Development of phenylpropanoid sucrose esters as antidiabetic lead compounds

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**NANYANG
TECHNOLOGICAL
UNIVERSITY**

SINGAPORE

**DEVELOPMENT OF PHENYLPROPANOID SUCROSE
ESTERS AS ANTIDIABETIC LEAD COMPOUNDS**

ONG LI LIN

INTERDISCIPLINARY GRADUATE SCHOOL

2019

**DEVELOPMENT OF PHENYLPROPANOID SUCROSE ESTERS AS
ANTIDIABETIC LEAD COMPOUNDS**

ONG LI LIN

Interdisciplinary Graduate School
NTU Institute for Health Technologies

A thesis submitted to the Nanyang Technological University in partial
fulfilment of the requirement for the degree of Doctor of Philosophy

2019

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I hereby certify that the work embodied in this thesis is the result of original research, is free of plagiarised materials, and has not been submitted for a higher degree to any other University or Institution.

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Abstract

Alpha-glucosidase inhibitors (AGIs), particularly Acarbose, have received increased attention in the treatment of Type 2 diabetes (T2D) due to their effectiveness in managing postprandial blood glucose and associated macrovascular and microvascular health complications. However, AGIs are commonly plagued with side effects such as flatulence, diarrhoea and abdominal discomfort due to their strong inhibition of α -amylase. To overcome this, there is a need to seek new AGIs that either inhibit α -glucosidase alone or offer higher inhibition selectivity for α -glucosidase over α -amylase. This work focuses on the development of phenylpropanoid sucrose esters (PSEs) as potential AGIs that offer improved or comparable efficacy, but with reduced or complete eradication of side effects.

A library of PSEs with variant structures needs to be synthesized in order to perform structure activity relationship (SAR) studies. As such, a defined synthetic strategy was developed so as to synthesize any structure we needed. The strategy starts with protecting sucrose with 2-methoxypropene to obtain 2,1':4,6-di-*O*-isopropylidene sucrose which contains four free hydroxyl groups. With this compound, tetra-substituted PSEs can be synthesized. However, the four free hydroxyl groups possess similar reactivities which poses a challenge for the synthesis of mono-substituted PSEs due to lack of regioselectivity. Hence, we developed an orthogonal protection scheme using *tert*-butyldimethylsilyl (TBS), carboxybenzyl (Cbz), *para*-nitrobenzoyl (PNB) and levulinoyl (Lev) moieties to selectively protect 2,1':4,6-di-*O*-isopropylidene sucrose. Following this strategy, a phenylpropanoid group can be selectively introduced at O-3, O-3', O-4' and O-6' to give four different series of mono-substituted PSEs.

SAR studies of tetra-substituted and mono-substituted PSEs were conducted using *in vitro* α -glucosidase and α -amylase enzyme assays. From the studies, functional group(s) such as hydroxyl group, isopropylidene rings and the positions of the substituents at O-3, O-3', O-4' were identified as key structural features affecting the inhibitory activities. The *in vitro* findings allowed us to design and synthesize a PSE, Hybrid 1, which only inhibits α -glucosidase.

Hybrid 1, alongside with 4CaS (PSE with $\geq 90\%$ inhibition for both enzymes), were selected for *in vivo* studies using a diet induced diabetic mouse model. Administration of these drugs orally via water showed controlled blood glucose levels following an intraperitoneal injection of 20% glucose solution in mice. Both PSEs are deemed to be as effective as standard drug Acarbose in reducing the surge in blood glucose after a glucose load in test mice. In addition, Hybrid 1 exhibited higher effectiveness in weight management as compared to 4CaS and Acarbose as well.

Overall, this work has given us deep insight into the design and synthesis of PSEs and proved PSEs as effective AGIs. Moving forward, these synthesized lead compounds will be further evaluated to validate any common side-effects aforementioned.

List of Abbreviations

T1D	Type 1 diabetes	Tol	Toluene
T2D	Type 2 diabetes	CAN	Ceric ammonium nitrate
GDM	gestational diabetes mellitus	DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
FBG	fasting blood glucose	DIPEA	N, N-diisopropylethylamine
HbA1c	glycated haemoglobin	TMEDA	tetramethylethylenediamine
OGTT	oral glucose tolerance test	DCC	N, N'-Dicyclohexylcarbodiimide
IPGTT	intraperitoneal glucose tolerance test	EDC	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
GLP-1	glucagon-like peptide 1	TFA	trifluoroacetic acid
GLP-1 RA	glucagon-like peptide 1 receptor agonist	Bn	benzyl
DPP-4	dipeptidylpeptidase 4	Bz	benzoyl
TZD	thiazolidinedione	Ph	phenyl
SGLT-2	sodium glucose cotransporter 2	DMF	dimethylformamide
ATP	adenosine triphosphate	DIAD	diisopropyl azodicarboxylate
TBDPS	<i>tert</i> -butyldiphenylsilyl	TMS	trimethylsilyl
TBDPSCI	<i>tert</i> -butyl(chloro)diphenylsilane	TMSOTf	trimethylsilyl trifluoromethanesulfonate
PMB	<i>para</i> -methoxybenzyl	TBAF	tetra-n-butylammonium fluoride
PMBCl	<i>para</i> -methoxybenzyl chloride	THF	tetrahydrofuran
Lev	levulinate	NPAc	2-nitrophenylacetyl
LevOH	levulinic acid	NBS	N-bromosuccinimide
Fmoc	fluorenylmethyloxycarbonyl	Ac	acetyl
FmocCl	fluorenylmethyloxycarbonyl chloride	DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
NAP	1-naphthylmethyl	Boc	<i>tert</i> -butyloxycarbonyl
SEM	[β -(trimethylsilyl)ethoxy]methyl	(Boc)₂O	di- <i>tert</i> -butyl dicarbonate
SEMCl	2-(trimethylsilyl)ethoxymethyl chloride	BOM	benzyloxymethyl
Alloc	alloxycarbonyl	BOMCl	benzyl chloromethyl ether
TBS	<i>tert</i> -butyldimethylsilyl	TBAI	tetra-n-butylammonium iodide
TBSCl	<i>tert</i> -butyldimethylsilyl chloride		

DMAP	4-dimethylaminopyridine	PPy	polypyrrole
TDS	thexyldimethylsilyl	DEIPS	Diethylisopropylsilyl
AcOH	acetic acid	Me	methyl
MeOH	methanol	EtOH	ethanol
Et	ethyl	EtOAc	ethyl acetate
NEt₃	triethylamine	MeCN	acetonitrile
OMe	methoxy	ClAc	chloroacetyl
Et₂O	diethyl ether	SAR	structure activity relationship
STZ	streptozotocin	NOD	non obese diabetic
PSE	phenylpropanoid sucrose ester	NZO	New Zealand obese
cinn	cinnamoyl	diOMe	dimethoxycinnamoyl
feru	feruloyl	triOMe	trimethoxycinnmoyl
coum	coumaroyl	caff	caffeoyl
sinap	sinapoyl	DABCO	1,4-diazabicyclo[2.2.2]octane
<i>p</i>-TsOH	<i>para</i> -toluenesulfonic acid	TLC	thin layer chromatography
HR-ESI-MS	high resolution electrospray ionisation mass spectrometry	S-	(4 <i>S-trans</i>)-2,2-dimethyl- $\alpha,\alpha,\alpha',\alpha'$ -
(R)-	2,6-bis[(4R)-(+)-isopropyl-2-	TADDOL-1	tetra(1-naphthyl)-1.3-dioxolane-4,5-dimethanol
PyBOX-1	oxazolin-2yl]pyridine	(S)-	2,6-bis[(4S)-(—)-isopropyl-2-
(R,R)-	(R,R) -(—)-N,N'-bis(3,5-di- <i>tert</i> -	PyBOX-2	oxazolin-2yl]pyridine
Salen-1	butylsalicylidene)-1,2-cyclohexanediamine	(S,S)-	(S,S)-(+)-N,N'-bis(3,5-di- <i>tert</i> -
CSA	camphorsulfonic acid	Salen-2	butylsalicylidene)-1,2-cyclohexanediamine
TBANO₂	tetra-butylammonium nitrite	Tf₂O	trifluoromethanesulfonic anhydride
PPTS	pyridinium <i>p</i> -toluenesulfonate	Bu	butyl
(Phth)₂O	phenolphthalein	Phth	phthalimide
DNSA	3,5-dinitro salicylic acid	Naphth	aphthalene
		PNPG	4-nitrophenyl α -D-glucopyranoside

The naming style of synthesized phenylpropanoid sucrose esters

A general introduction to the naming style of synthesized phenylpropanoid sucrose esters (PSEs)

1. For PSEs that were screened previously and the newly synthesized tetra-substituted PSEs

The compound name comprises of three fields:

- The first field is a number representing the degree of substitution, for instance '4' for tetra-substituted PSE, '3' for tri-substituted PSEs, '2' for di-substituted PSEs and '1' for mono-substituted PSEs.
- The second field represents the type of substituent groups, for instance 'Ci' for cinnamic acid, 'F' for ferulic acid, 'S' for sinapic acid and 'Co' for coumaric acid.
- The third field then represents the presence or absence of diisopropylidene ring on sucrose. For instance, 'I' denotes the presence of diisopropylidene rings and 'S' denotes the absence of diisopropylidene rings.
- In some PSEs, there is a fourth field – 'P'. This field 'P' represents the protection of all free hydroxyl groups on the PSEs with Ac groups.

2. For mono-substituted PSEs

The compound name comprises of two fields:

- The first field is a number representing the position at which the substituent is attached, for instance 3 for substituent attached to O-3, 3' for substituent attached to O-3', 4' for substituent attached to O-4' and 6' for substituent attached to O-6'
- The second field represents the type of substituent groups, for instance 'cinn' for cinnamoyl, 'coum' for coumaroyl, 'feru' for feruloyl, 'caff' for caffeoyl, 'diOMe' for dimethoxycinnamoyl, 'triOMe' for trimethoxycinnamoyl and 'sinap' for sinapoyl.

3. For mixed PSEs

The naming of the mixed PSEs will follow the naming style of mono-substituted PSEs

- The first field is a number representing the position at which the substituent is attached, for instance 3 for O-3, 3' for O-3', 4' for O-4' and 6' for O-6'
- The second field represents the type of substituent groups, for instance 'cinn' for cinnamoyl, 'coum' for coumaroyl, 'feru' for feruloyl, 'caff' for caffeoyl, 'diOMe' for dimethoxycinnamoyl, 'triOMe' for trimethoxycinnamoyl and 'sinap' for sinapoyl.

1 Introduction

1.1 Diabetes mellitus

Diabetes mellitus (diabetes) is a metabolic disorder characterized by chronic hyperglycaemia or high blood glucose. It is a consequence of either dysfunction in insulin secretion, insulin action, or both.¹ The World Health Organisation estimated a fourfold increase of individual with diabetes from 108 million in 1980 to 422 million in 2014.² Likewise, the International Diabetes Federation reported 425 million diagnosed diabetic cases worldwide in 2017, which was a 281% increase compared to 151 million cases in 2000.³ It is likely that the number of diagnosed cases will reach 629 million by 2045. Evidently, diabetes is on the rise and its high prevalence comes at a considerable socioeconomic cost such as low productivity, reduced quality of life, lower life expectancy and escalating medical bills.⁴

Typically, diabetes can be classified into 4 categories: type 1 diabetes (T1D), type 2 diabetes (T2D), gestational diabetes mellitus (GDM) and other specific types of diabetes due to other causes.⁵ To summarize, T1D is characterized by an absolute insulin deficiency caused by the destruction of pancreatic β -cells.⁵ On the other hand, T2D is a condition with varying degree of insulin resistance, impaired insulin secretion and increased glucose production.⁶ GDM is a condition that occurs in women during pregnancy, typically as a result of hormonal changes leading to body being unable to produce enough insulin.⁵ This condition is usually temporary but will lead to complications in the child if untreated. In addition, both mother and child are at a higher risk of developing T2D. Lastly, other specific types of diabetes due to other causes are less common causes of diabetes where the underlying cause of dysfunction in insulin secretion and action are identified.¹ Some examples include drug- or chemical-induced diabetes, diseases of the exocrine pancreas and genetic defects of β -cell function.

Amongst the 4 categories, T2D is the most common form of diabetes, accounting for more than 90% of all cases.⁷ Common symptoms of T2D include polydipsia, polyuria, glycosuria and weight gain.⁸ Previously, insulin resistance was thought to be the main cause of T2D while defects in insulin.⁹ However, this theory was changed with the discovery of a feedback loop that communicates between islet β -cells and insulin-sensitive tissues.¹⁰ In general, insulin released in the event of β -cells stimulation suppress glucose production in the liver and aids in the uptake of glucose, amino acid and fatty acids into insulin-sensitive tissues like muscle and adipose tissues. Subsequently, these tissues will feedback information to β -cells with regards to the needs for insulin (Figure 1).

Figure 1: Feedback loop between islet β -cells and insulin sensitive tissue¹⁰

When insulin resistance develops in tissues, β -cells will receive information to increase insulin production to maintain normal glucose homeostasis. In the event when β -cells fail to increase production of insulin, there will be an increase in blood glucose. This will lead to impaired glucose tolerance and eventually T2D.

Insulin resistance or β -cell dysfunction are generally caused by the interactions between genetic and environmental factors.¹⁰ Some of the key genes identified are PPARG, CAPN10, KCNJ11, TCF7L2, HHEXIIDE, KCNQ1, FTO, MC4R and sedentary lifestyle, increased consumption of sugar and saturated fats, age and excessive drinking of alcohol are examples of environmental factors.¹¹ Interactions between these factors can lead to impaired β -cell function, impaired insulin action and obesity. Obesity then leads to insulin resistance and β -cells dysfunction.¹² All these contribute to the development of T2D. In the long run, T2D can also lead to the development of microvascular and macrovascular complications like retinopathy with potential blindness, nephropathy with the possibility of renal failure, neuropathy with increased chances of foot ulcers, amputation and Charcot's joints and increased risks of cardiovascular, peripheral vascular and cerebrovascular diseases.¹

1.2 Diagnosis of T2D

T2D is generally diagnosed based on plasma glucose criteria. There are three common tests for diagnosis of T2D are summarized as follow:

- **Fasting blood glucose:** measures the amount of glucose in the blood under the fasting state. Fasting (no food consumption) for at least eight hours prior to testing is needed. A fasting blood glucose of more than 126 mg/dL or 7.0 mmol/L is indicative of diabetes.^{5, 13}
- **Oral glucose tolerance test (OGTT):** measures the blood glucose at the 2-h time point after consumption of a glucose load that is equivalent to dissolving 75.0 g of anhydrous glucose in water. A 2-h blood glucose more than 200 mg/dL or 11.1 mmol/L is indicative of diabetes.^{5, 13}

However, the use of OGTT is discouraged due to its inconvenience, high cost and poor reproducibility.¹⁴

- Glycated haemoglobin (HbA1c or A1C): measures the amount of glucose carried by haemoglobin, reflecting the average blood glucose levels over a period of 2 to 3 months.⁵ This test offers greater convenience (no fasting required), higher reproducibility and lesser variations due to daily stress and glycaemic fluctuations. However, this test is affected by conditions of red blood cells and offer unreliable results for patients suffering from anaemia and haemoglobin disorders.¹⁴ A HbA1c level of more than 6.5% is indicative of diabetes.^{5, 13}

Another indicator of T2D is postprandial blood glucose. This refers to the measurement of blood glucose concentrations after eating. The magnitude and time of peak blood glucose depend on various factors like timing, quantity and composition of the meal consumed.¹⁵ As such, postprandial blood glucose is measured over a period of time and it rarely exceeds 140 mg/dL for normal individuals. Blood glucose should return to normal (i.e. blood glucose before consumption) within two to three hours. For diabetic individuals, blood glucose will exceed 140 mg/dL and the state of hyperglycaemia will take a longer time (more than two to three hours) to return to normal. Research has reported that postprandial blood glucose is a stronger independent predictor of T2D and its associated microvascular and macrovascular complications such as retinopathy, neuropathy and cardiovascular events as compared to fasting blood glucose and HbA1c.¹⁶⁻¹⁸ Hence, postprandial blood glucose would be a relevant and important indicator for diagnosis of T2D as well. Moreover, optimal glycaemic control can be achieved with monitoring and management of postprandial blood glucose together than just fasting blood glucose and HbA1c alone.¹⁹⁻²⁰

1.3 Current Treatment of T2D

To manage T2D and prevent progression leading to severe health complications, individuals need to seek treatment to reduce blood glucose profiles and maintain glycaemic control. This will help manage the progression of T2D and prevent developments of long term microvascular and macrovascular complications. There are many options available for the treatment of T2D and they are grouped into three main categories: lifestyle modification, insulin injection and oral drugs.

1.3.1 Lifestyle modifications

Lifestyle modifications are one of the approaches to the treatment of T2D. Examples include regular physical exercise, less sugar and low-fat diet, reduction in the consumption of alcohol and smoking cessation. Studies reported that such modifications can help to improve blood glucose levels in diabetic patients and reduce risks of long term health complications associated with T2D.²¹⁻²² This approach can

be considered effective but can be difficult to maintain. Moreover, to complement the benefits of lifestyle changes, injectable or oral anti-diabetic drugs will still be required in most cases.¹¹

1.3.2 Injectable drugs

Most injectable drugs available for the treatment of T2D are injected subcutaneously (under the skin). These include insulin, Glucagon-like peptide-1 receptor agonists (GLP-1 RA) and amylin analogues.¹¹ In general, injectable drugs offer higher bioavailability and faster onset than oral drugs.¹⁴ However, reluctance, the pain experienced, the struggle with self-injection and the development of allergy/itch at the site of injection can lead to poor compliance in patients, thus limiting the effects of these injectable drugs in the long run.

1.3.2.1 Insulin

Insulin is the most effective treatment in terms of blood glucose control, resulting in a decrease of HbA1c of up to 2.5%.²³⁻²⁴ Insulin also exhibits higher sustained glycaemic improvements as compared to other drugs and has shown no adverse cardiovascular effects.^{11, 14} There are different types of insulin available commercially which differ in their half-lives. Examples are listed in the following table.

Table 1: Examples of insulin grouped according to half-lives¹¹

Half-life	Examples of drugs
Rapid acting	Aspart, Lispro, Glulisine
Short acting	Actrapid, Humulin S, Insuman rapid
Intermediate acting	Insulatard, Humulin I, Insuman basal
Long acting	Glargine, Detemir

The type of insulin prescribed, frequency and dosage amount will differ based on individuals' conditions.^{11, 14} Despite its effectiveness, insulin injection is commonly associated with many side effects like increased fluid retention, increased risk of hypoglycaemia (low blood glucose), weight gain and insulin shock.^{6, 11, 23} Patients may also develop resistance to insulin over time, reducing the effectiveness of insulin in the long run. Issues like reluctance and difficulties of self-injections, leading to poor adherence can also reduce the effectiveness of insulin therapy.

1.3.2.2 Glucagon-like peptide-1 receptor agonists

Glucagon-like peptide-1 (GLP-1) is an incretin hormone responsible for slowing gastric emptying, suppressing glucagon output and increasing glucose-dependent insulin release.¹⁴ Glucagon is a potent hyperglycaemic hormone that acts almost exclusively on the liver to increase hepatic glucose production within minutes.¹⁹ Hence, GLP-1 helps in reducing postprandial blood glucose, fasting blood

glucose and HbA1c. However, the half-life of GLP-1 is very short as it is rapidly degraded by dipeptidyl peptidase (DPP-4). This caused the development of glucagon-like peptide-1 receptor agonists (GLP-1 RA), a class of drugs that are structural analogues of the natural hormone. GLP-1 RA provides the same benefits as GLP-1 but have longer half-lives due to their resistance towards degradation by DPP-4.²⁵ Some examples of GLP-1 RA include Exenatide, Liraglutide, Albiglutide, Dulaglutide and Lixisenatide.

Different GLP-1 RAs have varying half-lives, ranging from approximately 6 hours to 1 week.²⁶ Depending on the half-lives, effects of GLP-1 RA on glucose profiles can differ.²⁶ Shorter acting GLP-1 RAs exhibit stronger effects on postprandial blood glucose but lesser effects on fasting blood glucose and HbA1c. On the other hand, longer acting GLP-1 RAs displays stronger effects on fasting blood glucose and HbA1c than postprandial blood glucose. Other beneficial effects include weight loss and improvement to metabolic dysfunction.²⁴

GLP-1 RAs are usually associated with gastrointestinal side effects like nausea and vomiting²⁵⁻²⁶. The severity and frequency of these side effects fluctuate with different GLP-1 RAs but can be minimized with gradual dose titration. As one of the most expensive drugs available for treatment of T2D²⁵, the high cost could deter patients, especially those from lower income groups, from usage. Likewise, poor compliance due to delivery via subcutaneous injection can limit the effectiveness of GLP-1 RAs. However, these issues were resolved with the introduction of oral GLP-1 RA, Semaglutide.²⁵

1.3.2.3 Amylin analogue

Amylin is a 37-amino acid peptide hormone secreted with insulin in 1:100 ratio and stored together.²⁷ The action of amylin is similar to GLP-1 (refer to 1.3.2.2) where it acts to induce satiety, delay gastric emptying and suppress pancreatic glucagon production. These, in turn, reduce hepatic glucose production and blood glucose level.²⁸ Circulating amylin, available in low amounts, can be a possible treatment for T2D patients. Even so, human amylin can form insoluble polymers and can cause beta-cell maturation and destruction.²⁷⁻²⁹ As such, an amylin analogue – pramlintide acetate (Pramlintide) was developed and introduced as the treatment instead.

Lys-Cys-Asn-Thr-Ala-Thr-Cys-Ala-Thr-Gln-Arg-Leu-Ala-Asn-Phe-Leu-Val-His-Leu-Ser-Asn-Asn-Phe-Gly-Ala-Ile-Leu-Ser-Ser-Thr-Asn-Val-Gly-Ser-Asn-Thr-Tyr

Amylin

Lys-Cys-Asn-Thr-Ala-Thr-Cys-Ala-Thr-Gln-Arg-Leu-Ala-Asn-Phe-Leu-Val-His-Leu-Ser-Asn-Asn-Phe-Gly-Pro-Ile-Leu-Pro-Pro-Thr-Asn-Val-Gly-Ser-Asn-Thr-Tyr

Pramlintide

Figure 2: Amino acid chains of Amylin and Pramlintide²⁷

Pramlintide is a soluble analogue of amylin, where the alanine (ala) and serine (ser) are replaced with proline (pro) at positions 25, 28 and 29 on the chain (Figure 2). Like amylin, Pramlintide can slow

gastric emptying, suppress glucagon secretion and reduce hepatic glucose production. Since it is soluble, Pramlintide is not associated with the adverse side effects linked with the insoluble amylin polymer.²⁸ Pramlintide helps to reduce postprandial blood glucose and does not induce weight gain or hypoglycaemia.²⁹ Gastrointestinal side effects such as nausea and vomiting are usually associated with this drug, but this can be reduced with adjustment to dosage quantity.²⁸⁻²⁹ Since this drug is also injected subcutaneously, poor compliance issues apply as well.

1.3.3 Oral Drugs

There are different types of oral anti-diabetic drugs available for treatment for T2D – Metformin, sulfonylureas, meglitinides, dipeptidyl peptidase-4 (DPP-4) inhibitors, thiazolidinediones (TZDs), sodium-glucose cotransporter-2 (SGLT-2) inhibitors and alpha-glucosidase inhibitors (AGIs).^{14, 23, 30} Oral drugs can reduce the possibility of poor adherence from patients as compared to injectable drugs. In some cases, oral drugs can entirely replace insulin. When insulin injection is still required, oral drugs can also help reduce the amount of insulin needed.³¹⁻³² Major benefits and drawbacks of each oral anti-diabetic drug are discussed in the following sub-sections.

1.3.3.1 Metformin

Metformin **1** is one of the oldest drugs used for the treatment of T2D. It remains as one of the first choices of therapy today. This drug works primarily by reducing hepatic glucose output through the inhibition of gluconeogenesis – a metabolic pathway producing glucose from non-carbohydrate carbon structures like lactate, glycerol and glucogenic amino acids.²³

1 Metformin

Figure 3: Structure of Metformin

The use of Metformin **1** effectively lowers fasting blood glucose by 20% and HbA1c by 1.5%.³⁰ It does not cause hypoglycaemia and weight gain. It has favourable effects on several cardiovascular risk factors, such as lipid profile, blood pressure and platelet function.¹⁴ One side effect of metformin **1** is gastrointestinal upset like nausea, abdominal discomfort and diarrhoea. However, this can be reduced by dosage adjustment.³⁰ Other side effects include vitamin B12 deficiency and less commonly, lactic

acidosis^{a, 14} Metformin **1**, with a half-life of approximately five hours, is absorbed via organic cationic transporters, remains unmetabolized in body and distributed into intestine, liver and kidney tissues.²⁴ It is cleared from the body through the kidneys and thus should be avoided for patients with renal impairment.

1.3.3.2 Sulfonylureas

Sulfonylureas are a class of drugs that contain an S-arylsulfonyllurea group (SO₂NHCONH). They act by binding to the specific sulfonylurea receptor on the surface of the β -cells, inhibiting the flow of potassium through the adenosine triphosphate(ATP)-dependent ion channel which depolarizes the cell membrane and thus stimulates insulin production.¹⁴

Figure 4: Examples of Sulfonylureas³³

Some examples of sulfonylureas are Glibenclamide **2**, Glimepiride **3**, Glipizide **4**, Gliclazide **5** and Gliquidone **6** (Figure 4). Sulfonylureas are fast acting and effective in lowering blood glucose concentration. Furthermore, its low cost makes it affordable to T2D patients from lower income groups.

^a a medical condition caused by the accumulation of lactate in the body, resulting in an excessively low pH in the bloodstream.

Side effects of sulfonylureas include hypoglycaemia (low blood glucose) weight gain, headache, dizziness, nausea, hypersensitivity reactions (allergies) and higher cardiovascular risks.^{14, 24, 33} As sulfonylureas act on β -cells, this class of drugs will not have an effect on T2D patients with an insufficient amount or total loss of β -cells.

1.3.3.3 Meglitinides

Meglitinides or glinides stimulate insulin release in a similar manner as sulfonylureas but binds to a different part of the sulfonylurea receptors on the surface of β -cells.³⁴ Like sulfonylureas, meglitinides are also fast acting and effective in lowering blood glucose concentration. However, meglitinides have shorter half-lives as compared to sulfonylureas.³⁵ Some examples of meglitinides include Nateglinide **7**, Repaglinide **8** and Mitiglinide **9** (Figure 5).

Figure 5: Examples of Meglitinides

Meglitinides are considered to be less effective in comparison to sulfonylureas but offer greater flexibility in treatment even though more frequent doses are required.²⁴ Nevertheless, shorter half-lives of meglitinides reduce the occurrence of side effects (also reported for sulfonylurea) like weight gain, hypoglycaemia and the risks of cardiovascular complications.¹⁴

1.3.3.4 Dipeptidyl peptidase-4 inhibitors

Dipeptidyl peptidase-4 (DPP-4) inhibitors are a class of drugs that inhibit the enzyme DPP-4, which is responsible for the degradation of GLP-1 (Section 1.3.2.2).¹⁴ A few examples of DPP-4 inhibitors are Sitagliptin **10**, Vildagliptin **11**, Saxagliptin **12**, Linagliptin **13** and Alogliptin **14** (Figure 6).^{14, 36}

Figure 6: Examples of DPP-4 inhibitors

DPP-4 inhibitors can effectively reduce the plasma activity of DPP-4 by up to 90%, which increases GLP-1 concentration by four times.³⁷ A higher concentration of circulating GLP-1 will then lead to a decrease in postprandial blood glucose as GLP-1 stimulates insulin release, suppresses glucagon secretion and slows gastric emptying. An advantage of DPP-4 inhibitors is that they do not possess the risk of hypoglycaemia.¹⁴ Moreover, DPP-4 inhibitors have no effect on weight and are free from cardiovascular complications. All DPP-4 inhibitors except Linagliptin **13** are excreted via the kidney, hence dosage adjustment will be necessary for patients with renal impairment.²⁴ In decreased dosage, DPP-4 inhibitors are considered safe for use in patients with moderate to severe renal impairment.

Like GLP-1 RA, DPP-4 inhibitors are associated with gastrointestinal side effects such as nausea and vomiting. In certain cases, DPP-4 inhibitors can cause skin rashes as well. Other reported side effects include upper respiratory tract infection, headache and pancreas inflammation.²⁴ In addition, their high costs also limit the clinical usage of DPP-4 inhibitors.

