การสังเคราะห์และความสัมพันธ์ระหว่างโครงสร้างและฤทธิ์ทางชีวภาพของน้ำตาล-เอสเทอร์

นางสาวจิตตินันท์ ม่วงจีน

SYNTHESIS AND STRUCTURE-BIOACTIVITY RELATIONSHIP OF SUGAR-ESTERS

MissJittinan Maungjeen

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จิตตินันท์ ม่วงจีน: การสังเคราะห์และความสัมพันธ์ระหว่างโครงสร้างและฤทธิ์ทางชีวภาพ ของน้ำตาลเอสเทอร์ (SYNTHESIS AND STRUCTUER-BIOACTIVITY RELATIONSHIP OF SUGAR-ESTERS) อ. ที่ปรึกษา: ผศ. ดร.วรินทร ชวศิริ, 112 หน้า . ISBN 974-17-6194-5.

งานวิจัยนี้เกี่ยวข้องกับการสังเคราะห์และศึกษาความสัมพันธ์ระหว่างโครงสร้างและฤทธิ์ ต้านอนุมูลอิสระของน้ำตาลเอสเทอร์ ได้สังเคราะห์น้ำตาลเอสเทอร์ 28 ตัวจากปฏิกิริยาเอสเทอริฟิเค ชันโดยใช้ DMAP และ DCC และได้พิสูจน์เอกลักษณ์ของผลิตภัณฑ์โดยวิธีทางสเปกโทรสโกปี มีสารใหม่ทั้งหมด 14 ตัว (**1C, 2C, 3C, 4C, 5C, 8C, 2D, 4D, 6D, 7D, 3E, 5E, 2I** และ **2J**) ได้ศึกษาปัจจัยสี่ปัจจัยต่อความสัมพันธ์ของโครงสร้างและฤทธิ์ทางชีวภาพรวมถึงชนิดของหมู่แทน ที่, ชนิดของหมู่บนกรดเบนโซอิก, ตำแหน่งและหมู่ไฮดรอกซีบนกรดเบนโซอิก, และจำนวน อนุพันธ์ของกรดเบนโซอิกบนโครงสร้างของน้ำตาลเอสเทอร์ 1,2,3,4,6-Penta-*O*-(3,4-dihydro xybenzoyl) glucopyranoside (**2J**) มีฤทธิ์ต้านอนุมูลอิสระต่อ DPPH สูงกว่า BHA และ เทียบเท่ากับกรดแกลลิค น้ำตาลเอสเทอร์ที่มีหมู่แทนที่เป็น เบนซิล, พิแวโลอิล, แอซิทิล และเมทอก ซีไม่มีฤทธิ์ หมู่ไฮดรอกซีที่ตำแหน่งออร์โททำให้ฤทธิ์ในการต้านอนุมูลอิสระของน้ำตาลเอสเทอร์ เพิ่มขึ้น ตัวอย่างเช่น น้ำตาลโมโนเอสเทอร์ที่มีส่วนของกรดคาร์บอกซิลิกเป็นกรด 3,4-dihydro xybenzoic acid (**3D**) ให้ฤทธิ์สูงกว่าน้ำตาลเอสเทอร์ของกรด 3,5-dihydroxybenzoic acid (**4D**) และกรด 2,4-dihydroxybenzoic acid (**2D**) ซึ่งมีผลมาจากอิทธิพลของเรโซแนนท์และ พันธะไฮโดรเจนของหมู่ไฮดรอกวิลที่ตำแหน่งออร์โท

ภาควิชา.........เคมี.........   ลายมือชื่อนิสิต...
สาขาวิชา........เคมี.........   ลายมือชื่ออาจารย์ที่ปรึกษา...
ปีการศึกษา......2547.......   ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.................-...................

This research involved the synthesis of sugar esters and structure-antioxidant relationship study. Twenty-eight sugar esters as glycoconjugate were manipulated through esterification using DMAP and DCC. The derived products were well-characterized by various spectroscopic methods. Fourteen compounds (**1C**, **2C**, **3C**, **4C**, **5C**, **8C**, **2D**, **4D**, **6D**, **7D**, **3E**, **5E**, **2I** and **2J**) were identified as new substances. Four parameters were set up to explore the relationship of structure and activity including types of substituents, types of substituents on a benzoic acid ring, effects of hydroxyl group on a benzoic ring and numbers of benzoic acid derivatives on the sugar ester structure. 1,2,3,4,6-Penta-*O*-(3,4-dihydroxybenzoyl)glucopyranoside (**2J**) revealed higher antioxidant activity as a radical scavenger againts DPPH radical than BHA but comparable to gallic acid. Sugar esters bearing as benzyl, pivaloyl, acetyl and methoxy groups were inactive. The arrangement of phenolic dihydroxyl groups as *ortho* to one another substantially increased the antioxidant activity for the parent sugar esters. For instance, monosubstituted 3,4-dihydroxybenzoic acid sugar ester (**3D**) expressed higher activity than 3,5-dihydroxybenzoic acid (**4D**) and 2,4-dihydroxybenzoic acid (**2D**) sugar esters. This could be stemmed from the resonance effect and H-bonding of hydroxyl groups at the *ortho* position.

Department…..Chemistry……   Student's signature………………………...
Field of study…Chemistry…..   Advisor's signature……………………..
Academic year….2004………   Co-advisor's signature………..-..……………

ACKNOWLEDGEMENTS

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LIST OF ABBREVIATIONS

br s	broad singlet (NMR)
BHA	butyrated hydroxy anisole
°C	degree of celcius
δ	chemical shift
J	coupling constant (NMR)
d	doublet (NMR)
DCC	dicyclohexylcarbodiimide
DMAP	2,2-diphenyl-1-picryhydrazyl
eq	equivalent (s)
Fig	Figure
g	gram (s)
Hz	hertz
IC_{50}	50 % inhibitory concentration
IR	infrared
lit.	literature
m.p.	melting point
mL	milliliter (s)
mmol	millimole (s)
m	multiplet (NMR)
NMR	nuclear magnetic resonance
ppm	part per million
q	quartet (NMR)
R_f	retardation factor
sep	septet (NMR)
s	singlet (NMR)
t	triplet (NMR)
TLC	thin layer chromatography
cm^{-1}	unit of wavenumber

CHAPTER I

INTRODUCTION

Carbohydrates play an important role in the cell function of multicellular organism and in biological molecular recognition. Sugars distribute as a vast source in natural products and possess a soluble water material which has benefits in pharmaceutical. Nutritionists recommend that about half of our energy should be supplied by carbohydrate. The majority of this should be from starchy food like cereals, rice, potatoes, pasta or bread, with the recommender coming from sugars.

Sugars are of great important as intermediates in carbohydrate chemistry. Sugar esters in the nature have variety benefit in pharmaceutical. Some of them are biological active compounds, such as tannins.

Tannins are high molecular weight polyphenolics found in higher plants including many plants used as food and feed. Tannins ingested with the diet by humans or animals may affect protein utilization by forming insoluble complexes with proteins, iron utilization by complexing with iron, and biological antioxidant status by participating in redox reaction. Tannin may contribute to the chemical defenses that minimize damage to plant by insect and mammalian herbivores. Their importance in nutrition and in ecological processes makes qualitative and quantitative analysis of tannin critical to studies of plant nutritional quality and plant ecology. Constraints on effective chemical analysis include the variable composition of tannin within plant, which includes variation with species, season, and environmental stresses such as herbivory.[1]

The search for highly active and at the same time acceptable and inexpensive compounds is one of the most important aims in pharmaceutical research. The isolation of secondary metabolites from natural sources, especially bacteria, fungi, and higher plants, has become increasingly important in the search for new compounds. In the search for biologically active compounds from plant extracts a large number of tannins with various activities could be identified and characterized

since the early 1980's. Extensive tests showed that many of tannins possess antibacterial, antiviral, and antitumoral properties. The observed high selectivity of tannin is caused frequently by the inhibition of specific enzymes. Therefore, this class of natural products has great potential for the development of new pharmaceuticals, especially in AIDS and cancer therapy.

Many papers have appeared recently on the biosynthesis, isolation, and biological activity of tannin. Access to pure tannins by isolation from natural sources frequently is cumbersome and yields only small quantities of the pure natural products. Therefore, it is a great challenge for the preparative chemist to provide synthetic access to this substance class, on the one hand to obtain sufficient quantities of pure biologically active compounds, on the other hand to optimize the biological activity (*e.g.* lowering cytotoxicity, improving absorption, or improving selectivity) by derivatization or modification.[2]

Sugar esters of carbohydrates have variety benefits as biologically active compounds, antioxidant, antiviral, anti-tumor, important in pharmaceutical research and natural surfactants. Fatty acid sugar esters are one of the compounds synthesized by means of enzymatic catalysis. These molecules have several applications particularly in cosmetics and food industry.[3] The selective derivation of multiple hydroxy function in carbohydrate molecules is a challenging problem in organic synthesis. The most widely approach involves multistep procedures, that is, the preliminary synthesis of selectively protected intermediates, following by esterification of the remaining free hydroxyl and subsequent removal of the protecting groups.[4]

1.1 Classification of Tannins
1.1.1 Condensed tannins

Proanthocyanidins (condensed tannins) are polymeric flavanoides. The flavanoides are a diverse group of metabolites based on a heterocyclic ring system derived from phenylalanine (B) and polyketide biosynthesis (A).[5a] Although the biosynthetic pathways for flavonoid synthesis are well understood, the steps leading to condensation and polymerization have not been elucidated.

The most widely studied condensed tannins are based on the flavan-3-ol: epicatechin and (+)-catechin.

epicatechin   catechin

The best characterized condensed tannins are linked *via* a carbon-carbon bond between C8 of the terminal unit and C4 of the extender. The four common modes of coupling are illustrated by dimers isolated by Haslam, and originally named B-1, B-2, B-3 and B-4. The more complete names specify the position and stereochemistry of the interflavan bond completely. In addition to these dimers, related dimer link by C6 of the terminal unit and C4 of the extender have been isolated.

1.1.2 Hydrolysable Tannins

The hydrolysable tannin family of secondary plant metabolites (gallotannins and ellagitannins) constitutes a vast array of polyphenolic natural products which are in evidence in approximately 41% of all orders of dicotyledonous plants. Despite this widespread occurrence and a long history of study, their role in the plant's life processes remains a matter of speculation. Current dogma favors an interpretation wherein the gallotannin (and possibly the ellagitannins) are, at very least, prominent members of the plant's chemical arsenal against predation by both herbivores and pathogens.

Hydrolysable tannins are derivatives of gallic acid (3,4,5-trihydroxy benzoic acid). Gallic acid is esterified to a core polyol, and the galloyl groups may be further esterified or oxidatively crosslinked to yield more complex hydrolysable tannins.

1.1.2.1 Gallotannins

Gallotannins are heterogeneous glycosidic esters of gallic acid and its *o*-linked derivatives (*meta*-depside linkage), which are secondary metabolites of higher

plants. The prototypical gallotannin is pentagalloy glucose (β-1,2,3,4,6-pentagalloyl-
O-D-glucopyranose). Pentagalloyl glucose, or PGG, has five identical ester linkages
that involve aliphatic hydroxyl groups of the core in gallotannins is D-glucose,
although other monosaccharides (D-hamamelose or D-fructose and a nonsugar polyol
core (quinic acid) have been found. The alpha anomer is not common in nature.
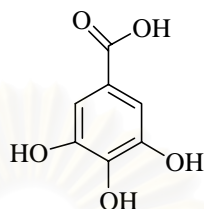
gallic acid
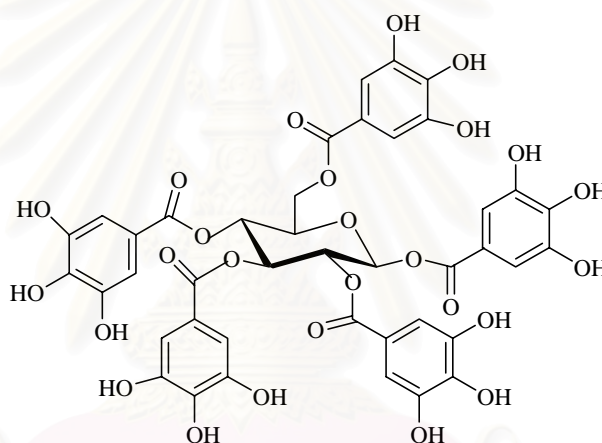
β-1,2,3,4,6-pentagalloyl-*O*-D-glucopyranoside

1.1.2.2 Ellagitannin

Ellagitannins are galloyl esters of glucose that contain at least one chiral
biaryl (digalloyl) subunit, in most cases, to a glucopyranose scaffold by ester
functions. Oxidative coupling of galloyl groups converts gallotannin to related
ellagitannins. The simple ellagitannins are esters of hexahydroxydiphenic acid
(HHDP). HHDP spontaneously lactonizes to ellagic acid in aqueous solution.

gallotannin $\longrightarrow$

gallic acid
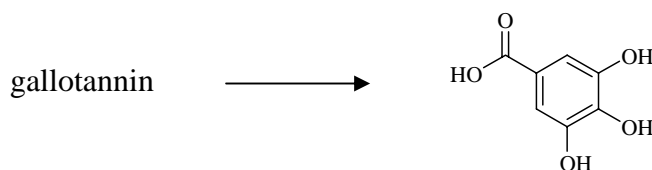
ellagitannin → hexahydroxydiphenic acid → ellagic acid
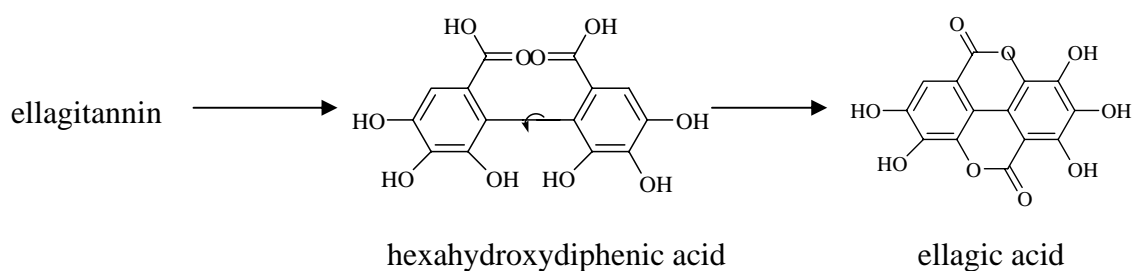
1.2 Literature Reviews

Sugar esters (hydrolysable tannins) could be isolated from natural sources. The most important aims involve in pharmaceutical research and as biologically active compounds from plant extracts with various activities. The selective derivation of multiple hydroxy function in carbohydrate molecules is a challenging problem in organic synthesis. In multistep syntheses of complex natural products, the selection of the most suitable protecting group for each hydroxy function is very important and sometime holds the key to success.[5b] The most widely approach involves multistep procedures, that is, the preliminary synthesis of selectively protected intermediates, following by esterification of the remaining free hydroxyl and subsequent removal of the protecting groups.

The synthesis and isolation of biologically active sugar esters from natural resources have been recently reported. For instance, in 1984 Nonaka and coworkers isolated two hydrolysable tannins from green tea, and their structures were characterized by chemical and spectral means as 1,4,6-tri-*O*-galloyl-β-D-glucose and 1-*O*-galloyl-4,6-(-)-hexahydroxydiphenoyl-β-D-glucose and a proanthocyanidin as epigallocatechin-(4β→ 8)-3-*O*-galloylepicatechin.[6] In the same year Kashiwada and coworkers isolated polymeric proanthocyanidin gallates named rhatannins, which exhibited the activity to decrease urea-nitrogen concentration in rat serum, as well as several lower-molecular-weight galloyl esters, *i.e.* galloyl proanthocyanidin dimers, galloyl glucoses and gallic acid glucoside from commercial rhubarb (Batei-Daio and Imo-Daio).[7]

In 1988, Kashiwada and coworkers isolated 4 new classes of gallotannins having a sucrose core from two different types of commercial rhubarbs, each produced in China and North Korea. The gallotannins were established as 6′-O-, 4′-O-, 6-O, 1′-O- and 2-O monogalloylsucrose.[8] After that Kashiwada and coworkers isolated and characterised five hydroxycinnamoyl and galloyl esters with glucose from commercial rhubarbs, produced in China, North Korea and Japan.[1]

In 1990, Saijo and coworkers isolated four new gallotannins (1-4), 3, 4-di-O-, 4,11-di-O and 3,4,11-tri-O-galloylbergenins and 4-O-galloylnorbergenin from the barks and leaves of *Mallotus japonicus*. In addition, the occurrence of thirteen known gallotannins and related compounds was demonstrated.[9]
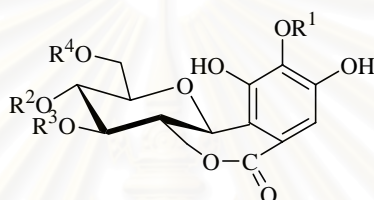
	R^1	R^2	R^3	R^4	
1	Me	G	G	H	
2	Me	H	G	G	G=galloyl
3	Me	G	G	G	
4	H	H	G	H	

In 1990, Ishimaru and coworkers have succeeded to establish the root culture of *Sanguisorba officinalis* and analyzed for the production of tannins as gallic acid, (+)-catechin, 1,2,3,6-tetra-O-galloyl-β-D-glucose, 1,2,3,4,6-penta-O-galloyl-β-D-glucose, sanguiins H-6 and H-11.[10] In the same year, Hatano and coworkers isolated nine polyphenolic compounds from *O. erythrosepala*, and have found that the main constituent, oneothein B, was a new dimeric, hydrolysable tannin in a macrocyclic structure which was the first example of this class.[11]
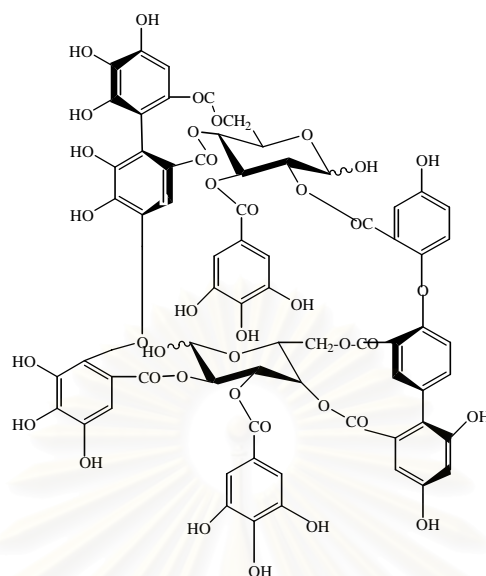
oneothein B

In 1991, Ishimaru and Shimomura isolated nine tannins and related compounds: gallic acid, ellagic acid, (+)-catechin, β-glucogallin, 1,6-di-*O*-, 1,2,3,6-tetra-*O*-, 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose, corilagin and geraniin from the hairy root culture of *Geranium thunbergii*.[12]

In 1996, Pakulski and Budzianowski isolated known compounds plumbagin, chloroplumbagin and 8,8′-biplumbagin as naphthoquinones, 1-O-β-galloylglucose, ellagic acid, 3-*O*-methylellagic acid, 3,3′-di-O-methylellagic acid and its 4-*O*-glucoside, a new compound, the 4,4′-di-O-glucoside of 3,3′-di-*O*- methylellagic acid from *Dionaea muscipula*, obtained by *in vitro* culture. *Dionaea muscipula* Eill was a carnivorous plant considered to be a source of an anticancer drug.[13]

In 1998, Bonnlander and coworkers isolated glycoside of Riesling wine by the use of multiplayer coil countercurrent chromatography (MLCCC). After acetylation and subsequent purification by high-performance liquid chromatography (HPLC), the glucose esters of *(E)*-2,6-dimethyl-6-hydroxyocta-2,7-dienoic acid and *(2E,6E)*-10,11-dihydroxy-3,7,11-trimethyl-2,6-dodecadienoic acid were gained.[14]
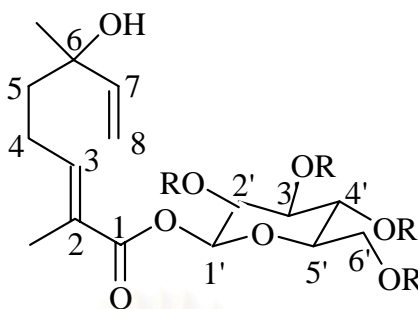
(E)-2,6-dimethyl-6-hydroxyocta-2,7-dienoic acid
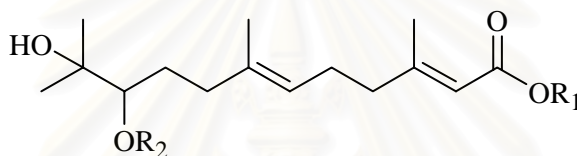
(2E,6E)-10,11-dihydroxy-3,7,11-trimethyl-2,6-dodecadienoic acid

In the same year, Kim and coworkers afforded a new flavonol glycoside gallate ester from the ethyl acetate extract of the leaves of *Acer okamotoanum* integrase, quercetin 3-*O*-(2″,6″-*O*-digalloyl)-β-D-galactopyranoside together with six known flavonol glycosides and three known phenolic compounds. The most active compounds were quercetin 3-*O*-(2″-galloyl)-α-L-arabinopyranoside and quercetin 3-*O*-(2″,6″-*O*-digalloyl)-β-D-galactopyranoside which exhibited IC$_{50}$ values of 18.1 ± 1.3 and 24.2 ± 6.6 μg/mL, respectively, against HIV-1 integrase.[15] Later, Kernan and coworkers presented three new phenylpropanoid glycosides, named luteoside A (**c**), luteoside B (**d**), and luteoside C (**e**), with the known compounds verbascoside (**a**) and isoverbascoside (**b**) from the roots of the medicinal plant *Markhamia lutea*. The plant *Markhamia lutea* Seemann ex Baillor (bignoniaceae) was identified as a potential treatment for viral respiratory infection, including RSV.[16]
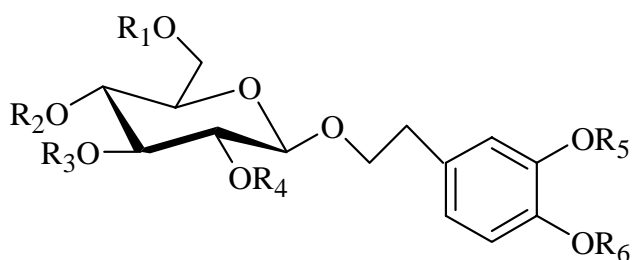
	R_1	R_2	R_3	R_4	R_5	R_6
a	H	Caf	Rha	H	H	H
b	Caf	H	Rha	H	H	H
c	Ac	Caf	Rha	Api	H	H
d	Caf	H	Rha	Api	H	H
e	Fer	H	Rha	Api	H	H
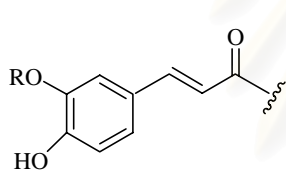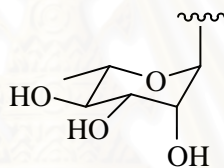
R= H, caffeoyl   rhamnosyl   apiosyl
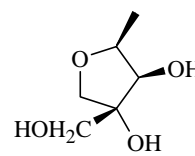
R= CH$_3$, feruloyl

In 1999, Chevalley and coworkers isolated seven compounds from methanol extract of whole *Saxifraga stellaris* (Saxifragaceae) plants. The new compounds as 6-*O*-galloyl-fructose (**1**), together with 3-*O*-[2-*O*-(β-D-xylopyranosyl)-β-D-galacto-pyranosyl]-kaempferol (**2**), 3-*O*-[2-*O*-(β-D-xylopyranosyl)-β-D-galactopyranosyl]-quercetin (**3**), trifolin (**4**), hyperin (**5**), resveratrol-3-*O*-glucoside (**6**), triandrin (**7**), by chemical and spectroscopic methods. Their free radical scavenging properties were also described.[17]
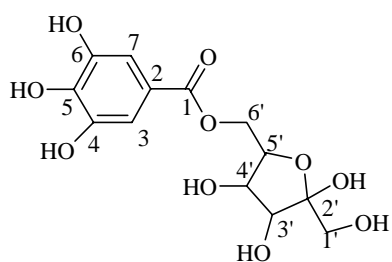
1
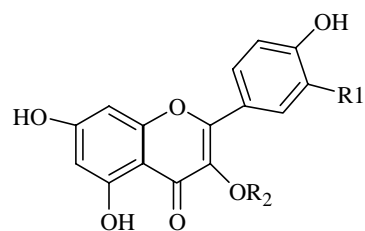
	R_1	R_2
2	-H	-Gal$_2$-Xyl
3	-OH	-Gal$_2$-Xyl
4	-H	-Gal
5	-OH	-Gal
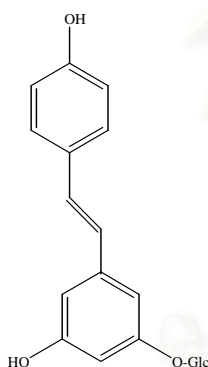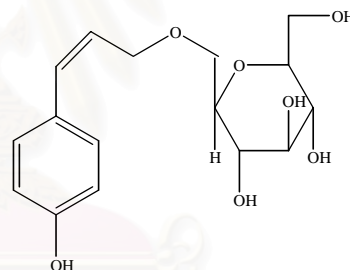
6   **7**

In the same year, Abou-Zaid and Nozzolillo addressed the isolation and characterization of 1-*O*-galloyl-α-L-rhamnose from *Acer rubrum*.[18]

In 2001, Abe and coworkers isolated ellagitannin from various plant sources as well as newly synthesized *n*-alkyl (C1-C18) esters of hexahydroxydiphenyl (HHDP) dicarboxylic acid. All isolated compounds were evaluated as enzyme inhibitors of recombinant rat squalene epoxidase, a rate-limiting enzyme of cholesterol biosynthesis.[19]

In 1980, Yoshimoto and coworkers reported the regioselective syntheses of all isomers of mono-*O*-tetradecanoyl derivatives of methyl α- and β-D-glucopyranosides.

The structures of the synthesized compounds were confirmed by ^{1}H- and ^{13}C NMR spectra.[20]

In 1989, Mikamo synthesized per-*O*-acetyl-α- and -β-D-gluco and D-galactopyranoses, α-D-mannopyranose and β-D-xylopyranose by selectively deacetylation at the anomeric position with ammonium carbonate in DMF in good yields to the corresponding acylated aldopyranoses.[21] Cammann and coworkers studied the biosynthesis of gallotannin *via* acyltransferase which was detected in young leaves of pedunculate oak *(Quercus robur)* that catalyzed the formation of 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose, the common precursor of gallotannins and the related ellagitannins.[22]

In 1993, Lee and coworkers investigated the effects of gallotannins and ellagitannins on Ca^{2+}-activated hyaluronidase activity.[23] In the same year, Hagena and Gross reported on the synthesis of rare trigalloylglucose ester and the conversion to 1,2,3,6-tetragalloylglucose by enzymatic processes.[24]

In 1994 Feldman and coworkers synthesized ellagitannin natural product, Tellimagrandin I.[25] In the same year, Feldman and Ensel studied the construction of strictly (*S*)-hexahydroxydiphenyl (HHDP) unit *via* biomimetic cyclization of suitably protected glucose-derived digalloyl esters which has been achieved in good yield.[26] Nalson and Meyers reported later the first asymmetric synthesis of (*S*)-hexamethoxydiphenic acid, an ubiquitous subunit in ellagitannins, *O*-permethyl tellimagrandin I.[27]
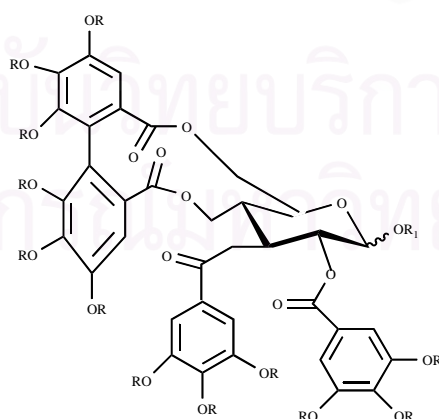
Tellimagrandin I   R = H , R_1 = H

Tellimagrandin II   R = H , R_1 = (β)-galloyl

O- Permethyl Tellimagrandin I   R = Me, R_1 = (α)-Me.

Xia and Hui reported in 1995 the synthesis of 6-acyl-D-glycopyranosides from non protected glycopyranoside using easily available reagents, acyl-*p*-nitrothiophenol esters and acyl-2,4-dinitrophenol esters, as acylating reagents.[4] In the same year Itoh, and Chika investigated the esterification of racemic biphenyl-dicarboxylic acid with a glucose derivative occurred diastereoselectively.[28] Feldman and Sambandam carried out the biomimetic synthesis of Sanguiin H-5 through the diastereoselective formation of the crucial biphenyl carbon-carbon bond between galloyl moieties at the O(2) and O(3) positions of an appropriately protected glucose-derived precursor.[29]
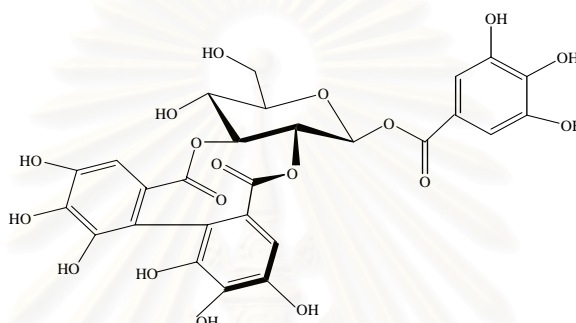
Sanguiin H-5

In 1996, Xia and Hui developed new methodologies to synthesize bio-active sugar esters, 6-acyl-D-glycopyranoside from non protected glycopyranoside by means of chlorophosphoric acid diethyl ester as a condensing reagent, resulting in high yield.[30] Later Xia and Hui developed a new proceduce for clevage of benzylidene acetals from glycopyranosides using tin (II) chloride which does not affect other protecting groups such as benzoyl, acetyl, benzyl, and acetonide.[31] In the same year, Feldman and Smith biomimetically synthesized 2,3- and 4,6-coupled ellagitannin pedunculagin through the sequential diastereoselective formation of two biphenyl C-C bonds.[32] In the same year, Itoh and coworkers synthesized trideca-O-methyl-α-pedunculagin a simple sequence.[33]
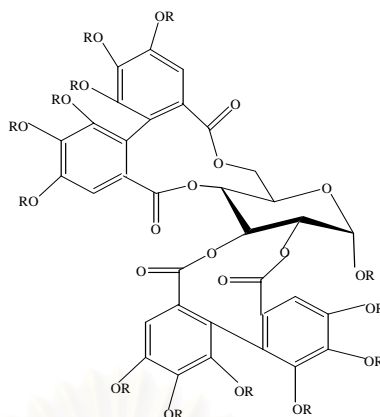
Trideca-*O*-methyl-α -pedunculagin   (R= Me)

In 1997 Kawamoto and Nakatsubo studied the effects of environmental factors on two-stage co-precipitation of tetragalloylglucose with three different proteins [bovine serum albumin (BSA), lysozyme, and myoglobin].[34] In the same year, Khanbabaee and Lotzerich synthesized *via* a short route of the natural products 2,3,4,6-tetra-O-galloyl-D-glucopyranose, 1,2,3,4,6-penta-O-galloyl-β-D-glucopyranose and the unnatural 1,2,3,4,6-penta-O-galloyl-α-D-glucopyranose utilizing an efficient esterification reaction of the benzylated gallic acid with α,β-glucopyranose *via* 4-*N,N*-dimethylaminopyridine (DMAP) and dicyclohexyl- carbodiimide (DCC).[35]

In 1998, Dai and Martin manipulated the internal biaryl coupling of carbohydrate derivatives carrying two 2-iodo-3,4,5-trimethoxybenzoyl groups under Ullmann conditions.[36] In the same year Khanbabaee and Lotzerich synthesized the enantiomerically pure unusual ellagitannins 1,4,6-tri-O-galloyl-2,3-(*R*)-hexahydroxydiphenoyl-β-D-glucopyranoside and 4,6-di-O-galloyl-2,3-(*R*)-hexahydroxydiphenoyl-D-glucoside.[37] Niemetz and Gross isolated the enzyme extracted from the leaves of staghorn sumac (Rhus typhina) that catalysed the galloylation of 1,2,3,4,6-penta-O-galloyl-β-D-glucose to the gallotannin, 3-O-digalloyl-1,2,4,6-tetra-O-galloyl-β-D-glucose. β-Glucogallin (1-O-galloyl-β-D-glucopyranose) served as activated acyl donor in this conversion.[38]

In 1999, Potier and coworkers reported the straightforward chemical synthesis of 6,6′-digalloylsucrose (SG2), 3′,4′,6′-trigalloylsucrose (SG3), 1′,2,3,3′,4′,6′-hexagalloylsucrose (SG6) and octagalloylsucrose (SG8).[39]
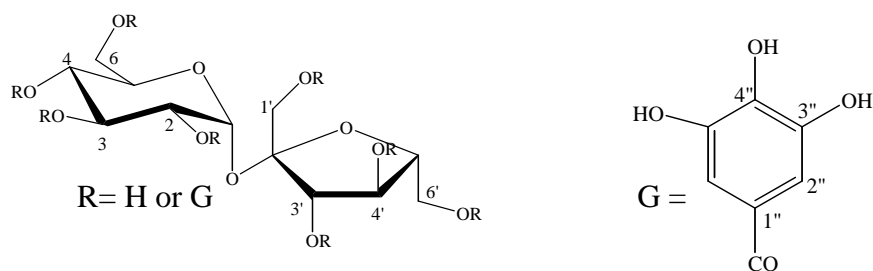
SG$_2$: R=G at O6 and O6′   SG$_3$: R=G at O3′, O4′ and O6′

SG$_6$: R=G at O1′, O2, O3, O3′, O4′ and O6′   SG$_8$: R=G

In the same year Feldman and Sahasrabudhe reported the synthesized of Tellimagrandin II.[40]

In 2000 Barros and coworkers synthesized three monogalloylsucroses, namely 6′-*O*-(tri-*O*-methylgalloyl)-2,3,4,6,1′,3′,4′-hepta-*O*-acetylsucrose, 6′-*O*-(tri-*O*-methyl galloyl)-2,3,4,6,1′,3′,4′-hepta-*O*-benzoylsucrose and 6,6′-di-*O*-*tert*-butyldiphenyl silyl-1′-*O*-(tri-*O*-methylgalloyl)-2,3,4,3,3′,4′-penta-*O*-acetylsucrose in four short sequences from sucrose.[41]
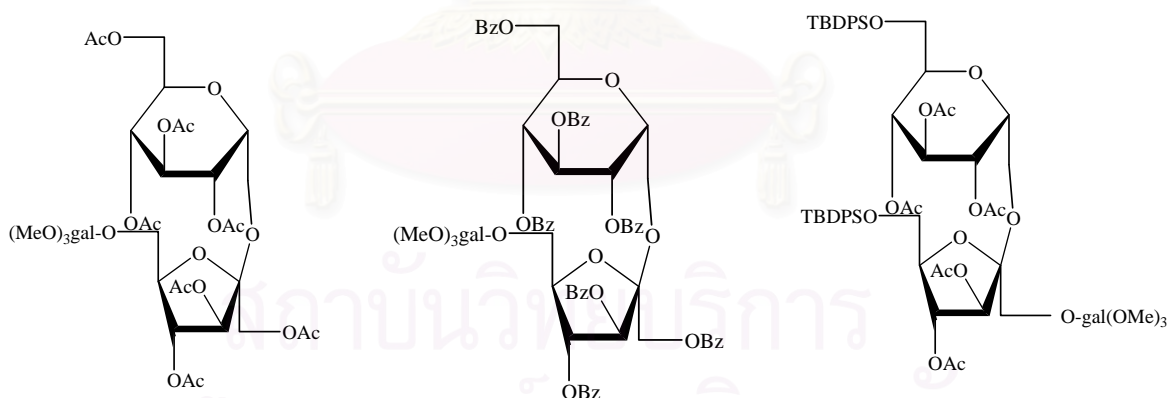
6′-*O*-(tri-*O*-methylgalloyl)-

2,3,4,6,1′,3′,4′-hepta

-*O*-acetylsucrose

6′-*O*-(tri-*O*-methylgalloyl)

- 2,3,4,6,1′,3′,4′-hepta

-*O*-benzoylsucrose

6,6′-di-*O*-tert-

butyldiphenylsilyl-1′

-*O*-(tri-*O*-methylgalloyl)-

2,3,4,3,3′,4-penta-

O- acetylsucrose

In the same year, Baker and coworkers displayed that sugar fatty acid esters could be used as nonionic surfactant by varying the different-sized sugar head groups (glucose, sucrose, or raffinose) and different lengths and numbers of alkyl chains [lauric (C_{12}) or palmitic (C_{16}) acid].[42]

In 2001, Holmberg addressed the natural surfactants from sugar esters. In the same year, Soultani and coworkers synthesized *via* lipase-catalyzed synthesis of fatty acid sugar esters through direct esterification in 2-methyl-2-butanol. Fructose and saturated fatty acids were used as substrates and the reaction was catalyzed by immobilized *Cavdida antarctica* lipase.[3]

In 2002, Maruyama and coworkers enzymatically synthesized sugar amino acid esters in polar organic solvents using surfactant-enzyme complexes, which were previously developed as a highly active biocatalyst in organic solvents.[43] In the same year, Dofour and coworkers reported three tests of increasing complexity to assess the antioxidant activity of five synthetic gallic esters of sucrose bearing 3,6,7, or 8 galloyl units.[44] In the same year, Khanbabaee and Grober reported the synthesis natural 1,3-di-O-galloyl-4,6-O-(*S*)-hexahydroxydiphenoyl-β-D-glucopyranoside.[45]

In 2003 Feldman and coworkers examined the stability and reactivity of 2,4-HHDP-containing glucopyranose system.[46]

Undesirable pharmaceutical and biopharmaceutical properties often hinder clinical development of biologically active compounds. One approach that has been used to improve the physicochemical properties is preparation of ester prodrugs. Recent investigations have revealed that glycoconjugates possessing an ester bond between the bioactive moiety and one of the sugar hydroxyls are valuable new tools in biomedical research. These monosaccharide esters seem to be able to improve antiviral and antibacterial activity, increase the intestinal permeability, alter receptor-selectivity, or they may be used as monomers in polycondensation reaction as well as building blocks for the solid phase combinatorial synthesis of libraries of novel glycopeptides and in peptide templated glycosylation reactions.[47]

1.3 Synthesis of Sugar Esters

Numerous methods applied for the synthesis of sugar esters since they possess various biological activities such as antioxidant, antiviral, antitumor and natural surfactants. These molecules have several applications particularly in the cosmetic and food industry.[3] The early reported routes to synthesized these compounds are, for instance, *via* modified Steglich esterification between polyol acid, DMAP and DCC in dry CH_2Cl_2 was purged with N_2 and heated at reflux.[2] Recent reports on the methodology used for the synthesis of sugar esters two different patterned routes. The first route utilizing a solution of glucose derivative and acid chloride, a catalytic amount of dry triethylamine and dry CH_2Cl_2 was refluxed under argon. The second route relied on esterificaion between a solution for glucose derivative and carboxylic acid, DCC, DMAP in dry CH_2Cl_2 at reflux under argon. Most sugar esters were obtained in good yield. The latter method was generally more common than the former because various starting materials required are commercially available.

