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ABSTRACT

Part I

A novel preparation of regiospecifically substituted 2-hydroxy-benzocyclobutenones is presented. The sequence involved conversion of *ortho*-bromobenzaldehydes via their cyanohydrins into TBS protected mandelate esters and subsequent cyclization by a halogen metal exchange reaction. Deprotection afforded 2-hydroxybenzocyclobutenones. Overall yield for the sequence was approximately 50%, and was improved by converting the ethyl esters into Weinreb amides prior to halogen metal exchange. Addition of aryl Grignard reagents to the 2-hydroxybenzocyclobutenones followed by oxidation and thermolysis yielded the target anthraquinones. The difficulties associated with the oxidation reaction are discussed since the best results were obtained using DMSO/SO_3-pyridine complex with a maximum yield of 50% for the compound with no substituents on the aromatic ring; yields were lower for substituted compounds. Alkylidene and bis-alkylidene benzocyclobutenones were synthesized by reacting the hydroxybenzocyclobutenones with Wittig type reagents. Reaction of 2-hydroxybenzocyclobutenone with Lawesson's reagent resulted in the formation of a ring expanded dithiolactone.

Part II

The synthesis of a series of podophyllotoxin derivatives is presented. The first type result from nucleophilic substitution of the 4 - hydroxy group under Lewis acid conditions using trimethylsilylcyanide or allyltrimethylsilane as the nucleophiles. The resulting 4-allyl-4-deoxypodophyllotoxin was reacted under hydroboration conditions to

yield the 4-(hydroxypropyl) derivative, oxidation gave the corresponding carboxylic acid. These were demethylated to give the required free hydroxy group. The second type result from addition of organolithium reagents to podophyllotoxone or its 4'- demethyl analogue. A tertiary alcohol of this type with a phenylethyl substituent in the C4 position was prepared. The most active of these compounds in vitro was the hydroxypropyl derivative which was chosen for in vivo study. In vivo testing showed the compound to be inactive.

Part III

Conjugates of alpha - terthienyl and bovine serum albumin were prepared using both reductive amination techniques and the mixed anhydride process. The biological and photochemical characterization of these conjugates is presented. The bioassays showed the conjugates to be very toxic to yeast and brine shrimp while the photochemical studies showed the conjugates were highly fluorescent and produced sufficient singlet oxygen to cause significant cell damage. However, the lower quantum yield as compared to the free α-T indicated a possibility of causing damage to the protein as well, thus altering its conformation. The mixed anhydride process was also used to prepare conjugates of α - T with a mouse antibody and with the human antibody MRK 16 which is specific to p-glycoprotein.

ACKNOWLEDGEMENTS

I would like to thank Professor Tony Durst for his guidance and support over the course of my graduate program.

I thank Shawna Mackinnon for her friendship, support and for just listening many times over the past five years.

I thank the members of the Durst group for many lively conversations, support, and sharing of limitless advice about chemistry and life. I especially thank Terry Connolly for many enlightening conversations regarding many facets of synthetic chemistry.

Finally, I thank my family for their support, not only for the last five years, but in all the years leading up to this point in my life.

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LIST OF ABBREVIATIONS

°C	degrees Celsius
α-T	α-terthienyl
BSA	bovine serum albumin
BuLi	butyllithium
d	doublet
Da	daltons
dd	doublet of doublets
DMF	dimethylformamide
DMSO	dimethyl sulfoxide
eq	equivalents
h	hours
hν	photochemical irradiation
HOMO	highest occupied molecular orbital
HRMS	high resolution mass spectrometry
Hz	hertz
IR	infrared
J	coupling constant
LDA	lithium diisopropylamide
LUMO	lowest unoccupied molecular orbital
m	multiplet
M	molar

M+	molecular ion
MDR	multidrug resistance
min	minutes
mmol	millimoles
mp	melting point
NMR	nuclear magnetic resonance
NOE	nuclear Overhauser effect
P-gp	p-glycoprotein
ppm	parts per million
q	quartet
t	triplet
T/C	test/control
TBAF	tetrabutylammonium fluoride
TBSCl	t-butyldimethylsilyl chloride
TBSCN	t-butyldimethylsilyl cyanide
THF	tetrahydrofuran
THP	tetrahydropyran
TMSCN	trimethylsilyl cyanide
UV	ultraviolet
VM-26	teniposide
VP-16	etoposide

Organizational Preface

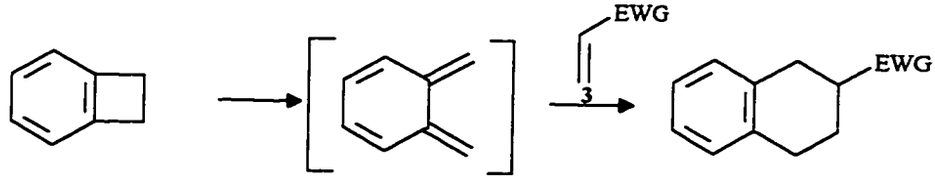
Organic chemists today are faced with a variety of challenging problems. This thesis is written in three distinct parts, each describing an approach taken toward a different chemical problem. The first deals with a synthetic problem, the design and synthesis of regiospecifically substituted 2-hydroxybenzocyclobutenones and their conversion to members of the anthraquinone family of natural products. The second part describes a derivatization of the natural product podophyllotoxin in an attempt to find more potent analogues of the commercial anti-cancer drug Etoposide. The third part is a brief account of a study involving the construction of a photactivated antibody probe. The three parts are treated as independent units, each with a separate introduction, structure numbers, and references.

Part I Benzocyclobutene Chemistry

Chapter 1 Introduction

The first record of a benzocyclobutene dates back to 1909 when Finkelstein, a graduate student at the time in Germany, reported the formation of dibromo-benzocyclobutene as a footnote in his doctoral thesis.[1] The subject was brought to light by Cava in 1956.[2] The chemistry expanded dramatically so that by 1969 when the first major review of benzocyclobutenones was published,[1] at least 134 reports on the chemistry of benzocyclobutenes had been published, and the area has continued to expand. The versatility of the compounds, **1**, is due to the strain inherent in the four membered ring, which serves as a springboard to the intermediate ortho-quinodimethane, **2**. This short-lived intermediate is a highly reactive diene in Diels Alder reactions due to its propensity toward rearomatization. (Scheme 1) The intermediate ortho-quinodimethane reacts in situ with an appropriate dienophile **3** to yield the desired product, **4**.

Scheme 1



1 2 4 4

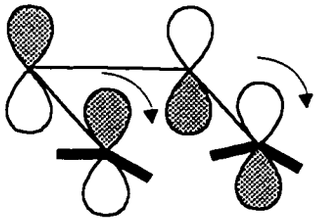
The ring opening reaction itself is governed by a number of factors.[3] In general, ring opening is facilitated by the presence of electron donating substituents on the four-membered ring, as is apparent from the difference in temperature required to open the cyclobutane ring.[4] (Table 1)

Table 1 Effect of substituents on cyclobutane ring opening temperatures

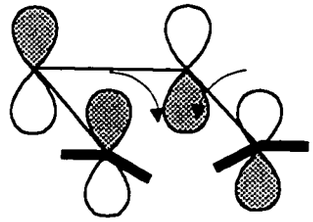
Substituent	NH_2	OH	NH(CO)R	C=O	CH_2	H
Temperature(°C)	25	80	110	150	180	200

From a synthetic standpoint, it is important to appreciate that the ring-opening of mono-substituted cyclobutenes and benzocyclobutenes is highly stereoselective. It has been shown that the process is conrotatory, meaning the groups on the saturated carbons of the cyclobutane ring rotate in the same direction, either clockwise or counterclockwise on ring opening.[4] The HOMO, ψ_2, for butadiene is shown below. (Figure 1) The conrotatory closure results in the formation of a bonding orbital, while the disrotatory closure results in formation of an antibonding orbital. The same principles apply for the reverse process.[5]

Figure 1



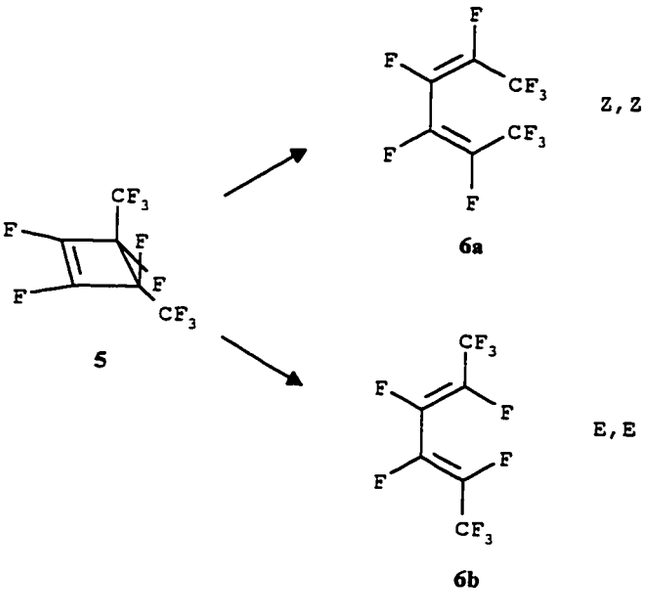
Conrotatory closure



Disrotatory closure

It has also been shown that π–electron donating substituents tend to rotate outward, while electron withdrawing groups tend to rotate inward, regardless of steric factors.[6,7] This is best illustrated by the case of the perfluorocyclobutene. Inward rotation of the two bulky trifluoromethyl groups of perfluoro-trans-3,4-dimethyl-cyclobutene **5** gives the sterically crowded Z,Z product, **6a**, which has an activation energy 19.2 kcal/mol less than the outward rotation process which gives the E,E isomer, **6b**. (Scheme 2) This effect has been explained on the basis of molecular orbital theory.[7] There is a greater stabilization of the transition state resulting in better overlap of the donor orbital of the fluorine substituent with the LUMO on outward rotation than on inward rotation. As well, there is a larger destabilization by donor orbital overlap with the HOMO on inward rotation than on outward rotation. These trends are reversed for electron withdrawing groups.

Scheme 2



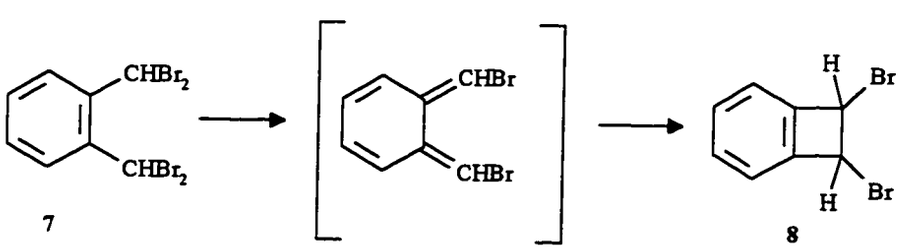
These effects can be used to predict the stereochemical outcome of ring opening reactions, with the corollary that in the intermediates only relative stereochemistry need be considered. This will be demonstrated by the applications later in this chapter.

Benzocyclobutenes and their derivatives have been prepared by a variety of methods. The area has been extensively reviewed.[8] The major methods and examples of each method follow:

1. Electrocyclizations

This method was the earliest used to generate benzocyclobutenes.[2] The tetrabromoxylene 7 was treated with excess sodium iodide in refluxing ethanol for two days to give the benzocyclobutene 8. (Scheme 3)

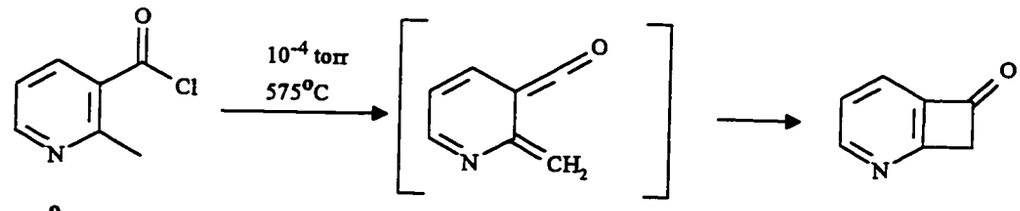
Scheme 3



7 8

Benzocyclobutenone has been prepared via the flash vacuum pyrolysis of o-toluoyl chloride.[9] A recent example illustrates this same method to prepare the pyridine analogue of benzocyclobutenone, **10**.[10] (Scheme 4)

Scheme 4

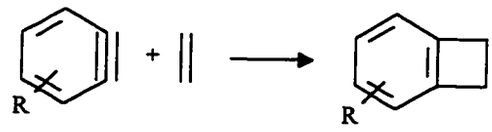


9 10

2. [2+2] cyclization of benzynes with alkenes

In order to prepare benzocyclobutanes via a [2+2] method, the appropriate benzyne must be generated in the presence of an alkene. (Scheme 5)

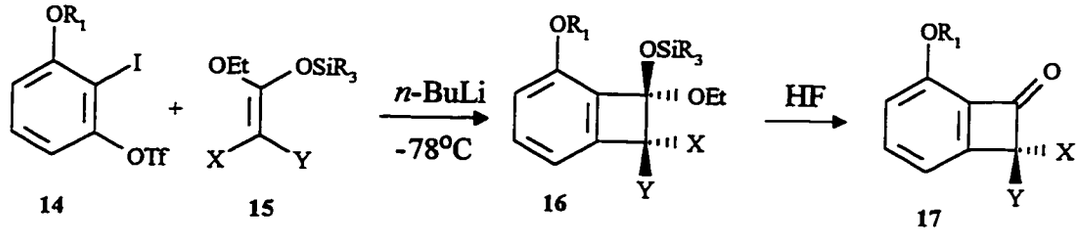
Scheme 5



11 12 13

Suzuki[11,12] is the most recent to report the reaction of benzynes with ketene silyl acetal as a route to benzocyclobutene derivatives. (Scheme 6) The benzyne is generated from the appropriate ortho-iodo triflate **14** in the presence of the ketene silyl acetal **15**.

Scheme 6



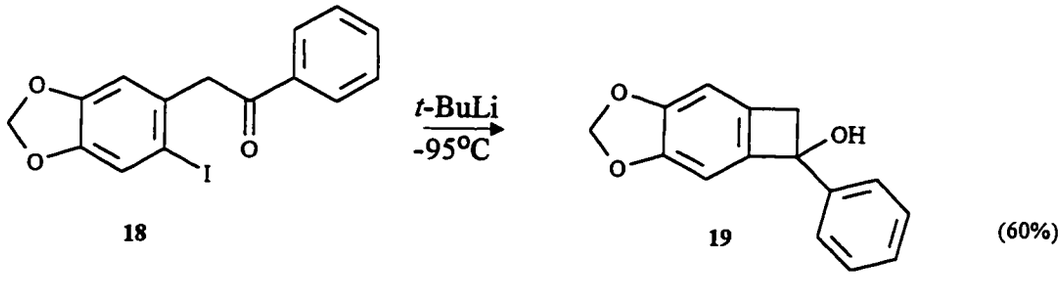
14 15 16 17

In this case, X and Y were either H, OR, or alkyl groups. In the case where both X and Y were OEt, hydrolysis yielded the corresponding benzocyclobutenedione. The regiochemical outcome results from the polarization of the aryne by the inductive electron withdrawing effect of the alkoxy group which directs the nucleophilic attack of the ketene silyl acetal.

3. Cyclizations

When an appropriately chosen ortho-halo aryl compound is treated with t-BuLi at low temperature, it is possible to isolate benzocyclobutene and its derivatives. For example, when the ortho-iododeoxybenzoin **18** is treated with *t*-BuLi at -95°C, the result is the aryl benzocyclobutenol **19**.[13] (Scheme 7)

Scheme 7

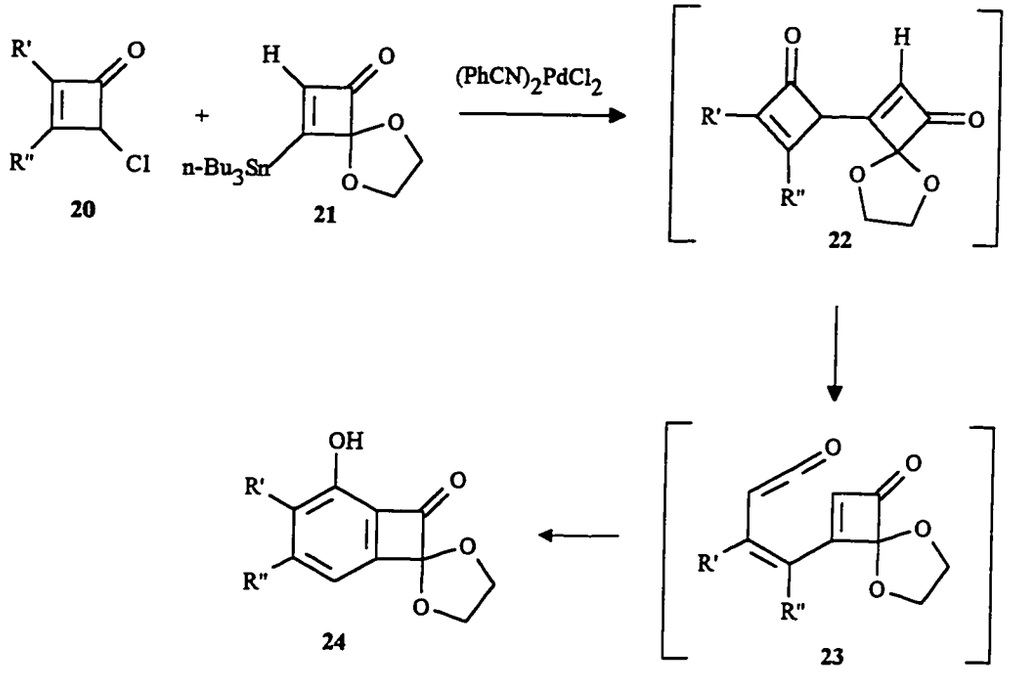


18 19 (60%)

4. Ring expansion

Liebeskind has recently developed a method to generate benzocyclobutenone derivatives by a Stille coupling of cyclobutenones followed by rearrangement.[14] (Scheme 8) This route provides a pathway towards substituted benzocyclobutenedione monoacetals such as **24**. This reaction proceeds via the coupled intermediate **22** which opens to the ketene intermediate **23**. Predictably, the cyclobutene ring with donor groups opens preferentially. The double bond rotates inward to facilitate ring closure.

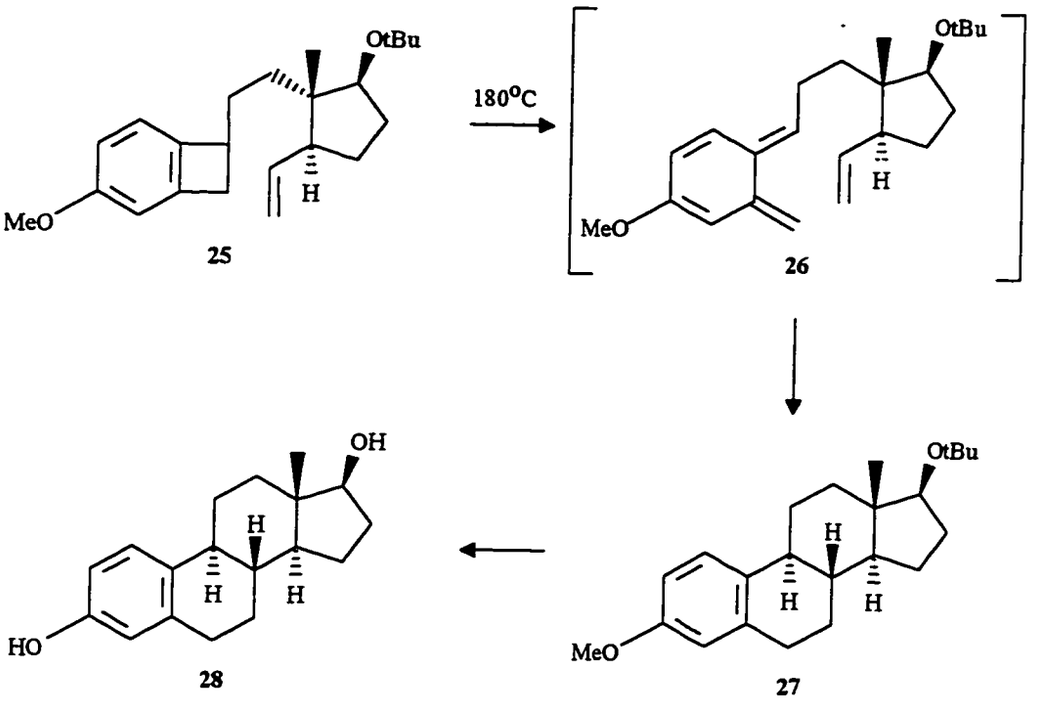
Scheme 8



Benzocyclobutenes in Synthesis

Benzocyclobutenes and their derivatives have been used to synthesize many polycyclic natural and non-natural products. An early very successful application of this method is Kametani's synthesis of estradiol **27**.[15] (Scheme 9) Thermolysis of **25** yielded **26** via an outward rotation of the substituent on the cyclobutene ring. Diels Alder reaction of **26** afforded only **27**.

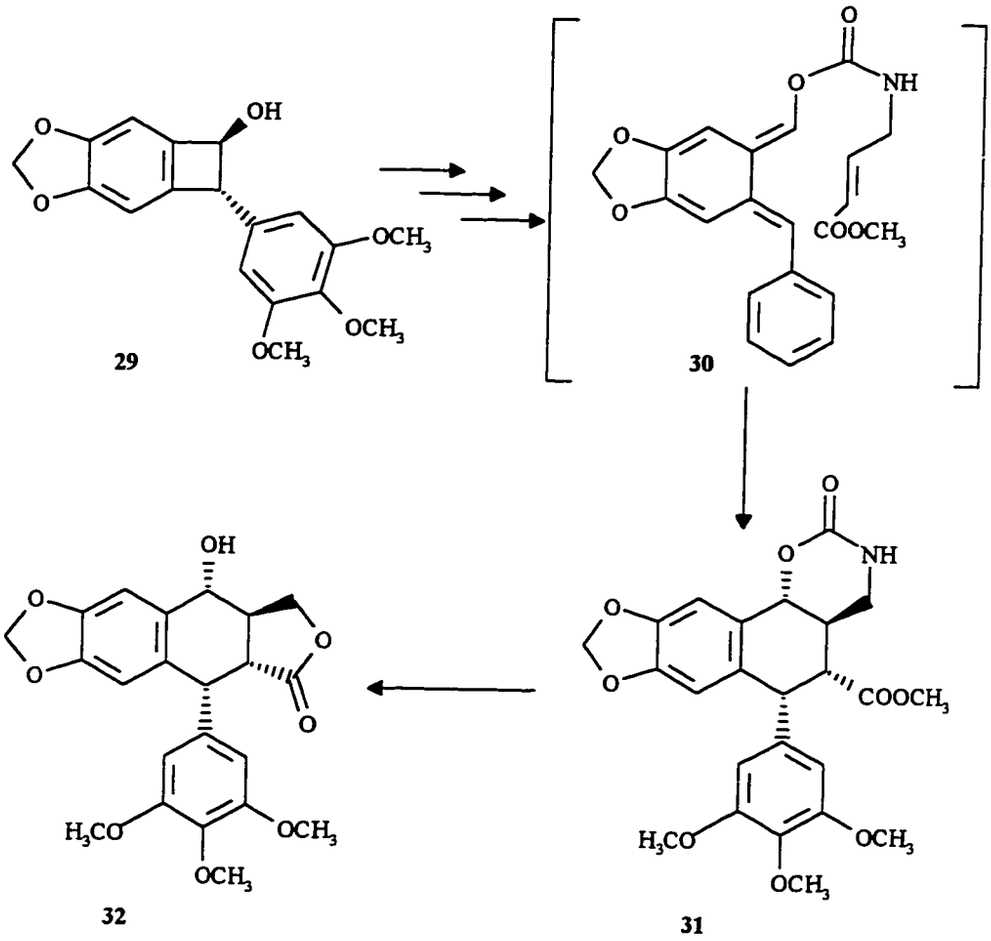
Scheme 9



Another example involoved the use of benzocyclobutenols is the synthesis of podophyllotoxin. [16] (Scheme 10) Intramolecular reaction of the appropriately tethered

dienophile with the thermally generated orthoquinodimethane in **30** gave rise to the correct stereochemistry at the four stereocenters on the C-ring of podophyllotoxin **32**. It should be noted that the stereochemistry of podophyllotoxin **32** represents one of the thermodynamically least favourable configurations.

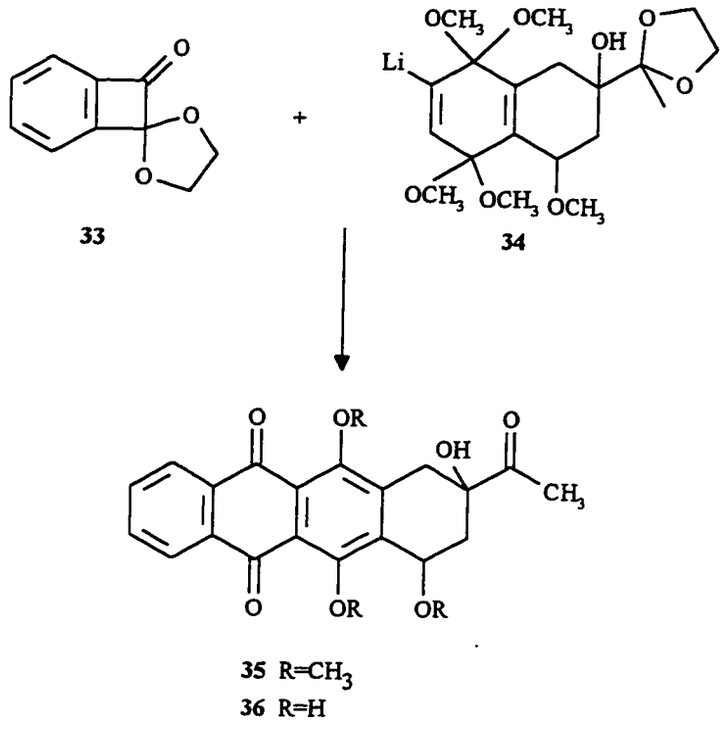
Scheme 10



Benzocyclobuten-1,2-diones have also been used as precursors to anthraquinones.[17] In 1979, Swenton used the benzocyclobutenedione monoacetal **33** as a

precursor for the efficient synthesis of 4-demethoxydaunomycinone **36**. (Scheme 11) The lithiated species **34** is added to the unprotected ketone of the benzocyclobutenedione, which opens to form an orthoquinodimethane. Electrocyclization and acid hydrolysis of the protecting groups afforded **36** in 63% yield for the two steps.

Scheme 11

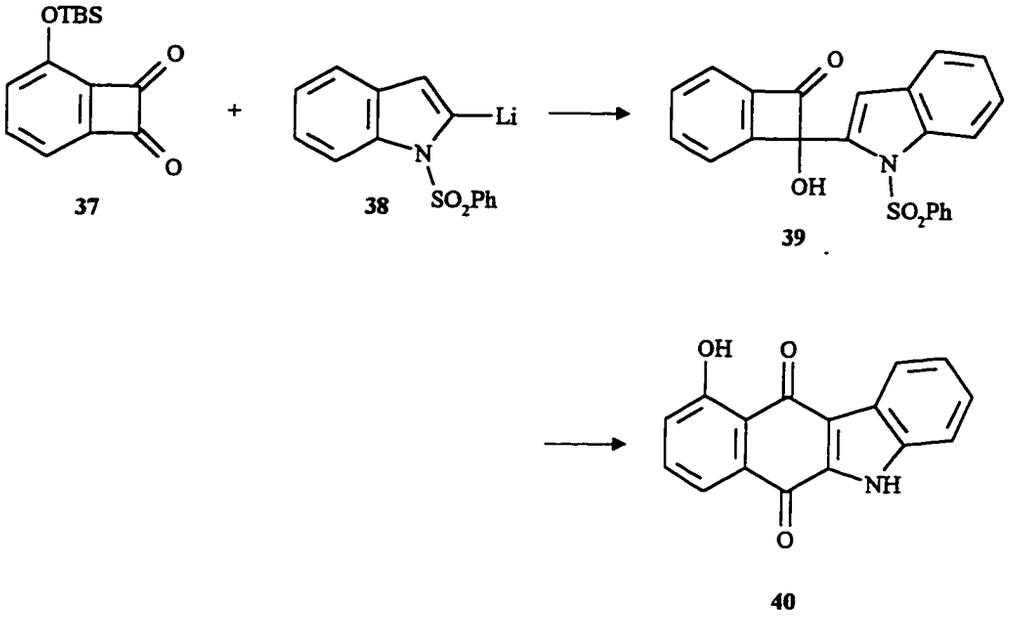


35 R=CH_3
36 R=H

Liebeskind has used also used benzocyclobuten-1,2-diones as precursors to other quinones.[18] (Scheme 12) Little selectivity toward one of the two carbonyl groups of the dione can be expected with a 'meta' substituted benzocylobuten-1,2-dione. However, a large ortho substituent, for example a t-butyldimethylsilyl group as in **37**, gives good regioselectivity. Thus, the addition of **38** to **37** led to a 13:1 mixture of regioisomers in

favour of attack at the less hindered carbonyl group giving the intermediate **39** which was subsequently thermolyzed to **40**.

Scheme 12

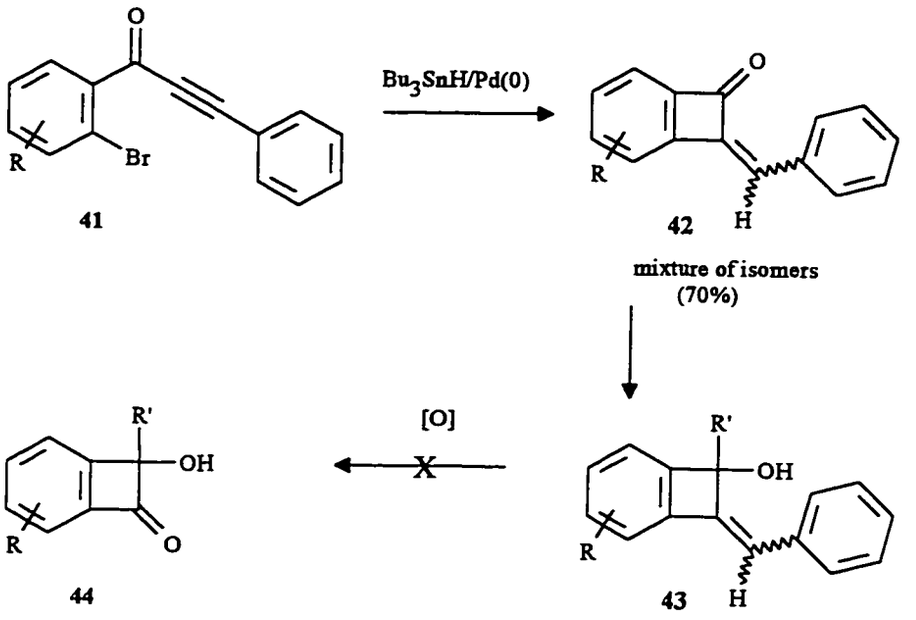


Chapter 2 Synthesis of 2-Hydroxybenzocyclobutenones

The first chapter showed that benzocyclobutenes and their derivatives are valuable intermediates in organic synthesis. It was also illustrated that benzocyclobutenedione monoacetals are useful intermediates in forming polycyclic natural products. A method was needed which would allow access to substituted benzocyclobutenediones with one of the carbonyl moieties masked to allow differentiation between the two carbonyl groups of those compounds which were not symmetrically substituted. Ideally, the method would allow for addition at one of the carbonyl groups, followed by unmasking of the second carbonyl group, and further transformation if necessary.

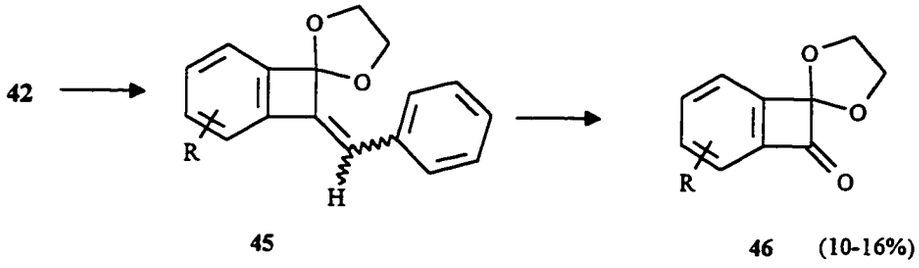
Bradley and Durst envisaged that the 2-benzylidenebenzocyclobutenone **42** might serve as an unsymmetrical benzocyclobuten-1,2-dione equivalent.[19] (Scheme 13) They showed that palladium(0) catalyzed cis addition of tributyltin hydride to the acetylenic ketone **41** followed by a Stille coupling of the intermediate vinyl stannane-aryl bromide afforded **42** in acceptable yields. Addition of organolithiums or Grignard reagents occurred exclusively by a 1,2 pathway to give **43**. The usefulness of **43** as a synthetic intermediate suffered greatly since **43** was oxidized to **44** in only poor yields. Furthermore, fragmentation of **43**, prior to oxidation of the benzylidene group to a carbonyl function, led to a variety of ring enlargement products.

Scheme 13



The possibility of first protecting the ketone in **42** as a ketal to give **45**, then ozonolyzing the benzylidene group to reveal the other carbonyl group to give **46** was also investigated, but unfortunately the latter step proceeded in only poor yield. (Scheme 14)

Scheme 14



Since it had already been shown that benzocyclobutenols could be made by halogen metal exchange, it was envisioned that this method could be applied to the

synthesis of benzocyclobutenones as well. It was necessary to generate a substrate which had a halogen ortho to a "functionalized arm" containing two oxygen functionalities; one as an ester to serve as an electrophile in the cyclization process and the other to generate the benzocyclobutenone. (Scheme 15) The chosen method should tolerate a variety of substituents on the aromatic ring, in particular methoxy and methylenedioxy groups. This was especially important since many of the targets envisioned contained a number of such oxygenated functions. The following route, if successful, should satisfy these criteria and yield specifically substituted 2-hydroxybenzocyclobutenones or precursors.

Scheme 15

47 48

P=protecting group, L=leaving group, X=halogen

Results

Piperonal 49 was treated with bromine in acetic acid to give the known 6-bromo-piperonal 50.[20] (Scheme 16) In order to obtain the chain extension and the functionality shown in 47, the *ortho*-bromobenzaldehyde was converted to the cyanohydrin 51. Several methods were used to obtain the cyanohydrin. These included conversion of the aldehyde to a bisulfite adduct using sodium bisulfite followed by displacement using cyanide ion.

This method had the advantage that the bisulfite adduct is water soluble, so that the low solubility of the cyanide ion in organic solvent is not a problem. However, the method adopted was the formation of the trimethylsilyl protected cyanohydrin using trimethylsilyl cyanide.[21] This method involved simply adding the reagent to the aldehyde in dichloromethane with catalytic zinc iodide and stirring at room temperature for one hour. The reaction was reproducibly quantitative, and the product was isolated by evaporating the solvent. The zinc iodide may be removed by quickly passing the reaction mixture through a short silica plug, but this proved unnecessary in most cases. The ^{1}H NMR of the compound showed the complete disappearance of the aldehyde proton at 10 ppm, with the appearance of a benzylic proton at 5.67 ppm, and a singlet at 0.23 ppm corresponding to the nine hydrogen atoms of the trimethylsilyl group. (Figure 2)

The trimethylsilyl cyanohydrin was heated in ethanol containing HCl to yield the corresponding hydroxy ester **52**. Of the various reaction conditions used which included *p*-toluenesulfonic acid in methanol or ethanol, sulfuric acid in ethanol, or hydrochloric acid in ethanol, hydrochloric acid in ethanol gave the highest yield of the desired ethyl ester. Prolonged reflux, more than 3 hours, led to lower yields. The ^{1}H NMR spectrum showed clean conversion to product in most cases. The trimethylsilyl singlet at 0.23 ppm had disapperared, the ethyl ester showed a triplet at 1.22 ppm as a doublet of quartets at 4.21 ppm. (Figure 3) The benzylic proton shifted to 5.47 ppm. ^{13}C NMR spectroscopy showed the carbonyl signal at 173.3 ppm. (Figure 4) The IR spectrum showed peaks at 1731 cm^{-1} for the ester carbonyl group and 3528 cm^{-1} for the hydroxy group. HRMS gave the expected mass for compound **52**.

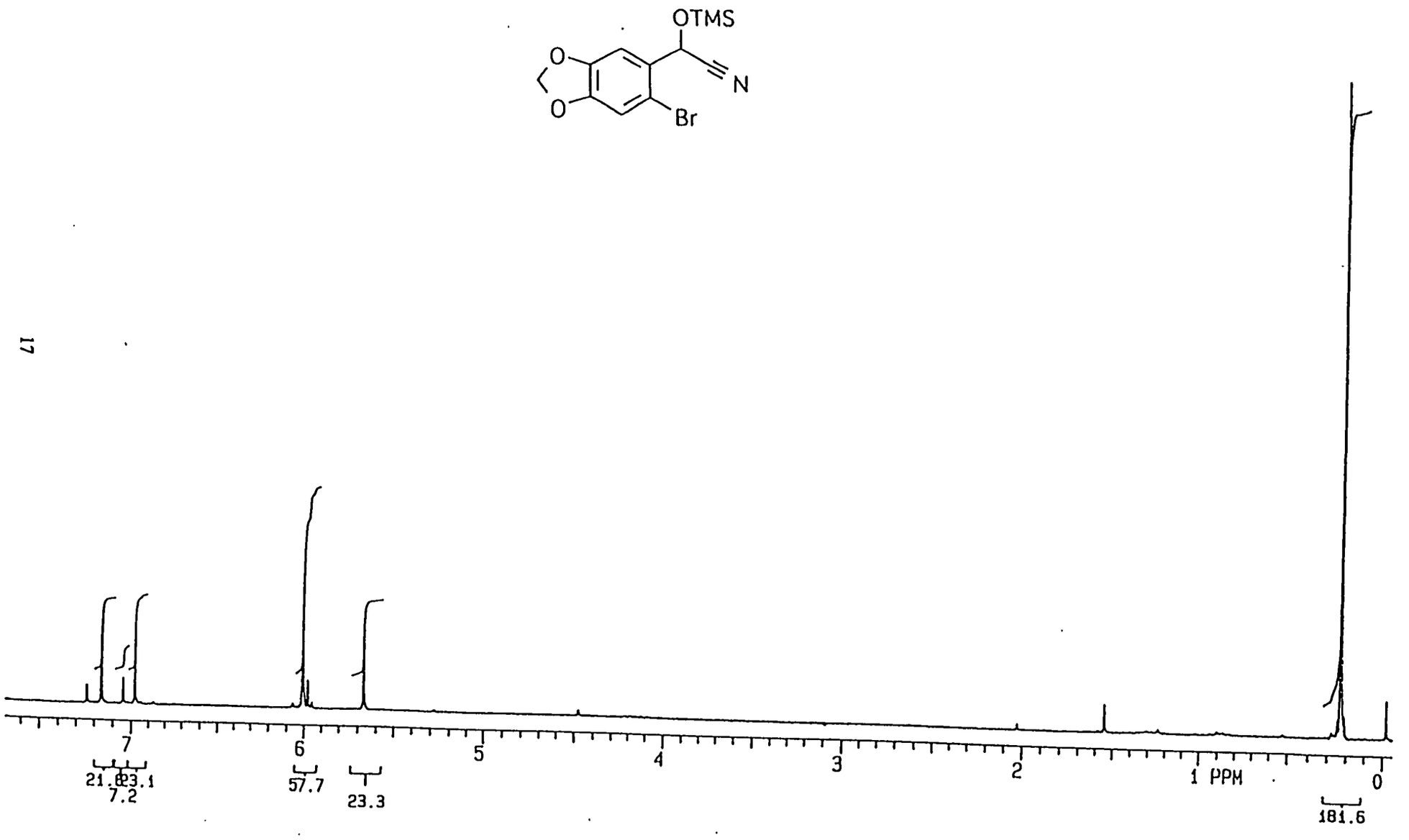


Fig. 2: ^{1}H NMR spectrum of **51**

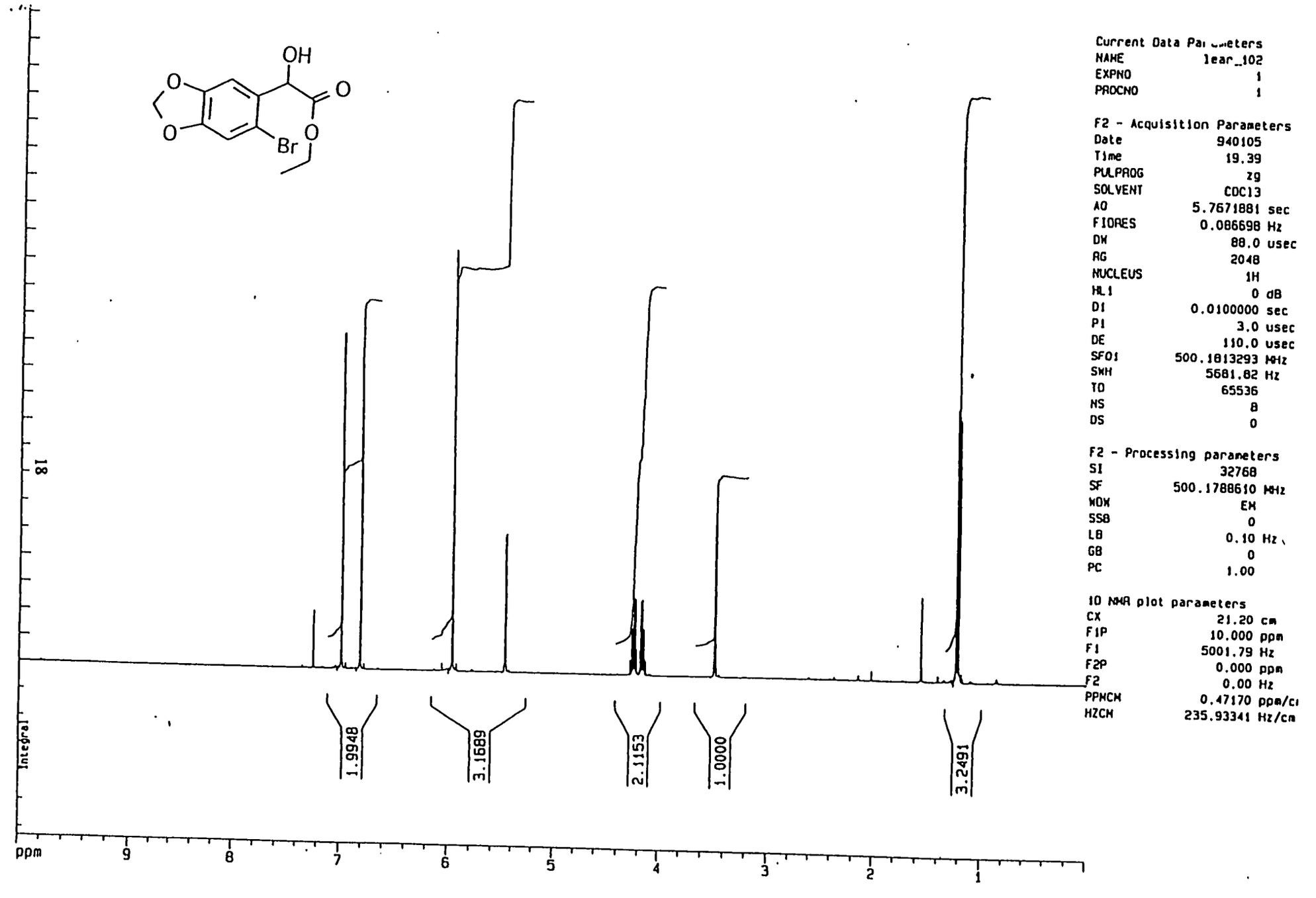


Fig. 3: ^{1}H NMR spectrum of **52**

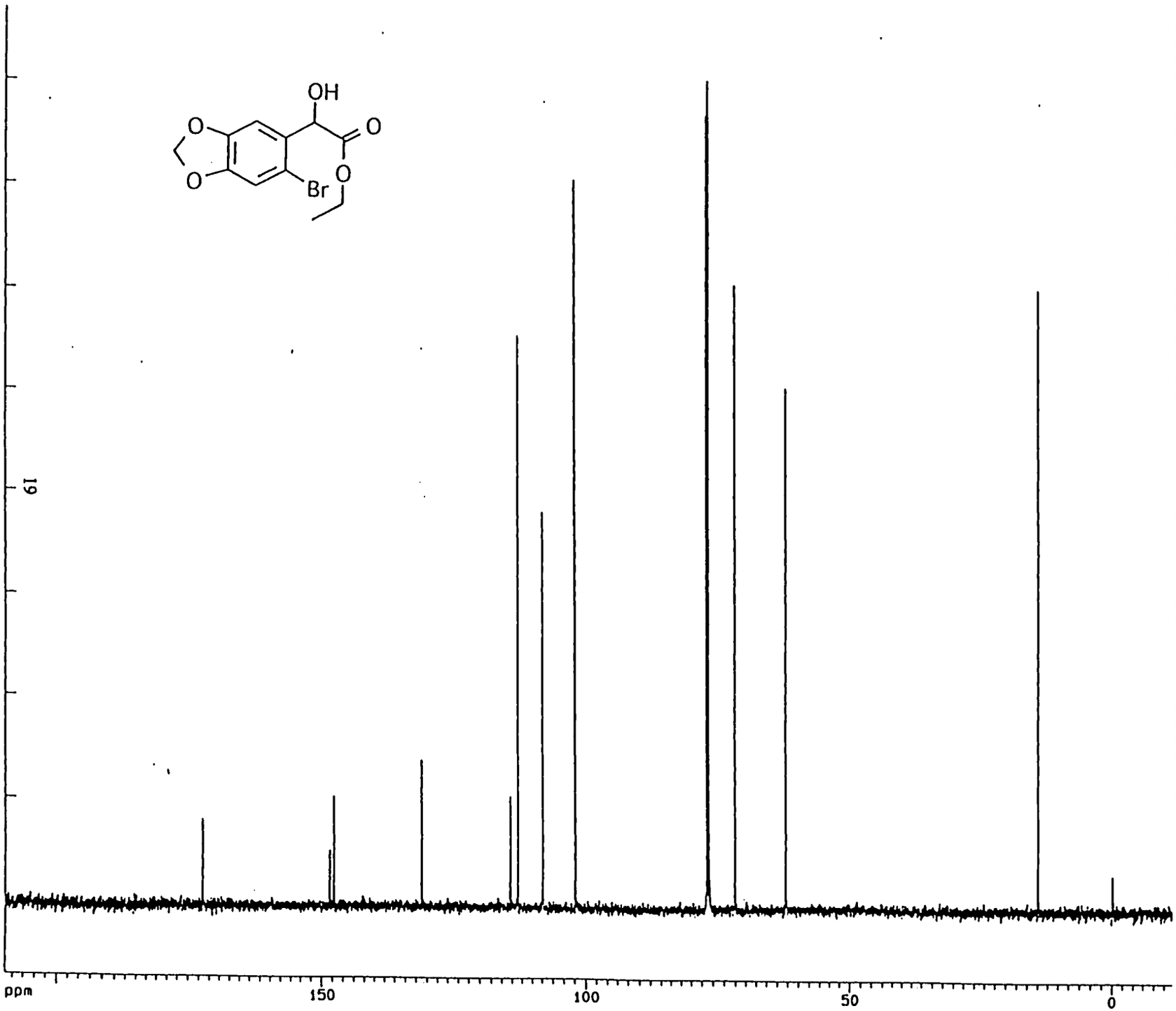


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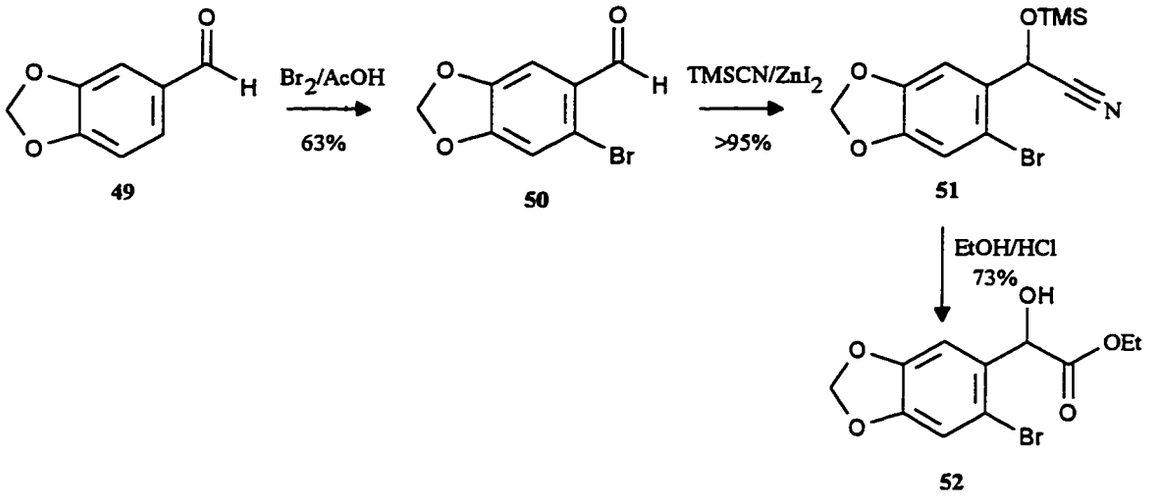
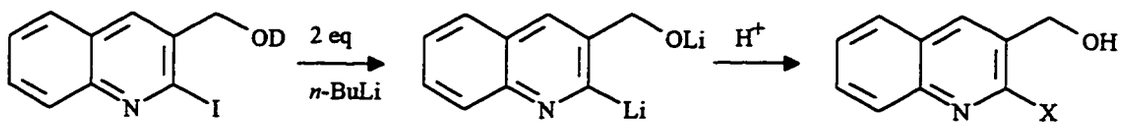
Fig. 4: ^{13}C NMR spectrum of 52