1.3.3.5 Thiazolidinediones

Thiazolidinediones (TZDs) or glitazones are drugs that act as insulin sensitizers which work via the peroxisome proliferator-activated receptor- γ (PPAR- γ) distributed in fat cells, skeletal muscle, liver and pancreatic β -cells.¹⁴ Usage of TZDs has been controversial since the recall of Troglitazone (a first-generation TZD) due to its liver toxicity.²³ Current TZDs available commercially are Rosiglitazone **15** and Pioglitazone **16** (Figure 7). Both drugs stimulate the production of proteins which improve insulin sensitivity and inhibit transcription of proteins accountable for insulin resistance and inflammation in target organs.¹⁴ They are effective in reducing HbA1c, possess no risk of hypoglycaemia and potentially reduce blood pressure as well.¹¹

Figure 7: Examples of TZDs

Despite its benefits, Rosiglitazone is not commonly used in treatment now due to potential adverse cardiovascular outcomes such as heart failure.^{11, 23} Other reported side effects of TZDs include weight gain, fluid retention and bone fractures.

1.3.3.6 Sodium-glucose Cotransporter-2 inhibitors

Sodium-glucose Cotransporter-2 (SGLT-2) inhibitors are a new class of drugs that reduce blood glucose in a glucose and insulin-independent pathway.^{24, 38} SGLT-2 is a transporter found in the kidney proximal tubule and is responsible for 90% of renal glucose reabsorption.¹⁴ By inhibiting SGLT-2, there will be reduced renal glucose absorption, leading to higher amount of urinary glucose excretion and thus reducing blood glucose.²³ Examples of SGLT-2 inhibitors include Dapagliflozin **17**, Canagliflozin **18** and Empagliflozin **19** (Figure 8).

Figure 8: Examples of SGLT-2 inhibitors

Since SGLT-2 inhibitors work via a glucose-independent mechanism, these drugs can be used in T2D patients with permanent loss of β -cells.²⁴ Other benefits include no risk of hypoglycaemia, a reduction in total body weight and a decrease in blood pressure.

The main side effect linked to the use of SGLT-2 inhibitors is urinary or genital tract infection, which is more predominant in women.²³ This class of drugs is also less effective in patients with serious renal impairment as a reduced dosage is required. Other reported side effects include bone fractures and an increased risk of peripheral vascular disease.²³

1.3.3.7 Alpha-glucosidase inhibitors

Alpha-glucosidase inhibitors (AGIs) is a class of pseudo carbohydrates that competitively blocks α -glucosidase enzymes located in the brush border of the small intestine.¹⁴ α -glucosidase enzymes are responsible for breaking down polysaccharides and oligosaccharides into absorbable monosaccharides like glucose.³⁹ The inhibition of such enzymes will slow down glucose absorption after a meal, lowering postprandial blood glucose and insulin levels. Examples of AGIs are Acarbose **20**, Miglitol **21** and Voglibose **22** (Figure 8).⁴⁰ Out of the three, Acarbose **20** is the most commonly used AGIs for the treatment of T2D.

Figure 9: Examples of AGIs

Differences in mechanisms of action and excretion exist amongst the three drugs. Acarbose **20** competes with polysaccharides and oligosaccharides and exhibits inhibitory activity towards glucoamylase, sucrase, maltase, dextranase (various classes of α -glucosidase), salivary and pancreatic α -amylase.³⁹⁻⁴⁰ On the other hand, Miglitol **21** and Voglibose **22** compete with disaccharides and inhibit disaccharide-digestive enzymes.⁴⁰ Both drugs also do not exhibit any inhibitory effect on the pancreatic α -amylase. Moreover, amongst the three drugs, only Miglitol **21** has minor inhibitory activity on β -glucosidase enzymes like lactase. Acarbose **20** and Voglibose **22** are poorly absorbed in the intestine and excreted through faeces while Miglitol **21** is completely absorbed in the intestine and cleared unchanged by the kidneys.³⁹

AGIs were proven to be effective and efficient in reducing postprandial blood glucose, fasting blood glucose and HbA1c.⁴⁰ They do not cause weight gain and hypoglycaemia. In addition, they improve insulin sensitivity and secretion. They also reduce the risk of associated cardiovascular outcomes.^{14, 39-}

⁴⁰ AGIs are usually consumed together with the first bite of a meal, bringing convenience to patients as well. Side effects of AGIs are gastrointestinal disturbance such as flatulence, diarrhoea and abdominal

discomfort.^{14, 39-40} These are caused by the breakdown of unabsorbed complex carbohydrates in the intestine by intestinal microflora. These side effects can be reduced via dosage adjustment. Furthermore, it is important to note that the effectiveness of AGIs in reducing postprandial blood glucose depends on the amount of complex carbohydrates in the meal.³⁹

1.3.4 Combination Therapy

The use of a single treatment – be it an insulin injection or an oral drug like Metformin **1**, AGIs and GLP-1 RAs will be ineffective over time due to the varying and progressive nature of T2D.¹¹ Combination therapy involving two or three drugs with different mechanisms of action is often required. Most of the oral drugs mentioned can be used together for combination therapy, except sulfonylurea and meglitinides due to their similar modes of action. A few examples of combined therapy include metformin with Empagliflozin **19** (SGLT-1 inhibitor) and Linagliptin **13** (DPP-4 inhibitor)⁴¹, Metformin **1** with Acarbose **20** (AGI)⁴² and Acarbose **20** with Sitagliptin **10** (DPP-4 inhibitor)⁴³. Combination therapy offers personalised treatment where T2D patients can be prescribed different combinations of drugs/treatments depending on their conditions.

1.3.5 Development of new therapeutic agents

Glucagon receptor antagonists are a class of new drug that reduces fasting blood glucose and HbA1c with lower risk hypoglycaemia, that is currently being evaluated in long term trials.²³ Example of other glucose lowering drugs in development include selective peroxisome proliferator-activated receptor (PPAR) modulators, cellular glucocorticoid inhibitors, adiponectin receptor agonists and fibroblast growth factor 21 analogues.⁴⁴

Within the current therapeutic agents available, research is also ongoing in screening and developing potential new lead compounds that offer improved efficacy and reduced side effects. Amongst these, AGIs have received increased attention due to their effectiveness in managing postprandial blood glucose – which was a stronger independent predictor of T2D and its associated health complications (refer to 1.2) ¹⁶⁻¹⁸ Hence, there are many ongoing research projects involving screening and/or isolating natural and synthetic compounds as lead compounds as potential AGIs.⁴⁵⁻⁵⁴ A few examples reported in the last two years include novel oxazolxanthone derivatives⁵⁵, dimeric acylphenol extracted from *Myristica cinnamomea* King⁵⁶ and Pu-erh tea polysaccharides⁵⁷.

Alongside other ongoing research, this work focuses on the screening of phenylpropanoid sucrose esters (both natural and synthetic compounds) for the identification of potential lead compounds for further development as AGIs.

1.4 Phenylpropanoid sucrose esters

Phenylpropanoid sucrose esters (PSEs) belongs to a class of natural compounds, phenylpropanoid glycosides, found commonly in plant species such as *Arecaceae*, *Liliaceae*, *Polygonaceae*, *Rosaceae*, *Brassicaceae* and *Smilacaceae*.⁵⁸ In general, PSEs contain a sucrose core that is randomly connected to one or more phenylpropanoid groups (Figure 10). These groups are generally *trans*- cinnamoyl, coumaroyl feruloyl, caffeoyl, sinapoyl, dimethoxycinnamoyl and trimethoxycinnamoyl groups. To date, more than 150 PSEs have been isolated and characterised.⁵⁸

Figure 10: General structure of PSEs

PSEs are used in many traditional medicines. They possess a diverse arrays of biological activities such as antiproliferation,⁵⁹⁻⁶² antitumor,⁶³⁻⁶⁴ antioxidation,⁶⁵⁻⁷² anti-inflammation⁷³⁻⁷⁶ and in particular, α -glucosidase, β -glucosidase and α -amylase inhibition^{69, 77-78}. Some of the reported PSEs even showed higher inhibitory activity as compared with standard drug Acarbose **20**, showing the potential of PSEs as AGI for treatment of T2D.

1.4.1 PSEs as potential AGIs

Fan *et al* reported four PSEs extracted from *Polygonaceae* with inhibitory activities against both α - and β -glucosidases (Table 2).⁶⁹ In particular, Lapathoside D **24** with IC₅₀ of 0.113 mM demonstrated a stronger α -glucosidase inhibition as compared to standard drug Acarbose with IC₅₀ of 0.375 mM.

Table 2: Inhibition of α - and β -glucosidases of four PSEs extracted from *Polygonaceae*

Entry	Compound ^a	α -glucosidase inhibition (%) ^b	β -glucosidase inhibition (%) ^b
1	Lapathoside D 24	77 $\pm$ 4	30 $\pm$ 1
2	Lapathoside C 25	25 $\pm$ 2	24 $\pm$ 6
3	Hydropiperoside 26	38 $\pm$ 4	21 $\pm$ 2
4	Vanicoside B 27	24 $\pm$ 7	21 $\pm$ 8

Mean values $\pm$ S.D. are shown

^a structures are shown in Figure 11

^b Concentration of PSE tested = 225 μ g/ml

Figure 11: PSEs with reported enzyme inhibitory activities

From Figure 11, Lapathoside D **24** is a di-substituted PSEs with *p*-coumaroyl groups attached to the sucrose core at O-3' and O-6'. Lapathoside C **25**, in contrast, is a tri-substituted PSEs with the same structure as Lapathoside D **24** but has an additional feruloyl group attached at O-6. Likewise, Hydropiperoside **26** is a tri-substituted PSE that has the same structure as Lapathoside D **24** but with

additional coumaroyl group attached at O-1'. Vanicoside B **27** is a tetra-substituted PSE with one feruloyl group attached at O-6 and three coumaroyl groups attached at O-1', O-3' and O-6' respectively.

Similar inhibitory activities for β -glucosidase by all four compounds (Table 2, Entry 1-4) suggest that the number of phenylpropanoid group on the sucrose core and the type of phenylpropanoid group attached do not contribute to β -glucosidase inhibition. However, this is different for α -glucosidase inhibition. With an additional phenylpropanoid attached, the % inhibition of α -glucosidase dropped from 77 ± 4 to 25 ± 2 and 38 ± 4 respectively (Table 2, Entry 1 & 3). A plausible hypothesis could be that tri-substituted PSEs are too bulky and cannot fit well into the active binding pockets on the enzyme. The difference of 14% between tri-substituted PSE **26** and tetra-substituted PSE **27** follows this general trend as well.

Comparison of tri-substituted PSE **25** with tetra-substituted PSE **27** showed no appreciable difference in their α -glucosidase inhibition (Table 2, Entry 2 and 4). This observation does not follow the trend described earlier where an additional phenylpropanoid group caused a decrease in α -glucosidase inhibitory activity. In addition, a difference of 13% in α -glucosidase inhibition between both tri-substituted PSEs **25** and **26** was observed as well. These suggest that either the type of phenylpropanoid group or the position at which the phenylpropanoid group is attached to may have contributions to their α -glucosidase inhibitory activities as well.

In another study by Kawai *et al*, Vanicoside A **28** was also reported to have shown β -glucosidase inhibitory activity.⁷⁸ The structures of Vanicoside A **28** and Vanicoside B **27** are slightly different as there is an additional acetyl group attached to the sucrose core at O-2 position for Vanicoside A **28** (Figure 11). Both Vanicoside A **28** and Vanicoside B **27** showed β -glucosidase inhibitory activities with IC_{50} of 60 ± 2 μ M and 48 ± 1 respectively. With a lower IC_{50} , Vanicoside B **27** has a significantly higher activity than Vanicoside A **28** and this suggests that the presence of the acetyl group in sucrose may have induced the decrease in activity. Unfortunately, this study did not test Vanicoside A **28** for α -glucosidase inhibition, hence it cannot be determined if the presence of an acetyl group will have any effect on α -glucosidase inhibitory activity.

In the same study, testing of *p*-coumaric acid and ferulic acid also showed β -glucosidase inhibitory activity with IC_{50} of 1134 ± 88 and 1306 ± 73 respectively.⁷⁸ This means that *p*-coumaric acid has higher β -glucosidase inhibitory activity than ferulic acid and the difference between IC_{50} values of both acids implies that the type of acids on the sucrose core may affect the β -glucosidase inhibitory activity.

To add on, Adisakwattana and coworkers reported the testing of cinnamic acid and derivatives for sucrase and maltase (α -glucosidase) inhibitory activities.⁷⁹ The structures of cinnamic acid and its derivatives are shown in Figure 12. It was reported that acids with one or two methoxy groups on the phenyl ring showed no inhibitory activity against both sucrase and maltase. Maltase inhibitory activities of the

remaining acids can be arranged in the following manner: cinnamic acid **30** < *m*-coumaric acid **32** $\approx$ *p*-coumaric acid **33** < *o*-coumaric acid **31** < caffeic acid **37** < ferulic acid **39** $\approx$ isoferulic acid **40**, while sucrose inhibitory activities of the remaining acids can be arranged: cinnamic acid **30** < *m*-coumaric acid **32** $\approx$ *p*-coumaric acid **33** < *o*-coumaric acid **31** $\approx$ caffeic acid **37** < ferulic acid **39** $\approx$ isoferulic acid **40**.

Figure 12: Structures of cinnamic acid and its derivatives

Hence for higher sucrose and maltase inhibitory activities, it is preferable to have hydroxyl group rather than methoxy group on the phenyl ring instead. The number of hydroxyl groups on the phenyl rings may or may not influence the inhibitory activity, and this is highly dependent on the enzyme. For this case, having an additional hydroxyl group on the phenyl ring only increases inhibitory activity against maltase but against sucrose. Differences in the positions of substituents on phenyl rings can also contribute to sucrose and maltase inhibitory activities as *o*-coumaric acid **31** showed higher inhibition against both sucrose and maltase than *m*-coumaric acid **32** and *p*-coumaric acid **33**.

Furthermore, it was observed that the combination of one methoxy group and one hydroxyl group on the phenyl rings works best for inhibition of both sucrose and maltase. However, this is different from the previous result where *p*-coumaric acid showed higher β -glucosidase inhibitory activity than ferulic acid.⁷⁸ Hence, it is not possible to determine which acid performs best as it seems that activities deviate depending on the enzyme used. However, based on these two studies, it can be concluded that the type of phenylpropanoid group can indeed affect the enzyme inhibitory activities of PSEs and that phenylpropanoid groups with no substituent or at least one hydroxyl group(s) on the phenyl rings are generally more potent than those with methoxy group(s).

Another PSE, Diboside A **29** (Figure 11), was reported to show inhibitory activity against α -amylase with an IC_{50} of 27 ± 1 and against sucrase (α -glucosidase) with an IC_{50} of 72 ± 7 .⁸⁰ Diboside A **29** and Vanicoside B **27** are positional isomers where both share the same molecular formulas as but differ in the arrangement of the phenylpropanoid groups (Figure 11). Vanicoside B **27** has one feruloyl group at O-6 and three coumaroyl groups at O-1', O-3' and O-6', while Diboside A **29** has one feruloyl group at O-6' and three coumaroyl groups at O-6, O1-1' and O-3'. However, it is not possible to compare inhibitory activities of these two compounds since Diboside A **29** was only tested for inhibition against α -amylase and sucrase. Therefore, it is difficult to conclude whether a difference in substitution patterns can affect inhibitory activities against α -amylase and α -glucosidase. Screenings of these structural isomers for their inhibitory activities on both α -amylase and α -glucosidase should be done to attain a better understanding.

It is evident from these reported works that PSEs exhibit α -glucosidase and α -amylase inhibitory activities. Amongst all reported PSEs, Lapathoside D **24** showed a stronger α -glucosidase inhibition as compared to standard drug, Acarbose. Hence, PSEs do possess the potential as AGIs for the treatment of T2D. However, a larger library of PSEs will need to be screened to identify lead compound(s) that are better than Acarbose for further development as AGI(s). Based on the observations from these reported studies, one can expect that the structural differences between PSEs – either varying the type of phenylpropanoid group, the position or the degree of substitution, can affect their inhibitory activities against both α -amylase and α -glucosidase. Hence, one can look deeper into screening PSEs with different substituents, degrees of substitution and substitution patterns.

Dr Surabhi Devaraj, a researcher previously from the same laboratory group, had screened a series of PSEs for their inhibitory activities against both α -glucosidase and α -amylase.⁸¹ Structures of the screened PSEs with inhibitory activities are shown in Figure 13 and their percentage inhibition against α -glucosidase and α -amylase are shown in Figure 14. A preliminary SAR was established based on these experimental results.

Prior to the discussion of the results, it is important to understand the naming style of the PSEs screened first. The compound name comprises of three fields:

- The first field is a number representing the degree of substitution, for instance '4' for tetra-substituted PSE, '3' for tri-substituted PSEs, '2' for di-substituted PSEs and '1' for mono-substituted PSEs.
- The second field represents the type of substituent groups, for instance 'Ci' for cinnamic acid, 'F' for ferulic acid, 'S' for sinapic acid and 'Co' for coumaric acid.
- The third field then represents the presence or absence of diisopropylidene ring on sucrose. For instance, 'I' denotes the presence of diisopropylidene rings and 'S' denotes the absence of diisopropylidene rings.

In some PSEs, there is a fourth field – ‘P’. This field ‘P’ represents the protection of all free hydroxyl groups on the PSEs with Ac groups.

Figure 13: Structures of PSEs with inhibitory activities for α -glucosidase and α -amylase

Note: all the PSEs were tested at 50 μ g/ml except cinnamoyl (Ci) PSEs which were tested at 25 μ g/ml

Figure 14: Graphs showing % α -glucosidase inhibition & α -amylase of different PSEs

Increased α -glucosidase activity was observed for two groups of feruloyl PSEs - 2FI **46**, 3FI **47**, 4FI **48** and 2FS **56**, 3FS **57** and 4FS **58** (Figure 14a). This suggests that as the number of feruloyl groups increases, α -glucosidase inhibitory activity of PSE increases as well. However, this was not the case for the cinnamoyl PSEs since 2CiI **41** and 3CiI **42** did not show a proportional increase in their α -

glucosidase inhibitory activities like that of the PSEs with feruloyl substituents. This deviation suggests that the type of phenylpropanoid group can indeed influence α -glucosidase inhibitory activity. The appreciable jump in α -glucosidase inhibitory activities between 3CiI **42** & 4CiI **43** and 3CiS **54** & 4CiS **55** still follows the trend observed in that of the PSEs with feruloyl substituents. Hence, it can still be concluded that tetra-substituted PSEs showed the highest α -glucosidase inhibitory activities amongst di-substituted and tri-substituted PSEs.

As for the inhibition of α -amylase, no appreciable difference was observed between di-substituted products and tri-substituted products like 2CiI **41** & 3CiI **42** and 2FI **46** & 3FS **47** (Figure 14b). Only 2FS **56** and 3FS **57** seemed to show an appreciable increase which follows the trend, suggesting that higher activities can be attained with increased number of substituents. Like the inhibitory activities against α -glucosidase, tetra-substituted PSEs such as 4CiI **43**, 4FI **48**, 4CiS **55**, 4FS **58** also showed higher inhibitory activities against α -amylase than their di-substituted and tri-substituted counterparts (Figure 14b). Hence, it can be concluded that tetra-substituted PSEs are more potent inhibitors of α -amylase.

From Figure 14a and b, comparison between 4FI **48** and 4SI **52** showed a decrease in inhibitory activity against both α -amylase and α -glucosidase. A similar observation was drawn from the comparison of 4FS **58** and 4SS **60** as well. These suggest that the presence of additional methoxy groups on the aromatic rings of PSEs do not aid in their inhibitory activities against both α -amylase and α -glucosidase.

Tetra-substituted sinapoyl PSEs are less potent than tetra substituted cinnamoyl PSEs since both 4SI **52** and 4SS **60** exhibited poorer inhibitory activities against both α -glucosidase and α -amylase as compared to both 4CiI **43** and 4CiS **55** even at higher concentrations. However, it is not possible to determine if the poorer activities of sinapoyl PSEs were a result of the presence of methoxy groups or hydroxyl group on the aromatic rings of PSEs since sinapoyl group contains both methoxy and hydroxyl groups.

The presence of acetyl (Ac) groups on the aromatic rings of the phenylpropanoid group has an impact on α -glucosidase inhibitory activity as well. The decreased activities observed for 4FI **48** & 4FIP **49**, 4SI **52** & 4SIP **53**, 4FS **58** & 4FSP **59** and 4SS **60** & 4SSP **61** (Figure 14a) imply that the presence of an acetyl group may have reduced α -glucosidase inhibitory activity. Hence to achieve a higher inhibition of α -glucosidase, having free hydroxyl groups on the phenyl rings of the phenylpropanoid groups would be a better option. This is the same for the inhibition of α -amylase, since decreased activities were also observed for 4FI **48** & 4FIP **49** and 4FS **58** & 4FSP **59** (Figure 14b).

A slight increase (less than 15%) in α -glucosidase inhibitory activities for 2CiI **41** & 2CiIP **44** and 3CiI **42** & 3CiIP **45** suggests that the presence of acetyl groups on the sucrose core may increase α -glucosidase inhibitory activities. The same applies for inhibition of α -amylase, as well since a slight increase (less than 10%) in α -amylase inhibitory activities were observed for both 2CiI **41** & 2CiIP

44 and **3CiI 42** & **3CiIP 45**. However, this result is inconclusive since only PSEs with cinnamoyl substituents were screened.

In addition, similar α -glucosidase inhibitory activities observed for the three pairs of feruloyl PSEs, **2FI 46** & **2FS 56**, **3FI 47** & **3FS 57** and **4FI 48** & **4FS 58** (Figure 14a) suggest that the presence of isopropylidene rings has no impact on inhibition of α -glucosidase. Similar activities for the two pairs of cinnamoyl PSEs, **3CiI 42** & **3CiS 54** and **4CiI 43** and **4CiS 55** (Figure 14a) also support this observation. The only exception was **4SI 52** & **4SS 60**, which showed a decrease in inhibitory activity with the absence of isopropylidene rings. Since this is the only exception observed, more screenings are required to determine if this is a one-time anomaly.

In the case of α -amylase inhibition, a significant difference in inhibitory activity was observed between **4FI 48** & **4FS 58**. With no isopropylidene rings, **4FS 58** showed a higher inhibitory activity against α -amylase than **4FI 48** (Figure 14b). This suggests that the presence of isopropylidene rings can lead to a decrease in α -amylase inhibitory activity. However, similar α -amylase inhibitory activities between **4CiI 43** and **4CiS 55** do not support this observation. One plausible theory could be that cinnamoyl and feruloyl PSEs bind differently in the active sites of α -amylase since the cinnamoyl group lacks free hydroxyl groups on its phenylpropanoid substituents. Further screening of PSEs with other phenylpropanoid groups is needed to draw a conclusion on the role of isopropylidene rings on inhibition of α -amylase.

Structural modifications to PSEs were done to determine the importance of the *trans*-alkenyl C=C bond and the phenyl ring on the phenylpropanoid moieties for the inhibition of α -amylase and α -glucosidase.⁸¹ Examples of modified PSEs are shown in Figure 15.

Figure 15: Structures of modified PSEs

Compounds **62** to **65** were synthesized from hydrogenation of 2CiI **41**, 4CiI **43**, 2CoIP **50** and 3FI **47** while compound **66** was synthesized from acylation of sucrose with 3-cyclohexanepropionic acid. Comparison of the modified PSEs (compounds **62-66**) with the original PSEs showed a decrease in both α -amylase and α -glucosidase inhibitory activities for all compounds. From this screening, it can be concluded that the presence of both *trans*-alkenyl C=C bond and phenyl ring are essential for the inhibition of α -amylase and α -glucosidase.

Other than the preliminary SAR study, IC₅₀ of PSEs that showed close to 100% inhibition for either α -glucosidase, α -amylase or both were calculated and tabulated in Table 3.

Table 3: IC₅₀ of PSEs and Acarbose for α -glucosidase and α -amylase

Entry	Compound	IC ₅₀ (μ g/ml) for α -glucosidase	IC ₅₀ (μ g/ml) for α -amylase
1	4FI 48	4 $\pm$ 2	-
2	4FS 58	5 $\pm$ 2	2 $\pm$ 1
3	4CiI 43	8 $\pm$ 3	1 $\pm$ 0.2
4	4CiS 55	8 $\pm$ 3	0.8 $\pm$ 0.1
5	Acarbose	212 $\pm$ 5	5 $\pm$ 0.1

Evidently, one can observe that all PSEs had lower IC₅₀ values in comparison to Acarbose for the inhibition of α -glucosidase (Table 3, Entries 1 to 5). These values, which are around 25 to 40 times lower than Acarbose, indicated that PSEs are much more potent inhibitors of α -glucosidase. As for inhibition of α -amylase, all three PSEs (Table 3, Entries 2 to 3) showed IC₅₀ values that are comparable to Acarbose (Table 3, Entry 5). This implied that these PSEs are just as potent as Acarbose for inhibition of α -amylase.

Other than that, comparison of the IC₅₀ values between 4FI **48** and 4CiI **43** showed no appreciable difference. This indicated that both PSEs are equally potent inhibitors of α -glucosidase, implying that the presence of additional methoxy and hydroxyl groups on the aromatic rings of PSEs do not aid in their inhibitory activities against α -glucosidase. Likewise, comparison between 4FS **58** and 4CiS **55** also followed the same trend. However, this contradicted with the trend observed from comparison between tetra-substituted sinapoyl PSEs (4SI **52** & 4SS **60**) and tetra-substituted cinnamoyl PSEs (4CiI **43** & 4CiS **55**). Hence, the role of methoxy and hydroxyl groups on the inhibition of α -glucosidase will need to be clarified.

As for α -amylase, comparison of IC₅₀ values between 4FS **58** and 4CiS **55** showed no appreciable difference as well. Since both PSEs are equally potent inhibitors of α -amylase, it can be implied that the presence of additional methoxy and hydroxyl groups do not contribute to their inhibitory activities against α -amylase. However, comparison between 4FI **48** and 4CiI **43** showed contradictory result. 4FI **48** was less potent inhibitor of α -amylase than 4CiI **43**, implying that the presence of methoxy and hydroxyl groups do contribute to their inhibitory activities against α -amylase. The same trend was observed from comparison between tetra-substituted sinapoyl PSEs (4SI **52** & 4SS **60**) and tetra-substituted cinnamoyl PSEs (4CiI **43** & 4CiS **55**) as well. With the contradiction, there is a need to clarify the role of methoxy and hydroxyl groups on the inhibition of α -amylase.

In summary, the preliminary screening and IC₅₀ values of PSEs showed that

- The degree of substitution and the type of phenylpropanoid groups can affect both α -amylase and α -glucosidase inhibitory activities.

- Amongst all compounds tested, tetra-substituted PSEs showed the highest inhibitory activities against both α -amylase and α -glucosidase.
- The presence of acetyl groups on the aromatic rings of the phenylpropanoid groups reduces both α -amylase and α -glucosidase inhibitory activities.
- The presence of isopropylidene rings does not have any impact on inhibition of α -glucosidase in most cases, with an exception for one pair of PSEs – 4SI **52** and 4SS **60**.
- The presence of the phenyl ring and alkenyl C=C bond on the phenylpropanoid group is essential for the inhibitory activities of PSEs against α -amylase and α -glucosidase.

Likewise, the following need to be further explored:

- The role of isopropylidene rings on the inhibition of α -amylase and α -glucosidase
- The role of acetyl groups on sucrose core on the inhibitory activities against both α -amylase and α -glucosidase
- The role of methoxy groups on inhibition of both α -amylase and α -glucosidase and whether having additional methoxy group will affect inhibition of both α -amylase and α -glucosidase as well
- The role of hydroxyl groups on inhibition of both α -amylase and α -glucosidase and whether having additional hydroxyl group will affect inhibition of both α -amylase and α -glucosidase as well

These four points will mean that further screenings of PSEs are needed to draw a more comprehensive understanding and establish SAR of PSEs and their inhibitory activities for both α -amylase and α -glucosidase. However, the information derived from this preliminary SAR study still lays a strong foundation for subsequent work and can be used in the synthesis of other PSEs for further screening and the development of potential lead compound(s) as AGI for treatment of T2D.

Overall, PSEs certainly showed potential for development as AGIs for T2D treatment. Since current screenings of PSEs mostly focuses on PSEs with cinnamoyl, feruloyl and sinapoyl as substituents, there is a need to screen a larger library of compounds to achieve a comprehensive understanding of their SAR, such that lead compound(s) can be identified for further development as AGI(s) for T2D treatment. This includes the screening of PSEs with caffeoyl, coumaroyl, dimethoxycinnamoyl, trimethoxycinnamoyl as substituents, PSEs with substituents at different position and PSEs with different substitution patterns. The synthesis of PSEs in laboratory, the challenges faced, and the proposed solution are discussed in the following section.

