# NOVEL SYNTHETIC APPROACHES TOWARD PROCYANIDINS AND BIFLAVONOIDS

MATTHEW CHILAKA ACHILONU

# NOVEL SYNTHETIC APPROACHES TOWARD PROCYANIDINS AND BIFLAVONOIDS

*Thesis submitted in fulfillment of the requirements for the degree*

**PHILOSOPHIAE DOCTOR**

*in the*

Department of Chemistry
Faculty of Natural and Agricultural Sciences

*at the*

University of the Free State
Bloemfontein

*by*

**MATTHEW CHILAKA ACHILONU**

**Promoter: Prof. Jan H. van der Westhuizen**
**Co-Promoter: Dr. Susan L. Bonnet**

*March 2009*

# ACKNOWLEDGEMENTS

To my amazingly good promoter, Prof. J. H. Westhuizen, thank you. I deeply appreciate all I learnt from you – brilliance and a never-give-up approach to research work. Thank you for your support, unalloyed advice, encouragement and wonderful ideas. Thank you for sacrificing some of your lunch breaks and weekends to look at my work and double-check my NMR spectra. Your insistence on high quality work has given the project and thesis a good value. You inspired me in more ways than the academic work.

My-co-promoter, Dr S.L. Bonnet, thank you for your invaluable input, advice, encouragement, and time spent in perusing my thesis. Thanks for unreserved contributions to our second publication. It demonstrated your hard earned talent. I endorse my earnest appreciation to you.

Prof. Daneel Ferreira, thank you for the time you squeezed out of your tight schedules during your annual visits to look at the progress of my work. Thank you for all your eye-opening ideas, advice and contributions.

Thank you to the Mimosa Central Co-operative Limited and the South African National Research Foundation for financial assistance, without which this thesis would never have materialized.

Co-students and workers, particularly Rosinah Montsho and Charles Enow, thanks for the good working atmosphere all of you helped to create. Anette Allemann, thank you for the CD spectra. Prof Louis Ackerman, I am grateful for the fatherly advice and encouragement you gave.

Thank you, Professor M. J. van der Merwe for the mass spectrometry.

To my friends, particularly Lawrence Amadi, and Celestine Amaechi and their families, thank you for the moral and financial support. That strengthened my spirit for the job well done.

I will not forget my beloved family: my wife, Matšeliso; my daughter, Chinedu; and my son, Ekene, for their continued love, support and understanding. It meant a lot. I thank you for absorbing the frustration transferred to you after the day's failed experiments.

Finally, most thanks go to Almighty God for leading the way.

M.C Achilonu 2009

# PUBLICATIONS AND PATENTS

We have published two papers and obtained two provisional patents from this research work:

1. 1-(4-Methoxybenzyl)pyridinium *p*-toluenesulfonate.

   *Acta Cryst*, (**2007**), *E63*, o4326.

   Matthew .C. Achilonu, J. Marthinus Janse van Rensburg, Jan H. van der Westhuizen and Andreas Roodt.*

2. Synthesis of Proanthocyanidins Part 1. The First Oxidative Formation of the Interflavanyl Bond in Procyanidins.

   *Org. Lett.*, **2008**, 10 *(17)*, pp 3865–3868

   Matthew. C. Achilonu, Susan L. Bonnet, Jan H. van der Westhuizen. *

3. PCT Application number **PCT/IB2009/052396** based on ZA Provisional Patent Application **2008/04953** Title: *"Method for The Preparation of C-4 Coupled Flavonoids, Proanthocyanidins and Analogues Thereof."* A provisional international patent filed on the 5 June 2009 in the name of The University of the Free State.

   The inventors are Matthew. C. Achilonu, Susan L. Bonnet, and Jan H. van der Westhuizen. *

4. South African Patent Application number **2009/05765** on *"The Synthesis of 3-Aryl Flavenes and C-3 Coupled Biflavonoids."* A Provisional patent application filed on the 19 August 2009 in the name of The University of the Free State.

   The inventors are Matthew. C. Achilonu, Susan L. Bonnet, and Jan H. van der Westhuizen. *

# TABLE OF CONTENTS

## INTRODUCTION

**CHAPTER 1:** GENERAL INTRODUCTION, CLASSIFICATION, AND NOMENCLATURE

| | | |
|---|---|---|
| 1.0 | Polyphenols and tannins | 1-2 |
| 2.0 | Classification of polyphenols | 2-3 |
| 3.0 | Hydrolyzable tannins | 3-4 |
| 4.0 | Flavonoids | 4-8 |
| 5.0 | Lignins | 8-10 |
| 6.0 | Proanthocyanidins (Condensed tannins) | 10-16 |
| 6.1 | Proanthocyanidin Classification | 10-12 |
| 6.2 | Types of proanthocyanidins | 12-16 |
| 6.2.1 | A-Type proanthocyanidins | 12-13 |
| 6.2.2 | B-Type proanthocyanidins | 13-14 |
| 6.2.3 | C-Type proanthocyanidins | 15 |
| 6.3 | Systematic nomenclature of proanthocyanidins | 16-19 |
| 6.4 | Determination of the stereochemistry of the C-ring of proanthocyanidins | 19-23 |
| 6.4.1 | Nuclear magnetic resonance spectroscopy (NMR) | 20-21 |
| 6.4.2 | Mass spectrometry | 21 |
| 6.4.3 | Circular dichroism (CD) | 22 |
| 6.4.4 | Crystallography | 22 |
| 7.0 | Tannins | 24-26 |
| 8.0 | Bi- and Triflavonoids | 27-28 |
| References | | 29-33 |

## LITERATURE SURVEY

**CHAPTER 2:** SYNTHESES OF PROANTHOCYANIDINS


| | | |
|---|---|---|
| 2.1 | Introduction | 34-35 |
| 2.2 | A review of synthetic methods | 35 |
| 2.2.1 | Methods based on flavan-4-ol and flavan-3,4-diol precursors to generate a C-4 flavanyl carbocation or an equivalent quinone methid | 36-51 |
| 2.2.2 | Methods nased on flavan-4-oxo monomers (C-4 ketoprocyanidin) as prcursors with an electrophilic C-4 carbon (nucleophilic addition) | 51-57 |
| 2.2.3 | Methods based on proanthocyanidin dimers and oligomers to generate C-4 flavanyl carbocation monomers via cleavage of the interflavanyl C-C bonds | 57-62 |
| 2.2.4 | Methods based on the introduction of functionality at C-4 of flavan-3-ols (activation of the benzylic position with a good leaving group) | 63-96 |
| 2.2.5.1 | Control of the degree of polymerization | 96-98 |
| 2.2.5.2 | Activation of the 'hard' acetate unit | 98-99 |
| 2.2.5.3 | Activation of the soft thiophenyl unit | 99-101 |
| 2.2.5.4 | Protection at C-8 | 101-102 |
| 2.2.5.5 | Orthogonal coupling reactions | 102-104 |
| References | | 106-109 |


**CHAPTER 3:** SYNTHESES OF BI- AND TRIFLAVONOIDS


| | | |
|---|---|---|
| 3.1 | Introduction | 110-112 |
| 3.2 | Syntheses of bi- and triflavonoids | 112 |
| 3.2.1 | Classes of bi- and triflavonoids (according to the nature | |

and position of the interflavonoid bond) 113-122

3.2.2 Classes of biflavonoids according to the chemistry employed to synthesize each class. 123-130

References 131-134

**CHAPTER 4: FLAVENES**

4.1 Introduction 135-136

4.2 Synthesis of flavenes from flavylium salts 136-137

4.3 Synthesis of flavenes *via* a cyclizable moiety 137-139

References 140-141

## DISCUSSION

**CHAPTER 5:** GENERAL CHEMISTRY OF FLAVONOIDS (Complex $C_6$-$C_3$-$C_6$ secondary metabolite monomers and oligomers)

5.1 Introduction 142-143

5.2 Chemistry of the hydroxyl groups on the aromatic A- and B-rings 143-144

5.3 Chemistry of the aromatic C-H groups 144-145

5.4 Chemistry of the C-2 145-147

5.5 Reaction at the C-3 position 147

5.6 Reaction at the C-4 position 147-148

References 149

**CHAPTER 6: SYNTHESIS OF FLAVENES AND 3-COUPLED BIFLAVONOIDS**

6.1 Introduction 150-151

6.2 $S_N2$ Substitution of the C-3 hydroxy group 151-152

6.3 Elimination of a tosyl or mesyl group at C-3 (Stereoselective synthesis of flav-3-enes and synthesis of flav-2-enes) 153-156

6.4 Condensation of 3-ketocatechin with an aryl moiety 156-163

6.5 Synthesis of I-3, II-6/8 biflavonoids 163

6.6 Future work 163-165

6.8 Conclusion 165

Reference 166-167

**CHAPTER 7:** NOVEL SYNTHESIS OF PROANTHOCYANIDINS WITH PREDOMINANTLY 3,4-CIS STEREOCHEMISTRY: THE FIRST CARBON-CARBON BOND FORMATION WITH SILVER TETRAFLUOROBORATE 168-186

References 187-188

## EXPERIMENTAL

**CHAPTER 8**: STANDARD EXPERIMENTAL METHODS

8.1 Chromatography 189-190

8.2 Spray reagent 190

8.3 Spectroscopical methods 190

8.3.1 Nuclear magnetic resonance spectroscopy (NMR) 190-191

8.3.2 Circular dichroism 191

8.3.3 Mass spectrometry 191

8.4 Anhydrous solvent and reagents 192

8.5 Chemical methods 192-193

8.5.1 Standard work up procedures 192

8.5.2 Acetylation 192-193

8.6 Freeze-drying 193

8.7 Melting point 193

8.8 Abbreviations 193-194

**CHAPTER 9: EXPERIMENTS**

9.1 **Synthesis of 1-(4-methoxybenzyl)-pyrimidinium; toluene-4-sulfate (8)** 195-96

**9.2 Syntheses of 3-*O*-derivatives of catechin.(Chap. 6)**

9.2.1 Synthesis of 5,7,3′,4′-tetra-*O*-methylcatechin (**1**) 196

9.2.2 Synthesis of 3-(*p*-toluenesulfonyl)-5,7,3′,4′-tetra-*O*-methylcatechin (**2**) 196-197

9.2.3 Synthesis of 3-*O*-methylsulfonyl-5,7,3′,4′-tetra-*O*-methylcatechin (**3**) 197-198

9.2.4 Synthesis of 3-*O*-trimethylsilyl-5,7,3′,4′-tetra-*O*-methylcatechin (**4**) 198-199

**9.3 Flavene preparation revisited: a convenient method of preparing flav-3-ene (Chap. 6)**

9.3.1 Synthesis of flav-2-ene (**11**) and flav-3-ene (**9**) from 3-(*p*-toluenesulfonyl)-5,7,3′,4′-tetra-*O*-methylcatechin 200

9.3.2 Synthesis of flav-2-ene (**11**) from 3-(*p*-toluenesulfonyl)-5,7,3′,4′-tetra-*O*-methylcatechin 201-202

9.3.3 Synthesis of flav-3-ene (**9**) from 3-*O*-methylsulfonyl-5,7,3′,4′-tetra-*O*-methylcatechin 202

**9.4 Synthesis of (*2R*)-5,7,3′,4′-tetra-*O*-methylflavan-3-one (16) (Chap. 6)** 202-203

**9.5 Synthesis of 3-coupled biflavonoids (Chap. 6)**

9.5.1 Synthesis of 3-(1,3,5-tri-O-methylphloroglucinol)-5,7,3,4-tetra-O-methylflavan-3-ene **(24)** 203

9.5.2 Attempts to couple phloroglucinol, free-phenolic catechin or tetra-*O*-methylcatechin to C-3 of tetra-*O*-methyl-3-oxocatechin 203-205

9.5.3 Investigation of temperature effect on the synthesis of 3-(1,3,5-tri-*O*-methylphloroglucinol)-5,7,3′4′-tetra-*O*-methyl-flavan-3-ene 205-206

9.5.4 Investigation of quantity of $SnCl_4$ (solution of 1 M $SnCl_4$ in dichloromethane) and solvent effect on the synthesis of 3-(1,3,5-tri-*O*-methylphloroglucinol)-5,7,3′4′-tetra-*O*-methylflavan-3-ene 206-207

9.5.5 Investigation of the activation ability of some other Lewis acids (LA) ($InCl_3$, $TiCl_4$, $Yb(OTf)_3$) on the synthesis of 3-(-1,3,5-tri-*O*-methylphloroglucinol)-5,7,3′4′-tetra-*O*-methylflavan-3-ene 207

**9.6 Syntheses of proanthocyanidins (Chap. 7)**

9.6.1 Synthesis of (*2R,4S*)- **(13)** and (*2R,2S*)-4-(1,3,5-tri-*O*-methylphloroglucinol)-5,7,3′,4′-tetra-*O*-methylflavan-3-one **(14)** 207-209

9.6.2 Synthesis of (*2R,3S,4S*)- **(22)** and (*2R,3S,4R*)-4-(1,3,5-tri-*O*-methylphloroglucinol)-5,7,3′,4′-tetra-*O*-methylflavan-3-ol **(24)** 209-210

9.6.3 Synthesis of (*2R,4S:2R,3S*)-5,7,3′,4′-tetra-*O*-methylflavan-3-one-4β→8-5,7,3′,4′-tetra-*O*-methylflavan-3-ol **(26)** and

(*2R,4R:2R,3S*)-5,7,3′,4′-tetra-*O*-methylflavan-3-one-
4α→8-5,7,3′,4′-tetra-*O*-methylflavan-3-ol **(27)** 211-213

9.6.4 Synthesis of (*2R,3S,4S:2R,3S*)-5,7,3′,4′-tetra-*O*-
methylflavan-3-ol-4β→8-5,7,3′,4′-tetra-*O*-methylflavan-3-ol **(28)** 213-215

References 216

**PHYSICAL DATA**

**APPENDIX A: NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY**

NMR plates 217-319

**APPENDIX B: REPRESENTATIVE CD SPECTRA**

CD plates 319-334

# SUMMARY


In this thesis we investigate procedures to introduce functional groups into the C-ring of flavonoids. These include the introduction of a double bond, aryl coupling on the C-3 position to obtain biflavonoids and aryl coupling on the C-4 position to obtain proanthocyanidins.

We observed that treatment of tetra-*O*-methyl-3-*O*-mesylcatechin with base yielded the corresponding flav-3-ene. We optimized conditions to obtain yields close to 100% (DBU, reflux in acetonitrile for 24 hours, mesyl derivative). Retention of configuration at C-2 was observed. Treatment of tetra-*O*-methyl-3-*O*-tosylepicatechin with DBU gives a mixture (*ca* 1:1 ratio) of the corresponding flav-2-ene and flav-3-ene in high yield. The C-2 hydrogen of epicatechin is *trans* relative to the tosyl/mesyl group at C-3 and base catalysed *trans* elimination to a flav-2-ene is feasible. This contrasts with tetra-*O*-methyl-3-*O*-mesylcatechin where the C-2 hydrogen is *cis* relative to the tosyl/mesyl group at C-3 and the only available *trans* hydrogen is at C-4, resulting in the flav-3-ene exclusively. Flav-2-enes and flav-3-enes are normally difficult to synthesize due to the ease with which they oxidize to anthocyanidins. We have thus developed an efficient and high yielding method that yields the first stereoselective access to optically active flav-3-enes.

Treatment of tetra-*O*-methyl-3-oxocatechin with tri-*O*-methylphloroglucinol in the presence of $SnCl_4$ affords facile coupling of the phloroglucinol analogue at C-3 *via* a carbon-carbon bond to give the 3-aryl-3,4-dehydrocatechin. The 3-arylcatechin intermediate could not be isolated, presumably because water elimination is encouraged by the formation of a stilbene-type conjugated system between the electron rich A-and D-rings. CD data confirm retention of configuration at C-2. We have thus developed proof of concept for the first stereoselective synthetic access to I-3,II-6/8 biflavonoids. This represents a significant contribution towards flavonoid synthesis.

Treatment of tetra-*O*-methyl-3-oxocatechin with tri-*O*-methylphloroglucinol in the presence of $AgBF_4$ in THF afforded the C-4β- and C-4α phloroglucinol-3-oxocatehin

adducts in 45% and 13% yields, respectively. Subsequent reduction with $NaBH_4$ in aqueous NaOH/MeOH afforded the C-4β and C-4α arylflavan-3-ol derivatives in 98% and 95% yields, respectively.

The requirement of an excess of $AgBF_4$ and the observation of a silver mirror (reduction of $Ag^1$ to $Ag^0$) indicate a two-electron oxidative mechanism. No self condensation or further condensation products were evident, probably due to the deactivation of the nucleophilic properties of the A-ring of the 3-oxocatechin *via* the enolic tautomer of the C-ring.

The $AgBF_4$-catalyzed condensation reaction between tetra-*O*-methyl-3-oxocatechin and tetra-*O*-methylcatechin afforded the anticipated C-4β and C-4α dimers in 38% and 6% yields, respectively, with [2*R*,4*S* (C-ring):2*R*,3*S* (F-ring)] and [2*R*,4*R* (C-ring):2*R*,3*S* (F-ring)] configurations, respectively, based on NMR coupling constants and NOESY data.

We thus developed a novel and facile method for the introduction of a phenolic unit at unfunctionalized C-4 of per-*O*-methylcatechin and hence to synthesize procyanidin B-3 type dimer derivatives. Our method has the following advantages:

1. It does not require pre-functionalization of the C-4 position of flavan-3-ols. We have developed direct oxidative C-6/8→C-4 bond formation between two flavonoid monomers. We could not find any previous usage of $AgBF_4$ to effect C-C bond formation.

2. Self condensation and the formation of homo-polymers are not observed. We believe that the carbonyl group on the C-3 position of our one starting material conjugates with the aromatic A-ring via its enol. This lowers the HOMO energy and deactivates the A-ring to such an extent that it does not act as a nucleophile.

3. The synthetic methods described so far rely on an electrophilic flavan-3-ol with a hydroxy group at C-3. The configuration of the hydroxy group at C-3 controls the stereochemistry at C-4 and 3,4-*trans* proanthocyanidins are

usually isolated as the major product. In our method, C-3 is a planar $sp^2$ carbonyl and the stereochemistry at C-4 is controlled by the stereochemistry at C-2 (configuration of the B-ring). We thus have access to both 3,4-*cis* and 3,4-*trans* proanthocyanidins depending on the configuration of the B-ring at C-2.

We have thus developed a unique and facile synthesis of catechin dimer derivatives that circumvents the need for C-4 functionalization, avoids competing polymerization and allows stepwise formation of hetero-oligomers. This method, based upon oxidative C-C interflavanyl bond formation will contribute significantly to ready synthetic access to proanthocyanidin analogues, especially procyanidins with 3,4-cis congifured catechin chain extension units.

# SAMEVATTING

Hierdie tesis ondersoek die verskillende metodes om die C-ring van flavonoïede te funksionaliseer. Die resultate sluit in die vorming van ’n dubbelbinding in die C-ring, koppeling van ’n arielgroep op C-3 om biflavonoïede te lewer, en arielkoppeling op C-4 wat aanleiding gee tot proantosianidiene.

Ons het waargeneem dat die behandeling van tetra-*O*-metiel-3-*O*-mesielkatesjien met basis die ooreenstemmende flav-3-een lewer. Die reaksiekondisies is geoptimiseer en opbrengste van feitlik 100% is verkry (DBU, refluks in asetonitriel vir 24 uur, mesielderivaat). Retensie van konfigurasie by C-2 is waargeneem. Behandeling van tetra-*O*-metiel-3-*O*-tosielepikatesjien met DBU het ’n mengsel (*ca* 1:1 ratio) van die ooreenstemmende flav-2-een en flav-3-een in hoë opbrengste gelewer. Die C-2 waterstof van die C-3 gefunksionaliseerde epikatesjien (*O*-mesiel of *O*-tosiel) is *trans* relatief tot die mesiel/tosielgroep op C-3 en dit maak basisgekataliseerde *trans* eliminasie na die flav-2-een waarskynlik. In teenstelling hiermee, is die C-2 waterstof van die C-3 gefunksionaliseerde katesjien (*O*-mesiel of *O*-tosiel) *cis* relatief tot die mesiel/tosielgroep op C-3 en dus is *trans* eliminasie nie moontlik nie. Die C-4 waterstof is egter *trans* relatief tot die mesiel/tosielgroep op C-3 en dus word slegs die flav-3-een gevorm. Flav-2- en flav-3-ene is gewoonlik moeilik om te sintetiseer aangesien oksidasie na antosianidiene met gemak plaasvind. Ons het dus nie slegs die eerste stereoselektiewe sintese vir opties aktiewe flav-3-ene ontwikkel nie, maar die sintese lewer ook hoë opbrengste.

Behandeling van tetra-*O*-metiel-3-oksokatesjien met tri-*O*-metielfloroglusinol in die teenwoordigheid van $SnCl_4$ lewer die 3-ariel-3,4-dehidrokatesjien d.m.v. koppeling van die floroglusinolderivaat met C-3 *via* die vorming van ’n koolstof-koolstof binding. Die intermediêre C-3-fluoroglusinol alkoholderivaat is nie geïsoleer nie. Eliminasie van water word waarskynlik bevorder deur die vorming van ’n stilbeen tipe gekonjugeerde sisteem tussen die elektronryk A- en D-ringe van die 3-ariel-3,4-dehidrokatesjien. Die retensie van konfigurasie by C-2 is bevestig deur SD data. Ons het dus die konsep vir die eerste stereoselektiewe sintetiese toegang tot I-3,II-6/8 biflavanoïede bewys.

Behandeling van tetra-*O*-metiel-3-oksokatesjien met tri-*O*-metielfloroglusinol in die teenwoordigheid van $AgBF_4$ in THF het beide die C-4β- en C-4α-floroglusinol derivate gelewer met opbrengste van 45% en 13% onderskeidelik. Die 3-C karbonielgroep is vervolgens met waterige NaOH/MeOH gereduseer om die C-4β- en C-4α-arielflavan-3-ol derivate te lewer in feitlik kwantitatiewe opbrengste.

’n Oormaat $AgBF_4$ word benodig vir die sintese en tydens die reaksie word ’n silwer spieël teen die wande van die reaksiefles gevorm (reduksie van $Ag^1$ na $Ag^0$). Hierdie twee waarnemings dui op ’n twee-elektron, oksidatiewe reaksiemeganisme. Geen selfkondensasie- of verdere kondensasie oligomere is waargeneem nie, waarskynlik omdat die nukleofiele eienskappe van die A-ring van die 3-oksokatesjien gedeaktiveer word via die enoliese tautomeer van die C-ring.

The $AgBF_4$ gekataliseerde kondensasiereaksie tussen tetra-*O*-metielkatesjien en tetra-*O*-metiel-3-oksokatesjien het die verwagte dimere [4β (38%) en 4α (6%)] gelewer met [2*R*,4*S* (C-ring):2*R*,3*S* (F-ring)] en [2*R*,4*R* (C-ring):2*R*,3*S* (F-ring)] konfigurasies onderskeidelik, gebaseer op KMR koppelingskonstantes en NOESY data.

Ons het dus ’n nuwe en eenvoudige metode ontwikkel om ’n fenoliese eenheid aan die ongefunksionaliseerde C-4 van per-*O*-metielkatesjien te koppel en dus om prosianidien B-3 tipe dimeer derivate te sintetiseer. Hierdie metode het die volgende voordele:

1. Dit is nie nodig om die C-4 posisie van flavan-3-ols vooraf te funksionaliseer nie. Ons het ’n direkte oksidatiewe bindingsvorming tussen twee monomeriese flavanoïede bewerkstellig. Geen vorige verwysings oor die gebruik van $AgBF_4$ vir C-C-bindingsvorming is beskryf nie.

2. Selfkondensasie en homo-polimeervorming is nie waargeneem nie. Ons glo dat die karbonielgroep op C-3 van ons een uitgangstof met die aromatiese A-ring konjugeer *via* die enol. Dit verlaag die HOMO energie en deaktiveer die A-ring sodoende dat dit nie as ’n nukleofiel kan optree nie.

3. Die sintetiese metodes wat tot dusver beskryf is maak staat op die elektrofiliese flavan-3-ol met ’n hidroksiegroep op C-3. Die konfigurasie van die hidroksiegroep op C-3 beheer die stereochemie van C-4 en die 3,4-*trans* proantosianidiene word gewoonlik as die hoofproduk geïsoleer. Ons metode maak staat op die planêre C-3 wat $sp^2$ gehibridiseer is en dus word die stereochemie op C-4 beïnvloed deur die stereochemie op C-2 (i.e. op relatiewe stereochemie van die B-ring). Ons het dus toegang verkry tot beide die 3,4-*cis* en 3,4-*trans* proantosianidiene, afhangend van die konfigurasie op C-2. In die geval van katesjien derivate vorm die 3,4-*cis* produk dus as hoofproduk.

Ons het dus ’n unieke en eenvoudige sintese van katesjien dimere ontwikkel wat C-4 funksionalisering uitskakel, kompeterende polimerisasie verhoed en die stapsgewyse vorming van hetero-oligomere moontlik maak. Hierdie metode, gebaseer op oksidatiewe C-C-interflavaniel bindingsvorming sal grootliks bydrae tot sintetiese toegang tot proantosianidien analoë, veral die prosianidiene met 3,4-*cis* stereochemiese katesjien ketting eenhede.

# Chapter 1

## GENERAL INTRODUCTION, CLASSIFICATION AND NOMENCLATURE.

The primary aim of the thesis is to advance our understanding of the synthesis of proanthocyanidins and biflavonoids. This requires formation of carbon-carbon bonds between flavanoid monomers.

The complex nature of flavonoids has spawned a complex classification and terminology. Our purpose in this chapter is to explain our use of the following terms: *polyphenol, hydrolysable tannin, complex tannin, tannin, condensed tannin, proanthocyanidin, procyanidin, anthocyanidin, anthocyanin, flavonoid, flavanoid, bi- and triflavonoid,* etc.

Because the stereochemistry of the C-ring plays such an important role in this thesis, we briefly discuss the use of NMR, mass spectrometry and circular dichroism to establish the relative and absolute stereochemistry of the C-ring of flavonoids.

Chapter two is a literature review of published syntheses of proanthocyanidins and chapter three reviews bi- and triflavonoid syntheses. In our efforts to synthesize proanthocyanidins and biflavonoids we made important contributions to the synthesis of flavenes. To put this in perspective, chapter four reviews the syntheses of flavenes.

## 1.0 POLYPHENOLS AND TANNINS

Naturally occurring polyphenols and tannins have traditionally played an important role in human life as ingredients in food and herbal medicines.[1-3] They play an essential role in the organoleptic properties of tea and wine. The industrial use of polyphenols from agricultural sources for the production of lignosulfonates[4,5] (from lignin), the tanning of leather[6] (with tannins), and the production of adhesives[7,8,9] (from proanthocyanidins) is of growing importance because crude oil based raw materials are being depleted. Leather tanning with chromium salts is causing serious environmental concerns,[10,11] particularly regarding the disposal of the waste from the tanning process and used leather products. More recently, it has been realized that flavonoids and polyphenols do not only act as antioxidants in the human body but interact specifically with biomolecules, including proteins and enzymes, to induce powerful biological activities. This has stimulated the search for biologically active entities that can be developed into pharmaceuticals.

## 2.0 CLASSIFICATION OF POLYPHENOLS

Polyphenols are secondary metabolites (natural products) that contain more than one phenolic hydroxy group per molecule. They are classified into two major groups:

1. Hydrolysable tannins (glucose and other sugar esters of gallic acid) and;
2. Phenylpropanoids.

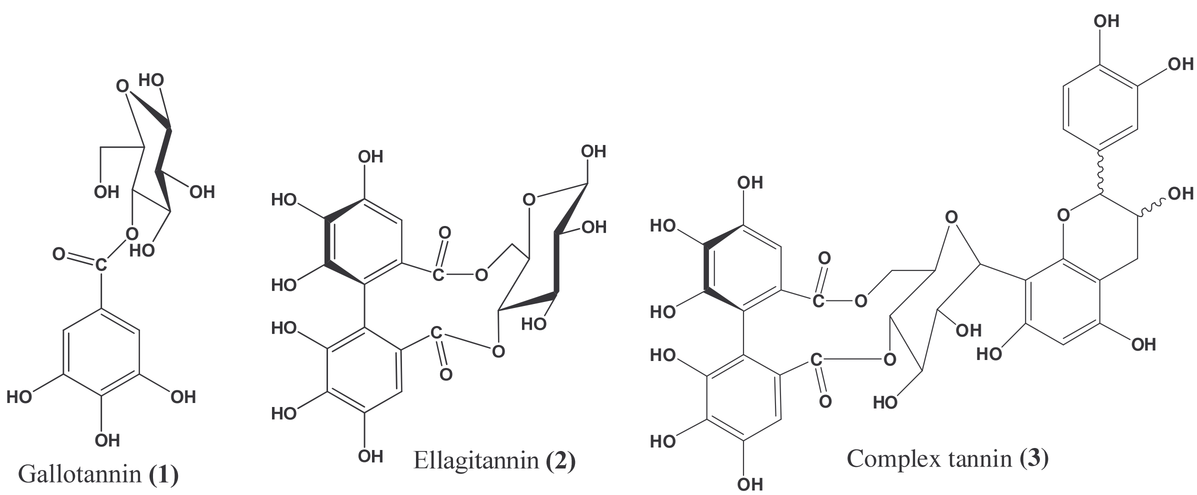
The phenylpropanoids are further subclassified into:

3. Flavonoids,
4. Lignins, and
5. Condensed tannins (also called proanthocyanidins).

This classification of the phenylpropanoids is based on the biosynthesis pathways in the shikimate cycle[12] responsible for each class, and their importance to certain fields of study. The condensed tannins were traditionally studied to obtain a better understanding of the leather tanning processes,[13] lignins to understand soil and paper chemistry, and flavonoids to understand the interaction between plants and insects (e.g. to repel plant-eating insects and attract pollinating insects).[3] The uniqueness of condensed tannins lies not only in their polyphenolic character but also in their relatively large molecular size (MW up to 20,000 amu).[14] Nowadays, condensed tannins are intensively studied because of their industrial use as adhesives, and flavonoids for their importance to human health.[3] When proanthocyanidins are bonded to sugar molecules, they are simply called proanthocyanidin glycosides.

# 3.0 HYDROLYZABLE TANNINS

The hydrolysable tannins can be classified into gallotannins **(1)** (sugar esters of gallic acids) and ellagitannins **(2)** (sugar esters of two gallic acid units C-C linked to each other). If the gallotannin or ellagitannin is linked *via* a carbon-carbon bond to a flavan-3-ol, the molecule is described as a complex tannin. Complex tannins are thus a combination of hydrolysable and condensed tannins **(3)** (Figure 1).[6]



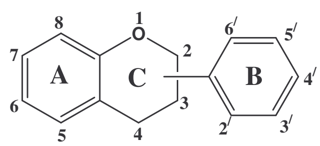
Gallotannin **(1)** Ellagitannin **(2)** Complex tannin **(3)**

**Figure 1:** Types of hydrolysable tannins.

This classification should not be confused with Ferreira's[15] use of the term "complex $C_6$-$C_3$-$C_6$ secondary metabolites" that refers to the two major classes of condensed tannins i.e. proanthocyanidins and bi- and triflavonoids (vide infra).

# 4.0 FLAVONOIDS

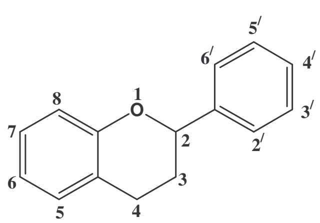
The term "flavonoid" is generally used to describe a broad collection of plant secondary metabolites whose chemical structures are based on a $C_6$-$C_3$-$C_6$ carbon system. The skeleton **(4)** is composed of 15 carbon atoms which consist of a benzopyran (chroman) ring bearing a second aromatic ring (ring-B) in position 2, 3 or 4 (Figure 2).



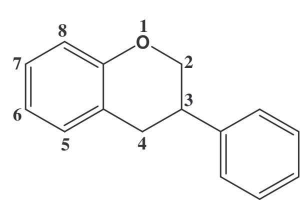

**(4)**

**Figure 2:** Flavonoid representative structure.

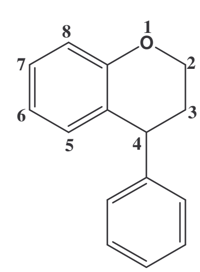
Depending on the position of the B-ring on the chroman moiety, the structure may be classified as a flavonoid (2-phenylbenzopyran) **(5)**, isoflavonoid (3-phenylbenzopyran) **(6)** or a neoflavonoid (4-phenylbenzopyran) **(7)** (Figure 3)**.**[16]



**(5)**



**(6)**

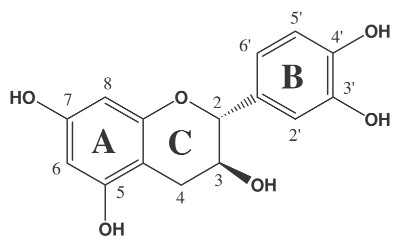


**(7)**

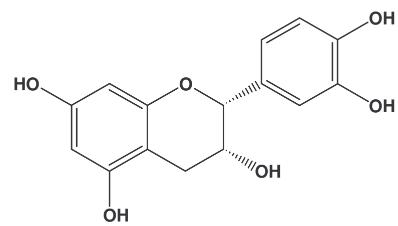
**Figure 3:** Classes of flavonoids.

There is ambiguity in the use of the word "flavonoid". It can either be used generally to describe all $C_6$-$C_3$-$C_6$ secondary metabolites or specifically to describe $C_6$-$C_3$-$C_6$ secondary metabolites with a carbonyl group at C-4. For this report, the $C_6$-$C_3$-$C_6$ secondary metabolites without a C-4 carbonyl group are referred to as flavanoids.

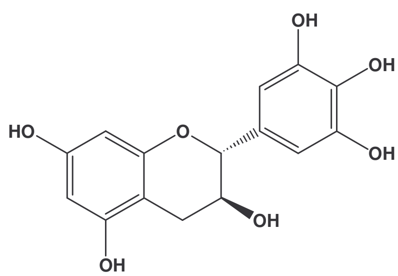
Flavan-3-ols (or flavanols) are a subgroup of flavanoids, which includes catechin **(8)**, epicatechin **(9),** gallocatechin **(10),** and epigallocatechin **(11)**, and their 3-*O*-gallates **(12)** and **(13)**.



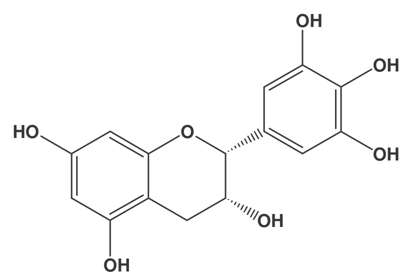

Catechin **(8)**



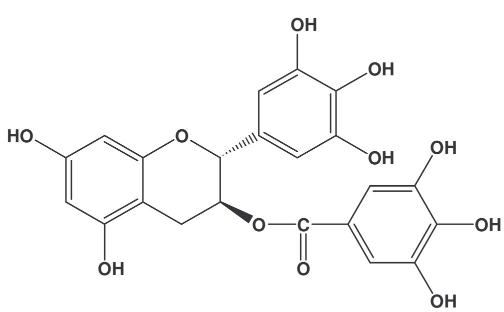
Epicatechin **(9)**



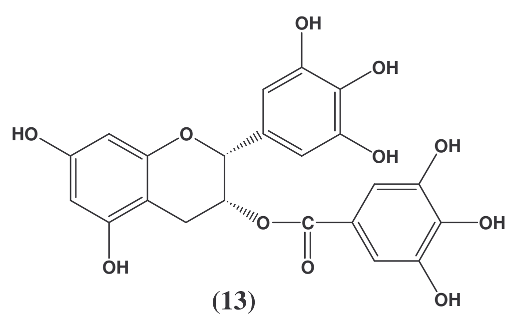
Gallocatechin **(10)**



Epigallocatechin **(11)**



**(12)**
Gallocatechin gallate (GCG)

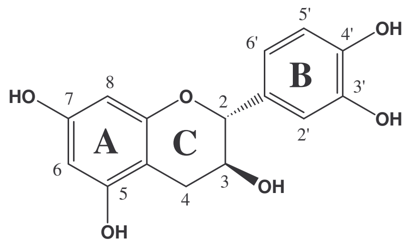


**(13)**
Epigallocatechin gallate (EGCG)

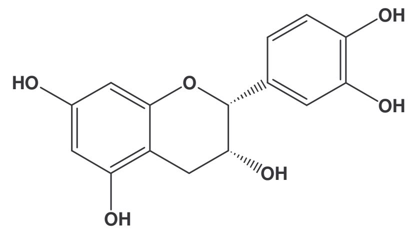
**Figure 4:** Flavan-3-ols.

Flavanoids are abundant in many plant species[17] and are the building blocks for proanthocyanidins.

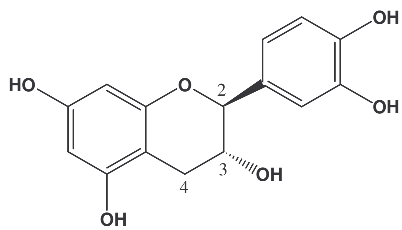
*Ent*-catechin **(14)** and *ent*-epicatechin **(15)** are rare in nature,[18,19] and co-exist with catechin **(8)** and epicathechin **(9)** (Figure 5). In the same manner, fisetinidol **(16)** is often found together with its enantiomer, *ent*-fisetinidol **(17)** in nature (Figure 6).[18-20]



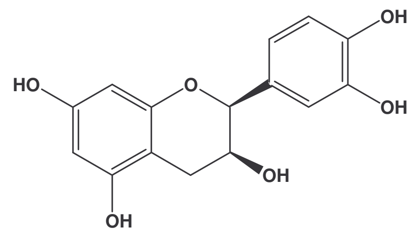
Catechin (**8**)



Epicatechin (**9**)

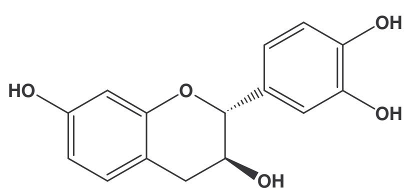


*ent*-Catechin (**14**)

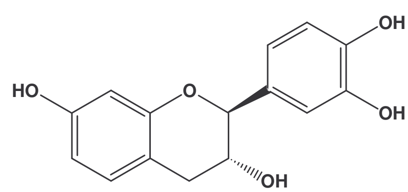


*ent*-Epicatechin (**15**)

**Figure 5:** Isomers of Catechin .



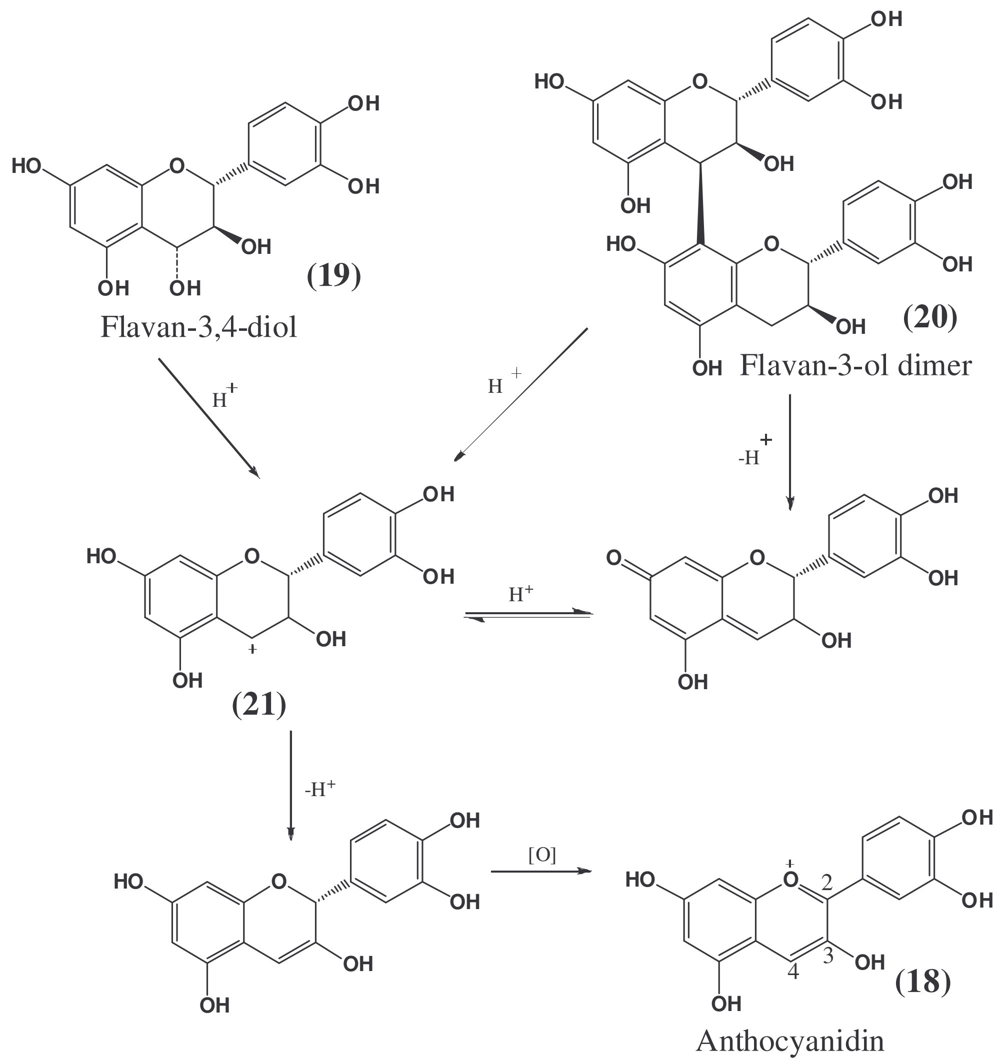
Fisetinidol (**16**)



*ent*-Fisetinidol (**17**)

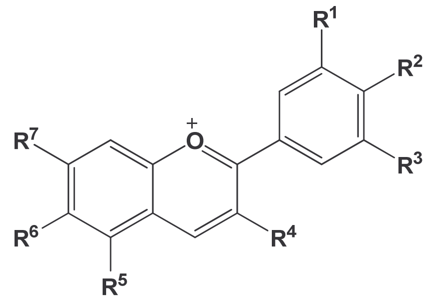
**Figure 6:** Fisetinidol and *ent*-Fisetinidol.

Certain colourless plant materials develop deep red colours when treated with mineral acid.[21] In 1920, Rosenheim[22] called these pigments *anthocyanidins* and the progenitors, *leucoanthocyanidins*. He concluded from his results that the *leucoanthocyanidins* were glycosides of the "pseudo-bases" of the corresponding *anthocyanidins* (**18**). Since then, various workers have examined the properties of the acclaimed *leucoanthocyanidins*, and in contrast with Rosenheim, concluded that flavan-3,4-diols **(19)**, and flavan-3-ol dimers **(20)**, and higher oligomers are principally responsible for the exhibited properties.[21] Weinges et al.[23] proposed that the flavan-3,4-diols should be named *leucoanthocyanidins*, while flavan-3-ol dimers **(e.g. 20)** and higher oligomers be called *proanthocyanidins* (Scheme 1).




**Scheme 1**: Formation of *anthocyanidin*.

Table 1 shows the major anthocyanidins as depicted by the hypothetical structure **(18)** (Figure 7).



Anthocyanidin **(18)**

**Figure 7:** Hypothetical structure of anthocyanidins.

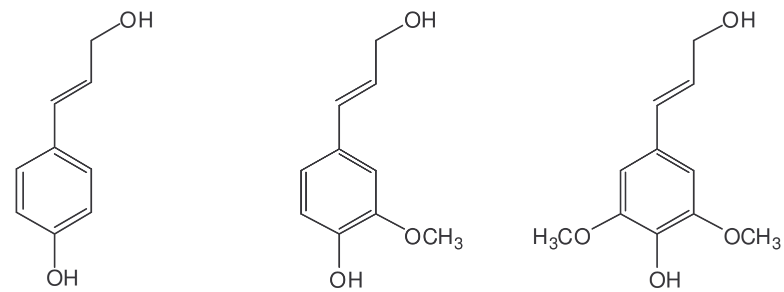
**Table 1:** Major Anthocyanidins (monomers).

| **Anthocyanidin** | **R[1]** | **R[2]** | **R[3]** | **R[4]** | **R[5]** | **R[6]** | **R[7]** | **Colour** |
|---|---|---|---|---|---|---|---|---|
| Aurantinidin | -H | -OH | -H | -OH | -OH | - | -OH | orange |
| Cyanidin | -OH | -OH | -H | -OH | -OH | -H | -OH | Orange-red |
| Delphinidin | -OH | -OH | -OH | -OH | -OH | -H | -OH | Blue-red |
| Europinidin | $-OCH_3$ | -OH | -OH | -OH | $-OCH_3$ | -H | -OH | Blue-red |
| Luteolinidin | -OH | -OH | -H | -H | -OH | -H | -OH | orange |
| Pelargonidin | -H | -OH | -H | -OH | -OH | -H | -OH | orange |
| Malvidin | $-OCH_3$ | -OH | $-OCH_3$ | -OH | -OH | -H | -OH | Blue-red |
| Peonidin | $-OCH_3$ | -OH | -H | -OH | -OH | -H | -OH | Orange-red |
| Petunidin | -OH | -OH | $-OCH_3$ | -OH | -OH | -H | -OH | Blue-red |
| Rosinidin | $-OCH_3$ | -OH | -H | -OH | -OH | -H | $-OCH_3$ | Red |

When $R_4$ = *O*-glycoside, **(18)** is called an anthocyanin

# 5. LIGNINS

Lignin is the major constituent of wood and an integral part of the secondary cell walls of plants.[4] The term is derived from the Latin word *lignum*,[24] meaning wood. It is one of the most abundant organic polymers on earth, superseded only by cellulose. Thirty percent of non-fossil organic carbon[4] on earth occurs in lignin. As a biopolymer, lignin is unusual because of its heterogeneity and lack of a defined primary structure. They are complex polymers, mainly a condensation of *p*-coumaryl alcohol **(22)**, coniferyl alcohol **(23)**, and sinapyl alcohol **(24)** (Figure 8)**.** The molecular masses are in excess of 10,000 amu. Figure 9 gives an example of a small lignin molecule.



*p*-Coumaryl alcohol    Coniferyl alcohol    Sinapyl alcohol

**Figure 8:** Monomeric units of lignin molecules.

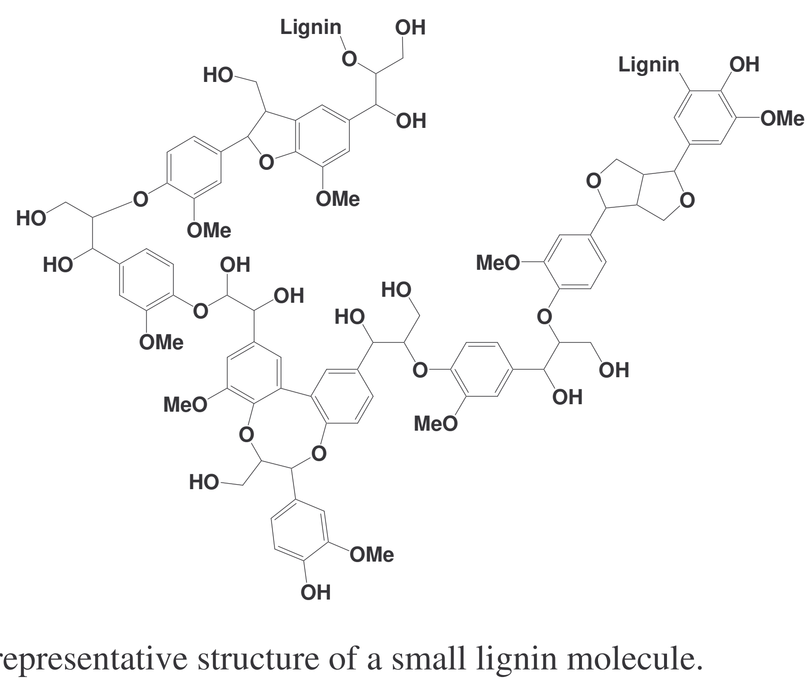


**Figure 9:** A representative structure of a small lignin molecule.

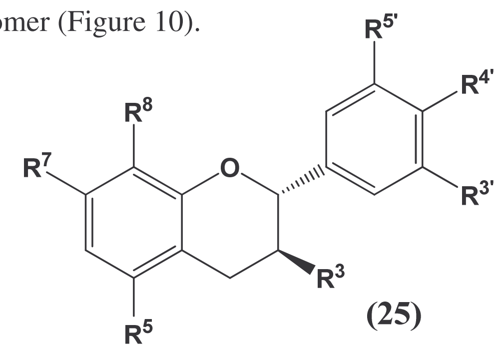
Lignins are responsible for newsprint's yellowing with age,[24] and must be removed as lignosulfonates from the pulp before high-quality bleached paper can be manufactured. The lignosulfonates are used as binding agents in pellets, in leather tanning, in dye baths, as emulsifiers in animal feed, and as raw material in the production of vanillin.

# 6.0 PROANTHOCYANIDINS (CONDENSED TANNINS)

Proanthocyanidins are flavan-3-ol oligomers that yield anthocyanidins **(18)** on heating with mineral acid by cleavage of a C-C interflavanyl bond, e.g. the prodelphinidins yield delphinidins under these conditions.[21] Proanthocyanidins are thought to be the products of ionic coupling at an electrophilic C-4 position (C-ring) of a flavanyl unit. The electrophile is generated from a flavan-3,4-diol or flavan-4-ol. The nucleophilic unit is the aromatic ring of another flavanyl unit, often a flavan-3-ol. Dimers linked by C-O-C interflavanyl bonds and trimers linked by both C-C and C-O-C bonds have been isolated and are also classified as proanthocyanidins.[15]

## 6.1 Proanthocyanidin Classification

Table 2 gives the names of monomer units,[15] the hydroxylation pattern, and the name of the corresponding dimers. The compound **(25)** is a representative general structure for the proanthocyanidin monomer (Figure 10).




**(25)**

**Figure10:** General structure of a proanthocyanidin monomer unit.

**TABLE 2:** Major proanthocyanidins.

| Proanthocyanidin | Monomer | Hydroxylation Pattern of monomer | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | $R^3$ | $R^5$ | $R^7$ | $R^8$ | $R^{3'}$ | $R^{4'}$ | $R^{5'}$ |
| Procassinidin | Cassiaflavan | H | H | OH | H | H | OH | H |
| Probutinidin | Butiniflavan | H | H | OH | H | OH | OH | H |
| Proapigenidin | Apigeniflavan | H | OH | OH | H | H | OH | H |
| Proluteolinidin | Luteoliflavan | H | OH | OH | H | OH | OH | H |
| Protricetinidin | Tricetiflavan | H | OH | OH | H | OH | OH | OH |
| Prodistenidin | Distenin | OH | OH | OH | H | H | H | H |
| Propelargonidin | Afzelechin | OH | OH | OH | H | H | OH | H |
| Procyanidin | Catechin | OH | OH | OH | H | OH | OH | H |
| Proguibourtinidin | Guibourtinidol | OH | H | OH | H | H | OH | H |
| Profisetinidin | Fisetinidol | OH | H | OH | H | OH | OH | H |
| Prorobinetinidin | Robinetinidol | OH | H | OH | H | OH | OH | OH |
| Proteracacinidin | Oritin | OH | H | OH | OH | H | OH | H |
| Promelacacinidin | Mesquitol | OH | H | OH | OH | OH | OH | H |
| Propeltogynidin | Peltogynol | $OCH_2$- | H | OH | H | H | OH | OH |
| Promopanidin | Mopanol | $OCH_2$- | H | OH | H | OH | OH | H |

The leucoanthocyanidins are monomeric flavonoids (flavan 3,4-diols and flavan-4-ols) that produce anthocyanidins **(18)** on heating with mineral acid by the cleavage of a C-O bond. The leucoanthocyanidins, flavan-4-ols and flavan-3,4-diols, act as electrophilic chain-extender units in the synthesis of proanthocyanidins, and the flavans and flavan-3-ols as nucleophilic chain-terminating units (Figure 11).

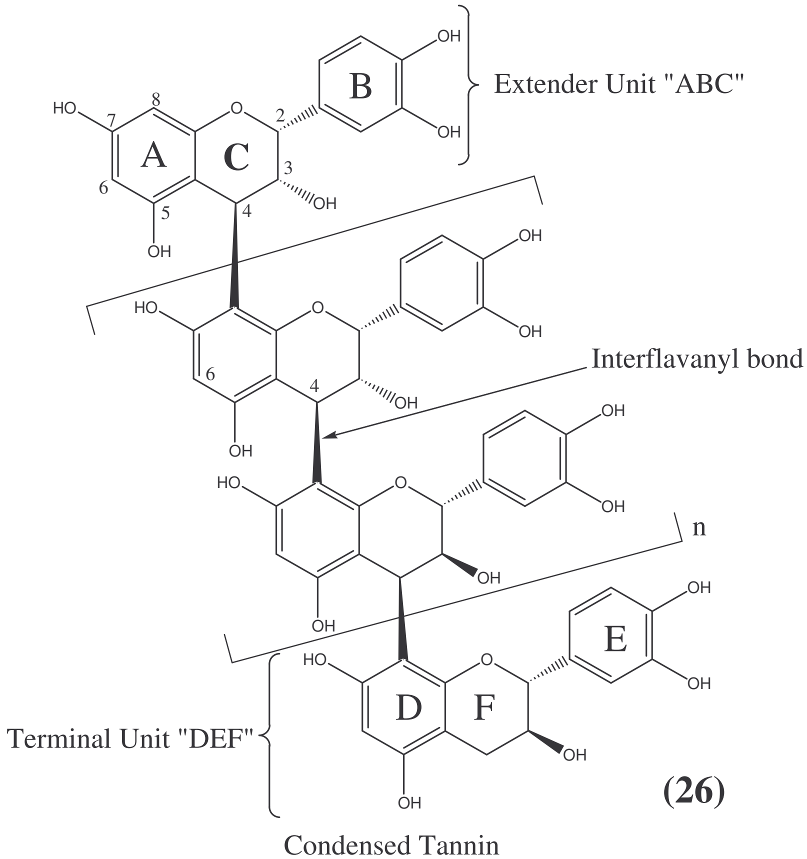
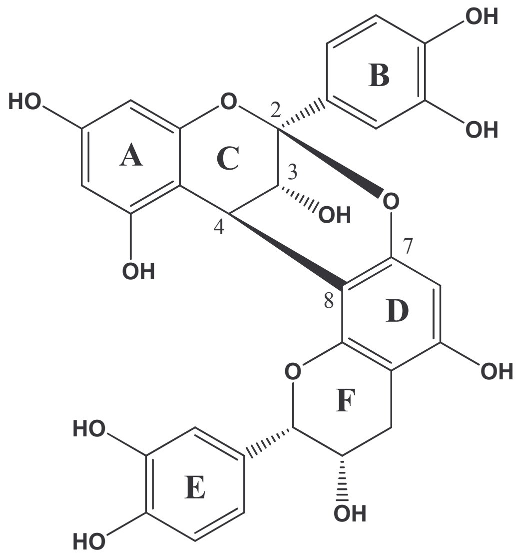



**Figure 11:** General structure of a proanthocyanidin.

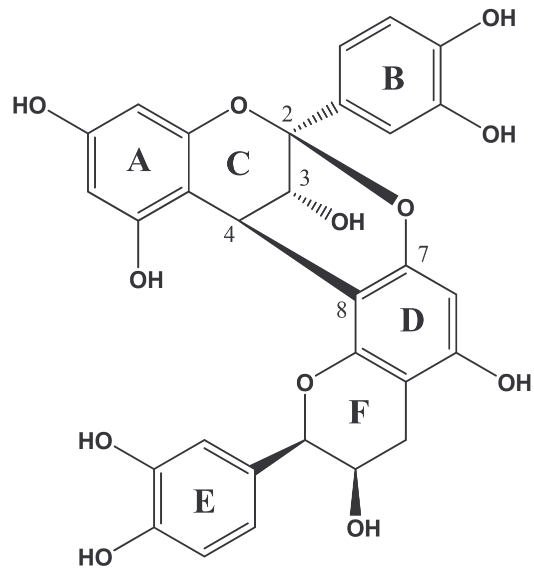
## 6.2 Types of proanthocyanidins

### 6.2.1 <u>A-Type Proanthocyanidins</u>

In contrast to B-type proanthocyanidins where the constituent flavanyl units are linked *via* a single bond, the A-type proanthocyanidins (Figure 12) have an ether linkage from the aromatic D-ring of the bottom unit to C-2 of the heterocyclic C-ring of the top unit. This second bond introduces a high degree of conformational stability and a characteristic AB-doublet ($^{3}J_{3,4}$= 3,4 Hz) for both 3,4-*trans* and 3,4-*cis* protons. Figure 12 give two examples of A-type proanthocyanidins **(27)** and **(28)**.



Procyanidin **A-1 (28)**

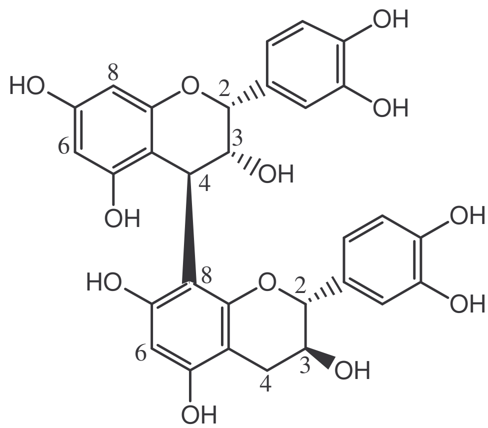


Procyanidin **A-2 (27)**

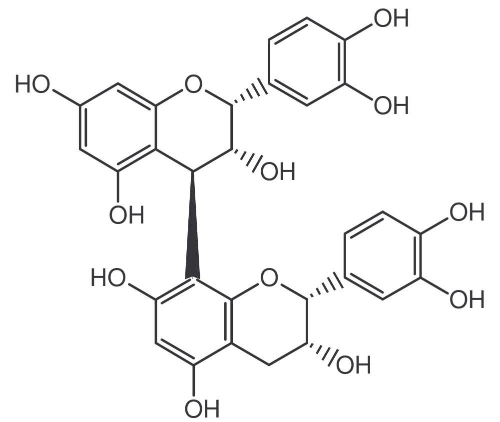
**Figure 12:** A-type proanthocyanidins.

#### 6.2.2 <u>B-Type Proanthocyanidins</u>

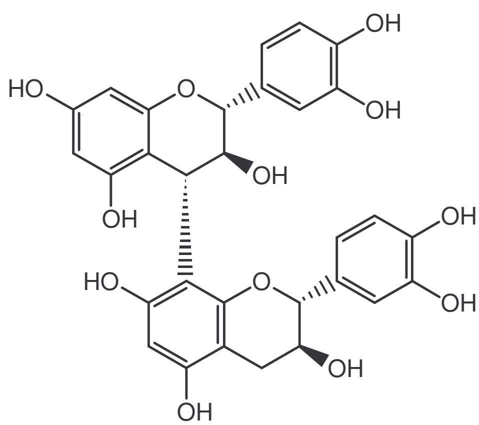
B-type proanthocyanidins (Figure 13) are depicted by a single C-C bond between the benzylic C-4 (C-ring) of the chain extender flavan-3-ol unit and C-8 or C-6 of the D-ring (terminal flavan-3-ol). They are classified according to the hydroxylation pattern of the chain extension unit (Table 2).



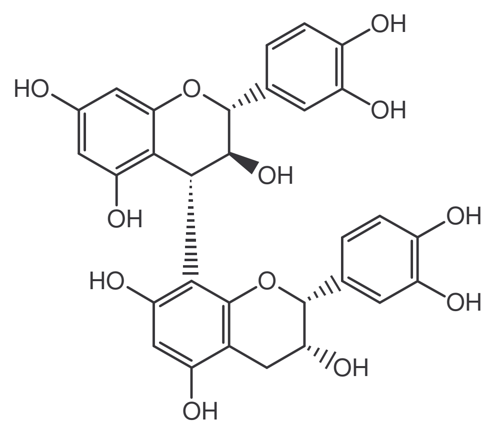
Procyanidin **B-1 (29)**



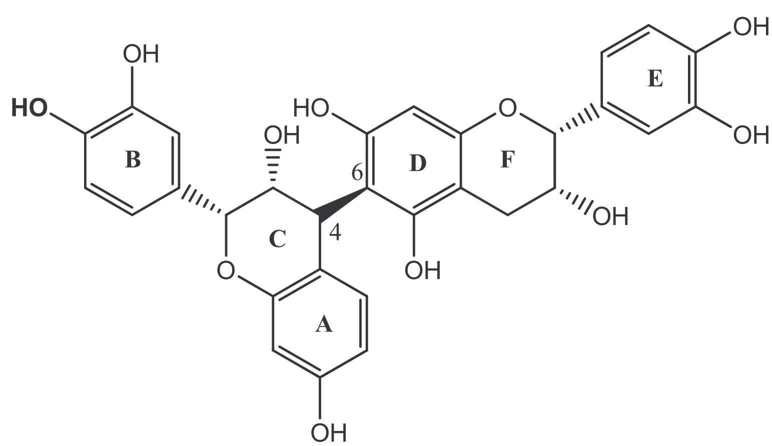
Procyanidin **B-2 (30)**



Procyanidin **B-3 (31)**



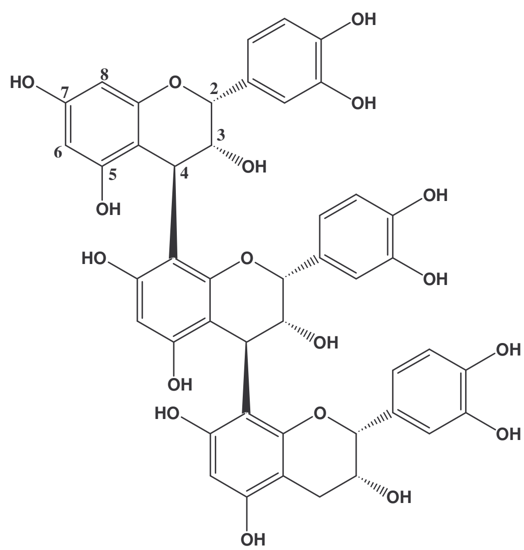
Procyanidin **B-4 (32)**



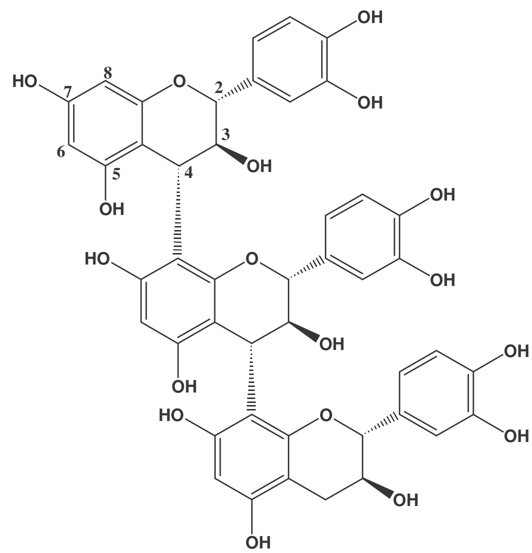
Procyanidin **B-5 (33)**

**Figure 13:** B-type proanthocyanidins.

#### 6.2.3 C-Type Proanthocyanidins



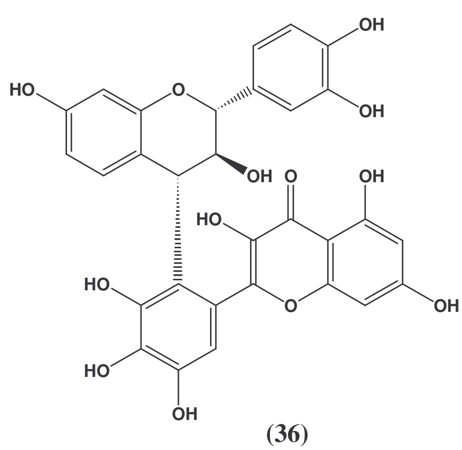

Procyanidin **C1 (34)**




Procyanidin **C2 (35)**

**Figure 14:** C-type proanthocyanidins.

Proanthocyanidins together with bi- and triflavonoids constitute the two major classes of complex $C_6$-$C_3$-$C_6$ secondary metabolites. Growing numbers of “mixed” dimers[15] e.g. flavan-3-ol→flavonol {fisetinidin-(4α→2′)-myricetin **(36)**} originating from ionic coupling of flavonols to flavan-3,4-diols have been isolated and belong to both classes (Figure 15).




**(36)**

**Figure 15:** “Mixed” dimer.

The bi- and triflavonoids (e.g. **(37)**, and **(38)**) consistently have a carbonyl group or equivalent at the C-4 position of every constituent monomeric unit (Figure 16).[15]

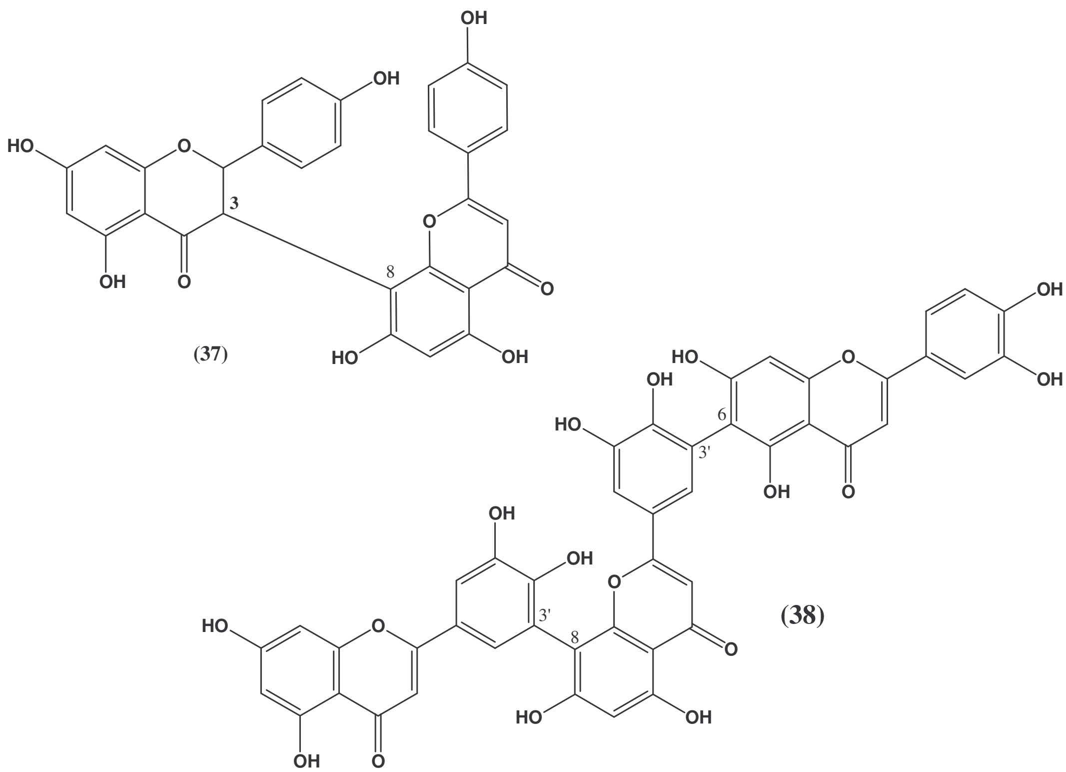



**Figure 16:** Bi- and triflavonoids.

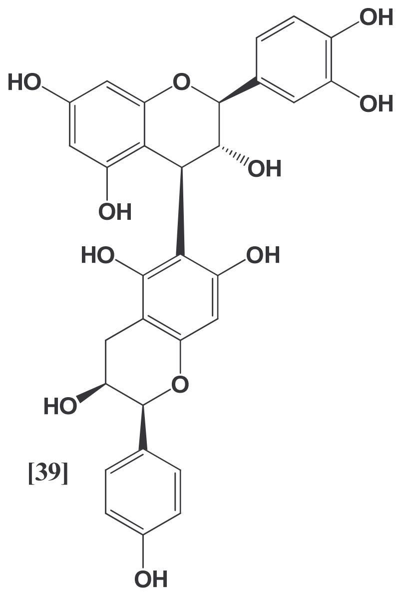
## 6.3 Systematic Nomenclature of Proanthocyanidins

Hemingway et al.[25] proposed a system of nomenclature for proanthocyanidins that was extended by Porter.[17,26] It is summarized as follows:[15]

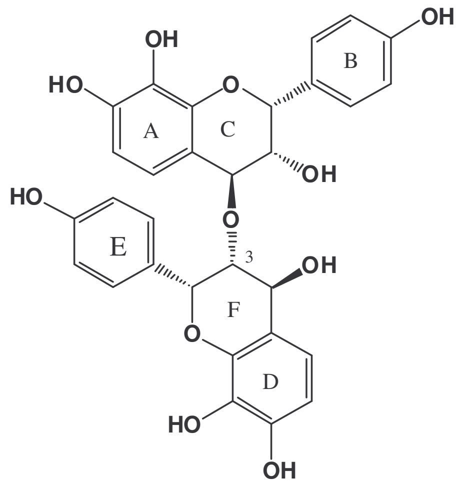
1. The names of the basic flavan units (the monomeric units of the proanthocyanidins) are given in Table 2.
2. All flavan-3-ols in the list possess 2*R*,3*S* absolute configuration, for example catechin (8). Flavan-3-ols with 2*R*,3*R* absolute configuration are prefixed "epi", for example

epicatechin (9). Units with a 2*S* absolute configuration are prefixed “*ent*” for example *ent*-epicatechin (15). All flavans in the list possess 2S configuration.

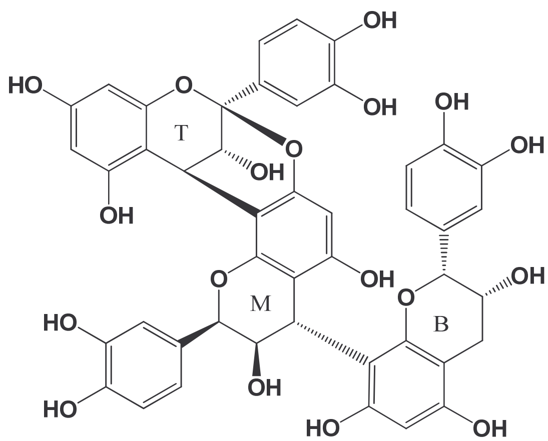
3. The flavanoid skeleton is drawn and numbered as illustrated by (4).
4. The location of the interflavanyl bond is denoted within parenthesis as in the case of carbohydrates.
5. The orientation of the interflavanyl bond is, as in the IUPAC rules, denoted α or β e.g. epicatechin-(4β→8)-catechin (29), epicatechin-(4β→8)-epicatechin (30) and *ent*-catechin-(4β→6)-*ent*-epiafzelechin (39) (Figure 17).
6. The rules are also used to name ether-linked proanthocyanidins e.g. epioritin-(4β→*O*-3)-epioritin-4β-ol (40) (Figure 17).
7. The constituent units of the oligomers may be differentiated by using ABC, DEF etc. Porter[17] proposed alternative T, M, P (top, middle and bottom) designation. This alternative system allows the same numbering system to be used for each monomeric unit e.g. (41) (Figure 17).
8. B-type proanthocyanidins are characterised by single links between the flavanyl units, usually between C-4 of the chain-extension unit and C-6 or C-8 of the chain-terminating moiety.
9. A-type proanthocyanidins possess an additional ether-linkage to C-2 of the T-unit. They are often incorrectly named due to the fact that the M moiety is rotated through 180 degrees. The system is used, cognizant of this fact, to name e.g., epicatechin-(2β→*O*-7,4β→8)-epicatechin (42) and epicatechin-(2β→O-7,4β→8)-epicatechin-(4β→8)-epicatechin (41).



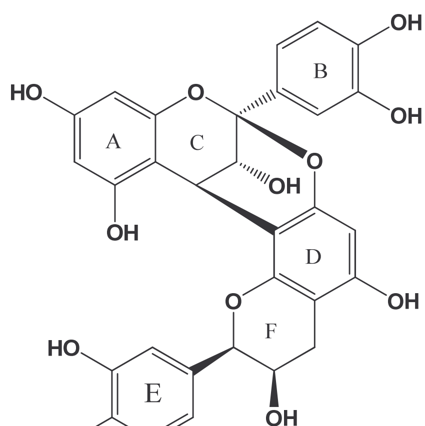
[39]



[40]



(41)



(42)

**Figure 17.** Structures exemplifying the nomenclature rules.

**TABLE 3:** Trivial names of common procyanidin B-1 dimers.[27]

| Common Types | Structural Name |
|---|---|
| Procyanidin B-1 | Epicatechin-(4β→8)-catechin |
| Procyanidin B-2 | Epicatechin-(4β→8)-epicatechin |
| Procyanidin B-3 | Catechin-(4α→8)-catechin |
| Procyanidin B-4 | Catechin-(4β→8)-epicatechin |
| Procyanidin B-5 | Epicatechin-(4β→6)-epicatechin |
| Procyanidin B-6 | Catechin-(4α→6)-catechin |
| Procyanidin B-7 | Epicatechin-(4β→6)-catechin |
| Procyanidin B-8 | Catechin-(4α→6)-epicatechin |
| Procyanidin A-1 | Epicatechin-(2β→*O*-7,4β→8)-catechin |
| Procyanidin A-2 | Epicatechin-(2β→*O*-7,4β→8)-epicatechin |

## 6.4 Determination of the stereochemistry of the C-ring of proanthocyanidins

A variety of techniques have been used for structure elucidation of proanthocyanidins. We summarize a few important guiding principles. Because the stereochemistry of the C-ring plays such an important role in this thesis, we briefly discuss the use of NMR, mass spectrometry and circular dichroism to establish the relative and absolute stereochemistry of the C-ring of flavonoids.

### 6.4.1 NMR

$^1$H NMR coupling constants of the C-ring resonances are used to distinguish between the four diastereoisomeric pairs of 4-arylflavan-3-ols. The characteristic coupling constants for 2,3-*trans*-3,4-*trans* isomers are $J_{2,3} = 10$ and $J_{3,4} = 8.5 – 9.8$ Hz. For 2,3-*trans*-3,4-*cis* isomers it is 8 – 10 and 5.0 – 6.5 Hz; for the 2,3-*cis*-3,4-*cis*-isomers it is 1.2 and 4.75 – 4.9 Hz and for 2,3-*cis*-3,4-*trans* it is < 1 and 1.9 – 3.8 Hz, respectively[28] (Table 5). Flexibility of the heterocyclic rings allows rapid equilibrium between the pseudo-axial and pseudo-equatorial forms of dimers, resulting in average values for C-ring coupling constants that complicate unambiguous assignment of configuration.

Slow rotational exchange around the interflavanyl bond causes many proanthocyanidins to occur as a mixture of two rotamers. This leads to line broadening and/or doubling of resonances. High temperature NMR is essential in these cases to simplify NMR spectra. The energy difference between rotamers is relatively small and efforts to separate rotamers, even with countercurrent chromatography, invariably fail.

Weinges[29] concluded that for restricted rotation about the carbon-carbon link from the C-4 position ($sp^3$) of the C-ring to a carbon on the 4-aryl substituent ($sp^2$) to be observed, two conditions must be fulfilled:

1. Both the *ortho* positions of the "lower unit" (4-aryl moiety) must be substituted.
2. The "upper unit" must have a substituent at either C-3 or C-5.

Haslam and co-workers[30] added a further condition for restricted rotation about the interflavanyl linkage:

3. The “upper unit” should have a 2,3-*trans*-configuration with the C-3 hydroxy group in a quasi-equatorial position.

Porter and Foo confirmed this rule.[31] Procyanidin units with a 2,3-*cis* configuration have the appending 3-ol unit in a pseudo-axial orientation with a considerable lower barrier to rotation around the interflavanyl bond than in dimers with a 2,3-*trans* configuration. Dimers with 2,3-*trans* configuration experiences steric hinderance from the 5- and 7-sustituents.[32,33]

This second bond in A-type proanthocyanidins introduces a high degree of conformational stability in the C-ring. Two characteristic AB-doublets ($^3J_{3,4}$= 3.4 Hz) for both 3,4-*trans* and 3,4-*cis* protons are observed.[34-38]

### 6.4.2 Mass spectrometry

It is generally assumed that mass spectrometry does not provide useful stereochemical information. The $M^+/M^+$ - 60 ratio in 3-*O*-acetyl derivatives of 4-arylflavan-3-ols has, however, been used to distinguish unambiguously between 3,4-*trans* and 3,4-*cis* isomers (Table 4 and 5). (The 4-proton is lost in the McLafferty rearrangement and not the 2-proton).[28]

**Table 4:** Correlation between MS & 4-arylflav-3-*O*-acetates configurations.

| (2,3 – 3,4) Configuration | $M^+/M^+ - 60$ **ratio** |
|---|---|
| *trans – trans* | 67 – 83 |
| *trans – cis* | 2.6 – 3.3 |
| *cis – trans* | 40 – 50 |
| *cis – cis* | 1.4 – 2.5 |

### 6.4.3 CD Spectropolarimetry

Botha and co-workers[39] studied a series of 4-arylflavan-3-ols and concluded that the absolute configuration of the diaryl moiety at the 4-position determines the sign of the high amplitude Cotton effect (220 to 240 nm). They concluded that configuration at C-2 and C-3 played a lesser role. The following rule regarding the absolute configuration at C-4 of 4-aryl-flavan-3-ols, biflavonoids and triflavonoids was established:

- A positive Cotton effect reflects a β-oriented C-4 aryl flavanyl substituent.
- A negative Cotton effect reflects an α-oriented C-4 aryl flavanyl substituent.

Van der Westhuizen et al.[40] found that deviations from the rule were correlated with compounds exhibiting abnormal $J_{2,3}$ and $J_{3,4}$ $^{1}$H NMR coupling constants and concluded that the rule only applies if the C-ring is in a half-chair conformation.

Advances in electronic circular dichroism calculations allow assignment of the absolute configuration of chiral molecules. Ferreira and co-workers determined the absolute configuration of 3,8''-biflavonoids unequivocally using time-dependant density functional theory. Extensive conformational analysis is a prerequisite.[41]

### 6.4.4 Crystallography

X-ray crystallography is of limited use because of the poor crystallizability of proanthocyanidins and their derivatives.[42]

### 6.4.5

Table 5 shows EI Mass Spectrometry, $J_{2,3}$ and $J_{3,4}$ NMR coupling constants and the sign of the Cotton effects of 4-aryl-flavan 3- acetates.[28]

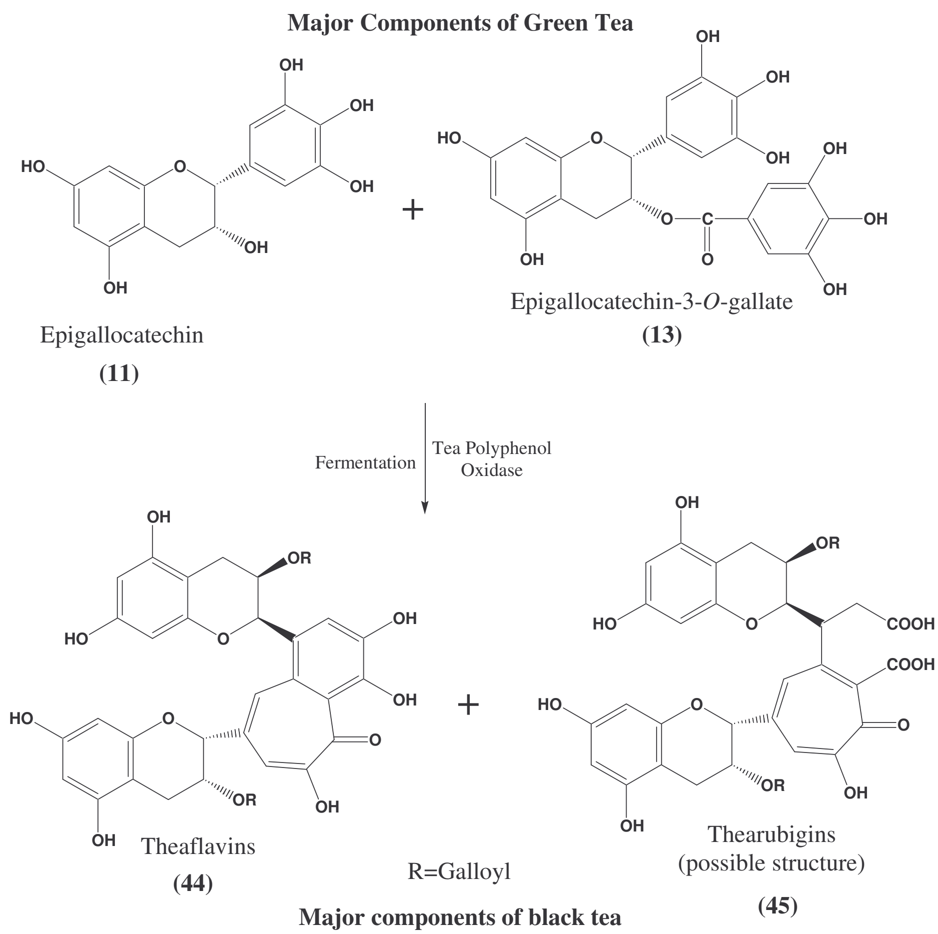
*Table 5 to be printed separately and inserted.*

# 7.0 TANNINS

Tannins are astringent, bitter-tasting plant polyphenols that bind and precipitate proteins.[43-45] Tannins are plant secondary metabolites that were traditionally used to tan leather. The term "tannin" comes from an ancient Celtic word, *tan*, for oak tree.[46] Oak trees were noted as an abundant source of extracts traditionally used in converting animal hides to leather. This age-old practice was employed by the prehistoric tribes[6] to make their clothes (hides and skins) last longer. The ability to complex with proteins via hydrogen bonds explains the use of tannins[6,32] for leather tanning.[47] Tannin extraction from the bark of black wattle trees (*Acacia mearnsii*, South Africa) and quebracho (*Schinopsis lorentzii*, South America) is an important industry that supplies raw materials for leather tanning.

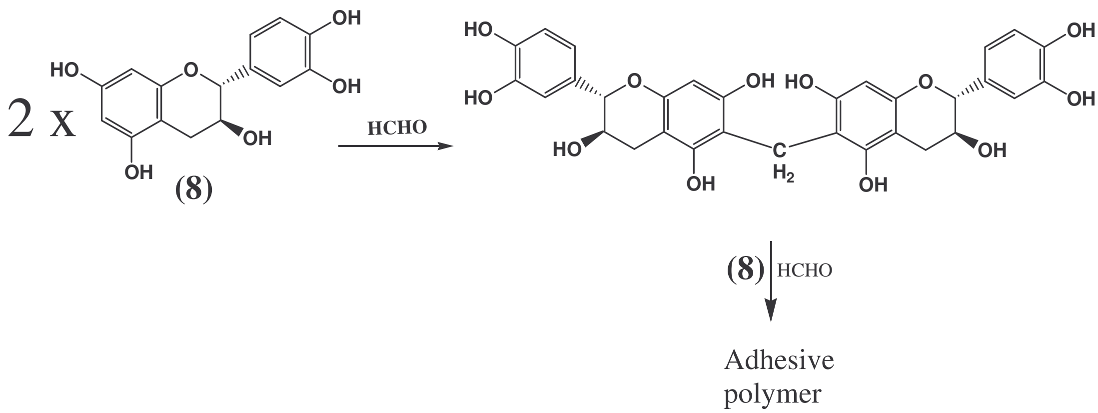
Gallotannins, elagitannins, complex tannins and condensed tannins are included but lignins and flavonoids (monomeric phenylpropanoids) are excluded from the term *tannin*. The terms *polyphenol* (which includes lignins and flavonoids) and *tannins* (which excludes lignins and flavonoids) should thus not be used interchangeably. Mineral tanning compounds such as chromium and alum are not classified as tannins.

Tannins are responsible for much of the taste and flavour properties of tea, a popular worldwide beverage made from leaves of *Camellia sinensis*, a tropical climate ever-green plant. In Chinese tea, also known as green tea, the fresh tea leaves are heated immediately after picking. This is followed by drying to destroy enzymes and preserve monomeric constituents. Chinese tea contains mostly monomers of which epigallocatechin is the most abundant.[48] The manufacturing[49,50] of black tea involves crushing the tea leaves to promote enzymatic oxidation and subsequent condensation of the tea polyphenols (theaflavins **(44)** and thearubigins **(45)**) through a fermentation process (Scheme 2). Addition of milk to the tea reduces the astringency by precipitating the tannins with milk proteins. This activity makes the tea more palatable by ameliorating the excessively bitter taste. Teas, like other tannin containing plants, are recorded to have possible beneficial health effects, such as the prevention of cancer and cardiovascular diseases. The structures of some of the major components of green tea and black tea are shown (Scheme 2).



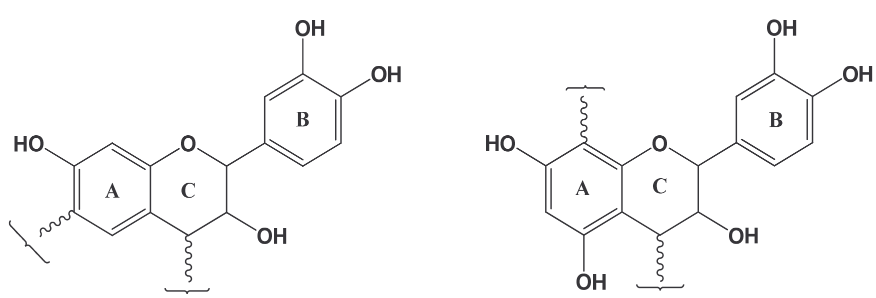

**Scheme 2:** Major components of green and black tea.[50]

Tannins are used as adhesives.[7,8,9] They have phenolic aromatic rings that react with formaldehyde to polymerize (Scheme 3). The reactive sites are, however, limited, resulting in weak and brittle adhesives. Much of the applied research on the chemistry of tannins has revolved around efforts to create additional reactive sites for polymerization for cold set adhesive applications[9] *via* cleavage of the heterocyclic C-ring.




**Scheme 3:** Model reaction for polymerization of polyphenol to adhesive polymer.

The tannin extract composition dictates the type and applications of the adhesive manufactured from it.[7] For example, resorcinol-type tannins **(46)** found in black wattle (South Africa) or quebracho (South America) extracts require hot-pressing to manufacture adhesives. Phloroglucinol-type tannins **(47)** found in pine bark extracts, polymerise at ambient temperature and act as cold-set adhesives (Figure 18). The additional hydroxy group on the phloroglucinol of the A-ring enhances its reactivity.[7]



**Resorcinolic tannin**
**(46)**

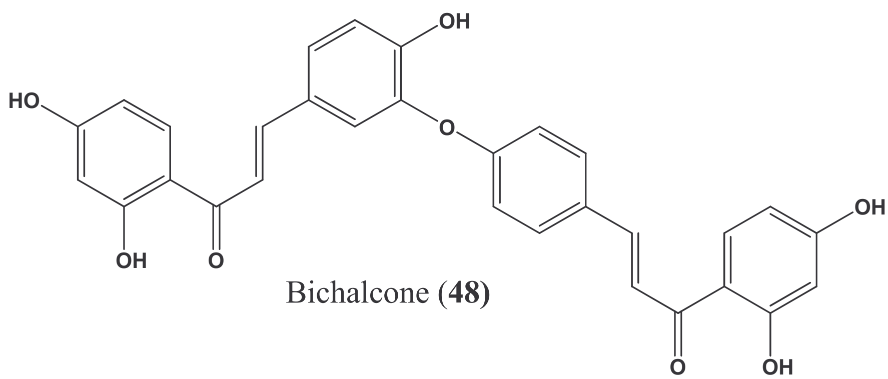
**phloroglucinolic tannin**
**(47)**

**Figure 18:** Tannin extracts composition and adhesive-type manufacture.

# 8.0 BI- AND TRIFLAVONOIDS

Bi-and triflavonoids are thought to be the products of phenol oxidative coupling of chalcones, aurones, auronols, isoflavones, flavanones, dihydroflavanols, flavanols and flavones. This contrasts with the putative C-4 electrophile (C-4 carbocationic intermediate, from flavan-4-ol) origin of proanthocyanidins. Due to the oxidative nature of carbon-carbon bond formation, the substituent at C-4 of the starting materials, are normally preserved and this class predominantly has a carbonyl group or equivalent at C-4. Together with proanthocyanidins, the bi- and triflavonoids constitute the two major classes of complex $C_6$-$C_3$-$C_6$ secondary metabolites.

Many compounds with a carbonyl group on C-4 but not arising from phenol oxidative coupling of the aforementioned classes of monomeric flavonoids have also been reported as bi- or tri-flavonoids (**48**).[51]



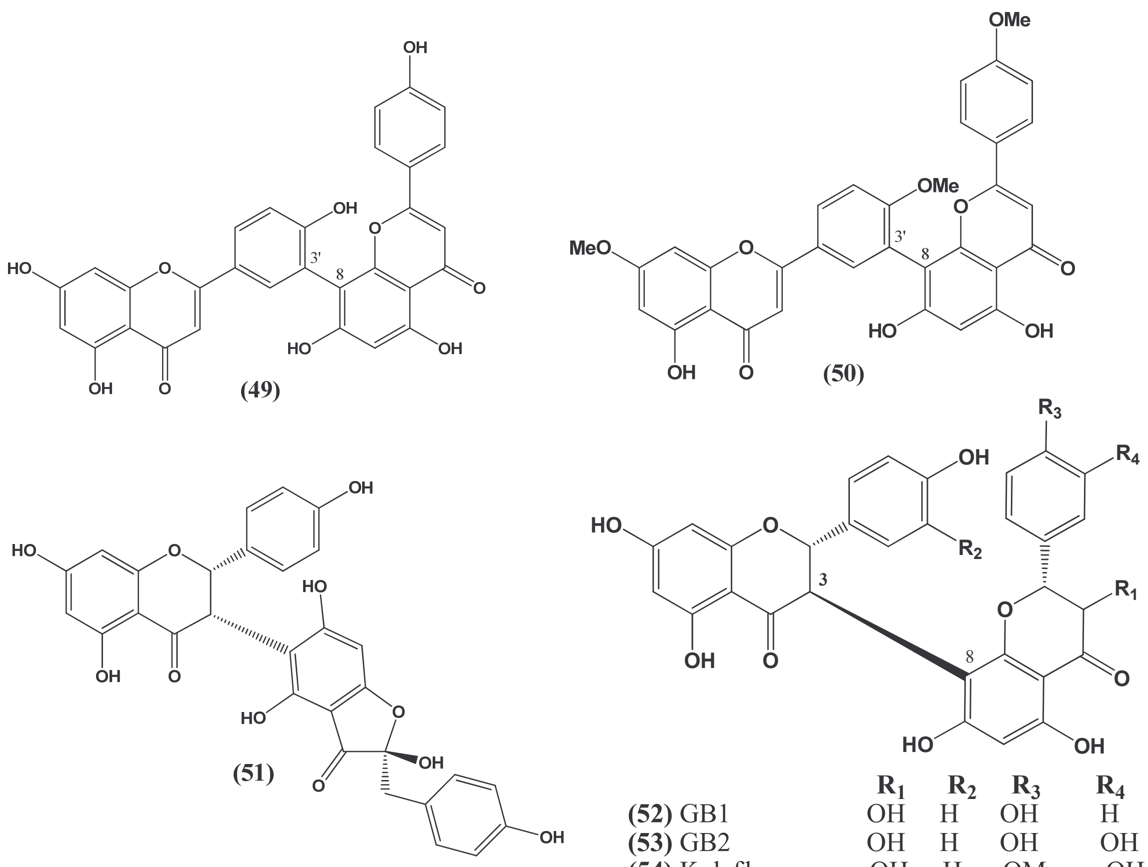

Bichalcone (**48**)

Tetra-, penta-, and hexaflavonoids have also been isolated and identified and these compounds are also included in the class of bi- and triflavonoids.[15]

Locksley[51] proposed a system of nomenclature[15] to clean up the disarray that had developed in the absence of a commonly accepted system and the absence of a trivial nomenclature. He proposed that the position of substitution in the upper (I) and lower (II) unit in a biflavonoid be defined from the usual numbering of the parent flavonoid. Each unit is described from the parent monomer.

Thus, amentoflavone **(49)** is named apigeninyl-(I-3',II-8)-apigenin, the tri-*O*-methyl derivative **(50)**, 7,4'-di-*O*-methylnaringeninyl-(I-3',II-8)-4'-*O*-methylnarenginin, and the flavone-auronol **(51)**, (2*S*)-naringeninyl-(I-3α,II-5)-(2*R*)-maesopsin (Figure 19).[15]

Garcinia biflavonoid 1 (GB1), Garcinia biflavonoid 2 (GB2), and kolaflavanones are natural compounds isolated from bitter kola nuts (found in Nigeria).[53,54] Typical
GB-FLAVONES [(I-3,II-8)-coupling] structures are **(52)**, **(53)**, and **(54)** (Figure 19)..




| | $R_1$ | $R_2$ | $R_3$ | $R_4$ |
|---|---|---|---|---|
| **(52)** GB1 | OH | H | OH | H |
| **(53)** GB2 | OH | H | OH | OH |
| **(54)** Kolaflavanone | OH | H | OMe | OH |

**Figure 19:** Typical biflavonoids.

# REFERENCES


1. Ferreira, D.; Li, X. C. *Natural Products Report* **2000**, *17*, 193-212.
2. Hertog, M. G.; Kromhout, D.; Aravanis, C.; Blackburn, H.; Buzina, R.; Fidanza, F.; Giampaoli, S.; Jansen, A.; Menotti, A.; Nedeljkovic, S. *Arch. Intern. Med.* **1995**, *155*, 381-386.
3. Harborne, J. B.; Williams, C.A. *Phytochemistry* **2000**, *55*, 481-504.
4. Goheen, D. W.; Hoyt, C. H. *Kirk-Othmer Encyclopedia of Chemical Technology*, *John Wiley & Sons, Inc*. **1981**, *14*, 294-312.
5. Martín-Ortiz, D.; Hernández-Apaolaza, L.; Gárate, A. *J. Agric. Food Chem.* **2007**, *57*, 226-231.
6. Khanbabaee, K.; Van Ree, T. *Nat. Prod. Rep.* **2001,** *18*, 641-649.
7. Pizzi, A.; Orovan, E.; Cameron, F. A. *Holz als Roh- und Werkstoff* **1998**, *46*, 67-71.
8. Pizzi, A.; Mittal, K. L. *Handbook of Adhesive Technology, Revised and expanded*, CRC press, **2003**, 581.
9. Grigsby, W.; Warnes, J. *Holz als Roh- und Werkstoff* **2004**, *62*, 433-438 and refs. therein.
10. Sundar, V. J.; Rao, R. J.; Muralidharan, C. *J. Cleaner Prod.* **2002**, *10*, 69-74.
11. Morera, J. P.; Bacardit, A.; Ollé, L.; Bartolí, E.; Borràs, M. B. *Chemosphere* **2007**, *69*, 1728-1733.
12. Dewick, P. M. *Medicinal Natural Products. A Biosynthetic Approach, 2nd Edition, John Wiley and Sons* **2002**, 121-130.
13. Oh, H. I.; Hoff; J. E.; Armstrong, G. S.; Haff, L. A. *J. Agric. Food Chem*. **1980**, *28 (2)*, 394-398.

14. Haslam, E. *Chemistry and Pharmacology of Natural Products. Plant Polyphenols: Vegetable Tannins Revisited, Cambridge University Press, Sydney* **1989**, 8, 28, 44, 165.

15. Ferreira, D; Slade, D; Marais, J. P. J. *Flavonoids Chemistry, Biochemistry and Applications, edited by ∅yvind M. Andersen Kenneth R. Markhams, CRC press* **2006**, 553-615; 1101-1128.

16. Marais, J.P.J.; Deavours, B.; Dixon, R.A.; Ferreira, D. *The Science of Flavonoids, (ed. Erich Grotewold); Springer Science* **2006**; 1-2.

17. Porter, L.J. *The Flavonoids: Advances in Research Since 1986, (ed. Harborne, J.B.); Chapman and Hall, London* **1994**, 23.

18. Ellis, C.J.; Foo, L.Y.; Porter, L.J. *Phytochemistry* **1983**, *22*, 483.

19. Drewes, S.E.; Roux, D.G. *J. Chem. Soc. (C),* **1966**, 1644.

20. Steenkamp, J.A.; Malan, J.C.S.; Roux, D.G.; Ferreira, D. *J. Chem. Soc. Perkin Trans I* **1988**, 1325.

21. Thompson, R.S.; Jacques, D.; Haslam, E.; Tanner, R.J.N. *J. Chem. Soc., Perkin I* **1972**, 1387-1399.

22. Rosenheim, O. *Biochem. J*. **1920**, *14*,178.

23. Weinges, K.; Bahr, W.; Ebert, W.; Goritz, K.; Marx, H.-D. *Konstitution, Ensteliung und Betleutung der Flavonoid – Gerbstoffe. Fortschritte Chem. Org. Naturstoffe* **1969, *27,*** 158.

*24. Sjöström, E.* Wood Chemistry: Fundamentals and Applications. Academic Press ***1993***.

25. Hemingway, R.W.; Foo, L.Y.; Porter, L.J. *J. Chem. Soc., Perkin Trans.*1 **1982,** 1209.

26. Porter, L.J. *The Flavonoids: Advances in Research Since 1980, (red. Harborne, J.B.); Chapman and Hall, London* **1988**, 21.

27. Feucht, W.; Treutter, D. *Principles and Practices in Plant Ecology. Allelochemical Interactions.* Eds. Dakshini, K.M.M.; Foy, L. C. CRC press, Boca Raton **1999**, chp. 9.

28. Van der Westhuizen, J. H. ‘n Nuwe Reeks Fotochemiese Reaksies in Flavonoïedsintese, Ph. D Thesis, UFS, **1979**, 99.

29. Weinges, K.; Marx, H. D.; Goritz, K. *Chem. Ber*. **1970**, *103*, 2336.

30. Thompson, R. S.; Jacques, D.; Haslam, E. *J. Chem. Soc., Perkin Trans.1* **1972**, 1387-1399.

31. Foo, L. Y.; Porter, L. J. *J. Chem. Soc., Perkin Trans.1* **1983**, 1535.

32. Haslam, E. *Phytochemistry* **1977**, *16*, 1625-1640.

33. Fletcher, A. C.; Porter, L. J.; Haslam, E.; Gupta, R. K. *J. Chem. Soc., Perkin Trans.1* **1977**, 1628.

34. Cronje, A.; Steynberg, Brandt, E. V.; Young, D. A.; Ferreira, D. *J. Chem. Soc, Perkin Trans. 1*, **1993**, 2467.

35. Barrett, M. W.; Klyne, W.; Scopes, P. M.; Fletcher, A. C.; Porter, L. J.; Haslam, E. *J. Chem. Soc, Perkin Trans. 1*, **1979**, 2375.

36. Jacques, D.; Haslam, E.; Bedford, G. F.; Greatbanks, D. *J. Chem. Soc, Perkin Trans. 1*, **1974**, 2663.

37. Cronje, A.; Burger, J. F.; Brandt, E. V.; Kolodziej, H.; Ferreira, D. *Tetrahedron Lett.*, **1990**, *31*, 3789.

38. Blade, A. M.; Pieters, L. A.; Gergely, A. Kolodziej, H.; Claeys, M.; Vlietinck, A. J. *Phytochemistry* **1991**, *30 (1)*, 337-342.

39. Botha, J. J.; Young, D. A.; Ferreira, D.; Roux, D. G. *J. Chem. Soc., Perkin Trans.1* **1981**, 1213-1219.

40. Van der Westhuizen, J. H.; Ferreira, D.; Roux, D.G. *J. Chem. Soc., Perkin Trans.1* **1981**, 1221-1226.

41. Ding, Y.; Li, X. –C.; Ferreira, D. *J. Org. Chem.* **2007**, *72 (24)*, 9010-9017.

42. Kozikowski, A. P.; Tuckmantel, W.; Hu, Y. *J. Org. Chem*. **2001**, *66*, 1287-1296.

43. Eberhardt, T. L.; Young, R. A. *J. Agric. Food chem*. **1994**, *42*, 1704-1708.

44. Hernes, P. J.; Bernner, R.; Colvie, G. L.; Goni, M. A.; Bergamanschi, B. A.; Hedges, J. I. *Geochimica et Cosmochima Acta* **2001**, *65(18)*, 3109-3122.

45. Ham, Y. M.; Baik, J. S.; Hyun, J. W.; Lee, N. H. *Bull. Korean Chem. Soc.* **2007**, *28(9)*, 1595.

46. Haslam, E. *Chemistry and Pharmacology of Natural Products. Plant Polyphenols: Vegetable Tannins Revisited, Cambridge University Press, Sydney* **1989**, 6.

47. Okuda, T.; Hatano T.; Yazaki, K. *Chem. Pharm. Bull*. **1993**, *31*, 333.

48. Kakiuchi, N.; Hattori, M.; Nishizawa, M.; Yamagishi, T.; Okuda T.; Namba, T. *Chem. Pharm. Bull*. **1986**, *34*,720.

49. Yang, C. S.; Lambert, J. D.; Ju, J.; Lu, G.; Sang, S. *Toxicology and Applied Pharmacology* **2007**, *224*, 265-273.

50. Yang, C. S.; Wang, Z. –Y. *J. Natl Cancer Inst.***1993**, *85(13),* 1038-1048.

51. De Carvalho, M. G.; Alvesa, C. C. F.; da Silvaa, K. G. S.; Marcos N. Eberlinb, M. N.; Werlec, A. A. *J. Braz. Chem. Soc*. **2004**, *15 (1)*, 146-149.

52. Rahman, M.; Riaz, M.; Desai, U. R. *Chemistry and Biodiversity* **2007**, *4*, 2495-2527; and refs therein.

53. Adaramoye, O.A.; Nwaneri, V.O.; Anyanwu, K.C.; Farombi, E.O.; Emerole G.O. *Clinical and Exptl. Pharmacol. and Physiol.* **2005**, *32*, 40-46.

54. Han, Q. –B.; Lee, S. –F.; Qiao, C. –F.; He, Z. D.; Song, J. –Z.; Sun, H. –D.; Xu, H. –X. *Chem. Pharm. Bull.* **2005**, *53(8),* 1034—1036.

# Chapter 2

## SYNTHESES OF PROANTHOCYANIDINS

### 2.1 Introduction

The search for biological active proanthocyanidins and their development into commercial pharmaceutical drugs requires pure and structurally defined molecules. Natural sources are unsatisfactory as these sources (mostly plant extracts) produce mixtures of closely related compounds that are difficult to separate, even with modern chromatographic methods. This has challenged synthetic organic chemists to develop synthetic methods that would provide homogeneous entities with well defined stereo- and regiochemistry and degree of oligomerisation.

Naturally occurring proanthocyanidins are thought to be the products of ionic coupling between an electrophilic C-4 carbocation (on the C-ring) of an electrophilic flavanyl unit and the nucleophilic aromatic ring of another flavanyl unit, often a flavan-3-ol. Synthetic methods described in the literature follow a similar strategy and are thus biomimetic in nature. Four general synthetic strategies have been described to obtain the required electrophilic C-4-flavanyl moiety:

1. Starting with flavan-4-ol and flavan-3,4-diol precursors from natural sources to generate the required C-4-flavanyl carbocation (nucleophilic substitution) under acidic conditions.[1-3]

2. Starting with flavan-4-oxo-monomers (flavonoids) from natural sources as precursor and a lithiated nucleophile (nucleophilic addition).[4-7]

3. Starting with proanthocyanidin oligomers from natural sources to generate C-4-flavanyl carbocation monomers *via* cleavage of the interflavanyl C-C bonds.[8-12]

4. Introducing functionality at C-4 (e.g. a halogen, oxygen or sulfur leaving group) in flavan-3-ol precursors followed by nucleophilic substitution of the C-4 functional group (activation of the benzylic position with a good leaving group).[13-17]

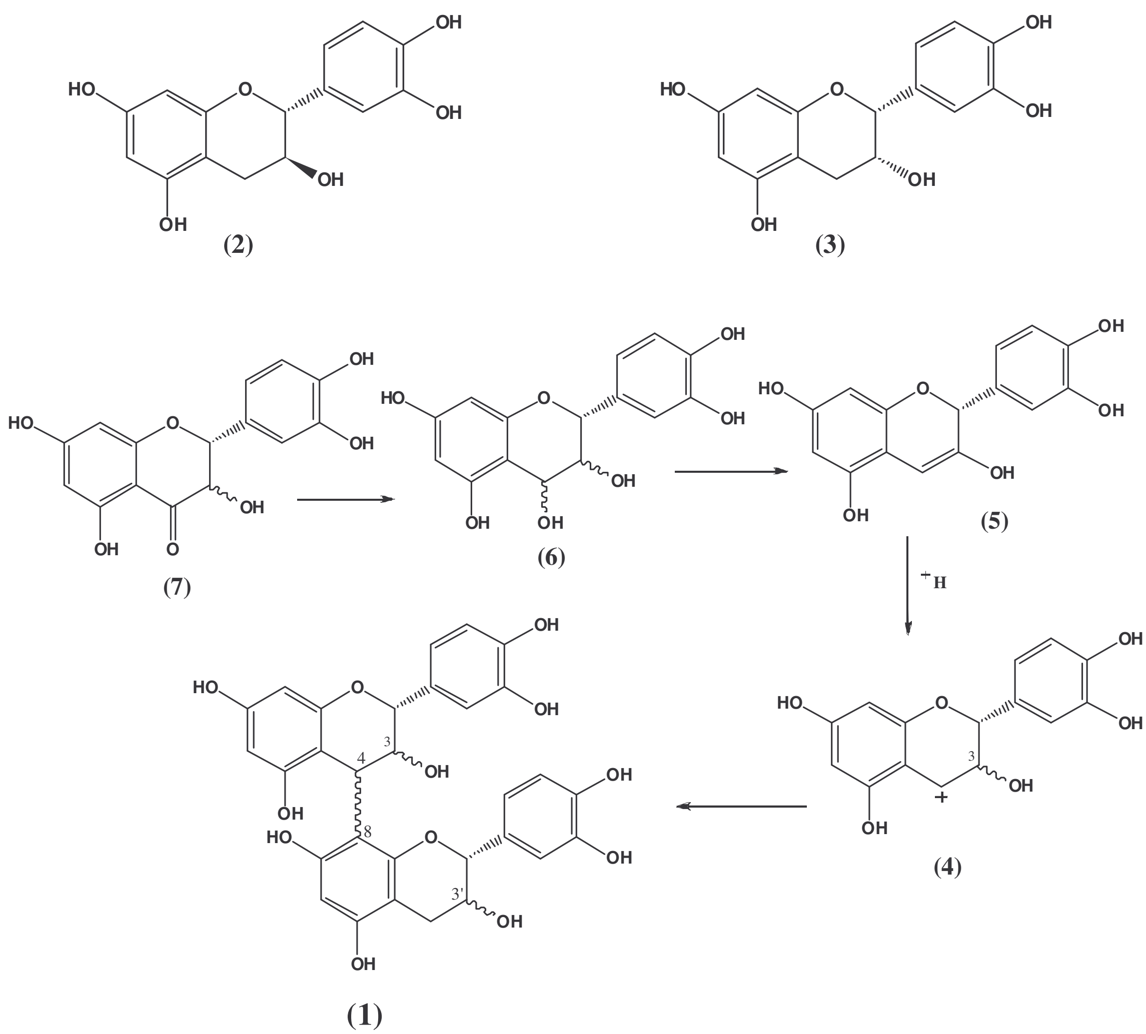
Protection of the phenolic functionalities is considered essential to achieve regioselectivity. This can be achieved with methyl, acetyl, or benzyl groups. Benzylic protection is normally the method of choice. The benzylic ethers are stable under acidic and basic conditions. The benzyilic groups can often be removed simultaneously with a suitable C-8 protecting group that is often used to prevent polymerization. *C*-benzylation may compete with *O*-benzylation during the protection step and careful control of reaction conditions (choice of solvent and temperature control) is often required. Methods traditionally used to methylate phenolic hydroxy groups with dimethyl sulfate ($K_2CO_3$, acetone, reflux) completely fail with benzyl bromide. Modified conditions include benzyl bromide, $K_2CO_3$, DMF; and NaH, benzyl bromide, DMF.[18-22]

## 2.2 A Review of Synthetic Methods

We review the different methods used in the synthesis of proanthocyanidins. Some of these methods were aimed at structural elucidation and do not necessarily yield pure compounds in good yields. We also discuss some of the stereochemical and other information obtained from synthetic procedures to assist with unambiguous structure elucidation of our own results. The older publications did not necessarily give complete stereochemical information (e.g. did not distinguish between 4α- and 4β-configuration).[1,4-6] In such cases, our Schemes and Figures appear without configurational information.

### 2.2.1 Methods based on flavan-4-ol and flavan-3,4-diol precursors to generate a C-4 flavanyl carbocation or an equivalent quinone methide

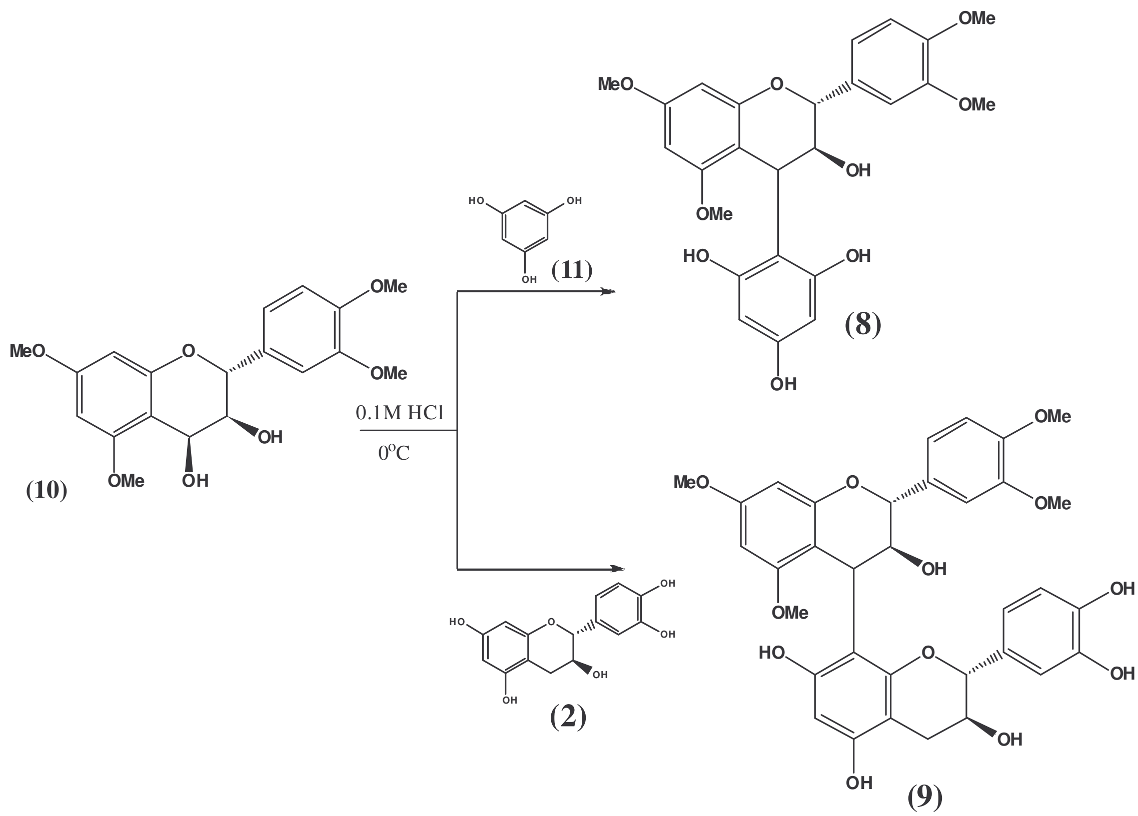
In 1977, Haslam and co-workers[2] determined with feeding experiments, based on labelled [$^{3}$H, $^{14}$C] cinnamic acids, that procyanidin dimers,  e.g. **(1)** are biosynthesized from two distinct units, a flavan-3-ol (catechin **(2)** or epicatechin **(3)**) and a C-4 carbocation **(4)**. They postulated that the carbocation was generated from a flav-3-en-3-ol **(5)**. The flav-3-en-3-ol originated from a putative flavan-3,4-diol **(6)** that is produced from reduction of a dihydroflavone-3-ol **(7)** (Scheme 1).




**Scheme 1:** Biosynthetic generation of C-4 flavanyl carbocation.

Condensation of flavan-3-ols and 4-substituted electrophilic flavans have been extensively investigated without phenol protection in a mildly acidic medium. Mixtures of regio- (4→8 and 4→6 isomers) and sometimes stereoisomers (α- and β- isomers) and higher oligomers were obtained despite the use of excess nucleophilic building blocks. HPLC and TLC are unsuitable to purify these mixtures because silica gel forms irreversible bonds with unprotected polyphenols. Time consuming chromatography on Sephadex LH-20 was the only solution. Non-protected 4-substituted catechins and epicatechins are not easily available and are synthesized by reduction of expensive taxifolin (4-ketone) **(7)**,[18,23] or by in situ degradation,[9,10,18] or thiolytic degradation[9] of proanthocyanidin polymer fractions from rare natural sources. Single stereoisomers with 3,4-*trans* (4β) configuration are generally obtained. Backside shielding of the 4-carbocation by the 3α-hydroxy group during alkylation has been used to explain the exclusive 3,4-*trans* (4β) configuration of the alkylation products. This coincidentally represents alkylation at the less congested face of the pyran ring opposite to the 2-aryl substituent. In the catechin series, where steric influence from the 2-aryl substituent and the 3-hydroxy group oppose each other, mixtures of 4α- and 4β- stereoisomers are obtained.[21,24,25]

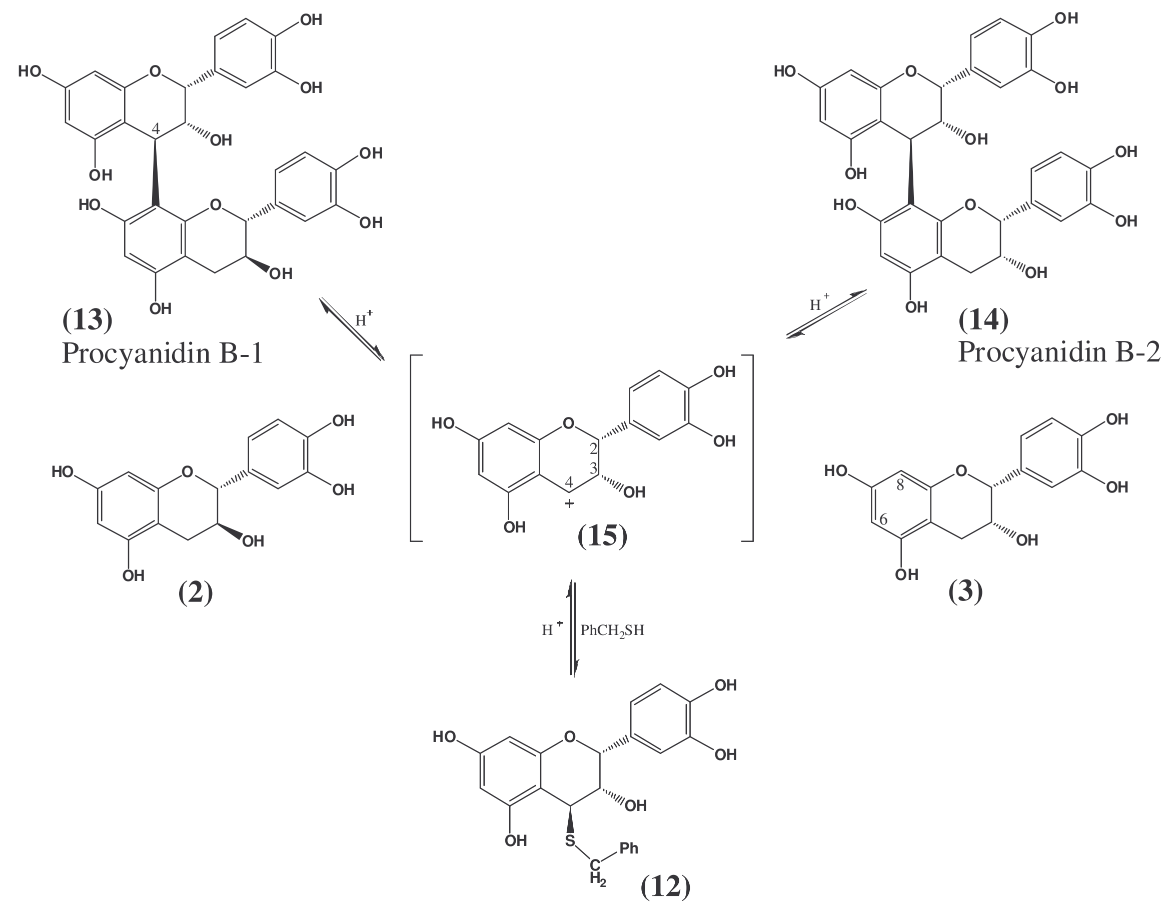
Geissman and Yoshimura[1] (in 1966), reported the first synthesis of a 4-arylflavan-3-ol **(8)** and a proanthocyanidin (C-4→C-8-coupled biflavanoid) **(9)** (Scheme 2). Treatment of a flavan-3,4-diol **(10)** (an incipient electrophile) and an equimolar amount of phloroglucinol **(11)** or catechin **(2)** with 0.1 M HCl in dioxane-water at 0 °C led to isolation of **(8)** or **(9)**. In both cases, an approximately equal amount of another product was detected by TLC. These were assumed to be their C-4 epimers. Purification of **(8)** involved vacuum sublimation of the trimethylsilyl ether followed by passage through a short silica gel column. Compound **(9)** was purified with preparative thin layer chromatography.




**Scheme 2:** The first synthesis of a 4-arylflavan-3-ol and a proanthocyanidin.

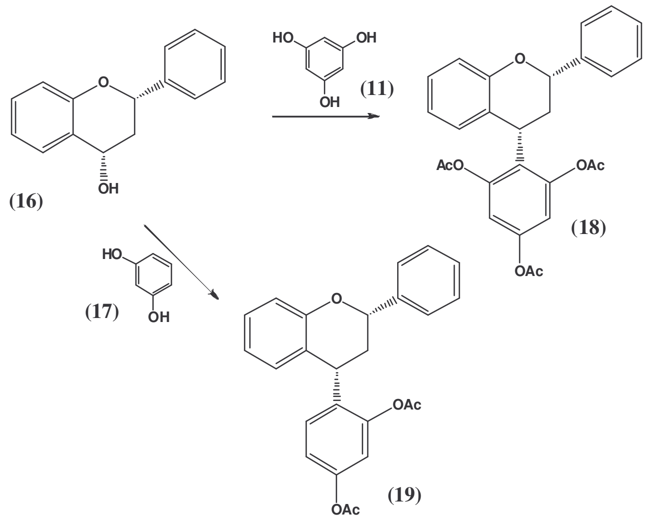
Treatment of the thioether **(12)** (1.7 mM), an acid catalyzed degradation product[26] from **(13)**, in aqueous acidic dioxane (1N HCl) with epicatechin **(3)** (3.8 mM) at room temperature, gave predominantly procyanidin B-2 **(14)** (25% yield based on epicatechin consumed) and also two other C-4 coupled isomers of procyanidins B-2 (combined yield of 8%). One of these isomers was identified as procyanidin B-5. This is an equilibrium process as the same ratio is obtained upon treatment of pure procyanidin B-2 **(14)** with acid. The same ratio is also observed in plant extracts in which epicatechin is the dominant flavan-3-ol. Replacement of epicatechin with catechin **(2)** gave procyanidin B-1 **(13)** (23%) and two stereoisomers (total yield 10%). These isomers are equivalent to the isomers obtained with epicatechin and are also formed in an equilibrium ratio upon treatment of pure procyanidin B-1 **(13)** with acid

(Scheme 3). The coupling is assumed to have taken place *via* an intermediary carbocation **(15)**.




**Scheme 3:** Synthesis of procyanidins *via* an intermediary carbocation.

Reaction of synthetic flavan-4α-ol **(16)** with phloroglucinol **(11)** or resorcinol **(17)** in acidic ethanol yielded, after acetylation, the condensation products **(18)** and **(19)** (Scheme 4).[27] $^1$H NMR analysis ($J_{3ax,4}$ = 3.8 Hz, $J_{3eq,4}$ = 4.0 Hz) indicated 2,4-*trans*-configuration.



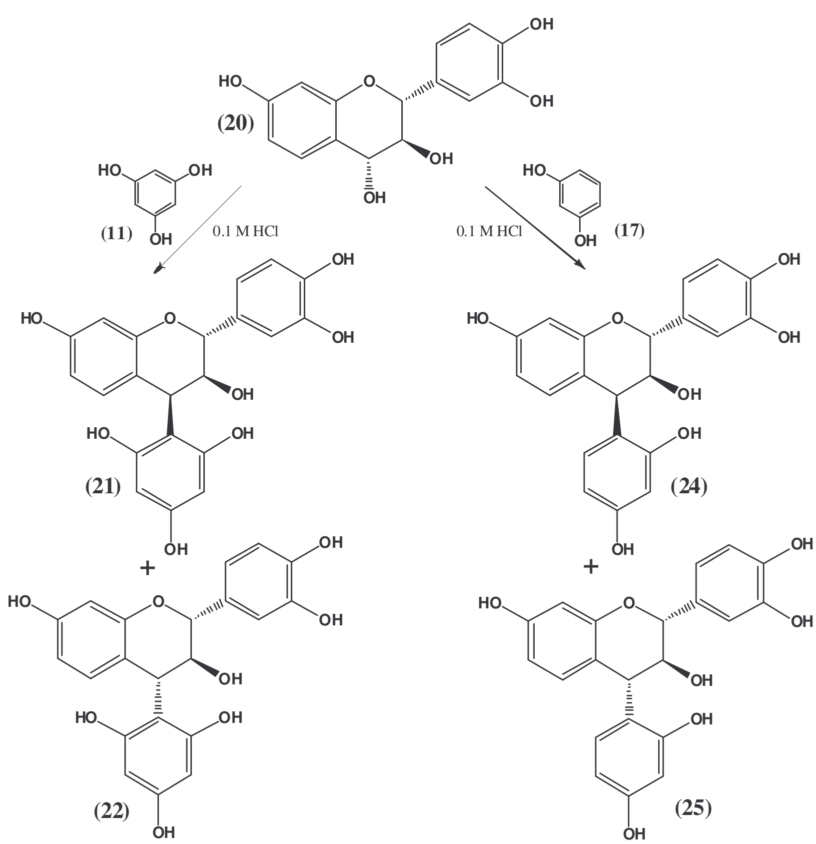

**Scheme 4:** Condensation of flavan-4α-ol with phloroglucinol or resorcinol in acidic ethanol to afford the corresponding 4α-coupled products.

Assignment of relative configuration at C-4, the point of the interflavanyl linkage, has been problematic, mainly because the conformational instability of the C-ring made assignments based on $J_{2,3}$ and $J_{3,4}$ coupling constants ambiguous. These coupling constants, based on the Karplus equation, change when the conformation of the C-ring changes, as is prevalent with sterically hindered 2,3-*cis*-3,4-*cis* isomers.

To overcome uncertainty about the absolute configuration at C-4, the point of the interflavan linkage, Roux and coworkers[3] synthesized a series of optically pure 4-arylflavan-3-ols *via* 4-carbocations, generated from flavan-3,4-diols of known absolute configuration. The sign of the high intensity Cotton effect,[3,28] caused by the aryl chromophore at C-4, correlated with the absolute configuration at C-4 of 2,3-*trans*-3,4-*trans*, 2,3-*trans*-3,4-*cis*, and 2,3-*cis*-3,4-*trans*-isomers.[3] This chiroptical rule was confirmed by Haslam.[27]

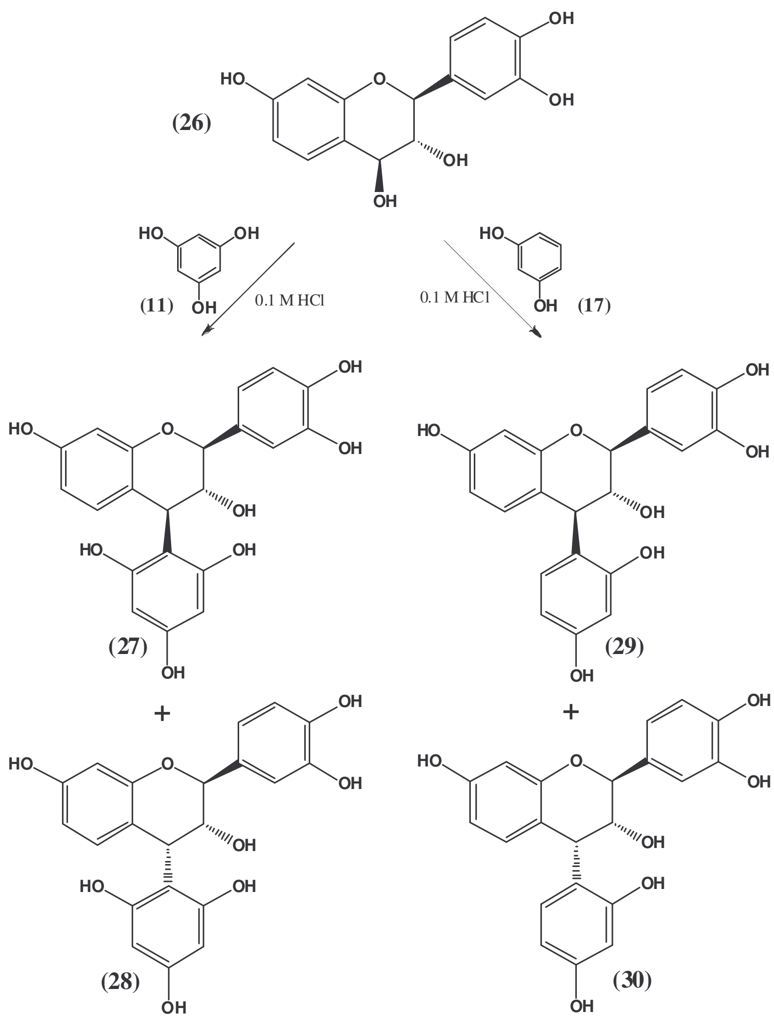
Van der Westhuizen[29] found deviations from this rule with 2,3-*cis*-3,4-*cis* isomers. These deviations had abnormal $J_{2,3}$ and $J_{3,4}$ coupling constants (6.5 and 5.5 Hz, respectively), indicating deviation from the anticipated heterocyclic half chair conformation. The rule consequently requires knowledge of the C-ring conformation for unambiguous application. The non-conforming CD spectra do not demonstrate the expected smooth Cotton effect but rather a hump with the "wrong" sign.

Condensation of fisetinidol-4α-ol **(20)** with phloroglucinol **(11)** (eight fold molar excess) or resorcinol **(17)** (eight fold molar excess) gave the products indicated in Scheme 5 [**(21)** and **(22)**], and [**(24)** and **(25)**], stereoselectively. The combined yields were about 83% with phloroglucinol and 60% with resorcinol. In each instance, a ratio of about 2:1 between 3,4-*trans* and 3,4-*cis* isomers were observed.



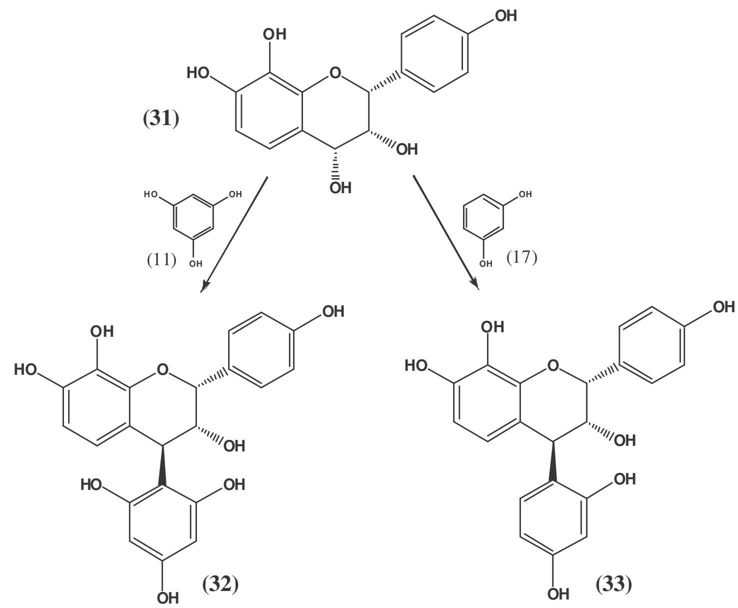

**Scheme 5:** Condensation of fisetinidol-4α-ol with phlorogucinol and resorcinol.

Condensation of *ent*-fisetinidol-4β-ol **(26)** with phloroglucinol **(11)** (eight fold molar excess) or resorcinol **(17)** (eight fold molar excess) gave the enantiomers indicated in Scheme 6 [**(27)** and **(28)**], and [**(29)** and **(30)**], stereoselectively.




**Scheme 6:** Condensation of *ent*-fisetinidol-4β-ol with phlorogucinol and resorcinol.

In contrast with the results in Scheme 5, epioritin-4α-ol **(31)** (2,3-*cis*-3,4-*cis*-flavan-3,4-diol) condenses with phloroglucinol and resorcinol, respectively, to form the 3,4-*trans*-arylflavan analogues **(32)** and **(33)** exclusively (Scheme 7).[3]




**Scheme 7:** Condensation of epioritin-4α-ol with phlorogucinol and resorcinol.

Partial retention of configuration during condensation at C-4 with 2,3-*trans*-flavan-3,4-diols **(20)** and **(26)** and inversion at C-4 with the 2,3-*cis*-flavan-3,4-diol **(31)** indicate that the 2,3-configuration influences the stereochemical outcome at C-4 of the condensation reaction.[3] The intermediary carbocation probably possesses a sofa conformation (Figure 1). The 2,3-*cis*-carbocation [**(34)**, *axial* 3-OH] will be attacked from the less hindered "upper face" to give an exclusive product with inverted configuration at C-4 **(32)**. The 2,3-*trans* carbocation [**(35)**, *equatorial* 3-OH] will experience a limited degree of interfering stereocontrol by the *equatorial* 3-OH. Unfavourable 1,3-diaxial interactions between the nucleophile and *axial* 2-H will cause net retention of configuration.

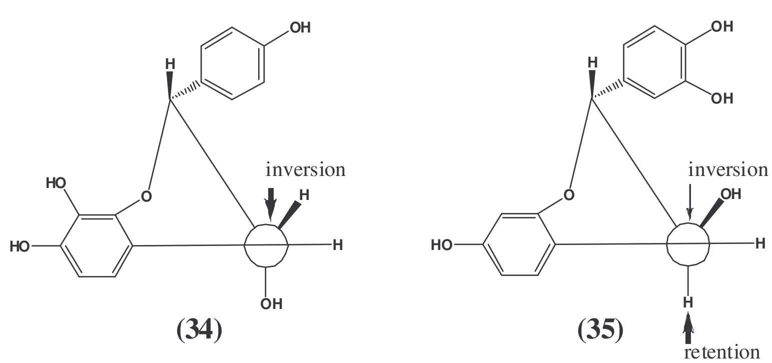
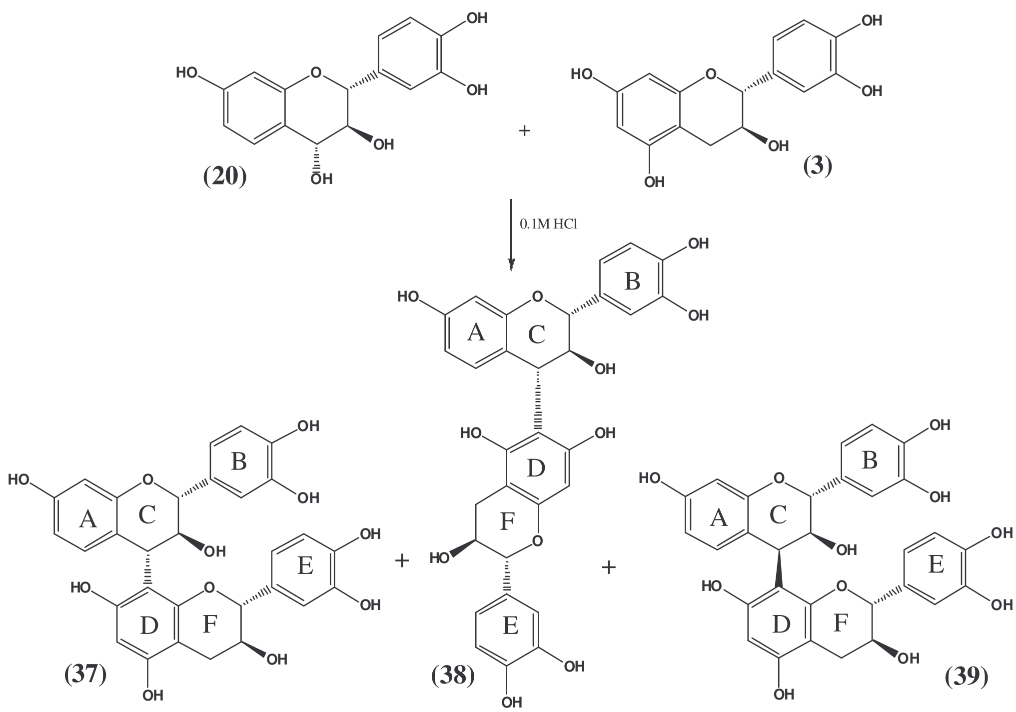


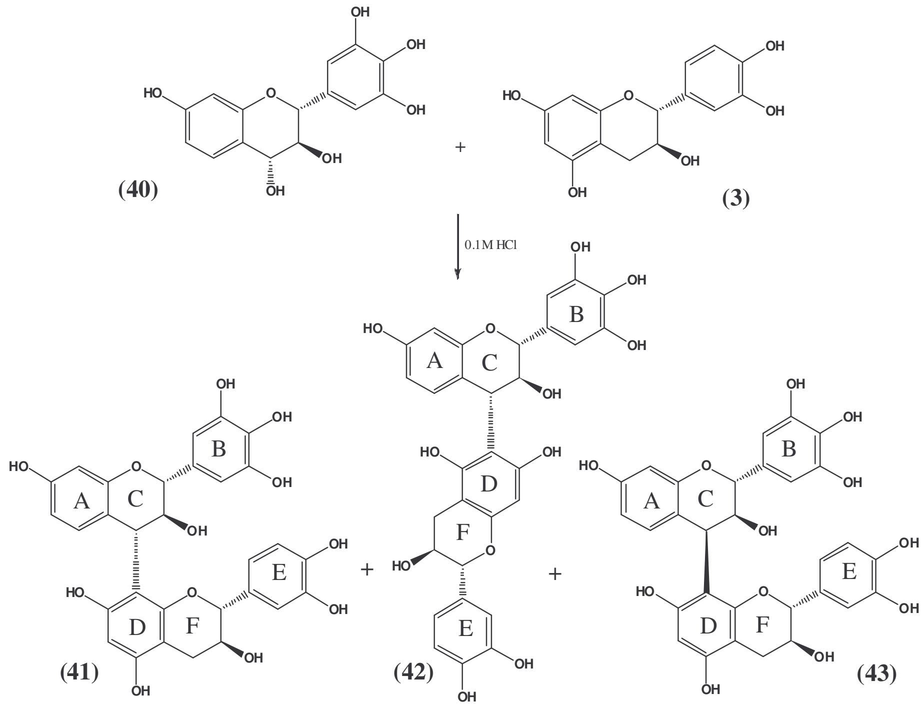

**Figure 1.**

Roux and co-workers[3] extended the synthesis of 4-arylflavan-3-ols to the synthesis of (4→6)- and (4→8)-profisetinidins. Treatment of fisetinidol-4α-ol **(20)** and catechin **(3)** with aqueous 0.1 M HCl at ambient temperatures for 2 hours gave a mixture of three profisetinidin dimers **(37)**, **(38)** and **(39)** in 25, 5.5 and 16.5% yields, respectively (Scheme 8).



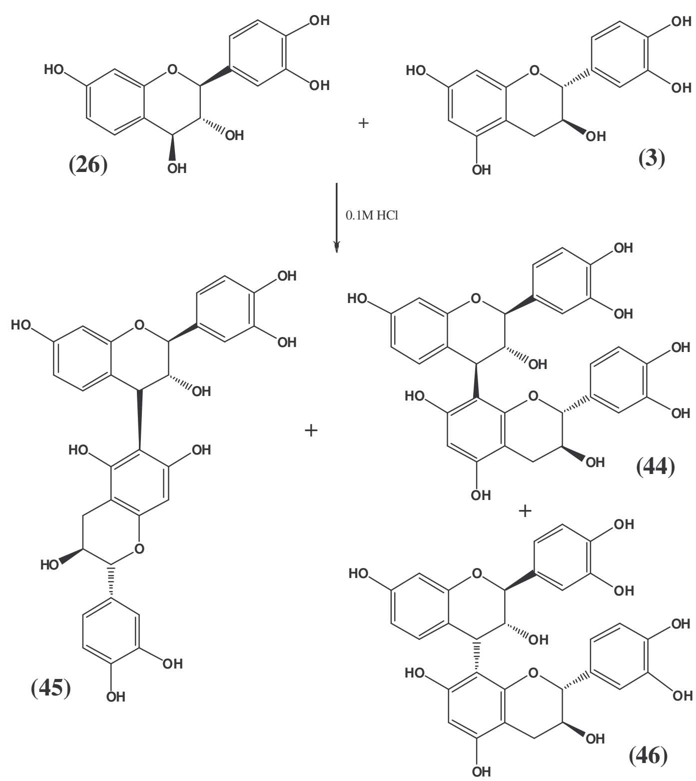

**Scheme 8:** Reaction of fisetinidol-4α-ol and catechin.

Similarly, treatment of robinetinidol-4α-ol **(40)** and catechin **(3)** with aqueous 0.1M HCl at ambient temperatures for 2 hours gave a mixture of two *trans-trans* prorobinetinidin dimers **(41)** and **(42)** and the *trans-cis* dimer **(43)** in 23, 3.3 and 11.2% yields, respectively (Scheme 9).



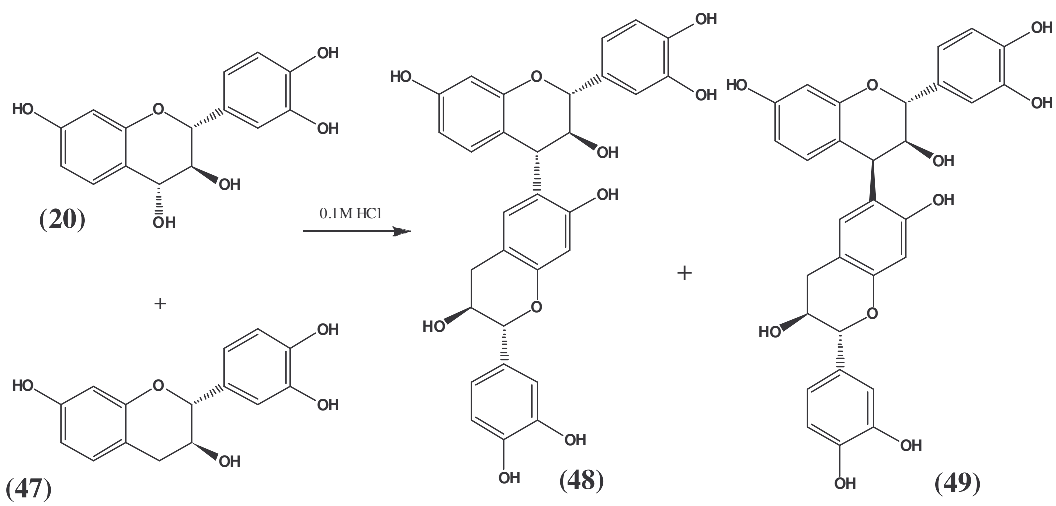
Scheme 9: Reaction of robinetinidol-4α-ol and catechin.

Condensation of *ent*-fisetinidol-4β-ol **(26)**, the enantiomer of fisetinidol-4α-ol **(20)**, with catechin **(3)**, yielded the dimers **(44)**, **(45)** and **(46)** in 24.8, 1.9, and 19.6% yields, respectively (Scheme 10).[24]



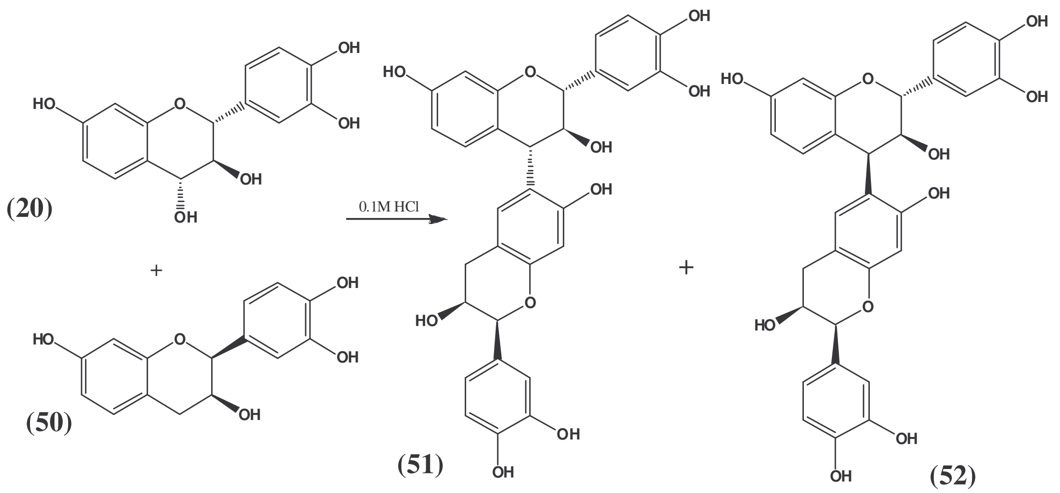
**Scheme 10:** Condensation of *ent*- fisetinidol-4β-ol and catechin.

