Synthesis and anticancer activity of phenylpropanoid sucrose esters

Parthasarathi Panda

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**NANYANG
TECHNOLOGICAL
UNIVERSITY**

**SYNTHESIS AND ANTICANCER ACTIVITY OF
PHENYLPROPANOID SUCROSE ESTERS**

PARTHASARATHI PANDA

SCHOOL OF CHEMICAL AND BIOMEDICAL ENGINEERING

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ABSTRACT

The research work presented in this thesis focuses on the synthesis, characterization and antiproliferative activities of natural and unnatural phenylpropanoid sucrose esters. To date, approximately, 150 natural PSEs have been isolated from various plant species of the families Arecaceae, Brassicaceae, Liliaceae, Polygonaceae, Polygalaceae and Rosaceae. The extracts of these plants have been used in traditional or folk medicines for the treatment of many diseases and disorders such as cancer, tumor, inflammation, viral, lung, cardiovascular, central nervous system disorders, ulcers, purgatives, syphilis, gonorrhoea, gout, rheumatic arthritis, boils, nephritis, diarrhoea, carbuncles and hair loss, etc. With the exception of niruriside, there are no existing synthetic routes to describe the laboratory synthesis of this class of compounds due to their structural complexity and the associated difficulties normally observed in sucrochemistry.

In the current work, we have successfully demonstrated the first total synthesis of four natural phenylpropanoid sucrose esters namely helonioside A, lapathoside C, lapathoside D and 3',4',6'-tri-*O*-feruloylsucrose, together with 40 unnatural phenylpropanoid sucrose esters starting from sucrose as cheap and sustainable material. The newly synthesized compounds were thoroughly characterized by ^1H NMR, ^{13}C NMR, DEPT, COSY, HMBC, HMQC experiments, ESI-MS, HR-ESI-MS, IR spectroscopy and elemental analysis. Regio- and chemoselective esterification of 2,1':4,6-di-*O*-isopropylidene sucrose with cinnamoyl chloride, *p*-acetoxycinnamoyl chloride and *p*-acetoxyferuloyl chloride afforded mono-, di-, tri- and tetra- variants in moderate yields. The hydroxyl groups reactivities in 2,1':4,6-di-*O*-isopropylidene sucrose were found to be in the order of 6'-OH > 3'-OH > 4'-OH > 3-OH. We also found that the reaction temperature, time and the nature of acylating agent have a slight effect on the selectivity but dramatically affect the product purity since increased intractable products are obtained as the reaction temperature and/or time is increased.

The selected synthesized PSEs were tested for *in vitro* cytotoxicity against human cervical epitheliod carcinoma cells (HeLa) and human umbilical vein endothelial cells (HUVEC) using MTS assay method. The preliminary MTS screening results indicated that nearly 22 out of the 31 screened synthetic PSEs showed significant antiproliferative activity against HeLa cells at 48 h drug exposure with their IC_{50} values ranging from 0.05 to 7.63 μM in comparison with camptothecin ($\text{IC}_{50} = 0.40 \mu\text{M}$) as a positive control. The structure-activity-relationship correlation studies revealed that the type, number and position of the

phenylpropanoid units on the sucrose core influence the antiproliferative activity against HeLa cells. Di-*O*-isopropylidene group, acetyl groups directly attached to the sucrose core and the number of phenylpropanoid units on the sucrose moiety play an important role in enhancing the antiproliferative activity. At the time of MTS course study, few compounds were selected for the evaluation of cytotoxicity at two different time points of drug exposure and it was found that these compounds exhibited time-dependent antiproliferative activities. The preliminary MTS study on normal human cell lines of few selected PSEs indicated that these PSEs have less cytotoxic effects on HUVAC cells than HeLa cells compared with known anticancer drug camptothecin. The preliminary MTS screening suggests that the PSEs may serve as a potentially valuable source of new potent anticancer drug candidates.

LIST OF ABBREVIATIONS

Å	Angstrom (s)
Ac	Acetyl
Ac ₂ O	Acetic anhydride
AcOH	Acetic acid
aq	Aqueous
app	Apparent
Anal.	Combustion elemental analysis
Bn	Benzyl
Bz	Benzoyl
BzCl	Benzoyl chloride
BzOH	Hydroxybenzoyl
br	Broad
°C	Degrees Celsius
calcd	Calculated
CAN	Ceric Ammonium Nitrate
Caff	Caffeloyl
CDCl ₃	Deuterated chloroform
Cinn	Cinnamoyl
CinnCl	Cinnamoyl chloride
CinnOH	Cinnamic acid
Cinn ₂ O	Cinnamic anhydride
Coum	Coumaroyl
CoumAc	4-Acetylcoumaroyl
¹³ C NMR	Carbon Nuclear Magnetic Resonance
cm ⁻¹	Wavenumber (s)
COSY	¹ H- ¹ H Correlation Spectroscopy
CPT	Camptothecin
δ	Chemical shift in parts per million downfield from tetramethylsilane
d	Day(s); doublet (spectral)
DABCO	1,4-Diazabicyclo[2.2.2]octane

DEPT	Distortionless Enhancement by Polarization Transfer
DIAD	Diisopropyl azodicarboxylate
Dihydroferu	Dihydroferuloyl
DMAP	<i>N,N</i> -Dimethylaminopyridine
DMF	<i>N,N</i> -Dimethylformamide
DMSO	Dimethyl sulfoxide
DMSO- <i>d</i> ₆	Deuterated dimethyl sulfoxide
DPPH	1,1-Diphenyl-2-picrylhydrazyl
dd	Doublet of doublet
Et	Ethyl
EtOH	Ethanol
EtOAc	Ethyl acetate
ESI-MS	Electrospray Ionization Mass Spectroscopy
equiv	Equivalent
EGM	Endothelial Growth Medium
Feru	Feruloyl
FeruAc	4-Acetylferuloyl
g	Gram (s)
Glc-feru	4- <i>O</i> - β -glucopyranosylferuloyl
h	Hours
Hz	Hertz
¹ H NMR	Proton Nuclear Magnetic Resonance
HMBC	Heteronuclear (¹ H- ¹³ C) Multiple Bond Correlation
HMQC	Heteronuclear (¹ H- ¹³ C) Multiple Quantum Correlation
HR-ESI-MS	High-resolution Electrospray Ionization Mass Spectroscopy
HeLa	Human Cervical Epitheliod Carcinoma cells
HUVAC	Human Umbilical Vein Endothelial cells
IC ₅₀	Concentration of samples that induces 50% growth inhibition compared with untreated control

<i>J</i>	Coupling constant (in NMR spectrometry)
min	Minutes
mol	Mole
Me	Methyl
MeOH	Methanol
MeOD	Deuterated methanol
MEM	Minimum Essential Medium
m	Multiplet
M	Molar
<i>m/z</i>	Mass/Charge
MTS	3-(4,5-Dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium
nm	Nanometer
PSE	Phenylpropanoid Sucrose Ester (s)
ppm	Parts per million
py	Pyridine
PTLC	Preparative Thin Layer Chromatography
<i>R_f</i>	Retention factor
rt	Room temperature
Sinap	Sinapoyl
SC ₅₀	Concentration of samples required to scavenge 50% of DPPH free radicals
<i>p</i> -TsOH	<i>p</i> -Toluenesulfonic acid
TBDMSCl	<i>tert</i> -Butyl-dimethylsilyl chloride
TBDPHCl	<i>tert</i> -Butyl-diphenylsilyl chloride
TLC	Thin Layer Chromatography
TMC	3,4,5-Trimethoxycinnamoyl
THF	Tetrahydrofuran
t	Triplet
UV	Ultraviolet
μg	Microgram
μM	Micromolar

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Chapter One: Introduction

1. Phenylpropanoid sucrose esters

Natural products provide the most prolific source of lead compounds for drug discovery and development due to their structural diversities and broad array of biological activities. Medicinal herbs which have been used in traditional Chinese medicine, ayurvedic medicine (India), jamu medicine (Indonesia), phytotherapy and homeopathy (Europe) etc. constitute a key source of lead compounds with potential therapeutic uses. Successful approaches by which lead compounds from natural sources have been developed into drugs have been described in a number of reviews.¹⁻⁸ Drugs such as amphotericin B, rapamycin, taxol, etoposide, vinblastin and colchicine have been obtained from natural sources or through structural modification of natural products.

Plant species of the families Arecaceae, Brassicaceae, Liliaceae, Polygonaceae, Polygalaceae, Rosaceae and Smilacaceae whose extracts have traditionally been used for the treatment of different diseases and disorders like cancer, inflammation, viral diseases, hair loss etc⁹⁻¹² gave various phenylpropanoid sucrose esters (PSEs) which are thought to be the main bioactive component. Surprisingly, despite the wide availability of PSEs in many plant species, their rich chemistry, biological activities and potential as lead compounds have not been well explored. This introduction aims to provide an up-to-date account of the known naturally occurring PSEs focusing on their structures, biological and pharmacological activities. Specifically, we will focus on PSEs having sucrose as the core structure where at least one OH group is substituted by a phenylpropanoid unit. The phenyl group of the phenylpropanoid may be substituted or unsubstituted (Figure 1.1). In the literature, the core and substituents of this large class of compounds are presented in variable atom numbering. The atom numbering shown in Figure 1.1 will be used throughout this thesis for consistency and easy reference.

Figure 1.1. The core structure of PSEs with atom numbering

Phenylpropanoid sucrose esters (PSEs) belong to the phenylpropanoid glycoside (glycoconjugates) class of compounds. As the name indicates, phenylpropanoid sucrose esters have a sucrose core connected to one or more Ph-CH=CH-CO- moieties through hydroxyl group of sucrose. The ester-forming moieties include substituted/unsubstituted cinnamic, coumaric, ferulic, caffeic and sinapic acids etc. The Vinylic double bond in majority of the PSEs is mostly present in the *trans* configuration. During the past 3-4 decades, nearly 150 PSEs have been isolated from various medicinal plant species of the families Arecaceae, Brassicaceae, Liliaceae, Polygonaceae, Polygalaceae, Rosaceae and Smilacaceae.⁹⁻¹² They have been identified by their spectroscopic data and chemical conversion methodologies.

1.1. Classification of phenylpropanoid sucrose esters

As of today, no acceptable classification exists for this diverse group of compounds. Here, various PSEs are categorized based on the number and position of the phenylpropanoid substituents (Tables 1.1-1.9 and Figures 1.2 & 1.3), as follows:

1.1.1. Mono-substituted phenylpropanoid sucrose esters

- i. 3'-*O*-Mono-*O*-substituted phenylpropanoid sucrose esters (Table 1.1)
- ii. 4'-*O*-Mono-*O*-substituted phenylpropanoid sucrose esters (Figure 1.2)
- iii. 6'-*O*-Mono-*O*-substituted phenylpropanoid sucrose esters (Table 1.2)
- iv. 6'-*O*-Mono-*O*-substituted phenylpropanoid sucrose esters (Table 1.3)

1.1.2. Di-substituted phenylpropanoid sucrose esters

- i. 3',6'-Di-*O*-substituted phenylpropanoid sucrose esters (Table 1.4)
- ii. 3',6'-Di-*O*-substituted phenylpropanoid sucrose esters (Table 1.5)
- iii. Di-*O*-substituted phenylpropanoid sucrose esters other than compound substituted at 3',6 and 3',6' positions (Table 1.6)

1.1.3. Tri-substituted phenylpropanoid sucrose esters

- i. 1',3',6'-Tri-*O*-substituted phenylpropanoid sucrose esters (Table 1.7)
- ii. Tri-*O*-substituted phenylpropanoid sucrose esters other than compound substituted at 1',3',6' positions (Table 1.8)

1.1.4. 1',3',6, 6'-Tetra-substituted phenylpropanoid sucrose esters (Table 1.9)

1.1.5. Phenylpropanoid sucrose esters with complex substituent (Figure 1.3)

The plant sources, phenylpropanoid units and pharmacological activities of the reported PSEs along with the references are given in Tables 1.1-1.9 and section 1.1.5.

1.1.1. Mono-substituted phenylpropanoid sucrose esters

Mono-substituted PSEs at 3', 6 and 6' of sucrose are summarized in Tables 1.1, 1.2 and 1.3, respectively, while 4-mono-substituted PSE is shown in Figure 1.2. PSEs substituted at 3' of sucrose constitute the largest group among these mono-substituted PSEs. The sucrose core is mainly esterified with coumaric, ferulic, caffeic, sinapic and trimethoxycinnamic acids. Other non-phenylpropanoid substituents include acetyl, benzoyl and *p*-hydroxybenzoyl groups. These mono-substituted PSEs have been isolated from the plant roots, seeds, rhizomes, stone-fruits, aerial parts, bark, wood, callus cultures and also from the whole plant of the families Aristolochiaceae, Asclepiadaceae, Bignoniaceae, Brassicaceae, Caryophyllaceae, Chenopodiaceae, Compositae, Globulariaceae, Liliaceae, Lamiaceae, Polygalaceae, Plantaginaceae, Rosaceae and Sparganiaceae. Unless otherwise indicated, the phenylpropanoid double bond stereochemistry is *trans*.

Table 1.1. C-3' Substituted phenylpropanoid sucrose esters

Compound		β -D-Fructose unit			α -D-Glucose unit				Source	Biological Activity	Ref.
		R ^{1'}	R ^{3'}	R ^{6'}	R ²	R ³	R ⁴	R ⁶			
No.	Name										
1	2,3,4,6- <i>O</i> -Tetra- <i>O</i> -acetyl-3'- <i>O</i> -coumaroylsucrose	H	Coum	H	Ac	Ac	Ac	Ac	<i>Prunus padus</i>	-	12
2	1',2,3,6- <i>O</i> -Tetra- <i>O</i> -acetyl-3'- <i>O</i> -coumaroylsucrose	Ac	Coum	H	Ac	Ac	H	Ac	<i>Prunus padus</i>	-	12
3	1',3,4,6- <i>O</i> -Tetra- <i>O</i> -acetyl-3'- <i>O</i> -coumaroylsucrose	Ac	Coum	H	H	Ac	Ac	Ac	<i>Prunus padus</i>	-	12
4	1',3,6- <i>O</i> -Tri- <i>O</i> -acetyl-3'- <i>O</i> -coumaroylsucrose	Ac	Coum	H	H	Ac	H	Ac	<i>Prunus padus</i>	-	12
5	1',6',2,4,6- <i>O</i> -Penta- <i>O</i> -acetyl-3'- <i>O</i> -coumaroylsucrose	Ac	Coum	Ac	Ac	H	Ac	Ac	<i>Prunus maximowiczii</i>	-	13
6	1',6',2,3,6- <i>O</i> -Penta- <i>O</i> -acetyl-3'- <i>O</i> -coumaroylsucrose	Ac	Coum	Ac	Ac	Ac	H	Ac	<i>Prunus maximowiczii</i>	-	13
7	1',6',2,6- <i>O</i> -Tetra- <i>O</i> -acetyl-3'- <i>O</i> -coumaroylsucrose	Ac	Coum	Ac	Ac	H	H	Ac	<i>Prunus maximowiczii</i>	-	13
8	6',2,4,6- <i>O</i> -Tetra- <i>O</i> -acetyl-3'- <i>O</i> -coumaroylsucrose	H	Coum	Ac	Ac	H	Ac	Ac	<i>Prunus maximowiczii</i>	-	13
9	1',2,6- <i>O</i> -Tri- <i>O</i> -acetyl-3'- <i>O</i> -coumaroylsucrose	Ac	Coum	H	Ac	H	H	Ac	<i>Prunus maximowiczii</i>	-	13
10	1',6',2- <i>O</i> -Tri- <i>O</i> -acetyl-3'- <i>O</i> -coumaroylsucrose	Ac	Coum	Ac	Ac	H	H	H	<i>Prunus maximowiczii</i>	-	13
11	6',2,6- <i>O</i> -Tri- <i>O</i> -acetyl-3'- <i>O</i> -coumaroylsucrose	H	Coum	Ac	Ac	H	H	Ac	<i>Prunus maximowiczii</i>	-	13
12	1',6',2,4,6- <i>O</i> -Penta- <i>O</i> -acetyl-3'- <i>O</i> - <i>cis</i> -coumaroylsucrose	Ac	<i>Cis</i> -Coum	Ac	Ac	H	Ac	Ac	<i>Prunus maximowiczii</i>	-	13
13	1',6',2,6- <i>O</i> -Tetra- <i>O</i> -acetyl-3'- <i>O</i> - <i>cis</i> -coumaroylsucrose	Ac	<i>Cis</i> -Coum	Ac	Ac	H	H	Ac	<i>Prunus maximowiczii</i>	-	13

(Table 1.1). Contd.....

Compound		β -D-Fructose unit			α -D-Glucose unit				Source	Biological Activity	Ref.
		R ^{1'}	R ^{3'}	R ^{6'}	R ²	R ³	R ⁴	R ⁶			
No.	Name										
14	3'-O-Feruloysucrose or Sibiricose A ₅	H	Feru	H	H	H	H	H	<i>Trillium kamtschaticum</i> <i>Lindelofia stylosa</i> <i>Polygala sibirica</i> <i>Polygala tenuifolia</i> <i>Polygala arillata</i>	Free radical-scavenging and antidepressant	14-19
15	6-O-Acetyl-3'-O-feruloysucrose or Regaloside A	H	Feru	H	H	H	H	Ac	<i>Trillium kamtschaticum</i>	Free radical-scavenging	16
16	1'-O-Acetyl-3'-O-feruloysucrose	Ac	Feru	H	H	H	H	H	<i>Polygala chamaebuxus</i>	-	20
17	1',2,4,6-O-Tetra-O-acetyl-3'-O-feruloysucrose	Ac	Feru	H	Ac	H	Ac	Ac	<i>Sparganium stoloniferum</i>	-	21
18	1',2,3,6-O-Tetra-O-acetyl-3'-O-feruloysucrose	Ac	Feru	H	Ac	Ac	H	Ac	<i>Sparganium stoloniferum</i>	-	21
19	1',2,3,6-Tetra-O-acetyl-3'-cis-feruloysucrose	Ac	Cis-Feru	H	Ac	Ac	H	Ac	<i>Sparganium stoloniferum</i>	Weak antitumor	22
20	2,3,4,6-O-Tetra-O-acetyl-3'-O-caffeoylsucrose	H	Caff	H	Ac	Ac	Ac	Ac	<i>Prunus ssiori</i>	-	23
21	3'-O-Sinapoylsucrose or Sibiricose A ₆	H	Sinap	H	H	H	H	H	<i>Polygala sibirica</i> <i>Polygala tricornis</i> <i>Polygala tenuifolia</i> <i>Polygala arillata</i>	-	17-19, 24
22	Glomeratose A	H	TMC	H	H	H	H	H	<i>Polygala sibirica</i> <i>Polygala glomerata</i> <i>Polygala tricornis</i>	-	17, 24, 25

(Table 1.1). Contd.....

Compound		β -D-Fructose unit			α -D-Glucose unit				Source	Biological Activity	Ref.
		R ^{1'}	R ^{3'}	R ^{6'}	R ²	R ³	R ⁴	R ⁶			
No.	Name										
23	Reiniose C or 6- <i>O</i> -Benzoyl-3'- <i>O</i> -feruloylsucrose	H	Feru	H	H	H	H	Bz	<i>Polgala reinii</i>	-	26
24	Reiniose B or 4- <i>O</i> -Benzoyl-3'- <i>O</i> -feruloylsucrose	H	Feru	H	H	H	Bz	H	<i>Polgala reinii</i>	-	26
25	Tenuifoliside A	H	TMC	H	H	H	<i>p</i> -BzOH	H	<i>Polygala tenuifolia</i> <i>Polygala sibirica</i> <i>Polygala hongkongensis</i>	Antidepressant	17, 18, 27-29
26	Tenuifoliside B	H	Sinap	H	H	H	<i>p</i> -BzOH	H	<i>Polygala tenuifolia</i>	-	27
27	6- <i>O</i> -Benzoyl-3'- <i>O</i> -sinapoylsucrose	H	Sinap	H	H	H	H	Bz	<i>Polygala sibirica</i> <i>Polygala tricornis</i>	-	17, 24
28	Tricornose A	H	TMC	H	H	H	H	Ac	<i>Polygala tricornis</i>	-	24
29	Tricornose B	H	TMC	H	H	H	Ac	Bz	<i>Polygala tricornis</i>	-	24
30	6- <i>O</i> -Benzoyl-3'- <i>O</i> -3,4,5-trimethoxycinnamoyl sucrose	H	TMC	H	H	H	H	Bz	<i>Polygala tricornis</i> <i>Polygala reinii</i> <i>Polygala glomerata</i> <i>Polygala wattersii</i>	-	24-26, 30
31	4- <i>O</i> -Benzoyl-3'- <i>O</i> -3,4,5-trimethoxycinnamoyl sucrose	H	TMC	H	H	H	Bz	H	<i>Polygala tricornis</i> <i>Polygala reinii</i>	-	24, 26

Figure 1.2. 4-*O*-Mono-*O*-substituted phenylpropanoid sucrose ester

Reiniose D **32** was isolated from the the plant *Polygala reinii* (Polygalaceae). So far, it is the only 4 mono-substituted PSE isolated and characterized.

Table 1.2. C-6 Substituted phenylpropanoid sucrose esters

Compound		R ⁶	Source	Biological Activity	Ref.
No.	Name				
33	6- <i>O</i> -Cinnamoylsucrose or Sibirioside A	Cinn	<i>Scrophularia ningpoensis</i>	-	31
34	6- <i>O</i> -Coumaroylsucrose or Acretoside	Coum	<i>Aristolochia cretica</i> <i>Kigelia pinnata</i>	-	32-34
35	6- <i>O</i> -Feruloylsucrose or Arillatoses B	Feru	<i>Kigelia pinnata</i> <i>Globularia orientalis</i> <i>Polygala arillata</i> <i>Lilium speciosum</i> forma <i>vestale</i> <i>Veronica pulvinaris</i> <i>Scrophularia ningpoensis</i> <i>Beta vulgaris</i>	Antioxidant	31, 19, 34-38
36	6- <i>O</i> -Caffeoylsucrose or Arillatose B	Caff	<i>Scrophularia ningpoensis</i> <i>Aristolochia cretica</i> <i>Kigelia pinnata</i> <i>Globularia orientalis</i> <i>Salvia officinalis</i>	Antioxidant	31-35, 39
37	Segetoside A or 6-dihydroferuloylsucrose	Dihydroferu	<i>Vaccaria segetalis</i>	-	40
38	Sibiricose A ₁	Sinap	<i>Polygala sibirica</i> <i>Cynanchum amplexicaule</i> <i>Iberis amara</i> <i>Cynanchum hancockianum</i> <i>Polygala arillata</i>	Antioxidant	17-19, 41- 43
39	Sibiricose A ₂	TMC	<i>Polygala sibirica</i>	-	17

Table 1.3. C-6' Substituted phenylpropanoid sucrose esters

Compound		β -D-Fructose unit	α -D-Glucose unit			Phenylpropanoid unit	Source	Biological Activity	Ref.
		R ^{1'}	R ²	R ⁴	R ⁶	R ^{3''}			
No.	Name								
40	6'-O-Coumaroylsucrose	H	H	H	H	H	<i>Bidens parviflora</i> <i>Canna edulis</i>	Anti-inflammatory	44, 45
41	6-O-Acetyl-6'-O-feruloylsucrose	H	H	H	Ac	OCH ₃	<i>Smilax bracteata</i>	-	46
42	1',2,4,6-Tetra-O-acetyl-6'-O-feruloylsucrose	Ac	Ac	Ac	Ac	OCH ₃	<i>Sparganium stoloniferum</i>	-	47

1.1.2. Di-substituted phenylpropanoid sucrose esters

Di-substituted PSEs are the largest group among the PSEs with substituents such as cinnamic, coumaric, ferulic, caffeic, sinapic and trimethoxycinnamic acids etc. Non-phenylpropanoid substituents include acetyl and benzyl groups. Majority of the disubstituted PSEs are substituted at either 3',6 (Table 1.4) or at 3',6' positions of sucrose (Table 1.5). Di-substituted PSEs other than compounds substituted at positions 3',6 (Table 1.4) and 3',6' (Table 1.5) are listed in Table 1.6. Di-substituted PSEs have been isolated from the plant roots, seeds, rhizomes, aerial parts, wood and also from the whole plant of the families Boraganiaceae, Brassicaceae, Cannaceae, Celastraceae, Euphorbiaceae, Liliaceae, Polygonaceae, Polygalaceae, Sparganiaceae and Smilacaceae.

Table 1.4. 3',6-Disubstituted phenylpropanoid sucrose esters

Compound		β -D-Fructose unit			α -D-Glucose unit			Source	Biological Activity	Ref.
		R ^{3'}	R ^{4'}	R ^{6'}	R ³	R ⁴	R ⁶			
No.	Name									
43	3',6-Di- <i>O</i> -coumaroylsucrose	Coum	H	H	H	H	Coum	<i>Lilium mackliniae</i>	-	48
44	3',6-Di- <i>O</i> -feruloylsucrose	Feru	H	H	H	H	Feru	<i>Lilium speciosum</i> var. <i>rubrum</i> <i>Lindelofia stylosa</i> <i>Lilium longiflorum</i> <i>Lilium henryi</i> <i>Lilium mackliniae</i> <i>Lilium speciosum</i> forma <i>vestale</i>	-	14, 15, 36, 48-51
45	4'- <i>O</i> -Acetyl-3',6-di- <i>O</i> -feruloylsucrose	Feru	Ac	H	H	H	Feru	<i>Lilium longiflorum</i> <i>Lilium henryi</i> <i>Lilium speciosum</i> var. <i>rubrum</i> <i>Lilium mackliniae</i>	-	48-51
46	4- <i>O</i> -Acetyl-3',6-di- <i>O</i> -feruloylsucrose	Feru	H	H	H	Ac	Feru	<i>Lilium speciosum</i> var. <i>rubrum</i> <i>Lilium henryi</i>	-	49, 51
47	6'- <i>O</i> -Acetyl-3',6-di- <i>O</i> -feruloylsucrose	Feru	H	Ac	H	H	Feru	<i>Lilium speciosum</i> var. <i>rubrum</i>	-	49
48	4',6'-Di- <i>O</i> -acetyl-3',6-di- <i>O</i> -feruloylsucrose	Feru	Ac	Ac	H	H	Feru	<i>Lilium speciosum</i> var. <i>rubrum</i>	-	49
49	3,6'-Di- <i>O</i> -acetyl-3',6-di- <i>O</i> -feruloylsucrose	Feru	H	Ac	Ac	H	Feru	<i>Lilium speciosum</i> var. <i>rubrum</i>	-	49
50	3,4',6'-Tri- <i>O</i> -acetyl-3',6-di- <i>O</i> -feruloylsucrose	Feru	Ac	Ac	Ac	H	Feru	<i>Lilium speciosum</i> var. <i>rubrum</i>	-	49
51	4,4'-Di- <i>O</i> -acetyl-3',6-di- <i>O</i> -feruloylsucrose	Feru	Ac	H	H	Ac	Feru	<i>Lilium mackliniae</i>	-	48

(Table 1.4). Contd.....

Compound		β -D-Fructose unit			α -D-Glucose unit			Source	Biological Activity	Ref.
		R ^{3'}	R ^{4'}	R ^{6'}	R ³	R ⁴	R ⁶			
No.	Name									
52	3'-O-Feruloyl-6-O-(4-O- β -glucopyranosyl)feruloylsucrose	Feru	H	H	H	H	Glc-feru	<i>Lilium mackliniae</i> <i>Lilium henryi</i>	-	48, 51
53	4'-Acetyl-3'-feruloyl-6-(4-O- β -glucopyranosyl)feruloylsucrose	Feru	Ac	H	H	H	Glc-feru	<i>Lilium longiflorum</i> <i>Lilium mackliniae</i>	-	48, 50
54	6-O-Coumaroyl-3'-O-feruloylsucrose	Feru	H	H	H	H	Coum	<i>Lindelofia stylosa</i>	-	14, 15
55	6-O-Caffeoyl-3'-O-feruloylsucrose	Feru	H	H	H	H	Caff	<i>Lindelofia stylosa</i>	-	14, 15
56	3'-O-Feruloyl-6-O-sinapoylsucrose	Feru	H	H	H	H	Sinap	<i>Polygala reinii</i> <i>Polygala wattersii</i>	-	26, 30
57	3'-O-Sinapoyl-6-O-feruloylsucrose	Sinap	H	H	H	H	Feru	<i>Ruta graveolens</i> <i>Polygala wattersii</i>	-	30, 52
58	Reiniose A	TMC	H	H	H	H	Feru	<i>Polygala reinii</i> <i>Polygala wattersii</i>	-	26, 30
59	3',6-Di-O-sinapoylsucrose	Sinap	H	H	H	H	Sinap	<i>Polygala reinii</i> <i>Polygala virgata</i> <i>Polygala tenuifolia</i> <i>Raphanus sativus</i> <i>Polygala sibirica</i> <i>Ruta graveolens</i> <i>Polygala glomerata</i> <i>Polygala wattersii</i> <i>Polygala tricornis</i> <i>Polygala hongkongensis</i> <i>Securidaca longipedunculata</i>	Antidepressant	11, 17, 18, 24- 30, 52- 54

(Table 1.4). Contd.....

Compound		β -D-Fructose unit			α -D-Glucose unit			Source	Biological Activity	Ref.
		R ^{3'}	R ^{4'}	R ^{6'}	R ³	R ⁴	R ⁶			
No.	Name									
60	3-Acetyl-3',6-di- <i>O</i> -sinapoylsucrose	Sinap	H	H	Ac	H	Sinap	<i>Polygala virgata</i>	-	11
61	4-Acetyl-3',6-di- <i>O</i> -sinapoylsucrose	Sinap	H	H	H	Ac	Sinap	<i>Polygala virgata</i>	-	11
62	Tenuifoliside C	TMC	H	H	H	H	Sinap	<i>Polygala tenuifolia</i> <i>Polygala japonica</i> <i>Polygala tricornis</i> <i>Polygala reinii</i> <i>Polygala glomerata</i>	-	24-27, 55
63	Glomeratose B	Sinap	H	H	H	H	Coum	<i>Polygala glomerata</i>	-	25
64	Glomeratose C	TMC	H	H	H	H	Coum	<i>Polygala glomerata</i>	-	25
65	Glomeratose D	TMC	H	H	H	H	TMC	<i>Polygala glomerata</i> <i>Polygala hongkongensis</i>	-	25, 29

Table 1.5. 3',6'-Disubstituted phenylpropanoid sucrose esters

Compound		β -D-Fructose unit			α -D-Glucose unit				Source	Biological Activity	Ref.
No.	Name	R ^{1'}	R ^{3'}	R ^{6'}	R ²	R ³	R ⁴	R ⁶			
66	Niruriside	Ac	Cinn	Cinn	Ac	H	Ac	Ac	<i>Phyllanthus niruri</i>	Anti-HIV	56
67	Lapathoside D	H	Coum	Coum	H	H	H	H	<i>Polygonum lapathifolium</i> <i>Polygonum sachalinensis</i> <i>Polygonum perfoliatum</i>	Antioxidant, antitumour and α -glucosidase inhibitory	57-59
68	6-O-Acetyl-3',6'-di-O-coumaroylsucrose	H	Coum	Coum	H	H	H	Ac	<i>Canna edulis</i>	-	45
69	Helonioside A	H	Feru	Feru	H	H	H	H	<i>Heloniopsis orientalis</i> <i>Polygonum perfoliatum</i> <i>Bistorta manshuriensis</i> <i>Trillium kamtschaticum</i> <i>Smilax glabra</i> <i>Smilax china</i> <i>Smilax bracteata</i> <i>Paris polyphylla</i> var. <i>yunnanensis</i> <i>Rumex dentatus</i>	Antioxidant and cytotoxic on LA 795	16, 58, 60-67
70	Helonioside B	H	Feru	Feru	H	H	H	Ac	<i>Heloniopsis orientalis</i> <i>Bistorta manshuriensis</i> <i>Smilax bracteata</i> <i>Polygonum perfoliatum</i> <i>Smilax china</i> <i>Heterosmilax erythrantha</i>	Antioxidant	58, 61, 62, 66, 68-70
71	Smiglaside C	H	Feru	Feru	Ac	H	Ac	Ac	<i>Smilax glabra</i> <i>Polygonum perfoliatum</i>	-	60, 68

(Table 1.5). Contd.....

Compound		β -D-Fructose unit			α -D-Glucose unit				Source	Biological Activity	Ref.
		R ^{1'}	R ^{3'}	R ^{6'}	R ²	R ³	R ⁴	R ⁶			
No.	Name										
72	1',2,6-Tri- <i>O</i> -acetyl-3',6'-di- <i>O</i> -feruloylsucrose	Ac	Feru	Feru	Ac	H	H	Ac	<i>Sparganium stoloniferum</i> <i>Polygonum perfoliatum</i>	-	47, 68
73	2,6-Di- <i>O</i> -acetyl-3',6'-di- <i>O</i> -feruloylsucrose	H	Feru	Feru	Ac	H	H	Ac	<i>Polygonum perfoliatum</i> <i>Smilax china</i> <i>Heterosmilax erythrantha</i>	Antioxidant	68-70
74	3,6-Di- <i>O</i> -acetyl-3',6'-diferuloylsucrose	H	Feru	Feru	H	Ac	H	Ac	<i>Smilax glabra</i>	-	60
75	Smilaside A	H	Feru	Feru	H	H	Ac	Ac	<i>Smilax china</i>	-	69
76	Smilaside B	H	Feru	Feru	Ac	H	H	H	<i>Smilax china</i>	-	69
77	1',3,4,6-Tetra- <i>O</i> -acetyl-3',6'-diferuloylsucrose	Ac	Feru	Feru	H	Ac	Ac	Ac	<i>Sparganium stoloniferum</i>	-	71
78	1',2,4,6-Tetra- <i>O</i> -acetyl-3',6'-diferuloylsucrose	Ac	Feru	Feru	Ac	H	Ac	Ac	<i>Sparganium stoloniferum</i> <i>Polygonum perfoliatum</i>	-	21, 68, 71
79	1',2,3,6-Tetra- <i>O</i> -acetyl-3',6'-diferuloylsucrose	Ac	Feru	Feru	Ac	Ac	H	Ac	<i>Sparganium stoloniferum</i>	-	21, 71
80	2,3,4,6-Tetra- <i>O</i> -acetyl-3',6'-diferuloylsucrose	H	Feru	Feru	Ac	Ac	Ac	Ac	<i>Bhesa paniculata</i>	-	72
81	Bistoroside A	H	<i>Cis</i> - Feru	<i>Cis</i> - Feru	H	H	H	H	<i>Bistorta manshuriensis</i>	-	61
82	Bistoroside B	H	<i>Cis</i> - Feru	<i>Cis</i> - Feru	H	H	H	Ac	<i>Bistorta manshuriensis</i>	-	61
83	Parispolyside F	H	Coum	Feru	H	H	H	H	<i>Paris polyphylla</i> var. <i>yunnanensis</i>	-	73
84	6- <i>O</i> -Acetyl-3'- <i>O</i> -coumaroyl-6'- <i>O</i> -feruloylsucrose	H	Coum	Feru	H	H	H	Ac	<i>Canna edulis</i>	-	45
85	Tenuifoliside E	Ac	Feru	Sinap	Ac	H	Ac	Ac	<i>Polygala tenuifolia</i>	-	74
86	Helonioside C	H	Feru	Glc-feru	H	H	H	H	<i>Heloniopsis orientalis</i>	-	62
87	Helonioside D	H	Feru	Glc-feru	H	H	H	Ac	<i>Heloniopsis orientalis</i>	-	62

Table 1.6. Di-*O*-substituted phenylpropanoid sucrose esters at positions other than 3',6- and 3',6'

Compound		β -D-Fructose unit			α -D-Glucose unit				Source	Biological Activity	Ref.
		R ^{1'}	R ^{3'}	R ^{6'}	R ²	R ³	R ⁴	R ⁶			
No.	Name										
88	6,6'-Di- <i>O</i> -coumaroylsucrose	H	H	Coum	H	H	H	Coum	<i>Bidens parviflora</i>	Anti-inflammatory	44
89	6,6'-Di- <i>O</i> -sinapoylsucrose	H	H	Sinap	H	H	H	Sinap	<i>Cynanchum amplexicaule</i>	-	41
90	Sibiricose A ₄	H	Sinap	H	H	H	Sinap	H	<i>Polygala sibirica</i>		17
91	Heterosmilaside	H	H	Feru	H	Feru	H	H	<i>Heterosmilax erythrantha</i>	Antioxidant	70
92	3',4-Di- <i>O</i> -coumaroylsucrose	H	Coum	H	H	H	Coum	H	<i>Lilium mackliniae</i>	-	48
93	2-Acetyl-3',4-di- <i>O</i> -coumaroylsucrose	H	Coum	H	Ac	H	Coum	H	<i>Lilium mackliniae</i>	-	48
94	3-Acetyl-3',4-di- <i>O</i> -coumaroylsucrose	H	Coum	H	H	Ac	Coum	H	<i>Lilium mackliniae</i>	-	48

(Table 1.6). Contd.....

Compound		β -D-Fructose unit			α -D-Glucose unit				Source	Biological Activity	Ref.
		R ^{1'}	R ^{3'}	R ^{6'}	R ²	R ³	R ⁴	R ⁶			
No.	Name										
95	3'-O-(3,4,5-Trimethoxycinnamoyl)-4-O-(p-hydroxybenzoyl)sucrose	H	TMC	H	H	H	p- BzOH	H	<i>Polygala reinii</i>	-	26
96	1'-O-Coumaroyl-6'-O-feruloylsucrose	Coum	H	Feru	H	H	H	H	<i>Smilax bracteata</i>	-	46, 66
97	1'-O-Sinpoyl-3'-O-feruloylsucrose	Sinap	Feru	H	H	H	H	H	<i>Polygala chamaebuxus</i>	-	20
98	1',3'-Di-O-sinpoylsucrose	Sinap	Sinap	H	H	H	H	H	<i>Polygala chamaebuxus</i>	-	20

1.1.3. Tri-substituted phenylpropanoid sucrose esters

1',3',6'-Trisubstituted PSEs (Table 1.7) are the largest group among the tri-substituted PSEs. Other tri-substituted PSEs are summarized in Table 1.8. The sucrose moieties of the tri-substituted PSEs are mostly esterified with coumaric or/and ferulic acids. Acetyl groups are the only observed non-phenylpropanoid substituents. These tri-substituted PSEs have been isolated from the plant roots, stems, rhizomes, aerial parts and also from the whole plant of the families Amaranthaceae, Arecaceae, Liliaceae, Polygonaceae, Polygalaceae, Smilacaceae and Rutaceae.

Table 1.7. 1',3',6'-Trisubstituted phenylpropanoid sucrose esters

Compound		<i>α</i> -D-Glucose unit				Phenylpropanoid unit			Source	Biological Activity	Ref.
		R ^{4'}	R ²	R ⁴	R ⁶	R ^{3''}	R ^{3'''}	R ^{3''''}			
No.	Name										
99	Hydropiperoside	H	H	H	H	H	H	H	<i>Polygonum hydropiper</i> <i>Polygonum pensylvanicum</i> <i>Polygonum perfoliatum</i> <i>Polygonum cuspidatum</i> <i>Polygonum sachalinense</i> <i>Polygonum lapathifolium</i>	Antitumor and antifertility	57-59, 75-79

(Table 1.7). Contd.....

Compound		α -D-Glucose unit				Phenylpropanoid unit			Source	Biological Activity	Ref.
		R ^{4'}	R ²	R ⁴	R ⁶	R ^{3''}	R ^{3'''}	R ^{3'''}			
No.	Name										
100	Vanicoside C	H	Ac	H	H	H	H	H	<i>Polygonum pensylvanicum</i> <i>Polygonum perfoliatum</i>	-	58, 75
101	Smilaside C	H	H	H	H	H	OCH ₃	OCH ₃	<i>Smilax china</i> <i>Smilax bracteata</i>	Antitumor	46, 69
102	Smilaside D	Ac	H	H	H	H	OCH ₃	OCH ₃	<i>Smilax china</i>	Antitumor	69
103	Smilaside E	H	H	H	Ac	H	OCH ₃	OCH ₃	<i>Smilax bracteata</i> <i>Smilax china</i>	Antitumor	66, 69
104	Smilaside F	H	Ac	H	Ac	H	OCH ₃	H	<i>Smilax china</i>	Antitumor	69
105	Smilaside G	H	H	H	H	H	OCH ₃	H	<i>Smilax bracteata</i>	Radical scavenging	66
106	Smilaside H	H	Ac	H	H	H	OCH ₃	H	<i>Smilax bracteata</i>	Radical scavenging	66
107	Smilaside I	H	H	H	Ac	H	OCH ₃	H	<i>Smilax bracteata</i>	Radical scavenging	66
108	Smilaside J	H	H	H	H	OCH ₃	OCH ₃	H	<i>Smilax bracteata</i>	Radical scavenging	66
109	Smilaside K	H	Ac	H	H	H	OCH ₃	OCH ₃	<i>Smilax bracteata</i>	-	66
110	Smilaside L	H	H	H	H	OCH ₃	OCH ₃	OCH ₃	<i>Smilax bracteata</i> <i>Bistorta manshuriensis</i>	-	61, 66
111	Smiglaside A	H	Ac	Ac	Ac	OCH ₃	OCH ₃	OCH ₃	<i>Smilax glabra</i>	-	60
112	Smiglaside B	H	Ac	H	Ac	OCH ₃	OCH ₃	OCH ₃	<i>Smilax glabra</i>	-	60
113	Smiglaside D	H	Ac	Ac	Ac	H	OCH ₃	OCH ₃	<i>Smilax glabra</i>	-	60
114	Smiglaside E	H	Ac	H	Ac	H	OCH ₃	OCH ₃	<i>Smilax china</i> <i>Smilax glabra</i>	-	60, 69

Table 1.8. Tri-*O*-substituted phenylpropanoid sucrose esters at positions other than at 1',3',6'

Compound		β -D-Fructose unit				α -D-Glucose unit			Source	Biological Activity	Ref.
		R ^{1'}	R ^{3'}	R ^{4'}	R ^{6'}	R ²	R ⁴	R ⁶			
115	Hydropiperoside A	Coum	H	H	Coum	H	H	Feru	<i>Polygonum hydropiper</i>	-	78
116	Lapathoside C	H	Coum	H	Coum		H	Feru	<i>Polygonum lapathifolium</i> <i>Polygonum cuspidatum</i> <i>Polygonum sachalinense</i>	-	57, 59, 76
117	3',4',6'-Tri- <i>O</i> -feruloylsucrose	H	Feru	Feru	Feru	H	H	H	<i>Smilax riparia</i>	-	80
118	6-Mono- <i>O</i> -coumaroyl-3',4'-di- <i>O</i> -feruloylsucrose	H	Feru	Feru	H	H	H	Coum	<i>Monnina obtusifolia</i>	-	81
119	6-Mono- <i>O</i> -caffeoyl-3',4'-di- <i>O</i> -feruloylsucrose	H	Feru	Feru	H	H	H	Caff	<i>Monnina obtusifolia</i>	-	81

(Table 1.8). Contd.....

Compound		β -D-Fructose unit				α -D-Glucose unit			Source	Biological Activity	Ref.
		R ^{1'}	R ^{3'}	R ^{4'}	R ^{6'}	R ²	R ⁴	R ⁶			
No.	Name										
120	1',4-Di- <i>O</i> -acetyl-2,3',6'-tri- <i>O</i> -coumaroylsucrose	Ac	Coum	H	Coum	Coum	Ac	H	<i>Froelichia floridana</i>	-	82
121	3',4',6-Tri- <i>O</i> -sinapoylsucrose	H	Sinap	Sinap	H	H	H	Sinap	<i>Securidaca longipedunculata</i> <i>Ruta corsica</i>	-	53, 83
122	Quiquesetinerviuside A	H	Feru	H	Feru	H	Feru	H	<i>Calamus quiquesetinervius</i>	Radical scavenging	84
123	Quiquesetinerviuside B	H	Feru	H	Feru	H	Feru	Ac	<i>Calamus quiquesetinervius</i>	Radical scavenging	84
124	Quiquesetinerviuside C	H	Feru	H	Feru	Ac	Feru	H	<i>Calamus quiquesetinervius</i>	Radical scavenging	84
125	Quiquesetinerviuside D	H	Feru	H	Feru	H	Coum	Ac	<i>Calamus quiquesetinervius</i>	Radical scavenging	84
126	Quiquesetinerviuside E	H	Feru	H	Feru	Ac	Coum	H	<i>Calamus quiquesetinervius</i>	Radical scavenging	84

1.1.4. 1',3',6,6'-Tetra-substituted phenylpropanoid sucrose esters

Interestingly, the reported tetra-substituted PSEs are esterified only with coumaric and/or ferulic acid at 1',3',6 and 6' positions (Table 1.9). Acetyl groups are the only observed non-phenylpropanoid substituents. These tetra-substituted PSEs have been isolated from the plant roots, rhizomes, aerial parts, stems, leaves and also from the whole plant of the families Brassicaceae, Liliaceae, Rosaceae, Polygonaceae and Polygalaceae.

Table 1.9. 1',3',6,6'-Tetra-substituted phenylpropanoid sucrose esters

Compound		<i>α</i> -D-Glucose unit			Phenylpropanoid unit			Source	Biological Activity	Ref.
		R ²	R ³	R ⁴	R ^{3''}	R ^{3'''}	R ^{3''''}			
No.	Name									
127	Vanicoside A	Ac	H	H	OCH ₃	H	H	<i>Polygonum hydropiper</i> <i>Polygonum pensylvanicum</i> <i>Polygonum perfoliatum</i> <i>Polygonum cuspidatum</i> <i>Polygonum sachalinense</i>	Protein kinase C and β-glucosidase inhibitory and antitumor antioxidant	68, 75, 76, 78, 79, 85

(Table 1.9). Contd.....

Compound		α -D-Glucose unit			Phenylpropanoid unit			Source	Biological Activity	Ref.
No.	Name	R ²	R ³	R ⁴	R ^{3''}	R ^{3'''}	R ^{3''''}			
128	Vanicoside B	H	H	H	OCH ₃	H	H	<i>Polygonum hydropiper</i> <i>Polygonum pensylvanicum</i> <i>Polygonum perfoliatum</i> <i>Polygonum cuspidatum</i> <i>Polygonum sachalinense</i> <i>Polygonum lapathifolium</i>	Protein kinase C β -glucosidase and AChE inhibitory antitumor	57-59, 68, 75, 76, 78, 79, 85
129	Vanicoside D	H	H	H	H	H	H	<i>Polygonum pensylvanicum</i> <i>Triplaris americana</i>	-	75, 86
130	Vanicoside E	Ac	H	Ac	OCH ₃	H	H	<i>Polygonum pensylvanicum</i> <i>Polygonum hydropiper</i>	Moderate antioxidant	75, 78
131	Vanicoside F	H	Ac	H	OCH ₃	H	H	<i>Polygonum pensylvanicum</i> <i>Polygonum perfoliatum</i>	-	58, 75
132	Lapathoside A	H	H	H	OCH ₃	OCH ₃	H	<i>Polygonum lapathifolium</i> <i>Polygonum cuspidatum</i> <i>Polygonum sachalinense</i> <i>Fagopyrum dibotrys</i>	Antioxidant and antitumor	59, 76, 87
133	Lapathoside B	H	H	H	OCH ₃	OCH ₃	OCH ₃	<i>Polygonum lapathifolium</i>	-	59
134	Hydropiperoside B	Ac	H	H	OCH ₃	OCH ₃	H	<i>Polygonum hydropiper</i>	Antioxidant	78
135	Diboside A	H	H	H	H	H	OCH ₃	<i>Fagopyrum dibotrys</i>	Antioxidant	87

1.1.5. Phenylpropanoid sucrose esters having complex substituents

PESs that fall under this category (Figure 1.3) are limited in number and variety. 6,6'-sucrose ester of (1 α ,2 α ,3 β ,4 β)-3,4-bis(4-hydroxyphenyl)-1,2-cyclodicarboxylic acid **136** was isolated from the whole plant, *Bidens parviflora* (Compositae).⁴⁴ Impecyloside or 6-acetyl-1-1,3-(4,4'-dihydroxy-3,3'-dimethoxy- β -truxinyl)sucrose **137** was isolated from the rhizomes of *Imperata cylindrical* (Gramineae).⁸⁸ Shegansu C or 3-*O*-acetyl-3-*O*-[4-*O*-(3,4-dimethoxycynamoyl)-5-*O*-feruloyl]-caffeoyl]sucrose **138** was isolated from the rhizome of *Belamcanda chinensis* (Iridaceae).⁸⁹ Glomeratose E **139** was isolated from the roots of *Polygala glomerata* (Polygalaceae).²⁵

Figure 1.3. Phenylpropanoid sucrose esters with complex substituents

1.2. Biological activities of PSEs

About 150 PSEs have been isolated from plants having medicinal values and their structures have been characterized. Interestingly, the biological activities, mechanism of action and structure-activity relationships (SAR) of only very few PSEs have been explored to date. Since this project also deals with exploring the anticancer activity of PSEs, an

overview of the pharmacological activities of the PSEs may serve as valuable indication for exploration of their full therapeutic potentials.

1.2.1. Pharmacological activities of the plants (as a whole or parts) and their extracts

Plant species of the Polygalaceae, Polygonaceae and Liliaceae families are the major sources of PSEs. Other families including Aristolochiaceae, Asclepiadaceae, Bignoniaceae, Boragniaceae, Brassicaceae, Caryophyllaceae, Celastraceae, Chenopodiaceae, Compositae, Euphorbiaceae, Globulariaceae, Lamiaceae, Rosaceae, Rutaceae and Sparganiaceae were also reported to contain various PSEs. The pharmacological activities of these plants based on traditional or folk medicines are summarized according to their family for easy reference.

1.2.1.1. Polygalaceous Plants

Polygala genera of the Polygalaceae family are rich sources for mono- and di-substituted PSEs. These plant species have tremendous significance in traditional and folk medicines. The roots of *Polygala* genera of the Polygalaceae family such as *P. tenuifolia*,^{18, 27, 74, 90} *P. arillata*,¹⁹ *P. sibirica*,¹⁷ *P. tricornis*,²⁴ *P. japonica*,⁵⁵ *P. reinii*²⁶ and *P. wattersii*³⁰ are used in traditional medicine as expectorant, tranquilizer, tonic, sedative and for the treatment of amnesia. The roots of *P. tenuifolia* are well-known in China as Yuan Zhi or Radix Polygalae and in Japan as Onji.^{27, 28, 74} “Yuan Zhi” is an important herb with wide-array of pharmacological activities. It has been widely used in traditional Chinese medicine for the treatment of insomnia, neurasthenia, amnesia, palpitations with anxiety, restlessness, disorientation and also to cure dementia and memory failure.^{28, 91} The root of *P. glomerata* is well-known in China as Jin Bu Huan and is used for the treatment of coughs and hepatitis.²⁵ The herb *P. hongkongensis* has been used in folk medicine for various therapeutic purposes such as heat-clearing, detoxification, removing food retention, improving blood flow and expelling phlegm to arrest coughing.²⁹ A Senegalese crude drug prepared from the bark of *Securidaca longipedunculata* of this family has been used as anti-inflammatory and antibacterial agents.⁵³ The herbs of *Monnina obtusifolia* are used in traditional folk medicine for heat-clearing, detoxicating, removing food retention, increasing blood flow and expelling phlegm to arrest coughing.⁸¹

1.2.1.2. Polygonaceous Plants

The Polygonaceous plants are a rich source for PSEs, chiefly tri- and tetra-substituted PSEs. The *Polygonum* genus of the Polygonaceous plants^{92, 93} are widely used in traditional and folk medicines for the treatment of various diseases. For examples the juice of *Polygonum amphibium* is used to treat nasal polyps.⁹³ *P. aviculare* has been used in Russian folk medicine for the treatment of external tumors whereas in northern and middle Africa used as a substitute for quinine.⁹³ The plant *P. sachalinense* has been used in China as a traditional and herbal medicine to treat arthralgia, jaundice, amenorrhea, coughs, scalds, burns, traumatic injuries, carbuncles, sores and as emmenagogue, hydragogue and an aperient agent. In Japan, it is used as analgesic and for haemostatic purposes.^{57, 85} The leave extracts of this plant has fungicidal activities against powdery mildew and the flower extracts possess significant antioxidant activities while the rhizomes showed β -glucosidase inhibitory activity.⁵⁷ *P. lapathifolium* has been used in China to treat dysentery, articular pain and also to reduce inflammation.⁵⁹ *P. perfoliatum* is a vine-type weed and is called as speedweed or mile-a-minute plant. This plant is used in traditional medicine in Asia for increasing white blood cells and platelet counts.⁶⁸ The whole plant of *P. hydropiper* is used as a hot-tasting spice in Japan, China and Europe and used as a folk medicine to treat cancer and as haemostatics.^{77, 78} The ethanolic extracts of the root of *P. hydropiper* showed antifertility activities against female albino rats.⁷⁷ The stems and leaves of *P. hydropiper* are used in Vietnam for the treatment of snake-bites and also as diuretic and anthelmintic agents. Different compounds isolated from this plant have also been found to possess strong insect antifeedant, antibacterial, antioxidant activities as well as aldose reductase and tyrosinase inhibitory activities.⁷⁸ The plant *P. cuspidatum* is known in China as Hu Zhang, in Japan as Kojo Kon and in Europe and North America as Mexican Bamboo, Japanese Bamboo or Japanese Knotweed.⁷⁶ It has been used for the treatment of various inflammatory diseases, favus, hepatitis, suppurative dermatitis, tumors, hyperlipemia, gonorrhoea, athletes foot and diarrhoea.^{76, 92} *P. pensylvanicum* has been used for many years to treat various conditions such as oral haemorrhages, piles and internal disorders.⁹³ Besides these, other species of *Polygonum* genus have been widely used to treat gastric cancer, hair loss, diarrhoea and as modulators of human mesangial cell proliferation.⁹³ The ethanolic extracts of *P. pensylvanicum* exhibited significant protein kinase C (PKC) inhibitory activity with an IC_{50} of 38 μ g/mL and also showed β -glucosidase inhibitory activities.⁸⁵ Fan *et al.*⁵⁷ showed that

the methanolic extracts of the leaves and flowers of *P. sachalinense* exhibited significant inhibitory activities on AChE, α/β -glucosidase and DPPH. Beside *Polygonum* genus, other genera of Polygonaceae family are important source of PSEs and have promising activities. For example, the perennial herb *Bistorta manshuriensis* is a Korean medicinal plant well-known as Bum-ko-ri and is used traditionally to treat diarrhoea. The rhizomes of *B. manshuriensis* have been used in Chinese folk medicine for the treatment of dysentery with bloody stools in acute gastroenteritis, acute respiratory infection and venomous snake bite.⁶¹ The plant *Fagopyrum dibotrys* is an erect perennial Polygonaceous herb, growing mainly in China, India, Vietnam, Thailand and Nepal. In China, the rhizome of this plant has been used for the treatment of various diseases such as lung diseases, dysentery and rheumatism.⁸⁷ It was also used for the treatment of colic and choleraic diarrhoeal fluxes in India.⁹⁴ The aqueous acetone extracts of the rhizomes of *F. dibotrys* showed significant antioxidant activities on stable free radical DPPH.⁸⁷ Extracts from the dried bark of *Triplaris americana* (pau-de-formiga', 'formigueiro' and 'pau-de-novato), which is used in Bolivia and Peru as a cure-all, exhibited significant antimalarial and antioxidant activities.^{86, 95, 96}

1.2.1.3. Liliaceous Plants

The bulbs of various species of *Lilium*, a member of Liliaceae, are rich source of di-substituted PSEs. The bulbs of various species of *Lilium* including *L. mackliniae*, *L. longiflorum*, *L. henryi* and *L. speciosum* have been used in traditional Chinese medicine as sedative, antitussive, anti-inflammatory, general tonic and as nutrients.^{48-51, 97} Lily bulbs have also been used in folk medicine for the treatment of burns or swelling in Europe.⁵¹ *L. henryi* is well-known in Japan as kikanoko-yuri and the strongly bitter bulbs are famous for their strong resistance to viral disease.⁵¹ The crude drug "Bai-he" which is used in traditional Chinese medicine is prepared from the bulbs of *Lilium* species and is frequently used in China for the treatment of lung diseases.⁹⁷ The whole plant *Paris polyphylla var yunnanensis* (Liliaceae) has been used in traditional Chinese medicine to treat lung, liver and laryngeal carcinoma.^{64, 73} The extracts of *Heloniopsis orientalis* of this family exhibited potent cytotoxicity against solid carcinoma cell lines: lung (A549) with an IC₅₀ of 4.6 $\mu\text{g}/\text{mL}$ and colon (Col2) with an IC₅₀ of 4.5 $\mu\text{g}/\text{mL}$.⁹⁸ *Smilax* genus of Liliaceous plants are extensively distributed in East Asia and North America and are major source of tri-substituted PSEs. The dried rhizome of the medicinal herb *S. glabra* is traditionally called in China as tufuling and

has been widely used for the treatment of various diseases such as syphilis, furunculosis, eczema, acute dysentery, cystitis, acute and chronic nephritis, brucellosis, dermatitis and mercury and silver poisoning as well as antipyretic, diuretic and detoxifying agent.^{46, 60, 99} The tuber of *S. china* is commonly known in China as Ba Qia or Jin Gang Teng and has been used in traditional Chinese medicine to treat various ailments such as tumor, lumbago, gout, rheumatic arthritis and inflammatory diseases and as diuretic and detoxicant.⁶³ This plant exhibited significant anti-inflammatory and antitumor activities.⁶³ The rhizome extracts of *S. glabra* and root extracts of *S. china* have various pharmacological activities such as hypoglycaemia, free radical scavenging, immunomodulatory and antioxidant enzyme fortifying activities.⁹⁹ It was reported that the ethanolic extracts of *S. bracteata* exhibited antioxidative effects.⁶⁶ The Chinese crude drug “Niu-wei-Cai” which is prepared from the rhizomes and roots of *S. Riparia* has been used commonly to treat bronchitis, lumbago of renal asthenia and traumatic injury as well as asthenia edema and bronchial dilation agents.⁸⁰

Kuo *et al.* isolated di- and tri-substituted PSEs from the ethanolic extracts of the perennial herbaceous plant *Smilax china* of the family Smilacaceae, which is most closely related to Liliaceae.⁶⁹ The *Smilax* plants of the family Smilacaceae such as *S. china* are widely distributed in China, Taiwan and Japan and have been used traditionally in Taiwan for the treatment of syphilis, gout and rheumatism.^{66, 69} The crude extracts of *S. china* possess antimutagenic, antioxidant and antitumor activities and were useful in arthritis adjuvant therapy.⁶⁹ The plant *Heterosmilax erythrantha* of the family Smilacaceae is widely distributed in Vietnam, India and China and its roots are used for the treatment of lumbago, rheumatism, arthralgia, boils, impetigo, osteodynia and prurigo.⁷⁰

1.2.1.4. Plant species of various families

Plants species of the families Aristolochiaceae, Asclepiadaceae, Bignoniaceae, Boraganiaceae, Brassicaceae, Caryophyllaceae, Celastraceae, Chenopodiaceae, Compositae, Euphorbiaceae, Globulariaceae, Lamiaceae, Rosaceae, Rutaceae and Sparganiaceae are important sources for mono- and di-substituted PSEs and have wide pharmacological activities. For example, the roots of the Greek endemic species *Aristolochia cretica* of the family Aristolochiaceae have been used in folk medicine as analgesic, expectorant, emmenagogue and to treat arthritis, snakebite, pruritus and fever.^{32, 33} The *Cynanchum* genus of the family Asclepiadaceae is well-known in Chinese folk medicine for their enormous medicinal values. For example, *C. amplexicaule* is used to treat rheumatoid arthritis, hectic

fevers and abscesses.⁴¹ The plant *C. hancockianum* has antitumour and insect antifeedant activities.⁴³ It was reported that *Kigelia pinnata* of the Bignoniaceae family has broad-spectrum pharmacological activities such as anti-implantation, molluscicidal, antimicrobial and cytotoxic activities.³⁴ The fruits of this plant are used in traditional medicines as dressings for ulcers, purgatives and as a lactagogue while its bark is used for the treatment of sexually transmitted diseases such as syphilis and gonorrhea.³⁴ The whole plant of *Lindelfia stylosa* of the family Boraganiaceae is useful for the treatment of lung and cardiovascular diseases.^{14, 15} The dried seeds of the plant *Iberis amara* (Brassicaceae) are used in traditional medicine for the treatment of digestive, hepatic and vesicle diseases. It has been reported that the hydroalcoholic extracts of the whole fresh plant have anti-inflammatory activities.⁴² The dried and powdered seeds of *Vaccaria segetalis* (Caryophyllaceae) are used in Chinese folk medicine for promoting diuresis, activating blood circulation and relieving carbuncles.⁴⁰ The bark of the plant *Bhesa paniculata* (Celastraceae), known as “gonggang” in Indonesia, is used to treat vomiting and diarrhea.¹⁰⁰ *Beta vulgaris* species of the family Chenopodiaceae has been used in folk medicines to treat liver and kidney diseases and also to stimulate the immune and haematopoietic system.¹⁰¹ The leaves of *B. vulgaris* are strong natural antioxidant and have good nutritional values because of the significant amounts of vitamins A, C and B, calcium, iron and phosphorous. It has significant hypoglycaemic activities and also used in the treatment of cancer as a special diet.^{101, 102} The plant, *Bidens parviflora* (Compositae) is known in Chinese folk medicine as Xiaohua-Guizhencao and has been used for its antipyretic, anti-inflammatory and anti-rheumatic benefits.⁴⁴ The butanolic extracts of *Bidens parviflora* exhibited significant PGE₂ production inhibition activity.⁴⁴ The perennial herb *Phyllanthus niruri* of the family Euphorbiaceae is traditionally known in India as stone breaker and in Nigeria as Enyikwonwa. The whole plant, fresh leaves and fruits of this plant are used for the treatment of various diseases such as jaundice, diabetes, dysentery, influenza, vaginitis, tumors, kidney stones, dyspepsia, hepatitis B and as diuretics, antihepatotoxic, antiviral, antibacterial and antihyperglycemic.^{56, 103, 104} The methanolic extract of both the aerial and underground parts of the plant *Globularia orientalis* of the family Globulariaceae (Turkish flora) showed significant antioxidative effects against stable free DPPH radical.³⁵ Snow hebes has been the subject of many biological study.³⁷ *Salvia officinalis* (Lamiaceae) which is commonly known as sage (Dalmatian sage) has been used for flavoring and seasoning of food while its extracts are well-known for their antioxidative activities.³⁹ The plant *Prunus padus* (Rosaceae) is known as bird cherry and has been used traditionally for the treatment of coughs and enhancement of complexion and eyesight. This plant extracts

showed moderate free radical scavenging activities against DPPH and significant antibacterial activities.¹⁰⁵ The bark of the plant *Prunus ssiori* (Rosaceae) has bitter taste and is used traditionally in Europe and the United States to treat coughs, headaches, heart and intestinal disorders and as a sedative.²³ There are no reports concerning the pharmacological activities of the plant *Prunus maximowiczii*, but Shimazaki *et al* reported that PSEs are responsible for the very bitter taste of the fruits of this plant.¹³ *Ruta graveolens* (Rutaceae) has been used as an abortifacient or emmenagogue.⁵² The fresh aerial part of *R. graveolens* is famous in Taiwan for the treatment of palpitation of the cardio-vascular diseases.⁵² The roots of *R. corsica* of this family has been traditionally used as a substitute for *R. graveolens*. This plant additionally has phototoxic properties.⁸³ The rhizome of *Sparganium stoloniferum* (Sparganiaceae) is one of the main constituent of the Chinese folk medicine and is known as ‘San Leng’ and has been used as emmenagogue, galactagogue and antispasmodic agent.^{21, 22, 47, 71} The rhizome of *S. stoloniferum* was reported to possess anti-tumor activities.²² The dried roots of *Scrophularia ningpoensis* of the Scrophulariaceae family which is known as Xuanshen have been used in traditional Chinese medicine for the treatment of fever, laryngitis, tonsillitis, carbuncles, constipation, pharyngitis and inflammation.^{31, 106} It was reported that some phenylpropanoid glycosides and iridoid, which are the main active constituents of this plant, possess antioxidant, antibacterial, antihypertensive, antiinflammatory, neuroprotective and antidiabetic activities.^{31, 106}

The endemic rattan *Calamus quiquesetinervius* (Arecaceae) is widely found in Taiwan and is a rich source of tri-substituted PSEs. The stems and roots of this plant have been used in traditional herbal medicine for the treatment of various diseases such as hypertension, hepatitis and skin disease.⁸⁴ The plant species of the families Gramineae, Iridaceae are sources of PSEs having complex substituent. The rhizomes of the aggressive, rhizomatous, perennial grass *Imperata cylindrical* (Gramineae) are used as diuretic, anti-inflammatory and antipyretic agent in Korean herbal medicines. Neuroprotective compounds were isolated from the methanolic extracts of this plant.⁸⁸ The plant *Belamcanda chinensis* of Iridaceae family is used in Chinese traditional medicine as an antitussive, antiinfective and expectorant.⁸⁹

1.2.2. Pharmacological activities of the isolated PSEs

The pharmacological activities of the isolated and structurally well-characterized PSEs will be discussed in the following sections. Majority of the PSEs have been explored

for their antioxidant and anticancer activities. For easy reference, the PSEs were grouped based upon the type of pharmacological activity.