1.4.2 The synthesis of PSEs

Despite their potential as new therapeutics, the exploitation of PSEs has been impeded by their low abundance from natural sources and challenging synthesis. The total synthesis of PSEs has been limited due to the inherent complexity of their structures in which one or more phenylpropanoid groups are randomly attached to a sucrose core. The key challenge hence lies in achieving regioselective introduction of phenylpropanoid groups to the desired hydroxyl groups of sucrose **67**.

Figure 16: Structures of sucrose **67 and 2,1':4,6-di-O-isopropylidene sucrose **68****

Sucrose **67** has eight free hydroxyl groups with similar reactivities – 3 primary (6-OH, 1'-OH, 6'-OH) and 5 secondary. Out of the three primary hydroxyl groups, 6-OH and 6'-OH are the most reactive as 1'-OH is more hindered due to its proximity to quaternary carbon C-2. The reactivity order of hydroxyl groups can follow two divergent pathways during treatment with bulky acylating agents like pivaloyl chloride⁸²: (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. However, for the same reaction, a slightly different reactivity order: 6-OH $\approx$ 6'-OH > 1'-OH > 3'-OH > 2-OH > 3-OH > 4-OH was reported by Queneau *et al* and Jarosz *et al*.⁸³⁻⁸⁴ It is also interesting to note that reactivity of primary hydroxyl groups can differ when treated with different acylating agents. The reaction with a bulky reagent like pivaloyl chloride gives reactivity in the order of 6-OH $\approx$ 6'-OH > 1'-OH while reaction with a less bulky reagent like benzoyl chloride gives reactivity in the order of 6-OH > 1'-OH $\approx$ 6'-OH.⁸⁴⁻⁸⁵ Taking steric factor into consideration, various synthetic methods giving the selective synthesis of primary sucrose esters at the less hindered hydroxyl groups have been developed. Some examples include Mitsunobu-type esterification, metal chelate directed esterification and protease catalysed esterification.⁸⁶⁻⁸⁸

Other than primary hydroxyl groups, direct acylation can also be achieved at 2-OH when the relative acidity is taken into consideration. Under basic conditions, reactivity order of hydroxyl groups on sucrose can be changed to 2-OH $\gg$ 1'-OH > 3'-OH > 6-OH $\approx$ 6'-OH.⁸⁵ Since 2-OH acts as hydrogen bond acceptor, this hydroxyl group is the most acidic. Activation of 2-OH with a strong base such as NaH or LiOH can lead to the formation of a highly stabilized and nucleophilic 2-oxyanion, thus making it more susceptible to acylation.⁸⁵ In addition, metal chelate directed esterification can also give selective acylation at 2-OH and 3'-OH. It was reported that coordination with nickel, copper, zinc or mercury favours acylation at 2-OH, while manganese or cobalt favours acylation at 3'-OH.⁸⁹⁻⁹⁰ Wu and

coworkers reported a total synthesis of Tenuifolide B **72** which involved regioselective introductions of acyl groups at 3'- and 6'-OHs of sucrose **67** by metal chelate-directed acylation and Mitsunobu esterification respectively (Scheme 1).⁸⁶

Scheme 1: Total synthesis of Tenuifolide B **72**⁸⁶

In conclusion, varying reagents and reaction conditions can give selectivity for the least hindered primary hydroxyl groups 6'-OH, 6-OH, 1'-OH or the more acidic secondary alcohol 2'-OH and 3-OH. However, acylation will be restricted to these five positions. This will limit the range of PSEs that can be synthesized, and so it is necessary to investigate other approaches that can overcome this limitation.

Another approach for the synthesis of PSEs is the use of 2,1':4,6-di-*O*-isopropylidene sucrose **68** as the starting material (Figure 16). Duynstee and coworkers⁹¹ reported the first synthesis of PSE, Niruriside **77**, by direct acylation of 2,1':4,6-di-*O*-isopropylidene sucrose **68** with cinnamoyl chloride via two different synthetic pathways (Scheme 2).

Reagents and conditions: i. *trans*-cinnamoyl chloride (2.2 equiv), pyridine (5 equiv), CH₂Cl₂, -30°C, 1.5 h; ii. TBSCl (2.2 equiv), imidazole (10 equiv), DMF, rt, 4 h; iii. ethylene glucol (2.2 equiv), CH₂Cl₂, cat. TsOH, rt, 48 h; iv. acetic anhydride in pyridine; v. 1 M Bu₄NF and 1 M AcOH in THF, 40°C, 24 h; vi. BnBr (5 equiv), NaH (7.5 equiv), DMF, rt, 2 h; vii. 60% AcOH, 50°C, 20 mins; viii. H₂, Pd/C, MeOH, rt, 48 h; ix. *trans*-cinnamoyl chloride (2.1equiv), pyridine (5 equiv), CH₂Cl₂, 0°C, 45 mins;

Scheme 2: Total synthesis of Niruriside 77

Using a similar approach, Dr Parthasarathi Panda, a previous researcher from our group, disclosed the total synthesis of Lapathoside D **24**, Lapathoside C **25**, Helonioside A **88**, 3',4',6'-tri-*O*-feruloylsucrose **89** and their analogues.^{60, 63} The synthetic pathway of these four natural PSEs is shown in Scheme 3.

(Substituted) cinnamoly moiety

Scheme 3: Synthesis of Lapathoside D 24, Lapathoside C 25, Helonioside A 88, 3',4',6'-tri-O-feruloylsucrose 89

Other PSEs (structural analogues of the natural compounds) can be synthesized by varying the equivalents of (substituted) cinnamoyl chloride to give different degree of substitution. Like the direct acylation of sucrose, this approach also gives mixtures of products. As a result, tedious purification is involved, and lower yields of desired products are obtained. In addition, regioselective introduction of phenylpropanoid group(s) at desired OH(s) on sucrose core will be difficult as the outcome of the reactions can be unpredictable due to the similar reactivities of the remaining four hydroxyl groups on 2,1':4,6-di-O-isopropylidene sucrose **68**. Hence, this approach will be very complex and inferior for the synthesis of mono-substituted PSEs at specific position such as O-4', O-3', O-3, and mixed PSEs where different phenylpropanoid groups are attached on the sucrose core.

To resolve the key challenge faced in the total synthesis of PSEs, orthogonal protection in which reactive hydroxyl groups are temporarily 'masked' with different protecting groups that can be selectively introduced and removed is considered. Further details are discussed in the following section.

1.5 Orthogonal Protection for total synthesis of PSEs

In this project, we envisioned the orthogonal protection of 2,1':4,6-di-O-isopropylidene sucrose **68** to give a versatile intermediate **91** that enables the introduction of the phenylpropanoid group(s) at specific position(s) (O-3, O-3', O-4', O-6') via selective deprotection and acylation (Figure 17). The use of

2,1':4,6-di-*O*-isopropylidene sucrose **68** instead of sucrose in this design was primarily based on the critical positive role which the isopropylidene rings played in the inhibition of α -amylase and α -glucosidase, as demonstrated by the SAR study done previously (section 1.4.1).⁸¹ Moreover, the complexity in number of protecting groups required is also reduced since of 2,1':4,6-di-*O*-isopropylidene sucrose **68** only have four free hydroxyl groups.

Figure 17: Proposed synthetic route to simple and mixed PSEs via orthogonal protection of 2,1':4,6-di-*O*-isopropylidene sucrose **68**

This approach will overcome the lack of regioselectivity in previous synthetic pathways, thereby providing a convenient and divergent access to wide range of simple and mixed PSEs with different degrees of substitution and different substitution patterns. With a greater library of compounds for SAR studies, it will be possible to identify the effects of phenylpropanoid groups on their biological activities so that better lead compounds can be synthesized. Although this approach is long, it is necessary to obtain the different types of PSEs required for the SAR studies in this thesis.

To the best of my knowledge, an effective strategy for orthogonal protection/deprotection of 2,1':4,6-di-*O*-isopropylidene sucrose **68** or sucrose **67** in general, remains elusive despite potential for the synthesis of highly functionalized sucrose derivatives. However, orthogonal protection/deprotection of monosaccharides and other disaccharides has been reported.⁹² The protecting groups used in these reported schemes will be discussed in the next section as they are good starting points to consider when devising our strategy for orthogonal protection of 2,1':4,6-di-*O*-isopropylidene sucrose **68**. In addition, the discussion will only be limited to reported work that demonstrated the use of three or more orthogonal protecting groups on a single carbohydrate molecule since 2,1':4,6-di-*O*-isopropylidene sucrose **68** requires four protecting groups to achieve a successful orthogonal protection strategy.

1.5.1 The orthogonal protection of monosaccharides

Wong and coworkers reported an orthogonally protected galactose thioglycoside **96** as shown in Scheme 4.⁹³ The protecting groups that were used include *tert*-butyldiphenylsilyl (TBDPS), *p*-methoxybenzyl (PMB), chloroacetate and levulinate (Lev) groups.

Reagents and conditions: i. TBDPSCI, imidazole, DMF, rt; ii. a) Bu_2SnO , toluene/benzene, reflux b) PMBCl, Bu_4NI , DMF, 60°C ; iii. ClCH_2COCl , NEt_3 , CH_2Cl_2 , -20°C to rt; iv. LevOH, DCC, DMAP, CH_2Cl_2 .

Scheme 4: Orthogonal protection of galactose thioglycoside **92**

Successful selective deprotection of individual protecting groups on the orthogonally protected galactose thioglycoside **96** was also illustrated in the reported work (Scheme 5).

Reagents and conditions: i. HF-pyridine, AcOH/THF(1:4), rt; ii. NaHCO₃, MeOH/H₂O (5:1), 60°C; iii. TFA, CH₂Cl₂, -20°C; iv. NH₂NH₂/AcOH, THF/MeOH (10:1), rt.

Scheme 5: Selective deprotection of orthogonally protected galactose thioglycoside **96**

This orthogonally protected galactose thioglycoside **96** was subsequently employed as a building block for the synthesis of many oligosaccharides such as pentasaccharides with a fully branched inner galactose core unit.⁹³

Ágoston and coworkers also reported an orthogonal protection scheme for the galactose thioglycoside (Scheme 6).⁹⁴ In this scheme, phenyl 4,6-*O*-(1-naphthyl)methylene-1-thio-β-D-galactopyranoside **100** was used as the starting material. Like the previous strategy, chloroacetate and Lev groups were used as protecting groups. However, fluorenylmethyloxycarbonyl (Fmoc) and 1-naphthylmethyl (Nap) groups were used instead of TBDPS and PMB for the remaining two hydroxyl groups. The synthetic route of the orthogonally protected galactose thioglycoside **104** is shown in Scheme 6.

Reagents and conditions: i. ClCH_2COCl , CuCl_2 , NaH , THF, 15°C , 1 h; ii. FmocCl, pyridine, CH_2Cl_2 , rt, 3 h; iii. $\text{BH}_3\cdot\text{THF}$, TMSOTf, CH_2Cl_2 , rt, 3 h; iv. LevOH, DCC, DMAP, CH_2Cl_2 , rt, 2 h.

Scheme 6: Orthogonal protection of galactose thioglycoside 100

The orthogonality of the scheme was also tested out via the selective removal of specific protecting groups on the orthogonally protected galactose thioglycoside **104**. Scheme 7 shows the successful orthogonal deprotection of all four protecting groups, hence demonstrating the orthogonality of this scheme. However, no application was shown for this work.

Reagents and conditions: i. $\text{NH}_2\text{NH}_2/\text{AcOH}$, CH_2Cl_2 , MeOH, rt, 30 mins; ii. Thiourea, toluene, MeOH, 80°C , 45 mins; iii. DBU, CH_2Cl_2 , rt, 2 mins; iv. CAN, MeCN, H_2O , rt, 2 h.

Scheme 7: Selective deprotection of orthogonally protected galactose thioglycoside 104

The next example reported by Compostella *et al* showed an orthogonal protection scheme for the galactopyranoside compound.⁹⁵ In this scheme, all five hydroxyl groups on the galactopyranoside compound are protected with different protecting groups - TBDPS, PMB, [β-(trimethylsilyl)ethoxy]methyl (SEM), Lev and Allyl. Using 3-*O*-*p*-methoxybenzyl-α-D-galactopyranoside **108** as the starting material, the subsequent steps involved are shown in Scheme 8.

Reagents and conditions: i. TBDPSCI, CH₂Cl₂/Pyridine; ii. LevOH, EDC, DMAP, CH₂Cl₂; iii. SEMCl, DIPEA, CH₂Cl₂.

Scheme 8: Synthesis of orthogonally protected galactose 111

Successful selective removal of TBDPS, SEM, Lev, PMB and Allyl groups from the orthogonally protected galactose **111** are also demonstrated in the reported work (Scheme 9), proving their orthogonality. The orthogonally protected galactose **111** was then used in the synthesis of a series of sulfated D-galactosyl ceramides.⁹⁵

Reagents and conditions: i. TBAF, THF/AcOH; ii. MgBr₂·Et₂O, Et₂O/CH₃NO₂; iii. NH₂NH₂·AcOH, CH₂Cl₂/MeOH; iv. DDQ, CH₂Cl₂/H₂O; v. Ir catalyst, THF then NBS, THF/H₂O

Scheme 9: Orthogonal deprotection of orthogonally protected galactose scaffold 111

Besides galactopyranoside, orthogonal protection schemes for glucosamine have also been reported. Hernández-Torres *et al* reported a seven-step synthesis route for the orthogonal protection of glucosamine **116** to give an orthogonally protected glucosamine intermediate **123** that can be used in the synthesis of other oligosaccharides (Scheme 10).⁹⁶ The hydroxyl protecting groups used in this strategy were *tert*-butyldimethylsilyl (TBS), PMB and alloxycarbonyl (Alloc).

Reagents and conditions: i. a) NaOMe, MeOH, rt b) Phth₂O, Et₃N, rt c) Ac₂O, pyridine, rt; ii. PhSH, TMSOTf, CH₂Cl₂, rt; iii. NaOMe, MeOH/CH₂Cl₂ (3:2), -10°C; iv. *p*-anisaldehyde dimethyl acetal, CSA, toluene, 90°C; v. TBSCl, AgNO₃, Et₃N, CH₂Cl₂, rt; vi. BH₃·THF, TMSOTf, CH₂Cl₂, -25°C to 0°C; vii. allyl chloroformate, Et₃N, DMAP, CH₂Cl₂, rt

Scheme 10: Orthogonal protection of glucosamine 116

Even though an orthogonally protected intermediate was successfully made, this work did not go on to prove the orthogonality of the strategy since selective deprotection of specific protecting groups were not shown. Thus, it is difficult to conclude whether this orthogonal protection strategy is successful or not. In addition, there was no application of this intermediate in the synthesis of other oligosaccharides.

Hou and coworkers reported an orthogonal protection scheme for tigogenyl glycoside **124**, a key intermediate for the preparation of saponin compounds.⁹⁷ The free hydroxyl groups on the glucosamine core were protected with TBS, benzyl (Bn) and chloroacetate groups as shown in Scheme 11.

Reagents and conditions: i. 0.1 M MeONa-MeOH, reflux; ii. *para*-methoxybenzaldehyde dimethyl acetal, *p*-TsOH, DMF, 50°C; iii. BnBr, NaH, DMF, rt; iv. 80% AcOH, 70°C; v. TBSCl, DMAP, imidazole, DMF, rt; vi. CO(CH₂Cl)₂, pyridine, rt.

Scheme 11: Orthogonal protection of tigogenyl glycoside 124

Selective deprotection of individual protecting groups on the orthogonally protected tigogenyl glycoside **129** was shown in Scheme 12. After proving the orthogonality of all protecting groups, tigogenyl glycoside intermediate **124** was used in the synthesis of six tigogenyl disaccharides and saponins with branched sugar moieties.⁹⁷

Reagents and conditions: i. thiourea, CH_2Cl_2 ; ii. H_2 , 10% Pd/C, $\text{CH}_2\text{Cl}_2/\text{EtOH}$; iii. CAN, $\text{CH}_2\text{Cl}_2/\text{MeOH}$.

Scheme 12: Selective deprotection of orthogonally protected tigogenyl glycoside 129

An orthogonal protection scheme was also developed for an oleanoyl glycoside, which was used as an intermediate for the synthesis of saponins.⁹⁸ In this strategy, free hydroxyl groups on the glucosamine core were protected with Lev, 2-nitrophenylacetyl (NPAc), and TBDPS groups. Though stepwise synthesis of the orthogonally protected oleanoyl glycoside **132** was not shown, the orthogonality of the strategy was clearly illustrated (Scheme 13).

Reagents and conditions: i. $\text{NH}_2\text{NH}_2 \cdot \text{AcOH}$, $\text{CH}_2\text{Cl}_2/\text{MeOH}$, rt; ii. Zn , NH_4Cl , THF/MeOH , rt; iii. TBAF/AcOH , THF , rt; iv. 3% AcCl in MeOH , CH_2Cl_2 , rt.

Scheme 13: Selective deprotection for orthogonally protected oleanoyl glycoside 132

It is interesting to note that the attempt to deprotect the TBDPS group with TBAF/AcOH in THF at room temperature (Scheme 13, iii) gave selective removal of TBDPS but at the same time, led to the migration of the NPAC group from O-4 to O-6 position. Another set of deprotection reagent and conditions was used for selective removal of TBDPS group instead (Scheme 13, iv). This observation pointed out the importance of selecting the correct reagent and conditions such that selective deprotection of an individual protecting group can be attained without affecting the other groups.

Orthogonal protection strategies were also reported for D-mannose. Reina and Rojo reported a fully orthogonally protected mannosyl glycoside with an azido group linker at its anomeric position for subsequent reactions with other carbohydrates.⁹⁹ The synthetic route for development of the orthogonally protected mannosyl glycoside **142** is shown in Scheme 14, using acetylated mannose as the starting material. The protecting groups used for the temporary masking of the hydroxyl groups were TBDPS, PMB and Lev groups. In addition, Bn group was introduced as a permanent protecting group in this scheme and hence was not selectively removed in subsequent deprotection reaction.

Reagents and conditions: i. a) $\text{HOCH}_2\text{CH}_2\text{Br}$, $\text{BF}_3 \cdot \text{Et}_2\text{O}$, CH_2Cl_2 , 0°C to rt, 24 h, b) NaN_3 , DMF, 50°C , 15 h, c) NaOMe , MeOH, rt, 15 mins; ii. TBDPSCl, imidazole, DMF, rt; iii. a) 2,2'-dimethoxypropane, PPTS, acetone, b) BnBr , NaH, DMF, c) TFA, CH_2Cl_2 ; iv. a) Bu_2SnO , toluene, 110°C , b) PMBCl , Bu_4NI ; v. LevOH, DCC, DMAP, CH_2Cl_2

Scheme 14: Development of orthogonally protected mannosyl glycoside 142

The orthogonality of this regime was demonstrated by the successful selective removal of individual protecting groups of orthogonally protected mannosyl glycoside **142** as shown in Scheme 15.

Reagents and conditions: i. Bu_4NF , THF, rt or HF, pyridine, AcOH/THF, rt; ii. TFA, CH_2Cl_2 , -20°C ; iii. $\text{NH}_2\text{NH}_2 \cdot \text{AcOH}$, MeOH, rt.

Scheme 15: Selective deprotection of orthogonally protected mannosyl glycoside 142

With an orthogonally protected mannosyl glycoside **142**, complex mannose derivatives can be synthesized. Additionally, hydrogenation of the azido linker will give an amine group at the anomeric position, ready for attachment to another carbohydrate scaffold.⁹⁹

Emmadi and Kulkarni reported an orthogonal protection scheme for phenyl 1-thio- β -D-mannopyranoside **145** which can be rapidly converted into orthogonally protected 2-azido-2-deoxyglucose thioglycoside **150** and 2-azido-2-deoxygalactose thioglycoside **152**.¹⁰⁰

Reagents and conditions: i. Me_2SnCl_2 (0.05 equiv), BzCl or AcCl (1.1 equiv), DIPEA (2 equiv), THF/ H_2O (19:1), rt, 2.5 h; ii. $\text{PhCH}(\text{OMe})_2$, cat. CSA, MeCN, rt, 10 mins; iii. a) Tf_2O , pyridine, CH_2Cl_2 , 0°C , 10 mins, b) NaN_3 , DMF, rt, 1 h; iv. a) 80% aq. AcOH, 80°C , 1.5 h, b) TBPDS (1.1 equiv), imidazole (2.5 equiv), MeCN, rt, 10 mins; v. Boc_2O , pyridine, cat. DMAP, CH_2Cl_2 , rt, 15 mins; vi. a) Tf_2O , pyridine, CH_2Cl_2 , 0°C , 10 mins, b) TBANO_2 , MeCN, rt, 1 h.

Scheme 16: Orthogonal protection of phenyl 1-thio- β -D-mannopyranoside **145**

The protecting groups used in this scheme were TBPDS, acetyl (Ac), benzoyl (Bz) and *tert*-butyloxycarbonyl (Boc). It is also interesting to note that epimerization from 2-azido-2-deoxyglucose thioglycoside **149** to 2-azido-2-deoxygalactose thioglycoside **151** can be achieved without affecting other protecting groups on the sugar core. Despite developing fully orthogonally protected from 2-azido-2-deoxyglucose thioglycoside **150** to 2-azido-2-deoxygalactose thioglycoside **152**, the

orthogonality was not proven as selective deprotection of individual protecting groups was not demonstrated. As such, it is hard to draw a conclusion to the orthogonality of this strategy.

The last example reported by Muramatsu and coworkers showed a highly regioselective and stepwise synthetic route for orthogonal protection of D-glucose derivatives (Scheme 17).¹⁰¹ The hydroxyl groups on glucose core was protected with TBDPS, isobutyrate, benzyloxymethyl (BOM) and Boc groups respectively. It was observed that screening of different protecting groups was done to select one group that offered higher regioselectivity and yield. Likewise, the same approach can be adopted in the development of the orthogonal protection strategy of 2,1':4,6-di-*O*-isopropylidene sucrose **68**.

Reagents and conditions: i. Isobutyric anhydride, collidine, CHCl_3 , -20°C ; ii. TBDPSCl, imidazole, DMF, 0°C ; $(\text{Boc})_2\text{O}$, cat. PPY, collidine, CH_2Cl_2 , -10°C ; iv. BOMCl, cat. TBAI, DIPEA, CH_2Cl_2 , reflux.

Scheme 17: Orthogonal protection of glucose derivative 153

The orthogonality of this strategy was also proven via successful selective deprotection of the individual protecting groups on the orthogonally protected glucose derivative **153** (Scheme 18).

Reagents and conditions: i. TBAF, THF; ii. o-dichlorobenzene, 180°C; iii. DIBAL-H, THF, -40°C; iv. Pd(OH)₂/C, H₂ (3 atm).

Scheme 18: Selective deprotection of orthogonally protected glucose derivative 153

This scheme uses an unusual deprotection condition that was not reported previously. The Boc group was selectively cleaved from the glucose core via thermal decomposition. This reaction condition used may be compatible with derivatives that are easily hydrolysed under acidic or basic conditions. With the orthogonally protected glucose derivative **153**, other glucose derivatives were synthesized in a chemoselective and regioselective manner via selective deprotections.

1.5.2 The orthogonal protection of disaccharides

Orthogonal protection strategies have also been reported for disaccharides. One example reported by Castoldi and coworkers showed an orthogonal protection scheme for a lactose derivative (Scheme 19).¹⁰² In this strategy, Bz, PMB, thexyldimethylsilyl (TDS) and Allyl groups were used as temporary protecting groups while Bn group and benzylidene acetal groups are used as permanent protecting groups.

Reagents and conditions: i. MeONa, MeOH, rt; ii. Bu₂Sn=O, MeOH, reflux, Allyl Bromide, TBAI, toluene, 60°C; iii. BnOH, TfOH, MeCN, 3-Å molecular sieves, 0°C; iv. PhCH(OMe)₂, CAS, MeCN, rt; v. TBSOTf, collidine, DMF, 0°C; vi. NaOH, EtOH, 40°C; vii. BnBr, NaH, DMF, rt; viii. TBAF, THF, -30°C; ix. TDS-Cl, imidazole, DMF, 0°C; x. BzCN, MeCN, NEt₃; xi. PMBCl, DMF, KI, NaH, -30°C.

Scheme 19: Orthogonal protection of lactose derivative 161

With an orthogonally protected lactose derivative **161**, Castoldi and coworkers proceeded to test the orthogonality of the scheme via selective deprotection. Individual protecting groups were selectively removed under different reaction conditions as shown in Scheme 20.

Reagents and conditions: i. TBAF, THF, -30°C; ii. NaOH, EtOH, rt; iii. DDQ, CH₂Cl₂/H₂O; iv. PdCl₂, AcONa, AcOH/H₂O.

Scheme 20: Orthogonal deprotection of orthogonally protected lactose derivative 172

Using this strategy, one can get selective access to the O-3 and O-6 positions on the glucose core and the O-2 and O-3 positions on the galactose core. Using this orthogonally protected lactose derivative **172**, synthesis of lactose derivatives with different substitution patterns can be achieved.

Boltje *et al* reported the chemical synthesis of a β -D-Man-(1 $\rightarrow$ 4)- α -D-Man disaccharide **176** that is functionalized with a set of orthogonal protecting groups – diethylisopropylsilyl (DEIPS), 1-naphthylmethyl (Nap), Lev and Allyl at O-1, O-2, O-2' and O-3' respectively.¹⁰³ The orthogonality of the strategy was tested by selectively removing individual protecting groups under different reaction conditions (Scheme 21).

Reagents and conditions: i. DDQ, CH₂Cl₂, H₂O; ii. TBAF, AcOH, THF; iii. NH₂NH₂·AcOH, Toluene/EtOH; iv. PdCl₂, NaOAc, AcOH, H₂O.

Scheme 21: Orthogonal Deprotection of 176

This strategy allows the selective introduction of glycosides at O-1, O-2, O-2' and O-3' and was used to synthesize a library of *F. tularensis* oligosaccharides with different substitution patterns.

Zhang and coworkers reported another orthogonal protection of a disaccharide which is made up of two glucosamine units (Scheme 22), which can be utilized in the synthesis of various tetra-acylated lipids.¹⁰⁴ The hydroxyl groups were protected with temporary protecting groups – Alloc, TDS and Lev groups. Bn group and benzylidene acetal were used as permanent protecting groups, which were not removed during deprotection. The orthogonality of this strategy was proven by the selective removal of TDS, Lev and Alloc groups.

Reagents and conditions: i. $\text{NH}_2\text{NH}_2 \cdot \text{AcOH}$, $\text{CH}_2\text{Cl}_2/\text{MeOH}$; ii. $\text{Pd}(\text{PPh}_2)_4$, HCOOH , $n\text{-BuNH}_2$, THF ; iii. Bu_4NF , AcOH , THF .

Scheme 22: General scheme for selective deprotection of 181

Other orthogonal protection schemes for disaccharides were also developed for the synthesis of heparin and heparin sulphate.¹⁰⁵⁻¹¹² Only two schemes will be discussed in detail as these are the only two schemes that demonstrated full orthogonality of the strategies prior to synthesis of heparin and heparin sulphate. The others failed to demonstrate full orthogonality of their strategies and focused mostly on the synthesis of their desired library of compounds.

Reagents and conditions: i. HF·pyridine, MeCN, rt; ii. NEt₃, CH₂Cl₂, 18 h, rt; iii. PdCl₂, AcOH, NaOAc; iv. NH₂NH₂, AcOH, MeOH, CH₂Cl₂.

Scheme 23: Orthogonal deprotection of orthogonally protected disaccharide 185

Prabhu and coworkers reported a disaccharide **185** protected with four orthogonal protecting groups – TBDPS, Fmoc, Allyl and Lev groups (Scheme 23). Bn groups are used as permanent protecting groups and hence not removed in subsequent deprotection steps. Several reaction conditions were attempted for the selective removal of TBDPS group before selecting HF·pyridine in MeCN as the optimal reaction condition. The use of TBAF in THF and HF·pyridine in THF both resulted in cleavage of Fmoc group alongside with removal of TBDPS group, which did not fulfil the requirement of orthogonality. Another noteworthy point is the use of unusual deprotection condition for selective removal of Fmoc group. The use of NEt₃ in CH₂Cl₂ are mild conditions that are compatible with compounds that hydrolyse easily under acidic and basic conditions.

