mmol) of hydroxysulfone (103) in 2.5 l of anhydrous THF. After stirring for 16 hours, 400 ml of methanol was added to the mixture. Solvents were removed under vacuo and the residue was treated with 500 ml of 12 M HCl at 50°C for 3 min. The solution was then diluted in 1 l of water and extracted three times with 300 ml of dichloromethane. The combined organic layer was washed twice with water, once with brine and dried over $MgSO_4$. After removal of solvents, flash chromatography of the residue gave sultine (246) as a yellow solid in 47% yield. (MP: 152-153°C). ^1H NMR (200 MHz, $CDCl_3$) δ:1.58 (d,3H,J=6.2 Hz, CH_3), 3.49 (d,1H,J=14.8 Hz,CH), 3.79 (s,3H,OCH_3), 3.80 (s,3H, OCH_3), 4.29 (d,1H, J=14.8 Hz,CH), 5.63 (q,1H,J=6.2 Hz, $CH_3C\underline{H}$), 6.82 (s,2H, ArH). CMR (75.44 MHz,$CDCl_3$) δ: 15.3, CH_3; 52.2, CH_2; 55.7, OCH_3; 59.9, CH; 110.1, 110.6, aryl CH; 123.6, 123.8, 149.8, 149.9, aryl C. MS (CI, NH_3,137°C). m/e: 246 (100, $M+NH_4$). The 4,7-dimethoxy-1-methyl-1,3- dihydrobenzo [2,3-C]thiophene-2,2-dioxide was obtained in 7% yield. (MP: 198-199°C). ^1H nmr (200 MHz, $CDCl_3$) δ: 1.58 (d,3H,J= 7.2 Hz,CH_3), 3.78 (s,3H,OCH_3), 3.79 (s,3H, OCH_3),

4.22 (dd,2H,J=17.2 Hz,CH_2), 4.33 (q,1H,J=7.2 Hz, C<u>H</u>CH_3), 6.76 (dd,2H,J=9.0 Hz,ArH). HRMS calculated for $C_{11}H_{14}O_4S$: [M+·] 242.0613 found 242.0587.

Cis and trans p-nitrobenzyl (5,8-dimethoxy-1- methylisochroman-3-yl) formate (247 and 248)

These 1-methylated isochromans were obtained by following the same procedure as described for isochroman (216). Thus, the reaction between 4 g (16.5 mmol) of 1-methylated sultine (246) and 18.76 g (83 mmol) of p-nitrobenzylglyoxalate hydrate resulted in a black residue which after flash chromatography (10% ethyl acetate - 25% toluene - 65% cyclohexane) gave the two diastereomeric isochromans (247) and (248) in a 3:1 ratio. The trans isomer (248) (2.8g, 44% yield) had: (MP:110-111°C). ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.53 (d,3H, J=6.8 Hz, CH_3), 2.85 (dd,1H,J=9.8, 16.8 Hz, Hc<u>ha</u>), 3.07 (dd,1H,J= 4.4, 16.7 Hz, HC<u>he</u>), 3.78 (s,3H,OCH_3), 3.79 (s,3H,OCH_3), 4.69 (dd,1H,J=4.7, 9.9 Hz, OCH), 5.29 (q,1H,J=6.7 Hz, OC<u>H</u>CH_3), 5.33 (bs,2H, OCH_2), 6.68 (s,2H,H-C=C-H), 7.49 (d,2H,J=8.9 Hz, ArH), 8.22 (d,2H,J=8.9 Hz,ArH). CMR (75.44 MHz, $CDCl_3$) δ: 19.6, CH_3; 25.5, CH_2; 55.3, 55.4, OCH_3; 65.0, OCH_2; 67.2, 68.6, OCH; 107.8, 123.6, 128.2, aryl CH; 121.0, 128.0, 142.8, 147.6, 149.2, 150.6, aryl C; 171.4, ester C=O.

IR (FT, $CDCl_3$) νmax: 1756, ester C=O, 1216, C-O. MS (CI, NH_3, 191°C) m/e: 405 (52, $M+NH_4$), 270 (100, $M+NH_4-C_7H_5NO_2$). The cis isomer (247) (0.9g, 14% yield, oil) had: ^1H NMR (200 MHz, $CDCl_3$) δ: 1.62 (d,3H, J=6.4Hz,CH_3, 2.73 (ddd,1H,J=2.1, 11.4, 16.0Hz, HCHa), 3.17 (dddd,1H,J= 1.2, 2.4, 16.0Hz, HCHe), 3.79 (s,3H, OCH_3), 3.80 (s,3H, OCH_3), 4.23 (dd,1H,J=2.4, 11.4, OCH), 5.09 (bq,1H,J=6.5 Hz, CHCH$_3$), 5.36 (bs,2H,OCH_2), 6.71 (bs,2H,ArH), 7.80 (d,2H,J=8.9 Hz, ArH), 8.25 (d,2H,J=8.9 Hz,ArH). CMR (75.44 MHz, $CDCl_3$) δ: 21.5, CH_3; 26.7, CH_2; 55.2, 55.6, OCH_3; 65.0, OCH_2, 71.5, 72.1, OCH; 108.4, 123.7, 128.3, aryl CH_2; 107.9, 122.9, 142.8 148.1, 149.9, 150.4, aryl C; 170.8, ester C=O. IR (FT, $CDCl_3$) νmax: 1755, ester C=O, 1219, C=O. HRMS calculated for $C_{20}H_{21}NO_7$ 387.1318 found 387.1299.

cis p-Nitrobenzyl(5,8-dioxo-1-methyl-3,4,5,8-tetrahydro-benzo [2,3-c]pyran-3-yl) formate (251)

To 400 mg (1.0 mmol) of isochroman (247) in 10 ml of acetonitrile was added, dropwise over 5 min and with stirring, a solution containing 1.96 g (3.6 mmol) of ceric ammonium nitrate[320,323] in 10 ml of water. After five minutes the mixture was diluted with 25 ml of water and then extracted three times with 50 ml of methylene chloride.

The combined organic layer was washed once with 50 ml of water, once with 25 ml of brine, and then dried over $MgSO_4$. Following evaporation of solvent, the oily yellow residue was found to be pure (95% yield) isochromandione (251). Flash chromatography, with 20% ethyl acetate in toluene, reduced the yield (65%) considerably without significantly increasing the purity. ^1H NMR (200 MHz, $CDCl_3$) δ:1.57 (d,3H,J=6.7 Hz,CH_3), 2.57 (ddd,1H,J= 4.3, 10.8, 18.4 Hz, HCHa), 2.98 (bdt,1H,J= 2.8, 18.4Hz, HCHe), 4.19 (dd,1H,J=2.75, 10.8 Hz,O-CH), 4.81 (m, 1H, OCHCH$_3$), 5.36 (bs,2H,OCH_2), 6.76 (dd,2H,J=10.1 Hz, ArH), 7.57 (d,2H,J=9.0 Hz,ArH), 8.24 (d,2H,J=9.0 Hz, ArH). CMR (75.44 MHz,$CDCl_3$) δ:20.2, CH_3; 25.2, CH_2: 65.3, OCH_2; 70.2, 71.1, OCH; 123.6, 128.4, aryl CH; 135.7, 136.8, CH; 138.4, 142.2, 143.4, 147.6, quaternary C; 169.2, ester C=O; 185.2, 185.4, quinone C=O.

trans-p-Nitrobenzyl (5,8-dioxo-1-methyl-5,8-dihydro-isochroman-3-yl) formate (252)

Oxidative demethylation of 160 mg (0.4 mmol) of trans-p-nitrobenzyl (5,8-dimethoxy-1-methylisochroman- 3-yl) formate with 780 mg (1.4 mmol) of ceric ammonium nitrate[320,323], as described for compound (251), gave 137 mg of isochromandione (252) as a yellow oil.

^{1}H NMR (200 MHz,$CDCl_3$) δ:1.5 (d,3H,J=6.8 Hz,CH_3), 2.67 (ddd,1H,J=2.2, 8.9, 19.0 Hz, HCHa), 2.89 (ddd,1H,J=1.3, 4.6, 19.0 Hz, HCHe), 4.59 (dd,1H,J=4.7, 8.9 Hz,OCH), 5.04 (bq,1H,J=6.8 Hz,OCHCH$_3$), 5.33 (bs,2H,OCH_2), 6.75 (dd,2H, J=10.1 Hz,H-C=C-H), 7.54 (d,2H,J=8.9 Hz,ArH), 8.24 (d,2H,J= 8.9 Hz,ArH). CMR (75.44 MHz,$CDCl_3$) δ: 19.4, CH_3; 24.4, CH_2; 65.5, OCH_2; 66.7, 67.1, OCH; 123.8, 128.6, 136.0, 136.5, aryl CH; 137.3, 142.2, 143.5, 146.7, aryl C; 185.0, 185.3, quinone C=O.

5,8-dimethoxy-3-trichloromethyl-1-methylisochroman (258)

To a refluxing solution containing 1.2g (8 mmol) of chloral in 10 ml of benzene was added dropwise over two hours 500 mg (2 mmol) of 1-methylsultine (246) dissolved in 25 ml of benzene. Reflux was continued for two hours and then the solvent was removed in vacuo. Flash chromatography of the residue gave 306g (47% yield) of a mixture of cis and trans isochromans (258). (MP: 83.0-84.5°C) (mixture). Cis isomer: ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.62 (d,3H,J= 6.4Hz), 2.78 (ddd,1H,J=1.6,10.9,16.4 Hz,HCHa), 3.38 (ddd,1H, J=1.2, 2.6, 16.3 Hz,HCHe), 3.79 (s,3H,OCH_3), 3.80 (s,3H, OCH_3), 4.03 (dd,1H,J=2.6,10.8 Hz,OCH), 5.21 (bq,1H, J= 6.5 Hz, CHCH$_3$), 6.71 (s,2H,ArH). CMR (75.44 MHz, $CDCl_3$) δ: 21.6, CH_3, 25.3,CH_2;55.4,55.7, $ArOCH_3$; 72.8, 83.5, $ArOCH_3$; 100.6, CCl_3;108.2,108.4, aryl CH;122.5,128.3,149.9,150.8, aryl C.

Trans isomer: ^{1}H NMR (200MHz, $CDCl_3$) δ: 1.60 (d,3H,J= 6.2 Hz,CH_3), 2.81 (dd,1H,J=10.7,17.2 Hz,HCHa), 3.26 (dd, 1H,J=3.4,17.2 Hz,HCHe), 3.82 (s,6H,2XOCH_3), 4.43 (dd, J= 3.5, 10.8 Hz,OCH), 5.36 (q,1H,J=6.3 Hz,C<u>H</u>CH_3), 6.72 (s,2H, ArH). CMR (75.44 MHz,$CDCl_3$) δ: 18.9, CH_3; 24.7, CH_2; 55.4, 55.5, ArOCH_3; 70.5, 77.6, OCH; 101.0, CCl_3; 107.7, 107.9, aryl CH; 121.1, 127.9, 149.2 151.0, aryl C. IR (FT,$CDCl_3$) νmax: 1484, aromatic C=C; 1259, C-O. MS (CI,NH_3, 119°C) m/e: 342 (100,M+NH_4), 327 (M+NH_4-1S).

Methyl (5,8-dimethoxyisochroman-3-yl) formate (260)

A solution containing 500 mg (1.3 mmol) of p-nitrobenzyl (5,8-dimethoxyisochroman-3-yl) formate (219) in 10 ml of dry THF was stirred with 15 ml of a 0.14M methanolic sodium methoxide solution for ten minutes. The mixture was then quenched with 1 ml of saturated ammonium chloride and then diluted with 50 ml of a 1:1 CH_2Cl_2/H_2O solvent system. The organic phase was separated and the aqueous layer was extracted three times with 25 ml of CH_2Cl_2. Washing of the combined organic layers was done successively with 50 ml aliquots of water and brine. After drying and evaporation of solvent, the residue was flash chromatographed with 10% ethyl acetate in toluene to give 282 mg (83.5% yield) of isochromanyl formate (260). (MP: 89-90°C.)