1.4 Goal of This Research

The aim of this research is to synthesize, investigate and develop sugar esters that possess antioxidant activity. The approach involves the synthesis of monosubstituted glucopyranosides at an anomeric position. The structure-activity relationship (SAR) study of sugar esters was performed which would permit a logical opportunity to predict the relationship of other molecules and antioxidant activities. Therefore, the goal of this research can be summarized as follows:

1. To synthesize sugar esters mainly substituted at an anomeric position by varying the position of substitutents on glucose and related compounds.

2. To study the relationship between sugar esters and related compounds and antioxidant activity.

CHAPTER II

EXPERIMENTAL

2.1 Instruments and Equipment

All melting points were determined with Fisher-Johns melting point apparatus or Electrothermal digital melting point apparatus model IA 9100. Column chromatography was carried out on silica gel (Merck Kieselgel 60, 70-230 mesh). Thin layer chromatography (TLC) was performed on aluminum sheets precoated with silica gel (Merck Kieselgel 60 PF254) and thin layer chromatography (TLC) was performed on aluminum sheets precoated with C-18 reversed phase (Merck RP-18 F_{254S}). The FT-IR spectra were recorded on a Nicolet Fourier Transform Infrared Spectrophotometer model Impact 410: solid samples were incorporated to potassium bromide to form a pellet. The ^{1}H, ^{13}C-NMR and COSY spectra were performed in deuterated chloroform ($CDCl_3$) or acetone-d_6 or deuterated dimethylsulfoxide (DMSO-d_6) with tetramethylsilane (TMS) as an internal reference on a Bruker model ACF 200 spectrometer which operated at 400 MHz for ^{1}H and 50.32 MHz for ^{13}C nuclei. The chemical shifts were assigned by comparison with residue solvent protons. $CDCl_3$/DMSO-d_6 means that DMSO-d_6 is added dropwise to a suspension of the compound in $CDCl_3$ until a clear solution is obtained.

2.2 Chemicals

All solvents used in this research were purified prior to use by standard methodology except for those which were reagent grades. The reagents used for synthesizing the precursors, sugar esters and other compounds were purchased from Fluka Chemical Company or otherwise stated and were used without further purification.
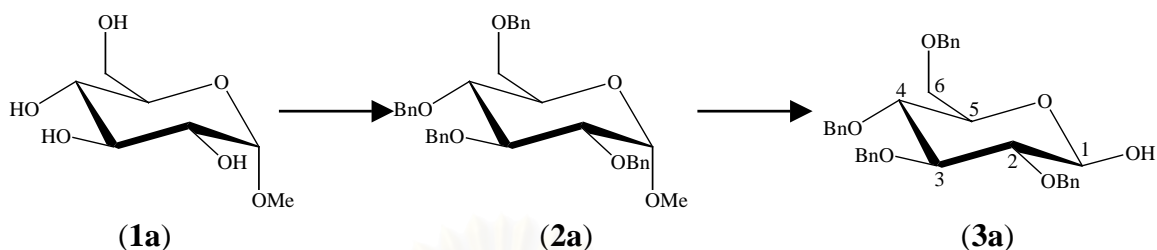
2.3 Dipping Reagent

Potassium permanganate and potassium carbonate in water were used for detecting spots of synthesized sugar compounds.

2.4 Synthesis

2.4.1 Synthesis of Starting Materials

2.4.1.1 Synthesis of 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranose

(**1a**)   (**2a**)   (**3a**)

Method 1 Sodium hydride powder (140 mmol) was added gradually to a solution of 1.98g (10 mmol) of methyl-α-D-glucopyranoside (**1a**) in 60 mL DMF in a 250 mL round-bottomed flask, and the suspension was swirled for 30 min at ~25°C. The mixture was then cooled to 0°C and 13 mL (100 mmol) of benzyl bromide was added. The reaction mixture became solid in about 40 min and was held at ~25°C until a clear solution formed (24-48 hours). Dry methanol (10 mL) was added and the solution was concentrated to dryness. To the residue were added 50 mL of dichloromethane and 50 mL of water. The organic layer was removed, washed with three 40-mL portion of water, dried over anhydrous magnesium sulfate and filtered. The solvent was removed *in vacuo* to give a yellow oil. Column chromatography of the crude product on silica gel yielded 3.17 g (59%) of (**2a**). The syrupy (**2a**) was dissolved in a mixture of acetic acid 65 mL and hydrochloric acid (2 M, 26 mL) was heated at reflux for one day at 80°C. The mixture was cooled and poured into 20 liters of water affording further product upon cooling. The crude product was recrystallized from methanol to give 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranose (**3a**). [48]

Method 2 Methyl-α-D-glucopyranoside (**1a**) (50 g) was suspended in 150 mL of dry dioxane with 250 g of powdered potassium hydroxide, and the mixture was stirred and gently boiled under reflux while 318 mL of benzyl chloride was added in dropwise over a period of 0.5 h at ~40°C. Boiling under reflux and stirring were continued for 0.5 h after the addition was complete; dioxane was then allowed to distill from the stirred mixture over the course of 3 h. The residue was cooled and sufficient water is added to dissolve the crystalline mass, the sugar derivative then being extracted with ether. After being washed with water, the combined extracts were dried over sodium sulfate, filtered through a charcoal, and concentrated under

diminished pressure to a syrup. Benzyl alcohol and dibenzyl ether were removed by distillation at 0.05 torr (200°C bath) to give 135 g (95%) methyl 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranoside (**2a**). The syrupy was dissolved in 2.5 L of hot glacial acetic acid, and the solution was then diluted with 540 mL of boiling 2 *N* sulfuric acid. After 2 h on the steam bath, the reaction was further diluted with 540 mL of boiling 2 *N* sulfuric acid and heating on the steam bath was continued for 24 h. The mixture was cooled and poured into 20 L of water and left at ~25°C for 2 days. After collection by filtration, the crude product was recrystallized from methanol to give pure 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranose (**3a**) 97.3 g (70% from (**1a**)).[48]

Methyl 2,3,4,6-tetra-O-benzyl-α-D-glucopyranoside (**2a**): colorless syrupy (59%), R_f 0.55 (hexane:ethyl acetate; 1:1), IR (NaCl, cm^{-1}): 3027, 1603, 1449 and 1197; ^{1}H-NMR (CDCl$_3$) δ(ppm): 7.42-7.13 (m, 20H, Ar-H), 5.03 (d, *J*=10.55 Hz, 1H, H-1), 4.88-4.83 (m, 3H, H-2, PhCH$_2$), 4.73-4.64 (m, 3H, H-4, PhCH$_2$), 4.54-4.49 (m,1H, H-3), 4.04 (t, *J*=8.80 Hz, 1H, H-6), 3.80-3.76 (m, 2H, PhCH$_2$), 3.62 (m, 2H, PhCH$_2$), 3.62 (dd, *J*=9.60 , 3.52 Hz, 1H, H-5) and 3.45 (s, 3H, OCH$_3$).

2,3,4,6-tetra-O-benzyl-D-glucopyranose (**3a**): white needle (80%), m.p. 151-152 ^{o}C , R_f 0.57 (ethyl acetate), α/β anomeric mixture (α/β ratio 10:3), IR (KBr, cm^{-1}) 3346, 2914, 2855, 1454 and 1084; ^{1}H-NMR (CDCl$_3$) δ(ppm): 7.38-7.19 (m, 20H, Ar-H), 7.20 (d, *J*= 6.45 Hz, 1H, H-1 (β-anomer)), 6.66 (d, *J*= 4.69 Hz, 1H, H-1 (α-anomer)), 5.23 (t, *J*= 4.10 Hz, 1H, H-2), 4.76-4.62 (m, 4H, 2PhCH$_2$), 4.53-4.44 (m, 4H, 2PhCH$_2$), 3.89-3.81 (m, 2H, H-4, H-6), 3.65-3.57 (m, 1H, H-5) and 3.48-3.33 (m, 2H, H-3, H-6′).

2.4.1.2 Synthesis of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside

(**1a**)   (**4a**)
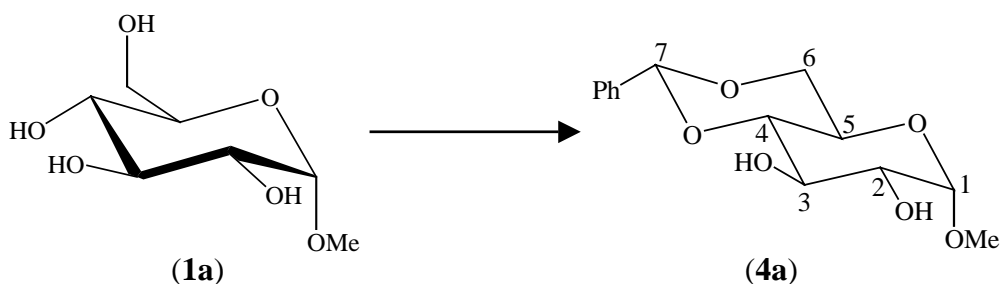
General Procedure: A mixture of methyl-α-D-glucopyranoside (**1a**) 12 g (62 mmol), powdered zinc chloride 9 g and benzaldehyde 30 mL was stirred at room temperature for a period of 48 hours. The resulting pale-yellow, cloudy reaction mixture was poured slowly, with stirring, into 500 mL of cold water, stirred for an additional 10 min, and refrigerated overnight. Hexane 50 mL was added and the resulting mixture is stirred for 0.5 hours. The product was separated upon filtration on a buchner funnel, washed twice with 100 mL of cold water, and dried under vacuum at room temperature overnight and the product was achieved upon recrystallization from chloroform-ether.[49]

Methyl 4,6-O-benzylidene-α-D-glucopyranoside (**4a**): white needle (63%), m.p. 163-165°C; R_f 0.11 (hexane:ethyl acetate 1:1), IR (KBr, cm^{-1}): 3167, 1637 and 1073; ^{1}H-NMR (DMSO) δ (ppm): 7.46-7.39 (m, 5H, aromatic), 5.59 (s, 1H, H-7), 5.22 (d, *J*=4.69 Hz, 1H, H-1), 5.04 (d, *J*=7.04 Hz, 1H, H-6), 4.18 (dd, *J*= 5.28 , 4.69 Hz, 1H, H-3), 3.70 (t, *J*=9.97 Hz, 1H, H-4), 3.62-3.57 (m, 2H, H-2 and H-5),), 3.41-3.37 (m, 1H, H-6) and 3.33 (s, 3H, OCH$_3$).

2.4.1.3 Synthesis of benzoic acid derivatives
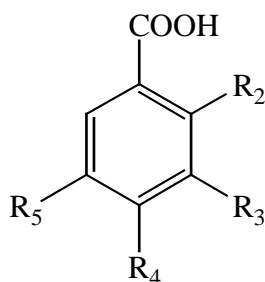
Compounds	R_2	R_3	R_4	R_5
1b	H	H	OBn	H
2b	OBn	H	OBn	H
3b	H	OBn	OBn	H
4b	H	OBn	H	OBn
5b	H	OBn	OBn	OBn
6b	H	H	OMe	H
7b	H	OMe	OMe	Ome

General Procedure:

Method I: (for **1b, 2b, 3b**)

Benzylation

Sodium hydride (28.96 mmol) was added gradually to a solution of (7.24 mmol) of benzoic acid derivatives in 30 mL DMF in a 250 mL round-bottomed flask, and the suspension was swirled for 30 min at ~25°C. The mixture was then cooled to 0°C and 7 mL (20.68 mmol) of benzyl bromide is added. The reaction mixture became solid in about 40 min and was held at ~25°C until a clear solution formed (24-48 hours). Dry methanol (10 mL) is added and the solution was concentrated to dryness. To the residue are added 30 mL of dichloromethane and 30 mL of water. The organic layer was removed, washed with three 40-mL portions of water, dried over anhydrous magnesium sulfate. The solvent was removed to give benzyl ester product.[48]

Saponification

A mixture of benzyl ester with 30 mL of 10% KOH in EtOH was refluxed for 3 h. The solvent was then removed to give a crude product. Ethyl acetate and water

were added and water phase was separated and then added concentrated HCl to furnish the product upon filtration.

.

Method II: (for **4b** and **5b**)

Benzylation

A mixture of 10 mmol benzoic acid derivative, 5 mL benzyl chloride, potassium carbonate 6.35 g and 100 mL THF was refluxed for 2 h. After cooling to room temperature, water was added, filtered and triturated at room temperature for 3 h with a solution of 12 g potassium hydroxide in 500 mL of a 4:1 methanol-water mixture, filtered, washed with methanol and water, dried and recrystallized from benzene-ligroin mixture to yield a benzyl ester product.[48]

Saponification

The benzyl ester was suspended in 50 mL boiling methanol and over 1 h was added in portions 30 mL of 20% potassium hydroxide solution. After the solution was cooled and acidified with dilute HCl. The benzyloxy benzoic acid was filtered out and washed with a little cold water. After drying, the product was obtained from recrystallization with a mixture of petroleum ether and ethyl acetate.[49]

4-Benzyloxybenzoic acid [50] (**1b**): pale yellow solid (73%), m.p. 185-186°C, R_f 0.33 (hexane:ethyl acetate 1:1); IR (KBr, cm^{-1}): 3621-3247, 3027, 2914, 1690, 1603, 1511 and 1429; ^{1}H-NMR (DMSO-d$_6$) δ (ppm): 7.91 (d, *J*= 8.58 Hz, 2H, Ar-H, ortho to COOH), 7.49-7.38 (m, 5H, Ar-H), 7.12 (d, *J*= 8.58 Hz, 2H, Ar-H, meta to COOH) and 5.20 (s, 2H, PhCH$_2$).

2,4-Dibenzyloxybenzoic acid [51] (**2b**): white solid (70%), m.p. 118-119°C, R_f 0.47 (hexane:ethyl acetate 1:1); IR (KBr, cm^{-1}): 3649-3260, 2918, 1692, 1606 and 1186; ^{1}H-NMR (CDCl$_3$) δ (ppm): 8.20-6.73 (m, 13H, Ar-H), 5.23 (s, 2H, PhCH$_2$) and 5.15 (s, 2H, PhCH$_2$).

3,4-Dibenzyloxybenzoic acid [50] (**3b**): white solid (90%), m.p. 182-184°C, R_f 0.31 (hexane:ethyl acetate 1:1); IR (KBr, cm^{-1}): 3610-3144, 1675, 1593, 1439 and 1280; ^{1}H-NMR (CDCl$_3$) δ (ppm): 7.75-6.98 (m, 13H, Ar-H), 5.28 (s, 2H, CH$_2$) and 5.25 (s, 2H, PhCH$_2$).

3,5-Dibenzyloxybenzoic acid [49] (**4b**): white solid (77.4%), m.p. 202-205°C; R_f 0.35 (hexane:ethyl acetate 1:1), IR (KBr, cm^{-1}): 3467, 1685, 1598 and 1163; ^{1}H-NMR

(DMSO-d$_6$) δ (ppm): 7.48-7.35 (m, 10H, Ar-H), 7.61 (s, 2H, Ar-H), 6.94 (s, 1H, Ar-H) and 5.16 (s, 4H, CH$_2$ ×2).

3,4,5-Tribenzyloxybenzoic acid [50] (**5b**): white solid (53%), m.p. 191-193°C; R$_f$ 0.33 (hexane:ethyl acetate 1:1), IR (KBr, cm^{-1}): 3472, 1685, 1593, 1434 and 1122; ^{1}H-NMR (DMSO-d$_6$) δ (ppm): 7.30-7.48 (m, 20H, Ar-H) and 5.18-5.19 (s, 6H, PhCH$_2$).

Method III: for trimethylgallic acid

Methylation

To a cold solution of 80 g (2 moles) of sodium hydroxide in 500 mL of water in a flask was added 50 g (0.27 mole) of carboxylic acid. The flask was immediately tightly stopped, and the mixture was shaken occasionally until all the acid dissolved; 89 g (67 mL) of dimethyl sulfate (0.71 mole) was then added and the flask was shaken for twenty minutes, being cooled by means of cold water in order that the temperature did not raise above 30-35°C. A second portion of 89 g (67 mL) of dimethyl sulfate was then added and shaking was continued for another ten minutes. The flask was then fitted with a reflux condenser and the contents were boiled for 2 h. In order to saponify the small amount of ester produced, a solution of 20 g of sodium hydroxide in 30 mL of water was then added and boiling was continued for two additional hours. The reaction mixture was then cooled and acidified with dilute hydrochloric acid to give the precipitated product which was then purified upon recrystallization.[52]

Trimethylgallic acid [52] (**7b**): colorless needles (30%), m.p.165-168°C, R$_f$ 0.27 (heaxane:ethyl acetate 1:1); IR (KBr,cm^{-1}): 1684, 1587, 1505 and 1458; ^{1}H-NMR (CDCl$_3$) δ (ppm): 7.19-7.50 (s, 2H, Ar-H) and 3.80-3.72 (m, 9H, 3OCH$_3$); ^{13}C-NMR (CDCl$_3$) δ (ppm): 166.9 (1C, C=O), 152.6 (1C, Ar-C to COOH), 141.3, 125.9, 106.5 (3C, Ar-C), 60.1, 55.9 (2C, Ar-C-O), 39.8, 39.4 and 39.0 (3C, OCH$_3$).

2.4.2 Synthesis of Sugar Esters

2.4.2.1 <u>Synthesis of 1-monosubstituted glucopyranoside</u>

<u>Step I</u>
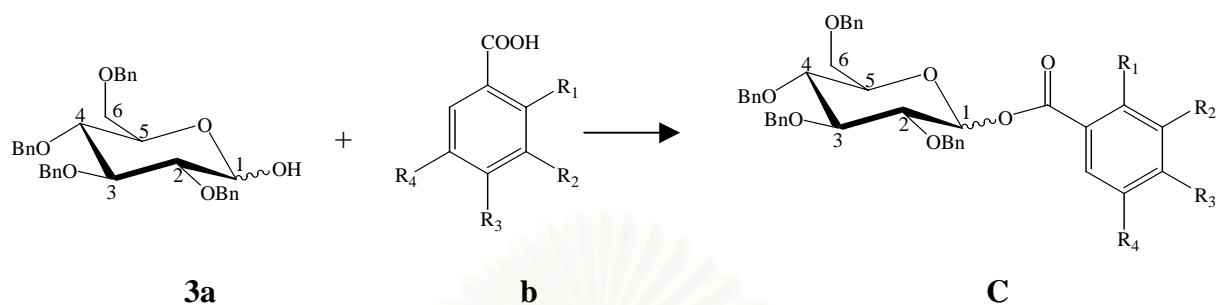
Compounds	Benzoic acid derivatives
1C	4-Benzyloxy benzoic acid (**1b**)
2C	2,4-Dibenzyloxy benzoic acid (**2b**)
3C	3,4-Dibenzyloxy benzoic acid (**3b**)
4C	3,5-Dibenzyloxy benzoic acid (**4b**)
5C	3,4,5-Tribenzyloxy benzoic acid (**5b**)
6C	*p*-Anisic acid (**6b**)
7C	3,4,5-Trimethoxy benzoic acid (**7b**)
8C	Stearic acid
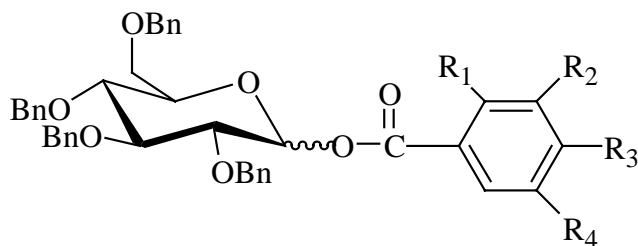
Compounds	R_1	R_2	R_3	R_4
1C	H	H	OBn	H
2C	OBn	H	OBn	H
3C	H	OBn	OBn	H
4C	H	OBn	H	OBn
5C	H	OBn	OBn	OBn
6C	H	H	OMe	H
7C	H	OMe	OMe	OMe

8C

Esterification: General Procedure A

A solution of sugar derivative, acid, DCC, DMAP in dry CH_2Cl_2 (50 mL) was refluxed under argon for 12-36 h (followed by TLC). The reaction mixture was allowed to cool to room temperature, and the white solid (dicyclohexylurea) was filtered off. The solvent was removed *in vacuo* to give a crude product. The crude product was redissolved in CH_2Cl_2, washed with water (2×50 mL), dried over anhydrous Na_2SO_4, and the solvent was removed *in vacuo*. The residue was purified by column chromatography with hexane and ethyl acetate to furnish the desired esters.[35,45]

4-Benzyloxy benzoyl-2,3,4,6-tetra-O-benzyl glucopyranoside (**1C**): yellow solid (85%), m.p. 93-95°C; R_f 0.58 (hexane:ethyl acetate 7:3) as an anomeric mixture ($\alpha/\beta \approx 1:10$); IR (KBr, cm^{-1}): 3021, 2914, 1731, 1608 and 1075; ^{1}H-NMR (CDCl$_3$) δ (ppm): 8.06 (d, $J= 8.58$ Hz, 2H, Ar-H), 7.18-7.49 (m, 25H, Ar-H ×5), 7.03 (d, $J= 9.36$ Hz, 2H, Ar-H), 6.62 (d, $J= 3.12$ Hz, 1H, H-1, α-anomer), 5.90 (d, $J= 7.80$ Hz, 1H, H-1, β-anomer), 5.17 (s, 2H, PhCH$_2$), 4.79-4.97 (m, 8H, PhCH$_2$ ×4) and 3.84-3.69 (m, 6H, H-glucose); ^{13}C-NMR (CDCl$_3$) δ(ppm): 164.5 (1C, COOR), 163.0 (1C, C-4′), 138.4-137.8 (4C, C-1″×4), 128.7-127.6 (25C, OBn ×5), 121.8 (1C, C-1′), 114.6 (2C, C-3′, C-5′), 94.5 (1C, C-1) and 84.9-68.1 (10C, C-glucose, CH$_2$×5).

2,4-Dibenzyloxy benzoyl-2,3,4,6-tetra-O-benzyl glucopyranoside (**2C**): white solid (52%) , m.p. 79°C; R_f 0.56 (hexane:ethyl acetate 8:2), as β-anomer, IR (KBr, cm^{-1}): 3011, 2931, 1691, 1602, 1503 and 1178; ^{1}H-NMR (CDCl$_3$) δ (ppm): 8.01 (d, $J= 8.58$ Hz, 1H, Ar-H), 7.18-7.55 (m, 30H, Ar-H ×6), 6.60-6.64 (m, 2H, Ar-H), 5.94 (d, $J= 7.80$ Hz, 1H, H-1 (β anomer), 4.51-4.94 (m, 8H, PhCH$_2$ × 4) and 3.52-4.00 (m, 6H, H-glucose); ^{13}C-NMR (CDCl$_3$) δ (ppm): 169.8 (1C, COOR), 163.9, 163.3 (2C, C-4′, C-6′), 138.6, 138.2, (2C, C-1″x 2), 138.1, 138.0, 136.4, 136.3 (4C, C-1″×4), 134.8 (1C, C-2′), 128.8-127.3 (30C, OBn×5), 106.2 (2C, C-1′, C-3′), 101.3 (1C, C-5′), 94.2 (1C, C-1) and 85.0-70.3 (11C, C-glucose, CH$_2$×6).

3,4-Dibenzyloxy benzoyl-2,3,4,6-tetra-O-benzyl glucopyranoside (**3C**): white solid (35%), m.p. 145-146°C, R_f 0.53 (hexane:ethyl acetate 7:3) as an anomeric mixture ($\alpha/\beta \approx 1:10$), IR (KBr, cm^{-1}): 3011, 2924, 1721, 1598 and 1075; ^{1}H-NMR (CDCl$_3$) δ (ppm): 7.70-7.72 (m, 2H, Ar-H), 7.31-7.51 (m, 30H, Ar-H × 6), 6.99 (d, $J= 9.36$ Hz, 1H, Ar-H), 6.60 (d, $J=3.12$ Hz, 1H, H-1 α-anomer), 5.90 (d, $J=7.02$ Hz, 1H, H-1 β-anomer), 5.22-5.29 (m, 4H, PhCH$_2$ × 2), 4.88-5.04 (m, 2H, PhCH$_2$), 4.52-4.72 (m, 4H, PhCH$_2$ × 2) and 3.68-3.90 (m, 6H, H-glucose); ^{13}C-NMR (CDCl$_3$) δ (ppm): 164.5 (1C, COOR), 153.4 (1C, C-4′), 148.3 (1C, C-3′), 137.8-138.4 (4C, C-1″of glucose), 136.4-136.7 (2C, C-1″of acid), 128.7-127.2 (30C, Ar-C), 124.8 (1C, C-1′), 122.0 (1C, C-6′), 115.7 (1C, C-2′), 113.1 (1C, C-5′), 94.6 (1C, C-1) and 68.1-84.9 (11C, CH$_2$× 6, C-glucose).

3,5-Dibenzyloxy benzoyl-2,3,4,6-tetra-O-benzyl glucopyranoside (**4C**): white solid (34%), m.p. 88°C, R_f 0.31 (dichloromethane) as an anomeric mixture ($\alpha/\beta \approx$

4:10); IR (KBr, cm^{-1}): 3016, 2919, 1736, 1598 and 1075; ^{1}H-NMR (CDCl$_3$) δ (ppm): 7.24-7.48 (m, 30H, Ar-H), 6.64 (d, *J*= 3.90 Hz, 1H, H-1 (α-anomer)), 5.95 (d, *J*=7.80 Hz, 1H, H-1 (β-anomer), 4.53-5.14(m, 12H, PhCH$_2$× 6), 4.10 (t, *J*= 9.36 Hz, 1H, H-6) and 3.70-4.12 (m, 5H, H-glucose); ^{13}C-NMR (CDCl$_3$) δ (ppm): 164.7 (1C, COOR), 159.8 (2C, C-3′, C-5′), 137.8-138.4 (4C, C-1′′×4), 136.4 (2C, C-1′′×2), 127.5-128.7 (30C, OBn×5), 108.9 (1C, C-4′), 91.0 (1C, C-1) and 68.1-84.9 (11C, C-glucose, CH$_2$×6).

3,4,5-Tribenzyloxy benzoyl-2,3,4,6-tetra-O-benzyl glucopyranoside (**5C**): colorless syrup (44%), R$_f$ 0.56 (hexane:ethyl acetate 7:3) as an anomeric mixture (α/β ≈ 7:10); IR (KBr, cm^{-1}) : 3016, 2924, 1731, 1588 and 1106; ^{1}H-NMR (CDCl$_3$) δ (ppm): 7.45-7.18 (m, 35H, Ar-H), 6.57 (d, broad, 1H, α-anomer), 5.91-5.89 (d, *J*=7.80 Hz, 1H, β-anomer), 5.20-5.12 (m, 6H, PhCH$_2$-acid), 5.00-4.52 (m, 8H, PhCH$_2$), 4.00 (t, *J*=8.18 Hz, 1H, H-6$^{'}$), 3.87-3.77 (m, 4H, H-2, H-4, H-5, H-6) and 3.70-3.65 (t, *J*=9.36 Hz, 1H, H-3); ^{13}C-NMR (CDCl$_3$) δ (ppm): 164.7 (1C, COOR), 152.6, 152.7 (2C, C-3′, C-5′), 142.9 (1C, C-4′), 138.7-127.6 (35C, OBn x 7), 94.9 (1C, C-1) and 84.9-53.8 (C-glucose, CH$_2$ x 7)
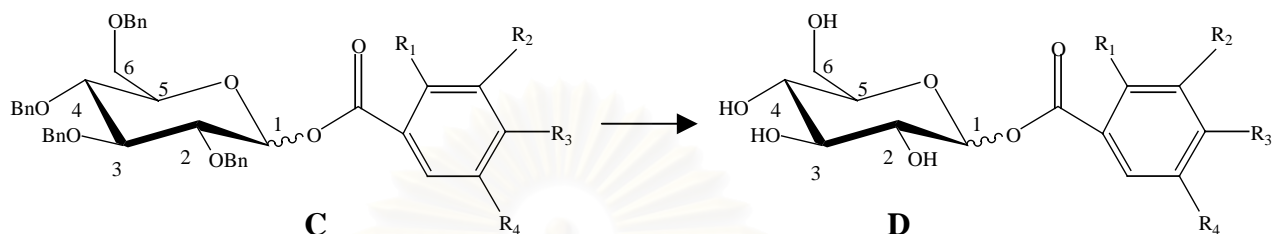
4-Methoxy benzoyl-2,3,4,6-tetra-O-benzyl glucopyranoside[53](**6C**): white solid (87%); m.p. 96-99°C; R$_f$ 0.60 (hexane:ethyl acetate 7:3) as an anomeric mixture (α/β ≈ 3:5); IR (KBr, cm^{-1}): 3021, 2910, 1726, 1598, 1511 and 1081; ^{1}H-NMR (CDCl$_3$) δ (ppm): 8.08 (d, *J*= 8.21 Hz, 2H, Ar-H), 7.40-7.18 (m, 20H, Ar-H ×4), 6.97 (d, *J*= 8.80 Hz, 2H, Ar-H), 6.63 (d, *J*=2.93 Hz, 1H, H-1, α-anomer), 5.91 (d, *J*= 7.62 Hz, 1H, H-1 β-anomer), 4.51-5.06 (m, 8H, PhCH$_2$×4) and 3.69-4.14 (m, 6H, H-glucose).

3,4,5-Trimethoxy benzoyl-2,3,4,6-tetra-O-benzyl glucopyranoside[54] (**7C**): colorless syrup (59%), R$_f$ 0.40 (hexane:ethyl acetate 7:3) as an anomeric mixture (α/β ≈1:10), IR (KBr, cm^{-1}): 3016, 2934, 1726, 1593 and 1219; ^{1}H-NMR (CDCl$_3$) δ (ppm): 7.94 (d, *J*=7.04 Hz, 2H, Ar-H), 7.60-7.26 (m, 20H, PhCH$_2$O×4), 6.61 (d, *J*= 3.12 Hz, 1H, H-1, α-anomer), 5.95 (d, *J*= 6.24 Hz, 1H, H-1, β-anomer), 4.56-5.11 (m, 8H, CH$_2$×4) and 3.71-4.16 (m, 15H, OCH$_3$×3, H-glucose).

Steroyl benzoyl-2,3,4,6-tetra-O-benzyl glucopyranoside (**8C**): white solid (89%); m.p. 63-64°C; R$_f$ 0.58 (hexane:ethyl acetate 8:2) as an anomeric mixture (α/β ≈ 3:5), IR (KBr, cm^{-1}): 2921, 1754, 1653, 1520 and 1081; ^{1}H-NMR (CDCl$_3$) δ (ppm): 7.19-7.33 (m, 20H, Ar-H), 6.45 (s, 1H, H-1, α-anomer), 5.68 (d, *J*=8.00 Hz, 1H, H-1,

β-anomer), 4.51-5.02 (m, 8H, CH$_2$×4) and 3.53-4.19 (m, 6H, H-glucose)**;** ^{13}C-NMR (CDCl$_3$) δ (ppm): 12.3 (1C, COOR), 129.0-127.7 (20C, OBn x 4), 84.8 (1C, C-1), 81.7-68.1 (26C, CH$_2$ x 2, C-glucose, 16C aliphatic) and 55.8 (1C, OCH$_3$).

Step II

Compounds	Debenzylation compounds
4-Benzyloxy benzoyl-2,3,4,6-tetra-*O*-benzyl glucopyranoside (**1C**)	**1D**
2,4-Dibenzyloxy benzoyl-2,3,4,6-tetra-*O*-benzyl glucopyranoside (**2C**)	**2D**
3,4-Dibenzyloxy benzoyl-2,3,4,6-tetra-*O*-benzyl glucopyranoside (**3C**)	**3D**
3,5-Dibenzyloxy benzoyl-2,3,4,6-tetra-*O*-benzyl glucopyranoside (**4C**)	**4D**
3,4,5-Tribenzyloxy benzoyl-2,3,4,6-tetra-*O*-benzyl glucopyranoside (**5C**)	**5D**
4-Methoxy benzoyl-2,3,4,6-tetra-*O*-benzyl glucopyranoside (**6C**)	**6D**
3,4,5-Trimethoxy benzoyl-2,3,4,6-tetra-*O*-benzyl glucopyranoside (**7C**)	**7D**

Deprotection: General Procedure B

A suspension of benzylated sugar ester, 10% Pd/C and dry THF was degassed with argon (3 times) and treated with hydrogen at 40°C for 24-48 h. The reaction mixture was allowed to cool to room temperature, the solid was filtered off through celite, and the celite was washed with acetone (30 mL). The combined organic phases were removed in *vacuo* to give a crude.[35,45]

4-Hydroxybenzoyl glucopyranoside[55] (**1D**): brown syrup (70%); R_f 0.67 (methanol: water 1:1) as an anomer mixture ($\alpha/\beta \approx 3/10$), IR (KBr, cm^{-1}): 3120-3704, 1710, 1637 and 1217; ^{1}H-NMR (acetone-d$_6$/D$_2$O): 7.92 (d, *J*= 8.74 Hz, 2H, Ar-H), 6.87 (d, *J*= 8.75 Hz, 2H, Ar-H), 6.23 (d, *J*= 3.64 Hz, 1H, H-1 (α-anomer), 5.63 (d, *J*=7.93 Hz, 1H, H-1(β-anomer)) and 3.25-3.83 (m, 6H, H-glucose).

2,4-Dihydroxybenzoyl glucopyranoside (**2D**): brown syrup (75%); R_f 0.58 (methanol:water 8:2); ^{1}H-NMR (acetone-d$_6$/D$_2$O): 10.62 and 8.13 (2H, OH), 7.86-7.66 (m, 1H, Ar-H), 6.37-6.46 (m, 2H, Ar-H), 5.72 (d, *J*=7.21 Hz, 1H, H-1 (β-anomer), 3.10-3.86 (m, 6H, H-glucose); ^{13}C-NMR (Acetone-d$_6$/D$_2$O): δ (ppm) 168.0 (1C, C=O), 165.0 and 163.0 (2C, Ar-C to OH), 133.0 (1C, Ar-C to C=O), 109.0, 102.0 and 95.0 (3C, Ar-C), and 57.7-77.4 (6C, C-glucose).

3,4-Dihydroxybenzoyl glucopyranoside [56] (**3D**): purple syrup (75 %), R_f 0.44 (methanol:water 8:2) as an anomeric mixture ($\alpha/\beta \approx 1:1.4$); IR (KBr, cm^{-1}): 3116-3669, 1707, 1629 and 1213; ^{1}H-NMR (acetone-d$_6$/D$_2$O) δ (ppm): 7.35-7.49 (m, 2H, Ar-H), 6.84-6.89 (m, 1H, Ar-H), 6.22 (d, *J*= 2.53 Hz, 1H, H-1 (α-anomer)), 5.65 (d, *J*= 7.26 Hz, 1H, H-1 (β-anomer)) and 3.16-4.02 (m, 6H, H-glucose).

3,5-Dihydroxybenzoyl glucopyranoside (**4D**): brown syrup (90%), R_f 0.80 (methanol:water 7:3) as an anomeric mixture ($\alpha/\beta \approx 1:1.2$), IR (KBr, cm^{-1}): 3149-3646, 3016, 1721, 1598 and 1050; ^{1}H-NMR (acetone-d$_6$/D$_2$O) δ (ppm): 8.93-8.82 (OH), 7.05-7.09 (m, 2H, Ar-H), 6.60-6.64 (m, 1H, Ar-H), 6.34-6.35 (d, 1H, *J*= 3.58 Hz, H-1 α-anomer), 5.73 (d, *J*= 7.52 Hz, 1H, H-1 β-anomer) and 3.10-4.05 (m, 6H, H-glucose); ^{13}C-NMR (acetone-d$_6$/D$_2$O): δ (ppm): 165.0 (1C, C=O), 158.6 (d, 2C, Ar-C to OH), 108.0, 107.9 and 107.8 (3C, Ar-C) and 58.0-77.0 (6C, C-glucose).

3,4,5-Trihydroxybenzoyl glucopyranoside [57] (**5D**): brown syrup (75%), R_f 0.82 (methanol:water 1:1) as an anomeric mixture ($\alpha/\beta \approx 1:1.8$) , IR (KBr, cm^{-1}): 3032-3641, 1695, 1629 and 1045; ^{1}H-NMR (acetone-d$_6$/ D$_2$O) δ (ppm): 7.11-7.18 (m, 2H, Ar-H), 6.25 (d, *J*=3.33 Hz, 1H, H-1, α-anomer), 5.64 (d, *J*= 7.58 Hz, 1H, H-1, β-anomer) and 3.18-4.66 (m, 6H, H-glucose).

4-Methoxybenzoyl glucopyranoside (**6D**): white solid (90%), m.p. 157-160°C, R_f 0.40 (metanol:water 1:1) as an anomeric mixture ($\alpha/\beta \approx 3.5:10$), IR (KBr, cm^{-1}): 3190-3560, 2968, 1711, 1688 and 1602; ^{1}H-NMR (acetone-d$_6$/ D$_2$O) δ(ppm): 8.03 (d, *J*= 8.74 Hz, 2H, Ar-H), 7.02 (d, *J*= 8.81 Hz, 2H, Ar-H), 6.26 (d, *J*=3.57 Hz, 1H, H-1

(α-anomer)), 5.65 (d, J=7.58 Hz, 1H, H-1 (β-anomer)) and 3.17-3.85 (m, 6H, H-glucose); ^{13}C-NMR (acetone-d$_6$/D$_2$O) δ (ppm): 166.0 (1C, C=O), 164.0 (1C, Ar-C to OMe), 132.2, 131.8, 114.0 and 113.8 (4C, Ar-C), 94.5 (1C, C-1) and 55.4-77.2 (7C, C-1′, C-glucose)

3,4,5-Trimethoxybenzoyl glucopyranoside (**7D**): White solid (90%), m.p. 144°C, R$_f$ 0.18 (metanol:water 1:1) as an anomeric mixture (α/β ≈ 1.7/10), IR (KBr, cm^{-1}): 3518, 3011, 1705, 1588 and 1075; ^{1}H-NMR (acetone-d$_6$/D$_2$O): δ (ppm) 7.41-7.42 (s, 2H, Ar-H), 6.34 (d, J= 6.24 *Hz*, 1H, H-1 (α-anomer)), 5.76 (d, *J*=7.80 *Hz*, 1H, H-1 (β-anomer)) and 3.93 (s, 9H, OCH$_3$); ^{13}C-NMR (acetone-d$_6$/D$_2$O) δ (ppm): 165.0 (1C, C=O), 152.0, 124.0 (3C, Ar-C to OMe), 107.4 (2C, Ar-C), 95.1 (1C, C-1), 77.0, 72.0, 69.0, and 60.0 (4C, C-glucose).