1.2.2.1. Antioxidative and free radical scavenging capabilities

The role of antioxidants in controlling several deleterious activities of free radicals that play a major part in the development of chronic and degenerative illness such as cancer, atherosclerosis, ischemia/reperfusion injury, hypertension, diabetes mellitus, Parkinsonism, Alzheimer's disease and neurodegenerative, inflammatory, pulmonary and hematological diseases is well-documented.¹⁰⁷⁻¹¹³ Plant polyphenols are an important class of such antioxidants. Many isolated PSEs possess phenolic moieties and are thought to be act as potential antioxidants. Of late, DPPH radical scavenging test has become a reference point for the *in vitro* antioxidant activity evaluation.¹¹⁴⁻¹¹⁹

6-Feruloylsucrose **35** was reported to exhibit significant antioxidant activities against DPPH radicals.³⁵ Arillatose B **36** exhibited moderate free radical-scavenging activity against DPPH free radical with a SC_{50} of 20.1 μ M.^{35, 39} Lapathoside D **67** exhibited significant free radical-scavenging activity against DPPH free radical with a SC_{50} of 0.088 mM in comparison with the strong antioxidant activity of caffeic acid which showed a SC_{50} of 0.045 mM.⁵⁷ Ono *et al.* reported that 3'-*O*-feruloylsucrose **14**, 6-*O*-acetyl-3'-*O*-feruloylsucrose **15** and helonioside A **69** showed significant antioxidant activities against stable DPPH free radical at a concentration of 0.02 mM. The antioxidant capacity of helonioside A **69** was almost the same as that of α -tocopherol.¹⁶ Helonioside B **70** and 2,6-di-*O*-acetyl-3',6'-di-*O*-feruloylsucrose **73** and heterosmilaside **91** showed significant antioxidant activities against stable free DPPH radicals as compared to that of α -tocopherol. The SC_{50} values of these compounds are summarized in Table 1.10.⁷⁰

Table 1.10. Antioxidant activities of α -Tocopherol and compounds **70**, **73** and **91**.⁷⁰

Compound	SC_{50} (μ g/mL)
α -Tocopherol	8.4
Helonioside B 70	9.1
2,6-Di- <i>O</i> -acetyl-3',6'-di- <i>O</i> -feruloylsucrose 73	8.7
Heterosmilaside 91	12.7

Zhang *et al.* reported that Smilaside G-L **105-110** exhibited significant scavenging activities against free DPPH radicals as compared with α -tocopherol. The SC_{50} values are summarized in Table 1.11.⁶⁶

Table 1.11. Antioxidant activities of α -Tocopherol and Smilaside G-L (**105-110**).⁶⁶

Compound	SC_{50} (10^{-5} M)
α -Tocopherol	2.820
Smilaside G 105	7.193
Smilaside H 106	7.935
Smilaside I 107	6.847
Smilaside J 108	2.667
Smilaside K 109	3.021
Smilaside L 110	3.270

Hydropiperoside B **134** and vanicoside A **127** showed significant antioxidant activities against stable free DPPH radicals whereas vanicoside E **130** showed moderate antioxidant activity, compared to that of standard antioxidant ascorbic acid (Table 1.12).⁷⁸ The acetyl groups in these molecules were suspected to be responsible for the antioxidant activities.⁷⁸

Table 1.12. Antioxidant activities of ascorbic acid and compounds **127**, **130** and **134**.⁷⁸

Compound	SC_{50} (μ g/mL)
Ascorbic acid	22.0
Hydropiperoside B 134	23.4
Vanicoside A 127	26.7
Vanicoside E 130	49.0

Diboside A **135** and lapathoside A **132** showed lower free radical scavenging activities against stable free DPPH radicals with SC_{50} values of 199.48 and 165.52 μ M, respectively, in comparison to that of ascorbic acid (SC_{50} value of 30.79 μ M).⁸⁷

Quiquesetinerviuside A-E **122-126** exhibited weak free radical scavenging activities against stable free DPPH radicals with their SC_{50} values ranging from 62.5-99.6 μ M. However, these compounds **122-126** showed potent $\cdot$ OH radical scavenging activities with their SC_{50} values of 6.8, 7.4, 3.6, 8.4 and 5.5 μ M, respectively, in comparison with Trolox as positive control (SC_{50} = 4.31 μ M). Quiquesetinerviuside D **125** and E **126** exhibited potent

inhibition of LPS-stimulated NO (nitric oxide) production with IC₅₀ values of 9.5 and 9.2 μ M, respectively, in comparison with positive control, quercetin which exhibited an IC₅₀ value of 34.5 μ M.⁸⁴

1.2.2.2. Cytotoxic and antiproliferative effects

Smilaside A-F (**75**, **76**, **101-104**) were evaluated against different cancer cell lines such as human oral epithelium carcinoma (KB), human cervical carcinoma (HeLa), human colon tumor (DLD-1), human breast adenocarcinoma (MCF-7), human lung carcinoma (A-549), and human medulloblastoma (Med) cells using MTT cytotoxicity assay by Kuo *et al.*⁶⁹ Smilaside D-F **102-104** showed significant cytotoxicity against DLD-1 cells (IC₅₀ = 2.7-5.0 μ g/mL) whereas smilaside A **75** exhibited weak cytotoxicity against the same cells with an IC₅₀ value of 11.6 μ g/mL. Most of these compounds, except for smilaside C **101**, showed weak cytotoxicity (IC₅₀ = 5.1-13.0 μ g/mL) against other human tumor KB, HeLa, A-549 and Med cell lines. It was proposed that the acetate group in the sucrose unit of these compounds might be responsible for mediating cytotoxicity.⁶⁹ Yan *et al.*^{64, 65} showed that helonioside A (**69**) exhibited cytotoxic effects in a dose-dependent manner against the mice lung adenocarcinoma cell line (LA 795). Vanicoside A **127** and B **128** exhibited cytotoxicity against MCF cell line at submicromolar dose levels.⁷⁹ 1',2,3,6-Tetra-*O*-acetyl-3'-*cis*-feruloylsucrose **19** which was isolated from the rhizome of *Sparganium stoloniferum* exhibited weak cytotoxic effect against mice lung adenocarcinoma cell line (LA 795) and showed an IC₅₀ value of 116 μ g/mL in comparison with the positive control, cyclophosphamide (IC₅₀ = 7.75 μ g/mL) using MTT assay.²² This suggested that the anti-tumor activities associated with the extracts of rhizome of *S. stoloniferum* may be due to the presence of PSE's in these extracts.

Epstein-Barr virus (EBV) is a cancer causing virus of the herpes virus family. EBV causes infectious mononucleosis and is associated with the development of different cancers like Burkitt's lymphoma, Hodgkin's disease, non-Hodgkin's lymphoma in immunocompetent individuals, nasopharyngeal carcinoma, gastric carcinoma, breast cancer, leiomyosarcomas and EBV-associated lymphomas in immunocompromised individuals.^{120, 121} Lapathoside A **132**, lapathoside D **67**, vanicoside B **128** and hydropiperoside **99** exhibited significant inhibitory effects on the Epstein-Barr virus early antigen (EBV-EA) activation by tumor-promoters such as 12-*O*-tetradecanoylphorbol-13-acetate (TPA) in Raji cells.^{59, 122} Takasaki

*et al.*¹²² reported that vanicosides B **128** and lapathoside A **132** showed significant anti-tumor-promoting effects on mouse two-stage skin carcinogenesis induced by 7,12-dimethylbenz[a]anthracene (DMBA, as an initiator) and TPA as a promoter. Vanicoside B **128** exhibited remarkable inhibitory effect on two-stage carcinogenesis test of mouse skin tumors initiated with an NO donor and NOR-1 ((±)-(E)-methyl-2-[(E)-hydroxyimino]-5-nitro-6-methoxy-3-hexenamide).^{59, 122}

Protein Kinase C (PKC) is a family of serine/threonine specific protein kinases that is activated by Ca^{2+} , phospholipids and diacylglycerol and has different isozymes such as α , βI , βII , γ , δ , ϵ , θ , η etc that are involved in signal transduction from membrane receptors to the nucleus. PKC isozymes have various functions in our body such as signal transduction pathways leading to synaptic transmissions activation, secretion, differentiation, proliferation and ion fluxes activation. It also plays important roles in cell cycle control, tumorigenesis, antitumor drug resistance and apoptosis. PKC is associated with the development of different malignancies such as CNS (central nervous system) tumors, breast cancer, pituitary and thyroid tumors, leukemias, skin cancer, colon tumors and prostate cancer.^{123-126,127} Vanicoside A **127** and B **128** have significant inhibition of PKC activity with an IC_{50} of 44 and 31 $\mu\text{g/ml}$, respectively. The free hydroxyl or phenol groups in these molecules are believed to be responsible for mediating their activities.^{75, 79}

1.2.2.3. Anti-Inflammatory and immunomodulating activities

Wang *et al.*⁴⁴ reported that 6'-O-coumaroylsucrose **40** exhibited significant PGE_2 production inhibition activity. 6'-O-Coumaroylsucrose **40**, 6,6'-di-O-coumaroylsucrose **88** and compound **136** had stronger inhibitory effects on histamine release (IC_{50} value of 21.7, 23.5 and 41.2 $\mu\text{g/mL}$, respectively) than non-steroidal anti-inflammatory drug indomethacin ($\text{IC}_{50} = 89.5 \mu\text{g/mL}$).⁴⁴ It was reported that shegansu C **138** has potent antagonism of lipoxygenase D_4 receptor with an IC_{50} of 10^{-5} mol/L .⁸⁹

1.2.2.4. Miscellaneous activities

Glucosidase inhibitors are recently of interest because of their promising therapeutic potential for the treatment of various diseases such as diabetes, human immunodeficiency virus (HIV) infection, metastatic cancer and lysosomal storage disorder. Lapathoside D **67**

was found to exhibit stronger α -glucosidase inhibition activities with an IC_{50} value of 11.3 μ M than standard drug acarbose ($IC_{50} = 37.5 \mu$ M).⁵⁷ Vanicosides A **127** and B **128** showed significant β -glucosidase inhibitory activities with the IC_{50} values of 59.8 μ g/ml and 48.3 μ g/ml, respectively.⁸⁵ *p*-Coumaric acid ($IC_{50} = 1133.7 \mu$ g/ml) and ferulic acid ($IC_{50} = 1306.0 \mu$ g/ml) showed a very little inhibitory effect and did not exhibit synergistic effect in their mixtures. Thus, it was suggested that the molecular structures of vanicosides and the acetyl moiety in sucrose might be responsible for mediating the β -glucosidase inhibitory activity.⁸⁵

Acetylcholinesterase (AChE) inhibitors are used for the treatment of Alzheimer's disease, myasthenia gravis, Lewy Body dementia and act by inhibiting cholinesterase enzyme from breaking down acetylcholine, increasing both the level and duration of the neurotransmitter acetylcholine in the synapses.⁵⁷ Vanicoside B **128** showed acetylcholine esterase inhibitory activity with an IC_{50} value of 62.0 μ M as compared to the well known inhibitor galanthamine ($IC_{50} = 0.9 \mu$ M). Fan *et al.*⁵⁷ suggested that the ester bonds in vanicoside B **128** might be responsible for mediating such activity.

Niruriside **66** was found to be a novel specific inhibitor of HIV REV (regulation of virion expression) protein to REV responsive element (RRE) RNA with an IC_{50} value of 3.3 μ M.⁵⁶

The anti-angiogenic activity of the butanolic extract of *Monnina obtusifolia* and the isolated PSEs **118** and **119** was investigated by Lepore *et al.*⁸¹ The butanolic extract were found to exhibit significant inhibition activity of vascular endothelial growth factor-A (VEGF-A) interaction with Flt-1 membrane receptor whereas compounds **118** and **119** did not show any activity. This observation may be due to the presence of a combination of compounds acting synergistically or as vehicles thus increasing the biological activity of the extracts.⁸¹

1.3. Physicochemical attributes of sucrose **140**

Since sucrose **140** is the core structure of all the PSEs mentioned before, a brief overview of its properties and reactivity towards electrophiles under various esterification reaction conditions is discussed in sections 1.3.1, 1.3.2 and 1.3.3.

1.3.1. Properties of sucrose **140**

Sucrose or β -D-fructofuranosyl- α -D-glucopyranoside **140** (Figure 1.4) is a natural non-reducing disaccharide with unique structure containing nine chiral centers and is produced from sugar beet or sugar cane on an industrial scale.¹²⁸

Figure 1.4. Structure of sucrose **140** showing atom numbering

Sucrose **140** is a cheap, pure, stable and chemically reactive substrate. Chemical modification (sacrochemistry) of sucrose **140** presents an immense challenge due to its functional richness with eight reactive hydroxyl groups and two anomeric carbons. Most chemical transformations are prone to give complex mixtures. In order to design viable synthetic routes for various PSEs, it is required to realize the relative reactivity of the various functional groups of sucrose **140** and to control their transformations. Sucrose **140** is practically soluble only in a limited number of solvents including water, *N,N*-dimethylformamide (DMF), dimethyl sulfoxide (DMSO) and pyridine. The interglycosidic bond in sucrose **140** which is quite acid-sensitive is hydrolyzed rapidly at pH 4. As a result, acid-catalyzed transformations that would conserve the disaccharide backbone are normally difficult to accomplish. On the other hand, enzyme-catalyzed cleavage can convert sucrose **140** efficiently into a mixture of glucose and fructose or to other derivatives by transglycosylation.¹²⁹

The eight hydroxyl groups of sucrose **140**, including three primary hydroxyl groups at carbons 6, 1' and 6' and five secondary hydroxyl groups at carbons 2, 3, 4, 3' and 4' are all available for reaction. The possible combinations for substitution at all -OH positions can produce as many as 255 different substituted compounds. However, the reactivities of different primary and secondary hydroxyl groups vary slightly towards electrophiles. Of the three primary hydroxyl groups, the most reactive are 6-OH and 6'-OH while the neopentyl-like 1'-OH being the least reactive. During treatment with bulky acylating agents like pivaloyl chloride, the reactivity order of the hydroxyl groups is i) 6-OH $\approx$ 6'-OH > 1'-OH > 4'-OH > 2-OH > 4-OH > 3'-OH > 3-OH; ii) 6-OH $\approx$ 6'-OH > 1'-OH > 3'-OH > 3-OH > 4'-OH > 2-OH > 4-OH.^{130, 131} But, for the same reaction, Jarosz *et al.*¹³² and Queneau *et al.*¹²⁹ found slightly different reactivity order of the hydroxyl groups towards the sucrose pivaloylation as 6-OH $\approx$

6'-OH > 1'-OH > 3'-OH > 2-OH > 3-OH > 4-OH. The reactivity of primary hydroxy groups towards hindered acyl chlorides such as pivaloyl chloride, long chain fatty acid chlorides is reported in the order as 6-OH $\approx$ 6'-OH > 1'-OH while the reactivity towards benzoyl chloride is 6-OH > 1'-OH, 6'-OH.^{132, 133}

When one of the eight hydroxyl groups of sucrose **140** shows greater reactivity towards an electrophilic partner, the second substitution occurs at a relatively slower pace. If the reaction occurs under kinetic control, the regioselectivity is the effect of a chemoselectivity of one hydroxyl group compared to the other hydroxyl groups. The regioselectivity of sucrose **140** may be controlled by the structural and electronic factors of the acylating agents.¹³⁴ The nucleophilic reactivity of sucrose **140** depends on the nature of the electrophilic species, catalysts used and the reaction conditions thus providing two types of selectivities, namely, the degree of substitution and the regiochemistry. The product distribution also depends on whether the reactions are kinetically or thermodynamically controlled.¹²⁹

It has been reported that the conformational structure of sucrose **140** is fundamentally based on the intramolecular hydrogen-bonding network that connects the hydroxyl groups within the glucose and the fructose moieties (Figure 1.5).^{129, 131, 134}

Figure 1.5. Conformational behavior of sucrose **140** in the solid (A) and in solution (B, C) states¹²⁹

The hydroxyl groups at positions 2, 1', and 3' are more reactive due to the electron-withdrawing effects and the hydrogen bonds connecting 2-OH, 1'-OH, and 3'-OH.¹²⁹

1.3.2. Sucrose esters and their properties

Sucrose esters are excellent non-ionic surface active agents (surfactants) since they have excellent surface activity and a very wide arbitrary hydrophilicity and lipophilicity balance (HLB). They are used as fat substitutes, bleaching boosters and emulsifiers in the food and cosmetic industries.¹³⁵ They are becoming more useful in the pharmaceutical industry and in the area of drug discovery because of their biodegradability and biocompatibility. Their properties depend solely on their compositions in terms of the kind of substituent and the degree and regiochemistry of substitution. Sucrose esters are unstable under certain conditions because intramolecular transesterification (acyl group migrations

from one position to another) readily occurs.¹³⁶ As a result, at times, it is difficult to relate the observed product distribution with the relative reactivity of hydroxyl groups. Partially substituted sucrose derivatives are very difficult to isolate in pure form because mixtures of esters are usually obtained through esterification or transesterification of sucrose due to many hydroxyl groups.¹³⁷ For this reasons, peracetylation is commonly used on partially substituted sucrose derivatives upon treatment with Ac₂O in pyridine for isolation and identification of the products.¹²⁹

Since this work will explore the acylation reaction of sucrose **140** to prepare various PSEs, an overview of different sucrose esterification methods is given in the next section.

1.3.3. Esterification of sucrose **140**

1.3.3.1. Carboxylic esters of sucrose **140**

1.3.3.1.1. Esterification at primary positions

The three primary hydroxyl groups of sucrose **140** usually react first when bulky electrophilic species such as chlorotrimethylsilane or highly substituted silyl chlorides such as *tert*-butyldiphenylsilyl chloride are used as acylating agent.¹³⁸⁻¹⁴² For example, the reaction of sucrose **140** with 3 mol equiv of sterically hindered pivaloyl chloride in pyridine at -40 °C for 6 h and then at rt for 24 h yielded 6,1',6'-tri-*O*-pivaloylsucrose **147** and 6, 6'-di-*O*-pivaloylsucrose **148** in 42% and 22% respectively,^{130, 131} while the reaction using 2.2 mol equiv of pivaloyl chloride in dry pyridine at rt for 12 h yielded 6, 6'-di-*O*-pivaloylsucrose **148** in 40% yield (Scheme 1.1).¹³⁰ These pivalic esters were used for the synthesis of chloro, azido, and anhydro derivatives and also could be converted into various epoxides *via* nucleophilic displacement reactions.¹⁴³ Khan reported that 6-*O*-acetylsucrose **146** could be obtained in 40% yield by treatment of sucrose **140** with 1.1 mol equiv of acetic anhydride in pyridine at -40 °C (Scheme 1.1).¹³¹ Some tin compounds such as dibutyltin oxide show significant selectivity towards position 6.^{144, 145} A polymer-supported butyltin (IV) reagent was also used to control the regioselectivity in acetylation of sucrose **140**.¹⁴⁶ Wang *et al.*¹⁴⁷ showed that 6-*O*-acylsucrose **149** and 6, 3'-di-*O*-acylsucrose **152** were obtained in highly purified state with good yields during the regioselective acylation of sucrose **140** using dibutylstannylene acetal method (Scheme 1.1). When esterification was carried out under the Mitsunobu conditions^{148, 149} (diethyl azodicarboxylate, Ph₃P), only esters at the primary positions C-6 and C-6' were produced (Scheme 1.1). Isolation of 6-, 6'-monoesters and the 6,6'-diesters could be achieved at ease.¹⁵⁰⁻¹⁵³ For e.g. Moliner *et al.*¹⁵¹ reported that acylation

of unprotected sucrose **140** with 2.5 equiv of different fatty acids such as octanoic, stearic, palmitic and lauric acids under the Mitsunobu conditions¹⁴⁸ (Scheme 1.1) provided 6, 6'-di-*O*-acylsucrose **151** in good selectivity along with small amounts of 6-monosubstituted **149** and 6'-monosubstituted **150** esters (85:15 ratio). The Mitsunobu reaction was also used to prepare sucrose phosphates and phosphonates.^{154, 155} Andrade *et al.*¹⁵⁶ investigated the microwave assisted esterification of sucrose bromides that allowed for the attachment of vinyl ester type side chains at the primary positions. Tribenzoates at various positions, such as 6,1',6'- (**153**), 6,1',3'- (**154**), 2,6,1'- (**155**), 2,6,6'- (**156**), 1',3',6'- (**157**), 2,1',6'- (**158**) and 6,3',6'-tri-*O*-benzoylsucrose **159** were successfully synthesized when sucrose **140** was treated with benzoyl chloride (Scheme 1.1).^{157, 158}

Scheme 1.1. Partial esterification of unprotected sucrose **140** at primary positions

1.3.3.1.2. Esterification at the secondary positions

Esterification of sucrose **140** was demonstrated to occur at the secondary hydroxyls, principally at 2-OH, when an acylating agent such as *N*-acylthiazolidinethione was used, giving the 2-monoester **160** by using catalytic sodium hydride (NaH) as the base (Scheme 1.2).^{133, 136, 153} Selective esterification at 2-OH was also obtained during the formation of tosyl derivatives. It was reported that a cyclic hydroxymethyl alkyl acetal involving 2-OH and 3-OH was obtained as a major product along with the Williamson ethers at 2-OH and 1'-OH by reacting sucrose **140** with chloropinacolone in DMF under basic catalysis (NaOH, K₂CO₃).¹⁵⁹ Esterification of unprotected sucrose in the presence of metal chelates afforded variations in the distribution, with the 3-acylsucrose **161** or 3'-acylsucrose **162** as the major products and also in the degree of substitution depending upon the affinity of carbohydrates for metal cation species (Scheme 1.2).^{129, 160}

Scheme 1.2. Partial esterification of unprotected sucrose **140** at secondary positions

1.3.3.1.3. Enzymatic esterifications

Selective acylation of sucrose **140** has been accomplished by enzymatic approaches, in particular with proteases and lipases (Scheme 1.3).¹⁶¹⁻¹⁶⁵ This approach can result in the mono- and/or- diesters at the primary 6-, 6' and 1'-OH, or at the secondary 2-OH. A main disadvantage of these methods is that many biological catalysts are inactivated by the polar solvents (DMSO, DMF and DMA) where sucrose **140** is soluble. It has been reported that several proteases of the subtilisin-family were able to catalyze selectively the acylation of the primary 1'-OH in the fructose ring of sucrose moiety to give **163** (Scheme 1.3).^{166, 167} These reactions are usually performed in DMF and DMSO despite the ability of DMSO to denature the proteins. Variations in selectivity can sometimes be observed. For example, the reaction of sucrose **140** with vinyl laurate catalyzed by protease AL-89 yielded the 2-OH derivative, while subtilisin A catalyzed the formation of 1'-OH monoester.¹⁶⁸ Once bearing some substituents, the decrease of polarity of the sucrose derivatives makes them more soluble in less-polar solvents, such as acetone or *tert*-butanol, in which some lipases are able to catalyze

esterification reactions. The lipases from *Pseudomonas* species or *Candida antarctica* exhibit regiospecificity for the hydroxyl 6'-OH¹⁶¹ allowing the synthesis of mixed 1',6'-diesters **164** (Scheme 1.3).^{162, 169} For some lipases, the regiochemistry was observed to depend on the chain-length.¹⁶⁵ A series of specifically substituted sucrose fatty acid esters with variations in the chain length, the level of saturation, and the position on the sugar backbone can be achieved by using combinations of enzyme-mediated and purely chemical esterification methods.^{129, 163, 170}

Scheme 1.3. Enzymatic esterification of sucrose **140**

1.3.3.1.4. Esterification in aqueous media

Monosubstituted sucrose esters and mixed carbonates were synthesized by reaction with acid chlorides or alkyl chloroformates in aqueous media (Scheme 1.4).¹⁷¹ A cosolvent such as tetrahydrofuran (THF) or 2-propanol, and an acylation catalyst such as 4-dimethylaminopyridine (DMAP), were added to decrease the strength of the cohesive energy density of water in order to limit the polyesterification and low substitution. DMAP helps in incorporation of the acyl chain within the aqueous phase. It was revealed that significant initial esterification takes place rapidly at the secondary hydroxyl groups and almost total subsequent migration towards primary positions occurs. These reactions provide new chemical evidence for the intrinsic pre-eminent reactivity of the hydroxyl groups of sucrose and particularly the 2-OH (**165**).^{129, 134, 159, 172} Esterification of selectively substituted mono- (**166**) or di-esters (**167**) with galloyl residues led to a series of polyesters containing gallate units (at the 6- and 6'-O; 3'-, 4'-, and 6'-; 1', 2, 3, 3', 4', and 6'- positions) (Scheme 1.4).^{173, 174}

Scheme 1.4. Esterification of sucrose (**140**) in aqueous condition

1.3.3.1.5. Partially esterified sucrose by deprotection of sucrose derivatives

Selective removal of acetyl groups from octa-*O*-acetyl sucrose for the preparation of partially acetylated sucrose has been studied chemically¹⁷⁵ and enzymatically.¹⁷⁶ Enzymatic methods were found to be more selective than chemical methods.¹⁷⁷ The use of different enzymes resulted in the formation of partially acylated sucrose derivatives with specific free hydroxyl groups (Scheme 1.5).^{132, 178} Hexa- and hepta-*O*-acetyl sucroses were thus synthesized under either basic catalysis with $\text{Al}_2\text{O}_3\text{--K}_2\text{CO}_3$, primary amines or by using enzymes.

Scheme 1.5¹³². Enzymatic deprotection of octa-*O*-acetylsucrose

A multistep route, based on selective desilylations of trisilylated sucrose derivatives was described to provide heptaesters having the 1'-position unprotected.¹⁷⁹ It was reported

that 4,6-orthoesters which were prepared by reaction of sucrose **140** with ethyl orthoacrylate can be opened to form either the 6- or 4- esters. These compounds provided suitable starting materials for the preparation of biodegradable polymers.¹⁸⁰

1.3.3.2. Sucrose esters other than carboxylic esters

Sulfuric esters of sucrose such as the aluminum salt of the octasulfate or sucralfate **168** used as an antiulcer drug, was synthesized by reaction of unprotected sucrose **140** with the SO₃–pyridine complex, in either pyridine or DMF (Scheme 1.6). Sulfonylation of unprotected sucrose with 3 equiv of SOCl₂ in pyridine yielded the 1',6,6'-tri-*O*-tritosylated sucrose **169** in moderate yield along with 6,6'-di-*O*-tritosylated sucrose as well as tetra- and penta-substituted derivatives (Scheme 1.6). The 1',6,6'-tri-*O*-trisulfonylated derivative **169** was synthesized using the more bulky mesitylenesulfonyl chloride in moderate yield (Scheme 1.6).¹²⁹ Direct regioselective 2-*p*-toluenesulfonylation of sucrose with *N*-(*p*-toluenesulfonyl)imidazole to give monosubstituted sucrose **170** has also been achieved (Scheme 1.6).¹⁸¹ Selective sulfonylation of 1',6,6'-tri-*O*-tritylsucrose (prepared using Bu₂SnO) with methanesulfonyl chloride in benzene provided the 3-mesylated derivative **171**, whereas the same reaction performed in toluene provided the 4-mesylate derivative **172** in moderate yield (Scheme 1.6) indicating the importance of solvent effects on the regioselectivity during substitution. When triflic anhydride was used as the sulfonating reagent, the 4-triflate derivative **173** was obtained in low yield (Scheme 1.6).¹²⁹

Scheme 1.6. Esterification of sucrose **140** with reagents other than carboxylic acids

1.3.3.3. Sucrose esters via isopropylidene acetal intermediates

Cyclic acetals or ketals are obtained when the hydroxyl groups of carbohydrates react with specific carbonyl substrates under acid catalysis. In case of sucrose **140**, only very reactive carbonyl substrates such as acetone, 2-methoxypropene and dimethoxypropane provide good yields of the isopropylidenated products because of the sensitivity of the glycosidic bond to acid catalysts. Isopropylidination of sucrose under thermodynamic control reaction conditions is very selective towards the 4-OH and 6-OH group and produce six-membered ring containing mono-isopropylidene acetal **174** whereas kinetically controlled reaction conditions provide a second isopropylidene ring utilizing the 2-OH and 1'-OH groups and produce di-isopropylidene acetal **175** by forming eight-membered ring (Scheme 1.7). These sucrose isopropylidene acetals (mono **174** and di **175**) are important synthetic intermediates, particularly for selective esterification and etherification of sucrose.^{129, 182}

Scheme 1.7. Sucrose isopropylidene acetals

1.4. Motivation behind this research project

Phenylpropanoid sucrose esters were isolated from various medicinal plants whose extracts exhibited promising antioxidant, anticancer, α -glucosidase inhibition activities among other activities. These compounds occur in minute quantities from their natural sources. Interestingly, except for niruriside, there are no synthetic routes to describe their laboratory synthesis.¹⁸³ This may be due to: (i) structural complexity and peculiar reactivity of sucrose **140**; (ii) the need to control the regio- and chemoselectivities during the multi-step protection/deprotection synthesis strategies; (iii) normally low yields are obtained (iv) purification is laborious and time consuming. Hence for aforementioned reasons, it is an immense challenge and time consuming process to synthesize these compounds. As described in sections 1.1 and 1.2, it is clear that PSEs have potential to be lead drug candidates. In this project, it is contemplated that robust chemical synthesis of such useful natural products would provide enough materials and varieties for evaluation of their anticancer activities and studying the structure-activity-relationship (SAR) to establish their usefulness for further investigation and development. Thus, it was envisaged that not only natural but also unnatural phenylpropanoid esters of sucrose would be synthesized and be useful for this cause.

1.5. Objectives

The main objectives of this research project were:

- Develop a robust synthetic methodology for phenylpropanoid sucrose esters using sucrose as cheap starting material.
- Synthesize various natural and unnatural phenylpropanoid sucrose esters
- Study the anticancer activities and the structure-activity-relationship of selected synthesized phenylpropanoid sucrose esters.

Chapter Two: Synthesis of natural and unnatural phenylpropanoid sucrose esters

2.1. Introduction

A major objective of this project is to establish a robust synthesis of natural and unnatural PSEs. Such PSEs will be screened for anticancer activities in Chapter 3. In particular, we are interested in the synthesis of:

1. Model cinnamoyl PSEs.
2. Lapathoside D **67** and C **116** and their analogues.
3. Helonioside A **69** and its analogues.

Before developing a synthetic strategy, we noted the following critical points:

1. In the isolated natural phenylpropanoid sucrose esters, sucrose core is acylated primarily at four positions: the 6, 1', 3' and 6' hydroxyls. Consequently, selective acylation at these positions would provide nearly all of the natural PSE's.
2. It has been well established that reactions of native sucrose are complex since chemo- and regio-selection between the eight hydroxyl groups is poor. To reduce the complexity of the reaction products, protection methodologies are normally followed.
3. Cyclic acetals or ketals are achieved when the hydroxyl groups of carbohydrates react with carbonyl groups under acid catalysis. In case of sucrose **140**, only very reactive carbonyl substrates such as acetone, 2-methoxypropene and dimethoxypropane provide good yields of the isopropylidenated products, because of the sensitivity of the glycosidic bond to acid catalysts. Isopropylidination of sucrose under thermodynamic control reaction conditions is very selective towards the 4-OH and 6-OH group whereas kinetically controlled reaction conditions provide a second isopropylidene ring with 2-OH and 1'-OH. Both monosaccharide moieties of sucrose **140** are doubly connected through eight-membered rings.
4. The sucrose isopropylidene acetals (mono and di) are important synthetic intermediates, particularly for selective esterification of sucrose.
5. 2,1':4,6-Di-*O*-isopropylidene sucrose **175** is an important synthetic intermediate for synthesizing various sucrose esters and its structure has been well established by chemical transformation and by NMR spectroscopy.¹⁸⁴⁻¹⁸⁸ For example, 6'-phosphate sucrose **176** was successfully synthesized in 35% yield by Kim *et al.*¹⁸⁹ by reacting diacetone **175** with excess phosphorus oxychloride in presence of water and pyridine at 0-2 °C followed by cleavage of the isopropylidene group (Scheme 2.1).

Clode *et al.*¹⁹⁰ investigated the partial benzoylation of diacetone **175** with 1.1-3.1 mol equiv of benzoyl chloride and obtained 6'-*O*-benzoyl- **177**, 3'-*O*-benzoyl- **178**, 3',6'-di-*O*-benzoyl- **179**, 3',4',6'-tri-*O*-benzoyl- **180**, 3,3',6'-tri-*O*-benzoyl- **181** and 3,3',4',6'-tetra-*O*-benzoyl-2,1':4,6-di-*O*-isopropylidenesucrose **182** in different ratios depending on the amount of benzoyl chloride utilized (Scheme 2.1). Khan *et al.*¹³¹ reported that benzoylation of diacetone **175** with excess benzoyl chloride (3.78 equiv) at 0 °C gave three derivatives: compounds **179** (36% yield), **180** (9% yield) and **181** (8% yield) (Scheme 2.1). It was suggested that the reactivity order of the hydroxyl groups in diacetone **175** is OH-6' > OH -3' > OH-4' > OH-3 and the greater reactivity of OH-3' compared with other secondary hydroxyl groups was reasoned based on the *cis*-arrangement of the OH-3' and the glycosidic oxygen O-2.^{131, 190} Duynstee *et al.*¹⁸³ reported that cinnamoylation of diacetone **175** with 2.2 equiv of *trans*-cinnamoyl chloride at -30 °C in pyridine-CH₂Cl₂ for 90 min led to the formation of the 3',6'-di-*O*-cinnamoyl derivative **183** in 73% yield (Scheme 2.1).

Scheme 2.1. Partial esterification of diacetone **175**

Among the reported 150 natural phenylpropanoid sucrose esters (PSEs), only niruriside **66** (Scheme 2.1) was successfully synthesized in the laboratory by Duynstee *et al* using a sucrose isopropylidene acetal intermediate.¹⁸³

In order to develop a viable synthetic strategy towards PSEs, we initially planned this strategy by synthesizing model PSEs using simple unsubstituted cinnamoyl phenylpropanoid moieties to avoid any complication due to substituents at the phenyl ring.

2.2 Synthesis of model cinnamoyl PSEs

The simplest phenylpropanoid substituent is the unsubstituted cinnamoyl group. Therefore, we have started this investigation by exploring the synthesis of cinnamoyl derivative 3',6'-di-*O*-cinnamoylsucrose **184** (Figure 2.1) using similar conditions used to synthesize niruriside **66**. It is important to note that the developed strategy should be applicable to the synthesis of other PSEs especially lapathosides (Table 1.5, 1.8), heloniosides (see Table 1.5) and their analogues.

Figure 2.1. The structure of the targeted model cinnamoly compound

Based on the above esterification precedents in section 2.1, it was anticipated that reaction of diacetone **175** with different equivalents of cinnamoyl chloride followed by deprotection of the acetonoid groups should furnish the initial target compound **184** with high selectivity according to Scheme 2.2.

Scheme 2.2. Proposed synthesis of cinnamoyl **184**

Hence, we attempted to synthesize compounds **175** and **184**.

2.2.1. Synthesis of 2,1':4,6-di-*O*-isopropylidene sucrose **175**

A number of acetonation methods have been described in the literature for the synthesis of compound **175**. Kinetically controlled products were obtained when 2,2-dimethoxypropane^{184, 191} or 2-alkoxypropene^{189, 192-195} in dry DMF, in the presence of *p*-toluenesulfonic acid (*p*-TsOH), were used as acetonating agents. Bazin *et al.*¹⁹⁶ reported that 2,1':4,6-di-*O*-isopropylidene sucrose **175** and 4,6-mono-*O*-isopropylidene sucrose **174** were obtained in 46% and 45% yield respectively using 12.1 mol equiv of 2,2-dimethoxypropene as acetonating agent in the presence of *p*-TsOH in dry DMF for 2 h. Kim *et al.*¹⁸⁹ reported that 2,1':4,6-di-*O*-isopropylidene sucrose **175** was obtained in 43% yield using 4.5 mol equiv of 2-methoxypropene and drierite as drying agent in the presence of catalytic amount of *p*-TsOH in dry DMF for 45 min. Poschalko *et al.*¹⁹⁵ reported the preparation of diacetone **175** by using 4.5 mole equiv of 2-methoxypropene in the presence of catalytic amount of *p*-TsOH in dry DMF at 70 °C for 40 min to give crude mixture which contained 74% diacetone **175**, 8% 4,6-mono-*O*-isopropylidene sucrose **174** and 17% of unidentified byproduct. Interestingly, purification was performed after treating the reaction mixture with acetic anhydride in pyridine to acetylate the free hydroxyl groups of each compound in the mixture. Thus, the obtained reaction mixture was subjected to column chromatography followed by recrystallization and deacetylation using sodium methoxide to provide 40% of pure diacetone **175**.¹⁹⁵ Sato *et al.*¹⁹⁷ successfully synthesized 2,1':4,6-di-*O*-isopropylidene sucrose **175** in 70% yield employing acetone dimethylacetal and CAN¹⁹⁸ under mild reaction conditions.

Synthesis of 2,1':4,6-di-*O*-isopropylidene sucrose **175** according to the literature methods described by Fanton,¹⁹³ Kim,¹⁸⁹ Bazin,¹⁹⁶ Poschalko,¹⁹⁵ Sato¹⁹⁷ were attempted in this research project. We found that Poschalko's (Scheme 2.3) gave reproducible yields of compound **175** and was easy to perform on large scale.

Scheme 2.3. Synthesis of diacetone **175**

However, when purification of diacetone **175** relied solely on column chromatography as described in the literature, it proved to be tedious, time consuming and problematic especially for large scale synthesis. Therefore, an alternative simple and fast purification route that is amenable for large scale was sought and developed. In our modified purification procedure, the crude syrupy product obtained after extraction was initially subjected to flash column chromatography to remove the non-polar impurities and the more polar mixture was recrystallized using EtOAc to give a white solid (R_f value of 0.20, EtOAc) in 56% yield leaving behind various by-products in the solvent. This procedure was extremely suitable and convenient for large scale purification (*ca* 200-300 g). The ESI-mass spectrum showed a molecular ion at m/z value of 446.33 corresponding to $(M + Na + H)^+$ thus supporting the molecular formula $C_{18}H_{30}O_{11}$ (m/z calcd for $C_{18}H_{30}O_{11}Na$ ($M + Na$) $^+$: 445.42). Its 1H NMR spectrum showed three new singlets at δ 1.45, 1.49, 1.52 ppm corresponding to the four methyl groups. The anomeric proton (H-1) signal appeared at δ 6.26 ppm. The ^{13}C NMR spectrum and DEPT analysis confirmed the presence of four methyl carbon at δ 19.1, 24.3, 25.2, 29.0 ppm and two quaternary carbons at δ 102.6 and 103.0 ppm. These signals were assigned to the two isopropylidene moieties. Furthermore, comparison of the 1H NMR and ^{13}C NMR spectral data with the published literature data¹⁹⁵ confirmed the product to be compound **175**. On continued recrystallization of the polar mixture from EtOAc, a second crystalline crop with an R_f value of 0.25 (EtOAc) was obtained in 10% yield. The NMR data of the solid from the second crop showed typical signals for an isopropylidene group. But the signals for the anomeric proton at δ 5.74 ppm as well as other sucrose protons revealed that this compound is different from sucrose **140** and compound **175**. Its ESI-mass spectrum supported the molecular formula $C_{15}H_{26}O_{11}$ (m/z calcd for $C_{15}H_{26}O_{11}Na$ ($M + Na$) $^+$: 405.36; Found: 406.48). From the above evidence and also from the literature description,¹⁹⁵ the compound was assigned as structure **174**. Compound **174** was widely used for the synthesis of 6-*O*-acyl sucrose,^{196, 199} but to-date, its NMR data has not been reported.

2.2.2. Acylation of diacetone **175** with cinnamoyl chloride

Excluding niruriside, all of the discovered natural PSEs have substituted phenyl rings with either OH, COMe, MeO or combination of these groups. In order to avoid any complication during the acylation of sucrose **140**, it was decided to explore model reactions using cinnamoyl group since it lacks the free OH substituents on the aromatic ring. The established reaction conditions would then be extended to the coumaroyl and feruloyl

counterparts to prepare the natural PSEs. Regio- and chemoselective acylation of sucrose **140** with acid chlorides^{147, 173, 183, 200, 201} or acid anhydride¹³⁴ in presence of base catalyst such as pyridine, NEt₃, DMAP is widely used for synthesizing sucrose esters. Since diacetone **175** has 4 free OH groups, it was expected that a product distribution will be obtained when it is reacted with cinnamoyl chloride. The OH groups, like the parent sucrose **140**, were expected to have slight differences in their reactivities. The most pronounced difference is expected to be between the primary 6'-OH and the rest of the secondary 3-OH, 3'-OH and 4'-OH. In order to explore their reactivities diacetone **175** was reacted with variable number of moles of cinnamoyl chloride.

(i) *Acylation using 1.1 moles:*

At the outset, when diacetone **175** was treated with 1.1 mole equiv of cinnamoyl chloride at rt for 9 days (Scheme 2.4), after workup and chromatographic purification using a gradient of CH₂Cl₂-EtOAc as eluent, the major product was obtained as a white solid in 40% yield (R_f value of 0.09, 3:1 EtOAc-hexanes), mp 126-129 °C, along with *ca* 16% yield of a minor product (R_f value of 0.24, 3:1 EtOAc-hexanes).

Scheme 2.4. Acylation of diacetone **175** with 1.1 mole equiv CinnCl

The HR-ESI-MS of the major product suggested a molecular formula C₂₇H₃₆O₁₂ based on a molecular ion peak at *m/z* 575.2092 [M + Na]⁺ (calcd 575.2099 for C₂₇H₃₆O₁₂Na). Its IR spectrum showed absorption band for an α,β-unsaturated ester at 1712 cm⁻¹ (carbonyl group) and 1638 cm⁻¹ (double bond (PhCH=CH)). The product was extensively analyzed with the help of ¹H NMR, ¹³C NMR, DEPT, ¹H-¹H COSY and HMQC experiments. The ¹H NMR spectrum indicated proton signals characteristic for 2,1:4,6-di-*O*-isopropylidene sucrose moiety along with a *trans*-cinnamoyl moiety. The *trans*-cinnamoyl moiety was indicated by 5 aromatic proton signals at δ 7.27-7.37 (m, 3H, H-3'', H-4'', H-5'') and 7.48-7.55 ppm (m, 2H, H-2'', H-6'') and two *trans*-olefinic proton signals at δ 6.46 (d, 1H, *J* = 16.2 Hz, H-8'') and 7.69 ppm (d, 1H, *J* = 16.2 Hz, H-7''). Moreover, the ¹³C NMR and DEPT spectra of this major product revealed new 9 additional carbon signals besides the 2,1:4,6-di-

O-isopropylidene sucrose moiety, including an ester carbonyl carbon, one pair of double-bond carbons, five methines and one quaternary aromatic carbon that were in agreement with values for one *trans*-cinnamoyl moiety. The above data confirmed that compound **175** was successfully esterified with one *trans*-cinnamoyl moiety. To confirm the position of the cinnamoyl group, the COSY spectrum of the major product was assigned to find out the significant shifts on proton NMR. The anomeric proton (H-1) signal at δ 6.21 ppm correlated to H-2 proton signal at δ 3.77 ppm which in turn correlated to H-3 proton at δ 4.10 ppm. This latter correlated with H-4 proton signal at δ 3.60 ppm which in turn correlated with H-5 proton overlapping signal with H-6b proton at δ 3.94 ppm. The H-3' proton signal at δ 3.94 ppm correlated with H-4' proton which is overlapped with H-5' proton signal at δ 4.21 ppm. H-5' proton correlated with H-6'b proton signal at δ 4.54 ppm which overlapped with H-6'a proton signal at δ 4.34 ppm. Thus, the ester carbonyl was assigned to be connected to C-6' of the product based on the strong downfield chemical shift of H₂-6' (δ 4.54, 4.34 ppm) in comparison to that of **175** (δ 3.84, 3.61 ppm) and also from the correlation peaks between H₂-6' protons (δ 4.54, 4.34 ppm) and α,β -unsaturated carbonyl carbon C-9'' (δ 167.2 ppm) in the HMBC spectrum of the product. Based on these spectroscopic data, the major product was assigned to be 6'-mono-*O*-cinnamoyl-2,1':4,6-di-*O*-isopropylidene sucrose **185**.

The ESI-MS of the minor product indicated a molecular ion peak at m/z 575.26 [$M + Na$]⁺ (calcd 575.22 for C₂₇H₃₆O₁₂Na), consistent with the same chemical formula as **185**, C₂₇H₃₆O₁₂. Similar to compound **185**, its IR spectrum showed absorption bands for α,β -unsaturated ester with sharp peaks at 1718 and 1636 cm⁻¹ corresponding to the carbonyl and double bond groups, respectively. In addition to signals corresponding to the 2,1':4,6-di-*O*-isopropylidene sucrose moiety, the ¹H NMR showed signals corresponding to one *trans*-cinnamoyl moiety, represented by 5 aromatic proton signals at δ 7.39-7.45 (m, 3H, H-3'', H-4'', H-5'') and 7.60-7.63 ppm (m, 2H, H-2'', H-6'') and 2 *trans*-double bond signals at δ 6.55 (d, 1H, J = 16.2 Hz, H-8'') and 7.82 ppm (d, 1H, J = 16.2 Hz, H-7''). The ¹³C NMR and DEPT spectra of this minor product revealed new 9 additional carbon signals, the same as described for compound **185**, indicating the presence of one *trans*-cinnamoyl moiety. But, the sugar carbon signals showed different positions from **185**. Moreover, the major difference found in the ¹H NMR spectra between this minor product and that of compound **185** in comparison to starting compound **175** was that the H-3' and H-4' proton signals were shifted to downfield instead of H₂-6' protons at δ 5.03 (d, 1H, J = 7.5 Hz, H-3') and 4.84-4.95 ppm

(m, 1H, H-4') respectively (where in compound **175**, H-3' and H-4' proton signals were observed at δ 3.96 and 4.58 ppm, respectively). This change was further confirmed by the inspection of the COSY spectrum in a similar manner as described for compound **185** which showed the correlation between H-3' (δ 5.03 ppm) and H-4' (δ 4.84-4.95 ppm) which in turn correlated to H-4' proton signal at δ 4.13 ppm. Again, the cinnamoyl was assigned to be connected to C-3' of compound **175** based on the correlation cross peaks between H-3' (δ 5.03 ppm) and C-9'' (δ 167.6 ppm) in the HMBC spectrum. Based on these spectroscopic data, it was confirmed that compound **175** was successfully esterified with one *trans*-cinnamoyl moiety at C-3' and the minor product was assigned to be 3'-mono-*O*-cinnamoyl-2,1':4,6-di-*O*-isopropylidene sucrose **186**.

(ii) *Acylation using 2.2 moles:*

When diacetonide **175** was treated with 2.2 mole equiv of cinnamoyl chloride at rt for 5 days (Scheme 2.5) and the product purified, a white solid in 31% yield (R_f value of 0.73, 3:1 EtOAc-hexanes) and mp 118-120 °C was obtained as the sole product.

Scheme 2.5. Acylation of diacetonide **175** with 2.2 mole equiv CinnCl

The HR-ESI-MS spectrum of this product showed a molecular ion peak at m/z 705.2502 $[M + Na]^+$ representing a molecular formula $C_{36}H_{42}O_{13}$ (calcd m/z 705.2518 for di-cinnamoyl substituted diacetonide **175**). The IR spectrum displayed absorption bands for α,β -unsaturated ester with a carbonyl group absorption at 1715 cm^{-1} and a *trans*-vinyl ($CH=CH$) group at 1637 cm^{-1} . The 1H NMR spectrum of the product showed ten aromatic proton signals at δ 7.37-7.43 (m, 6H, H-3'', H-4'', H-5''), 7.51-7.54 and 7.59-7.62 (2 x m, 4H, H-2'', H-6'') ppm together with two pairs of *trans*-double bond signals at δ 6.48, 6.54 (2 x d, 2H, $J = 16.2\text{ Hz}$) and 7.71, 7.82 (2 x d, 2H, $J = 16.2\text{ Hz}$) ppm. The ^{13}C NMR and DEPT spectra of the product revealed eighteen new carbons in comparison to compound **175**, including two ester carbonyl carbons, two pair of *trans*-double bond carbons, ten methines and two quaternary aromatic carbons, indicating the successfully esterification of compound

175 and that the product contains two *trans*-cinnamoyl substituents. The notable changes in the ^1H NMR spectra of the new product in comparison to compounds **185** and **186** were the presence of signals for the two cinnamoyl groups, the characteristic anomeric signal was shifted to δ 6.13 (d, 1H, J = 3.6 Hz, H-1) ppm and strong down field shifts for H-3' at δ 4.95 (d, 1H, J = 6.3 Hz) and for H-4', H-5' & H₂-6' at δ 4.50 (m, 2H, H-4', H-6'b) and 4.39 (m, 2H, H-5', H-6'a) ppm. This change was further confirmed from the COSY spectrum of the product. The important correlations noted are that H-3' (δ 4.95 ppm) correlated with H-4' proton overlapping signal with H-6'b proton at δ 4.50 ppm which in turn overlapped with H-5' and H-6' proton signals at δ 4.39 ppm. The two ester carbonyls were assigned to be connected to C-3' and C-6' of **175** unit based on the correlation peaks between the H-3' proton (δ 4.95 ppm) and C-9'' (δ 167.7 ppm) and also between the H₂-6' protons (δ 4.50, 4.39 ppm) and C-9'' (δ 166.8 ppm) in the HMBC spectrum of the new product. Therefore, this product was assigned to be 3',6'-di-*O*-cinnamoyl-2,1':4,6-di-*O*-isopropylidene sucrose **183**. Compound **183** was already synthesized by Duynstee *et al.*¹⁸³

We tried to synthesize compound **183** on treatment of diacetone **175** with 2.2 equiv of *trans*-cinnamoyl chloride at -30 °C in pyridine-CH₂Cl₂ according to the method reported by Duynstee *et al.*¹⁸³ (Scheme 2.1). After 90 min, TLC analysis (3:1 EtOAc-hexanes) revealed the presence of the starting material, even after stirring the reaction for more than 5 h (Scheme 2.5). In this system, we could not find any remarkable selectivity difference between the two different conditions.

(iii)Acylation using 3.3 moles:

In order to see the effect of higher amounts of cinnamoyl chloride, a solution of diacetone **175** was treated with 3.3 mole equiv of cinnamoyl chloride at rt for 3 days (Scheme 2.6). After workup and chromatographic purification of the crude product using a gradient of CH₂Cl₂-EtOAc as eluent two fractions were obtained. One of the products was found to be compound **183** (12% yield) while the other white solid (17% yield) proved to be a new compound with R_f value of 0.80, 3:1 EtOAc-hexanes and mp 116-120 °C.

Scheme 2.6. Acylation of diacetone **175** with 3.3 mole equiv CinnCl

The ESI-MS of the new product displayed a molecular ion at m/z 835.34 $[M + Na]^+$ (calcd 835.30 for $C_{45}H_{48}O_{14}Na$) while the HR-ESI-MS spectrum showed a molecular ion at m/z 835.2954 $[M + Na]^+$ (calcd 835.2936 for $C_{45}H_{48}O_{14}Na$) thus indicating a molecular formula of $C_{45}H_{48}O_{14}$. The IR spectrum of the product showed absorption bands for an α,β -unsaturated ester carbonyl group (1723 cm^{-1}) and a $CH = CH$ double-bond (1635 cm^{-1}). In addition to the 2,1':4,6-di-*O*-isopropylidene sucrose moiety, the 1H NMR spectrum showed *trans*-cinnamoyl moiety as described for compounds **183**, **185** and **186**, except that the new product showed three *trans*-cinnamoyl moieties, represented by fifteen aromatic proton signals at δ 7.26-7.38 (m, 9H, H-3'', H-4'', H-5'') and 7.47-7.49, 7.57-7.60 (2 x m, 6H, H-2'', H-6'') ppm and three pairs of *trans*-double bond signals at δ 6.43, 6.45, 6.55 (3 x d, 3H, $J = 16.2\text{ Hz}$, H-8'') and 7.69, 7.71, 7.82 (3 x d, 3H, $J = 16.2\text{ Hz}$, H-7'') ppm. In addition, the ^{13}C NMR and DEPT spectra of the product revealed 27 new signals, including three ester carbonyl carbons, three pair of double-bond carbons, fifteen methine and three aromatic quaternary carbons thus confirming the presence of 3 cinnamoyl moieties. The distinguished correlations observed in the COSY spectrum of the new product for describing the significant chemical shifts in the proton NMR were as δ 5.61 (H-3') and δ 5.37 (H-4'); H-4' and δ 4.54 (H-5'), this latter was overlapped with δ 4.54 (H-6'a); H-6'a and δ 4.61 (H-6'b) ppm. Besides, the remarkable change observed between the 1H NMR spectra of the new product and that of starting compound **175** was that the the chemical shifts of the anomeric proton shifted from δ 6.27 to δ 6.14 ppm and the H-3', H-4', H-5' and H₂-6' proton signals shifted to downfield at δ 5.61 (dd, 1H, $J = 3.6\text{ Hz}$, 4.8 Hz , H-3'), δ 5.37 (d, 1H, $J = 5.4\text{ Hz}$, H-4'), δ 4.61 (m, 1H, H-6'b) and 4.54 (m, 2H, H-5', H-6'a) ppm relative to their positions in diacetone **175** (H-3', H-4', H-5' and H₂-6' proton signals were observed at δ 3.96, 4.61, 4.03, 3.84 and 3.61 ppm, respectively). Further, the three ester carbonyls were assigned to be at C-3', C-4' and C-6' of **175** based on the HMBC cross peaks from H-3' (δ 5.61 ppm) and C-9'' (δ 165.8 ppm); from H-4' (δ 5.37 ppm) and C-9'' (δ 165.7 ppm) and from H₂-6' (δ 4.61, 4.54 ppm) and C-9'' (δ 166.3 ppm). Hence, the new product was assigned to be 3',4',6'-tri-*O*-cinnamoyl-2,1':4,6-di-*O*-isopropylidene sucrose **187**.

(iv) *Acylation using 4.4 moles:*

Addition of more equiv of cinnamoyl chloride to **175** was envisioned to produce the tetracinnamoyl derivative. Thus, further increase in the amount of cinnamoyl chloride to 4.4 mole equiv (Scheme 2.7), followed by workup and chromatographic purification using a

gradient of CH₂Cl₂-EtOAc as eluent, gave a white solid (*R_f* value of 0.92, 3:1 EtOAc-hexanes) in 36% yield, mp 88-93 °C along with compound **187** (21% yield).