Condensation of fisetinidol-4α-ol **(20)** and fisetinidol **(47)** gave dimers **(48)** and **(49)** regiospecifically (exclusive coupling at C-6 of the resorcinol-type flavan-3-ol) in yields of 27 and 19%, respectively. This represented the first synthetic access to natural dimers with both A-ring moieties of the resorcinol type (Scheme 11). No line broadening due to rotational isomerism was observed in the NMR spectra of the hexamethyl ether diacetates.




**Scheme 11:** Condensation of fisetinidol-4α-ol and fisetinidol.

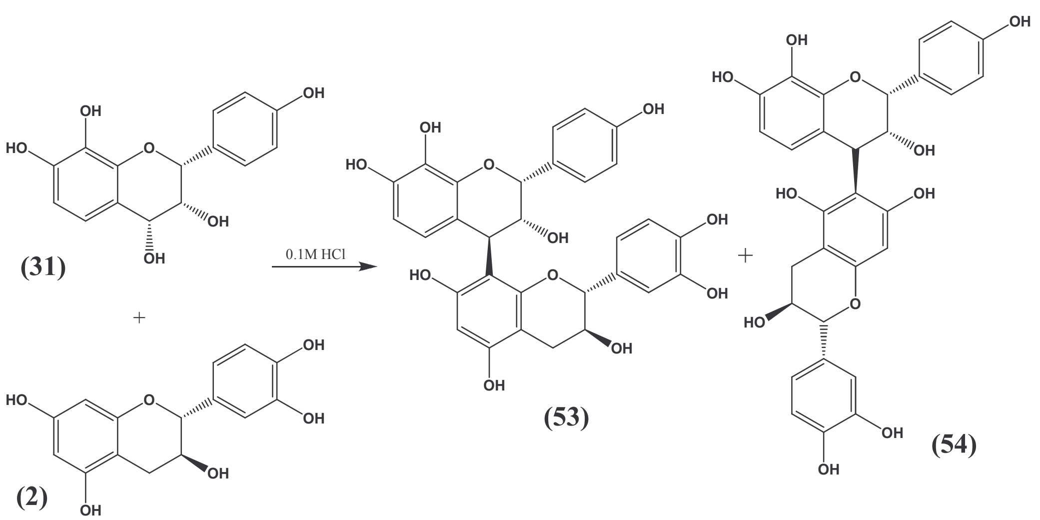
Condensation of **(20)** with *ent*-epifisetinidol **(50)** gave regiospecific coupling at C-6 to yield **(51)** and **(52)** in 21 and 16% yields, respectively. The absence of a 5-OH substituent in the resorcinol type A-ring of **(20)** and **(46)** renders the 6-position sterically less hindered than the 8-position, resulting in exclusive 6-coupling. The absence of a 5-OH group also allows free rotation around the interflavanyl bond, explaining the absence of line broadening in the NMR spectra. These dimers with 3,4-*cis* configuration in the "bottom unit" were later isolated from *Colophospermum mopane*[24] (Scheme 12).



(20) (50) (51) (52)

**Scheme 12:** Condensation of fisetinidol-4α-ol with *ent*-epifisetinidol.

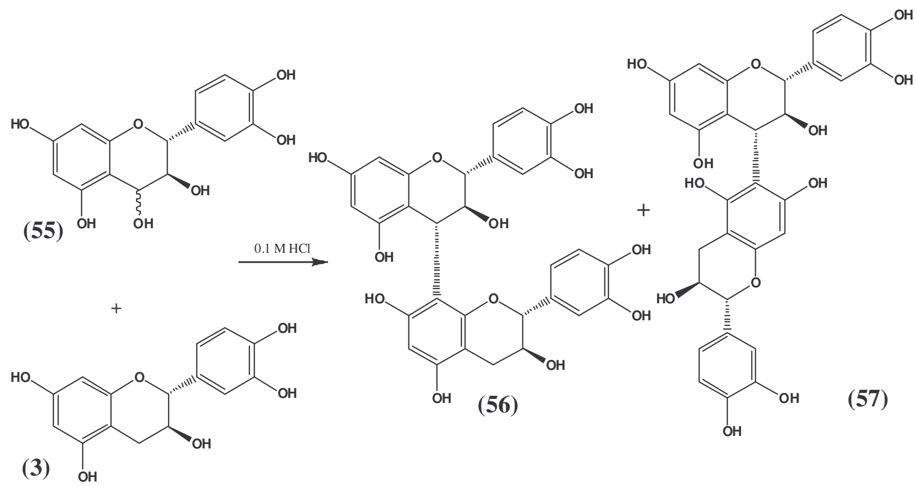
Condensation of epioritin-4α-ol[3] **(31)** and catechin **(2)** gave **(53)** and **(54)** (Scheme 13).



(31) (2) (53) (54)

**Scheme 13:** Condensation of epioritin-4α-ol and catechin.

Condensation of catechin **(2)** with flavan-3,4-diol **(55)**, obtained from reduction of dihydroquercetin **(7)**, yielded **(56)** and **(57)** (Scheme 14).[3]

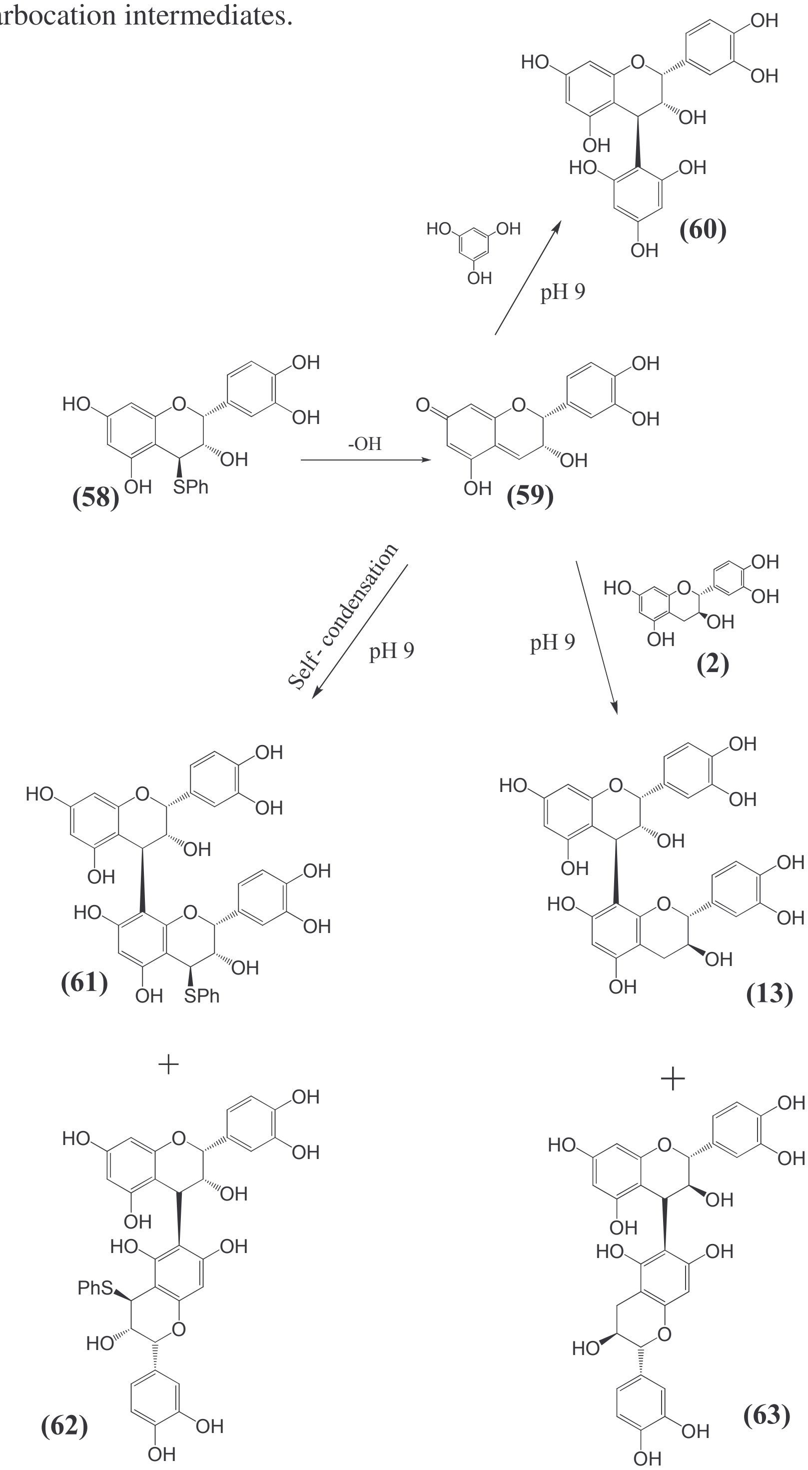


**Scheme 14:** Condensation of catechin with the reduction product of dihydroquercetin.

These results demonstrate the versatility of this method which is based on flavan-3,4-diols. The mild conditions prevent anthocyanidin formation. The association of dimers and flavan-3,4-diols in natural plant sources supported a carbocation mediated mechanism in the biosynthetic formation of dimers[1,26,30-35] rather than the quinone methide or flav-3-en-3-ol mechanism proposed by Hemingway and co-workers.[36]

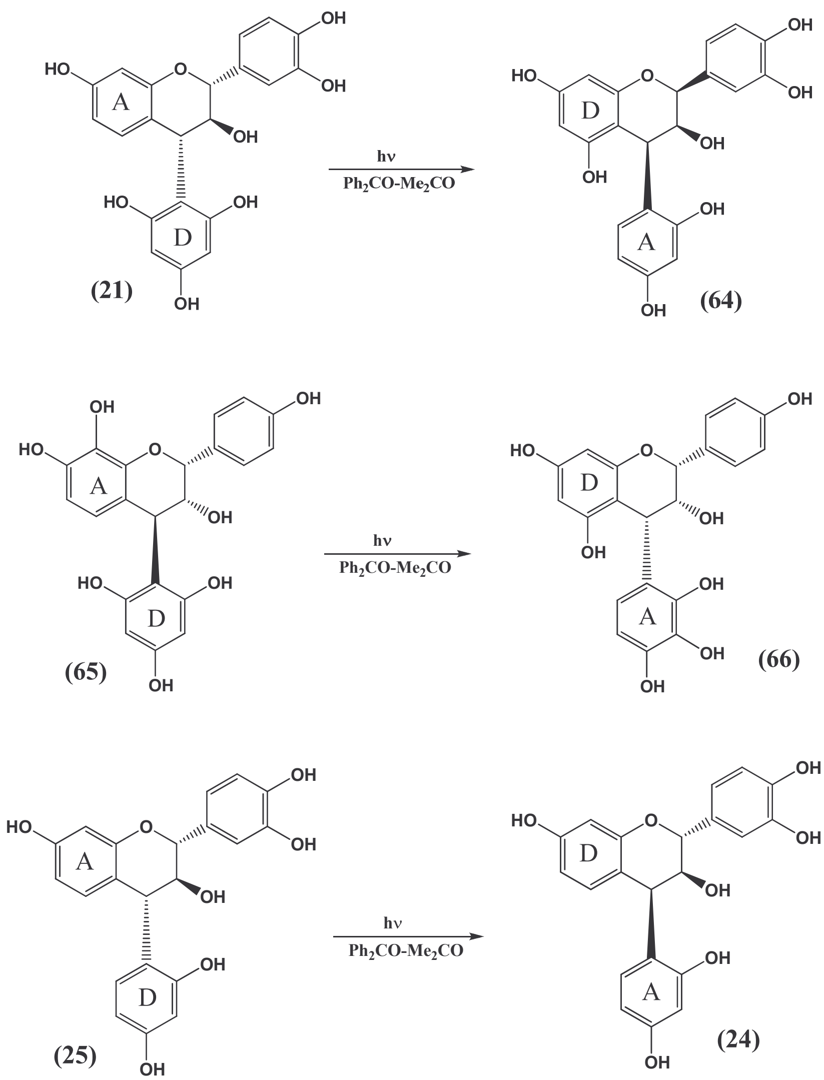
Ferreira and co-workers extended this methodology to trimers and tetramers.[37-39] A complete survey of this complex and important work is beyond the scope of this review.

Base-catalysed condensation of a flavan-4-ylthioether **(58)** with a variety of nucleophiles was demonstrated by Hemingway and Foo.[36] A solution of **(58)** in acetone-water (1:1, v/v) with the pH adjusted to 9.0 with a sodium hydrogen carbonate-sodium carbonate buffer yielded self-condensation products **(61)** and **(62)** and polymeric materials after 30 minutes at ambient temperature. Addition of phloroglucinol **(11)** to the reaction mixture yielded **(60)** after 45 minutes and addition of catechin **(2)** yielded **(13)** and **(63)** (Scheme 15). Repetition of the reaction in acetone-water at pH 3 yielded no products after 8 hours. No epimerization at C-2 was observed at pH 9. Preservation of configuration at C-4 (only 3,4-*trans* products were isolated) ruled out $S_N2$ substitution at C-4. These results were claimed to indicate that reactions *via* a quinone methide **(59)** are much faster than reactions *via* acid catalyzed generated carbocation intermediates.




**Scheme 15:** Base-catalysed condensation of a flavan-4-yl-thioether with a variety of nucleophiles.

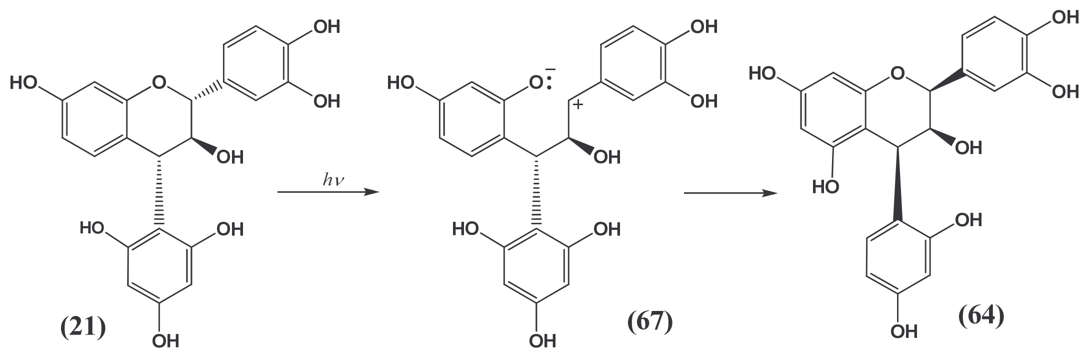
Benzophenone sensitized photolytic rearrangement of 2,3-*trans*-3,4-*trans*- **(21)** and **(25)** and 2,3-*cis*-3,4-*trans*-4-arylflavan-3-ols **(65)**, where the nucleophilicity of the D-ring (4-aryl ring) hydroxy group exceeds that of the A-ring, gave the first access to 2,3-*cis*-3,4-*cis*-diastereoisomers **(64)** and **(66)** ( Scheme 16).[29]




**Scheme 16:** Photolytic rearrangement of 4-arylflavan-3-ol analogues.

In the absence of benzophenone, the 4-arylflavan-3-ols **(21)**, **(65)**, and **(25)** were photo-stable in methanol, ethyl acetate and water, presumably indicating that transfer of triplet energy is a prerequisite for the above conversions. Alternatively, hydrogen abstraction by benzophenone is required.

These photolytic rearrangements require formal heterolytic cleavage of the heterocyclic ether bond and simultaneous intramolecular recyclization *via* a possible zwitterion **(67)** involving the stronger nucleophilic hydroxy group of the phloroglucinol D-ring. Inversion of configuration at C-4 is due to 180$^{o}$ rotation about the C-3-C-4 bond (Scheme 17).[29] The configuration at C-2 is retained and gives 2,3-*trans*-3,4-*cis*-diastereoisomer **(24)**



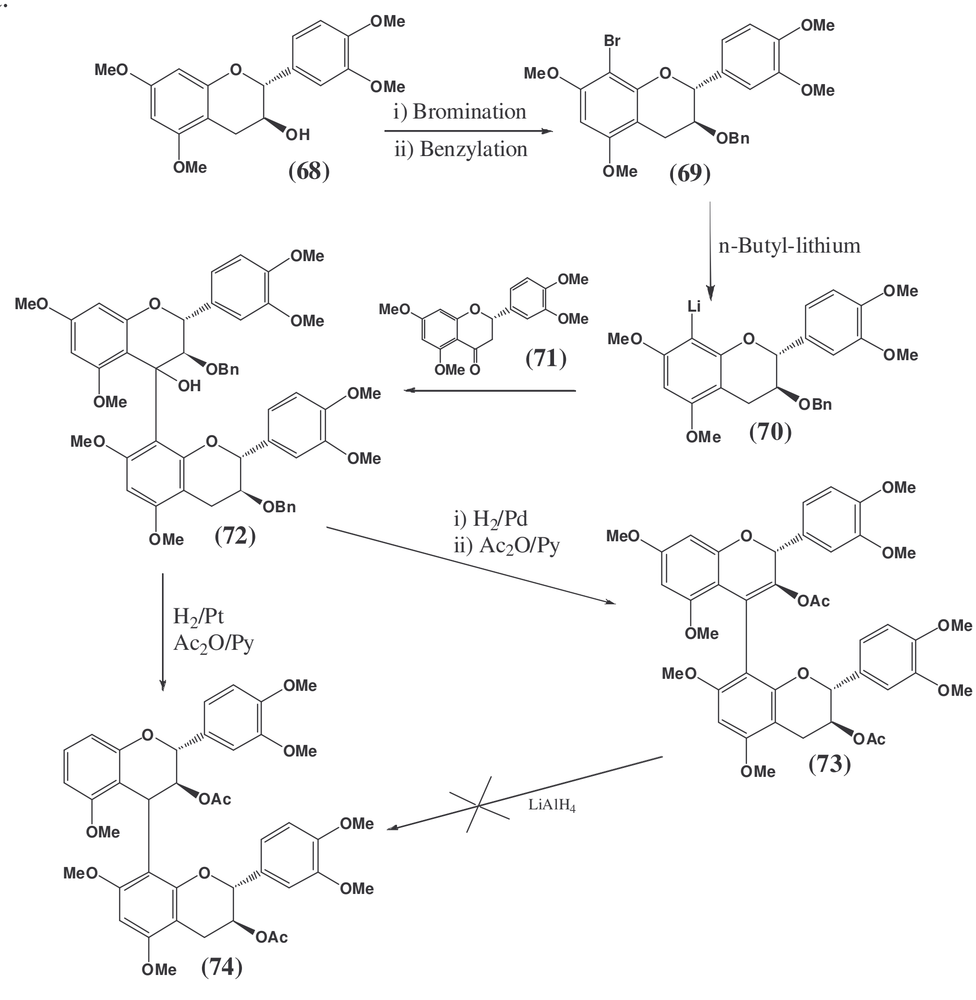

**Scheme 17:** Photolytic cleavage of 4-arylflavan-3-ol.

The only difference between **(21)** that undergoes inversion at C-2 and **(25)** that retains its configuration at C-2 during the photolytic process is the presence [**(21)**] or absence [**(25)**] of an extra hydroxy group in the *ortho* position of the D-ring of the starting material.

### 2.2.2 Methods based on flavan-4-oxo monomers (C4 ketoprocyanidin) as precursors with an electrophilic C4 carbon (nucleophilic addition)

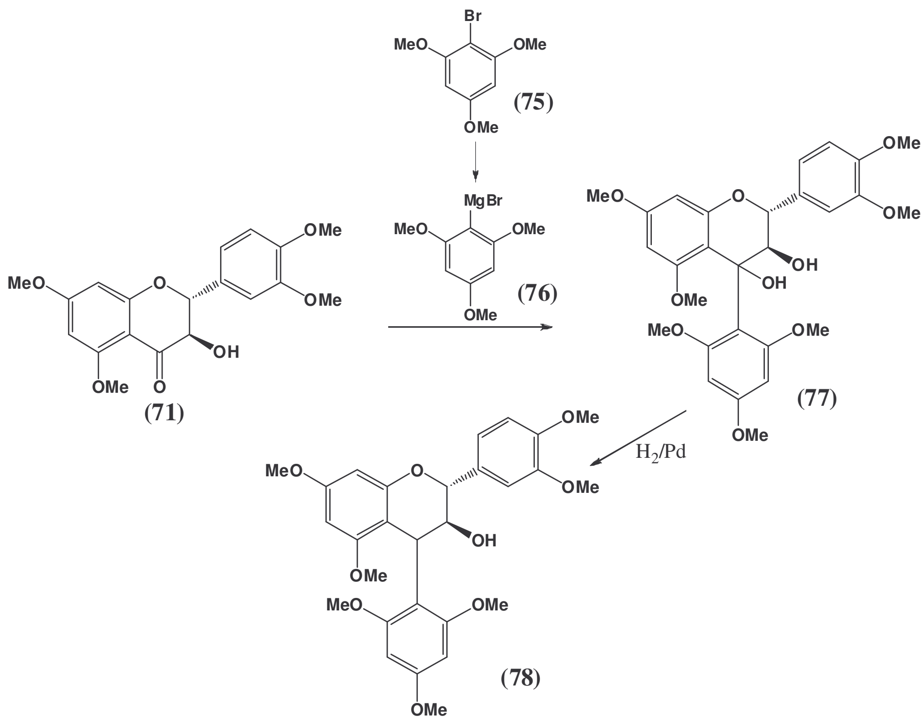
Weinges and coworkers (1967, 1970)[4-6] synthesized procyanidin B-3 **(74)** in 5% yield from 8-lithium-3′,4′,5,7-tetramethoxyflavan-3-*O*-benzylate **(70)** [prepared from brominated tetramethoxycatechin **(69)**] and 3′,4′,5,7-tetramethyltaxifolin **(71)**. This is the same C-4→C-8 linked product **(9)** that Geissman produced from a flavan-3,4-diol and catechin. The biflavan-4-ol intermediate **(72)** was not isolated. Hydrogenation of **(72)** with $H_2$/Pt gives the desired product **(74)** but hydrogenation with $H_2$/Pd leads to water elimination and isolation of the flav-3-ene dimer **(73)**. Efforts to convert **(73)** to **(74)** with $LiAlH_4$ failed. Replacement of catechin with epicatechin yielded a product that could not be obtained in pure form but was believed to be the dimer with epicatechin in the lower position.

This synthesis confirmed the structure of a procyanidin isolated from Cola nuts as suggested on the basis of the NMR and mass spectra of the crystalline derivative of the isolated natural product.[4]



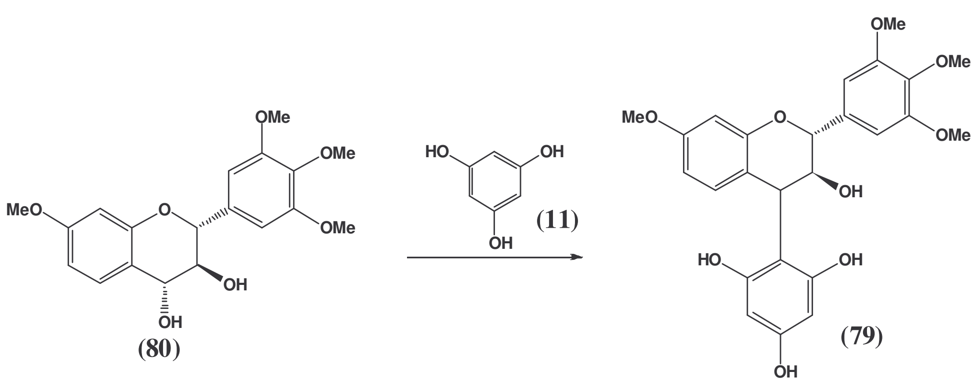

**Scheme 18:** Synthesis of octa-*O*-methyl-diacetyl procyanidin B-3.

Weinges and co-workers[5,6] similarly synthesized the 4-arylflavan-3-ol **(78)** in 5% yield from tetra-*O*-methyltaxifolin **(71)** and bromophloroglucinol-tri-*O*-methylether [*via* the Grignard reagent **(76)**]. Hydrogenation of **(77)** with $H_2$/Pd yielded the desired product **(78)**.




**Scheme 19:** Synthesis of 4-arylflavan-3-ol *via* a Grignard reaction.

For the synthesis of **(79)**, also in 5% yield, Weinges[5] used the Geissman method,[1] starting from the tetra-*O*-methylflavan-3,4-diol **(80)** and phloroglucinol **(11)** (Scheme 20).

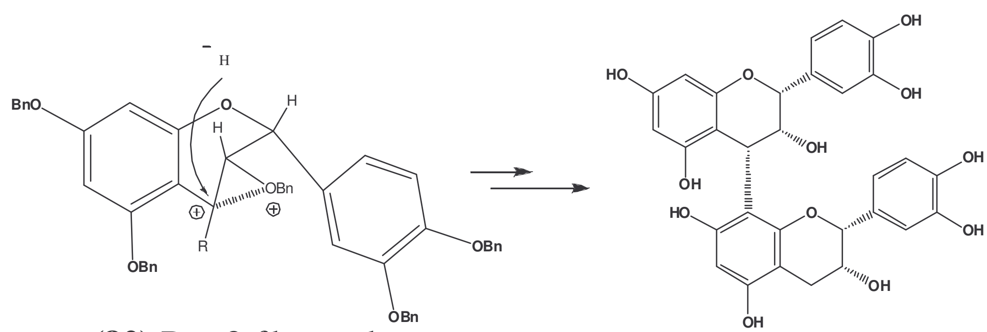



**Scheme 20:** Synthesis of 4-arylflavan-3-ol *via* the flavan-3,4-diol derivative.

Weinges and coworkers required these 4-arylflavan and 4-arylflavan-3-ol products as model compounds to study atropisomerism in procyanidins (see also Chapter 1). They concluded that for restricted rotation about the carbon-carbon link from the C-4 position ($sp^3$) of the C-ring to a $sp^2$ carbon on the 4-aryl substituent to be observed, two conditions must be fulfilled:

1. Both the *ortho* positions of the "lower unit" (4-aryl moiety) must be substituted.
2. The "upper unit" must have a substituent at either C-3 or C-5.

Kozikowski and et al.[7] set out to synthesize a procyanidin dimer **(81)** with a 4α-linked epicatechin unit. Synthesis of **(81)** *via* a 4-carbocation intermediate had not been achieved before, presumably because the 2-arylgroup and 3-oxygen directs the approaching nucleophile towards the β-face. They concluded that, by merely modifying reaction conditions, the 4α-stereoisomer would remain inaccessible. They postulated that a carbocation **(82)** (Figure 2) that has the flavanyl unit already in place would be attacked by a hydride nucleophile from the β-face, forcing the 4-flavanyl unit into the α-position.

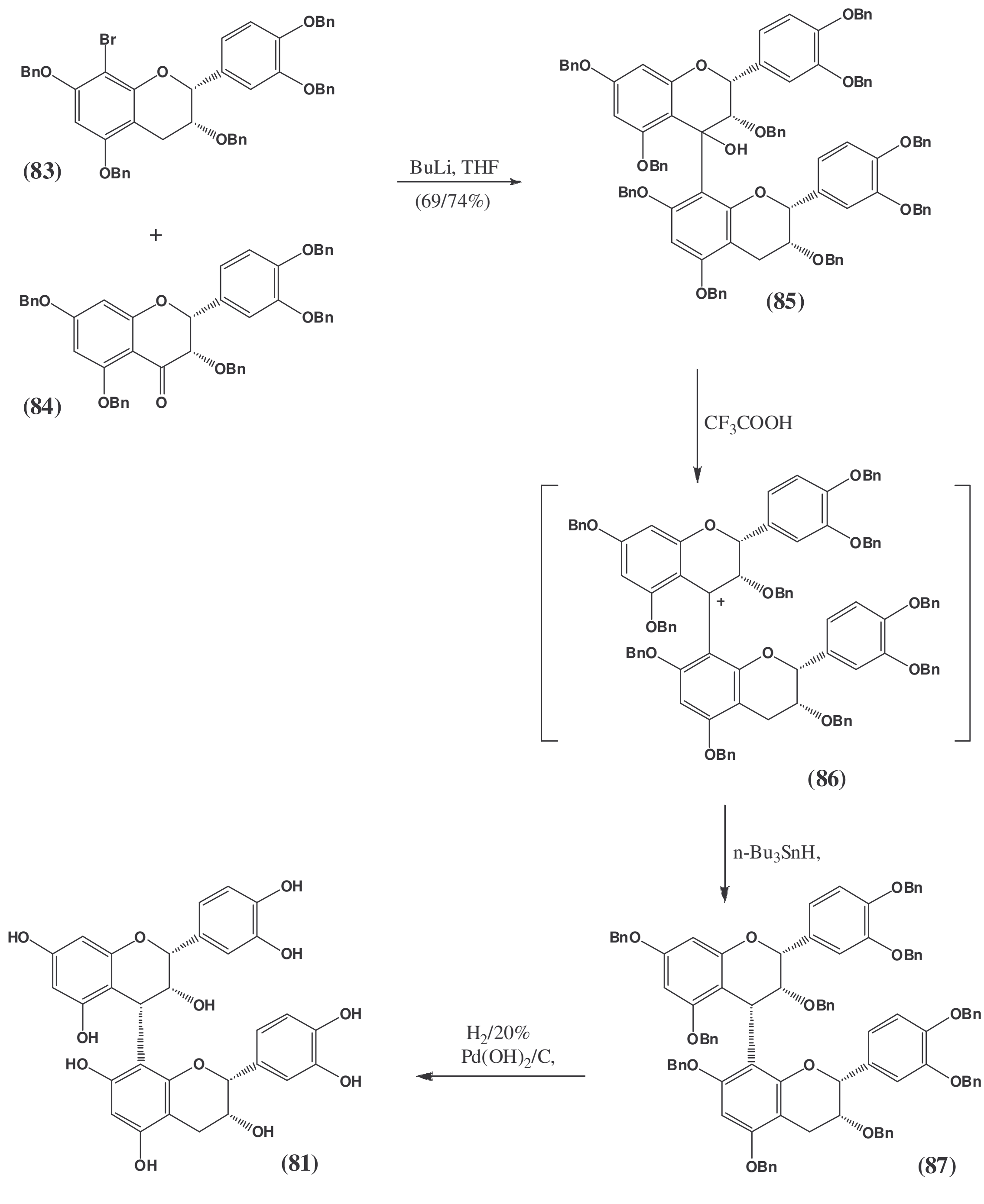



**(82)** R = 8-flavanyl **(81)**

**Figure 2:** Synthesis of **(81)** *via* a 4-carbocation intermediate.

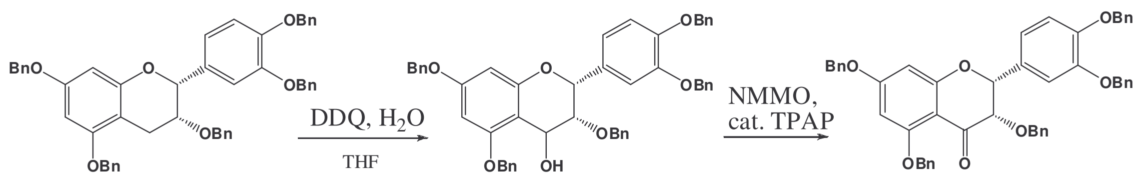
Condensation of a 8-lithiated nucleophile obtained from **(83)**, and a 4-ketone **(84)** gave a tertiary alcohol **(85)**. This alcohol is analogue to **(72)** and generates the required carbocation

**(86)** upon treatment with trifluoroacetic acid. Their stereoselective synthesis of the unnatural epicatechin-(4α→8)-epicatechin dimer **(81)** is depicted in Scheme 21.



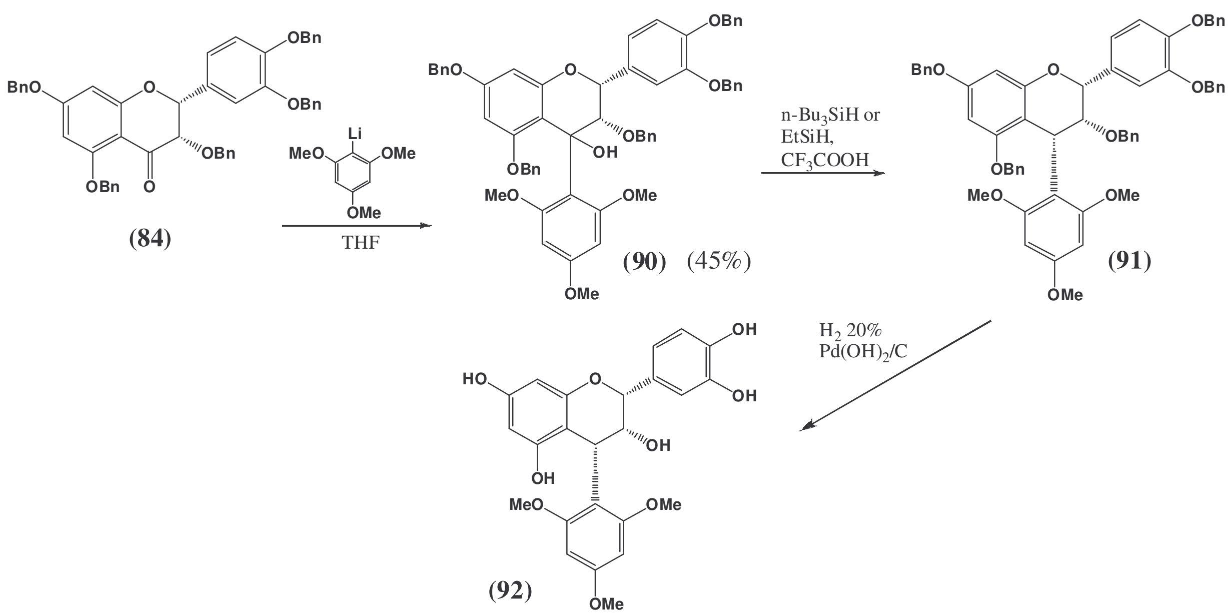

**Scheme 21:** Synthesis of epicatechin-(4α→8)-epicatechin dimer.

The electrophile ketone **(84)** was obtained by oxidation of perbenzylated catechin **(88)**, first with DDQ in water and DMF to the 4-hydroxy intermediate **(89)** followed by oxidation of the benzylic hydroxy group to a carbonyl group using Ley's method[40] (Scheme 22).




**Scheme 22:** Synthesis of 4-ketone.

Kozikowski et al.[7] also synthesized 2,3-*cis*-3,4-*cis*-4-aryl-flavan-3-ol **(92)** (Scheme 23).



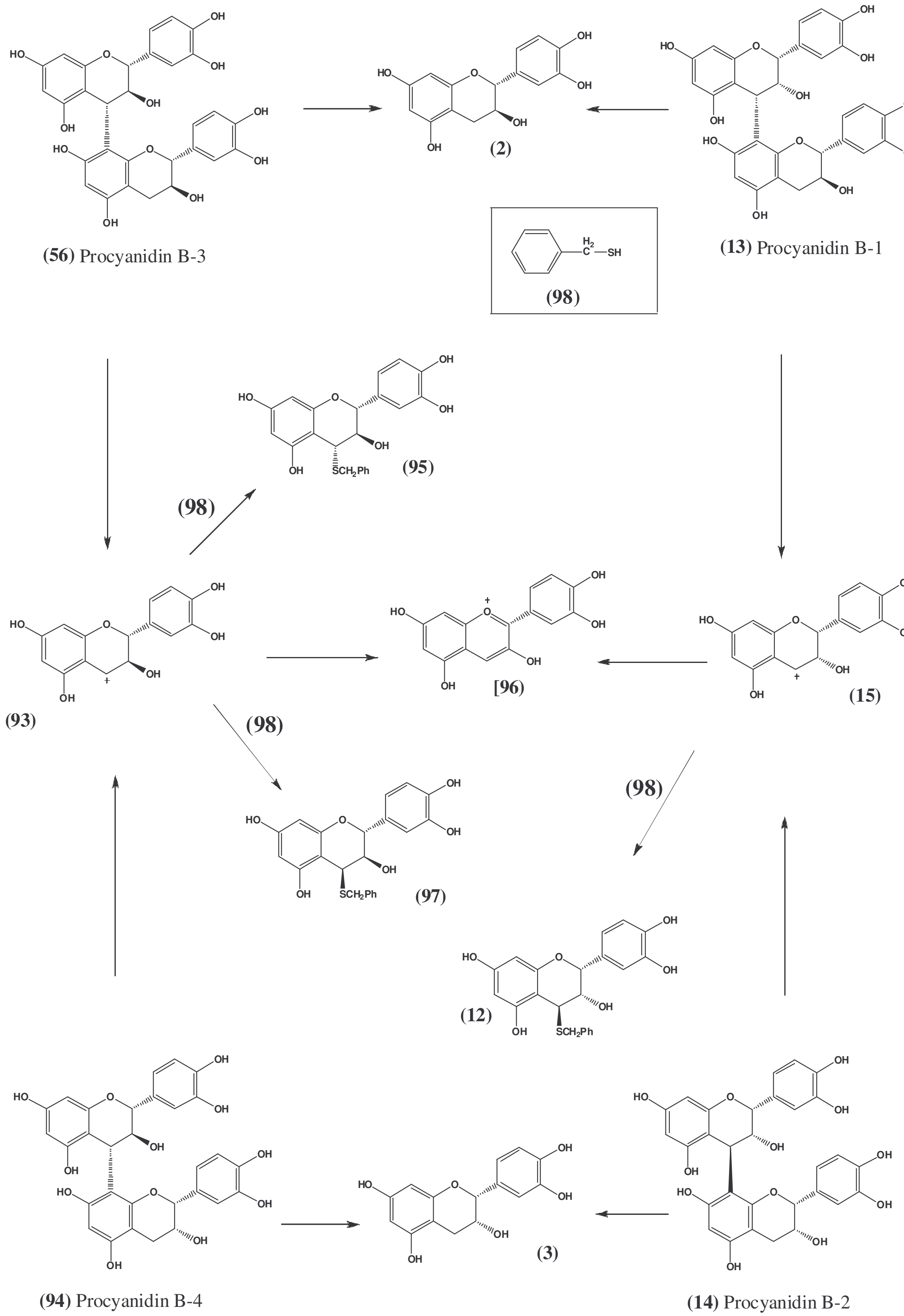

**Scheme 23:** Synthesis of 2,3-*cis*-3,4-*cis*-4-aryl-flavan-3-ol.

Protection of the 3-hydroxy group was achieved with either benzylation or silylation (NaH, benzyl bromide, DMF (73%) or TBDMS-Cl, imidazole, DMF). Deprotection of the silyl group with *n*-$Bu_4NF$ gave a complex mixture but aqueous HF in acetonitrile gave good results. Alternatively, the two protected products **(87)** and **(91)** were hydrogenated with 1 bar $H_2$, 20% $Pd(OH)_2/C$ in an EtOAc/MeOH mixture in quantitative yields. Smooth reduction of the tertiary alcohols **(85)** and **(90)** requires *n*-$Bu_3SnH$ in $CF_3COOH$ whilst $Et_3SiH$ in $CF_3COOH$ caused elimination and cleavage of the interflavanyl bond, hence the lower reported yields of 79/77% and 87/68%, respectively (Schemes 21 and 23).

### 2.2.3. Methods based on proanthocyanidin dimers and oligomers to generate C-4 flavanyl carbocation monomers via cleavage of the interflavanyl C-C bonds

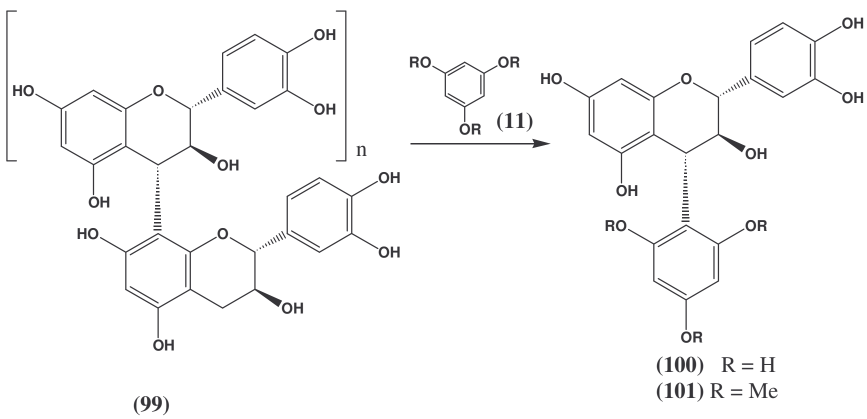
The interflavanyl carbon-carbon bond is a relatively weak bond. The name proanthocyanidin, in fact, refers to the ease with which this bond is broken under mild acidic conditions to yield coloured anthocyanidins. The breaking of this bond in the presence of nucleophiles yields 4-arylsubstituted proanthocyanidins.

Treatment of the procyanidin dimers[8] B-1 **(13)** and B-2 **(14)** with toluene-α-thiol **(98)** and acetic acid in ethanol gave catechin **(2)** and epicatechin **(3),** respectively, and a 4-benzylthioflavan **(12)**. The procyanidin dimers B-3 **(56)** and B-4 **(94)**, gave catechin **(2)** and epicatechin **(3)**, respectively, under the same conditions and also two 4-benzylthioflavans **(95)** and **(97)**. The flavan-3-ols (catechin and epicatechin) is derived from the “lower half” of the dimer and the transient carbocations **(15)** and **(93)** from the ABC-moieties that are trapped by the thiol **(98)** to give the 4-benzylthioflavans (Scheme 24). Formation of two thioethers **(95)** and **(97)** from the carbocation with 2,3-*trans* configuration **(93)** and stereospecific formation of only one thioester **(12)** from the carbocation with 2,3-*cis* configuration **(15)** is supported by analogous results from the solvolysis of 3,4-diols. Solvolysis of 2,3-*trans*-flavan-3,4-diols give mixtures of 3,4-*trans*- and 3,4-*cis*-ethers under thermodynamic control whilst 2,3-*cis*-flavan-3,4-diols give 3,4-*trans*-ethers only.[41,42] Yields of the thioesters were between 53 and 75%.



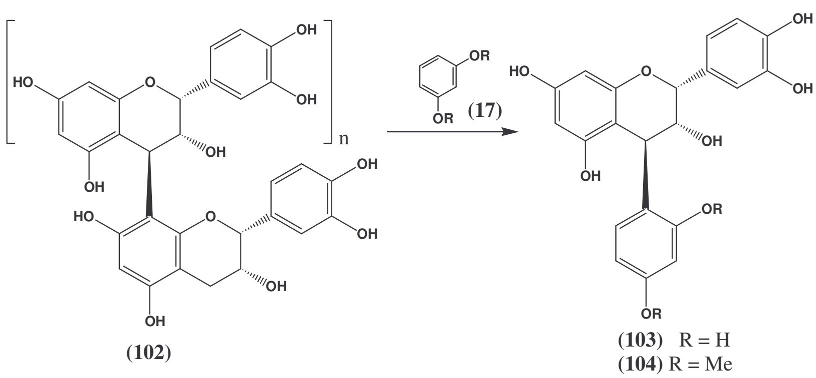

**Scheme 24**: Acid-catalysed degradation of procyanidins.

Treatment of polymeric procyanidins[9] **(99)**, from *Salix caprea catkins,* with phloroglucinol **(11)** yielded 4-arylflavan-3-ol derivatives where the unsubstituted flavan unit **(18)** (Scheme 4) is replaced by its catechin analogue **(100)** (Scheme 25).




**Scheme 25:** Synthesis of 4-aryflavan-3-ol derivatives from **(99)**.

Structure **(102)**, isolated from *Butea frondosa* gum, yielded the epicatechin analogue **(103)** on treatment with resorcinol **(17)** (Scheme 26).




**Scheme 26:** Synthesis of 4-aryflavan-3-ol derivatives **(102)** isolated from *Butea frondosa* gum.

To solve the problem of differentiating between C-4→C-6″ and C-4→C-8″ linked proanthocyanidins, where the 'terminal' unit possesses a phloroglucinol A-ring (e.g. catechin), Roux[11,12] and co-workers synthesized both 6- and 8-bromo-tetra-*O*-methyl-catechins **(105)** and **(106)** (Figure 4). Chemical shift data for the residual A-ring protons at C-

6 and C-8 fall into narrow ranges that did not overlap [δ ($CDCl_3$) 6.1-6.22 and 6.32-6.47, respectively]. X–ray diffraction analysis was used to unambiguously distinguish **(105)** from **(106)**.

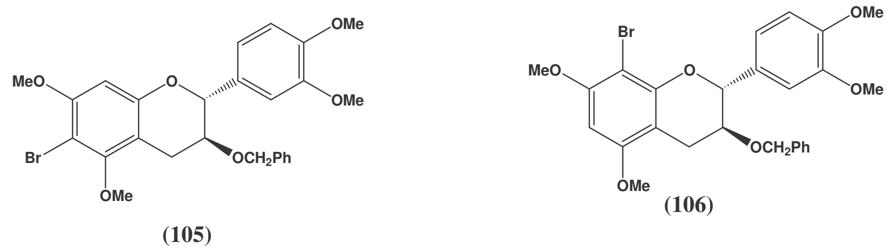
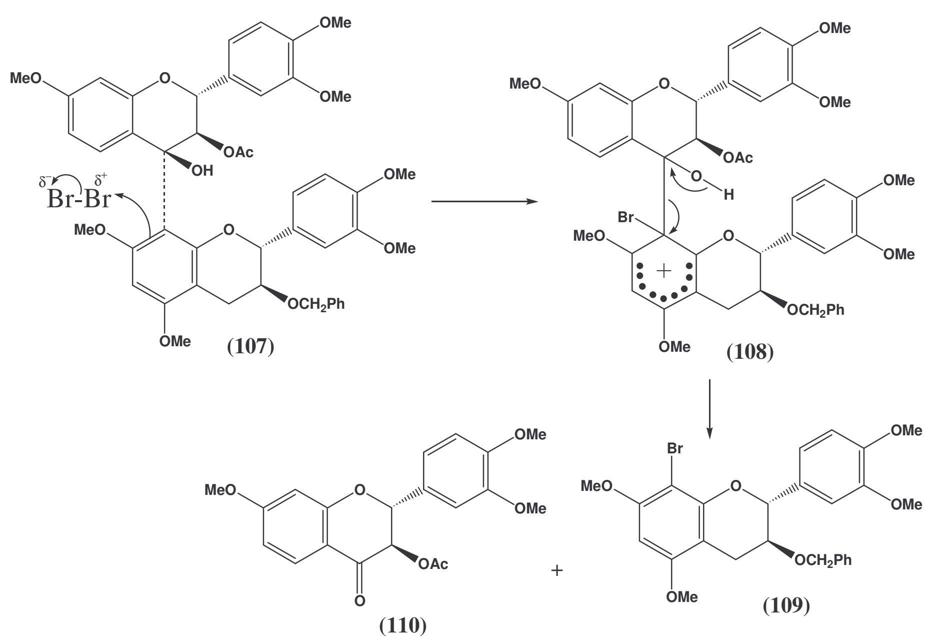


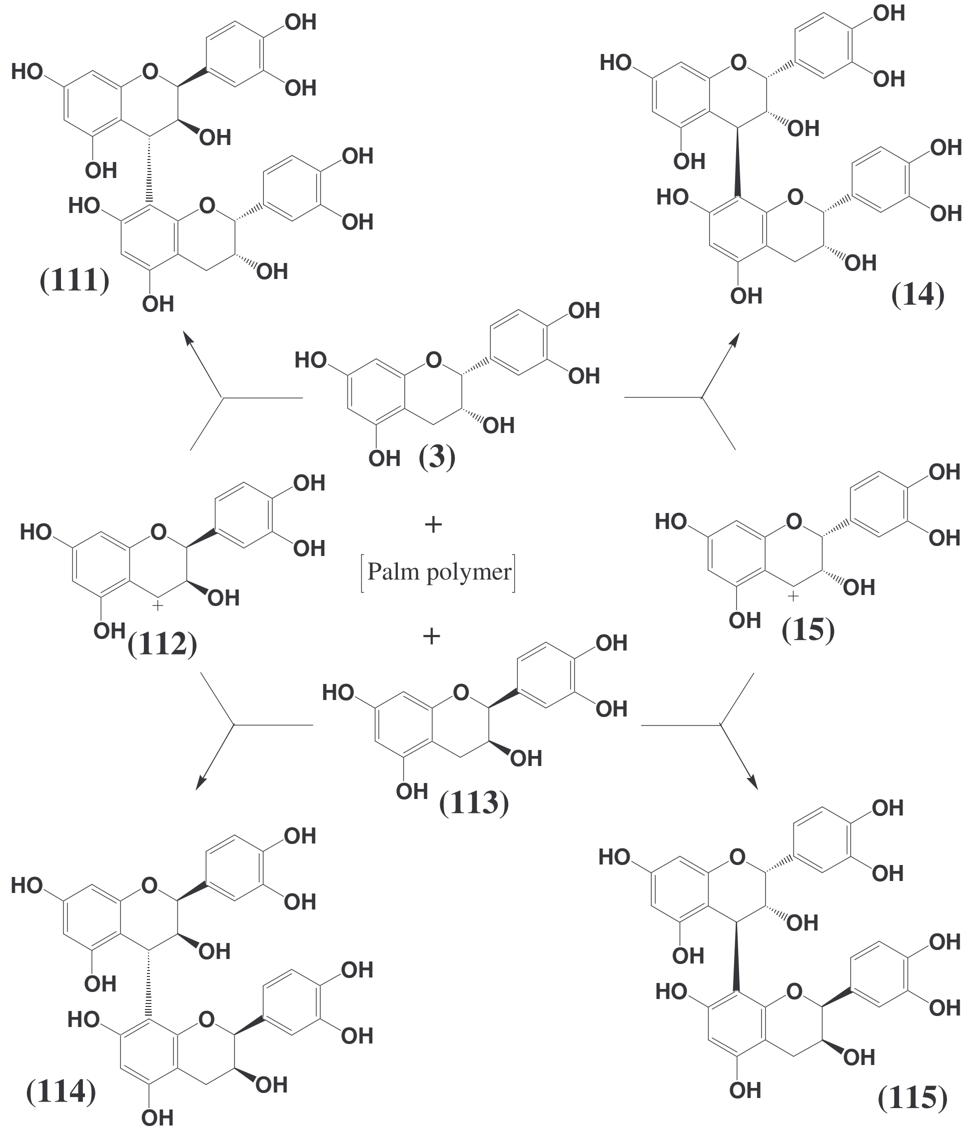

**Figure 3:** 6- and 8-Bromo-tetra-*O*-methylcatechins.

These chemical shifts were used to distinguish between flavanyl [6→4]- and flavanyl-[8→4]-flavan-3-ols and to establish the absolute configuration of the constituent monomers. The primary driving force behind degradation of the C4→C6″ and C4→C8″ linked proanthocyanidins and formation of diagnostic 6- or 8-bromo-flavan-3-ol **(105)** or **(106)** is postulated to be electrophilic attack by $Br^+$ at the $sp^2$ – carbon involved in linkage between flavanoid units (Scheme 27).



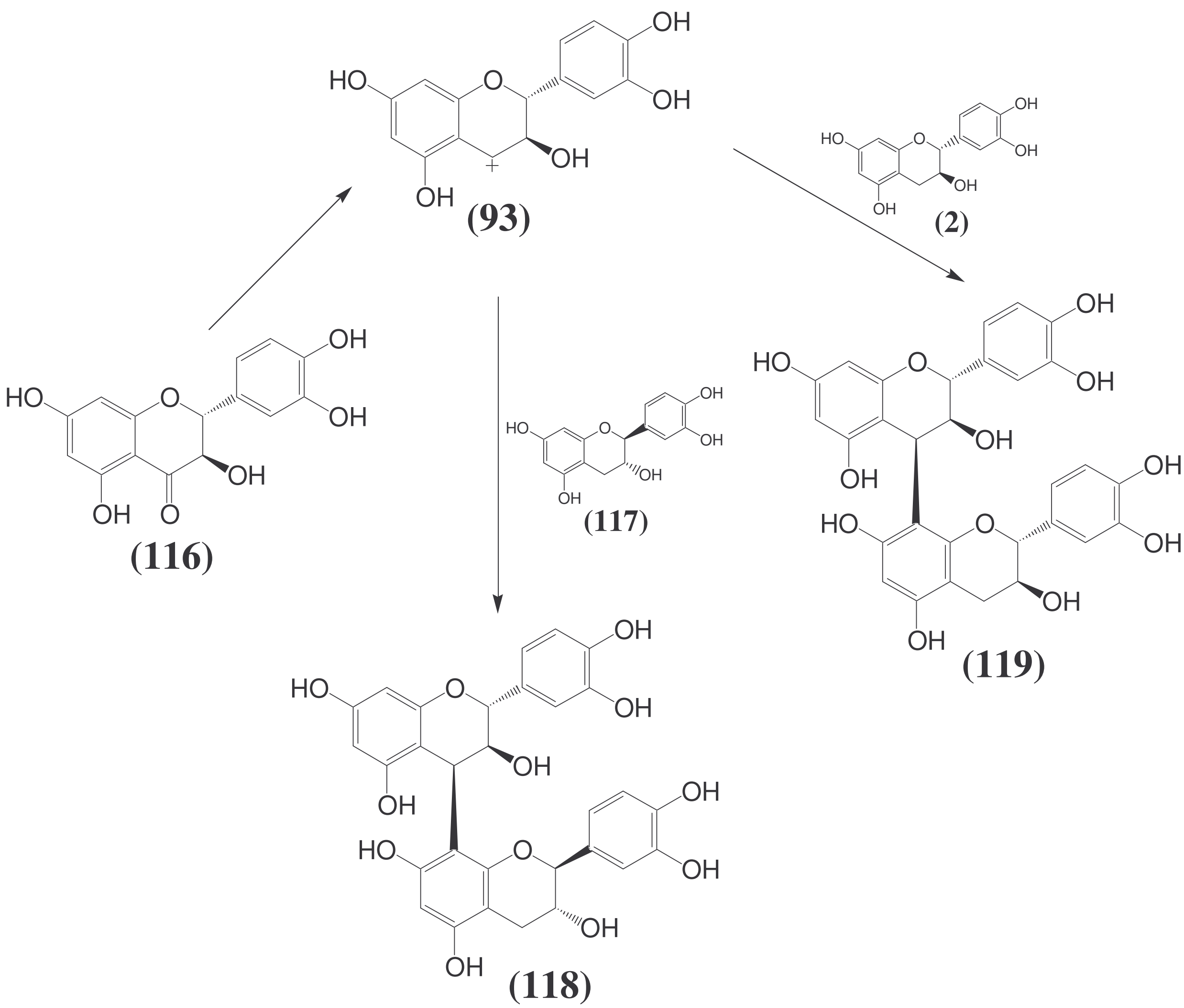

**Scheme 27:** Mechanism for the formation of 8-bromo-tetra-*O*-methylcatechin.

Most proanthocyanidins isolated prior to 1983 possessed units with *(2R)* configuration. Palm polymer (procyanidin polymers from *Phoenix canariensis*) contains 2,3-*cis* polymers with both *(2R)* and *(2S)* configurations.[10,43] Reaction of the palm polymer in mild acid solution with epicatechin **(3)** produced a mixture of procyanidin dimers **(14)** and **(111).** Replacement of epicatechin with *ent*-epicatechin **(113)** yielded **(114)** and **(115)**. The diastereoisomers **(114)** and **(14)**, and **(111)** and **(115)** are enantiomers (Scheme 28).[10] Larger amounts of **(115)** were obtained by treating horse chestnut polymer [containing exclusively (2*R*)-2,3-*cis*-procyanidin units].




**Scheme 28:** Synthesis of 2,3-*cis*-procyanidin diastereoisomers.

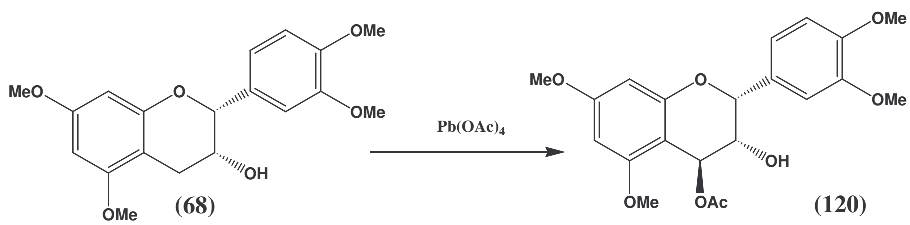
Treatment of 4-carbocation **(93)** (obtained by reduction of taxifolin **(116)** with $NaBH_4$) with catechin **(2)** or ent-catechin **(117)** yielded 2,3-*trans*-procyanidin diastereoisomers **(118)** and **(119)** respectively (Scheme 29).[10]




**Scheme 29:** Synthesis of 2,3-*trans*-procyanidin diastereoisomers.

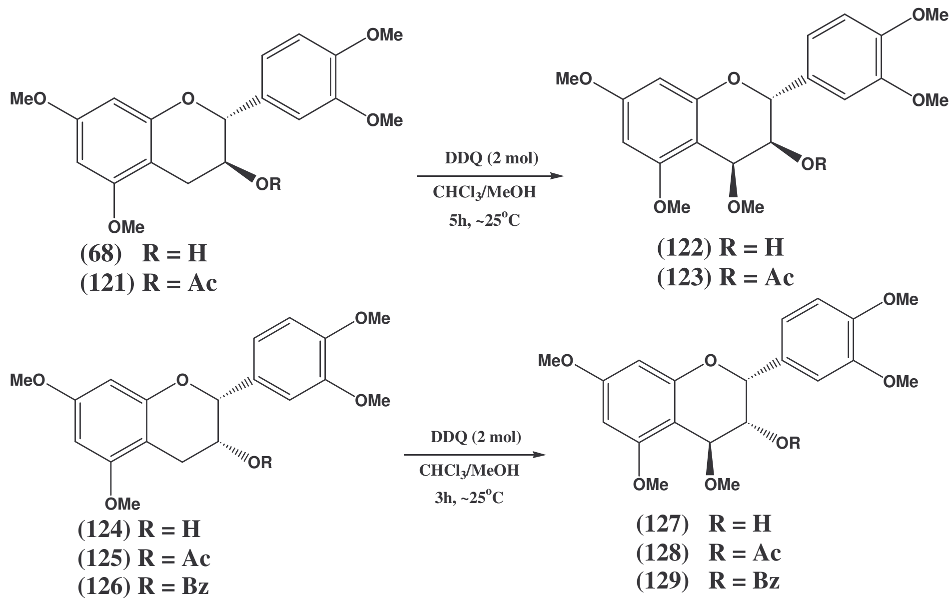
### 2.2.4 Methods based on the introduction of functionality at C-4 of flavan-3-ols (activation of the benzylic position with a good leaving group)

Brown and coworkers[13] reported acyloxylation of the 4-benzylic position of epicatechin tetra-*O*-methyl ether with lead tetra-acetate in benzene (33% yield after 24-28 hours, Scheme 30).



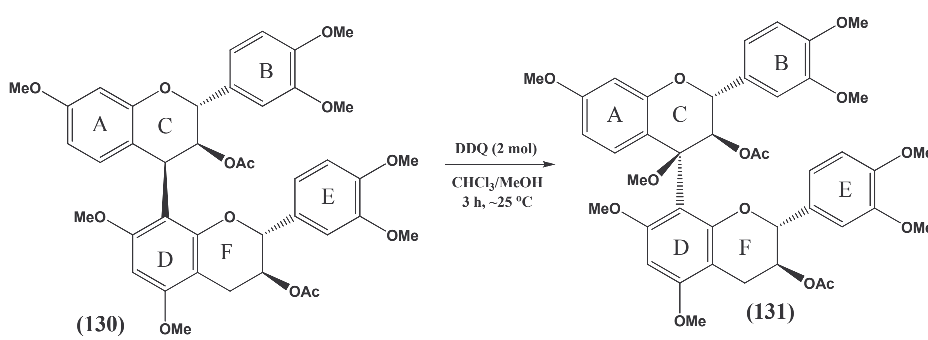

**Scheme 30:** Acyloxylation of the C-4 of epicatechin tetramethyl ether.

Roux and co-workers[14] overcame the long reaction times and low yields by using DDQ. Catechin tetra-*O*-methyl ether **(68)** and its 3-*O*-acetate **(121)** yielded the 3,4-*cis* 4-methyl ether **(122)** and **(123)** in chloroform with MeOH as trapping agent (ca. 50% yield after 5 hours) (Scheme 31). Epicatechin tetra-*O*-methylether **(124)**, its 3-*O*-acetate **(125)** and 3-*O*-benzylate **(126)** yielded the 2,3-*cis*-3,4-*trans* 4-methyl ether **(127)**, **(128)** and **(129)** (c.a 50% yield after 3 hours, Scheme 31). The high degree of stereoselectivity is remarkable. Neighbouring group participation by the 3-oxygen containing moiety does not seem to play a role as **(122)** has 3,4-*cis* configuration and **(127)** has 3,4-*trans* configuration. It is more likely that the 2-aryl moiety plays a role as in both **(122)** and **(127)** the introduced 4-methyl ether is *trans* to this moiety. A double molar excess of DDQ allows hydride ion abstraction to compete with side reactions. Excess DDQ must be neutralized by reduction with $NaBH_4$ immediately after a specific reaction time to avoid anthocyanidin formation.



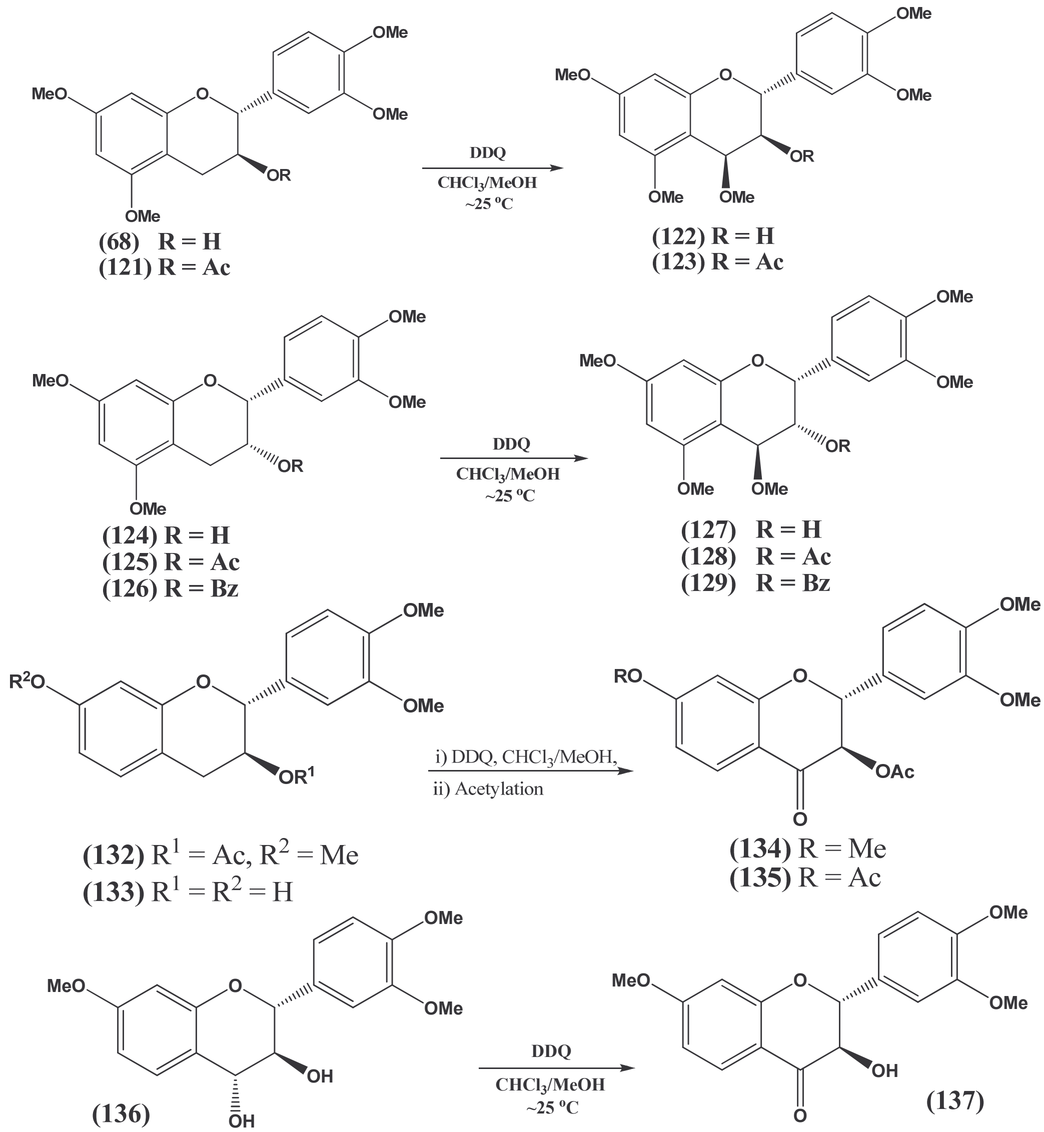

**Scheme 31:** Synthesis of the 4-methyl ether of catechin and epicatechin tetra-*O*-methyl ether.

The dimer **(130)** with two benzylic positions was oxidized selectively with DDQ to the 4-methoxy derivative **(131)** (25% yield after 3 hours) (Scheme 32). Notable is the configurational inversion at C-4. Oxidation of the 2,3-*trans*-3,4-*trans* diastereoisomer (4-α) of **(130)** gave poor yields. This indicates that hydride ion abstraction by DDQ requires that H-4(C) be in the quasi-*equatorial* position. Similar to **(122)** and **(127)** the introduced 4-methyl ether is *trans* to the 2-aryl moiety.



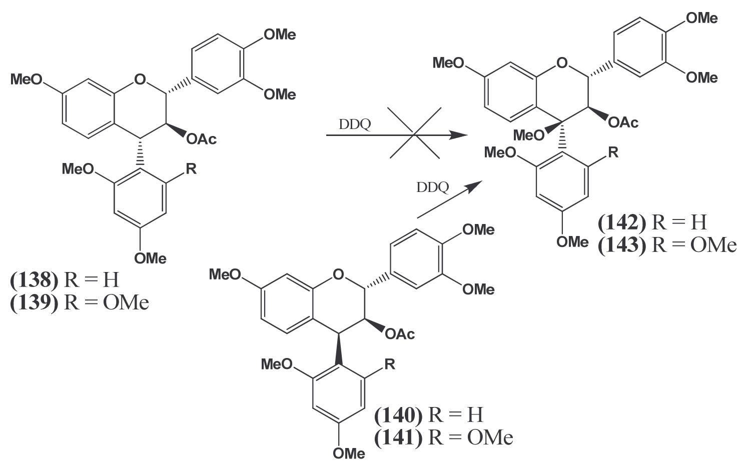

**Scheme 32:** Oxidation of double benzylic position of profisetinidin **(130)** with DDQ.

The development of biomimetic methods to synthesize proanthocyanidins based on flavan-3,4-diol starting materials and the poor availability of these flavan-3,4-diols, particularly diols with C-5 oxygenation, stimulated further research into oxidation of the C-4 position of catechin and epicatechin with DDQ by Ferreira and co-workers[15] (Scheme 33).



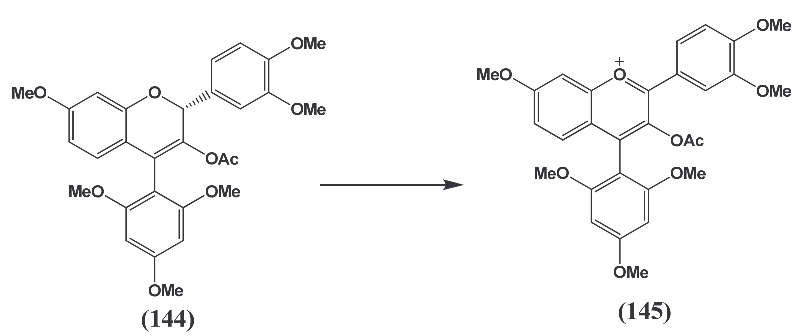
**Scheme 33:** C-4 functionalization of flavan-3-ols.

The high degree of regio- and stereoselectivity was attributed to the ability of DDQ to form charge transfer complexes with aromatic substrates.[44,45] The phloroglucinol-type A-ring has a higher electron density than the pyrocatechol-type B-ring, explaining regioselective oxidation at C-4 and not at C-2. Formation of a deep blue complex with DDQ, which disappears as the reaction progresses, supports the putative formation of a DDQ complex. It was further postulated that the axial H-2 prevents formation of a bulky DDQ complex from the β-face. This would allow selective removal of the pro-*R* diastereotopic benzylic hydrogen as a hydride ion within a tight complex. The incipient carbocation is then attacked by the nucleophile (MeOH) from the opposite side (i.e. in an $S_N2$ fashion) to give exclusively 2,4-*trans* products. During oxidation of 4α- and 4β-arylflavan-3-ols **(138)**, **(139), (140)** and **(141),** only the 4β-isomers are oxygenated at the 4-position [to give **(142)** and **(143)**] as only in these isomers are the axial H-2 proton opposite to the 4-aryl substituent (the DDQ on the same side as the single remaining H-4) (Scheme 34).



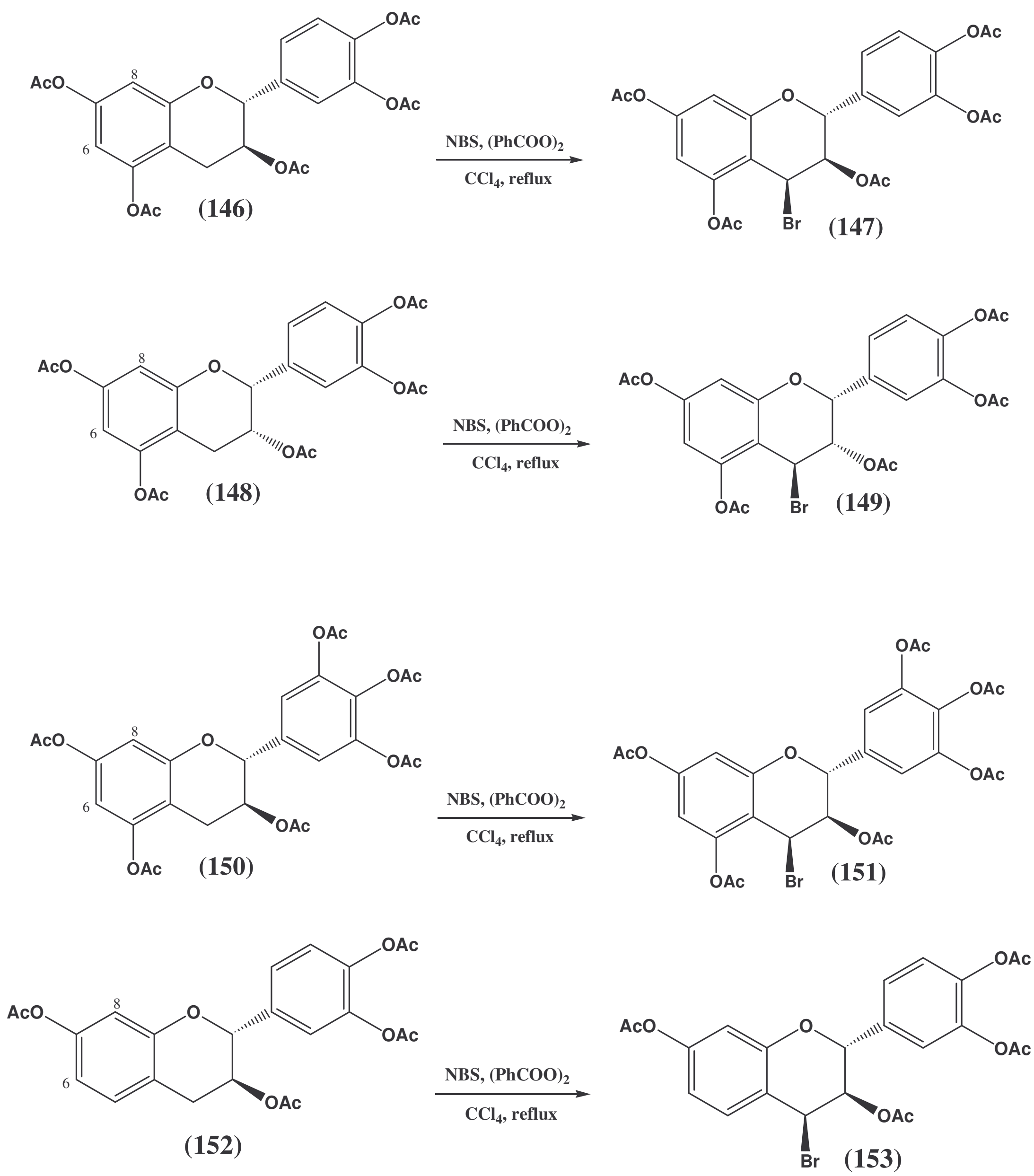

**Scheme 34:** Oxidation of 4α- and 4β-arylflavan-3-ols with DDQ.

The 4β-methoxy derivative **(143)** was accompanied by flav-3-en-3-ol derivative **(144)**, presumably the first step towards the formation of an anthocyanidin **(145)** (Scheme 35), which explains the considerable degree of reddening observed in the oxidations.



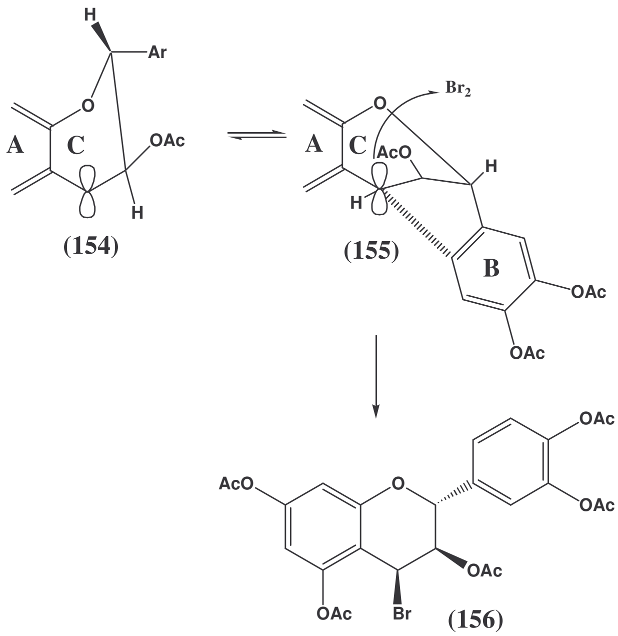

**Scheme 35:** Formation of anthocyanidin derivative.

Ferreira and coworkers[16] brominated the peracetates of catechin **(146)**, epicatechin **(148)**, robinetinidol **(150)** and fisetinidol **(152)** at the benzylic position (4-carbon) with NBS in the presence of benzoyl peroxide in refluxing $CCl_4$ (Scheme 36).




**Scheme 36:** Bromination at C-4 of peracetates of flavanoids.

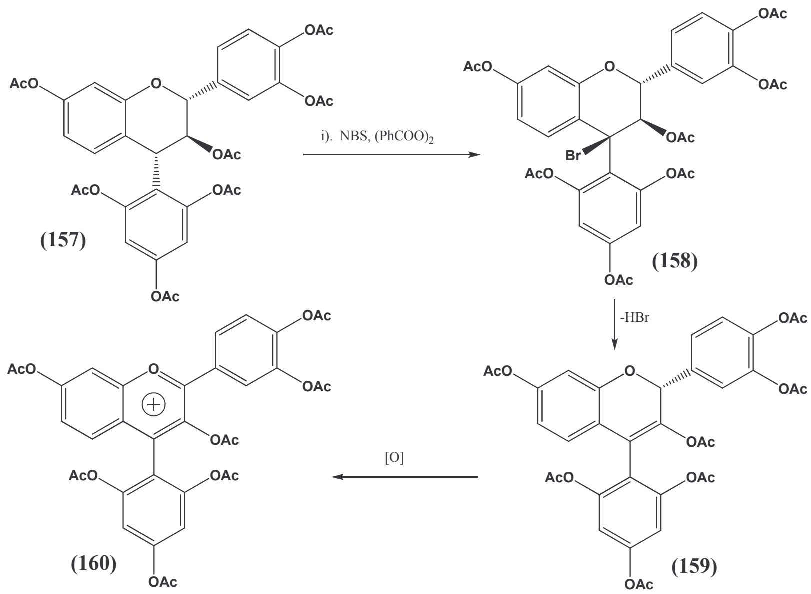
Exclusive formation of 4β-bromo analogues was rationalized by stereoselective abstraction of the β-face diastereotopic methylene hydrogen on C-4. This was attributed to neighbouring group participation of the π-system of the B-ring at C-2 *via* the non-preferred conformation **(155)** (Scheme 37).




**Scheme 37:** Neighbouring group participation of the π-system of the B-ring.

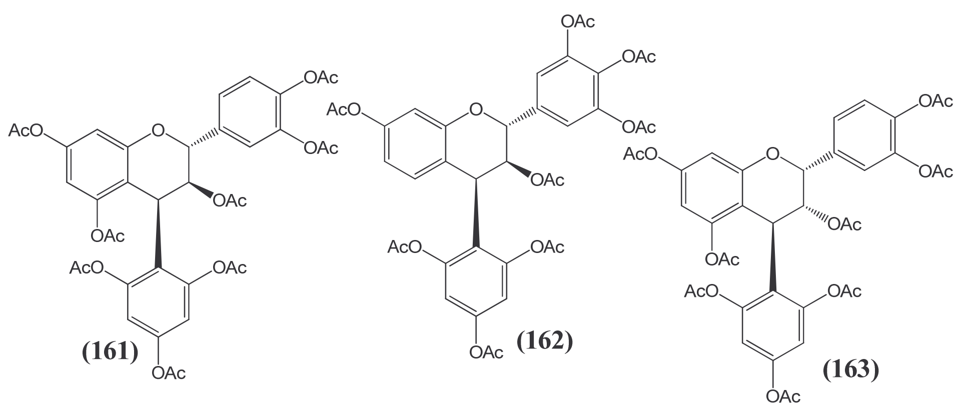
B-ring involvement is substantiated by the increased rate and yield with which (-)-robinetinidol, with an electron-rich tri-oxygenated type B-ring, were brominated. The stereoselectivity of C-4 bromination is paralleled by previous observations of substitution at C-4, which invariably afforded 4β-products.[46] The rate of bromination of peracetates with tri-oxygenated phloroglucinol-type A-rings **(146)** and **(148)** was enhanced compared to **(152)** with a di-oxygenated resorcinol-type A-ring presumably due to stabilization of the benzylic radical by the A-ring.

Bromination of 4-arylflavan-3-ol **(157)** leads to the disappearance of the starting material and the appearance of a deep red colour. It was postulated that the highly activated double benzylic C-4-bromo derivative **(158)** undergoes rapid HBr elimination to give the flav-3-ene derivative that was rapidly oxidized under the reaction conditions to the anthocyanidin **(160)** (Scheme 38).



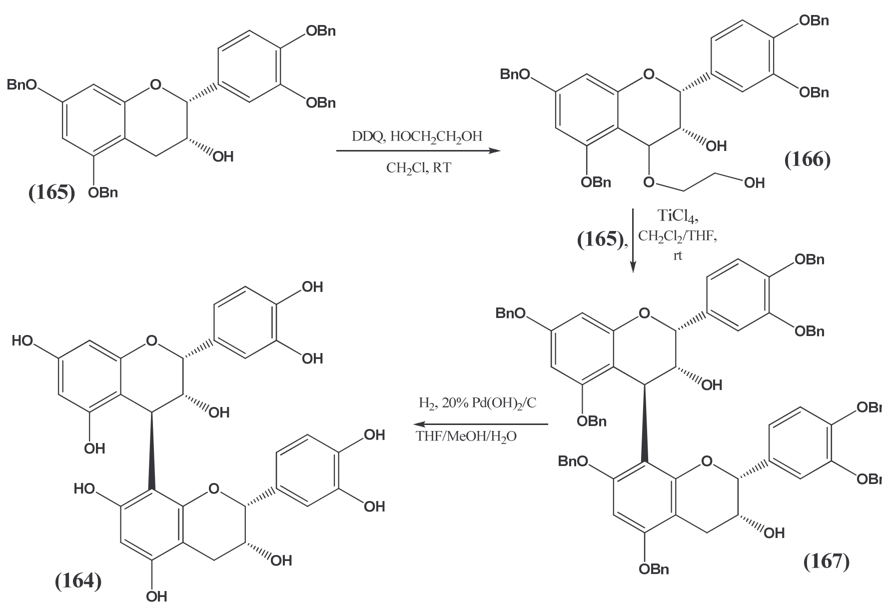

**Scheme 38:** HBr elimination and formation of anthocyanidin.

The brominated peracetates **(147), (149)** and **(153)** were used to synthesize the 4-arylflavan 3-acetates **(161), (162)** and **(163)** (Scheme 39).



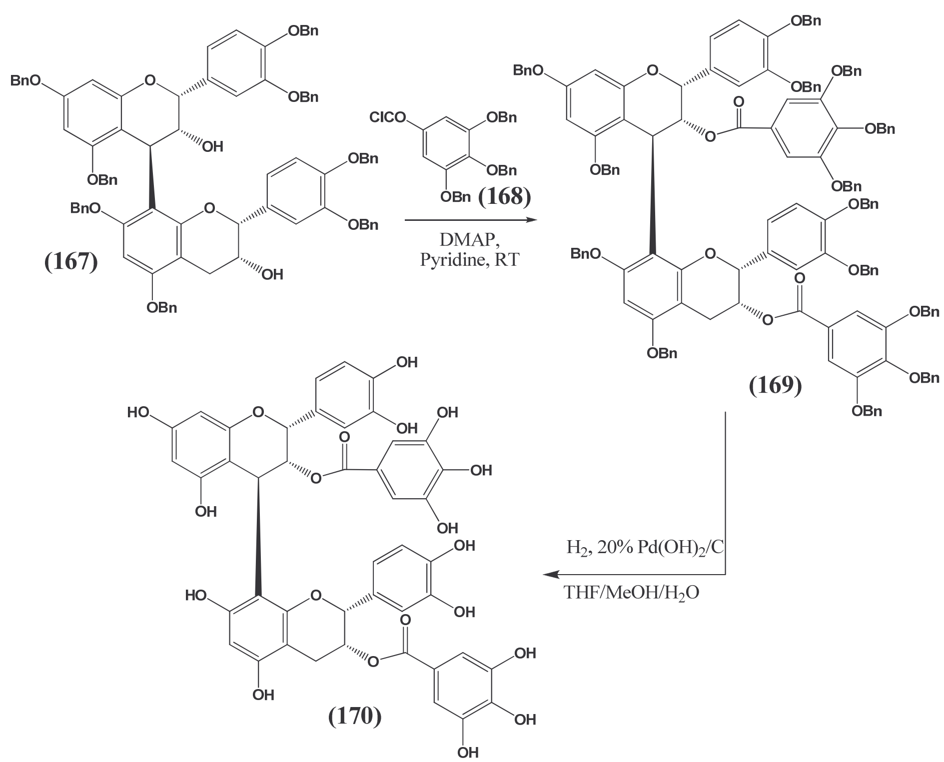

**Scheme 39:** Synthesis of 4-arylflavan-3-*O*-acetates *via* brominated peracetates.

Tückmantel and coworkers[18] prepared procyanidin B-2 **(164)** according to the procedure in Scheme 40. Ethylene glycol in $CH_2Cl_2$ was used as nucleophile during the oxidation of **(165)** with DDQ and $TiCl_4$ was used as Lewis acid in the subsequent condensation step.




**Scheme 40:** Condensation of **(166)** and tetra-*O*-benzylflavan-3-ol **(165).**

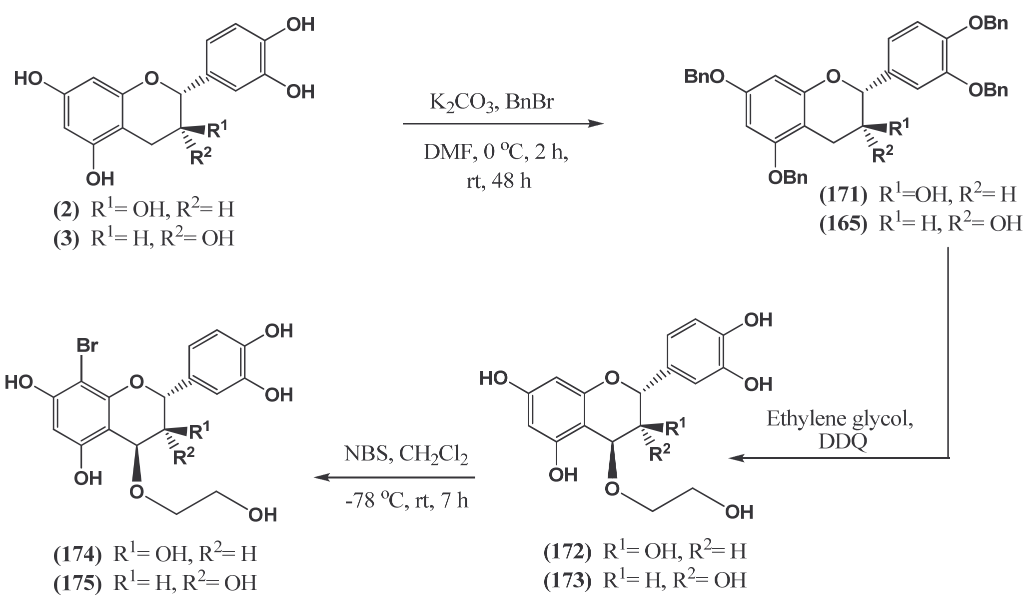
They also prepared the bisgallate **(170)** in good yield (Scheme 41).




**Scheme 41:** Synthesis of 3-*O*-galloylepicatechin-(4β→8)-3-*O*-galloylepicatechin.

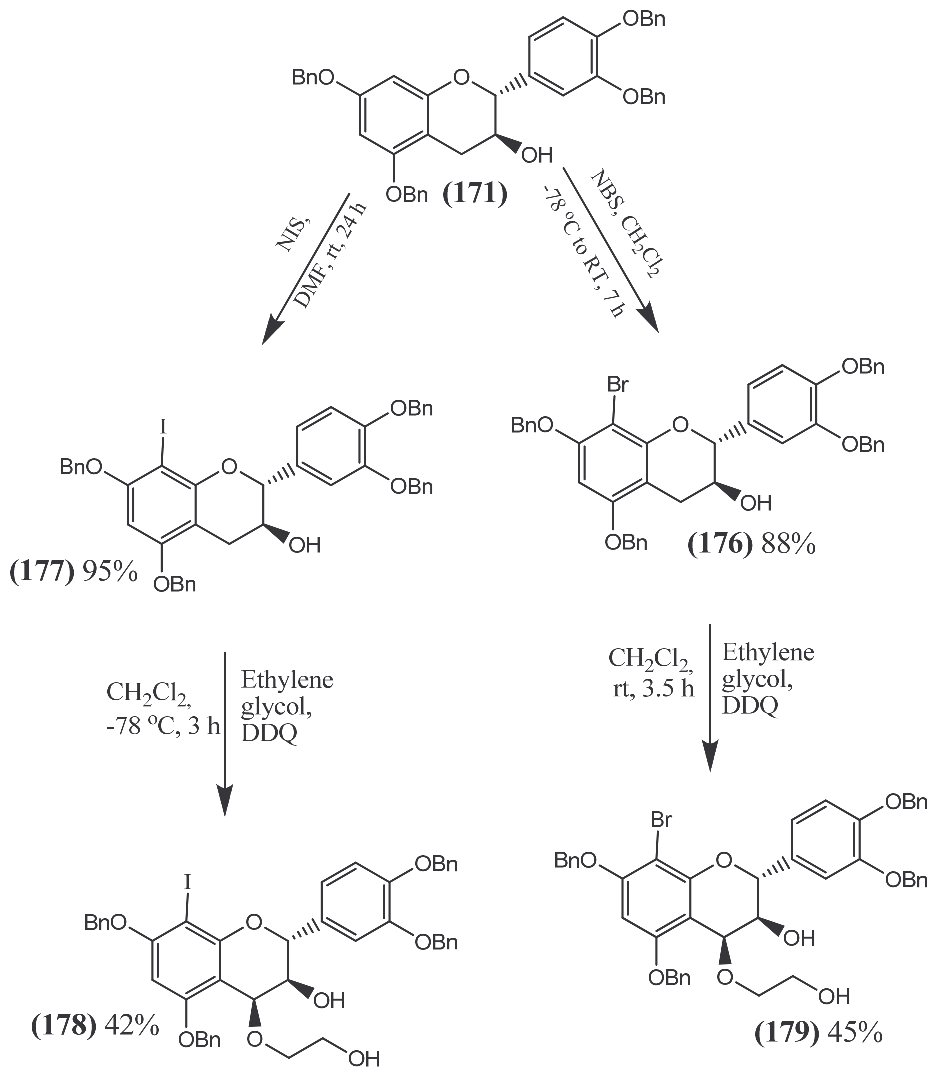
As there is little electronic difference between C-6 and C-8 on the A ring of **(165)**, the exclusive formation of 8-coupled dimers was attributed to steric influences. The 8-position is favoured because the adjacent alkoxy group in the pyran C-ring is tied back compared to the flexible benzyloxy groups at C-5 and C-7. This explanation is supported by the observance of coupling at both the 6- and 8- position at elevated temperatures when benzyl protection is replaced by methyl protecting groups. The stereoselective oxidation at C-4 supported a suggestion that the approach of DDQ was controlled by the configuration at C-2 and occurs at the bottom face of the C-ring to avoid interaction with the axial $H_2$ atom.[18]

Bromination of the 8-position is commonly used to prevent self condensation of electrophilic units and uncontrolled polymerization. This is normally done with NBS in $CH_2Cl_2$ after oxidative functionalization of the 4-position (Scheme 42).[17]



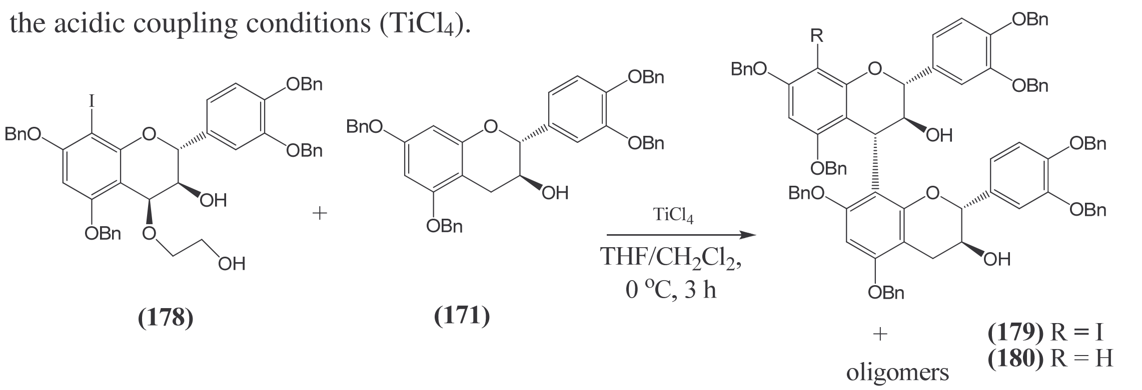

**Scheme 42:** Bromination at C-8 of catechin and epicatechin.

Kiehlman and coworkers[47] attempted bromination or iodination before oxidative functionalization of the 4-position and obtained significantly reduced yields (Scheme 43). This was attributed to a lack of reactivity of the charge-transfer complex formed between DDQ and the halogenated A-rings of **(176)** and **(177)** as the substrates.




**Scheme 43:** Bromination or iodination before oxidative functionalization at C-4.

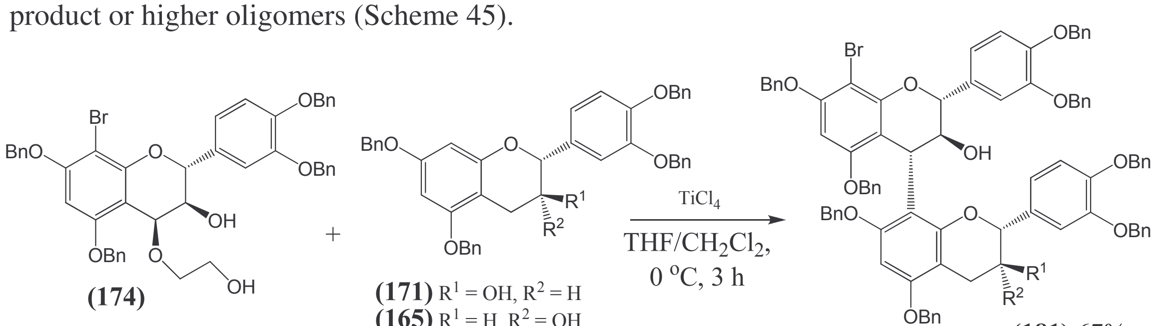
Stereoselectivity at C-4 of catechin is enhanced by replacing the C-4 hydroxy group by a methoxy or other alkoxy groups including the hydroxyethoxy group. All these methods require a large excess (5-10 equivalents) to avoid self condensation. Efforts by Barathieu and co-workers[17,48] to couple with equimolecular amounts of **(178)** and **(171)** (Scheme 44) gave the expected dimers **(179)** and **(180)** (42%) accompanied by some trimeric (17%), tetrameric (6%), and pentameric (3%) oligomers. The presence of equimolecular amounts of iodinated **(179)** and non-iodinated product **(180)** indicated that the carbon-iodine bond cannot survive the acidic coupling conditions ($TiCl_4$).




**(178)** **(171)** **(179)** R = I
**(180)** R = H

**Scheme 44:** Coupling reaction with 8-iodoflavan-3,4-dioxy derivative **(178)**.

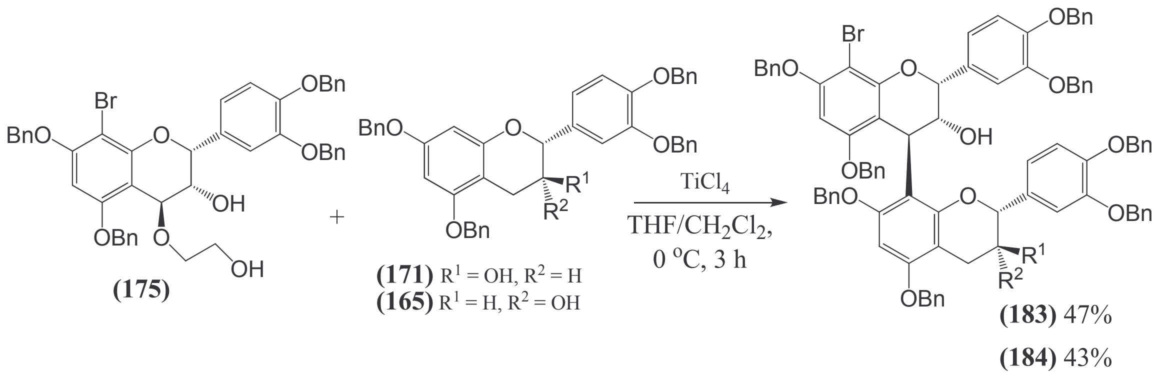
Repetition of the reaction with the brominated analogue **(174)** (which was thought to be resistant to acidic conditions) gave the procyanidin dimers B-3 **(181)** and B-4 **(182)** with inversion of configuration at the attacked C-4 carbon and with no trace of debrominated product or higher oligomers (Scheme 45).




**(174)** **(171)** $R^1 = OH, R^2 = H$
**(165)** $R^1 = H, R^2 = OH$
**(181)** 67%
**(182)** 53%

**Scheme 45:** $TiCl_4$-mediated coupling procedure with bromide **(174)**.

Starting with **(175)**, the procyanidin dimers B-1 **(183)** and B-2 **(184)** were obtained with complete retention of configuration at C-4 (Scheme 46).

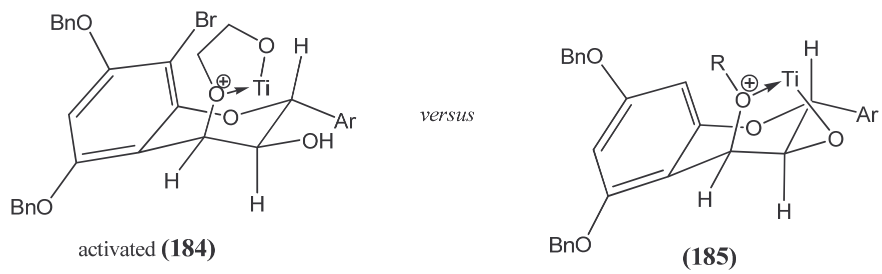



**Scheme 46:** $TiCl_4$-mediated coupling procedure with bromide **(175)**.

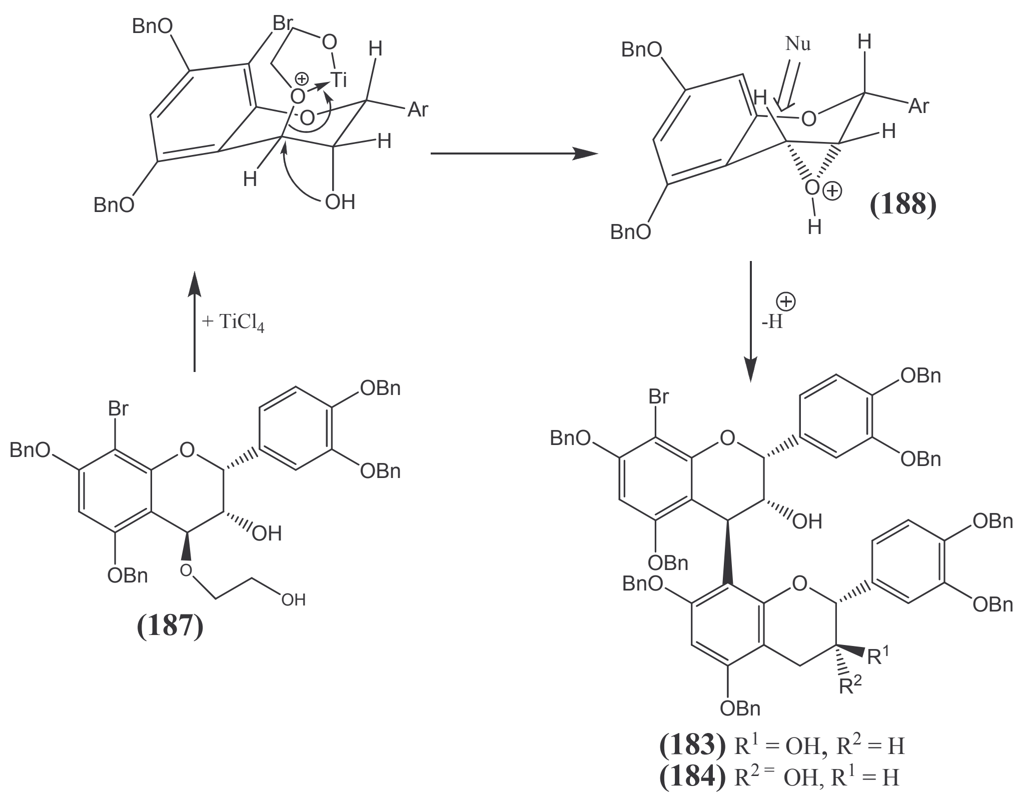
Pearlman's catalyst[49] [$Pd(OH)_2$ / C (20% Pd on carbon)] was found to be more efficient than Pd/C and $H_2$ in a one pot procedure for hydrodebromination and removal of benzyl groups to yield B-1, B-2, B-3 and B-4 free phenolic procyanidin dimers. Triethylamine was required as an additive. It reduces the catalyst *in situ* to $Pd^0$ and prevents degradation of the free phenolic procyanidin dimers by trapping the generated hydrobromic acid.[17]

Reversal of the stereoselectivity at C-4 observed by replacing **(174)** with its epimer **(175)** indicates that the configuration at the C-3 carbon controls stereoselectivity (Scheme 46). The C-3 hydroxy group is not engaged in the coordination sphere of the titanium, and is available to provide anchimeric assistance as a result of the axial-axial position of the neighbouring groups.

Vercauteren and Kawamoto and their coworkers,[50,51] however, observed that reactions carried out under similar conditions and with the same Lewis acid, led only to partial stereocontrol depending on the C-4 leaving group (3:2 ratio with hydroxy leaving group and 2:1 ratio with methoxy leaving group). In view of the bidentate nature of the hydroxy-ethoxy substituent and the preferential bis-chelated state of $TiCl_4$, the origin of total stereoinduction was rationalized by the activated complexes **(185)** and **(187)**. In the case of **(187)**, the transient protonated epoxide **(188)** explained retention of configuration in compounds **(183)** and **(184)** (Scheme 47 and 48).

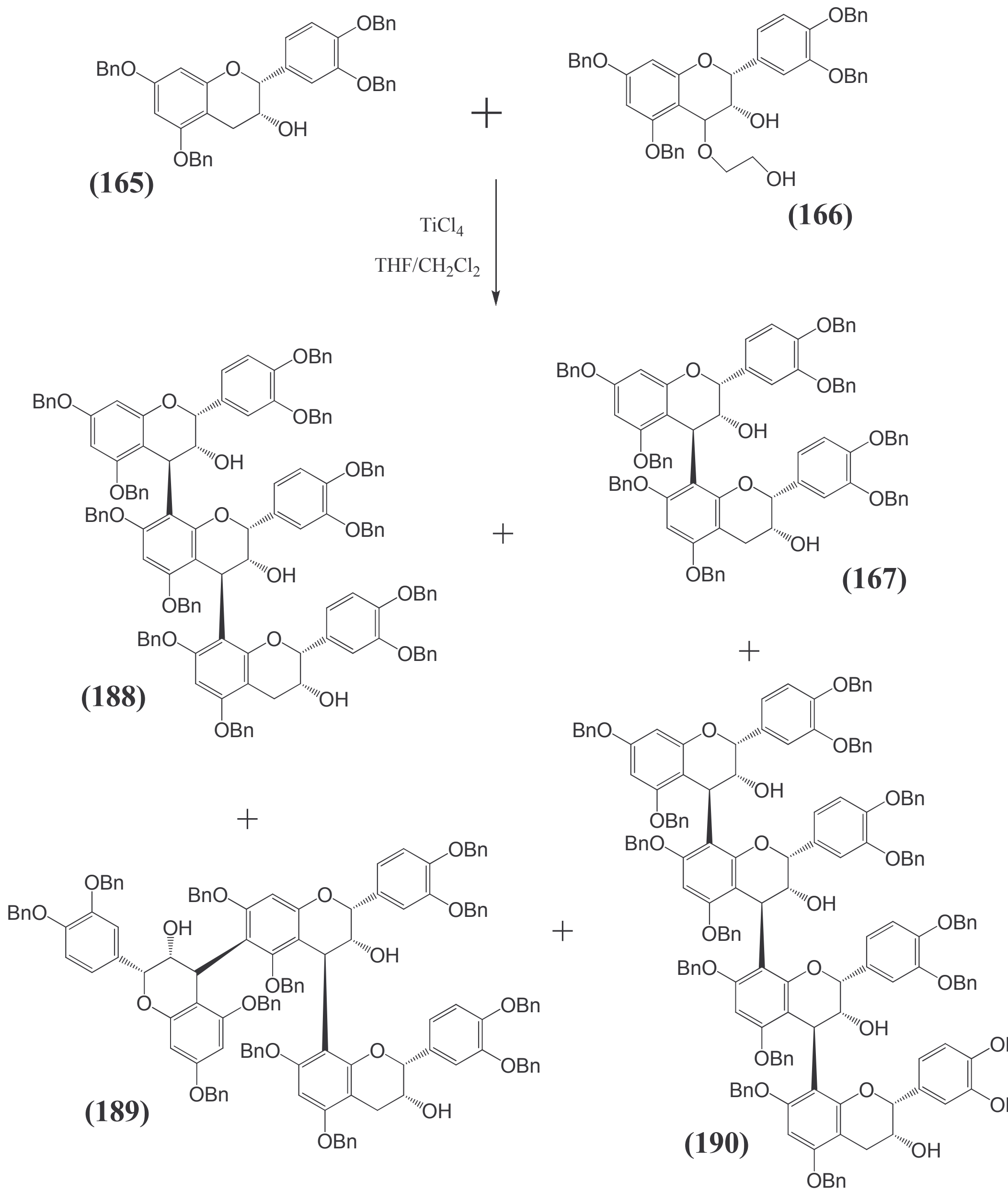



**Scheme 47:** Comparison of mono- and bidentate activating groups.




**Scheme 48:** Nucleophilic substitution mechanism with **(187)**.

Kozikowski and co-workers[52] synthesized trimeric, tetrameric, pentameric, and higher oligomeric epicatechin derived procyanidins with (4β→8)-interflavan connectivity. Condensation of **(165)** and **(166)** with $TiCl_4$ gave **(167), (188)**, **(189)**, **(190)** and trace amounts of higher oligomers (Scheme 49).



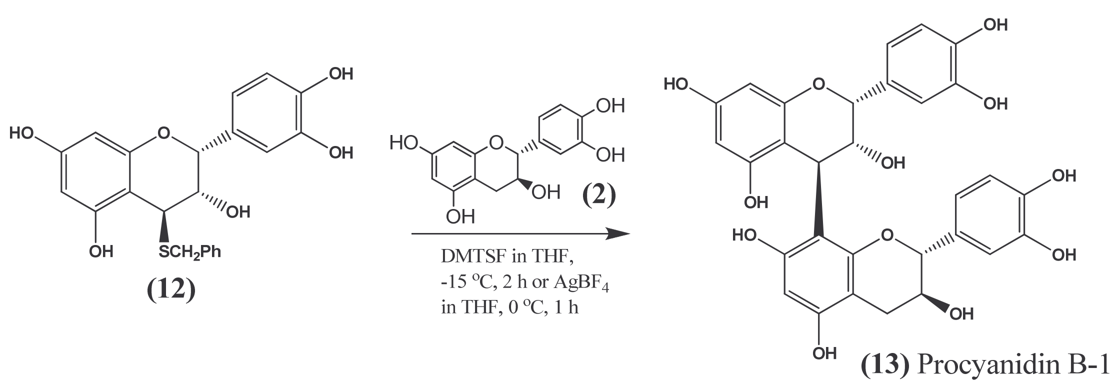

**Scheme 49:** $TiCl_4$-mediated condensation of epicatechin and C-4 functionalized epicatechin derivatives.

The same products were obtained starting from the dimer **(167)** and condensing it with the electrophile **(166)**. Condensation of the trimer **(188)** with **(166)** gave not only the tetramer, pentamer, and small amounts of higher oligomers, but also degradation of the trimer to the monomer and dimer, giving rise to regioisomeric oligomers such as **(189).** This degradation and consequent formation of "branched" trimer **(189)** indicated that a different approach to oligomerisation was required.

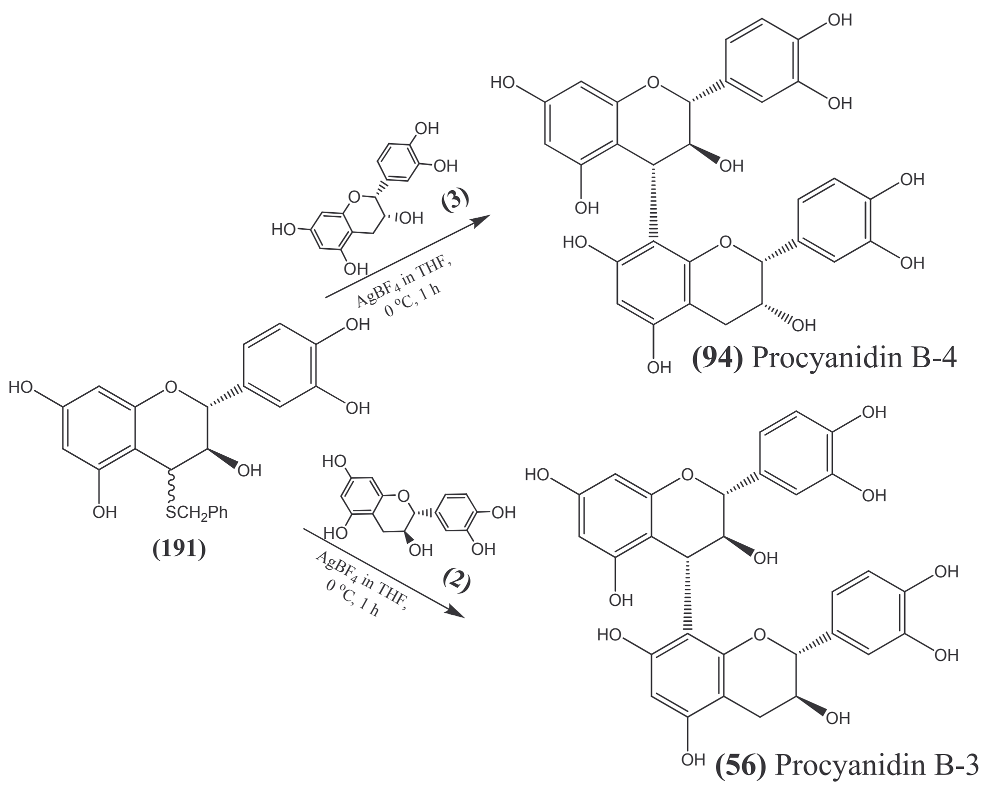
Stirring of **(165)** (4 equivalents) and **(166)** in $CH_2Cl_2$ with commercial acidic clay (Bentonite K-10) gave the dimer **(167)** in 90% isolated yield and some trimer **(188)**. A high reactivity differential between monomer **(165)** and dimer **(167)** presumably discourages the dimer from entering into further chain extension condensations. Reaction of the dimer **(167)** with the electrophile (**166)** yielded only 40% trimer and tetramer formation was reduced to such an extent that oligomer synthesis *via* the bentonite protocol was considered impractical.

Romanczyk and coworkers[53] scaled up the synthesis of epicatechin-(4β→8)-epicatechin **(14)** and its digallate **(170)** to kilogram scale through the manipulation of the chemistry described earlier by Kozikowski and co-workers.[7,18,52] Their improved debenzylation procedure, utilizing a biphasic system that permits the debenzylated compounds to go into the water phase, constitutes an important contribution. Debenzylation without disproportionation of the interflavanyl bond is not a trivial matter.[54]

Ferreira and coworkers[38] described a chain extension protocol based on the activation of the C4-S bond in the 4-thiobenzylcatechin **(191)** and 4-thiobenzylepicatechin **(12)** with thiophilic Lewis acids, dimethyl(methylthio)-sulfonium tetrafluoroborate (DMTSF) or silver tetrafluoroborate ($AgBF_4$) towards nucleophiles, to generate interflavanyl bonds under neutral conditions. They synthesised procyanidin B-1 **(13)** (22% yield), B-4 **(94)** (35%) and B-3 **(56)** (51%) without the need for protection of the phenolic hydroxy groups (Scheme 50 and 51). This procedure avoided acidic or basic conditions which break the labile interflavanyl bond and lead to an equilibrium between substrates and products, hampering progressive construction of procyanidin oligomers. Problems were experienced due to the moisture sensitivity of DMTSF and $AgBF_4$. The coupling protocol using DMTSF compares favourably with the classical acid-catalysed condensation of catechin-4α-ol which gave a mixture of procyanidin, trimer, including its 4→6 regioisomer, and tetraflavanoid analogues. However, $AgBF_4$ gave overall better results including control of the level of oligomerization. The free phenolic 4β-benzenesulfanylepicatechin **(12)** was available *via* thiolysis of Loblolly pine purified tannin.[36,39,55,56] The 4-benzylsulfanylcatechin **(191)** was obtained as a mixture of 4β- and 4α-epimers (4:1) *via* reduction of 2,3-*trans*-dihydroquercetin with $NaBH_4$ in ethanol[24] and acid catalyzed thiolysis of the unstable catechin-4-ol intermediate with phenylmethanethiol acetic acid. Both the 4β- and 4α-benzenesulfanylcatechin epimers **(191)** gave the same intermediate transient C-4 carbocation and follows the same stereochemistry during interflavanyl coupling to yield 3,4-*trans* dimer and oligomers.

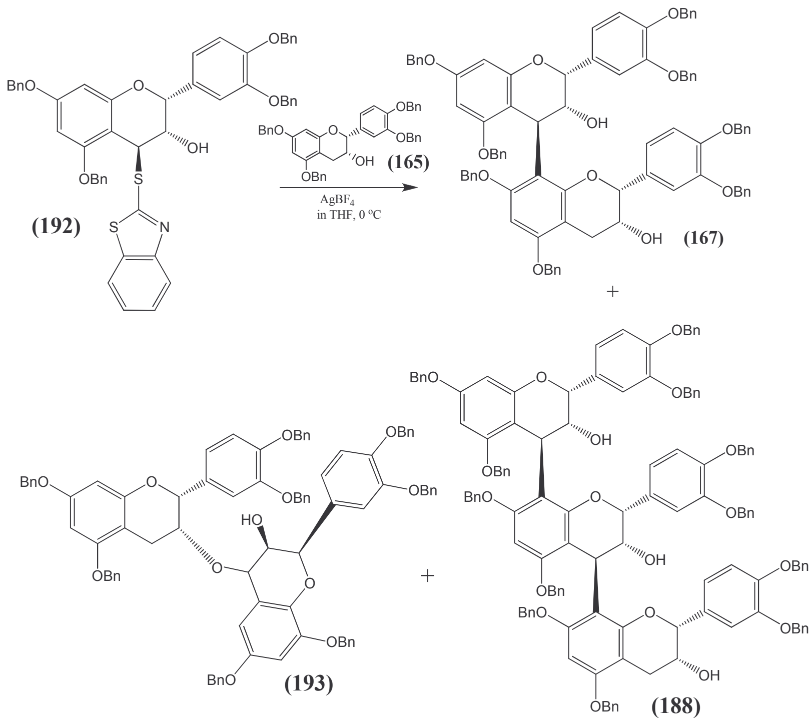



**Scheme 50:** Procyanidin synthesis using Lewis acid activation of **(12)**.



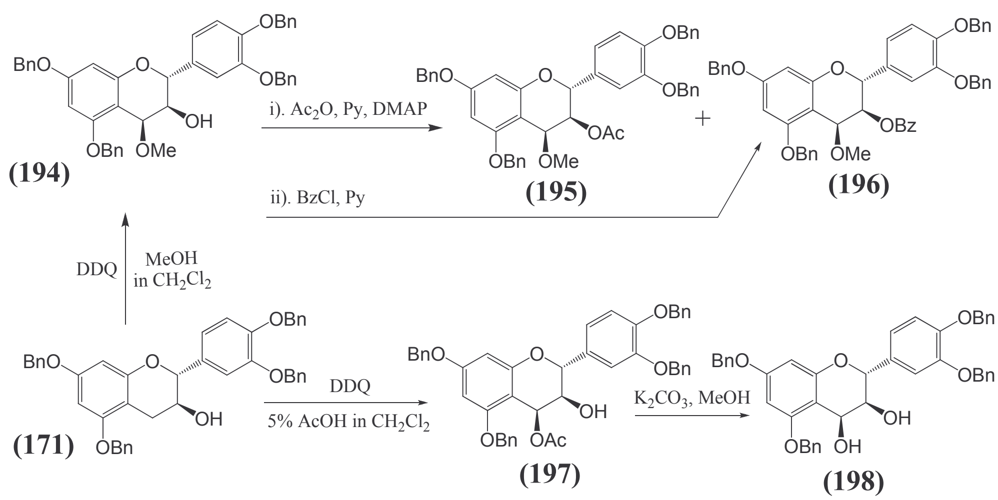
**Scheme 51:** Procyanidin synthesis using Lewis acid activation of **(191)**.

Kozikowski and co-workers[52] reinvestigated the potential of 4-thioderivatives of catechin and epicatechin. They chose to work with benzyl protected starting materials due to the ease of handling (including chromatography) and better stability compared to unprotected phenolic compounds and the poor accessibility of non-protected 4-substituted epicatechins. They also decided to avoid using benzyl mercaptan analogues due to their noxious nature. They were replaced with a nonvolatile, odourless heterocyclic thiol, 2 mercaptobenzothiazole analogues **(192).** Addition of $AgBF_4$ to a solution of **(165)** and **(192)** in THF resulted in the formation of **(167)** (56%), **(188)** (14%) and a 3-*O*-4-dimer **(193)** (Scheme 52).



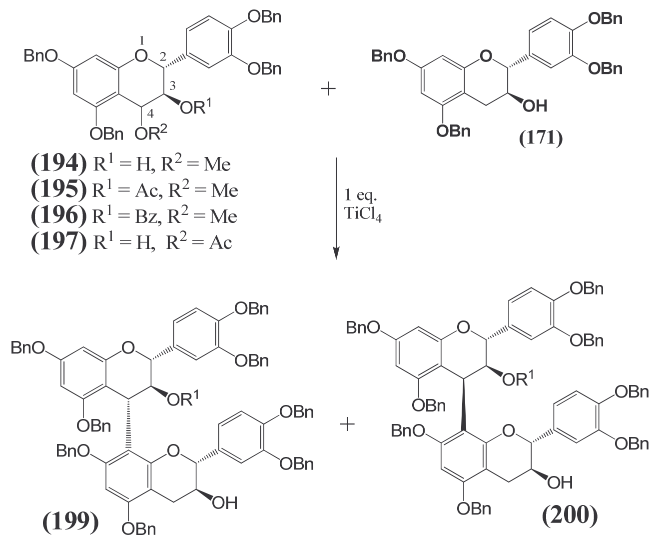
**Scheme 52:** Synthesis with 4-{(2-benzothiazolyl)thio} derivative.

Saito and co-workers[57] obtained the 4-methoxycatechin derivative **(194)** by oxidizing tetra-*O*-benzylcatechin **(171)** with DDQ in $CH_2Cl_2$ in the presence of 10% MeOH. Acetylation and benzoylation of **(194)** gave acetate **(195)** and benzoate **(196)**, respectively. Treatment of **(171)** with DDQ in the presence of 5% acetic acid in $CH_2Cl_2$ afforded 4-acetoxycatechin **(197).** Hydrolysis of **(197)** yielded the flavan-3,4-diol **(198)** quantitatively (Scheme 53).



**Scheme 53:** Synthesis of C-4 functionalized electrophiles.

Condensation of **(194)** or **(197)** with **(171)**, using $TiCl_4$ as Lewis acid, yielded a benzylated procyanidin B-3 (4α) **(199)** as the major product and a 4β-isomer **(200)** as minor product (Scheme 54 and Table 1). Hydrogenolysis with $Pd(OH)_2$ and purification with Sephadex LH-20 yielded pure free phenolic procyanidin B-3 **(56)**.

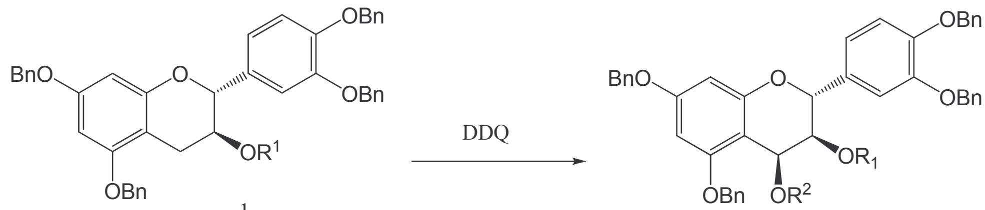


**Scheme 54:** Stereoselective condensation of the electrophiles with **(171)**.

**Table 1:** Stereoselective condensation of the electrophiles with **(170)**.[57]

| Entry | Electrophile No. | $R^1$ | $R^2$ | Solvent | $TiCl_4$ Equiv. | Temp. (°C) | Coupling yield (%) | Selectivity **200**(4α):**201**(4β) |
|---|---|---|---|---|---|---|---|---|
| 1 | **198** | H | H | $CH_2Cl_2$ | 1 | 0 | 80 | 1.5:1 |
| 2 | **194** | H | Me | $CH_2Cl_2$ | 1 | 0 | 74 | 2.0:1 |
| 3 | **195** | Ac | Me | $CH_2Cl_2$ | 1 | 0 | 81 | 7.4:1 |
| 4 | **195** | Ac | Me | $CH_2Cl_2$ | 1 | -20 | 83 | 23:1 |
| 5 | **195** | Ac | Me | $CH_2Cl_2$ | 1 | -40 | 35 | 55:1 |
| 6 | **195** | Ac | Me | $CH_2Cl_2$ | 2 | -40 | 64 | 9.1:1 |
| 7 | **195** | Ac | Me | Toluene | 1 | 0 | 0 | - |
| 8 | **195** | Ac | Me | THF | 1 | 0 | 0 | - |
| 9 | **196** | Bz | Me | $CH_2Cl_2$ | 1 | 0 | 70 | 20:1 |

Saito and coworkers[58] investigated oxidation of **(171)** in five different alcohols. In *tert*-butyl alcohol, 2,4-bis oxidation occurred in preference to mono oxidation. The highest yield was obtained in 2-ethoxyethanol (98%). The yield in ethylene glycol was 74%. DDQ oxidation of 3-acetylated catechin **(202)** gave similar yields but at a slower rate (Scheme 55, Table 2).




**(171)**: $R^1 = H$
**(202)**: $R^1 = Ac$

**(203 - 208)**

**Scheme 55:** DDQ oxidation of **(171)** and **(202)**.

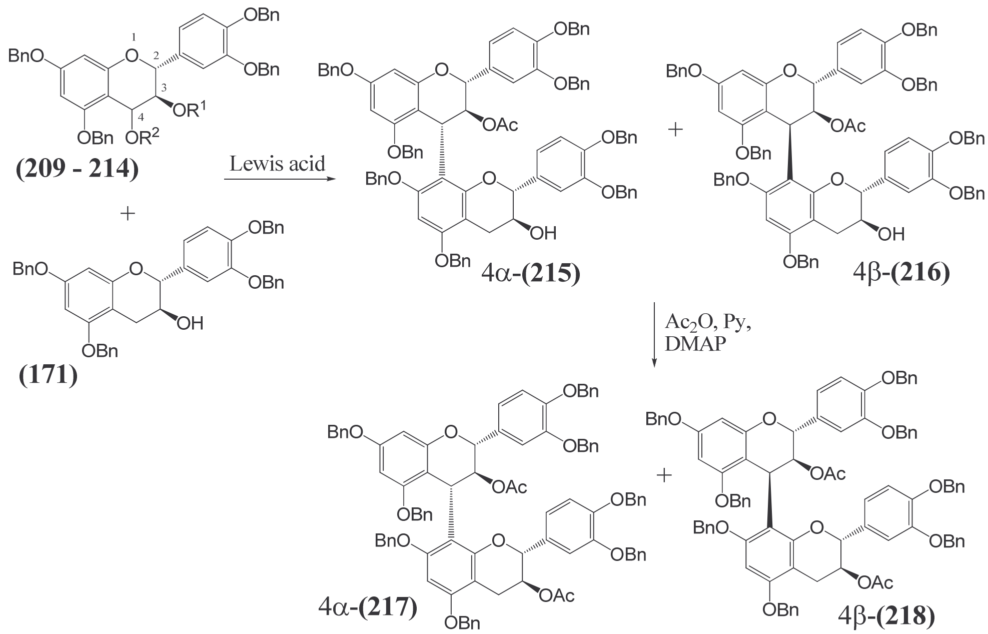
**Table 2:** DDQ oxidation of **(171)** and **(202)**.[58]

| Entry | Nucleophile | Catechin $R^1$ | Time (h) | Products $R^2$ | Products No. | Isolation yield (%) |
|---|---|---|---|---|---|---|
| 1 | Methanol | **171** | 4 | $CH_3$ | **203** | 52 |
| 2 | Isopropyl alcohol | **171** | 8 | $CH(CH_3)_2$ | **204** | 58 |
| 3 | Ethylene glycol | **171** | 6 | $CH_2CH_2OH$ | **205** | 74 |
| 4 | Ethylene glycol | **202** | 12 | $CH_2CH_2OH$ | **206** | 70 |
| 5 | 2-Ethoxyethanol | **171** | 2 | $CH_2CH_2OCH_2CH_3$ | **207** | 98 |
| 6 | Benzyl alcohol | **171** | 12 | $CH_2Ph$ | **208** | 86 |

The results of oxidation studies with benzylated 4-*O*-alkyl derivatives **(203)** to **(208)** and tetra-*O*-benzylcatechin **(171)** with a variety of Lewis acids are given in Table 3. Neighbouring group participation of the 3-*O*-acetyl moiety ($R_3$ = Ac) improved selectivity (enhances the 4α/4β ratio). The highest yield (100%) and selectivity (4α/4β ratio of 32/1) was achieved with TMSOTf as catalyst at -78 °C. They achieved gram scale stereoselective synthesis of protected procyanidin B-3 (Scheme 56).

**Table 3.** Condensation studies of catechin electrophiles and benzylated catechin in the presence of Lewis acid.[58]

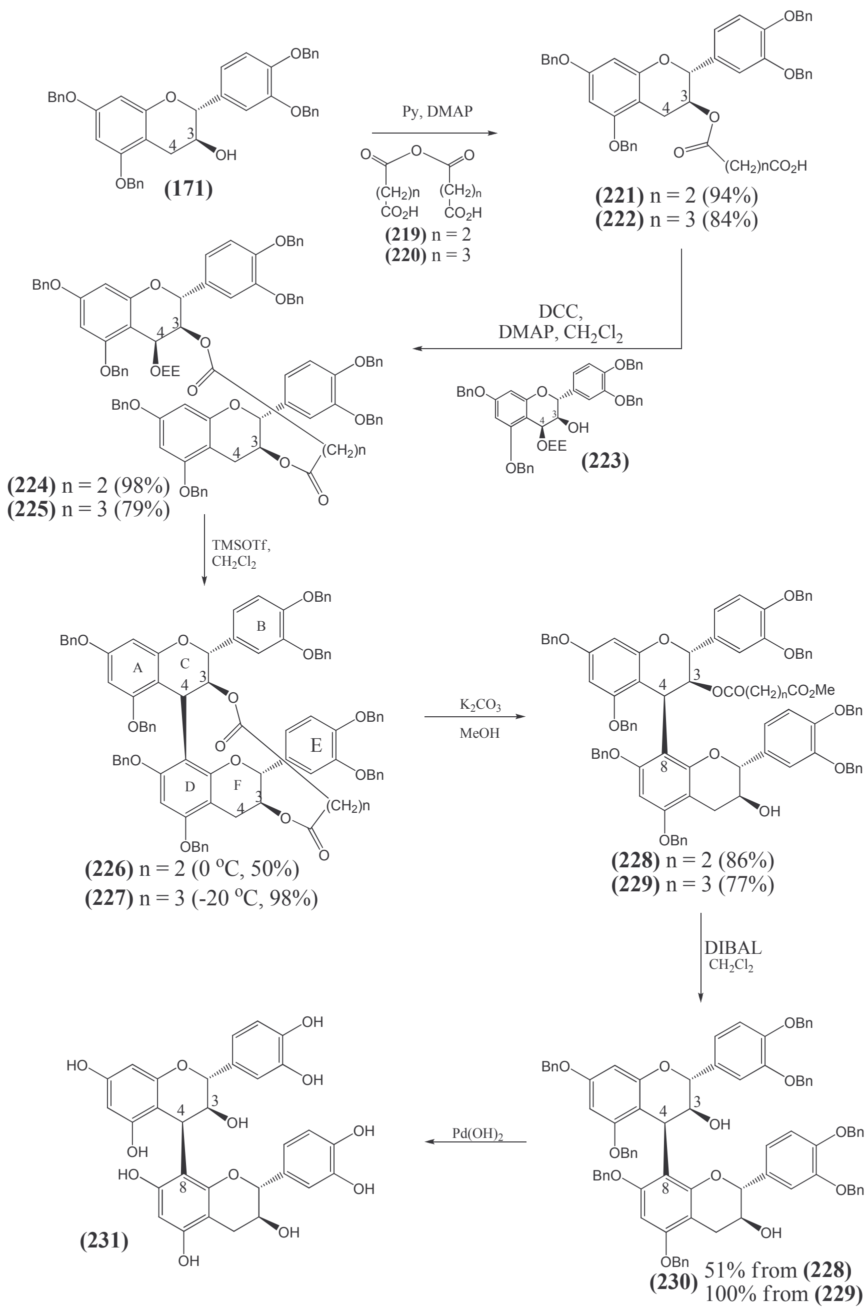
| Entry | Electrophile | | | Solvent | Lewis acid | Temp. (°C) | Coupling yield (%) | Selectivity (4α)-4/(4β)-4 |
|---|---|---|---|---|---|---|---|---|
| | No. | $R^1$ | $R^2$ | | | | | |
| 1 | **209** | H | Me | $CH_2Cl_2$ | $TiCl_4$ | 0 | 84 | 2.1:1 |
| 2 | **210** | Ac | Me | $CH_2Cl_2$ | $TiCl_4$ | 0 | 81 | 7.4:1 |
| 3 | **210** | Ac | Me | $CH_2Cl_2$ | $TiCl_4$ | -40 | 35 | 55:1 |
| 4 | **210** | Ac | Me | $CH_2Cl_2$ | $BF_3.OEt_2$ | -78 | 55 | 10:1 |
| 5 | **210** | Ac | Me | $CH_2Cl_2$ | $SnCl_4$ | -78 | 87 | 11:1 |
| 6 | **210** | Ac | Me | $CH_2Cl_2$ | TMSOTf | -78 | Quant. | 32:1 |
| 7 | **210** | Ac | Me | Toluene | $BF_3.OEt_2$ | 0 | 85 | 4.0:1 |
| 8 | **210** | Ac | Me | Toluene | $SnCl_4$ | 0 | Quant. | 4.4:1 |
| 9 | **210** | Ac | Me | Toluene | TMSOTf | 0 | 89 | 7.0:1 |
| 10 | **211** | Ac | iPr | $CH_2Cl_2$ | TMSOTf | -78 | Quant. | 40:1 |
| 11 | **212** | Ac | $(CH_2)_2OH$ | $CH_2Cl_2$ | TMSOTf | -78 | Quant. | 46:1 |
| 12 | **213** | Ac | EE | $CH_2Cl_2$ | TMSOTf | -78 | Quant. | >48:1 |
| 13 | **214** | Ac | Bn | $CH_2Cl_2$ | TMSOTf | -78 | 88 | 25:1 |




**Scheme 56:** Condensation studies of catechin electrophiles and benzylated catechin in the presence of Lewis acid.

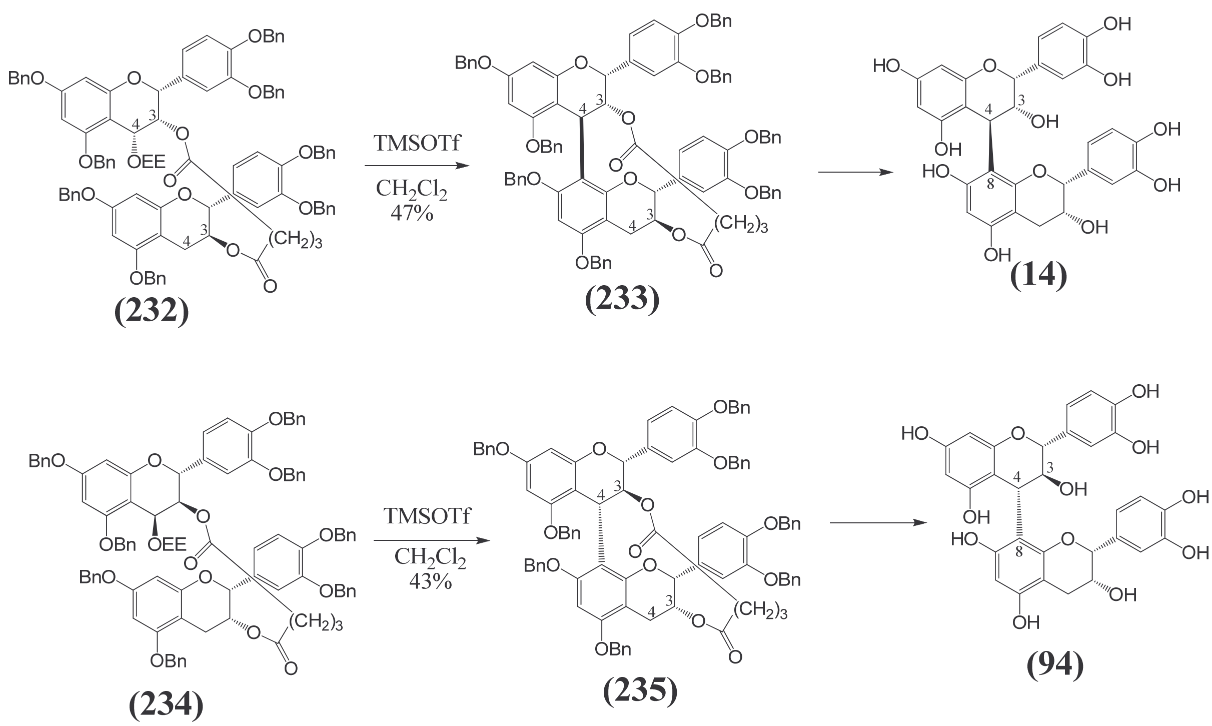
Most methods of proanthocyanidin synthesis require a 4 to 5 fold excess of the nucleophile to avoid self condensation of the electrophilic flavanyl carbocation. Neighbouring group participation by the 3-ol- or 3-acetoxy group of the electrophile leads to a predominance of coupled products with 3,4-*trans*-configuration.

Saito and coworkers[59] developed intramolecular coupling of monomeric units bound by a temporary di-ester linker that requires the nucleophile and electrophile in equimolecular amounts. Good yields of procyanidin B-1 and procyanidin B-3 dimers with 3,4-*trans* configuration were obtained. Esterification of tetra-*O*-benzylcatechin **(171)**, with succinyl or glutaryl anhydride **(219)** or **(220)** gives a carboxylic mono-ester **(221)** or **(222)**. These esters can be coupled to an electrophilic unit, 4-*O*-ethoxyethyl (OEE) derivative **(223)**, using DCC to give the di-esters **(224)** and **(225)** in 94 and 84% yields, respectively. Treatment of the di-esters with TMSOTf in dichloromethane gives the (4→8)-condensed product **(226)** or **(227)** with 3,4-*cis* configuration. The best yield (98%) was obtained with the glutaryl diester **(226)** at 20 $^oC$. The succinyl linker was best removed by a two step procedure. $K_2CO_3$ in methanol cleaves the less hindered F-ring ester, followed by DIBAL treatment that cleaves the C-ring ester (Scheme 57). $Pd(OH)_2$ catalyzed hydrogenolysis gave the 3,4-*cis*-catechin-(4β→8)-catechin dimer **(231).** This compound is the C-4 (C-ring) diastereoisomer of procyanidin B-3.



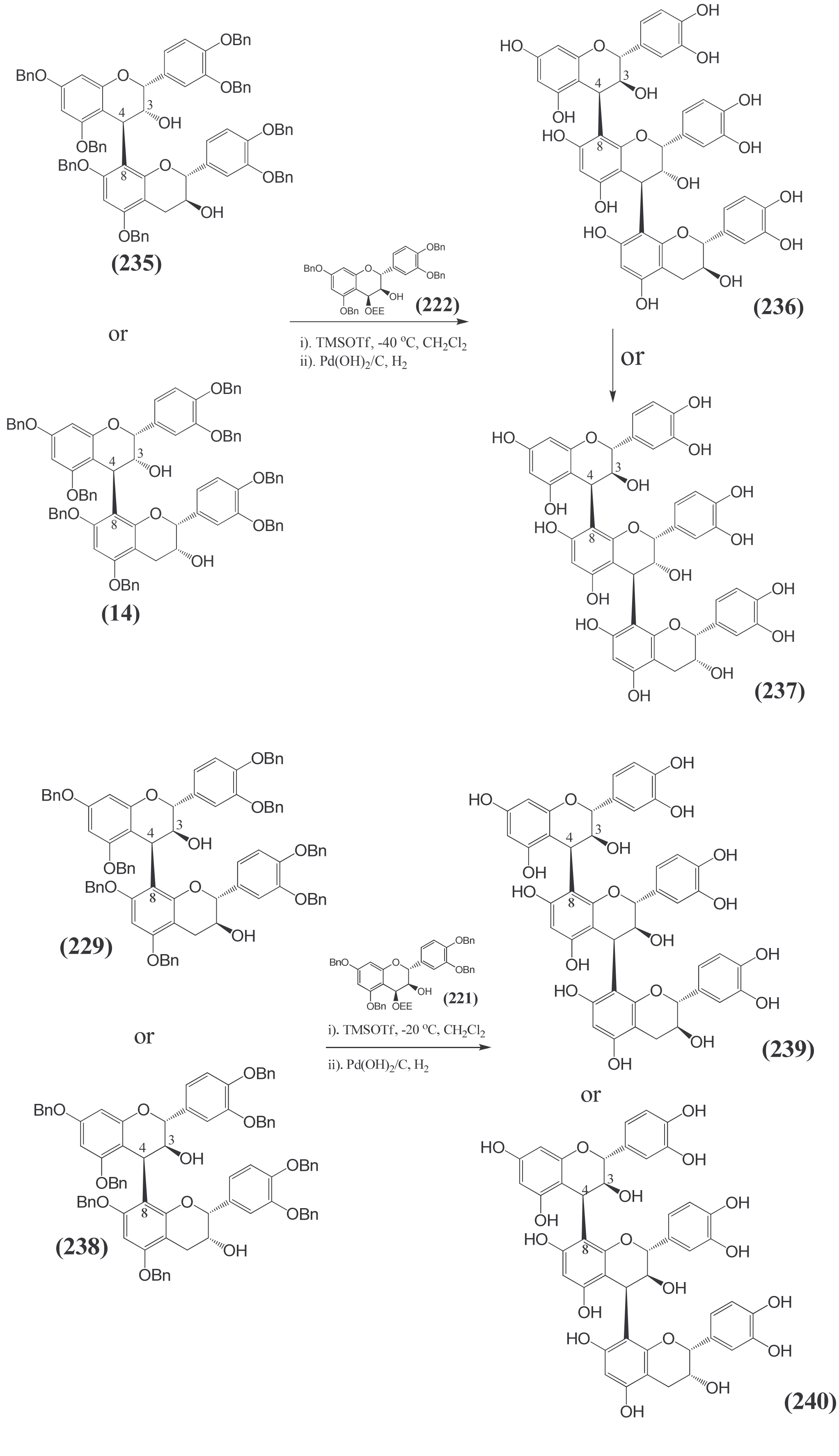

**Scheme 57:** 3,4-*cis* catechin and catechin condensation by TMSOTf-catalyzed intramolecular coupling.

Intramolecular condensation of the epicatechin-catechin **(232)** and catechin-epicatechin **(234)** combinations gave products with 3,4-*trans* configuration in 47 and 43% yields, respectively (Scheme 58). Hydrogenolysis gave **(14)** and **(94)**. The method failed to be of general use. No product with 3,4-*cis* configuration was detected and the configuration of the C-3 moieties prevented synthesis of procyanidin B-2 and B-4 dimers (e.g. epicatechin-epicatechin condensation did not take place).



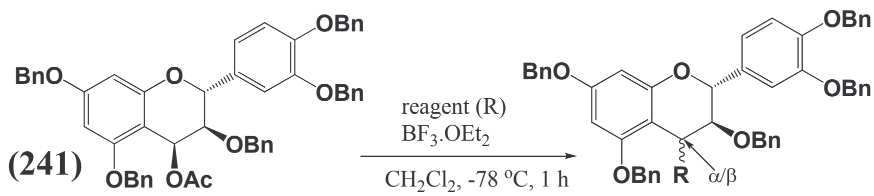

**Scheme 58:** 3,4-*Trans* catechin and catechin condensation by TMSOTf-catalyzed intramolecular coupling.

Saito and co-workers[60] synthesized trimers **(236)**, **(237)**, **(239)** and **(240)** using their usual methodology (TMSOTf as catalyst), Scheme 59. The best yields (90 – 100%) were obtained at -40 $^{o}$C.



**Scheme 59:** Synthesis of epicatechin/catechin trimers.

Ohmori, Ushirmaru, and Suzuki[61] described an effective method for introducing a variety of substituents to the C-4 position of catechin and epicatechin. The C-4 acetoxylated catechin and epicatechin derivatives, **(241)** and **(242)**, smoothly reacted with a range of nucleophiles under Lewis acidic conditions (such as $BF_3.OEt_2$), to yield C-4 elaborated flavan-3-ols (Scheme 60, Table 4; Scheme 62, Table 5).




**Scheme 60:** Catechin series.

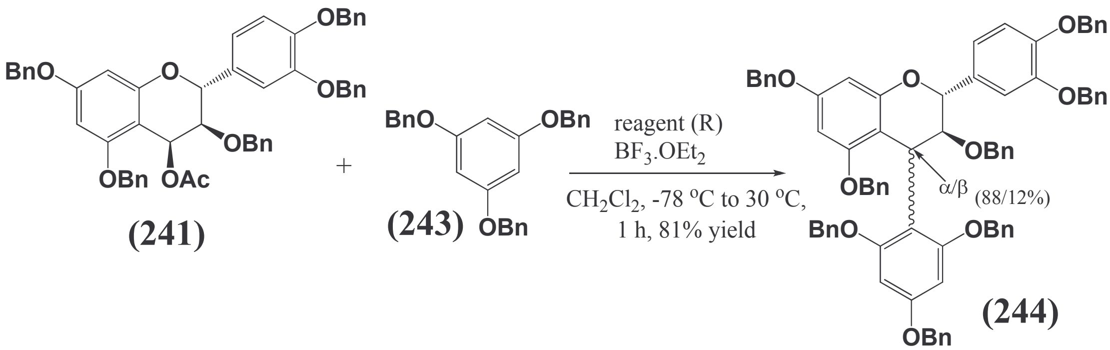
The results in Table 4 indicated that the chemical yields depended on the nucleophilicity of the reagents.