Scheme 16



Beak found that when a compound with an acidic hydrogen, such as a hydroxy proton, underwent halogen metal exchange, the acidic proton is generally removed before halogen metal exchange takes place.[22] (Scheme 17) When 2-iodo-3-(deuterioxymethyl) quinoline was treated with two equivalents of *n*-butyllithium at -78°C, stirred for 10 minutes and then quenched with methanol, both **55** and **55a** were isolated. The compound **55** was formed when the dianion **54** was quenched with methanol. The compound **55a** was formed when the dianion abstracted a deuterium from another molecule of **53**. Based on the relative amounts of **55** and **55a** formed under various conditions, it was concluded that deprotonation occurred before halogen-metal exchnage. In light of Beak's results, the hydroxy ester **52** was protected using *t*-butyldimethylsilyl chloride in DMF using imidazole as base and **56** was isolated in 87% yield. (Scheme 18)

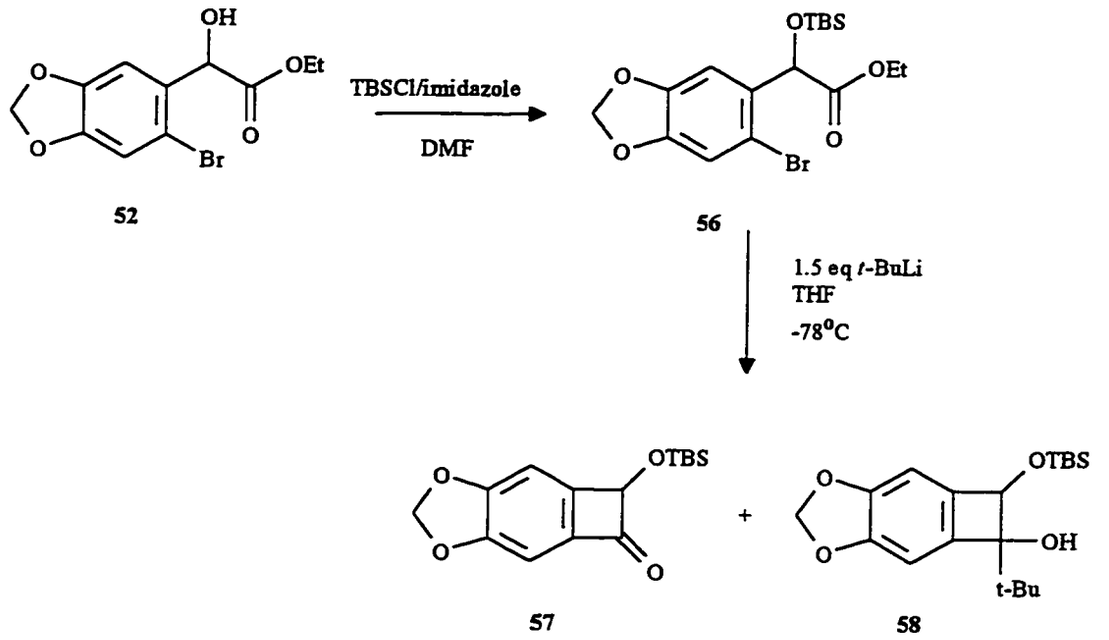
Scheme 17



53 54 55 X=H 55a X=D

Halogen metal exchange reactions are generally carried out using two equivalents of butyllithium, one to do the exchange reaction and the other is consumed as base causing an elimination from the butyl bromide formed from the reaction.[23] When the protected ester **56** was treated with two equivalents of *t*-butyllitium at -78°C, the desired benzocyclobutenone **57** was formed, but the tertiary alcohol **58**, resulting from addition of *t*-butyllithium to **57**, was also formed. With 1.1 equivalents of *t*-BuLi, ^{1}H NMR spectroscopy showed approximately 60% of the starting material had been converted to the desired benzocyclobutenone. The use of 1.5 equivalents of *t*-butyllithium at -78°C for fifteen minutes followed by quenching at the same temperature led to a 95% yield of the desired benzocyclobutenone. The product was purified by silica gel chromatography when the reactions produced mixtures, or simply by recrystallization from hexanes/ ethyl acetate when the reaction went cleanly. The ^{1}H NMR spectrum showed the disappearance of the ethyl group of the ester and the shift of the benzylic proton to 5.56 ppm. (Figure 5) The ^{13}C NMR spectrum showed the ketone at 187.5 ppm, while the IR spectrum showed the ketone at 1757 cm^{-1}, values which are similar to those of known benzocyclobutenones.[19] (Figure 6) HRMS and combustion analysis also agreed with the structure of the hydroxybenzocyclobutenone.

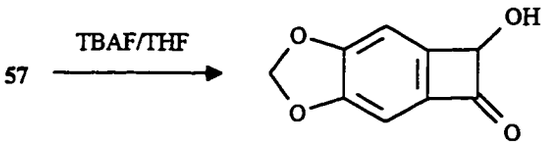
Scheme 18



52 56 57 58

It was found that the hydroxybenzocyclobutenone could be deprotected using tetrabutylammonium fluoride in THF in 80% yield to afford 59. (Scheme 19) With the conversion of the *o*-bromobenzaldehyde **49** to the 2-hydroxybenzocyclobutenone **59** accomplished, the next step was to extend the method to other *ortho*-bromobenzaldehydes to determine the scope of the sequence.

Scheme 19



59

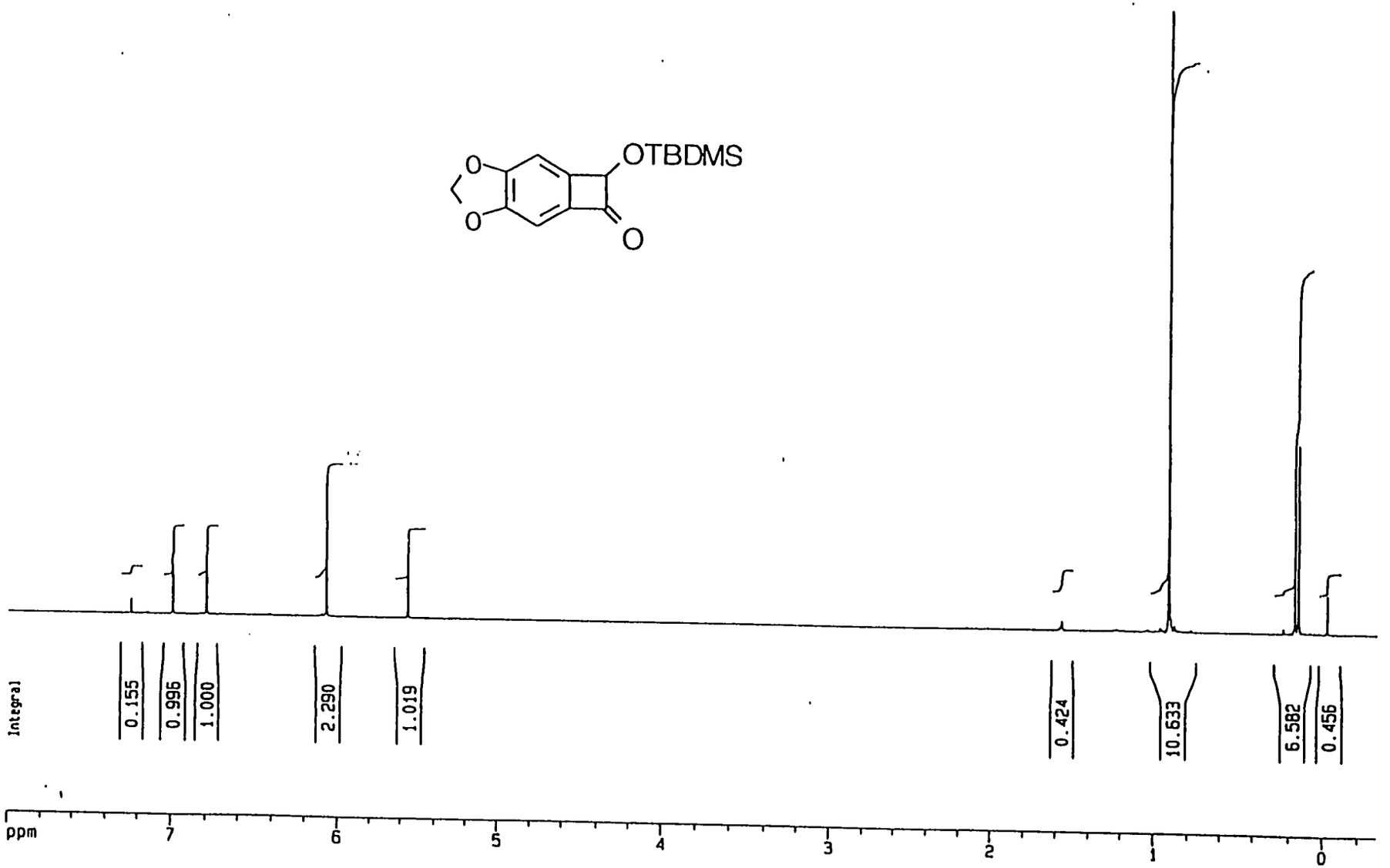


Fig. 5: ^{1}H NMR spectrum of **57**

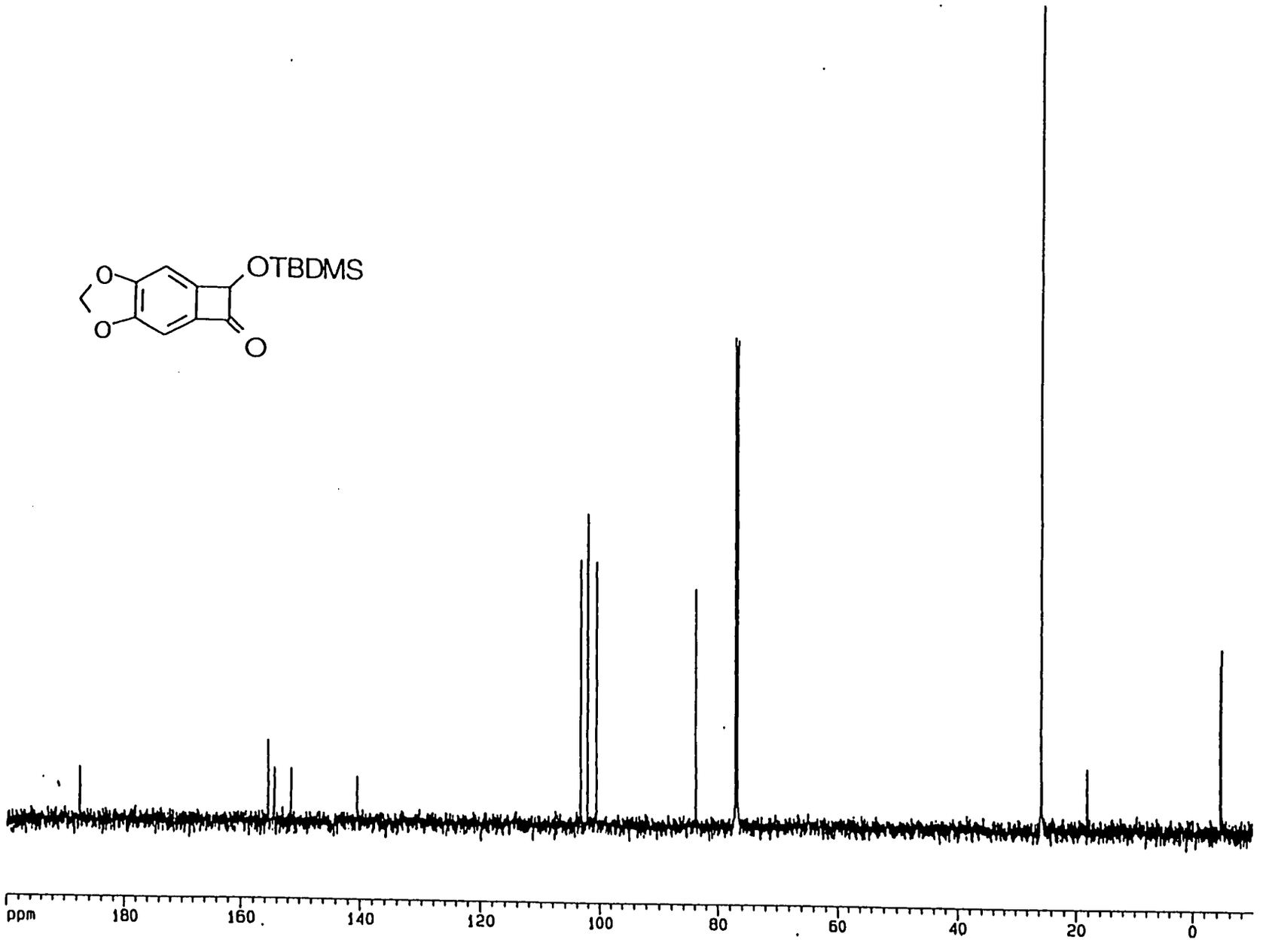


Fig. 6: ^{13}C NMR spectrum of **57**

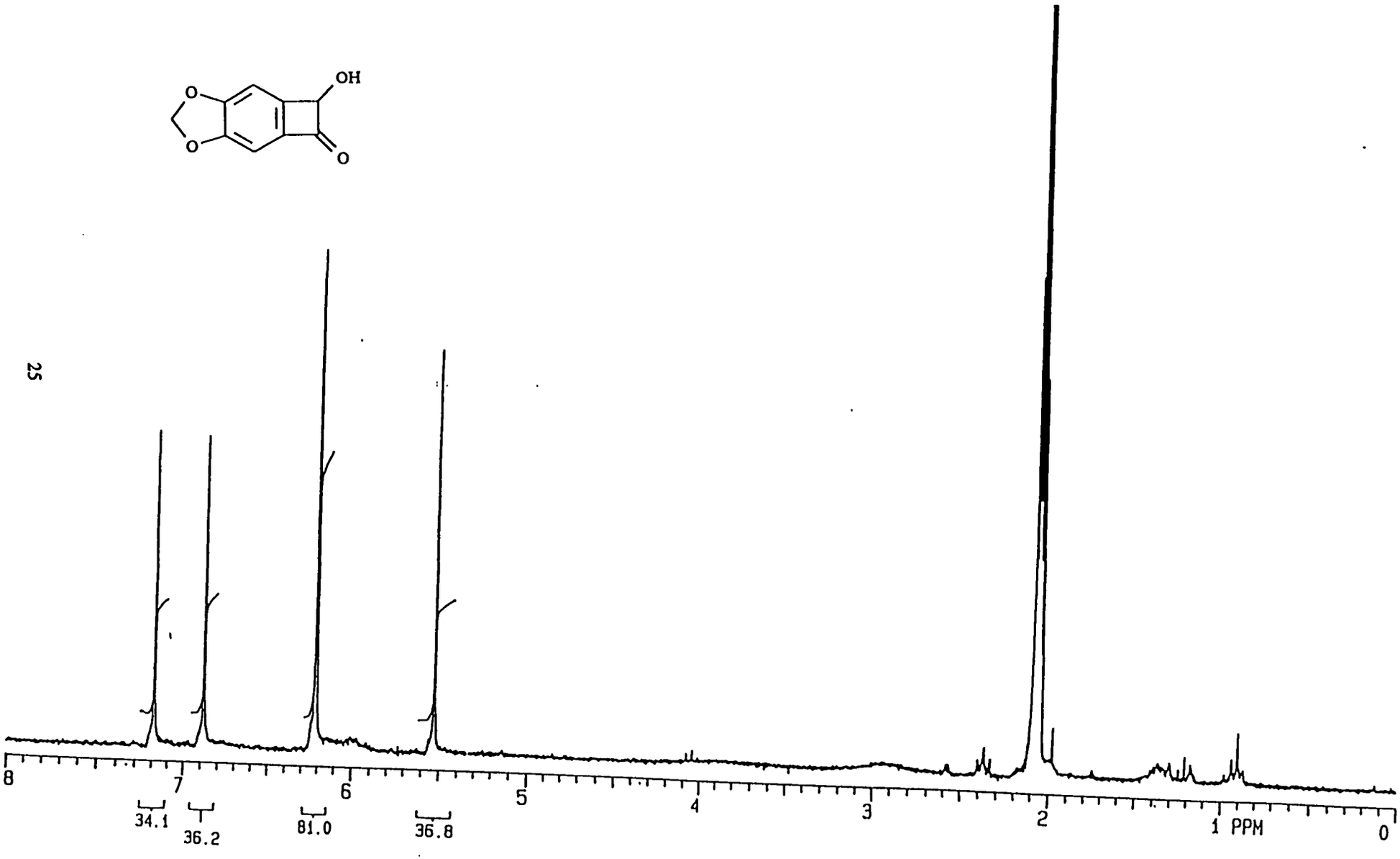


Fig. 7: ^{1}H NMR spectrum of 59

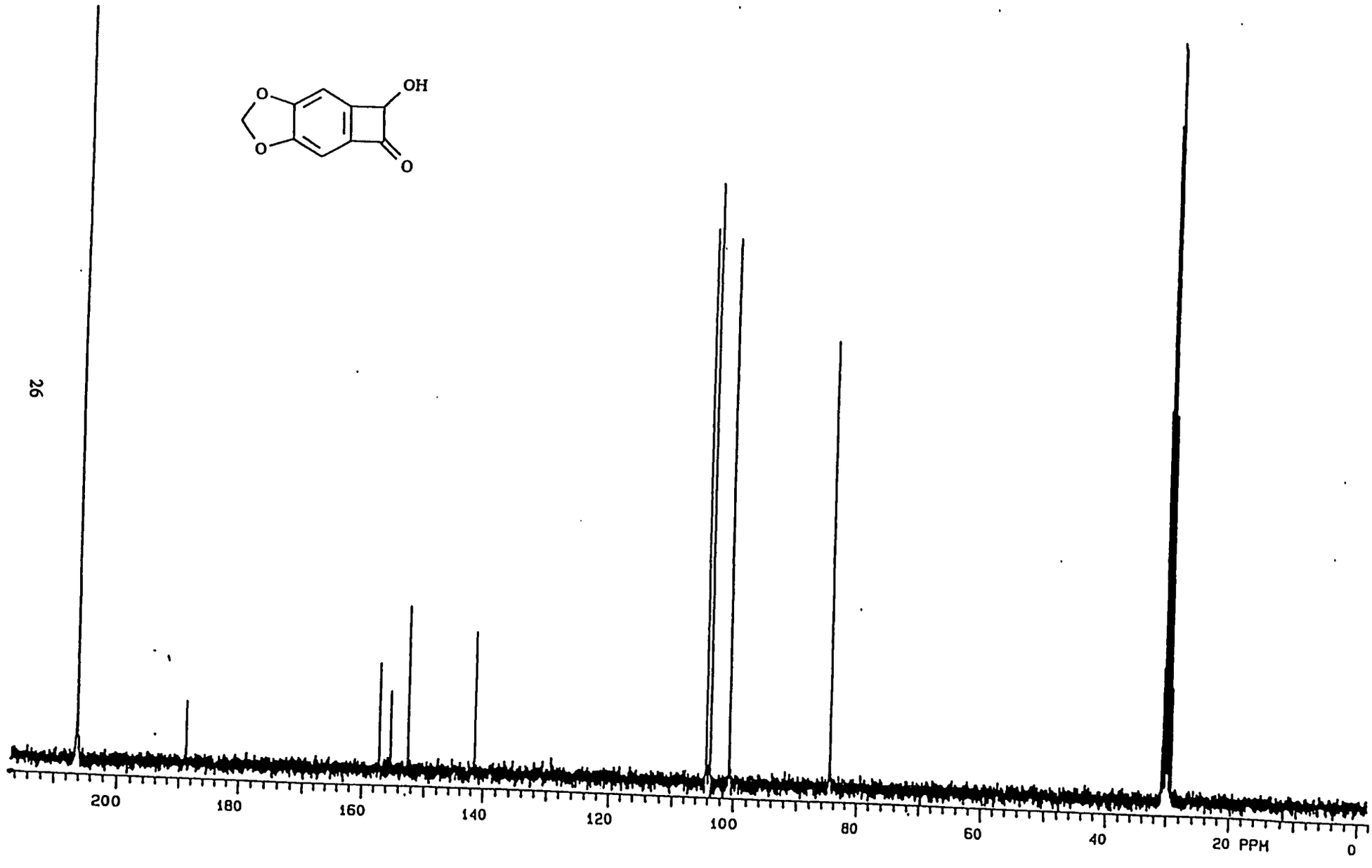
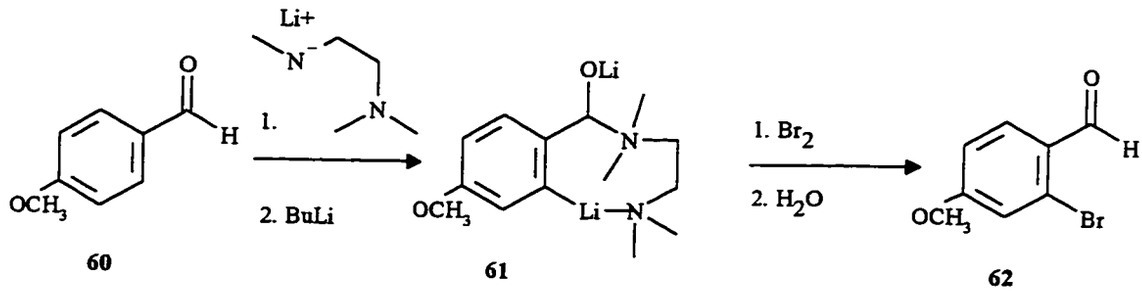


Fig. 8: ^{13}C NMR spectrum of 59

The starting materials used were 2-bromobenzaldehyde, *m*-anisaldehyde, *p*-anisaldehyde, 3-bromophenol, and 3-thiophenecarboxaldehyde, all of which were commercially available. *m*-Anisaldehyde was brominated in the 2 position using the same method as for piperonal, bromine in acetic acid. *p*-Anisaldehyde and 3-thiophenecarboxaldehyde were brominated using Comins' procedure.[24,25] (Scheme 20) *p*-Anisaldehyde **60** was treated with the anion of trimethylethylenediamine, followed by *n*-butyllithium to give the stabilized ortho-lithio derivative **61**. Quenching of a solution of **61** with a source of bromine, in the present case carbon tetrabromide, gave a moderate 54% yield of **62**. Iodination of 3-thiophenecarboxaldehyde using the same method, but quenching with molecular iodine, afforded 2-iodo-3-thiophenecarboxaldehyde in 25% yield.

Scheme 20



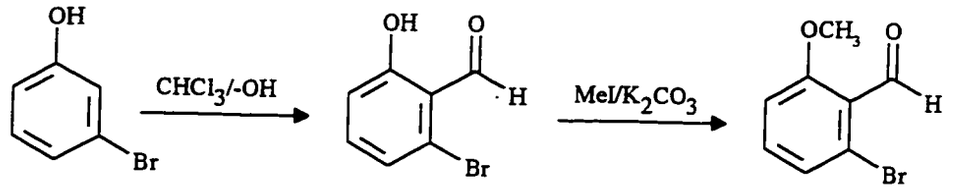
60 **61** **62**

Since the same sequence can not be carried out on *ortho*-anisaldehyde due to the instability of the intermediate *ortho*-lithiated species **63**,[25] *m*-bromophenol **64** was formylated to give the necessary compound.[26] Formylation of phenols by most methods

affords the *para*-hydroxyphenol. The method chosen, the Reimer-Tiemann reaction,[27] involves the generation of dichlorocarbene which adds preferentially *ortho* to the aldehyde. (Scheme 21) The best yield of **65** obtained when this method was applied to *m*-bromophenol was about 12%, however it allowed access to the compound necessary for the study. The phenol was methylated to afford **66** in >95% yield using potassium carbonate and methyl iodide in acetone.

63

Scheme 21



64 **65** **66**

All of the *ortho*-bromobenzaldehydes were converted in >95% yield to the corresponding trimethylsilyl protected cyanohydrins **70-74** using trimethylsilyl cyanide as previously described. (Scheme 22, Table 2) However, the reactivities of the substrates toward ethanolysis varied somewhat. When the cyanohydrin **73** from 2-bromo-4-

methoxybenzaldehyde **62** was reacted under the conditions previously described for the piperonal system, a 1:1 mixture of the hydroxy ester **78** and the ethoxy ether ester **78a** was formed. (Scheme 23) This difference in reactivity may be due to the methoxy group *para* to the benzylic alcohol, which makes the benzylic hydroxy group susceptible to exchange by the ethanol solvent. This reaction was suppressed by using hydrogen chloride gas bubbled through anhydrous ethanol instead of concentrated aqueous hydrochloric acid. This led to the exclusive formation of the desired hydroxy ester in 87% yield. The formation of the other hydroxy esters was carried out using aqueous acid with yields ranging from 60-90%. (See Experimental Section)

Scheme 22

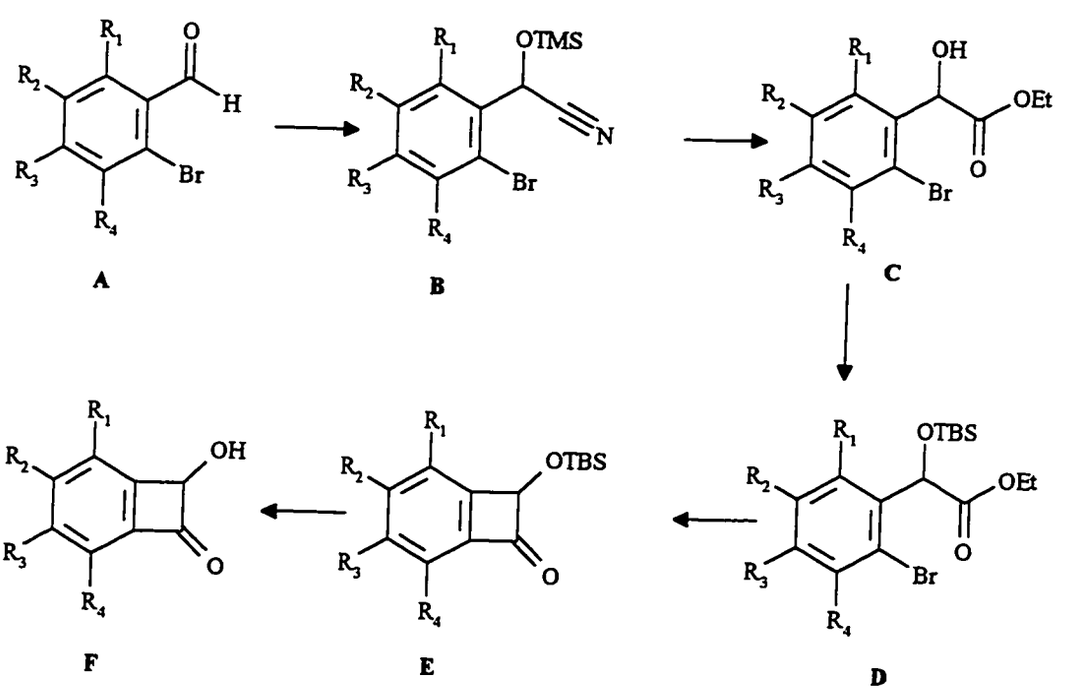
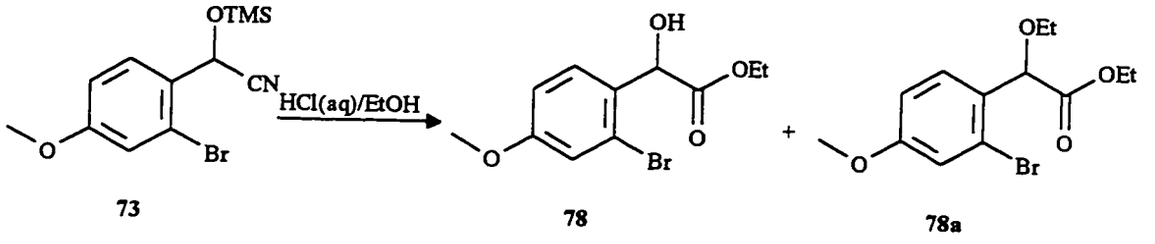


Table 2 Structure numbers for benzocyclobutenone synthesis

R_1	R_2	R_3	R_4	A	B	C	D	E	F
H	H	H	H	67	70	75	80	85	90
OCH_3	H	H	H	66	71	76	81	86	91
H	OCH_3	H	H	68	72	77	82	87	92
H	H	OCH_3	H	62	73	78	83	88	93
thiophene	(see	below)		69	74	79	84	89	-

Scheme 23



73 78 78a

The α-hydroxy esters were protected as *t*-butyldimethylsilyl (TBS) ethers using either TBSCl with imidazole as base, or *t*-butyldimethylsilyl trifluoromethanesulfonate with 2,6-lutidine as base. For the ethyl ester of 2-methoxy-*o*-bromomandelic acid, **76**, protection using TBSCl with imidazole was incomplete after three days, necessitating the use of the triflate, which gave **81** in >95% yield in thirty minutes.

With the protected esters in hand, the next step was to determine whether the cyclization reaction would work with the substrates chosen. The problem in optimizing

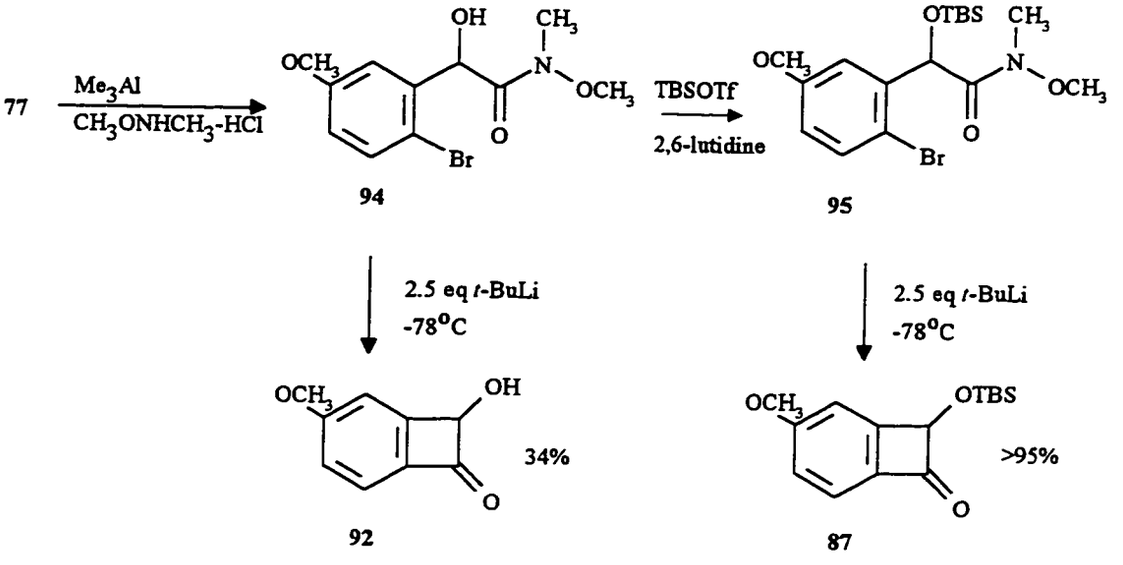
this reaction again was in maximizing the cyclization while minimizing the addition of t-butyllithium to the benzocyclobutenone.

The simplest case was the unsubstituted benzocyclobutenone. The intermediate **98** was treated with two equivalents of *t*-butyllithium at -78°C to afford the cyclized product in 90% isolated yield. Compounds bearing methoxy groups, however, required only 1.2-1.5 equivalents of *t*-butyllithium, as did the piperonal derivative. This difference can be attributed to the electron donating groups on the aromatic ring. The anion formed by halogen metal exchange in these compounds is more reactive, and therefore the cyclization reaction rate becomes competitive with the elimination of HBr from *t*-butyl bromide.

When cyclization reactions were carried out on mandelic esters **82** and **83** however, the ^{1}H NMR spectra of the crude reaction mixtures generally showed starting material, the desired benzocyclobutenone, and a third compound in which t-butyllithium had added to the benzocyclobutenone. This indicates that the addition of *t*-butyllithium to the benzocyclobutenone product begins before halogen metal exchange is complete. Slower addition of *t*-butyllitium and/or lowering the reaction temperature did not improve the selectivity. The reactions were optimized with yields in the 60-70% range. This problem was overcome by converting the mandelic ester **77** to the corresponding Weinreb amide **95**. When this ester was reacted with 2.5 equivalents of *t*-butyllithium at -78°C, the desired benzocyclobutenone **87** was produced in essentially quantitative yield. (Scheme 24) The amide **94** was produced in 75% yield using trimethylaluminum and N,O-dimethylhydroxylamine hydrochloride.[28,29] The ^{1}H NMR spectrum showed two new methyl singlets while the ^{13}C NMR spectrum showed the amide resonance at 172.7 ppm.

(Figures 9 and 10) The compound **94** was protected using TBS-triflate in the presence of 2,6-lutidine to afford the TBS ether **95** in quantitative yield. Attempts to shorten the sequence by direct cyclization of the unprotected hydroxy ester **94** using 2.5 equivalents of *t*-butyllithium at -78°C led to the cyclized product **92** in only 34% yield, along with four other side products which were not identified.

Scheme 24



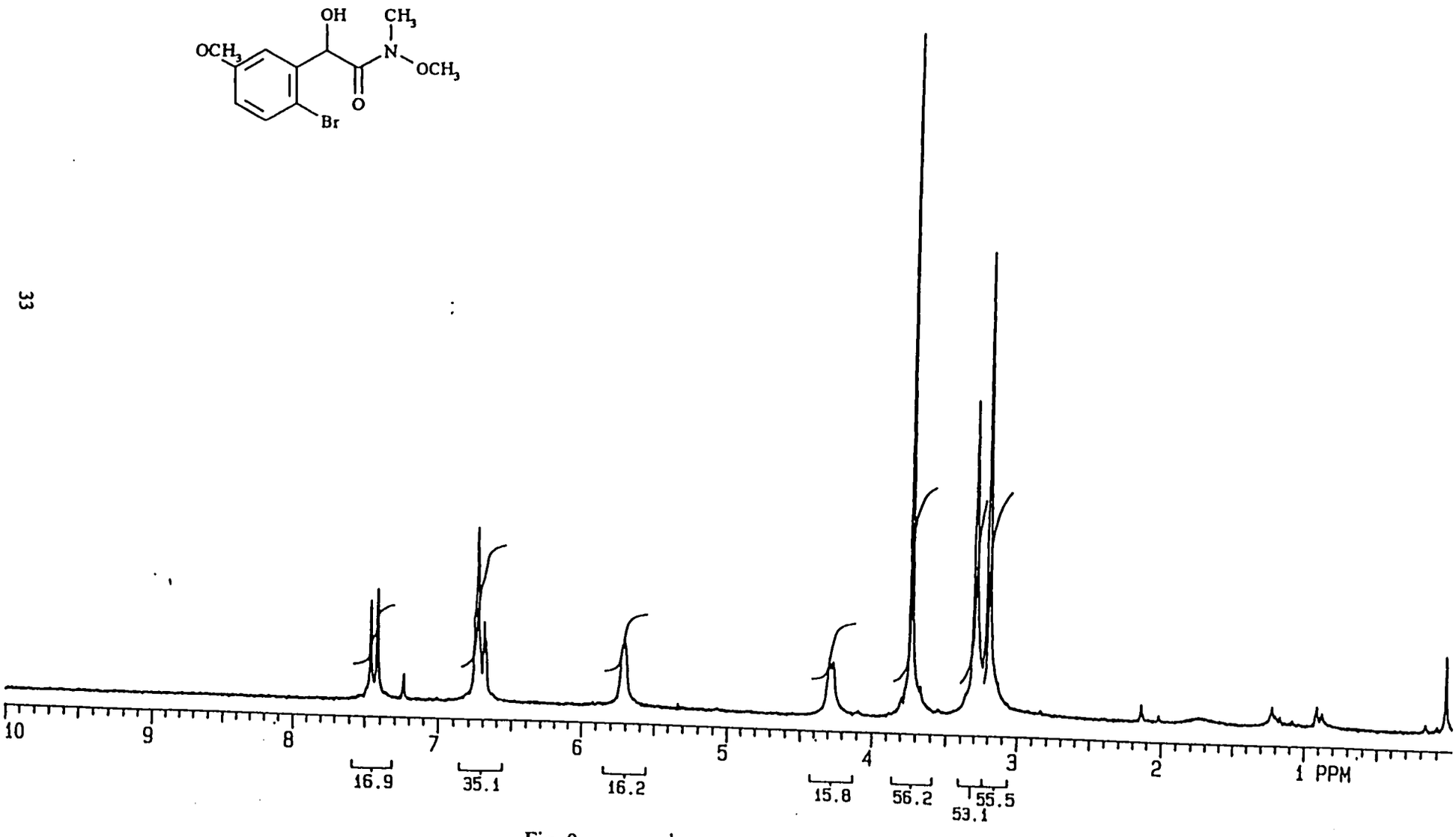


Fig. 9: ^{1}H NMR spectrum of **94**

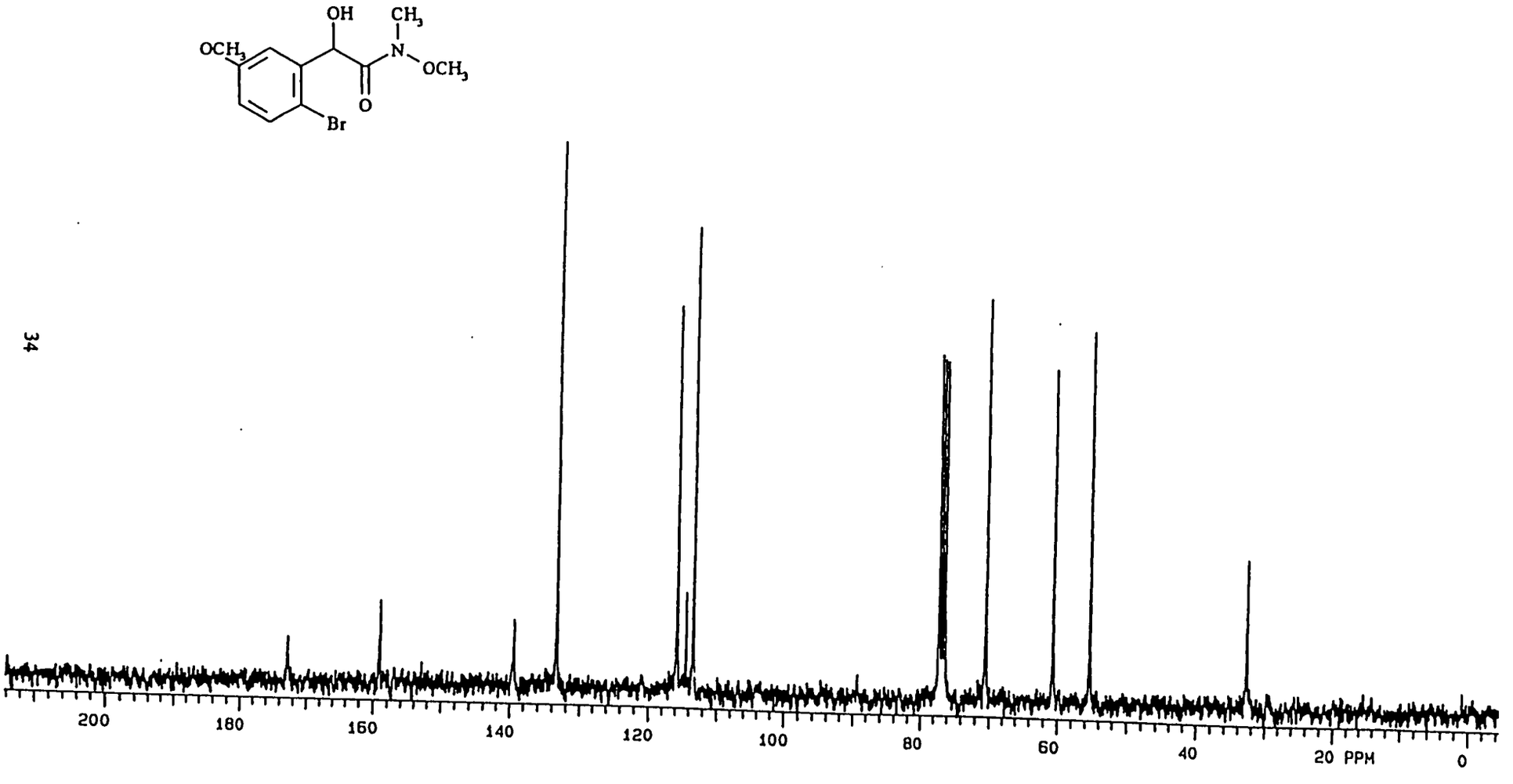


Fig. 10: ^{13}C NMR spectrum of 94

The synthesis of thiophene analogue **89** was also examined. (Scheme 25) While the formation of the intermediate ester **84** was straightforward, the cyclization step afforded none of the desired cyclobutenone **89**. Instead, only the compound **89a** was isolated. This indicated that while halogen metal exchange proceeded, cyclization was unsuccessful and the organolithium quenched on aqueous workup. This can be attributed to the geometrical differences between the benzene and thiophene rings. The distance between the lithium and the ester carbonyl would be greater in the thiophene than in the analogous benzene derivative. This can be seen by comparing the distances between the methyl carbons of *o*-xylene and 2,3 dimethylthiophene. (Figure 11) For the thiophene, the C(2)-C(3)-CH_3 bond angle is 123° and the C(3)-C(2)-CH_3 bond angle is 127°, compared to the C-C-CH_3 bond angle of 120° for *o*-xylene. This difference in bond angles translates into a greater distance between the two methyl groups in each compound when the distances are calculated using the known bond lengths for the two molecules. This difference in distance makes it more difficult to form a four membered ring fused to a thiophene rather than a benzene ring. A survey of the Ring Index in Chemical Abstracts revealed no 5,4 ring systems with an aromatic 5 membered ring, even though there are many examples with saturated systems.

The only step remaining was the deprotection of the TBS derivatives of the 2-hydroxybenzocyclobutenones using tetrabutylammonium fluoride in THF at 25°C and quenching immediately with saturated ammonium chloride solution. Yields for this step

ranged from 65-90%. For the benzocyclobutenone 93, it was necessary to add acetic acid to the mixture to effect the deprotection to avoid base catalysed ring opening.