Scheme 2.7. Acylation of diacetone **175** with 4.4 mole equiv CinnCl

The ESI-MS spectrum of the white solid showed a molecular ion peak at m/z 965.36 $[M + Na]^+$ (calcd 965.35 for C₅₄H₅₄O₁₅Na) corresponding to the elemental formula C₅₄H₅₄O₁₅ while confirmation of this formula came from the HR-ESI-MS spectrum which displayed a molecular ion at m/z 965.3326 $[M + Na]^+$, calcd 965.3355 for C₅₄H₅₄O₁₅Na. Its IR spectrum displayed the typical absorption band for an α,β -unsaturated ester at 1719 cm⁻¹ (carbonyl), and 1636 cm⁻¹ (CH = CH). Similarly, as described for compounds **183**, **185**, **186** and **187**, analysis of the white solid using ¹H NMR spectrum indicated the presence of four *trans*-cinnamoyl moieties, represented by twenty aromatic proton signals at δ 7.35-7.40 (m, 12H, H-3'', H-4'', H-5''), 7.49-7.52 and 7.63-7.75 (2 x m, 8H, H-2'', H-6'') ppm and four pairs of *trans*-double bond signals at δ 6.43, 6.45, 6.46, 6.63 (4 x d, 4H, J = 15.9 Hz, H-8'') and 7.63-7.75 (m, 3H, H-7''), 7.96 (d, 1H, J = 15.9 Hz, H-7'') ppm. Additionally, ¹³C NMR and DEPT spectra of the white solid revealed 36 new signals besides carbon signals for the 2,1':4,6-di-*O*-isopropylidene sucrose moiety, including four ester carbonyl carbons, four pair of double bond carbons, twenty aromatic methine carbons and four aromatic quaternary carbons that were in agreement with four *trans*-cinnamoyl moieties. Detail analysis of the COSY spectrum and ¹H NMR spectrum of the white solid revealed that the anomeric proton signal shifted from δ 6.27 to δ 6.20 ppm and the H-3', H-3, H-4', H-5' and H₂-6' proton signals shifted strong downfield at δ 5.62 (dd, 1H, J = 3.3 Hz, 5.1 Hz, H-3'), δ 5.37-5.43 (m, 2H, H-3, H-4') and δ 4.53-4.61 (m, 3H, H-5', H-6'a, H-6'b) ppm relative to their positions in diacetone **175** (H-3, H-3', H-4', H-5' and H₂-6' proton signals were noticed at δ 4.12, 3.96, 4.61, 4.03, 3.84 and 3.61 ppm, respectively). Based on the above facts, we can conclude that compound **175** was successfully esterified with four *trans*-cinnamoyl moieties. The four ester carbonyls were further assigned to be connected at C-3, C-3', C-4' and C-6' of diacetone **175** unit based on the long-range correlation peaks between H-3' (δ 5.62 ppm) and C-9'' (δ

165.8 ppm); H-3 and H-4' (δ 5.40 ppm) and C-9''' (δ 165.8, 166.2 ppm) and also between H₂-6' (δ 4.57 ppm) and C-9'' (δ 166.5 ppm) in the HMBC spectrum of the white solid. Considering all of the above data, the white solid was assigned to be 3,3',4',6'-tetra-*O*-cinnamoyl-2,1':4,6-di-*O*-isopropylidene sucrose **188**.

2.2.3. Acetylation of compounds **183**, **187**, **188** with Ac₂O

In order to further confirm the degree and position of cinnamoylation of **175** under the reaction conditions as described in Schemes 2.6-2.8, selected compounds **183**, **187** and **188** were subjected to acylation with Ac₂O and the products were characterized. This acetylation was also done to prepare compounds to investigate the effect of acetyl groups on the anticancer activities of PSEs and to study the structure activity relationship (SAR) (discussed in the Chapter 3).

Thus, compound **183** was acetylated with excess Ac₂O (7.58 equiv) in dry pyridine at rt for 24 h (Scheme 2.8).

Scheme 2.8. Acetylation of compound **183** with Ac₂O

Acetylation of **183** proceeded smoothly to give a new product with a higher R_f value (0.91) as revealed by TLC analysis (3:1 EtOAc-hexanes). The new compound was obtained as a white solid in 22% yield, mp 88-90 °C after workup and chromatographic purification using hexane-EtOAc (2:1) as eluent. This compound was analyzed by ¹H NMR, ¹³C NMR, DEPT, ¹H-¹H COSY and HMQC experiments. In addition to the signals obtained for compound **183**, two acetyl group signals in the ¹H NMR spectrum at δ 2.02, 2.11 ppm (2 x s, 6H, -COCH₃) and four carbon signals in the ¹³C NMR spectrum (2 methyl and 2 carbonyl signals) at δ 20.8, 21.0 (2 x C-11'') and 169.7, 170.1 (2 x C-10'') ppm, respectively, were observed. The significant difference noticed between the new product and the starting material **183** by detailed analysis of the ¹H NMR and COSY spectra was that the H-3 and H-4' proton signals shifted to lower field at δ 5.20-5.28 ppm and δ 5.45 ppm, respectively

relative to their positions in compound **183** (H-3 and H-4' signals were observed at δ 3.90 and δ 4.50 ppm respectively). Again, the two acetyl carbonyls were assigned to be at C-3 and C-4' of sucrose core based on the long-range correlation between H-3 proton (δ 5.20-5.28 ppm) and the carbonyl carbon of the acetyl moiety (δ 169.7 ppm) and also between the H-4' proton (δ 5.45 ppm) and the carbonyl carbon of the acetyl moiety (δ 170.1 ppm) in the HMBC spectrum. The molecular formula of this compound was deduced to be $C_{40}H_{46}O_{15}$ based on the molecular ion peak in the ESI-mass spectrum at m/z 789.34 $[M + Na]^+$ (calcd 789.28 for $C_{40}H_{46}O_{15}Na$) and on the HR-ESI-MS spectrum where an ion peak at m/z 789.2727 $[M + Na]^+$ (calcd 789.2729 for $C_{40}H_{46}O_{15}Na$) was observed. Hence, it was concluded that **183** has 2 free OH groups that are available for acylation and that **183** has been completely and successfully acylated. Therefore, the compound was assigned to 3,4'-di-*O*-acetyl-3',6'-di-*O*-cinnamoyl-2,1':4,6-di-*O*-isopropylidene sucrose **189**.

Similarly, compound **187** was acetylated with excess Ac_2O (5 equiv) in dry pyridine at rt for 24 h (Scheme 2.9) in order to confirm the degree of cinnamoylation of **175** under the reaction conditions as described in Scheme 2.6.

Scheme 2.9. Acetylation of compound **187** with Ac_2O

After acylation according to Scheme 2.9, workup and chromatographic purification using hexanes-EtOAc (2:1) as eluent, a white solid (R_f value 0.94, 3:1 EtOAc-hexanes) was obtained in 46% yield, mp 132-134 °C. The molecular formula of the acylated product was assigned as $C_{47}H_{50}O_{15}$ based on the HR-ESI-MS spectrum which showed a molecular ion peak at m/z 877.3051 $[M + Na]^+$ (calcd 877.3042 for $C_{47}H_{50}O_{15}Na$) and the ESI-MS of the product displayed a molecular ion peak at m/z 877.36 $[M + Na]^+$ (calcd 877.31 for $C_{47}H_{50}O_{15}Na$). Again, this compound was characterized with the help of 1H NMR, ^{13}C NMR, DEPT, 1H - 1H COSY and HMQC experiments in a similar fashion to compound **189**. Here, only one acetyl group was observed in its 1H NMR spectrum represented by the proton signal at δ 2.02 ppm (1s, 3H, $-COCH_3$) while the ^{13}C NMR spectrum showed signals corresponding to the methyl & carbonyl signals at δ 21.0 (C-11'') and 169.7 (C-10''), respectively. The acetyl carbonyl carbon was assigned to be at C-3 of compound **187** based on the strong

downfield chemical shift of H-3 proton at δ 5.25 (dd, 1H, J = 9.6 Hz, 9.3 Hz) ppm compared to the same signal in compound **187** (H-3, multiplet signal at δ 3.80-3.86 ppm) and also based on the long-range correlation peaks between H-3 proton (δ 5.25 ppm) and the carbonyl carbon of the acetyl moiety (δ 169.7 ppm) in the HMBC spectrum of the new compound. We can conclude that **187** has one free OH group available for acylation. Consequently, the new compound was assigned as 3-mono-*O*-acetyl-3',4',6'-tri-*O*-cinnamoyl-2,1':4,6-di-*O*-isopropylidene sucrose **190**.

On the other hand, when **188** was subjected to acetylation according to Scheme 2.10, the starting material was recovered unchanged as evident by TLC and NMR analysis, even after extended reaction times (66 h). This result indicates that compound **188** has no free OH groups available for acetylation.

Scheme 2.10. Acetylation of compound **188** with Ac_2O

Thus, these results indicated that compounds **183**, **187** and **188** have two, three and four cinnamoyl groups, respectively and can be acylated with ease.

2.2.4. Cleavage of the isopropylidene groups of compounds **183**, **187** and **188**

A crucial step in the synthetic methodology was the deprotection of the isopropylidene (acetal) groups. Several acetal deprotection conditions such as 1.18 *N* HClO_4 -THF solutions,²⁰² ethylene glycol (2.2 equiv) in presence of cat. *p*-TsOH,¹⁸³ 60% aq. AcOH at 50 °C for 20 min¹⁸³ or at 80 °C for 10 min¹⁹⁹ and 0.1 M HCl in MeOH solution¹⁹⁷ as well as using a Lewis acid such as ferric chloride hexahydrate,²⁰³ montmorillonite K10,²⁰⁴ ceric ammonium nitrate²⁰⁵ and cation-exchange resin¹⁸⁵ have been reported with variable success based upon the nature of the starting material. In our hands, acetal deprotection using 60% aq. AcOH at 80 °C for 20 min was found to be very convenient and a high yielding method. Consequently, this method was used in the present study for cleavage of the isopropylidene groups of compound **183**, **187** and **188**.

Scheme 2.11. Acetal deprotection of compound **183**

After deprotection of diacetone **183** according to Scheme 2.11, TLC analysis (3:1 EtOAc-hexanes) indicated the formation of a new compound having a lower R_f value (0.06, 3:1 EtOAc-hexanes) than the starting material **183**. The new product was obtained in 54% yield, mp 159-161 °C, after recrystallization from EtOAc. It showed lower solubility in organic solvents such as EtOAc and CH_2Cl_2 due to its increased hydrophilicity. The HR-ESI-MS suggested an elemental formula $\text{C}_{30}\text{H}_{34}\text{O}_{13}$ based on the molecular ion at m/z 625.1879 $[\text{M} + \text{Na}]^+$ (calcd 625.1892 for $\text{C}_{30}\text{H}_{34}\text{O}_{13}\text{Na}$) while the ESI-MS spectrum showed a molecular ion peak at m/z 625.23 $[\text{M} + \text{Na}]^+$ (calcd 625.20 for $\text{C}_{30}\text{H}_{34}\text{O}_{13}\text{Na}$). Spectroscopic analysis of the new compound using ^1H and ^{13}C NMR spectra revealed the loss of the characteristic signals for the two isopropylidene moieties, represented by the proton signal of the methyl group signals at δ 1.39, 1.42, 1.52, 1.53 ppm (4 x s, 12H, $(\text{CH}_3)_2\text{C}$) and carbon signals at δ 19.1, 24.1, 25.5, 29.1 ppm (4 x $(\text{CH}_3)_2\text{C}$) and also two quaternary carbons at δ 99.9, 101.8 (2 x $(\text{CH}_3)_2\text{C}$) ppm. In the ^1H NMR spectrum of the new product, it was noticed that the anomeric proton peak at δ 5.45 ppm and the sugar proton peaks were shifted to higher field compared to the corresponding peaks in compound **183** (anomeric proton peak was observed at δ 6.13 ppm). Therefore, it was concluded that the isopropylidene groups of compound **183** was cleaved successfully. Hence, the new compound was assigned to be 3',6'-di-*O*-cinnamoyl sucrose **184**. This is our basic model compound that we planned to synthesize and on which the methodology was developed.

After successful deprotection of compound **183**, di-*O*-isopropylidene deprotection of diacetone **187** was achieved in a similar fashion according to Scheme 2.12.

Scheme 2.12. Acetal deprotection of compound **187**

After removing excess AcOH by co-distillation with toluene, recrystallization of the crude product using EtOAc afforded a new white solid in 69% yield showing a lower R_f value (0.11, 3:1 EtOAc-hexanes) compared to its parent compound **187**. Analysis of this new compound was done in a similar fashion to compound **184**. The characteristic signals for the two isopropylidene moieties were missing in the ^1H and ^{13}C NMR spectra of the new compound. Beside this, the ^1H NMR spectrum revealed that the characteristic anomeric (H-1) signal changed its position from δ 6.14 ppm to δ 5.40 ppm. The ESI-MS of the new product exhibited a molecular ion at m/z 755.23 $[\text{M} + \text{Na}]^+$, calcd 755.24 for $\text{C}_{39}\text{H}_{40}\text{O}_{14}\text{Na}$ and its chemical formula was found to be $\text{C}_{39}\text{H}_{40}\text{O}_{14}$ by the HR-ESI-MS spectrum based on the observed molecular ion at m/z 755.2310 $[\text{M} + \text{Na}]^+$, calcd 755.2310 for $\text{C}_{39}\text{H}_{40}\text{O}_{14}\text{Na}$. Therefore, we can conclude that the two isopropylidene groups of compound **187** were successfully cleaved. Hence, the new compound was assigned to be 3',4',6'-tri-*O*-cinnamoylsucrose **191**.

Similarly, when compound **188** was subjected to cleavage (Scheme 2.13), a new product with a lower R_f value of 0.72 (EtOAc-hexanes) compared to **188** was obtained as a white solid in 54% yield.

Scheme 2.13. Acetal deprotection of compound **188**

Again, the ^1H and ^{13}C NMR spectra of the white solid indicated the loss of the characteristic two isopropylidene peaks. The other significant difference observed in the ^1H NMR spectra of the white solid and the starting compound **188** was the characteristic **188** anomeric (H-1) signal shifted upfield from δ 6.20 ppm to δ 5.54-5.61 ppm. The ESI-MS of the new compound displayed molecular ion peak at m/z 885.27 $[\text{M} + \text{Na}]^+$ (calcd 885.28 for $\text{C}_{48}\text{H}_{46}\text{O}_{15}\text{Na}$) corresponding to the expected molecular formula $\text{C}_{48}\text{H}_{46}\text{O}_{15}$ while the HR-ESI-MS spectrum showed a molecular ion at m/z 885.2725 $[\text{M} + \text{Na}]^+$ (calcd 885.2729 for $\text{C}_{48}\text{H}_{46}\text{O}_{15}\text{Na}$) confirming the same formula. Based on the above facts, it was confirmed that the two isopropylidene groups of compound **188** were cleaved successfully. Hence, the new compound was assigned as 3,3',4',6'-tetra-*O*-cinnamoyl sucrose **192**.

2.2.5. Summary

Regio- and chemoselective esterification of 2,1':4,6-di-*O*-isopropylidene sucrose (**175**) with cinnamoyl chloride at the four free hydroxyl groups (3-OH, 3'-OH, 4'-OH and 6'-OH) was achieved successfully in moderate yields. The reactivities of these hydroxyl groups were found to be in the order of 6'-OH > 3'-OH > 4'-OH > 3-OH. A similar reactivity trend was also observed by Clode *et. al.*,¹⁹⁰ when benzylation was carried out on 2,1':4,6-di-*O*-isopropylidene sucrose **175**. Acetylation of compounds **183**, **187** and **188** with Ac₂O confirmed that these compounds have two, three and four cinnamoyl groups, respectively, and also confirmed the positions of the cinnamoyl groups. Our model target compound **184** was successfully synthesized in moderate yield upon deprotection of acetonoid protection groups.

Having established this protocol for a simple model compound **184**, we can now move ahead with confidence with synthesizing more complex natural and unnatural PSEs.

2.3. Synthesis of Lapathoside D and its analogues

Lapathoside D or 3',6'-di-*O*-coumaroylsucrose **67** (Figure 2.2) was isolated from various herbal plants whose extracts were used traditionally as folk or traditional medicine to treat different diseases and conditions. In addition, isolated Lapathoside D **67** was found to have broad array biological activities including antioxidant, antitumor and α -glucosidase inhibition activities (see details in Chapter 1). Intrigued by its remarkable therapeutic intervention in a wide range of diseases and conditions, synthesis of this natural product along with its analogues was of interest.

Figure 2.2. Structure of lapathoside D **67**

In lapathoside D (Figure 2.2), the *p*-hydroxycinnamoyl moiety is the phenylpropanoid unit and located on the 3' and 6' position of the sucrose core. Thus, the synthetic strategy

described for the preparation of 3',6'-di-*O*-cinnamoyl sucrose **184**, our model compound, in section 2.2 could be applied for the synthesis of lapathoside D **67**. Consequently, *p*-hydroxycinnamoyl chloride was needed to achieve this aim.

2.3.1. Synthesis of *p*-acetoxycinnamoyl chloride **195**

p-Acetoxycinnamoyl chloride **195** was synthesized according to the reported literature procedure.²⁰⁶ The phenolic hydroxyl group of *p*-coumaric acid **193** was first protected by acetylation with acetic anhydride (Scheme 2.14) in order to prevent polymerization reaction during the conversion to acid chloride with SOCl₂.²⁰⁷ *p*-Acetoxycinnamic acid **194** was obtained as a white solid in 69% yield, mp 205-211 °C (reported mp 205-211 °C) after recrystallization of the crude reaction product from MeOH (Scheme 2.14).²⁰⁶ Acid chloride **195** was synthesized by refluxing a mixture of carboxylic acid **194** and SOCl₂ in benzene for 5 h (Scheme 2.14). Recrystallization from hot toluene gave the required *p*-acetoxycinnamoyl chloride **195** in 80% yield, mp 118-121 °C (reported mp 118.5-121.5 °C).²⁰⁶

Scheme 2.14. Preparation of *p*-acetoxycinnamoyl chloride **195**

2.3.2. Acylation of diacetoneide **175** with *p*-acetoxycinnamoyl chloride **195**

Initial experiments using the conditions established for the synthesis of compound **184** gave a complex mixture of products. Therefore, regio- and chemoselective acylation of diacetoneide **175** was performed using variable equivalents of acid chloride **195** in order to obtain the desired compound as well as establish the optimal reaction conditions for the highest yield.

i. Acylation with 1.1 mole equiv of 195:

At the outset, when diacetoneide **175** was reacted with 1.1 mol equiv of *p*-acetoxycinnamoyl chloride **195** at rt for 9 days (followed by TLC) (Scheme 2.15), and after workup and chromatographic purification using a gradient of CH₂Cl₂-EtOAc as eluent, a mixture of three new compounds were obtained: first fraction in 30% yield, second fraction

in 10% yield and third fraction in 6% yield along with 40% of recovered diacetone **175** (entry 1, Table 2.1).

Scheme 2.15. Acylation of diacetone **175** with 1.1 mole equiv acid chloride **195**

Table 2.1. Acylation of diacetone **175** using 1.1 mole equiv of acid chloride **195**

Entry	175 : 195 (equiv)	Reaction condition	Reaction time (days)	Product (yield)	Total yield
1 ^[a]	1:1.1	rt	9	196 (30%) 197 (10%) 198 (6%)	36%
2 ^[a]	1:1.1	50 °C	14	196 (20%) 198 (4%)	24%

^[a] 40% of diacetone **175** was recovered.

In the ESI-MS spectrum, the first fraction showed a molecular ion peak at m/z 633.26 $[M + Na]^+$ (calcd 633.23 for $C_{29}H_{38}O_{14}Na$) while the HR-ESI-MS showed a molecular ion at m/z 633.2144 $[M + Na]^+$ (calcd 633.2154 for $C_{29}H_{38}O_{14}Na$) confirming the molecular formula to be $C_{29}H_{38}O_{14}$. The IR spectrum of this fraction showed absorption bands for the α,β -unsaturated ester with the carbonyl group showing stretching at 1702 cm^{-1} and the (CH=CH) group showing stretching at 1636 cm^{-1} . In addition to the signals for the 2,1':4,6-di-*O*-isopropylidene sucrose moiety, the ^1H NMR spectrum indicated the presence of a *trans*-acetoxycinnamoyl moiety represented by one set of 1,4-disubstituted aromatic ring proton signals at δ 7.11 and 7.52 ppm showing an A_2B_2 spin system, one *trans*-double bond signals at δ 6.41 (d, 1H, $J = 15.9\text{ Hz}$, H-8'') ppm and 7.66 (d, 1H, $J = 15.9\text{ Hz}$, H-7'') and one acetyl group at δ 2.32 (s, 3H, H-11'') ppm. In addition, ^{13}C NMR spectrum of this fraction revealed 29 new signals, including two ester carbonyl carbons, one pair of double bond carbons, four aromatic methine carbons, two quaternary aromatic carbons and one methyl carbon indicating the presence of one acetoxycinnamoyl residue. Correlations observed in the COSY spectrum

of this fraction for α -D-glucose: δ 6.19 (H-1) and δ 3.73 (H-2); H-2 and δ 4.07 (H-3); H-3 and δ 3.61 (H-4); H-4 and δ 3.92 (H-5), this latter with δ 3.92 (H-6b) and 3.73 (H-6a); β -D-fructose: δ 3.50 (H-1'a) and δ 4.30 (H-1'b); δ 3.92 (H-3') and δ 4.19 (H-4'); H-4' was overlapped with δ 4.19 (H-5'), this latter with δ 4.52 (H-6'b) and 4.30 (H-6'a). The chemical shifts of most of the sugar protons and carbons were shifted downfield. The anomeric signal at δ 6.19 ppm (d, 1H, J = 3.0 Hz, H-1) showed a slight downfield shift compared to that of diacetone **175** which appeared at δ 6.26 ppm. Based on the above analysis, it was confirmed that compound **175** was successfully esterified with one *trans*-acetoxycinnamoyl moiety. The ester carbonyl was assigned to be at C-6' of diacetone **175** based on the strong downfield shift of the signal for H₂-6' (δ 4.52, 4.31 ppm) compared to the same signal in diacetone **175** (δ 4.12, 3.96 ppm) and the correlation peaks between the H₂-6' (δ 4.52, 4.31 ppm) and C-9'' (δ 167.1 ppm) in the HMBC spectrum of the compound. Based on these data, the first fraction was assigned to be 6'-mono-*O*-acetoxycinnamoyl-2,1':4,6-di-*O*-isopropylidene sucrose **196**.

The ESI-MS of the second fraction showed molecular ion peak at m/z 633.18 [M + Na]⁺ (calcd 633.23 for C₂₉H₃₈O₁₄Na) and the HR-ESI-MS spectrum suggested the molecular formula C₂₉H₃₈O₁₄ based on the molecular ion peak at m/z 633.2151 [M + Na]⁺ (calcd 633.2154 for C₂₉H₃₈O₁₄Na). Its IR spectrum displayed the characteristic absorption bands for α,β -unsaturated ester carbonyl group (1718 cm⁻¹) and CH=CH (1636 cm⁻¹). Similar to compound **196**, the ¹H NMR spectrum of the second fraction showed the presence of signals corresponding to 2,1':4,6-di-*O*-isopropylidene sucrose moiety and signals corresponding to one *trans*-acetoxycinnamoyl moiety, represented by one set of 1,4-disubstituted aromatic ring proton signals at δ 7.16 and 7.63 ppm, displayed as an A₂B₂ spin system, one *trans*-double bond signals at δ 6.51 (d, 1H, J = 15.9 Hz, H-8'') and 7.79 ppm (d, 1H, J = 15.9 Hz, H-7'') and one acetyl group at δ 2.32 ppm (1s, 3H, H-11''). The main differences observed in the ¹H NMR spectrum of this fraction compared to compounds **196** and **175** was the strong downfield shifts for H-3' what was observed at δ 5.04 (d, 1H, J = 7.8 Hz) ppm and also for H-4' which was observed at δ 4.86 (dd, 1H, J = 7.2 Hz, 7.5 Hz) ppm. This change was further confirmed from the correlations observed in the COSY spectrum for this fraction as H-3' proton signal (δ 5.04 ppm) correlated to H-4' (δ 4.86 ppm) which in turn correlated with H-5' proton signal (δ 4.13 ppm). The ester carbonyl was assigned to be at C-3' of diacetone **175** unit based on the long-range correlation between the H-3' proton (δ 5.04 ppm) and C-9'' (δ

167.4 ppm) in the HMBC spectrum. With the help of these data, the second fraction was assigned to be 3'-mono-*O*-acetoxycinnamoyl-2,1':4,6-di-*O*-isopropylidene sucrose **197**.

At this stage, we could not obtain enough material to characterize the third fraction. (Note: the third fraction was proved to be compound **198** by comparison with the fraction obtained when using 2.2 mol equiv of **195** as indicated below in Scheme 3.3)

Attempts to drive the reaction to completion by raising the reaction temperature to 50 °C and by stirring the reaction for extended time (up to 14 days) were not successful as compound **196** and the third fraction (which was later proved to be **198**), were obtained only in 20% and 4% yields, respectively (Table 2.1, entry 2) along with mixtures of intractable products. We observed that at higher reaction temperature, various decomposition products were obtained indicating the instability of the products at elevated temperature.

ii. Acylation with 2.2 mole equiv of 195:

Treatment of a pyridine solution of diacetone **175** with 2.2 mole equiv of *p*-acetoxycinnamoyl chloride **195** at rt for 24 h gave two new fractions: a major white solid in 51% yield (R_f value of 0.62, 3:1 EtOAc-hexanes), mp 109-111 °C along with a minor fraction which was identified to be compound **196** (*ca* 5%). Unreacted diacetone **175** (20%) was also recovered in this case (entry 1, Table 2.2) (Scheme 2.16).

Scheme 2.16. Acylation of diacetone **175** with 2.2 mole equiv acid chloride **195**

Table 2.2. Acylation of diacetone **175** with 2.2 mole equiv acid chloride **195**

Entry	175 : 195 (equiv)	Reaction condition	Reaction time (days)	Product (yield)	Total yield
1 ^[a]	1 : 2.2	rt	1	196 (<i>ca</i> 5%) 198 (51%)	56%
2 ^{[a],[b]}	1 : 2.2	50 °C	14	198 (20%)	20%

^[a] 20% of diacetone **175** was recovered. ^[b] no improvement in the yield of product **198** was observed from day 2 to day 14 (analysis of TLC and ¹H NMR spectrum of the crude reaction mixtures), while the intractable reaction products started appearing as reaction time increased.

The major fraction displayed a molecular ion peak at m/z 821.31 $[M + Na]^+$ in the ESI-MS spectrum (calcd 821.27 for $C_{40}H_{46}O_{17}Na$) and the HR-ESI-MS revealed a molecular ion at m/z 821.2618 $[M + Na]^+$, calcd 821.2627 for $C_{40}H_{46}O_{17}Na$, thus confirming its molecular formula to be $C_{40}H_{46}O_{17}$. The IR spectrum of this fraction showed an ester carbonyl group (1768 cm^{-1}) as well as an α,β -unsaturated ester functional group (1715 cm^{-1} for the carbonyl and 1637 cm^{-1} for $CH=CH$). The 1H NMR spectrum of this fraction indicated the presence of signals for the 2,1':4,6-di-*O*-isopropylidene sucrose moiety and signals for two *trans*-acetoxycinnamoyl moieties, represented by two sets of 1,4-disubstituted aromatic ring proton signals at δ 7.12, 7.17, 7.52 and 7.62-7.66 ppm as an A_2B_2 spin system, two *trans*-double bond proton signals at δ 6.43, 6.49 (2 x d, 2H, $J = 15.9\text{ Hz}$, H-8'') and 7.73, 7.79 (2 x d, 2H, $J = 15.9\text{ Hz}$, H-7'') ppm and two acetyl groups at δ 2.32 (1s, 6H, H-11'') ppm. Additionally, the ^{13}C NMR spectrum revealed 40 new signals, including four ester carbonyl carbons, two pairs of double-bond carbons, eight aromatic methine carbons, four quaternary aromatic carbons and two methyl carbons indicating the presence of two *trans*-acetoxycinnamoyl residues. Upon inspection of the COSY spectrum of the new product, the significant correlations observed for β -D-fructose: δ 3.67 (H-1'a) and δ 4.07 (H-1'b); δ 4.91 (H-3') and δ 4.45 (H-4'); H-4' and δ 4.38 (H-5'), this latter with δ 4.52 (H-6'b) and 4.38 (H-6'a) ppm. The notable changes in the 1H NMR spectrum of the new fraction compared to compounds **196** and **197** were the strong downfield chemical shifts for H-3' at δ 4.91 (d, 1H, $J = 6.3\text{ Hz}$) ppm and also for H-5' and H₂-6' at δ 4.52 (m, 1H, H-6'b) and at δ 4.38 (m, 2H, H-5', H-6'a) ppm. The two ester carbonyls were assigned to be at C-3' and C-6' of diacetonide **175** unit based on the correlation peaks between the H-3' proton (δ 4.91 ppm) and C-9'' (δ 167.2 ppm) and between the H₂-6' protons (δ 4.52, 4.38 ppm) and C-9'' (δ 166.7). Thus, the new fraction was assigned to be 3',6'-di-*O*-acetoxycinnamoyl-2,1':4,6-di-*O*-isopropylidene sucrose **198**.

Attempts to drive the reaction in Scheme 2.16 to completion by raising the reaction temperature to 50 °C and by stirring the reaction for extended time (up to 14 days) were not successful as compound **198** was obtained only in 20% yield (Table 2.2, entry 2) along with mixtures of decomposition products. Here, it is important to note that as the reaction time increased, more intractable reaction mixtures were formed providing low yield of **198** and making the purification very difficult.

iii. Acylation with 3.3 mole equiv of **195**:

We then attempted to increase the yield of compound **198**, the precursor for lapathoside D **67**, by reacting diacetone **175** with 3.3 equiv of acid chloride **195** at rt (Scheme 2.17). The reaction gave two new fractions along with the previously obtained compound **198**.

Scheme 2.17. Acylation of diacetone **175** with 3.3 mole equiv acid chloride **195**

The first new fraction (R_f value of 0.67, 3:1 EtOAc-hexanes) showed a molecular ion peak at m/z 1009.31 $[M + Na]^+$ while the HR-ESI-MS spectrum showed a molecular ion peak at m/z 1009.3087 $[M + Na]^+$ (calcd 1009.3101 for $C_{51}H_{54}O_{20}Na$) corresponding to the molecular formula $C_{51}H_{54}O_{20}$. Its IR spectrum indicated the presence of the absorption bands for the ester carbonyl group (1768 cm^{-1}) and an α,β -unsaturated ester group (1718 cm^{-1} for the carbonyl and 1636 cm^{-1} for the $CH = CH$). The 1H NMR spectrum of this fraction confirmed the presence of 2,1':4,6-di-*O*-isopropylidene sucrose moiety and *trans*-acetoxycinnamoyl moiety as described for previous compounds **196-198**. The 1H NMR spectrum of this fraction showed new signals corresponding to three *trans*-acetoxycinnamoyl moieties, represented by three sets of 1,4-disubstituted aromatic ring proton signals at δ 7.06-7.14 (m, 6H, H-3'', H-5'') ppm and 7.48-7.51, 7.60-7.69 (2 x m, 6H, H-2'', H-6'') ppm and three pairs of *trans*-double bond signals at δ 6.38, 6.39, 6.49 (3 x d, 3H, $J = 15.9\text{ Hz}$, H-8''), 7.60-7.69 (m, 2H, H-7'') and 7.78 (d, 1H, $J = 15.9\text{ Hz}$, H-7'') ppm and three acetyl groups at δ 2.29 (s, 9H, H-11'') ppm. In addition, the ^{13}C NMR and DEPT spectra of this fraction revealed the appearance of 33 new signals besides the signals for 2,1':4,6-di-*O*-isopropylidene sucrose moiety, including six ester carbonyl carbons, three pairs of double-bond carbons, twelve aromatic methine carbons, six aromatic quaternary carbons and three methyl carbons that confirmed the presence of three *trans*-acetoxycinnamoyl residues. The 1H NMR spectrum of this fraction showed the anomeric proton at δ 6.11 (d, 1H, $J = 3.3\text{ Hz}$, H-1) ppm, the H-4' at 5.33 (d, 1H, $J = 5.1\text{ Hz}$, H-4') ppm and H-3' at δ 5.57 (m, 1H, H-3') ppm, H-6'b at δ 4.60 (m, 1H, H-6'b) ppm while H-5' and H-6'a at 4.50 (m, 2H, H-5', H-6'a)

ppm. This significant change was again confirmed by the COSY spectrum of this fraction which exhibited that H-3' proton signal (δ 5.57) correlated to H-4' at δ 5.33 ppm which in turn correlated with H-5' and H-6'a protons at overlapping signal δ 4.50 ppm. This latter was correlated with H-6'b proton at δ 4.60 ppm. The three ester carbonyls were assigned to be at C-3', C-4' and C-6' of diacetone **175** unit based on the HMBC cross peaks from H-3' (δ 5.57 ppm) and C-9'' (δ 165.7 ppm); H-4' (δ 5.33 ppm) and C-9'' (δ 165.8 ppm) and from H-6' (δ 4.60, 4.50 ppm) and C-9'' (δ 166.3 ppm). Therefore, this fraction was assigned to be 3',4',6'-tri-*O*-acetoxycinnamoyl-2,1':4,6-di-*O*-isopropylidene sucrose **199**.

The ESI-MS spectrum of a second new fraction (R_f value of 0.89, 3:1 EtOAc-hexanes) showed a molecular ion peak at 1197.36 $[M + Na]^+$ while the HR-ESI-MS spectrum showed a molecular ion peak at m/z 1197.3598 $[M + Na]^+$ (calcd 1197.3574 for $C_{62}H_{62}O_{23}Na$) corresponding to the molecular formula $C_{62}H_{62}O_{23}$. The IR spectrum of this fraction displayed the absorption bands for the ester carbonyl of the acetyl group at 1764 cm^{-1} and the carbonyl and $CH = CH$ at 1718 cm^{-1} and 1635 cm^{-1} , respectively for the α,β -unsaturated ester group. Analysis of this compound was done in a similar fashion to compounds **196-199**. The 1H NMR spectrum indicated four sets of 1,4-disubstituted aromatic ring proton signals at δ 7.04-7.14 (m, 8H, H-3'', H-5'') and 7.45-7.55 and 7.60-7.75 (2 x m, 8H, H-2'', H-6''), four pairs of *trans*-double bond signals at δ 6.38, 6.40, 6.41, 6.58 (4 x d, 4H, $J = 15.9\text{ Hz}$, H-8'') and 7.60-7.75 (m, 3H, H-7''), 7.93 (d, 1H, $J = 15.9\text{ Hz}$, H-7'') and four acetyl groups at δ 2.31 (1s, 12H, H-11'') ppm. Additionally, the ^{13}C NMR and DEPT spectra revealed 62 new signals, including eight ester carbonyl carbons, four pairs of double-bond carbons, sixteen aromatic methine carbons, eight aromatic quaternary carbons and four methyl carbons that were in good agreement with those values for four *trans*-acetoxycinnamoyl residues. Correlations observed in the COSY spectrum of this fraction for α -D-glucose: δ 6.18 (H-1) and δ 3.93 (H-2); H-2 and δ 5.37 (H-3); H-3 and δ 3.72 (H-4); H-4 and δ 3.93 (H-5), this latter was correlated with δ 4.03 (H-6b); H-6b and 3.67 (H-6a); β -D-fructose: δ 3.62 (H-1'a) and δ 4.24 (H-1'b); δ 5.59 (H-3') and δ 5.37 (H-4'); H-4' and δ 4.54 (H-5'), this latter was overlapped with 4.54 (H-6'a and H-6'b) ppm. The four ester carbonyls were assigned to be at C-3, C-3', C-4' and C-6' of diacetone **175** unit based on the HMBC cross peaks from H-3' (δ 5.59 ppm) and C-9'' (δ 166.0 ppm); H-3 and H-4' (δ 5.37 ppm) and C-9''' (δ 165.7 ppm) and from H-2-6' (δ 4.54 ppm) and C-9'' (δ 166.3 ppm). Thus, this fraction

was assigned to be 3,3',4',6'-tetra-*O*-acetoxycinnamoyl-2,1':4,6-di-*O*-isopropylidene sucrose **200**.

Table 2.3. Acylation of diacetone **175** with 3.3 mole equiv acid chloride **195**

Entry	175 : 195 (equiv)	Reaction condition	Reaction time (days)	Product (yield)	Total yield
1	1 : 3.3	rt	9	198 (5%)	47%
				199 (33%)	
				200 (9%)	
2	1 : 3.3	50 °C	2	198 (5%)	49%
				199 (31%)	
				200 (13%)	

The reaction progress was followed by ^1H NMR spectrum where crude samples were taken every 12 h for 9 days to examine the distribution of the products (Table 2.3). Analysis of the ^1H NMR spectrum indicated that diacetone **175** was consumed within 24 h to give a distribution of three products: di-acylated **198**, tri-acylated **199** and tetra-acylated **200** products. The ratios of the products were observed to change as the reaction time increased especially within the first two days, after which there was little change observed. At any instance, the ratio of the tri-acylated product **199** seems to dominate the reaction products. After reacting for nine days, the tri-acylated product **199** was obtained as the major reaction product in 33% yield along with di-acylated product **198** (5%) and tetra-acylated product **200** (9%) (Table 2.3, entry 1). It should be noted that as the reaction time increased, more intractable reaction mixtures were formed making the purification very difficult. When the same reaction was conducted at 50 °C for 2 d, again tri-acylated product **199** was formed as the major product in 31% yield along with products **198** (5%) and **200** (13%) (Table 2.3, entry 2).

iv. *Acylation with 4.4 mole equiv of 195:*

Diacetone **175** on treatment with 4.4 mole equiv of *p*-acetoxycinnamoyl chloride **195** for 4 days (Scheme 2.18) provided compound **200** in 50% yield, mp 138-140 °C together with compound **199** (22% yield). When the same reaction was repeated at 50 °C, the yield of tetra-acylated product **200** improved to 67% while the yield of tri-esterified product **199** remained at 24% (Table 2.4, entry 2). This result indicates that the tetra-substituted compound **200** has greater stability compared to the mono- **196**, **197**, di- **198** and tri-substituted **199** compounds.

Scheme 2.18. Acylation of diacetone **175** with 4.4 mole equiv acid chloride **195**

Table 2.4. Acylation of diacetone **175** with 4.4 mole equiv acid chloride **195**

Entry	175 : 195 (equiv)	Reaction condition	Reaction time (days)	Product (yield)	Total yield
1	1:4.4	rt	4	199 (22%) 200 (50%)	72%
2	1:4.4	50 °C	2	199 (24%) 200 (67%)	91%

2.3.3. Preparation of lapathoside D (**67**)

Successful deprotection of di-*O*-isopropylidenes of compounds **183**, **187** and **188** in the section 2.2.4 utilizing 60% aq. AcOH prompted us to utilize the same reaction conditions for removal of the di-*O*-isopropylidenes of compound **198**. Consequently, diacetone **198** was treated with 60% aq. AcOH at 80 °C for 20 min (Scheme 2.19) and the crude product obtained was recrystallized from EtOAc. A new product was obtained in 49% yield with an R_f value of 0.61 (15:1 EtOAc-MeOH), mp 108-110 °C.

Scheme 2.19. Preparation of lapathoside D (**67**)

The new product showed much lower solubility compared to its parent compound **198** in organic solvents such as EtOAc and CH_2Cl_2 . The ^1H and ^{13}C NMR spectra of the new product indicated the absence of the characteristic signals for the two isopropylidene moieties compared to compound **198**. Again, the distinguished difference noticed between the ^1H

NMR spectra of the new product and the starting compound **198** was that the anomeric (H-1) proton signal shifted to lower field at δ 5.35 ppm corresponding to the same signal in compound **198** at δ 6.12 ppm. The ESI-MS spectrum of the new product showed a molecular ion peak at m/z 741.22 $[M + Na]^+$ corresponding to the expected molecular formula $C_{34}H_{38}O_{17}$ while the HR-ESI-MS showed a molecular ion peak at m/z 741.2001 $[M + Na]^+$ (calcd 741.2001 for $C_{34}H_{38}O_{17}Na$) thus confirming the molecular formula $C_{34}H_{38}O_{17}$. Therefore, it was confirmed that the isopropylidene groups of compound **198** was cleaved successfully and the new product was assigned to be 3',6'-di-*O*-acetoxycinnamoyl sucrose (**201**).

Helm *et. al.*²⁰⁶ reported that 95% ethanolic solution of piperidine or pyrrolidine was very convenient for the removing of acetate protecting groups without affecting the hydroxycinnamate in the fully protected *L*-arabinofuranoside. Subsequently, cleavage of the acetyl groups of compound **201** was achieved based on this method using an ethanolic pyrrolidine suspension of **201** (Scheme 2.19). The crude reaction mixture was passed through a column of strongly acidic ion-exchange resin using 95% EtOAc as eluent to give a new product with a lower R_f value (0.55, 15:1 EtOAc-MeOH) compared to compound **201**. The product was obtained in 70% yield as a white solid, mp 98-100 °C. The ESI-MS spectrum of the new product showed a molecular ion peak at m/z 657.1 $[M + Na]^+$ corresponding to the expected molecular formula $C_{34}H_{38}O_{17}$ while the HR-ESI-MS spectrum showed a molecular ion at m/z 657.1786 $[M + Na]^+$ (calcd 657.1790 for $C_{30}H_{34}O_{15}Na$) thus confirming the molecular formula. The 1H (Figure 3.2) and ^{13}C NMR spectra indicated the loss of the characteristic signals for the two acetyl moieties, represented by the proton signals of the acetyl signals at δ 2.28 (s, 6H, H-11'') and carbon signal at δ 21.0 (2 x C-11'') and also two acetyl ester quaternary carbons at δ 170.9 ppm (2 x C-10''). The success of the deprotection was further indicated in the IR spectrum of the product where peaks corresponding to the acetyl ester carbonyl group at 1764 cm^{-1} have disappeared. The structure of new product was confirmed to be lapathoside D **67** (Figure 2.3) by the spectroscopic analysis and also by comparison to the data reported for the isolated natural product (Table 2.5).⁵⁹

Figure 2.3. ^1H NMR spectrum of lapathoside D **67** (300 MHz, CD_3OD)

Table 2.5. Comparison table for ^1H & ^{13}C data of the isolated & synthetic lapathoside D (300 MHz, CD_3OD , δ in ppm, J in Hz)

Position	Isolated Natural Product ⁵⁹		Synthesized Product 67	
	^1H	^{13}C	^1H	^{13}C
1	5.44 (d, $J = 4.0$)	93.2	5.44(d, $J = 3.6$)	93.2
2	3.43 (dd, $J = 9.7, 4.0$)	73.2	3.44 (m)	73.2
3	3.63 (dd, $J = 9.7, 9.5$)	75.0	3.63 (m)	75.0
4	3.41 (m)	71.5	3.41 (m)	71.4
5	3.98 (m)	74.5	3.91 (m)	74.4
6	3.92 (m), 3.82 (m)	62.6	3.91 (m), 3.80 (m)	62.6
1'	3.62 (2H, m)	65.1	3.61 (2H, m)	65.1
2'		105.1		105.1
3'	5.49 (d, $J = 8.1$)	79.2	5.49 (d, $J = 7.8$)	79.3
4'	4.43 (dd, $J = 8.1, 8.0$)	75.0	4.43 (dd, $J = 7.8, 8.1$)	75.0
5'	4.16 (m)	81.2	4.15 (m)	81.2
6'	4.54 (2H, m)	66.4	4.56 (2H, m)	66.4
1''		127.1		127.2
2'', 6''	7.51 (d, $J = 8.6$)	131.5	7.52 (d, $J = 9.0$)	131.5
3'', 5''	6.80 (d, $J = 8.6$)	116.9	6.81 (d, $J = 8.4$)	116.9
4''		161.6		161.3
7''	7.71 (d, $J = 15.9$)	147.5	7.72 (d, $J = 15.9$)	147.6
8''	6.41 (d, $J = 15.9$)	114.7	6.41 (d, $J = 15.9$)	114.6
9''		168.4		168.4
1'''		127.1		127.2
2''', 6'''	7.48 (d, $J = 8.6$)	131.3	7.48 (d, $J = 9.0$)	131.3
3''', 5'''	6.80 (d, $J = 8.6$)	116.9	6.81 (d, $J = 8.4$)	116.9
4'''		161.5		161.3
7'''	7.66 (d, $J = 15.9$)	147.0	7.67 (d, $J = 15.9$)	147.0
8'''	6.36 (d, $J = 15.9$)	114.8	6.37 (d, $J = 15.9$)	114.8
9'''		169.1		169.1

2.3.4. Deacetylation of compounds **196**, **198** and **199**

Due to the promising biological activities of PSEs, it was of interest to deacetylate compounds **196**, **198** and **199** to study the effect of the acetyl groups on the biological activities.

Consequently, cleavage of the acetyl group in compound **196** was successfully achieved by stirring an ethanolic suspension of this compound with pyrrolidine²⁰⁶ for 15 min at rt followed by passing the crude reaction mixture through a column of strongly acidic ion-exchange resin using 95% EtOAc as eluent (Scheme 2.20). The solvent was then evaporated under reduced pressure to give a syrup which was subjected to silica gel column chromatography using a gradient of CH₂Cl₂-EtOAc as eluent. Evaporation of the solvent gave a white solid (50% yield).

Scheme 2.20. Deacetylation of compound **196**

The ESI-MS spectrum of the new product showed a molecular ion peak at m/z 591.24 $[M + Na]^+$ corresponding to the expected molecular formula C₂₇H₃₆O₁₃ while the HR-ESI-MS spectrum showed a molecular ion peak at m/z 591.2064 $[M + Na]^+$ (calcd 591.2048 for C₂₇H₃₆O₁₃Na) thus confirming the molecular formula C₂₇H₃₆O₁₃. The IR spectrum of this product showed the disappearance of the absorption bands for the acetyl ester carbonyl group at 1768 cm⁻¹ indicating the successful deprotection of the acetyl group. The ¹H and ¹³C NMR spectra indicated the disappearance of the characteristic signals for the acetyl moiety for *trans*-acetoxycinnamoyl residue, represented by the proton signal at δ 2.31 ppm (s, 3H, H-11'') and carbon signal at δ 21.1 ppm (C-11'') and also one acetyl ester quaternary carbon at δ 169.2 ppm (C-10''). Therefore, the new compound was assigned to be 6'-mono-*O*-coumaroyl-2,1':4,6-di-*O*-isopropylidene sucrose **202**.

Deprotection of the acetyl group in compound **198** was successfully achieved similarly according to Scheme 2.21. Purification of the compound on silica gel column chromatography using a gradient of CH₂Cl₂-EtOAc gave a white solid in 63% yield.

Scheme 2.21. Deacetylation of compound **198**

The ESI-MS spectrum of the white solid showed a molecular ion peak at m/z 737.27 $[M + Na]^+$ corresponding to the expected molecular formula $C_{36}H_{42}O_{15}$ while the HR-ESI-MS spectrum showed a molecular ion peak at m/z 737.2418 $[M + Na]^+$ (calcd 737.2416 for $C_{36}H_{42}O_{15}Na$) thus confirming the molecular formula. The 1H and ^{13}C NMR spectra of the new compound indicated the absence of the characteristic signals for the two acetyl moieties for the *trans*-acetoxycinnamoyl residue, represented by the proton signals at δ 2.32 ppm (s, 6H, H-11'') and carbon signals at δ 21.1 ppm (2 x C-11'') and also two acetyl ester quaternary carbons at δ 169.0, 169.1 ppm (2 x C-10'') compared to compound **198**. Therefore, the new compound was assigned to be 3',6'-di-*O*-coumaroyl-2,1':4,6-di-*O*-isopropylidene sucrose **203**.

Similarly, stirring an ethanolic suspension of compound **199** with pyrrolidine gave a new product as a white solid in 46% yield (Scheme 2.22).

Scheme 2.22. Deacetylation of compound **199**

The HR-ESI-MS spectrum of the new product suggested the molecular formula $C_{36}H_{42}O_{15}$ based on a molecular ion peak at m/z 883.2793 $[M + Na]^+$ (calcd 883.2784 for $C_{45}H_{48}O_{17}Na$). The 1H and ^{13}C NMR spectra of the new compound indicated the loss of the characteristic signals for the three acetyl moieties for *trans*-acetoxycinnamoyl residue, represented by the proton signals at δ 2.29 (s, 9H, H-11'') and carbon signals at δ 21.1 (3 x C-11'') and also three acetyl ester quaternary carbons at δ 169.0, 169.1 ppm (3 x C-10''). Thus,

the new compound was assigned to be 3',4',6'-tri-*O*-coumaroyl-2,1':4,6-di-*O*-isopropylidene sucrose **204**.

2.3.5. Summary

From the above results, it is evident that selectivity could be achieved during the acylation of the free hydroxyl groups of diacetone **201** using *p*-acetoxycinnamoyl chloride **195**. The order of reactivities of these free hydroxyl groups towards *p*-acetoxycinnamoyl chloride **195** were in good agreement with those orders for the cinnamoyl chloride. Therefore, di-, tri- and tetra- variants of diacetone **175** could be synthesized with ease in moderate yields.

It was found that a lower temperature such as -30 °C or a higher temperature like 50 °C had very little effect on selectivity in comparison to room temperature. On the other hand, the reaction time did not change the ratio of reaction products. Rather, prolonged reaction time produced more intractable reaction mixtures making the purification very difficult.

The first total synthesis of lapathoside D **67** was successfully achieved in four synthetic steps starting from sucrose **140** in moderate yield. Di-*O*-isopropylidene PSEs analogues **202-204** were successfully prepared by deprotection of the acetyl groups of compounds **196**, **198** and **199** in moderate yield.

2.4. Synthesis of Helonioside A and its analogues

Helonioside A (**69**, Figure 2.4.) was isolated from various medicinal plants and has promising biological activities such as antioxidant and anticancer activities. 3',6'-Di-*O*-feruloylsucrose derivatives are the largest class among the di-substituted PSEs (see Chapter 1). Similar to lapathoside D **67** and compound **184**, helonioside A **69** is a di-substituted PSE but has a feruloyl group as the phenylpropanoid unit attached to the 3',6'-hydroxyls of sucrose **140**. Due to its promising activities, synthesis of 3',6'-di-*O*-feruloylsucrose (helonioside A **69**) and its analogues were attempted. Synthesis of the analogues is important since they are needed to study the anticancer activities.

Figure 2.4. Structure of helonioside A **69**

To perform regioselective acylation on sucrose (as in sections 2.2 and 2.3), protected feruloyl chloride **207** was needed.

2.4.1. Preparation of *p*-acetoxyferuloyl chloride **207**

p-Acetoxyferuloyl chloride **207** was synthesized according to the reported literature procedure.²⁰⁷ The phenolic group of *trans*-ferulic acid **205** was first protected by acetylation with Ac₂O (Scheme 2.23) in order to prevent polymerization after addition of SOCl₂.²⁰⁷ Crystallization of the crude product from 95% EtOH afforded *p*-acetoxyferulic acid **206** in 79% yield, mp 201-204 °C (reported mp 201-204 °C).²⁰⁷

Scheme 2.23. Preparation of *p*-acetoxyferuloyl chloride **207**

Acid chloride **207** was prepared by refluxing a mixture of **206** and SOCl₂ in benzene for 2 h (Scheme 2.4.1). recrystallization from hot toluene afforded the *p*-acetoxyferuloyl chloride **207** in 81% yield, mp 130-133 °C (reported mp 130-133 °C).²⁰⁷

2.4.2. Acylation of diacetone **175** with *p*-acetoxyferuloyl chloride **207**

Regio- and chemoselective acylation of diacetone **175** with *p*-acetoxyferuloyl chloride **207** was attempted for the synthesis of the helonioside A **69** and the desired analogues by using the optimal reaction conditions established for the synthesis of compound **184** and lapathoside D **67**.

Variable mol equiv of compound **207** were used to examine the reactivity of acid chloride **207** towards diacetone **175** and also to obtain various analogues of helonioside A **69**.

(i) *Acylation using 1.1 mol equiv of 207:*

Reaction of diacetone **175** with 1.1 mole equivalent of *p*-acetoxyferuloyl chloride **207** at rt for 3 days (Scheme 2.24), gave three fractions: the major fraction in 31% yield with R_f value of 0.12 (3:2 EtOAc-CH₂Cl₂), mp 147-150 °C was obtained as a white solid and two minor fractions with a higher R_f values (0.21 and 0.6, 3:2 EtOAc-CH₂Cl₂) in 11% and 12% yield respectively.

Scheme 2.24. Acylation of diacetone **175** with 1.1 mol equiv of acid chloride **207**

The HR-ESI-MS spectrum of the first fraction indicated a molecular formula of C₃₀H₄₀O₁₅ based on the molecular ion peak at m/z 663.2268 [M + Na]⁺, calcd 663.2259 for C₃₀H₄₀O₁₅Na. Its IR spectrum indicated the presence of absorption bands for an ester carbonyl group at 1766 cm⁻¹ and an α,β -unsaturated aromatic ester group with the carbonyl group stretching at 1712 cm⁻¹ and *trans*-vinylene (CH = CH) stretching at 1636 cm⁻¹. In addition to the presence of signals corresponding for the 2,1':4,6-di-*O*-isopropylidene sucrose moiety, the ¹H NMR spectrum revealed one set of 1,3,4-trisubstituted aromatic ring proton signals at δ 7.00-7.19 ppm (m, 3H, H-2'', H-5'', H-6''), one *trans*-double bond signals at δ 6.41 ppm (d, 1H, J = 15.9 Hz, H-8'') and 7.64 ppm (d, 1H, J = 15.9 Hz, H-7''), one methoxyl group at δ 3.85 ppm (s, 3H, OCH₃) and one acetyl group at δ 2.32 ppm (1s, 3H, H-11''). Moreover, the ¹³C NMR and DEPT spectra of this major product revealed 12 new signals besides 2,1':4,6-di-*O*-isopropylidene sucrose moiety, including two ester carbonyl carbons, one pair of double-bond carbons, three aromatic methine carbons, three aromatic quaternary carbons, one methoxyl carbon and one methyl carbon that were consistent with one *trans*-acetoxyferuloyl residue. The above data confirmed that compound **175** was successfully

esterified with one *trans*-acetoxyferuloyl moiety. To confirm the position of the *trans*-acetoxyferuloyl moiety, the COSY spectrum of this fraction was measured. The anomeric proton (H-1) signal at δ 6.19 ppm correlated to the H-2 proton at δ 3.73 ppm which in turn correlated to H-3 proton signal at δ 4.05 ppm. The H-3 proton signal was correlated to the H-4 proton at δ 3.59 ppm which in turn correlated to H-5 proton overlapping H-6b proton signal at δ 3.93 ppm. The H-3' proton signal at δ 3.93 ppm correlated to the overlapping H-4' and H-5' proton signals at δ 4.23 ppm. This latter correlated to the overlapping H₂-6' proton signals at δ 4.51 and 4.30 ppm. The ester carbonyl was assigned to be connected at C-6' of the diacetone **175** unit based on the strong downfield shift of H₂-6' (δ 4.51, 4.30 ppm) in comparison to that of diacetone **175** (δ 3.84, 3.61 ppm) and the correlation peaks between H₂-6' (δ 4.51, 4.30 ppm) and an α,β -unsaturated carbonyl carbon C-9'' (δ 167.0 ppm) in the HMBC spectrum of this fraction. Based on these spectroscopic data, the major product was assigned to be 6'-mono-*O*-acetoxyferuloyl-2,1':4,6-di-*O*-isopropylidene sucrose **208**.

The HR-ESI-MS of the minor product having a R_f value of 0.21 and mp 130-132 °C suggested a molecular formula C₃₀H₄₀O₁₅ based on the molecular ion at m/z 663.2255 [M + Na]⁺ (calcd 663.2259 for C₃₀H₄₀O₁₅Na). Similar to compound **208**, its IR spectrum showed the absorption bands for the acetyl ester carbonyl at 1765 cm⁻¹ and α,β -unsaturated ester carbonyl group at 1716 and 1638 cm⁻¹. In addition to signals corresponding to the 2,1':4,6-di-*O*-isopropylidene sucrose moiety, the ¹H spectrum of this product showed proton signals characteristic for one *trans*-acetoxyferuloyl moiety, represented by one set of 1,3,4-trisubstituted aromatic ring proton signals at δ 7.09 and 7.16-7.22 ppm, one *trans*-double bond signal at δ 6.50 (d, 1H, J = 15.9 Hz, H-8'') and 7.77 (d, 1H, J = 15.9 Hz, H-7''), one methoxyl group at δ 3.91 (s, 3H, OCH₃) and one acetyl group at δ 2.05 (1s, 3H, H-11''). Moreover, similar to compound **208**, the ¹³C NMR and DEPT spectra of this minor product revealed 12 new signals besides 2,1':4,6-di-*O*-isopropylidene sucrose moiety that were in agreement with values for one *trans*-acetoxyferuloyl residue. The major differences in the ¹H NMR spectrum of the product compared to compounds **208** and **175** were the characteristic anomeric signal at δ 6.22 (d, 1H, J = 3.6 Hz, H-1) and doublet signal for H-3' at δ 5.03 (d, 1H, J = 7.8 Hz, H-3') with a strong downfield chemical shift and multiplet signal for H-4' at δ 4.87 ppm. The COSY spectrum of the new product showed the H-3' proton signal at δ 5.03 ppm was correlated to the H-4' proton at δ 4.87 ppm and the H-1 proton signal at δ 6.22 ppm correlated to the H-2 proton at δ 3.77 ppm. The feruloyl moiety was assigned to be connected

at C-3' of diacetone **175** unit based on the HMBC cross peaks from H-3' (δ 5.03 ppm) and C-9'' (δ 167.2 ppm). Based on these spectroscopic data, it was concluded that compound **175** was successfully esterified with one *trans*-acetoxyferuloyl moiety at C-3' and this minor product was assigned to be 3'-mono-*O*-acetoxyferuloyl-2,1':4,6-di-*O*-isopropylidene sucrose **209**.

The molecular formula of the last minor fraction with a R_f value of 0.61 and mp 109-110 °C was deduced to be $C_{42}H_{50}O_{19}$ by the HR-ESI-MS spectrum based on the molecular ion peak at m/z 881.2860 $[M + Na]^+$ (calcd 881.2839 for $C_{42}H_{50}O_{19}Na$). The IR spectrum of this product displayed the absorption band for an acetyl carbonyl group absorption at 1766 cm^{-1} and an α,β -unsaturated ester (carbonyl group at 1716 cm^{-1} and $(CH = CH)$ at 1637 cm^{-1}). In the 1H NMR spectrum of this product two sets of 1,3,4-trisubstituted aromatic ring proton signals at δ 7.03-7.12, 7.20 (2 x m, 6H, H-2'', H-5'', H-6''), two *trans*-double bond signals at δ 6.43, 6.48 (2 x d, 2H, $J = 15.9$ Hz, H-8'') and 7.66, 7.77 (2 x d, 2H, $J = 15.9$ Hz, H-7'') ppm, two methoxyl groups at δ 3.87, 3.92 (2 x s, 6H, OCH_3) ppm and two acetyl groups at δ 2.33 (s, 6H, H-11'') ppm. Additionally, the ^{13}C NMR and DEPT spectra of this fraction revealed twenty-four new signals, including four ester carbonyl carbons, two pairs *trans*-double bond carbons, six aromatic methine carbons, six aromatic quaternary carbons, two methoxyl carbons and two acetyl carbons confirming the presence of two *trans*-acetoxyferuloyl residues. In the COSY spectrum of the new compound, the anomeric proton (H-1) signal at δ 6.13 ppm correlated to the H-2 proton at δ 3.79 ppm which in turn correlated to H-3 proton signal at δ 3.88 ppm. The H-3 proton signal was correlated to the H-4 proton at δ 3.63 ppm which in turn correlated to H-5 proton overlapping H-3 proton signal at δ 3.88 ppm. The H-3' proton signal at δ 4.92 ppm was correlated to the H-4' proton at δ 4.46 ppm which in turn correlated to H-5' proton signal at δ 4.38 ppm. This latter was correlated to the overlapping H₂-6' proton signals at δ 4.53 and 4.38 ppm. Since, the H-3' and H₂-6' proton signals were all shifted downfield from their positions in the proton spectrum of diacetone **175**, then 3' and 6' hydroxyls were confirmed acylated. Based on the analysis of the HMBC spectrum of the product, the long-range correlations observed between the ester carbonyl carbons (C-9'') of two *trans*-acetoxyferuloyl groups (δ 167.7 and 166.6 ppm) and H-3' (δ 4.92 ppm) and H₂-6' (δ 4.53, 4.38 ppm) suggested the assignment of the two feruloyl groups at C-3' and C-6' of the diacetone **175** unit. Therefore, this product was assigned to be 3',6'-di-*O*-acetoxyferuloyl-2,1':4,6-di-*O*-isopropylidene sucrose **210**.