Reagents and conditions: i. TBAF (30 equiv) in THF, adjusted to pH 10, 24 h, rt; ii. CAN in 90% *aq* MeCN (3 equiv), 12 h, 0°C; $\text{NH}_2\text{NH}_2 \cdot \text{H}_2\text{O}$ (10 equiv) in pyridine/AcOH (3:2), 6 h, rt; iv. $\text{MgBr}_2 \cdot \text{Et}_2\text{O}$ (10 equiv), CH_3NO_2 (20 equiv), Et_2O , 6 h, rt; v. $\text{Mg}(\text{OMe})_2$, (15 equiv) in $\text{MeOH}/\text{CH}_2\text{Cl}_2$ (1:1), 12 h, rt.

Scheme 24: Orthogonal deprotection of disaccharide 190

Another orthogonal protection scheme was also developed for a disaccharide **190** used in the synthesis of a heparin sulphate component (Scheme 24).¹⁰⁶ The orthogonal protecting groups used were TBDPS, PMB, Lev, Ac and SEM. In this scheme, existing deprotection conditions for each protecting group were adopted but with modifications to ensure the stabilities of other protecting groups and compatibility with existing *O*-sulphate ester groups.

1.5.3 Summary

Following the literature review on the orthogonal protection of monosaccharides and disaccharides, the following points should be taken into consideration when devising a synthetic route for orthogonal protection of 2,1':4,6-di-*O*-isopropylidene sucrose **68**.

- Primary hydroxyl groups are generally protected with bulky protecting groups such as TBDPS, TBS and TDS.
- Protecting groups for secondary hydroxyl groups include Boc, Bn, Bz, PMB, allyl, Fmoc, Lev, Ac and many others are successful in the reported schemes

- To determine which protecting groups to use, screening of a few groups on each of the secondary hydroxyl groups can be done to select an optimum one that offers higher regioselectivity and yield.
- For an orthogonal protection scheme to be determined as successful, orthogonality of the strategy needs to be proven by the demonstrations of selective removal of all the protecting groups used.
- In addition, deprotection conditions should also be compatible with existing substituents on the sugar core, in this case, phenylpropanoid moieties
- Migration or simultaneous cleavage of other protecting groups can also result from using the wrong choice of reagents and reaction conditions during selective deprotection

1.6 SAR studies

Structure-activity relationship (SAR) is an idea established over 150 years ago and suggests a correlation between the chemical structure of a compound and its biological activities.¹¹³ The development of SAR is key to many areas of drug discovery and development such as primary screening and lead optimization. An SAR study of a library of compounds begins by identifying if a relationship exists between the chemical structures and their biological activities. Using this information, structural modifications can be made to the potential lead compounds to optimize their biological activities or properties like toxicity, bioavailability and solubility.¹¹⁴⁻¹¹⁵ Essentially, an understanding of SAR for a library of compounds allows one to identify key functional groups on a compound which are responsible for their biological activities.

SAR studies can be done via two approaches: (i) computational chemistry and molecular modelling (ii) non-computational methods. Previously, Dr Surabhi Devaraj performed molecular modelling studies of PSEs on α -amylase and α -glucosidase using six cinnamoyl and six feruloyl PSEs – 2CiI **41**, 3CiI **42**, 2CiIP **44**, 3CiIP **45**, 3CiS **54**, 4CiS **55**, 2FI **46**, 3FI **47**, 4FI **48**, 2FS **56**, 3FS **57**, 4FS **58** (Figure 13).⁸¹ Based on this study, the importance of having a substituent on the O-3 position and isopropylidene rings on PSEs for α -amylase and α -glucosidase inhibitory activities were established. However, this work was not conclusive as it only screened a limited range of feruloyl and cinnamoyl PSEs. A wider range of PSEs will need to be screened to further the study and establish a more comprehensive SAR of PSEs.

Hence, this work will focus on the synthesis of many structural variants of PSEs using different phenylpropanoid moieties, at different positions – O-3, O-3', O'4' and O-6' and with different substitution patterns. Examples of these PSEs include tetra-substituted caffeoyl, coumaroyl, dimethoxycinnamoyl and trimethoxycinnamoyl PSEs and mono-substituted PSEs with different phenylpropanoid groups at O-3, O-3', O'4' and O-6' position. However, a non-computational approach will be adopted in this work and these newly synthesized PSEs will be screened for their inhibitory

activities using *in vitro* α -amylase and α -glucosidase enzyme assays instead. Through this work, we aim to identify key functional group(s) and position(s) like O-3, O-3', O-4' and O-6' which contribute to their α -amylase and α -glucosidase inhibitory activities. SAR of PSEs and their inhibitory activities against both α -amylase and α -glucosidase can be established with these correlations. Conclusions drawn from SAR study can then serve as guidelines for the synthesis of potential lead compound(s) with optimised activities for further development as AGIs for the treatment of T2D. If *in vitro* results of these lead compound(s) are positive, *in vivo* testing using a mouse model will be considered as well.

1.7 Animal Model

The use of animal models is common in pharmaceutical and biotechnology research. Researchers employ animal models for many purposes such as studying the etiology of diseases and illness, testing and validating novel treatments or surgical procedures and screening of toxicity and safety.¹¹⁶ For the study of diabetes, animal models can be adopted as well. This is due to the common occurrence of spontaneous diabetes in many animals and the availability of a wide range of experimental procedures that can render animals diabetic.¹¹⁷ Even though the disease pathway is not identical to humans, the animal model can still aid in understanding of the complex *in vivo* interactions for diabetes and provide relevant information in situations where the genetics and molecular pathways are similar. Amongst all the animals, rodents like mice are most commonly used in research due to ease of manipulation and a relatively lower cost than other animals.¹¹⁸ There are many different types of mouse model available for the study of diabetes (both T1D and T2D). Examples of mouse models for T1D include a low dose streptozotocin (STZ) mouse model, a high dose STZ mouse model and a non obese diabetic (NOD) model, while examples of mouse models for T2D include a New Zealand obese (NZO) mouse model and a high fat diet induced diabetic mouse model.¹¹⁹ In this research work, a T2D mice model will be employed to test the *in vivo* effects of lead PSEs as potential AGIs in treatment of T2D. This is discussed in Chapter 6.

Nevertheless, experimentation on animals remains controversial. Many people still regard animal experimentation as an unjustified way to obtain knowledge and that it is ethically inappropriate to subject animals to procedures which can cause any degree of discomfort since they are neither able to provide informed consent nor benefit in any way from the studies.¹¹⁸ In addition, animal experimentation requires skilled manpower and incurs high cost. As such, alternative methods are explored and examples include computer models, bioinformatics tools, *in vitro* cell cultures, enzymatic screens and mini-organs on a chip.¹¹⁶ However, these methods cannot replace animal experimentation completely as they cannot mimic the fully complex *in vivo* environment yet. As such, animal work remained indispensable in research work. However, to minimize controversial issues, rules and regulations were set in place. Currently, the use of animal in experimentation is governed by three Rs:

refinement, replacement and reduction.¹¹⁶ Refinement refers to the use of methods that minimise pain and discomfort in animals which ensure humane handling and care. Replacement refers to the use of alternative methods, as mentioned above, whenever possible. Finally, reduction means minimizing the numbers of animals used in research without compromising the quality of data. Prior to the experiment, all protocols will need approval from Institutional Animal Care and Use Committee (IACUC) to ensure compliance with the mentioned guidelines to avoid possible ethical issues and misappropriate experimentation in the research facility. Prior to testing in the research facility, procedures for the mice experimentation in this work were subjected to IACUC approval.

1.8 Research motivation

Diabetes is a chronic illness that affects 425 million people worldwide as of 2017, and amongst these cases, more than 90% are T2D.² These rapidly rising numbers and high global prevalence comes at a huge socioeconomic costs in terms of low productivity, increased risks of associated health complications, reduced quality of life and escalating medical bills.

To manage T2D and prevent associated health complications, a combination of lifestyle modifications and drug therapy is required. Though current treatment options (refer to 1.3.2 & 1.3.3) aids in improving hyperglycaemia, they are commonly plagued with side effects such as gastrointestinal discomfort, kidney failure and liver damage. Not to mention, increased resistance to current treatment options can also limit their effectiveness in the treatment of T2D. There is an urgent demand to explore new treatment options that offer either improved/comparable efficacy, elimination of side effects or both. Amongst all T2D treatment options, AGIs have garnered increased attention as this class of drug delays the absorption of sugar and carbohydrate after a meal, effectively managing postprandial blood glucose.^{14, 39} Effective management of postprandial blood glucose is good for T2D management since it was reported to be a stronger independent predictor of T2D and its associated health complications as compared to fasting blood glucose and HbA1c. In addition, monitoring and management of postprandial blood glucose together with fasting blood glucose and HbA1c will give optimal glycaemic control.

The most commonly used AGI now is Acarbose and its associated side effects include abdominal pain, flatulence and diarrhoea.¹⁴ These side effects are likely a result of its strong inhibition of α -amylase, leading to accumulation of undigested polysaccharides and oligosaccharides in the gut.⁵⁷ These accumulated carbohydrates will subsequently be broken down by intestinal microflora and this process produces gas which leads to the gastrointestinal discomforts as mentioned. To overcome this issue, this project aims to develop a PSE that exhibits high α -glucosidase inhibition but minimal or no α -amylase inhibition. To derive this lead compound for further development, an SAR study of the PSEs with

variant structures will need to be established first to identify key functional group(s) and position(s) that contribute to their inhibitory activities. Conclusions drawn from the SAR study will then serve as guidelines for synthesis of potential lead compound(s) that only inhibit α -glucosidase. Subsequently, the lead compound will be subjected to *in vitro* testing using enzyme assays and *in vivo* testing using a diabetic mouse model to determine its efficacy and side effects as compared to standard drug, Acarbose.

1.9 Research objectives

The overall objective of this thesis was to develop PSEs as potent inhibitors of α -glucosidase and as weak (or even non) inhibitors of α -amylase. We aim:

1. To synthesize tetra-substituted PSEs and examine their *in vitro* α -amylase and α -glucosidase inhibitory activities to establish SARs.
2. To develop strategy for the orthogonal protection of 2,1':4,6-di-*O*-isopropylidene sucrose **68** to achieve regioselective acylation in the synthesis of PSEs
3. To synthesize a series of mono-substituted PSEs having substituents at different positions (O-3, O-3', O-4', O-6') and examine their *in vitro* α -amylase and α -glucosidase inhibitory activities to establish SARs.
4. To develop PSE(s) that only inhibit α -glucosidase
5. To test the efficacy of lead PSE(s) using *in vitro* enzyme assays in comparison to the standard drug, Acarbose
6. To test the efficacy of lead PSE(s) as AGI in a diabetic mouse model in comparison to the standard drug, Acarbose

2 Tetra-substituted PSEs

2.1 Introduction

Previously, a range of cinnamoyl, feruloyl and sinapoyl PSEs were screened for their α -amylase and α -glucosidase inhibitory activities.⁸¹ Based on the results obtained, several conclusions to the SAR of PSEs were made. However, the conclusions drawn from this preliminary screening are not comprehensive since the screenings were limited to only cinnamoyl, sinapoyl and feruloyl PSEs. To further the SAR study, caffeoyl, coumaroyl, dimethoxycinnamoyl and trimethoxycinnamoyl PSEs will be synthesized and their inhibition activities screened. Since tetra-substituted PSEs showed the highest α -glucosidase or α -amylase inhibitory activities as compared to PSEs with lesser degrees of substitution (refer to 1.4.1),⁸¹ this work focuses on the synthesis of tetra-substituted caffeoyl, coumaroyl, dimethoxycinnamoyl and trimethoxycinnamoyl PSEs. Prior to that, inconclusive observations from previously screened PSEs are discussed. The structures of previously screened tetra-substituted PSEs are represented in Figure 18 and the values obtained from these PSEs are shown in Figure 19.

Figure 18: Structured of previously screened tetra-substituted PSEs

Inhibitory activities of tetra-substituted PSEs

Note: All testing was done at 50 μ g/ml, except 4CiI and 4CiS (25 μ g/ml)

Figure 19: % α -glucosidase and α -amylase inhibition of tetra-substituted PSEs

From Figure 19, tetra-substituted PSEs with an additional methoxy group on aromatic rings such as 4SI **52** and 4SS **60** showed lower inhibitory activities against α -glucosidase and α -amylase as compared to 4FI **48** and 4FS **58**. This implied that the presence of an additional methoxy group may reduce inhibitory activities of PSEs against both α -glucosidase and α -amylase.

In addition, 4SI **52** and 4SS **60** were poorer inhibitors of α -glucosidase and α -amylase than 4CiI **43** and 4CiS **55** despite being tested at higher concentrations. However, since sinapoyl groups contain both hydroxyl and methoxy groups on its aromatic rings, it is hard to determine which group may have contributed to the decrease in inhibitory activities.

4FI **48** was a poorer inhibitor of α -amylase than 4FS **58** (Figure 19), indicating that the presence of isopropylidene rings impede the inhibitory activity against α -amylase. However, comparison between 4CiI **43** and 4CiS **55** (Figure 19) showed no appreciable difference with or without the presence of isopropylidene rings. Hence, the role of isopropylidene rings on α -amylase inhibition is unclear since the inhibitory activities of 4FI **48** & 4FS **58** and 4CiI **43** and 4CiS **55** contradicted each other.

Comparison between two pairs of PSEs – 4FI **48** & 4FS **58** (Figure 19) and 4CiI **43** & 4CiS **55** (Figure 19) showed no appreciable difference in their inhibitory activities against α -glucosidase. These implied

that the presence of isopropylidene rings do not contribute to their inhibitory activities against α -glucosidase. However, comparison between 4SI **52** & 4SS **60** (Figure 19) contradicted with the observation. 4SS **60** was a poorer inhibitor of α -glucosidase than 4SI **52**, suggesting that the presence of isopropylidene rings contributed to their inhibitory activities against α -glucosidase. Likewise, the role of isopropylidene rings on α -glucosidase inhibition needs to be clarified as well.

To summarize, the following points need to be determined to achieve a more comprehensive understanding:

- The effect and number of methoxy group(s) on the aromatic rings of tetra-substituted PSEs on the inhibition of both α -amylase and α -glucosidase
- The effect and number of hydroxyl group(s) their number on the aromatic rings of tetra-substituted PSEs on the inhibition of both α -amylase and α -glucosidase
- The effect of isopropylidene rings on inhibition of α -amylase and α -glucosidase

Hence to further the SAR study, tetra-substituted PSEs with caffeoyl, coumaroyl, dimethoxycinnamoyl and trimethoxycinnamoyl substituents will be synthesized and tested for their α -glucosidase or α -amylase inhibitory activities. The synthesis of these tetra-substituted PSEs will be discussed in the following section.

Similarly, the newly synthesized tetra-substituted PSEs will follow the same naming style which has been developed previously. The compound name comprises of three fields:

- The first field is a number representing the degree of substitution, for instance '4' for tetra-substituted PSE
- The second field represents the type of substituent groups, for instance 'Co' for coumaric acid, 'Ca' for caffeic acid, 'D' for dimethoxycinnamic acid and 'T' for trimethoxycinnamic acid
- The third field represents the presence or absence of diisopropylidene ring on sucrose. For instance, 'I' denotes the presence of diisopropylidene rings and 'S' denotes the absence of diisopropylidene rings

2.2 The synthesis of tetra-substituted PSEs

The synthesis of tetra-substituted PSEs with caffeoyl, coumaroyl, dimethoxycinnamoyl and trimethoxycinnamoyl groups is shown in the following subsections:

2.2.1 The synthesis of 2,1':4,6-di-*O*-isopropylidene sucrose **68**

Synthesis of 2,1':4,6-di-*O*-isopropylidene sucrose **68** was first achieved using the same procedure reported by Dr Parthasarathi Panda⁵⁸ with slight modifications (Scheme 25). Sucrose **67** was treated with 4.5 equivalents of 2-methoxypropene and *p*-TsOH in dry dimethylformamide (DMF). Thin layer chromatography (TLC) analysis of the reaction mixture showed the presence of two spots, corresponding to the product and the starting material sucrose. Upon purification via column chromatography, a white solid was obtained in 65% yield.

Reagents and conditions: 2-methoxypropene (4.5 equiv), *p*-TsOH (0.1 equiv), dry DMF, rt, 1 week

Scheme 25: Synthesis of 2,1':4,6-di-*O*-isopropylidene sucrose **68**

¹H NMR analysis of the spectrum of the product revealed the characteristic peaks from δ 1.49 to 1.54 ppm and δ 6.26 ppm, which corresponded to the two isopropylidene rings and the anomeric proton at C-1 respectively. Comparison with reported values in literature⁵⁸ also confirmed its identity to be 2,1':4,6-di-*O*-isopropylidene sucrose **68**. This product was then used as the starting material for the synthesis of tetra-substituted PSEs.

2.2.2 The synthesis of tetra-substituted caffeoyl PSEs

2.2.2.1 The synthesis of protected caffeic acid

Prior to the synthesis of tetra-substituted caffeoyl PSEs, there is a need to protect the two free hydroxyl groups on the aromatic ring of caffeic acid **37**. This is to avoid potential reaction that may produce undesired acylation by-products. The free hydroxyl groups on caffeic acid were protected using three different temporary protecting groups – Ac, allyl and TBS groups.

For protection with Ac groups, caffeic acid **37** was reacted with 2.5 equivalents of acetic anhydride in 4N NaOH at room temperature for 12 hours (Scheme 26). Upon completion, the mixture was adjusted to pH 2 using HCl. After filtration, the residue was recrystallised using hot EtOH. Finally, a white solid was obtained in 86% yield.

Reagents and conditions: i. Acetic anhydride (2.5 equiv), 4N NaOH, rt, 12 h then adjust to pH 2 using HCl

Scheme 26: Synthesis of 3,4-diacetoxycinnamic acid **196**

^1H NMR of this product revealed characteristic Ac proton peaks at δ 2.14 and 2.15 ppm. Integration of these peaks showed six protons which indicated the presence of two Ac groups. Other than the Ac proton peaks, *trans*-alkenyl and aromatic proton peaks were also observed from δ 6.31 to 7.53 ppm. Integration of these peaks showed five protons, which confirmed the presence of the diacetoxycinnamoyl group. Hence, the product was confirmed as 3,4-diacetoxycinnamic (OAc-caff) acid **196**. Comparison with reported values in the literature further confirmed the product to be OAc-caff acid **196**.¹²⁰

For protection with allyl groups, caffeic acid **37** was reacted with 2.5 equivalents of allyl bromide with K_2CO_3 in acetone under reflux for 12 hours (Scheme 27). Likewise, reaction was adjusted to pH 2 using HCl upon completion. After filtration, the residue was recrystallised using hot EtOH. A yellowish solid was obtained in 80% yield.

Reagents and conditions: i. Allyl bromide (2.5 equiv), K_2CO_3 (5 equiv), acetone, reflux, 12 h then adjust to pH 2 using HCl

Scheme 27: Synthesis of 3,4-bis(allyloxy)cinnamic acid **197**

Its structure was confirmed by ^1H NMR. Characteristic signals of the allyl group could be seen from δ 4.63 to 6.07 ppm. Integration of these peaks showed a total of eight protons, corresponding to the presence of two allyl groups. Other than that, integration of the *trans*-alkenyl and aromatic proton peaks from δ 6.10 to 7.71 ppm showed five protons. This confirmed the presence of the diallyloxycinnamoyl moiety. Based on these data, the product was confirmed as 3,4-bis(allyloxy)cinnamic (OAllyl-caff) acid **197**. Comparison with reported values in literature further confirmed the identity of the product to be OAllyl-caff acid **197**.¹²¹

Lastly, for protection with TBS groups, caffeic acid **37** was reacted with 2.5 equivalents of *tert*-butyldimethylsilyl chloride (TBSCl) with *N,N*-diisopropylethylamine (DIPEA) in CH_2Cl_2 at room

temperature for 12 hours (Scheme 28). Upon completion, the reaction was diluted with EtOAc, washed twice with 1N HCl and dried with anhydrous MgSO₄. Subsequently, the crude product obtained was dissolved in THF/H₂O (9:1) and K₂CO₃ was added to the solution. After stirring for another 4 hours, the mixture was diluted with EtOAc and washed twice again with 1N HCl before drying with anhydrous MgSO₄. EtOAc was removed and hexane was added to the residue. The mixture was shaken prior to filtration. Finally, a white solid was collected in 91% yield.

Reagents and conditions: i. (a) TBSCl (2.5 equiv), DIPEA (5.0 equiv), CH₂Cl₂, rt, 12 h, (b) K₂CO₃ (0.1 equiv), 9:1 THF/H₂O, rt, 4h

Scheme 28: Synthesis of 3,4-di-*tert*-butyldimethylsiloxycinnamic acid 198

¹H NMR of the product revealed distinctive peaks of TBS groups at δ 0.13 and 0.91 ppm. Integration of these two peaks showed 12 and 18 protons respectively, corresponding to the presence of two TBS groups. Other than that, integration of the *trans*-alkenyl and aromatic proton peaks from δ 6.00 to 7.48 ppm showed five protons. This confirmed the presence of the di-*tert*-butyldimethylsiloxycinnamoyl moiety. Hence, the product was confirmed as 3,4-di-*tert*-butyldimethylsiloxycinnamic (OTBS-caff) acid **198**. Comparison with reported values in the literature¹²² also confirmed its structure. With protected caffeic acids, the synthesis of 4CaI **201** and 4CaS **202** was attempted next.

2.2.2.2 The synthesis of 4CaI **201** and 4CaS **202**

The synthesis of 4CaI **201** was first attempted by reacting 2,1':4,6-di-*O*-isopropylidene sucrose **68** with 6.5 equivalents of OAc-caff acid **196** in the presence of DCC and DMAP in CH₂Cl₂ at room temperature for two days. After which, the reaction mixture was evaporated, and the resultant solid was dissolved in cold diethyl ether. The mixture was then filtered and subjected to column chromatography. However, ¹H NMR analysis of the products obtained after purification revealed the formation of undesired products instead.

Subsequent attempts to synthesize 4CaI **201** using OAc-caff acid chloride (made from OAc-caff **196** using sulfonyl chloride) in pyridine and OAc-caff **196** with oxalyl chloride, pyridine in CH₂Cl₂, also failed to give the desired product. Due to the numerous failed attempts in the synthesis of 4CaI **201** using with OAc-caff **196**, the protecting groups on caffeic acid were changed. Hence, Oallyl-caff acid **197** was used instead.

PSEs with different numbers of allyl groups attached on the aromatic rings, suggesting different degrees of removal. Furthermore, the yield of products obtained was extremely low due to numerous attempts of separation. In addition, characteristic peaks from the allyl groups overlapped with the proton peaks on sucrose core as seen from the spectrum of tetra-substituted PSE **199**. These overlapping peaks made it challenging to analyse the chemical shifts of protons on the sucrose core. Due to these difficulties, the protecting groups on caffeic acid were changed again. OTBS-caff acid **198** was used instead.

To synthesize 4CaI **201**, 2,1':4,6-di-*O*-isopropylidene sucrose **68** was reacted with 6.5 equivalents of OTBA-caff acid **198** in the presence of DCC and DMAP in CH₂Cl₂ at room temperature for two days (Scheme 29). Upon completion, the mixture was evaporated, and the crude product obtained was dissolved in cold diethyl ether. After filtration and column chromatography, a yellowish solid was obtained in 76% yield. The structure of the product obtained was confirmed by ¹H NMR, ¹³C NMR and high-resolution electrospray ionisation mass spectrometry (HR-ESI-MS).

Characteristic protons peaks from δ 0.00 to 0.97 ppm indicated the presence of TBS groups. Integration of these peaks showed 48 and 72 protons respectively, which implied the presence of eight TBS groups. With two TBS groups on each caffeoyl substituent, the presence of eight TBS groups would denote the presence of four caffeoyl groups. Moreover, *trans*-alkenyl and aromatic protons corresponding to the OTBS-caffeoyl groups from δ 6.19 to 7.65 ppm were observed. Integration of these peaks showed 20 protons, which confirmed the presence of four OTBS-caffeoyl groups as well.

In addition, ¹³C NMR showed new signals from δ -5.83 to 0.00 ppm and δ 164.1 to 166.0 ppm, corresponding to the presence of silyl carbon and ester carbon respectively. These signals confirmed the presence of OTBS-caffeoyl groups. Other than that, the HR-ESI-MS of the product showed a molecular ion *m/z* value of 2005.9867, corresponding to [M+Na]⁺. This supported the molecular formula C₁₀₂H₁₆₆O₂₃Si₈Na (*m/z* calcd. for C₁₀₂H₁₆₆O₂₃Si₈Na: 2005.9872). All these data confirmed the product as tetra-substituted PSE **200**.

With tetra-substituted PSE **200** synthesized successfully, the next step would be to remove all TBS groups to obtain 4CaI **201**. Therefore, tetra-substituted PSE **200** was treated with 3.0 equivalents of 1.56 M 3HF·NEt₃ and 2.0 equivalents of NEt₃ in pyridine at room temperature.¹²⁴⁻¹²⁵ To ensure a clean removal of all eight TBS groups, this reaction was left to stir for 48 hours before drying off pyridine. Subsequently, the crude mixture was purified using column chromatography. In the end, a yellow solid was obtained in 84% yield.

¹H NMR of the product revealed the absence of characteristic TBS peaks at δ 0.00 and 1.00 ppm. This indicated the successful removal of all eight TBS groups. In addition, the integration of characteristic protons peaks of isopropylidene rings from δ 1.19 to 1.48 ppm showed 12 protons, confirming the presence of the two isopropylidene rings. Furthermore, the integration of the *trans*-alkenyl and aromatic

proton peaks from δ 6.25 to 7.83 ppm showed 20 protons which confirmed the presence of four caffeoyl substituents. These implied that both caffeoyl substituents and isopropylidene rings were not affected during the deprotection reaction. Comparison of the ^1H NMR spectrum to that of tetra-substituted PSE **200** also indicated that no transesterification or migration has occurred.

^{13}C NMR of the product showed the absence of characteristic silyl carbon peaks at around δ -5.00 ppm, which corresponded to the carbons from TBS groups. This implied the successful removal of all TBS groups. Furthermore, the presence of four ester carbon signals from δ 166.5 to 167.2 ppm confirmed that the caffeoyl substituents were not affected during the deprotection reaction. Finally, the HR-ESI-MS of the product also showed a molecular ion m/z value of 1071.3175 which corresponded to $[\text{M}+\text{H}]^+$. This supported the molecular formula $\text{C}_{54}\text{H}_{55}\text{O}_{23}$ (m/z calcd. for $\text{C}_{54}\text{H}_{55}\text{O}_{23}$: 1071.3134). Based on these data, the product was confirmed as 4CaI **201**. Furthermore, comparison with reported literature values of similar compound such as 4CiI **43** and 4FI **48** confirmed the formation of 4CaI **201**.¹²⁶

Unlike OAllyl-caffeoyl, OTBS-caffeoyl does not have any overlapping peaks with the protons on the sucrose core. In comparison, this makes the analysis easier. Furthermore, a clean removal of TBS groups can also be achieved in high yield without involving tedious purification steps. Therefore, OTBS-caff acid **198** is certainly a better alternative to OAllyl-caff acid **197**.

The synthesis of 4CaS **202** was then attempted by treating 4CaI **201** with 0.2 equivalent of $\text{Yb}(\text{OTf})_3 \cdot \text{H}_2\text{O}$ in MeCN at room temperature for 12 hours (Scheme 30). To avoid removal of phenylpropanoid groups during reaction, $\text{Yb}(\text{OTf})_3 \cdot \text{H}_2\text{O}$ is used to remove the isopropylidene rings since it was reported to have shown high chemoselectivity in the presence of other acid sensitive functional groups.¹²⁷

Upon completion, the reaction was diluted with EtOAc. Subsequently, the mixture was washed twice with water and dried with over anhydrous MgSO_4 . In the end, a yellow gel was attained in 78% yield. The structure of product was confirmed by ^1H NMR, ^{13}C NMR and HR-ESI-MS.

Reagents and conditions: i. Yb(OTf)₃·H₂O (0.2 equiv), MeCN, rt, 12 h

Scheme 30: Synthesis of 4CaS 202

Its ¹H NMR spectrum showed the absence of distinctive isopropylidene peaks from δ 1.19 to 1.48 ppm. In addition, the proton peak which corresponded to the anomeric proton at C-1 was shifted to around δ 4.57 ppm as compared to that in 4CaI **201** (δ 6.00 ppm). These confirmed the successful removal of both isopropylidene rings. Furthermore, the integration of *trans*-alkenyl and aromatic proton peaks from δ 6.29 to 8.53 ppm showed 20 protons, which was the same as that of 4CaI **201**. This confirmed the presence of four caffeoyl substituents, indicating that these four caffeoyl substituents were not affected during the deprotection reaction. Hence, it was concluded that the removal of both isopropylidene rings was achieved successfully without affecting the caffeoyl substituents. The comparison of the ¹H NMR spectrum to that of 4CaI **201** also indicated that no migration has occurred during the deprotection reaction.

