SYNTHESIS AND APPLICATIONS OF EPICATECHIN AND EPIAFZELECHIN DERIVATIVES FROM PROANTHOCYANIDINS

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Abstract

Proanthocyanidins (PAs), one of the most ubiquitous groups of plant polyphenols, are the natural polymer widespread throughout the plant kingdom but their application as raw materials for fine chemicals are largely unexplored. This thesis documented the results on the effort towards chemical conversion of PAs into epicatechin and epiafzelechin derivatives with potential application such as multidentate chiral ligands.

Mangosteen pericarps proanthocyanidins (MPPs) were extracted with 0.66% yield (dry matter) from mangosteen peels, agricultural waste in South East Asian countries. $^{13}C\{^{1}H\}$ and ^{1}H NMR signals showed the presence of predominantly procyanidins together with a few prodelphinidin units along with small amounts of stereoisomers of afzelechin/epiafzelechin and gallocatechin/ epigallocatechin. Depolymerization of MPPs with benzylmercaptan indicated that the mean degree of polymerization (mDP) is 6.6. The electrospray ionization–mass spectrometry (ESI-MS) and matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectra revealed the dominant B type oligomers with mainly epicatechin units and with a small amount of A-type oligomers.

MPPs and CPs extracted in our lab were compared with commercial pine bark proanthocyanidins (PBPs) and grape seed proanthocyanidins (GSPs) via MALDI-TOF-MS and thiolysis analysis. Both thiolysis and MALDI-TOF-MS results indicated that MPPs were ideal for synthesis of the new chiral ligands due to the higher mDP and ratio of epicatechin units.

MPPs were depolymerized with selective carbon and sulfur nucleophiles. The products were purified by column chromatography and characterized with ESI-

MS/MS and NMR spectrometry. After screening the selective protecting reagents of the B-ring ortho-dihydroxyl groups, the depolymerized products were applied for one or two step reaction and finally sixteen chiral epicatechin derivatives were synthesized.

Dethiolation was observed in two sulfur-containing epicatechin derivatives. In order to synthesize stable sulfur-containing ligands, proanthocyanidins from the rhizomes *Selliguea feei* (FSPs), were extracted and characterized. FSPs are mainly A-type propelargonidin dimer and trimers. Then, three sulfur-containing epiafzelechin derivatives were synthesized together with a rare alkaloidal A-type propelargonidin.

Kinetics of the dethiolation of three sulfur-containing epicatechin derivatives under weakly basic conditions were investigated and the mechanism of the base-catalyzed condensation was proposed. The results indicated that epicatechin derivative **8** is an ideal intermediate for synthesis of bioactive natural products such as procyanidin B2 and some rare alkaloidal flavonoid under very mild conditions.

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List of Abbreviations

Symbols	Description
PAs	proanthocyanidins
DP	degree of polymerization
MS	mass spectroscopy
mDP	mean degree of polymerization
HPLC	high-performance liquid chromatography
ESI-MS	electrospray ionization mass spectroscopy
MALDI-TOF-MS	matrix-assisted laser desorption / ionization - time-of-flight mass spectroscopy
FIA-MS	flow injection analysis – mass spectra
RDA	retro Diels-Alder
QM	Quinone-Methide
HRF	heterocyclic ring fission
DHB	2, 5-Dihydroxybenzoic acid
IAA	trans-3-indolacrylic acid
DPPH	1,1-diphenyl-2-picrylhydrazyl

ABTS	2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid
IC50	the concentration required to inhibit DPPH radical formation by 50%
TEAC	Trolox equivalent antioxidant capacity
LDL	low-density lipoproteins
nNOS	neuronal nitric oxide synthases
UTIs	urinary tract infections
MIC	the minimum inhibitory concentration
EDR	endothelium-dependent relaxation
ACE	angiotensin converting enzyme
ODC	ornithine decarboxylase
PhIP	2- amino-1-methyl-6-phenylimidazo [4, 5-b] pyridine
TGF	transforming growth factor
HSV	herpes simplex virus
HIV	human immunodeficiency virus
IC_{50}	the half maximal inhibitory concentration
ROS	reactive oxygen species
MPPs	mangosteen pericarps proanthocyanidins

CPs	cocoa proanthocyanidins
PBPs	pine bark proanthocyanidins
GSPs	grape seed proanthocyanidins
CD	circular dichroism
BINOL	1,1'-Bi-2-naphthol
DMAP	4-dimethylaminopyridine
MeOH	methanol

Chapter 1

Literature Review

1.1 Structures, sources, and bioavailability of proanthocyanidins

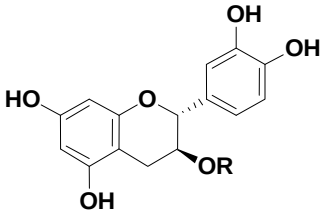
Proanthocyanidins (PAs), also known as condensed tannins, are a class of oligomeric and polymeric flavan-3-ols which are widespread throughout the plant kingdom, where they accumulate in many different tissues to provide protection against predation. The chemistry of proanthocyanidins has been studied for many decades. The proanthocyanidins obtained their name from the characteristic oxidative depolymerization reaction in acidic medium, which yields colored anthocyanidins [1].

1.1.1 Structure of proanthocyanidins

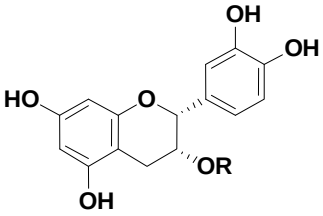
Proanthocyanidins consist of an electrophilic flavanyl unit, generated from a flavan-4-ol or a flavan-3,4-diol (leucoanthocyanidin), coupled to a nucleophilic flavanyl unit, often a flavan-3-ol [2-4]. Because of variation in monomeric units, stereochemistry at the three chiral centers at C2, C3, and C4 positions, and the location and type of interflavan linkage, proanthocyanidins exhibit rich structural complexity and diversity [5].

Figure 1.1 showed the common monomeric units in proanthocyanidins, i.e. catechin/epicatechin, gallocatechin/epigallocatechin, afzelechin/epiafzelechin and their gallates. The most studied monomeric units are flavan-3-ols (+)-catechin and (-)-epicatechin. Many plants such as pine bark and apple mainly contain these monomeric units [6, 7]. Other important flavan-3-ols are (+)-gallocatechin, (-)-epigallocatechin, and (-)-epigallocatechin gallate. The latter two compounds are monomeric units of green tea proanthocyanidins [8]. Afzelechin and epiafzelechin units are rich in red kidney bean and strawberry [9]. The most common substituent bound as an ester is gallic acid as 3-O-gallates. (epi)gallocaechin 3-O-gallates are the major units of grape seed proanthocyanidins [10]. In addition, derivatisations, such as

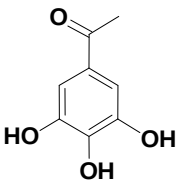
O-methylation, C- and O-glycosylation, and O-galloylation are frequently reported [2, 11]. Several glycosylated proanthocyanidin oligomers have been identified, with the sugar linked to the C3 or the C5 position as the most widely distributed glycosylation [2, 3, 12].



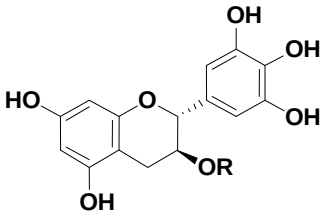
Catechin (R = H) or
Catechin gallate (R = G)



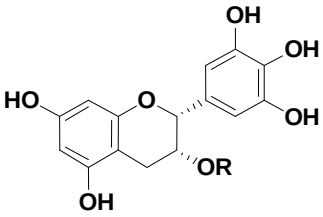
Epicatechin (R = H) or
Epicatechin gallate (R = G)



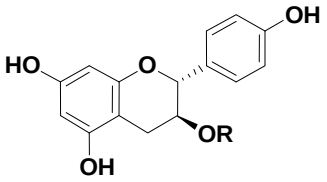
G: Galloyl group



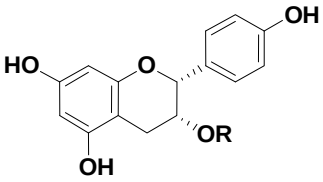
Gallocatechin (R = H) or
Gallocatechin gallate (R = G)



Epigallocatechin (R = H) or
Epigallocatechin gallate (R = G)



Afzelechin (R = H) or
Afzelechin gallate (R = G)



Epiafzelechin (R = H) or
Epiafzelechin gallate (R = G)

Figure 1.1 Chemical structures of typical flavan-3-ol units in proanthocyanidins

Proanthocyanidins are classified into several subgroups according to their hydroxylation pattern in the extension units, including procyanidins (3,5,7,3',4'-OH), prodelphinidins (3,5,7,3',4',5'-OH), propelargonidins (3,5,7,4'-OH), profisetinidins (3,7,3',4'-OH), prorobinetinidins (3,7,3',4',5'-OH), proguibourtinidins (3,7,4'-OH), proteracacinidins (3,7,8,4'-OH), and promelacacinidins (3,7,8, 3',4'-OH) (examples

shown in Figure 1.2). In addition, in red wines colored proanthocyanidins with anthocyanin units in polymeric alliance have also been identified [13].

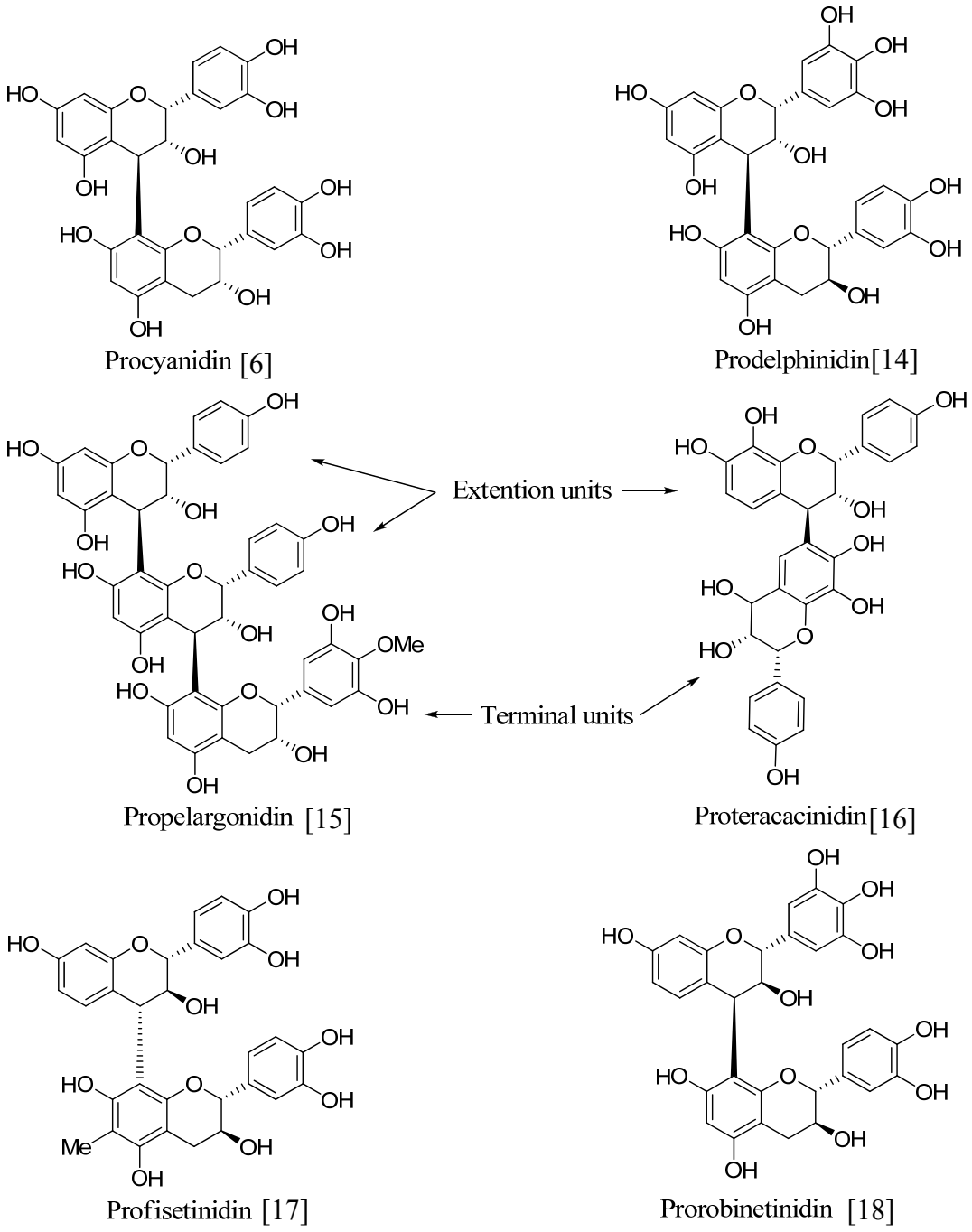


Figure 1.2 Reported examples of subgroups of proanthocyanidins

Procyanidins are the most common group of naturally occurring proanthocyanidins. Pine (Pinus radiate) bark proanthocyanidins are entirely procyanidin of degree of polymerization (DP) up to 10 [6]. While apple (*Malus*

silvestris) tannin are mainly procyanidins of low molecular mass [7]. Pecan nut (*Carya illionensis*) pith proanthocyanidins are predominantly prodelphinidins (prodelphinidins: procyanidin = 6:1) [14]. Strawberry is a typical food containing the propelargonidins [9].Quebracho (*Schinopsis balansae var. chaqueno*) wood proanthocyanidins are predominantly profisetinidins/prorobinetinidins [19]. 5-Deoxy [(epi)robinetinidol or (epi)fisetinidol] is also known [17, 20].

Another structural feature of proanthocyanidins is that there are lots of chiral centers, which increase with the increasing DP. Besides the two chiral center appear in monomeric units, the proanthocyanidins have one additional chiral center at the 4-position for each extension unit in the polymer, created by the interflavonoid bond (Figure 1.3). To describe the stereochemistry of the interflavonoid bond, a nomenclature system using α and β has been adopted. The α designation is given to proanthocyanidins with the 'lower' group *cis* to the B-ring of the 'upper' unit while the β designation is given to proanthocyanidins with the 'lower' group *trans* to the B-ring of the 'upper' group [21, 22].

There are two types of linkages between successive units in proanthocyanidins (Figure 1.3). B-type interflavan linkage, the familiar linkages between successive units is between the 4-position of the 'upper' unit and the 8-position or 6-position of the 'lower' unit. A-type proanthocyanidins possess an additional ether linkage between C-2 of the upper unit and a 7- and/or 5-OH of the lower unit [6], this subclass has two hydrogen atoms less compared to the B-type. Cranberry proanthocyanidins are typical proanthocyanidins possessing A-type interflavan linkage [9].

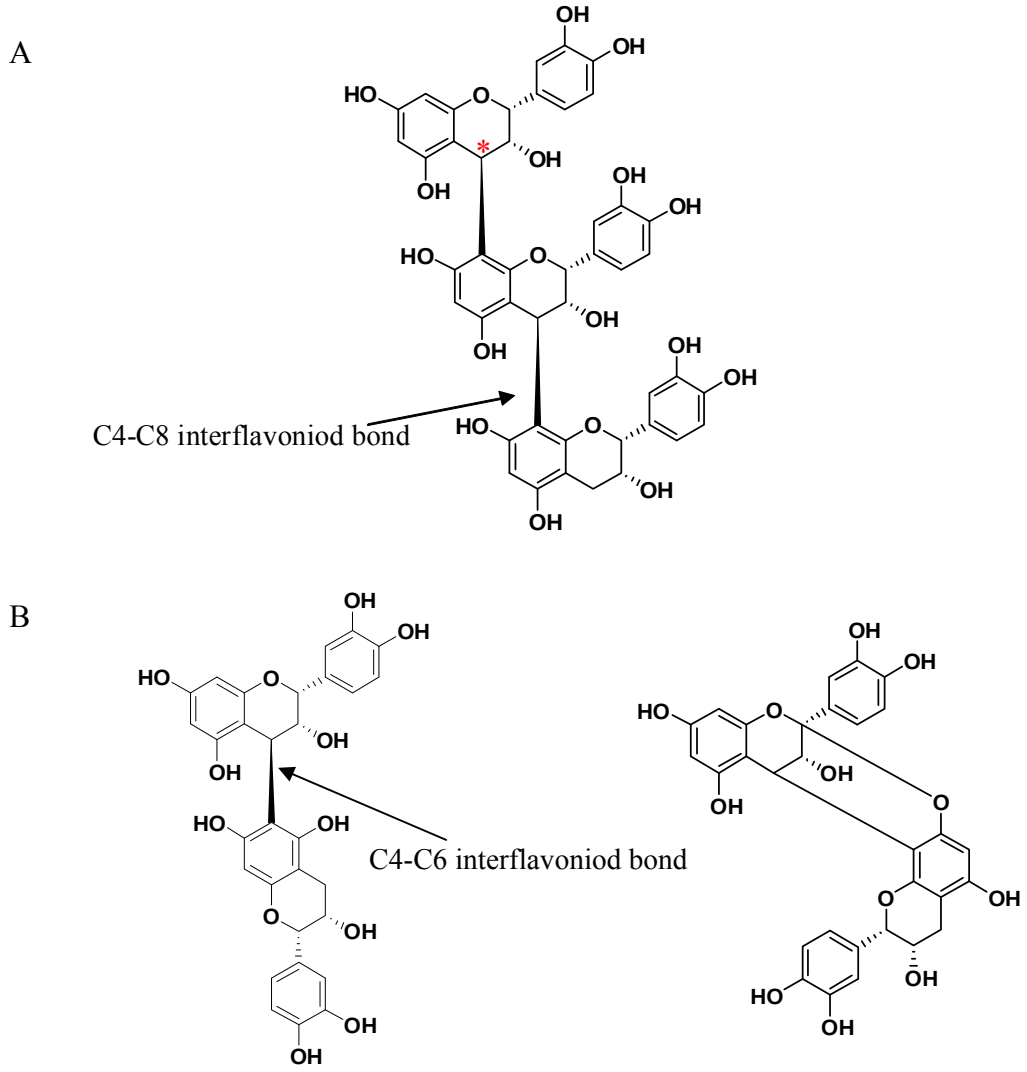


Figure 1.3 Typical procyanidins showing different interflavan linkage. A, the trimer epicatechin-(4β→8)-epicatechin-(4β→8)-epicatechin; B, the dimer epicatechin-(4β→6)- epicatechin and C, the A-type dimmer epicatechin-(4β→8, 2β→O→7)-epicatechin. *, the additional chiral center at the 4-position in the polymer.

1.1.2 Analytical methods of proanthocyanidins

Due to the linkage complexity and structural diversity of monomeric units, the characterization of highly polymerized proanthocyanidins thus is challenging, and proanthocyanidins were considered to be the final frontier of flavonoid research [23]. Various techniques including NMR and mass spectroscopy (MS) have been used to characterize proanthocyanidins and summary herein are the technologies in context to proanthocyanidins.

NMR spectroscopy

NMR spectroscopy is an important tool for characterization of proanthocyanidins. ^{1}H NMR may give the information about the mean degree of polymerization (mDP) of proanthocyanidins. The mDP can be estimated by integrating the A-ring proton signals between 5.8 and 6.5 ppm and comparing them to the intensity of the H4 signals of the terminal units between 2.4 and 3.0 ppm [24]. Alternatively, mDP can also be determined by high-performance liquid chromatography (HPLC) after depolymerizing with benzyl mercaptan or phloroglucinol [25-26]. Monomeric units and the structural diversity of the linkage (A and B type) can be indicated from ^{13}C NMR spectrum. A type linkages often showed the signals at 151–152 ppm due to C5 and C7 of the A ring involved in the double linkage. The average molecular weight can be determined from the spectra by comparing the areas of the C3 resonances of the terminal and extension flavan-3-ol units in B-type proanthocyanidins [27]. The determination of the ratio of the 2, 3-cis to 2, 3-trans stereochemistry could be achieved through ^{13}C NMR by virtue of the distinct differences in their respective C2 chemical shifts [27].

Procyanidins show very broad ^{1}H signals at room temperature due to atropisomerism; this makes it difficult to determine the linkage of the interflavanoid bond by NMR. Atropisomerism results from steric interactions in the vicinity of the interflavanoid bond, which does not allow the flavanoid units to rotate rapidly under ^{1}H NMR time scale. Tarascou et al. [28] reported a ratio of up to 55:45 of two rotamers observed via ^{1}H-NMR. To handle the problem of atropisomerism, a method is to compare ^{13}C-NMR chemical shifts of each unit after the cleavage of the interflavanoid bond by thiolysis [29] or reaction with phloroglucinol [30]. The chemical shift of the H2 of the terminal unit (lower unit) of a dimeric procyanidin

with the 2, 3-trans configuration can help to evaluate the position of the interflavanoid bond (4β→8 or 4β→6) of underivatized procyanidins [31]. In case of a 4β→ 6 bond, the chemical shift of this proton is around 4.58 ppm, while for a 4β→8 bond it is found at 4.91–4.97 ppm. Another similar method was to use the chemical shift of H6 or H8 (E-ring) of an acetylated or methylated procyanidin obtained at temperatures of 100–170 ^{o}C. The interflavanoid bond fastly rotates under this condition. The H6 (E-ring) exhibits a chemical shift of 6.06–6.16 ppm in the 4β→ 8-linked dimers, whereas the H8 (E-ring) shows a 6.20–6.38 ppm shift in the 4β→6-linked dimers [32]. Interestingly, low temperature ^{1}H-NMR resulted in sharp and resolved peaks while the spectra recorded at ambient temperature showed the broadening of ^{1}H NMR signals due to atropisomerism [33].

ESI-MS and MALDI-TOF-MS

Because quantitative data regarding to the polymerization profile of proanthocyanidins can not be reliably obtained by NMR spectrum, further characterization is usually achieved by electrospray ionization mass spectroscopy (ESI-MS) and matrix-assisted laser desorption / ionization - time-of-flight mass spectroscopy (MALDI-TOF-MS). ESI-MS spectrum can show the units and linkage character via a series of molecular ions or fragments. It is a gentle, sensitive, and the most often used method to date [34]. Analyzing procyanidins in grape extracts, Wu et al. [35] determined a limit of quantification of about 40 ng/ml for flavanol monomers such as catechin/epicatechin and estimated the concentration levels of proanthocyanidin dimer to heptamer in grape wine and grape juice, by comparing their flow injection analysis – mass spectra (FIA-MS) peak areas. Some additional experiments using the mass spectrometer i.e., using a triple quadruple (MS/MS) or an ion trap for MS^2 or MS^n experiments, can lead to specific reaction products and hence

helping to get higher sensitivity. Cheynier et al. [36] detected double and triple charged ions that could be interpreted as a lower DP. Friedrich et al. [37] described a technique for elucidating the structures of unknown oligomeric procyanidins. Multiple MS experiments using an LCQ IT were used and the retro Diels-Alder (RDA) fission in the T-unit was found to be the most important fragmentation [37]. Gu et al applied MS/MS analysis for determination of proanthocyanidins in many foods and reported the main fragmentation pathway, i.e. Quinone-Methide (QM) cleavage, RDA and heterocyclic ring fission (HRF) as shown in Figure 1.4 [9].

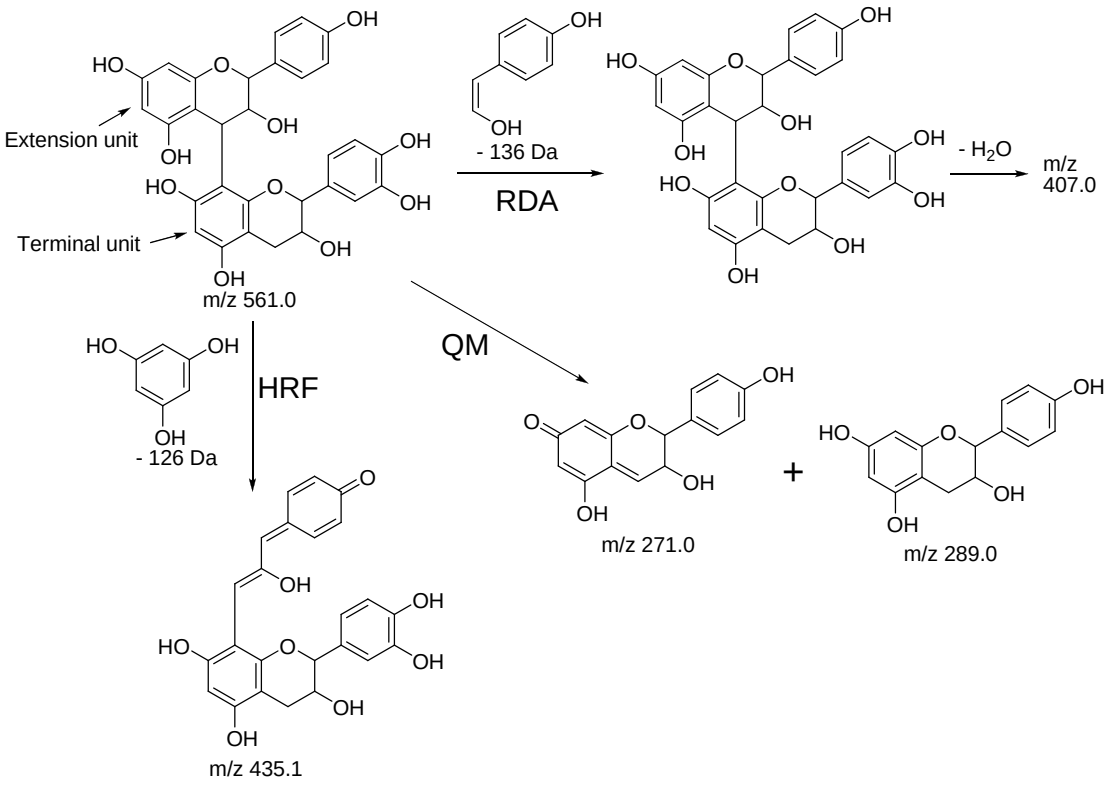


Figure 1.4 Fragmentation pathway of a propelargonidin dimer detected in strawberry [9].

However, the limited range imposed by the quadruple analyzer as well as the possible presence of multiply-charged ions for the larger molecules, inducing peak dispersion and frequent overlapping, resulted in an increased difficulty of

interpretation of the signals when using ESI-MS to higher DP proanthocyanidins. A complementary spectroscopic technique to limit the production of multiply charged species is MALDI-TOF MS, allowing the analysis of polymers and revealing information about their chain lengths [38, 39].

The application of the MALDI-TOF-MS for analysis of oligomeric procyanidins has been introduced within the last 10 years. In the first publication, the range of polymerization of proanthocyanidins in apples from dimer to pentadecamer was determined [40]. Recently, polymers up to a DP of 25 can be detected [41]. MALDI-TOF MS is very suitable for proanthocyanidins since this method produces only a singly charged molecular ion for each parent molecule and allows detection of high mass with precision [42] 2, 5-Dihydroxybenzoic acid (DHB) is the matrix best suited for proanthocyanidin analysis in the reflectron mode. DHB provides the broadest mass range with the least background noise and hence is superior r to other commonly used matrix systems, such as trans-3-indolacrylic acid (IAA), R-cyano-4-hydroxycinnamic acid, sinapinic acid, 9-nitroanthracene, 5-chlorosalicylic acid, 2-(4-hydroxyphenylazo)benzoic acid and dithranol [12]. In fact, IAA matrix can provide a mass range similar to DHB, however, the signals from proanthocyanidin dimers is easy to be blocked out because DHB often generates a very high background noise in the mass range below 500 [41, 43]. In addition, Yang and Chien recommended using MALDI-TOF not only for characterization but also for quantification of procyanidins in grapes [43]. In order to promote the formation of a single ion adduct, cesium trifluoroacetate or sodium chloride sometimes was added to the matrix/sample solution [44-46].

X-ray analysis

As a matter of fact, X-ray analysis is the most direct method to show the structure of chemicals directly. However, only the structure of procyanidin B1 was unequivocally confirmed by X-ray analysis of its deca-O-acetyl derivative (Figure 1.5) [47]. Stereochemistry at the various chiral centers in proanthocyanidins might be responding to the difficulty of applying X-ray analysis to indicate the structure and investigate the structure-activity relationship of proanthocyanidins.

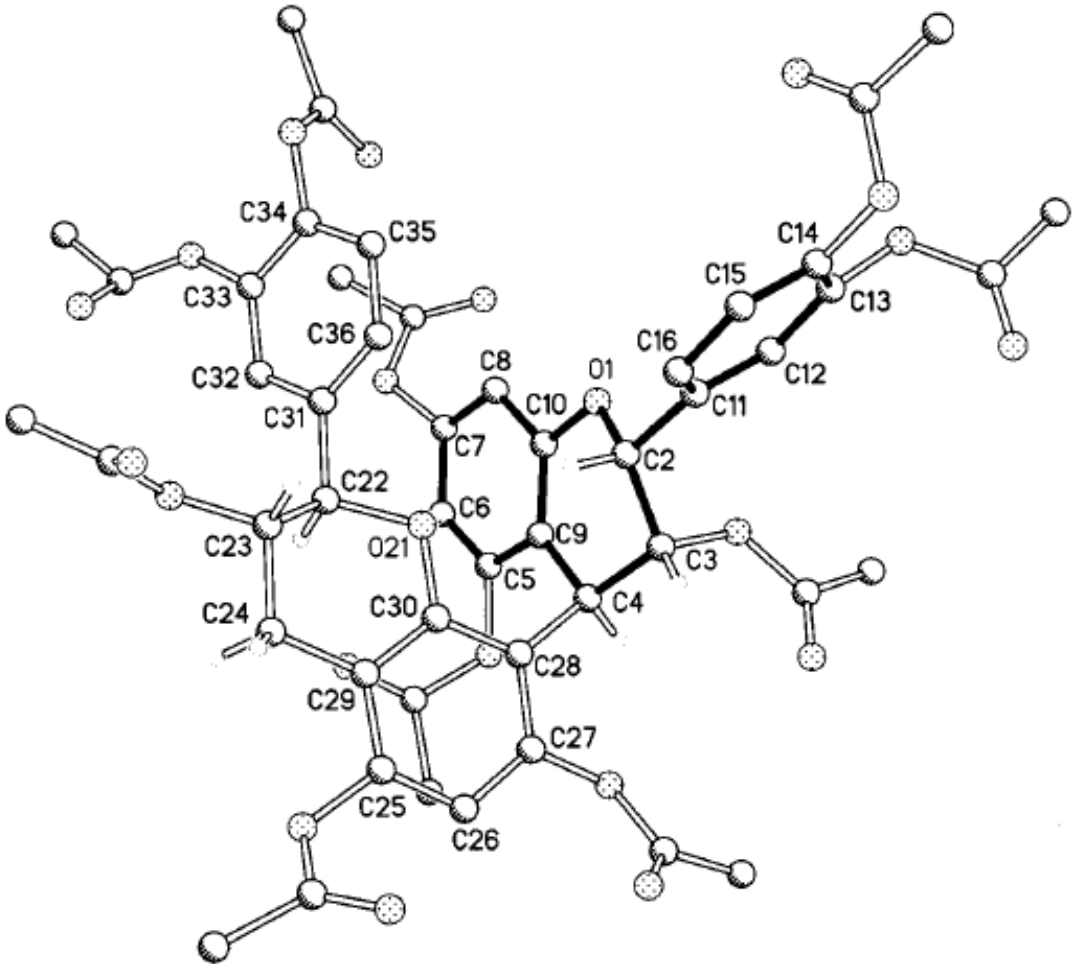


Figure 1.5 Molecular structure of procyanidin B1 [47]. (Some H-atoms are omitted for clarity.)

Theoretically, the dimeric procyanidins of group B have five stereogenic centers, so that 32 optically active forms are possible. The number is reduced in that only

polyhydroxy- flavanols with 2R-configuration like (+)-catechin and (-)-epicatechin occur in nature. However, without taking into account the configuration of C-4, there are still four combinations of the two halves of the molecule responding to procyanidin dimers. So, it is difficult to get a single crystal even for dimer. With the increasing degree of polymerization, the molecule weight becomes large and the possible optically active forms become numerous and make it more difficult to obtain the single crystals, this is the limiting factor for broad application of this method.

1.1.3 Sources and content of proanthocyanidins.

Apart from lignin, proanthocyanidins represent the most abundant class of natural phenolic compounds [48, 49]. The proanthocyanidins were mainly detected in fruits and berries, but also nuts, beans, cereals (such as barley and sorghum), the spices (e.g. curry and cinnamon). The wide presence of proanthocyanidins in plants makes them an important part of the human diet, and they are also found in the beverages derived from proanthocyanidins rich plant-based products. The knowledge about the distribution and nature of proanthocyanidins in foods represented an important progress with work in Gu's group only very recently [9]. The content of proanthocyanidins in human foods has been thoroughly investigated in a screening of 88 different kinds of foods for proanthocyanidins, including oligomers and polymers. Out of the 88 plant-based foods investigated using LC-MS/MS after thiolytic degradation, 39 were found to contain proanthocyanidins. In a later study, Gu et al. [50] furthermore investigated the concentration of proanthocyanidins in all the foods that were found to contain proanthocyanidins in the first study. The investigated results showed that ground cinnamon contained the highest amount of proanthocyanidins at 8108 mg/100 g (fresh weight foods) followed by dry grape seed

and sorghum bran with the content of 3965 and 3532 mg/100 g (fresh weight foods), respectively [50].

Most of plant-based foods tested contain exclusively the homogeneous B-type procyanidins, while few also contain the heterogeneous propelargonidin or prodelphinidin. A-type proanthocyanidins were only found in curry, cinnamon, cranberry, peanut, plums, and avocado [9]. However, the content of A-type proanthocyanidins was determined to be very high in these foods. In curry and cinnamon between 84–90% of the total amount of proanthocyanidins were A-type. While in cranberry and peanut about 51–65% was A-type proanthocyanidins [9]. The majority of A-type proanthocyanidins found in nature contain only one additional A-type interflavan bond primarily in between the extension units or as an A-type terminal unit.

19 vegetables were investigated for the content of proanthocyanidins and the results indicated that vegetables are not an important source of proanthocyanidins. In fact, only Indian squash contained proanthocyanidins. In addition, no proanthocyanidins were detected in citrus fruits, pineapple, watermelon, oat, rice, corn, or in 13 other spices [9].

1.1.4 Absorption and bioavailability of proanthocyanidins

Proanthocyanidins are an important contributor of health promotion; Gu et al reported that the mean daily intake of proanthocyanidins (DP ≥ 2) in the population of all ages (>2 y old) in the United States is about 53.6 mg/person [50]. However, to produce a biological effect *in vivo*, it is essential that sufficient quantities reach the target tissues. The uptake and metabolism of the complex mixtures of proanthocyanidins presenting in our food were studied with the advance of the new analytical techniques. Though the proanthocyanidins found in food cover a wide

range of mDP, only the low-molecular-weight oligomers (DP < 3) are absorbed intact in the gastrointestinal tract. Isotope labeled procyanidin dimers and trimers are absorbed to a similar extent as (+)-catechin in experiments obtained from a caco-2 cell line, which is used as an *in vitvo* model of the human intestinal epithelium [51]. Unlike the lower oligomers, proanthocyanidins with DP > 3 are difficult to be absorbed directly from the gastrointestinal lumen but are thought to depolymerize into mixtures of flavanol monomer and dimers in the gastrointestinal tract [52].What's more, the higher the polymerization degree, the more readily the oligomers were cleaved [53, 54]. A time-dependent decomposition of oligomers (trimer to hexamer) to mixtures of epicatechin monomer and dimers were observed when procyanidins were exposed to gastric juice [54]. However, cocoa procyanidins are stable in the stomach environment in six healthy volunteers, which suggested that most of the ingested procyanidins reach the small intestine intact [55].

Déprez et al. found that proanthocyanidins can be degraded by the human colonic microflora [56].Approximately 50% of the proanthocyanidins were degraded by human colonic flora after 10 hours of incubation in anoxic conditions. Almost all proanthocyanidins were degraded after 48 hours incubation. The degradation products, phenylacetic, nylpropionic, and phenylvaleric acids, have been suggested to be the major metabolites of oligomeric and polymeric proanthocyanidins in healthy humans [57].

Richelle et al. reported that the epicatechin plasma concentration has a marked increase after chocolate consumption, which indicated the resorption of procyanidins – either unchanged or as flavan-3-ols [58]. Epicatechin concentration can reach its maximal level two to three hours after chocolate ingestion. In another study, Spencer et al. found that epicatechin is the primarily bioavailable form of the procyanidin

dimers B2 and B5 after transferring across the small intestine [59]. However, it was demonstrated in rats that procyanidin B2 is absorbed and partially excreted in urine with some procyanidin B2 degraded to epicatechin [60].

1.2. Bioactivities of proanthocyanidins

A large body of literature has emerged supporting potential health beneficial effects of various crude and purified proanthocyanidin fractions from plant fruits, leaves and bark, especially green tea, grapes and cranberry.

1.2.1 Antioxidant activities

A significant percentage of work on heath effects of proanthocyanidins focused on antioxidant activities. Lots of flavan-3-ols and proanthocyanidins were documented for their scavenging activities against free radical including 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS•+) [61], O_2•- [62, 63], 1,1-diphenyl-2-picrylhydrazyl (DPPH•) [62, 64-66], and HO• [67]. Different group at B-ring and position 3 affected the antioxidant activities in several studies. The highest scavenging activity of all proanthocyanidins tested was found for galloylated procyanidins [61-65]. While, introduction of a gallic acid function at position 3 increases significantly the radical scavenging activity, and glycosylation of the position 3 of proanthocyanidins units decreases the scavenging ability [61, 64, 65] somehow. In addition, the doubly linked A-type proanthocyanidin dimers were less effective than their B-type counterparts [61] With respect to the effect of the stereochemistry in B-type procyanidins on the scavenging activity, the results are ambiguous. A series of tannins and flavonoids were isolated from several oriental medicinal herbs. Their antioxidant activities were analyzed with a DPPH radical-generating system. The micromolar concentration required to inhibit DPPH radical

formation by 50% (IC_{50}) of procyanidin B2 is 3.42 μM while that of procyanidin B3 is 4.85 μM [64]. Hatano et al reported that IC_{50} for procyanidin B2 was 1.4 μM while that of procyanidin B5 is 2.3 μM for DPPH radical [66]. In contrast to the former studies, in Trolox equivalent antioxidant activity (TEAC) assay, no significant difference was found in activity between six B-type procyanidins [65]. Effect of the degree of polymerization on scavenging activity has a better consensus. Polymerization up to trimers will increase free radical scavenging activity, while further polymerization will decrease scavenging activity [61, 67].

The flavan-3-ols and proanthocyanidins were reported as inhibitors of lipid peroxidation in several studies. However, there is no unambiguous structure-activity relationship [61, 63, 68, 69-71]. The antioxidant activity of different proanthocyanidins on oxidation of human low-density lipoproteins (LDL) was investigated and the results showed that the number of hydroxyl groups or the degree of polymerization is related to the antioxidant activity. Antioxidant activity of different proanthocyanidins and flavan-3-ols decreased in the following order: cinnamtannin A2 ~ procyanidin C1 > procyanidin B2 > (+)-catechin > (-)-epicatechin [70]. Equimolar concentrations of individual procyanidins were used in a copper – catalyzed LDL system and the results indicated that the antioxidant activity of PAs was proportional to DP [71]. Though lipid peroxidation was initiated through different mechanisms, which could explain the diverse results, it can be concluded that proanthocyanidins are good inhibitors of lipid peroxidation, with similar or higher inhibitory activities than Trolox™ and vitamin E, the standard antioxidants [63, 67, 70].

Inhibition of oxidases was another mechanism on antioxidant activity of proanthocyanidins. A mammalian reticulocyte-type 15-lipoxygenase, which is an important catalyst for lipid peroxidation of biomembranes and plasma lipoproteins, was inhibited by (-)-epicatechin and cocoa procyanidins [72]. Inhibitory activities decreased and increased again up to decamers. One study described the inhibition of cycooxygenase-1and 5-lipoxygenase by oligomeric proanthocyanidins from cocoa at concentrations similar to indomethacin, a drug used for the same purpose [73]. In another study, it was demonstrated that procyanidin dimers, trimers, tetramers and pentamers inhibited recombinant human 5-lipoxygenase, while hexamers and higher DP procyanidins were almost inactive [74]. In a recent study on a series of hop proanthocyanidins, it was found that procyanidin B2 and B4 and (-)-epigallocatechin gallate were the most active inhibitors of neuronal nitric oxide synthases (nNOS), while procyanidin B3, (+)-catechin, and (-)-epicatechin were inactive [75]. It suggested that dimeric procyanidins possessing epicatechin as terminal flavan-3-ol unit are stronger inhibitors of nNOS activity than dimers in which catechin represents the terminal unit. Additionally, procyanidin also inhibit the activities of xanthine oxidase and horseradish peroxidase [76]

Procyanidins oligomers (DP < 10) from *Theobroma cacao L.* were more effective than (-) - epicatechin by molarity to protect against peroxynitrite ($ONOO^-$) -dependent oxidation of dihydrorhodamine and nitration of tyrosine in two studies [77-78]. The tetramer showed the highest protecting activity against oxidation and nitration reactions [77]. It was suggested that procyanidins most likely react with oxidizing/nitrating intermediates, instead of reacting directly with $ONOO^-$ [77].

1.2.2 Antimicrobial Activities

The inhibitory activity of proanthocyanidins on bacteria and fungi has been recognized for a long time. Until now, the most interesting antibacterial activity of proanthocyanidins is related to cranberry proanthocyanidins. It was showed that consumption of cranberry or its products is effective in the prevention of urinary tract infections (UTIs) according to anecdotal observations and critical evaluation of scientific literature. The antiadhesion activity of cranberry (Vaccinium macrocarpon Ait.) juice was related to the presence of proanthocyanidins with at least one A-type linkage [79, 80]. It was reported that proanthocyanidins from cranberry might inhibit P-fimbriated *E. coli* from adhering to uroepithelial cells [81]. A-type proanthocyanidin trimers isolated from cranberries were more effective inhibitors of adherence than A-type proanthocyanidin dimers, while a B-type proanthocyanidins was not active at all [81]. A recent study showed that a cranberry fraction is able to inhibit adhesion of three strains of *Helicobacter pylori*, indicating a potential role in preventing peptic ulcers [82]. Further studies are underway to determine the preventive and curative capacity of A-type proanthocyanidins in UTIs and in other infectious diseases [83]. A cranberry fraction containing A-type proanthocyanidins inhibits several oral bacteria from adherence to teeth [84].

In addition, in two recent studies, a series of proanthocyanidins were evaluated for their activity against several Gram-positive and Gram-negative bacteria and against the yeasts *Candida albicans* and *Cryptococcus neoformans* [85, 86]. Both studies indicated that all proanthocyanidins tested showed only a moderate to weak antibacterial activity. In contrast to cranberry proanthocyanidins, B-type proanthocyanidins were more active than A-type proanthocyanidins against *C. albicans* and *C. neoformans*, with the minimum inhibitory concentration (MIC) values ranging from 250 to 1000 μg/ml [87].

Antiviral [herpes simplex virus (HSV) and human immunodeficiency virus (HIV)] and antibacterial activities of several proanthocyanidins were tested [88] The results indicated that more pronounced activities were obtain with epicatechin-containing dimers for anti-HSV and anti-HIV. The presence of ortho-trihydroxyl groups in the B-ring was important in compounds exhibiting anti-HSV effect. Double interflavan linkages gave rise to interesting antiviral effects both for HSV and HIV [88]. In another study, it was reported that epigallocatechin- (4β→8, 2β→O→7)-epicatechin inhibited HIV-1 protease at 70 μg/ml, while procyanidin A2 was not active at concentrations up to 100 μg/ml [89]. A structure-antiviral activity relationship study indicated that the anti-HIV-1 activity of procyanidin A2 higher than that of A1 [88]. In addition, a proanthocyanidins mixture mainly containing (-) epicatechin gallate-dimers, -trimers, and –tetramers, was evaluated for its antiviral activity against feline calicivirus F9 strain (FCV/F9) and coxsackievirus A7 strain (Cox.A7). The results suggested that proanthocyanidin may be an effective disinfectant against enteroviruses such as noroviruses [90].

Pyrogallol function affected the antiviral activities. (-)-epigallocatechin had a better antiherpetic activity than some dimers, such as B3 and B4. Another affecting factor was the interflavan linkage. A-type procyanidins or 4→6 linkage was preferred. Procyanidin C1 and an extracellular anti-HIV-1 activity, at a maximal non-toxic dose of 100 μg/ml [91].

Some interesting results were once reported on *in vitro* activity against HSV-1 and -2 of SP-303, a proanthocyanidin oligomer isolated from the latex of the plant *Croton lechleri* [92]. The mechanism appeared to act through inhibition of virus penetration into cells. The safety and effectiveness of a topical formulation of SP-303 was evaluated in a phase II study for the treatment of AIDS patients with recurrent

genital and perianal herpetic lesions [93]. However, the clinical results were disappointing and hence the development of the formulation was suspend in 1998[93].

A small number of studies were documented on the antiprotozoal activity of proanthocyanidins. A series of proanthocyanidins were tested for *in vitro* activities against *L. donovani amastigotes* and *promastigotes* and all of the proanthocyanidins tested significantly inhibited the intracellular survival of *L. donovani amastigotes* [94], with the half maximal inhibitory concentration (IC_{50}) values ranging from 0.7 to 7.7 nM, which can be similar with positive drugs sodium stibogluconate and amphotericin. However, none of the proanthocyanidins were active against the extracellular form. Specially, 4α→8-coupled dimers were more active than their corresponding 4β→8-dimers. The factors for enhancing the antieishmanial activity included increasing in molecular weights, galloylation of constituent units or the presence of predominantly 2,3-cis flavanyl chain extension units [94].

Two A-type proanthocyanidins from *Geranium niveum*, epiafzelechin-(4β→8, 2β→O→7)-afzelechin or geranin A and epicatechin-(4β→8, 2β→O→7)-afzelechin or geranin B were isolated and tested for antiprotozoal activity. Geranins A and B showed IC_{50} values of respectively 2.4 and 6.0 μg/ml for *G. lamblia* and of respectively 184.7 and 13.6 μg/ml for *E. histolytica* [95, 96]. However, the activity is much weaker than existing drug, Metronidazole, which exhibited much lower IC_{50} values of 0.21 and 0.04 μg/ml for respectively *G. lamblia* and *E. histolytica* [96].

1.2.3 Cardioprotective properties

Cardioprotective properties of proanthocyanidins were documented in many papers. However, there are different mechanisms of action, including inhibition of LDL oxidation, endothelium-dependent relaxation of blood vessels, inhibition of

platelet aggregation and thrombosis, and protection against ischemia-reperfusion injury [97-104].

Lots of studies have demonstrated for an interesting endothelium-dependent relaxation (EDR) activity in blood vessels of procyanidins with a structure-activity relationship [97-99]. The activity of the procyanidins increased with polymerization degree, epicatechin content, and number of galloyl units Procyanidins cause endothelium-dependent vasorelaxation and the effects are a result of increased production of NO by endothelial cells [100]. Furthermore, several proanthocyanidins and preparations containing them inhibited angiotensin converting enzyme (ACE) activity in both *in vitro* and *in vivo* experiments [101-103]. In another study, it was found that the ACE inhibitory activity of procyanidins increased with the polymerization degree. The trimeric procyanidin C1 was almost twice as active as the dimeric procyanidin B2 [103]. Recently, one investigation demonstrated *in vitro* that proanthocyanidins could inhibit angiotensin II binding to the AT1 receptor and the degree of polymerization affected the inhibitory activity [104]. The proanthocyanidins pentamers and hexamers have maximal activity.

1.2.4 Anticancer and antiinflammatory activity

Proanthocyanidins are effective inhibitor of several tumors. The 3,3'-di-O-gallate of procyanidin B2 possessed cytotoxicities against human leukemic cells and a melanoma cell line [105]. Inhibition of 12-*O* -tetradecanoylphorbol-13-acetate-induced mouse skin tumor promotion was demonstrated for proanthocyanidin rich extracts in several studies [106, 107]. These protective effects of proanthocyanidins were related to their antioxidant activity [68] and their inhibitory activity of ornithine decarboxylase (ODC), a rate-limiting enzyme in the biosynthesis of polyamines [108, 109]. A high expression of ODC is an important characteristic of tumor cells and

tumor development and ODC inhibitory activity of procyanidins was changed with the DP. ODC inhibitory activity decreased in the following order: trimer (procyanidin C1) > dimer (procyanidins B1, B2, and B4) > monomer ((+) - catechin and (-)-epicatechin) [107].

Cacao proanthocyanidins showed strong antimutagenic effects on 2- amino-1-methyl-6-phenylimidazo [4, 5-b] pyridine (PhIP)-induced mutagenesis *in vitvo* and on *in vivo* carcinogenesis in female Sprague-Dawley rats [110]. They also inhibited significantly rat pancreatic carcinogenesis in the initiation stage, but not mammary carcinogenesis. In the same study, *in vitvo* experiments suggested proanthocyanidins also directly inhibited the mutagenic activity of PhIP, perhaps through nonspecific binding [110].

There are several reports about the antiinflammatory activities of proanthocyanidins. Degree of polymerization affected the anti-inflammatory activities. In interferon γ-stimulated macrophages, (+) - catechin, (-)-epicatechin, procyanidins B1 and B2 decreased the transcription factor nuclear factor-κB-dependent gene expression, however, procyanidin C2 increased the expression [111]. Similar results were obtained for NO• production and tumor necrosis factor-α secretion. It was documented that dimer and tetramer proanthocyanidins isolated from cocoa compared to higher polymers (DP > 5), greatly stimulated transforming growth factor (TGF) β1 production in *in vitvo* studies with peripheral blood mononuclear cells isolated from human subjects that had low production of TGF- β1 [112].

1.2.5 Other bioactivities

Recently, more bioactivities of proanthocyanidins were reported. Cocoa proanthocyanidins fed to diabetes-induced rats nearly completely inhibited cataract formation which is a common complication of type 2 diabetes [113]. Cinnamon

proanthocyanidins, containing a series of trimers and tetramers of flavan-3-ol, each with an A-type linkage, significantly reduced fasting blood glucose in a group of type 2 diabetic patients [114]. In addition, *in vitro* studies with peripheral blood mononuclear cells (PBMCS), isolated from human subjects that had low production of TGF- β1, proanthocyanidins are also helpful to the immune system. Interleukin-2 and Interleukin -5 secretions were unresponsive to isolated proanthocyanidins treatment [115-116].

1.3 Epicatechin derivatives from proanthocyanidins and their bioactivities

Proanthocyanidins have enormous potential waiting to be explored, especially for producing bioactive agents and economical, sustainable, and novel chiral ligands replacing the costly synthetic ligands. There are some studies reported on bioactivities of epicatechin derivatives. However, no report was found on applying epicatechin derivatives as fine chemicals.

As a matter of fact, there is increasing public awareness of the need to minimize wastes by fostering the integral exploitation of natural resources and to design sustainable processes based on renewable materials. Ideally, sustainability must be economically viable, apart from being environmentally advantageous. The efficient recovery of, proanthocyanidins by converting it into high value-added products is a challenge for scientists and technologists alike.

Currently, a variety of health-promoting products obtained from plant byproducts are on the market and a great deal of research efforts is being devoted to testing the putative beneficial effects of grape, tea, and pine bark polyphenols including proanthocyanidins. Lluis et al synthesized three thio-containing epicatechin molecules

from proanthocyanidins (Figure 1.6), which can be separated from the crude mixtures by combination cation exchange resin and HPLC [117, 118].

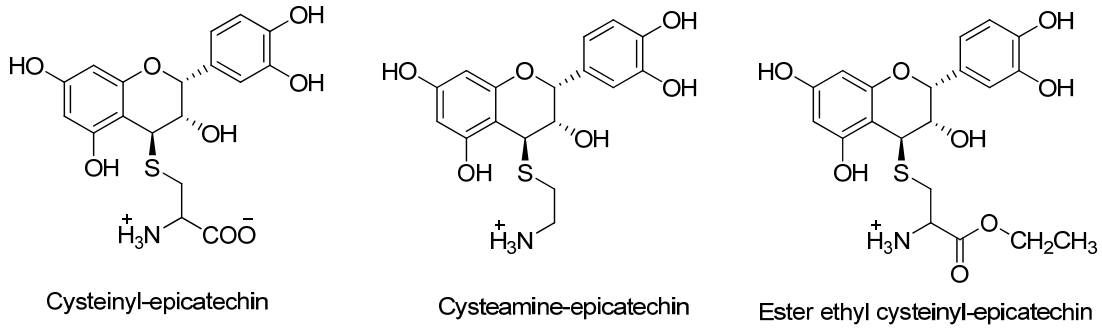


Figure 1.6 Three sulfur-containing epicatechin derivatives from proanthocyanidins

The thiocontaining epicatechin derivatives were valuable compounds. They are active in protecting HT-22 nerve cells from oxidative stress-induced death by a mechanism of maintaining the cellular levels of GSH, the tripeptide consisting of the amino acids cysteine, glutamate, and glycine, instead of scavenging mitochondrial reactive oxygen species (ROS) [119]. In addition, the galloylated conjugates may have other, potentially harmful, effects such as prooxidation by redox cycling and induction of apoptosis. It was suggested that the epicatechin thio-conjugates are good candidates for safe neuroprotective agents [120]. It is very interesting that the non-phenolic part of the molecule enhanced the capability to penetrate biological membranes, as well as the layers of the skin [121, 122].

There was no report on the bioactivity of epicatechin derivatives obtained from depolymerization of proanthocyanidins with carbon nucleophiles, although they can be easily obtained by depolymerizing proanthocyanidins with carbon nucleophile phloroglucinol [26]. Other carbon nucleophiles such as 3,5-dimethoxyphenol, 2,3-dimethylpyrazole are worthy candidates as nucleophile, so do pyrrole and its derivatives..

1.4. Stereochemistry of epicatechin derivatives

Environmentally benign and sustainable catalytic processes are the main theme of current chemical research in response to the demand for greener chemical processes with minimal energy consumption. Full utilization of natural products in fine chemicals is increasingly important because natural products are structurally diverse and complex and would typically take many steps to be "total synthesized" from fossil fuel derived basic chemicals and hence are renewable, sustainable, and greener. Some shining examples are the privileged asymmetric catalysts such as cinchona alkaloids and tartaric acid derivatives [123-126]. There are lot more under-utilized natural products particularly those from agricultural and forestry wastes and one of them is proanthocyanidins. Surprisingly, their potential as ligands for transition metal catalysts has never been explored presumably due to the potential complication of chelating modes. In the hope to take advantage of the rich source of proanthocyanidins as raw materials for fine chemical synthesis, we plan to convert proanthocyanidins into wide range of chiral multidentate ligands through acid depolymerization and simple transformations because the interflavanoid links in most proanthocyanidins may be broken under acidic conditions.

The possible mechanism is that the proanthocyanidin interflavonoid bonds are hydrolyzed to form cationic intermediates, which can be captured by other nucleophiles (Figure 1.7) [127]. Numerous nucleophiles have been used so far, these include including thiolacetic acid, benzene-p-sulphinic acid, thiophenol, benzyl mercaptan, 2-mercaptoethanol, or phloroglucinol [128-132]. Benzyl mercaptan is frequently used while phloroglucinol is sometimes preferred to the sulfur nucleophile because it is odorless. However, the conversion yield of phloroglucinol is much lower

than that of $ArCH_2SH$ [26].

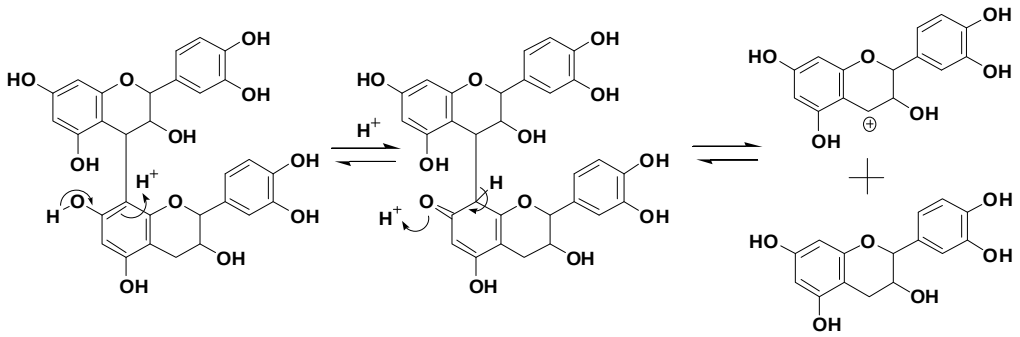


Figure 1.7 Mechanism for the acid catalyzed degradation of proanthocyanidins [127].

When procyanidins B1-B4 were depolymerized with mercaptan, only one epicatechin thioether isomer is obtained from B2 (epicatechin-(4β→8)-epicatechin) and B4 (epicatechin-(4β→8)-catechin), while there are two catechin thioether isomers from B1 (catechin-(4β→8)-epicatechin) and B3 (catechin-(4β→8)-catechin) (Figure 1.8) [127].

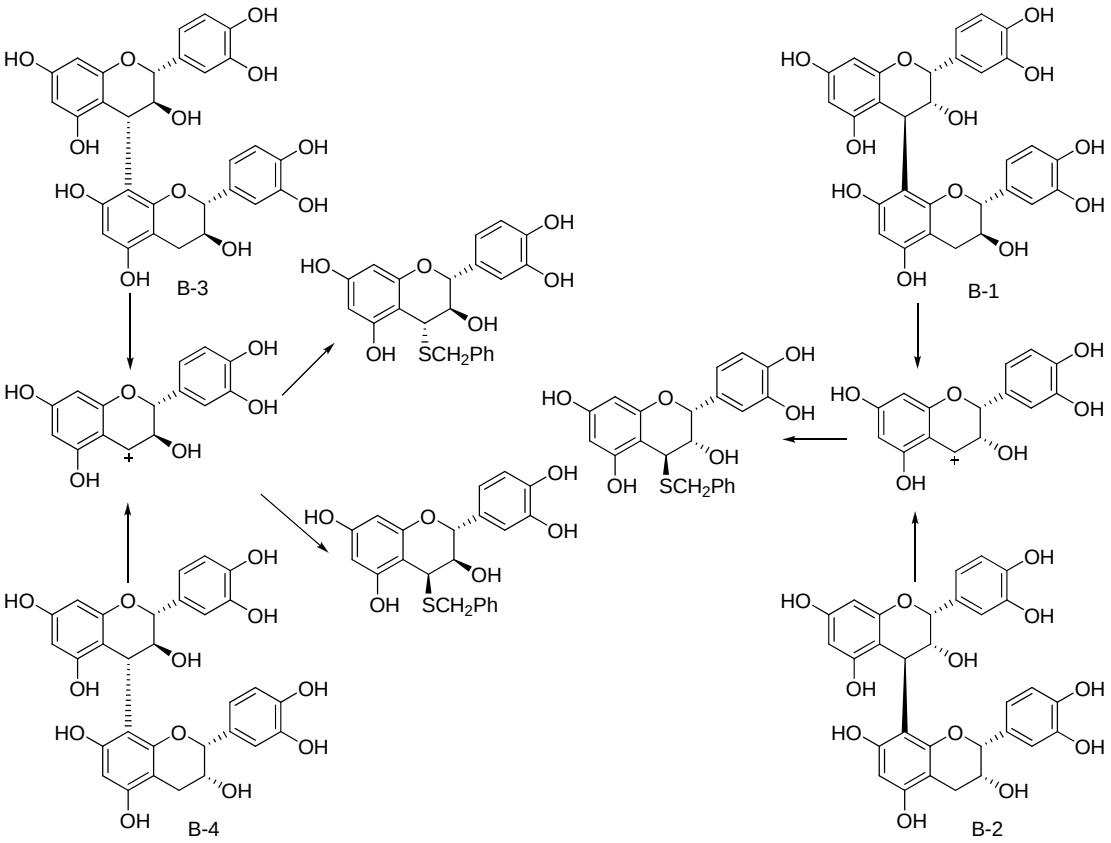


Figure 1.8 Acid-catalyzed degradation of procyanidins B1-B4 [127].

This is an exciting result and we can synthesize chiral ligands with single enantiomer by one step direct depolymerization of proanthocyanidins possessing epicatechin as extension flavan-3-ol unit. This appearance of the only one isomer is most readily explicable if it is assumed that the benzylthio-group occupies the quasiaxial 4-pro-S-position in the heterocyclic ring. The deshielding of H-2 is then due to the sulphur atom in the 1,3-diaxial relationship. Participation of the neighbouring axial 3-hydroxy-group is believed to control the stereochemistry of these reactions.

1.5 The aim of this research

Proanthocyanidins are one of the most ubiquitous groups of natural products with application as raw materials for fine chemicals being largely unexplored. To explore the potential of the utilization of proanthocyanidins, this thesis work aims to carry out the following research tasks:

(1) Separate of proanthocyanidins from biomasses especially low value agricultural byproducts. Because mangosteen pericarps were composes about two-thirds of the whole fruit weight as agricultural waste and proanthocyanidins from this source are not characterized, so the first step of my research is to isolate, purify and identify the mangosteen pericarps proanthocyanidins (MPPs).

(2) Extract and characterized cocoa proanthocyanidins (CPs). Then MPPs and CPs were compared with commercial pine bark proanthocyanidins (PBPs) and grape seed proanthocyanidins (GSPs) via MALDI-TOF-MS and thiolysis analysis to find the ideal source for synthesis of epicatechin derivatives which possess potential application such as multidentate chiral ligands.

(3) Depolymerize the selective proanthocyanidins, with sulfur and carbon nucleophiles and get epicatechin derivatives. Then a convenient way to selective protect the ortho-diphenol group in epicatechin derivates need to be developed to avoid competitive binding of metals by the ortho-dihydroxyl groups.

(4) Investigate some possible applications of the new proanthocyanidins and the flavanol derivatives both as functional foods or drugs and for fine chemical.

Chapter 2

Isolation, Purification and Characterization of Mangosteen Pericarps Proanthocyanidins

2.1 Introduction

Garcinia mangostana Linn, commonly known as mangosteen, is a tropical fruit belonging to the Guttiferae family [133]. It is commonly found in Thailand, Malaysia and Indonesia with worldwide production about 150,000 tons per annum [134]. Due to its popularity; mangosteen is considered as "the queen of the tropical fruit" in ASEAN countries. The edible portion of mangosteen is milky white, whereas the pericarp is dark red and compose of *ca.* two third of the whole fruit weight as an agricultural waste. However, the non-edible pericarps have been used for treating diarrhoea, wounds and skin infection in traditional Thai medicine [135].