**Table 4:** Catechin series.[61]

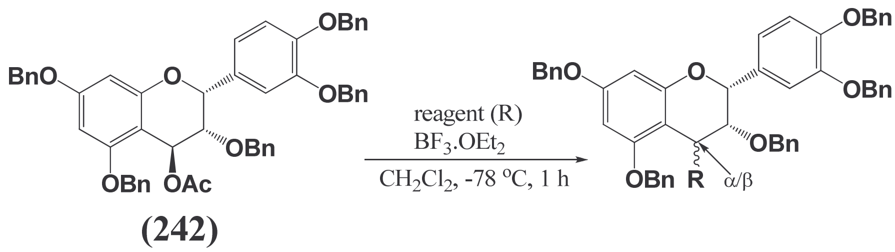
| Run | Reagent[a] | Yield/% | α/β |
|---|---|---|---|
| 1 | $-C(Me)_2CO_2$i-pr | 87 | β |
| 2 | -CH2C(O)Ph | 88 | 12/88 |
| 3 | $-CH_2CH{=}CH_2$ | 7 | 8/92 |
| 4 | $-CH_2CH{=}CH_2$ | 22 | β |
| 5 | -SPh | 92 | β |
| 6 | $-N_3$ | 81 | β |

a. reagent (3 mol equiv.), $BF_3.OEt_2$ (1 mol equiv.).

The reaction of acetate **(241)** with tri-*O*-benzylphloroglucinol (3 mol equiv., $CH_2Cl_2$, -78 $^oC$, 1 h) gave the 4α→2-tri-*O*-benzyl phloroglucinol catechin derivative as the major product (Scheme 61).

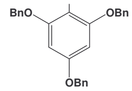



**Scheme 61:** Synthesis of the 4-arylcatechin derivative.



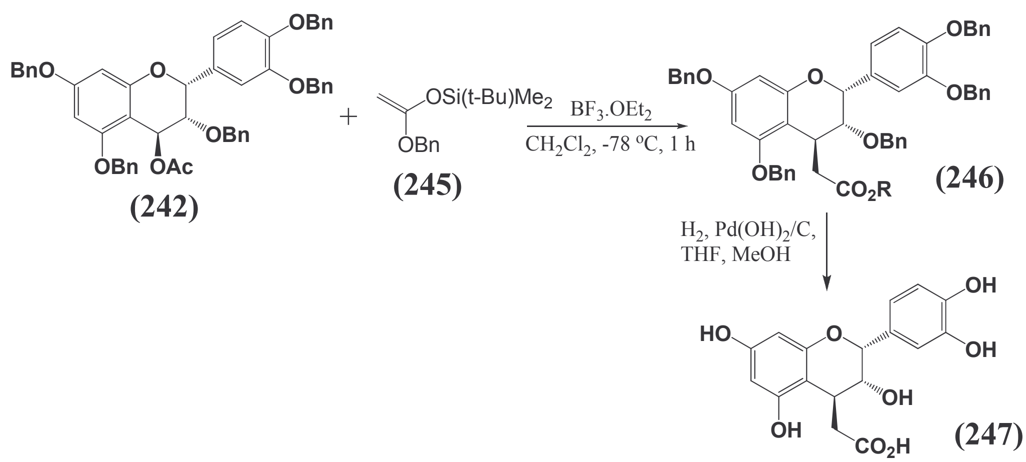

**Scheme 62:** Epicatechin series.

**Table 5:** Epicatechin series.[61]

| Run | Reagent (R) | Yield/% | α/β |
|---|---|---|---|
| 1 | $-CH_2CO_2Et$ | 89 | β |
| 2 | $-C(Me)_2CO_2$i-pr | 34 | β |
| 3 | $-CH_2C(O)Ph$ | 80 | β |
| 4 | $-CH_2CH=CH_2$ | -[b] | - |
| 5 | $-CH_2CH=CH_2$ | 2 | β |
| 6 | -SPh | 88 | β |
| 7 | $-N_3$ | 59 | β |
| 8[c] |  | 72 | β |

a. reagent (3 mol equiv.), $BF_3.OEt_2$ (1 mol equiv.).
b. the reaction gave only a mixture of unidentified side products
c. at -78 $^oC \rightarrow 30$ $^oC$

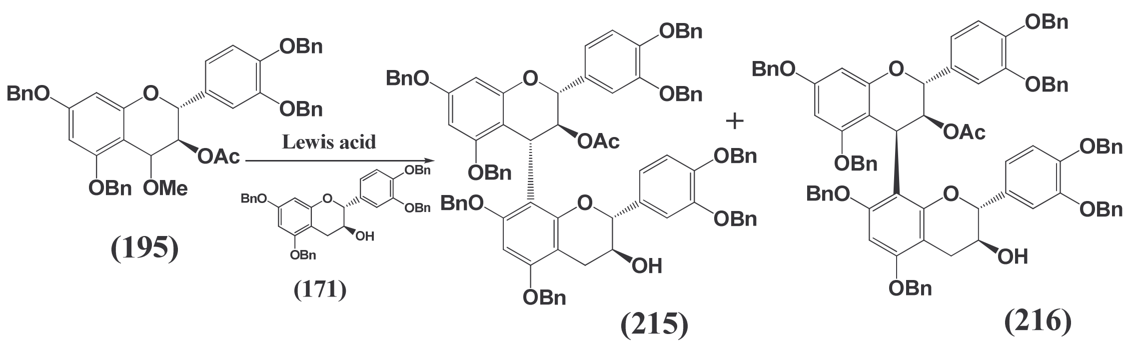
The method was used to synthesize the natural product dryopteric acid **(247)** (Scheme 63).[61]




**Scheme 63:** Synthesis of dryopteric acid.

In most coupling reactions, a large excess of nucleophile (up to 5 fold) is required. This means that the coupled product is only a small part of the reaction mixture and a large amount of the starting material has to be removed by chromatography.

Mohri and co-workers[61] investigated equimolar condensation of **(171)** and **(195)** with a variety of Lewis acids (Table 6) and obtained the best results (64% yield) with $Yb(OTf)_3$ (Scheme 64).




**Scheme 64:** Lewis acid mediated coupling reaction between **(171)** and **(195)**.

**Table 6:** Equimolar coupling reaction of **(171)** and **(195)** by Lewis acids.[62]

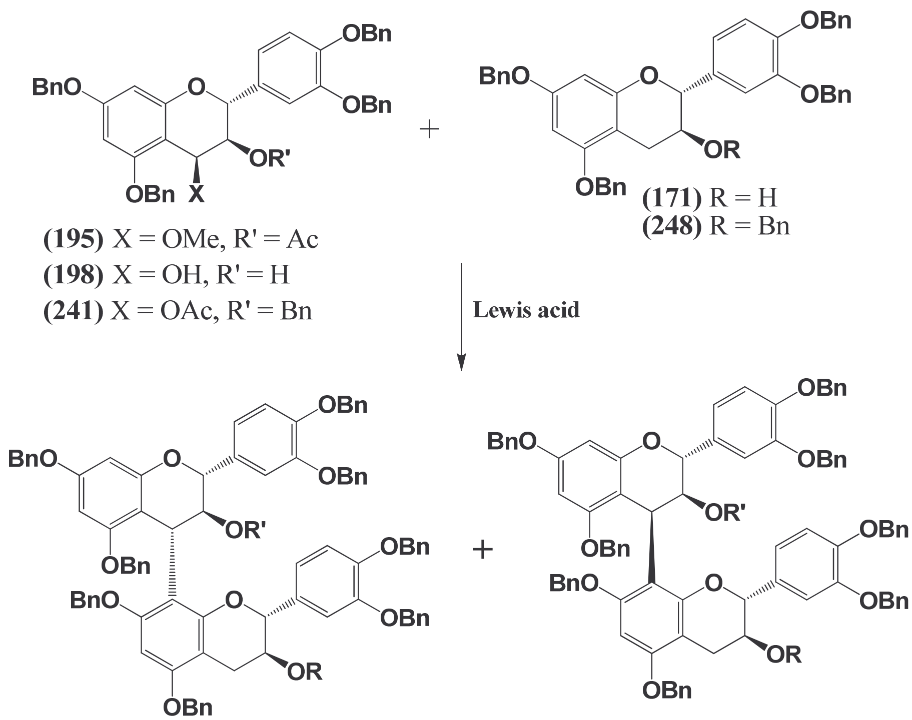
| **Lewis acids** | **Time** | **Yield (%)** | **Selectivity (α:β)** |
|---|---|---|---|
| $TiCl_4$ | 0.5 | 36 | 75:25 |
| $BF_3.Et_2O$ | 3 | ND | - |
| $B(C_6H_5)_3$ | 2 | 38 | 89:11 |
| $AgBF_4$ | 7.5 | 50 | 98:2 |
| $Cu(OTf)_3$ | 0.5 | 43 | 91:9 |
| $In(OTf)_3$ | 0.5 | 45 | 91:9 |
| $Sc(OTf)_3$ | 0.5 | 50 | 67:33 |
| $La(OTf)_3$ | 72 | 34 | 98:2 |
| $Yb(OTf)_3$ | 2 | 64 | 98:2 |
| 10 mol % of $Yb(OTf)_3$ | 12 | 42 | 91:9 |

ND = not determined

**Table 7**: Examples of condensation of the catechin nucleophile (Nu) and electrophile (E) by Lewis acids.[62]

| Entry | Nu | E | Nu/E | Lewis acids | T (°C) | Prod. | Yield (%) | Selectivity (α:β) | Reference |
|---|---|---|---|---|---|---|---|---|---|
| 1 | **171** | **198** | 5 | $TiCl_4$ | 0 | **179** | 92 | 60:40 | Kawamoto et al[21] |
| 2 | **171** | **195** | 4.5 | $TiCl_4$ | -20 | **214** | 83 | 96:4 | Saito et al[57,58] |
| 3 | **171** | **195** | 4.5 | TMSOTf | -78 | **214** | Quant | 97:3 | Saito et al[57,58] |
| 4 | **171** | **195** | 10 | $Yb(OTf)_3$ | rt | **214** | 64 | 98:2[a] | Mohri et al[62] |
| 5 | **88** | **241** | 3 | $BF_3$·Et2O | -30 | **247** | 94 | 90:10 | Ohmori et al[63] |
| 6 | **88** | **241** | 12 | $BF_3$·Et2O | -30 | **247** | 59 | 90:10 | Ohmori et al[63] |

[a] The selectivity was determined by $^1H$ NMR analysis of C-3 of diacetate derivative of α-**(7)** (5.80 and 5.83 ppm) and β-**(7)** (5.33 and 5.58 ppm) according to the reported procedure.[58]



**(195)** X = OMe, R' = Ac
**(198)** X = OH, R' = H
**(241)** X = OAc, R' = Bn

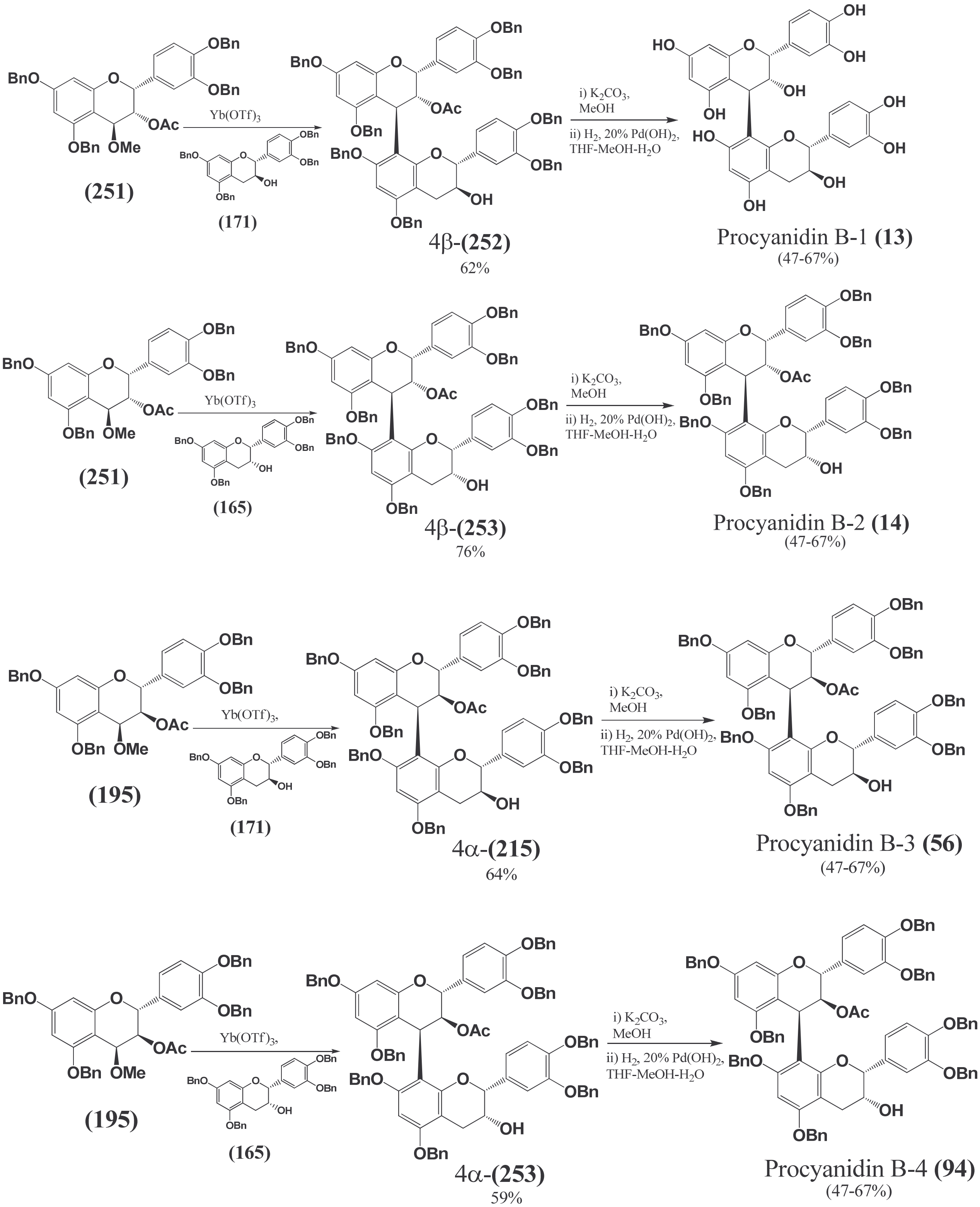
**(171)** R = H
**(248)** R = Bn

α-**(215)** R = H, R' = Ac
α-**(180)** R = R' = H
α-**(249)** R = Bn, R' = Bn

β-**(216)** R = H, R' = Ac
β-**(229)** R = R' = H
β-**(250)** R = Bn, R' = Bn

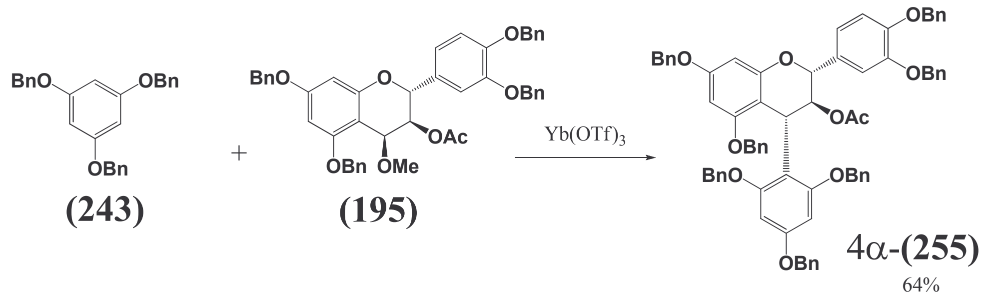
**Scheme 65:** Examples of Lewis acid mediated coupling reactions between catechin nucleophiles and catechin electrophiles.

Table 7 compares Mohri's results (entry 4) with other published results described so far. The high α/β selectivity (98:2) and nucleophile/electrophile ratio of entry 1 compensates for the lower yield with $Yb(OTf)_3$ (64%) (Scheme 65). The method was used to synthesize procyanidin B-1, B-2, B-3 and B-4 (Scheme 66).




**Scheme 66:** Equimolar coupling of catechin and epicatechin derivatives towards synthesis of procyanidin B-1 (**13**) – B-4 (**94**) derivatives.

Replacement of the catechin or epicatechin nucleophiles **(171)** or **(165)** with tri-*O*-benzylphloroglucinol **(243)** gave reduced selectivity (75:25 ratio). This correlates with the smaller size of **(243)** compared to **(171)** and **(165)** (Scheme 67).




**Scheme 67:** Equimolar coupling of benzylated phloroglucinol with **(195)**.

Inspired by the Suzuki report[61] that the protecting group of the C-3 hydroxy group influences stereoselectivity and reactivity at C-4 due to steric effects and neighbouring group participation, Kondo and co-workers[64] designed two monomers, 3-*O*-acetyl- and 3-*O*-TBS-4-acetoxyper-*O*-benzylcatechin **(248)** and **(249)** (Figure 4) as key building monomers for procyanidin synthesis.

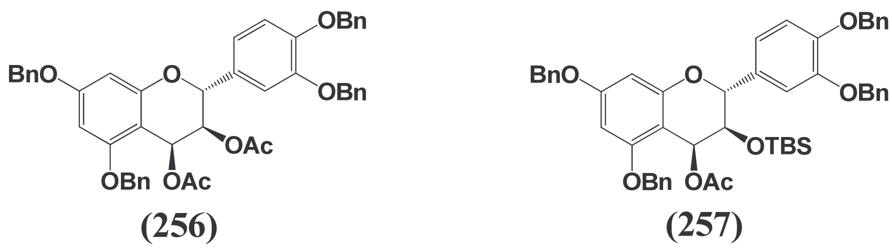
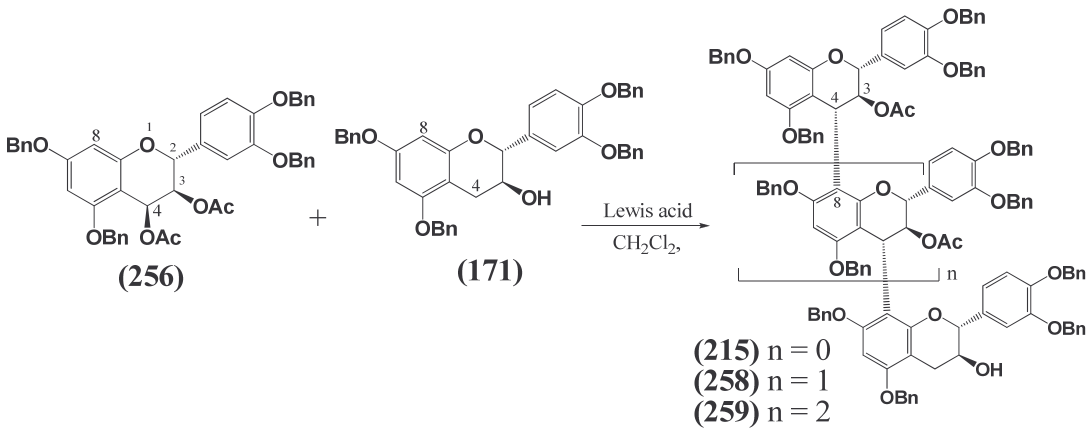



**Figure 4:** Building monomers for procyanidin synthesis.

Treatment of **(257)** with 1.5 equivalents **(171)** and $BF_3.Et_2O$ yielded traces of partly desilylated dimers, probably due to steric hindrance of TBS. Under the same conditions the diacetoxy building block **(256)** smoothly condenses at 0 $^{o}$C in 5 minutes to dimer **(215)** (57%), trimer **(258)** (20%) and tetramer **(259)** (3%), all with 3,4-*trans* configuration (Table 8). With one equivalent TMSOTf at -78 $^{o}$C procyanidin B-3 **(56)** was obtained after deacetylation and careful hydrogenolysis with 82% yield and high stereoselectivity (α/β ratio of 97:3) (Scheme 68 and Table 8).



**Scheme 68:** Condensation of **(171)** and **(256)** in the presence of different Lewis acids.

**Table 8:** Condensation of **(171)** and **(256)** in the presence of different Lewis acids.

| Entry | Lewis acid (equiv.) | Temp ($^{o}$C) | Time | Yield (%)[a] | | |
|---|---|---|---|---|---|---|
| | | | | Dimer **(215)** (n = 0) | Trimer **(258)** (n = 1) | Tetramer **(259)** (n = 2) |
| 1 | $BF_3.OEt_2$ (1) | 0 | 5 min | **57** | **20** | **3** |
| 2 | TMSOTf (1) | -78 | 5 min | **82**[b] | **10** | **Trace** |
| 3 | TMSOTf (0.20) | -78 | 1h | **49** | **33** | **10** |
| 4 | $Sc(OTf)_3$(0.1) | 0 to rt | 1h | **49** | **20** | **4** |
| 5 | $Sc(OTf)_3$(0.1) | Rt | 30 min | **61** | **22** | **4** |
| 6 | $Yb(OTf)_3$(1) | 0 to rt | 1h | **45** | **20** | **Trace** |
| 7 | $In(OTf)_3$(0.1) | 0 to rt | 5h | **44** | **19** | **2** |
| 8 | $La(OTf)_3$(1) | 0 to rt | 21h | **40** | **24** | **Trace** |
| 9 | $Cp_2HfCl_2$(1) | 0 to rt | 1h | **Trace** | **0** | **0** |
| 10 | $B(C_6F_5)_3$(0.1) | 0 | 3.5h | **60** | **23** | **4** |

[a] Stereoselectivity was not determined except for entry 2.

[b] Stereoselectivity (α/β = 97/3) was determined by $^{1}$H NMR data.

With 10 mole % $B(C_6F_5)_3$, **(256)** self-condensed at 0 $^oC$ for 5 h to give a mixture of procyanidin oligomers in 93% yield.

### 2.2.5.1 Control of the degree of polymerization

The central problem in all the strategies described so far, apart from the difficulty of obtaining pure starting materials from natural sources, is uncontrolled polymerisation due to self condensation.

Efforts to synthesize homo-oligomers (constructed from a single starting material to obtain an oligomer with the same repeating or monomeric unit in all positions) yield a mixture of oligomers with different chain lengths.[63,64] The oxygen groups on the A-ring that encourage formation of a benzylic cation at C-4, also enhance the nucleophilicity of the A-ring of the same molecule at C-6 or C-8. The outcome is uncontrolled self condensation. Some success has been achieved by varying reaction time, reaction temperature and using dilute solutions, followed by chromatography.

Syntheses of hetero-oligomers (constructed with more than one starting material to obtain an oligomer with different repeating units of different stereochemistry and oxidation patterns in different positions) pose an even bigger challenge. A solution is to use a large excess of the nucleophilic building block to avoid self condensation of the electrophilic building block. This inelegant solution requires considerable effort.

Suzuki and co-workers[63] recognised the similarity in $S_N1$ reactivity of the anomeric position of sugars and the C-4 position of polyphenols and called it the "sugar-flavonoid analogy" (figure 5).[61,65,66]

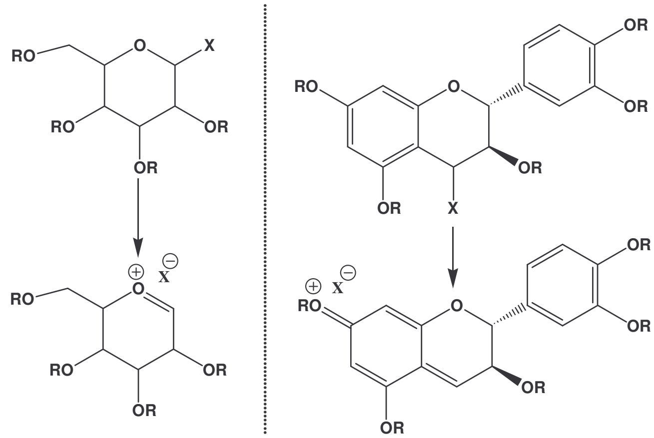
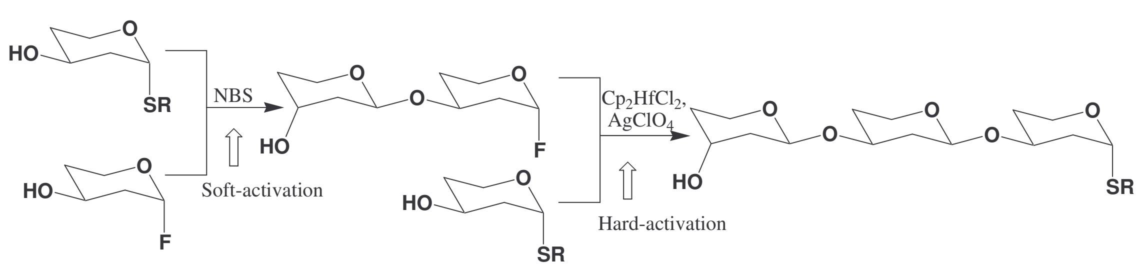



**Figure 5:** Structural analogy of sugar and flavonoid.

In sugar chemistry, a thio group at the anomeric position is selectively activated by soft thiophilic promoters (e.g. *N*-bromosuccinimide, NBS) in the presence of monomers with a fluoro group in the anomeric position. This allows the thio-sugar to act as electrophile and the fluoro sugar to act as a nucleophile. In the next step, the fluoro group in the anomeric position is selectively activated by hard Lewis acid conditions (e.g. $Cp_2HfCl_2$-$AgClO_4$) in the presence of monomers with a thio group in the anomeric position. This allows the fluoro sugar to act as an electrophile and the thio-sugar to act as a nucleophile. This ortogonal strategy allows efficient assembly of complex saccharides in a few steps without the need for protection and deprotection (Scheme 69).[67]

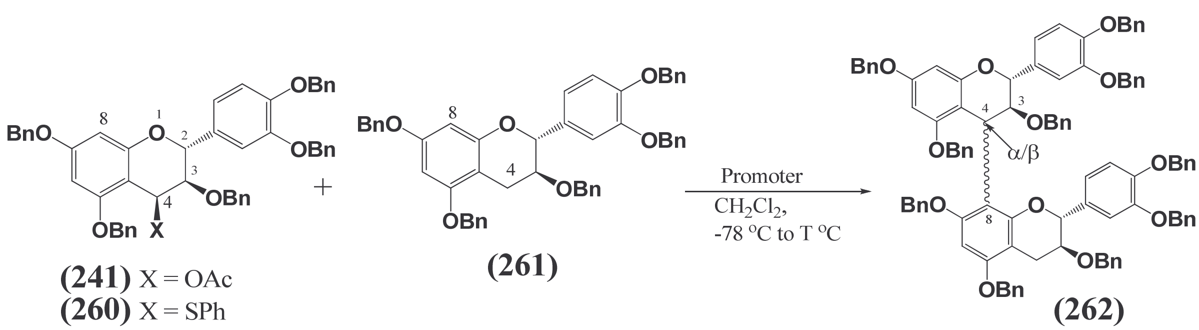



**Scheme 69:** Orthogonal strategy in carbohydrate synthesis.

Suzuki and co-workers[63] developed an iterative step-by-step strategy, similar to orthogonal oligosaccharide synthesis to synthesize oligomeric catechin analogues of proanthocyanidins. They did not succeed in preparing C-4 fluoro catechin building blocks for hard Lewis acid activation of the benzylic C-4 position to a carbocationic electrophile and attributed this to the poor nucleophilicity of fluoride. They replaced fluoride with an acetate group that could be activated by $BF_3{\cdot}OEt_2$ (a hard Lewis acid). They achieved soft activation at C-4 *via* the use of a phenylsulfide and the use of $AgBF_4$ or N-iodosuccinimid (NIS) as soft Lewis acids (Scheme 70).

### 2.2.5.2 Activation of the 'hard' acetate unit

Activation of the 'hard' acetate unit **(241)** with $BF_3{\cdot}OEt_2$ in the presence of an excess of nucleophilic catechin unit **(261)** (without the leaving group at C-4), the reaction proceeded smoothly to give the corresponding dimeric product **(262)** (entries 1 and 2, Table 9).




**Scheme 70:** Hard and soft activation of electrophilic catechin derivatives.

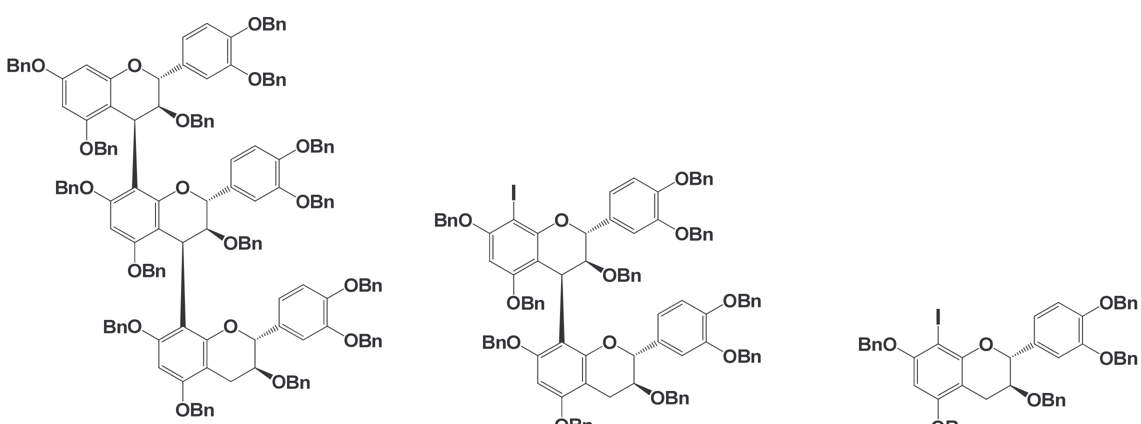
**Table 9:** Hard and soft activation of electrophilic catechin derivatives.

| Entry | Electrophilic unit | Promoter (mol. equiv.) | **5**/mol equiv. | Time/h | T (°C) | Yield/% | α/β[c] |
|---|---|---|---|---|---|---|---|
| 1 | **(241)** | $BF_3.OEt_2$ (1) | 3 | 0.5 | -30 | 94 | 9:1 |
| 2 | **(241)** | $BF_3.OEt_2$ (1) | 1.2 | 0.5 | -30 | 59[a] | 9:1 |
| 3 | **(260)** | $AgBF_4$ (2) | 3 | 4 | -10 | 77 | 9:1 |
| 4 | **(260)** | NIS (2) | 3 | 0.5 | -30 | 40[b] | 9:1 |

[a]Trimer **(263)** was obtained in 31% yield. [b]Iodine derivatives **(264)** and **(265)** were obtained in 19% and 17% yields, respectively. [c]Ratios of diastereomers were determined by $^1$H NMR data.

### 2.2.5.3 Activation of the soft thiophenyl unit

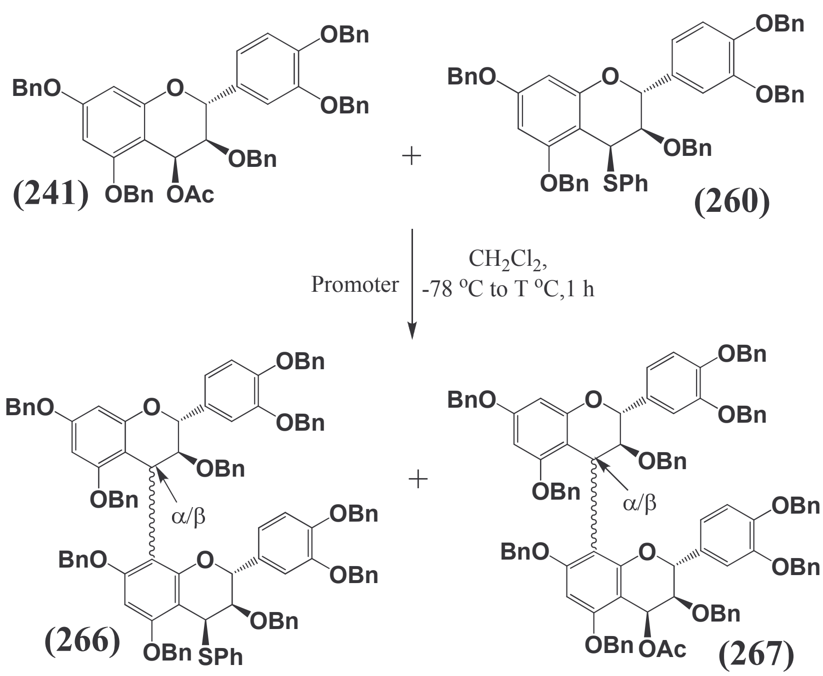
Soft activation of phenylsulfide **(260)** was achieved by using $AgBF_4$ or NIS as a promoter in the presence of 3 mol equivalent of nucleophilic substituent **(261)** to obtain dimer **(262)** in 77% and 40% yield, respectively (entries 3 and 4, Table 9). The lower yield in the latter case was due to the concomitant formation of iodinated by-products, **(264)** (19%) and **(265)** (17%) which, however, offered an important hint for masking the nucleophilic C-8 position of the flavan skeleton.




**(263)** **(264)** **(265)**

**Scheme 71:** Hard and soft activation of electrophilic catechin derivatives.

However, selective activation of the sulfide **(260)** with $AgBF_4$ (soft Lewis acid promoter) in the presence of the 4-acetoxy derivative **(241)** was unfruitful and polymers derived from **(260)** were isolated. Therefore, self condensation of **(260)** prevailed over cross condensation (Scheme 72 and Table 10).




**Scheme 72:** Comparing nucleophilicities of **(241)** and **(260)**.

**Table 10:** Comparing nucleophilicities of **(241)** and **(260)**.

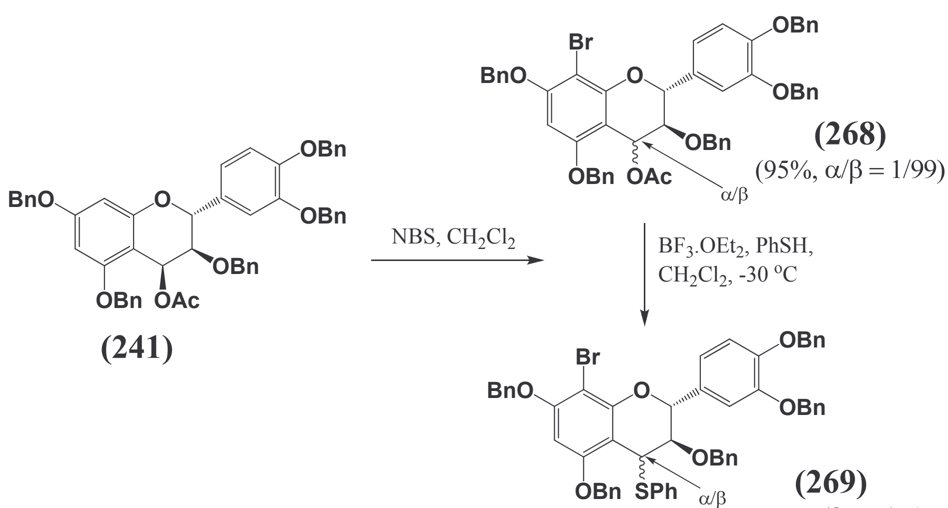
| Entry | Electrophilic unit | Nucleophilic unit | Promoter | T | Yield/% | α/β |
|---|---|---|---|---|---|---|
| 1 | **(241)** (1 mol equiv.) | **(260)** (3 mol equiv.) | $BF_3{\cdot}OEt_2$[a] | -30 | 90 | 9:1 |
| 2 | **(260)** (1 mol equiv.) | **(241)** (3 mol equiv.) | $AgBF_4$[b] | -15 | - | - |

[a] 1.0 mol equiv., [b] 2.5 mol equiv.

The acetate **(241)** was unsuitable as a nucleophilic partner and, as indicated above, the fluoride could not be synthesized. It was suggested that the electron-withdrawing acetoxy group in the C-4 position decreases the nucleophilicity of the adjacent A-ring relative to the A-ring with a 4-phenylthio group. This requires capping of the reactive 8-position of the more reactive 4-phenylthio starting monomer to avoid cross coupling and to ensure a successful orthogonal synthesis. Notable is the absence of any C-6 coupled products, even without capping of this position with bromine, demonstrating the relative electron rich nature of the 8-position.

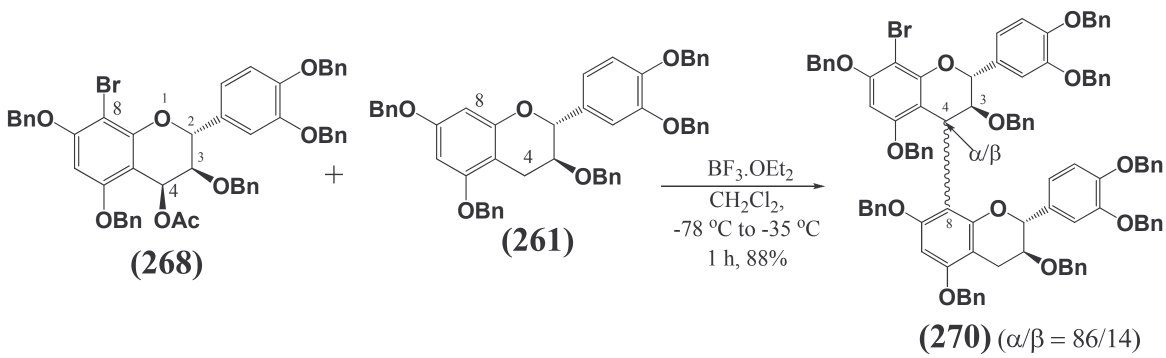
### 2.2.5.4 Protection at C-8

The C-8 position was masked with bromine (Scheme 73), thereby reducing the nucleophilicity of the A-ring and preventing self-condensation to achieve regioselectivity of the 4-acetoxy-8-bromocatechin derivative **(268).** The corresponding C-4-phenylthio derivative **(269)** was obtained by treatment of **(268)** with PhSH in the presence of $BF_3{\cdot}OEt2$ (Scheme 73).




**Scheme 73:** C-8 bromination of catechin derivative (**241**).

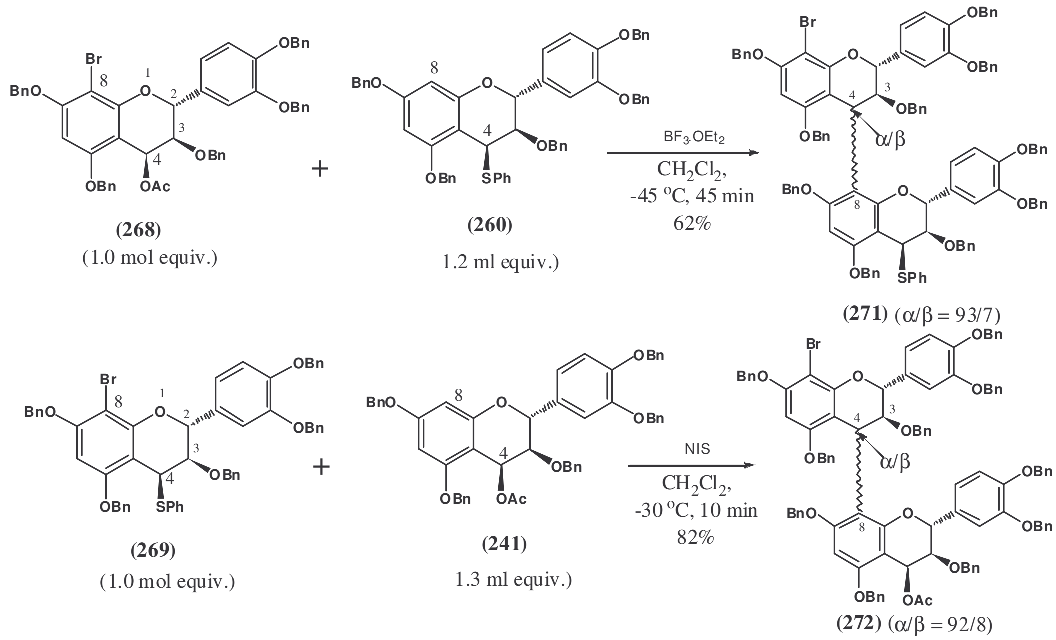
These C-8 bromo derivatives were excellent coupling partners, providing an efficient entry into controlled oligomer formation. The requirement of C-8 bromo protection of the 4-phenyl thio compound has certain disadvantages. The phenylthio starting material **(269)**, in contrast to the acetoxy starting material **(268)**, cannot be brominated directly. The 4-phenylthio-8-bromo starting material is synthesized from the 4-acetoxy-8-bromo monomer **(268)**, albeit in high overall yield (Scheme 73). On treatment of the mixture of the bromoacetate **(268)** (1.0 mol equivalent) and nucleophilic partner **(261)** (1.2 mol equiv.) with $BF_3{\cdot}OEt_2$ (1.0 mol equiv., $CH_2Cl_2$, -78→-35 °C, 1 h), the desired C-C bond formation clearly occurred to give the corresponding dimer **(270)** in 88% yield (Scheme 74). Notably, they proved that equimolar coupling is feasible through this bromo-capping technology. This achievement was remarkable, because the corresponding reaction for non-bromo derivative **(241)** gave a lower yield (59%) when only a slight excess (1.2 mol equivalents) of nucleophilic partner **(261)** was used (Scheme 70; Table 9, entry 2).




**Scheme 74:** Equimolar coupling of bromoacetate **(268)** and **(261)**.

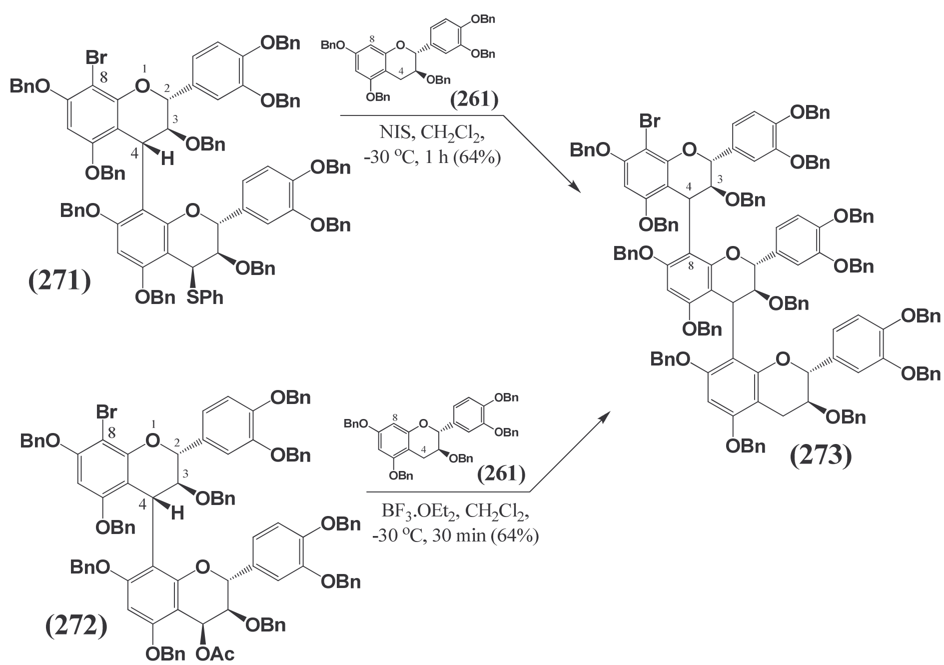
### 2.2.5.5 Orthogonal coupling reactions

The effectiveness of bromo-capped substrates **(268)** and **(269)** was further demonstrated through orthogonal coupling reactions shown in Scheme 75. (NIS activates the sulfur-leaving group and did not attack the aromatic rings).



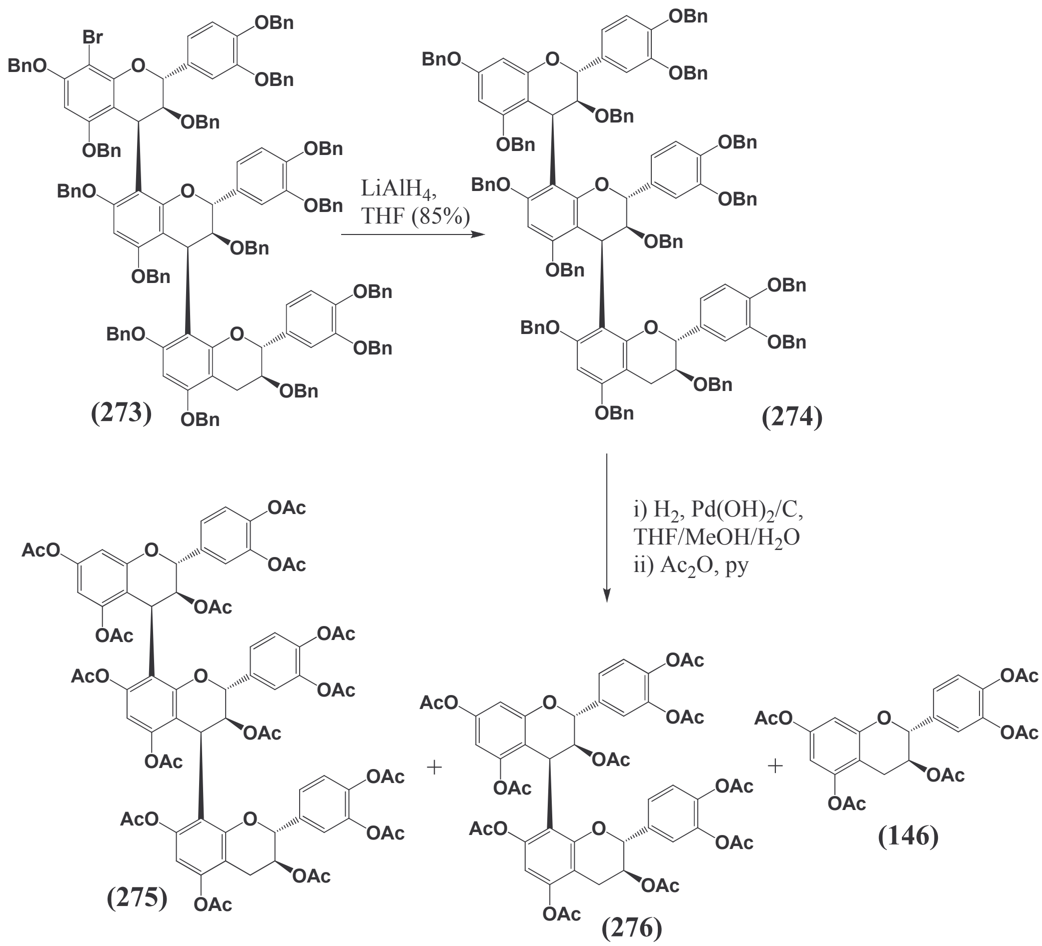

**Scheme 75:** Orthogonal activation of bromo-capped derivatives.

The trimer **(273)** was formed through the use of dimeric acetate **(272)** or dimeric sulfide **(2271)** (Scheme 76).




**Scheme 76:** Iterative activation of dimer **(271)** and **(272)**.

The final dimer, trimer, etc., were firstly debrominated to **(274)** with $LiAlH_4$/THF/25 $^oC$/4 h, because hydrogen bromide liberated during catalytic debenzylation of the oligomers causes complete scission of all interflavan bonds. The benzyl protecting groups were then removed under nonacidic conditions by hydrogenolysis over 20% $Pd(OH)_2$/C in THF/MeOH (6:1) at 25 $^oC$ for 5 hours. The crude product was acetylated ($Ac_2O$, pyridine, 0 $^oC$, 21 h) to afford the peracetates **(275)** (33%), **(276)** (9%) and **(146)** (34%) samples for NMR spectroscopy (Scheme 77).




**Scheme 77:** Removal of bromo and benzyl groups.

Despite these problems, the work of Suzuki represents an important contribution to our ability to synthesize proanthocyanidins with known configuration and degree of polymerization.

In summary, synthesis of proanthocyanidin dimers, trimers and oligomers of known stereochemistry, chain length (degree of polymerisation) and in good yields has made steady progress over the past 40 years.

The work of Roux and Ferreira provided us for the first time with dimers, trimers and tetramers with known stereochemistry, but relied on difficult to obtain flavan-3,4-diols and suffered from uncontrolled polymerisation and self-condensation. This required extensive chromatography.

Subsequent oxidative methods to introduce a leaving group in the C-4 position allowed the use of relatively abundant flavan-3-ol starting materials but still could not solve the problem of uncontrolled polymerisation and self-condensation, and the associated inability to construct hetero-oligomers.

Suzuki's orthogonal strategy that relies on the alternating use of hard and soft activation of the C-4 leaving groups and the armed/disarmed concept, allowed much improved control of polymerisation and self-condensation and allowed synthesis of hetero-oligomers. This method was however not fully successful as tedious protection and deprotection of the nucleophilic A-ring via bromination was still required.

The need for direct introduction of a flavonoid monomer at C-4 without pre-functionalisation of this position, simple control of the degree of polymerisation, the ability to construct hetero-oligomers, removal of the need to protect the A-ring, and access the 3,4-*cis* stereochemistry has remained a desirable target.

# REFERENCES


1. Geissman, T. A.; Yoshimura, N. N. *Tetrahedron Lett.* **1966**, *24*, 2669-2673.
2. Jacques, D.; Opie, C. T.; Porter, L. J.; Haslam, E. *J. Chem. Soc., Perkin Trans. I* **1977**, 1637-1643.
3. Botha, J. J.; Young, D. A.; Ferreira, D.; Roux, D. G. *J. Chem. Soc., Perkin Trans. I* **1981**, 1213-1219.
4. Weinges, K.; Perner, J. *J Chem. Soc., Chem. Commun*. **1967**, 351-352.
5. Weinges, K.; Perner, J.; Marx, H.D. *Chem. Ber*. **1970**, *103*, 2344-2349.
6. Weinges, K.; Marx, H. –D.; Groritz, K. *Chem. Ber*. **1970**, *103*, 2336-2343.
7. Kozikowiski, P. A.; Tuckmantel, W.; Hu, Y. *J. Org. Chem*. **2001**, *66*, 1287-1296.
8. Thompson, R. S.; Jacques, D.; Haslam, E. *J. Chem. Soc., Perkin Trans. I* **1972**, 1387-1399.
9. Fletcher, A. C.; Porter, L. J.; Haslam, E.; Gupta, R. K. *J. Chem. Soc., Perkin Trans. I* **1977**, 1628.
10. Foo, L. Y.; Porter, L. *J. Chem. Soc., Perkin Trans. I* **1983**, 1535.
11. Hundt, H. K. L.; Roux, D. G. *J. Chem. Soc., Chem. Commun.* **1978**, 696-698.
12. Hundt, H. K. L.; Roux, D. G. *J. Chem. Soc., Chem. Commun.* **1981**, 1227-1235.
13. Betts, M. J.; Shaw, M. R.; Brown, B. R. *J. Chem. Soc. (C)* **1969**, 1178-1184.
14. Steenkamp, J. A.; Ferreira, D.; Roux, D. G. *Tetrahedron Lett.* **1985**, *26*, 3045-3048.
15. Steenkamp, J. A.; Monton, H. C.; Ferreira, D. *Tetrahedron* **1991**, *47*, 6705-6716.
16. Steenkamp, J. A.; Malan, J. C. S.; Ferreira, D. *J. Chem. Soc., Perkin Trans. I* **1988**, 2179-2183.
17. Tarascou, I.; Barathieu, K.; André, Y.; Pianet, I.; Dufourc, E. J.; Fouquet, E. *Eur. J. Org. Chem.* **2006**, 5367-5377.

18. Tückmental, W.; Kozikowski, A. P.; Romanczk Jr, L. J. *J. Am. Chem. Soc*. **1999**, *121*, 12073-12081.

19. Konstanecki, St. V.; Tambor, J. *Chem. Ber*. **1902**, *35*, 1867.

20. Mehta, P. P.; Whalley, W. B. *J. Chem. Soc*. **1963**, 5327.

21. Kawamoto, H.; Nakatsubo, F.; Murakami, K. *Mokuzani Gakkaishi* **1991**, *37*, 488; *Chem. Abstr*. **1991**, *115*, 279648y.

22. Miura, S.; Midorikawa, T.; Awata, N. *Radioisotopes* **1983**, *32*, 225; *Chem. Abstr*. **1983**, *99*, 158183r.

23. Porter, L. J.; Foo, L. Y. *Phytochemistry* **1982**, *21*, 2947.

24. Botha, J. J.; Ferreira, D.; Roux, D. G. *J. Chem. Soc., Perkin Trans. I* **1981**, 1235.

25. Brown, B. R.; Shaw, M. R. *J. Chem. Soc., Perkin Trans. I* **1974**, 2036-2049.

26. Haslam, E. *J. Chem. Soc., Chem. Commun*. **1974**, 594-595.

27. Fletcher, A. C.; Porter, L. J.; Haslam, E. *J. Chem. Soc.,Chem. Commun.* **1976**, 627-629.

28. Botha, J. J.; Ferreira, D.; Roux, D. G. *J. Chem. Soc., Chem. Commun.* **1978**, 698-700.

29. Van der Westhuizen, J. H.; Ferreira, D.; Roux, D. G. *J. Chem. Soc., Perkin Trans. I* **1981**, 1220-1226.

30. Greasey, L. L.; Swain, T. *Nature* **1965**, *208*, 151.

31. Roux, D. G.; Drewes, S. E. *Biochem. J*. **1964**, *90*, 343.

32. Roux, D. G.; Drewes, S. E. *Biochem. J*. **1964**, *92*, 555.

33. Roux, D. G.; Drewes, S. E. *Biochem. J*. **1965**, *94*, 982.

34. Roux, D. G.; Drewes, S. E. *Biochem. J*. **1966**, *98*, 493.

35. Jacques, D.; Haslam, E. *J. Chem. Soc., Chem. Commun.* 1974, 231.

36. Hemingway, R. W.; Foo, L. Y. *J. Chem. Soc., Chem. Commun.* **1983**, 1035-1036.

37. Delcour, J. A.; Ferreira, D.; Roux, D. G. *J. Chem. Soc., Perkin Trans. I* **1983**, 1711-1717.

38. Viviers, P. M.; Young, D. A.; Botha, J. J.; Ferreira, D.; Roux, D. G. *J. Chem. Soc., Perkin Trans. I* **1982**, 535-540.

39. Steynberg, P. J.; Nel, R. J. J.; Van Rensburg, H.; Benzuidenhoudt, B. C. B.; Ferreira, D. *Tetrahedron* **1998**, *54*, 8153-8158.

40. Griffith, W. P.; Ley, S. V.; Whitcombe, G. P.; White, A. D. *J. Chem. Soc., Chem. Commun.* **1987**, 1625.

41. Clark-Lewis, J. W.; Williams, L. R. *Austral. J. Chem*.,**1967**, *20*, 2151.

42. Du Preez, I. C.; Ferreira, D.; Roux, D. G. *J. Chem. Soc. (C)* **1971**, 336.

43. Foo, L. Y.; Porter, L. J. *J. Chem. Soc., Chem. Commun.* **1982**, 241.

44. Turner, A. B. In: *'Synthetic Reagents'*, vol. 3, Ed. J. S. Pizey, Ellis Horwood, Chichester **1977**, p. 193-225.

45. Lemaire, M.; Guy, A.; Imbert, D.; Guette, J. P. *J.Org. Chem., Chem. Commun*. **1986**, 741.

46. Kolodziej, H.; Ferreira, D.; Roux, D. G. *J. Chem. Soc., Perkin Trans. I*, **1984**, 343.

47. Kiehlmann, E.; Lehto, N.; Cherniwchan, D. *Can. J. Chem*., **1988**, 66, 2431-2439.

48. Barathieu, K.; Ph.D. Thesis, Bordeaux 1 University **2002**, 77-80 (unpublished results).

49. Pearlman, W. M. *Tetrahedron Lett*. **1967**, *17*, 1663-1664.

50. Kawamoto, H.; Nakatsubo, F.; Murakami, K. *J. Wood Chem. Technology* **1990**, *10*, 59-74.

51. Arnaudinaud, V.; Nay, B.; Verger, S.; Nuhrich, A.; Deffieux, G.; Merillon, J. M.; Monti, J. P.; Vercauteren, J. *Tetrahedron Lett.* **2001**, *42*, 5667-5671.

52. Kozikowski, A. P.; Tückmantel, W.; Böttcher, G.; Romanczyk, L. J. *J. Org. Chem.* **2003**, *68*, 1641-1658.

53. Sharma, P. K.; Kolchinski, A.; Shea, H. A.; Nair, J. J.; Gou, Y.; Romanczyk, L. J.; Schmitz, H. H. *Org. Process Res. Develop.* **2007**, *11*, 422-430.

54. Hemingway, R. W. Personal communication.

55. Hemingway, R. W.; Foo, L. Y.; Porter, L. J. *J. Chem. Soc. Perkin Trans. I*. **1982**, 1209.

56. Foo, L. Y.; Karchesy, J. J. *Phytochemistry* **1989**, *28*, 3185.

57. Saito, A.; Nakajima, N.; Tanaka, A.; Ubukata, M. *Biosci. Biotechnol. Biochem*. **2002**, *66*, 1764-1767.

58. Saito, A.; Nakajima, N.; Tanaka, A.; Ubukata, M. *Tetrahedron* **2002**, *58*, 7829-7837.

59. Saito, A.; Nakajima, N.; Tanaka, A.; Ubukata, M. *Tetrahedron Lett.* **2003**, *44*, 5449-5452.

60. Saito, A.; Doi, Y.; Tanaka, A.; Matsuura, N.; Ubukata, M.; Nakajima, N. *Bioorg. Med. Chem.* **2004**, *12*, 4783-4790.

61. Ohmori, K.; Ushimaru, N.; Suzuki, K. *Tetrahedron Lett.* **2002**, *43*, 7753-7756.

62. Mohri, Y.; Sagehashi, M.; Yamada, T.; Hattori, Y.; Morimura, K.; Kamo, T.; Hirota, M.; Makabe, H. *Tetrahedron Lett.* **2007**, *48*, 5891-5894.

63. Ohmori, K.; Ushimaru, N.; Suzuki, K. *Proc. Natl., Acad. Sci. U.S.A*. **2004**, *101*, 12002-12007.

64. Oyama, K.; Kuwano, M.; Ito, M.; Yoshida, K.; Kondo, T. *Tetrahedron Lett.* **2008**, *49*, 3176-3180.

65. Ohmori, K.; Hatakeyama, K.; Ohrui, H.; Suzuki, K. *Tetrahedron* **2004**, *60*, 1365-1373.

66. Ohmori, K.; Ohrui, H.; Suzuki, K. *Tetrahedron Lett.* **2000**, *41*, 5537-5541.

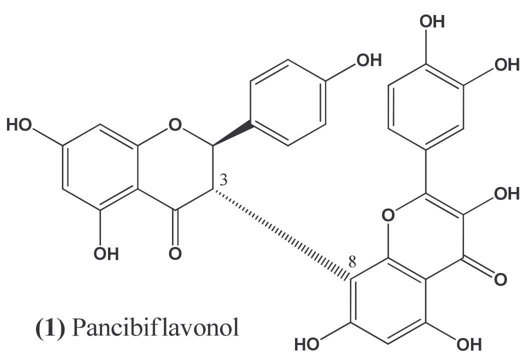
67. Kanie, O.; Ito, Y.; Ogawa, T. *J. Am. Chem. Soc*. **1994**, 12073-12074.

# Chapter 3

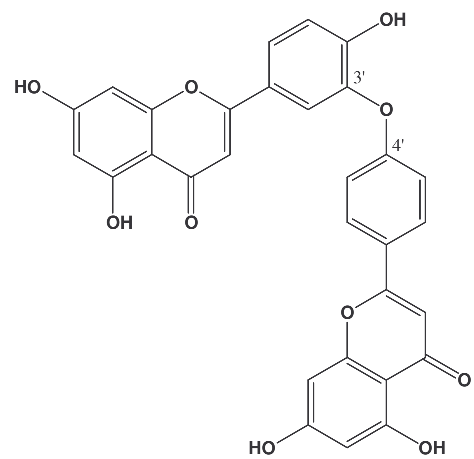
## SYNTHESES OF BI- AND TRIFLAVONOIDS

## 3.1 Introduction

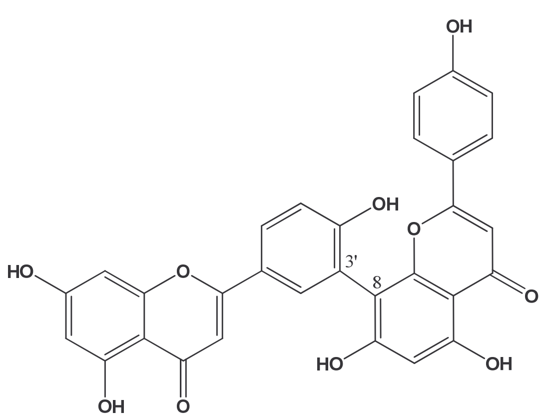
"Bi- and triflavonoids" represent a diverse family of dimers, trimers, and oligomers of flavonoid monomers that are not linked via the C-4 heterocyclic carbon and are consequently not classified as proanthocyanidins. Unlike the proanthocyanidins, they do not form coloured anthocyanidins upon treatment with acids. The flavonoids, characteristically have a carbonyl group or equivalent at the C-4 position (Figure 1). Together with proanthocyanidins, the bi- and triflavonoids constitute the two major classes of "complex $C_6$-$C_3$-$C_6$ secondary metabolites".[1]



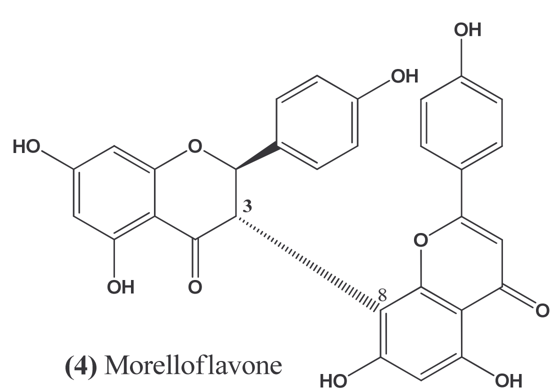
**(1)** Pancibiflavonol



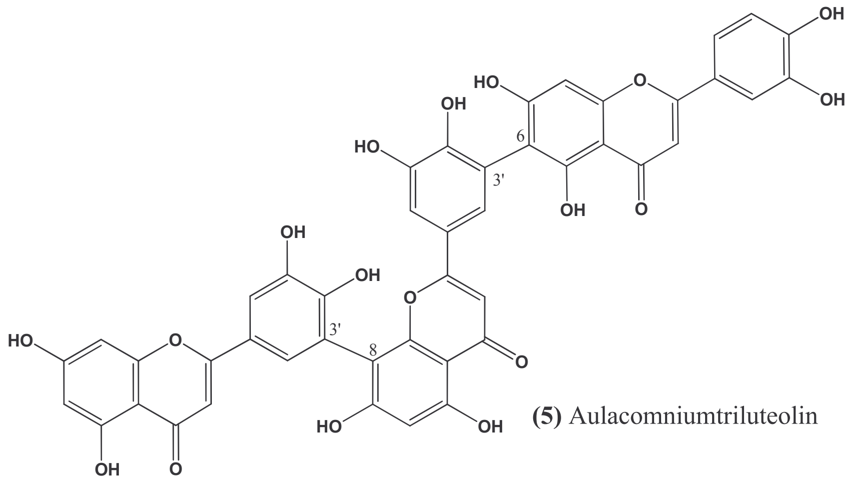
**(2)** Ochnaflavone



**(3)** Amentoflavone



**(4)** Morelloflavone



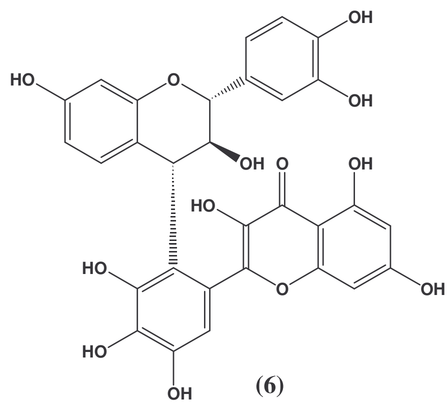

**(5)** Aulacomniumtriluteolin

**Figure 1:** The structures of some bi- and triflavonoids.

Bi-and triflavonoids that occur in nature are thought to be the products of phenol oxidative coupling of chalcones, aurones, auronols, isoflavones, flavanones, dihydroflavanols, flavanols and flavones. This contrasts with natural proanthocyanidins that are thought to be products of nucleophilic substitution of an hydroxy leaving group at C-4 of flavan-3,4-diols (*via* a C-4 carbocationic intermediate or via an $S_N2$ reaction). The oxidative nature of carbon-carbon bond formation leads to preservation of the substituents at C-4 of the starting materials. Tetra-, penta-, and hexaflavonoids have also been isolated and identified, and these compounds are also included in the class of bi- and triflavonoids. We will follow the Locksley system[2,3] of nomenclature for bi- and triflavonoids as described in Chapter 1 (pp 28 - 30 ). We will, however, not follow his suggestion that biflavonoids be called biflavanoids.

Compounds with a carbonyl group at C-4 that do not arise from phenol oxidative coupling of monomeric flavonoids, including compounds linked *via* carbon-oxygen bonds, have been reported as bi- or tri-flavonoids (e.g. compound **(50)**, Chapter 1, pp 29).

A growing number of "mixed" dimers[1] e.g. flavan-3-ol→flavonol (e.g. fisetinidol-[4α→2′]-myricetin (**6**)) originating from oxidative coupling of flavan-3-ols to flavonoids has been isolated and belongs to both classes.



(**6**)

**Figure 2:** Fisetinidol-[I-4α][II-2′]-myricetin.

The absolute configuration of optically active bi- and triflavonoids is often ignored, despite availability of sufficient chiroptical data to use empirical methods. Ferreira and co-workers[4] recently published an important paper on the use of computational chiroptical results to unambiguously interpret experimental chiroptical data.

Reviewing of all the bi-and triflavonoids that have been isolated from natural sources, their bioactivity and classification of these structurally diverse compounds into subgroups, falls outside the scope of this work. Further studies in these areas are available in the literature.[1,5-14]

## 3.2 Syntheses of bi- and triflavonoids

The structural diversity of bi- and triflavonoids[1] makes the development of general synthetic methods, as is the case with proanthocyanidins, impossible. For simplicity, we classify bi-and triflavonoids into four classes, depending on the nature and position of the interflavonoid bond.

### 3.2.1 Classes of bi- and triflavonoids (according to the nature and position of the interflavonoid bond).

**Class 1.** Bi- and triflavonoids where the carbon-carbon link is between aromatic rings. A plethora of synthetic methods exists to construct carbon-carbon inter-aromatic bonds (including Suzuki[15,16] and Stille[17] coupling reactions). These can be applied directly to bi-and triflavonoid synthesis.

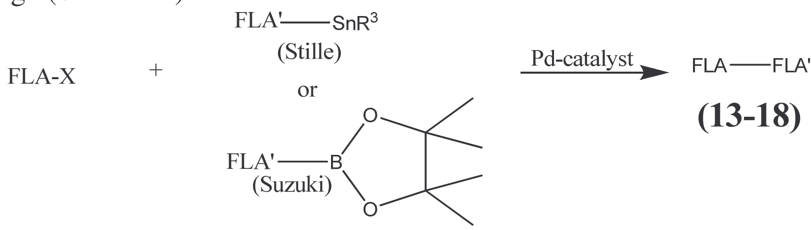
**Class 2.** Bi- and triflavonoids where the carbon-carbon link between the aromatic rings are replaced with a carbon-oxygen-carbon bond. Many synthetic methods exist to construct these inter-aromatic bonds (including the Ullmann coupling).[18-20] These can be applied directly to bi-and triflavonoid synthesis.

**Class 3.** Bi- and triflavonoid is where the carbon-carbon link is between the aromatic ring of one constituent monomer and the heterocyclic C-ring of another constituent monomer. As coupling of an aromatic ring on the 4-position of one constituent monomer will result in a proanthocyanidin (synthetic methods were extensively reviewed in the previous chapter), it leaves only C-2 and C-3 to couple. (I-3 and I-2 biflavones, including GB-flavones [I-3, II-8]-coupling and I-2 and I-3 bi-isoflavones). No I-2 biflavones have ever been isolated, and unless one considers A-type proanthocyanidins as biflavonoids, I-3 bi-isoflavones have not been isolated. Despite the interest that these I-2 and I-3 flavones have received and their considerable pharmaceutical potential,[4,21-27] no methods exists to synthesize any examples from this class.

**Class 4.** Bi- and triflavonoids where the carbon-carbon link is between the heterocyclic C-rings.

### 3.2.1.1 Class 1

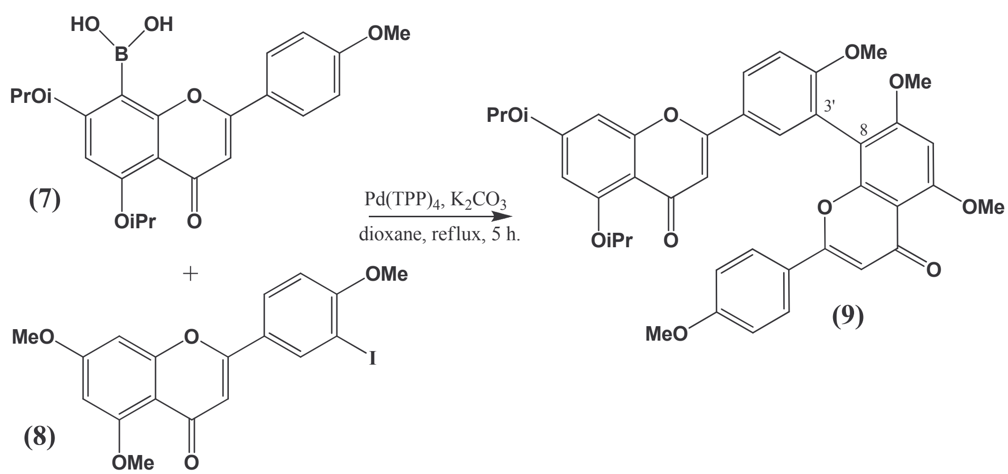
The Suzuki[15,16] and Stille[17] coupling methods, based on transition metal-catalyzed cross coupling methodology, were employed in biflavonoid synthesis to connect two flavone units via a biaryl linkage (Scheme 1).




**(13-18)**

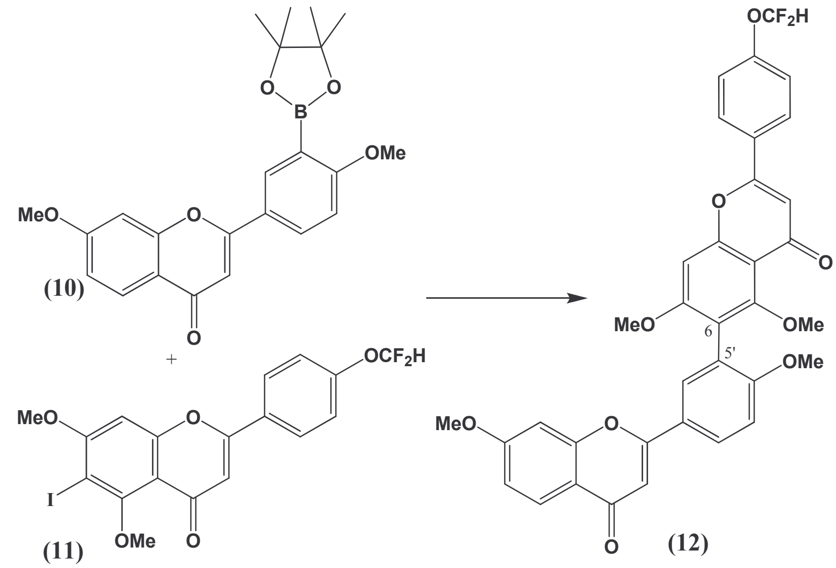
**Scheme 1:** Typical Stille and Suzuki cross-coupling reactions for C-C biflavones.

Muller and Fleury[16] synthesized amentoflavone 3',8-biflavonoid analogues (e.g. (**9**)) from 8-flavone-boronoic acids and 3'-iodoflavones (Scheme 2).



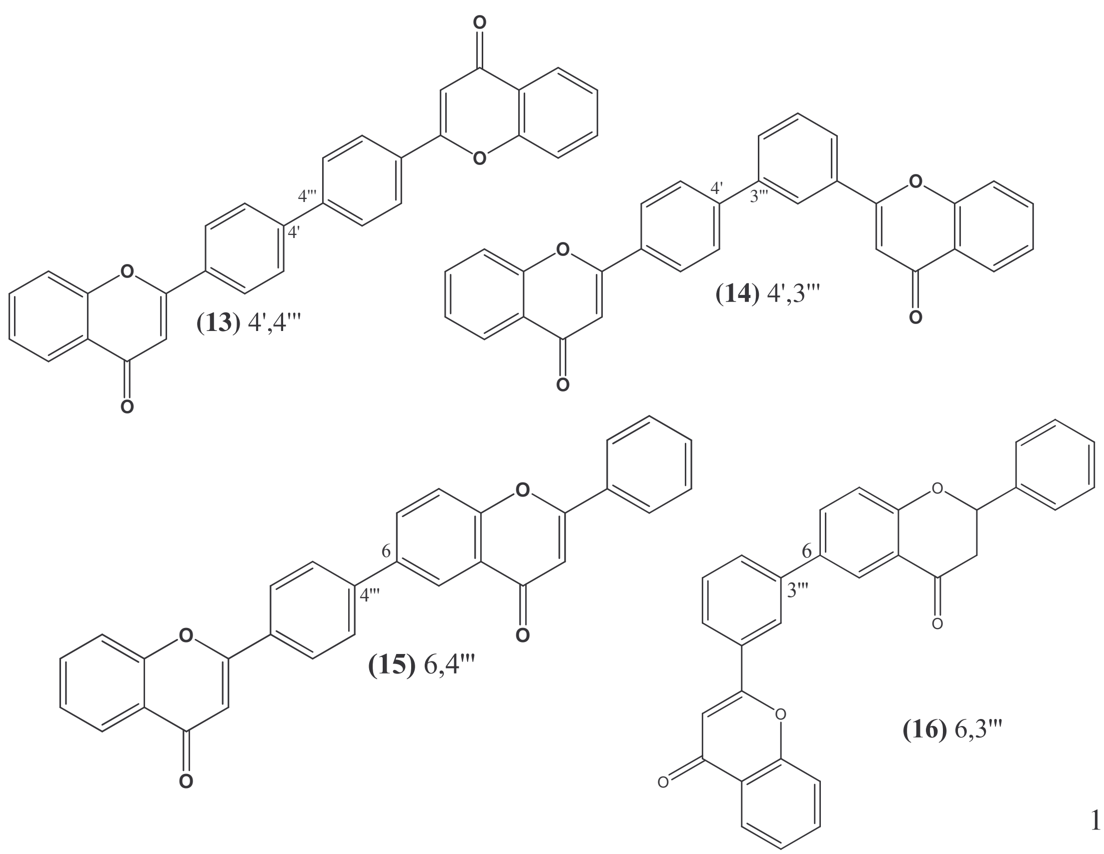

**Scheme 2:** Synthesis of amentoflavone ether with the Suzuki coupling reaction.

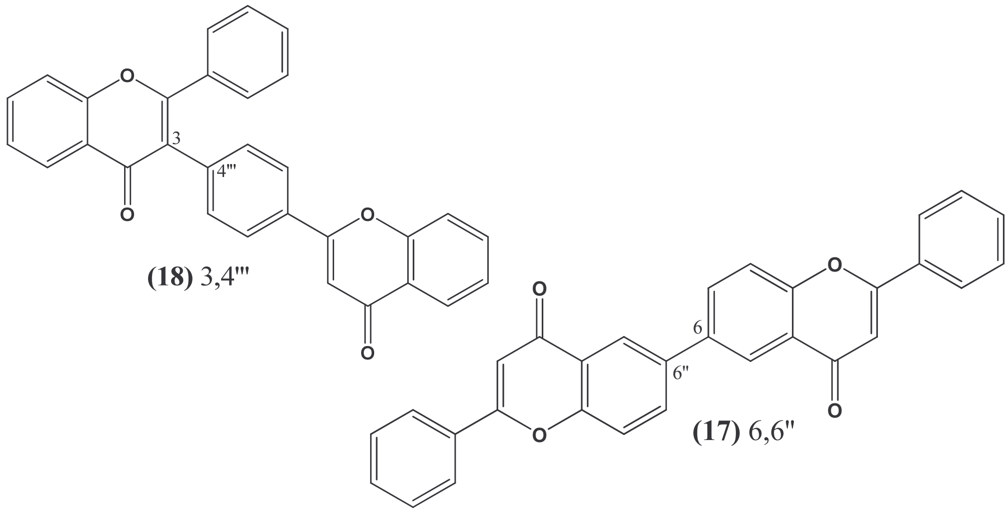
Qing and co-workers[9] synthesized a *gem*-difluoromethylated biflavonoid via the Suzuki coupling reaction (Scheme 3).




**Scheme 3:** Synthesis of *gem*-difluoromethylenated biflavonoid with the Suzuki reaction.

Park and co-workers[5,8] synthesized six C-C biflavonoid classes (Scheme 1) *via* Stille and Suzuki conditions (based on the way the flavone subunits are linked, Figure 3) for pharmaceutical screening and to study structure – activity relationship (SAR).[7,26,27]





**(18)** 3,4'''

**(17)** 6,6''

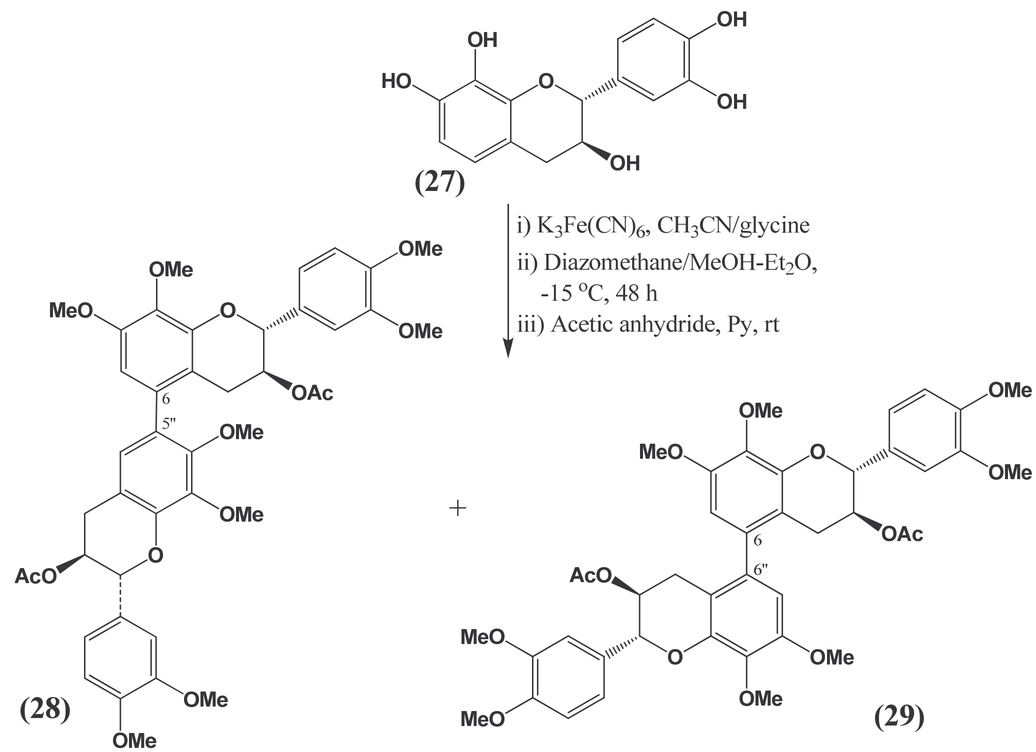
**Figure 3:** Biflavonoid classes synthesized by Park and co-workers *via* Stille and Suzuki conditions.

Four of the classes **(13, 14, 16, 17)** were prepared under Stille conditions,[5,8,17] which involved treatment of 1.3 equivalent of tributyltinflavones, obtained from the corresponding bromoflavones with bromoflavones (3-, 6-, 3′-, and 4′-) in refluxing toluene with $Pd(PPh_3)_4$) in 25 – 50% yield (Scheme 1). Chromatography was required.

The remaining two classes **(15)** and **(18)** were prepared under Suzuki conditions[5,8,15] (which involved treatment of 1,2 equivalents of pinacolatoboronate with bromoflavones (3′- and 3-, 1.0 equiv.) at 90 $^{o}C$ with 5 mol % $Pd(PPh_3)_4$, NaOH (4 equiv.) in DMF-$H_2O$ (9:1)) in 31 and 21% yield, respectively (Scheme 1).[8]

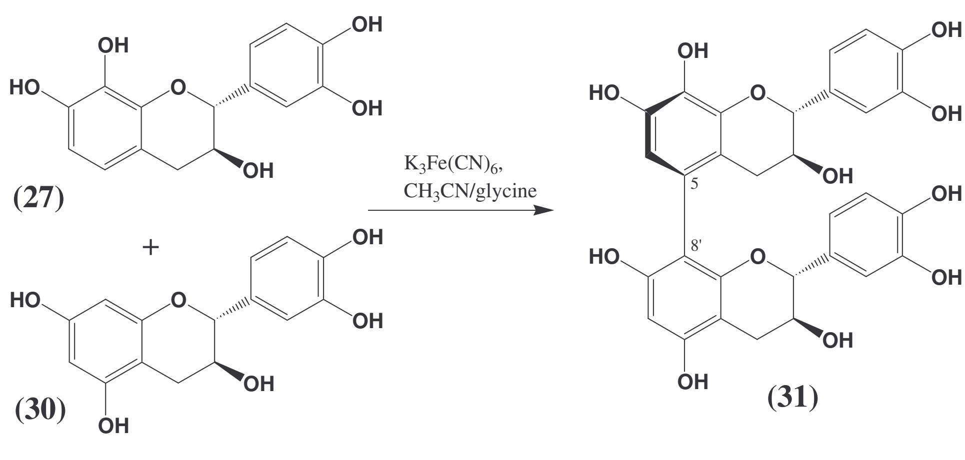
Roux and co-workers[28] demonstrated oxidative dimerization of flavan-3-ols (Scheme 4). Treatment of mesquitol **(27)** with $K_3Fe(CN)_6$ in an acetonitrile-glycine buffer[29] gave **(28)** and **(29)** after methylation and acetylation in a ratio of 2.5:1.

.



**Scheme 4:** Oxidative dimerization of mesquitol.

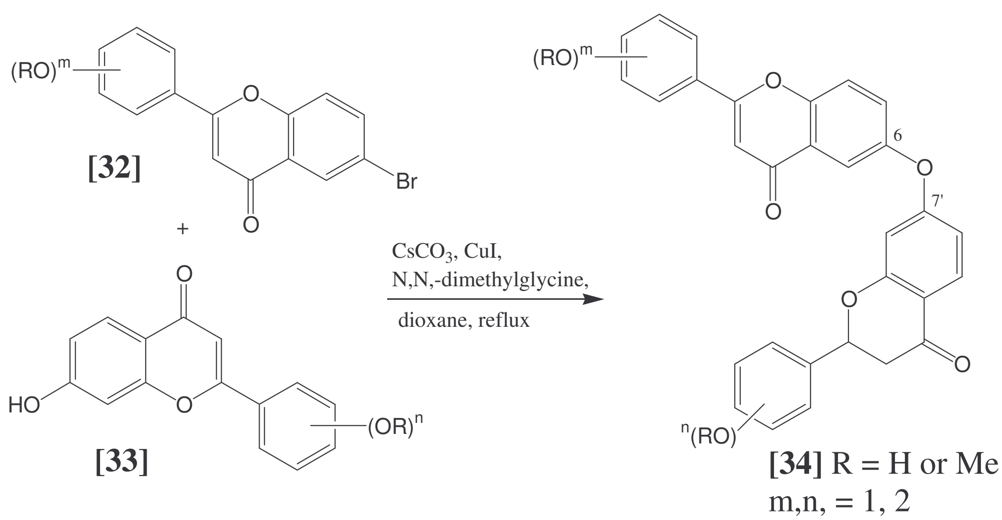
Treatment of equimolar quantities of catechin **(30)** and mesquitol **(27)** under the same conditions[28] gave the atropisomer **(31)** as the major product (Scheme 5).



**Scheme 5:** Oxidative condensation of mesquitol and catechin.

### 3.2.1.2 Class 2

Park and co-workers,[30] in 2008, employed intermolecular Ullmann ether synthesis methodology[31,32] ($CsCO_3$, CuI, *N,N*-dimethylglycine, dioxane, reflux), to synthesize 17 C-O-C biflavones with 6-*O*-7'' linkages from 6-bromo- **(32)** and 7-hydroxyflavones **(33)** (Scheme 6). Deprotection of the methylethers with $BBr_3$ in $CHCl_3$ yielded free phenolic analogues for testing their effects on cyclo-oxygenase-2 and nitric oxide synthase enzymes.

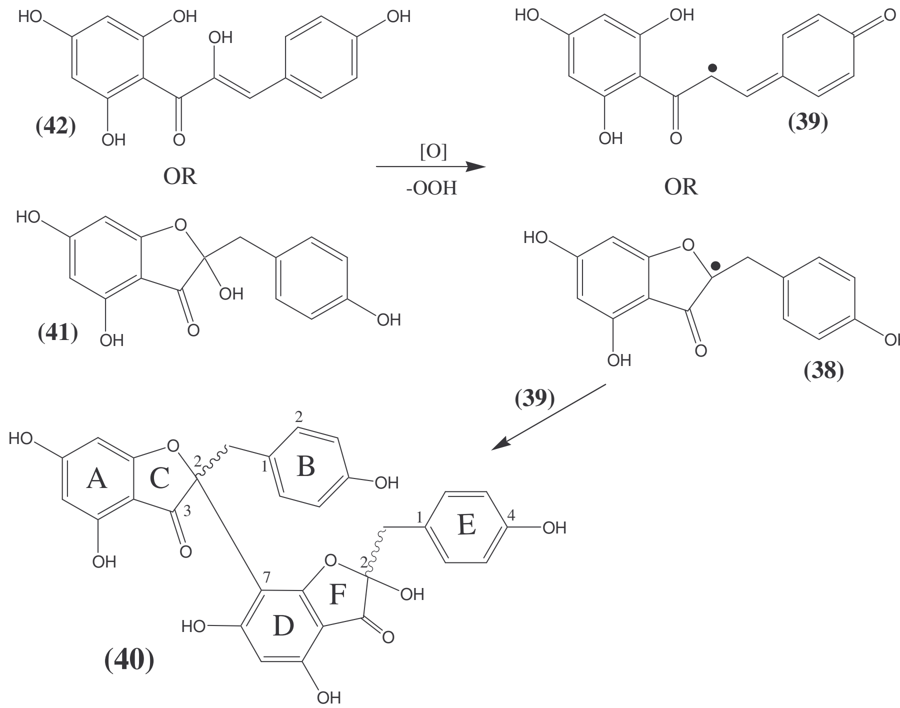



**Scheme 6:** Typical intermolecular Ullmann ether syntheses for 6-*O*-7″ biflavones.

### 3.2.1.3 Class 3.

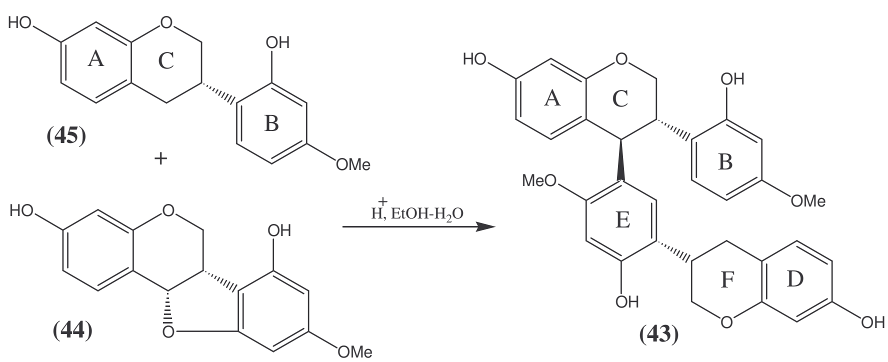
We could not find any literature describing synthetic methods to obtain examples of this class, despite the fact that morelloflavone **(4)** exhibits anti-inflammatory activity.

Ferreira and co-workers[33] speculated about the intermediacy of a quinomethane radical[34] **(38)** or **(39)** in the biosynthesis of naturally occurring 2,7-bibenzofuranoids **(40)** from maesopsin **(41)** or an α-hydroxychalcone **(42)** (Scheme 8).



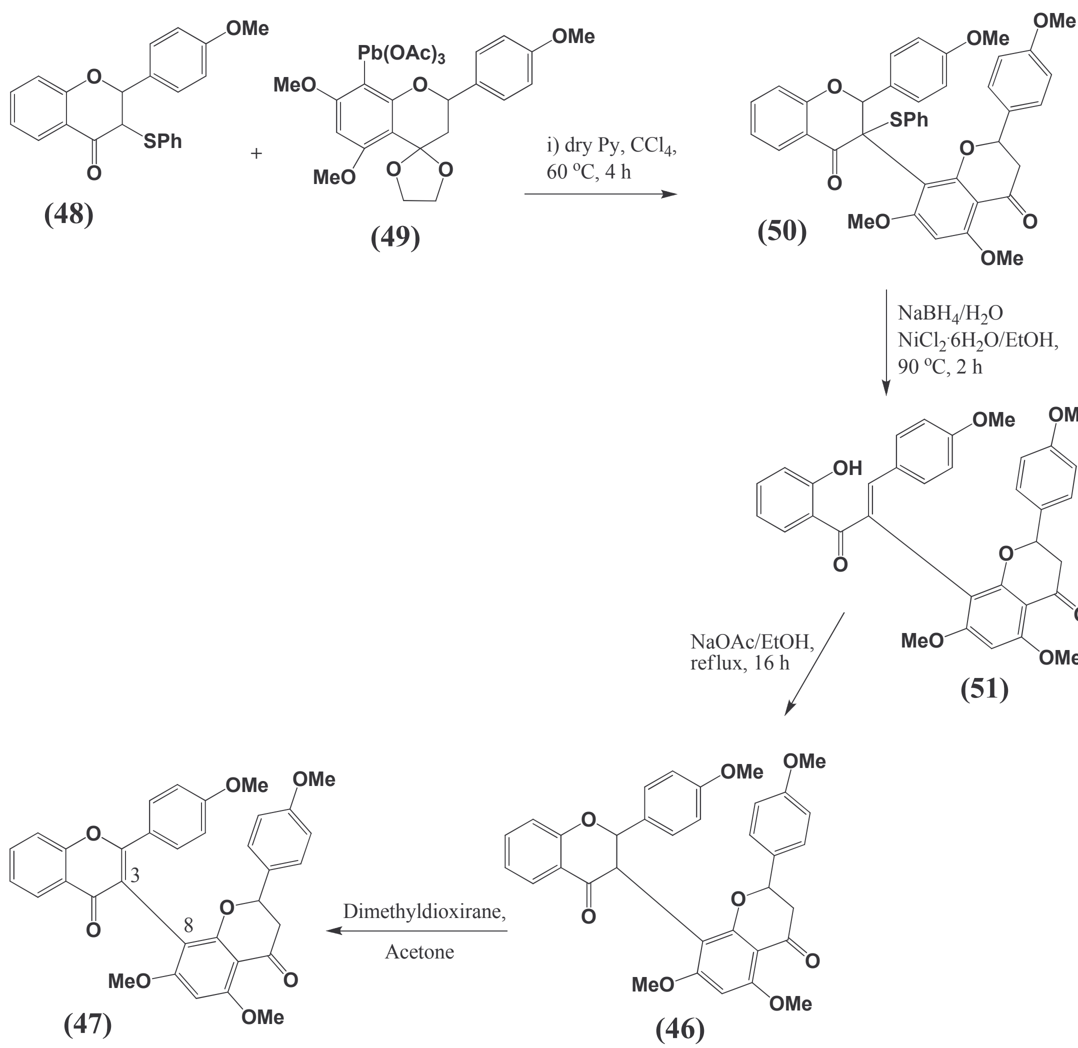
**Scheme 8:** Proposed biosynthesis of bibenzofuranoids.

We consider the synthesis of the first natural [I-4,II-3′′] bi-isoflavonoid **(43)**, isolated from *Dalbergia nitidula* and synthesized by Roux and co-workers[35,36] *via* nucleophilic attack on an isoflavanyl-4-carbocation [generated from a pterocarpan **(44)**], as a special case of the C-4 arylsubstituted proanthocyanidin syntheses discussed in the previous chapter. The B-ring of the isoflavanyl nucleophile is more oxygenated and reactive than its A-ring and a C4→C3'-linkage is formed (Scheme 9).



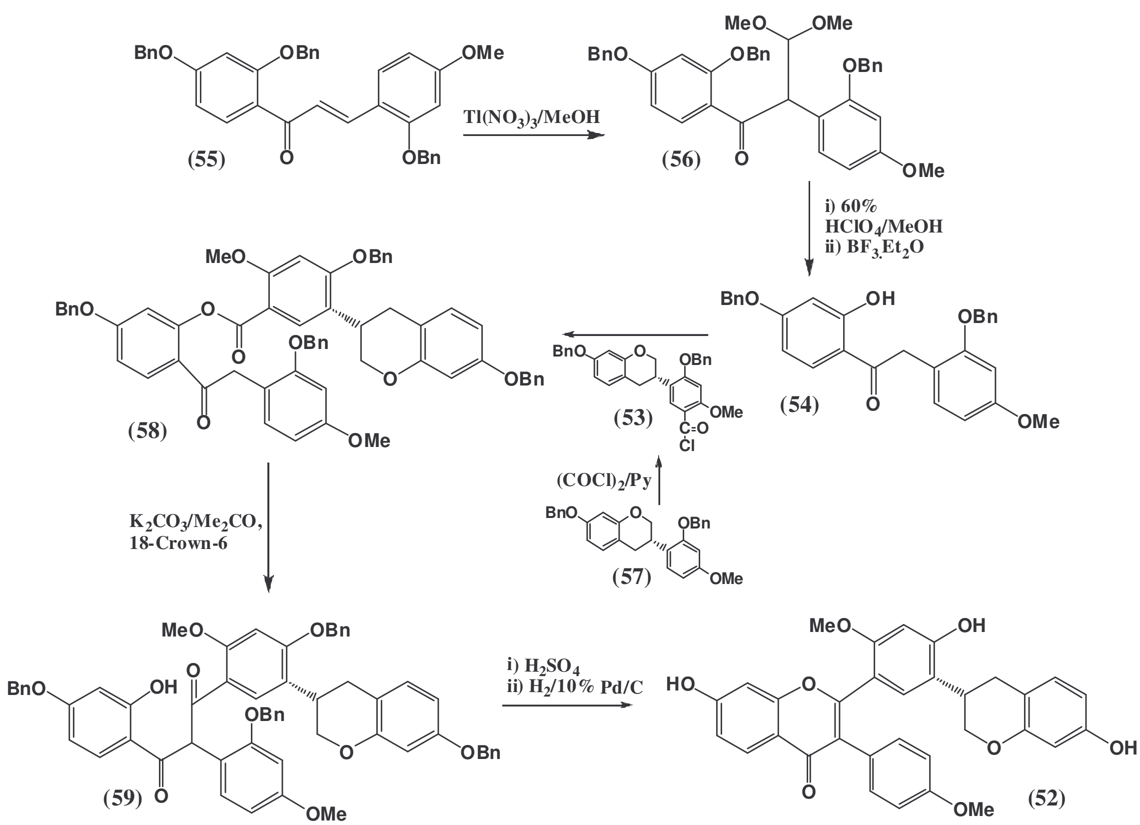
**Scheme 9:** Synthesis of a bi-isoflavonoid via nucleophilic substitution at C-4.

Donnelly and co-workers[37] synthesized the biflavonoids **(46)** and **(47)** *via* arylation of a 3-phenylsulfanylflavanone **(48)** with an 8-triacetoxyplumbylflavan derivative **(49)** (2:1 mixture of *cis* and *trans*). The dioxolane ring of the intermediate **(50)** was cleaved during acid workup. Desulfurization of the intermediate **(50)** with nickel boride ($NaBH_4/H_2O$, $NiCl_2{\cdot}6H_2O/EtOH$) yielded a chalcone **(51)** (in a 1.1:1 ratio of *E*/*Z*) that was cyclised with anhydrous sodium acetate in refluxing ethanol to the 3,8- biflavanone **(46)** in 73% yield (only the 2,3-*trans* isomer was isolated from a mixture of diastereoisomers with an α/β ratio of 1.4:1). Oxidation of **(46)** with dimethyldioxirane in acetone yielded **(47)** in 47% yield (Scheme 10).




**Scheme 10:** Aryllead triacetate-mediated synthesis of biflavonoids.

Synthesis of the isoflavone-isoflavan dimer **(52)**, isolated from *Dalbergia nitidula* posed a challenge. It is linked *via* an electron deficient β-carbon in an α,β-unsaturated carbonyl moiety and no simple biomimetic equivalent of the proanthocyanidin syntheses described in the previous chapter was available. Ferreira and co-workers[38] tried a variety of options, including coupling of nucleophilic phenolic units to aromatic oxygenated isoflavone-2,3-epoxides,[39] direct coupling of phenolic units to isoflavones in a 1,4-Michael fashion, linkage of phenolic units to the acetal-type electrophilic centre of the intermediate in isoflavone synthesis prior to heterocycle construction (thallium(III)nitrate strategy)[40,41] and condensation of a $C_{16}$ (5'-formylated isoflavan) **(53)** with a $C_{14}$-unit (2-hydroxydeoxybenzoin) **(54)** in a modified Baker-Venkataraman reaction.[42] The last strategy succeeded in producing the target dimer **(52)** (Scheme 11).

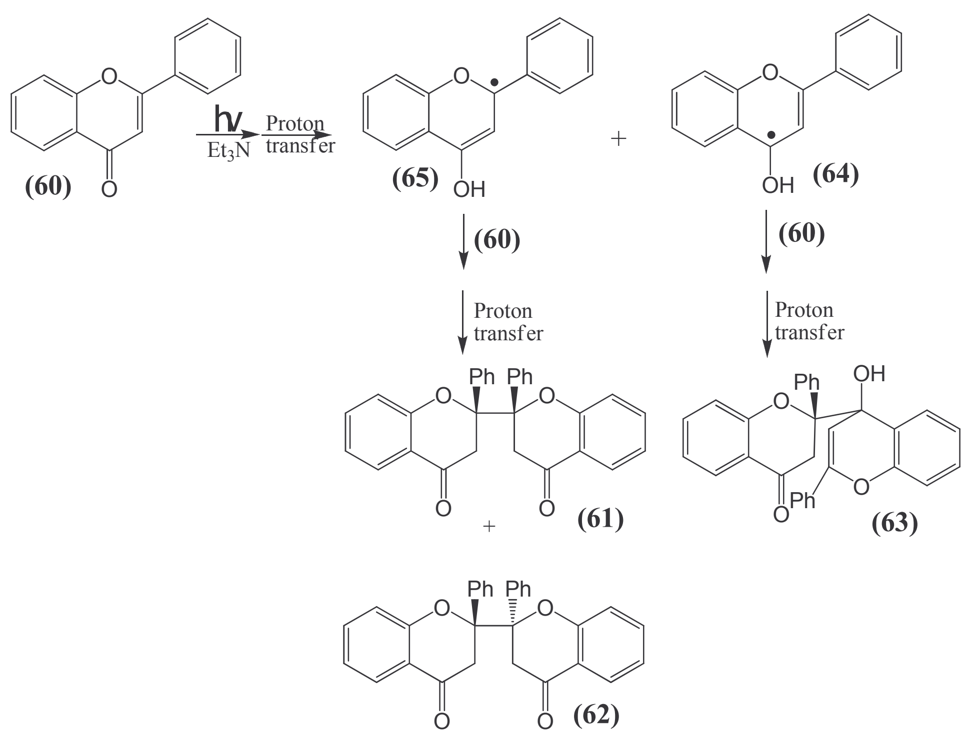



**Scheme 11:** Synthesis of a C-ring dimer *via* a modified Baker-Venkataraman process.

The carboxylic moiety in **(53)** (the $C_{16}$ unit) was introduced *via* formylation of the B-ring of **(57)** with the photochemical Reimer-Tieman reaction.[43,44] The deoxybenzoin **(54)** (the $C_{14}$ unit) was obtained from oxidation of the chalcone **(55)** with $Tl(NO_3)_3$ to **(56)** followed by decarbonylation with perchloric acid.

### 3.2.1.4 Class 4

Xu and co-workers[45] described a photo-induced single electron transfer (SET) dimerization of flavone **(60)**. This amine catalyzed photochemical reaction yielded 2,4'- **(61)** and **(62)**, and 2,2'-biflavanoids **(63)** in good yields (77% in acetonitrile and 94% in benzene). The mechanism was postulated to take place *via* addition of a 4-ketyl- **(64)** or 2-ketyl radical **(65)** to the flavone **(60)** (Scheme 12).

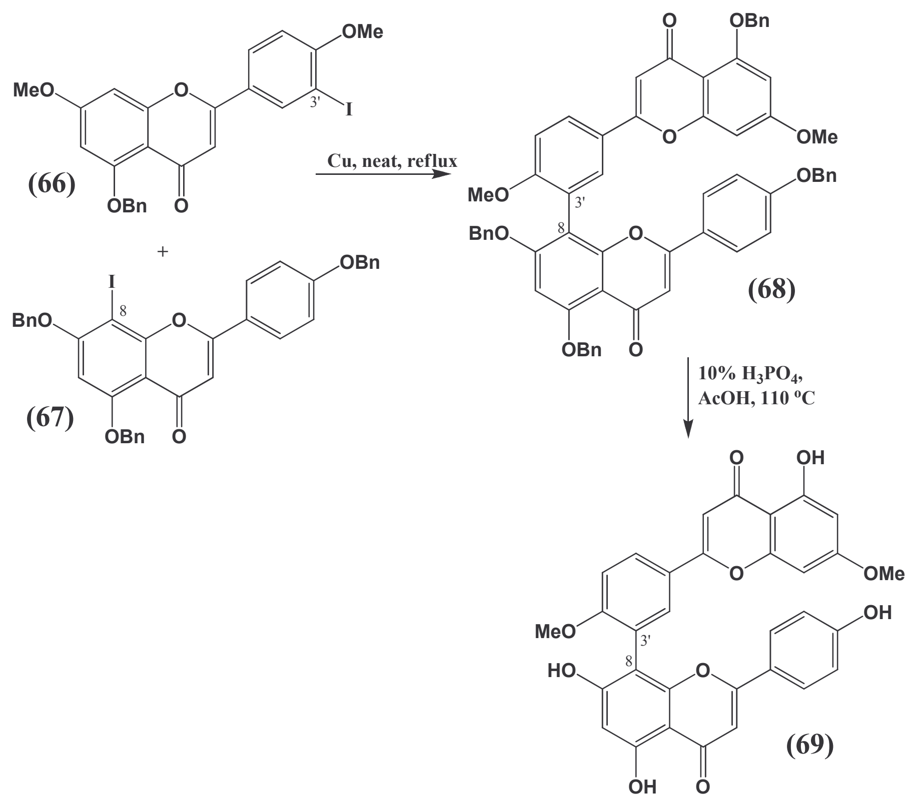



**Scheme 12:** Synthesis of biflavonoids linked *via* their heterocyclic rings.

### 3.2.2 Classes of biflavonoids according to the chemistry employed to synthesize each class

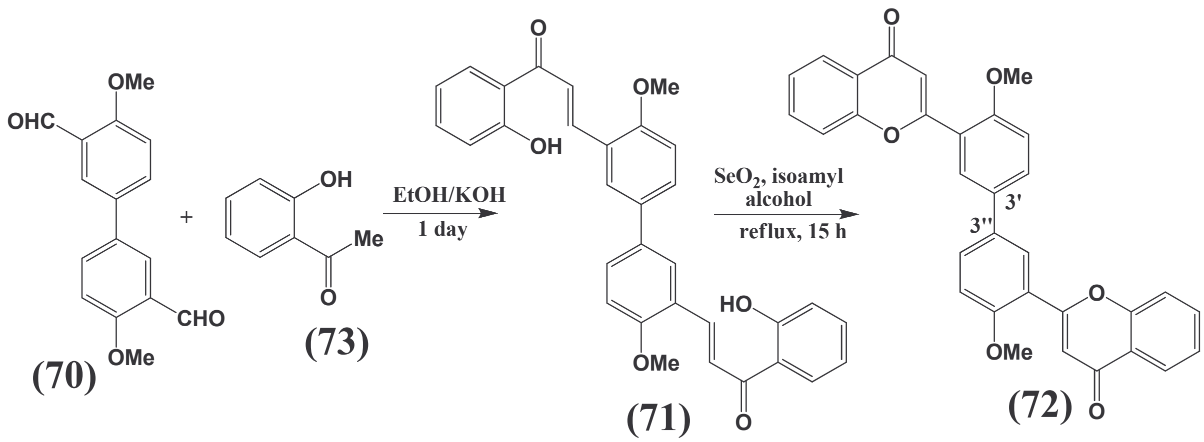
Desai and co-workers[3] classified biflavonoids into nine classes depending on the chemistry involved in their synthesis:

**1. Ullmann coupling of halogenated flavones** (this Ullmann reaction gives carbon-carbon bonds and should be distinguished from the Ullmann ether synthesis that gives biflavonoids linked via carbon-oxygen-carbon bonds as described in class 2 above). This method gives predominantly symmetrical biflavonoids. This reaction requires high temperatures (260-300 $^{o}$C) and consequently suffers from poor yields due to decomposition and resin formation. Examples include the synthesis of ginkgetin **(69)** (Scheme 15)[18].




**Scheme 13:** Synthesis of ginkgetin by Ullmann Coupling.

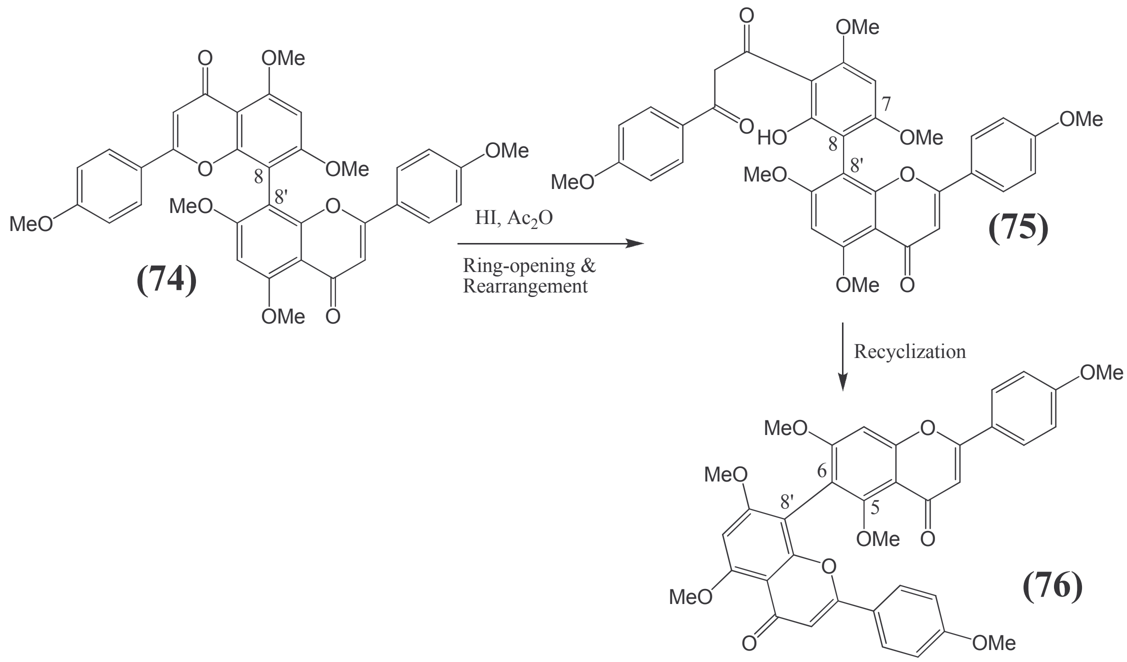
**2. Using biphenyls as precursors.** An example of this method is given in Scheme 14 where a biphenyl **(70)** is used to synthesize a chalcone **(71)** that is cyclized to a biflavonoid **(72)**[46].




**Scheme 14:** Synthesis of biflavonoids from biphenyl precursors.

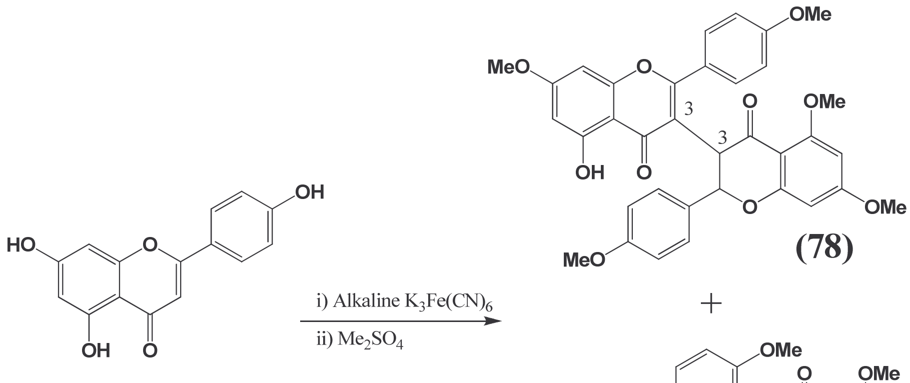
**3. Metal-catalyzed cross coupling of flavones.** Various examples using Stille or Suzuki methodology to link two aromatic rings were given under class 1 above, Schemes 1 and 2).

**4. Wesseley-Moser rearrangements.**[47] This reaction does not create new interflavonoid bonds but can be used to change the substitution pattern on the A-ring of an existing biflavonoid (*via* opening and closing of the C-ring). Scheme 15 gives the synthesis (rearrangement) of **(74)** to **(76)**. The rearrangement is characterized by the restructuring of 5,7,8-substituents to a 5,6,7-substitution pattern under acidic conditions.




**Scheme 15:** Synthesis of a biflavonoid using the Wesseley – Moser rearrangement.[47]

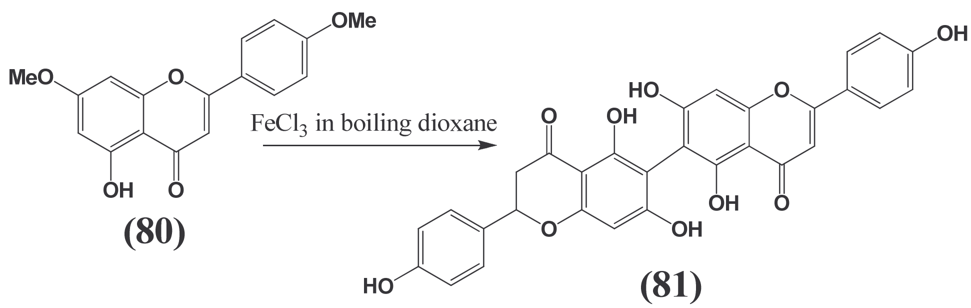
**5. Oxidative coupling of phenols.** This biomimetic method probably follows the natural biosynthetic process. Examples of the oxidative dimerization of apigenin **(77)** are given in Scheme 16.[48] This method does not require protection of free phenolic hydroxy groups and only the B-ring is involved in dimerization. The inter-flavone linkages may be formed *via* coupling of two radicals. Electron spin-resonance studies,[43] however, indicate that an unpaired electron at the C4'–OH of apigenin **(77)** can be delocalized to C-3', C-1' and C-3 only (not the A-ring). Electron rich A-ring (at C-6 or C-8) attacks the delocalized electron to give a biflavonoid (*via* electrophilic substitution and not radical pairing).




(77)

**Scheme 16:** Synthesis of biflavonoids **(78)** and **(79)** *via* oxidative coupling.

It was suggested that protection of the OH groups at C-4'- and C-7 of apigenin would allow radical generation on the A-ring. Apigenin-4'-dimethyl ether **(80)** is oxidized with $FeCl_3$ in boiling dioxane to the biflavone **(81)** in 6 % yield (Scheme17).[49]

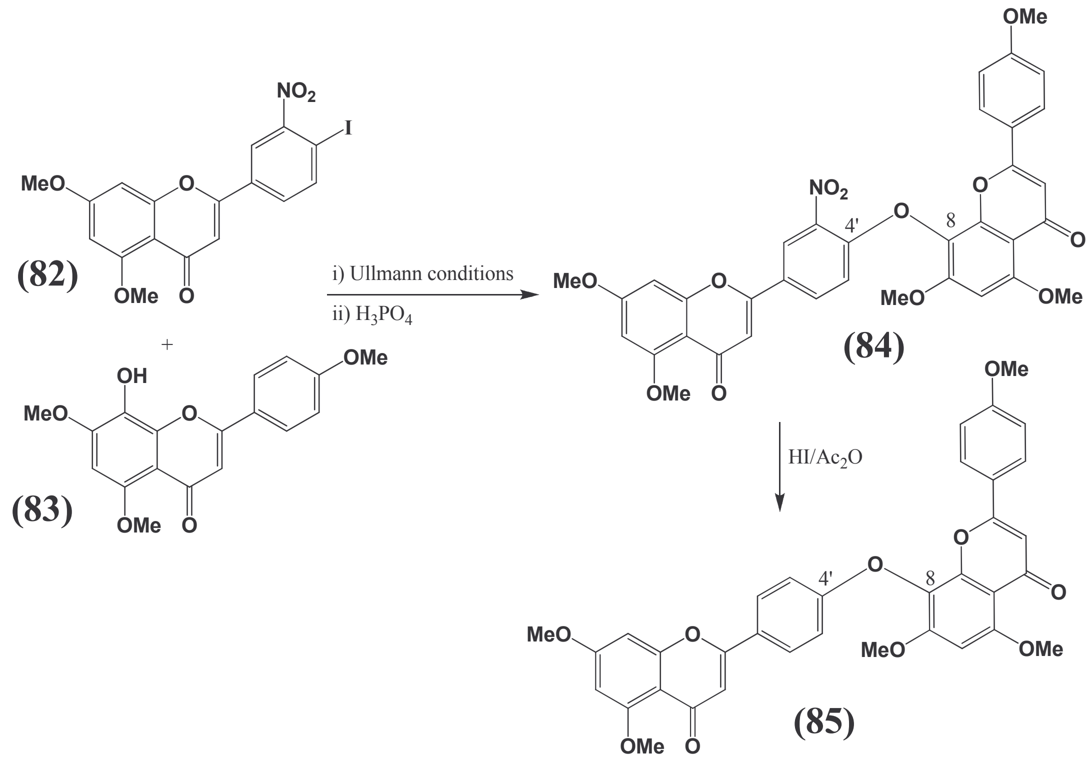



(80)

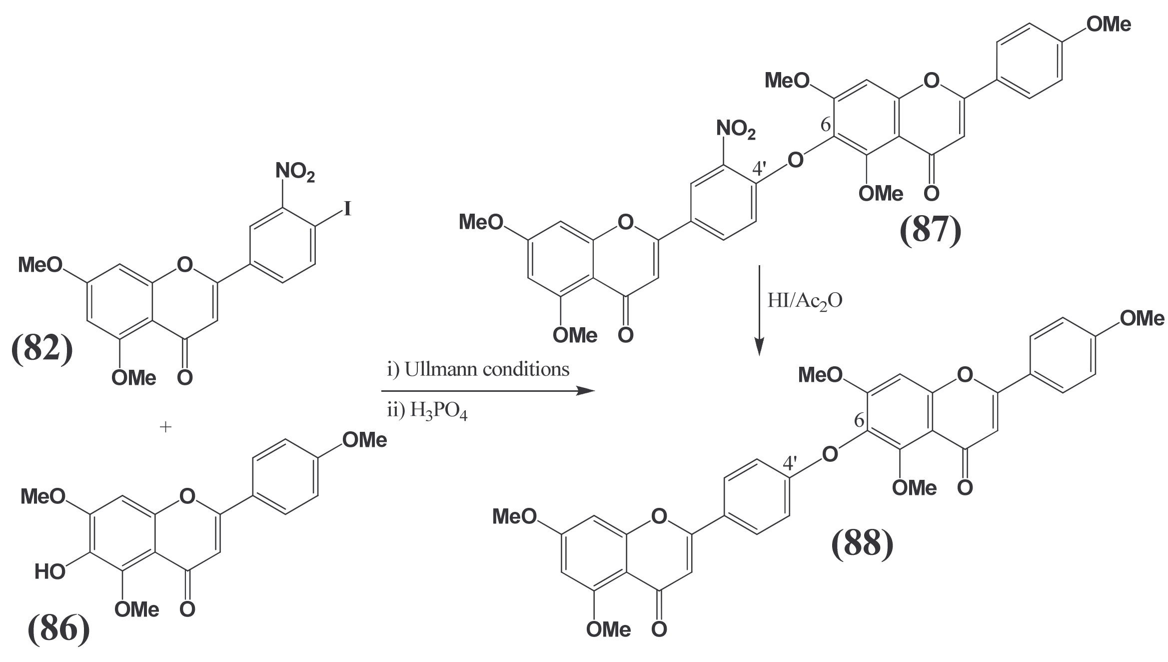
(81)

**Scheme 17:** Synthesis of biflavonoid **(81)** *via* oxidative coupling on the A-ring.

**6. Ullmann condensation** (Cu-catalyzed coupling of flavone salts and halogenated flavones). This reaction has the potential to provide an ether-linked biflavonoid in a single step. The reaction is depicted in Schemes 18 and 19,[50] and gives yields in excess of 70%. The reactivity of the halogen atom was enhanced by the electron-withdrawing $NO_2$ group *ortho* to the halogen atom.



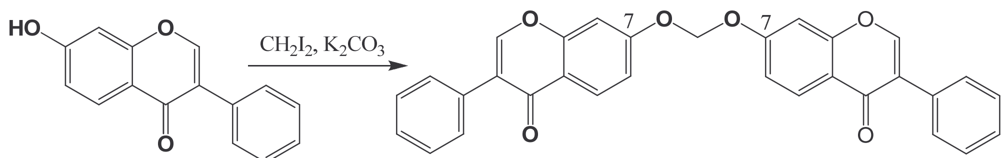

**Scheme 18:** Synthesis of biflavonoid (**85**) *via* Ullmann condensation.




**Scheme 19:** Synthesis of biflavonoid (**88**) *via* Ullmann condensation.

## 7. Nucleophilic substitution.

Methylenated phenolic groups are common in nature. Scheme 20[3] gives an example of the synthesis of the symmetrical methylene linked biflavonoid **(89)** *via* nucleophilic substitution of the iodine atoms on $CH_2I_2$ with a flavone aromatic hydroxy group.

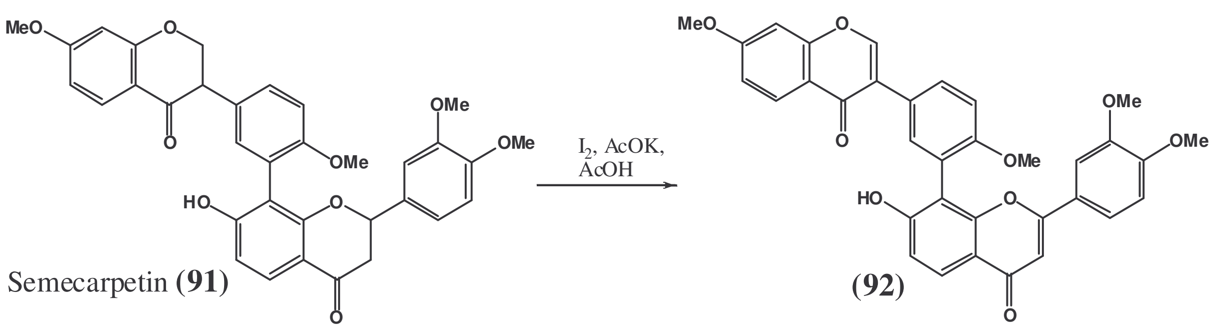


**(90)** **(89)**

**Scheme 20:** Synthesis of bi-isoflavonoid **(89)** *via* nucleophilic substitution.

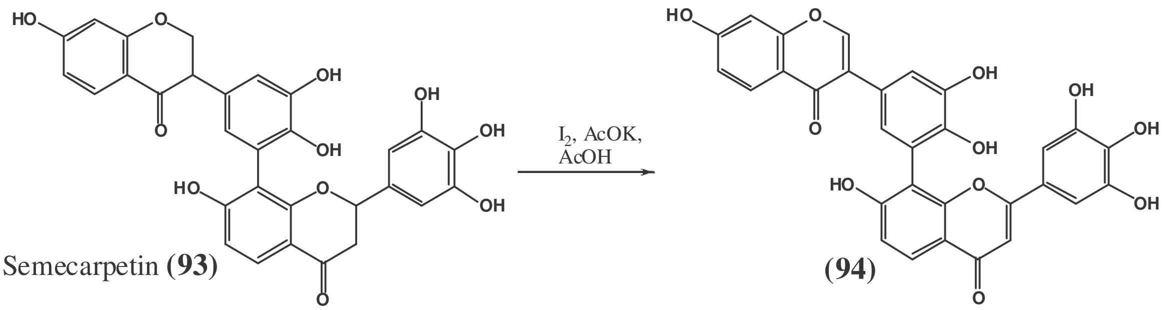
## 8. Dehydrogenation of biflavanones to biflavones.

Dehydrogenation of the α,β-unsaturated moiety of biflavanone dimers to their biflavone equivalents have been achieved with Fentons reagent, alkaline potassium ferricyanide, $SeO_2$, or N-bromosuccinimide.[3] Murthy[51] , however, achieved dehydrogenation of semicarpetin **91** and galluflavanone **93** with $I_2$ and AcOK in AcOH under reflux to yield **92** and **94**, respectively (Schemes 21 and 22).



Semecarpetin **(91)** **(92)**

**Scheme 21:** Dehydrogenation of a biflavanone to a biflavone.



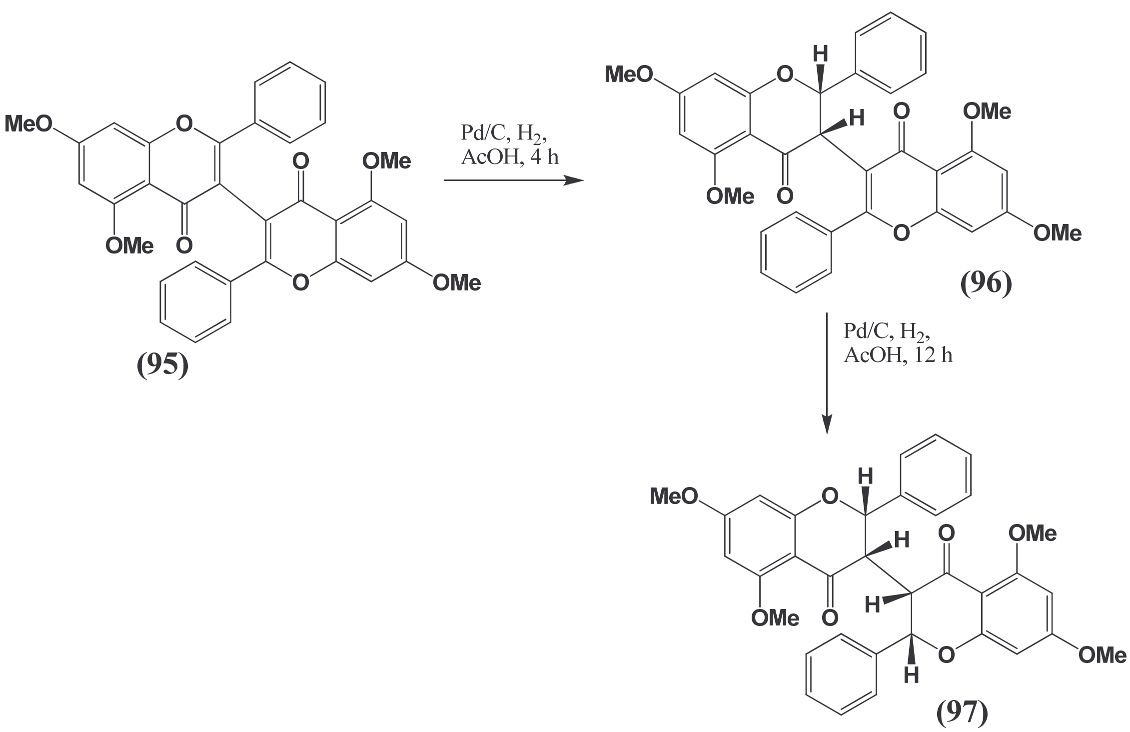
Semecarpetin **(93)**

**(94)**

**Scheme 22:** Dehydrogenation of a biflavanone to a biflavone.

### 9. Hydrogenation of biflavones to biflavanones

This is the reverse of dehydrogenation and it is possible to produce mono-dihydrogenated biflavonoids selectively (Scheme 23).[52] Yields are usually low (about 35%). Li and co-workers[52] tried various hydrogenating conditions but could not increase yields.



**(95)**

**(96)**

**(97)**

**Scheme 23:** Synthesis of biflavanoids **(96)** and **(97)**.

## 10. Conclusion

The various approaches developed through recent years to synthesize biflavonoids include the synthesis of symmetrical biflavonoids via the Ullmann coupling procedure and their unsymmetrical counterparts by using the Stille or Suzuki coupling methods. The diether-linked biflavonoids are synthesized via a nucleophilic substitution approach, while the Wessely-Moser protocol leads to rearrangment of certain biflavonoids. The range of biflavonoid structures is further expanded by dehydrogenation-based or hydrogenation-based syntheses. Yet, it is clear that most of these synthetic approaches deliver low yields, and a more rapid and robust protocol is needed to readily generate structurally diverse biflavonoids for structure-activity studies. The interesting biological properties of biflavonoids have stimulated research to synthesize these molecules successfully, and an improvement in the synthetic strategies should lead to the discovery of more potent biflavonoids.

# REFERENCES


1. Ferreira, D; Slade, D; Marais, J. P. J. *Flavonoids Chemistry, Biochemistry and Applications, edited by ∅yvind M. Andersen K. R. Markhams, CRC press* **2006**, 553-615, 1102-1135.
2. Locksley, H. D. *Fortschr. Chem. Org. Naturst*. **1973**, *30*, 207.
3. Rahman, M.; Riaz, M.; Desai, U. R. *Chem. & Biodiv*. **2007**, *4*, 2495-2527; and refs therein.
4. Ding, Y.; Li, X. -C.; Ferreira, D. *J. Org. Chem.* **2007**, *7*, 9010-9017.
5. Chen, J.; Chang, H. W.; Kim, H. P.; Park, H. *Bioorg. Med. Chem. Lett*. **2006**, *16*, 2373-2375.
6. Svenningsen, A. B.; Madsen, K. D.; Liljefors, T.; Stafford, G.I.; van Staden, J.; Jäger, A. K. *J. Ethnopharmcology* **2006**, *103*, 276-280.
7. Suh, S. J.; Chung, T. -W.; Son, M. -J.; Kim, S. -H.; Moon, T. C.; Son, K. H.; Kim, H. P.; Chang, H. W.; Kim, C. -H. *Arch. Biochem. Biophys*. **2006**, *447*, 136-146.
8. Moon, T. C.; Quan, Z.; Kim, J.; Kim, H. P.; Kudo, I.; Murakami, M.; Park, H.; Chang, H. W. *Bioorg. Med. Chem. Lett*. **2007**, *15*, 7138-7143.
9. Zheng, X.; Meng, W. -D.; Qing, F. -L. *Tetrahedron lett.* **2004**, *45*, 8083-8085.
10. Lee, M. K.; Lim, S. W.; Yang, H.; Sung, S. H.; Lee, H.-S.; Park, M. J.; Kim, Y. C. *Bioorg. Med. Chem. Lett*. **2006**, *16*, 2850-2854.
11. Gil, B.; Sanz, M. J.; Terenncio, M. C.; Gunasegaran, R.; Paya, M.; Alcaraz, M. J. *Biochem. Pharmacol.* **1997**, *53*, 733-740.
12. O'Leary, K. A.; de Pascual-Tereasa, S.; Needs, P. W.; Bao, Y. -P.; O'Brien, N. M.; Williamson, G. *Mutation Res.* **2004**, *551*, 245-254.

13. Lee, C. -W.; Choi, H. -J.; Kim, H. -S.; Kim, D. -H.; Chang, I. -S.; Moon, H. T.; Lee, S. -Y.; Oh, W. K.; Woo, E. –R. *Bioorg. Med. Chem. Lett*. **2008**, *16*, 732-738.

14. Harborne, J. B.; Williams, C. A. *Phytochemistry* **2000**, *55*, 481-504.

15. Miyaura, N.; Suzuki, A. *Chem. Rev*. **1995**, *95*, 2457.

16. Muller, D.; Fleury, J. -P. *Tetrahedron lett.* **1991**, *32*, 2229-2232.

17. Stille, J. K. *Angew. Chem., Intl. Ed. Engl*. **1986**, *25*, 508.

18. Nakazawa, K.; Ito, M. *Chem. Pharm. Bull*. **1963**, 283.

19. Ahmed, S.; Razaq, S. *Tetrahedron* **1976**, *32*, 503.

20. Zhang, F. -J.; Lin, G. -Q.; Huang, Q. -C. *J. Org. Chem*. **1995**, *60*, 6427.

21. Rucksthl, M.; Beretz, A.; Anton, R.; Landry, Y. *Biochem. Pharmacol*. **1979**, *28*, 535.

22. Iwu, M. M.; Igboko, O. A.; Okunji, C. O.; Tempesta, M. S. *J. Pharm. Pharmacol*. **1990**, *42*, 290.

23. Amella, M.; Bronne, C.; Briancon, F.; Hagg, M.; Anton, R.; Landry, Y. *Planta Med.* **1985**, *51*, 16.

24. Sun, C. -M.; Syu, W. -J.; Huang, Y. -T.; Chen, C. -C.; Ou, J. -C. *J. Nat. Prod*. **1997**, *60*, 382.

25. Lin, Y. -M.; Flavin, M. T.; Cassidy, C. S.; Mar, A.; Chen, F. C. *Bioorg. Med. Chem. Lett*., **2001,** 11, 2101.

26. Chang, H. W.; Baek, S. H.; Chung, K. W.; Son, K. H.; Kim, H. P.; Kang, S. S. *Biochem. Biophys. Res. Commun*. **1994**, *205*, 843.

27. Gil, B.; Sanz, M. J.; Terencio, M. C.; Gunasegaran, R.; Paya, M.; Alcaraz, M. *J. Biochem. Pharmacol*. **1997**, *53*, 733.

28. Young, E.; Brandt, E. V.; Young, D. A.; Ferreira, D.; Roux, D. G. *J. Chem. Soc., Perkin Trans. I* **1986**, 1737-1749.

29. Hundt, H. K. L.; Roux, D. G. *J. Chem. Soc., Perkin Trans. I* **1981**, 1227-1235.

30. Che, H.; Park, B. K.; Lim, H.; Kim, H. P.; Chang, H. W.; Jeong, J. -H.; Park, H. *Bioorg. Med. Chem. Lett*. **2009**, *19*, 74 – 76.

31. Sawyer, J. S. *Tetrahedron* **2000**, *56*, 5045.

32. Frlan, R.; Kikelj, D. *Synthesis* **2006**, *14*, 2271.

33. Bekker, R.; Ferreira, D.; Swart, K. J.; Brandt, E. V. *Tetrahedron* **2000**, *56*, 5297-5302.

34. Jackson, B,; Locksley, H. -D.; Scheinmann, F.; Wolstenholme, W. A. *J. Chem. Soc. (C)* **1971**, 3791.

35. Brandt, E. V.; Bezuidenhoudt, B. C. B.; Roux, D. G. *J. Chem. Soc., Chem. Commun.* **1982**, 1408-1410.

36. Bezuidenhoudt, B. C. B.; Brandt, E. V.; Roux, D. G. *J. Chem. Soc., Perkin Trans. I* **1984**, 2767-2778.

37. Donnelly, D. M. X.; Fitzpatrick, B. M.; Ryan, S. M.; Finet, J. -P. *J. Chem. Soc., Perkin Trans. I* 1994, 1794-1801.

38. Bezuidenhoudt, B. C. B.; Brandt, E. V.; Steenkamp, J. A.; Roux, D. G.; Ferreira, D. *J. Chem. Soc., Perkin Trans. I* **1988**, 1227-1235.

39. Bezuidenhoudt, B. C. B.; Brandt, E. V.; Ferreira, D. *J. Chem. Soc., Perkin Trans. I* **1987**, 1081.

40. Farkas, L.; Gottsegen, A.; Nogradi, M.; Antus, S. *J. Chem. Soc., Perkin Trans. I* **1974**, 305.

41. Antus, S.; Farkas, L.; Gottsegen, A.; Kardos-Balogh, Z.; Nogradi, M. *Chem. Ber*. **1976**, *109*, 3811.

42. Wagner, H.; Farkas, L. In: *'The Flavonoids'*, ed. J. B. Harborne, T. J. Mabry, and H. Mabry, Chapman and Hall, London, **1975**, 138.

43. Hirao, K.; Yonnemitsu, O. *J. Chem. Soc., Chem. Commun*. **1972**, 812.

44. Hirao, K.; Ikegame, M.; Yonnemitsu, O. *Tetrahedron* **1974**, *30*, 2301.

45. Chen, C. -F.; Zhu, Y.; Liu, Y. -C.; Xu, J. -H. *Tetrahedron Lett.* **1995**, *36*, 2835-2838.

46. Mathai, K. P.; Sethna, J. *J. Indian Chem. Soc.* **1964**, *41*, 347.

47. Pelter, A.; Warren, R.; Handa, B. K.; Chexal, K. K.; Rahman, W. *Indian J. Chem.* **1971**, *9*, 98.

48. Molyneux, R. J.; Waiss, A. C.; Haddon, W. F. *Tetrahedron* **1970**, *26*, 1409.

49. Locksley, H. D. *Prog. Chem.Org. Nat. Prod.* **1973**, *30*, 208.

50. Nakazawa, K. *Chem. Pharm. Bull*. **1968**, *16*, 2503.

51. Murthy, S. S. N. *Natl. Acad. Sci. Lett. (India)* **1985**, *8*, 79.

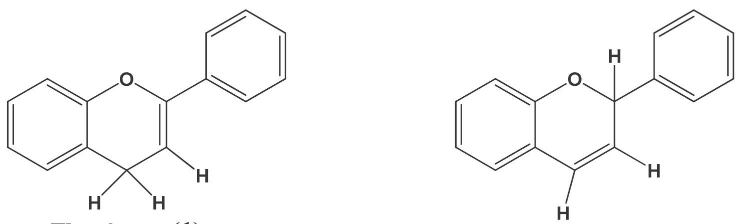
52. Wang, Q.; Zhu, J.; Li, Y. *Chin. Sci. Bull*. **1990**, *35*, 744.

# Chapter 4

# FLAVENES

## 4.1 Introduction

The flav-2-enes **(1)** and flav-3-enes **(2)** constitute a small class of flavonoids (Figure 1). Flavenes are colourless compounds which produce flavylium salts when treated with acid and readily undergo air oxidation.[1,2] When flavylium salts bear free phenolic hydroxyl groups, they are reported to be extremely unstable and short-lived compounds.[3]

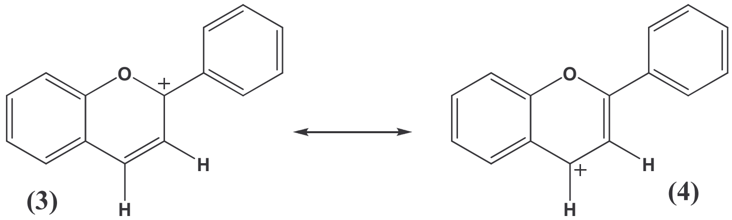


**Flav-2-ene (1)**　　**Flav-3-ene (2)**

**Figure 1:** Two types of flavenes.

A range of biological activities that are associated with the 2-phenyl-2*H*-chromene core of flav-3-enes has been described.[4-9] Accordingly, the development of facile synthetic strategies to access such heterocyles is of considerable interest and is briefly reviewed here.

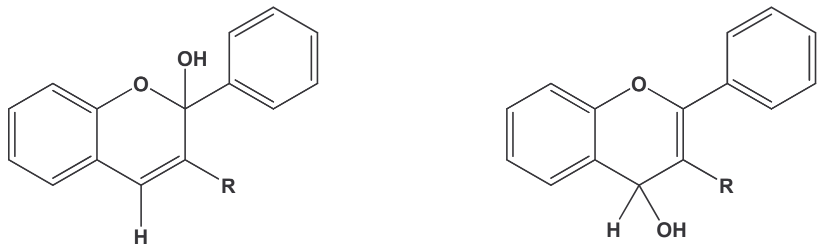
One of the few early sources of the flavenes is flavylium salts.[1,3] Flavylium salts possess resonating carbonium ion structures of types **(3)** and **(4)** (Figure 2).



**(3)**　　**(4)**

**Figure 2:** Resonating structures of flavylium salt.

Flavylium salts can be hydrolyzed to colourless products called pseudo-bases. From the resonating flavylium ion structures, there should be two possible pseudo-base structures [(**5**) and (**6**)] of the hydroxyflavene type.

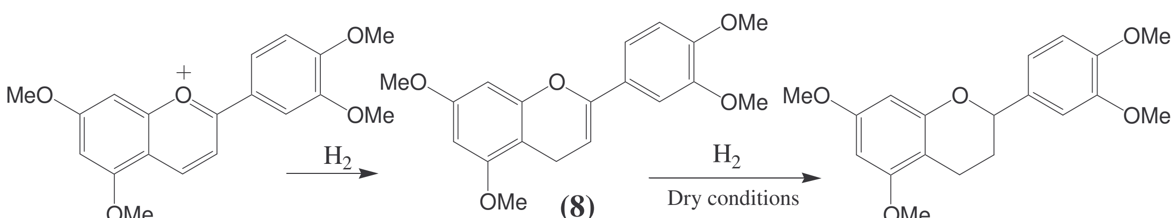



**(5)** **(6)**

**Figure 3:** Two possible pseudo-base structures of flavenes.

# 4.2 Syntheses of flavenes from flavylium salts

Catalytic hydrogenation of flavylium salts gives colourless flavans[2,3] by a two-step reaction. In the first step the flavylium salt **(7)** rapidly takes up one mole of hydrogen to give the corresponding flav-2-ene **(8)** and, in the absence of water, the flav-2-ene slowly takes up a second mole of hydrogen to give the flavan **(9)** (Scheme 1).[2]

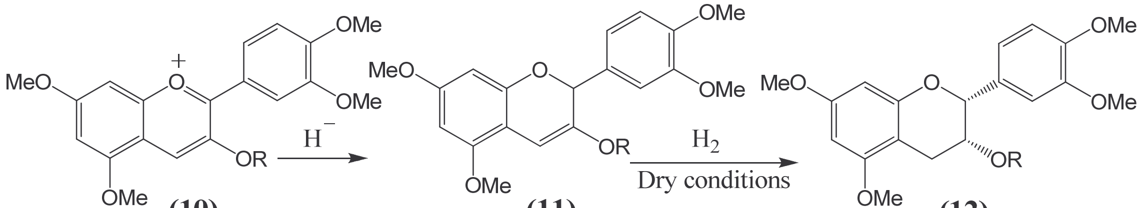



**(7)** **(8)** **(9)**

**Scheme 1:** Reduction of a flavylium salt *via* flav-2-ene to a flavan.

Clark-Lewis and Baig[10] described the synthesis of flav-2-enes based on metal hydride reduction of flavylium salts. Reduction of the flavylium salt **(10)** with lithium aluminum hydride gives the flav-3-ene compound **(11)**[3,11] *via* hydride ion attack at C-4 of the salt.