Other than that, the ¹³C NMR spectrum of the product indicated the absence of signals from δ 19.2 to 36.2 ppm (as seen in the spectrum of 4CaI **201**) which corresponded to the carbons of the isopropylidene rings. This implied the successful removal of the isopropylidene rings as well. The spectrum also revealed signals at δ 166.7, 166.8, 167.1 and 171.0 ppm, which corresponded to ester carbons. The presence of these signals confirmed the presence of caffeoyl substituents and that these substituents were not affected during the deprotection reaction.

Aside from the NMR spectra, the HR-MS-ESI of the product showed a molecular ion *m/z* value of 1013.2330 corresponding to [M+Na]⁺, which supported the molecular formula of C₄₈H₄₆O₂₃Na (*m/z* calcd. for C₄₈H₄₆O₂₃Na: 1013.2328). Based on these data, the product was identified as 4CaS **202**. Comparison with reported literature values of similar compounds such as 4CiS **55** and 4FS **58** further confirmed the formation of 4CaS **202**.¹²⁶

2.2.3 The synthesis of tetra-substituted coumaroyl PSEs

The tetra-substituted coumaroyl PSEs 4CoI **205** and 4CoS **206** were previously synthesized by Dr Parthasarathi Panda, a former researcher in our research lab. However, these compounds were not included in the previous screenings of PSEs. To gain a comprehensive understanding for SAR of tetra-substituted PSEs, these two compounds were synthesized again for screening. Instead of adopting the experimental procedure demonstrated by Dr Parthasarathi Panda which involved the use of coumaroyl chloride, Steglich esterification was employed. Details of the synthesis is discussed in the following subsection.

2.2.3.1 The synthesis of silylated coumaric acid

Free hydroxy group on coumaric acid **33** needs to be protected before the acylation reaction to avoid formation of undesired by-product(s) during the Steglich reactions. Since the TBS protecting group was easily and efficiently removed (refer to 2.2.2.2), it was chosen as the temporary protecting group for coumaric acid as well.

The synthesis of 4-*tert*-butyldimethylsilylcinnamic (OTBS-coum) acid **203** was attempted by reacting coumaric acid **33** with 1.5 equivalents of TBSCl and N,N-diisopropylethylamine (DIPEA) in CH₂Cl₂ at room temperature (Scheme 31). After 12 hours of stirring, reaction mixture was diluted in EtOAc, washed twice with 1N HCl and dried with anhydrous MgSO₄. After removal of EtOAc, the crude product was dissolved in 9:1 THF/H₂O before adding K₂CO₃. The reaction mixture was left to stir for another four hours before diluting with EtOAc, washed twice again with 1N HCl before evaporating off the solvent. Hexane was added to the crude mixture and shaken prior to filtration. Finally, a white solid was obtained in 86% yield.

Reagents and conditions: i. (a) TBSCl (1.5 equiv), DIPEA (3 equiv), CH₂Cl₂, rt, 12 h (b) K₂CO₃ (0.1 equiv), 9:1 THF/H₂O

Scheme 31: Synthesis of 4-*tert*-butyldimethylsilylcinnamic acid **203**

¹H NMR of this product revealed characteristic peaks of TBS group at δ 0.20 and 1.97 ppm. Integration of these peaks showed six and nine protons, corresponding to the presence of one TBS group. In addition, integration of the *trans*-alkenyl and aromatic proton peaks from δ 6.27 to 7.74 ppm showed six protons, which confirmed the presence of the *tert*-butyldimethylsilyloxycinnamoyl moiety. Hence, the product was confirmed as OTBS-coum acid **203**. Comparison with reported values in the literature confirmed the product as OTBS-coum acid **203**.¹²⁸

2.2.3.2 The synthesis of 4CoI **205** and 4CoS **206**

Reagents and conditions: i. acetylated coumaric acid **203** (6.5 equiv), DCC (6.5 equiv), DMAP (0.1 equiv), CH₂Cl₂, rt, 48 h; ii. 1.56 M 3HF·NEt₃ in pyridine (3.0 equiv), NEt₃ (2.0 equiv), pyridine, 12 h

Scheme 32: Synthesis of 4CoI **205**

To synthesize 4CoI **205**, 2,1':4,6-di-*O*-isopropylidene sucrose **68** was reacted with 6.5 equivalents of OTBS-coumaric acid **203** in the presence of DCC and DMAP in CH₂Cl₂ at room temperature for two days (Scheme 32). Upon completion, the solvent CH₂Cl₂ was removed and the crude mixture obtained was dissolved in cold diethyl ether. After that, filtration was done, and the filtrate collected was then purified using column chromatography. A white solid was obtained in 75% yield.

¹H NMR of this product revealed distinctive proton peaks at δ 0.00 and 0.77 ppm, which corresponded to the TBS groups. Integration of these peaks showed 24 and 36 protons respectively, indicating the presence of four TBS groups. With one TBS group on each coumaroyl moiety, the presence of four TBS groups confirmed the presence of four coumaroyl substituents. In addition, *trans*-alkenyl and aromatic proton peaks corresponding to that of the coumaroyl groups can also be observed from δ 6.05 to 7.63 ppm. Integration of these peaks showed 24 protons, which confirmed the presence of four coumaroyl substituents as well.

In addition, its ¹³C NMR spectrum revealed new carbon signals at δ 165.5, 165.7, 166.0 and 166.5 ppm. These signals corresponded to the ester carbons, which hence implied the presence of OTBS-coumaroyl

substituents. New signals were also observed at δ -5.00 ppm. These signals corresponded to the silyl carbons, hence confirming the presence of TBS groups as well.

Furthermore, the HR-ESI-MS of the product showed a molecular ion m/z value of 1464.6785, which corresponded to $[M+H]^+$. This supported the molecular formula of $C_{78}H_{111}O_{19}Si_4$ (m/z calcd. for $C_{78}H_{111}O_{19}Si_4$: 1463.6752). Based on the data, the product was identified as tetra-substituted PSE **204**. Comparison of 1H NMR with the reported values of a similar compound synthesized by Dr Parthasarathi Panda¹²⁶ also confirmed the synthesis of tetra-substituted PSE **204**.

Removal of TBS groups on tetra-substituted PSE **204** was attempted by treating tetra-substituted PSE **204** with 3.0 equivalents of 1.56 M $3HF \cdot NEt_3$ and 2.0 equivalents of NEt_3 in pyridine at room temperature for 12 hours (Scheme 32). Upon completion, pyridine was removed. The product obtained was then purified via column chromatography. A white solid was obtained in 82% yield.

1H NMR of the product confirmed the successful removal of TBS groups since no distinctive proton peaks corresponding to TBS groups were observed at δ 0.00 and 1.00 ppm. Characteristic proton peaks corresponding to the isopropylidene rings were still observed in the spectrum at δ 1.14, 1.19, 1.42 and 1.44 ppm. Integration of these peaks showed 12 protons which confirmed the presence of two isopropylidene rings. These implied that both isopropylidene rings were not affected during removal of TBS groups.

Furthermore, integration of the *trans*-alkenyl and aromatic proton peaks from δ 6.35 to 7.87 ppm showed 24 protons, which was the same as compared to that in tetra-substituted PSE **204**. This indicated that there were still four coumaroyl substituents attached to the sucrose core. No migration was observed as well since the proton shift remained the same as compared to that in tetra-substituted PSE **204**. These spectroscopic data confirmed the product to be 4CoI **205**. Comparison with reported values by Dr Parthasarathi Panda¹²⁶ further confirmed the formation of 4CoI **205**.

The isopropylidene rings on 4CoI **205** were then removed by treating 4CoI **205** with 0.2 equivalent Yb(OTf)₃·H₂O in MeCN at room temperature for 12 hours (Scheme 33). Upon completion, MeCN was removed and the crude product was purified via column chromatography. A white solid was obtained in 78% yield.

Reagents and conditions: i. Yb(OTf)₃·H₂O (0.2 equiv), MeCN, rt, 12 h

Scheme 33: Synthesis of 4CoS **206**

¹H NMR of the product confirmed the absence of distinctive proton peaks between δ 1.10 and 1.50 ppm. The absence of these peaks which corresponded to the isopropylidene rings, indicated the successful removal of both isopropylidene rings. In addition, integration of *trans*-alkenyl and aromatic proton peaks from δ 6.10 to 7.81 ppm showed 24 protons, which was the same as that in 4CoI **205**. This confirmed that all four coumaroyl groups were not affected during the deprotection reaction. In addition, the comparison of ¹H NMR spectrum with that of 4CoI **205** showed no migration of the four coumaroyl groups. Hence, the structure of the product was confirmed as 4CoS **206**. Comparison with reported values from Dr Parthasarathi Panda¹²⁶ also confirmed its identity.

2.2.4 The synthesis of tetra-substituted dimethoxycinnamoyl PSEs

2.2.4.1 The synthesis of 3,4-dimethoxycinnamic acid

3,4-dimethoxycinnamic (diOMe) acid **38** can be synthesized by reacting ferulic acid **39** with 2.5 equivalents of dimethyl sulphate in 4N NaOH at room temperature (Scheme 34). After stirring for 12 hours, pH of the reaction was adjusted to 2 using HCl and the mixture was filtered. A pale-yellow solid was collected in 83% yield.

Reagents and conditions: i. $(\text{CH}_3)_2\text{SO}_4$ (1.5 equiv), 4N NaOH, rt, 12 h then adjust to pH 2 using HCl

Scheme 34: Synthesis of dimethoxycinnamic acid **38**

^1H NMR of the product revealed the characteristic singlet peak at δ 3.86 ppm. Integration of this peak showed 6 protons which corresponded to two methoxy ($-\text{OCH}_3$) groups. In addition, integration of the *trans*-alkenyl and aromatic proton peaks from δ 6.28 to 7.74 ppm showed five protons, confirming the presence of the dimethoxycinnamoyl moiety. Comparison with reported literature values also confirmed the product as diOMe acid **38**.¹²⁹

2.2.4.2 The synthesis of 4DI **207** and 4DS **208**

Likewise, synthesis of 4DI **207** was attempted by reacting 2,1':4,6-di-*O*-isopropylidene sucrose **68** with 6.5 equivalents of diOMe acid **38** in the presence of DCC and DMAP in CH_2Cl_2 at room temperature for two days (Scheme 35). Upon completion, CH_2Cl_2 was removed and the product obtained was dissolved in cold diethyl ether. After filtration, the filtrate was concentrated and purified by column chromatography. A white solid was obtained in 78% yield.

Reagents and conditions: i. diOMe acid **38** (6.5 equiv), DCC (6.5 equiv), DMAP (0.1 equiv), CH₂Cl₂, rt, 48 h;

Scheme 35: Synthesis of 4DI **207**

The structure of the product obtained was confirmed by ¹H NMR, ¹³C NMR and HR-ESI-MS. Its ¹H NMR spectrum showed characteristic methoxy peaks from δ 3.92 to 3.98 ppm. Integration of these peaks showed 24 protons, indicating the presence of eight methoxy groups. With two methoxy groups on each dimethoxycinnamoyl group, the presence of eight methoxy groups would confirm the presence of four dimethoxycinnamoyl moieties. Furthermore, the ¹H NMR spectrum showed characteristic *trans*-alkenyl and aromatic protons from δ 6.28 to 7.91 ppm, implying the presence of the dimethoxycinnamoyl moieties. Integration of these peaks showed 20 protons, which confirmed the presence of four dimethoxycinnamoyl groups.

The ¹³C NMR spectrum of the product also revealed new signals from δ 166.1 to 169.0 ppm. These signals which corresponded to the ester carbons confirmed the presence of dimethoxycinnamoyl substituents. Other than that, the HR-ESI-MS of the product showed a molecular ion *m/z* value of 1183.4359 corresponding to [M+H]⁺. This supported the molecular formula of C₆₂H₇₁O₂₃ (*m/z* calcd. for C₆₂H₇₁O₂₃: 1183.4386). From these data, the product was confirmed as 4DI **207**. Comparison with reported values for similar compounds as such 4CiI **43**, 4FI **48** and 4SI **52** also confirmed the structure of the product to be 4DI **207**.^{81, 126}

The synthesis of 4DS **208** was attempted by treating 4DI **207** with 0.2 equivalent of Yb(OTf)₃·H₂O in MeCN at room temperature for 12 hours (Scheme 36). Upon completion, MeCN was removed and the crude product was purified by column chromatography. Finally, a white solid was obtained in 71% yield.

Reagents and conditions: Yb(OTf)₃·H₂O (0.2 equiv), MeCN, rt, 12 h

Scheme 36: Synthesis of 4DS 208

¹H NMR spectrum of the product revealed the absence of distinctive proton peaks from δ 1.24 to 1.50 ppm, which corresponded to the isopropylidene rings. This proved the successful removal of both isopropylidene rings. Integration of the *trans*-alkenyl and aromatic proton peaks from δ 6.27 to 7.86 ppm showed 20 protons, which was the same as that of 4DI 207. This implied that none of the dimethoxycinnamoyl groups was affected during the deprotection reaction. Comparison of the ¹H NMR spectrum with that of 4CaI 201 showed that no migration or transesterification has occurred during the deprotection reaction as well.

¹³C NMR spectrum of the product also showed the absence of signals at δ 19.9, 24.6, 27.8 and 30.9 ppm (as seen in the analysis of 4DI 207), which corresponded to the carbons of isopropylidene rings. This further confirmed the successful removal of both isopropylidene rings. The presence of signals from δ 166.1 to 167.4 ppm, which corresponded to the ester carbons, also confirmed that the dimethoxycinnamoyl substituents were not removed during the deprotection reaction. Furthermore, the HR-MS-ESI of the product showed a molecular ion m/z value of 1125.3562, corresponding to [M+Na]⁺. This supported the molecular formula of C₅₆H₆₂O₂₃Na (m/z calcd. for C₅₆H₆₂O₂₃Na: 1125.3580). From these data, the product identified as 4DS 208. Comparison with reported values for similar compounds such as 4CiS 55, 4FS 58 and 4SS 60 further confirmed the formation of 4DS 208.^{81, 126}

2.2.5 The synthesis of tetra-substituted trimethoxycinnamoyl PSEs

2.2.5.1 The synthesis of 3,4,5-trimethoxycinnamic acid

Synthesis of 3, 4, 5-trimethoxycinnamic (triOMe) acid **210** was attempted by reacting sinapic acid **209** with 1.5 equivalents of dimethyl sulphate in 4N NaOH at room temperature (Scheme 37). After 12 hours, pH of the mixture was adjusted to 2 using HCl. Finally, the mixture was filtered and pale-yellow solid obtained was obtained in 80% yield.

Reagents and conditions: i. 4N NaOH, (CH₃)₂SO₄, rt, 12 h then adjust to pH 2 using HCl

Scheme 37: Synthesis of 3, 4, 5-trimethoxycinnamic acid 210

The identity of the product was determined by ¹H NMR. Its spectrum showed distinctive methoxy peak at δ 3.83 pm. Integration of this singlet showed 9 protons which corresponded to presence of three methoxy groups. Other than that, integration of the *trans*-alkenyl and aromatic proton peaks from δ 6.12 to 7.71 ppm showed four protons. This confirmed the presence of the trimethoxycinnamoyl moiety. Hence, the product was identified as triOMe acid **210**. Comparison with the reported values in the literature further confirmed the structure to be triOMe acid **210**.¹³⁰

2.2.5.2 The synthesis of 4TI **211** and 4TS **212**

The synthesis of 4TI **211** was attempted by reacting 2,1':4,6-di-*O*-isopropylidene sucrose **68** with 6.5 equivalents of triOMe acid **210** in the presence of DCC and DMAP in CH₂Cl₂ at room temperature for two days (Scheme 38). Upon completion, the solvent CH₂Cl₂ was removed. The crude product was then dissolved in cold diethyl ether. After filtration, the filtrate was concentrated and subjected to purification via column chromatography. A white solid was obtained in 74% yield.

Reagents and conditions: i. triOMe acid **206** (6.5 equiv), DCC (6.5 equiv), DMAP (0.1 equiv), CH₂Cl₂, rt, 48 h;

Scheme 38: Synthesis of 4TI **211**

From its ¹H NMR spectrum, characteristic methoxy peaks at around δ 3.87 ppm were observed. Integration of this multiplet showed 36 protons, indicating the presence of 12 methoxy groups. Since each trimethoxycinnamoyl group contains three methoxy groups on its aromatic ring, the presence of 12 methoxy groups would thus implied the presence of four trimethoxycinnamoyl groups. In addition, the spectrum showed the presence *trans*-alkenyl and aromatic proton peaks from δ 6.31 to 7.31 ppm, which corresponded to protons of trimethoxycinnamoyl moieties. Integration of these peaks showed 16 protons, which implied the presence of four trimethoxycinnamoyl substituents.

Furthermore, the ¹³C NMR spectrum of the product showed carbon signals from δ 165.8 to 167.4 ppm. These signals corresponding to the ester carbons confirmed the presence of trimethoxycinnamoyl groups as well. Furthermore, HR-ESI-MS of the product showed a molecular ion m/z value of 1303.4871 corresponding to [M+H]⁺. This supported the molecular formula of C₆₆H₇₉O₂₇ (m/z calcd. for C₆₆H₇₉O₂₇: 1303.4809). From the data collected, the product was confirmed as 4TI **211**. Comparison with reported values for similar compounds as such 4CiI **43**, 4FI **48** and 4SI **52** also confirmed the structure of the product to be 4TI **211**.^{81, 126}

To synthesize 4TS **212**, 4TI **211** was treated with 0.2 equivalent of Yb(OTf)₃·H₂O in MeCN at room temperature for 12 hours (Scheme 39). Upon completion, the solvent MeCN was removed and the crude product was purified by column chromatography to give a white solid in 78% yield.

Reagents and conditions: Yb(OTf)₃·H₂O (0.2 equiv), MeCN, rt, 12 h

Scheme 39: Synthesis of 4TS 212

The ¹H NMR spectrum of the product showed the absence of the distinctive isopropylidene rings from δ 1.10 to 1.50 ppm, which indicated the successful removal of both isopropylidene rings. Other than that, the presence of the *trans*-alkenyl and aromatic proton peaks from δ 6.23 to 7.63 ppm confirmed that the trimethoxycinnamoyl groups were not affected during the deprotection reactions. Integration of these peaks showed 16 protons, which was the same as compared to that in 4TI 211. This implied that the four trimethoxycinnamoyl groups were not affected during the deprotection reaction. Furthermore, comparison of the ¹H NMR spectrum with that of 4TI 211 concluded that no migration has occurred during the deprotection reaction.

¹³C NMR of the product also showed the absence of carbon signals from δ 19.1 to 30.0 ppm (as seen in 4TI 211) which corresponded to the carbons of the isopropylidene rings. This confirmed the successful removal of isopropylidene rings. In addition, the presence of carbon signals from δ 165.9 to 167.1 ppm which corresponded to the ester carbons, confirmed the presence of trimethoxycinnamoyl groups. This confirmed that the trimethoxycinnamoyl substituents were not affected during the deprotection reaction. Finally, the HR-ESI-MS of the product showed a molecular ion *m/z* value at 1223.4193, corresponding to [M+H]⁺. This supported the molecular formula of C₆₀H₇₁O₂₇ (*m/z* calcd. for C₆₀H₇₁O₂₇: 1223.4183). Based on these data, the product was confirmed as 4TS 212. Comparison with reported values for similar compounds as such 4CiI 43, 4FI 48 and 4SI 52 also confirmed the structure of the product to be 4TI 211.^{81, 126}

2.3 *In vitro* enzyme assays studies

To compare the current results with the previously screened tetra-substituted PSEs, the newly synthesized tetra-substituted PSEs were screened for their α -glucosidase (from *Saccharomyces cerevisiae*) and α -amylase (from porcine pancreas) inhibitory activities at the same concentration - 50 μ g/ml (except cinnamoyl PSEs which were screened at 25 μ g/ml) due to solubility issues. The methods and procedures involved in both enzyme assays can be found in the experimental section (refer to 8.3). The results obtained from the *in vitro* studies will be discussed in the following subsections.

2.3.1 Results and Discussion

2.3.1.1 α -Glucosidase inhibition assay

4CaI **201**, 4CaS **202**, 4CoI **205**, 4CoS **206**, 4DI **207**, 4DS **208**, 4TI **211** and 4TS **212** were screened for their α -glucosidase inhibitory activities at a concentration of 50 μ g/ml. The values obtained are shown in Table 4 and represented in Figure 20. The values obtained from previous screenings are also included for comparison (highlighted in red). The value obtained for Acarbose was also included for comparison (highlighted in blue).

Table 4: % α -glucosidase inhibition of tetra-substituted PSEs

Entry	Compound	% α -glucosidase inhibition
1	4CiI 43	77 $\pm$ 5
2	4FI 48	90 $\pm$ 8
3	4SI 52	43 $\pm$ 3
4	4CaI 201	99 $\pm$ 3
5	4CoI 205	98 $\pm$ 1
6	4DI 207	24 $\pm$ 14
7	4TI 211	15 $\pm$ 8
8	4CiS 55	74 $\pm$ 9
9	4FS 58	98 $\pm$ 4
10	4SS 60	21 $\pm$ 8
11	4CaS 202	99 $\pm$ 2
12	4CoS 206	6 $\pm$ 2
13	4DS 208	NA
14	4TS 212	NA
15	Acarbose	18 $\pm$ 6

Note: All testing was done at 50 μ g/ml, except 4CiI and 4CiS (25 μ g/ml)
NA = no inhibitory activity

●-glucosidase inhibition of tetra-substituted PSEs

Figure 20: % α -glucosidase inhibition by tetra-substituted PSEs

Based on the data from Table 4 and Figure 20, the following conclusions can be made:

1. Newly synthesized PSEs – 4CaI **201**, 4CoI **205** and 4CaS **202** gave higher % α -glucosidase inhibition values as compared to standard drug Acarbose (Figure 20). The % inhibition values of these three PSEs at 99 ± 3 , 98 ± 1 and 99 ± 2 respectively (Table 4, Entries 4, 5 & 11), were five times higher than that of Acarbose at 18 ± 6 (Table 4, Entry 15).
2. 4DI **207** and 4TI **211** gave % α -glucosidase inhibition values at 24 ± 14 and 15 ± 8 respectively (Table 4, Entries 6 & 7), which were comparable to Acarbose at 18 ± 6 (Table 4, Entry 15).
3. 4DI **207** and 4TI **211** gave % α -glucosidase inhibition values at 24 ± 14 and 15 ± 8 respectively (Table 4, Entries 6 & 7) which were lower in comparison to that of 4CiI at 77 ± 5 (Table 4, Entry 1), despite 4DI **207** and 4TI **208** being tested at higher concentration. This implies that the presence of methoxy groups on the aromatic rings of tetra-substituted PSEs can reduce the inhibition of α -glucosidase. Likewise, comparison of 4DS **208** and 4TS **212** to 4CiS **55** supported this observation as well since 4DS **208** and 4TS **212** showed no inhibitory activities despite being tested at higher concentrations as compared to 4CiS **55**.
4. 4TI **211** showed lower α -glucosidase inhibition value as compared to 4DI **207** (Table 4, Entries 6 & 7). This means that as the number of methoxy groups increases, α -glucosidase inhibition

decreases. This supports the observation between previously screened PSEs – 4FI **48** & 4SI **52** and 4FS **58** and 4SS **60** (Figure 20) as well.

5. Based on the high % α -glucosidase inhibitory activities shown 4CaI **201**, 4CoI **205** and 4CaS **202** at 99 ± 3 , 98 ± 1 and 99 ± 2 respectively (Table 4, Entry 4, 5 & 11), it can be concluded that the presence of hydroxyl groups on the aromatic rings of tetra-substituted PSEs contributes significantly to α -glucosidase inhibition. However, the comparable activities of 4CaI **201** and 4CoI **205** (Figure 20) suggests that the number of hydroxyl groups on the aromatic rings of tetra-substituted PSEs does not affect the α -glucosidase inhibition values.

However, there is an anomaly. Despite having hydroxyl groups on the aromatic rings, 4CoS **206** showed extremely low % α -glucosidase inhibition at 6 ± 2 (Table 4, Entry 12). This drastic decrease in activity as compared to 4CiI **43**, 4CaI **201**, 4CoI **205**, 4CiS **55** and 4CaS **202** (Figure 20) can be attributed to the absence of isopropylidene rings which is discussed in the next point.

6. The decrease in % α -glucosidase inhibition values of 4CoI **205** and 4CoS **206** from 98 ± 1 to 6 ± 2 suggests that the absence of isopropylidene rings on PSEs may decrease the inhibitory activities against α -glucosidase inhibition. Decreased activities between two pairs of PSEs – 4DI **207** & 4DS **208** and 4TI **211** and 4TS **212** (Figure 20) supports this observation. The same trend was also observed in the previously screened PSEs – 4SI **52** and 4SS **60** where a decrease in activity from 43 ± 3 to 24 ± 8 (Table 4, Entries 3 & 10) was observed. However, this was not the case for tetra-substituted caffeoyl, feruloyl and cinnamoyl PSEs. 4CaI **201** and 4CaS **202** gave same α -glucosidase inhibitory activities at 99 ± 3 and 99 ± 2 respectively (Table 4, Entries 4 & 11). Likewise, comparison of the pairs of PSEs – 4FI **48** & 4FS **58** and 4CiI **43** & 4CiS **55** also showed comparable activities at 90 ± 8 & 98 ± 4 (Table 4, Entries 2 & 9) and 77 ± 5 & 74 ± 9 respectively (Table 4, Entries 1 & 8). In these cases, the isopropylidene rings had no effect on the inhibition of α -glucosidase inhibition.

Based on the current results, it is hard to determine if the presence of isopropylidene rings has any effect on the inhibition of α -glucosidase. One plausible reason for this contradictory result can be due to the difference in binding interactions of tetra-substituted PSEs with α -glucosidase. This can be confirmed by performing a docking study using computational modelling software. Despite this contradiction, it can still be concluded that the presence of isopropylidene rings is important in developing PSEs with higher inhibitory activities against α -glucosidase.

2.3.1.2 α -Amylase inhibition assay

4CaI **201**, 4CaS **202**, 4CoI **205**, 4CoS **206**, 4DI **207**, 4DS **208**, 4TI **211** and 4TS **212** were also screened for their α -amylase inhibitory activities at a concentration of 50 μ g/ml. The inhibition values obtained are shown in Table 5 and represented in Figure 21. Likewise, the values obtained from the previous screenings (red) and for standard drug Acarbose (blue) are also included for comparison.

Table 5: % α -amylase inhibition of tetra-substituted PSEs

Entry	Compound	% α -amylase inhibition
1	4CiI 43	98 $\pm$ 2
2	4FI 48	54 $\pm$ 4
3	4SI 52	NA
4	4CaI 201	90 $\pm$ 4
5	4CoI 205	82 $\pm$ 3
6	4DI 207	NA
7	4TI 211	NA
8	4CiS 55	99 $\pm$ 1
9	4FS 58	98 $\pm$ 1
10	4SS 60	NA
11	4CaS 202	90 $\pm$ 2
12	4CoS 206	91 $\pm$ 5
13	4DS 208	NA
14	4TS 212	9 $\pm$ 4
15	Acarbose	95 $\pm$ 1

Note: All testing was done at 50 μ g/ml, except 4CiI and 4CiS (25 μ g/ml)
NA = no inhibitory activity

Figure 21: % α-amylase inhibition of tetra-substituted PSEs

Based on the inhibition values obtained (Table 5, Figure 21), the following observations can be made:

1. The newly synthesized PSEs – 4CaI **201**, 4CaS **202** and 4CoS **206** exhibited high % α-amylase inhibition values at 90 ± 4 , 90 ± 2 and 91 ± 5 respectively (Table 5, Entries 4, 11 & 12). These values are comparable to that of standard drug Acarbose at 95 ± 1 (Table 5, Entry 15).
2. The presence of methoxy groups on the aromatic rings of PSEs decreased the inhibition of α-amylase. This is evident from Figure 21 where PSEs with methoxy groups on their aromatic rings such as 4DI **207**, 4TI **211**, 4DS **208** and 4TS **212** showed no α-amylase inhibitory activities at all.
3. Since 4DI **207**, 4TI **211**, 4DS **208** and 4TS **212** all showed no inhibitory activities, comparison cannot be made between these two pairs of PSEs – 4DI **207** & 4TI **211** and 4DS **208** & 4TS **212**. As such, the effect of having an additional methoxy group on the aromatic rings of PSEs on α-amylase inhibition cannot be determined.
4. Evidently, PSEs with hydroxyl groups on their aromatic rings such as 4CaI **202**, 4CoI **205**, 4CaS **202** and 4CoS **206** all gave high % α-amylase inhibition at 90 ± 4 , 82 ± 3 , 90 ± 2 and 91 ± 5 respectively (Table 5, Entries 4, 5, 11 & 12). This implies that the presence of hydroxyl groups on the aromatic rings of PSEs contribute to the inhibition of α-amylase.