(ii) Acylation using 2.2 mol equiv of 207:

When a solution of diacetone **175** was treated with 2.2 mol equiv of *p*-acetoxyferuloyl chloride **207** at rt for 5 days (Scheme 2.25), two fractions were obtained. Upon analysis, the fractions proved to be compound **208** (ca 3% yield) and compound **210** which was obtained, as expected, as the major product in 30% yield.

Scheme 2.25. Acylation of diacetone **175** with 2.2 mole equiv acid chloride **207**

(iii) Acylation using 3.3 mol equiv of 207:

Subsequently, in order to prepare the tri-acylated compounds, diacetone **175** was reacted with 3.3 mole equiv of acid chloride **207** at rt for 2 days according to Scheme 2.26. After workup and chromatographic purification of the crude product using a gradient of CH₂Cl₂-EtOAc as eluent, two fractions were obtained. Di-acylated product **210** was found to be the major reaction product while the other white solid (26% yield) proved to be a new component with a higher R_f value 0.73 (3:2 EtOAc-CH₂Cl₂), mp 135-138 °C. Here it is important to note that when 3.3 equiv of acid chloride **195** was reacted with the diacetone **175**, tri-acylated compound was obtained as the major reaction product in 33% yield.

Scheme 2.26. Acylation of diacetone **175** with 3.3 mole equiv acid chloride **207**

The HR-ESI-MS spectrum of the new component suggested the molecular formula C₅₄H₆₀O₂₃ based on the molecular ion peak at *m/z* 1099.3401 [M + Na]⁺ (calcd 1099.3418 for C₅₄H₆₀O₂₃Na). Its IR spectrum displayed the absorption bands for the ester carbonyl group (1766 cm⁻¹) and the α,β-unsaturated ester carbonyl group (1721 cm⁻¹ for the carbonyl and

1638 cm^{-1} for the (CH = CH) group). In addition to the 2,1':4,6-di-*O*-isopropylidene sucrose moiety, the ^1H NMR spectrum of the new product indicated the presence of *trans*-acetoxyferuloyl moiety as described for compounds **208**, **209** and **210**, except that the new product showed new signals for three *trans*-acetoxyferuloyl moieties, represented by three sets of 1,3,4-trisubstituted aromatic ring proton signals at δ 7.02-7.13, 7.20-7.22 ppm (2 x m, 9H, H-2'', H-5'', H-6''), three *trans*-double bond signals at δ 6.40, 6.42, 6.52 (3 x d, 1H, J = 15.9 Hz, H-8'') and 7.66, 7.68, 7.79 ppm (3 x d, 1H, J = 15.9 Hz, H-7''), two methoxyl groups at δ 3.86, 3.88, 3.93 ppm (3 x s, 9H, OCH_3) and three acetyl groups at δ 2.34 ppm (s, 9H, H-11''). The ^{13}C NMR and DEPT spectra of product revealed 36 new signals besides the 2,1':4,6-di-*O*-isopropylidene sucrose moiety, including six ester carbonyl carbons, three pairs *trans*-double bond carbons, nine aromatic methines carbons, nine aromatic quaternary carbons, three methoxyl carbons and three methyl carbons thus confirming the presence of three *trans*-acetoxyferuloyl residues. Inspection of the COSY spectrum of new product in a similar manner as described for compounds **208**, **209** and **210**, resulted in the following being noted: correlations between H-3' (δ 5.60 ppm) and H-4' (δ 5.38 ppm); this latter is correlated with H-5' and H-6'a at (δ 4.51 ppm) which in turns correlated with δ 4.64 ppm (H-6'b). The notable changes in the ^1H NMR spectrum of the new product were the difference in chemical shift of the anomeric proton observed at δ 6.15 ppm (d, 1H, J = 3.3 Hz, H-1) and strong downfield chemical shifts for H-3' at δ 5.60 ppm (dd, 1H, J = 5.1 Hz, 3.6 Hz, H-3'), for H-4' at 5.38 ppm (d, 1H, J = 5.4 Hz, H-4') and for H-5' and H₂-6' observed at δ 4.64 (m, 1H, H-6'b) and 4.51 ppm (m, 2H, H-5', H-6'a) relative to their unacylated positions. The three ester carbonyls were assigned to be connected at C-3', C-4' and C-6' of diacetone **175** unit based on the HMBC cross peaks from H-3' (δ 5.60 ppm) and C-9'' (δ 165.7 ppm); H-4' (δ 5.38 ppm) and C-9'' (δ 165.8 ppm) and from H₂-6' (δ 4.64, 4.51 ppm) and C-9'' (δ 166.3 ppm). Based on these spectroscopic data, the new product was assigned to be 3',4',6'-tri-*O*-acetoxyferuloyl-2,1':4,6-di-*O*-isopropylidene sucrose **211**.

(iv) *Acylation using 4.4 moles:*

Addition of more equiv of *p*-acetoxyferuloyl chloride **207** to diacetone **175** was envisioned to achieve the tetra-acylated derivative. Thus, acylation of **175** with 4.4 mole equiv of acid chloride **207** for 4 days (Scheme 2.27), followed by workup and chromatographic purification of the crude product using a gradient of CH_2Cl_2 -EtOAc as eluent afforded two fractions: a new major fraction as a white solid with an R_f value of 0.88 (3:2 EtOAc- CH_2Cl_2) in 44% yield, mp 133-135 $^\circ\text{C}$ and compound **211** in 35% yield.

Scheme 2.27. Acylation of diacetone **175** with 4.4 mole equiv acid chloride **207**

The HR-ESI-MS spectrum of the new fraction showed a molecular ion peak at m/z 1317.3980 $[M + Na]^+$ (calcd 1317.3997 for $C_{66}H_{70}O_{27}Na$) corresponding to the molecular formula $C_{66}H_{70}O_{27}$. Its IR spectrum displayed the typical absorption bands for the acetyl carbonyl at 1767 cm^{-1} and the α,β -unsaturated ester carbonyl group at 1721 cm^{-1} and 1637 cm^{-1} . Similarly, as described for compounds **208**, **209**, **210** and **211**, analysis of the white solid using the ^1H NMR spectrum indicated the presence of four *trans*-acetoxyferuloyl moieties, represented by four sets of 1,3,4-trisubstituted aromatic ring proton signals at δ 7.02-7.15 and 7.28-7.33 ppm (2 x m, 12H, H-2'', H-5'', H-6''), four pairs *trans*-double bond signals at δ 6.38, 6.42, 6.43, 6.57 (4 x d, 4H, $J = 15.9\text{ Hz}$, H-8'') and 7.61, 7.66, 7.69, 7.93 ppm (4 x d, 4H, $J = 15.9\text{ Hz}$, H-7''), four methoxyl groups at δ 3.86, 3.87, 3.89, 3.92 (4 x s, 12H, OCH_3) and four acetyl groups at δ 2.33 ppm (each as s, 3H, H-11'') along with 2,1':4,6-di-*O*-isopropylidene sucrose moiety. In addition, the ^{13}C NMR and DEPT spectra of the product revealed new forty eight carbons, including eight ester carbonyl carbons, four pairs *trans*-double bond carbons, twelve aromatic methine carbons, twelve aromatic quaternary carbons, four methoxyl carbons and four methyl carbons that were in agreement with those values for four *trans*-acetoxyferuloyl units. The significant change between the ^1H NMR spectrum of the new product and that of the starting material **175** was that the sugar proton (3', 3, 4', 5' and 6' proton) signals were all shifted downfield from their unacylated positions. The anomeric proton signal was at δ 6.21 (d, 1H, $J = 3.6\text{ Hz}$, H-1) and 3', 3, 4', 5' and 6' proton signals were shifted downfield to δ 5.61 (m, 1H, H-3'), δ 5.42 (m, 2H, H-3, H-4') and δ 4.52-4.63 (m, 3H, H-5', H-6'b, H-6'a) ppm, respectively. The COSY spectrum of the new product showed the H-3' proton signal at δ 5.61 correlated to the H-4' proton at δ 5.42 which in turn correlated to H-5' proton signal at δ 4.58 ppm. This latter overlapped with H-2-6' proton signals at δ 4.52-4.63 ppm. On the other hand, the anomeric proton (H-1) signal at δ

6.21 correlated to the H-2 proton at δ 3.96 which in turn correlated to H-3 proton signal at δ 5.42 ppm. This latter correlated to the H-4 proton at δ 3.70 ppm which in turn correlated to H-5 proton overlapping H-2 proton signal at δ 3.96 ppm. It was concluded that compound **175** was successfully esterified with four *trans*-acetoxyferuloyl moieties. The four ester carbonyls were assigned to be at C-3, C-3', C-4' and C-6' of diacetonide **175** unit based on the HMBC cross peaks from H-3' (δ 5.61 ppm) and C-9'' (δ 166.3 ppm); H-3 and H-4' (δ 5.42 ppm) and C-9''' (δ 165.7 and 166.0 ppm) and from H₂-6' (δ 4.52-4.63 ppm) and C-9'' (δ 167.3 ppm). Therefore, the new product was assigned to be 3,3',4',6'-tetra-*O*-acetoxyferuloyl-2,1':4,6-di-*O*-isopropylidene sucrose **212**.

2.4.3. Acetal deprotection of diacetonides **208** and **210-212**

As described earlier, 60% aq. AcOH at 80 °C for 20 min was found to be very successful deprotection conditions for the acetal moieties. Consequently, this method was applied for the cleavage of the di-*O*-isopropylidene groups of diacetonides **208** and **210-212** according to Schemes 2.28-2.31.

After deprotection of the diacetonide **208** according to Scheme 2.28, TLC analysis (3:2 EtOAc-CH₂Cl₂) revealed the formation of a new compound having a lower R_f value of 0.08 (9:1 EtOAc-MeOH) than the starting material **208**. The new product was obtained as a white solid in 86% yield, mp 168-170 °C after recrystallization from EtOAc. This product showed lower solubility compared to compound **208** in organic solvents such as EtOAc and CH₂Cl₂ due to increased hydrophilicity (almost similar to sucrose).

Scheme 2.28. Acetal deprotection of the diacetonide **208**

The ESI-MS spectrum of the new product showed a molecular ion peak at m/z 583.10 [M + Na]⁺ (calcd 583.17 for C₂₄H₃₂O₁₅Na). The HR-ESI-MS spectrum confirmed the molecular formula C₂₄H₃₂O₁₅ based on a molecular ion peak at m/z 583.1628 [M + Na]⁺ (calcd 583.1633 for C₂₄H₃₂O₁₅Na). Detailed analysis of the ¹H and ¹³C NMR spectroscopic data of the new product with the help of DEPT, ¹H-¹H COSY, HMQC and HMBC

experiments revealed the disappearance of the characteristic signals for the two isopropylidene moieties as compared to compound **208**. The significant change between the ^1H NMR spectrum of the new product and that of the starting material compound **208**, was that the anomeric proton signal at δ 5.39 ppm and the sugar proton peaks were shifted upfield (in compound **208** anomeric proton peak was observed at δ 6.19 ppm). Therefore, it was confirmed that the di-*O*-isopropylidene groups of compound **208** were cleaved successfully. Hence, the new compound was assigned to be 6'-mono-*O*-acetoxyferuloylsucrose **213**.

After that, the cleavage of di-*O*-isopropylidene groups of the diacetonide **210** was achieved in a similar fashion according to Scheme 2.29. Recrystallization of the crude product using EtOAc furnished a new white solid in 89% yield showing a lower R_f value (0.56, 9:1 EtOAc-MeOH) compared to its parent compound **210**, mp 128-130 °C.

Scheme 2.29. Acetal deprotection of the diacetonide **210**

Again, the new product displayed lower solubility compared to compound **210** in organic solvents such as EtOAc and CH_2Cl_2 . The distinguished change between the ^1H NMR spectra of new product and that of the starting material **210** was that the characteristic signals for the two isopropylidene moieties were missing and the anomeric proton signal (H-1) shifted upfield to δ 5.45 ppm relative to compound **210** (where H-1 proton signal was at δ 6.13 ppm). The ESI-MS spectrum of compound **214** exhibited a molecular ion peak at m/z 801.25 $[\text{M} + \text{Na}]^+$ corresponding to the expected molecular formula $\text{C}_{36}\text{H}_{42}\text{O}_{19}$ while the HR-ESI-MS spectrum showed a molecular ion at m/z 801.2235 $[\text{M} + \text{Na}]^+$ (calcd 801.2213 for $\text{C}_{36}\text{H}_{42}\text{O}_{19}\text{Na}$). Therefore, it was concluded that the isopropylidene groups of compound **210** were cleaved successfully. Considering all these data, the new compound was assigned to be 3',6'-di-*O*-acetoxyferuloylsucrose **214**.

On the other hand, reaction of diacetonide **211** according to Scheme 2.30 followed by column chromatography of the crude mixture using a gradient of CH_2Cl_2 -EtOAc as eluent provided a white solid with lower R_f value of 0.49 (9:1 EtOAc-MeOH) in 67% yield, mp 128-130 °C.

Scheme 2.30. Acetal deprotection of the diacetone **211**

The ^1H and ^{13}C NMR spectra of the white solid indicated the loss of the characteristic signals for the two isopropylidene moieties. Again, the anomeric proton (H-1) signal shifted upfield to δ 5.49 ppm. The HR-ESI-MS spectrum showed the expected molecular ion peak at m/z 1019.2774 $[\text{M} + \text{Na}]^+$ (calcd 1019.2792 for $\text{C}_{48}\text{H}_{52}\text{O}_{23}\text{Na}$) corresponding to the expected molecular formula $\text{C}_{48}\text{H}_{52}\text{O}_{23}$ while the ESI-MS spectrum showed a molecular ion peak at m/z 1019.23 $[\text{M} + \text{Na}]^+$. Based on the above facts, we concluded that the isopropylidene groups of compound **211** were cleaved successfully. Hence, the white solid was assigned to be 3',4',6'-*O*-tri-*O*-acetoxyferuloylsucrose **215**.

Similarly, the di-*O*-isopropylidene deprotection of the diacetone **212** was achieved according to Scheme 2.31. A new product having a lower R_f value of 0.87 (9:1 EtOAc-MeOH) was obtained as a white solid in 87% yield (mp 127-129 $^{\circ}\text{C}$).

Scheme 2.31. Acetal deprotection of the diacetone **212**

Again, the ^1H and ^{13}C NMR spectra of the new product indicated the disappearance of the characteristic signals for the di-*O*-isopropylidene moieties. In the ^1H NMR spectrum, the anomeric proton (H-1) signal shifted upfield to δ 5.56 ppm. The molecular formula of the new product was assigned to be $\text{C}_{60}\text{H}_{62}\text{O}_{27}$ based on the HR-ESI-MS spectrum which showed a molecular ion peak at m/z 1237.3373 $[\text{M} + \text{Na}]^+$, calcd 1237.3371 for $\text{C}_{60}\text{H}_{62}\text{O}_{27}\text{Na}$. Hence, it was concluded that the di-*O*-isopropylidene groups of compound **212** were cleaved

successfully. Therefore, the new product was assigned to be 3,3',4',6'-*O*-tetra-*O*-acetoxyferuloylsucrose **216**.

2.4.4. Deacetylation of compounds 213-216

Several deacetylation methods such as methanolic ammonia at -10 °C,¹⁸⁶ NaOMe-MeOH (Zemplén method),²⁰⁸ sodium-dry MeOH,¹⁹⁵ primary amines,¹⁷⁵ pyrrolidine-95% ethanol (rt),²⁰⁶ piperidine-95% ethanol (rt or 50 °C)²⁰⁶ for fully or partially protected sugar esters are known in the literature. Hatfield *et. al.*²⁰⁷ reported the successful deacetylation of methyl-5-*O*-acetylferuloyl- α -*L*-arabinofuranoside with piperidine-95% ethanol (rt) in good yield. The success of the removal of acetyl protecting group from the feruloyl moiety is due to the fact that the ferulic acid ester bond is more strong than an acetate ester, which allows the differentiation between the two acyl groups.²⁰⁶ Hence, cleavage of the acetyl groups of compound **213** was successfully achieved by stirring an ethanolic suspension of this compound with piperidine for 7 h at rt (Scheme 2.32). After quenching with AcOH, the crude reaction mixture was subjected to silica gel column chromatography using a gradient of CH₂Cl₂-EtOAc-MeOH solvent system as eluent.

Scheme 2.32. Deacetylation of compound **213**

The solvent was evaporated under diminished pressure to furnish a white solid with a lower *R_f* value than compound **213** in 72% yield (mp 133-135 °C). The IR spectrum of the new product showed the disappearance of the absorption bands for the acetyl ester carbonyl group at 1754 cm⁻¹. The molecular formula of the new product was assigned to be C₂₂H₃₀O₁₄ by the HR-ESI-MS spectrum based on the observed molecular ion peak at *m/z* 541.1516 [M + Na]⁺ (calcd 541.1528 for C₂₂H₃₀O₁₄Na) while the ESI-MS spectrum showed the molecular ion peak at *m/z* 541.09 [M + Na]⁺ (calcd 541.16 for C₂₂H₃₀O₁₄Na). By the spectroscopic analysis with the help of DEPT, ¹H-¹H COSY, HMQC and HMBC experiments, the major difference between the ¹H and ¹³C NMR spectra of new product and that of the starting compound **213** was that the characteristic signals for one acetyl moiety of the *trans*-acetoxyferuloyl moiety, represented by the proton signal of acetyl group signals at δ 2.27 ppm (s, 3H, H-11'') and carbon signal at δ 20.5 ppm (C-11'') and also one acetyl ester

quaternary carbon at δ 170.6 ppm (C-10'') were disappeared. Based on these spectroscopic data, it was concluded that the deprotection of acetyl group of compound **213** was successfully achieved and the new product was assigned to be 6'-mono-*O*-feruloylsucrose **217**.

Similarly, an ethanolic suspension of compound **214** with piperidine for 3 h at rt (Scheme 2.33), followed by column chromatographic purification of the crude product using a gradient of CH₂Cl₂-EtOAc-MeOH solvent system as eluent afforded a white solid with a lower R_f value of 0.49 (9:1 EtOAc-MeOH) than the starting compound **214** in 68% yield, mp 154-156 °C.

Scheme 2.33. Deacetylation of compound **214**

The IR spectrum of the white solid indicated the absence of the absorption bands for the acetyl ester carbonyl group at 1762 cm⁻¹. The HR-ESI-MS of the white solid displayed a molecular ion at m/z 717.1984 [M + Na]⁺ (calcd 717.2001 for C₃₂H₃₈O₁₇Na) corresponding to the expected molecular formula C₃₂H₃₈O₁₇ while the ESI-MS spectrum showed molecular ion peak at m/z 717.21 [M + Na]⁺. Again, the ¹H and ¹³C NMR spectra of the white solid indicated that the disappearance of the characteristic signals for the two acetyl moieties of the *trans*-acetoxyferuloyl residue, represented by the proton signal of acetyl group signals at δ 2.24 ppm (s, 6H, H-11'') and carbon signals at δ 20.5, 20.9 ppm (2 x C-11'') and also two acetyl ester quaternary carbons at δ 170.5 ppm (2 x C-10''). Therefore, it was concluded that the deprotection of acetyl group of compound **214** was successfully achieved. The white solid was assigned to be 3',6'-di-*O*-feruloylsucrose or helonioside A **69** (Figure 2.5) by detailed spectroscopic analysis and by comparison to the data reported for the isolated natural product^{62, 64} (Table 2.6).

Figure 2.5. ^1H NMR spectrum of helonioside A **69** (300 MHz, CD_3OD)

Table 2.6. Comparison table for ^1H & ^{13}C data of synthetic & isolated helonioside A (300 MHz, CD_3OD , δ in ppm, J in Hz)

Position	Isolated Natural Product ^{62, 64}		Synthesized Product 69	
	^1H	^{13}C	^1H	^{13}C
1	5.45 (d, $J = 3.6$)	93.1	5.45 (d, $J = 3.6$)	93.1
2		73.1		73.2
3		75.0 ^a		75.0
4		71.4		71.4
5		74.4 ^a		74.4
6		62.7		62.7
1'		65.2		65.2
2'		105.1		105.1
3'	5.48 (d, $J = 8.0$)	79.3	5.50 (d, $J = 7.8$)	79.2
4'		75.0 ^a		75.0
5'		81.3		81.3
6'		66.2		66.2
1''		127.7		127.7
2''	7.16 (d, $J = 1.6$)	112.1	7.20 (d, $J = 1.5$)	112.1
3''		149.3		149.4
4''		150.7		150.7
5''	6.81 (d, $J = 8.4$)	116.5	6.82 (d, $J = 8.1$)	116.5
6''	7.08 (dd, $J = 1.6, 8.4$)	124.2	7.10 (dd, $J = 1.5, 8.4$)	124.2
7''	7.68 (d, $J = 16.0$)	147.8	7.72 (d, $J = 15.9$)	147.8
8''	6.44 (d, $J = 16.0$)	115.1	6.44 (d, $J = 15.9$)	115.1
9''		168.3		168.3
C-OCH ₃	3.88 (3H, s)	56.5	3.90 (3H, s)	56.5
1'''		127.7		127.7
2'''	7.20 (d, $J = 2.0$)	111.7	7.24 (d, $J = 1.5$)	111.7
3'''		149.3		149.4
4'''		150.7		150.7

Table 2.6. Contd.....

Position	Isolated Natural Product ^{62, 64}		Synthesized Product 69	
	¹ H	¹³ C	¹ H	¹³ C
5'''	6.81 (d, <i>J</i> = 8.4)	116.5	6.82 (d, <i>J</i> = 8.1)	116.5
6'''	7.12 (dd, <i>J</i> = 2.0, 8.4)	124.2	7.14 (dd, <i>J</i> = 1.5, 8.4)	124.2
7'''	7.62 (d, <i>J</i> = 16.0)	147.2	7.66 (d, <i>J</i> = 15.9)	147.3
8'''	6.36 (d, <i>J</i> = 16.0)	114.8	6.41 (d, <i>J</i> = 15.9)	114.9
9'''		169.0		169.1
C-OCH ₃	3.88 (3H, s)	56.5	3.90 (3H, s)	56.5

^a Signals bearing the same alphabetical superscript may be interchanged.

Having compound **215** in hand, the next step was carried out in similar fashion as described for compound **214** according to Scheme 2.34. Chromatographic purification of the crude product furnished a white solid with a lower *R_f* value (0.46, 9:1 EtOAc-MeOH) than the starting compound **215** in 65% yield (mp 99-101 °C).

Scheme 2.34. Deacetylation of compound **215**

Its IR spectrum revealed the loss of the absorption bands for the acetyl ester carbonyl group at 1764 cm⁻¹. The HR-ESI-MS of the white solid suggested the elemental formula C₄₂H₄₆O₂₀ based on the molecular ion at *m/z* 893.2478 [M + Na]⁺ (calcd 893.2475 for C₄₂H₄₆O₂₀Na) while the ESI-MS spectrum showed a molecular ion peak at *m/z* 893.23 [M + Na]⁺ (calcd 893.26 for C₄₂H₄₆O₂₀Na). The significant change between the ¹H and ¹³C NMR spectra of the white solid and that of starting compound **215**, was only the absence of the characteristic signals for the three acetyl moieties of the *trans*-acetoxyferuloyl residues, represented by the proton signal of acetyl group signals at δ 2.26 ppm (s, 9H, H-11'') and carbon signals at δ 20.5 ppm (3 x C-11'') and also three acetyl ester quaternary carbons at δ 170.4 ppm (3 x C-10''). Therefore, the above facts suggested the success of the deprotection of acetyl group of compound **215**. By considering all the above data and by comparison to the data reported for the isolated natural product⁸⁰ (Table 2.7), the white solid was assigned to be 3',4',6'-tri-*O*-feruloylsucrose **117** (Figure 2.6).

Figure 2.6. ^1H NMR spectrum of 3',4',6'-tri-*O*-feruloylsucrose **117** (300 MHz, CD_3OD)

Table 2.7. Comparison table for ^1H & ^{13}C data of synthetic & isolated 3',4',6'-tri-*O*-feruloylsucrose (300 MHz, CD_3OD , δ in ppm, J in Hz)*

Position	Isolated Natural Product ⁸⁰		Synthesized Product 117	
	^1H	^{13}C	^1H	^{13}C
1	5.52 (d, $J = 3.6$)	93.6	5.51 (d, $J = 3.3$)	93.6
2	4.02 (m)	74.4	3.52 (m)	73.2
3	3.45 (dd, $J = 9.8, 8.0$)	73.1	3.80 (m)	75.0
4	3.68 (dd, $J = 9.8, 8.2$)	75.0	3.52 (m)	71.2
5	3.41 (m)	71.7	4.05 (m)	74.6
6	3.84 (m), 3.89 (m)	62.8	4.05 (m), 3.90 (m)	62.4
1'	4.54 (m)	66.2 ^a	3.73 (2H, m)	64.7
2'		103.6		105.8
3'	5.61 (d, $J = 7.9$)	79.3 ^a	5.81 (m)	77.1
4'	4.56 (dd, $J = 1.6, 8.4$)	74.2 ^a	5.81 (m)	77.1
5'	4.21 (m)	81.1	4.47 (m)	78.9
6'	4.54 (m), 4.37 (m)	65.9 ^a	4.62 (2H, m)	65.7
1''		126.8		127.4
2''	7.10 (br s)	111.8	6.94 (m)	111.6
3''		149.3		149.2
4''		150.6		150.6
5''	6.80 (d, $J = 7.9$)	116.5	6.74 (m)	116.5
6''	7.01 (br d, $J = 7.9$)	124.3	6.94 (m)	124.3
7''	7.70 (d, $J = 15.9$)	148.1	7.70 (d, $J = 15.9$)	148.2
8''	6.44 (d, $J = 15.9$)	114.7	6.41 (d, $J = 15.9$)	114.4
9''		168.5		168.2
C-OCH ₃	3.81 (3H, s)	56.6	3.72 (3H, s)	56.5
1'''		127.6		127.5
2'''	7.16 (br s)	111.8	7.17 (br s)	111.8
3'''		149.3		149.2

Table 2.7. Contd.....

Position	Isolated Natural Product ⁸⁰	¹³ C	Synthesized Product 117	¹³ C
	¹ H		¹ H	
4'''		150.6		150.7
5'''	6.75 (d, <i>J</i> = 8.5)	116.5	6.74 (m)	116.5
6'''	7.10 (br d, <i>J</i> = 8.5)	124.3	7.08 (br d, <i>J</i> = 8.4)	124.4
7'''	7.64 (d, <i>J</i> = 15.9)	147.2	7.54 (d, <i>J</i> = 15.9)	147.4
8'''	6.40 (d, <i>J</i> = 15.9)	115.2	6.29 (m)	114.9
9'''		169.1		168.7
C-OCH ₃	3.84 (3H, s)	56.4	3.83 (3H, s)	56.4
1'''		127.7		127.6
2'''	7.16 (br s)	112.2	6.94 (m)	112.1
3'''		149.3		149.2
4'''		150.7		150.7
5'''	6.81 (d, <i>J</i> = 8.0)	116.5	6.74 (m)	116.4
6'''	7.08 (br d, <i>J</i> = 8.0)	124.3	6.94 (m)	124.4
7'''	7.64 (d, <i>J</i> = 15.9)	147.4	7.54 (d, <i>J</i> = 15.9)	147.4
8'''	6.36 (d, <i>J</i> = 15.9)	115.0	6.29 (m)	114.6
9'''		168.3		167.9
C-OCH ₃	3.84 (3H, s)	56.6	3.77 (3H, s)	56.4

^a Signals bearing the same alphabetical superscript may be interchanged.

*Interestingly, the ¹H and ¹³C NMR of the synthesized compound **117** did not match with data of the reported literature values.⁸⁰ At this point, we presume that the structure of the isolated natural product is wrongly assigned as the feruloyl groups present on C-3', C-4' and C-6' positions of sucrose. Further investigations might be needed to correctly assign the structure of isolated natural product.

Ethanol solution of compound **216** on treatment with piperidine for 4 h at rt (Scheme 2.35) provided a new product with a lower R_f value (0.73) than compound **216** as revealed by TLC analysis (9:1 EtOAc-MeOH) as a white solid in 76% yield, mp 123-125 °C.

Scheme 2.35. Deacetylation of compound **216**

The IR spectrum of the new product displayed the loss of the absorption bands for the acetyl ester carbonyl group at 1765 cm⁻¹. The HR-ESI-MS of the new product revealed the expected molecular formula C₅₂H₅₄O₂₃ from the observed molecular ion at *m/z* 1069.2926 [M + Na]⁺

(calcd 1069.2948 for $C_{52}H_{54}O_{23}Na$) while the ESI-MS spectrum showed a molecular ion peak at m/z 1069.25 $[M + Na]^+$ (calcd 1069.31 for $C_{52}H_{54}O_{23}Na$). Again, spectroscopic analysis using the 1H and ^{13}C NMR spectra of the new product indicated the disappearance of the characteristic signals for the four acetyl moieties of the *trans*-acetoxyferuloyl residues, represented by the proton signal of acetyl group signals at δ 2.29, 2.30, 2.31, 2.32 ppm (4 x s, 12H, H-11'') and carbon signals at δ 20.7 ppm (4 x C-11'') and also four acetyl ester quaternary carbons at δ 168.4, 168.7, 168.8 ppm (4 x C-10'') in comparison to compound **216**. Hence, the new product was assigned to be 3,3',4',6'-tetra-*O*-feruloylsucrose **218**.

2.4.5. Preparation of conformationally restricted PSEs analogues 219-221

Phenylpropanoid esters of sucrose natural products are reported to be potential leads for anticancer drugs. Selected diacetoneides **210-212** were subjected to deprotection of the acetyl groups to prepare the conformationally restricted PSEs analogues in order to investigate the effect of free phenolic hydroxyl groups on the anticancer activities of PSEs and also to study the structure activity relationship (SAR).

Thus, treatment on stirred ethanolic suspension of compound **210** with piperidine for 9 h at rt according to Scheme 2.36, followed by column chromatographic purification using a gradient of CH_2Cl_2 -EtOAc as eluent afforded a new product with a lower R_f values (0.25) than their parent compound **210** as revealed by TLC analysis (3:1 EtOAc-hexanes).

Scheme 2.36. Deacetylation of isopropylidene compound **210**

The new product was obtained as a white solid in 61% yield, mp 125-127 °C. The IR spectrum of the new product showed the disappearance of the absorption band for the acetyl ester carbonyl group at 1766 cm^{-1} . The notable change between the 1H and ^{13}C NMR spectra of new product and that of parent compound **210** was the loss of the characteristic signals for the two acetyl moieties of the *trans*-acetoxyferuloyl residue, represented by the proton signal of acetyl group signals at δ 2.33 ppm (s, 6H, H-11'') and carbon signal at δ 20.7 ppm (2 x C-11'') and also acetyl ester quaternary carbons at δ 168.8 ppm (2 x C-10''). The above data

indicated the successful deprotection of the two acetyl groups of the starting compound **210**. The HR-ESI-MS of the new product suggested the molecular formula $C_{38}H_{46}O_{17}$ based on the observed molecular ion peak at m/z 797.2636 $[M + Na]^+$ (calcd 797.2627 for $C_{38}H_{46}O_{17}Na$) while the ESI-MS spectrum showed a molecular ion peak at m/z 797.28 $[M + Na]^+$, calcd 797.27 for $C_{38}H_{46}O_{17}Na$. Based on these spectroscopic data analysis using the 1H and ^{13}C NMR, DEPT, 1H - 1H COSY, HMQC and HMBC experiments, the new product was assigned to be 3',6'-di-*O*-feruloyl-2,1':4,6-di-*O*-isopropylidene sucrose **219**.

Similarly, when an ethanolic suspension of compound **211** was treated with piperidine for 4 h at rt (Scheme 2.37) and the product purified, a new compound with a lower R_f value (0.27, 3:1 EtOAc-hexanes) was obtained as a white solid in 71% yield, mp 145-147 °C.

Scheme 2.37. Deacetylation of isopropylidene compound **211**

Its IR spectrum displayed the disappearance of the absorption band for the acetyl ester carbonyl group at 1766 cm^{-1} . The spectroscopic analysis using the 1H and ^{13}C NMR spectra of the new compound revealed that the characteristic signals for the three acetyl moieties of the *trans*-acetoxyferuloyl residue, represented by the proton signal of the acetyl group signals at δ 2.34 ppm (s, 9H, H-11'') and carbon signal at δ 20.7 ppm (3 x C-11'') and also acetyl ester quaternary carbons at δ 168.6, 168.7, 168.8 ppm (3 x C-10''). The above data indicated the success of the deprotection of acetyl groups of the starting compound **211**. The molecular formula of the new compound was assigned to be $C_{48}H_{54}O_{20}$ by the HR-ESI-MS spectrum based on the molecular ion peak at m/z 973.3078 $[M + Na]^+$ (calcd 973.3101 for $C_{48}H_{54}O_{20}Na$) and the ESI-MS spectrum displayed a molecular ion peak at m/z 973.27 $[M + Na]^+$ (calcd 973.32 for $C_{48}H_{54}O_{20}Na$). Hence, the new compound was assigned to be 3',4',6'-tri-*O*-feruloyl-2,1':4,6-di-*O*-isopropylidene sucrose **220**.

After successful deacetylation of compounds **210** & **211**, deprotection of the acetyl group of compound **212** was successfully achieved in a similar fashion according to Scheme 2.38. After workup and column chromatographic purification of the crude reaction mixture using a gradient of CH_2Cl_2 -EtOAc as eluent furnished a new white solid in 73% yield

showing a lower R_f value (0.45) than their parent compound **212** as revealed by TLC analysis (3:1 EtOAc-hexanes).

Scheme 2.38. Deacetylation of isopropylidene compound **212**

The IR spectrum of the new product indicated the disappearance of the absorption bands for the acetyl ester carbonyl group at 1767 cm^{-1} . The significant change in the ^1H and ^{13}C NMR spectra of the white solid in comparison to compound **212** was the disappearance of the characteristic signals for the four acetyl moieties of the *trans*-acetoxyferuloyl residue, represented by the proton signal of acetyl group signals at δ 2.33 ppm (s, 12H, H-11'') and carbon signal at δ 20.7 ppm (4 x C-11'') and also acetyl ester quaternary carbons at δ 168.6, 168.7, 168.8 ppm (4 x C-10''). The HR-ESI-MS spectrum of the white solid revealed the molecular formula $\text{C}_{58}\text{H}_{62}\text{O}_{23}$ based on the molecular ion peak at m/z 1149.3570 $[\text{M} + \text{Na}]^+$, calcd 1149.3574 for $\text{C}_{58}\text{H}_{62}\text{O}_{23}\text{Na}$. Therefore, the above data indicated the success of the deprotection of acetyl groups of the starting compound **212**, the white solid was assigned to be 3,3',4',6'-tetra-*O*-feruloyl-2,1':4,6-di-*O*-isopropylidene sucrose **221**.

2.4.6. Summary

Regio- and chemoselective esterification of 2,1':4,6-di-*O*-isopropylidene sucrose **175** with *p*-acetoxyferuloyl chloride **207** at four free hydroxyl groups was successfully achieved in moderate yields and their reactivities were in accordance with the order previously described for cinnamoyl chloride and *p*-acetoxycinnamoyl chloride **195** [$6'\text{-OH} > 3'\text{-OH} > 4'\text{-OH} > 3\text{-OH}$]. By inspection of the ^1H NMR spectra of the crude products during the esterification reactions of diacetone **175** with different mole equiv of cinnamoyl chloride, *p*-acetoxycinnamoyl chloride **195** and *p*-acetoxyferuloyl chloride **207**, we noticed the similar products distribution. Interestingly, it was found that purification process in the case of *p*-acetoxyferuloyl chloride **207** was clean compared to the other two acylating agents. For this reason, the mono-derivative 6'-mono-*O*-feruloylsucrose **217** and compound **221** were successfully achieved along with di-, tri- and tetra- variants of the diacetone **175** with ease

in moderate yields. On the other hand, at that moment we could not obtain pure 6'-mono-*O*-coumaroyl sucrose and 3,3',4',6'-tetra-*O*-coumaroyl-2,1':4,6-di-*O*-isopropylidene sucrose after tedious purification. Hence, it was concluded that the nature of acylating agent might play an important role in the purification process. The first total syntheses of helonioside A **69** and 3',4',6'-tri-*O*-feruloyl sucrose **117** were successfully achieved in four synthetic steps each starting from sucrose **140** in moderate yields. Conformationally restricted PSE analogues **219-221** were successfully prepared by deprotection of the acetyl groups of compounds **210-212** in moderate yields.

After successful syntheses of di-substituted natural PSEs and having 3',6'-di-*O*-acetoxycinnamoyl sucrose **201** in our hands, we then targeted with confidence the synthesis of 6,3',6'-tri-*O*-substituted PSE lapathoside C **116** and 6,1',3',6'-tetra-*O*-substituted natural PSEs vanicoside B **128**, vanicoside D **129** and lapathoside A **132**.

2. 5. Synthesis of Lapathoside C and its analogues

Sucrose **140**, the core molecule of the isolated natural PSEs (details described in Chapter 1) was acylated primarily at four positions – the 6, 1', 3', and 6' hydroxyls. Consequently, it was believed that selective acylation of 6, 1', 3', and 6' hydroxyls of sucrose would provide nearly all these natural phenylpropanoid sucrose esters. Interestingly to make things complicated, sucrose has 8 free hydroxyl groups - 3 primary (6, 1', 6') hydroxyls and 5 secondary (2, 3, 4, 3', 4') hydroxyls. Among the four possible positions, sucrose **140** was successfully acylated at 3' and 6' hydroxyls. Now, the reactivity of 6 and 1' hydroxyl groups towards acylation was of interest. As 3',6'-di-*O*-acetoxycinnamoyl sucrose **201** was in our hand and also the structure of lapathoside C **116**, lapathoside A **132**, vanicoside B **128** and vanicoside D **129** (Figure 2.7) are simpler than other tri- and tetra-substituted natural PSEs, it was then decided to target the synthesis of these compounds. These compounds also have promising biological activity.

Figure 2.7. Structure of natural products **116**, **128**, **129** & **132**

The widely used synthetic approach in carbohydrate chemistry involves the following steps: i) selective protection of the primary hydroxyls; ii) protection of the secondary hydroxyls; iii) deprotection of the primary hydroxyls; iv) acylation of the primary hydroxyls and finally, v) deprotection of the secondary hydroxyls. Natural products **116**, **128**, **129** & **132** can be synthesized from compound **201** by using the above approach. However, the following drawbacks were found: i) sufficient material is required for each step; ii) it is time consuming; iii) deprotection reaction is more complicated because sucrose esters are unstable and also labile to heat, acid or alkali.

It was well established that primary hydroxyl groups (6-OH and 1'-OH) of sucrose **140** are more reactive than other hydroxyls.^{132, 134} Based on this idea and also to reduce the complexity arising from multi-step synthesis, it was then decided to synthesize lapathoside C **116**, vanicoside B **128**, vanicoside D **129** and lapathoside A **132** directly from 3',6'-di-*O*-acetoxy-cinnamoyl sucrose **201** using controlled conditions as shown in Scheme 2.39.

Scheme 2.39. Proposed synthesis of natural products **116**, **128**, **129** and **132**

2.5.1. Synthesis of 6-*O*-acetoxyferuloyl-3',6'-di-*O*-acetoxy-cinnamoylsucrose **222** and 3,6-di-*O*-acetoxyferuloyl-3',6'-di-*O*-acetoxy-cinnamoylsucrose **226**

At the outset, when a solution of 3',6'-di-*O*-acetoxy-cinnamoyl sucrose **201** in dry CH₂Cl₂ was treated with 1.1 mole equiv of *p*-acetoxyferuloyl chloride **207** in the presence of 10 mole equiv dry pyridine and 4 Å molecular sieves powder (Scheme 2.40) for 24 h, TLC analysis (3:1 EtOAc-hexanes) revealed that the crude mixture had four components. After purified by column chromatography of the crude product using a gradient of CH₂Cl₂-EtOAc as eluent and followed by PTLC, the major product was obtained as a white solid with an R_f value 0.06 (3:1 EtOAc-hexanes) in 36% yield along with *ca* 7% yield of a minor product (R_f value of 0.11, 3:1 EtOAc-hexanes) and other two unidentified products.

Scheme 2.40. Synthesis of compounds **222** and **226**

The molecular formula of the major product was assigned as $C_{46}H_{48}O_{21}$ by the HR-ESI-MS spectrum based on the molecular ion peak at m/z 959.2549 $[M + Na]^+$, calcd 959.2580 for $C_{46}H_{48}O_{21}Na$ while the ESI-MS spectrum showed a molecular ion peak at m/z 959.14 $[M + Na]^+$ (calcd 959.27 for $C_{46}H_{48}O_{21}Na$). Its IR spectrum displayed the absorption bands for the ester carbonyl group (1767 cm^{-1}), the α,β -unsaturated aromatic ester carbonyl group (1710 cm^{-1}) and *trans*-vinylene ($CH = CH$) group (1636 cm^{-1}). The 1H and ^{13}C NMR spectra of the major product, extensively analyzed with the help of DEPT, 1H - 1H COSY and HMQC experiments, revealed the presence of new additional peaks for one *trans*-acetoxyferuloyl moiety besides the signals for compound **201** moiety. In the 1H NMR spectrum, the *trans*-acetoxyferuloyl moiety was indicated by one set of 1,3,4-trisubstituted aromatic ring proton signals at δ 6.94 (d, 1H, $J = 8.1\text{ Hz}$, H-5'') and 7.00-7.09 ppm (m, 2H, H-2'', H-6''), one pair *trans*-double bond signals at δ 6.41 ppm (d, 1H, $J = 15.9\text{ Hz}$, H-8'') and 7.65 ppm (d, 1H, $J = 15.9\text{ Hz}$, H-7''), one methoxyl group at δ 3.77 (s, 3H, OCH₃) and one acetyl group at δ 2.28 ppm (s, 3H, H-11''). In addition, the ^{13}C NMR and DEPT spectra, chemical shifts attributed to 12 new signals, including two ester carbonyl carbons, one pair *trans*-double bond carbons, three aromatic methines carbons, three aromatic quaternary carbons, one methoxyl carbon and one methyl carbon that were in accordance with those of one *trans*-acetoxyferuloyl moiety. The significant change between the 1H NMR spectrum of the new product and that of the starting compound **201** was that the proton signals of H-5 and

H₂-6 were shifted to higher field at δ 4.11 and δ 4.55 ppm, respectively corresponding to the same signals in parent compound **201** at δ 3.70 and 3.82 ppm respectively. This change was further confirmed by the detailed analysis of the COSY spectrum of major product. The correlations observed between the anomeric proton (H-1) signal at δ 5.42 ppm and H-2 proton at δ 3.54 ppm which in turn correlated to H-3 proton signal at δ 3.68 ppm. This latter signal correlated to the H-4 proton at δ 3.32 ppm which in turn correlated to H-5 proton at δ 4.11 ppm. The 5 proton correlated to H₂-6 proton signals at δ 4.55 ppm. The substitution of *trans*-acetoxyferuloyl and *trans*-acetoxycinnamoyl groups at C-6, C-3' and C-6' of the sucrose unit was deduced from the HMBC spectrum of the major product. Correlation peaks were observed between H₂-6 protons (δ 4.55 ppm) and an α,β -unsaturated carbonyl carbon, C-9'' (δ 167.4 ppm) of the *trans*-acetoxyferuloyl group. The carbonyl carbon at δ 167.4 ppm also showed the HMBC correlations with *trans*-olefinic protons, resonating at δ 6.41 (H-8'') and 7.65 ppm (H-7''). These HMBC correlations indicated a *trans*-acetoxyferuloyl unit was attached at the C-6 position of the α -glucose. The HMBC cross peaks between H-3' (δ 5.30 ppm) and C-9'' (δ 167.2 ppm), H₂-6' (δ 4.55 ppm) and C-9'' (δ 166.9 ppm) confirmed that two *trans*-acetoxycinnamoyl residues were esterified at C-3' and C-6' of the fructofuranosyl moiety. From the above facts, it was confirmed that compound **201** was successfully esterified with one *trans*-acetoxyferuloyl moiety at C-6. Based on these spectroscopic data, the major product was assigned to be 6-mono-*O*-acetoxyferuloyl-3',6'-di-*O*-acetoxycinnamoylsucrose **222**.

The HR-ESI-MS of the minor product exhibited a molecular ion peak at m/z 1177.3159 [M + Na]⁺ (calcd 1177.3159 for C₅₈H₅₈O₂₅Na) corresponding to the molecular formula C₅₈H₅₈O₂₅ while the ESI-MS spectrum displayed a molecular ion peak at m/z 1177.11 [M + Na]⁺ (calcd 1177.23 for C₅₈H₅₈O₂₅Na). The presence of an ester carbonyl group (1766 cm⁻¹), an α,β -unsaturated aromatic ester carbonyl group (1713 cm⁻¹) and a *trans*-vinylene (CH = CH) group (1637 cm⁻¹) absorption bands in the IR spectrum of the minor product suggested the presence of the ester substitution in the structure. The spectroscopic analysis using the ¹H NMR spectrum of the minor product and the starting compound **201**, indicated the proton signals corresponding to compound **201**, except for the appearance of the additional two *trans*-acetoxyferuloyl moieties, represented by two sets of 1,3,4-trisubstituted aromatic ring proton signals at δ 6.98-7.16 ppm (m, 6H, H-2'', H-5'', H-6''), two pairs *trans*-double bond signals at δ 6.51, 6.52 ppm (2 x d, 2H, J = 16.2 Hz, H-8''), 7.53-7.64 ppm (m,

1H, H-7'') and 7.79 ppm (d, 1H, $J = 16.2$ Hz, H-7''), two methoxyl groups at δ 3.81 ppm (s, 6H, OCH₃) and two acetyl groups at δ 2.30 ppm (s, 6H, H-11''). The ¹³C NMR and DEPT spectra of the minor product showed the presence of 24 new signals, including four ester carbonyl carbons, two pairs *trans*-double bond carbons, six aromatic methines carbons, six aromatic quaternary carbons, two methoxyl carbons and two methyl carbons suggesting the presence of two *trans*-acetoxyferuloyl moieties. The remarkable differences noticed in the ¹H NMR spectrum of minor product in comparison to compound **201** were that the anomeric proton signal was shifted from δ 5.35 to δ 5.57 ppm and 3, 5 & 6 proton signals shifted downfield to δ 5.25, 4.24-4.28 and 4.46-4.60 ppm respectively (wherein compound **201**, 3, 5 & 6 proton signals were observed at δ 3.60, 3.70 and 3.82 ppm respectively). Upon analysis of the COSY spectrum of the minor product, the following correlations were observed: the anomeric proton (H-1) signal at δ 5.57 ppm correlated to the H-2 proton at δ 3.74 ppm which in turn correlated to H-3 proton signal at δ 5.25 ppm. The latter correlated to the H-4 proton at δ 3.54 ppm which in turn correlated to H-5 proton signal at δ 4.24-4.28 ppm. The H-3' proton signal at δ 5.29 ppm correlated to the H-4' proton at δ 4.53 ppm which in turn correlated to H-5' proton signal at δ 4.24-4.28 ppm. Both the H-5 and H-5' proton signals correlated to the overlapping H₂-6 and H₂-6' proton signals, respectively, at δ 4.46-4.60 ppm. Thus, 3 and 6 hydroxyls of compound **201** were acylated. A long-range correlations between H-3 (5.25 ppm) and the α,β -unsaturated carbonyl carbon C-9'' (δ 168.2 ppm) and H₂-6 protons (δ 4.53 ppm) and C-9'' (δ 167.8 ppm) of the *trans*-acetoxyferuloyl group were found in the HMBC spectrum of the minor product. These correlations indicated that two *trans*-acetoxyferuloyl units were attached at the C-3 and C-6 positions of the glucopyranosyl moiety of compound **201**. Considering all these spectroscopic data, the minor product was assigned to be 3,6-*O*-di-*O*-acetoxyferuloyl-3',6'-di-*O*-acetoxycinnamoylsucrose **226**.

2.5.2. Synthesis of 6-mono-*O*-feruloyl-3',6'-di-*O*-coumaroylsucrose (lapathoside C, 116)

Deacetylation of compound **222** was performed with pyrrolidine²⁰⁶ in ethanol as solvent for 90 min (Scheme 2.41). The crude reaction mixture was passed through a column of strongly acidic ion-exchange resin using 95% EtOAc as eluent. The eluent was then evaporated under reduced pressure to give a syrup which was subjected to silica gel column chromatography using a gradient of CH₂Cl₂-EtOAc-MeOH as eluent. The appropriate

fractions were evaporated under diminished pressure to provide a new product as a white solid (75% yield) with a R_f value 0.55 (9:1 EtOAc-MeOH) and mp 125-127 °C.

Scheme 2.41. Synthesis of lapathoside C **116**

The notable change between the ^1H and ^{13}C NMR spectra of the new product and starting compound **222** was only the disappearance of the characteristic signals for the three acetyl moieties, represented by the proton signal of acetyl groups signals at δ 2.23, 2.26, 2.28 ppm (s, 9H, H-11'') and carbon signals at δ 20.6, 21.1 ppm (3 x C-11'') and also three acetyl ester quaternary carbons at δ 168.9, 169.2, 169.5 ppm (3 x C-10'') ppm. The IR spectrum of the new product showed the loss of the absorption bands for the acetyl ester carbonyl group at 1767 cm^{-1} . The elemental formula of the product was deduced as $\text{C}_{40}\text{H}_{42}\text{O}_{18}$ by the HR-ESI-MS spectrum based on the observed molecular ion at m/z 833.2283 $[\text{M} + \text{Na}]^+$ (calcd 833.2263 for $\text{C}_{40}\text{H}_{42}\text{O}_{18}\text{Na}$) while the ESI-MS exhibited the molecular ion peak at m/z 833.13 $[\text{M} + \text{Na}]^+$, calcd 833.24 for $\text{C}_{40}\text{H}_{42}\text{O}_{18}\text{Na}$. The above data indicated the success of the deprotection of acetyl groups of compound **222**. Based on spectroscopic data analysis and also by comparison to the data reported for the isolated natural product (Table 2.8),⁵⁹ the new product was assigned to be 6-mono-*O*-feruloyl-3',6'-di-*O*-coumaroylsucrose or lapathoside C **116** (Figure 2.8).

Figure 2.8. ^1H NMR spectrum of lapathoside C **116** (300 MHz, CD_3OD)**Table 2.8.** Comparison table for ^1H & ^{13}C data of synthetic & isolated lapathoside C (300 MHz, CD_3OD , δ in ppm, J in Hz)

Position	Isolated Natural Product ⁵⁹		Synthesized Product 116	
	^1H	^{13}C	^1H	^{13}C
1	5.51 (d, $J = 4.0$)	92.5	5.50 (m)	92.5
2	3.47 (dd, 9.6, 4.0)	73.1	3.48 (m)	73.1
3	3.64 (dd, 9.6, 9.0)	74.8	3.65 (m)	74.8
4	3.29 (m)	72.2	3.32 (m)	72.1
5	3.30 (m)	72.4	3.35 (m)	72.3
6	4.70 (m), 4.24 (m)	65.8	4.71 (m), 4.29 (m)	65.8
1'	3.61 (2 H, m);	65.4	3.60 (2H, m)	65.4
2'		104.9		104.9
3'	5.53 (d, $J = 8.1$)	79.0	5.54 (m)	79.0
4'	4.67 (m)	75.0	4.65 (m)	75.0
5'	4.17 (m)	81.1	4.18 (m)	81.1
6'	4.55 (2H, m)	65.8	4.55 (2H, m)	65.8
R ₁	(<i>p</i> -Feruloyl)			
1''		127.7		127.7
2''	7.21 (d, $J = 1.9$)	111.5	7.21 (br, s)	111.5
3''		149.3		149.3
4''		150.6		150.6
5''	6.75 (d, $J = 8.2$)	116.3	6.75-6.82 (m)	116.3
6''	7.01 (dd, $J = 8.2, 1.9$)	124.6	7.02 (d, $J = 7.5$)	124.5
7''	7.61 (d, $J = 16.1$)	147.2	7.62 (d, $J = 15.9$)	147.2
8''	6.48 (d, $J = 16.1$)	115.3	6.48 (d, $J = 15.9$)	115.3
9''		169.3		169.3
O-CH ₃	3.84 (3H, s)	56.4	3.84 (3H, s)	56.4
R ₂	<i>p</i> -Coumaroyl			
1'''		127.1		127.1
2''', 6'''	7.51 (d, $J = 8.7$)	131.5	7.52 (d, $J = 8.4$)	131.5
3''', 5'''	6.80 (d, $J = 8.7$)	116.8	6.75-6.82 (m)	116.8
4'''		161.4		161.4
7'''	7.71 (d, $J = 16.0$)	147.6	7.73 (d, $J = 15.9$)	147.6
8'''	6.43 (d, $J = 16.0$)	114.6	6.43 (d, $J = 15.9$)	114.6
9'''		168.4		168.4
R ₃	<i>p</i> -Coumaroyl			
1''''		127.1		127.1
2''', 6''''	7.33 (d, $J = 8.7$)	131.2	7.34 (d, $J = 8.4$)	131.2
3''', 5''''	6.76 (d, $J = 8.7$)	116.8	6.75-6.82 (m)	116.8
4''''		161.3		161.3
7''''	7.57 (d, $J = 16.0$)	146.8	7.62 (d, $J = 15.9$)	146.8
8''''	6.24 (d, $J = 16.0$)	114.8	6.24 (d, $J = 15.9$)	114.8
9''''		168.9		168.9

2.5.3. Synthesis of 3,6-di-*O*-feruloyl-3',6'-di-*O*-coumaroylsucrose **227**

Having compound **226** in our hand, the next step i.e. cleavage of the acetyl groups was successfully achieved by treating an ethanolic suspension of **226** with pyrrolidine for 3 h according to Scheme 2.42. After workup and column chromatographic purification of the crude product using a gradient of CH₂Cl₂-EtOAc-MeOH as eluent, a white solid with a lower *R_f* value (0.74, 9:1 EtOAc-MeOH) than the starting material was achieved in 47% yield, mp 135-138 °C.

Scheme 2.42. Synthesis of compound **227**

The spectroscopic analysis using the ¹H and ¹³C NMR spectra of the white solid revealed that the only change from compound **226** was the loss of the characteristic signals for the four acetyl moieties, represented by the proton signal of acetyl groups signals at δ 2.28, 2.30 ppm (s, 12H, H-11'') and carbon signal at δ 20.7, 21.1 ppm (4 x C-11'') and also four acetyl ester quaternary carbons at δ 168.8, 169.1 ppm (4 x C-10''). The IR spectrum of the white solid displayed the disappearance of the absorption band for the acetyl ester carbonyl group at 1767 cm⁻¹. The HR-ESI-MS spectrum of the white solid suggested a molecular formula C₅₀H₅₀O₂₁ based on the molecular ion at *m/z* 1009.2740 [M + Na]⁺ (calcd 1009.2737 for C₅₀H₅₀O₂₁Na) and the ESI-MS spectrum showed the molecular ion at *m/z* 1009.12 [M + Na]⁺ (calcd 1009.28 for C₅₀H₅₀O₂₁Na). Hence, the deprotection of acetyl group of compound **226** was successfully achieved and the white solid was assigned to be 3,6-di-*O*-feruloyl-3',6'-di-*O*-coumaroylsucrose **227**.

2.5.4. Synthesis of 6-mono-*O*-feruloyl-3,3',6'-tri-*O*-coumaroylsucrose 229

As lapathoside C **116** was successfully achieved and the structural difference between vanicoside B **128** and lapathoside C **116** (Figure 2.5) is only one coumaroyl group substitution at C-1' position of sucrose, then we targeted vanicoside B **128** in similar approach from compound **222**. Thus, treatment of a solution of compound **222** in dry CH₂Cl₂ with 0.92 mole equiv of 4-acetoxycinnamoyl chloride **195** according to Scheme 2.43 and followed by the chromatographic purification afforded a new component with a higher R_f value (0.43, 3:1 EtOAc-hexanes) than the starting material **222**. The new component was obtained as a white solid in 27% yield (mp 113-115 °C).

Scheme 2.43. Preparation of compound **229**

The HR-ESI-MS of the new component indicated a molecular formula of C₅₇H₅₆O₂₄ as determined by the observed molecular ion peak at m/z 1147.3036 [M + Na]⁺ (calcd 1147.3054 for C₅₇H₅₆O₂₄Na) while the ESI-MS showed a molecular ion peak at m/z 1147.17 [M + Na]⁺, calcd 1147.32 for C₅₇H₅₆O₂₄Na. The IR spectrum of the new component exhibited the absorption bands for ester with a carbonyl group absorption at 1765 cm⁻¹, an α,β -unsaturated aromatic ester carbonyl group at 1718 cm⁻¹ and a *trans*-vinylene (CH = CH) group at 1636 cm⁻¹. The spectroscopic analysis using ¹H NMR spectroscopy of the new

component indicated the presence of one *trans*-acetoxyferuloyl moiety, represented by one set of 1,3,4-trisubstituted aromatic ring proton signals at δ 6.93-7.17 ppm (m, 3H, H-2'', H-5'', H-6''), one pair *trans*-double bond signals at δ 6.52 ppm (d, 1H, J = 15.9 Hz, H-8'') and 7.77 ppm (d, 1H, J = 15.9 Hz, H-7''), one methoxyl group at δ 3.81 ppm (s, 3H, OCH₃) and one acetyl group at δ 2.30 ppm (s, 3H, H-11'') along with three *trans*-acetoxycinnamoyl moieties, represented by three sets of 1,4-disubstituted aromatic ring proton signals at δ 6.93-7.17 ppm (m, 6H, H-3'', H-5''), 7.47 (d, 4H, J = 8.1 Hz, H-2'', H-6'') and 7.57-7.67 ppm (m, 2H, H-2'', H-6''), three *trans*-double bond signals at δ 6.40, 6.41, 6.46 ppm (3 x d, 3H, J = 15.9 Hz, H-8'') and 7.57-7.67 ppm (m, 3H, H-7'') and three acetyl groups at δ 2.28, 2.29 ppm (s, 9H, H-11''). In addition, the ¹³C NMR and DEPT spectra of the new component revealed the presence of 45 new signals, including eight ester carbonyl carbons, four pairs *trans*-double bond carbons, fifteen aromatic methines carbons, nine aromatic quaternary carbons, one methoxyl carbon and four acetyl carbons that were in agreement with those values for one *trans*-acetoxyferuloyl and three *trans*-acetoxycinnamoyl moieties. After analysis of the ¹H NMR and COSY spectra of the minor product in a similar fashion described for compound **226**, the significant change between the ¹H NMR spectra of the new component compared to compound **222** was that H-1 and H-3 signals shifted to higher field (δ 5.42 to 5.57 and δ 3.68 to 5.25 ppm, respectively). The HMBC spectrum of the new component showed the long-range correlation peaks between H-3 proton (δ 5.25 ppm) and C-9'' (δ 167.8 ppm); H-3' proton (δ 5.30 ppm) and C-9'' (δ 167.3 ppm); H₂-6' protons (δ 4.55 ppm) and C-9'' (δ 166.9 ppm). These correlations indicated the three *trans*-acetoxycinnamoyl residues at C-3, C-3' and C-6' positions of the sucrose unit. The HMBC cross peaks between H₂-6 protons (δ 4.55 ppm) and C-9'' (δ 168.2 ppm) revealed that the *trans*-acetoxyferuloyl unit was esterified at C-6 of the glucopyranosyl moiety. From the above evidence, it was confirmed that compound **222** was successfully esterified with one *trans*-acetoxycinnamoyl moiety at C-3. Therefore, the new component was assigned to be 6-*O*-mono-*O*-acetoxyferuloyl-3,3',6'-tri-*O*-acetoxycinnamoylsucrose **228**.