This product, like the flav-2-ene compound **(8)** referred to above, can be catalytically hydrogenated to a flavan **(12)**, the corresponding epicatechin derivative (Scheme 2).[11,12]



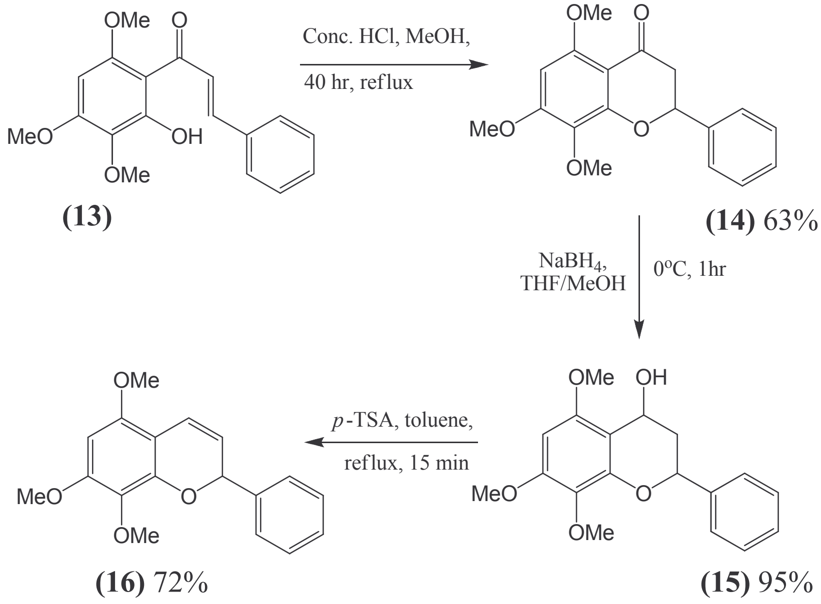

**(10)** **(11)** **(12)**

**Scheme 2:** Reduction of a flavylium salt *via a* flav-3-ene to a flavan.

In general, flavylium salts bearing an OR group at the 3-position afford the flav-3-enes **(11)**, while flavylium salts unsubstituted in the 3-position give flav-2-enes **(8)**.[12]

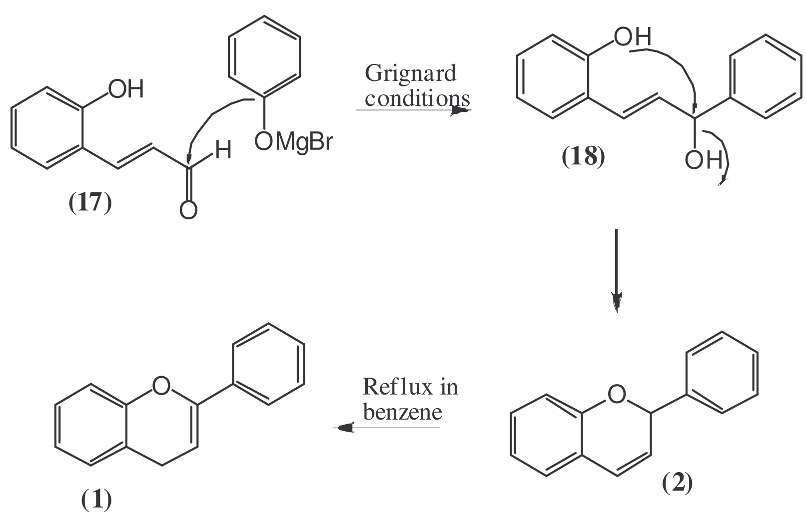
## 4.3 Syntheses of flavenes *via* a cyclizable moiety

Clark-Lewis and Jemison[13] described the first synthesis of racemic flav-3-enes based on chalcone starting materials. Zaveri,[14] Pelter and Stainton,[15] Nay and co-workers,[16] and Deodlar and co-workers[17] used similar cyclizations of chalcones **(13)** to synthesize flav-3-enes. These reactions rely on reduction of the C-4 carbonyl group **(14)** to a 4-hydroxy group **(15)** that eliminates water to yield the racemic flav-3-ene **(16)** (Scheme 3).[17]



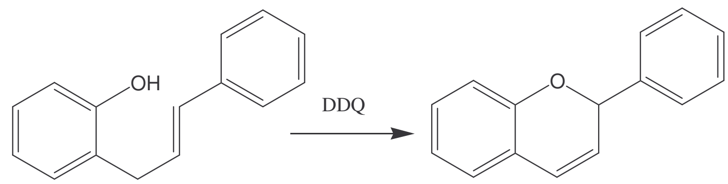

**Scheme 3:** Synthesis of a flav-3-ene *via* cyclization of chalcone.

Casiraghi and Casnati[18] used cinnamaldehyde and alkyl derivatives of phenoxymagnesium bromides as starting material to obtain racemic flav-3-enes (**2**). They found that flav-3-enes isomerize to flav-2-enes (**1**) when heated under reflux in benzene in the presence of the corresponding phenoxymagnesium bromide (Scheme 4).




**Scheme 4:** Synthesis of a flav-3-ene by reaction of 2-hydroxy-cinnamaldehyde and phenoxymagnesium bromide.

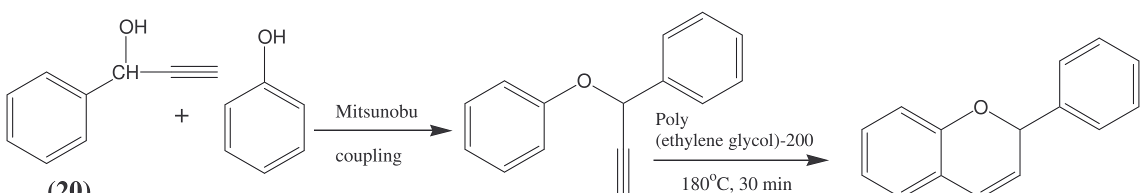
Cardillo and coworkers[19] described a biogenetic-like synthesis of racemic flav-3-enes from *o*-cinnamylphenols (19) *via* DDQ dehydrogenation (Scheme 5).




**(19)** **(2)**

**Scheme 5:** Synthesis of a flav-3-ene by dehydrogenation of *o*-cinnamylphenol with DDQ.

Subramanian and Balasubramanian[20] used a facile Claisen rearrangement to synthesize racemic flav-3-enes from 1-arylprop-2-ynyl aryl ethers **(21)** in good yields (Scheme 6).




**(20)** **(21)** **(22)** **(2)** 93%

**Scheme 6:** Synthesis of a flav-3-ene from a 1-Arylprop-2-ynyl aryl ether by a Claisen rearrangement.

In conclusion, a paucity of synthetic methods has been published to synthesize flavenes. None of these methods is stereoselective.

# REFERENCES


1. Trotter, P. C. *Doctorate Dissertation: The Action of Sodium Hydrosulfite on Selected Flavonoid Compounds,* Lawrence College, Wisconsin, **1961**, 8-10.
2. Freudenberg, K.; Harder, M. *Ann.* **1927**, *451*, 213-22.
3. Freudenberg, K.; Weinges, K. *Ann*. **1954**, *590*, 140-54.
4. Lee, M. H.; Choi, E. T.; Lee, Y. M.; Kim, D.; Park, Y. S. *Bull. Korean Chem. Soc.,* **2008**, *29*, 1299-1300, and refs. therein.
5. Lee, C. -W.; Choi, H. -J.; Kim, H. -S.; Kim, D. -H.; Chang, I. -S.; Moon, H. T.; Lee, S. -Y.; Oh, W. K.; Woo, E. -R. *Bioorg. Med. Chem. Lett*. **2008**, *16*, 732-738.
6. Zheng, X.; Meng, W. -D.; Qing, F. -L. *Tetrahedron Lett.* **2004**, *45*, 8083-8085.
7. Gil, B.; Sanz, M. J.; Terencio, M. C.; Gunasegaran, R.; Paya, M.; Alcaraz, M. *J. Biochem. Pharmacol*. **1997**, *53*, 733.
8. Lee, M. K.; Lim, S. W.; Yang, H.; Sung, S. H.; Lee, H.-S.; Park, M. J.; Kim, Y. C. *Bioorg. Med. Chem. Lett*. **2006**, *16*, 2850-2854.
9. Chen, J.; Chang, H. W.; Kim, H. P.; Park, H. *Bioorg. Med. Chem. Lett*. **2006**, *16*, 2373-2375.
10. Clark-Lewis, J. W.; Baig, M.I. *Austral. J. Chem.* **1971**, *24*, p. 2581.
11. Karrer, P.; Seyhan, M.; *Helv. Chim. Acta*, **1950**, *33*, 2209-10.
12. Iacobucci, G. A.; Sweeny, J. G. *Tetrahedon*, **1983**, *39*, 3005-3038.
13. Clark-Lewis, J. W.; Jemison, R. W. *Aust. J. Chem.* **1970,** *23*, 315-321.
14. Zaveri, T. N. *Organic Lett.* **2001**, *3*, 843-846.
15. Pelter, A.; Stainton, P. *J. Chem. Soc*. (C), **1967**, 1933-1937.
16. Nay, B.; Arnaudinaud, V.; Peyrat, J. -F.; Nurich, A.; Deffieux, G.; Merillon, J. -M.; Vercauteren, J. *Eur. J. Org. Chem*. **2000**, 1279-1283.
17. Deodlar, M.; Black, D. St C.; Kumar, N. *Tetrahedron*, **2007**, *63*, 5227-5235.

18. Casiraghi, G.; Casnati, G. *J. Chem. Soc., Chem. Commun*. **1970**, 321.

19. Cardillo, G.; Cricchio, R.; Merlini, L. *Tetrahedron Lett.* **1969**, 907-908.

20. Subramanian, R. S.; Balasubramanian, K. K. *Tetrahedron Lett*. **1988**, *29,* 6797-6800.

# Chapter 5

## GENERAL CHEMISTRY OF FLAVONOIDS

**(Complex $C_6$-$C_3$-$C_6$ secondary metabolite monomers and oligomers)**

## 5.1 Introduction

The deceptively simple $C_6$-$C_3$-$C_6$ formula (one heterocyclic and two aromatic rings) of the monomeric building blocks of flavonoids gives rise to almost intractable complex extracts and many synthetic challenges remain to be resolved.

The large number of reactive positions on the monomers available for condensation results in an extremely complex mixture of dimers, trimers, tetramers, oligomers and polymers linked at different positions. The possibility of branching and rearrangement of monomer subunits further increases the complexity.

The free phenolic nature of flavonoids renders these compounds prone to oxidation. Strong adsorption on silica gel reduces resolution, leads to poor recovery rates and makes it difficult to isolate pure compounds. The high number of chiral centers (three stereogenic centers on the C-ring of each monomeric building block) leads to a plethora of stereoisomers. The conformation of the heterocyclic ring, which is highly sensitive towards substitution patterns on both the heterocyclic and aromatic rings, influences the stereochemical outcome of many reactions.

To simplify the complexity, we classify chemical reactivity of flavonoids or free polyphenolic $C_6C_3C_6$ compounds according to the nature of the carbon involved:

1. Hydrogen substituted carbons on the aromatic A- or B-rings (aromatic C-H bonds).

2. Hydroxy substituted carbons on the aromatic A- or B-rings (aromatic C-OH bonds).

3. The C-2 carbon on the heterocyclic C-ring (a benzylic ether).

4. The C-3 carbon (most often a secondary alcohol).

5. C-4 position (C-H, C-OH or a carbonyl).

## 5.2 Chemistry of the hydroxyl groups on the aromatic A- and B-rings.

Hydrogen bonding of the hydroxyl groups on the aromatic A- and B-rings is responsible for the water solubility of monomeric flavonoids. As the size of flavonoids increases, the hydrogen bonding becomes insufficient to keep the molecule in solution and large polymers are insoluble in water.

Hydrogen bonding of the hydroxyl groups on the aromatic rings of flavonoids with amino acids in leather protein is responsible for the age-old process of leather tanning with tannins. This protein-polyphenol interaction renders skin resistant to water penetration and durable and it becomes leather. The "feel" is also improved. Despite the age of this process, the precise nature of this protein-polyphenol interaction is only vaguely understood.[1]

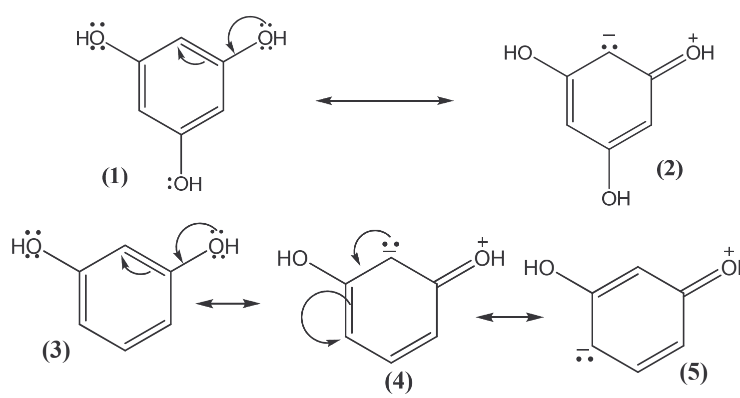
Tannins are responsible for much of the taste and flavour properties of tea, the popular beverage made from leaves of *Camellia sinensis.* During the manufacturing[2,3] of black or Indian tea *via* a fermentation process, flavonoid monomers condense oxidatively to astringent theaflavins and thearubigins. Addition of milk to tea reduces astringency by precipitating bitter-tasting tannins *via* hydrogen bonding with milk proteins.

Traditional silica based chromatography is not well suited for purification of free phenolic flavonoids and tannins because of the strong adsorption of these compounds *via* hydrogen bonds on silica gel and subsequent low recovery rates. Protection of the hydroxyl groups renders free phenolic flavonoids more “organic’’ and allows high resolution purification with silica gel. It also allows better region- and stereochemistry control.

Protection of the phenolic hydroxy groups can be achieved with methyl, acetyl or benzyl groups. Benzylic protection is normally the method of choice. The benzylic ethers are stable under acidic and basic conditions and can be removed by catalytic hydrogenation. C-benzylation may compete with *O*-benzylation during the protection step and careful control of reaction conditions (choice of solvent and temperature control) is often required. Methods traditionally used to methylate phenolic hydroxyl groups with dimethyl sulfate ($K_2CO_3$, acetone, reflux) fail completely with benzyl bromide. Modified conditions include benzyl bromide, $K_2CO_3$ and DMF, and NaH, benzyl bromide and DMF.[4,5]

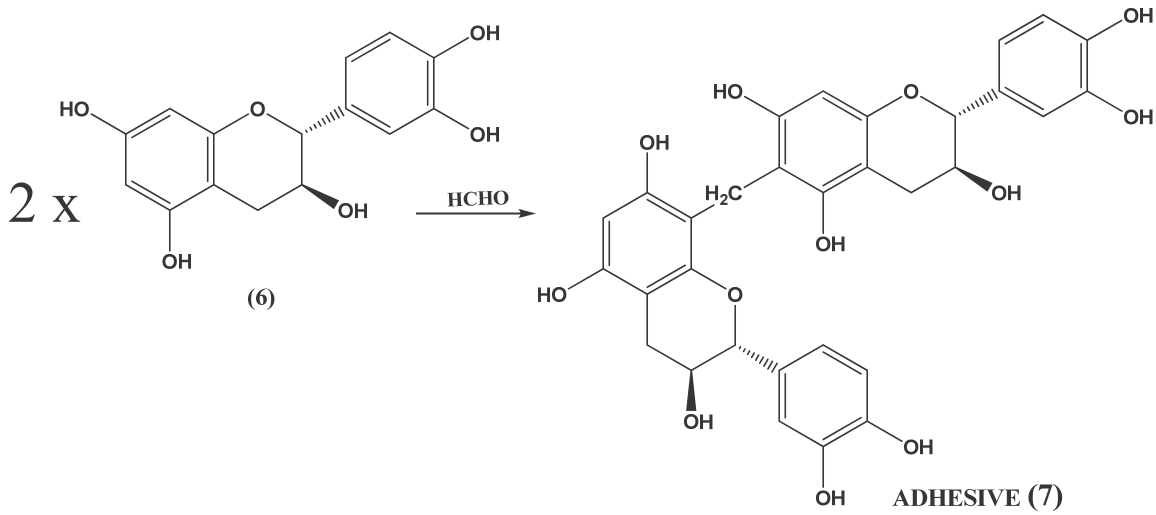
## 5.3 Chemistry of the aromatic C-H groups

The hydroxy groups and other *O*-derivative substituents (such as *O*-alkyl or *O*-benzyl) of phloroglucinol and other polyhydroxybenzenes donate electrons to the aromatic ring to increase the nucleophilicity of the aromatic carbons. Both substituents are *ortho* and *para* directors (Scheme1). The ring carbons can thus react like carbanions with the ability to form new carbon-carbon bonds with suitable electrophiles.




**Scheme 1:** Resonance stabilized nucleophilicity of the A-ring.

The nucleophilicity of the A-ring towards formaldehyde forms the basis of adhesive manufacturing. Scheme 2 gives a simplified example of formaldehyde based polymerization. This nucleophilicity of the A-ring forms the basis of the proanthocyanidin syntheses discussed in Chapter two.

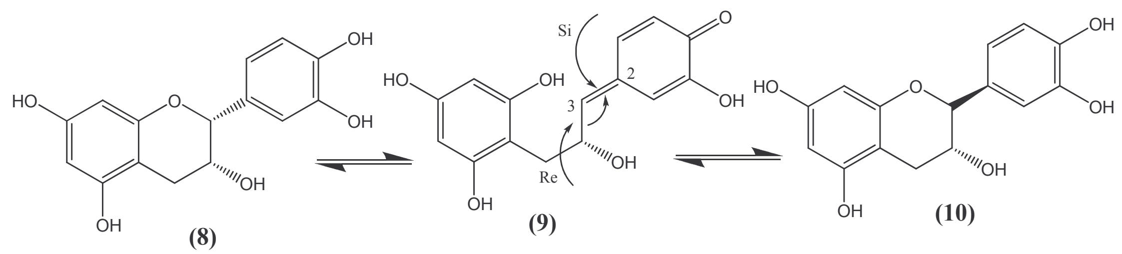



**Scheme 2:** Preparation of tannin-formaldehyde adhesives.

## 5.4 Chemistry of the C-2

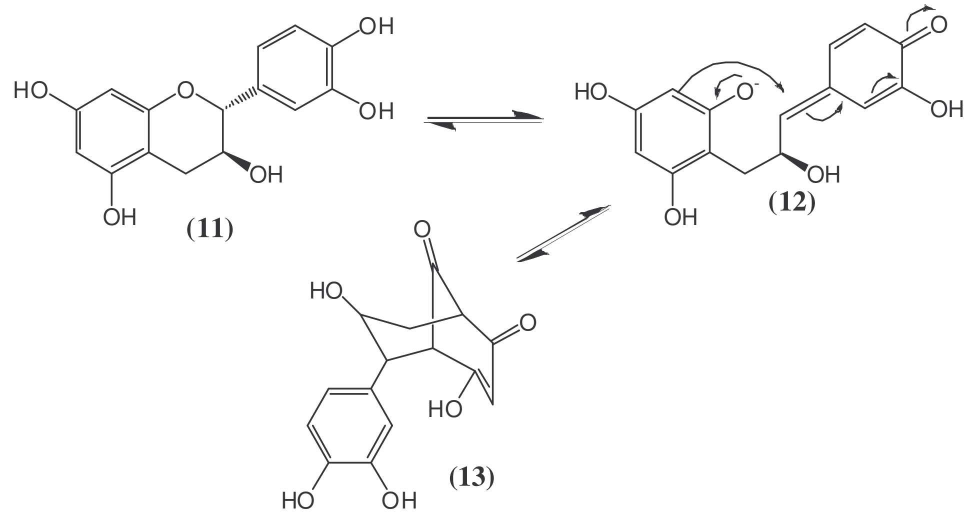
The flavonoid heterocyclic ring is susceptible to opening under basic conditions at C-2 to give a B-ring quinone methide **(9)**. The quinone methide is an important intermediate in phlobatannin formation and opens the possibility of attaching substituents at C-2. This reaction probably requires a free phenolic hydroxyl in the 4-position of the B-ring and may proceed *via* either an ionic or a free radical process. The following are examples of reactions at this position:

1. Epimerization of epicatechin **(8)** to an equilibrium mixture of ent-catechin **(10)** (thermodynamically more stable) and epicatechin (thermodynamically less stable) (Scheme 3).[6]



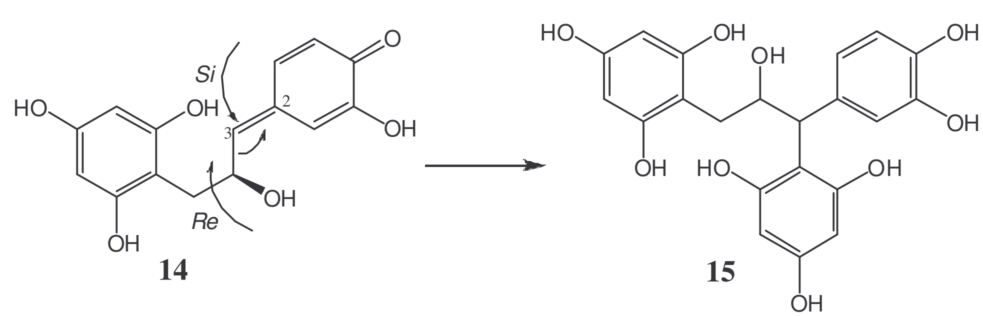

**Scheme 3:** Epimerization of epicatechin

2. Attack of the A-ring on the quinone methide (base catalysed) to form catechinic acid **(13)** (Scheme 4).[7]




**Scheme 4:** Formation of catechinic acid.

3. Trapping of the quinone methide with phloroglucinol or other polyphenolic nucleophiles (Scheme 5).[6,8]




**Scheme 5:** Trapping of the quinone methide with aryl compound.

Polyphenolic tannins are widely used as they have many reactive phenolic aromatic rings that react with formaldehyde to polymerize and form adhesives[9-13] for wood laminating. The reactive sites are, however, limited and much of the applied research on the chemistry of tannins has revolved around efforts to cleave the heterocyclic C-ring and create additional reactive sites for polymerization for adhesive applications.[13]

## 5.5 Reaction at the C-3 position

C-3 usually carries a hydroxyl group but may also be $sp^2$ hybridised (in flav-2- or 3-enes, and anthocyanidins or anthocyanins). Efforts to replace the secondary hydroxyl group at C-3 usually fail, probably because the anti-bonding orbital involved in the $S_N2$ reaction is "hidden" inside the C-ring and cannot overlap sufficiently with the HOMO of the approaching nucleophile. Chapter 6 will describe our efforts to make this hydroxyl group a better leaving group and introduce a flavonoid nucleophile into this position to synthesize optically active I-3,II-6/8 biflavonoids, flav-2-enes and optically active flav-3-enes.

## 5.6 Reaction at the C-4 position

C-4 is in a benzylic position relative to the A ring. The reactivity of this carbon is strongly influenced by the presence or absence of hydroxylation at C-5 (A-ring), assuming that C-7 carries the usual phenolic hydroxyl group. This position may exist in three different oxidation levels (C-H, C-OH or carbonyl). The reactivity of this position was extensively discussed in chapter 2 (proanthocyanidin syntheses) and can be classified as follows:

1. Oxidation of the $CH_2$ group to a CHOH group or derivative thereof.
2. Substitution of the benzylic C-OH group with a nuceophile, usually a polyhydroxy aromatic ring. This can take place *via* a quinone methide (assuming that C-7 contains the

usual hydroxyl group), a carbocation ($S_N1$ mechanism) or concerted substitution ($S_N2$ mechanism). The stereochemistry of reactions at C-2 is profoundly influenced by the stereochemistry at C-2 and C-3.

3. Attack of the carbonyl group at C-4 by a nucleophile.

The chemistry of this position was extensively discussed in Chapter 2 (proanthocyanidin Syntheses). Our successful effort to introduce a flavonoid nucleophile oxidatively into this position and to develop novel syntheses of proanthocyanidins will be discussed in Chapter 5.

# REFERENCES


1. Coving , A. D. *Chem. Soc. Rev. 1997*, 111-125.

2. Bohm, B. A.; Harnone, J. B., Ed.; *In the Flavonoids – Advances in Research Since 1986*, Chapman & Hall: London, **1994**, 23.

3. Graham, H. N. *PubMed*. **1992**, *21 (3)*, 334-350.

4. Mehta, P. P.; Whalley, W. B. *J. Chem. Soc*. **1963**, 5327.

5. Tückmental, W.; Kozikowski, A. P.; Romanczk Jr, L. J. *J. Am. Chem. Soc*. **1999**, *121*, 12073-12081.

6. Laks, P. E.; Hemingway, R. W.; Conner, A. H. *J. Chem. Soc., Perkin Trans I*, **1987**, 1875-1881.

7. Kiatgrajai, P.; Wellons, J. D.; Gollob, L., White, J. D. *J. Org. Chem.*, **1982**, *47*, 2910-2912

8. Laks, P. E.; Hemingway, R. W. *J. Chem. Soc., Perkin Trans. I*, **1987**, 465.

9. Pizzi, A. *Holz als Roh- und Werkstoff*, **1982**, *40*, 293-301.

10. Pizzi, A. *Forest Prod. J.*, **1978**, *28*, 42-47.

11. Kreibich, R. E.; Hemingway, R. W. *Forest Prod. J.*, **1985**, *35*, 23-25.

12. Santana, M. A. E.; Baumann, M. G. D.; Conner, A. H. *Holzforschung*, **1995**, *49*, 146-152.

13. Cetin, N. S.; Ozmen, N. *International Journal of Adhesion and Adhesives*, **2002**, *22*, 477-480.

# Chapter 6

## SYNTHESIS OF FLAVENES AND 3-COUPLED BIFLAVONOIDS

### 6.1 Introduction

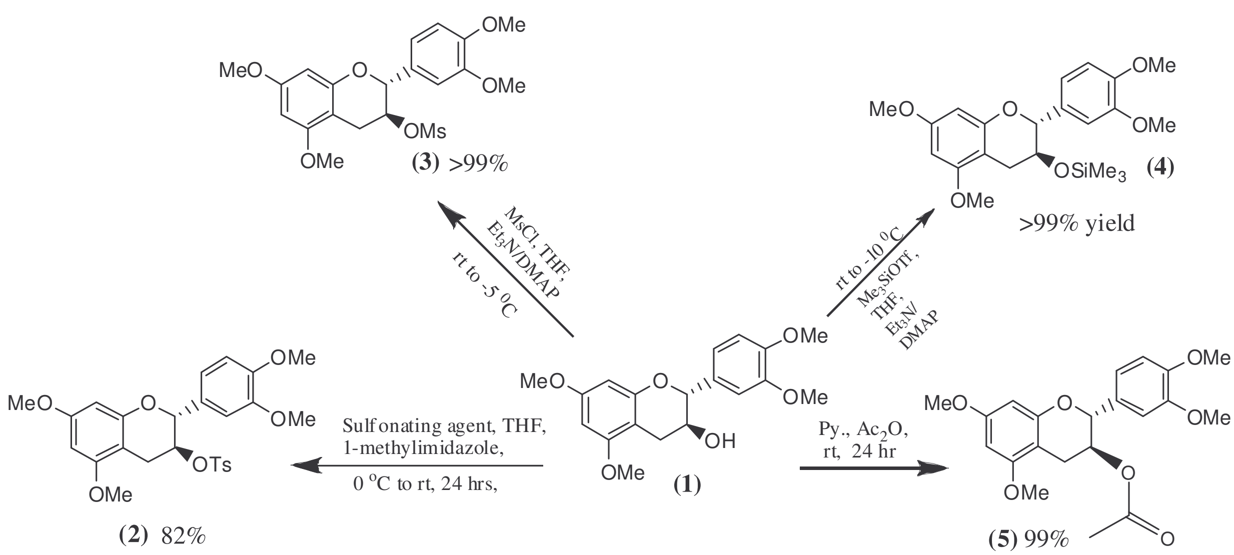
The replacement of the 3-hydroxy group of flavan-3-ols with a carbon-carbon bond remains an elusive goal. This would open the way to a plethora of new classes of flavonoids, including naturally occurring 3-coupled biflavonoids (I-3, II-6/8 biflavonoids). This class of biflavonoids has been proved to have biological activity in a variety of screens.[1-5] Progress in drug development is, however, hampered because no stereoselective synthetic access exists to obtain larger quantities of pure compounds and all research is based on molecules isolated in small quantities from natural sources.

In contrast to primary alcohols (that are readily displaced by an $S_N2$ mechanism), tertiary alcohols (that are smoothly replaced by an $S_N1$ mechanism), and benzylic and allylic alcohols (that are readily displaced because the intermediary carbocations are resonance stabilized by the adjacent π-systems), secondary alcohols normally require the OH to be converted into a group with better leaving properties. This weakens and further polarizes the C-O bond before displacement of the modified hydroxy group by nucleophilic attack on the α-carbon can take place.

Weakening and further polarization of the C-O bond can be achieved by protonation to convert the hydroxy group (R-OH) into an oxonium ion ($R\text{-}OH_2^+$). Tertiary alcohols react rapidly with cold concentrated aqueous acids by an $S_N1$ mechanism but replacement of the protonated hydroxy group from secondary alcohols *via* an $S_N2$ mechanism requires heat and additional assistance such as hexamethylphosphoric triamide[6] (HMPT) solvent, a phase transfer catalyst, or HI-$H_3PO_4$[7]. Tosylates and mesylates can be isolated and stored and can be used to convert secondary alcohols (to halides, for example) under mild conditions with inversion of configuration. The Mitsunobu reaction allows the conversion of secondary alcohols to esters, phenyl ethers, thioethers and various other compounds. The nucleophile employed should be resistant to acid, since one of the reagents, DEAD (diethyl azodicarboxylate) must be protonated during the course of the reaction to prevent side reactions.

## 6.2 $S_N2$ Substitution of the C-3 hydroxy group.

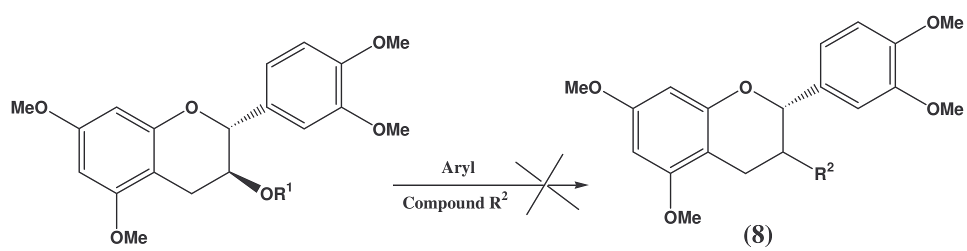
We have synthesized the following 3-*O*-derivatives of tetra-*O*-methylcatechin (Scheme 1) to enhance the susceptibility of the 3-hydroxy group towards $S_N2$ substitution with a nucleophile:




**Scheme 1:** Synthesis of 3-*O*-derivatives of catechin.

The $^{1}H$ NMR spectra (**plates 1.1**, **2.1**, and **3.1**) of the 3-*O*-derivatives **(2)**, **(3)**, and **(4)** clearly indicates the presence of an aromatic ABX- (dd, J = 2.0, 8.0 Hz; d, J = 2.0 Hz; d, J = 8.0 Hz) as well as an AB resonance system (J = 2.0 Hz) which represent the catechol and phloroglucinol character of the B- and A-rings, respectively. The CD spectra of **(3)** and **(4)** suggest that optical activity at C-2 and C-3 has been maintained. Our present understanding does not allow any conclusions from the CD spectra (**CD-plate 1** and **CD-plate 2**) except that these products are optically active.

In our hands, all efforts to replace the 3-hydroxy group of catechin or its 3-*O*-derivatives (in Scheme 2), which should be better leaving groups under $S_N2$ conditions, with aryl compounds to form a carbon-carbon bonded aryl moiety have failed. These failures, including the use of phloroglucinol and resorcinol as nucleophiles, are in sharp contrast with the ease with which a 4-hydroxy group is replaced, under very mild conditions.



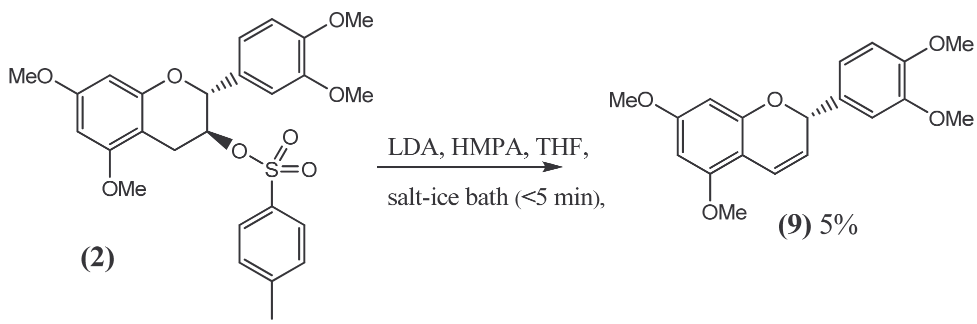

$OR^1$ = Hydroxy, mesyl, tosyl, silyl
$R^2$ = Aryl moiety

**Scheme 2:** Attempted replacement of 3-hydroxy group or its 3-*O*-derivatives on catechin.

We attribute this to the inaccessibility of the C-O antibonding orbital. Steric factors, compounded by the bulkiness of the 3-*O*-derivatives with better leaving group properties that prefer to occupy equatorial positions, force the heterocyclic C-ring to a chair conformation. The antibonding orbital is then "hidden'' on the inside of the heterocyclic C-ring and cannot be reached by the approaching electron-rich nucleophilic bonding orbitals.[8]

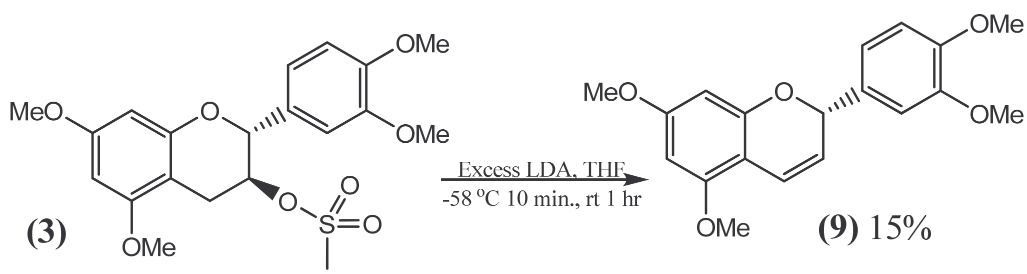
## 6.3 Elimination of a tosyl or mesyl group at C-3 (Stereo-selective synthesis of flav-3-enes and synthesis of flav-2-enes)

Under basic conditions, our tosylates and mesylates underwent elimination to flav-3-enes (Schemes 3, 4 and 5).




**(2)** **(9)** 5%

**Scheme 3:** Synthesis of flav-3-ene from catechin tosylate *via* base elimination of the tosyl group at C-3.



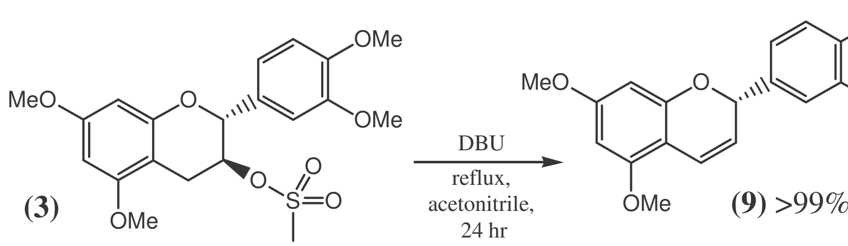

**(3)** **(9)** 15%

**Scheme 4:** Synthesis of flav-3-ene from catechin mesylate *via* base elimination of the mesyl group at C-3.

The flav-3-ene **(9)** has three different one-proton resonances on the C-ring that exhibit a complex ABX system. The olefinic protons on C-3 and C-4 (H-3 and H-4) are coupled by *cis*-coupling ($J_{3,4}$ = 10 Hz) and the proton on C-2 (H-2) is coupled to both H-3 and H-4 ($J_{2,3}$ = 3 Hz, and $J_{2,4}$ = 2 Hz) [$^{1}$H NMR **plate 4.1** and COSY **plate 4.4**]. H-2 is benzylic and adjacent to an ether oxygen, and resonates at $\delta_H$ 5.76 as a doublet of doublets (J = 2.0, 3.0 Hz). Flav-3-

enes have a chiral carbon on C-2. The HMBC correlation between C-2/C-5 and H-4 in HMBC-**plate 4.7** allows us to distinquish H-4 from H-3. **Plate 4.3** ($^{13}$C APT), and **plate 4.6** (HSQC) reveals the carbon C-2 overlapped by the chloroform solvent signal in the $^{13}$C NMR **plate 4.2**. The HSQC correlation between H-4 and C-4 in **plate 4.5** allows differentiation between C-3 and C-4 in the **plate 4.2**. The COSY experiment (**plate 4.4**) confirms the vinyl nature of the C-ring. Our product demonstrates a clear negative Cotton effect at $\lambda$ = 228 nm (**CD-plate 3**).

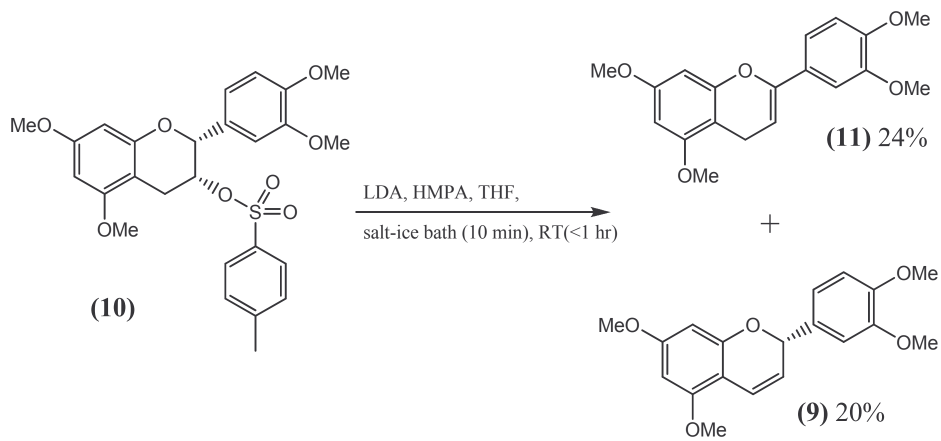
We optimised conditions to obtained yields close to 100% (DBU, reflux in acetonitrile for 24 hours, mesyl derivative). THF gave lower yields under similar conditions (reflux).




**Scheme 5:** Synthesis of flav-3-ene from catechin mesylate by elimination of the mesyl group at C-3 with DBU.

As retention of configuration at C-2 was observed, we have developed an efficient and high yielding method to synthesize optically active flav-3-enes.

Repetition of the reaction with epicatechin **(10)** yielded a mixture of a flav-2-ene **(11)** and the anticipated flav-3-ene **(9)** (Scheme 6).



**Scheme 6:** Synthesis of a flav-2-ene and a flav-3-ene from epicatechin tosylate *via* base elimination of the tosyl group at C-3.

The flav-2-ene **(11)** has two different proton resonances on the C-ring. It is characterised by a two-proton doublet ($J_4$ = 3.8 Hz) on C-4 ($CH_2$ group) and a one proton triplet bonded to C-3 (H-3) that resonate at $\delta_H$ 5.40. The $CH_2$ (H-$4_{\alpha/\beta}$ and C-4) is both allylic and benzylic and resonates at $\delta_H$ 3.34 ($^1$H NMR **plate 5.1),** and $\delta_C$ 19.49 ($^{13}$C NMR **plate 5.2**). C-2 resonates at $\delta_C$ 101.25 ($^{13}$C NMR **plate 5.2,** and HMBC **plate 5.5**). The COSY spectrum (**plate 5.3**) shows strong correlation between the H-3 and the H-4. Flav-2-enes do not possess chiral carbons and no optical activity is possible.

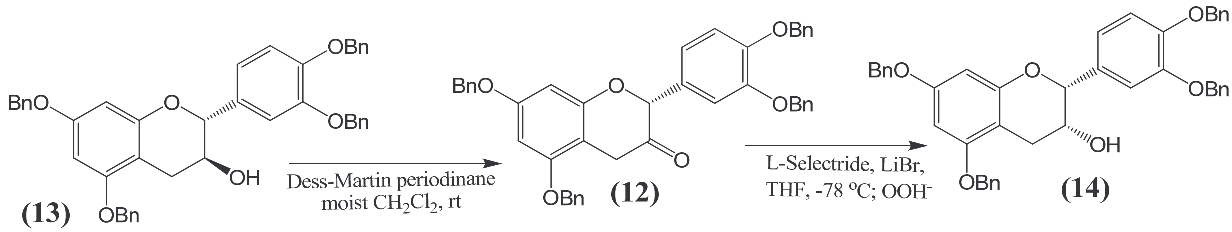
In the case of tetra-*O*-methyl-3-*O*-tosyl-epicatechin **(10)** the hydrogen at C-2 is *trans* to the tosyl group at C-3 and base-catalysed *trans* elimination to a flav-2-ene is feasible. This contrasts with tetra-*O*-methyl-3-*O*-tosyl-catechin **(3)** where the 2-hydrogen is *cis* to the tosyl group at C-3 and the only available *trans* hydrogen is at C-4, resulting in the exclusive formation of the flav-3-ene and retention of configuration at C-2. Flav-2-enes and flav-3-enes are normally difficult to synthesize due to the ease with which they oxidize to anthocyanidins.

With DBU in refluxing acetonitrile, the combined yields of flav-2- and flav-3-enes are in excess of 80% in a ratio of about 1:1. We have thus developed the first stereoselective access to flav-3-enes. Our yields are close to 100%. We observed that about 5% of the flav-3-ene **(9)** that was stored at room temperature for six months rearranged to the flav-2-ene **(11)**. This indicates that the flav-3-ene is thermodynamically less stable than the flav-2-ene. It agrees with the work of Casiraghi and Casnati[9] that 3-unsubstituted flav-3-enes are regioselectively reduced to flav-2-enes. We will investigate the influence of temperature and solvent on the ratio between **(9)** and **(11)** to enhance regioselectivity.

## 6.4 Condensation of 3-ketocatechin with an aryl moiety.

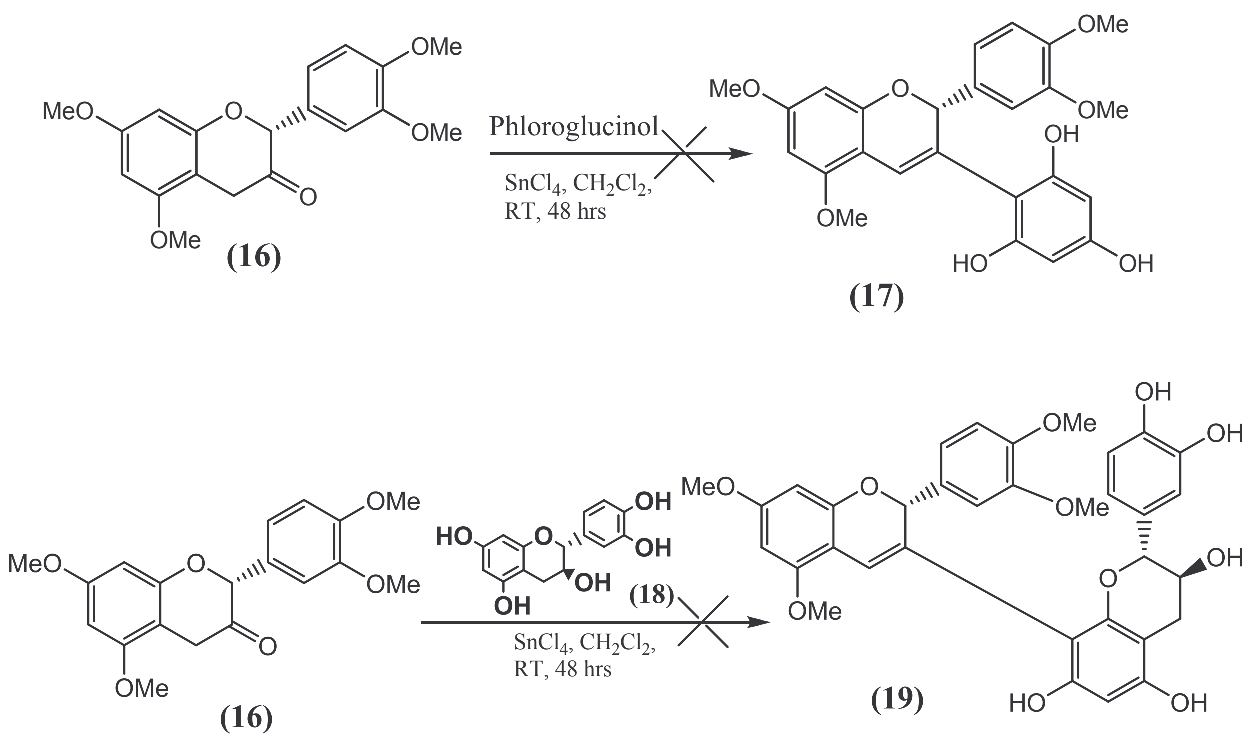
The ready availability of protected 3-oxocatechin analogues *via* Dess-Martin periodinane oxidation[10] and a new method to benzylate the phenolic groups of flavan-3-ols in high yields[11] with retention of configuration at C-2, opened new approaches towards nucleophilic attack at C-3. In contrast to a C-3 functionality that is $sp^3$ functionalised and tetrahedral, the $sp^2$ functionalized 3-oxo-group is planar. This reduces steric influences and allows nucleophilic attack on the carbonyl carbon from either the α- or the β-face. It is expected that the C-2 configuration will play an important role in the stereoselectivity of the planar C-3 carbonyl group.

The utility of the 3-keto functionality was recently demonstrated by reduction of the 3-ketoflavan **(12)**, obtained from tetra-*O*-benzyl catechin **(13)**, with L-selectride (a bulky hydride donor) to tetra-*O*-benzylepicatechin **(14)**.[10] This reaction allows transformation of inexpensive catechin into expensive epicatechin in a good yield (Scheme 7). The configuration at C-3 is based on stereoselective $H^-$ attack from the β-face (opposite to the α-3-arylgroup).



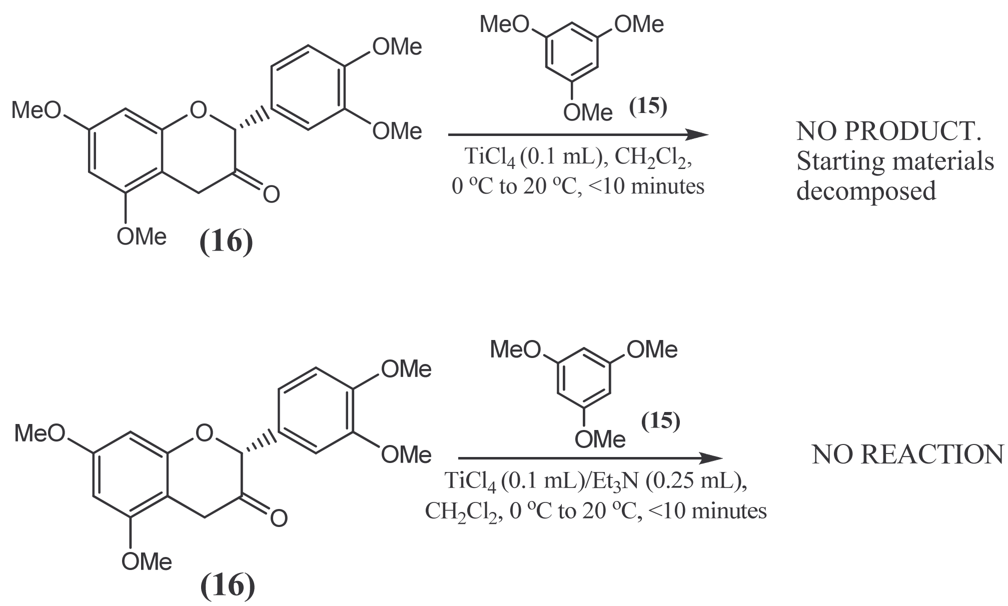
**Scheme 7:** Conversion of protected catechin to protected epicatechin *via* the 3-keto analogue.

Our attempts to couple phloroglucinol or free-phenolic catechin to C-3 of tetra-*O*-methyl-3-oxocatechin (**16**), was unsuccessful (Scheme 8).

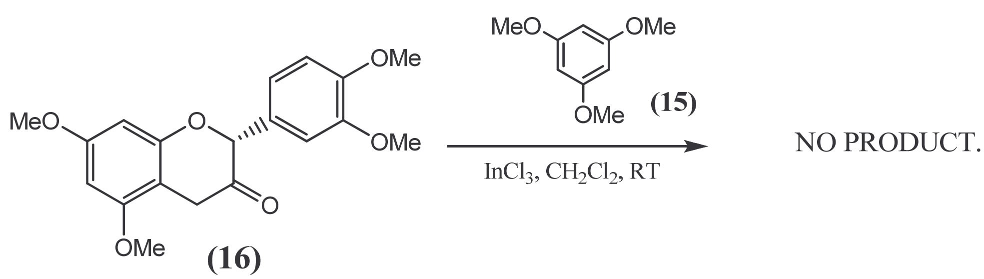


**Scheme 8:** Attempted synthesis of biflavonoids with free phenolic moieties.

We tried a variety of Lewis acids [$TiCl_4$ (Scheme 9), $InCl_3$ (Scheme 10), $Yb(OTf)_3$ (Scheme 11)] to catalyze coupling at C-3. Most of these efforts proved fruitless.

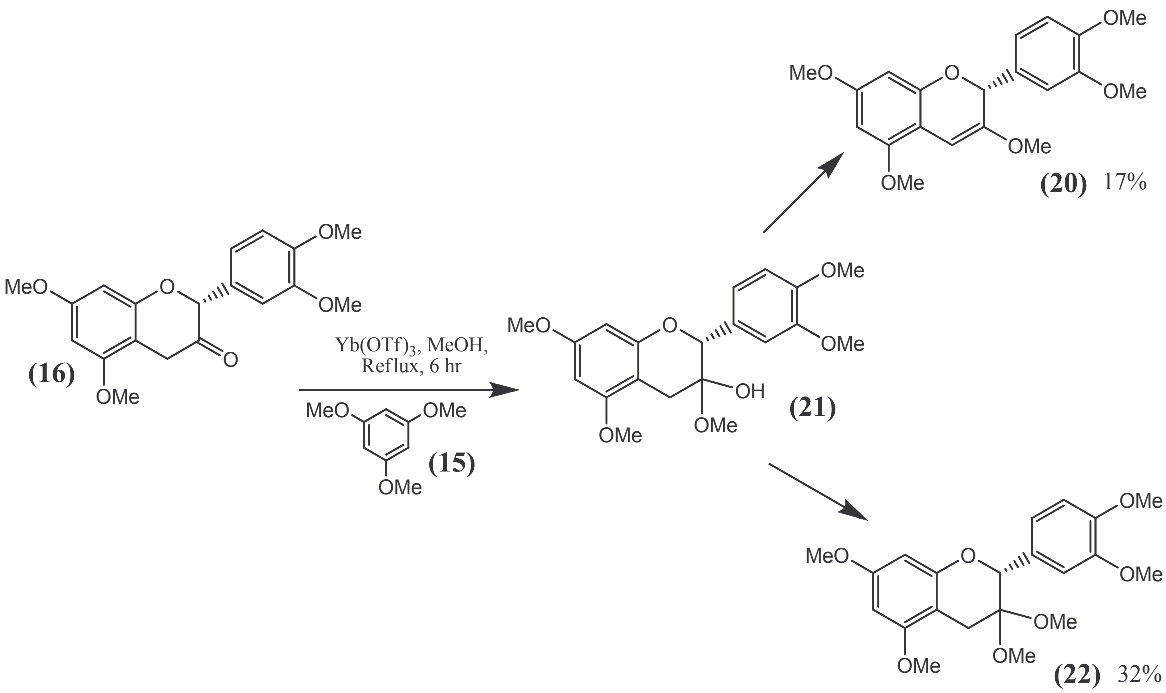



**Scheme 9:** $TiCl_4$ mediated attempt to couple aryl moiety at C-3 of **(16)**.




**Scheme 10:** $InCl_3$ mediated attempt to couple aryl moiety at C-3 of **(16)**.

Ytterbium trifluoromethanesulfonate [$Yb(OTf)_3$] did not dissolve in the solvents that we used with the other Lewis acids and we had to use methanol. This led to formation of 3-*O*-methylflavan-3-ene **(20)** with its double bond conjugated with the more electron rich phloroglucinol A-ring (compared to the catechol B-ring) and a methyl acetal **(22)**. A hemiacetal intermediate **(21)** is probably involved (Scheme 11). Both products demonstrated Cotton effects in the wavelength range 260-284 nm (**CD-plate 4** and **CD-plate 5**), indicating that configuration at C-2 was not affected. This contrasts with racemic products synthesized by Clark-Lewis and coworkers.[12]

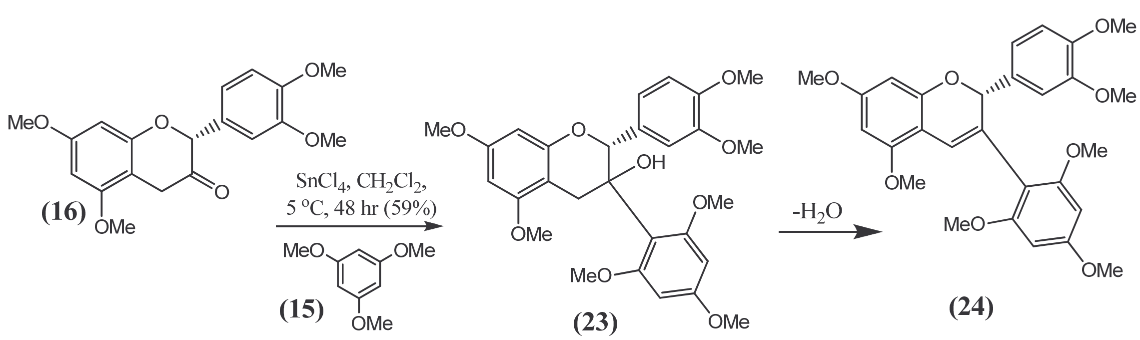



**Scheme 11:** Synthesis of 3-*O*-methylflav-3-ene and 3,3-dimethoxy flavan.

The enol ether **(20)** is characterised by the disappearance of the carbonyl resonance in the $^{13}C$ NMR spectrum of the starting material **(16)** at *ca* 206.1 ppm ($^{13}C$ NMR **plate 6.2**) and the appearance of a methoxy resonance at $\delta_H$ 3.73 ppm in the $^1H$ NMR spectrum ($^1H$ NMR **plate 6.1** and COSY **plate 6.4**). The two enol carbons resonate at 150.7 (C-3) and 77.2 (C-2) (APT **plate 6.3** and HSQC **plate 6.7**). The two benzylic protons C-4 in the starting material **(16)** disappear and are replaced by an olefinic resonance at $\delta_H$ 5.60 ppm ($^1H$ NMR **plate 6.1**). The structure elucidation is further supported by mass spectrometry (expected $[M+H]^+$ ion at m/z 359.35) corresponding to the addition of a methyl group and the loss of a hydrogen). The CD spectrum (**CD-plate 4**) shows a positive Cotton effect in the $\lambda$ = 241 nm range.

The ketal **(22)** is characterised by the disappearance of the starting material's **(16)** carbonyl resonance at about $\delta_C$ 206.1 ppm chemical shift, and the appearance of two additional methoxy resonances at $\delta_H$ 3.34 and 3.31 ($^1$H NMR **plate 7.1**). The ketal carbon (C-3) resonates at $\delta_C$ 101.2 ($^{13}$C NMR **plate 7.2**). This is further supported by mass spectrometry (expected $[M+H]^+$ ion at m/z 391.29) corresponding to the addition of two methoxy groups (and the loss of an OH group). NOESY demonstrates correlations between the two benzylic C-4 protons (4α and 4β) and the A-ring and the C-2-proton. There is NOESY between H-4β and H-2. H-4β shows NOESY with H-2′ and H-6′ (NOESY **plate 7.4** and **plate 7.5**). The two methoxy groups on C-3 crowd the C-ring and line broadening in the $^1$H NMR spectrum ($^1$H NMR **plate 7.1**) possibly indicates conformational correlations (NOESY **plate 7.5**) between $H_\alpha$-4 and H-2′/H-6′ suggesting a significant population of the A-conformer[13] (B-ring in the axial position). The CD spectrum (**CD-plate 5**) shows a positive Cotton effect at $\lambda$ = 239 nm.

Our breakthrough was attained with protected phloroglucinol and $SnCl_4$. Treatment of tetra-*O*-methyl-3-oxocatechin **(16)** with tri-*O*-methylphloroglucinol **(15)** in the presence of $SnCl_4$ affords facile coupling of the phloroglucinol analogue at C-3 *via* a carbon-carbon bond to afford the 3-arylcatechin product **(24)** (Scheme 12).




**Scheme 12:** Synthesis of 3-arylflav-3-ene.

The effect of temperature on the coupling of tetra-*O*-methyl-3-oxocatechin under mediation of $SnCl_4/CH_2Cl_2$ promoter was investigated.

For the reactions carried out at low temperatures,

(i) From -80 $^{o}C$ to -40 $^{o}C$ showed no indication of product formation after 3 hours.

(ii) From -40 $^{o}C$ to 0 $^{o}C$ did not show any trace of product after 3 hours.

(iii) From about +5 $^{o}C$, the reaction mixture showed indication of product formation after 1 hour. The reaction was continued for 48 hours. The reaction was relatively clean and afforded **(24)** (69 mg, 59%) as fine yellow crystals.

Thus, after investigation of various reaction conditions [temperature, quantity of $SnCl_4$, (Table 1); solvent effects, (Table 2)], the conclusion was reached that optimal reaction conditions were: temperature +5 $^{o}C$ → room temperature and 2.6 equivalents of $SnCl_4$ in dichloromethane.

**Table 1:** Investigation of quantity of $SnCl_4$ (solution of 1M $SnCl_4$ in dichloromethane) used in the reaction.

| **Reagent** | **Quantity** | | | |
|---|---|---|---|---|
| **$SnCl_4$** | **1 mL (1.7 equiv.)** | **1.5 mL (2.6 equiv.)** | **2.0 mL (3.5 equiv.)** | **2.5 mL (4.3 equiv.)** |
| Tetra- methoxflavan-3-one | 200 mg | 200 mg | 200 mg | 200 mg |
| Tri-*O*-methylphloroglucinol | 150 mg | 150 mg | 150 mg | 150 mg |
| Dichloromethane | 15 mL | 15 mL | 15 mL | 15 mL |
| Time | 60 hours | 24 hours | 4 hours | 2 hours |
| **Yield** | **40 mg** | **101 mg** | **38 mg** | **32 mg** |

**Table 2:** Investigation of solvent effect used in the reaction

| Solvent | MC-K | $SnCl_4$ | Time | | |
|---|---|---|---|---|---|
| DCM | 100 mg | 0.75 ml | 48 hours | 71 mg (50%) | Product isolated |
| THF | 100 mg | 0.75ml | 48 hours | - | No product, starting materials (SM) decomposed |
| DMF | 100 mg | 0.75 ml | 48 hours | - | No product, SM decomposed |
| Toluene | 100 mg | 0.75 ml | 48 hours | - | Trace of product not isolated |
| Acetonitrile | 100 mg | 0.75 ml | 1 hour | | No product, SM decomposed |

MC-K = Tetra-methoxyflavan-3-one

The intermediate **(23)** could not be isolated, presumably because water elimination is encouraged by the formation of a stilbene-type conjugated system between the electron rich A- and D-rings (Scheme 12).

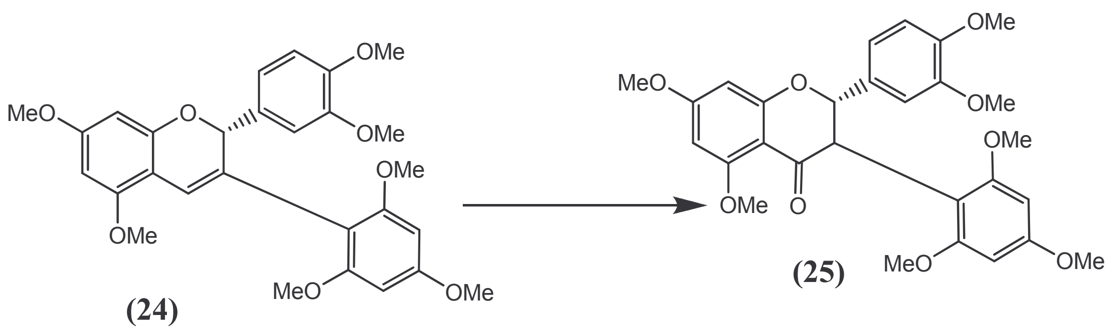
The 3-arylsubstituted flav-3-ene **(24)** has a $^{13}C$ NMR spectrum that clearly indicates the presence of six additional carbons ($^{13}C$ NMR **plate 8.2**), and an $^{1}H$ NMR spectrum with three additional methoxy groups and two additional aromatic protons ($^{1}H$ NMR **plate 8.1**). The presence of the additional aromatic moiety is confirmed by mass spectrometry (expected $[M+H]^+$ ion at m/z 495.29). The C-ring has only two protons at $\delta_H$ 5.83 (H-2, broadened), and 6.03 (H-4, $J_2$ = 1.0 Hz). Broadening of H-2 and H-4 spin is attributed to benzylic long range coupling to H-2.[14] The COSY spectrum (COSY **plate 8.3**) indicates that H-4 correlates with H-6, and H-2 correlates with H-2′ and H-6′, as well as the D-ring (the additional aromatic moiety) protons H-3″ and H-6″. As in the case of the ketal **(22)** and the enol ether **(20)**, the carbonyl resonance associated with the starting material **(16)** is not present. No rotational isomerism is observed.

The CD spectrum (**CD-plate 6**) of the 3-arylsubstituted flav-3-ene **(24)** shows a positive Cotton effect at $\lambda = 244$ nm.

## 6.5 Synthesis of I-3, II-6/8 biflavonoids

We consider synthesis of (**24**) as a proof of concept for a novel highly stereoselective biflavonoid synthesis that can be expanded to tri-, tetra-, and higher oligomers if we so wish.

Our initial efforts to oxidize the double bond between C-3 and C-4 to a carbonyl group on C-4 failed (Scheme 13). These include efforts to epoxidize or hydroxylate the conjugated double bond.

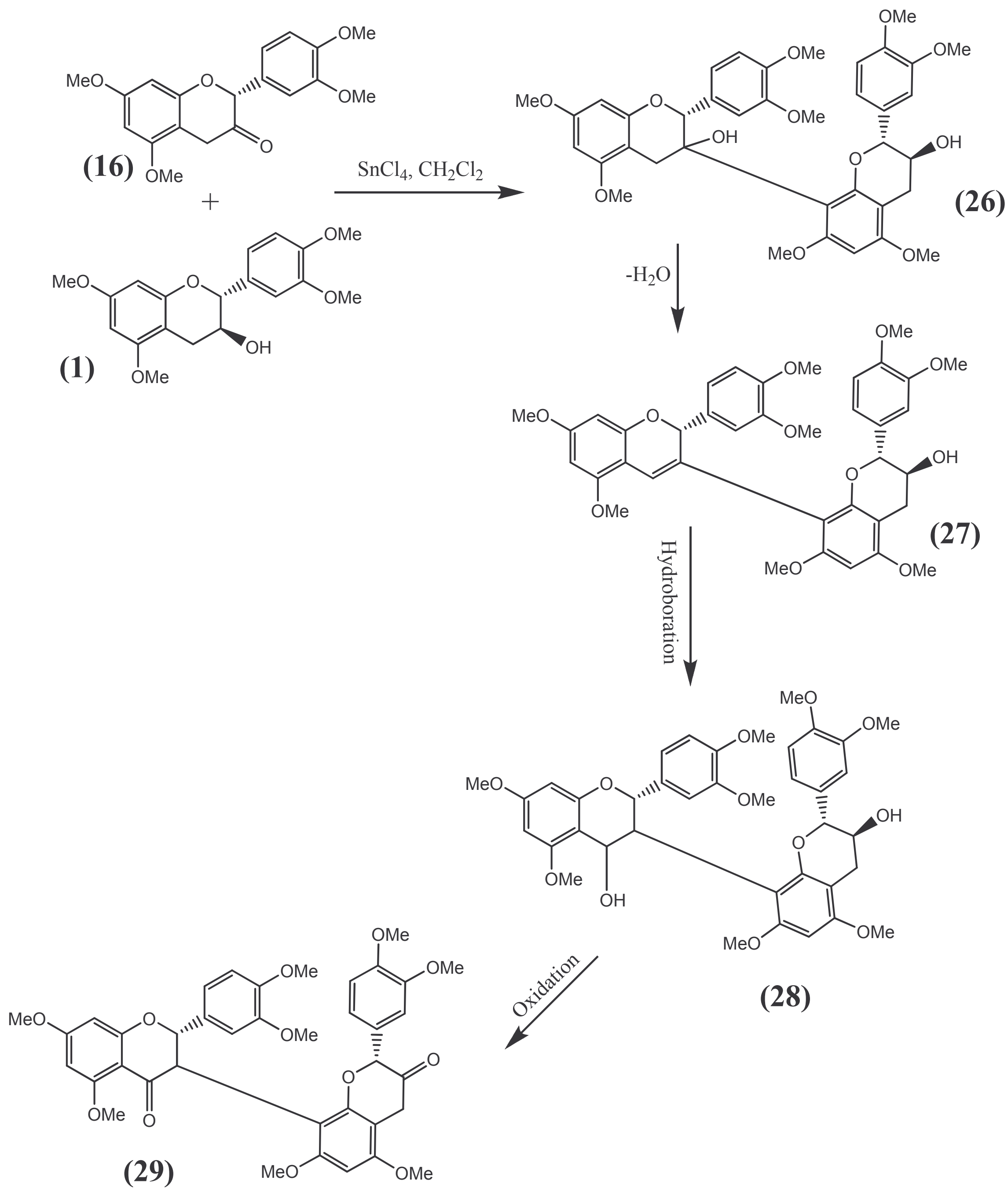



**Scheme 13:** Attempted oxidation of **(24)** to a biflavonoid (**25**).

These results are in agreement with Clark-Lewis's conclusion that flavenes with electron-releasing substituents in conjugated positions in the A-ring [e.g. 3′,4′,5,7-tetra-*O*-methylflav-3-ene (**9**)] do not give epoxides under acidic or basic conditions.[15]

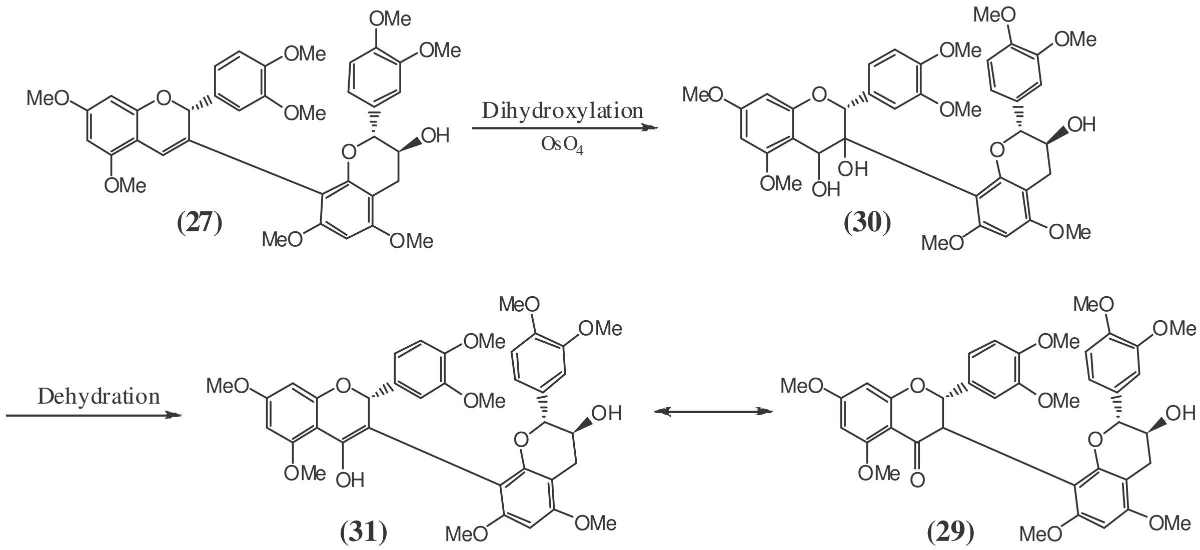
## 6.6 Future work.

Future work should concentrate on transforming the 3,4 double bond into a carbonyl group in the 4-position. We believe that a hydroboration-oxidation reaction sequence will provide facile access to the 3-aryl-4-keto compound as a model for the naturally occurring I-3, II-6/8 series of biflavonoids and subsequent oligomeric forms (Scheme 14).




**Scheme 14:** Prospective synthesis of the I-3, II-6/8 series of biflavonoids 1.

Alternatively, subjecting the 3-arylflav-3-ene to $OsO_4$ dihydroxylation, followed by dehydration will yield an enol that will rearrange spontaneously to form an I-3, II-6/8 biflavonoid compound having an oxo group at its C-4 carbon (Scheme 15).




**Scheme 15:** Prospective synthesis of the I-3, II-6/8 series of biflavonoids 2.

We will also investigate the transformation of flav-3-ene to flav-2-ene in accordance with the work by Casiraghi and Casnati[9] in order to achieve high yield access to flav-2-enes.

## 6.8 Conclusion.

In this chapter we have developed the first stereoselective synthesis of flav-3-enes and we have developed proof of concept for the first stereoselective synthetic access to I-3, II-6/8 biflavonoids. Both these are significant contributions towards flavonoid synthesis. We have also developed an alternative synthesis for flav-2-enes.

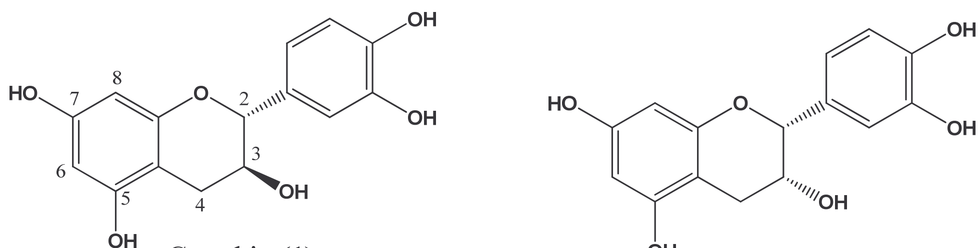
# REFERENCES


1. Lee, M. H.; Choi, E. T.; Lee, Y. M.; Kim, D.; Park, Y. S. *Bull. Korean Chem. Soc.,* **2008**, *29*, 1299-1300, and refs. therein.
2. Lee, C. -W.; Choi, H. -J.; Kim, H. -S.; Kim, D. -H.; Chang, I. -S.; Moon, H. T.; Lee, S. -Y.; Oh, W. K.; Woo, E. -R. *Bioorg. Med. Chem. Lett*. **2008**, *16*, 732-738.
3. Zheng, X.; Meng, W. -D.; Qing, F. –L. *Tetrahedron Lett.* **2004**, *45*, 8083-8085.
4. Gil, B.; Sanz, M. J.; Terencio, M. C.; Gunasegaran, R.; Paya, M.; Alcaraz, M. *J. Biochem. Pharmacol*. **1997**, *53*, 733.
5. Lee, M. K.; Lim, S. W.; Yang, H.; Sung, S. H.; Lee, H.-S.; Park, M. J.; Kim, Y. C. *Bioorg. Med. Chem. Lett*. **2006**, *16*, 2850-2854.
6. Gasnati, G.; Crisafulli, M.; Rica, A. *Tetrahedron Lett*. **1965**, 243.
7. Gasnati, G.; Crisafulli, M.; Conia, M.; Sartori, G.; Ungaro, K. *J. Chem. Soc., Perkin 1* **1974**, 2077.
8. Ferreira, D.; Slade, D.; Marais, J. P. J. *Flavonoids: Chemistry, Biochemistry and Applications,* Eds. Andersen, Q. M.; Markham, K. R.; CRC press Boca Raton, **2006**, 553-616
9. Casiraghi, G.; Casnati, G. *J. Org. Chem., Chem. Commun*. **1970**, 321.
10. Tückmantel, W.; Kozikowski, A. P.; Romanczyk, L. J. *J. Am. Chem. Soc.* **1999**, *121*, 12073–12081.
11. Mustafa, J.; Khan, R.; Khan, I. A.; Ferreira, D. *Org. Prep. Proced. Int*., **2004**, *36 (1)*, 61-68.
12. Clark-Lewis, J. W.; Jemison, R. W. *Aust. J. Chem.* **1970,** *23*, 315-321.
13. Steynberg, P.J.; Steynberg, J. P.; Bezuidenhoudt, B. C. B.; Ferreira, D. *J. Chem. Soc. Perkin Trans. I* **1995**, 3005-3012.
14. Marais, J. P. J. *Ph. D. Thesis*, *University of the Free State,* **2002**, 64.

15. Clark-Lewis, J. W.; McGarry, E. J.; Ilsley, A. H. *Aust. J. Chem.* **1974,** *27*, 865-877.

# NOVEL SYNTHESIS OF PROANTHOCYANIDINS WITH PREDOMINANTLY 3,4-*CIS* STEREO-CHEMISTRY: THE FIRST CARBON-CARBON BOND FORMATION WITH SILVER TETRAFLUOROBORATE

Condensed tannins or proanthocyanidins are ubiquitous in plants and are important constituents of the human diet. A wide range of potentially significant biological activities including antioxidant,[1] antiatherosclerotic,[2,3] anti-inflammatory,[4,5] antitumor,[6] antiosteoporotic,[7] and antiviral[8] effects have been attributed to this class of compounds. The procyanidins consist of oligo- and polymers having catechin (**1**) or epicatechin (**2**) (Figure 1) as constituent units and linked *via* the 4- and 8- or 4- and 6-positions.




Catechin (**1**)

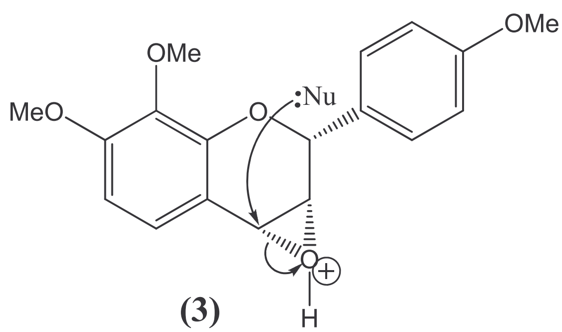
Epicatechin (**2**)

**Figure 1:** Structures of catechin and epicatechin.

Progress in the chemistry and biology of these compounds has been slow due to the difficulty of isolating and synthesizing pure free phenolic compounds. Chapter 1 gives a summary of the nomenclature of polyphenols, including a section on proantocyanidins (pp. **17-20**). Attention is also given to structure elucidation (pp. **20-24**).

In contrast with a secondary 3-hydroxy group on the heterocyclic ring (e.g. catechin), that resists nucleophilic substitution (see previous chapter), a flavonoid 4-hydroxy group is readily substituted with nucleophiles. This is attributed to the benzylic nature of this position that allows both $S_N2$ and $S_N1$ substitutions.

Ferreira and co-workers[9] postulated that weak nucleophiles attack *via* an $S_N1$ mechanism (intermediacy of a C-4 carbocation or quinone methide) and strong nucleophiles *via* an $S_N2$ mechanism (overlap of the HOMO of the approaching nucleophile with the antibonding orbital of the leaving group at C-4). They also postulated that a strong polar leaving group on the C-4 carbon favours an $S_N1$ mechanism.[9] In the case of carbocations, neighbouring group participation by the C-3-OH *via* a protonated oxirane **(3)** favours a 3,4-*trans* product (Figure 2).

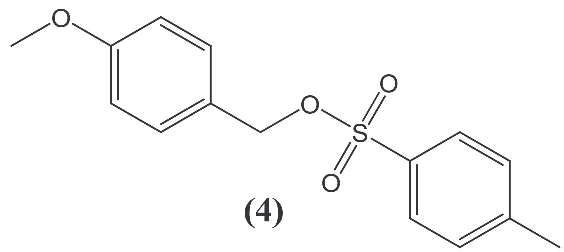



**(3)**

**Figure 2:** Neighbouring group participation by the C3-OH *via* a protonated oxirane (**3**).

In the case of an $S_N2$ mechanism, intermolecular hydrogen bonding between the C-3-OH and the approaching nucleophile may, in rare cases, guide the HOMO of the approaching nucleophile towards the σ*-antibonding orbital of the leaving group to yield products with 3,4-*cis* configuration.

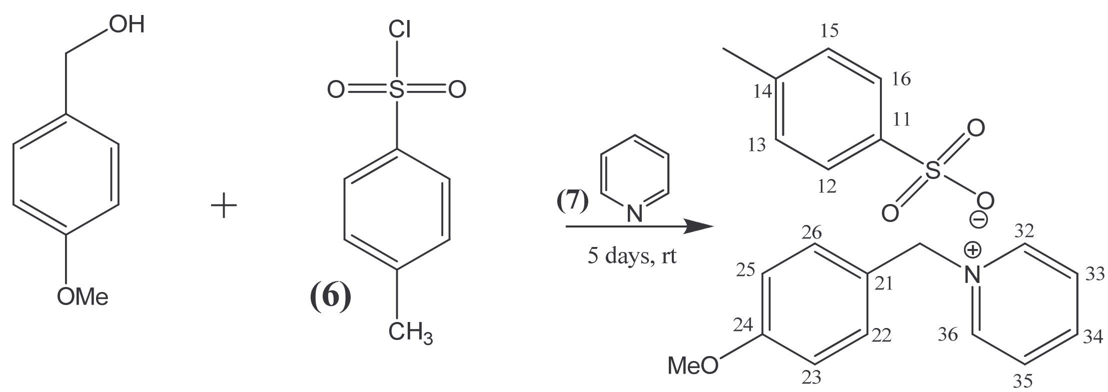
The ease with which a benzylic hydroxyl group is eliminated was demonstrated by our effort to synthesize 4-methoxybenzyltosylate **(4)** as a model compound (Figure 3).



**(4)**

**Figure 3:** Suspected structure of **(4)**.

Tosylation of *p*-methoxybenzylalcohol **(5)** with tosylchloride **(6)** in dry pyridine **(7)** yielded 1-(4-methoxybenzyl) pyridinium toluene-*4*-sulfonate **(8)** as a crystalline product in quantitative yield. The benzylic hydroxyl group is replaced by pyridine to give a quaternary ammonium cation that is stabilized by a sulfonate anion (Scheme 1).



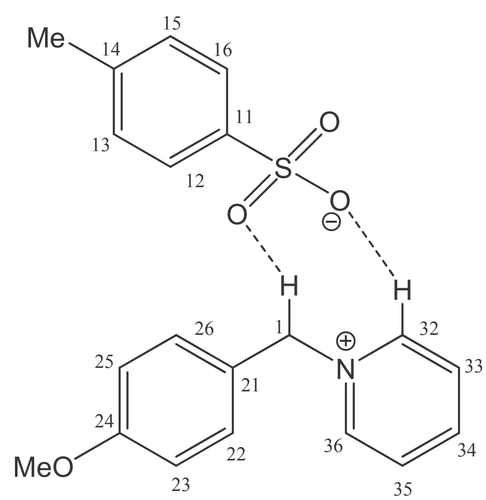

(5) (6) (7) (8)

**Scheme 1:** Synthesis of 1-(*4*-methoxybenzyl)-pyridinium; toluene-*4*-sulfonate.

This product **(8)**, exhibited not only NMR absorptions ($^{1}$H NMR **Plate 9.1** and $^{13}$C NMR **Plate 9.2**) corresponding with the initially anticipated benzylic tosylate **(4)**, but also absorptions from a pyridine moiety [$\delta_H$ = 9.29 (s, 2H, H-32/36); 8.24 (t, 1H, J = 7.7 Hz, H-

34); 7.85 (t, 2H, J = 6.6 Hz, 2H, H-33/35)] in a 1:1 ratio. We initially thought the pyridine was an impurity, but all efforts to remove it by chromatography or heating under vacuum failed and the 1:1 ratio persevered.

The compound **(8)**, $C_{13}H_{14}NO^{+}\cdot C_7H_7O_3S^{-}$, is evidently held together by intermolecular C–H···O hydrogen bonds[10] as shown in figure 4.




**(8)**

**Figure 4:** The intermolecular hydrogen bonds of compound **(8)**.

This conclusion is supported by electrospray mass spectrometry that shows an m/e = 200.3 base peak in positive mode corresponding to a 1-(4-methoxybenzyl)-pyridinium cation and an m/e 171.2 base peak in negative mode corresponding to a toluene-4-sulfonic acid anion. The structure of this product was subsequently proved unequivocally with crystallography (Figure 5).[10]

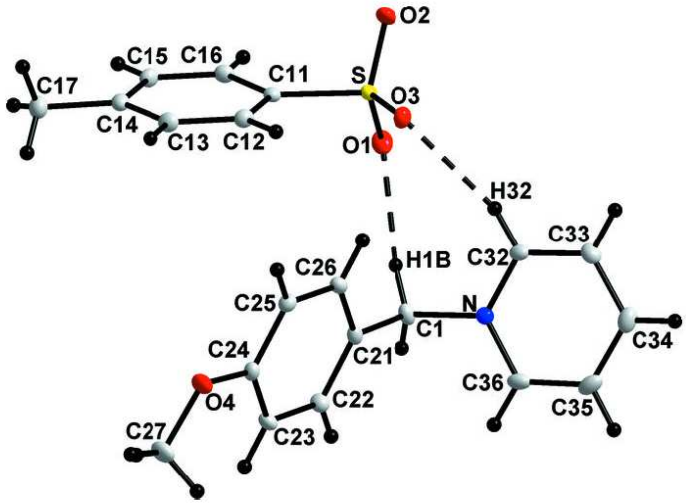



**Figure 5:** Crystallographical structure of 1-(4-methoxybenzyl)-pyridinium; toluene-*4*-sulfonate

The introduction of phenolic and flavanyl moieties at C-4 of flavan-3,4-diols is consequently easily effected by stereoselective acid-catalyzed condensation of the appropriate nucleophilic and electrophilic flavanoid units.[11] These initial stereoselective synthetic methods played an important role in the structure elucidation of the economically important profisetinidins and prorobinetinidins from *Acacia mearnsii* (Black Wattle) and *Schinopsis spp* up to the tetrameric level.[15-18] However, such methods were hampered by the laborious extraction procedures required to obtain the naturally occurring optically active starting materials with a 4-hydroxy leaving group. The compounds occur in low concentrations in plant material. (Chapter 2 gives a review of all published methods to synthesize proanthocyanidins from flavonoids with a hydroxyl or other leaving group at C-4 (pp 40-57)). An alternative strategy involving nucleophilic attack at a C-4 carbonyl group (Chapter 2, pp 58-64) also suffers from poor availability of starting materials.

Subsequent developments of methods to generate electrophilicity at the C-4 benzylic position of commercially available catechin **(1)** and epicatechin **(2)** by introducing a C-4 oxygen leaving group greatly enhanced the synthetic access to procyanidin dimers and oligomers.[19-24] These efforts are comprehensively described in Chapter 2.

Selective bromination at C-4 of compounds **(1)** and **(2)** was the first described method to introduce a leaving group at C-4. This was, however, only possible with peracetates where the reactivity of the aromatic rings toward competing bromination is supressed by electron-withdrawing acetate groups.[25]

The bromination methodology was replaced by oxidation of the C-4 hydrogen of flavan-4-ols with DDQ and a variety of other oxidation reagents and trapping of the intermediates by the solvent to yield a variety of alkoxy leaving groups at C-4. A large number of Lewis acids were used to catalyze the replacement of the alkoxy groups with aryl moieties. These efforts are comprehensively described in Chapter 2 (pp 70-96).

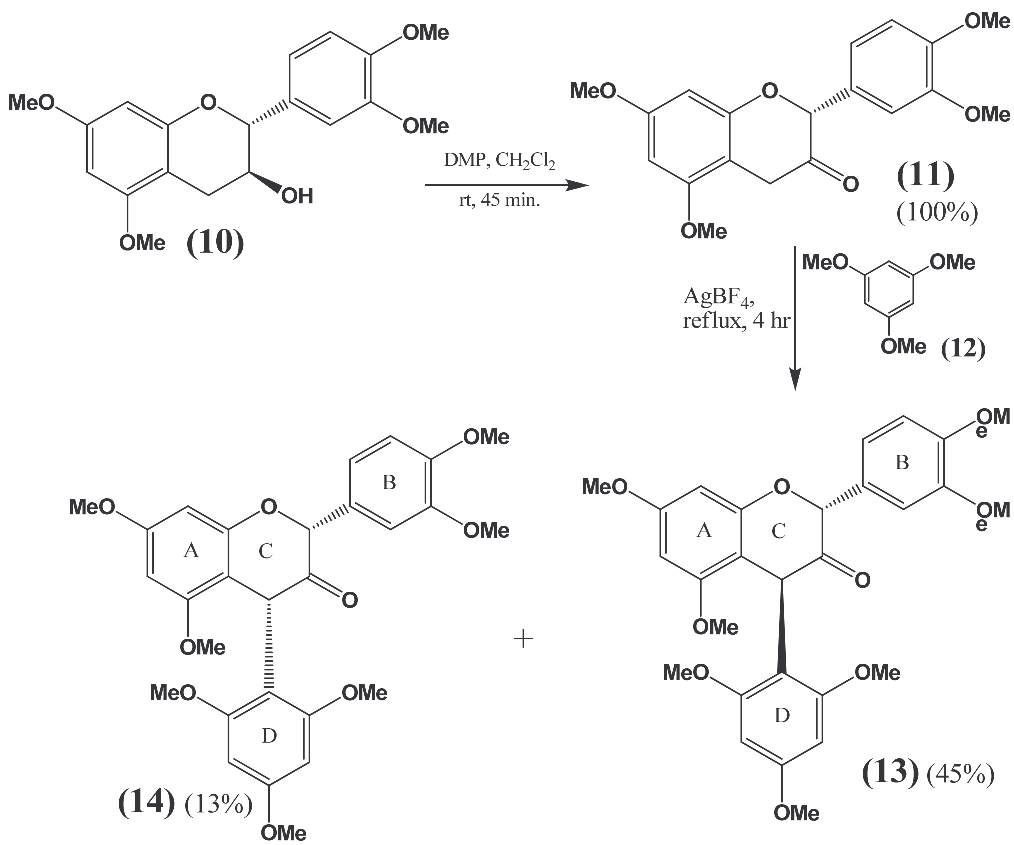
A major disadvantage of using starting materials with a leaving group at C-4 was self condensation and the formation of homo-oligomers. An equally serious problem was that the degree of polymerization could not be controlled and a mixture of di-, tri-, tetra- and higher oligomers were formed. This causes low yields of a specific target oligomer and requires high-resolution chromatography.

A variety of strategies were developed to obtain hetero-oligomers where the monomeric units are not the same. This includes protection of the reactive C-8 position *via* halogenation. Suzuki recognized the “sugar-flavonoid” analogy (Chapter 2, pp 106-115) and developed his "orthogonal strategy" that was based on oligosaccharide synthetic methods. This involves the alternating use of hard and soft leaving groups at C-4 and corresponding hard and soft catalysts. Despite Suzuki's claim that his method solved the problem of uncontrolled polymerization and formation of homo-polymers, few subsequent reported proanthocyanidin syntheses used his method. His method also requires protection at C-8 or C-6. Chapter 2 gives a comprehensive review of methods to introduce functionality at C-4 and efforts to control homo-oligomerisation and uncontrolled polymerization.

In this thesis and chapter, we report a novel and facile method for the introduction of a phenolic unit at unfunctionalized C-4 of per-*O*-methylcatechin and hence to synthesize procyanidin B-3 type dimer derivatives. Our method has the following advantages:

1. It does not require pre-functionalization of the C-4 position of flavan-3-ols. We have developed direct oxidative C-6/8→C-4 bond formation between two flavonoid monomers. We could not find any previous use of carbon-carbon bond formation with $AgBF_4$
2. Self condensation and the formation of homo-polymers are not observed. We believe that the carbonyl group on the C-3 position of our one starting material conjugates with the aromatic A-ring *via* its enol. This lowers the HOMO energy and deactivates the A-ring of the carbonyl containing congener to such an extent that it does not act as a nucleophile.
3. The synthetic methods described so far relies on an electrophilic flavan-3-ol with a hydroxyl group at C-3. The configuration of the hydroxy group at C-3 controls the stereochemistry at C-4 and 3,4-*trans* proanthocyanidins are usually isolated as the major product. In our method, C-3 is a planar $sp^2$ carbonyl and the stereochemistry at C-4 is controlled by the stereochemistry at C-2 (configuration of the B-ring). We thus have access to both 3,4-*cis* and 3,4-*trans* proanthocyanidins, depending on the configuration of the B-ring at C-2.

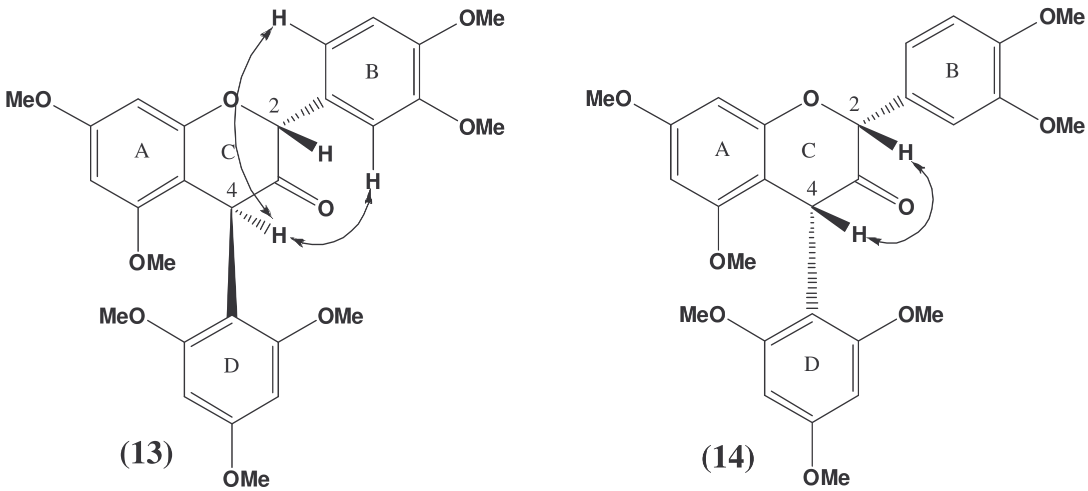
Treatment of tetra-*O*-methyl-3-oxo-catechin **(11)**, available almost quantitatively from tetra-*O*-methylcatechin **(10)** via Dess-Martin periodinane (DMP) oxidation,[20] with 1,3,5-tri-*O*-methylphloroglucinol **(12)** in the presence of $AgBF_4$ in THF afforded the C-4 phloroglucinol adducts **(13)** (45%) and **(14)** (13%) (Scheme 2).[26]




**Scheme 2:** Condensation reaction between **(11)** and **(12)**.

The two 4-coupled oxo products **(13)** and **(14)** have NMR spectra that clearly indicate the presence of six additional aromatic carbons ($^{13}C$ NMR **Plate 10.2** and **Plate 11.2**, respectively), three additional methoxy groups ($^{1}H$ NMR **Plate 10.1** and **Plate 11.1**, respectively) and two additional aromatic protons ($^{1}H$ NMR **Plate 10.1** and **Plate 11.1**, respectively). The presence of the additional aromatic moiety is confirmed by mass spectrometry ($M^+$: m/z= 511.25). The C-ring has only two protons [$\delta_H$ 5.10 (H-4), and 5.63 (H-2) for **(13)**, and 5.23 (H-4), and 5.36 (H-2) for **(14)**]. The HMBC spectrum of **(13)** confirms that H-2 is coupled to the B-ring carbons (correlation between H-2 and C-2′ and C-6′) (HMBC **Plate 10.6**). Correlations of H-4 with C-10 and C-1″ confirm the connection of H-4 with both the A- and D-rings (HMBC **Plate 10.6**). Similar correlations for (14) confirmed the structure. The carbonyl resonances associated with the starting material **(11)** remain at about $\delta_C$ 206 for both **(13)** and **(14)** ($^{13}$C NMR **Plate 10.2** and **Plate 11.2**, respectively). The hydrogen and carbon resonances of the A-and B-rings of **(13)** and **(14)** are similar to those of the starting material. No rotational isomerism is observed. The isolation of the two diastereoisomers **(13)** and **(14)** that demonstrates opposite Cotton effects at about wavelength 237 nm (**CD-Plate 7** and **CD-Plate 8**) indicates that the C-2 configuration has been maintained.

NOESY experiments (NOESY **Plate 10.3** and **Plate 11.3**) allows us to distinguish between the 4-α- **(14)** and 4-β-aryl **(13)** products and to assign (2*R*,4*S*)- and (2*R*,4*R*)- configurations, respectively. The 4-β-aryl product **(13)** has the aromatic B- and D-rings on the opposite face and H-2 and H-4 *trans* to each other. No through space coupling between H-2 and H-4 is observed. However, observed NOESY between H-4 and H-2′/6′ confirms that H-4 and the B-ring are on the same face (NOESY **Plate 10.3**) (Figure 5). The 4-α-aryl product has the aromatic B- and D-rings on the same face and H-2 and H-4 *cis* (on the same face) to each other. Through space coupling, NOESY between H-2 and H-4 is thus observed (NOESY **Plate 11.3**). This conclusion is further supported by the presence of *W*–coupling between H-2 and H-4 in **(14)** ($^1$H NMR **Plate 11.1**) and the absence of *W*-coupling in **(13)** ($^1$H NMR **Plate 10.1**).

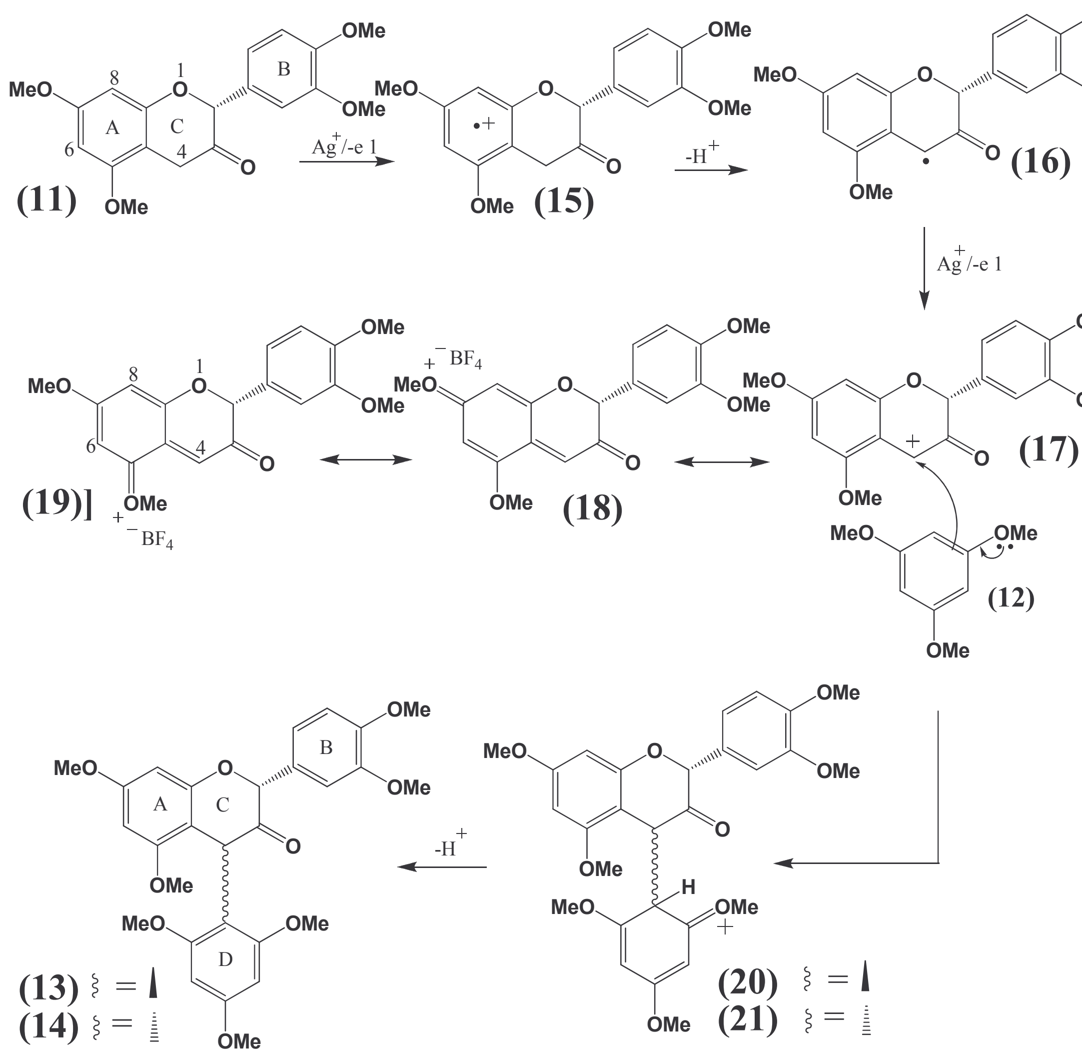


(13) (14)

**Figure 5:** Observed NOE correlations between H-4 and H-2′/6′ of **(13)**, and between H-2 and H-4 of **(14)**.

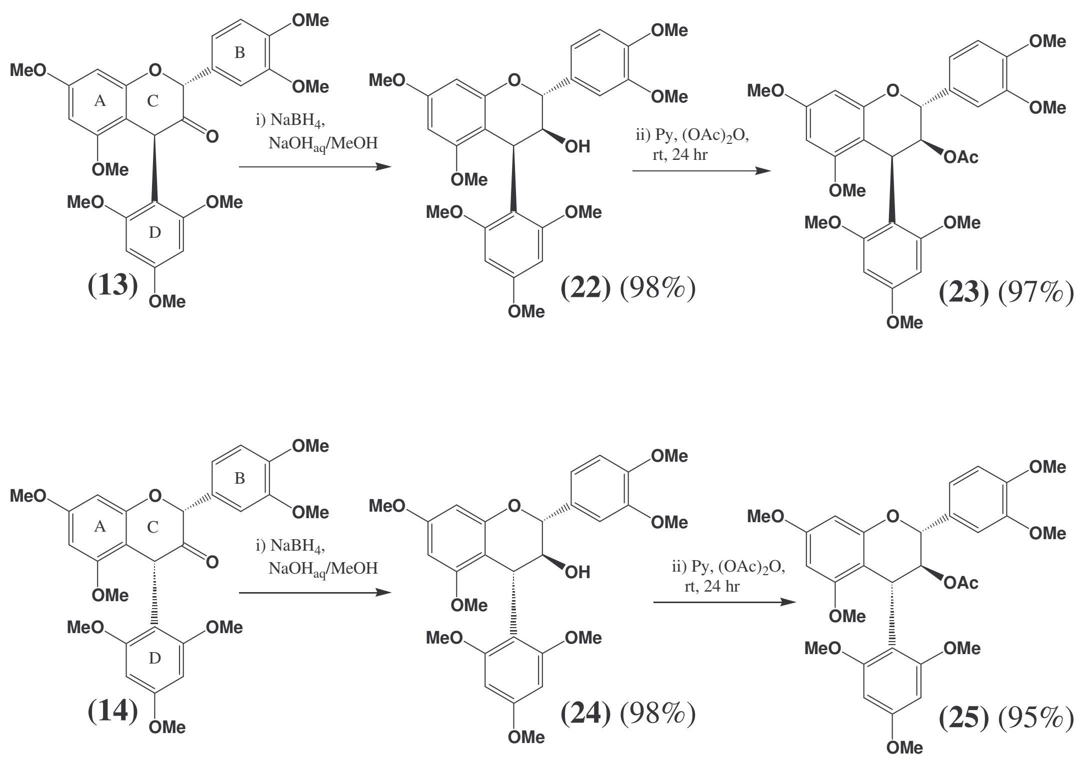
The requirement of an excess of $AgBF_4$ and the observation of a silver mirror (reduction of $Ag^1$ to $Ag^0$) indicate a two-electron oxidative mechanism (Scheme 3). The $BF_4^-$ counterion probably assists in stabilising the 4-carbocation **(17)** *via* the quinone methide tautomer **(18)**.

No self-condensation or further condensation products were evident, probably due to the deactivation of the nucleophilic properties of the A-ring of **(11)** *via* the enolic tautomer of the C-ring.



**Scheme 3:** Proposed mechanism for the oxidative formation of the interflavanyl bond.

Reduction of the 3-carbonyl of the 4-aryl products with $NaBH_4$ in aqueous NaOH/MeOH, takes place stereoselectively and in high yields (above 95%). We observed $H^-$ attack from the α-face exclusively, resulting in two 4-arylflavan-3-ol epimers **(22)** and **(24)** from **(13)** and **(14)**, respectively (Scheme 4). Both products have 3*S* configuration i.e. (2*R*,3*S*,4*S*) for **(22),** and (2*R*,3*S*,4*R*) absolute configuration for **(24)**. This stereoselectivity is attributed to steric influences from the B-ring on C-2.



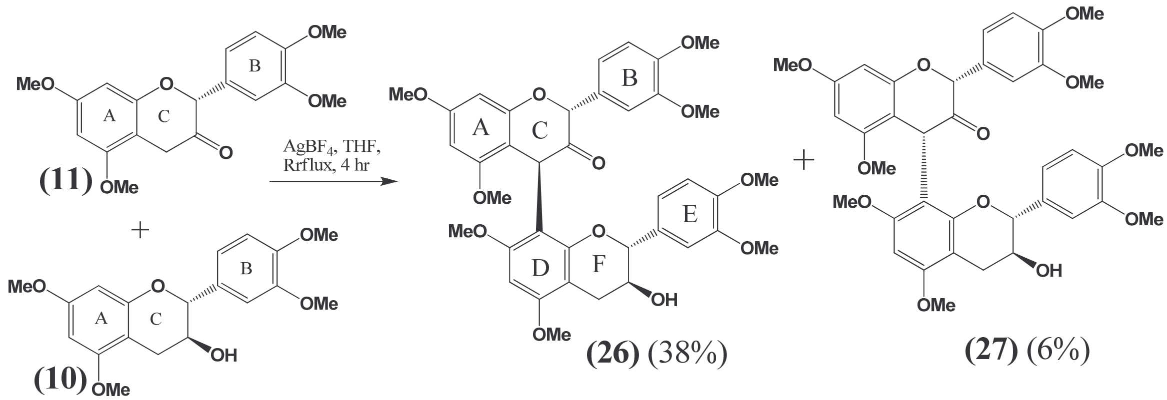

**Scheme 4:** Reduction of **(13)** and **(14)** using $NaBH_4$, and subsequent acetylation with pyridine/acetic anhydride.

The 2,3-*trans*-3,4-*cis*-epimer **(22)** has $J_{3,4}$ = 6.3 Hz ($^1$H NMR **Plate 12.1**), and the 3,4-*trans* epimer **(24)** has $J_{3,4}$ = 6.5 Hz ($^1$H NMR **Plate 14.1**). The 2,3-*trans* coupling constant is 9 Hz in the **(22)** epimer ($^1$H NMR **Plate 12.1**) and $J_{2,3}$ = 10.4 Hz for the **(24)** epimer ($^1$H NMR **Plate 14.1**). These coupling constants are in agreement with the characteristic coupling constants in Chapter 1, p 23.[27]

Acetylation of **(22)** and **(24)** yielded the acetates **(23)** and **(25)**, respectively (Scheme 4). The acetate moiety resonates as a three proton singlet at $\delta_H$ 1.71 for **(23)** and at $\delta_H$ 1.71 for **(25)** ($^1$H NMR **Plate 13.1** and **15.1**, respectively). The mass spectra agree with the expected addition of an m/z = 43 fragment, associated with an additional $CH_3CO$ moiety.

The CD spectra and associated Cotton effects at about 240 nm of **(23)** and **(25)** (**CD-plates 10** and **11**) are in agreement with the empirically established CD rules in Chapter 1, Table 5[27].

The $AgBF_4$-catalyzed condensation reaction between the monomers **(10)** and **(11)** afforded the anticipated dimers **(26)** (38%) and **(27)** (6%) with [2*R*,4*S* (C-ring):2*R*,3*S* (F-ring)] and [2*R*,4*R* (C-ring):2*R*,3*S* (F-ring)] configurations, respectively (Scheme 5).

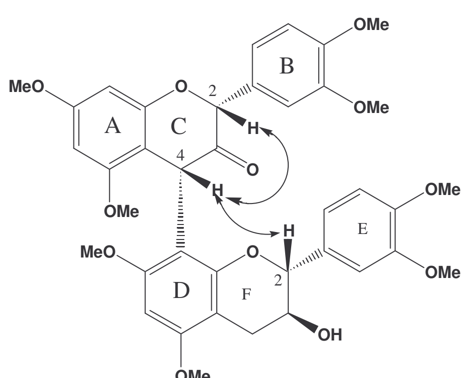



**Scheme 5:** Condensation reaction between **(10)** and **(11)**.

The two 4-coupled oxo dimers **(26)** and **(27)** have [1]H NMR spectra that clearly indicate four additional methoxy groups, four additional aromatic protons ([1]H NMR **Plate 16.1** and **16.2**, and **17.1** and **17.2**, respectively), and the presence of twelve additional aromatic, and three additional heterocyclic carbons ([13]C NMR **Plate 16.3** and **17.3**, respectively). The presence of the additional flavan-3-ol moiety for **(26)** and **(27)** was confirmed by mass spectrometry ($M^+$: m/z = 688). Two pyran-rings (C-ring and F-ring) are observed. The C-ring has only two aliphatic singlet protons [$\delta_H$ 5.09 (H-4) and 5.39 (H-2) for **(26)**, and $\delta_H$ 5.42 (H-4) and 5.19 (H-2) for **(27)**]. The F-ring is a typical ABX system, associated with flavan-3-ols (the "bottom unit"). The carbonyl resonances associated with the starting material **(11)** remain at

$\delta_C$ 206.2 for **(26)** (**Plate 16.3**), and $\delta_C$ 204.8 for **(27)** (**Plate 17.3**), and the hydrogen and carbon resonances of the A- and B-rings remain similar to those of the starting material for both **(26)** and **(27)**. No rotational isomerism is observed. The isolation of two diastereoisomers **(26)** and **(27)** that demonstrate opposite Cotton effects at wavelength of about 240 nm (**CD-plates 12** and **13**, respectively) indicates that the C-2 configuration has been maintained.

A NOESY experiment (Noesy plates **Plate 17.4** and **Plate 17.5**) allows us to distinguish between the 4-α- **(27)** and 4-β-dimers **(26)** with 2*R*,3*S*,*4S* and 2*R*,3*S*,4*R* configuration respectively. The 4-α-aryl product has the aromatic B- and D-rings on the same face and H-2 and H-4 *cis* to each other. Through space coupling between H-2 and H-4 is observed. The 4-β-aryl product has the aromatic B- and D-rings on opposite faces and H-2 and H-4 *trans* to each other and no through space coupling between H-2 and H-4 is thus observed (Figure 6). The 4-α configuration of **(27)** is further supported by the presence of *W*-coupling (J = 1.7 Hz, **plate 17.2**). *W*-coupling is not possible in the 4-β-isomer **(26)** and H-2 and H-4 occur as singlets (**Plate 16.2**).

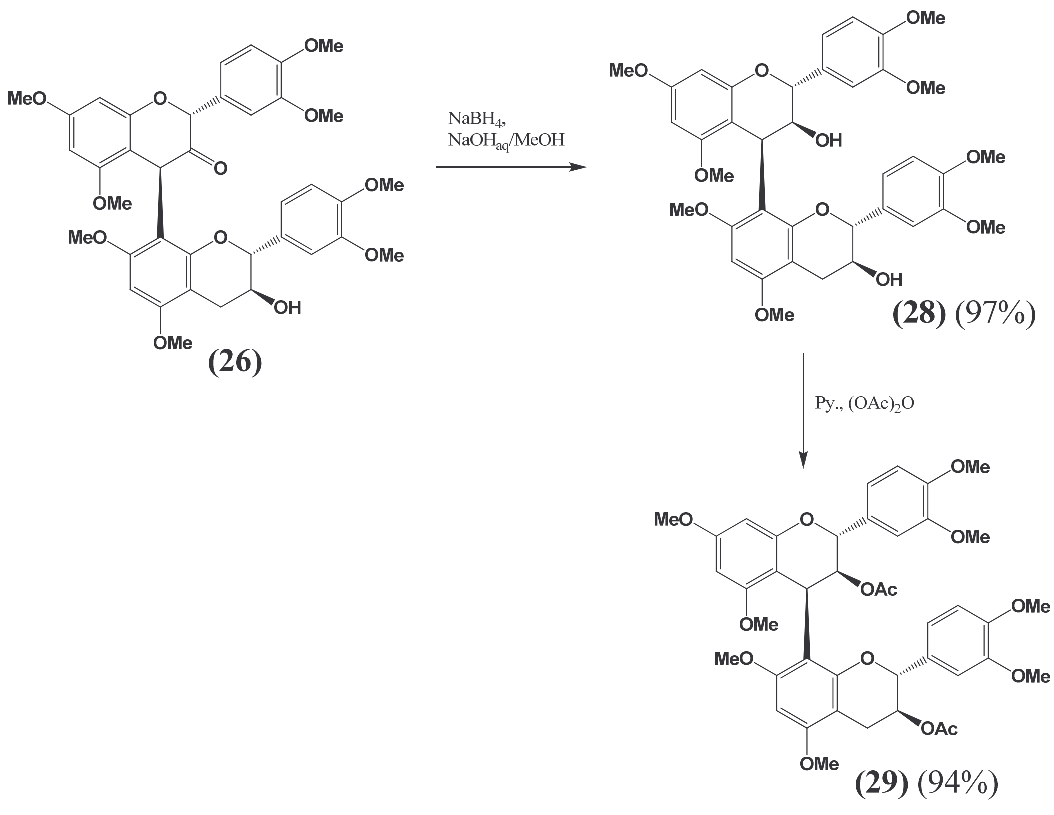



**(27)**

**Figure 6:** Observed NOESY interactions between H-4(C) and H-2(C) and H-2(F), respectively, for **(27)**.

The 8-position of **(11)** is less hindered than the 6-position. The heterocyclic ring folds C-2 away from the 8-position. The two methoxy groups on each side of the 6-position are not folded away. Expected coupling on the 8-position for **(27)** is confirmed by a NOESY experiment that shows coupling between H-4 (C) and H-4 (F) (Noesy **Plate 17.5**) (Figure 6). This interaction is not possible with coupling on the 6-position.

Reduction of **(26)** with $NaBH_4$ takes place stereoselectively in high yields (above 95%). We observed $H^-$ attack from the $\alpha$-face exclusively, resulting in the 2,3-*trans*-3,4-*cis* proanthocyanidin epimer **(28)** with 2*R*,3*S*,4*S* configuration (NMR **Plates 18**). The stereoselective reduction of the carbonyl group is attributed to steric influences from the B-ring on C-2 (Scheme 6).[27,28]




**Scheme 6:** Reduction of **(26)** using $NaBH_4$, and subsequent acetylation with pyridine/acetic anhydride.

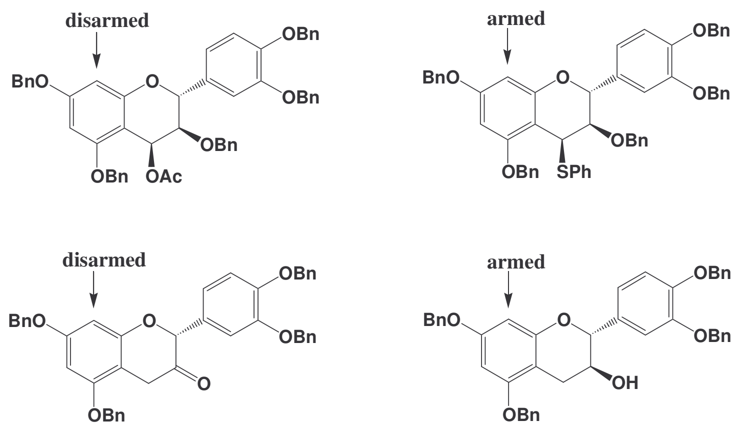
Acetylation of **(28)** yielded the di-acetates **(29).** The $^{1}H$ NMR spectra of both **(28)** and **(29)** show the effects of rotational isomerism ($^{1}H$ NMR **Plates 18.1 – 18.3** and Plates **19.1 – 19.3**, respectively). The two acetate moieties of the main rotational isomer of **(29)** resonate as two proton singlets at $\delta_H$ 1.65 and 1.73 (**Plate 19.1** and **Plate 19.2**). The mass spectra agree with the expected addition of two m/z = 43 fragments, associated with two additional $CH_3CO$ moieties. The CD spectrum of **(29)** (**CD-plate 15**) is in agreement with Table 5 (Chapter 1, p 23, ref.28).

This epimer with 2,3-*trans*-3,4-*cis*-configuration on the "upper unit" **(29)** has coupling constants of $J_{2,3}$ = 8.9 Hz and $J_{3,4}$ = 6.3 Hz ($^{1}H$ NMR **Plate 19.1** and **Plate 19.2**). These coupling constants are in agreement with the characteristic coupling constants in Chapter 1, p. 23[27] and similar to the results obtained with the 4β-aryl product **(23)**. The "bottom unit" remains the same and corresponds closely with that of the catechin starting material ($J_{2,3}$= 10.4 Hz, $J_{3,4}$ = 6.3 Hz).

The relatively low yields are explicable in terms of poor recovery for silica chromatography substrates, possible oxidation of the enol form of the 3-keto flavan to the corresponding anthocyanidin, and the small scale of the reaction. We are currently investigating protocols to increase yields.

In contrast with existing methods where self-condensation of the C-4-functionalized precursors cannot be avoided, and a complex mixture of dimers, trimers, tetramers, and higher oligomers can be formed, our 3-oxocatechin precursor does not undergo self-condensation and allows isolation of the dimer as the sole product. Deactivation of the nucleophilicity of the A-ring by oxidising the 3-OH to a 3-oxo group parallels the use of a 4-acetoxy group by

Suzuki and co-workers.[23] Suzuki used the term arm/disarm (Figure 7) to illustrate his strategy to avoid uncontrolled polymerization and allow exclusive formation of hetero-oligomers. In our work we do not protect the 8-position *via* bromination. This indicates that a 3-oxo group is more deactivationg than a 4-acetoxy group. In both our and Suzuki's work, no coupling in the 6-position was observed.




**Scheme 7:** Strategies to manipulate the reactivity of the A-ring.

Oxidation of the 3″-hydroxyl group in the catechin moiety of the dimer **(28)** to a 3″-oxo group would allow introduction of a second catechin molecule and isolation of a trimer. We envisage a stepwise controlled synthesis of oligomers of catechin based on repeated cycles of oxidation and condensation.

Surprisingly, and in contrast to the 3-hydroxy dimers **(22), (24),** and **(28)**, respectively, the 3-oxo dimers **(13)**, **(14)**, **(26)** and **(27)** did not demonstrate rotational isomerism in their NMR spectra. Presumably the additional planarity of the C-ring, introduced by the planar $sp^2$ carbonyl on C-3, forces the 4-aryl moiety into an *axial* position, away from the methoxy group on C-5.

Previously, formation of procyanidin B-3 derivatives from 4-functionalized flavan-3-ol precursors yielded predominantly the 3,4-*trans* isomer and only minute quantities of the 3,4-*cis* isomer. Stereochemistry is controlled by the 3-hydroxy substituent that directs attack from the anti position to give 3,4-*trans* configuration. In our reaction, the tetrahedral $sp^3$ 3-hydroxy substituent has been replaced by a planar $sp^2$ carbonyl group, and stereochemistry is now controlled by the 2-aryl substituent. We observed the opposite distribution of diastereoisomers with a β:α ratio ranging from about 3:1 (Scheme 3) to about 6:1 (Scheme 5). Such a preference for β face diastereoselectivity and hence the favoring of procyanidins with 3,4-*cis* interflavanyl bonds is, no doubt, caused by the α-orientated B-ring in intermediate **(17)** (Scheme 3).

Tetra-*O*-methylcatechin **(10)** is much larger than tri-*O*-methylphloroglucinol **(12)** and should experience a greater steric directing effect into the β-position during coupling by the α-orientated B-ring of the oxo-congener **(11)**. This is indeed confirmed by a β:α ratio that changes from about 3:1 to about 6:1 when tri-*O*-methylphloroglucinol **(12)** is replaced by tetra-*O*-methylcatechin **(10)** in our coupling step.

## Conclusion

We have thus developed a unique and facile synthesis of catechin dimer derivatives which circumvents the need for C-4 functionalization, protection of reactive positions on the A-ring, avoids competing polymerization, and will allow stepwise formation of hetero-oligomers. This method, based upon oxidative C-C interflavanyl bond formation, will contribute significantly to ready synthetic access to proanthocyanidin analogues, especially procyanidins with 3,4-*cis* configured catechin chain extension units.

## Future work

Work is in progress to synthesize the free phenolic analogues of these dimers *via* benzyl ethers. In the longer term we will synthesize tri- and tetramers with predominantly 3,4-cis stereochemistry for bio-activity testing.

# REFERENCES


1. Korkina, L. G.; Afanas'ev, I. B. *ADV Pharmacol.* **1997**, *38*, 151–163.
2. Hertog, M. G.; Kromhout, D.; Aravanis, C.; Blackburn, H.; Buzina, R.; Fidanza, F.; Giampaoli, S.; Jansen, A.; Menotti, A.; Nedeljkovic, S. *Arch. Intern. Med.* **1995**, *155*, 381386;
3. Knekt, P.; Jarvinen, R.; Reunanen, A.; Matela, J. *BMJ* **1996**, *312*, 478–481.
4. Laughton, M. J.; Evans, P. J.; Morony, M.A.; Hoult, J. R.; Halliwell, B. *Biochem. Pharmacol.* **1991**, *42*, 1673–1681.
5. Yoshimoto,T.; Furukawa, M.; Yamamoto, S.; Horie, T.; Watanabi-Kohno, S. *Biochem. Biophys. Res. Commun.* **1983**, *116*, 612–618.
6. Stefani, E. D.; Boffetta, P.; Deneo-Pellegrini, H. *Nutr. Cancer* **1999**, *34*, 100–110.
7. Hegarty, V. M.; May, H. M.; Khaw, K. T. *Am. J. Clin. Nutr.* **2000**, *71*, 1003–1007.
8. Wang, H. K.; Xia, Y.; Yang, Z. Y.; Natschke, S. L.; Lee, K. H. *Adv. Exp. Med. Biol.* **1998**, *439*, 191–225.
9. Coetzee, J.; Malan, E.; Ferreira, D. *Tetrahedron*, **1999**, *55*, 9999-10004.
10. Achilonu, M. C.; Van Rensburg, J. M.; Van der Westhuizen, J. H.; Roodt, A. *Acta Cryst.,* **2007**, *E63*, 04326.
11. Geissman, T. A.; Yoshimura, N. N. *Tetrahedron Lett.* **1966**, *7* (24), 2669–2773.
12. Barrett, M. W.; Klyne, W.; Scopes, P. M.; Fletcher, A. C.; Porter, L. J.; Haslam, E. *J. Chem. Soc., Perkin Trans. I* **1979**, 2376.
13. Botha, J. J.; Ferreira, D.; Roux, D. G. *J. Chem. Soc., Chem. Commun.* **1978**, 698–513.
14. Botha, J. J.; Young, D. A.; Ferreira, D.; Roux, D. G. *J. Chem. Soc., Perkin Trans. 1* **1981**, 1213–1219.
15. Botha, J. J.; Ferreira, D.; Roux, D. G. *J. Chem. Soc., Perkin Trans. 1* **1981**, 1235–1244.
16. Delcour, J. A.; Ferreira, D.; Roux, D. G. *J. Chem. Soc., Perkin Trans. 1* **1983**, 1711–1717.

17. Botha, J. J.; Viviers, P. M.; Young, D. A.; Du Preez, I. C.; Ferreira, D.; Roux, D. G.; Hull, W. E. *J. Chem. Soc., Perkin Trans. 1* **1982**, 527–533.

18. Young, D. A.; Kolodziej, H.; Ferreira, D.; Roux, D. G. *J. Chem. Soc., Perkin Trans. 1* **1985**, 25372544..

19. Steynberg, P. J.; Nel, R. J. J.; Van Rensburg, H.; Bezuidenhoudt, B. C. B.; Ferreira, D. *Tetrahedron* **1998**, 8153–8158.

20. Tückmantel, W.; Kozikowski, A. P.; Romanczyk, L. J. *J. Am. Chem. Soc.* **1999**, *121*, 12073–12081.

21. Kozikowski, A. P.; Tückmantel, W.; George, C. *J. Org. Chem.* **2000**, *65*, 5371–5381.

22. Saito, A.; Nakajima, N.; Tanaka, A.; Ubukata, M. *Biosci. Biotechnol. Biochem.* **2002**, *66* (8), 1764–1767.

23. Ohmori, K.; Nakajima, N.; Suzuki, K. *PNAS* **2004**, *101* (33), 12002–12007.

24. Mohri, Y.; Sagehashi, M.; Yamada, T.; Hattori, Y.; Morimura, K.; Kamo, T.; Hirota, M.; Makabe, H. *Tetrahedron Lett.* **2007**, *48*, 5891–5894.

25. Steenkamp, J. A.; Malan, J. C. S.; Ferreira, D. *J. Chem. Soc., Perkin Trans. 1* **1988**, *217*, 9–2183.

26. Standard coupling method. A solution of tetra-*O*-methyl-catechin (0.435 mmol) in THF (3 mL) was added dropwise to a mixture of $AgBF_4$ (1.1 mmol) and tetra-*O*-methyl-3-oxocatechin (0.145 mmol) in THF (3 mL) and refluxed under nitrogen for 4 hr. Filtration on $SiO_2$ and silica gel TLC yielded the desired products.

27. Van der Westhuizen, J. H. *'n Nuwe Reeks Fotochemiese Reaksies in Flavonoïedsintese, Ph. D Thesis* **1979**, 90-100.

28. Van der Westhuizen, J. H.; Ferreira, D.; Roux, D. G. *J. Chem. Soc., Perkin Trans. I* **1981**, *122*, 0–1226.

# Chapter 8

# STANDARD EXPERIMENTAL TECHNIQUE

Unless specified otherwise, the following techniques are applied throughout the course of this study.

## 8.1 CHROMATOGRAPHY

### 8.1.1 Thin- layer chromatography

**Qualitative** thin layer chromatography (TLC) was conducted on Merck TLC – Aluminum – backed Silica Gel $F_{254}$ (0.2 mm layer) sheets divided into strips of *ca*. 3 x 6 cm. $R_f$ values reported as those observed in the qualitative TLC assessments.

**Preparative scale:** Thin-layer chromatography (TLC) was conducted on glass plates (20 x 20 cm) coated with a layer of inactivated Merck Kieselgre 60 $PF_{254}$ (1.0 mg) and dried at room temperature overnight. Each plate was charged with about 20 mg of the crude product and developed in about 100 ml of pre-determined development solvent. After development in the appropriate eluent, the plates were dried in a fast stream of air and the bands were identified by either UV- light (254 nm) or by the appropriate spraying reagent.

The carefully marked out and collected bands were eluted with acetone (in some cases, acetone/methanol mixture). Then, the product was obtained by removing the solvent under reduced pressure (rotary evaporator) at about 40 $^{o}$C.

### 8.1.2 Column chromatography

**Flash column chromatography (**FCC) was performed on a glass column (5 cm diameter) charged with 10 0g of Merck Kieselgel 60 (230- 400 mesh) for every 1 g of the crude product. The crude product was dissolved in a minimum amount of the appropriate solvent and carefully applied to the column. A flow of nitrogen (about 50 kPa pressure) through the appropriate solvent separated the sample into clean products. The pure products were collected in fractions, and the solvent was removed by rotary evaporation.

## 8.2 SPRAY REAGENTS

### Formaldehyde/sulfuric acid

Thin-layer chromatograms were sprayed with a 2% v/v solution of formaldehyde (40% solution in water) and concentrated sulfuric acid, and heated to about 120 $^{o}C$ to effect maximum development of colour.

## 8.3 SPECTROSCOPICAL METHODS

### 8.3.1 Nuclear magnetic resonance spectroscopy (NMR)

NMR spectra were recorded on a Bruker Avance spectrometer (600 MHz). Tetramethylsilane was added as reference to all samples. The solvents used were deuteriochloroform ($CDCl_3$: $\delta_H$ 7.24), deuteriobenzene ($C_6D_6$: $\delta_H$ 7.15), deuteriodimethylformamide (DMF-$d_7$: $\delta_H$ 8.31) and deuterioacetone $(CD_3)_2CO$/acetone-$d_6$: $\delta$ 2.04). Chemical shifts ($\delta$) are reported in parts per million (ppm) on the $\delta$-scale and coupling constants (*J*) were measured in Hz.

**Table 1**: Abbreviations used in describing $^1$H NMR signal multiplicities.

| *Abbreviations* | *Signal multiplicity* |
|---|---|
| s | singlet |
| d | doublet |
| t | triplet |
| multiplet | multiplet |
| dd | doublet of doublets |
| ddd | doublet of doublet of doublets |
| b | broadened |

### 8.3.2 Circular dichroism (CD)

CD spectra were recorded on a Jasco J-710 specropolarimeter in spectrophotometric grade methanol (about 1 mg /10 mL MeOH). The formula used to calculate the molecular ellipticity $[\theta]$ is:

$$[\theta] = \frac{\text{(L) (Scale)(molecular weight)(100)}}{\text{[length of tube (cm)][concentration 9 g/L)]}}$$

where L is the difference (at any given wavelength) between the reading (in cm) of the compound in solution and the reading (in cm) of pure solvent.

### 8.3.3 Mass spectrometry

All fast atom bombardment (FAB) and electron ionizarion (EI) mass spectra were recorded on a VG70 SEQ mass spectrometer with a MASPEC II data system and inotech saddlefield FAB gum. $M^+$ denotes the molecular ion.

## 8.4 ANHYDROUS SOLVENT AND REAGENTS

**Acetone** was left over dry $K_2CO_3$ (oven dried 24 hours, 200 $^oC$) for 24 hours. The $K_2CO_3$ was filtered off and the solvent distilled over 3Å molecular sieves and stored under $N_2$.

**Benzene, diethyl ether, THF, triethylamine,** and **diisopropylamine** were refluxed over sodium wire/benzophenone under nitrogen until a dark blue colour persisted with subsequent fresh distillation under nitrogen prior to use.

Dry THF was also obtained through the following alternative protocol:

Commercially available THF (2.0 L) was dried over sodium (from 6-10 cm sodium rod) overnight, benzophenone indicator (50-70 g) was added and the dry THF obtained by simple distillation process under an inert atmosphere.

**Acetonitrile, dichloromethane, *N*-methylimidazole,** and **DMF** were refluxed over $CaH_2$ under nitrogen for 12 hours with subsequent fresh distillation under nitrogen before use.

## 8. 5 CHEMICAL METHODS

### 8.5.1 Standard work up procedures

Unless specified to the contrary, where appropriate, water was added to the reaction mixture and the aqueous phase extracted with diethyl ether or ethyl acetate. The organic extract was washed with water, dried ($Na_2SO_4$ or $MgSO_4$) and the ether or ethyl acetate removed under reduced pressure at about 40 $^oC$. Purification via PLC or FCC afforded the product.

### 8.5.2 Acetylation

Dry phenolic material was dissolved in the minimum volume of pyridine and twice the same amount of the acetic anhydride was added. After about 8-12 hours at ambient temperature the reaction was terminated by adding ice and the excess pyridine removed from the precipitate by repetitive washing with cold water.

## 8.6 FREEZE – DRYING

Phenolic material in aqueous solution was freeze-dried using a Virtis Freezemobile 12 SL (40 millitorr).

## 8.7 MELTING POINTS

Melting points were determined with a Reichert Thermopan microscope with a Koffler hot–stage and are uncorrected.

## 8.8 ABBREVIATION

The following abbreviations for solvents are used throughout the experimental section:

| | | |
|---|---|---|
| DCM | = | dichloromethane |
| DMF | = | *N,N*-dimethylformamide |
| EtOH | = | ethanol |
| MeOH | = | methanol |
| T | = | toluene |
| THF | = | tetrahydrofuran |
| H | = | n-Hexane |
| EtOAc | = | Ethyl acetate |
| DMP | = | Dess-Martin Periodinane |
| MS | = | Mass Spectroscopy |
| NMR | = | Nuclear Magnetic Resonance |
| IR | = | Infra Red spectroscopy |

| | | |
|---|---|---|
| CD | = | Circular Dichroism |
| $AgBF_4$ | = | Silver tetrafluoroborate |
| $Cp_2HfCl_2$-$AgClO_4$ | = | Promoter employed in activation of glycosyl fluoride. |
| | | (Cp = cyclopentadiene, Hf = Hafnium) |
| NMO or NMMO | = | *N*-methylmorpholine-*N*-oxide |
| TPAP | = | Tetrapropylammoniumperruthenate |
| LDA | = | Lithium diisoprpylamide |
| HMPA | = | Hexamethylphosphoramide |
| DBU | = | 1,8-diazabicyclo(5,4,0)undec-7-ene |

# Chapter 9

# EXPERIMENTAL

## 9.1 Synthesis of 1-(4-methoxybenzyl)-pyrimidinium; toluene-4-sulfate (8)

*p*-Toluenesulfonyl chloride (3.0 g, 15.7 mmol) and 4-methoxybenzyl alcohol (2.0 g, 14.7 mmol) were dissolved in dry pyridine (3.5 mL). The reaction was left for 5 days at room temperature with a $CaCl_2$ tube fitted for protection against atmospheric moisture. The resulting solidified reaction mixture was washed with hexane. Recrystallization of the product from dichloromethane yielded 1-(4-methoxybenzyl)-pyridinium *p*-toluene-4-sulfonate[1] (4.91 g, 90%) as colourless cuboid crystals; mp 128–131°C.

**MS:** m/*z*: (positive mode) 201.3 ($[M+1]^+$, 10%), 200.3 ($M^+$, 60%), 122.2 (24%), 121.2(100%); m/*z* (negative mode) 172.2 ($[(M\text{-}1]^-$, 8%), 171.2 ($M^+$, 100%).

**$^1$H NMR:** **Plate 9.1:** $\delta_H$ (300 MHz; $CDCl_3$; $Me_4Si$) 9.30 (dd, J = 1.0, 8.0 Hz, 2H, H-36, H-32); 8.73 (t, J = 8.0 Hz, 2H, H-35, H-33); 8.22 (t, J = 8.0 Hz, 1H, H-34); 7.50 (d, J = 9.0 Hz, 2H, H-25, H-23); 6.75 (d, J = 9.0 Hz, 2H, H-26, H-22); 7.10 (d, J = 8.0 Hz, 2H, H-15, H-13); 7.77 (d, J = 8.0 Hz, 2H, H-16, H-12); 5.91 (s, $–CH_2–$, 2H, H-1); 3.74 (s, Ar-$OCH_3$, 3H, H-27); 2.34 (s, Ar—$CH_3$, 3H, H-17).

**$^{13}$C NMR:** **Plate 9.2:** $\delta_C$ (75 MHz; $CDCl_3$) 160.9 (C-24), 145.4 (C-32 and C-36), 144.2 (C-34), 142.6 (C- 11'), 139.8 (C-14), 131.6(C-22 and C-26), 129.2 (C-13 and C-15), 128.6 (C-33 and C-35), 126.3 (C-12 and C-16), 125.7 (C-21), 115.1 (C-

23 and C-25), 64.5 (–CH2–, C-1), 55.7 (Ar-OCH3, C-27), and 21.7 (Ar-$CH_3$, C-17).

## 9.2 Syntheses of 3-*O*-derivatives of catechin (Chap. 6)

### 9.2.1 Synthesis of 5,7,3′,4′-tetra-*O*-methylcatechin (1)

Dried catechin (10 g, 35 mmol) was dissolved in dry acetone (250 mL) under an inert atmosphere ($N_2$). $K_2CO_3$ (38 g, 276 mmol, dried at 240 $^oC$ overnight) was added and suspended in the reaction mixture. After stirring for 1 hour, dimethylsulfate (87 mg, 276 mmol) was added slowly over a period of 30 min. and the reaction mixture refluxed for 2 hours. The $K_2CO_3$ was filtered off, the acetone removed under reduced pressure and the excess $(CH_3)_2SO_4$ destroyed with cold ammonia (80 mL, 25% $NH_3/H_2O$, v/v). The reaction mixture was subsequently extracted with ethyl acetate (2 x 100 mL), washed with water (2 x 70 mL) and brine (70 mL), dried over $MgSO_4$ and the solvent removed under reduced pressure. The title compound **(1)** was obtained as an off-white, amorphous solid (11.9 g, 99%). Physical data corresponded with published data.[2]

### 9.2.2 Synthesis of 3-(*p*-toluenesulfonyl)-3′,4′,5,7-tetra-*O*-methylcatechin (2)

**Step I: Synthesis of 1-(*p*-toluenesulfonyl)imidazole**

To a stirred solution of imidazole (3.6 g, 52.9 mmol) in dry THF (10 mL) under a cloud of nitrogen, a solution of *p*-toluenesulfonyl chloride (5 g, 26.2 mmol) in dry THF (8 mL) was added dropwise over a period of 10 minutes. After 1 hour, the mixture was filtered, washed with THF, and the solvent concentrated under reduced pressure to afford 1-(*p*-toluenesulfonyl)imidazole as white needle-like crystals. The crystals was washed with hexane and used in the next reaction step without further purification or characterization.

**Step II: "Sulfonating agent"**

To a 10 mL THF solution of 1-(*p*-toluenesulfonyl)imidazole (266 mg, 1.20 mmol) (Step I) cooled to -5 $^{o}$C under a nitrogen cloud, was added in drops methyl triflate (180 mg, 1.10 mmol). The reaction mixture continued at -5 $^{o}$C for a further hour to afford the "sulfonating agent" for the next reaction step.

**Step III: Tosylation**

3-Hydroxy-3′,4′,5,7-tetra-*O*-methylepicatechin (346 mg, 1.0 mmol) was dissolved in dry THF (10 mL) under a nitrogen cloud and *N*-methylimidazole (82 mg, 0.8 mL, 1.00 mmol) was added. The mixture was added to the creamy sulfonating reagent synthesized in step II. After a further 10 minutes, the cooling system was removed and the reaction continued at room temperature for 24 hours. The mixture was chromatographed on silica gel with toluene/ethyl acetate 1:1 as the development solution, to obtain the title compound **(2)** in 82% (410 mg) yield, $R_f$ 0.75, as a white amorphous solid. Physical data corresponded with published information.[3]

**$^{1}$H NMR:** **Plate 1.1:** $\delta_H$ ($CDCl_3$)

**$^{13}$C NMR:** **Plate 1.2:** $\delta_C$ ($CDCl_3$)

### 9.2.3 Synthesis of 3-*O*-methylsulfonyl-3′,4′,5,7-tetra-*O*-methylcatechin (3)

A mixture of dried 5,7,3′,4′-tetra-*O*-methylcatechin **(1)** (346 mg, 1.0 mmol) and 4-dimethylaminopyridine (DMAP) (12.2 mg, 0.1 mmol) was dissolved in dry THF (5 mL) under a nitrogen atmosphere. Triethylamine (0.28 mL, 2.0 mmol) was added to the stirring solution and cooled in a salt-ice bath for 10 minutes, before a solution of methylsufonyl chloride (172 mg, 0.28 mL, 1.5 mmol) in 0.5 mL dry THF was carefully added dropwise. After 30 minutes, the reaction mixture was diluted with 10 mL ethyl acetate, washed with water (2 x 5 mL), brine 5 mL, and dried over anhydrous sodium sulfate. The ethyl acetate was removed under reduced pressure and the crude product purified with silica gel PLC (toluene/acetone 9:1, v/v) to obtain the title compound **(3)** in 99% (420 mg) yield as white needle-like crystals. $R_f$ 0.33 (toluene/acetone 9:1, v/v).

**MS:** Found: $\underline{M}^+$ 424.11690, $C_{20}H_{24}O_8S$ requires $\underline{M}^+$ 424.11919, $(\underline{M}+H)^+$ 425.11690 (24), m/z 328.19(100), 424.46 (30), 178.94 (78), 150 (44).

**$^1$H NMR:** **Plate 2.1:** $\delta_H$ ($CDCl_3$) 2.43 (2 x s, $SO_2C\mathbf{H}_3$), 2.85 (dd, J = 7.4, 16.5 Hz, 1H, H-4β), 3.09 (ddd, J = 1.7, 5.3, 16.5 Hz, 1H, H-4α), 4.90 (d, J = 7.7 Hz, 1H, H-2), 4.94 (m, 1H, H-3), 6.04 (d, J = 2.0 Hz, 1H, H-6), 6.08 (d, J = 2.0 Hz, 1H, H-8), 6.80 (d, J = 1.7, 8.2 Hz, 1H, H-5′), 6.90 (d, J = 1.7 Hz, 1H, H-2′), 6.91 (d, J = 1.7, 8.2 Hz, 1H, H-6′).

**$^{13}$C NMR:** **Plate 2.2:** $\delta_C$ ($CDCl_3$) 26.5 (C-4), 38.1 (1 x $SO_2\mathbf{C}H_3$), 55.4 – 56.1 (4 x $O\mathbf{C}H_3$), 77.5 (C-3), 78.3 (C-2), 92.2 (C-6), 93.2 (C-8), 100.1 (C-1), 110.0 (C-5′), 111.3 (C-2′), 119.8 (C-6′), 129.8 (C-9), 149.4 (C-3′), 149.6 (C-4′), 154.7 (C-10), 158.6 (C-7), 160.2 (C-5).

**CD:** **CD-plate 1:** λ nm (θ), 212.00 ($2.288 \times 10^4$), 227.00 ($-9.810 \times 10^4$), 234.00 ($1.190 \times 10^3$), 238.00 ($-1.141 \times 10^4$), 244.00 ($5.382 \times 10^2$).

### 9.2.4 Synthesis of 3-*O*-trimethylsilyl-3′,4′,5,7-tetra-*O*-methylcatechin (4)

Under inert atmosphere (nitrogen), a mixture of 3-hydroxy-3′,4′,5,7-tetra-*O*-methylcatechin **(1)** (346 mg, 1.0 mmol) and 4-dimethylaminopyridine (13 mg, 0.1mmol) was dissolved in 4.0

mL of dry THF and placed in a salt-ice bath to cool for 5minutes. To the stirring solution, triethylamine (0.28 mL, 2.0 mmol) was added, followed by trimethylsilyltrifluoromethane sulfonate (333 mg, 0.27 mL, 1.5 mmol), carefully introduced dropwise. The reaction mixture was concentrated under a stream of nitrogen, flushed through a plug of silica gel with toluene/ethyl acetate 7:3 v/v, to obtain 3-oxo-trimethylsilyl-3′,4′,5,7-tetra-*O*-methylcatechin **(4)**, 99% (367mg), $R_f$ 0.73, as off-white crystals.

**MS:** Found: $\underline{M}^+$ 418.18171, $C_{22}H_{30}O_6Si$ requires $\underline{M}^+$ 418.18117, $(\underline{M}+H)^+$ 419 (6), m/z 252 (100), 239 (16), 151 (14), 73 (15).

**$^{1}$H NMR:** **Plate 3.1:** $\delta_H$ ($CDCl_3$) -0.16 (s, 9H, 1 x Si[C**H**$_3$]$_3$), 2.58 (dd, J = 9.7, 16.0 Hz, 1H, H-4α), 3.01 (dd, J = 5.9, 16.0 Hz, 1H, H-4β), 3.77 – 3.91 (3 x s, 12H, 4 x OC**H**$_3$), 3.93 (m, 1H, H-3), 4.62 (d, J = 8.8 Hz, 1H, H-2), 6.10 (d, J = 2.3 Hz, 1H, H-6), 6.14 (d, J = 2.3 Hz, 1H, H-8), 6.88 (d, J = 8.2 Hz, 1H, H-5′), 6.96 (d, J = 1.9 Hz, 1H, H-2′), 6.99 (dd, J = 1.9, 8.2 Hz, 1H, H-6′).

**$^{13}$C NMR:** **Plate 3.2:** $\delta_C$ ($CDCl_3$) 0.00 (s, 1 x –Si[**C**H$_3$]$_3$), 30.3 (C-4), 55.6 – 56.2 (4 x s, 4 x O**C**H$_3$), 69.59 (C-3), 82.2 (C-2), 92.0 (C-6), 93.2 (C-8), 102.3 (C-9), 110.8 (C-5′), 111.0 (C-2′), 120.3 (C-6′), 132.0 (C-1′), 148.9 (C-3′), 149.1 (C-4′), 155.7 (C-10), 158.8 (C-7), 159.9 (C-5).

**CD:** **CD-plate 2:** λ nm (θ), 206.00 ($9.846 \times 10^4$), 214.00 ($-4.290 \times 10^4$), 221.00 ($8.217 \times 10^3$), 230.00 ($1.021 \times 10^4$), 239.00 ($-1.292 \times 10^3$), 247.00 ($2.692 \times 10^3$), 254.00 ($2.761 \times 10^3$), 273.00 ($6.101 \times 10^3$), 284.00 ($5.307 \times 10^3$), 296.00 ($4.857 \times 10^3$), 319.00 ($4.030 \times 10^3$).

## 9.3 Flavene preparation revisited: a convenient method of preparing flav-3-ene (Chap. 6)

### 9.3.1 Synthesis of flav-2-ene (11) from 3-(*p*-toluenesulfonyl)-3′,4′,5,7-tetra-*O*-methylcatechin (2).

Under a nitrogen cloud, dry THF (10 mL) was cooled to -5 °C before adding LDA (0.6 mL) followed by HMPA (0.05 mL). After 10 minutes, 3-(*p*-toluenesulfonyl)-3′,4′,5,7-tetra-*O*-methylcatechin (200 mg, 0.4 mmol) was added. The reaction mixture was stirred for an hour, allowed to warm to room temperature, and then stirred for another hour. The reaction solvent was removed under reduced pressure and the product purified on silica gel PLC (DCM/n-Hexane/EtOAc 2:8:1). Flav-2-ene **(11)**, 6% (6 mg) yield, was obtained and 49 % (98 mg) of the tosylate starting material was recovered.

**Compound (11):**

**MS:** Found: $\underline{M}^+$ 328.13238, $C_{19}H_{20}O_5$ reqiues $\underline{M}^+$ 328.13108, $(\underline{M}+H)^+$ 329 (20), 313 (15), 297 (15), 165 (65), 77 (5).

**$^1$H NMR:** **Plate 5.1:** $\delta_H$ ($CDCl_3$) 3.3 (d, J = 3.9 Hz, 2H, H-4), 3.79 – 3.95 (4 x s, 4 x OC**H**$_3$), 5.40 (t, J = 3.9 Hz, 1H, H-3), 6.15 (d, J = 2.3 Hz, 1H, H-6), 6.21 (d, J = 2.3 Hz, 1H, H-8), 6.87 (d, J = 8.4 Hz, 1H, H-5′), 7.23 (d, J = 2.0 Hz, 1H, H-2′), 7.24 (dd, J = 2.0, 8.4 Hz, 1H, H-6′).

**$^{13}$C NMR:** **Plate 5.2:** $\delta_C$ ($CDCl_3$) 19.5 (C-4), 55.5 – 55.9 (3 x s, 4 x O**C**H$_3$), 93.0 (C-8), 93.3 (C-6), 95.7 (C-3), 101.2 (C-9), 107.7 (C-2′), 110.7 (C-5′), 117.2 (C-6′), 127.6 (C-1′), 148.1 (C-4′), 148.6 (C-3′), 149.1 (C-2), 152.8 (C-10), 158.1 (C-7), 159.5 (C-5).