5. Comparing inhibitory activities of 4CaI **201** and 4CoI **205** shows a slight increase from 82 ± 3 to 90 ± 4 (Table 5, Entries 4 & 5). This suggests that having additional hydroxyl groups on the aromatic rings of PSEs may lead to slight increase in the inhibition of α -amylase. However, the inhibition values obtained from 4CaS **202** and 4CoS **206** (Figure 20) do not support this observation. Hence, the effect of having additional hydroxyl groups on α -amylase inhibition remains inconclusive. With these contradictory results, it is possible that the increased in activity observed between 4CoI **205** and 4CaI **201** could be due to other reasons.
6. Comparing inhibitory activities of 4CoI **205** and 4CoS **206** shows a slight increase in the value from 82 ± 3 to 91 ± 5 (Table 5, Entries 5 & 12). This supports the observation from previously screened PSEs – 4FS **58** and 4FI **48** where the presence of isopropylidene rings led to an increase in the inhibition of α -amylase. However, comparison of 4CaI **201** and 4CaS **202** contradicted with this observation since both PSEs gave comparable % inhibition at 90 ± 4 and 90 ± 2 respectively (Table 5, Entries 4 & 11). This observation was the same as previously observed with 4CiI **43** and 4CiS **55** where comparable activities were observed even with the absence of the isopropylidene rings. Hence, the effect of isopropylidene rings on inhibition of α -amylase remained inconclusive. The difference in the observations may be a result of the difference in binding interactions with α -amylase.

However, it can be concluded that the presence of isopropylidene rings on PSEs is important for the development of PSEs that only inhibits α -glucosidase since absence of the isopropylidene rings either has no effects or decreases α -amylase inhibitory activity.

2.4 Summary

The use of Steglich esterification in the synthesis of tetra-substituted caffeoyl, coumaroyl, dimethoxycinnamoyl and trimethoxycinnamoyl PSEs was successful in giving desired products in good yields. This method offers an advantage over the method demonstrated by Dr Parthasarathi Panda¹²⁶ since there is no need to convert (substituted) cinnamic acids into their respective chlorides for subsequent acylation reactions.

Moreover, temporary protecting groups on the (substituted) cinnamic acid may influence the outcomes of the reactions, especially for the synthesis of tetra-substituted caffeoyl PSEs. Evidently, the use of OAc-caff acid **196** failed to give desired products despite numerous attempts using different reaction conditions. In addition, the use of TBS protecting group is better in comparison to allyl protecting group

since TBS group does not have any overlapping peaks with the protons on sucrose core and a clean removal can be achieved efficiently in high yields.

Furthermore, the use of $\text{Yb}(\text{OTf})_3 \cdot \text{H}_2\text{O}$ in the cleavage of isopropylidene rings offers high chemoselectivity in the presence of acid labile groups. Evidently from the ^1H NMR spectra of the newly synthesized PSEs, clean removal of isopropylidene rings on PSEs can be attained without affecting any of the phenylpropanoid groups.

The inhibitory activities of the newly synthesized tetra-substituted PSEs against both α -glucosidase and α -amylase are represented in Figure 22.

Inhibitory activities of newly synthesized tetra-substituted PSEs

Figure 22: inhibitory activities of tetra-substituted PSEs for both α -glucosidase and α -amylase

The *in vitro* inhibition studies using the tetra-substituted PSEs having different phenylpropanoid groups provided the following information:

1. PSEs with hydroxyl groups on their aromatic rings (caffeoyl and coumaroyl PSEs) showed higher inhibitory activities than PSEs with methoxy groups on their aromatic rings (dimethoxycinnamoyl and trimethoxycinnamoyl PSEs).
2. The number of methoxy group(s) and hydroxyl group(s) has minimal or no effect on their inhibitory activities.

3. The role of the isopropylidene rings on both α -glucosidase and α -amylase remains unclear. However, the presence of isopropylidene rings on PSEs is important in developing PSEs that may give higher α -glucosidase inhibition and reduced α -amylase inhibition.

4CaI **201** and 4CaS **202** showed the highest inhibitory activities for both α -amylase and α -glucosidase amongst all the newly synthesized PSEs (Figure 21). 4CaI **201** and 4CaS **202** showed comparable α -amylase inhibitory activities as Acarbose, but their α -glucosidase inhibitory activities are around five times higher than that of Acarbose. Hence, both PSEs showed potential to be developed as AGIs for the treatment of T2D. Nevertheless, common side effects associated with the inhibition of α -amylase will still be present in these two potential lead compounds. To eliminate side effects, there is still a need to synthesize a PSE that only inhibit α -glucosidase.

Amongst all the newly synthesized PSEs, 4CoS **206** is the most interesting since it exhibited more than 90% α -amylase inhibition but only showed 6% α -glucosidase inhibition at 50 μ g/ml. This drastic difference in inhibitory activities for both enzymes provided the possibility of synthesizing a PSE compound that only inhibits α -glucosidase. However, SAR of PSEs will need to be established further.

Computational docking studies done previously showed the importance of having a substituent at the O-3 position for both feruloyl and cinnamoyl PSEs. Using this as a basis, it was hypothesized that synthesizing mono-substituted PSEs at different positions – O-3, O-3', O-4' and O-6' can identify key position(s) and key functional group(s) that contribute to inhibition of either α -amylase, α -glucosidase or both. Information derived from the SAR study of mono-substituted PSEs will be a useful guideline for the synthesis of simple or mixed PSEs that inhibits α -glucosidase alone. However, the similar reactivities of the four free hydroxyl groups – 3-OH, 3'-OH, 4'-OH and 6'-OH on 2,1':4,6-di-*O*-isopropylidene sucrose **68** makes the synthesis of mono-substituted PSEs at different positions challenging due to problems of regioselectivity. Thus prior to this, orthogonal protection of 2,1':4,6-di-*O*-isopropylidene sucrose **68** needs to be achieved. Details of the orthogonal protection strategy will be discussed in the next chapter.

3 Orthogonal Protection and Deprotection of 2,1':4,6-di-*O*-isopropylidene sucrose **68** for the Synthesis of PSE

3.1 Introduction

To resolve the key challenge faced in the total synthesis of simple and mixed PSEs at different positions and with different substitution patterns, we proposed orthogonal protection of 2,1':4,6-di-*O*-isopropylidene sucrose **68** to give a versatile intermediate which allows the selective introduction of the phenylpropanoid group(s) at the specific position (O-3, O-3', O-4', O-6'). To achieve this, the following conditions need to be fulfilled:

- regioselective protection of *O*-3, *O*-3', *O*-4' and *O*-6'
- regioselective deprotection of any desired protecting groups without affecting the integrity of the other protecting moieties
- maintain the integrity of the phenylpropanoid groups and avoid their removal and/or reduction of their double bonds during deprotection
- maintain the integrity of the isopropylidene rings under the reaction conditions employed during protection and deprotection and during the purification processes.

Therefore, the choice of the protecting groups, their order of introduction onto 2,1':4,6-di-*O*-isopropylidene sucrose **68** and the choice of reagents and reaction conditions for their selective removal becomes critical in this design. For instance, hydrogenation or hydrolysis under acidic or basic conditions should be avoided in order not to affect the phenylpropanoid groups on the sucrose core.

Based on our earlier studies and reported results, the order of reactivity of the remaining four hydroxyl groups in of 2,1':4,6-di-*O*-isopropylidene sucrose **68** is 6'-OH > 3'-OH > 4'-OH > 3-OH.^{60, 63} However, it is important to note this order of reactivity can change to 6'-OH > 3'-OH > 3-OH > 4'-OH, depending on the reaction conditions and acylating agent used. After an extensive search and preliminary experiments, we proposed the use of the following set of protecting groups - *tert*-butyldimethylsilyl (TBS), *p*-nitrobenzoyl (PNB), levulinoyl (Lev) and *tert*-butyloxycarbonyl (Boc). The proposed synthetic route is shown in Scheme 40 and details of the synthesis are discussed in the next section.

Scheme 40: Proposed orthogonally protected 2,1':4,6-di-*O*-isopropylidene sucrose 68

3.2 Orthogonal Protection Route 1

3.2.1 Selective Protection of the 6'-OH

Out of the four free hydroxyl groups on 2,1':4,6-di-*O*-isopropylidene sucrose **68**, only the 6'-OH is a primary hydroxyl group. Based on a literature review (refer to 1.5.3), the primary hydroxyl group is usually selectively protected with a bulky reagent due to lesser steric hindrance in comparison to secondary hydroxyl groups. Hence in this orthogonal protection strategy, the 6'-OH of 2,1':4,6-di-*O*-isopropylidene sucrose **68** will be protected with the bulky TBS group.

To achieve that, 2,1':4,6-di-*O*-isopropylidene sucrose **68** was treated with 1.2 equivalents of TBSCl in the presence of DMAP and NEt₃ in CH₂Cl₂ at room temperature for 12 hours (Scheme 41). A yellow solid was obtained in 95% yield and its structure was confirmed by ¹H NMR, ¹³C NMR and HR-ESI-MS.

Reagents and conditions: i. TBSCl (1.2 equiv), NEt₃ (2.4 equiv), DMAP (0.1 equiv), CH₂Cl₂, rt, 12 h

Scheme 41: Selective protection of 6'-OH with TBSCl

From ¹H NMR spectrum of the product, characteristic signals of TBS group were observed at δ 0.00 and 0.83 ppm. Integration of these peaks showed six and nine protons respectively, thus confirming the presence of one TBS group. Moreover, comparison with reported literature data of a similar sucrose compound¹³¹ confirmed the TBS group is attached to the primary hydroxyl group at C-6'.

Other than that, ¹³C NMR spectrum of the product showed new carbon signals at δ -5.43 and -5.22 ppm. These new signals corresponded to the silyl carbons, which confirmed the presence of TBS moiety. The

HR-ESI-MS of the product also showed a molecular ion m/z value of 537.2734, corresponding to $[M+H]^+$. This supported the molecular formula of $C_{24}H_{45}O_{11}Si$ (m/z calcd. for $C_{24}H_{45}O_{11}Si$: 537.2731). From these data, the product obtained was confirmed as 6'-*O*-TBS **214**.

3.2.2 Selective protection of the 3'-OH

Next, selective protection of 3'-OH on 6'-*O*-TBS **214** was attempted by reacting 6'-*O*-TBS **214** with 1.5 equivalents of PNBCL in the presence of NEt_3 and DMAP in CH_2Cl_2 at room temperature (Scheme 42). However, this reaction was not very successful since a mixture of products was obtained as indicated by TLC. After chromatographic purification, the reaction gave three products in 32%, 29% and 30% yield respectively. The identities of these products were confirmed by 1H NMR.

Reagents and conditions: PNBCL (1.5 equiv), NEt_3 (3.0 equiv), DMAP (0.1 equiv), CH_2Cl_2 , rt, 12 h

Scheme 42: Selective protection of 3'-OH with PNBCL

The spectra of all three products revealed characteristic aromatic proton peaks of PNB group from δ 8.00 to 8.40 ppm. Integration of these peaks for the first and second products showed four protons each, suggesting the presence of one PNB group. Integration of these peaks for the third product showed eight protons, indicating the presence of two PNB groups instead.

Subsequently, COSY NMR were done for the first two products to determine the position(s) at which PNB group was attached. From Figure 23, a correlation between the doublet at δ 5.10 ppm and the triplet at δ 4.50 ppm was observed. This implied that they are neighbouring protons. As such, the doublet should correspond to the proton at C-3' while the triplet should correspond to the proton at C-4'. The proton at C-3' forms a doublet as it couples with the neighbouring proton at C-4', while the proton at C-4' forms a triplet as it couples with two neighbouring protons at C-3' and C-5'. The proton at C-3' shifted to δ 5.10 ppm as compared to that in 6'-*O*-TBS **214** (δ 3.50 to 4.20 ppm). This downfield shift can be attributed to the electron withdrawing effect of PNB group, thus implying that the PNB group of the first product was attached to O-3'. Since the proton at C-4' is coupled to the proton at C-3', it shifted to δ 4.50 ppm as compared to that in 6'-*O*-TBS **214** (δ 3.50 to 4.20 ppm). Therefore, the first product was identified as 3'-*O*-PNB **215**.

Only correlation between protons of C-3' and C-4' was highlighted

Figure 23: COSY spectrum of 3'-O-PNB 215

From Figure 24, it was evident that the triplet at δ 5.54 ppm did not show any correlation to the doublet which corresponded to the proton at C-3', unlike the previous product. Hence, this triplet should correspond to the proton at C-3 rather than C-4'. The proton at C-3 forms a triplet as it couples with two neighbouring protons at C-2 and C-4. In the second product, the proton at C-3 shifted to δ 5.54 ppm as compared to that in 6'-O-TBS **214** (δ 3.50 to 4.20 ppm). Likewise, this downfield shift can be attributed to the strong electron withdrawing effects of PNB group, implying that the PNB group on the second product was attached to O-3. Hence, the second product was confirmed as 3-O-PNB **216**.

Figure 24: COSY spectrum of 3-O-PNB 216

¹H NMR spectrum of the third products showed two triplets at around δ 5.10 and 5.40 ppm. From its COSY NMR spectrum, a correlation was observed between the triplet at δ 5.10 and the doublet at δ 4.70 ppm. This implied that they are neighbouring protons, hence the triplet at δ 5.10 ppm should correspond to the proton at C-3', while the doublet at δ 4.70 ppm should correspond to the proton at C-4'. As for the other triplet at δ 5.40 ppm, no correlation to the proton at C-3' (the doublet at δ 4.70 ppm) was observed. As such, this triplet should correspond to the proton at C-3. Both protons at C-4' and C-3 shifted downfield as compared to that in 6'-O-TBS **214** (δ 3.50 to 4.20 ppm). These downfield shifts can be attributed to the electron withdrawing effects of PNB groups, hence implying that the two PNB

groups on the third product were attached to O-4' and O-3' respectively. Therefore, the third product as di-*O*-PNB **217**.

Because of the poor selectivity, the desired compound 3'-*O*-PNB **215** and the other two side products were obtained in an approximately 1:1:1 ratio. This inefficiency in reaction generates greater amount of waste and lower yield of desired product. In the hope of improving selectivity of PNBCl towards 3'-OH, the reaction was repeated at a lower temperature of 4°C using an ice bath. This reaction also gave three products but in different ratios as compared to the previous reaction. At 4°C, 3'-*O*-PNB **215** was obtained in 65% yield, while 3-*O*-PNB **216** and di-*O*-PNB **217** were obtained in 16% and 11% yield respectively. This significant improvement in the yield of desired product implied that the reaction temperature can enhance regioselectivity. With an improved yield of 3'-*O*-PNB **215**, selective protection of the 4'-OH was attempted next.

3.2.3 Selective protection of the 4'-OH

To selectively protect the 4'-OH, 3'-*O*-PNB **215** was reacted with 1.2 equivalents of LevOH in the presence of DCC and DMAP in CH₂Cl₂ at room temperature (Scheme 43). Upon purification, two products were obtained in 85% and 5% yield.

Reagents and conditions: LevOH (1.2 equiv), DCC (1.2 equiv), DMAP (0.1 equiv), CH₂Cl₂, rt, 12 h

Scheme 43: Selective protection of 4'-OH with LevOH

The structures of the products were confirmed by ¹H NMR. From the spectrum of the major product, characteristic signals of Lev groups at around δ 2.10 ppm and δ 2.50 to 2.75 ppm were observed. Integration of these peaks showed three and four protons respectively, indicating the presence of one Lev group. In addition, the triplet which corresponded to the proton at C-4' shifted to δ 5.62 ppm as compared to that in 3'-*O*-PNB **215** (δ 4.50 ppm). This downfield shift can be attributed to the electron withdrawing effect of the Lev group, hence implying that the Lev group was attached to O-4'. Based on the spectroscopic data, the major product was identified as 4'-*O*-Lev **218**.

The spectrum of the minor product showed characteristic signals of Lev group at δ 2.10 ppm and δ 2.50 to 2.75 ppm as well. However, integration of these peaks showed six and eight protons respectively,

indicating the presence of two Lev moieties. Like the first product, the triplet which corresponded to the proton at C-4' shifted downfield as well. However, unlike the first product, the ^1H NMR spectrum showed that an additional triplet at around δ 5.60 ppm. From the COSY NMR spectrum, this triplet does not show a correlation with the doublet which corresponded to the proton at C-3'. This implied that the additional triplet should correspond to the proton at C-3. This triplet shifted downfield as compared to that in 3'-*O*-PNB **215** (δ 3.50 to 4.20 ppm). These downfield shifts can be attributed to the electron withdrawing effects of Lev groups, hence implying that the two Lev groups were attached to O-3 and O-4' respectively. Hence, the minor product was confirmed as di-*O*-Lev **219**.

3.2.4 Protection of the 3-OH

To protect the last hydroxyl group, 4'-*O*-Lev **218** was treated with di-*tert*-butyl dicarbonate ((Boc) $_2$ O) in the presence of tetramethylethylenediamine (TMEDA) and DMAP in CH_2Cl_2 at reflux (Scheme 44). A stronger base and a higher temperature were used to drive the reaction since (Boc) $_2$ O is an extremely bulky reagent. After chromatographic purification, a white solid was obtained in 40% yield.

Reagents and conditions: (Boc) $_2$ O (1.5 equiv), TMEDA (3 equiv), DMAP (0.1 equiv), CH_2Cl_2 , reflux, 48 h

Scheme 44: Protection of 3-OH with (Boc) $_2$ O

The ^1H NMR spectrum of the product obtained revealed a multiplet at δ 1.10 - 2.10 ppm, which corresponded to the protons from the *tert*-butyl of TBS group, the *tert*-butyl of Boc group and from the isopropylidene rings. Integration of the multiplet showed 30 protons, indicating a successful protection with one Boc group. Since 3-OH is the only free hydroxyl group available for reaction, the Boc group was inferred to be attached to O-3 position. The product was then confirmed as orthogonally protected 2,1':4,6-di-*O*-isopropylidene sucrose (OP-diisopropylidene) **213**.

The low yield obtained from this reaction can be attributed to the low conversion since 45% of the starting compound was recovered. One plausible reason for this low conversion could be strong steric hindrance at O-3 with the presence of the isopropylidene ring. This resulted in the lower ability for 3-OH to access bulky (Boc) $_2$ O. There is certainly a need to optimize the reaction conditions to obtain a better conversion rate. However, the orthogonality of the scheme should be assessed first to ensure the successful selective removal of specific protecting groups.

3.2.5 Selective deprotection of PNB group

The orthogonality of the scheme is only proven by the successful selective deprotection of individual protecting groups. To selectively remove PNB group, OP-diisopropylidene **213** was reacted with 0.1 equivalents of $\text{Mg}(\text{OMe})_2$ in 8:2 MeOH/THF at room temperature for one hour.¹³² However, this reaction was unsuccessful as ^1H NMR of the product obtained showed simultaneous cleavage of both Lev and PNB moieties. This denoted the failure of the proposed orthogonal protection strategy and prompted a re-examination of the strategy to achieve a successful orthogonal protection scheme for 2,1':4,6-di-*O*-isopropylidene sucrose **68**. The newly proposed scheme is discussed in the next section.

3.3 Orthogonal Protection Route 2

The newly proposed scheme is as shown in Scheme 45. In this new scheme, the same protecting groups were used but they were repositioned with the following considerations. We proposed to attach the Boc group at the O-3' position to achieve higher conversion due to reduced steric hindrance. Other than that, PNB and Lev groups were also proposed to be at O-4' and O-3, respectively. Hopefully, simultaneous removal of Lev and PNB groups will not happen with these two groups attached on two different monosaccharides. Details of the synthesis are discussed in the following subsections.

Scheme 45: Proposed orthogonally protected 2,1':4,6-di-*O*-isopropylidene sucrose 220

Since selective protection of 6'-OH with TBSCl was already discussed (refer to 3.2.1), discussion will focus on selective protection of 3'-OH with $(\text{Boc})_2\text{O}$.

3.3.1 Selective protection of the 3'-OH

To achieve regioselective protection of 3'-OH with Boc group, 6'-O-TBS **214** was reacted with 1.5 equivalents of (Boc)₂O in the presence of TMEDA and DMAP in CH₂Cl₂ under reflux (Scheme 46). Even after two days, majority of the starting material remained unchanged. Moreover, the formation of multiple product spots (based on TLC analysis) indicated that the reaction was not very successful in attaining selective protection of 3'-OH. After purification, 65% of the starting material was recovered and three other products were obtained in 9%, 8% and 8% yield. The structures of these products were confirmed by ¹H NMR.

Reagents and conditions: (Boc)₂O (1.5 equiv), TMEDA (3.0 equiv), DMAP (0.1 equiv), CH₂Cl₂, reflux, 48 h

Scheme 46: Selective Protection of 3'-OH with (Boc)₂O

The spectra of all three products revealed multiple peaks from δ 0.70 to 2.10 ppm which corresponded to the protons from TBS, Boc and isopropylidene rings. Integration of these peaks for the first two products showed 30 protons, indicating the presence of a single Boc group. Integration of these peaks for the third product showed 39 protons, corresponding to the presence of two Boc moieties.

Likewise, COSY experiments were done for the first two products to determine the position at which the Boc group was attached.

Figure 25: COSY spectrum of 3'-O-Boc 221

From Figure 25, a correlation between the triplet at δ 4.36 ppm and the doublet at δ 4.63 ppm was observed. This implied that they are neighbouring protons. As such, the triplet should correspond to the proton at C-4' while the doublet should be the proton at C-3'. The proton at C-4' formed a triplet as it couples with the two neighbouring protons at C-3' and C-5' while the proton at C-3' formed a doublet as it couples with the neighbouring proton at C-4'. The proton at C-3' shifted to δ 4.63 ppm as compared to that in 6'-O-TBS **214** (δ 3.50 to 4.20 ppm). This slight downfield shift was a result of the electron withdrawing effect of Boc group, hence implying that the Boc group was attached to O-3'. Since proton at C-4' is coupled to the proton at C-3', it shifted to δ 4.36 ppm as compared to that in 6'-O-TBS **214**

(δ 3.50 to 4.20 ppm) as well. Based on the spectroscopic data, the product was confirmed as 3'-*O*-Boc **221**.

Only correlation between protons of C-3' and C-4' will be highlighted

Figure 26: COSY spectrum of 3-*O*-Boc **222**

From Figure 26, it was observed that the triplet at δ 4.96 ppm showed no correlation to the doublet at C-3', unlike that of the first product. Hence, this triplet should correspond to the proton at C-3 rather than C-4'. The proton at C-3 is a triplet as it couples with the neighbouring protons at C-2 and C-4. The proton at C-3 shifted to δ 4.96 ppm as compared to that in 6'-*O*-TBS **214** (δ 3.50 to 4.20 ppm) due to the electron withdrawing effect of Boc group. This implied that the Boc group was attached to O-3. Based on the spectroscopic data, the second product was confirmed as 3-*O*-Boc **222**.

As for the third product which has two Boc groups, its ^1H NMR spectrum revealed one doublet and one triplet at δ 4.61 and 4.89 ppm respectively. From its COSY NMR spectrum, the triplet at δ 4.89 ppm showed no correlation to the doublet at δ 4.61 ppm, while the doublet at δ 4.61 ppm showed a correlation to another triplet at δ 4.31 ppm. Since they are neighbouring protons, the doublet at δ 4.61 ppm should correspond to the proton at C-3' while the triplet at δ 4.31 ppm should correspond to the proton at C-4'. As for the triplet at δ 4.89 ppm, it should correspond to the proton at C-3. Due to the electron withdrawing effects of Boc groups, both protons at C-3 and C-3' shifted downfield as compared to that in 6'-O-TBS **214** (δ 3.50 to 4.20 ppm). This implied that the two Boc groups were attached to O-3' and O-3 respectively. Hence, the third product was assigned as di-O-Boc **223**.

With low conversion and poor selectivity towards the 3'-OH, this reaction was considered inefficient and ineffective. Hence, there was a need to optimize the reaction to obtain improved rate of conversion as well as selectivity. Since solvent is one of the many factors which can influence a reaction¹³³, the reaction was repeated using different solvents to examine their effects on selectivity and conversion. Since the solvents chosen had higher boiling points than CH_2Cl_2 , the reactions were carried out at a higher temperature of around 75°C .

Table 6: Reaction of 6'-O-TBS **214 with $(\text{Boc})_2\text{O}$ using different solvent**

Reagents and conditions: $(\text{Boc})_2\text{O}$ (1.5 equiv), TMEDA (3.0 equiv), DMAP (0.1 equiv), 75°C , 48 h

Entry	Solvent	Yield ^a (ratio)			Conversion ^b
		221	222	223	
1	Toluene	1	1	1	11
2	Benzene	1	1	1	10
3	Dimethylformamide	1	1	1	42
4	Acetonitrile	1	1	1	40

^a based on TLC analysis

^b calculated based on the recovery of starting material 6'-O-TBS **214** from column chromatography

The use of different solvents did not have an improved effect on the selectivity of the reaction since a mixture of products was still obtained in similar yields and ratios (Table 6, Entries 1 to 4). However, the change in solvent did affect the conversion. Maximum conversion of 40% was obtained in polar solvents such as acetonitrile (MeCN) and dimethylformamide (DMF) (Table 2, Entries 3 & 4) while

non-polar solvent like toluene and benzene gave only 10% conversion (Table 2, Entries 1 & 2). This implied that the polarity of the solvent can influence the conversion and for this reaction, polar solvent such as MeCN or DMF should be used instead. Though both MeCN and DMF showed similar conversion, MeCN was selected as the solvent for subsequent reactions since it is easier to remove as compared to DMF and thus easier to work with. However, with only 40% of conversion achieved, there was a need to further optimize the reaction so that improved regioselectivity in MeCN and yield of desired product can be achieved.

Other than solvent, a change in catalyst was also investigated to see if selectivity towards the 3'-OH and the conversion for the reaction can be improved. Since the use of chiral copper (II) complex and lithium chloride have been reported to achieve selectivity at specific hydroxyl groups on carbohydrates¹³⁴⁻¹³⁵, several of these catalysts were then examined to gauge their influence on the outcome of this reaction (Table 7).

Table 7: Reaction of 6'-O-TBS 214 with (Boc)₂O using different catalyst

Reagents and conditions: (Boc)₂O (1.5 equiv), TMEDA (3.0 equiv), catalyst (0.1 equiv), ligand (0.1 equiv) if any, MeCN, 75°C, 48 h

Entry	Catalyst	Ligand	Yield ^a (ratio)		
			221	222	223
1	LiCl ₂	-	1	1	1
2	CuCl ₂	-	1	1	1
3	CuCl ₂		5	1	4
4	CuCl ₂		5	1	4
5	CuCl ₂		1	1	1
6	CuCl ₂		1	1	1
7	CuCl ₂		1	1	1

^a based on TLC analysis

^b calculated based on the recovery of starting material 6'-O-TBS 214 from column chromatography

Despite changing the catalyst, no improvement was observed for conversion as around 60% of the starting material – 6'-O-TBS **214** were recovered from each reaction (unchanged).

As for selectivity, we observed no improvement for the reactions done with LiCl₂ and CuCl₂ (Table 7, Entry 1 & 2) since all three products were obtained in 1:1:1 ratio. Likewise, reactions done with

complexes of CuCl₂ and ligands such as (R,R)-Salen-1, (S,S)-Salen-2 and S-TADDOL-1 gave all three products in the same ratio as well (Table 7, Entries 5 to 7).

However, an improvement in the selectivity was seen for reactions done with complexes of CuCl₂ and (R)-PyBOX-1 and (S)-PyBOX-2 ligands (Table 7, Entries 3 & 4). However, both reactions gave 3-*O*-Boc **222** and di-*O*-Boc **223** as the major products, with desired product 3'-*O*-Boc **221** as the minor product. This implied that improvement to selectivity was achieved towards 3-OH instead of 3'-OH. As such, this approach was discontinued.