Scheme 25

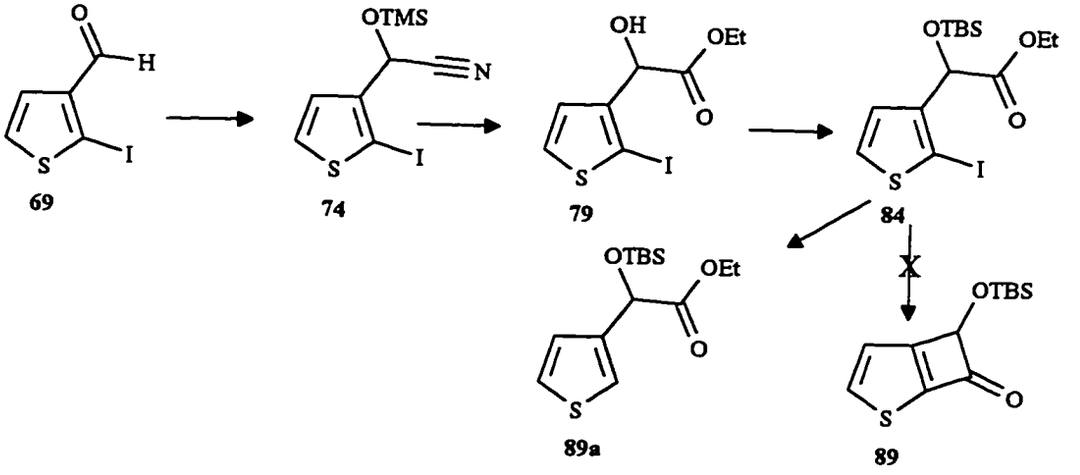
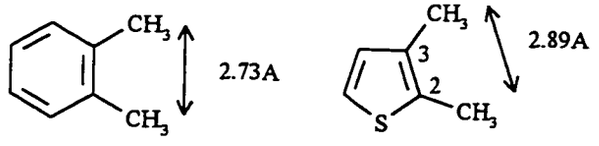
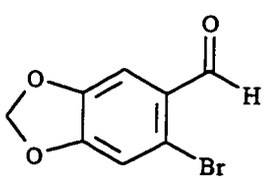


Figure 11



Experimental Section

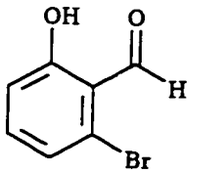
General: Tetrahydrofuran (THF) was freshly distilled from sodium and benzophenone before use. Hexanes and ethyl acetate used for chromatography and distillation were routinely distilled before use. Melting points were determined using a Thomas Scientific melting point apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on either a Varian Gemini 200, Varian XL-300 or Bruker AMX 500 spectrometer in deuterated chloroform unless otherwise stated. IR spectra were recorded on a Bomem Michelson FTIR as solutions in CH_2Cl_2, then the solvent spectrum was subtracted.



50

The literature procedure was carried out as follows: Piperonal (15 g, 0.10 mol) was stirred with 100 mL glacial acetic acid and bromine (6 mL, 0.12 mol) for 18 h. Water (300 mL) was added to precipitate the product. The solid was collected on a Buchner funnel and recrystallized from ethanol as colorless needles of **50** (14.3 g, 63 mmol, 63% yield).

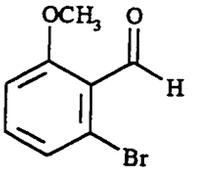
mp 126-128°C (lit[20]127-129°C) **^{1}H NMR:** δ 6.10 (s, 2H), 7.13 (s, 1H), 7.35 (s, 1H), 10.15 (s, 1H).



66a

A mixture of m-bromophenol (5 g, 29 mmol), calcium hydroxide (14 g, 0.19 mol), sodium carbonate (16 g, 0.13 mol), and 100 mL water was stirred and heated to 70°C. Chloroform (7 mL, 10.4 g, 87 mmol) was added at a rate such that a gentle reflux was maintained, over about 90 minutes. Stirring was continued for 2 h. The mixture was acidified with concentrated sulfuric acid, extracted with CH_2Cl_2, dried with $MgSO_4$ and the solvent evaporated. Chromatography on silica gel (9:1 hexane/ethyl acetate) gave **66a** (660 mg, 3.3 mmol, 12%yield), 600 mg of the other ortho isomer, 2-hydroxy-4-bromobenzaldehyde, and about 1.2 g of starting material.

mp 50-51°C (lit 52.5°C) **^{1}H NMR:** δ 6.92 (dt, J=8.9, 0.8 Hz, 1H), 7.14 (dd, J=6.7, 0.8Hz, 1H), 7.30 (dd, 6.7, 8.9 Hz, 1H), 10.31 (s, 1H), 11.96 (s, 1H) [31]

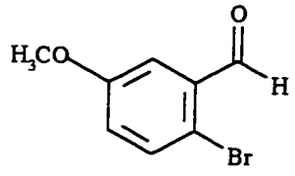


66

A mixture of **66a** (380 mg, 2 mmol), potassium carbonate (1 g, 7.2 mmol), methyl iodide (2 mL, 4.56 g, 32 mmol) and 10 mL acetone was stirred for 20 h, diluted with 20

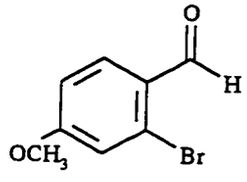
mL water, and extracted with ether. The organic extracts were dried with anhydrous $MgSO_4$ and evaporated to afford 400 mg of crystalline **66** (2 mmol, >95% yield) which was pure by ^1H NMR.

mp 52-53°C **^{1}H NMR:** δ 3.87 (s, 3H), 6.89 (dd, J=1.1, 8.1 Hz, 1H), 7.16-7.32 (m, 2H), 10.36 (s, 1H) **^{13}C NMR:** δ 55.86, 110.71, 124.29, 126.08, 131.07, 134.58, 161.61, 190.29 **IR:** 1585, 1697 cm^{-1}. **HRMS:** Calc'd for $C_8H_7O_2Br$: 213.9629. Found: 213.9631.



68

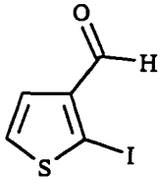
The procedure was the same as that used to prepare **50**. Isolated yield: 16.5 g of **68** (70% yield). mp 71-73°C (lit 74°C[32]) **^{1}H NMR**: δ 3.83 (s,3H), 7.01 (dd, J=8.8, 3.0 Hz, 1H), 7.40 (d, J=3.0 Hz, 1H), 7.45 (d, J=3.0Hz, 1H), 10.29 (s, 1H).



62

To a stirred solution of trimethylethylenediamine (4.6 mL, 3.7 g, 36 mmol) in 80 mL THF at -20°C was added dropwise n-BuLi (13 mL 2.5M, 32 mmol). The solution was stirred for 15 min, then p-anisaldehyde (3.7 mL, 4.4 g, 33 mmol) was added and stirred for 15 min. n-BuLi (39 mL, 2.5M, 97.5 mmol) was added and the solution was stirred, then let stand in a freezer (0°C) for 22 h. The solution was cooled to -78°C, carbon tetrabromide (30 g, 90 mmol) was added and the solution was allowed to warm to 25°C. A 10% HCl solution (300 mL) was added and extraction carried out with CH_2Cl_2. The combined organic extracts were washed with sodium thiosulfate until no cloudiness was imparted to the aqueous layer, then washed with water and brine. The organic solution was dried with anhydrous $MgSO_4$ and the solvent evaporated. The product was isolated by silica gel column chromatography (9:1 hexanes/ethyl acetate) and recrystallized from ethanol to afford 350 mg **62** (54% yield).

mp 70-71°C **^{1}H NMR**: δ 3.79 (s, 3H), 6.82 (dd, 1H), 7.12 (d, 1H), 7.80 (d, 1H), 10.11 (s, 1H) **^{13}C NMR:** δ 55.52, 113.74, 118.13, 126.59, 128.32, 130.93, 164.13, 190.12 **IR:** 1595, 1686 cm^{-1} **HRMS:** Calc'd for $C_8H_7O_2Br$: 213.9629. Found: 213.9622.

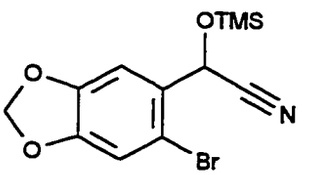


69

The compound was prepared following a literature procedure.[24] A solution of trimethylethylenediamine (0.46 mL, 0.37 g, 3.6 mmol) and n-BuLi (1.45 mL, 2.5 M, 3.6 mmol) in THF was stirred for five min at -78°C. 3-Thiophenecarboxaldehyde (0.26 mL, 0.33 g, 3.0 mmol) was added and the mixture was stirred for 5 min. n-BuLi (2.6 mL, 2.5M, 6.5 mmol) was added and the solution stored in a freezer (0°C) for 20 h. Iodine (0.8 g, 3.1 mmol) was added and the solution allowed to warm to 25°C. The solution was washed with saturated aqueous sodium thiosulfate and water, then dried with anhydrous $MgSO_4$ and the solvent removed. The product was isolated by silica gel column chromatography (1:1 CH_2Cl_2/hexanes) to afford 175 mg of **69**, a colorless oil (25% yield). **^{1}H NMR:** 7.53 (d, 1H), 7.98 (d, 1H), 9.79 (s, 1H)

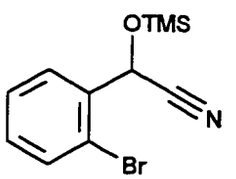
CYANOHYDRINS

The cyanohydrins were prepared according to Gassman's procedure.[21] The cyanohydrin was stirred for one hour with 1.2 equivalents of trimethylsilyl cyanide and catalytic ZnI_2 in CH_2Cl_2. The product was isolated either by evaporating the solvent and using the crude product directly or by passing the reaction mixture through a short silica gel plug to remove the catalyst and then evaporating the solvent. The yields for all cyanohydrins was >95%, on scales from 100 mg to 15 g.



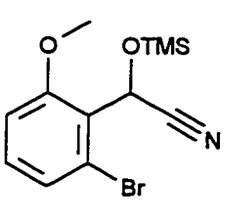
51

^{1}H NMR: δ 0.23 (s, 9H), 5.67 (s, 1H), 6.01 (s,2H), 6.98 (s,1H), 7.16 (s,1H)



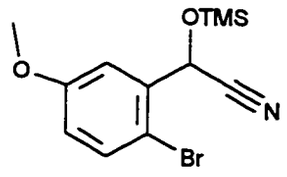
70

^{1}H NMR: δ 0.18 (s, 9H), 5.68 (s, 1H), 7.16 (dd, 1H), 7.32 (dd, 1H), 7.48 (d, 1H), 7.65 (d, 1H)



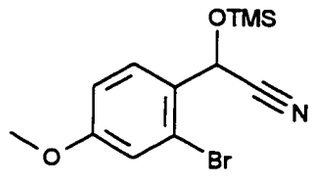
71

^{1}H NMR: δ 0.18 (s, 9H), 3.91 (s, 3H), 6.16 (s, 1H), 6.87-6.91 (m, 1H), 7.18-7.21 (m, 2H)



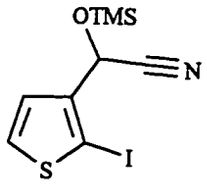
72

^{1}H NMR: δ 0.16 (s, 9H), 3.72 (s, 3H), 5.61 (s, 1H), 6.71(dd, 1H), 7.15 (d, 1H), 7.34 (d, 1H)



73

^{1}H NMR: δ 0.22 (s, 9H), 3.80 (s, 3H), 5.72 (s, 1H), 6.94 (dd, J=2.6,8.7 Hz, 1H), 7.10 (d, J=2.6,8.7 Hz, 1H)



74

^{1}H NMR: δ 0.23 (s, 9H), 5.35 (s, 1H), 7.15 (s, 1H), 7.31(s, 1H)

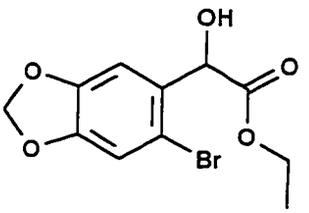
α-HYDROXY ESTERS

Procedure A: The cyanohydrin **B** in a solution of 3:2 ethanol/concentrated hydrochloric acid (1g **B** in 15 mL acid solution) was refluxed for one hour, the solution neutralized with sodium bicarbonate or sodium hydroxide, the ethanol evaporated, and extraction carried out with ether. The organic extracts were combined, dried with anhydrous $MgSO_4$ and the solvent was evaporated.

Procedure B: As for Procedure A except reflux was continued for 20 h.

Procedure C: Anhydrous hydrogen chloride was bubbled through a solution of B in absolute ethanol for 30 min. Workup was as for Procedure A.

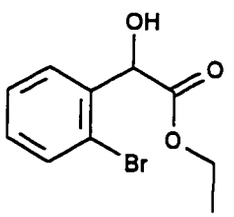
The crude product was generally clean by ^1H NMR, but could be purified by silica gel column chromatography (9:1 hexanes/ethyl acetate). The reactions were carried out on scales from 100 mg to 15 g with no decrease in yield.



52

The compound was prepared by Procedure A starting with 7.2 g of **51** (22 mmol); the yield of **52** was 4.9 g (16 mmol, 73% yield).

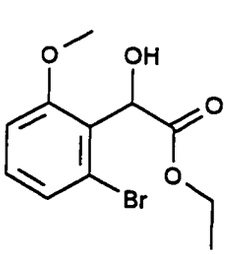
mp 56-58°C **^{1}H NMR:** δ 1.22 (t, J=7.1 Hz, 3H), 3.49 (d, J=4.7 Hz, 1H), 4.21 (m, 2H), 5.47 (d, J=4.7 Hz, 1H), 5.96 (s, 2H), 6.82 (s, 1H), 6.99 (s, 1H) **^{13}C NMR:** δ 14.0, 62.5, 72.1, 102.0, 108.0, 112.9, 114.4, 131.0, 147.7, 148.5, 173.3 **IR:** 1731, 3528 cm^{-1} **HRMS:** Calc'd for $C_{11}H_{11}O_5Br$: 301.9789. Found: 301.9779.



75

The compound was prepared by Procedure A starting with 1.42 g of **70** (5 mmol); the yield of **75** was 1.13 g (4.3 mmol, 87% yield).

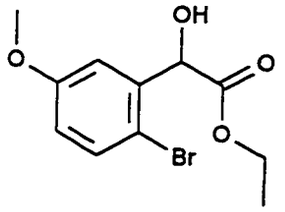
mp oil **^{1}H NMR:** δ 1.11 (t, J=7.1 Hz, 3H), 3.40-3.60 (br, 1H), 3.95-4.25 (m, 2H), 5.45 (s, 1H), 7.06-7.11 (m, 1H), 7.15-7.35 (m, 2H), 7.46 (dd, J=8.7 Hz, 1H) **^{13}C NMR:** δ 13.6, 62.0, 72.0, 123.2, 127.4, 128.4, 129.5, 132.8, 137.5, 172.8 **IR:** 3693, 1703 cm^{-1} **HRMS:** Calc'd for $C_{10}H_{11}O_3Br$: 257.9891. Found: 257.9890.



76

The compound was prepared by Procedure B from 540 mg of **71** (1.7 mmol); the yield of **76** was 280 mg (0.97 mmol, 57% yield)

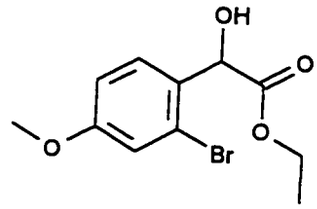
mp 102-103°C **^{1}H NMR:** δ 1.19 (t, J=7.6 Hz, 3H), 3.62 (d, J=1.7, 6.8 Hz, 1H), 3.79 (s, 3H), 4.21(q, J=7.6 Hz, 2H), 5.66 (d, J=6.8 Hz, 1H), 6.81 (dd, J=1.7, 7.7 Hz, 1H), 7.10-7.24 (m, 2H) **^{13}C NMR:** δ 14.1, 55.9, 61.8, 70.7, 110.4, 125.5, 127.2, 130.4, 158.3, 173.2 **IR:** 1737 cm^{-1} **MS(CI,M+1):** Calc'd for $C_{11}H_{13}O_4Br$: 289.0. Found: 288.9



77

The compound was prepared by Procedure A from 13.9 g of **72**(44 mmol); the yield of **77** was 10.9 g (38 mmol, 86% yield).

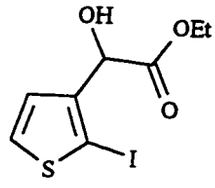
mp 60-62°C **^{1}H NMR:** δ 1.19 (t, J=7.1 Hz, 3H), 3.70 (br, 1H), 3.73 (s, 3H), 4.09-4.31 (m, 2H), 5.60 (s, 1H), 6.70 (dd, J=3.0, 8.8 Hz, 1H), 6.88 (d, J=3.0 Hz, 1H), 7.40 (d, J=8.8 Hz, 1H) **^{13}C NMR:** δ 13.6, 55.1, 62.1, 72.0, 113.4, 113.5, 115.7, 133.4, 138.3, 158.8, 172.7 **IR:** 3517, 1732 cm^{-1} **HRMS:** Calc'd for $C_{11}H_{13}O_2Br$: 287.9997. Found: 287.9998



78

The compound was prepared by Procedure C from 100 mg of **73**(0.32 mmol); the yield of **78** was 81 mg (0.28 mmol, 88% yield).

mp 58-59°C **^{1}H NMR:** δ 1.23 (t, J=7.1 Hz, 3H), 3.48 (d, J=5.0 Hz), 3.78 (s, 3H), 4.21 (m, 2H), 5.46 (d, J=5.0 Hz, 1H), 6.84 (dd, J=2.7, 8.7 Hz, 1H), 7.10 (d, J=2.7 Hz, 1H), 7.24 (d, J=8.7 Hz, 1H) **^{13}C NMR:** δ 13.6, 55.2, 61.9, 71.6, 113.4, 117.9, 129.0, 129.6, 159.6, 173.1, 190.3 **IR:** 3519, 1732 cm^{-1} **HRMS:** Calc'd for $C_{11}H_{13}O_2Br$: 287.9997. Found: 287.9985.



79

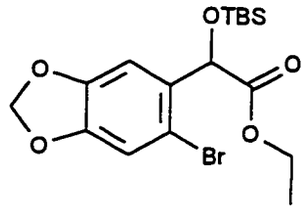
The compound was prepared by Procedure A from 220 mg of **74** (0.65 mmol), the yield of **79** was 175 mg (0.56 mmol, 86% yield).

^{1}H NMR: δ 1.21 (t, 3H), 3.5 (br, 1H), 4.15 (q, 2H), 5.05 (s, 1H), 7.10-7.30 (m, 2H)

TBS PROTECTED ESTERS

Procedure A: The hydroxy ester was treated with 1.2 equivalents of t-butyldimethylsilyl chloride and 2.2 equivalents of imidazole in DMF and stirred at 25°C for 24 h. The reaction mixture was diluted with water, extracted with CH_2Cl_2, and the organic extracts washed with water until no concentration gradients were visible when water was added. The organic extracts were dried with anhydrous $MgSO_4$ and the solvent was evaporated. The compounds were isolated and purified by either silica gel column chromatography or reduced pressure distillation as indicated. This reaction was carried out on scales from 100 mg to 15 g with no effect on the yield.

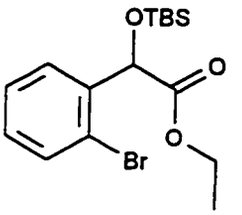
Procedure B: The hydroxy ester was stirred with 1.5 equivalents of t-butyldimethylsilyl trifluoromethanesulfonate and 2 equivalents of 2,6-lutidine in CH_2Cl_2 at 25°C for 30 min. Water was added and extraction carried out with CH_2Cl_2. The organic extracts were dried with anhydrous $MgSO_4$ and the solvent was evaporated. The crude material was purified by flash chromatography on silica gel (9:1 hexane/ethyl acetate)



56

The compound was prepared by Procedure A from 2.65 g of **52** (8.7 mmol); the yield of **56** was 3.19 g (7.5 mmol, 87% yield).

bp oil, distilled 160°C / 1.5 mtorr **^{1}H NMR:** δ 0.03 (s, 3H), 0.11(s, 3H), 0.89 (s, 9H), 1.21 (t, J=7.1 Hz, 3H), 4.13 (q, J=7.1 Hz, 2H), 5.48 (s, 1H), 5.96 (s, 1H), 6.94 (s, 1H), 7.07 (s, 1H) **^{13}C NMR:** δ -5.1, -5.0, 14.1, 18.2, 25.7, 25.8, 61.2, 73.4, 101.8, 108.4, 112.2, 113.0, 132.4, 148.2, 171.2 **IR:** 1743 cm^{-1} **LRMS(M+ - t-Bu)** Calc'd for $C_{13}H_{16}O_5BrSi$: 359.0. Found 358.8.

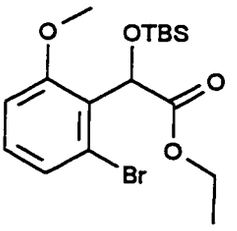


80

The compound was prepared by Procedure A from 2.90 g of **75** (11 mmol); the yield of **80** was 3.74 g (9.9 mmol, 90% yield).

bp oil, distilled 130°C /0.5 mtorr **^{1}H NMR:** δ 0.03 (s, 3H), 0.12 (s, 3H), 0.89 (s, 9H), 1.20 (t, J=7.1 Hz, 3H), 4.13 (q, J=7.1 Hz, 2H), 5.57 (s, 1H), 7.15 (dd, J=1.8,9.4 Hz, 1H), 7.31 (m, 1H), 7.49 (d, J=1.3, 9.4 Hz, 1H), 7.59 (dd, J=1.8, 7.8 Hz, 1H)

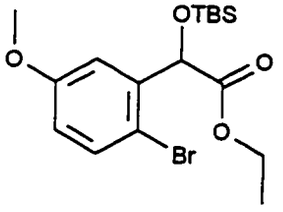
^{13}C NMR: δ 13.6, 17.9, 25.2, 25.3, 60.8, 73.1, 122.1, 127.2, 128.5, 129.1, 132.1, 138.7, 170.8 **IR:** 3661, 1738 cm^{-1} **LRMS(M+ - t-Bu):** Calc'd for $C_{12}H_{16}O_3BrSi$: 315.0. Found: 315.0.



81

The compound was prepared by Procedure B from 60 mg of **76** (0.2 mmol), which afforded 60 mg of **81** (0.15 mmol, 75% yield) after flash chromatography on silica gel (9:1 hexanes/ ethyl acetate as eluent).

mp oil **^{1}H NMR:** δ -0.05 (s, 3H), 0.14 (s, 3H), 0.85 (s, 9H), 1.18 (t, J=7.1 Hz, 3H), 3.77 (s, 3H), 4.16 (dq, J=3.2, 7.1 Hz, 2H), 5.74 (s, 1H), 6.80 (dd, J=2.0, 7.6 Hz, 1H), 7.08-7.20 (m, 2H) **^{13}C NMR:** δ -4.89, -4.61,14.3, 18.3, 25.8, 56.4, 61.0, 70.1, 110.9, 125.0, 125.6, 129.2, 130.1, 158.6, 172.6 **IR:** 1753 cm^{-1} **MS(CI,M$^+$+1,-t-Bu):** Calc'd for $C_{17}H_{27}O_4BrSi$: 403.1. Found 402.9.

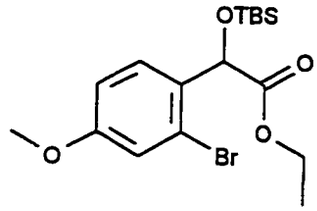


82

The compound was prepared by Procedure A from 1.25 g of **77** (4.3 mmol); the yield of **82** was 1.59 g (3.9 mmol, 89% yield) after silica gel column chromatography.

mp oil **^{1}H NMR:** δ 0.04 (s, 3H), 0.12 (s, 3H), 0.90 (s, 9H), 1.21 (t, J=7.1 Hz, 3H), 3.77 (s, 3H), 4.08-4.18 (dq, J=7.1, 0.8 Hz, 2H), 5.50 (s, 1H), 6.71 (dd, J=3.3, 8.8 Hz, 1H), 7.15 (d, J=3.3 Hz, 1H), 7.38 (d, J=8.8 Hz, 1H) **^{13}C NMR:** δ 13.6, 17.9, 24.8, 25.3, 55.0, 60.8, 73.1, 112.4, 113.1, 115.7, 132.6, 139.5, 158.7, 170.6 **IR:** 1742 cm^{-1}

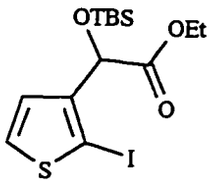
HRMS: (M^+-t-Bu) Calc'd for $C_{13}H_{18}O_4BrSi$: 345.0157. Found 345.0163.



83

The compound was prepared by Procedure B on 130 mg of **78** (0.45 mmol); the yield of **83** was 180 mg (0.45 mmol, >95% yield) after flash chromatography on silica gel.

mp oil **^{1}H NMR:** δ 0.01 (s, 3H), 0.10 (s, 3H), 0.88 (s, 9H), 1.20 (t, J=7.1 Hz, 3H), 3.77 (s, 3H), 4.12 (q, J=7.1 Hz, 2H), 5.50 (s, 1H), 6.86 (dd, J=2.6, 8.8 Hz, 1H), 7.04 (d, J=2.6 Hz, 1H), 7.48 (d, J=8.8 Hz, 1H) **^{13}C NMR:** δ 14.0, 18.2, 25.5, 25.6, 55.4, 61.0, 73.0, 113.8, 117.4, 122.7, 129.4, 131.1, 159.7, 171.4 **IR:** 1722 cm^{-1} **MS:** Calc'd for $C_{17}H_{27}O_4SiBr$: 402.1(M^+), 345.0 for M^+-t-Bu. Found: CI(M^++1) 403.0, EI(M^+-t-Bu) 345.0



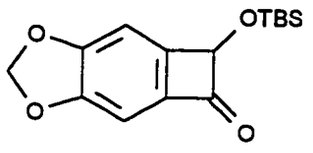
84

The compound was prepared by Procedure A from 165 mg of **79** (0.62 mmol); the yield of **84** was 126 mg (0.33 mmol, 53% yield) after flash chromatography on silica gel.

^{1}H NMR: δ -0.02 (s, 3H), 0.02 (s, 3H), 0.82 (s, 9H), 1.18 (t, 3H), 4.08 (q, 2H), 5.12 (s, 1H), 7.15 (br, 1H), 7.21 (s, 1H)

2-t-BUTYLDIMETHYLSILYLOXYBENZOCYCLOBUTENONES

General Method: *t*-Butyllithium (equivalents as indicated for each compound) was added to a solution of ester D in THF under nitrogen atmosphere at -78°C and stirred for 15 min. The solution was quenched with saturated NH_4Cl solution and allowed to warm to room temperature. Extraction was carried out with diethyl ether, the organic extracts dried with anhydrous $MgSO_4$, and the solvent was evaporated. Purification was carried out using flash chromatography on silica gel (9:1 hexanes/ethyl acetate as eluent) Recrystallization was carried out as noted for each compound.

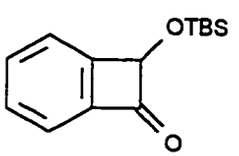


57

The compound was prepared by the general method using 1.5 equivalents of t-butyllithium and 1.9 g of **56** (4.5 mmol) as starting material, the yield of **57** was 1.35 g (4.5 mmol, >95%) isolated as colorless prisms after recrystallization from hexanes/ethyl acetate.

mp 104-105°C **^{1}H NMR:** δ 0.15 (s, 3H), 0.17 (s, 3H), 0.92 (s, 9H), 5.56 (s, 1H), 6.07 (s, 2H), 6.80 (s, 1H), 6.99 (s, 1H) **^{13}C NMR:** δ -4.7, -4.5, 18.3, 25.8, 83.9, 100.7, 102.2, 103.4, 140.5, 151.5, 154.4, 155.5, 187.5 **IR:** 1757 cm^{-1} **HRMS:** Calc'd for $C_{15}H_{20}O_4Si$: 292.1131. Found 292.1108.

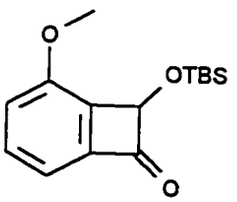
Anal. Calc'd for $C_{15}H_{20}O_4Si$: 61.61%C, 6.90%H. Found: 61.48%C, 6.86%H.



85

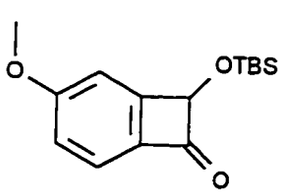
The compound was prepared by the general method using two equivalents of t-butyllithium and 1.0 g of **80** (2.6 mmol), the yield of **83** was 0.62 g (2.5 mmol, 96%) after purification by radial chromatography (9:1 hexanes/ethyl acetate)

mp oil **^{1}H NMR:** δ 0.20 (s, 3H), 0.22 (s, 3H), 0.96 (s, 9H), 5.78 (s, 1H), 7.44-7.68 (m, 4H), **^{13}C NMR:** δ -4.8, -4.5, 18.3, 25.8, 86.2, 121.6, 123.6, 131.0, 135.4, 146.9, 158.0, 190.7 **IR:** 1765 cm^{-1} **HRMS:** Calc'd for $C_{14}H_{20}O_2Si$: 248.1233. Found 248.1204.



86

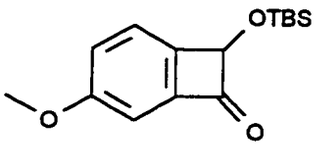
The compound was prepared by the general method using 1.2 equivalents t-butyllithium and 750 mg of **81** (1.6 mmol), 386 mg of **86** (1.4mmol, 88% yield) was isolated after purification by silica gel chromatography (9:1 hexanes/ethyl acetate). **mp** oil **^{1}H NMR:** δ 0.18 (s, 3H), 0.19 (s, 3H), 0.91 (s, 9H), 4.00 (s, 3H), 5.83 (s, 1H), 7.01 (m, 2H), 7.41 (m, 1H) **^{13}C NMR:** δ -4.5, -3.8, 18.8, 26.3, 57.8, 86.6, 112.9, 113.9, 121.8, 133.6, 142.6, 149.3, 157.1, 191.4 **IR:** 1769 cm^{-1} **HRMS:** Calc'd for $C_{15}H_{22}O_3Si$: 278.1329. Found 278.1350.



87

The compound was prepared by the General Method using 1.5 equivalents of t-butyllithium and 1 g of **82** (2.5 mmol), the yield of **87** was 470 mg (1.7 mmol, 68%) Purification was carried out by flash chromatography on silica gel (9:1 hexanes/ethyl acetate).

mp 43-44°C **^{1}H NMR:** δ 0.18 (s, 3H), 0.21 (s, 3H), 0.95 (s, 9H), 3.91 (s, 3H), 5.68 (s, 1H), 7.00-7.08 (m, 1H), 7.05 (s, 1H), 7.36 (dd, J=1.7, 7.5 Hz) **^{13}C NMR:** δ -4.8, -4.5, 18.3, 25.7, 55.8, 85.1, 105.9, 120.7, 123.6, 138.9, 160.4, 165.6, 188.1 **IR:** 1754 cm^{-1} **HRMS:** Calc'd for $C_{15}H_{22}O_3Si$: 278.1329. Found 278.1315.



88

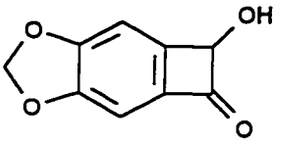
The compound was prepared by the General Method using 1.5 equivalents of t-butyllithium and 120 mg of **83** (3.0 mmol); the yield of **88** was 50 mg (1.8 mmol, 60% yield) after flash chromatography on silica gel.

mp oil **^{1}H NMR:** δ 0.15 (s, 3H), 0.17 (s, 3H), 0.92 (s, 9H), 3.80 (s, 3H), 5.67 (s, 1H), 6.87 (d, J=2.0 Hz, 1H), 7.13 (dd, J=2.0, 8.2 Hz, 1H), 7.53 (d, J=8.2 Hz, 1H) **^{13}C NMR:** δ -4.7, -4.5, 18.3, 25.8, 55.7, 85.0, 102.6, 124.6, 125.4, 148.3, 151.3, 162.3, 190.2 **IR:** 1762 cm^{-1} **HRMS:** Calc'd for $C_{15}H_{22}O_3Si$: 278.1329. Found 278.1352.

2-HYDROXYBENZOCYCLOBUTENONES

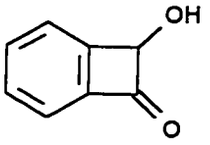
General Method: Tetrabutylammonium fluoride solution in THF (1 equivalent) was added to a stirred solution of the benzocyclobutenone in THF and immediately quenched with saturated aqueous NH_4Cl. The mixture was extracted with diethyl ether, the organic extracts dried with anhydrous magnesium sulfate and the solvent evaporated. The

compounds were purified by flash chromatography on silica gel (1:1 hexanes/ethyl acetate as eluent)



59

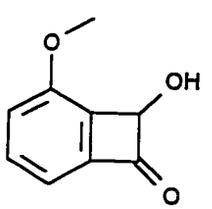
The compound was prepared by the general method on 420 mg of **57** (1.4 mmol); the yield of **59** was 190 mg (1.1 mmol, 79%) after silica gel column chromatography. **mp** 155-157°C **^{1}H NMR**($d_6$acetone): δ 5.35 (br, 1H), 5.50 (s, 1H), 6.19 (s, 2H), 6.86 (s, 1H), 7.16 (s, 1H) **^{13}C NMR**($d_6$acetone): δ 84.6, 100.6, 103.5, 104.2, 141.4, 152.5, 155.3, 157.1, 188.8 **IR:** 1756, 3581 cm^{-1} **HRMS:** Calc'd for $C_9H_6O_4$: 178.0266. Found 178.0279. **Anal.** Calc'd for $C_9H_6O_4$: 60.68%C, 3.39%H. Found 60.49%C, 3.25%H.



90

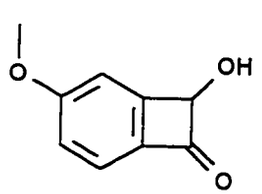
The compound was prepared using the general method on 380 mg of **85** (1.5 mmol); the yield of **90** was 132 mg (0.99 mmol, 66%) after silica gel column chromatography.

mp 52.5-53.5°C **^{1}H NMR:** δ 3.05 (br, 1H), 5.79 (s, 1H), 7.49-7.62 (m, 3H), 7.75 (m, 1H) **^{13}C NMR**($d_6$acetone): δ 87.1, 121.7, 124.6, 131.8, 136.2, 148.0, 159.6, 192.2 **IR:** 1766, 3582 cm^{-1} **HRMS:** Calc'd for $C_8H_6O_2$: 134.0368. Found 134.0379. **Anal.** Calc'd for $C_8H_6O_2$: 71.64%C, 4.51%H. Found 71.93%C, 4.29%H.



91

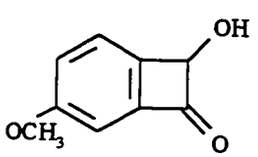
The reaction was performed on 50 mg of **86** (0.18 mmol); 20 mg **91** (0.12mmol, 67% yield) which crystallized on standing was isolated after column chromatography. **mp** 70-72°C **^{1}H NMR:** δ 3.50 (br, 1H), 4.03 (s, 3H), 5.85 (br, 1H), 7.01-7.07 (m, 2H), 7.45 (m, 1H) **^{13}C NMR:** δ 57.3, 85.7, 113.5, 121.7, 133.4, 141.3, 148.9, 156.6, 191.3 **IR:** 1767, 3575 cm^{-1} **HRMS:** Calc'd for $C_9H_8O_3$: 164.0473. Found 164.0487.



92

The compound was prepared using the general method on 470 mg of **87** (1.7 mmol); the yield of **92** was 240 mg (1.5 mmol, 88%) after silica gel column chromatography.

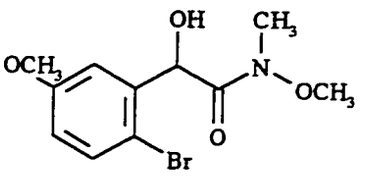
mp 95.0-95.5°C **^{1}H NMR**($d_6$acetone): δ 3.95 (s, 3H), 5.63 (s, 1H), 7.12 (dd, J=2.0, 8.5 Hz, 1H), 7.27 (d, J=2.0 Hz, 1H), 7.38 (dd, J=0.7, 8.5 Hz, 1H) **^{13}C NMR**($d_6$acetone): δ 56.4, 86.0, 107.1, 121.3, 123.7, 139.9, 162.2, 166.5, 189.5 **IR:** 1758, 3587 cm^{-1} **HRMS:** Calc'd for $C_9H_8O_3$: 164.0473. Found 164.0453.



93

TBAF (1M, 0.16 mL, 0.16 mmol) was added to a solution of 11 mg of **88** (0.040 mmol) and 100 μL of acetic acid in 5 mL of THF. The solution was stirred for one hour, washed with saturated NH_4Cl, saturated $NaHCO_3$, and brine, then dried with anhydrous $MgSO_4$ and the solvent was evaporated. Column chromatography on silica gel (ethyl acetate needed to remove **93**) afforded approximately 2 mg of the desired hydroxy ketone **93** (0.01 mmol, ~30% yield).

^{1}H NMR: 3.82 (s, 3H), 5.69 (br s, 1H), 6.88 (dd, J=2.2, 0.4 Hz, 1H), 7.19 (dd, J=8.3, 2.2 Hz, 1H), 7.62 (dd, J=8.3, 0.4 Hz, 1H) **^{13}C NMR:** 55.8, 85.1, 102.7, 124.9, 125.8, 148.8, 150.5, 162.7, 189.2. **HRMS:** Calc'd for $C_9H_8O_3$: 164.0473. Found:164.0453

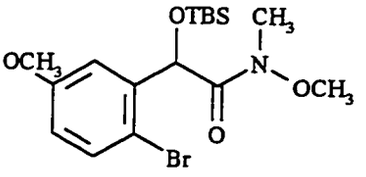


94

Trimethylaluminum (4.3 mL, 2.0M, 8.6 mmol) was added to a solution of N,O-dimethylhydroxylamine hydrochloride (370 mg, 3.8 mmol) in 10 mL of dry CH_2Cl_2 under nitrogen atmosphere and the solution was stirred for 15 min at 25°C. A solution of 77 (1.0 g, 3.5 mmol) in dry CH_2Cl_2 was added and the solution was refluxed for 24 h. Dilute HCl(aq) was CAREFULLY added dropwise through the condenser until gas evolution ceased and all remaining white precipitate had dissolved. The layers were separated and the aqueous layer extracted with CH_2Cl_2. The organic extracts were combined, washed with water and brine, dried with anhydrous $MgSO_4$, and the solvent evaporated. Yield: ^{1}H NMR showed 60% conversion; 450 mg was isolated, 73% based on conversion of starting material.

mp 71.0-71.5°C **^{1}H NMR**: δ 3.20 (s, 3H), 3.29 (s, 3H), 3.73 (s, 3H), 4.28 (br, 1H), 5.71 (br, 1H), 6.68-6.76 (m, 2H), 7.44 (d, J=8.6 Hz, 1H) **^{13}C NMR**: δ 55.1, 60.5, 70.4, 89.2, 113.2, 114.3, 115.6, 133.3, 139.3, 159.0, 172.7 **IR:** 1662, 3447 cm^{-1}

HRMS: Calc'd for $C_{11}H_{14}NO_4$ (M^+-Br) 224.0923. Found: 224.0914.



95

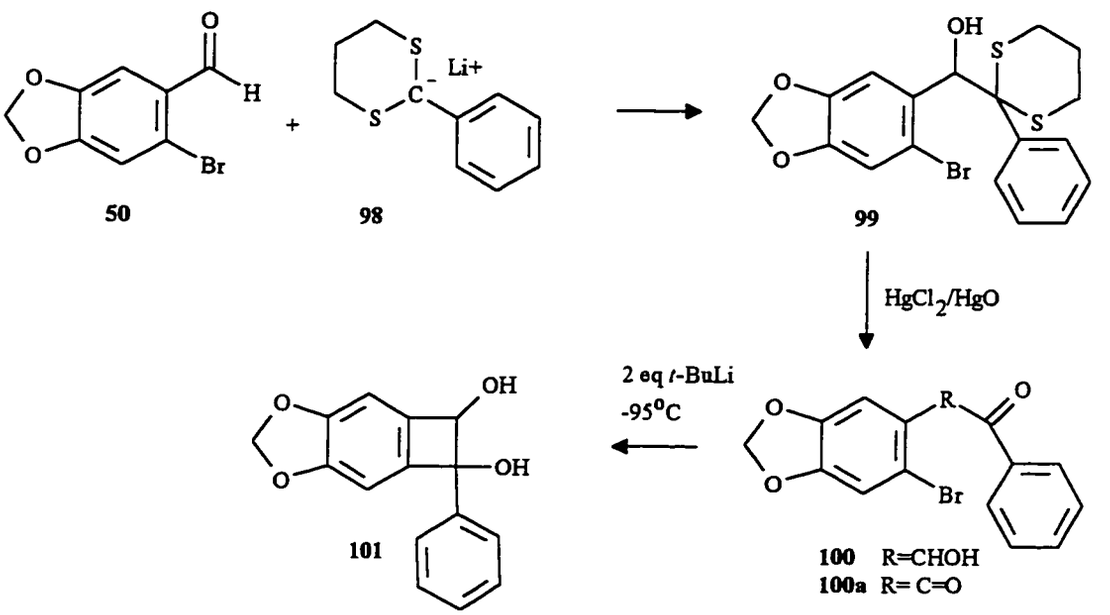
A solution of **94** (135 mg, 0.44 mmol), t-butyldimethylsilyl triflate (154 μL, 0.66 mmol), and 2,6-lutidine (105 μL, 0.88 mmol) in 10 mL of dry CH_2Cl_2 was stirred for 30 min at 25°C. The solution was diluted with water (10 mL) and extracted with CH_2Cl_2. The combined organic extracts were dried with anhydrous $MgSO_4$ and the solvent was evaporated leaving 179 mg (0.43 mmol, >95% yield) of a pale yellow crystalline solid which was pure by ^1H NMR.

mp 81.0-81.5°C **^1H NMR**: δ -0.01 (s, 3H), 0.12 (s, 3H), 0.86 (s, 9H), 3.13 (s, 3H), 3.48 (s, 3H), 3.75 (s, 3H), 5.83 (s, 1H), 6.68-6.71 (m, 1H), 7.09-7.11 (m, 1H), 7.35-7.38 (m, 1H). **^{13}C NMR**: δ -4.8, -4.6, 18.2, 25.8, 32.7, 55.5, 61.0, 71.8, 113.3, 114.5, 116.3, 132.9, 140.0, 159.1, 171.9 **IR**: 1677 cm^{-1} HRMS: Calc'd for $C_{13}H_{19}BrNO_4Si$ (M^+-t-Bu) 360.0267. Found: 360.0279.

Chapter 3 Other approaches toward benzocyclobutenones

While the approach outlined in the previous chapter was by far the most successful method of generating benzocyclobutenones, a number of other approaches via halogen metal exchange were investigated. The first involved the generation of an *ortho* brominated benzoin as a key intermediate. (Scheme 28)

Scheme 28



The anion of 2-phenyl-1,3-dithiane **98** was added to 6-bromopiperonal **50** to give the protected benzoin **99**. Deprotection of the dithiane with mercuric chloride and mercuric oxide afforded the benzoin **100** in approximately 75% yield after the two steps. The deprotection was carried out under nitrogen atmosphere. When the same reaction was carried out in air, the diketone **100a** was instead isolated.

Halogen metal exchange was carried out on the benzoin **100** using two equivalents of *t*-butyllithium at -95°C. It was assumed that the first equivalent of butyllithium would deprotonate the alcohol and the second carrry out the exchange reaction. The reaction afforded the diol **101** as a white solid which was recrystallized from hexanes/ethyl acetate. The IR spectrum showed the disappearance of the carbonyl absorption and appearance of two hydroxy peaks. ^{1}H NMR spectroscopy showed a shift in the benzylic proton from 6.28 ppm to 4.88 ppm due to the loss of the carbonyl group. (Figure 12) High resolution mass spectral data also confirmed the molecular weight and composition. The best yield obtained for the reaction was 61%, and was poorly reproducible, presumably due to the problems associated with the halogen metal exchange in compounds which also contain exchangeable hydrogens (see page 21). When the reaction was carried out at -78°C, poorer yields were obtained. Attempts to cyclize the diketone **100a** directly were unsuccessful since reaction with one equivalent of butyllithium at -100°C led to formation of at least five compounds without consuming all of the starting material.

This approach was not pursued further for two reasons. First because the cyclization step was not easily reproducible, and secondly because this approach is less convergent than the method described in Chapter 2, requiring both a substituted 2-bromobenzaldehyde and a suitably substituted aryl dithiane in the first step of the sequence. The approach presented in Chapter 2, however, required only that the 2-bromobenzaldehyde have the appropriate substituents. The second substituted aryl group could be added later in the sequence, allowing for greater variability in the final products.

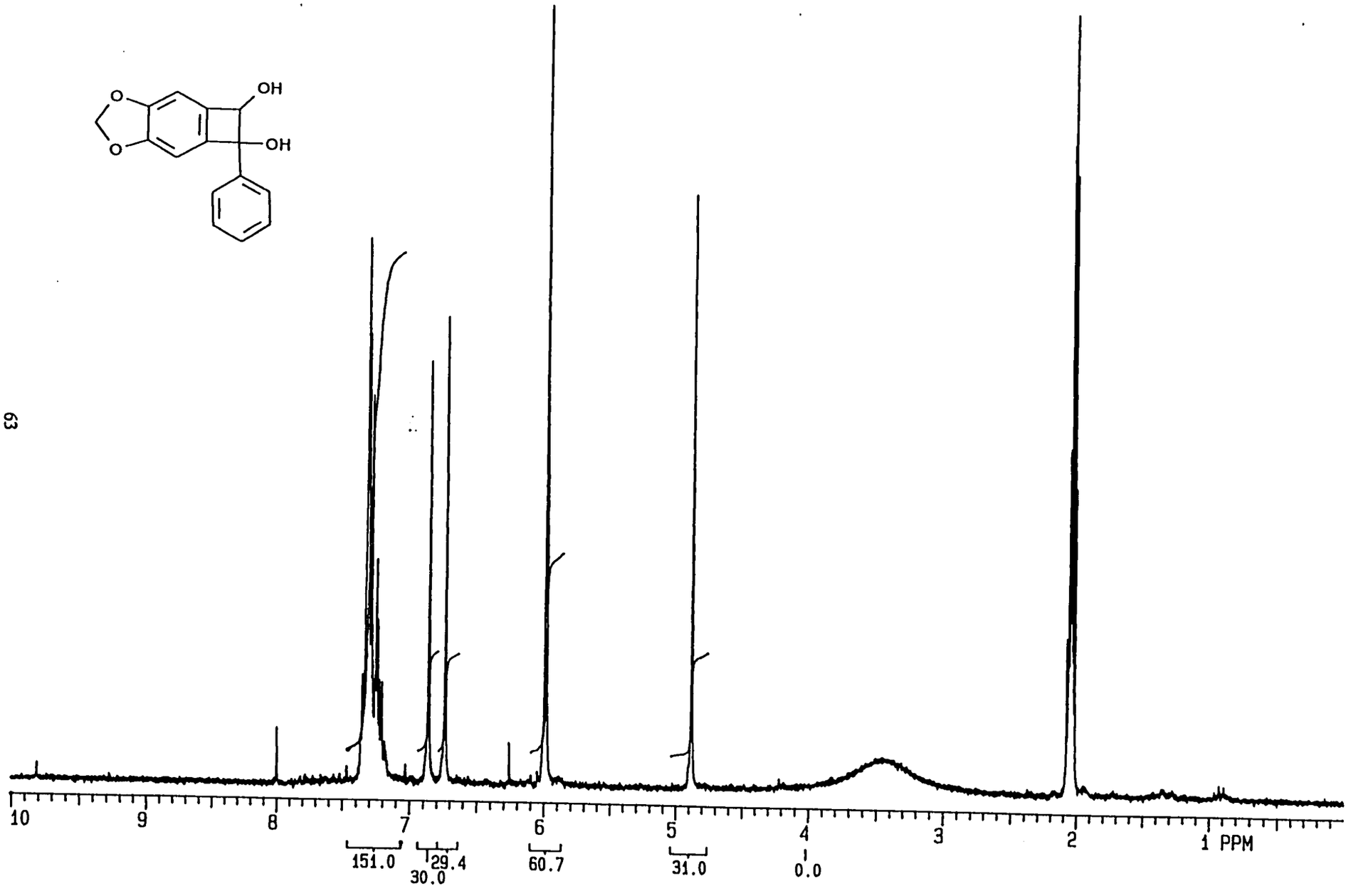
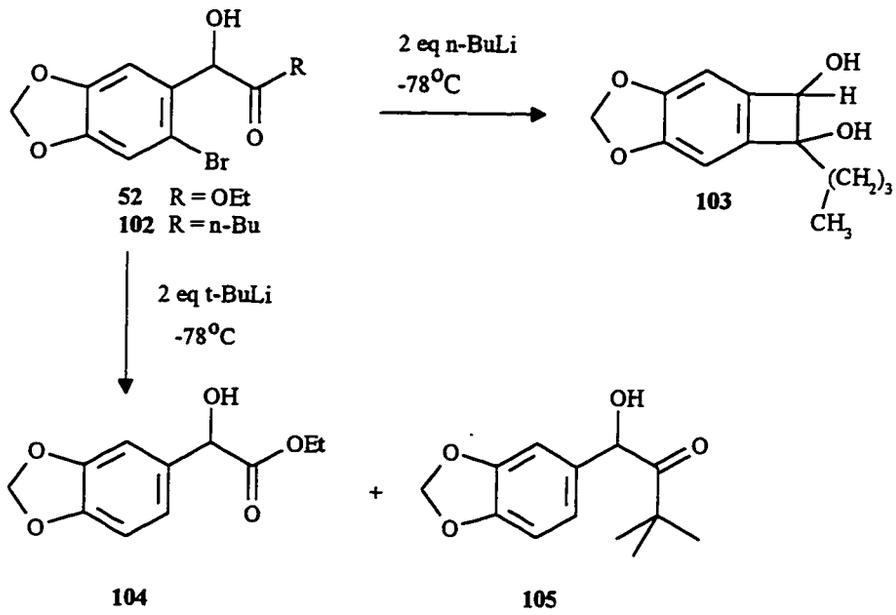


Fig. 12: ^{1}H NMR spectrum of **101**

Attempts were made to shorten the sequence used in Chapter 2. In order to determine whether or not the protection step was necessary, the hydroxy ester **52** was subjected to cyclization conditions. (Scheme 29) The ester **52** was treated with two equivalents of *n*-butyllithium at -78°C. The major product isolated was **103**, a product which resulted from cyclization and addition of the second equivalent of butyllithium to the desired benzocyclobuten-2-ol. The question which remained was whether the addition or the cyclization had occurred first.