### 9.3.2 Synthesis of flav-3-ene (9) from 3-*O*-methylsulfonyl-3′,4′,5,7-tetra-*O*-methylcatechin (3).

Under a nitrogen atmosphere, dried 3-*O*-methylsulfonyl-3′,4′,5,7-tetra-*O*-methylcatechin (30 mg, 0.07 mmol) was dissolved in acetonitrile (1.5 mL) and DBU (0.2 mL, 0.22 mmol) was added dropwise. The mixture was refluxed for 24 hours. The reaction solvent was removed on a rotary evaporator. The crude product was collected with ether (10 mL), washed with water (2 x 5 mL), and brine (5 mL). The organic phase was dried over $Na_2SO_4$ and the solvent removed under reduced pressure to obtain the abovementioned product **(9)** in a 100% yield and pure enough for subsequent reactions.

**Compound (9):**

**MS:** Found: $\underline{M}^+$ 328.13225, $C_{19}H_{20}O_5$ reqiues $\underline{M}^+$ 328.13108, $(\underline{M} + H)^+$ 329 (20), 313 (15), 297 (15), 165 (65), 77 (5).

**$^1$H NMR:** **Plate 4.1:** $\delta_H$ ($CDCl_3$) 3.75 – 3.89 (4 x s, 4 x OC**H**$_3$), 5.61 (dd, J = 3.0, 10.0 Hz, 1H, H-4), 5.79 (t, J = 2.0, 3.0 Hz, 1H, H-2), 6.04 (d, J = 2.2 Hz, 1H, H-6), 6.06 (d, J = 2.2 Hz, 1H, H-8), 6.83 (dd, J = 2.0, 10.0 Hz, 1H, H-3), 6.86 (d, J = 8.1 Hz, 1H, H-5′), 7.01 (dd, J = 2.0, 8.1 Hz, 1H, H-6′), 7.03 (d, J = 2.0 Hz, 1H, H-2′).

**$^{13}$C NMR:** **Plate 4.2:** $\delta_C$ ($CDCl_3$) 55.35 – 55.94 (4 x OC**H**$_3$), 77.2 (C- 2), 91.9 (C-6), 93.8 (C-8), 104.5 (C-9), 110.6 (C-2′), 111.0 (C-5′), 119.0 (C-3), 119 (C-6′), 119.9 (C-4), 133.4 (C-1′), 149.1 (C-4′), 149.2 (C-3′), 154.9 (C-10), 156.3 (C-5), 161.3 (C-7).

**CD:** **CD-plate 3:** λ nm (θ) 209.00 ($5.423 \times 10^3$), 228.50 ($-2.356 \times 10^3$), 266.00 ($1.178 \times 10^3$), 288.00 ($-1.084 \times 10^2$), 347.00 ($8.848 \times 10^2$), 486.00 ($3.215 \times 10^3$).

### 9.3.3 Synthesis of flav-2-ene (11) and flav-3-ene (9) from 3-(*p*-toluene-sulfonyl)-3′,4′,5,7-tetra-*O*-methylepicatechin (10).

Under a nitrogen cloud, dry THF (5 mL) was cooled to -5 °C before adding LDA (0.5 mL), followed by HMPA (0.05 mL). After 10 minutes, 3-(*p*-toluenesulfonyl)-3′,4′,5,7-tetra-*O*-methylepicatechin **(2)** (120 mg, 0.2400 mmol) was added and the cooling system removed. The reaction ran at room temperature for another hour, after which the reaction solvent was removed under reduced pressure. The crude product was purified on silica gel PLC (DCM/Hex/EtOAc 2:8:1, x 2). Flav-2-ene **(11)** and flav-3-ene **(9)** were obtained in yields of 24% (19 mg) and 20% (16 mg), respectively. Characterisation information of the product **(9)** and **(11)** agree with subsection 9.3.1 and 9.3.2.

## 9.4 Synthesis of (2R)-5,7,3′4′-Tetra-*O*-methylflavan-3-one (16)

Under a $N_2$ atmosphere, Dess-Martin periodinane (7.5 mL, 0.3 M solution of DMP in DCM) was added to a solution of **(1)** (560 mg, 1.6 mmol) in dry $CH_2Cl_2$ (5 mL). After stirring for 5 min., moistened $CH_2Cl_2$ (20 mL) was added dropwise over 45 min resulting in a cloudy reaction mixture. The reaction mixture was extracted with ether (2 x 30 mL), washed with a mixture of 10% $Na_2S_2O_3$ and saturated $NaHCO_3$ (2 x 30 mL, 1:1, v/v) and the two layers separated. The water phase was extracted with ether (20 mL), and the combined organic phases washed with water and brine, dried over anhydrous sodium thiophosphate, and the solvent removed under vacuum. The crude product was filtered over $SiO_2$ (4 x 4 cm, H:EtOAc 6:4) and crystallized from the eluent as white needles. Yield: 95% (451 mg, 85 mg **(1)** recovered). The spectral data was identical to those reported in the literature.[4]

**MS:** Found: $\underline{M}^+$ 344.12545, $C_{19}H_{20}O_6$ requires $\underline{M}^+$ 344.12599, $(\underline{M}+H)^+$ 345 (20), 343 (4), 342 (6).

## 9.5 Synthesis of 3-Coupled Biflavonoids (Chap. 6)

### 9.5.1 Attempts to couple phloroglucinol, free-phenolic catechin or tetra-*O*-methylcatechin to C-3 of tetra-*O*-methyl-3-oxocatechin

**(I).** Reaction of tetra-*O*-methyl-3-oxocatechin with phloroglucinol or free phenolic catechin under the same reaction conditions as in section 9.2.1 did not give the expected corresponding compounds **(17)** or **(19)** (see Chapter 6, p. 157)**.** The starting materials decomposed.

**(II).** Reaction of tetra-*O*-methyl-3-oxocatechin with tetra-*O*-methylcatechin under the same reaction conditions as in section 9.2.1, yielded flav-3-ene (4%) after 48 hours.

### 9.5.2 Investigation of the activation ability of some Lewis acids (LA) ($InCl_3$, $TiCl_4$, $Yb(OTf)_3$) on the synthesis of 3-(-1,3,5-tri-*O*-methyl-phloroglucinol)-5,7,3′4′-tetra-*O*-methyl-flavan-3-ene

**General procedure**

To a dry, stirred DCM (10 mL) solution of tetra-*O*-methyl-3-oxocatechin (100 mg, 0.29 mmol) and 1,3,5-trimethylphloroglucinol (120 mg, 0.71 mmol, 2.5 equiv.) at 0 $^oC$ under a nitrogen atmosphere, was added LA (0.1 mL) in drops. For the $TiCl_4$, in less than 10 minutes, the starting materials decomposed and silica gel TLC showed no presence of products. For the $InCl_4$, the reaction proceeded to room temperature for hours without indication of formed product even after refluxing for 1 hour.

For the $Yb(OTf)_3$ systems, in DCM and THF solvents there was no noticeable reaction possibly because the catalyst did not dissolve in both solvents even after refluxing for 10 hours. When the solvent was changed to methanol and the mixture refluxed for 6 hours, two products [**(20)** and **(22)**] were isolated in 17% and 32% yields, respectively (Chapter 6, p. 159).

**Compound 20:**

**MS:** Found: $\underline{M}^+$ 358.14140, $C_{20}H_{22}O_6$ requires $\underline{M}^+$ 358.14164, $[\underline{M}+H]^+$ = 359.35, m/z = 343.35 (10), 327.35 (12), 360.34 (25).

**$^1$H NMR:** **Plate 6.1:** $\delta_H$ ($CDCl_3$): 3.71-3.83 (s, 15H, 5 x OC**H**$_3$), 5.59 (s, 1H, H-2), 5.93 (s, 1H, H-4), 6.06 (d, J = 2.2 Hz, 1H, H-6), 6.07 (d, J = 2.2 Hz, 1H, H-8), 6.78 (d, J = 8.2 Hz, 1H, H-5′), 6.92 (dd, J = 2.0, 8.2 Hz, 1H, H-6′), 6.95 (d, J = 2.0 Hz, 1H, H-2′).

**$^{13}$C NMR:** **Plate 6.2:** $\delta_C$ ($CDCl_3$): 55.3 – 56.1 (5 x s, 5 x O**C**H$_3$), 77.2 (C-2), 88.1 (C-4), 92.3 (C-6), 94.1 (C-8), 104.7 (C-9), 110.7 (C-2′), 111.0 (C-5′), 119.6 (C-6′), 131.4 (C-1′), 149.0 (C-4′), 149.2 (C-3′), 150.9 (C-3), 151.1 (C-10), 154.9 (C-5), 159.0 (C-7).

**CD:** **CD-plate 4:** $\lambda$ nm ($\theta$), 204.00 ($2.039 \times 10^4$), 216.00 ($-2.566 \times 10^4$), 241.00 ($3.111 \times 10^4$), 285.50 ($-8.995 \times 10^3$).

**Compound 22:**

**MS:** Found: $\underline{M}^+$ 390.16759, $C_{21}H_{26}O_9$ requires $\underline{M}^+$ 390.16785, $[\underline{M}+H]^+$ = 391.39, m/z = 345.39 (8), 327.41 (25), 360.34 (22), 392.34 (10).

**$^1$H NMR:** **Plate 7.1:** $\delta_H$ ($CDCl_3$): 2.50 (d, J = 16.3 Hz, 1H, H-4α), 2.95 (dd, J = 2.0, 16.3 Hz, 1H, H-4β), 3.32 – 3.85 (s, 18H, 6 x OC**H**$_3$), 5.19 (d, 1H, J = 2.0 Hz, 1H, H-2), 6.09 (d, J = 2.3 Hz, 1H, H-6), 6.21 (d, J = 2.3 Hz, 1H, H-8), 6.77 (d, J = 8.4 Hz, 1H, H-5′), 6.97 (dd, J = 2.0 Hz, 8.4 Hz, 1H, H-6′), 7.02 (d, J = 2.0 Hz, 1H, H-2′).

**$^{13}$C NMR:** **Plate 7.2:** $\delta_C$ ($CDCl_3$): 25.9 (C-4), 48.6 – 55.8 (5 x s, 6 x O**C**H$_3$), 76.8 (C-2), 91.6 (C-6), 93.1 (C-8), 98.2 (C-10), 101.2 (C-3), 110.7 (C-5′), 111.2 (C-2′), 119.7 (C-6′), 130.7 (C-1′), 148.3 (C-3′), 148.5 (C-4′), 154.6 (C-9), 158.3 (C-7), 159.9 (C-5).

**CD:** **CD-plate 5:** λ nm (θ), 216.50 ($-3.312 \times 10^2$), 242.50 ($3.310 \times 10^3$), 274.50 ($-1.562 \times 10^3$), 294.00 ($3.428 \times 10^2$).

### 9.5.3 Synthesis of 3-(1,3,5-tri-*O*-methylphloroglucinol)-5,7,3′4′-tetra-*O*-methylflavan-3-ene (24)

Dry tetra-*O*-methyl-3-oxocatechin **(16)** (100 mg, 0.29 mmol) was dissolved in dry dichloromethane (8.0 mL) under nitrogen. To the solution was carefully added tin(IV)-chloride (1.0 M solution in dichloromethane 0.5 mL, 0.005 mmol, 0.017 equiv.) in drops over a period of 5 minutes. This was followed by addition of a dichloromethane (7 mL) solution of 1,3,5-trimethylphloroglucinol (120 mg, 0.71 mmol, 2.5 equiv.). The reaction mixture was stirred at room temperature for 48 hours. The mixture was filtered through a neutral aluminum oxide pack (3 cm x 10 cm, H: EtOAc 6:4) and subsequently purified on neutral alumina plc (H:EtOAc 8.5:1.5) to afford 3-(1,3,5-*O*-methylphloroglucinol)-5,7,3′,4′-tetra-O-methylflavan-3-ene **(24)** ($R_f$ 0.26, 70.2 mg, 49%) as a white amorphous solid.

**MS:** Found: $\underline{M}^+$ 494.30320, $C_{28}H_{30}O_8$ requires 494.30381, $(\underline{M}+H)^+$ 495 (100), m/z 402 (50), 327 (35), 317 (30).

**$^1$H NMR:** **Plate 8.1:** $\delta_H$ ($CDCl_3$) 3.64-3.80 (s, 21H, 7 x $OC\mathbf{H_3}$), 5.83 (br, 1H, 4-H), 6.03 (ddd, J = 0.8, 1.3, 2.0 Hz, 1H, 2-H), 6.26 (s, 2H, 3″/5″-H), 6.33 (d, J = 2.0 Hz, 1H, 6-H), 6.69 (dd, J = 0.6, 2.0 Hz, 1H, 8-H), 6.78 (dd, J = 2.0, 8.3Hz, 1H, 6′-H), 6.85 (d, J = 8.3Hz, 1H, 5′-H), 6.90 (d, J = 2 Hz, 1H, 2′-H).

**$^{13}$C NMR:** **Plate 8.2:** δc (DMSO) 40.52 (C-2), 55.67-56.30 (s, 21H, 7 x $OCH_3$), 88.95 (C-8), 92.11 (C-3″/5″), 94.23 (C-6), 100.10 (C-4), 110.35 (C-1″), 111.98 (C-5′), 112.07 (C-10), 113.16 (C-2′), 121.26 (C-6′), 134.00 (C-1′), 155.91 (C-9), 158.70 (C-3), 147.85-152.75, 158.34, 158.97, 160.49 (7 x **Ar**-$OCH_3$).

**CD:** **CD plate 6:** λ nm (θ), 208.00 (-5.851 x $10^4$), 214.00 (7.491 x $10^4$), 220.00 (-1.931 x $10^5$), 228.00 (1.796 x $10^5$), 244.00 (5.621 x $10^4$), 254.00 (4.889 x $10^4$), 271.00 (5.375 x $10^4$), 286.00 (5.883 x $10^4$), 302.00 (4.622 x $10^4$), 323.00 (3.776 x $10^4$).

### 9.5.4 Investigation of temperature effect on the synthesis of 3-(-1,3,5-tri-*O*-methylphloroglucinol)-5,7,3′4′-tetra-*O*-methylflavan-3-ene (24)

The reaction was carried out according to the reaction conditions explained in section 9.4.1. However, the temperature was varied from -80 °C to room temperature over 48 hours. The progress of the reaction was monitored hourly with silica gel TLC (H/EtOAc 8.5:1.5).

(i) At temperatures between -80 to -40 $^{o}$C, there was no indication of formed product observed on the TLC sheets after the first 3 hours.

(ii) From -40 $^{o}$C to 0 $^{o}$C, TLC did not show any trace of product after 3 hours.

(iii) At about +5 $^{o}$C, the reaction mixture showed indication of a product being formed after 1 hour at this temperature. The reaction progressed for 48 hours. The reaction mixture was worked up and purified according to the procedure in section 9.5.3. The reaction was relatively clean and afforded product **(24)** (169 mg, 59%) as fine yellow crystals. The NMR data agreed with the earlier result (section 9.5.3).

### 9.5.5 Investigation of quantity of $SnCl_4$ (solution of 1 M $SnCl_4$ in dichloromethane) and solvent effect on the synthesis of 3-(1,3,5-tri-*O*-methylphloroglucinol)-5,7,3′4′-tetra-*O*-methylflavan-3-ene

The reactions were conducted according to the procedure and reaction conditions stated in section 9.5.3. For the results, see Tables 1 and 2 in Chapter 6, p. 161 and 162.

## 9.6 Syntheses of Proanthocyanidins (Chap. 7)

### 9.6.1 Synthesis of (2*R*,4*S*)- (13) and (2*R*,4*R*)-4-(1,3,5-tri-*O*-methyl-phloroglucinol)-5,7,3′4′-tetra-*O*-methyl-flavan-3-ones (14).

Under a nitrogen atmosphere 5,7,3′4′-tetra-*O*-methyl-flavan-3-ol (50 mg, 0.145 mmol) and $AgBF_4$ (215 mg, 1.1 mmol) were dissolved in THF (2 mL). A solution of 1,3,5-tri-*O*-methylphloroglucinol (150 mg, 0.893 mmol) in THF (3 mL) was added dropwise and the reaction mixture refluxed for one hour. After concentrating the reaction mixture under vacuum, it was filtered on $SiO_2$ (2 cm x 4 cm, H:EtOAc 6:4) and subsequently separated on $SiO_2$ (T:EtOAc 8:2) to afford **(13)** ($R_f$ 0.40, 22 mg, 45%) and **(14)** ($R_f$ 0.30, 6 mg, 13%).

**Compound (13)**:

**MS:** Found: <u>M</u>$^+$ 510.18842, $C_{28}H_{30}O_9$ requires <u>M</u>$^+$ 510.18898, <u>(M+H)</u>$^+$ 511 (43), m/z 343 (100), 315 (22), 287 (10).

**IR:** $\nu_{max}$ 2959, 1724, 1593 cm$^{-1}$.

**$^1$H NMR:** **Plate 10.1:** $\delta_H$ ($CDCl_3$) 3.51-3.84 (s, 21H, 7 x O**CH$_3$**), 5.10 (s, 1H, 4-H), 5.63 (d, J = 0.5 Hz 1H, 2-H), 5.99 (d, J = 2.0 Hz, 1H, 6-H), 6.10 (s, 2H, 3″/5″-H), 6.31 (d, J = 2.0 Hz, 1H, 8-H), 6.77 (d, J = 8.0 Hz, 1H, 5′-H), 6.90 (dd, J = 2.0, 8.0 Hz, 1H, 6′-H), 6.94 (d, J = 2.0 Hz, 1H, 2′-H).

**$^{13}$C NMR:** **Plate 10.2:** $\delta_C$ ($CDCl_3$) 38.1 (C-4), 55.2-55.9 (7 x O**C**$H_3$), 82.8 (C-2), 91.0 (C-3″/5″), 92.8 (C-6), 94.1 (C-8), 106.1 (C-□), 109.4 (C-2'), 111.1 (C-5'), 111.3 (C-6′), 118.3 (C-1″), 128.1 (C-1′), 148.8 (C-3′), 149.0 (C-4′), 154.1 (C-□), 158.4 (C-2″/6″), 158.7, 160.0 (C-4″), 160.1 (C-7), 206.4 (C-3).

**CD:** **CD-plate 7:** λ nm (θ), 184.60 (6.573 x $10^3$), 194.20 (7.232 x $10^3$), 200.40 (-8 x $10^3$), 211.60 (6.762 x $10^4$), 237.20 (3.221 x $10^4$), 258.60 (5.108 x $10^3$), 290.00 (8.759 x $10^3$).

**Compound (14):**

**MS:** Found: <u>M</u>$^+$ 510.18826, $C_{28}H_{30}O_9$ requires <u>M</u>$^+$ 510.18898, <u>(M+H)</u>$^+$ 511 (68), m/z 343 (100), 315 (82), 287 (53).

**IR:** $\nu_{max}$ 2925, 1731, 1594 cm$^{-1}$.

**$^1$H NMR:** **Plate 11.1:** $\delta_H$ ($CDCl_3$) 3.58-3.89 (s, 21H, 7 x $OCH_3$), 5.24 (d, J = 2 Hz, 1H, 4-H), 5.34 (d, J = 2 Hz, 1H, 2-H), 6.07 (d, J = 2.0 Hz, 1H, 6-H), 6.08 (s, 2H, 3″/5″-H), 6.27 (d, J = 2.0 Hz, 1H, 8-H), 6.89 (d, J = 8.0 Hz, 1H, 5′-H), 6.96 (d, J = 2.0 Hz, 1H, 2′-H), 6.98 (dd, J = 2.0, 8.0 Hz, 1H, 6′-H).

**$^{13}$C NMR:** **Plate 11.2:** $\delta_C$ ($CDCl_3$) 29.4, 40.4 (C-4), 54.9-55.9 (7 x $OCH_3$), 84.8 (C-2), 91.2 (C-3″/5″), 93.4 (C-6), 93.8 (C-8), 107.8 (C-α), 110.9 (C-5'), 111.2 (C-1″), 111.7 (C-2′), 121.5 (C-6′), 127.8 (C-1′), 148.9 (C-4′), 149.4 (C-3′), 156.5 (C-□), 158.5 (C-2″/6″), 158.8 (C-4″), 159.7 (C-5), 160.1 (C-7), 205.2 (C-3).

**CD:** **CD-plate 8:** λ nm (θ), 210.50 ($-5.362 \times 10^4$), 233.00 ($-1.464 \times 10^4$), 273.50 ($5.963 \times 10^3$), 293.00 ($6.651 \times 10^3$), 344.50 ($4.432 \times 10^3$).

### 9.6.2 (2*R*,3*S*,4*S*)- (22) and (2*R*,3*S*,4*R*)-4-(1,3,5-tri-*O*-methylphloro-glucinol)-5,7,3′4′-tetra-*O*-methylflavan-3-ol (24)

An aqueous NaOH solution (10 mL, 2.00 M) was added to ethanol (200 mL) followed by $NaBH_4$ (7.71 g, 0.20 mol). To a solution of **(13)** (20 mg, 0.04 mmol) in ethanol (5 mL) was slowly added the $NaBH_4$ solution (1 mL) prepared above. After stirring the reaction mixture for 5 min. the ethanol was removed under reduced pressure, the excess $NaBH_4$ destroyed with water (1 mL) and the subsequent mixture extracted with ether (2 x 10 mL). The organic phase was washed with water (10 mL), dried over $MgSO_4$ and evaporated to dryness under vacuum to yield **(22)** (98%, 20 mg) as a white, amorphous solid. Compound **(24)** was prepared in exactly the same way.

**Compound (22)**

**MS:** Found: $\underline{M}^+$ 512.20408, $C_{28}H_{32}O_9$ requires $\underline{M}^+$ 512.20463, $(\underline{M}+H)^+$ 513 (58), m/z 345 (76), 333 (100), 317 (72), 303 (20), 191 (22)

**IR:** $\nu_{max}$ 3515, 2936, 2836, 1607 $cm^{-1}$.

**$^1$H NMR:** **Plate 12.1:** δ ($CDCl_3$) 3.35 – 3.87 (7 x s, 21H, 7 x $OCH_3$), 4.23 (dd, J = 6.3, 9.0 Hz, 1H, 3-H), 4.94 (d, J = 6.3 Hz, 1H, 4-H), 4.96 (d, J = 9.0 Hz, 1H, 2-H), 6.01 (d, J = 2.4 Hz, 1H, 6-H), 6.14 (very br. s, 1H, H-3″/5″(D)) 6.21 (very br. s, 1H, H-3/5(D)), 6.19 (d, J = 2.4 Hz, 1H, 8-H), 6.23 (very br. s, 1H, 3″/5″-H), 6.85 (d, J = 9.0 Hz, 1H, 5′-H), 6.98 (d, J = 2.0 Hz, 1H, 2′-H), 6.99 (dd, J = 2.0, 9.0 Hz, 1H, 6′-H).

**$^{13}$C NMR:** **Plate 12.2:** $^{13}$C NMR: δ ($CDCl_3$) 32.2 (C-4), 55.2 – 56.3 (6 x s, 7 x $OCH_3$), 71.8 (C-3), 78.2 (C-2), 91.4 (C-8), 91.9 (C-3″/5″), 92.7 (C-6), 93.6 (C-3″/5″), 105.5, 109.8, 110.4 (C-2′), 111.0 (C-5′), 120.2 (C-6′), 132.0, 148.9, 149.0, 156.3, 158.2, 159.5, 160.0.

**Compound (24)**

**MS:** Found: $\underline{M}^+$ 512.20406, $C_{28}H_{32}O_9$ requires $\underline{M}^+$ 512.20463, $(\underline{M} +H)^+$ 513 (60), m/z 345 (80), 333 (100), 317 (75), 191 (21).

**IR:** $\nu_{max}$ 3526, 2941, 2828, 1598 $cm^{-1}$.

**$^1$H NMR:** **Plate 14.1:** δ (DMSO) 3.28 – 3.78 (7 x s, 21H, 7 x $OCH_3$), 3.98 (m, 1H, 3-H), 4.21 (d, J = 6.0 Hz, 1H, 3-OH), 4.81 (d, J = 6.5 Hz, 1H, 4-H), 4.85 (d, J = 10.0 Hz, 1H, 2-H), 5.99 (d, J = 2.3 Hz, 1H, H-6), 6.02 (d, J = 10.0 Hz, 1H, 8-H), 6.14 (br s, 1H, 3″/5″-H), 6.25 (br s, 1H, 3″/5″-H), 6.86 (dd, J = 1.8, 8.3 Hz, 1H, 6′-H), 6.90 (d, J = 1.8 Hz, 1H, 2′-H), 6.91 (d, J = 8.3 Hz, 1H, 5′-H).

**$^{13}$C NMR:** **Plate 14.2:** δ (DMSO): 32.1 (C-4), 55.4 – 56.9 (6 x s, 7 x $OCH_3$), 70.40 (C-3), 78.4 (C-2), 91.15 (C-6), 92.7 (C-3″/5″), 93.0 (C-8), 93.8 (C-3″/5″), 106.1, 111.4, 111.7, 112.0 (C-5′), 120.8 (C-6′), 133.4, 148.7, 148.8, 156.5, 158.2, 159.4, 159.5, 160.0, 160.6.

### 9.6.3 Synthesis of (2*R*,4*S*:2*R*,3*S*)-5,7,3′4′-tetra-*O*-methylflavan-3-one-4β→8-5,7,3′,4′-tetra-*O*-methyl-flavan-3-ol (26) and (2*R*,4*R*:2*R*,3*S*)-5,7,3′4′-tetra-*O*-methylflavan-3-one-4α→8-5,7,3′,4′-tetra-*O*-methylflavan-3-ol (27).

Under a $N_2$ atmosphere 5,7,3′4′-tetra-*O*-methyl-flavan-3-one (50 mg, 0.145 mmol) and $AgBF_4$ (215 mg, 1.1 mmol) were dissolved in THF (3 mL). A solution of 5,7,3′4′-tetra-*O*-methyl-flavan-3-ol (150 mg, 0.435 mmol) in THF (3 mL) was added dropwise and the reaction mixture refluxed for 4 hours. The reaction mixture was filtered on $SiO_2$ (2 x 4 cm, T:A 7:3) and subsequently separated by PLC (T:A 7:3) to afford **(26)** ($R_f$ 0.32, 36 mg, 38%) and **(27)** ($R_f$ 0.12, 6 mg, 6%), respectively.

**Compound (26)**

**MS:** Found: $\underline{M}^+$ 688.23363, $C_{38}H_{40}O_{12}$ requires $\underline{M}^+$ 688.25198, $[M+H]^+$ 689 (96) m/e 509 (52), 343 (100), 315 (28), 287 (19).

**IR:** $\nu_{max}$ 2939, 2838, 1720, 1611 $cm^{-1}$.

**$^1$H NMR:** **Plate 16.2:** δ ($CDCl_3$) 2.57 [dd, J = 9.4, 16.2 Hz, 1H, 4-H(F)], 3.07 [dd, J = 5.6, 16.2 Hz, 1H, 4-H(F)], 3.54-3.90 (8 x s, 24H, 8 x OC**H₃**), 3.95 [m, 1H, 3-H (F)], 4.51 [d, J = 8.5, 1H, 2-H (F)], 5.10 [s, 1H, 4-H(C)], 5.40 [s, 1H, 2-H(C)], 5.99 [d, J = 2.4 Hz, 1H, 6-H(A)], 6.10 [d, J = 2.4 Hz, 1H, H-8(A)], 6.12 [s, 1H, 6-H (D)], 6.72 [d, J = 8.4 Hz, 1H, 5-H(B)] 6.80 [ddd, J = 1.0, 2.0, 8.4 Hz, 1H, 6-H(B)], 6.84 [d, J = 2.0 Hz, 1H, 2-H(B)], 6.89 [d, J = 8.8 Hz, 1H, 5-H(E)], 6.96 [dd, J = 2.0, 8.8 Hz, 1H, 6-H(E)], 6.96 [d, J = 2.0 Hz, 1H, 2-H(E)].

**$^{13}$C NMR:** **Plate 16.3:** δ ($CDCl_3$) 28.9 (C-4(F)), 38.1 (C-3(F)), 55.2-56.2 (8 x O**C**$H_3$), 68.8 (C-4(C)), 81.6 (C-2(F)), 82.6 (C-2(C)), 88.7 (C-6(D)), 92.8 (C-8(A)), 94.1 (C-6(A)), 101.9, 106.1 (, 109.4 (C-2(B)), 110.1, 110.2 (C-2(E)), 111.0 (C-

5(E)), 111.2 (C-5(B)), 118.3 (C-6(B)), 119.6 (C-6(E)), 128.2 (C-1(B)), 130.7 (C-1(E)), 148.7 (C-3(B)), 148.9 (C-4(B)), 149.1 (C-3(E)), 149.2 (C-4(E)), 152.4 (C-8(D)), 154.2 (C-5(D)), 156.9, 157.1 (C-7(D)), 158.6 (C-5(A)), 159.9 (C-7(A)), 206.2 (C-3).

**CD:** **CD-plate 12**: λ nm (θ), 202.00 ($4.076 \times 10^4$), 214.20 ($1.445 \times 10^5$), 237.60 ($6.305 \times 10^4$), 259.00 ($1.141 \times 10^4$), 289.20 ($1.659 \times 10^4$), 348.20 ($-1.932 \times 10^3$).

**Compound (27)**

**MS:** Found: $\underline{M}^+$ 688.24550, $C_{38}H_{40}O_{12}$ requires $\underline{M}^+$ 688.25198, $[M+H]^+$ 689 (100) m/e 509 (30), 343 (86), 315 (20), 287 (14).

**IR:** $\nu_{max}$ 2935, 2838, 1720, 1612, 1517 $cm^{-1}$.

**$^1$H NMR:** **Plate 17.2:** δ ($CDCl_3$) 2.64 [dd, J = 7.3, 16.5 Hz, 1H, 4-H(F)], 2.85 [dd, J = 5.2, 16.5 Hz, 1H, 4-H(F)], 3.63-3.88 (8 x s, 24H, 8 x O**CH$_3$**), 4.04-4.09 [m, 1H, 3-H(F)], 4.86 [d, J = 6.5 Hz, 1H, 2-H(F)], 5.21 [d, J = 1.4 Hz, 1H, 2-H(C)], 5.44 [d, J = 1.4 Hz, 1H, 4-H(C)], 6.08 [d, J = 2.4 Hz, 1H, 6-H(A)], 6.09 [s, 1H, 6-H(D)], 6.14 [d, J = 2.4 Hz, 1H, 8-H(A)], 6.71 [dd, J = 1.9, 8.3 Hz, 1H, 6-H(E)], 6.77 [d, J = 8.3 Hz, 1H, 5-H(E)], 6.80 [d, J = 1.90 Hz, 1H, 2-H(E)], 6.78 [dd, J = 1.6, 8.1 Hz, 1H, 6-H(B)], 6.96 [d, J = 8.1 Hz, 1H, 5-H(B)], 6.88 [d, J = 1.6 Hz, 1H, 2-H(B)].

**$^{13}$C NMR:** **Plate 17.3:** δ ($CDCl_3$) 26.5 (C-4(F)), 30.9 (C-3(F)), 40.6 (C-4(C)), 55.2-56.2 (8 x O**C**H$_3$), 81.6 (C-2(F)), 84.7 (C-2(C)), 88.9 (C-6(D)), 93.5 (C-6(A)), 94.3 (C-8(A)), 101.2, 107.6, 109.4 (C-2(E)), 109.9, 110.9 (C-5(B)), 111.0 (C-5(E)), 111.9 (C-2(B)), 119.0 (C-6(E)), 121.4 (C-6(B)), 127.6, 131.0, 149.0, 149.0, 149.1, 149.4, 152.4, 156.6, 156.7, 157.3, 158.8, 159.7, 206.2 (C-3).

**CD:** **CD-plate 13:** λ nm (θ), 183.20 ($3.958 \times 10^3$), 188.80 ($-1.789 \times 10^3$), 192.60 ($7.21 \times 10^2$), 195.00 ($-6.758 \times 10^2$), 202.20 ($4.926 \times 10^4$), 211.80 ($-4.122 \times 10^3$), 289.80 ($1.573 \times 10^4$).

### 9.6.4 (2R,3S,4S:2R,3S)-5,7,3′4′-tetra-*O*-methylflavan-3-ol-4β→8-5,7,3′,4′-tetra-*O*-methyl-flavan-3-ol (28)

An *aq*NaOH solution (10 ml, 2.00 M) was added to ethanol (200 mL), followed by $NaBH_4$ (7.71 g, 0.20 mol). To a solution of **(22)** (18 mg, 0.03 mmol) in methanol (5 mL) the above mentioned $NaBH_4$ solution (1 mL) was slowly added. After stirring the reaction mixture for 5 min. the methanol was removed under reduced pressure, the excess $NaBH_4$ destroyed with water (1 mL) and the subsequent mixture extracted with ethyl acetate (2 x 10 mL). The organic phase was washed with water (10 mL), dried over $MgSO_4$ and evaporated to dryness under vacuum. PLC (toluene:ethyl acetate; 5:5) yielded **(28)** (21 mg, 97%, $R_f$ 0.42) as a white, amorphous solid. Acetylation of **(28)** afforded the octamethyl ether diacetate **(29)** as a white amorphous solid (23 mg, 100%).

**Compound (28)**

**MS:** Found: $\underline{M}^+$ 690.26917, $C_{38}H_{42}O_{12}$ requires $\underline{M}^+$ 690.26763**,** $[M+H]^+$ 691 (100) m/e 511 (32), 495 (12), 345 (35).

**IR:** $\nu_{max}$ 2927, 2837, 1700, 1595, 1518 $cm^{-1}$.

**$^1$H NMR:** **Plate 18.2** and **18.3:** δ (DMSO, 145 $^oC$) 2.52 (s, 3H, OC**H₃**), 2.53 (dd, J = 6.9, 16.2 Hz, 1H, 4-H(F)$_{ax}$), 2.90 (dd, J = 5.1, 16.2 Hz, 1H, 4-H(F)$_{eq}$), 3.46-3.84 (7 x s, 21H, 7 x OC**H₃**), 3.89-3.96 [m, 1H, 3-H(F)], 4.06 [dd, J = 6.4, 9.2 Hz, 1H, 3-H(C)], 4.32 [br s, 1H, 2-H(F)], 4.84 [d, 1H, J = 6.4 Hz, 4-H(C)], 4.89 [d, 1H, J = 9.2 Hz, H-2(C)], 5.68 [br s, 1H, 6-H(D)], 6.00 [br s, 1H, 6/8-H(A)], 6.30 [br s, 1H, 6/8-H(A)], 6.80-6.96 [m, 6H, 2,5,6-H(E)/(D)].

**$^{13}$C NMR:** **Plates 18.4:** δ (DMSO) 29.0 (C-4(F)), 32.6 (C-4(C)), 40.8 (1 x O**C**$H_3$), 55.5-57.1 (7 x O**C**$H_3$), 67.2 (C-3(F)), 70.7 (C-3(C)), 78.3 (C-2(C)), 79.1 (C-6/8(A)), 79.3 (C-6/8(A)), 79.5, 82.2, 92.0, 92.5, 94.2, 107.0, 110.4, 113.2, 113.4, 113.5, 113.8, 120.6, 121.1, 133.0, 133.9, 149.6, 154.3, 156.3, 157.0, 158.6, 158.7, 159.6.

**CD:** **CD-plate 14:** λ nm (θ), 205.50 (-1.924 x $10^2$), 212.00 (1.204 x $10^3$), 221.00 (-1.051 x $10^3$), 227.50 (6.870 x $10^1$), 233.00 (-1.796 x $10^3$), 242.5.80 (2.583 x $10^4$), 277.00 (3.277 x $10^3$).

**Compound (29)**

**MS:** Found $\underline{M}^+$ 774.28859, $C_{38}H_{42}O_{12}$ requires $\underline{M}^+$ 774.28876**,** $\underline{M}^+$ 774 (78) m/e 714 (100), 654 (31), 511 (35), 345 (18).

**IR:** $\nu_{max}$ 2927, 2837, 1700, 1595, 1518 $cm^{-1}$.

**$^1$H NMR:** **Plate 19.2:** δ ($CDCl_3$, 25 °C) 1.74 and 1.81 (2 x s, 2 x 3H, 2 x COC**$H_3$**), 1.85 and 2.00 (2 x s, 2 x 3H, 2 x COC**$H_{3rot.}$**), 2.60 (dd, J = 8.8, 16.5 Hz, 1H, 4-H(F)$_{ax}$), 2.71 (dd, J = 5.2, 17.6 Hz, 1H, 4-H(F)$_{ax\ rot.}$), 2.84 (dd, J = 2.6, 17.6 Hz, 1H, 4-H(F)$_{eq\ rot.}$), 3.18 (dd, J = 6.5, 16.5 Hz, 1H, 4-H(F)$_{eq}$), 3.34-3.88 (15 x s, 48H, 16 x OC**$H_3$**), 4.90 [d, J = 8.9 Hz, 1H, 2-H(F)], 5.02 [d, 1H, J = 6.3 Hz, 4-H(C)], 5.16 (d, 1H, J = 6.0 Hz, 4-H(C)$_{rot.}$), 5.15-5.19 [m, 1H, 3-H(F)], 5.22 (d, J = 3.6 Hz, 1H, 2-H(F)$_{rot.}$), 5.27 (d, 1H, J = 10.4 Hz, 2-H(C)$_{rot.}$), 5.32 [d, J = 2.4 Hz, 1H, 6/8-H(A)], 5.32 [d, 1H, J = 10.4 Hz, 2-H(C)], 5.34-5.36 (m, 1H, 3-H(F)$_{rot.}$), 5.47 [dd, J = 6.3, 10.4 Hz, 1H, 3-H(C)], 5.51 (dd, J = 6.0, 10.4 Hz, 1H, 3-H(C)$_{rot.}$), 5.85 [d, J = 2.4 Hz, 1H, 6/8-H(A)], 6.04 (d, J = 2.3 Hz, 1H, 6/8-H(A)$_{rot.}$), 6.12 (s, 1H, 6-H(D)$_{rot.}$), 6.20 (d, J = 2.3 Hz, 1H, 6/8-H(A)$_{rot.}$), 6.21 [s, 1H, 6-H(D)], 6.65 [dd, J = 2.0, 8.2 Hz, 1H, 6-H(E)], 6.69 [d, J = 2.0 Hz, 1H, 2-H(E)], 6.73 [d, J = 8.2 Hz, 1H, 5-H(E)], 6.81 [dd, J = 1.8, 8.3 Hz, 1H, 6-H(B)], 6.81 (d, J = 8.3 Hz, 1H, 5-H(B)$_{rot}$), 6.82 (d, J = 8.3 Hz, 1H, 5-H(E)$_{rot}$), 6.88 [d, J = 1.8 Hz, 1H, 2-H(B)], 6.88 (d, J = 1.8 Hz, 1H, 2-H(E)$_{rot.}$), 6.93 (dd, J = 1.8, 8.3 Hz, 1H, 6-H(B)$_{rot.}$), 6.94 (d, J = 1.8 Hz, 1H, 2-H(B)$_{rot}$), 6.97 [d, J = 8.3 Hz, 1H, 5-H(B)], 6.98 (dd, J = 1.8, 8.3 Hz, 1H, 6-H(E)$_{rot.}$).

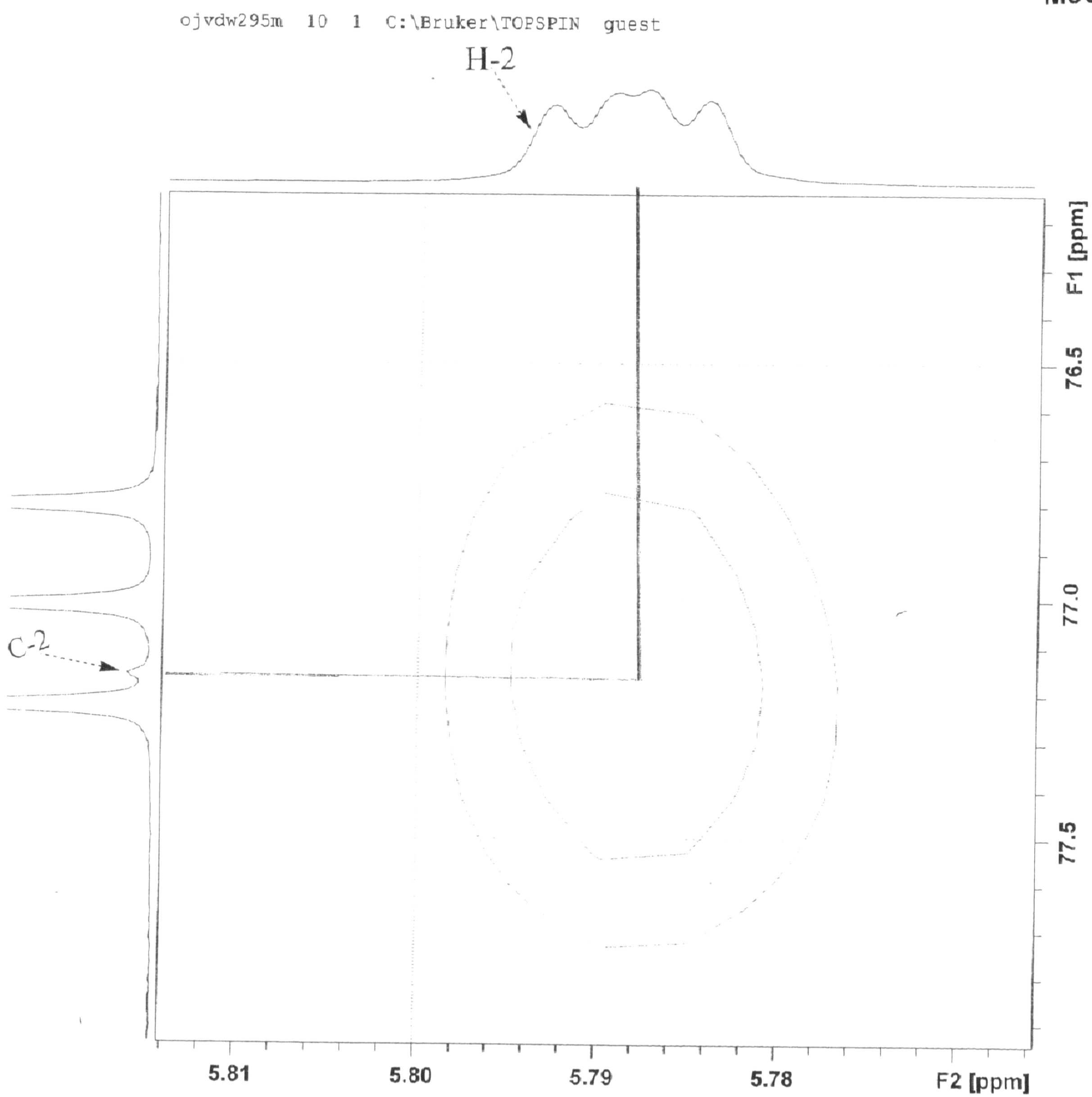
**$^{13}$C NMR:** **Plate 19.3:** δ (CDCl3, 25$^{o}$C) 20.6 – 21.3 (2 x CO**C**$H_3$ and 2 x CO**C**$H_{3rot.}$), 21.8 (C-4(F)$_{rot.}$), 26.7 (C-4(F)), 30.2 (C-4(C)$_{rot.}$), 30.4 (C-4(C)), 53.8 – 56.6 (8 x O**C**$H_3$ and 8 x O**C**$H_{3rot.}$), 69.2 (C-3(F)$_{rot}$), 70.0 (C-3(F)), 71.9 (C-3(C)), 72.2 (C-3(C)$_{rot.}$), 75.5 (C-2(C)), 75.9 (C-2(C)$_{rot.}$), 77.8 (C-2(F)$_{rot.}$), 79.4 (C-2(F)), 78.3 (C-2(C)), 88.7 (C-6(D)$_{rot.}$), 91.0 (C-6(D)), 91.5 (C-6/8(A)$_{rot.}$), 91.6 (C-6/8(A)), 92.0 (C-6/8(A)), 92.7 (C-6/8(A)$_{rot.}$), 100.1, 103.0, 104.8, 105.3, 109.0, 109.2, 109.5 (C-2(B)), 110.0 (C-2(E)), 110.4 (C-5(E)), 110.4, 110.7 (C-2(E)$_{rot.}$), 110.7, 110.8 (C-6(B)), 111.0, 118.1, 119.6 (C-6(E)), 120.4 (C-6(B)rot.), 120.4 (C-6(E)$_{rot.}$), 129.8, 131.1, 131.2, 131.2, 148.3 (**C**-O$CH_3$), 148.6, 148.7 (2 x **C**-O$CH_3$), 148.8, 148.9 (3 x s), 169.3 - 170.3 (4 x **C**O$CH_3$).

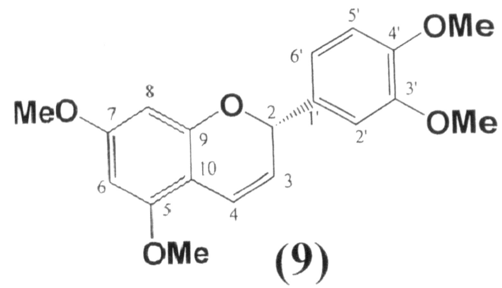
**CD:** **CD-plate15:** λ nm (θ), 213.50 ($-3.878 \times 10^{1}$), 221.00 ($2.202 \times 10^{2}$), 230.50 ($-4.873 \times 10^{2}$), 248.00 ($7.377 \times 10^{3}$), 288.00 ($-7.828 \times 10^{3}$), 330.00 ($2.544 \times 10^{1}$).

# REFERENCES


1. Achilonu, M. C.; Van Rensburg, J. M.; Van der Westhuizen, J. H.; Roodt, A. *Acta Cryst.,* **2007**, *E63*, 04326.
2. Marais, J.P.J. *PhD Thesis*, **2002**, University of the Free State.
3. O'Connell, J. F.; Rapoport, H. *J. Org. Chem*. **1992**, *57*, 4775-4777.
4. Kozikowski, A.P.; Tückmantel, W.; George, C. *J. Org. Chem*., **2000**, *65*, 5371-5381.

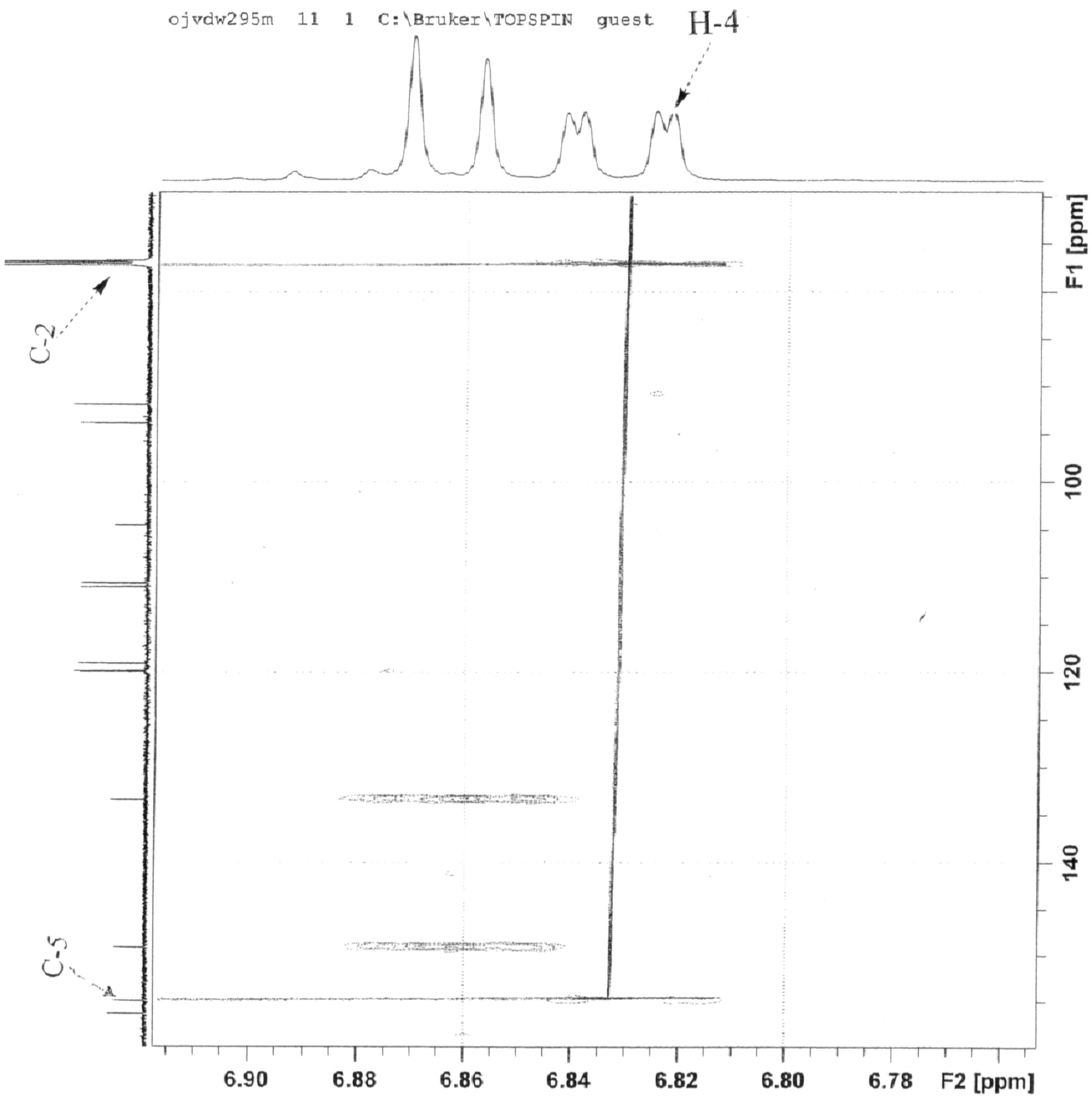
**Plate 4.6:** HSQC NMR ($CDCl_3$, 298K)

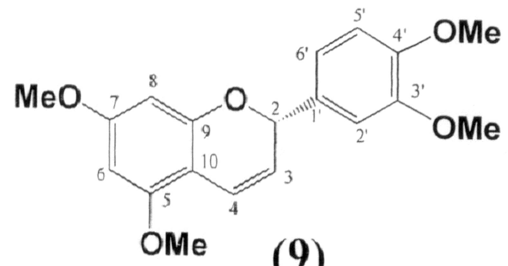



**(9)**

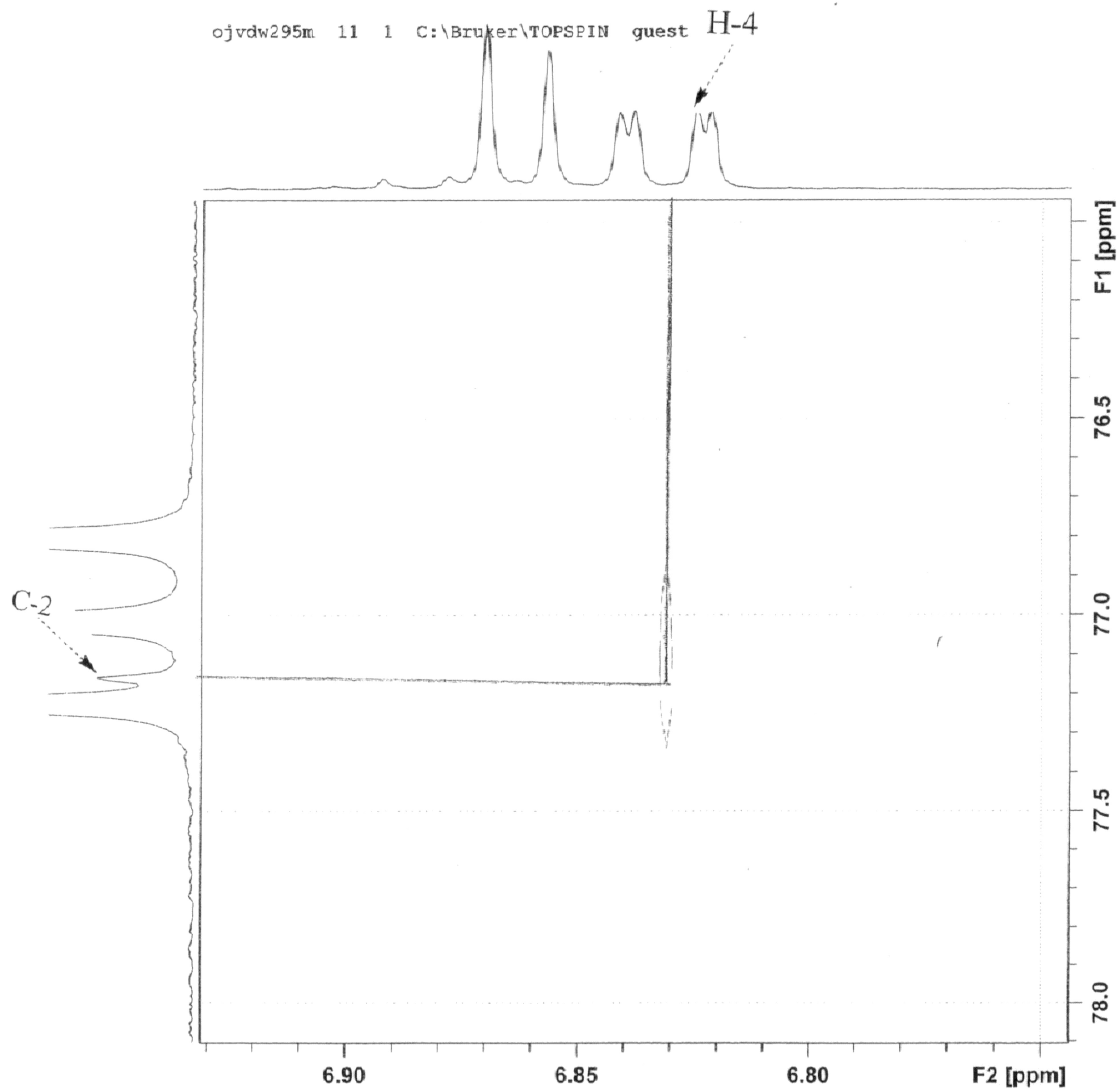
**Plate 4.7:** HMBC NMR ($CDCl_3$, 298K)

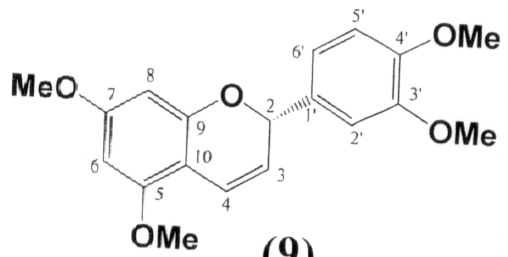



**(9)**

Plate 4.8: HMBC NMR ($CDCl_3$, 298K)




**(9)**

**Plate 5.1:** $^1$H NMR ($CDCl_3$, 298K)

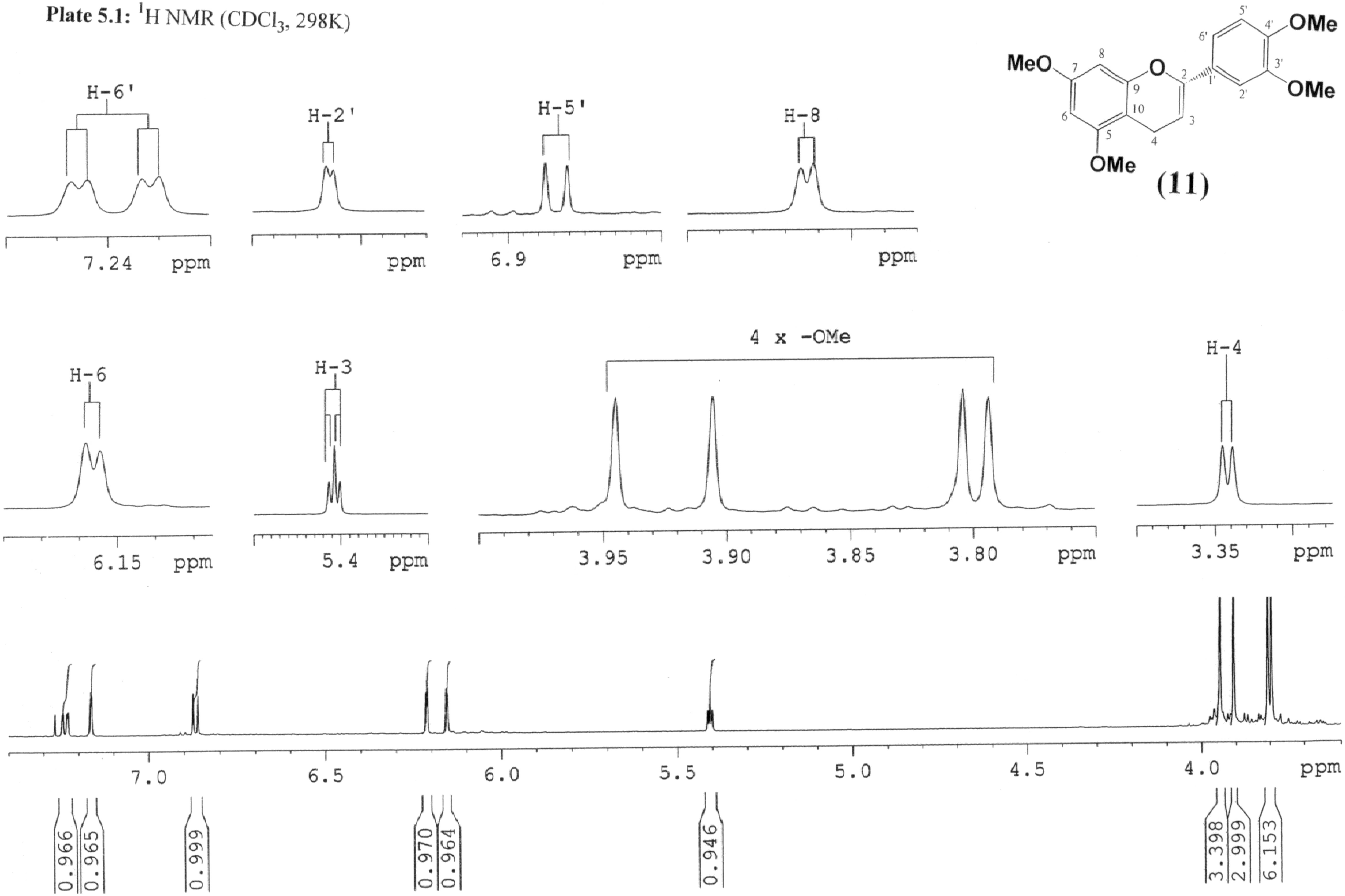
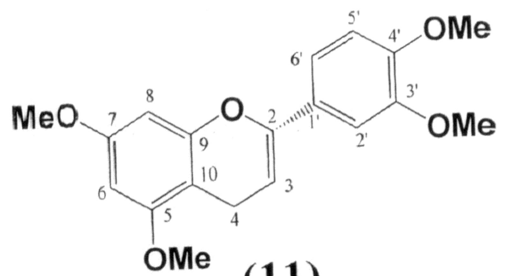


Plate 5.2: $^{13}$C NMR ($CDCl_3$, 298K)




(11)

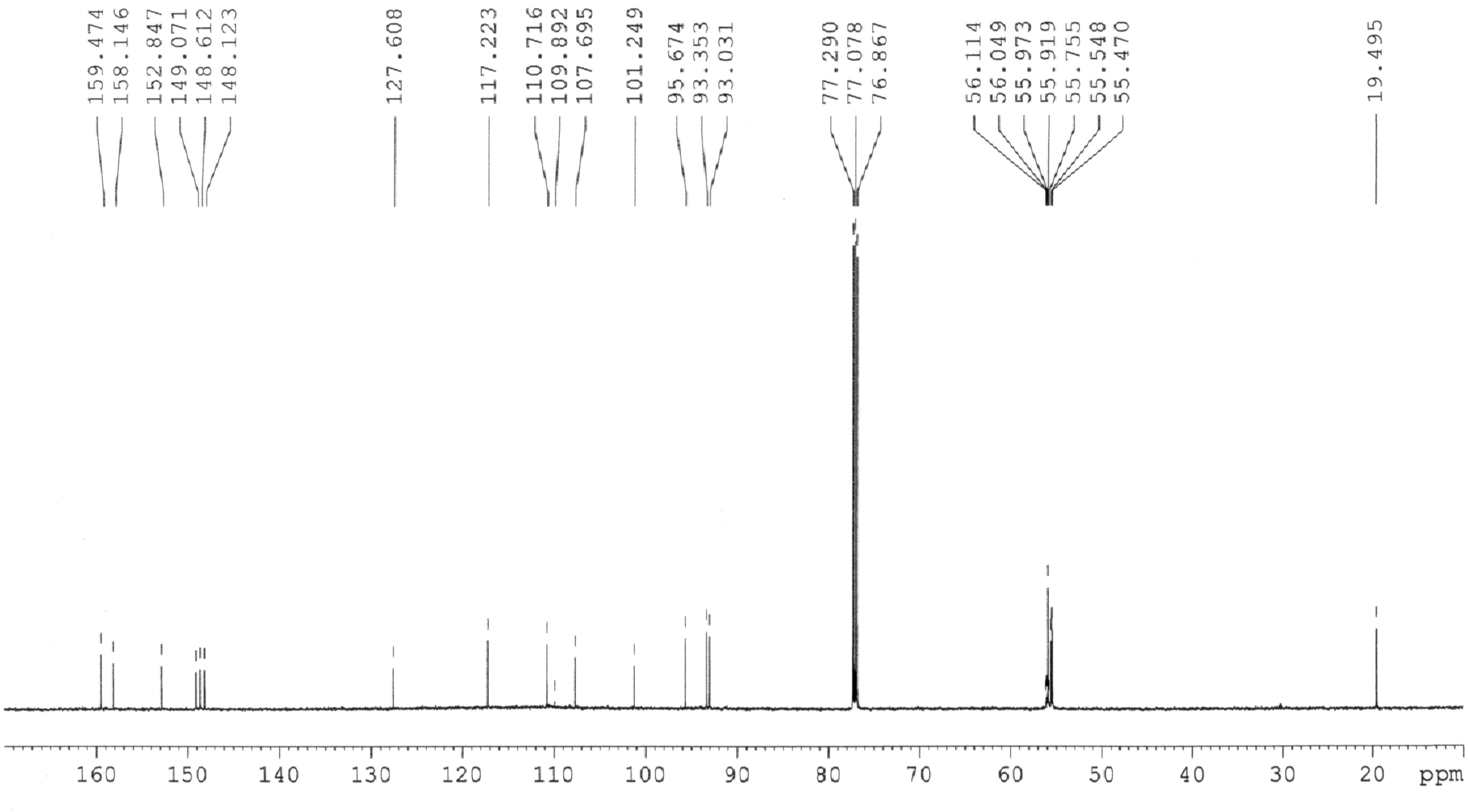
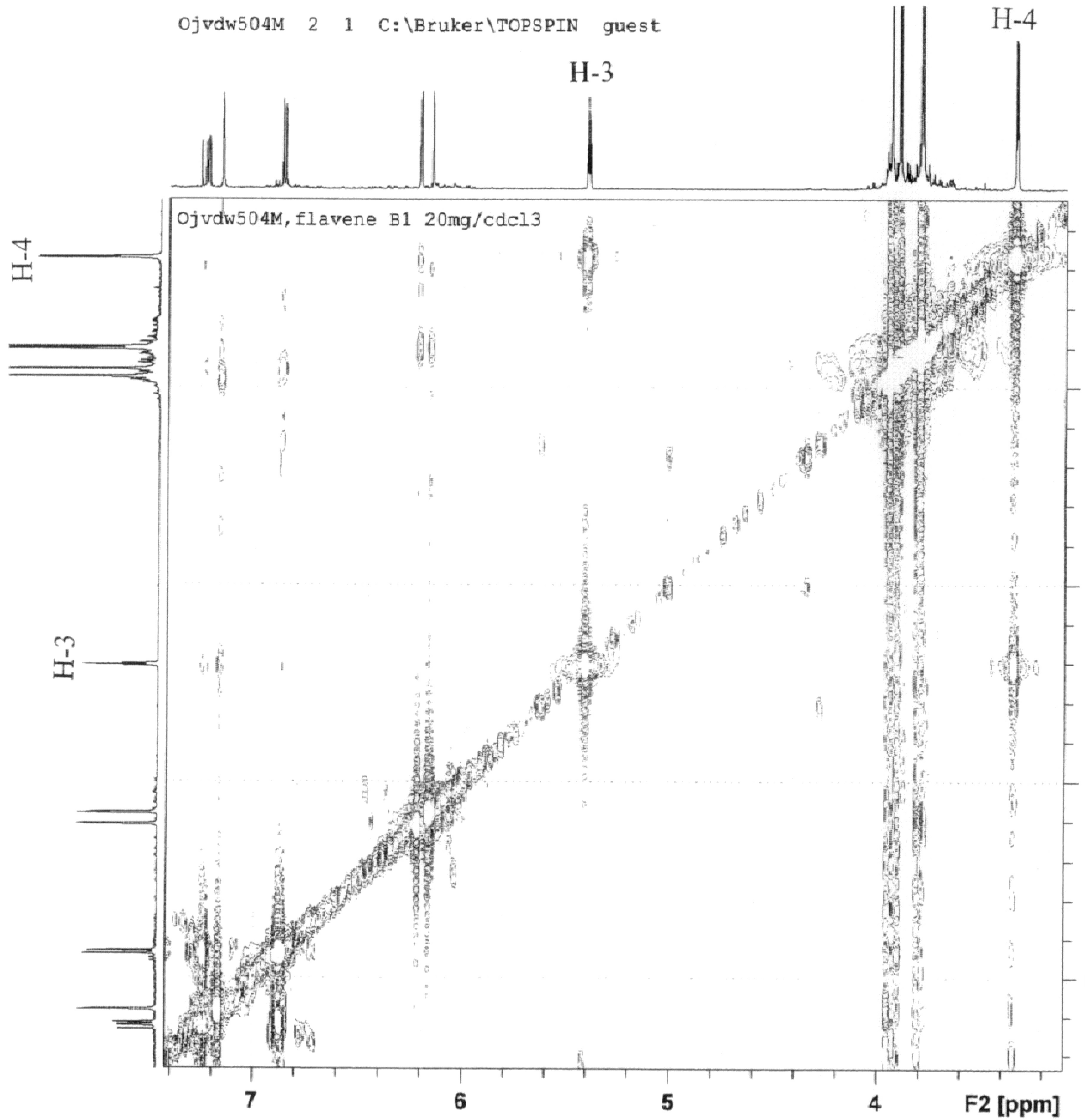
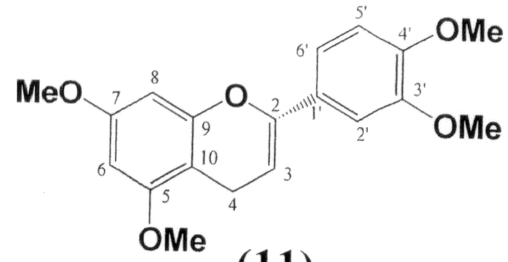


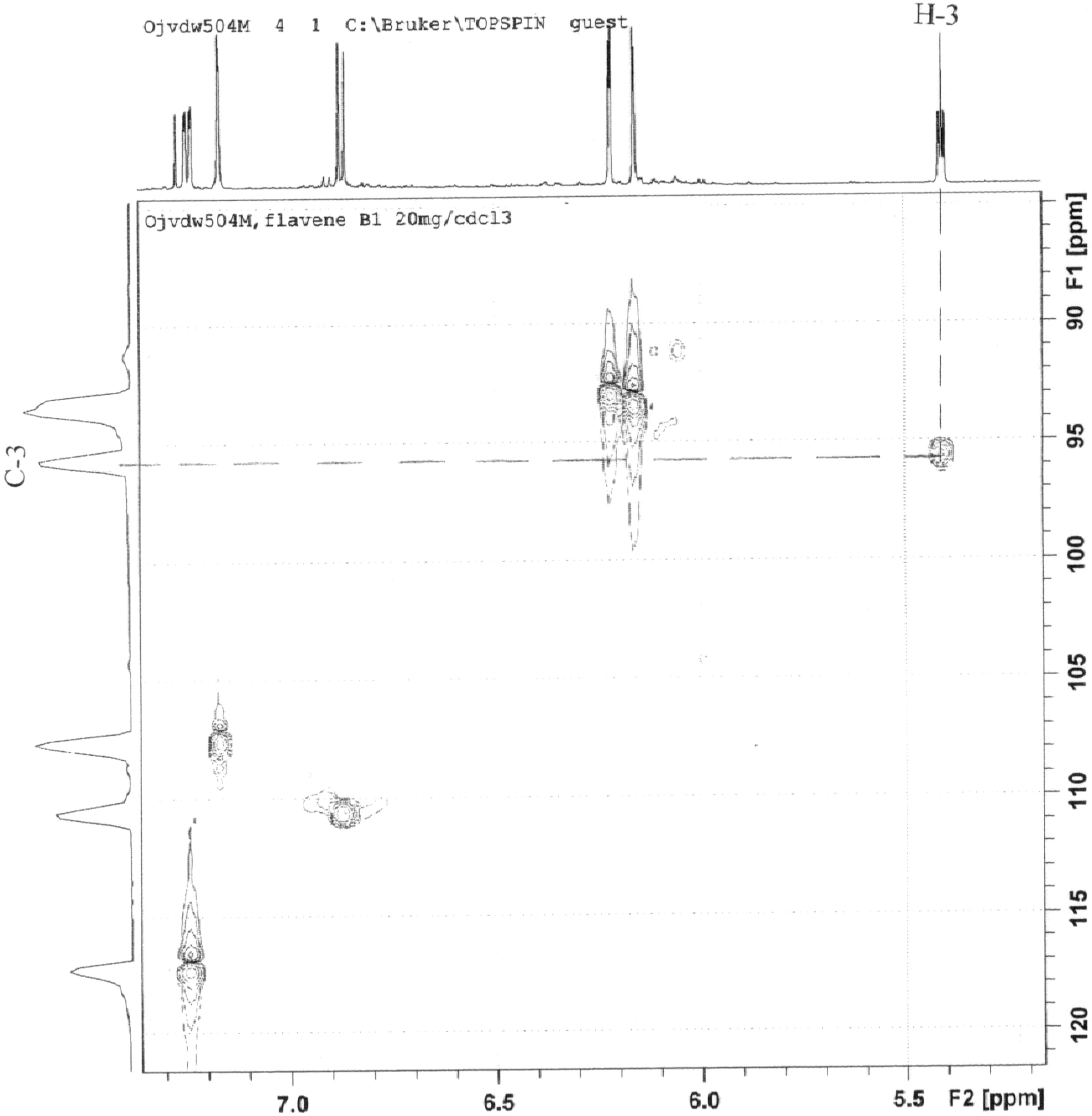
**Plate 5.3:** COSY NMR ($CDCl_3$, 298K)

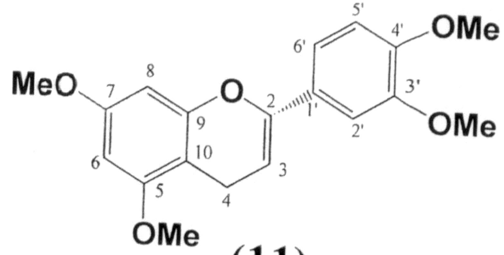



**(11)**

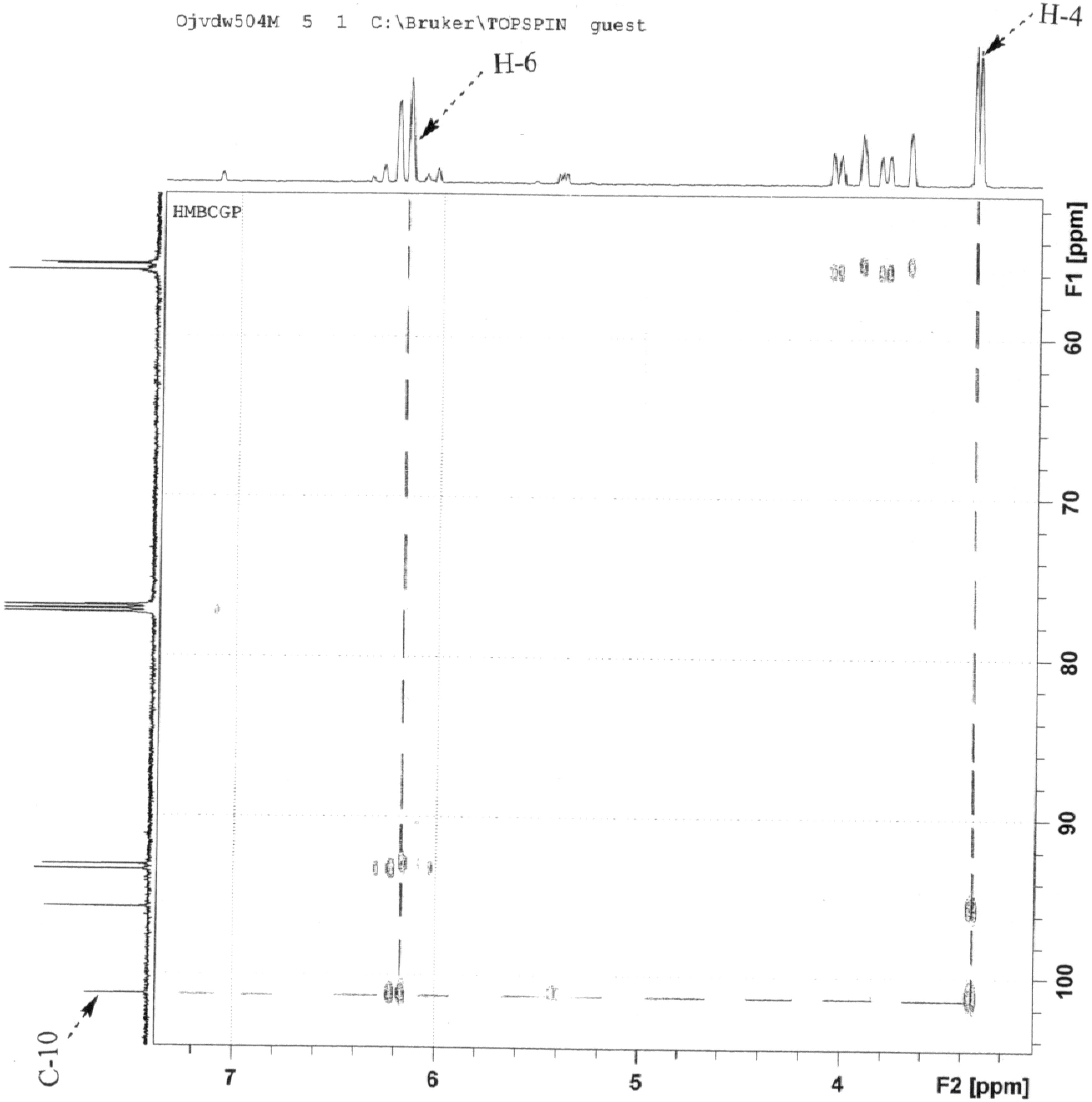
**Plate 5.4:** HSQC NMR ($CDCl_3$, 298K)

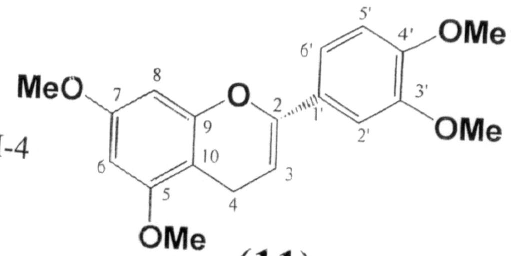



**(11)**

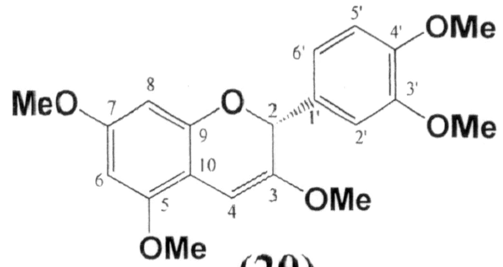
**Plate 5.5:** HMBC NMR ($CDCl_3$, 298K)



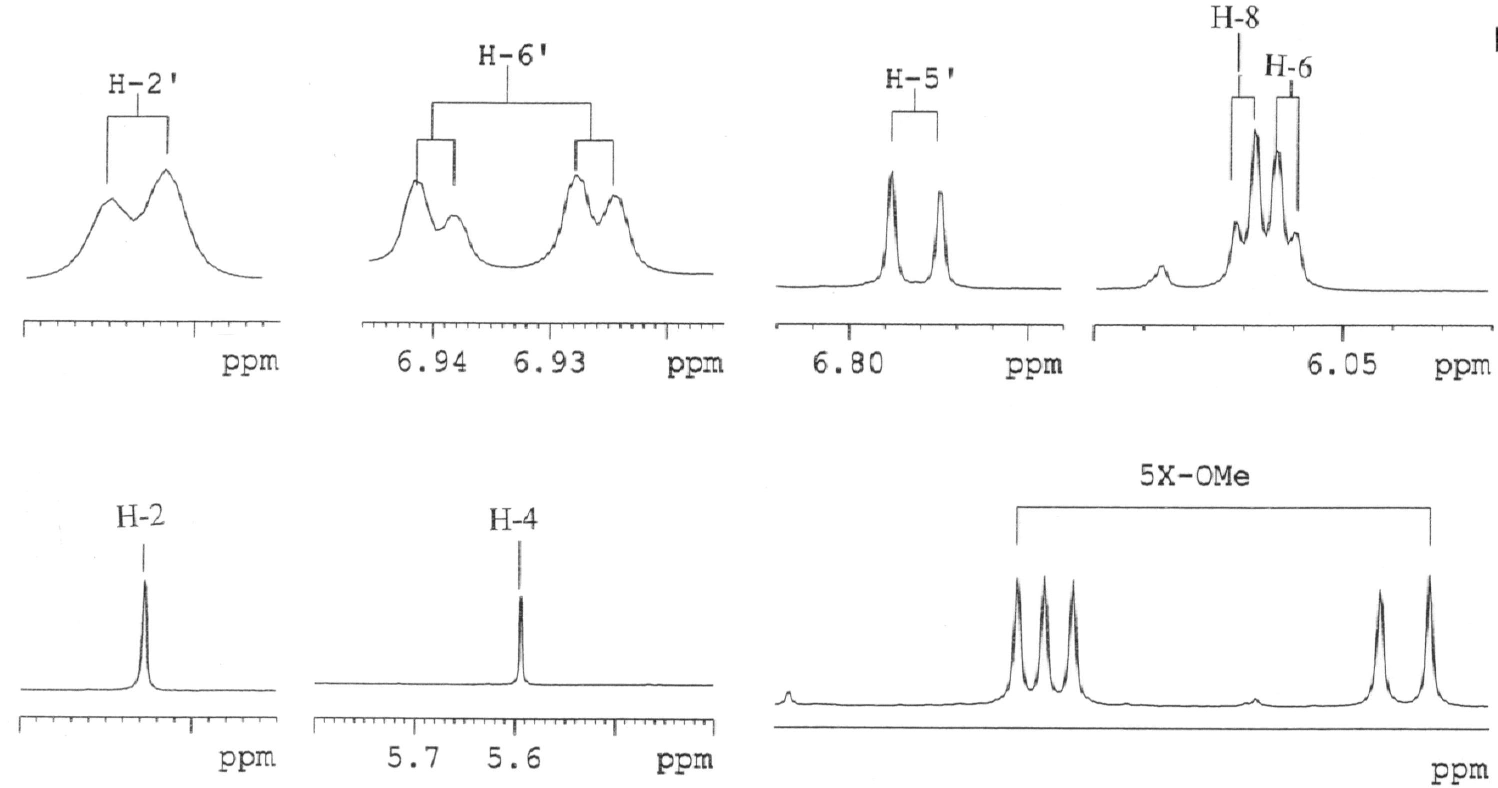




**(11)**

**Plate 6.1** $^1$H NMR ($CDCl_3$, 298K)




**(20)**



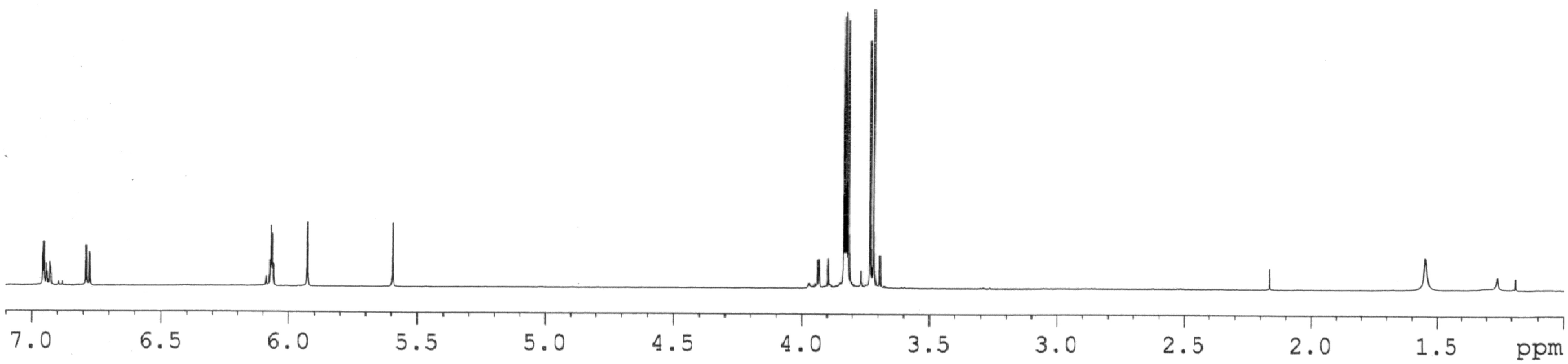
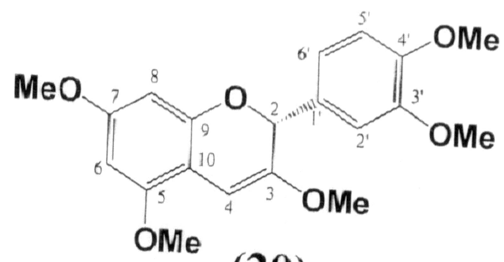



**Plate 6.2**: $^{13}C$ NMR ($CDCl_3$, 298K)



**(20)**

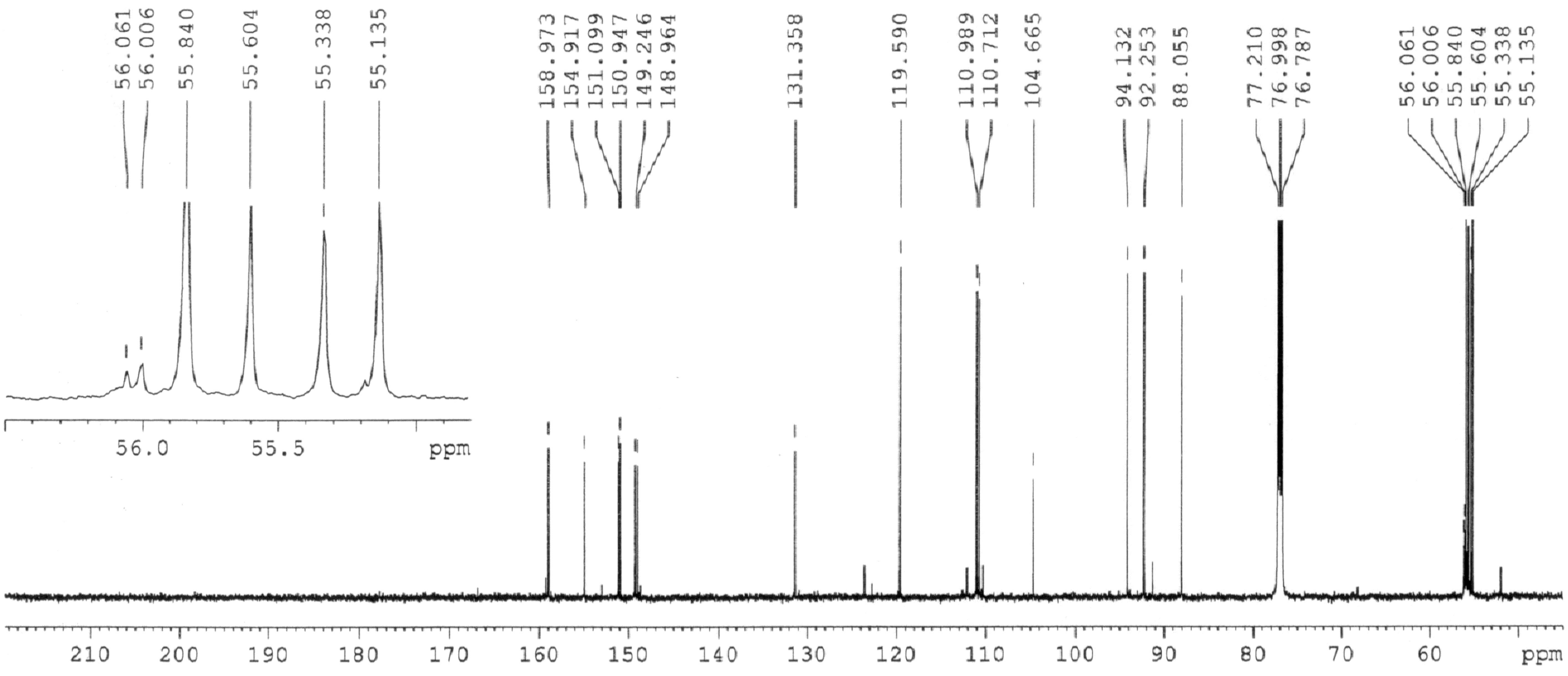
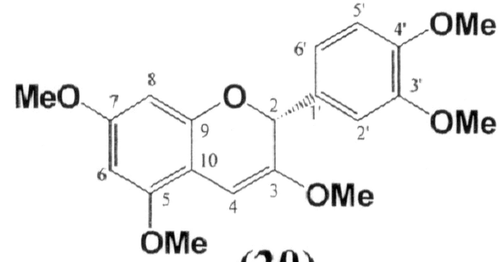


**Plate 6.3:** APT NMR ($CDCl_3$, 298K)




**(20)**

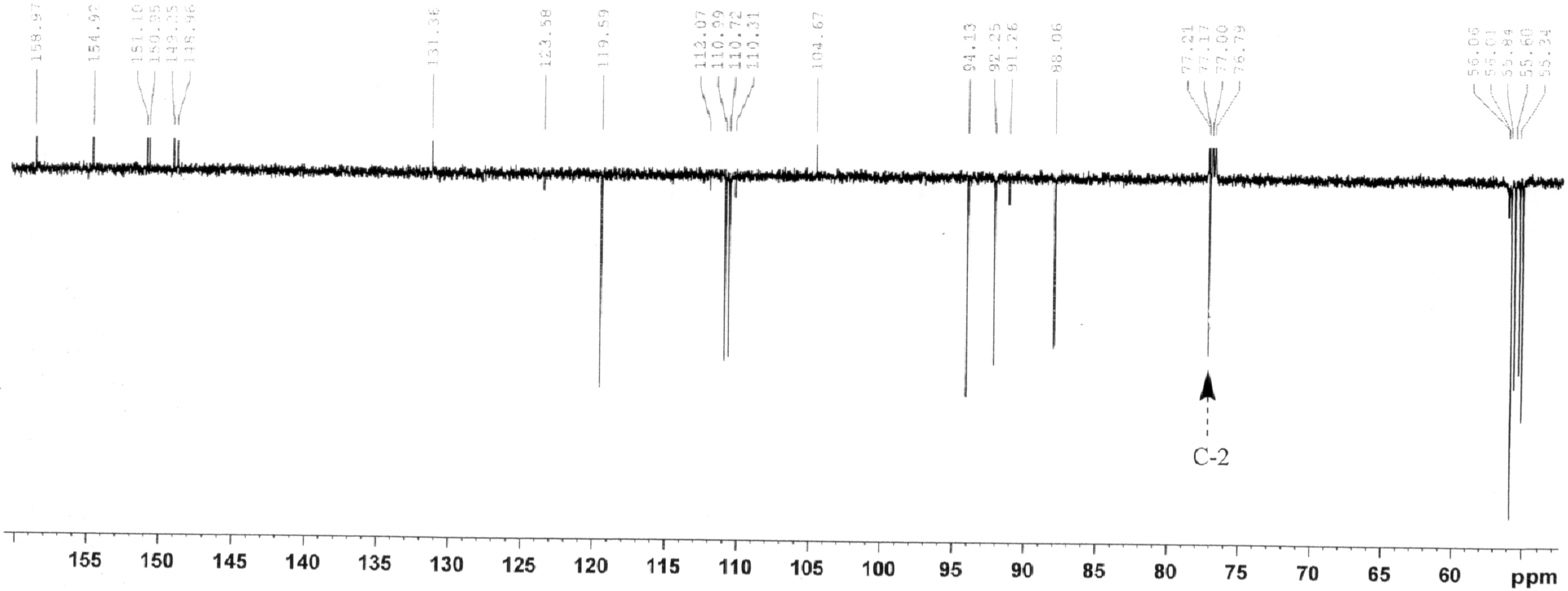
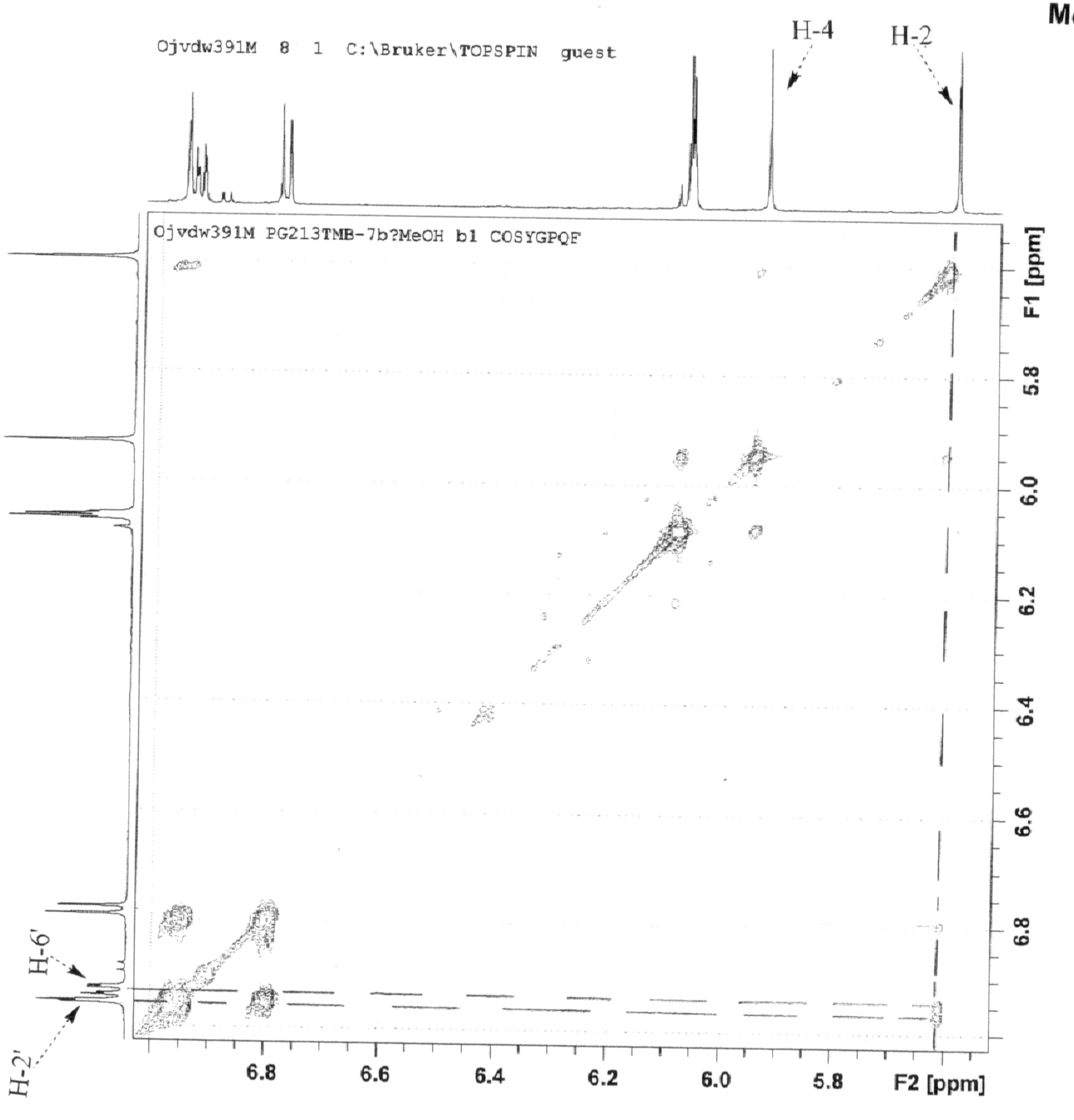
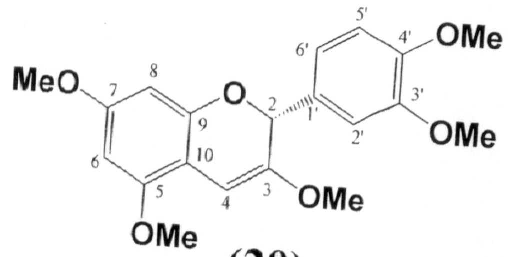


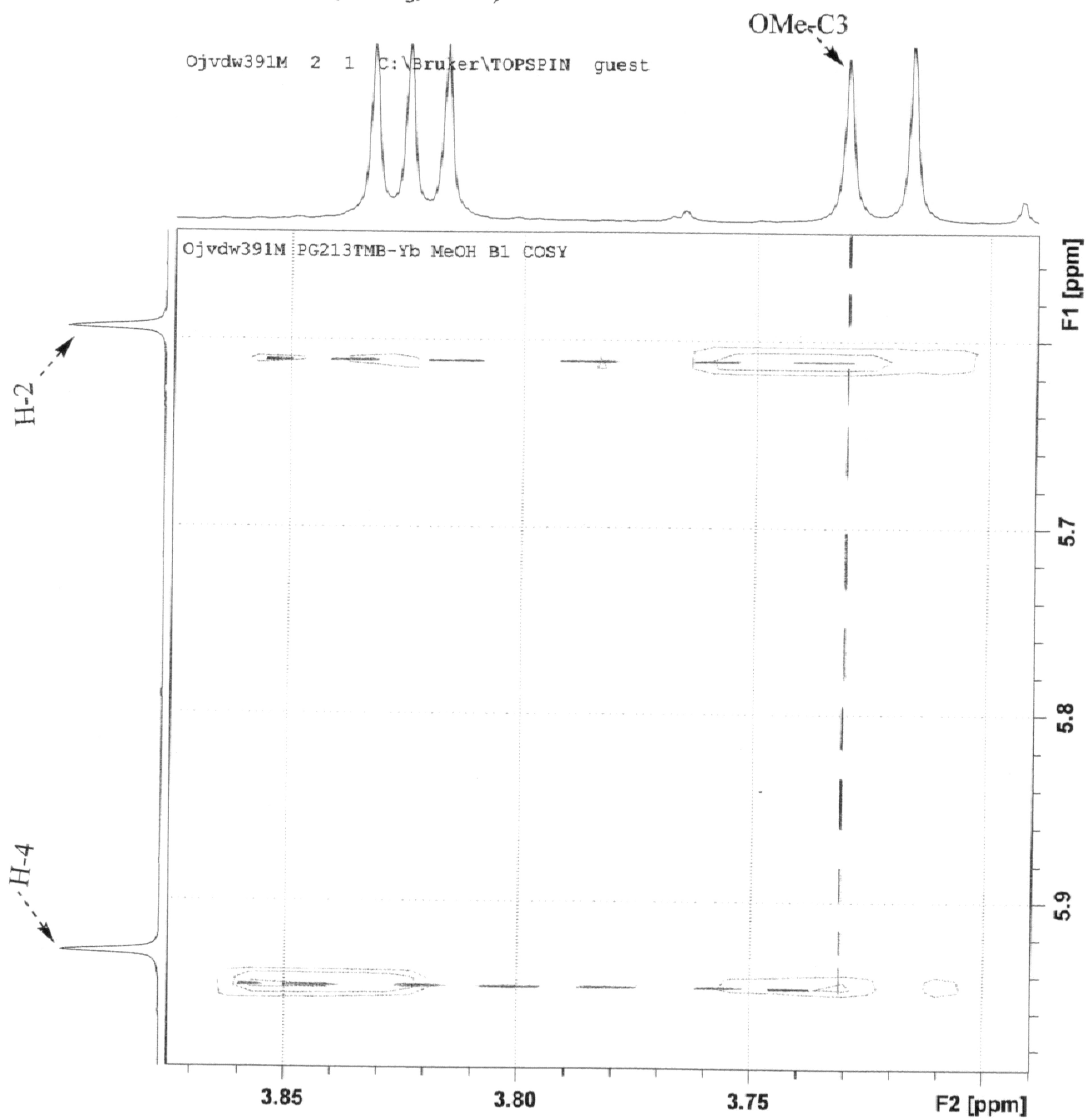
Plate 6.4: COSY NMR ($CDCl_3$, 298K)

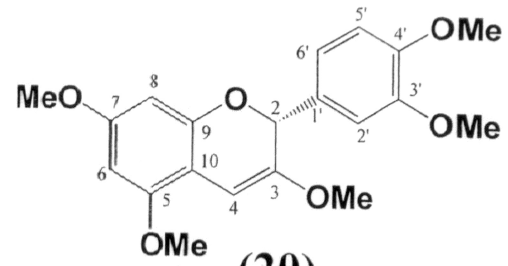



(20)

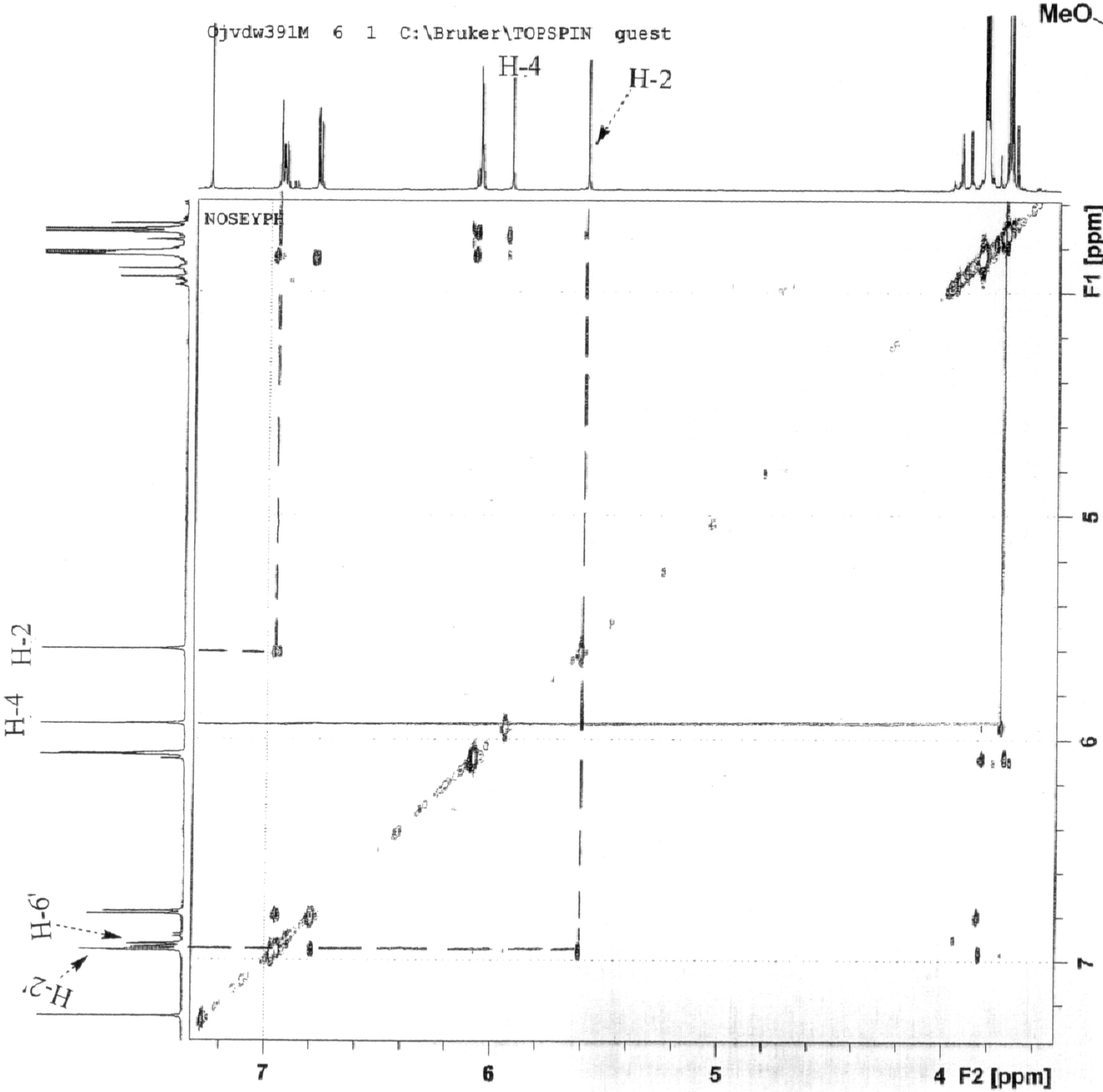
**Plate 6.5:** COSY NMR ($CDCl_3$, 298K)

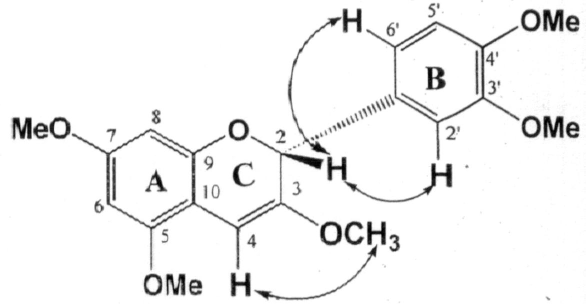



**(20)**

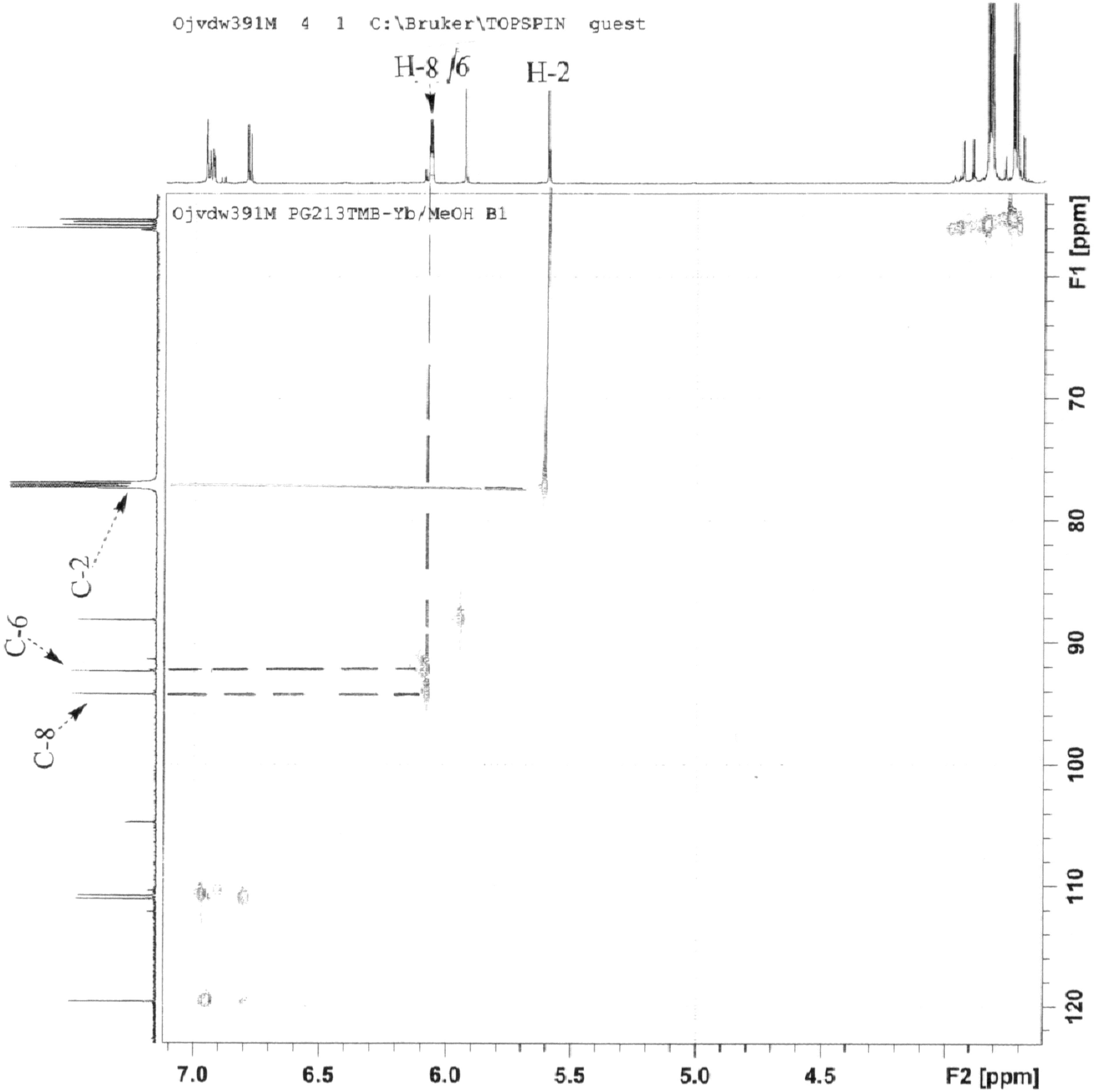
**Plate 6.6:** NOESY NMR ($CDCl_3$, 298K)

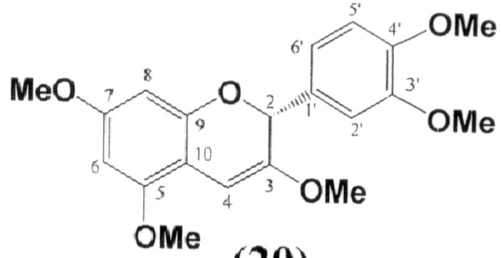


(20)

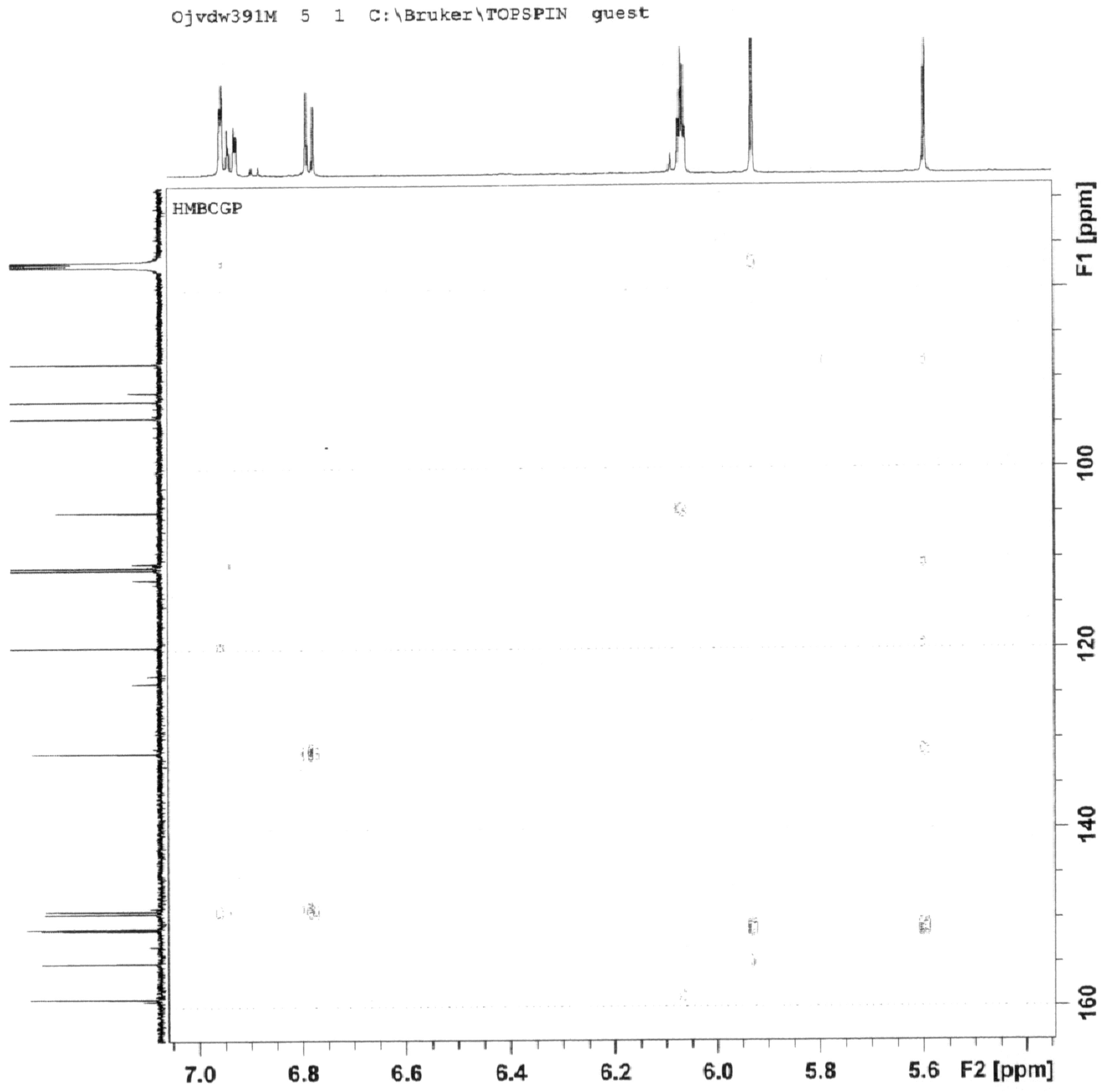
Plate 6.7: HSQC NMR ($CDCl_3$, 298K)

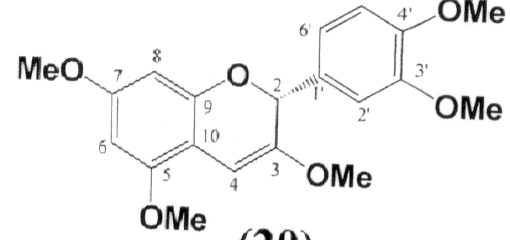



**(20)**

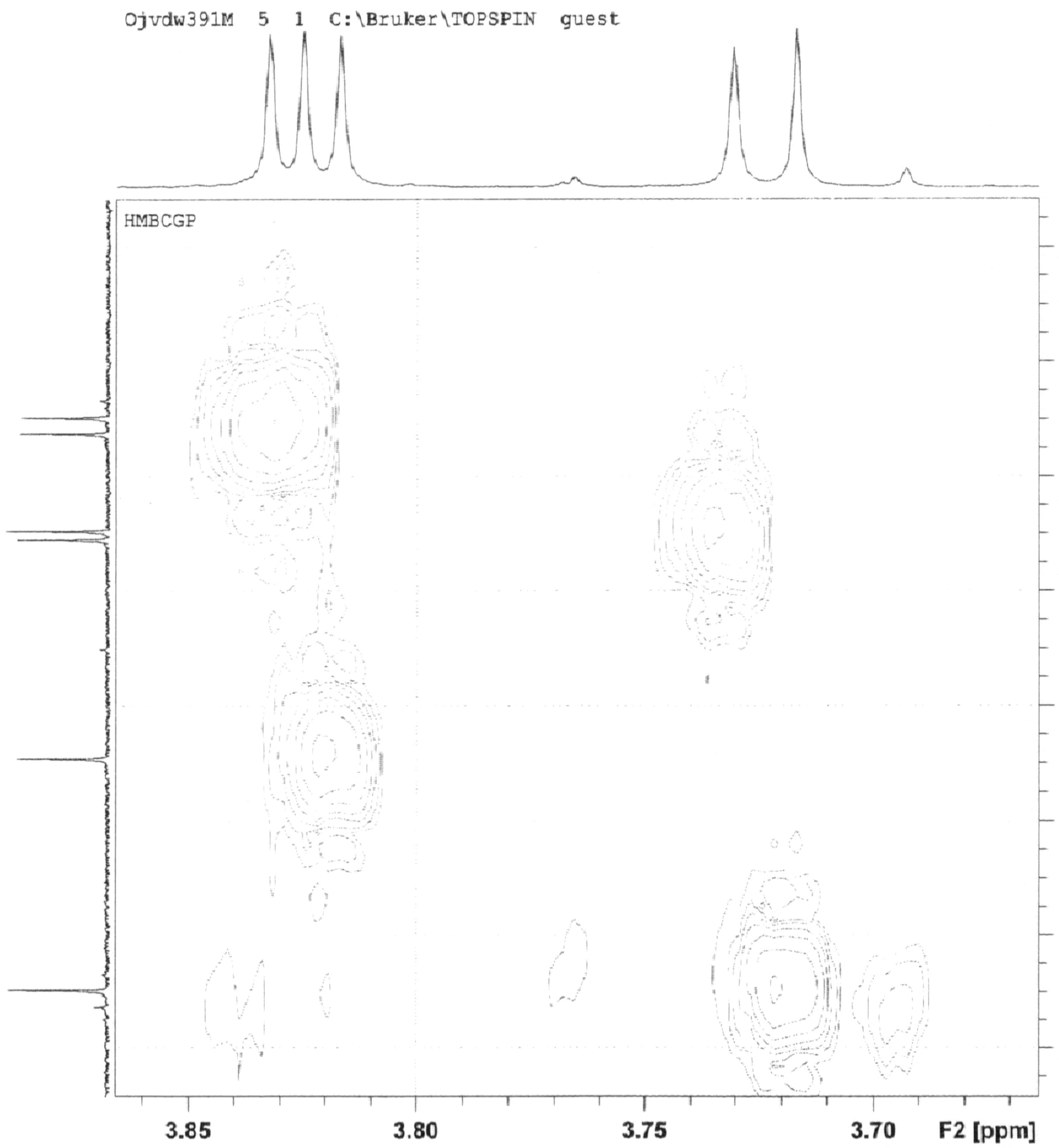
Plate 6.8: HMBC NMR ($CDCl_3$, 298K)

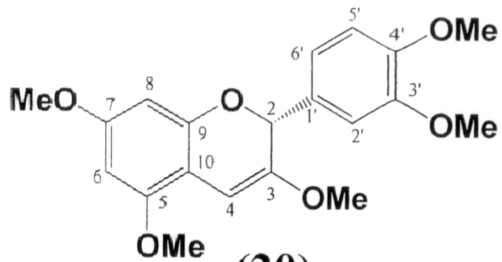



(20)

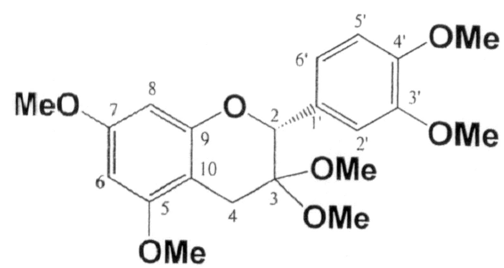
**Plate 6.9:** HMBC NMR ($CDCl_3$, 298K)



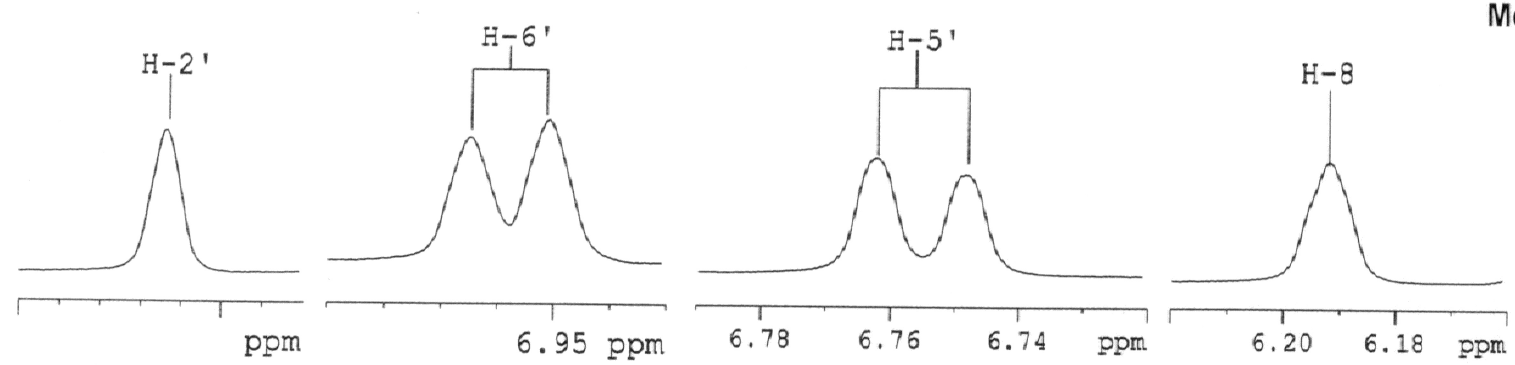




**(20)**

**Plate 7.1:** $^{1}H$ NMR ($CDCl_3$, 298K)



(22)



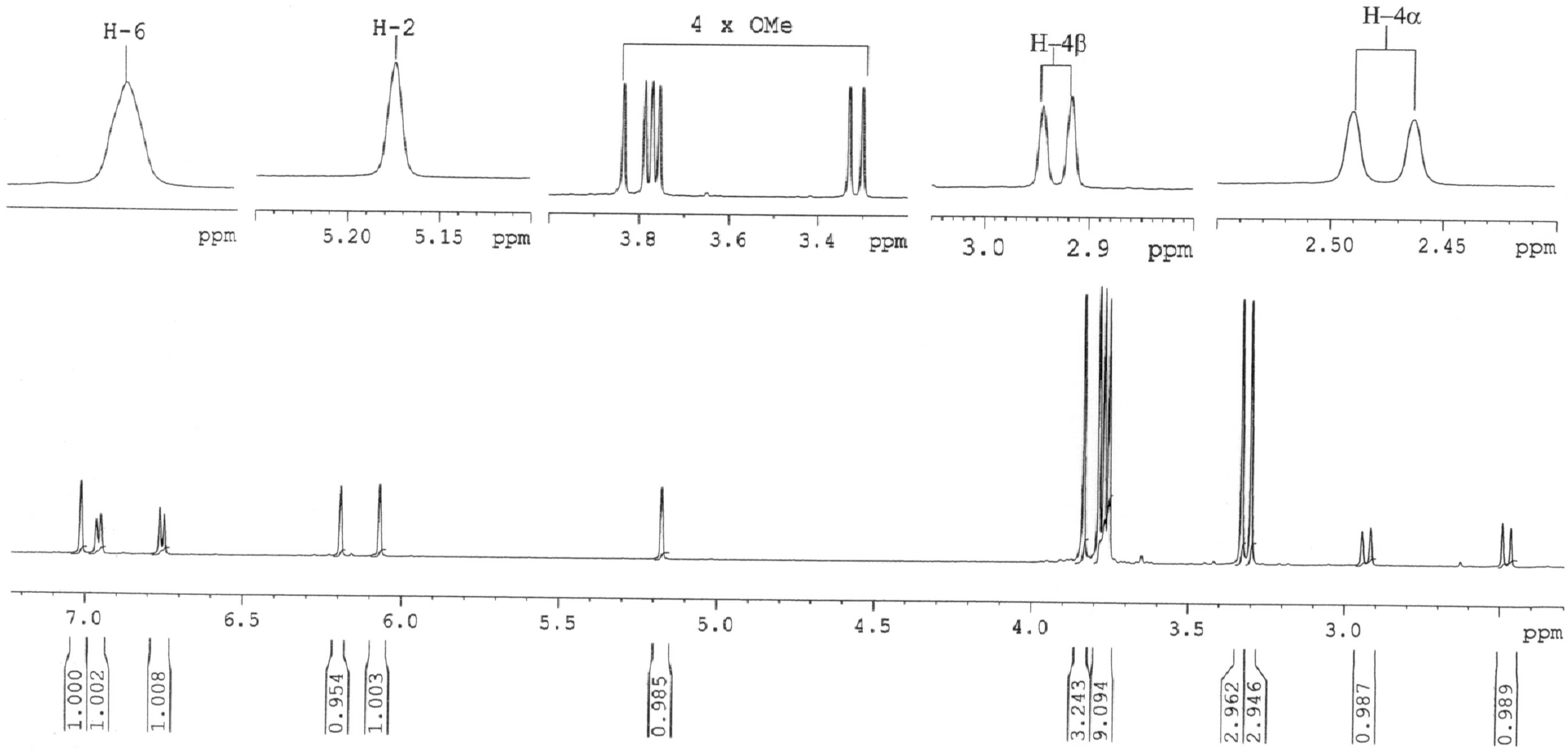
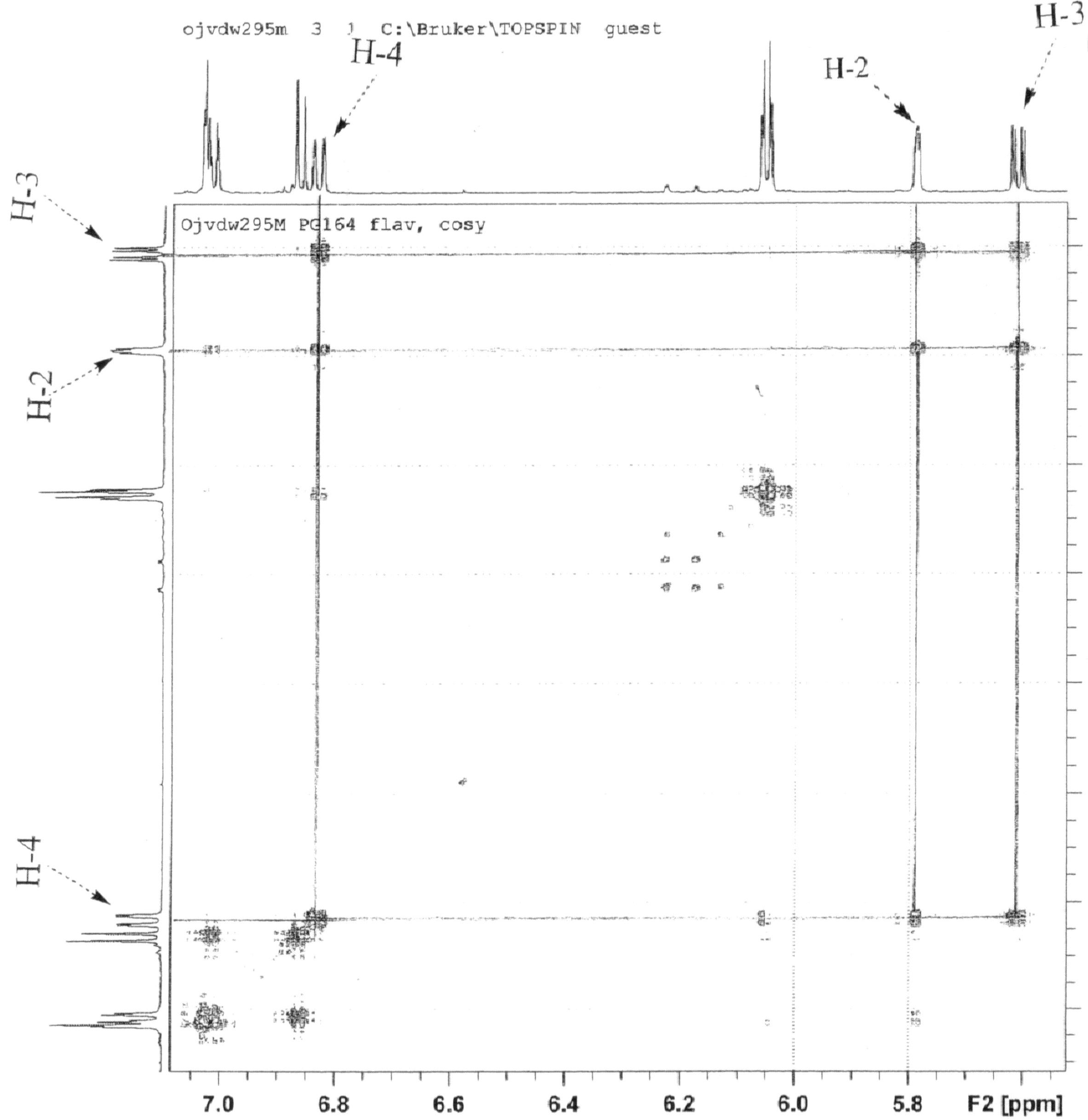
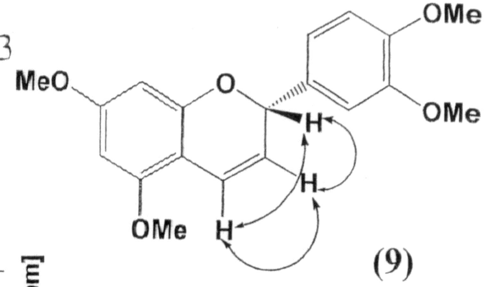



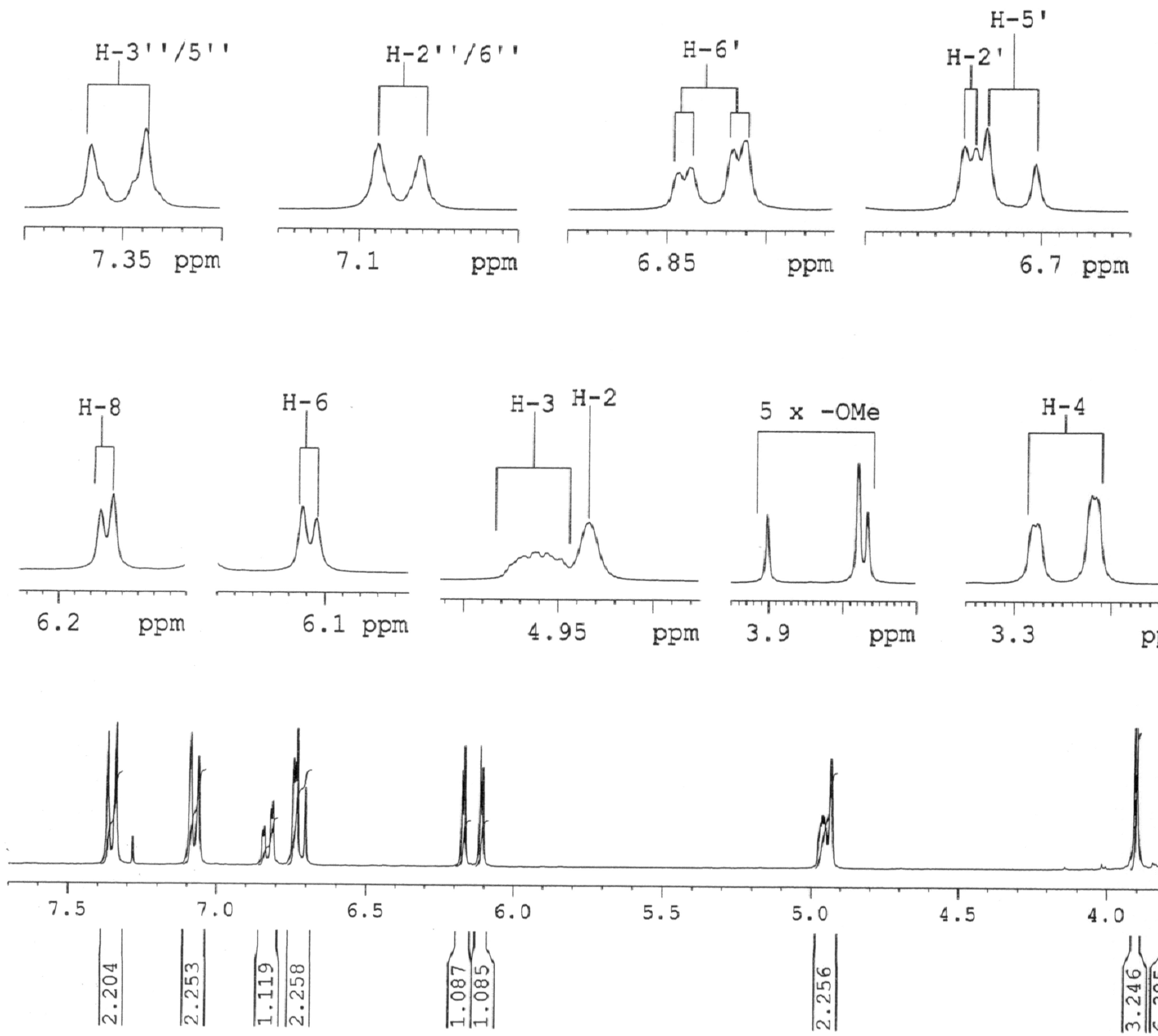
**Plate 4.4:** COSY NMR ($CDCl_3$, 298K)

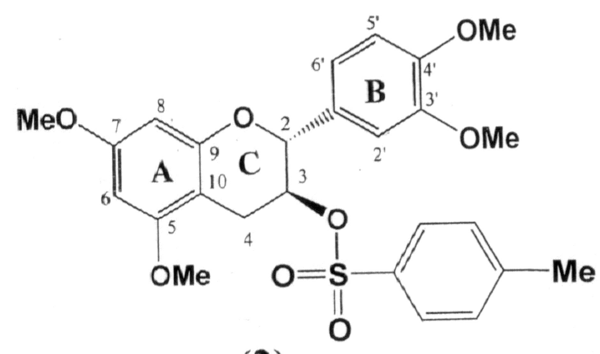



(9)

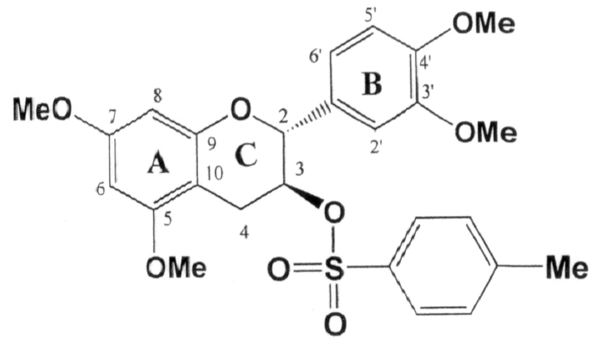
Plate 1.1: $^1$H NMR ($CDCl_3$, 298K)




**(2)**

**Plate 1.2**: $^{13}$C NMR ($CDCl_3$, 298K)




(2)

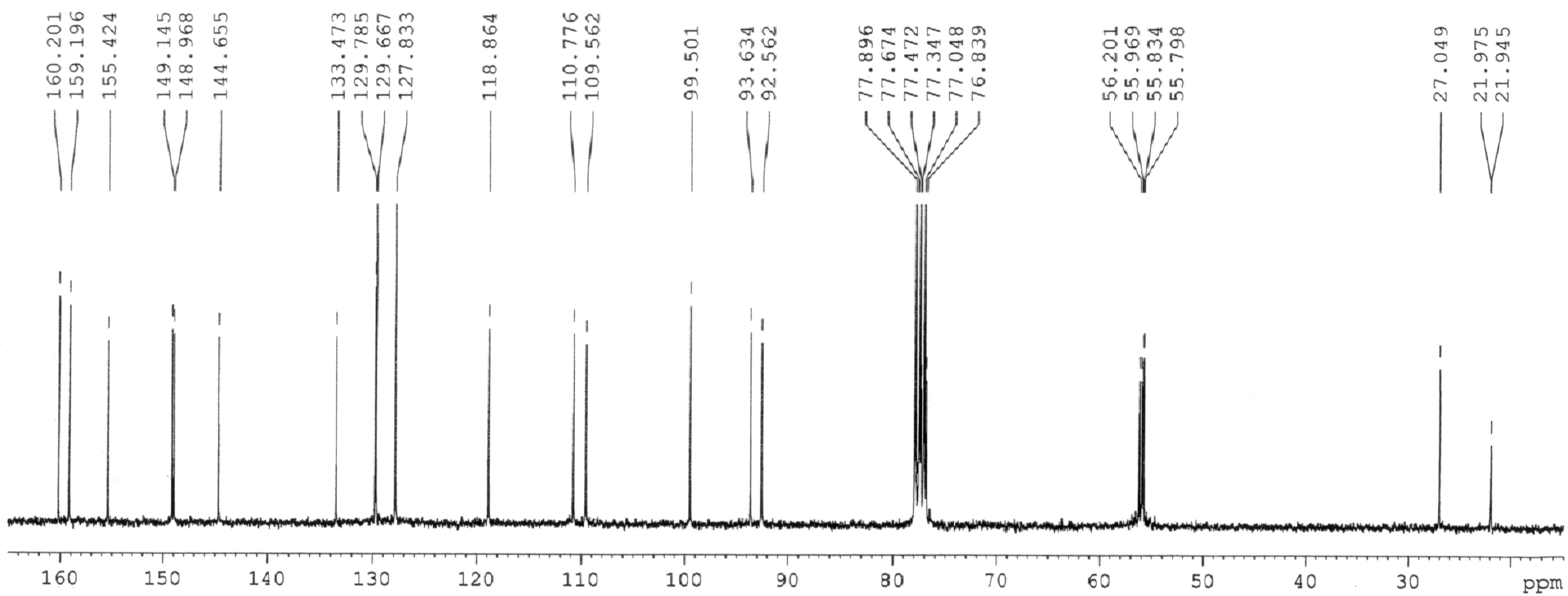
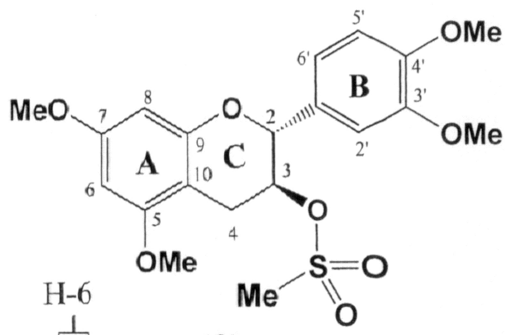


**Plate 2.1** $^{1}$H NMR ($CDCl_3$, 298K)




**(3)**

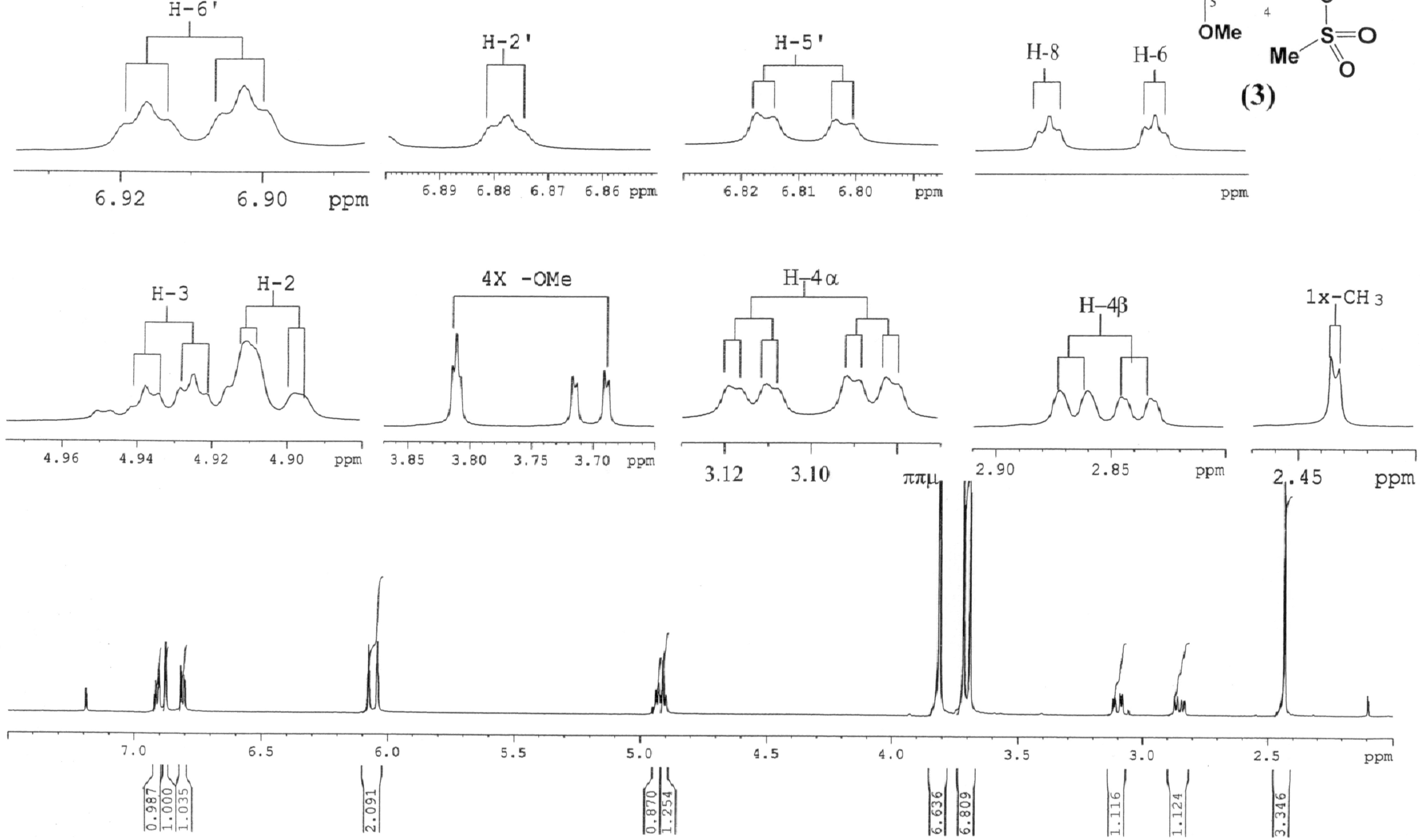
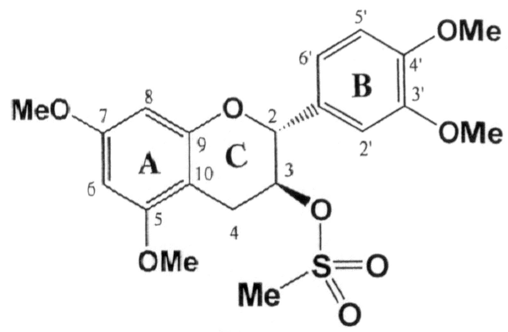


**Plate 2.2**: $^{13}C$ NMR ($CDCl_3$, 298K)




(3)