Since numerous attempts at this reaction to improve the conversion and selectivity towards the 3'-OH were proven to be futile, it would be better to seek an alternative protecting group to replace the Boc group. This group should give higher efficiency and better selectivity in comparison.

3.4 Orthogonal Protection Route 3

From the extensive search, the carboxybenzyl (Cbz) group was selected as an alternative protecting group to replace the Boc group. Like Boc, Cbz is a protecting group that can be used to temporary mask hydroxyl groups.¹³⁶ However, its reactivity is much better as compared to Boc since it is not as bulky. The newly proposed approach is shown in Scheme 47:

Scheme 47: Proposed orthogonally protected 2,1':4,6-di-*O*-isopropylidene sucrose **224**

Details of the synthesis will be discussed in the following subsections. Since selective protection of 6'-OH with TBSCl was already discussed (refer to 3.2.1), selective protection of 3'-OH with benzyl chloroformate (CbzCl) was discussed first.

3.4.1 Selective Protection of the 3'-OH

Reaction of 6'-*O*-TBS **214** with 1.5 equivalents of CbzCl were attempted using different bases such as NEt₃, DIPEA, 1,4-diazabicyclo[2.2.2]octane (DABCO) and TMEDA as it was reported that reaction with CbzCl is highly dependent on the nature of base.¹³⁷⁻¹³⁸ While reactions using NEt₃, DIPEA and DABCO were sluggish, the reaction using TMEDA efficiently gave two products in 75% and 20% yield (Scheme 48).

Reagents and conditions: i. CbzCl (1.5 equiv), TMEDA (3 equiv), DMAP (0.1 equiv), CH₂Cl₂, rt, 12 h

Scheme 48: Selection protection of 3'-OH with CbzCl

The structures of the products were confirmed with NMR experiments. The ¹H NMR spectra of both products revealed characteristic proton signals of Cbz group at δ 5.17 and 7.35 ppm. For the major product, integration of the Cbz peaks showed two and five protons respectively, indicating the presence of one Cbz moiety. The position of the Cbz group on the major product was determined by COSY NMR (Figure 27).

Only correlation between protons of C-3' and C-4' was highlighted

Figure 27: COSY spectrum of 3'-O-Cbz 225

From Figure 27, a correlation between the doublet at δ 4.76 ppm and the triplet at δ 4.38 ppm was observed. This implied that they are neighbouring protons. Hence, the doublet should correspond to the proton at C-3' while the triplet should correspond to the proton at C-4'. The proton at C-3' is a doublet as it couples with the neighbouring proton at C-4' while the proton at C-4' is a triplet as it couples with the two neighbouring protons at C-3' and C-5'. From the spectrum, the proton at C-3' shifted to δ 4.76 ppm as compared to that in 6'-O-TBS **214** (δ 3.50 – 4.20 ppm). This downfield shift can be attributed to the electron withdrawing effect of the Cbz group, implying that the Cbz group is attached to O-3'. Since the proton at C-4' couples with the proton at C-3', the proton at C-4' shifted to δ 4.38 ppm as compared to that in 6'-O-TBS **214** (δ 3.50 – 4.20 ppm) as well.

Other than ^1H and COSY NMR, ^{13}C NMR spectrum of the major product revealed aromatic carbon signals at δ 128.4, 128.7, 128.7 and 134.7 ppm and ester carbon at δ 155.2 ppm, indicating the presence of Cbz moiety. Furthermore, HR-ESI-MS of the major product showed a molecular ion m/z value of 671.3102 corresponding to $[\text{M}+\text{H}]^+$. This supported the molecular formula of $\text{C}_{32}\text{H}_{51}\text{O}_{13}\text{Si}$ (m/z calcd. for $\text{C}_{32}\text{H}_{51}\text{O}_{13}\text{Si}$: 671.3099). Based on the data acquired, the major product was identified as 3'-*O*-Cbz **225**.

Only correlation between protons of C-3' and C-4' was highlighted

Figure 28: COSY spectrum of di-O-Cbz 226

On the other hand, integration of the Cbz peaks for the minor product gave four and ten protons. This indicated the presence of two Cbz moieties, instead of one. Subsequently, the position of the Cbz groups the minor product was determined by COSY NMR (Figure 28).

From Figure 28, a correlation was observed between the triplet at δ 5.30 ppm and the doublet at δ 4.90 ppm. This confirmed that they are neighbouring protons. Likewise, the doublet at δ 4.90 ppm should correspond to the proton at C-3' while the triplet at δ 5.30 ppm should correspond to the proton at C-4'. The proton at C-4' shifted to δ 5.30 ppm as compared to that in 3'-O-Cbz **224** (δ 4.38 ppm) due to the electron withdrawing effect of the Cbz group. This indicated that the additional Cbz group was attached to O-4'. Since the proton at C-3' couples with the proton at C-4', the proton at C-3' shifted from δ 4.76 ppm in 3'-O-Cbz **224** to δ 4.90 ppm as well.

Furthermore, ^{13}C NMR spectrum of the minor product also revealed new signals at δ 128.3, 128.4, 128.6, 128.7, 134.8 and 134.8 ppm, which corresponded to the aromatic carbons. Signals at δ 154.0 and 154.5 ppm which corresponded to the ester carbons were also observed. These carbon peaks indicated the presence of two Cbz moieties. HR-ESI-MS of the minor product also showed a molecular ion m/z value of 805.3452, which corresponded to $[\text{M}+\text{H}]^+$. This confirmed the molecular formula of $\text{C}_{40}\text{H}_{57}\text{O}_{15}\text{Si}$ (m/z calcd. for $\text{C}_{40}\text{H}_{57}\text{O}_{15}\text{Si}$: 805.3467). From these data, the minor product was confirmed as di-O-Cbz **226**.

Unlike the reaction with $(\text{Boc})_2\text{O}$ (refer to 3.3.1), the reaction of 6'-O-TBS **214** with CbzCl exhibited greater selectivity towards the 3'-OH. A higher conversion of >95% and a higher selectivity towards the 3'-OH at room temperature made the Cbz group a better alternative to the Boc group. With the successful synthesis of 3'-O-Cbz **225**, selective protection of the 4'-OH with PNBCl was attempted next.

3.4.2 Selective protection of 4'-OH

Selective protection of the 4'-OH was attempted by reacting 3'-O-Cbz **225** with 1.5 equivalents of PNBCl. Three products were obtained in 51%, 10% and 19% yield, respectively (Scheme 49).

4'-O-PNB 227: R³ = H, R^{4'} = PNB 70%

3-O-PNB 228: R³ = PNB, R^{4'} = H 10%

di-O-PNB 229: R³ = R^{4'} = PNB 19%

Reagents and conditions: i. PNBCl (1.5 equiv), NEt₃ (3.0 equiv), DMAP (0.1 equiv), CH₂Cl₂, -4°C (ice bath), 12 h.

Scheme 49: Selective protection of 4'-OH using PNBCl

Characteristic aromatic proton signals corresponding to the PNB group were observed from δ 8.00–8.50 ppm in the ¹H NMR spectra of the three products. These indicated the presence of PNB group(s) on all three products.

Integration of the PNB proton peaks of the first product showed four aromatic protons, which indicated the presence of one PNB group. The position of the PNB on the first product was then determined with COSY NMR (Figure 29).

Only correlation between protons of C-3' and C-4' was highlighted

Figure 29: COSY spectrum of 4'-O-PNB 227

From Figure 29, a correlation between the triplet at δ 5.71 ppm and the doublet at δ 4.98 ppm was observed. This implied that they are neighbouring protons. Hence, this triplet should correspond to the proton at C-4' and the doublet should correspond to the proton at C-3'. Due to the electron withdrawing effect of the PNB group, the proton at C-4' shifted to δ 5.71 ppm as compared to that in 3'-O-Cbz 225 (δ 4.38 ppm). This implied that the PNB group was attached to O-4'. Since the proton at C-3' couples with the proton at C-4', it shifted to δ 4.98 as compared to that in 3'-O-Cbz 225 (δ 4.76 ppm) as well.

Other than ^1H and COSY NMR, ^{13}C NMR of the first product also revealed additional aromatic carbon signals from δ 123.6 to 134.7 ppm as compared to that of 3'-O-Cbz 225. These additional aromatic

carbon peaks indicated the presence of a PNB group. Furthermore, the additional signal at δ 154.6 ppm, which corresponded to the carbon with the nitro group attached, confirmed the presence of the PNB moiety as well. HR-ESI-MS of the first product showed a molecular ion m/z value of 820.3218, corresponding to $[M+H]^+$. This supported the molecular formula of $C_{39}H_{54}NO_{16}Si$ (m/z calcd. for $C_{39}H_{54}NO_{16}Si$: 820.3212). From the data, the first product was identified as 4'-O-PNB **227**.

Figure 30: COSY spectrum of 3-O-PNB 228

Integration of the PNB proton peaks of the second product also showed four protons, indicating the presence of a single PNB moiety. Likewise, the position of the PNB moiety was determined with COSY NMR (Figure 30).

From Figure 30, a correlation between the doublet at δ 4.72 ppm and the triplet at δ 4.40 ppm was observed. This indicated that they are neighbouring protons. Hence, the triplet at δ 4.40 ppm should

correspond to the proton at C-4' while the doublet at δ 4.72 ppm should correspond to the proton at C-3'. Comparison with ^1H NMR spectrum of 3'-O-Cbz **225** showed no change to the chemical shifts of both protons at C-3' and C-4'. This implied that the PNB group was not attached to O-4' or O-3'.

From Figure 30, another triplet at δ 5.46 ppm was also observed. This triplet showed no correlation to the doublet which corresponded to the proton at C-3'. As such, this triplet should correspond to the proton at C-3 instead of C-4'. The proton at C-3 shifted to δ 5.46 ppm as compared to that in 3'-O-Cbz **225** (δ 3.50 - 4.20 ppm). This downfield shift can be attributed to the strong electron withdrawing effect of PNB group, thus implying that the PNB group of the second product is attached to O-3.

The ^{13}C NMR spectrum of the second product also revealed additional aromatic carbon signals from δ 123.0 to 135.1 ppm as compared to that of 3'-O-Cbz **225**, confirming the presence of PNB moiety. Likewise, an additional signal at δ 154.3 ppm which corresponded to the carbon with nitro group attached confirmed the presence of PNB moiety as well. Other than that, HR-ESI-MS of the second product showed a molecular ion m/z value of 820.3216, which corresponded to $[\text{M}+\text{H}]^+$. This supported the molecular formula of $\text{C}_{39}\text{H}_{54}\text{NO}_{16}\text{Si}$ (m/z calcd. for $\text{C}_{39}\text{H}_{54}\text{NO}_{16}\text{Si}$: 820.3212). Based on all the data acquired, the structure of the second product was assigned as 3-O-PNB **228**.

Finally, integration of the PNB proton peaks of the third product showed eight protons, which indicated the presence of two PNB moieties. Its ^1H NMR revealed a doublet at δ 4.99 ppm and a triplet at δ 5.35 ppm. From the COSY NMR spectrum, a correlation between the doublet at δ 4.99 ppm and the triplet at δ 5.35 ppm was observed, implying that they are neighbouring protons. As such, the doublet should correspond to the proton at C-3' and the triplet should correspond to the proton at C-4'. The proton at C-4' shifted to δ 5.35 ppm as compared to that in 3'-O-Cbz **225** (δ 3.50 - 4.20 ppm). This downfield shift can be attributed to the electron withdrawing effect of PNB group, hence implying that one of the PNB groups was attached to O-4'. Since the proton at C-3' couples with the proton at C-4', it shifted to δ 4.99 ppm as compared to that in 3'-O-Cbz **225** (δ 4.76 ppm) as well.

The ^1H NMR spectrum also showed a triplet at δ 5.72 ppm. This triplet showed no correlation to the doublet which corresponded to the proton at C-3'. As such, the triplet at δ 5.72 ppm should correspond to the proton at C-3. The proton at C-3 shifted to δ 5.72 ppm as compared to that in 3'-O-Cbz **225** (δ 3.50 - 4.20 ppm). This downfield shift can be attributed to the electron withdrawing effect of PNB, suggesting that the other PNB group was attached to O-3. Based on its COSY NMR spectrum, the two PNB groups were attached to O-3 and O-4' respectively.

The ^{13}C NMR spectrum of the product also showed new aromatic carbon signals from δ 123.5 to 135.9 ppm. The presence of these new aromatic carbon signals confirmed the presence of PNB moieties. Other than that, the HR-ESI-MS of this third product showed a molecular ion m/z value of 969.3352,

corresponding to $[M+H]^+$. It supported the molecular formula of $C_{46}H_{57}N_2O_{19}Si$ (m/z calcd. for $C_{46}H_{57}N_2O_{19}Si$: 969.3325). From the data acquired, the third product was confirmed as di-*O*-PNB **229**.

To improve the yield of desired product, the acylation reaction was repeated at a lower temperature of 4°C using an ice bath. The reaction at 4°C increased the yield of the desired 4'-*O*-PNB **227** from 51% to 70% (Scheme 49). This reaction also saw a decrease in the yield of di-*O*-PNB **229** from 36% to 19% but did not affect the yield of 3-*O*-PNB **218** which remained constant at 10%. With an improved yield of 70%, the last step of protection was attempted next.

3.4.3 Selective protection of 3-OH

To protect the last remaining 3-OH, 4'-*O*-PNB **227** was reacted with 1.2 equivalents of LevOH, in the presence of DCC/DMAP in CH_2Cl_2 (Scheme 50). After column chromatographic purification, a single product was obtained in 85% yield.

Reagents and conditions: i. LevOH (1.2 equiv), DCC (1.2 equiv), DMAP (0.1 equiv), CH_2Cl_2 , rt, 12 h

Scheme 50: Protection of 3-OH with LevOH

The structure of the product was confirmed by 1H NMR which revealed characteristic proton signals of Lev group at δ 2.15 and 2.60 ppm. Integration of these peaks showed three and four protons respectively, indicating the presence of one Lev group. Other than that, integration of the multiplet at δ 5.23 ppm showed a total of three protons, corresponding to the $-CH_2$ group on Cbz group and the proton at C-3. The proton at C-3 shifted to δ 5.23 ppm as compared to that of 4'-*O*-PNB **227** (δ 3.50 - 4.20 ppm). This downfield shift can be attributed to the presence of electron withdrawing Lev group, hence confirming that the Lev group was attached to O-3. ^{13}C NMR also showed additional signal at δ 206.4 ppm, which corresponded to the ester carbon on Lev moiety. This confirmed the presence of Lev group as well.

In addition, HR-ESI-MS of the product showed a molecular ion m/z value of 918.3589 corresponding to $[M+H]^+$. This supported the molecular formula of $C_{44}H_{60}NO_{18}Si$ (m/z calcd. for $C_{44}H_{60}NO_{18}Si$: 918.3580). Based on all these data, the product was identified as orthogonally protected 2,1':4,6-di-*O*-isopropylidene sucrose (OP-diisopropylidene) **224**.

3.4.4 Selective deprotection of OP-diisopropylidene **224**

To prove the orthogonality of OP-diisopropylidene **224**, selective deprotection of TBS, Cbz, PNB and Lev groups need to be achieved. Details of the deprotection conditions are discussed in the following subsections.

3.4.4.1 Selective deprotection of the TBS group

Selective removal of TBS group was first attempted by treating OP-diisopropylidene **224** with 1.6 equivalents of tetra-*n*-butylammonium fluoride (TBAF) at pH 7 potassium buffer/THF.¹³⁹ The reason why buffered medium was used is to prevent possible migration of the secondary O-3' Cbz or O-4' PNB groups to primary O-6' position during the deprotection. However, this reaction still gave multiple product spots as indicated by TLC analysis. The formation of multiple products may be due to transesterification and/or removal of other moieties. To resolve this issue, selective removal of the TBS group was attempted again by treating OP-diisopropylidene **224** with 3.0 equivalents of 1.56 M of 3HF·NEt₃ in pyridine at room temperature for 12 hours (Scheme 51).¹⁴⁰ A single product was obtained in 86% yield after column chromatography.

Reagents and conditions: i. 1.56 M 3HF·NEt₃ in pyridine, NEt₃ (2.0 equiv), pyridine, rt, 12 h

Scheme 51: Selective deprotection of TBS

The ¹H NMR of the product obtained showed the absence characteristic TBS peaks from δ 0.00 to 1.00 ppm (Figure 31). This indicated the successful removal of TBS group. In addition, the presence of characteristic Cbz, PNB, Lev and isopropylidene peaks indicated that the other protecting groups were not affected under these deprotection conditions. Migration of the protecting groups to primary 6'-OH did not occur since the proton peaks corresponding to the protons at C-3', C-4' and C-3 from δ 5.00 to 5.80 ppm were not shifted as compared to that in OP-diisopropylidene **224**.

Figure 31: ^1H NMR spectrum of compound **230**

^{13}C NMR also showed an absence of characteristic TBS carbon signals from δ -5.50 to 0.00 ppm. This confirmed the successful removal of TBS groups as well. Other than ^1H and ^{13}C NMR, the HR-ESI-MS of the product showed a molecular ion m/z value 826.2546, corresponding to $[\text{M}+\text{Na}]^+$. This supported the molecular formula of $\text{C}_{38}\text{H}_{45}\text{NO}_{18}\text{Na}$ (m/z calcd. for $\text{C}_{38}\text{H}_{45}\text{NO}_{18}\text{Na}$: 826.2534). Hence, the product was confirmed as compound **230**. Therefore, selective deprotection of the TBS group was thus achieved successfully without affecting other protecting groups. As such, selective removal of the Cbz group was attempted next.

3.4.4.2 Selective deprotection of the Cbz group

To avoid potential reduction of *trans*-alkenyl group on phenylpropanoid groups which will be introduced later during the synthesis of PSEs, traditional methods for removal of Cbz by hydrogenation are not possible. Instead, the selective removal of the Cbz group was attempted by thermal treatment of OP-diisopropylidene **224** in water or 1,4-dioxane at 100 $^\circ\text{C}$.¹³⁶ Reaction in water failed as OP-diisopropylidene **224** was insoluble in water. Reaction in 1,4-dioxane, on the other hand, had no solubility issues. However, the reaction failed as well since OP-diisopropylidene **224** had degraded instead. As such, another set of deprotection conditions was needed.

Using conditions reported by Coleman and coworkers,¹⁴¹ the selective removal of the Cbz from OP-isopropylidene **224** was attempted by treating it with 1.6 equivalents of Et₃SiH in the presence of Pd(OAc)₂ and NEt₃ in CH₂Cl₂ at room temperature for 12 hours (Scheme 52). In the end, a single product was obtained in 68% yield.

Reagents and conditions: i. Pd(OAc)₂ (0.1 equiv), NEt₃ (0.16 equiv), Et₃SiH (1.6 equiv), CH₂Cl₂, rt, 12 h

Scheme 52: Selective deprotection of Cbz group

The structure of the product was confirmed by ¹H NMR. From Figure 32, the absence of characteristic Cbz at δ 5.17 and 7.35 ppm indicated the successful removal of Cbz group. Characteristic TBS, Lev, PNB and isopropylidene peaks were also observed in the spectrum, indicating the successful removal of Cbz group without affecting the other protecting groups.

Figure 32: ¹H NMR spectrum of compound 231

From its COSY NMR spectrum (Figure 33), a correlation between the triplet at δ 5.54 ppm and the doublet at δ 4.21 ppm was observed. This implied that they are neighbouring protons. Hence, the triplet at δ 5.54 ppm should correspond to the proton at C-4' while the doublet should correspond to the proton

at C-3'. Proton at C-3' shifted to δ 4.21 ppm as compared to that in OP-diisopropylidene **224** (δ 4.98 ppm). This upfield shift can be attributed to the removal of the electron withdrawing Cbz group. Since the proton at C-4' couples with the proton at C-3', the proton at C-4' shifted to δ 5.54 ppm as compared to that in OP-diisopropylidene **224** (δ 5.71 ppm) as well. All these indicated the Cbz group at O-3' was removed successfully and that no migration nor transesterification has occurred.

Only correlation between protons of C-3' and C-4' was highlighted

Figure 33: COSY spectrum of compound 231

^{13}C NMR of the product also showed absence of some aromatic carbon signals from δ 122.0 to 133.6 ppm, which corresponded to the carbons of the Cbz group. This implied the successful removal of Cbz group. The HR-ESI-MS of the product also showed a molecular ion m/z value at 806.3079, which corresponded to $[\text{M}+\text{Na}]^+$. This supported the molecular formula of $\text{C}_{36}\text{H}_{53}\text{NO}_{16}\text{NaSi}$ (m/z calcd. for $\text{C}_{36}\text{H}_{53}\text{NO}_{16}\text{NaSi}$: 806.3031). Based on the data, the product was assigned as compound **231**. With the successful selective deprotection of the Cbz group, selective deprotection of PNB group was attempted next.

3.4.4.3 Selective deprotection of the PNB group

Selective removal of PNB was attempted via treatment of OP-diisopropylidene **224** with 0.1 equivalent of $\text{Mg}(\text{OMe})_2$ in $\text{MeOH}:\text{THF}$ (8:2)¹³² at 4 °C (ice bath) for one hour (Scheme 53). Since $\text{Mg}(\text{OMe})_2$ is a weak base, the reaction was kinetically driven in the hope to selectively remove PNB in the presence of other acyl groups (i.e. Cbz and Lev). Upon completion (TLC), the reaction was immediately quenched with 1N HCl. Subsequently, the CH_2Cl_2 was removed and the crude product was purified using column chromatography. Two products were obtained in 70% and 15% yield.

Reagents and conditions: i. $\text{Mg}(\text{OMe})_2$ (0.1 equiv), $\text{MeOH}:\text{THF}$ (8:2), ice bath (4°C), 1 h

Scheme 53: Selective removal of PNB group

The ^1H NMR spectrum of the first product (Figure 34) revealed absence of characteristic PNB peaks from δ 8.00 to 8.50 ppm. This indicated the successful removal of PNB group. In addition, the presence of characteristic TBS, Lev, Cbz and isopropylidene peaks implied that the selective deprotection of the PNB group was achieved without affecting the other groups.

Figure 34: ^1H NMR spectrum of compound 232

From its COSY NMR spectrum (Figure 35), a correlation was observed between the doublet at δ 4.71 ppm and the triplet at δ 4.38 ppm. This implied that they are neighbouring protons. Therefore, the doublet should correspond to the proton at C-3' while the triplet should correspond to the proton at C-4'. Due to the removal of the electron withdrawing PNB group, the proton at C-4' position shifted to δ 4.38 ppm as compared to that in OP-diisopropylidene **224** (δ 5.71 ppm). This upfield chemical shift could be attributed to the removal of the electron withdrawing PNB group, implying that the PNB group at O-4' was successfully removed and that no transesterification has occurred during the reaction. Since the proton at C-3' couples with the proton at C-4', the proton at C-3' shifted to δ 4.71 ppm as compared to that in OP-diisopropylidene **224** (δ 4.98 ppm) as well. As for the proton at C-3, no change in the chemical shift was observed.

Only correlation between protons of C-3' and C-4' was highlighted

Figure 35: COSY spectrum of compound 232

^{13}C NMR of the product also showed absence of signals corresponding to the aromatic carbons of PNB group from δ 128.5 to 134.9 ppm. This implied the successful removal of PNB group. Other than that, the HR-ESI-MS of the product showed a molecular ion m/z value of 769.3460 corresponding to $[\text{M}+\text{Na}]^+$. This supported the molecular formula of $\text{C}_{37}\text{H}_{57}\text{NO}_{15}\text{Si}$ (m/z calcd. for $\text{C}_{37}\text{H}_{57}\text{NO}_{15}\text{Si}$: 769.3467). Based on the data, the product was identified as compound **232**.

As for the second product, the ^1H NMR spectrum also revealed an absence of the characteristic PNB peaks from δ 8.00 to 8.50 ppm (Figure 36). However, unlike the first product, an absence of characteristic Cbz peaks at δ 5.20 ppm and δ 7.20 to 7.50 ppm were observed as well. This implied a simultaneous removal of both PNB and Cbz groups on the second product. However, the other two protecting groups – TBS and Lev were not affected since their proton signals were still observed in the spectrum (Figure 36).

Figure 36: ^1H NMR spectrum of compound 233

Since the triplet at δ 5.20 ppm which corresponded to the proton at C-3 was not shifted as compared to that in compound **232**, it was implied that Lev group was still attached to O-3 and that no transesterification has occurred. Hence, the minor product was assigned as compound **233**.

Since compound **232** was obtained as the major product in 70% yield, the reaction was still considered a success since selective deprotection of PNB group could still be achieved. Since longer reaction times would lead to simultaneous cleavage of both PNB and Cbz groups, this reaction need to be monitored closely by TLC and quenched with 1N HCl immediately once the starting material had been consumed completely. Alternatively, the reaction can be extended to remove both PNB and Cbz groups if needed. With the successful removal of PNB group selectively, selective removal of Lev group was attempted next.

3.4.4.4 Selective deprotection of the Lev group

Selective removal of the Lev group was attempted by treating OP-diisopropylidene **224** with 2.2 equivalents of NaBH_4 in 1,4-dioxane at room temperature (Scheme 54).¹⁴² Upon completion, 1,4-dioxane was removed and the crude product was purified via column chromatography. A single product was obtained in 72% yield.

Reagents and conditions: i. NaBH₄ (2.2 equiv), 1,4-dioxane, rt, 3 h

Scheme 54: Selective removal of NaBH₄

The structure of the product was also confirmed using ¹H NMR. The spectrum revealed an absence of characteristic Lev peaks at δ 2.00 to 2.80 ppm and the presence of characteristic TBS, Cbz, PNB and isopropylidene peaks (Figure 37). These indicated the successful selective removal of the Lev group without affecting other protecting groups.

Figure 37: ¹H NMR Spectrum of compound 227

Comparison of the ¹H NMR spectrum of the product with that of 4'-O-PNB **227** confirmed the product to be 4'-O-PNB **227**. Hence, the selective removal of the Lev group was successful.

3.5 Summary

An orthogonal protection scheme was successfully developed. Orthogonal protection of 2,1':4,6-di-*O*-isopropylidene sucrose **68** was achieved in four steps using TBS, Cbz, PNB and Lev groups with an overall yield of 43% (Scheme 55).

Reagents and conditions: i. TBSCl (1.2 equiv), NEt₃ (2.4 equiv), DMAP (0.1 equiv), CH₂Cl₂, rt, 12 h; ii. CbzCl (1.5 equiv), TMEDA (3.0 equiv), DMAP (0.1 equiv), CH₂Cl₂, rt, 12 h; iii. PNBCl (1.5 equiv), NEt₃ (3.0 equiv), DMAP (0.1 equiv), CH₂Cl₂, ice bath at 4 °C, 12 h; iv. LevOH (1.2 equiv), DCC (1.2 equiv), DMAP (0.1 equiv), CH₂Cl₂, rt, 12 h

Scheme 55: Synthesis of OP-diisopropylidene 224

Subsequently, the orthogonality of this strategy was proven successful since selective removal of TBS, Cbz, PNB and Lev groups was achieved without affecting the other protecting groups and the diisopropylidene rings (Scheme 56).

Reagents and conditions: i. 1.56 M 3HF·NEt₃ in pyridine (3.0 equiv), NEt₃ (2.0 equiv), pyridine, rt, 12 h; ii. Pd(OAc)₂ (0.1 equiv), Et₃SiH (1.6 equiv), NEt₃ (0.16 equiv), CH₂Cl₂, rt, 12 h; iii. Mg(OMe)₂, (0.1 equiv), MeOH:THF (8:2), rt, 1 h; iv. NaBH₄ (2.2 equiv), 1,4-dioxane, rt, 3 h

Scheme 56: Selective deprotection of OP-diisopropylidene 224

OP-diisopropylidene **224** can be utilized as the starting material in subsequent acylation reactions to synthesize simple or mixed PSEs with different degrees of substitution, substituents at different positions (3'-, 4', 3- and 6'-positions) and different substitution patterns. This would give access to a wide range of both natural and synthetic PSE compounds, thus expanding the library of compounds for further screenings. The synthesis of mono-substituted PSEs at different positions following this orthogonal protection strategy will be discussed in the next chapter.

4 SAR Study: Mono-substituted PSEs

4.1 Introduction

To eliminate the side effects commonly associated with strong inhibition of α -amylase, we aimed to synthesize PSEs that only showed strong inhibition of α -glucosidase. To achieve this, a SAR study needs to be done to determine if any correlation exists between positions (O-3, O-3', O-4' and O-6') as well as the type of substituents and their inhibitory activities on both α -glucosidase and α -amylase. Using this information as guidelines, PSE(s) that inhibited α -glucosidase alone can be proposed and synthesized for further evaluation.