2.4.2.2 Synthesis of 2,3-disubstituted sugar esters

Step I
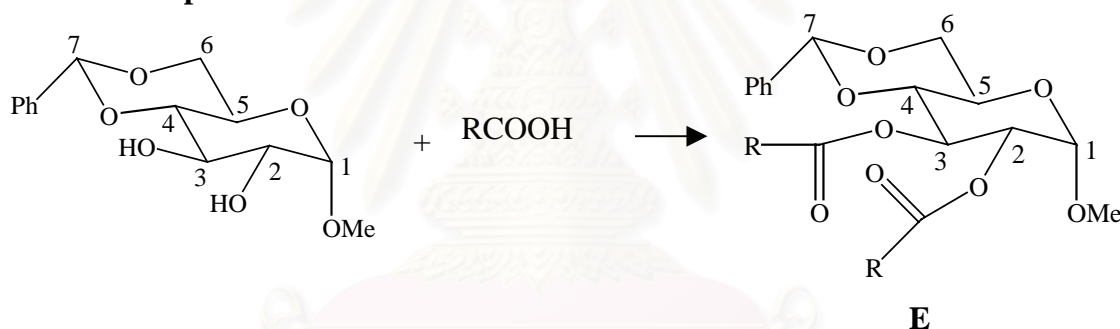
Compounds	RCOOH
1E	Acetic acid
2E	Pivalic acid
3E	4-Methoxybenzoic acid
4E	3,4,5-Trimethoxybenzoic acid
5E	3,4-Dibenzyloxybenzoic acid (**3b**)

Esterification: General Procedure A

A solution of sugar derivative, acid, DCC, DMAP in dry CH$_2$Cl$_2$ (50 mL) was refluxed under argon for 12-36 h. The reaction mixture was allowed to cool to room temperature, and the white solid (dicyclohexylurea) was filtered off. The solvent was removed *in vacuo* to give a crude product. The crude product was redissolved in

CH$_2$Cl$_2$, washed with water (2×50 mL), dried over anhydrous Na$_2$SO$_4$ and the solvent was removed *in vacuo*. The residue was purified by column chromatography with hexane and ethyl acetate to elute the desired esters.

Methyl-2,3-di-O-acetyl-4,6-O-benzylidene-α-D-glucopyranoside[58] (**1E**): white solid (78%), mp.104-105°C, R$_f$ 0.60 (hexane:ethyl acetate 1:1); IR (KBr, cm^{-1}): 2933, 1742 and 1061; ^{1}H-NMR (CDCl$_3$) δ (ppm): 7.36-7.46 (m, 5H, Ar-H), 5.51 (s, 1H, H-7), 5.58 (t, *J*= 9.38 Hz, 1H, H-3), 4.89-4.98 (m, 2H, H-1, H-2), 4.30-4.33 (m, 1H, H-6), 4.07-4.19 (m, 1H, H-5), 3.60 (t, *J*= 9.97 Hz, 1H, H-6), 3.67 (t, *J*= 9.38 Hz, 1H, H-4), 3.42 (s, 3H, OCH$_3$), 2.10 and 2.05 (s, 6H, CH$_3$).

Methyl-2,3-di-O-pivaloyl-4,6-O-benzylidene-α-D–glucopyranoside[59] (**2E**): white solid (20%), m.p. 154-155°C; R$_f$ 0.68 (ethyl acetate); IR (KBr, cm-1): 2976, 1723, 1458 and 1287; ^{1}H-NMR (CDCl$_3$) δ (ppm): 7.39-7.58 (m, 5H, Ar-H), 5.60 (s, 1H, H-7), 4.99 (d, *J*= 4.10 Hz, 1H, H-1), 4.78 (dd, *J*=10.00, 3.56 Hz, 1H, H-2), 4.38 (dd, *J*=10.00, 4.69 Hz ,1H, H-6), 4.25 (t, *J*=9.37 Hz, 1H, H-3), 3.89-4.00 (m, 1H, H-5), 3.84 (t, *J*=9.37 Hz, 1H, H-6′), 3.63 (t, *J*=9.37 Hz, 1H, H-4), 3.40 (s, 3H, OCH$_3$) and 1.25 (s, 18H, CH$_2$).

Methyl-2,3-di-O-(p-methoxy)benzoyl-4,6-O-benzylidene-α-D-glucopyranoside (**3E**): white solid (53%), m.p. 147-148°C, R$_f$ 0.64 (hexane:ethyl acetate 1:1), IR (KBr, cm^{-1}): 3021, 2934, 1716, 1644, 1593, 1465 and 1224; ^{1}H-NMR (CDCl$_3$) δ (ppm): 7.77 (d, *J*=8.21 Hz, 4H, Ar-H), 7.30-7.40 (m, 5H, Ar-H), 6.62 (t, *J*= 8.80 Hz, 5H, H-7, Ar-H), 5.97 (t, *J*=9.97 Hz, 1H, H-3), 5.52-5.53 (m, 2H, H-1, H-2), 4.40-4.50 (m, 1H, H-5), 4.00-4.10 (m, 2H, H-6), 3.90-4.00 (m, 2H, H-6, H-4) and 3.43 (s, OCH$_3$); ^{13}C-NMR (CDCl$_3$) δ (ppm): 165.8, 65.3 (2C, C=O), 163.7 (1C, C-4′), 163.3 (1C, C-4′), 136.9 (1C, C-1″), 131.8-132.1 (4C, C-2″×2, C-6″×2), 129.0 (1C, C-4″), 128.2 (2C, C-3″, C-5″), 126.2 (2C, C-2″,C-6″), 122.1(1C, C-1′), 121.4 (1C,C-1′), 113.5-113.7 (4C, C-3′×2, C-5′×2), 101.6 (1C, C-7), 97.9 (1C, C-1), 62.5-79.5 (5C, glucose), 55.5, 55.4 and 55.4 (3C, OCH$_3$).

Methyl-2,3-di-O-(trimethoxy)benzoyl-4,6-O-benzylidene-α-D-glucopyrano-side[27](**4E**): white solid (72%), m.p. 148-152°C; R$_f$ 0.51 (hexane:ethyl acetate 1:1); IR (KBr, cm^{-1}): 3021, 2934, 1716, 1644, 1593, 1465 and 1224; ^{1}H-NMR (CDCl$_3$) δ (ppm): 7.36-7.47 (m, 5H, Ar-H), 7.24-7.27 (m, 4H, Ar-H), 6.05 (t, *J*= 9.96 Hz, 1H, H-3), 5.25 (d, *J*= 3.52 Hz, 1H, H-1), 5.18 (dd, *J*=10.00, 3.52 Hz, 1H, H-2), 4.40-4.42

(m, 1H, H-5), 4.08-4.18 (m, 1H, H-6), 3.86-3.98 (m, 2H, H-6, H-4),   3.94-3.98 (m, 18H, OCH$_3$) and 3.49 (s, 3H, OCH$_3$).

Methyl-2,3-di-O-(3,4-dihydroxy)benzoyl-4,6-O-benzylidene-α-D-glucopyrano-side (**5E**): white solid (57%), m.p. 58-60°C; R$_f$ 0.56 (hexane:ethyl acetate 1:1); IR (KBr, cm^{-1}): 3030, 2925, 1715, 1602 and 1516; ^{1}H-NMR (CDCl$_3$) δ (ppm): 6.8-7.74 (m, 31H, Ar-H), 6.85 (m, 1H, H-7), 5.72 (t, *J*= 9.57 Hz, 1H, H-3), 5.07-5.29 (m, 12H, 2H-glucose, CH$_2$×5, H-1,H-2), 4.83-4.78 (dd, *J*=4.37, 4.44 Hz, 1H, H-6), 4.60-4.63 (m, 1H, H-6′), 4.22-4.10 (m, 1H, H-5), 3.72-3.84 (m, 1H, H-4) and 3.47 (s, 3H, OCH$_3$); ^{13}C-NMR (CDCl$_3$) δ (ppm): 168.2, 165.7 (2C, C=O), 153.3, 153.2, 148.4, 148.3, 136.7 , 136, 136.4, 136.4, 128.6, 128.6, 128.5, 128.1, 128.0, 130.0, 127.5, 127, 127.4, 127.2, 127.1, 124.5, 124.4, 124.4, 122.5, 121.9, 115.7, 115.4, 115.3, 113.2 and 113.2 (38C, Ar-C), 113.1 (1C, C-7) and 97.2 (1C, C-1).

Step II
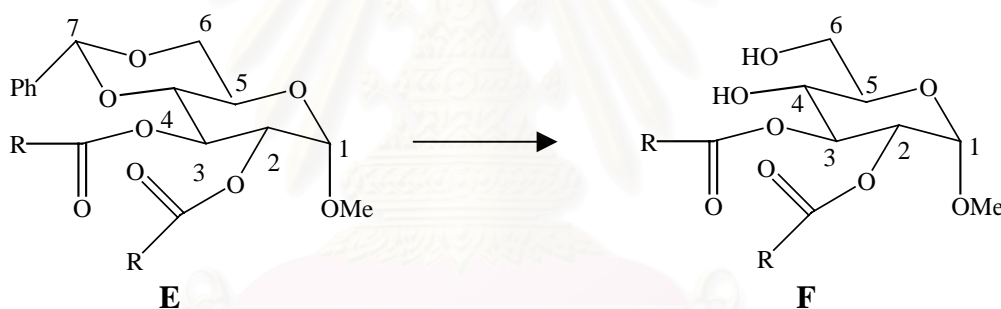
Compounds	Deprotected product
Methyl-2,3-di-*O*-pivaloyl-4,6-*O*-benzylidene-α-D-glucopyranoside (**2E**)	**2F**
Methyl-2,3-di-*O*-(p-methoxy)benzoyl-4,6-*O*-benzylidene-α-D-glucopyranoside (**3E**)	**3F**
Methyl-2,3-di-*O*-(trimethoxy)benzoyl-4,6-*O*-benzylidene-α-D-glucopyranoside (**4E**)	**4F**

Deprotection: General Procedure C

A solution of benzylidene acetal in THF (50 mL) was treated dropwise at 60°C with 2 N HCl (65 ml). The reaction mixture was refluxed at 78°C for 24 hours. After cooling to room temperature, the reaction mixture was carefully neutralized with a saturated NaHCO$_3$ solution and extracted five times with ethyl acetate (80 ml). The combined organic phases were dried over anhydrous sodium sulfate and filtered, and the solvent was removed *in vacuo* to give a residue. The residue was purified by silica gel column chromatography with hexane and ethyl acetate to elute the desired esters.

Methyl-2,3-di-O-pivaloy-α-D-glucopyranoside [60] (**2F**): white solid (65 %), m.p. 133-135°C; R$_f$ 0.31 (ethyl acetate), IR (KBr, cm^{-1}): 3436, 2960, 1726, 1357 and 1045; ^{1}H-NMR (CDCl$_3$) δ (ppm): 5.14 (t, J=5.46 Hz, 1H, H-6), 4.69 (d, J=3.90 Hz, 1H, H-1), 4.58 (t, J= 6.24 Hz, 1H, H-4), 4.40 (dd, J=10.40, 3.90 Hz, 1H, H-2), 3.61-3.69 (m, 2H, H-3,H-5), 3.46-3.52 (m, 1H, H-6′) and 3.27 (s, 3H, OCH$_3$).

Methyl-2,3-di-O-(p-methoxy)benzoyl-α-D-glucopyranoside [54] (**3F**): white solid (72%), R$_f$ 0.11 (hexane:ethyl acetate 1:1), IR (KBr, cm^{-1}): 3451, 3011, 1715, 1602, 1509, 1217; ^{1}H-NMR (CDCl$_3$) δ (ppm): 7.89 (d, J=9.36 Hz, 2H, Ar-H), 7.78 (d, J=9.36 Hz, 2H, Ar-H), 6.98-7.02 (m, 2H, Ar-H), 5.54-5.61 (m, 2H, H-3, H-4), 5.04 (d, J=3.12 Hz, 1H, H-1), 4.96 (m, 3.90 Hz, 1H, H-2), 4.7-4.78 (m, 1H, H-6), 3.80 (s, 9H, OCH$_3$), 3.67-3.76 (m, 1H, H-5) and 3.58-3.62 (m, 1H, H-6′).

Methyl-2,3-di-O-(trimethoxy)benzoyl-α-D-glucopyranoside [27] (**4F**): pale yellow syrup (64%), R$_f$ 0.35 (ethyl acetate); IR (KBr, cm^{-1}): 3392, 2941, 1715, 1657 and 1590; ^{1}H-NMR (400 MHz, CDCl$_3$): δ (ppm) 8.06 (s, 2H, Ar-H), 7.95 (s, 2H, Ar-H), 6.46-6.48 (d, J=6.45 Hz, 1H, H-4), 6.37-6.42 (t, J=9.97 Hz, 1H, H-3), 5.95 (d, J=3.52 Hz, 1H, H-1), 5.71-5.74 (dd, J=2.93, 3.52 Hz, 1H, H-2), 5.58-5.61 (t, J=5.86 Hz, 1H, H-6), 4.55-4.65 (m, 1H, H-5) and 4.46-4.52 (m, 1H, H-6′).

2.4.2.3 Synthesis of tetrasubstituted sugar esters

1a   **H**
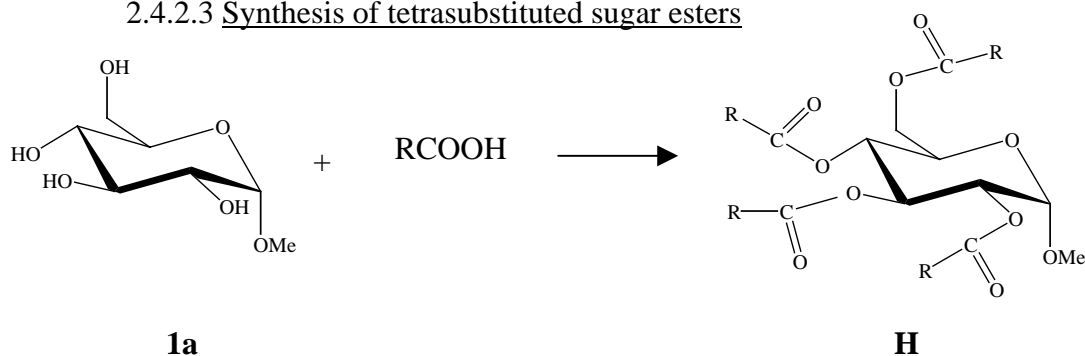
Compounds	RCOCl or RCOOH	Methods
Methyl-2,3,4,6-tetra-*O*-benzyl-glucopyranoside (**1H**)	Benzoyl chloride	B
Methyl-2,3,4,6-tetra-*O*-stearyl-glucopyranoside (**2H**)	Stearic acid	A

Esterification: General Procedure A

A solution of methyl α-D-glucopyranoside (**1a**), acid, DCC, DMAP in dry dichloromethane (50 mL) was refluxed under argon for 12-36 h. The reaction mixture was allowed to cool to room temperature, and the white solid of dicyclohexylurea was filtered off. The solvent was removed *in vacuo* to give a crude product, which was then redissolved in dichloromethane, washed with water (2×50 mL), dried over anhydrous sodium sulfate, and the solvent was removed *in vacuo* to give a crude product. The residue was purified by column chromatography with hexane and ethyl acetate to elute the desired esters.

Methyl-2,3,4,6-tetra-O-benzyl-glucopyranoside[61] (**1H**): white solid (52%), m.p. 110°C, R_f 0.55 (dichloromethane); IR (KBr, cm^{-1}): 3015, 2934, 1725, 1597, 1451 and 1275; ^{1}H-NMR (CDCl$_3$) δ (ppm): 7.30-8.10 (m, 30H, Ar-H), 6.22 (t, *J*= 9.97 Hz, 1H, H-3), 5.72 (t, *J*= 9.97 Hz, 1H, H-4), 5.32-5.36 (m, 1H, H-2), 5.29 (d, *J*= 3.52 Hz, 1H, H-1), 4.65 (dd, *J*=12.00 , 3.52 Hz, 1H, H-5), 4.45-4.55 (m, 2H, H-6, H-6′) and 3.53 (s, 3H, OCH$_3$).

Methy-2,3,4,6-tetra-O-stearyl-glucopyranoside[54] (**2H**): white solid (15%), m.p. 139°C; R_f 0.82 (hexane: ethyl acetate 8:2); IR (KBr, cm^{-1}): 2924, 1741, 1470 and 1214; ^{1}H-NMR (CDCl$_3$) δ (ppm): 5.56 (t, *J*= 9.85 Hz, 9.82 Hz, 1H, H-3), 5.11 (t, *J*= 9.80 Hz, 1H, H-4), 4.99 (d, *J*= 3.56 Hz, 1H, H-1), 4.96 (dd, *J*= 10.40, 3.66 Hz,

1H, H-2), 4.25 (dd, J= 12.40, 4.76 Hz, 1H, H-6), 4.13-4.17 (m, 1H, H-6), 4.00-4.03 (m, 1H, H-5), 3.43 (s, 3H, OCH$_3$), 2.23-2.40 (m, 8H, CH$_2$) and 1.29 (s, long chain).

2.4.2.4 Synthesis of pentasubstituted sugar esters
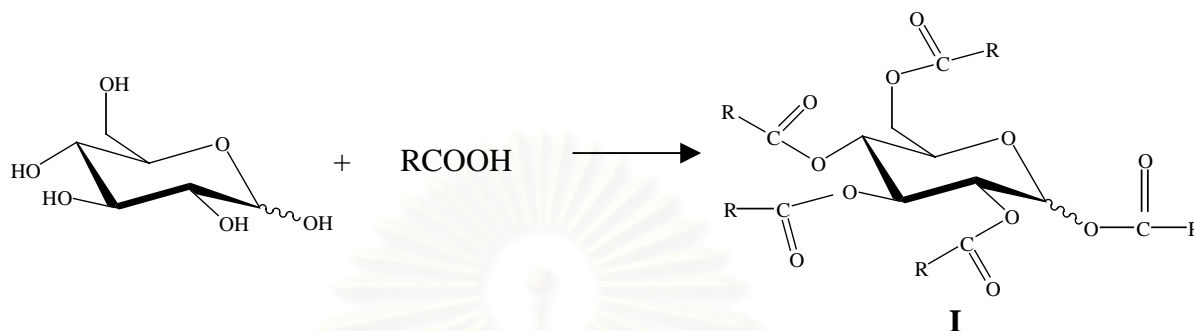
Compounds	RCOCl or RCOOH	Methods
1,2,3,4,6-penta-*O*-benzoyl-glucopyranoside (**1I**)	Benzoyl chloride	B
1,2,3,4,6-penta-*O*-(*3,4-dibenzyloxy) benzoyl* glucopyranoside (**2I**)	3,4-Dibenzyloxy benzoic acid (**3b**)	A

Esterification: General Procedure B

A solution of sugar derivative, acid chloride, a catalytic amount of dry triethylamine (6 drops) and dry dichloromethane (50 mL) was cooled in an ice-salt bath. The reaction mixture was stirred while maintained the temperature of the reaction below 10°C. Allow the reaction mixture to stand at 0°C for 24 h. The mixture was diluted in dichloromethane, washed with dilute H$_2$SO$_4$, saturated NaHCO$_3$ solution, water (2×50 mL), dried over anhydrous sodium sulfate and the solvent was removed *in vacuo*. The residue was separated by column chromatography using hexane and ethyl acetate as eluent.

1,2,3,4,6-Penta-O-benzoyl-glucopyranoside[62] (**1I**): white solid (57%); m.p. 184-186°C; R$_f$ 0.44 (CH$_2$Cl$_2$); IR (KBr, cm^{-1}): 3062, 2957, 1731, 1598, 1450 and 1267; ^{1}H-NMR (CDCl$_3$) δ (ppm): 7.20-8.20 (m, 25H, Ar-H), 6.85 (d, J= 4.10 Hz, H-1), 6.32 (t, J = 9.97 Hz, H-3), 5.86 (t, J= 9.97 Hz ,1H, H-4), 5.68 (dd, J= 10.55 ,3.52 Hz, 1H, H-2), 4.61 (m, 2H, H-5) and 4.48 (m,1H, H-6).

1,2,3,4,6-Penta-O-(3,4-dibenzyloxy)benzoyl glucopyranoside (**2I**): white solid (30%), R_f 0.20 (hexane:ethyl acetate 8:2), IR (KBr, cm^{-1}): 3027, 2921, 1719, 1598 and 1267; ^{1}H.NMR (CDCl$_3$) δ (ppm): 7.38-7.78 (m, 50H, Ar-H), 6.84-6.96 (m, 15H, Ar-H), 6.34-6.40 (m, 1H, H-1), 5.91-5.94 (m, 2H, H-2, H-4), 5.10-5.31 (m, 20H, CH$_2$), 4.79-4.87 (m, 1H, H-6) and 44-4.75 (m, 2H, H-5, H-6′).

1,2,3,4,6-Penta-O-(3,4-dihydroxyl)benzoyl glucopyranoside (**2J**): brown syrup (45%); R_f 0.20 (MeOH); IR (KBr, cm^{-1}): 3650-3350, 1727, 1513, 1427 and 1272; ^{1}H. NMR (acetone-d$_6$ and D$_2$O) δ (ppm): 7.30-7.58 (m, 15H, Ar-H), 6.36 (d, *J*=8.05 Hz, 1H, H-1), 5.57-6.07 (m, 2H, H-glucose) and 3.52-4.77 (m, 4H, H-glucose); ^{13}C-NMR (acetone-d$_6$ and D$_2$O) δ (ppm): 168.5, 168.0, 167.8, 167.0, 164.2 (5C, C=O), 154.2, 154.0, 153.8, 153.7, 153.4, 148.2, 148.0, 147.9, 131.6, 131.3, 131.0, 126.6, 126.4, 126.2, 124.7, 129.9, 123.1, 120.3, 121.1, 118.5, 118.0 and 117.6 (30C, Ar-C) and 95.8, 76.4, 75.8, 74.2, 73.9 and 73.0 (6C, C-glucose).

2.5 Antioxidant Experiments

Scavenging Effects on DPPH Radicals

2,2-Diphenyl-1-picryhydrazyl (DPPH) radical is a stable radical with a purple color (λ_{max} 517 nm). Upon reduction by a scavenger, the extensive conjugation is disrupted and the compound turns yellow.

2.5.1 TLC Autographic Assay

After developing and drying, TLC plates were sprayed with a 0.2% DPPH in methanolic solution. TLC plates were examined 5 minutes after spraying. Active compounds appeared as yellow spots against purple background.

The preliminary results of free radical scavenging activity of synthesized sugar esters are tabulated in Table 2.1.

Table 2.1 Free radical scavenging activity of synthesized sugar esters
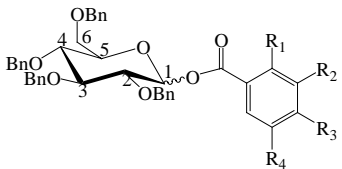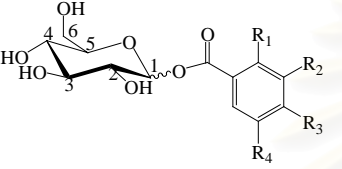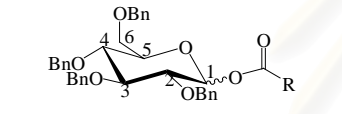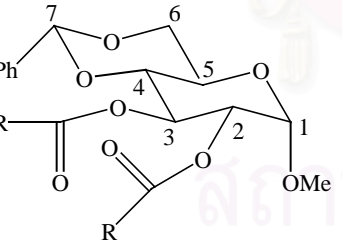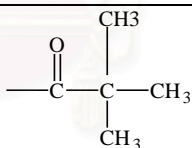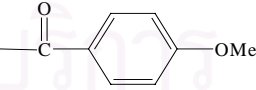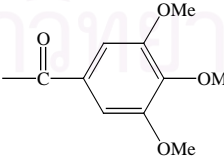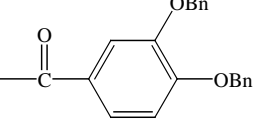
Structure	Compound	R				Antioxidant
		R_1	R_2	R_3	R_4	
	1C	H	H	OBn	H	-
	2C	OBn	H	OBn	H	-
	3C	H	OBn	OBn	H	-
	4C	H	OBn	H	OBn	-
	5C	H	OBn	OBn	OBn	-
	6C	H	H	OMe	H	-
	7C	H	OMe	OMe	OMe	-
	1D	H	H	OH	H	-
	2D	OH	H	OH	H	-
	3D	H	OH	OH	H	**
	4D	H	OH	H	OH	-
	5D	H	OH	OH	OH	***
	6D	H	H	OMe	H	-
	7D	H	OMe	OMe	OMe	-
	8C	$CH_3(CH_2)_{16}COOH$				-
	1E	$-H_3C$				-
	2E					-
	3E					-
	4E					-
	5E					-

Table 2.1 (cont)

Structure	Compound	R	Antioxidant
	2F		-
	3F		-
	4F		-
	1H		-
	2H	$CH_3(CH_2)_{16}COOH$	-
	1I		-
	2I		-
	2J		***

*   : positive results observed after 30 minutes
**   : positive results observed after 15 minutes
***   : positive results observed immediately

2.5.2 Spectrophotometric Assay

Samples of various concentrations (0.5 mL) were added to a 1 mL methanolic solution of DPPH radical (final concentration of DPPH was 0.2 mM). The mixture was shaken vigorously and then left for 30 minutes. The absorbance of the resulting was measured at 517 nm with a spectrophotometer. All tests and analyses were run in three replicates and averaged. The percentage of radical scavenging was calculated by the following equation.

$$\% \text{ radical scavenging} = (1 - A_{sample}/A_{control}) \times 100$$

A_{sample} = Absorbance of sample solution with DPPH

$A_{control}$ = Absorbance of only DPPH and used solvent

The results of free radicals scavenging activity synthesized of sugar esters and analogues are presented in Table 2.2.

Table 2.2 Free radicals scavenging activity of sugar esters and analogues.
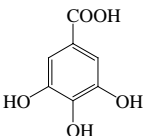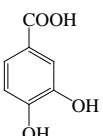
Compounds	Conc (mM)	Absorbance	% Radical Scavenging	IC$_{50}$
Gallic acid	1.00	0.0986	91.99	
	0.50	0.0967	92.15	
	0.25	0.1106	91.02	
	1.25×10^{-1}	0.0924	92.49	
	6.25×10^{-2}	0.3205	73.96	
	3.13×10^{-2}	0.8046	34.64	**3.69×10^{-2}**
	1.56×10^{-2}	1.0200	17.14	
	7.81×10^{-3}	1.0819	12.11	
	3.90×10^{-3}	1.0957	10.99	
	1.95×10^{-3}	1.0599	13.90	
	1.00	0.1085	90.49	
	0.50	0.0978	91.43	
	0.25	0.1154	89.88	**8.83×10^{-2}**
	1.25×10^{-1}	0.3675	67.79	
	6.25×10^{-2}	0.8492	25.58	

Table 2.2 (cont)
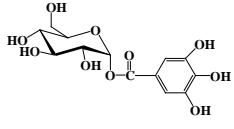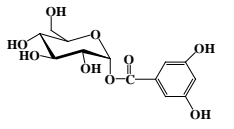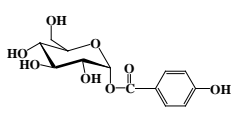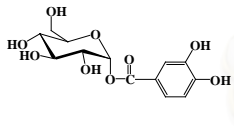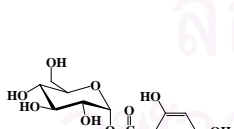
Compounds	Conc (mM)	Absorbance	% Radical Scavenging	IC_{50}
5D	1.00	0.1077	90.83	**6.76×10^{-2}**
	0.50	0.0923	92.14	
	0.25	0.0944	91.97	
	1.25×10^{-1}	0.2374	79.79	
	6.25×10^{-2}	0.8564	27.11	
4D	1.00	1.0777	8.28	-
	0.50	1.1051	5.95	
	0.25	1.0906	7.18	
	1.25×10^{-1}	1.0994	6.43	
	6.25×10^{-2}	1.0871	7.48	
1D	1.00	1.1490	2.17	-
	0.50	1.0910	7.15	
	0.25	1.1166	4.97	
	1.25×10^{-1}	1.0915	7.11	
	6.25×10^{-2}	1.1327	3.60	
3D	1.00	0.0742	93.97	**25.63×10^{-2}**
	0.50	0.1267	89.71	
	0.25	0.8312	32.47	
	1.25×10^{-1}	1.0102	17.93	
	6.25×10^{-2}	1.0872	11.68	
2D	1.00	1.1794	-0.11	-
	0.50	1.1145	5.39	
	0.25	1.1293	4.14	
	1.25×10^{-1}	1.1600	1.53	
	6.25×10^{-2}	1.1833	-0.44	

Table 2.2 (cont)

Compounds	Conc (mM)	Absorbance	% Radical Scavenging	IC$_{50}$
2J	1.00	0.1239	89.47	
	0.50	0.1023	91.31	
	0.25	0.0852	92.76	
	1.25×10^{-1}	0.0880	92.53	
	6.25×10^{-2}	0.0890	92.44	**2.44×10^{-2}**
	3.13×10^{-2}	0.1948	83.46	
	1.56×10^{-2}	0.8122	31.05	
	7.81×10^{-3}	1.0320	12.41	
	3.90×10^{-3}	1.0621	9.85	
	1.95×10^{-3}	1.1247	4.54	

CHAPTER III

RESULTS AND DISCUSSION

Among plant-derived biologically active compounds, tannin has been reported to posses various bioactivities such as antiviral, antitumor, antibacterial and antioxidant.[20] Sugar esters are in fact a virtually basic skeleton of hydrolysable tannin.[33] According to the literature reviews, there is an evidence that the structure of sugar esters (or sometime referred as glycoconjugated compounds) dealed closely to their biological activities and mainly played the pivotal roles in biological processes. These processes intimately ranged from cell growth and differentiation, cell-cell communication, modulation of protein function to pathological processes namely cancer metastasis lysosomal storage diseases, chronic inflammation and microbial infections. For instance, oenothein B and eight accompanying compounds isolated from *O. erythrosepala* have been found to be of noticable biological activity, such as antitumor and antiviral,[11] glucoconjugates of (±)-ibuprofen displayed anti-inflammatory activity far higher than (±)-ibuprofen.[63] Additionally, recent investigation has revealed that glycoconjugates possessing an ester bond between the bioactive moiety and one of the sugar hydroxyls are valuable new tools in biomedical research. These monosaccharide esters seem to be able to improve antiviral and antibacterial activity, increase the intestinal permeability, alter receptor-selectivity, or they may be used as monomer in polycondensation reaction as well as building blocks for the solid phase combinatorial synthesis of libraries of novel glycopeptides and in peptide templated glycosylation reaction.[47] Consequently, sugar esters both from natural sources and synthesis have been scrutinized. Almost sugar esters in this research were synthesized from the esterification between 2,3,4,6-tetra-*O*-benzyl-glucopyranoside and interested carboxylic acids. DMAP and DCC were selected as a coupling reagent since the by-products (DHU or dicyclohexylurea) were easily removed by filtration. The benzyl groups of the sugar esters derived were further
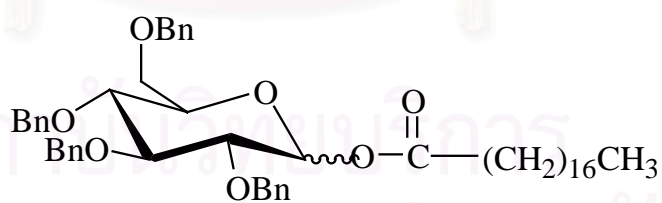
deprotected to the hydroxyl groups by catalytic hydrogenation. Finally, the mixture could be purified by reverse phase chromatography. The overview of the preparation of sugar ester in this research work can be depicted as followings:
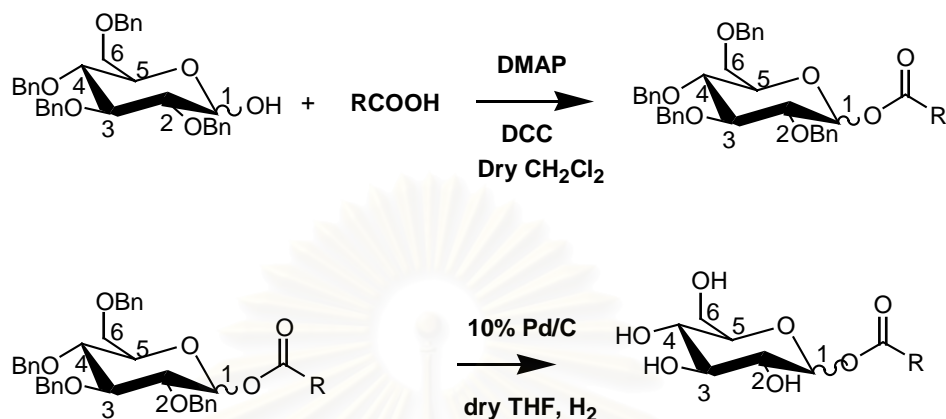
This research focused on the syntheses and structure-bioactivity relationship study of sugar esters mainly of those substituted at an anomeric position and their analogues. Thirty-eight compounds were synthesized which could be categorized into five groups: 1) nine starting materials including three glucose derivatives and six benzoic acid derivatives 2) fifteen monosubstituted glucose derivatives at an anomeric position 3) eight disubstituted glucose derivatives at positions -2,3 4) two tetrasubstituted glucose derivatives at positions- 2,3,4,6 and 5) three pentasubstituted glucose derivatives. Among those synthesized compounds, fourteen compounds (**1C, 2C, 3C, 4C, 5C, 8C, 2D, 4D, 6D, 7D, 3E, 5E, 2I** and **2J**) were disclosed to be new substances based upon no report of those compounds available in chemical literature (structures presented in Fig 3.1). The structures of all synthesized compounds were well characterized using various spectroscopic techniques including IR, ^{1}H-NMR, ^{13}C-NMR and ^{1}H-^{1}H COSY.

.

Compounds	R	R₁	R₂	R₃	R₄
1C	Bn	H	H	OBn	H
2C	Bn	OBn	H	OBn	H
3C	Bn	H	OBn	OBn	H
4C	Bn	H	OBn	H	OBn
5C	Bn	H	OBn	OBn	OBn
2D	H	OH	H	OH	H
4D	H	H	OH	H	OH
6D	H	H	H	OMe	H
7D	H	H	OMe	OMe	OMe

8C

Figure 3.1 New synthetic sugar esters and analogoues.

Compounds	R
3E	MeO—⟨benzene⟩—
5E	BnO,BnO—⟨benzene⟩—
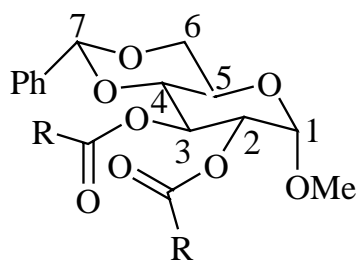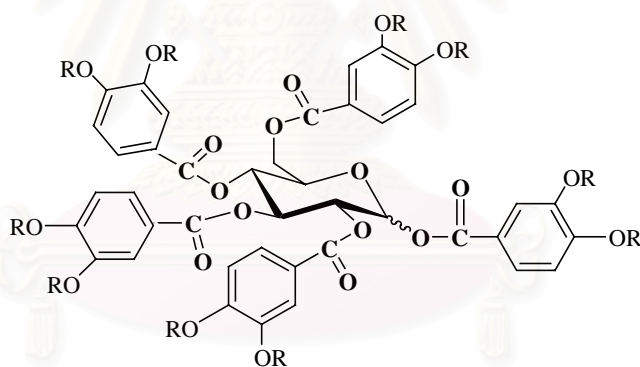
Compounds	R
2I	Bn
2J	H

Figure 3.1 (cont.)

3.1 Syntheses

3.1.1 Synthesis of Starting Materials

3.1.1.1 <u>Protecting of sugar derivatives.</u>

2,3,4,6-tetra-O-benzyl-α-D-glucopyranose

The methodology to synthesize 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranose **(3a)** has been previously reported.[57] The syntheses of the mentioned compound comprised two steps from methyl α-D-glucopyranoside **(1a)** (Scheme 3.1). The first step was the benzylation of hydroxyl groups using benzyl chloride and potassium hydroxide in dry dioxane. The protected compound was obtained in moderate yield (58 %). The signals of the benzyl group were clearly observed in ^{1}H-NMR as multiplet around 7.13-7.42 ppm, an anomeric proton was observed as doublet around 5.02-5.04 ppm (*J*= 10.55 Hz) and 6H of glucose were observed as multiplet around 3.60-4.88 ppm. The methoxy protons were detected as singlet at 3.45 ppm. Demethylation was then carried out to accomplish the desired product using concentrated acetic acid and 2 M hydrochloric acid under reflux condition. The ultimate product was obtained in moderate yield (70%). According to the ^{1}H-NMR spectrum of this compound, the anomeric proton was found as doublet at 6.66 ppm (*J*= 4.69 Hz), representing α-anomer and at 7.20 ppm (*J*= 6.45 Hz), representing β-anomer. This could be clearly stated that the product was definitely a mixture of α- and β-anomer. The integration ratio of both anomers revealed the presence of α-/β-form in the ratio of 10:3. Moreover, it must be noted that the chemical shift of both anomers was slightly appeared higher filed than that of proton of the starting sugar.

Step 1:

OH

HO
HO
HO
OH
OMe

(1a)

BnCl, KOH
dry dioxane
reflux

OBn

BnO
BnO
OBn
OMe

(2a)

Step 2:

OBn

BnO
BnO
OBn
OMe

(2a)

2 M HCl

OBn

BnO
BnO
OBn
OH

(3a)
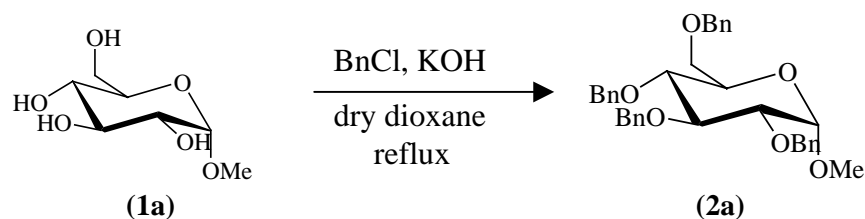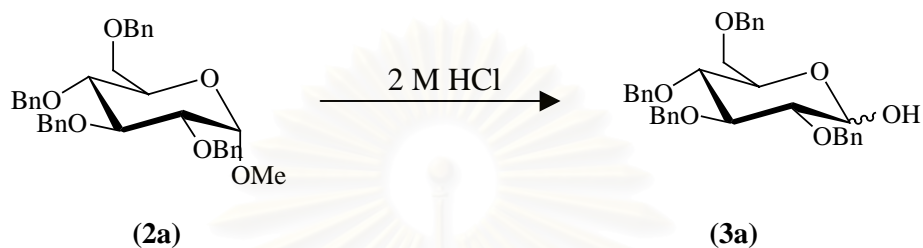
Scheme 3.1 General procedure for the synthesis of 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranose **(3a)**

Methyl 4,6-O-benzylidene-α-D-glucopyranoside **(4a)**

This compound was generally synthesized by the reaction of methyl α-D-glucopyranoside **(1a)**, benzaldehyde and powdered zinc chloride.[49] The mixture was stirred at room temperature and the insoluble product was easily separated by filtration to gain the desired product in moderate yield (63%).