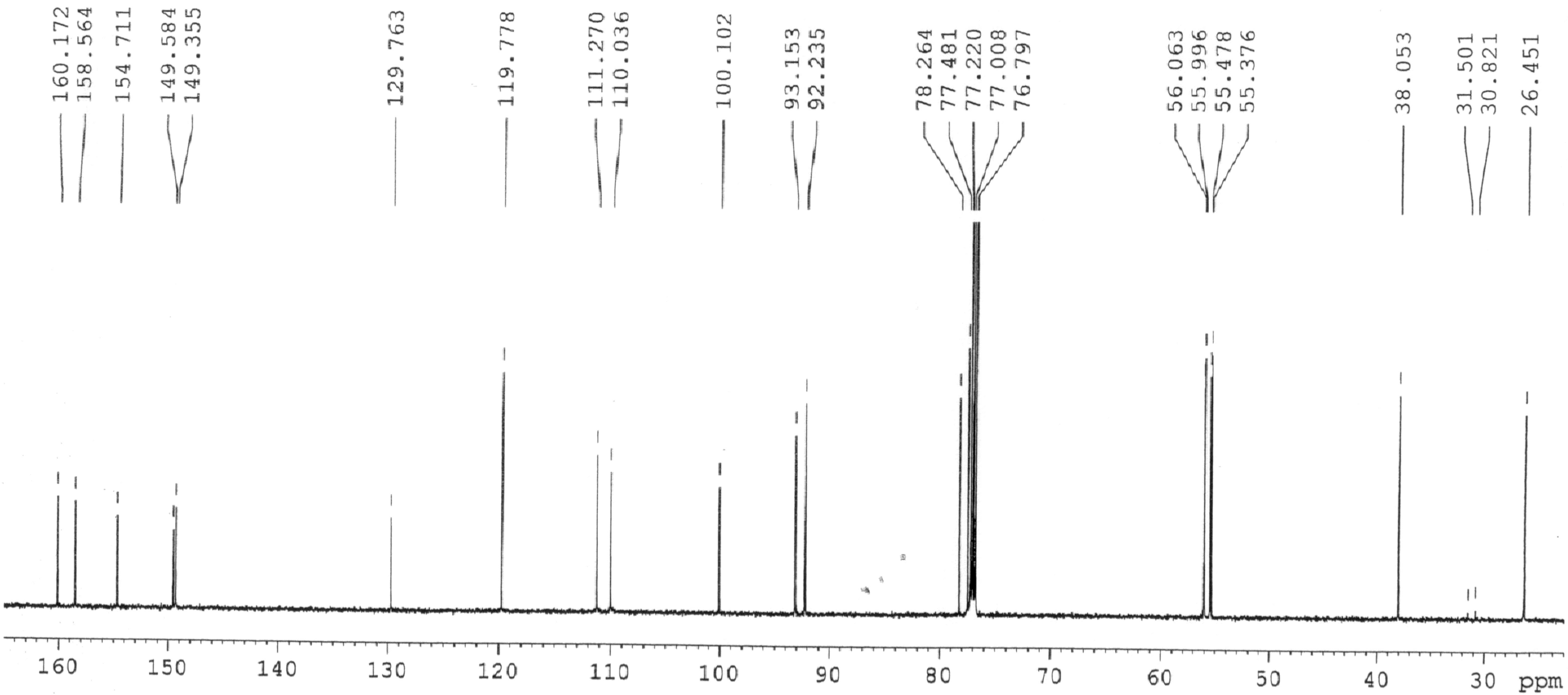
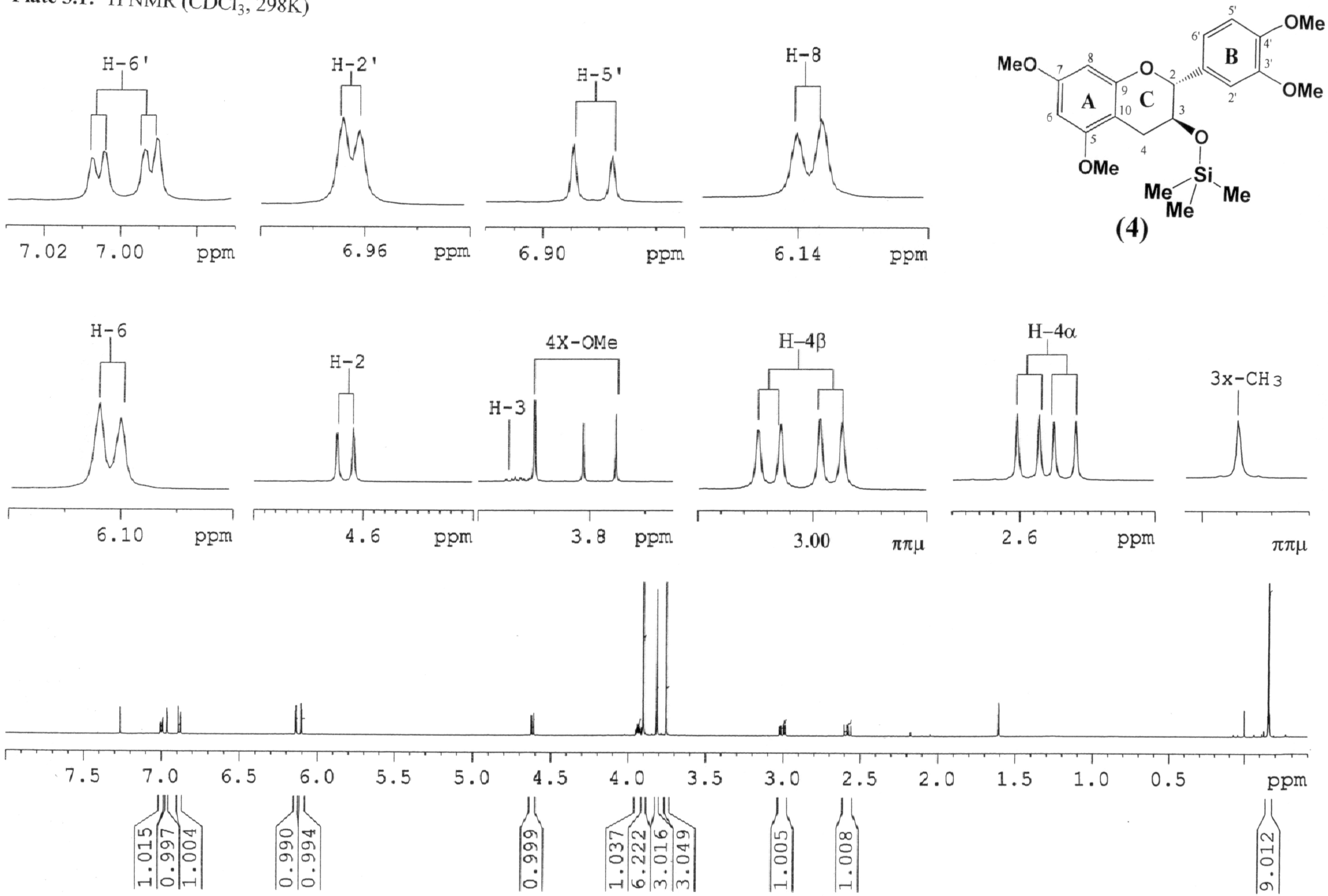
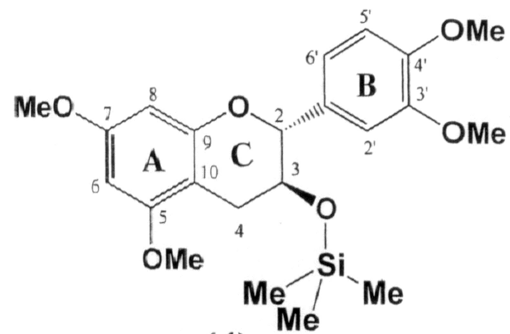


**Plate 3.1:** $^{1}H$ NMR ($CDCl_3$, 298K)




**(4)**

Plate 3.2: $^{13}C$ NMR ($CDCl_3$, 298K)



(4)

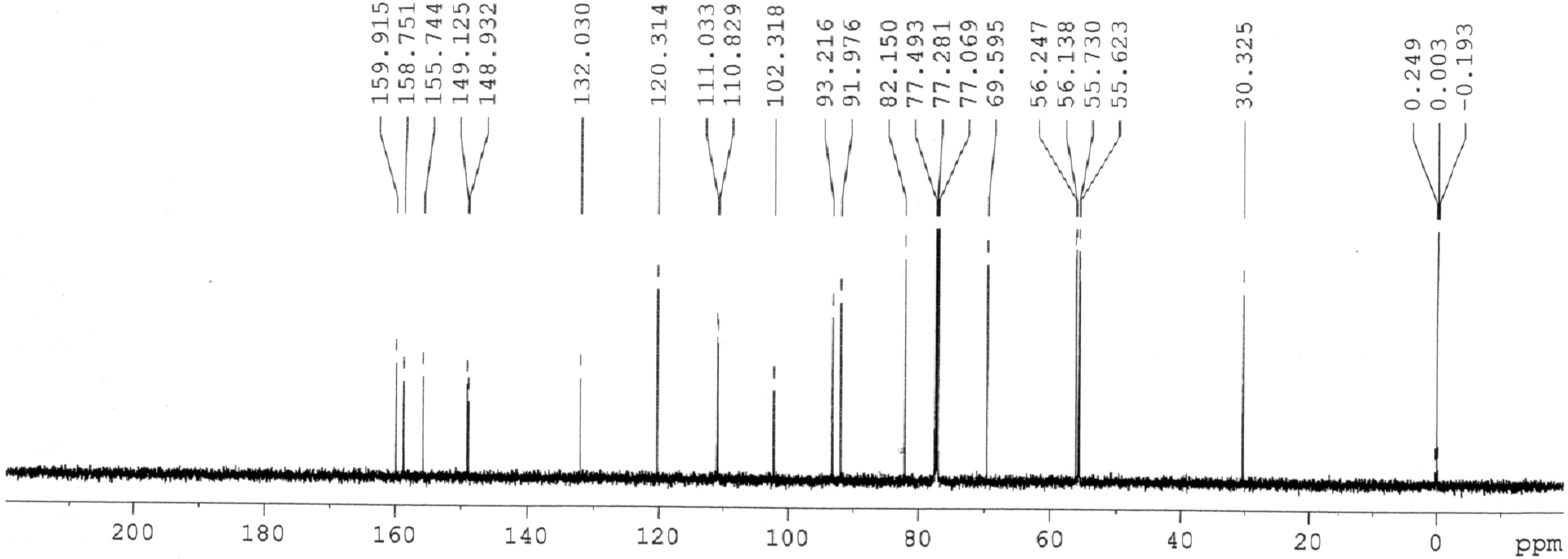
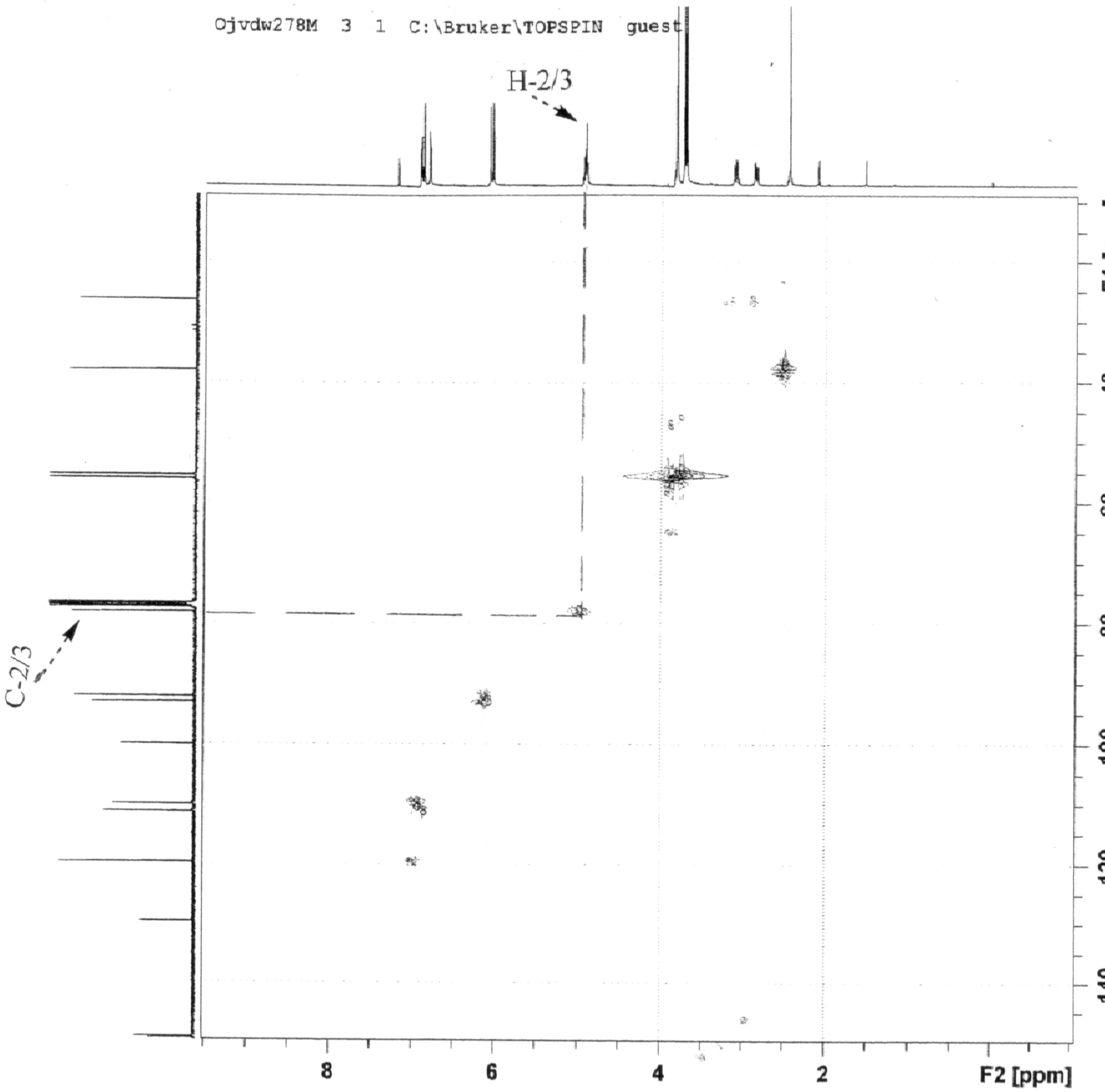
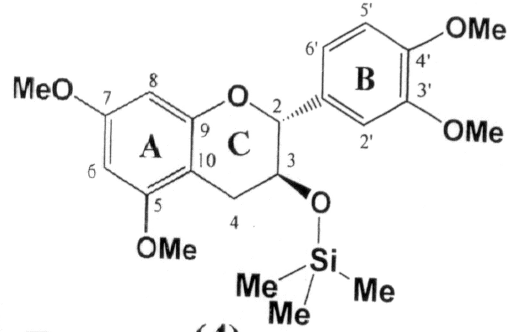


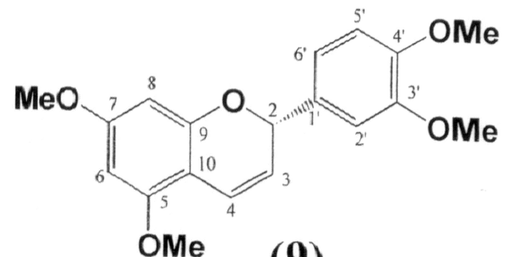
**Plate 3.3:** HSQC NMR ($CDCl_3$, 298K)




**(4)**

**Plate 4.1:** $^{1}$H NMR ($CDCl_3$, 298K)




(9)

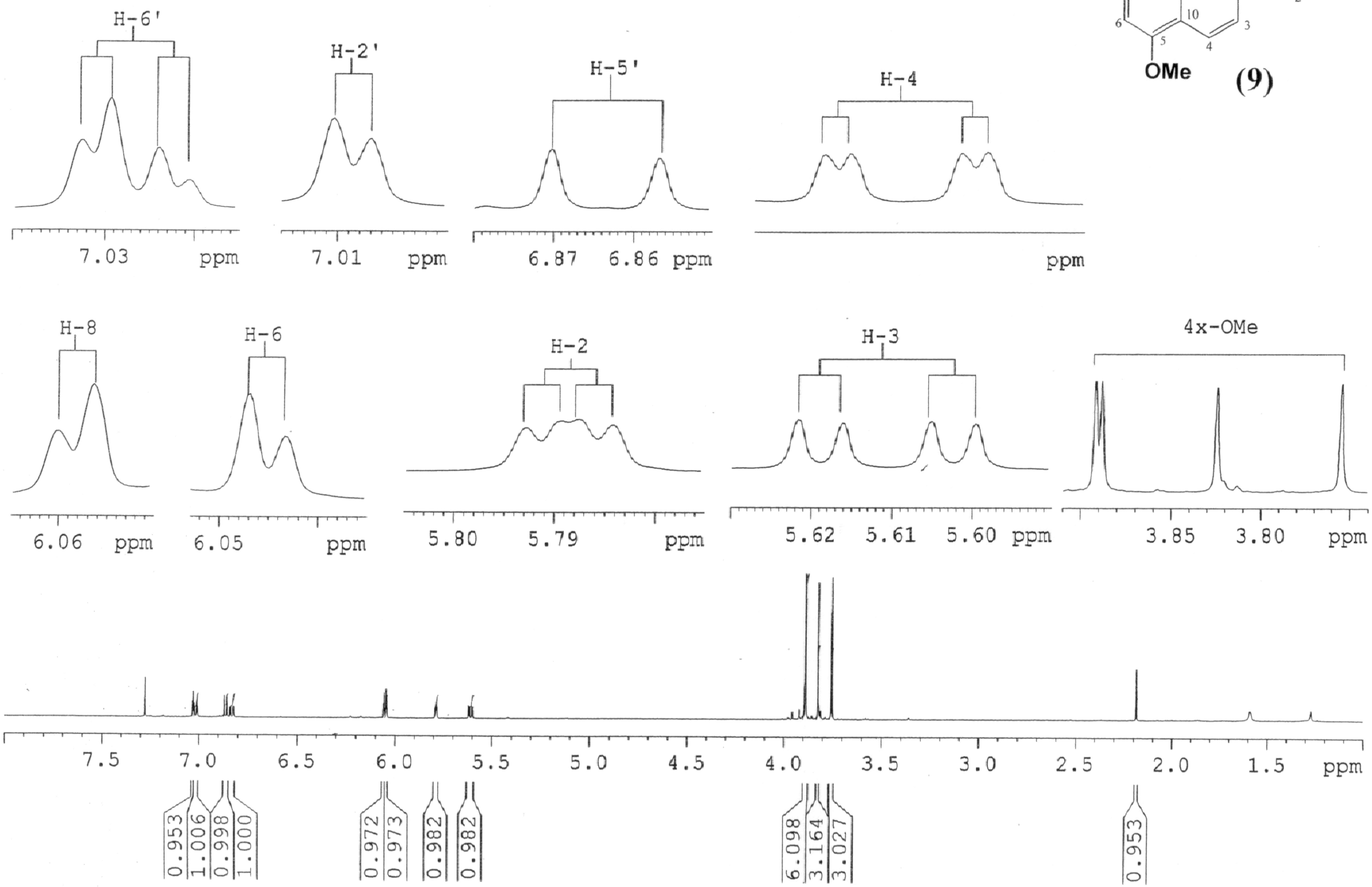
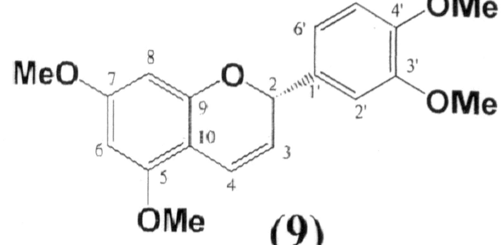


Plate 4.2 $^{13}C$ NMR ($CDCl_3$, 298K)




(9)

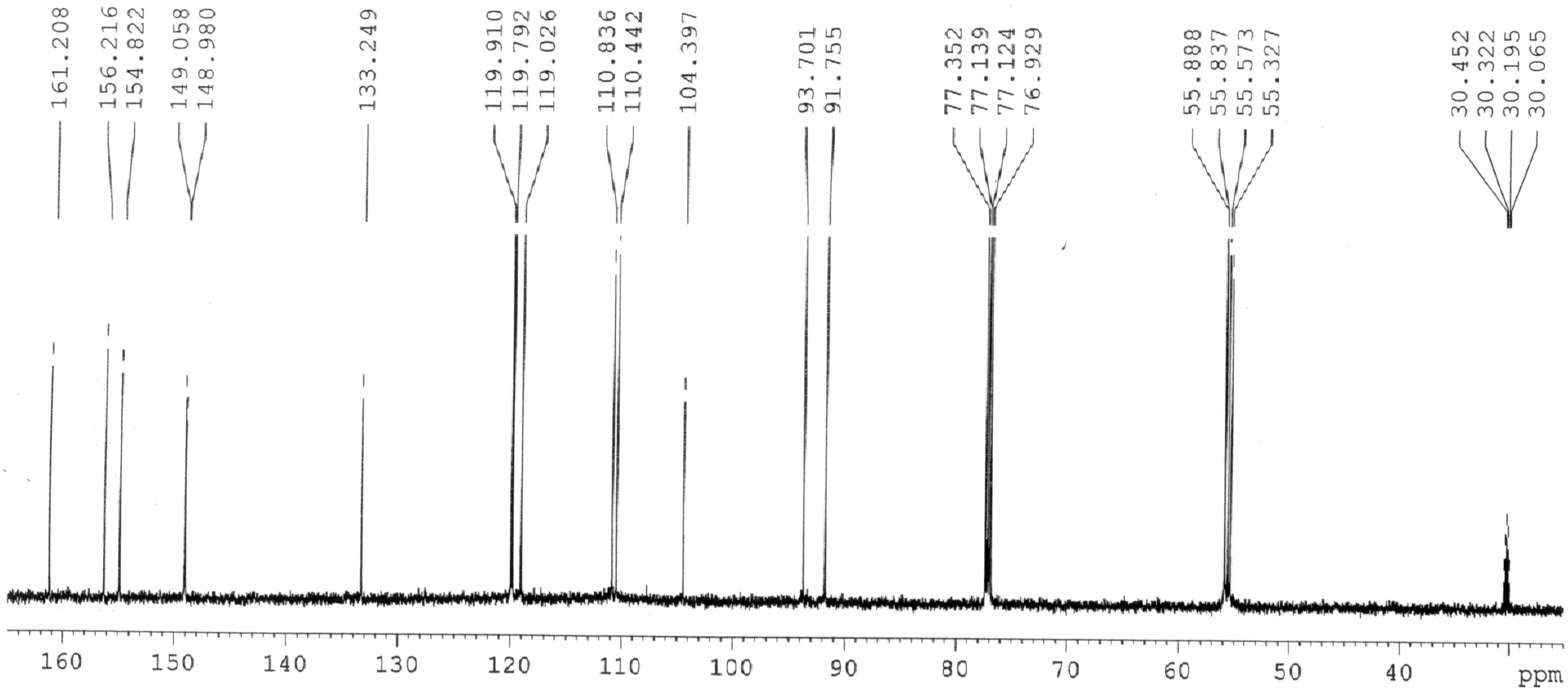


**Plate 4.3**: APT-NMR ($CDCl_3$, 298K)

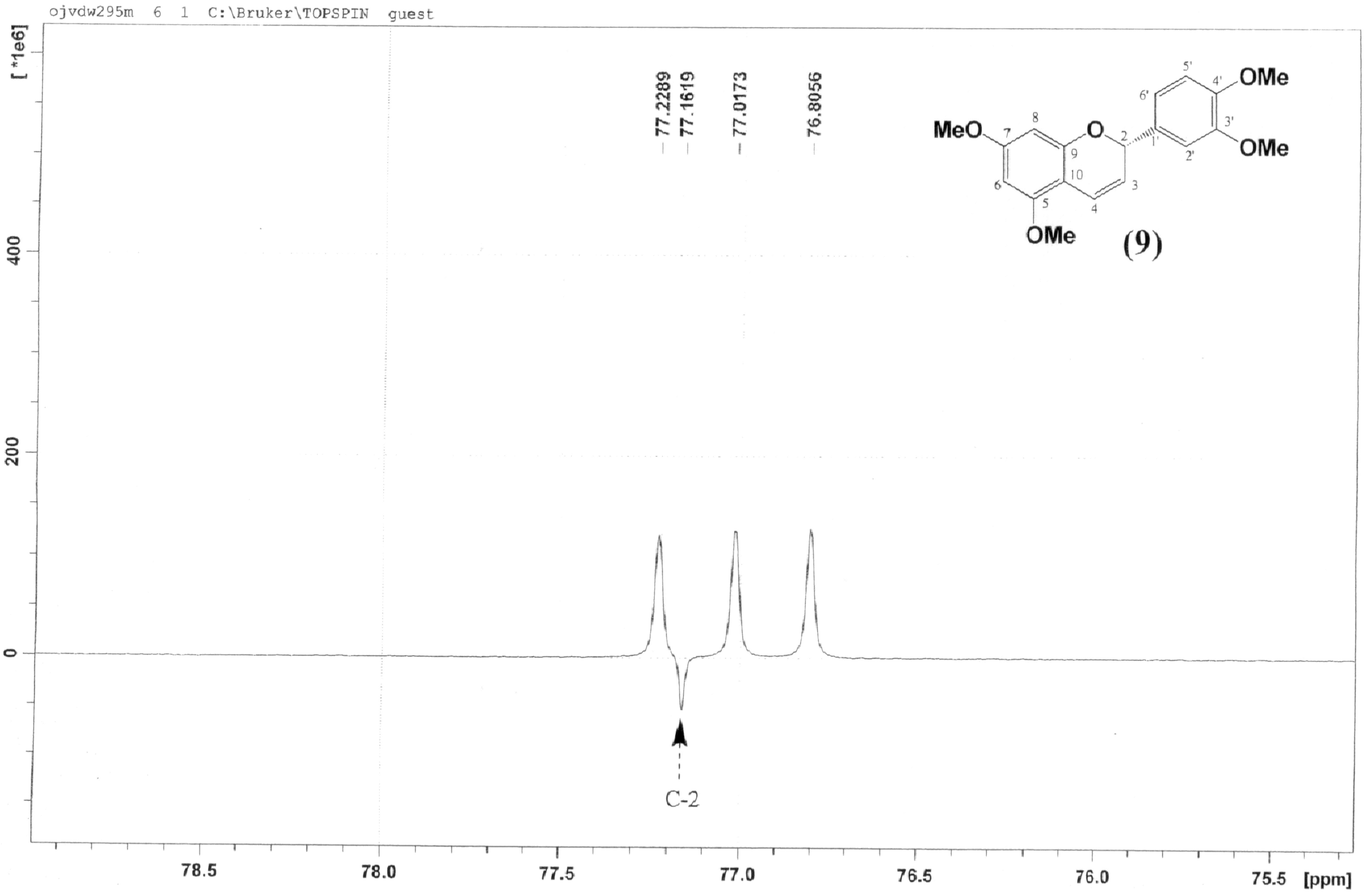
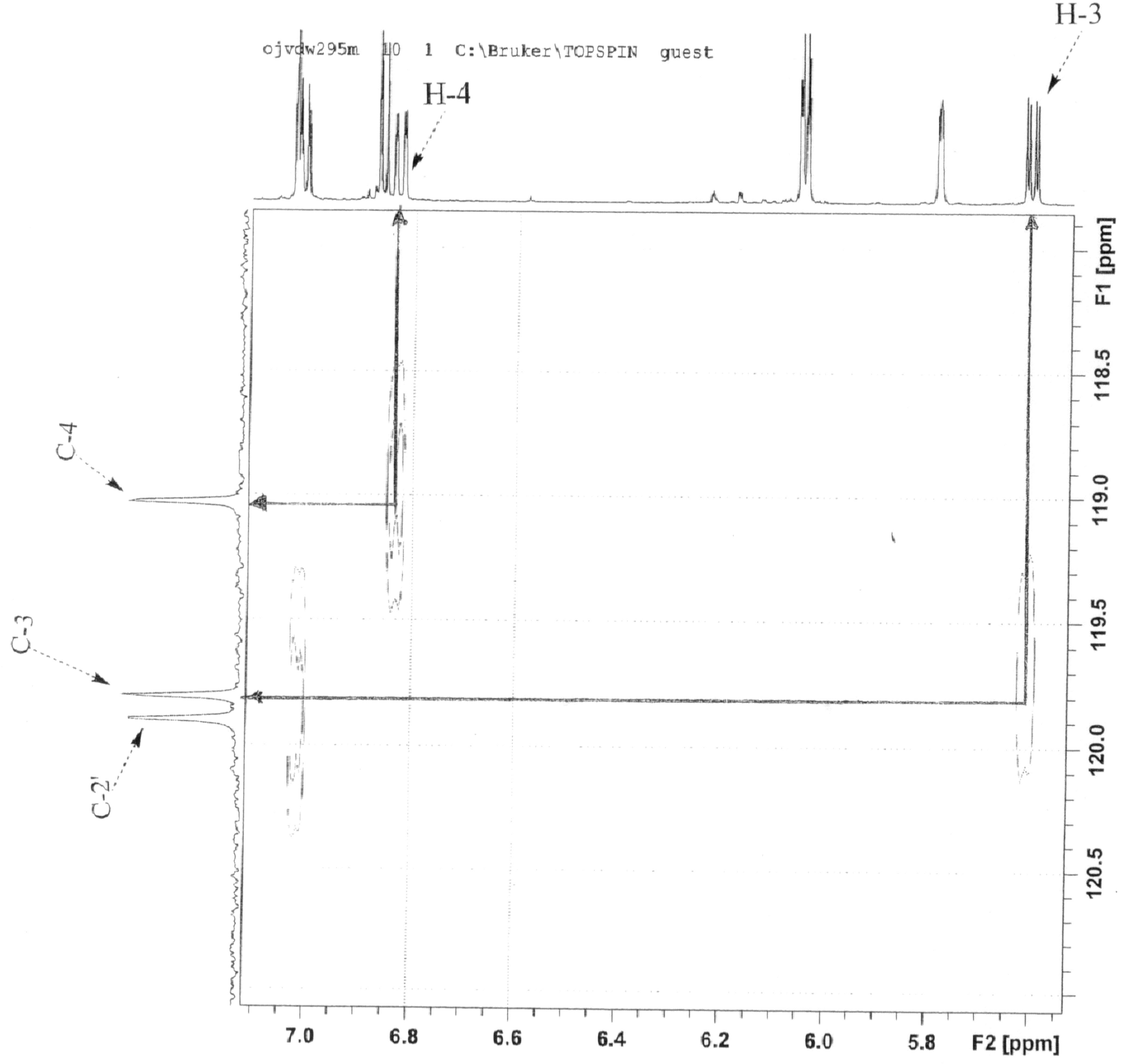
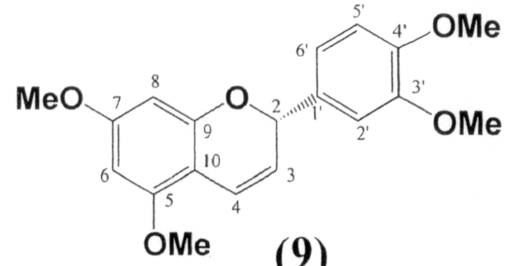


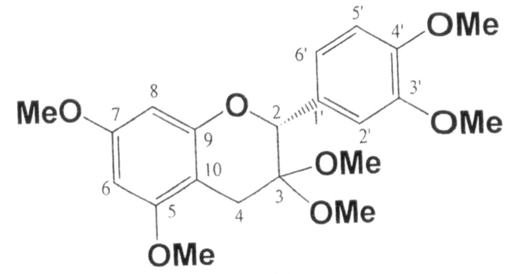
**Plate 4.5:** HSQC NMR ($CDCl_3$, 298K)




**(9)**

Plate 7.2: $^{13}C$ NMR ($CDCl_3$, 298K)




**(22)**

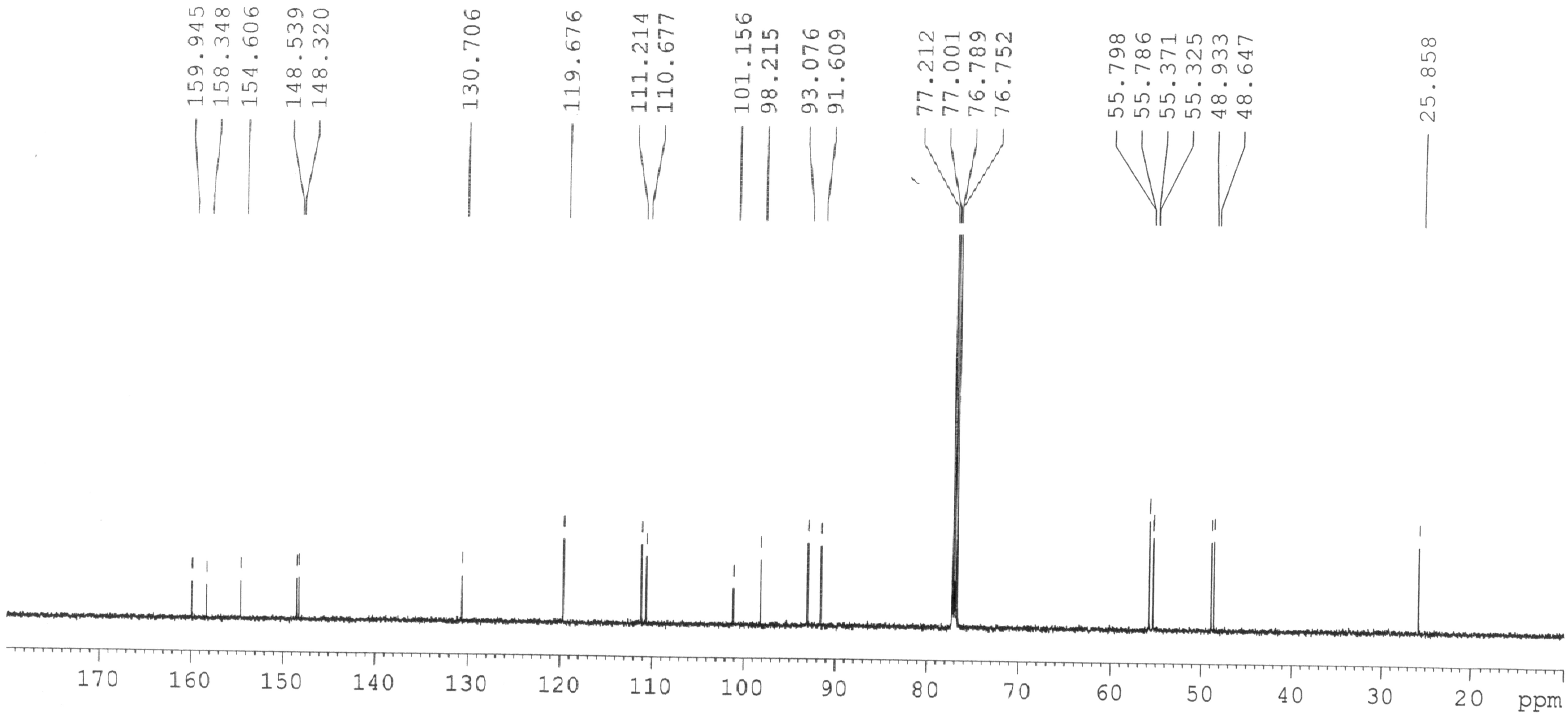
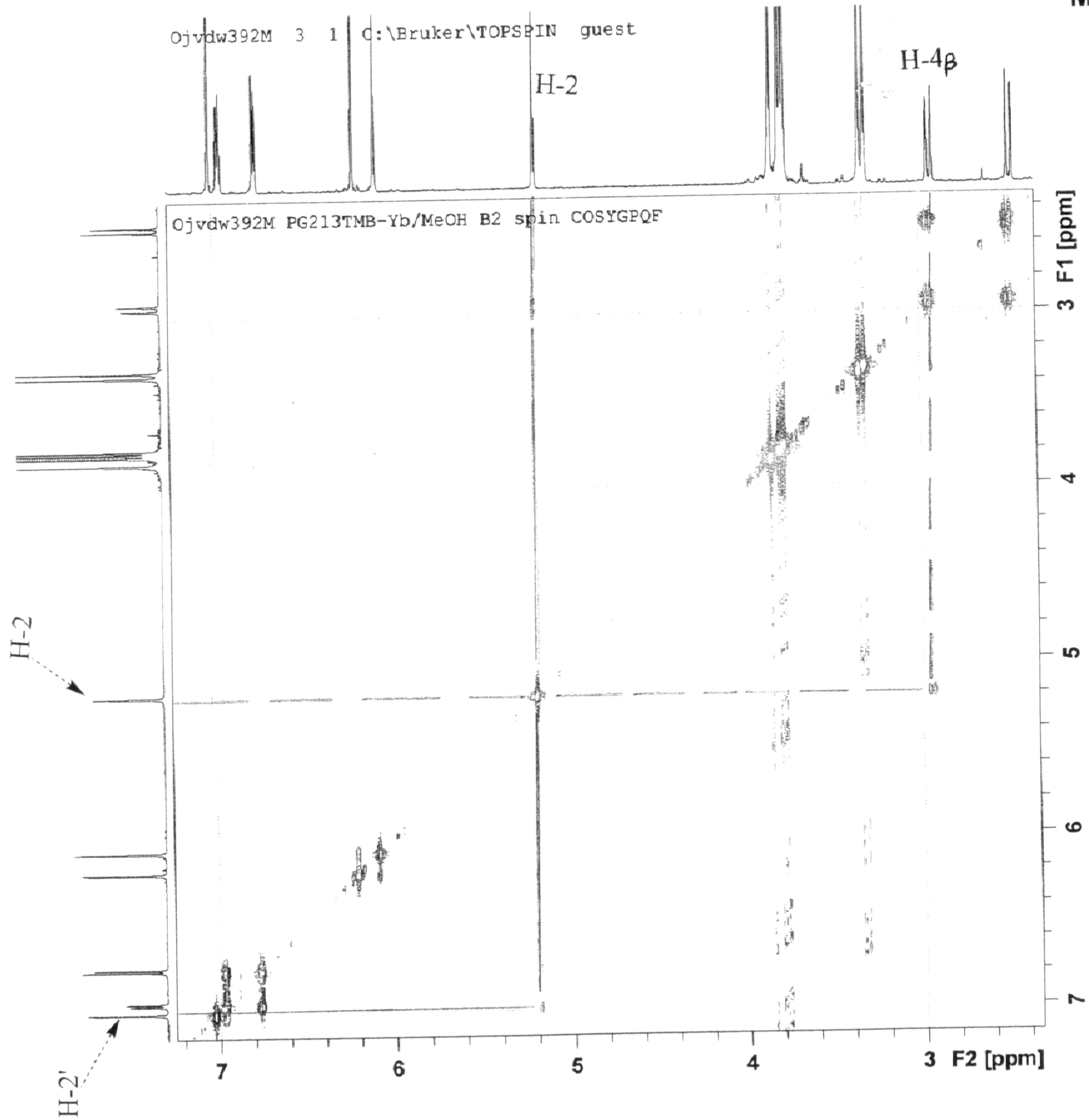
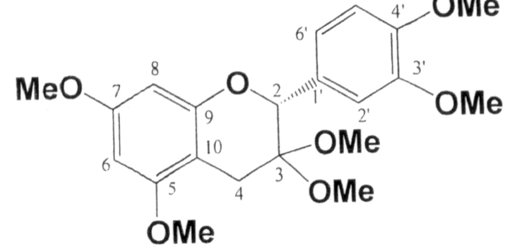


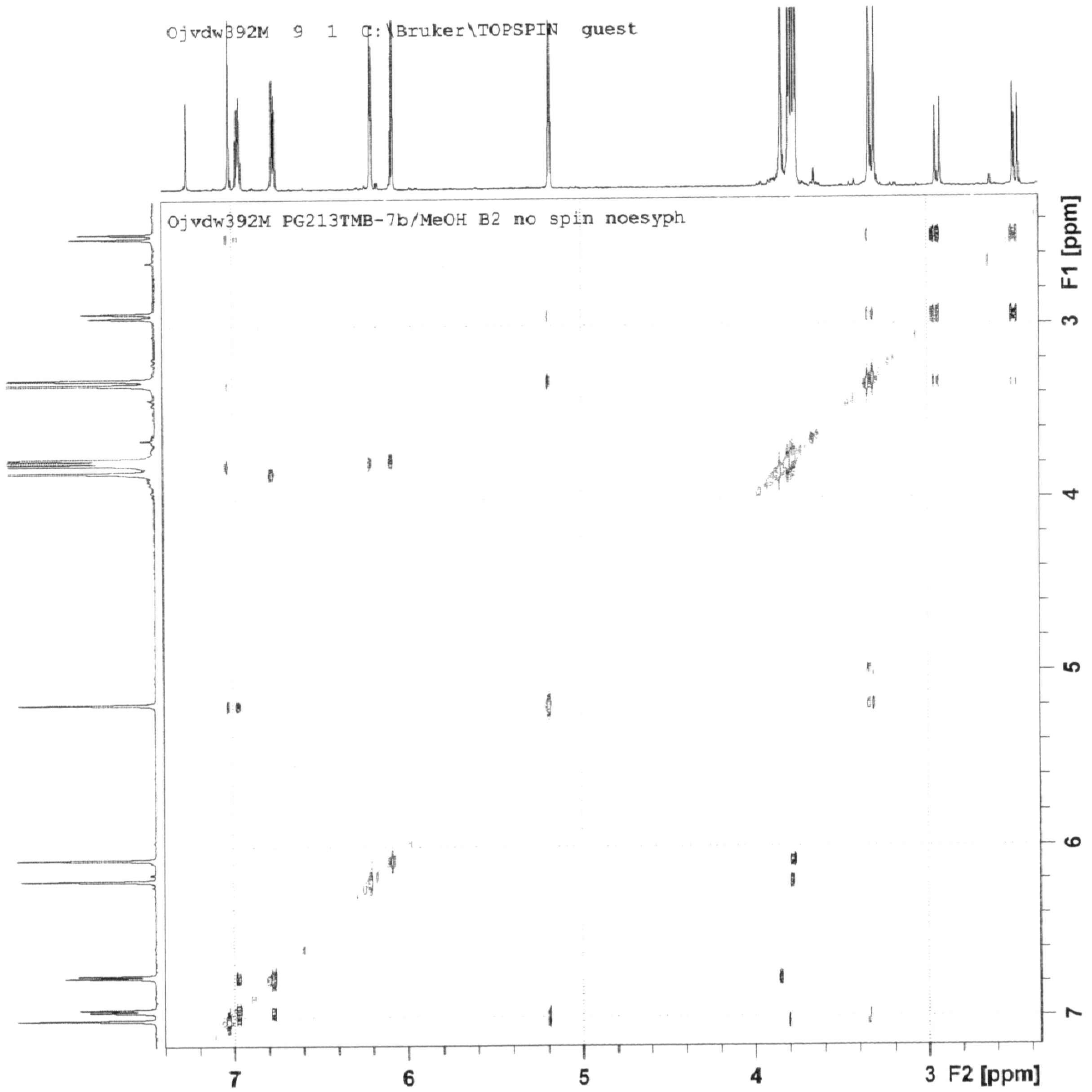
Plate 7.3: COSY NMR ($CDCl_3$, 298K)

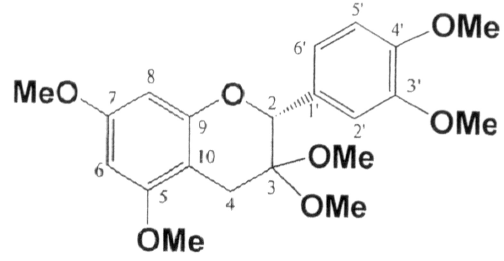


(22)

**Plate 7.4:** NOESY NMR ($CDCl_3$, 298K)

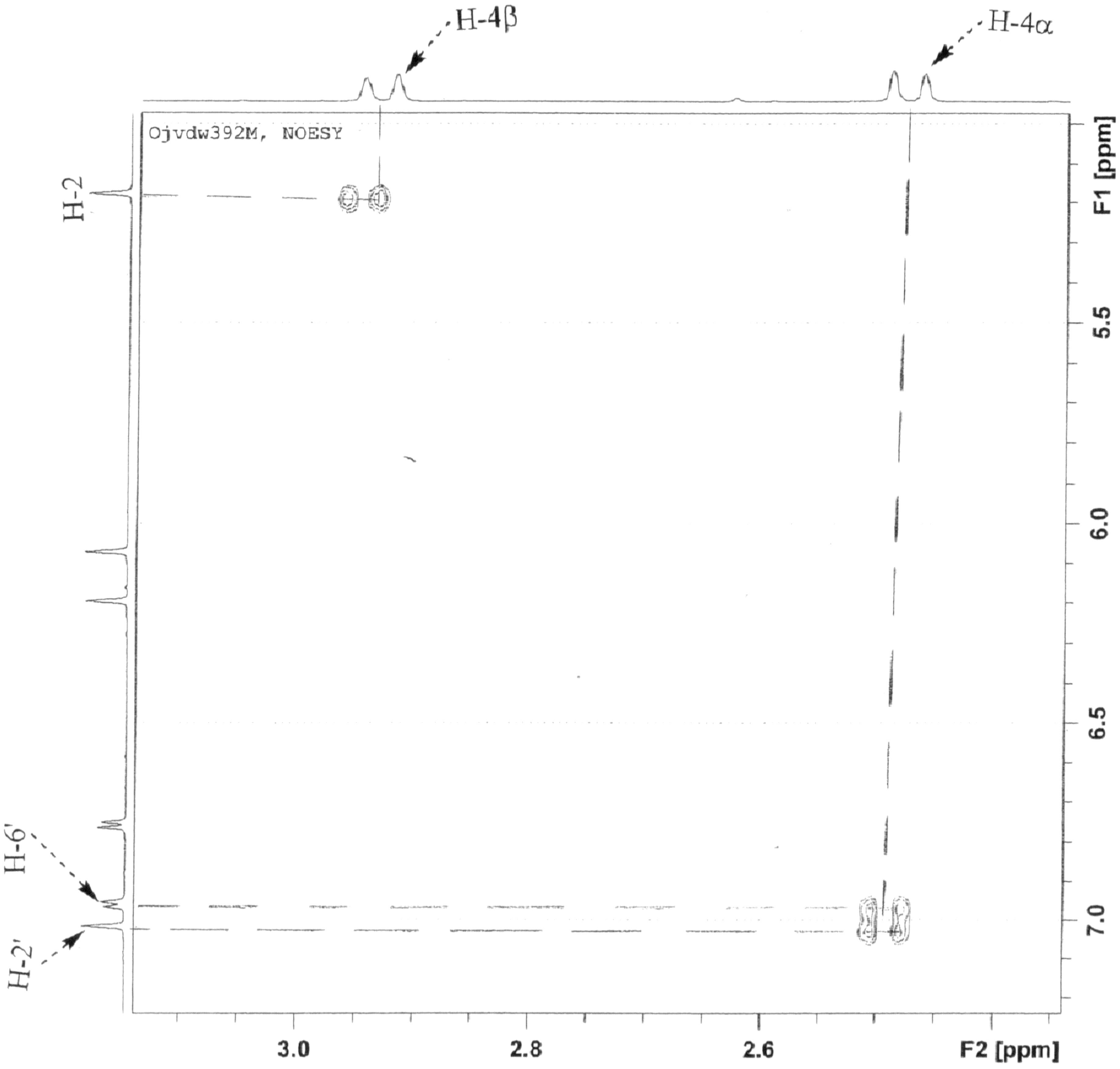


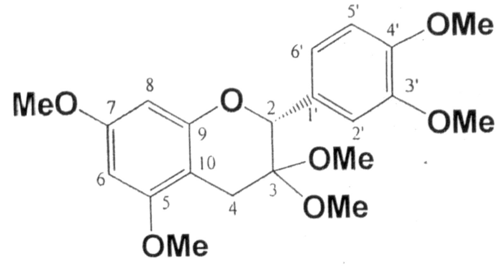




**(22)**

**Plate 7.5**: NOESY NMR ($CDCl_3$, 298K)

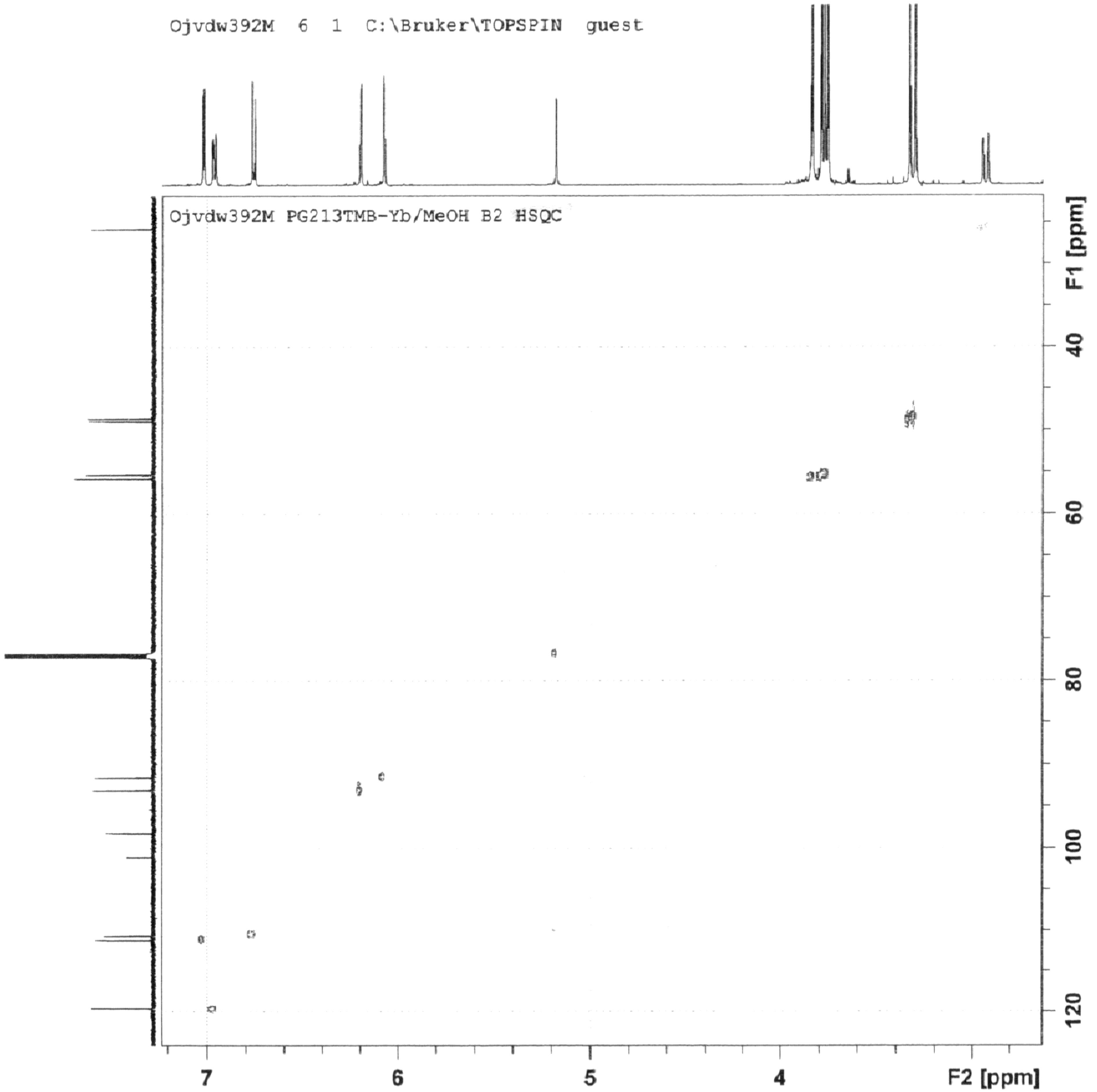
Ojvdw392M 9 1 C:\Bruker\TOPSPIN guest

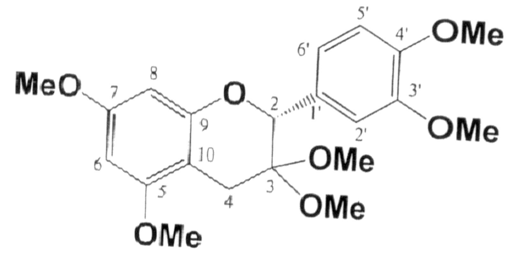



**(22)**

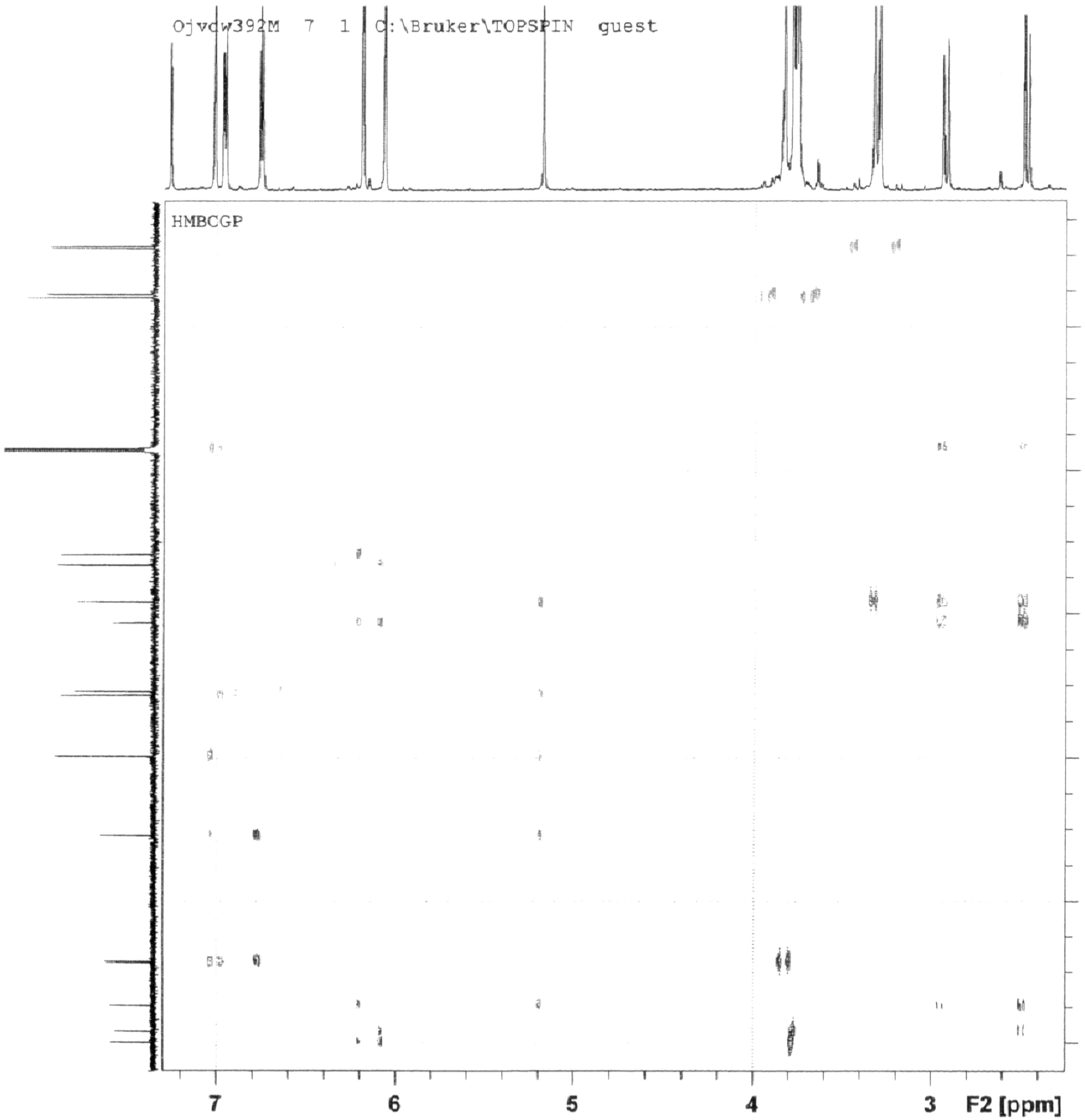
**Plate 7.6:** HSQC NMR (CDCl$_3$, 298K)

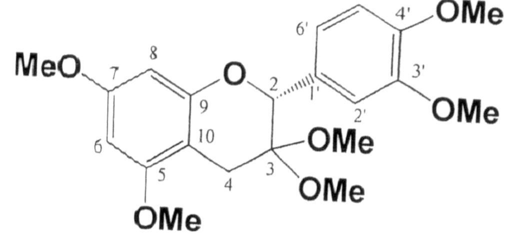



**(22)**

Plate 7.7: HMBC NMR ($CDCl_3$, 298K)




**(22)**

**Plate 8.1:** $^1H$ NMR (DMSO, 298K)

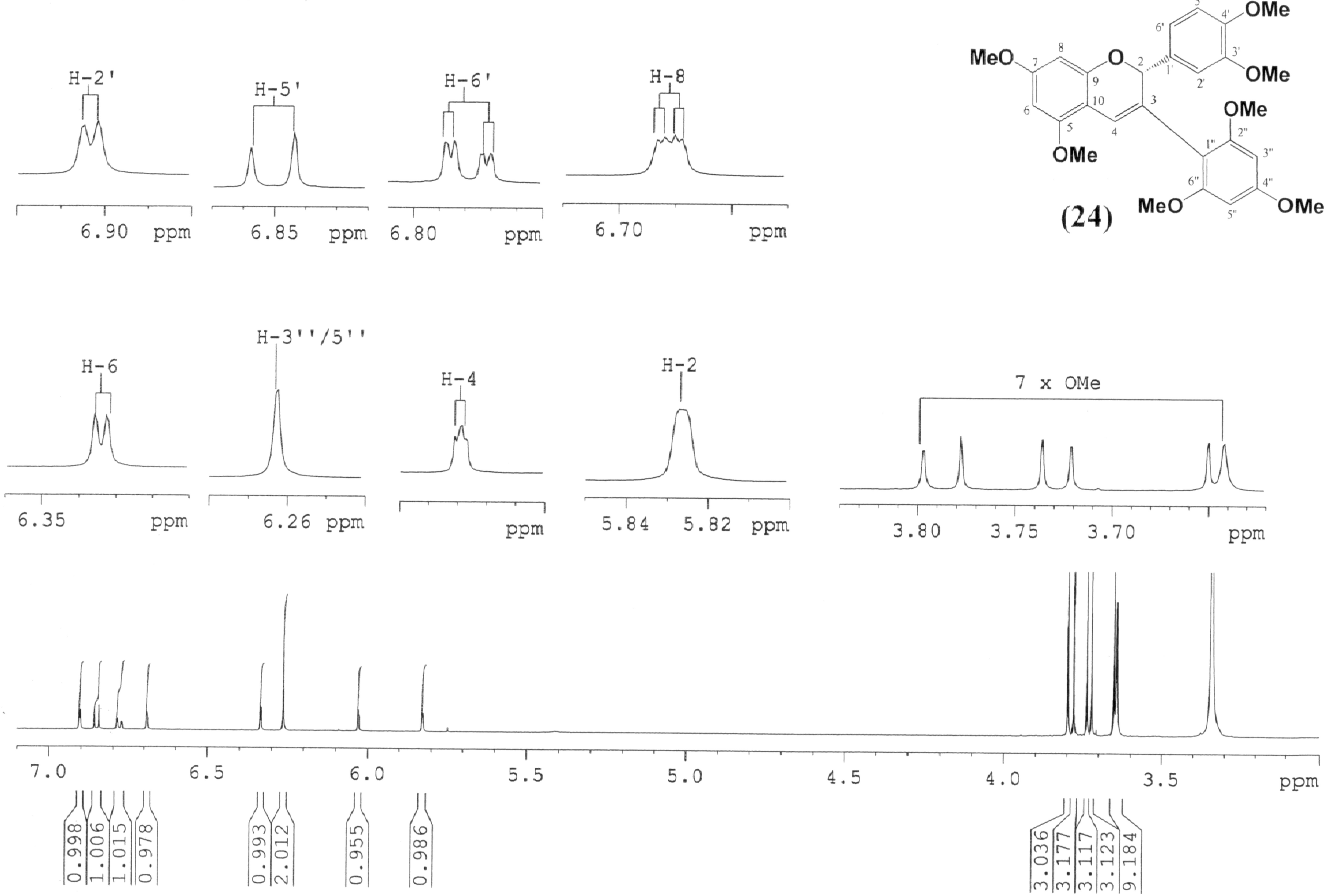
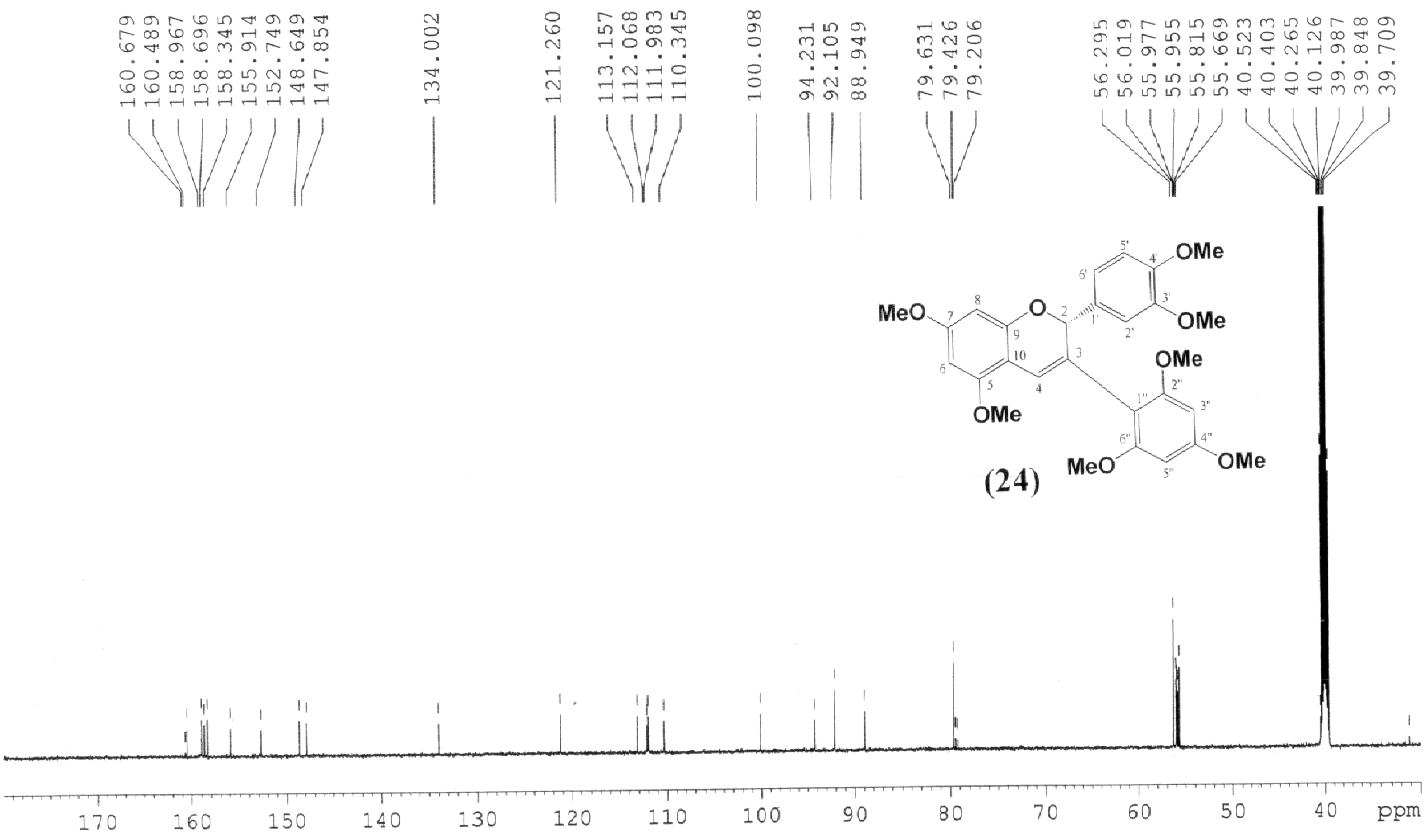
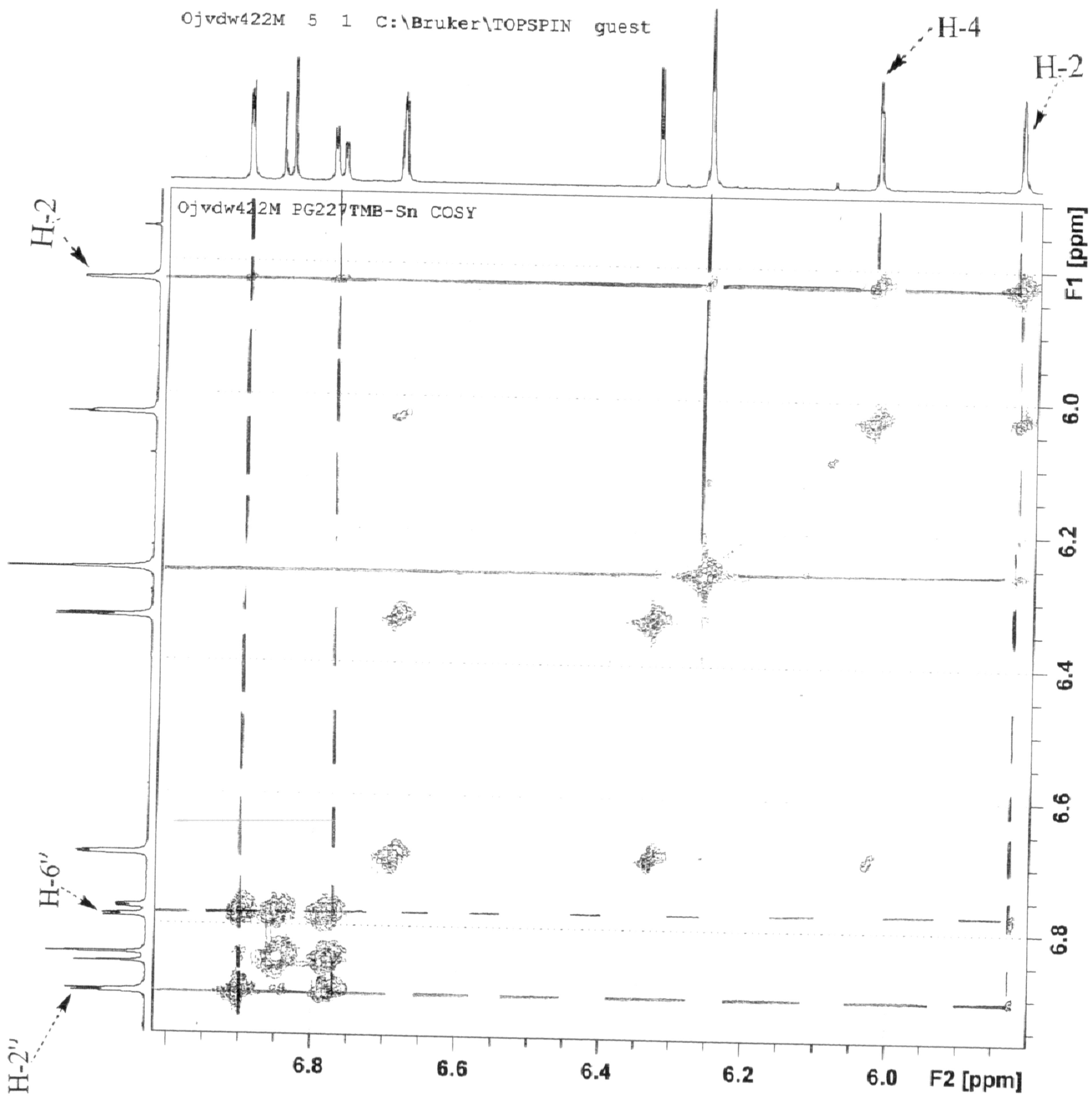


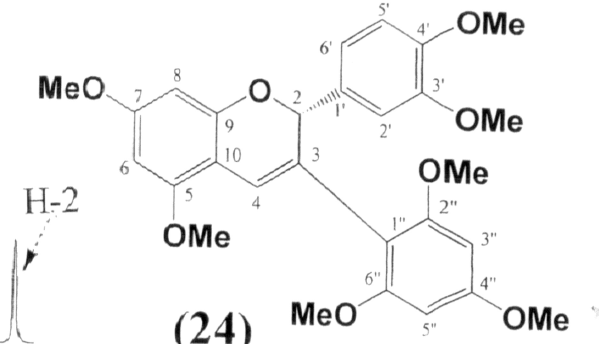
**Plate 8.2:** $^{13}C$ NMR (DMSO, 298K)




(24)

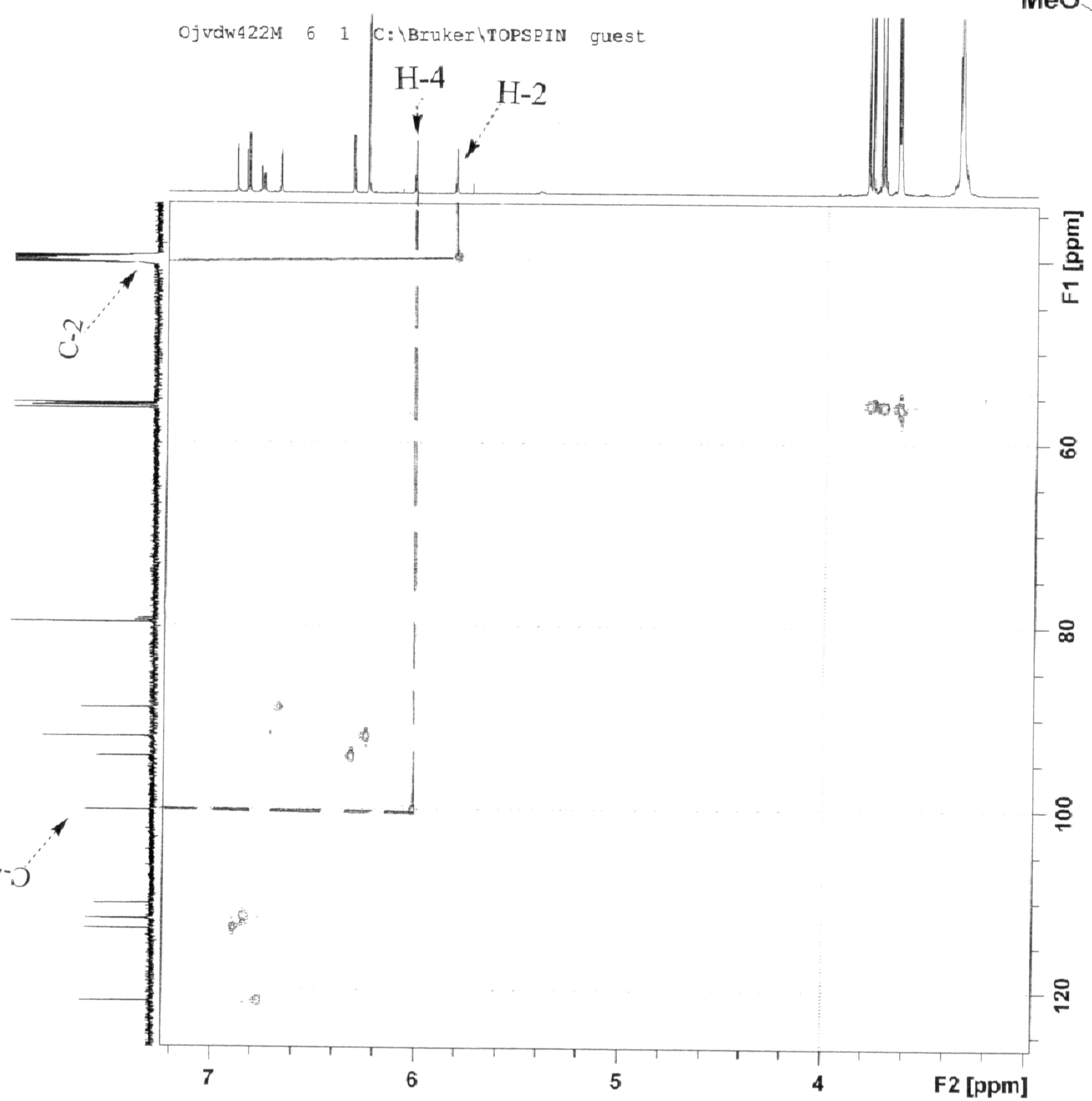
**Plate 8.3**: COSY NMR (DMSO, 298K)

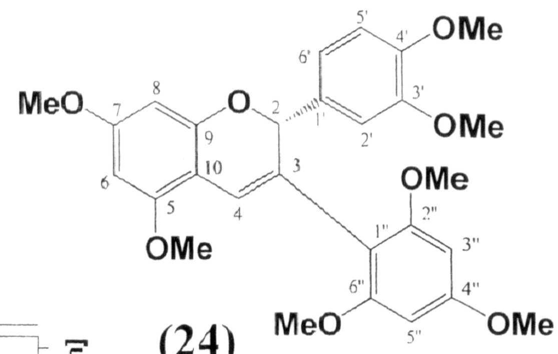


**(24)**

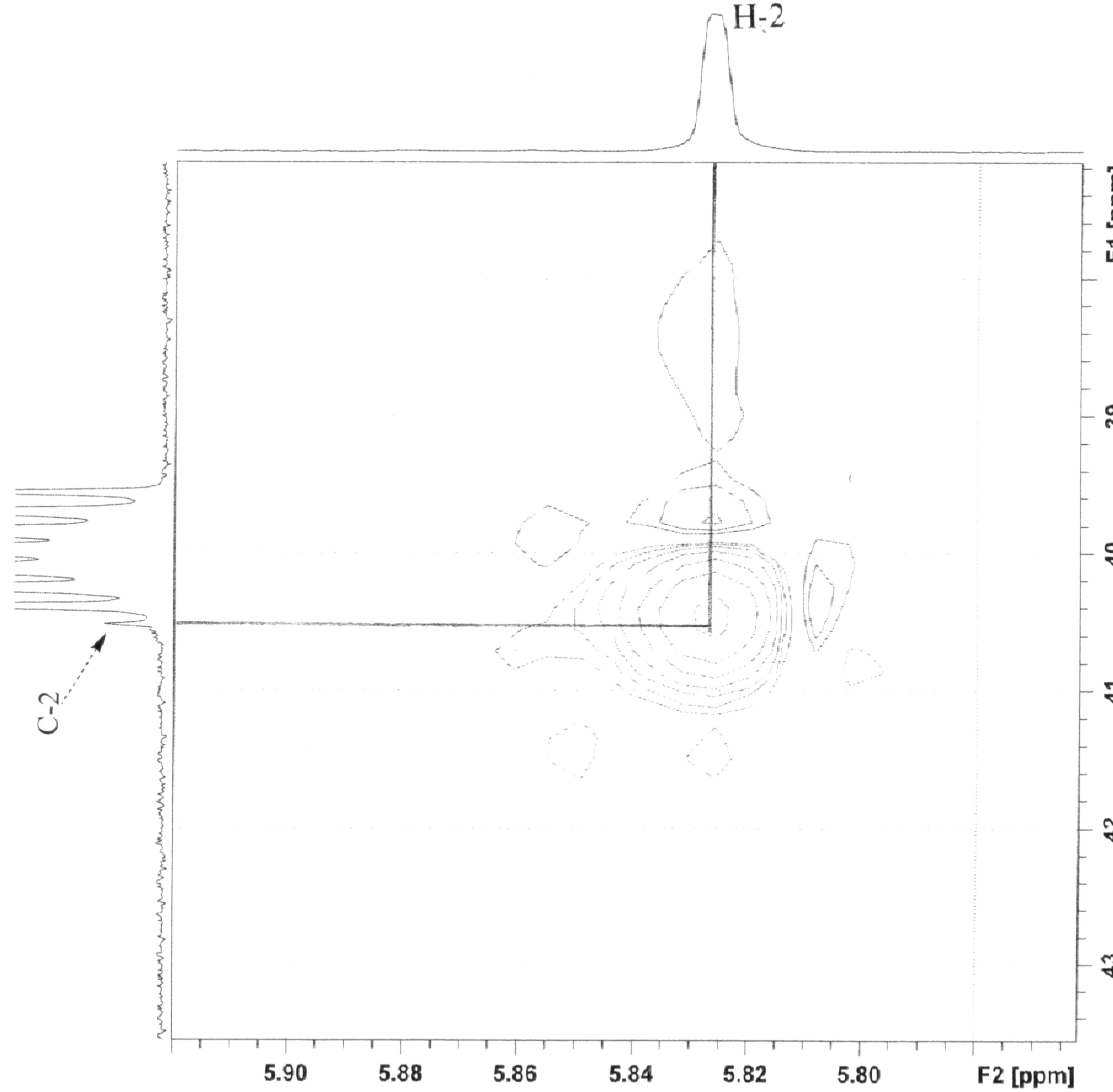
**Plate 8.4:** HSQC NMR (DMSO, 298K)

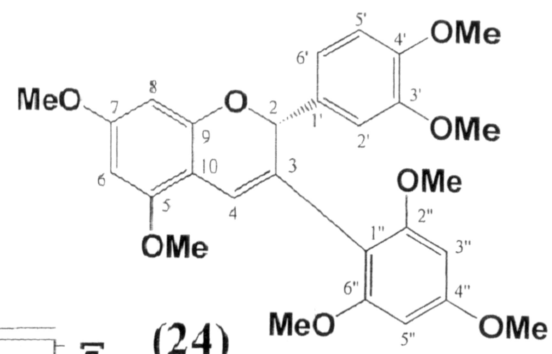



**(24)**

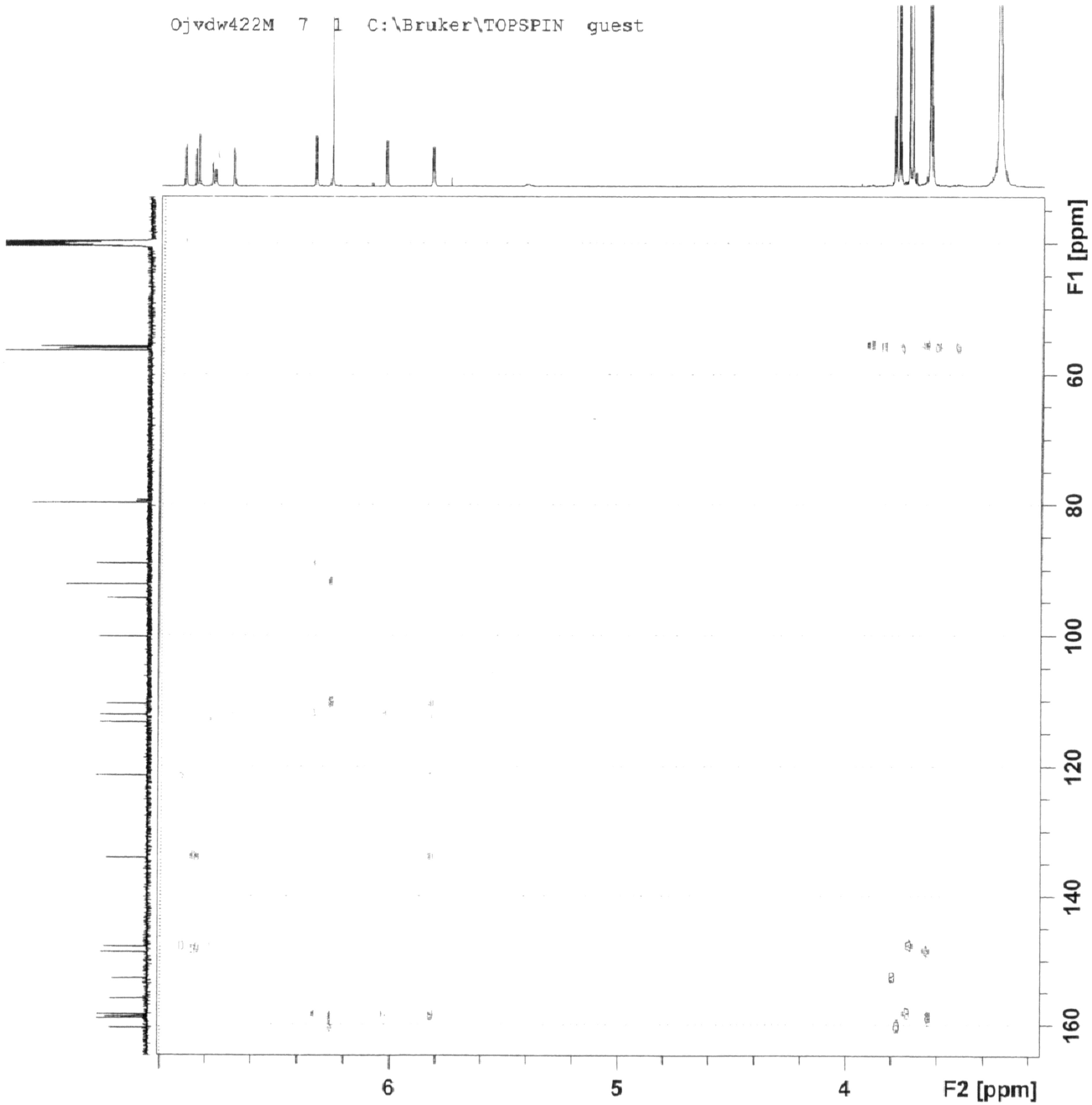
**Plate 8.5:** HSQC NMR (DMSO, 298K)

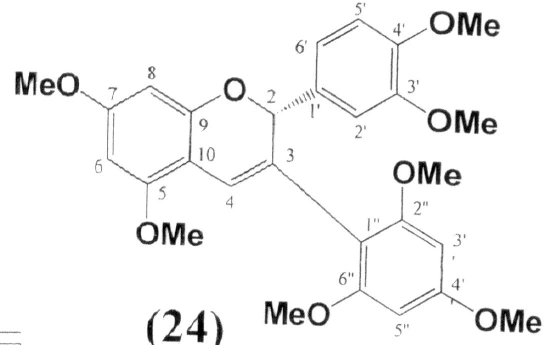



**(24)**

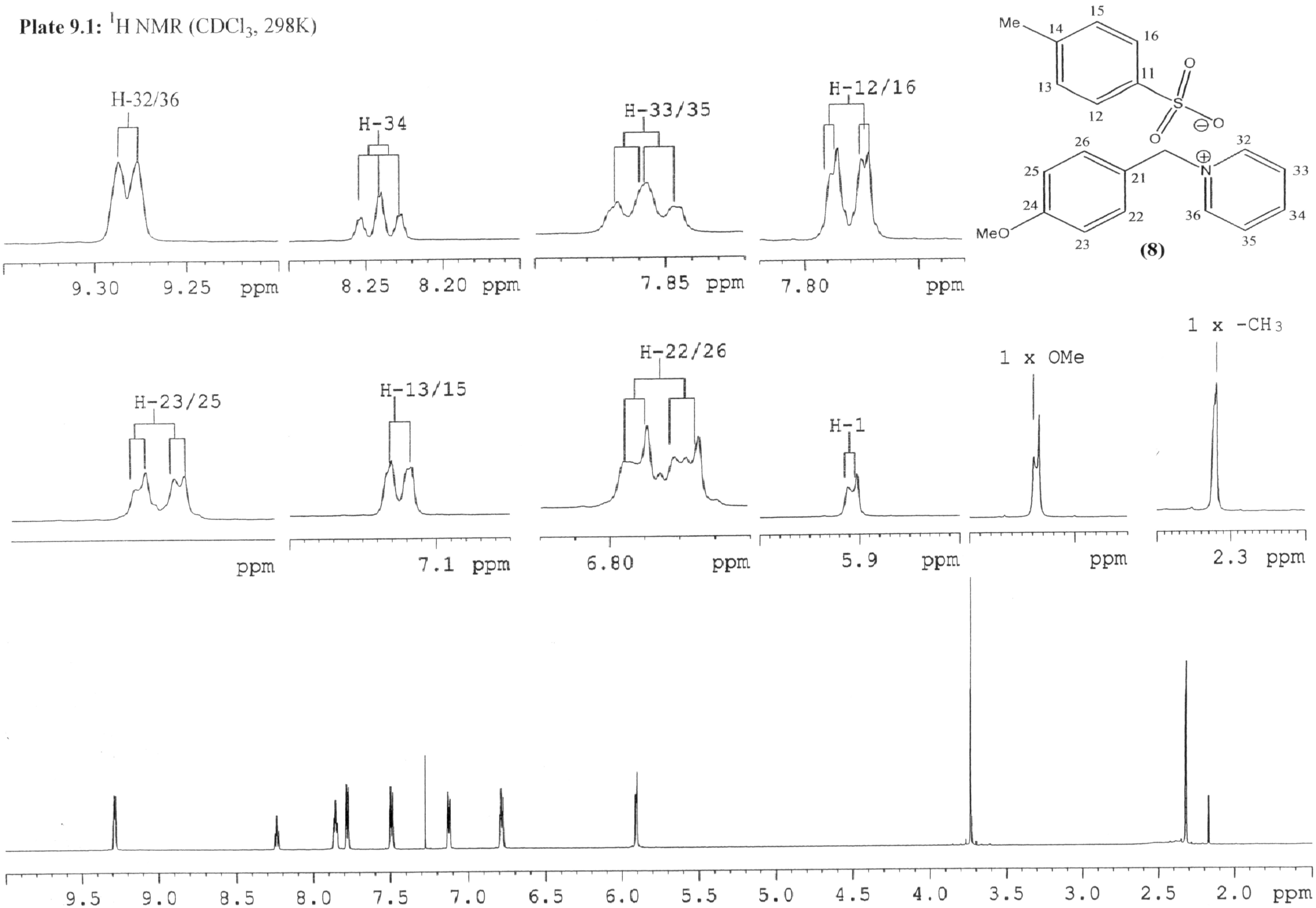
Plate 8.6: HMBC NMR (DMSO, 298K)




(24)

**Plate 9.1:** $^1$H NMR ($CDCl_3$, 298K)




**(8)**

**Plate 9.2** $^{13}C$ NMR ($CDCl_3$, 298K)

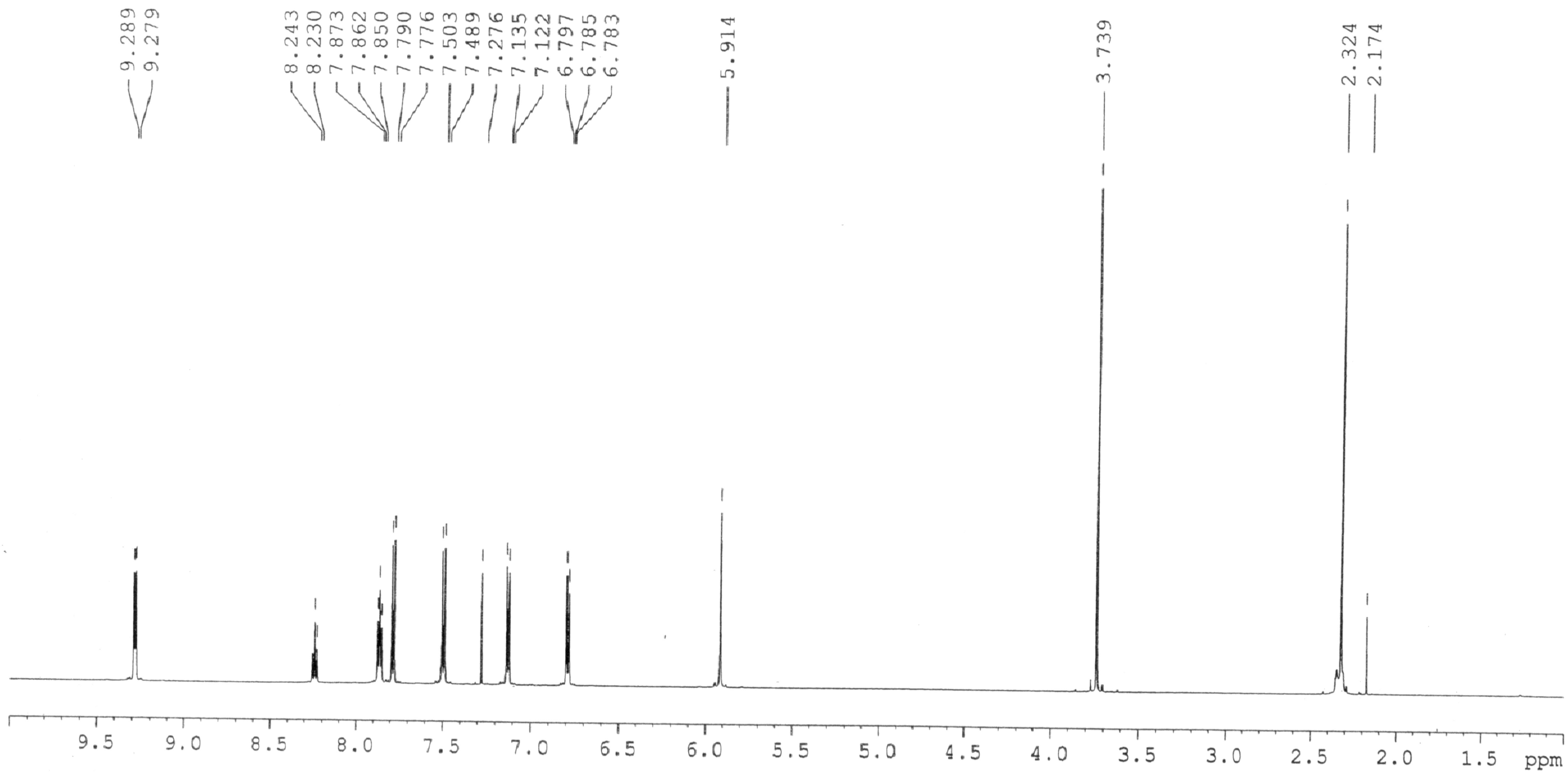
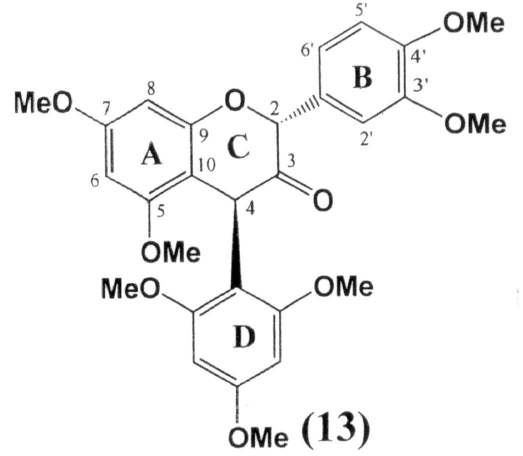
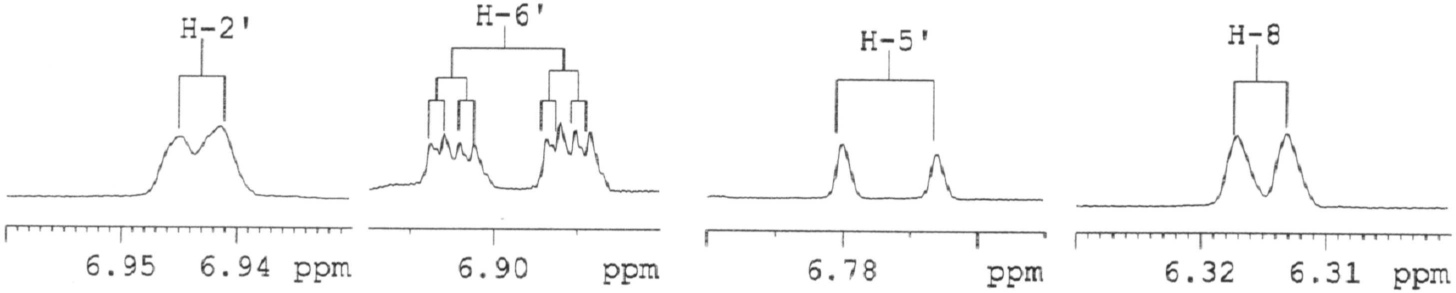


**Plate** 10.1: $^1$H NMR ($CDCl_3$, 298K)



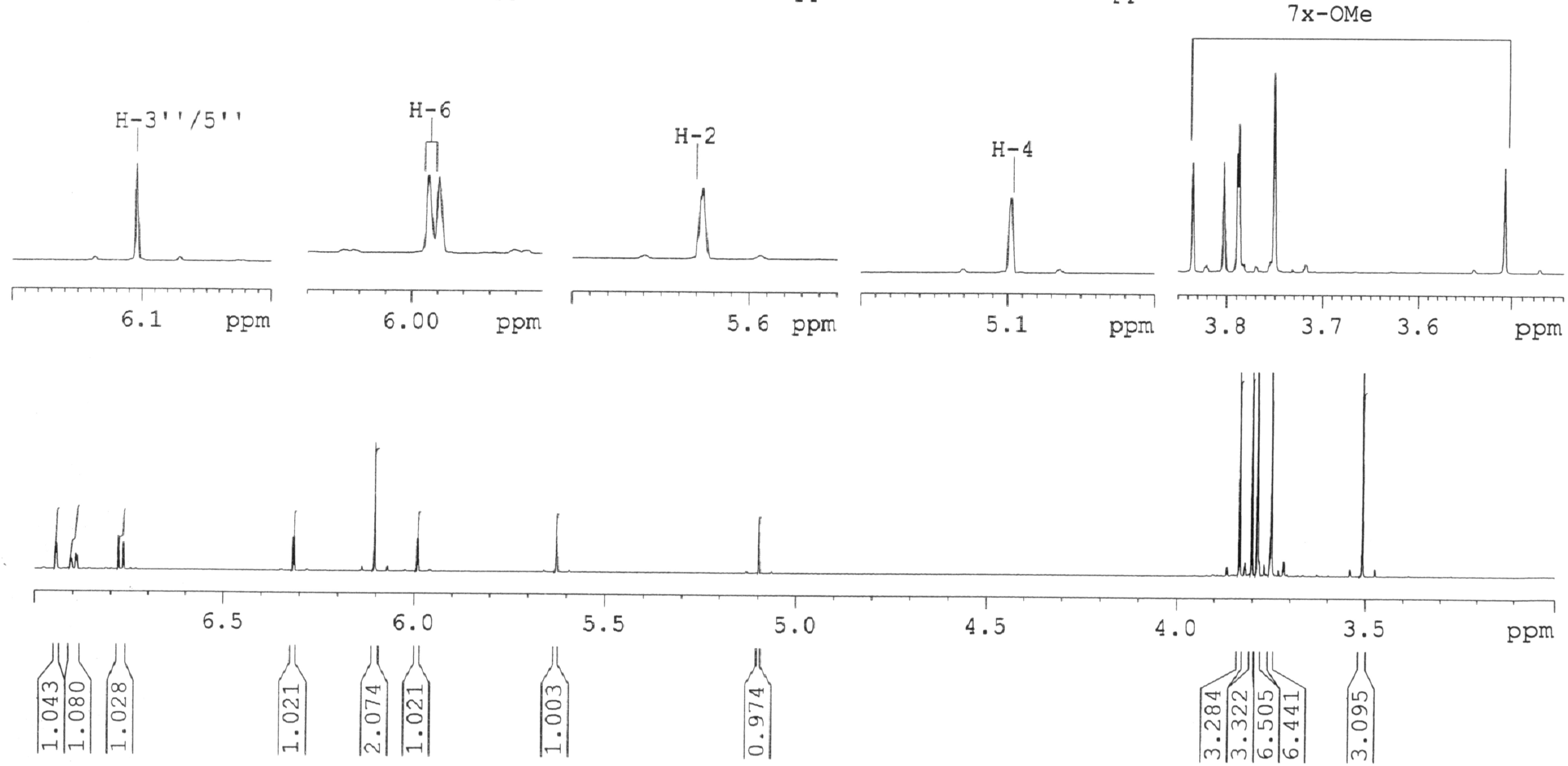
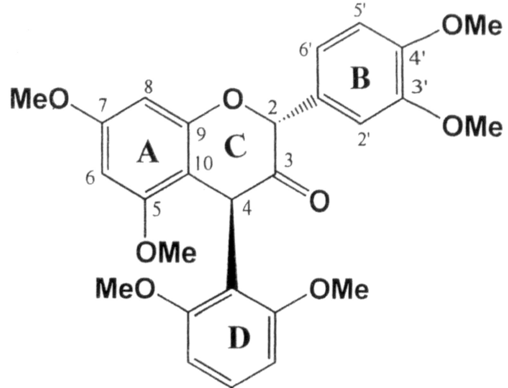



Plate 10.2: $^{13}C$ NMR ($CDCl_3$, 298K)




(13)

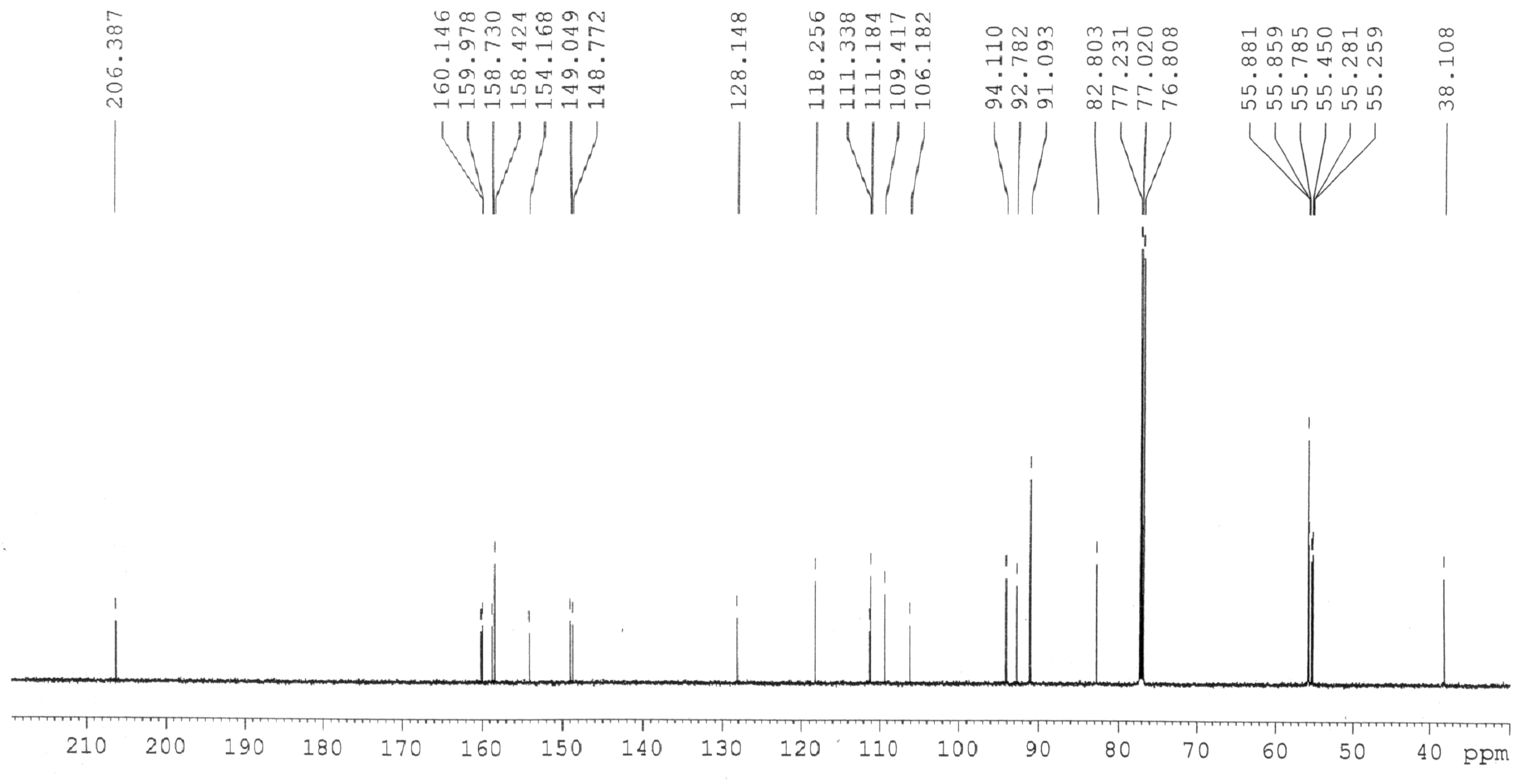
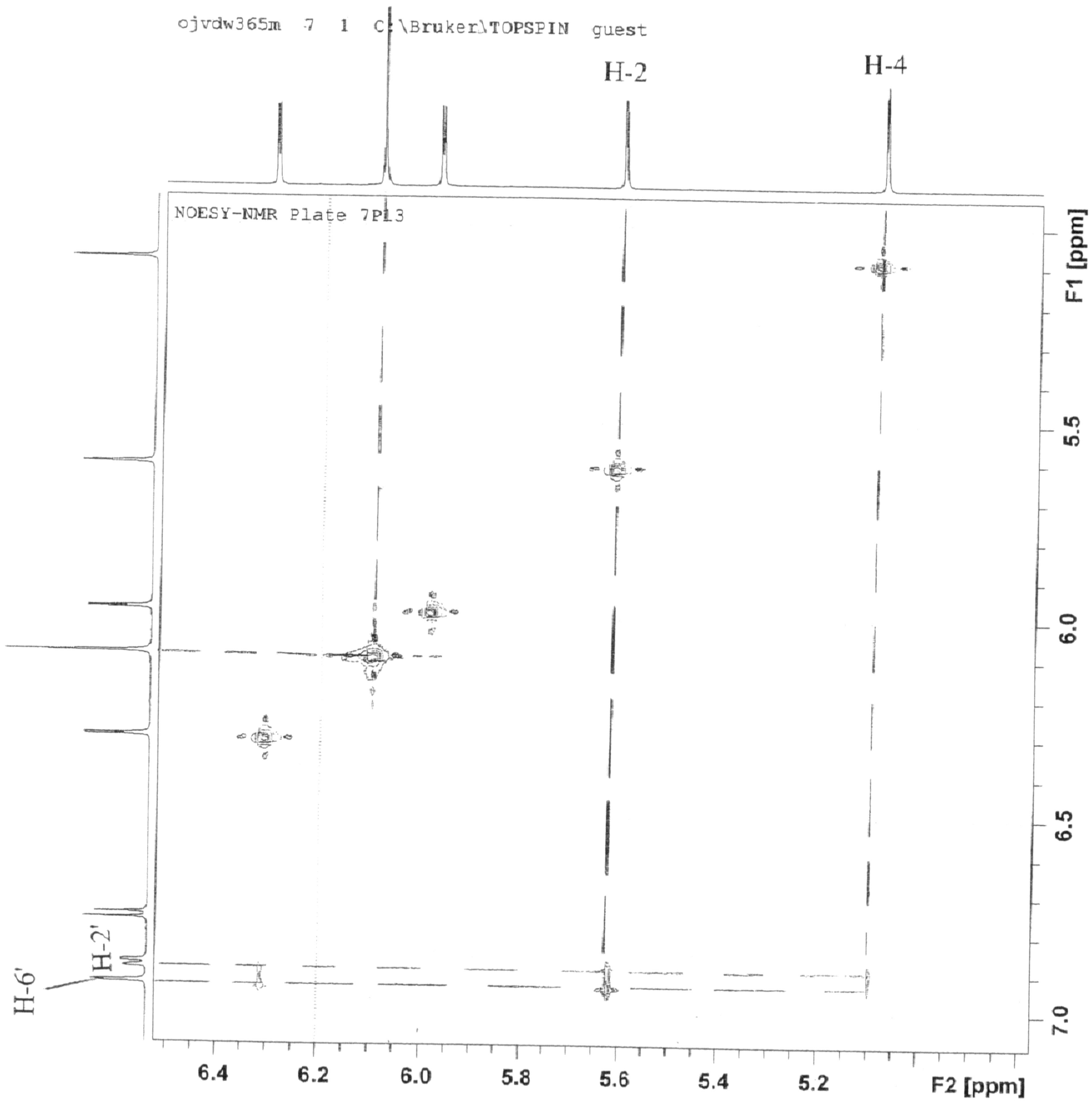
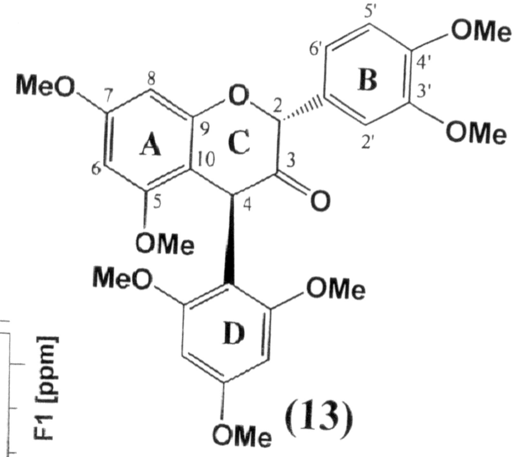


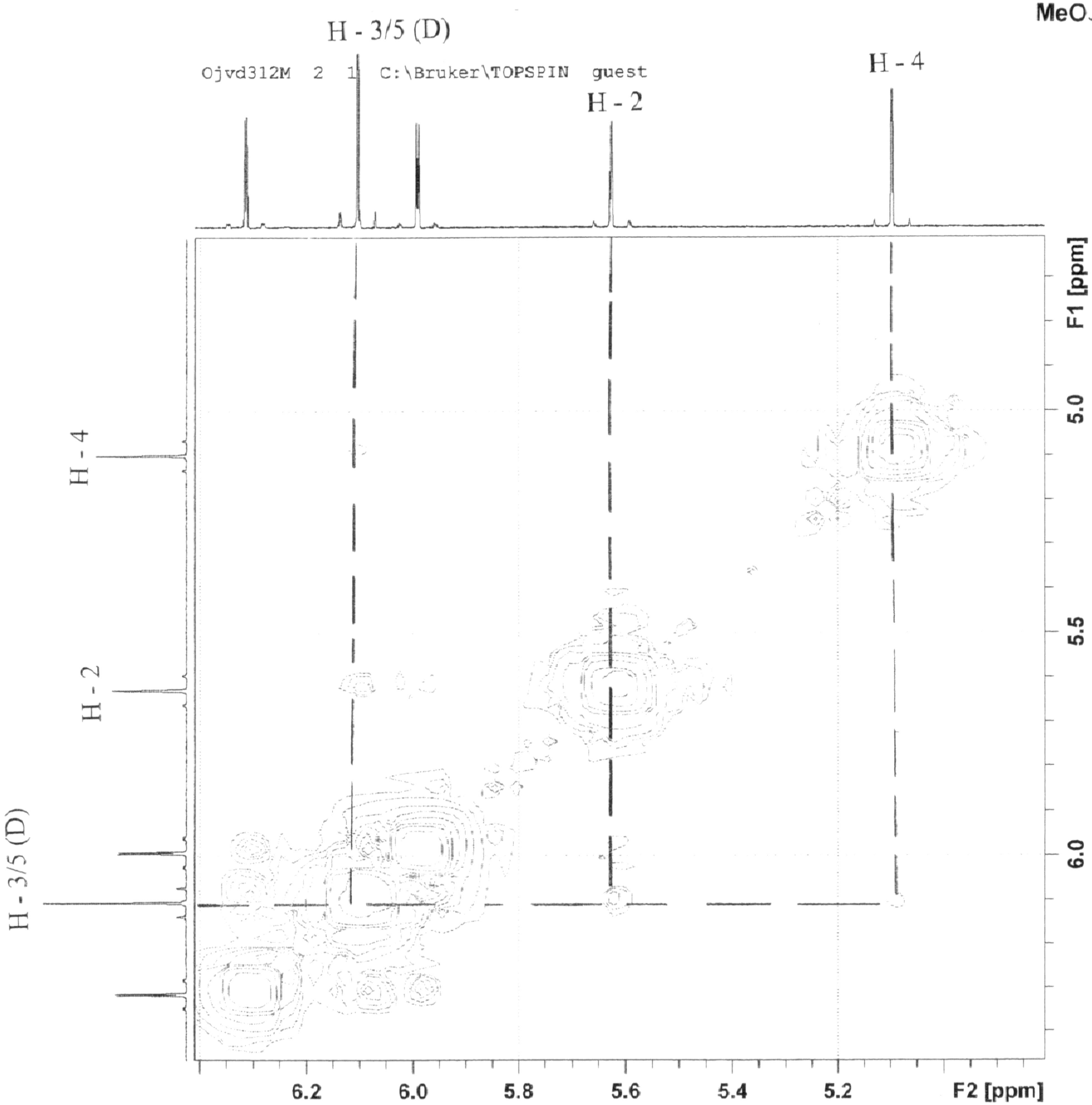
**Plate 10.3:** NOESY NMR ($CDCl_3$, 298K)

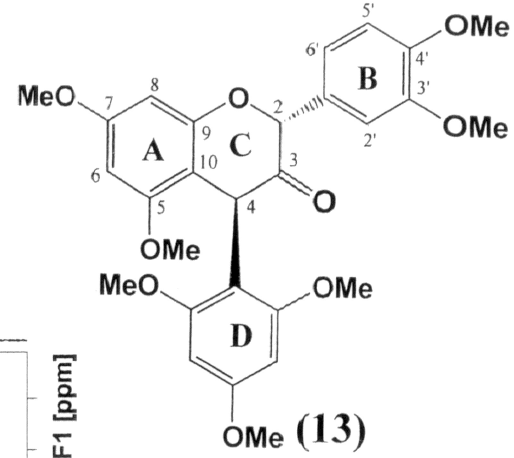


**(13)**

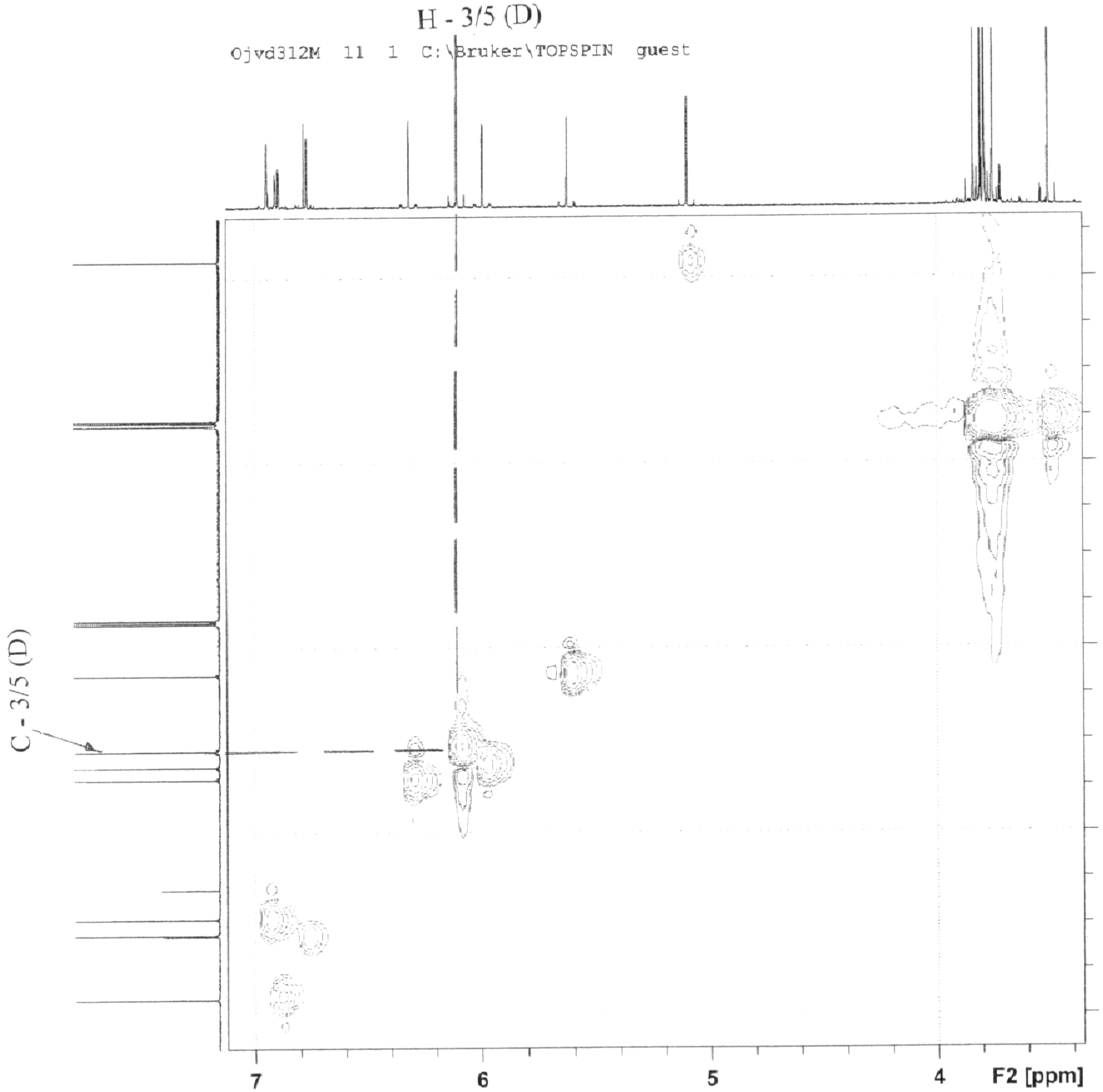
**Plate 10.4:** COSY NMR ($CDCl_3$, 298K)

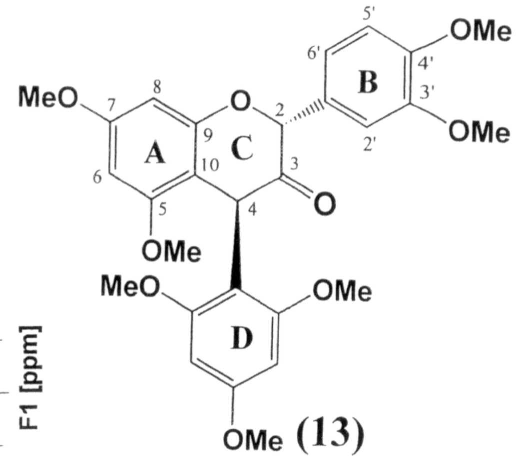



(13)

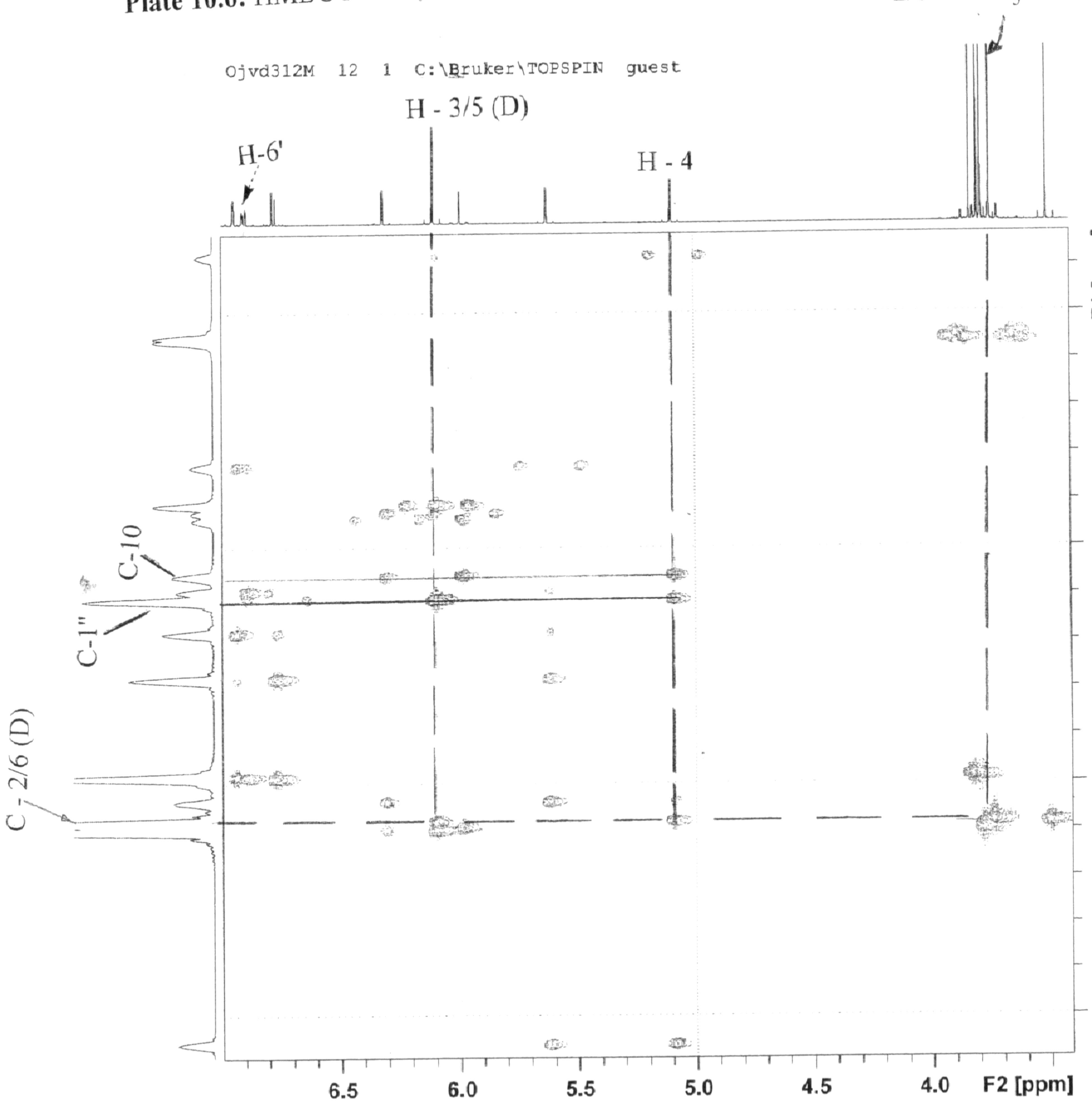
**Plate 10.5:** HSQC ($CDCl_3$, 298K)

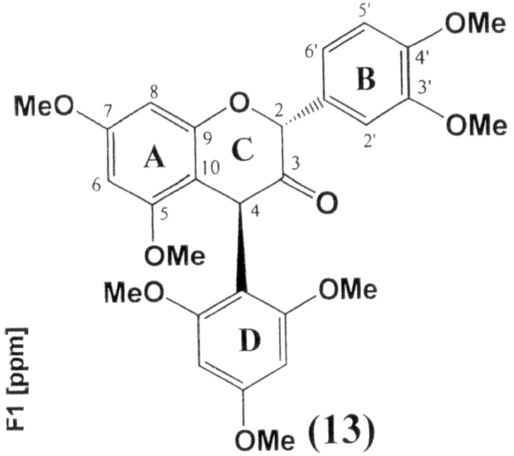



(13)

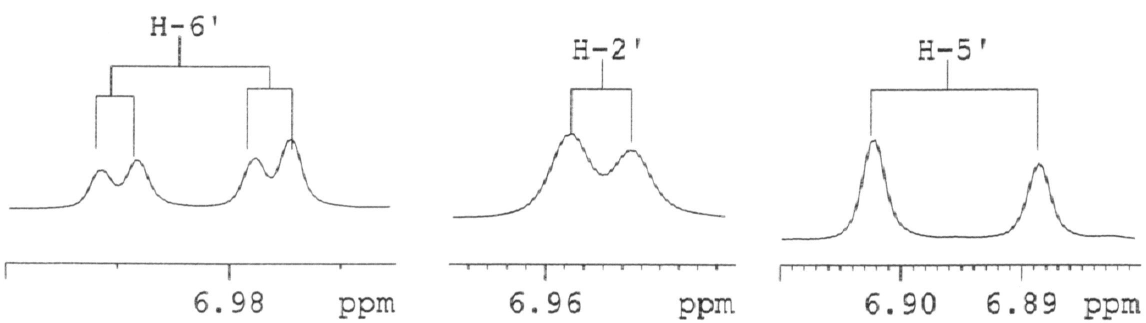
**Plate 10.6:** HMBC NMR ($CDCl_3$, 298K)

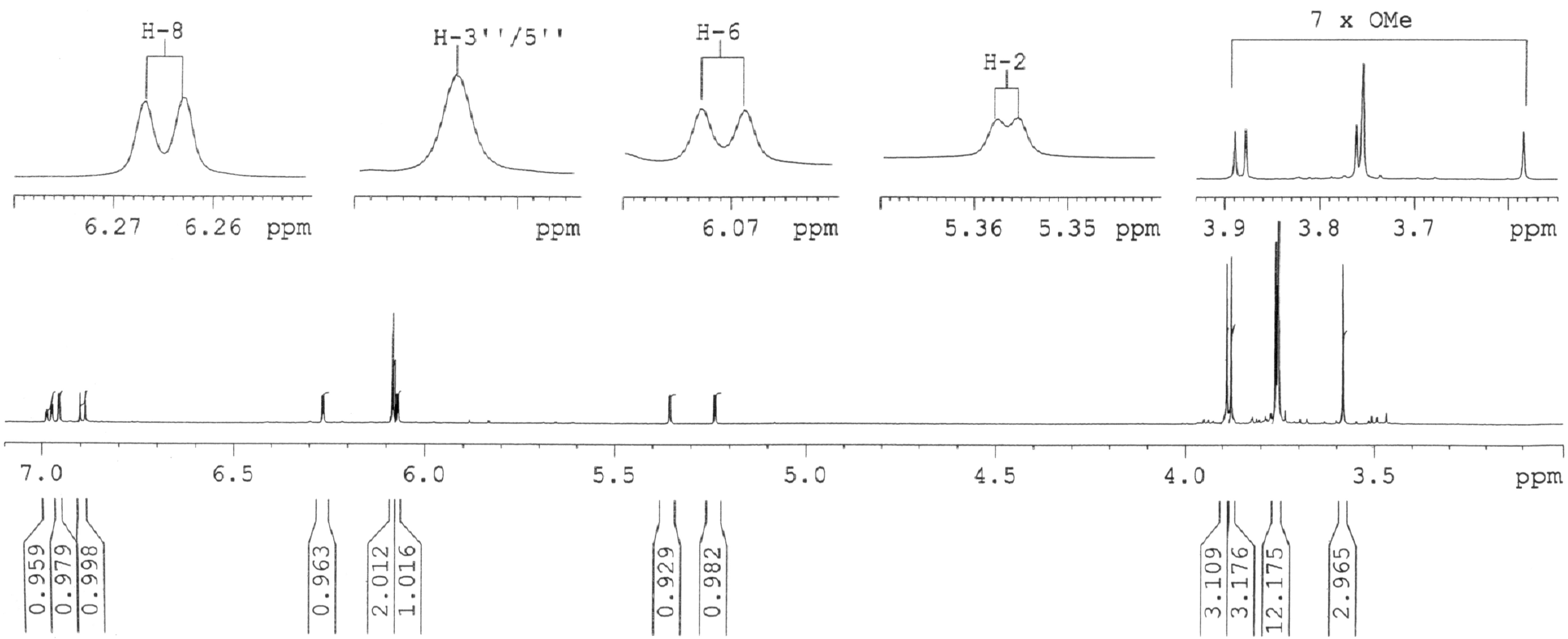


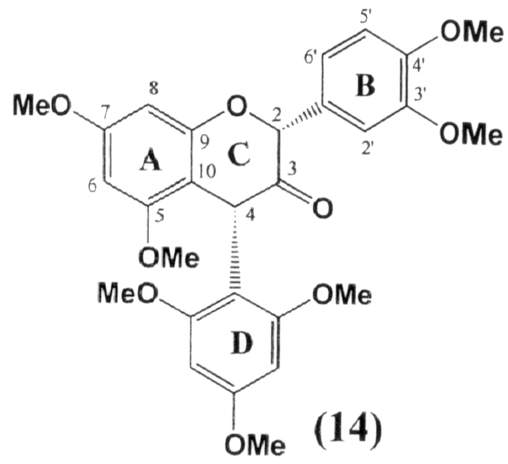




**(13)**

**Plate 11.1:** $^{1}$H NMR ($CDCl_3$, 298K)

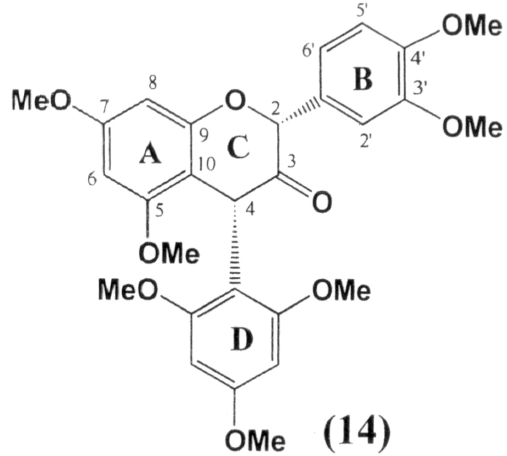



**(14)**

**Plate 11.2:** $^{13}C$ NMR ($CDCl_3$, 298K)




**(14)**

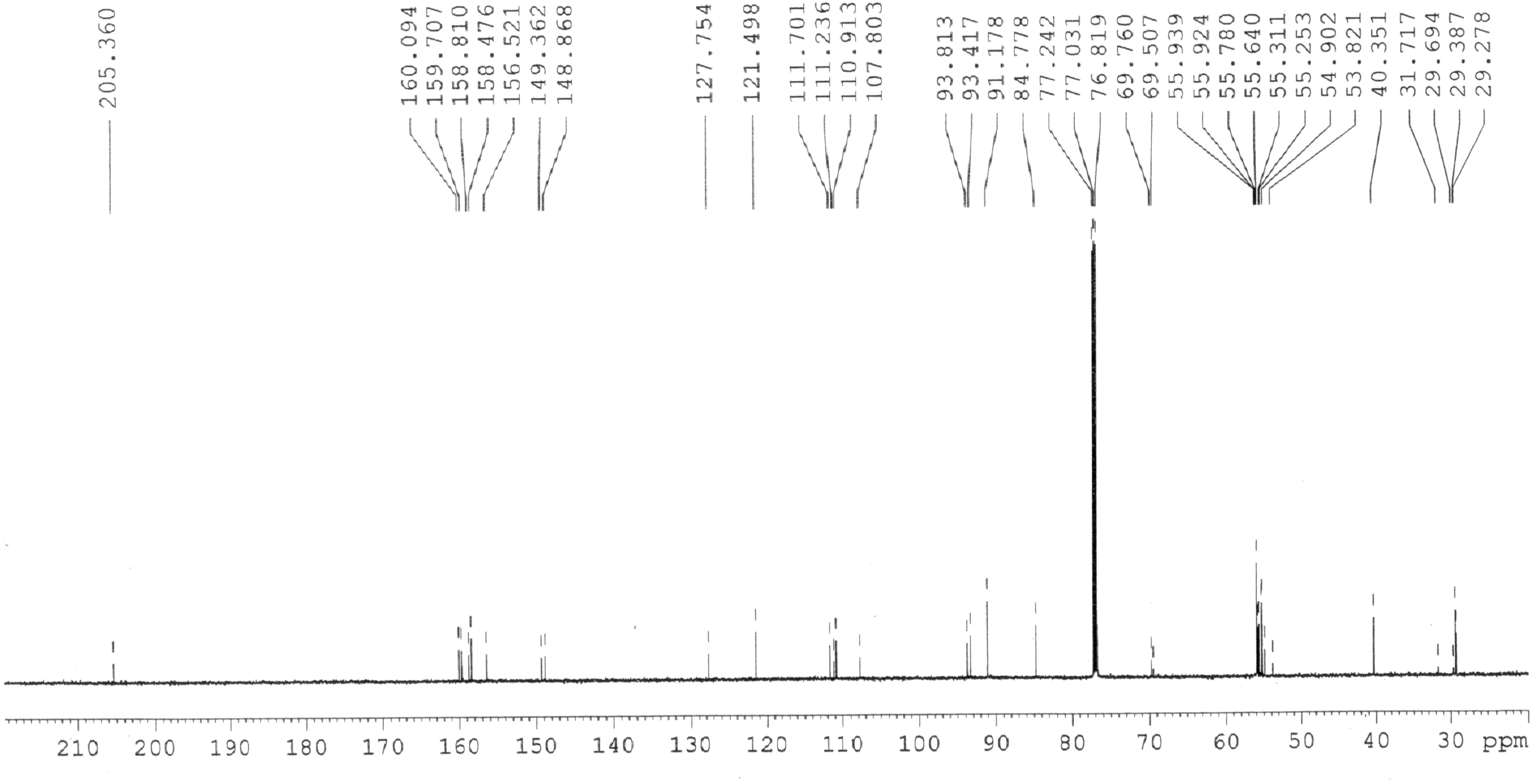
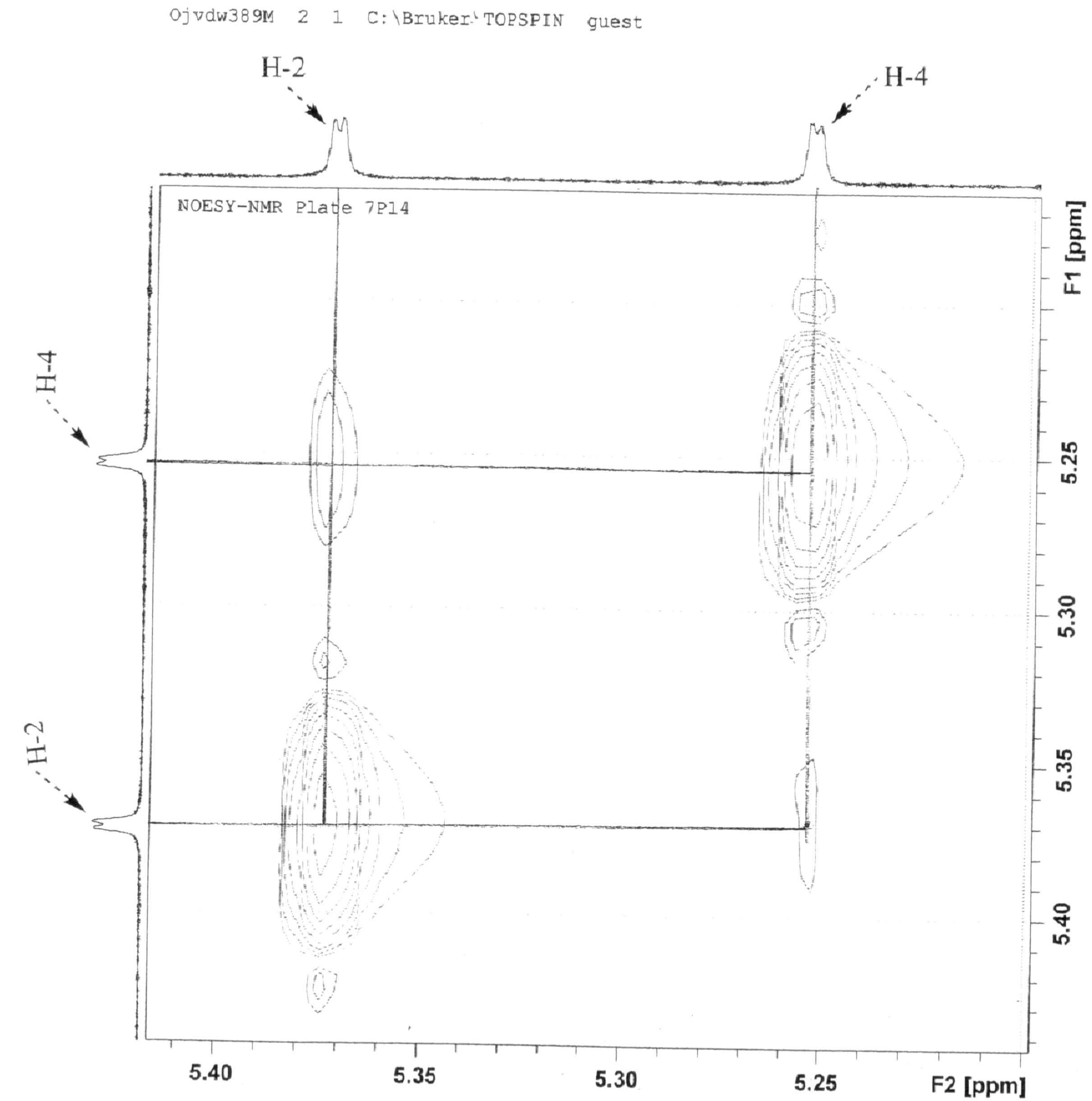
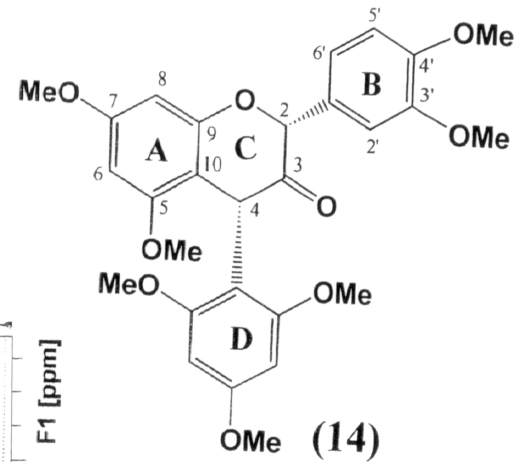


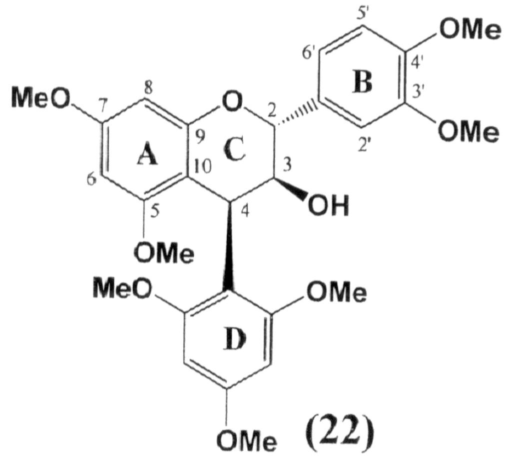
**Plate 11.3:** NOESY NMR ($CDCl_3$, 298K)



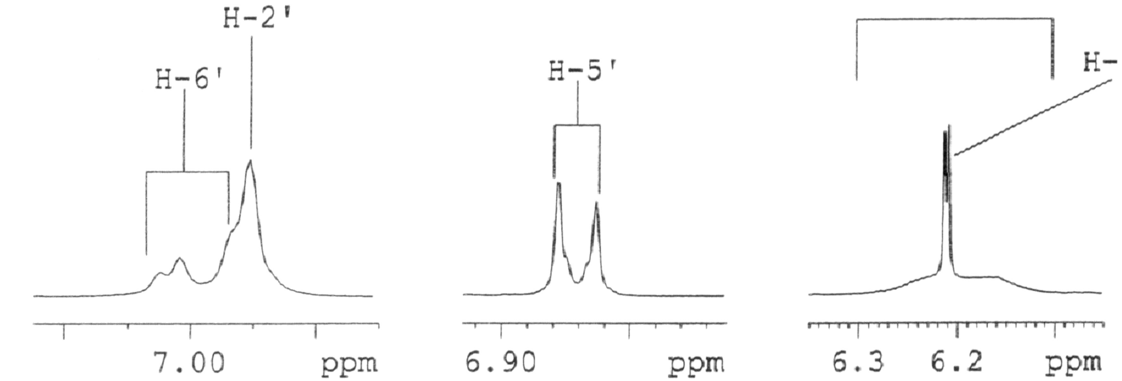




**(14)**

**Plate 12.1:** $^{1}$H NMR ($CDCl_3$, 298K)



(22)



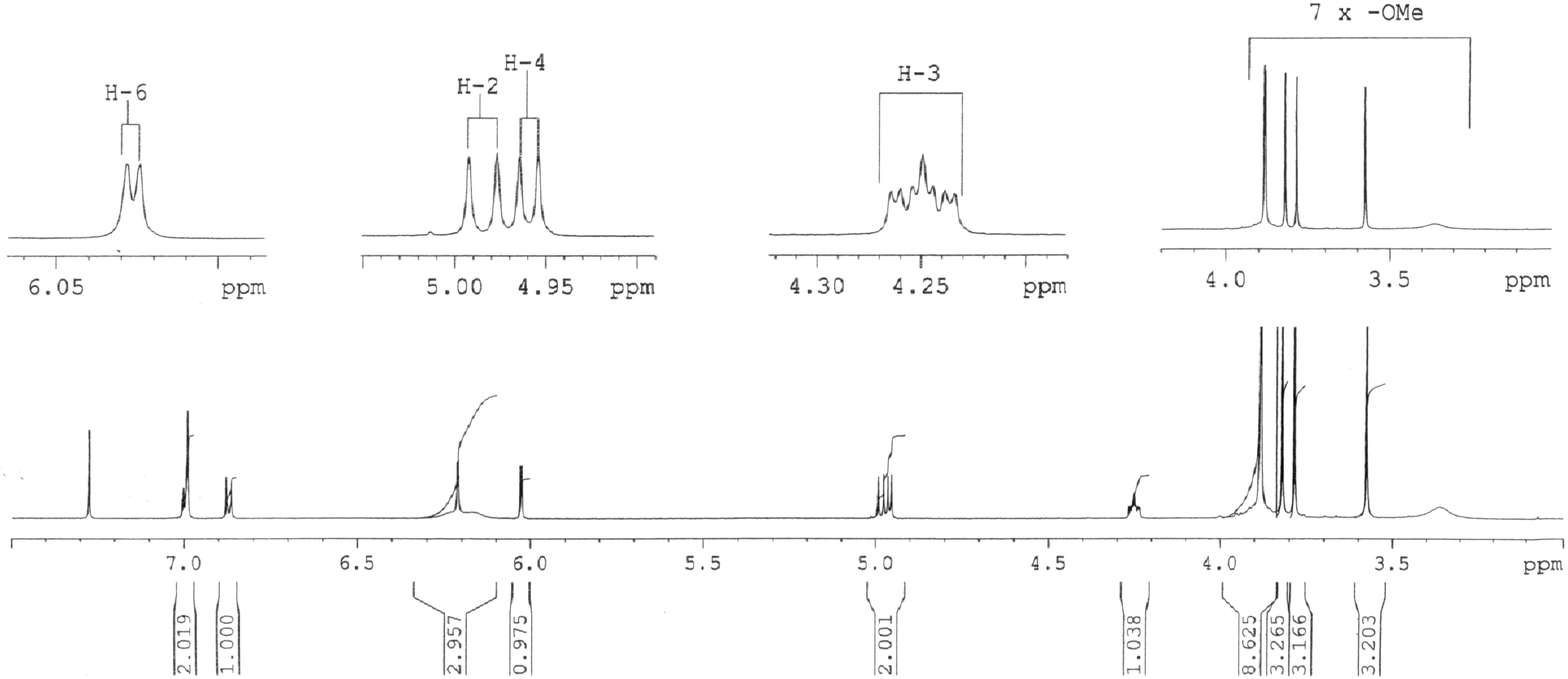
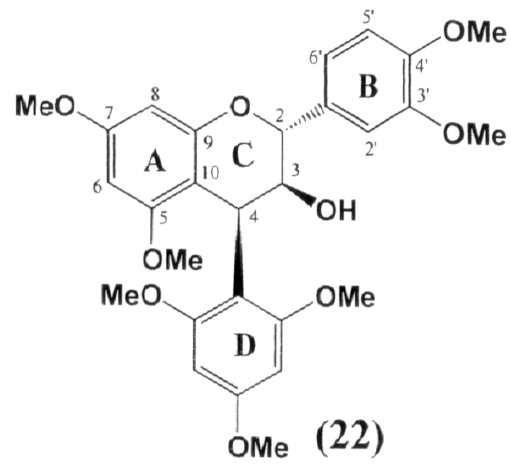



Plate 12.2: $^{13}C$ NMR ($CDCl_3$, 298K)




(22)

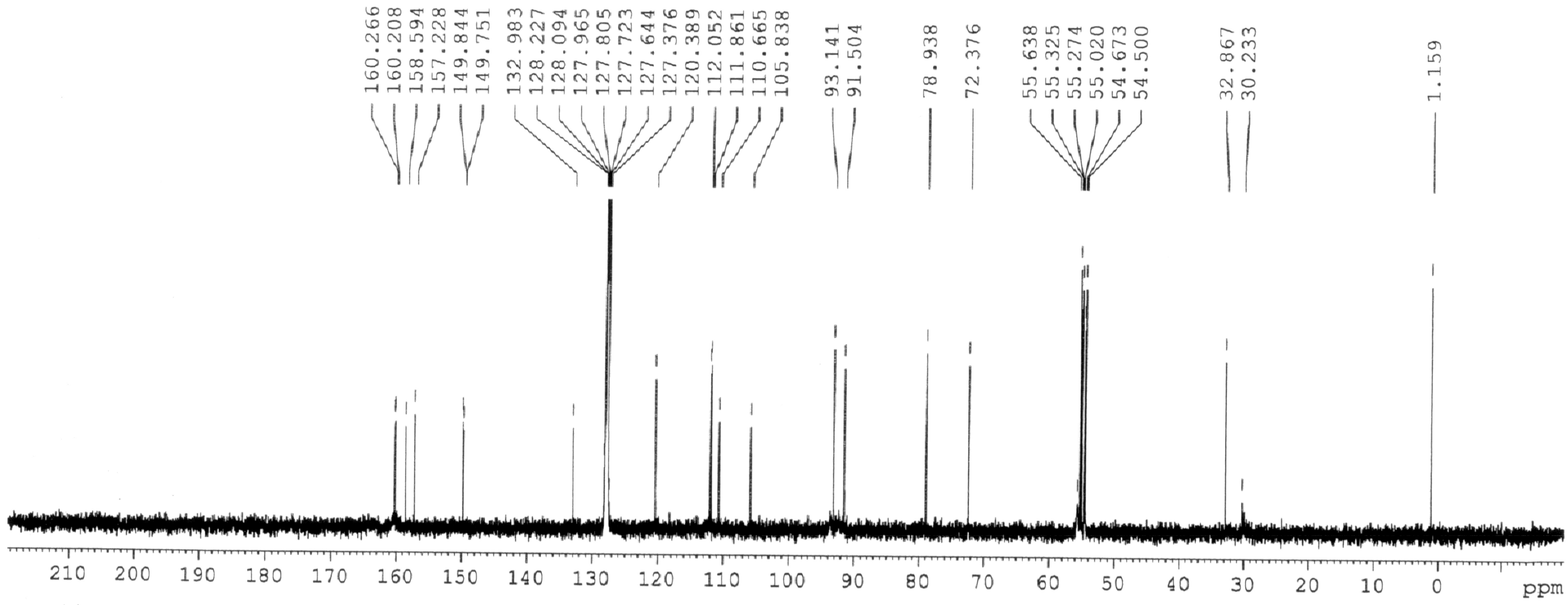
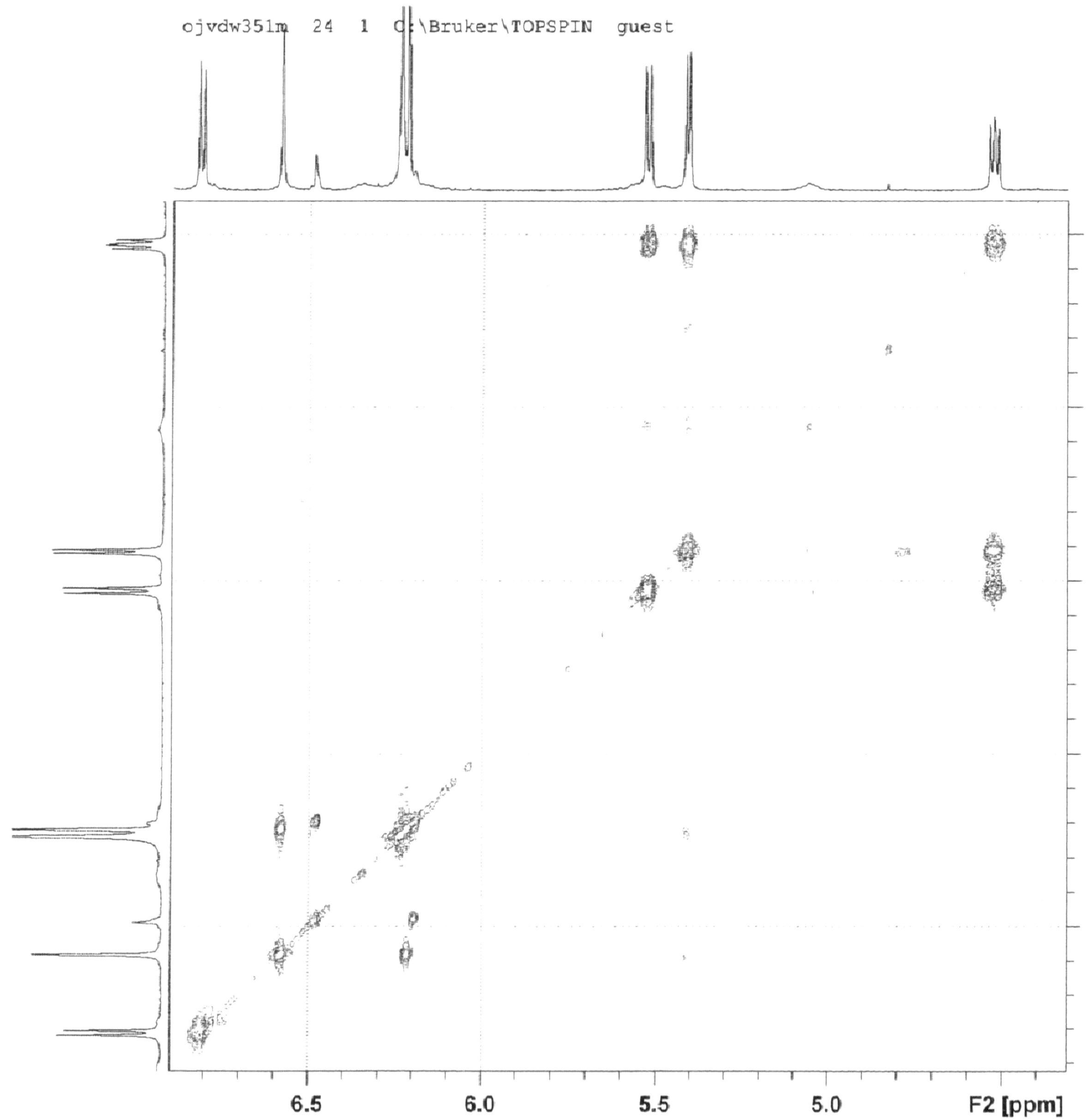
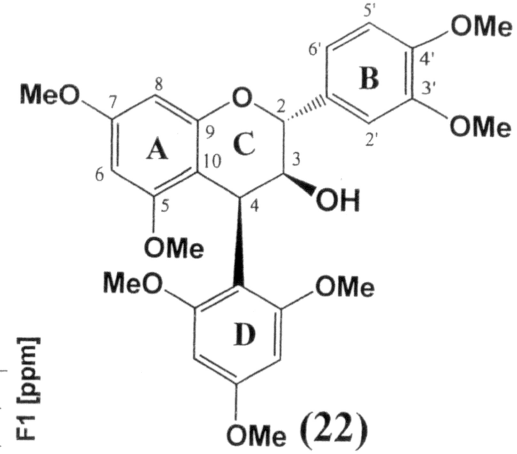