Mangosteen pericarp contains xanthones, which are exclusively found in plants of the Guttiferae and Gentianaceae [136]. There are over ten different xanthones isolated from the pericarps and some of them have been shown to possess broad spectrum of bioactivities including antifungal [137], antibacterials [138, 139], antioxidant (peroxynitrite scavenging) [140],and antiproliferation activity *in vitro* against human breast cancer cell [141]. Mangosteen pericarps are also rich in anthocyanins including primarily cyanidin-3-sophoroside with smaller amounts of cyanidin-3-glucoside [142]. The high amounts of anthcyanidins in the pericarp indicate that there are proanthocyanidins as well; yet, they have not yet been characterized. Therefore, in searching for a convenience source of proanthocyanidins, we extracted the PAs from mangosteen peels and characterized its chemical composition and structure.

2.2 Results and discussion

2.2.1 Isolation of proanthocyanidins from mangosteen pericarps. Typical solvent extraction and fractionation on Sephadex LH-20 gave 4.2 g of proanthocyanidin

mixtures from 2.0 kg of fresh mangosteen pericarps, corresponding to 0.21% yield-based fresh weight. Taking into account the moisture in the mangosteen pericarps (68.3%), the proanthocyanidins content in mangosteen is 0.66% and rather low in comparison with that in cocoa and grape seed [6, 10]. Besides proanthocyanidins, xanthones and anthocyanins are the major secondary metabolites in the pericarps obtained in large quantity. Xanthones are lipid soluble and mainly retained in the hexane solution and dichloromethane extraction. We estimated that xanthones are composed of about 6.6% of the dry pericarps, about 10 times higher than that of proanthocyanidins. The mangosteen pericarp tastes very astringent due to the presence of proanthocyanidins. The UV/vis spectrum of the mangosteen pericarp proanthocyanidins (MPPs) shows high similarity to catechin, indicating that the major unit is catechin or its diastereomer epicatechin (Figure 2.1). Using catechin as a reference standard, the catechin content of the MPPs is estimated to be 1300 mg catechin equivalent per gram sample; the higher catechin equivalence based on UV/vis absorbance is likely due to the higher absorbance coefficiency of the proanthocyanidins. The absorption maxima of proanthocyanidins at ~280 nm overlap well with that of catechin, indicative of a homogeneous procyanidin structure [143]. The UV/vis method can serves as a quick estimate of the PAs contents in a sample.

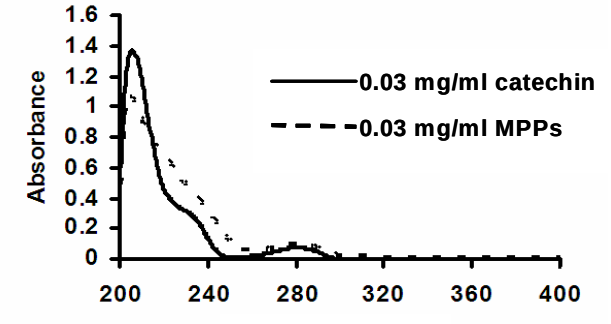


Figure 2.1 UV-vis spectrum of proanthocyanidins from mangosteen pericarps

2.2.2 Structural characterization. The purified condensed tannin mangosteen pericarp proanthocyanidins (MPP) were analyzed by ^{1}H and ^{13}C NMR spectroscopy, and the signal assignment was performed according to the method of Czochanska et al. [27] The ^{13}C NMR spectrum (Figure 2.2) of the MPPs in CD_3OD shows characteristic ^{13}C peaks in consistence with that of condensed tannins with dominant procyanidin (PC) units with a minor amount of prodelphinidins (PD). The structural diversity of the linkage (A and B type) and stereoisomer of catechin and epicatechin units is apparent from the spectrum.

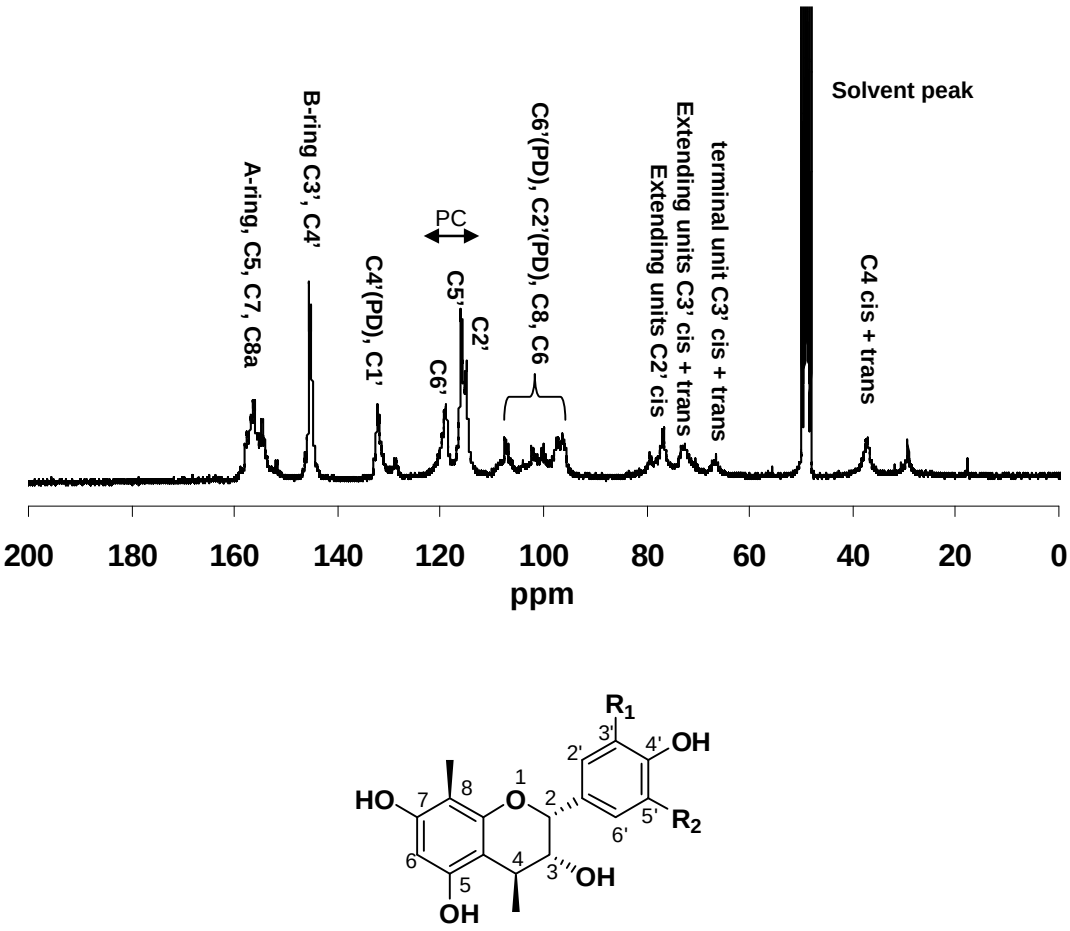


Figure 2.2 $^{13}C\{^{1}H\}$ NMR spectrum of proanthocyanidins from mangosteen pericarps; solvent, CD_3OD; room temperature; and 75 MHz. Identity of the structures: R_1 = H, R_2 = H, epiafzelechin; R_1 = H, R_2 = OH, epicatechin; and R_1 = OH, R_2 = OH, epigallocatechin.

Specifically, C5, C7, and C8a carbons of procyanidins appear at 160 to 150 ppm. Peaks at 145.3 and 145.5 belong to C3' and C4' of procyanidin units. Small amount of prodelphinidins are also detected as its C4' peak appears at 131 ppm, overlapping with the chemical shifts of C1' (PC). The clusters of peaks between 110 to 90 ppm is assigned to C8 and C6 of PC and C6' (PD) and C2' (PD). The region between 70 and 90 ppm is sensitive to the stereochemistry of the C ring. The ratio of the 2,3-*cis* to 2,3-*trans* isomers could be determined through the distinct differences in their respective C2 chemical shifts. C2 gives a resonance at 75.3 ppm for the *cis* form and 79 ppm for the *trans* form. From the peak areas, it is estimated that the *cis* isomer is dominant. C3 of both *cis* and *trans* isomers occurs at ~71.4 ppm. A sharp line at 64 ppm is due to C3 of the terminal unit. The C4 atoms of the extension units showed a broad peak at 36 ppm, while the terminal C4 exhibits multiple lines at 29 and 27 ppm [27]. There are also some A-type linkages indicated from the signals at 151–152 ppm due to C5 and C7 of the A ring involved in the double linkage. The chemical shift of the ketal carbon (C2) formed as a result of this additional bond observed at 104.7 ppm provided further support for A-type linkage albeit in trace quantities [27].

Although the ^{13}C NMR spectrum reveals complex structural characteristics of the mangosteen proanthocyanidins, quantitative data regarding the degree of polymerization (DP) can not be reliably obtained. Further characterization was achieved by electron spray ionization–mass spectrometry (ESI-MS). Analysis was performed in the negative ion mode as proanthocyanidin molecules are better detected than in the positive ion mode due to the acidity of the phenolic protons. Figure 2.3 depicts the ESI-MS spectra of the proanthocyanidins.

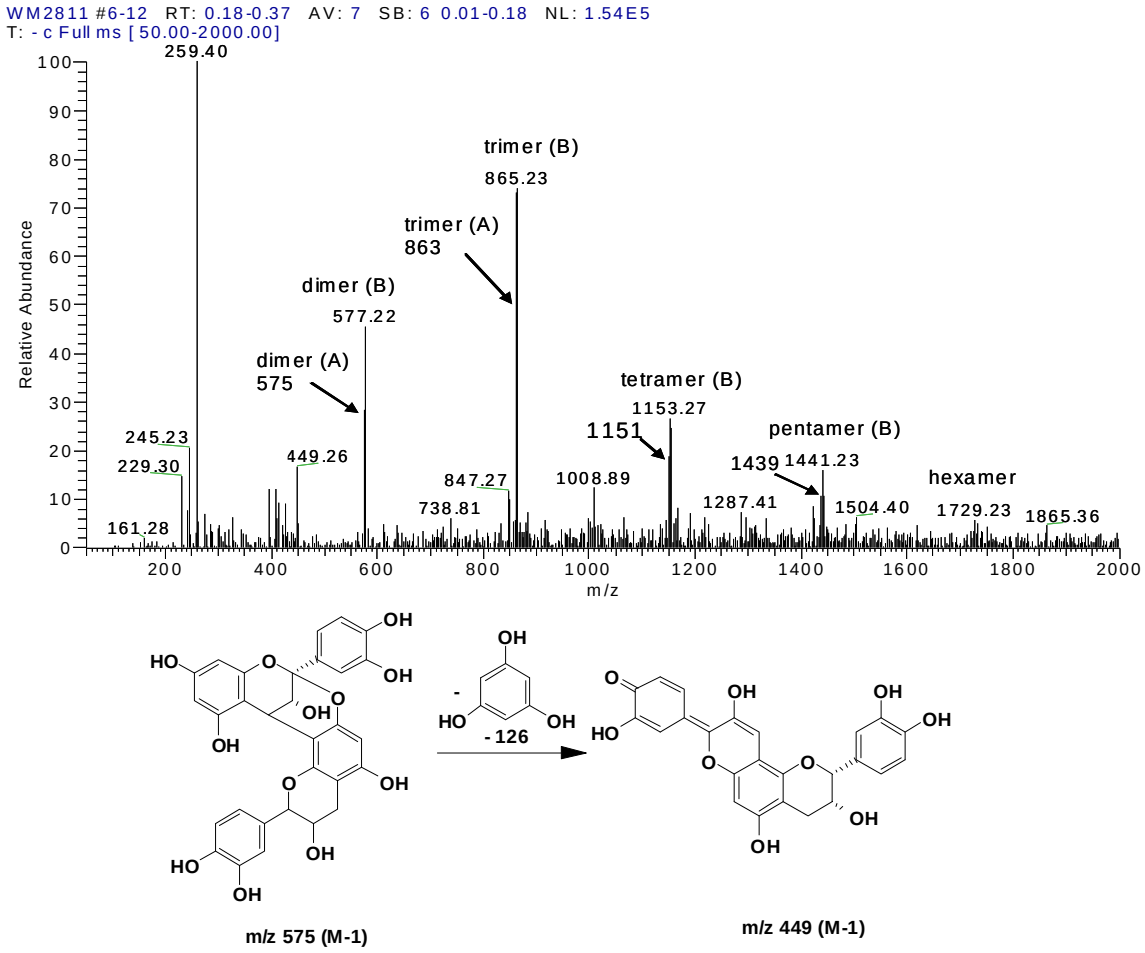


Figure 2.3 ESI-MS spectra of mangosteen pericarp proanthocyanidins recorded in the negative ion mode and possible fragmentation pathway.

Among the observed signals, a first series of abundant ions separated by 288 Da were observed from *m*/*z* 577 to 1729, corresponding to the molecular masses of procyanidins with DPs of 2–6; this is in consistence with the ^{13}C NMR spectrum, which reveals that the dominant species are procyanidins. Peaks 449 can be accounted for from fragmentation of procyanidin dimer (*m*/*z* 575) after heterocyclic ring fission as shown. Other less intense signals were also observed in the higher *m*/*z* values. Mass spectra also provided evidence for the doubly charged ions $[M - 2H]^{2-}$ of odd polymerization degree. The peak at *m*/*z* 1009 was 144 mass units higher than trimer ion (*m*/*z* 865) and 144 lower than tetramer (*m*/*z* 1153). Hence, it can be attributed to

the doubly charged species of heptameric procyanidins. Signals corresponding to trimeric (*m*/*z* 847) and pentameric (*m*/*z* 1425) oligomers with only one afzelechin/epiafzelechin unit (-16 Da) were also observed but were less abundant. Mass spectra also showed a series of compounds that were two units less than dimeric (*m*/*z* 577), trimeric (*m*/*z* 865), tetrameric (*m*/*z* 1153), pentameric (*m*/*z* 1441), and hexameric (*m*/*z* 1729). These masses might represent a series of compounds in which an A-type interflavan ether linkage occurs (4→8, 2→O→7) between adjacent flavan-3-ol units because two hydrogen atoms (Δ2 amu) are lost in the formation of this interflavanyl bond. It agreed with the NMR results. However, the limited range imposed by the quadruple analyzer as well as the possible presence of multiple-charged ions for the larger molecules, inducing peak dispersion and frequent overlapping, resulted in an increased difficulty of interpretation of the signals when using ESI-MS to higher DP proanthocyanidins. Singly charged ions of higher molecular weight proanthocyanidins are often observed with a weak intensity and are difficult to detect with good precision in ESI-MS.

A complementary spectroscopic technique to limit the production of multiply charged species is matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), allowing the analysis of polymers and revealing information about their chain lengths due to the production of only a singly charged molecular ion for each parent molecule and allowing for the detection of high mass with precision. Figure 2.4 shows the MALDI-TOF mass spectra of the MPPs, recorded in the positive reflectron ion mode. A series of polyflavan-3-ols extending from the dimer (*m*/*z* 785) to DP13 (*m*/*z* 3785) were observed. In addition, there were many peaks with mass signals having a difference of 16 Da more than homoprocyanidins, corresponding to the addition of *n* gallocatechin/epigallocatechin

units. Therefore, MALDI-TOF MS indicated the coexistence of procyanidin polymers and polymers with different ratios of procyanidins and prodelphinidins. The major peak assignments are listed in Table 2.1. Monomeric units, type of linkage and degree of polymerization were indicted.

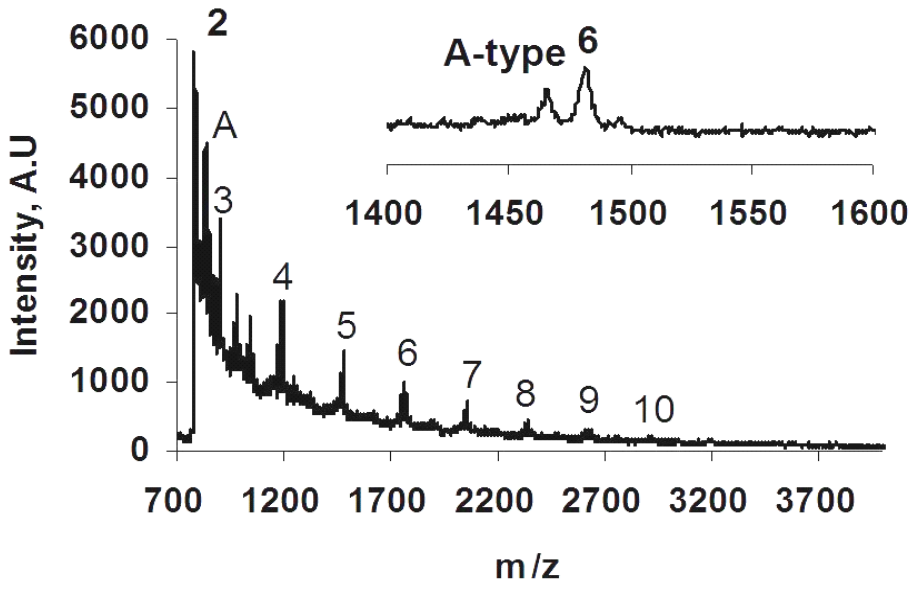


Figure 2.4 MALDI-TOF mass spectrum of mangosteen proanthocyanidins.

Table 2.1 Observed masses of mangosteen proanthocyanidins by MALDI-TOF MS

m/z	*Polymer*	*Monomeric unit*	*Interflavanoid linkage*
785	Dimmer	1(epi)gallocatechin+1(epi)gallocatechin gallate	B-type
905	Trimer	2(epi)catechin+1(epi)gallocatechin	B-type
985	Trimer	2(epi)gallocatechin+1chlorogenic acid	1A-type
1041	Trimer	2(epi)catechin+1(epi)catechin gallate	B-type
1193	Trimer	1(epi)catechin+2(epi)catechin gallate	B-type
1329	Tetramer	3(epi)catechin+1(epi)catechin gallate	B-type
1465	pentamer	5(epi)catechin	B-type

1481	pentamer	4(epi)catechin+1(epi)gallocatechin	B-type
1753	Hexamer	5(epi)catechin+1(epi)gallocatechin	B-type
2057	heptamer	6(epi)catechin+1(epi)gallocatechin	B-type
2165	heptamer	5(epi)catechin+1(epi)catechin gallate+1afzelechin	6A-type
2345	Octamer	7(epi)catechin+1(epi)gallocatechin	B-type
2633	nonamer	8(epi)catechin+1(epi)gallocatechin	B-type
2905	Decamer	10(epi)catechin	B-type
3207	undecamer	10(epi)catechin+1(epi)gallocatechin	1A-type
3481	dodecamer	12(epi)catechin	B-type
3785	13-mer	12(epi)catechin+1(epi)gallocatechin	B-type

2.2.3 Thiolysis. To further investigate if the proanthocyanidins are composed of epicatechin and catechin and measure the mDP, depolymerization through thiolysis reaction was carried out by standard conditions using benzyl mercaptan. The reaction mixture was analyzed by HPLC as shown in Figure 2.5. The major product observed was the expected epicatechin 4-benzylsulfide along with a significant amount of epicatechin and a much smaller peak for catechin. This result suggests that that there are significant amounts of epicatechin extension units in mangosteen proanthocyanidins. The mean degree of depolymerization (mDP) of mangosteen proanthocyanidins was calculated to be 6.6 by comparing the peak areas based on the following equation [144]

$$\text{mDP} = 1 + \frac{\text{area under the curve of benzyl thioether derivative of catechins}}{\text{area under the curve of catechin and epicatechin}}$$

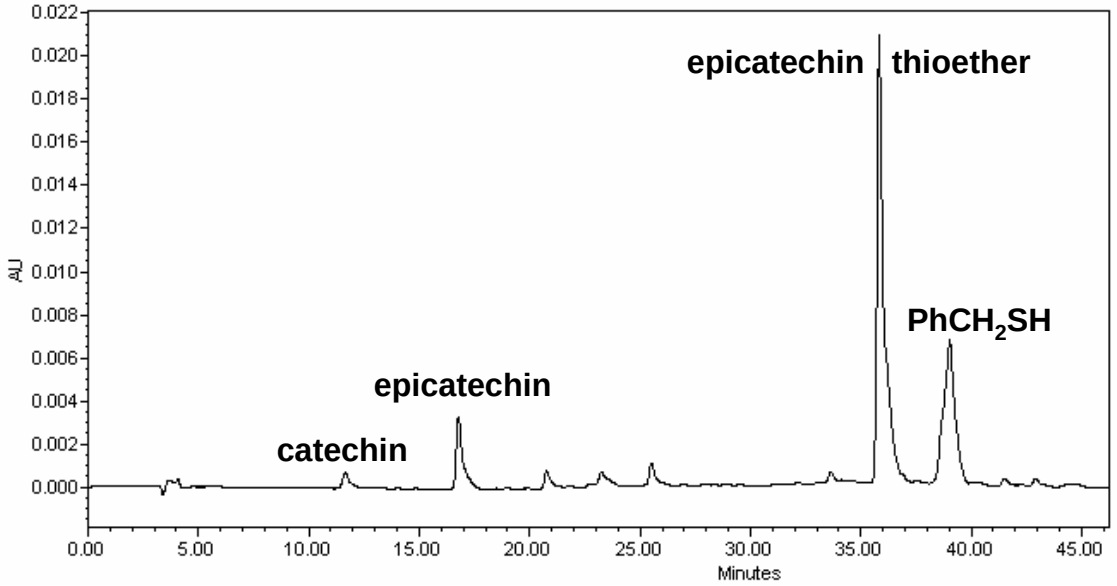


Figure 2.5 HPLC chromatogram of thiolytic products of proanthocyanidins by benzyl mercaptan. Column: 250 mm × 4.6 mm i.d., 5 μm C18 column (Shimadzu); detector set at 280 nm.

In consistence with this data, the ^{1}H NMR peak integration of terminal C (4)H2 and extending unit C4 (H) gave an mDP of 7.0. Because there are A-type proanthocyanidins that do not react with benzyl mercaptan and A-type dimer thioether did not show peak in the chromatogram, the mDP determined by thiolysis is lower than that determined by ^{1}H NMR. Chemically, most C-C bonds are not easy to be cleaved, the facile cleavage of inter-flavanol C-C bonds of B-type proanthocyanidins by nucleophiles such as thiol reagents can lead to catechin derivatives with different functional groups. One successful example is the preparation of antioxidant cysteinyl-epicatechin, cysteamine-epicatechin and ester ethyl cysteinyl-epicatechin by Lluis et al [117-121]. The bioactivities of these novel compounds are still largely unexplored and have great potential as antioxidants and therapeutic agents.

In summary, mangosteen pericarps is a rich source of secondary metabolites including xanthones, the colored pigment, mainly cyaniding 3-glucoside, and monomeric and oligomeric proanthocyanidins (Figure 2.6).

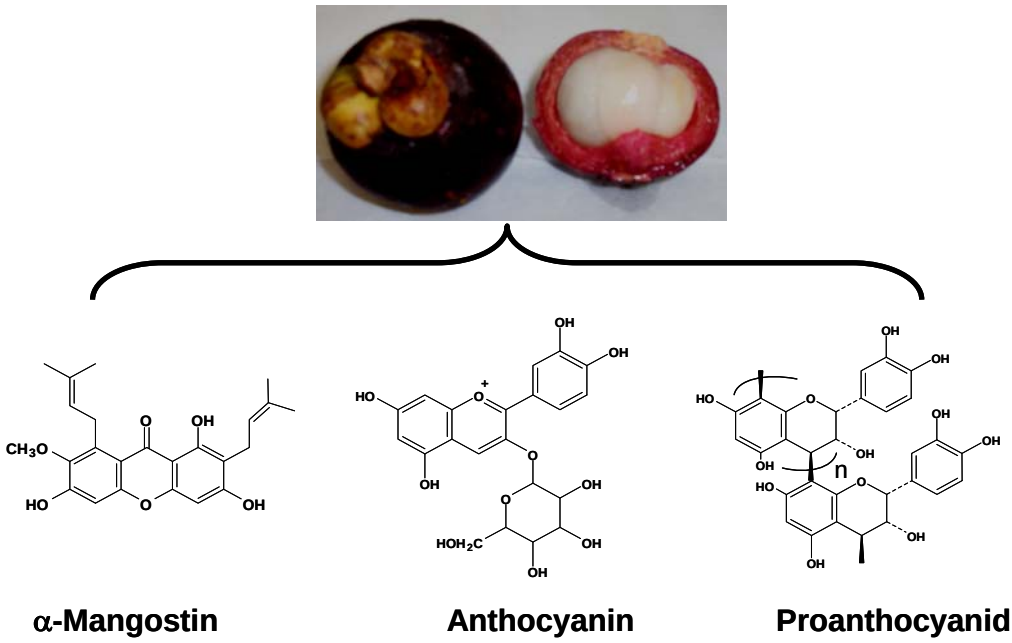


Figure 2.6 Major secondary metabolites of mangosteen pericarps.

For mangosteen and many other fruits, the pericarps are not edible. Utilization of these micronutrients will then rely on chemical extractions and separations. The fruit pericarps or skin contain high amount of bioactive compounds, many of them are phytoalexins in response to micriobial or other forms of environmental stress. The interests on the nutritional benefits of these phytoalexins as rekindled lately upon the discovery that, resveratrol (phytoalexin found in grape skin) can be used as calorie restriction mimetics for chronic disease prevention. The secondary metabolites found in mangosteen pericarps warrants further investigation regarding to their therapeutic and chronic disease prevention potential and mechanisms.

Chapter 3

Comparison among

Proanthocyanidins from Different Sources

3.1 Introduction

Proanthocyanidins has been studied for many decades and a rapidly growing body of evidence suggested that the proanthocyanidins may act as potent antioxidants or modulate key biological pathways [61-70, 90-104]. Because proanthocyanidins attracted a considerable amount of attention in the fields of nutrition, health and medicine, lots of proanthocyanidins were extracted from different plants and some of them are commercially available. Two popular commercial sources of proanthocyanidins are grape seed proanthocyanidins (GSPs) and pine bark proanthocyanidins (PBPs), which are found in a variety of commercial products ranging from capsules to sports drinks and cosmetics [145]. To be an ideal starting material for the preparation of epicatechin derivatives, proanthocyanidins should have three features: i) dominantly B-type linkage, the depolymerizable interflavanyl bond; ii) high mean degree of polymerization, and iii) dominating epicatechin as extension units. Fresh cocoa beans (Theobroma cacao seeds) contain approximately 2% w/w epicatechin and almost exclusively epicatechin based procyanidin oligomers and polymers [146], might be a promising source for synthesis of epicatechin derivatives. However, to the best of our knowledge, no commercial cocoa proanthocyanidins (CPs) were found. So, CPs were extracted in present study and then MPPs and CPs extracted in our lab were compared with commercial PBPs and GSPs were compared with to get ideal proanthocyanidin source for preparation of epicatechin derivatives.

3.2 Results and discussion.

3.2.1 Extraction and NMR analysis of cocoa proanthocyanidins. Typical solvent extraction and fractionation on Sephadex LH-20 gave 5.8 g of proanthocyanidin mixtures from 200 kg dry cocoa powder. The ^{13}C NMR spectrum (Figure 3.1) of the

cocoa proanthocyanidins (CPs) in CD_3OD shows characteristic ^{13}C peaks in consistence with that of condensed tannins with exclusively procyanidin (PC). The structural diversity of the linkage (A and B type) and stereoisomer of catechin and epicatechin units is apparent from the spectrum.

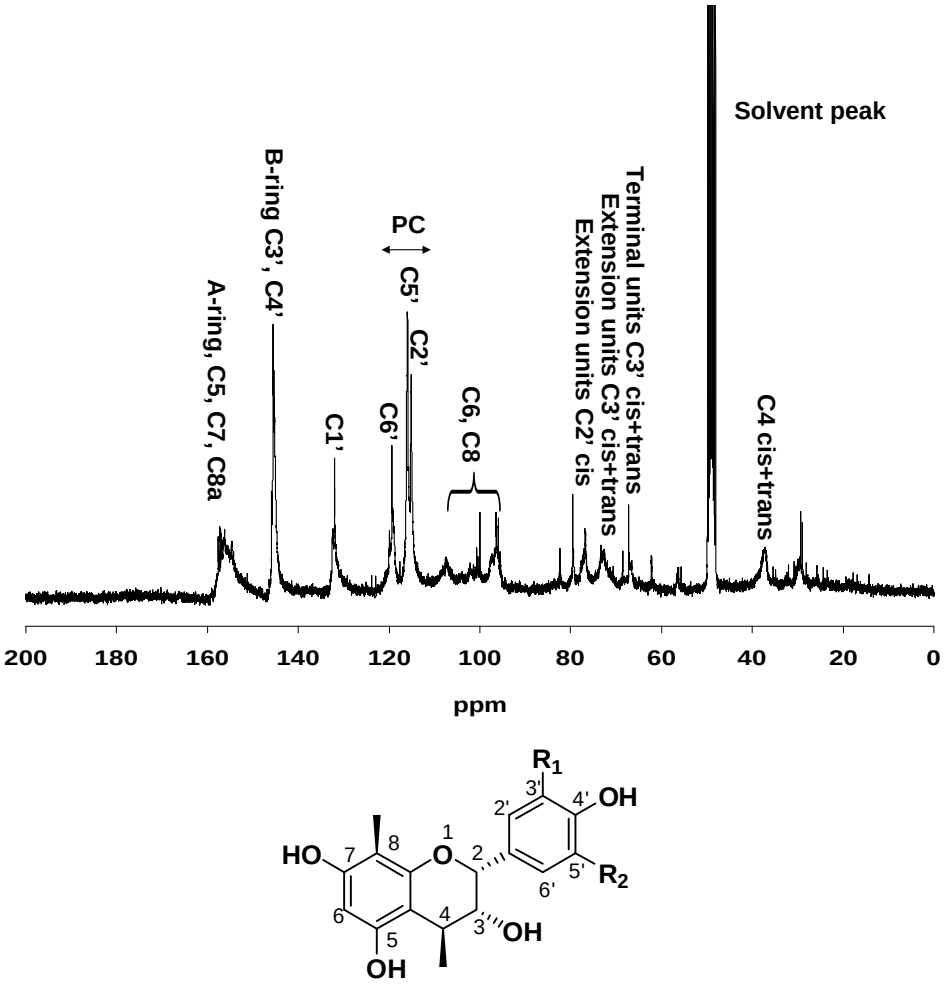
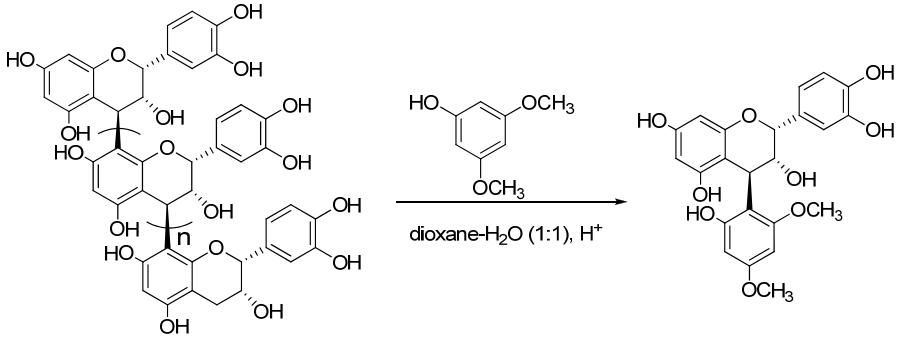


Figure 3.1 ^{13}C NMR spectrum of cocoa proanthocyanidins; solvent, CD_3OD; room temperature; and 75 MHz. Identity of the structures: R_1 = H, R_2 = H, epiafzelechin; R_1 = H, R_2 = OH, epicatechin; and R_1 = OH, R_2 = OH, epigallocatechin.

Specifically, the resonance line at 146 ppm represents a typical indicator for the presence of prodelphinidin (PD) units (gallocatechin/epigallocatechin) [27]. The absence of this signal in the spectra of the CPs reveals that they are comprised of only PC monomers. C5, C7, and C8a carbons of procyanidins appear at 160 to 150 ppm.

Peaks at 145.3 and 145.5 belong to C3' and C4' of procyanidin units. The clusters of peaks between 110 to 90 ppm are assigned to C8 and C6 of PC. The region between 70 and 90 ppm is sensitive to the stereochemistry of the C ring. The ratio of the 2,3-*cis* to 2,3-*trans* isomers could be determined through the distinct differences in their respective C2 chemical shifts. C2 gives a resonance at 75.3 ppm for the *cis* form and 79 ppm for the *trans* form. From the peak areas, it is estimated that the *cis* isomer is dominant. C3 of both *cis* and *trans* isomers occurs at ~71.4 ppm. A sharp line at 64 ppm is due to C3 of the terminal unit. The C4 atoms of the extension units showed a broad peak at 36 ppm, while the terminal C4 exhibits multiple lines at 29 and 27 ppm [27]. There is no obvious signal of A-type linkages both at 151–152 ppm and 104.7 ppm

3.2.2 Synthesis of *(2R,3R,4S)-2-(3,4-dihydroxyphenyl)-4-(2-hydroxy-4,6-dimethoxyphenyl)-3,4-dihydro-2H-chromene-3,5,7-triol,* 1. Because it is odorless, phloroglucinol was previously used to measure the mean degree of polymerization (mDP) of proanthocyanidins in several samples [26]. In order to depolymerization mangosteen pericarp proanthocyanidins (MPPs) with different carbon nucleophiles, substituted analog of phloroglucinol, 3,5-dimethxoyphenol were used to fix on the reaction conditions. Under acidic conditions, MPPs were depolymerized with 3,5-dimethoxyphenol in dioxane/H_2O (1: 1) (Scheme 3.1).



Scheme 3.1 Depolymerization of MPPs with 3,5-dimethoxyphenol

The epicatechin derivative **1** was obtained after purification with silica gel. The molecular weight of the compound is determined from high resolution mass spectroscopy (HRMS) as 441.1190 from the anionic mode. The normal ESI-MS/MS spectrum of **1** was shown in Figure 3.2 and the fragmentation peaks at *m/z* 423, 397 and 315 in MS/MS spectra correspond to the loss of the H_2O, CO_2 and phloroglucinol group.

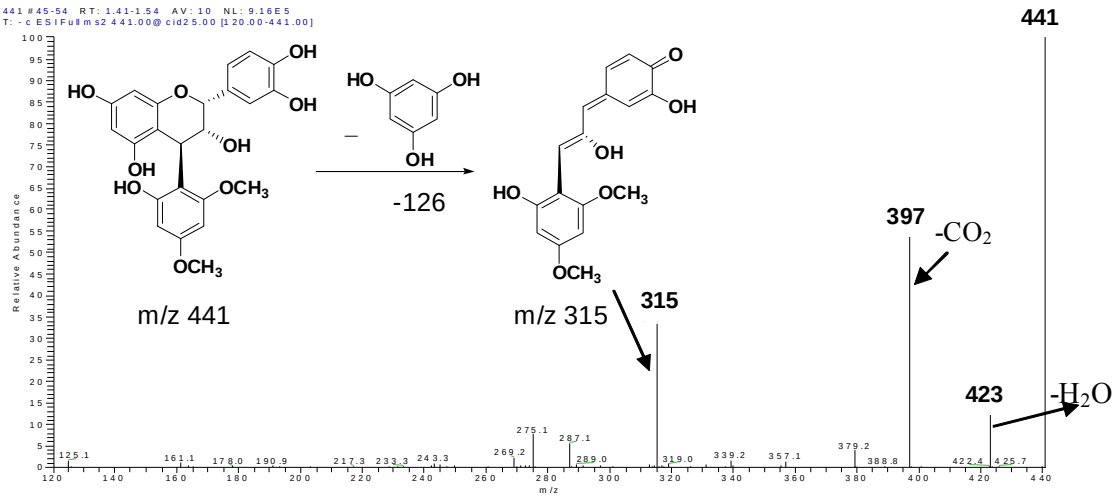
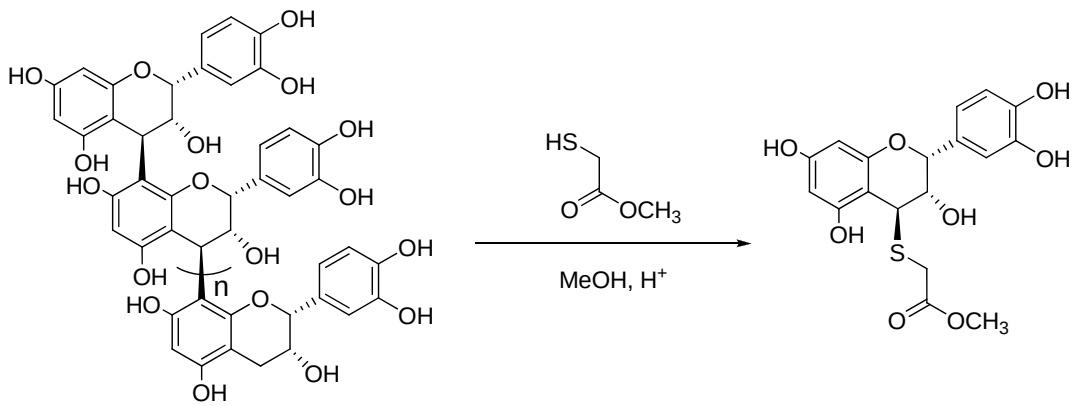


Figure 3.2 ESI-MS/MS spectrum of **1** recorded in the negative ion mode and possible fragmentation pathway.

3.2.3 Optimization of the depolymerization condition for 3, 5-dimethoxyphenol. When phloroglucinol was used to measure the mDP of proanthocyanidins, the yields of depolymerized products were rather low (3-10%), only ¼ of those obtained by degradation with benzyl mercaptan [26]. Similar to phloroglucinol, the isolated yield of **1** is only 6.6 g/100 g MPPs. In order to optimize the reaction conditions, different solvents, such as acetonitrile, methanol, dioxane/H_2O (1:1), tetrahydrofuran, n-butanol, i-propanol and acetone, were tested and reaction mixture was analyzed by HPLC using purified **1** as standard. The HPLC results indicated that the highest conversion was obtained with methanol. However, when using methanol as solvent,

the ethyl acetate fraction after liquid -liquid extraction was dark-black and it is very difficult to purify **1** with silica gel. Mixture of dioxane/H_2O (1:1) was previously used for depolymerizing proanthocyanidins [26] and was proven to be an ideal solvent for convenient purification of **1**. The ethyl acetate fraction after liquid-liquid extraction was red and **1** can be purified by column chromatography with silica gel. However, when we use commercial PBPs as the starting material, the isolated yield of **1** is only 3.2 g/100 g PBPs and repeated purification with silica gel need to be carried out to get the purified products. If the starting material is commercial GSPs, the purification is so difficult that it is hard to obtain **1** with a purity of >90% even after several times of purifications. Therefore, MPPs is so far the best relatively speaking but the overyield is not practical.

3.2.4 Depolymerization of MPPs with methyl thioglycolate. Before comparing different proanthocyanidins, we planned to synthesize sulfur-containing epicatechin derivative as a standard because thiolysis is smooth and easily completed. Under acidic conditions, we depolymerized the MPPs with methyl thioglycolate under 50 ^oC (Scheme 3.2). The produced thioether **2** was obtained at milligram scale after purification with column chromatography with silica gel.



Scheme 3.2 Depolymerization of MPPs with methyl thioglycolate

The molecular weight of **2** is determined from ESI-MS spectroscopy to be 393 from the anionic mode MS. The major fragmentation peak is at *m/z* 287 corresponding to the loss of the sulfanyl group. This result indicates that the C-S bond is the weakest bond in the compound. The SCH_2 protons show an AB pattern at 3.52 ppm with coupling constant of 15.5 Hz, this is similar to CH_2 in the benzylthioether analog (AB pattern, J = 12.0 Hz) [27]. The small coupling constant (1.8 Hz) between C(4)-H and C(3)-H in the ^{1}H NMR spectroscopy is also comparable to the benzylthioether compound (2.0 Hz). Therefore we concluded that **2** had a 4β linkage. The chromophores of **1** and **2** give a positive circular dichroism (CD) band at 220-240nm region (Figure 3.3) and hence the absolute configuration at C4 is β, agree with the NMR spectra. For the undetected 4α isomer, one would expect the coupling constant between C(4)-H and C(3)-H to be much larger (~ 5.5 Hz).

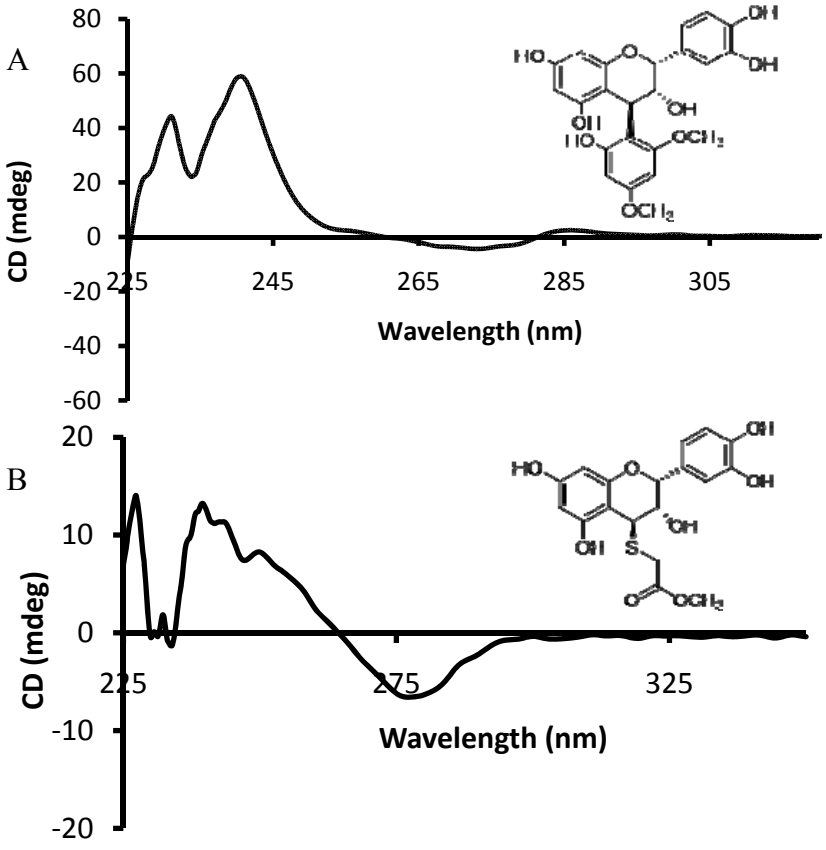


Figure 3.3 CD spectra of **1** (A) and **2** (B) in methanol at 25^o

Compound **2** may be a tridentate ligand for complexation of metal ions through the C-5 hydroxyl group, sulfur atom, and the carboxylate group. We planned to get gram-scale of this ligand in order to apply for further reaction. Luckily, we found that it can be separated after one-pot depolymerization extraction from mangosteen pericarps directly, according to thin layer chromatography (TLC) analysis. Hence preparative quantity of the **2** was achieved conveniently through conventional column chromatography to give moderate yield (4.5%) based on the mangosteen pericarps. Commercially available pine bark proanthocyanidins gave lower yield while usage of the grape seed extracts yielded mixture of compounds that made the separation of **2** complicated.

3.2.5 Comparison among proanthocyanidins from four sources. The commercial pine bark proanthocyanidins (PBPs), grape seed proanthocyanidins (GSPs), cocoa powder proanthocyanidins (CPs), and mangosteen pericarps proanthocyanidins (MPPs) were evaluated by thiolysis and MALDI-TOF MS analysis. These four proanthocyanidins were depolymerized with methyl thioglycolate and the reaction mixtures were analyzed by HPLC using **2** as standard. The HPLC chromatogram of thiolytic mixtures were shown in Figure 3.4. Mean degrees of polymerization (mDP) of different proanthocyanidins were quantified from the integration of the catechins and **2** from the HPLC chromatograms based on the following equation [144]. LC-MS were used to analysis the new thiolytic products in reaction mixture of GSPs.

$$\text{mDP} = 1 + \frac{\text{area under the curve of 2}}{\text{area under the curve of catechin and epicatechin}}$$

The yield of **2** and the mDP of proanthocyanidins from different botanical sources were listed in Table 3.1. The results indicated that the MPPs were the best source as it gave the highest yield of **2.** The LC-MS results showed that, for grape

seeds, another main thiolytic product was catechin/epicatechin gallate-thioether.

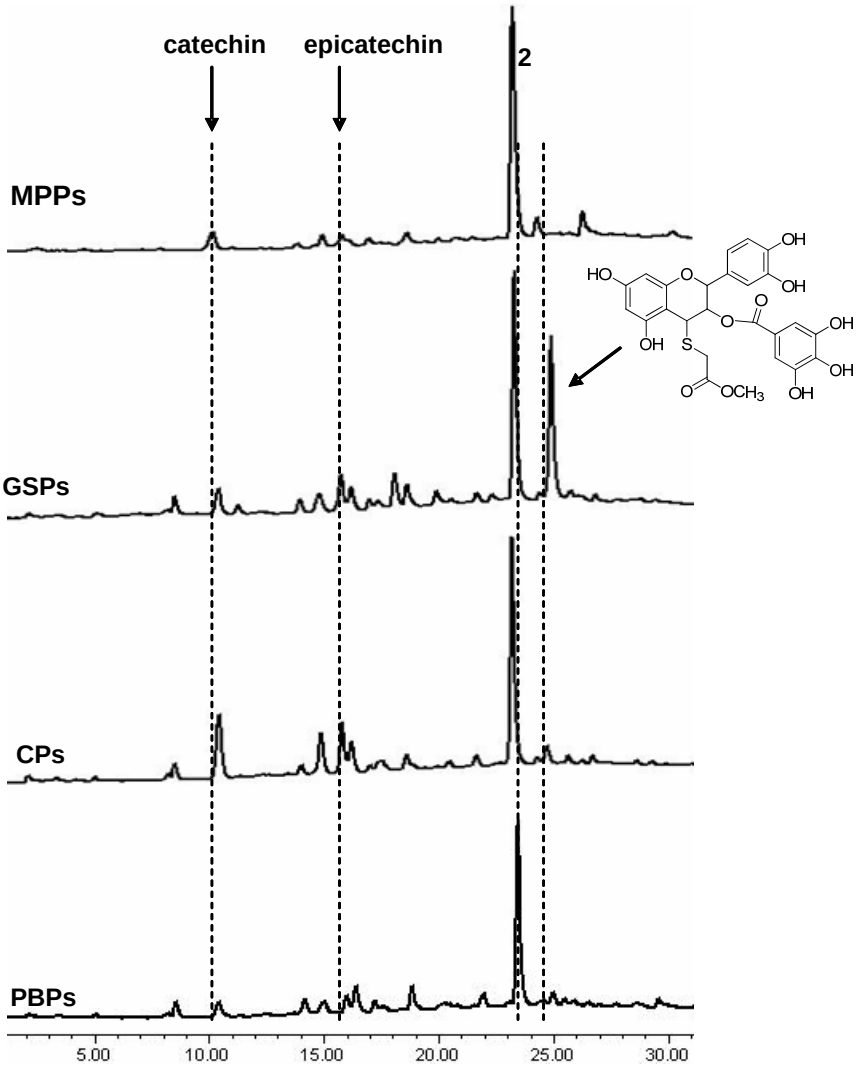


Figure 3.4 HPLC chromatogram of thiolytic products of proanthocyanidin from four types of plants with methyl thioglycolate. Detector wavelength set at 280nm.

Table 3.1. The thiolytic yield and the degree of polymerization of proanthocyanidins from different botanical sources

Proanthocyanidin source	Yield of 2	Degree of Polymerization
Mangosteen pericarp	51%	6.8
Grape seed	36%	3.1
Pine bark	32%	3.5
Cocoa bean	37%	2.4

In agreement with the results, MALDI-TOF-MS spectrum of the same sample showed that the GSPs contain fairly large amount of gallate groups (Figure 3.5**)** illustrated by groups of peaks correspondingly.

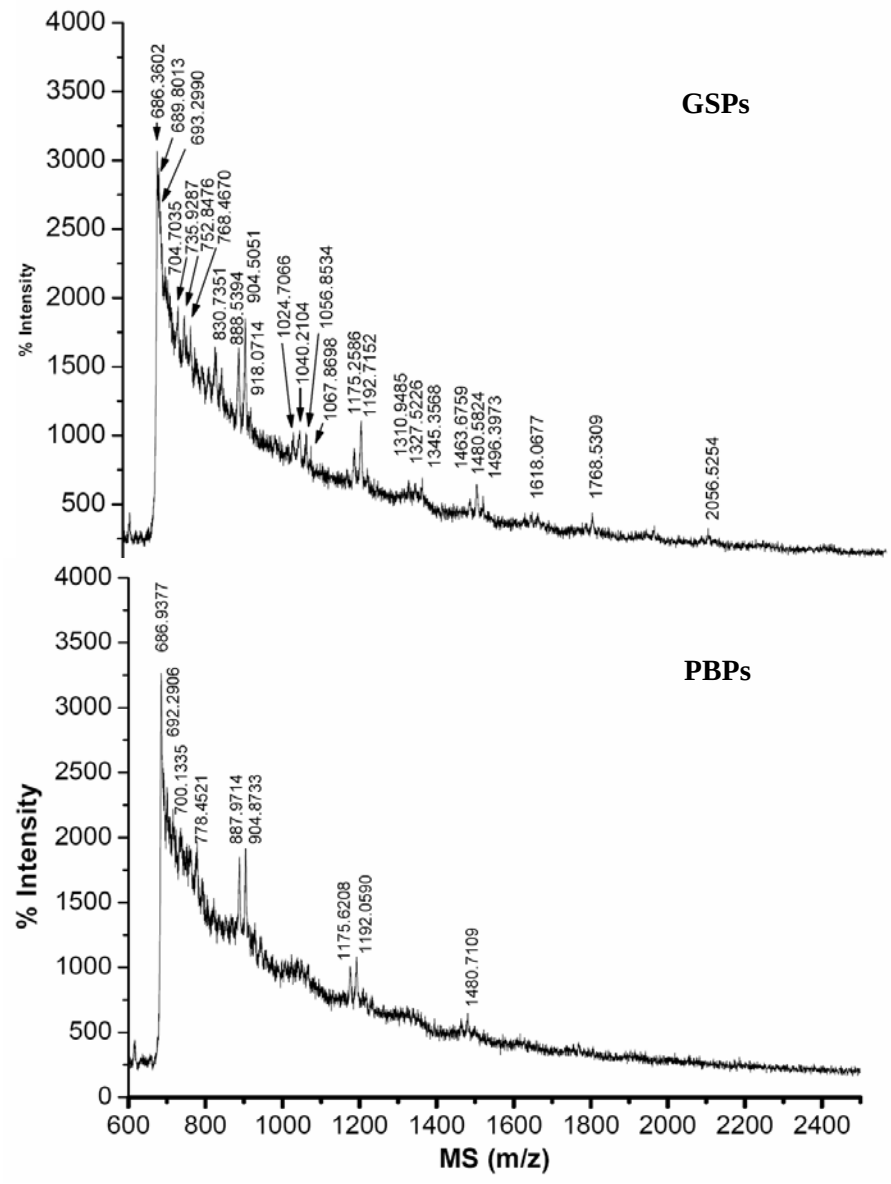


Figure 3.5 MALDI-TOF MS spectra of commercial pine bark proanthocyanidins (PBPs) and grape seed proanthocyanidins (GSPs).

Specifically, the signals that can be assigned to gallated (G) units are 753 (dimer + 1G), 905 (dimer + 2G), 1040 (trimer + 1G), 1193 (trimer + 2G), 1345 (trimer + 3G), 1328 (tetramer + 1G), 1481 (tetramer + 2G). Un-gallated oligomers are also detected at *m/z* values of 889 (trimer), 1175 (tetramer –2H, one A-type linkage), 1464

(pentamer) along with numbers of low molecular weight peaks, which may be dominant in the sample as revealed from the low degree of polymerization at 3.1. Our data are in consistence with literature report [10] with some subtle differences which may be due to the difference source of grape seeds. The PBPs, on the other hand, showed lesser number of peaks of un-gallated oligomers (887 trimer, 1176 (tetramer) and gallated oligomers (904, trimer + 1G; 1193, trimer + 2 G; 1481, tetramer + 2G) (Figure 3.5). However, from the HPLC chromatograms, there is undetectable amount of thiolated gallocatechin, indicating that the gallated oligomers are minor components in PBPs. No polymer with higher DP greater than 7 was found in the two samples.

In summary, mangosteen pericarp proanthocyanidins (MPPs) and cocoa proanthocyanidins extracted in our lab were compared with commercial pine bark proanthocyanidins (PBPs) and grape seed proanthocyanidins (GSPs) via MALDI-TOF-MS and thiolysis analysis. The mean degree of polymerization (mDP) quantified from the HPLC chromatograms showing the mDP of mangosteen pericarps > cocoa bean > grape seeds > pine bark. Both thiolysis and MALDI-TOF-MS results indicated that MPPs were superior for synthesis the new chiral ligands due to the higher mDP and ratio of epicatechin units.

Chapter 4

Synthesis of epicatechin derivatives

4.1 Introduction

Proanthocyanidins are known as potent metal chelators through the phenolic groups [147]. Surprisingly, their potential as ligands for transition metal catalysts has never been explored presumably due to the potential complication of chelating modes. Proanthocyanidins seemed to be a promising precursor for synthesizing flavanol derivatives which may be applied as chiral ligands and mangosteen pericarp proanthocyanidins (MPPs) are a good source because it contains dominantly B type interflavanol linkage and epicatechin as the monomeric unit with relatively high degree of polymerization. It has been known for long time that acid catalyzed depolymerization of the proanthocyanidins in the presence of carbon (phloroglucinol) or thiol (benzylmercaptan) nucleophiles led to C-4 substituted epicatechin derivatives [25, 26]. Furthermore, it was reported that epicatechin thioether with single enantiomer was obtained by depolymerization of proanthocyanidins possessing epicatechin as extension flavan-3-ol unit with benzyl mercaptan [25]. By selecting proper carbon and sulfur nucleophiles, we should be able to obtain a number of novel epicatechin derivatives with potential application to asymmetric reaction via depolymerizing MPPs with the nucleophiles. In order to reduce the potential number of metal chelating sites and get more new chiral ligands, selective protection of the ortho-dihydroxyl groups were performed on the depolymerized products after screen for the regioselective protecting reagents.

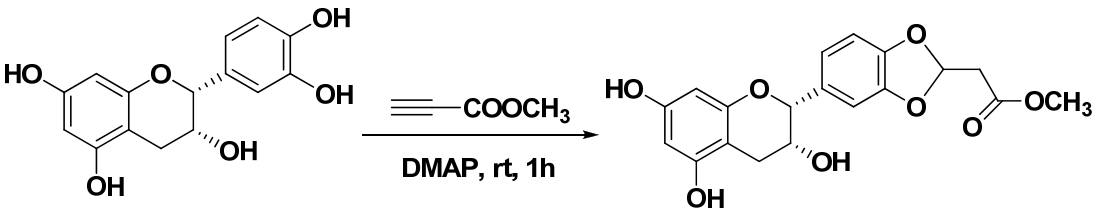
4.2 Results and discussion

When investigating the antioxidant mechanism of catechin via NMR analysis of the reaction media of catechin and DPPH, the proposed antioxidation mechanism was based on the change of the B-ring to an o-quinone [148]. In order to use the

epicatechin derivatives to asymmetric reaction, it is very important to selectively protect the ortho-dihydroxyl groups on the B ring to keep the epicatechin derivatives stable and avoid competitive binding of metals. We have tried many strategies known to protect the phenol groups but only one method worked reasonably well when using catechin/epicatechin as model compound.

4.3.1 Regioselective protecting of catechins. Catechin/epicatechin is the good model molecule to screen the regioselective protectors due to the commercial availability. However, the choice of the reagent is rather limited for epicatechin comparing with other catechols. Because for epicatechin, it is imperative to exclude any protective groups requiring particularly drastic pH conditions during their introduction and removal to avoid epimerization and rearrangement [149-150]. Many known methods aiming to protect o-dihydroxyphenyl group did not work in catechin-type compounds according to other's reports and our primary study. Cécile Cren-Olivé et al investigated a lot of catechol-protecting reagents including P_2O_5, phosgene, borate and benzyl carbonate, but all protections were failed [151]. Then, they reported a method to protect the o-dihydroxyphenyl group in catechin/epicatechin with dichlorodiphenylmethane but the yield of the protected products were too low (~20%) [151].

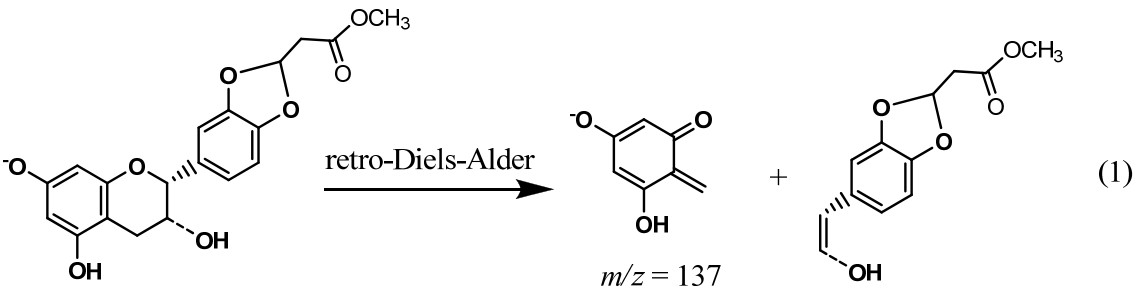
In my study, selective protection of the B-ring phenolic groups in epicatechin is accomplished by reaction of epicatechin with equimolar methyl propiolate in the presence of 4-dimethylaminopyridine (DMAP) at room temperature for 8 hours (Scheme 4.1). The crude product was purified by typical column chromatography to give **3** in satisfactory isolated yield of 43%.



epicatechin **3**

Scheme 4.1 Selective protection of epicatechin

The ^{1}H NMR spectrum of **3** features two partially overlapping doublet of doublets at 6.48−6.52 ppm for C_9-H. This indicates two diastereomers formed in the reaction due to the chiral centre at C-9 and the chirality of epicatechin itself. The relative ratio of the two products is 1:1 according to the integration of the peaks. However, the peaks for C_{10}-H do not show much difference between the isomers. In consistence with the NMR data, the position of the substitution is confirmed from the ESI-MS^2 spectrum which shows a retro-Diels-Alder fragmentation of 137 when the molecular ion is fragmented (eq. 1).

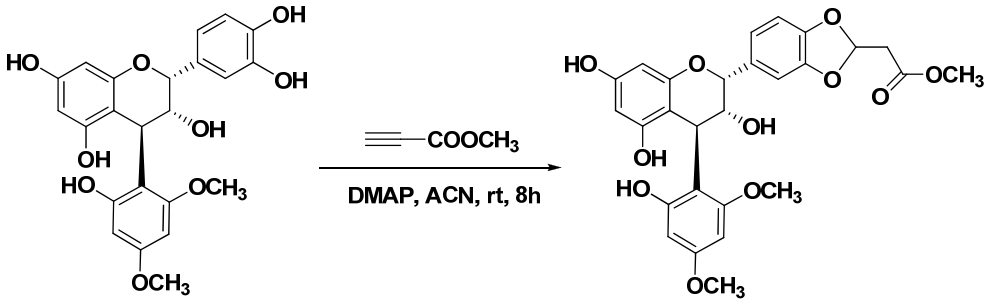


(1)

Besides methyl propiolate, phenylboronic acid, which was known to react with catechols effectively even without a solvent [152], is a good option because it reacted with catechin in CD_3CN produced a 50% conversion within one hour of mixing. However, further tests showed that the product is sensitive to H_2O. It did not work to make the reaction inreversible by removing of water from the reaction mixture with activated 4Å molecular sieves. Substitute phenylboronic acids did yet not give the satisfied results. In addition, it was reported that reaction of catechin with di-*tert*-

butyldichlorosilane and triethylamine in acetonitrile gave a high yield (85%) of the corresponding di-*tert*butylsilylene derivative but only gave hydroxysilyl mono ethers mixture of the parent catechol due to rapid hydrolysis of di-*tert*butylsilylene derivative during TLC separation on silica [151]. Kobayashi and co-workers [147] designed a complex titanium (IV) molecule which selectively chelates to the ortho-hydroxyl group on catechin at a 75% yield but this method is not economical enough. Chloroformate was found to be the best for the protection of phenolic groups on the A-ring but not selective for the protection of phenolic groups on the B-ring in our research. These methods may be promising with more improvements.

4.3.2 Synthesis of epicatechin derivatives from MPPs According to the above-mentioned reaction, we found methyl propiolate was ideal reagent for catechin-type compounds. Then, a milligram-scale of test via reaction of **1** with equimolar methyl propiolate in the presence of 4-dimethylaminopyridine (DMAP) was performed at room temperature for 8 hours (Scheme 4.2). The crude product was purified with typical column chromatography to give **4** in isolated yield of 41%. So, methyl propiolate can work in epicatechin derivatives obtained by depolymerization of MPPs with carbon nucleophiles.



1 **4**

Scheme 4.2 Selective protection of **1**

Then, using MPPs as the raw materials, sixteen epicatechin derivatives were synthesized (Figure 4.1).