A new product with a lower R_f value (0.76, 9:1 EtOAc-MeOH) than the starting compound **228** was obtained as a white solid in 35% yield by treating an ethanolic suspension of **228** with pyrrolidine for 3 h according to Scheme 2.43 and following the general purification procedure as described for compounds **116** and **227**. The major difference between the ¹H and ¹³C NMR spectra of the new product and compound **228** was that the characteristic signals for the four acetyl moieties, represented by the proton signal of acetyl

groups signals at δ 2.28, 2.29, 2.30 ppm (3 x s, 12H, H-11'') and carbon signals at δ 20.7, 21.1 ppm (4 x C-11'') and also four acetyl ester quaternary carbons at δ 168.8, 169.1 ppm (4 x C-10'') were lost. The IR spectrum of the new product showed the absence of the absorption bands for the acetyl ester carbonyl group at 1765 cm^{-1} . The above data indicated the success of the deprotection of acetyl group of compound **228**. The HR-ESI-MS of the new product revealed the expected molecular formula $\text{C}_{49}\text{H}_{48}\text{O}_{20}$ based on the observed molecular ion at m/z 979.2612 $[\text{M} + \text{Na}]^+$ (calcd 979.2631 for $\text{C}_{49}\text{H}_{48}\text{O}_{20}\text{Na}$) while the ESI-MS showed the molecular ion at m/z 979.13 $[\text{M} + \text{Na}]^+$, calcd 979.27 for $\text{C}_{49}\text{H}_{48}\text{O}_{20}\text{Na}$. Therefore, the new product was assigned to be 6-mono-*O*-feruloyl-3,3',6'-tri-*O*-coumaroylsucrose **229**.

2.5.5. Synthesis of 3,6,3',6'-tetra-*O*-coumaroylsucrose **231**

When a solution of compound **201** in dry CH_2Cl_2 was treated with 1.31 mole equiv of 4-acetoxycinnamoyl chloride **195** in presence of 10 mole equiv dry pyridine and 4 Å molecular sieves powder (Scheme 2.44), after 24 h TLC analysis (3:1 EtOAc-hexanes), revealed the presence of the new components that have different R_f value compared to the starting material **201**. The crude mixture was subjected to column chromatography using a gradient of CH_2Cl_2 -EtOAc as eluent and further, purified by PTLC to furnish a white solid with a R_f value of 0.43 (3:1 EtOAc-hexanes) in 12% yield while other two compounds remained unidentified because of their complicated spectral behaviour.

Scheme 2.44. Preparation of compound **231**

The molecular formula of the white solid was deduced to be $C_{56}H_{54}O_{23}$ from an observed molecular ion peak at m/z 1117.15 $[M + Na]^+$ (calcd 1117.31 for $C_{56}H_{54}O_{23}Na$) in the HR-ESI-MS spectrum while the ESI-MS exhibited a molecular ion peak at m/z 1117.2918 $[M + Na]^+$ (calcd 1117.2948 for $C_{56}H_{54}O_{23}Na$). Its IR spectrum showed the absorption bands for the ester carbonyl group at 1768 cm^{-1} , an α,β -unsaturated aromatic ester carbonyl group at $1718, 1704\text{ cm}^{-1}$ and a *trans*-vinylene ($CH=CH$) group at 1636 cm^{-1} . The 1H NMR spectrum of the white solid indicated the presence of four *trans*-acetoxycinnamoyl moieties, represented by four sets of 1,4-disubstituted aromatic ring proton signals at δ 7.05 (d, 8H, $J = 8.4\text{ Hz}$, H-3'', H-5'') and 7.44-7.49, 7.57 ppm (2 x m, 8H, H-2'', H-6''), four *trans*-double bond signals at δ 6.38, 6.40, 6.44, 6.51 ppm (4 x d, 4H, $J = 15.9\text{ Hz}$, H-8''), 7.61, 7.62, 7.64 (3 x m, 3H, H-7'') and 7.76 ppm (d, 1H, $J = 15.9\text{ Hz}$, H-7'') and four acetyl groups at δ 2.27 ppm (s, 12H, H-11'') together with a sucrose moiety. Additionally, the ^{13}C NMR and DEPT chemical shifts attributed to 44 new signals, including eight ester carbonyl carbons, four pairs *trans*-double bond carbons, sixteen aromatic methines carbons, eight aromatic quaternary carbons and four acetyl carbons, thus confirming the presence of four *trans*-acetoxycinnamoyl moieties. The distinguished difference found in the 1H NMR spectrum of

the white solid in comparison to compound **201** was that the anomeric proton (H-1), 3, 5, 6 proton signals shifted to higher field at δ 5.56 (H-1), 5.27 ppm (dd, 1H, $J = 9.0$ Hz, 9.3 Hz, H-3), 4.26 ppm (m, H-5, H-6a) and 4.53 ppm (m, H-6b) compared to the corresponding signals in compound **201** (the 1, 3, 5, 6 proton signals were observed at δ 5.35, 3.60, 3.70 and 3.82 ppm respectively). This change was further confirmed by analysis of the COSY spectrum of the white solid in a similar approach described for compound **226**. The important correlations observed that the anomeric proton (H-1) signal (δ 5.56 ppm) correlated to H-2 proton signal at δ 3.78 ppm which in turn correlated to H-3 proton signal at δ 5.27 ppm. The H-3 proton signal correlated to H-4 proton signal at δ 3.60 ppm. This latter correlated to H-5 proton signal at δ 4.26 which in turn correlated to H₂-6 proton signals at δ 4.53 and δ 4.26 ppm. Again, the esterification positions of the *trans*-acetoxycinnamoyl groups at C-3, C-6, C-3' and C-6' of the sucrose unit were identified by the long-range correlation peaks found in the HMBC spectrum of the white solid between H-3 (5.27 ppm) and carbonyl carbon C-9'' (δ 169.2 ppm), H₂-6 (δ 4.53, 4.26 ppm) and C-9'' (δ 169.2 ppm) in the glucopyranosyl moiety and between H-3' (δ 5.27 ppm) and C-9'' (δ 169.2 ppm), H₂-6' (δ 4.53 ppm) and C-9'' (δ 169.2 ppm) in the fructofuranosyl moiety. From the above data, it was concluded that compound **201** was successfully esterified with two *trans*-acetoxycinnamoyl moieties at C-3 and C-6 positions of the glucopyranosyl moiety. Therefore, the white solid was assigned to be 3,6,3',6'-tetra-*O*-acetoxycinnamoylsucrose **230**.

In order to cleave of the acetyl groups in compound **230**, an ethanolic suspension of **230** was reacted with pyrrolidine for 30 min (Scheme 2.44). After workup and column chromatographic purification of the crude product using a gradient of CH₂Cl₂-EtOAc-MeOH as eluent, a new product with a lower R_f value (0.76, 9:1 EtOAc-MeOH) than compound **230** was obtained as a white solid in 71% yield, mp 149-151 °C. The major difference between the ¹H and ¹³C NMR spectra of the new product and that of parent compound **230**, involved only the disappearance of the characteristic signals for the four acetyl moieties, represented by the proton signal of acetyl groups signals at δ 2.27 ppm (s, 12H, H-11'') and carbon signals at δ 21.1 ppm (4 x C-11'') and also four acetyl ester quaternary carbons at δ 169.2 ppm (4 x C-10''). The IR spectrum of the new product showed the disappearance of the absorption band for the acetyl ester carbonyl group at 1768 cm⁻¹ that indicated the success of the deprotection of acetyl groups in compound **230**. The HR-ESI-MS of the new product revealed a molecular formula of C₄₈H₄₆O₁₉ as assigned by the observed molecular ion at m/z 949.2494 [M + Na]⁺ (calcd 949.2526 for C₄₈H₄₆O₁₉Na) while the ESI-MS displayed the

molecular ion peak at m/z 949.14 $[M + Na]^+$, calcd 949.26 for $C_{48}H_{46}O_{19}Na$. By considering all the above data, the new product was assigned to be 3,6,3',6'-tetra-*O*-coumaroylsucrose **231**.

After spectroscopic analysis, it has been noticed that the 3-hydroxyl of compounds **201** and **222** was acylated instead of the primary hydroxyl (OH-1'). Therefore, one important conclusion from these acylation reactions is, though OH-1' is a primary hydroxyl, reactivity is very low due to its neo-pentyl arrangement. The next available reactive hydroxyl in these systems is OH-3.

2.5.6. Summary

Lapathoside C **116** and its analogues **227**, **229** and **231** were synthesized successfully from 3',6'-di-*O*-acetoxycinnamoylsucrose **201** in 2-3 steps in moderate yields. It should be noted that compounds **227**, **229** and **231** are tetra-acylated sucrose esters like lapathoside A **132**, vanicoside B **128** and vanicoside D **129**, but the substitution pattern is different. The hydroxycinnamic acids in the natural products vanicoside B **128**, vanicoside D **129** and lapathoside A **132** are substituted at C-6, C-1', C-3' and C-6' positions of the sucrose unit, whereas in case of these synthesized compounds **227**, **229** and **231**, substituent positions are at the C-6, C-3, C-3' and C-6' of the sucrose unit. Interestingly, it has been found the reactivities of the remaining free hydroxyl groups of 3',6'-di-*O*-acetoxycinnamoylsucrose **201** were in the order of 6-OH > 3-OH. That is totally different from our expected order of reactivities (6-OH > 1'-OH). Although the yields of the final compounds are not satisfactory for industrial applications, the work carried out is novel and these compounds were successfully achieved from sucrose **140** in a short synthetic route.

Chapter 3: *In Vitro* cytotoxicity studies of selected phenylpropanoid sucrose esters synthesized in Chapter 2 using MTS assay method

3.1. Introduction

Cancer as a leading disease in the human population is becoming a huge health problem today. Some of the major problems in cancer treatment are medication toxicity, low specificity and high cost. The hope is to provide medication that has greater antitumor activity with diminished toxicities and side effects at an affordable price. Moreover, cancer medications require constant observation of the patients during the therapy. At the molecular level, molecular biology now offers the opportunity to dissect the molecular mechanisms and pathophysiology underlying cancer and allow us to identify genes and proteins which are responsible for this disease. Many of these mechanisms have been exploited as new targets for drug development.²⁰⁹

The vast structural diversity of natural products and their broad pharmacological activities can serve as potential sources for lead compounds.¹⁻⁸ Such compounds have been successfully developed into drugs through methodical research and development. For example, plactaxel (taxol) from *Taxus brevifolia*, camptothecin from *Camptotheca acuminata*, vincristine and vinblastin from *Catharanthus roseus*, podophyllotoxin and its analogues etoposide, teniposide from *Podophyllum peltatum* have been developed into successful anticancer drugs. Molecular modification of the functional groups of lead compounds has the ability to produce semisynthetic analogues having higher pharmacological activities and fewer adverse effects.^{1, 4, 6, 7} For example, Docetaxel developed from plactaxel itself was found to be more potent and with less toxicity. Similarly, topotecan, irinotecan were obtained from camptothecin.¹

As described in details in Chapter 1, plant species such as *Kigelia pinnata*³⁴, *Cynanchum hancockianum*⁴³, *Phyllanthus niruri*^{56, 103, 104}, *Smilax china*,⁶³ *Polygonum aviculare*⁹³, *Polygonum hydropiper*^{77, 78} and *Polygonum cuspidatum*^{76, 92} which are known to be rich in PSEs have been used in traditional or folk medicine for the treatment of various tumors and cancers. The whole plant *Paris polyphylla* var. *yunnanensis* has been used in traditional Chinese medicine to treat lung, liver and laryngeal carcinoma.^{64, 73} The leaves of *Beta vulgaris* have been used as a special diet in the treatment of cancer.^{101, 102} The rhizome of *Sparganium stoloniferum* possesses anti-tumour activities.²² The extracts of *Heloniopsis orientalis* exhibited potent cytotoxicity against solid carcinoma cell lines: lung (A549) with an IC₅₀ value of 4.6 µg/mL and colon (Col2) with an IC₅₀ value of 4.5 µg/mL.⁹⁸ The tuber of

Smilax china showed significant cytotoxicity against various tumour cell lines.^{63, 69} Different compounds isolated from various species of *Polygonum* genus possess anticancer activities.⁹² The ethanolic extract of *Polygonum pensylvanicum* exhibited significant protein kinase C (PKC) inhibitory activity with an IC₅₀ value of 38 µg/mL.⁸⁵

Natural PSEs extracted and identified from various plant species were reported to have antiproliferative activities against different human cancer cell lines (See Chapter 1 for details). Briefly, smilaside D-F **102-104** showed significant cytotoxicity against human colon tumour cells (DLD-1) whereas smilaside A **75** exhibited weak cytotoxicity against the same cells. Smilaside A, B, D-F **75, 76, 102-104** showed weak cytotoxicity against human oral epithelium carcinoma (KB), human cervical carcinoma (HeLa), human breast adenocarcinoma (MCF-7), human lung carcinoma (A-549) and human medulloblastoma (Med) cells.⁶⁹ The acetate groups in the sucrose core of the PSEs are suspected to play important role in mediating the cytotoxicity.⁶⁹ Helonioside A **69** and 1',2,3,6-Tetra-*O*-acetyl-3'-*cis*-feruloylsucrose **18** exhibited dose-dependent and weak cytotoxicity against LA 795 cells, respectively.^{22, 64, 65} Vanicoside A **127** and B **128** exhibited cytotoxicity against MCF cell line at submicromolar dose levels.⁷⁹ Lapathoside A **132**, lapathoside D **67**, vanicoside B **128** and hydropiperoside **99** exhibited significant inhibitory effects on the EBV-EA activation by tumour-promoters such as TPA in Raji cells.^{59, 122} Vanicosides B **128** and lapathoside A **132** showed significant anti-tumour-promoting effects on mouse two-stage skin carcinogenesis.^{59, 122} Vanicoside A **127** and B **128** are potent PKC inhibitor. Free hydroxyl or phenol groups in these molecules are believed to be responsible for mediating their anti-tumour activities.^{79, 122} Its worth noting that very little work concerning the mechanism of action and structure activity relationships (SAR) studies using natural or synthetic PSEs has been done.

It is evident based on the above summary (and details mentioned in Chapter 1) that PSEs have great potential to be very promising and useful anticancer lead compounds. It is hoped that further investigation will shed more light on their potential for further development to new anticancer drugs / drug candidates. Therefore, we investigated the *In Vitro* cytotoxicity of selected phenylpropanoid sucrose esters synthesized in Chapter 2 using the MTS assay method.

3.2. Experimental section

3.2.1. MTT and MTS methods

The microculture tetrazolium assay (MTA) is a colorimetric assay used for measuring the activity of enzymes of the metabolically active cells that reduce the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) or the related tetrazolium dyes such as XTT, MTS and WSTs to the water-insoluble, purple-coloured formazan dye. DMSO as a solvent aids in the solubilization of cellular-generated MTT-formazan solid dye to produce purple coloured solution whose absorbance can be measured and quantified.²¹⁰⁻²¹² The MTA is well established and is widely used for *in vitro* anticancer drug screening to assess the viability and the proliferation of cells and to determine cytotoxicity of drugs.^{210, 212}

The Cell Titer 96 Aqueous One Solution Assay (Promega) contains a standard tetrazolium substrate, the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) and an electron coupling reagent the phenazine ethosulfate (PES). The MTS compound is bio-reduced by viable cells into a colored formazan. The quantity of formazan product as measured by the amount of 490 nm absorbance is directly proportional to the number of living cells in culture. The major advantage of the MTS assay is that it requires one less step (solubilization of water-insoluble formazan product) than the MTT assay because viable cells convert MTS compound to a water-soluble formazan.²¹³

3.2.2. Chemicals and reagents

The Cell Titer 96 Aqueous One Solution Assay (MTS) was purchased from Promega Pte. Ltd, Singapore. Plant cell culture tested DMSO (minimum 99.5% GC) was purchased from Sigma-Aldrich (Singapore). Minimum essential medium (MEM) 1X, Endothelial growth medium (EGM), penicillin-streptomycin-glutamine (100X), fetal bovine serum, phosphate buffer saline 1X pH 7.4 (without calcium chloride and magnesium chloride), 0.5% Trypsin-EDTA 10X were purchased from Invitrogen Corporation (Gibco, USA).

3.2.3. Cell line and culture

Human cervical epitheliod carcinoma cells (HeLa) and Human Umbilical Vein Endothelial cells (HUVEC) were cultured in MEM and EGM media, respectively,

supplemented with 10% v/v fetal bovine serum (FBS) and 1% v/v penicillin-streptomycin in a CO₂ incubator in a humidified condition with 5% CO₂ and 95% air at 37 °C.

3.2.4. *In vitro* cytotoxicity of selected PSEs

In Vitro cytotoxicity activities of the selected PSEs against HeLa and HUVEC cell lines were evaluated by measuring the metabolism of a standard tetrazolium substrate, MTS at 24 and 48 h of drug exposure.

3.2.4.1. Cytotoxicity against cancerous cells (HeLa)

3.2.4.1.1. Sample preparation

Stock solutions (50 mM) of the test compounds (i.e. selected synthesized PSEs and camptothecin (as positive control) were first prepared in DMSO and then diluted with MEM (1% v/v penicillin-streptomycin was added; no FBS was added) to the desired final concentrations prior to the experiment. The final DMSO concentration was 0.2% in each well. This DMSO concentration exhibited no interference with the biological activities tested.

3.2.4.1.2. Cell seeding and sample addition

The cells (1×10^4 cells per well) were incubated in 96-well plates in which each cell well contained 100 µl of the MEM media. After 24 h of incubation, the medium was removed and replaced with 100 µl of test solution of varying concentration (0.001-100 µM). The samples were again incubated for 24 h and 48 h at 37 °C.

3.2.4.1.3. Measurement of sample

After incubation, the samples were removed and 100 µl of the medium containing MTS at conc. of 5:1 without serum was added into the wells and then incubated at 37 °C for 4 h. After 4 h of incubation, the absorbencies of the samples were measured at 490 nm using Infinite M200 micro plate reader controlled by Magellan and i-control softwares (Tecan Group Ltd, Männedorf, Switzerland). The samples containing media with and without cells were also analyzed and labelled as ‘control’ and ‘blank’, respectively. Subtracting the average 490 nm absorbance from the “no-cell” control from all other absorbance values yielded the correct absorbance. All experiments were performed in triplicate.

3.2.4.1.4. IC_{50} calculation

The percentage of cytotoxicity (or growth inhibition) was calculated as $(1 - (\text{Net } A_{490} (\text{testing drug}) / \text{Net } A_{490} (\text{control}))) \times 100\%$. Hence, the negative control was set to 100% survival or 0% toxicity. IC_{50} is the concentration that induces 50% growth inhibition compared with untreated control cells. The mean and the IC_{50} value of the screened PSEs were calculated from the dose-response curve by non-linear regression analysis using the data analysis software (Prism) from three independent experiments.

3.2.4.1.5. Statistical analysis

Comparisons between multiple groups were carried out using one-way analysis of variance (one-way ANOVA) with Bonferroni's correction. Differences were considered statistically significant when $P < 0.05$.

3.2.4.2. Cytotoxicity against normal cells (HUVEC)

In Vitro cytotoxicity activity of selected PSEs on HUVEC cell line was carried out similarly according to the method as described above for HeLa cells at 48 h of drug exposure. In this case, HUVEC cells were used and seeded in EGM medium. Samples were prepared in EGM medium. For the preliminary screening, only one concentration (1 μM) of the test solutions were used for the experiments. Here, to plot the graph, the percentage cell viability was used. The percentage cell viability was calculated as $(\text{Net } A_{490} (\text{testing drug}) / \text{Net } A_{490} (\text{control})) \times 100\%$.

3.3. Cytotoxicity studies

In this chapter, the *in vitro* screening of selected PSEs synthesized in Chapter 2 will be discussed and their potential as drug lead compounds for future development and optimization will be assessed. The selected PSEs shown in Tables 3.1, 3.2, 3.3, 3.4 and 3.5 were subjected to *in vitro* cytotoxicity studies against human cervical epitheliod carcinoma cells (HeLa) and human umbilical vein endothelial cells (HUVEC) using microculture tetrazolium assay (MTA) method as described in the experimental section 3.2.^{69, 214-217} Compounds containing ferulic acid skeleton have the potential for high anti-carcinogenesis²¹⁸ while *trans*-cinnamic acid induces cytostasis and a reversal of the malignant properties of human tumour cells.^{219, 220, 221, 222} The PSEs were selected to examine the effect of various

structural features especially the effects of (i) number of the aromatic rings attached to the core sucrose structure, (ii) position at the sucrose core (iii) type of the aromatic ring (i.e. cinnamoyl, coumaroyl and feruloyl) and substituent at the aromatic ring, (iv) acetyl groups, and (v) isopropylidene groups.

It should be emphasized that this is a preliminary study which aims to shed light on the potential of the synthesized PSEs as lead drug candidates. Hence, not all of the synthesized PSEs in Chapter 2 were screened and no mechanistic studies were conducted at this early stage.

3.3.1. Cytotoxicity studies using HeLa cell lines

Simple PSEs having cinnamoyl groups as the phenylpropanoid substituents were first investigated. Therefore, compounds **183**, **184** and **187-192** were screened for *in vitro* cytotoxicity against HeLa cell lines using the MTS assay method at 48 h exposure and the results compared with camptothecin (CPT) as a positive control. The IC₅₀ values of these compounds are shown in Table 3.1. 3',6'-Di-*O*-cinnamoylsucrose **184** did not show any appreciable activity upto 100 µM concentration (entry 1, Table 3.1), but its tri- **191** and tetra- **192** variants were found to have significant antiproliferative activity with the IC₅₀ values of 4.10 and 0.47 µM, respectively (entries 2 and 3, Table 3.1). With these encouraging results, it was of interest to find the antiproliferative activity of the corresponding di-*O*-isopropylidene compounds **183**, **187** and **188**. It was anticipated that the di-*O*-isopropylidene group in compounds **183**, **187** and **188** would provide restricted conformation and may have an influence on the antiproliferative activity. Indeed, di-*O*-isopropylidene compounds **183** (0.97 µM), **187** (1.52 µM) and **188** (0.25 µM) showed better IC₅₀ values compared to their counterparts **184** (>100 µM), **191** (4.10 µM) and **192** (0.47 µM), respectively (entries 4-6 vs entries 1-3 Table 3.1, respectively). These results underscore the positive influence of the di-*O*-isopropylidene on the antiproliferative activity.

It was reported that vanicosides possess a variety of biological activities and Kawai *et al.* suggested that the acetyl moiety on the sucrose core of these compounds might be responsible for mediating the observed activities.⁸⁵ Kuo *et al.* also mentioned that the acetate group on the sucrose core of PSEs might be responsible for mediating the observed cytotoxicities.⁶⁹ These reports encouraged us to synthesize compounds having acetyl groups attached to the sucrose moiety. Thus, compounds **183** and **187** were acetylated to provide

compounds **189** and **190**, respectively, in the hope that these compounds would have better antiproliferative activity. To our delight, compounds **189** (0.58 μM) and **190** (0.05 μM) showed enhanced cytotoxicities (entries 7 and 8, Table 3.1) in comparison to their corresponding parent compounds **183** (0.97 μM) and **187** (1.52 μM) (entries 7 and 8, Table 3.1).

The above results suggested that the acetyl and di-*O*-isopropylidene groups directly attached to the sucrose core play a positive role in mediating the cytotoxicities of PSEs. This finding will be examined with more compounds.

Table 3.1. IC₅₀ values of selected synthesized cinnamoyl PSEs **183**, **184** and **187-192** along with CPT at 48 h exposure

Entry	Compound Name	Code	IC ₅₀ (μM) ^a
1	3',6'-Di- <i>O</i> -cinnamoylsucrose	184	>100
2	3',4',6'-Tri- <i>O</i> -cinnamoylsucrose	191	4.10
3	3,3',4',6'-Tetra- <i>O</i> -cinnamoylsucrose	192	0.47
4	3',6'-Di- <i>O</i> -cinnamoyl-2,1':4,6-di- <i>O</i> -isopropylidene sucrose	183	0.97
5	3',4',6'-Tri- <i>O</i> -cinnamoyl-2,1':4,6-di- <i>O</i> -isopropylidene sucrose	187	1.52
6	3,3',4',6'-Tetra- <i>O</i> -cinnamoyl-2,1':4,6-di- <i>O</i> -isopropylidene sucrose	188	0.25
7	3,4'-Di- <i>O</i> -acetyl-3',6'-di- <i>O</i> -cinnamoyl-2,1':4,6-di- <i>O</i> -isopropylidene sucrose	189	0.58
8	3- <i>O</i> -Acetyl-3',4',6'-tri- <i>O</i> -cinnamoyl-2,1':4,6-di- <i>O</i> -isopropylidene sucrose	190	0.05
9	Camptothecin	CPT	0.40

^aIC₅₀ is the concentration that induces 50% growth inhibition compared with untreated control cells.

In Table 3.1, sucrose was functionalized with simple unsubstituted cinnamoyl groups representing the phenylpropanoid units. Therefore, a second set of compounds (Table 3.2) with coumaroyl groups as the phenylpropanoid units were synthesized utilizing our general synthetic protocol for the preparation of PSEs to assess the effects of the (i) coumaroyl phenolic OH group, (ii) acetyl (COCH₃) group on the phenyl ring, and (iii) di-*O*-isopropylidene groups. Thus, coumaroyl functionalized PSEs-lapathoside D **67** and its analogues **202-205**, **208**, **245** and **246** were selected and screened against HeLa cells. The results are shown in (Table 3.2).

Lapathoside D (3',6'-di-*O*-coumarylsucrose) **67** did not show any appreciable antiproliferative activity upto 100 μM concentration (entry 1, Table 3.2). This result is similar to that of 3',6'-di-*O*-cinnamoylsucrose **184** (entry 1, Table 3.1) which did not show any appreciable activity. The presence of phenolic functionality in the phenyl ring did not change the antiproliferative activity. Similarly, compound **201** in which the phenolic groups were protected with acetyl group did not show appreciable antiproliferative activity as the IC₅₀ value obtained was >100 μM (entry 2, Table 3.2). At this point, it seemed that the phenolic and acetyl groups did not influence the antiproliferative activities. With the knowledge acquired from the cinnamoyl PSEs (Table 3.1), the presence of the di-*O*-isopropylidene groups might play an important part in influencing the activity. Thus, compound **198**, which is the counterpart of compound **201** was synthesized and its cytotoxicity evaluated.

Pleasingly and as expected, compound **198** showed significant antiproliferative activity with an IC_{50} value of 2.46 μ M (entry 3, Table 3.2). Similarly, tri- and tetra- variants **199** and **200** were synthesized and found to have significant cytotoxicity with their IC_{50} values 5.26 and 0.56 μ M, respectively (entries 4 and 5, Table 3.2). A closer look, reveals a trend in the antiproliferative activities of the synthesized cinnamoyl (Table 3.1) and coumaroyl (Table 3.2) PSEs:

- (i) Cinnamoyl di-*O*-isopropylidene sucrose esters: **188** (tetra-, IC_{50} = 0.25 μ M) > **183** (di-, IC_{50} = 0.97 μ M) > **187** (tri-, IC_{50} = 1.52 μ M).
- (ii) Coumaryl di-*O*-isopropylidene sucrose esters: **200** (tetra-, IC_{50} = 0.56 μ M) > **198** (di-, IC_{50} = 2.46 μ M) > **199** (tri-, IC_{50} = 5.26 μ M)

Next, to further examine the effect of the acetyl groups on the phenyl ring on the IC_{50} values i.e. free phenolic *vs* acetyl protected phenolic groups, compound **204** was compared to compound **199** and compound **231** was compared to compound **230**. Compound **204** which has free phenolic groups showed lower antiproliferative activity with an IC_{50} value of 7.63 μ M (entry 6, Table 3.2) compared to the corresponding acetylated compound **199** (IC_{50} = 5.26 μ M) (entry 4, Table 3.2). In comparison to the compounds **204** and **199**, both compounds **230** and **231** showed similar but higher antiproliferative activities with IC_{50} values of 1.86 and 1.70 μ M, respectively (entries 4 and 6 *vs* entries 7 and 8, Table 3.2). The higher values for compounds **230** and **231** are consistent with the fact that tetra-substituted PSEs show higher antiproliferation activities compared to tri-substituted compounds.

In comparison to the results shown in Table 3.1, in general, cinnamoyl PSEs showed better IC_{50} values compared to coumaroyl and acetyl coumaroyl PSEs (Table 3.2). The di-*O*-isopropylidene group containing compounds again exhibited significant cytotoxicity thus underscoring the positive effect of the di-*O*-isopropylidene group as observed in Table 3.1. The exact role of the phenolic and acetyl groups seems to be variable with the structure of the PSE. It seems that both phenolic and acetyl groups don't play a major role in mediating the cytotoxic activities of the PSEs shown in Table 3.2.

Table 3.2. IC₅₀ values of selected synthesized coumaroyl PSEs **67**, **198-201**, **204**, **230** and **231** along with CPT at 48 h of exposure

No	Compound Name	Code	IC ₅₀ (μM) ^a
1	Lapathoside D	67	>100
2	3',6'-Di- <i>O</i> -acetoxycinnamoylsucrose	201	>100
3	3',6'-Di- <i>O</i> -acetoxycinnamoyl-2,1':4,6-di- <i>O</i> -isopropylidene sucrose	198	2.46
4	3',4',6'-Tri- <i>O</i> -acetoxycinnamoyl-2,1':4,6-di- <i>O</i> -isopropylidene sucrose	199	5.26
5	3,3',4',6'-Tetra- <i>O</i> -acetoxycinnamoyl-2,1':4,6-di- <i>O</i> -isopropylidene sucrose	200	0.56
6	3',4',6'-Tri- <i>O</i> -coumaroyl-2,1':4,6-di- <i>O</i> -isopropylidene sucrose	204	7.63
7	3,6,3',6'-Tetra- <i>O</i> -acetoxycinnamoyl sucrose	230	1.86
8	3,6,3',6'-Tetra- <i>O</i> -coumaroyl sucrose	231	1.70
9	Camptothecin	CPT	0.40

^aIC₅₀ is the concentration that induces 50% growth inhibition compared with untreated control cells.

A more complex phenylpropanoid unit was then examined to see the effect of more substitution on the IC₅₀ value. Therefore, PSEs with a more complex feruloyl group (i.e. with a phenolic and methoxy functionalities in the phenyl ring) compared to the cinnamoyl (Table 3.1) and coumaroyl (Table 3.2) were examined (Table 3.3). Thus, natural product helonioside A **69** and its analogues PSEs **117**, **208**, **210-212**, **214**, **215**, **218** and **220** (Table 3.3) were synthesized and screened against HeLa cells. The IC₅₀ values along with the value for CPT are shown in Table 3.3. The objective here was to examine the effect of the (i) methoxy groups, (ii) number of feruloyl substituents, and (iii) di-*O*-isopropylidene on the IC₅₀ value.

As expected, helonioside A **69** and its corresponding compound **214** in which the phenolic groups are acetylated (entries 1 and 2, Table 3.3, respectively) did not show any appreciable activities up to 100 µM concentration. Here again, acetylation of the phenolic groups proved to be ineffective in enhancing the antiproliferative activity. Moreover, at this stage, the methoxy groups seem to have no appreciable positive effects on the IC₅₀ value if we compare the IC₅₀ values of **69** with **67** and **201** with **214**. In all cases the IC₅₀ is >100 µM. However, introducing one or two more feruloyl groups to helonioside A **69** enhanced the activity significantly compared to compounds **69** and **214**. Thus, compound **117**, a tri-feruloyl sucrose ester showed improved cytotoxicity with an IC₅₀ value of 22.35 µM (entry 3, Table 3.3) whereas tetra-feruloyl sucrose ester **218** exhibited significant cytotoxicity with an IC₅₀ value of 1.62 µM (entry 4, Table 3.3). Now, PSEs with di-*O*-isopropylidene groups were of interest as this group proved to greatly enhance the antiproliferative activities as seen in Tables 3.1 and 3.2. Consequently, compounds **210**, **211** and **212** were examined carefully. Pleasingly and as expected, those compounds showed significant antiproliferative activities with their IC₅₀ values of 6.01, 0.16 and 3.22 µM, respectively, compared to their parent analogues **210** vs **214** and **211** vs **215** (entries 5 vs 8 and 6 vs 8, Table 3.3). The trend of the antiproliferative activity of 3',4',6'-tri-*O*-feruloylsucrose analogues are: **211** (IC₅₀ = 0.16 µM) > **215** (IC₅₀ = 1.70 µM) > **220** (IC₅₀ = 4.20 µM) > **117** (IC₅₀ = 22.35 µM).

From the above results, it was noticed that the completely acetylated di-*O*-isopropylidene variants like compound **211** have greater activity while deacetonide (di-*O*-isopropylidene-free) **215** or deacetylated product **220** showed lower activities. Additionally, when acetyl and di-*O*-isopropylidene groups of compound **211** were completely removed (deprotected) to give the counterpart compound **117**, the activity was dramatically reduced (entries 3 & 6, Table 3.3). The reason could be attributed to differences in the lipophilicity of these compounds. It has been found in the literature that increased lipophilicity of molecules

might be responsible for enhanced cytotoxicity in the MTT model.²¹⁵ The presence of free phenolic and alcoholic (due to a lack of the di-*O*-isopropylidene group) groups may have resulted in decreased lipophilicity of these PSEs which account for reduced activity. But in case of the tetra-substituted PSEs, free phenolic and alcoholic groups did not dramatically affect the cytotoxicity because of the presence of four phenylpropanoid moieties. When the number of phenylpropanoid moieties in the sucrose molecule is increased, the lipophilicity of the molecule is also increased. Thus, the trend in tetra-cinnamoyl derivatives is: **188** ($IC_{50} = 0.25 \mu M$) > **192** ($IC_{50} = 0.47 \mu M$) and in tetra-feruloyl derivatives is: **218** ($IC_{50} = 1.62 \mu M$) > **212** ($IC_{50} = 3.22 \mu M$).

It was anticipated that compound **208** with a mono phenylpropanoid group would show weak activity. Instead, this compound did not show any antiproliferative activity up to 100 μM concentration (entry 10, Table 3.3). In this case, the positional effect at the sucrose core seems to have a great effect. We suspect that substituents at the C6' position play no major role in the antiproliferative activity.

Again here, the di-*O*-isopropylidene groups play an important positive role in enhancing the cytotoxicity. The effect of the di-*O*-isopropylidene is much more prominent compared to the acetyl groups.

Table 3.3. IC₅₀ values of selected synthesized feruloyl PSEs **69**, **117**, **208**, **210-212**, **214**, **215**, **218** and **220** along with CPT at 48 h of exposure

No	Compound Name	Code	IC ₅₀ (μM) ^a
1	Helonioside A	69	>100
2	3',6'-Di- <i>O</i> -acetoxyferuloylsucrose	214	>100
3	3',4',6'-Tri- <i>O</i> -feruloylsucrose	117	22.35
4	3,3',4',6'-Tetra- <i>O</i> -feruloylsucrose	218	1.62
5	3',6'-Di- <i>O</i> -acetoxyferuloyl-2,1':4,6-di- <i>O</i> -isopropylidene sucrose	210	6.01
6	3',4',6'-Tri- <i>O</i> -acetoxyferuloyl-2,1':4,6-di- <i>O</i> -isopropylidene sucrose	211	0.16
7	3,3',4',6'-Tetra- <i>O</i> -acetoxyferuloyl-2,1':4,6-di- <i>O</i> -isopropylidene sucrose	212	3.22
8	3',4',6'-Tri- <i>O</i> -acetoxyferuloylsucrose	215	1.70
9	3',4',6'-Tri- <i>O</i> -feruloyl-2,1':4,6-di- <i>O</i> -isopropylidene sucrose	220	4.20
10	6'-Mono- <i>O</i> -acetoxyferuloyl-2,1':4,6-di- <i>O</i> -isopropylidene sucrose	208	>100
11	Camptothecin	CPT	0.40

^aIC₅₀ is the concentration that induces 50% growth inhibition compared with untreated control cells.

So far, all the screened natural and unnatural phenylpropanoid sucrose esters in Table 3.1-3.3 had the same phenylpropanoid units in the PSE molecule. Obviously, the next goal was to test the antiproliferative activity of “mixed” PSEs having a combination of two different phenylpropanoid units in a single molecule. Table 3.4 showed the efforts towards this goal.

Table 3.4. IC₅₀ values of selected synthesized “mixed” PSE’s **116**, **222**, **226**, **227** and **229** along with CPT at 48 h of exposure

No	Compound Name	Code	IC ₅₀ (μM) ^a
1	Lapathoside C	116	12.61
2	3,6-Di- <i>O</i> -acetoxyferuloyl-3',6'-di- <i>O</i> -acetoxy-cinnamoylsucrose	226	3.14
3	3,6-Di- <i>O</i> -feruloyl-3',6'-di- <i>O</i> -coumaroylsucrose	227	1.67
4	6-Mono- <i>O</i> -feruloyl-3,3',6'-tri- <i>O</i> -coumaroylsucrose	229	3.12
5	6-Mono- <i>O</i> -acetoxyferuloyl-3',6'-di- <i>O</i> -acetoxy-cinnamoylsucrose	222	>100
6	Camptothecin	CPT	0.40

^aIC₅₀ is the concentration that induces 50% growth inhibition compared with untreated control cells.

As expected tetra-phenylpropanoid sucrose esters **226**, **227** and **229** (entries 2-4, Table 3.4) showed better antiproliferative activities compared to the tri-phenylpropanoid sucrose esters **116** and **222** (entries 1 and 5, Table 3.4). Acetylation of **116** to give compound

222 did not improve the IC₅₀ value (entry 1 vs 5, Table 3.4). Replacing one of the coumaroyl substituents at C3 of **229** with a feruloyl group as in **227** improved the IC₅₀ value from 3.12 to 1.67 μ M (entry 4 vs 3, Table 3.4). Compound **227** (entry 3, Table 3.4) showed superior activity compared to its acetylated product **226** (entry 2, Table 3.4).

In conclusion, di-*O*-isopropylidene group proved to be essential for enhancing the cytotoxicity while acetyl and methoxy groups had much lower effects. Lipophilicity of the examined PSEs seems to also influence the cytotoxicity in MTS model.

To examine if the cytotoxicity is time dependent, a few compounds were selected for evaluation of cytotoxicity at two different time intervals of drug exposure by MTS assay (Table 3.5).

Table 3.5. IC₅₀ values of selected synthesized PSEs **117**, **191**, **198**, **199**, **210** and **218** along with CPT at 24 h and 48 h of exposure

No	Compound		IC ₅₀ (μ M) ^a	
	Name	Code	24 h	48 h
1	3',4',6'-Tri- <i>O</i> -cinnamoylsucrose	191	7.64	4.10
2	3',6'-Di- <i>O</i> -acetoxycinnamoyl-2,1':4,6-di- <i>O</i> -isopropylidene sucrose	198	19.44	2.46
3	3',4',6'-Tri- <i>O</i> -acetoxycinnamoyl-2,1':4,6-di- <i>O</i> -isopropylidene sucrose	199	12.22	5.26
4	3',6'-Di- <i>O</i> -acetoxiferuloyl-2,1':4,6-di- <i>O</i> -isopropylidene sucrose	210	27.92	6.01
5	3',4',6'-Tri- <i>O</i> -feruloylsucrose	117	55.07	22.35
6	3,3',4',6'-Tetra- <i>O</i> -feruloylsucrose	218	7.90	1.62
	Camptothecin	CPT	1.57	0.40

^aIC₅₀ is the concentration that induces 50% growth inhibition compared with untreated control

In the MTS time course of study, the selected PSEs shown in Table 3.5 exhibited time-dependent antiproliferative activities with the IC_{50} values as shown. In all cases, using compounds **117**, **191**, **198**, **199**, **210** and **218**, the IC_{50} values increased as the time increased from 24 to 48 h exposure.

3.3.2. Cytotoxicity studies using HUVEC cell lines

To examine the cytotoxic effects on normal non-cancerous human cells, compounds **199**, **210**, **218** and **231** were selected for screening against HUVAC cells at 1 μ M concentration. The % cell viability of CPT as positive control and selected PSEs at 1 μ M concentrations against both HeLa and HUVAC cells was plotted in Figures 3.1 for preliminary comparisons of the cytotoxic effects between normal non-cancerous and cancerous human cells. The known anticancer drug camptothecin (CPT) showed similar cytotoxicity activity against both HeLa and HUVAC cell lines. From the Figure 3.1, it has been found that compounds **218** and **231** exhibited almost similar cell growth as like control on HUVAC cells whereas in cancerous (HeLa) cells, they showed 55% and 75% cell viability respectively.

Figure 3.1. The % cell viability of CPT and selected PSEs against HeLa and HUVAC cells

Compounds **199** and **210** indicated less cytotoxicity on HUVAC cells (~90% cell viability as shown in Figure 3.1) than cancerous cells (nearly 45% and 65% cell viability).

Based on this preliminary MTS result and by comparison with positive control CPT, we concluded that our synthesized PSEs might have less cytotoxic effects on normal cells.

3.4. Summary

Preliminary screening results indicated that 22 out of 31 screened synthesized PSEs showed significant antiproliferative activities against HeLa cells at 48 h drug exposure with their IC₅₀ values ranging from 0.05 to 7.63 μ M. The structure activity relationship correlation studies reveal that the *type, number and position* of the phenylpropanoid units on the sucrose core influence the antiproliferative activity against HeLa cells. Preliminary MTS studies on normal human cell lines indicated that PSEs have less cytotoxic effects on HUVAC cells than HeLa cells compared with CPT. This study has provided important information regarding the SAR for future research:

- (i) Di-*O*-isopropylidene groups positively influenced the IC₅₀ values to nanomolar levels. Such groups have the most pronounced effects.
- (ii) As the number of phenylpropanoid units on the sucrose moiety increased, antiproliferative activity improved.
- (iii) Acetyl groups directly attached to the sucrose core improved the antiproliferative activity.
- (iv) Free phenolic group seems to enhance the activity to different extents.
- (v) Acetyl groups on the phenyl moieties seem to have minor effect on the antiproliferative activity.

Based upon the MTS screening results, PSEs proved to be valuable and potential source for new potent anticancer drug candidates. Detailed investigation of the cytotoxic mechanism of these PSEs along with screening using different human cancer cell lines such as colon cancer (DLD-1), lung carcinoma (A-549), skin cancer etc should be examined.

Chapter 4. Experimental

General

All commercial materials used in this work were obtained from Sigma-Aldrich, Acros, Merck and Fisher scientific and were used as received unless indicated. Melting points were determined by Barnstead electrothermal apparatus (9100) and were uncorrected. IR spectra were recorded in KBr on a DIGILAB FTIR-FTS 3100 spectrometer. Routine ^1H NMR spectra were recorded at 300 MHz on a Bruker AC300F spectrometer. Unless otherwise stated, data refer to solutions in CDCl_3 or CD_3OD or $(\text{CD}_3)_2\text{CO}$ or $\text{DMSO}-d_6$ with the residual solvent protons as internal references. ^1H NMR multiplicities were designated as singlet (s), doublet (d), doublet of doublet (dd), doublet of doublet of doublet (ddd), triplet (t), quartet (q), multiplet (m), broad (br), apparent (app). ^{13}C NMR spectra were measured at 75.47 MHz on a Bruker AC300F spectrometer. Fully coupled or decoupled carbon spectra were carried out on approximately 0.01 M solutions in CDCl_3 or CD_3OD or $(\text{CD}_3)_2\text{CO}$ or $\text{DMSO}-d_6$ at 300K using the Bruker AC300F spectrometer operating at 75.47 MHz. Chemical shifts (δ) were in parts per million (ppm) relative to the central solvent peaks. C-H correlations were performed on the Bruker AC300F spectrometers at 300K using the Bruker automation program XHCORR.AU. H-H correlations were recorded on the same instrument using the DQF-COSY program. Elemental analysis was carried out on vario EL III elemental analyzer.

Routine mass spectra were recorded on LCQ mass spectrometer from Thermo, using ESI positive mode spectrometer at 25 eV ionising potential and 4.2 KV accelerating voltage with an ion source temperature of 350 °C. The principle ion peaks m/z are reported together with their percentage intensities relative to the base peak. HR mass spectra were recorded on Finnigan MAT95XL-T, using ESI positive mode spectrometer at 70 eV ionising potential and 3.8 KV accelerating voltage with an ion source temperature of 220 °C.

Flash chromatography and column chromatography were carried out using Merck silica gel 60 (Art. No. 9385) 230-400 mesh. The products on the TLC plate were visualized under UV light (254 nm) or by using a solution of 5% H_2SO_4 in EtOH (v/v).

All reactions carried out using anhydrous solvents were performed under argon atmosphere and the dry solvents were prepared as follows - dimethylformamide (DMF) was distilled under water aspirator pressure from calcium hydride and stored over 4Å molecular sieves. Pyridine was stored over sodium hydroxide.

4.1. Synthesis of cinnamoyl sucrose derivatives

4.1.1. 2,1':4,6-Di-*O*-isopropylidene sucrose **175**

2,1':4,6-di-*O*-isopropylidene sucrose **175** was prepared in accordance with the method reported previously.¹⁹⁵ A mixture of sucrose **140** (65.0 g, 190.1 mmol) and drierite* (36.0 g) in dry DMF (700 mL) was stirred at 70°C under nitrogen for 30 min. 2-Methoxypropene (86.0 mL, 898.1 mmol) and *p*-TsOH (82.0 mg) were added to the above mixture and stirred for another 100 min at the same temperature. The reaction was then quenched by addition of NEt₃ (7.0 mL). The reaction mixture was filtered under vacuum to remove the drierite and the solvent was evaporated to dryness under reduced pressure. The yellow slurry obtained was suspended in water (652.0 mL), AcOH (1.7 mL) with stirring followed by addition of Na₂CO₃ (16.3 g). The mixture was then evaporated to dryness and the residue was dissolved in EtOAc (403.0 mL). The solution was dried over anhydrous MgSO₄, filtered and the solvent was removed under reduced pressure. TLC analysis in EtOAc showed two major spots. The crude product was subjected to column chromatography initially to remove the non-polar impurities. The remaining mixture on the column was flushed and this crude mixture was concentrated and recrystallized in EtOAc to provide 4,6-mono-*O*-isopropylidene sucrose **174** and 2,1':4,6-di-*O*-isopropylidene sucrose **175** as white solid in 10% (7.5 g) and 56% (45.0 g) yield, respectively.

Analytical data for **175**: R_f = 0.20 (EtOAc); ¹H NMR (300 MHz, CDCl₃): δ 1.45, 1.50, 1.52 (3 x s, 12H, (CH₃)₂C), 3.50 (d, 1H, J = 12.3 Hz, H-1'a), 3.55 (app t, 1H, J = 9.6 Hz, H-4), 3.61 (m, 1H, H-6'a), 3.69 (d, 1H, J = 10.8 Hz, H-6a), 3.77 (d, 1H, J = 3.6 Hz, 9.0 Hz, H-2), 3.84 (dd, 1H, J = 5.4 Hz, 5.1 Hz, H-6'b), 3.90 (m, 2H, H-5', H-6b), 3.96 (m, 1H, H-3'), 4.03 (m, 1H, H-5'), 4.12 (m, 1H, H-3), 4.34 (d, 1H, J = 12.3, H-1'b), 4.61 (dd, 1H, J = 8.1 Hz, 8.1 Hz, H-4'), 6.27 (d, 1H, J = 3.6 Hz, H-1); ¹³C NMR (75.48 MHz, CDCl₃): δ 19.1, 24.3, 25.2, 29.0 (4 x (CH₃)₂C), 61.3 (C-6'), 62.1 (C-6), 64.0 (C-5), 66.5 (C-1'), 68.7 (C-3), 72.9 (C-4'), 73.3 (C-4), 73.7 (C-2), 78.6 (C-5'), 82.2 (C-3'), 91.2 (C-1), 100.1 (C-2'), 102.6, 103.0 (2 x (CH₃)₂C); ESI-Mass (positive mode): m/z 446.33 [M + Na]⁺, calcd 445.42 for C₁₈H₃₀O₁₁Na.

* Drierite is widely used as drying agent. The chemical name is calcium sulfate, anhydrous and particle size 8 mesh

4.1.2. Acylation of diacetone 175 with cinnamoyl chloride

General procedure

Diacetonide **175** was dissolved in dry pyridine under nitrogen atmosphere. The solution was then cooled to 0 °C in an ice bath. Cinnamoyl chloride was added slowly at 0 °C and the reaction was left to stir while warming to rt. The reaction was monitored by TLC (3:1 EtOAc-hexanes). Stirring was continued until the reaction was complete. The resulting mixture was poured into vigorously stirred ice-water (100 mL) and a white solid precipitated and was filtered. The precipitate was redissolved in EtOAc (25 mL) and washed once with 1N HCl (50 mL). The aqueous layer was back extracted with EtOAc (50 mL) and combined with the original organic layer. The organic solution was then successively washed with 5% NaHCO₃ (50 mL) and brine (25 mL) and then dried over anhyd. MgSO₄. The EtOAc solution was concentrated to residue that was subjected to column chromatography using a gradient of CH₂Cl₂-EtOAc as eluent.

4.1.2.1. 6'-Mono-*O*-cinnamoyl-2,1':4,6-di-*O*-isopropylidene sucrose **185** and 3'-mono-*O*-cinnamoyl-2,1':4,6-di-*O*-isopropylidene sucrose **186**

Following the general procedure, reaction between diacetone **175** (0.5 g, 1.2 mmol) and cinnamoyl chloride (0.2 g, 1.3 mmol) in dry pyridine (5 mL) for 9 days gave compound **185** as a white solid in 40% (0.25 g) yield and **186** as a white solid in 16% (0.10 g) yield.

Analytical data for **185**: R_f = 0.09 (3:1 EtOAc-hexanes); mp 126-129 °C; FT-IR (KBr) $\nu_{\max}$: 3445, 2994, 2939, 1712, 1638, 1451, 1384, 1312, 1270, 1204, 1173, 1135, 1069, 943, 859, 770, 716 cm⁻¹;

¹H NMR (300 MHz, CDCl₃): δ 1.43, 1.45, 1.46, 1.51 (4 x s, 12H, (CH₃)₂C), 3.52 (d, 1H, J = 12.6 Hz, H-1'a), 3.60 (dd, J = 9.3 Hz, 9.5 Hz, 1H, H-4), 3.70 (m, 1H, H-6a), 3.77 (dd, 1H, J = 3.3 Hz, 8.7 Hz, H-2), 3.89-3.98 (m, 3H, H-3', H-5, H-6b), 4.10 (dd, 1H, J = 9.3 Hz, 9 Hz, H-3), 4.21 (m, 2H, H-4', H-5'), 4.28 (m, 1H, H-1'b), 4.34 (m, 1H, H-6'a), 4.54 (dd, 1H, J = 4.2 Hz, 11.4 Hz, H-6'b), 6.21 (d, 1H, J = 3.3 Hz, H-1); *trans*-cinnamoyl units: 6.46 (d, 1H, J = 16.2 Hz, H-8''), 7.27-7.37 (m, 3H, H-3'', H-4'', H-5''), 7.48-7.55 (m, 2H, H-2'', H-6''), 7.69 (d, 1H, J = 16.2 Hz, H-7''); ¹³C NMR (75.48 MHz, CDCl₃): δ 19.2, 24.2, 25.2, 29.0 (4 x (CH₃)₂C), 62.3 (C-6), 63.7 (C-5), 65.9 (C-6'), 66.5 (C-1'), 69.2 (C-3), 73.4 (C-4), 73.9 (C-2),

77.3 (C-4'), 78.9 (C-3'), 79.6 (C-5'), 91.0 (C-1), 100.1, 102.3 (2 x (CH₃)₂C), 103.6 (C-2'), *trans*-cinnamoyl units: 117.6 (C-8''), 128.2 (C-2'', C-6''), 128.9 (C-3'', C-5''), 130.4 (C-4''), 134.3 (C-1''), 145.5 (C-7''), 167.2 (C-9''); ESI-Mass (positive mode): m/z 575.20 [M + Na]⁺, calcd 575.22 for C₂₇H₃₆O₁₂Na; HR-ESI-MS (positive mode): found m/z 575.2092 [M + Na]⁺, calcd 575.2099 for C₂₇H₃₆O₁₂Na.

Analytical data for **186**: R_f = 0.24 (3:1 EtOAc-hexanes); FT-IR (KBr) $\nu_{\max}$: 3468, 2993, 2927, 1718, 1636, 1576, 1507, 1496, 1452, 1384, 1331, 1269, 1205, 1171, 1093, 1069, 1012, 944, 860, 768 cm⁻¹;

¹H NMR (300 MHz, CDCl₃): δ 1.39, 1.45, 1.52, 1.53 (4 x s, 12H, (CH₃)₂C), 3.60 (m, 2H, H-1'a, H-2), 3.70 (m, 2H, H-6a, H-6'a), 3.77 (m, 1H, H-4), 3.85 (m, 2H, H-5, H-6b), 3.93 (m, 2H, H-3, H-6'b), 4.07 (d, 1H, J = 12.3 Hz, H-1'b), 4.13 (m, 1H, H-5'), 4.84-4.95 (m, 1H, H-4'), 5.03 (d, 1H, J = 7.5 Hz, H-3'), 6.21 (d, 1H, J = 3.6 Hz, H-1); *trans*-cinnamoyl units: 6.55 (d, 1H, J = 16.2 Hz, H-8''), 7.39-7.45 (m, 3H, H-3'', H-4'', H-5''), 7.60-7.63 (m, 2H, H-2'', H-6''), 7.82 (d, 1H, J = 16.2 Hz, H-7''); ¹³C NMR (75.48 MHz, CDCl₃): δ 19.1, 24.2, 25.4, 29.0 (4 x (CH₃)₂C), 61.2 (C-6), 61.9 (C-5), 64.0 (C-6'), 66.5 (C-1'), 70.1 (C-3), 71.7 (C-4'); 72.7 (C-2), 73.5 (C-4), 80.4 (C-3'), 84.3 (C-5'), 91.0 (C-1), 100.0, 102.0 (2 x (CH₃)₂C), 103.5 (C-2'); *trans*-cinnamoyl units: 116.6 (C-8''), 128.5 (C-2'', C-6''), 129.0 (C-3'', C-5''), 130.9 (C-4''), 133.9 (C-1''), 147.1 (C-7''), 167.6 (C-9''); ESI-Mass (positive mode): m/z 575.26 [M + Na]⁺, calcd 575.22 for C₂₇H₃₆O₁₂Na.

4.1.2.2. 3',6'-Di-*O*-cinnamoyl-2,1':4,6-di-*O*-isopropylidene sucrose **183**

Following the general procedure, reaction between the diacetone **175** (2.2 g, 5.2 mmol) and cinnamoyl chloride (1.9 g, 11.5 mmol) in dry pyridine (20 mL) for 5 days gave compound **183**¹⁸³ as a white solid in 31% (1.1 g) yield. Analytical data for **183**: R_f = 0.73 (3:1 EtOAc-hexanes); mp 118-120 °C;

FT-IR (KBr) $\nu_{\max}$: 3479, 3418, 2992, 2941, 1715, 1637, 1578, 1497, 1451, 1384, 1312, 1270, 1204, 1169, 1070, 1011, 944, 860, 768, 712, 684, 658 cm^{-1} ; ^1H NMR (300 MHz, CDCl_3): δ 1.39, 1.42, 1.52, 1.53 (4 x s, 12H, $(\text{CH}_3)_2\text{C}$), 3.60 (m, 1H, H-1'a), 3.67 (m, 1H, H-4), 3.75 (m, 2H, H-2, H-6a), 3.83 (dd, 1H, $J = 9.9$ Hz, 4.5 Hz, H-5), 3.90 (m, 1H, H-3), 3.97 (dd, 1H, $J = 4.8$ Hz, 9.9 Hz, H-6b), 4.08 (m, 1H, H-1'b), 4.39 (m, 2H, H-5', H-6'a), 4.50 (m, 2H, H-4', H-6'b), 4.95 (d, 1H, $J = 6.3$ Hz, H-3'), 6.13 (d, 1H, $J = 3.6$ Hz, H-1); *trans*-cinnamoyl units: R_1 : 6.48 (d, 1H, $J = 16.2$ Hz, H-8''), 7.37-7.43 (m, 3H, H-3'', H-4'', H-5''), 7.51-7.54 (m, 2H, H-2'', H-6''), 7.71 (d, 1H, $J = 16.2$ Hz, H-7''); R_2 : 6.54 (d, 1H, $J = 16.2$ Hz, H-8''), 7.37-7.43 (m, 3H, H-3'', H-4'', H-5''), 7.59-7.62 (m, 2H, H-2'', H-6''), 7.82 (d, 1H, $J = 16.2$ Hz, H-7''); ^{13}C NMR (75.48 MHz, CDCl_3): δ 19.1, 24.1, 25.5, 29.1 (4 x $(\text{CH}_3)_2\text{C}$), 62.1 (C-6), 63.8 (C-5), 65.7 (C-6'), 65.9 (C-1'), 70.3 (C-3), 72.9 (C-4), 73.8 (C-2), 76.6 (C-4'), 81.2 (C-3'), 81.4 (C-5'), 90.9 (C-1), 99.9, 101.8 (2 x $(\text{CH}_3)_2\text{C}$), 104.5 (C-2'), *trans*-cinnamoyl units: R_1 : 116.5 (C-8''), 128.2 (C-2'', C-6''), 128.9 (C-3'', C-5''), 130.4 (C-4''), 133.8 (C-1''), 145.4 (C-7''), 166.8 (C-9''); R_2 : 117.6 (C-8''), 128.5 (C-2'', C-6''), 129.0 (C-3'', C-5''), 131.0 (C-4''), 134.3 (C-1''), 147.3 (C-7''), 167.7 (C-9''); ESI-Mass (positive mode): m/z 705.32 $[\text{M} + \text{Na}]^+$, calcd 705.26 for $\text{C}_{36}\text{H}_{42}\text{O}_{13}\text{Na}$; HR-ESI-MS (positive mode): found m/z 705.2502 $[\text{M} + \text{Na}]^+$, calcd 705.2518 for $\text{C}_{36}\text{H}_{42}\text{O}_{13}\text{Na}$; Anal. Calcd for $\text{C}_{36}\text{H}_{42}\text{O}_{13}$: C, 63.33; H, 6.20; found: C, 62.51; H, 6.12.

4.1.2.3. 3',4',6'-Tri-*O*-cinnamoyl-2,1':4,6-di-*O*-isopropylidene sucrose **187**

Following the general procedure, reaction between the diacetone **175** (0.5 g, 1.2 mmol) and cinnamoyl chloride (0.7 g, 3.9 mmol) in dry pyridine (5 mL) for 3 days afforded compound **183** (0.10 g, 12% yield) and compound **187** as a white solid (0.16 g, 17% yield).