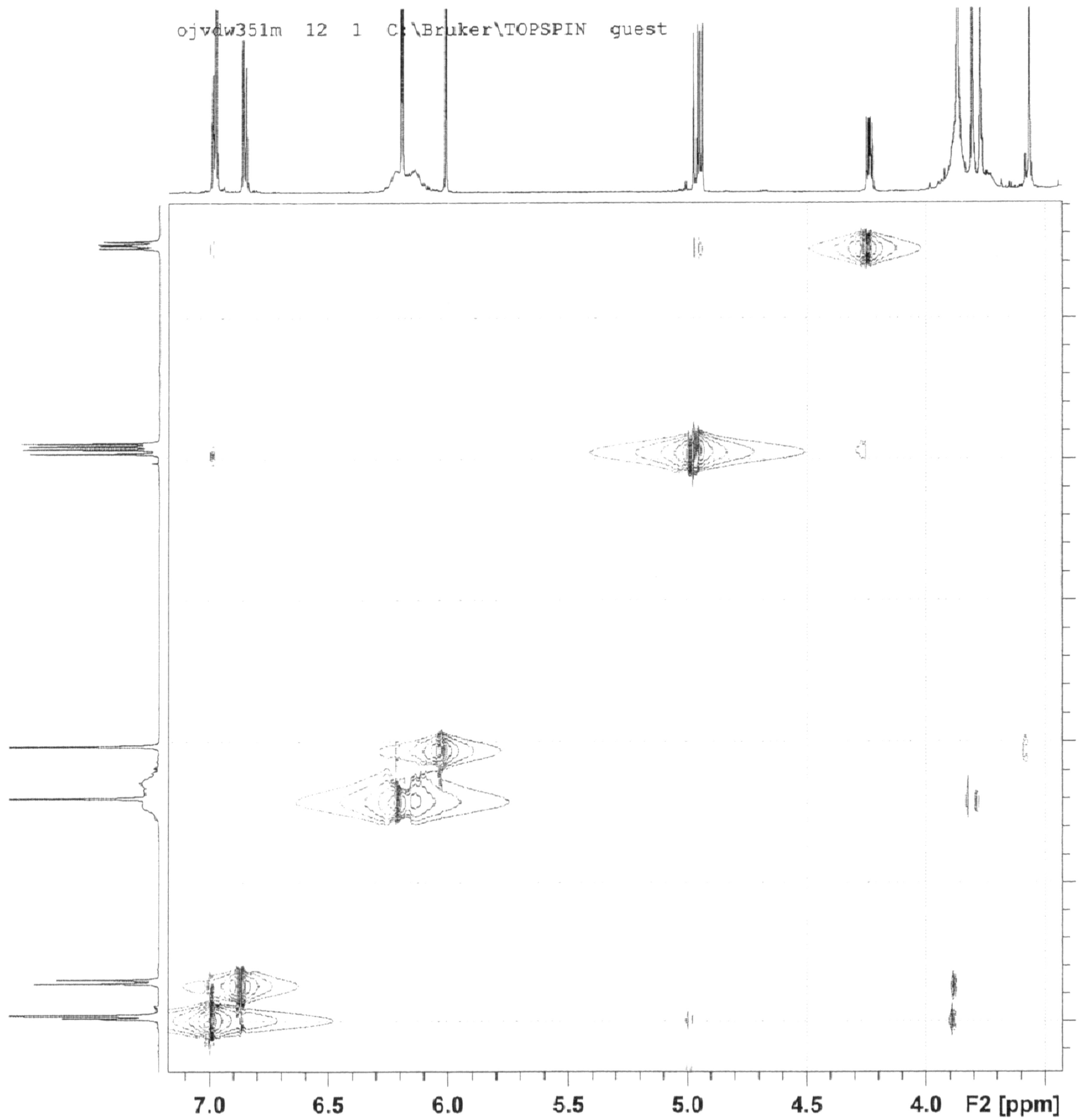
**Plate 12.3:** COSY NMR ($CDCl_3$, 298K)

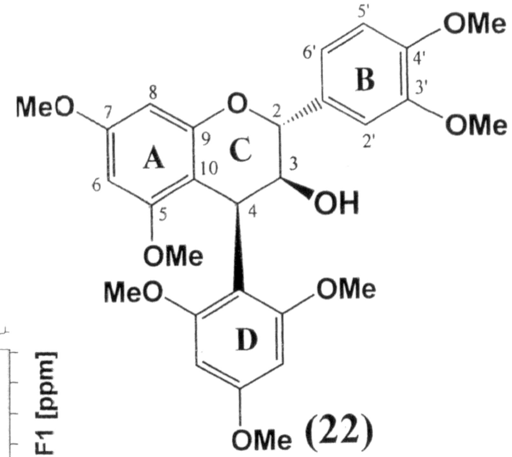



**(22)**

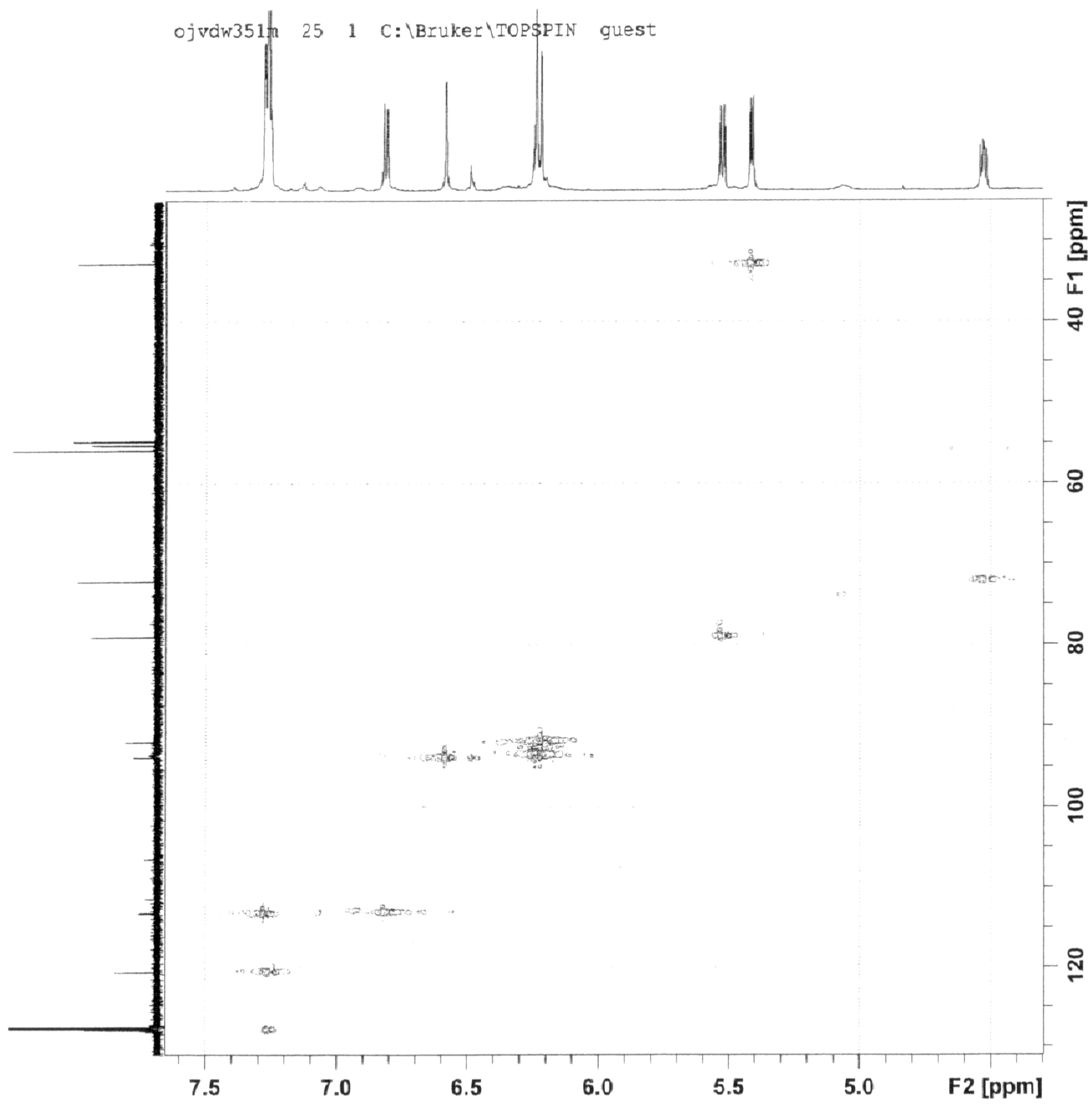
**Plate 12.4:** NOESY NMR ($CDCl_3$, 298K)

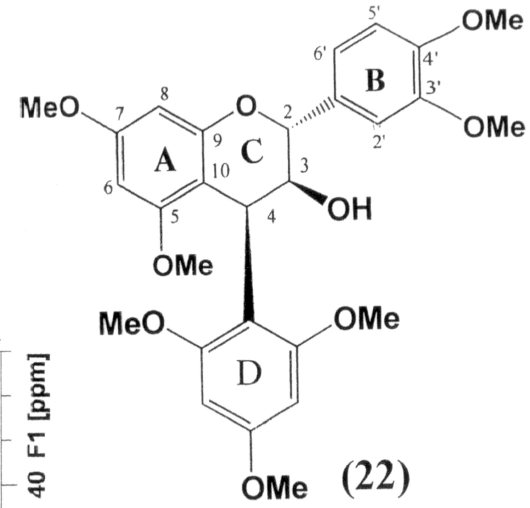


(22)

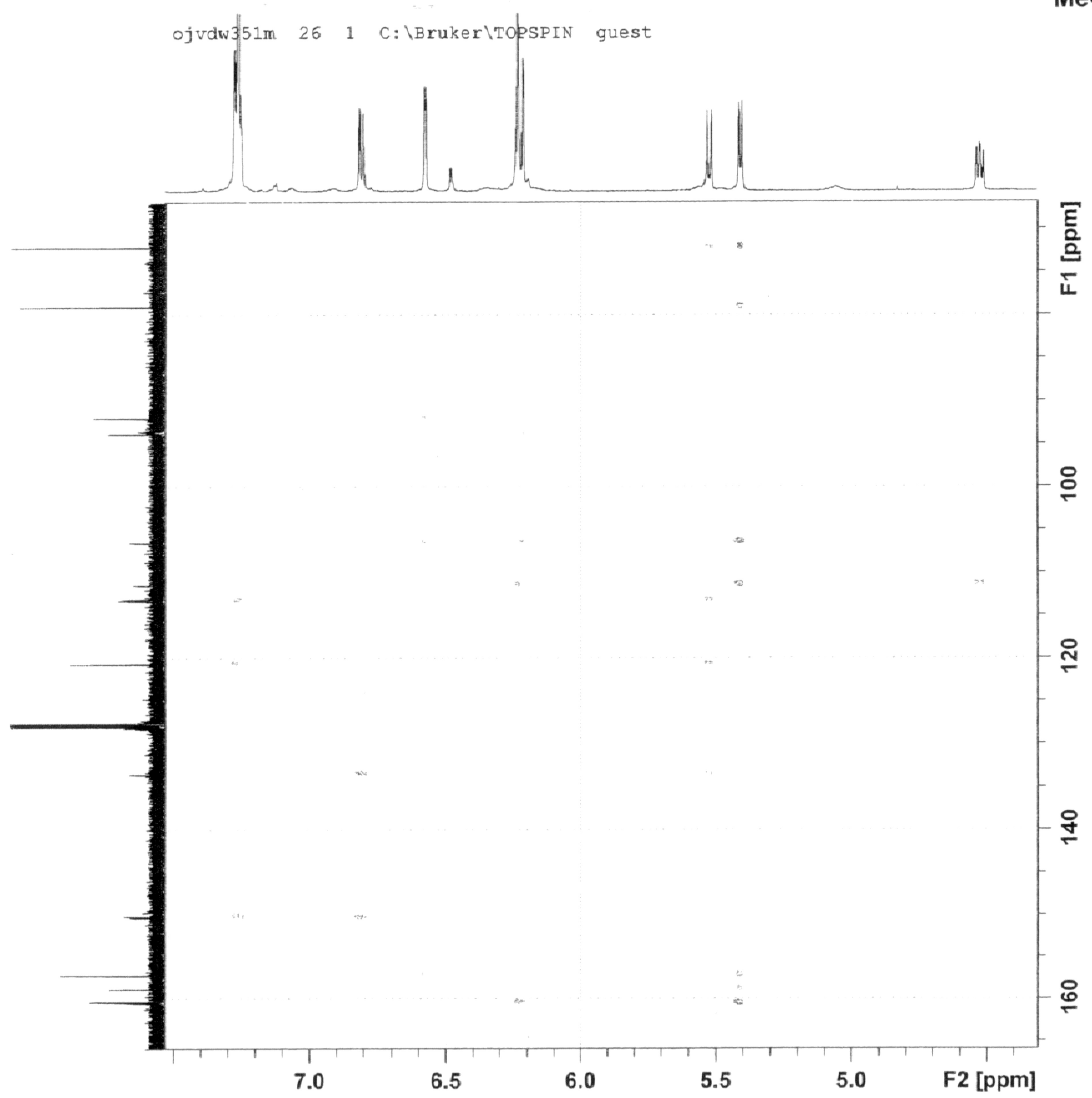
**Plate 12.5:** HSQC NMR ($CDCl_3$, 298K)

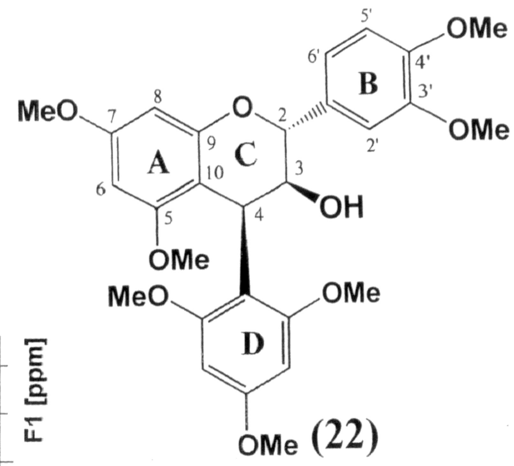



(22)

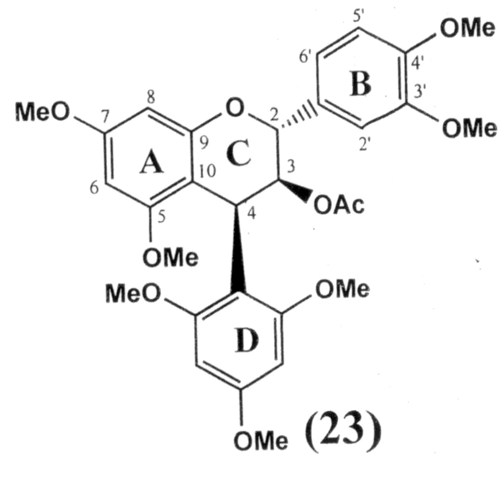
**Plate 12.6:** HMBC NMR ($CDCl_3$, 298K)




(22)

**Plate 13.1** $^{1}$H NMR ($CDCl_3$, 298K)




(23)

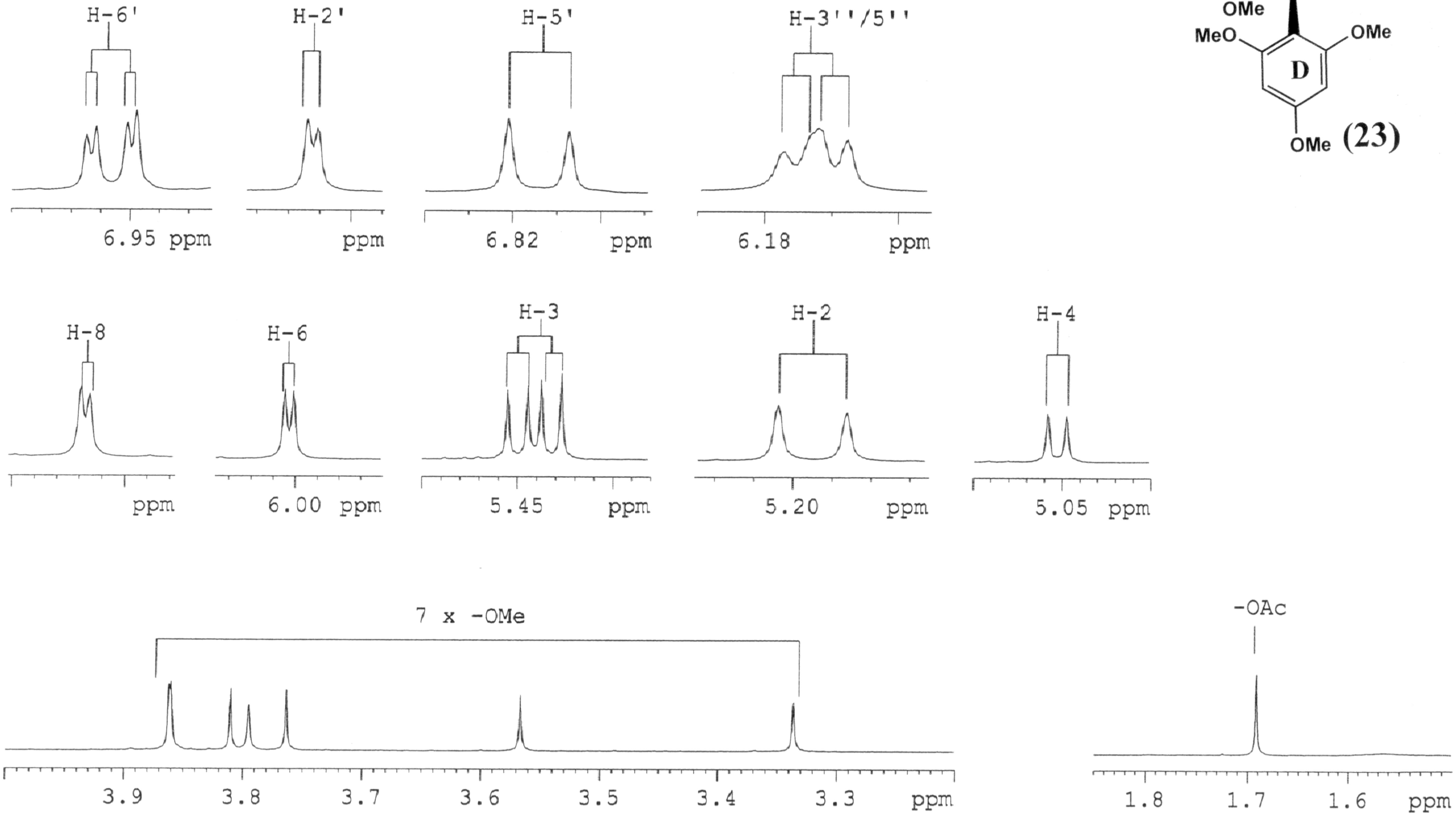
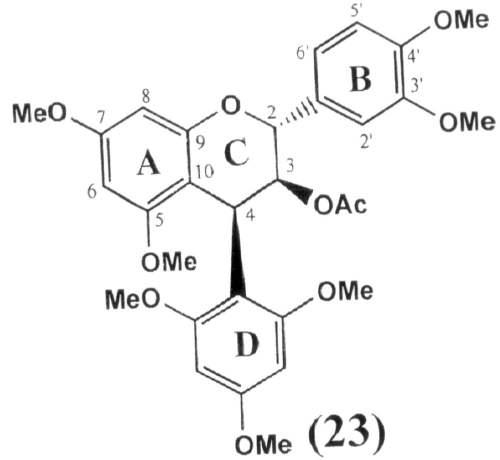


Plate 13.2: $^{1}$H NMR ($CDCl_3$, 298K)




(23)

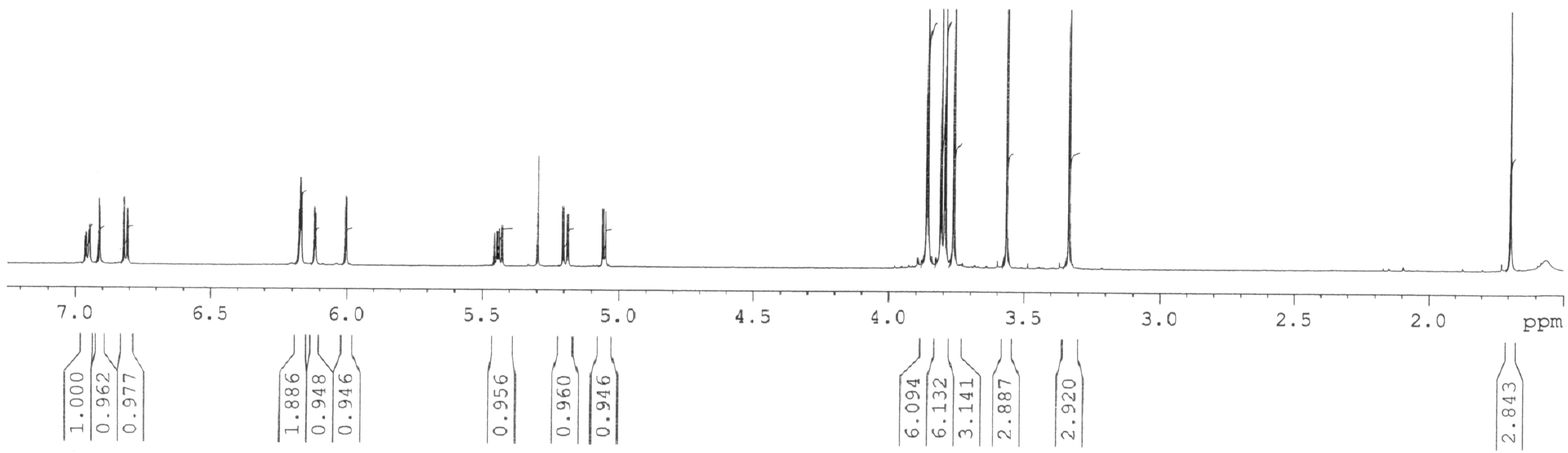
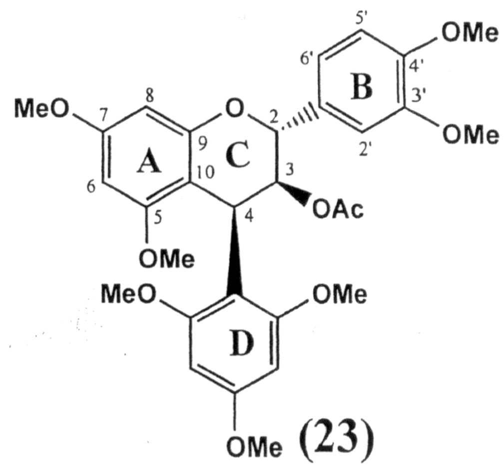


Plate 13.3 $^{13}C$ NMR ($CDCl_3$, 298K)




(23)

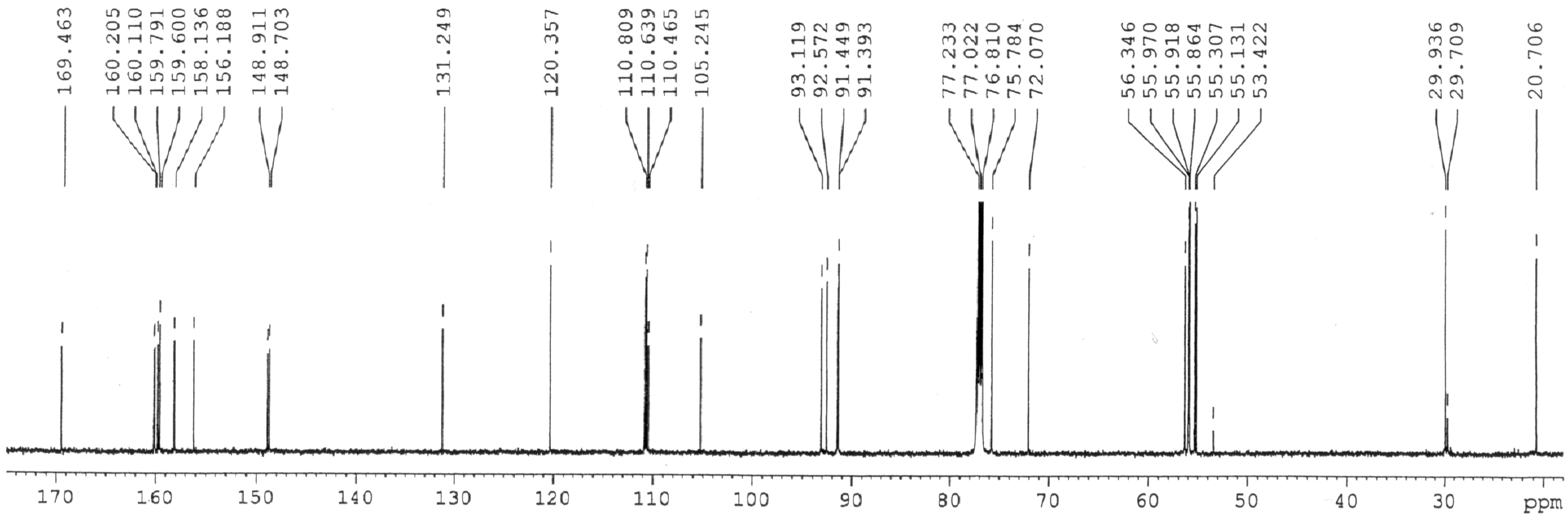
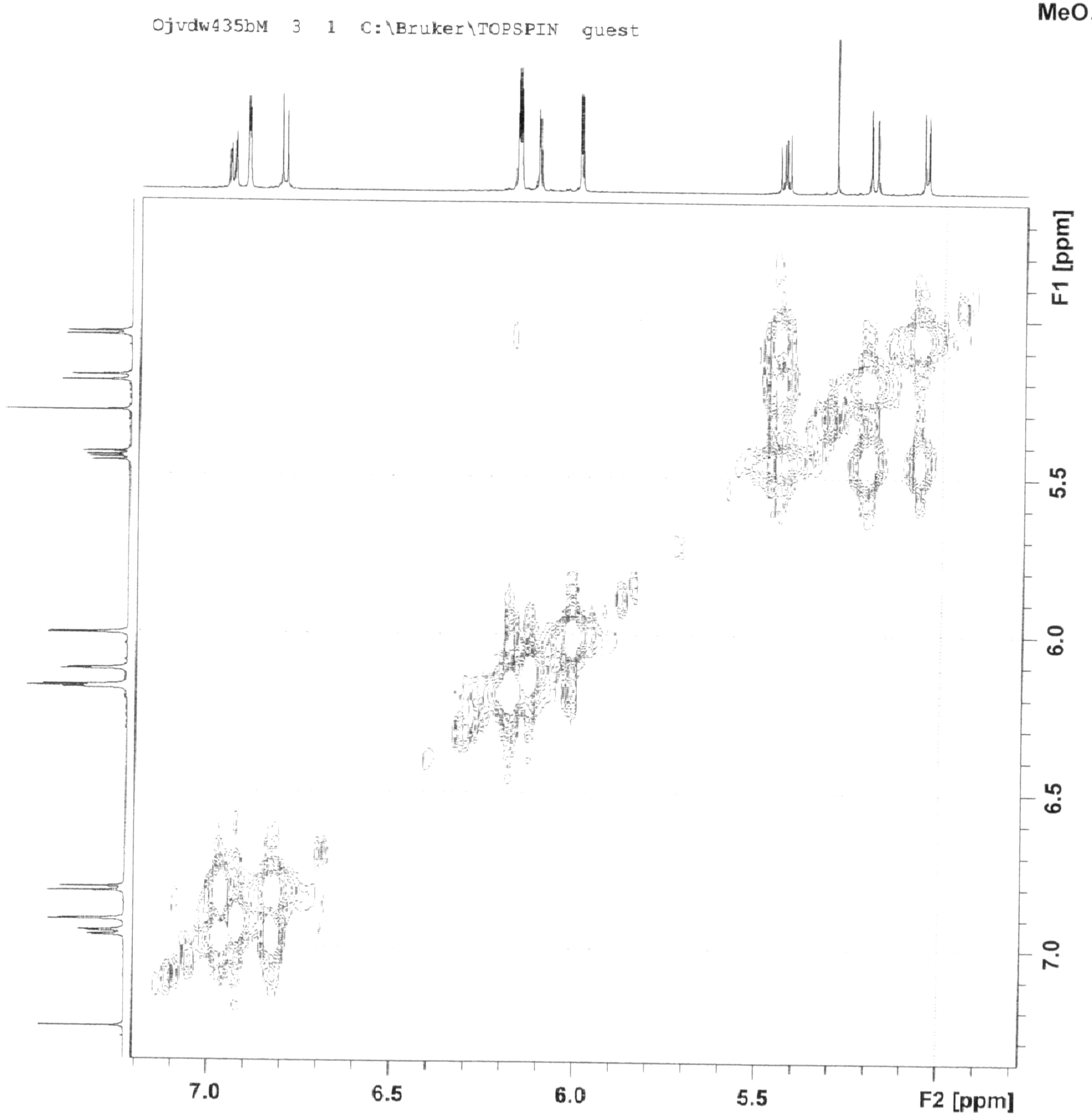
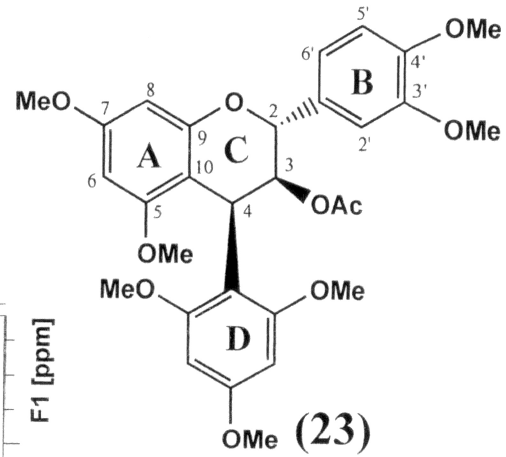


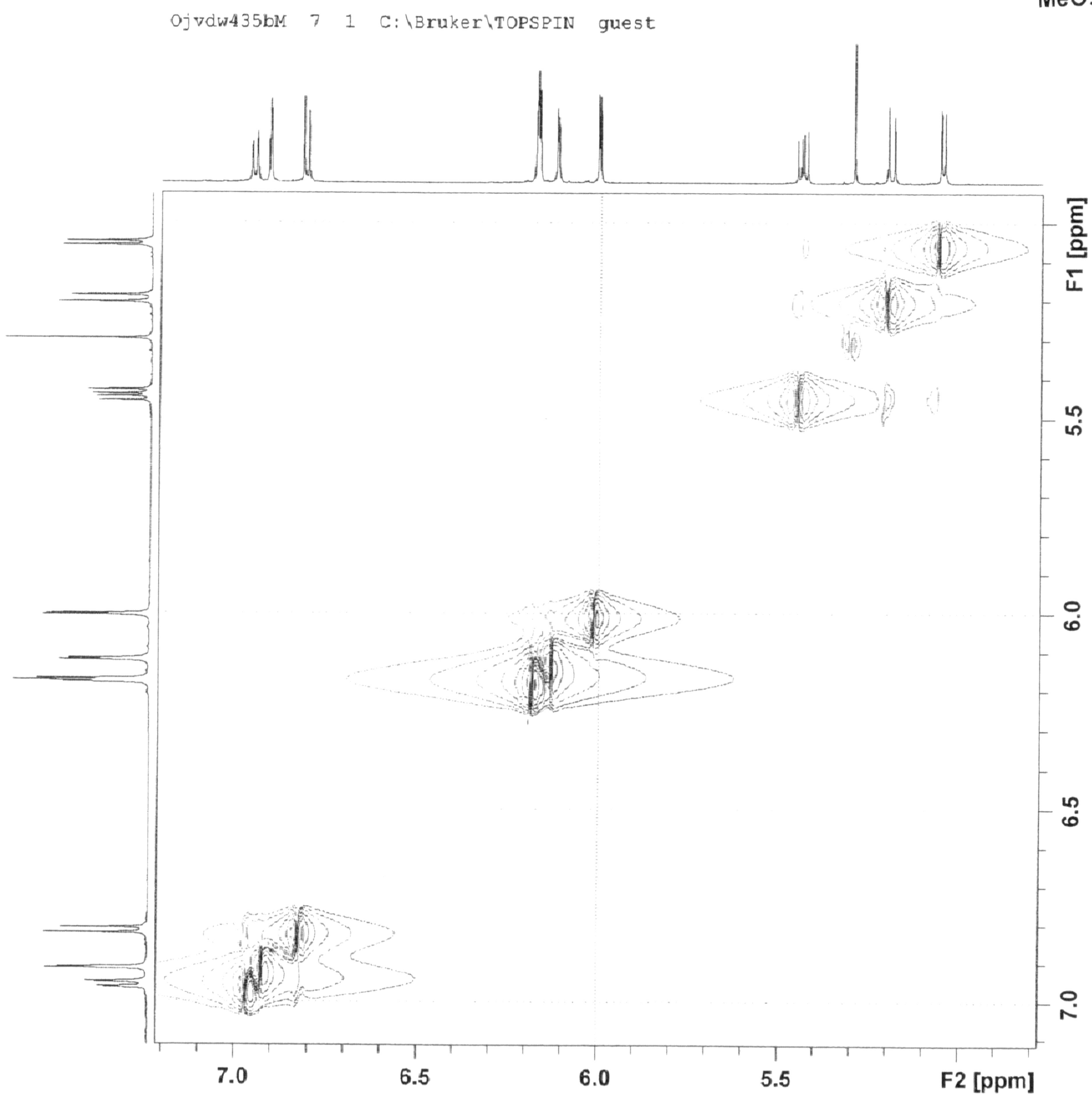
**Plate 13.4:** COSY NMR ($CDCl_3$, 298K)

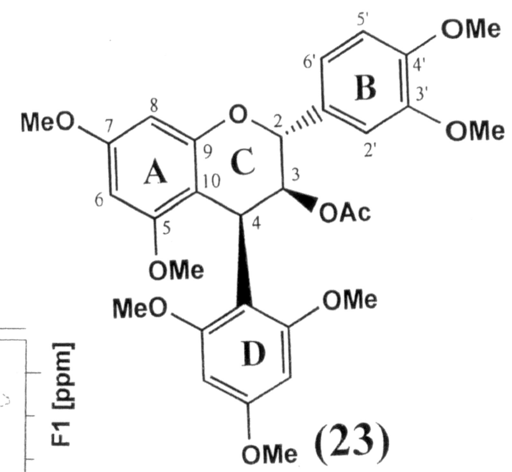



**(23)**

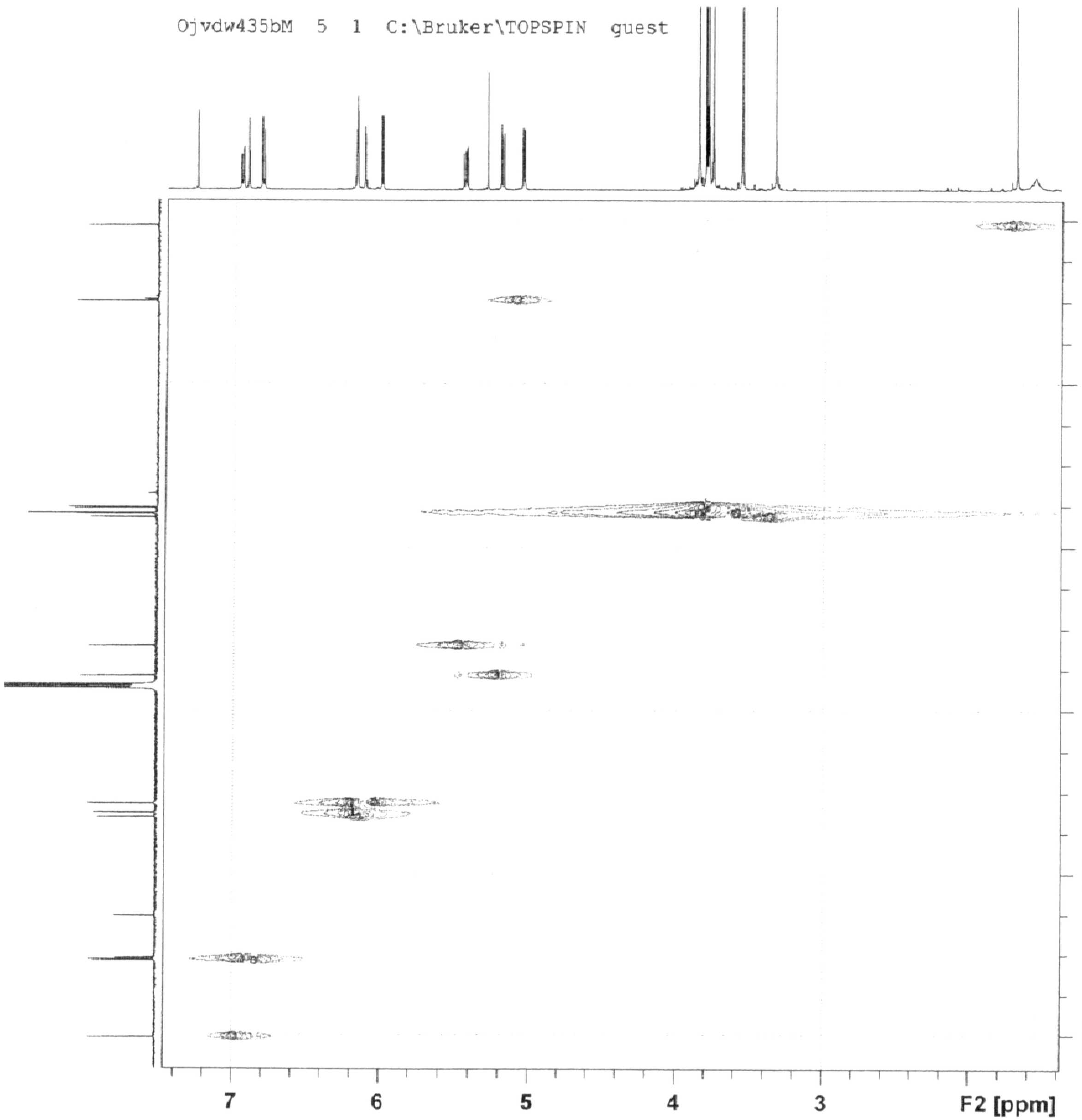
**Plate 13.5**: NOESY NMR ($CDCl_3$, 298K)

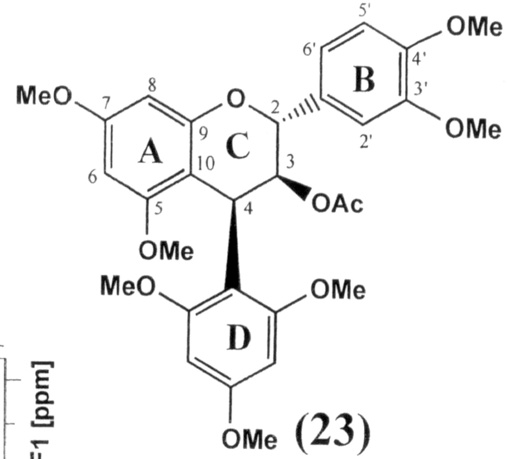



(23)

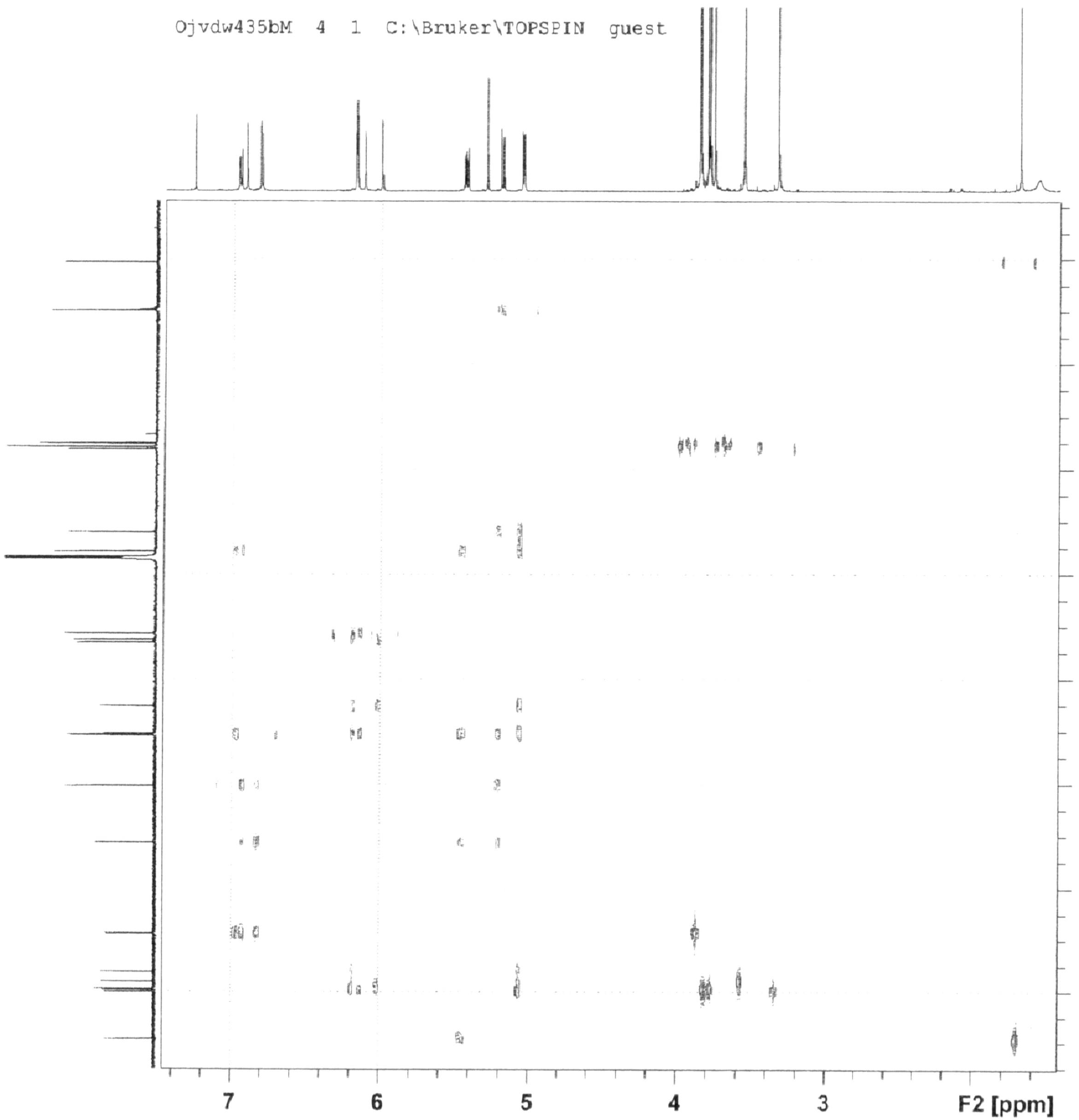
**Plate 13.6:** HSQC NMR ($CDCl_3$, 298K)

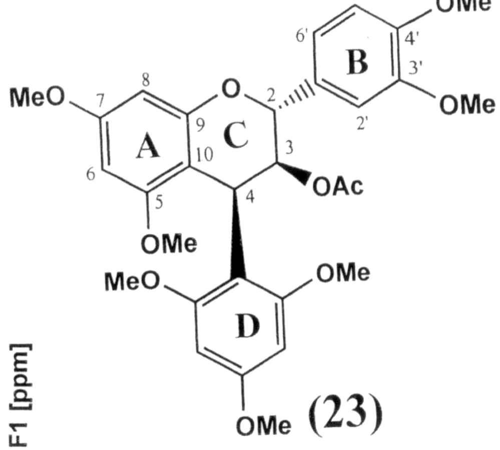



**(23)**

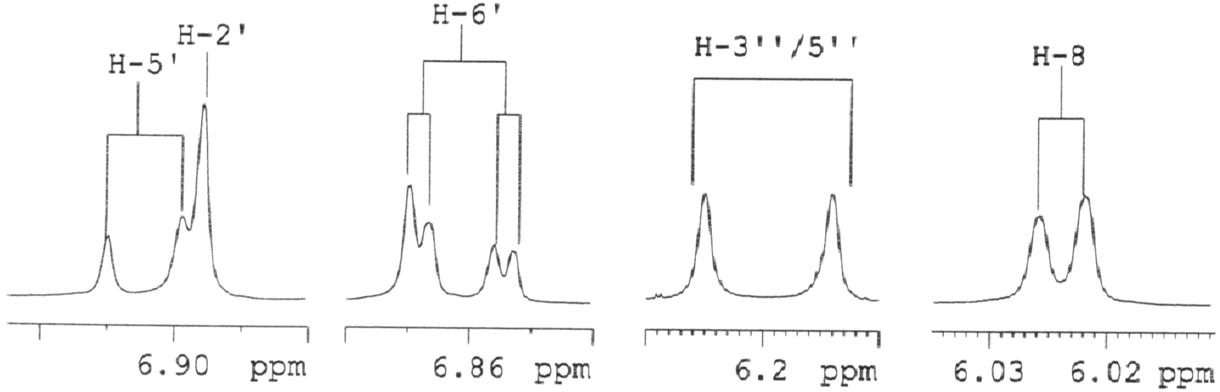
**Plate 13.7:** HMBC NMR ($CDCl_3$, 298K)

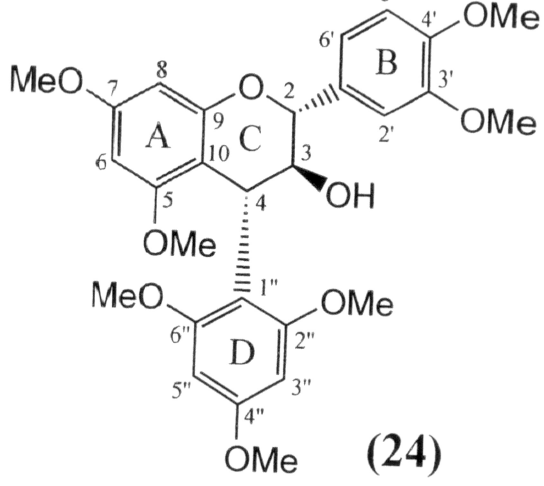


(23)

Plate 14.1: $^{1}$H NMR (DMSO, 302K)




(24)

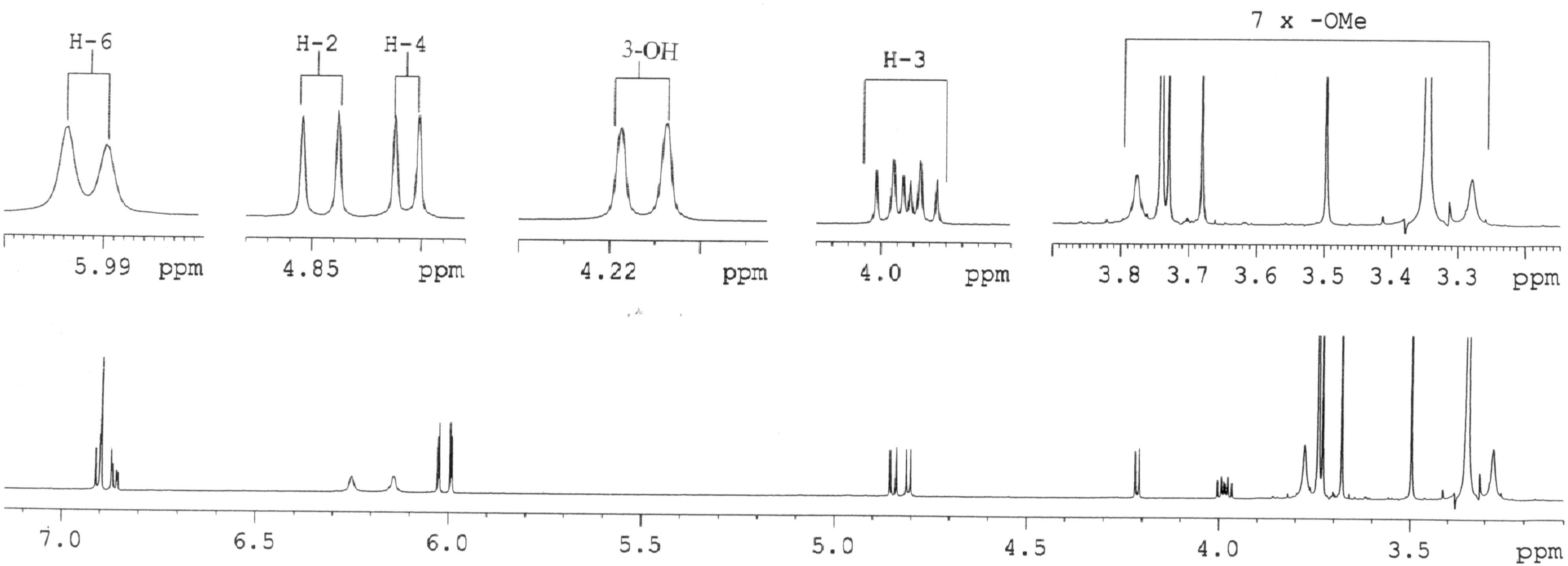
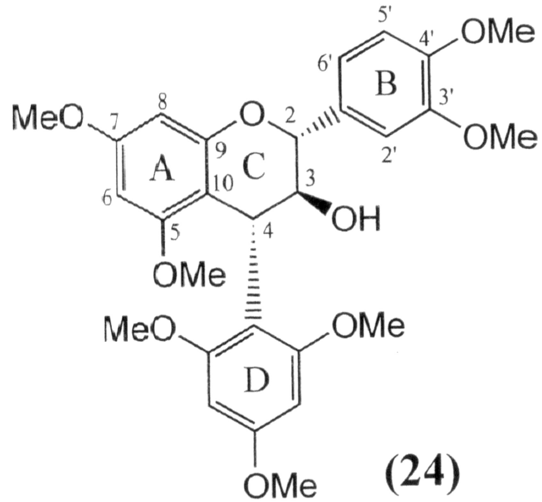


**Plate 14.2:** $^{13}C$ NMR (DMSO, 302K)




**(24)**

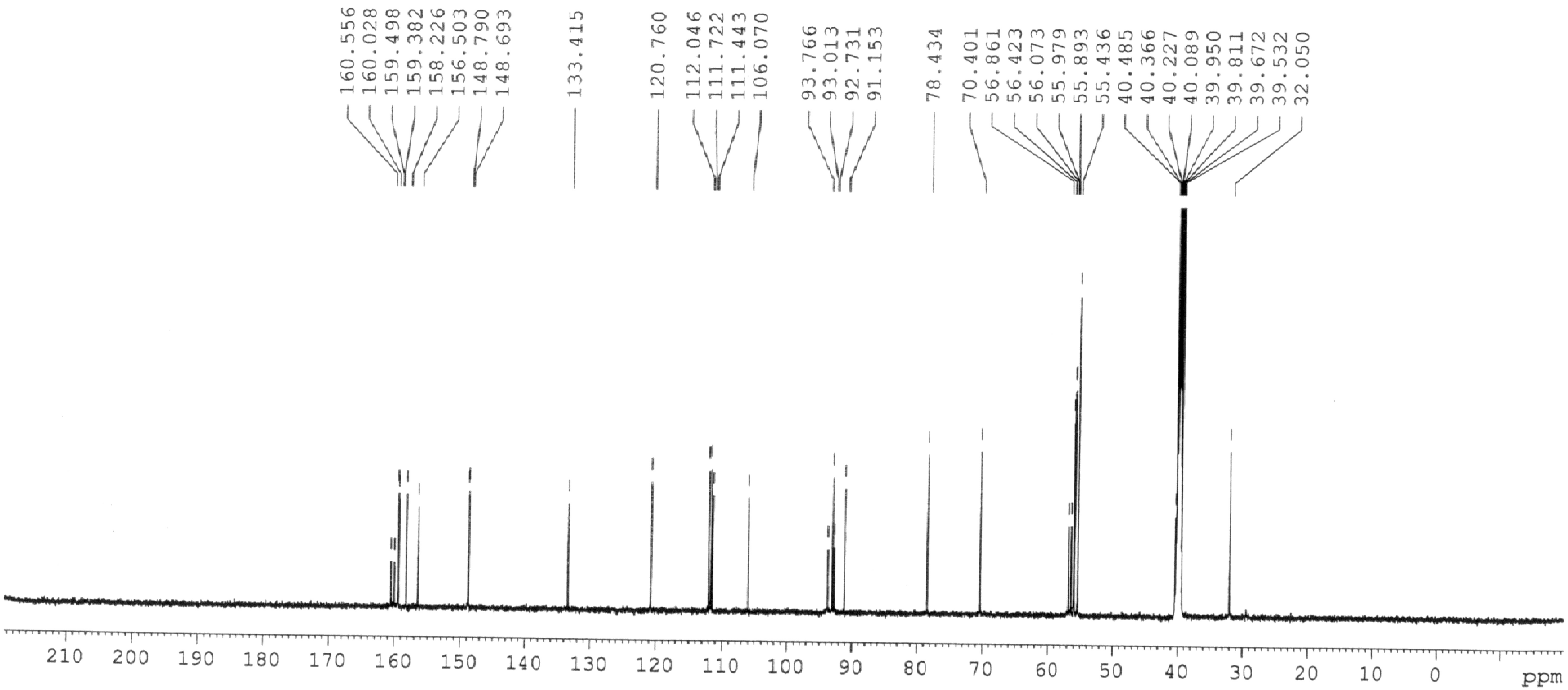
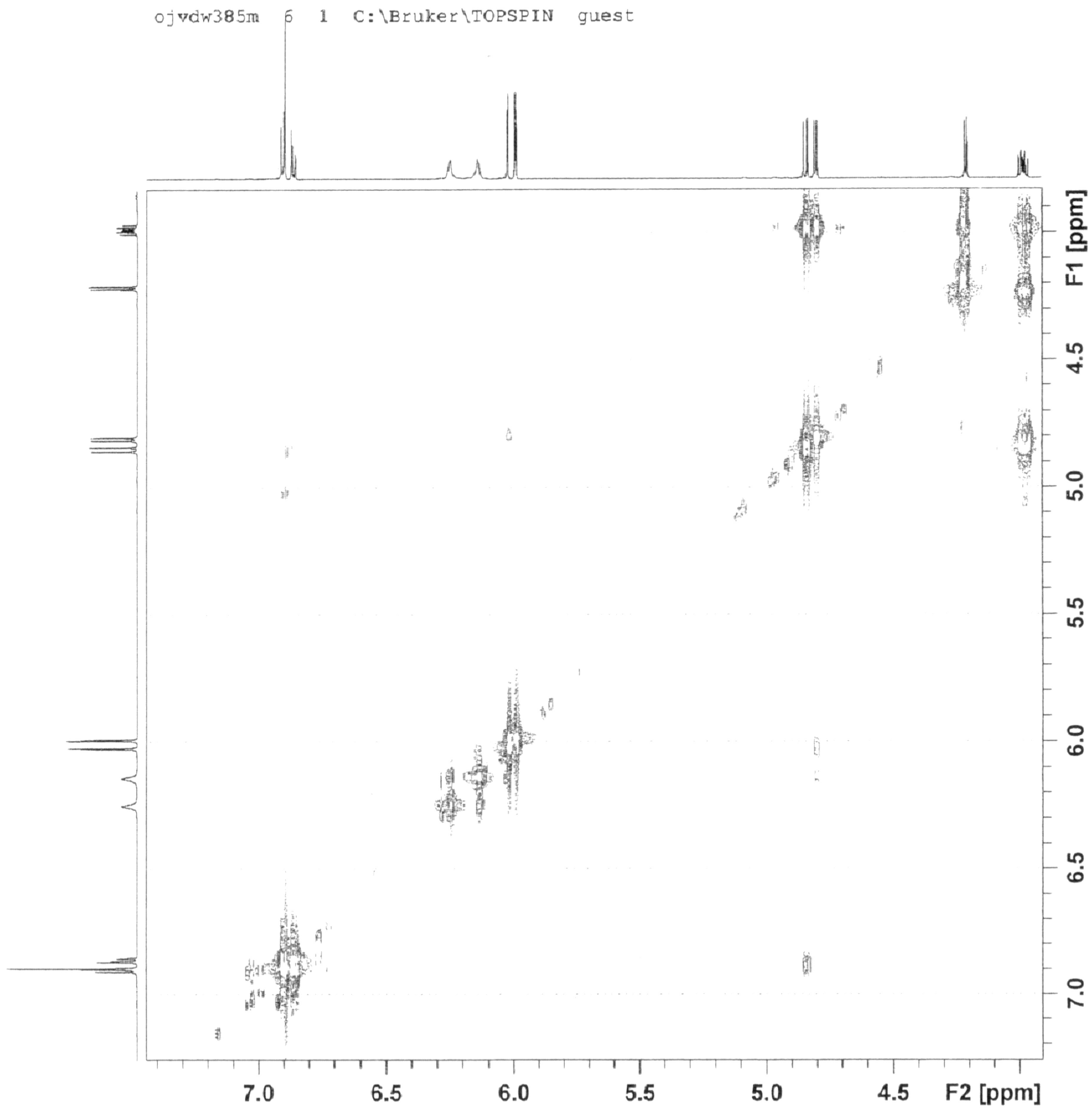
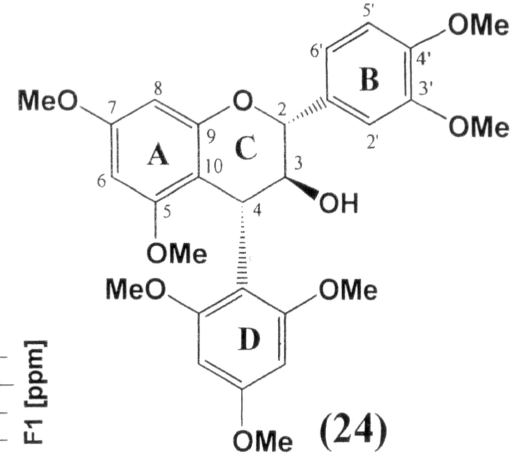


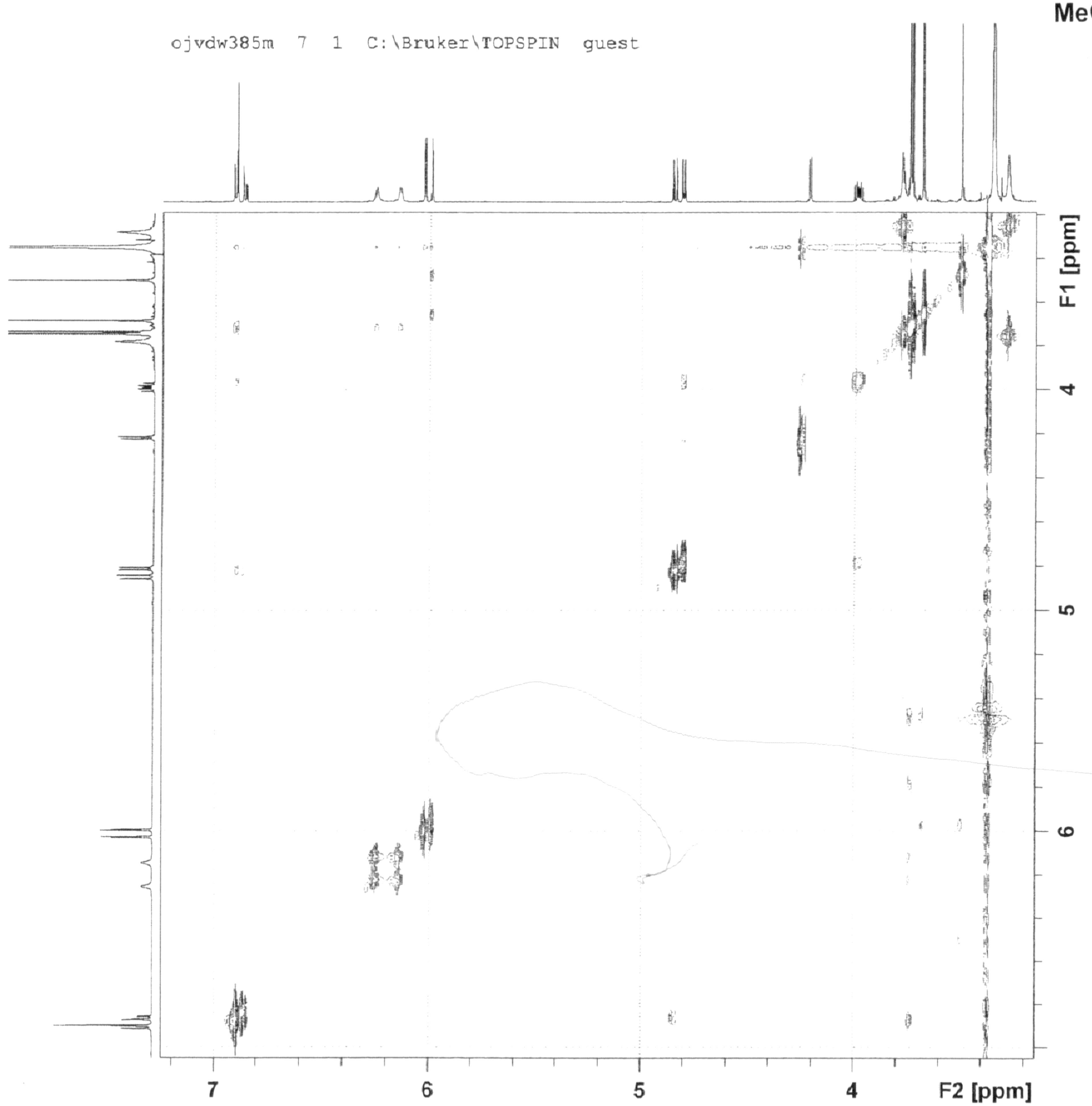
Plate 14.3: COSY NMR (DMSO, 302K)

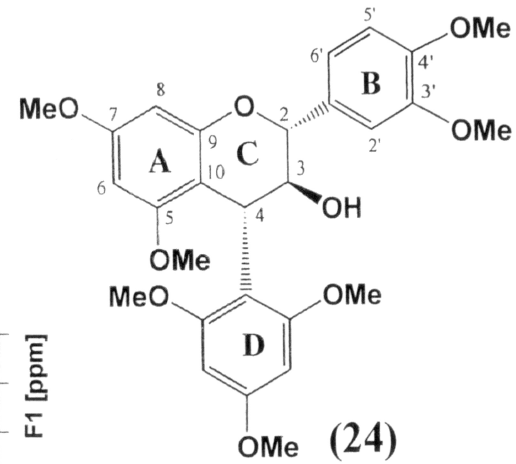



(24)

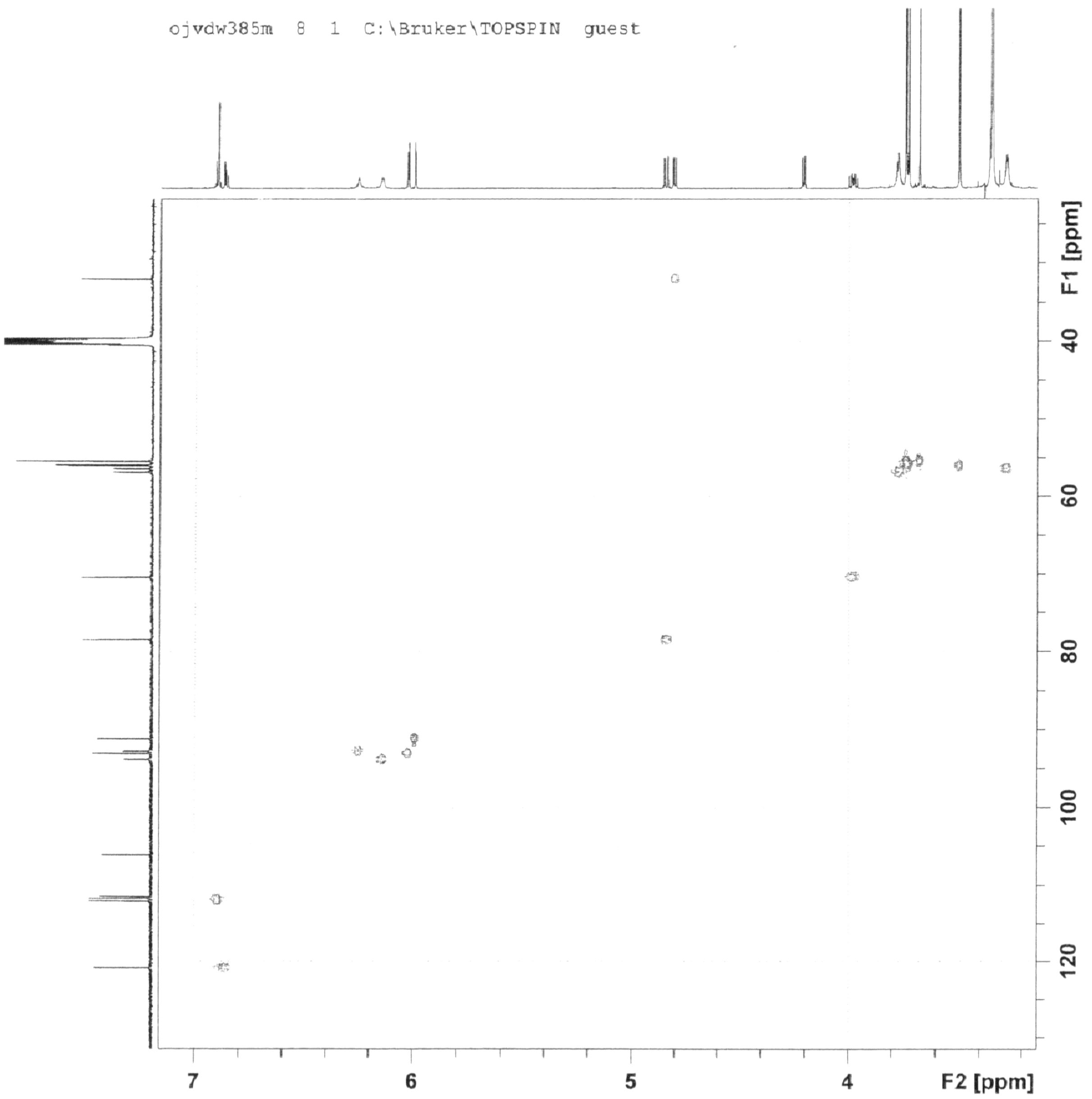
Plate 14.4: NOESY NMR (DMSO, 302K)

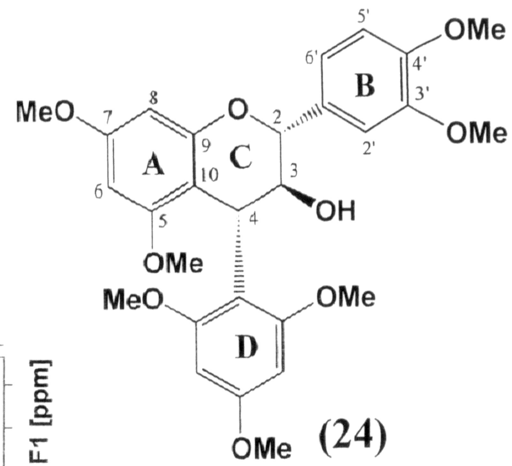



**(24)**

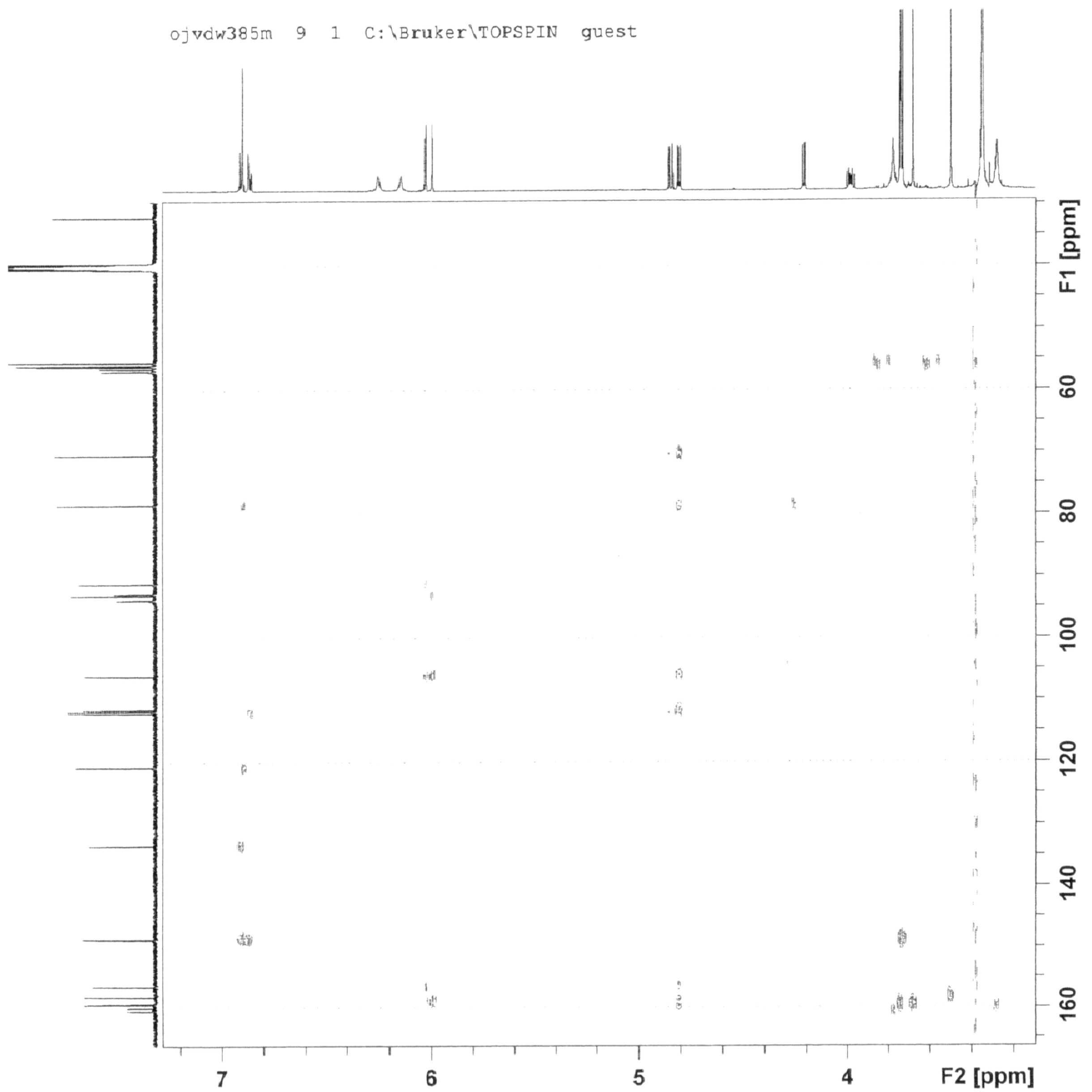
Plate 14.5: HSQC NMR (DMSO, 302K)

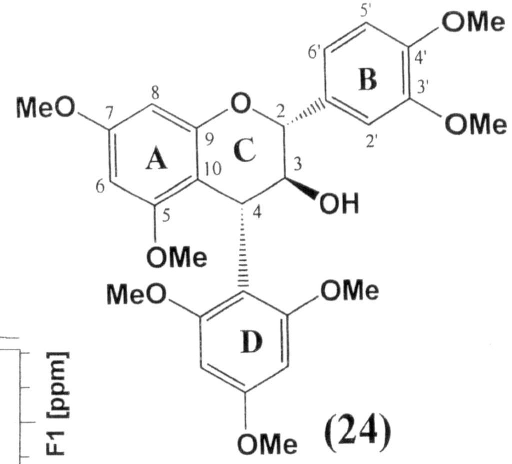



(24)

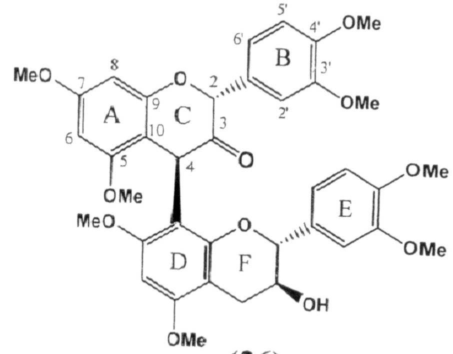
Plate 14.6: HMBC NMR (DMSO, 302K)




(24)

**Plate 16.1:** $^1$H NMR ($CDCl_3$, 298K)



**(26)**

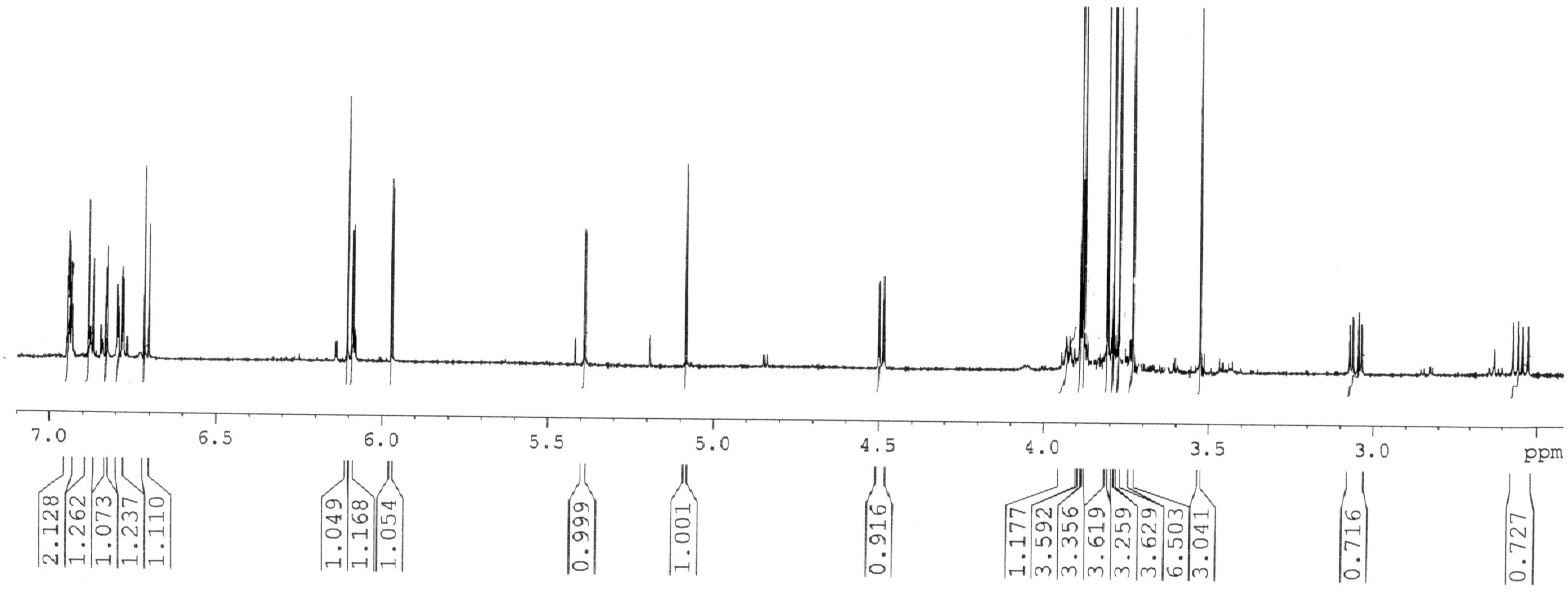
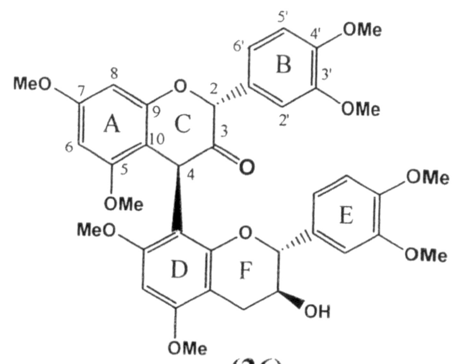
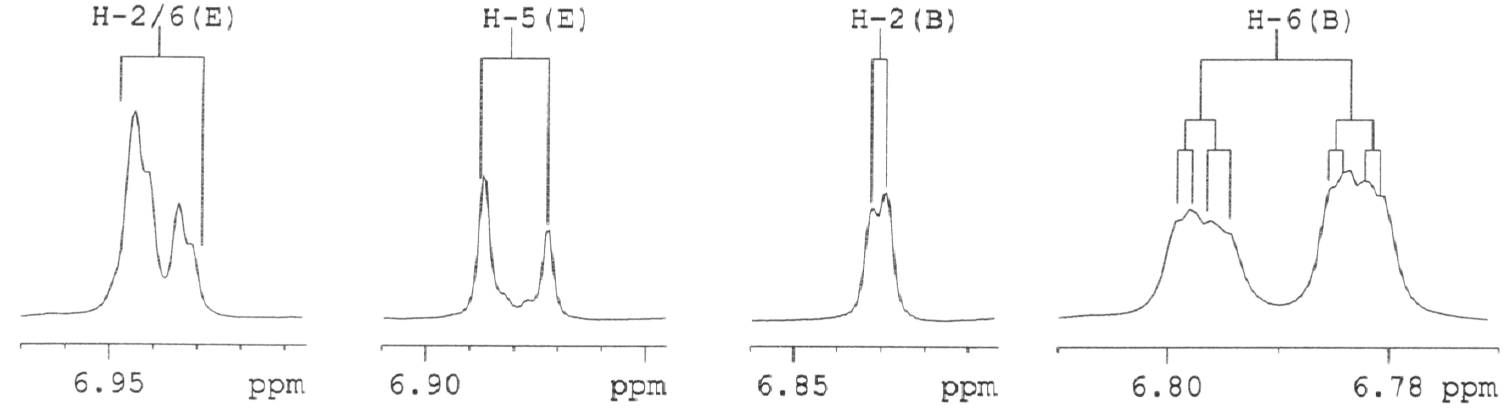


**Plate 16.2** $^1H$ NMR ($CDCl_3$, 298K)




**(26)**



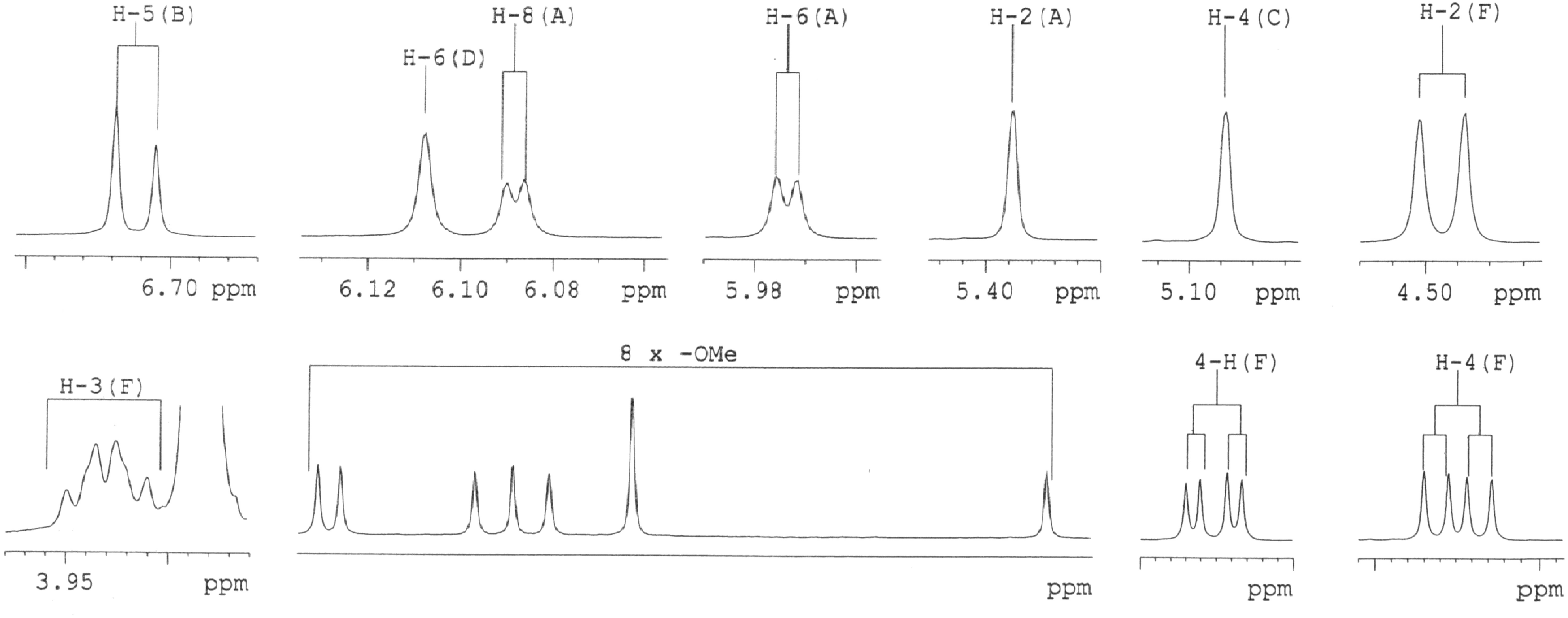
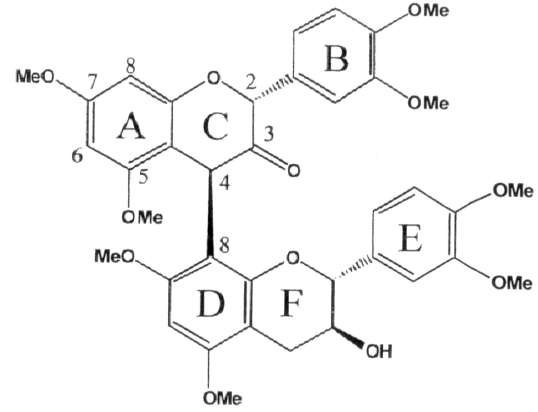



**Plate 16.3:** $^{13}$C NMR ($CDCl_3$, 298K)



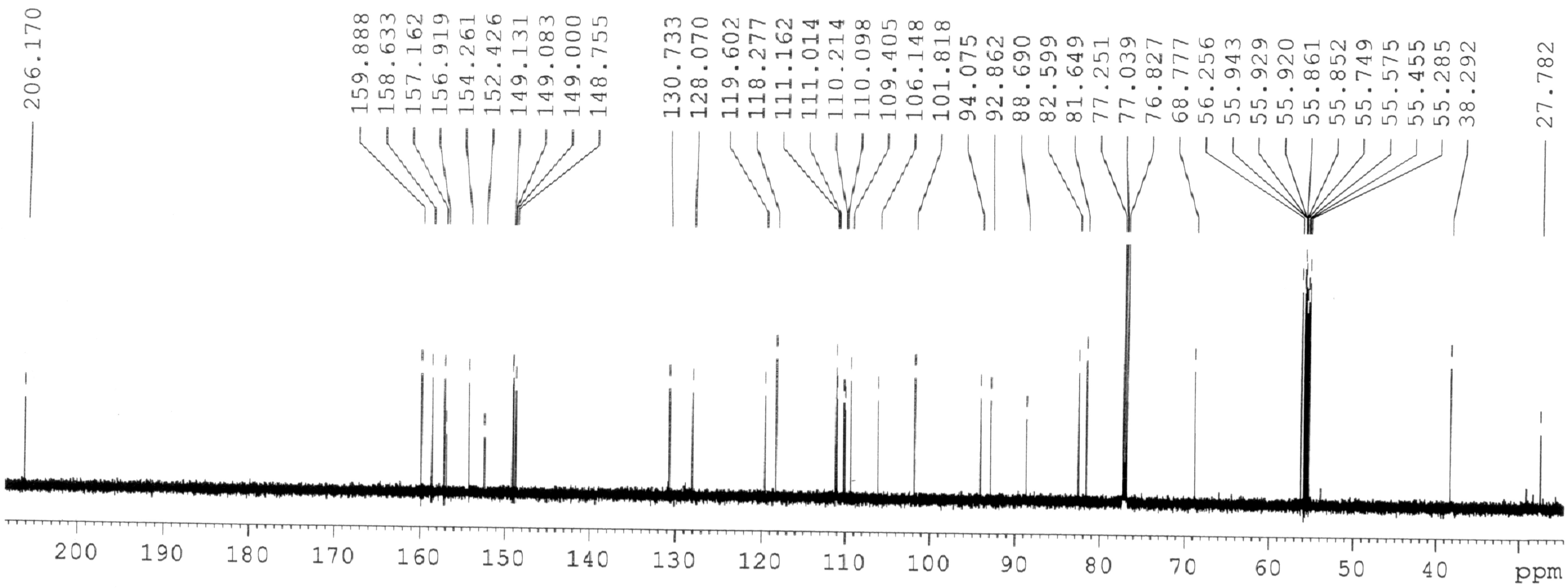
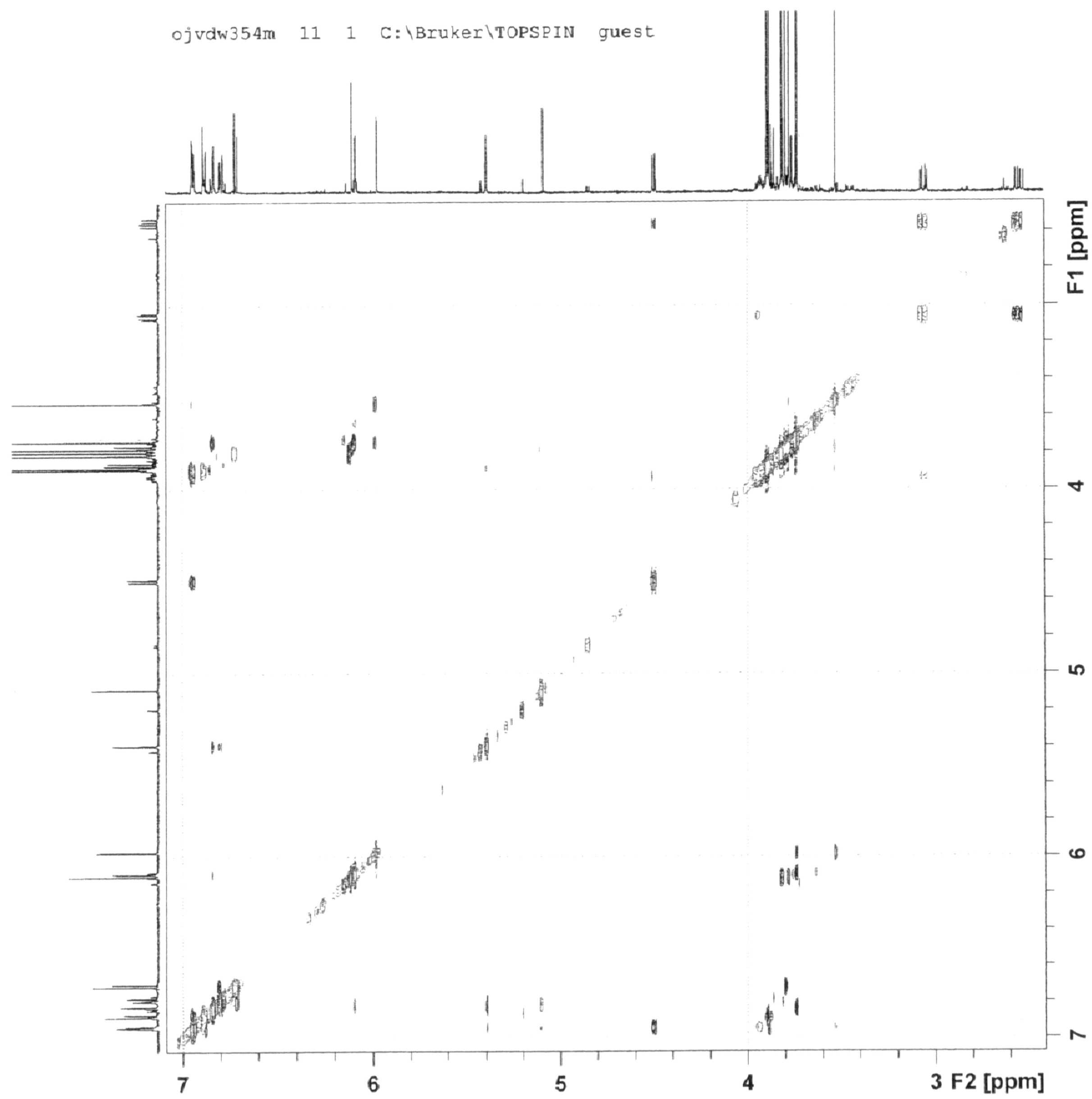
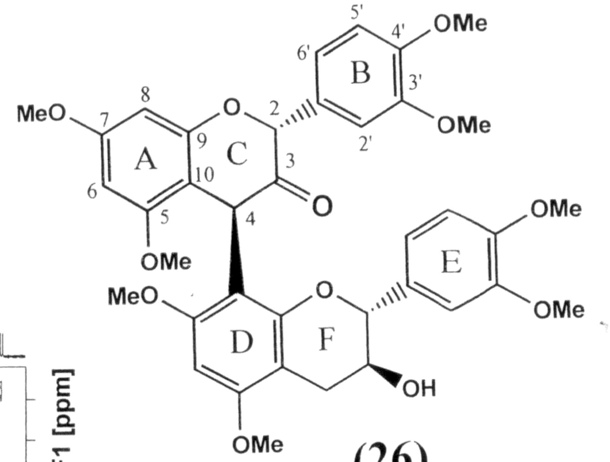



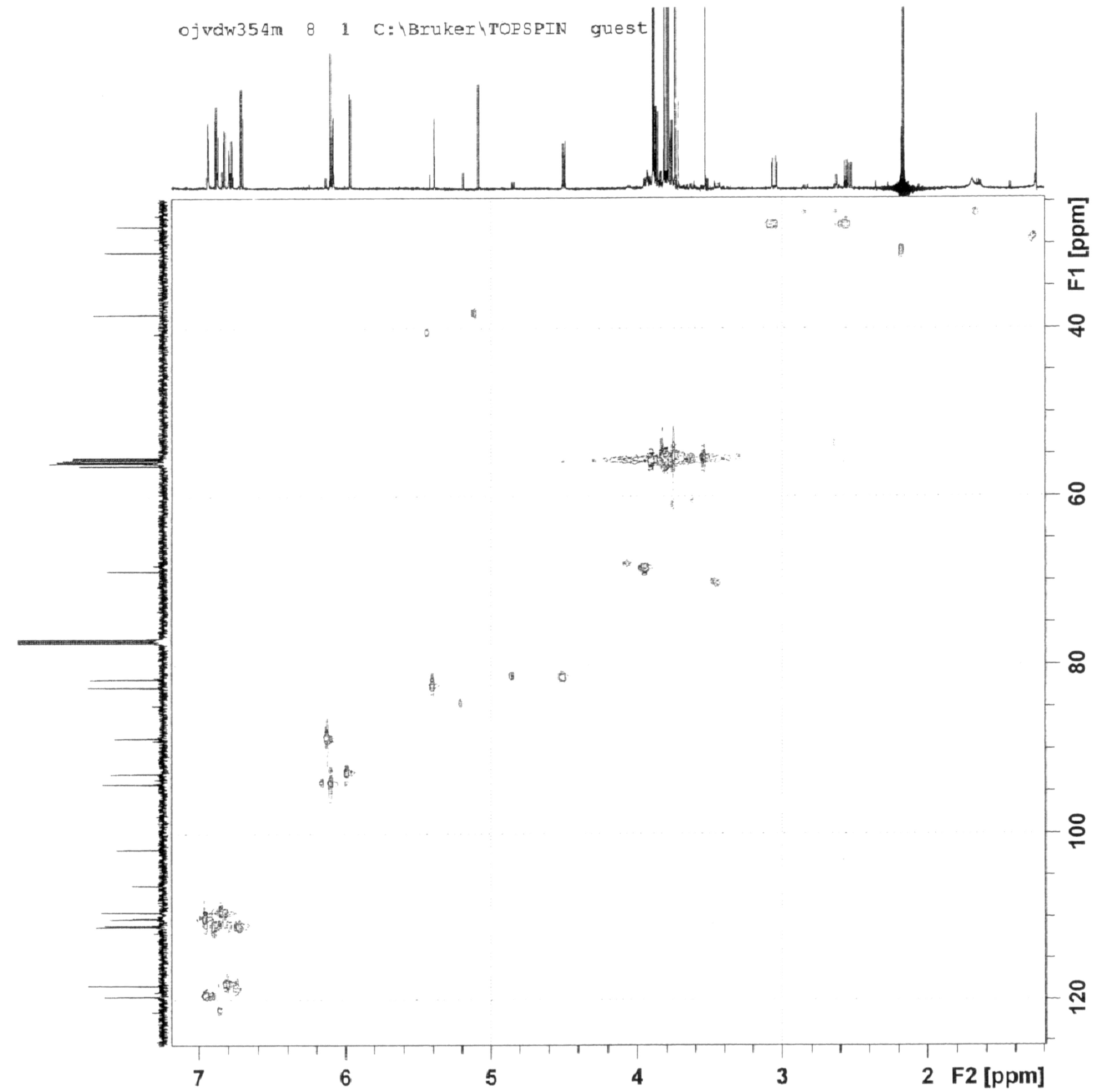
**Plate 16.4:** COSY NMR ($CDCl_3$, 298K)

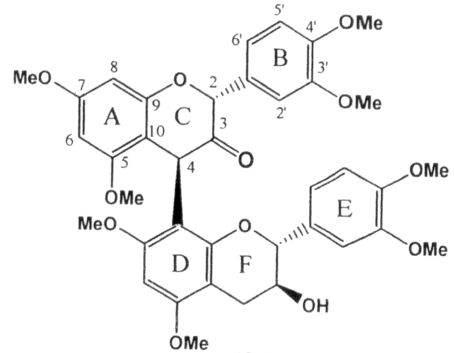



**(26)**

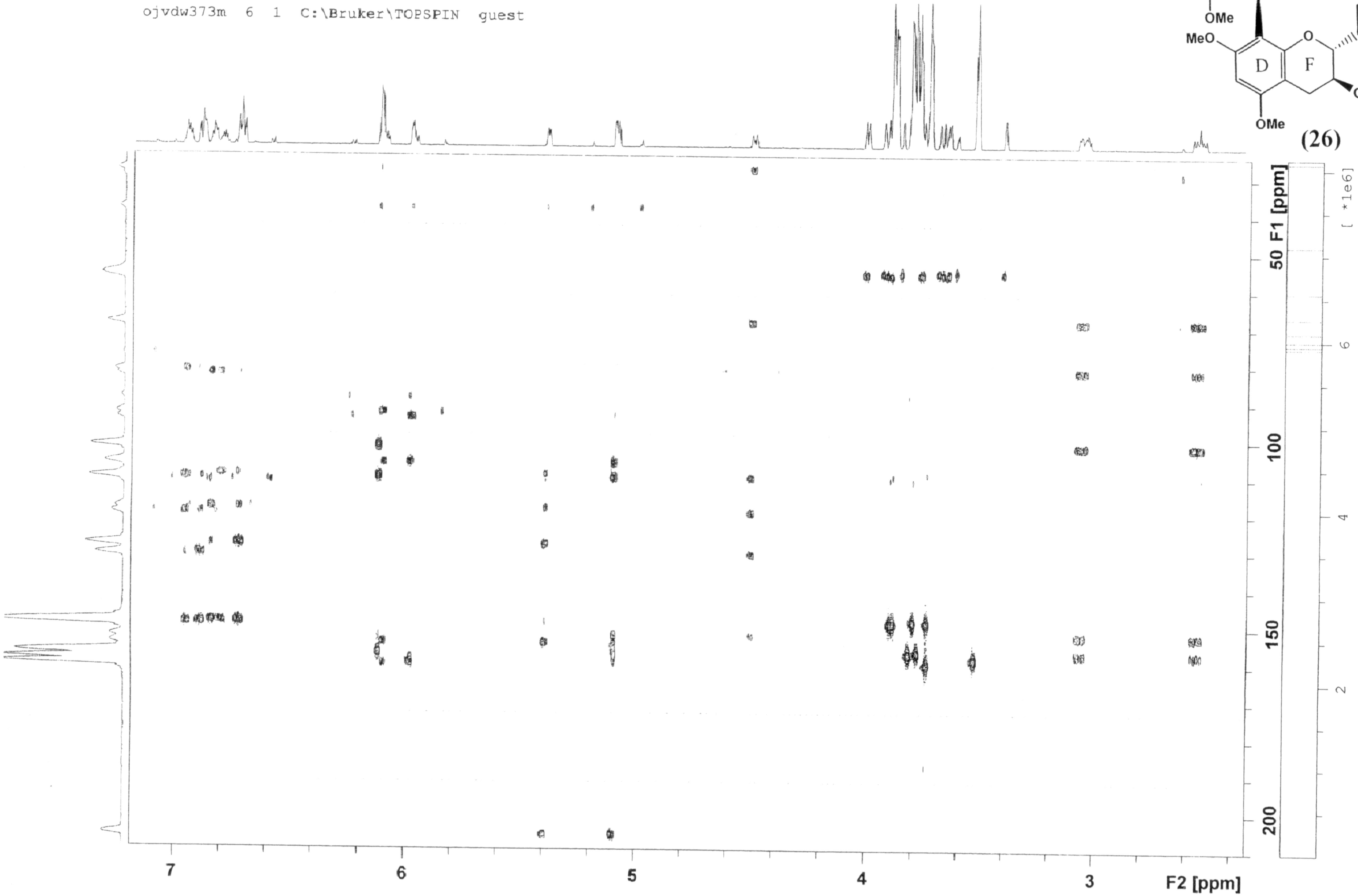
Plate 16.5: HSQC NMR ($CDCl_3$, 298K)

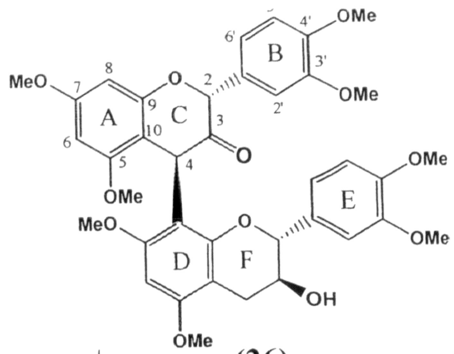



(26)

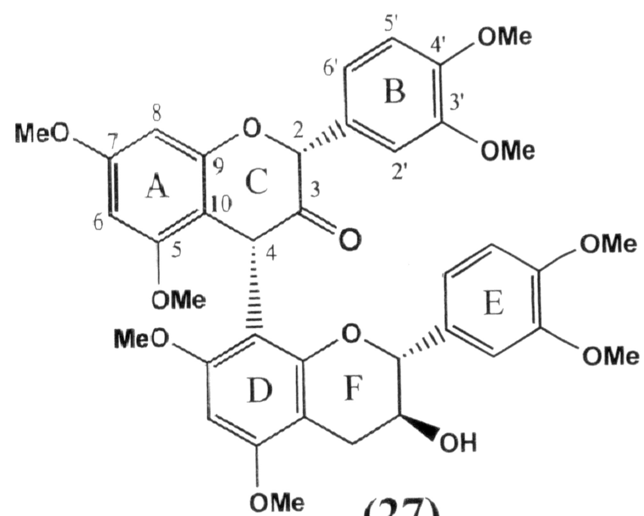
Plate 16.6: HMBC ($CDCl_3$, 298K)




(26)

Plate 17.1: $^{1}$H NMR ($CDCl_3$, 298K)




(27)

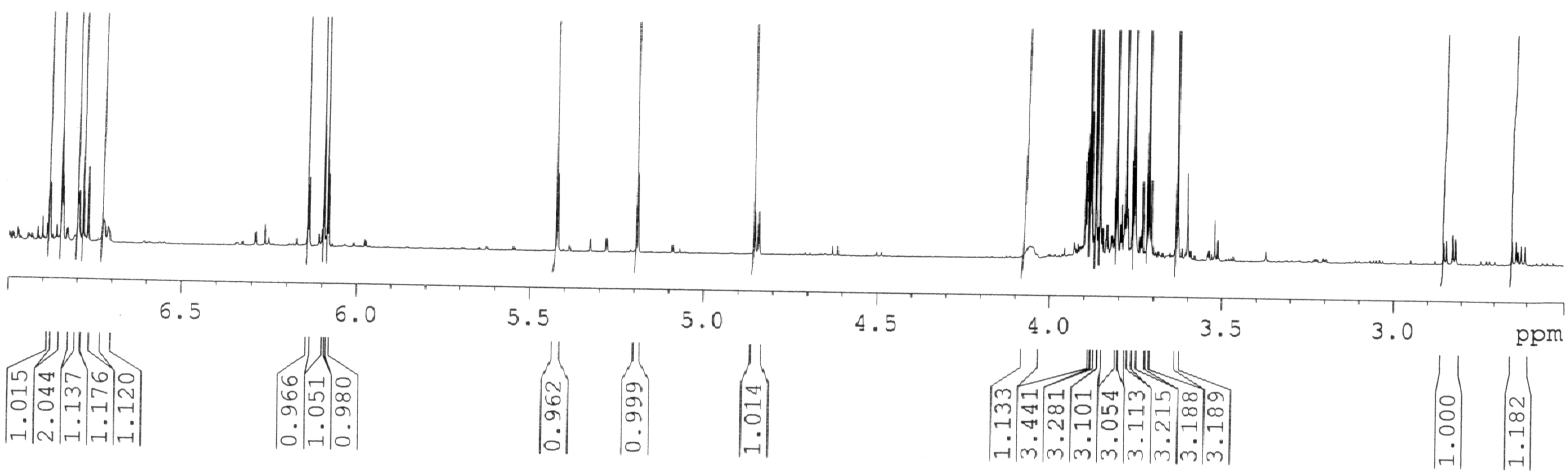
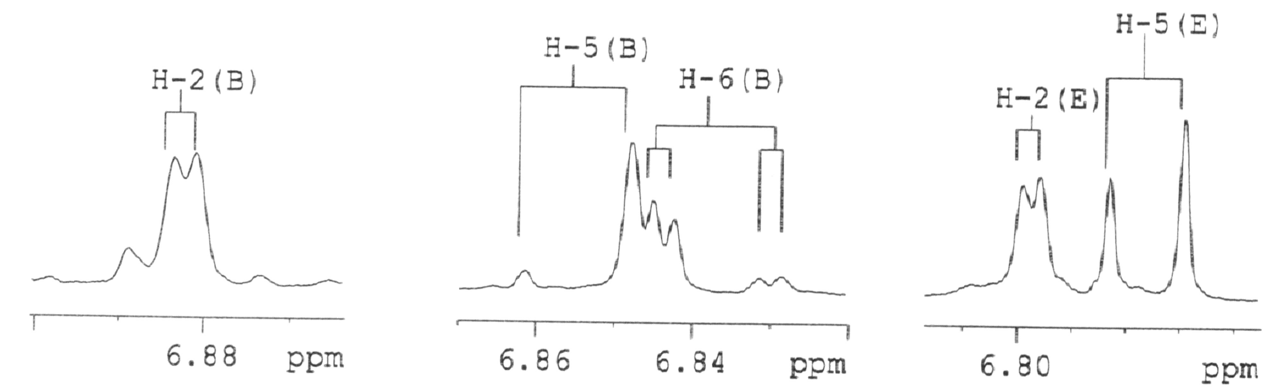
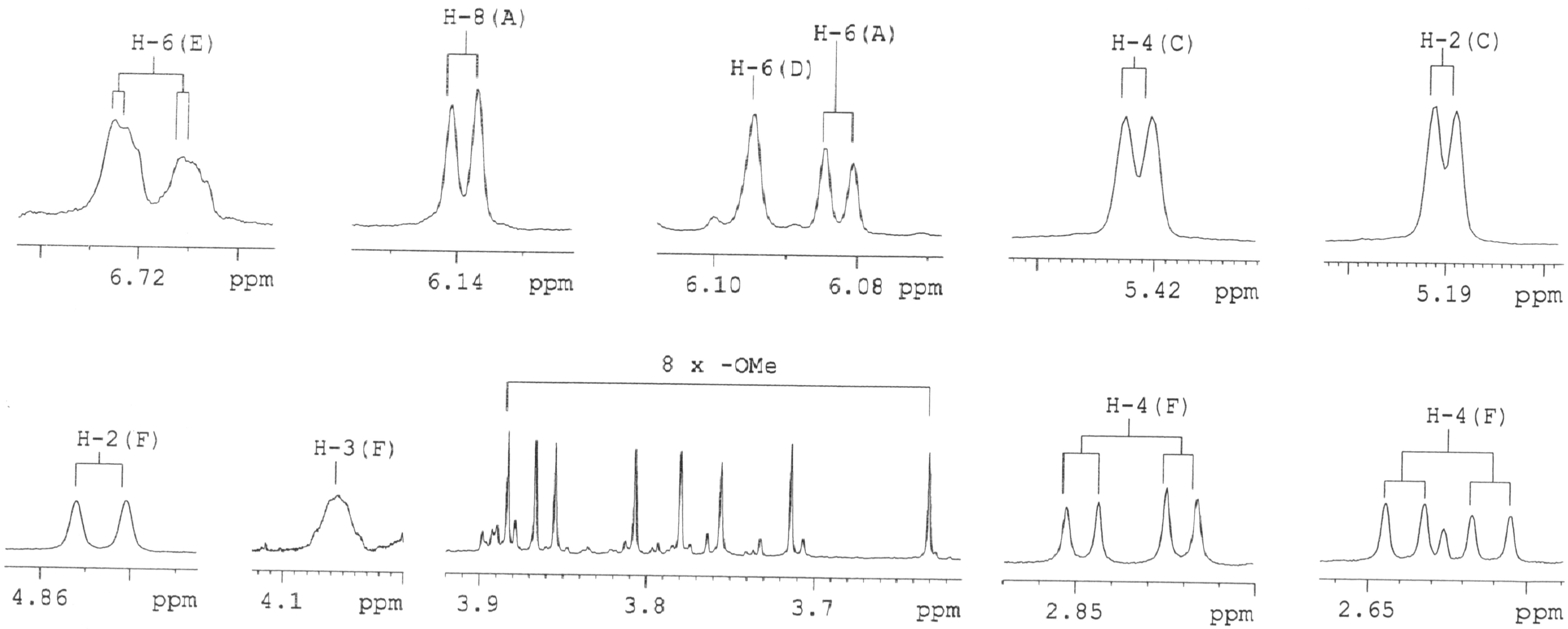
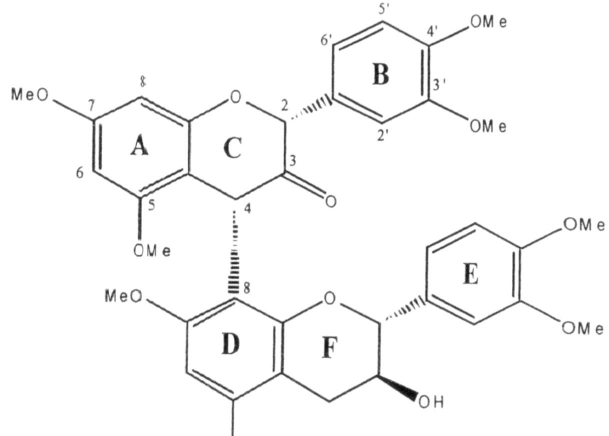


**Plate 17.2:** $^1H$ NMR ($CDCl_3$, 298K)

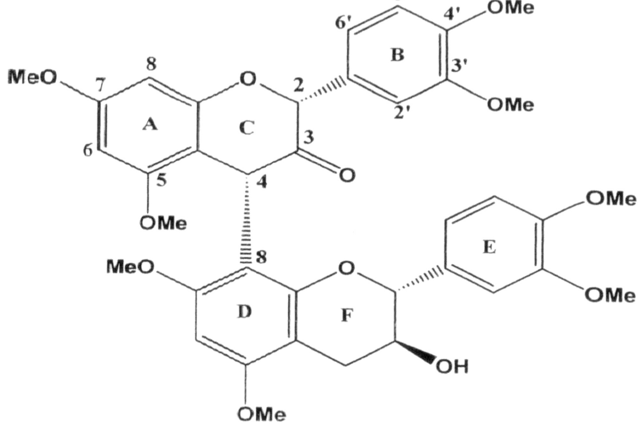



**(27)**

**Plate 17.3:** $^{13}C$ NMR ($CDCl_3$, 298K)



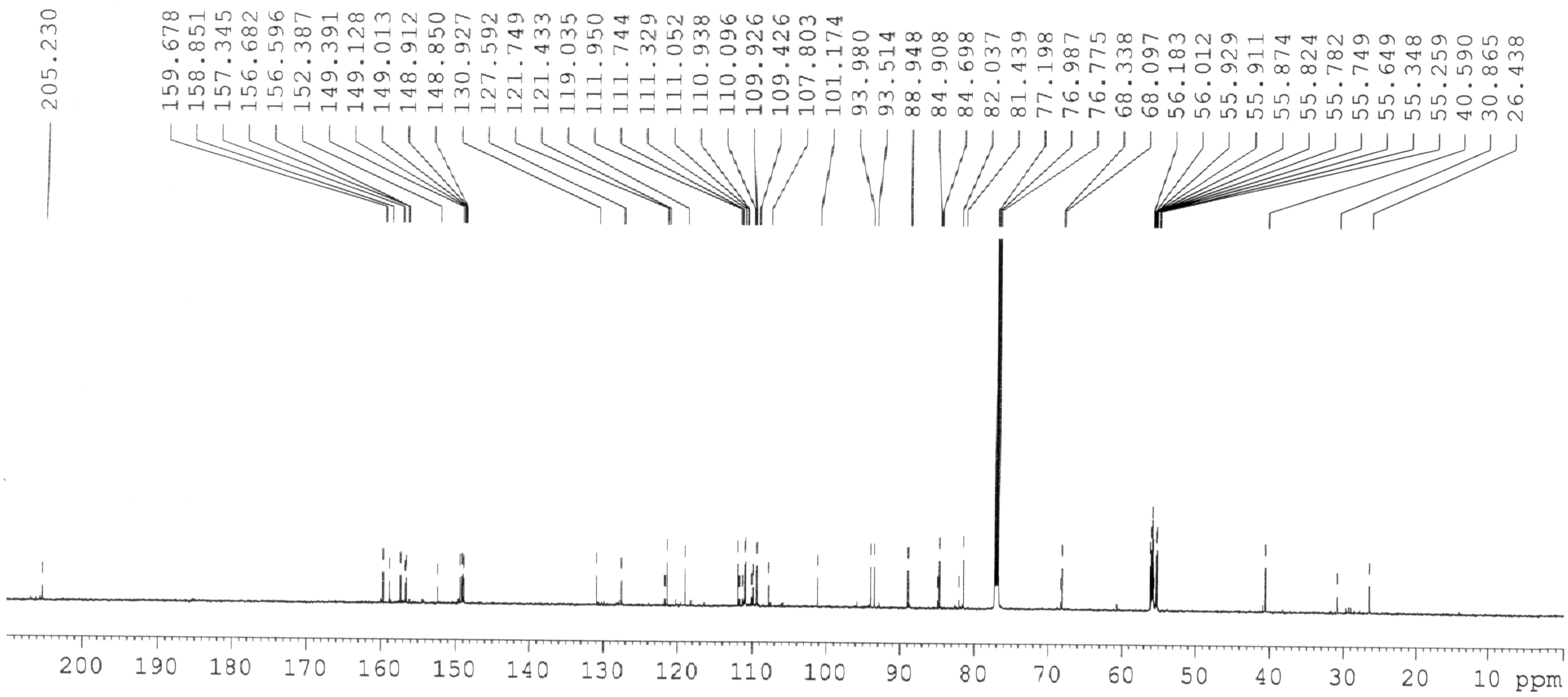
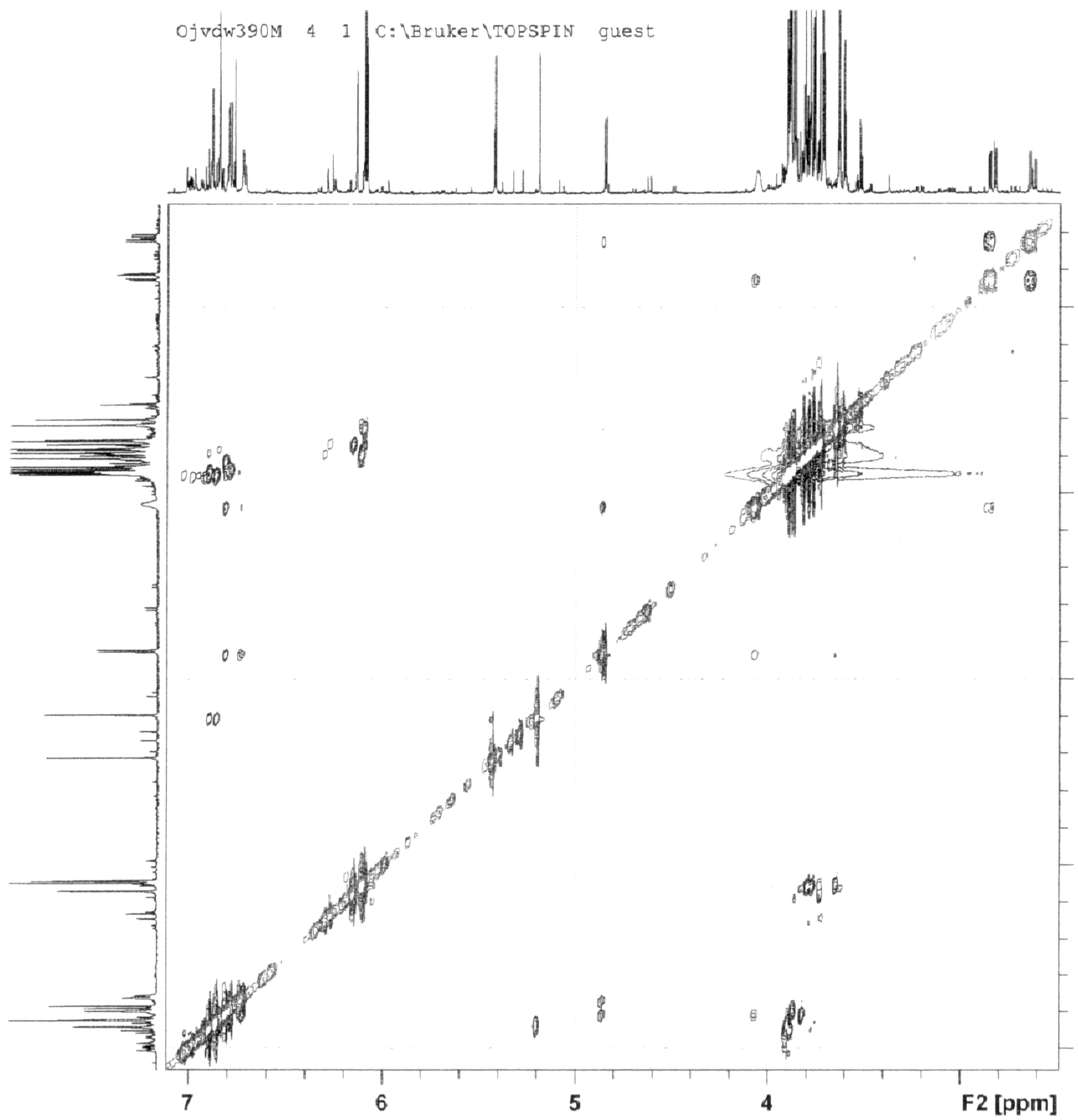
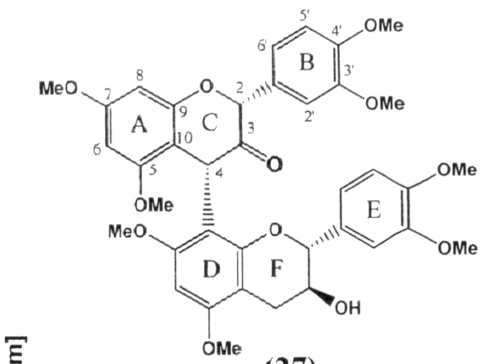



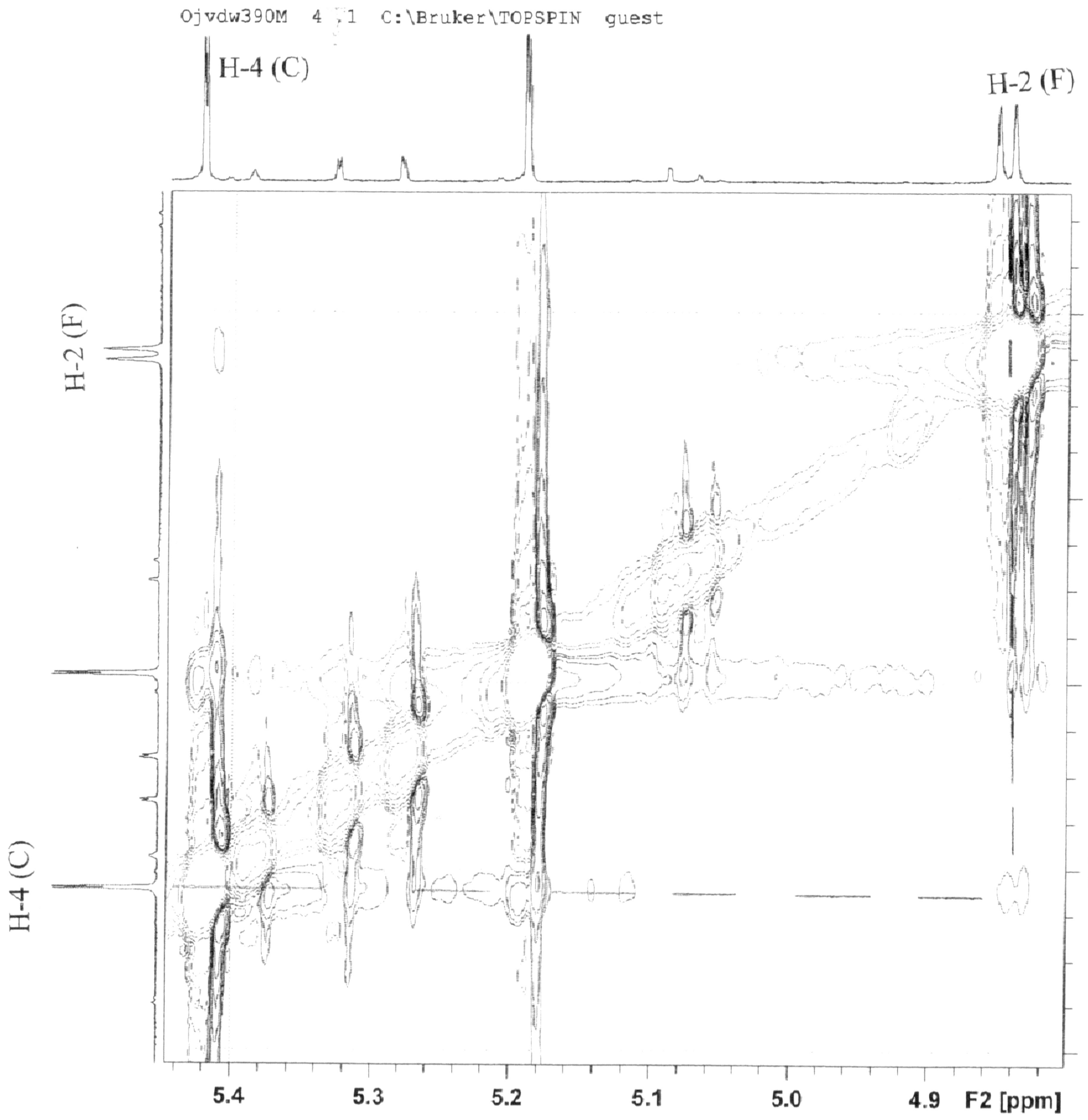
**Plate 17.4:** NOESY NMR ($CDCl_3$, 298K)

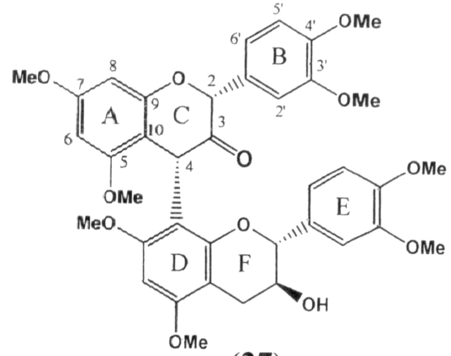



**(27)**

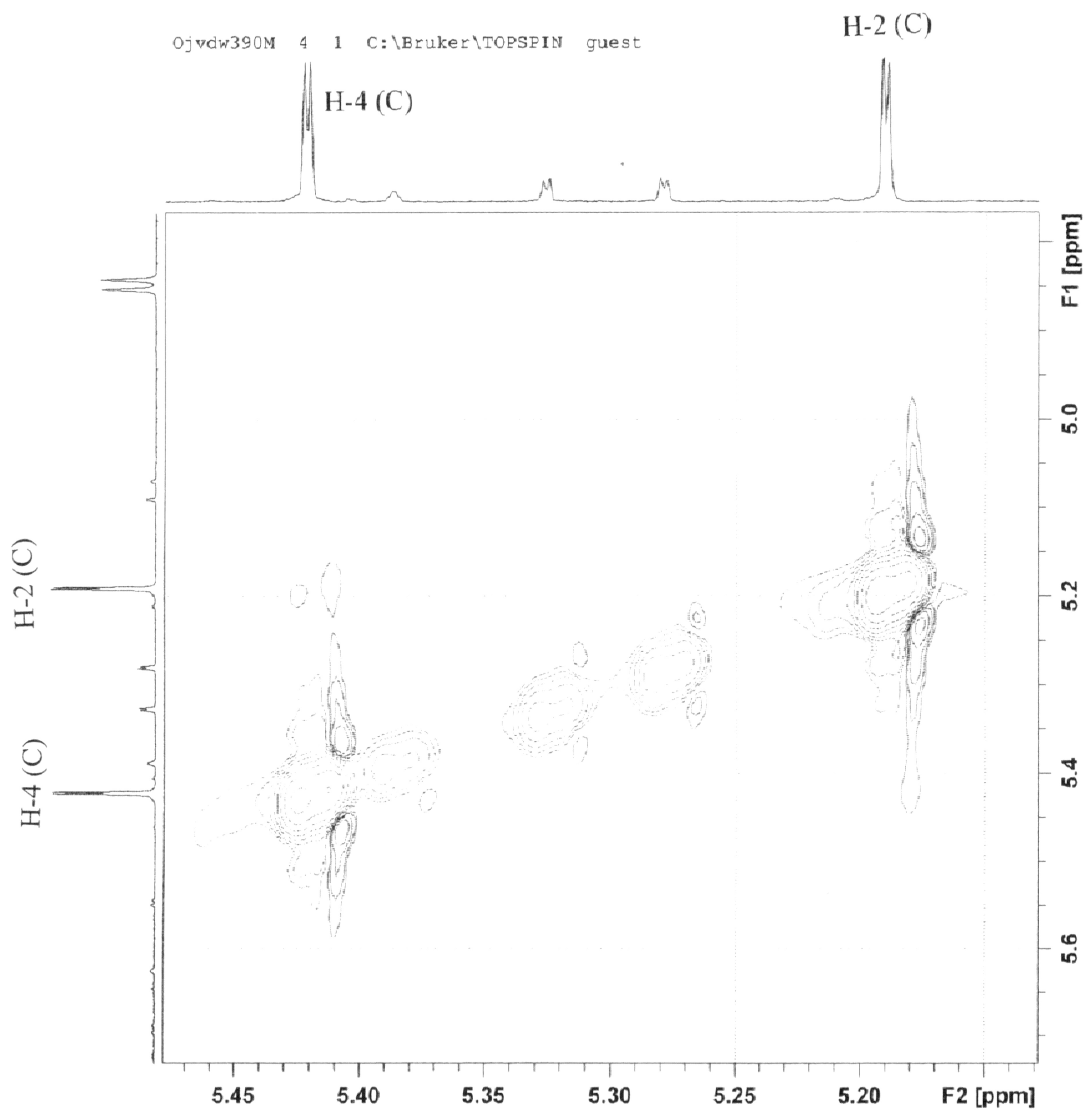
**Plate 17.5:** NOESY NMR ($CDCl_3$, 298K)

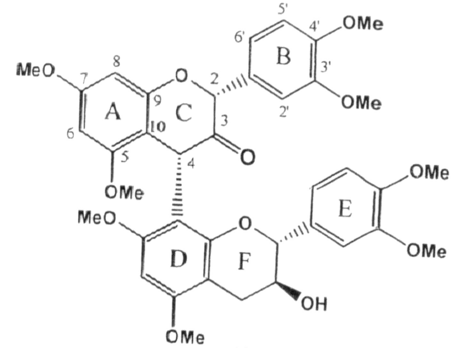



**(27)**

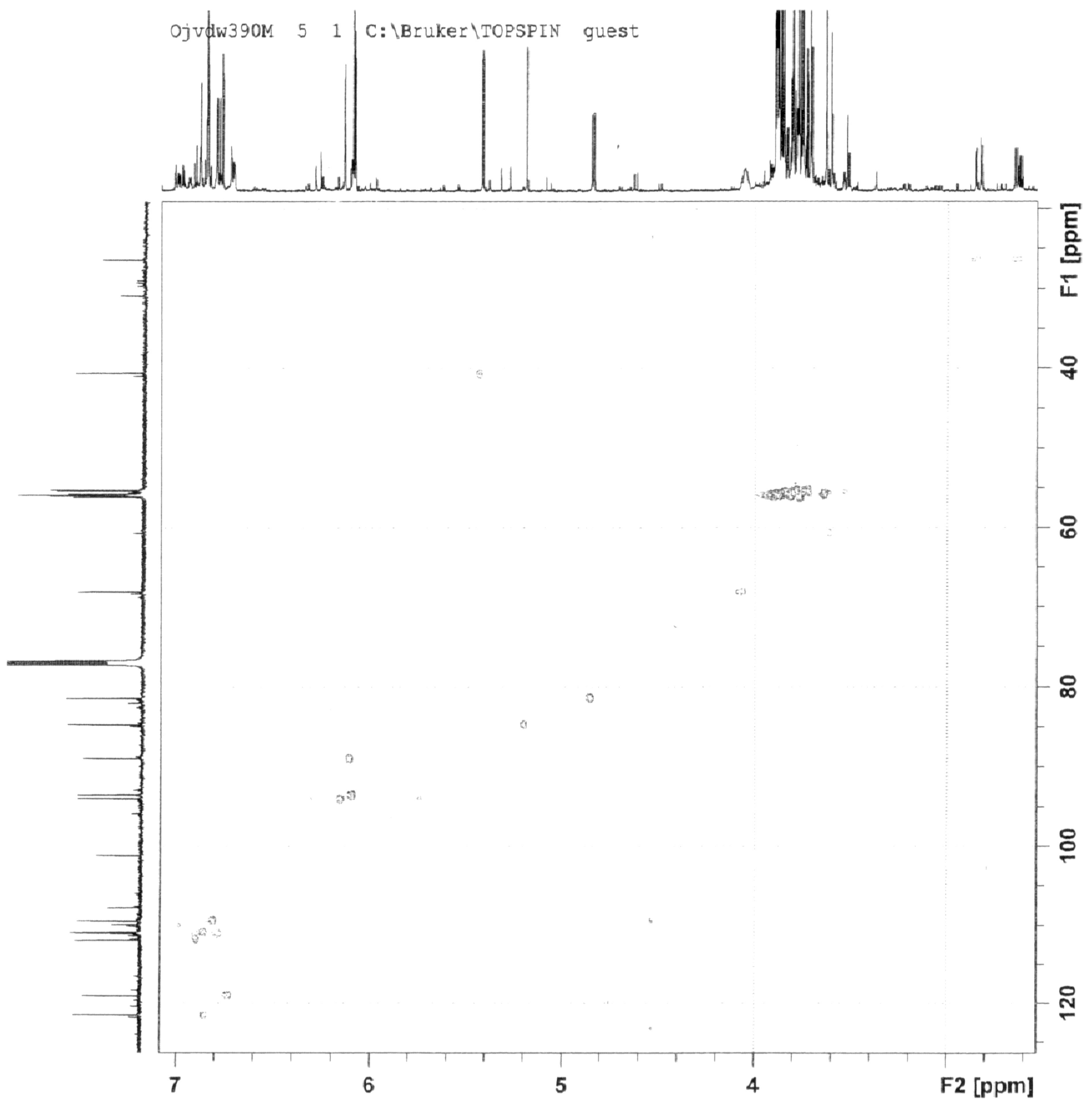
**Plate 17.6:** NOESY NMR ($CDCl_3$, 298K)

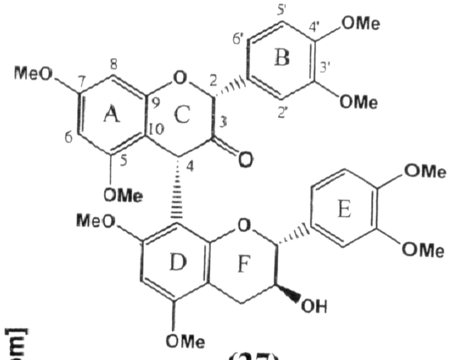



**(27)**

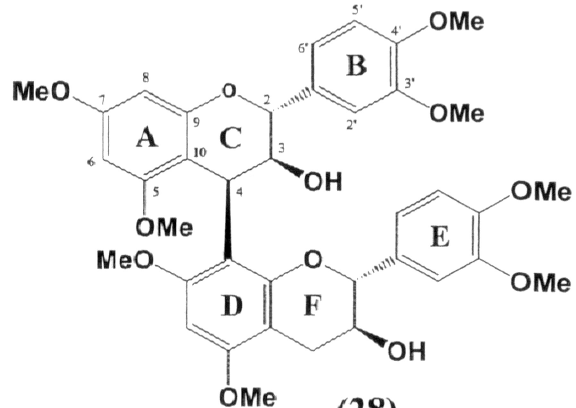
**Plate 17.7:** HSQC NMR ($CDCl_3$, 298K)




**(27)**

Plate 18.1: $^1$H NMR (DMSO, 298K)




(28)

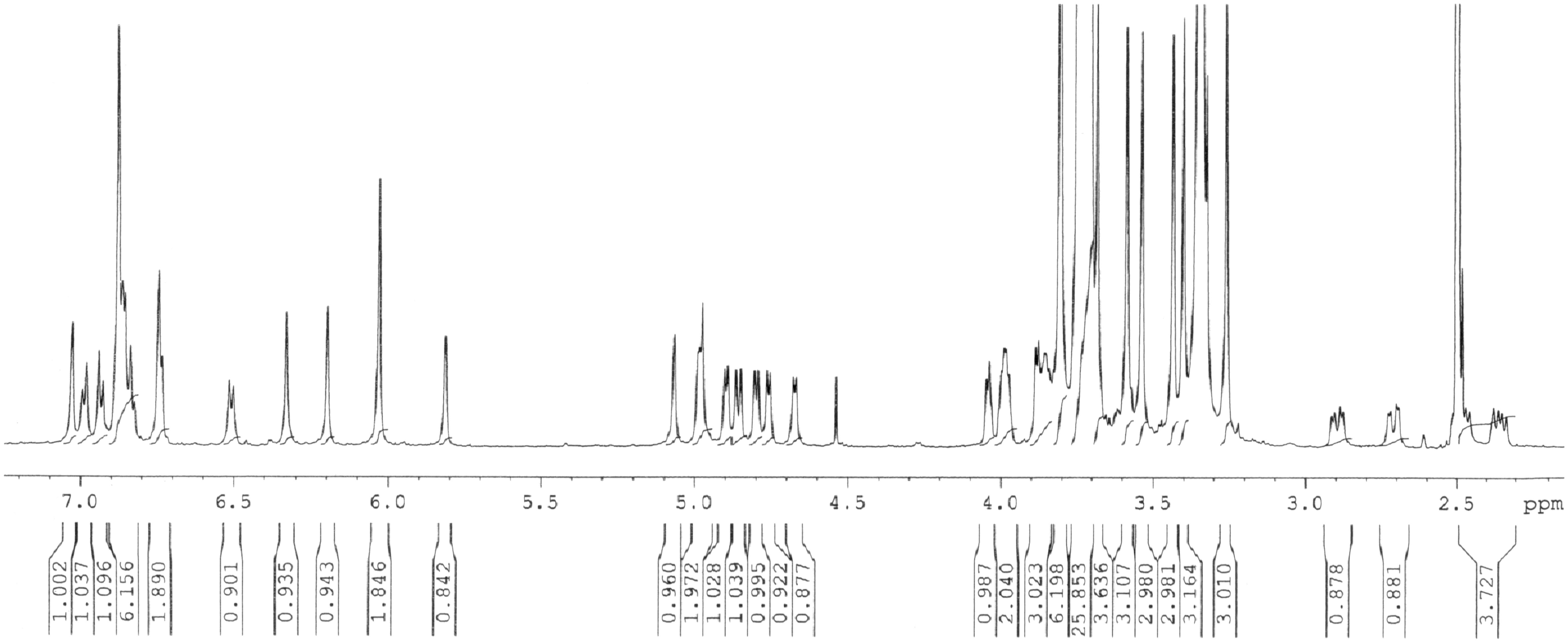
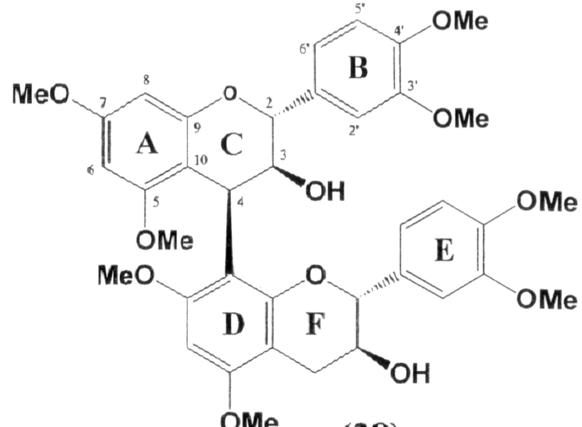
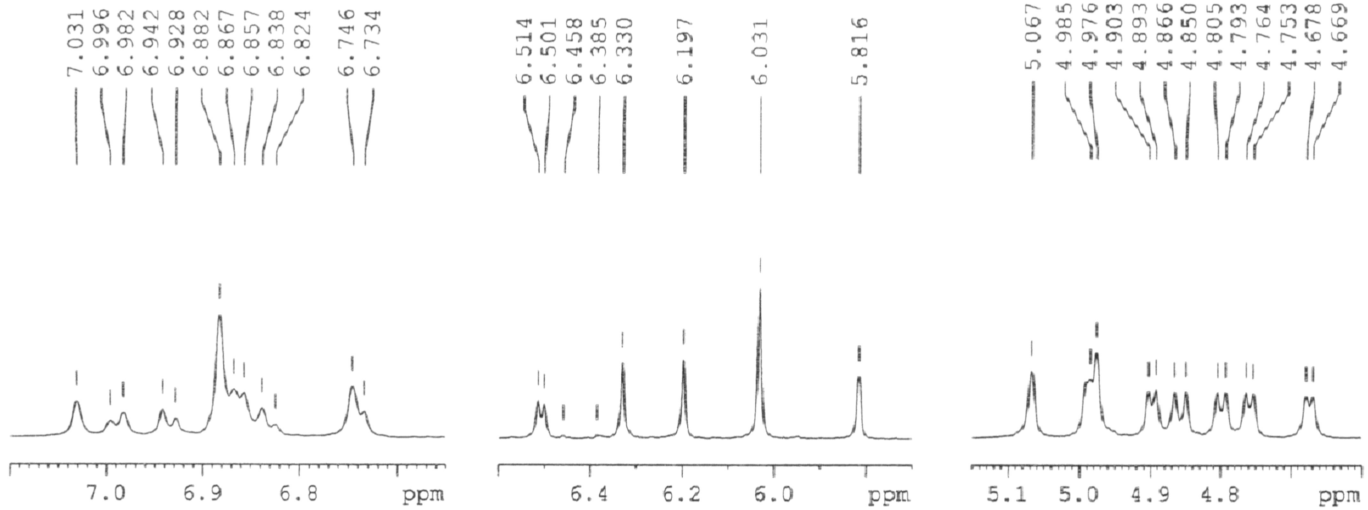


Plate 18.2: $^{1}$H NMR (DMSO, 298K)



(28)



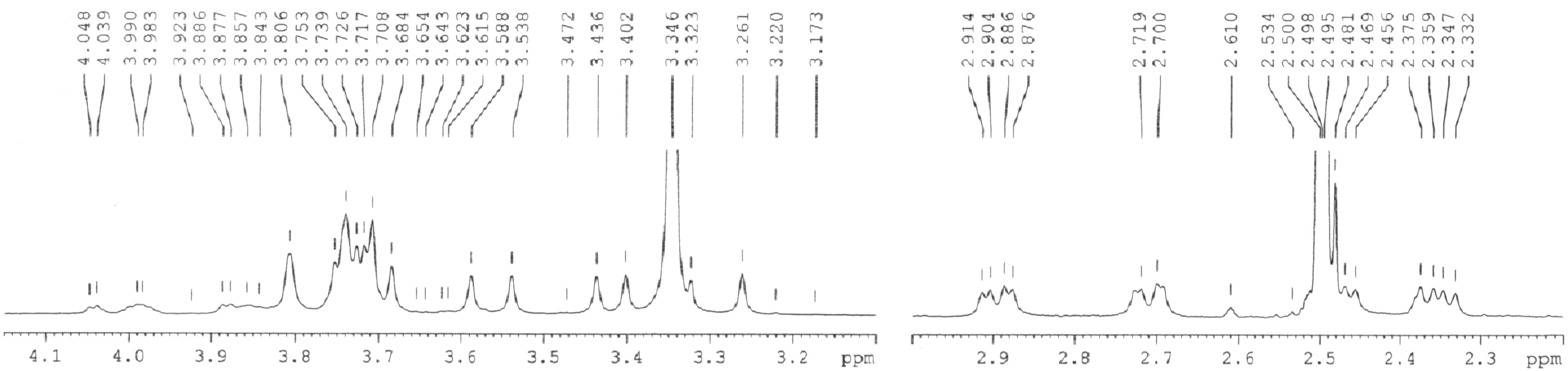
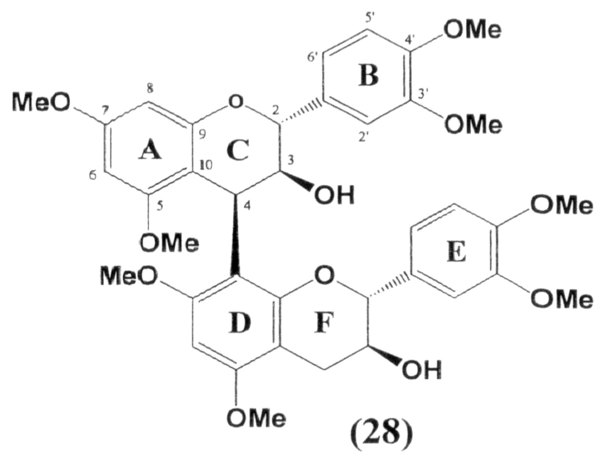
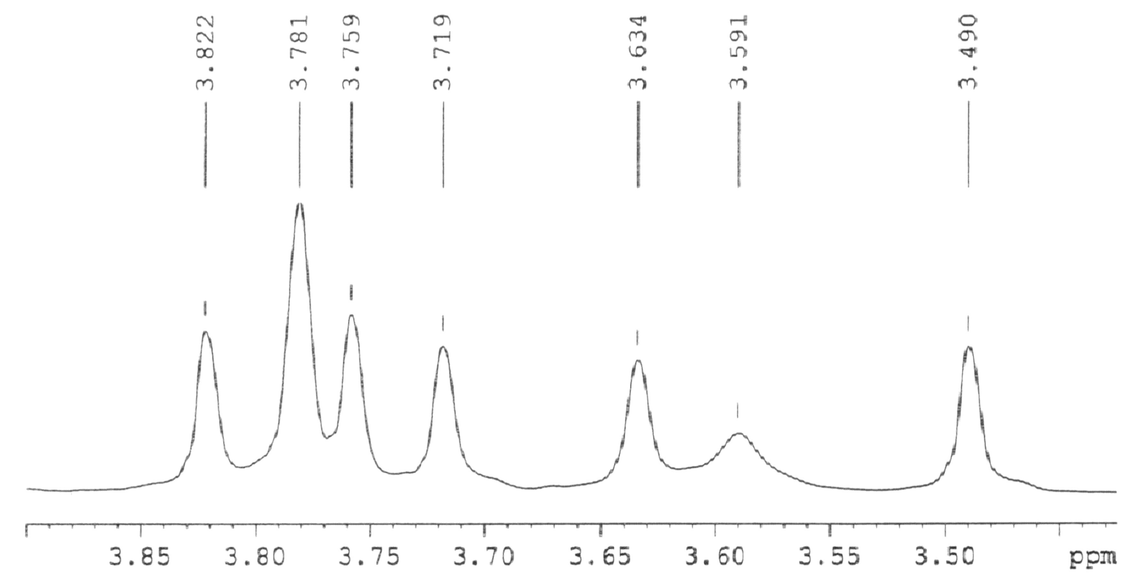