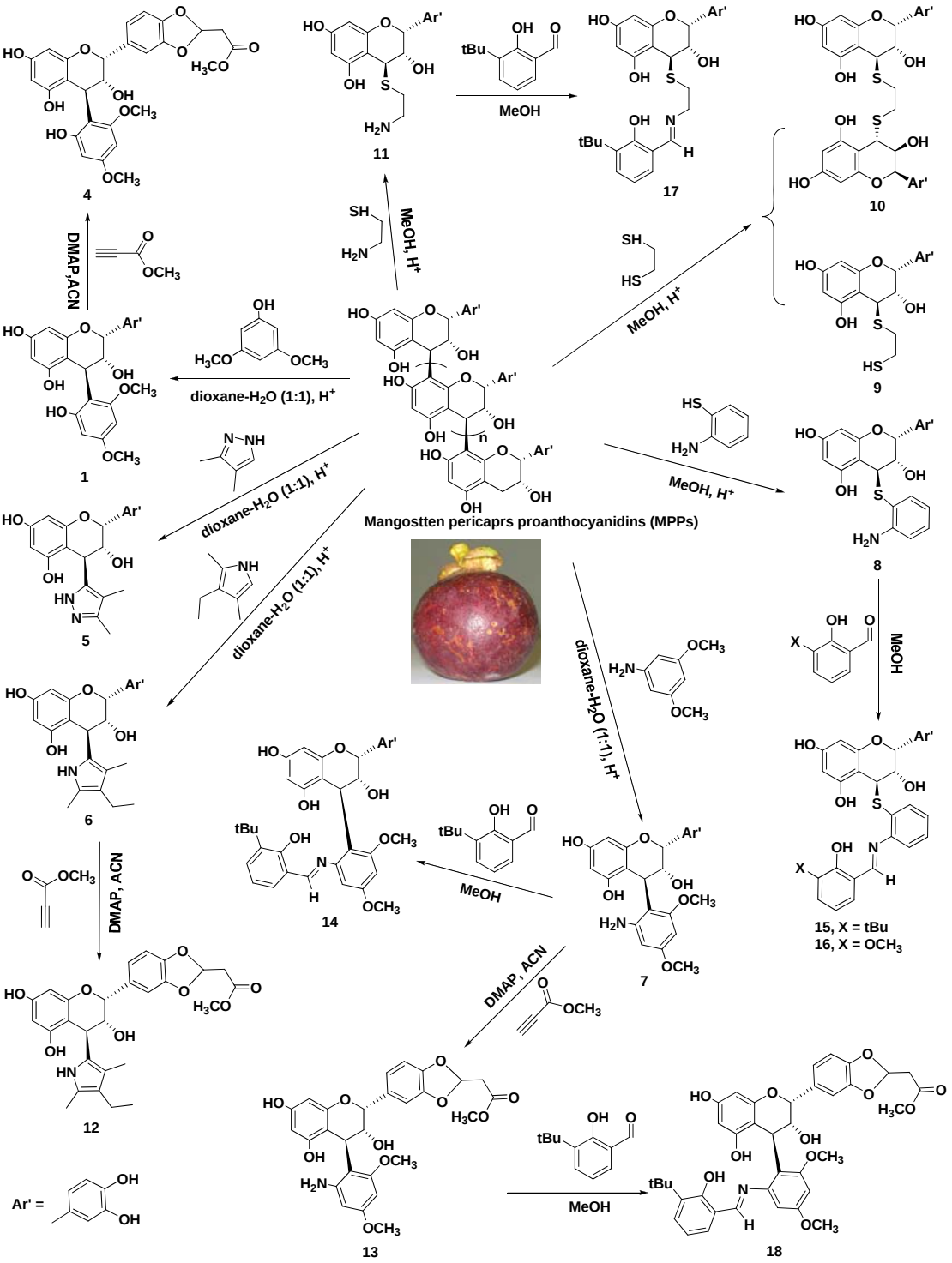


Figure 4.1 Synthesis of epicatechin derivatives from mangosteen pericarp proanthocyanidins (MPPs).

We attempted to use different nucleophiles in acid mediated depolymerization of MPPs. In the presence of un-substituted pyrrole, a potent carbon nucleophile, the reaction only gave black mixture likely due to polymerization or unselective nucleophilic substitution on α or β-carbon. Substituted analogs, 2, 3-dimethylpyrazole and 3-ethyl-2,4-dimethylpyrrole successfully yielded **5** and **6**. The products were isolated conveniently by normal phase silica column chromatography. The HPLC chromatograms of the compounds all gave rise to one sharp peak indicating a single enantiomer for **5** and **6** in consistence with epicatechin derivatives. The stereochemistry of C4 is determined by comparing the ^{1}H NMR spectra pattern of C ring protons with known compounds. Epicatechin derivatives **5** and **6** are rare examples of alkaloidal flavonoids. Naturally, there are only three reported examples, i.e. lotthanongine [153] (flavonoidal indole derivative), ficine/isoficine [154], and phyllospadine [155]. The bioactivity of these compounds remains largely unexplored. Our method in preparing **5** and **6** is very mild and straightforward for large quantity synthesis for further investigation of their bioactivity. Regarding to metal chelating potential, **1** and **2** have two bidentate sites, one chiral (N, O) and the other achiral (catecholic unit on B ring) and thus have potential as ligands to prepare bimetallic complexes. Complexes formed between oxotitanium(IV) phthalocyanine and catechins or apple procyanidins were synthesized in Kobayashi's lab [147]. The structures of complexes formed between oxotitanium(IV) phthalocyanine and epicatechin or one procyanidin trimer were shown in Figure 4.2 [147]. We can find that the titanium regioselectively chlated the catecholic unit on B ring in catechin or every monomeric units in the apple procyanidin trimer.

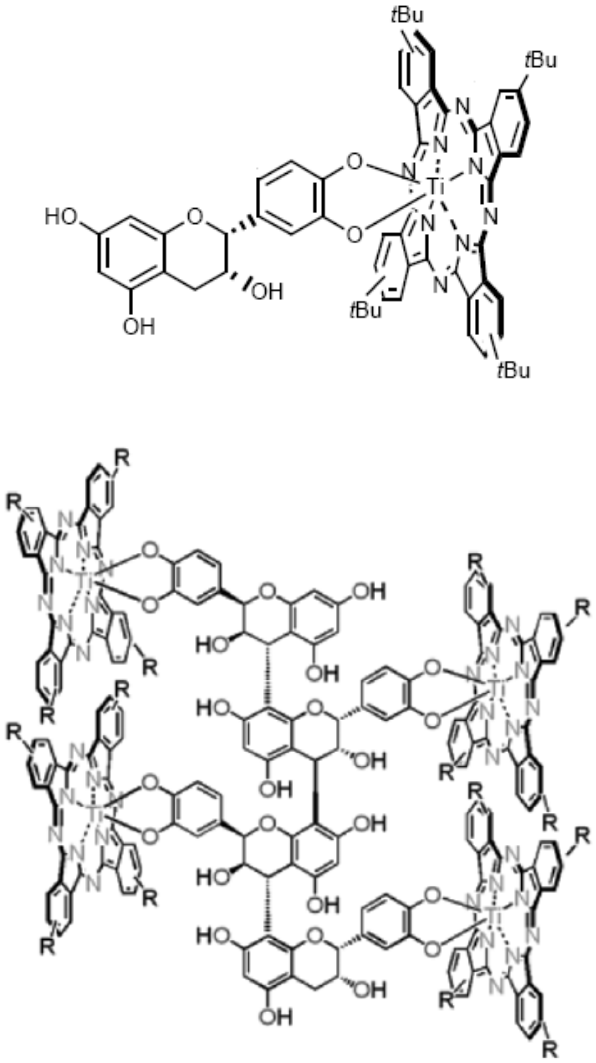
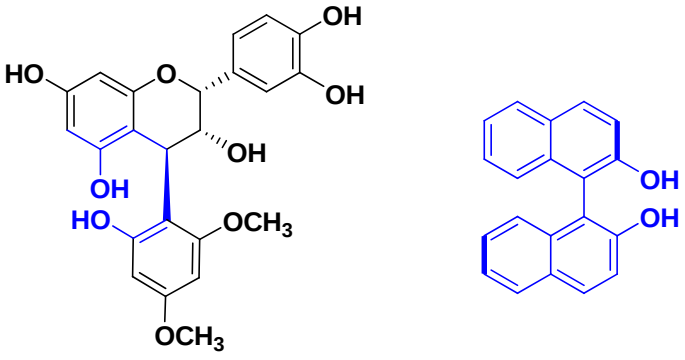


Figure 4.2 Structures of complexes formed between oxotitanium(IV) phthalocyanine and epicatechin (upper one) or procyanidin trimer (lower one) [147]

Weaker carbon nucleophiles like 3, 5-dimethoxyphenol and 3,5-dimethoxyaniline also led to depolymerization products **1** and **7** in a lower yield. Structurally, **1** has some similarity with (R or S) -1,1'-Bi-2-naphthol (BINOL), a versatile chiral ligand found many application in asymmetric organic reactions [156]. The hydroxyl groups (shown in blue in structure shown in Figure 4.3) of two monomer units are positioned ideally for chelating transition metals forming chiral complexes. In addition, BINOL has axial chirality and the two enantiomers can be readily separated and are stable toward racemisation while **3** has three chiral centers at C2, C3 and C4. 3-hydroxy-

group possibly affects stereoselectivity when using **3** in asymmetric synthesis. With simple protection of the ortho-dihydroxyl groups on the B ring from competitive binding of metals, a new chiral ligand will be obtained and its effect will be examined on catalyzing C-C bond forming organic reactions such as ene reaction, Diels-Alder reaction, etc.



3 **BINOL**

Figure 4.3 Structural similarity of 3 and BINOL

Thiols are strong nucleophiles and even the highly odorous benzylmercaptan has been widely used for determination of degree of polymerization of proanthocyanidins because the yield was almost quantitative. Herein, we depolymerized the MPPs with selective thiols, 2-aminothiophenol, 1,2-ethanedithiol and cysteamine to prepared multidentate ligands **8**, **9**, **10** and **11**. The odorless 2-aminothiophenol was an excellent nucleophile in depolymerization of MPPs as it gave **8** with high yield and easy isolation. Compound **8** are not only chiral ligands but also the intermediates for synthesis of Schiff base. By using 1, 2-dithiolethanol as nucleophile for depolymerization of MPPs, we were able to isolate mono and disubstituted product, **9** and **10** in the same reaction. Increasing the ratio of 1, 2-dithiolethanol, the yield of **9** can increase but there still are some **10** in the reaction media. Compound **10** is particularly interesting as tetradentate (O_2S_2) ligand with C2 symmetry. Analyzing

with HPLC, it was found the highest yield of **10** was obtained when the molar ratio of MPPs to 1,2-ethanedithiol equals to 3:1 (w/v). Because the absorption of the carbon nucleophile-epicatechin derivatives on silica gel, we used the conversion ratio to detect the reaction. Table 4.1 indicated the conversion ratio established with HPLC. **10** was analyzed at the optimal condition (MPPs-1,2-ethanedithiol (3:1, w/v).

Table 4.1 Conversion of MPPs to epicatechin derivatives

Nucleophiles	Products	Conversion(%)
3,5-dimethoxyphenol	**1**	42
3,5-dimethoxyaniline	**5**	47
3,-ethyl-2,4-dimethylpyrrole	**6**	50
2,3-dimethylpyrazole	**7**	56
2-aminothiopheol	**8**	63
1,2-ethanedithiol	**9**	49
	10	52
cysteamine hydrochloride	**11**	63

Torres *et al* synthesized **11** via depolymerization of pine bark proanthocyanidins and this depolymerization reaction has been optimized [117-118]. Combination of semi-preparative cation-exchange chromatography or resin exchange with preparative HPLC separation was required for purification of the product. In our presented study, gram-scale of **11** can be directly purified only using column chromatography with reverse silica gel. It was found that quenching the reaction mixture with pH 6.5 Na_2HPO_4 buffer, followed by filtering the salt produced and concentration of the filtrate was the best extraction procedure. The eluent that gave the best separation was

a gradient elution of 0.4% aq. AcOH/MeOH (1:1, v/v), and then 0.4% aq. AcOH/MeOH (4:1, v/v)). Addition of AcOH was found to be important in obtaining a pure product. The pure fractions obtained from column chromatography had to be neutralized as high acidity may cause decomposition of the product. The identity was established by comparison with the reported ^{1}H NMR data [117]. An interesting point to note was that second order spectra were obtained for the peaks corresponding to S-C$\underline{H}_2$-C$\underline{H}_2$-N in the 3.30 – 2.80 ppm region due to the presence of the chiral centre at C4 (Figure 4.4).

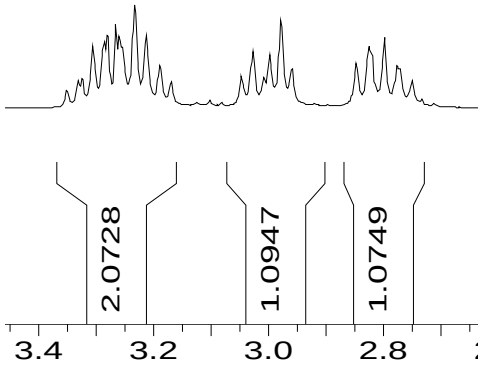


Figure 4.4 ^{1}HMR spectrum of peaks corresponding to S-C$\underline{H}_2$-C$\underline{H}_2$-N

After getting these epicatechin derivatives, the catecholic unit on B ring can be blocked selectively with methyl propiolate in the presence of dimethylaminopyridine (DMAP) under mild conditions for carbon nucleophile products (e.g. **1 → 4).** However, sulfur-containing ligands decomposed under this reaction condition. **4** has two diastereomers due to two equally populated configuration of *CH-(CH_2) indicated by two equal intensity ^{1}H NMR resonance signals around 6.52-6.48 ppm. Both of **5** and **6** can be further modified by protection of the catecholic units to give **12** and **13**. **13** was an N,O-bidentate chiral ligand and the intermediates for synthesis of Schiff base **18**.

Multidentate Schiff base **14, 15, 16, 17 and 18** was also obtained readily. **14** has only one set of ESI-MS signal, however, there are two set of peaks in its NMR spectra. We supposed that the possible reason is due to the hindered rotary of the C-4 bond. The hindered rotation of interflavanol C-C bond has been observed in catechin dimmer, procyanidin B1 previously [28]. In order to confirm the interconversion of two set of signals and prove the thought, the ^{1}H NMR spectra of **14** were tested at different temperature. The peak of the t-butyl group changed with the temperature (Figure 4.5) and the results indicated that the two set of peaks is due to two isomeric rotamers.

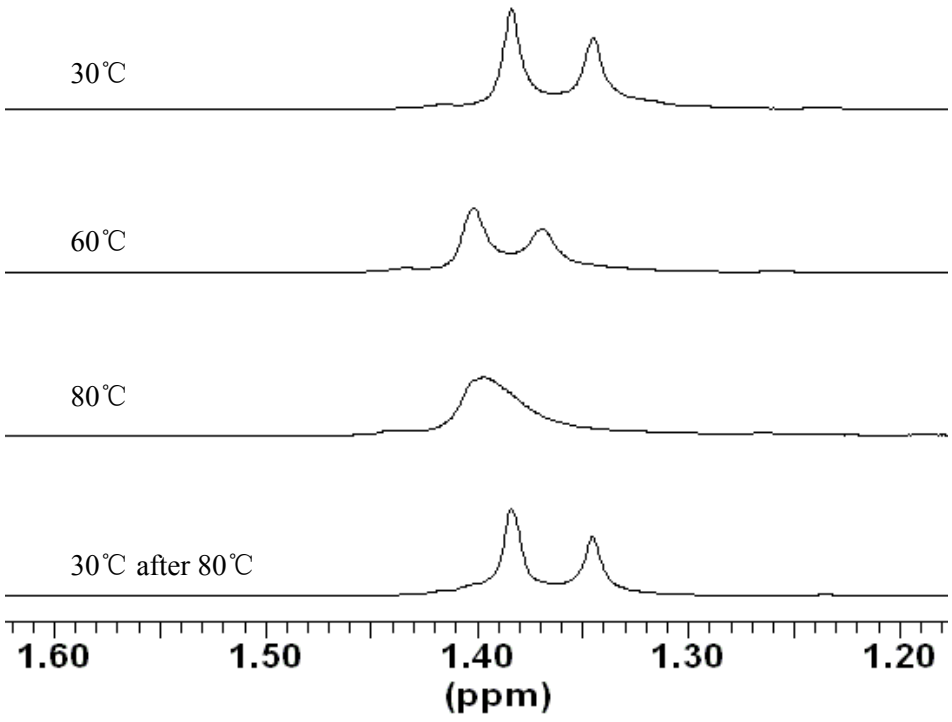


Figure 4.5. A portion of ^{1}H NMR spectrum of **14** at different temperature demonstrating the existence of interconveting rotamers.

Tarascou et al investigated the three-dimensional structures of 5 procyanidin dimers, epicatechin-catechin (B1), epicatechin-epicatechin (B2), catechin-catechin (B3), catechin-epicatechin (B4) and epicatechin-epicatechin gallate [157]. They found that all procyanidins investigated exist in two conformations that are in slow exchange

in the NMR timescale; one is compact, the other extended. Two rotomers of B2 were obvious [Figure 4.6]

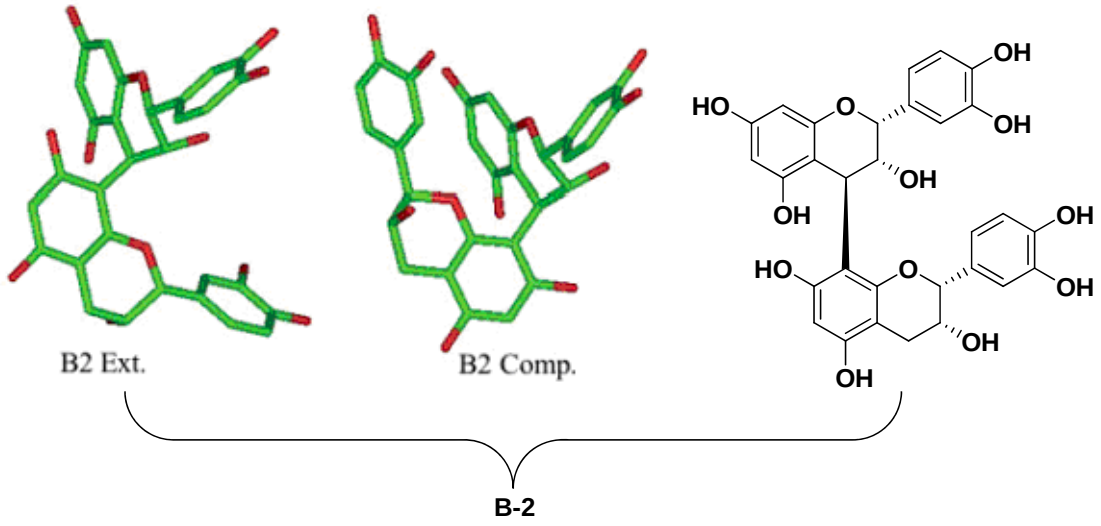


Figure 4.6 3D representations of procyanidin B2 [157]

Due to the same exchange, there are two set of peaks in NMR spectra of **14**. The conformations of two rotational isomers about the interflavanyl linkages of **14** were shown in Figure 4.7. In fact, a series of methyl ether acetate derivatives of dimeric profisetinidin diastereomers showed the rotational conformation [2, 4]

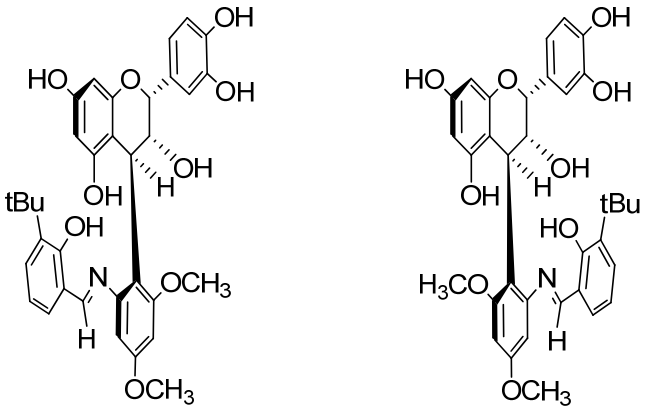


Figure 4.7 Two rotational isomers of **14**

Surprisingly, although **15** and **16** are stable in acidic conditions and stable on

silica gel, they readily eliminate thiol in CD_3OD after 2 days. In NMR tube, Schiff base **15** and **16** slowly loses thiol and, the resulting thiols were oxidized to disulfides under aerial conditions. However, there is no elimination of thiol observed when placing Schiff base **17** in CD_3OD even for five days. The results suggested that less nucleophilic Ar-S^- may contribute to sensitivity of **15** and **16**.

Both the two disulfides readily crystallized. The disulfide **19** from **15** is a novel compound. The detailed structural knowledge was provided by using crystallographic results (Figure 4.8). The skewed C–S–S–C bridge is the central part of the molecule. S–S bond character, molecular conformation around the central part, and hydrogen bonding are of major structural importance in this type of Schiff base disulfide ligands. The S–S bond length in **19** was found as 2.037(5) Å. This bond length is in agreement with those reported for analogous structures [158-160]. The comparison of S–S bond lengths and the other main structural parameters around S–S bond are given inTable 4.2

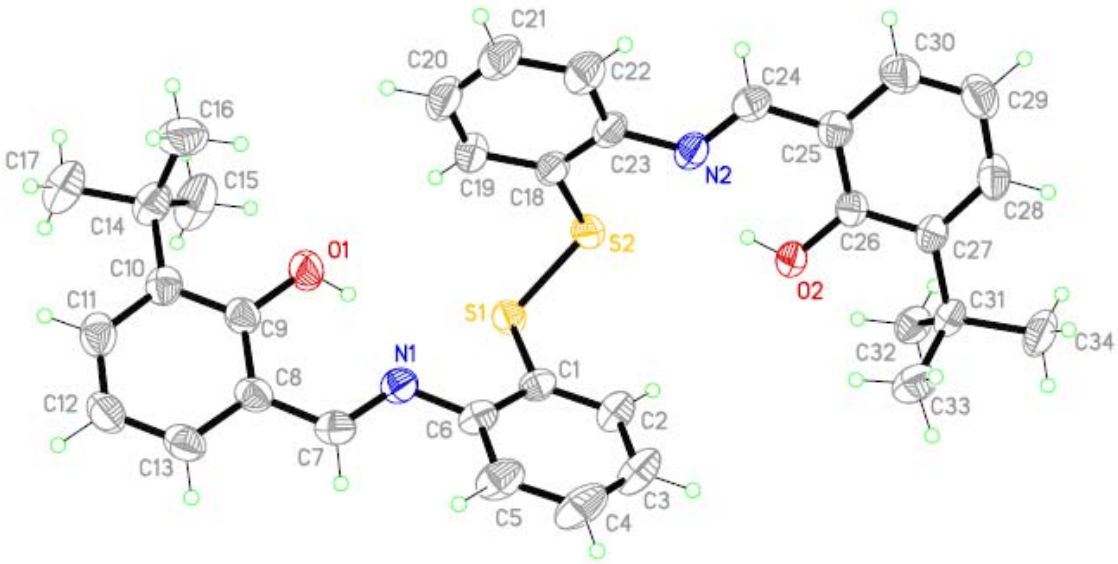


Figure 4.8 Molecular configuration and atomic numbering scheme for **19** showing 50% probability displacement ellipsoids(ORTEP III)

Table 4.2 Some geometric parameters for the similar disulfide compounds

Compound	S–S (Å)	C–S (Å)	C'–S' (Å)	Literature
11	2.037(5)	1.780(14)	1.787(16)	
N,N'-bis-(4-chlorobenzylidene)-2,2'-diamino-diphenyl disulfide	2.032(2)	1.781(4)	1.770(4)	[158]
N,N'-bis-(2-methoxybenzylidene)-2,2'-diaminodiphenyl disulfide	2.032(2)	1.786(6)	1.779(7)	[159]
N,N'-bis-(2-thenylidene)-2,2'-diaminodiphenyl disulfide	2.037(2)	1.783(4)	1.773(5)	[160]

Compound **20** was synthesized and characterized by other group previously [161] without the crystallographic results. The possible route for formation of **20** from **16** in my study was shown in Figure 4.9. The molecular structure of **20** is shown in Figure 4.10. Intramolecular hydrogen bonding occurs between the benzothiazole and phenol ring systems. Similar bonding were observed in 3-(1,3-Benzothiazol-2-yl)-2-naphthol previously [162].

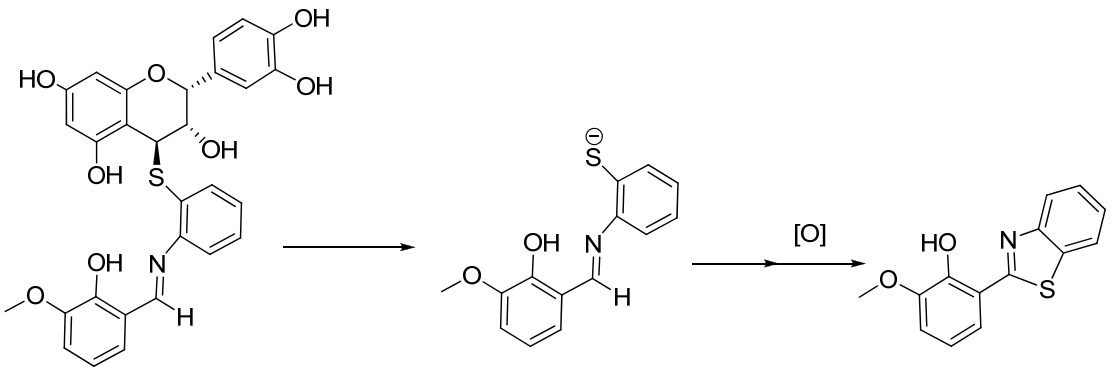


Figure 4.9 The possible route for formation of **20** from **16**

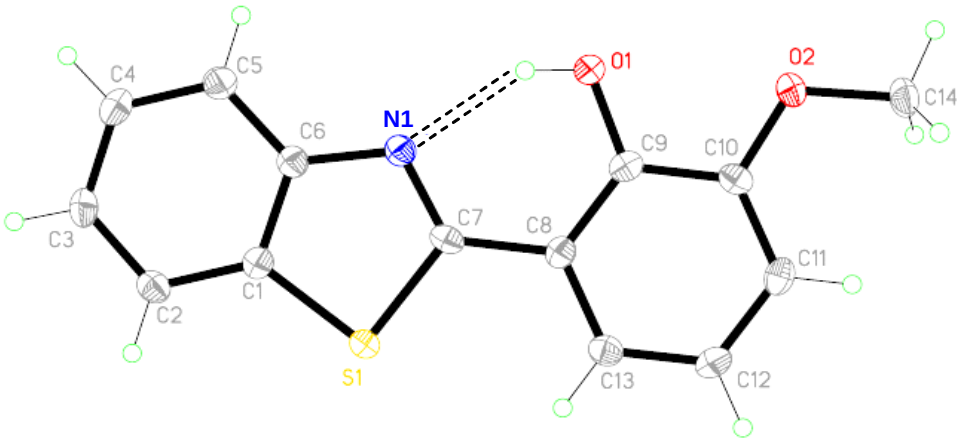


Figure 4.10 The molecular structure of **20**, with 40% probability displacement ellipsoids (arbitrary spheres for H atoms). Dashed lines indicate hydrogen bonding.

In conclusion, we have demonstrated that mangosteen pericarps proanthocyanidins can be sustainable and renewable materials for a wide range of epicatechin derivatives. The chemistry of these compounds warrants extensive study both as potential therapeutic agents and as ligands in asymmetric catalysis. Once these compounds are proven effective for important industrial applications, the large utilization of proanthocyanidins will then take place.

Chapter 5

Isolation, Identification and Modification of Proanthocyanidins from the Rhizomes of Selliguea feei

5.1. Introduction

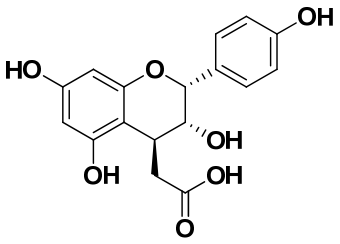
It was necessary to find proanthocyanidins possessing A-type afzelechin as extension units since the decomposition were observed in sulfur-containing epicatechin derivatives (described in chapter 4). One promising source is of *Selliguea feei.*

Selliguea feei is a species of fern belonging to the polypodiaceae. It is a perennial plant that inhabits the forests of Indonesia and Philippines. In particular, it has been reported as a traditional medicinal plant in West Java where tea made from the rhizomes of this fern is used for the treatment of rheumatism and also prevention of urinary tract infection [163].

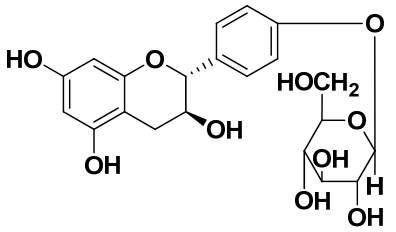
In a continuing search for plant-derived sweet compounds, Beak et al isolated and spectroscopically characterized an A-type propelargonidin trimer, Selligueain A (Figure 5.1) [163]. The sweetness intensity of Selligueain A was ranked as about 35 times sweeter than sucrose according to an evaluation of a small taste panel. Selligueain A was shown to be nontoxic in preliminary acute toxicity tests in mice. In addition, in the dose range of 0.01-10 mg/ml. Selligueain A was not mutagenic for *Salmonella typbimurium* strain TM677 [162]. In another study, it was reported that Selligueain A possessing anti-inflammation and analgesic activities. The results suggested that this compound can be used to treat rheumatism [164].

Besides Selligueain A, Beak and the coworkers continued to isolate other four compounds, Selligueain B, 3'-deoxydryopteric, (+)-afzelechin-O-β-4'-D-glucopyranoside and kaempferol-3-O-β-D-glucopyranoside-7-O-α-L-rhamnopyranoside (Figure 5.1) [165]. Interestedly, Selligueain B was neither sweet not bitter together. In addition, kaempferol-3-O-β-D-glucopyranoside-7-O-α-L-

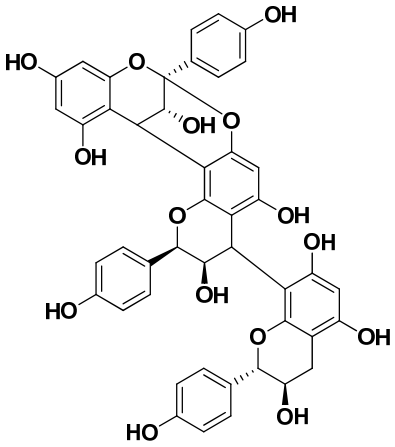
rhamnopyranoside is a bitter-tasting flavonoid glycoside [165].



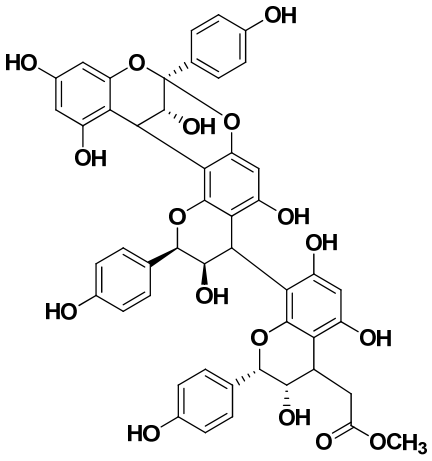
(-)- 4β-carboxymethyl epiafzelechin



(+)-afzelechin-O-β-4'-D- glucopyranoside



Selligueain A



Selligueain B

Figure 5.1 Flavanol monomers and proanthocyanidins reported in *Selliguea feei*

However, the profile of proanthocyanidins from the rhizomes of *Selliguea feei* (SFPs) is not clear. According to the reported compounds shown in Figure 5.1, it is likely that that the SFPs were mainly consisted of A-type propelargonidins. It would be attractive to us for synthesizing the stable chiral ligands with one step depolymerization after we obtained and identified the proanthocyanidins. In this present study, we reported the results about the isolation, purification and characterization of the proanthocyanidins and synthesis of four epiafzelechin derivatives.

5.2 Results and discussion

5.2.1 Isolation and characterization of proanthocyanidins from the rhizomes of *Selliguea feei* (SFPs). Typical solvent extraction and fractionation on Sephadex LH-20 gave 3.93 g of proanthocyanidin mixtures from 200 g fresh rhizomes of *Selliguea feei*, corresponding to 1.96% yield-based fresh weight. Taking into account the moisture in the *Selliguea feei* root (80.9%), the proanthocyanidins content in 2.43% and ideal for further depolymerization to get epiafzelechin derivatives.

Proanthocyanidins from the rhizomes of *Selliguea feei* (SFPs) were revealed to be oligomeric proanthocyanidins (OPCs) according to the ^{1}H NMR in CD_3OD (Figure 5.2) spectrum. The mean degree of polymerization (mDP) of the *Selliguea feei* OPCs was calculated to be 2.6 by integrating the A-ring proton signals between 5.8 and 6.5 ppm and comparing them to the intensity of the H4 signals of the terminal units between 2.4 and 3.0 ppm based on the following equation [24].

$$\text{mDP} = 2 \times \frac{\text{(H-6 + H-8) signal area}}{\text{(H-4) signal area}} - 1$$

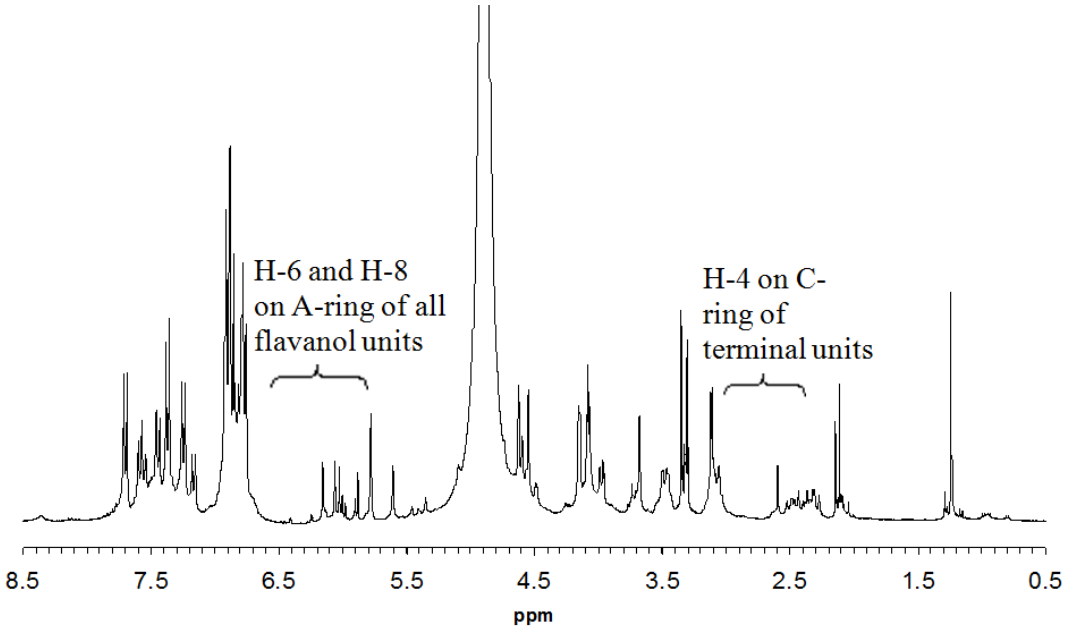


Figure 5.2. ^{1}H NMR spectrum of proanthocyanidins from the rhizomes of *Selliguea feei*; solvent, CD_3OD; room temperature; and 300 MHz.

The ^{13}C NMR spectrum (Figure 5.3) of the SFPs in CD_3OD shows characteristic ^{13}C peaks of propelargonidin. Indeed, procyanidin units (catechin/epicatechin) generally showed a typical resonance at 145 ppm while prodelphinidin units (gallocatechin/epigallocatechin) generally showed a typical resonance at 146 ppm [27]. The absence of a clear signal with such a chemical shift in the spectra of the *Selliguea feei* proanthocyanidins revealed that they are mostly composed of propelargonidin units (afzelechin/epiafzelechin). The structural diversity of the linkage (A and B type) and stereochemistry is apparent from the spectrum. Specifically, C5, C7, C8a and C4' carbons of propelargonidin appear at 160 to 150 ppm. The cluster peaks around 131 ppm belong to C1' and peak at 116 ppm is assigned to C3' and C5'. The peaks between 110 and 90 ppm is assigned to C8, C6, C6', and C2'. The region between 70 and 90 ppm is sensitive to the stereochemistry of the C ring. The ratio of the 2,3-cis to 2,3-trans isomers could be determined through the distinct differences in their respective C2 chemical shifts. C2 gives a resonance line at 76 ppm for the cis and at 84 ppm for the trans form. The latter is clearly visible in the spectrum indicating that here both stereoisomers (afzelechin/epiafzelechin) are present. The C3 in terminal units generally have their chemical shift around 67 ppm. The C4 atoms of the extension units showed at 37 ppm, while the terminal C4 exhibits multiple lines around 29 ppm [27]. There are obvious A-type linkages indicated from the signals at 151–152 ppm due to C5 and C7 of the A ring involved in the double linkage. The chemical shift of the ketal carbon (C2) formed as a result of this additional bond observed at 104.7 ppm provided further support for A-type linkage. Interestingly, strong signal of ketone carbon was observed at 176.5 ppm which confirm the FSPs contained (-)- 4β-carboxymethyl epiafzelechin.

Meanwhile, it may reveal that there is other proanthocyanidins possessing a carboxyl or ketone group.

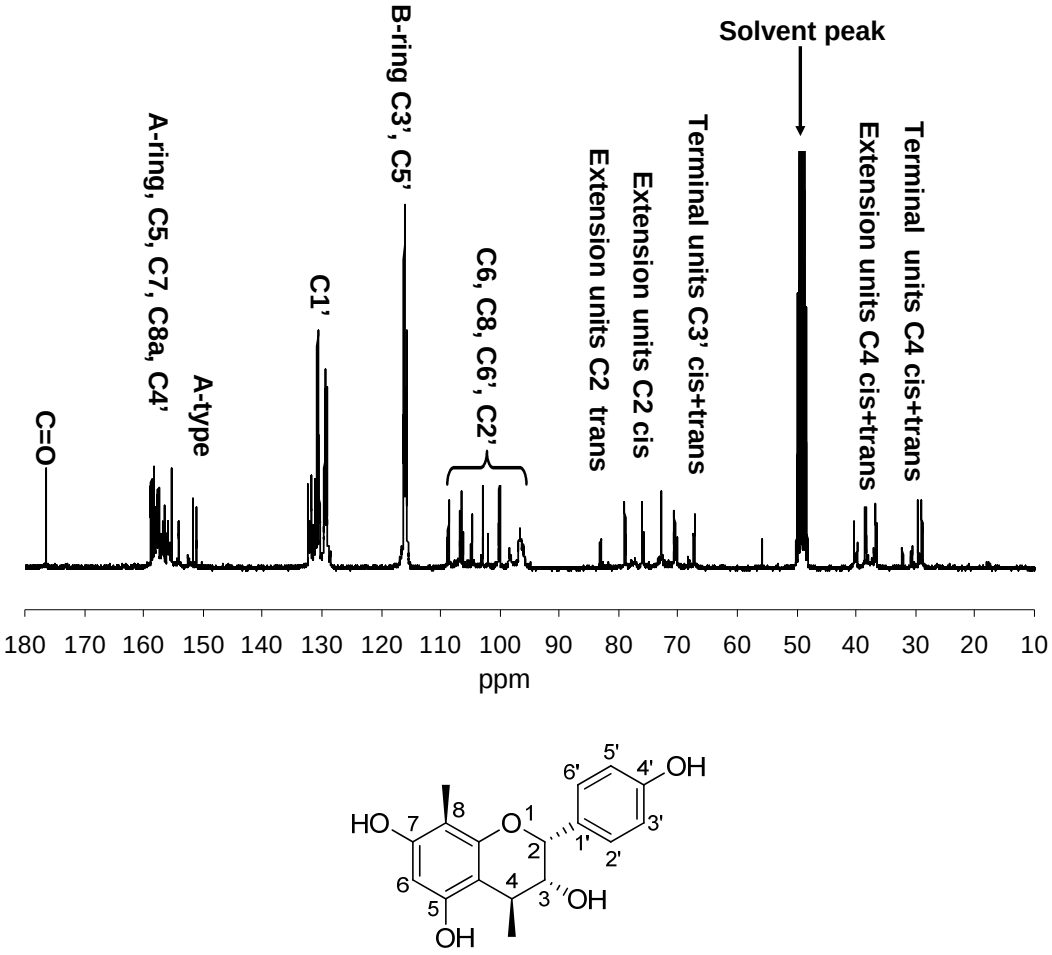


Figure 5.3. ^{13}C NMR spectrum of proanthocyanidins from the rhizomes of *Selliguea feei*; solvent, CD_3OD; room temperature; and 75 MHz.

In order to reveal the profile of the FSPs, further characterization was continued by means of ESI/MS spectrometry. Analysis was performed in the negative ion mode as proanthocyanidin molecules are thereby better detected than in the positive ion mode due to the acidity of the phenolic protons. Figure 5.4 depicts the ESI-MS spectra of the proanthocyanidins. In consistence with the ^{1}H NMR spectrum, the abundant ions were observed from *m*/*z* 331 to 873, corresponding to the molecular masses of propelargonidins with DP 1-3. The peak at m/z = 815 is due to Selligueain A (trimer) while the peak at m/z = 889 is from Selligueain B. (+)-afzelechin-O-β-4'-

D-glucopyranoside and (-)- 4β-carboxymethyl epiafzelechin are present at m/z = 473 and m/z = 331 respectively. Peak at m/z = 603 may be due to A-type propelargonidin dimer acetic acid while 541 can be from fragmentation of Selligueain A (m/z 815) after Quinone-Methide (QM) cleavage of the interflavanyl bond as show in Figure 5.3.

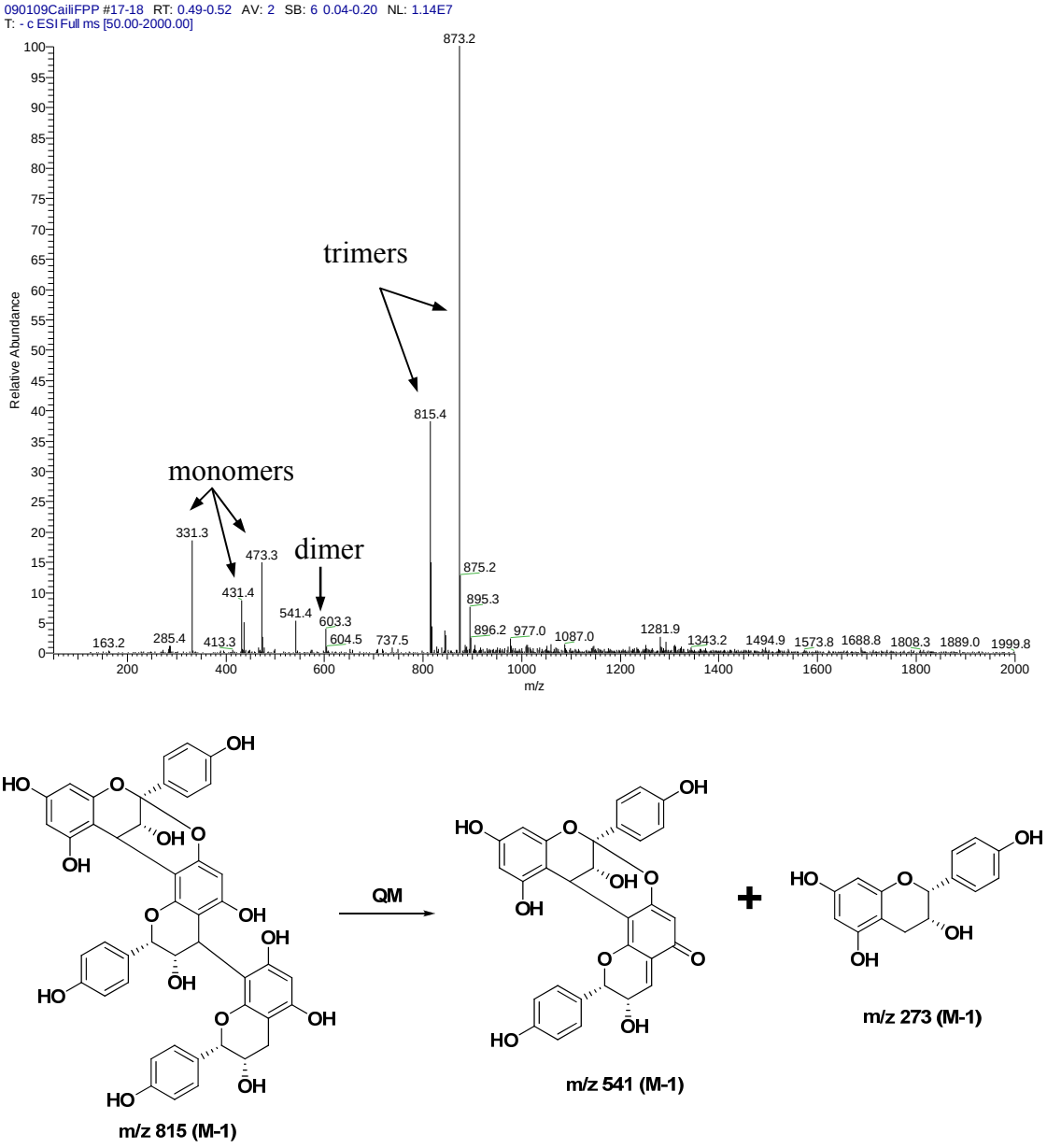
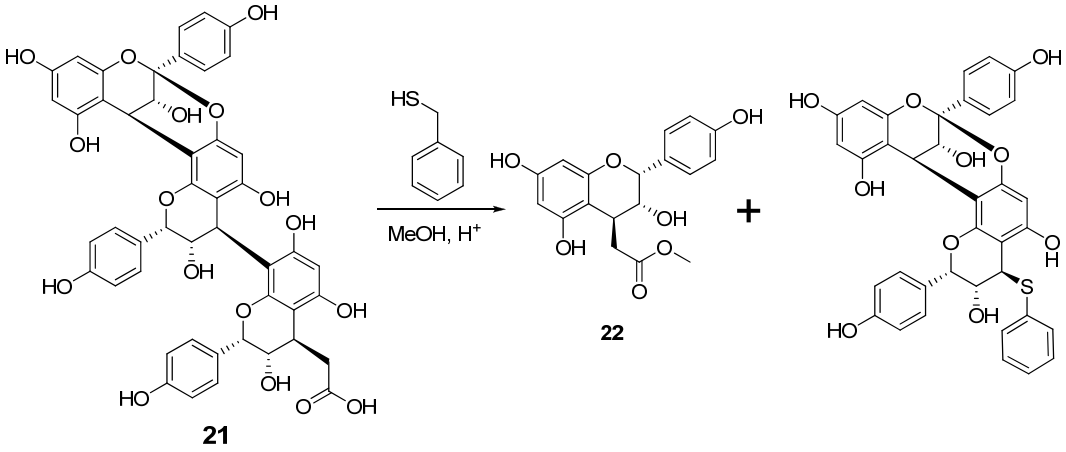


Figure 5.4. ESI/MS spectra of proanthocyanidins from the rhizomes of *Selliguea feei* recorded in the negative ion mode and the possible fragmentation pathway for Selligueain A.

In addition, there is another proanthocyanidin trimer present as seen from the

peak at *m/z* = 873. It is responding to the most abundant ion. In fact, higher molecular weight proanthocyanidins, are more difficult to be detected with a good precision in same spectra as their singly charged ions are often observed with a weak intensity, so, this ion should be responding to a proanthocyanidins with high ratio. According to the ^{13}C NMR spectra of PFPs, we proposed the structure of this trimer to be epiafzelechin-(2 β→O→ 7, 4 β→8)-epiafzelechin-(4 β→8)-epiafzelechin-4 β-acetic acid, a rare trimer found only in another fern previously [166]. After purification with silica gel, this trimer **21** exhibits spectral (UV, IR, ^{1}H NMR, ^{13}C NMR, ESI-MS) data comparable to published values [166]. There is almost no proanthocyanidins signal after m/z 900. MALDI-TOF MS results indicated that there is no obvious signal responding to higher molecule weight polymer, which agree with the ^{1}H and ^{13}C NMR spectra.

Thiolysis of **21** with benzyl mercaptan produced a new monomer, **22** (Scheme 5.1). The molecular weight of **22** is determined from ESI-MS spectroscopy as 345 from the anionic mode MS. It indicated that when the thiolysis was carried out in methanol the desired terminal unit was not obtained. Instead, the methyl ester was isolated as a new compound. Apparently under the acidic methanolic conditions, the depolymerization and esterification occurred in one pot to afford observed product. The unesterified product can be generated via depolymerization in none-alcoholic media such as 1,4-dioxane. This new monomer and the trimer were used as model compound to test the antioxidant activities together with a propelargonidin dimer derivative, in order to analyze the effect of mean degree of polymerization on antioxidant activities.



Scheme 5.1 Depolymerization of propelargonidin 21 with benzyl mercaptan

Proanthocyanidins isolated from different plants show various degree of polymerizations and characteristics of monomeric units. Besides the Selliguea feei proanthocyanidins, red kidney bean and strawberry are typical foods containing the propelargonidin units [9]. Proanthocyanidins in red kidney bean have been found to contain the highest proportion of (epi)afzelechin (14.6%) in 88 different kinds of foods. The propelargonidin oligomers were also identified in pinto bean, small red bean, red kidney bean, strawberry, almond, raspberry, and cinnamon [9]. On the basis of the peak area on the normal-phase HPLC, about 5.4-7.8% of the proanthocyanidins in strawberry contained at least one (epi)afzelechin subunit. A similar proportion was observed for almond. Propelargonidins contribute about 8.2-11.6% of the proanthocyanidins in pinto bean, small red bean, and red kidney bean. The principal proanthocyanidins in these foods are, however, procyanidins [9]. Proanthocyanidins only consisting of afzelechin/epiafzelechin unit are unfamiliar. A highly sweet proanthocyanidin trimer from the rhizomes of *Selliguea feei*, Selligueain A, has been reported to be anti-inflammatory and analgesic which could be used to treat rheumatism. As an unusual secondary metabolites, proanthocyanidins from the

rhizomes of *Selliguea feei* warrant further investigation regarding their therapeutic and chronic disease prevention potential or as food additives.

5.2.2 Synthesis four epiafzelechin derivatives. Proanthocyanidins from rhizomes of *Selliguea feei* (SFPs) are ideal source for chiral flavanol derivatives especially for the sulfur-containing ligands. There is only one hydroxyl group in the B-ring and E-ring and the 7-OH in the lower epiafzelechin unit was protected. These can make the produced chiral ligands stable to oxygen and weekly basic condition. So, the selective carbon nucleophile, 2, 4-dimethyl-3-ethyl pyrrole and sulfur nucleophiles, methyl thioglycolate and 2-aminothiophenol, were used for depolymerization the SFPs and four chiral ligands were synthesized (Figure 5.5).

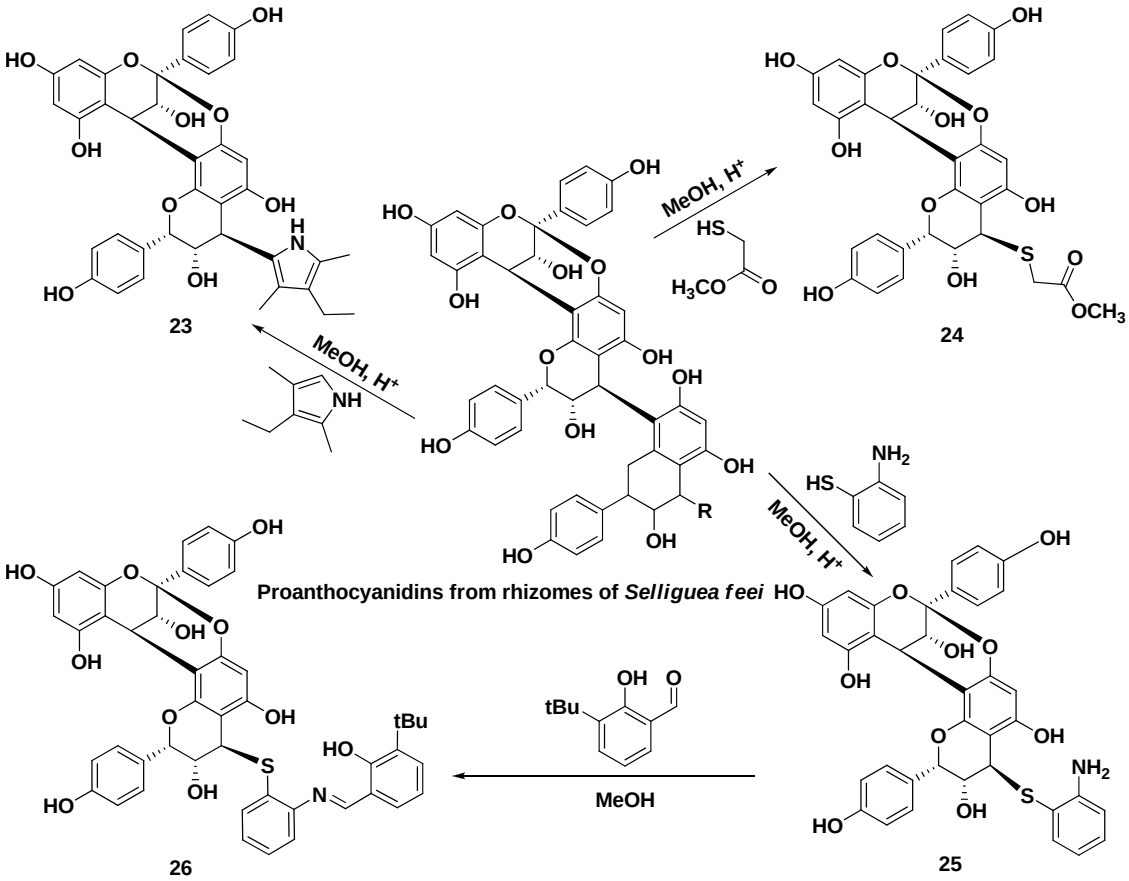


Figure 5.5 Epiafzelechin derivatives from depolymerization of proanthocyanidins from rhizomes of *Selliguea feei* (FSPs)

Depolymerization of the proanthocyanidins from rhizomes of *Selliguea feei* in the

presence of the selective nucleophile, 3-ethyl-2, 4-dimethylpyrrole, methyl thioglycolate and 2-aminothiophenol successfully yielded chiral ligands **23**, **24** and **25**. The products were isolated conveniently by normal phase silica column chromatography. Weaker carbon nucleophiles like 3, 5-dimethoxyphenol and 3, 5-dimethoxyaniline also led to depolymerization products according to MS analysis. However, the yield seemed to be not satisfied from the TLC results. Compound **23** was alkaloidal A-type propelargonidin. The bioactivity of this compound is quite promising due to the combination of several functional groups. In addition, the hydroxyl groups (shown in blue in Figure 5.6) on the D ring and the nitrogen atom in G ring are positioned ideally for chelating transition metals forming chiral complexes. There is only one hydroxyl group on the B ring and hence the competitive binding of metals can be avoided. After we get this ligand, its effect will be examined on catalyzing C-C bond forming organic reactions such as ene reaction, Diels-Alder reaction, etc.

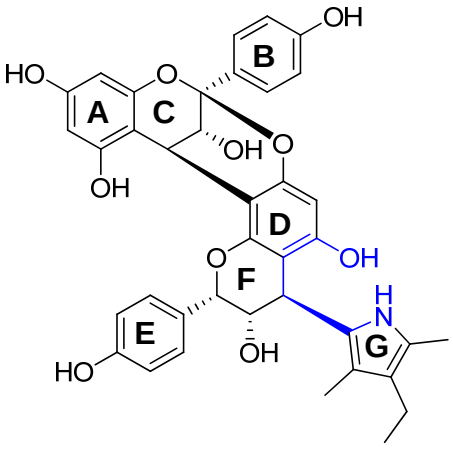


Figure 5.6 Potential chelating position of metal in compound **23**

What's more, this compound can be an organocatalyst itself. Organocatalysis, the acceleration of organic reactions by non-metallic catalysts, which has been known and described for more than a century [167], fills the gap between metal- and enzyme-

catalysis. Organocatalysis can offer highly stereoselective transformations of versatile starting materials, using easy procedures. For organocatalysis, the catalysts can be simply handled and stored and many of them are now commercially available. Proline-based compounds and cinchona alkaloids are the successful stars [123, 124, 168, 169]. As a chiral alkaloid from natural product, compound **23** can be promising for asymmetric organocatalysis.

Thiols are strong nucleophiles and two thios are selected to use in our presented study. Firstly, a tridentate ligand **24** was synthesized via depolymerization the proanthocyanidins from rhizomes of *Selliguea feei* with methyl thioglycolate. The molecular weight of compound **24** is determined from ESI-MS spectroscopy as 647 from the anionic mode MS (Figure 5.7). Besides the peak of molecule ion, a higher peak appeared at m/z 541, it was corresponding to the fragmentation of 24 (m/z 647) after Quinone-Methide (QM) cleavage of C-S bond (Figure 5.7)

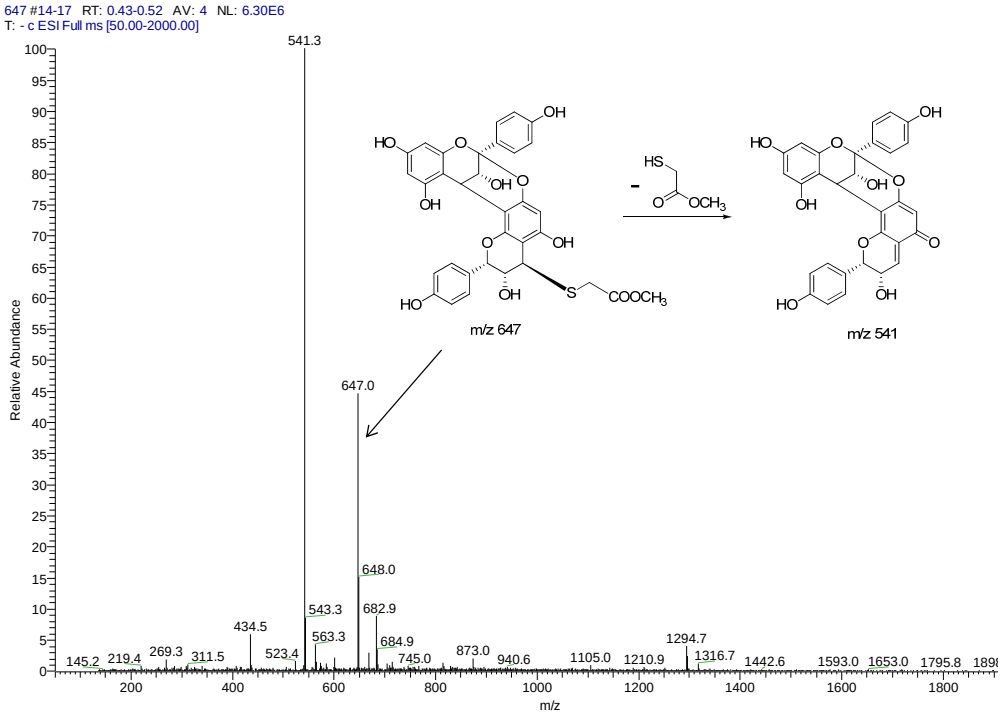


Figure 5.7 ESI-MS spectra of **24** recorded in the negative ion mode and possible fragmentation pathway.

Then, the odorless o-thioaniline was used and the depolymerized product, **25** was get with gram scale. Because the 7-OH group on the lower epiafzelechin unit was protected via the 2-O-7 bond and there is only one hydroxyl group on the B ring and E ring, compound **25** is both stable in air and under weak basic condition. It can make compound **25** more useful. In fact, compound **25** not only a chiral ligand but also the intermediate for synthesis of Schiff base **26**. By purification with silica gel after reaction **25** and 3-tert-butyl-2-benzylaldihyde, Schiff base **26** was obtained readily. For this tetradentate chiral ligand, we can find that its structure has some similarity with a successful ligand B (Figure 5.8) [170]. Ligand B is a very excellent chiral salan ligand possessing two donating amino groups and amino protons that might participate in hydrogen bonding with the substrate and regulate the transition state structure. Complex between this salan and Fe was used to catalyze asymmetric oxidative coupling of various 2-Naphthols with high enantioselectivities greater than 90% ee [170]. Complex between salan B and niobium used to catalyze asymmetric epoxidation of allylic alcohols with high enantioselectivity ranging from 83 to 95% ee using aqueous hydrogen peroxide as an oxidant [171]. Compound **26** might be used to try the asymmetric reaction catalyzed by compound B.

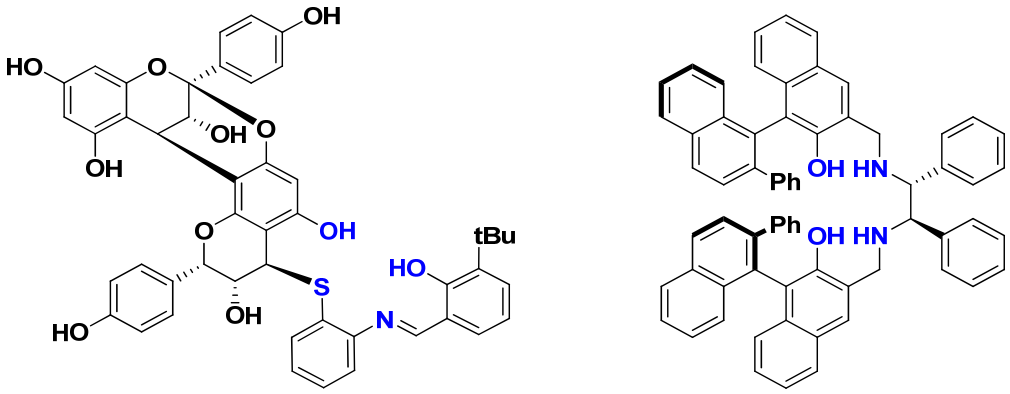


Figure 5.8 structural similarities between compound **26** and reported chiral ligand B

In summary, *Selliguea feei* were extracted and characterized to be mainly A-type propelargonidin dimer and trimers via electron spray ionization–mass spectrometry (ESI-MS) and NMR spectrometry. Then, stable tridentate and tetradentate sulfur ligands were synthesized. The 7-OH protected help to prevent the sulfur-containing ligands from the decomposition. The proanthocyanidins from *Selliguea feei* also depolymerized with 2,4-dimethyl-3-ethylpyrrole and get a chiral ligand which is rare alkaloidal flavonoids.

Chapter 6

Applications of Sulfurcontaining Epicatechin Derivatives for Proanthocyanidins Synthesis

6.1 Introduction

The facile deattachmend of the sulfur ligands from the C-4 epicatechin derivative was observed in sulfur-containing epicatechin derivatives (described in chapter 4). To resolve this problem, one method is to use proanthocyanidins from the rhizomes of *Selliguea feei* (SFPs) replacing mangosteen pericarps proanthocyanidins (MPPs) to avoid the decomposition in order to prepare broad range of chiral ligands from proanthocyanidins (described in chapter 5). However, as a matter of fact, the labile nature of the sulfur moiety of the sulfur-containing epicatechin derivatives also enlightened us to find a new method to synthesize some interested bioactive compounds using these sulfur-containing epicatechin derivatives as intermediate.