Scheme 29



The reaction was repeated on **52** using one equivalent of *t*-butyllithium. The major products isolated when the reaction was carried out at -78°C were two compounds, **104** and **105**, along with a significant amount of unreacted starting material. Both of these compounds formed by exchanging one equivalent of *t*-butyllithium with the bromine atom.

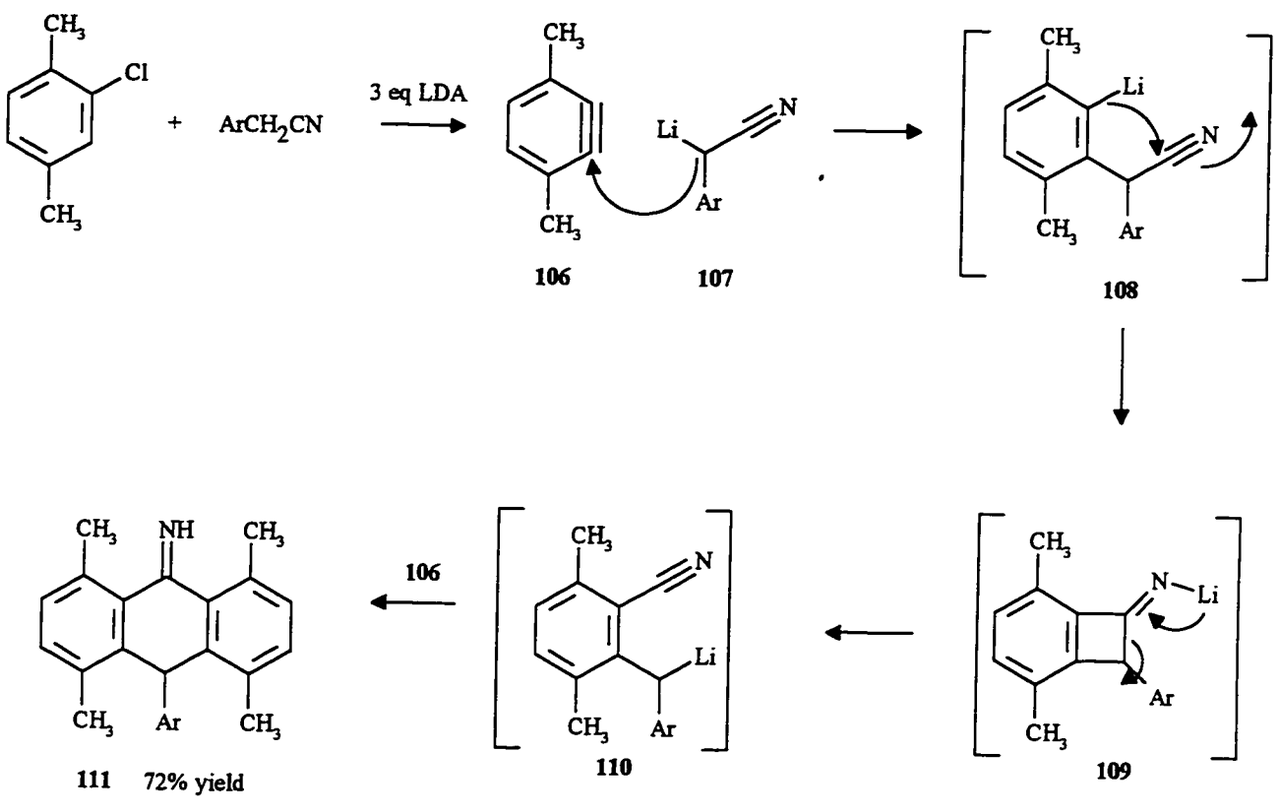
The compound **105** also underwent displacement of the ethoxy group by a second reaction with *t*-butyllithium. No benzocyclobutenone cyclization products were detected. This suggested that the formation of **103** involved initial conversion of **52** to an α-hydroxy *n*-butyl ketone **102** followed by halogen metal exchange and cyclization. When *t*-butyllithium was used, however, the ketone was too sterically hindered to undergo cyclization, hence on workup the anions formed by halogen metal exchange were quenched affording **104** and **105**.

The carbon homologue of the aldehyde needed for cyclization was introduced in the first step via the formation of the cyanohydrin. It was envisioned that perhaps direct cyclization of the cyanohydrin or a protected version would afford the same compounds in two steps. There exists a literature precedence that the desired cyclization does occur. Biehl has reported that reaction of the lithio nitrile **107** with an in situ generated benzyne **110** furnished the anthrone imine **111**.[33] The proposed pathway is shown in Scheme 30. Initial trapping of the benzyne **106** with **107** would afford the α-lithiated nitrile **108**, cyclization of which should give the N-lithiated benzocyclobutanone imine **109**. The latter can cyclorevert to afford the anion **110** which is doubly benzylic. This intermediate was subsequently trapped with another molecule of benzyne to form the anthrone imine **111**.

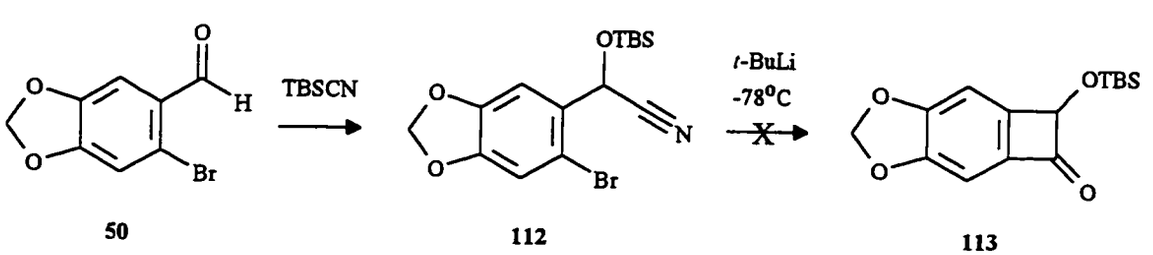
This sequence was investigated using the *t*-butyldimethylsilyl protected cyanohydrin **112** of 6-bromopiperonal, **50**, the unprotected cyanohydrin **114**, and the THP protected cyanohydrin **116**. (Schemes 31 and 32) In each case, it was hoped that the cyclization would proceed as in the literature, but that the absence of the second phenyl ring and its replacement by an -OTBS or -OTHP group would prevent the ring opening

reaction. The cyanohydrin **112** was generated by adding t-butyldimethylsilyl cyanide to 6-bromopiperonal with catalytic zinc iodide. (Scheme 31) The aldehyde was completely converted to the cyanohydrin, and passed through silica gel to remove the catalyst.

Scheme 30



Scheme 31



Halogen metal exchange was carried out on **112**, however only a complex mixture of products resulted it was when treated with one or two equivalents of t-butyllithium at -78°C. Since it appeared that part of the compound had been deprotected, a different protecting group was used to determine its effect on the reaction. This time, the cyanohydrin was formed by reacting the bisulfite adduct of 2-bromopiperonal with cyanide ion to give the free cyanohydrin **114**. (Scheme 32) Direct reaction of **114** with two equivalents of t-butyllithium at -78°C led to complete conversion to the compound **115**, which resulted from halogen metal exchange but no cyclization. The tetrahydropyranyl ether of the cyanohydrin was formed by reacting **114** with dihydropyran under acidic conditions. The isomer mixture of **116** was then subjected to halogen metal exchange conditions. When **116** was treated with one equivalent of *t*-butyllithium at -78°C, the major compound isolated was **118**. The structure was deduced based on the ^{1}H NMR which showed an AB pattern between 4.5 and 4.9 ppm integrating for two benzylic hydrogens, and only two aromatic singlets, indicating a tetrasubstituted aromatic compound. (Figure 13) The IR spectrum showed no carbonyl absorption as would be expected of the desired product, and the high resolution mass spectrum showed the correct molecular weight and molecular composition for **118**. This can be explained by the formation of the intermediate **117**, which formed via cyclization. Iminium ion promoted ring opening led to the final product **118**.

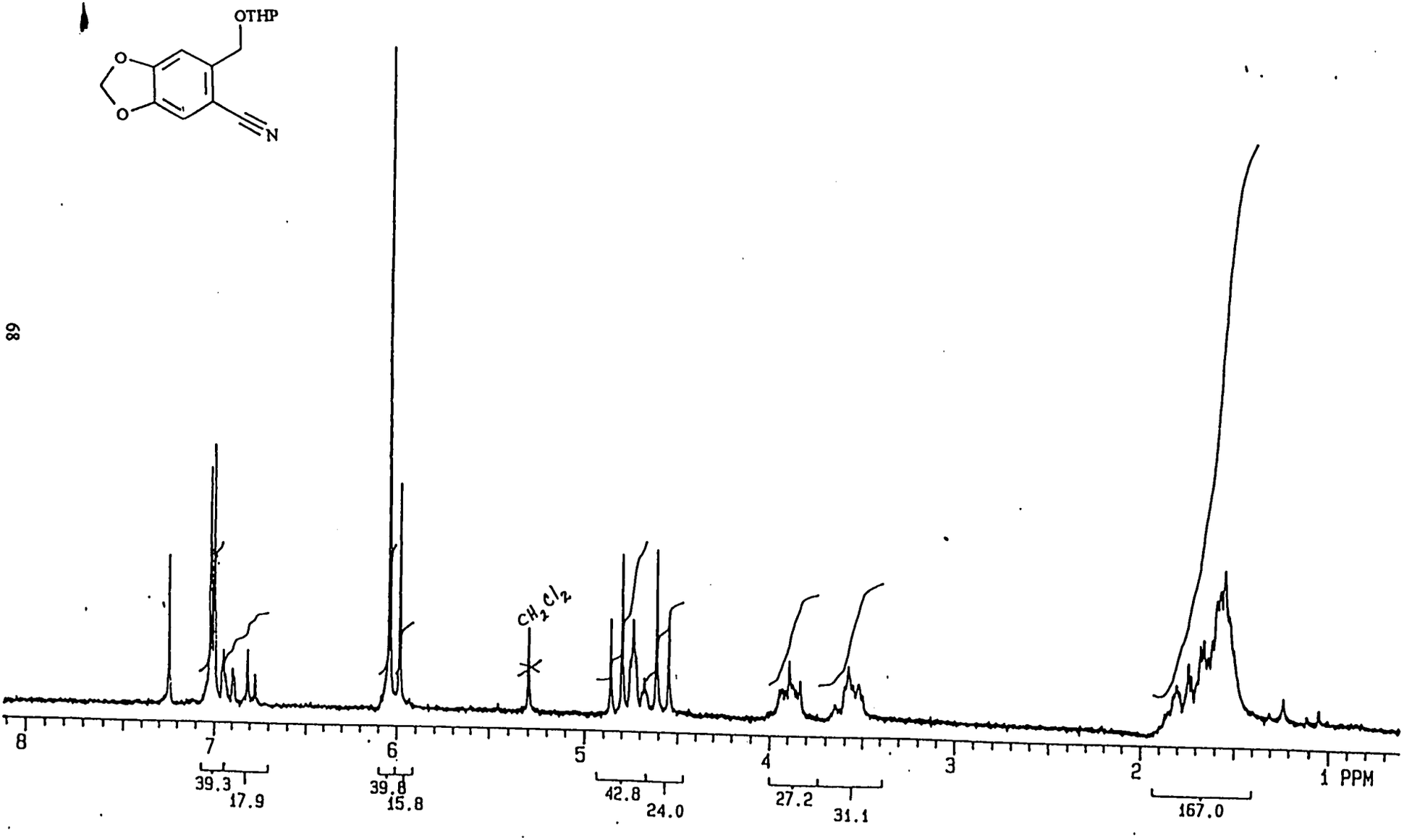
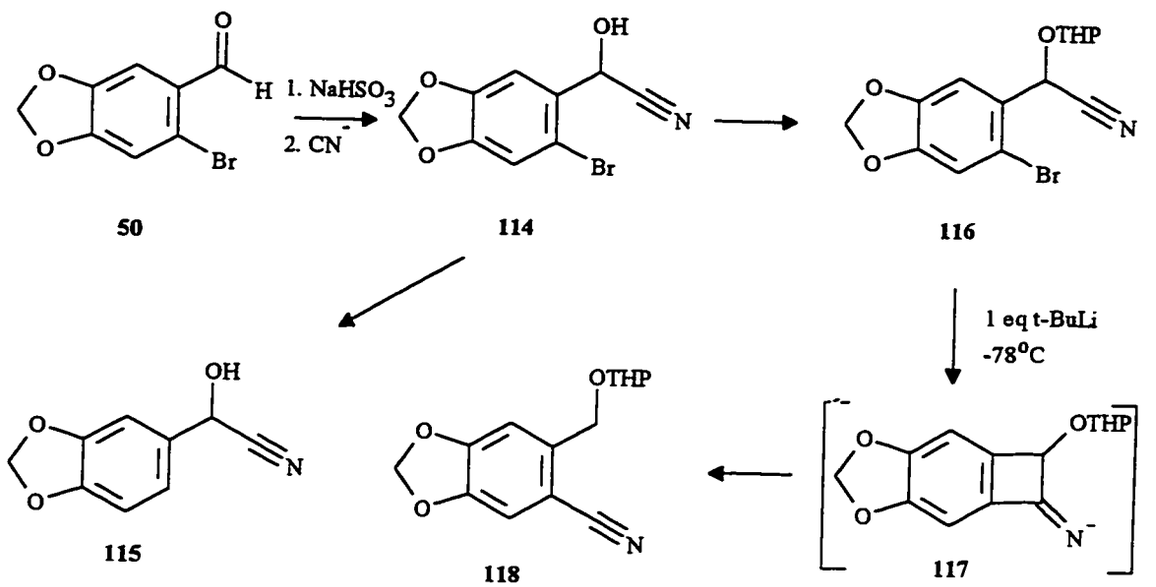


Fig. 13: ^{1}H NMR spectrum of **118**

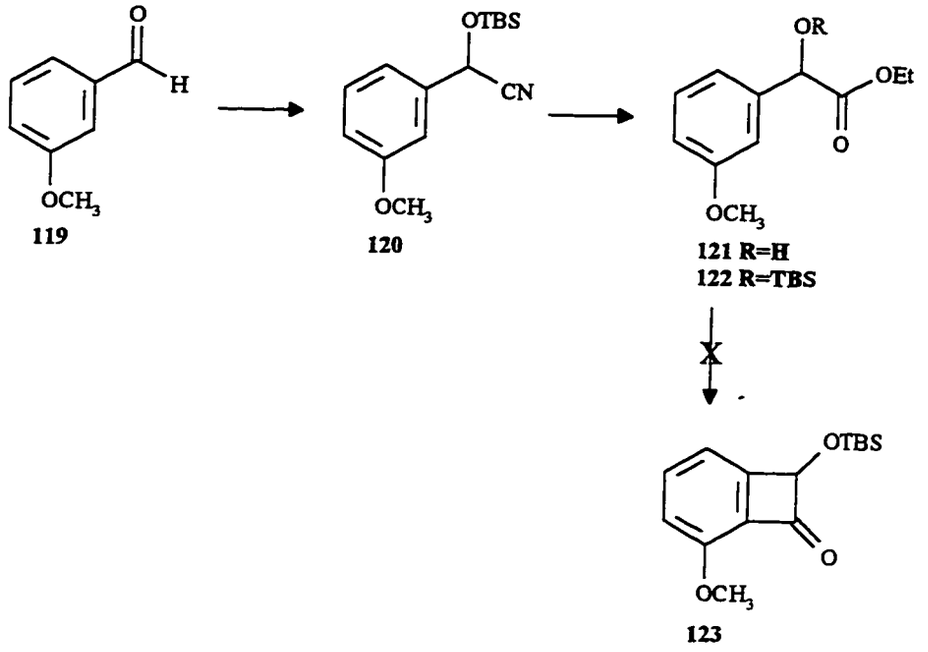
Scheme 32



50 114 116 115 118 117

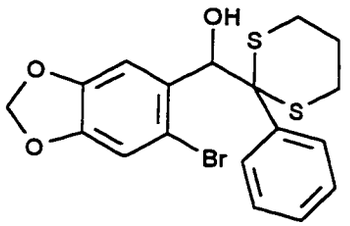
The possibility of generating benzocyclobutenones such as **123** via an ortho-metallation procedure and thus avoiding the extra step of *ortho*-bromination was briefly investigated using **122** as substrate. This compound was prepared in a routine fashion from meta-anisaldehyde. When **122** was reacted with *n*-butyllithium in THF at -78°C a complex mixture of products was formed which was not further investigated. Perhaps a stronger DOM group such as $-OCONEt_2$ might have been successful. This was not attempted since it involves an additional protection - deprotection sequence to generate the methoxy derivatives.

Scheme 33


O
H
OCH3
119
OTBS
CN
OCH3
120
OR
OEt
O
OCH3
121 R=H
122 R=TBS
X
OTBS
O
OCH3
123

Experimental Section

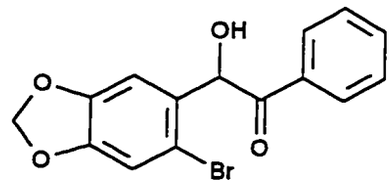
The general methods used were the same as those given at the beginning of Chapter 2.



99

t-Butyllithium (1.6 mL, 2.3 M, 3.7 mmol) was added to a solution of 2-phenyl-1,3-dithiane (0.60 g, 3.0 mmol) in THF (5 mL) at 0°C and stirred for 15 min turning the solution dark red. 6-Bromopiperonal (0.70 g, 3.0 mmol) in THF was added and stirred for 15 min at 0°C. The reaction was quenched with saturated NH_4Cl solution and allowed to warm to 25°C. The reaction mixture was extracted with diethyl ether, dried with anhydrous $MgSO_4$ and the solvent was evaporated. The crude mixture was recrystallized from acetone to yield 1.2 g of **99** (2.8 mmol, 93 % yield).

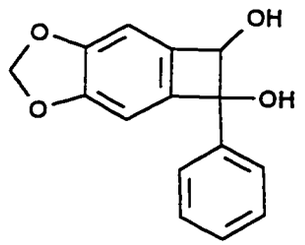
mp 175.0-175.5°C **^{1}H NMR:** δ 1.83-1.95 (m, 2H), 2.48-2.79 (m, 4H), 2.86 (d, J=3.3 Hz, -OH), 5.40 (d, J=3.3 Hz, 1H), 5.91 (d, J=1.4 Hz, 1H), 5.94 (d, J=1.4 Hz, 1H), 6.66 (s, 1H), 6.82 (s, 1H), 7.29-7.33 (m, 3H), 7.78-7.83 (m, 2H) **^{13}C NMR:** δ 24.7, 26.9, 27.6, 67.1, 78.6, 101.7, 110.2, 111.8, 115.7, 127.7, 128.3, 130.3, 130.8, 137.3, 146.5, 148.1 **IR:** 1480, 3683 cm^{-1} **HRMS**(M+ -H_2O): Calc'd for $C_{18}H_{15}O_2BrS_2$: 405.9697. Found: 405.9715



100

A mixture of 970 mg of **99** (2.3 mmol), mercuric chloride (1.36 g, 5 mmol), and mercuric oxide (0.54 g, 2.5 mmol) in 35 mL of 80% aqueous acetonitrile was refluxed for 90 min under nitrogen atmosphere. The mixture was cooled and filtered through Celite. The filter cake was washed with 1:1 hexanes/dichloromethane. The organic extracts were washed sequentially with 5M aqueous ammonium acetate, water and brine, then dried with $MgSO_4$ and the solvent was evaporated. The crude mixture was recrystallized from methanol to precipitate the oxidized diketone impurity. The solvent was again evaporated and the solid recrystallized from 3:1 hexanes/ethyl acetate to yield 650 mg of pure **100** (1.9 mmol, 83% yield). When the reaction was carried out under air, the major product isolated was the oxidized diketone form of **100**.

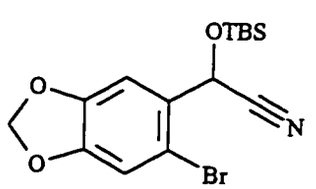
mp 104.5-106.0°C **^{1}H NMR:** δ 4.50 (br, -OH), 5.87 (d, J=1.3 Hz, 1H), 5.91 (d, J=1.3 Hz, 1H), 6.28 (br, 1H), 6.47 (s, 1H), 7.02 (s, 1H), 7.33-7.59 (m, 3H), 7.89-7.94 (m, 2H) **^{13}C NMR:** δ 75.1, 102.1, 108.1, 113.2, 115.2, 128.8, 129.0, 131.5, 133.1, 134.2, 148.1, 148.8 **IR:** 1681, 3464 cm^{-1} **HRMS:** Calc'd for $C_{15}H_{11}O_4Br$: 333.9840. Found: 333.9847



101

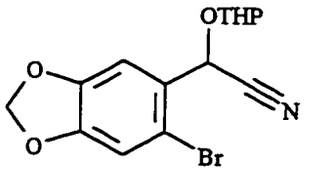
A solution of 1.1 g of **100** (3.3 mmol) in 25 mL of THF was cooled to -95°C. *t*-BuLi (2.9 mL, 2.37M, 6.9 mmol) was added over 10 min, and stirring continued for 5 min, allowing the temperature to rise to -70°C. The reaction was quenched with saturated aqueous NH_4Cl and allowed to warm to 25°C. Water (10 mL) and dichloromethane (5 mL) were added, the layers separated and the aqueous layer extracted with another 5 mL of dichloromethane. The organic extracts were dried with anhydrous $MgSO_4$ and the solvent was evaporated. The oily residue was recrystallized from 15 mL of 3:1 hexanes/ethyl acetate to afford 510 mg **101** (2.0 mmol, 61% yield).

mp 136.5-138.0°C **^{1}H NMR**(acetone-d_6): δ 3.2-3.7 (br, -OH), 4.88 (s, 1H), 5.99 (s, 2H), 6.74 (s, 1H), 6.86 (s, 1H), 7.15-7.38 (m, 5H) **^{13}C NMR**(acetone-d_6): δ 77.2, 79.3, 98.9, 101.6, 102.9, 123.8, 125.0, 126.1, 137.9, 140.8, 142.2, 147.3, 147.5 **IR:** 3597, 3683 cm^{-1} **HRMS:** Calc'd for $C_{15}H_{12}O_4$: 256.0736. Found: 256.0744



112

The procedure was the same as for the TMS cyanohydrins in Chapter 2, but the TMSCN was replaced by TBSCN. **^{1}H NMR**: δ 0.14 (s, 3H), 0.23 (s, 3H), 0.92 (s, 9H), 5.68 (s, 1H), 6.01 (s, 2H), 6.98 (s, 1H), 7.14 (s, 1H)

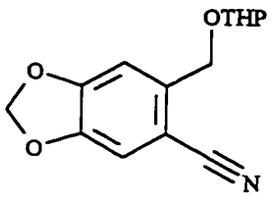


116

A solution of 0.4 g sodium bisulfite (3.8 mmol) in 3 mL of water was added to an ice-cooled solution of 0.5 g of 6-bromopiperonal (2.2 mmol) in 25 mL THF and was stirred for 30 min at 0°C. The white precipitate was filtered and washed with THF, then dissolved in water. Potassium cyanide(200 mg, 3 mmol) in 0.5 mL water was added and stirred for 5 min. Extraction was carried out using CH_2Cl_2, the organic extracts were dried using anhydrous $MgSO_4$, and the solvent was evaporated leaving an oily residue. Dihydropyran (300 μL, 277 mg, 3.3 mmol) and one drop of concentrated HCl were added and refluxed for 1 h. The mixture was diluted with CH_2Cl_2, and sodium bicarbonate was added until pH 7 was reached. The aqueous layer was extracted with CH_2Cl_2, the organic extracts dried with magnesium sulfate, and the solvent was evaporated, leaving a brown oily residue. Purification was carried out by flash chromatography on silica gel, affording 300 mg of a mixture of isomers of **116** (0.88 mmol, 40% yield).

mp oil **^{1}H NMR:** δ 1.4-1.9 (m, 12H), 3.4-4.1 (m, 4H), 4.80 (m, 1H), 5.10 (m, 1H), 5.63 (s, 1H), 5.78 (s, 1H), 5.99 (s, 4H), 6.98 (s, 2H), 7.12 (s, 1H), 7.15 (s, 1H) (integration reflects total number of protons for both isomers) **HRMS:** Calc'd for $C_{14}H_{14}O_4BrN$: 339.0106.

Found: 339.0100.



118

107 (300 mg, 0.88 mmol) in 10 mL of THF was cooled to -78°C and t-butyllithium (550 μL, 1.7M, 0.94 mmol) was added and stirred for 15 min. The reaction was quenched with saturated NH_4Cl solution and allowed to warm to 25°C. Extraction was carried out with CH_2Cl_2, the solvent dried with anhydrous $MgSO_4$, and the solvent was evaporated. The major compound isolated after chromatography on silica gel was **118**.

mp oil **^{1}H NMR:** δ 1.4-1.9 (m, 6H), 3.45-3.63 (m,1H), 3.82-3.97 (m, 1H), 4.58 (d, J=18 Hz,1H), 4.72-4.78 (m, 1H), 4.82 (d, J=18 Hz, 1H), 5.98 (s, 1H), 6.04 (s, 2H), 6.98 (s, 1H), 7.02 (s, 1H) **HRMS:** Calc'd for $C_{14}H_{15}O_4N$: 261.1002. Found: 261.1008

Chapter 4 Synthesis of Regiospecifically Substituted Anthraquinones

With the benzocyclobutenones from Chapter 2 in hand, the next step was to convert these compounds to anthraquinones. The second aryl ring required for anthraquinone formation would be introduced as an aryl magnesium reagent. Depending on the stereochemistry of the diols thus formed, ring opening and subsequent internal trapping might occur directly. Alternatively, the secondary alcohol would have to be oxidized to the ketone prior to ring expansion with a suitable oxidizing agent. These issues are the subject of this chapter.

A number of diols were obtained by reacting Grignard reagents with the benzocyclobutenones. Two equivalents of reagent were required, since the first deprotonates the free alcohol, and the second adds to the ketone. This reaction went in good to high yield, as expected. (Scheme 34, Table 3)

Scheme 34

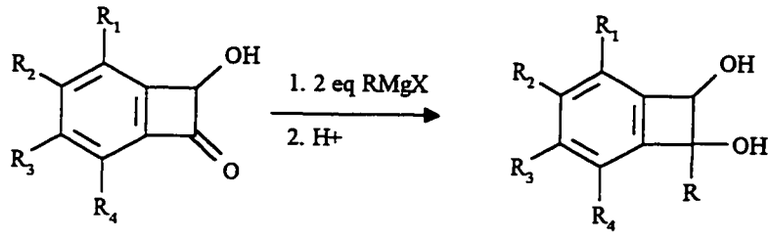


Table 3 Formation of benzocyclobutene diols

R_1	R_2	R_3	R_4	RMgX	Product	% yield
H	H	H	H	p-tolylmagnesium bromide	**124**	95
H	OCH_3	H	H	p-tolylmagnesium bromide	**125**	88
H	OCH_2O		H	p-tolylmagnesium bromide	**126**	90
H	OCH_2O		H	vinyl magnesium bromide	**127**	50
H	OCH_2O		H	m-tolylmagnesium chloride	**128**	96
OCH_3	H	H	H	p-tolylmagnesium bromide	**129**	70

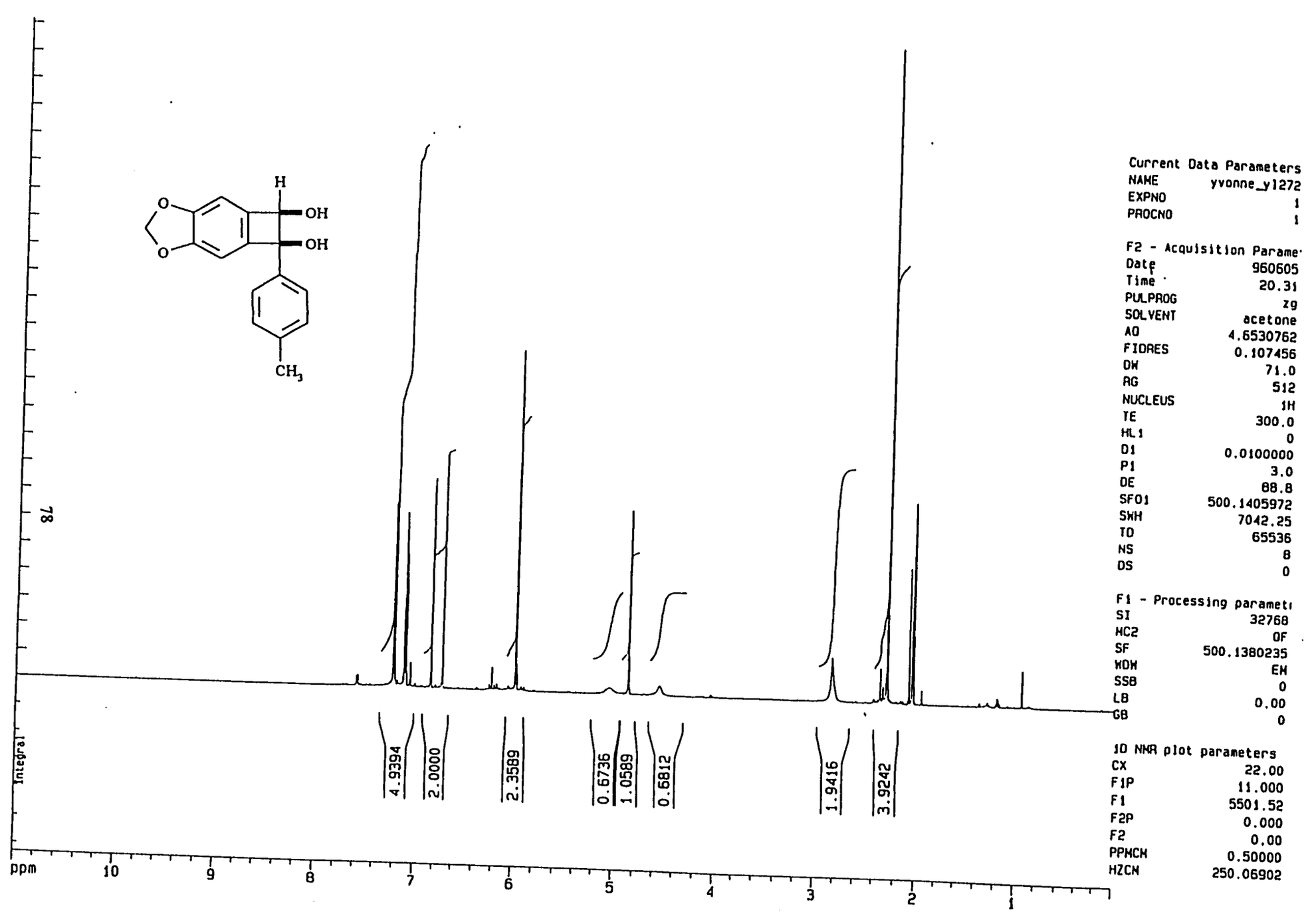
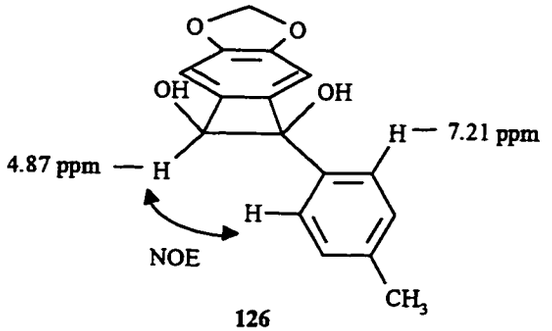


Fig. 14: ^{1}H NMR spectrum of **126**

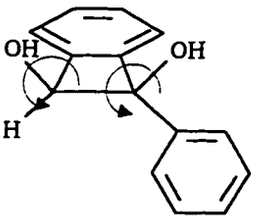
The relative stereochemistry of the two hydroxy groups was determined by NOE studies of **126**. (Figure 14 and 15) Irradiation of the benzylic proton at 4.87 ppm led to enhancement of the aromatic protons at 7.21 ppm, which correspond to the ortho protons on the aromatic ring. The experiment was also reversed, so that irradiation of the ortho protons at 7.21 ppm also led to enhancement of the benzylic proton. This indicates the proton and the pendant aromatic ring are on the same side of the four membered ring, meaning the two hydroxy groups are cis to each other.

Figure 15



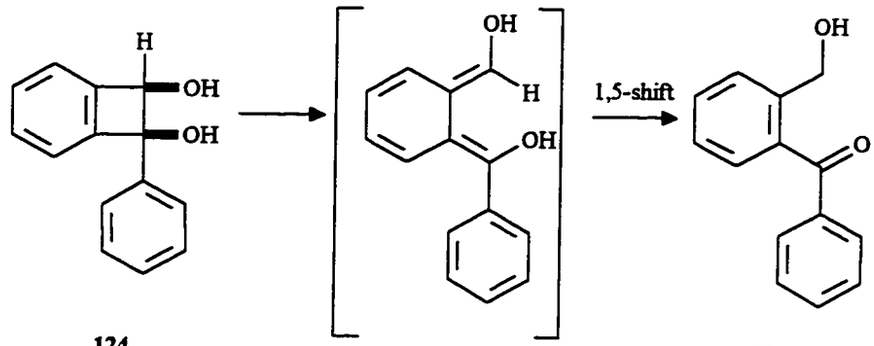
126

Figure 16



The formation of the cis-1,2 diol in the addition of Grignard reagents to the hydroxy ketones was considered unfortunate in the subsequent thermolysis reaction. The conrotatory ring opening of the cis 1,2-diol places one of the OH groups on the interior of the 1,3 diene system and it is thus liable to undergo a 1,5 hydrogen shift to yield an ortho-ketobenzylic alcohol. (Scheme 35)

Scheme 35



124 124a

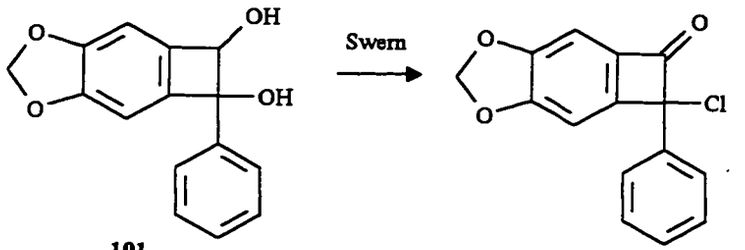
This was the result when the diol **124** was refluxed in p-xylene for 2.5h. The diol **124** was converted cleanly to **124a** as evidenced by the doublets at 7.65 and 7.37 ppm, indicative of an aromatic ring bearing one strongly electron withdrawing group, and the appearance of a singlet at 4.98 ppm which integrated for two protons.

Since thermolysis of the diol did not give the anthraquinone directly, the next step was to oxidize the diol to the hydroxy ketone, since it is known that thermolysis of these compounds at 110°C affords the desired anthraquinones.[18,38] The diol was subjected to a variety of oxidation conditions. As expected, many common transition metal based oxidizing agents such as chromium reagents and manganese dioxide cleaved the 1,2 dihydroxy compounds to afford dicarbonyl derivatives, as did the non-metallic Dess-Martin reagent.

The next set of reagents to be investigated were various forms of activated dimethyl sulfoxide, of which oxalyl chloride, or Swern reagent, is most common. Oxidation of the diol **101**, described in the previous chapter, by Swern oxidation furnished

the 2-chlorobenzocyclobutenone **101a** in 67% yield, however this yield was difficult to reproduce. (Scheme 36) It was thought that other activated DMSO reagents might give more satisfactory results.

Scheme 36



101 **101a**

One of the mildest ways of activating dimethyl sulfoxide is by using sulfur trioxide.[39] The most convenient way of introducing this reagent is through the sulfur trioxide-pyridine complex, which is a commercially available solid. When the diol **124** was treated with SO_3-pyridine activated DMSO, the desired ketone **131** was isolated in 50% yield. (Scheme 37) Similarly, the diol **126** was oxidized to the ketone **132** in 27% yield, and the diol **129** to the ketone **133** in 41% yield. The compound **132** showed, for example, the expected IR peak at 1758 cm^{-1} and a ^{13}C NMR peak at 188.6 for the cyclobutenone carbonyl group. (Figure 17) When the vinylic diol **144** was subjected to the same conditions, only a complex mixture of compounds was obtained.

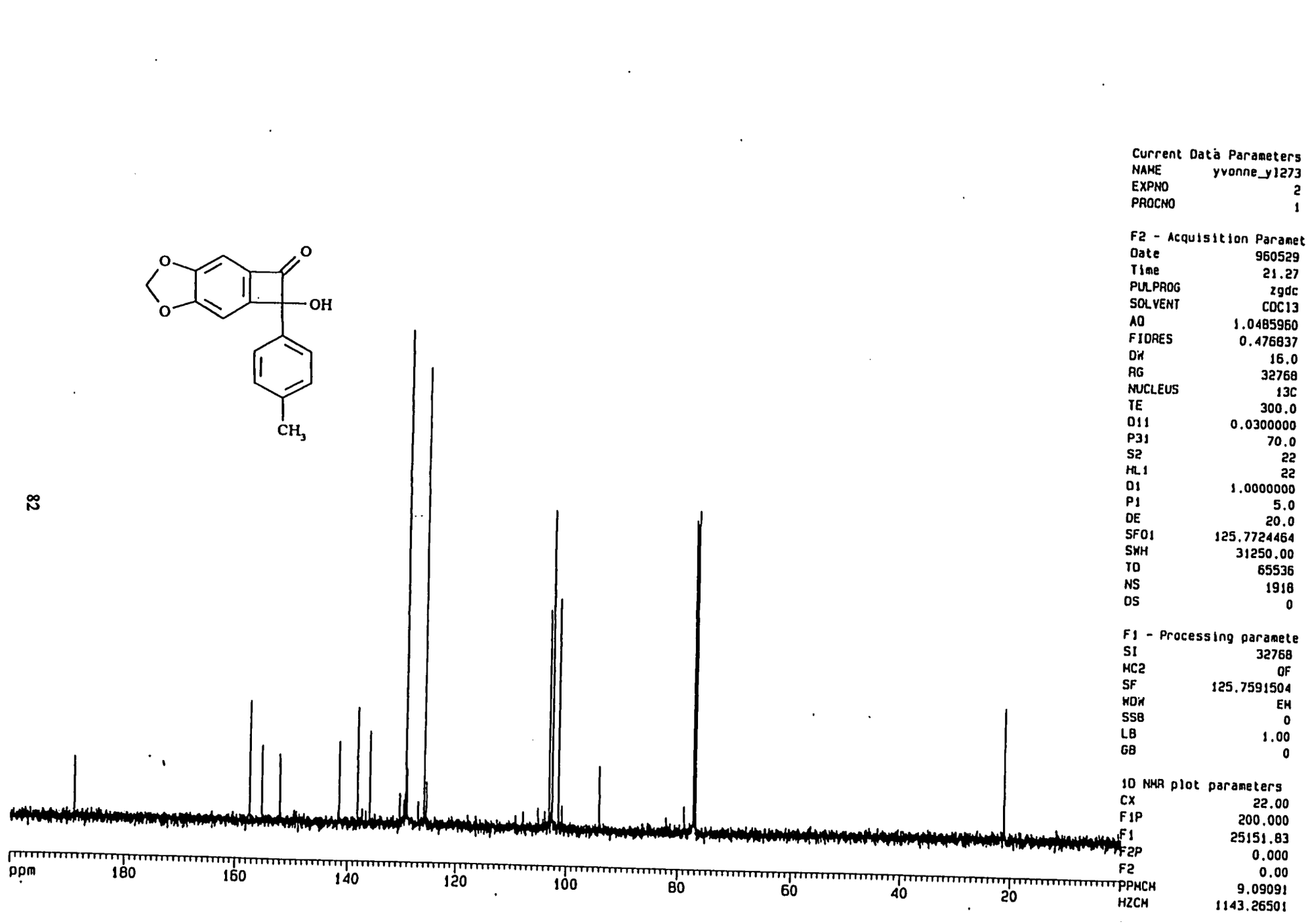
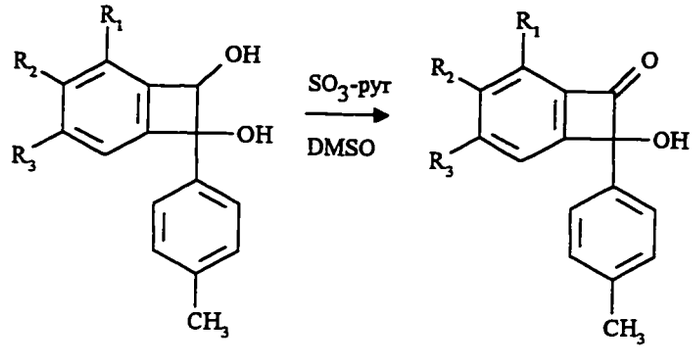


Fig. 17: ^{13}C NMR spectrum of **132**

Scheme 37



124 $R_1 = R_2 = R_3 = H$
126 $R_1 = H$, $R_2, R_3 = OCH_2O$
129 $R_1 = OCH_3$, $R_2 = R_3 = H$

131 $R_1 = R_2 = R_3 = H$ (50%)
132 $R_1 = H$, $R_2, R_3 = OCH_2O$ (27%)
133 $R_1 = OCH_3$, $R_2 = R_3 = H$ (41%)

Interestingly, when the diols **125** and **128** were subjected to the same conditions, the desired ketones did not form. (Scheme 38) The major products isolated were bright yellow solids. The compound which resulted from oxidation of **128** showed a doublet at 8.88 ppm in the ^1H NMR and the absence of any signals between the methyl peak at 2.41 ppm and the methylene dioxy peak at 6.03 ppm, indicating aromatization. (Figure 18) The mass spectrum of the compound which resulted from the oxidation of **128** showed a very stable molecular ion with odd mass, indicating the incorporation of nitrogen into the structure. The infrared spectrum showed an intense peak at 1634 cm^{-1} and the absence of a strained carbonyl group in the 1760 cm^{-1} range. The ^1H NMR which showed a low field doublet at δ = 8.88 ppm, an aromatic methyl group at δ = 2.41 ppm, the methylenedioxy hydrogens at δ = 6.03 ppm and 9 aromatic hydrogens agreed with the proposed structure **135**.