^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.80 (broad dd,1H, J=16.9, 10.8 Hz, HC<u>Ha</u>CHC=O), 3.12 (ddd, 1H, J=17.0, 3.9, 1.4 Hz, HC<u>He</u>CHC=O), 3.78 (s,3H,OCH_3), 3.81 (s,3H,OCH_3), 3.84 (s, 3H,$COOCH_3$), 4.30 (dd,1H,J=10.8, 3.9 Hz,OCHC=O), 4.70 (dt,1H,J=16.2,1.5 Hz,HC<u>Ha</u>O), 5.08 (d,1H,J=16.2Hz,HC<u>He</u>O) 6.67 (dd,2H,ArH). CMR (75.44 MHz, $CDCl_3$) δ: 25.7, CH_2; 52.3, OCH_3; 55.4, 55.6, $ArOCH_3$; 64.7, OCH_2, 72.8, OCH; 107.2, 107.7, aryl CH; 122.1, 123.7, 149.3, 150.8, aryl C; 171.1, ester CO. HRMS calculated for $C_{13}H_{16}O_5$ [M+·] 251.0920 found 251.0963.

Methyl 5,8-dioxo-5,8-dihydroisochroman-3-yl-formate (294)

To a solution containing 265 mg (1.0 mmol) of isochoman in 5 ml of acetonitrile was added dropwise a solution of 1.726g of cerric ammonium nitrate[320,323] in 5 ml of water. After stirring for ten minutes, the mixture was diluted with 50 ml of methylene chloride. The organic phase was separated and the aqueous layer was extracted twice with 25 ml of CH_2Cl_2. The combined organic extract were washed once with water, once with brine and then dried over $MgSO_4$. Evaporation of solvents gave 228 mg (98%) of residue which was found to be 95% pure pyranoquinone. (MP: 55-58°C)

^{1}H NMR (200 MHz,$CDCl_3$) δ: 2.68 (dddd,1H,J=19.0, 9.0 3.7,2.8Hz,HCHaCHC=O), 2.93 (d septet, 1H,J=19.0OHz,HCHeCHC=O), 3.84 (s,3H,OCH_3), 4.31 (dd,1H,J=9.0,4.4Hz,CH), 4.52 (dt,1H,J=18.7, 3.3 Hz, HCHa0), 4.86 (ddd,1H,J=18.9, 2.8, 1.6 Hz,HCHe0), 6.78 (dd,2H,HC=CH). CMR (75.44 MHz,$CDCl_3$) δ: 24.0, CH_2, 52.4, OCH_3; 62.2, OCH_2; 71.3, OCH; 136.0, 136.3, CH; 137.8, 139.4, quaternary C; 170.2, ester CO; 185.0, 185.1, quinone CO.

5,8-dimethoxyisochroman-3-yl carboxylic acid (261).

A solution containing 200 mg (0.5 mmol) of the p-nitrobenzylisochroman formate (219) in 10 ml of THF was added 10 ml of a 1M aqueous sodium hydroxide solution. After stirring at room temperature for 0.5 hour, the mixture was evaporated down to 5 ml and then diluted with 25 ml of water. The aqueous layer was extracted three times with 20 ml aliquots of CH_2Cl_2, then acidified with concentrated aqueous HCl and reextracted four times with 50 ml of ethyl acetate. Only the combined ethyl acetate layers were kept, and after washing twice with water, evaporation of solvent gave 125 mg (99% yield) of the isochromanyl acid (261). (MP:217-218°C).

^{1}H NMR (200 MHz, DMSO-d_6) δ: 2.88 (broad ddt,1H,J= 9.9, 17 Hz,HCHaCH), 3.15 (ddd,1H,J=1.4,4.6, 17 Hz,HCHeCH), 3.91 (s,3H,OCH_3), 3.93 (s,3H,OCH_3), 4.46 (dd,1H,J=4.5, 9.9, OCHC=O), 4.75 (broad dt,1H,J=16.4 Hz,ArH, CHaO), 5.05 (broad dd,1H,J=16.5 Hz,ArHCHeO), 6.92 (dd,2H,J=11.2 Hz,ArH).

CMR (75.44 MHz, $CDCl_3$) δ: 26.7, CH_2; 49.9, OCH_2; 55.9, 56.1, $ArOCH_3$; 65.3, OCH; 108.7, 109.1, aryl CH; 123.1, 124.5, 150.6, 152.3, aryl CH; 210.2, CO_2H.

Methyl (5,8-dimethoxyisochroman-3-yl) ketone (259)

Procedure 1

To a solution containing 420 mg (2.0 mmol) of isochroman (260) in 25 ml of diethyl ether was added dropwise at -78°C under argon, 1.40 ml of a 1.4M methyl lithium in ether solution. After stirring for 2 hours, 2.0 ml of methanol was added. The mixture was combined with 50 ml of water extracted three times with 50 ml of ether and the combined organic phase was washed once with 50 ml of water, 50 ml of brine and dried over $MgSO_4$. After removal of solvent flash chromatography of the residue (10% ethyl acetate in toluene) gave 150 mg (37% yield of isochroman (259). (MP 84-85 °C).

^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.32 (s,3H,$COCH_3$), 2.59 (bdd,1H,J=11.3,17.0Hz, HC<u>Ha</u>), 3.04 (ddd,1H,J=1.5, 3.8, 17.1, HC<u>He</u>), 3.78 (s,3H,OCH_3), 3.79 (s,3H,OCH_3), 4.08 (dd,1H, J= 3.8, 11.4 Hz,O-CH), 4.66 (bd,1H,J=15.9 Hz,HC<u>Ha</u>-O), 5.04 (bd,1H,J=15.9 Hz,HC<u>He</u>-O), 6.66 (dd,2H,J=9.0 Hz,ArH). CMR (75.44 MHz,$CDCl_3$) δ: 24.7, CH_2; 25.9, CH_3; 55.4, 55.6, ArOCH_3; 64.7, OCH_2; 79.1, OCH; 107.2, 107.7, aryl CH; 122.2, 123.9, 149.2, 151.0, aryl C; 208.4, C=O. IR ($CDCl_3$) νmax: 1717, C=O. The tertiary alcohol was also isolated in 44% yield (.185g) (MP: 155-160°C).

^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.20 (s,3H,CH_3), 1.24 (s,3H,CH_3), 2.48 (bdd,1H,J=11.3,16.9 Hz,HC<u>Ha</u>), 2.67 (ddd, 1H,J=1.5,3.3,16.9 Hz,HC<u>He</u>), 3.33 (dd,1H,J=3.4,11.3Hz,O- CH), 3.70 (s,3H,OCH_3), 3.73 (s,3H, OCH_3), 4.54 (bd,1H,J= 15.7 Hz, HC<u>Ha</u>-O), 4.94 (bd,1H,J=15.6 Hz,HC<u>He</u>-O), 6.58 (dd,2H, J= 9.2 Hz,ArH), CMR (75.44 MHz,$CDCl_3$) δ: 23.2, CH_2; 23.6, 26.0, CH_3; 55.5, 55.6, ArOCH_3; 65.2, OCH_2; 72.0, C-OH; 80.6, OCH; 106.8, 107.5, aryl CH; 123.7, 124.6, 149.4, 151.2, aryl C. 1R ($CDCl_3$) νmax: 3155, broad, OH; 1215, C-O. HRMS calculated for $C_{13}H_{16}O_4$: [M+·] 236.1049 found 236.1027.

Procedure 2.

To a solution containing 150 mg (6.3 mmol) of isochromanyl acid (261) in 15 ml of anhydrous THF was added dropwise, over 5 minutes at -78°C and under argon, 0.94 ml of a 1.4M methyl lithium in ether solution. The solution was stirred for 10 minutes at -78°C then warmed to room temperature and stirred for two more hours. Methanol (1ml) was then added, followed with 25 ml of water, and the mixture was extracted three times with 50 ml of dichloromethane. The combined organic layer was washed with 25 ml water, 25 ml of saturated aqueous NaCl, and dried over $MgSO_4$. After evaporation of solvent, flash chromatography of the residue (10% ethyl acetate in toluene) gave 110 mg (74%) of the isochroman ketone (259).

Procedure 3

A 40% aqueous solution of pyruvic aldehyde (100 ml in 200 ml of benzene) was refluxed untill no more water could be collected in a Dean-Stark water separator. Then a solution containing 12g (5.3 mmol) of sultine (167) in 200 ml of benzene was added dropwise at reflux under argon, over three hours. The mixture was refluxed two more hours, cooled, and the residue obtained after evaporation of solvent was flash chromatographed (5% ethyl acetate in hexanes). The titled compound was obtained in 25% yield. Dimethoxybenzosulfone (168) was isolated in 50% yield.

Cis-p-nitrobenzyl(5,12-dioxo-1-methyl-3,4,5,12-tetrahydroanthraceno[2,3-c]pyran-3-yl)formate (253)

This compound was obtained by following the same procedure described for tetracycle (239). Thus, reaction of 250mg (1.5mmol) of sultine (236) with 268mg (0.7mmol) of quinone (251) gave 157mg (49 % yield) of tetracycle (253). MP: 118-120°C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.69 (d, 3H, J=6.5 Hz, CH_3), 2.74 (ddd, 1H, J=3.7, 10.8, 18.5 Hz, HCHa), 3.24 (dt, 1H, J=2.8, 18.5 Hz, HCHe), 4.26 (dd, 1H, J=2.9, 10.9 Hz, OCH), 5.04 (m, 1H, $CHCH_3$), 5.38 (bs, 2H, OCH_2), 7.59 (d, 2H, J=8.9 Hz, ArH), 7.7 (m, 2H, ArH), 8.05 (m, 2H, ArH), 8.26 (d, 2H, J=8.9 Hz, ArH), 8.60 (s, 1H, ArH), 8.61 (s, 1H, ArH). CMR (75.44 MHz, $CDCl_3$) δ: 20.6, CH_3; 26.2, CH_2; 65.5, OCH_2; 71.0, 71.4, OCH; 123.9, 128.6, 129.9, 129.7, 130.2, aryl CH; 127.8, 127.9, 128.2, 134.7, 141.1, 142.2, 146.7, 147.2, aryl C; 169.7, ester C=O; 182.2, 182.5, quinone C=O.

Trans-p-nitrobenzyl(5,12-dioxo-1-methyl-3,4,5,12-tetrahydroanthraceno[2,3-c]pyran-3-yl)formate (255)

Following the procedure to prepare (239), the reaction between 175mg (1.1mmol) of sultine (236) with 187mg (0.5mmol) of quinone (252) gave 119mg (52% yield) of the titled compound (255). MP: 131-132°C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.62 (d, 3H, J=6.8 Hz, CH_3), 2.89 (ddd, 1H, J=2.0,

8.9, 19.0 Hz, HC<u>Ha</u>), 3.11 (ddd, 1H, J=1.0, 4.6, 19.1 Hz, HC<u>He</u>), 4.69 (dd, 1H, J=4.7, 8.8 Hz, OCH), 5.30 (bq, 1H, J=6.8 Hz, C<u>H</u>CH_3), 5.35 (bs, 2H, OCH_2), 7.55 (d, 2H, J=8.7 Hz, ArH), 7.7 (m, 2H, ArH), 8.05 (m, 2H, ArH), 8.20 (d, 2H, J=8.7 Hz, ArH), 8.58 (bs, 2H, ArH). CMR (75.44 MHz, $CDCl_3$) δ: 19.6, CH_3; 25.3, CH_2; 65.5, OCH_2; 67.0, 67.7, OCH; 123.9, 128.7, 128.8, 129.6, 130.2, aryl CH; 127.9, 128.0, 128.2, 134.8, 141.1, 142.3, 146.7, 147.6, aryl C; 170.3, ester C=O; 182.8, 182.9, quinone C=O.