Analytical data for **187**: $R_f = 0.80$ (3:1 EtOAc-hexanes); mp 116-120 $^{\circ}\text{C}$; FT-IR (KBr) $\nu_{\max}$: 3475, 2992, 2943, 1723, 1635, 1539, 1507, 1330, 1312, 1255, 1204, 1158, 1093, 1066, 1010, 943, 860, 767, 710, 684, 668 cm^{-1} ; ^1H NMR (300 MHz, CDCl_3): δ 1.29, 1.38, 1.47, 1.50 (4 x s, 12H, $(\text{CH}_3)_2\text{C}$), 3.58 (app t, 1H, $J = 9.3$ Hz, H-4); 3.68 (m, 2H, H-6a, H-1'a), 3.73 (dd, 1H, $J = 3.3$ Hz, 5.4 Hz, H-2), 3.80-3.86 (m, 2H, H-3, H-5), 4.02 (dd, 1H, $J = 5.1$ Hz, 10.2 Hz, H-6b), 4.19 (d, 1H, $J = 12.3$ Hz, H-1'b), 4.54 (m, 2H, H-5', H-6'a), 4.61 (m, 1H, H-6'b), 5.37 (d,

1H, $J = 5.4$ Hz, H-4'), 5.61 (dd, 1H, $J = 3.6$ Hz, 4.8 Hz, H-3'), 6.14 (d, 1H, $J = 3.3$ Hz, H-1); *trans*-cinnamoyl units: R₁: 6.55 (d, 1H, $J = 16.2$ Hz, H-8''), 7.26-7.38 (m, 3H, H-3'', H-4'', H-5''), 7.47-7.49 (m, 2H, H-2'', H-6''), 7.82 (d, 1H, $J = 16.2$ Hz, H-7''); R₂: 6.45 (d, 1H, $J = 16.2$ Hz, H-8''), 7.26-7.38 (m, 3H, H-3'', H-4'', H-5''), 7.47-7.49 (m, 2H, H-2'', H-6''), 7.71 (d, 1H, $J = 16.2$ Hz, H-7''); R₃: 6.43 (d, 1H, $J = 16.2$ Hz, H-8''), 7.26-7.38 (m, 3H, H-3'', H-4'', H-5''), 7.57-7.60 (m, 2H, H-2'', H-6''), 7.69 (d, 1H, $J = 16.2$ Hz, H-7''); ¹³C NMR (75.48 MHz, CDCl₃): δ 19.0, 24.0, 25.4, 28.8 (4 x (CH₃)₂C), 62.0 (C-6), 63.8 (C-5), 64.8 (C-6'), 66.2 (C-1'), 70.1 (C-3), 72.8 (C-4), 73.8 (C-2), 77.3 (C-4'), 77.7 (C-3'), 80.1 (C-5'), 91.3 (C-1), 99.6, 101.7 (2 x (CH₃)₂C), 104.8 (C-2'); *trans*-cinnamoyl units: R₁: 116.4 (C-8''), 128.0 (C-2'', C-6''), 128.8 (C-3'', C-5''), 130.2 (C-4''), 133.9 (C-1''), 145.1 (C-7''), 165.7 (C-9''); R₂: 116.6 (C-8''), 128.2 (C-2'', C-6''), 128.8 (C-3'', C-5''), 130.6 (C-4''), 134.0 (C-1''), 146.4 (C-7''), 165.8 (C-9''); R₃: 117.6 (C-8''), 128.4 (C-2'', C-6''), 128.8 (C-3'', C-5''), 130.7 (C-4''), 134.3 (C-1''), 147.0 (C-7''), 166.3 (C-9''); ESI-Mass (positive mode): m/z 835.34 [M + Na]⁺, calcd 835.30 for C₄₅H₄₈O₁₄Na; HR-ESI-MS (positive mode): found m/z 835.2954 [M + Na]⁺, calcd 835.2936 for C₄₅H₄₈O₁₄Na; Anal. Calcd for C₄₅H₄₈O₁₄: C, 66.49; H, 5.95; found: C, 67.12 ; H, 6.37.

4.1.2.4. 3,3',4',6'-Tetra-*O*-cinnamoyl-2,1':4,6-di-*O*-isopropylidene sucrose **188**

Following the general procedure, reaction between the diacetone **175** (0.5 g, 1.2 mmol) and cinnamoyl chloride (0.9 g, 5.2 mmol) in dry pyridine (5 mL) for 2 days furnished compound **188** as a white solid (0.40 g, 36% yield) along with compound **188** (0.20 g, 21% yield).

Analytical data for **188**: R_f = 0.92 (3:1 EtOAc-hexanes); mp 88-93 °C; FT-IR (KBr) ν_{max}: 2992, 2941, 1719, 1636, 1578, 1497, 1450, 1384, 1310, 1270, 1203, 1155, 1072, 1008, 944, 861, 767, 708, 683 cm⁻¹; ¹H NMR (300 MHz, CDCl₃): δ 1.20, 1.28, 1.44, 1.47 (4 x s, 12H, (CH₃)₂C), 3.64 (d, 1H, $J = 12.3$ Hz, H-1'a); 3.68-3.78 (m, 2H, H-4, H-6a), 3.92-4.00 (m, 2H, H-2, H-5), 4.05 (dd, 1H, $J = 5.1$ Hz, 10.2 Hz, H-6b), 4.24 (d, 1H, $J = 12.6$ Hz, H-1'b), 4.53-4.61 (m, 3H, H-5', H-6'a, H-6'b), 5.37-5.43 (m, 2H, H-3, H-4'), 5.62 (dd, 1H, $J = 3.3$ Hz, 5.1 Hz, H-3'), 6.20 (d, 1H, $J = 3.6$ Hz, H-1); *trans*-cinnamoyl units: R₁: 6.43 (d, 1H, $J = 15.9$ Hz,

H-8''), 7.35-7.40 (m, 3H, H-3'', H-4'', H-5''), 7.49-7.52 (m, 2H, H-2'', H-6''), 7.63-7.75 (d, 1H, $J = 15.9$ Hz, H-7''); R_2 : 6.45 (d, 1H, $J = 15.9$ Hz, H-8''), 7.35-7.40 (m, 3H, H-3'', H-4'', H-5''), 7.49-7.52 (m, 2H, H-2'', H-6''), 7.63-7.75 (m, 1H, H-7''); R_3 : 6.46 (d, 1H, $J = 15.9$ Hz, H-8''), 7.35-7.40 (m, 3H, H-3'', H-4'', H-5''), 7.49-7.52 (m, 2H, H-2'', H-6''), 7.63-7.75 (m, 1H, H-7''); R_4 : 6.63 (d, 1H, $J = 15.9$ Hz, H-8''), 7.35-7.40 (m, 3H, H-3'', H-4'', H-5''), 7.63-7.75 (m, 2H, H-2'', H-6''), 7.96 (d, 1H, $J = 15.9$ Hz, H-7''); ^{13}C NMR (75.48 MHz, CDCl_3): δ 19.0, 23.9, 25.5, 28.8 (4 x $(\text{CH}_3)_2\text{C}$), 62.1 (C-6), 64.3 (C-5), 65.0 (C-6'), 66.2 (C-1'), 71.0 (C-3), 71.6 (C-4), 71.9 (C-2), 77.4 (C-4'), 77.8 (C-3'), 80.2 (C-5'), 91.7 (C-1), 99.6, 101.5 (2 x $(\text{CH}_3)_2\text{C}$), 105.0 (C-2'); *trans*-cinnamoyl units: R_1 : 116.5 (C-8''), 128.1 (C-2'', C-6''), 128.7 (C-3'', C-5''), 130.2 (C-4''), 134.0 (C-1''), 144.7 (C-7''), 165.8 (C-9''); R_2 : 116.8 (C-8''), 128.2 (C-2'', C-6''), 128.8 (C-3'', C-5''), 130.3 (C-4''), 134.3 (C-1''), 145.2 (C-7''), 165.9 (C-9''); R_3 : 117.7 (C-8''), 128.3 (C-2'', C-6''), 128.9 (C-3'', C-5''), 130.5 (C-4''), 134.4 (C-1''), 146.4 (C-7''), 166.2 (C-9''); R_4 : 118.2 (C-8''), 128.3 (C-2'', C-6''), 128.9 (C-3'', C-5''), 130.7 (C-4''), 134.5 (C-1''), 147.4 (C-7''), 166.5 (C-9''); ESI-Mass (positive mode): m/z 965.36 $[\text{M} + \text{Na}]^+$, calcd 965.35 for $\text{C}_{54}\text{H}_{54}\text{O}_{15}\text{Na}$; HR-ESI-MS (positive mode): found m/z 965.3326 $[\text{M} + \text{Na}]^+$, calcd 965.3355 for $\text{C}_{54}\text{H}_{54}\text{O}_{15}\text{Na}$; Anal. Calcd for $\text{C}_{54}\text{H}_{54}\text{O}_{15}$: C, 68.78; H, 5.77; found: C, 68.80; H, 6.09.

4.1.3. Acetylation of cinnamoylated compounds 183, 187 and 188

General procedure

To a stirred solution of cinnamoylated compounds in dry pyridine was added separately excess Ac_2O . The reaction mixture was stirred at rt for 24 h. After this time, TLC (3:1 EtOAc-hexanes) analysis revealed complete disappearance of the starting material. Water was added to the crude reaction mixture and the solution was extracted three times with EtOAc. Pyridine and Ac_2O were removed by repeated coevaporation with toluene (3 x 100 mL). The solvent was evaporated to dryness and was subjected to column chromatography using hexanes-EtOAc (2:1) as eluent to furnish the acetylated compounds as a white solid.

4.1.3.1. 3,4'-Di-*O*-acetyl-3',6'-di-*O*-cinnamoyl-2,1':4,6-2,1':4,6-di-*O*-isopropylidene sucrose **189**

Following the general procedure, reaction between **183** (0.5 g, 0.7 mmol) and acetic anhydride (0.5 mL, 0.6 g, 5.6 mmol) in pyridine (2.5 mL) for 24 h gave compound **189** as a white solid (0.12 g, 22% yield). Analytical data for **189**: $R_f = 0.91$ (3:1 EtOAc-hexanes); mp 88-90 °C;

FT-IR (KBr) $\nu_{\max}$ cm^{-1} : 3471, 3418, 2993, 2942, 1753, 1720, 1637, 1579, 1497, 1451, 1371, 1312, 1233, 1203, 1159, 1072, 1049, 990, 946, 893, 860, 769, 712, 685; ^1H NMR (300 MHz, CDCl_3): δ 1.20, 1.29, 1.42, 1.46 (4 x s, 12H, $(\text{CH}_3)_2\text{C}$), 2.02, 2.11 (2 x s, 6H, $-\text{COCH}_3$), 3.60 (m, 2H, H-1'a, H-4), 3.68 (d, 1H, $J = 10.5$ Hz, H-6a), 3.80-3.92 (m, 2H, H-2, H-5), 4.00 (dd, 1H, $J = 5.1$ Hz, 10.2 Hz, H-6b), 4.18 (d, 1H, $J = 12.3$ Hz, H-1'b), 4.42 (m, 1H, H-6'a), 4.51 (m, 2H, H-5', H-6'b), 5.20-5.28 (m, 2H, H-3', H-3), 5.45 (dd, 1H, $J = 3.6$ Hz, 5.1 Hz, H-4'), 6.14 (d, 1H, $J = 3.3$ Hz, H-1); *trans*-cinnamoyl units: R_1 : 6.46 (d, 1H, $J = 16.2$ Hz, H-8''), 7.36-7.40 (m, 3H, H-3'', H-4'', H-5''), 7.51-7.54 (m, 2H, H-2'', H-6''), 7.71 (d, 1H, $J = 16.2$ Hz, H-7''); R_2 : 6.58 (d, 1H, $J = 16.2$ Hz, H-8''), 7.36-7.40 (m, 3H, H-3'', H-4'', H-5''), 7.63-7.66 (m, 2H, H-2'', H-6''), 7.90 (d, 1H, $J = 16.2$ Hz, H-7''); ^{13}C NMR (75.48 MHz, CDCl_3): δ 19.0, 23.9, 25.5, 28.8 (4 x $(\text{CH}_3)_2\text{C}$), 20.8, 21.0 (2 x C-11''), 62.1 (C-6), 64.2 (C-5), 64.9 (C-6'), 66.1 (C-1'), 70.7 (C-3), 71.5 (C-4), 71.8 (C-2), 77.29 (C-4'), 77.5 (C-3'), 80.2 (C-5'), 91.6 (C-1), 99.5, 101.4 (2 x $(\text{CH}_3)_2\text{C}$), 104.9 (C-2'); *trans*-cinnamoyl units: R_1 : 116.4 (C-8''), 128.1 (C-2'', C-6''), 128.8 (C-3'', C-5''), 130.3 (C-4''), 134.2 (C-1''); 145.2 (C-7''); 166.1 (C-9''); R_2 : 117.7 (C-8''), 128.7 (C-2'', C-6''), 128.9 (C-3'', C-5''), 130.5 (C-4''), 134.4 (C-1''); 147.4 (C-7''); 166.5 (C-9''), 169.7, 170.1 (2 x C-10''); ESI-Mass (positive mode): m/z 789.34 $[\text{M} + \text{Na}]^+$, calcd 789.28 for $\text{C}_{40}\text{H}_{46}\text{O}_{15}\text{Na}$; HR-ESI-MS (positive mode): found m/z 789.2727 $[\text{M} + \text{Na}]^+$, calcd 789.2729 for $\text{C}_{40}\text{H}_{46}\text{O}_{15}\text{Na}$; Anal. Calcd for $\text{C}_{40}\text{H}_{46}\text{O}_{15}$: C, 62.65; H, 6.05; found: C, 62.22; H, 6.33.

4.1.3.2. 3-*O*-Acetyl-3',4',6'-tri-*O*-cinnamoyl-2,1':4,6-di-*O*-isopropylidene sucrose **190**

Following the general procedure, reaction between **187** (0.4 g, 0.5 mmol) and acetic anhydride (0.3 mL, 0.3 g, 2.9 mmol) in pyridine (5 mL) for 24 h gave compound **190** as a white solid (0.19 g, 46% yield).

Analytical data for **190**: $R_f = 0.94$ (3:1 EtOAc-hexanes); mp 132-134 °C; FT-IR (KBr) $\nu_{\max}$: 3336, 2927, 1765, 1701, 1636, 1601, 1507, 1374, 1323, 1284, 1209, 1167, 1063, 994, 915, 860, 838, 794, 694, 650 cm^{-1} ;

^1H NMR (300 MHz, CDCl_3): δ 1.19, 1.31, 1.42, 1.47 (4 x s, 12H, $(\text{CH}_3)_2\text{C}$), 2.02 (1s, 3H, $-\text{COCH}_3$), 3.57 (m, 1H, H-4), 3.64 (m, 1H, H-1'a), 3.70 (d, 1H, $J = 10.5$ Hz, H-6a), 3.85 (dd, 1H, $J = 3.3$ Hz, 9.3 Hz, H-2), 3.92 (m, 1H, H-5), 4.04 (dd, 1H, $J = 5.1$ Hz, 10.5 Hz, H-6b), 4.23 (d, 1H, $J = 12.3$ Hz, H-1'b), 4.54 (m, 2H, H-5', H-6'a), 4.61 (m, 1H, H-6'b), 5.25 (dd, 1H, $J = 9.6$ Hz, 9.3 Hz, H-3), 5.38 (d, 1H, $J = 5.4$ Hz, H-4'), 5.61 (br dd, 1H, $J = 4.8$ Hz, 3.3 Hz, H-3'), 6.18 (d, 1H, $J = 3.3$ Hz, H-1); *trans*-cinnamoyl units: R_1 : 6.45 (d, 1H, $J = 16.2$ Hz, H-8''), 7.30-7.39 (m, 3H, H-3'', H-4'', H-5''), 7.48-7.52 (m, 2H, H-2'', H-6''), 7.70 (d, 1H, $J = 16.2$ Hz, H-7''); R_2 : 6.45 (d, 1H, $J = 16.2$ Hz, H-8''), 7.30-7.39 (m, 3H, H-3'', H-4'', H-5''), 7.48-7.52 (m, 2H, H-2'', H-6''), 7.72 (d, 1H, $J = 16.2$ Hz, H-7''); R_3 : 6.60 (d, 1H, $J = 15.9$ Hz, H-8''), 7.30-7.39 (m, 3H, H-3'', H-4'', H-5''), 7.62-7.67 (m, 2H, H-2'', H-6''), 7.92 (d, 1H, $J = 15.9$ Hz, H-7''); ^{13}C NMR (75.48 MHz, CDCl_3): δ 19.0, 23.9, 25.4, 29.1 (4 x $(\text{CH}_3)_2\text{C}$), 21.0 (C-11''), 62.1 (C-6), 64.3 (C-5), 65.0 (C-6'), 66.2 (C-1'), 70.7 (C-3), 71.5 (C-4), 71.8 (C-2), 77.4 (C-4'), 77.7 (C-3'), 80.2 (C-5'), 91.6 (C-1), 99.5, 101.4 (2 x $(\text{CH}_3)_2\text{C}$), 105.0 (C-2'); *trans*-cinnamoyl units: R_1 : 116.4 (C-8''), 128.1 (C-2'', C-6''), 128.7 (C-3'', C-5''), 130.3 (C-4''), 134.0 (C-1''), 145.2 (C-7''), 165.9 (C-9''), R_2 : 116.7 (C-8''), 128.1 (C-2'', C-6''), 128.8 (C-3'', C-5''), 130.5 (C-4''), 134.3 (C-1''), 146.4 (C-7''), 166.1 (C-9''), R_3 : 117.7 (C-8''), 128.3 (C-2'', C-6''), 128.9 (C-3'', C-5''), 130.7 (C-4''), 134.4 (C-1''), 147.4 (C-7''), 166.4 (C-9''), 169.7 (C-10''); ESI-Mass (positive mode): m/z 877.36 $[\text{M} + \text{Na}]^+$, calcd 877.31 for $\text{C}_{47}\text{H}_{50}\text{O}_{15}\text{Na}$; HR-ESI-MS (positive mode): found m/z 877.3051 $[\text{M} + \text{Na}]^+$, calcd 877.3042 for $\text{C}_{47}\text{H}_{50}\text{O}_{15}\text{Na}$; Anal. Calcd for $\text{C}_{47}\text{H}_{50}\text{O}_{15}$: C, 66.03; H, 5.90; found: C, 66.45; H, 6.27.

Compound **188** remained unchanged as revealed by TLC and NMR analysis when a solution of **188** (0.5 g, 0.5 mmol) in pyridine (5 mL) was treated with acetic anhydride (0.3 mL, 0.3 g, 2.9 mmol), even after 66 h reaction time.

4.1.4. Cleavage of isopropylidene group of compounds **183**, **187** and **188**

General Procedure

Separate solutions of diacetone cinnamoylated compounds in 60% aq. AcOH were heated at 80 °C until the reactions were completed. The reactions were monitored by TLC (3:1 EtOAc-hexanes). The reaction solutions were then evaporated to dryness under reduced pressure by co-distillation with toluene (3 x 100 mL). The deacetone products were obtained as a white solid by recrystallization in EtOAc.

4.1.4.1. 3',6'-Di-*O*-cinnamoylsucrose **184**

Following the general procedure, a solution of compound **183** (0.4 g, 0.6 mmol) was stirred with 60% aq. AcOH (26 mL) at 80 °C for 20 min to afford compound **184** as a white solid in 54% (0.2 g) yield.

Analytical data for **184**: $R_f = 0.06$ (3:1 EtOAc-hexanes); mp 159-161 °C;

FT-IR (KBr) $\nu_{\max}$: 3453, 2925, 1712, 1693, 1640, 1496, 1451, 1353, 1313, 1283, 1206, 1156, 1066, 1032, 997, 924, 865, 766, 709, 681 cm^{-1} ; ^1H NMR (300 MHz, CD_3OD): δ 3.41 (m, 1H, H-4), 3.45 (m, 1H, H-2), 3.62 (m, 2H, H-1'b, H-1'a), 3.70 (m, 1H, H-3), 3.81 (dd, 1H, $J = 4.8$ Hz, 12 Hz, H-6a), 3.91-3.95 (m, 2H, H-6b, H-5), 4.17-4.23 (m, 1H, H-5'), 4.47 (app t, 1H, $J = 8.1$ Hz, H-4'), 4.57 (m, 2H, H-6'a, H-6'b), 5.45 (d, 1H, $J = 3.6$ Hz, H-1), 5.53 (d, 1H, $J = 7.8$ Hz, H-3'); *trans*-cinnamoyl units: R_1 : 6.57 (d, 1H, $J = 15.9$ Hz, H-8''), 7.40-7.42 (m, 3H, H-3'', H-4'', H-5''), 7.60-7.67 (m, 2H, H-2'', H-6''), 7.74 (d, 1H, $J = 15.9$ Hz, H-7''); R_2 : 6.62 (d, 1H, $J = 15.9$ Hz, H-8''), 7.40-7.42 (m, 3H, H-3'', H-4'', H-5''), 7.60-7.67 (m, 2H, H-2'', H-6''), 7.80 (d, 1H, $J = 15.9$ Hz, H-7''); ^{13}C NMR (75.48 MHz, CD_3OD): δ 62.7 (C-6), 65.1 (C-1'), 66.6 (C-6'), 71.5 (C-4), 73.2 (C-2), 74.5 (C-5), 75.0 (C-4', C-3), 79.5 (C-3'), 81.2 (C-5'), 93.2 (C-1), 105.1 (C-2'), *trans*-cinnamoyl units: R_1 : 118.5 (C-8''), 129.4 (C-3'', C-5''), 130.1 (C-2'', C-6''), 131.7 (C-4''), 135.7 (C-1''), 146.8 (C-7''), 167.8 (C-9''); R_2 : 118.6 (C-8''), 129.6 (C-3'', C-5''), 130.1 (C-2'', C-6''), 131.7 (C-4''), 135.8 (C-1''), 147.3 (C-7''), 168.5 (C-9''); ESI-Mass (positive mode): m/z 625.23 $[\text{M} + \text{Na}]^+$, calcd 625.20 for $\text{C}_{30}\text{H}_{34}\text{O}_{13}\text{Na}$; HR-ESI-MS (positive mode): found m/z 625.1879 $[\text{M} + \text{Na}]^+$, calcd 625.1892 for $\text{C}_{30}\text{H}_{34}\text{O}_{13}\text{Na}$; Anal. Calcd for $\text{C}_{30}\text{H}_{34}\text{O}_{13}$: C, 59.80; H, 5.69; found: C, 59.73; H, 5.82.

4.1.4.2. 3',4',6'-Tri-*O*-cinnamoylsucrose **191**

Following the general procedure, a solution of compound **187** (0.3 g, 0.4 mmol) was treated with 60% aq. AcOH (21 mL) at 80 °C for 20 min to furnish compound **191** as a white solid in 69% (0.2 g) yield. Analytical data for **191**: R_f = 0.11 (3:1 EtOAc-hexanes); mp 127-129 °C;

FT-IR (KBr) $\nu_{\max}$ cm^{-1} : 3061, 3028, 2927, 1716, 1636, 1578, 1497, 1450, 1313, 1283, 1255, 1204, 1170, 1002, 864, 710, 683; ^1H NMR (300 MHz, CD_3OD): δ 3.52 (m, 2H, H-1'a, H-4), 3.61 (m, 2H, H-2, H-6a), 3.72 (m, 2H, H-1'b, H-3), 3.84 (m, 2H, H-6b, H-5), 4.27 (m, 2H, H-5', H-6'a), 4.44 (m, 1H, H-6'b), 5.40 (br s, 1H, H-1), 5.55 (br s, 2H, H-3', H-4'); *trans*-cinnamoyl units: 6.22-6.40 (m, 1H, H-8''), 7.17-7.30 (m, 3H, H-3'', H-4'', H-5''), 7.32-7.39 (m, 2H, H-2'', H-6''), 7.56-7.65 (m, 1H, H-7''), R_2 : 6.22-6.40 (m, 1H, H-8''), 7.17-7.30 (m, 3H, H-3'', H-4'', H-5''), 7.32-7.39 (m, 2H, H-2'', H-6''), 7.56-7.65 (m, 1H, H-7''), R_3 : 6.22-6.40 (m, 1H, H-8''), 7.17-7.30 (m, 3H, H-3'', H-4'', H-5''), 7.32-7.39 (m, 2H, H-2'', H-6''), 7.56-7.65 (m, 1H, H-7''); ^{13}C NMR (75.48 MHz, CD_3OD): δ 61.3 (C-6), 64.3 (C-1', C-6'), 69.4 (C-4), 71.7 (C-2), 73.1 (C-5), 73.9 (C-3), 76.7 (C-4'), 77.2 (C-3'), 79.4 (C-5'), 92.4 (C-1), 105.5 (C-2'); *trans*-cinnamoyl units: R_1 : 116.6 (C-8''), 128.3 (2C, C-3'', C-5''), 128.8 (2C, C-2'', C-6''), 130.4 (C-4''), 134.0 (C-1''), 145.9 (C-7''), 165.8 (C-9''), R_2 : 116.6 (C-8''), 128.4 (C-3'', C-5''), 128.9 (C-2'', C-6''), 130.7 (C-4''), 134.1 (C-1''), 146.7 (C-7''), 166.1 (C-9''), R_3 : 117.2 (C-8''), 128.6 (C-3'', C-5''), 128.9 (C-2'', C-6''), 130.7 (C-4''), 134.2 (C-1''), 147.1 (C-7''), 166.8 (C-9''); ESI-Mass (positive mode): m/z 755.23 $[\text{M} + \text{Na}]^+$, calcd 755.24 for $\text{C}_{39}\text{H}_{40}\text{O}_{14}\text{Na}$.

4.1.4.3. 3,3',4',6'-Tetra-*O*-cinnamoylsucrose **192**

Following the general procedure, a solution of compound **188** (0.5 g, 0.5 mmol) was reacted with 60% aq. AcOH (32 mL) at 80 °C for 20 min to give compound **192** as a white solid (0.25 g, 54% yield).

Analytical data for **192**: $R_f = 0.72$ (3:1 EtOAc-hexanes); mp 91-94 °C; FT-IR (KBr) $\nu_{\max}$ cm^{-1} : 3061, 3028, 2992, 1716, 1636, 1578, 1497, 1450, 1384, 1313, 1270, 1255, 1204, 1170, 1002, 943, 861, 708, 683;

^1H NMR (300 MHz, CD_3OD): δ 3.64-3.68 (m, 1H, H-4), 3.84 (m, 4H, H-1'b, H-1'a, H-2, H-6a), 4.06 (m, 2H, H-6b, H-5), 4.49-4.59 (m, 2H, H-5', H-6'a), 4.69 (dd, 1H, $J = 7.2$ Hz, 11.1 Hz, H-6'b), 5.16 (app t, 1H, $J = 9.6$ Hz, H-3), 5.54-5.61 (m, 2H, H-1, H-3'), 5.77 (app t, 1H, $J = 5.1$ Hz, H-4'); *trans*-cinnamoyl units: R_1 : 6.44 (d, 1H, $J = 15.9$ Hz, H-8''), 7.35-7.38 (m, 3H, H-3'', H-4'', H-5''), 7.45-7.51 (m, 2H, H-2'', H-6''), 7.72 (d, 1H, $J = 15.9$ Hz, H-7''), R_2 : 6.45 (d, 1H, $J = 15.9$ Hz, H-8''), 7.35-7.38 (m, 3H, H-3'', H-4'', H-5''), 7.45-7.51 (m, 2H, H-2'', H-6''), 7.73 (d, 1H, $J = 15.9$ Hz, H-7''), R_3 : 6.47 (d, 1H, $J = 16.2$ Hz, H-8''), 7.35-7.38 (m, 3H, H-3'', H-4'', H-5''), 7.45-7.51 (m, 2H, H-2'', H-6''), 7.74 (d, 1H, $J = 16.2$ Hz, H-7''), R_4 : 6.62 (d, 1H, $J = 15.9$ Hz, H-8''), 7.35-7.38 (m, 3H, H-3'', H-4'', H-5''), 7.60-7.64 (m, 2H, H-2'', H-6''), 7.89 (d, 1H, $J = 15.9$ Hz, H-7''); ^{13}C NMR (75.48 MHz, CD_3OD): δ 62.4 (C-6), 64.3 (C-6'), 64.6 (C-1'), 69.6 (C-4), 70.6 (C-2), 73.6 (C-5), 76.4 (C-4'), 77.1 (C-3), 78.0 (C-3'), 79.6 (C-5'), 92.5 (C-1), 105.5 (C-2'); *trans*-cinnamoyl units: R_1 : 116.2 (C-8''), 128.3 (C-3'', C-5''), 128.7 (C-2'', C-6''), 130.6 (C-4''), 133.9 (C-1''), 146.1 (C-7''), 165.9 (C-9''), R_2 : 116.4 (C-8''), 128.3 (C-3'', C-5''), 128.9 (C-2'', C-6''), 130.6 (C-4''), 134.0 (C-1''), 146.3 (C-7''), 166.9 (C-9''), R_3 : 117.1 (C-8''), 128.4 (C-3'', C-5''), 128.9 (C-2'', C-6''), 130.8 (C-4''), 134.1 (C-1''), 146.9 (C-7''), 167.0 (C-9''), R_4 : 117.2 (C-8''), 128.4 (C-3'', C-5''), 129.0 (C-2'', C-6''), 130.8 (C-4''), 134.1 (C-1''), 148.0 (C-7''), 168.6 (C-9''); ESI-Mass (positive mode): m/z 885.27 $[\text{M} + \text{Na}]^+$, calcd 885.28 for $\text{C}_{48}\text{H}_{46}\text{O}_{15}\text{Na}$; HR-ESI-MS (positive mode): found m/z 885.2725 $[\text{M} + \text{Na}]^+$, calcd 885.2729 for $\text{C}_{48}\text{H}_{46}\text{O}_{15}\text{Na}$.

4.2. Synthesis of Lapathoside D and its analogues

4.2.1. 4-Acetoxybenzoyl chloride **195**

p-Coumaric acid **193** (30.0 g, 182.7 mmol) was acetylated in dry pyridine (57.0 mL) with Ac₂O (49.6 mL, 525.8 mmol). The mixture was left for 20 h and quenched by pouring into ice-water (1200 mL) with stirring. A white precipitate that fell out during mixing was filtered, washed with water and air-dried.

Crystallization from MeOH afforded *p*-acetoxybenzoic acid **194** (25.81 g, 69% yield).

Analytical data for **194**: mp 205-211 °C; ¹H NMR (300 MHz, DMSO-*d*₆): δ 2.25 (1s, 3H, H-11), 6.51 (d, 1H, *J* = 15.9 Hz, H-8), 7.16 (m, 2H, H-3, 5), 7.60 (d, 1H, *J* = 15.9 Hz, H-7), 7.70-7.82 (m, 2H, H-2, 6); ¹³C NMR (75.48 MHz, DMSO-*d*₆): δ 20.9 (C-11), 119.4 (C-8), 122.4 (C-3,5), 129.5 (C-2,6), 132.0 (C-1), 143.0 (C-7), 151.9 (C-4), 167.7 (C-9), 169.1 (C-10).

The acid chloride **195** was prepared by refluxing a mixture of the 4-*O*-acetoxybenzoic acid **194** (19.4 g, 94.0 mmol) and SOCl₂ (28 mL, 368.2 mmol) in benzene (200 mL) for 5 h. The resulting clear solutions were evaporated to a solid, redissolved in toluene and evaporated to a solid again.

Crystallization from hot toluene gave the *p*-acetoxybenzoyl chloride **195** (16.9 g, 80% yield).

Analytical data for **195**: mp 118-121 °C; ¹H NMR (300 MHz, (CD₃)₂CO): δ 2.33 (1s, 3H, H-11), 6.61 (1d, 1H, *J* = 15.6 Hz, H-8), 7.19 (m, 2H, H-3, 5), 7.60 (m, 2H, H-2, 6), 7.82 (d, 1H, *J* = 15.6 Hz, H-7); ¹³C NMR (75.48 MHz, (CD₃)₂CO): δ 21.2 (C-11), 117.4 (C-8), 122.2 (C-3, 5), 129.5 (C-2, 6), 131.8 (C-1), 145.7 (C-7), 154.0 (C-4), 169.2 (C-9, C-10). Spectral data of compounds **194** and **195** was the same as reported previously.²⁰⁶

4.2.2. Acylation of diacetone 175 with *p*-acetoxycinnamoyl chloride 195

General procedure

Diacetonide **175** was dissolved in dry pyridine under a nitrogen atmosphere. The solution was then cooled to 0 °C in an ice bath. *p*-Acetoxycinnamoyl chloride **195** was then added slowly at 0 °C and the reaction was left to stir while warming to rt. Stirring was continued at rt or 50 °C until the reaction was completed. Reaction was monitored by TLC analysis (3:1 EtOAc-hexanes). The resulting mixture was poured into vigorously stirred ice-water (100 mL) and the white solid precipitated was obtained after decantation and filtration. The precipitate was redissolved in EtOAc (25 mL) and washed once with 1N HCl (50 mL). The aqueous layer was back extracted with EtOAc (50 mL) and combined with the original organic layer. The organic solution was then successively washed with 5% NaHCO₃ (50 mL) and brine (25 mL) and then dried with anhyd. MgSO₄. The EtOAc solution was concentrated to residue that was subjected to column chromatography using a gradient of CH₂Cl₂-EtOAc as eluent.

4.2.2.1. 6'-Mono-*O*-acetoxycinnamoyl-2,1':4,6-di-*O*-isopropylidene sucrose (**196**) and 3'-mono-*O*-acetoxycinnamoyl-2,1':4,6-di-*O*-isopropylidene sucrose **197**

Following the general procedure, the reaction between diacetone **175** (1.0 g, 2.4 mmol) and *p*-acetoxycinnamoyl chloride **195** (0.6 g, 2.7 mmol) in dry pyridine (10 mL) for 9 days at rt afforded compound **196** as a white solid (0.44 g, 30% yield) along with compounds **197** and **198** in 10% (0.15 g) and 6 % (0.11 g) yield, respectively.

Analytical data for **196**: R_f = 0.10 (3:1 EtOAc-hexanes); mp 127-129 °C; FT-IR (KBr) $\nu_{\max}$: 2994, 2934, 1702, 1636, 1558, 1507, 1374, 1319, 1206, 1167, 1134, 1069, 1014, 942, 857, 836, 700, 649 cm⁻¹;

¹H NMR (300 MHz, CDCl₃): δ 1.45, 1.52 (2s, 12H, (CH₃)₂C), 3.50 (m, 1H, H-1'a), 3.61 (dd, 1H, J = 9.3 Hz, 9.0 Hz, H-4), 3.73 (m, 2H, H-2, H-6a), 3.92 (m, 3H, H-3', H-5, H-6b), 4.07 (m, 1H, H-3), 4.19 (m, 2H, H-4', H-5'), 4.30 (m, 2H, H-1'b, H-6'a), 4.52 (m, 1H, H-6'b), 6.19 (d, 1H, J = 3.0 Hz, H-1); *trans*-*p*-coumaroyl units: δ 2.31 (1s, 3H, H-11''), 6.41 (d, 1H, J =

15.9 Hz, H-8''), 7.11 (d, 2H, H-3'', H-5''), 7.52 (d, 2H, H-2'', H-6''), 7.66 (d, 1H, $J = 15.9$ Hz, H-7''); ^{13}C NMR (75.48 MHz, CDCl_3): δ 19.2, 24.3, 25.3, 29.1 (4 x $(\text{CH}_3)_2\text{C}$), 62.3 (C-6), 63.8 (C-5), 65.9 (C-6'), 66.5 (C-1'), 69.4 (C-3), 73.4 (C-4), 73.9 (C-2), 77.5 (C-4'), 79.0 (C-3'), 79.6 (C-5'), 91.0 (C-1), 100.1, 102.3 (2 x $(\text{CH}_3)_2\text{C}$), 103.6 (C-2'); *trans-p*-coumaroyl units: 21.1 (C-11''), 117.8 (C-8''), 122.2 (C-3'', C-5''), 129.4 (C-2'', C-6''), 132.0 (C-1''), 144.4 (C-7''), 152.2 (C-4''), 167.1 (C-9''), 169.2 (C-10''); ESI-Mass (positive mode): m/z 633.26 $[\text{M} + \text{Na}]^+$, calcd 633.23 for $\text{C}_{29}\text{H}_{38}\text{O}_{14}\text{Na}$; HR-ESI-MS (positive mode): found m/z 633.2144 $[\text{M} + \text{Na}]^+$, calcd 633.2154 for $\text{C}_{29}\text{H}_{38}\text{O}_{14}\text{Na}$; Anal. Calcd for $\text{C}_{29}\text{H}_{38}\text{O}_{14}$: C, 57.04; H, 6.27; found: C, 54.78; H, 6.07.

Analytical data for **197**: $R_f = 0.16$ (3:1 EtOAc-hexanes); mp 120-124 °C; FT-IR (KBr) ν_{max} : 3485, 2994, 2925, 1768, 1718, 1636, 1602, 1508, 1419, 1373, 1322, 1269, 1206, 1167, 1091, 1069, 1016, 946, 856, 754, 729, 655;

^1H NMR (300 MHz, CDCl_3): δ 1.39, 1.45, 1.52, 1.53 (4 x s, 12H, $(\text{CH}_3)_2\text{C}$), 3.54 (m, 1H, H-1'a), 3.64 (m, 1H, H-4), 3.68 (m, 2H, H-6a, H-6'a), 3.77 (dd, 1H, $J = 3.6$ Hz, 9.0 Hz, H-2), 3.90 (m, 4H, H-3, H-5, H-6b, H-6'b), 4.07 (m, 1H, H-1'b), 4.13 (m, 1H, H-5'), 4.86 (dd, 1H, $J = 7.5$ Hz, 7.2 Hz, H-4'), 5.04 (d, 1H, $J = 7.8$ Hz, H-3'), 6.21 (d, 1H, $J = 3.6$ Hz, H-1); *trans-p*-coumaroyl units: δ 2.32 (1s, 3H, H-11''), 6.51 (d, 1H, $J = 15.9$ Hz, H-8''), 7.16 (d, 2H, H-3'', H-5''), 7.63 (d, 2H, H-2'', H-6''), 7.79 (d, 1H, $J = 15.9$ Hz, H-7''); ^{13}C NMR (75.48 MHz, CDCl_3): δ 19.1, 24.2, 25.4, 29.0 (4 x $(\text{CH}_3)_2\text{C}$), 61.2 (C-6), 61.9 (C-6'), 64.0 (C-5), 66.5 (C-1'), 70.1 (C-3), 71.6 (C-4'), 72.7 (C-4), 73.5 (C-2), 80.45 (C-3'), 84.3 (C-5'), 90.8 (C-1), 99.9, 102.0 (2 x $(\text{CH}_3)_2\text{C}$), 103.1 (C-2'), *trans-p*-coumaroyl units: δ 21.2 (C-11''), 116.8 (C-8''), 122.3 (C-3'', C-5''), 129.7 (C-2'', C-6''), 131.6 (C-1''), 145.9 (C-7''), 152.6 (C-4''), 167.4 (C-9''), 169.1 (C-10''); ESI-Mass (positive mode): m/z 633.18 $[\text{M} + \text{Na}]^+$, calcd 633.23 for $\text{C}_{29}\text{H}_{38}\text{O}_{14}\text{Na}$; HR-ESI-MS (positive mode): found m/z 633.2151 $[\text{M} + \text{Na}]^+$, calcd 633.2154 for $\text{C}_{29}\text{H}_{38}\text{O}_{14}\text{Na}$.

4.2.2.2. 3',6'-Di-*O*-acetoxycinnamoyl-2,1':4,6-di-*O*-isopropylidene sucrose **198**

Following the general procedure, the reaction between diacetone **175** (1.0 g, 2.4 mmol) and *p*-acetoxycinnamoyl chloride **195** (1.2 g, 5.2 mmol) in dry pyridine (10 mL) for 1 day at rt gave compound **198** as a white solid (0.96 g, 51% yield) along with compound **196** in 5% (0.07 g) yield.

Analytical data for **198**: $R_f = 0.62$ (3:1 EtOAc-hexanes); mp 109-111 °C; FT-IR (KBr) $\nu_{\max}$: 3486, 2993, 2942, 1768, 1715, 1637, 1602, 1508, 1418, 1372, 1322, 1270, 1166, 1068, 1013, 945, 912, 858, 837, 792, 724, 652 cm^{-1} ;

^1H NMR (300 MHz, CDCl_3): δ 1.39, 1.43, 1.49, 1.52 (4 x s, 12H, $(\text{CH}_3)_2\text{C}$), 3.67 (m, 2H, H-1'a, H-4), 3.74 (m, 2H, H-2, H-6a), 3.84 (m, 2H, H-3, H-5), 3.96 (m, 1H, H-6b), 4.07 (m, 1H, H-1'b), 4.38 (m, 2H, H-5', H-6'a), 4.45 (m, 1H, H-4'), 4.52 (m, 1H, H-6'b), 4.91 (d, 1H, $J = 6.3$ Hz, H-3'), 6.13 (d, 1H, $J = 3.3$ Hz, H-1); *trans-p*-coumaroyl units: R_1 : 2.32 (s, 3H, H-11''), 6.43 (d, 1H, $J = 15.9$ Hz, H-8''), 7.12 (d, 2H, H-3'', H-5''), 7.54 (d, 2H, H-2'', H-6''), 7.73 (d, 1H, $J = 15.9$ Hz, H-7''), R_2 : 2.32 (s, 3H, H-11''), 6.49 (d, 1H, $J = 15.9$ Hz, H-8''), 7.17 (d, 2H, H-3'', H-5''), 7.62-7.66 (m, 2H, H-2'', H-6''), 7.79 (d, 1H, $J = 16.2$ Hz, H-7''); ^{13}C NMR (75.48 MHz, CDCl_3): δ 19.1, 24.1, 25.4, 29.1 (4 x $(\text{CH}_3)_2\text{C}$), 62.1 (C-6), 63.8 (C-5), 65.8 (C-6'), 66.0 (C-1'), 70.3 (C-3), 73.0 (C-4), 73.8 (C-2), 76.3 (C-4'), 80.8 (C-3'), 81.3 (C-5'), 91.0 (C-1), 99.8, 101.7 (2 x $(\text{CH}_3)_2\text{C}$), 104.4 (C-2'); *trans-p*-coumaroyl units: R_1 : 21.1 (C-11''), 116.7 (C-8''), 122.1 (C-3'', C-5''), 129.3 (C-2'', C-6''), 131.6 (C-1''), 144.2, 145.9 (C-7''), 152.1 (C-4''), 166.7 (C-9''), 169.0 (C-10''); R_2 : 21.1 (C-11''), 117.8 (C-8''), 122.2 (C-3'', C-5''), 129.7 (C-2'', C-6''), 132.0 (C-1''), 145.9 (C-7''), 152.6 (C-4''), 167.2 (C-9''), 169.1 (C-10''); ESI-Mass (positive mode): m/z 821.31 $[\text{M} + \text{Na}]^+$, calcd 821.27 for $\text{C}_{40}\text{H}_{46}\text{O}_{17}\text{Na}$. HR-ESI-MS (positive mode): found m/z 821.2618 $[\text{M} + \text{Na}]^+$, calcd 821.2627 for $\text{C}_{40}\text{H}_{46}\text{O}_{17}\text{Na}$. Anal. Calcd for $\text{C}_{40}\text{H}_{46}\text{O}_{17}$: C, 60.14; H, 5.80; found: C, 59.24; H, 6.04.

4.2.2.3. 3',4',6'-Tri-*O*-acetoxycinnamoyl-2,1':4,6-di-*O*-isopropylidene sucrose **199**

Following the general procedure, the reaction between diacetone **175** (1.0 g, 2.5 mmol) and *p*-acetoxycinnamoyl chloride **195** (1.9 g, 8.2 mmol) in dry pyridine (10 mL) for 9

days at rt afforded compound **199** as a white solid (0.80 g, 33% yield) along with compounds **198** and **200** in 5% (0.10 g) and 9% (0.25 g) yield, respectively.

Analytical data for **199**: $R_f = 0.67$ (3:1 EtOAc-hexanes); mp 135-138 °C; FT-IR (KBr) $\nu_{\max}$: 2993, 2942, 1768, 1718, 1636, 1602 1559, 1507, 1419, 1372, 1322, 1205, 1165, 1065, 1012, 945, 912, 858, 837, 726, 653 cm^{-1} ;

^1H NMR (300 MHz, CDCl_3): δ 1.29, 1.37, 1.47, 1.49 (4 x s, 12H, $(\text{CH}_3)_2\text{C}$), 3.56 (m, 2H, H-1'a, H-4), 3.62 (m, 1H, H-6a), 3.77 (m, 1H, H-2), 3.85 (m, 2H, H-3, H-5), 4.00 (dd, 1H, $J = 4.8$ Hz, 10.2 Hz, H-6b), 4.17 (d, 1H, $J = 12.6$ Hz, H-1'b), 4.50 (m, 2H, H-5', H-6'a), 4.60 (m, 1H, H-6'b), 5.33 (d, 1H, $J = 5.1$ Hz, H-4'), 5.57 (m, 1H, H-3'), 6.11 (d, 1H, $J = 3.3$ Hz, H-1); *trans-p*-coumaroyl units: R_1 : 2.29 (s, 3H, H-11''), 6.38 (d, 1H, $J = 15.9$ Hz, H-8''), 7.06-7.14 (m, 2H, H-3'', H-5''), 7.48-7.51 (m, 2H, H-2'', H-6''), 7.60-7.69 (m, 1H, H-7''), R_2 : 2.29 (s, 3H, H-11''), 6.39 (d, 1H, $J = 15.9$ Hz, H-8''), 7.06-7.14 (m, 2H, H-3'', H-5''), 7.48-7.51 (m, 2H, H-2'', H-6''), 7.60-7.69 (m, 1H, H-7''), R_3 : 2.29 (s, 3H, H-11''), 6.49 (d, 1H, $J = 15.9$ Hz, H-8''), 7.06-7.14 (m, 2H, H-3'', H-5''), 7.60-7.69 (m, 2H, H-2'', H-6''), 7.78 (d, 1H, $J = 15.9$ Hz, H-7''); ^{13}C NMR (75.48 MHz, CDCl_3): δ 19.1, 24.1, 25.5, 29.0 (4 x $(\text{CH}_3)_2\text{C}$), 62.0 (C-6), 63.9 (C-5), 64.9 (C-6'), 66.3 (C-1'), 70.2 (C-3), 72.9 (C-4), 73.8 (C-2), 77.4 (C-4'), 77.8 (C-3'), 80.1 (C-5'), 91.4 (C-1), 99.7, 101.8 (2 x $(\text{CH}_3)_2\text{C}$), 104.8 (C-2'); *trans-p*-coumaroyl units: R_1 : 21.1 (C-11''), 116.6 (C-8''), 122.1 (C-3'', C-5''), 129.3 (C-2'', C-6''), 131.6 (C-1''), 144.1 (C-7''), 152.1 (C-4''), 165.7 (C-9''), 169.0 (C-10''), R_2 : 21.1 (C-11''), 116.8 (C-8''), 122.1 (C-3'', C-5''), 129.4 (C-2'', C-6''), 131.7 (C-1''), 145.4 (C-7''), 152.4 (C-4''), 165.8 (C-9''), 169.0 (C-10''), R_3 : 21.1 (C-11''), 117.8 (C-8''), 122.2 (C-3'', C-5''), 129.7 (C-2'', C-6''), 132.1 (C-1''), 145.9 (C-7''), 152.5 (C-4''), 166.3 (C-9''), 169.1 (C-10''); ESI-Mass (positive mode): m/z 1009.31 $[\text{M} + \text{Na}]^+$, calcd 1009.32 for $\text{C}_{51}\text{H}_{54}\text{O}_{20}\text{Na}$; HR-ESI-MS (positive mode): found m/z 1009.3087 $[\text{M} + \text{Na}]^+$, calcd 1009.3101 for $\text{C}_{51}\text{H}_{54}\text{O}_{20}\text{Na}$; Anal. Calcd for $\text{C}_{51}\text{H}_{54}\text{O}_{20}$: C, 62.06; H, 5.51; found: C, 61.68; H, 5.91.

4.2.1.4. 3,3',4',6'-Tetra-*O*-acetoxycinnamoyl-2,1':4,6-di-*O*-isopropylidene sucrose **200**

Following the general procedure, the reaction between diacetone **175** (1.0 g, 2.4 mmol) and *p*-acetoxycinnamoyl chloride **195** (2.4 g, 10.7 mmol) in dry pyridine (10 mL) for

4 days at rt furnished compound **200** as a white solid (1.40 g, 50% yield) along with compound **199** in 22% (0.51 g) yield.

Analytical data for **200**: $R_f = 0.89$ (3:1 EtOAc-hexanes); mp 138-140 °C; FT-IR (KBr) $\nu_{\max}$: 2992, 2943, 1764, 1718, 1635, 1601, 1559, 1507, 1419, 1374, 1319, 1204, 1165, 1047, 1011, 946, 911, 860, 836, 669, 650 cm^{-1} ;

^1H NMR (300 MHz, CDCl_3): δ 1.19, 1.27, 1.43, 1.47 (4 x s, 12H, $(\text{CH}_3)_2\text{C}$), 3.65 (m, 2H, H-1'a, H-6a), 3.74 (d, 1H, $J = 9.9$ Hz, H-4), 3.89-3.97 (m, 2H, H-2, H-5), 4.00-4.06 (m, 1H, H-6b), 4.24 (d, 1H, $J = 12.3$ Hz, H-1'b), 4.48-4.59 (m, 3H, H-5', H-6'a, H-6'b), 5.33-5.40 (m, 2H, H-3, H-4'), 5.59 (br dd, 1H, $J = 3.0$ Hz, 5.4 Hz, H-3'), 6.18 (d, 1H, $J = 3.6$ Hz, H-1); *trans-p*-coumaroyl units: R_1 : 2.31 (s, 3H, H-11''), 6.38 (d, 1H, $J = 15.9$ Hz, H-8''), 7.04-7.14 (m, 2H, H-3'', H-5''), 7.45-7.55 (m, 2H, H-2'', H-6''), 7.60-7.75 (m, 1H, H-7''), R_2 : 2.31 (s, 3H, H-11''), 6.40 (d, 1H, $J = 15.9$ Hz, H-8''), 7.04-7.14 (m, 2H, H-3'', H-5''), 7.45-7.55 (m, 2H, H-2'', H-6''), 7.60-7.75 (m, 1H, H-7''), R_3 : 2.31 (s, 3H, H-11''), 6.41 (d, 1H, $J = 15.9$ Hz, H-8''), 7.04-7.14 (m, 2H, H-3'', H-5''), 7.45-7.55 (m, 2H, H-2'', H-6''), 7.60-7.75 (m, 1H, H-7''), R_4 : 2.31 (s, 3H, H-11''), 6.58 (d, 1H, $J = 15.9$ Hz, H-8''), 7.04-7.14 (m, 2H, H-3'', H-5''), 7.60-7.75 (m, 2H, H-2'', H-6''), 7.93 (d, 1H, $J = 15.9$ Hz, H-7''); ^{13}C NMR (75.48 MHz, CDCl_3): δ 19.0, 23.9, 25.5, 28.8 (4 x $(\text{CH}_3)_2\text{C}$), 62.1 (C-6), 64.3 (C-5), 65.0 (C-6'), 66.2 (C-1'), 70.9 (C-3), 71.5 (C-4), 71.9 (C-2), 77.5 (C-4'), 77.8 (C-3'), 80.2 (C-5'), 91.7 (C-1), 99.6, 101.5 (2 x $(\text{CH}_3)_2\text{C}$), 105.0 (C-2'), *trans-p*-coumaroyl units: R_1 : 21.1 (C-11''), 116.6 (C-8''), 122.0 (C-3'', C-5''), 129.2 (C-2'', C-6''), 131.7 (C-1''), 143.6 (C-7''), 152.0 (C-4''), 165.7 (C-9''), 169.0 (C-10''), R_2 : 21.1 (C-11''), 116.9 (C-8''), 122.1 (C-3'', C-5''), 129.3 (C-2'', C-6''), 132.0 (C-1''), 144.0 (C-7''), 152.1 (C-4''), 165.7 (C-9''), 169.1 (C-10''), R_3 : 21.1 (C-11''), 117.8 (C-8''), 122.1 (C-3'', C-5''), 129.4 (C-2'', C-6''), 132.1 (C-1''), 145.3 (C-7''), 152.3 (C-4''), 166.0 (C-9''), 169.1 (C-10''), R_4 : 21.1 (C-11''), 118.3 (C-8''), 122.2 (C-3'', C-5''), 130.0 (C-2'', C-6''), 132.2 (C-1''), 146.2 (C-7''), 152.4 (C-4''), 166.3 (C-9''), 169.2 (C-10''); ESI-Mass (positive mode): m/z 1197.36 $[\text{M} + \text{Na}]^+$, calcd 1197.37 for $\text{C}_{62}\text{H}_{62}\text{O}_{23}\text{Na}$; HR-ESI-MS (positive mode): found m/z 1197.3598 $[\text{M} + \text{Na}]^+$, calcd 1197.3574 for $\text{C}_{62}\text{H}_{62}\text{O}_{23}\text{Na}$; Anal. Calcd for $\text{C}_{62}\text{H}_{62}\text{O}_{23}$: C, 63.37; H, 5.32; found: C, 63.29; H, 5.60.

4.2.3. Synthesis of Lapathoside D 67

4.2.3.1. 3',6'-Di-*O*-acetoxycinnamoylsucrose 201

A solution of compound **198** (1.6 g, 2.2 mmol) in 60% aq. AcOH (103 mL) was kept at 80 °C for 20 min. The reaction was monitored by TLC analysis (EtOAc). The reaction solution was then evaporated to dryness under reduced pressure by codistillation with toluene (3 x 100 mL). It was then evaporated to dryness under reduced pressure. The product **201** was obtained from recrystallization in EtOAc as a white solid (0.70 g, 49% yield).

Analytical data for **201**: $R_f = 0.61$ (15:1 EtOAc-MeOH); mp 108-110 °C; FT-IR (KBr) $\nu_{\max}$: 3326, 2969, 2930, 1764, 1699, 1635, 1601, 1558, 1539, 1507, 1419, 1371, 1323, 1208, 1166, 1062, 995, 917, 862, 833, 700, 650 cm^{-1} ;

^1H NMR (300 MHz, CD_3OD): δ 3.39-3.48 (m, 2H, H-2, H-4), 3.66 (m, 3H, H-1'a, H-1'b, H-3), 3.80 (m, 1H, H-6a), 3.95 (m, 2H, H-6b, H-5), 4.16-4.24 (m, 1H, H-5'), 4.47 (dd, 1H, $J = 8.1$ Hz, 8.4 Hz, H-4'), 4.58 (m, 2H, H-6'b, H-6'a), 5.45 (br s, 1H, H-1), 5.53 (d, 1H, $J = 7.8$ Hz, H-3'); *trans-p*-coumaroyl units: R_1 : 2.28 (s, 3H, H-11''), 6.55 (d, 1H, $J = 15.9$ Hz, H-8''), 7.16 (d, 2H, H-3'', H-5''), 7.63-7.73 (m, 2H, H-2'', H-6''), 7.79 (d, 1H, $J = 15.9$ Hz, H-7''), R_2 : 2.28 (s, 3H, H-11''), 6.60 (d, 1H, $J = 15.9$ Hz, H-8''), 7.16 (d, 2H, H-3'', H-5''), 7.63-7.73 (m, 2H, H-2'', H-6''), 7.79 (d, 1H, $J = 15.9$ Hz, H-7''); ^{13}C NMR (75.48 MHz, CD_3OD): δ 62.7 (C-6), 65.2 (C-1'), 66.6 (C-6'), 71.5 (C-4), 73.2 (C-2), 74.6 (C-5), 75.1 (C-4', C-3), 79.5 (C-3'), 81.3 (C-5'), 93.2 (C-1), 105.1 (C-2'); *trans-p*-coumaroyl units: R_1 : 21.0 (C-11''), 118.7 (C-8''), 123.5 (C-3'', C-5''), 130.6 (C-2'', C-6''), 133.5 (C-1''), 145.7 (C-7''), 154.0 (C-4''), 167.7 (C-9''), 170.9 (C-10''), R_2 : 21.0 (C-11''), 118.8 (C-8''), 123.5 (C-3'', C-5''), 130.8 (C-2'', C-6''), 133.5 (C-1''), 146.2 (C-7''), 154.0 (C-4''), 168.4 (C-9''), 170.9 (C-10''); ESI-Mass (positive mode): m/z 741.22 $[\text{M} + \text{Na}]^+$, calcd 741.21 for $\text{C}_{34}\text{H}_{38}\text{O}_{17}\text{Na}$; HR-ESI-MS (positive mode): found m/z 741.2001 $[\text{M} + \text{Na}]^+$, calcd 741.2001 for $\text{C}_{34}\text{H}_{38}\text{O}_{17}\text{Na}$. Anal. Calcd for $\text{C}_{34}\text{H}_{38}\text{O}_{17}$: C, 56.28; H, 5.33; found: C, 56.23; H, 5.49.

4.2.3.2. Synthesis of lapathoside D or 3',6'-di-*O*-hydroxycinnamoylsucrose **67**

Compound **205** (0.5 g, 0.7 mmol) was suspended in 95% EtOH (21 mL) and pyrrolidine (325.0 μ L, 281.4 mg, 4.0 mmol) was added (which caused the solution to turn yellow). The starting material typically dissolved within 15 min and the reaction was allowed to continue for a total of 2 h. The mixture was added directly to a column of strongly acidic ion-exchange resin [Amberlite IRA-120 (H^+), 66.0 g, washed and packed in 95% EtOH]. The appropriate fractions were concentrated to a residue that was filtered off and washed with EtOAc. The filtrate was evaporated under diminished pressure to afford compound **67** as a white solid in 70% (0.31 g) yield.

Analytical data for **67**: R_f = 0.55 (15:1 EtOAc-MeOH); mp 98-100 $^{\circ}$ C; FT-IR (KBr) ν_{max} : 3304, 2938, 1706, 1636, 1606, 1516, 1438, 1330, 1264, 1204, 1171, 1062, 1002, 863, 831 cm^{-1} ;

1H NMR (300 MHz, CD_3OD): δ 3.41 (m, 1H, H-4), 3.44 (dd, 1H, J = 9.7 Hz, 5.1 Hz, H-2), 3.61 (m, 2H, H-1'b, H-1'a), 3.63 (m, 1H, H-3), 3.80 (m, 1H, H-6a), 3.91 (m, 2H, H-6b, H-5), 4.15 (m, 1H, H-5'), 4.43 (dd, 1H, J = 7.8 Hz, 8.1 Hz, H-4'), 4.54 (m, 2H, H-6'b, H-6'a), 5.44 (d, 1H, J = 3.6 Hz, H-1), 5.49 (d, 1H, J = 8.1 Hz, H - 3'); *trans-p*-coumaroyl units: R_1 : 6.41 (d, 1H, J = 15.9 Hz, H-8''), 6.81 (d, 2H, H-3'', H-5''), 7.52 (d, 2H, H- 2'', H-6''), 7.72 (d, 1H, J = 15.9 Hz, H-7''); R_2 : 6.37 (d, 1H, J = 15.9 Hz, H-8''), 6.81 (d, 2H, H-3'', H-5''), 7.48 (d, 2H, H-2'', H-6''), 7.67 (d, 1H, J = 15.9 Hz, H-7''); ^{13}C NMR (75.48 MHz, CD_3OD): δ 62.6 (C-6), 65.1 (C-1'), 66.4 (C-6'), 71.4 (C-4), 73.2 (C-2), 74.4 (C-5), 75.0 (C-4', C-3), 79.3 (C-3'), 81.2 (C-5'), 93.2 (C-1), 105.1 (C-2'); *trans-p*-coumaroyl units: R_1 : 114.6 (C-8''), 116.9 (C-3'', C-5''), 127.2 (C-1''), 131.5 (C-2'', C-6''); 147.6 (C-7''), 161.3 (C-4''), 168.4 (C-9''), R_2 : 114.8 (C-8''), 116.9 (C-3'', C-5''), 127.2 (C-1''), 131.3 (C-2'', C-6''); 147.0 (C-7''), 161.3 (C-4''), 169.2 (C-9''); ESI-Mass (positive mode): m/z 657.1 $[M + Na]^+$, calcd 657.1 for $C_{30}H_{34}O_{15}Na$; HR-ESI-MS (positive mode): found m/z 657.1786 $[M + Na]^+$, calcd 657.1790 for $C_{30}H_{34}O_{15}Na$; Anal. Calcd for $C_{30}H_{34}O_{15}$: C, 56.78; H, 5.40; found: C, 55.31; H, 6.12. Spectral data of compound **67** was the same as reported previously.⁵⁹

4.2.4. Deacetylation of compounds 196, 198 and 199

General Procedure

To a stirred separate suspension of diacetone coumaroyl derivatives in 95% EtOH, pyrrolidine was added dropwise (which caused the solution to turn yellow). The starting material typically dissolved within 15 min and the reaction was allowed to continue for a total of 1 h. Upon the disappearance of starting material (determined by TLC analysis using EtOAc-hexanes (3:1) as solvent system), the crude reaction mixture was added directly to a column of strongly acidic ion-exchange resin [Amberlite IRA-120 (H^+), washed and packed in 95% EtOH]. The appropriate fractions were then concentrated to a residue that was filtered off and washed with EtOAc. The filtrate was evaporated under diminished pressure to give a syrup. It was subjected to silica gel column chromatography using a gradient of CH_2Cl_2 -EtOAc as eluent. The solvent was evaporated under diminished pressure to furnish deacetylated diacetone products as a white solid.