3.1.1.2 Protecting of benzoic acid derivatives

Numerous methods to convert the hydroxyl groups of benzoic acid derivative to the benzyl groups have been reported.[48,49] For instance, by sodium hydride and excess benzyl bromide in THF (Method I)[48] or using potassium carbonate and benzyl chloride in DMF (Method II).[49] General produre is presented as shown in Scheme 3.2.

O
‖
C—OH

OH

B₁

O
‖
C—OBn

OBn

B₂

O
‖
C—OH

OBn

B₃
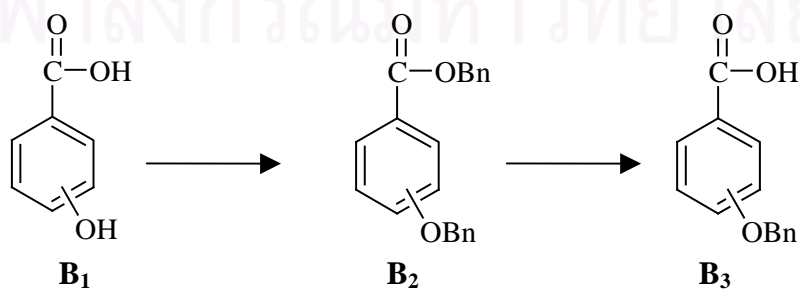
Scheme 3.2 Procedure for the synthesis of benzoic acid derivatives.

Deterring to Scheme 3.2, both hydroxyl group of benzoic acid B_1 and phenolic group were converted to phenoxy benzyl ester B_2 (Methods I or II). It should also be noted that the obtained product B_2 was easily purified by silica gel column chromatography in Method I, comparing to recrystallization in Method II. After that the benzyl ester was removed by saponification using potassium hydroxide and acidification with dilute hydrochloric acid. The desired products were normally obtained in moderate to high yield.

The comparative results of the synthesized compounds are tabulated in Table 3.1 and their structures are shown below.

(**1b**)   (**5b**)

(**2b**)   (**3b**)   (**4b**)
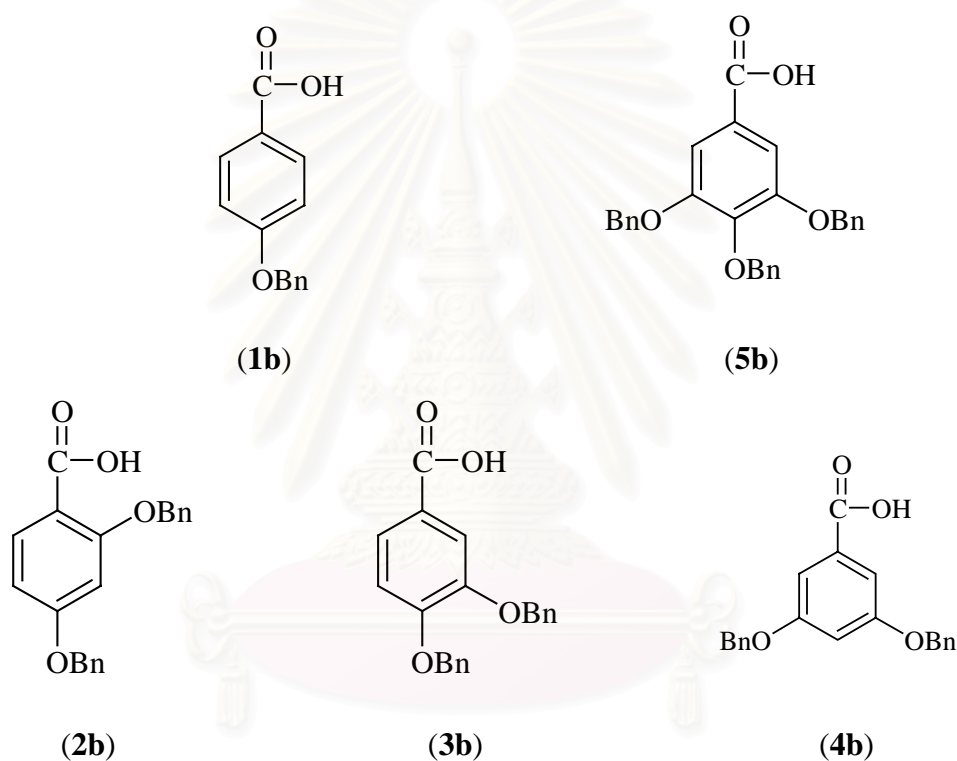
Table 3.1 Physical properties and % yield of synthesized benzoic acid derivatives.

Compounds	Physical Property		% Yield	Method
	Appearance	m.p. (^{o}C)		
1b	pale yellow solid	185-186	73	I
2b	white solid	118-119	70	I
3b	white solid	182-184	90	II
4b	white solid	202-205	77	II
5b	white solid	191-193	53	II

3.1.2 Synthesis of Sugar Esters

Synthesized sugar esters could be divided into 6 subgroups depending on the number of substituents on a sugar ester. Two monosubstituted, two disubstituted, a tetrasubstituted and a penta-substituted sugar esters are designated as series **C, D, E, F, H** and **I** respectively.

3.1.2.1 Synthesis of monosubstituted sugar esters.

The esterification between sugar and protected benzoic acid derivatives was performed by using DMAP and DCC in dry dichloromethane. This method was convenient and gave the corresponding products in excellent yield. Fourteen monosubstituted derivatives of glucose bearing at anomeric position were synthesized. Among them, nine new compounds (**1C, 2C, 3C, 4C, 5C, 2D, 4D, 6D** and **7D**) based upon no report in chemical literature available were successfully synthesized (Scheme 3.3). Their structures were well characterized using various spectroscopic techniques including IR, ^{1}H-, ^{13}C-NMR and ^{1}H-^{1}H COSY.

Step I
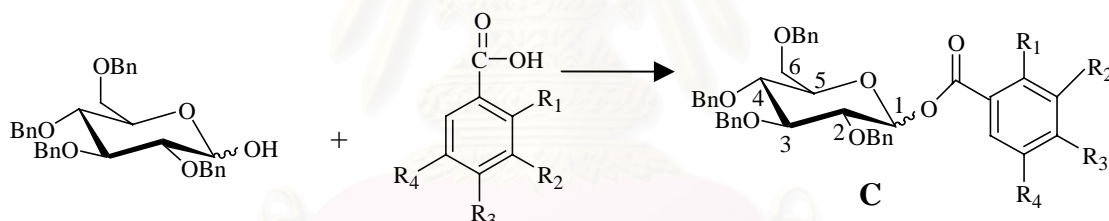
Step II
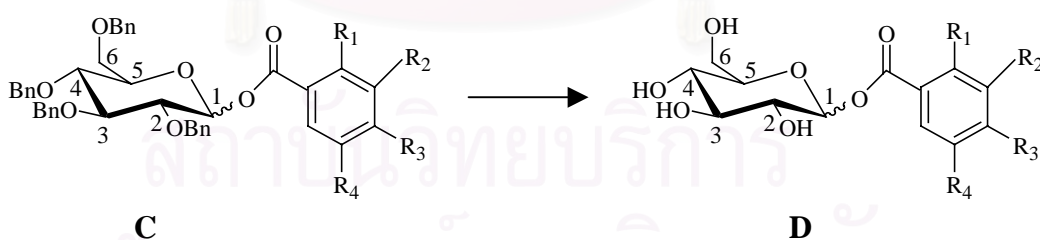
Scheme 3.3 General procedure for the synthesis of 1-monosubstituted glucopyranoside.

3.2 Spectroscopy of Glucopyranosides and Analogues

3.2.1 Monosubstituted Sugar Esters

Series C
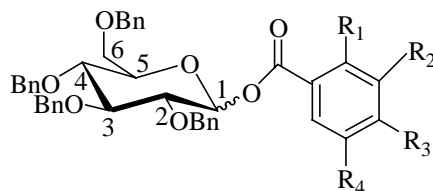
Sugar esters in series C included **1C, 2C, 3C, 4C** and **5C** displayed common characteristics in the IR pattern, such as C-H stretching of aromatic vibration around 3016-3021 cm^{-1} and that of aliphatic at 2914-2925 cm^{-1}. C=O stretching vibration of sugar ester at 1691-1736 cm^{-1} and that of C=C stretching of aromatic ring at 1588-1608 cm^{-1} and C-O stretching vibration at 1076-1178 cm^{-1} were also visualized.

The synthesis of 1-monosubstituted glucopyranosides in series **C** was achieved and the desired product was obtained as an α/β mixture while a major product being a β-anomer. This was manifestly endorsed by the information from aromatic proton signal appeared as multiplet around 7.18-8.06 ppm. For the proton signal of α-anomer, it was observed as doublet around 6.60-6.64 ppm (J = 2.53-3.90 Hz) while that of β-anomer proton was detected as doublet around 5.90-6.03 ppm (J = 6.24-8.00 Hz). Four methylene protons of the benzyl group of glucose derivative were detected as multiplet around 4.51-5.11 ppm and those of benzyl group of benzoic acid moiety were observed at higher chemical shift than those of glucose derivative around 4.52-5.29 ppm. The remaining proton signals of glucose were visualized as multiplet around 3.52-4.16 ppm.

The ^{13}C-NMR spectrum of this series showed the important carbon signals of carbonyl group at 164.5-170.1 ppm. The aromatic carbons of benzyl protecting groups were around 124.3-129.1 ppm. Normally, the aromatic carbons of acid could be easily observed around 145.3-163.0 ppm if they were connected with electron donation group and appearred at chemical shift higher than aromatic carbons without any substituent. For anomeric carbons, it was observed at 91.0-94.6 ppm. The other carbons of glucose derivative and methylene carbons could be seen around 68.1-85.0 ppm.

The identities of six new compounds in the C series (**1C, 2C, 3C, 4C, 5C** and **8C**) were confirmed by ^{13}C-NMR spectrum as presented in Table 3.2.

Table 3.2 The ^{13}C-NMR spectral assignment of new compounds in series **C**

Compounds	Chemical shift (ppm)			
	C=O	Ar-C (Bn)	C-1	C-glucose , CH$_2$
1C	164.5	127.6-128.7	94.5	68.1-84.9
2C	170.0	127.2-128.8	94.2	70.3-85.0
3C	164.5	127.2-128.7	94.6	68.9-84.9
4C	164.6	127.6-128.7	91.0	68.1-84.9
5C	164.7	124.3-129.1	94.9	68.1-89.0
8C	172.2	127.6-138.6	94.0	68.1-84.8

Series **D**
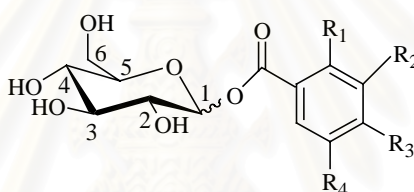
The debenzylation products of series C fell into the compounds in series **D**. The derived products were still obtained in a mixture form of α/β which the major product being β-anomer. For IR absorption pattern, four compounds in series **D** displayed the characteristics of common functional groups: O-H stretching vibration around 3093-3704 cm^{-1}, C=O stretching vibration of sugar ester at 1695-1721 cm^{-1} and C=C of aromatic ring stretching at 1588-1637 cm^{-1}.

The ^{1}H-NMR spectra of 1-monosubstituted glucopyranosides clearly supported the occurrence of α/β mixture with the β-anomer as a major product. The aromatic protons were observed as multiplet around 7.05-7.49 ppm. The signal belonging to α-anomer was observed as doublet around 6.21-6.38 ppm (J=2.53-6.24 Hz) while H-1 signal observed as doublet around 5.64-5.76 ppm (J=6.26-7.80 Hz) could be assigned for β-anomer. The proton signals of glucose were clearly observed as multiplet around 3.16-4.66 ppm.

The ^{13}C-NMR spectra of this series showed the important carbon signals of carbonyl group at 165.0-168.0 ppm whereas the anomeric carbon was observed

around 95.0-95.1 ppm. The other carbons of glucose appeared at 55.3-77.6 ppm while the aromatic carbons were detected at 102.0-108.1 ppm.

In addition, four new compounds in this series were confirmed their structures by means of ^{13}C-NMR as tabulated in Table 3.3.

Table 3.3 The^{13}C-NMR spectral assignment of new compounds in series **D**

Compounds	Chemical shift (ppm)			
	C=O	C-1	Ar-C (OBn)	C-glucose
2D	168.0	95.0	163.0, 133.0	57.7-77.4
4D	165.0	95.0	158.0, 159.0	58.7-77.4
6D	166.0	94.5	132.0, 131.0	55.0-77.0
7D	165.0	95.1	153.0, 124.0	60.0-77.0

3.2.2 2,3-Disubstituted sugar esters

E   **F**
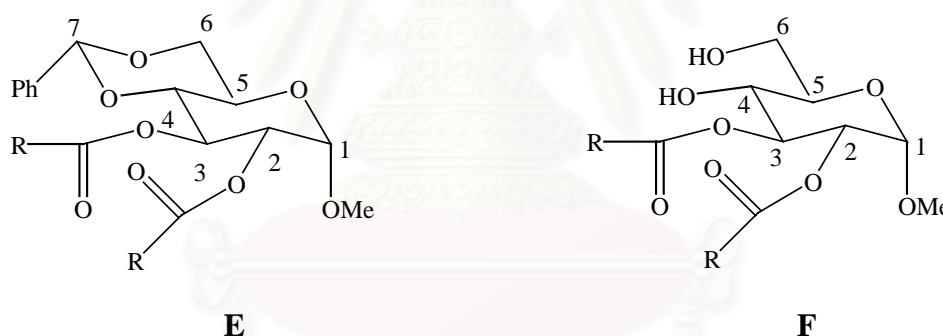
These compounds were generally synthesized *via* esterification utilizing similar manner described earlier (Chapter II). The corresponding products were obtained in moderate yield (53-78 %), except for **2E** in low yield (20%).

Among nine compounds synthesized including five 4,6-*O*-benzylidene glucopyranosides and four 2,3-disubstituted sugar esters, compounds **3E** and **5E** were disclosed to be two new substances in this series. According to the IR spectra, the spectral pattern of 2,3-disubstituted glucopyranoside (series **E**) with 4,6-O-benzylidene and methoxy protecting group of sugar esters and 2,3-disubstituted of glucopyranosides (series **F**) bearing methoxy protecting groups at anomeric of sugar ester displayed the characteristics of common functional group. To illustrate this, C-H stretching vibration of aromatic was presented around 3011-3090 cm^{-1} and that of aliphatic at 2929-2975 cm^{-1} was detected. C=O Stretching vibration of sugar ester at

1715-1742 cm^{-1} and that of C=C of aromatic ring stretching at 1509-165 cm^{-1} were also found. Other absorption peaks involved C-O stretching vibration were visualized at 1287-1061cm^{-1}.

The ^{1}H-NMR spectra of the compounds in this series, generally exhibited multiplet with 1H integration at 6.87-7.77 ppm which could be assigned as aromatic protons and multiplet with 1H integration at 5.51-6.85 ppm which could be denoted for H-7. Another signal detected approximately 3.63-6.05 ppm could be designated for glucose protons and a singlet at 3.40-3.47 ppm as protons of methoxy group.

The ^{1}H-NMR spectral assignments for new synthetic sugar esters in Series **E** are shown in Table 3.4.

Table 3.4 The ^{1}H-NMR spectral assignment of new compounds in series **E**

Compounds	Chemical shift (ppm)		
	Ar-H	H-glucose	H-OCH$_3$
3E	7.77, 7.30-7.40, 6.62	6.62 (H-7), 5.97 (H-3), 5.52-5.53 (H-1, H-2), 4.40-4.50 (H-5), 4.00-4.10 (H-6) and 3.90-4.00 (H-4, H-6)	3.43
5E	6.87-7.74	6.85 (H-7), 5.72 (H-3), 5.07-5.29 (H-1, H-2), 4.78-4.83 (H-6), 4.60-4.63 (H-6), 4.00-4.22 (H-5) and 3.77-3.84 (H-4)	3.47

The ^{13}C-NMR spectra exhibited a signal of a carbonyl ester around 165.3-168.2 ppm. The signals of aromatic carbons were detected in the range of 113.2-153.3 ppm. The signals of anomeric carbons were observed at 97.2 and 97.9 ppm. Another set of signal assigned to glucose protons as C-2, C-3, C-4, C-5, C-6 and methylene carbon could be assigned in the range of 62.5-79.5 ppm.

The ^{13}C-NMR spectral assignments of new synthetic compounds in this series are accumulated in Table 3.5.

Table 3.5 The ^{13}C-NMR spectral assignment of new compounds in series **E**

Compounds	Chemical shift (ppm)			
	C=O	Ar-C	C-1	C-glucose, CH$_2$
3E	165.8, 165.3	113.5-136.9	97.9	62.5-79.5
5E	168.2, 165.7	113.2-153.3	97.2	63.4-74.0

3.2.3 2,3,4,6-Tetrasubstituted and 1,2,3,4,6-Pentasubstituted Sugar Esters

These compounds were generally synthesized by esterification between methyl α-D-glucopyranoside or D-glucose with interested carboxylic acids as exhibited in Chapter II. Five compounds were fruitfully synthesized in these two series and two new compounds **(2I and 2J)** were achieved. The structures of all synthesized compounds in these series were well characterized using various spectroscopic techniques.
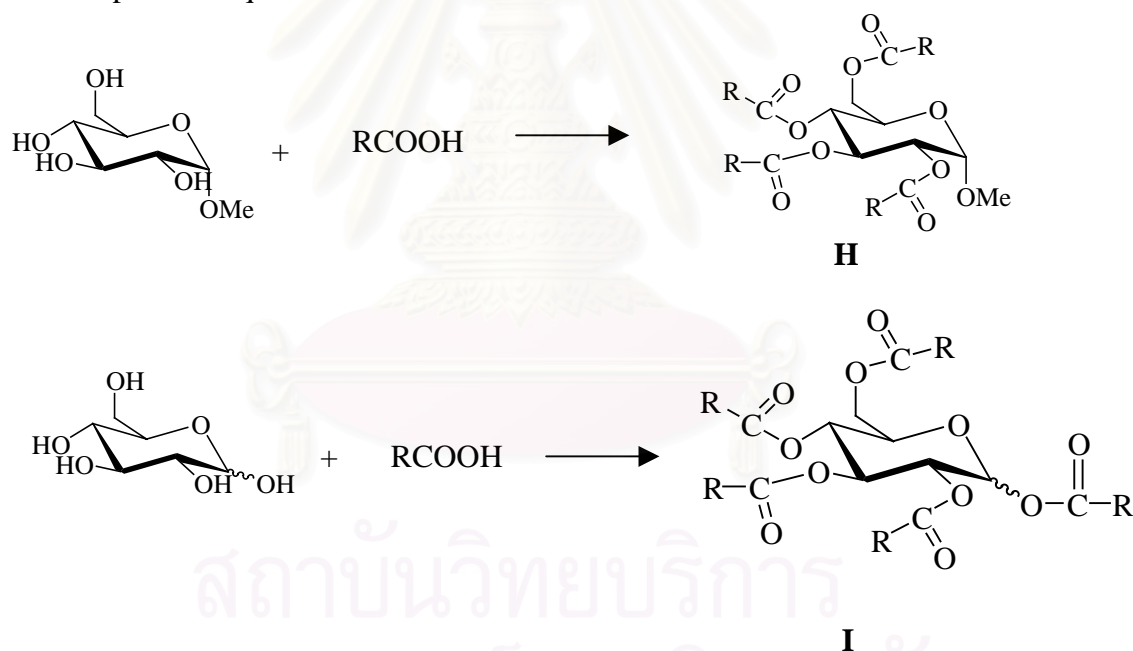
Scheme 3.4 General procedure for the synthesis 2,3,4,6-tetrasubstituted and 1,2,3,4,6-penta substituted sugar esters

Spectroscopic techniques including IR, ^{1}H, ^{13}C-NMR and ^{1}H-^{1}HCOSY of 2,3,4,6-tetrasubstituted and 1,2,3,4,6-pentasubstituted sugar esters clearly confirmed the structures of these compounds. Both of them are different at an anomeric position, *i.e.*, the presence of a methoxy group for 2,3,4,6-tetrasubstituted sugar esters and another ester moiety for 1,2,3,4,6-pentasubstituted sugar esters. The IR absorption

another ester moiety for 1,2,3,4,6-pentasubstituted sugar esters. The IR absorption pattern for all 2,3,4,6-tetrasubstituted and 1,2,3,4,6-pentasubstituted of glucopyranosides were displayed the characteristics of common function groups. For example, C-H stretching of aromatic vibration was presented around 3015-3062 cm^{-1} and that of aliphatic at 2924-2957 cm^{-1} was detected. C=O stretching vibration sugar ester at 1725-1741 cm^{-1} and that of C=C of aromatic ring stretching at 1598-1597 cm^{-1} were also observed.

The ^{1}H-NMR spectra of all 2,3,4,6-tetrasubstituted and 1,2,3,4,6-penta-substituted of glucopyranosides displayed the aromatic protons as multiplet around 7.20-8.20 ppm. The aromatic protons of 2,3,4,6-tetrasubstituted and 1,2,3,4,6-pentasubstituted glucopyranosides displayed the existence of configuration. The glucose protons were observed as multiplet around 4.00-6.35 ppm and those belonged to a methoxy group was observed as singlet around 3.43-3.53 Hz.

The ^{1}H-NMR spectral assignment for two new compounds are shown in Table 3.6.

Table 3.6 The ^{1}H-NMR spectral assignment of **2I** and **2J**

Compounds	Chemical shift (ppm)		
	H-1	Ar-H	H-glucose, CH$_2$
2I	6.34-6.40	7.38-7.78	6.34-6.40, 6.10-6.16, 5.91-5.97, 4.8-4.79, 4.47-4.75, 5.10-5.31
2J	6.36	7.30-7.58	5.57-6.07, 3.52-4.77

Two new compounds were further confirmed their structures by ^{13}C-NMR. Five carbonyl ester signals were clearly observed. The ^{13}C-NMR spectral assignments of **2J** are presented in Table 3.7.

Table 3.7 The ^{13}C-NMR spectral assignment of **2J**

Compound	Chemical shift (ppm)	
	C=O	C-glucose
2J	168.5, 168.0, 167.8, 167.0, 164.2	73.0-95.8

3.3 Antioxidant Activity of Sugar Esters

TLC Autographic Assay

Antioxidant assay was accomplished by the protocol described in Chapter II. The chromatograms of sugar esters before and after spraying with DPPH radical reagent are demonstrated in Figure 3.2.
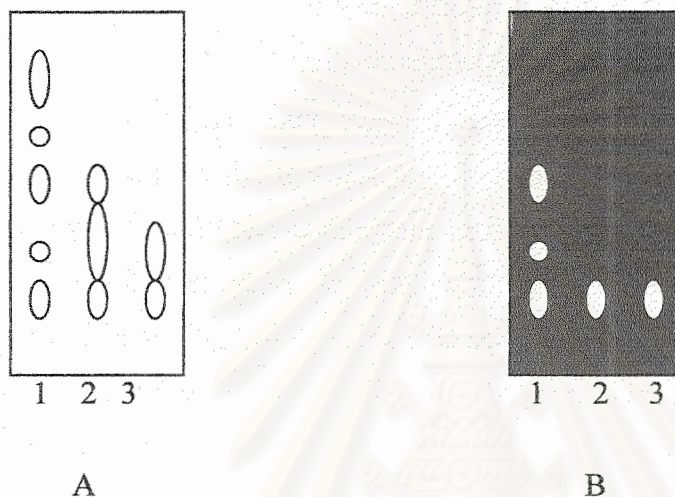
Figure 3.2   TLC autographic assay for DPPH radical scavenger assay

(A) TLC chromatogram before spraying with DPPH reagent

(B) TLC chromatogram after spraying with DPPH reagent

1,2,3 : Sugar esters

Figure 3.2A shows the TLC of all sugar esters which were developed in 30% ethyl acetate:hexane solvent system before spraying with DPPH. After sprayed with DPPH reagent, the active components were visualized as yellow spot against purple background (Figure 3.2B).

3.3.1 The Structure-Antioxidant Activity Relationship Study of Sugar Esters

A comparison of antioxidant activity of all synthesized sugar esters and the reference compounds such as gallic acid and 3-*tert*-butyl-4-hydroxyanisole (BHA) revealed that gallic acid was of the highest antioxidant activity, unfortunately, this compound could not totally dissolve in water. It should be noted that the synthesized

Regarding to monosubstituted sugar esters at an anomeric position, it was observed that the compound **5D** showed the highest antioxidant activity compared with those for compounds **3D**, **4D**, **2D** and **1D.**
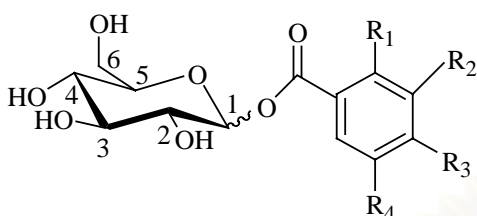
These results were in the same trend as the work of Sroka, and Cisowski who performed a comparative study of free radical scavenging of some phenolic acids with 3,4,5-trihydroxybenzoic acid (gallic acid) and 1,2,3-trihydroxybenzene (pyrogallol).

Gallic acid substituted sugar ester showed the highest antioxidant activity. Additionally, based on this study, it was observed that 3,4-dihydroxybenzoic acid substituted sugar ester showed the antioxidant activity higher than 2,4-dihydroxy-benzoic acid and 3,5-dihydroxybenzoic acid substituents. The lowest antioxidant activity was observed from 4-hydroxybenzoic acid substituent.

Fourteen synthesized mono-substituted glucopyranosides were taken to an antioxidant test. For qualitative antioxidant test, it was found that the hydroxy groups in mono-substituted glucopyranosides were crucial for the antioxidant activity against DPPH. Moreover, 1-(3,4-dihydroxybenzoyl)-glucopyranoside **(3D)** was strongly active to DPPH in a very short time, while 1-(2,4-dihydroxybenzoyl)-glucopyranoside **(2D)** and 1-(3,5-dihydroxybenzoyl)-glucopyranoside **(4D)** expressed against DPPH only after 15 minute delaying times.

Generally, the position, amount and type of substituent on benzoic acid moiety directly influenced the antioxidant activity. To make the SAR study more comprehendible, the comparison of various substituents of fourteen glucopyranosides with their activity could be summarized as follows:

1. Types of substituents on glucose derivatives
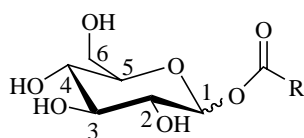
Regarding to the results achieved, the antioxidant activity depended on the substituents (R) on the aromatic ring. Furthermore, the hydroxy substituent showed a

higher better antioxidant activity than acetyl, pivaloyl, long chain hydrocarbon (stearic acid) and benzoic acid derivative substituents.

2. Types of substituent on the aromatic ring of glucose derivatives
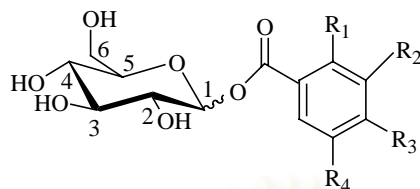
Among a variety of substituents, the methoxy and benzyloxy groups on the aromatic ring did not show any antioxidant activity, while some activity was observed where the aromatic ring beared a hydroxy group.

3. Effects of hydroxyl group on the aromatic ring

This investigation confirmed that the number of hydroxy group on the aromatic ring was highly influenced the antioxidant activity of the compound. Regarding to the result in Table 2.1, the three hydroxy groups on the aromatic ring gave a better antioxidant in antioxidant activity than the di- and monohydroxy substituents . Moreover, the position of hydroxy groups on the aromatic ring could affect the antioxidant activity. If the hydroxy groups were located "*ortho*" to each other, the compound showed stronger activity than those located at *meta* and *para* positions. For these reasons, compound **5D** revealed the best result as a antioxidant compared with the other monosubstituted compounds synthesized.

For a set of dihydroxybenzoic acid substituent 3,4-Dihydroxybenzoic acid expressed a higher antioxidant activity than 3,5- and 2,4-dihydroxybenzoic acid substituents. That was because the structure of 3,4-dihydroxybenzoic acid with *ortho* substitution of two hydroxyl groups was possible the most important factor for the antioxidant activity. Thus, 3,5-dihydroxybenzoic acid showed a little stronger ability for scavenging DPPH radical than 2,4-dihydroxybenzoic acid. The lowest antioxidant activity was observed for 4-hydroxybenzoic acid substituent. The arrangement as *para* substituent of hydroxyl groups to the aromatic ring seemed to be disadvantageous for the antioxidant activity of dihydroxybenzoic acid substituents. This was resulted the from resonance effect and hydrogen bonding of hydroxyl groups at *ortho* dihydroxy position of benzoic acid derivative as presented below.
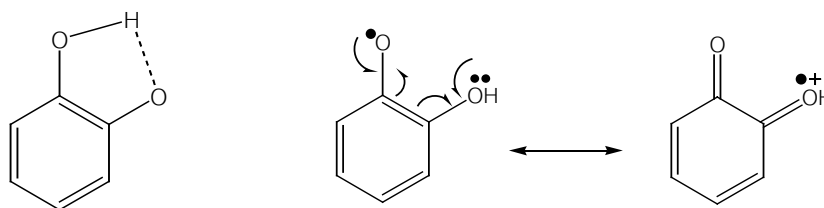
Number of hydroxyl substituent; order of activity

trihydroxyl > dihydroxyl > monohydroxyl substituent

Position of two hydroxyl substituent; order of activity

ortho > meta

4. Number of benzoic acid derivative on glucose

The comparison of the antioxidant activity for penta-(3,4-dihydroxy benzoyl) substituent and mono-(3,4-dihydroxybenzoyl) substituent on the D glucose observed revealed that pentasubstituents gave a higher the activity than mono-(3,4-dihydroxybenzoyl) substituent did.

Number of benzoic acid derivative substituent; order of activity

pentasubstituent > monosubstituent

In addition, it should be mentioned that only the compounds **3D**, **2J** and **5D**, revealed the antioxidant activity higher than the reference antioxidant, BHA.

Nevertheless, this research could beneficially be as an inventory of a search for the appropriated carrier, sugar ester, as antioxidant delivery. As a matter of fact, most antioxidants could not dissolve in biological media such as blood, thus limiting the use of those compounds in the body. The strategies to and the appropriate carrier linkage to the antioxidant can over come get rid of the problem. A water soluble carrier can increase the solubility of the antioxidant in the media. This solution in therefor useful for a search of new pharmaceutical drugs and also their delivery method.

CHAPTER IV

CONCLUSION

During the course of this research, the synthesis of sugar esters as glycoconjugates was carried out with the aim to comprehend the structure-bioactivity relationship (SAR) of these analogue compounds and antioxidant activity. The synthesis of the desired compounds was accomplished by employing esterification between glucose and derivatives of carboxylic acids in the presence of DMAP and DCC. Twenty-eight sugar esters were synthesized in low to high yield. Seven starting materials consisted of one sugar derivative and six benzoic acid derivatives were obtained in medium to high yield. The six benzoic acid derivatives were synthesized using benzylation of the benzoic acid derivatives. The monosubstituted products were detected as a mixture of α- and β- anomer. For antioxidant activity test, it was disclosed that the phenolic hydroxyl groups were essential. Monosubstituted sugar esters in series D revealed an advantage property that they could be soluble in water and polar organic solvents which propably could be utilized as a prodrug antioxidant delivery.

The structures of all synthesized compounds were endorsed by physical properties and spectroscopic evidences such as IR, ^{1}H-NMR and ^{1}H-^{1}H COSY and in some cases ^{13}C-NMR. There are new fourteen compounds, namely ((4-benzyloxy)benzoyl-2,3,4,6-tetra-O-benzyl glucopyranoside), ((2,4-dibenzyloxy)benzoyl-2,3,4,6-tetra-O-benzyl glucopyranoside), ((3,4-dibenzyloxy)benzoyl-2,3,4,6-tetra-O-benzyl glucopyranoside), ((3,5-dibenzyloxy)benzoyl-2,3,4,6-tetra-O-benzyl glucopyranoside), ((3,4,5-tribenzyloxy)benzoyl-2,3,4,6-tetra-O-benzyl glucopyranoside), (steroyl-2,3,4,6-tetra-O-benzyl glucopyranoside), ((2,4-dihydroxy)benzoyl glucopyranoside), ((3,5-dihydroxy)benzoyl glucopyranoside), ((4-methoxy)benzoyl glucopyranoside), ((3,4,5-trimethoxy)benzoyl glucopyranoside), (methyl-2,3-di-O-(p-methoxy)benzoyl-4,6-O-benzylidene-α-D-glucopyranoside), (methyl-2,3-di-O-(3,4-dihydroxy)benzoyl-4,6-O-benzylidene-α-D–glucopyranoside), (1,2,3,4,6-penta-O-(3,4-dibenzyloxy)benzoyl glucopyranoside and (1,2,3,4,6-penta-O-(3,4-dihydroxy)benzoyl

glucopyranoside) that have not been reported in chemical literatures. The structures of these new compounds are depicted below:

Compounds	R	R_1	R_2	R_3	R_4
1C	Bn	H	H	OBn	H
2C	Bn	OBn	H	OBn	H
3C	Bn	H	OBn	OBn	H
4C	Bn	H	OBn	H	OBn
5C	Bn	H	OBn	OBn	OBn
2D	H	OH	H	OH	H
4D	H	H	OH	H	OH
6D	H	H	H	OMe	H
7D	H	H	OMe	OMe	OMe

8C

Compounds	R
3E	MeO—⟨benzene⟩—
5E	BnO, BnO—⟨benzene⟩—
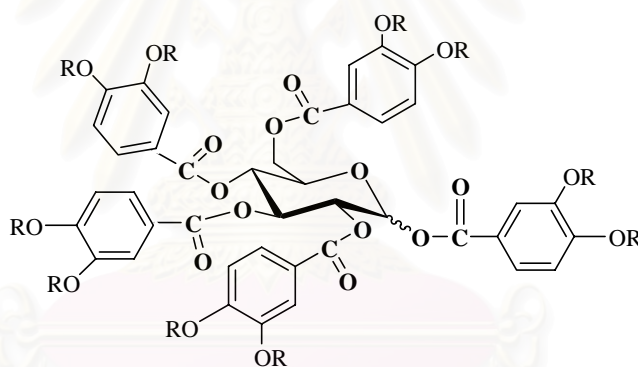
Compounds	R
2I	Bn
2J	H

The sugar ester and analogues were tested for antioxidant activity with 1,1-diphenyl-2-picrylhydrazyl radical (DPPH). The test was carried out in comparison with two commercially used antioxidant; gallic acid and BHA. The most active compound, 1,2,3,4,6-penta (3,4-dihydroxybenzoyl)glucopyranoside (**2J**) displayed an equal activity as gallic acid; however higher than BHA. The sugar esters fully bearing (3,4-dihydroxy)benzoyl glucopyranoside bond on sugar exhibited higher antioxidant activity than the monosubstituted sugar esters did.

Among the antioxidant activity of 1-monosubstituent of glucose examined, dihydroxy compound **5D** bearing gallic acid showed the highest activity. These compounds have three hydroxyl groups bonded to the aromatic at *ortho* position to each other. Compound **3D** bearing two hydroxy groups next to one another had the highest activity, while compound **4D** with two *meta*-hydroxy groups showed lower activity. The sugar ester with hydroxyl groups on an aromatic ring at *ortho* displayed higher activity than that of *meta* position. The addition of a hydroxyl group at *para* position on aromatic ring showed less activity than the dihydroxyl substituents. Generally when a number of phenolic hydroxy substituents on aromatic ring increased, the activity was indeed significantly increased.

Moreover, sugar esters containing only an ester as acetyl, pivaloyl, long chain hydrocarbon, and methoxy groups revealed no antioxidant activity.