Cis-p-nitrobenzyl(5,12-dioxo-7,10-dimethoxy-1-methyl-3,4,5,12-tetrahydroanthraceno[2,3-c]pyran-3-yl)formate (255)

Following the procedure to prepare (239), the reaction between 95mg (0.4mmol) of sultine (167) with 71mg (0.2mmol) of quinone (251) gave 42mg (41% yield) of tetracycle (254). MP: 154-156°C. ^1H NMR (200 MHz, $CDCl_3$) δ: 1.68 (d, 3H, J=7.6 Hz, CH_3), 2.75 (ddd, 1H, J=3.6, 10.7, 18.6 Hz, HC<u>Ha</u>), 3.27 (bdt, 1H, J=2.8, 18.6 Hz, HC<u>He</u>), 4.00 (s, 3H, OCH_3), 4.01 (s, 3H, OCH_3), 4.28 (dd, 1H, J=2.8, 10.7 Hz, OCH), 5.06 (m, 1H, OC<u>H</u>CH_3), 5.38 (bs, 2H, OCH_2), 6.90 (s, 2H, ArH), 7.59 (d, 2H, J=8.9 Hz, ArH), 8.26 (d, 2H, J=8.9 Hz, ArH), 8.95 (s, 1H, ArH), 8.98 (s, 1H, ArH). IR (FT, $CDCl_3$) ν_{MAX}: 1757, ester C=O; 1664, quinone C=O. MS (DCI, 240, NH_3, m/e: 517 (100, M^+), 382 (68, $M^+\cdot - C_7H_5NO_2$).

Trans-p-nitrobenzyl(5,12-dioxo-7,10-dimethoxy-1-methyl-3,4,5,12-tetrahydroanthraceno[2,3-c]pyran-3-yl)formate (256)

Following the same procedure as described for (239), the reaction between 70mg (0.3mmol) of sultine (167) and 53mg (0.15mmol) of quinone (252) gave 30mg (44% yield) of tetracycle (256). MP: 180-182°C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.57 (d, 3H, J=6.8 Hz, CH_3), 2.84 (ddd, 1H, J=2.1, 9.1, 19.0 Hz, HCHa), 3.06 (ddd, 1H, J=1.2, 4.6, 18.9 Hz, HCHe), 3.95 (s, 3H, OCH_3), 3.96 (s, 3H, OCH_3), 4.63 (dd, 1H, J=4.69, 8.9 Hz, OCH), 5.29 (m, 1H, $OCHCH_3$), 5.30 (bs, 2H, OCH_2), 6.86 (s, 2H, ArH), 7.50 (d, 2H, J=8.8 Hz, ArH), 8.16 (d, 2H, J=8.8 Hz, ArH), 8.91 (s, 1H, ArH), 8.92 (s, 1H, ArH). CMR (75.44 MHz, $CDCl_3$) δ: 19.7, CH_3; 25.4, CH_2; 55.9, 2XOCH_3; 65.5, OCH_2; 67.1, 67.8, OCH; 107.6, 123.4, 123.9, 128.7, aryl CH; 127.4, 127.5, 128.6, 140.9, 142.3, 146.8, 150.9, aryl C; 170.4, ester C=O; 182.8, 182.9, quinone C=O. IR (FT, $CDCl_3$) ν_{MAX}: 1757, ester C=O; 1664, quinone C=O.

Methyl (5,8-dioxo-5,8-dihydroisochroman-3-yl) ketone (262)

To a stirred solution containing 700 mg (3 mmol) of isochroman (259) in 20 ml of acetonitrile was added dropwise over 5 minutes, at room temperature, a solution containing 2.0g (3.6 mmol) of ceric ammonium nitrate in 20 ml of water. Stirring was continued for five minutes and then 100 ml of dichloromethane was added to the mixture. Successive washings of the organic layer were done with 50 ml of water and 50 ml of brine. After drying over $MgSO_4$, evaporation of solvent gave 560 mg (92% yield) of the yellow isochromandione (262), as a dark yellow oil.

^1H NMR (300 MHz, $CDCl_3$) δ: 2.34 (s,3H,$COCH_3$), 2.48 (dddd, 1H,J=2.8, 4.0, 10.1, 19.2 Hz, HCHa), 2.87 (dm,1H,J= 19.2Hz,HCHe), 4.06 (dd,1H,J=4.0, 10.1Hz, OCH), 4.52 (dt,1H, J=3.3, 18.6 Hz, HCHa-O), 4.83 (ddd,1H,J=1.0, 2.7, 18.6 Hz. HCHe-O), 6.80 (dd,2H,J=10.2 Hz, HC=CH).

CMR (75.44 MHz, $CDCl_3$) δ: 23.1, CH_2; 25.8, CH_3; 62.7, OCH_2; 77.7, OCH; 136.0, 136.4, CH; 183.3, 139.6, quaternary C; 185.17, 185.24, quinone C=O, 206.4, $COCH_3$.

IR ($CDCl_3$) νmax: 1722, $COCH_3$; 1659, quinone C=0.

(1S, 3S) and (1R, 3R) p-nitrobenzyl [1-hydroxy-5,12-dioxo-3,4,5,12-tetrahydroanthraceno [2,3-c] pyran-3-yl] formate (263)

From a modification of Honek procedure[251], a mixture containing 164 mg (0.37 mmol) of the pyranoanthracyclinone from step 2, 65 mg (0.37 mmol) of N-bromosuccinimide and 10 mg of AIBN in 25 ml of CCl_4 was refluxed for 2 hours. The solvent was then removed and the residue was stirred with 35 ml of a 7:3 solution of THF in water for ten hours. The mixture was then extracted three times with 25 ml aliquots of CH_2Cl_2. The combined organic layer was washed once with 10 ml of water, 10 ml of brine, and then dried over $NaSO_4$. After evaporation of solvents, flash chromatography of the residue gave 118 mg (69%) of the desired pyranoanthraquinone aglycone. (MP:275°C decomposes). ^1H NMR (200 MHz, DMSO $-d_6$) δ:2.68 (dd,1H,J=19.1, 11.5 Hz, HC<u>Ha</u>CHC=O), 3.00 (dd,1H,J=19.4, 4.4 Hz, HC<u>He</u>CHC=O), 4.89 (dd,1H,J=11.4, 4.1 Hz, OCHC=O), 5.39 (broad s, 2H,$ArCH_2$), 5.98 (d,1H,J=6.3 Hz, C<u>H</u>OH), 7.40 (d,1H,J=6.1, exchangeable OH), 7.7 (d,2H,ArH), 7.8 (m,2H,ArH), 8.27 (d,2H, ArH), 8.29 (m,2H,ArH), 8.66 (s,1H,ArH), 8.67 (s,1H,ArH). CMR (75.44 MHz, DMSO-d_6) δ:29.3, CH_2; 64.4, OCH_2, 64.7, OCH_2; 123.1, 127.7, 128.4, 129.3, 129.8: aryl CH; 127.58, 127.60, 134.0, 134.1, 142.1, 143.0, 144.5, 147.1:aryl C; 169.4, ester CO; 180.9, 182.8, quinone CO. IR ($CDCl_3$) νmax: 3505, broad, OH; 1741, ester CO; 1654, 1666, quinone CO.

(1'S,1R,3S) and (1'S,1S,3R)-p-nitrobenzyl [1-(2',3',6'-trideoxyacetamido-4'-O-p-nitrobenzoyl-L-lyxohexopyranose)-5,12-dioxo-3,4,5,12-tetrahydroanthraceno (2,3-c) pyran-3-yl] formate (269) and (270).

These glycosides were obtained in 75% overall yield by following the same procedure as described for glycosides (200) and (201) and by using the aglycone (263). (MP:192-195°C for the less polar 1'S,1S,3R and 173-174°C for the more polar 1'S,1R,3S). ^{1}H NMR (200 MHz, $CDCl_3$) of the (1'S,1S,3R) diastereomer (269) δ: 1.12 (d,3H,J= 6.3 Hz, H_3C-6'), 2.09 (m,2H,H_2C-2'), 2.83 (dd,1H, J=19.4, 11.7 Hz, HCHaCHC=0), 3.26 (dd,1H,J=19.1,3.7 Hz, HCHeCHC=0), 4.4 (broad q, 1H,J=6.1 Hz,HC-5'), 4.65 (m,1H,HC-3'), 4.89 (dd, 1H,J=11.8, 3.6 Hz,OCHC=0), 5.42 (broad s,3H,HC-4' and aral CH_2), 5.82 (broad s,1H,W_h<6 Hz,HC-1'), 6.13 (s,1H, W_h<0.7 Hz, OCH-0), 6.40 (d,1H,J=7.3 Hz, NH), 7.28 (d,2H, p-nitrobenzyl-H), 7.73 (m,2H,ArH), 8.09 (m,2H,ArH), 8.29 (d, 2H,p-nitrobenzyl-H), 8.31 (dd,4H,benzoyl H), 8.64 (s,1H, ArH), 8.69 (s,1H,ArH). CMR (75.44 MHz, $CDCl_3$) δ: 16.5, CH_3; 24.2, CH_2; 29.5, CH_2; 45.3, CH; 65.9, 66.5, OCH; 71.6, OCH; 76.8, OCH_2; 92.3, 98.3, O-CH-0; 115.4, quartet, J=287.5Hz, CF_3; 123.9, 124.2, 128.7, 129.0, 129.1, 129.3, 129.6, 131.2, aryl CH; 127.1, 128.0, 130.4, 134.5, 135.0, 135.1, 140.6, 142.2, 143.1, 151.0, aryl C; 157.2, quartet, J=37.6Hz, $\underline{C}OCF_3$; 164.8, 169.4, ester C=0; 182.4, 183.5, quinone C=0. IR (FT, $CDCl_3$), νmax: 1737 (broad, carbonyls), 1667 (quinone C=O).

^{1}H NMR (200 MHz, $CDCl_3$) of the (1'S,1R,3S), diastereomer (270) δ: 1.42 (d,3H,J=6.4 Hz,H_3C-6'), 2.09 (m,2H, H_2C-2'), 2.85 (dd,1H,J=19.4,11.3 Hz, HCHaCHC=O), 3.25 (dd, 1H,J=19.4,4.3 Hz,HCHeCHC=O), 4.63 (m,1H,HC-3'), 4.84 (overlaped, m, 2H,HC-5' and OCH=O), 5.41 (broad s,2H, aral CH_2), 5.47 (s,1H,W_h=5 Hz,HC-4'), 5.69 (s,1H, W_h<0.7Hz, O-CH-O), 6.57 (d,1H,J=7.2 Hz,NH), 7.73 (d,2H, p-nitrobenzyl-H), 7.75 (m,2H,ArH), 8.11 (m,2H,ArH), 8.33(d, 2H, p-nitrobenzyl-H), 8.37 (dd,4H,p-nitrobenzoyl-H), 8.69 (s,2H, ArH). IR ($CDCl_3$) νmax: 1738 (broad, carbonyls), 1669 (quinone C=O). CMR (75.44 MHz, $CDCl_3$) δ: 17.0, CH_3, 25.0, CH_2; 29.7, CH_2; 45.7, CH; 65.7, 66.7, OCH; 72.5, OCH; 76.8, OCH_2; 87.7, 92.4, O-CH-O; 123.9, 124.0, 128.7, 129.3, 129.4, 129.9, 130.0, 131.1, aryl CH; 128.0, 128.9, 131.0, 134.3, 134.9, 135.0, 139.9, 142.1, 143.9, 151.0, aryl C; 115.4, q, J=287.1Hz, CF_3; 157.5, J=37.8Hz, $\underline{C}OCF_3$; 165.0, 169.4, ester CO; 181.7, 183.2, quinone CO.