4.2.4.1. 6'-Mono-*O*-coumaroyl-2,1':4,6-di-*O*-isopropylidene sucrose **202**

Following the general procedure, a suspension of compound **196** (0.3 g, 0.4 mmol) in 95% EtOH (11 mL) was treated with pyrrolidine (84.5 μ L, 73.2 mg, 1.0 mmol) for 15 min to afford compound **202** as a white solid

(0.12 g, 50% yield). Analytical data for **202**: R_f = 0.54 (9:1 EtOAc-MeOH); mp 151-153 $^{\circ}C$;

FT-IR (KBr) ν_{max} : 3453, 3417, 2994, 2939, 1702, 1606, 1515, 1443, 1384, 1268, 1204, 1171, 1134, 1068, 1014, 942, 857, 836, 700, 649 cm^{-1} ; 1H NMR (300 MHz, CD_3OD): δ 1.36, 1.45, 1.49 (3 x s, 12H, $(CH_3)_2C$), 3.44 (d, 1H, J = 12.0 Hz, H-1'a), 3.55 (dd, 1H, J = 9.0 Hz, 8.7 Hz, H-4), 3.69-3.73 (m, 3H, H-2, H-3', H-6a), 3.83-3.86 (m, 3H, H-3, H-5, H-6b), 4.06-4.09 (m, 2H, H-4', H-5'), 4.13 (d, 1H, J = 12.3 Hz, H-1'b), 4.24 (m, 1H, H-6'a), 4.43 (d, 1H, J = 11.4 Hz, H-6'b), 6.04 (d, 1H, J = 2.7 Hz, H-1); *trans-p*-coumaroyl units: 6.36 (d, 1H, J = 15.9 Hz, H-8''), 6.81 (d, 2H, H-3'', H-5''), 7.46 (d, 2H, H-2'', H-6''), 7.64 (d, 1H, J = 15.9 Hz, H-7''); ^{13}C NMR (75.48 MHz, CD_3OD): δ 19.4, 24.5, 25.7, 29.6 (4 x $(CH_3)_2C$), 63.5 (C-6), 64.7 (C-5), 67.0 (C-1'), 67.7 (C-6'), 71.1 (C-3), 74.9 (C-4), 75.4 (C-2), 78.1 (C-4'), 80.2 (C-3'), 81.3 (C-5'), 92.5 (C-1), 101.0, 103.0 (2 x $(CH_3)_2C$), 105.3 (C-2'); *trans-p*-coumaroyl units: 115.1

(C-8''), 117.0 (C-3'', C-5''), 127.3 (C-1''), 131.4 (C-2'', C-6''), 146.9 (C-7''), 161.4 (C-4''), 169.1 (C-9''); ESI-Mass (positive mode): m/z 591.24 $[M + Na]^+$, calcd 591.22 for $C_{27}H_{36}O_{13}Na$; HR-ESI-MS (positive mode): found m/z 591.2064 $[M + Na]^+$, calcd 591.2048 for $C_{27}H_{36}O_{13}Na$.

4.2.4.2. 3', 6'-Di-*O*-coumaroyl-2,1':4,6-di-*O*-isopropylidene sucrose **203**

Following the general procedure, a suspension of compound **198** (0.3 g, 0.3 mmol) in 95% EtOH (11 mL) was treated with pyrrolidine (167.5 μ L, 145.0 mg, 2.0 mmol) for 1 h to give compound **203** as a white solid (0.14 g, 63% yield).

Analytical data for **203**: R_f = 0.78 (9:1 EtOAc-MeOH); mp 159-162 °C; FT-IR (KBr) ν_{max} : 3404, 2993, 2942, 1708, 1632, 1606, 1516, 1443, 1374, 1330, 1266, 1204, 1170, 1068, 1012, 943, 858, 832, 7452, 724, 655 cm^{-1} ;

1H NMR (300 MHz, CD_3OD): δ 1.26, 1.33, 1.45 (3 x s, 12H, $(CH_3)_2C$), 3.52-3.58 (m, 2H, H-1'a, H-4), 3.66-3.75 (m, 4H, H-2, H-3, H-5, H-6a), 3.88-3.92 (m, 1H, H-6b), 4.05-4.12 (m, 1H, H-1'b), 4.23-4.34 (m, 2H, H-5', H-6'a), 4.44 (m, 1H, H-4'), 4.49 (m, 1H, H-6'b), 5.05 (d, 1H, J = 5.7 Hz, H-3'), 6.05 (br s, 1H, H-1); *trans-p*-coumaroyl units: R_1 : 6.36 (d, 1H, J = 15.9 Hz, H-8''), 6.81 (d, 2H, H-3'', H-5''), 7.46 (d, 2H, H-2'', H-6''), 7.65 (d, 1H, J = 15.9 Hz, H-7''), R_2 : 6.43 (d, 1H, J = 15.9 Hz, H-8''), 6.81 (d, 2H, H-3'', H-5''), 7.52 (d, 2H, H-2'', H-6''), 7.75 (d, 1H, J = 15.9 Hz, H-7''); ^{13}C NMR (75.48 MHz, CD_3OD): δ 19.4, 24.3, 25.7, 29.4 (4 x $(CH_3)_2C$), 63.3 (C-6), 65.2 (C-5), 66.6 (C-6'), 67.6 (C-1'), 71.3 (C-3), 74.6 (C-4), 75.2 (C-2), 76.6 (C-4'), 80.7 (C-3'), 82.5 (C-5'), 92.7 (C-1), 101.0, 102.9 (2 x $(CH_3)_2C$), 105.9 (C-2'); *trans-p*-coumaroyl units: R_1 : 114.3 (C-8''), 116.9 (C-3'', C-5''), 127.1 (C-1''), 131.3 (C-2'', C-6''), 146.9 (C-7''), 161.3 (C-4''), 168.4 (C-9''), R_2 : 115.0 (C-8''), 116.9 (C-3'', C-5''), 127.2 (C-1''), 131.7 (C-2'', C-6''), 148.0 (C-7''), 161.6 (C-4''), 169.0 (C-9''); ESI-Mass (positive mode): m/z 737.27 $[M + Na]^+$, calcd 737.25 for $C_{36}H_{42}O_{15}Na$; HR-ESI-MS (positive mode): found m/z 737.2418 $[M + Na]^+$, calcd 737.2416 for $C_{36}H_{42}O_{15}Na$.

4.2.4.3. 3',4',6'-Tri-*O*-coumaroyl-2,1':4,6-di-*O*-isopropylidene sucrose **204**

Following the general procedure, a suspension of compound **199** (0.1 g, 0.1 mmol) in 95% EtOH (4.0 mL) was treated with pyrrolidine (97.5 μ L, 84.4 mg, 1.2 mmol) for 1 h to furnish compound **204** as a white solid (0.04 g, 46% yield).

Analytical data for **204**: R_f = 0.57 (9:1 EtOAc-MeOH); mp 115-118 $^{\circ}$ C; FT-IR (KBr) $\nu_{\max}$: 3290, 1700, 1632, 1605 1515, 1443, 1374, 1330, 1263, 1204, 1170, 1106, 1057, 995, 864, 830 cm^{-1} ;

^1H NMR (300 MHz, CD_3OD): δ 1.24, 1.33, 1.44 (3 x s, 12H, $(\text{CH}_3)_2\text{C}$), 3.52 (m, 1H, H-4); 3.68 (m, 4H, H-2, H-3, H-1'a, H-6a), 3.78 (m, 1H, H-5), 3.96 (m, 1H, H-6b), 4.20 (m, 1H, H-1'b), 4.46 (m, 2H, H-5', H-6'a), 4.59 (m, 1H, H-6'b), 5.33 (br s, 1H, H-4'), 5.60 (br s, 1H, H-3'), 6.09 (br s, 1H, H-1); *trans-p*-coumaroyl units: R_1 : 6.26-6.42 (m, 1H, H-8''), 6.72-6.80 (m, 2H, H-3'', H-5''), 7.39 (m, 2H, H-2'', H-6''), 7.63 (m, 1H, H-7''), R_2 : 6.26-6.42 (m, 1H, H-8''), 6.72-6.80 (m, 2H, H-3'', H-5''), 7.39 (m, 2H, H-2'', H-6''), 7.63 (m, 1H, H-7''), R_3 : 6.26-6.42 (m, 1H, H-8''), 6.72-6.80 (m, 2H, H-3'', H-5''), 7.52 (m, 2H, H-2'', H-6''), 7.75 (m, 1H, H-7''); ^{13}C NMR (75.48 MHz, CD_3OD): δ 19.5, 24.4, 25.8, 29.5 (4 x $(\text{CH}_3)_2\text{C}$), 63.3 (C-6), 65.4 (C-5), 66.1 (C-6'), 67.6 (C-1'), 71.3 (C-3), 74.6 (C-4), 75.1 (C-2), 79.0 (C-4'), 79.3 (C-3'), 81.2 (C-5'), 93.1 (C-1), 101.0, 103.0 (2 x $(\text{CH}_3)_2\text{C}$), 106.5 (C-2'); *trans-p*-coumaroyl units: R_1 : 114.0 (C-8''), 116.9 (C-3'', C-5''), 127.1 (C-1''), 131.4 (C-2'', C-6''), 147.0 (C-7''), 161.3 (C-4''), 168.0 (C-9''), R_2 : 114.2 (C-8''), 117.0 (C-3'', C-5''), 127.1 (C-1''), 131.7 (C-2'', C-6''), 148.1 (C-7''), 161.6 (C-4''), 168.3 (C-9''), R_3 : 115.0 (C-8''), 117.0 (C-3'', C-5''), 127.2 (C-1''), 131.9 (C-2'', C-6''), 148.5 (C-7''), 161.6 (C-4''), 168.8 (C-9''); HR-ESI-MS (positive mode): found m/z 883.2793 $[\text{M} + \text{Na}]^+$, calcd 883.2784 for $\text{C}_{45}\text{H}_{48}\text{O}_{17}\text{Na}$.

4.3. Synthesis of Helonioside A and its analogues

4.3.1. Preparation of *p*-acetoxyferuloyl chloride **207**

trans-Ferulic acid **205** (26.0 g, 134.0 mmol) was acetylated in dry pyridine (47 mL) with Ac_2O (44.0 mL, 446.3 mmol). The mixture was stirred for 22 h at room temperature and quenched with 95% EtOH (33 mL) while stirring. Upon cooling, the solution deposited

crystalline *p*-acetoxyferulic acid **206**. The filtrate was evaporated to a syrup and any remains of pyridine were removed by co-distillation with toluene. Redissolution of the resulting syrup in 95% EtOH gave a second crop of crystals. Crystallization of the combined extracts from 95% EtOH afforded *p*-acetoxyferulic acid

206 (25.0 g, 79%). Analytical data for **206**:
mp 201-204 °C;

^1H NMR (300 MHz, DMSO- d_6): δ 2.25 (1s, 3H, H-11), 3.81 (s, 3H, -OCH₃), 6.60 (d, 1H, J = 15.9 Hz, H-8), 7.10 (d, 1H, J = 8.4 Hz, H-5), 7.24 (dd, 1H, J = 8.4 Hz, H-6), 7.45 (br s, 1H, H-2), 7.62 (d, 1H, J = 15.9 Hz, H-7); ^{13}C NMR (75.48 MHz, DMSO- d_6): δ 20.2 (C-11), 56.1 (-OCH₃), 111.7 (C-2), 119.4 (C-8), 121.2 (C-6), 123.1 (C-5), 133.2 (C-1), 140.8 (C-4), 143.3 (C-7), 151.1 (C-3), 167.6 (C-9), 168.4 (C-10).

The acid chloride **207** was prepared by refluxing a mixture of the *p*-acetoxyferulic acid **206** (9.9 g, 42.0 mmol) and SOCl₂ (13 mL, 179.0 mmol) in benzene (186 mL) for 2 h in an oil bath (95 °C). The resulting clear solutions were evaporated to a solid, redissolved in toluene and evaporated to a solid again.

Recrystallization from hot toluene afforded the *p*-acetoxyferuloyl chloride **207** (8.7 g, 81% yields). Analytical data for **207**: mp 130-133 °C;

^1H NMR (300 MHz, (CD₃)₂CO): δ 2.33 (s, 3H, H-11), 3.88 (s, 3H, -OCH₃), 6.59 (d, 1H, J = 15.9 Hz, H-8), 7.11 (dd, 2H, J = 2.7 Hz, 8.1 Hz, H-5, H-6), 7.14 (dd, 1H, J = 2.1 Hz, 8.1 Hz, H-2), 7.79 (d, 1H, J = 15.9 Hz, H-7); ^{13}C NMR (75.48 MHz, (CD₃)₂CO): δ 20.4 (C-11), 56.4 (-OCH₃), 113.3 (C-2), 122.9 (C-8), 123.9 (C-6), 124.4 (C-5), 132.8 (C-1), 144.0 (C-4), 151.5 (C-7), 152.8 (C-3), 166.2 (C-9), 168.7 (C-10). Spectral data of compounds **206** and **207** was the same as reported previously.²⁰⁷

4.3.2. Acylation of diacetone 175 with *p*-acetoxyferuloyl chloride **207**

General procedure

Diacetone **175** was dissolved in dry pyridine under a nitrogen atmosphere. The solution was then cooled to 0 °C in an ice bath. *p*-Acetoxyferuloyl chloride **207** was then added slowly at the same temperature and the reaction was left to stir while warming to rt.

Stirring was continued at rt until the reaction was completed. Reaction was monitored by TLC analysis (3:1 EtOAc-hexanes). The resulting mixture was poured into vigorously stirred ice-water (100 mL) and a white solid precipitated was obtained after decantation and filtration. The precipitate was redissolved in EtOAc (25 mL) and washed once with 1N HCl (50 mL). The aqueous layer was extracted with EtOAc (50 mL). The combined organic layers were then successively washed with 5% NaHCO₃ (50 mL) and brine (25 mL) and then dried over anhyd. MgSO₄. The EtOAc solution was concentrated to residue that was subjected to column chromatography using a gradient of CH₂Cl₂-EtOAc as eluent.

4.3.2.1. 6'-Mono-*O*-acetoxyferuloyl -2,1':4,6-di-*O*-isopropylidene sucrose **208** and 3'-mono-*O*-acetoxyferuloyl-2,1':4,6-di-*O*-isopropylidene sucrose **209**

Following the general procedure, the reaction between diacetone **175** (1.0 g, 2.4 mmol) and *p*-acetoxyferuloyl chloride **207** (0.7 g, 2.6 mmol) in dry pyridine (10 mL) for 3 days afforded compound **208** as a white solid (0.47 g, 30% yield) along with compounds **209** and **210** in 11% (0.16 g) and 12% (0.25 g) yield, respectively.

Analytical data for **208**: $R_f = 0.12$ (3:2 EtOAc-CH₂Cl₂); mp 147-150 °C; FT-IR (KBr) $\nu_{\max}$: 3452, 2993, 2941, 1766, 1712, 1636, 1601, 1511, 1417, 1372, 1260, 1199, 1157, 1127, 1069, 1013, 943, 858, 718, 651 cm⁻¹;

¹H NMR (300 MHz, CDCl₃): δ 1.45, 1.51 (2 x s, 12H, (CH₃)₂C), 3.50 (m, 1H, H-1'a), 3.60 (m, 1H, H-4), 3.73 (m, 2H, H-2, H-6a), 3.93 (m, 3H, H-3', H-5, H-6b), 4.05 (m, 1H, H-3), 4.23 (m, 2H, H-4', H-5'), 4.30 (m, 2H, H-1'b, H-6'a), 4.51 (m, 1H, H-6'b), 6.19 (br d, 1H, $J = 1.8$ Hz, H-1); *trans-p*-feruloyl units: 2.32 (1s, 3H, H-11''), 3.85 (s, 3H, -OCH₃), 6.41 (d, 1H, $J = 15.9$ Hz, H-8''), 7.00-7.19 (m, 3H, H-2'', H-5'', H-6''), 7.64 (d, 1H, $J = 15.9$ Hz, H-7''); ¹³C NMR (75.48 MHz, CDCl₃): δ 19.2, 24.2, 25.3, 29.1 (4 x (CH₃)₂C), 62.3 (C-6), 63.8 (C-5), 65.9 (C-6'), 66.5 (C-1'), 69.3 (C-3), 73.4 (C-4), 73.9 (C-2), 77.5 (C-4'), 79.0 (C-3'), 79.7 (C-5'), 91.0 (C-1), 100.1, 102.2 (2 x (CH₃)₂C), 103.7 (C-2'); *trans-p*-feruloyl units: δ 20.7 (C-11''), 55.9 (-OCH₃), 111.3 (C-2''), 117.9 (C-8''), 121.4 (C-5''), 123.2 (C-6''), 133.2 (C-1''), 141.5 (C-3''), 144.7 (C-7''), 151.4 (C-4''), 167.0 (C-9''), 168.8 (C-10''); ESI-Mass (positive

mode): m/z 663.22 $[M + Na]^+$, calcd 663.24 for $C_{30}H_{40}O_{15}Na$; HR-ESI-MS (positive mode): found m/z 663.2268 $[M + Na]^+$, calcd 663.2259 for $C_{30}H_{40}O_{15}Na$.

Analytical data for **209**: $R_f = 0.21$ (3:2 EtOAc/ CH_2Cl_2); mp 130-132 °C; FT-IR (KBr) ν_{max} : 2994, 2936, 1765, 1716, 1638, 1590, 1510, 1467, 1421, 1373, 1333, 1260, 1199, 1156, 1125, 1069, 1033, 1015, 946, 855, 650 cm^{-1} ;

1H NMR (300 MHz, $CDCl_3$): δ 1.40, 1.44, 1.53 (3 x s, 12H, $(CH_3)_2C$), 3.58 (m, 2H, H-1'a, H-4), 3.68 (m, 2H, H-6a, H-6'a), 3.77 (m, 1H, H-2), 3.87 (m, 4H, H-3, H-5, H-6b, H-6'b), 4.10 (m, 2H, H-1'b, H-5'), 4.87 (m, 1H, H-4'), 5.03 (d, 1H, $J = 7.8$ Hz, H-3'), 6.22 (d, 1H, $J = 3.6$ Hz, H-1); *trans-p*-feruloyl units: δ 2.05 (1s, 3H, H-11''), 3.91 (s, 3H, $-OCH_3$), 6.50 (d, 1H, $J = 15.9$ Hz, H-8''), 7.09 (d, 1H, $J = 7.8$ Hz, H-5''), 7.16-7.22 (m, 2H, H-5'', H-6''), 7.77 (d, 1H, $J = 15.9$ Hz, H-7''); ^{13}C NMR (75.48 MHz, $CDCl_3$): δ 19.0, 24.1, 25.3, 29.0 (4 x $(CH_3)_2C$), 61.2 (C-6), 61.8 (C-6'), 63.9 (C-5), 66.5 (C-1'), 70.0 (C-3), 71.4 (C-4'), 72.7 (C-2), 73.4 (C-4), 80.0 (C-3'), 84.0 (C-5'), 91.0 (C-1), 99.9, 102.0 (2 x $(CH_3)_2C$), 103.48 (C-2'); *trans-p*-feruloyl units: 20.6 (C-11''), 56.0 ($-OCH_3$), 111.3 (C-2''), 116.8 (C-8''), 122.0 (C-5''), 123.4 (C-6''), 132.9 (C-1''), 141.9 (C-3''), 146.3 (C-7''), 151.5 (C-4''), 167.2 (C-9''), 168.7 (C-10''); ESI-Mass (positive mode): m/z 663.19 $[M + Na]^+$, calcd 663.24 for $C_{30}H_{40}O_{15}Na$; HR-ESI-MS (positive mode): found m/z 663.2255 $[M + Na]^+$, calcd 663.2259 for $C_{30}H_{40}O_{15}Na$.

4.3.2.2. 3',6'-Di-*O*-acetoxyferuloyl-2,1':4,6-di-*O*-isopropylidene sucrose **210**

Following the general procedure, the reaction between diacetone **175** (1.0 g, 2.4 mmol) and *p*-acetoxyferuloyl chloride **207** (1.3 g, 5.2 mmol) in dry pyridine (10 mL) for 5 days gave compound **210** as a white solid (0.6 g, 30% yield) along with compound **208** in 3% (0.05 g) yield.

Analytical data for **210**: $R_f = 0.61$ (3:2 EtOAc- CH_2Cl_2); mp 109-110 °C; FT-IR (KBr) ν_{max} : 3486, 2993, 2942, 1766, 1716, 1637, 1601, 1511, 1417, 1372, 1332, 1259, 1198, 1069, 1032, 1012, 947, 906, 858, 655 cm^{-1} ;

^1H NMR (300 MHz, CDCl_3): δ 1.40, 1.41, 1.53 (3 x s, 12H, $(\text{CH}_3)_2\text{C}$), 3.63 (m, 2H, H-1'a, H-4), 3.74 (m, 1H, H-6a), 3.79 (m, 1H, H-2), 3.88 (m, 2H, H-3, H-5), 3.94 (m, 1H, H-6b), 4.08 (d, 1H, $J = 12.3$ Hz, H-1'b), 4.38 (m, 2H, H-5', H-6'a), 4.46 (m, 1H, H-4'), 4.53 (m, 1H, H-6'b), 4.92 (d, 1H, $J = 6.3$ Hz, H-3'), 6.13 (d, 1H, $J = 3.3$ Hz, H-1); *trans-p*-feruloyl units: R_1 : 2.33 (s, 3H, H-11''), 3.87 (s, 3H, $-\text{OCH}_3$), 6.43 (d, 1H, $J = 15.9$ Hz, H-8''), 7.03-7.12, 7.20 (2 x m, 3H, H-2'', H-5'', H-6''), 7.66 (d, 1H, $J = 15.9$ Hz, H-7''), R_2 : 2.33 (s, 3H, H-11''), 3.92 (s, 3H, $-\text{OCH}_3$), 6.48 (d, 1H, $J = 15.9$ Hz, H-8''), 7.03-7.12, 7.20 (2 x m, 3H, H-2'', H-5'', H-6''), 7.77 (d, 1H, $J = 15.9$ Hz, H-7''); ^{13}C NMR (75.48 MHz, CDCl_3): δ 19.1, 24.1, 25.4, 29.1 (4 x $(\text{CH}_3)_2\text{C}$), 62.1 (C-6), 63.8 (C-5), 65.7 (C-6'), 65.9 (C-1'), 70.3 (C-3), 72.9 (C-4), 73.8 (C-2), 76.5 (C-4'), 81.3 (C-3'), 81.4 (C-5'), 90.9 (C-1), 99.9, 101.8 (2 x $(\text{CH}_3)_2\text{C}$), 104.5 (C-2'); *trans-p*-feruloyl units: R_1 : 20.7 (C-11''), 55.9 ($-\text{OCH}_3$), 111.2 (C-2''), 116.5 (C-8''), 121.4 (C-5''), 123.4 (C-6''), 132.8 (C-1''), 141.6 (C-3''), 144.6 (C-7''), 151.4 (C-4''), 166.6 (C-9''), 168.8 (C-10''); R_2 : 20.7 (C-11''), 56.1 ($-\text{OCH}_3$), 111.4 (C-2''), 117.9 (C-8''), 122.1 (C-5''), 123.4 (C-6''), 133.3 (C-1''), 142.0 (C-3''), 146.6 (C-7''), 151.5 (C-4''), 167.7 (C-9''), 168.8 (C-10''); ESI-Mass (positive mode): m/z 881.35 $[\text{M} + \text{Na}]^+$, calcd 881.29 for $\text{C}_{42}\text{H}_{50}\text{O}_{19}\text{Na}$; HR-ESI-MS (positive mode): found m/z 881.2860 $[\text{M} + \text{Na}]^+$, calcd 881.2839 for $\text{C}_{42}\text{H}_{50}\text{O}_{19}\text{Na}$.

4.3.2.3. 3',4',6'-Tri-*O*-acetoxyferuloyl-2,1':4,6-di-*O*-isopropylidene sucrose **211**

Following the general procedure, the reaction between diacetone **175** (1.0 g, 2.4 mmol) and *p*-acetoxyferuloyl chloride **207** (2.0 g, 7.8 mmol) in dry pyridine (10 mL) for 2 days furnished compound **211** as a white solid (0.67 g, 26% yield) along with compound **210** in 44% (0.89 g) yield. Analytical data for **211**: $R_f = 0.73$ (3:2 EtOAc- CH_2Cl_2);

mp 135-138 $^{\circ}\text{C}$; FT-IR (KBr) ν_{max} : 3507, 2993, 2942, 1766, 1721, 1638, 1601 1510, 1467, 1418, 1371, 1332, 1259, 1198, 1155, 1124, 1067, 1032, 1011, 944, 904, 858, 837, 726, 650 cm^{-1} ; ^1H NMR (300 MHz, CDCl_3): δ 1.33, 1.41, 1.51, 1.53 (4 x s, 12H, $(\text{CH}_3)_2\text{C}$), 3.62 (m, 2H, H-1'a, H-4), 3.70 (m, 1H, H-6a), 3.76 (m, 1H, H-2), 3.84 (m, 2H, H-3, H-5), 4.03 (m, 1H, H-6b), 4.19 (d, 1H, $J = 12.0$ Hz, H-1'b), 4.51 (m, 2H, H-5', H-6'a), 4.64 (m, 1H, H-6'b), 5.38 (d, 1H, $J = 5.4$ Hz, H-4'), 5.60 (br dd, 1H, $J = 5.1$ Hz, 3.6 Hz, H-3'), 6.15 (d, 1H, $J = 3.3$ Hz,

H-1); *trans-p*-feruloyl units: R₁: 2.34 (s, 3H, H-11''), 3.86 (s, 3H, -OCH₃), 6.40 (d, 1H, *J* = 15.9 Hz, H-8''), 7.02-7.13 (m, 3H, H-2'', H-5'', H-6''), 7.66 (d, 1H, *J* = 15.9 Hz, H-7''); R₂: 2.34 (s, 3H, H-11''), 3.88 (s, 3H, -OCH₃), 6.42 (d, 1H, *J* = 15.9 Hz, H-8''), 7.02-7.13 (m, 2H, H-5'', H-6''), 7.20-7.22 (m, 1H, H-2''), 7.68 (d, 1H, *J* = 15.9 Hz, H-7''); R₃: 2.34 (s, 3H, H-11''), 3.93 (s, 3H, -OCH₃), 6.52 (d, 1H, *J* = 15.9 Hz, H-8''), 7.08 (d, 2H, *J* = 8.7 Hz, H-5'', H-6''), 7.20-7.22 (m, 1H, H-2''), 7.79 (d, 1H, *J* = 15.9 Hz, H-7''); ¹³C NMR (75.48 MHz, CDCl₃): δ 19.1, 24.1, 25.5, 29.0 (4 x (CH₃)₂C), 62.0 (C-6), 63.9 (C-5), 64.9 (C-6'), 66.3 (C-1'), 70.2 (C-3), 72.8 (C-4), 73.8 (C-2), 77.3 (C-4'), 77.6 (C-3'), 80.1 (C-5'), 91.4 (C-1), 99.7, 101.8 (2 x (CH₃)₂C), 104.8 (C-2'); *trans-p*-feruloyl units: R₁: 20.7 (C-11''), 55.9 (-OCH₃), 111.2 (C-2''), 116.5 (C-8''), 121.4 (C-5''), 123.2 (C-6''), 132.9 (C-1''), 141.4 (C-3''), 144.4 (C-7''), 151.3 (C-4''), 165.7 (C-9''), 168.6 (C-10''); R₂: 20.7 (C-11''), 56.0 (-OCH₃), 111.3 (C-2''), 116.8 (C-8''), 121.5 (C-5''), 123.3 (C-6''), 132.9 (C-1''), 141.8 (C-3''), 144.8 (C-7''), 151.4 (C-4''), 165.8 (C-9''), 168.7 (C-10''); R₃: 20.7 (C-11''), 56.0 (-OCH₃), 111.4 (C-2''), 117.9 (C-8''), 122.1 (C-5''), 123.4 (C-6''), 133.3 (C-1''), 141.9 (C-3''), 146.4 (C-7''), 151.5 (C-4''), 166.3 (C-9''), 168.8 (C-10''); ESI-Mass (positive mode): *m/z* 1099.35 [M + Na]⁺, calcd 1099.35 for C₅₄H₆₀O₂₃Na; HR-ESI-MS (positive mode): found *m/z* 1099.3401 [M + Na]⁺, calcd 1099.3418 for C₅₄H₆₀O₂₃Na.

4.3.2.4. 3,3',4',6'-Tetra-*O*-acetoxyferuloyl-2,1':4,6-di-*O*-isopropylidene sucrose **212**

Following the general procedure, the reaction between diacetone **175** (1.0 g, 2.4 mmol) and *p*-acetoxyferuloyl chloride **207** (2.7 g, 10.4 mmol) in dry pyridine (10 mL) for 4 days afforded compound **212** as a white solid (1.34 g, 44% yield) along with compound **211** in 35% (0.9 g) yield. Analytical data for **212**: R_f = 0.88 (3:2 EtOAc-CH₂Cl₂);

mp 133-135 °C; FT-IR (KBr) ν_{max}: 3629, 2993, 2942, 1767, 1721, 1637, 1601, 1467, 1419, 1371, 1327, 1259, 1198, 1153, 1123, 1070, 1033, 1010, 944, 903, 856, 832, 796, 727, 649 cm⁻¹; ¹H NMR (300 MHz, CDCl₃): δ 1.20, 1.30, 1.45, 1.49 (4 x s, 12H, (CH₃)₂C), 3.65 (m, 1H, H-1'a), 3.70 (m, 2H, H-4, H-6a), 3.96 (m, 2H, H-2, H-5), 4.07 (m, 1H, H-6b), 4.25 (d,

1H, $J = 12.3$ Hz, H-1'b), 4.52-4.63 (m, 3H, H-5', H-6'a, H-6'b), 5.42 (m, 2H, H-3, H-4'), 5.61 (m, 1H, H-3'), 6.21 (d, 1H, $J = 3.6$ Hz, H-1); *trans*-feruloyl units: R₁: 2.33 (s, 3H, H-11"), 3.86 (s, 3H, -OCH₃), 6.38 (d, 1H, $J = 15.9$ Hz, H-8"), 7.02-7.15 and 7.28-7.33 (2 x m, 3H, H-2", H-5", H-6"), 7.61 (d, 1H, $J = 15.9$ Hz, H-7"), R₂: 2.33 (s, 3H, H-11"), 3.88 (s, 3H, -OCH₃), 6.42 (d, 1H, $J = 15.9$ Hz, H-8"), 7.02-7.15 and 7.28-7.33 (2 x m, 3H, H-2", H-5", H-6"), 7.66 (d, 1H, $J = 15.9$ Hz, H-7"), R₃: 2.33 (s, 3H, H-11"), 3.89 (s, 3H, -OCH₃), 6.43 (d, 1H, $J = 15.9$ Hz, H-8"), 7.02-7.15 and 7.28-7.33 (2 x m, 3H, H-2", H-5", H-6"), 7.69 (d, 1H, $J = 15.9$ Hz, H-7"), R₄: 2.33 (s, 3H, H-11"), 3.92 (s, 3H, -OCH₃), 6.57 (d, 1H, $J = 15.9$ Hz, H-8"), 7.02-7.15 and 7.28-7.33 (2 x m, 3H, H-2", H-5", H-6"), 7.93 (d, 1H, $J = 15.9$ Hz, H-7"); ¹³C NMR (75.48 MHz, CDCl₃): δ 19.0, 23.9, 25.4, 28.8 (4 x (CH₃)₂C), 62.1 (C-6), 64.3 (C-5), 65.0 (C-6'), 66.2 (C-1'), 70.9 (C-3), 71.5 (C-4), 71.9 (C-2), 77.4 (C-4'), 77.7 (C-3'), 80.3 (C-5'), 91.7 (C-1), 99.6, 101.5 (2 x (CH₃)₂C), 105.0 (C-2'); *trans-p*-feruloyl units: R₁: 20.6 (C-11"), 55.9 (-OCH₃), 111.2 (C-2"), 116.8 (C-8"), 121.2 (C-5"), 123.0 (C-6"), 132.9 (C-1"), 141.4 (C-3"), 144.1 (C-7"), 151.3 (C-4"), 165.7 (C-9"), 168.7 (C-10"), R₂: 20.6 (C-11"), 55.9 (-OCH₃), 111.2 (C-2"), 116.9 (C-8"), 121.3 (C-5"), 123.2 (C-6"), 133.3 (C-1"), 141.4 (C-3"), 144.4 (C-7"), 151.4 (C-4"), 165.7 (C-9"), 168.8 (C-10"), R₃: 20.6 (C-11"), 56.0 (-OCH₃), 111.3 (C-2"), 117.9 (C-8"), 121.5 (C-5"), 123.2 (C-6"), 133.3 (C-1"), 141.6 (C-3"), 145.7 (C-7"), 151.4 (C-4"), 166.0 (C-9"), 168.8 (C-10"), R₄: 20.6 (C-11"), 56.0 (-OCH₃), 112.4 (C-2"), 118.2 (C-8"), 121.7 (C-5"), 123.3 (C-6"), 133.4 (C-1"), 141.8 (C-3"), 146.6 (C-7"), 151.4 (C-4"), 166.3 (C-9"), 168.8 (C-10"); ESI-Mass (positive mode): m/z 1317.35 [M + Na]⁺, calcd 1317.41 for C₆₆H₇₀O₂₇Na; HR-ESI-MS (positive mode): found m/z 1317.3980 [M + Na]⁺, calcd 1317.3997 for C₆₆H₇₀O₂₇Na.

4.3.3. Acetal deprotection of the diacetonoides 208, 210-212

General Procedure

A separate solution of diacetonoid feruloyl derivatives in 60% aq. AcOH was kept at 80 °C until the reaction was completed. The reaction was monitored by TLC (3:2 EtOAc-CH₂Cl₂). The reaction solution was then evaporated to dryness under reduced pressure by codistillation with toluene (3 x 100 mL). The products were obtained from recrystallization in EtOAc and/ or by column chromatography using a gradient of CH₂Cl₂-EtOAc as eluent.

4.3.3.1. 6'-Mono-*O*-acetoxyferuloylsucrose **213**

Following the general procedure, a solution of compound **208** (0.1 g, 0.2 mmol) was treated with 60% aq. AcOH (6.4 mL) at 80 °C for 20 min. Recrystallization in EtOAc gave compound **213** as a white solid (0.09 g, 86% yield).

Analytical data for **213**: $R_f = 0.08$ (9:1 EtOAc-MeOH); mp 168-170 °C; FT-IR (KBr) $\nu_{\max}$: 3324, 2927, 1754, 1712, 1687, 1640, 1600, 1547, 1514, 1467, 1421, 1372, 1332, 1262, 1218, 1187, 1158, 1123, 1062, 1032, 995, 917, 902, 834 cm^{-1} ;

^1H NMR (300 MHz, CD_3OD): δ 3.33 (m, 1H, H-4), 3.37-3.43 (m, 1H, H-2), 3.64 (m, 2H, H-1'a, H-1'b), 3.69-3.75 (dd, 2H, H-6a, H-3), 3.84-3.90 (m, 2H, H-5, H-6b), 3.99-4.01 (m, 1H, H-5'), 4.06-4.14 (m, 2H, H-3', H-4'), 4.42-4.52 (m, 2H, H-6'b, H-6'a), 5.39 (d, 1H, $J = 3.6$ Hz, H-1); *trans-p*-feruloyl units: δ 2.27 (s, 3H, H-11''), 3.87 (s, 3H, -OCH₃), 6.57 (d, 1H, $J = 15.9$ Hz, H-8''), 7.05-7.09 (m, 1H, H-6''), 7.19-7.24 (m, 1H, H-5''), 7.32-7.34 (m, 1H, H-2''), 7.71 (d, 1H, $J = 15.9$ Hz, H-7''); ^{13}C NMR (75.48 MHz, CD_3OD): δ 62.6 (C-6), 63.9 (C-1'), 67.0 (C-6'), 71.6 (C-4), 73.4 (C-2), 74.3 (C-5), 74.8 (C-3), 76.9 (C-4'), 79.0 (C-3'), 80.8 (C-5'), 93.6 (C-1), 105.7 (C-2'); *trans-p*-feruloyl units: 20.5 (C-11''), 56.6 (-OCH₃), 112.8 (C-2''), 119.1 (C-8''), 122.4 (C-5''), 124.4 (C-6''), 134.8 (C-1''), 143.1 (C-3''), 146.0 (C-7''), 153.1 (C-4''), 168.5 (C-9''), 170.6 (C-10''); ESI-Mass (positive mode): m/z 583.10 $[\text{M} + \text{Na}]^+$, calcd 583.17 for $\text{C}_{24}\text{H}_{32}\text{O}_{15}\text{Na}$; HR-ESI-MS (positive mode): found m/z 583.1628 $[\text{M} + \text{Na}]^+$, calcd 583.1633 for $\text{C}_{24}\text{H}_{32}\text{O}_{15}\text{Na}$.

4.3.3.2. 3',6'-Di-*O*-acetoxyferuloylsucrose **214**

Following the general procedure, a solution of compound **210** (1.1 g, 1.3 mmol) was treated with 60% aq. AcOH (66 mL) at 80 °C for 20 min. After recrystallization of the crude product in EtOAc gave compound **214** as a white solid (0.90 g, 89% yield). Analytical data for **214**: $R_f = 0.56$ (9:1 EtOAc-MeOH); mp 128-130 °C;

FT-IR (KBr) $\nu_{\max}$: 3411, 2930, 1762, 1706, 1635, 1601, 1508, 1420, 1371, 1330, 1261, 1220, 1194, 1159, 1125, 1060, 1031, 996, 938, 848, 792, 691, 650 cm^{-1} ; ^1H NMR (300 MHz, CD_3OD): δ 3.40-3.46 (m, 2H, H-2, H-4), 3.62 (m, 2H, H-1'a, H-1'b), 3.70 (m, 1H, H-3), 3.80 (m, 1H, H-6a), 3.94 (m, 2H, H-6b, H-5), 4.16-4.22 (m, 1H, H-5'), 4.47 (app t, 1H, $J = 7.8$ Hz, H-4'), 4.53-4.61 (m, 2H, H-6'a, H-6'b), 5.45 (d, 1H, $J = 3.6$ Hz, H-1), 5.53 (d, 1H, $J = 7.8$ Hz, H-3'); *trans-p*-feruloyl units: R₁: 2.27 (s, 3H, H-11''), 3.86 (s, 3H, -OCH₃), 6.58 (d, 1H, $J = 16.2$ Hz, H-8''), 7.07 (d, 1H, $J = 8.1$ Hz, H-6''), 7.22 (dd, 1H, $J = 1.5$ Hz, 8.4 Hz, H-5''), 7.35 (d, 1H, $J = 13.8$ Hz, H-2''), 7.72 (d, 1H, $J = 16.2$ Hz, H-7''), R₂: 2.27 (s, 3H, H-11''), 3.86 (s, 3H, -OCH₃), 6.61 (d, 1H, $J = 16.2$ Hz, H-8''), 7.07 (d, 1H, $J = 8.1$ Hz, H-6''), 7.22 (dd, 1H, $J = 1.5$ Hz, 8.4 Hz, H-5''), 7.35 (d, 1H, $J = 13.8$ Hz, H-2''), 7.78 (d, 1H, $J = 16.2$ Hz, H-7''); ^{13}C NMR (75.48 MHz, CD_3OD): δ 62.7 (C-6), 65.2 (C-1'), 66.5 (C-6'), 71.5 (C-4), 73.2 (C-2), 74.5 (C-5), 75.0 (C-4', C-3), 79.5 (C-3'), 81.3 (C-5'), 93.2 (C-1), 105.1 (C-2'); *trans-p*-feruloyl units: R₁: 20.9 (C-11''), 56.6 (-OCH₃), 112.7 (C-2''), 118.7 (C-8''), 122.4 (C-5''), 124.3 (C-6''), 134.8 (C-1''), 143.1 (C-3''), 146.0 (C-7''), 153.0 (C-4''), 167.6 (C-9''), 170.5 (C-10''), R₂: 20.9 (C-11''), 56.6 (-OCH₃), 113.2 (C-2''), 118.9 (C-8''), 122.4 (C-5''), 124.3 (C-6''), 134.8 (C-1''), 143.1 (C-3''), 146.6 (C-7''), 153.1 (C-4''), 168.4 (C-9''), 170.5 (C-10''); ESI-Mass (positive mode): m/z 801.25 $[\text{M} + \text{Na}]^+$, calcd 801.23 for $\text{C}_{36}\text{H}_{42}\text{O}_{19}\text{Na}$; HR-ESI-MS (positive mode): found m/z 801.2235 $[\text{M} + \text{Na}]^+$, calcd 801.2213 for $\text{C}_{36}\text{H}_{42}\text{O}_{19}\text{Na}$.

4.3.3.3. 3',4',6'-Tri-*O*-acetoxyferuloylsucrose **215**

Following the general procedure, a solution of compound **211** (0.8 g, 0.7 mmol) was reacted with 60% aq. AcOH (49 mL) at 80 °C for 20 min after column chromatography using a gradient of CH_2Cl_2 -EtOAc as eluent afforded compound **215** as a white solid (0.50 g, 67% yield). Analytical data for **215**: R_f = 0.49 (9:1 EtOAc-MeOH); mp 128-130 °C;

FT-IR (KBr) $\nu_{\max}$: 3425, 2938, 1764, 1713, 1638, 1601, 1510, 1465, 1418, 1371, 1332, 1300, 1260, 1218, 1199, 1155, 1124, 1031, 1012, 903, 836, 650 cm^{-1} ; ^1H NMR (300 MHz, CD_3OD): δ 3.46-3.52 (m, 2H, H-2, H-4), 3.67 (m, 2H, H-1'a, H-3), 3.82 (m, 1H, H-1'b), 3.88

(m, 1H, H-6a), 3.99-4.08 (m, 2H, H-6b, H-5), 4.47-4.51 (m, 1H, H-5'), 4.61-4.65 (m, 2H, H-6'b, H-6'a), 5.49 (d, 1H, $J = 3.6$ Hz, H-1), 5.82-5.87 (m, 2H, H-3', H-4'); *trans-p*-feruloyl units: R₁: 2.26 (s, 3H, H-11''), 3.74 (s, 3H, -OCH₃), 6.47 (d, 1H, $J = 15.9$ Hz, H-8''), 6.93-7.10 (m, 2H, H-5'', H-6''), 7.20-7.25 (m, 1H, H-2''), 7.63 (d, 1H, $J = 15.9$ Hz, H-7''), R₂: 2.26 (s, 3H, H-11''), 3.78 (s, 3H, -OCH₃), 6.53 (d, 1H, $J = 15.9$ Hz, H-8''), 6.93-7.10 (m, 2H, H-5'', H-6''), 7.20-7.25 (m, 1H, H-2''), 7.64 (d, 1H, $J = 15.9$ Hz, H-7''), R₃: 2.26 (s, 3H, H-11''), 3.84 (s, 3H, -OCH₃), 6.57 (d, 1H, $J = 15.9$ Hz, H-8''), 6.93-7.10, 7.20-7.25 (2 x m, 2H, H-5'', H-6''), 7.34 (br s, 1H, H-2''), 7.76 (d, 1H, $J = 15.9$ Hz, H-7''); ¹³C NMR (75.48 MHz, CD₃OD): δ 62.3 (C-6), 64.6 (C-1'), 65.8 (C-6'), 71.2 (C-4), 73.1 (C-2), 74.6 (C-5), 75.0 (C-3), 77.3 (C-4'), 77.4 (C-3'), 78.9 (C-5'), 93.6 (C-1), 105.8 (C-2'); *trans-p*-feruloyl units: R₁: 20.5 (C-11''), 56.5 (-OCH₃), 112.5 (C-2''), 118.1 (C-8''), 122.5 (C-5''), 124.3 (C-6''), 134.4 (C-1''), 143.0 (C-3''), 146.2 (C-7''), 152.9 (C-4''), 167.2 (C-9''), 170.4 (C-10''), R₂: 20.5 (C-11''), 56.6 (-OCH₃), 112.8 (C-2''), 118.3 (C-8''), 122.5 (C-5''), 124.3 (C-6''), 134.5 (C-1''), 143.1 (C-3''), 147.0 (C-7''), 152.9 (C-4''), 167.5 (C-9''), 170.4 (C-10''), R₃: 20.5 (C-11''), 56.6 (-OCH₃), 113.2 (C-2''), 118.6 (C-8''), 122.6 (C-5''), 124.3 (C-6''), 134.7 (C-1''), 143.2 (C-3''), 147.0 (C-7''), 152.9 (C-4''), 168.0 (C-9''), 170.4 (C-10''); ESI-Mass (positive mode): m/z 1019.23 [M + Na]⁺, calcd 1019.29 for C₄₈H₅₂O₂₃Na; HR-ESI-MS (positive mode): found m/z 1019.2774 [M + Na]⁺, calcd 1019.2792 for C₄₈H₅₂O₂₃Na.

4.3.3.4. 3,3',4',6'-Tetra-*O*-acetoxyferuloylsucrose **216**

Following the general procedure, a solution of compound **212** (0.6 g, 0.4 mmol) was treated with 60% aq. AcOH (32 mL) at 80 °C for 35 min after column chromatography using a gradient of CH₂Cl₂-EtOAc as eluent yielded compound **216** as a white solid (0.45 g, 87% yield). Analytical data for **216**: R_f = 0.87 (9:1 EtOAc-MeOH); mp 127-129 °C;

FT-IR (KBr) ν_{max} : 3482, 2963, 2941, 1765, 1717, 1637, 1601, 1510, 1467, 1420, 1371, 1325, 1260, 1154, 1123, 1032, 1008, 945, 904, 832, 797, 704, 649 cm⁻¹; ¹H NMR (300 MHz, CDCl₃): δ 3.61 (dd, 1H, $J = 9.6$ Hz, 9.3 Hz, H-4); 3.73 (m, 1H, H-2), 3.84 (m, 3H, H-1'b, H-

1'a, H-6a), 4.01 (m, 1H, H-6b), 4.12 (m, 1H, H-5), 4.50 (m, 1H, H-5'), 4.56 (m, 1H, H-6'a), 4.67 (dd, 1H, $J = 7.2$ Hz, 11.4 Hz, H-6'b), 5.13 (dd, 1H, $J = 9.6$ Hz, 9.3 Hz, H-3), 5.56 (d, 1H, $J = 2.7$ Hz, H-1), 5.59 (d, 1H, $J = 5.4$ Hz, H-3'), 5.71 (app t, 1H, $J = 4.8$ Hz, H-4'); *trans-p*-feruloyl units: R₁: 2.29 (s, 3H, H-11''), 3.82 (s, 3H, -OCH₃), 6.39 (d, 1H, $J = 15.9$ Hz, H-8''), 7.00-7.09 (m, 3H, H-2'', H-5'', H-6''), 7.59 (d, 1H, $J = 15.9$ Hz, H-7''), R₂: 2.30 (s, 3H, H-11''), 3.83 (s, 3H, -OCH₃), 6.39 (d, 1H, $J = 15.9$ Hz, H-8''), 7.00-7.09 (m, 3H, H-2'', H-5'', H-6''), 7.68 (d, 1H, $J = 15.9$ Hz, H-7''), R₃: 2.31 (s, 3H, H-11''), 3.83 (s, 3H, -OCH₃), 6.42 (d, 1H, $J = 15.9$ Hz, H-8''), 7.00-7.09 (m, 3H, H-2'', H-5'', H-6''), 7.19 (d, 1H, $J = 8.4$ Hz, H-2''), 7.69 (d, 1H, $J = 15.9$ Hz, H-7''), R₄: 2.32 (s, 3H, H-11''), 3.85 (s, 3H, -OCH₃), 6.57 (d, 1H, $J = 15.9$ Hz, H-8''), 7.00-7.09 (m, 1H, H-6''), 7.19 (d, 1H, $J = 8.4$ Hz, H-5''), 7.24 (br s, 1H, H-2''), 7.84 (d, 1H, $J = 15.9$ Hz, H-7''); ¹³C NMR (75.48 MHz, CDCl₃): δ 62.5 (C-6), 64.4 (C-1'), 64.5 (C-6'), 69.6 (C-4), 70.6 (C-2), 73.6 (C-5), 76.5 (C-4'), 77.0 (C-3), 78.1 (C-3'), 79.8 (C-5'), 92.5 (C-1), 105.5 (C-2'); *trans-p*-feruloyl units: R₁: 20.7 (C-11''), 55.9 (-OCH₃), 111.3 (C-2''), 116.4 (C-8''), 121.4 (C-5''), 123.2 (C-6''), 132.8 (C-1''), 141.6 (C-3''), 145.4 (C-7''), 151.3 (C-4''), 165.7 (C-9''), 168.4 (C-10''), R₂: 20.7 (C-11''), 55.9 (-OCH₃), 111.4 (C-2''), 116.5 (C-8''), 121.5 (C-5''), 123.2 (C-6''), 132.9 (C-1''), 141.7 (C-3''), 145.6 (C-7''), 151.4 (C-4''), 165.7 (C-9''), 168.7 (C-10''), R₃: 20.7 (C-11''), 55.9 (-OCH₃), 111.4 (C-2''), 117.4 (C-8''), 121.6 (C-5''), 123.3 (C-6''), 133.0 (C-1''), 141.8 (C-3''), 146.2 (C-7''), 151.4 (C-4''), 166.7 (C-9''), 168.7 (C-10''), R₄: 20.7 (C-11''), 56.0 (-OCH₃), 112.0 (C-2''), 117.4 (C-8''), 121.9 (C-5''), 123.3 (C-6''), 133.0 (C-1''), 141.9 (C-3''), 147.2 (C-7''), 151.5 (C-4''), 166.8 (C-9''), 168.8 (C-10''); ESI-Mass (positive mode): m/z 1237.28 [M + Na]⁺, calcd 1237.35 for C₆₀H₆₂O₂₇Na; HR-ESI-MS (positive mode): found m/z 1237.3373 [M + Na]⁺, calcd 1237.3371 for C₆₀H₆₂O₂₇Na.

4.3.4. Deacetylation of compounds 213-216

General Procedure

To a separate suspension of acetoxysteruloyl compounds in 95% EtOH, piperidine were added, whereupon the solution turned yellow. After dissolving the starting material completely dissolved the reaction was allowed to continue until the disappearance of the starting material. The reaction was monitored by TLC analysis (9:1 EtOAc-MeOH). The mixture was quenched with AcOH and was evaporated to a syrup. It was subjected to silica

gel column chromatography using a gradient of CH_2Cl_2 -EtOAc-MeOH as eluent. The solvent was evaporated under diminished pressure to furnish deacetylated products as a white solid.

4.3.4.1. 6'-Mono-*O*-feruloylsucrose **217**

Following the general procedure, a suspension of compound **213** (0.2 g, 0.3 mmol) in 95 % EtOH (13 mL) was treated with piperidine (63.5 μL , 54.7 mg, 0.6 mmol) for 7 h to give compound **217** as a white solid (0.12 g, 72% yield).

Analytical data for **217**: $R_f = 0.08$ (9:1 EtOAc-MeOH); mp 133-135 $^{\circ}\text{C}$; FT-IR (KBr) ν_{max} : 3443, 3360, 2958, 2932, 2890, 1726, 1691, 1637, 1603, 1517, 1467, 1433, 1373, 1322, 1293, 1279, 1259, 1181, 1134, 1102, 1074, 1027, 999, 953, 933, 878, 838, 680 cm^{-1} ; ^1H NMR (300 MHz, CD_3OD): δ 3.38 (d, 1H, $J = 9.6$ Hz, H-4), 3.44 (dd, 1H, $J = 3.6$ Hz, 9.9 Hz, H-2), 3.66 (m, 2H, H-1'a, H-1'b), 3.71-3.77 (m, 2H, H-6a, H-3), 3.86 (m, 2H, H-5, H-6b), 4.03 (m, 1H, H-5'), 4.08 (m, 1H, H-4'), 4.14 (m, 1H, H-3'), 4.44-4.50 (m, 2H, H-6'b, H-6'a), 5.41 (d, 1H, $J = 3.6$ Hz, H-1); *trans-p*-feruloyl units: δ 3.88 (s, 3H, $-\text{OCH}_3$), 6.37 (d, 1H, $J = 15.9$ Hz, H-8''), 6.81 (d, 1H, $J = 8.1$ Hz, H-6''), 7.06 (d, 1H, $J = 8.4$ Hz, H-5''), 7.16 (br s, 1H, H-2''), 7.63 (d, 1H, $J = 15.9$ Hz, H-7''); ^{13}C NMR (75.48 MHz, CD_3OD): δ 62.5 (C-6), 63.9 (C-1'), 66.8 (C-6'), 71.5 (C-4), 73.3 (C-2), 74.3 (C-5), 74.7 (C-3), 76.9 (C-4'), 79.1 (C-3'), 80.7 (C-5'), 93.5 (C-1), 105.5 (C-2'); *trans-p*-feruloyl units: δ 56.5 ($-\text{OCH}_3$), 111.8 (C-2''), 115.2 (C-8''), 116.5 (C-5''), 124.2 (C-6''), 127.7 (C-1''), 147.2 (C-7''), 149.4 (C-3''), 150.7 (C-4''), 169.2 (C-9''); ESI-Mass (positive mode): m/z 541.09 $[\text{M} + \text{Na}]^+$, calcd 541.16 for $\text{C}_{22}\text{H}_{30}\text{O}_{14}\text{Na}$; HR-ESI-MS (positive mode): found m/z 541.1516 $[\text{M} + \text{Na}]^+$, calcd 541.1528 for $\text{C}_{22}\text{H}_{30}\text{O}_{14}\text{Na}$.

4.3.4.2. 3',6'-Di-*O*-feruloylsucrose (Helonioside A, **69**)

Following the general procedure, a suspension of compound **214** (0.7 g, 0.9 mmol) in 95% EtOH (47 mL) was treated with piperidine (335.0 μL , 0.3 g, 3.4 mmol) for 3 h to afford compound **69** as a white solid (0.40 g, 68% yield).

Analytical data for **69**: $R_f = 0.49$ (9:1 EtOAc-MeOH); mp 154-156 °C; FT-IR (KBr) $\nu_{\max}$: 3417, 2938, 1720, 1691, 1632, 1519, 1454, 1431, 1379, 1273, 1151, 1057, 1030, 995, 938, 841, 822, 788, 696 cm^{-1} ;

^1H NMR (300 MHz, CD_3OD): δ 3.39 (m, 1H, H-4), 3.44 (dd, 1H, $J = 3.6$ Hz, 6.0 Hz, H-2), 3.61 (m, 2H, H-1'b, H-1'a), 3.69 (m, 1H, H-3), 3.80 (dd, 1H, $J = 4.2$ Hz, 11.7 Hz, H-6a), 3.88 (m, 1H, H-6b), 3.96 (m, 1H, H-5), 4.16 (m, 1H, H-5'), 4.45 (dd, 1H, $J = 7.6$ Hz, 7.8 Hz, H-4'), 4.57 (m, 2H, H-6'b, H-6'a), 5.45 (d, 1H, $J = 3.6$ Hz, H-1), 5.50 (d, 1H, $J = 7.8$ Hz, H-3'), *trans-p*-feruloyl units: R_1 : 3.90 (s, 3H, $-\text{OCH}_3$), 6.41 (d, 1H, $J = 15.9$ Hz, H-8''), 6.82 (d, 1H, $J = 8.1$ Hz, H-5''), 7.14 (dd, 1H, $J = 1.5$ Hz, 8.4 Hz, H-6''), 7.24 (d, 1H, $J = 1.5$ Hz, H-2''), 7.66 (d, 1H, $J = 15.9$ Hz, H-7''), R_2 : 3.90 (s, 3H, $-\text{OCH}_3$), 6.44 (d, 1H, $J = 15.9$ Hz, H-8''), 6.82 (d, 1H, $J = 8.1$ Hz, H-5''), 7.10 (dd, 1H, $J = 1.5$ Hz, 8.4 Hz, H-6''), 7.20 (d, 1H, $J = 1.5$ Hz, H-2''), 7.72 (d, 1H, $J = 15.9$ Hz, H-7''); ^{13}C NMR (75.48 MHz, CD_3OD): δ 62.7 (C-6), 65.2 (C-1'), 66.2 (C-6'), 71.4 (C-4), 73.2 (C-2), 74.4 (C-5), 75.0 (C-4', C-3), 79.2 (C-3'), 81.3 (C-5'), 93.1 (C-1), 105.1 (C-2'); *trans-p*-feruloyl units: R_1 : 56.5 ($-\text{OCH}_3$), 111.7 (C-2''), 114.9 (C-8''), 116.5 (C-5''), 124.3 (C-6''), 127.7 (C-1''), 147.3 (C-7''), 149.4 (C-3''), 150.7 (C-4''), 168.3 (C-9''), R_2 : 56.5 ($-\text{OCH}_3$), 112.1 (C-2''), 115.1 (C-8''), 116.5 (C-5''), 124.3 (C-6''), 127.7 (C-1''), 147.8 (C-7''), 149.4 (C-3''), 150.7 (C-4''), 169.1 (C-9''); ESI-Mass (positive mode): m/z 717.21 $[\text{M} + \text{Na}]^+$, calcd 717.21 for $\text{C}_{32}\text{H}_{38}\text{O}_{17}\text{Na}$; HR-ESI-MS: found m/z 717.1984 $[\text{M} + \text{Na}]^+$, calcd 717.2001 for $\text{C}_{32}\text{H}_{38}\text{O}_{17}\text{Na}$. Spectral data of helonioside A **69** was the same as reported for the isolated natural product.^{62, 64}

4.3.4.3. 3',4',6'-Tri-*O*-feruloyl sucrose **117**

Following the general procedure, a suspension of compound **215** (0.4 g, 0.4 mmol) in 95% EtOH (28 mL) was treated with piperidine (232.0 μL , 0.2 g, 2.3 mmol) for 4 h to afford compound **117**⁸⁰ as a white solid (0.22 g, 65% yield). Analytical data for **117**: $R_f = 0.46$ (9:1 EtOAc-MeOH); mp 99-101 °C;

FT-IR (KBr) $\nu_{\max}$: 3355, 2969, 1715, 1699, 1653, 1595, 1517, 1457, 1430, 1272, 1157, 1034, 992, 845, 815 cm^{-1} ; ^1H NMR (300 MHz, CD_3OD): δ 3.52 (m, 1H, H-2, H-4), 3.73 (m, 2H, H-1'b, H-1'a), 3.80 (m, 1H, H-3), 3.90 (m, 1H, H-6a), 4.05 (m, 2H, H-6b, H-5), 4.47 (m, 1H, H-5'), 4.62 (m, 2H, H-6'b, H-6'a), 5.51 (d, 1H, $J = 3.3$ Hz, H-1), 5.81 (m, 2H, H-3', H-4'), *trans-p*-feruloyl units: R₁: 3.72 (s, 3H, $-\text{OCH}_3$), 6.29 (m, 1H, H-8''), 6.74 (m, 1H, H-5''), 6.94 (m, 2H, H-2'', H-6''), 7.54 (d, 1H, $J = 15.9$ Hz, H-7''), R₂: 3.77 (s, 3H, $-\text{OCH}_3$), 6.29 (m, 1H, H-8''), 6.74 (m, 1H, H-5''), 7.08 (br d, 1H, $J = 8.4$ Hz, H-6''), 7.17 (br s, 1H, H-2''), 7.54 (d, 1H, $J = 15.9$ Hz, H-7''), R₃: 3.83 (s, 3H, $-\text{OCH}_3$), 6.41 (d, 1H, $J = 15.9$ Hz, H-8''), 6.74 (m, 1H, H-5''), 6.94 (m, 2H, H-2'', H-6''), 7.70 (d, 1H, $J = 15.9$ Hz, H-7''); ^{13}C NMR (75.48 MHz, CD_3OD): δ 62.4 (C-6), 64.8 (C-1'), 65.7 (C-6'), 71.2 (C-4), 73.2 (C-2), 74.6 (C-5), 75.0 (C-3), 77.1 (C-3', C-4'), 78.9 (C-5'), 93.6 (C-1), 105.8 (C-2'); *trans-p*-feruloyl units: R₁: 56.4 ($-\text{OCH}_3$), 111.6 (C-2''), 114.4 (C-8''), 116.5 (C-5''), 124.3 (C-6''), 127.4 (C-1''), 148.2 (C-7''), 149.2 (C-3''), 150.6 (C-4''), 168.2 (C-9''), R₂: 56.4 ($-\text{OCH}_3$), 111.8 (C-2''), 114.6 (C-8''), 116.5 (C-5''), 124.4 (C-6''), 127.5 (C-1''), 147.4 (C-7''), 149.2 (C-3''), 150.7 (C-4''), 168.7 (C-9''), R₃: 56.5 ($-\text{OCH}_3$), 112.1 (C-2''), 114.9 (C-8''), 116.4 (C-5''), 124.4 (C-6''), 127.6 (C-1''), 147.4 (C-7''), 149.2 (C-3''), 150.7 (C-4''), 167.9 (C-9''); ESI-Mass (positive mode): m/z 893.23 $[\text{M} + \text{Na}]^+$, calcd 893.26 for $\text{C}_{42}\text{H}_{46}\text{O}_{20}\text{Na}$; HR-ESI-MS (positive mode): found m/z 893.2478 $[\text{M} + \text{Na}]^+$, calcd 893.2475 for $\text{C}_{42}\text{H}_{46}\text{O}_{20}\text{Na}$.