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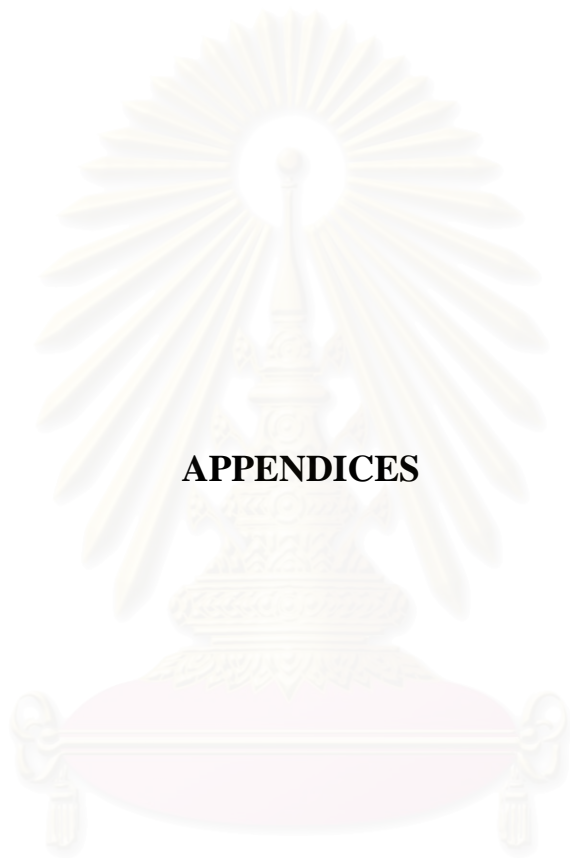
APPENDICES
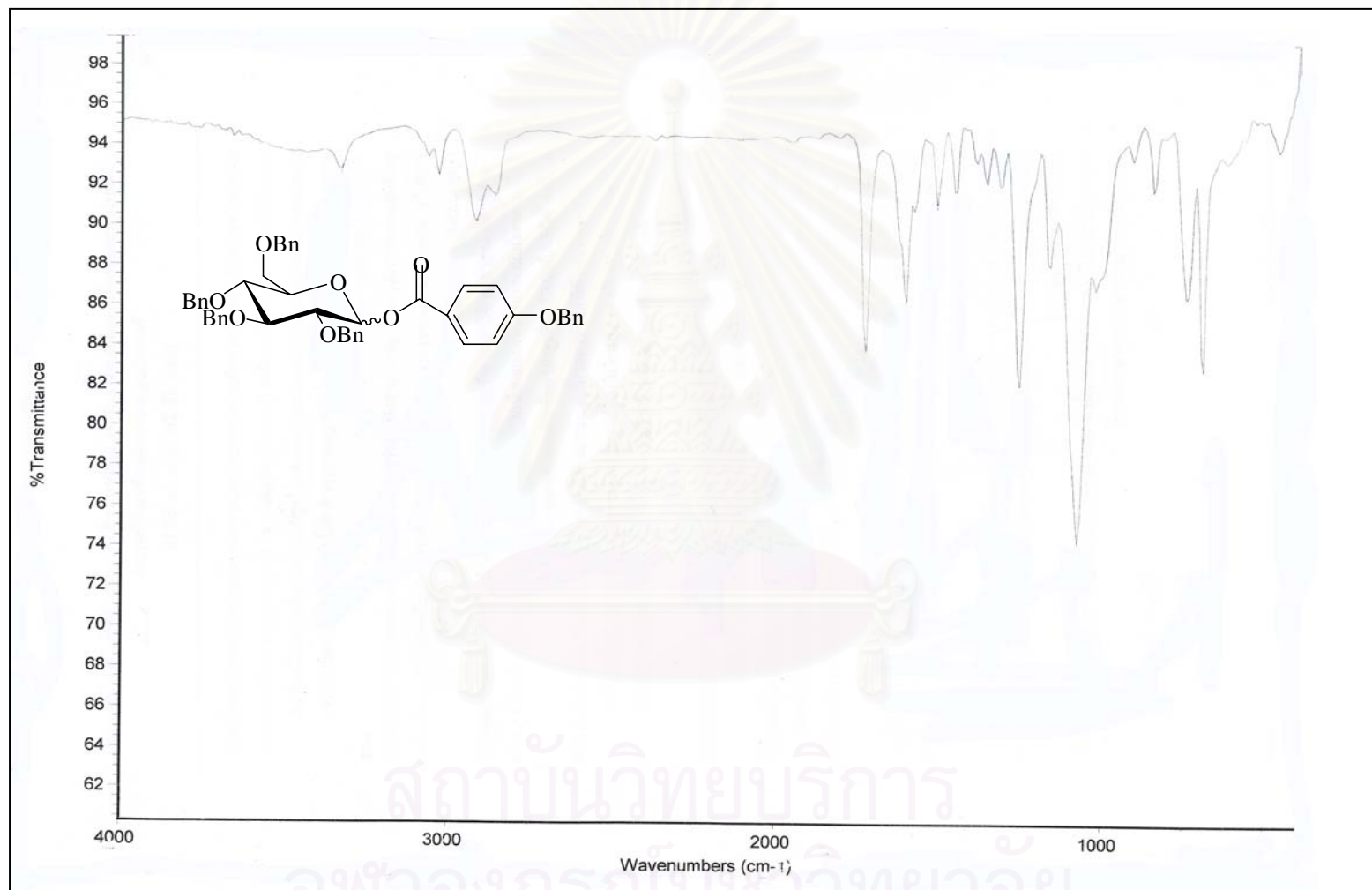
Figure A.1 The IR spectrum of compound **1C**
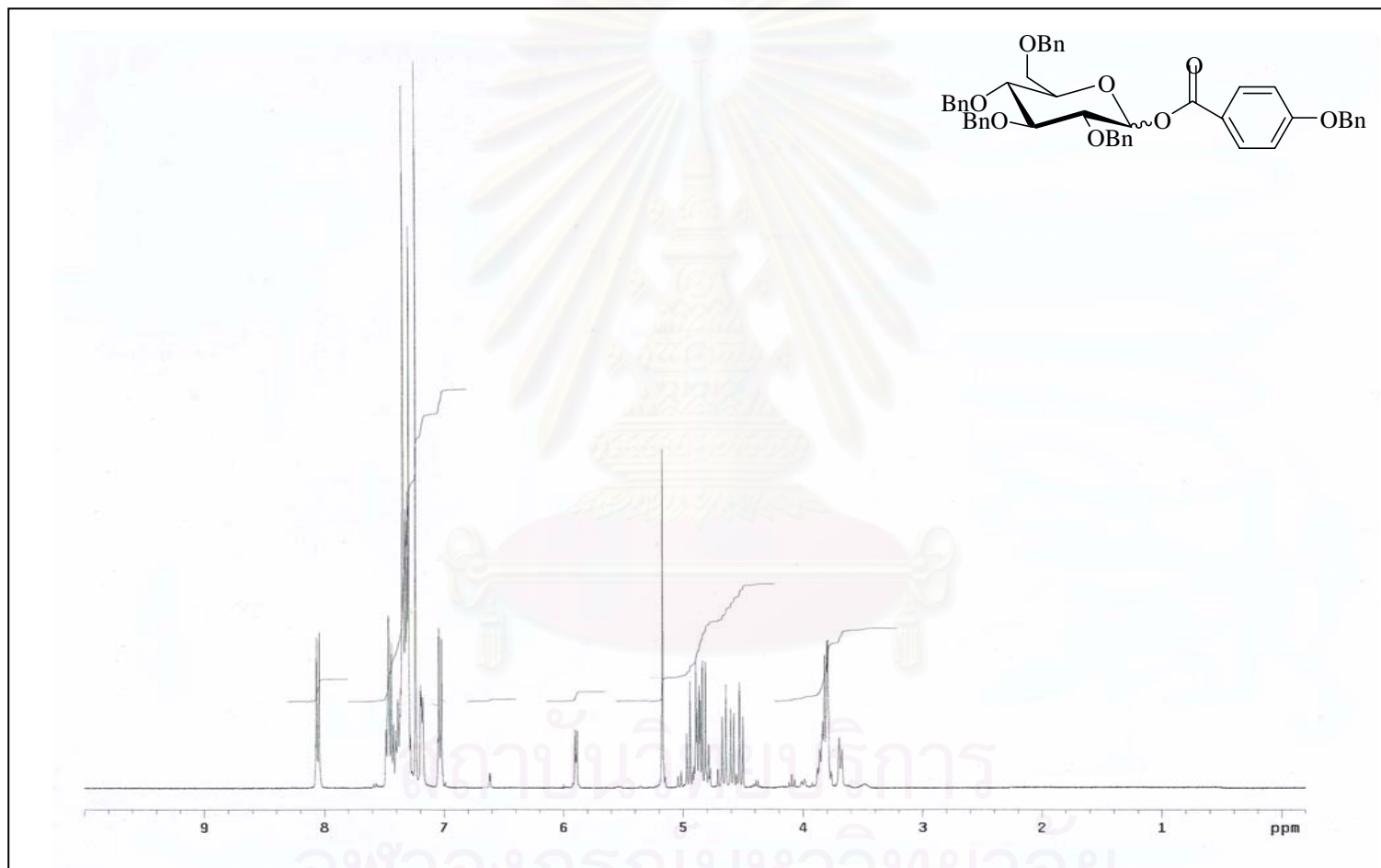
Figure A.2 The ^{1}H-NMR spectrum of compound **1C**
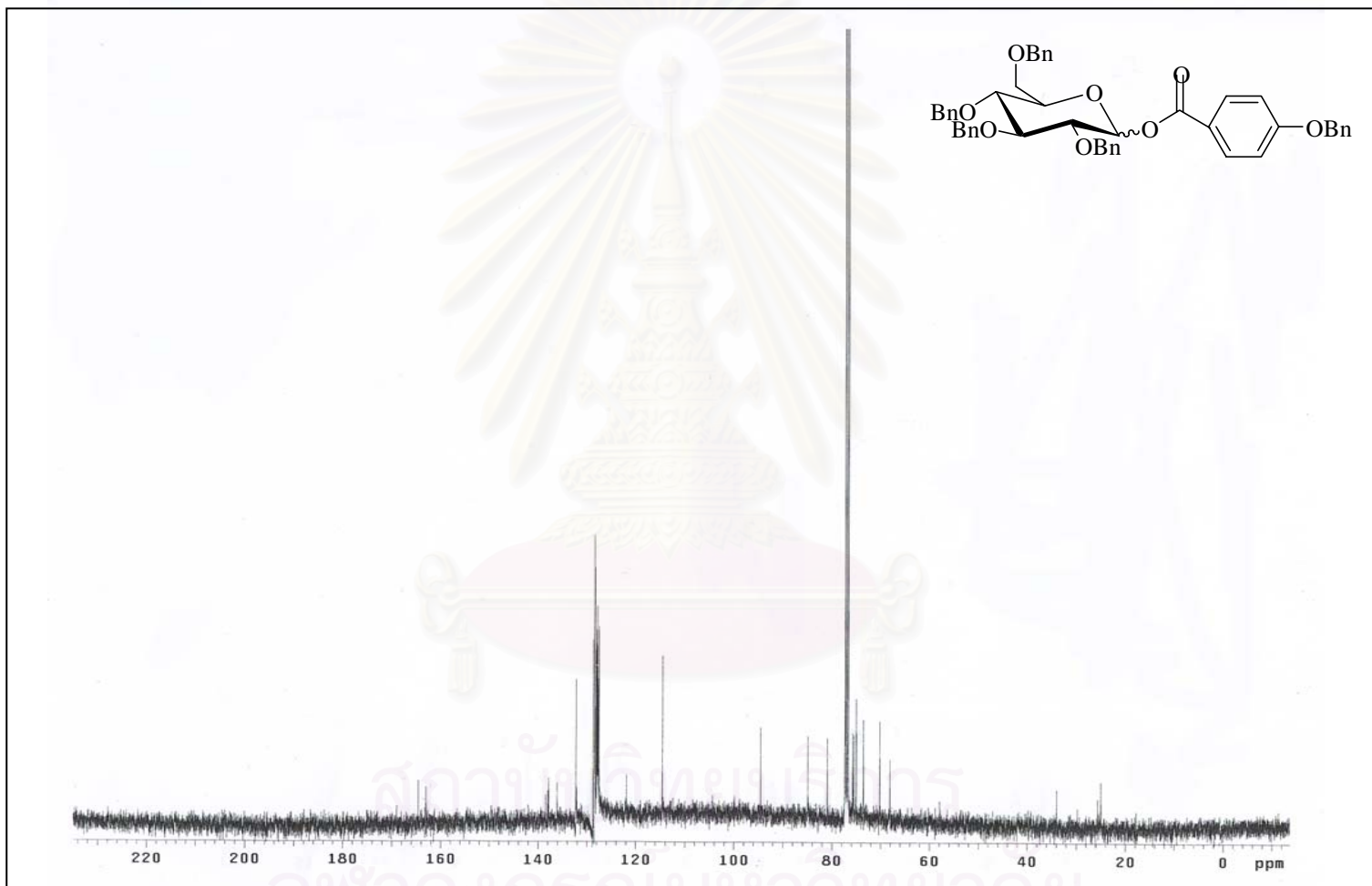
Figure A.3 The ^{13}C-NMR spectrum of compound **1C**
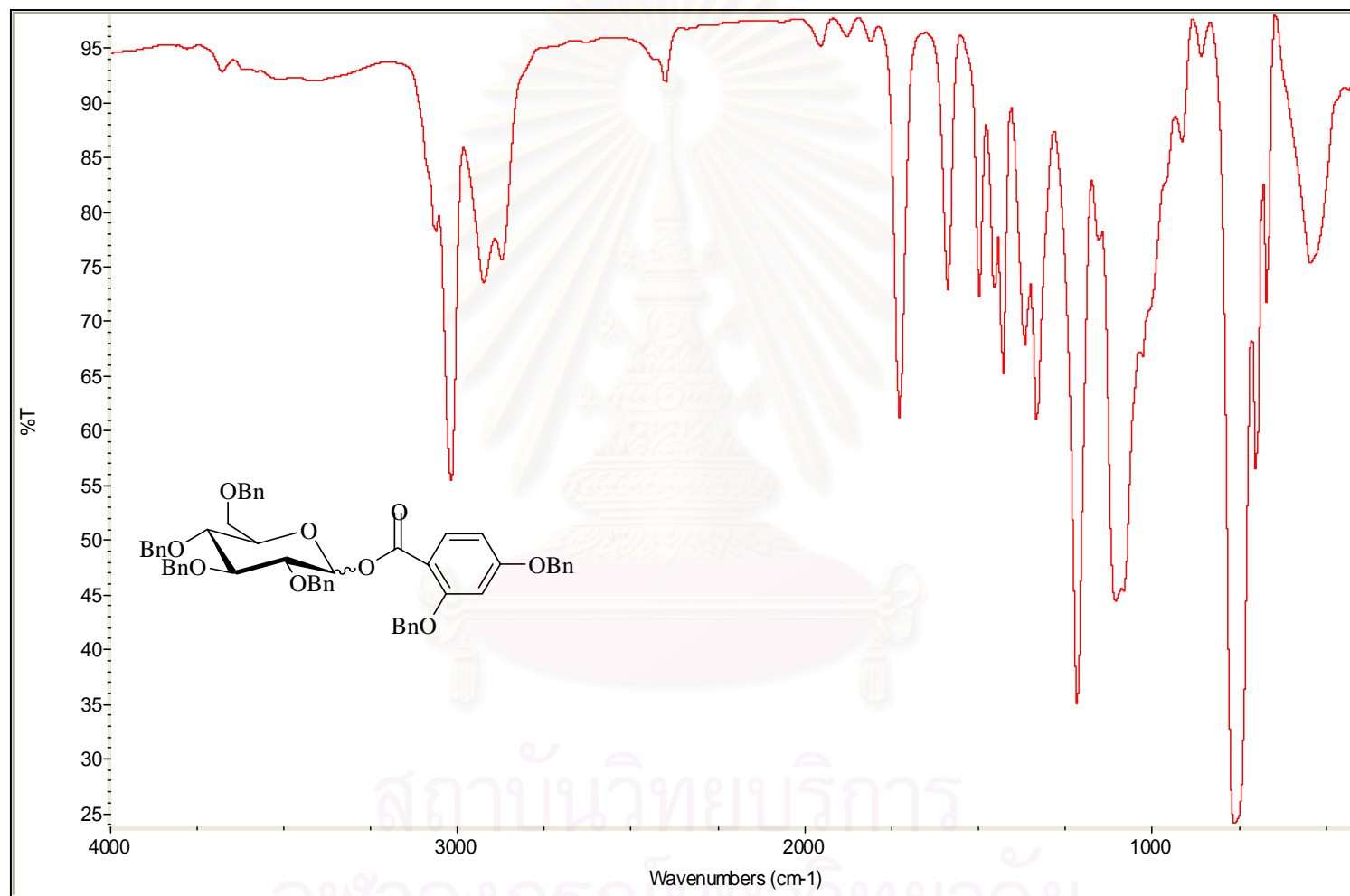
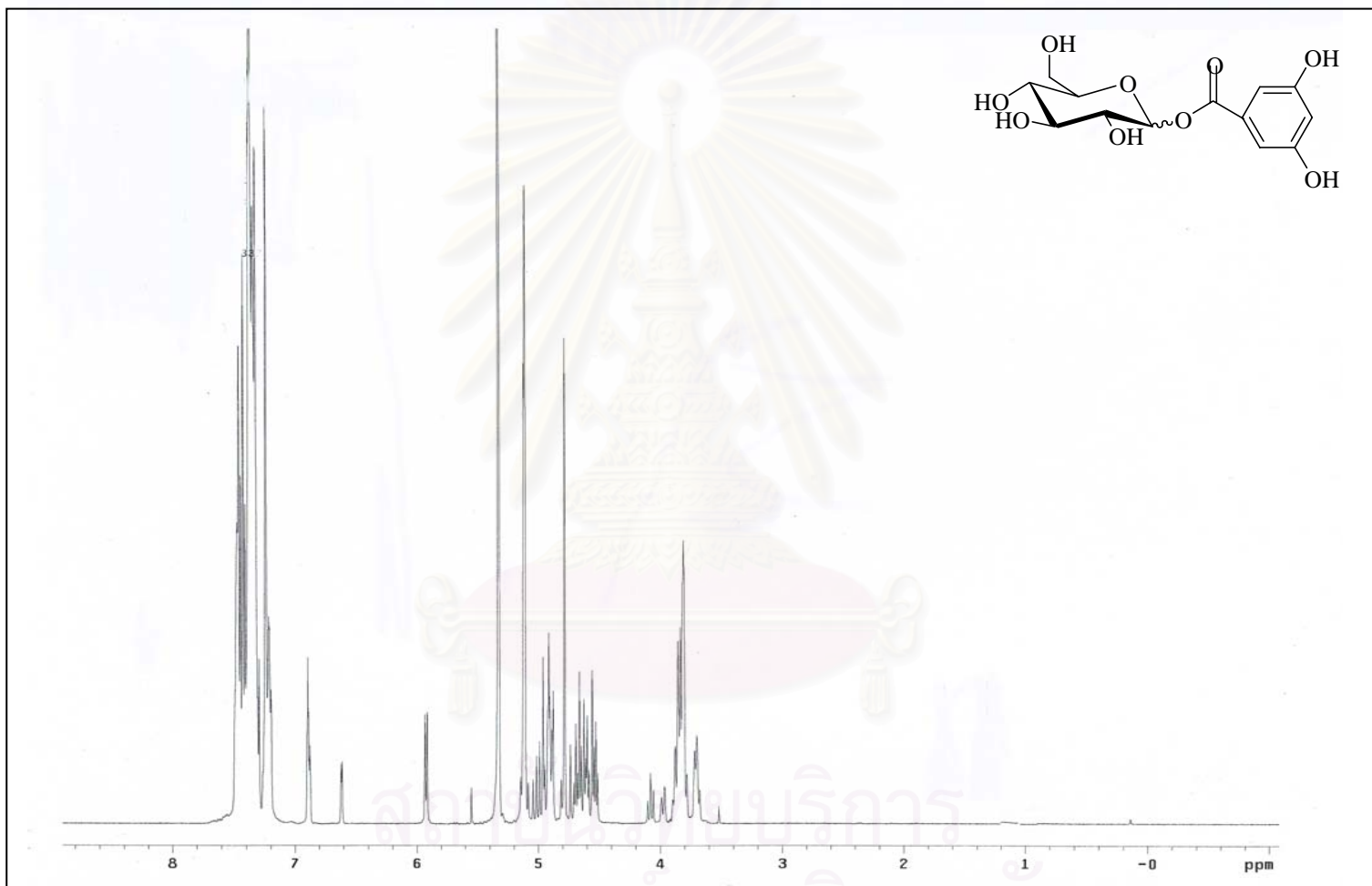**Figure A.4** The IR spectrum of compound **2C**
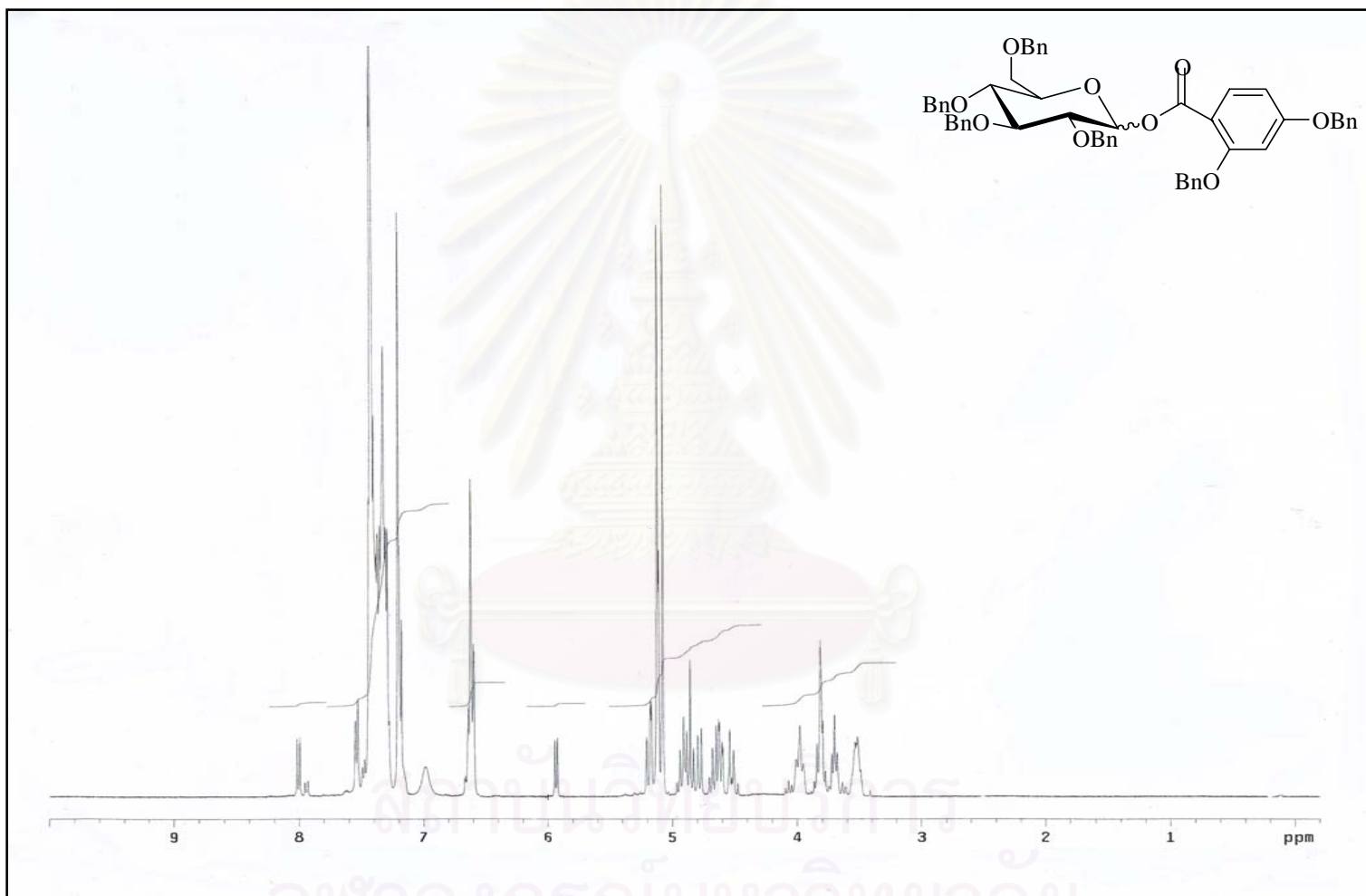
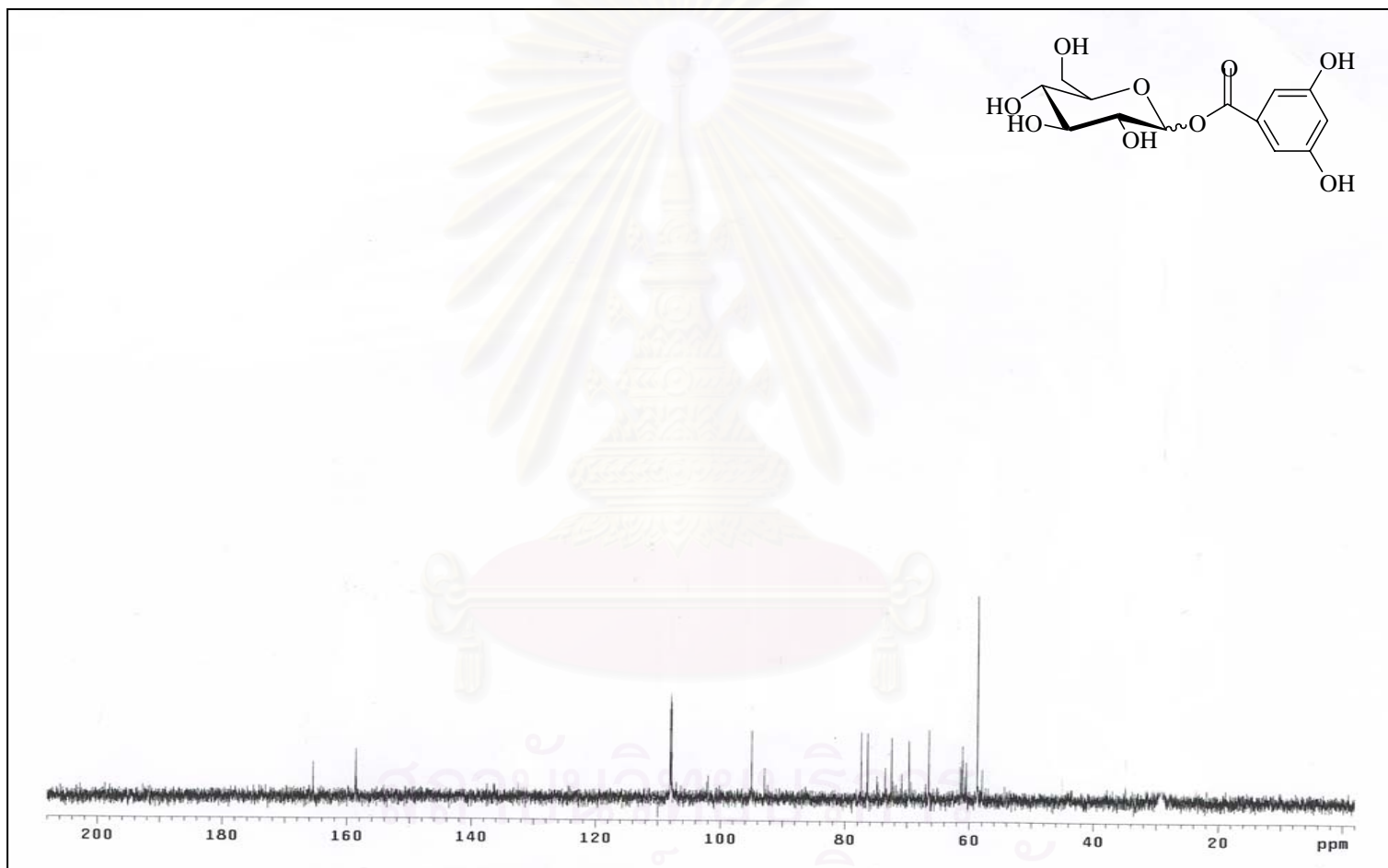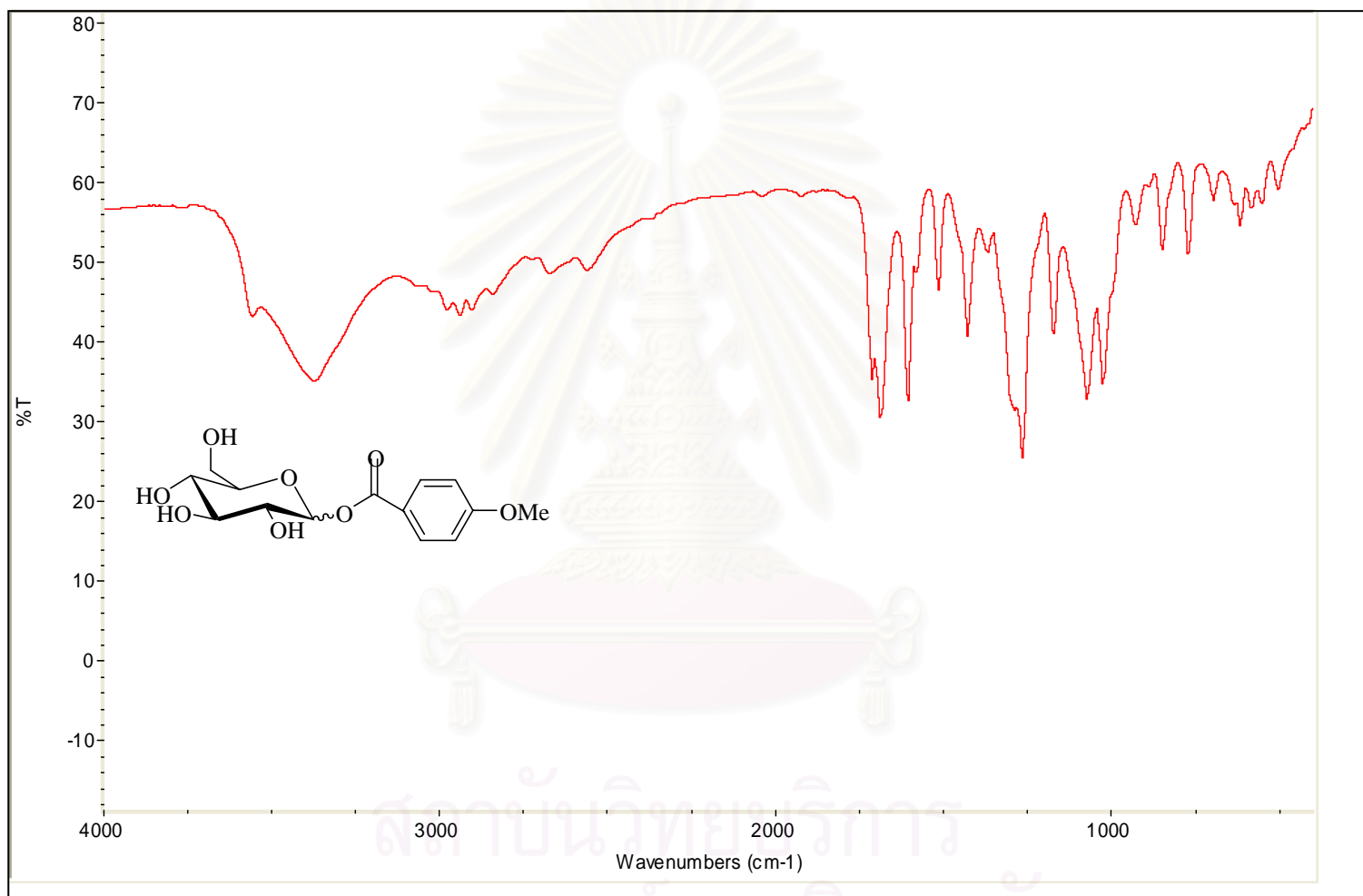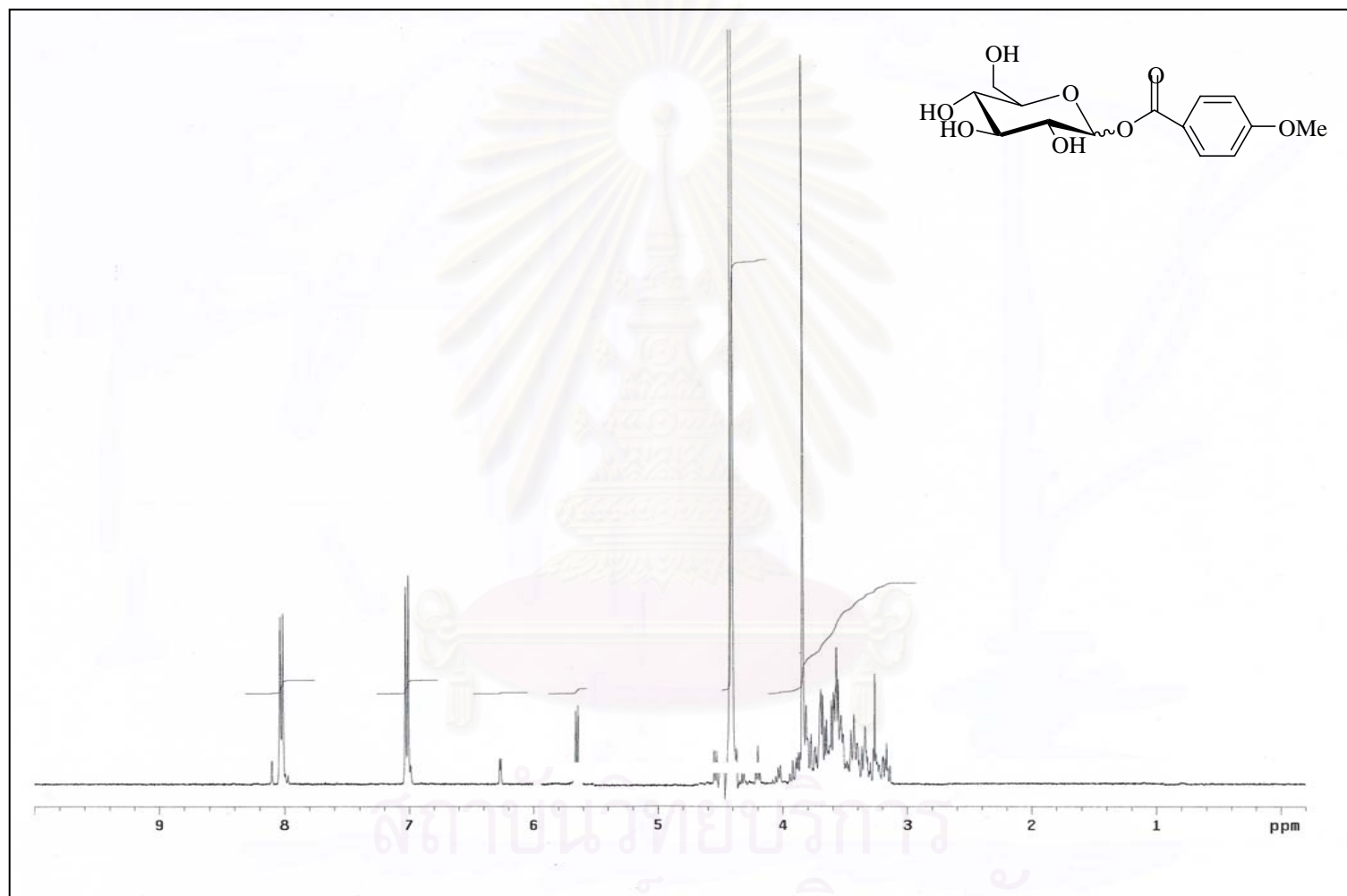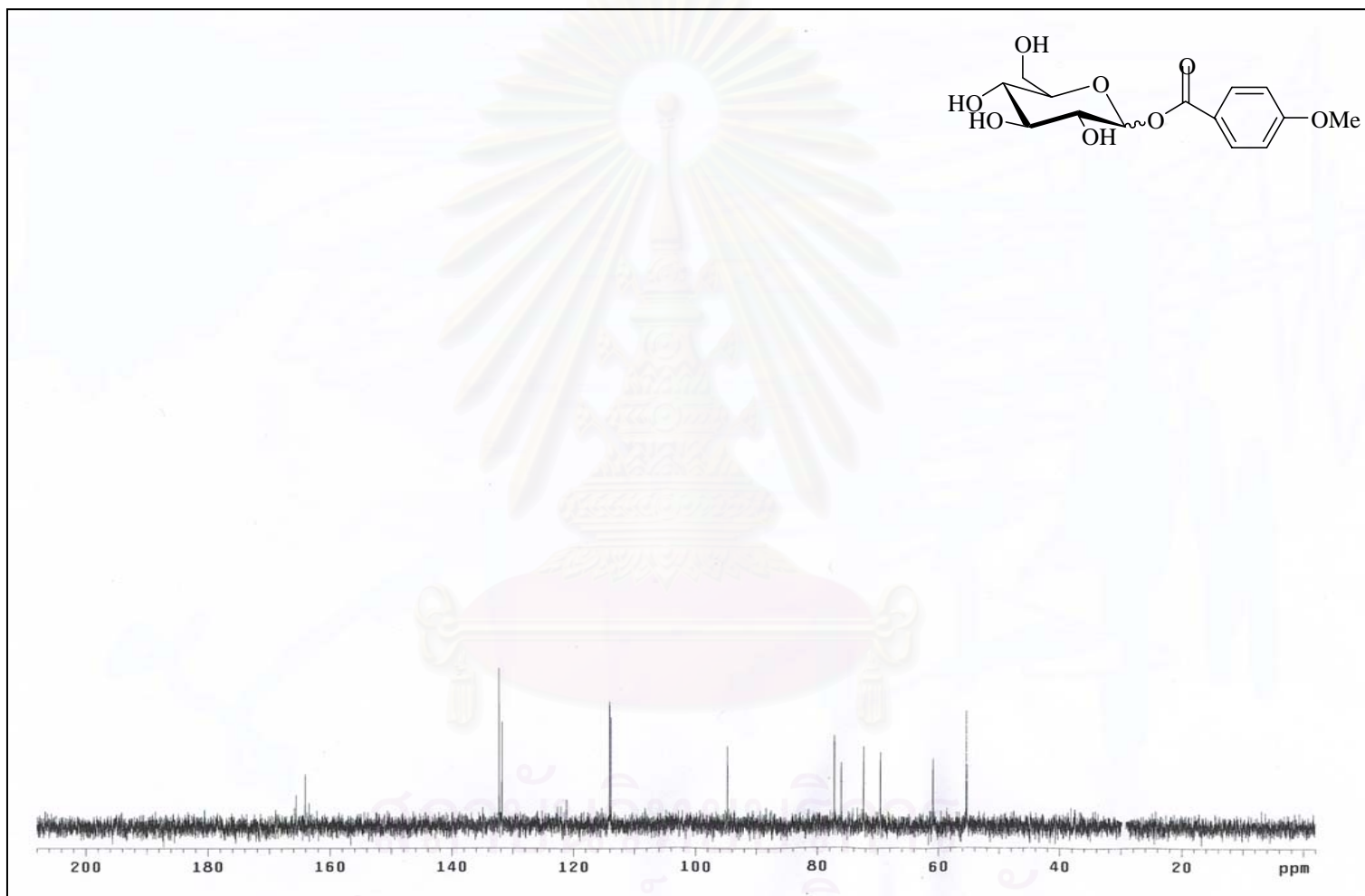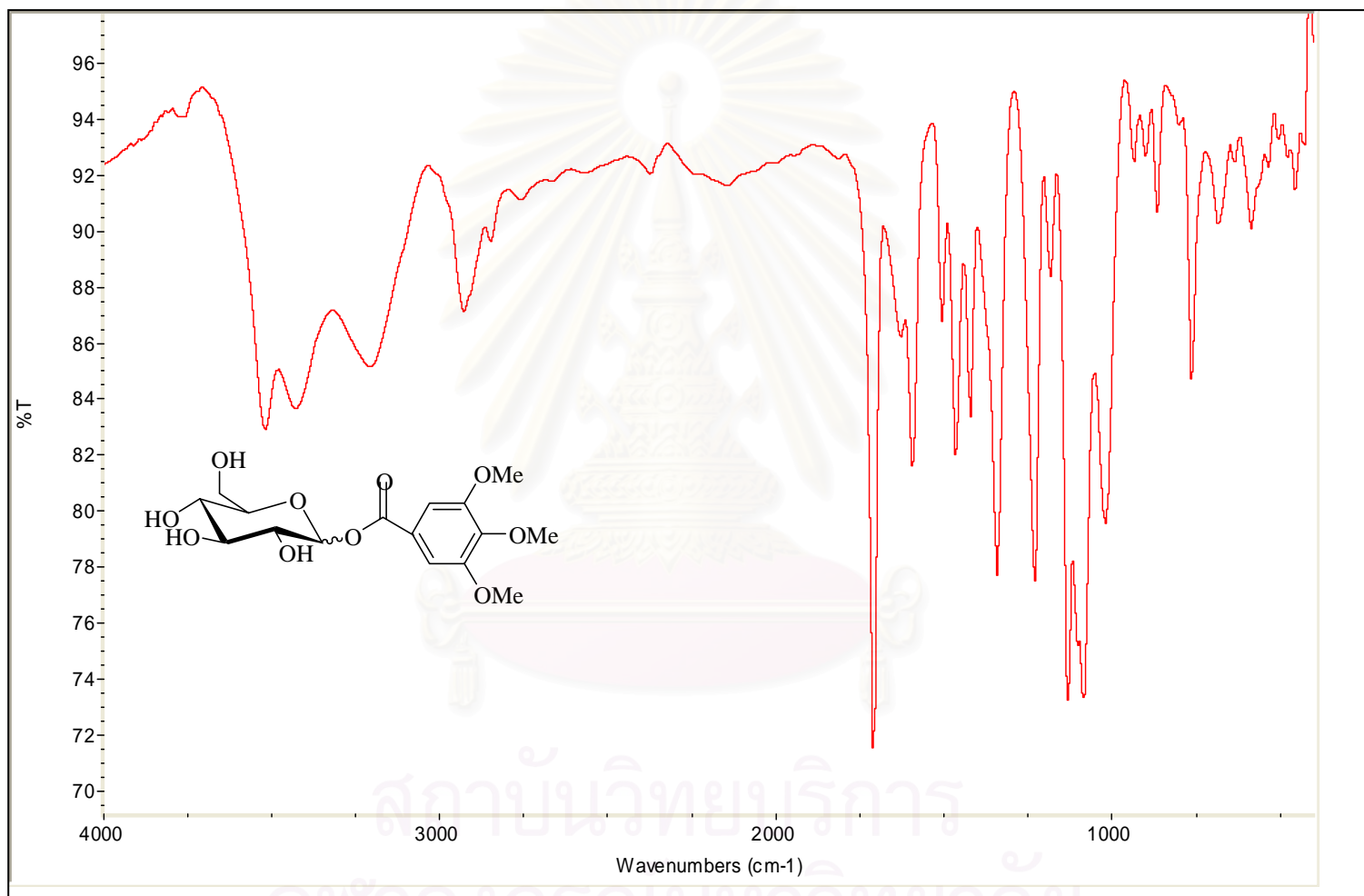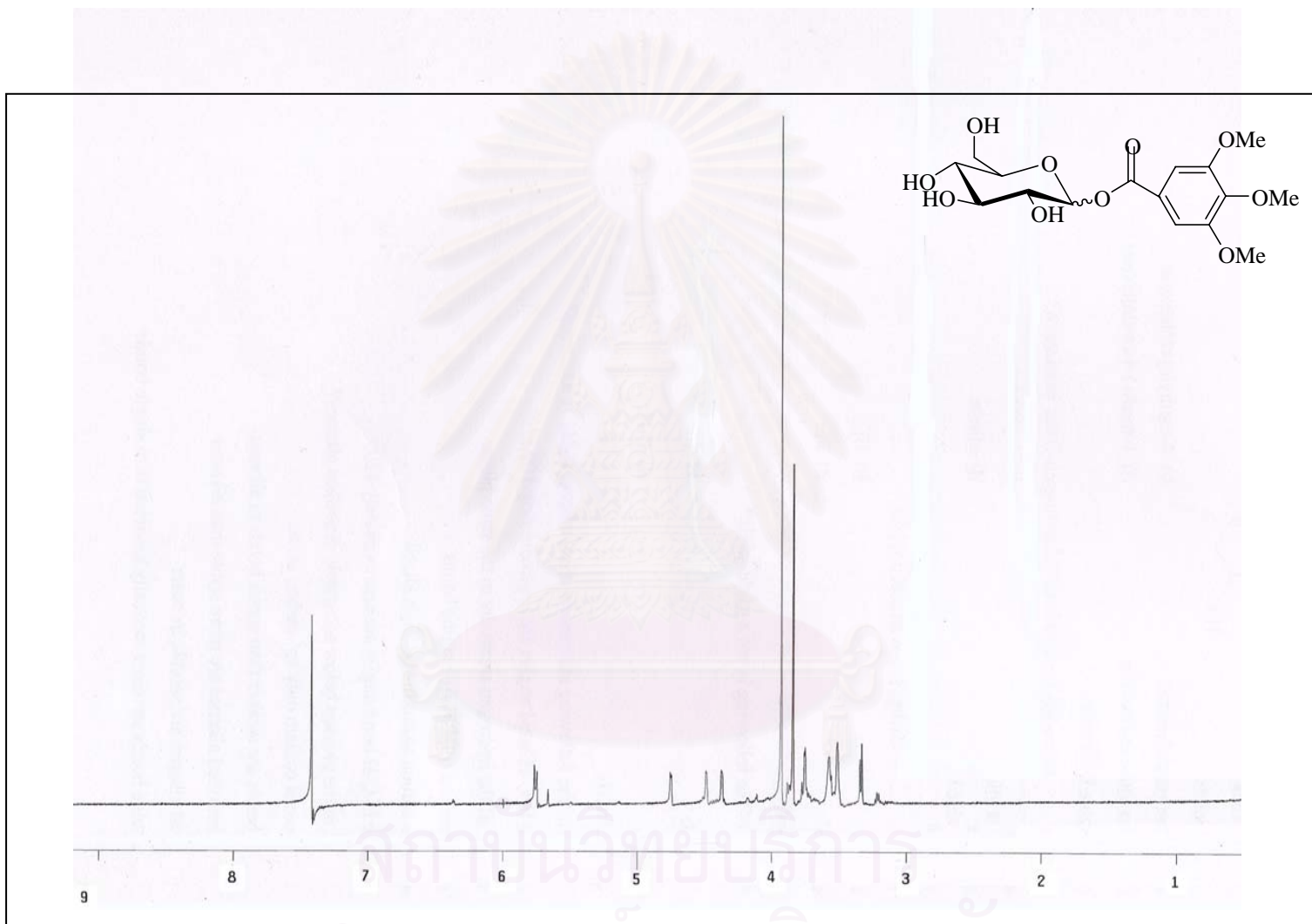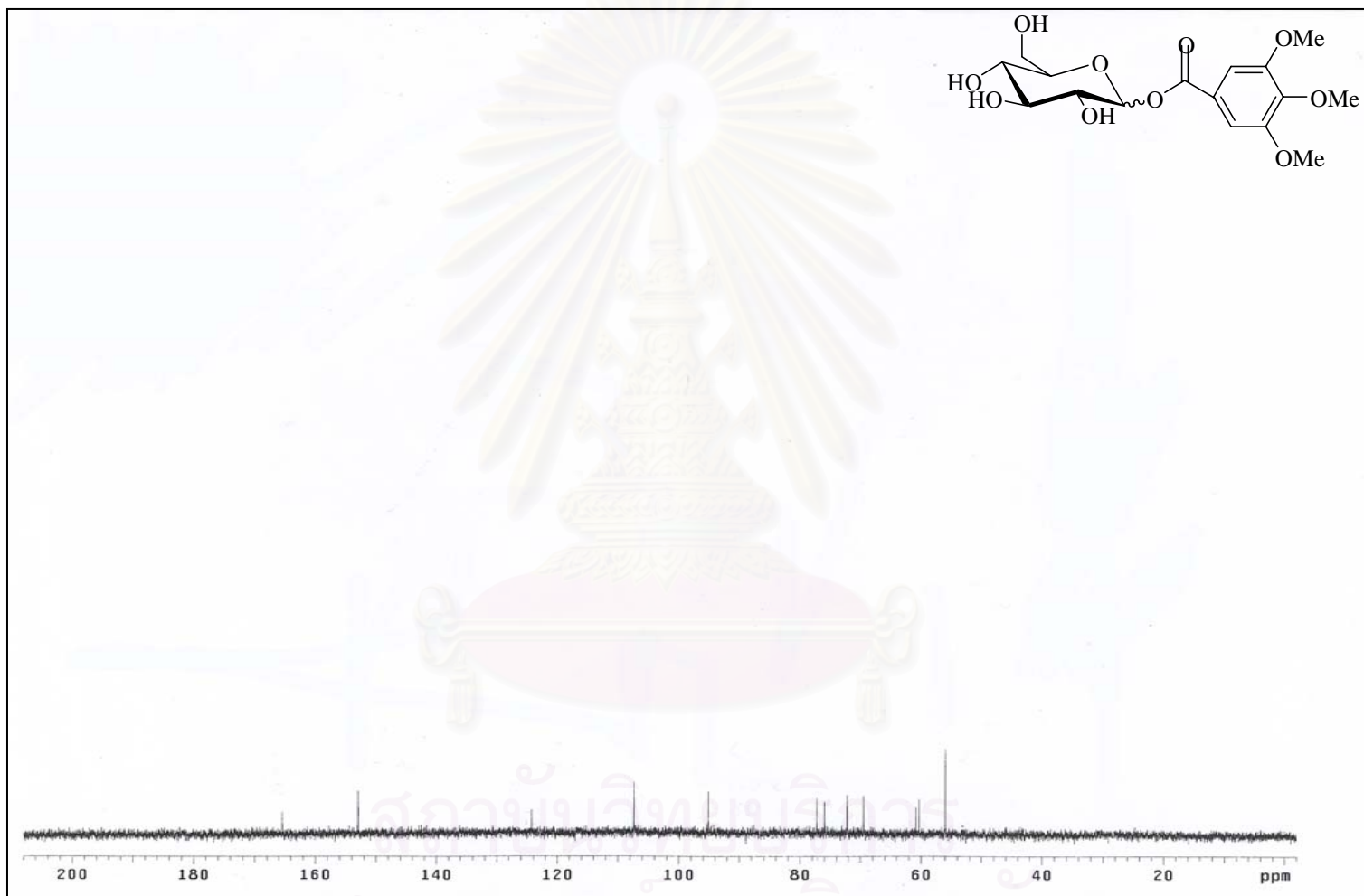**Figure A.5** The ^{1}H-NMR spectrum of compound **2C**
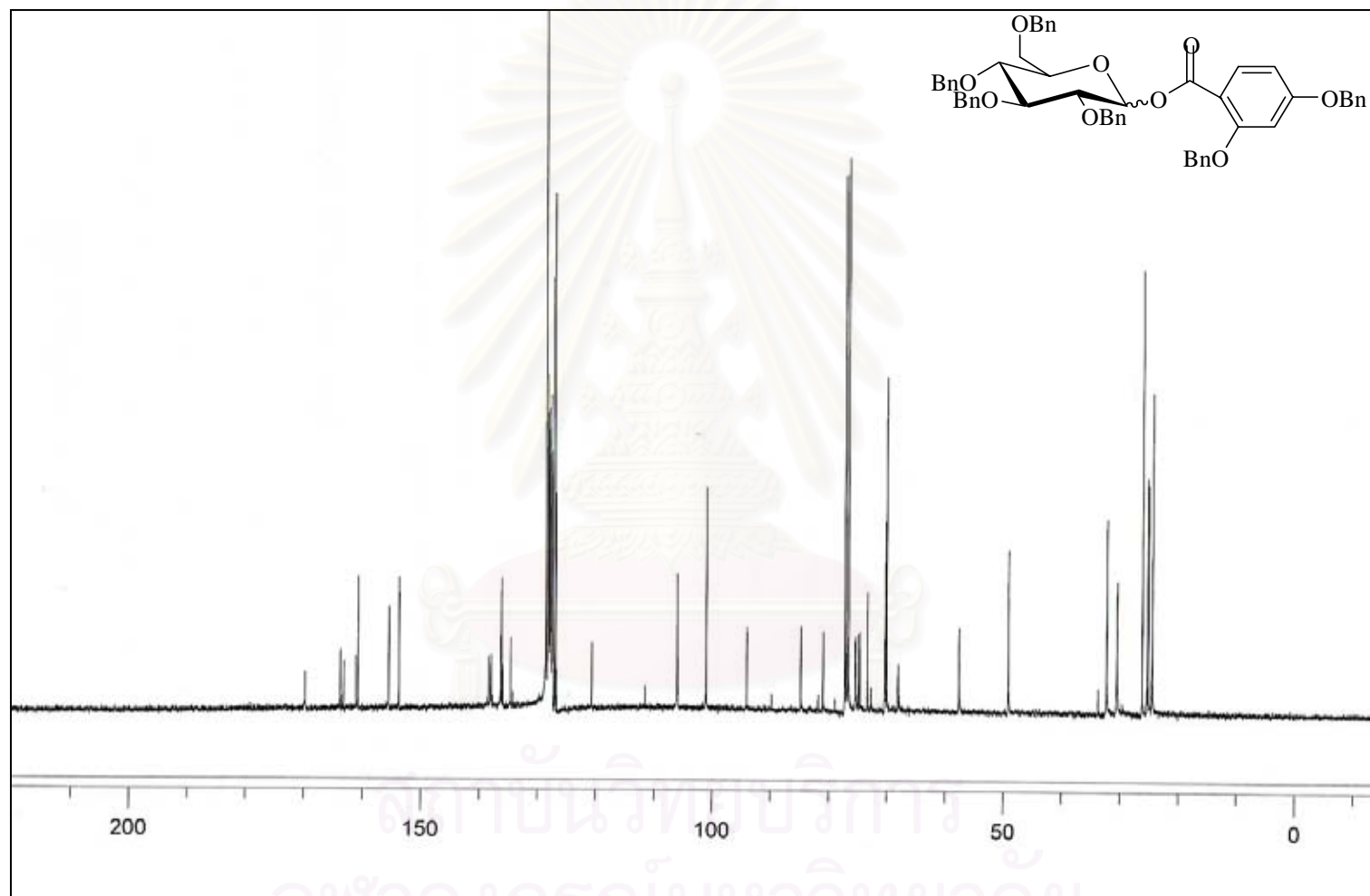