1,4-Dimethoxy-2-bromomethyl-3-methylquinizarin (282)

A mixture containing 3.80g (12.8 mmol) of quinizarin derivative (281), 2.05g (11.5 mmol) of NBS and 15 mg of AIBN in 300 ml of deoxygenated CCl_4 was refluxed under argon for one hour and then cooled to 40°C. An additional 0.23g of NBS with 10 mg of AIBN was added and reflux was continued for one more hour. The mixture was then cooled, filtered

and evaporated to dryness. Flash chromatography of the residue with dichloromethane gave 3.0g (62% yield) of monobrominated quinizarin (282). MP:(118-119°C). ^1H NMR (200 MHz, $CDCl_3$) δ: 2.46 (s,3H,CH_3), 3.88 (s,3H,OCH_3), 4.06 (s,3H,OCH_3), 4.67 (s,2H, CH_2 Br), 7.64 (m,2H,ArH), 8.20 (m,2H,ArH). CMR (75.44 MHz, $CDCL_3$) δ: 12.3, CH_3; 24.0, CH_2Br; 61.6, 62.8, OCH_3; 126.4, 133.5, aryl CH; 124.7, 127.0, 133.8, 133.9, 139.4, 142.0, 155.1, 155.3, aryl C: 182.2, 182.7, quinone C=0. IR (FT, $CDCl_3$) νmax: 1672, quinone C=0. MS (EI, 70eV, 90°C). m/e: 374 (5,M+·), 295 (85,M+·-Br).

1,4-dimethoxy-2-formyl-3-methylquinizarin (283)

Following the same procedure as described for (101), treatment of 2.63g (7.0 mmol) of the bromomethylquinizarin (282) with 8.59g (1.2 mmol) of bis-tetrabutylammonium dichromate gave 1.09g (50% yield) of quinizarin aldehyde (283) after flash chromatography with 10% ethyl acetate in toluene. (MP: 201-202°C).

^1H NMR (200 MHz, $CDCl_3$) δ: 2.60 (s,3H,CH_3), 3.95 (s,3H,OCH_3), 4.15 (s,3H,OCH_3), 7.81 (m,2H,ArH), 8.25 (m,2H,ArH), 10.68 (s,1H,CHO). CMR (75.44 MHz, $CDCl_3$) δ: 13.0, CH_3; 61.7, 64.3, OCH_3; 126.5, 126.6, 133.7, 133.8,

aryl CH; 125.2, 129.9, 135.3, 143.7, 155.4, 155.6, aryl C; 181.7, 182.6, quinone C=0; 192.3, CHO. IR (FT, $CDCl_3$) ν_{max}: 1698, aldehyde C=0; 1676, quinone, C=0. UV (ethanol) max: 357, 337, 262. MS (EI, 70eV, 95°C) m/e: 310 (100,$M^{+\cdot}$), 295 (49, $M^{+\cdot}-CH_3$), 281 (21, $M^{+\cdot}$-HCO), 264 (30, $M^{+}-CH_3$-HCO).

Ethyl (5,8-dimethoxyisochroman-3-yl) formate (304)

In a 250 ml triple necked round bottom flask, equipped with a Dean-Stark, was refluxed a solution containing 30.6g (0.3 mmol) of ethylglyoxalate[356] in 100 ml of benzene until no more water could be separated. A solution containing 4.39g (19.2 mmol) of the sultine (167) in 75 ml of benzene was then added dropwise over 3 hours. During the addition argon was bubbled in the reaction mixture. Reflux was continued overnight and after cooling, the excess glyoxalate was extracted from the mixture with four portions of 200 ml water. The benzene layer was dried and evaporated to give a residue from which was separated 3.13g (61%) of dimethoxyisochroman (304) after flash chromatogrpahy from ethyl acetate/toluene. (MP: 60-61°C). ^1H NMR (200 MHz, $CDCl_3$) δ: 1.34 (s,3H,J=7.1 Hz, CH_3), 2.78 (broad dd,1H,J=17.0, 10.8, 1.3 Hz, HC$\underline{H}_a$CHC=O), 3.10 (ddd,1H,J=16.9, 3.9, 1.44 Hz, HC$\underline{H}_e$CHC=O), 3.77 (s,3H, OCH_3), 3.80 (s,3H,

OCH_3), 4.26 (dd,1H,J=10.8, 3.9Hz, OCHC=O), 4.30 (q,2H,J= 7.1 Hz,-OCH_2), 4.68 (broad dt,1H, J=16.0, 1.4, 1.3, ArHCHaO), 5.07 (broad d,J=16.0Hz, ArHCHeO), 6.66 (dd,J=8.9H, ArH). CMR (75.44 MHz, $CDCl_3$) δ: 14.1, CH_3; 25.6, CH_2; 55.3, 55.5, $ArOCH_3$; 61.1, 64.5, OCH_2; 72.7, OCH; 107.1, 107.6, aryl CH; 122.0, 123.6, 149.1, 150.7, aryl C; 171.2, ester CO. HRMS calculated for $C_{14}H_{18}O_5$: [M+·] = 266.1154 found 266.1163.

Ethyl (5,8-dioxo-5,8-dihydroisochroman-3-yl) formate (305)

Oxidative demethylation of 1.45g of isochroman (305) with ceric ammonium nitrate, as described for (221), gave a 94% yield of quinone (305) as a yellow product. (MP: 75-76°C). ^1H NMR (200 MHz, $CDCl_3$) δ: 1.30 (t, 3H, J=7.1 Hz, CH_3), 2.64 (ddt,1H,J=3.5, 9.4, 19.1 Hz, HCHa), 2.88 (dm, 1H,J=19.1 Hz, HCHe), 4.27 (overlapped of and dd,3H,OCH_2 and OCH), 4.48 (dt,1H,J=3.4, 19.0 Hz,OHCHa), 4.82 (dm,1H,J= 19.0 Hz,OHCHe), 6.75 (dd,2H,J=10.0 Hz,HC=CH). IR (Et, $CDCl_3$) νmax: 1748, ester C=O; 1659, quinone C=O. IR (FT, $CDCL_3$) νmax: 1756, ester C=O; 1258, C-O. MS (CI,NH_3, 138°C) m/e: 405 (100, M+NH_4).

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Ethyl [6-hydroxy-5,12-dioxo-3,4,5,12-tetrahydroanthraceno (2,3-c) pyran-3-yl] formate (309)

Following a slight modification of Tamura's method[355], a solution of 2.5M n-butyl lithium (.20 mmol) was added under argon at 0°C to a stirred solution of 0.07 ml of dry diisopropylamine in 2 ml of THF and stirred for 0.5 hour at -78°C. To the LDA was added dropwise over several minutes a solution of 73 mg (0.45 mmol) of homophthtalic anhydride in 2ml of THF and then 100 mg (0.45 mmol) of the pyranoquinone (305) dissolved in 3 ml of THF. The resulting mixture was stirred 20 minutes at -78°C, warmed to room temperature, and stirred for one hour. After quenching with 5 ml of saturated aqueous ammonium chloride the mixture was partitioned between 5 ml of 5% HCl and 50 ml CH_2Cl_2. The organic layer was separated, washed with 10 ml of brine and dried over Na_2SO_4. Flash chromatography of the residue (10% EtOAc in toluene) obtained after evaporation of solvents, gave pyranotetracycle (309) in 15% yield. (MP: 150-152°C.). ^1H NMR (200 MHz, $CDCl_3$) δ: 1.36 (t, 3H, J=7.1 Hz, CH_3), 2.84 (ddt,1H,J=19.1, 9.1, 3.2 Hz, HCHaCHC=O), 3.13 (d m,1H,J=19.1 Hz,HCHeCHC=O), 4.32 (q,2H, J=7.1 Hz,OCH_2), 4.34 (dd,J=9.1,4.3 Hz,OCHC=O), 4.67 (dt, 1H,J=19.0,3.3 Hz, HCHa-O), 5.06 (broad d,1H,J=19.0Hz,HCHe-O), 7.71 (multiplet, 2H, ArH), 7.94 (multiplet, 1H, ArH), 8.11 (s,1H,ArH), 8.47 (multiplet, 1H, ArH), 13.7 (s,1H, exchangeable OH).

CMR (75.44 MHz, $CDCl_3$) δ: 14.0, CH_3; 24.4, CH_2; 61.6, 63.2, OCH_2; 71.7, OCH; 121.9, 125.0, 129.4, 130.7, 131.5, aryl CH; 127.2, 127.6, 131.0, 136.0, 141.7, 144.2, 162.7, aryl C; 170.4, ester C=O; 183.5, 187.5, quinone C=O. IR (FT, $CDCl_3$) νmax: 3405, bs, OH; 1748, ester C=O; 1660, 1644, quinone C=O, 1609, C=C. HRMS calculated for $C_{20}H_{16}O_6$: [M+·]= 352.0947 found 352.0997.

The less polar ethyl [11-hydroxy-5,12-dioxo-3,4,5,12-tetrahydroanthraceno (2,3-C) pyran-3-yl] formate (308) was obtained in 32% yield. (MP: 149-150°C). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.35 (t,3H, J=7.1 Hz, CH_3), 2.78 (ddt,1H, J=3.4,9.1,19.0 Hz,HCHa), 3.07 (d m,1H, J=19.0 Hz, HCHe), 4.31 (overlaped of with dd,3H,OCH_2 and OCH), 4.65 (dt, 1H, J=3.3,18.8 Hz,HCHa) 5.04 (bd,1H,J= 18.8 Hz, HCHe), 7.71 (m, 2H,ArH), 7.93 (dd,1H,J=1.3,7.3 Hz,ArH), 8.07 (s,1H, ArH), 8.43 (dd,1H,J=1.2,7.2 Hz,ArH). CMR (75.44 MHz, $CDCl_3$) δ: 13.9, CH_3; 24.8, CH_2; 61.6, 62.6, OCH_2; 71.6, OCH; 122.1, 124.8, 129.3, 130.6, 131.4, aryl CH; 106.1, 127.0, 127.6, 135.7, 142.6, 143.0, 162.6, CH; 170.3, ester C=O; 182.0, 186.0, quinone C=O. IR (FT, $CDCl_3$) νmax: 3590, broad, OH; 1748, ester C=0; 1662, 1645, quinone C=O, 1607, C=C. HRMS calculated for $C_{20}H_{16}O_6$: [M+·]=352.0947 found 352.0946.

Ethyl [6-acetoxy-5,12-dioxo-3,4,5,12-tetrahydroanthraceno (2,3-c) pyran-3-yl] formate (311).

A mixture containing 60 mg (0.18 mmol) of pyranotetracycle (309) 0.25 ml acetic anhydride, 0.3 ml pyridine and 6 mg of dimethylaminopyridine in 20 ml of CH_2Cl_2 was stirred overnight at room temperature under argon atmosphere. The mixture was then diluted with 25 ml CH_2Cl_2 and washed consecutively twice with 15 ml of water, twice with 10 ml of 1N HCl, once with 15 ml of water and dried over $NaSO_4$. Flash chromatography of the residue obtained after flash chromatography yielded 55 mg (81%) of the titled acetylated pyranotetracycle. (MP: 196-198°C). ^1H NMR (300 MHz, $CDCl_3$), δ: 1.34 (t, 3H, J=7.2 Hz, CH_3), 2.63 (s,3H, $COCH_3$), 2.78 (ddt, 1H, J=3.3, 9.1, 18.7 Hz, HCHa), 3.10 (dm,1H,J=19.0 Hz, HCHe), 4.29 (q,2H, J=7.2 Hz, OCH_2), 4.31 (dd,1H,J=4.2, 9.3 Hz,OCH), 4.61 (dt, 1H,J=3.2, 19.0 Hz, HCHa-O), 5.02 (bd,1H,J=19.0 Hz,HCHe-O), 7.73 (m,2H, ArH), 8.05 (m,1H,ArH), 8.13 (m,1H,ArH), 8.58 (s,1H,ArH). ^1H NMR (200 MHz, $CDCl_3$) δ: 1.34 (t,3H,J=7.1 Hz, CH_3), 2.60 (s, 3H, $COCH_3$), 2.81 (ddt,1H, J=18.7, 9.1, 3.3 Hz, HCHaCHC=O), 3.10 (dm,1H,J=19.0Hz, HCHeCHC=O) 4.61 (q,2H, J= 7.1 Hz,OCH_2), 4.31 (dd,1H, J=9.3, 4.2 Hz,CH), 4.61 (dt,1H, J=19.0,3.2 Hz,HCHa-O), 5.02 (broad d,1H,J=19.0 Hz,HCHe-O), 7.73 (m,2H,ArH), 8.05 (m,1H,ArH), 8.13 (m,1H,ArH), 8.58 (s, 1H,ArH). IR (FT,$CDCl_3$) ν_{MAX}: 1773, 1751, ester C=0;1667, 1644, quinone C=0; 1618, C=C. HRMS calculated for $C_{22}H_{18}O_7$: [M+] 394.1053 found 394.1020.