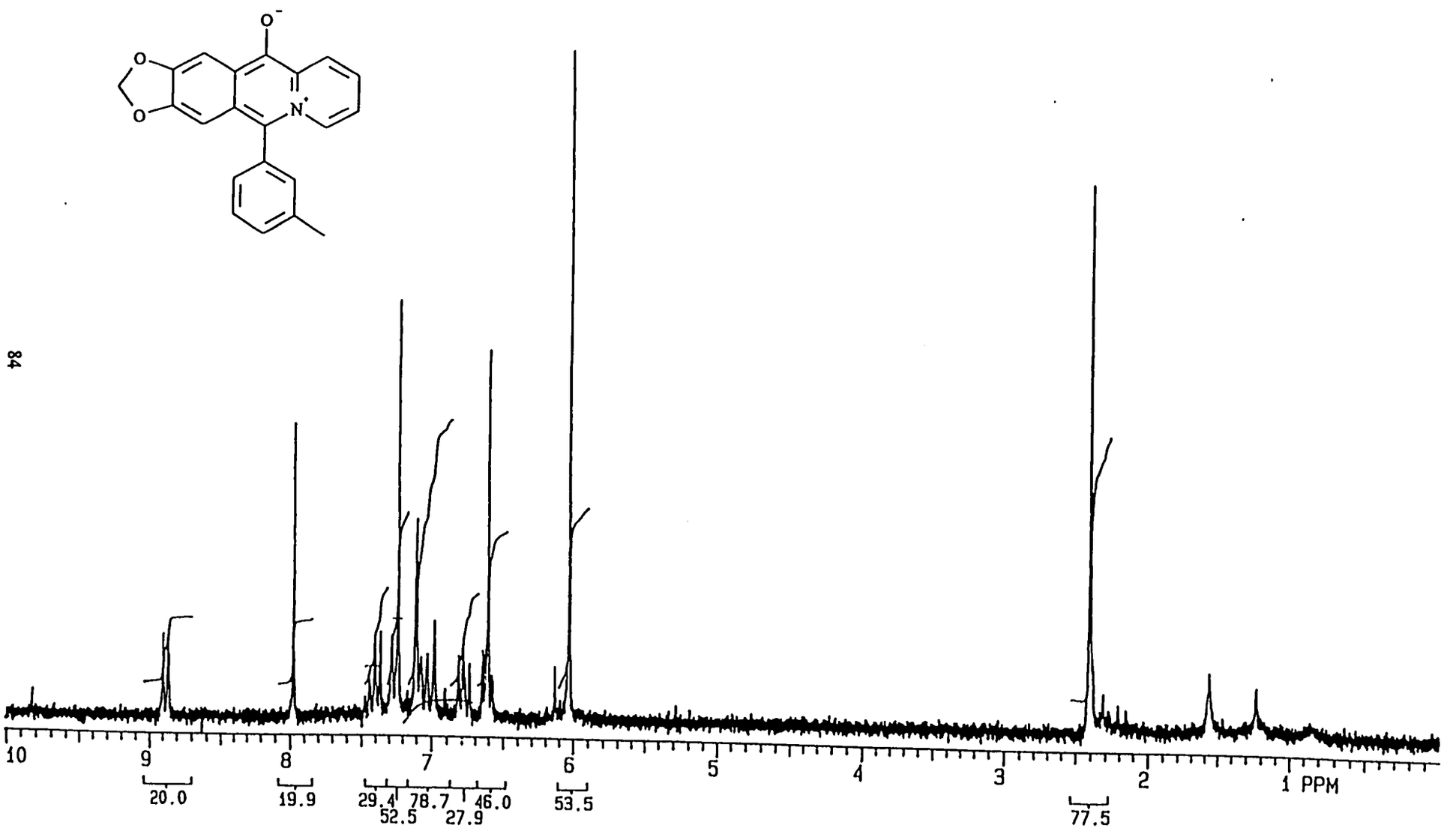
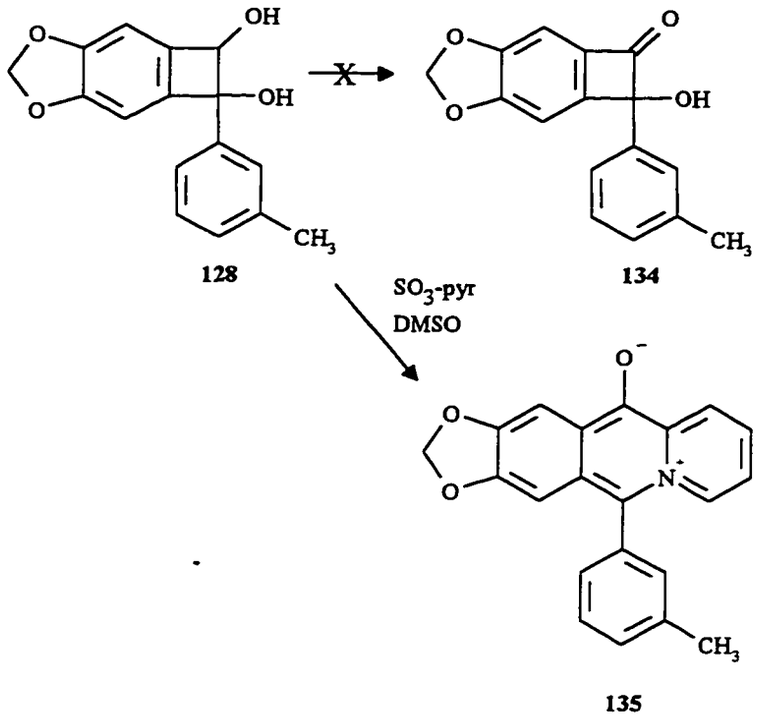


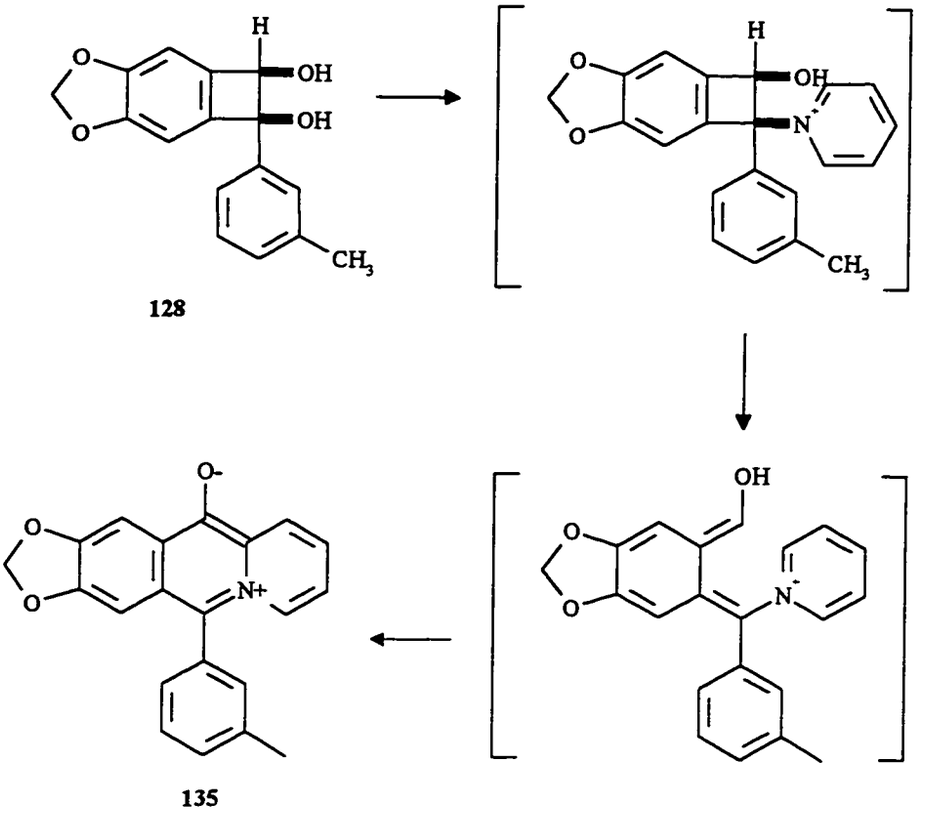
Fig. 18: ^{1}H NMR spectrum of 135

Scheme 38



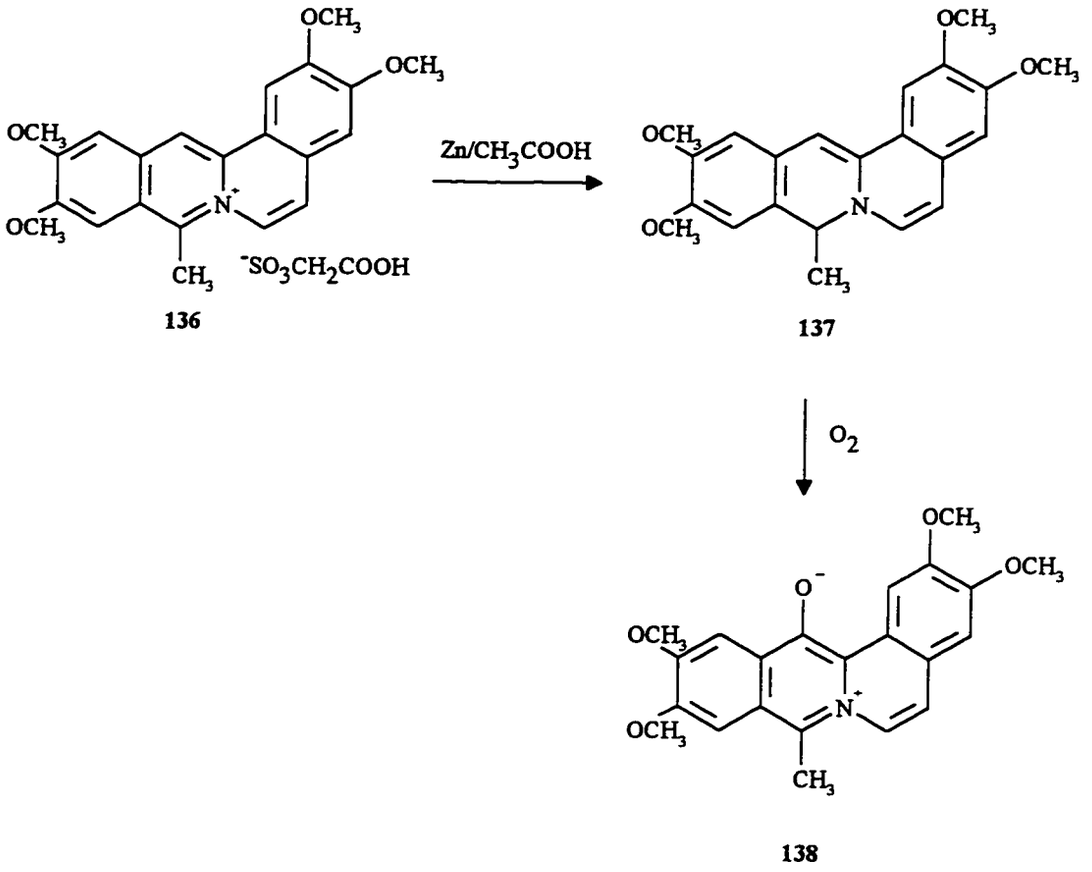
This compound would result from an exchange of the hydroxy group by pyridine, and subsequent ring opening and intramolecular trapping of the pyridinium moiety. (Scheme 39) A similar result was observed for **125**, however the yield was much lower since a mixture of compounds was formed.

Scheme 39



These compounds exhibit a tricyclic core resembling that of 13-hydroxyberberinium phenolbetaine **138**.[40] (Scheme 40) Kondo reported that the coralyne salt **136** was reduced with zinc in acetic acid to give the dihydrocoralyne **137**. The dihydrocoralyne was oxidized to give the betaine **138**. Coralyne alkaloid salts exhibit antileukemic activity. These experiments were conducted to try to elucidate the mechanism of oxidation of these berberine alkaloids by enzymes. It has been shown that **137** is oxidized to **138** under physiological conditions. This oxygenation represents a possible nonenzymic model of dioxygenase.

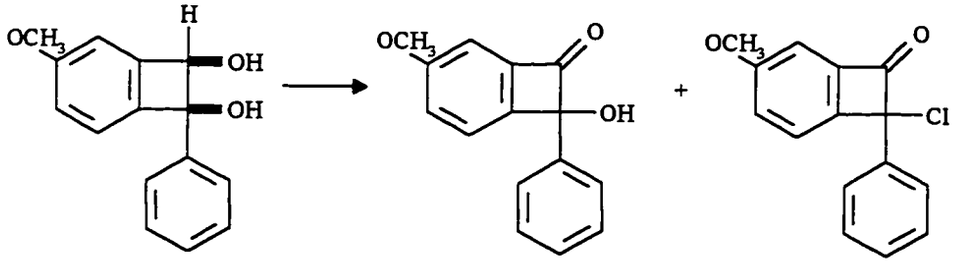
Scheme 40



136 137 138

The diol **125** was oxidized using oxalyl chloride and dimethyl sulfoxide, however a mixture of 30% of the desired hydroxy ketone **136** and 20% of the keto chloride **137** was obtained. (Scheme 41)

Scheme 41



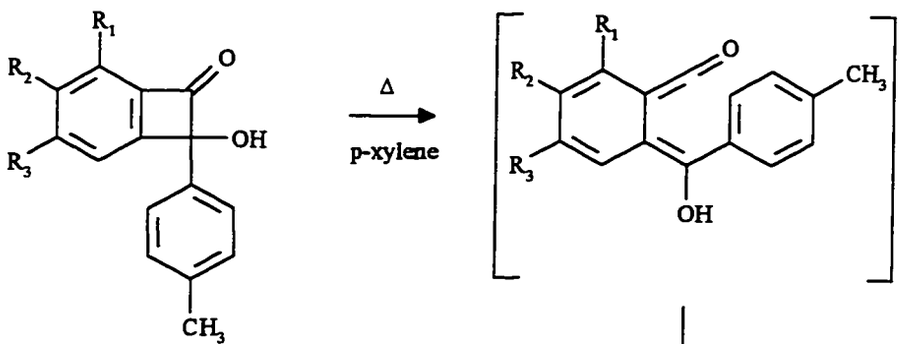
125 136 137

The final step was to confirm that the oxidized products could be converted by thermolysis to the corresponding anthraquinones. Thermolysis of the hydroxy ketones in refluxing p-xylene led to the exclusive formation of the anthraquinones as shown in Scheme 42. Yields ranged from 40-70%, however, these are generally unoptimized as a result of the difficulties encountered in preparing the precursors by oxidation. The ^1H NMR spectra showed a downfield shift in the aromatic protons as a result of the quinone. The compounds were typically yellow, crystalline solids with sharp melting points.

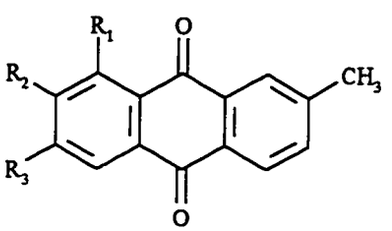
While the goal of synthesizing anthraquinones was realized by this method, a number of issues are worth revisiting. Since the key drawback of the route is the low yield for the oxidation step, it would be worth attempting the oxidation by other methods. Work is now underway to investigate the use of TEMPO as the oxidizing agent to carry out this reaction.

Another possibility is to revisit the direct cyclization of the nitrile. The results presented here confirm the cyclization of the nitrile did take place, but that the intermediate underwent ring opening. Performing the reaction at a lower temperature or under conditions which would prevent the iminium species from ring opening may be possible, if the iminium ion can somehow be stabilized.

Scheme 42



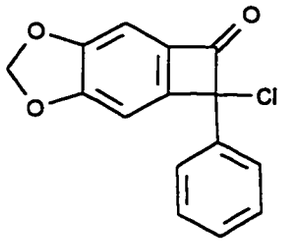
131 $R_1=R_2=R_3=H$
136 $R_1=R_3=H$, $R_2=OCH_3$
132 $R_1=H$, $R_2,R_3=OCH_2O$
133 $R_1=OCH_3$, $R_2=R_3=H$



138 $R_1=R_2=R_3=H$ (59% yield)
139 $R_1=R_3=H$, $R_2=OCH_3$ (59% yield)
140 $R_1=H$, $R_2,R_3=OCH_2O$ (67% yield)
141 $R_1=OCH_3$, $R_2=R_3=H$ (42% yield)

Experimental Section

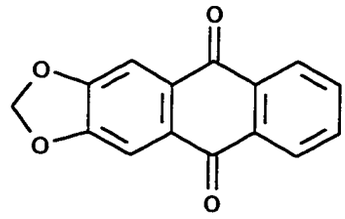
The general methods used can be found in the Experimental Section of Chapter 2.



101a

Oxalyl chloride (77 μL, 0.88 mmol) in 2 mL of dichloromethane was cooled to -55°C. Dimethyl sulfoxide (125 μL, 1.76 mmol) was added and stirred for 2 min. **117** (100 mg, 0.4 mmol) was added in 0.5 mL dichloromethane and was stirred for 15 min. Triethylamine (487 μL, 3.5 mmol) was added and the solution allowed to warm to 25°C. The organic solution was washed sequentially with water, 1% sulfuric acid, water, 5% sodium bicarbonate, and water. The solution was dried with anhydrous $MgSO_4$ and the solvent was evaporated. The resulting solid was recrystallized from 3:1 hexanes/ethyl acetate to give 70 mg of **102** (0.26 mmol, 65% yield).

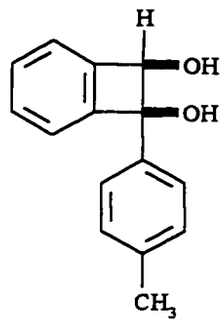
mp darkens 135-140°C **^{1}H NMR:** δ 6.16 (s, 2H), 6.87 (s, 1H), 7.23 (s, 1H), 7.32-7.42 (m, 2H), 7.51-7.57 (m, 2H) **IR:** 1768 cm^{-1} **HRMS:** Calc'd for $C_{15}H_9O_3Cl$: 272.0241. Found: 272.0127



101b

A solution of 50 mg of **101a** (1.8 mmol) was refluxed in 3 mL p-xylene for 105 min when TLC showed most of the starting material had been consumed, while a baseline spot was becoming intense. The solution was passed through a silica gel plug to remove baseline impurities and the solvent was removed under vacuum. The remaining solid was recrystallized from 3:1 hexanes/ethyl acetate to give yellow needles of **103** (20 mg, 40% yield).

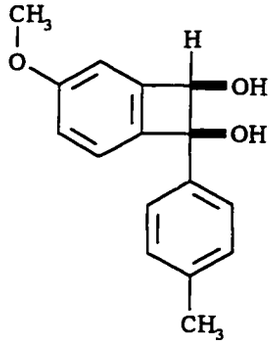
mp 222-224°C (lit 225-226°C[41]) **^{1}H NMR:** δ 6.17 (s, 2H), 7.68 (s, 2H), 7.70-7.79 (m, 2H), 8.24-8.29 (m, 2H)



124

p-Tolyl magnesium bromide (1.4 mL, 1M, 1.4 mmol) was added to a solution of 76 mg of **90** (0.57 mmol) at 0°C in 10 mL of THF, the cold bath was removed and stirring was continued for 1h. The reaction was quenched with saturated NH_4Cl, diluted with water, and extracted with diethyl ether. The organic extracts were washed with brine, dried with anhydrous $MgSO_4$, and the solvent was evaporated. The diol was purified by chromatography on silica gel (3:1 hexanes/ethyl acetate) to afford 130 mg of **124** (0.57 mmol, >95% yield).

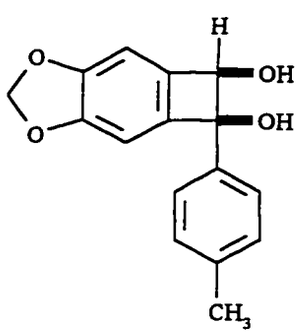
mp 93-94°C **^{1}H NMR:** δ 2.32 (s, 3H), 5.18 (s, 1H), 7.11-7.15 (m, 2H), 7.25-7.40 (m, 2H) **^{13}C NMR:** δ 21.0, 80.1, 83.0, 122.8, 124.0, 125.5, 128.8, 129.8, 130.2, 137.0, 139.3, 146.0, 148.4 **IR:** 3579 cm^{-1} **HRMS:** Calc'd for $C_{15}H_{14}O_2$: 226.0994. Found: 226.0992



125

The procedure was the same as that used to prepare **124**, using **92** as the starting material. **92** (0.49 mmol, 80 mg) was reacted with 2 equivalents of p-tolyl magnesium bromide to afford 110 mg of **125** (0.43 mmol, 88% yield)

mp 104-106°C **^{1}H NMR:** δ 2.31 (s, 3H), 3.1-3.7 (br, 1-OH), 3.78 (s, 3H), 5.07 (s, 1H), 6.90-6.97 (m, 2H), 7.09-7.13 (m, 2H), 7.21-7.25 (m, 3H) **^{13}C NMR:** δ 21.1, 55.5, 79.6, 82.2, 107.9, 118.4, 124.2, 125.5, 129.0, 129.3, 137.2, 139.5, 140.0, 147.2, 161.4 **IR:** 3571, 3682 cm^{-1} **HRMS:** Calc'd for $C_{16}H_{16}O_3$: 256.1100. Found: 256.1114

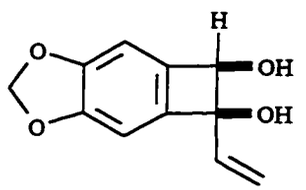


126

The same procedure was used as described for **124**, but the starting material used was **59**. **59** (0.73 mmol, 130 mg) afforded 190 mg of **126** (0.75 mmol, >95% yield) after recrystallization from chloroform.

mp 140-142°C (dec) **^{1}H NMR:** δ 2.28 (s, 3H), 4.60 (br, 1H), 4.87 (s, 1H), 5.08 (br, 1H), 5.98 (d, J=1 Hz), 6.73 (s, 1H), 6.85 (s, 1H), 7.11 (d, J=8.5 Hz, 2H), 7.21 (d, J=8.5 Hz, 2H)

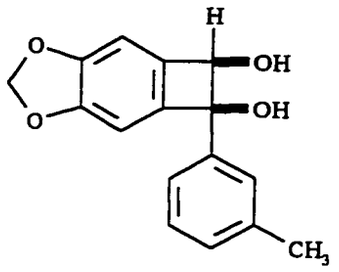
^{13}C NMR: δ 21.0, 79.8, 81.9, 101.6, 104.2, 105.5, 126.4, 129.4, 137.1, 140.6, 141.9, 143.6, 149.9, 150.1 **IR:** 3432 cm-1(br) **HRMS:** Calc'd for $C_{16}H_{14}O_4$: 270.0892 Found: 270.0907



127

Vinyl magnesium bromide (1.9 mL, 1M, 1.9 mmol) was added to a solution of 156 mg of **59** (0.88 mmol) in 8 mL of THF at 0°C. The cold bath was removed and the solution was stirred for 1 h. The reaction was cooled to 0°C and quenched with saturated NH_4Cl. The mixture was diluted with water, extracted with diethyl ether, dried with anhydrous $MgSO_4$, and the solvent was evaporated. Chromatography on silica gel yielded 80 mg of **127** (0.39 mmol, 45% yield).

mp oil **^{1}H NMR:** δ 3.8-4.20(br,1H), 4.83(s,1H), 5.10-5.24(m,2H), 5.90(d,2H), 5.90-6.10(m,1H), 6.67(s,1H), 6.74(s,1H) **^{13}C NMR:** δ 77.4, 81.1, 100.6, 103.7, 105.0, 115.1, 138.3, 138.7, 140.5, 149.1 **IR:** 3574 cm^{-1} **HRMS:** Calc'd for $C_{11}H_{10}O_4$: 206.0579. Found: 206.0591

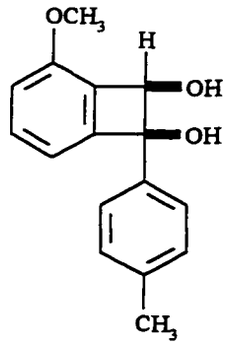


128

m-Tolyl magnesium chloride (1.7 mL, 1M, 1.7 mmol) was added to a solution of 140 mg of **59** (0.79 mmol) in 8 mL of THF at 0°C. The cold bath was removed and the solution stirred for 1 h. The reaction was cooled to 0°C and quenched with saturated NH_4Cl. The mixture was diluted with water, extracted with diethyl ether, dried with anhydrous $MgSO_4$ and the solvent was evaporated. Chromatography on silica gel (3:1 hexanes/ethyl acetate) afforded 190 mg of **128** (0.70 mmol, 89% yield)

mp 108-109°C **^{1}H NMR:** δ 2.30 (s, 3H), 3.4-3.9 (br, 1H), 4.95 (s, 2H), 5.92 (s, 2H), 6.72 (s, 1H), 6.79 (s, 1H), 7.05-7.26 (m, 4H) **^{13}C NMR:** δ 21.4, 78.7, 81.4, 119.2, 113.4, 111.4, 116.0, 122.6, 126.1, 128.2, 137.9, 138.8, 141.4, 142.2, 149.2, 149.4

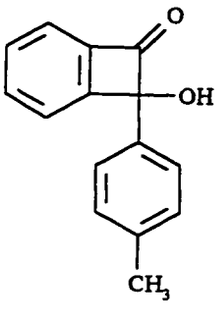
IR: 3563 cm^{-1} **HRMS:** Calc'd for $C_{16}H_{14}O_4$: 270.0892. Found: 270.0909



129

p-Tolylmagnesium bromide (1.5 mL, 1M, 1.5 mmol) was added to a solution of 110 mg of **91** (0.67 mmol) in dry THF. The solution was stirred at 25°C for 1h, quenched with saturated NH_4Cl, extracted with diethyl ether, the organic extracts dried with anhydrous $MgSO_4$ and the solvent was evaporated. Column chromatography on silica gel yielded 119 mg of **129** (0.46 mmol, 69%).

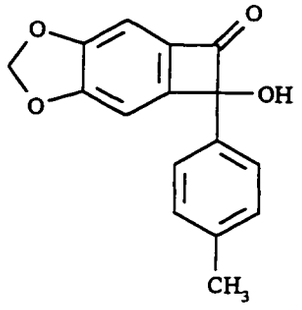
mp oil **^{1}H NMR:** δ 2.42(s,3H), 4.03(s,3H), 5.29(s,1H), 6.93(dd,1H), 7.21(d,2H), 7.32-7.40(m,5H) **^{13}C NMR:** δ 21.1, 57.0, 79.8, 82.6, 114.6, 116.0, 125.6, 129.0(2), 132.1, 137.2, 139.2, 150.3, 155.8 **IR:** 3582, 3681 cm^{-1} **HRMS:** Calc'd for $C_{16}H_{16}O_3$: 256.1100. Found: 256.1085.



131

A solution of 0.21 g of sulfur trioxide pyridine complex (1.3 mmol) in 1 mL of dimethyl sulfoxide (DMSO) was added to a solution of 100 mg of **124** (0.44 mmol) and triethylamine (0.4 mL, 29 mmol) in 0.5 mL of DMSO at 25°C. Water (10 mL) was added and the mixture was extracted with diethyl ether. ^{1}H NMR spectroscopy showed the product to be pure **131** (50 mg, 0.22 mmol, 50% yield).

mp oil **^{1}H NMR:** δ 2.32 (s, 3H), 7.15 (dd, 2H), 7.33 (dd, 2H), 5.59 (m, 2H), 7.70 (m, 1H), 7.80 (dd, 1H) **IR:** 1738, 3684 cm^{-1} **LRMS:** Calc'd for $C_{15}H_{12}O_2$: 224.1. Found: 224.0.

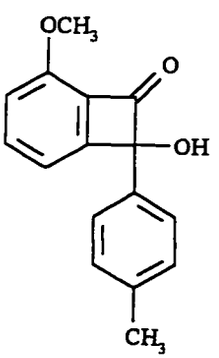


132

The procedure used was the same as for the preparation of **131**. Thus 110 mg **126** (0.4 mmol) afforded 30 mg of **132** (0.1 mmol, 25% yield).

mp 134-135°C **^{1}H NMR:** δ 2.31 (s, 3H), 3.0-3.4 (br, 1-OH), 6.11 (s, 2H), 6.89 (s, 1H), 7.09 (s, 1H), 7.14 (d, J=8.3 Hz, 2H), 7.29 (d, J=8.3 Hz, 2H) **^{13}C NMR:** δ 21.1, 93.9, 101.3, 102.5, 103.0, 125.9, 129.2, 135.85, 138.2, 141.4, 151.9, 155.2, 157.3, 188.6

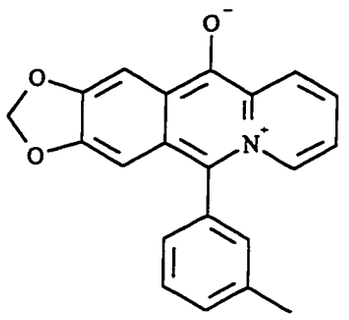
IR: 1758, 3570 cm^{-1} **HRMS:** Calc'd for $C_{16}H_{12}O_4$: 268.0736. Found: 268.0735



133

A solution of 225 mg of sulfur trioxide pyridine complex (1.4 mmol) in 1 mL of dimethyl sulfoxide (DMSO) was added to a solution of 120 mg of **129** (0.47 mmol) and triethylamine (0.50 mL, 3.6 mmol) in 0.5 mL of DMSO at 25°C. Water (10 mL) was added and the mixture was extracted with diethyl ether. ^{1}H NMR spectroscopy showed 70% conversion of the starting material to the desired ketone. Silica gel column chromatography (3:1 hexanes/ethyl acetate) yielded 50 mg of **133** as a yellow oil (0.20 mmol, 43% yield)

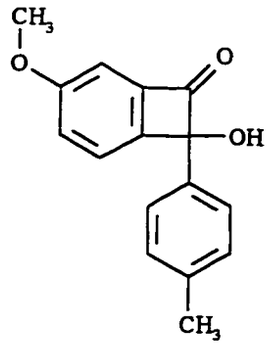
mp oil **^{1}H NMR**: δ 2.32(s,3H), 2.9-3.8(br,1H), 4.09(s,3H), 6.97(dd,J=8.3,0.6Hz,1H), 7.15(d,J=7.8Hz,2H), 7.24(d,J=7.2,0.6Hz,1H), 7.33(d,J=7.8Hz,2H), 7.55(dd, J=7.2,8.3 Hz,1H) **^{13}C NMR:** δ 21.1, 59.8, 95.1, 114.4, 117.8, 126.1, 129.2, 132.5, 135.5, 138.3, 138.9, 155.1, 159.0, 187.8 **IR:** 1764, 3570 cm^{-1}. **HRMS:** Calc'd for $C_{16}H_{14}O_3$: 254.0943. Found: 254.0943.



135

The compound was prepared in the same manner as **131.** Diol **128** (70 mg, 0.26 mmol) afforded 25 mg of **135** (0.076 mmol, 29% yield).

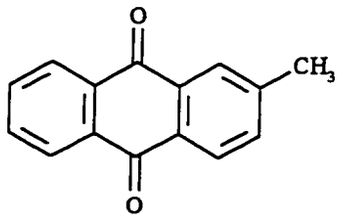
mp 185°C (dec) **^{1}H NMR:** δ 2.41 (s, 3H), 6.03 (s, 2H), 6.61 (s, 1H), 6.59-6.63 (td, J=6.2, 1.3 Hz, 1H), 6.77-6.80 (ddd, J=9.4, 6.2, 1.3 Hz, 1H), 6.99-7.02 (m, 1H), 7.06-7.11 (m, 2H), 7.26 (d, J=7.3 Hz, 1H), 7.40 (t, J=7.3 Hz, 1H), 7.98 (s,1H), 8.88 (dt, J=7.6, 1.1 Hz, 1H) **IR:** 1634 cm^{-1} **HRMS:** Calc'd for $C_{21}H_{15}NO_3$: 329.1053. Found: 329.1040.



136

Oxalyl chloride (75 μL, 0.86 mmol) in 2 mL of dichloromethane was cooled to -55°C. Dimethyl sulfoxide (120 μL, 1.69 mmol) was added and stirred for 2 min. **125** (100 mg, 0.39 mmol) was added in 0.5 mL of dichloromethane and stirred for 15 min. Triethylamine (500 μL, 3.6 mmol) was added and the solution allowed to warm to 25°C. The organic solution was washed sequentially with water, 1% sulfuric acid, water, 5% sodium bicarbonate, and water. The solution was dried with anhydrous $MgSO_4$ and the solvent was evaporated. Column chromatography on silica gel (hexanes/ethyl acetate) yielded 20 mg of the keto chloride (20% yield) and 30 mg of the desired hydroxyketone **136** (30% yield).

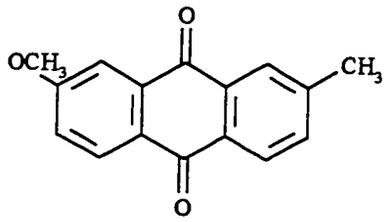
mp oil **^{1}H NMR:** δ 2.30 (s, 3H), 3.82 (s, 3H), 6.94 (s, 1H), 7.10-7.30 (m, 5H), 7.60-7.70 (m, 1H) **^{13}C NMR:** δ 21.1, 55.8, 95.3, 103.4, 124.4, 126.0, 126.3, 129.3, 136.0, 138.2, 149.5, 152.9, 162.7, 191.0 **IR:** 1774, 3400(br) cm^{-1} **HRMS:** Calc'd for $C_{16}H_{14}O_3$: 254.0943. Found: 254.0954.



138

124 (50 mg, 0.22 mmol) was refluxed for 1h in p-xylene when the reaction was complete by TLC. The solvent was removed by distillation under low pressure and the yellow solid was purified by silica gel column chromatography (9:1 hexanes/ethyl acetate) to give 30mg of a yellow crystalline solid (0.13 mmol, 59% yield)

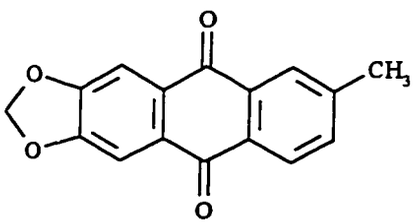
mp 174-176°C (lit 177°C[42]) **^{1}H NMR:** 2.52 (s, 3H), 7.57 (dd, J=7.9, 0.73 Hz, 1H), 7.75-7.79 (m, 2H), 8.09 (s, 1H), 8.18 (d, J=8.0 Hz, 1H), 8.26-8.31 (m, 2H).



139

125 (15 mg, 0.059 mmol) was refluxed in p-xylene for 1h and the solvent was evaporated. Silica gel column chromatography (9:1 hexanes/ethyl acetate) afforded 9 mg of **139** (0.035 mmol, 59% yield).

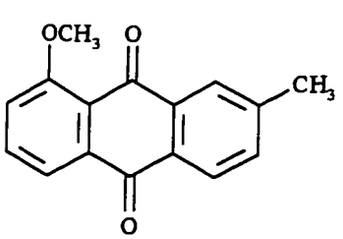
mp 160-162°C (lit 162-163°C[43]) **^{1}H NMR:** δ 2.52 (s, 3H), 3.98 (s, 3H), 7.24 (dd, J=8.3, 2.6 Hz, 1H), 7.58 (d, J=8.0 Hz, 1H), 7.72 (d, J=2.6 Hz, 1H), 8.08 (s, 1H), 8.19 (d, J=8.0, 1H), 8.25 (d, J=8.3 Hz, 1H).



140

132 (25 mg, 0.09 mmol) was refluxed in 3 mL of p-xylene for 1h. The solvent was evaporated. The crude mixture was purified by silica gel column chromatography (9:1 hexanes/ethyl acetate) and recrystallized from ethyl acetate yielding 16 mg of yellow, crystalline **140** (0.06 mmol, 67% yield)

mp 249-250°C **^{1}H NMR:** δ 2.50 (s, 3H), 6.14 (s, 2H), 7.53 (dd, J=7.8, 1.1 Hz, 1H), 7.65 (s, 2H), 8.03 (s, 1H), 8.13 (d, J=7.8 Hz, 1H). **^{13}C NMR:** δ 21.5, 102.2, 104.7, 106.0, 126.9, 127.1, 130.4, 130.5, 130.7, 132.8, 134.2, 144.6, 152.1, 152.2, 181.6, 182.0. **HRMS:** Calc'd for $C_{16}H_{10}O_4$: 266.0579 Found: 266.0589



141

133 (46 mg, 0.18 mmol) was refluxed for 8h in p-xylene. The solvent was evaporated. ^{1}H NMR showed about 75% conversion of the starting material, however a significant amoung of baseline material was apparent by both ^{1}H NMR and TLC.

Separation by silica gel column chromatography (3:1 hexanes/ethyl acetate) returned 6 mg of the starting material and 19 of mg **141** (0.075 mmol, 42% yield).

mp 165-166°C **^{1}H NMR:** δ 2.49 (s, 3H), 4.02 (s, 3H), 7.30(dd, J=8.4, 1.0Hz, 1H), 7.50 (m, 1H), 7.68 (m, 1H), 7.92 (dd, J=7.7, 1.2 Hz, 1H), 8.07 (m, 2H).

^{13}C NMR: δ 22.0, 56.5, 117.8, 119.7, 121.6, 126.8, 127.5, 130.2, 134.1, 134.9, 135.8, 145.4, 160.3, 182.8, 183.2. **IR:** 1586, 1670 cm^{-1}. **HRMS:** Calc'd for $C_{16}H_{12}O_3$: 252.0787. Found: 252.0803.

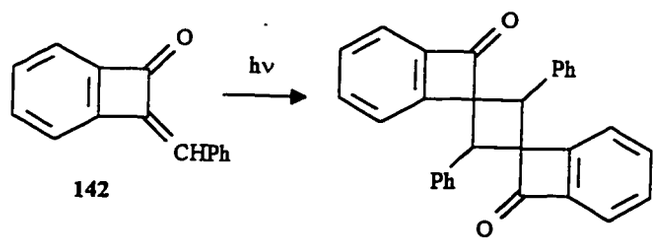
Chapter 5 Reactions of 2-Hydroxybenzocyclobutenones

A number of other reactions were carried out on the 2-hydroxy-benzocyclobutenones prepared in Chapter 2. These reactions are outlined in the following sections.

A. Preparation of Mono- and Bis-Alkylidene Benzocyclobutenones

Alkylidene benzocyclobutenones have been prepared during previous work in our lab as described in Chapter 2. These compounds exhibited interesting photochemical properties. (Scheme 43) It was found that when the Z isomer of **142** was irradiated at 250 or 350 nm, a photostationary mixture of E and Z isomers was obtained. When irradiation was continued at 350 nm for several hours, dimers formed as a result of [2+2] cyclization. The major dimer was the 'head to tail' product **143**.

Scheme 43

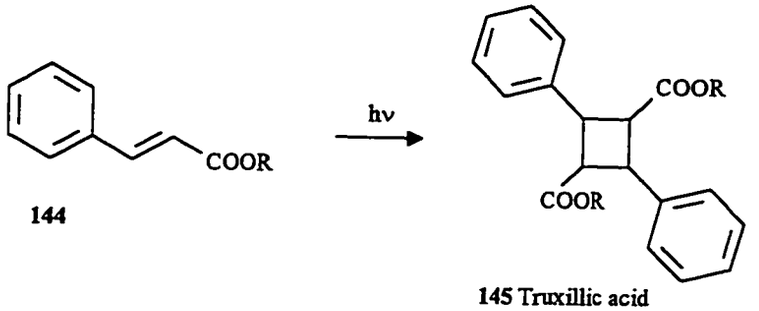


142 **143**

It was expected that replacement of the phenyl group in **142** by others, in particular an ester group, would give rise to **144**, based on the experiments described

above and the analogy of the photochemical dimerization of cinnamic acids and their esters to truxillic acid derivatives such as **145**. (Scheme 44)

Scheme 44



144 **145** Truxillic acid

Reaction of carbomethoxymethylenetriphenylphosphorane with **57** afforded an 8:1 mixture of alkylidene benzocyclobutenes. The stereochemistry of the major isomer was established by NOE which showed enhancement of the aromatic proton at 7.32 ppm upon irradiation of the vinyl proton at 5.60 ppm. (Figures 19 and 20) The assignment of the vinyl proton and the benzylic proton adjacent to the silyl ether was based on HMQC data. Base catalyzed ring opening of **146** occurred in essentially quantitative yield upon treatment with TBAF in THF for 30 minutes at 25°C to afford **147**. The stereochemistry of **147** was evident from the trans coupling constant of 16 Hz between Ha and Hb. This confirmed the stereochemistry of **146** was Z, since this reaction has been shown to proceed with retention of configuration.[44] (Scheme 45)

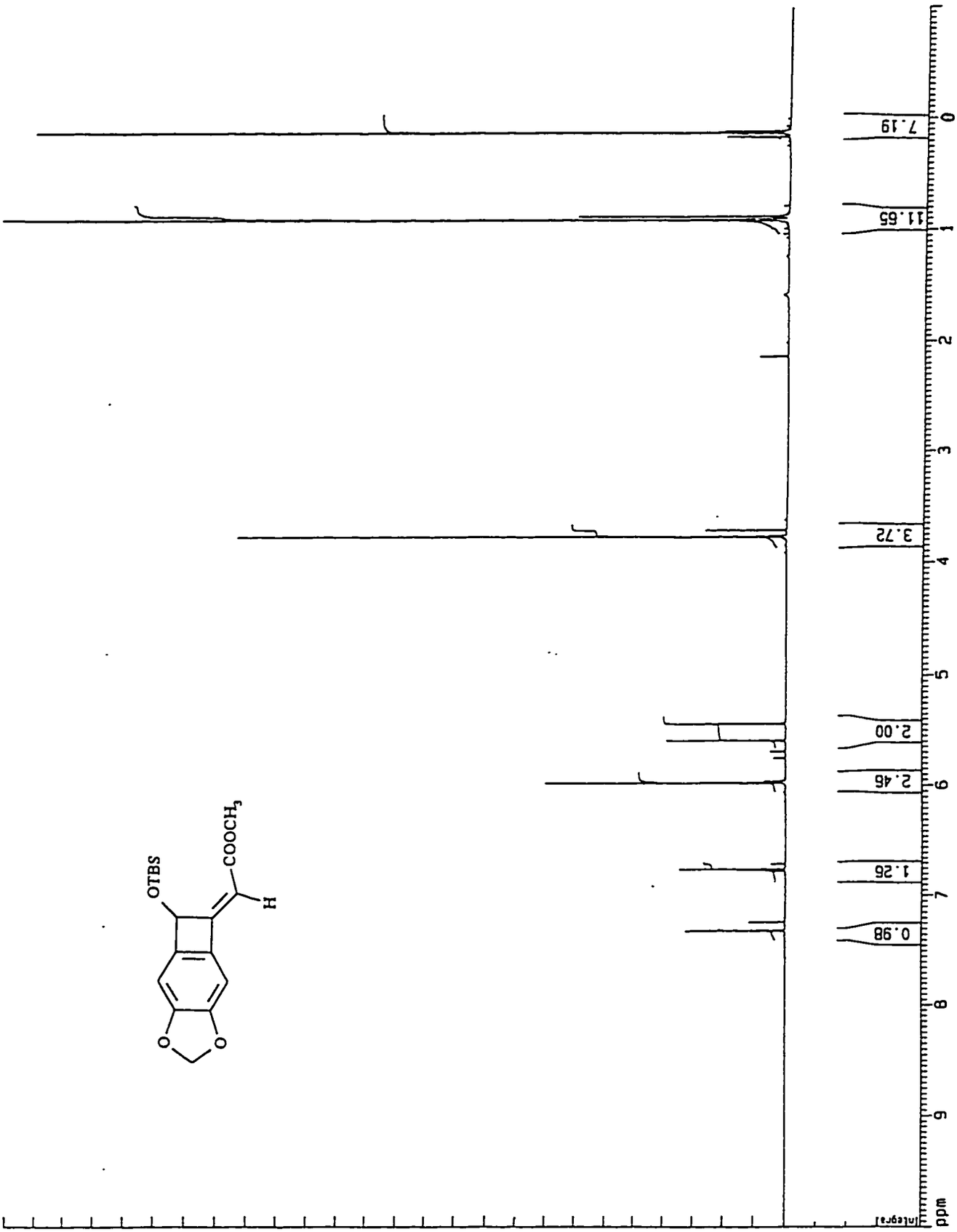


Fig. 20: ^{1}H NMR spectrum of 146 Z/E = 8/1

Scheme 45

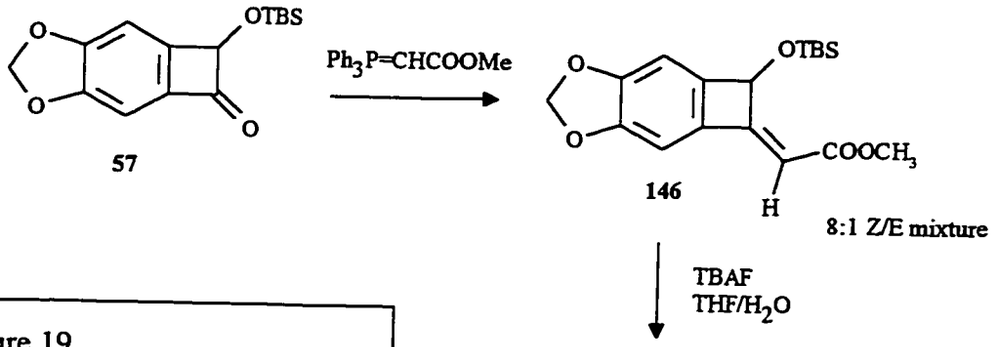
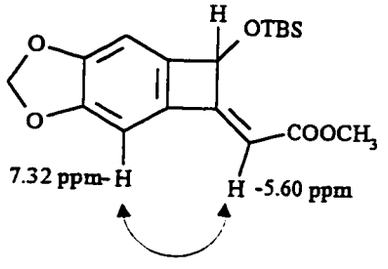
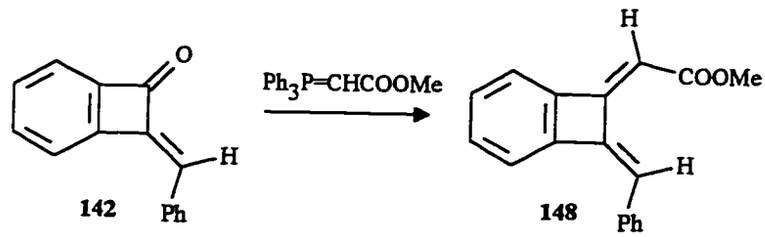


Figure 19



Accessibility to alkylidene benzocyclobutenones such as **142**, either via Stille coupling reaction or from reaction of the 2-silyloxybenzocyclobutenones with Wittig reagents followed by deprotection and oxidation allows one to prepare unsymmetrical bis-alkylidene benocyclobutenones such as **148**. This represents the first example of such compounds. It is evident that starting with the protected 2-hydroxybenzocyclobutenones, a wide variety of unsymmetrical bis-alkylidene benzocyclobutenones could be prepared. The compound **142** was treated with a stabilized Wittig reagent to afford **148**. (Scheme 46) **142** had been previously prepared via Stille coupling as described in Chapter 2.