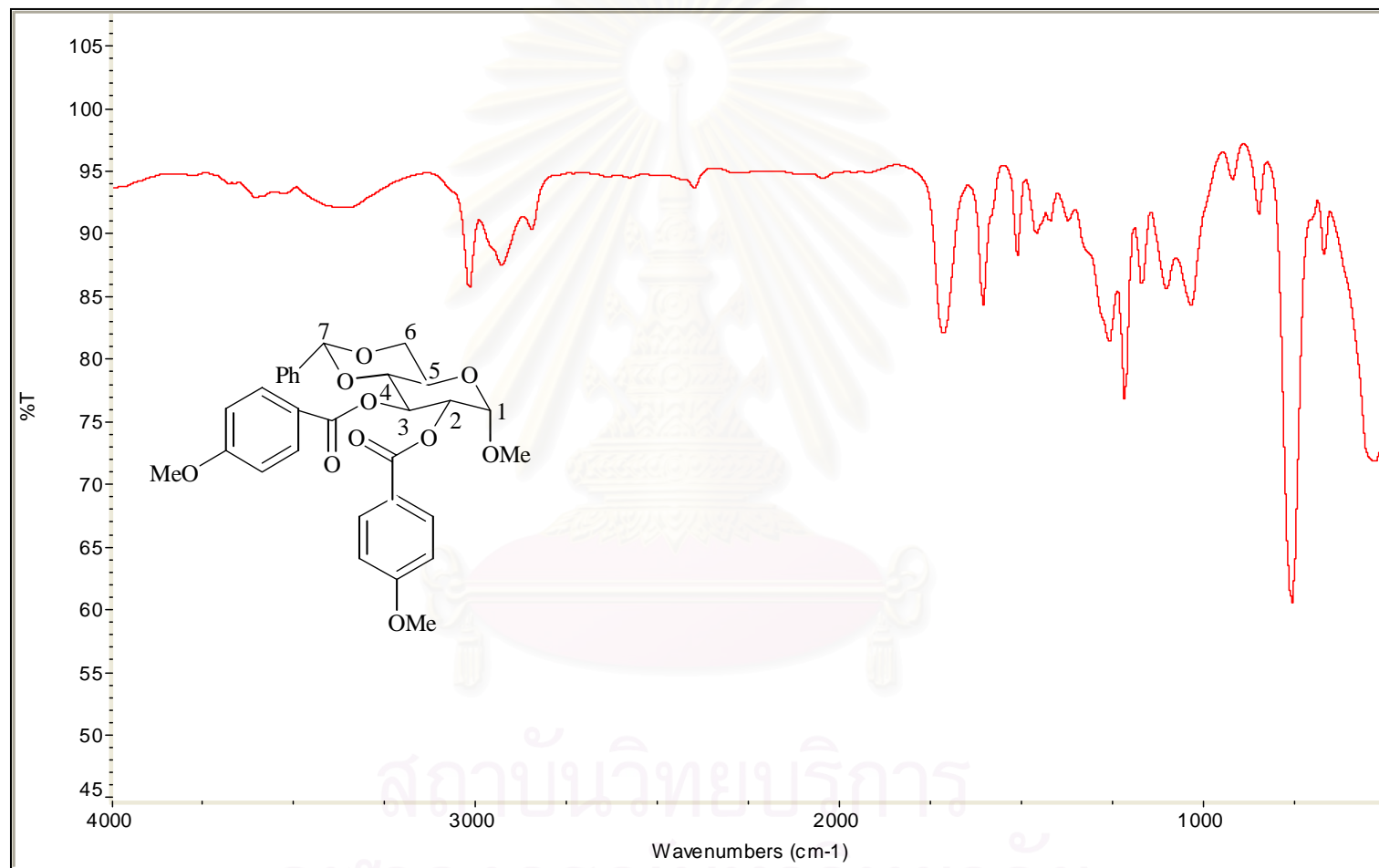**Figure A.6** The ^{13}C-NMR of compound **2C**
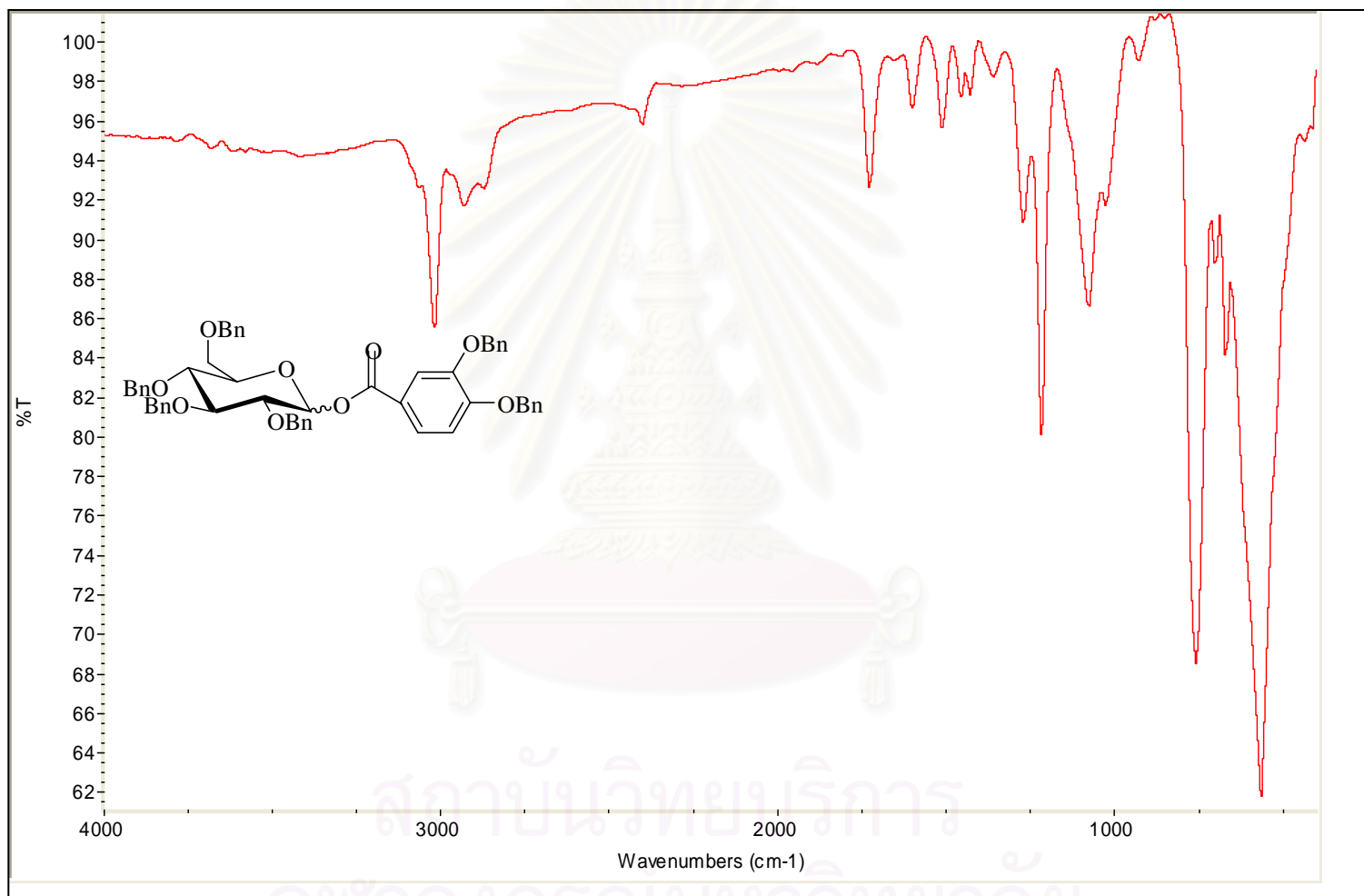
Figure A.7 The IR spectrum of compound **3C**
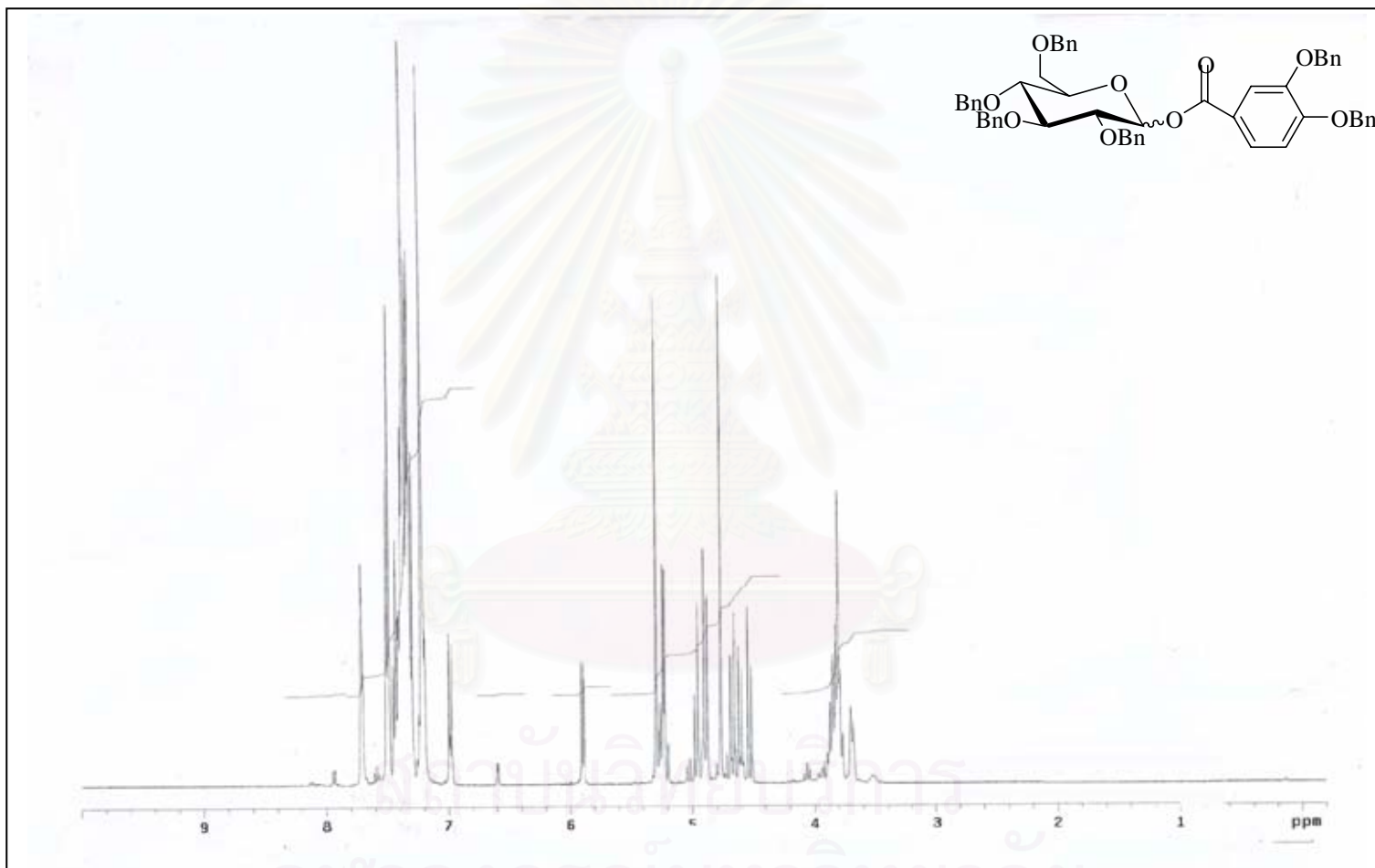
Figure A.8 The ^{1}H-NMR spectrum of compound **3C**
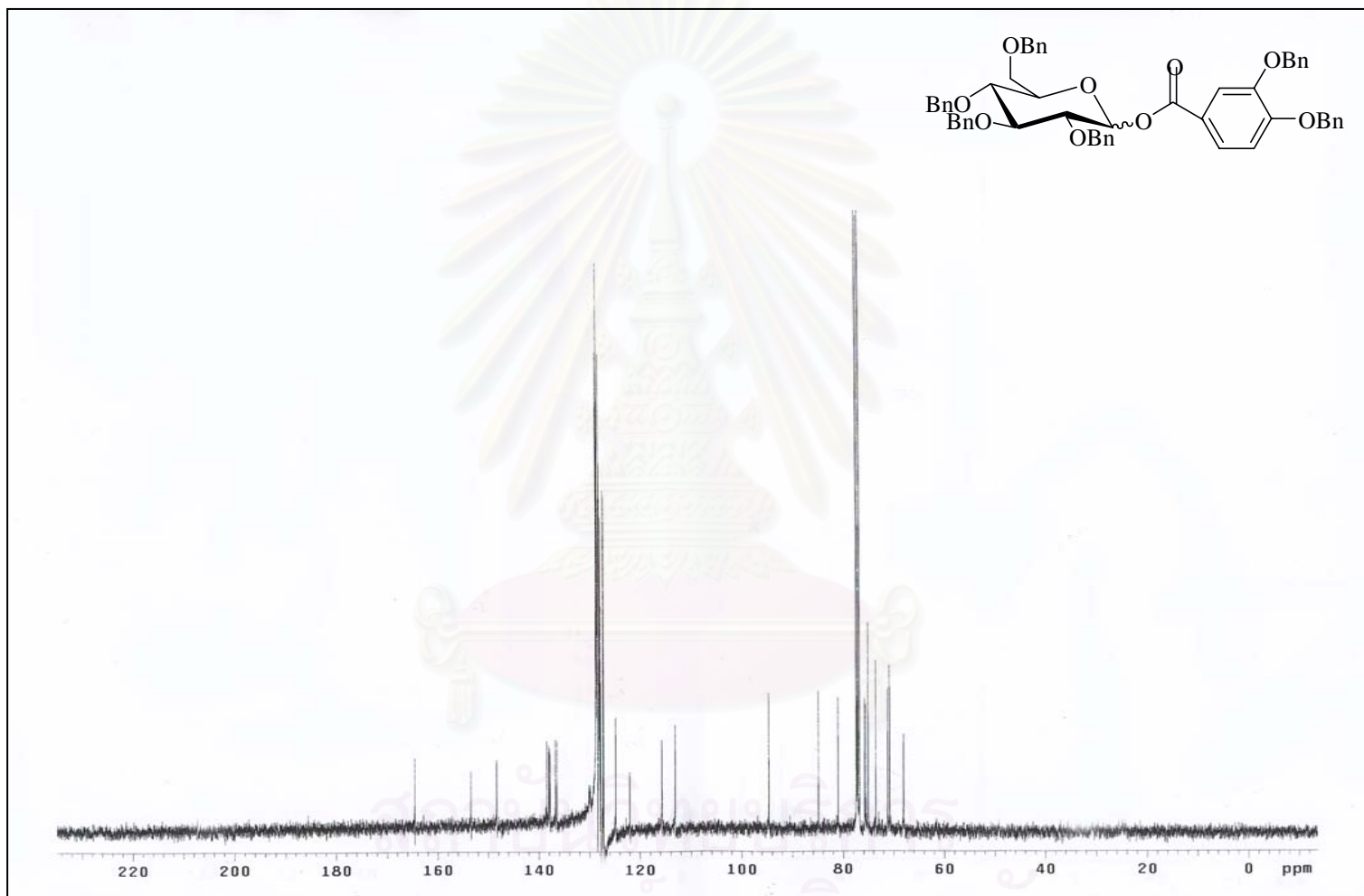
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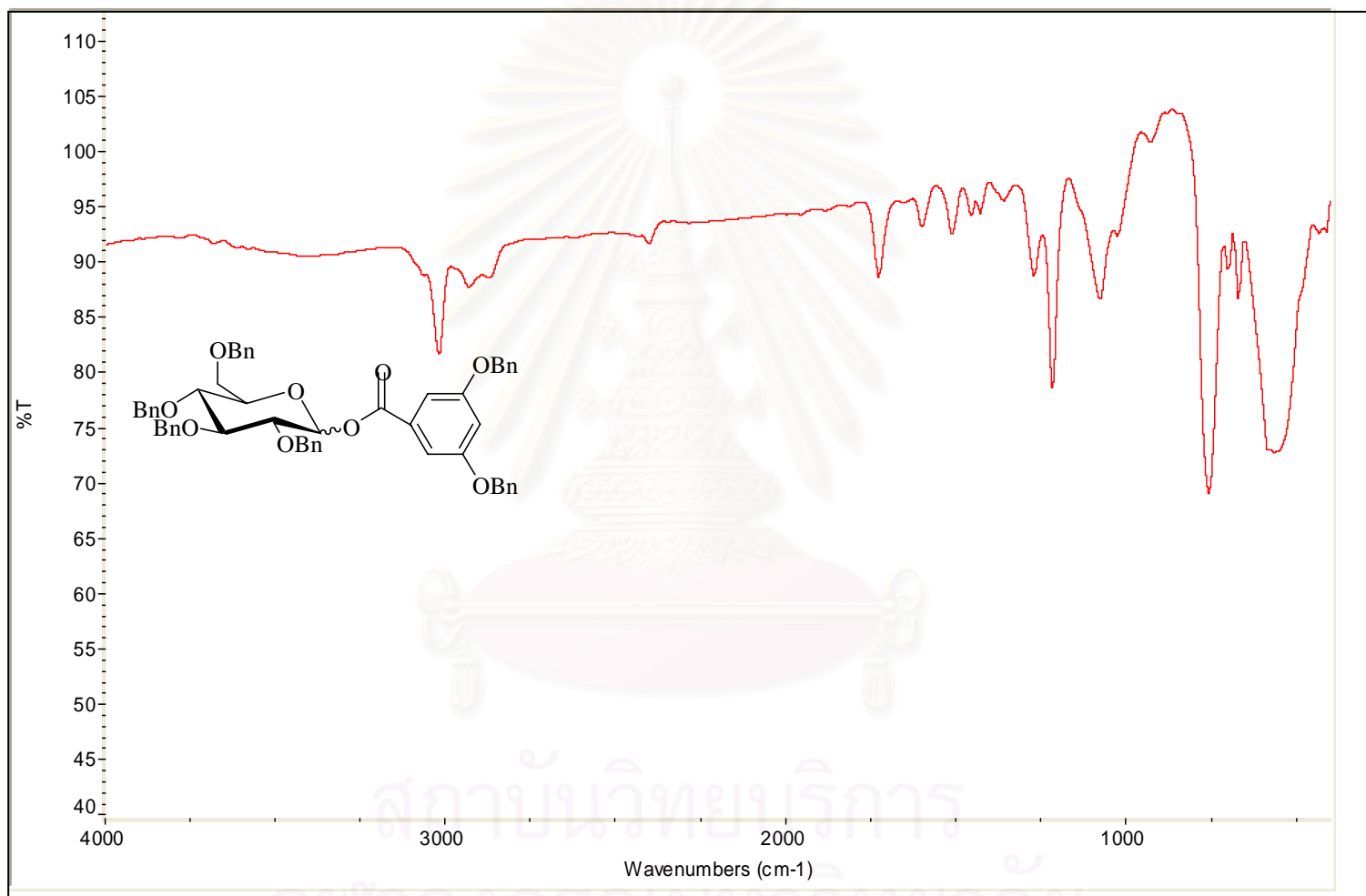
Figure A.10 The IR spectrum of compound **4C**
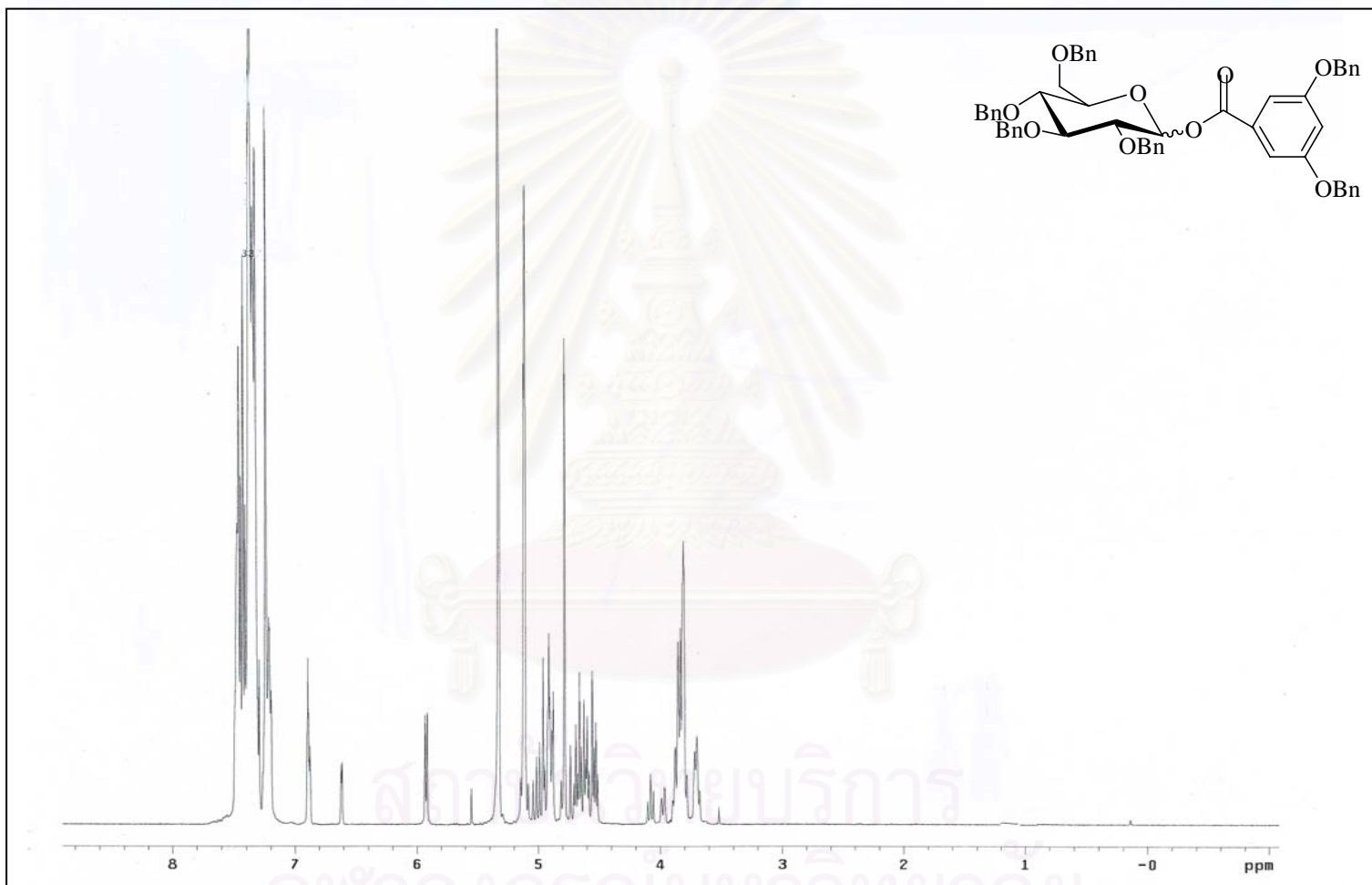
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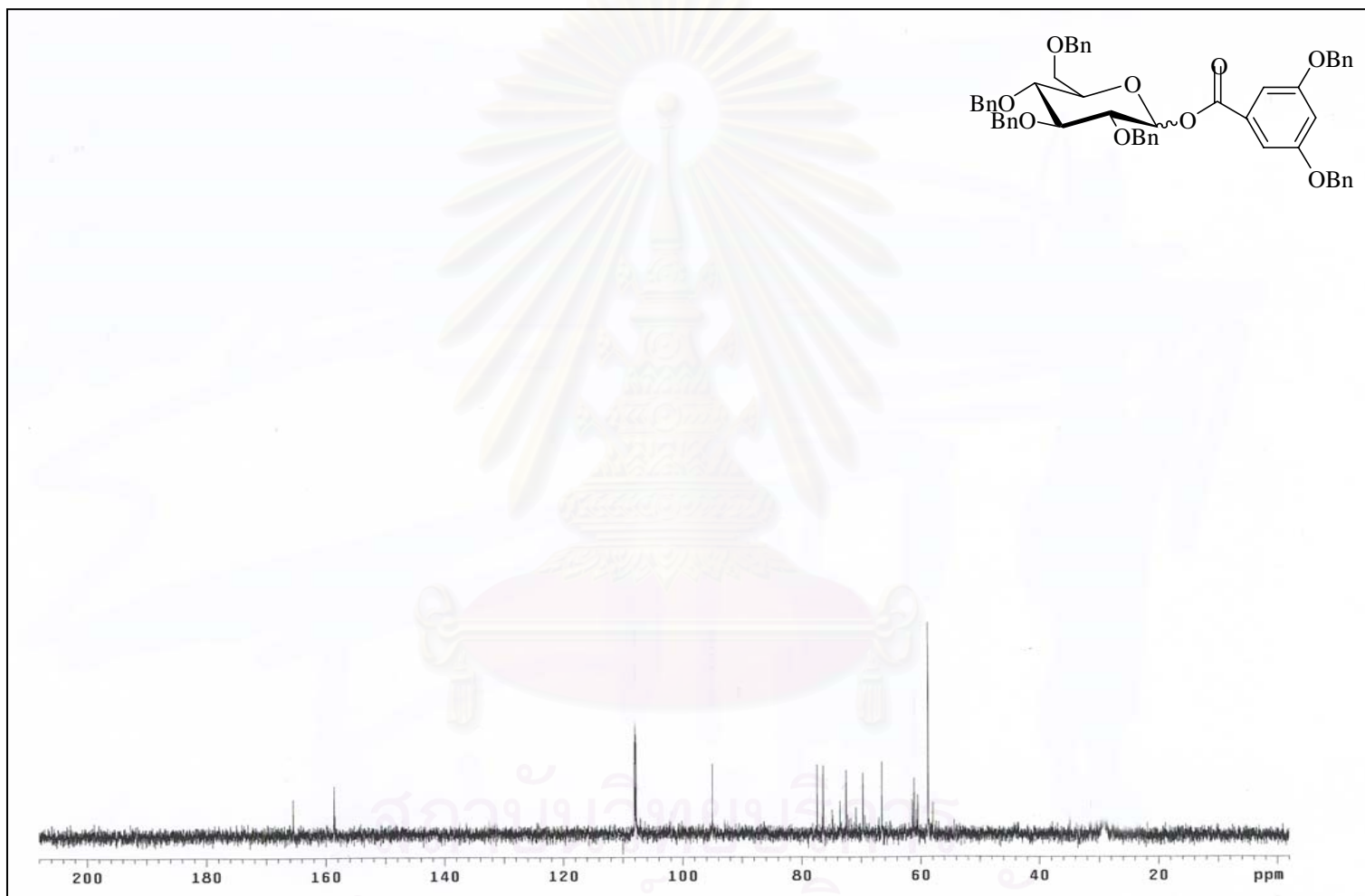
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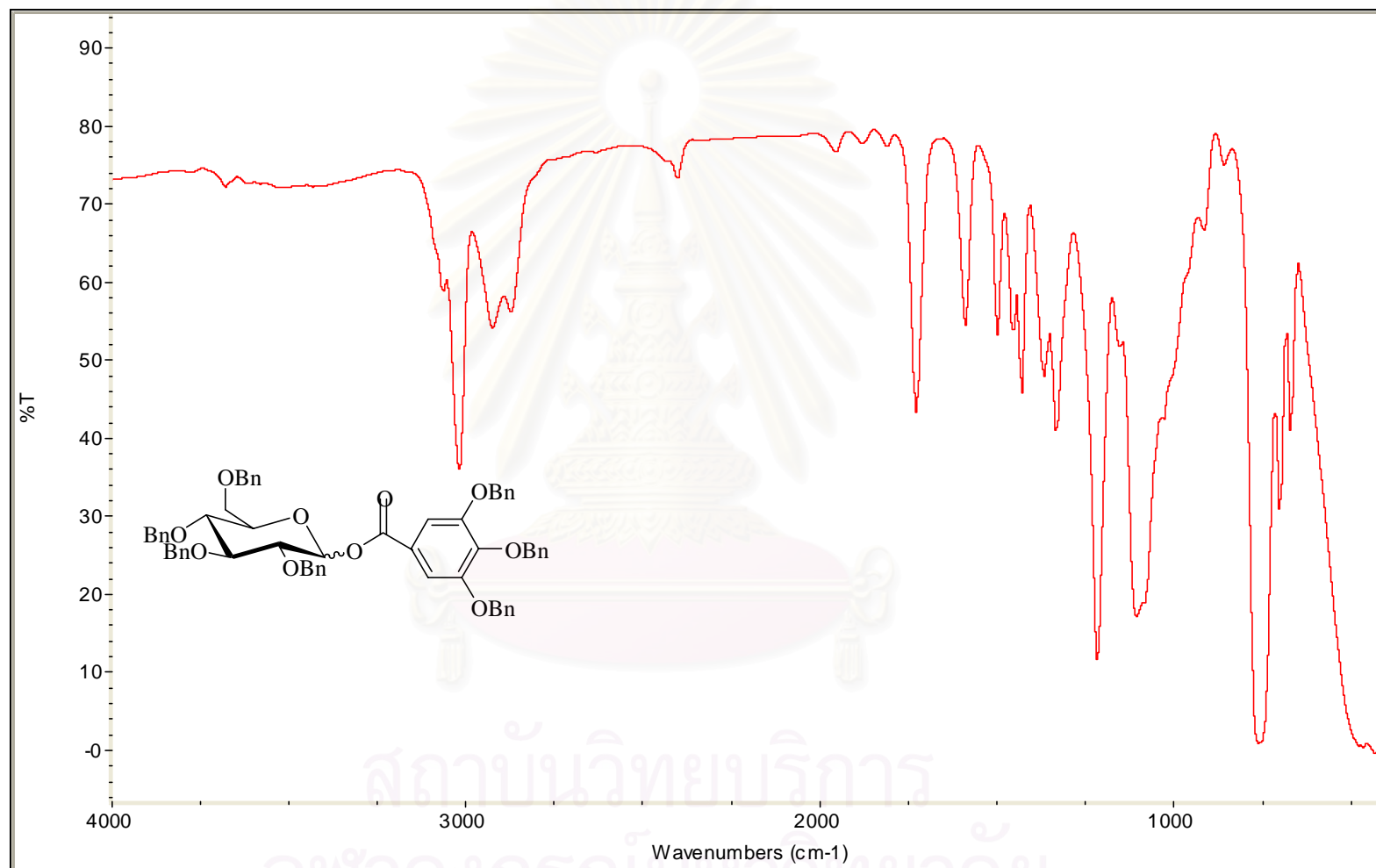
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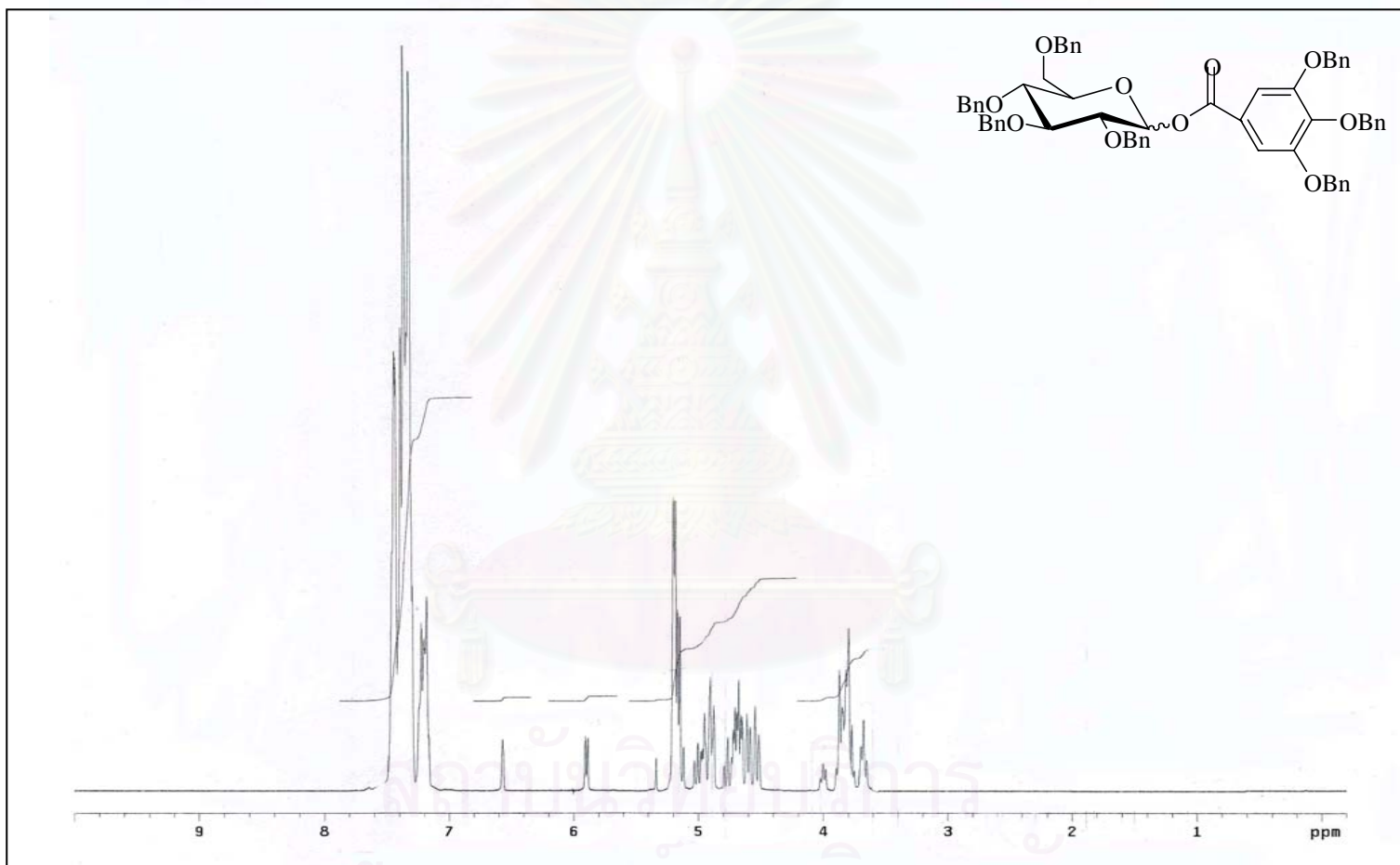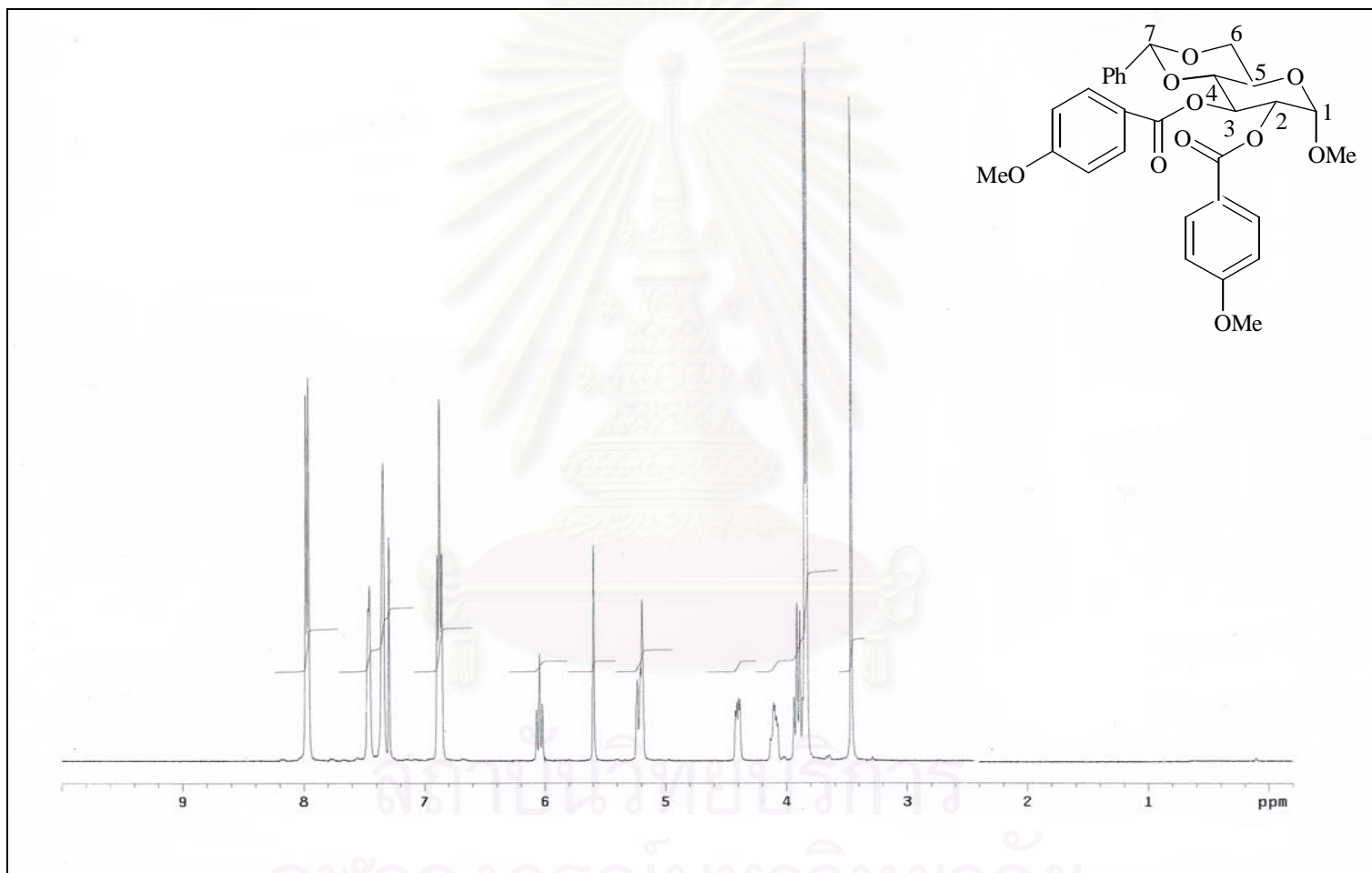
Figure A.14 The ¹H-NMR spectrum of compound **5C**
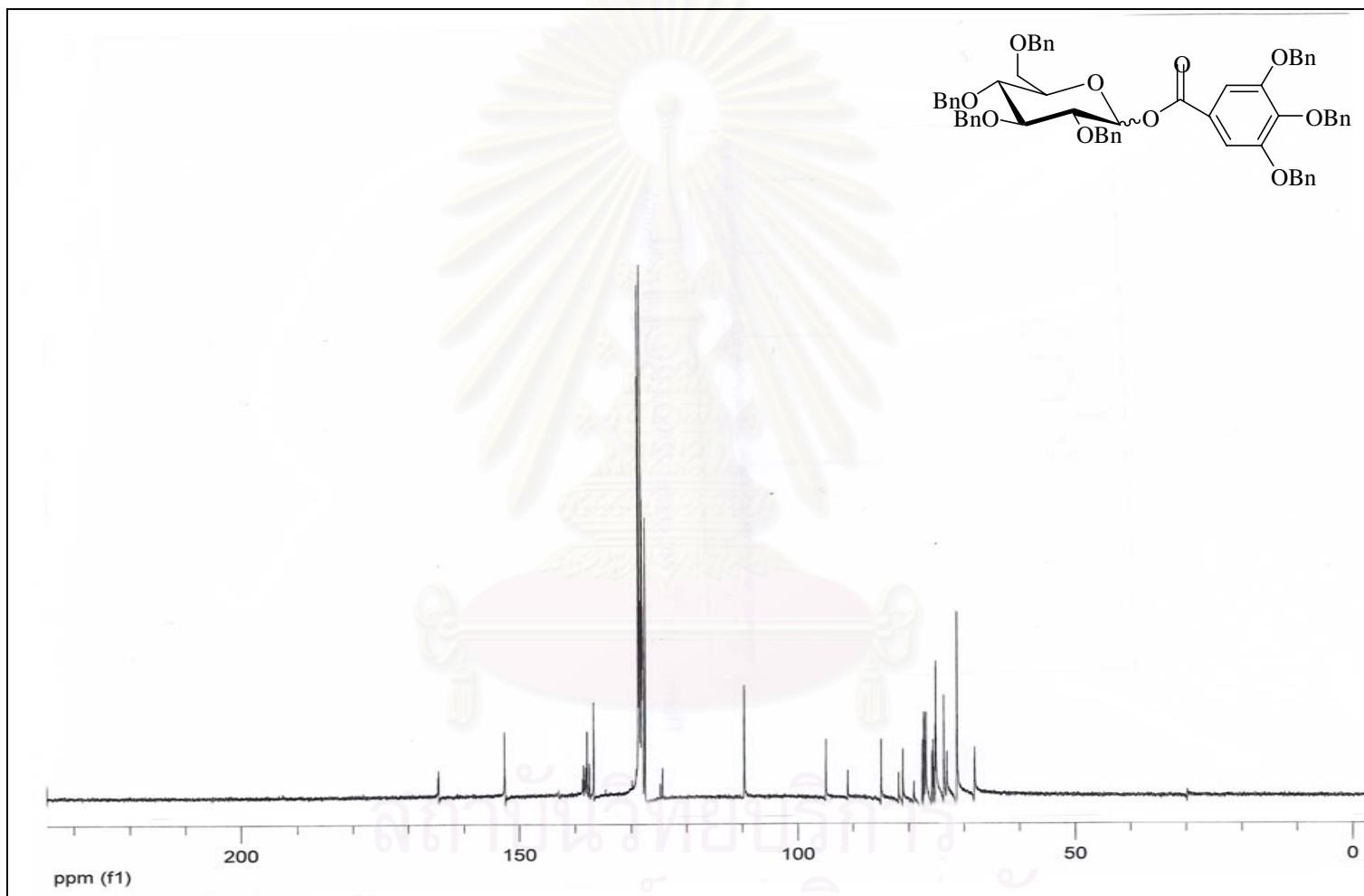