The cleavages of C-S bonds in epicatechin-(4β)-phenyl sulphide under basic condition (pH 9.0) have been documented. Under this condition, (4β)-epicatechin phenyl sulfide eliminated benzenethiol and the resulting undetected methide intermediate condensed with epicatechin-(4β→6)-catechin to give branched procyanidin trimer [172]. What's more, leucocyanidin reacted with excess of phloroglucinol gave catechin-(4α→2)-phloroglucinol at pH 9.0 and the base-catalyzed reaction is more rapid than that of the acid-catalyzed reaction [173]. So, rapid base-catalyzed condensation is promising between the sulfur-containing epicatechin derivatives synthesized in our lab and catechins or phloroglucinol analogs such as 3,5-dimethoxyphenol and 3,5-dimethoxyaniline. Two nitrogen containing nucleophiles, 2,3-dimethylpyrazole and 3-ethyl-2,4-dimethylpyrrole were worth of use for synthesis of alkaloidal flavonoids. Additionally, epimerization at C-2 in catechin/epicatechin is well-known to occur in alkaline solutions, so, the more weakly basic condition, the broader the range of the base-catalyzed condensation reaction will

be. Because **8** lose thiol under neutral condition, it seemed to be a good starting material which can be used for this type condensation under more weakly basic conditions. In the present study, three sulfur-containing epicatechin derivatives, **2**, **8** and **11**, were chosen to perform the kinetic analysis to find the best intermediate. The possible mechanism and effect of group on the reaction were also reported here.

6.2 Results and discussion

6.2.1 Kinetics of the dethiolation of three sulfur-containing epicatechin derivatives. While the C-C bond is sensitive in acidic conditions but stable in moderate basic conditions, this is in sharp contrast with the C-S bond in epicatechin derivatives including **2**, **8** and **11**, which cleaves in weakly basic solution (pH > 8.5). This phenomenon enlightened us that these compounds may be useful intermediate for synthesis bioactive compounds such as procyanidin B2. Because two Schiff bases derived from **8** lost thiol in MeOD under aerial conditions while no elimination of thiol was observed in Schiff base of **11**(described in chapter 5), we supposed that the difficulty of cleavage of C-S in these compound changed with electron donating activities of substituent group at C-4. In order to screen the best intermediate for synthesis of natural procyanidins and rare alkaloidal flavonoids, kinetic analysis was performed via dissolving **2**, **8** and **11** in acetone-water (1: 1, v/v) solution and the pH adjusted to 8.5 with sodium hydrogen carbonate-sodium carbonate buffer. The reaction mixture were analyzed by HPLC at different reaction time and the results showed that **8** reduced to about 70% in 1hrs while **2** and **11** need more than 5h (Figure 6.1). The results indicated that the methyl thioglycolate in **2** and cysteamine in **11** is not good leaving groups in this weak base-catalyzed reaction. The electron donating activities of substituent group at C-4 in epicatechin derivatives markedly affected the

facile dethiolation. **8** might be the best intermediate for further base-catalyzed condensation among the three sulfur-containing epicatechin derivatives.

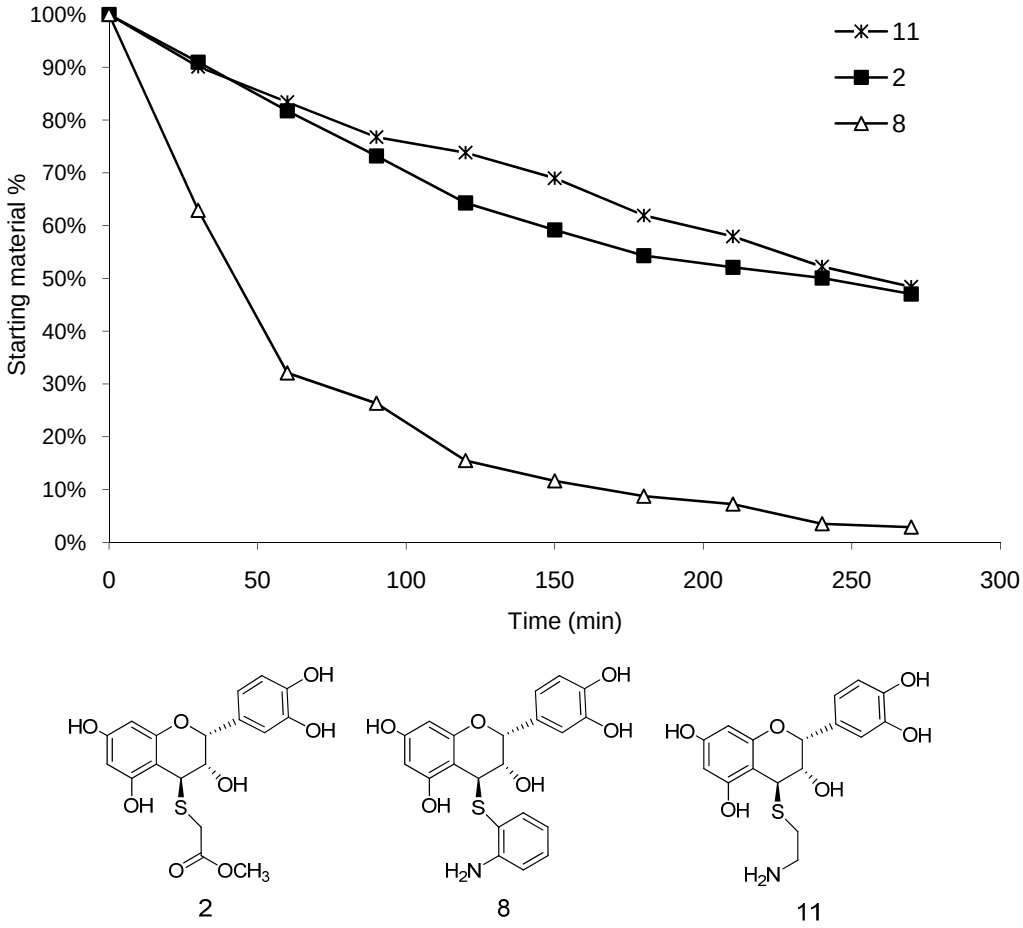


Figure 6.1 Cleavage rate of C-S bond in epicatechin derivatives **2**, **8** and **11**.

After dissolving **8** in pH8.5 acetone-water (1: 1, v/v) solution for 2 h, the reaction mixture was analyzed by ESI-MS at anionic mode. The spectra showed two main peaks at 700 and 988 corresponding to the dimeric and trimeric epicatechins with $SC_6H_4NH_2$ at the terminal unit (Figure 6.2). It seemed that self-oligomerization happened due to nucleophilicity of the upper phloroglucinol ring in **8**. Compound **2** was dissolved in acetone-water (1: 1, v/v) and the pH adjusted to 8.8 with sodium hydrogen carbonate-sodium carbonate buffer. Similar results were obtained by HPLC analysis after 2h.

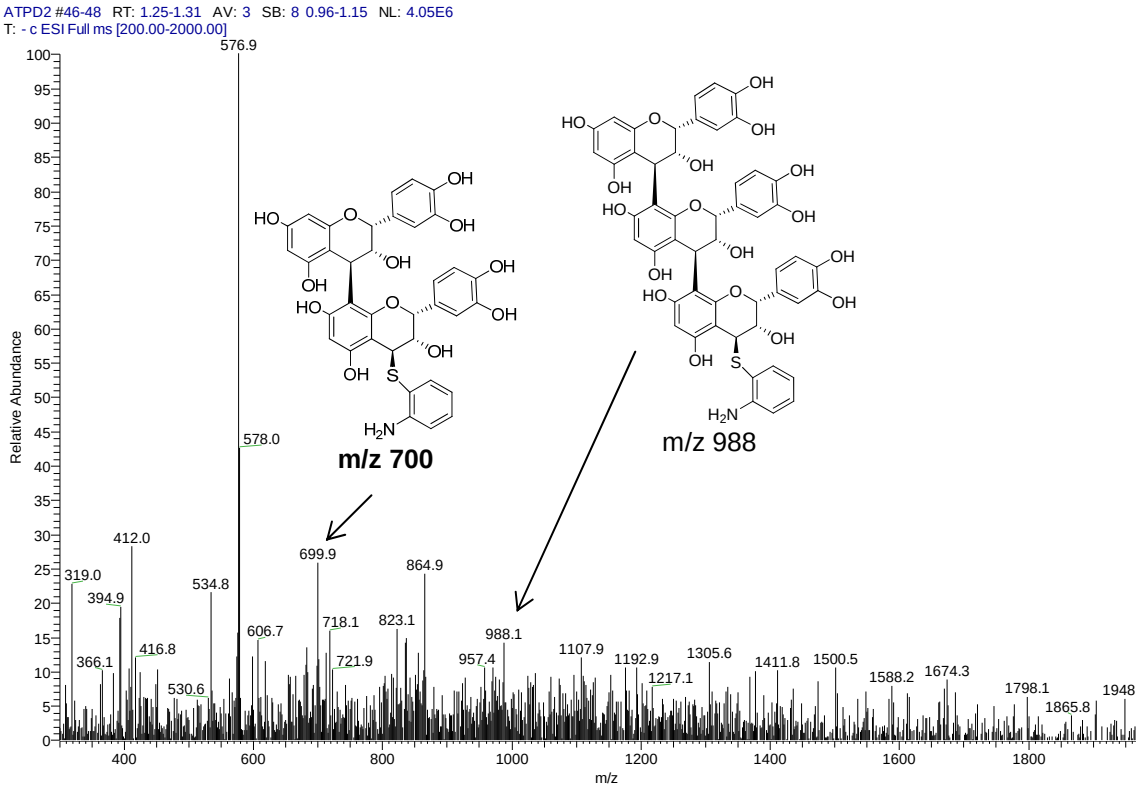


Figure 6.2 ESI-MS spectra of the dethiolation mixture of **8** recorded in the negative ion mode ESI conditions: 250 °C and 4.5 kV, full scan *m*/*z* 50–2000.

6.2.2 Synthesis of procyanidin B2 via base-catalyzed condensation. When equal molar epicatechin and **8** was mixed at pH 8.5, B2 was formed over the course of 6 hours as the major product at conversion rate of 51%. No oligomers of **8** were detected at *m/z* 700 and 988. The reaction finished much faster at pH 9.0 buffer, but the yield of B2 decreased significantly to 30%. Increasing the molar ratio of epicatechin and **8** from equimolar to 4:1 did not improve the yield, indicating that epicatechin is not the limiting factor for B2 formation. We propose the dissociation of proton at 7-hydroxy group under weakly basic conditions leads to cleavage of the S-C bond to form quinone methide intermediate. In the meantime, the phenolate form by deprotonation of epicatechin undergo nucleophilic addition with the quinone methide forming B2 (Figure 6.3). On the other hand, under acidic conditions, procyanidin B2

or other polymers were protonated on the oxygen of the 7-ketone form followed by C-C bond cleavage.

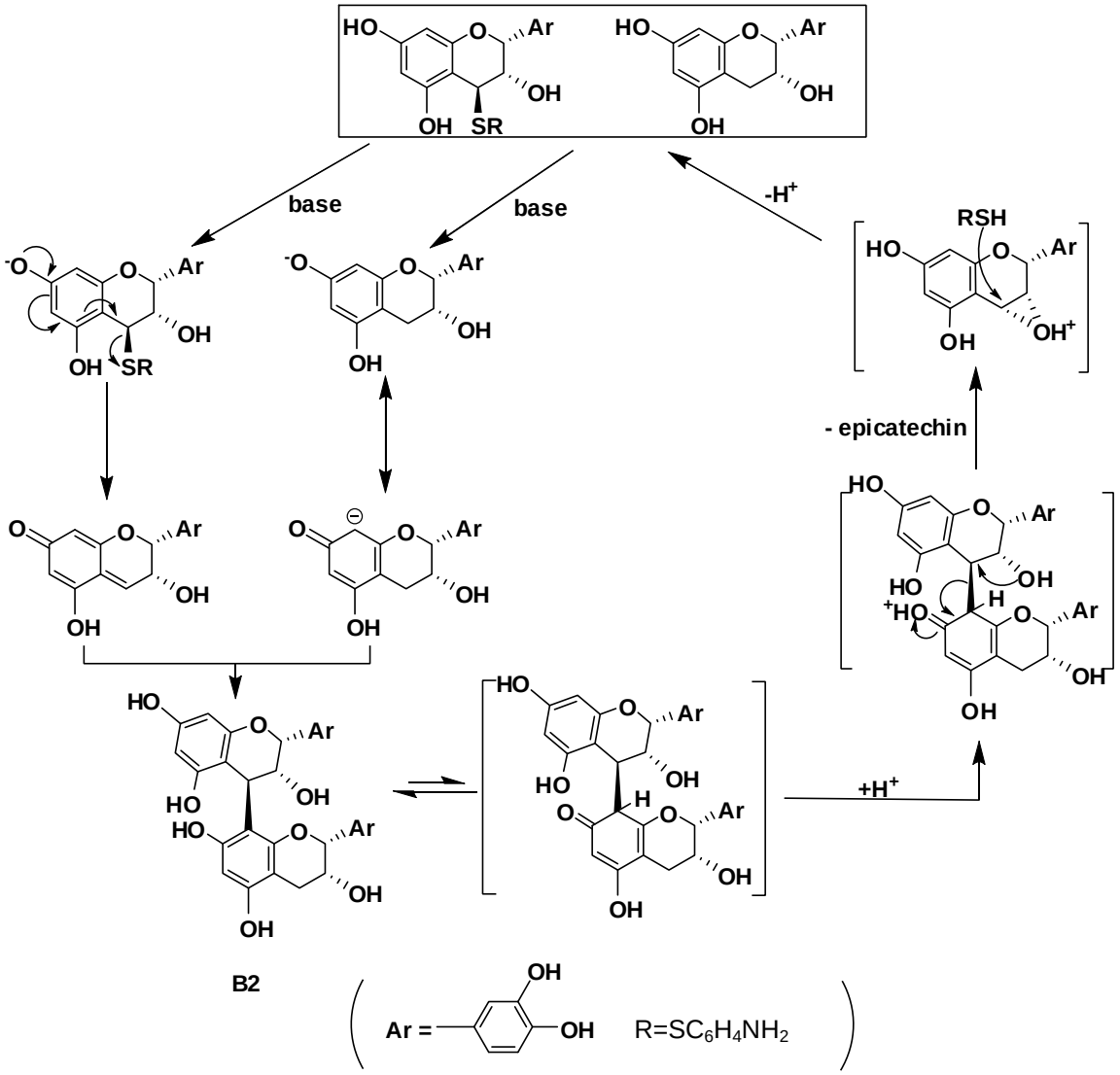
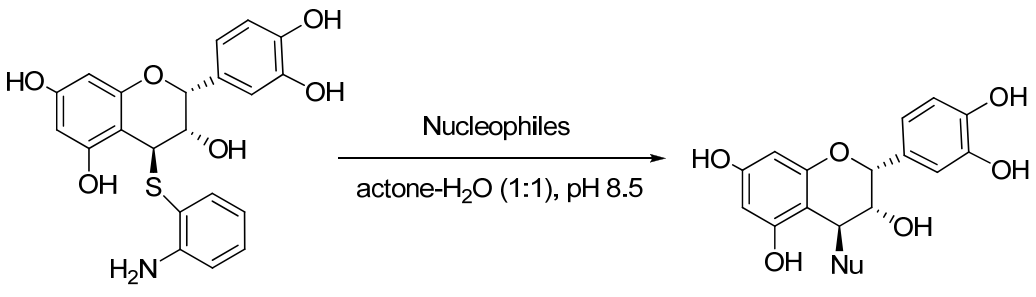


Figure 6.3 Proposed mechanism of B2 thiolysis and formation through acid-base catalysis.

The cleavages of C-S bonds in thiolated (epi)catechin have been documented, but the facile dethiolation of **8** is unprecedented. Under basic conditions, a branched procyanidin trimer, epicatechin-(4β→8)-catechin-(6 β→4)-epicatechin was synthesized by condensation of (4β)-epicatechin phenyl sulfide with epicatechin-(4β→6)-catechin [172]. Thiophilic Lewis acid including silver salt can promote the

dethiolation of the C-S bond in 4-(benzyl)thiol ether of (epi)catechin. This feature was utilized in synthesis of medium-sized (dimer to decamer) protected oligomeric proanthocyanidins from thiolated precursor, 3-*O*-acetyl-4-[(2-benzothiazolyl)thio]-5,7,3'4'-tetra-O-benzylepicatechin, in the presence of silver tetrafluoroborate. The protected oligomeric proanthocyanidins were isolated and then hydrogenolyzed to give free proanthocyanidins identical to those isolated from cocoa [174]. However, the synthesis of the 2-benzothiazolyl thioderived (epi)catechins involves usage of highly air and moisture sensitive trimethyl aluminum. The facile preparation of **8** may offer certain advantage if its value as synthetic block toward oligomeric proanthocyanidins or epicatechin derivatives can be fully materialized.

6.2.3 Synthesis of epicatechin alkaloids. With the same mechanism of B2 formation under weak basic condition, **8** can reaction with several carbon nucleophiles including 3,5-dimethoxyphenol 3,5-dimethoxyaniline, 3-ethyl-2,4-dimethylpyrrole and 2,3-dimethylpyrazole (Scheme 6.1) The corresponding products were analyzed with HPLC using **1**, **5**, **6** and **7** synthesized in our lab (described in chapter 4) as standard and the conversion were shown in Table 6.1. Products **6** and **7** are epicatechin alkaloids. We can synthesize other special epicatechin alkaloids following the same procedures.



8

Scheme 6.1 Reaction between **8** with selective nucleophiles

Table 6.1 Conversion of 1, 5, 6, 7 from 8 by base-catalyzed condensation

Nucleophiles	Products	Conversion(%)
3,5-dimethoxyphenol	**1**	28
3,5-dimethoxyaniline	**5**	25
3,-ethyl-2,4-dimethylpyrrole	**6**	62
2,3-dimethylpyrazole	**7**	59

For 3,5-dimethoxyphenol and 3,5-dimethoxyaniline, the yields for the monosubstituted products are lower. ESI-MS spectra of the reaction mixtures for 3,5-dimethoxyphenol showed additional peaks corresponding to disubstituted products [Figure 6.4]. With further purifications, we found that there is only one spot in TLC plate at several conditions corresponding to the product with m/z 729. But the NMR spectra of the product were still complex. The results suggested that the products may be the mixture of two isomers [Figure 6.4]**.**

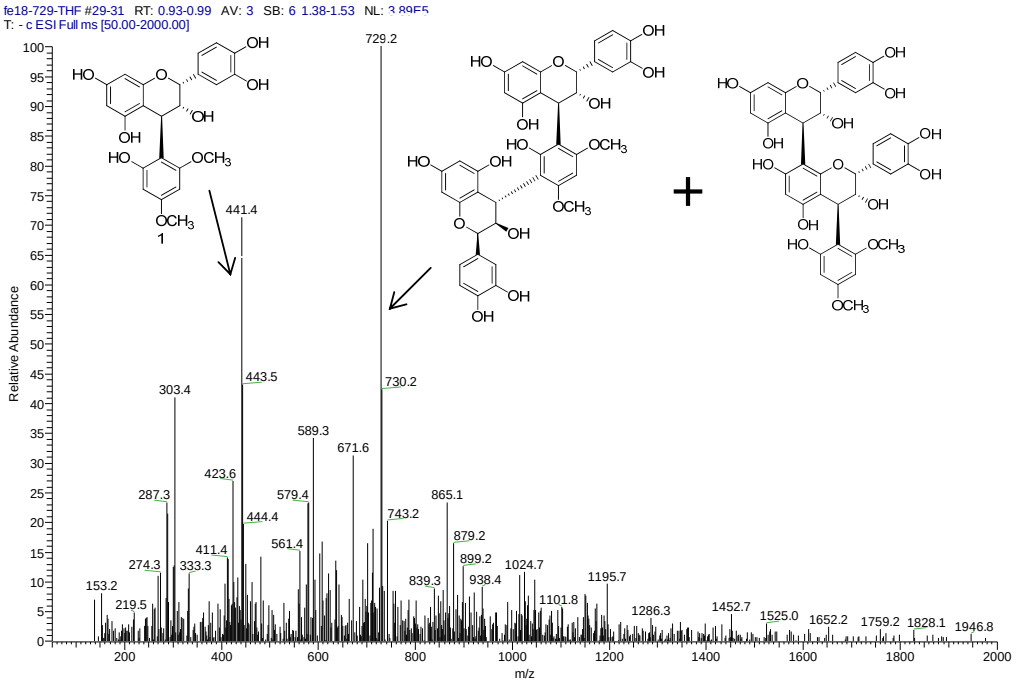


Figure 6.4 ESI-MS spectra of reaction mixture of **8** with 3,5-dimethoxyphenol recorded in the negative ion.

Similar results were found when using 3,5-dimethoxyaniline as carbon nucleophiles [Figure 6.5]. The results suggested that the nucleophilicity of the upper phloroglucinol ring in **5** is closed to the 3,5-dimethoxyaniline. It is promising to get only C-2 symmetric product when stronger carbon nucleophiles with similar structure, for example, 3,4-Dimethylpyrrole being used.

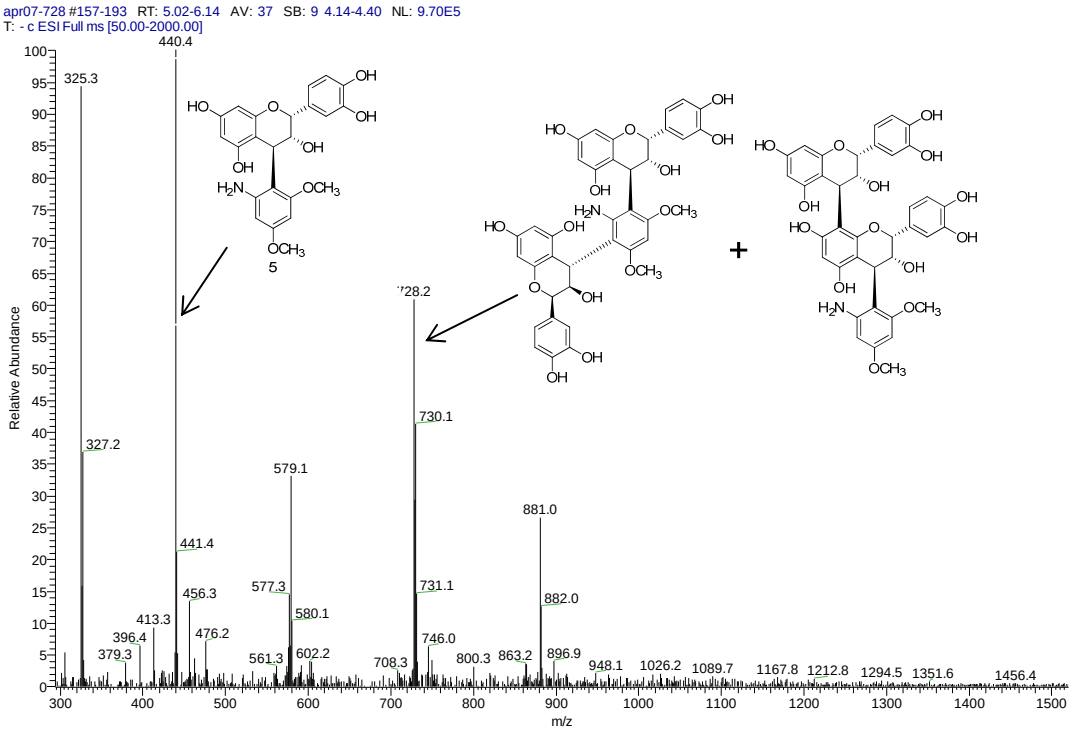


Figure 6.5 ESI-MS spectra of reaction mixture of **8** with 3,5-dimethoxyaniline recorded in the negative ion.

In conclusion, difference of the cleavage speed for different sulfur-containing epicatechin derivatives indicated the best intermediate for synthesis of procyanidin B2 was **8.** Epicatechin derivative **8** can be used to synthesize proanthocyanidins polymers and special epicatechin alkaloids. The possible mechanism was discussed in the present study.

Two procyanidin dimers, epicatechin-(4β→8)-catechin and epicatechin-(4 β→6)-catechin did not react with phloroglucinol after several hours at pH 9.0 and ambient

temperature [175]. It indicated that the lower flavanoid unit is not a good leaving group in base-catalyzed reactions. For proanthocyanidins, it is difficult to cleave interflavanoid bond to form a quinone methide under weakly basic condition. So, **8** might be an ideal source to produce functional quinine methide with epicatechin-like structure.

Chapter 7

Conclusions and

Suggestions for Further Work

7.1 Conclusions

Proanthocyanidins (PAs), one of the most ubiquitous groups of plant polyphenols, are the natural polymer widespread throughout the plant kingdom but their application as raw materials for fine chemicals are largely unexplored. This thesis documented the results on the effort towards chemical conversion of PAs into epicatechin derivatives with potential application such as multidentate chiral ligands. Research work was centered on characterization of the proanthocyanidins from mangosteen pericarps and rhizomes of *Selliguea feei,* and synthesis of the chiral ligands. Comparison among different proanthocyanidins was also preformed in order to get ideal source for preparing the epicatechin derivatives.

Mangosteen pericarps proanthocyanidins (MPPs) were extracted and fractionated by a Sephadex LH-20 column to give 0.66% yield (dry matter). ^{13}C and ^{1}H NMR signals showed the presence of predominantly procyanidins together with a few prodelphinidin units along with small amounts of stereoisomers of afzelechin/epiafzelechin and gallocatechin/ epigallocatechin. Depolymerization with benzylmercaptan indicated that the mean degree of polymerization (mDP) equals to 6.6. The electron spray ionization–mass spectrometry (ESI-MS) and matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectra revealed the dominant B type oligomers with mainly epicatechin units and with a small amount of A-type oligomers.

Cocoa proanthocyanidins (CPs) and MPPs extracted in our lab were compared with commercial pine bark proanthocyanidins (PBPs) and grape seed proanthocyanidins (GSPs) via MALDI-TOF-MS and thiolysis analysis. The mean degree of polymerization (mDP) quantified from the HPLC chromatograms of

thiolytic media showing the mDP of mangosteen pericarps > cocoa bean > grape seeds > pine bark. Both thiolysis and MALDI-TOF-MS results indicated that MPPs were ideal source for synthesis of the new chiral ligands due to the higher mDP and ratio of epicatechin units.

Then, MPPs were depolymerized with selective carbon and sulfur nucleophiles. The products were purified by column chromatography and characterized with ESI-MS/MS and NMR spectrometry. After screening the selective protecting reagents of the B-ring ortho-dihydroxyl groups, the depolymerized products were applied for one or two step reaction and finally sixteen chiral epicatechin derivatives were synthesized. X-ray single crystal analysis showed the there was unexpected decomposition in two sulfur-containing epicatechin derivatives under aerial conditions.

In order to overcome the decomposition, the A-type proplargonidins are rather worth of use and hence the proanthocyanidins from rhizomes of *Selliguea feei* (SFPs) were extracted with .2.43% yield (dry matter). NMR and ESI-MS spectra showed that SFPs were mainly A-type propelargonidin dimer and trimers. Then, stable tridentate and tetradentate sulfur ligands were synthesized together with one alkaloidal A-type propelargonidin. All products are stable to oxygen and weakly basic condition.

Kinetics of the dethiolation of three sulfur-containing epicatechin derivatives under weakly basic conditions were investigated and the mechanism of the base-catalyzed condensation was proposed. The results indicated that epicatechin derivative **8** is an ideal intermediate for synthesis of bioactive natural products such as procyanidin B2 and some rare alkaloidal flavonoid under very mild synthetic conditions.

7.2 Suggestions for further work

The encouraging findings obtained from this research work certainly serve as a solid starting point that could potentially branch out to various exciting areas.

Proanthocyanidins are ubiquitous constituents in plants and most byproducts generated by agricultural activities, forestry, and related industries contain proanthocyanidins, which have putative applications as food antioxidants and preventative agents against numerous diseases. New proanthocyanidins and a series of flavanol derivatives were obtained in this study, the bioactivities such as antitumor, antidiabetic activities shall be invested for these compounds in future work.

Meanwhile, some flavanol derivatives synthesized in our lab include ionic groups, which may be used to modulate their action within different physicochemical and biological environments [122]. Whether these products will exhibit same activities in biological environments, is a question remained to be answered by more *in vitro* and *in vivo* works in order to efficiently recover of bioactive species in mangosteen pericarps and rhizomes of *Selliguea feei* converted into high value-added products

In addition, these epicatechin derivatives may be with potential application such as multidentate chiral ligands for asymmetric reaction. Structurally, chiral ligands derived from MPPs have some similarity with BINOL, or some recently successful ligands. The effectiveness of epicatechin-type ligands in asymmetric reactions shall be examined in further work, in combination transition metals, especially Fe and (Ti (IV)) to get the ideal parameter with high enantioselectivities. For the nitrogencontaining ligands, it is also worth of performing some organocatalytic reaction following procedure of proline-based or cinchona alkaloid-based catalysis.

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Chapter 8

Experimental Procedures

8.1 Instruments and Reagents

^{1}H and ^{13}C NMR spectra were recorded on a Bruker ACF300 (300MHz) or AMX500 (500MHz) spectrometer. Circular dichroism (CD) spectra were measured on a JASCO J-725 spectrodichrometer using 1 mm quartz cells at 25^{o}. **1**, **2** and **11** were dissolve in methanol with sample concentration of 1.0mg/ml.Chemical shifts are reported in parts per million (ppm). The residual solvent peak was used as an internal reference. The electrospray ionization mass spectra (ESI-MS) were obtained from a Finnigan/MAT LCQ ion trap mass spectrometer (San Jose, CA) equipped with an ESI source. The heated capillary and voltage were maintained at 250 ^{o}C and 4.5 kV, respectively. The full-scan mass spectra from *m*/*z* 50 to 2000 were recorded. Samples were dissolved in methanol, and the solution was introduced into the ion spray source with a syringe (100 μL). Liquid chromatography/mass spectrometry (LC/MS) spectra were acquired using Finnigan/MAT LCQ ion trap mass spectrometer equipped with a TSP 4000 high-performance liquid chromatography (HPLC) system, which includes an UV6000LP photodiode array detector, P4000 quaternary pump, and AS3000 autosampler. The heated capillary and spray voltage were maintained at 250 ^{o}C and 4.5 kV, respectively. Nitrogen was operated at 80 psi for the sheath gas flow rate and 20 psi for the auxiliary gas flow rate. The full scan mass spectra from *m*/*z* 50 to 2000 were acquired in both positive and negative ion modes with a scan speed of 1 s/scan. Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectra were collected on an Applied Biosystems Voyager-DE STR mass spectrometer (Foster City, CA) equipped with delayed extraction and a N_2 laser set at 337 nm. The length of one laser pulse was 3 ns. The measurements were carried out using the following conditions: positive polarity, linear flight path with 21 kV acceleration

voltage, and 100 pulses per spectrum. The samples were dissolved in methanol (4 mg/ mL). Sodium chloride and 2, 5-dihydroxybenzoic acid as the matrix were used to enhance ion formation (*25*). An aqueous solution of sodium chloride (1.0 μL, 0.1 M) was added to the sample solution (1.0 mL) followed by addition of an equal volume of methanol solution of 2, 5- dihydroxybenzoic acid (10 mg/mL). The resulting solution (1.0 μL) was evaporated and introduced into the spectrophotometer. UV/vis spectra were recorded using a Shimadzu UK1601 spectrophotometer (Kyoto, Japan) fitted with a quartz cell. All high resolution mass spectra were obtained on a Finnigan/MAT 95XL-T spectrometer. Infrared spectra were recorded on a BIO-RAD FTS 165 FTIR spectrometer. Analytical thin layer chromatography (TLC) was performed with Merck pre-coated TLC plates (silica gel 60F-254, layer thickness 0.25 mm). Flash chromatography separations were performed on Merck 60 (0.040 - 0.063mm) mesh silica gel. Sephadex LH-20 was purchased from GE Healthcare BioSciences AB (Uppsala, Sweden).

All solvents used were of reagent grade unless otherwise specified. Ripe mangosteens with dark brown pericarps (cultivated in Malaysia) were purchased from a local market. The rhizomes of *Selliguea feei* (cultivated in Indonesia) were purchased from a local market. Commercial pine bark proanthocyanidins and grape seed proanthocyanidins were purchased from Chengdu SanHerb Plant Extract Co. Ltd (Chengdu, Sichuan, China). Benzyl mercaptan, methyl thioglycolate, 3-tert-Butyl-2-hydroxybenzaldehyde, 3-Methoxysalicylaldehyde, 3, 5-dimethoxyphenol 3, 5-dimethoxyaniline, cysteamine hydrochloride, 3,-ethyl-2,4-dimethylpyrrole, 2,3-dimethylpyrazole, 2-aminothiopheol, 1,2-ethanedithiol, (+)-catechin (98%), epicatechin, methyl propiolate, dichlorodiphenylmethane, 2-,3-,4-methoxyphenylboronic acid, boric acid, phenylboronic acid (≥97.0% HPLC) and

benzyl chloroformate (50% in toluene) (referred to as CbzCl) were purchased from Sigma- Aldrich Chemical Co. (St. Louis, MO). 4-Dimethylaminopyrid- ine (99%) was from Lancaster Synthesis Ltd (Morecambe, England). Potassium phosphate dibasic and triethylamine were from Fisher Scientific Company; Pyridine was purchased from Analar Chemicals; Oxo-titanium (IV) tert-butylated phthalo- cyanine was purchased from Dr. Kobayashi and Dr. Muranaka at Tokohu University. All chemical solvents were used without further purification.

8.2 Characterization of mangosteen pericarp proanthocyanidins.

8.2.1 Isolation, purification of mangosteen pericarp proanthocyanidins The mangosteen pericarps (2.0 kg, fresh) were ground and Soxhlet defatted with hexane (3 × 1500 mL). The remaining solids were subsequently extracted by a mixture of acetone/ water (7:3, 3 × 4000 mL) for 4 h. The mixture was filtered, and the filtrate was pooled. The acetone in the filtrate was evaporated to yield slurry, which was centrifuged at 3000*g* for 15 min. The supernatant was collected and liquid–liquid extracted with dichloromethane (3 × 500 mL) to further remove xanthones and other lipophilic compounds. The water phase was collected and concentrated to 60 mL. The crude proanthocyanidin fraction (20 mL) was filtered through a Sartorius Minisart 45 μm porosity filter (Epsom, United Kingdom) and then loaded on a Sephadex LH-20 column containing 50 g of LH-20 equilibrated with MeOH/water (1:1) for 4 h. The column was washed with MeOH/water (1:1) until the eluent turned colorless. The adsorbed proanthocyanidins were then eluted with aqueous acetone (70%, 500 mL). The acetone was removed on a rotary evaporator at 40 °C, and the resulting residue was freeze-dried to give a light brown powder (4.2 g overall yield). The moisture content in mangosteen was determined to be 68.3%, and thus, the yield of the

proanthocyanidins was 0.66% of dry matter. The "purity" measured by UV/vis colorimetric methods analysis showed that the extract contains over 99% (wt) epicatechin (standard) equivalents.

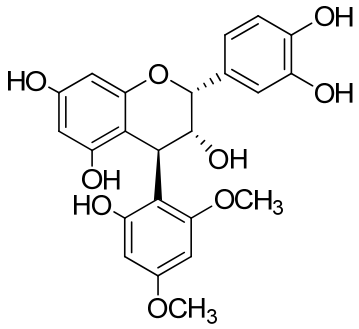
8.2.2 Thiolysis of mangosteen pericarp proanthocyanidins. This was done by following a reported method (*26*). In a small glass vial, proanthocyanidins solution (50 μL, 2.0 mg/mL in methanol) was mixed together with methanol acidified with concentrated HCl (50 μL, 3.3%, v/v) and 100 μL of benzyl mercaptan (5% v/v in methanol). The vial was sealed with an inert Teflon cap. The reaction was carried out at 40 °C for 30 min and then kept at room temperature for 10 h; then, the reaction mixtures were kept in the freezer (-20 °C) until 10 μL was injected directly for reverse-phase HPLC analysis. The thiolysis media were further analyzed using LC/MS with a Shimadzu 250 mm × 4.6 mm i.d., 5 μm C18 column (Kyoto, Japan). The binary mobile phases consisted of A (2% acetic acid in water, v/v) and B (methanol), which were delivered in a linear gradient of B from 15 to 80% (v/v) in 45 min. The flow rate was set at 1.0 mL/min.

8.3 Comparison among different proanthocyanidins

8.3.1 Extraction of cocoa proanthocyanidins. The dry cocoa powder (200 g) was Soxhlet defatted with hexane (3 × 1500 mL). The remaining solids were subsequently extracted by a mixture of acetone/ water (7:3, 3 × 4000 mL) for 4 h. The mixture was filtered, and the filtrate was pooled. The acetone in the filtrate was evaporated to concentrate to 60 mL. 60ml MeOH was added and then the crude proanthocyanidin fraction (20 mL) was filtered through a Sartorius Minisart 45 μm porosity filter (Epsom, United Kingdom) and then loaded on a Sephadex LH-20 column containing 100 g of LH-20 equilibrated with MeOH/water (1:1) for 4 h. The column was washed

with MeOH/water (1:1) until the eluent turned colorless. The adsorbed proanthocyanidins were then eluted with aqueous acetone (70%, 500 mL). The acetone was removed on a rotary evaporator at 40 °C, and the resulting residue was freeze-dried to give a brown powder (5.8 g overall yield).

8.3.2 Depolymerization of MPPs with 3,5-dimethoxyphenol. Under nitrogen atmosphere, 9 g mangosteen pericarp proanthocyanidins (MPPs) was mixed with dioxane/H_2O (2 00mL), hydrochloric acid (36%, 2 mL), and 3, 5-dimethoxyphenol (3 g). The mixture was heated at 65 °C for 12 h with stirring. The filtrate was neutralized with 0.1M $NaHCO_3$ to pH 7.0 before it was extracted with ethyl acetate. The combined organic fraction was dried over anhydrous sodium sulphate. Evaporation of the ethyl acetate gave dark brown residue, which was purified with column chromatography (silica gel, eluted with dichloromethane-methanol 8:1 and EtOAc-hexanes 2:1, respectively) to afford **1** as a light yellow solid (593mg, 6.2%).

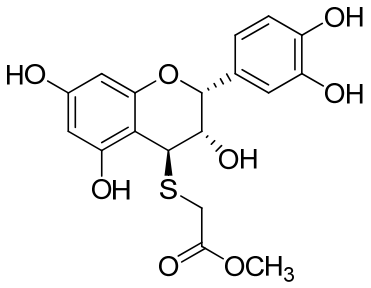


1

*(2R,3R,4S)-2-(3,4-dihydroxyphenyl)-4-(2-hydroxy-4,6-dimethoxyphenyl)-3,4-dihydro-2H-chromene-3,5,7-triol, (**1**).* MS (ESI, -c): 441 $[M-H]^-$. ^{1}H-NMR (300 MHz, acetone-d_6): δ = 6.98 (d, *J* = 1.8 Hz, 1H, C(2')-H), 6.75 (d, *J* = 8.0, 1H, C(6')-H), 6.72 (d, *J* = 1.8 Hz, 1H, C(5')-H), 5.97 (s, 1H, C(6)-H), 5.91 (m, 3H, C(8)-H, C(3'')-H, C(5'')-H), 5.04 (s, 1H, C(2)-H), 4.61 (s, 1H, C(4)-H), 3.91 (s, 1H, C(3)-H), 3.74 (s,

6H, OCH_3). $^{13}C\{^1H\}$NMR (75 MHz, acetone-d_6): δ = 161.18, 158.33-158.81, 146.06, 145.84, 133.10, 119.81, 116.15, 115.84, 96.79, 96.05, 92.85, 77.51, 73.35, 56.91, 55.95, 37.38. IR: 3391, 2938, 1615, 1516, 1466, 1361, 1282, 1202, 1146, 1092, 1056, 1018, 818, 632, 540 cm^{-1}. HRMS: calcd. for $C_{23}H_{21}O_9$ 441.1180; found 441.1190.

8.3.3 Synthesis of 2-((2R,3S,4S)-2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxychroman-4-ylthio)acetate 2 from MPPs. Under nitrogen atmosphere, the proanthocyanidins (1 g) was mixed with MeOH (2 00mL), hydrochloric acid (36%, 2 mL), and methyl thioglycolate (2ml). The mixture was heated at 65 °C for 12 h with stirring. The filtrate was neutralized with 0.1M $NaHCO_3$ to pH 7.0 before it was extracted with ethyl acetate. The combined organic fraction was dried over anhydrous sodium sulphate. Evaporation of the ethyl acetate gave dark brown residue, which was purified with column chromatography (silica gel, eluted with dichloromethane-methanol 9:1) to afford **2** as a light yellow solid (318mg, 31.8%).



2

2-((2R,3S,4S)-2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxychroman-4-ylthio)acetate. (**2**). HRMS calcd. for C18H18O8 23Na32S: 417.0615, found, 417.0621. ^1H NMR (300 MHz, CD_3OD): δ = 7.00 (d, J = 1.8 Hz, 1H, C(2')–H), 6.84 (dd, J1 = 1.8, J2 = 8.2, 1H, C(6')–H), 6.79 (d, J = 8.2 Hz, 1H, C(5')–H), 5.97 (d, J = 2.3 Hz, 1H, C(8)–H), 5.91 (d, J = 2.3 Hz, 1H, C(6)–H), 5.24 (s, 1H, C(2)–H), 4.12 (d, J = 2.1 Hz, 1H, C(4)–H), 4.05 (d, J = 2.1 Hz, 1H, C(3)–H), 3.75 (s, 3H, OCH3), 3.54 and 3.49 (two AB, J = 15.5 Hz,

2H, SCH2). $^{13}C\{1H\}$ NMR (75 MHz, CD3OD): δ = 173.5, 159.4, 159.0, 157.2, 145.9, 145.8, 131.9, 119.3, 116.0, 115.3, 99.4, 96.9, 95.8, 75.5, 71.5, 53.0, 44.4, 34.7. MS (ESI, -c): 393 $[M-H]^-$. IR (KBr): 3392, 2956, 2923, 2852, 1716, 1629, 1605, 1520, 1471, 1440, 1379, 1287, 1150, 825 cm^{-1}.

8.3.4 Gram-scale synthesis of 2 from mangosteen pericarps directly. To analyze the mean degree of polymerization (mDP) in different proanthocyanidins and use for further reaction, **2** was synthesized and purified from mangosteen pericarps directly. In a flask (2.0 l), the freeze-dried mangosteen pericarps powder (100 g) was mixed with methanol (1.0 l), hydrochloric acid (36%, 10 ml), and methyl thioglycolate (25 ml). The mixture was heated at 65 _C for 12 h with stirring. The filtrate was neutralized with 0.1 M $NaHCO_3$ to pH 7.0 before it was extracted with ethyl acetate (4 x 500 ml). The combined organic fraction was dried over anhydrous sodium sulphate. Evaporation of the ethyl acetate gave dark brown residue, which was purified with column chromatography (silica gel, eluented with dichloromethane–methanol 8:1 and EtOAc- hexanes 2:1, respectively) to afford 2 (4.5 g, 4.5%) as a light yellow highly hydroscopic and slightly air-sensitive solid.

8.4 Screening regioselective protecting reagents of epicatechin derivatives.

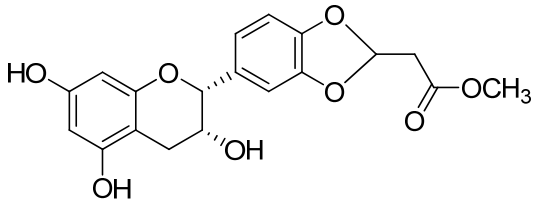
8.4.1 Reaction between (+)-Catechin and phenylboronic acid. (+)-Catechin (5 mg, 0.017 mmol) was dissolved in CD_3CN (0.75 mL) followed by addition of $PhB(OH)_2$ (2.1 mg, 0.017 mmol, 1 equiv). The mixture was shaken for 5 min and its ^1H NMR spectra was recorded 1 h later without further purification.

^1H NMR (300MHz, CD_3CN) Spectra showed downfield shift of peaks corresponding to catechin boronate ester product. Integration of peaks shows 50% conversion in the

first hour of reaction. δ 2.40 (1H, dd, *J* 16.3 and 8.2, C-$H_{4\beta}$), 2.52 (1H, dd, *J* 17.0 and 8.5, ester-$H_{4\beta}$), 2.76 (1H, dd, *J* 15.5 and 5.6, C-$H_{4\alpha}$), 2.89 (1H, dd, *J* 15.9 and 5.6, ester-$H_{4\alpha}$), 4.00 (1H, m, C-H_3), 4.10 (1H, m, ester-H_3), 4.57 (1H, d, *J* 7.7, C and ester-H_2), 4.77 (1H, d, *J* 8.1, ester-H_2), 5.98 (4H, m, C and ester-H_8, H_6), 6.88 (6H, m, C and ester-$H_{2'5'6'}$), 7.42 (5H, m, Ph). ESI-MS (-) m/z (%) 954.8 (12%) $[2C + M]^+$, 665.2 (100%) $[C + M]^+$, 375.4 (10%)$[M]^+$. However, the conversion did not increase with further increasing of reaction time. It seemed that the product is sensitive to water. Next, attempts to remove water were performed to increase the conversion and separate product.

8.4.2 Reaction between (+)-catechin and n-methoxyphenylboronic acid. This reaction type follows general procedure: (+)-Catechin (1 mg, 0.0034 mmol) was dissolved in acetone (400 μL) followed by the addition of 2-, 3-, or 4-methoxyphenylboronic acid (0.5 mg, 0.0034 mmol, 1 equiv). The reaction was allowed to stir for 1 day at room temperature. There is almost no reaction.

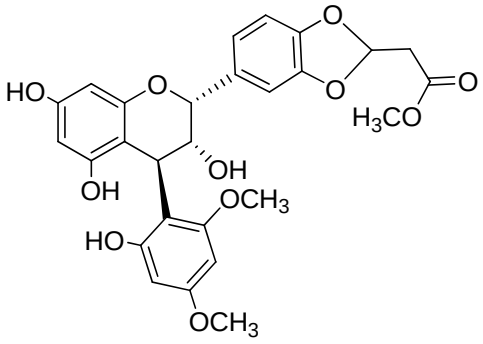
8.4.3 Reaction between epicatechin and methyl propiolate. Under nitrogen atmosphere, to a 10 mL CH_3CN solution of epicatechin (100 mg, 0.34 mmol) and 4-dimethylaminopyridine (DMAP) (63 mg, 0.52 mmol) was added methyl propiolate (34 μL, 0.38 mmol). The mixture was stirred at room temperature for 8 h. The volatiles were removed under reduced pressure and the residue was purified by column chromatography on silica gel (1:2 hexanes-EtOAc) to afford **3** (50 mg, 43%).



3

*methyl 2-(5-((2R,3R)-3,5,7-trihydroxychroman-2-yl)benzo[d][1,3]dioxol-2-yl)acetate, (**3**).* ^{1}H-NMR (300 MHz, CD_3OD): δ = 7.01 (s, 1H, C(2')-H), 6.94 (d, *J* = 8.1Hz, 1H, C(6')-H), 6.78 (d, *J* = 8.1 Hz, 1H, C(5')-H), 6.50 (dd, *J* = 5.2, 2.5 Hz, 0.5 H, C(9)-H), 6.48 (dd, *J* = 5.2, 2.5 Hz, 0.5H, C(9)-H), 5.95 (d, *J* = 2.2 Hz, 1H, C(8)-H), 5.92 (d, *J* = 2.2 Hz, 1H, C(6)-H), 5.48 (s, 1H, C(2)-H), 4.17 (s, 1H, C(3)-H), 3.73 (s, 3H, C(12)-3H), 3.00 (dd, *J* = 5.2, 1.8 Hz, 2H, C(10)-2H), 2.78 (dd, *J* = 16.9, 4.5 Hz, 1H, C(4α)-H), 2.74 (dd, *J* = 16.9, 2.6 Hz, 1H,C(4β)-H). ^{13}C NMR (75 MHz, CD_3OD): δ = 170.4 (C-11), 158.0, 157.7 (C-5 and C-7), 157.2 (C-8a), 148.3, 147.8 (C-3' and C-4'), 134.9 (C-1'), 121.2 (C-9), 109.4 (C-6'), 108.74, 108.67 (C-5' and C-2'), 100.0 (C-4a), 96.5 (C-6), 95.9 (C-8), 79.8 (C-2), 67.4 (C-3), 52.5 (C-12), 40.8 (C-10), 29.3 (C-4).MS (ESI, anionic mode), *m/z* 373 $[M-H]^-$. MS^2 (*m/z* = 137, RDA fragment).

8.4.4 Selective protection compound 1 with methyl propiolate. Under nitrogen atmosphere, to a 10 mL CH_3CN solution of **1** (88 mg, 0.2 mmol) and 4-dimethylaminopyridine (DMAP) (63 mg, 0.52 mmol) was added methyl propiolate (34 μL, 0.38 mmol). The mixture was stirred at room temperature for 8 h. The volatiles were removed under reduced pressure and the residue was purified by column chromatography on silica gel (10:1 DCM-MeOH) to give **4** as a light yellow solid.



4

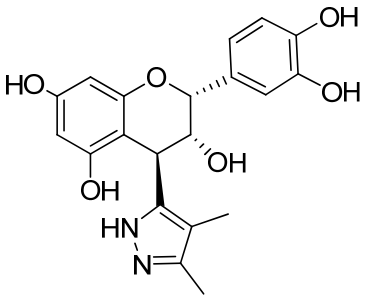
Methyl 2-(5-((2R, 3R, 4S)-3, 5, 7-trihydroxy-4-(2-hydroxy-4, 6-dimethoxyphenyl)chroman-2-yl)benzo[d][1, 3]dioxol-2-yl)acetate ***(4)*****: 4** was purified with column chromatography (silica gel, EtOAc- hexanes 3:2) as a white solid. MS (ESI, -c): 525 $[M-H]^-$. ^{1}H-NMR (300 MHz, acetone-d_6): δ = 7.06 (s, 1H, C(2')-H), 6.79 (d, J = 8.0, 1H, C(6')-H), 6.79 (d, J = 8.0 Hz, 1H, C(5')-H), 6.51 (t, J = 5.3 Hz, 1H, C(11)-H), 6.12 (s, 1H, C(6)-H), 6.01 (s, 3H, C(6)-H, C(3'')-H, C(5'')-H), 5.10 (s, 1H, C(2)-H), 4.62 (s, 1H, C(4)-H), 3.90 (s, 1H, C(3)-H), 3.72 (m, 9H, OCH3), 3.01 (dd, J_1 = 0.8, J_2 = 5.3, 2H, C(12)-H). ^{13}C{^{1}H}NMR (75 MHz, acetone-d_6): δ = 169.83, 162.83, 161.25, 158.21-159.42, 148.45, 147.88, 135.89, 121.74, 109.00-109.71, 96.82, 96.11, 92.81, 77.64, 73.34, 56.94, 55.96, 52.78, 41.09, 37.47. IR (KBr): 3415, 3001, 2953, 2843, 1736, 1619, 1498, 1465, 1442, 1363, 1316, 1251, 1203, 1174, 1148, 1094, 1049, 1038, 949, 857, 816, 789, 754, 535, 537, 497 cm^{-1}. HRMS: calcd. for $C_{27}H_{25}O_{11}$ 525.1391; found 525.1400.

8.5 Synthesis of epicatechin derivatives

8.5.1 General Procedure for the acid mediated depolymerization of proanthocyanidins in the presence of carbon and sulfur nucleophiles. Synthesis of the chiral ligands **5, 6, 7, 8, 9, 10** and **11**:

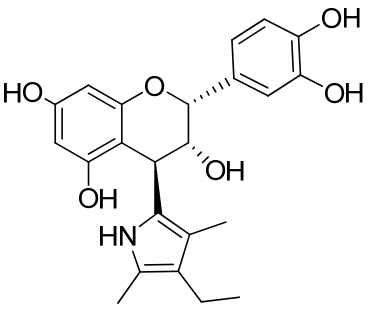
Under nitrogen atmosphere, the mangosteen proanthocyanidins (MPPs) (9.0 g) was mixed with MeOH for sulfur nucleophiles, or dioxane-H_2O (1:1) for carbon nucleophiles (2 00mL), hydrochloric acid (36%, 2 mL), and nucleophiles (5 equal). The mixture was heated at 50 °C for 8 hrs with stirring. The filtrate was neutralized with 0.1M $NaHCO_3$ to pH 7.0 before it was extracted with ethyl acetate. The combined organic fraction was dried over anhydrous sodium sulphate. Evaporation of the ethyl acetate gave dark brown residue, which was purified with column

chromatography (detailed conditions were described under individual compounds) to afford the chiral ligands **5, 6, 7, 8, 9, 10 and 11.**



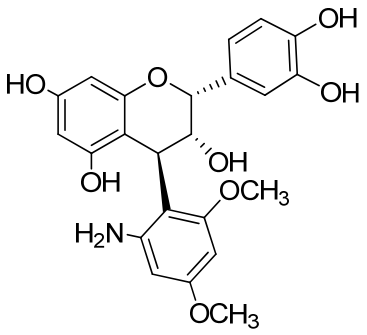
5

(2R, 3R, 4R)-2-(3', 4'-dihydroxyphenyl)-4-(3'', 4''-dimethyl-1H-pyrazol-5-yl)chroman-3, 5, 7-triol **(5): 5** was purified with column chromatography (silica gel, EtOAc- hexanes 2:1 and then dichloromethane-methanol 8:1) as a yellow solid. MS (ESI, -c): 383 $[M-H]^-$. ^{1}H-NMR (300 MHz, acetone-d_6): δ = 6.86(d, 1H, C(2')-H, J = 1.8), 6.73(d, 1H, C(5')-H, J = 8.6), 6.65(d, 1H, C(6')-H, J = 8.6), 6.04(d, 1H, C(6)-H, J = 2.3), 6.03(d, 1H, C(8)-H, J = 2.3), 5.34(d, 1H, C(2)-H, J = 2.3), 4.59(s, 1H, C(4)-H), 4.22(brs, 1H, C(3)-H), 2.18(s, 3H, -CH_3), 1.94(s, 3H, -CH_3). ^{13}C{^{1}H}NMR (75 MHz, acetone-d6): δ = 159.2, 158.4, 157.0, 147.4, 144.5, 129.6, 128.1, 117.8, 114.5, 113.8, 112.8, 95.7, 95.2, 94.3, 74.1, 69.5, 56.8, 10.0, 6.9. IR (KBr): 3368, 2969, 1619, 1519, 1448, 1283, 1154, 1109, 1075, 842, 795, 764, 668, 630, 535 cm^{-1}. HRMS: calcd. for $C_{20}H_{21}O_6N_2$ 385.1394; found 385.1400.



6

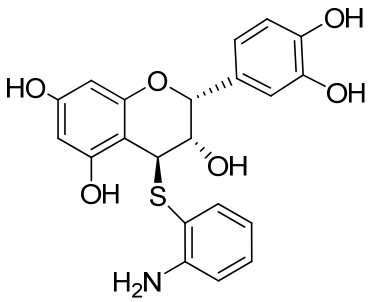
(2R, 3R, 4R)-2-(3, 4-dihydroxyphenyl)-4-(4-ethyl-3, 5-dimethyl-1H-pyrrol-2-yl)chroman-3, 5, 7-triol **(6): 6** was purified with column chromatography (silica gel, EtOAc- hexanes 2:1 and then dichloromethane-methanol 9:1) as a red solid. MS (ESI, -c): 410 $[M-H]^-$. ^{1}H-NMR (300 MHz, acetone-d_6): δ = 6.97 (s, 1H, C(2')-H), 6.76 (d, *J* = 8.2, 1H, C(6')-H), 6.71 (d, *J* = 8.2 Hz, 1H, C(5')-H), 6.00 (s, 1H, C(6)-H), 5.98 (s, 1H, C(8)-H), 4.81 (s, 1H, C(2)-H), 4.29 (s, 1H, C(4)-H), 3.98 (s, 1H, C(3)-H), , 2.36(dd, J_1= 7.3, J_2= 7.5, 2H, C(10)-H), 2.06(s, 3H, C(9)-H), 1.99(s, 3H, C(12)-H), 1.03(t, J= 7.5, 3H, C(11)-H). ^{13}C{^{1}H}NMR (75 MHz, acetone-d_6): δ = 157.41, 157.14, 156.64, 144.44, 144.25, 131.26, 125.45, 120.63, 120.03, 114.61, 111.77, 99.31, 95.67, 94.85, 74.91, 71.4, 37.15, 17.39, 15.30, 9.98, 8.55. IR (KBr): 3367, 2968, 1619, 1519, 1497, 1446, 1374, 1284, 1153, 1108, 1062, 1021, 822, 767, 672, 544 cm^{-1}.



7

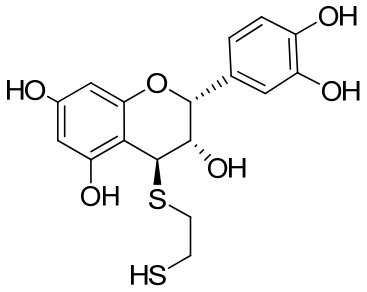
(2R, 3R, 4S)-4-(2-amino-4, 6-dimethoxyphenyl)-2-(3, 4-dihydroxyphenyl)chroman-3, 5, 7-triol **(7): 7** was purified with column chromatography (silica gel, EtOAc- hexanes 2:1 and then dichloromethane-methanol 9:1) as a light brown solid. MS (ESI, -c): 440 $[M-H]^-$. ^{1}H-NMR (300 MHz, acetone-d_6): δ = 6.98 (d, *J* = 1.8 Hz, 1H, C(2')-H), 6.75 (d, *J* = 8.0, 1H, C(6')-H), 6.72 (d, *J* = 1.8 Hz, 1H, C(5')-H), 5.97 (s, 1H, C(6)-H), 5.91 (m, 3H, C(8)-H, C(3'')-H, C(5'')-H), 5.04 (s, 1H, C(2)-H), 4.61 (s, 1H, C(4)-H), 3.91 (s, 1H, C(3)-H), 3.74 (s, 6H, OCH_3). ^{13}C{^{1}H}NMR (75 MHz, acetone-d_6): δ = 159.6 (2C, C-2'', C-6''), 155.6-156.1 (4C, C-5, C-7, C-8a, C-4''), 144.1, 143.8 (C-3', C-4'),

131.0 (C-1'), 127.9 (C-6'), 118.4 (C-5'), 114.7 (C-2'), 113.9 (C-4a), 95.2-94.2 (4C, C1'', C5'', C6, C8), 89.1 (C-3''), 76.4 (C-2), 71.9 (C-3), 55.4 (C-10), 54.5 (C-9), 36.1 (C-4). IR (KBr): 3368, 2938, 2841, 1607, 1516, 1465, 1341, 1283, 1244, 1204, 1150, 1116, 1091, 1061, 1018, 933, 821, 792, 667, 632, 542, 494 cm^{-1} HRMS: calcd. for $C_{23}H_{22}O_8N$ 440.1351; found 440.1340.



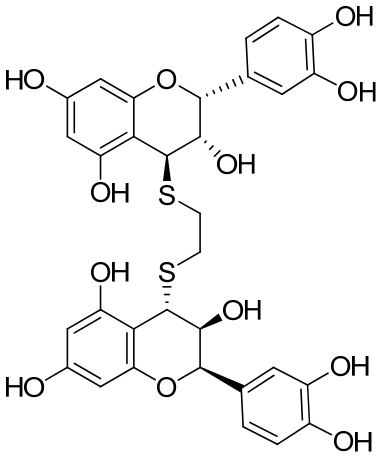
8

(2R, 3S, 4S)-4-(2-aminophenylthio)-2-(3, 4-dihydroxyphenyl)chroman-3, 5, 7-triol **(8):** **8** was purified with column chromatography (silica gel, EtOAc- hexanes 2:1 and then dichloromethane-methanol 8:1) as a yellow solid. MS (ESI, -c): 412 $[M-H]^-$. ^{1}H-NMR (300 MHz, acetone-d_6): δ = 7.41 (dd, J= 1.7, 1.7, 1H, C(13)-H), 6.77 (d, J = 1.7, 1H, C(2')-H), 7.12 (dd, J= 0.7, 1.7, 1H, C(11)-H), 6.94 (dd, J = 1.5, 1.7, 1H, C(12)-H), 6.86 (m, 2H, C(5')-H, C(6')-H), 6.57 (m, C(10)-H), 6.60 (d, J= 2.3, 1H, C(6)-H), 6.00 (d, J= 2.3, 1H, C(8)-H), 5.66 (s, 1H, C(2)-H), 4.34 (s, 1H, C(4)-H), 3.94 (s, 1H, C(3)-H). ^{13}C{^{1}H}NMR (75 MHz, acetone-d_6): δ = 159.80, 159.32, 158.38, 152.29, 146.20, 146.04, 138.74, 132.78, 132.01, 119.98, 118.06, 116.68, 116.33, 116.13, 115.98, 99.91, 97.36, 996.65, 75.79, 71.12, 45.25. IR (KBr): 3360, 1692, 1607, 1517, 1476, 1447, 1367, 1283, 1187, 1148, 1092, 1062, 1040, 1017, 973, 852, 821, 787, 754, 704, 671, 537 cm^{-1}.



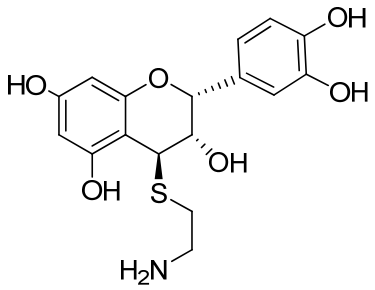
9

(2R, 3S, 4S)-2-(3, 4-dihydroxyphenyl)-4-(2-mercaptoethylthio)chroman-3, 5, 7-triol **(9). 9** was purified with column chromatography (silica gel, EtOAc- hexanes 2:1 and then dichloromethane-methanol 9:1) as a red solid. MS (ESI, -c): 381 $[M-H]^-$. ^{1}H-NMR (300 MHz, CD_3OD): δ = 6.99 (d, J = 1.65 Hz, 1H, C(2')-H), 6.83 (dd, J_1 = 1.65 Hz, J_2 = 8.0 Hz, 1H, C(6')-H), 6.78 (d, J = 8.0 Hz, 1H, C(5')-H), 5.96 (d, J = 2.31 Hz, 1H, C(8)-H), 5.90 (d, J = 2.31 Hz, 1H, C(6)-H), 5.26 (s, 1H, C(2)-H), 4.02 (d, J = 2.31 Hz, 1H, C(4)-H), 3.98 (dd, J_1 = 0.9 Hz, J_2 = 2.31 Hz, 1H, C(3)-H), 2.80-3.08 (m, 4H, SCH_2CH_2S). $^{13}C\{^1H\}$NMR (75 MHz, CD_3COCD_3): δ = 159.17, 158.67, 157.34, 145.76, 145.68, 132.19, 119.62, 115.84, 115.63, 100.20, 96.90, 95.93, 75.56, 72.04, 43.32, 37.32, 25.88. IR (KBr): 3369, 1626, 1516, 1470, 1280, 1146, 1091, 1059, 1016, 852, 821, 783 cm^{-1}. HRMS: calcd. for $C_{17}H_{17}O_6{}^{32}S_2$ 383.0618; found 383.0599.