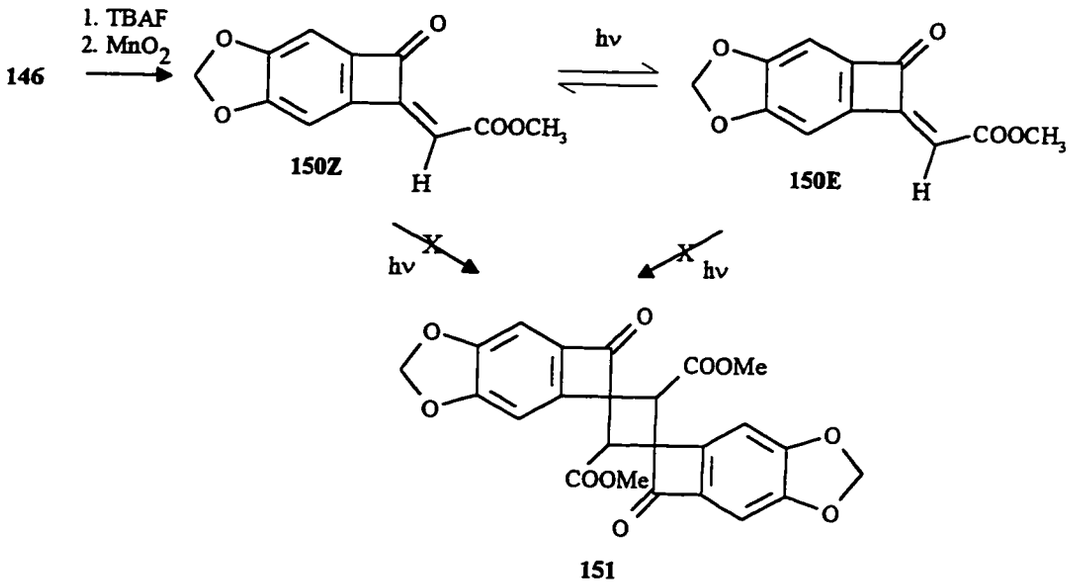
Scheme 46



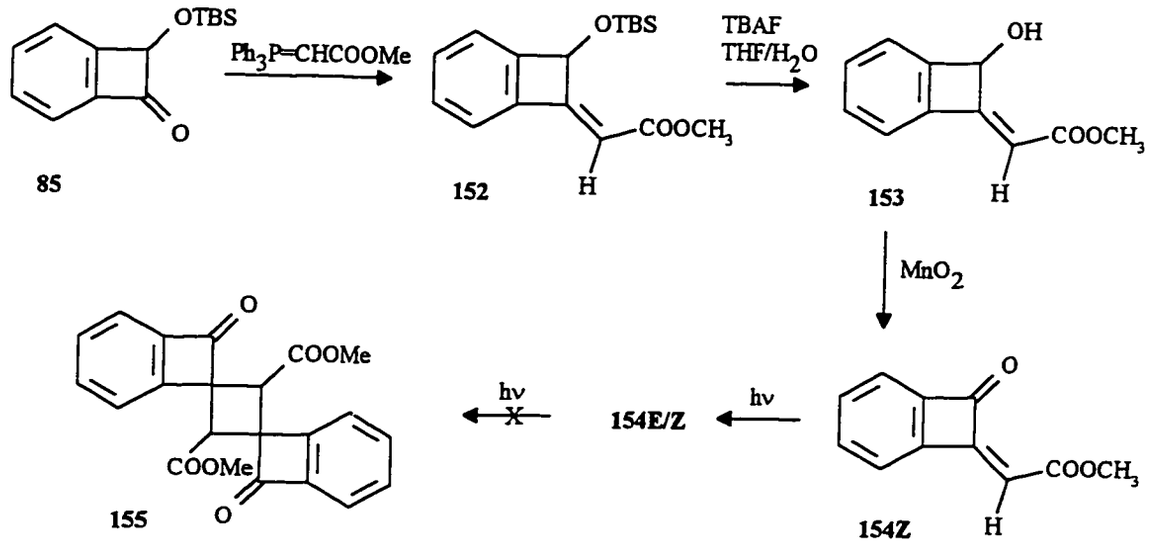
The ester **146** was deprotected with tetrabutylammonium flouride to afford the free alcohol which was oxidized with manganese dioxide to **150**. (Scheme 47) This compound was degassed and irradiated at 350 nm in an attempt to form the dimer **151**, however, only isomerization about the double bond to give a 3/1 mixture of of Z/E isomers was observed as evidenced by the appearance of a new singlet slightly upfield of the vinylic proton. (Figure 21, shown for **154**) The isomer mixture was also separated to confirm the identity of the second isomer by HRMS and NMR studies. Prolonged exposure led to substantial decomposition of the starting material.

An analogue of **150**, the unsubstituted **154**, was also prepared to determine whether the methylenedioxy group was having an effect on the photochemistry. The benzocyclobutenone **85** was reacted with the Wittig reagent, deprotected with TBAF, and oxidized with manganese dioxide. The same result was obtained on irradiation, isomerization to give a 3:1 photostationary mixture of Z/E isomers but no dimerization to **155** took place. (Scheme 48)

Scheme 47



Scheme 48



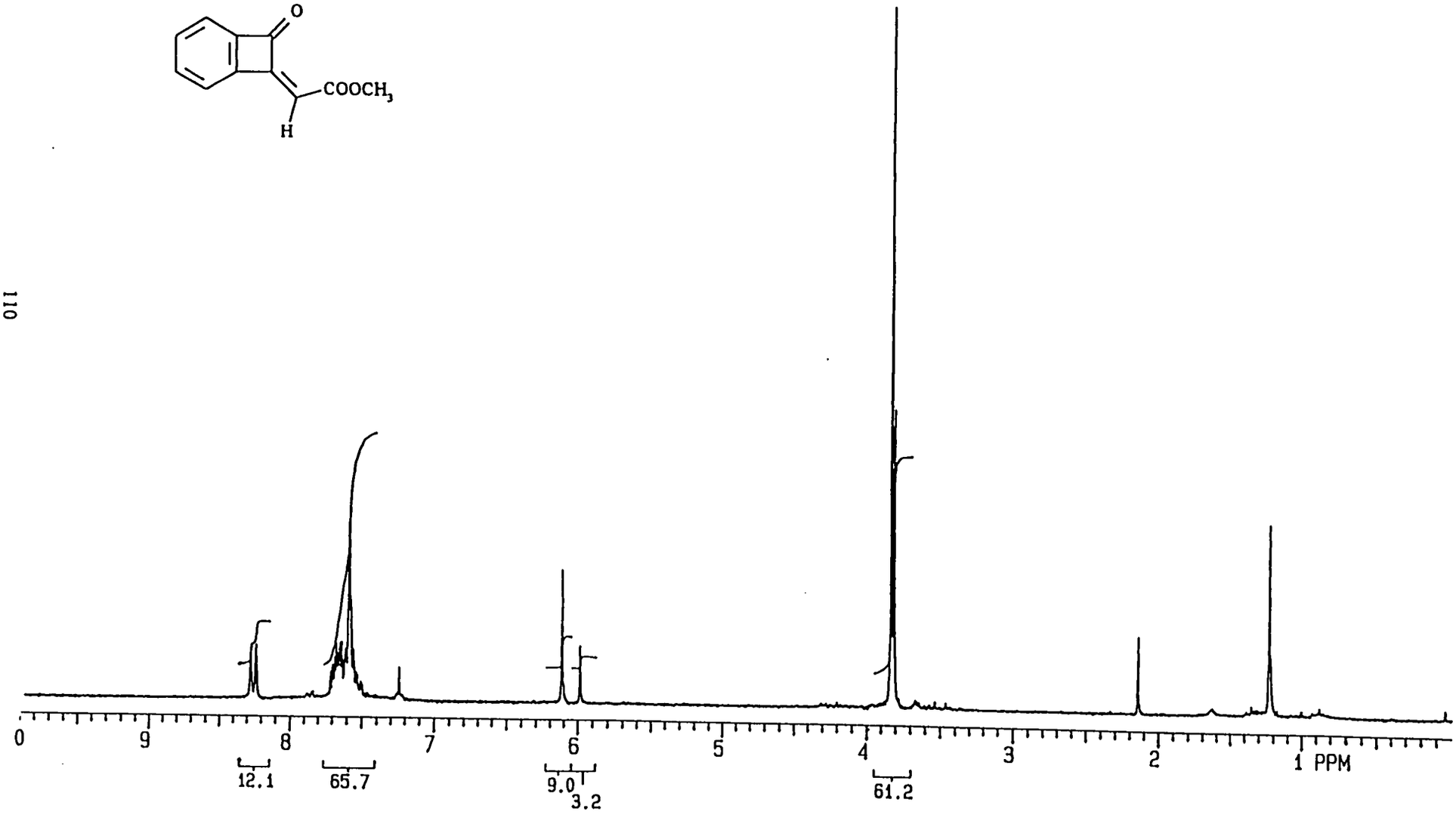


Fig. 21: ^{1}H NMR spectrum of 154 after irradiation

B. Reaction with Lawesson's Reagent

Lawesson's reagent has been used extensively in the conversion of ketones to thioketones.[45] Generally only aryl or highly hindered aliphatic thioketones are stable enough to be isolable. Often only dimers and trimers of the thioketones are isolated. The 2-silyloxybenzocyclobutenone **85** was treated with Lawesson's reagent in refluxing toluene. We had anticipated formation of the corresponding thiolactone as a stable entity, since such a compound was assumed to be sufficiently hindered to prevent the formation of dimers or trimers. As well, thioenolization was not an option.

The major product, isolated in 28% yield, could be recrystallized from methanol as bright red prisms and melted at 70°C. The spectroscopic properties appeared to fit that expected for the thioketone, except for the ^{1}H NMR resonance at 6.81 ppm which appeared to be too far downfield to correspond to the benzylic proton. The ^{13}C NMR showed a resonance at 226 ppm, which agrees with the expected value for the thioketone calculated from Lawesson's formula. (Figure 22) The IR showed the disappearance of the carbonyl peak and a new peak at 1210 cm^{-1}. Mass spectral data indicated the presence of a second sulfur atom in the molecule. The product was identified as the dithiolactone **156** by X-ray crystallography of a single crystal. (Figure 23)

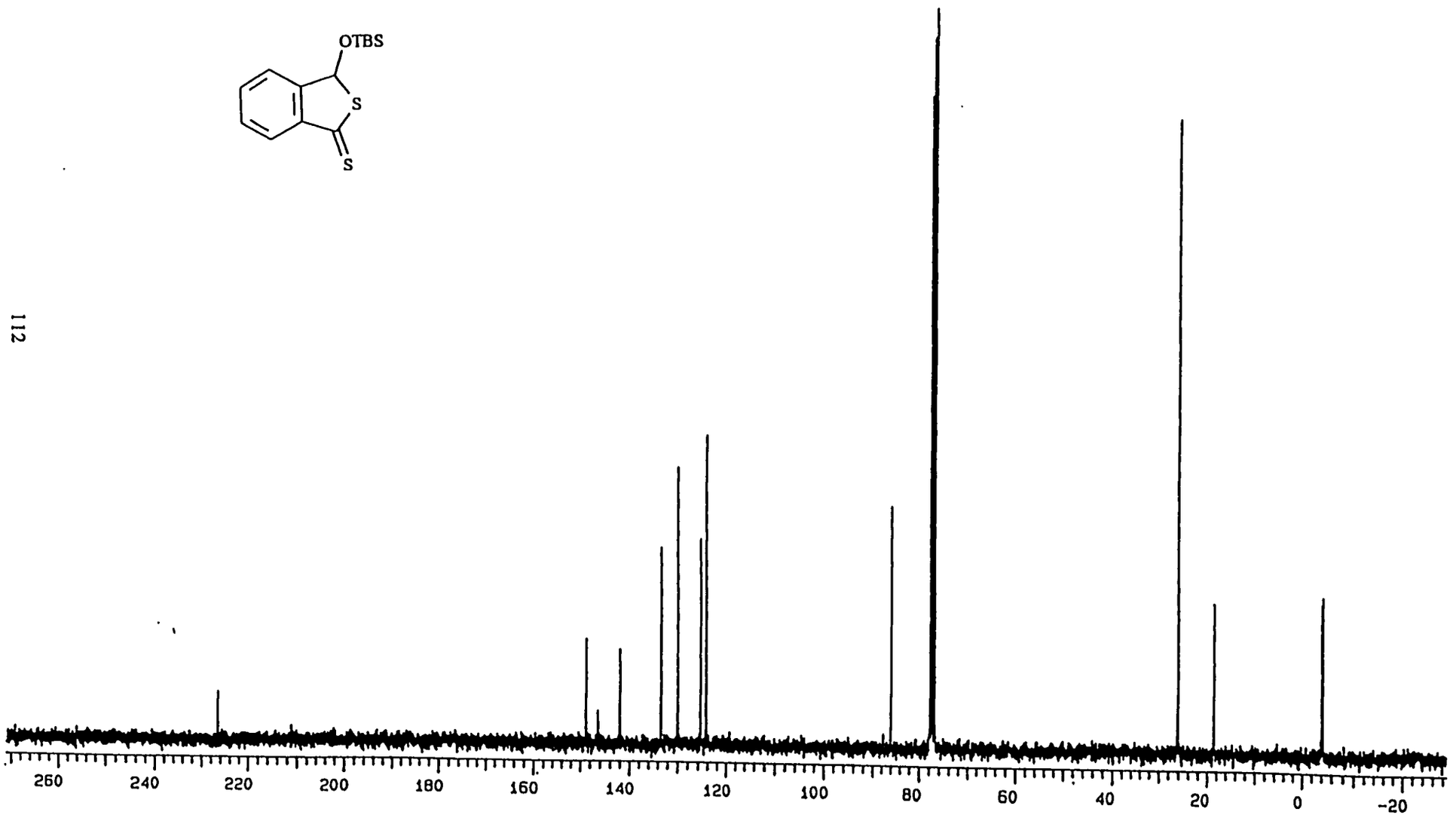


Fig. 22: ^{13}C NMR spectrum of 156

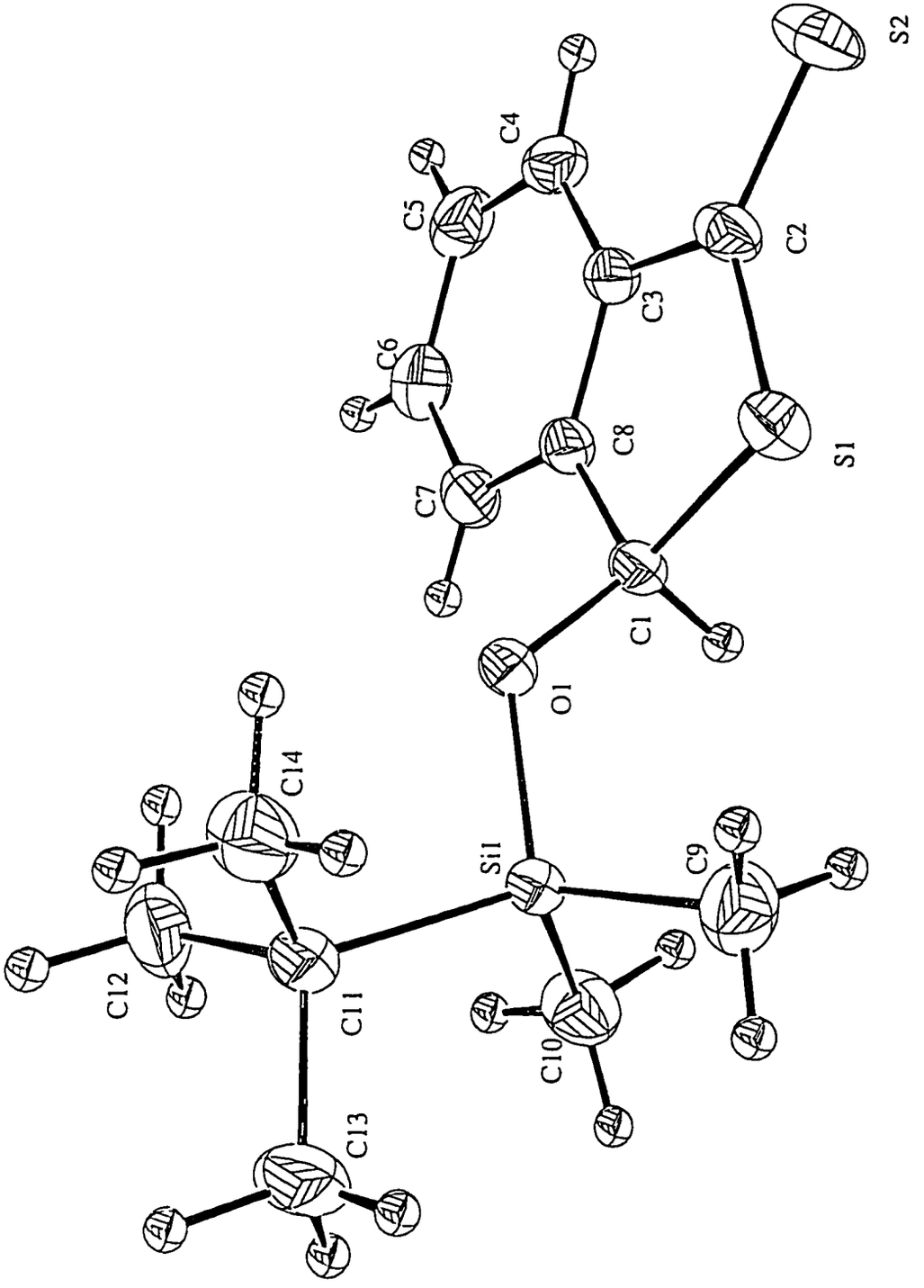
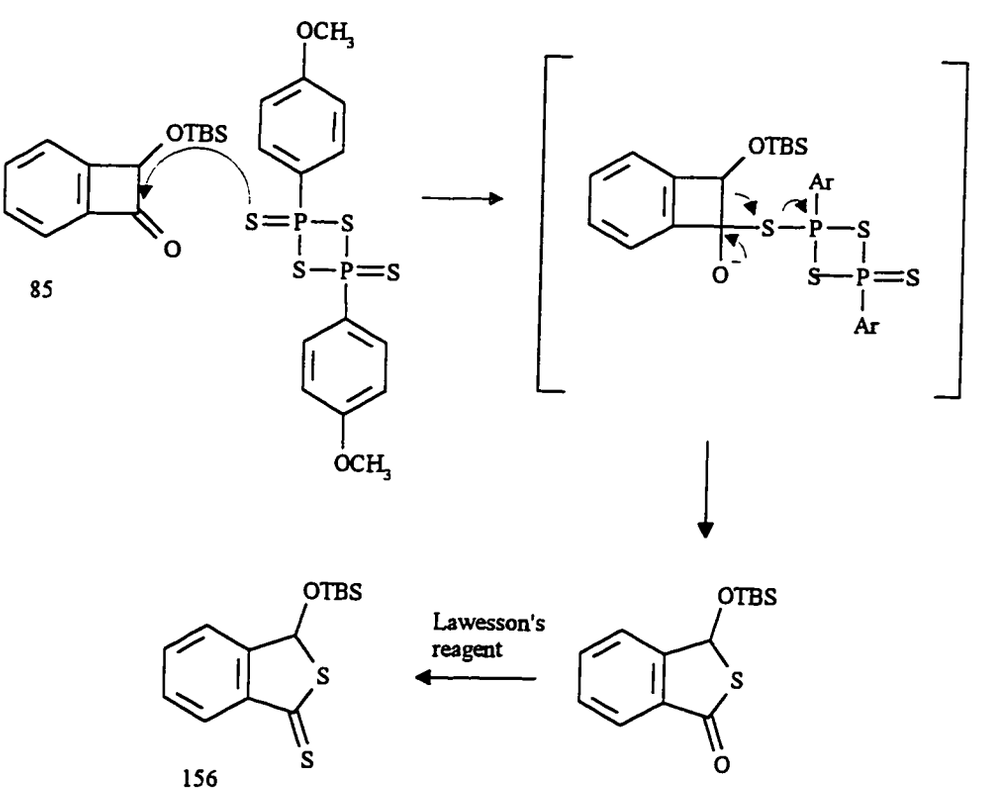


Fig. 23: X-ray structure of **156**

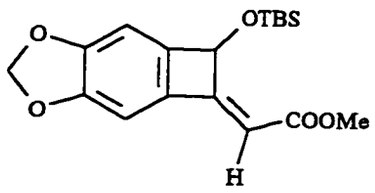
A plausible mechanism for the formation of **156** which involves a ring expansion is shown in Scheme 48. The sulfur of the Lawesson reagent attacks the carbonyl group and the resultant alkoxy anion, instead of attacking the phosphorus centre to generate a thione, fragments the cyclobutene ring to yield the thiolactone. Further thiation should generate the observed dithiolactone. This project will be continued in our laboratory to determine the mechanism of the initial reaction with Lawesson's reagent. Since only one equivalent of reagent was used, it should be possible to increase the yield dramatically, since formation of **156** should require two equivalents of reagent. As well, it may be possible to isolate intermediates in the reaction and verify the proposed reaction pathway.

Scheme 48



Experimental Section

A description of general methods can be found in Chapter 2.

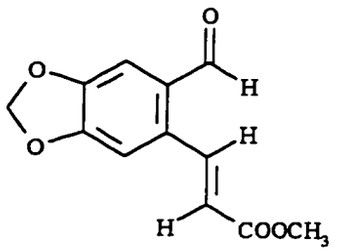


146

A solution of 450 mg of **57** (1.5 mmol) and 575 mg of (carbomethoxymethylene) triphenylphosphorane (1.7 mmol) in 15 mL of toluene was refluxed for 22 h. The solvent was removed by distillation under reduced pressure, and the residue was chromatographed on silica gel (9:1 hexanes/ethyl acetate) to afford 510 mg of **146** (95% yield).

mp 47-48°C **^{1}H NMR:** 0.13 (s, 6H), 0.92 (s, 9H), 3.77 (s, 3H), 5.44 (s, 1H), 5.60 (s, 1H), 5.98 (s, 2H), 6.76 (s, 1H), 7.32 (s, 1H) **^{13}C NMR:** -4.4, 18.2, 25.8, 51.2, 73.7, 74.6, 101.2, 103.1, 103.9, 104.0, 106.1, 136.0, 147.7, 149.9, 151.3, 158.0, 167.6

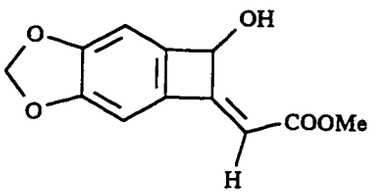
IR: 1670, 1707 cm^{-1} **HRMS:** Calc'd for $C_{18}H_{24}O_5Si$: 348.1393. Found: 348.1366.



147

A solution of 500 mg of **146** (1.4 mmol) and tetrabutylammonium fluoride (1.6 mL, 1M solution in THF) in 25 mL of THF was stirred for 30 min at 25°C. The solvent was evaporated and the residue chromatographed on silica gel (9:1 hexanes/ethyl acetate) to afford 330 mg **147** (1.4 mmol, >95% yield).

mp 182-183°C (dec) **^{1}H NMR:** 3.81 (s, 3H), 6.09 (s, 2H), 6.30 (d, J=16 Hz, 1H), 7.04 (s, 1H), 7.33 (s, 1H), 8.41 (d, J=16 Hz, 1H), 10.23 (s, 1H) **^{13}C NMR**: δ 51.9, 102.5, 106.8, 109.1, 121.8, 129.7, 133.7, 139.7, 149.6, 152.6, 166.6, 188.7 **IR:** 1683, 1697, 1715 cm^{-1} **HRMS:** Calc'd for $C_{12}H_{10}O_5$: 234.0528. Found: 234.0540

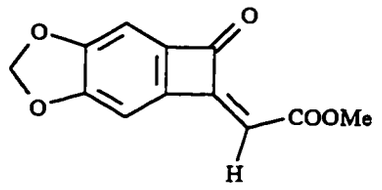


146a

A solution of 250 mg **of 146** (1.1 mmol) and tetrabutylammonium fluoride (780 μL, 1M) in 15 mL of THF was stirred for 5 min, then immediately quenched with saturated NH_4Cl. The mixture was extracted with diethyl ether, washed with brine, dried with anhydrous $MgSO_4$ and the solvent was evaporated. Chromatography on silica gel (hexanes/ethyl acetate) afforded 190 mg **146a** (0.81 mmol, 74% yield).

mp: 134-135°C **^{1}H NMR:** 2.50 (br, 1H), 3.76 (s, 3H), 5.37 (br, 1H), 5.68 (s, 1H), 6.00 (dd, J=2.2, 1.1 Hz, 2H), 6.84 (s, 1H), 7.29 (s, 1H) **^{13}C NMR:** 51.7, 74.5, 101.8, 103.7, 104.6, 106.4, 136.8, 147.9, 150.6, 151.9, 158.4, 168.1 **IR:** 1670, 1707, 3577 cm^{-1}

HRMS: Calc'd for $C_{12}H_{10}O_5$: 234.0538. Found: 234.0516



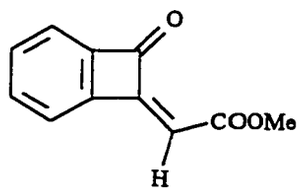
150

A solution of 65 mg of **146a** (0.3 mmol) and 65 mg of manganese dioxide (0.7 mmol) in 5 mL of $CHCl_3$ was refluxed for 4h, the solvent was evaporated and the residue chromatographed on silica gel (9:1 hexanes/ethyl acetate) to afford 40 mg of **150** (0.17 mmol, 57% yield).

mp: 184.5-185.5°C **^{1}H NMR:** 3.79 (s, 3H), 5.90 (s, 1H), 6.17 (s, 2H), 6.95 (s, 1H), 7.67 (s, 1H) **^{13}C NMR:** 51.6, 100.8, 102.0, 103.1, 105.7, 153.2, 155.3, 157.1, 161.4, 166.7, 180.9 **IR:** 1674, 1711, 1766 cm^{-1} **HRMS:** Calc'd for $C_{12}H_8O_5$: 232.0371. Found: 232.0380 **UV**(in CH_3CN, $\lambda(\varepsilon)$):202(3035),204(3912),250(6821), 304(3113), 356(5260), 372(5616) **EA**: calc'd for $C_{12}H_8O_5$ 62.07%C, 3.47%H, found 61.97%C, 3.31%H

151 (Procedure)

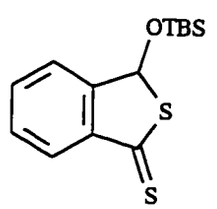
A solution of 50 mg of **150Z** (0.2 mmol) in 3 mL of $CHCl_3$ contained in an NMR tube was degassed with nitrogen for 5 min and irradiated at 350 nm for 3 h. ^{1}H NMR spectroscopy showed isomerization about the double bond, giving a 3:1 mixture of Z/E isomers. After an additional 2 h of irradiation, the isomer ratio was the same, but degradation was apparent both from the spectrum and the TLC.



154Z

The compound was prepared in the same manner as **150**, except the starting material used was **85**.

mp: 82-83°C **^{1}H NMR:** 3.84 (s, 3H), 6.12 (s, 1H), 7.56-7.67 (m, 3H), 8.25 (d, J=7.4 Hz, 1H) **^{13}C NMR:** 51.9, 106.2, 121.4, 125.9, 132.9, 136.0, 158.4, 159.4, 162.9, 166.2, 183.7 **IR:** 1714, 1777 cm^{-1} **HRMS:** Calc'd for $C_{11}H_8O_3$: 188.0473. Found: 188.0462



156

A heterogeneous mixture of **85** (0.5g, 2 mmol) and Lawesson's reagent (0.48 g, 1.2 mmol) in 3 mL of dry toluene was refluxed for 4h under nitrogen atmosphere, when TLC showed the disappearance of the starting material. Silica gel column chromatography using hexanes as eluent yielded a red solid which was recrystallized from methanol as 163 mg of **156** (0.55 mmol, 28% yield) as red transparent prisms.

mp 69-70°C **^{1}H NMR:** 0.12 (s, 3H), 0.24 (s, 3H), 0.96 (s, 9H), 6.81 (s, 1H), 7.5 (m, 2H), 7.64 (d, 1H), 7.98 (d, 1H) **^{13}C NMR:** -4.7, -4.5, 18.1, 25.7, 85.7, 124.0, 125.2, 129.7, 133.1, 141.7, 148.6, 226.4 **IR:** 1210 cm^{-1}

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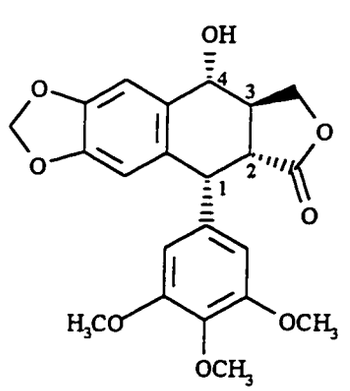
Part II: **Podophyllotoxin Derivatization**

Chapter 6 Derivatization of Podophyllotoxin

A Introduction

Podophyllotoxin, **1**, was first used for medicinal purposes in 1731.[1] At the time, the resin from the perennial herb, *podophyllum peltatum*, commonly known as mandrake or mayapple, which may contain 20% podophyllotoxin, was deemed an excellent emetic. In the mid-1800's, the resin was used topically against cancerous growths. Podophyllum resin was first used topically to treat condyloma acuminatum (genital warts) in 1942. The resin is still used topically in the treatment of benign epithelial growths such as warts, fibroids or papillomas.



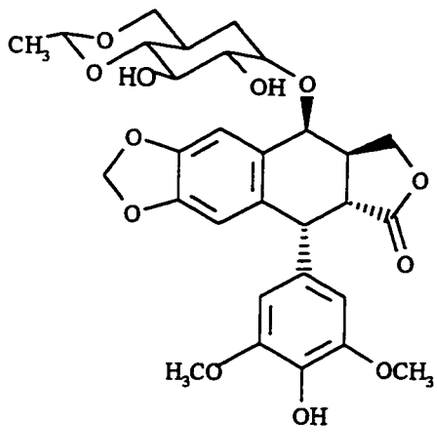
1

Podophyllotoxin is a potent cytotoxic agent.[2] Podophyllotoxin inhibits cell division in the metaphase stage of mitosis. Podophyllotoxin is a spindle poison which inhibits the polymerization of tubulin to microtubules, a process required for the formation

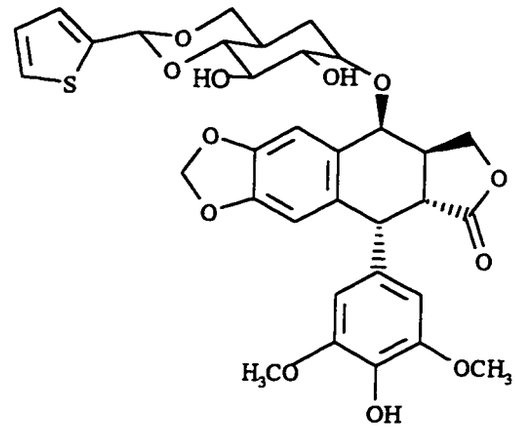
of the mitotic spindle. The lack of spindle fibers in mitosis produced a "c-mitosis" or colchicine mitosis. Metaphase cannot proceed so the chromosomes clump together rather than separate. The drawback with its use as a therapeutic agent is its toxicity. Death has resulted when as little as 350 mg has been swallowed. Even when used topically the drug should be removed within four hours. It is lipid soluble and is rapidly absorbed through the gastro-intenstinal system, the skin or mucous surfaces. Its toxic effects may become apparent in a few hours and are multisystemic, with hemoperfusion the only possible effective mode of therapy against poisoning.[1]

In the early 1950's, chemists at Sandoz began investigating podophyllum extracts more carefully, with the hopes of isolating natural glucosides of podophyllotoxin. It was hoped that such compounds would be less hydrophobic and less toxic than the parent compound. While this proved true when the glucosides were isolated, their cytostatic activity was also diminished. Derivatization of these glucosides was inititated. It was found that two derivatives showed most promising, Etoposide **2**, and Teniposide **3**. These new compounds did not arrest mitosis, but instead prohibited cells from entering mitosis.[3] While the exact mechanism of action for these compounds is unknown, it is known that cell-cycle progression is blocked in the late S and G_2 phases.[2,4] They are known to be nonintercalating DNA topoisomerase II poisons. It has been found that both result in sister chromatid exchanges and DNA strand breaks. Normally strand breaks are reversible due to the mediation of DNA topoisomerase II. In the presence of Teniposide or Etoposide, the process is no longer reversible. It appears that these drugs, like the intercalative antitumour agents such as anthracyclines and acridines among others,

stabilize a cleavable complex of the intermediate DNA after strand breakage, preventing the reverse reaction. Unlike the other classes of drugs, Teniposide and Etoposide do not themselves bind to the DNA, indicating they act directly on topoisomerase II.



2 etoposide



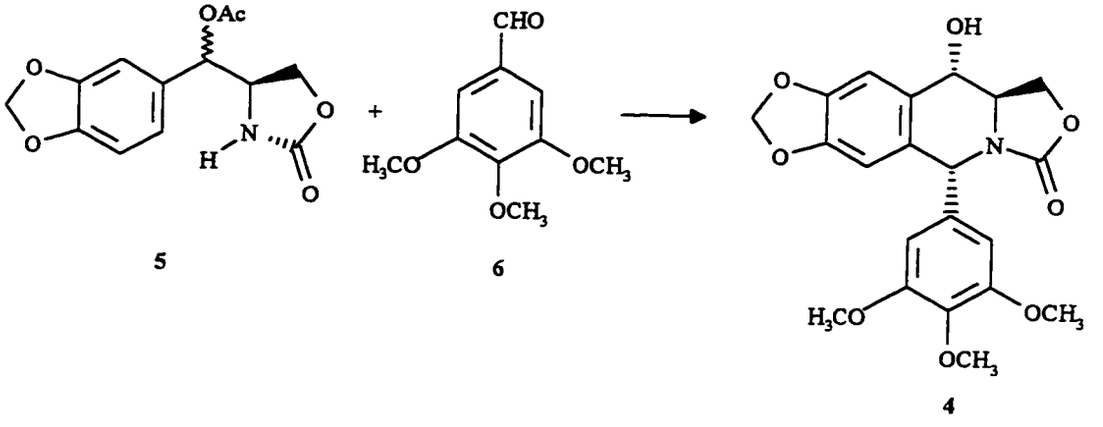
3 teniposide

A number of studies have elucidated structure-activity relationships for Etoposide-like activity in podophyllotoxin derivatives.[5] It has been found that it is necessary to have a 4'-phenolic hydroxy group, the β-configuration at the C-4 position, and the trans fused lactone and a cis arrangement between the substituents at C1 and C2. Such an arrangement places the pendant aryl group perpendicular to the the three fused rings. The relative configuration at C1, C2 and C3 is thermodynamically unstable, a point which needs to be addressed in any synthetic efforts involving either the synthesis of podophyllotoxin or the preparation of potentially active analogues.

Many groups continue to exert considerable effort into synthesizing new derivatives of podophyllotoxin which possess superior antitumour activity. These

modifications have included the replacement of the lactone ring with a non-epimerizable azapodophyllotoxin, **4**, which appeared to have comparable activity to **2** and **3** in *in vitro* and *in vivo* experiments.[6] This compound was prepared by condensation of the cyclic urethane **5** and 3,4,5-trimethoxybenzaldehyde **6**. (Scheme 1)

Scheme 1

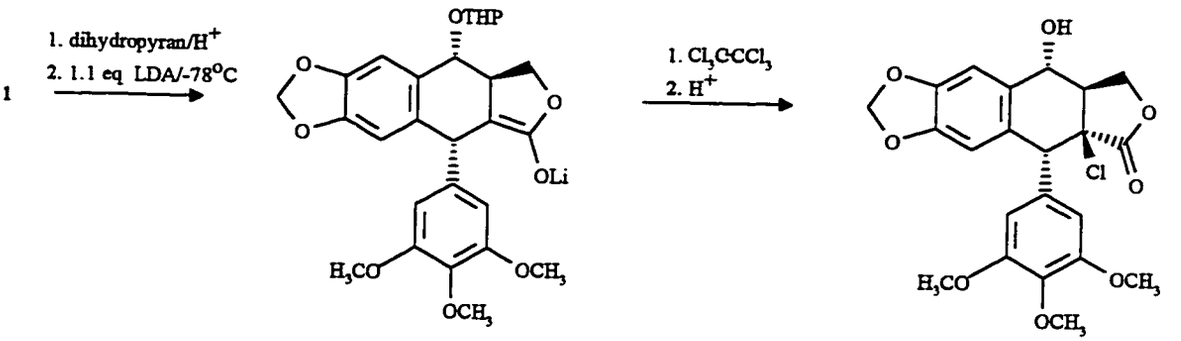


5 **6** **4**

Another example of a synthesis of a non-epimerizable derivative of podophyllotoxin was the synthesis of 2-chloropodophyllotoxin.[7] (Scheme 2) Podophyllotoxin was protected as the tetrahydropyran and reacted with two equivalents of LDA to generate the enolate **7**. Reaction of the enolate with hexachloroethane afforded the THP protected 2-Chloropodophyllotoxin in 74% yield. The direction of attack is from the front since the rear face is blocked by the THP group. The THP group was hydrolyzed to afford **8**. 2-chloropodophyllotoxin was tested in vivo against leukemia P388 by Bristol laboratories in mice. It was found to posess significant activity and lower

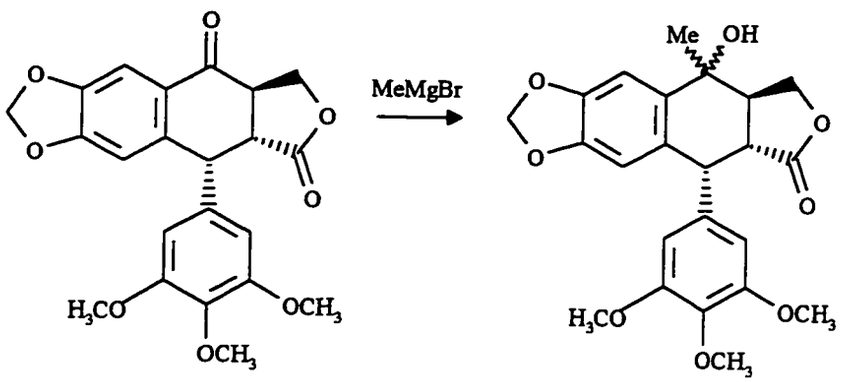
cyctotoxicity than podophyllotoxin. Thus, the non-epimerizable centre did lead to better activity.

Scheme 2



The majority of reported derivatives have involved changing the group at the C-4 centre of the molecule. These have included the synthesis of ethers, esters,[8] amines,[9,10] sulfides, sulfoxides and sulfones.[11,12] One report in 1960 decribed the compound **10** formed by the reaction of methylmagnesium bromide with podophyllotoxone **9**, but this is the only example of a C-4 carbon substituent.[13] (Scheme 3) The object of the following project was to synthesize new C-4 carbon substituted derivatives. Such derivatives should have greater chemical stability than those with heteroatoms since the C-4 heteroatom bond is susceptible to heterolytic cleavage which generates a secondary benzylic carbocation intermediate and hence either new substitution or elimination products.

Scheme 3

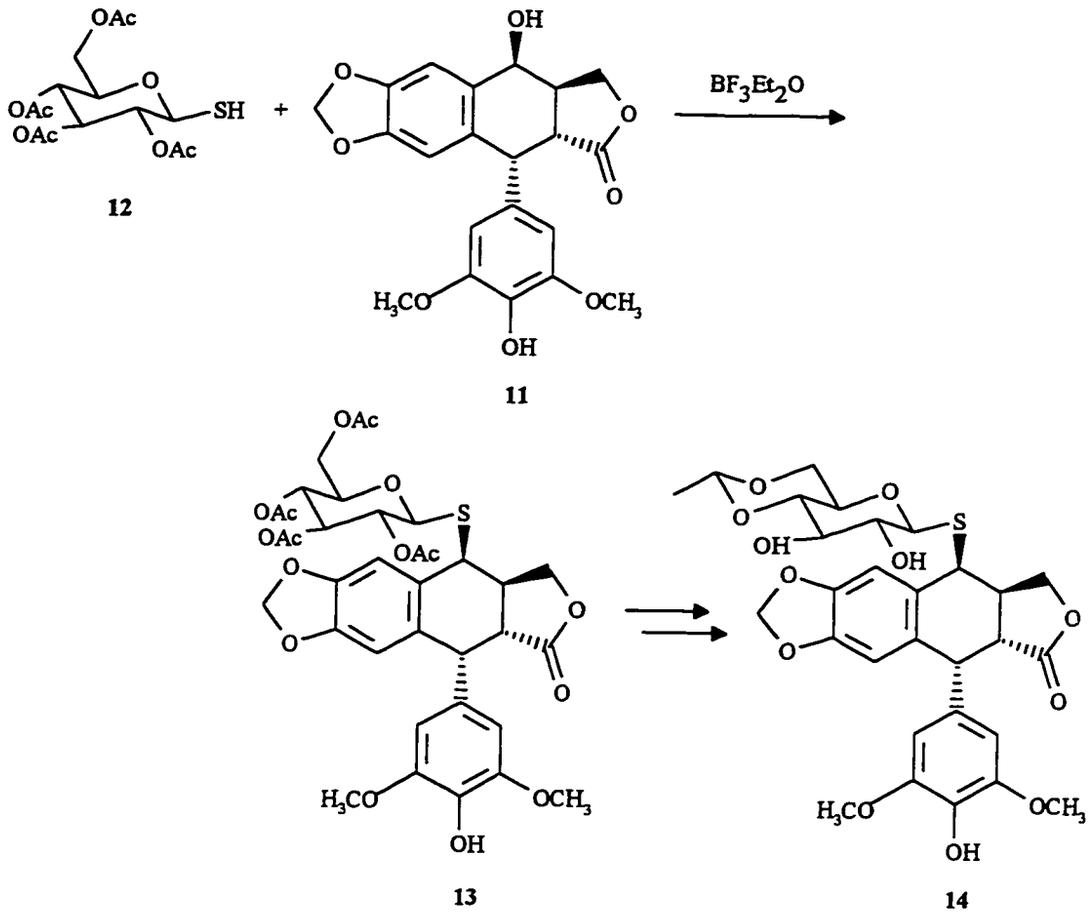


9 10

B Derivatives by Lewis Acid Assisted Substitution

Podophyllotoxin had already been modified using boron trifluoride etherate in the presence of a nucleophile.[11] (Scheme 4) Allevi reacted 4'-demethylpodophyllotoxin 11 with the thio sugar 12 in the presence of boron trifluoride etherate in the synthesis of the thioglucose analogue of Etoposide, 14. Under these conditions, the trans geometry of the lactone is preserved. The incoming nucleophile assumes the β-configuration, since the α-position is blocked by the pendant aromatic ring. Thus, boron trifluoride in the presence of carbon nucleophiles should generate derivatives with carbon at C-4 with the desired β-geometry.

Scheme 4



The nucleophiles chosen were allyltrimethylsilane and trimethylsilylcyanide.[14] Reaction of podophyllotoxin in the presence of boron trifluoride etherate with allyltrimethylsilane afforded deoxyallylpodophyllotoxin **15** in 95% yield after recrystallization from hexane/ethyl acetate. (Scheme 5) The β-geometry was confirmed by the the 5.8 Hz coupling constant between H3 and H4 which is consistent with a cis relationship for similar compounds.[15] (Figure 1) The trans geometry of the lactone was apparent in the coupling constant of 5.2 Hz between H1 and H2 and the coupling constant

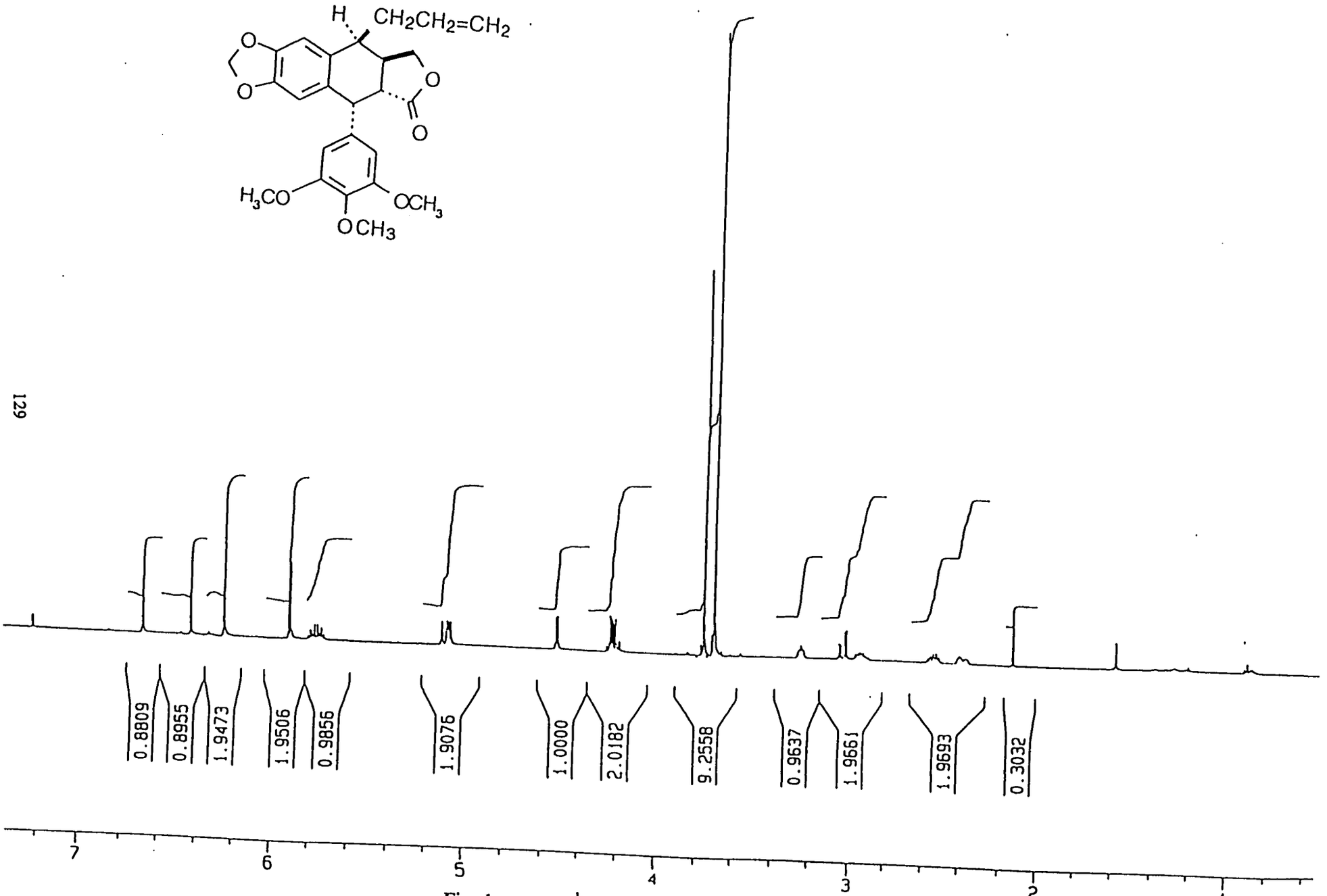
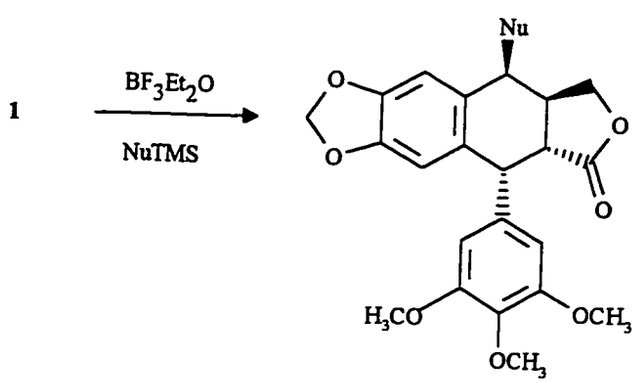


Fig. 1: ^{1}H NMR spectrum of **15**

of 14.3 Hz between H2 and H3. When the reaction was carried out using trimethylsilylcyanide as the nucleophile, the expected product **16** was isolated in 88% yield. The coupling constants were once again consistent wtih the desired geometry with $J_{3,4} = 5.1$Hz. The ^{13}C NMR and mass spectral data also agreed with the structure shown.