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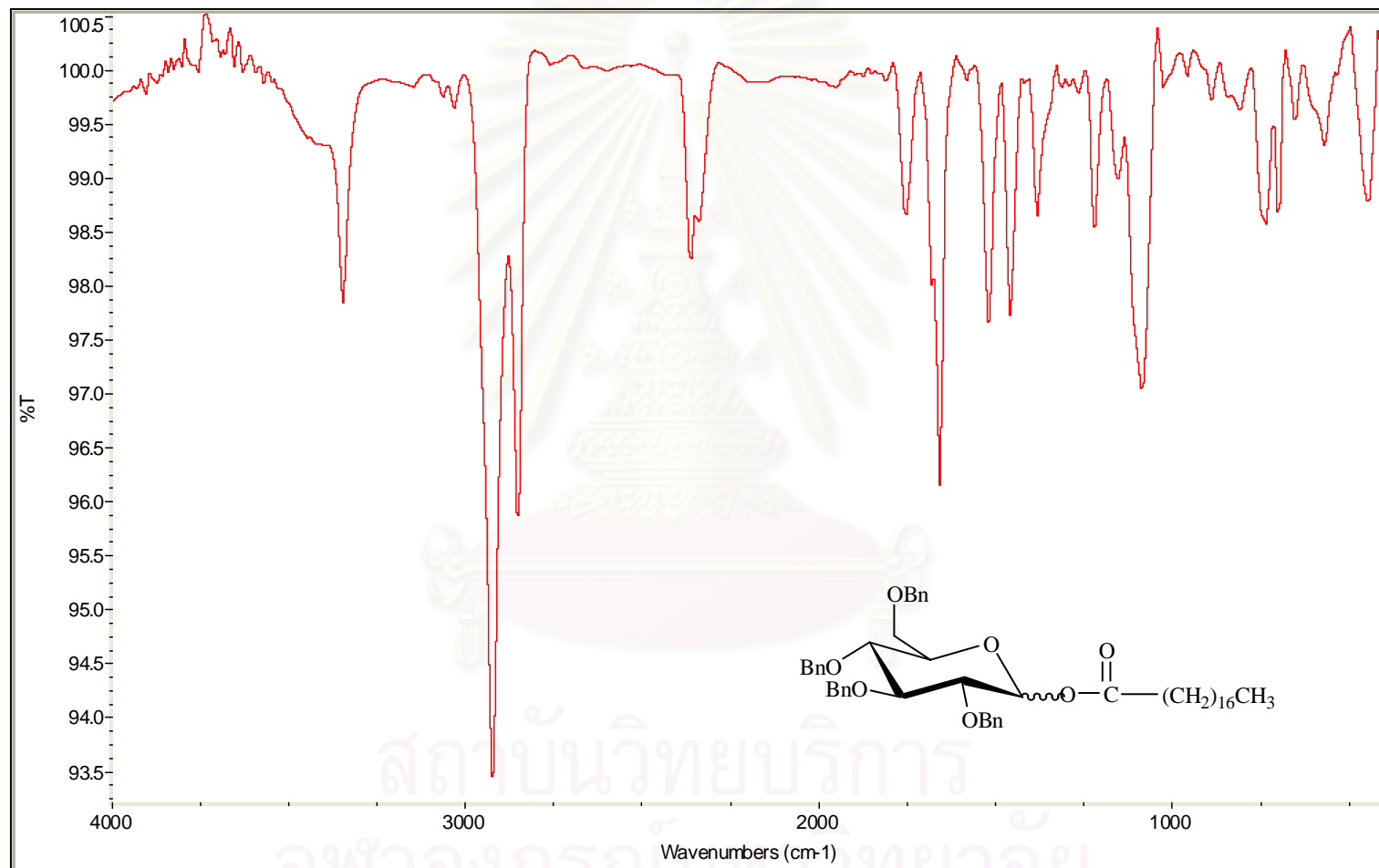
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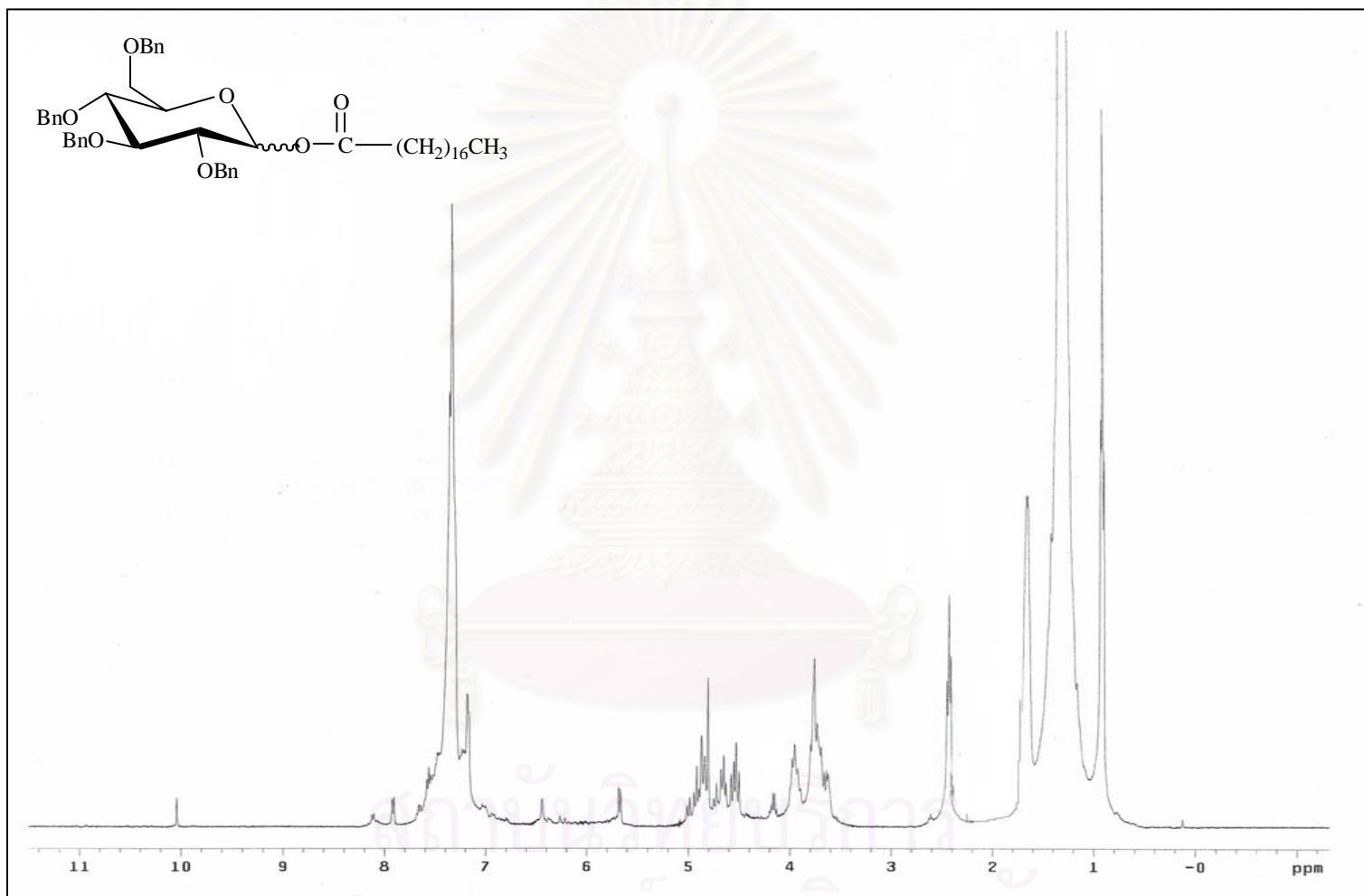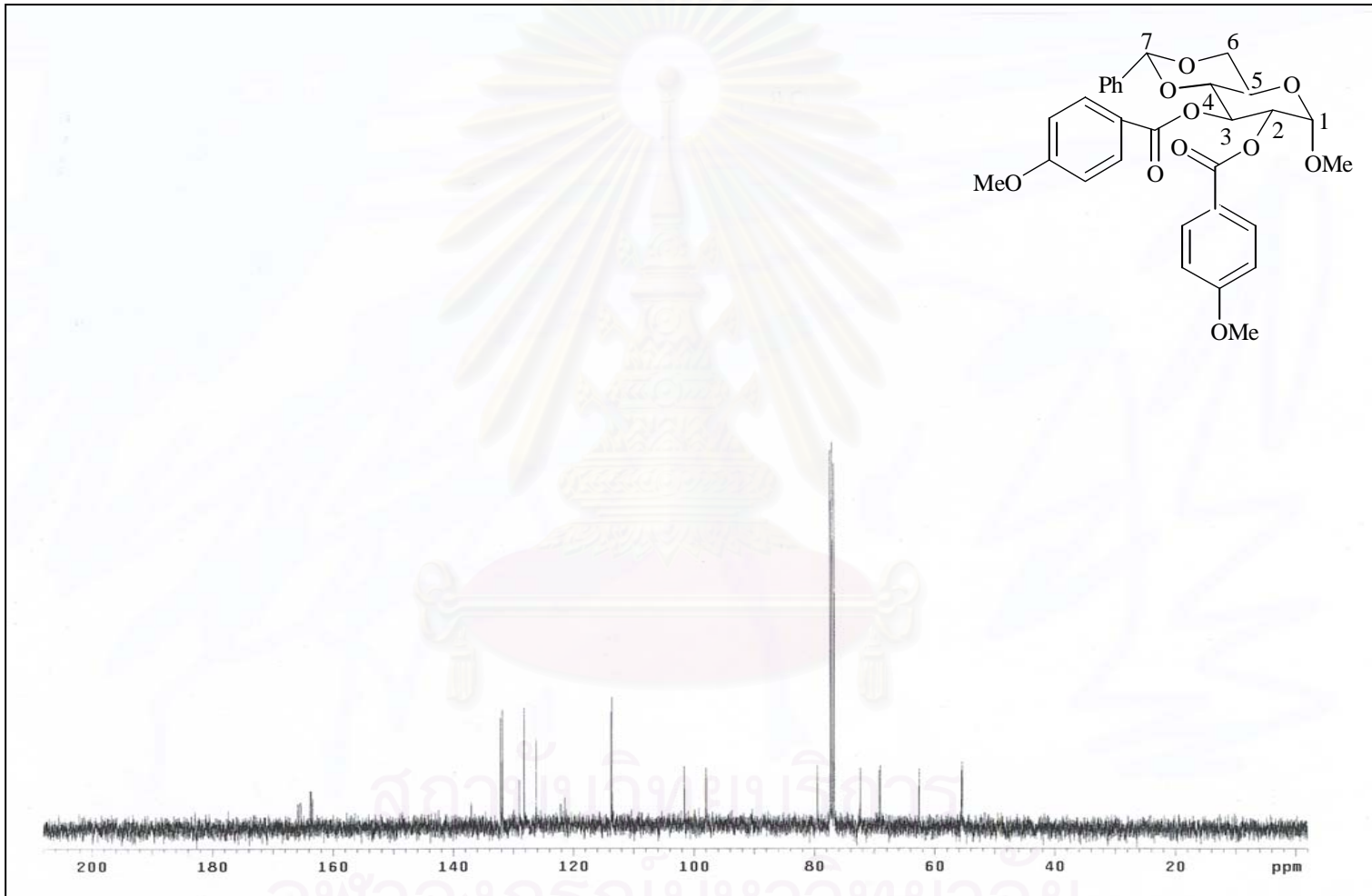
Figure A.17 The ^{1}H-NMR spectrum of compound **8C**
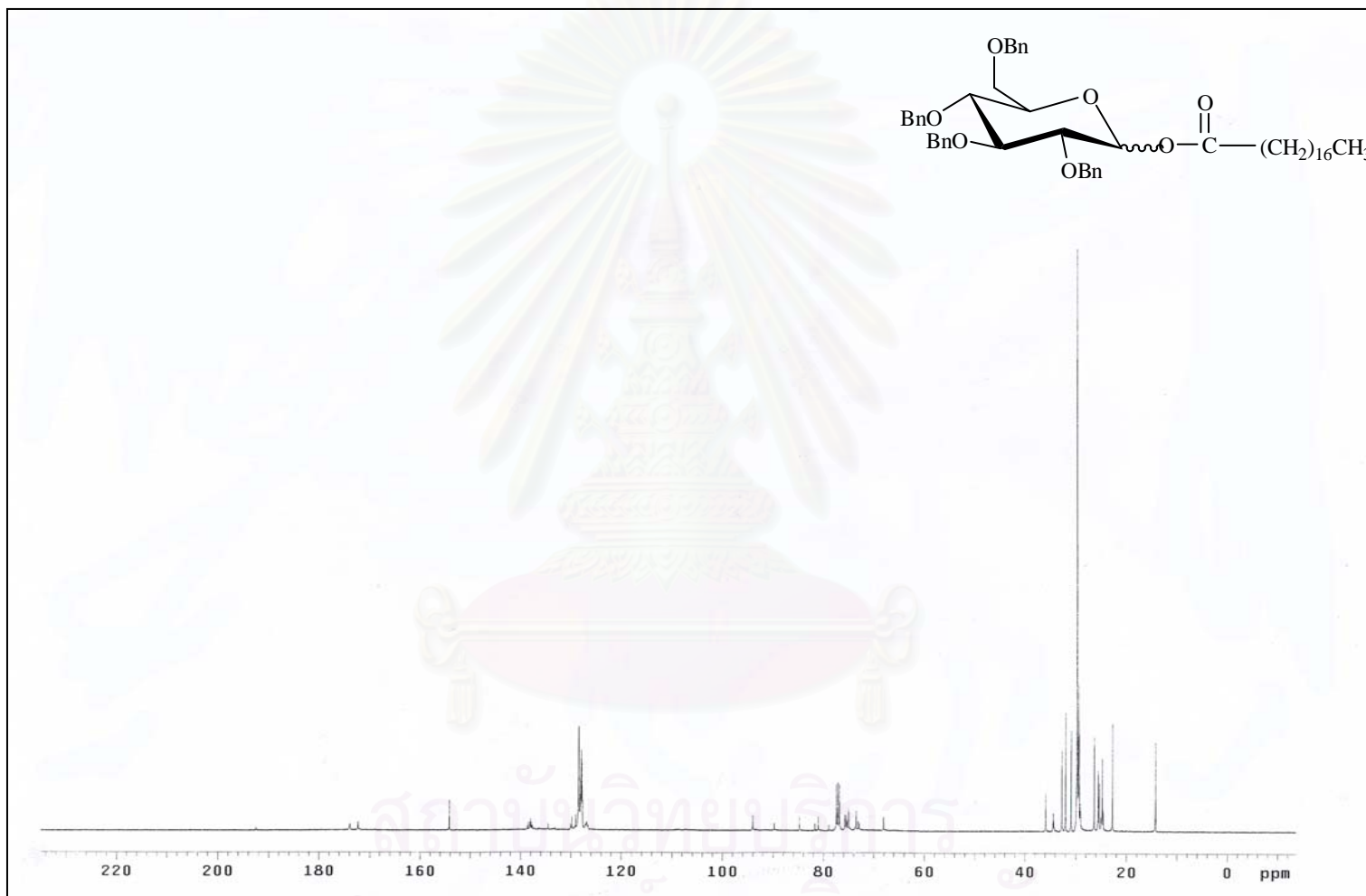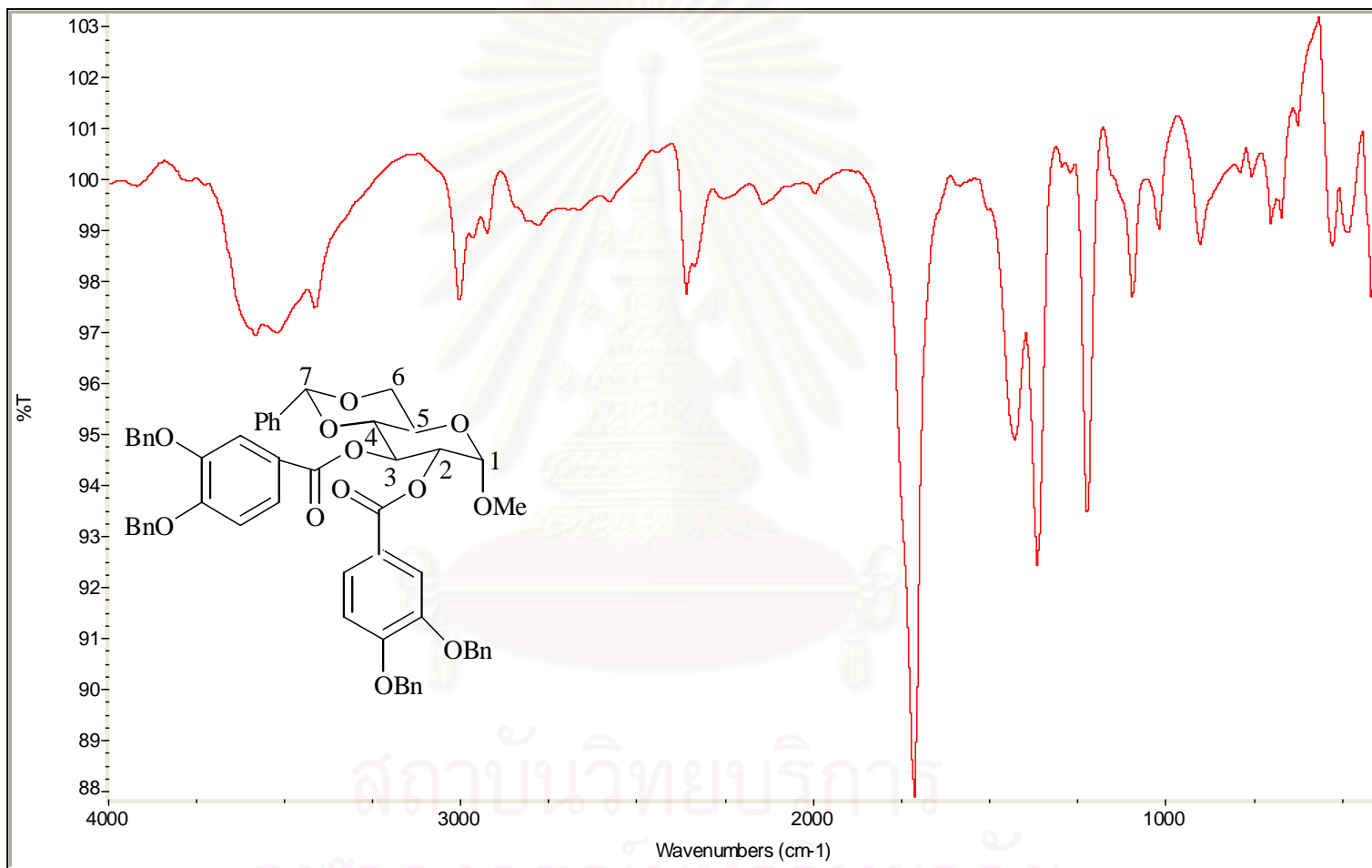
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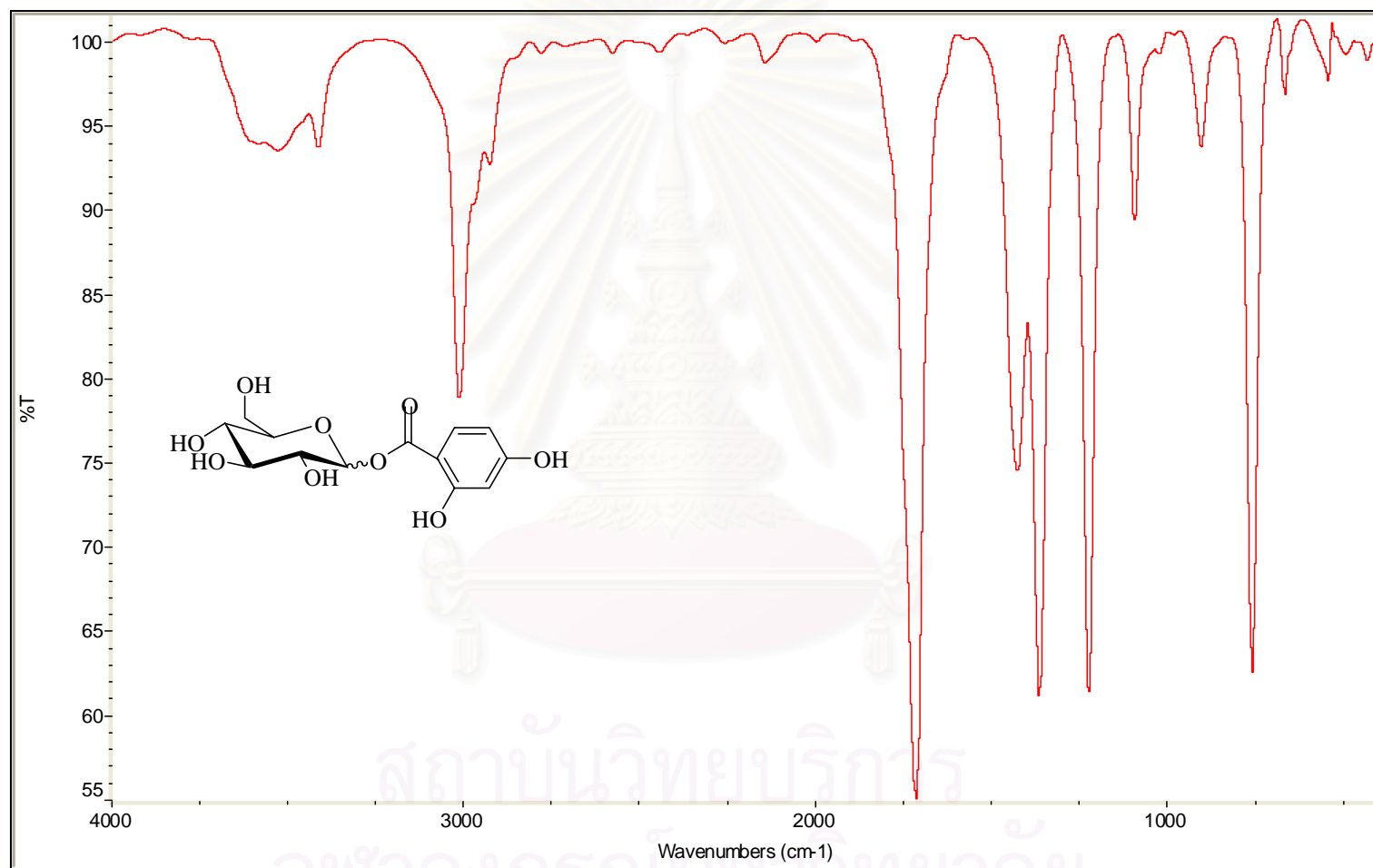
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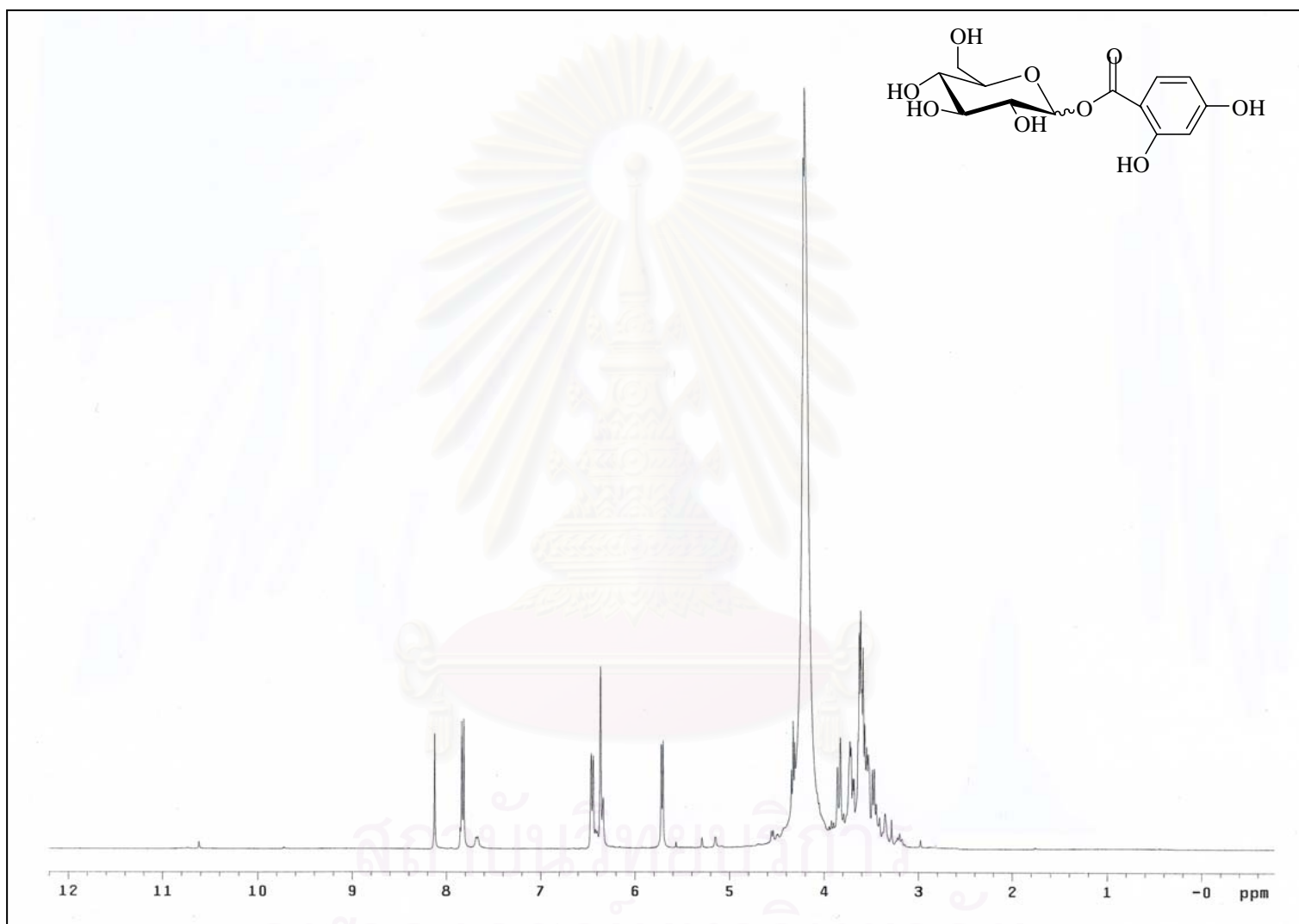
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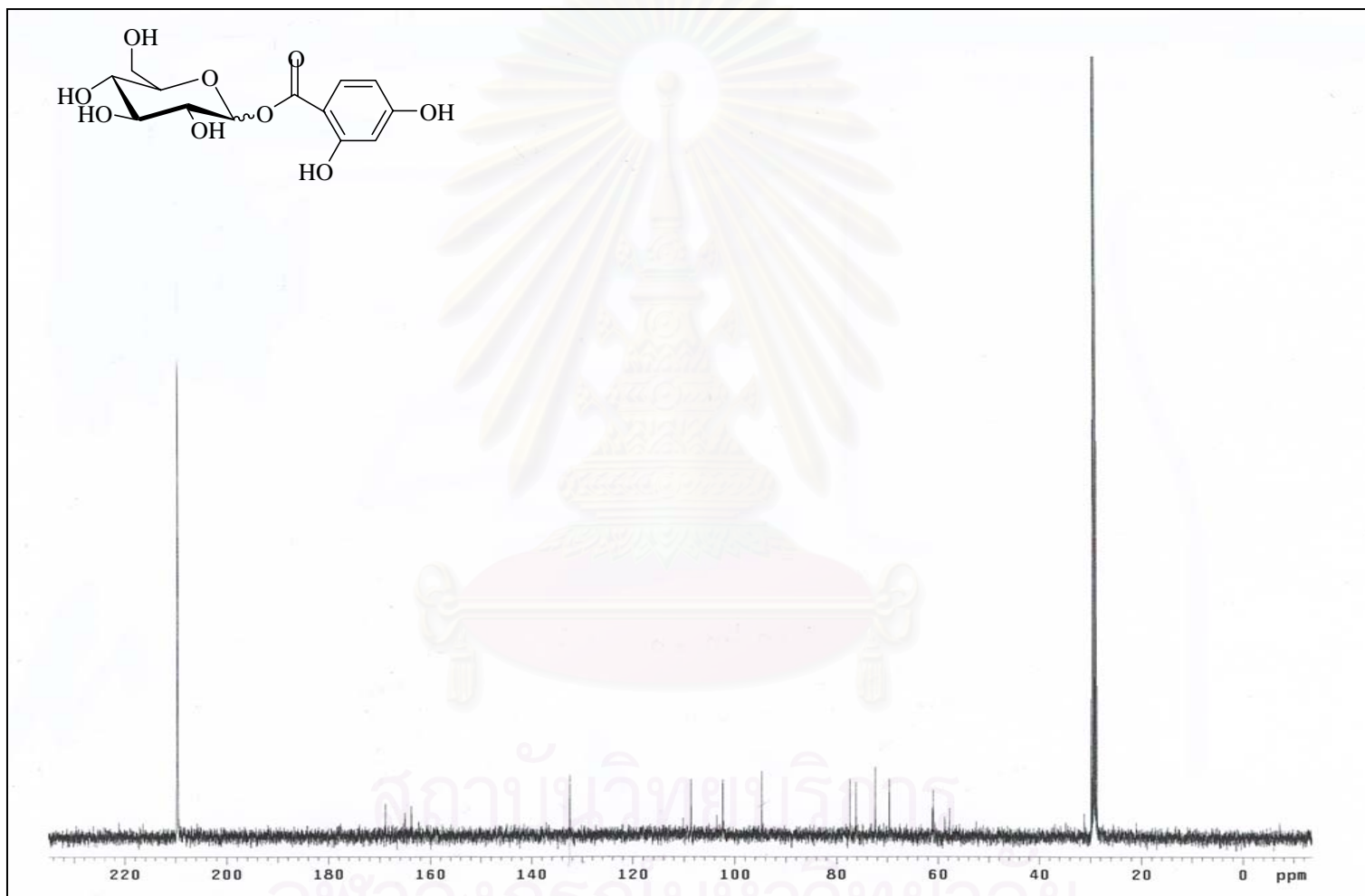
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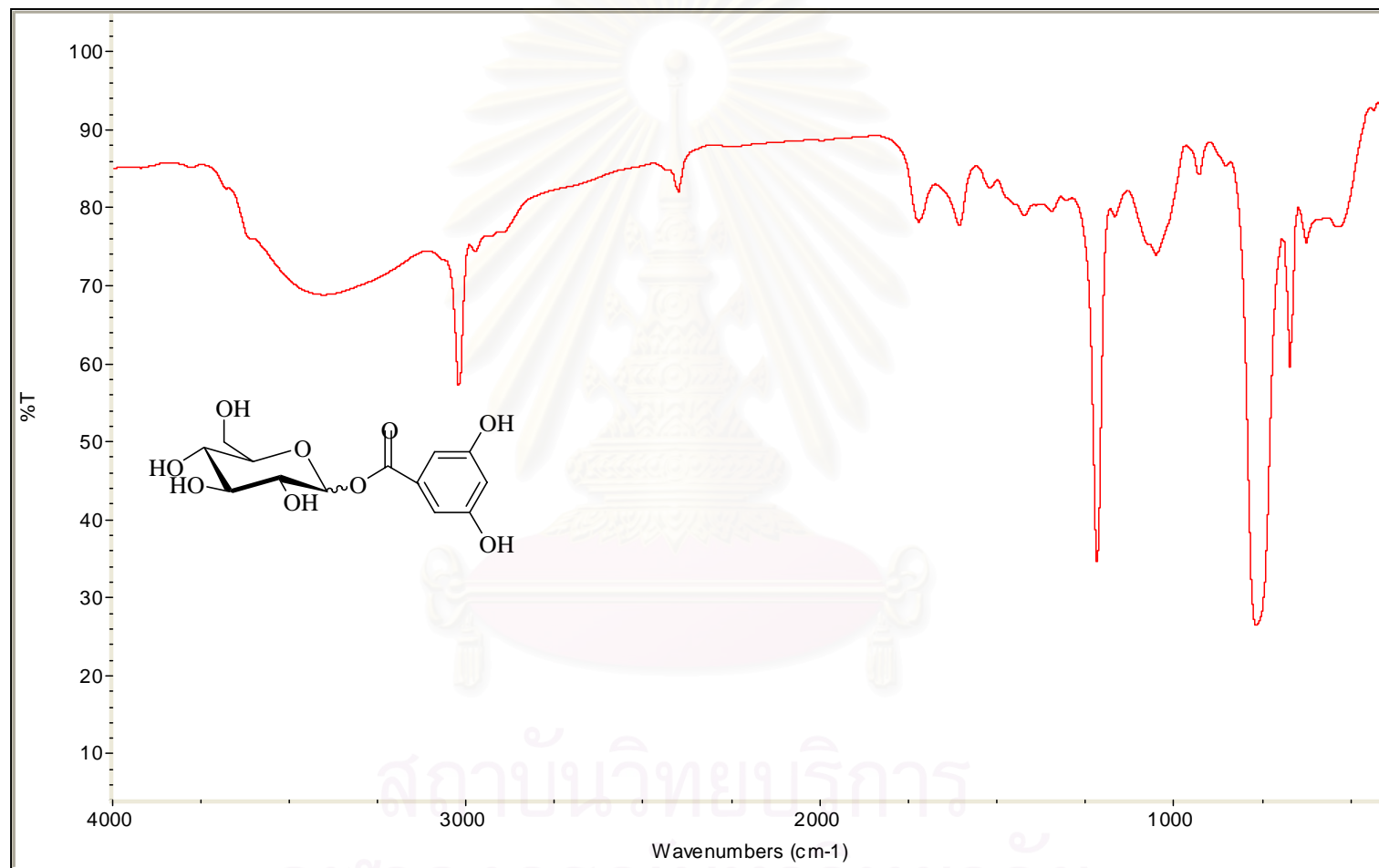
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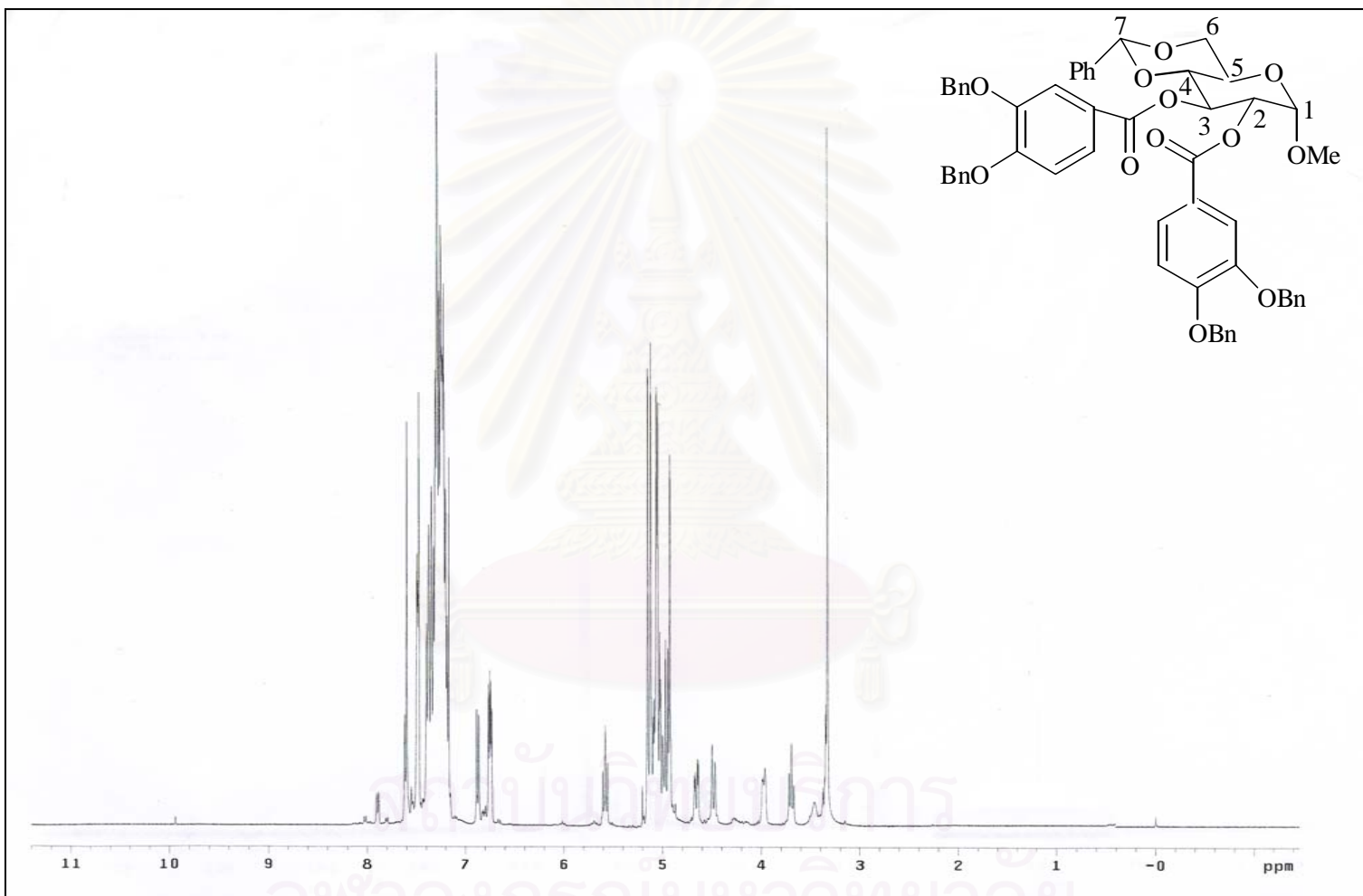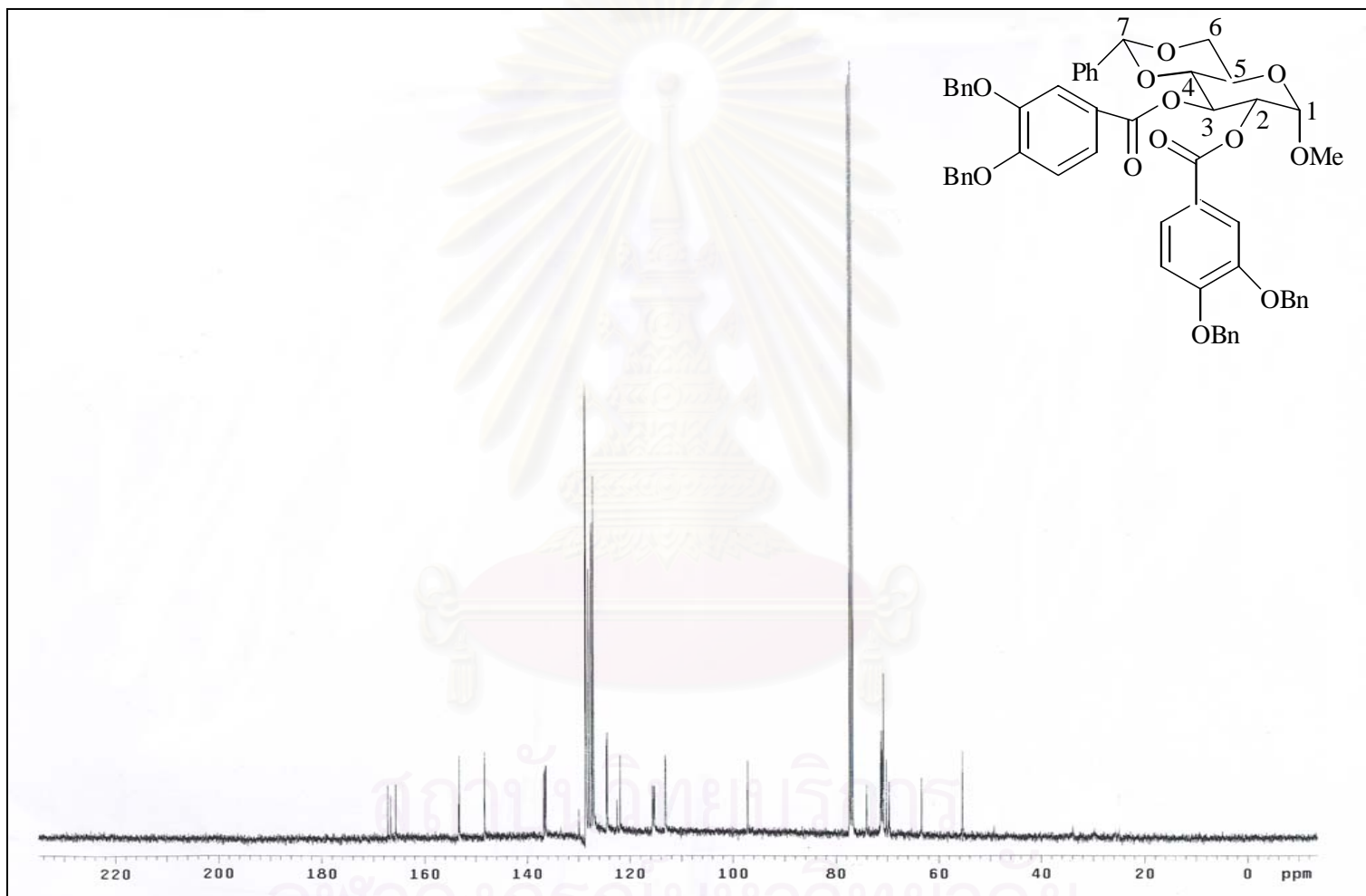
Figure A.23 The ^{1}H-NMR spectrum of compound **4D**