(1S, 3S) and (1R, 3R)-Ethyl-[6-acetoxy-1-hydroxy-5,12-dioxo-3,4,5,12-tetrahydroanthraceno (2,3-c) pyran-3-yl] formate (310)

A mixture containing 47 mg (0.12 mmol) of the acetylated pyranotetracycle (311) 0.23 mg of N-bromosuccinimide, and 0.1 mg of AIBN in 5 ml of CCl_4 was refluxed for two hours. The solvent was then removed in vacuo and to the residue was added 10 ml of a 3:1 THF-H_2O solvent mixture. After stirring for one hour at room temperature, the mixture was extracted with three 10 ml portions of CH_2Cl_2. The combined organic extracts were washed once with 10 ml of water and dried over Na_2SO_4. Flash chromatography of the residue obtained after removal of solvents gave 35 mg (71%) of pyranotetracyclic aglycone (310) (MP: 215-220°C decomposes). ^1H NMR (200 MHz, DMSO-d_6) δ: 1.35 (t,3H,J=7.1 Hz,CH_3), 2.60 (dd,1H,J=19.6,11.8 Hz, HCHaCHC=O), 2.91 (dd,1H, J=19.6,4.3 Hz,HCHeCHC=O), 4.32 (q,2H,J=7.1 Hz, OCH_2), 4.76 (dd,1H,J=11.5,4.5 Hz,OCHC=O), 5.91 (d,1H, J=6.2 Hz,CHOH), 7.34 (d,1H,J=6.3 Hz, exchangeable OH), 7.84 (m,2H,ArH), 8.3 (m, 2H,ArH), 8.65 (s,1H,ArH). CMR (75.44 MHz, DMSO-d_6) δ: 13.9, CH_3; 20.9, CH_3; 24.7, CH_2; 60.9, OCH_2; 64.6, OCH; 85.9, O-CH-O; 124.1, 126.7, 130.8, 130.9, 131.0, aryl CH; 118.1, 126.6, 129.7, 135.3, 141.1, 143.9, 147.6, aryl C; 169.3, 170.5, ester C=O; 181.3, 182.6, quinone C=O. IR (FT, $CDCl_3$) νmax: 3365, bs, OH; 1774, 1748, ester C=O; 1668, quinone C=O. HRMS calculated for $C_{22}H_{18}O_8$: [M+·] 410.1002 found 410.1009.

(1'S, 1R, 3S) and (1'S, 1S, 3R)-Ethyl [6-acetoxy-1-(2',3', 6'-trideoxy-3'-trifluoroacetamido-4'-O-p-nitrobenzoyl-L-lyxohexopyranose)-5,12-dioxo-3,4,5,12-tetrahydroanthraceno (2,3-c) pyran-3-yl] formate (312) and (313)

Glycosidation of the pyranoanthraquinone (310) could be carried out by following the procedure as described for (200) and (201). The titled pyranoanthraquinone glycoside could be obtained in an overall yield of 77% (MP: 158-160°C of 1's,1S,3R and 225-227°C of 1's,1R,3S). ^{1}H NMR (200 MHz, $CDCl_3$) of the (1'S,1S,3R) diastereomers, δ: 1.25 (d,3H,J=6.8 Hz,H_3C-6'), 1.36 (t,3H,J=7.0 Hz, CH_3), 2.06 (m,2H,H_2C-2'), 2.62 (s,3H,acetyl,CH_3), 2.77 (dd,1H,J= 19.5,11.3 Hz,HC<u>Ha</u>CHC=O), 3.19 (dd,1H,J=19.3,3.7 Hz, HC<u>He</u>CHC=O), 4.31 (q,2H,J-7.0 Hz, OCH_2), 4.42 (broad q,1H, J=6.9 Hz, HC-5'), 4.65 (m,1H,HC-3'), 4.78 (dd,1H,J=11.4,3.7 Hz, OCHC=O), 5.48 (broad s,1H,HC-4'), 5.76 (broad s,1H,HC-1'), 6.05 (s,1H,O-CH-O), 6.35 (d,1H,J=6.4 Hz,NH), 7.76 (m,2H,ArH) 8.12 (m,2H,ArH), 8.31 (dd,4H,p-nitrobenzoyl-H), 8.65 (s,1H, ArH). ^{1}H NMR (200 MHz, $CDCl_3$) of the (1'S,1R,3S) diastereomer, δ: 1.40 (d,3H,J=6.6 Hz,H_3C-6'), 1.37 (t, 3H, J= 7.0 Hz, CH_3), 2.06 (m,2H,H_2C-2'), 2.61 (s,3H, acetyl, CH_3), 2.81 (dd,1H,J=19.0,11.7, Hc<u>Ha</u>CHC=O), 3.19 (dd,1H, J=19.4, 3.9 Hz,HC<u>He</u>CHC=O), 4.31 (q,2H,J=7.0 Hz, OCH_2), 4.72 (broad m, 3H, overlaped HC-5', HC-3', OCHC=O), 5.46 (broad s,1H,HC-4'), 5.67 (broad s,1H,HC-1'),

6.26 (s,1H, O-CH-O), 6.41 (d,1H,J=8.0Hz,NH), 7.72 (m,2H, ArH), 8.18 (dm,2H,ArH), 8.35 (dd,4H, p-nitrobenzoyl-H), 8.67 (s,1H,ArH). CMR of the diastereomeric mixture of (312) and (313) (75.44 MHz, $CDCl_3$) δ: 14.3 and 14.5, $CO_2CH_2\underline{C}H_3$; 17.0 and 17.2, 6'-CH_3; 21.4, acyl CH_3; 24.9 and 25.4, 2'-CH_2; 29.9 and 30.0, 4-CH_2; 45.9 and 46.0, CHNH; 62.1 and 62.2, ester OCH_2; 66.2 and 66.8, 5'-OCH; 66.9 and 67.1, 4'-OCH; 72.2 and 72.8, 3-OCH; 88.0 and 92.7,1-O-CH-O; 92.8 and 98.7,1'-O-CH-O; 113.1, quartet, J=287.1 Hz, CF_3; aromatic CH: 124.5, 127.8, 128.1, 131.0, 131.2, 131.6, 131.7, 135.0, aromatic quaternary C: 118.5, 128.9, 130.9, 131.0, 131.2, 134.97, 138.16, 138.23, 139.5, 140.0, 144.9, 145.8, 148.85, 148.94, 151.7, 157.5, quartet J=37.3 Hz, $\underline{C}OCF_3$; 165.4, 165.7, 169.6, 169.7, 170.1, 170.5, ester C=O; 182.0, 182.3, 182.7, quinone C=O. IR (FT, $CDCl_3$)νmax: 1774, 1737, broad singlet, ester C=O; 1669, quinone C=O, 1532, amide.

Ethyl [6,11-diacetoxy-5,12-dioxo-3,4,5,12-tetrahydroanthraceno (2,3-c) pyran-3-yl] formate (322)

Following a modification of Tamura's method[355], a mixture containing 387 mg (1.1 mmol) of the unacetylated pyranotetracycle (308), 2.5g lead tetraacetate, 60 ml of acetic acid, and 30 ml of CH_2Cl_2 was stirred for 48 hours

under argon at room temperature. The mixture was then diluted with 100 ml of CH_2Cl_2, extracted twice with 50 ml of water and dried over Na_2SO_4. After removal of solvents, the residue was added to a solution containing 5 ml of acetic anhydride, 6 ml of pyridine and 60 mg of dimethylami- nopyridine in 50 ml of CH_2Cl_2. The mixture was stirred at room temperature overnight under argon and then added to 50g of ice. The aqueous layer was separated and extracted twice with 50 ml of CH_2Cl_2. The combined organic extracts were then consecutively washed once with 25 ml of water, twice with 25 ml of IN HCl, 25 ml of water, 25 ml of brine and then dried over Na_2SO_4. After evaporation of solvents, flash chromatography of the residue yielded 175 mg (35%) of the titled bisacetylated pyranoanthraquinone. (MP: 203-205°C). ^1H NMR (200 MHz, $CDCl_3$) δ: 1.36 (t,3H, J=7.1 Hz, CH_3), 2.50 (s,3H, $COCH_3$), 254 (S,3H, $COCH_3$), 3.05 (broad m,2H,$C\underline{H}_2CHC=O$, 4.35 (masked dd,1H, OCHC=O), 4.32 (q,2H,J=7.1 Hz, $OC\underline{H}_2CH_3$), 4.75 (broad d, 1H, J=16.7 Hz, $HC\underline{H}a$-O), 5.11 (broad d,1H,J=16.5 Hz,$HC\underline{H}e$-O), 7.75 (m,2H,ArH), 8.16 (m,2H, ArH). IR (FT, $CDCl_3$) νmax: 1771, broad s, ester CO; 1677, quinone C=O, 1591, C=C. HRMS calculated for $C_{24}H_{19}O_{19}$: 451.1029 found 451.1061.

(1S,3S) and (1R,3R) Ethyl [11-acetoxy-1,6-dihydroxy-5,12-dioxo-3,4,5,12-tetrahydroanthraceno (2,3-c) pyran-3-yl] formate (323).

A mixture containing 75 mg (0.17 mmol) of the pyranotetracycle (322), 32 mg (0.17 mmol) of N-bromosuccinimide, 1 mg AIBN in 15 ml of CCl_4 was refluxed under argon for 2.5 hours. After removal of solvent, 25 ml of a 4:1 THF-H_2O solvent mixture was added to the residue and stirred for 0.5 hour. The mixture was then extracted three times with 25 ml of CH_2Cl_2 and the combined extracts were washed with 25 ml of water, 25 ml of brine and dried over Na_2SO_4. After evaporation of solvent, flash chromatography of the residue gave 61 mg (77%) of the desired bis acetylated tetracyclic aglycone (323). (MP: 220-250°C decomposes). ^1H NMR (300 MHz, DMSO-d_6) δ: 1.25 (t,3H,J= 7.1 Hz, CH_3), 2.42 (s,3H,$COCH_3$), 2.35 (bm,1H,HCHa), 2.47 (bm,1H, HCHe), 4.21 (q,2H,J=7.1 Hz,OCH_2), 4.42 (m.1H,OCH), 6.11 (d, 1H,J= 5.8 Hz,O-CH-OH), 7.42 (d,1H,J=5.8 Hz, exchangeable, OH), 7.95 (m,2H,ArH), 8.12 (m,1H,ArH), 8.22 (m,1H,ArH), 13.3 (s, 1H, exchangeable, ArOH).

IR (FT, $CDCl_3$) νmax: 3690, OH; 3500, 3700, bs, OH; 1764, 1730, ester C=O; 1668, 1636, quinone C=O; 1601, C=C.

HRMS calculated for $C_{22}H_{18}O_9$: [$M^{+\cdot}$] 425.0951 found 425.0948.