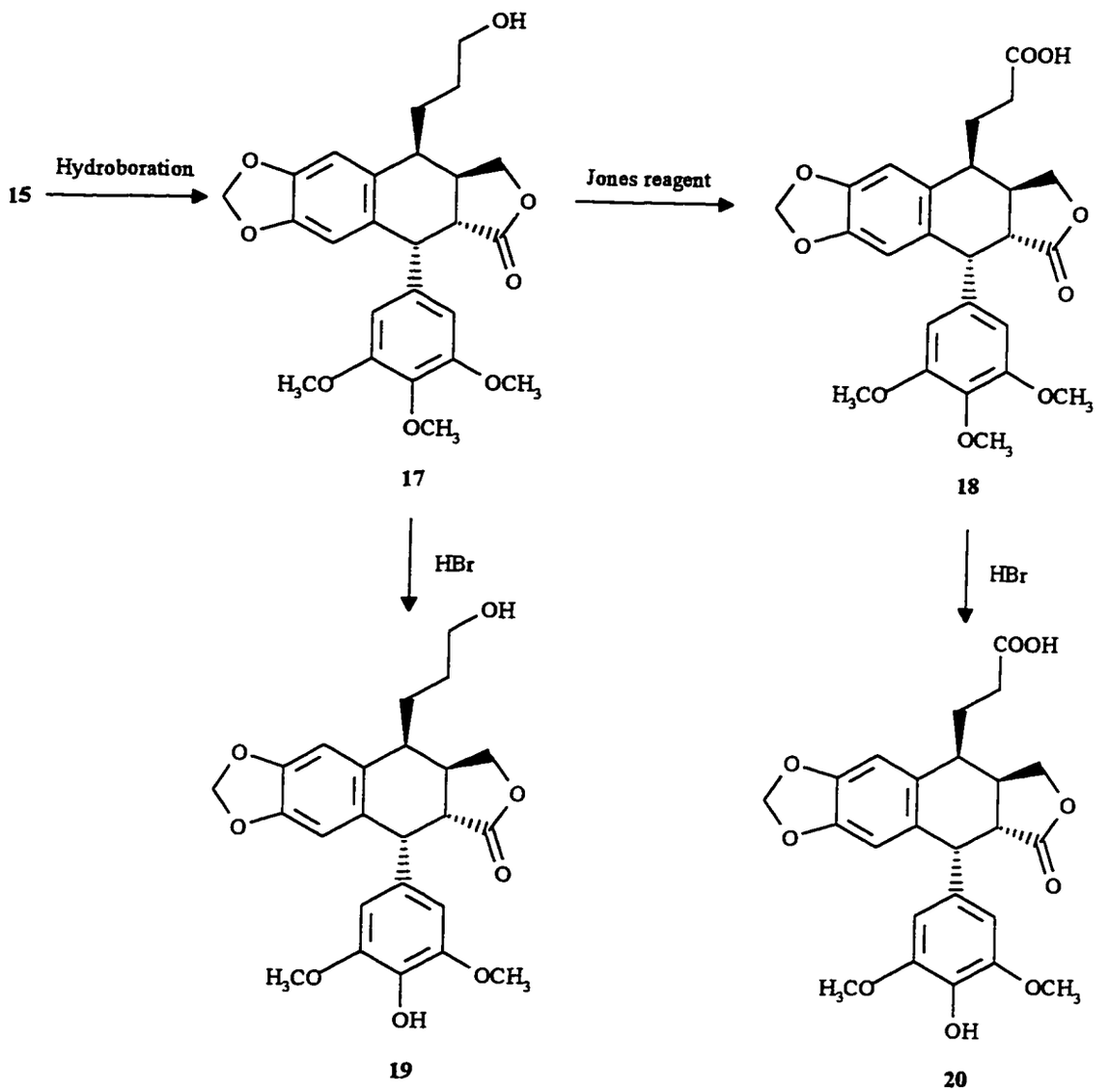
Scheme 5



15 Nu = $-CH_2CH{=}CH_2$
16 Nu = -CN

Since the active derivatives of podophyllotoxin all possessed a group containing a heteroatom at the C-4 position, and often a sugar, it was assumed that a group which could undergo binding was necessary for biological activity. The other requirement was a phenolic group at the C-4' centre. With these criteria in mind, the allyl derivative **15** was reacted under hydroboration conditions using borane methyl-sulfide with basic peroxide workup to afford the primary alcohol **17** in 90% yield. (Scheme 6) The compound showed a hydroxy group in the IR spectrum centred at 3612 cm^{-1}while the ^1H NMR

Scheme 6


15
Hydroboration
OH
O
O
O
O
H3CO
OCH3
OCH3
17
Jones reagent
COOH
18
HBr
19
HBr
20

showed the disappearance of the vinyl protons. (Figure 2) Oxidation of **17** to the carboxylic acid **18** using Jones reagent proceeded in 66% yield. The acid showed a broad peak in the IR spectrum from 2600-3300 cm^{-1} and the appropriate carbonyl peaks for the acid and the lactone at 1708 and 1776 cm^{-1}. Both the alcohol and the acid were demethylated using anhydrous HBr in dichloromethane to afford **19** and **20** respectively. It was possible to isolate the demethylated alcohol **19** and the demethylated acid **20** in yields of 63% and 20% respectively. This is easily seen in the ^1H NMR spectrum by the disappearance of one set of methyl protons. (Figure 3) Mass spectral data agreed with the structures proposed. The low yield obtained for **20** appeared to be largely due to its instability during recrystallization efforts and chromatography.

The cyano derivative **16** was hydrolyzed in ethanolic hydrochloric acid to afford the ester **21** in 57% yield. (Scheme 7) This was easily seen in the ^1H NMR by the appearance of the new ethoxy group and in the mass spectrum. Attempts to demethylate either of these compounds using HBr or boron tribromide have been unsuccessful.

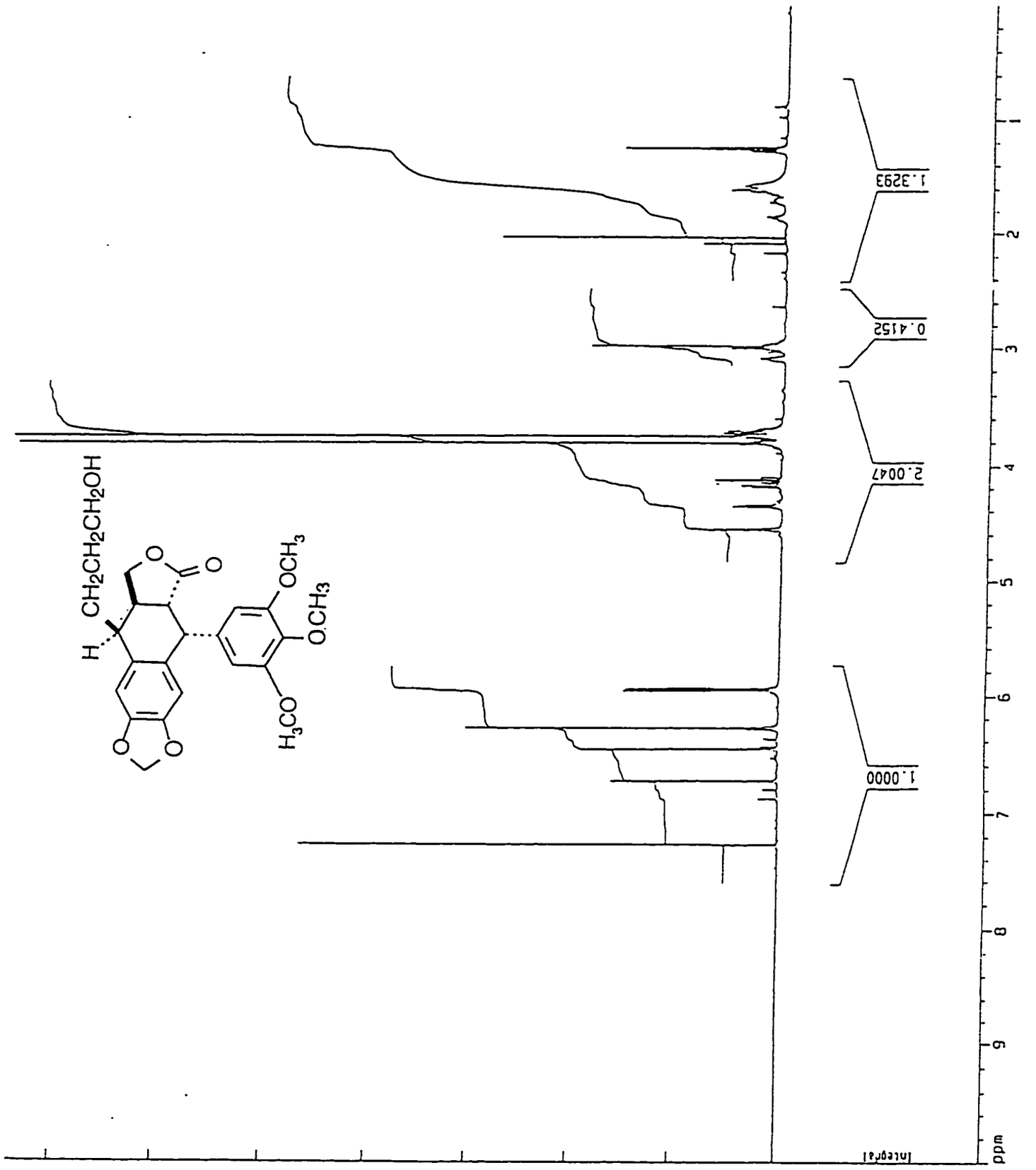


Fig. 2: ^{1}H NMR spectrum of 17

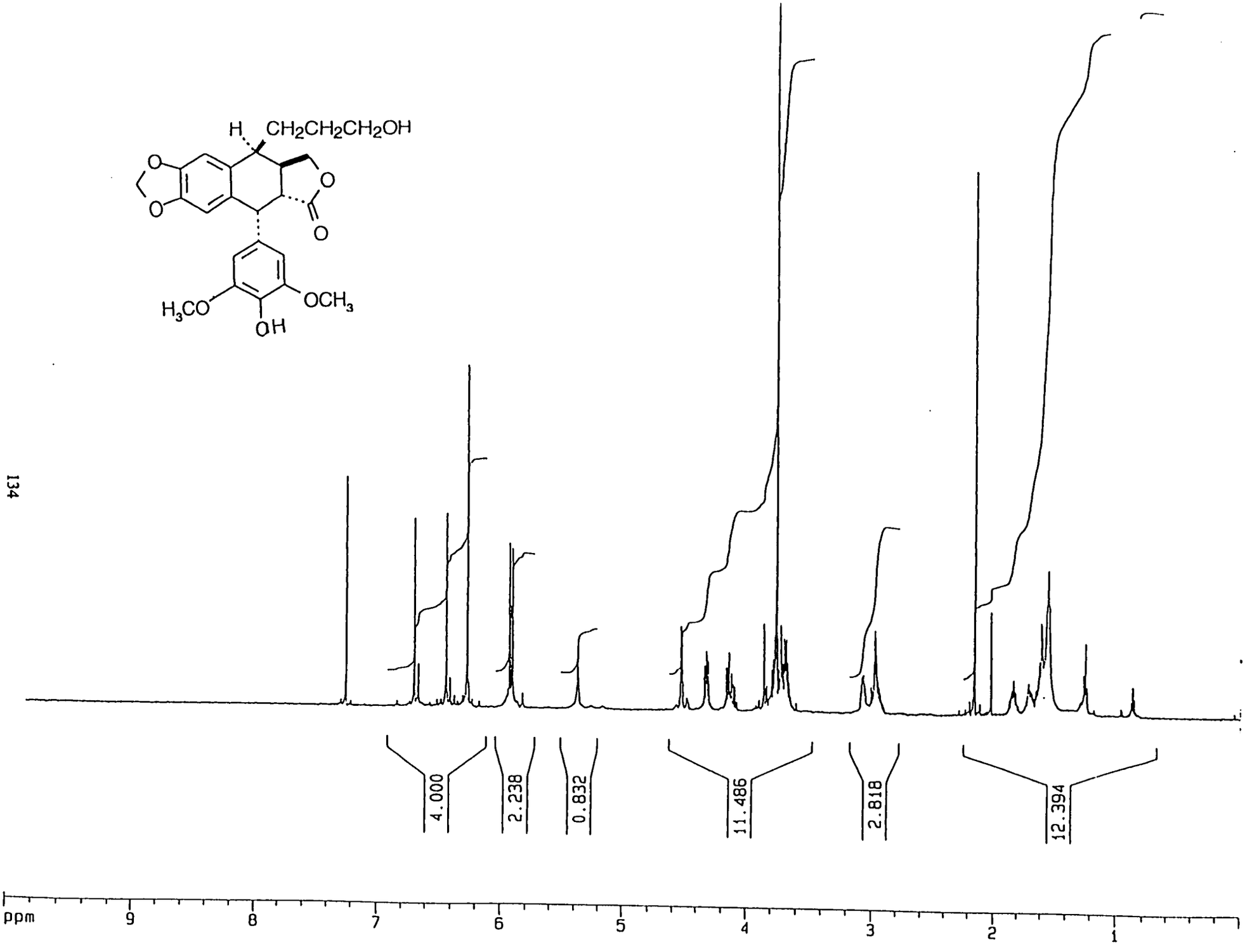
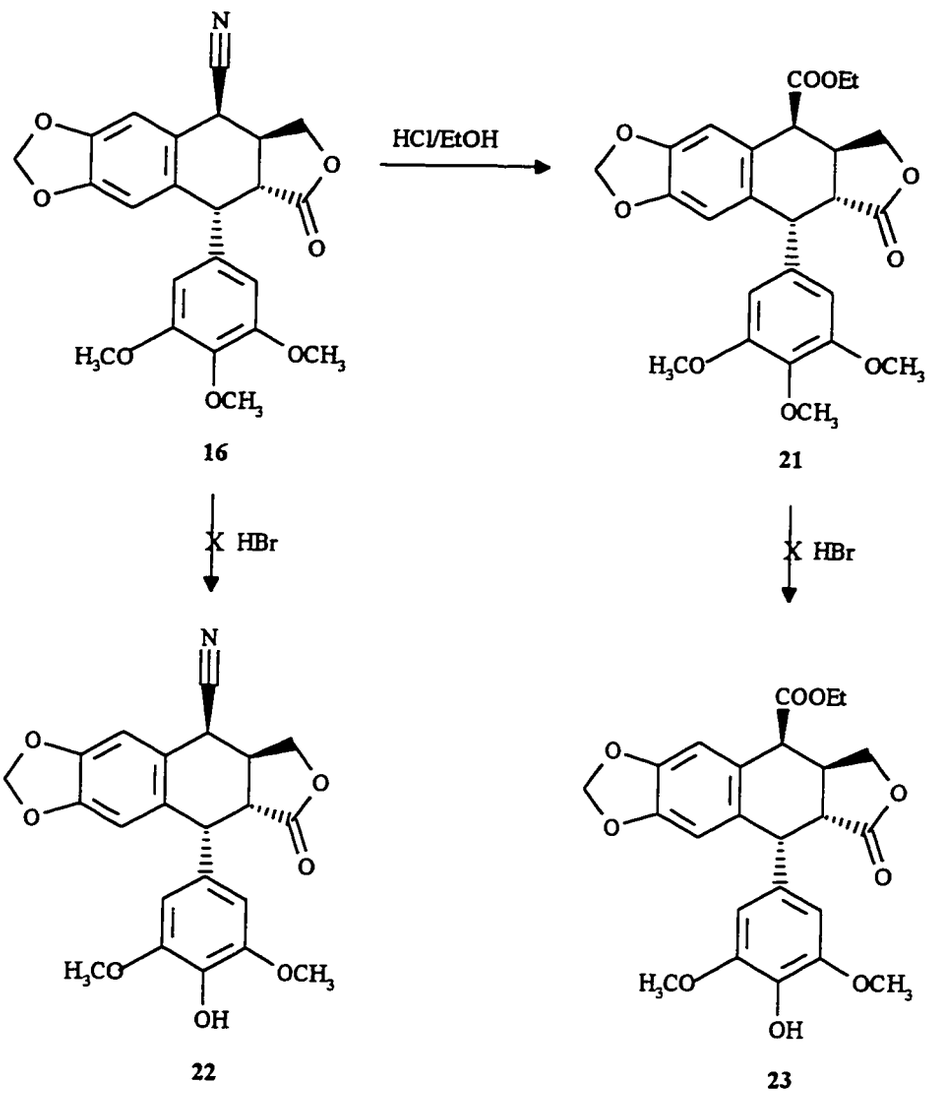


Fig. 3: ^{1}H NMR spectrum of 19

Scheme 7


N
HCl/EtOH
COOEt
H_3CO
OCH_3
OCH_3
16
21
X HBr
X HBr
N
COOEt
H_3CO
OCH_3
OH
22
23

C Derivatives via Addition of Organometallic Reagents to Podophyllotoxone

As previously described, another route toward C4-substituted podophyllotoxin analogs is via addition to podophyllotoxone, which is available by oxidation of podophyllotoxin using manganese dioxide in refluxing chloroform. (Scheme 8) Addition of 1.1 equivalents of *n*-butyllithium at -78°C to podophyllotoxone in THF resulted in a 40% yield of a 4/7 mixture of the isomers of the tertiary alcohol **24**. The two isomers were separated by silica gel column chromatography. The α-isomer was expected as the minor product; the assignment was confirmed by X-ray crystallography of the minor product which showed the α-isomer as expected. (Figure 4) Predictably, the ^{1}H NMR spectra of the two isomers are very similar and did not allow us to make definitive structure assignments. The major product results from the nucleophilic addition from the β-face of the 4-keto group, since the α-face is hindered by the pendant aromatic ring.

When lithium phenylacetylide was reacted with podophyllotoxone at -78°C, only one isomer was isolated, presumably the β-product **25**. The same reaction was performed using the THP protected anion of propargyl alcohol to afford presumably **26**. The mass spectral data and infrared spectra agreed with the structures proposed. Again the ^{1}H and ^{13}C spectra could not be used to verify the stereochemistry and thus the assignments are based on mechanistic grounds. It was hoped that hydrogenolysis of these compounds would afford the deoxygenated structures such as **27**; however, hydrogenolysis using a variety of reagents including triethylsilane with trifluoroacetic acid, or cyclohexene in the

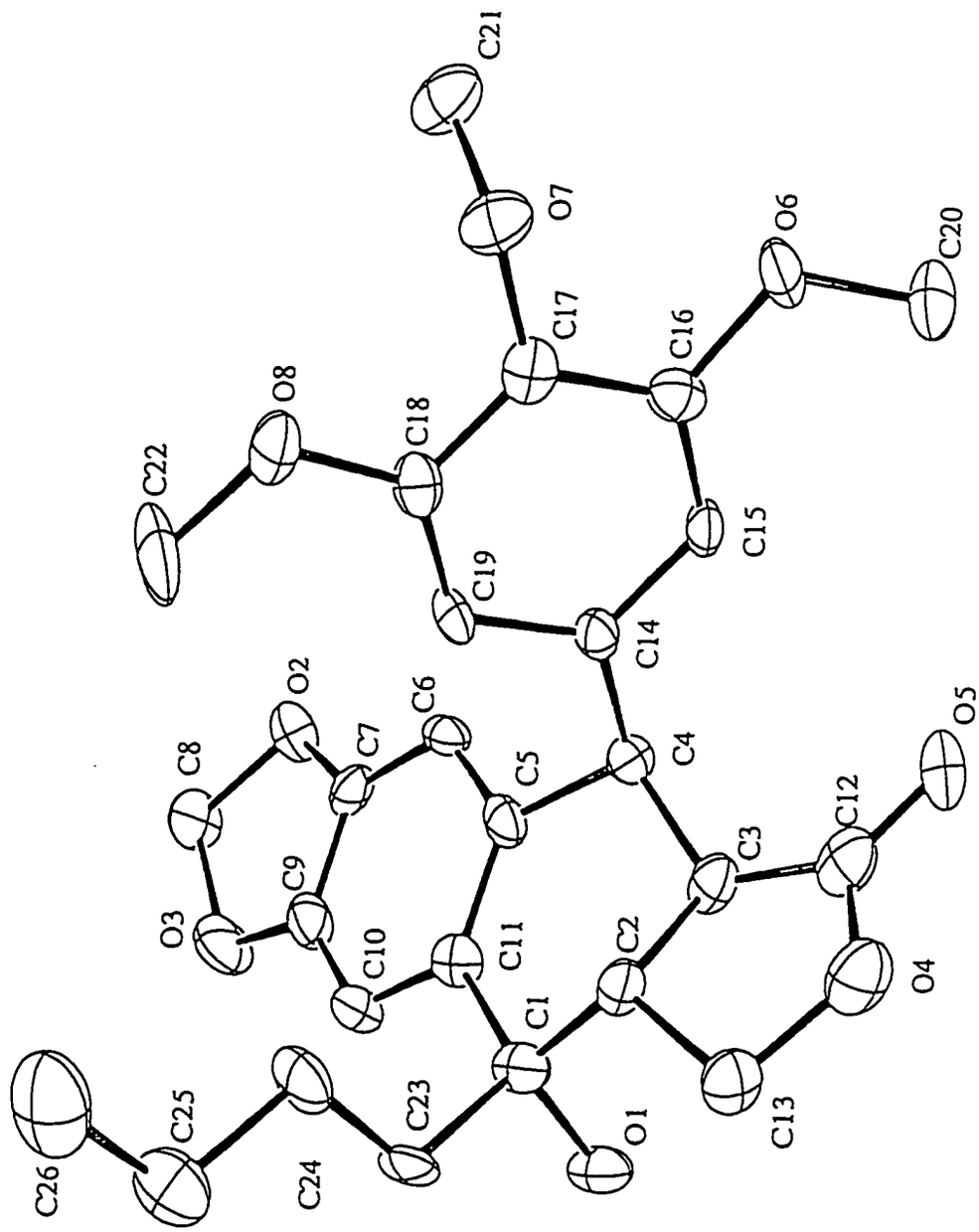
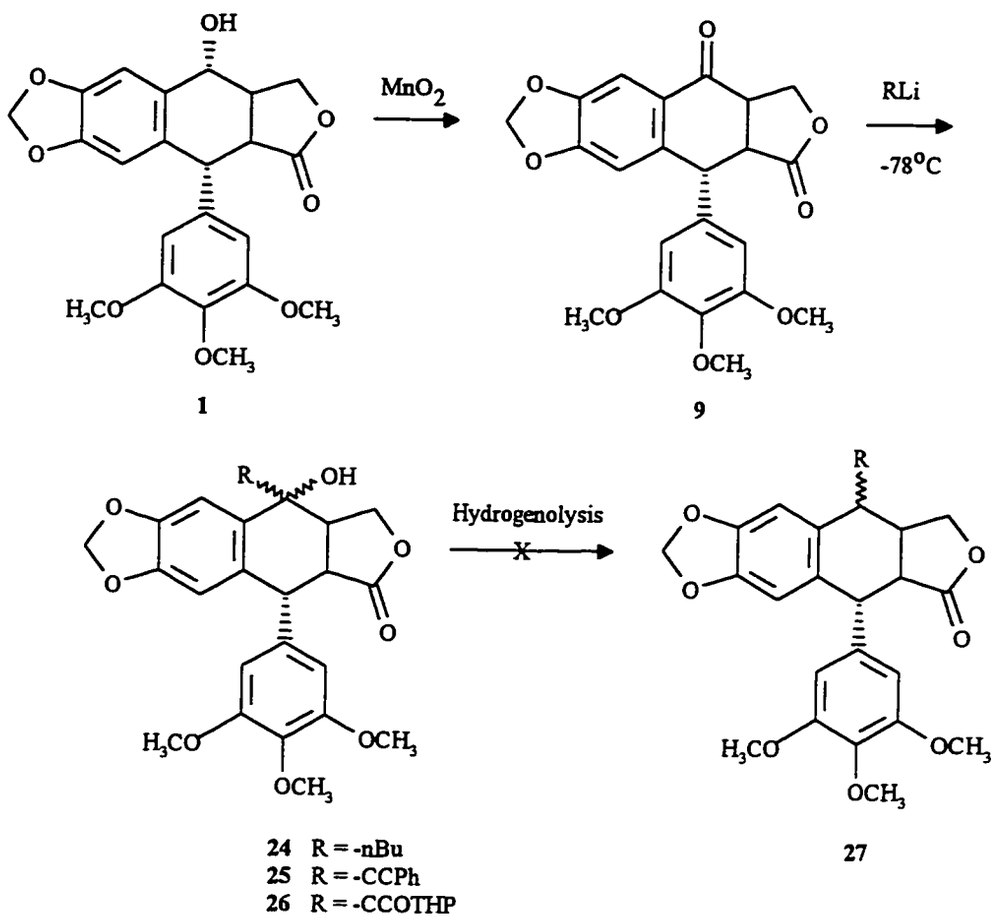


Fig. 4: X-ray structure of **24**

presence of palladium was unsuccessful. Demethylation of the C4' group to a phenol was unsuccessful using HBr or BBr_3.

Scheme 8



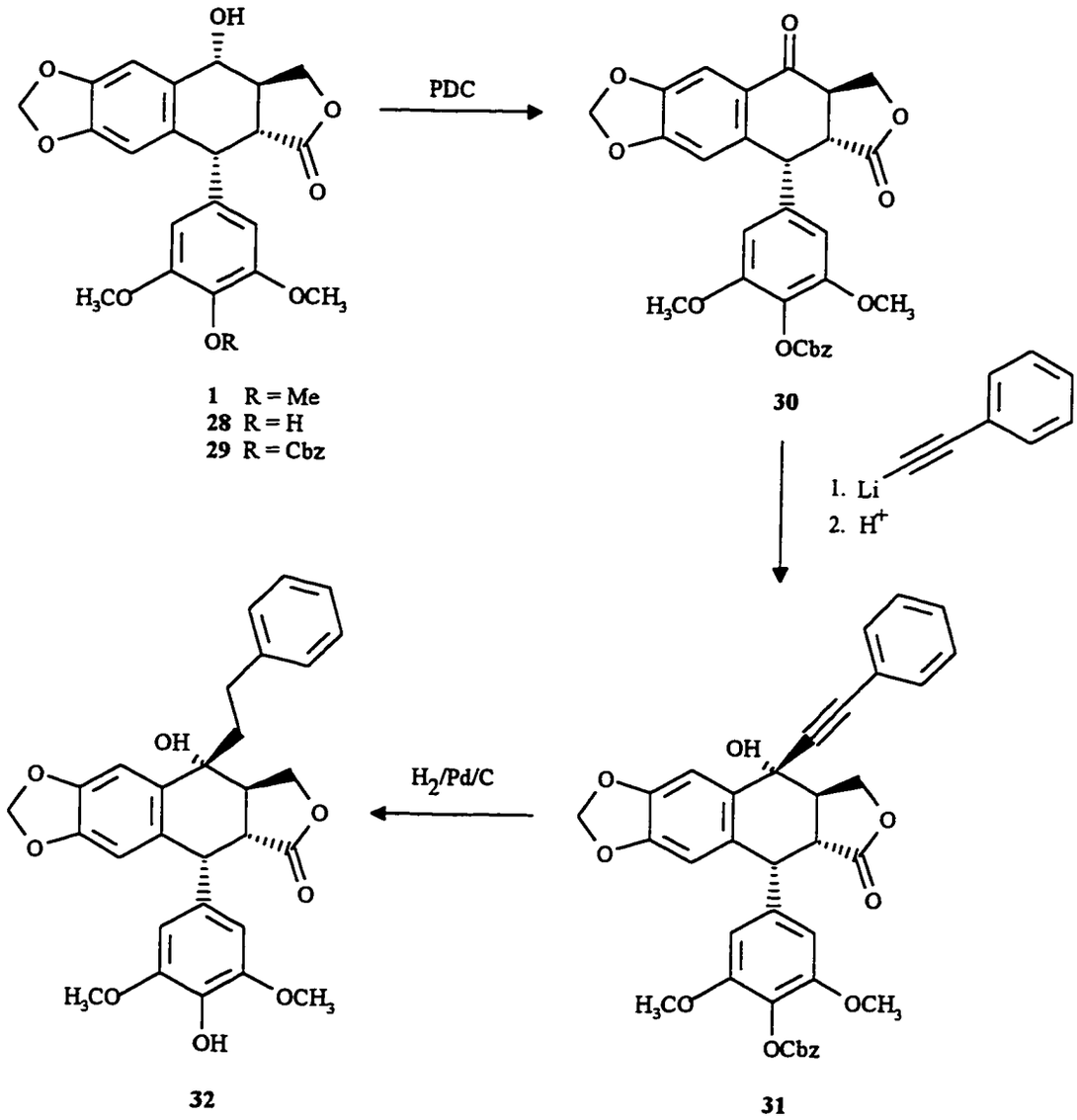
In order to obtain the 4'-demethylated analogs required for biological activity, the starting material used was the 4'-demethylated podophyllotoxin. The phenol was protected using benzyl chloroformate, and oxidation was carried out to afford the protected demethylated podophyllotoxone 30. (Scheme 9) The demethylation of podophyllotoxin is a known procedure,[16] however it was our experience that the methods

in the literature were not high yielding and poorly reproducible. We adopted a procedure reported to us by chemists at Bristol-Myers Squibb, who have been intensively involved in the derivatization of podophyllotoxin.[17] This method involved the addition of a saturated hydrogen bromide diethyl ether/dichloroethane solution to podophyllotoxin at 0°C, stirring for 20 minutes, stirring at 25°C for 4 hours, evaporating the solvent, adding an acetone solution of barium carbonate, and stirring for an additional 14 hours. This method afforded 4'-demethylpodophyllotoxin reproducibly in about 70% yield. Protection of the phenol using benzyl chloroformate proceeded as described[29] to give **29** in 75% yield. Oxidation of the protected 4'-demethylpodophyllotoxin with pyridinium dichromate led to the formation of the desired ketone **30** in 82% yield, as evidenced by the IR peak at 1733 cm^{-1}. The peak at 1773 cm^{-1} confirmed that the trans lactone was still intact. The ^1H NMR spectrum also showed $J_{1,2} = 4.3$ Hz and $J_{2,3} = 15.6$ Hz, which confirmed the retention of the desired lactone geometry. Somewhat surprisingly, oxidation using the manganese (IV) oxide, normally a very mild reagent, gave lower yields and mixtures of products. 1-Lithio-2-phenylacetylide was added to **30** to generate the tertiary alcohol **31** in 76% yield as a single compound by ^1H NMR spectroscopy.

Once again, the β-stereochemical assignment is based mainly on the mechanistic argument mentioned earlier. The carbobenzoxy group was removed by catalytic hydrogenation using palladium as the catalyst. This procedure also reduced the alkyne to the saturated alkane **32** in 38% yield. Once again, from the ^1H NMR spectrum it can be seen that the required geometry is intact with $J_{1,2} = 5.7$ Hz and $J_{2,3} = 14.9$ Hz. (Figure 5)

This provided an example of a tertiary alcohol at the C4 centre in a derivative which possessed the requisite free hydroxy group at C4'.

Scheme 9



1 R = Me
28 R = H
29 R = Cbz

30

31

32

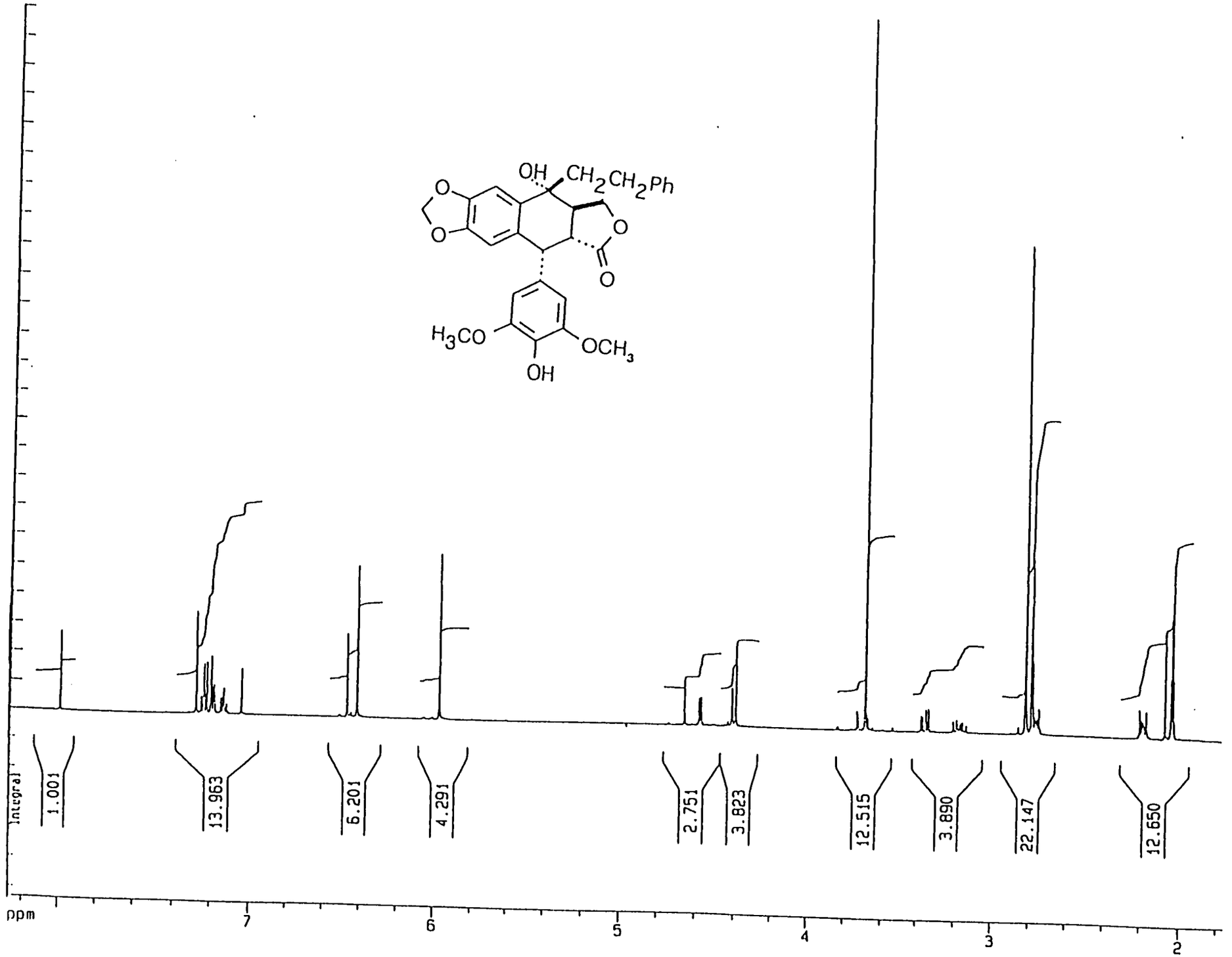


Fig. 5: ^{1}H NMR spectrum of **32**

D Biological Activity of Podophyllotoxin Derivatives

Three of the C4 carbon substituted compounds synthesized in the previous sections were chosen for in vitro testing, **19**, **20**, and **32**. These compounds all possessed the necessary trans fused lactone, a phenol at C4', a group at C4 capable of hydrogen bonding, and all had the desired β stereochemistry at C4.

The compounds were tested by Bristol Myers Squibb at Wallingford, Connecticut. The cytotoxicity of the compounds was assessed using three human colon carcinoma cell lines, HCT116, HCT116/VM46, and HCT116/VP35. The last two are resistant cell lines. HCT116/VM46 is resistant to VM-26 and expresses the multidrug resistance phenotype including resistance to lipophilic anticancer drugs such as taxol, VP-16 (Etoposide), VM-26 (Teniposide) doxorubicin and vinblastine. This cell line overexpresses p-glycoprotein, a cell surface drug efflux pump, which limits accumulation of the anticancer drugs mentioned. The cell line HCT116/VP35 was selected for its resistance to topoisomerase II drugs such as VP-16 (Etoposide), and VM-26 (Teniposide) due to reduced levels of the topoisomerase II enzyme.

In the cytoxicity assay, cells are plated and 24 hours later drugs are added and serially diluted. After 72 hours, the tetrazolium dye XXT is added. A dehydrogenase enzyme in live cells reduces the XXT to a form that absorbs light at 450 nm, and thus can be measured spectrophotometrically. Greater absorbance values result from greater numbers of live cells. The results are expressed as an IC_{50} which is the drug concentration

required to inhibit cell proliferation to 50% of that of untreated control cells. The results are summarized in Table 1.

Table 1 In Vitro Cytotoxicity of Podophyllotoxin Derivatives in Human Colon Carcinoma Cell Lines

	$IC_{50}(\mu m)$		
	HCT116	HCT116/VM46	HCT116/VP35
19	0.758	0.758	0.663
20	9.5	12.6	11.1
32	2.2	2.4	2.2
Etoposide	1.5	8.6	7.4
Teniposide	0.183	0.916	0.763

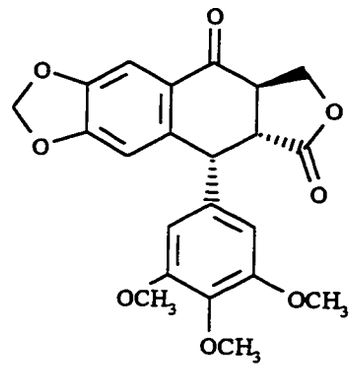
The most active compound tested was the hydroxypropanol derivative, **19**, with an IC_{50} of 0.758 μM. Surprisingly, unlike Etoposide and Teniposide, this compoound showed comparable results for the three cell lines. This indicates that it is not a substrate for the p-glycoprotein pump, nor does it interact with topoisomerase II. This in itself is quite remarkable, since one could imagine the mechanism of action for compounds such as **19** to be comparable to that of Etoposide, which acts on topoisomerase II. Compound **32**, the tertiary alcohol with the phenylethyl sidechain, was comparable to Etoposide toward sensitive cell line HCT 116 but about four times more active toward the resistant cell lines.

The activity of **32** was surprisingly high and challenged the notion of a necessary binding group in the β position at the C4 centre. This compound has it 4-hydroxy group in the α position, and no heteroatom in the group in the 4β position. Based on the in vitro results, compound **19** was selected to undergo *in vivo* testing.

The in vivo model used was a murine lung carcinoma (M109) subcutaneous implant model. The drug was administered to mice intraperitoneally as a solution in water-carboxymethoxy cellulose in doses ranging from 50-120 mg/kg. Survival times at 50 and 80 mg/kg were identical to that of the control group. (Test/control = 100-105) At the highest dosage employed, the T/C fell to 87 indicating toxicity. No further testing of the compounds was carried out. While the results in vivo were disappointing, there are a number of factors which affect the in vivo results other than the activity of the compound tested.[18] These include instability of the compound under physiological conditions, poor solubility of the compound in the medium used to deliver it, or poor absorbtion of the compound in the body. A different delivery system might have given more encouraging results.

Experimental Section

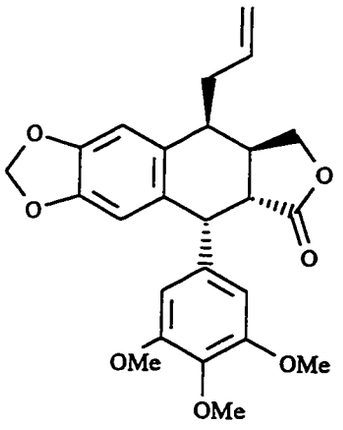
General methods can be found in Chapter 2.



9

The compound was prepared following a literature procedure.[13] A mixture of 950 mg of podophyllotoxin (2.3 mmol), 3 g of activated MnO_2, and 60 mL of $CHCl_3$ was refluxed for 2h and filtered. The MnO_2 was washed 3 times with hot $CHCl_3$. Evaporation of the solvent yielded an off-white solid (800 mg, 1.9 mmol, 83% yield).

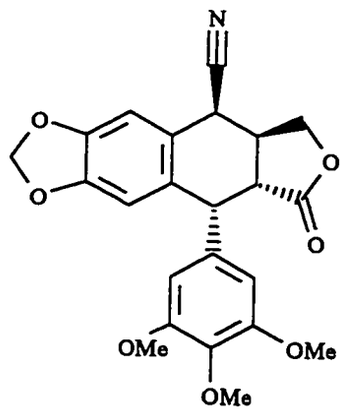
mp 189-190°C (lit 190-191.5°C)[13] **^{1}H NMR**: 3.24 (dd, J=15.4, 4.2 Hz, 1H), 3.41-3.58 (m, 1H), 3.72 (s, 6H), 3.79 (s, 3H), 4.32 (dd, J=10.1, 9.3Hz, 1H), 4.53 (dd, J=9.3, 7.7 Hz, 1H), 4.81 (d, J=4.2 Hz, 1H), 6.05 (d, J=1.1 Hz, 1H), 6.07 (d, J=1.1 Hz, 1H), 6.35 (s, 2H), 6.67 (s, 1H), 7.52 (s, 1H)



15

Podophyllotoxin (1.0 g, 2.4 mmol) was stirred with boron trifluoride etherate (750 μL, 6mmol) and allyltrimethylsilane (80 μL, 4.8 mmol) in 1 mL of dry CH_2Cl_2 at 25°C for 4 h. Water (3 mL) was added and the product was extracted with CH_2Cl_2, washed with water, dried with anhydrous $MgSO_4$, and the solvent was evaporated to afford a white solid. Chromatography on silica gel (1:1 hexanes/ethyl acetate) and recrystallization from hexane/ethyl acetate yielded 1.05 g of **15** (95% yield)

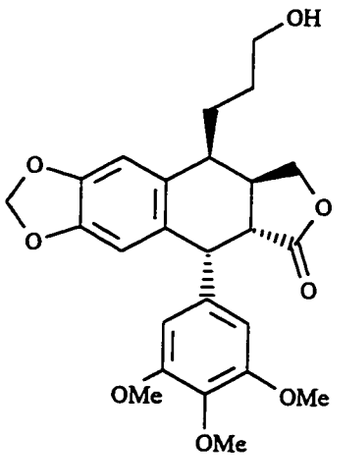
mp 137-138°C **^{1}H NMR:** 2.43-2.45 (m, 1H), 2.57-2.60 (m, 1H), 2.96-3.01 (m, $J_{2,3}$=14.3, $J_{3,4}$=5.8Hz, 1H), 3.05-3.09 (m, 1H), 3.27-3.40 (m, 1H), 3.71 (s, 1H), 3.77 (s, 3H), 4.23-4.30 (m, 2H), 4.56 (d, $J_{1,2}$=5.2Hz, 1H), 5.11-5.15(m, 2H), 5.81 (m, 1H), 5.94 (s,1H), 5.95 (s, 1H), 6.29 (s, 2H), 6.47 (s, 1H), 6.73 (s, 1H) **^{13}C NMR:** 36.1, 37.7, 38.5, 42.2, 44.0, 56.2, 60.7, 69.0, 101.2, 108.4, 108.7, 110.1, 116.9, 130.8, 133.0, 136.1, 137.0, 146.9, 147.0, 152.4, 175.1 **IR:** 1775 cm^{-1} **HRMS:** Calc'd for $C_{25}H_{26}O_7$: 438.1679 Found: 438.1681. **EA:** calc'd for $C_{25}H_{26}O_7$ 68.48%C, 5.98%H found 68.37%C, 5.97%H



16

Boron trifluoride etherate (120 μL, 0.96 mmol) was added to a solution of 200 mg of podophyllotoxin (0.48 mmol) and trimethylsilyl cyanide (70 μL, 0.53 mmol) in 3 mL of CH_2Cl_2 at 0°C. The solution was stirred for 20 min at 0°C and at 25°C for 1.5 h when TLC showed no starting material remained. Water (10 mL) was added and extraction carried out with CH_2Cl_2. The organic extracts were dried with anhydrous $MgSO_4$ and the solvent was evaporated to leave 180 mg of **16** as a yellow solid which was pure by NMR.