Figure A.24 The ^{13}C-NMR spectrum of compound **4D**

Figure A.25 The IR spectrum of compound **6D**

Figure A.26 The ^{1}H-NMR spectrum of compound **6D**

Figure A.27 The ^{13}C-NMR spectrum of compound **6D**
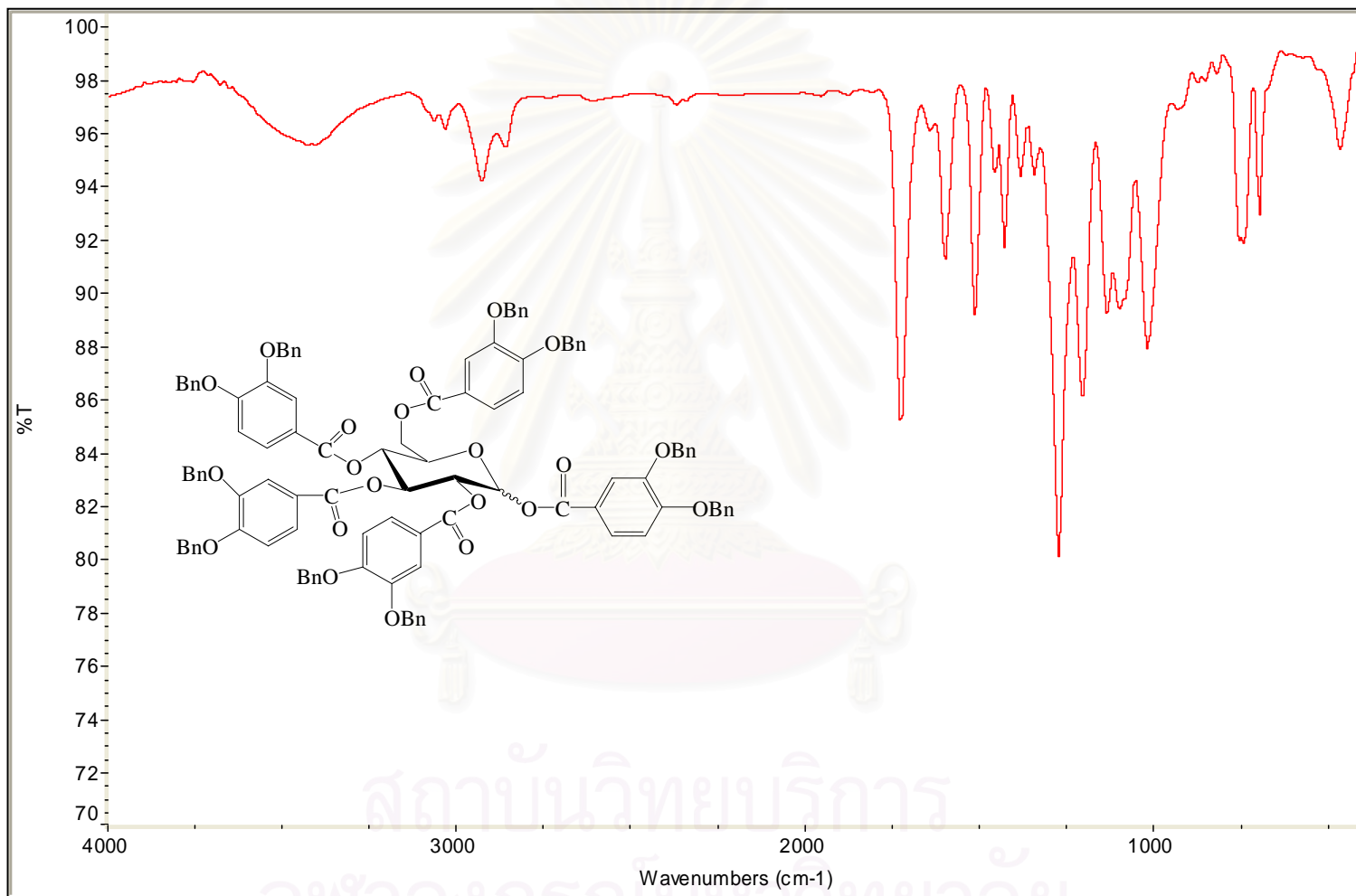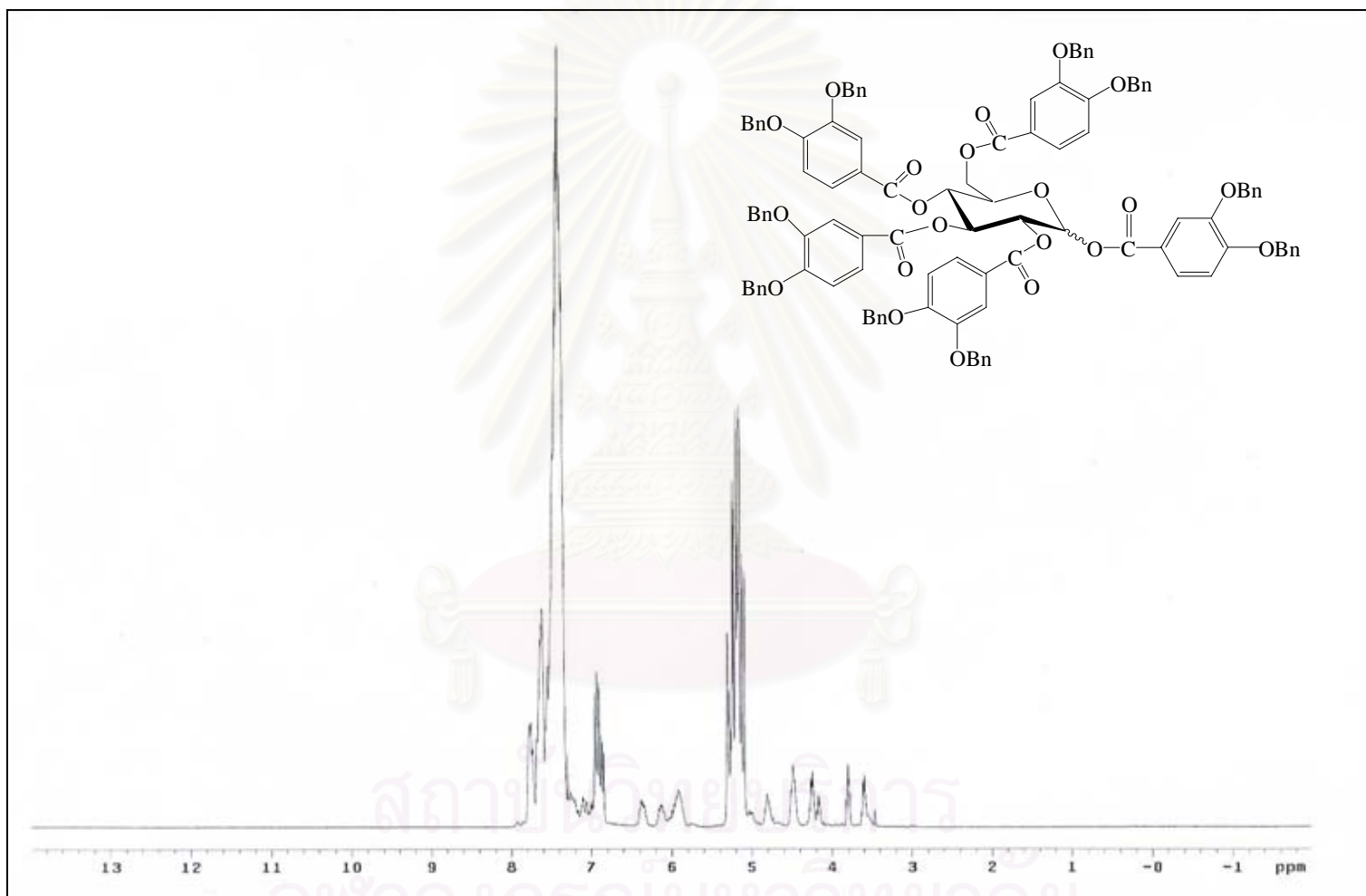
Figure A.28 The IR spectrum of compound **7D**

Figure A.29 The ^{1}H-NMR spectrum of compound **7D**

Figure A.30 The .13C-NMR spectrum of compound **7D**

Figure A.31 The IR spectrum of compound **3E**

Figure A.32 The ^{1}H-NMR spectrum of compound **3E**

Figure A.33 The ^{13}C-NMR spectrum of compound **3E**

Figure A.34 The IR spectrum of compound **5E**

Figure A.35 The ^{1}H-NMR spectrum of compound **5E**

Figure A.36 The ^{13}C-NMR spectrum of compound **5E**

Figure A.37 The IR spectrum of compound **2I**

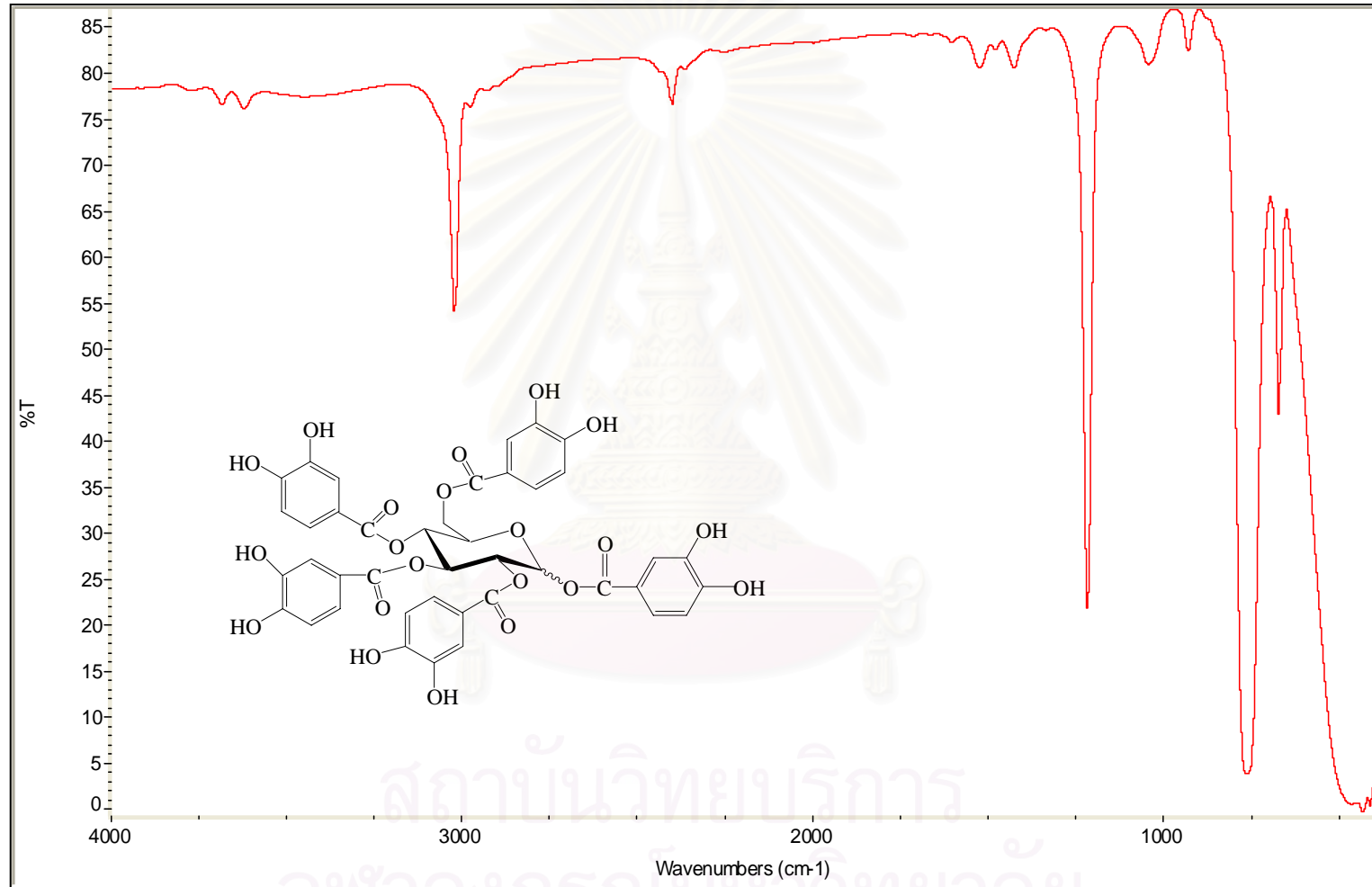**Figure A.38** The ^{1}H-NMR spectrum of compound **2I**
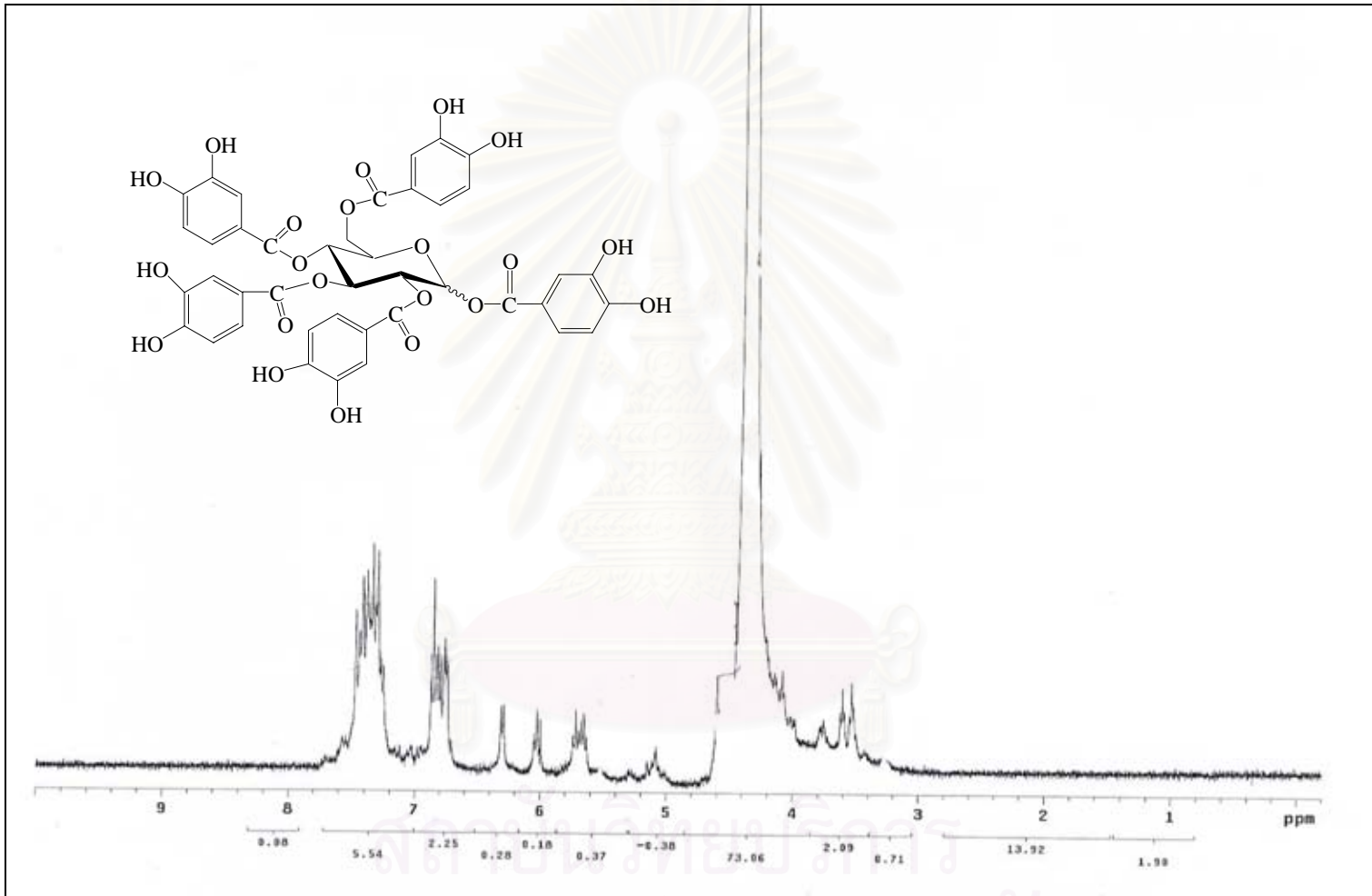
Figure A.39 The IR spectrum of compound **2J**

Figure A.40 The ^{1}H-NMR spectrum of compound **2J**
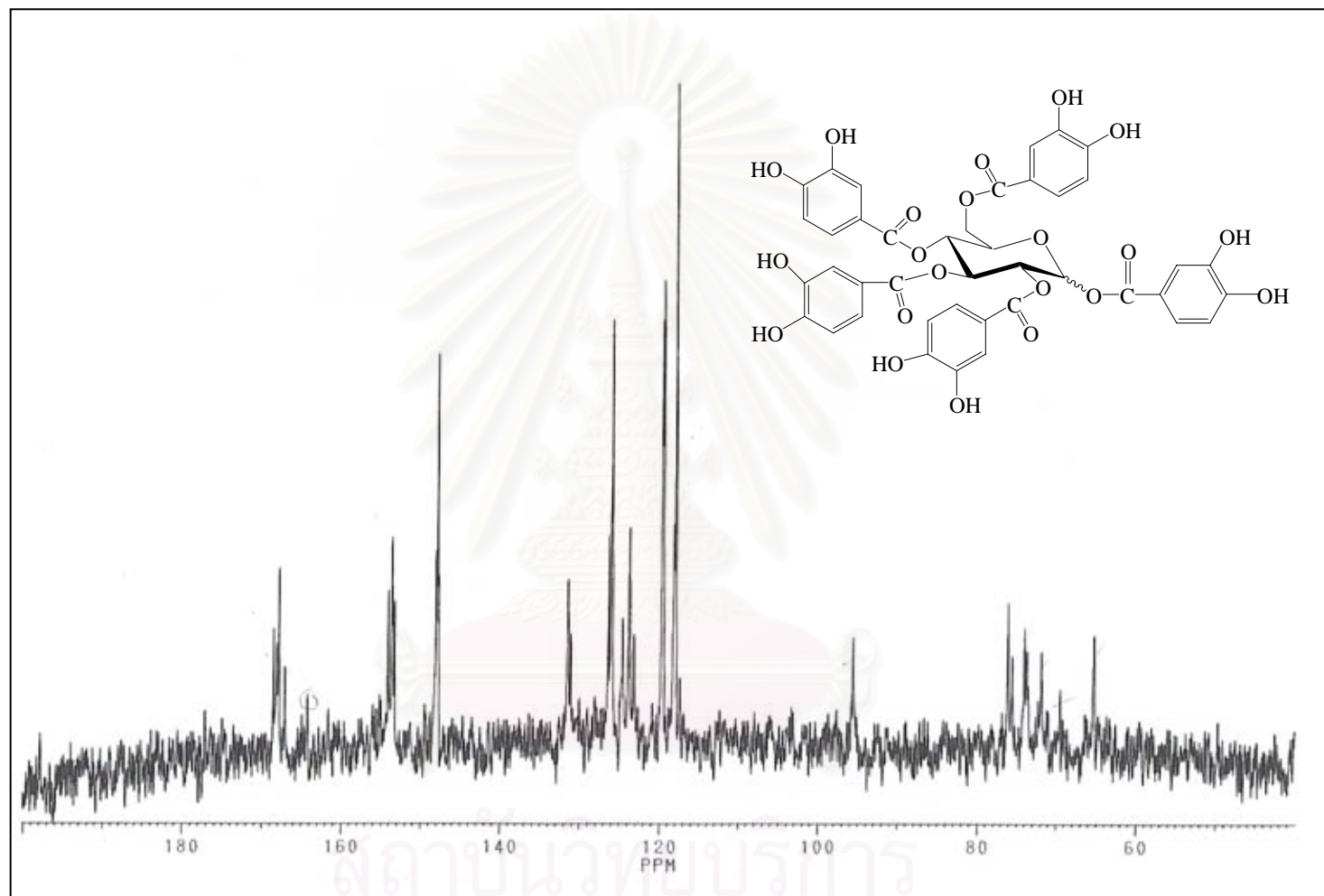
Figure A.41 The ^{13}C-NMR spectrum of compound **2J**

VITA

Miss Jittinan Maungjeen was born on June 22, 1979 in Bangkok, Thailand. She graduated with Bachelor Degree of Science in Chemistry from Srinakharinwirot University in 1997. In 2001, she has been a graduate student studying in Organic Chemistry at Chulalongkorn University. During her study towards the Master Degree, she was awarded as a teaching assistantship by the Faculty of Science, Chulalongkorn University and was also supported a research grant for her Master degree's thesis by Graduate School of Chulalongkorn University.