(1'S,1R,3S) and (1'S,1S,3R) Ethyl [11-hydroxy-6-acetoxy-1-(2',3',6'-trideoxy-3'-trifluoroacetamido-4'-O-p-nitrobenzoyl-L-lyxohexopyranose) 5,12-dioxo-3,4,5,12-tetrahydroanthraceno (2,3-C) pyran-3-yl] formate (324) and (325)

These compounds were obtained in 61% yield by following the same procedure as described for glycosides (200) and (201) and using the aglycone (323) with daunosamine (134). (MP: 155-158°C of 1'S,1S,3R and 182-184°C of 1'S,1R,3S). ^{1}H NMR (200 MHz,$CDCl_3$) of the less polar (1'S,1S,3R) diastereomer, δ: 1.26 (d,3H,J=6.5 Hz,H_3C-6'), 1.39 (t, 3H,J=7.1 Hz, CH_3), 2.21 (m,2H, H_2C-2'), 2.54 (s,3H,O=C- CH_3), 2.88 (m,1H,HCHaCHC=O), 3.13 (m,1H,HCHeCHC=O), 4.37 (q,2H,J=7.1 Hz, OCH_2), 4.49 (broad q, 1H,J=6.5 Hz, HC-5'), 4.67 (m,1H,HC-3'), 4.83 (dd,1H,J=11.6,4.45 Hz,OCHC=O), 5.49 (broad s,1H, HC-4'), 5.77 (broad s,1H,Wh<6 Hz,HC-1'), 6.21 (s,1H,O-CH-O), 6.24 (d,1H,J=9.1 Hz,NH), 7.84 (m,2H,ArH), 8.60 (m,2H,ArH), 8.33 (dd,4H,p-nitroaryl-H), 13.54 (s,1H, exchangeable, OH). ^{1}H NMR (200 MHz, $CDCl_3$) of the (1'S, 1R,3S) diastereomer δ: 1.29 (d,3H,J=6.5 Hz,H_3C-6'), 1.37 (t,3H,J=7.1 Hz, CH_3), 2.08 (m,2H,H_2C-2'), 2.88 (broad m,1H, HCHaCHC-O), 3.11 (broad m,1H,HCHeCHC=O), 4.25 (q,2H,J= 7.0 Hz,OCH_2), 4.67 (m,1H,HC-3'), 4.81 (m,1H, HC- 5'), 4.85 (m,1H,OCHC=O), 5.45 (broad s,1H,HC-4'), 5.71 (broad s,1H,H-C-1'), 6.37 (broad d,1H,J=9 Hz,NH), 6.39 (s, 1H,O-CH-O), 7.85 (m,2H,ArH), 8.30 (m,2H,ArH), 8.33 (dd,4H, p-nitroaryl-H), 13.66 (s,1H, exchangeable, OH). IR (FT, $CDCl_3$) νmax: 3431, OH; 1737, bs, ester C=O; 1674, quinone C=O; 1595, C=C.

Ethyl(7-chloro-5,8-dimethoxyisochroman-3-yl)formate (316) Under argon and at room temperature, was added dropwise 0.820g (7.6 mmol) of t-butylhypochlorite[357] to a stirred solution containing 1.973g (7.4 mmol) of the isochroman (304) in 75 ml of anhydrous CH_2Cl_2. The reaction mixture was stirred for three hours and then washed sucessively with 25 ml portions of saturated aqueous sodium thiosulfate, water and brine. After drying over Na_2SO_4, the organic layer was evaporated and the residue was flash chromatographed with 2.5% ethyl acetate in toluene as the eluting solvent mixture. The titled compound was obtained in 46% yield (1.02g). (MP: 94.0-95.0°C). ^1H NMR (200 MHz, $CDCl_3$) δ: 1.35 (t,3H,J=7.1 Hz,CH_3), 2.93 (broad dd, J=16.8,10.3 Hz,HC<u>Ha</u>CHC-O), 3.16 (broad dd, J=16.9, 3.2 Hz, HC<u>He</u>CHC=O), 3.78 (s,3H,OCH_3), 3.81 (s,3H, OCH_3), 4.27 (dd,J=10.2,3.3 Hz-CH), 4.31 (q,2H,J=7.1 Hz, OCH_2), 4.63 (broad d,1H,J=16.2 Hz,HCHaO), 5.01 (d,1H,J= 16.2 Hz,HCHeO), 6.73 (s,1H,ArH). HRMS calculated for $C_{14}H_{17}O_5Cl$: [M+·] 300.0765 found 300.0772. The 6-chloro isomer (317) was obtained in 48% yield (1.05g). (MP: 60-62°C). ^1H NMR (200 MHz, $CDCl_3$) δ: 1.34 (t,3H,J=7.1Hz,CH_3), 2.74 (dddd,1H,J=1.0, 1.6, 10.5, 17.1 Hz,HCHa), 3.05 (ddd,1H, J= 1.3, 4.2, 17.2 Hz,HCHe), 3.79 (s,3H,OCH_3), 3.81 (s,3H, OCH_3), 4.28 (overlaped q, 3H,OCH and OCH_2), 4.75 (ddd, 1H,J=1.0, 1.6, 16.0 Hz,HC<u>Ha</u>), 5.12 (bd,1H,J=15.9 Hz, HC<u>He</u>), 6.75 (s,1H,ArH). HRMS calculated for $C_{14}H_7O_5Cl$: [M+·] 300.0765 found 300.0754.

Ethyl(7-chloro-5,8-dioxo-5,8-dihydroisochroman-3-yl) formate (319)

To a stirred solution of 1.0g (3.7 mmol) of a 1:1 mixture of chloroisochromans (316) and (317) in 20 ml of acetonitrile was added dropwise a solution containing 6.25g (11.4 mmol) of cerric ammonium nitrate in 20 ml of water. The mixture was stirred overnight and then diluted with 50 ml of CH_2Cl_2. The organic layer was separated and the aqueous phase was extracted twice with 25 ml CH_2Cl_2. The combined organic layer was washed once with 50 ml H_2O, 50 ml brine and then dried over Na_2SO_4. After evaporation of solvents, flash chromatography of the residue with a solvent gradient of 5 to 20% ethyl acetate in toluene yielded 495 mg (55%) of the titled compound. (MP: 83.0 - 83.5°C). ^1H NMR (200 MHz, $CDCl_3$) δ: 1.27 (t,3H,J=7.1 Hz,CH_3), 2.58 (ddt,1H,J=19.0,8.8,3.2 Hz,HCHaCHC=O), 2.83 (d multiplets, 1H,HCHeCHC=O), 4.17 (dd,1H,J=8.7,3.3 Hz, OCHC=O), 4.20 (q,2H,J=7.0 Hz,OCH_2), 4.44 (dt,1H,J=18.8,3.4 Hz, HCHaO), 4.78 (d multiplet, 1H,J=18.8,2.6,1.7Hz,HCHeO), 6.95 (s,1H,C=CH). Ethyl(6-chloro-5,8-dioxo-1-hydroxy-5,8-dihydroisochroman-3-yl) formate (320) was also isolated in 25% yield as a thick yellow oil. ^1H NMR (200 MHz, $CDCl_3$) δ: 1.31 (t,3H,J=7.1 Hz,CH_3), 2.58 (ddd,1H,J= 1.1,11.8,19.5 Hz,HCHa), 3.01 (dd,1H,J=4.0, 19.5 Hz, HCHe), 4.32 (q,2H,J=7.1 Hz, OCH_2), 4.84 (dd,1H, J= 4.0, 11.6 Hz,

OCH), 4.98 (d,1H,J=5.3Hz, exchangeable, OH), 6.04 (d,1H,J= 5.0 Hz,O-CH-O), 6.99 (s,1H,ArH). IR (FT, $CDCl_3$) ν_{max}: 3691, OH; 1750, ester C=O; 1680, 1666, quinone C=O; 1595, C=C. HRMS calculated for $C_{12}H_{11}Cl_{06}$: [M+·] 286.0245 found 286.0250.

Ethyl [11-acetoxy-5,12-dioxo-3,4,5,12-tetrahydroanthraceno (2,3-c) pyran-3-yl] formate (326)

Following a modification of Tamura's procedure [255] to a cooled (0°C) and stirred solution of 203 mg of dry diisopropylamine in 7 ml of dry tetrahydrofuran under argon was added 0.74 ml of a 2.5M solution of n-butyl lithium in hexanes. The mixture was cooled to -78°C and stirring was continued for 1/2 hour. A solution containing 325 mg (2.0 mmol) of homophthalic anhydride in 7 ml THF was slowly added over five minutes. Next was added in one portion a solution containing 500 mg (1.85 mmol) of the chloroquinone (319) in 9 ml THF. The reaction mixture was then stirred for 20 minutes at -78°C, allowed to warm up to room temperature and stirred for one hour. The reaction was then quenched with 10 ml of saturated ammonium chloride, and partitioned between 10 ml of 5% aqueous HCl and 100 ml CH_2Cl_2. The organic layer was then separated and washed with 25 ml of water, 25 ml of brine and dried over Na_2SO_4.

Evaporation of solvents yielded the crude pyranoanthracyclinone (308) which was immediately acetylated in 90 ml of CH_2Cl_2 at room temperature for 10 hours with acetic anhydride (1.25ml) in the presence of 100 mg of dimethylaminopyridine and 1.5ml of pyridine. To this reaction mixture was then added 50g of ice, and the isolated organic layer was washed consecutively with 25 ml portions of 5% aqueous HCl, water and brine. The organic solution was then dried over Na_2SO_4 and evaporated. The residue was then subjected to flash chromatography (5% ethyl acetatein toluene) and gave 494 mg (48% yield) of the desired titled compound. (MP: 171-173°C).

^1H NMR (200 MHz, $CDCl_3$) δ: 1.38 (t,3H,J=7.1 Hz, CH_3), 2.64 (s,3H,$COCH_3$), 2.83 (ddt, 1H,J=19.1, 9.0, 3.0 Hz, HcHaCHC=O), 3.14 (d M,1H,J=18.9 Hz, HCHeCHC=O), 4.32 (dd,1H, J=9.0,4.8 Hz,OCHC=O), 4.34 (q,1H,J=7.1Hz,OCH_2), 4.65 (dt, 1H,J=18.9,3.2 Hz,HCHaO), 5.03 (broad d,1H,J=18.9 Hz,HCHeO), 7.76 (m,2H,ArH), 8.13 (m, 2H,ArH), 8.20 (s,1H, ArH). CMR (75.44 MHz, $CDCl_3$) δ: 13.9, CH_3; 20.9, CH_3; 24.6, CH_2; 61.6, 63.3, OCH_2; 71.1, OCH; 123.9, 127.4, 130.3, 130.5, 130.6, aryl CH; 117.9, 128.6, 130.4, 135.6, 140.9, 144.4, 148.2, aryl C; 166.7, 170.4, ester C=O; 181.7, 182.5, quinone C=O. IR (FT, $CDCl_3$) νmax: 1774 acetate C=O, 1750, PNB ester C=O; 1667, quinone C=O. HRMS calculated for $C_{22}H_{18}O_7$: [$M^{+\cdot}$] 394.1053 found 394.1067.