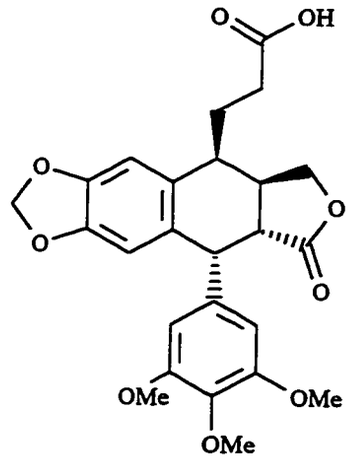
mp 158-159°C **^{1}H NMR:** 2.90-3.05 (m, 1H), 3.05-3.15 (m, 1H), 3.74 (s, 6H), 3.80 (s, 3H), 4.21 (d, $J_{3,4}$=5.1Hz, 1H), 4.35 (d, J=9.4Hz, 1H), 4.45-4.50 (m, 1H), 4.67 (d, $J_{1,2}$=4.6 Hz, 1H), 5.99 (s, 1H), 6.01 (s, 1H), 6.27 (s, 2H), 6.56 (s, 1H), 6.83 (s, 1H) **^{13}C NMR:** 33.0, 33.5, 43.1, 43.3, 56.0, 60.6, 68.5, 101.7, 107.9, 108.2, 110.7, 117.4, 122.1, 131.1, 134.4, 137.2, 147.7, 148.6, 152.4, 172.6 **IR:** 1784, 2308 cm^{-1} **HRMS:** Calc'd for $C_{23}H_{21}O_7N$: 423.1318 Found: 423.1314



17

Borane methyl sulfide complex (100 μL, 10M, 1 mmol) was added dropwise to a solution of **15** (925 mg, 2.1 mmol) in 6 mL of dry CH_2Cl_2 at 0°C. The solution was stirred at 25°C for 4 h, then diluted with 10 mL of ethanol. The pH of the solution was maintained at 8 using 3N aq NaOH while hydrogen peroxide (0.3 mL, 30% in water) was added at 0°C. Water (10 mL) was added and the product extracted with CH_2Cl_2, dried with anhydrous $MgSO_4$, and the solvent was evaporated leaving a white solid which was recrystallized from hexanes/ethyl acetate to give 915 mg **17** (95% yield).

mp 118-119°C **^{1}H NMR:** 2.85-2.95 (m, 2H), 2.95-3.00 (m, 1H), 3.72 (s, 6H), 3.77 (s, 3H), 4.08-4.13 (m, 1H), 4.36 (dd, J=8.5, 7.1 Hz, 1H), 4.53 (d, J=4.8 Hz, 1H), 5.90 (s, 1H), 5.92 (s, 1H), 6.25 (s, 2H), 6.43 (s, 1H), 6.69 (s, 1H) **^{13}C NMR:** 29.7, 32.1, 36.2, 39.3, 42.2, 44.1, 56.2, 60.7, 62.5, 69.0, 101.2, 108.5, 108.8, 110.2, 130.5, 133.8, 136.2, 137.1, 146.8, 147.0, 152.4, 175.1 **IR:** 1775, 3612 cm^{-1} **HRMS:** Calc'd for $C_{25}H_{28}O_8$: 456.1784 Found: 456.1773

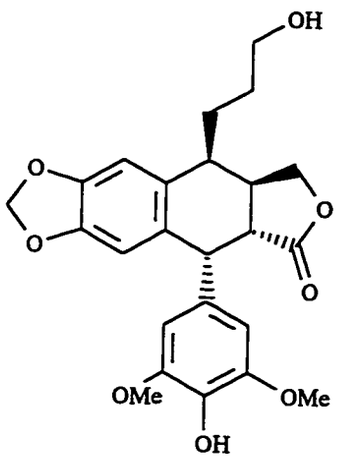


18

Jones reagent (2 mL, 0.4M, 0.8 mmol) was added dropwise to a solution of 160 mg of **15** in acetone until an orange colour persisted. Stirring was continued for 15 min. The reaction mixture was extracted using CH_2Cl_2. The organic layer was washed with water, dried with anhydrous $MgSO_4$, and the solvent was evaporated. The product was isolated by column chromatography on silica gel (12:6:1 ethyl acetate/hexanes/methanol) and recrystallized from hexane/ethyl acetate to afford 105 mg of **18** (66% yield).

mp 197-198°C **^{1}H NMR:** 1.86-1.90 (m, 1H), 2.02-2.07 (m, 1H), 2.41-2.49 (m, 2H), 2.98 (br, 1H), 3.11 (br, 1H), 3.71 (s, 6H), 3.77 (s, 3H), 4.12 (m, 1H), 4.36 (m, 1H), 4.54 (br, 1H), 5.91 (s, 1H), 5.93 (s, 1H), 6.24 (s, 2H), 6.44 (s, 1H), 6.77 (s, 1H)

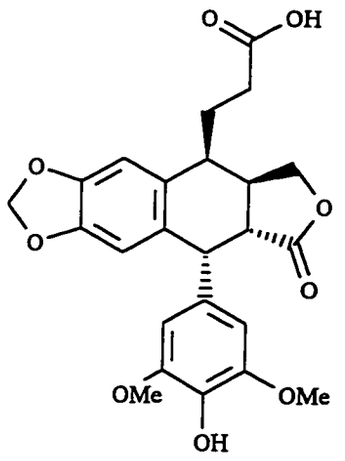
^{13}C NMR: 27.7, 32.7, 36.1, 38.5, 41.9, 44.1, 56.2, 60.8, 68.6, 101.3, 108.5, 108.8, 110.4, 130.3, 132.7, 136.1, 137.2, 147.1, 152.5, 174.7, 177.2 **IR:** 1708, 1776, 2600-3300 cm^{-1} **HRMS:** Calc'd for $C_{25}H_{26}O_9$: 470.1577, Found: 470.1590.



19

A solution of 10 mL of dichloroethane and 3 mL of diethyl ether saturated with anhydrous HBr at 0°C was added to 750 mg of **17** (1.6 mmol). The solution was stirred at 0°C for 30 min and at 25°C for 90 min. The solution was neutralized by adding solid potassium carbonate. The organic layer was diluted with 10 mL of CH_2Cl_2, washed with water and brine, dried with anhydrous $MgSO_4$, and the solvent was evaporated. The compound was isolated by radial chromatography on silica (ethyl acetate eluent) and recrystallized from CH_2Cl_2/hexane to yield 440 mg of **19** (1.0 mmol, 63%)

mp 129-130°C **^{1}H NMR:** 1.54-1.69 (m, 4H), 1.70 (m, 1H), 1.83 (m, 1H), 2.96 (m, 1H), 3.06 (m, 1H), 3.75 (s, 6H), 4.11-4.16 (m, 1H), 4.30-4.33 (m, 1H), 4.52 (d, $J_{1,2}$=4.7 Hz, 1H), 5.90 (s, 1H), 5.92 (s, 1H), 6.27 (s, 2H), 6.43 (s, 1H), 6.69 (s, 1H) **^{13}C NMR:** 14.6, 29.7, 32.1, 36.1, 39.3, 42.3, 44.0, 56.4, 62.6, 68.9, 101.2, 108.1, 108.8, 110.2, 131.7, 146.3. **IR:** 3000-3500 cm^{-1}(br) **HRMS:** Calc'd for $C_{24}H_{26}O_8$: 442.1628 Found: 442.1630

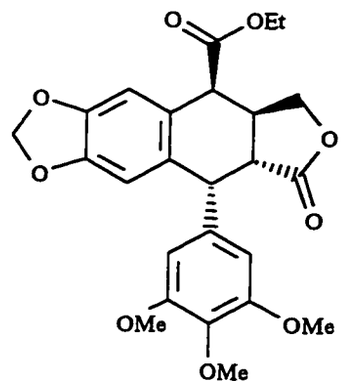


20

A solvent mixture of 25% diethyl ether in dichloroethane was saturated with HBr(g) at 0°C. The solvent mixture (10 mL) was added to 95 mg of **18** (0.2 mmol)and stirred at 0°C for 30 min and at 25°C for 2 h. The solution was brought to pH 7 with saturated ammonium bicarbonate, extracted with CH_2Cl_2, dried with anhydrous $MgSO_4$, and the solvent was evaporated. Chromatography on silica gel (12:6:1 ethyl acetate/hexanes/ methanol) by preparative TLC and recrystallization from hexane/ethyl acetate yielded 20 mg (0.04 mmol, 20% yield) of **20**, which decomposed substantially at every stage of purification.

mp 198-200°C **^{1}H NMR:** 2.94 (s, 1H), 2.97 (d, 1H), 3.05-3.08 (m, 1H), 3.75 (s, 6H), 4.12-4.18 (m, 1H), 4.31-4.33 (m, 1H), 4.53 (d, 1H), 5.90 (d, 1H), 5.92 (d, 1H), 6.27 (s, 2H), 6.44 (s, 1H), 6.69 (s, 1H) **^{13}C NMR:** 29.7, 32.1, 36.1, 39.3, 42.3, 44.0, 56.4, 62.6, 68.9, 101.2, 108.1, 108.8, 110.2, 131.0, 133.0, 134.2, 146.3, 175.3, 191.0

HRMS: Calc'd for $C_{24}H_{24}O_9$: 456.1420 Found: 456.1441

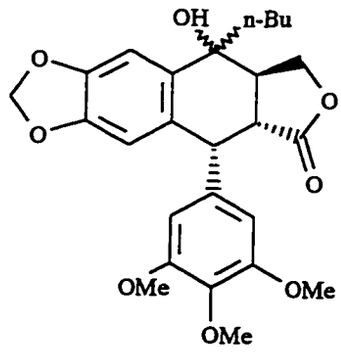


21

16 (150 mg, 0.35 mmol) was refluxed in a solution of 3 mL of conc HCl in 6 mL of ethanol for 1 h. The solution was brought to pH 7 with saturated sodium bicarbonate solution, extracted with CH_2Cl_2, dried with anhydrous $MgSO_4$, and the solvent evaporated to yield 95 mg of **21** as a white solid (0.20 mmol, 57% yield).

mp 138-139°C **^{1}H NMR:** 1.25 (t, 3H), 3.00 (dd, $J_{1,2}$=5.4 Hz, $J_{2,3}$=13.0 Hz, 1H), 3.15-3.20 (m, 1H), 3.72 (s, 6H), 3.77 (s, 3H), 3.78 (m, 1H), 3.83 (d, J=9.2 Hz, 1H), 4.10-4.20 (m, 1H), 4.44 (d, $J_{1,2}$=5.4 Hz, 2H), 5.92 (s, 1H), 5.94 (s, 1H), 6.07 (s, 1H), 6.47 (s, 1H), 7.11 (s, 1H) **^{13}C NMR:** 14.2, 25.7, 29.7, 32.1, 42.2, 46.4, 46.8, 56.2, 60.8, 61.0, 70.2, 101.3, 106.4, 108.9, 109.4, 121.7, 130.5, 135.6, 147.2, 147.7, 153.0, 171.5, 175.6

IR: 1732, 1776 cm^{-1} **HRMS:** Calc'd for $C_{25}H_{26}O_9$: 470.1577 Found: 470.1579



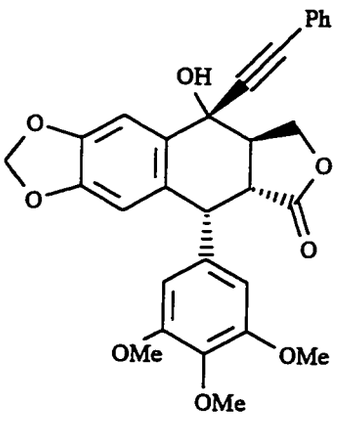
24

To a solution of podophyllotoxone (250 mg, 0.6 mmol) in 3 mL of dry THF at -78°C was added n-butyllithium (250 μL, 2.4M in hexanes, 0.6 mmol). The solution was stirred at -78°C for 15 min, then quenched with saturated NH_4Cl. Chromatography on silica gel (1:1 hexanes/ethyl acetate) afforded 40 mg of the α substituted isomer (0.085 mmol, 14% yield) and 70 mg of the β substituted isomer (0.14 mmol, 23% yield).

<u>α-isomer</u>: **mp** 105-106°C **^{1}H NMR:** 0.89 (t, 3H), 1.05-1.40 (m, 4H), 1.77-1.84 (m, 2H), 2.88-2.97 (m, 1H), 3.22 (dd, $J_{1,2}$=4.9 Hz, $J_{2,3}$=14.1 Hz, 1H), 3.71 (s, 6H), 3.79 (s, 3H), 4.27-4.38 (m, 2H), 4.58 (d, $J_{1,2}$=4.9 Hz, 1H), 5.96 (s, 1H), 6.00 (s, 1H), 6.31 (s, 2H), 6.52 (s, 1H), 6.98 (s, 1H) **^{13}C NMR:** 13.9, 23.1, 27.8, 39.3, 39.4, 41.7, 44.6, 55.9, 60.7, 67.1, 72.9, 101.5, 104.9, 107.8, 110.5, 132.5, 134.3, 135.1, 136.5, 147.9, 152.5, 175.4 **IR:** 1777, 3452(br) cm^{-1}

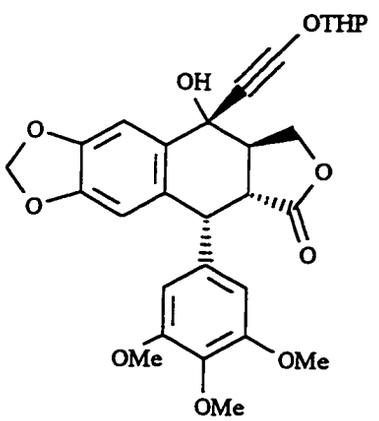
<u>β-isomer</u>: **mp** 110-111°C **^{1}H NMR:** 0.88 (t, 3H), 1.20-1.40 (m, 4H), 1.82-1.88 (m, 2H), 2.98 (m, 1H), 3.08 (dd, $J_{1,2}$=4.9 Hz, $J_{2,3}$=14.9 Hz, 1H), 3.73 (s, 6H), 3.78 (s, 3H), 4.27 (s, 1H), 4.45 (dd, 1H), 4.56 (d, $J_{1,2}$=4.9 Hz, 1H), 5.96 (s, 2H), 6.29 (s, 2H), 6.45 (s,

1H), 7.05 (s, 1H) **^{13}C NMR:** 13.9, 23.3, 27.2, 39.4, 44.4, 44.5, 44.6, 56.3, 60.7, 67.7, 74.6, 101.4, 106.0, 108.4, 109.6, 131.3, 135.4, 136.3, 137.2, 147.4, 147.7, 152.5, 174.5 **IR:** 1774, 3593(br) cm^{-1} **HRMS:** Calc'd for $C_{26}H_{30}O_8$ 470.1954 Found: 470.1967



25

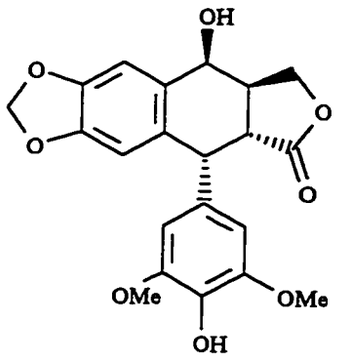
A solution of n-butyllithium (100 μL, 2.5M,0.25 mmol) and phenylacetylene (40 μL,0.36 mmol) generated at -78°C in THF was added via cannula to a solution of podophyllotoxone (100 mg, 0.25 mmol) in THF at -78°C. The solution was stirred for 30 min, quenched at -78°C with saturated NH_4Cl, and allowed to warm to 25°C. The reaction mixture was extracted with CH_2Cl_2, dried with anhydrous $MgSO_4$, and the solvent was evaporated. Separation by radial chromatography afforded the compound **25**. **mp** 115°C **^{1}H NMR:** 2.90-3.10 (m, 1H), 3.75 (s, 6H), 3.80 (s, 3H), 4.51-4.62 (m, 2H), 5.97 (d, 2H), 6.37 (s, 2H), 6.47 (s, 1H), 7.33 (s, 1H), 7.25-7.42 (m, 5H) **^{13}C NMR:** 29.7, 30.9, 43.3, 44.2, 44.7, 56.3, 60.7, 69.1, 71.8, 87.9, 89.9, 101.6, 106.3, 108.4, 109.6, 121.3, 128.4, 129.2, 130.5, 131.8, 135.0, 147.9, 148.3, 152.7, 174.2 **IR:** 1780, 3590 cm^{-1} **HRMS:** Calc'd for $C_{30}H_{26}O_8$ 514.1628 Found: 514.1625



26

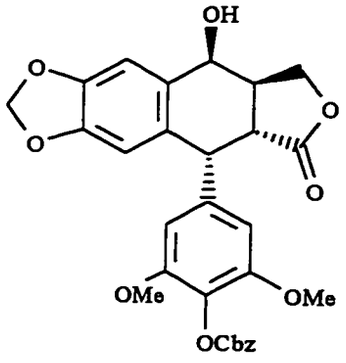
A solution of *n*-butyllithium (100 μL, 2.5M,0.25 mmol) and THP protected propargyl alcohol (32 mg, 0.25 mmol) in THF was added via cannula to a solution of podophyllotoxone (100 mg, 0.25 mmol) in THF at -78°C. The solution was stirred for 30 min, quenched at -78°C with saturated NH_4Cl, and allowed to warm to 25°C. The reaction mixture was extracted with CH_2Cl_2, dried with anhydrous $MgSO_4$, and the solvent was evaporated. Separation by radial chromatography afforded the compound **26** (35 mg, 0.06 mmol, 24% yield) (CH_2Cl_2/ethyl acetate gradient elution)

mp 110-111°C **^{1}H NMR:** 1.58-1.95 (m, 6H), 3.07-3.11 (m, 2H),3.55-3.60 (m, 1H), 3.73 (s, 3H), 3.78 (s, 6H), 4.27-4.31 (m, 1H), 4.44-4.50 (m, 2H), 4.56 (d, J=4.8 Hz, 1H), 4.70 (d, J=3.4 Hz, 1H), 5.95 (s, 1H), 5.97 (s, 1H), 6.34 (s, 2H), 6.45 (s, 1H), 7.17 (s, 1H) **^{13}C NMR:** 19.0, 25.2, 30.2, 43.0, 44.2, 44.6, 54.4, 56.2, 60.7, 62.2, 68.9, 71.3, 84.9, 86.4, 97.6, 101.6, 106.3, 108.4, 109.6, 130.5, 134.8, 135.0,137.3, 147.8, 148.2, 152.6, 174.2 **IR:** 1780, 3587 cm^{-1} **HRMS:** Calc'd for $C_{29}H_{30}O_9$ 552.1890 Found 552.2020



28

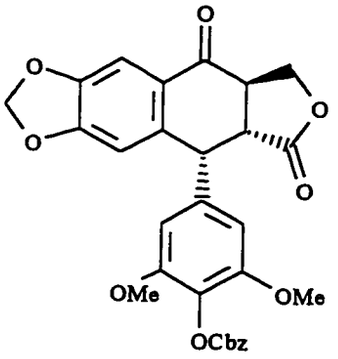
The procedure used was obtained from chemists at Bristol Myers Squibb.[17] A solution of diethyl ether (10 mL) and dichloroethane(30 mL) was saturated with HBr(g) at 0°C and added to podophyllotoxin (3.0 g, 7.2 mmol) and stirred for 20 min at 0°C. The solution was then stirred at 25°C for 16h and the solvent was evaporated. Acetone (80 mL), water (10 mL) and $BaCO_3$ (3.0g, 15 mmol) was added and the mixture stirred for 15h at 25°C. The acetone was evaporated and the remaining mixture was diluted with 50 mL of water and extracted with CH_2Cl_2. The organic extracts were dried with anhydrous $MgSO_4$, and the solvent was evaporated leaving a brown solid which was recrystallized from ethyl acetate to afford 2 g of 4'-demethylpodophyllotoxin (5 mmol, 69% yield). **mp** 224-226°C (lit value 224-226°C[17]) **^{1}H NMR**: 3.24 (dd, 1H), 3.75 (s, 6H), 3.83 (m, 1H), 4.29-4.34 (m, 2H), 4.58 (d, 1H), 4.81 (dd, 1H), 5.97 (dd, 2H), 6.25 (s, 1H), 6.52 (s, 1H), 6.83 (s, 1H).



29

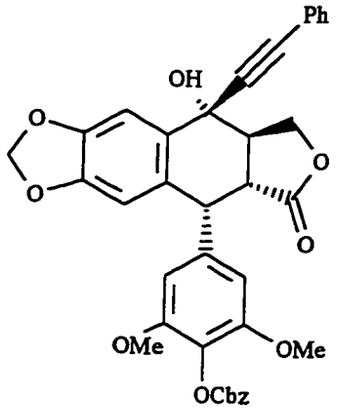
The compound was prepared by a literature procedure.[19] A solution of 4'-demethylpodophyllotoxin (2.0 g, 5.0 mmol), triethylamine (2.5 mL, 18 mmol), and benzyl chloroformate (2.5 mL, 18 mmol) in dry CH_2Cl_2 was prepared at 0°C and stirred at 25°C for 2 h, when the solution was deep purple. The solvent was evaporated and the compound purified by flash chromatography on silica gel to afford 1.9 g of **29** (3.6 mmol, 72% yield).

mp 205-207°C (lit value 205-207°C)[20] **^{1}H NMR**: 2.81 (m, 1H), 3.30 (dd, 1H), 3.74 (s, 6H), 4.34-4.41 (m, 2H), 4.64 (m, 1H), 4.86 (m, 1H), 5.26 (s, 2H), 5.98 (s, 1H), 6.00 (s, 1H), 6.31 (s, 2H), 6.55 (s, 1H), 6.88 (s, 1H), 7.33-7.43 (m, 5H).



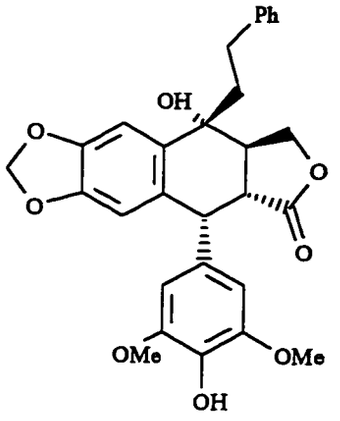
30

A solution of 100 mg of 4'-carbobenzyloxyepipodophyllotoxin [8] (0.19 mmol) and 300 mg of pyridinium dichromate (0.80 mmol) in 2 mL of dry CH_2Cl_2 was stirred at 25°C for 26 h. Separation by silica gel chromatography (1:1 hexanes/ethyl acetate) and recrystallization from CH_2Cl_2/hexanes afforded 82 mg of **30** (0.15 mmol, 79% yield) **mp** 99-100°C **^{1}H NMR:** 3.29 (dd, $J_{1,2}$=4.3 Hz, $J_{2,3}$=15.6 Hz, 1H), 3.45-3.51 (m, 1H), 3.71 (s, 1H), 4.34-4.38 (m, 1H), 4.56-4.59 (m, 1H), 4.87 (d, $J_{1,2}$=4.3 Hz, 1H), 5.27 (s, 2H), 6.09 (s, 1H), 6.11 (s, 1H), 6.42 (s, 2H), 6.70 (s, 1H), 7.34-7.44 (m, 5H), 7.56 (s, 1H) **^{13}C NMR:** 43.0, 44.3, 46.3, 55.9, 66.6, 70.1, 102.1, 105.8, 106.7, 109.4, 127.9, 128.1, 134.6, 134.9, 140.7, 147.8, 151.7, 152.8, 172.6, 191.9 **IR:** 1733, 1773 cm^{-1} **FABMS(M+1):** Calc'd for $C_{29}H_{24}O_{10}$ 533.1369 Found: 533.1397 **EA:** calc'd for $C_{29}H_{24}O_{10}$ 65.47%C, 4.55%H, found 65.56%C, 4.71%H



31

n-Butyllithium (60 μL, 2M in hexanes, 0.12 mmol) was added to a solution of phenylacetylene (20 μL, 0.18 mmol) in dry THF at -78°C. A solution of 90 mg of **30** (0.17 mmol) was added and stirring continued at -78°C for 30 min. The reaction was quenched at -78°C with saturated NH_4Cl. The mixture was allowed to warm to 25°C and extracted with CH_2Cl_2. The organic layer was washed with water, dried with anhydrous $MgSO_4$, and the solvent was evaporated leaving 82 mg of the white solid **31** (0.13mmol, 77% yield). The solid was used for the next step without further purification.



32

A mixture of 70 mg of **31** (0.13 mmol) and 25 mg 10% palladium on carbon in 5 mL of ethyl acetate was stirred under hydrogen atmosphere for 20 min when TLC showed no starting material remained. Filtration, evaporation of the solvent, and recrystallization from chloroform gave 25 mg of **32** (0.05 mmol, 38% yield)

mp 163-164°C **^{1}H NMR(acetone-d_6):** 2.17-2.21 (m, 1H), 2.75-2.78 (m, 1H), 3.14-3.21 (m, 1H), 3.36 (dd, $J_{2,3}$=14.9 Hz, $J_{1,2}$=5.7 Hz, 1H), 3.70 (s, 6H), 4.40 (s, 1H), 4.42 (d, J=1.5 Hz, 1H), 4.57 (d, $J_{1,2}$=5.7 Hz, 1H), 4.66 (s, 1H), 5.97 (s, 2H), 6.42 (s, 2H), 6.47 (s, 1H), 7.12-7.15 (m, 1H), 7.18-7.25 (m, 2H), 7.28 (s, 1H), 8.00 (s, 1H).

^{13}C NMR(acetone-d_6): 31.5, 41.4, 44.4, 45.0, 45.4, 56.5, 68.0, 74.2, 79.0, 101.9, 107.2, 110.0, 126.3, 128.9, 132.2, 132.6, 135.8, 138.1, 143.2, 147.5, 147.6, 147.9, 175.0

IR: 1778, 3529 cm^{-1} **HRMS**: Calc'd for $C_{29}H_{28}O_8$ 504.1784 Found: 504.1764

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Part III

Chapter 7 Synthesis and Photochemistry of α-Terthienyl Protein Conjugates

A Introduction

Many organisms have the ability to remove foreign materials from cells. Two very important examples of how this phenomenon affects humans are in the rejection of drugs in those undergoing cancer chemotherapy treatment, and in the rejection of pesticides by insects which are detrimental to the agricultural industry. In the case of rejection of chemotherapy drugs, Dr. Victor Ling of the Ontario Cancer Institute has discovered that cells develop resistance to alkaloids by overexpression of p-glycoprotein (P-gp).[1] P-gp exists as a phosphorylated, membrane bound glycoprotein weighing approximately 170,000 Da in molecular weight.[2] It consists of 12 transmembrane spanning regions and two cytoplasmic domains arranged in a repeating pseudo dimer. It has an extracellular loop that presents an N-linked site of glycosylation. It is this protein which expels the foreign chemicals from the cells.

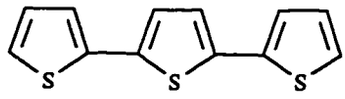
This p-glycoprotein pump has thus become the subject of study in the so-called multidrug resistance (MDR) phenomenon. It has been found that many non-resistant cells contain P-gp. Upon prolonged exposure to non-lethal concentration of a chemotherapy drug, the cells react by DNA amplification and mRNA overexpression of the p-glycoprotein gene, which gives rise to resistant cells. Because the drugs have already

destroyed the non-resistant cells, the problem becomes to destroy the new growth of cancerous cells, all of which have become resistant.

It is thought that a mechanism similar to the p-glycoprotein-based detoxification by the MDR cancer cells is most likely responsible for the extrusion of pesticides in insects. An alkaloid pump has been discovered in the tobacco hornworm, Manduca sexta.[3] It has been shown that p-glycoprotein - like immunoreactivity is intense in the Malpighian tubules of Manduca sexta, which are known to pump out nicotine. It became the objective of the biologists and biochemists involved in this project to characterize this pump in Manduca sexta and determine its relationship to the p-glycoprotein pump found in mammals. This objective included the isolation and characterization of the gene responsible for toxin resistance in the insects.

Work in the field of multidrug resistance has included the development of monoclonal antibodies which specifically bind to the p-glycoprotein pump in mammals.[4] In order to see if a p-glycoprotein pump was operating in insects, this antibody was administered to Manduca sexta. It has been shown that these antibodies do indeed bind to the Malpighian tubules of Manduca sexta, however, detection of the antibody has been difficult. It was therefore suggested that if a fluorescent molecule were attached to the antibody, it would be much easier to visualize the antibody in the insects, and thus further characterize the pump. It was also imagined if a phototoxic molecule were attached to the antibody, the conjugate could be photoactivated to specifically destroy the p-glycoprotein pump. Such antibody-targeted photolysis has been carried out in other laboratories and applied in clinical processes.[5] The phototoxic molecule chosen was α-terthienyl 1, or α-

T.[6] α-T is a natural product which is found in the plant family Asteraceae. It is produced in the plant as a secondary metabolite and serves as a chemical defense via its potent phototoxicity. It is highly fluorescent and is one of the most potent phototoxins known. It is also known to be an excellent sensitizer of singlet oxygen, which is most likely the reason for its toxicity.[7]



α-terthienyl 1

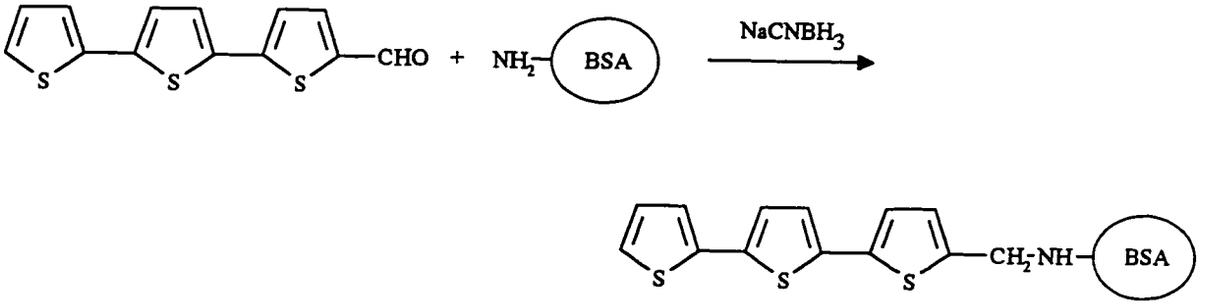
The next objective was to carry out the conjugation of the α-terthienyl to the antibody. There are a number of different methods of carrying out conjugation reactions between proteins and small molecules.[8] The methods take advantage of the functional groups on the surface of the protein to link on smaller molecules. In general, the groups available on a protein are the carboxyl groups of the aspartic and glutamic acid residues, amino groups of lysine residues, imidazo and phenolic groups of the histidine and tyrosine residues respectively, and the sulfhydryl group of the cysteine residues. Due to the variety of groups available in the protein, the method for conjugation is often chosen on the basis of the functional groups available on the molecule to be attached to the protein or antibody. Since a variety of α-T derivatives have been synthesized,[9] a choice of methods was available. The results of the conjugation reaction and the subsequent characterization

of the conjugates by biological and photochemical methods will be discussed in the next section.

B Synthesis of α-Terthienyl Conjugates

In order to develop methods for the conjugation reaction on a scale large enough to facilitate characterization, the protein bovine serum albumin (BSA) was chosen as a model compound for the P-gp antibody. The first method of conjugation was via reductive amination technique.[10] (Scheme 1)

Scheme 1



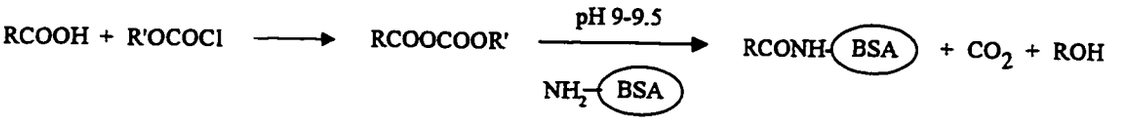
2-Terthiophenecarboxaldehyde was reacted with BSA in the presence of sodium cyanoborohydride for 7 days at 37°C in phosphate buffer saline solution. It was necessary to dissolve the aldehyde in a small volume of tetrahydrofuran before adding it to the solution since its solubility in aqueous solution is very low. The solution was dialysed for 20 hours against distilled water to remove the organic solvent, filtered to remove any

insoluble impurities, and freeze-dried to isolate the conjugate. Characterization of the conjugate by UV spectroscopy showed the conjugate contained approximately 12 molecules of α-T per molecule of protein. This was determined by using the free α-T aldehyde as a standard and assuming the extinction coefficient of the conjugated and free α-T would be approximately the same.

It was of interest to determine the nature of the binding of the α-T to the protein. There was a possibility that instead of becoming covalently bound to the protein, the α-T had only become associated with the protein. In order to determine whether or not this was the case, the conjugate was dialysed against 8M urea solution for 8 hours to denature or unwind the protein. The urea solution was extracted with ethyl acetate. No α-T was imparted to the ethyl acetate and the α-T conjugate retained its colour. From this experiment it was concluded that the α-T had been bound to the protein. When the same reaction was carried out using only α-T itself, not the aldehyde, only 3 molecules of α-T were bound to the protein by UV. This indicates the extent of association in the absence of covalent bonding, presumably the α-T became associated with the protein by intermolecular bonding only.

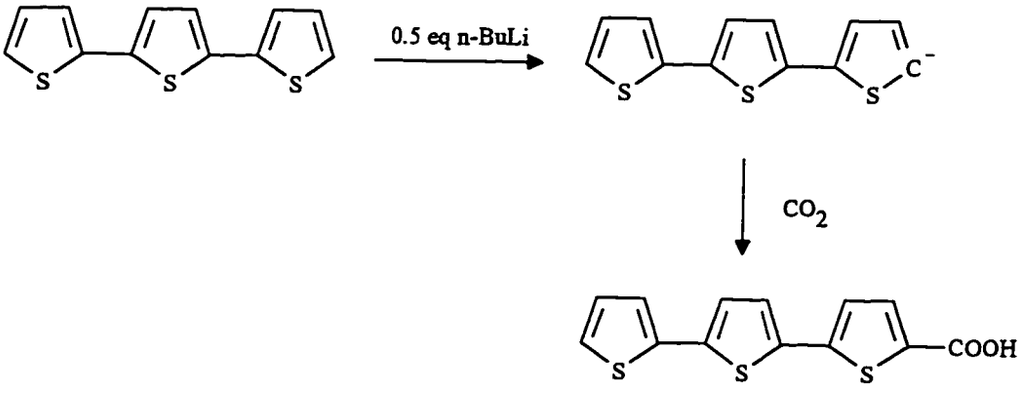
While this method provided α-T conjugates, it was desirable to carry out the reaction at lower temperatures and shorter time to decrease the probability of denaturing the protein, and eventually the antibody. The next method adopted was the mixed anhydride process.[7] (Scheme 2) This method involves reaction of a carboxylic acid with a chloroformate to generate a mixed anhydride. The mixed anhydride is then reacted with the amino groups of the protein to form amide bonds.

Scheme 2



In order to carry out this process between α-T and BSA, it was necessary to first obtain the 2-terthiophenecarboxylic acid. This molecule has been reported in the literature and is made by treating terthiophene with 0.5 equivalents of butyllithium to form the anion, followed by quenching with carbon dioxide to form the acid.[9] (Scheme 3) It has been shown that if one equivalent of base is used, an equilibrium mixture of the starting material, the monoanion and the dianion is created. When quenched, a mixture of the starting material, the monoacid and the diacid is formed. This must be avoided since the mono and diacids are quite difficult to separate.

Scheme 3



The 2-terthiophenecarboxylic acid thus obtained was reacted with methyl chloroformate in the presence of triethylamine in dioxane/THF at 10°C for 15 minutes. This solution was added to BSA in sodium bicarbonate solution and stirred for one hour at 10°C. The mixture was dialysed against distilled water for 24 hours, filtered and freeze-dried. Using this method, it was possible to vary the quantity of terthiophene mixed anhydride added and thus make conjugates with different numbers of α-T moieties on the protein. This was important since if too many hydrophobic molecules are attached to a protein, the configuration of the protein may be altered. In the case of an antibody this can dramatically affect its ability to bind to the desired target. It was possible to isolate conjugates with between 1 and 12 terthiophene moieties per molecule of BSA. These measurements were made by UV using the extinction coefficients for the corresponding amide of terthiophene.

This method was applied next to a mouse antibody to determine whether or not the same chemistry could be used. The same reaction was carried out using Mouse IgG purchased from Biocan. This conjugate was not freeze-dried, it was instead dialysed against phosphate buffer saline and appered to behave as the BSA, producing a yellow solution which was filtered to remove insoluble impurities. The same procedure was then repeated on the human antibody MRK16, which is a monoclonal antibody against p-glycoprotein. While the reaction appeared to proceed in the same manner as the previous examples, no experiments have as yet been carried out to determine whether or not the antibody remains active after the conjugation process.

C Characterization of BSA - α-T conjugates

1. Biological Activity

In order to determine the toxicity of the conjugated α-T in comparison to free α-T, yeast and brine shrimp bioassays were carried out using the BSA - α-T conjugates in the laboratory of Dr. J. T. Arnason in the Department of Biology.* For each bioassay, α-T was compared with protein conjugate at the same weight concentration, meaning the concentration of α-T in the conjugates is actually much lower. The conjugate used had approximately 12 α-T molecules per molecule of protein. The results for the yeast bioassay are summarized in Table 1. The results show the width of the area on the plate where yeast growth was inhibited. In the absence of UV light, no inhibition was visible. This is expected since upon irradiation the α-T produces singlet oxygen, which gives rise to the toxicity of the compound. Upon irradiation, the inhibition of yeast growth increases with increased concentration of α-T. It is interesting that when the same concentrations of the free α-T and the conjugated α-T are used, the toxicity of the conjugate is approximately 60-70% of that of the free molecule, when the concentration of α-T in the conjugate is roughly 10 times less than the free molecule. This suggests that the conjugate is a more efficient toxin than the free molecule.

* From the PhD Thesis of Gabriel R. Guillet, Department of Biology, University of Ottawa, 1997.

Table 1 Yeast Bioassay of α-T vs. α-T-BSA

μL stock solution (5 mg/mL)	*Inhibition Width (mm)*			
	+ UV		- UV	
	α-T	α-T-BSA	α-T	α-T-BSA
0.0	0.0	0.0	0.0	0.0
10.0	4.0	2.3	0.0	0.0
25.0	4.5	3.0	0.0	0.0
50.0	NA	3.0	NA	0.0

When both α-T and its BSA conjugate were evaluated in the brine shrimp bioassay, the conjugate with approximately 12 α-T molecules per molecule of BSA was used. The results are summarized in Table 2. The death rate was measured after 12, 24 and 36 hours. When the bioassay was performed under UV irradiation, all brine shrimp had died within 12 hours for both the conjugate and the free α-T. In the absence of UV, surprisingly, both forms were still quite toxic. As in the case of the yeast assay, the conjugate seemed to be a much more efficient toxin than the free α-T with death rates about 50-60% of that of the free α-T even though the effective concentration is about 10 times less than the free molecule. The bioavailability of the protein conjugate may be better than the free hydrophobic α-T itself.

Table 2 Brine Shrimp Bioassay of α-T vs. α-T-BSA without UV irradiation

[α-T] or [conj] (ppm)	d_{12}		d_{24}		d_{36}	
	α-T	α-T-BSA	α-T	α-T-BSA	α-T	α-T-BSA
0	0	0	0	0	0	0
25	10	0	63	30	93	50
50	34	22	73	46	93	58
100	NA	34	NA	63	NA	96

Both sets of biological results reflect the high toxicity of both the free α-T molecule and α-T conjugated to a protein. These results confirmed that the toxicity of the molecule was at least as good and probably better than the free molecule. This was an encouraging result since there was a significant possibility that the singlet oxygen produced by the α-T in the conjugate may have been quenched by the surrounding protein environment.

2. Photochemical Results

The photochemical characterization of α-T had already been carried out in the laboratory of Professor J.-C. Scaiano* in our department, where the studies described briefly in this section were also performed.[7] The goal was to compare the photochemical behaviour of α-T in a conjugate with that of free α-T and how the photochemical behaviour of α-T was affected by the surrounding protein environment. The differences between the photochemical behaviour of the α-T-BSA conjugate and α-T merely associated with the protein were also investigated.

Photoexcitation of α-terthienyl generates its triplet state, a potent electron donor and singlet oxygen sensitizer.[7] The triplet state can be monitored using laser flash photolysis techniques.[11,12] The lifetime of the strong triplet absorption at 460 nm in its transient absorption spectrum is about 27 μs in nitrogen saturated acetonitrile. The triplet is quenched with oxygen to generate singlet oxygen with quantum yields in the range 0.70-0.89 in a variety of solvents.[13] Electron transfer can be facilitated in the presence of good electron acceptors such as hydrophobic benzoquinone or water- soluble methyl viologen cation, resulting in the formation of the α-T radical cation, which absorbs at 530 nm. Because α-terthienyl is also highly fluorescent it was considered suitable as an antibody-conjugated photosensitizer.

* From the PhD Thesis of Ron Boch, Department of Chemistry, University of Ottawa, 1996.

The fluorescence of the conjugates proved to be very high. The intensities of the fluorescence emission spectra collected from 10 ppm α-T and 1 ppm α-T-BSA conjugate are roughly the same, even though the effective concentration of α-T molecules in the conjugate is much less. It was important that the conjugate could be detected at these low concentrations since this would be a concentration typically used in *in vivo* studies. The fluorescence of the conjugate has its maximum about 50 nm higher than α-T, presumably due to the increase in π conjugation due to the amide bond.

The transient absorption spectra for the conjugates were similarly shifted to higher wavelength. The triplet lifetime of protein-conjugated α-T was significantly longer than protein-free α-T in solution. While the lifetime of α-T in solution is only 27 μs, the lifetime of the triplet in the conjugate was about 100 μs. It was assumed that deactivation pathways normally present in homogeneous solution were restricted.

Quenching experiments were carried out with molecular oxygen. The α-T in the conjugate was quenched about an order of magnitude slower than α-T in homogeneous solution, with a rate constant of $3.6 \times 10^8\ M^{-1}s^{-1}$. Addition of oxygen shortened the triplet lifetime from about 100 μs to about 5 μs, indicating nearly complete quenching of the triplet with oxygen. The pattern of slower quenching was also observed in the associated complex of α-T with BSA. It was assumed that when α-T is either bound or associated with the protein, the protein may adapt a new conformation to accomodate the highly hydrophobic α-T. This reorganization causes the α-T to become buried in the protein and therefore it is not as accessible to oxygen.

The triplet quenching plots of the conjugate with benzoquinone and methyl viologen were dramatically different. The rate constants for associated and conjugated α-T with benzoquinone were 1.9 and 2.5 x $10^8 M^{-1}s^{-1}$ respectively, results similar to the quenching with oxygen in that they are slower by an order of magnitude. Again, it is assumed that the α-T molecules become buried within the protein and are thus not accessible to the quencher molecule. In contrast, the triplet quenching plot of the conjugate using methyl viologen, a water soluble electron acceptor, gave a two component quenching plot. The first componenet was fast due to triplets that were easily quenched, while the second was slower due to triplets that were not as easily quenched. When the same experiment was carried out on a solution of protein containing α-T which was not bound, but merely associated, no fast component was observed, indicating all of the α-T had been buried in the protein. This suggested the presence of two distinct populations of α-T in the conjugate. The fast component would arise from quenching at easily accessible sites on the surface of the protein, while the slower component would arise from less accessible, buried sites.

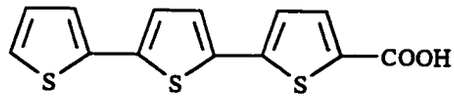
Singlet oxygen luminescence lifetime and quantum yield were also determined for the conjugate. In homogeneous solution, α-T and the known standard Rose Bengal are known to have similar quantum yields of singlet oxygen, about 0.8, with a lifetime of about 60 μs. The quantum yields of conjugates with 12 and 4 α-T molecules per molecule of protein are 0.2 and 0.1 respectively, with a lifetime of only about 40 μs. This was explained on the basis of quenching of singlet oxygen by the protein, meaning less singlet oxygen was able to escape to the solution. It had already been shown that singlet oxyen

reacts with tryptophan, tyrosine, histidine, methionine and cysteine at rates between 2 x 10^6 and 5 x 10^7 $M^{-1}s^{-1}$,[14] and since these amino acids are present in BSA, it is reasonable to assume they served as quenchers. It was encouraging to see that despite this behaviour, the quantum yields indicated that a significant amount of singlet oxygen does escape into solution. This trend is in agreement with a report where singlet oxygen yields were diminished for a series of chlorin e6 immunoconjugates, where the singlet oxygen yield decreased with increased conjugation.[5] The authors suggested this was due to higher photosensitizer aggregation.

To summarize, the conjugates were highly fluorescent, and thus would be easily detectable at very low concentrations. The triplet state was easily generated in the protein environment, and was quenched with oxygen, benzoquinone and methyl viologen. Its singlet oxygen production would be adequate to cause damage at the target site. The slower rates for quenching may be explained by reorganization of the protein around the hydrophobic α-T molecules. This is unfortunate since it suggests the protein has not maintained its original conformation, and when translated to the antibody case, this may result in loss of activity. In addition to this loss of binding capability, the lower quantum yield of singlet oxygen in the conjugate suggests the α-T has caused substantial damage to the protein, since the amino acid residues have most likely served as quenchers. This damage may also cause decreased antigenic specificity in the conjugate.

Experimental Section

General methods can be found in Chapter 2.



The compound was prepared using a literature procedure.[9] A solution of α-terthienyl (1g, 4 mmol) in THF at -78°C was treated with butyllithium (1.1 mL, 1.8 M, 2.0 mmol) and stirred for 15 min. The reaction mixture was quenched with $CO_{2(g)}$ and stirred for 15 min. The mixture was quenched with saturated NH_4Cl and allowed to warm to 25°C. The mixture was washed with water and extracted with THF. The organic extracts were dried with anhydrous $MgSO_4$ and the solvent was evaporated. The acid was isolated by silica gel column chromatography (2:1 ethyl acetate/hexane) and recrystallized from ethanol to yield 0.36 g terthiophenecarboxylic acid (0.12 mmol, 30% yield)

Conjugation of α-T to BSA by reductive amination technique

A solution of 3 mg of terthiophenecarboxaldehyde in 2.25 mL of THF was added to a solution of bovine serum albumin (25 mg), $NaCNBH_3$ (3.5 mg) and 2.5 mL of phosphate buffer saline in 20.5 mL of water and stirred at 37°C for 6 days. The solution was washed with ethyl acetate to remove any residual aldehyde. The solution was

dialysed against distilled water overnight and freeze dried. More than 95% of the total mass was recovered. UV analysis indicated the resulting conjugate contained ~12 molecules of α-T per molecule of protein.

Conjugation of α-T to BSA by mixed anhydride process

A solution of 2-thienylcarboxylic acid (30 mg, 0.1 mmol), methyl chloroformate (0.010 mL, 0.1 mmol), and triethylamine (0.015 mL, 0.11 mmol) in 0.80 mL of THF/dioxane (2:1) was stirred for 1 h at 0°C. An aliquot of this solution (10-500 μL) was added to a solution of 40 mg BSA (Sigma, essentially globulin free) in 4.0 mL 0.1 N aqueous sodium bicarbonate solution and stirred for 1 h at 10°C. The solution was then dialyzed against distilled water for 24 h and freeze dried. In order to obtain conjugates with varying degrees of substitution, the volume of the aliquot of α-T solution was varied. The extent of conjugation was measured by comparison to UV standards for 2-thienyl carboxamide.

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Claims to Original Research

1. The synthesis of regiospecifically substituted 2-hydroxybenzocyclobutenones was accomplished via halogen metal exchnage and cyclization of a 2-bromomandelate ester or the corresponding α-hydroxy Weinreb amide.

2. A 1-aryl-1,2-benzocyclobutenediol was synthesized via halogen metal exchange of the corresponding 2-bromobenzoin.

3. Synthesis of 1-aryl-cis-1,2-benzocyclobutenediols was accomplished.

4. 1-Aryl-cis-1,2-benzocyclobutenediols were oxidized in moderate yield to 2-aryl-2-hydroxybenzocyclobutenones and reacted under thermolysis conditions to furnish specifically substituted anthraquinones.

5. Mono and bis-alkylidene benzocyclobutenes were prepared regiospecifically in high yield.

6. A dithiolactone was prepared by reaction of 2-hydroxybenzocyclobutenone with Lawesson's reagent and characterized by x-ray crystallography.

7. A series of C-4 carbon substituted podophyllotoxin derivatives were prepared and tested for antitumour activity.

8. A series of terthiophene-bovine serum albumin conjugates were prepared by both reductive amination and mixed anhydride processes. These were characterized in terms of their biological activity and photochemical behaviour.

Publications from this Thesis

"Study of Photoinduced Energy and Electron Transfer in α-Terthienyl-Bovine Serum Albumin Conjugates: A Laser Flash Photolysis Study." Boch, R.; Mohtat, N.; Lear, Y.; Arnason, J. T.; Durst, T.; and Scaiano, J. C. *Photochem. Photobiol.* **64,** 92, (1996).

"Synthesis and Biological Evaluation of Carbon Substituted C-4 Derivatives of Podophyllotoxin." Lear, Y.; and Durst, T. *Can. J. Chem.* **74**, 1704, (1996).

"Synthesis of Regiospecifically Substituted 2-Hydroxybenzocyclobutenones." Lear, Y.; and Durst, T. *Can. J. Chem. in press*