4.3.4.4. 3,3',4',6'-Tetra-*O*-feruloyl sucrose **218**

Following the general procedure, a suspension of compound **216** (0.2 g, 0.2 mmol) in 95% EtOH (16 mL) was reacted with piperidine (150.0 μL , 0.1 g, 1.5 mmol) for 4 h to afford compound **218** as a white solid (0.15 g, 76% yield). Analytical data for **218**: $R_f = 0.73$ (9:1 EtOAc-MeOH); mp 123-125 $^\circ\text{C}$;

FT-IR (KBr) $\nu_{\max}$: 3423, 2964, 2940, 1708, 1631, 1594, 1515, 1452, 1431, 1372, 1271, 1158, 1032, 1003, 846, 819, 703, 603 cm^{-1} ; ^1H NMR (300 MHz, CD_3OD): δ 3.65-3.95 (m, 5H, H-1'b, H-1'a, H-2, H-4, H-6a), 4.03-4.07 (m, 1H, H-6b), 4.18-4.23 (m, 1H, H-5), 4.46-4.50 (m, 1H, H-5'), 4.58-4.64 (m, 2H, H-6'b, H-6'a), 5.45 (app t, 1H, $J = 9.6$ Hz, H-3),

5.58 (d, 1H, $J = 3.3$ Hz, H-1), 5.86 (m, 2H, H-3', H-4'); *trans-p*-feruloyl units: R₁: 3.76 (s, 3H, -OCH₃), 6.29 (d, 1H, $J = 15.9$ Hz, H-8''), 6.71 (d, 1H, $J = 8.1$ Hz, H-5''), 6.95-7.08 (m, 2H, H-2'', H-6''), 7.57 (d, 1H, $J = 15.9$ Hz, H-7''), R₂: 3.81 (s, 3H, -OCH₃), 6.34 (d, 1H, $J = 15.9$ Hz, H-8''), 6.76 (d, 1H, $J = 8.1$ Hz, H-5''), 6.95-7.08 (m, 2H, H-2'', H-6''), 7.59 (d, 1H, $J = 15.9$ Hz, H-7''), R₃: 3.87 (s, 3H, -OCH₃), 6.42 (d, 1H, $J = 15.9$ Hz, H-8''), 6.79 (d, 1H, $J = 8.1$ Hz, H-5''), 7.16 (m, 2H, H-2'', H-6''), 7.60 (d, 1H, $J = 15.9$ Hz, H-7''), R₄: 3.88 (s, 3H, -OCH₃), 6.46 (d, 1H, $J = 15.9$ Hz, H-8''), 6.81 (d, 1H, $J = 8.1$ Hz, H-5''), 6.95-7.08 (m, 1H, H-6''), 7.28 (br d, 1H, $J = 1.8$ Hz, H-2''), 7.74 (d, 1H, $J = 15.9$ Hz, H-7''); ¹³C NMR (75.48 MHz, CD₃OD): δ 62.0 (C-6), 64.5 (C-1'), 65.7 (C-6'), 69.3 (C-4), 71.7 (C-2), 74.8 (C-5), 77.0, 77.1 (C-3, C-3', C-4'), 78.8 (C-5'), 93.7 (C-1), 105.8 (C-2'); *trans-p*-feruloyl units: R₁: 56.4 (-OCH₃), 111.6 (C-2''), 114.4 (C-8''), 116.0 (C-5''), 124.0 (C-6''), 127.5 (C-1''), 146.9 (C-7''), 149.3 (C-3''), 150.6 (C-4''), 168.1 (C-9''), R₂: 56.5 (-OCH₃), 111.8 (C-2''), 114.8 (C-8''), 116.5 (C-5''), 124.3 (C-6''), 127.6 (C-1''), 147.5 (C-7''), 149.4 (C-3''), 150.7 (C-4''), 168.2 (C-9''), R₃: 56.5 (-OCH₃), 111.8 (C-2''), 115.0 (C-8''), 116.5 (C-5''), 124.4 (C-6''), 127.8 (C-1''), 147.5 (C-7''), 149.4 (C-3''), 150.7 (C-4''), 168.8 (C-9''), R₄: 56.6 (-OCH₃), 112.7 (C-2''), 115.0 (C-8''), 116.6 (C-5''), 124.5 (C-6''), 127.9 (C-1''), 148.3 (C-7''), 149.4 (C-3''), 150.9 (C-4''), 169.3 (C-9''); ESI-Mass (positive mode): m/z 1069.25 [M + Na]⁺, calcd 1069.31 for C₅₂H₅₄O₂₃Na; HR-ESI-MS (positive mode): found m/z 1069.2926 [M + Na]⁺, calcd 1069.2948 for C₅₂H₅₄O₂₃Na.

4.3.5. Deacetylation of isopropylidene acetals 210-212

General Procedure

Piperidine were added to a separate suspension of diacetone acetoxyferuloyl compounds in 95% EtOH, whereupon the solution turned yellow. After starting material was completely dissolved, the reaction was allowed to continue until the disappearance of the starting material as indicated by TLC-analysis (3:1 EtOAc-hexane). The reaction mixture was quenched with AcOH and was evaporated to a syrup. The reaction mixture was then dissolved in EtOAc (25 mL) and was washed with 1N HCl (2 x 50 mL). The aqueous layer was back extracted with EtOAc (25 mL) and combined with the original organic layer. The organic solution was then successively washed with 5% NaHCO₃ (2 x 100 mL) and brine (2 x 50 mL) and then dried over anhyd. MgSO₄. Thus, was subjected to silica gel column

chromatography using a gradient of CH₂Cl₂-EtOAc as eluent. The solvent was evaporated under diminished pressure to afford deacetylated diacetone compounds as a white solid.

4.3.5.1. 3',6'-Di-*O*-feruloyl-2,1':4,6-di-*O*-isopropylidene sucrose **219**

Following the general procedure, a suspension of compound **210** (0.4 g, 0.5 mmol) in 95% EtOH (30 mL) was treated with piperidine (92.0 μ L, 0.1 g, 0.9 mmol) for 9 h to furnish compound **219** as a white solid (0.22 g, 61% yield). Analytical data for **219**: R_f = 0.25 (3:1 EtOAc-hexanes); mp 125-127 °C;

FT-IR (KBr) $\nu_{\max}$: 2991, 2924, 2855, 1706, 1635, 1592, 1516, 1457, 1431, 1374, 1270, 1211, 1158, 1126, 1067, 1030, 944, 858, 819, 754, 655 cm⁻¹; ¹H NMR (300 MHz, CDCl₃): δ 1.40, 1.42, 1.53 (3 x s, 12H, (CH₃)₂C), 3.59 (m, 2H, H-1'a, H-4), 3.67 (m, 1H, H-6a), 3.75 (d, 1H, J = 9.0 Hz, H-2), 3.85 (m, 1H, H-5), 3.97 (m, 2H, H-3, H-6b), 4.08 (d, 1H, J = 12.6 Hz, H-1'b), 4.38 (m, 2H, H-5', H-6'a), 4.44 (m, 1H, H-4'), 4.51 (m, 1H, H-6'b), 4.91 (d, 1H, J = 6.6 Hz, H-3'), 6.13 (d, 1H, J = 3.3 Hz, H-1); *trans-p*-feruloyl units: R_1 : 3.93 (s, 3H, -OCH₃), 6.33 (d, 1H, J = 15.9 Hz, H-8''), 6.91 (d, 1H, J = 8.1 Hz, H-5''), 7.04-7.16 (m, 2H, H-2'', H-6''), 7.63 (d, 1H, J = 15.9 Hz, H-7''), R_2 : 3.99 (s, 3H, -OCH₃), 6.37 (d, 1H, J = 15.9 Hz, H-8''), 6.94 (d, 1H, J = 9.3 Hz, H-5''), 7.04-7.16 (m, 2H, H-2'', H-6''), 7.74 (d, 1H, J = 15.9 Hz, H-7''); ¹³C NMR (75.48 MHz, CDCl₃): δ 19.1, 24.1, 25.4, 29.0 (4 x (CH₃)₂C), 62.1 (C-6), 63.7 (C-5), 65.7 (C-6'), 66.0 (C-1'), 70.3 (C-3), 73.0 (C-4), 73.8 (C-2), 76.4 (C-4'), 80.7 (C-3'), 81.3 (C-5'), 90.9 (C-1), 99.9, 101.8 (2 x (CH₃)₂C), 104.4 (C-2'); *trans-p*-feruloyl units: R_1 : 55.9 (-OCH₃), 109.4 (C-2''), 113.5 (C-8''), 114.9 (C-5''), 123.2 (C-6''), 126.4 (C-1''), 146.9 (C-3''), 145.5 (C-7''), 148.1 (C-4''), 167.2 (C-9''), R_2 : 56.1 (-OCH₃), 109.5 (C-2''), 115.0 (C-8''), 114.9 (C-5''), 124.1 (C-6''), 126.8 (C-1''), 147.0 (C-3''), 147.4 (C-7''), 148.7 (C-4''), 168.0 (C-9''); ESI-Mass (positive mode): m/z 797.28 [M + Na]⁺, calcd 797.27 for C₃₈H₄₆O₁₇Na; HR-ESI-MS (positive mode): found m/z 797.2636 [M + Na]⁺, calcd 797.2627 for C₃₈H₄₆O₁₇Na.

4.3.5.2. 3',4',6'-Tri-*O*-feruloyl-2,1':4,6-di-*O*-isopropylidene sucrose **220**

Following the general procedure, a suspension of compound **211** (0.3 g, 0.3 mmol) in

95% EtOH (23 mL) was treated with piperidine (176.0 μ L, 0.2 g, 1.8 mmol) for 4 h to give compound **220** as a white solid (0.20 g, 71% yield).

Analytical data for **220**: R_f = 0.27 (3:1 EtOAc-hexanes); mp 145-147 $^{\circ}$ C; FT-IR (KBr) $\nu_{\max}$: 3067, 2991, 2939, 1715, 1632, 1593, 1516, 1465, 1431, 1383, 1271, 1211, 1154, 1065, 1031, 942, 856, 816, 724, 655, 603 cm^{-1} ;

^1H NMR (300 MHz, CDCl_3): δ 1.31, 1.37, 1.49 (3 x s, 12H, $(\text{CH}_3)_2\text{C}$); 3.62 (m, 3H, H-1'a, H-4, H-6a); 3.75 (m, 1H, H-2); 3.87 (m, 2H, H-3, H-5), 4.02 (m, 1H, H-6b), 4.14 (m, 1H, H-1'b), 4.49 (m, 2H, H-5', H-6'a), 4.57 (m, 1H, H-6'b), 5.35 (br d, 1H, J = 5.1 Hz, H-4'), 5.59 (br s, 1H, H-3'), 6.16 (m, 1H, H-1); *trans-p*-feruloyl units: R_1 : 3.84 (s, 3H, $-\text{OCH}_3$), 6.24 (d, 1H, J = 15.9 Hz, H-8''), 6.83-7.08 (m, 3H, H-2'', H-5'', H-6''), 7.59 (d, 1H, J = 15.9 Hz, H-7''), R_2 : 3.87 (s, 3H, $-\text{OCH}_3$), 6.26 (d, 1H, J = 15.9 Hz, H-8''), 6.83-7.08 (m, 3H, H-2'', H-5'', H-6''), 7.59 (d, 1H, J = 15.9 Hz, H-7''), R_3 : 3.92 (s, 3H, $-\text{OCH}_3$), 6.37 (d, 1H, J = 15.9 Hz, H-8''), 6.83-7.08 (m, 3H, H-2'', H-5'', H-6''), 7.71 (d, 1H, J = 15.9 Hz, H-7''); ^{13}C NMR (75.48 MHz, CDCl_3): δ 19.1, 24.1, 25.5, 28.9 (4 x $(\text{CH}_3)_2\text{C}$), 62.0 (C-6), 63.9 (C-5), 64.9 (C-6'), 66.3 (C-1'), 70.2 (C-3), 72.8 (C-4), 73.8 (C-2), 77.1 (C-4'), 77.3 (C-3'), 79.9 (C-5'), 91.4 (C-1), 99.8, 101.8 (2 x $(\text{CH}_3)_2\text{C}$), 104.7 (C-2'); *trans-p*-feruloyl units: R_1 : 55.8 ($-\text{OCH}_3$), 109.4 (C-2''), 113.6 (C-8''), 114.8 (C-5''), 123.2 (C-6''), 126.5 (C-1''), 145.2 (C-7''), 146.8 (C-3''), 148.0 (C-4''), 166.2 (C-9''), R_2 : 55.9 ($-\text{OCH}_3$), 109.4 (C-2''), 113.9 (C-8''), 114.9 (C-5''), 123.4 (C-6''), 126.6 (C-1''), 146.5 (C-7''), 146.8 (C-3''), 148.4 (C-4''), 166.4 (C-9''), R_3 : 56.0 ($-\text{OCH}_3$), 109.5 (C-2''), 113.9 (C-8''), 114.9 (C-5''), 124.1 (C-6''), 126.9 (C-1''), 146.9 (C-3''), 147.2 (C-7''), 148.5 (C-4''), 166.8 (C-9''); ESI-Mass (positive mode): m/z 973.27 $[\text{M} + \text{Na}]^+$, calcd 973.32 for $\text{C}_{48}\text{H}_{54}\text{O}_{20}\text{Na}$; HR-ESI-MS (positive mode): found m/z 973.3078 $[\text{M} + \text{Na}]^+$, calcd 973.3101 for $\text{C}_{48}\text{H}_{54}\text{O}_{20}\text{Na}$.

4.3.5.3. 3,3',4',6'-Tetra-*O*-feruloyl-2,1':4,6-di-*O*-isopropylidene sucrose **221**

Following the general procedure, a suspension of compound **212** (0.2 g, 0.2 mmol) in 95% EtOH (13 mL) was treated with piperidine (116.0 μ L, 0.1 g, 1.2 mmol) for 3 h to afford compound **221** as a white solid (0.12 g, 73% yield).

Analytical data for **221**: $R_f = 0.45$ (3:1 EtOAc-hexanes); mp 120-122 °C; FT-IR (KBr) $\nu_{\max}$: 2935, 2855, 1718, 1635, 1592, 1515, 1464, 1431, 1383, 1270, 1211, 1156, 1072, 1032, 983, 943, 852, 819, 755, 606 cm^{-1} ;

^1H NMR (300 MHz, CDCl_3): δ 1.24, 1.27, 1.45, 1.47 (4 x s, 12H, $(\text{CH}_3)_2\text{C}$), 3.59 (1H, $J = 12.3$ Hz, H-1'a), 3.68-3.81 (m, 2H, H-4, H-6a), 3.84-3.95 (m, 2H, H-2, H-5), 4.05 (dd, 1H, $J = 4.5$ Hz, 9.9 Hz, H-6b), 4.20 (d, 1H, $J = 12.6$ Hz, H-1'b), 4.45-4.60 (m, 3H, H-5', H-6'a, H-6'b), 5.33-5.44 (m, 2H, H-3, H-4'), 5.61 (br dd, 1H, $J = 4.5$ Hz, H-3'), 6.19 (d, 1H, $J = 3.3$ Hz, H-1); *trans-p*-feruloyl units: R_1 : 3.88 (s, 3H, $-\text{OCH}_3$), 6.27 (d, 1H, $J = 15.9$ Hz, H-8''), 6.88 (d, 1H, $J = 9.6$ Hz, H-5''), 6.99-7.11 (m, 2H, H-2'', H-6''), 7.57 (d, 1H, $J = 15.9$ Hz, H-7''), R_2 : 3.91 (s, 3H, $-\text{OCH}_3$), 6.28 (d, 1H, $J = 15.9$ Hz, H-8''), 6.88 (d, 1H, $J = 9.6$ Hz, H-5''), 6.99-7.11 (m, 2H, H-2'', H-6''), 7.61 (d, 1H, $J = 15.9$ Hz, H-7''), R_3 : 3.92 (s, 3H, $-\text{OCH}_3$), 6.30 (d, 1H, $J = 15.9$ Hz, H-8''), 6.91 (d, 1H, $J = 9.0$ Hz, H-5''), 6.99-7.11 (m, 2H, H-2'', H-6''), 7.62 (d, 1H, $J = 15.9$ Hz, H-7''), R_4 : 3.95 (s, 3H, $-\text{OCH}_3$), 6.47 (d, 1H, $J = 15.9$ Hz, H-8''), 6.91 (d, 1H, $J = 9.0$ Hz, H-5''), 7.17-7.23 (m, 2H, H-2'', H-6''), 7.84 (d, 1H, $J = 15.9$ Hz, H-7''); ^{13}C NMR (75.48 MHz, CDCl_3): δ 19.0, 23.9, 25.4, 28.8 (4 x $(\text{CH}_3)_2\text{C}$), 62.1 (C-6), 64.2 (C-5), 65.1 (C-6'), 66.1 (C-1'), 70.8 (C-3), 71.6 (C-4), 71.9 (C-2), 76.9 (C-4'), 77.3 (C-3'), 79.9 (C-5'), 91.6 (C-1), 99.6, 101.5 (2 x $(\text{CH}_3)_2\text{C}$), 104.8 (C-2'); *trans-p*-feruloyl units: R_1 : 55.9 ($-\text{OCH}_3$), 109.3 (C-2''), 113.9 (C-5''), 114.8 (C-8''), 123.0 (C-6''), 126.6 (C-1''), 144.8 (C-7''), 146.7 (C-3''), 148.0 (C-4''), 166.2 (C-9''), R_2 : 55.9 ($-\text{OCH}_3$), 109.4 (C-2''), 114.0 (C-5''), 114.8 (C-8''), 123.2 (C-6''), 127.0 (C-1''), 145.2 (C-7''), 146.7 (C-3''), 148.0 (C-4''), 166.2 (C-9''), R_3 : 55.9 ($-\text{OCH}_3$), 109.5 (C-2''), 114.6 (C-5''), 115.1 (C-8''), 123.4 (C-6''), 127.0 (C-1''), 146.4 (C-7''), 146.8 (C-3''), 148.2 (C-4''), 166.5 (C-9''), R_4 : 56.0 ($-\text{OCH}_3$), 110.4 (C-2''), 114.7 (C-5''), 115.4 (C-8''), 123.8 (C-6''), 128.8 (C-1''), 146.8 (C-3''), 147.4 (C-7''), 148.3 (C-4''), 166.7 (C-9''); ESI-Mass (positive mode): m/z 1146.60 $[\text{M} + \text{Na}]^+$, calcd 1149.37 for $\text{C}_{58}\text{H}_{62}\text{O}_{23}\text{Na}$; HR-ESI-MS (positive mode): found m/z 1149.3570 $[\text{M} + \text{Na}]^+$, calcd 1149.3574 for $\text{C}_{58}\text{H}_{62}\text{O}_{23}\text{Na}$.

4.4. Synthesis of Lapathoside C and its analogues

4.4.1. Preparation of 6-mono-*O*-acetoxyferuloyl-3',6'-di-*O*-acetoxyferuloylsucrose **222** and 3,6-di-*O*-acetoxyferuloyl-3',6'-di-*O*-acetoxyferuloylsucrose **226**

3',6'-Di-*O*-acetoxyferuloyl sucrose **201** (1.1 g, 1.5 mmol) was dissolved in dry CH₂Cl₂ (21 mL) to which 4 Å molecular sieves powder followed by dry pyridine (1.1 g, 1.2 mL, 14.4 mmol) was added. The solution was then cooled to 0 °C in an ice bath. *p*-Acetoxyferuloyl chloride **207** (0.4 g, 1.6 mmol) was then added slowly at the same temperature and the reaction was left to stir while warming to rt. Stirring was continued until the reaction was completed as indicated by TLC analysis (3:1 EtOAc-hexanes). After 24 h, the resulting mixture was poured into vigorously stirred ice-water (100 mL) and a white solid precipitated was obtained after decantation and filtration. The precipitate was redissolved in EtOAc (25 mL) and washed with 1N HCl (2 x 50 mL). The aqueous layer was extracted with EtOAc (25 mL). The combined organic layers were then successively washed with 5% NaHCO₃ (2 x 50 mL) and brine (25 mL) and then dried over anhyd. MgSO₄. The EtOAc solution was concentrated to residue that was subjected to column chromatography using a gradient of CH₂Cl₂-EtOAc as eluent and further, purified by

PTLC afforded compound **222** as a white solid (0.50 g, 36% yield) along with compound **226** in 7% (0.12 g) yield. Analytical data for **222**: R_f = 0.06 (3:1 EtOAc-hexanes); mp 108-110 °C; FT-IR (KBr) $\nu_{\max}$: 3457, 3423, 2925, 2852, 1767, 1710, 1636, 1602, 1508, 1457, 1419, 1371, 1323, 1282, 1261, 1206, 1165, 1056, 1015, 946, 912, 836, 794, 754, 649, 595 cm⁻¹;

¹H NMR (300 MHz, CDCl₃): δ 3.32 (m, 1H, H-4), 3.54 (m, 2H, H-2, H-1'a), 3.68 (m, 2H, H-1'b, H-3), 4.11 (m, 1H, H-5), 4.24 (m, 1H, H-5'), 4.46 (m, 1H, H-4'), 4.55 (m, 4H, H-6b, H-6a, H-6'b, H-6'a), 5.30 (d, 1H, J = 5.7 Hz, H-3'), 5.42 (br s, 1H, H-1); *trans-p*-feruloyl units: R_1 : 2.28 (s, 3H, H-11''), 3.77 (s, 3H, -OCH₃), 6.41 (d, 1H, J = 15.9 Hz, H-8''), 6.94 (d, 1H, J = 8.1 Hz, H-5''), 7.00-7.09 (m, 2H, H-2'', H-6''), 7.65 (d, 1H, J = 15.9 Hz, H-7''); *trans-p*-

coumaroyl units: R₂: 2.23 (s, 3H, H-11''), 6.39 (d, 1H, $J = 15.9$ Hz, H-8''), 7.00-7.09 (m, 2H, H-3'', H-5''), 7.40 (d, 2H, H-2'', H-6''), 7.56 (d, 1H, $J = 15.9$ Hz, H-7''), R₃: 2.26 (s, 3H, H-11''), 6.29 (d, 1H, $J = 15.9$ Hz, H-8''), 7.00-7.09 (m, 2H, H-3'', H-5''), 7.47-7.53 (m, 2H, H-2'', H-6''), 7.47-7.53 (m, 1H, H-7''); ¹³C NMR (75.48 MHz, CDCl₃): δ 64.3 (C-1'), 64.5 (C-6), 64.8 (C-6'), 70.3 (C-4), 71.0 (C-5), 71.7 (C-2), 73.9 (C-3), 74.7 (C-4'), 79.8 (C-3'), 80.9 (C-5'), 91.6 (C-1), 104.5 (C-2'); *trans-p*-feruloyl units: R₁: 20.6 (C-11''), 55.9 (-OCH₃), 111.5 (C-2''), 116.7 (C-8''), 121.6 (C-5''), 123.2 (C-6''), 133.1 (C-1''), 141.5 (C-3''), 146.1 (C-7''), 151.3 (C-4''), 167.4 (C-9''), 169.5 (C-10''); *trans-p*-coumaroyl units: R₂: 21.1 (C-11''), 117.4 (C-8''), 122.1 (C-3'', C-5''), 129.4 (C-2'', C-6''), 131.6 (C-1''), 144.5 (C-7''), 152.2 (C-4''), 166.9 (C-9''), 168.9 (C-10''), R₃: 21.1 (C-11''), 117.5 (C-8''), 122.2 (C-3'', C-5''), 129.8 (C-2'', C-6''), 131.8 (C-1''), 145.1 (C-7''), 152.4 (C-4''), 167.2 (C-9''), 169.2 (C-10''); ESI-Mass (positive mode): m/z 959.14 [M + Na]⁺, calcd 959.27 for C₄₆H₄₈O₂₁Na; HR-ESI-MS (positive mode): found m/z 959.2549 [M + Na]⁺, calcd 959.2580 for C₄₆H₄₈O₂₁Na. Analytical data for **226**: R_f = 0.11 (3:1 EtOAc-hexanes); mp 114-116 °C; FT-IR (KBr) $\nu_{\max}$: 3483, 2925, 2855, 2363, 2340, 1766, 1713, 1637, 1602, 1508, 1467, 1419, 1371, 1321, 1260, 1203, 1164, 1123, 1061, 1032, 1011, 909, 834, 652 cm⁻¹; ¹H NMR (300 MHz, CDCl₃): δ 3.54 (m, 1H, H-4), 3.74 (m, 3H, H-2, H-1'a, H-1'b), 4.24-4.28 (m, 2H, H-5, H-5'),

4.46-4.60 (m, 5H, H-4', H-6b, H-6a, H-6'b, H-6'a), 5.25 (m, 1H, H-3), 5.29 (d, 1H, $J = 7.2$ Hz, H-3'), 5.57 (br s, 1H, H-1), *trans-p*-coumaroyl units: R₁: 2.28 (s, 3H, H-11''), 6.40 (d, 1H, $J = 16.2$ Hz, H-8''), 6.98-7.16 (m, 2H, H-3'', H-5''), 7.47 (d, 2H, H-2'', H-6''), 7.53-7.64 (m, 1H, H-7''), R₂: 2.28 (s, 3H, H-11''), 6.46 (d, 1H, $J = 16.2$ Hz, H-8''), 6.98-7.16 (m, 2H, H-3'', H-5''), 7.53-7.64 (m, 3H, H-2'', H-6'', H-7''); *trans-p*-feruloyl units: R₃: 2.30 (s, 3H, H-11''), 3.81 (s, 3H, -OCH₃), 6.52 (d, 1H, $J = 16.2$ Hz, H-8''), 6.98-7.16 (m, 3H, H-2'', H-5'', H-6''), 7.53-7.64 (m, 1H, H-7''), R₄: 2.30 (s, 3H, H-11''), 3.81 (s, 3H, -OCH₃), 6.51 (d, 1H, $J = 16.2$ Hz, H-8''), 6.98-7.16 (m, 3H, H-2'', H-5'', H-6''), 7.79 (d, 1H, $J = 16.2$ Hz, H-7''); ¹³C NMR (75.48 MHz, CDCl₃): δ 63.7 (C-6), 64.5 (C-6'), 64.6 (C-1'), 69.1 (C-4), 70.6 (C-2), 71.4 (C-5), 74.4 (C-4'), 76.6 (C-3), 80.5 (C-3'), 80.6 (C-5'), 91.9 (C-1), 104.5 (C-2'); *trans-p*-

coumaroyl units: R₁: 21.1 (C-11''), 116.4 (C-8''), 122.1 (C-3'', C-5''), 129.4 (C-2'', C-6''), 131.6 (C-1''); 144.7 (C-7''), 152.2 (C-4''), 166.9 (C-9''), 168.8 (C-10''), R₂: 21.1 (C-11''), 116.4 (C-8''), 122.1 (C-3'', C-5''), 129.9 (C-2'', C-6''), 131.9 (C-1''), 145.3 (C-7''), 152.5 (C-4''), 167.3 (C-9''), 168.8 (C-10''); *trans-p*-feruloyl units: R₃: 20.7 (C-11''), 55.9 (-OCH₃), 111.4 (C-2''), 117.4 (C-8''), 121.5 (C-5''), 123.2 (C-6''), 133.1 (C-1''), 141.6 (C-3''), 145.6 (C-7''), 151.4 (C-4''), 167.8 (C-9''), 169.1 (C-10''), R₄: 20.7 (C-11''), 55.9 (-OCH₃), 111.4 (C-2''), 117.4 (C-8''), 121.5 (C-5''), 123.2 (C-6''), 133.1 (C-1''), 141.6 (C-3''), 146.5 (C-7''), 151.4 (C-4''), 168.2 (C-9''), 169.1 (C-10''); ESI-Mass (positive mode): *m/z* 1177.11 [M + Na]⁺, calcd 1177.23 for C₅₈H₅₈O₂₅Na; HR-ESI-MS (positive mode): found *m/z* 1177.3159 [M + Na]⁺, calcd 1177.3159 for C₅₈H₅₈O₂₅Na.

4.4.2. Preparation of 6-mono-*O*-feruloyl-3',6'-di-*O*-coumaroylsucrose **116** and 3,6-di-*O*-feruloyl-3',6'-di-*O*- coumaroylsucrose **227**

General Procedure

Pyrrolidine were added to a separate suspension of compounds **222** in 95% EtOH. Consequently, it caused the solution to turn yellow. The starting material typically dissolved within 15 min and the reaction was allowed to continue until the disappearance of the starting material as indicated by TLC analysis (EtOAc). This mixture was directly added to a column of strongly acidic ion-exchange resin [Amberlite IRA-120 (H⁺) washed and packed in 95% EtOH]. The appropriate fractions were concentrated under diminished pressure to a residue that was subjected to column chromatography using a gradient of CH₂Cl₂-EtOAc-MeOH afforded compound **116**. The similar approach was achieved for a compound **226** to obtain compound **227**.

4.4.2.1. 6-Mono-*O*-feruloyl-3',6'-di-*O*-coumaroylsucrose (Lapathoside C, **116**)

Following the general procedure, a suspension of compound **222** (0.2 g, 0.2 mmol) in 95 % EtOH (10 mL) was treated with pyrrolidine (155.0 μ L, 0.1 g, 1.9 mmol) for 90 min to furnish lapathoside C **116** as a white solid

(0.13 g, 75% yield). Analytical data for **116**: R_f = 0.55 (9:1 EtOAc-MeOH); mp 125-127 °C; FT-IR (KBr) $\nu_{\max}$: 3447, 3421, 2956, 2926, 2362, 2340, 1700, 1636, 1559, 1540, 1517, 1457, 1445, 1374, 1328, 1266, 1170, 1059, 997, 946, 831, 668 cm^{-1} ;

^1H NMR (300 MHz, CD_3OD): δ 3.32 (m, 1H, H-4), 3.35 (m, 1H, H-5), 3.48 (m, 1H, H-2), 3.60 (m, 2H, H-1'a, H-1'a), 3.65 (m, 1H, H-3), 4.18 (m, 1H, H-5'), 4.29 (m, 1H, H-6a), 4.55 (m, 2H, H-6'b, H-6'a), 4.65 (m, 1H, H-4'), 4.71 (m, 1H, H-6b), 5.50 (m, 1H, H-1), 5.54 (m, 1H, H-3'); *trans-p*-feruloyl units: R_1 : 3.84 (s, 3H, $-\text{OCH}_3$), 6.48 (d, 1H, J = 15.9 Hz, H-8''), 6.75-6.82 (m, 1H, H-5''), 7.02 (d, 1H, J = 7.5 Hz, H-6''), 7.21 (br s, 1H, H-2''), 7.62 (d, 1H, J = 15.9 Hz, H-7''); *trans-p*-coumaroyl units: R_2 : 6.43 (d, 1H, J = 15.9 Hz, H-8''), 6.75-6.82 (m, 2H, H-3'', H-5''), 7.52 (d, 2H, H-2'', H-6''), 7.73 (d, 1H, J = 15.9 Hz, H-7''), R_3 : 6.24 (d, 1H, J = 15.9 Hz, H-8''), 6.75-6.82 (m, 2H, H-3'', H-5''), 7.34 (d, 2H, H-2'', H-6''), 7.62 (d, 1H, J = 15.9 Hz, H-7''); ^{13}C NMR (75.48 MHz, CD_3OD): δ 65.4 (C-1'), 65.8 (C-6, C-6'), 72.1 (C-4), 72.3 (C-5), 73.1 (C-2), 74.8 (C-3), 75.0 (C-4'), 79.0 (C-3'), 81.1 (C-5'), 92.5 (C-1), 104.8 (C-2'); *trans-p*-feruloyl units: R_1 : 56.4 ($-\text{OCH}_3$), 111.5 (C-2''), 115.3 (C-8''), 116.3 (C-5''), 124.5 (C-6''), 127.7 (C-1''), 147.2 (C-7''), 149.3 (C-3''), 150.6 (C-4''), 169.3 (C-9''); *trans-p*-coumaroyl units: R_2 : 114.6 (C-8''), 116.8 (C-3'', C-5''), 127.1 (C-1''), 131.5 (C-2'', C-6''), 147.6 (C-7''), 161.4 (C-4''), 168.4 (C-9''), R_3 : 114.8 (C-8''), 116.8 (C-3'', C-5''), 127.1 (C-1''), 131.2 (C-2'', C-6''), 146.8 (C-7''), 161.3 (C-4''), 168.9 (C-9''); ESI-Mass (positive mode): m/z 833.13 $[\text{M} + \text{Na}]^+$, calcd 833.24 for $\text{C}_{40}\text{H}_{42}\text{O}_{18}\text{Na}$; HR-ESI-MS (positive mode): found m/z 833.2283 $[\text{M} + \text{Na}]^+$, calcd 833.2263 for $\text{C}_{40}\text{H}_{42}\text{O}_{18}\text{Na}$. Spectral data of lapathoside C **116** was the same as reported for the isolated natural product.⁵⁹

4.4.2.2. 3,6-Di-*O*-feruloyl-3',6'-di-*O*-coumaroylsucrose **227**

Following the general procedure, a suspension of compound **226** (0.1 g, 0.1 mmol) in 95 % EtOH (5 mL) was reacted with pyrrolidine (130.0 μ L, 0.1 g, 1.6 mmol) for 3 h to afford compound **227** as a white solid

(0.04 g, 47% yield). Analytical data for **227**: R_f = 0.74 (9:1 EtOAc-MeOH); mp 135-138 $^{\circ}$ C; FT-IR (KBr) $\nu_{\max}$: 3363, 2924, 2852, 2363, 2340, 1698, 1635, 1604, 1517, 1457, 1455, 1337, 1277, 1169, 1128, 1087, 1031, 987, 932, 831, 666 cm^{-1} ;

^1H NMR (300 MHz, CD_3OD): δ 3.56 (m, 1H, H-4), 3.65-3.74 (m, 3H, H-1'a, H-1'b, H-2), 4.15-4.21 (m, 1H, H-5'), 4.24-4.33 (m, 1H, H-6'b), 4.41 (dd, 1H, J = 8.7 Hz, 9.0 Hz, H-5), 4.52-4.59 (m, 2H, H-6b, H-6a), 4.67-4.74 (m, 2H, H-4', H-6'a), 5.34 (dd, 1H, J = 9.9 Hz, 9.0 Hz, H-3), 5.57-5.62 (m, 2H, H-1, H-3'); *trans-p*-coumaroyl units: R_1 : 6.26 (d, 1H, J = 16.2 Hz, H-8''), 6.74-6.82 (m, 2H, H-3'', H-5''), 7.36 (d, 2H, H-2'', H-6''), 7.54-7.65 (m, 1H, H-7''); R_2 : 6.49 (d, 1H, J = 16.2 Hz, H-8''), 6.74-6.82 (m, 2H, H-3'', H-5''), 7.54-7.65 (m, 2H, H-2'', H-6''), 7.75 (d, 1H, J = 16.2 Hz, H-7''), *trans-p*-feruloyl units: R_3 : 3.84 (s, 3H, $-\text{OCH}_3$), 6.42 (d, 1H, J = 16.2 Hz, H-8''), 6.74-6.82 (m, 1H, H-5''), 7.01 (dd, 1H, J = 1.5 Hz, 7.8 Hz, H-6''), 7.18 (dd, 1H, J = 9.9 Hz, 1.2 Hz, H-2''), 7.54-7.65 (m, 1H, H-7''), R_4 : 3.88 (s, 3H, $-\text{OCH}_3$), 6.48 (d, 1H, J = 16.2 Hz, H-8''), 6.74-6.82 (d, 1H, J = 7.8 Hz, H-5''), 7.07 (dd, 1H, J = 1.5 Hz, 7.8 Hz, H-6''), 7.18 (dd, 1H, J = 9.9 Hz, 1.2 Hz, H-2''), 7.54-7.65 (m, 1H, H-7''); ^{13}C NMR (75.48 MHz, CD_3OD): δ 65.3 (C-1'), 65.6 (C-6), 65.7 (C-6'), 70.5 (C-4), 71.6 (C-2), 72.5 (C-5), 74.7 (C-4'), 77.0 (C-3), 79.0 (C-3'), 81.2 (C-5'), 92.7 (C-1), 105.4 (C-2'), *trans-p*-coumaroyl units: R_1 : 114.7 (C-8''), 116.9 (C-3'', C-5''), 127.1 (C-1''), 131.3 (C-2'', C-6''), 146.9 (C-7''), 161.5 (C-4''), 168.7 (C-9''), R_2 : 114.9 (C-8''), 116.9 (C-3'', C-5''), 127.3 (C-1''), 131.7 (C-2'', C-6''), 147.0 (C-7''), 161.5 (C-4''), 169.0 (C-9''); *trans-p*-feruloyl units: R_3 : 56.5 ($-\text{OCH}_3$), 111.8 (C-2''), 115.3 (C-8''), 116.4 (C-5''), 124.1 (C-6''), 127.7 (C-1''), 147.4 (C-7''), 149.4 (C-3''), 150.7 (C-4''), 169.3 (C-9''), R_4 : 56.5 ($-\text{OCH}_3$), 111.8 (C-2''), 115.8 (C-8''), 116.6 (C-5''), 124.6 (C-6''), 127.9 (C-1''), 147.8 (C-7''), 149.5 (C-3''), 150.8 (C-4''), 169.3 (C-9''); ESI-Mass (positive mode): m/z 1009.12 $[\text{M} + \text{Na}]^+$, calcd 1009.28 for $\text{C}_{50}\text{H}_{50}\text{O}_{21}\text{Na}$; HR-ESI-MS (positive mode): found m/z 1009.2740 $[\text{M} + \text{Na}]^+$, calcd 1009.2737 for $\text{C}_{50}\text{H}_{50}\text{O}_{21}\text{Na}$.

4.4.3. Synthesis of 6-mono-*O*-feruloyl-3,3',6'-tri-*O*-coumaroylsucrose 229

4.4.3.1. Preparation of 6-mono-*O*-acetoxyferuloyl-3,3',6'-tri-*O*-acetoxyferuloylsucrose 228

Compound **222** (0.4 g, 0.4 mmol) was dissolved in dry CH₂Cl₂ (7 mL) to which 4 Å molecular sieves powder followed by dry pyridine (0.3 g, 0.3 mL, 3.8 mmol) was added. The solution was then cooled to 0 °C in an ice bath and then *p*-acetoxyferuloyl chloride **195** (0.08 g, 0.35 mmol) was added slowly at the same temperature and the reaction mixture was left to stir while warming to rt. Stirring was continued until the disappearance of the starting material as indicated by TLC (3:1 EtOAc-hexanes). After 24 h, the resulting mixture was poured into vigorously stirred ice-water (100 mL) and a white solid precipitated was obtained after decantation and filtration. The precipitate was redissolved in EtOAc (25 mL) and washed with 1N HCl (2 x 50 mL). The aqueous layer was extracted with EtOAc (25 mL). The combined organic layers were then successively washed with 5% NaHCO₃ (2 x 50 mL) and brine (25 mL) and then dried over anhyd. MgSO₄. The solvent was concentrated to residue that was subjected to column chromatography using a gradient of CH₂Cl₂-EtOAc as eluent furnished compound **228** as a white solid (0.12 g, 27% yield). Analytical data for **228**: R_f = 0.43 (3:1 EtOAc-hexanes); mp 113-115 °C;

FT-IR (KBr) ν_{max} : 3428, 2923, 2362, 2340, 1765, 1718, 1636, 1602, 1559, 1540, 1507, 1457, 1419, 1374, 1320, 1281, 1260, 1205, 1165, 1054, 1009, 987, 913, 839, 792, 649 cm⁻¹; ¹H NMR (300 MHz, CDCl₃): δ 3.61 (m, 1H, H-4), 3.78 (m, 3H, H-2, H-1'a, H-1'b), 4.27 (m, 2H, H-5, H-5'), 4.55 (m, 5H, H-4', H-6b, H-6a, H-6'b, H-6'a), 5.25 (m, 1H, H-3), 5.30 (m, 1H, H-3'), 5.57 (br s, 1H, H-1); *trans*-*p*-coumaroyl units: R₁: 2.28 (s, 3H, H-11''), 6.40 (d, 1H, *J* = 15.9 Hz, H-8''), 6.93-7.17 (m, 2H, H-3'', H-5''), 7.47 (d, 2H, H-2'', H-6''), 7.57-7.67 (m, 1H, H-7''), R₂: 2.29 (s, 3H, H-11''), 6.41 (d, 1H, *J* = 15.9 Hz, H-8''), 6.93-7.17 (m, 2H, H-3'', H-5''), 7.47 (d, 2H, H-2'', H-6''), 7.57-7.67 (m, 1H, H-7''), R₃: 2.29 (s, 3H, H-11''), 6.46 (d, 1H, *J* = 15.9 Hz, H-8''), 6.93-7.17 (m, 2H, H-3'', H-5''), 7.57-7.67 (m, 2H, H-2'', H-6''), 7.57-7.67

(m, 1H, H-7''); *trans-p*-feruloyl units: R₄: 2.30 (s, 3H, H-11''), 3.81 (s, 3H, -OCH₃), 6.52 (d, 1H, *J* = 15.9 Hz, H-8''), 6.93-7.17 (m, 3H, H-2'', H-5'', H-6''), 7.77 (d, 1H, *J* = 15.9 Hz, H-7''); ¹³C NMR (75.48 MHz, CDCl₃): R₁: 63.7 (C-6), 64.5 (C-6'), 64.7 (C-1'), 69.0 (C-4), 70.6 (C-2), 71.4 (C-5), 74.4 (C-4'), 76.6 (C-3), 80.5 (C-3'), 80.6 (C-5'), 91.9 (C-1), 104.5 (C-2'); *trans-p*-coumaroyl units: R₁: 21.1 (C-11''), 117.4 (C-8''), 122.1 (C-3'', C-5''), 129.4 (C-2'', C-6''), 131.6 (C-1''), 144.7 (C-7''), 152.2 (C-4''), 166.9 (C-9''), 168.8 (C-10''), R₂: 21.1 (C-11''), 117.4 (C-8''), 122.1 (C-3'', C-5''), 129.4 (C-2'', C-6''), 131.8 (C-1''), 145.2 (C-7''), 152.3 (C-4''), 167.3 (C-9''), 168.8 (C-10''), R₃: 21.1 (C-11''), 117.4 (C-8''), 122.1 (C-3'', C-5''), 129.9 (C-2'', C-6''), 131.9 (C-1''), 145.3 (C-7''), 152.5 (C-4''), 167.8 (C-9''), 169.1 (C-10''); *trans-p*-feruloyl units: R₄: 20.7 (C-11''), 55.9 (-OCH₃), 111.4 (C-2''), 116.5 (C-8''), 121.6 (C-5''), 123.2 (C-6''), 133.1 (C-1''), 141.6 (C-3''), 146.5 (C-7''), 151.4 (C-4''), 168.2 (C-9''), 169.1 (C-10''); ESI-Mass (positive mode): *m/z* 1147.17 [M + Na]⁺, calcd 1147.32 for C₅₇H₅₆O₂₄Na; HR-ESI-MS (positive mode): found *m/z* 1147.3036 [M + Na]⁺, calcd 1147.3054 for C₅₇H₅₆O₂₄Na.

4.4.3.2. Preparation of 6-mono-*O*-feruloyl-3,3',6'-tri-*O*-coumaroylsucrose **229**

A suspension of compound **228** (0.1 g, 0.1 mmol) in 95% EtOH (7 mL) was stirred with pyrrolidine (143.0 μL, 0.1 g, 1.7 mmol) which caused the solution to turn yellow. The starting material typically dissolved within 15 min and the reaction was allowed to continue for 3 h. After this time, the starting material was completely disappeared as indicated by TLC-analysis (EtOAc). The mixture was added directly to a column of strongly acidic ion-exchange resin [Amberlite IRA-120 (H⁺) washed and packed in 95% EtOH]. The appropriate fractions were concentrated under diminished pressure to a residue that was subjected to column chromatography using a gradient of CH₂Cl₂-EtOAc-MeOH to afford compound **229** as a white solid (0.03 g, 35% yield).

Analytical data for **229**: R_f = 0.76 (9:1 EtOAc-MeOH); mp 78-83 °C; FT-IR (KBr) ν_{max}: 3428, 2923, 2340, 2362, 2337, 1765, 1718, 1734, 1699, 1653, 1636, 1602, 1559, 1540, 1507, 1457, 1419, 1374, 1320, 1281, 1260, 1205, 1165, 1054, 1009, 987 cm⁻¹; ¹H NMR (300 MHz,

(CD₃)₂CO): δ 3.69 (m, 3H, H-4, H-1'a, H-1'b), 3.80 (m, 1H, H-2), 4.27 (m, 1H, H-5'), 4.35 (m, 1H, H-6a), 4.45 (m, 1H, H-5), 4.58 (m, 2H, H-6b, H-6'a), 4.70 (m, 2H, H-4', H-6'b), 5.43 (m, 1H, H-3), 5.57 (d, 1H, J = 8.1 Hz, H-3'), 5.62 (d, 1H, J = 3.0 Hz, H-1); *trans-p*-coumaroyl units: R₁: 6.36 (d, 1H, J = 15.9 Hz, H-8''), 6.83-6.91 (m, 2H, H-3'', H-5''), 7.50-7.67 (m, 3H, H-2'', H-6'', H-7''), R₂: 6.37 (d, 1H, J = 15.9 Hz, H-8''), 6.83-6.91 (m, 2H, H-3'', H-5''), 7.50-7.67 (m, 3H, H-2'', H-6'', H-7''), R₃: 6.49 (d, 1H, J = 15.9 Hz, H-8''), 6.83-6.91 (m, 2H, H-3'', H-5''), 7.50-7.67 (m, 3H, H-2'', H-6'', H-7''); *trans-p*-feruloyl units: R₄: 3.89 (s, 3H, -OCH₃), 6.56 (d, 1H, J = 15.9 Hz, H-8''), 6.83-6.91 (d, 1H, J = 8.4 Hz, H-5''), 7.13 (d, 1H, J = 6.9 Hz, H-6''), 7.36 (br s, 1H, H-2''), 7.77 (d, 1H, J = 15.9 Hz, H-7''); ¹³C NMR (75.48 MHz, (CD₃)₂CO): δ 64.9 (C-6), 65.3 (C-6'), 65.6 (C-1'), 70.0 (C-4), 71.4 (C-2), 72.2 (C-5), 74.7 (C-4'), 76.8 (C-3), 79.3 (C-3'), 81.0 (C-5'), 92.4 (C-1), 104.8 (C-2'); *trans-p*-coumaroyl units: R₁: 115.1 (C-8''), 116.7 (C-3'', C-5''), 126.9 (C-1''), 130.9 (C-2'', C-6''), 145.5 (C-7''), 160.6 (C-4''), 167.3 (C-9''), R₂: 115.2 (C-8''), 116.7 (C-3'', C-5''), 127.0 (C-1''), 131.1 (C-2'', C-6''), 145.9 (C-7''), 160.7 (C-4''), 167.5 (C-9''), R₃: 115.8 (C-8''), 116.8 (C-3'', C-5''), 127.0 (C-1''), 131.4 (C-2'', C-6''), 146.1 (C-7''), 160.8 (C-4''), 167.7 (C-9''); *trans-p*-feruloyl units: R₄: 56.3 (-OCH₃), 111.3 (C-2''), 115.9 (C-8''), 116.0 (C-5''), 124.2 (C-6''), 127.5 (C-1''), 146.6 (C-7''), 148.7 (C-3''), 150.1 (C-4''), 167.8 (C-9''); ESI-Mass (positive mode): m/z 979.13 [M + Na]⁺, calcd 979.27 for C₄₉H₄₈O₂₀Na; HR-ESI-MS (positive mode): found m/z 979.2612 [M + Na]⁺, calcd 979.2631 for C₄₉H₄₈O₂₀Na.

4.4.4. Synthesis of 3,6,3',6'-tetra-*O*-coumaroylsucrose 231

4.4.4.1. Preparation of 3,6,3',6'-tetra-*O*-acetoxycinnamoylsucrose 230

3',6'-Di-*O*-acetoxycinnamoyl sucrose **201** (1.1 g, 1.5 mmol) was dissolved in dry CH₂Cl₂ (21 mL) to which 4 Å molecular sieves powder followed by dry pyridine (1.2 g, 1.2 mL, 15.3 mmol) was added. The solution was cooled to 0 °C in an ice bath and *p*-acetoxycinnamoyl chloride (**195**, 0.5 g, 2.0 mmol) was added slowly at the same temperature and the reaction mixture was left to stir while warming to rt. Stirring was continued for 24 h. After this time, the starting material was completely disappeared as indicated by TLC (3:1 EtOAc-hexanes). The resulting mixture was poured into vigorously stirred ice-water (100 mL) and a white solid precipitated was obtained after decantation and filtration. The

precipitate was redissolved in EtOAc (25 mL) and washed with 1N HCl (2 x 50 mL). The aqueous layer was extracted with EtOAc (25 mL). The combined organic layers were then successively washed with 5% NaHCO₃ (2 x 50 mL) and brine (25 mL) and then dried over anhyd. MgSO₄. The EtOAc solution was concentrated to residue that was subjected to column chromatography using a gradient of CH₂Cl₂-EtOAc as eluent afforded compound **230** as a white solid (0.20 g, 12% yield). Analytical data for

230: R_f = 0.43 (3:1 EtOAc-hexanes);

mp 113-115 °C; FT-IR (KBr) ν_{max} : 3475, 2934, 2362, 2337, 1768, 1718, 1704, 1636, 1602, 1559, 1540, 1507, 1457, 1419, 1371, 1322, 1283, 1207, 1169, 1058, 1009, 946, 911, 836, 792, 649 cm⁻¹; ¹H NMR (300 MHz, CDCl₃): δ 3.60 (m, 1H, H-4), 3.68 (m, 1H, H-1'a), 3.78 (m, 2H, H-2, H-1'b), 4.26 (m, 3H, H-5, H-5', H-6a), 4.53 (m, 4H, H-4', H-6b, H-6'b, H-6'a), 5.27 (dd, 1H, J = 9.0 Hz, 9.3 Hz, H-3), 5.34 (d, 1H, J = 6.9 Hz, H-3'), 5.56 (br s, 1H, H-1); *trans-p*-coumaroyl units: R₁: 2.27 (s, 3H, H-11''), 6.38 (d, 1H, J = 15.9 Hz, H-8''), 7.05 (d, 2H, H-3'', H-5''), 7.44-7.49 (m, 2H, H-2'', H-6''), 7.61 (m, 1H, H-7''), R₂: 2.27 (s, 3H, H-11''), 6.40 (d, 1H, J = 15.9 Hz, H-8''), 7.05 (d, 2H, H-3'', H-5''), 7.44-7.49 (m, 2H, H-2'', H-6''), 7.62 (m, 1H, H-7''), R₃: 2.27 (s, 3H, H-11''), 6.44 (d, 1H, J = 15.9 Hz, H-8''), 7.05 (d, 2H, H-3'', H-5''), 7.44-7.49 (m, 2H, H-2'', H-6''), 7.64 (m, 1H, H-7''), R₄: 2.27 (s, 3H, H-11''), 6.51 (d, 1H, J = 15.9 Hz, H-8''), 7.05 (d, 2H, H-3'', H-5''), 7.57 (m, 2H, H-2'', H-6''), 7.76 (d, 1H, J = 15.9 Hz, H-7''); ¹³C NMR (75.48 MHz, CDCl₃): δ 63.8 (C-6), 64.5 (C-6'), 64.7 (C-1'), 69.0 (C-4), 70.6 (C-2), 71.4 (C-5), 74.5 (C-4'), 76.5 (C-3), 80.2 (C-3'), 80.6 (C-5'), 92.0 (C-1), 104.5 (C-2'); *trans-p*-coumaroyl units: R₁: 21.1 (C-11''), 116.6 (C-8''), 122.1 (C-3'', C-5''), 129.4 (C-2'', C-6''), 131.7 (C-1''), 144.6 (C-7''), 152.2 (C-4''), 166.9 (C-9''), 169.2 (C-10''); R₂: 21.1 (C-11''), 117.3 (C-8''), 122.1 (C-3'', C-5''), 129.4 (C-2'', C-6''), 131.8 (C-1''), 144.8 (C-7''), 152.2 (C-4''), 167.4 (C-9''), 169.2 (C-10''); R₃: 21.1 (C-11''), 117.5 (C-8''), 122.1 (C-3'', C-5''), 129.5 (C-2'', C-6''), 131.8 (C-1''), 145.0 (C-7''), 152.2 (C-4''), 167.6 (C-9''), 169.2 (C-10''); R₄: 21.1 (C-11''), 117.5 (C-8''), 122.1 (C-3'', C-5''), 129.9 (C-2'', C-6''), 131.9 (C-1''), 146.3 (C-7''), 152.4 (C-4''), 168.1 (C-9''), 169.2 (C-10''); ESI-Mass (positive mode): m/z

1117.15 $[M + Na]^+$, calcd 1117.31 for $C_{56}H_{54}O_{23}Na$; HR-ESI-MS (positive mode): found m/z 1117.2918 $[M + Na]^+$, calcd 1117.2948 for $C_{56}H_{54}O_{23}Na$.

4.4.4.2. Preparation of 3,6,3',6'-tetra-*O*-coumaroylsucrose **231**

Compound **230** (0.05 g, 0.05 mmol) was suspended in 95% EtOH (4.0 mL) and pyrrolidine (70.0 μ L, 0.1 g, 0.9 mmol) was added (which caused the solution to turn yellow). The starting material typically dissolved within 15 min and the reaction was allowed to continue for 15 min. Reaction was monitored by TLC-analysis (EtOAc). The mixture was directly added to a column of strongly acidic ion-exchange resin [Amberlite IRA-120 (H^+) washed and packed in 95% EtOH]. The appropriate fractions were concentrated under diminished pressure to a residue that was subjected to column chromatography using a gradient of CH_2Cl_2 -EtOAc-MeOH to furnish compound **231** as a white solid (0.03 g, 71% yield).

Analytical data for **231**: R_f = 0.76 (9:1 EtOAc-MeOH); mp 149-151 $^{\circ}C$; FT-IR (KBr) ν_{max} : 3415, 2957, 2922, 2850, 2363, 2340, 1700, 1635, 1604, 1559, 1516, 1441, 1369, 1327, 1266, 1169, 1060, 1004, 864, 831 cm^{-1} ; 1H NMR (300 MHz, CD_3OD): δ 3.59 (m, 1H, H-4), 3.68 (m, 2H, H-1'a, H-1'b), 3.73 (m, 1H, H-2), 4.14-4.19 (m, 1H, H-5'), 4.30 (m, 1H, H-6b), 4.38 (m, 1H, H-5), 4.50-4.57 (m, 2H, H-6a, H-6'b), 4.62-4.70 (m, 2H, H-4', H-6'a), 5.33 (app t, 1H, J = 9.6 Hz, H-3), 5.57 (d, 1H, J = 7.8 Hz, H-3'), 5.62 (d, 1H, J = 3.3 Hz, H-1), *trans-p*-coumaroyl units: R_1 : 6.29 (d, 1H, J = 15.9 Hz, H-8''), 6.73-6.81 (m, 2H, H-3'', H-5''), 7.38-7.46 (m, 2H, H-2'', H-6''), 7.54-7.66 (m, 1H, H-7''), R_2 : 6.38 (d, 1H, J = 15.9 Hz, H-8''), 6.73-6.81 (m, 2H, H-3'', H-5''), 7.38-7.46 (m, 2H, H-2'', H-6''), 7.54-7.66 (m, 1H, H-7''), R_3 : 6.40 (d, 1H, J = 15.9 Hz, H-8''), 6.73-6.81 (m, 2H, H-3'', H-5''), 7.38-7.46 (m, 2H, H-2'', H-6''), 7.54-7.66 (m, 1H, H-7''), R_4 : 6.48 (d, 1H, J = 15.9 Hz, H-8''), 6.73-6.81 (m, 2H, H-3'', H-5''), 7.54-7.66 (m, 2H, H-2'', H-6''), 7.76 (d, 1H, J = 15.9 Hz, H-7''); ^{13}C NMR (75.48 MHz, CD_3OD): δ 65.7 (C-6'), 65.8 (C-6), 65.9 (C-1'), 70.8 (C-4), 72.0 (C-2), 72.8 (C-5), 74.9 (C-4'), 77.3 (C-3), 79.4 (C-3'), 81.6 (C-5'), 93.2 (C-1), 105.6 (C-2'), *trans-p*-coumaroyl units: R_1 : 114.8 (C-8''), 117.4 (C-3'', C-5''), 127.2 (C-1''), 131.6 (C-2'', C-6''), 147.2 (C-7''), 162.3 (C-4''), 169.2 (C-9''), R_2 : 115.0 (C-8''), 117.4 (C-3'', C-5''), 127.3 (C-1''),

131.7 (C-2'', C-6''), 147.4 (C-7''), 162.4 (C-4''), 169.4 (C-9''), R₃: 115.1 (C-8''), 117.5 (C-3'', C-5''), 127.4 (C-1''), 131.9 (C-2'', C-6''), 147.4 (C-7''), 162.5 (C-4''), 169.8 (C-9''), R₄: 115.6 (C-8''), 117.6 (C-3'', C-5''), 127.4 (C-1''), 132.1 (C-2'', C-6''), 148.4 (C-7''), 162.6 (C-4''), 169.9 (C-9''); ESI-Mass (positive mode): m/z 949.14 [M + Na]⁺, calcd 949.26 for C₄₈H₄₆O₁₉Na; HR-ESI-MS (positive mode): found m/z 949.2494 [M + Na]⁺, calcd 949.2526 for C₄₈H₄₆O₁₉Na.

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