10

(2R, 2'R, 3S, 3'S, 4S, 4'S)-4, 4'-(ethane-1, 2-diylbis(sulfanediyl))bis(2-(3, 4-dihydroxyphenyl)chroman-3, 5, 7-triol) **(10). 10** was purified with column chromatography (silica gel, dichloromethane-methanol 5:1) as a red solid. MS (ESI, -c): 669 $[M-H]^-$. ^{1}H-NMR (300 MHz, CD_3OD): δ = 7.01 (d, *J* = 1.8 Hz, 2H, C(2')-H), 6.83 (dd, J_1 = 1.8 Hz, J_2 = 8.0 Hz, 2H, C(6')-H), 6.78 (d, *J* = 8.0 Hz, 2H, C(5')-H), 5.96 (s, 2H, C(8)-H), 5.91 (s, 2H, C(6)-H), 5.28 (s, 2H, C(2)-H), 4.60, (s, 2H, C(4)-H), 4.05 (s, 2H, C(3)-H), 3.11 (dq, *J* = 15.6 Hz, 4H, SCH_2CH_2S). ^{13}C NMR (75 MHz, CD_3OD): δ = 159.08, 158.8, 157.16, 146.00, 145.81, 132.05, 119.36, 116.05, 115.33, 100.30, 96.85, 95.75, 75.63, 72.26, 43.68, 33.94. IR (KBr): 3392, 1610, 1519, 1445, 1373, 1283, 1148, 1099, 1062, 820 cm^{-1}. HRMS: calcd. for $C_{32}H_{29}O_{12}{}^{32}S_2$: 669.1106, found, 669.1107.



11

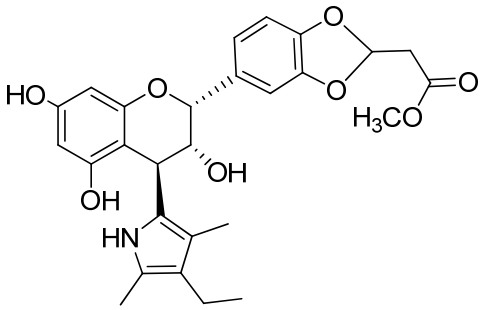
(2R, 3S, 4S)-4-(2-aminoethylthio)-2-(3, 4-dihydroxyphenyl)chroman-3, 5, 7-triol **(11):** **11** was purified with reversed phased column chromatography (gradient elution of $MeOH/H_2O$ (1:4, v/v), 0.4% aq. AcOH/MeOH (4:1, v/v), 0.4% aq. AcOH/MeOH (1:1, v/v),). The pure fractions were combined, neutralized using pH 6.5 Na_2HPO_4 buffer, and then concentrated. After extraction with acetone, the extract was dried in vacuum to furnish **15** as a red solid. MS (ESI, +c): 366 $[M+H]^+$. ^{1}H-NMR (300 MHz, D_2O): δ = 6.92 (d, 1H, C(2')-H), 6.82 – 6.77 (m, 2H, C(5')-H, and C(6')-H), 6.01 (d, *J* = 2.3 Hz, 1H, C(8)-H), 5.95 (d, *J* = 2.3 Hz, 1H, C(6)-H), 5.14 (s, 1H, C(2)-H), 4.00 (d, *J* =

2.3 Hz, 1H, C(4)-H), 3.86 (d, J = 2.3 Hz, 1H, C(3)-H), 3.30 – 2.80 (m, 4H, S-C$\underline{H}_2$-C$\underline{H}_2$-N); $^{13}C\{^1H\}$-NMR (75 MHz, D_2O): δ = 156.6, 156.2, 155.1, 143.7, 143.5, 130.3, 118.8, 115.9, 114.2, 98.7, 96.1, 95.2, 73.9, 70.1, 40.6, 38.4, 28.7. IR (KBr): 3352, 3189, 1620, 1519, 1468, 1384, 1284, 1192, 1150, 1094, 1063.

Conversion analysis. In a small glass vial, proanthocyanidins solution (50 μL, 2.0 mg/mL in methanol) was mixed together with methanol acidified with concentrated HCl (50 μL, 3.3%, v/v) and 100 μL of nucleophiles (5% v/v in methanol). The vial was sealed with an inert Teflon cap. The reaction was carried out at 40 °C for 30 min and then kept at room temperature for 10 h; then, the reaction mixtures were kept in the freezer (-20 °C) until 10 μL was injected directly for reverse-phase HPLC analysis. The depolymerization media were further analyzed using HPLC system with a Shimadzu 250 mm × 4.6 mm i.d., 5 μm C18 column (Kyoto, Japan). The binary mobile phases consisted of A (2% acetic acid in water, v/v) and B (methanol), which were delivered in a linear gradient of B from 15 to 80% (v/v) in 45 min. The flow rate was set at 1.0 mL/min.

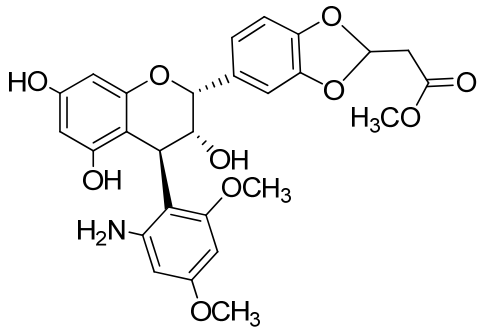
8.5.2 General Procedure for selective protection of the ortho-dihydroxyl groups in 6 and 7. Synthesis of the chiral ligands **12** and **13**:

Under nitrogen atmosphere, a 50 mL acetonitrile solution of **6** or **7** (1mmol) and methyl propiolate (1.1 mmol) was added 4-N-dimethylaminopyridine (DMAP) (1.5mmol). The mixture was stirred at room temperature for 8h. The volatiles were removed under reduced pressure and the residue was purified by column chromatography on silica gel to afford the chiral ligands **12 and 13**.



12

Methyl 2-(5-((2R, 3R, 4R)-4-(4-ethyl-3, 5-dimethyl-1H-pyrrol-2-yl)-3, 5, 7-trihydroxychroman-2-yl)benzo[d][1, 3]dioxol-2-yl)acetate **(12), 12** was purified with column chromatography (silica gel, dichloromethane-methanol 13:1) as a red solid. MS (ESI, -c): 494 $[M-H]^-$. ^{1}H-NMR (300 MHz, acetone-d_6): δ = 7.02 (s, 1H, C(2')-H), 6.77 (s, 2H, C(5')-H, C(6')-H), 6.50 (t, J= 5.3, 1H, C(13)-H), 6.01 (d, J= 2.4, 1H, C(6)-H), 5.97(d, J= 2.4, 1H, C(8)-H), 4.85 (s, 1H, C(2)-H), 4.29 (s, 1H, C(4)-H), 3.97 (s, 1H, C(3)-H), 3.65(s, 3H, C(16)-H), 3.04 (dd, J_1= 1.5, J_2= 3.8, 2H, C(14)-H), 2.35 (dd, J_1 = 7.5, J_2 = 7.5, 2H, C(10)-H), 2.06(s, 3H, C(9)-H), 1.98 (s, 3H, C(12)-H), 1.29 (s, 3H, C(11)-H). ^{13}C{^{1}H}NMR (75 MHz, acetone-d_6): δ = 164.5, 151.4, 152.6, 148.7, 131.8, 129.9, 122.7, 121.8, 110.1, 109.3, 109.2, 97.7, 96.6, 92.6, 83.0, 75.5, 52.8, 41.1, 25.6, 20.8, 13.8, 8.8, 5.9. IR (KBr): 3332, 2973, 2934, 1694, 1497, 1440, 1376, 1314, 1252, 1153, 1106, 1048, 991, 840, 765, 698, 633, 546 cm^{-1}.

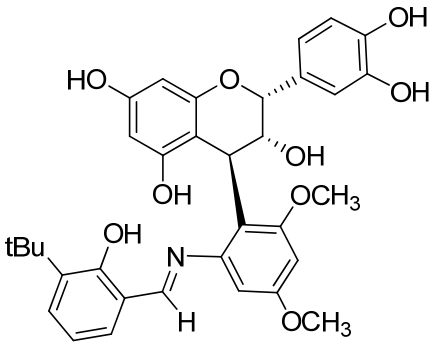


13

Methyl 2-(5-((2R, 3R, 4S)-4-(2-amino-4, 6-dimethoxyphenyl)-3, 5, 7-trihydroxychroman-2-yl)benzo[d][1, 3]dioxol-2-yl)acetate **(13): 13** was purified with column chromatography (silica gel, dichloromethane-methanol 11:1) as a yellow solid. MS (ESI, -c): 524 $[M-H]^-$. ^{1}H-NMR (300 MHz, acetone-d_6): δ = 6.95 (s, 1H, C(2')-H), 6.76 (d, J = 8.1, 1H, C(6')-H), 6.74 (d, J = 8.1 Hz, 1H, C(5')-H), 6.47 (m, 1H, C(11)-H), 6.02 (s, 1H, C(6)-H), 6.01 (s, 1H, C(6)-H), 5.96 (C(3'')-H), 5.82(C(5'')-H), 5.07 (s, 1H, C(2)-H), 4.59 (s, 1H, C(4)-H), 3.81 (s, 4H, C(3)-H, C(13)-H), 3.72 (s, 6H, C(14)-H, C(15)-H), 2.98 (dd, J_1 = 2.1, J_2 = 3.0, 2H, C(12)-H). ^{13}C{^{1}H}NMR (75 MHz, acetone-d_6): δ = 169.81, 161.06 , 158.99, 158.03, 157.75, 157.60, 148.40, 147.83, 135.75, 121.63, 109.67, 109.61, 109.46, 108.98, 96.96, 96.45, 96.21, 95.85, 77.28, 73.82, 55.72, 52.78, 41.04, 36.84. IR (KBr): 3368, 2936, 2841, 1731, 1607, 1497, 1440, 1236, 1202, 1147, 1036, 812, 788, 753, 631, 537 cm^{-1}. HRMS: calcd. for $C_{27}H_{26}O_{10}N$ 524.1562; found 524.1572.

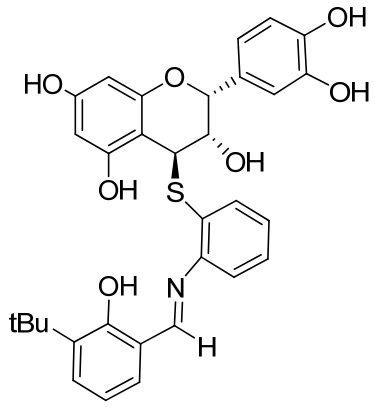
8.5.3 General Procedure the preparation of Schiff base of compounds 7, 8, 9 and 13. Synthesis of the chiral ligands **14, 15, 16, 17 and 18.**

To a solution of **7, 8, 9 or 13** (1 mmol) in MeOH (5 mL) was successively added 3-tert-Butyl-2-hydroxybenzaldehyde (1.1 mmol) and one drop of acetic acid only for **9**. The reaction mixture was refluxed for 8 h and the solvent was then removed under reduced pressure. The residue was purified by further to afford the multidentate chiral ligands **14, 15, 17 and 18**. One more reaction between **8** and 3-methyloxy-2-hydroxybenzaldehyde was performed following the same procedure to afford the Schiff base **16**.



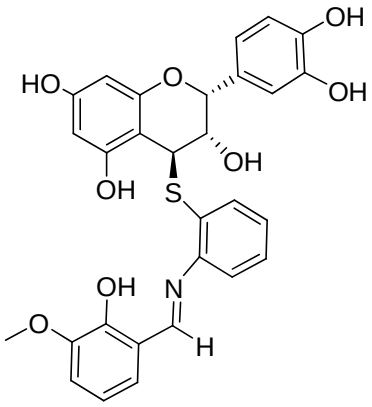
14

*(2R,3R,4S)-4-(2-((E)-3-tert-butyl-2-hydroxybenzylideneamino)-4,6-dimethoxyphenyl)-2-(3,4-dihydroxyphenyl)chroman-3,5,7-triol (**14**).* MS (ESI, -c): 600 $[M-H]^-$. ^{1}H-NMR (300 MHz, acetone-d_6): δ = 8.78 & 8.13 (2H, C(11)-H), 7.72 (m, 10H, -OH), 7.40 (m, 2H, C(12)-H), 7.23 & 7.07 (2H, C(14)-H), 7.04 & 6.98 (2H, C(13)-H), 6.89 (m, 2H, C(2')-H), 6.73 (m, 4H, C(5', 6')-H), 6.49 (2H, C(9)-H), 6.00 (m, 4H, C(10, 6)-H), 5.73 (2H, C(8)-H), 5.25 & 5.09 (2H, C(2)-H), 4.73 & 4.63 (2H, C(4)-H), 4.04 & 3.85 (m, 14H, C(3)-H, -OCH_3), 1.40 (18H, -tBu). ^{13}C{^{1}H}NMR (75 MHz, acetone-d_6): δ = 166.5 & 165.2, 161.5 & 161.3, 161.2 & 161.0, 158.3 & 158.1, 157.8 & 157.6, 153.4 & 151.5, 146.0, 145.8 & 145.7, 138.5 & 138.0, 133.3, 132.7 & 132.6, 131.6 & 131.0, 121.0 & 120.8, 119.9, 119.3, 116.2 & 116.1, 116.0, 104.1 & 103.3, 100.8 & 100.7, 99.5, 97.5 & 97.1, 96.4 & 95.9, 77.4 & 77.3, 73.7 & 73.6, 57.4 & 57.0, 56.4 & 56.3, 39.2 & 38.8, 36.0 & 35.9, 31.2. IR (KBr): 1599, 1576, 1517, 1458, 1433, 1363, 1310, 1280, 1199, 1145, 1092, 1018, 974, 876, 856, 823, 795, 751, 668, 630, 587, 540 cm^{-1}.



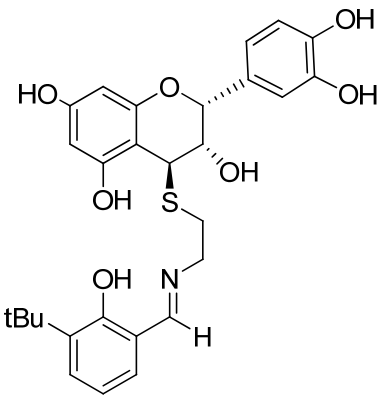
15

(2R, 3S)-4-(2-((E)-3-tert-butyl-2-hydroxybenzylideneamino)phenylthio)-2-(3, 4-dihydroxyphenyl)chroman-3, 5, 7-triol **(15): 15** was purified with column chromatography (silica gel, dichloromethane-methanol 12:1) as a yellow solid. MS (ESI, -c): 572 $[M-H]^-$. ^{1}H-NMR (300 MHz, acetone-d_6): δ =.8.85(s, 1H, C(13)-H), 7.92(d, J=2.6, 1H, C(14)-H), 7.43(m, 3H, C(9, 10, 16)-H), 7.36(m, 2H, C(11, 12)-H), 7.12(s, 1H, C(2')-H), 6.92(t, 1H, C(15)-H), 6.79(d, J=8.1, 1H, C(5')-H), 6.71(d, J=8.1, 1H, C(6')-H, 6.15(d, J=2.2, 1H, C(6)-H), 6.03(d, J=2.2, 1H, C(8)-H), 5.60(s, 1H, C(2)-H), 4.82(s, 1H, C(4)-H), 4.01(s, 1H, C(3)-H), 1.44(s, 9H, tBu). ^{13}C{^{1}H}NMR (75 MHz, acetone-d_6): δ = 164.12, 158.43, 157.85, 156.80, 147.32, 144.48, 137.05, 130.78, 129.79, 122.31, 118.62, 114.55, 114.54, 97.31, 95.91, 94.91, 74.67, 65.97, 44.85, 34.49, 29.65. IR (KBr): 3367, 2957, 1697, 1607, 1520, 1441, 1364, 1282, 1197, 1146, 1100, 1061, 1018, 973, 855, 821, 795, 751, 670, 540 cm^{-1}. HRMS: calcd. for $C_{32}H_{30}O_7N^{32}S$ 572.1748; found 572.1729. 1017, 988, 888, 825, 783, 656, 540, 482 cm^{-1}.



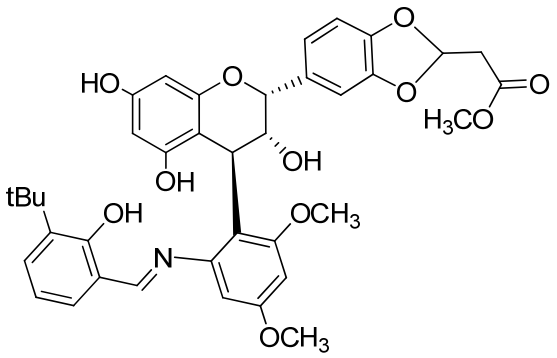
16

(2R,3S,4S)-2-(3,4-dihydroxyphenyl)-4-(2-((E)-2-hydroxy-3-methoxybenzylideneamino)phenylthio)chroman-3,5,7-triol **(16): 16** was purified with column chromatography (silica gel, dichloromethane-methanol 11:1) as a yellow solid. MS (ESI, -c): 546 $[M-H]^-$. ^{1}H-NMR (300 MHz, acetone-d_6): δ =.7.96(s, 1H, C(13)-H), 7.44(d, J=2.6, 1H, C(9)-H), 7.14(m, 2H, C(10, 11)-H), 6.85(m, 2H, C(12, 14)-H), 6.85(s, 1H, C(2')-H), 6.82(s, 1H, C(16)-H), 6.70(m, 1H, C(15)-H), 6.62(m, 1H, C(5')-H), 6.52(t, J = 8.1, 1H, C(6')-H, 6.12(d, J=2.2, 1H, C(6)-H), 5.98(d, J=2.2, 1H, C(8)-H), 4.52(s, 1H, C(2)-H), 4.35(s, 1H, C(4)-H), 3.96(s, 1H, C(3)-H), 3.85(s, 3H, -OCH_3). ^{13}C{^{1}H}NMR (75 MHz, acetone-d_6): δ = 161.8, 158.2, 157.6, 156.7 150.1, 143.6, 137.1, 130.6, 125.6, 115.7, 114.4, 110.2, 109,7, 95.7, 95., 74.2, 69.6, 55.37, 43.9, 41.2, 35.2. IR (KBr): 1728. 1017, 988, 540, 481 cm^{-1}.



17

(2R, 3S, 4S)-4-(2-((E)-3-tert-butyl-2-hydroxybenzylideneamino)ethylthio)-2-(3, 4 dihydroxyphenyl)chroman-3, 5, 7-triol **(17): 17** was purified via washing with hexane as a yellow solid. MS (ESI, +c): 526 $[M-H]^+$. ^{1}H-NMR (300 MHz, $(CD_3)_2CO$): δ = 8.59 (s, 1H, C(13)-H), 7.63 – 7.23 (m, 2H, C(6'')-H and C(4'')-H), 7.11 (s, 1H, C(5'')-H), 6.87 – 6.79 (m, 3H, C(6')-H, C(5')-H and C(2')-H), 6.05 (s, 1H, C(8)-H), 5.92 (s, 1H, C(6)-H), 5.34 (s, 1H, C(2)-H), 4.18 (d, J = 2.1 Hz, 1H, C(4)-H), 4.12 (s, 1H, C(3)-H), 3.87 (m, 2H, C(11)-H), 3.21 – 3.00 (m, 2H, C(10)-H), 1.42 (s, 9H, $(CH_3)_3$). ^{13}C{^{1}H}-NMR (75 MHz, $(CD_3)_2CO$): δ = 167.4, 157.9, 157.4, 156.2, 144.5, 144.4, 136.6, 131.0, 130.0, 118.8, 118.4, 117.8, 114.6, 114.4, 99.0, 95.6, 94.7, 74.4, 70.7, 58.5, 42.1, 34.3, 32.7. IR (KBr): 3370, 2957, 2739, 1703, 1610, 1518, 1437, 1375, 1281, 1198, 1145, 1092, 1062, 823, 753, 679, 545, 483 cm^{-1}.



18

Methyl 2-(5-((2R, 3R, 4S)-4-(2-((E)-3-tert-butyl-2-hydroxybenzylideneamino)-4, 6-dimethoxyphenyl)-3, 5, 7-trihydroxychroman-2-yl)benzo[d][1, 3]dioxol-2-yl)acetate **(18): 18** was purified via washing with hexane as a yellow solid. MS (ESI, -c): 684 $[M-H]^-$. ^{1}H-NMR (300 MHz, acetone-d6): δ = 8.96 (s, 1H, C(16)-H), 7.43 (s, 1H, C(18)-H), 7.40 (s, 1H, C(20)-H), 7.08 (s, 1H, C(2')-H), 6.90 (t, J = 7.7, C(19)-H), 6.76 (m, 3H, C(5'), C(6'), C(13)-H), 6.51(t, J = 2.3, 2H, 2H, C(11), C(23)-H), 6.00 (d, J = 2.3, 1H, C(6)-H), 5.96 (d, J = 2.3, 1H, C(8)-H), 5.18(C(2)-H), 4.75(C(4)-H), 3.89

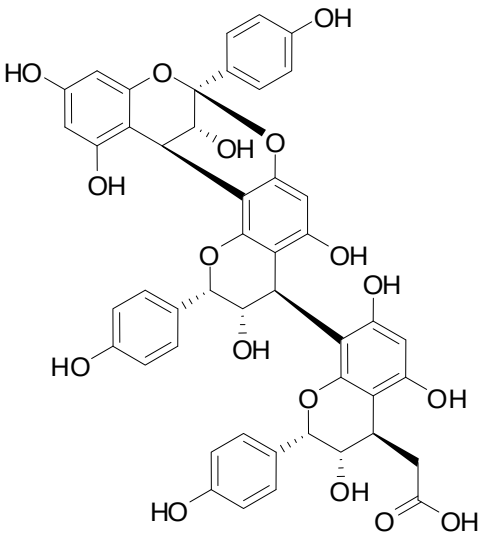
(s, 1H, C(3)-H), 3.84 (s, 3H, -OCH_3), 3.70 (s, 6H, -OCH_3), 3.01 (d, J = 5.3, C(24)-H), 1.46 (s, 9H, -tBu). $^{13}C\{1H\}$NMR (75 MHz, acetone-d6): δ = 168.13, 163.74, 160.30, 156.44, 156.17, 147.82, 146.92, 145.03, 134.48, 131.02, 130.00, 120.03, 118.30, 108.3, 107.87, 107.32, 94.74, 94.27, 75.96, 71.65, 55.78, 51.10, 39.43, 36.01, 34.42, 31.16. IR (KBr): 3368, 2936, 2841, 1731, 1607, 1497, 1440, 1236, 1202, 1147, 1036, 812, 788, 753, 631, 537 cm^{-1}. HRMS: calcd. for $C_{41}H_{36}O_8N_2$ 684.2477; found 684.2481.

8.6 Characterization of proanthocyanidins from the rhizomes of *Selliguea feei* and synthesis of epiafzelechin derivatives

8.6.1 Extraction and characterization of proanthocyanidins from the rhizomes of *Selliguea feei*

The rhizomes of Selliguea feei (200 g) were meshed and Soxhlet defatted with hexane (500 mL x 2). Condensed tannins were subsequently extracted from the residue by a mixture of acetone/water (7:3, 4000 mL x 3) for 4 hours. The mixture was filtered and the filtrate was pooled. The acetone in the filtrate was evaporated to yield slurry, which was centrifuged at 3000g for 15 min. The supernatant was collected and liquid-liquid extracted with dichloromethane (3 x 200 mL) to further remove other lipophilic compounds. The water phase was collected and concentrated to 40 mL. The crude proanthocyanidin fraction (20 mL) was filtered through a 45 micron porosity filter (Minisart) and then loaded on a Sephadex LH-20 column (50 grams of LH-20, equilibrated with MeOH/water (1:1) for 4h). The column was washed with MeOH / water (1:1) until the eluent turned colorless. The adsorbed proanthocyanidins were then eluted with aqueous acetone (70%, 500 mL). The acetone was removed on a rotary evaporator at 40 ^oC and the resulting residue freeze-dried to give a light brown

powder (3.93 g overall yield from the 200 g fresh the rhizomes of Selliguea feei). A portion (1.0g) of the OPCs was applied over sequential silica gel columns, eluting with dichloromethane–methanol (3: 1) and acetone-dichloromethane-hexane (5: 1: 1), respectively, to afford **21** (320mg). We named it as Selligueain C. This trimer was identified by comparison of its spectral data (UV, IR, ^{1}H and ^{13}C NMR) with those of published data [166].

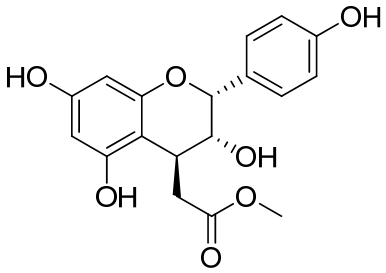


21

*Epiafzelechin-(4β→8, 2β→O→7)-epiafzelechin-(4β→8)-3'-deoxydryopteric acid (**21**),* MS (ESI, -c): 873 $[M-H]^{-}$. ^{1}H NMR (300 MHz, acetone-d_6): δ = 7.76(s, 1H, C(9'')–H),7.73(s, 1H, C(12'')–H), 7.40(s, 1H, C(9)–H), 7.37(s, 1H, C(12)–H), 7.34(s, 1H, C(9')–H), 7.32(s, 1H, C(12')–H), 6.89(m, 6H, C(10,11,10',11',10'',11'')–H), 6.22(s, 1H, C(6')–H), 6.01(d, J=2.4, 1H, C(8)–H), 5.93(d, J=2.4, 1H, C(6)–H), 5.85(s, 1H, C(6'')–H), 5.79(s, 1H, C(2')–H), 4.65(s, 1H, C(4'')–H), 4.56(s, 1H, C(4')–H), 4.18(s, 1H, C(3')–H), 3.98(d, J=3.3, 1H, C(4)–H), 3.73(s, 1H, C(2'')–H), 3.47(M, 1H, C(3'')–H), 3.20(d, J = 3.3, 1H, C(3'')–H), 3.03 and 2.29 (two dd, J_1 = 3.5, J_2 = 12, 2H, -CH_2). $^{13}C\{^{1}H\}$ NMR (75 MHz, acetone-d_6): δ = 174.80, 158.88, 158.80, 158.23, 158.05, 157.55, 156.40, 156.06, 155.92, 154.40,

151.91, 151.44, 132.47, 132.36, 131.58, 131.45, 130.00, 129.86, 129.75, 129.43, 116.45, 116.05, 115.88, 108.90, 106.81, 106.64, 104.99, 103.61, 100.28, 98.65, 97.35, 96.77, 96.40, 79.12, 76.50, 72.53, 70.60, 67.46, 40.35, 38.96, 37.00, 29.17. IR (KBr): 3368, 1898, 1703, 1615, 1518, 1448, 1370, 1234, 1174, 1144, 1119, 1096, 1009, 902, 835, 799, 609, 561 cm^{-1}.

Thiolysis for 21. In a flask (100 ml), the purified **21** (300mg) was mixed with methanol (30 ml), hydrochloric acid (36%, 0.3 ml), and methyl thioglycolate (0.3 ml). The mixture was heated at 65℃ for 12 h with stirring. The filtrate was neutralized with 0.1 M $NaHCO_3$ to pH 6.5 before it was extracted with ethyl acetate (4 × 50 ml). The combined organic fraction was dried over anhydrous sodium sulphate. Evaporation of the ethyl acetate gave dark brown residue, which was applied over sequential silica gel columns, eluting with ethyl acetate-hexane (2: 1) and dichloromethane–methanol (9:1), respectively, to afford methyl ester of terminal unit **22** (44 mg) as a light brown solid.



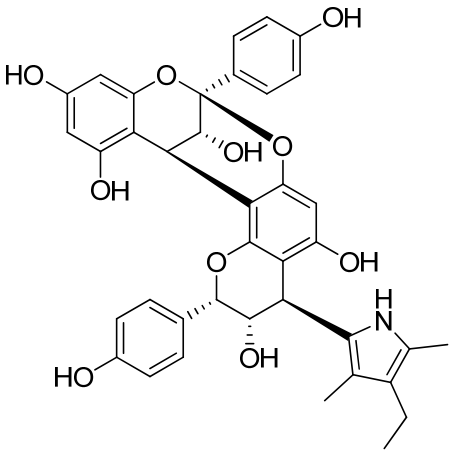
22

*Methyl 2-((2R, 3R, 4S)-3, 5, 7-trihydroxy-2-(4-hydroxyphenyl)chroman-4-yl)acetate (**2**):* MS (ESI, -c): 345 $[M-H]^-$. ^1H NMR (300 MHz, acetone-d_6): δ = 7.39 (d, *J* = 8.5 Hz, 2H, C(2', 6')–H), 7.37 (d, J = 8.5 Hz, 2H, C(3', 5')–H), 6.04 (d, J = 2.3, 1H, C(6)–H), 5.94 (d, J = 2.3 Hz, 1H, C(8)–H), 4.96 (s, 1H, C(2)–H), 3.94(d, J = 4.7 Hz, 1H, C(4)–H), 3.74 (d, J = 5.7 Hz, 1H, C(3)–H), 3.67 (s, 3H, C(11)–H), 3.06 and 2.52

(two dd, J_1 = 12.5 Hz, J_2 = 3.6 Hz, 2H, C(9)–H). $^{13}C\{1H\}$ NMR (75 MHz, acetone-d_6): δ = 172.2 (C-10), 157.0 (C-5), 156.9 (C- 7), 156.7 (C-8a), 156.0(C-4), 130.3(C-1'), 128.2 (C-2' and C-6'), 114.6 (C-3' and C-5'), 101.5 (C-4a), 95.5 (C-6), 94.9 (C- 8), 74.4 (C-2), 69.1 (C-3), 50.7 (C-11), 38.2 (C-4), 35.3 (C-9). IR (KBr): 3412, 2954, 1715, 1616, 1518, 1468, 1365, 1238, 1172, 1149, 1130, 1050, 1025, 818, 795 cm^{-1}.

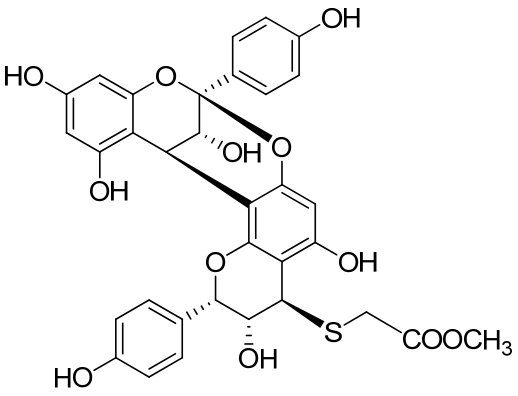
8.6.2. General Procedure for the acid depolymerization of proanthocyanidins from rhizomes of Selliguea feei in the presence of carbon and sulfur nucleophiles.

Synthesis of the chiral ligands **23**, **24** and **25**.Under nitrogen atmosphere, the proanthocyanidins from rhizomes of *Selliguea feei* (2.0 g) was mixed with MeOH (15 mL), hydrochloric acid (36%, 150 uL), and nucleophiles. The mixture was heated at 50 °C for 8 hrs with stirring. The filtrate was neutralized with 0.1M $NaHCO_3$ to pH 7.0 before it was extracted with ethyl acetate. The combined organic fraction was dried over anhydrous sodium sulphate. Evaporation of the ethyl acetate gave dark brown residue, which was purified with column chromatography (detailed conditions were described under individual compounds) to afford the chiral ligands **23, 24 and 25.**



23

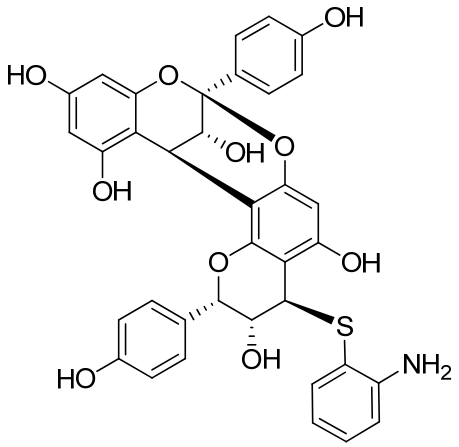
*2,4-dimethyl-3-ethylpyrrole A-type epiafzelechin-epiafzelechin (**23**).* **23** was purified with column chromatography (silica gel, eluent: dichloromethane-methanol 10:1 and then ethyl acetate-hexane 2:1) as a red solid (370 mg). MS (ESI, -c): 664 $[M-H]^{-1}$. H NMR (300 MHz, acetone-d_6): δ = 7.67 (d, *J* = 8.6 Hz, 2H, (C-2',6')-H), 7.55 (d, *J* = 8.7 Hz, 2H, C-(2",6")-H), 6.92 (d, *J* = 8.6 Hz, 2H, (C-3',5')-H), 6.87 (d, *J* = 8.7 Hz, 2H, (C-3",5")-H), 6.80 (s, 1H, C(13)-H), 6.09 (d, *J* = 2.3 Hz, 1H, C(6)-H), 6.00 (d, *J* = 2.3 Hz, 1H,C(8)-H), 5.37 (s, 1H, C(9)-H), 4.37 (d, *J* = 3.3 Hz, 1H,C(4)-H), 4.29 (d, *J* = 1.8 Hz, 1H, C(10)-H), 4.18 (d, *J* = 3.3 Hz, 1H,C(3)-H), 4.15 (d, *J* = 1.8 Hz, 1H, C(11)-H), 3.71 (s, C(18)-H).



24

*4β-(carboxymethyl)sulphanyl-epiafzelechin-(2 β→O→ 7, 4 β →8)-epiafzelechin methyl ester (**24**).* **24** was purified with column chromatography (silica gel, EtOAc-hexanes 2:1 and then dichloromethane-methanol 9:1) as a light yellow solid (656 mg). ^{1}H NMR (300 MHz, acetone-d_6): δ = 7.70 (s, 1H, (C2')-H), 7.67(s, 1H, (C6')-H), 7.57(s, 1H, (C2'')-H), 7.54(s, 1H, (C6'')-H), 6.94(s, 1H, (C3')-H), 6.91(s, 1H, (C3')-H), 6.89(s, 1H, (C3'')-H), 6.86(s, 1H, (C5'')-H), 6.17(s, 1H, (C13)-H), 6.09(d, J = 2.3, 1H, (C-6)-H), 6.99(d, J = 2.3, 1H, (C-8)-H), 5.36(s, 1H, C(9)-H), 4.37(d, J = 3.5, 1H, (C-4)-H), 4.29 (s, 1H, (C-10)-H), 4.17(s, 1H, (C-3)-H), 4.14(s, 1H, (C-11)-H) 3.74(m, 4H, (C-18, C-19)-H). ^{13}C{^{1}H} NMR (75 MHz, acetone-d_6): δ = 173.4 (C-16), 159.2

(C-4'), 158.6 (C-4''), 157.9 (C-12), 157.6 (C-7), 154.7 (C-5), 154.6 (C-14), 152.3 (C-8a, C-8a'), 132.1 (C-1", C-1'), 131.3 (C-2'', C-6"), 130.2 (C-2'), 130.0 (C-6'), 116.5 (C-3'', 5''), 115.9 (C-3',5'), 107.7 (C-8), 104.3 (C-4a), 102.0 (C-4a'), 100.7 (C-2), 98.8 (C-6), 98.2 (C-13), 96.9 (C-8), 78.1 (C-9), 71.1 (C-10), 68.0 (C-3), 53.7 (C-18), 45.1 (C-11), 34.9 (C-16), 27.8 (C-4). MS (ESI, -c): 647 $[M-H]^-$. IR (KBr): 3391, 2956, 1715, 1615, 1518, 1474, 1449, 1378, 1307, 1230, 1174, 1143, 1120, 1009, 966, 899, 880, 835, 781, 747 cm^{-1}..

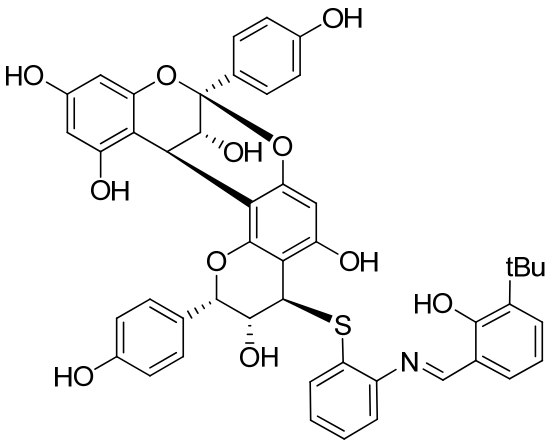


25

*2-aminothiophenol A-type epiafzelechin-epiafzelechin (**25**).* **25** was purified with column chromatography (silica gel, dichloromethane-methanol 9:1) as a light brown solid (791 mg). MS (ESI, -c): 666 $[M-H]^-$.^{1}H NMR (300 MHz, acetone-d_6): δ = 7.64 (d, *J* = 7.63 Hz,1H, C(2')-H), 7.61 (s,1H, C(2'')-H), 7.53 (s,1H, C(6')-H), 7.51 (s,1H, C(6'')-H), 7.34 (d, *J* = 4.6 Hz, 1H, C(16)-H), 7.11 (t, *J* = 4.6 Hz,1H, C(18)-H), 6.90 (s,1H, C(3')-H), 6.89 (s,1H, C(3'')-H), 6.85 (s,1H, C(5')-H), 6.83 (s,1H, C(5'')-H), 6.81 (d, *J* = 4.9 Hz,1H, C(17)-H), 6.59 (s,1H, C(19)-H), 6.17 (s,1H, C(13)-H), 6.09 (s,1H, C(6)-H), 6.03 (s,1H, C(8)-H), 4.84 (s,1H, C(9)-H), 4.47 (d, *J* = 2.3 Hz,1H, C(4)-H), 4.28 (s,1H, C(11)-H), 4.11 (s,1H, C(3)-H), 3.96 (s,1H, C(10)-H). ^{13}C{^{1}H} NMR (75 MHz, acetone-d_6): δ = 158.45, 158.21, 157.77, 157.25, 154.19, 154.08,

152.67, 151.87, 138.40, 131.77, 130.52, 130.11, 129.43, 118.29, 116.73, 116.27, 116.12, 115.57, 107.54, 103.91, 101.63, 100.32, 98.31, 96.99, 96.63, 77.22, 70.48, 68.05, 49.86, 29.40.

8.6.3 Synthesis of epiafzelechin Schiff base, 26. To a solution of **3 (**70 mg**)** in MeOH (5 mL) was successively added 3-*tert*-butyl-2-hydroxybenzaldehyde (1.1 mmol) mixture was refluxed for 8 h and the solvent was then removed under reduced pressure. The residue was purified by column chromatography (silica gel, dichloromethane-methanol 11:1) to afford the tetradentate chiral ligands **26** as a yellow solid (43 mg).



26

2-aminothiophenol A-type epiafzelechin-epiafzelechin Schiff base, ***26****.* ^{1}H NMR (300 MHz, acetone-d_6): δ = 8.84 (s, 1H, C(20)-H), 7.92 (d, *J* = 3.1 Hz, 1H, C(21)-H), 7.67 (d, *J* = 8.6 Hz, 2H, C(2',6')-H), 7.55 (m, 4H, C-(16, 17, 23, 19)-H), 7.45 (m 5H, C-(18, 2', 2'', 6', 6'')-H), 6.90 (d, *J* = 2.6, 1H, C(22)-H), 6.87 (m, 2H, (C-3',5')-H), 6.77 (m, 2H, (C-3",5")-H), 6.21 (s, 1H, C(13)-H), 6.09 (s, 1H, C(6)-H), 6.00 (s, 1H,C(8)-H), 4.77 (s, 1H, C(9)-H), 4.29 (s, 1H,C(4)-H), 4.26 (s, 1H, C(11)-H), 4.21 (s, 1H,C(10)-H), 4.03 (s, 1H, C(3)-H), 1.48 (s, 9H, -tBu). MS (ESI, -c): 826 $[M-H]^-$.

8.7 Applications of sulfur-containing epicatechin derivatives from proanthocyanidins

8.7.1 Kinetics of the dethiolation of three sulfur-containing epicatechin derivatives.

Three sulfur-containing derivatives were dissolved into 0.1M $NaHCO_3$ (pH 8.5) aqueous solution, the reaction mixture was injected directly for reverse-phase HPLC analysis every 30minites from 0 to 330 mins .

HPLC Analysis. The reaction media was further analyzed using LC-MS with a C18 column (250 × 4.6 mm, 5 um, a Shimadzu, Kyoto, Japan). The solvent system consisted of A (2% acetic acid in water, v/v) and B (methanol), which were delivered by an Agilent 1100 binary pump in a linear gradient of B from 15% to 80% (v/v) in 45 min. For hydrolysis products, solvent A was replaced with 5% (v/v) formic acid in water and the linear gradient was 15-55% B in 45 min. The detection wavelength of the diode array detector was set at 280 nm (6 nm breadth)

8.7.2 Reaction of 8 with Epicatechin and carbon nucleophiles. 8 (3.0 mg 7.6 umol) and epicatechin or nucleophiles, 3,5-dimethoxyphenol, 3,5-dimethoxyaniline, 2,3-dimethylpyrozzole and 2, 4-dimethyl-3-ethyl pyrrrole (10 umol) was dissolved in acetone-water solution (v/v = 1/1, 0.2 mL). Sodium hydrogen carbonate-sodium carbonate buffer (pH 8.5, 0.8 mL) was added to afford the basic condition. The mixture was stirred at room temperature for 5 h and then kept in the freezer (-18°C) until ESI-MS and HPLC analysis. The conversions were calculated according to the LC-MS results.

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List of Publications and Patent

1. Caili Fu, Wei Chen, Yi Ling Quek, Runyan Ni, Amylia Bte Abdul Ghani, Wendy Wen Yi Leong, Huaqiang Zeng, Dejian Huang. Sustainability from agricultural waste: chiral ligands from oligomeric proanthocyanidins via acid-mediated depolymerization. Tetrahedron Letters. 2010, 51(48), 6322-6324.
2. Wei Chen, Caili Fu, Yong Qin and Dejian Huang. One-pot depolymerizative extraction of proanthocyanidins from mangosteen pericarps. Food Chemistry. 2009, 114(3): 874–880.
3. Chen Wei, Tan Kheng Lin, Fu Caili, Qin Yong, Huang Dejian. Selectivity Protection of Hydroxyl Groups on Epicatechin and Catechin. Chinese Journal of Synthetic Chemistry. 2008, 133-135.
4. Caili Fu, Alvin Eng Kiat Loo, Fiona Ping Ping Chia and Dejian Huang. Oligomeric proanthocyanidins from mangosteen pericarps. Journal of Agriclture and Food Chemistry. 2007, 55(19):7689-7694.
5. Caili Fu, Wei Ling Ng, Lixia Song, Dejian Huang. Isolation and chemical modification of propelargonidins from the rhizomes of *Selliguea feei*. Food Chemistry. Submitted.
6. Caili Fu, Dejian Huang. Kinetics of the dethiolation of sulfur-containing epicatechin derivatives. Manuscript in preparation.
7. Huang Dejian et al. Derivatives of Epicatechin or its Oligomers, their Preparation Procedures, and Applications in Catalysis. US Provisional Patent (No. 61/287,281). 2009. Main inventor.

Appendix

1. Crystal data for **19**

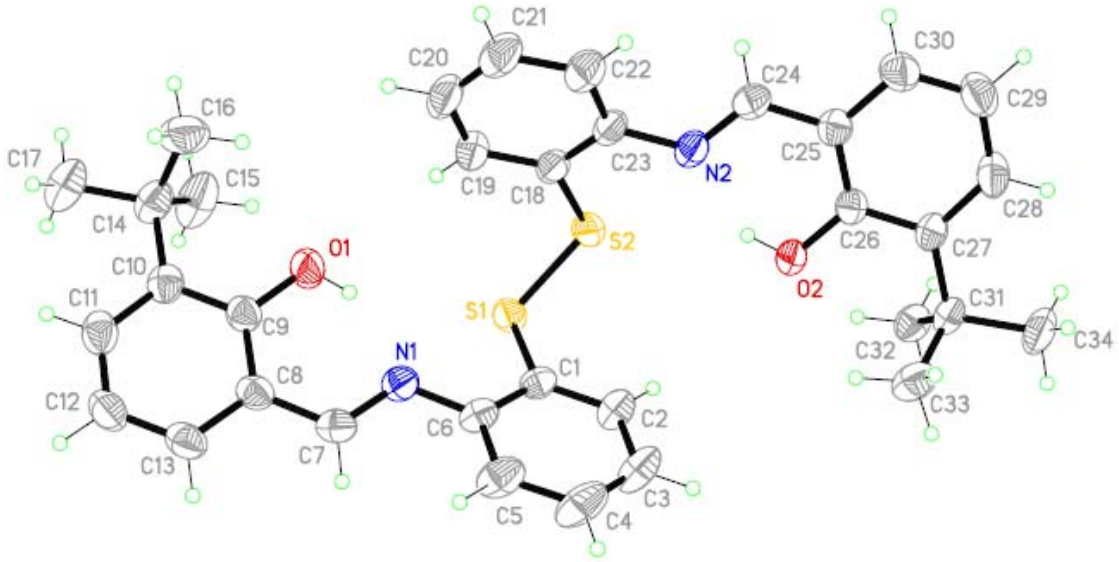


Table 1. Crystal data and structure refinement for **19**.

Identification code	19	
Empirical formula	C34 H36 N2 O2 S2	
Formula weight	568.77	
Temperature	223(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	P2(1)/n	
Unit cell dimensions	a = 12.2308(6) Å	a= 90°.
	b = 9.2242(4) Å	b= 100.6700(10)°.
	c = 27.3892(12) Å	g = 90°.
Volume	3036.6(2) $Å^3$	
Z	4	
Density (calculated)	1.244 Mg/m^3	
Absorption coefficient	0.208 mm^{-1}	
F(000)	1208	
Crystal size	0.60 x 0.60 x 0.30 mm^3	
Theta range for data collection	1.72 to 27.50°.	
Index ranges	-15<=h<=14, -	

	11<=k<=11, -35<=l<=26
Reflections collected	20706
Independent reflections	6946 [R(int) = 0.0288]
Completeness to theta = 27.50°	100.0 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.9401 and 0.8852
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	6946 / 0 / 369
Goodness-of-fit on F^2	1.034
Final R indices [I>2sigma(I)]	R1 = 0.0430, wR2 = 0.1139
R indices (all data)	R1 = 0.0506, wR2 = 0.1186
Largest diff. peak and hole	0.281 and -0.251 e.Å^{-3}

Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters (Å^{2}x 10^3) for **19**. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
S(1)	6242(1)	5612(1)	5351(1)	37(1)
S(2)	7152(1)	3768(1)	5335(1)	41(1)
N(1)	6624(1)	8524(1)	4994(1)	37(1)
N(2)	9393(1)	2858(1)	5311(1)	37(1)
O(1)	5537(1)	7612(1)	4146(1)	52(1)
O(2)	9616(1)	2879(1)	6264(1)	40(1)
C(1)	7249(1)	6853(2)	5663(1)	34(1)
C(2)	7915(1)	6498(2)	6117(1)	43(1)
C(3)	8639(2)	7512(2)	6367(1)	53(1)
C(4)	8697(2)	8876(2)	6169(1)	60(1)
C(5)	8042(2)	9244(2)	5722(1)	51(1)
C(6)	7296(1)	8236(2)	5461(1)	36(1)
C(7)	6287(1)	9804(2)	4863(1)	38(1)
C(8)	5642(1)	10116(2)	4375(1)	37(1)

C(9)	5274(1)	9004(2)	4029(1)	38(1)
C(10)	4624(1)	9329(2)	3560(1)	40(1)
C(11)	4407(2)	10782(2)	3456(1)	47(1)
C(12)	4792(2)	11892(2)	3785(1)	50(1)
C(13)	5390(1)	11559(2)	4243(1)	45(1)
C(14)	4204(2)	8127(2)	3183(1)	49(1)
C(15)	3501(2)	7019(3)	3412(1)	73(1)
C(16)	5201(2)	7391(2)	3022(1)	67(1)
C(17)	3455(2)	8738(2)	2716(1)	63(1)
C(18)	7816(1)	4025(2)	4812(1)	38(1)
C(19)	7291(2)	4687(2)	4377(1)	48(1)
C(20)	7845(2)	4825(2)	3983(1)	54(1)
C(21)	8908(2)	4284(2)	4018(1)	54(1)
C(22)	9440(2)	3619(2)	4449(1)	47(1)
C(23)	8893(1)	3474(2)	4850(1)	37(1)
C(24)	10179(1)	1927(2)	5350(1)	38(1)
C(25)	10734(1)	1394(2)	5831(1)	36(1)
C(26)	10458(1)	1926(2)	6277(1)	32(1)
C(27)	11045(1)	1452(2)	6743(1)	34(1)
C(28)	11856(1)	402(2)	6736(1)	47(1)
C(29)	12111(2)	-169(2)	6300(1)	54(1)
C(30)	11558(1)	337(2)	5851(1)	47(1)
C(31)	10774(1)	2055(2)	7230(1)	38(1)
C(32)	9591(1)	1617(2)	7280(1)	43(1)
C(33)	10879(2)	3713(2)	7243(1)	53(1)
C(34)	11569(2)	1459(2)	7686(1)	56(1)

Table 3. Bond lengths [Å] and angles [°] for **19**.

S(1)-C(1)	1.7801(14)
S(1)-S(2)	2.0373(5)
S(2)-C(18)	1.7873(16)
N(1)-C(7)	1.280(2)
N(1)-C(6)	1.4136(19)
N(2)-C(24)	1.280(2)
N(2)-C(23)	1.4152(18)
O(1)-C(9)	1.3476(18)

O(2)-C(26)	1.3492(17)
C(1)-C(2)	1.393(2)
C(1)-C(6)	1.396(2)
C(2)-C(3)	1.380(2)
C(3)-C(4)	1.378(3)
C(4)-C(5)	1.376(3)
C(5)-C(6)	1.402(2)
C(7)-C(8)	1.451(2)
C(8)-C(13)	1.399(2)
C(8)-C(9)	1.412(2)
C(9)-C(10)	1.412(2)
C(10)-C(11)	1.386(2)
C(10)-C(14)	1.537(2)
C(11)-C(12)	1.389(3)
C(12)-C(13)	1.363(2)
C(14)-C(16)	1.530(3)
C(14)-C(17)	1.535(2)
C(14)-C(15)	1.542(3)
C(18)-C(19)	1.387(2)
C(18)-C(23)	1.398(2)
C(19)-C(20)	1.381(3)
C(20)-C(21)	1.379(3)
C(21)-C(22)	1.381(2)
C(22)-C(23)	1.395(2)
C(24)-C(25)	1.451(2)
C(25)-C(30)	1.397(2)
C(25)-C(26)	1.413(2)
C(26)-C(27)	1.4131(19)
C(27)-C(28)	1.389(2)
C(27)-C(31)	1.537(2)
C(28)-C(29)	1.393(2)
C(29)-C(30)	1.369(2)
C(31)-C(32)	1.531(2)
C(31)-C(33)	1.534(2)
C(31)-C(34)	1.535(2)
C(1)-S(1)-S(2)	102.43(5)

C(18)-S(2)-S(1)	103.78(5)
C(7)-N(1)-C(6)	121.95(13)
C(24)-N(2)-C(23)	122.61(13)
C(2)-C(1)-C(6)	120.56(14)
C(2)-C(1)-S(1)	121.03(12)
C(6)-C(1)-S(1)	118.22(11)
C(3)-C(2)-C(1)	119.93(16)
C(4)-C(3)-C(2)	119.90(16)
C(5)-C(4)-C(3)	120.79(16)
C(4)-C(5)-C(6)	120.46(16)
C(1)-C(6)-C(5)	118.35(15)
C(1)-C(6)-N(1)	118.08(13)
C(5)-C(6)-N(1)	123.51(14)
N(1)-C(7)-C(8)	122.17(14)
C(13)-C(8)-C(9)	119.37(15)
C(13)-C(8)-C(7)	118.91(14)
C(9)-C(8)-C(7)	121.72(14)
O(1)-C(9)-C(8)	120.05(14)
O(1)-C(9)-C(10)	119.18(14)
C(8)-C(9)-C(10)	120.77(14)
C(11)-C(10)-C(9)	116.55(15)
C(11)-C(10)-C(14)	122.13(15)
C(9)-C(10)-C(14)	121.31(14)
C(10)-C(11)-C(12)	123.37(16)
C(13)-C(12)-C(11)	119.39(16)
C(12)-C(13)-C(8)	120.48(16)
C(16)-C(14)-C(17)	107.91(16)
C(16)-C(14)-C(10)	109.20(14)
C(17)-C(14)-C(10)	111.56(16)
C(16)-C(14)-C(15)	110.97(18)
C(17)-C(14)-C(15)	106.87(15)
C(10)-C(14)-C(15)	110.30(15)
C(19)-C(18)-C(23)	120.19(15)
C(19)-C(18)-S(2)	122.68(14)
C(23)-C(18)-S(2)	117.08(11)
C(20)-C(19)-C(18)	119.76(17)
C(21)-C(20)-C(19)	120.29(16)

C(20)-C(21)-C(22)	120.64(16)
C(21)-C(22)-C(23)	119.74(17)
C(22)-C(23)-C(18)	119.36(14)
C(22)-C(23)-N(2)	123.40(15)
C(18)-C(23)-N(2)	117.16(13)
N(2)-C(24)-C(25)	121.47(13)
C(30)-C(25)-C(26)	119.62(14)
C(30)-C(25)-C(24)	118.98(14)
C(26)-C(25)-C(24)	121.40(13)
O(2)-C(26)-C(27)	118.83(12)
O(2)-C(26)-C(25)	120.42(12)
C(27)-C(26)-C(25)	120.74(13)
C(28)-C(27)-C(26)	116.51(13)
C(28)-C(27)-C(31)	122.34(13)
C(26)-C(27)-C(31)	121.14(13)
C(27)-C(28)-C(29)	123.39(15)
C(30)-C(29)-C(28)	119.25(16)
C(29)-C(30)-C(25)	120.41(15)
C(32)-C(31)-C(33)	109.77(15)
C(32)-C(31)-C(34)	107.44(13)
C(33)-C(31)-C(34)	107.48(14)
C(32)-C(31)-C(27)	109.90(12)
C(33)-C(31)-C(27)	110.46(13)
C(34)-C(31)-C(27)	111.71(13)

Symmetry transformations used to generate equivalent atoms:

Table 4. Anisotropic displacement parameters ($Å^2 x\ 10^3$) for **19**. The anisotropic displacement factor exponent takes the form: $-2p^2[\ h^2\ a^{*2}U^{11} + ... + 2\ h\ k\ a^*\ b^*\ U^{12}\ ]$

	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
S(1)	34(1)	30(1)	44(1)	-3(1)	-2(1)	-2(1)
S(2)	56(1)	32(1)	36(1)	4(1)	9(1)	7(1)
N(1)	43(1)	34(1)	34(1)	-1(1)	8(1)	-6(1)
N(2)	42(1)	39(1)	29(1)	2(1)	3(1)	-2(1)
O(1)	70(1)	32(1)	46(1)	0(1)	-10(1)	1(1)
O(2)	44(1)	45(1)	28(1)	3(1)	2(1)	11(1)
C(1)	33(1)	38(1)	32(1)	-6(1)	7(1)	-7(1)
C(2)	43(1)	50(1)	34(1)	1(1)	3(1)	-9(1)
C(3)	53(1)	74(1)	31(1)	-1(1)	-1(1)	-21(1)
C(4)	66(1)	70(1)	41(1)	-11(1)	3(1)	-36(1)
C(5)	65(1)	47(1)	42(1)	-5(1)	9(1)	-25(1)
C(6)	39(1)	40(1)	31(1)	-5(1)	9(1)	-9(1)
C(7)	45(1)	34(1)	39(1)	-4(1)	16(1)	-6(1)
C(8)	42(1)	33(1)	40(1)	1(1)	17(1)	2(1)
C(9)	41(1)	34(1)	40(1)	3(1)	11(1)	0(1)
C(10)	39(1)	45(1)	38(1)	5(1)	12(1)	1(1)
C(11)	48(1)	54(1)	44(1)	12(1)	16(1)	10(1)
C(12)	66(1)	38(1)	53(1)	9(1)	24(1)	15(1)
C(13)	55(1)	35(1)	49(1)	-1(1)	23(1)	6(1)
C(14)	51(1)	55(1)	39(1)	2(1)	2(1)	-10(1)
C(15)	79(2)	76(1)	55(1)	15(1)	-9(1)	-35(1)
C(16)	74(1)	63(1)	60(1)	-24(1)	3(1)	3(1)
C(17)	59(1)	85(2)	42(1)	10(1)	-1(1)	-14(1)
C(18)	52(1)	31(1)	29(1)	0(1)	3(1)	-1(1)
C(19)	61(1)	45(1)	35(1)	4(1)	-1(1)	7(1)
C(20)	76(1)	50(1)	31(1)	8(1)	-1(1)	-1(1)
C(21)	72(1)	56(1)	33(1)	6(1)	11(1)	-8(1)
C(22)	54(1)	51(1)	36(1)	4(1)	8(1)	-5(1)
C(23)	48(1)	34(1)	27(1)	0(1)	1(1)	-5(1)
C(24)	41(1)	40(1)	32(1)	-1(1)	10(1)	-4(1)
C(25)	35(1)	38(1)	35(1)	3(1)	7(1)	-1(1)
C(26)	29(1)	34(1)	33(1)	2(1)	4(1)	-2(1)

C(27)	29(1)	40(1)	32(1)	3(1)	2(1)	-4(1)
C(28)	36(1)	61(1)	43(1)	12(1)	2(1)	9(1)
C(29)	47(1)	65(1)	53(1)	10(1)	14(1)	22(1)
C(30)	47(1)	54(1)	44(1)	3(1)	17(1)	10(1)
C(31)	38(1)	44(1)	29(1)	1(1)	-2(1)	-2(1)
C(32)	43(1)	52(1)	35(1)	1(1)	8(1)	-4(1)
C(33)	67(1)	48(1)	41(1)	-7(1)	5(1)	-14(1)
C(34)	51(1)	78(1)	34(1)	5(1)	-7(1)	5(1)

Table 5. Hydrogen coordinates (x 10^4) and isotropic displacement parameters ($Å^2$x 10^3) for **19**.

	x	y	z	U(eq)
H(1)	5933	7574	4426	78
H(2)	9329	3051	5971	60
H(2A)	7870	5569	6253	51
H(3)	9093	7272	6673	64
H(4)	9191	9564	6341	72
H(5)	8094	10178	5591	62
H(7)	6463	10568	5091	46
H(11)	3977	11029	3146	57
H(12)	4642	12865	3694	60
H(13)	5634	12305	4471	54
H(15A)	3212	6293	3165	109
H(15B)	2887	7513	3519	109
H(15C)	3961	6553	3695	109
H(16A)	4938	6663	2772	101
H(16B)	5659	6933	3307	101
H(16C)	5637	8111	2884	101
H(17A)	3873	9428	2557	95
H(17B)	2819	9220	2810	95
H(17C)	3199	7953	2488	95
H(19)	6562	5041	4350	58
H(20)	7495	5290	3690	64
H(21)	9273	4369	3746	64
H(22)	10169	3265	4471	56

H(24)	10403	1580	5061	45
H(28)	12253	60	7041	57
H(29)	12656	-893	6313	65
H(30)	11734	-31	5555	56
H(32A)	9063	2027	7007	65
H(32B)	9433	1978	7592	65
H(32C)	9529	568	7272	65
H(33A)	11608	3988	7182	79
H(33B)	10787	4067	7566	79
H(33C)	10310	4131	6988	79
H(34A)	11510	411	7692	84
H(34B)	11372	1859	7986	84
H(34C)	12326	1731	7668	84

Table 6. Hydrogen bonds for **19** [Å and °].

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
O(1)-H(1)...N(1)	0.83	1.85	2.5931(17)	148.9
O(2)-H(2)...N(2)	0.83	1.83	2.5759(15)	148.1

Symmetry transformations used to generate equivalent atoms:

2. Crystal data for **20**

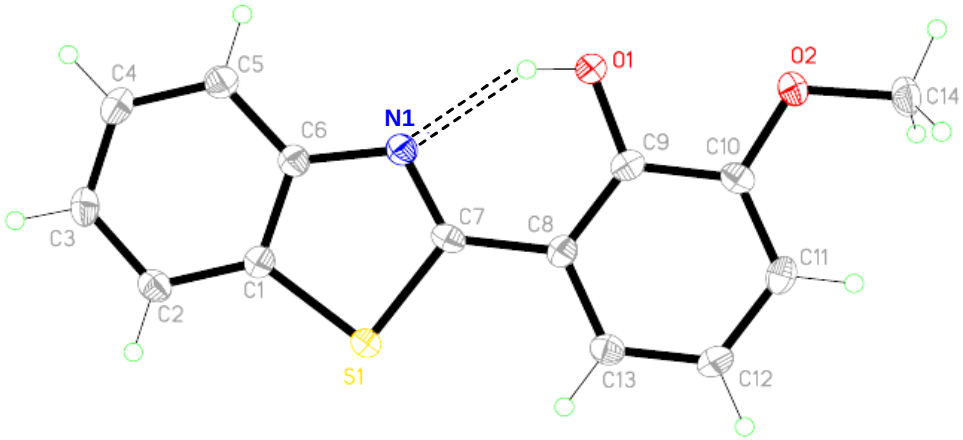


Table 1. Crystal data and structure refinement for **20**.