(1S,3S) and (1R,3R)-Ethyl [11-acetoxy-1-hydroxy-5,12-dioxo-3,4,5,12-tetrahydroanthraceno (2,3-c) pyran-3-yl] formate (327)

A mixture containing 257 mg (0.65 mmol) of the pyranotetracycle (326), 121 mg of N-bromosuccinimide, and 15 mg of AIBN in 25ml of carbon tetrachloride was refluxed for two hours. After cooling, the solvent was removed in vacuo and the residue was treated with 40 ml of a 1:1 THF/water solvent mixture for one hour. Most of the THF was then removed in a rotary evaporator and the residual aqueous mixture was extracted three times with 30 ml CH_2Cl_2. The combined organic layer was then washed with 25 ml aliquots of water and brine. After drying over Na_2SO_4, the solvent was removed and the residue was flash chromatographed to give 178 mg (67%) of the titled aglycone. (MP: 190-192°C). ^1H NMR (300 MHz, $CDCl_3$) δ: 1.25 (t,3H,J=7.1Hz,CH_3), 2.54 (s,3H,$OCOCH_3$), 2.59 (dd,1H,J=11.5,19.1Hz, HCHa), 2.89 (dd, 1H, J=4.0,19.1 Hz, HCHe), 4.21 (q,2H, J=7.1 Hz, OCH_2), 4.73 (dd,1H,J=4.1,11.6 Hz,OCH), 5.89 (bs, 1H,OCH-OH), 7.34 (bs,1H, exchangeable OH), 7.84 (m,2H,ArH), 8.25 (m,1H,ArH), 8.34 (m,1H,ArH), 8.63 (s,1H,ArH). CMR (75.44 MHz, DMSO-d_6) δ: 14.0, CH_3; 20.9, CH_2; 61.0, OCH_2; 64.4, OCH; 85.9, O-CH-OH; 120.0, 126.8, 130.8, 130.9, 135.1, aryl CH; 118.0, 128.5, 130.0, 142.1, 142.7, 147.7, aryl C; 169.3,

170.5, ester C=O; 180.8, 183.2, quinone C=O. IR (FT, $CDCl_3$) νmax: 3575,bs,OH; 1774, 1750, ester C=O; 1670, quinone C=O. HRMS Calculated for $C_{22}H_{18}O_8$: [M+·] 410.1002 found 410.1010.

(1'S,1R,3S) and (1'S,1S,3R)-Ethyl [11-acetoxy-1-(2',3',6'-trideoxy-3'-trifluoroacetamido-4'-O-p-nitrobenzoyl-L-lyxohexopyranose)-5,12-dioxo-3,4,5,12-tetrahydroanthraceno-(2,3-c) pyran-3-yl] formate (328) and (329)

Following a modification of Terashima procedure[292], to a stirred and cooled (-40°C) solution of 222 mg (0.41 mmol) of 2,3,6-trideoxy-3-trifluoroacetamido-1,4-di-O-p-nitrobenzoyl-α (or β)-L-lyxohexopyranose[254] in 20 ml of a 3:1 CH_2Cl_2-Et_2O solvent system was added 0.15 ml of trimethylsilyltrifluoromethane sulfonate under argon atmosphere and in the presence of 4A molecular sieves. The mixture was stirred one hour at -5°C and then cooled to -15°C. A solution containing 121 mg (0.30 mmol) of the pyranoaglycone (327) in 10 ml CH_2Cl_2 was added next and the mixture was stirred for 20 hours at -15°C. The reaction mixture was then poured in 50 ml of a 1:1 ethyl acetate saturated sodium bicarbonate solvent system, filtered and the separated organic layer was washed with 10 ml of water, 10 ml of brine and dried over Na_2SO_4.

After removal of solvents, the residue was flash chromatographed with 10% ethyl acetate in toluene. The desired titled pyranoanthra- cycline glycosides were obtained as a 1:1 diastereomeric mixture in 65% yield (239 mg). (MP: 160-162°C decomposes). ^{1}H NMR (200 MHz, $CDCl_3$) of the (1'S,1S,3R) diastereomer, δ: 1.24 (d,3H,J=6.5 Hz, H_3C-6'), 1.37 (t,3H,J= 7.1Hz, CH_3), 2.06 (m,2H, H_2C-2'), 2.58 (s,3H,acetyl,CH_3) 2.74 (dd,1H,J=17.5,12 Hz, HCHaCHC=O), 3.17 (dd,1H,J=19.4, 3.8 Hz, HCHeCHC=O), 4.32 (q,2H,J=7.0 Hz,OCH_2CH_3), 4.42 (broad q,1H,J=7.1 Hz, HC-5'), 4.66 (m,1H,HC-3'), 4.74 (dd,1H, J=11.9, 3.8 Hz, OCHC=O), 5.47 (broad s,1H,HC-4'), 5.75 (broad s,1H, HC-1'), 6.05 (s,1H,O-CH-O), 6.31 (d,1H,J=6.6 Hz,NH), 7.76 (m, 2H, ArH), 8.14 (dm,2H,ArH), 8.31 (dd,4H, p-nitrobenzoyl-H), 8.60 (s,1H,ArH). ^{1}H NMR (200 MHz, $CDCl_3$) of the (1'S,1R,3S) diastereomer, δ: 1.26 (d,3H,J=6.5 Hz, H_3C-6'), 1.35 (t,3H,J= 7.1 Hz, OCH_2CH_3), 2.06 (m,2H,H_2C-2'), 2.59 (s,3H,acetyl- CH_3), 2.76 (dd,1H,J=18.8,12 Hz, HCHaCHC=O), 3.15 (dd,1H,J= 19.4,4.1 Hz, HCHeCHC=O), 4.31 (q,2H,J=7.1 Hz, OCH_2CH_3), 4.66 (m,2H,overlaped HC-3' and HC-5'), 4.76 (dd,1H,J=11.9, 4.0 Hz,OCHC=O), 5.44 (broad s,1H,HC-4'), 5.65 (broad s, 1H, HC-1'), 6.42 (d,1H, J=7.4 Hz,NH), 7.76 (m,2H, ArH), 8.14 (dm, 2H,ArH), 8.32 (dd,4H, p-nitrobenzoyl- H), 8.60 (s,1H,ArH). CMR of the mixture of (328) and (329), (75.44 MHz, $CDCl_3$) δ: 13.9 and 14.0, $CO_2CH_2CH_3$; 16.6, 16.8, 6'-CH_3; 20.9, acyl CH_3; 24.1, 24.6, 2'-

CH_2; 29.4, 29.8, 4-CH_2; 45.4, 45.5, CHNH; 61.7, 61.8, ester OCH_2; 65.6, 66.3, 5'-OCH; 66.5, 4'-OCH; 71.8, 72.4, 3-OCH; 87.9, 92.7, 1-O-CH-O; 92.7, 98.0, 1'-O-CH-O; 115.6, quartet, J=289.2Hz, CF_3; 124.0, 127.57, 127.64, 130.5, 130.6, 130.7, 131.18, 131.22, 135.6, aryl CH; 118.0, 128.4, 134.6, 134.7, 141.0, 141.5, 142.2, 143.1, 148.3, 151.10, 151.14, aryl C; 157.1, quartet, J= 37.7, $\underline{C}OCF_3$; 164.8, 165.2, 169.2, 169.8, 170.2, ester C=O; 181.2, 183.2, quinone C=O. IR (FT, $CDCl_3$) νmax: 1775, 1737, bs, ester C=O; 1670, quinone C=O.

(2R,1R,3S) and (1'S,1S,3R)-Methyl [11-hydroxy-1-(2',3,'6'-trideoxy-3'-trifluoroacetamido-L-lyxohexopyranose)-5,12-dioxo 3,4,5,12-tetrahydroanthraceno (2,3-C) pyran-3-yl] formate (330) and (331)

Under argon, at room temperature a solution containing 115 mg (0.13 mmol) of glycosides (328) and (329) in 10 ml of dry methanol was treated with 0.16 ml of a 1.0M $NaOCH_3$ methanolic solution for two hours. The reaction mixture was then quenched with three drops of saturated aqueous NH_4Cl and the solvent was evaporated to dryness. The residue was stirred with pentane for five hours and then filtered. The pentane insoluble portion was then taken up in ether and filtered.

The ether was evaporated and the residue was flash chromatographed with a solvent gradient ranging from 50% ethyl acetate in toluene to 20% methanol in ethyl acetate. The titled heteroanthracycline glycosides were obtained in 74% yield (56 mg) as a 1:1 mixture (MP: 147-150°C)

^{1}H NMR (200 MHz, $CDCl_3$) of the (1'S,1R,3S) diastereomer, δ: 1.30 (d,3H,J=6.9 Hz, H_3C-6'), 1.88 (m,1H,Hac- 2'), 2.04 (m,1H,HeC-2'), 2.72 (dd,1H,J=19.0,12.0 Hz, HCHaCHC=O), 3.18 (dd,1H,J=19.0, 3.9 Hz, HCHeCHC=O), 3.66 (broad s,1H, HC-3'), 3.86 (s,3H,OCH_3) 4.33 (m,2H,HC-4'and HC-5'), 4.73 (dd,1H,J=11.8,3.9 Hz, O-CHC=O), 5.50 (broad s,1H,HC-1'), 5.88 (singlet, 1H,O-CH-O), 6.74 (broad d,1H,NH), 7.74 (m,2H,ArH), 7.98 (m,1H,ArH), 8.18 (s,1H,ArH), 8.50 (m,1H,ArH).

^{1}H NMR (300 MHz, $CDCl_3$) of the (1'S,1S,3R) diastereomer, δ: 1.34 (d,3H, J=7.0Hz, H_3C-6'), 1.88 (m,1H, HaC- 2'), 2.04 (m,1H,HeC-2'), 2.72 (dd,1H,J=19.0, 12.0 Hz,HCHaCHC= O), 3.18 (dd,1H,J=19.0, 3.9 Hz,HCHeCHC=O), 3.66 (broad s,1H, HC-3'), 3.87 (s,3H,OCH_3), 4.33 (m,1H,HC-4'), 4.59 (broad q, 1H,J=7.1 Hz,HC-5'), 4.73 (dd,1H,J=11.8,3.9 Hz,O-CHC-O), 5.60 (broad s,1H,HC-1'), 6.04 (s,1H,O-CH-O), 6.74 (broad d, 1H,NH), 7.74 (m,2H,ArH), 7.98 (m,1H,ArH), 8.18 (s,1H,ArH), 8.50 (m,1H,ArH). HRMS calculated for $C_{28}H_{26}F_3NO_{10}$: [M+] 579.1353, found 579.1358.

2,5-Dimethoxy-6-hydroxyethylbenzaldehydedioxane acetal (346).

To a cooled (-40°C) solution containing 1.68g (7.4 mmol) of 2,5-dimethoxybenzaldehydedioxane acetal in 50 ml of anhydrous diethyl ether was added, with stirring and under argon, 4.8 ml of a 2.5M n-butyl lithium in hexanes solution. The mixture was stirred for five hours at -5°C, 537 mg (6.0 mmol) of CuCN was then added, and stirring was continued for one more hour. To the mixture was then added 1.0g of ethylene oxide and stirred overnight at 4°C. The mixture was then washed with 20 ml of water, 20 ml of saturated aqueous sodium chloride and dried over $MgSO_4$. Flash chromatography of the residue obtained after removal of solvent gave 547mg (27% yield) of 2,5-dimethoxy-6-hydroxyethylbenzaldehyde dioxane acetal as a white solid. MP: 135-136°C. ^1H NMR (200 MHz, $CDCl_3$) δ:1.48 (bd, 1H, J=13.6 Hz, HC<u>He</u>), 2.32 (m, 1H, HC<u>Ha</u>), 3.48 (dt, 2H, J=4.76, 12.0 Hz, $ArCH_2$), 3.79 (s, 6H, $2XOCH_3$), 3.86-4.08 (overlapped m, 4H, 2XOHC<u>He</u> and CH_2OH), 4.29 (m, 2H, 2XOHC<u>Ha</u>), 6.28 (s, 1H, O-CH-O), 6.79 (dd, 2H, J=9.0 Hz, ArH). CMR (75.44 MHz, $CDCl_3$) δ : 25.9, 29.5, CH_2; 55.7, 56.3, OCH_3; 62.3, CH_2OH; 67.9, $2XOCH_2$; 97.6, O-CH-O; 109.5, 111.8, aryl CH; 126.3, 129.2, 150.8, 152.8, aryl C. IR (FT, $CDCl_3$) ν_{MAX}: 3350, 3550, bs, OH; 1257, 1089, C-O. HRMS calculated for $C_{14}H_{20}O_5$: [$M^{+\cdot}$] 268.1311 found 268.1316.