**Plate 18.3** $^{1}$H NMR (DMSO, 418K)




(28)



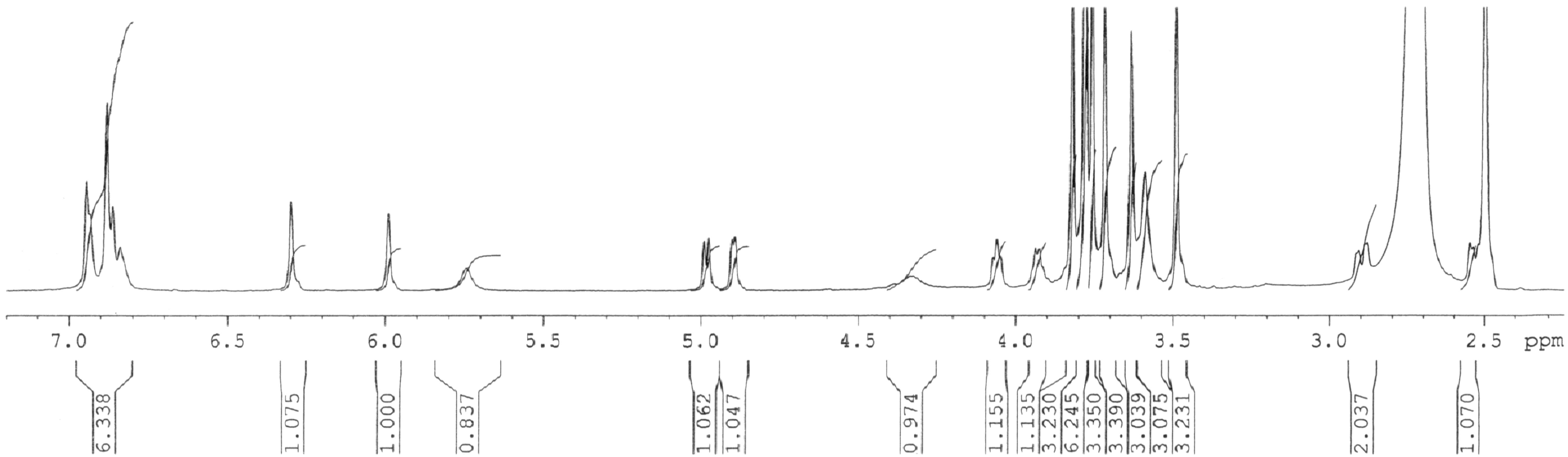
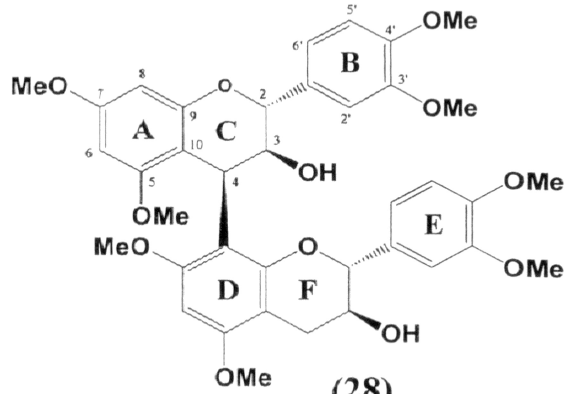



Plate 18.4: $^{13}C$ NMR (DMSO, 418K)




(28)

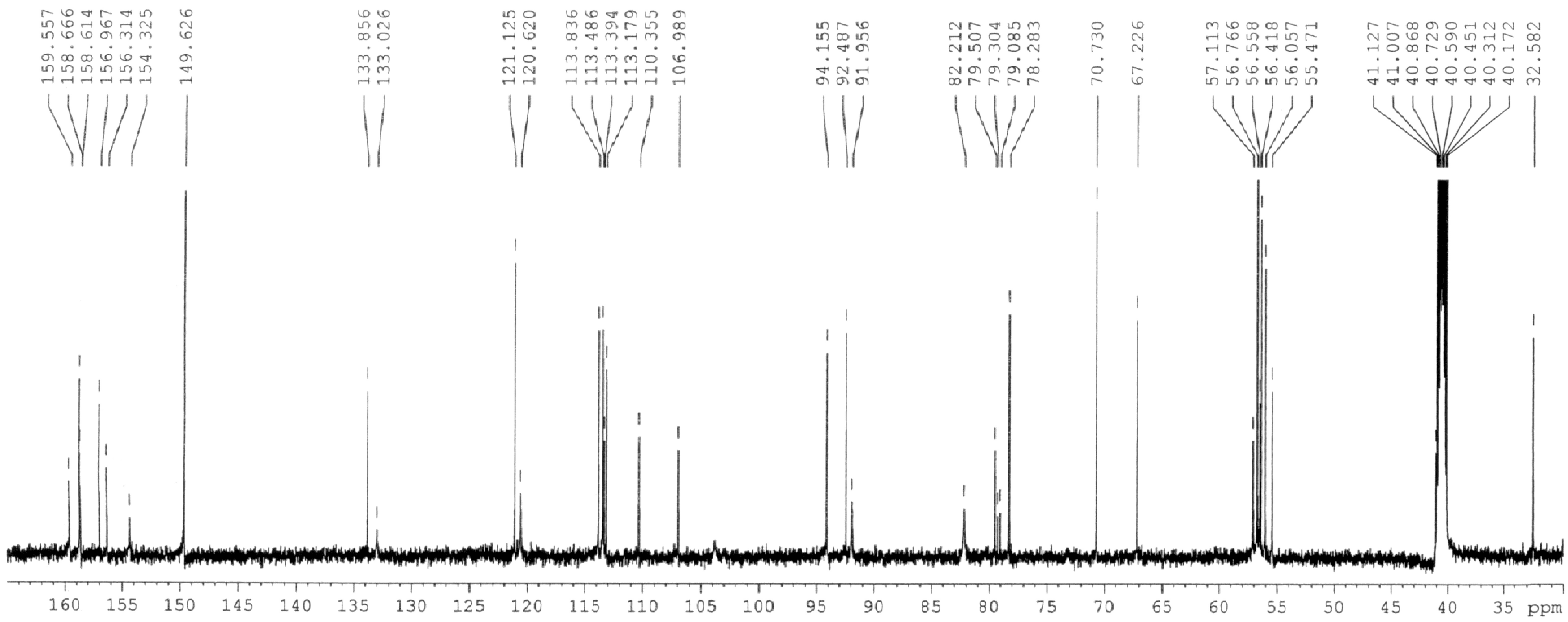
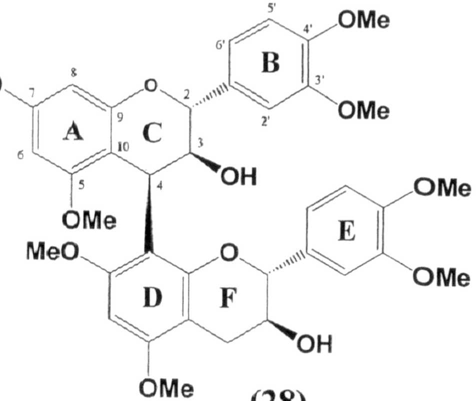
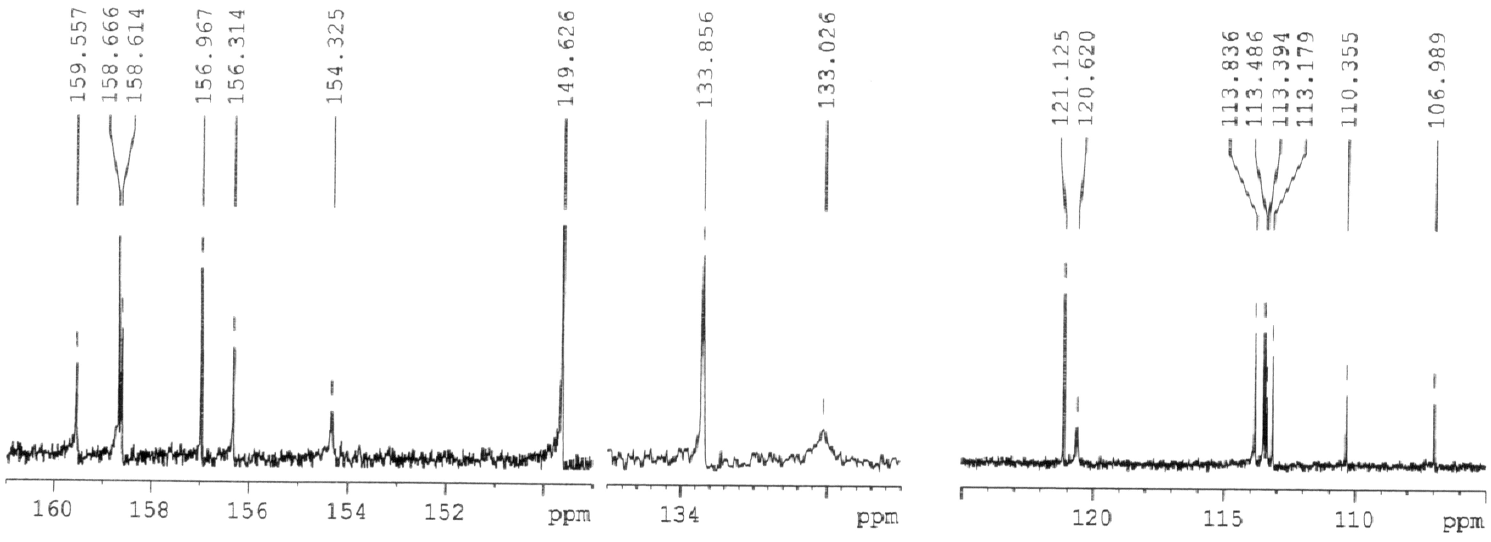


**Plate 18.5:** $^{13}C$ NMR (DMSO, 298K)



(28)



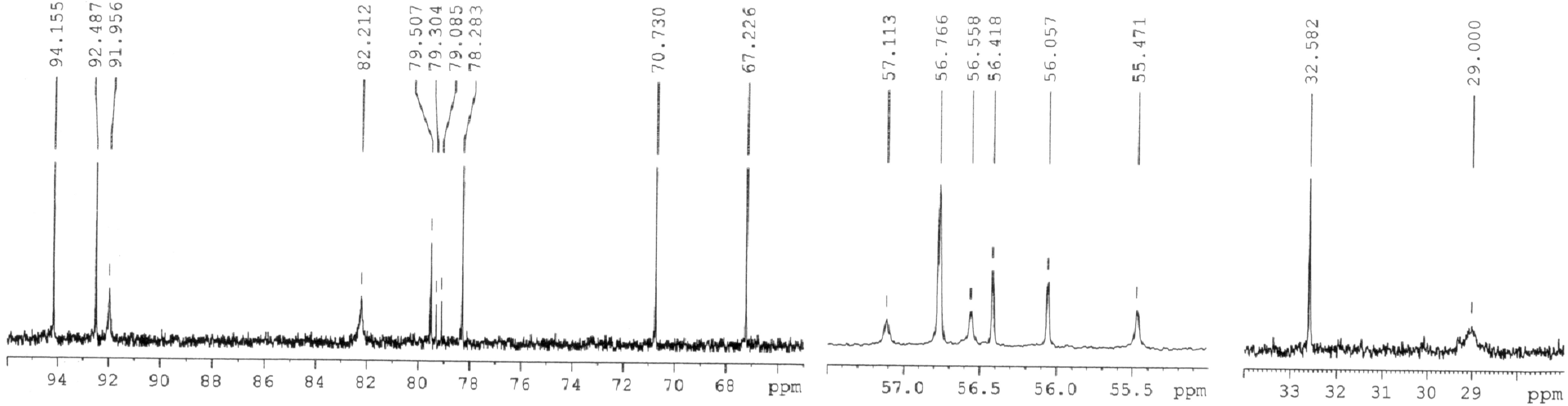
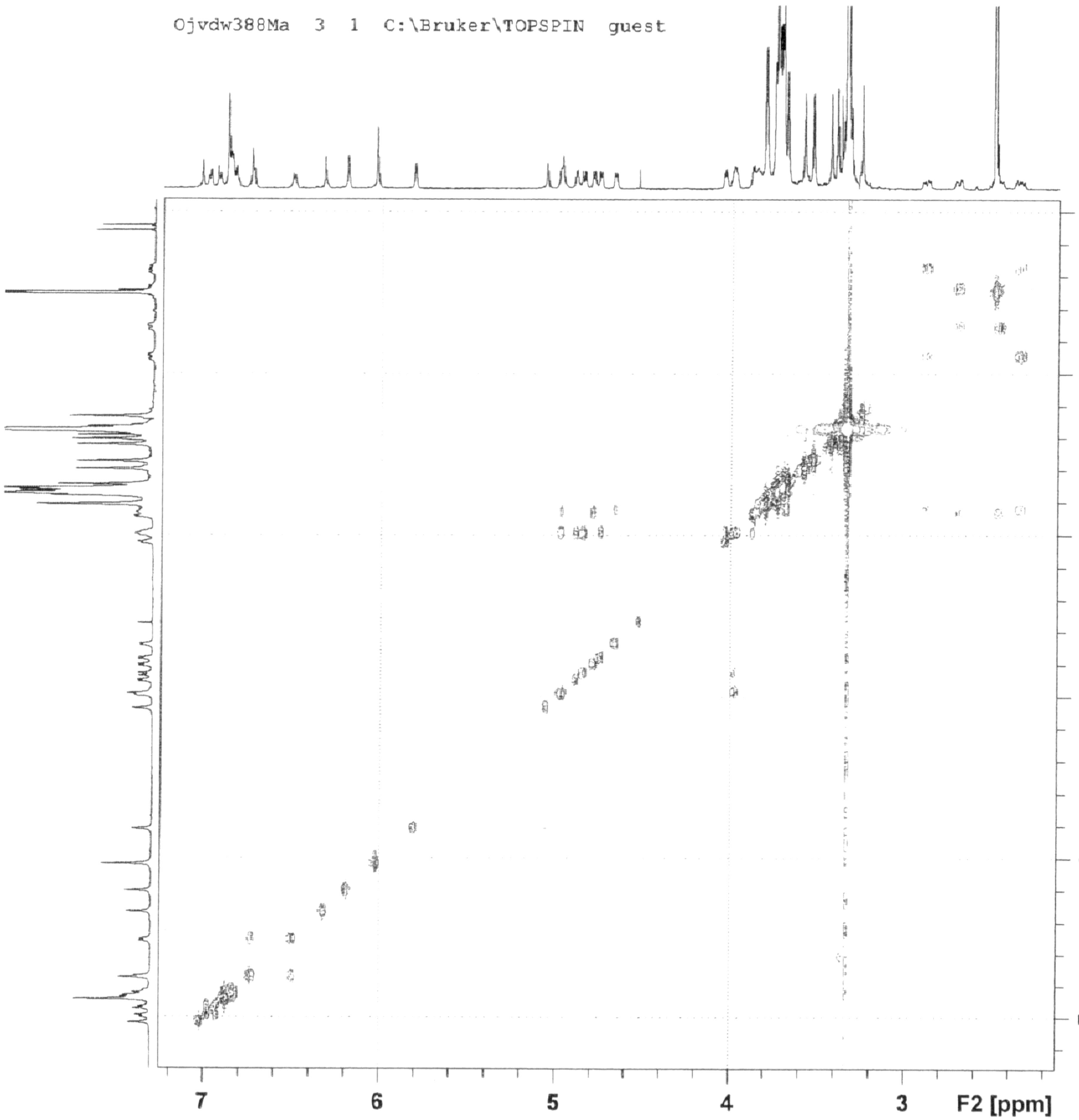
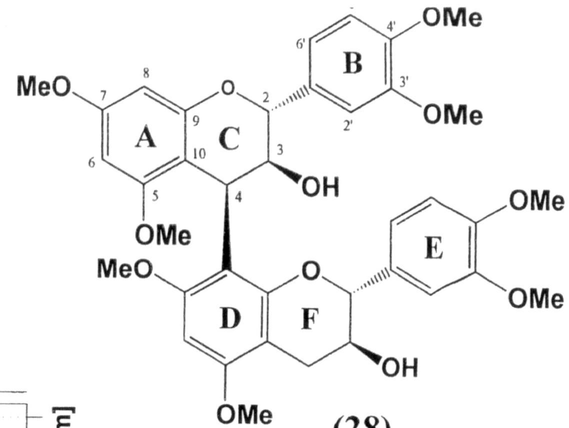



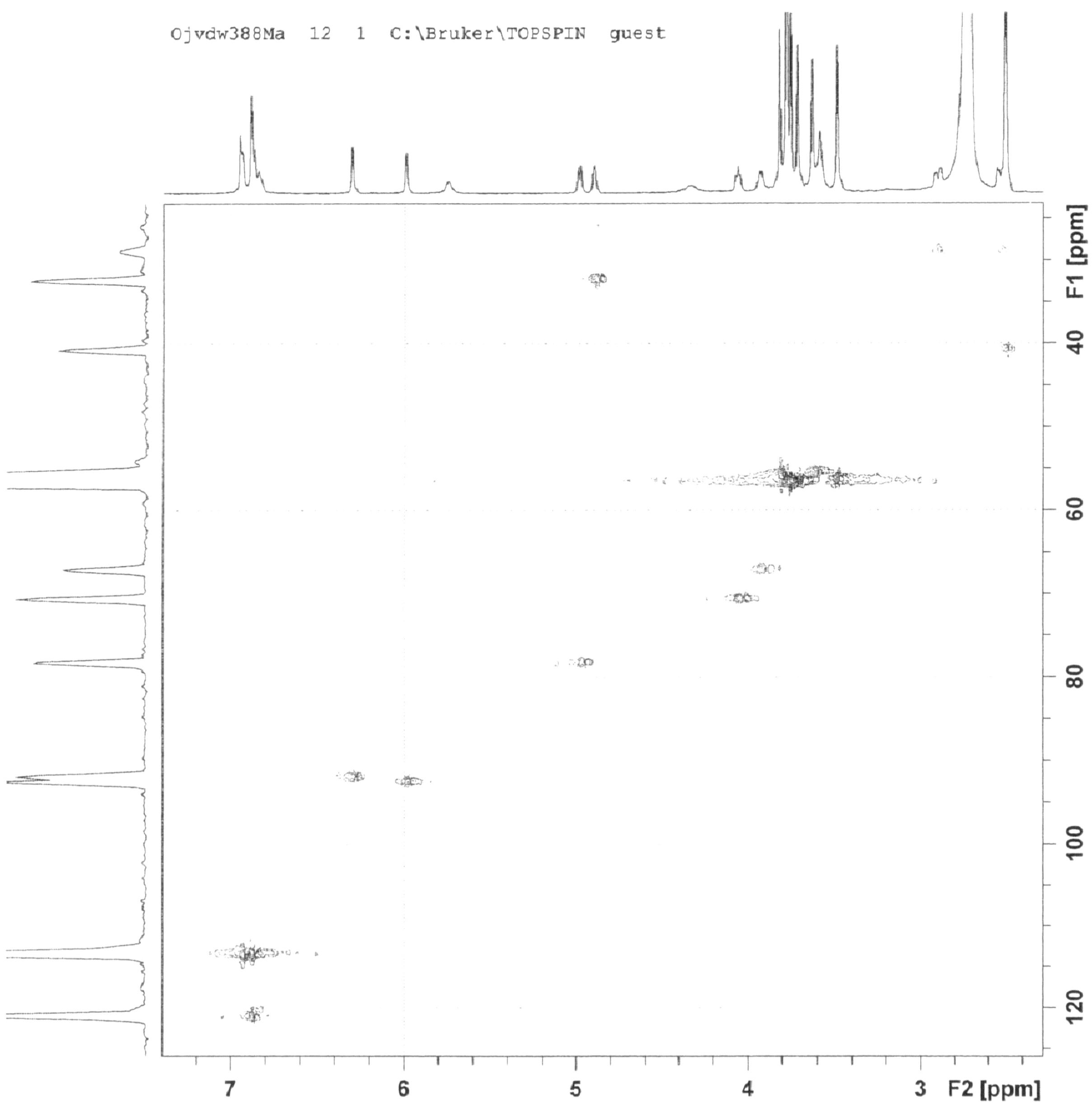
**Plate 18.6:** COSY NMR (DMSO, 298K)

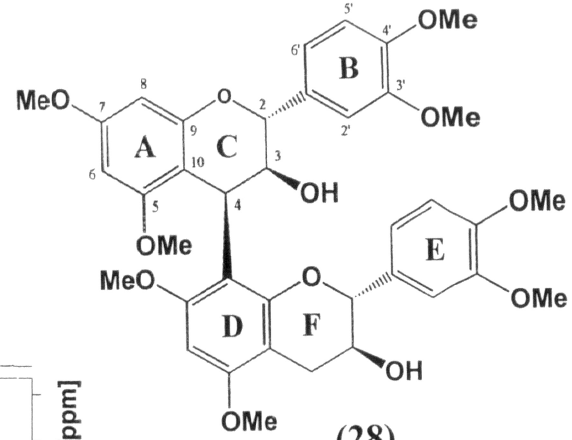



(28)

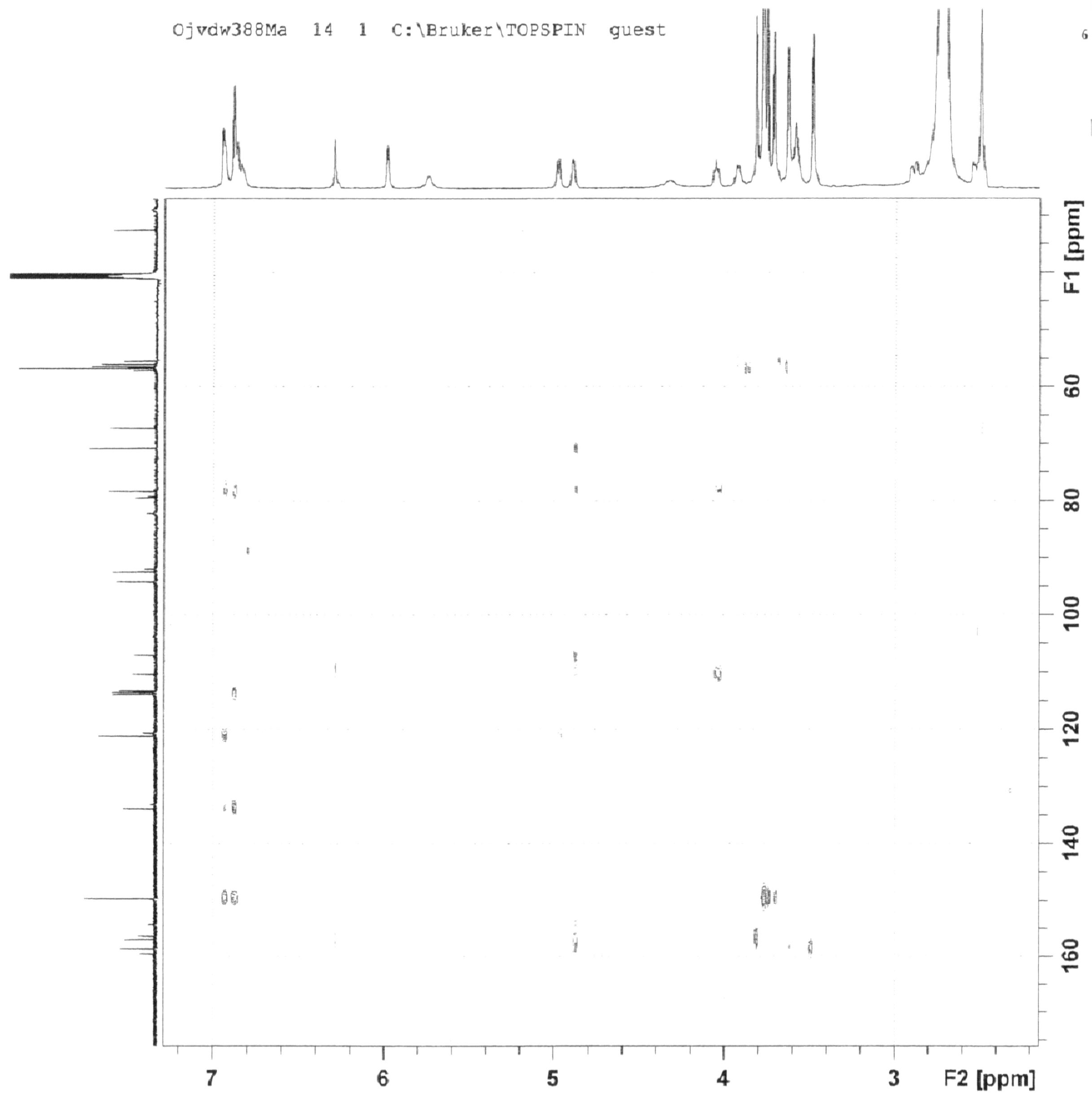
**Plate 18.7:** HSQC NMR (DMSO, 298K)

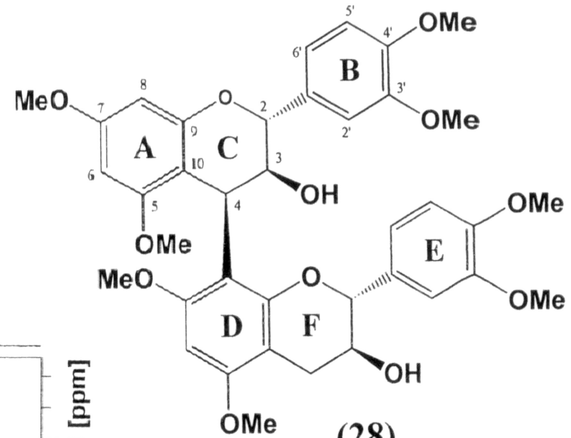


(28)

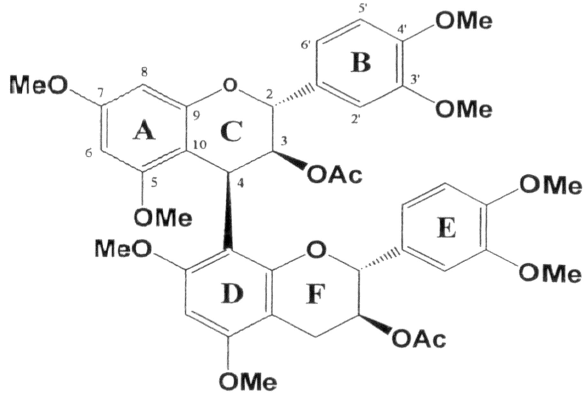
**Plate 18.8:** HMBC NMR (DMSO, 298K)




(28)

Plate 19.1 $^{1}$H NMR ($CDCl_3$, 298K)




(29)

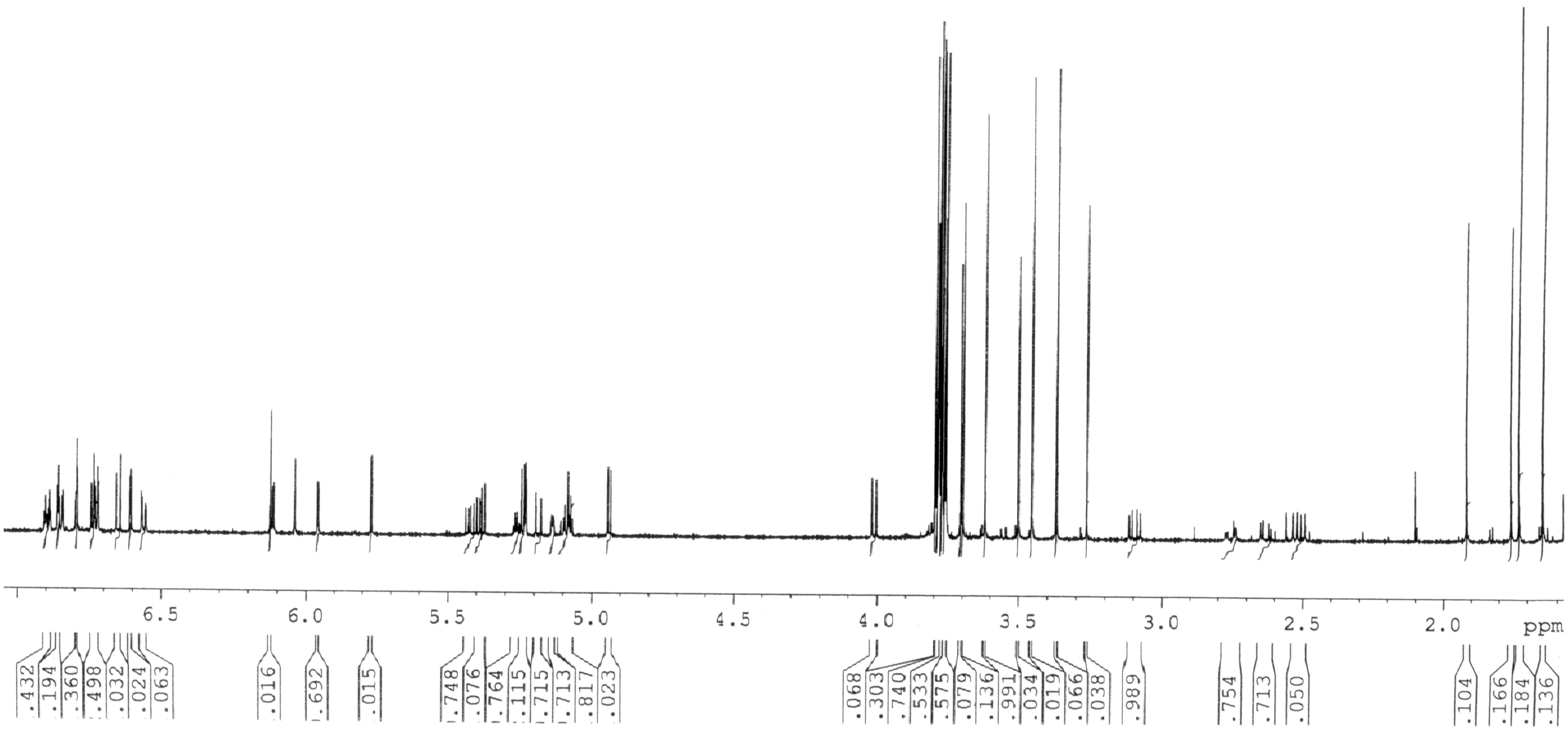
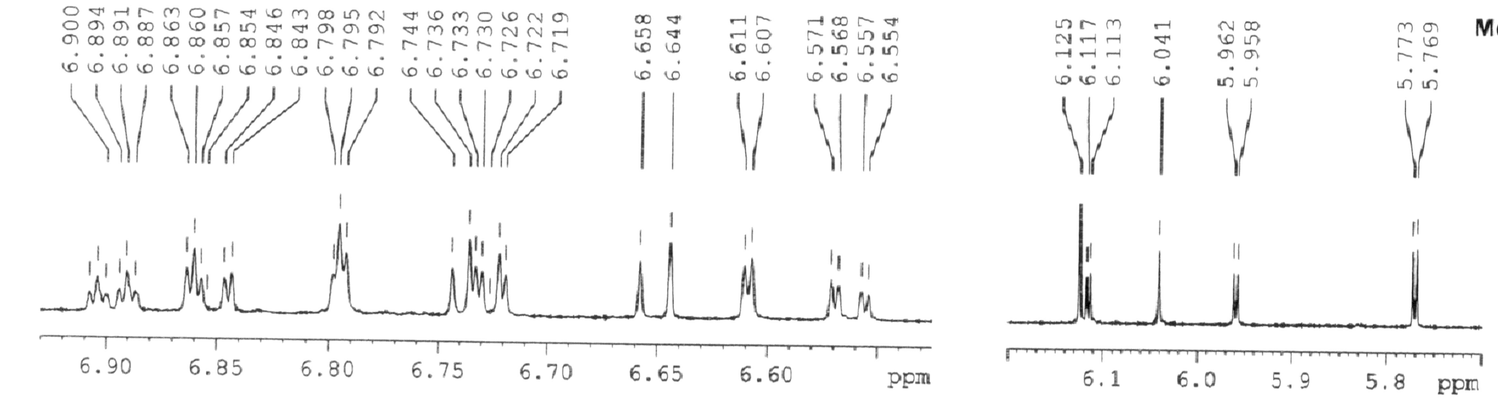
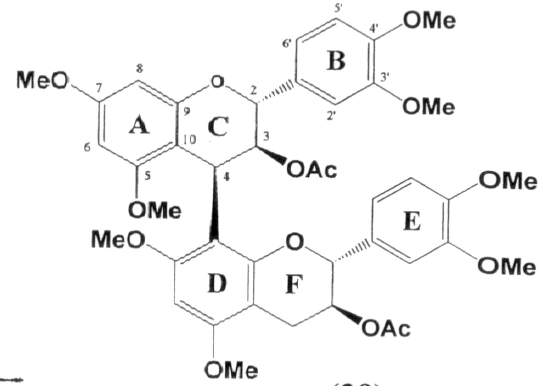


Plate 19.2: $^{1}$H NMR ($CDCl_3$, 298K)




(29)

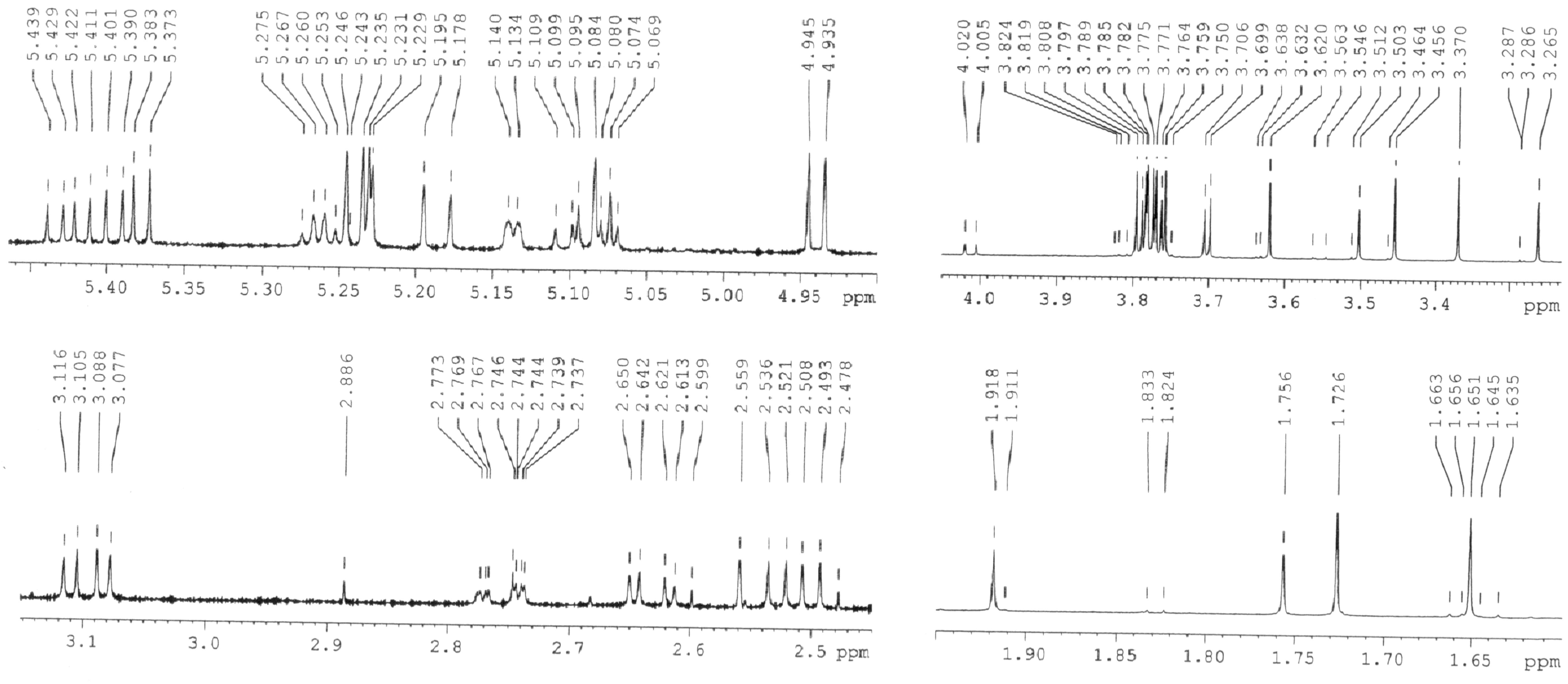
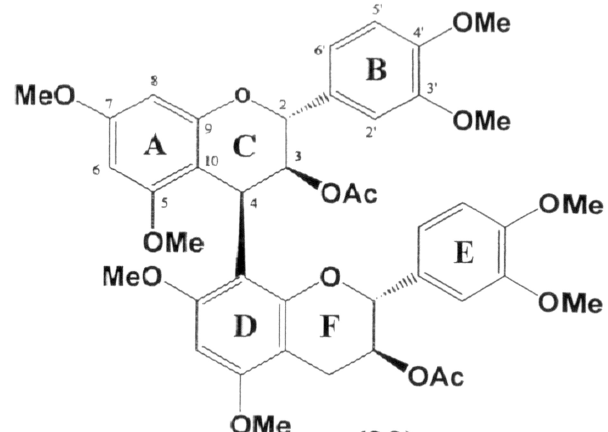


**Plate 19.3:** $^{13}C$ NMR ($CDCl_3$, 298K)




(29)

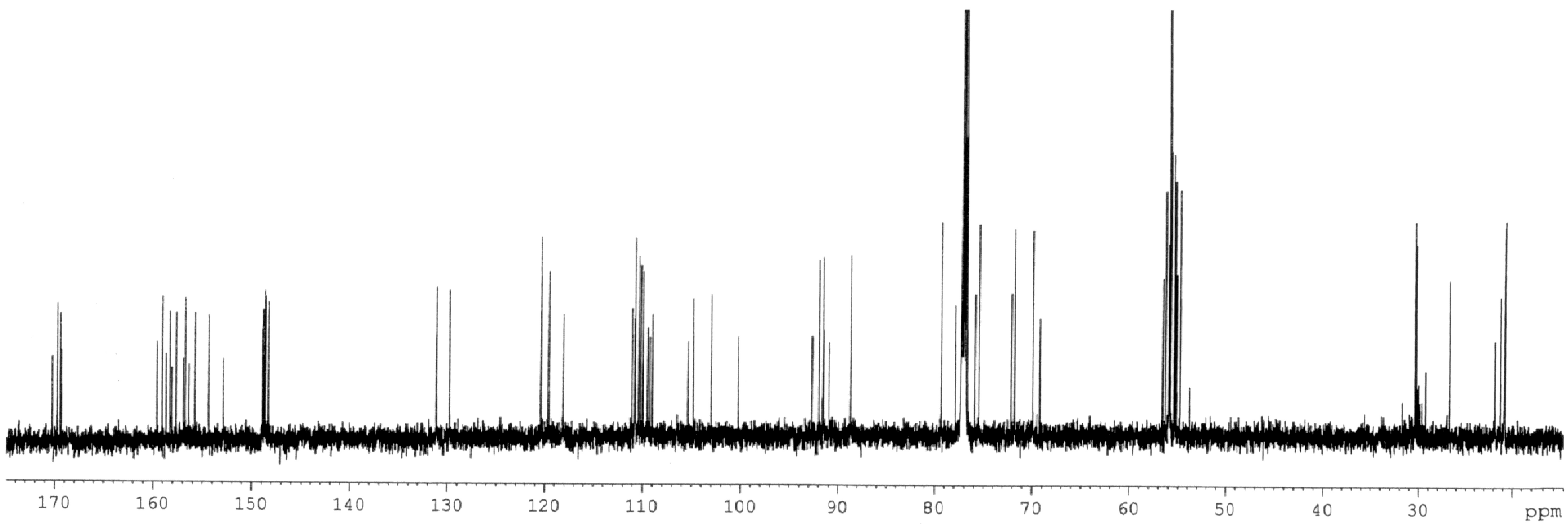
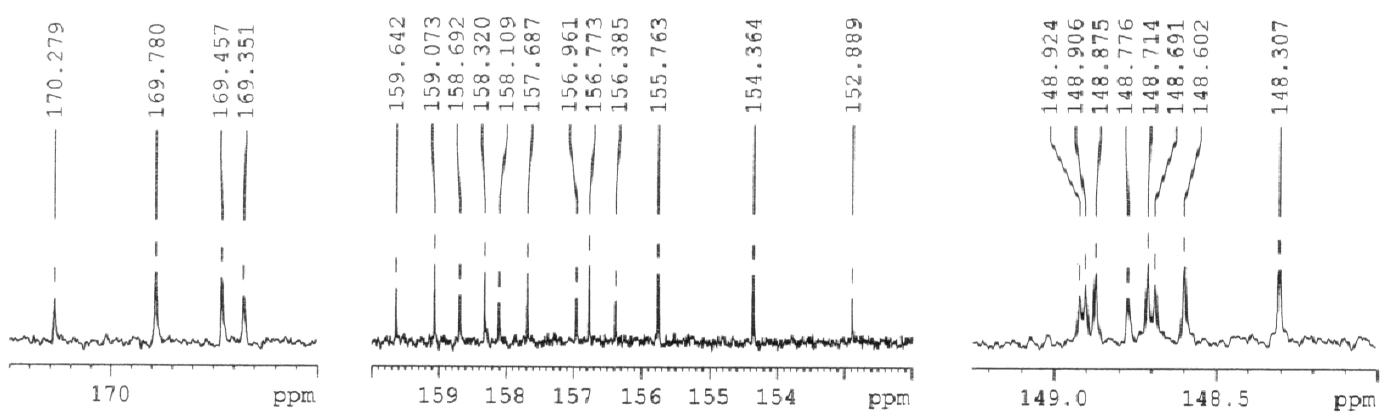
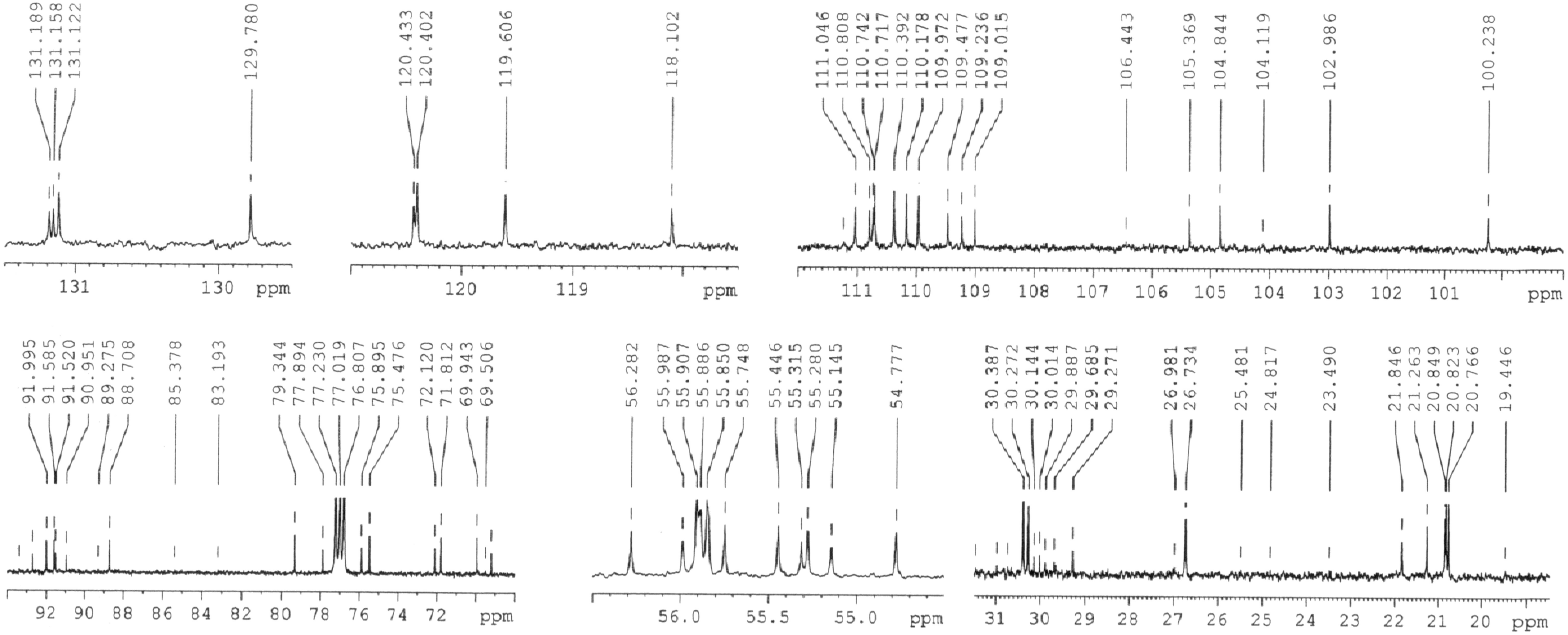
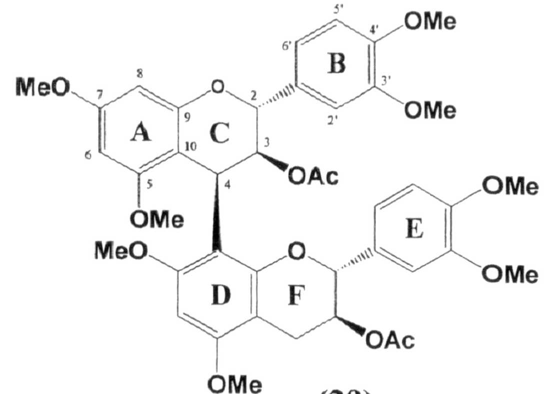


**Plate 19.4:** $^{13}C$ NMR ($CDCl_3$, 298K)

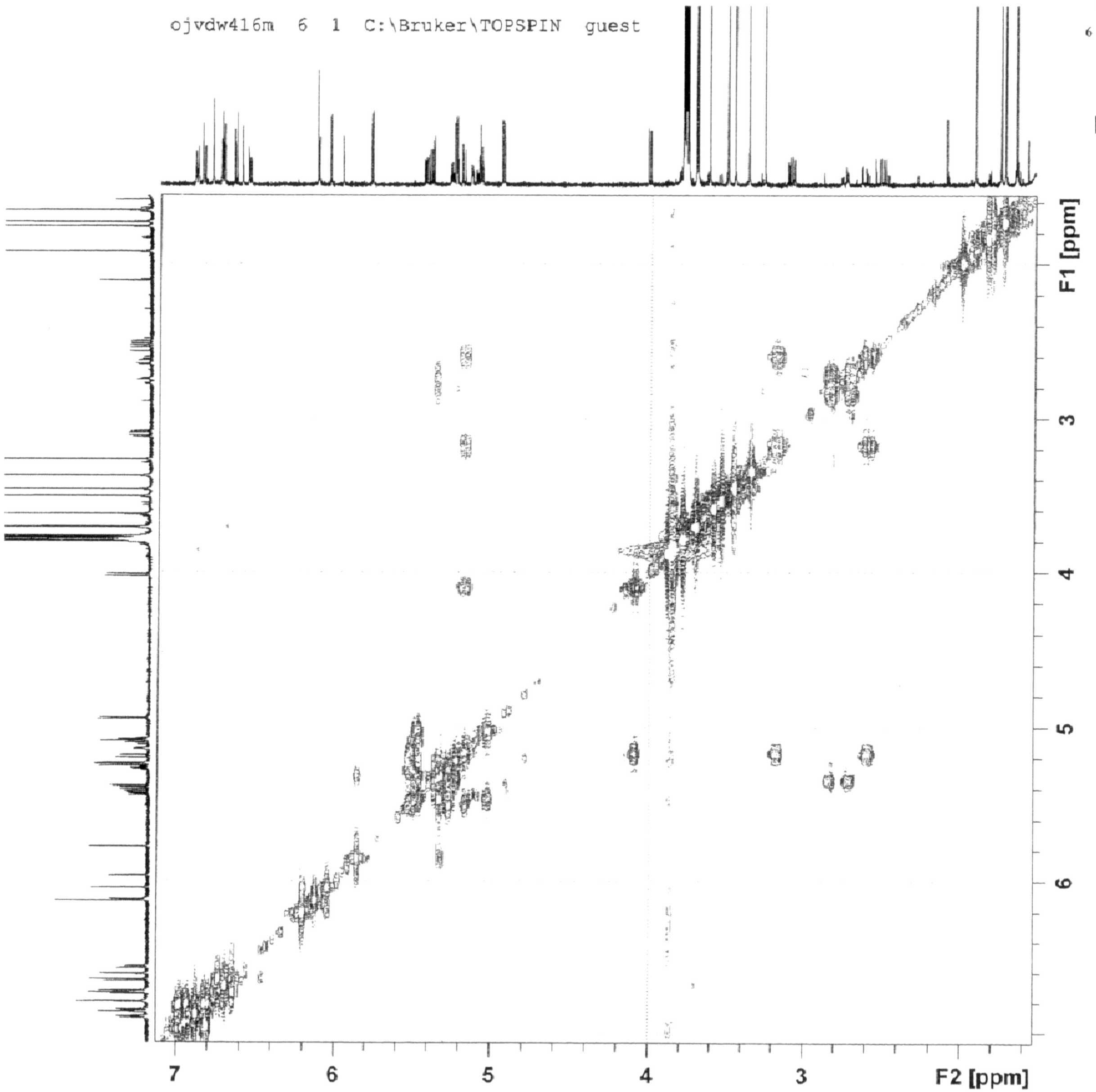


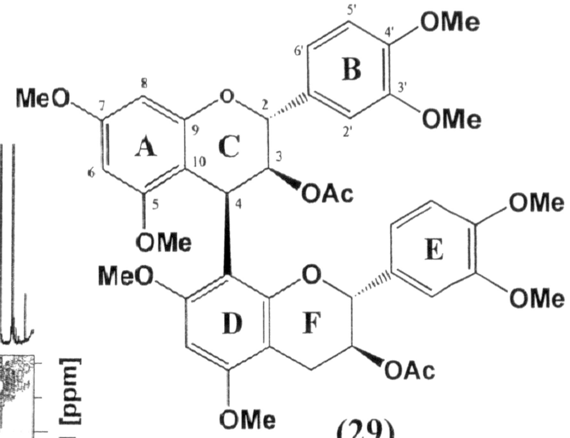




(29)

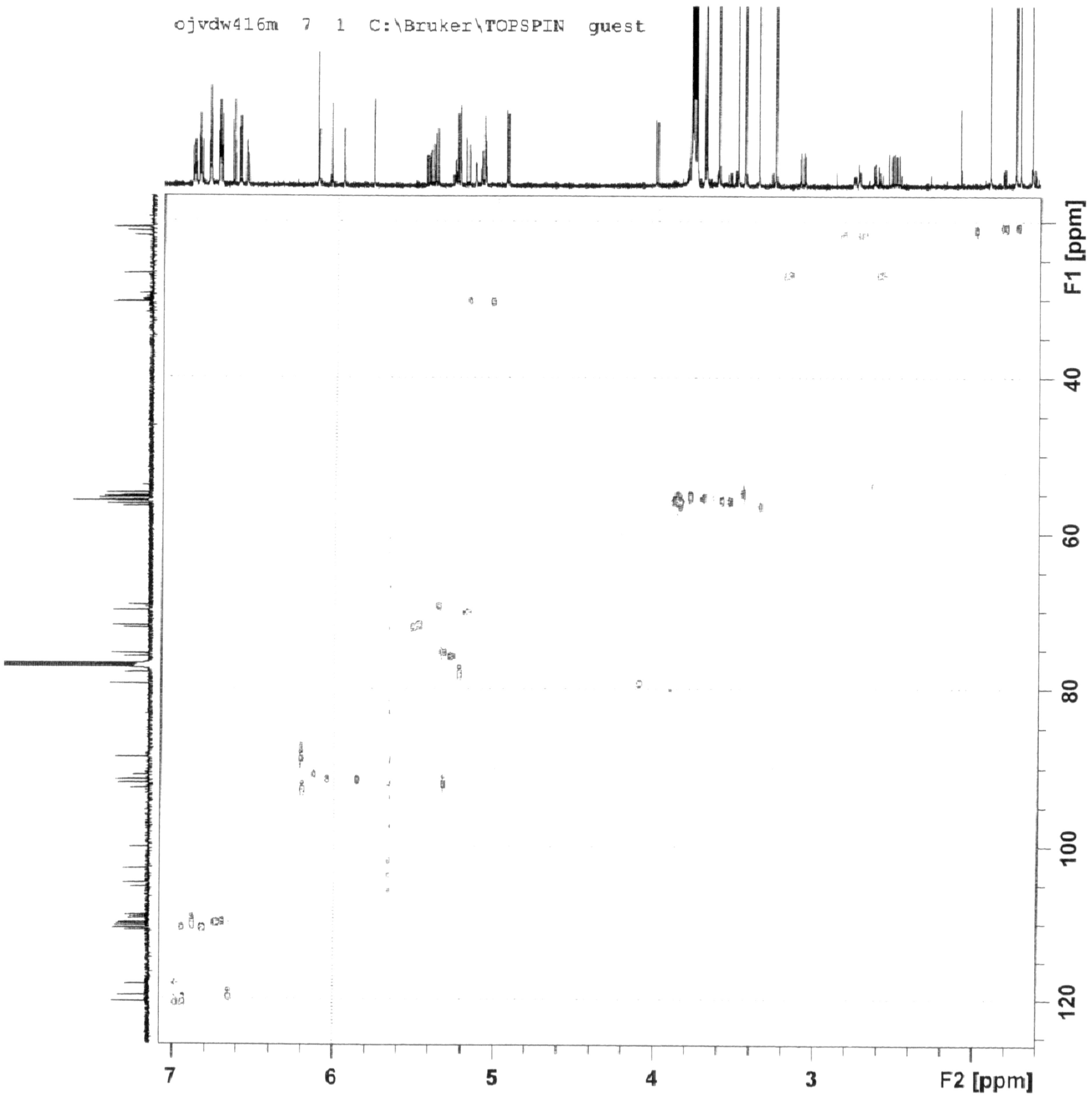
**Plate 19.5:** COSY NMR ($CDCl_3$, 298K)

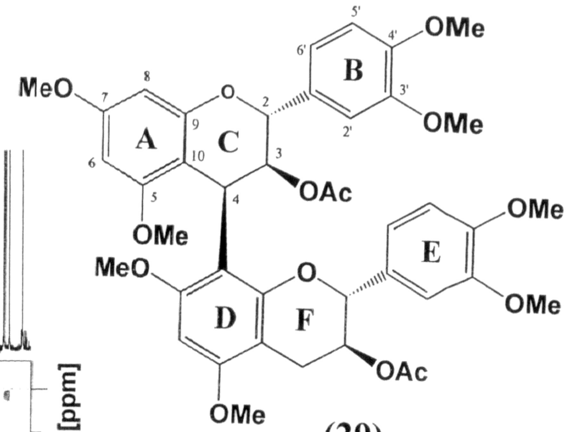


**(29)**

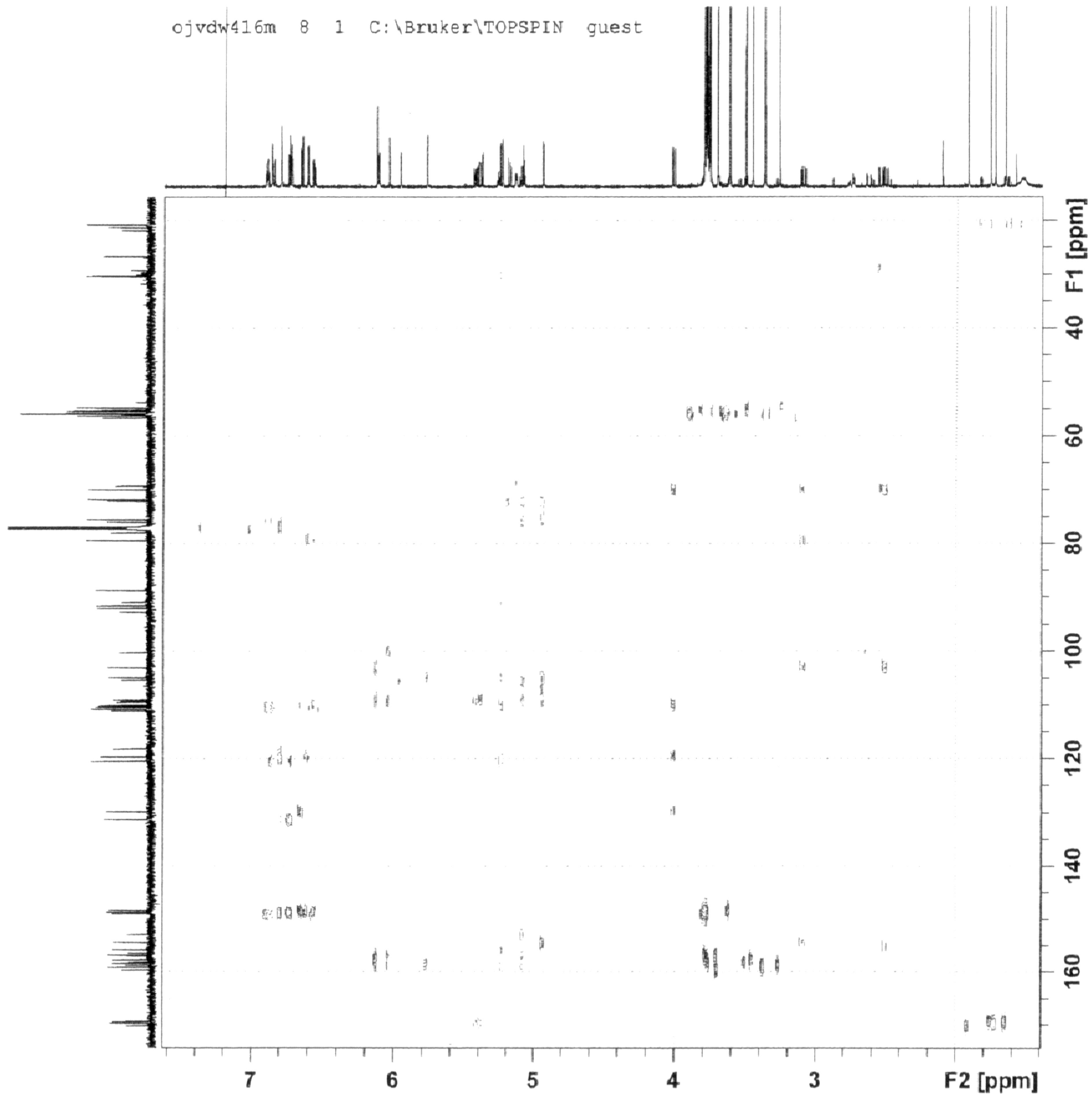
Plate 19.6: HSQC NMR ($CDCl_3$, 298K)

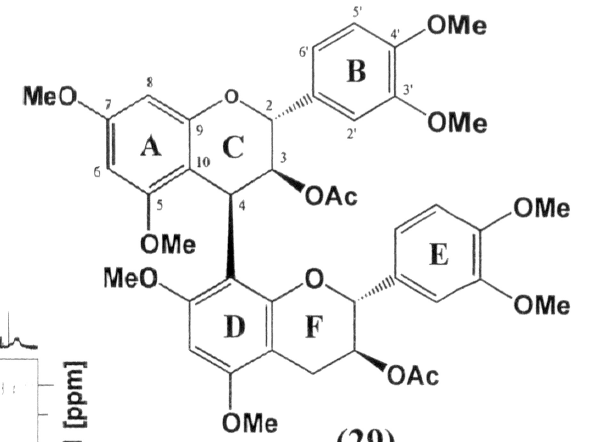



(29)

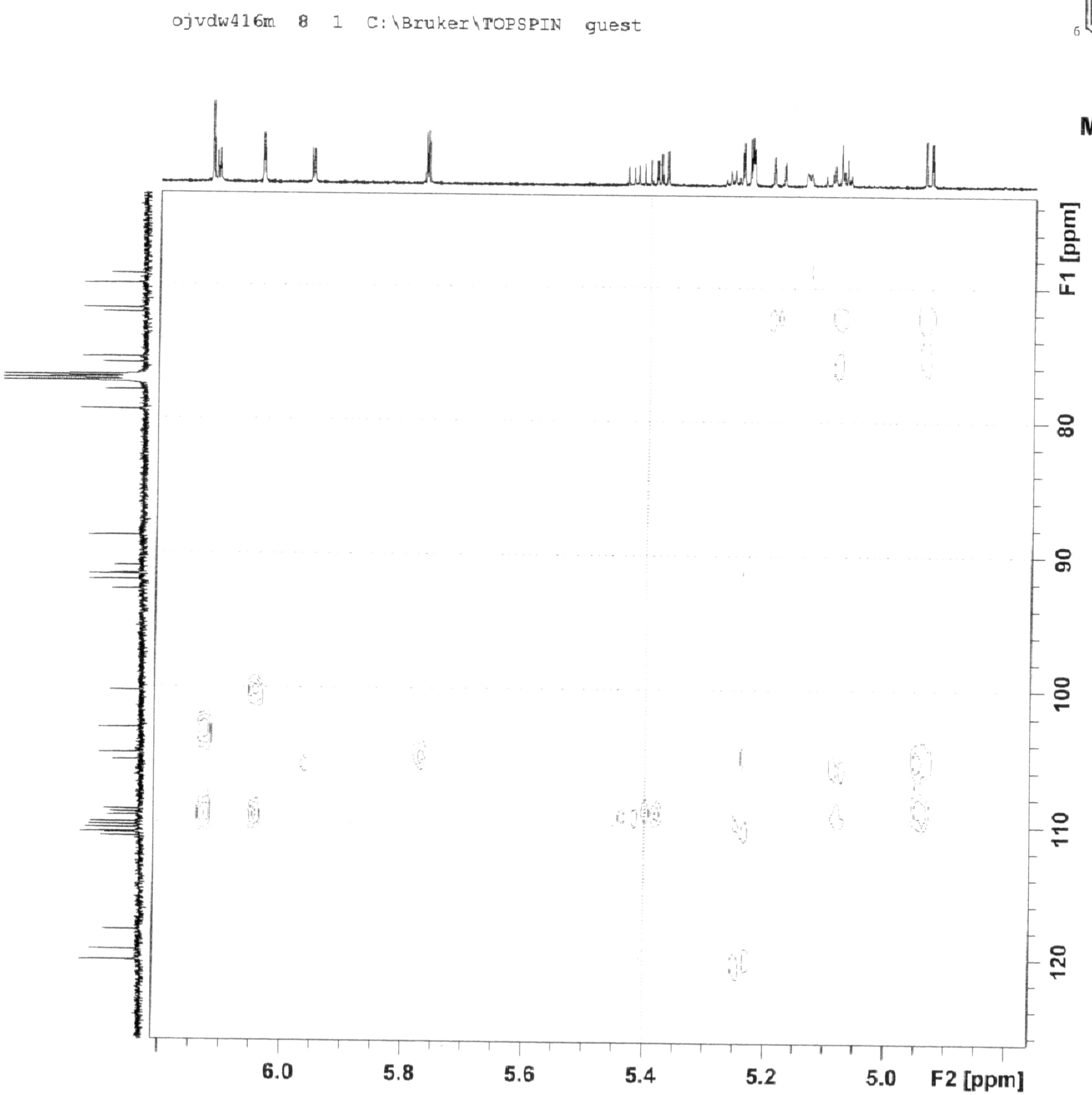
**Plate 19.7:** HMBC NMR ($CDCl_3$, 298K)

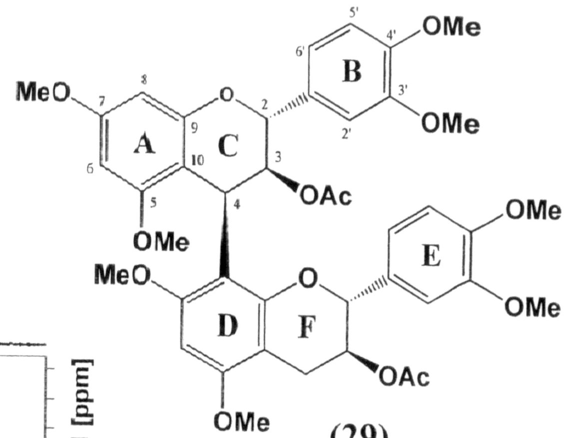



(29)

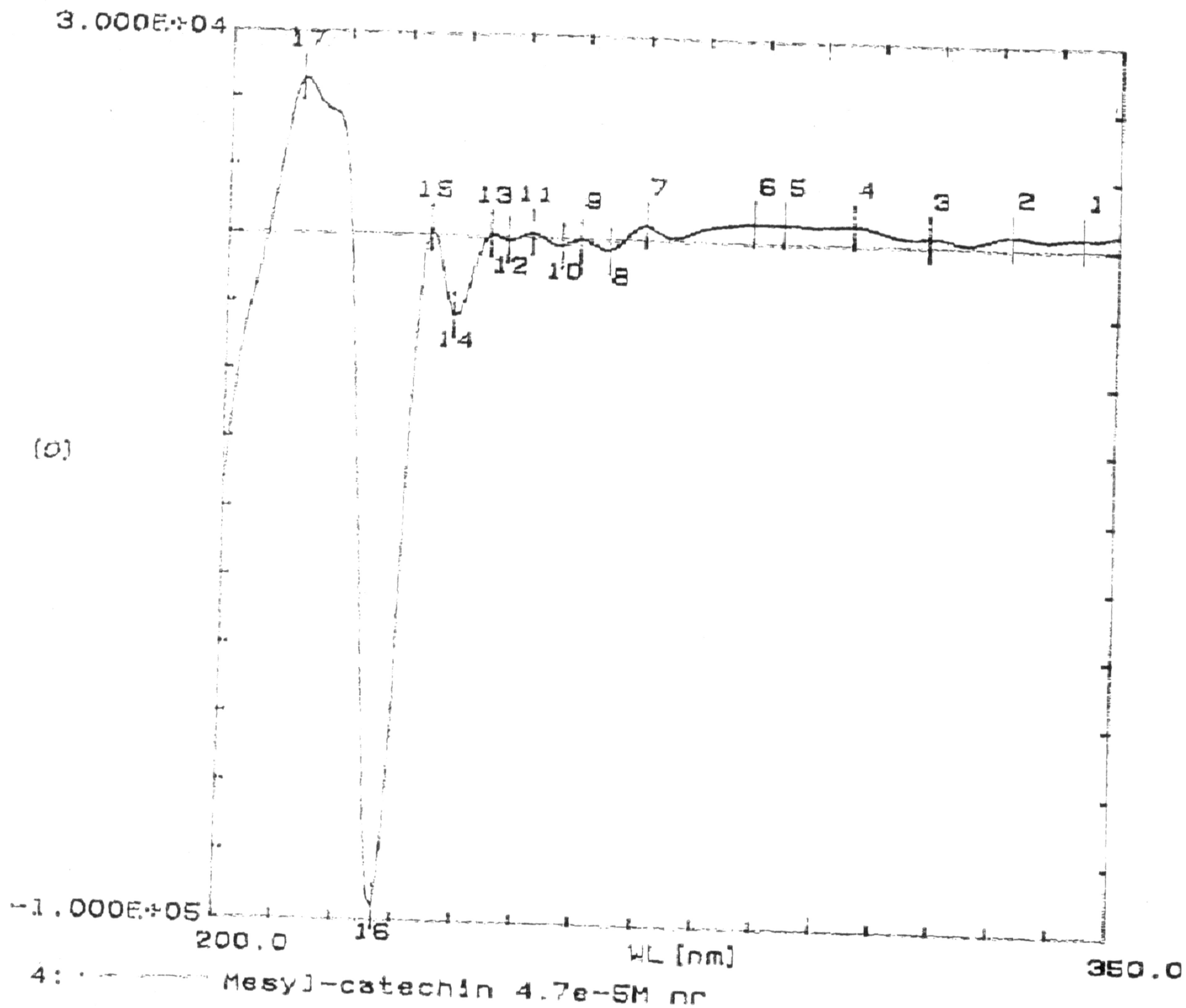
**Plate 19.8:** HMBC NMR ($CDCl_3$, 298K)

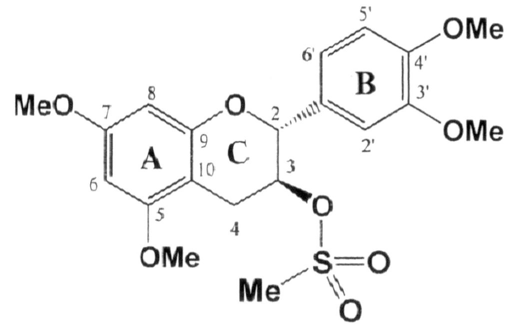



(29)

CD Plate 1:

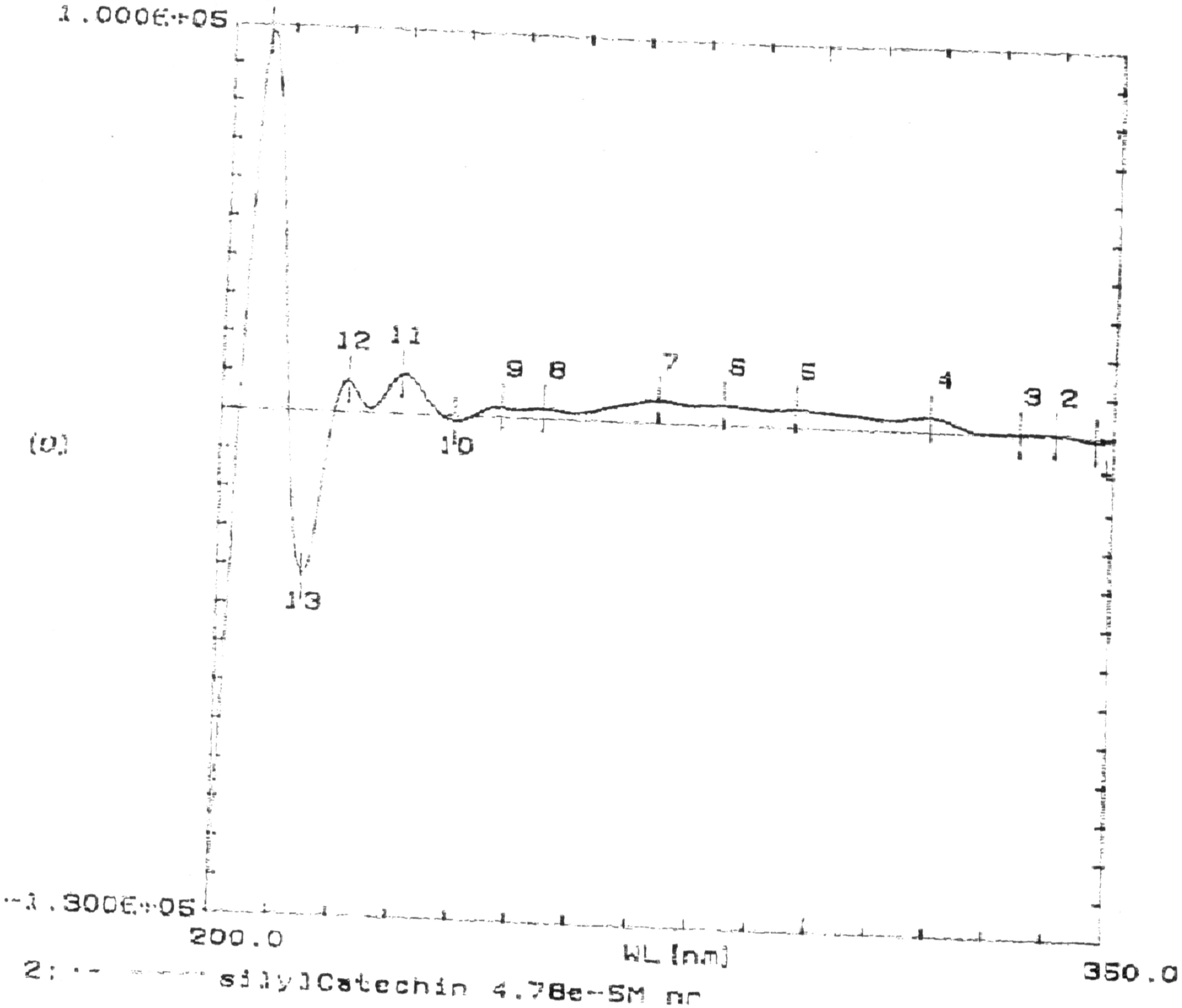


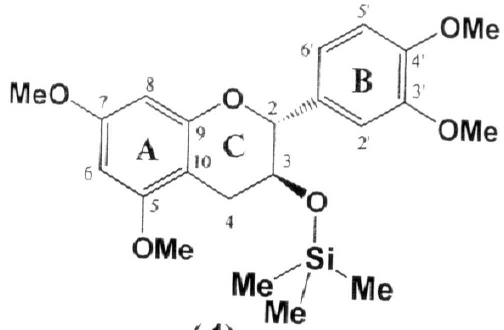




(3)

| No. | Wavelength | Value |
|---|---|---|
| 1 | 344.00 nm | 1.840E+03 |
| 2 | 332.00 nm | 2.172E+03 |
| 3 | 318.00 nm | 1.544E+03 |
| 4 | 305.00 nm | 3.084E+03 |
| 5 | 293.00 nm | 3.059E+03 |
| 6 | 288.00 nm | 3.048E+03 |
| 7 | 270.00 nm | 2.378E+03 |
| 8 | 264.00 nm | -1.433E+03 |
| 9 | 259.00 nm | 1.117E+02 |
| 10 | 256.00 nm | -8.578E+02 |
| 11 | 251.00 nm | 9.139E+02 |
| 12 | 247.00 nm | -3.031E+02 |
| 13 | 244.00 nm | 5.382E+02 |
| 14 | 238.00 nm | -1.141E+04 |
| 15 | 234.00 nm | 1.190E+03 |
| 16 | 227.00 nm | -9.810E+04 |
| 17 | 212.00 nm | 2.288E+04 |

## CD Plate 2:

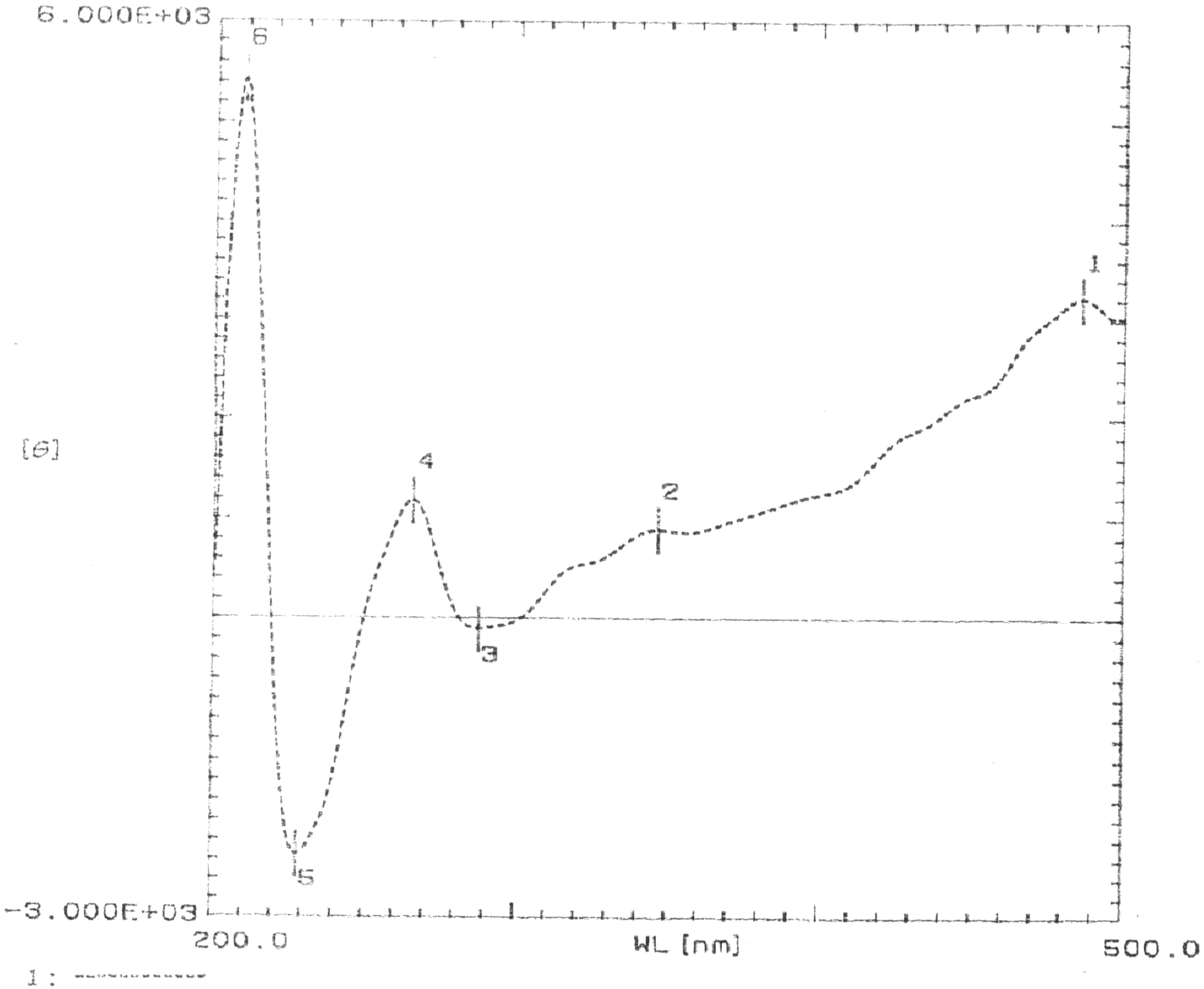



(4)

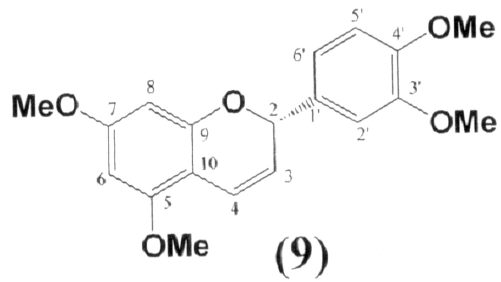
| No. | Wavelength | Value |
|---|---|---|
| 1 | 347.00 nm | -8.112E+02 |
| 2 | 340.00 nm | 6.355E+02 |
| 3 | 334.00 nm | 5.706E+02 |
| 4 | 319.00 nm | 4.030E+03 |
| 5 | 296.00 nm | 4.857E+03 |
| 6 | 284.00 nm | 5.307E+03 |
| 7 | 273.00 nm | 6.101E+03 |
| 8 | 254.00 nm | 2.761E+03 |
| 9 | 247.00 nm | 2.692E+03 |
| 10 | 239.00 nm | -1.292E+03 |
| 11 | 230.00 nm | 1.021E+04 |
| 12 | 221.00 nm | 8.217E+03 |
| 13 | 214.00 nm | -4.290E+04 |
| 14 | 206.00 nm | 9.846E+04 |

## CD Plate 3:



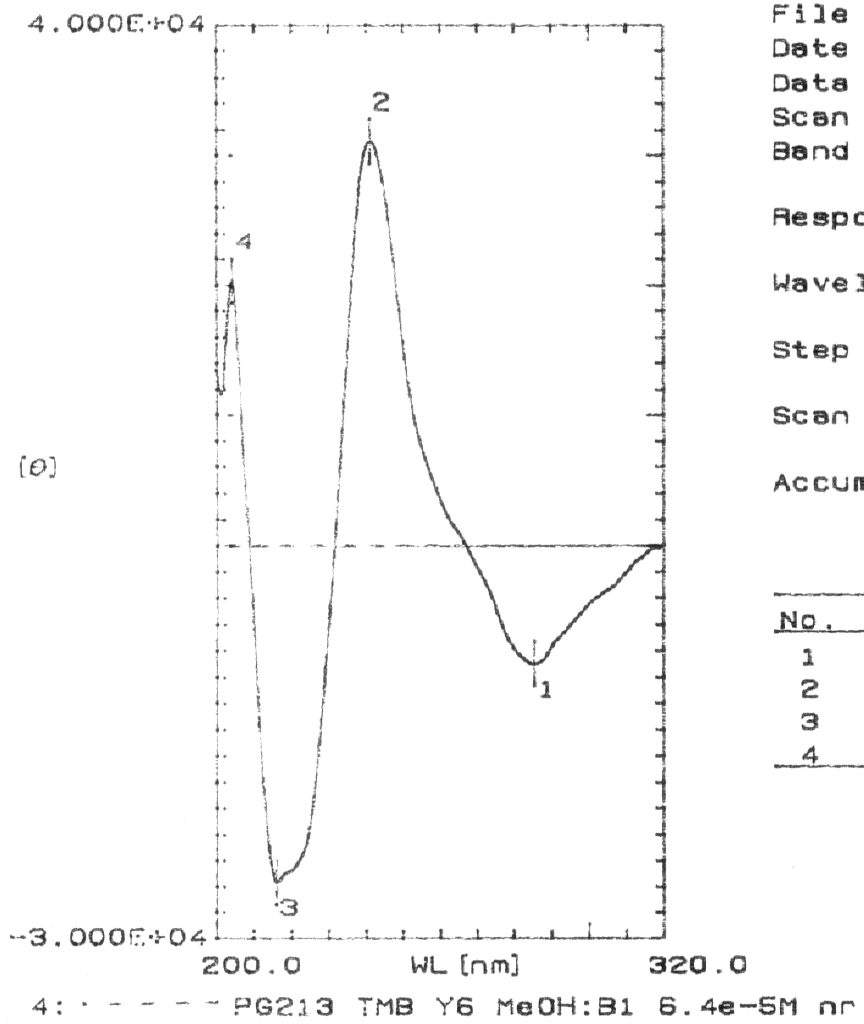

Conditions of Memory 1
File : MA012-2
Date : 80-52-10
Data : [θ]
Scan : WL
Band width :
1.0 nm
Response :
0.25 sec
Wavelength range :
500.0 - 200.0nm
Step resolution :
0.5nm/data
Scan speed :
50nm/min
Accumulation : 1

| No. | Wavelength | Value |
|---|---|---|
| 1 | 486.00 nm | 3.215E+03 |
| 2 | 347.00 nm | 8.848E+02 |
| 3 | 288.00 nm | -1.084E+02 |
| 4 | 266.00 nm | 1.178E+03 |
| 5 | 228.50 nm | -2.356E+03 |
| 6 | 209.00 nm | 5.423E+03 |



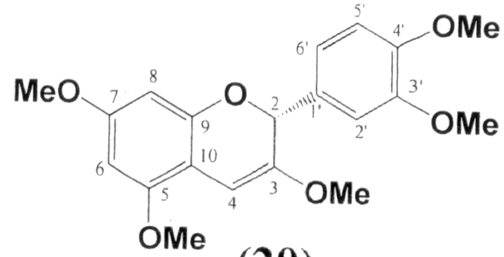

**(9)**

CD Plate 4:



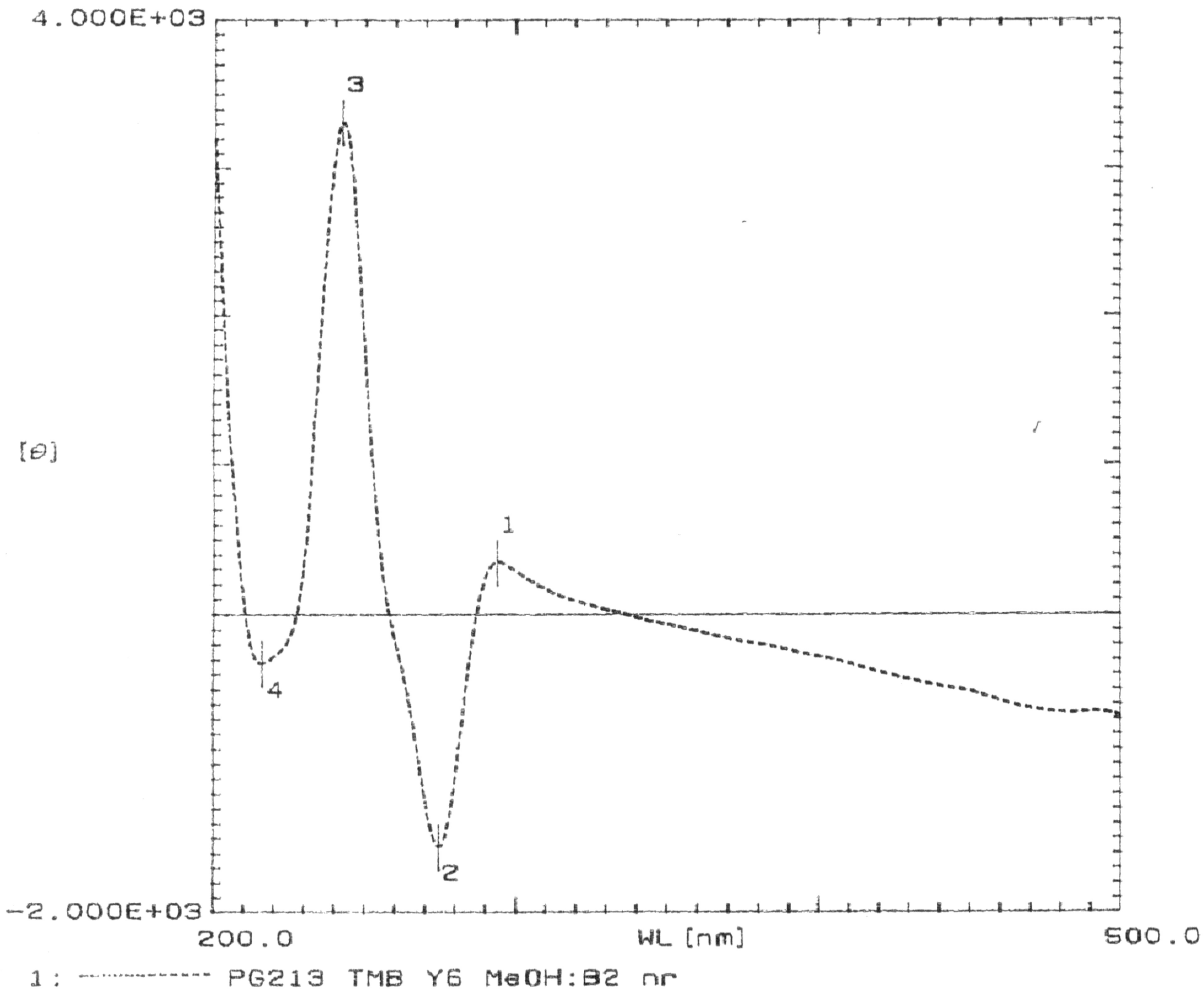

Conditions of Memory 4
File : MA007-4
Date : 71-13-10
Data : [θ]
Scan : WL
Band width :
1.0 nm
Response :
0.25 sec
Wavelength range :
320.0 - 200.0nm
Step resolution :
0.5nm/data
Scan speed :
50nm/min
Accumulation : 1

| No. | Wavelength | Value |
|---|---|---|
| 1 | 285.50 nm | -8.995E+03 |
| 2 | 241.00 nm | 3.111E+04 |
| 3 | 216.00 nm | -2.566E+04 |
| 4 | 204.00 nm | 2.039E+04 |

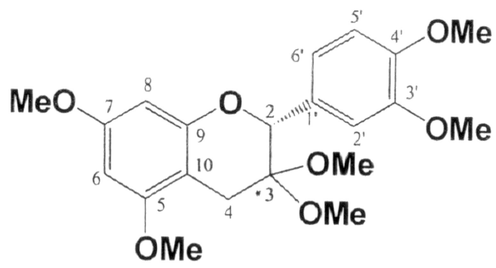



(20)

# CD Plate 5:

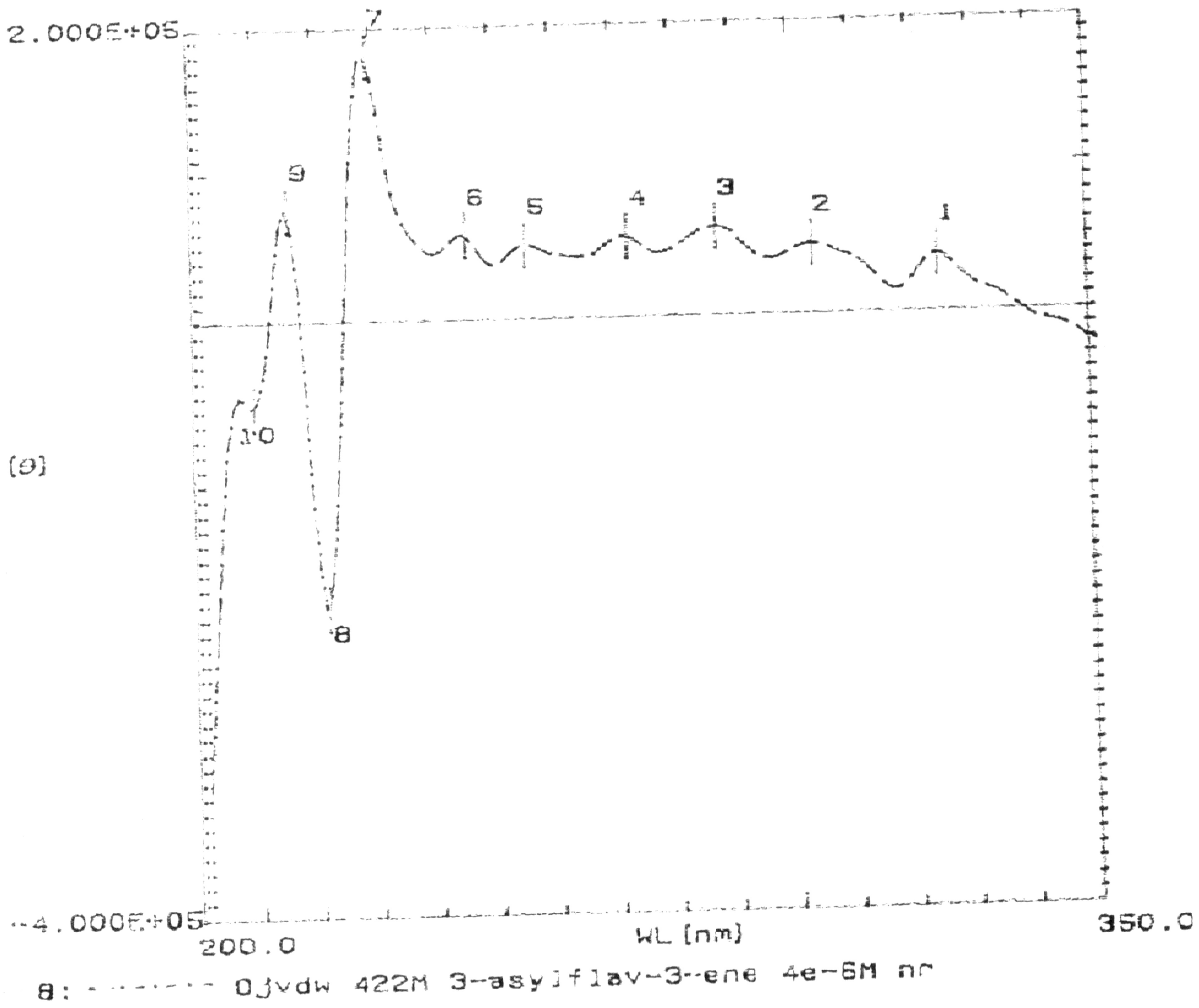


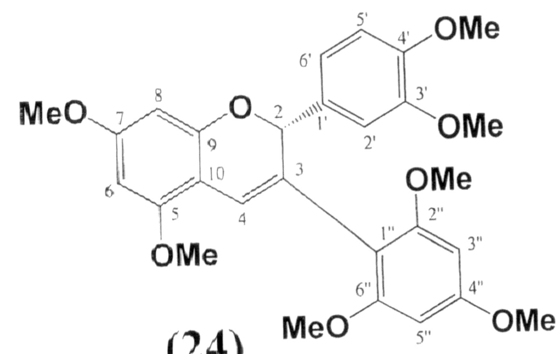

| No. | Wavelength | Value |
|---|---|---|
| 1 | 294.00 nm | 3.428E+02 |
| 2 | 274.50 nm | -1.562E+03 |
| 3 | 242.50 nm | 3.310E+03 |
| 4 | 216.50 nm | -3.312E+02 |




**(22)**

**CD Plate 6:**

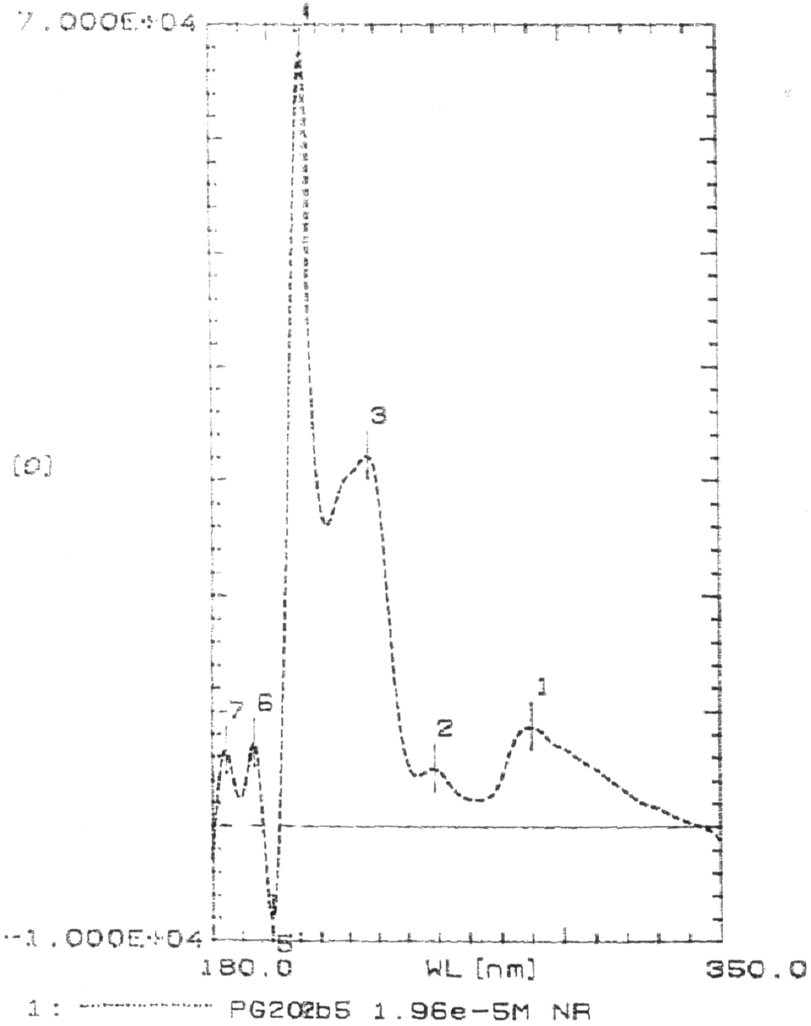


(24)

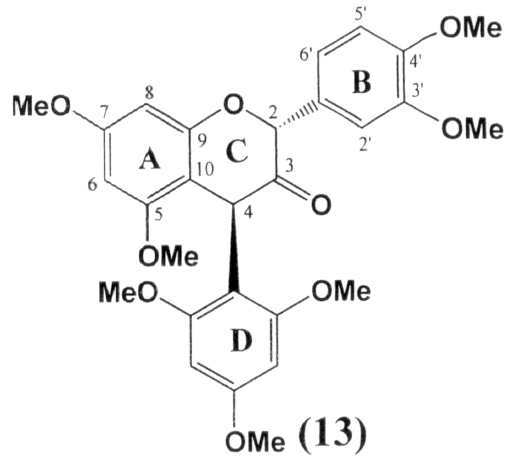
| No. | Wavelength | Value |
|---|---|---|
| 1 | 323.00 nm | 3.776E+04 |
| 2 | 302.00 nm | 4.622E+04 |
| 3 | 286.00 nm | 5.883E+04 |
| 4 | 271.00 nm | 5.375E+04 |
| 5 | 254.00 nm | 4.889E+04 |
| 6 | 244.00 nm | 5.621E+04 |
| 7 | 228.00 nm | 1.796E+05 |
| 8 | 220.00 nm | -1.931E+05 |
| 9 | 214.00 nm | 7.491E+04 |
| 10 | 208.00 nm | -5.851E+04 |

## CD Plate 7:



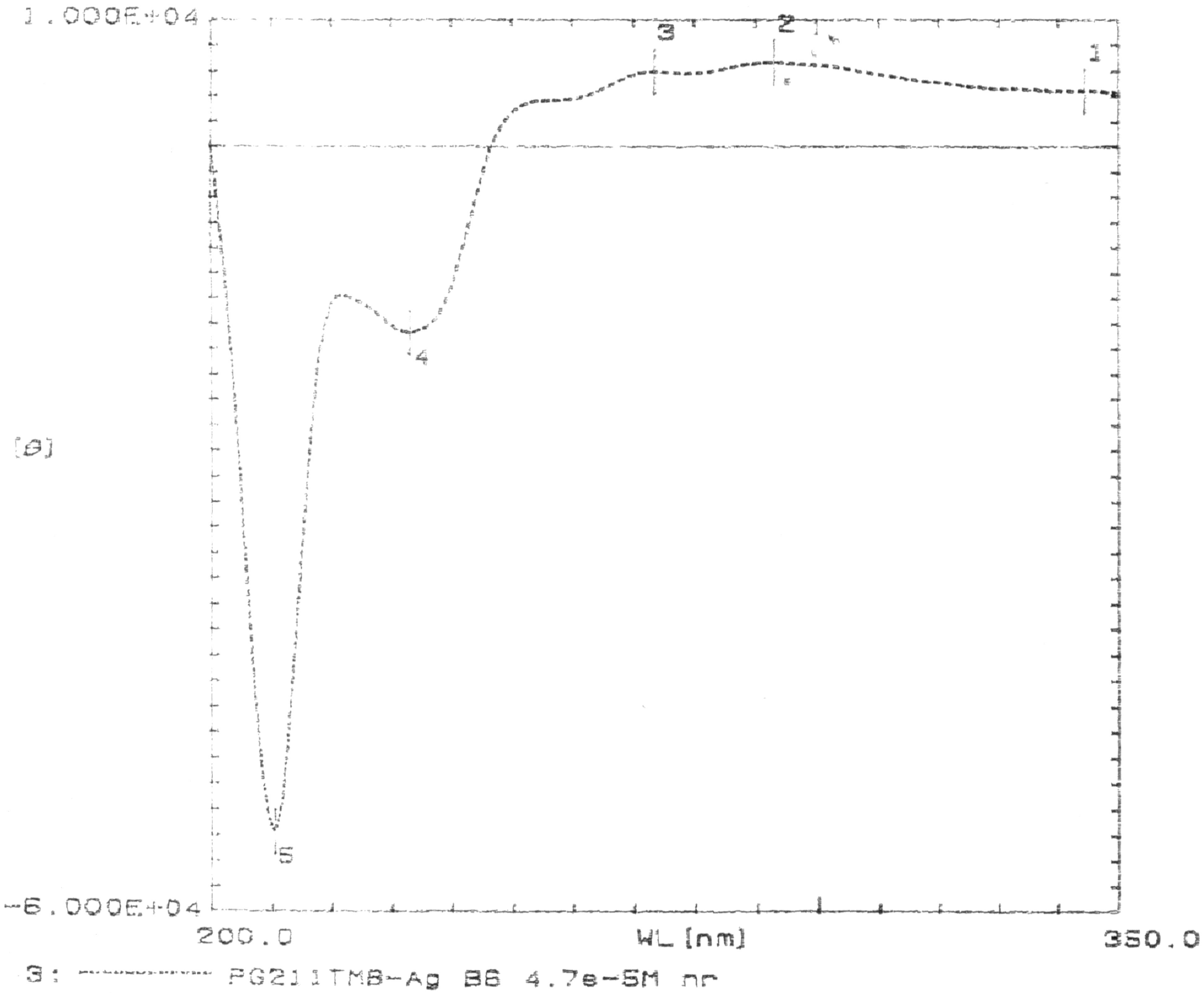

Conditions of Memory 1
File : MA002-2
Date : 71-10-10
Data : [θ]
Scan : WL
Band width :
1.0 nm
Response :
0.125 sec
Wavelength range :
350.0 - 180.0nm
Step resolution :
0.2nm/data
Scan speed :
10nm/min
Accumulation : 1

| No. | Wavelength | Value |
|---|---|---|
| 1 | 290.00 nm | 8.759E+03 |
| 2 | 258.60 nm | 5.108E+03 |
| 3 | 237.20 nm | 3.221E+04 |
| 4 | 211.60 nm | 6.762E+04 |
| 5 | 200.40 nm | -8.001E+03 |
| 6 | 194.20 nm | 7.232E+03 |
| 7 | 184.60 nm | 6.573E+03 |



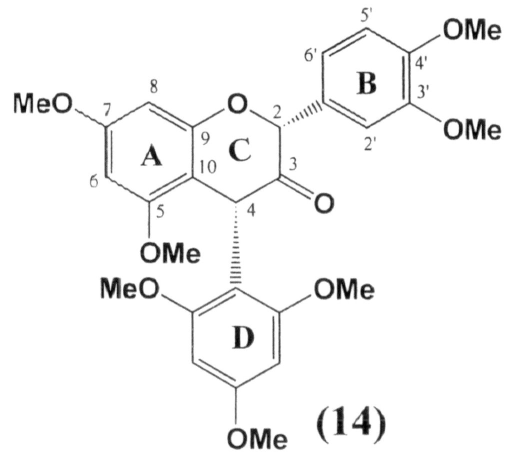
(13)

## CD Plate 8:



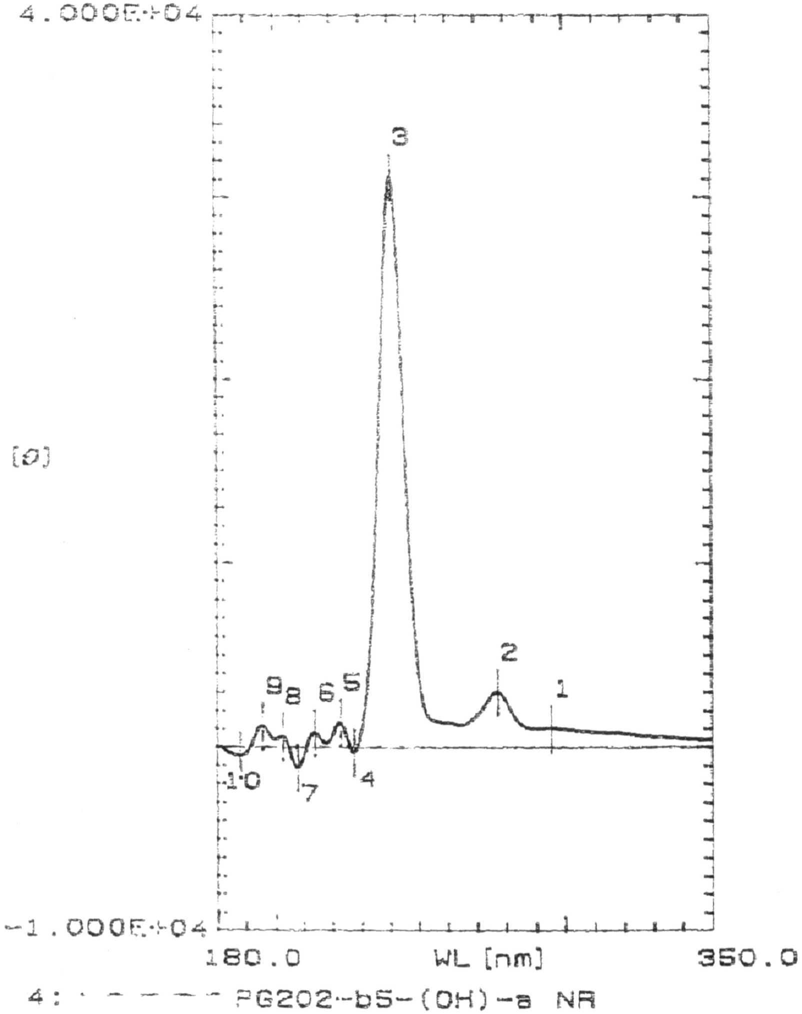

Conditions of Memory 3
File : MA009-3
Date : 71-22-10
Data : [θ]
Scan : WL
Band width :
1.0 nm
Response :
0.25 sec
Wavelength range :
350.0 – 200.0nm
Step resolution :
0.5nm/data
Scan speed :
50nm/min
Accumulation : 1

| No. | Wavelength | Value |
|---|---|---|
| 1 | 344.50 nm | 4.432E+03 |
| 2 | 293.00 nm | 6.651E+03 |
| 3 | 273.50 nm | 5.963E+03 |
| 4 | 233.00 nm | -1.464E+04 |
| 5 | 210.50 nm | -5.362E+04 |



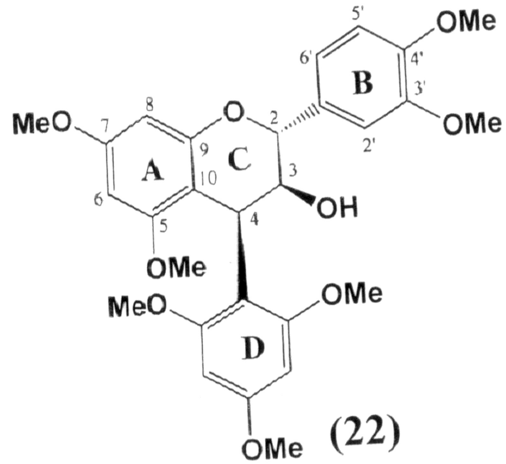

**(14)**

**CD Plate 9:**



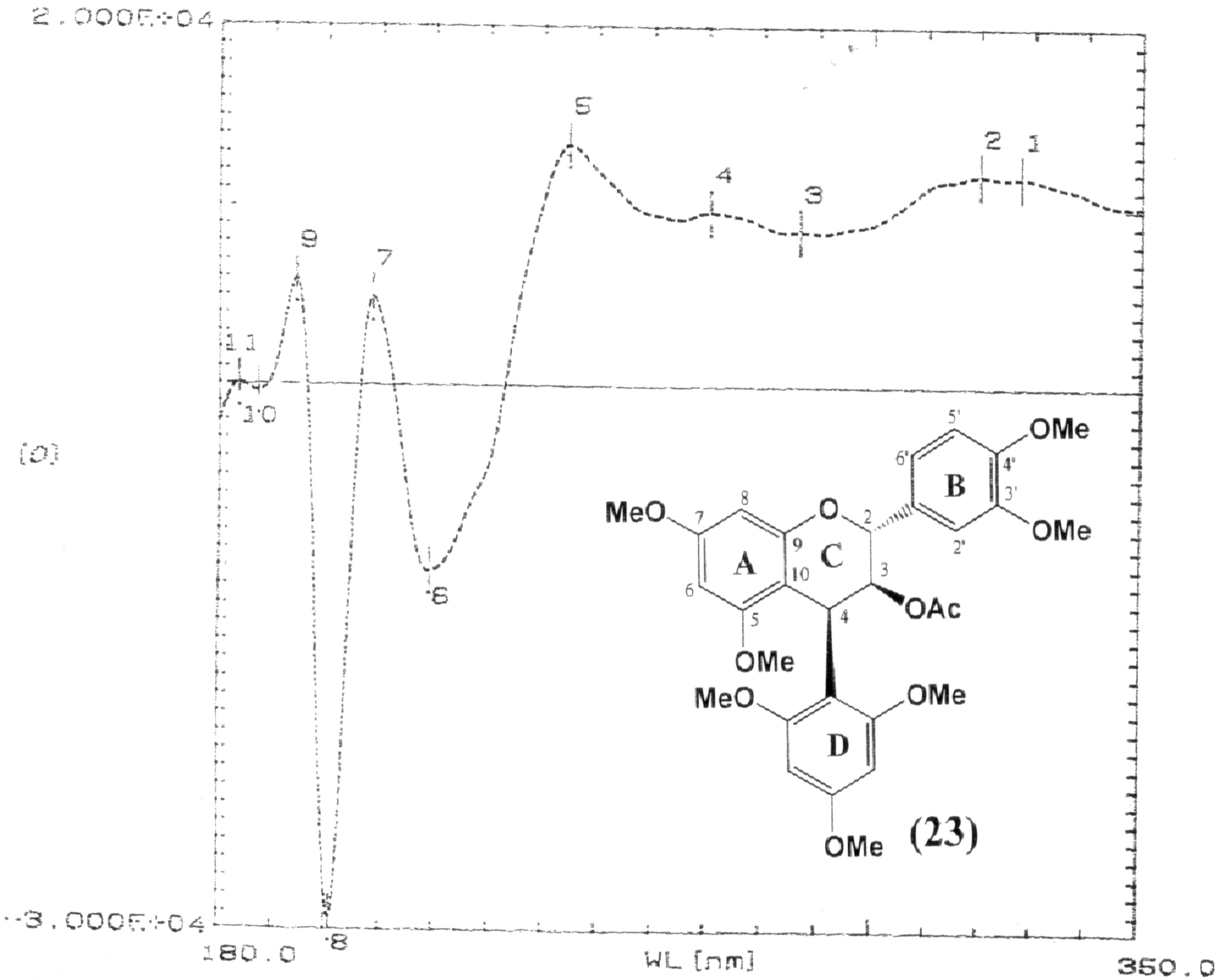

Conditions of Memory 4
File : MA006-4
Date : 71-11-10
Data : [θ]
Scan : WL
Band width :
1.0 nm
Response :
0.125 sec
Wavelength range :
350.0 - 180.0nm
Step resolution :
0.2nm/data
Scan speed :
10nm/min
Accumulation : 1

| No. | Wavelength | Value |
|---|---|---|
| 1 | 295.60 nm | 1.034E+03 |
| 2 | 277.20 nm | 3.005E+03 |
| 3 | 240.60 nm | 3.111E+04 |
| 4 | 227.80 nm | -2.565E+02 |
| 5 | 223.00 nm | 1.334E+03 |
| 6 | 214.20 nm | 7.761E+02 |
| 7 | 208.20 nm | -1.109E+03 |
| 8 | 202.60 nm | 5.937E+02 |
| 9 | 195.60 nm | 1.152E+03 |
| 10 | 188.00 nm | -4.404E+02 |




**(22)**

**CD Plate 10:**

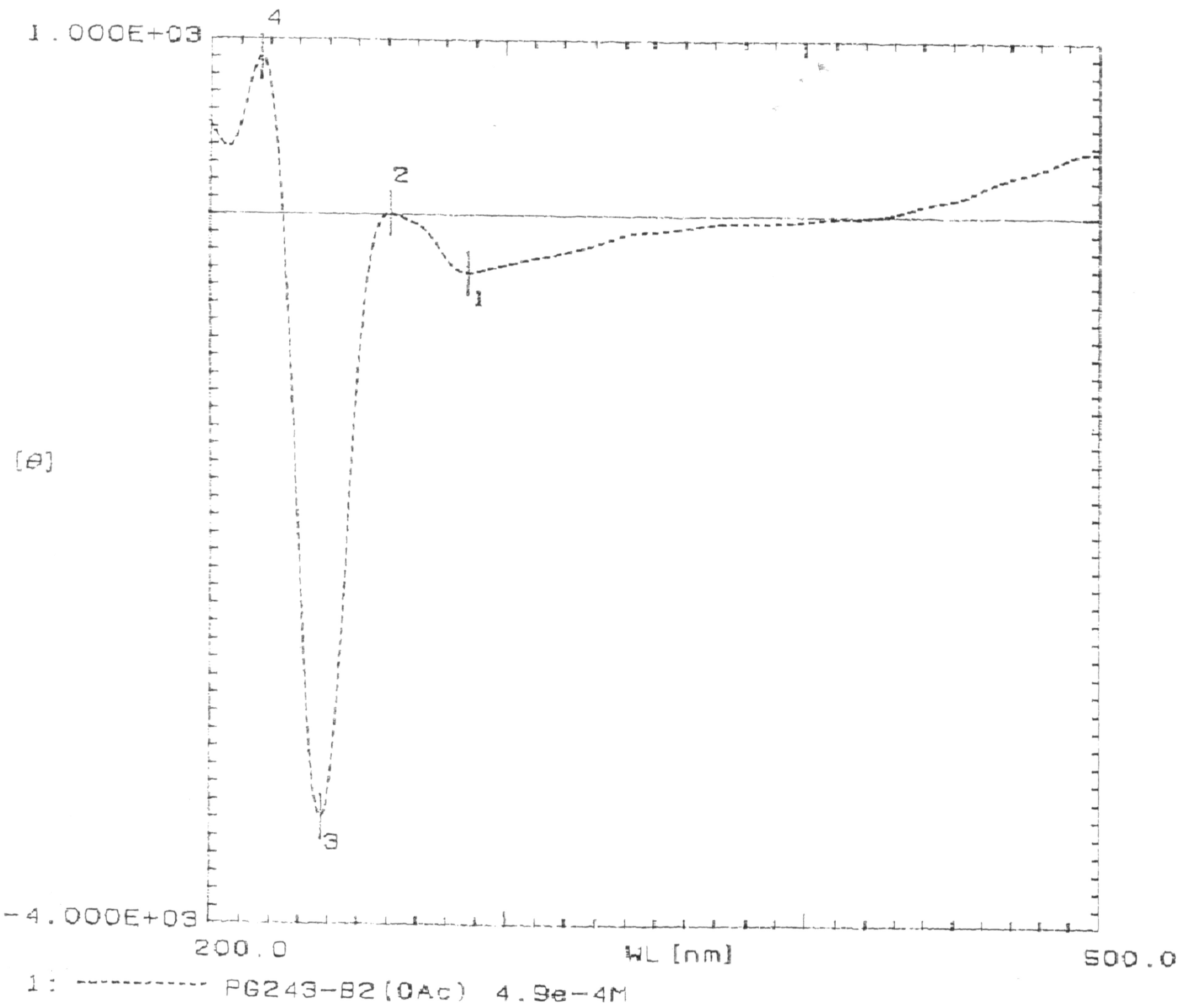



2: ............ PG202B5 (OAc) b1 1.81e-5M NR

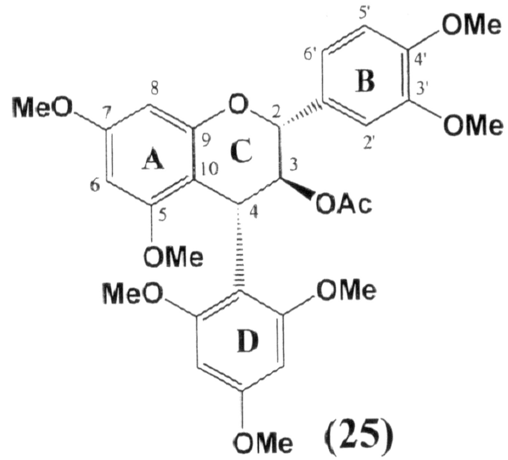
Conditions of Memory 2
File : MA003-2
Date : 71-10-10
Data : [θ]
Scan : WL
Band width :
1.0 nm
Response :
0.125 sec
Wavelength range :
350.0 - 180.0nm
Step resolution :
0.2nm/data
Scan speed :
10nm/min
Accumulation : 1

| No. | Wavelength | Value |
|---|---|---|
| 1 | 327.80 nm | 1.173E+04 |
| 2 | 320.00 nm | 1.183E+04 |
| 3 | 286.80 nm | 8.658E+03 |
| 4 | 270.40 nm | 9.701E+03 |
| 5 | 244.80 nm | 1.342E+04 |
| 6 | 219.20 nm | -1.023E+04 |
| 7 | 208.40 nm | 4.849E+03 |
| 8 | 201.00 nm | -2.932E+04 |
| 9 | 194.20 nm | 5.832E+03 |
| 10 | 187.00 nm | -4.093E+02 |
| 11 | 183.60 nm | 1.510E+01 |

**CD Plate 11:**



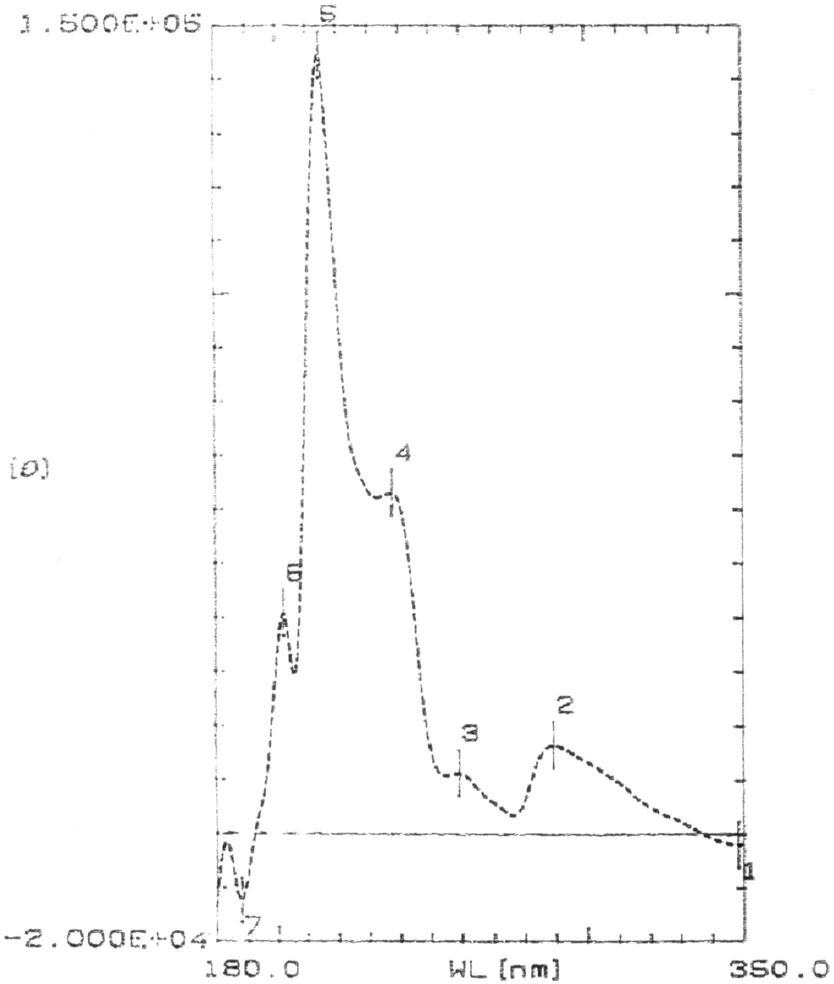

Conditions of Memory 1




**(25)**

| No. | Wavelength | Value |
|---|---|---|
| 1 | 287.00 nm | -3.312E+02 |
| 2 | 260.50 nm | 7.353E+00 |
| 3 | 237.50 nm | -3.397E+03 |
| 4 | 217.00 nm | 8.960E+02 |

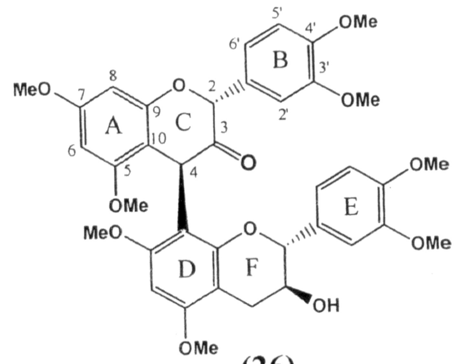
## CD Plate 12:




1: ------------ PG204MC-Ag-b1 1.16e-5M NR

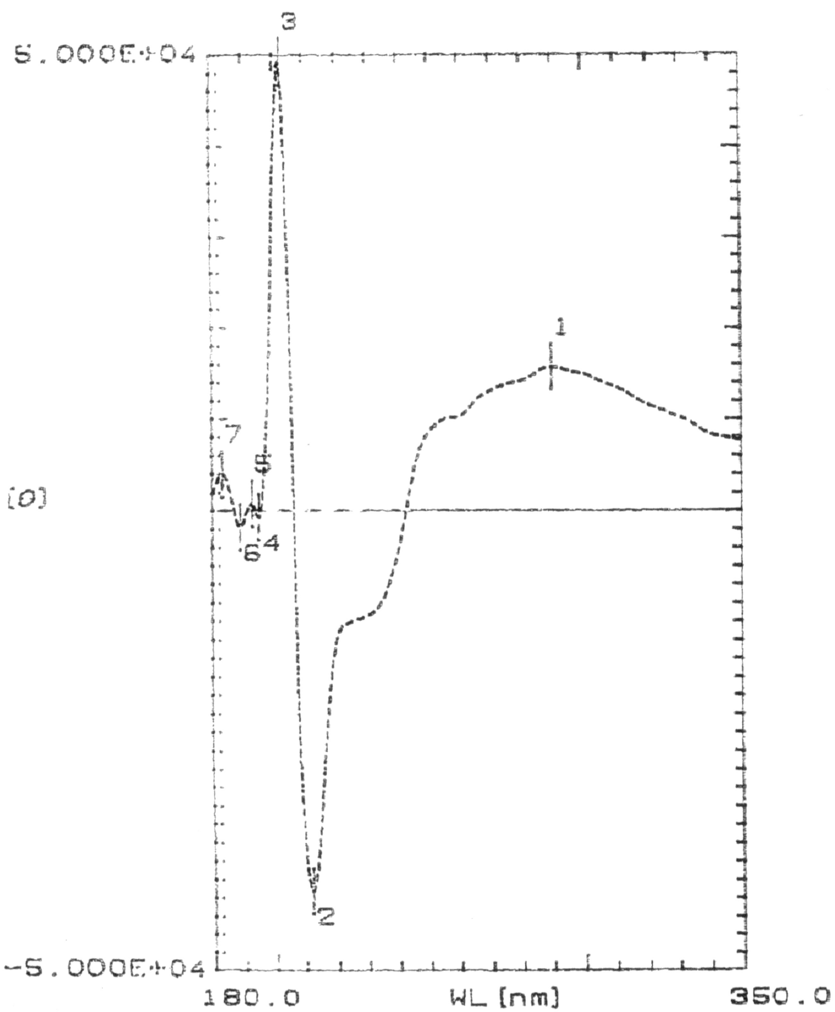
Conditions of Memory 1
File : MA005-2
Date : 71-10-10
Data : [θ]
Scan : WL
Band width :
1.0 nm
Response :
0.125 sec
Wavelength range :
350.0 – 180.0nm
Step resolution :
0.2nm/data
Scan speed :
10nm/min
Accumulation : 1

| No. | Wavelength | Value |
|---|---|---|
| 1 | 348.20 nm | -1.932E+03 |
| 2 | 289.20 nm | 1.659E+04 |
| 3 | 259.00 nm | 1.141E+04 |
| 4 | 237.60 nm | 6.305E+04 |
| 5 | 214.20 nm | 1.445E+05 |
| 6 | 202.00 nm | 4.076E+04 |
| 7 | 188.00 nm | -1.226E+04 |



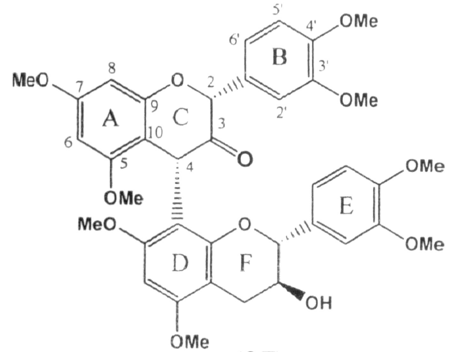

(26)

**CD Plate 13:**



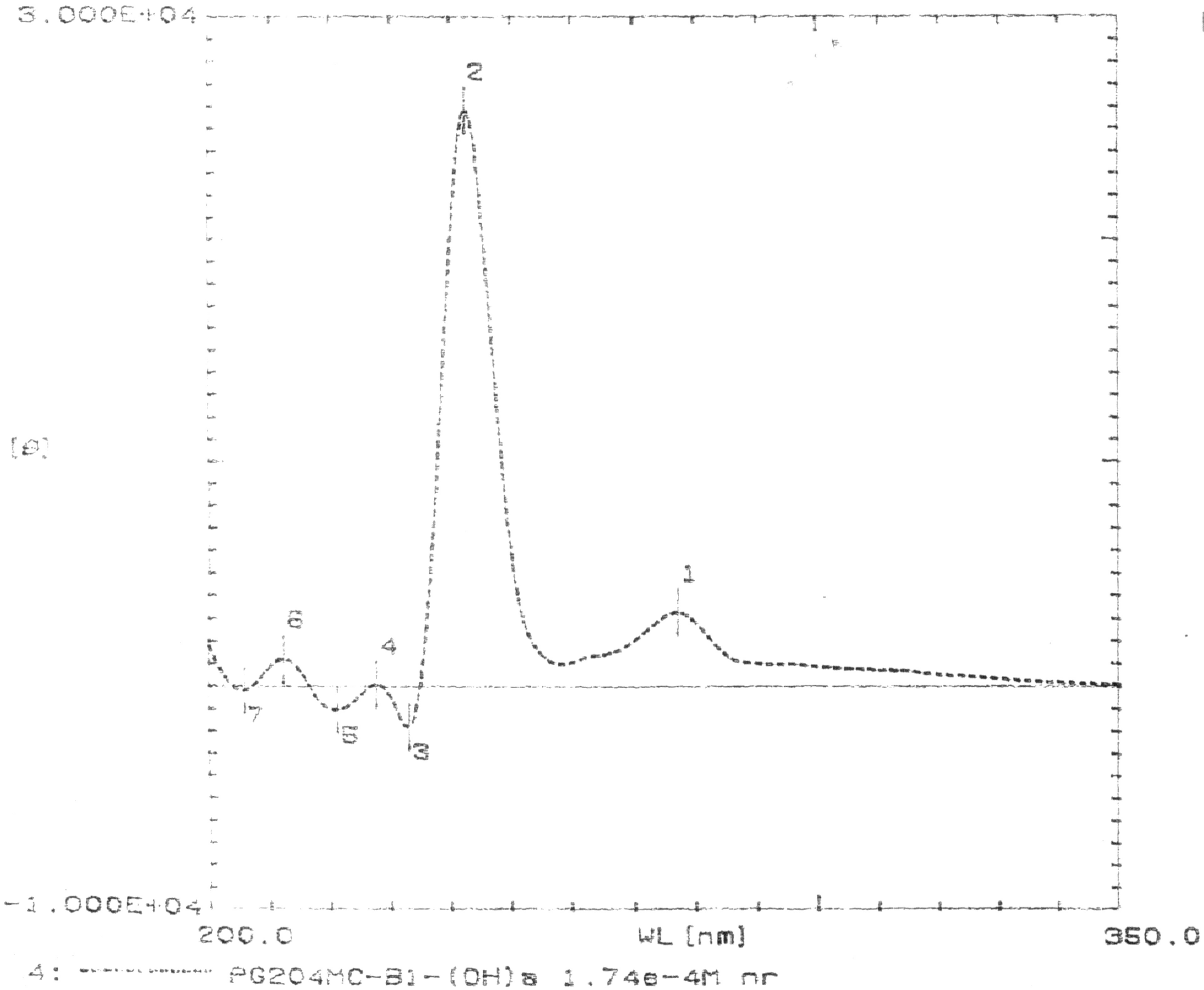

Conditions of Memory 1
File : MA004-2
Date : 71-10-10
Data : [θ]
Scan : WL
Band width :
1.0 nm
Response :
0.125 sec
Wavelength range :
350.0 - 180.0nm
Step resolution :
0.2nm/data
Scan speed :
10nm/min
Accumulation : 1

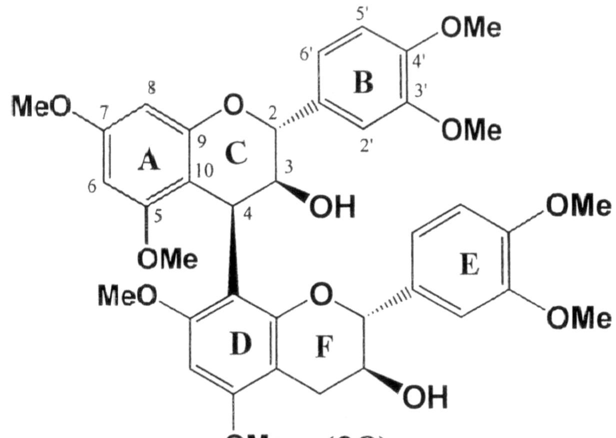
| No. | Wavelength | Value |
|---|---|---|
| 1 | 289.80 nm | 1.573E+04 |
| 2 | 211.80 nm | -4.122E+04 |
| 3 | 202.20 nm | 4.926E+04 |
| 4 | 195.00 nm | -6.758E+02 |
| 5 | 192.60 nm | 7.215E+02 |
| 6 | 188.80 nm | -1.789E+03 |
| 7 | 183.20 nm | 3.958E+03 |




(27)

# CD Plate 14:

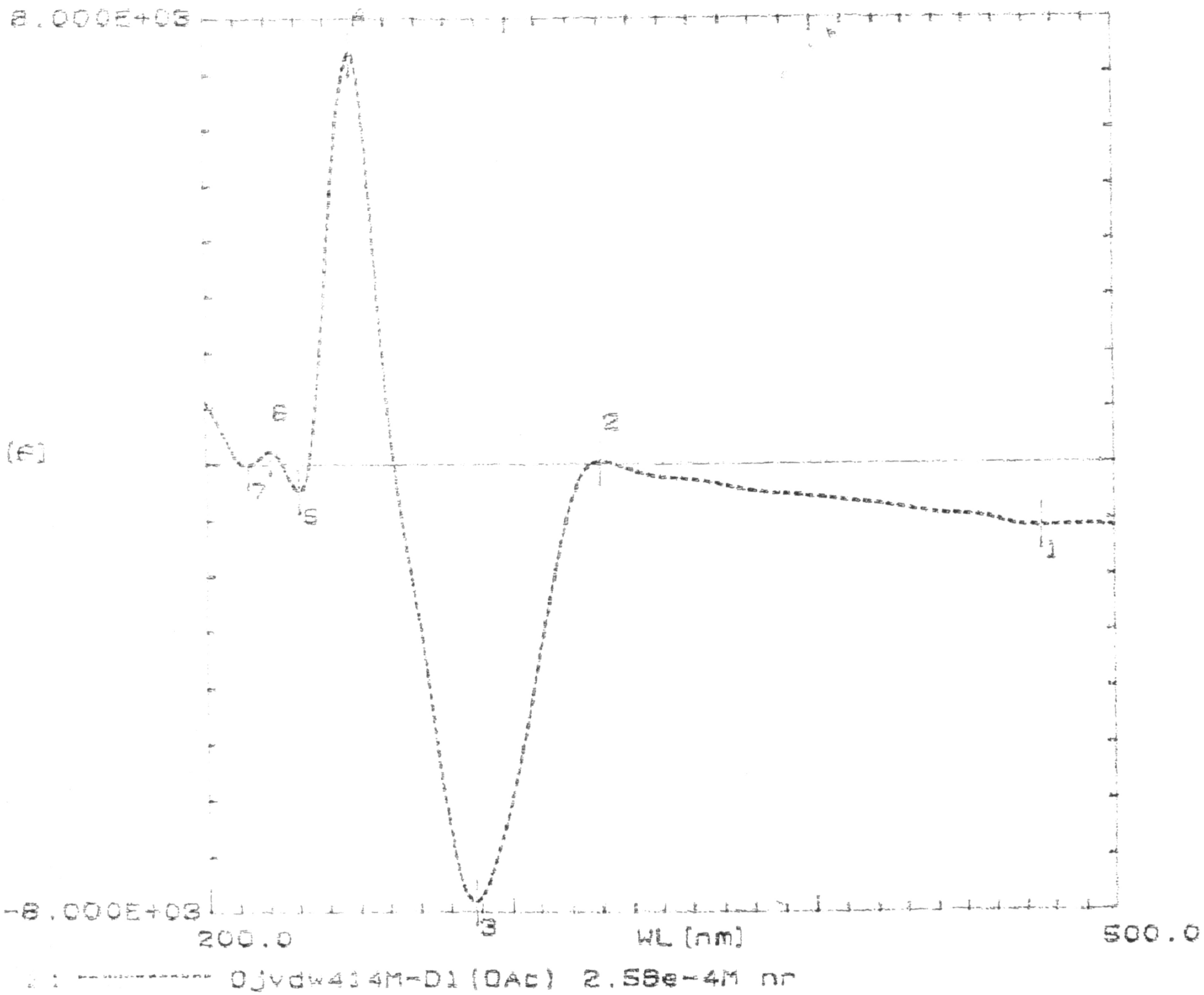


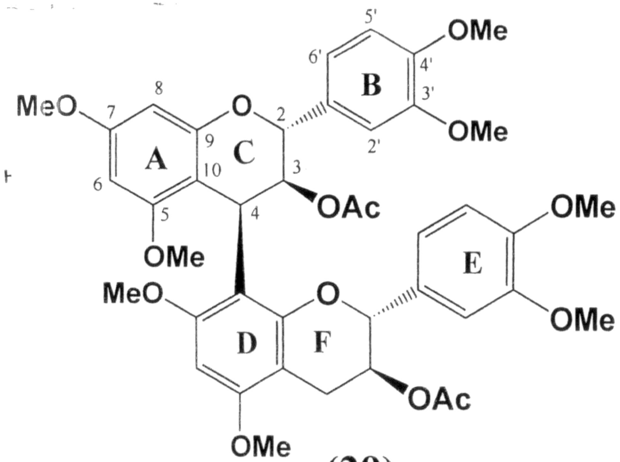
(28)

| No. | Wavelength | Value |
|---|---|---|
| 1 | 277.00 nm | 3.277E+03 |
| 2 | 242.50 nm | 2.583E+04 |
| 3 | 233.00 nm | -1.796E+03 |
| 4 | 227.50 nm | 6.870E+01 |
| 5 | 221.00 nm | -1.051E+03 |
| 6 | 212.00 nm | 1.204E+03 |
| 7 | 205.50 nm | -1.924E+02 |

## CD Plate 15:



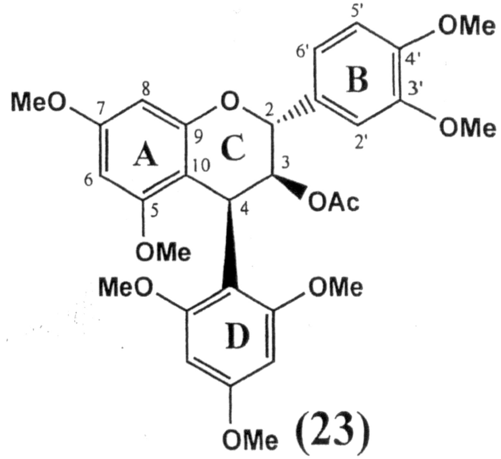

Conditions of Memory 1
File : MO10-1



(29)

| No. | Wavelength | Value |
|---|---|---|
| 1 | 475.50 nm | -1.149E+03 |
| 2 | 330.00 nm | 2.544E+01 |
| 3 | 288.00 nm | -7.828E+03 |
| 4 | 248.00 nm | 7.377E+03 |
| 5 | 230.50 nm | -4.873E+02 |
| 6 | 221.00 nm | 2.202E+02 |
| 7 | 213.50 nm | -3.878E+01 |

Plate 13.3 $^{13}C$ NMR ($CDCl_3$, 298K)




(23)

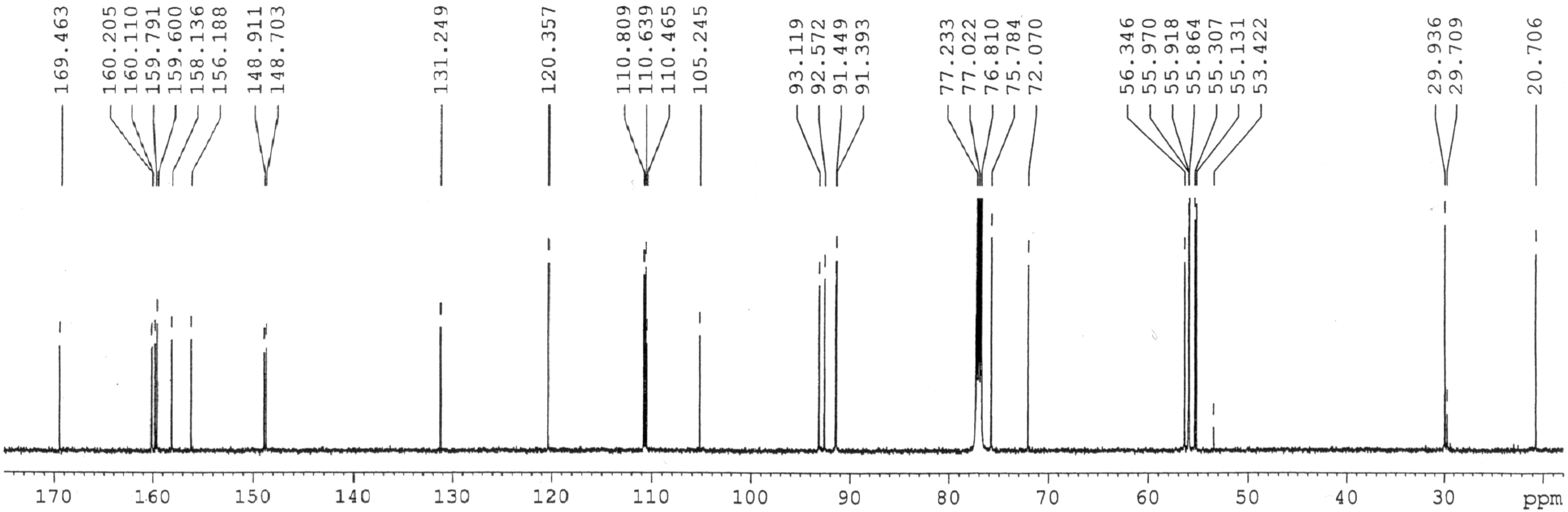
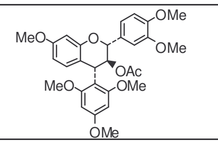
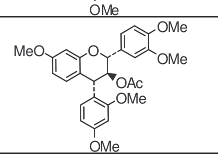
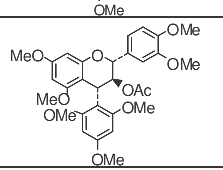
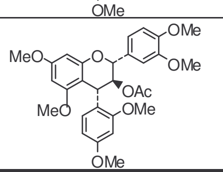
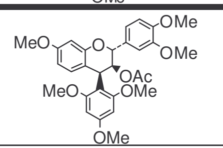
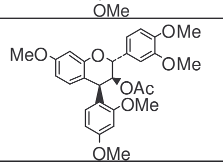
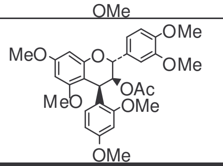
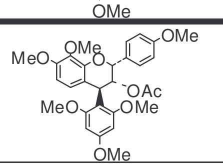
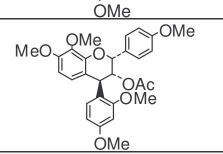
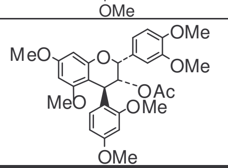
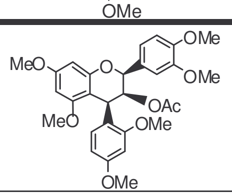
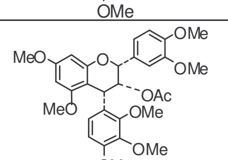


**Table 5**

**EI Mass Spectrometry, $J_{2,3}$ and $J_{3,4}$ NMR coupling constants and sign of cotton effects of 4-aryl-flav-3-*O*-acetates**

| | MS | | | $^{1}$H-NMR | | | | | CD |
|---|---|---|---|---|---|---|---|---|---|
| | Intensity | | $\frac{M^{+}-60}{M^{+}}$ | J | | δ | | | Signs of Cotton-effect |
| | $M^{+}$ | $M^{+}$-60 | | $J_{2,3}$ | $J_{3,4}$ | 2-H | 3-H | 4-H | |
| | | | | 2,3-*trans*-3,4-*trans* | | | | | |
|  | 1.3 | 100 | 77 | 10.20 | 9.80 | 4.90 | 6.02 | 4.85 | Negative |
|  | 1.2 | 100 | 83 | 10.00 | 9.00 | 5.00 | 5.77 | 4.62 | Negative |
|  | 1.3 | 100 | 77 | 10.00 | 8.50 | 4.68 | 5.73 | 4.69 | Negative |
|  | 1.5 | 100 | 67 | 6.50 | 5.50 | 5.00 | 5.65 | 4.62 | Negative |
| | | | | 2,3-*trans*-3,4-*cis* | | | | | |
|  | 9 | 30 | 3.3 | 9.50 | 6.50 | 5.16 | 5.46 | 4.88 | Positive |
|  | 26 | 75 | 2.9 | 8.00 | 5.00 | 5.13 | 5.53 | 4.83 | Positive |
|  | 21 | 55 | 2.6 | 10.00 | 5.80 | 4.94 | 5.45 | 5.04 | Positive |
| | | | | 2,3-*cis*-3,4-*trans* | | | | | |
|  | 2.5 | 100 | 40 | <1 | 3.8 | 5.44 | 5.47 | 4.53 | Positive |
|  | 2.5 | 100 | 40 | <1 | 1.9 | 5.02 | 5.37 | 4.41 | Positive |
|  | 2.0 | 100 | 50 | <1 | 2.3 | 4.93 | 5.39 | 4.48 | Positive |
| | | | | 2,3-*cis*-3,4-*cis* | | | | | |
|  | 8 | 20 | 2.5 | 1.20 | 4.75 | 5.18 | 5.52 | 5.09 | Negative |
|  | 11 | 15 | 1.5 | 1.25 | 4.90 | 5.17 | 5.39 | 4.90 | Positive |