Identification code	20	
Empirical formula	C14 H11 N O2 S	
Formula weight	257.30	
Temperature	293(2) K	
Wavelength	0.71073 Å	
Crystal system	Orthorhombic	
Space group	P2(1)2(1)2(1)	
Unit cell dimensions	a = 6.0482(5) Å	α= 90°.
	b = 12.1659(9) Å	β= 90°.
	c = 15.8957(13) Å	γ = 90°.
Volume	1169.63(16) $Å^3$	
Z	4	
Density (calculated)	1.461 Mg/m^3	
Absorption coefficient	0.268 mm^{-1}	
F(000)	536	
Crystal size	0.30 x 0.18 x 0.14 mm^3	
Theta range for data collection	2.56 to 27.49°.	
Index ranges	-6<=h<=7, -	
	15<=k<=15, -	
	17<=l<=20	
Reflections collected	8310	
Independent reflections	2681 [R(int) = 0.0325]	
Completeness to theta = 27.49°	99.9 %	
Absorption correction	Semi-empirical from	

	equivalents
Max. and min. transmission	0.9634 and 0.9238
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	2681 / 0 / 165
Goodness-of-fit on F^2	1.089
Final R indices [I>2sigma(I)]	R1 = 0.0362, wR2 = 0.0882
R indices (all data)	R1 = 0.0388, wR2 = 0.0897
Absolute structure parameter	0.02(7)
Largest diff. peak and hole	0.361 and -0.182 e.$Å^{-3}$

Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$x 10^3) for **20**. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
S(1)	-1557(1)	2037(1)	7221(1)	18(1)
O(1)	1749(2)	-1189(1)	6345(1)	20(1)
O(2)	5278(2)	-1216(1)	5429(1)	21(1)
N(1)	-1411(3)	-91(1)	7120(1)	17(1)
C(1)	-3583(3)	1294(1)	7748(1)	17(1)
C(2)	-5313(3)	1685(2)	8244(1)	20(1)
C(3)	-6685(3)	916(2)	8616(1)	21(1)
C(4)	-6343(3)	-210(2)	8503(1)	23(1)
C(5)	-4623(3)	-597(2)	8010(1)	21(1)
C(6)	-3217(3)	161(1)	7623(1)	17(1)
C(7)	-414(3)	802(1)	6862(1)	16(1)
C(8)	1547(3)	802(1)	6328(1)	16(1)
C(9)	2541(3)	-199(2)	6103(1)	16(1)
C(10)	4459(3)	-196(2)	5606(1)	17(1)
C(11)	5372(3)	789(2)	5335(1)	19(1)
C(12)	4361(3)	1789(2)	5557(1)	20(1)
C(13)	2477(3)	1794(2)	6043(1)	19(1)
C(14)	7208(3)	-1271(2)	4909(1)	24(1)

Table 3. Bond lengths [Å] and angles [°] for 20.

S(1)-C(1)	1.7383(19)
S(1)-C(7)	1.7496(18)
O(1)-C(9)	1.352(2)
O(2)-C(10)	1.366(2)
O(2)-C(14)	1.432(2)
N(1)-C(7)	1.309(2)
N(1)-C(6)	1.388(2)
C(1)-C(2)	1.394(3)
C(1)-C(6)	1.410(2)
C(2)-C(3)	1.383(3)
C(3)-C(4)	1.397(3)
C(4)-C(5)	1.385(3)
C(5)-C(6)	1.397(3)
C(7)-C(8)	1.459(3)
C(8)-C(9)	1.404(2)
C(8)-C(13)	1.407(3)
C(9)-C(10)	1.404(3)
C(10)-C(11)	1.388(3)
C(11)-C(12)	1.406(3)
C(12)-C(13)	1.377(3)
C(1)-S(1)-C(7)	89.34(9)
C(10)-O(2)-C(14)	117.17(15)
C(7)-N(1)-C(6)	111.11(14)
C(2)-C(1)-C(6)	122.08(17)
C(2)-C(1)-S(1)	128.59(14)
C(6)-C(1)-S(1)	109.31(14)
C(3)-C(2)-C(1)	117.45(18)
C(2)-C(3)-C(4)	121.32(18)
C(5)-C(4)-C(3)	121.17(18)
C(4)-C(5)-C(6)	118.78(18)
N(1)-C(6)-C(5)	125.97(16)
N(1)-C(6)-C(1)	114.83(15)
C(5)-C(6)-C(1)	119.20(17)
N(1)-C(7)-C(8)	123.81(15)

N(1)-C(7)-S(1)	115.40(14)
C(8)-C(7)-S(1)	120.78(13)
C(9)-C(8)-C(13)	119.40(17)
C(9)-C(8)-C(7)	119.79(15)
C(13)-C(8)-C(7)	120.81(16)
O(1)-C(9)-C(8)	123.28(16)
O(1)-C(9)-C(10)	117.08(16)
C(8)-C(9)-C(10)	119.63(16)
O(2)-C(10)-C(11)	125.19(17)
O(2)-C(10)-C(9)	114.38(16)
C(11)-C(10)-C(9)	120.43(17)
C(10)-C(11)-C(12)	119.70(17)
C(13)-C(12)-C(11)	120.28(17)
C(12)-C(13)-C(8)	120.55(17)

Symmetry transformations used to generate equivalent atoms:

Table 4. Anisotropic displacement parameters ($Å^2 x\ 10^3$) for 20. The anisotropic displacement factor exponent takes the form: $-2\pi^2[\ h^2\ a^{*2}U^{11} + ...\ + 2\ h\ k\ a^*\ b^*\ U^{12}\ ]$

	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
S(1)	17(1)	14(1)	22(1)	0(1)	2(1)	1(1)
O(1)	19(1)	16(1)	25(1)	0(1)	6(1)	-1(1)
O(2)	19(1)	20(1)	25(1)	-1(1)	6(1)	4(1)
N(1)	16(1)	17(1)	17(1)	0(1)	-1(1)	1(1)
C(1)	16(1)	19(1)	15(1)	1(1)	-2(1)	0(1)
C(2)	19(1)	19(1)	21(1)	-2(1)	-2(1)	2(1)
C(3)	16(1)	28(1)	19(1)	-4(1)	4(1)	0(1)
C(4)	20(1)	27(1)	22(1)	1(1)	3(1)	-5(1)
C(5)	20(1)	20(1)	23(1)	-2(1)	-1(1)	-1(1)
C(6)	15(1)	19(1)	18(1)	-1(1)	-2(1)	0(1)
C(7)	17(1)	14(1)	16(1)	-2(1)	-5(1)	3(1)
C(8)	12(1)	19(1)	16(1)	1(1)	-2(1)	-1(1)
C(9)	16(1)	18(1)	14(1)	1(1)	-4(1)	-3(1)
C(10)	18(1)	17(1)	16(1)	0(1)	-4(1)	3(1)
C(11)	14(1)	27(1)	17(1)	1(1)	0(1)	-2(1)

C(12)	20(1)	19(1)	22(1)	5(1)	-1(1)	-3(1)
C(13)	19(1)	17(1)	20(1)	1(1)	-2(1)	2(1)
C(14)	19(1)	27(1)	26(1)	-2(1)	7(1)	4(1)

Table 5. Hydrogen coordinates (x 10^4) and isotropic displacement parameters ($Å^2$x 10^3) for **20**.

	x	y	z	U(eq)
H(1)	632	-1102	6630	30
H(2)	-5536	2434	8322	23
H(3)	-7858	1153	8947	25
H(4)	-7287	-708	8764	27
H(5)	-4408	-1348	7938	25
H(11)	6647	788	5008	23
H(12)	4968	2450	5374	24
H(13)	1814	2458	6184	22
H(14A)	6924	-906	4385	36
H(14B)	7576	-2026	4805	36
H(14C)	8420	-917	5190	36

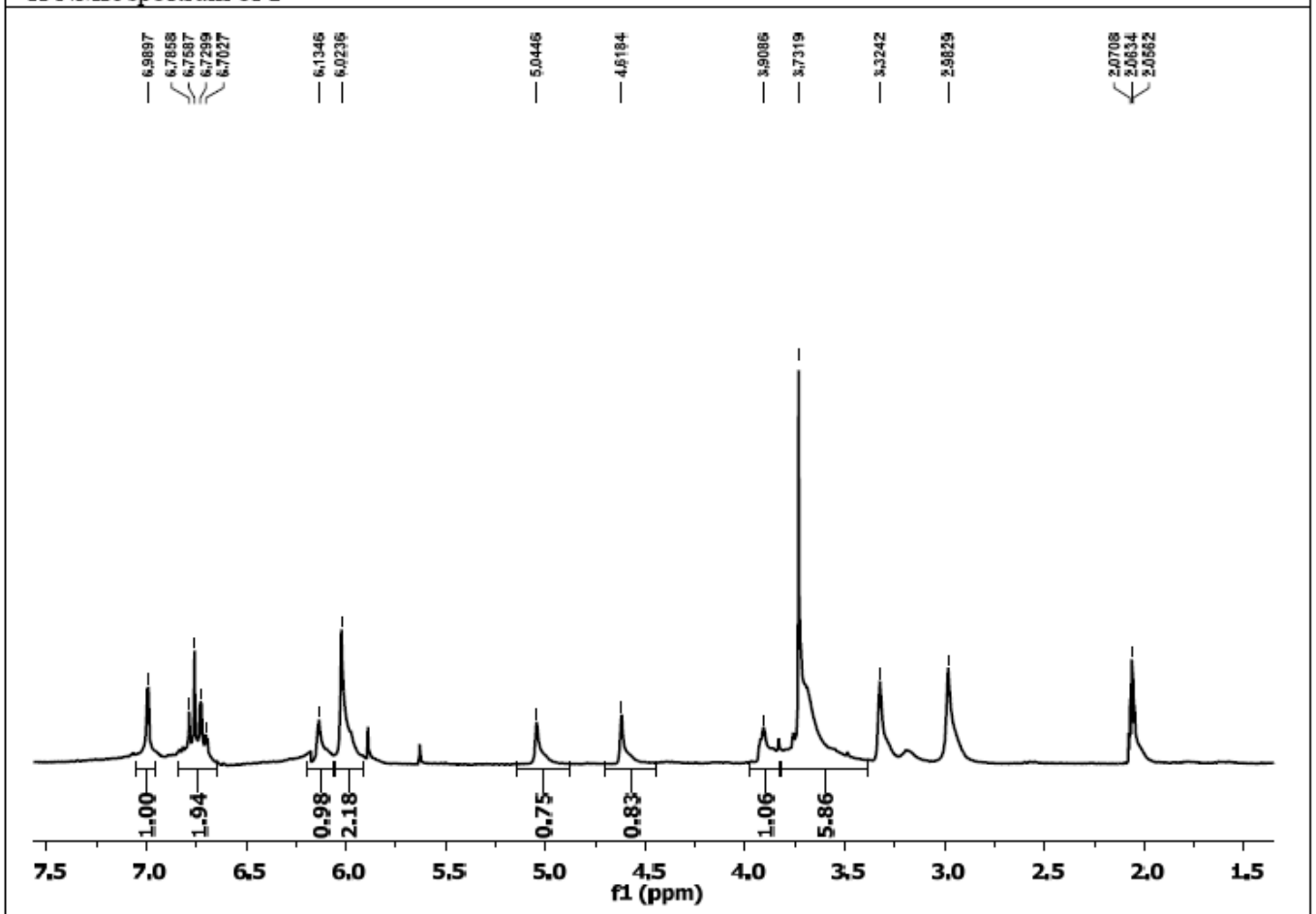
Table 6. Hydrogen bonds for **20** [Å and °].

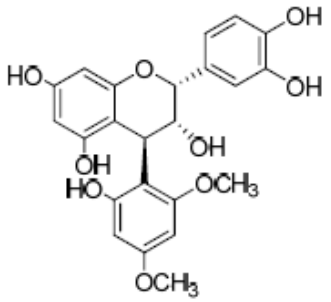
D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
O(1)-H(1)...N(1)	0.82	1.91	2.638(2)	147.3

Symmetry transformations used to generate equivalent atoms:

3. NMR spectra.

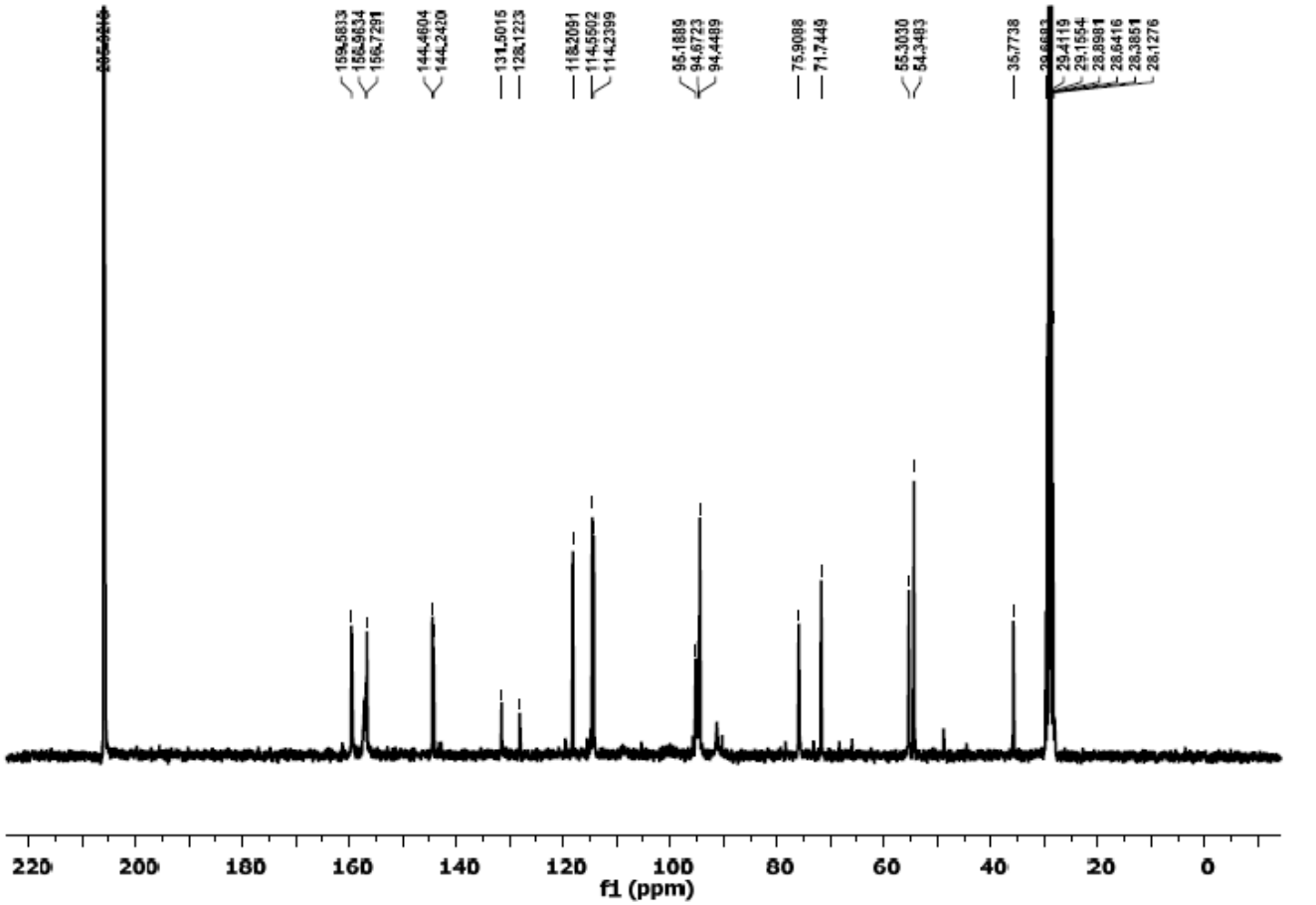
^{1}H NMR spectrum of **1**

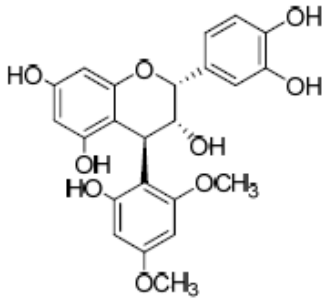




1

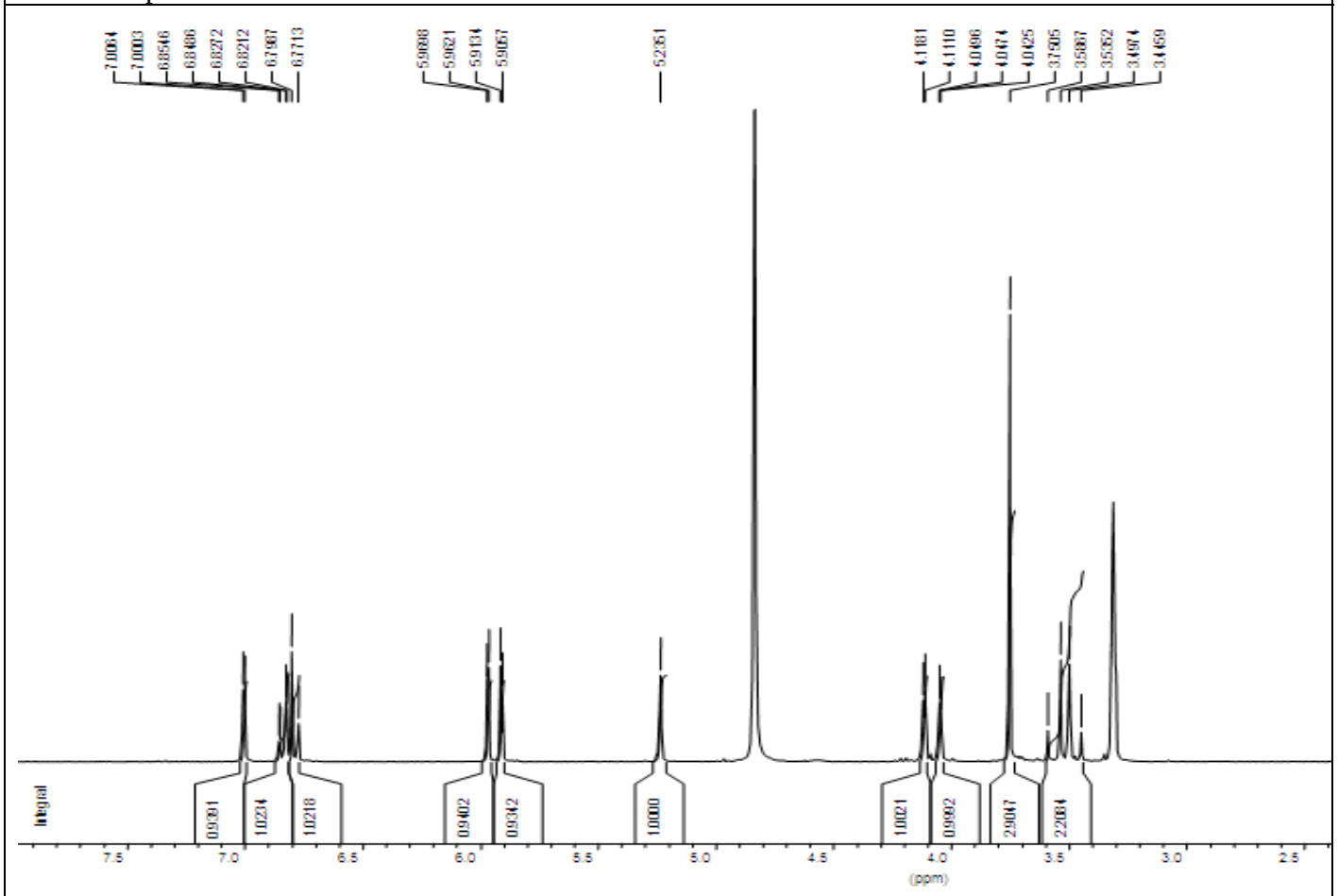
^{13}C NMR spectrum of **1**

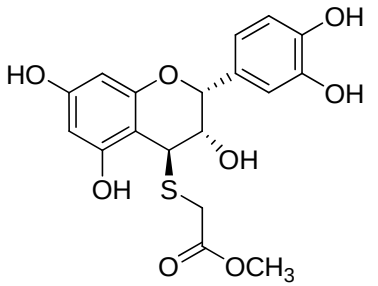




1

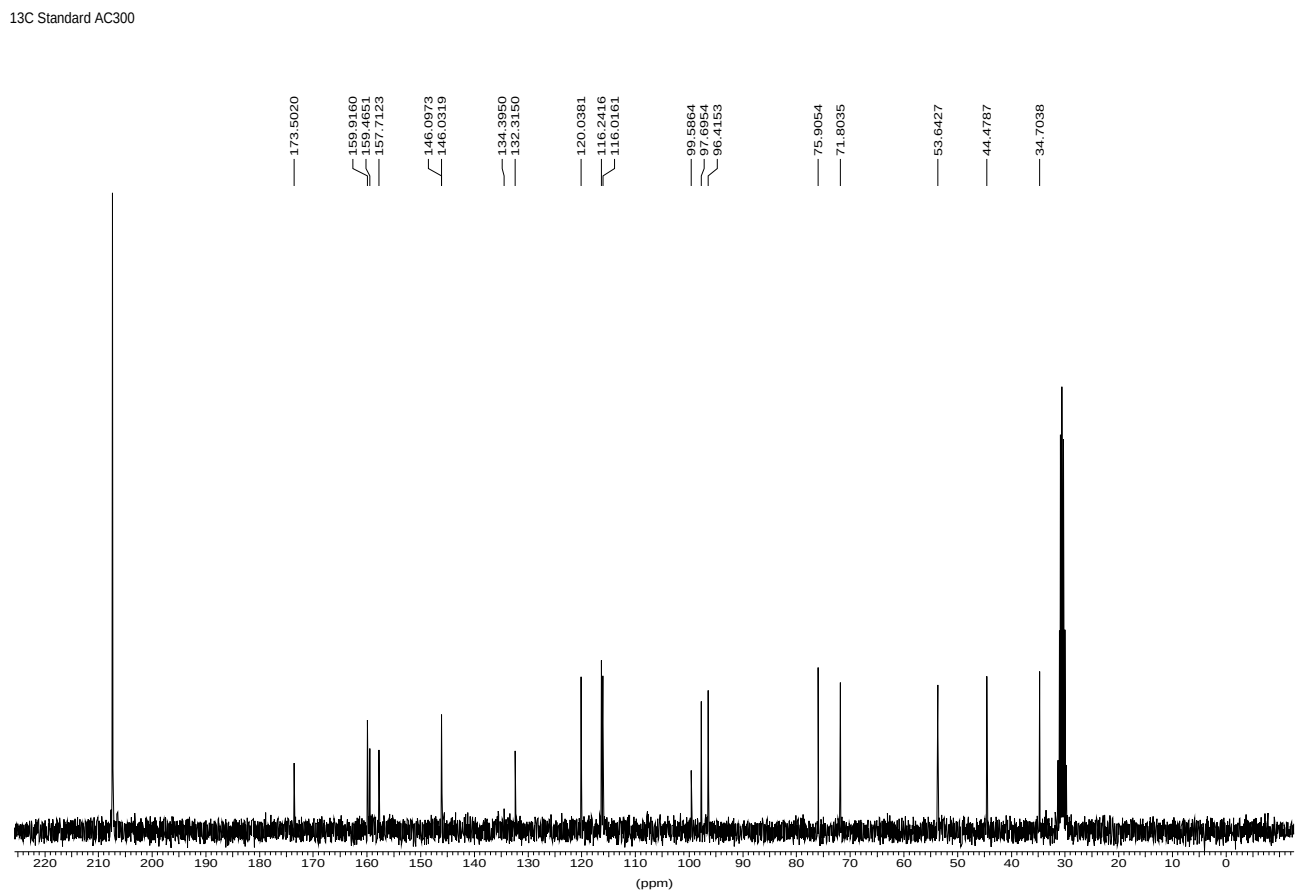
^{1}H NMR spectrum of **2**

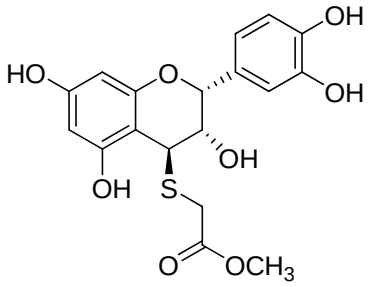




2

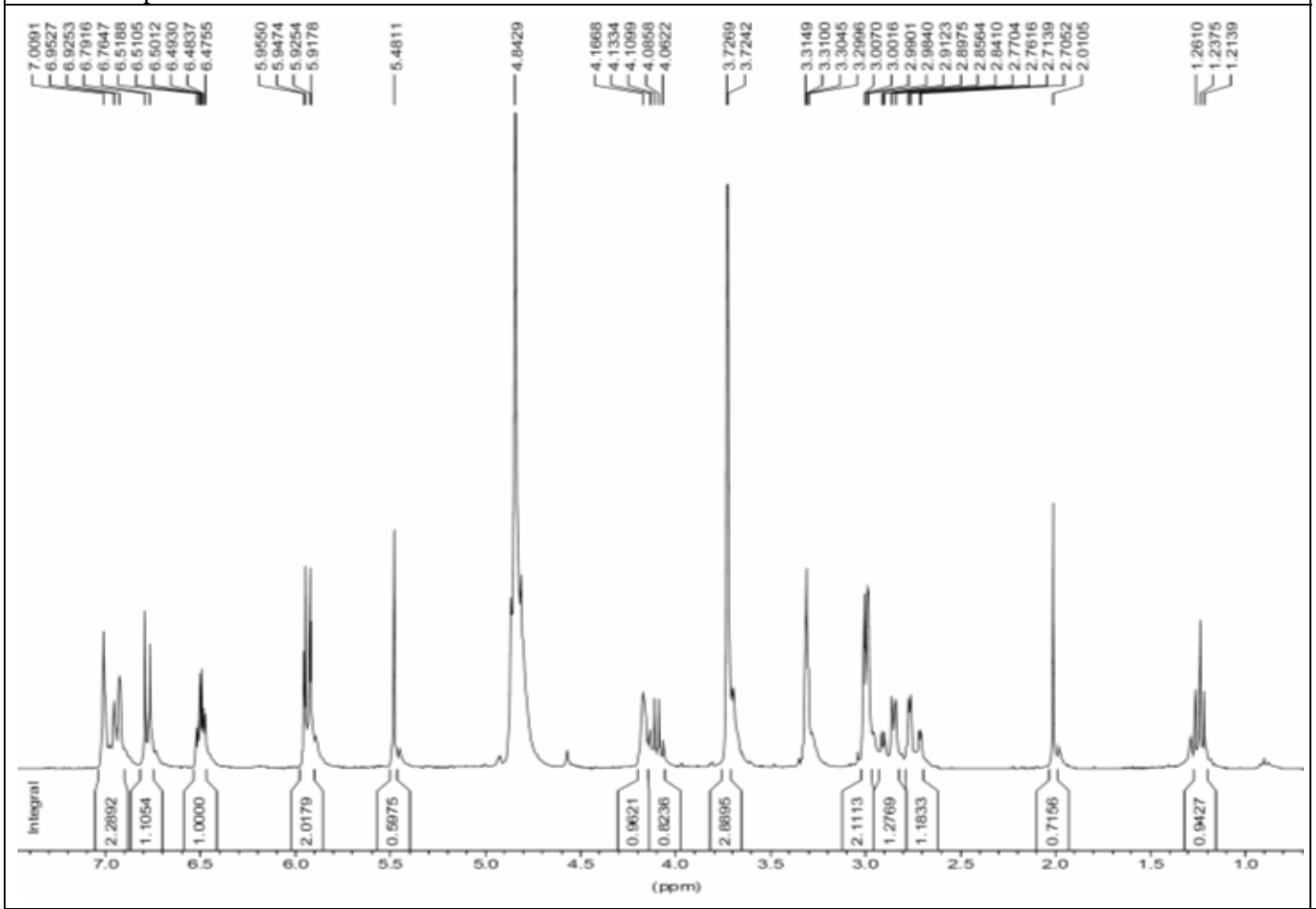
^{13}C NMR spectrum of **2**

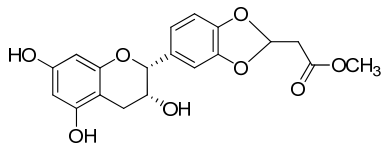




2

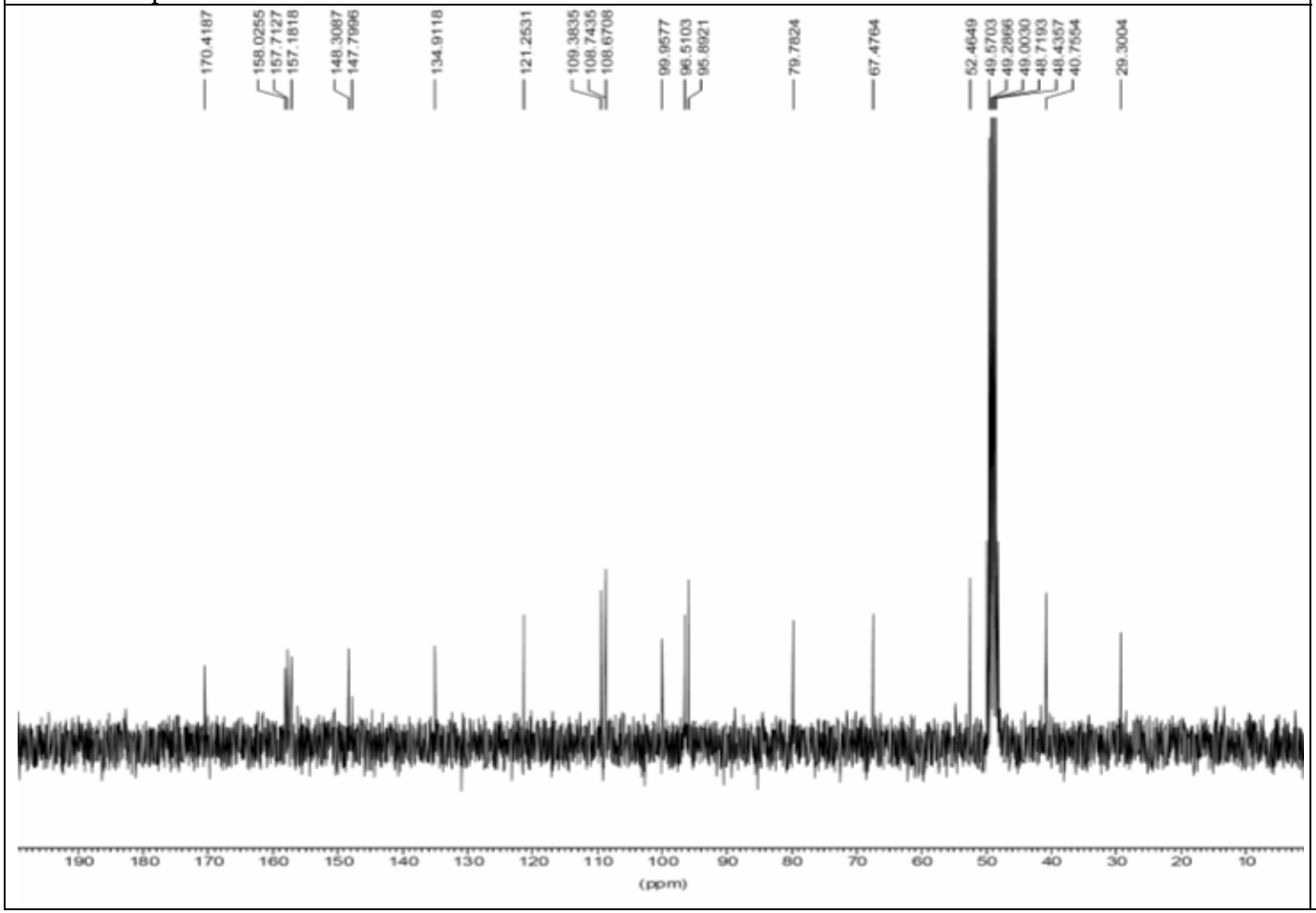
^{1}H NMR spectrum of **3**

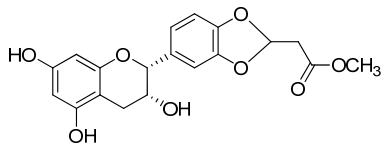




3

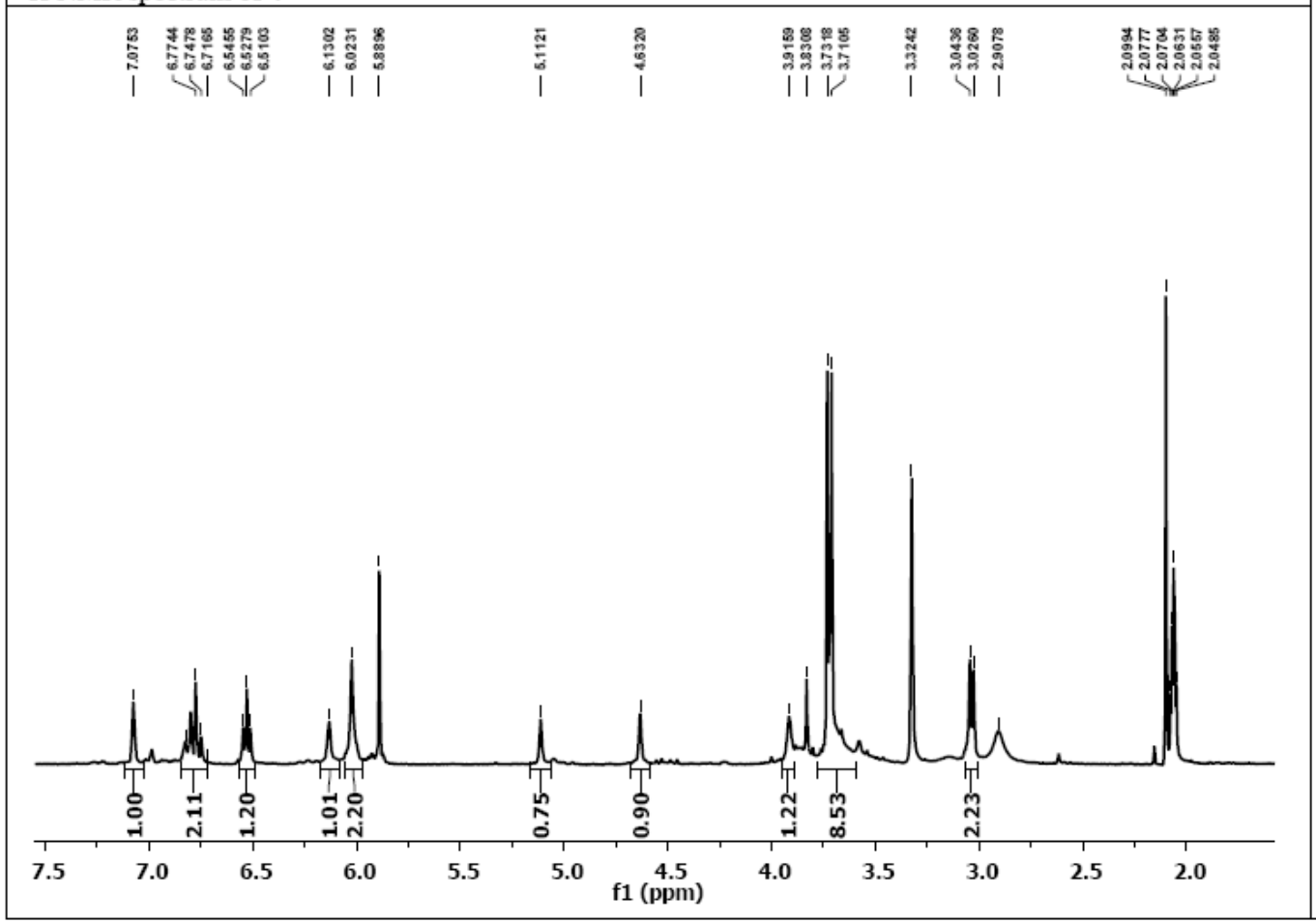
^{13}C NMR spectrum of **3**

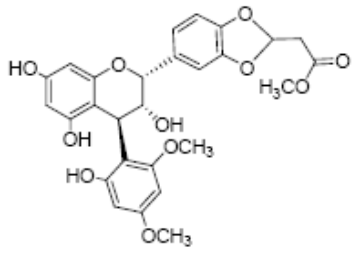




3

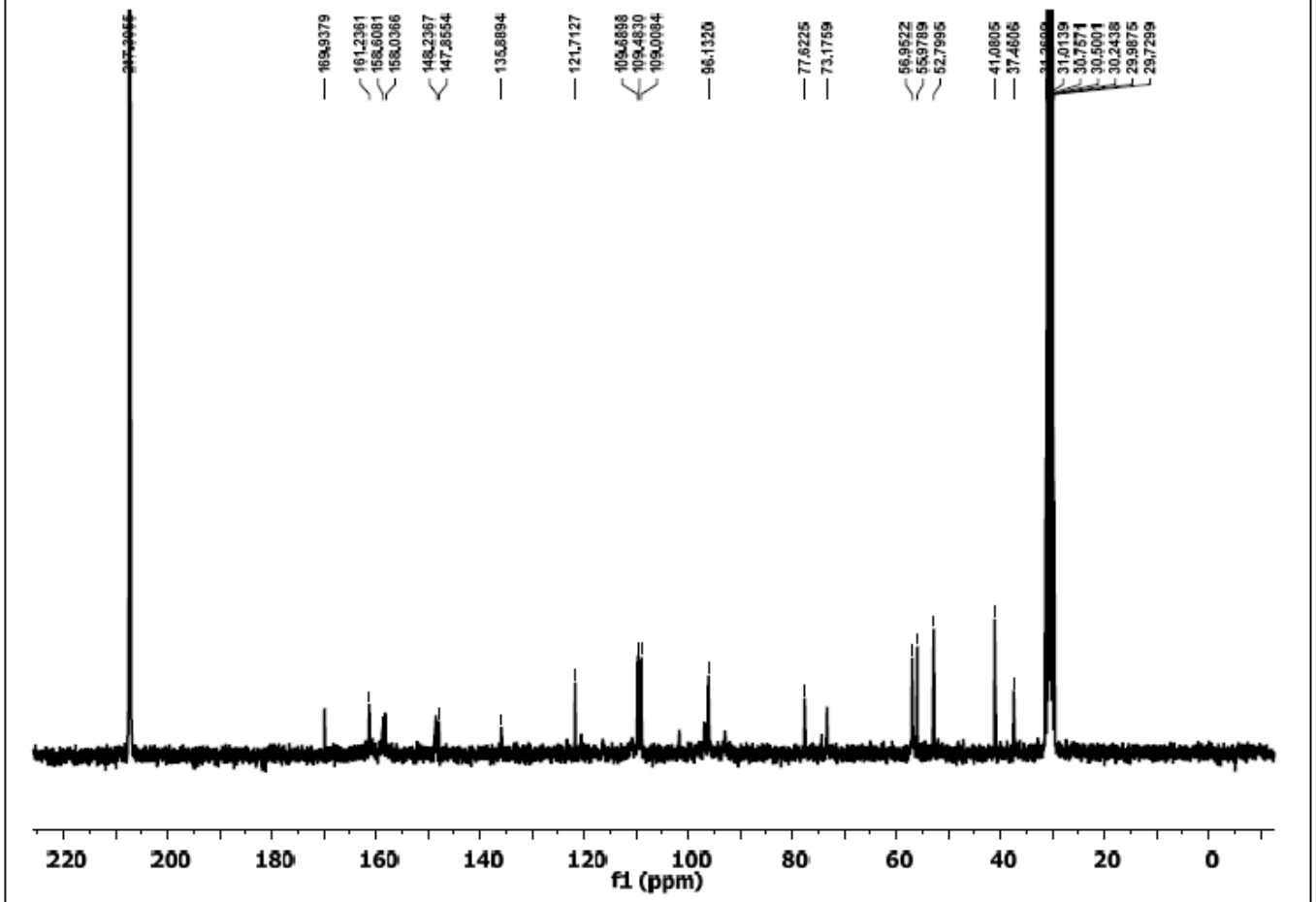
^{1}H NMR spectrum of **4**

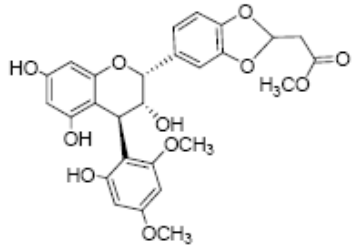




4

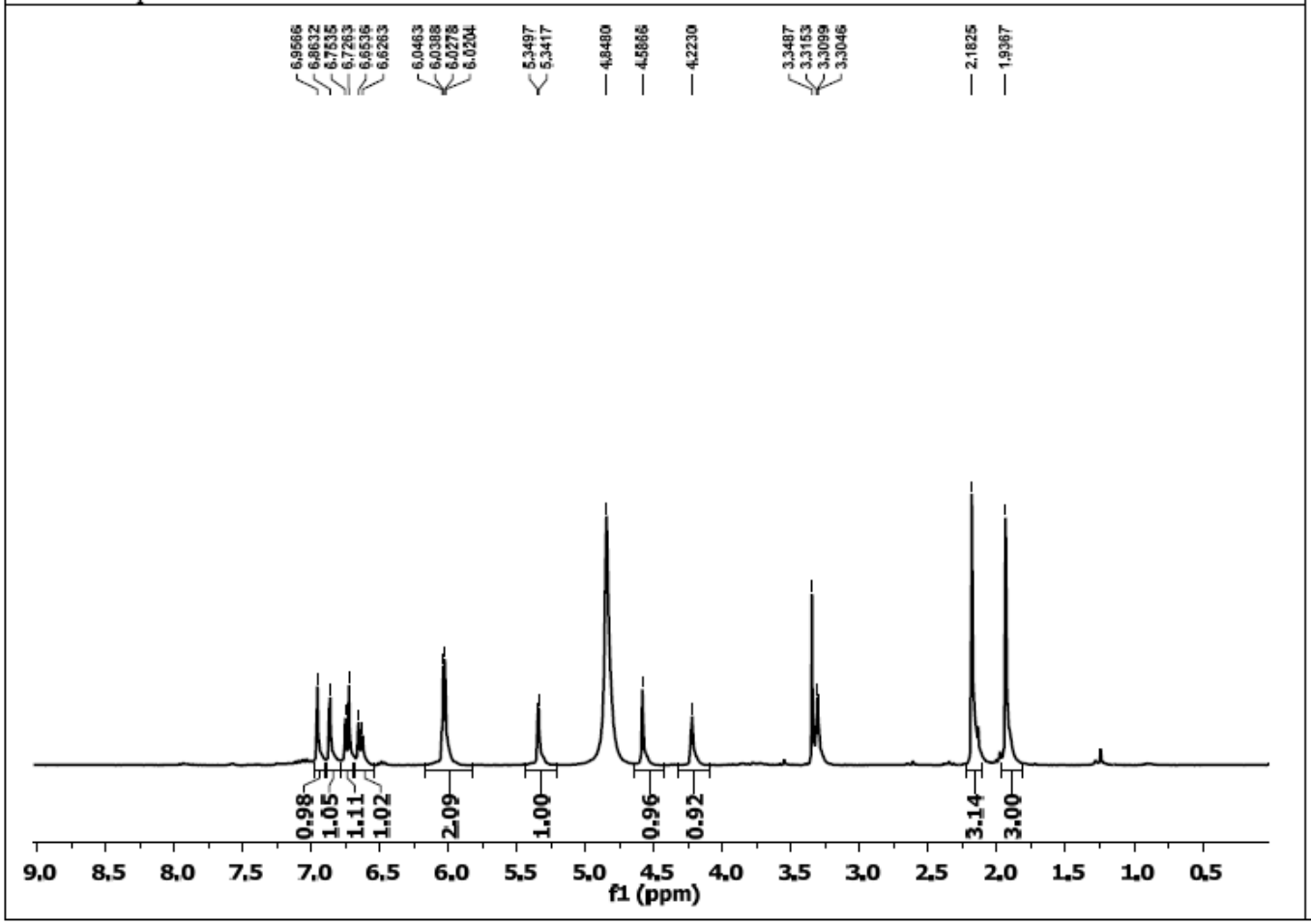
^{13}C NMR spectrum of **4**

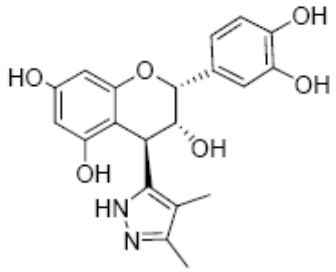




4

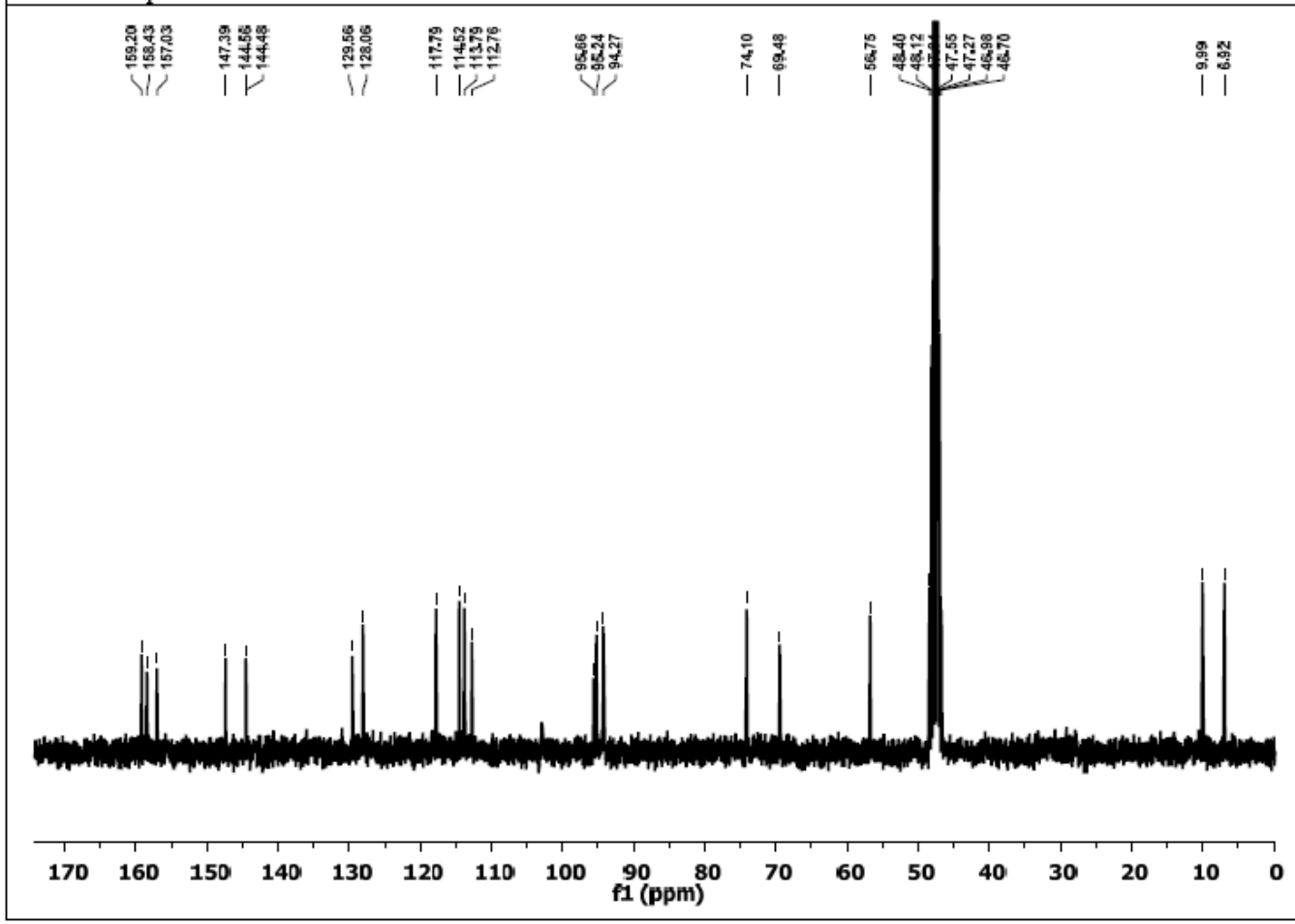
^{1}H NMR spectrum of 5

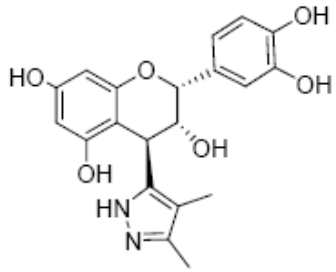




5

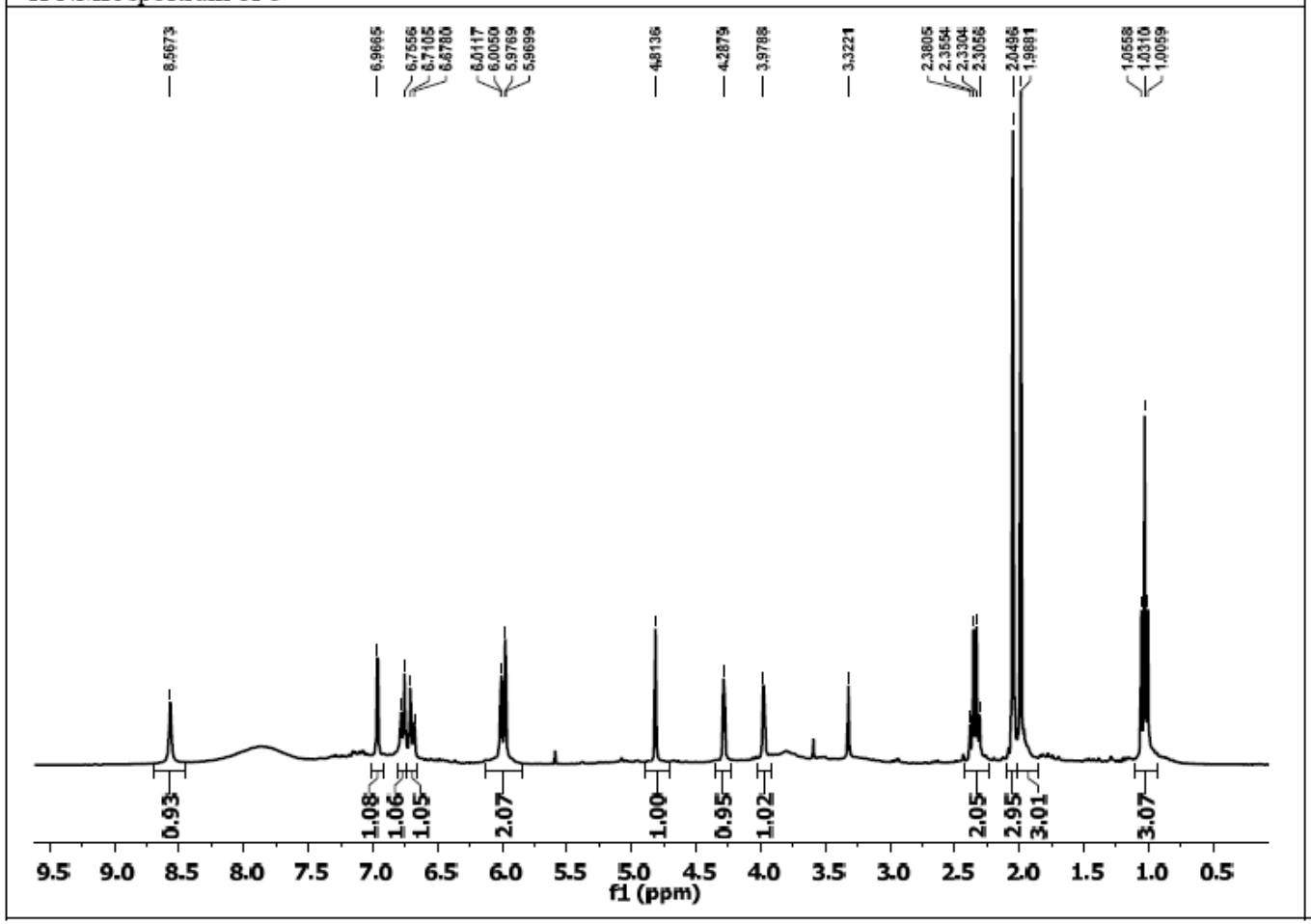
^{13}C NMR spectrum of 5

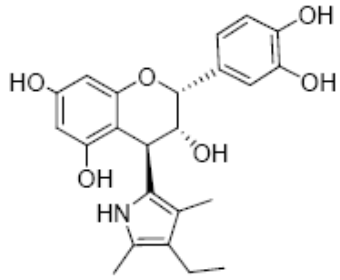




5

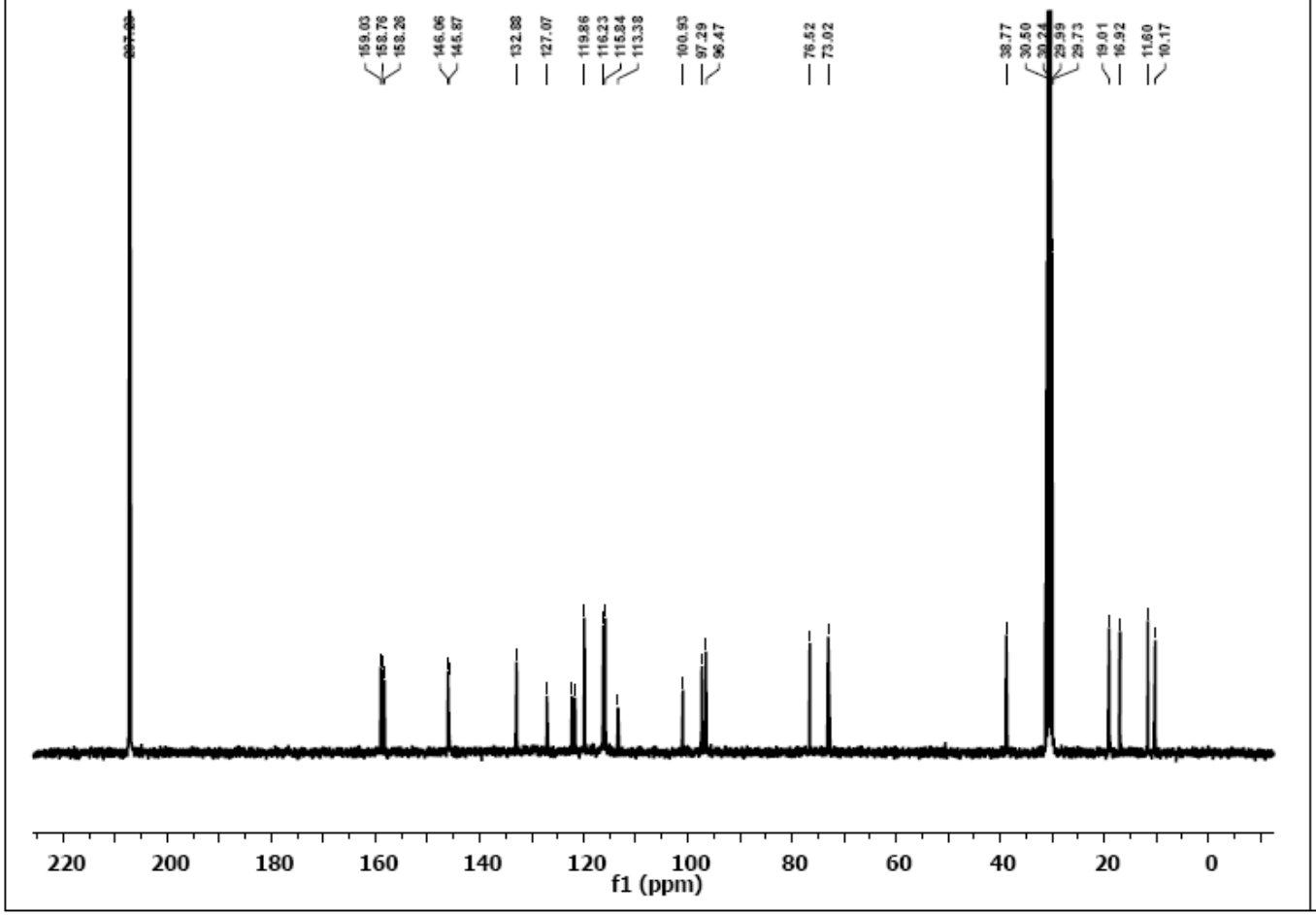
^{1}H NMR spectrum of **6**

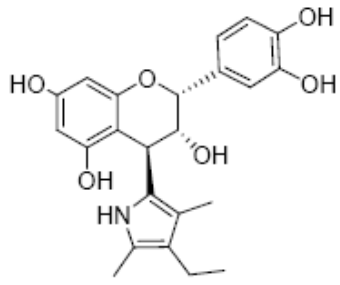




6

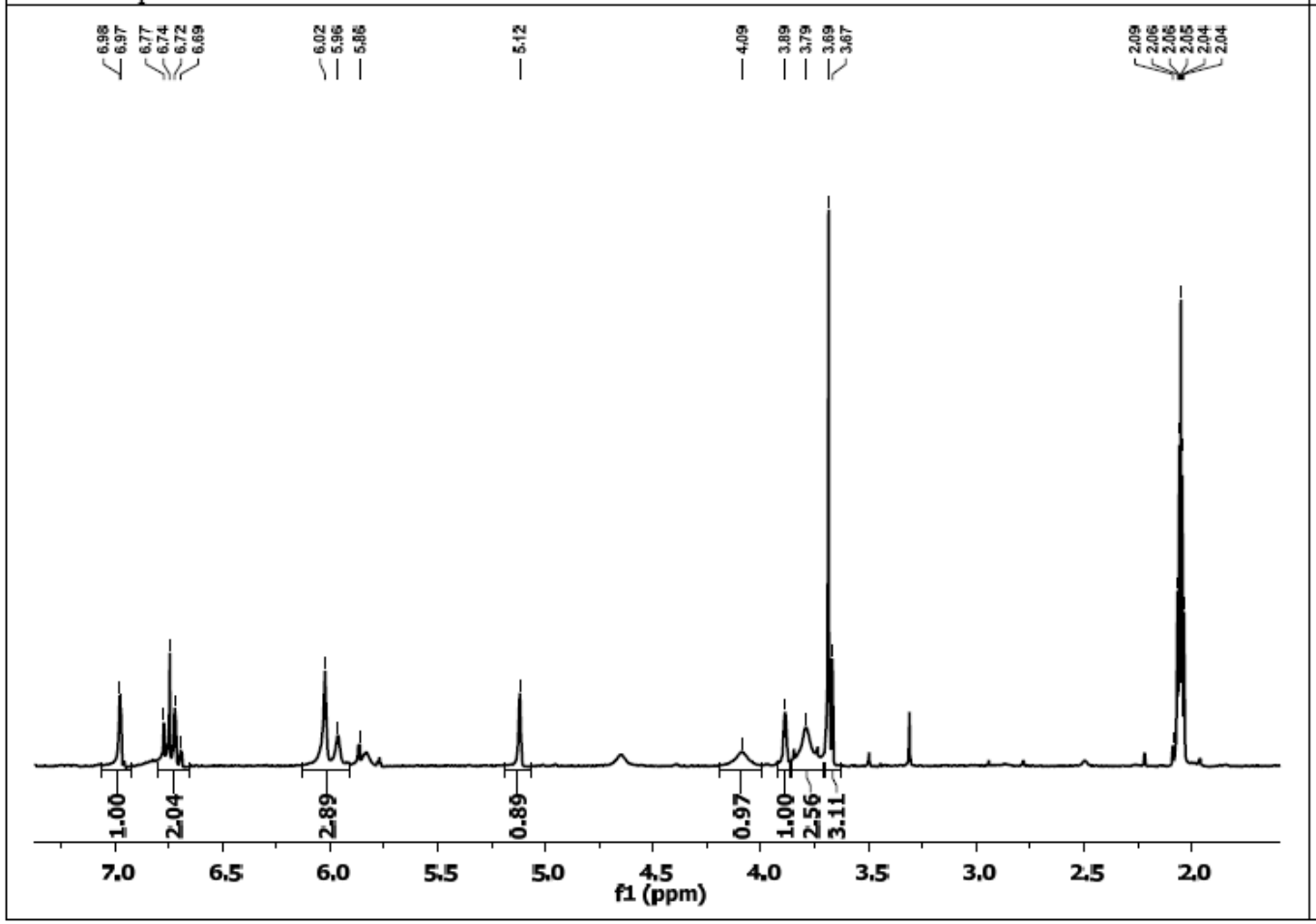
^{13}C NMR spectrum of **6**

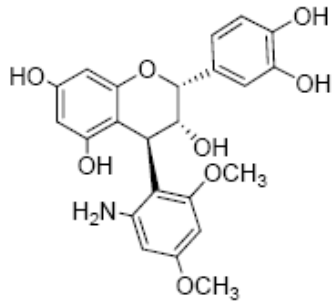




6

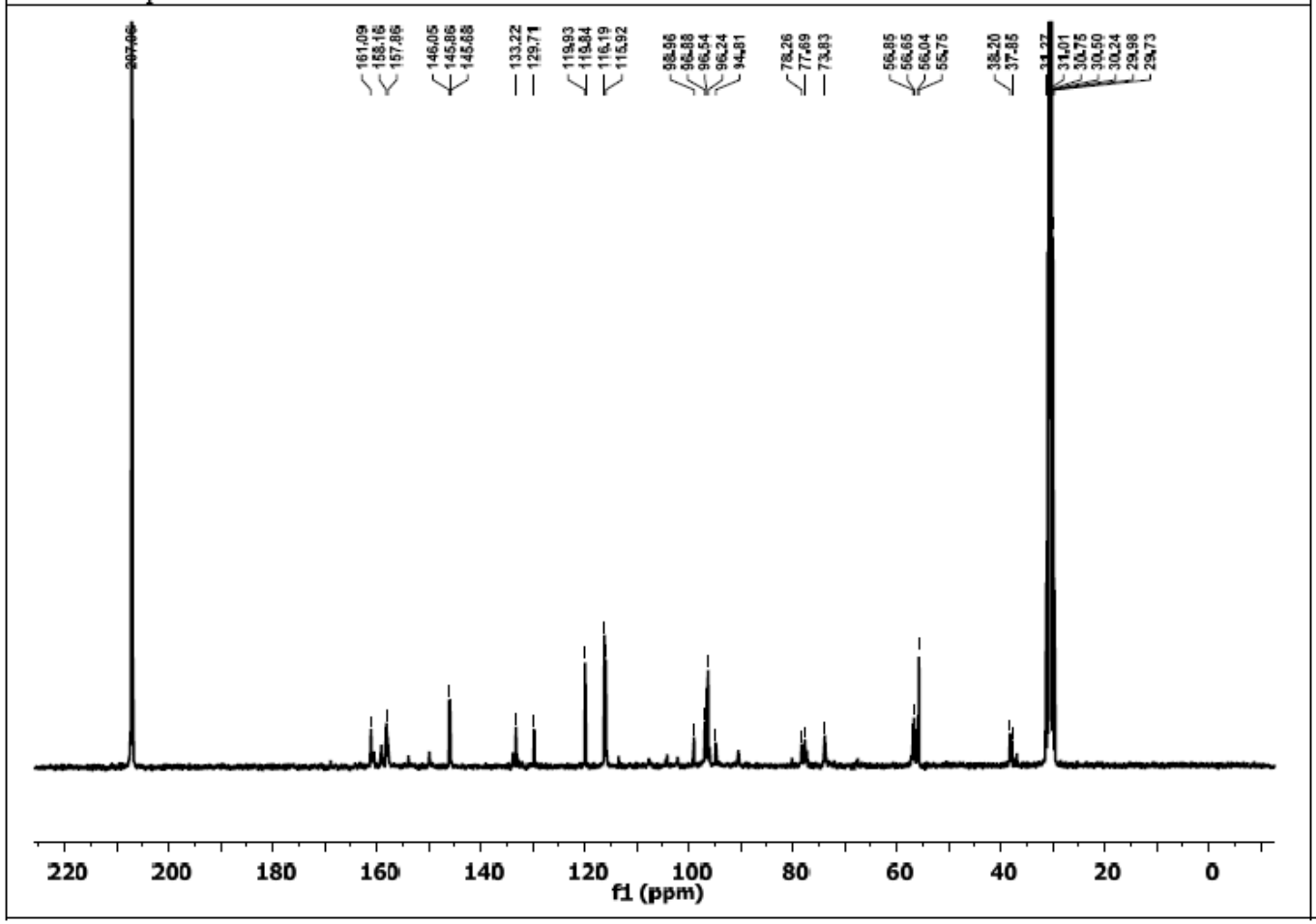
^{1}H NMR spectrum of 7

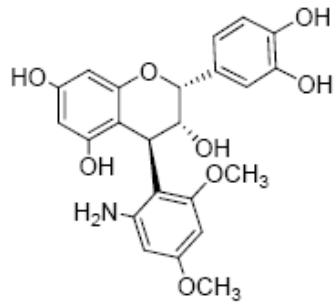




7

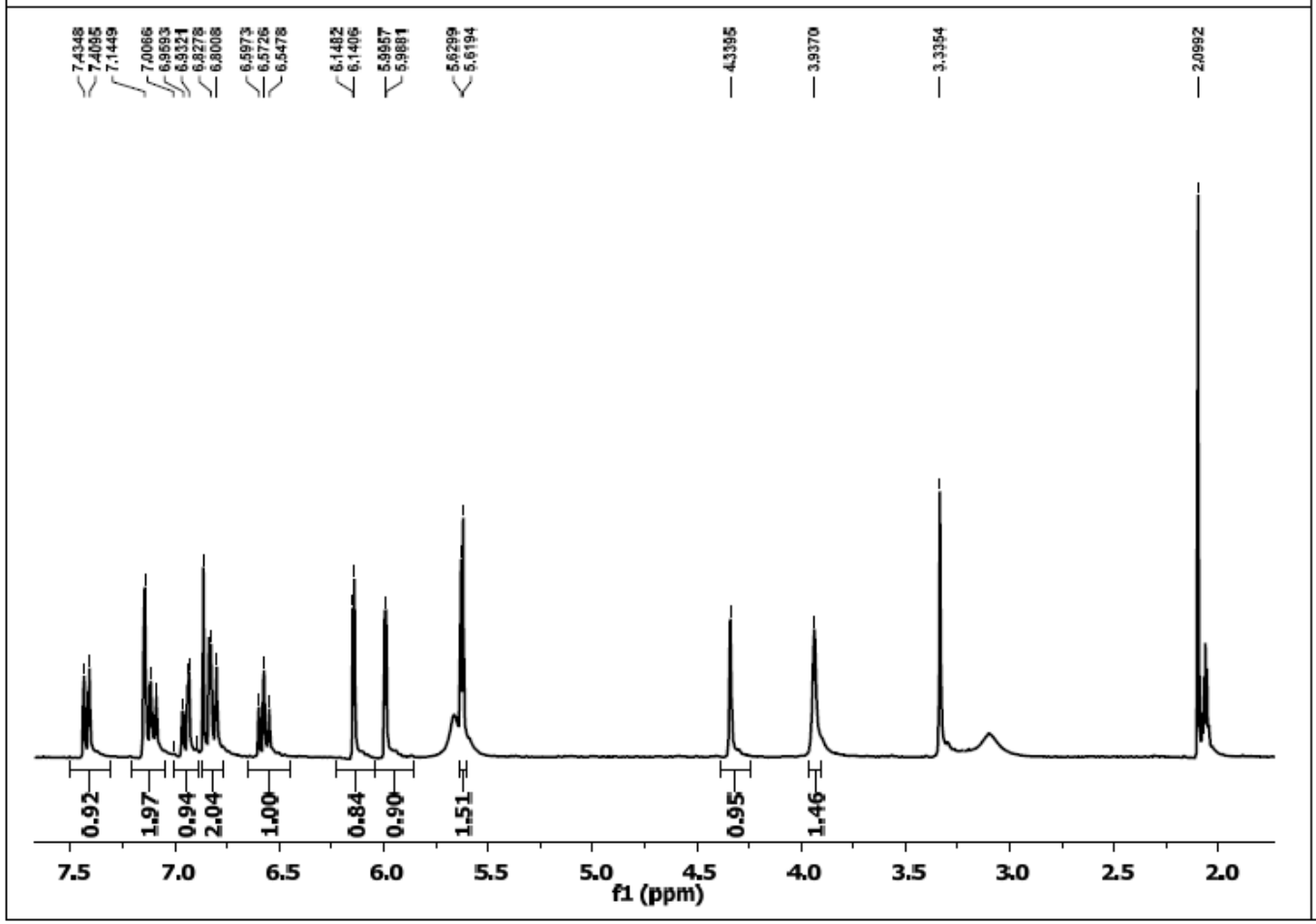
^{13}C NMR spectrum of 7

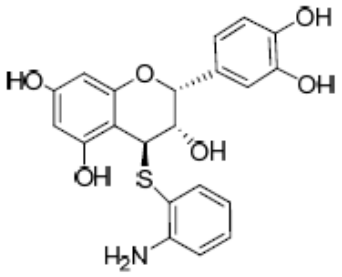




7

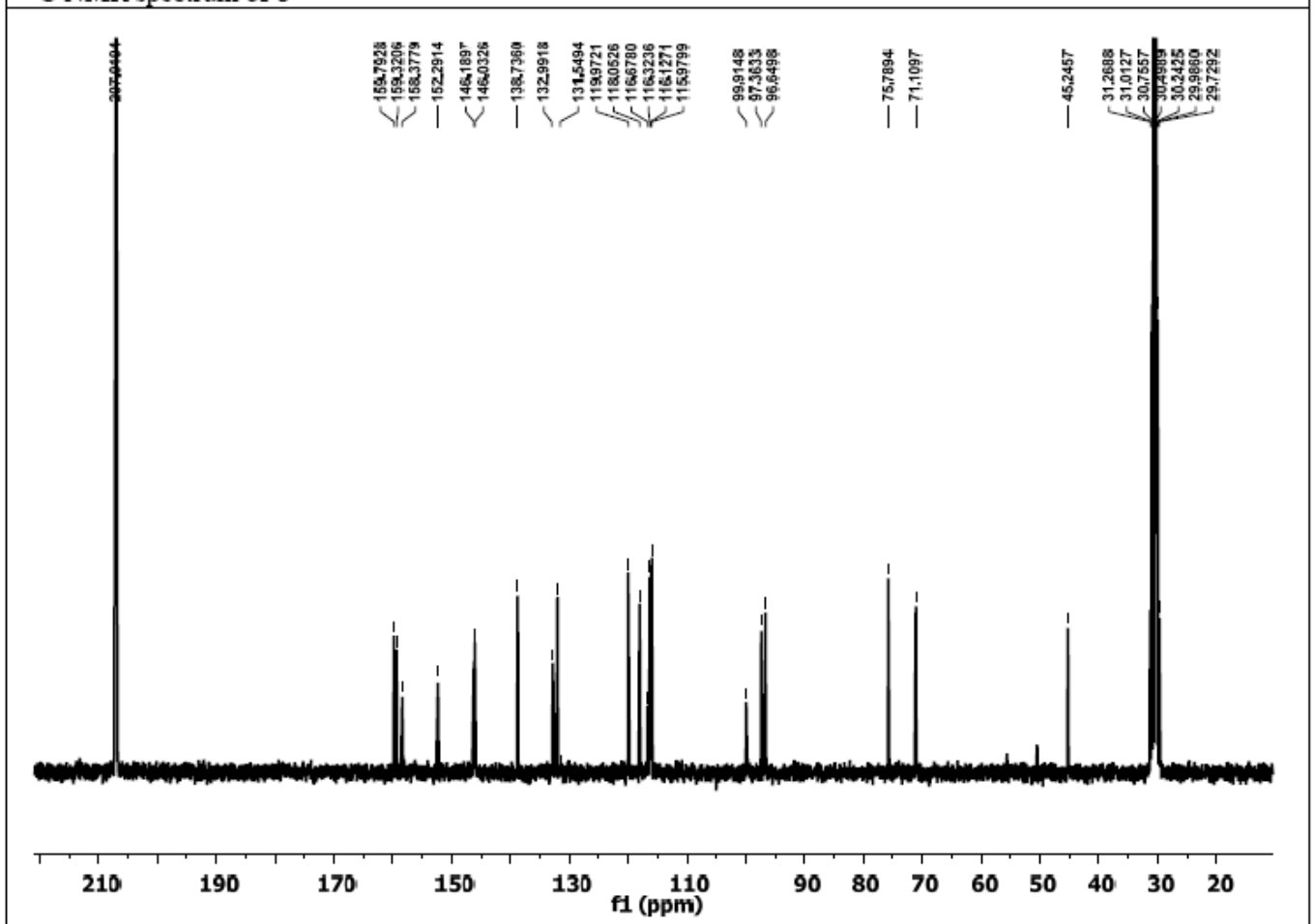
^{1}H NMR spectrum of 8

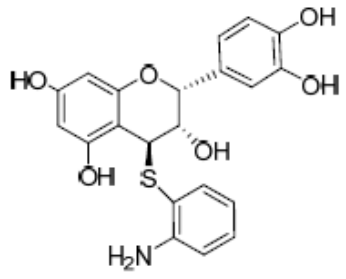




8

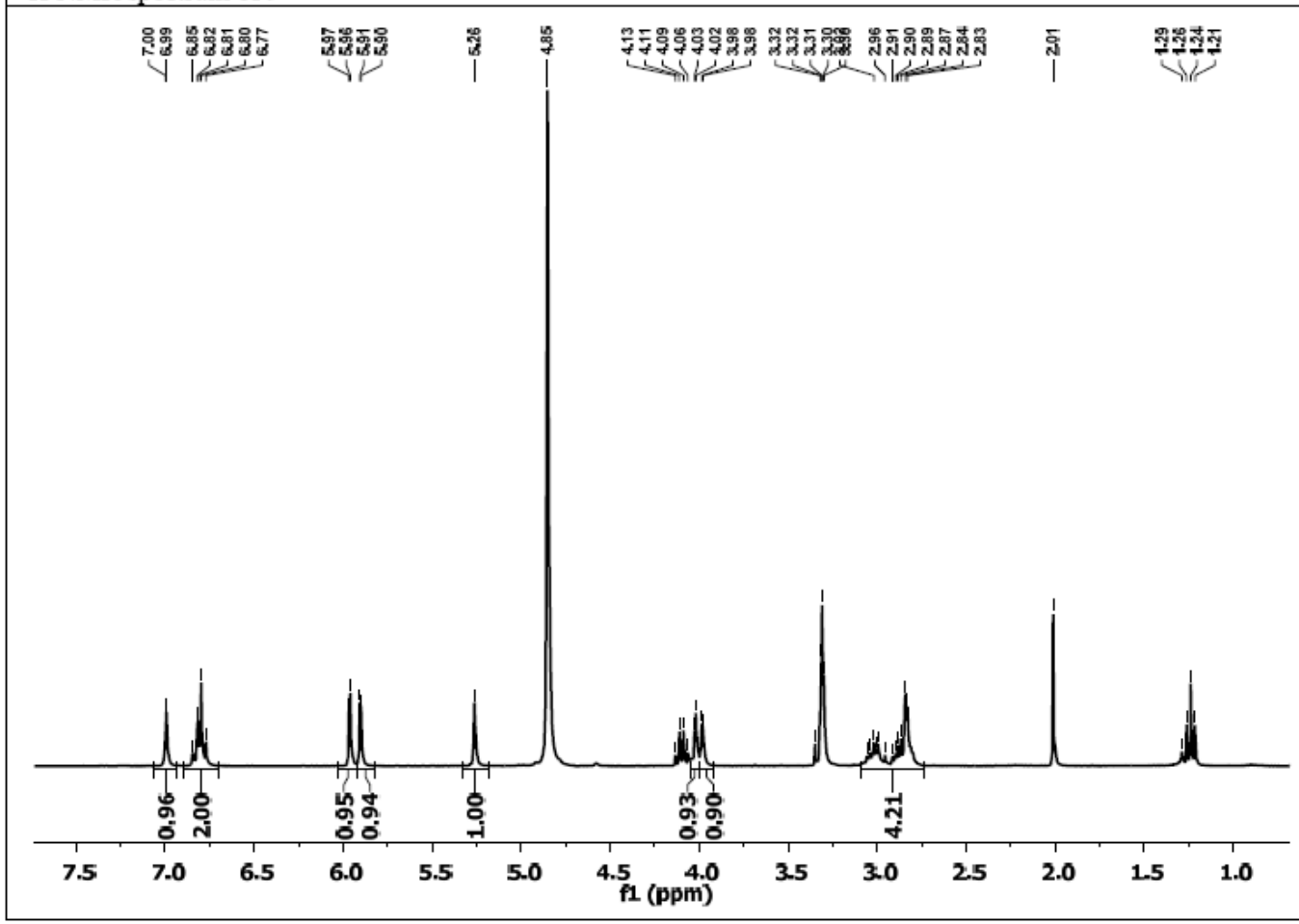
^{13}C NMR spectrum of **8**

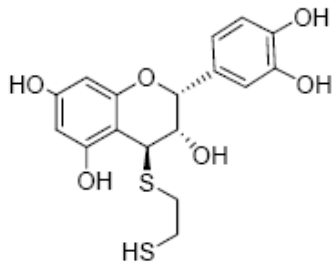




8

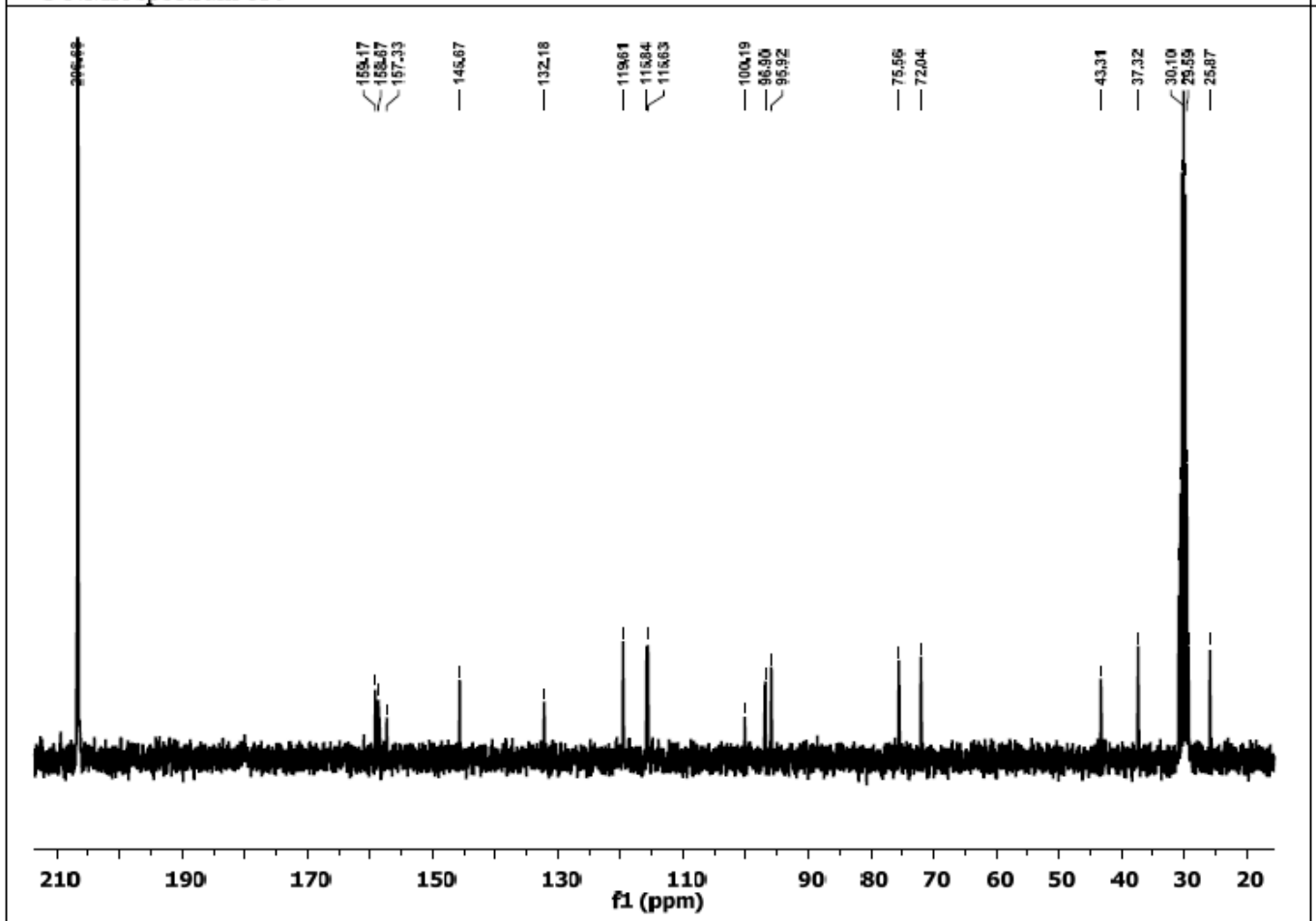
^{1}H NMR spectrum of **9**

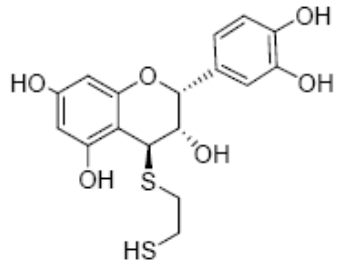




9

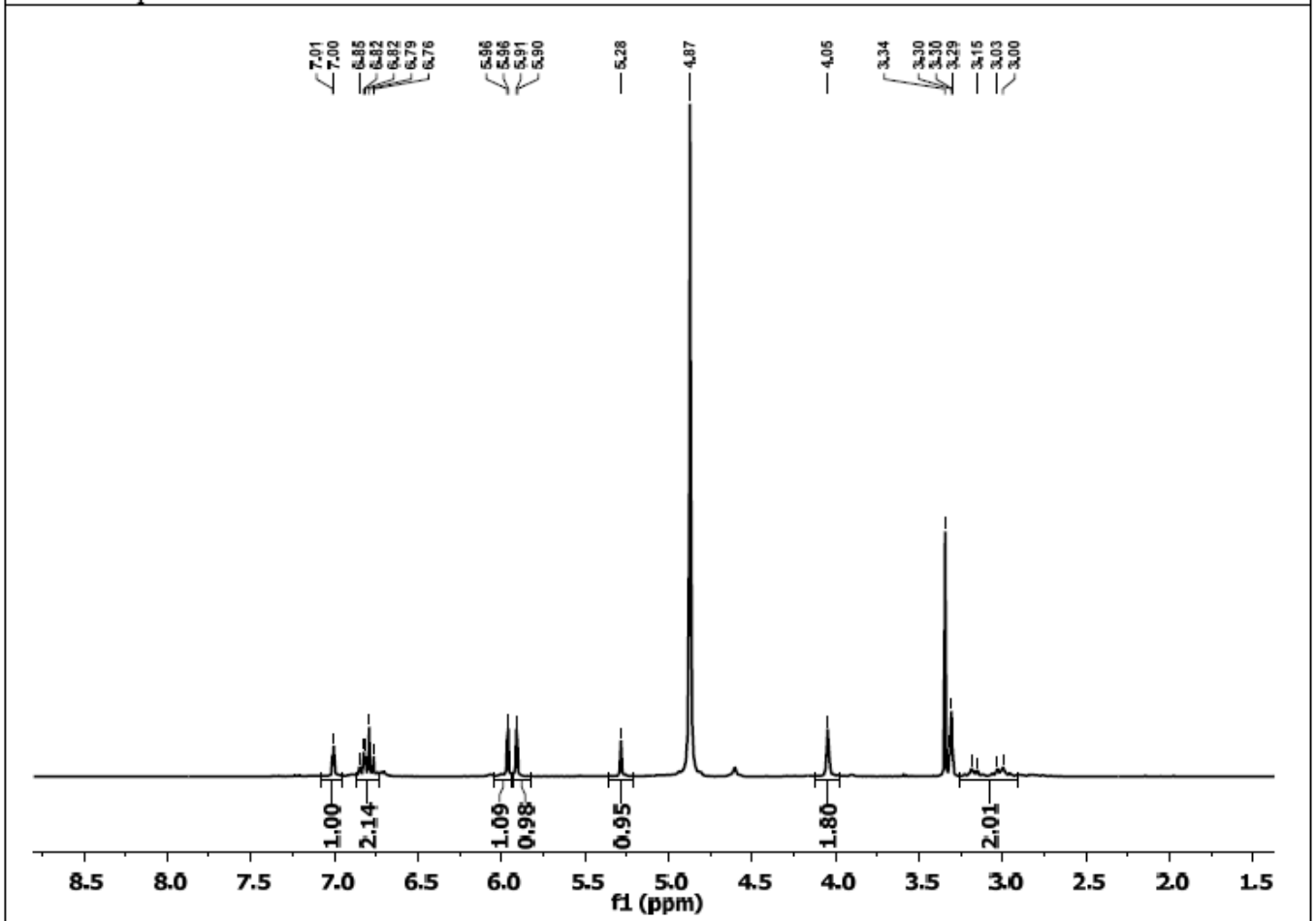
^{13}C NMR spectrum of **9**

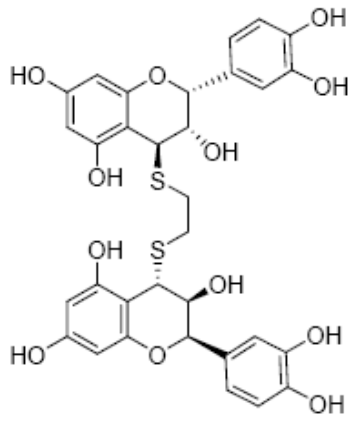




9

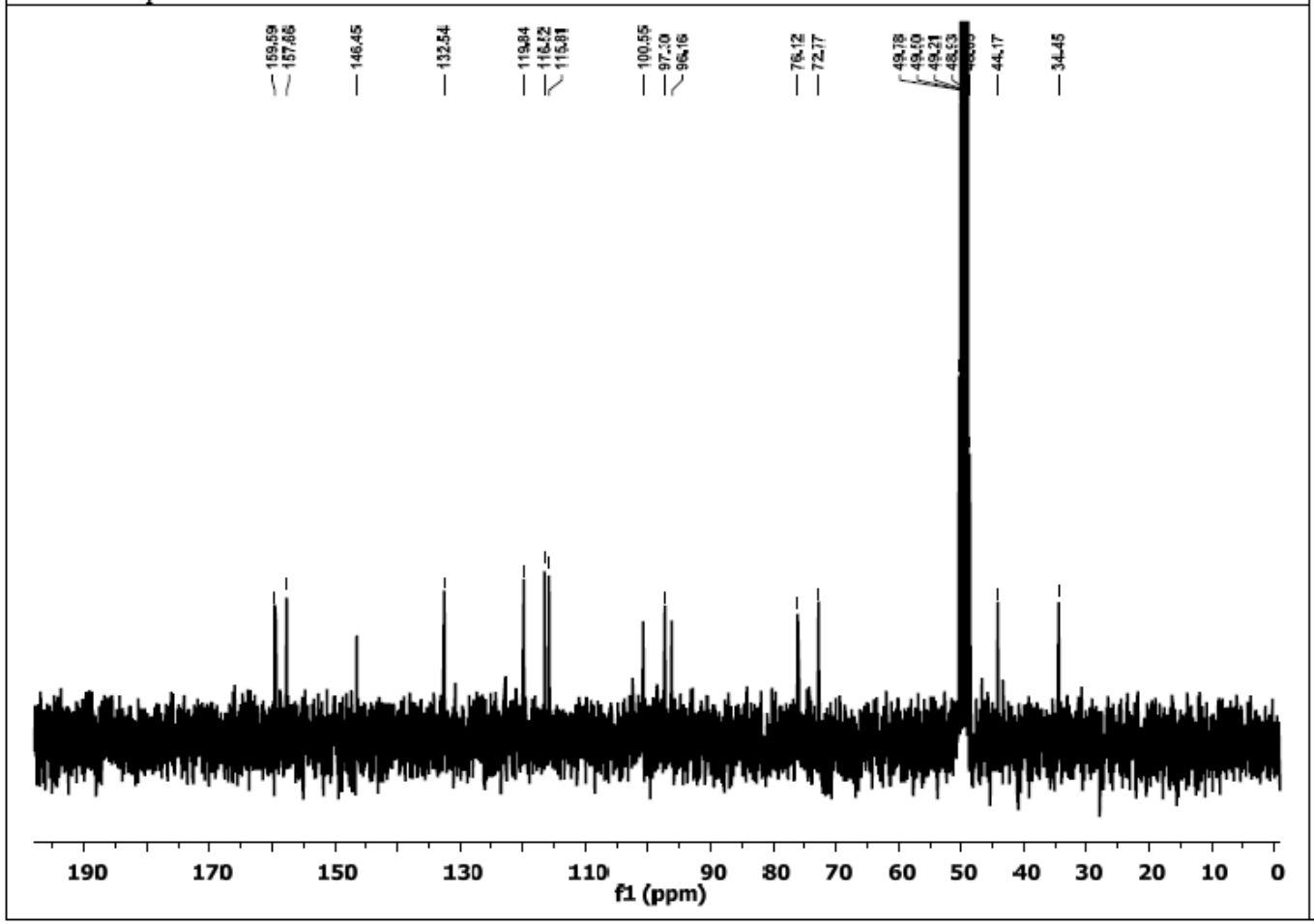
^{1}H NMR spectrum of **10**

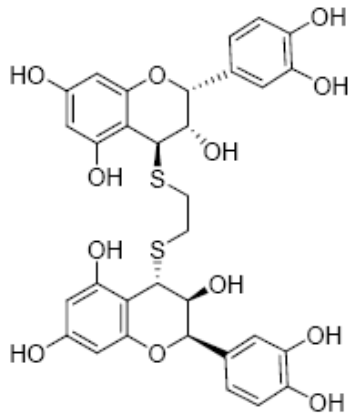




10

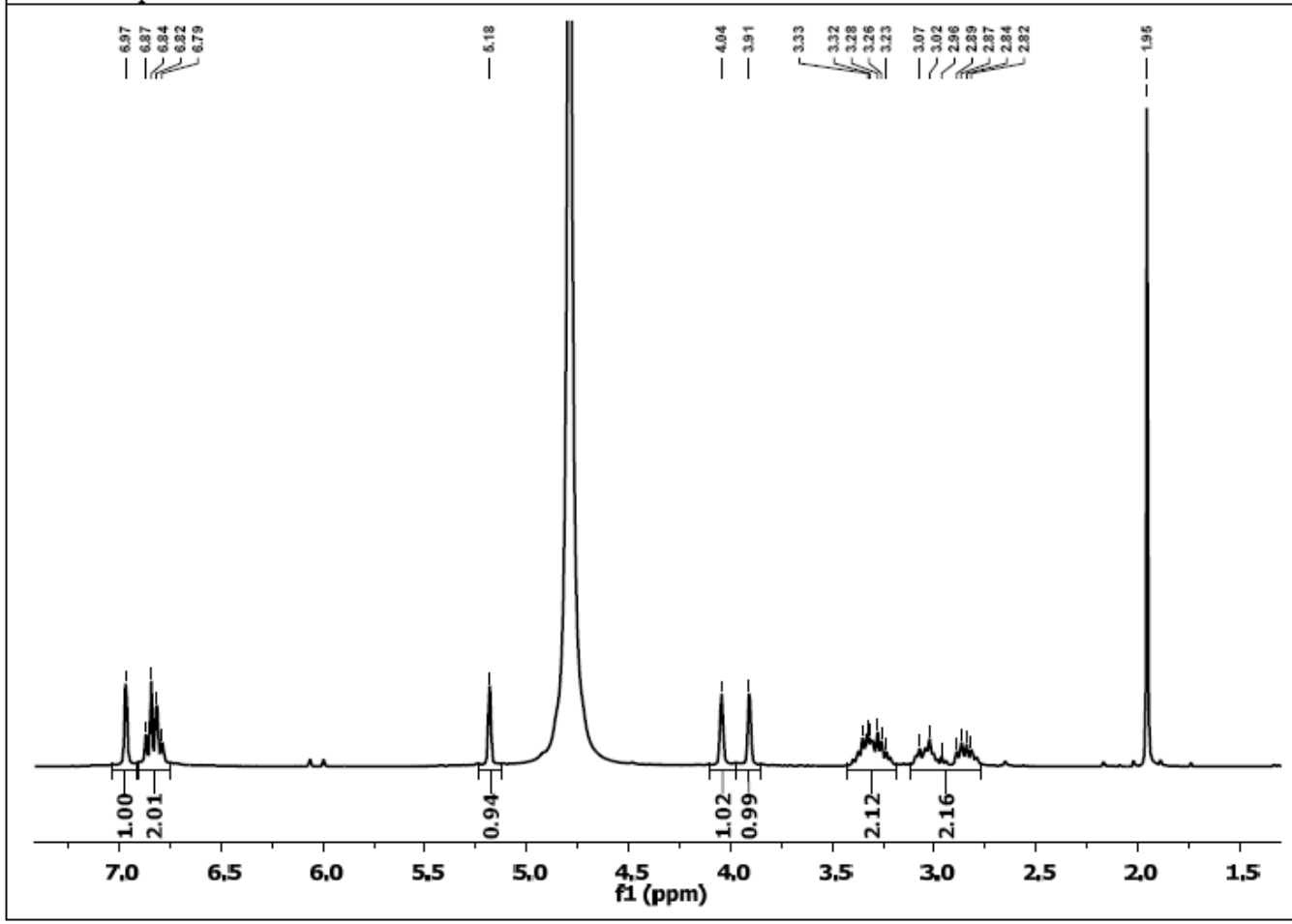
^{13}C NMR spectrum of **10**

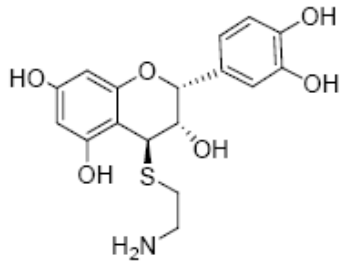




10

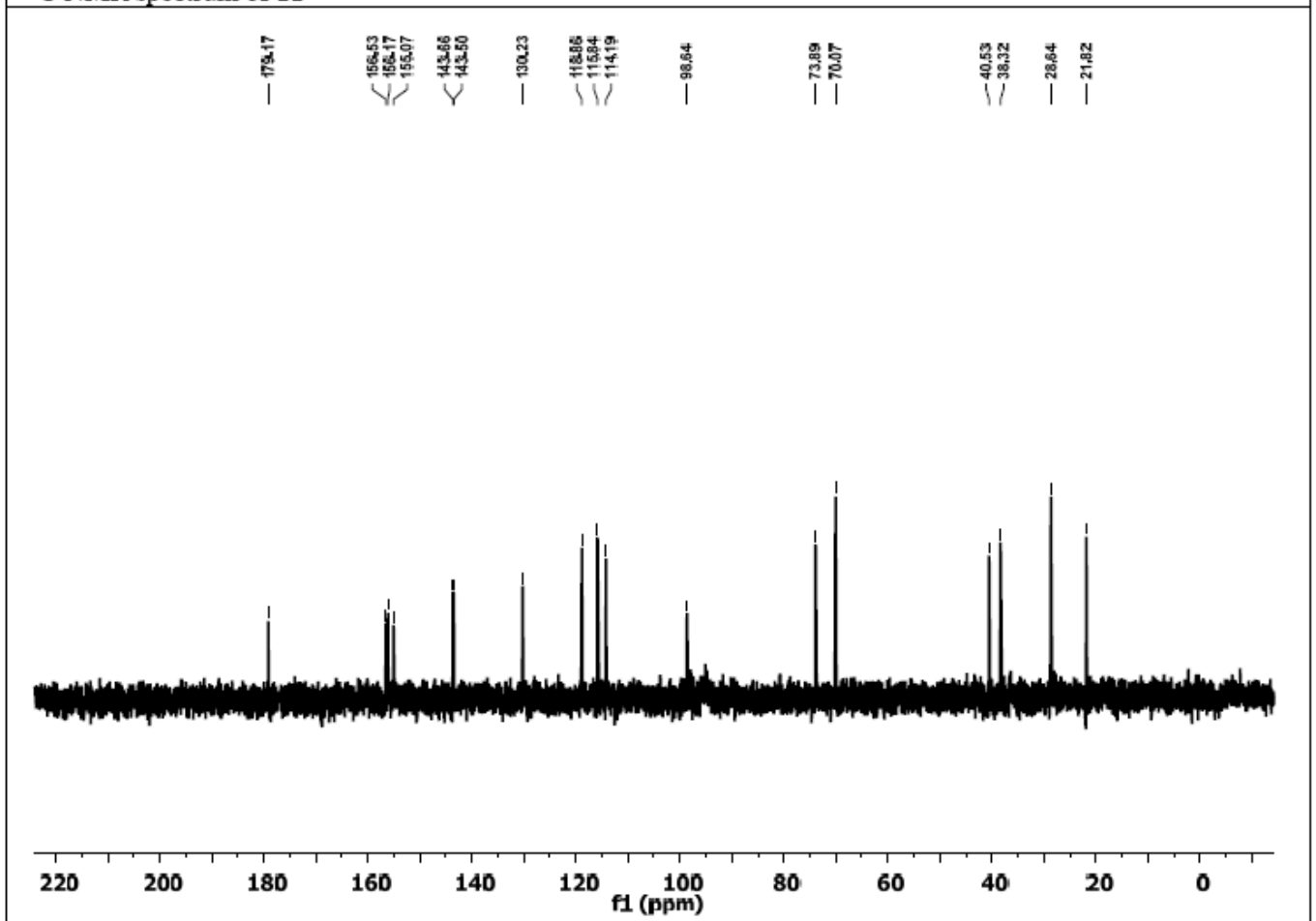
^{1}H NMR spectrum of **11**

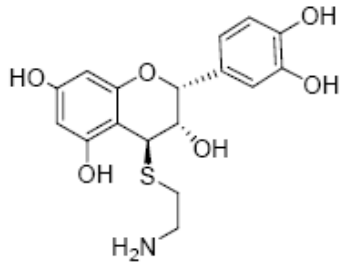




11

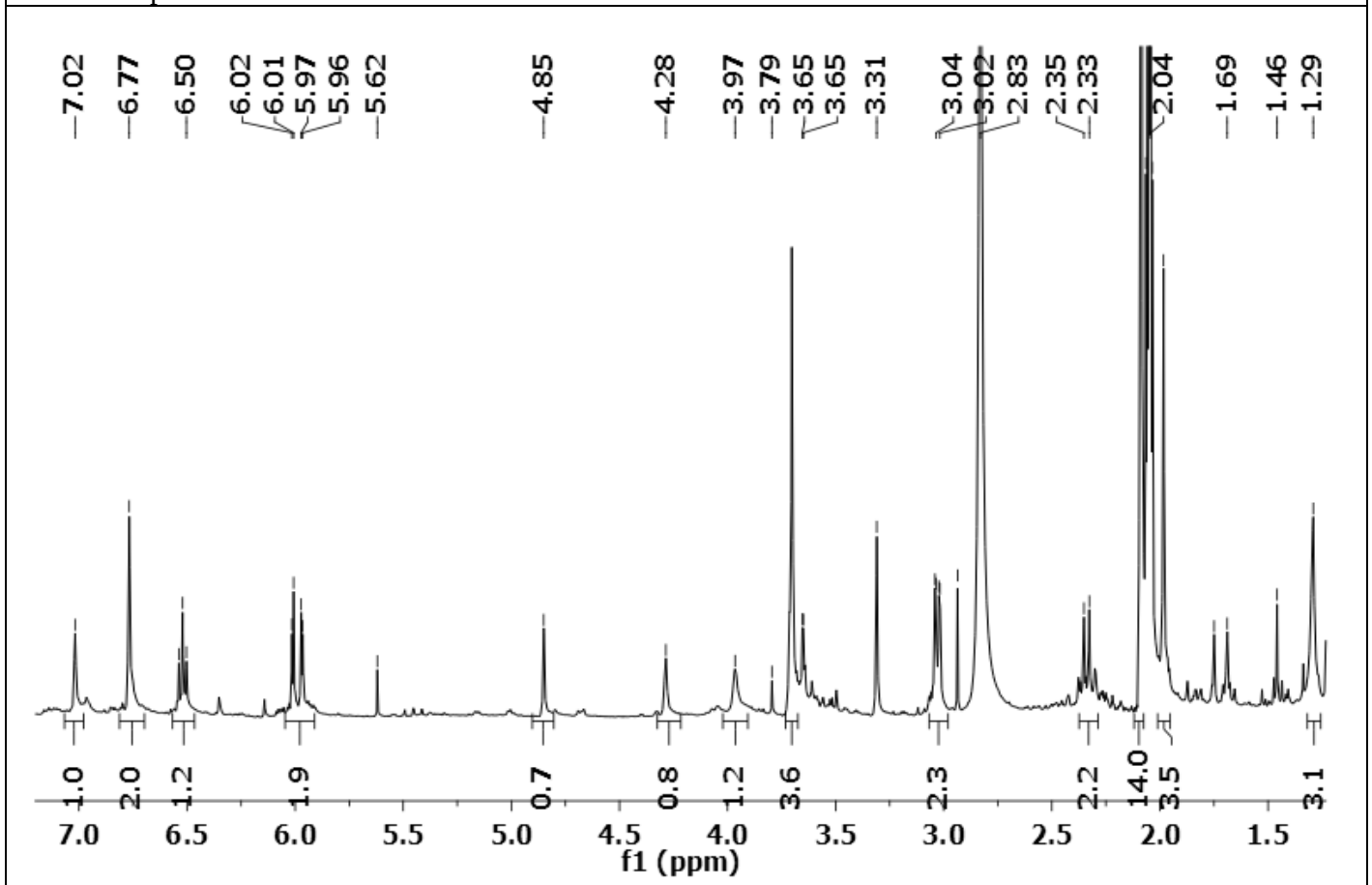
^{13}C NMR spectrum of **11**

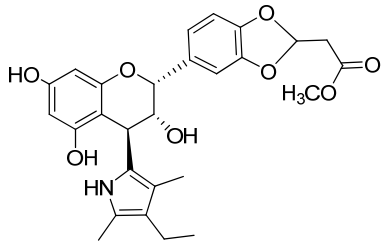