In addition, 74mg (4% yield) of 2,5-dimethoxy-4-hydroxyethylbenzaldehydedioxane acetal could be recovered as an oil from the chromatography. ^{1}H NMR (300 MHz, $CDCL_3$) δ: 1.47 (dm, 1H, HCHa), 1.60 (bs, 1H, exchangeable OH), 2.32 (m, 1H, HCHe), 2.88 (t, 2H, J=6.1 Hz, $ArCH_2$), 3.80 (t, 2H, J=6.1 Hz, $HOCH_2$), 3.81 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 4.01 (m, 2H, OHCH), 4.23 (m, 2H, OHCH), 5.85 (s, 1H, O-CH-O), 6.73 (s, 1H, ArH), 7.16 (s, 1H, ArH). CMR (75.44 MHz, $CDCl_3$) δ: 25.8, 34.4, CH_2; 55.9, 56.5, 2XOCH_3; 62.8, CH_2OH; 67.6, 2XOCH_2; 96.8, O-CH-O; 109.4, 114.5, aryl CH; 127.9, 128.6, 151.3, 153.7, aryl C. IR (FT, $CDCl_3$) ν_{MAX}: 3400-3700, bs, OH; 1214, 1045, C=O.

2,5-Dimethoxy-6-(2-hydroxypropyl)benzaldehydedioxane acetal (347).

Application of the procedure described for compound (346) gave 238mg (32% yield) of the desired benzaldehydedioxane acetal (347) from 739mg (3.3 mmol) of 2,5-dimethoxybenzaldehydedioxane acetal after flash chromatography from 5% ethyl acetate in toluene. MP: 160-161°C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.33 (d, 3H, J=6.1 Hz, CH_3), 1.49 (bd, 1H, J=13.7 Hz, HCHe), 2.30 (m, 1H, HCHa), 3.10 (dd, 1H, J=2.4, 14.1 Hz, HCH), 3.47 (dd, 1H, J=9.5, 13.9 Hz, HCH), 3.78 (s, 6H, OCH_3), 4.02 (overlapped m, 3H, HO-CH and 2XHCHe-O), 4.30 (m, 2H, 2XCH_2-O), 6.27 (s, 1H, O-

CH-O), 6.78 (dd, 2H, J=9.0 Hz, ArH). CMR (75.44 MHz, $CDCl_3$) δ: 25.1, CH_3; 25.8, 35.7, CH_2; 55.6, 56.3, OCH_3; 67.5, CHOH; 67.6, 67.7, OCH_2; 97.4, O-CH-O; 109.4, 111.7, aryl CH; 125.9, 129.3, 150.7, 152.7, aryl C. HRMS calculated for $C_{15}H_{22}O_5$: [$M^{+\cdot}$] 282.1467 found 282.1449. IR (FT, $CDCl_3$) ν_{MAX}: 3300-3550, bs, OH; 1257, 1094, C-O.

In addition to the recovery of 257mg (35% yield) of starting material, chromatography also yielded 62mg (8.4% yield) of 2,5-dimethoxy-4-(2-hydroxypropyl)benzaldehydedioxane acetal as a clear viscous oil. ^1H NMR (200 MHz, $CDCl_3$) δ: 1.13 (d, 3H, J=7.1 Hz, CH_3), 1.38 (m, 1H, HCHa), 2.30 (m, 1H, HCHe), 2.61 (dd, 1H, J=7.4, 13.2 Hz, HO-HCH), 2.77 (dd, 1H, J=3.3, 13.3 Hz, HO-HCH), 3.74 (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3), 4.06 (m, 2H, O-HCHa), 4.31 (m, 2H, O-HCHe), 5.79 (s, 1H, O-CH-O), 6.65 (s, 1H, ArH), 7.10 (s, 1H, ArH). CMR (75.44 MHz, $CDCl_3$) δ: 22.8, CH_3; 25.8, CH_2; 40.7, CH_2; 55.8, 56.4; OCH_3; 67.6, 2XOCH_2; 67.9, CHOH; 96.7, OCHO; 109.4, 114.8, aryl CH; 125.8, 128.5, 150.2, 151.7, aryl C. IR (FT, $CDCl_3$) ν_{MAX}: 3380-3700, bs, OH; 1212, 1094, C-O.

5,8-Dimethoxy-1-hydroxyisochroman (348)

A solution containing 150 mg (0.56mmol) of 2,5-dimethoxy-6-hydroxyethylbenzaldehydedioxane acetal in 5 ml of THF was stirred for one hour at room temperature with 5

ml of 0.2M aqueous HCl. The mixture was then diluted with 25 ml of dichloromethane, washed successively with 25 ml aliquots of aqueous sodium bicarbonate, water, brine and then dried over $MgSO_4$. Flash chromatography of the residue obtained after removal of solvent gave 97 mg (82% yield) of 1-hydroxyisochroman (348). MP: 217-219°C. ^1H NMR (200 MHz, $CDCl_3$) δ: 2.73 (m, 2H, CH_2), 3.09 (bs, 1H, exchangeable, OH), 3.84 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 4.03 (m, 1H, OHC*H*), 4.27 (m, 1H, O<u>H</u>CH), 6.12 (bs, 1H, OCH), 6.77 (d̈d, 2H, J=7.2 Hz, ArH). CMR (75.44 MHz, $CDCl_3$) δ: 22.2, CH_3; 55.5, 55.7, OCH_3; 56.8, OCH_2; 87.4, OCH; 107.9, 109.4, aryl CH; 124.2, 124.8, 150.4, 150.6, aryl C. IR (FT, $CDCl_3$) ν_{MAX}: 3650, bs, OH; 1260, C-O. HRMS calculated for $C_{11}H_{14}O_4$: [$M^{+}\cdot$] 210.0892 found 210.0871.

5,8-Dimethoxy-1-hydroxy-3-methylisochroman (349)

Application of the procedure described for isochroman (348) gave 215 mg (86% yield) of the 3-methyl substituted isochroman (349) from 250 mg of 2,5-dimethoxy-6-(2-hydroxypropyl)benzaldehydedioxane acetal after flash chromatography with 20% ethyl acetate in hexanes. MP: 240-241°C. ^1H NMR (200 MHz, $CDCl_3$) δ: 1.41 (d, 3H, J=6.2 Hz, CH_3), 2.34 (dd, 1H, J=11.4, 17.4 Hz, HC<u>Ha</u>), 2.81 (dd, 1H, J=3.25, 17.3Hz, HC<u>He</u>), 3.02 (d, 1H,exchangeable, J=3.4 Hz,

OH), 3.81 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 4.37 (m, 1H, OCH), 6.12 (d, 1H, J=3.3 Hz, O-CH-O), 6.74 (dd, 2H, J=8.9 Hz, ArH). CMR (75.44 MHz, $CDCl_3$) : 21.3, CH_3; 29.8, CH_2; 55.7, 55.8, OCH_3; 62.6, O-CH; 88.6, O-CH-O; 107.9, 109.5, aryl CH; 124.6, 150.47, 150.53, aryl C. IR (FT, $CDCl_3$) ν_{MAX}: 3630, bs, OH; 1260, C-O. HRMS calculated for $C_{12}H_{16}O_4$: [$M^{+\cdot}$] 224.1049 found 224.1036.

1,5,8-Trimethoxy isochroman (352)

A solution containing 650 mg of 2,5-dimethoxy-6-hydroxyethylbenzaldehydedioxane acetal and 5 mg of p-toluenesulfonic acid in 20 ml of anhydrous methanol was stirred under argon for ten minutes at room temperature. Solid bicarbonate (10 mg) was then added, the mixture was evaporated to dryness, and the residue was dissolved in 75 ml of CH_2Cl_2. The resulting organic solution was washed successively with 25 ml aliquots of water, 25 ml of saturated aqueous NaCl, and dried over $MgSO_4$. Evaporation of solvent gave 508 mg (94% yield) of pure 1,5,8-trimethoxyisochroman. MP: 191-192°C. ^1H NMR (200 MHz, $CDCl_3$) δ: 2.68 (m, 2H, CH_2), 3.56 (s, 3H, OCH_3), 3.79 (s, 3H, $ArOCH_3$), 3.82 (s, 3H, $ArOCH_3$), 4.03 (m, 2H, OCH_2), 5.55 (s, 1H, O-CH-O), 6.72 (dd, 2H, J=9.0 Hz, ArH). CMR (75.44 MHz, $CDCl_3$) δ : 22.3, CH_2; 55.4, 55.7, 56.1, OCH_3; 56.4,

OCH_2; 94.2, O-CH-O; 108.3, 109.7, aryl CH; 124.0, 124.3, 150.7, 150.8, aryl C. IR (FT, $CDCl_3$) ν_{MAX}: 1261, C-O.

1,3,5-Trimethoxy-1-methylisochroman (353)

This compound was prepared by using the procedure employed for (352). An 85% yield (201mg) was obtained from 294mg (1.0mmol) of benzaldehydedioxane acetal 9347). MP: 201-202°C. ^1H NMR (200 MHz, $CDCl_3$) δ: 1.38 (d, 3H, J=6.3 Hz, CH_3), 2.32 (dd, 1H, J=11.5, 17.4 Hz, HCHa), 2.78 (dd, 1H, J= 3.3, 17.4 Hz, HCHe), 3.78 (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 4.23 (m, 1H, $OCHCH_3$), 5.69 (s, 1H, O-CH-O), 6.71 (dd, 2H, J=8.8 Hz, ArH). IR (FT, $CDCl_3$) ν_{MAX}: 1261, C-O.

1-Hydroxy-5,8-dioxo-5,8-dihydroisochroman (350)

To a stirred solution containing 75 mg (0.36 mmol) 5,8-dimethoxy-1-hydroxyisochroman (348) in 2 ml of THF was added dropwise over four minutes, a solution containing 546 mg (1.0 mmol) of ceric ammonium nitrate in 2 ml of water. The mixture was stirred for five minutes and then diluted with 10 ml of dichloromethane. The separated organic phase was washed with 5 ml of water, 5 ml of brine and dried over

$MgSO_4$. Following evaporation of solvent, the yellow oil (62 mg, 96% yield) was found to be pure isochromandione (350). ^1H NMR (200 MHz, $CDCl_3$), δ: 2.54 (m, 2H, CH_2), 3.51 (d, 1H, J=4.4 Hz, exchangeable, OH), 4.09 (m, 2H, OCH_2), 5.88 (d, 1H, J=4.4 Hz, O-CH-O), 6.79 (dd, 2H, J=10.1 Hz, H-C=C-H). CMR (75.44 MHz, $CDCl_3$) δ: 21.5, CH_2; 56.4, OCH_2; 85.3, OCH; 136.3, 136.4, aryl CH; 138.4, 141.3, CH; 185.3, 186.4, quinone C=O.

1-Hydroxy-5,8-dioxo-3-methyl-5,8-dihydroisochroman (351)

Oxidative demethylation of 336 mg (1.5 mmol) of 5,8-dimethoxy-1-hydroxy-3-methylisochroman (349) with 2.94 g (5 mmol) of cerric ammonium nitrate as described for compound (350) gave 262 mg (90% yield) of isochromandione (351) as a yellow oil. ^1H NMR (200 MHz, $CDCl_3$) δ: 1.38 (d, 3H, J=6.3 Hz, CH_3), 2.15 (ddd, 1H, J=1.2, 11.0, 19.5 Hz, HC<u>Ha</u>), 2.62 (dd, 1H, J=3.3, 19.5 Hz, HC<u>He</u>), 3.16 (d, 1H, J=4.3 Hz, exchangeable, OH), 4.31 (m, 1H, O-C<u>H</u>CH_3), 5.91 (d, 1H, J=4.3 Hz, O-CH-O), 6.78 (dd, 2H, J=10.2 Hz, ArH).

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