11

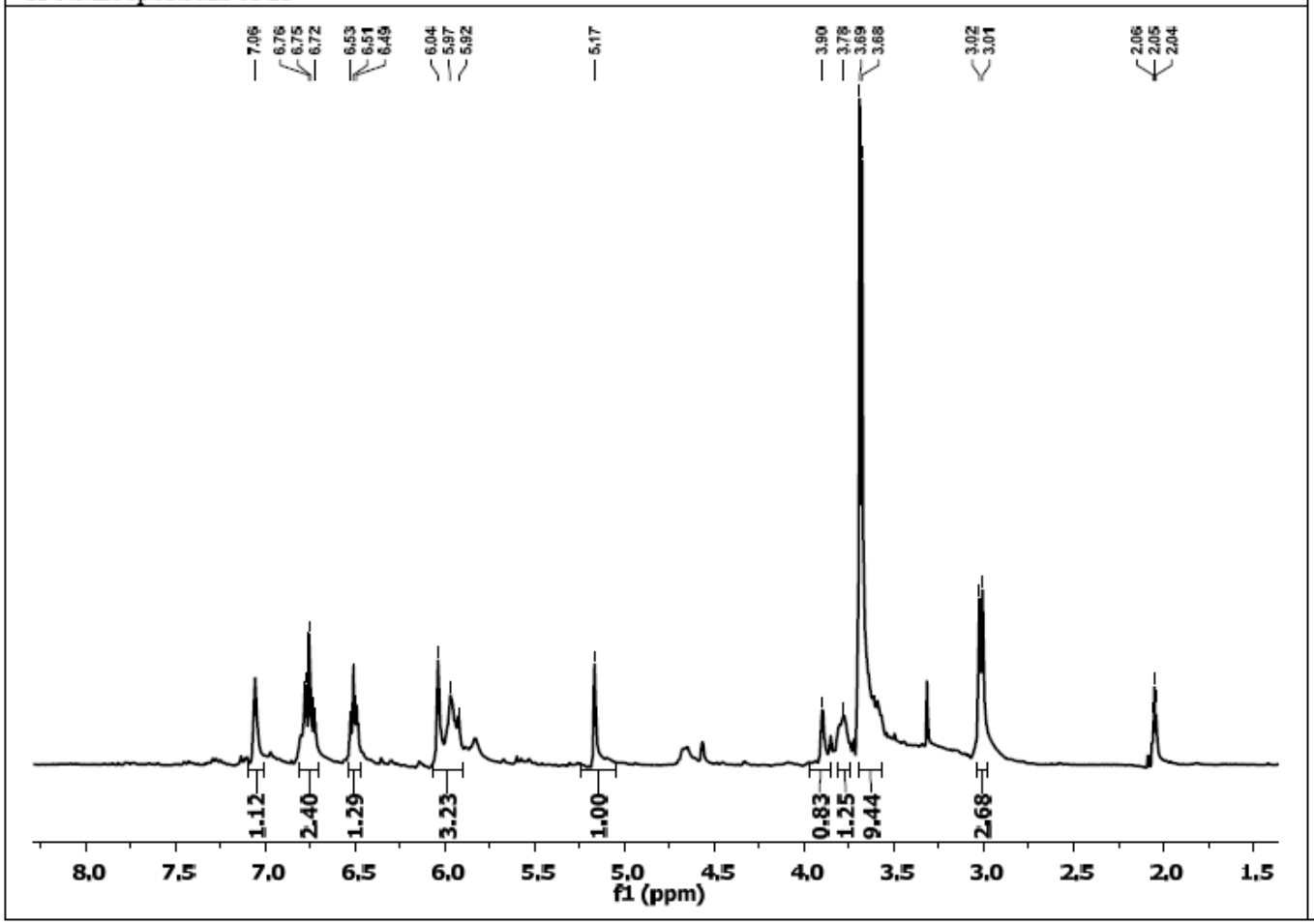
^{1}H NMR spectrum of **12**

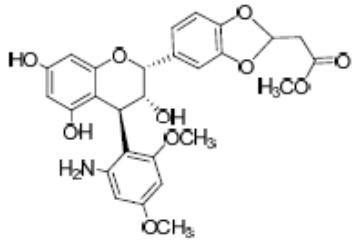




12

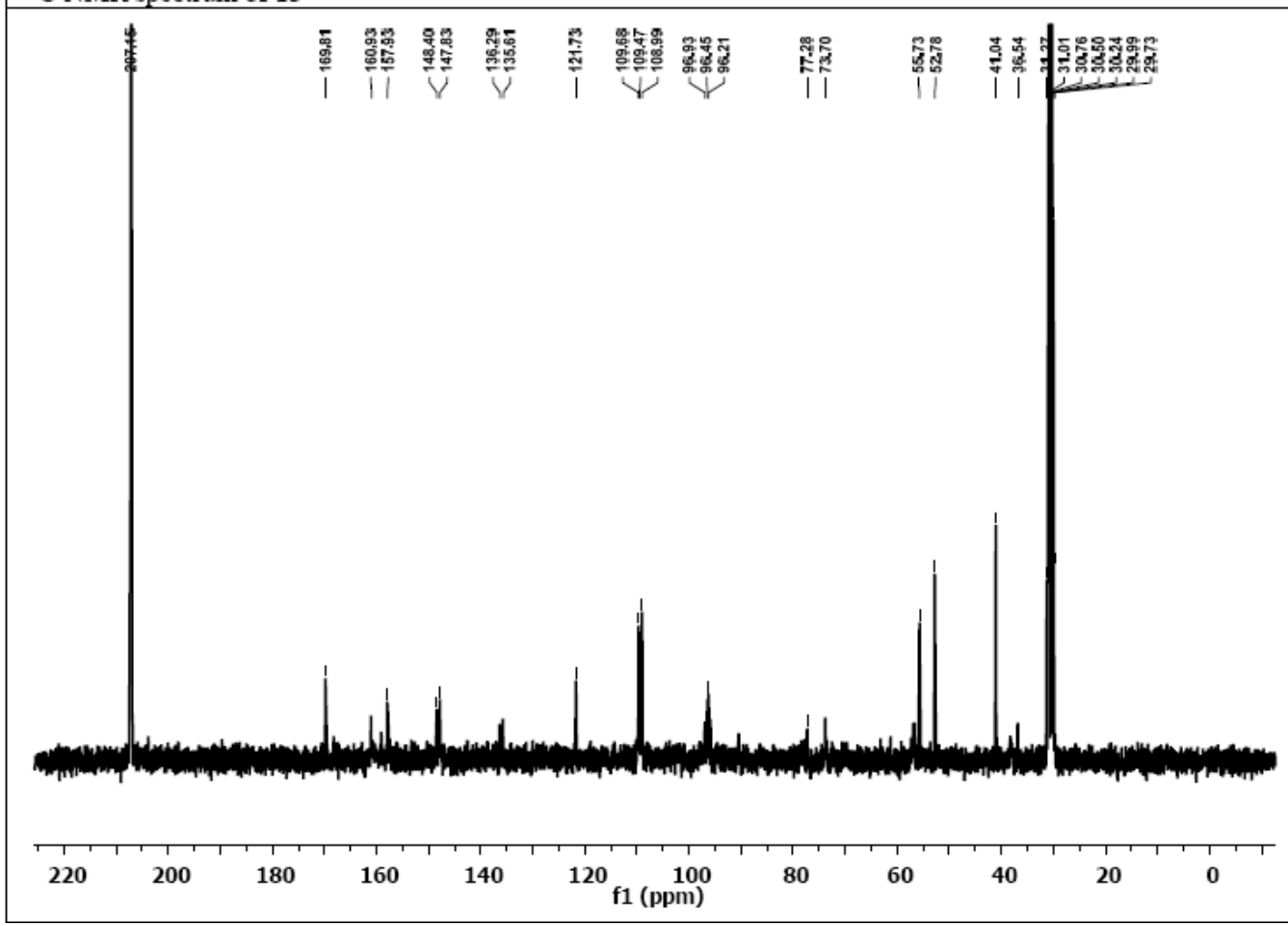
^{1}H NMR spectrum of **13**

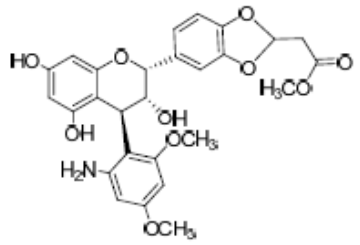




13

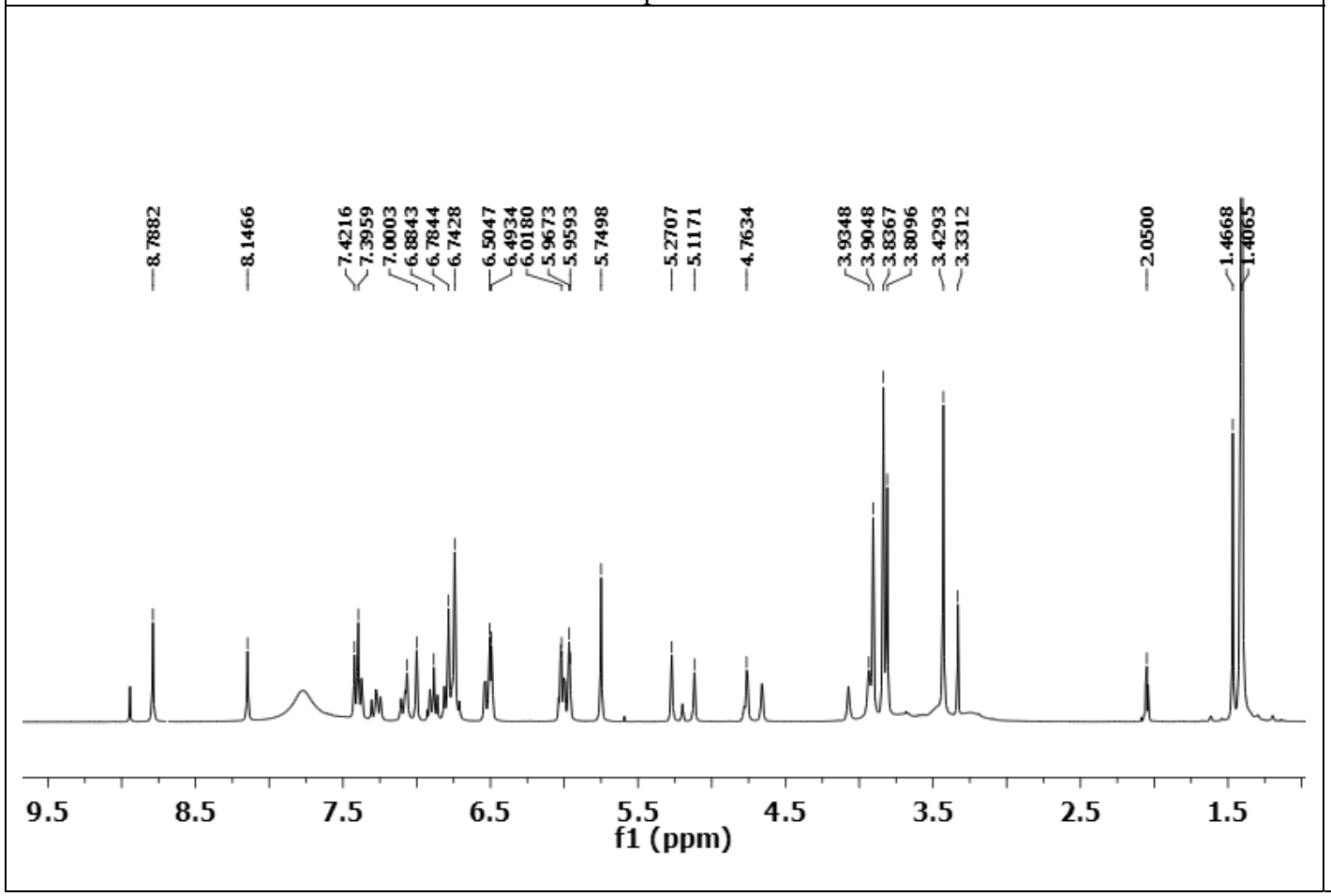
^{13}C NMR spectrum of **13**

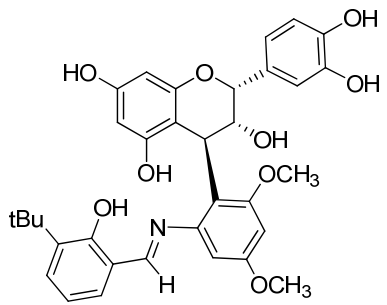




13

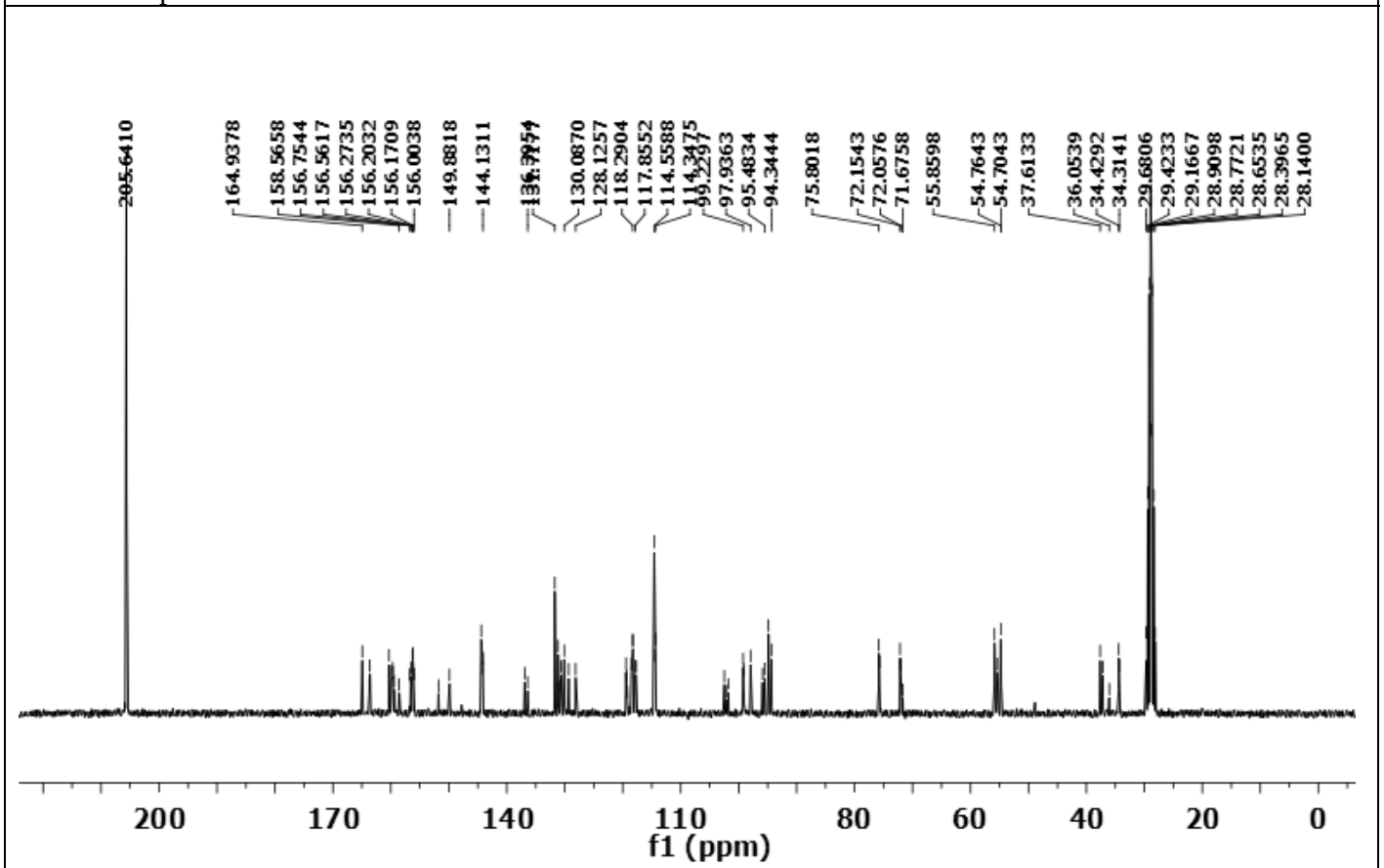
^{1}H NMR spectrum of **14**

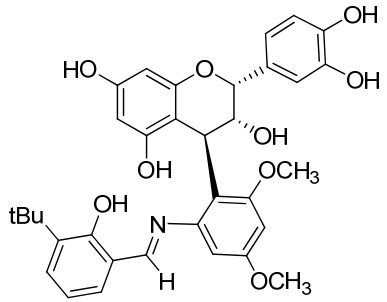




14

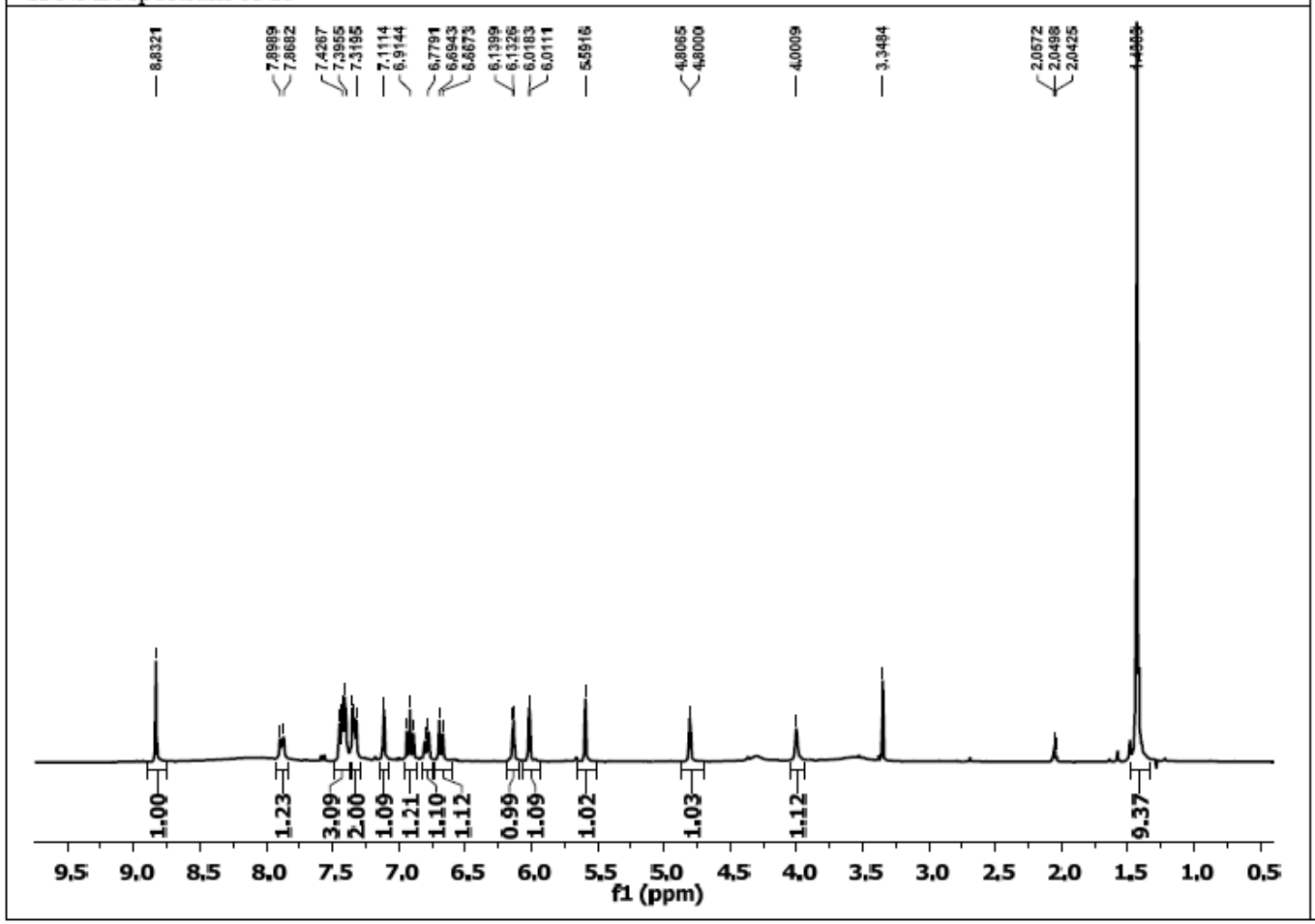
^{13}C NMR spectrum of **14**

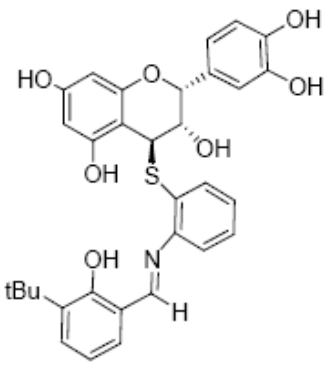




14

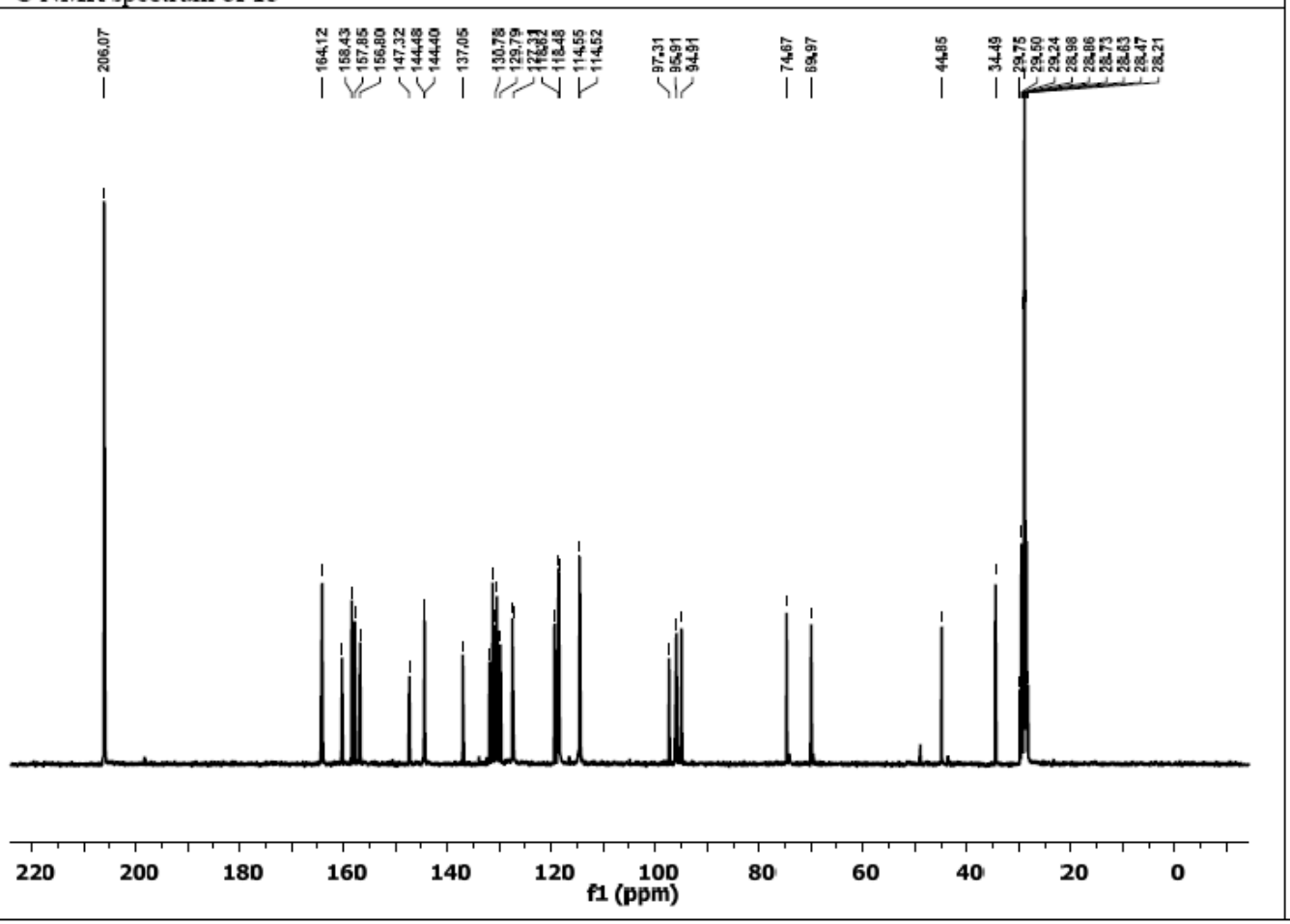
^{1}H NMR spectrum of **15**

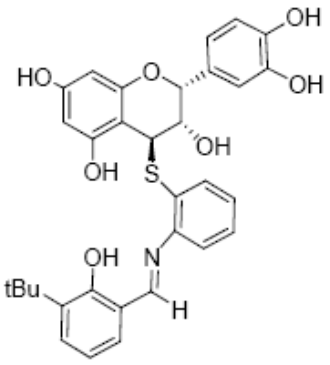




15

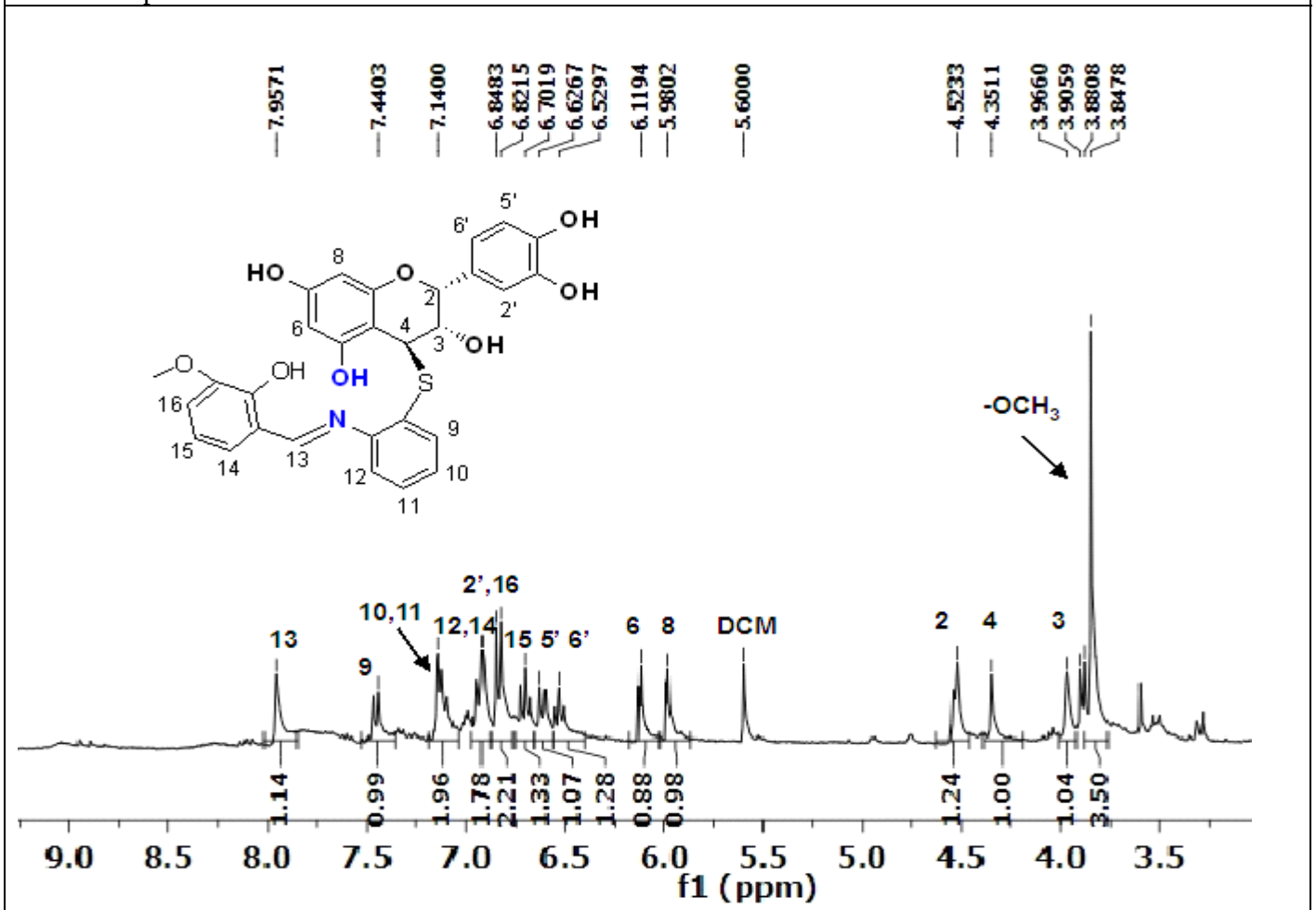
^{13}C NMR spectrum of **15**

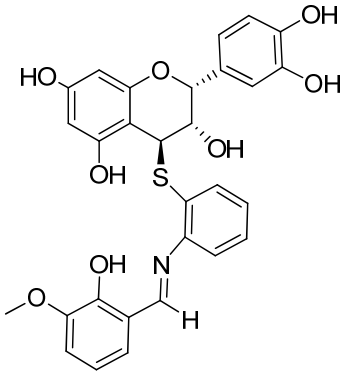




15

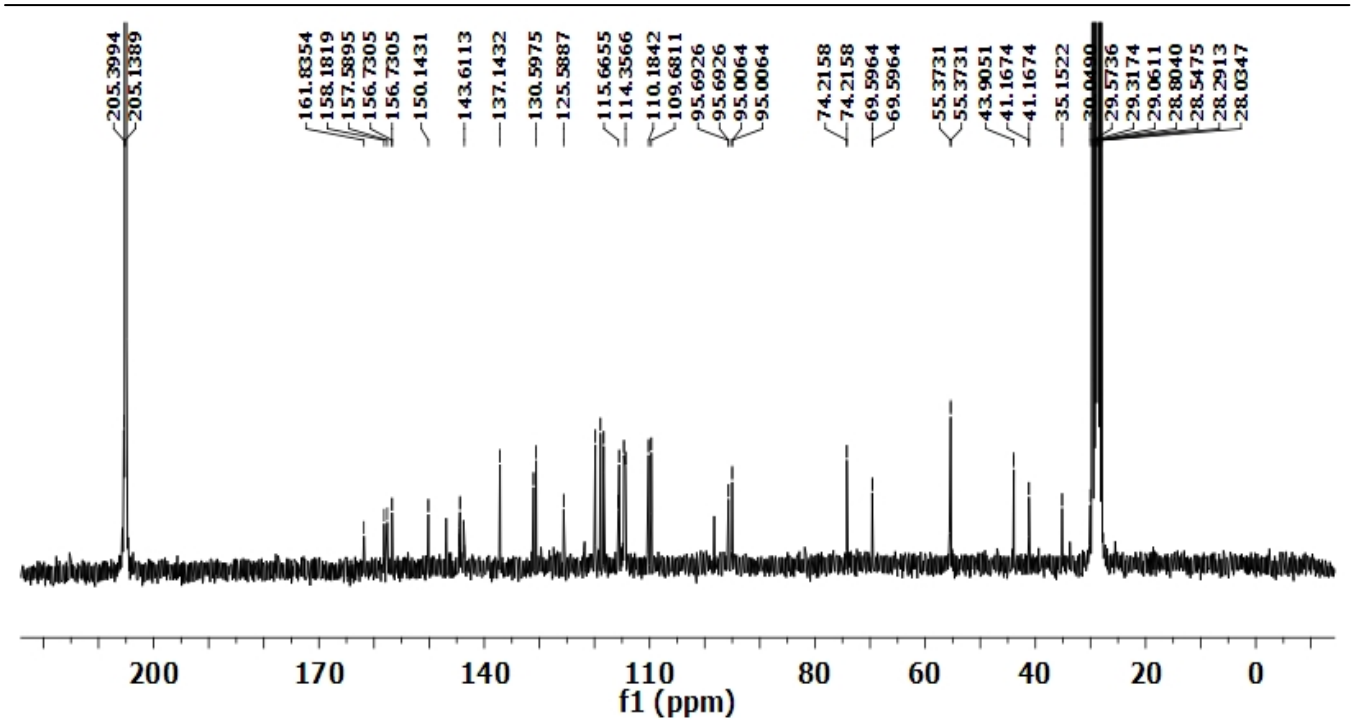
^{1}H NMR spectrum of **16**

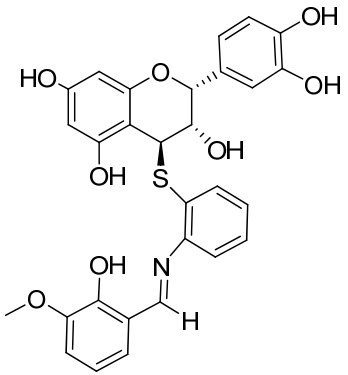




16

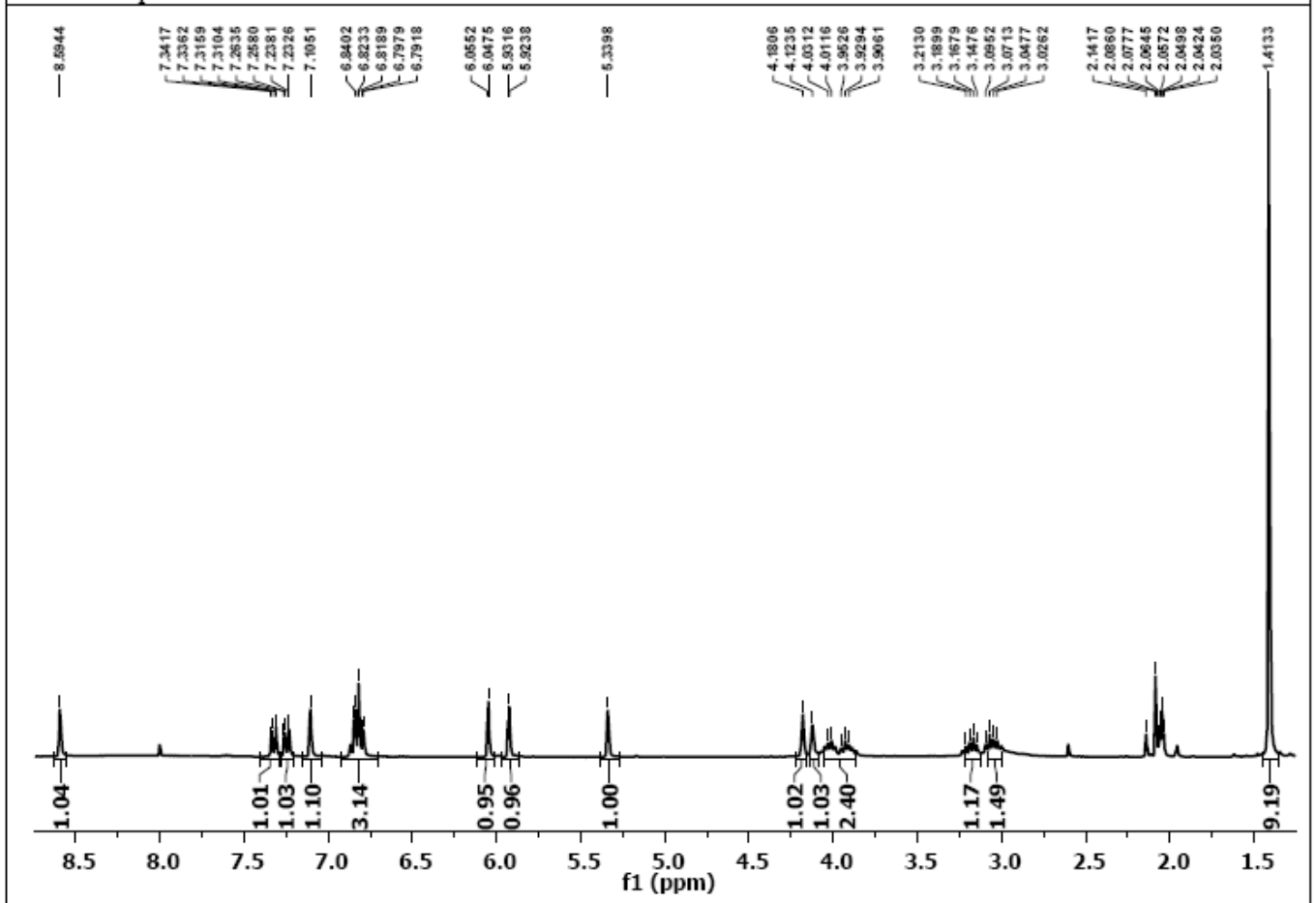
^{13}C NMR spectrum of **16**

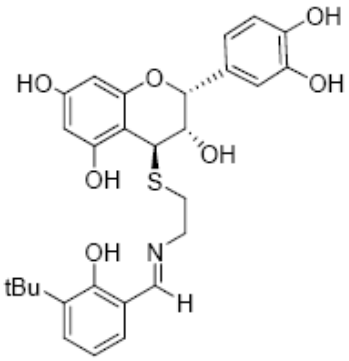




16

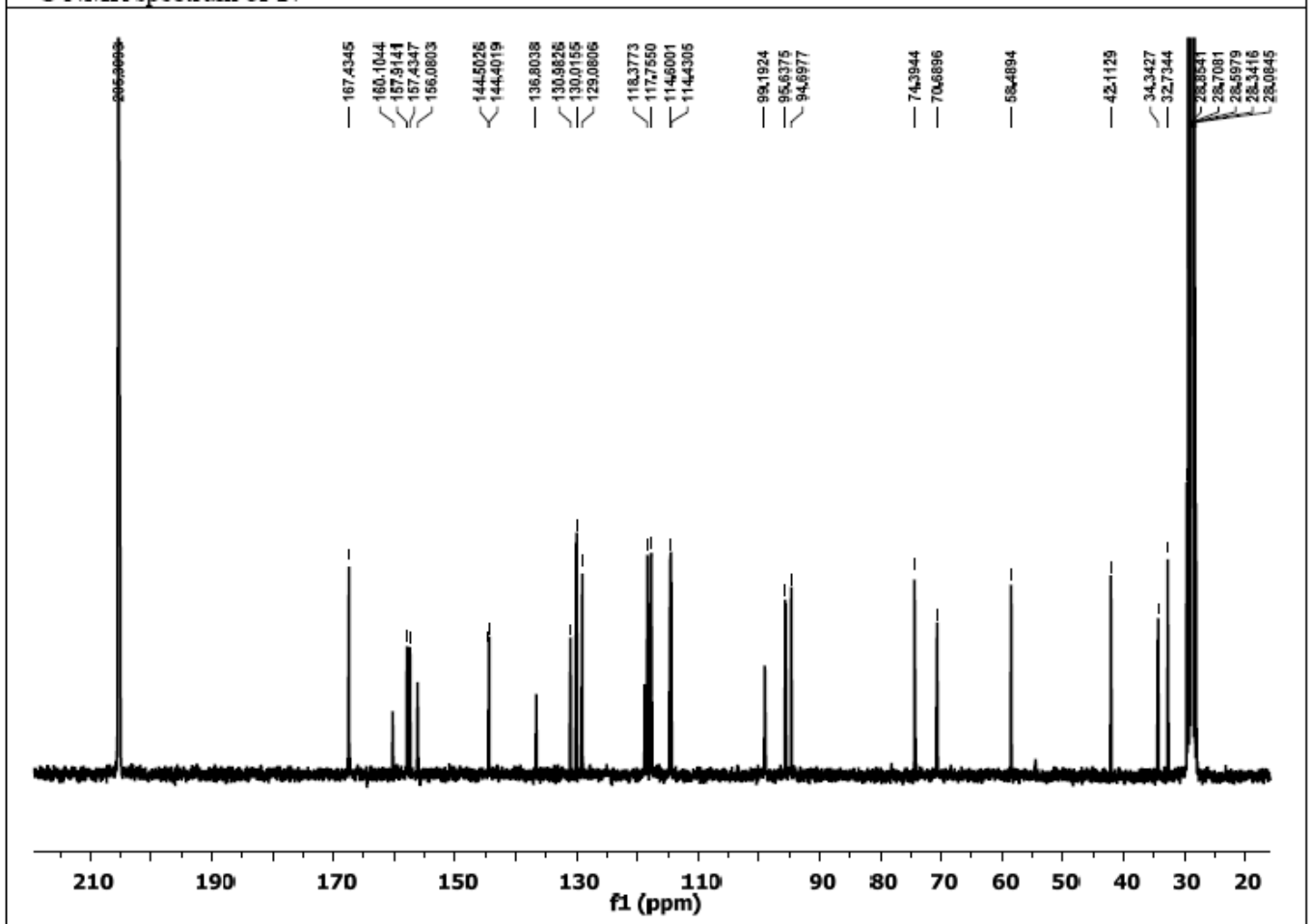
^{1}H NMR spectrum of **17**

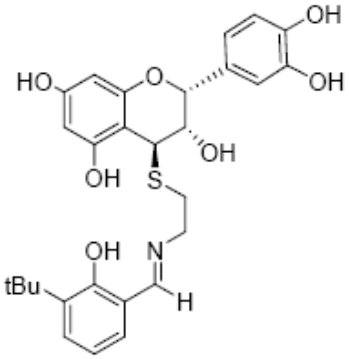




17

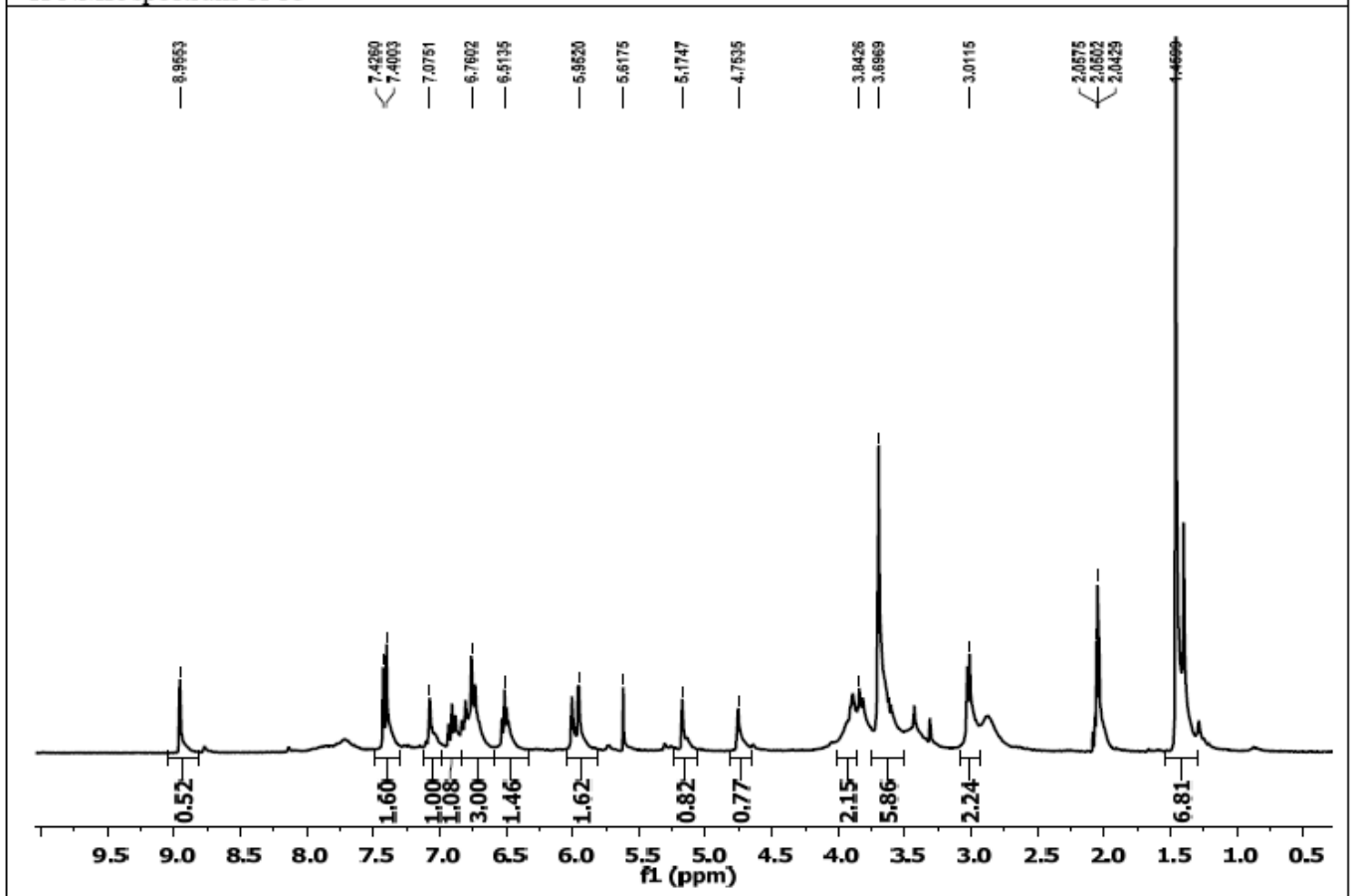
^{13}C NMR spectrum of **17**

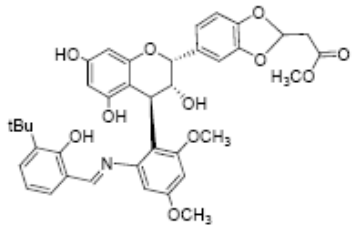




17

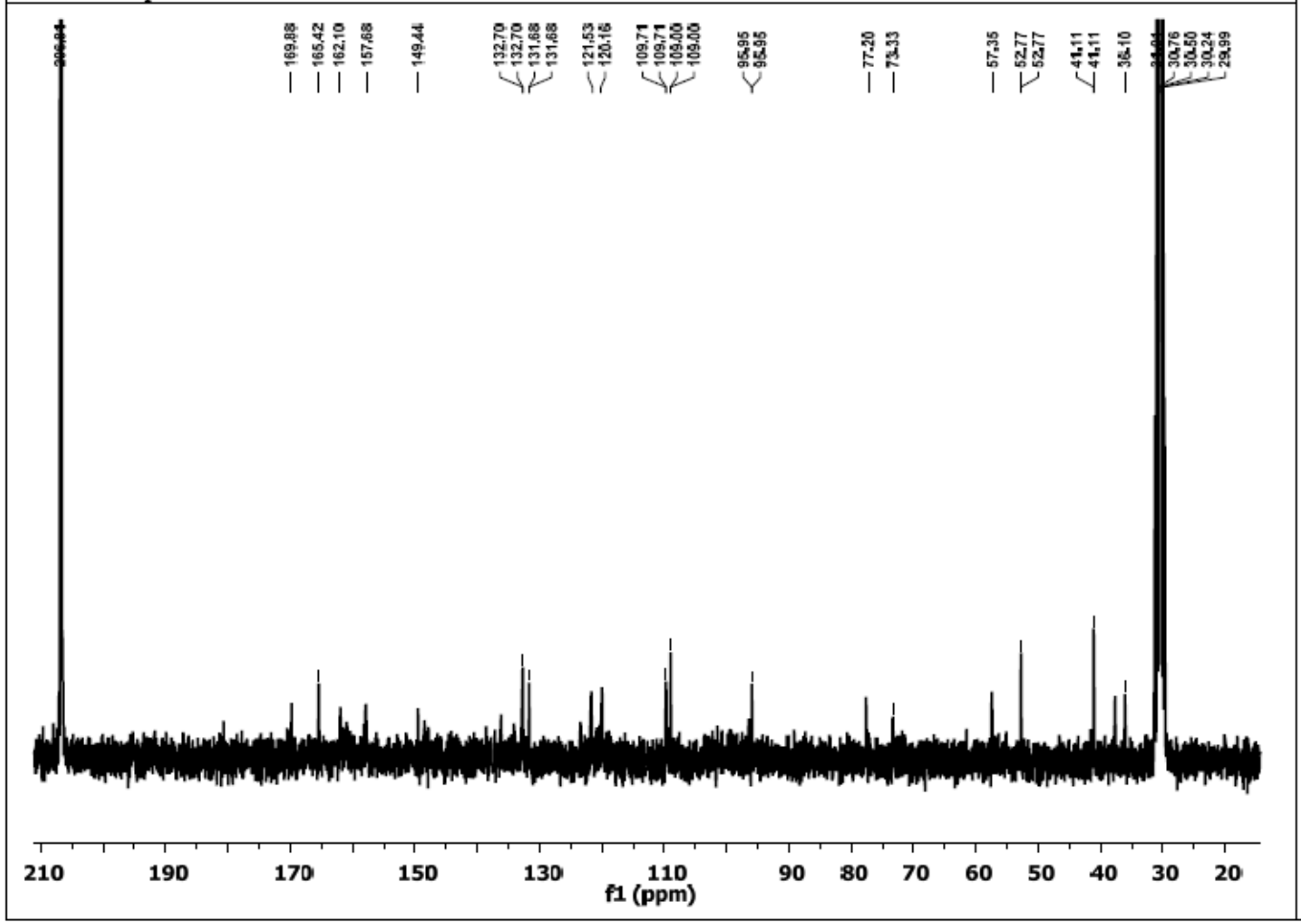
^{1}H NMR spectrum of 18

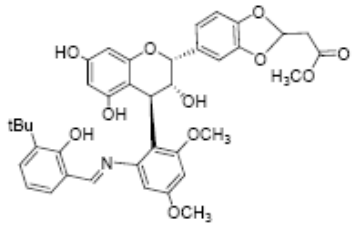




18

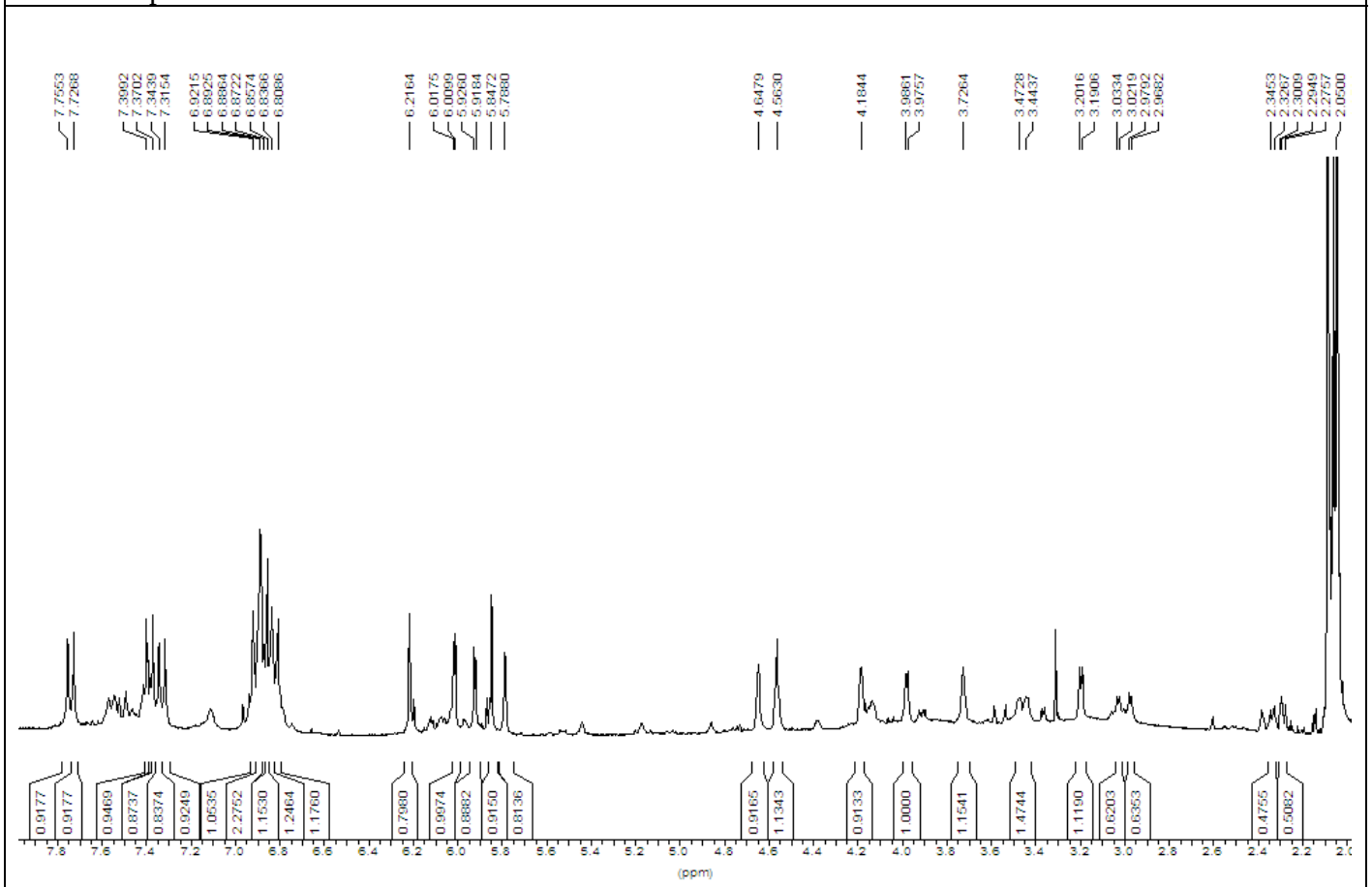
^{13}C NMR spectrum of **18**

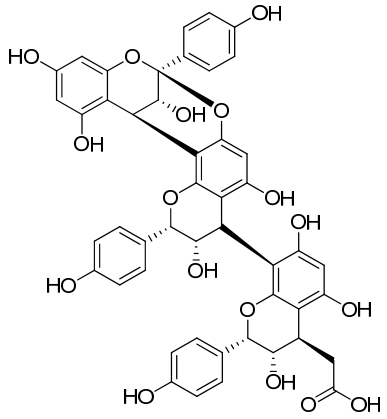




18

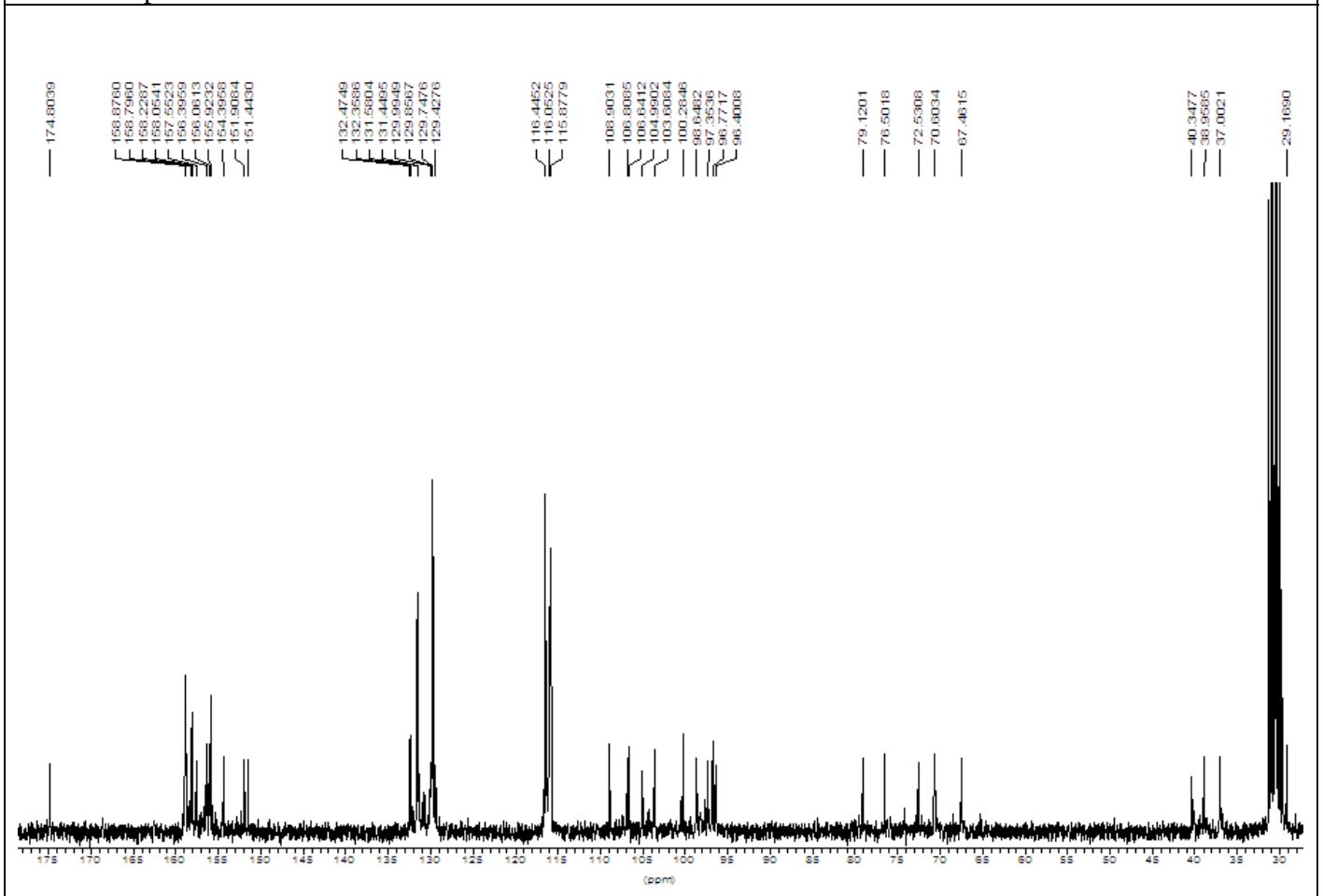
^{1}H NMR spectrum of **21**

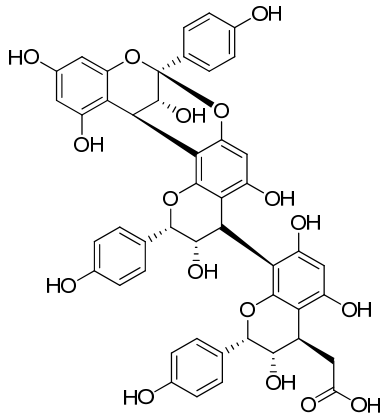




21

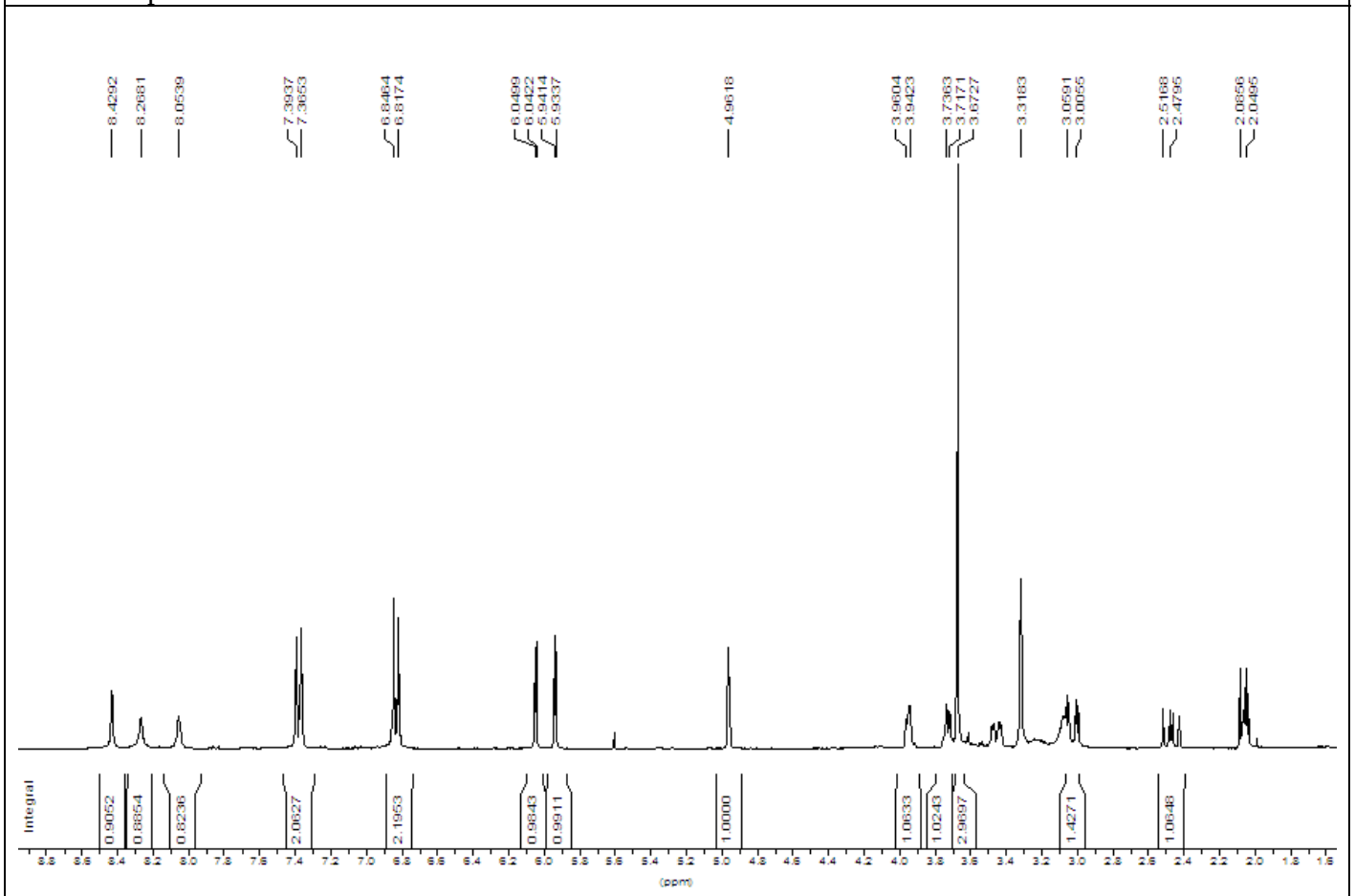
^{1}H NMR spectrum of **5**

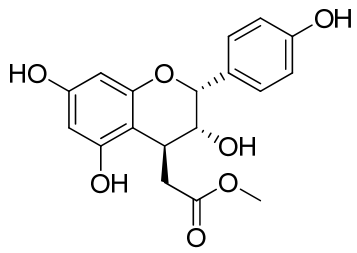




21

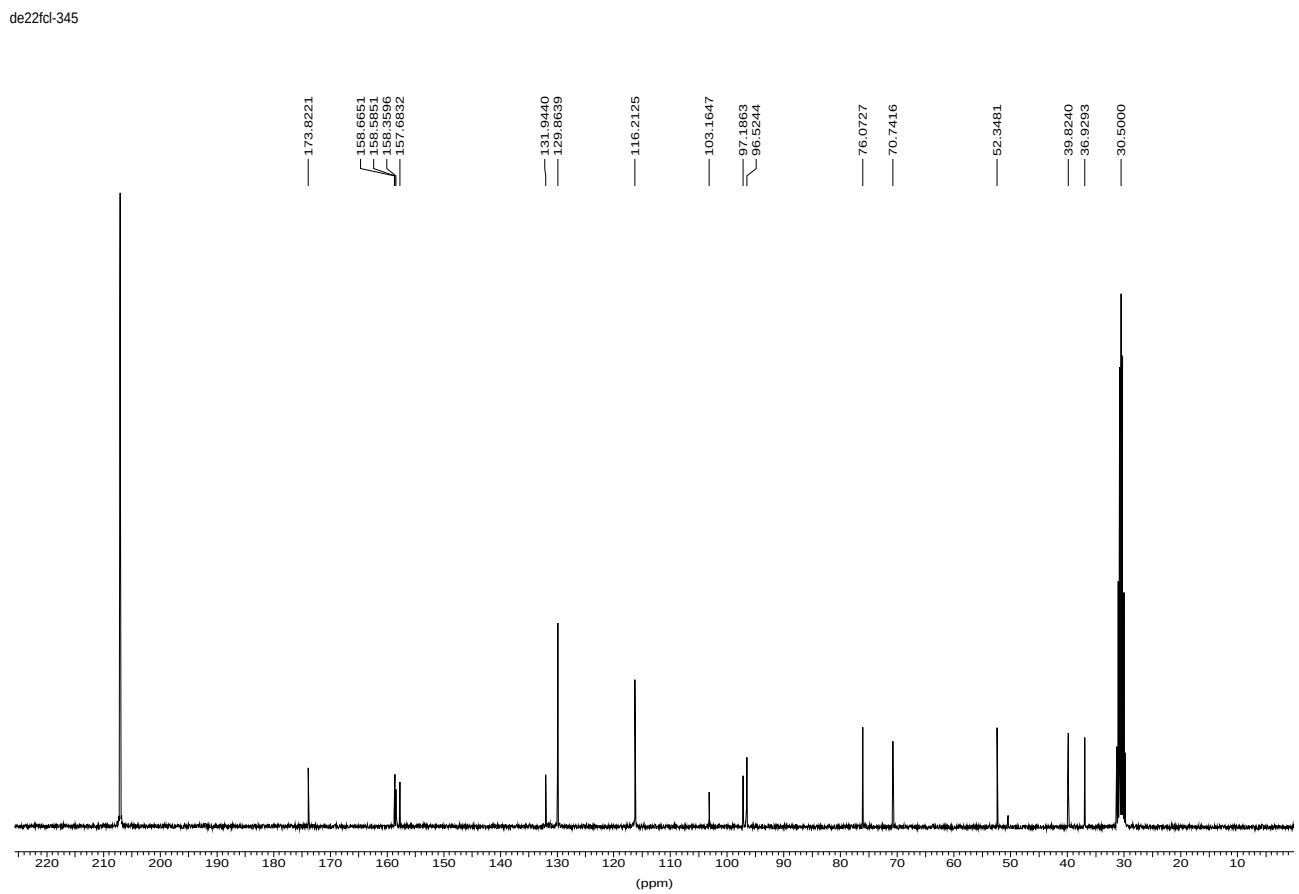
^{1}H NMR spectrum of **22**

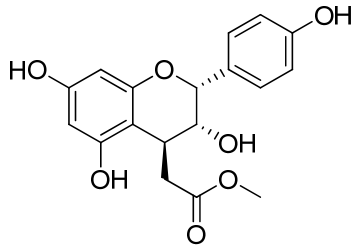




22

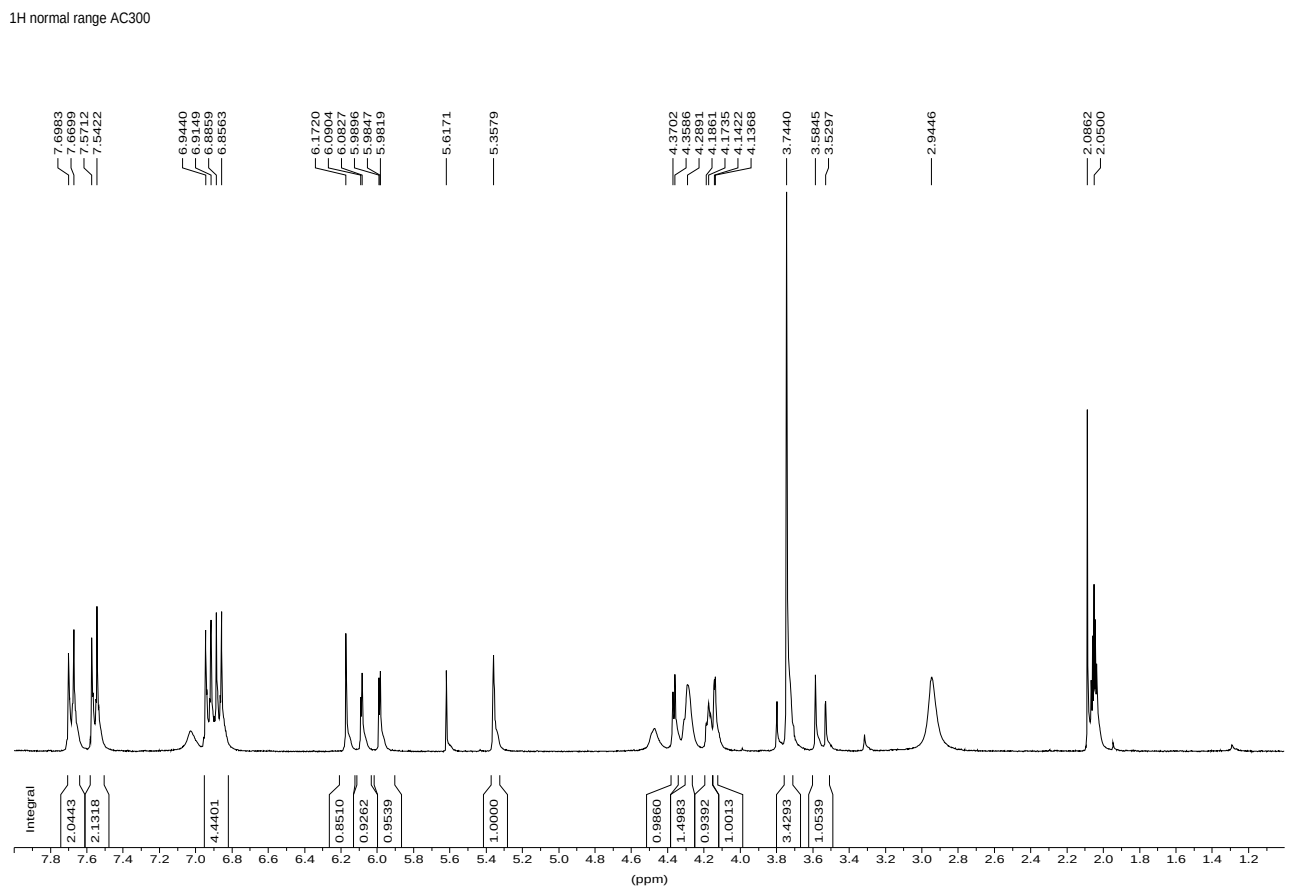
^{13}C NMR spectrum of **22**

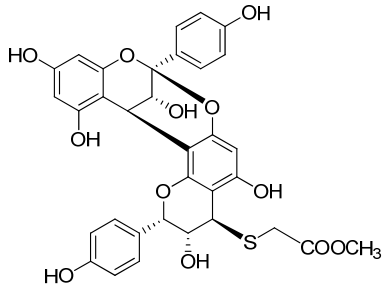




22

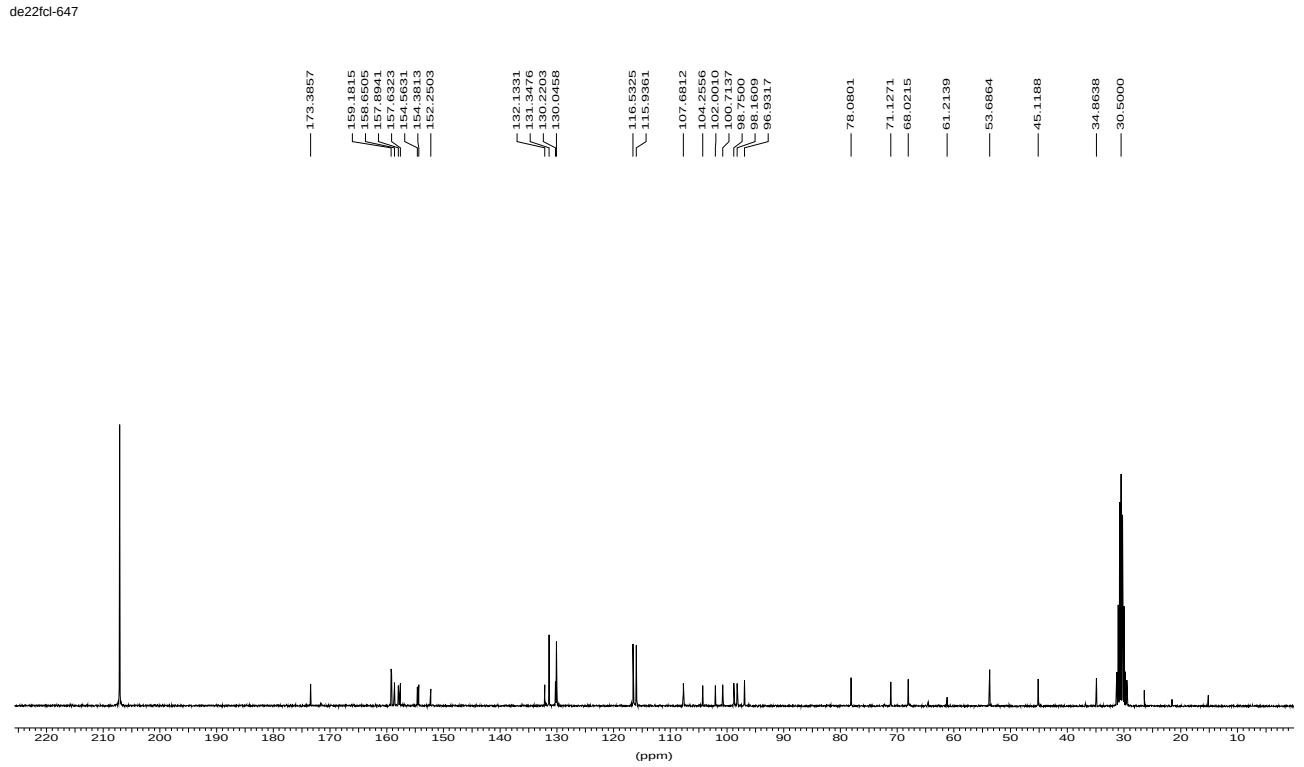
^{1}H NMR spectrum of **24**

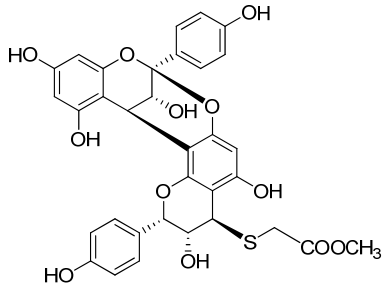




24

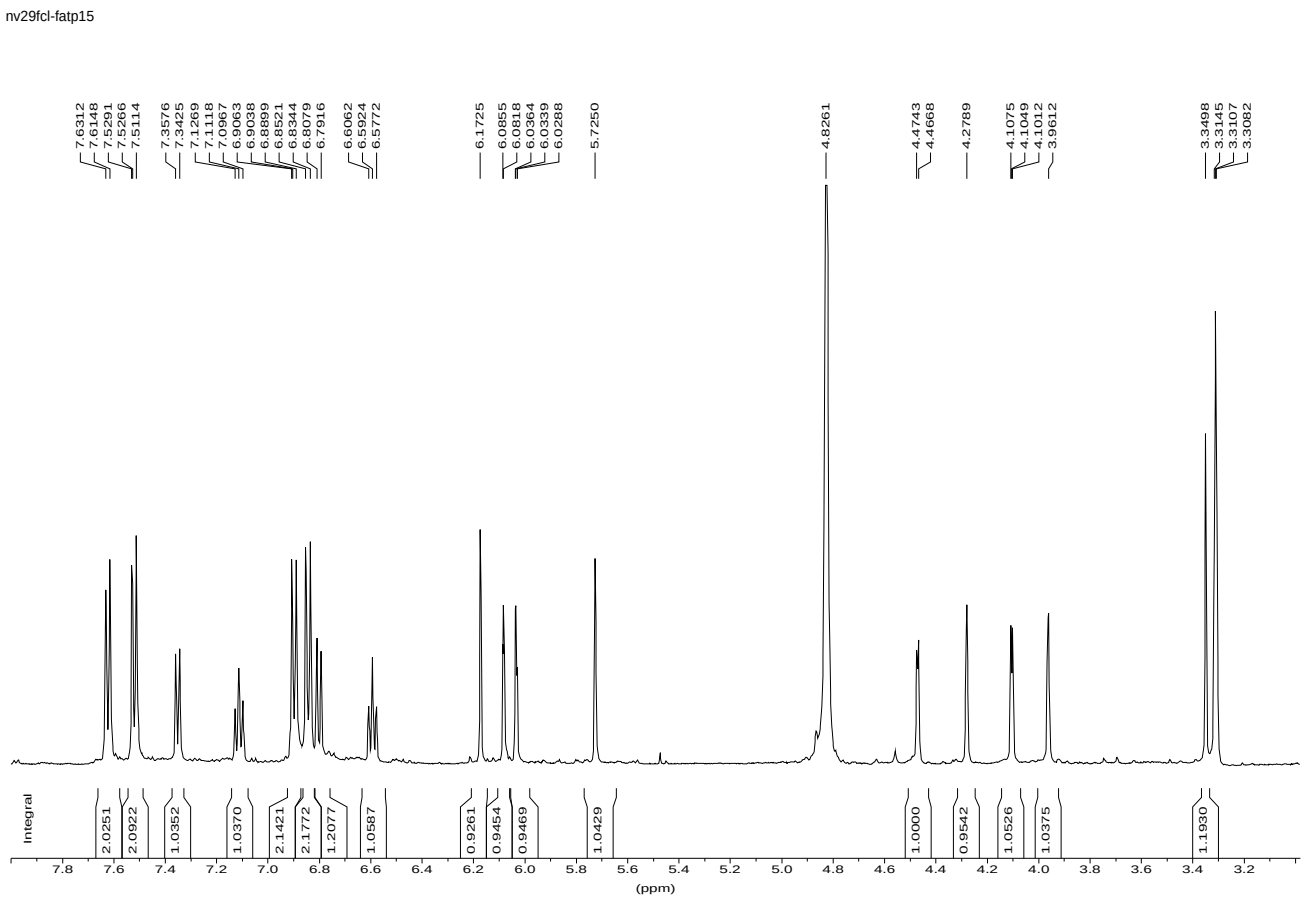
^{13}C NMR spectrum of **24**

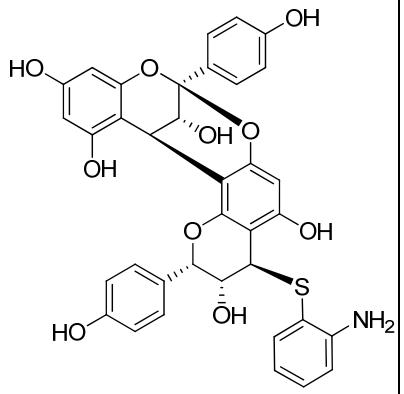




24

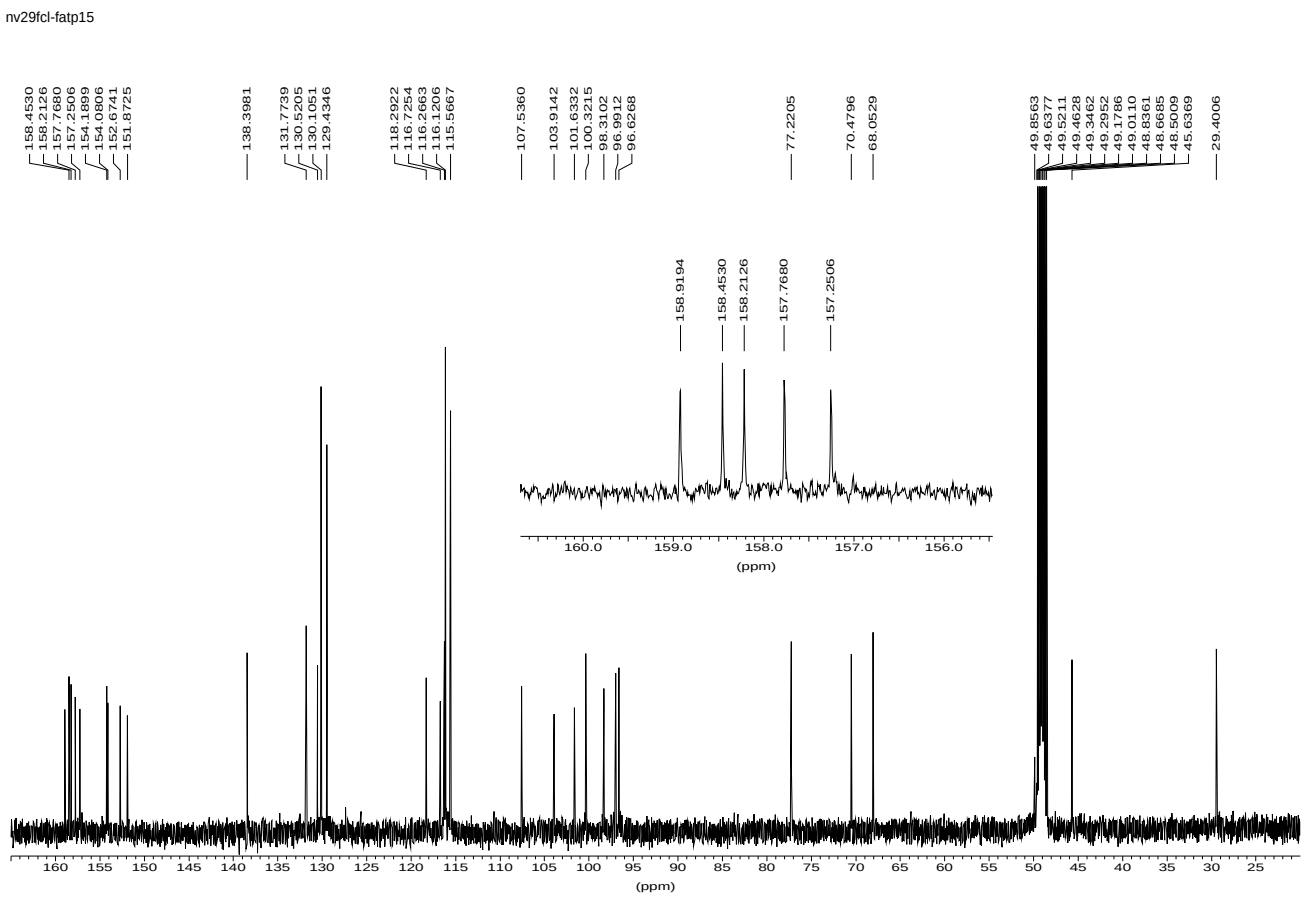
^{1}H NMR spectrum of **5**

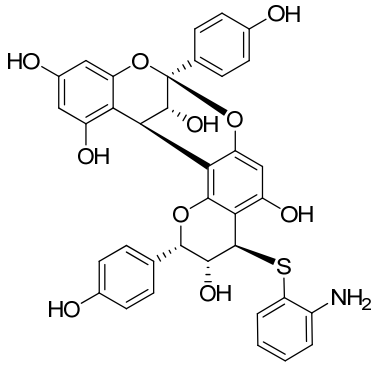




25

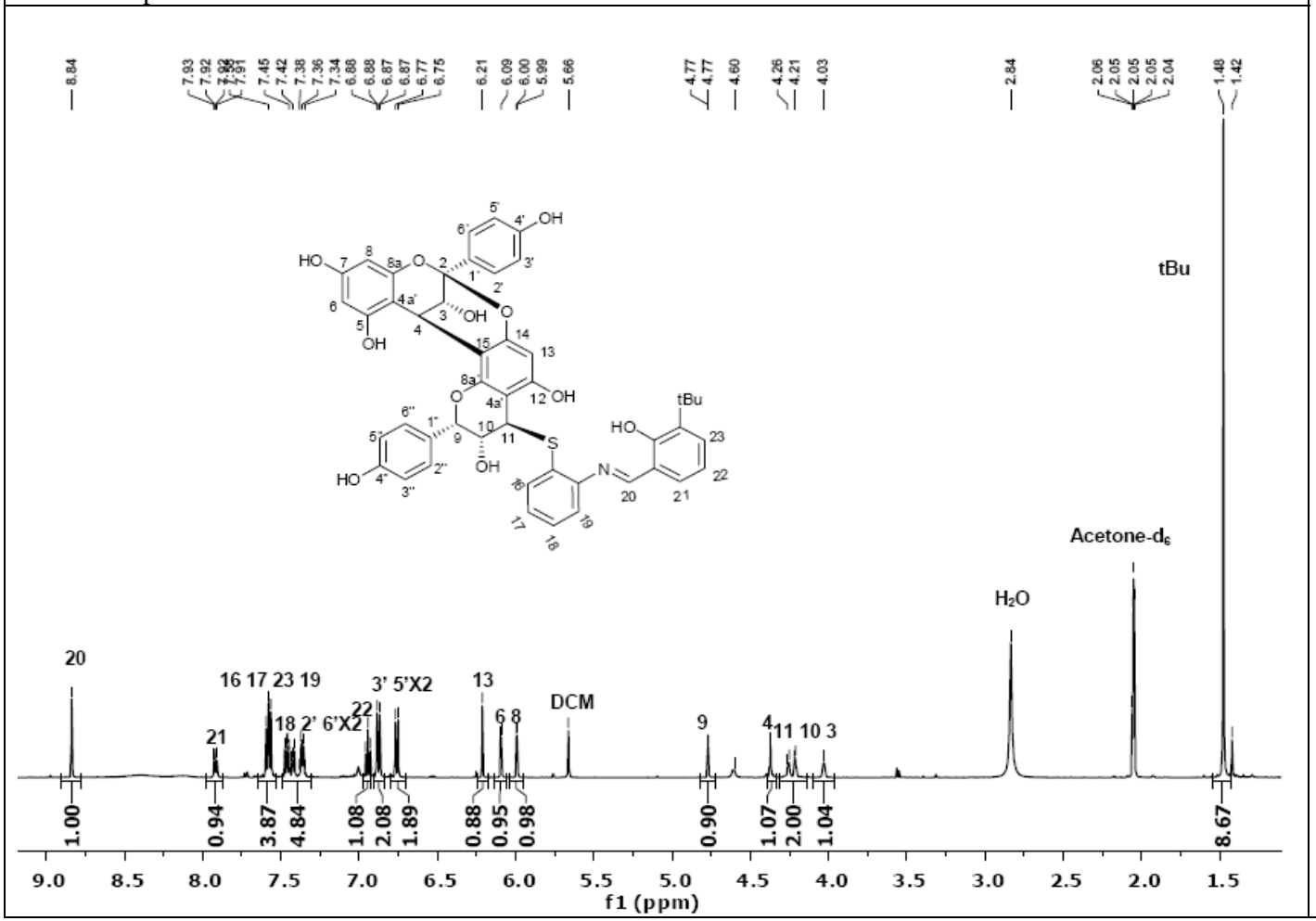
^{13}C NMR spectrum of 2**5**

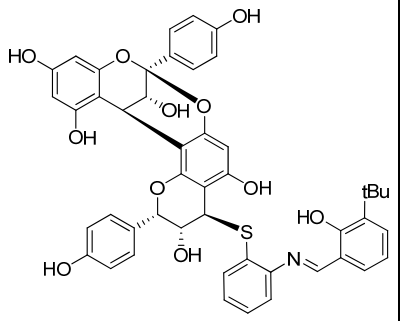




25

^{1}H NMR spectrum of 26





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