To start off, four series of mono-substituted PSEs with esters at different positions (O-3, O-3', O-4' and O-6') with different (substituted) cinnamoyl groups will be synthesized. After which, these mono-substituted PSEs will be tested for their inhibitory activities against both α -glucosidase and α -amylase using the *in vitro* enzyme assays (refer to experimental section). From the results obtained, the observations and the analysis will be made to establish SARs for mono-substituted PSEs.

Naming of the mono-substituted PSEs will not be following the same naming style that was developed previously. To highlight the position at which the substituent is attached, a new naming system was established. The new compound name comprises of two fields:

- The first field is a number representing the position at which the substituent is attached, for instance 3 for substituent attached to O-3, 3' for substituent attached to O-3', 4' for substituent attached to O-4' and 6' for substituent attached to O-6'
- The second field represents the type of substituent groups, for instance 'cinn' for cinnamoyl, 'coum' for coumaroyl, 'feru' for feruloyl, 'caff' for caffeoyl, 'diOMe' for dimethoxycinnamoyl, 'triOMe' for trimethoxycinnamoyl and 'sinap' for sinapoyl.

The naming system did not include an 'I' which denoted the presence the isopropylidene rings as all mono-substituted PSEs that were synthesized contained both isopropylidene rings on their sucrose cores. These isopropylidene rings were not removed since *in vitro* studies of tetra-substituted PSEs concluded the importance of having isopropylidene rings to achieve high α -glucosidase inhibition and low α -amylase inhibition.

4.2 The synthesis of mono-substituted PSEs

The synthesis of mono-substituted PSEs at different positions is challenging due to similar reactivities between 6'-OH, 3'-OH, 4'-OH and 3-OH. However, this issue had been resolved with the successful orthogonal protection scheme developed in Chapter 3. Using the orthogonal protection strategy, the synthesis of mono-substituted PSEs can be achieved at O-3, O-3', O-4' and O-6' respectively. The synthetic routes for all four series are described in the following subsections.

4.2.1 The protection of free hydroxyl groups on (substituted) cinnamic acid

Prior to the synthesis of mono-substituted PSEs, the free hydroxyl groups on coumaric acid **33**, caffeic acid **37**, ferulic acid **39** and sinapic acid **209** were protected to avoid reactions leading to undesired by-products. Since the synthesis of OTBS caff acid **198** and OTBS-coum acid **203** were already discussed previously (refer to 2.2.2.1 & 2.2.3.1), this section focused on the synthesis of other protected acids.

4.2.1.1 The synthesis of 4-*tert*-butyldimethylsilyloxy-3-methoxycinnamic acid

The TBS group can be used to temporary mask the hydroxyl group of ferulic acid **39**. To achieve this, ferulic acid **39** was reacted with 1.5 equivalents of TBSCl and DIPEA in CH₂Cl₂ at room temperature for 12 hours (Scheme 57). The reaction gave the product as white solid in 87% yield.

Reagents and conditions: i. (a) TBSCl (1.5 equiv), DIPEA (3.0 equiv), CH₂Cl₂, rt, 12 h; (b) K₂CO₃ (0.1 equiv), 9:1 THF/H₂O, rt, 4 h

Scheme 57: Synthesis of 4-*tert*-butyldimethylsilyloxy-3-methoxycinnamic acid

¹H NMR of the product revealed distinctive TBS peaks at δ 0.00 and 0.82 ppm. Integration of these peaks showed six and nine protons respectively, implying the presence of a single TBS group. The characteristic methoxy peak at δ 3.67 ppm was also observed and integration of this singlet showed three protons, confirming the presence of a single methoxy group. Based on its ¹H NMR spectrum, the product was identified as 4-*tert*-butyldimethylsilyloxy-3-methoxycinnamic (OTBS-feru) acid **234**. Comparison with reported values in literature further confirmed its structure¹⁴³.

4.2.1.2 The synthesis of 4- *tert*-butyldimethylsilyloxy-3,5-dimethoxycinnamic acid

The hydroxyl group of sinapic acid can also be protected with TBS group. To achieve this, sinapic acid **209** was reacted with 1.5 equivalents of TBSCl and DIPEA in CH₂Cl₂ at room temperature for 12 hours (Scheme 58). The reaction gave a white solid in 88% yield.

Reagents and conditions: i. (a) TBSCl (1.5 equiv), DIPEA (3.0 equiv), CH₂Cl₂, rt, 12 h (b) K₂CO₃ (0.1 equiv), 9:1 THF/H₂O

Scheme 58: 4- *tert*-butyldimethylsilyloxy-3,5-dimethoxycinnamic acid

The structure of the product was confirmed with ¹H NMR. The spectrum of the product revealed characteristic peaks of the TBS group at δ 0.13 and 0.993 ppm. Integration of these peaks showed six and nine protons respectively, indicating the presence of a single TBS group. The distinctive methoxy peak at δ 3.82 ppm was also observed. Integration of this peak showed six protons which confirmed the presence of two methoxy groups. Based on its spectroscopic data, the product was identified as 4- *tert*-butyldimethylsilyloxy-3,5-dimethoxycinnamic (OTBS-sinap) acid **235**. Comparison with the reported values in literature also confirmed the product to be OTBS-sinap acid **235**.¹⁴⁴

With all the free hydroxyl groups on (substituted) cinnamic acids masked with temporarily protecting groups, we proceeded with the synthesis of the mono-substituted PSEs.

4.2.2 The synthesis of 3-mono-substituted PSEs

4.2.2.1 The synthesis of 3-mono-substituted PSEs using 4'-O-PNB 227

The synthesis of 3-mono-substituted PSEs may be achieved following the proposed synthetic route as shown in Scheme 59.

Reagents and conditions: i. ROOH (2 equiv), DCC (2 equiv), DMAP (0.1 equiv), CH₂Cl₂, rt, 24 h; ii. Mg(OMe)₂ (0.5 equiv), 8:2 MeOH:THF, rt, 12 h; iii. 1.56 M 3HF·NEt₃ in pyridine (3.0 equiv), NEt₃ (2.0 equiv), pyridine, rt, 12 h;

Scheme 59: Proposed synthetic route for 3-monosubstituted PSEs

The synthesis of 3-mono-substituted PSEs with cinnamoyl, dimethoxycinnamoyl, trimethoxycinnamoyl, OTBS-caffeoyl, OTBS-feruloyl, OTBS-coumaroyl and OTBS-sinapoyl as substituent were attempted by treating 4'-O-PNB 227 with 2.0 equivalents of each (substituted) cinnamic acid in the presence of DCC and DMAP in CH₂Cl₂ at room temperature for 24 hours (Scheme 60). A single product was obtained for each reaction and the structures of the products obtained from each reaction were confirmed by ¹H NMR, ¹³C NMR and HR-ESI-MS.

Reagents and conditions: i. ROOH (2 equiv), DCC (2 equiv), DMAP (0.1 equiv), CH₂Cl₂, rt, 12 h

For 1st reaction: ROOH = cinnamic acid 30	236: R = cinn	76%
For 2nd reaction: ROOH = diOMe acid 38	237: R = diOMe	80%
For 3rd reaction: ROOH = triOMe acid 210	238: R = triOMe	81%
For 4th reaction: ROOH = diOTBS-caff acid 198	239: R = OTBS-caff	80%
For 5th reaction: ROOH = OTBS-feru acid 234	240: R = OTBS-feru	80%
For 6th reaction: ROOH = OTBS-coum acid 203	241: R = OTBS-coum	80%
For 7th reaction: ROOH = OTBS-sinap acid 235	242: R = OTBS-sinap	80%

Scheme 60: Acylation of 4'-O-PNB 227 with (substituted) cinnamic acid

The ¹H NMR spectra of all the products obtained from each reaction revealed characteristic peaks of the TBS, Cbz and PNB groups from δ 0.00 to 1.00 ppm, δ 5.30 & 7.20 ppm and δ 8.00 to 8.40 ppm respectively. Moreover, characteristic isopropylidene rings peaks were also observed from δ 1.23 to 1.46 ppm for each product. These confirmed that acylation reactions did not affect the protecting groups that were present on the sucrose core.

Other observations made for individual spectrum are shown in the following table (Table 8). Additional signals observed in ¹³C NMR spectrum and the HR-ESI-MS of each product are also shown in the same table.

Table 8: NMR and HR-ESI-MS analysis of compounds 236 to 242

No	Reaction with	¹ H NMR	¹³ C NMR	HR-ESI-MS	Product
1	Cinnamic acid 30	<p>trans-alkenyl and aromatic protons: δ 6.41 to 7.70 ppm</p> <p>Integration of these peaks showed 7 protons, confirming the presence of one cinnamoyl group.</p> <p>Due to the electron withdrawing effect of cinnamoyl group, the proton at C-3 shifted to around δ 5.35 ppm from that of 4'-O-PNB 227 (δ 3.50 – 4.20 ppm).</p>	New carbon signal at δ 165.9 ppm was observed. This peak corresponded to the ester carbon, hence confirming the presence of one cinnamoyl group.	The HR-ESI-MS of the product showed a molecular ion m/z value of 972.3431, which corresponded to [M+Na] ⁺ . This supported the molecular formula of C ₄₈ H ₅₉ NO ₁₇ NaSi (m/z calcd. for C ₄₈ H ₅₉ NO ₁₇ NaSi: 972.3450)	236

		Hence, the cinnamoyl group was attached to O-3.			
2	diOMe acid 38	<p>Methoxy protons: around δ 3.85 ppm Integration of the multiplet showed 6 protons which confirmed the presence of 2 methoxy groups. This indicated the presence of one dimethoxycinnamoyl group.</p> <p>trans-alkenyl and aromatic protons: δ 6.27 – 7.45 ppm Integration of these peaks showed five protons, confirming the presence of one dimethoxycinnamoyl group.</p> <p>Due to the electron withdrawing effect of dimethoxycinnamoyl group, the proton at C-3 shifted to around δ 5.35 ppm from that of 4'-O-PNB 227 (δ 3.50 – 4.20 ppm). Hence, the dimethoxycinnamoyl group was attached to O-3.</p>	New carbon signal at δ 165.6 ppm was observed. This peak corresponded to the ester carbon, hence confirming the presence of one dimethoxycinnamoyl group.	The HR-ESI-MS of the product showed a molecular ion m/z value of 1032.3684, which corresponded to $[M+Na]^+$. This supported the molecular formula of $C_{50}H_{63}NO_{19}NaSi$ (m/z calcd. for $C_{50}H_{63}NO_{19}NaSi$: 1032.3661)	237
3	triOMe acid 210	<p>Methoxy protons: around δ 3.86 ppm Integration of the multiplet showed 9 protons which confirmed the presence of 3 methoxy groups. This indicated the</p>	New carbon signal at δ 165.8 ppm was observed. This peak corresponded to the ester carbon, hence confirming the presence of one trimethoxycinnamoyl group.	The HR-ESI-MS of the product showed a molecular ion m/z value of 1062.3792, which corresponded to $[M+Na]^+$. This supported the molecular formula of $C_{51}H_{65}NO_{20}NaSi$	238

		<p>presence of one trimethoxycinnamoyl group.</p> <p><i>trans</i>-alkenyl and aromatic protons: δ 6.31 – 7.56 ppm Integration of these peaks showed four protons, confirming the presence of one trimethoxycinnamoyl group.</p> <p>Due to the electron withdrawing effect of trimethoxycinnamoyl group, the proton at C-3 shifted to around δ 5.33 ppm from that of 4'-O-PNB 227 (δ 3.50 – 4.20 ppm). Hence, the trimethoxycinnamoyl group was attached to O-3.</p>		<p>(m/z calcd. for $C_{51}H_{65}NO_{20}NaSi$: 1062.3767)</p>	
4	OTBS-caff acid 198	<p>Additional TBS proton peaks at: δ 0.00, 0.01 ppm and 0.77, 0.78 ppm. Integration of these peaks showed 12 and 18 protons respectively, suggesting the presence of two TBS groups. This confirmed the presence of one OTBS-caffeoyl group.</p> <p><i>trans</i>-alkenyl and aromatic protons: δ 6.01 – 7.39 ppm Integration of these peaks showed five protons, confirming the presence of one</p>	<p>New carbon signal at δ 163.3 ppm was observed. This peak corresponded to the ester carbon, hence confirming the presence of one OTBS-caffeoyl group.</p>	<p>The HR-ESI-MS of the product showed a molecular ion m/z value of 1232.5082, which corresponded to $[M+Na]^+$. This supported the molecular formula of $C_{60}H_{87}NO_{19}NaSi_3$ (m/z calcd. for $C_{60}H_{87}NO_{19}NaSi_3$: 1232.5078)</p>	239

		<p>OTBS-caffeoyl group.</p> <p>Due to the electron withdrawing effect of OTBS-caffeoyl group, the proton at C-3 shifted to around δ 5.15 ppm from that of 4'-O-PNB 227 (δ 3.50 – 4.20 ppm). Hence, the OTBS-caffeoyl group was attached to O-3.</p>			
5	OTBS-feru acid 234	<p>Additional TBS proton peaks at: δ 0.00 ppm and 0.83 ppm. Integration of these peaks showed six and nine protons respectively, suggesting the presence of one TBS group. This confirmed the presence of one OTBS-feruloyl group.</p> <p>trans-alkenyl and aromatic protons: δ 6.12 – 7.49 ppm. Integration of these peaks showed five protons, confirming the presence of one OTBS-feruloyl group.</p> <p>Due to the electron withdrawing effect of OTBS-feruloyl group, the proton at C-3 shifted to around δ 5.20 ppm from that of 4'-O-PNB 227 (δ 3.50 – 4.20 ppm). Hence, the OTBS-feruloyl group was attached to O-3.</p>	<p>New carbon signal at δ 166.1 ppm was observed. This peak corresponded to the ester carbon, hence confirming the presence of one OTBS-feruloyl group.</p>	<p>The HR-ESI-MS of the product showed a molecular ion m/z value of 1132.4365, which corresponded to $[M+Na]^+$. This supported the molecular formula of $C_{55}H_{75}NO_{19}NaSi_2$ (m/z calcd. for $C_{55}H_{75}NO_{19}NaSi_2$: 1132.4370)</p>	240

6	OTBS-coum acid 203	<p>Additional TBS proton peaks at: δ 0.20 ppm and 0.96 ppm. Integration of these peaks showed six and nine protons respectively, suggesting the presence of one TBS group. This confirmed the presence of one OTBS-coumaroyl group.</p> <p>trans-alkenyl and aromatic protons: δ 6.28 – 7.66 ppm. Integration of these peaks showed six protons, confirming the presence of one OTBS-coumaroyl group.</p> <p>Due to the electron withdrawing effect of OTBS-coumaroyl group, the proton at C-3 shifted to around δ 5.37 ppm from that of 4'-O-PNB 227 (δ 3.50 – 4.20 ppm). Hence, the OTBS-coumaroyl group was attached to O-3.</p>	New carbon signal at δ 157.8 ppm was observed. This peak corresponded to the ester carbon, hence confirming the presence of one OTBS-coumaroyl group.	The HR-ESI-MS of the product showed a molecular ion m/z value of 1102.4264, which corresponded to $[M+Na]^+$. This supported the molecular formula of $C_{54}H_{73}NO_{18}NaSi_2$ (m/z calcd. for $C_{54}H_{73}NO_{18}NaSi_2$: 1102.4232)	241
7	OTBS-sinap acid 235	<p>Additional TBS proton peaks at: δ 0.00 ppm and 0.87 ppm. Integration of these peaks showed six and nine protons respectively, suggesting the presence of one TBS group. This confirmed the presence of one</p>	New carbon signal at δ 166.1 ppm was observed. This peak corresponded to the ester carbon, hence confirming the presence of one OTBS-sinapoyl group.	The HR-ESI-MS of the product showed a molecular ion m/z value of 1162.4419, which corresponded to $[M+Na]^+$. This supported the molecular formula of $C_{56}H_{77}NO_{20}NaSi_2$ (m/z calcd. for $C_{56}H_{77}NO_{20}NaSi_2$: 1162.4475)	242

		<p>OTBS-sinapoyl group.</p> <p><i>trans</i>-alkenyl and aromatic protons: δ 6.16 to 7.50 ppm. Integration of these peaks showed four protons, confirming the presence of one OTBS-sinapoyl group.</p> <p>Due to the electron withdrawing effect of OTBS-sinapoyl group, the proton at C-3 shifted to around δ 5.24 ppm from that of 4'-O-PNB 227 (δ 3.50 – 4.20 ppm). Hence, the OTBS-sinapoyl group was attached to O-3.</p>			
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Based on the data (Table 8), the products formed from each reaction were confirmed as compounds **236** to **242** respectively. After acylation, the next step would be the removal of Cbz, PNB and TBS moieties.

Reagents and conditions: i. $\text{Mg}(\text{OMe})_2$ (0.5 equiv), 8:2 MeOH/THF, rt, 24 h; ii. $3\text{HF} \cdot \text{NEt}_3$ (3.0 equiv), NEt_3 (2.0 equiv), pyridine, rt, 12 h

Scheme 61: Removal of Cbz, PNB and TBS groups

Removal of Cbz and PNB groups on 3-mono-substituted PSEs were attempted by first treating compound **236** with 0.5 equivalent of $\text{Mg}(\text{OMe})_2$ in 8:2 MeOH/THF at room temperature for 24 hours (Scheme 61). This was the same reaction conditions shown in the selective deprotection of the PNB group (refer to 3.4.4.3), except that a higher equivalent of $\text{Mg}(\text{OMe})_2$ was used and that reaction times were longer. These were done to ensure that clean removal of both Cbz and PNB groups can be obtained.

An initial TLC analysis (after 1 hour of reaction) of the reaction showed two new spots. One of the spots should correspond to the product with the PNB group removed while the other spot should be the

product with both PNB and Cbz groups removed. After the completion of the reaction (where only the 2nd product was remaining), the reaction was quenched with 1.0 equivalent of 1N HCl before removing MeOH/THF. After which, we proceeded straight to the removal of TBS group(s) without any work-up nor purification.

The crude mixture was then dissolved in pyridine before adding 3.0 equivalents of 3HF·NEt₃ and 2.0 equivalents of Et₃N (Scheme 61). Subsequently, all the reactions were left to stir for 12 hours at room temperature. At the end of the reactions, pyridine was removed, and each reaction was subjected to chromatographic purification. A single product was obtained in 70% yield. The identity of the product was confirmed by ¹H NMR, ¹³C NMR and HR-ESI-MS.

From its ¹H NMR spectrum, absence of characteristic TBS, Cbz and PNB peaks from δ 0.00 to 1.00 ppm, δ 5.30 & 7.30 ppm and δ 8.00 to 8.40 ppm respectively confirmed the successful removal of all temporary protecting groups. In addition, the distinctive proton peaks corresponding to isopropylidene rings from δ 1.34 to 1.50 ppm were also observed, suggesting that the two isopropylidene rings were not affected during the deprotection reaction. The presence of *trans*-alkenyl and aromatic protons from δ 6.44 to 7.73 ppm confirmed that the cinnamoyl substituent was not affected during the deprotection reactions as well. Integration of these peaks showed seven protons, confirming the substituent as the cinnamoyl group.

Only correlation between protons of C-3' and C-4' will be highlighted

Figure 38: COSY spectrum of 3-cinn 243

From the COSY spectrum (Figure 38) of this product, the triplet at δ 5.44 ppm showed no correlation to the doublet which corresponded to C-3', suggesting that the triplet should correspond to the proton at C-3. This implied that no migration has occurred and that the cinnamoyl group was still attached to O-3.

Its ^{13}C NMR spectrum also confirmed the removal of TBS, Cbz and PNB moieties with the absence of characteristic carbon signals that corresponded to the carbons of these temporary protecting groups. Furthermore, the presence of the carbon signal at δ 166.3 ppm, which corresponded to an ester carbon, confirmed that the cinnamoyl moiety was not removed during the deprotection reaction. Moreover, the

HR-ESI-MS of the product showed a molecular ion m/z value of 553.2278, which corresponded to $[M+H]^+$. This supported the molecular formula of $C_{27}H_{37}O_{12}$ (m/z calcd. for $C_{27}H_{37}O_{12}$: 553.2285). Based on these data, the product was confirmed as 3-cinn **243**.

Reagents and conditions: i. $Mg(OMe)_2$ (0.5 equiv), 8:2 MeOH/THF, rt, 24 h; ii. $3HF \cdot NEt_3$ (3.0 equiv), NEt_3 (2.0 equiv), pyridine, rt, 12 h

237: R = diOMe, R^1 = PNB

238: R = triOMe, R^1 = PNB

239: R = OTBS-caff, R^1 = PNB

240: R = OTBS-feru, R^1 = PNB

241: R = OTBS-coum, R^1 = PNB

242: R = OTBS-sinap, R^1 = PNB

244: R = diOMe 69%

245: R = triOMe 71%

246: R = caff 62%

247: R = feru 70%

248: R = coum 67%

250: R = sinap 62%

Scheme 62: Removal of TBS, Cbz and PNB groups

Based on the successful synthesis of 3-cinn **243**, removal of TBS, Cbz and PNB groups were also attempted for compounds **237** to **242**. Each compound was treated with 0.5 equivalent of $Mg(OMe)_2$ in 8:2 MeOH/THF at room temperature for 24 hours respectively (Scheme 62). Upon completion, all reactions were quenched with 1.0 equivalent of 1N HCl. After which, MeOH/THF was removed and each of the crude mixtures was then 3.0 equivalents of 1.56 M $3HF \cdot NEt_3$ and 2.0 equivalents of NEt_3 in pyridine at room temperature for 12 hours (Scheme 62). A single product was obtained from each reaction. The structures of the products obtained were confirmed by 1H NMR, ^{13}C NMR and HR-ESI-MS.

1H NMR spectra of these products revealed similar observations as that of 3-cinn **243**. The spectra of the products obtained revealed the absence of characteristic TBS, Cbz and PNB proton peaks from δ 0.00 to 1.00 ppm, δ 5.30 & 7.30 ppm and δ 8.00 to 8.40 ppm respectively, implying the successful removal of all three temporary protecting groups. In addition, the presence of proton peaks corresponding to the isopropylidene rings from δ 1.10 to 1.50 ppm confirmed that the isopropylidene rings were not affected during the deprotection reactions. Other observations made from individual spectrum of the products obtained were noted in the following table (Table 9).

Table 9: NMR and HR-ESI-MS analysis of compounds 244 to 250

No	Starting material	¹ H NMR / COSY NMR	¹³ C NMR	HR-ESI-MS	Product
1	237	<p>Methoxy peak: δ 3.80 ppm Integration of this peak showed six protons which indicated the presence of two methoxy groups. This confirmed the presence of one dimethoxycinnamoyl group</p> <p>trans-alkenyl and aromatic protons: δ 6.38 to 7.62 ppm. Integration of these peaks showed five protons which confirmed the presence of one dimethoxycinnamoyl group</p> <p>COSY spectrum confirmed the triplet at δ 5.31 ppm to be the proton at C-3. Hence no migration was observed.</p>	<p>¹³C NMR spectrum of the product showed the absence of distinctive carbon signals corresponding to carbons of TBS, Cbz and PNB groups.</p> <p>The presence of the signal at δ 170.9 ppm, which corresponded to an ester carbon, confirmed that the dimethoxycinnamoyl moiety was not removed during deprotection</p>	<p>HR-ESI-MS of the product showed a molecular ion <i>m/z</i> value of 613.2468, which corresponded to [M+H]⁺. This supported the molecular formula of C₂₉H₄₁O₁₄ (<i>m/z</i> calcd. for C₂₉H₄₁O₁₄: 613.2496).</p>	3-diOMe 244
2	238	<p>Methoxy peak: δ 3.81 ppm Integration of this multiplet showed nine protons which indicated the presence of three methoxy groups. This confirmed the presence of one trimethoxycinnamoyl group</p> <p>trans-alkenyl and aromatic protons: δ 6.20 to 7.50 ppm Integration of these peaks showed four protons which</p>	<p>¹³C NMR spectrum of the product showed the absence of distinctive carbon signals corresponding to carbons of TBS, Cbz and PNB groups.</p> <p>The presence of the signal at δ 169.1 ppm, which corresponded to an ester carbon, confirmed that the trimethoxycinnamoyl moiety was not removed during</p>	<p>HR-ESI-MS of the product showed a molecular ion <i>m/z</i> value of 665.2421, which corresponded to [M+Na]⁺. This supported the molecular formula of C₃₀H₄₂O₁₅Na (<i>m/z</i> calcd. for C₃₀H₄₂O₁₅Na: 665.2421).</p>	3-triOMe 245

		confirmed the presence of one trimethoxycinnamoyl group COSY spectrum confirmed the triplet at δ 5.37 ppm to be the proton at C-3. Hence no migration was observed.	deprotection reactions		
3	239	No distinctive proton peaks corresponding to TBS group were observed. This confirmed that the TBS groups on caffeoyl moiety was removed successfully. trans-alkenyl and aromatic protons: δ 6.12 to 7.44 ppm Integration of these peaks showed five protons which confirmed the presence of one caffeoyl group COSY NMR confirmed the triplet at δ 5.19 ppm to be proton at C-3. Hence no migration was observed.	^{13}C NMR spectrum of the product showed the absence of distinctive carbon signals corresponding to carbons of TBS, Cbz and PNB groups. The presence of the signal at δ 165.9 ppm, which corresponded to an ester carbon, confirmed that the caffeoyl moiety was not removed during deprotection reactions.	HR-ESI-MS of the product showed a molecular ion m/z value of 607.2007, which corresponded to $[\text{M}+\text{Na}]^+$. This supported the molecular formula of $\text{C}_{27}\text{H}_{36}\text{O}_{14}\text{Na}$: (m/z calcd. for $\text{C}_{27}\text{H}_{36}\text{O}_{14}\text{Na}$: 607.2003).	3-caff 246
4	240	No distinctive proton peaks corresponding to TBS group were observed. This confirmed that the TBS groups on feruloyl moiety was removed successfully. Methoxy peak: δ 3.86 ppm Integration of this multiplet showed three protons, which	^{13}C NMR spectrum of the product showed the absence of distinctive carbon signals corresponding to carbons of TBS, Cbz and PNB groups. The presence of the signal at δ 166.5 ppm, which corresponded to an ester carbon, confirmed that the	HR-ESI-MS of the product showed a molecular ion m/z value of 621.2175, which corresponded to $[\text{M}+\text{Na}]^+$. This supported the molecular formula of $\text{C}_{28}\text{H}_{38}\text{O}_{14}\text{Na}$: (m/z calcd. for $\text{C}_{28}\text{H}_{38}\text{O}_{14}\text{Na}$: 621.2159).	3-feru 247

		<p>indicated one methoxy group. This confirmed the presence of one feruloyl group.</p> <p>trans-alkenyl and aromatic protons: δ 6.17 to 7.56 ppm Integration of these peaks showed five protons which confirmed the presence of one feruloyl group</p> <p>COSY NMR confirmed the triplet at δ 5.31 ppm to be the proton at C-3. Hence no migration was observed.</p>	feruloyl moiety was not removed during deprotection reactions.		
5	241	<p>No distinctive proton peaks corresponding to TBS group were observed. This confirmed that the TBS groups on coumaroyl moiety was removed successfully.</p> <p>Methoxy peak: δ 4.00 ppm Integration of this multiplet showed six protons, which indicated two methoxy groups. This confirmed the presence of one sinapoyl group.</p> <p>trans-alkenyl and aromatic protons: δ 6.17 to 7.55 ppm Integration of these peaks showed four protons which confirmed the presence of one sinapoyl group</p>	<p>^{13}C NMR spectrum of the product showed the absence of distinctive carbon signals corresponding to carbons of TBS, Cbz and PNB groups.</p> <p>The presence of the signal at δ 165.8 ppm, which corresponded to an ester carbon, confirmed that the coumaroyl moiety was not removed during deprotection reactions.</p>	HR-ESI-MS of the product showed a molecular ion m/z value of 569.2232, which corresponded to $[\text{M}+\text{H}]^+$. This supported the molecular formula of $\text{C}_{27}\text{H}_{37}\text{O}_{13}$: (m/z calcd. for $\text{C}_{27}\text{H}_{37}\text{O}_{13}$: 569.2234).	3-coum 248

		<p>COSY NMR confirmed the triplet at δ 5.34 ppm to be the proton at C-3. Hence no migration was observed.</p>			
6	242	<p>No distinctive proton peaks corresponding to TBS group were observed. This confirmed that the TBS groups on sinapoyl moiety was removed successfully.</p> <p><i>trans</i>-alkenyl and aromatic protons: δ 6.30 to 7.62 ppm Integration of these peaks showed six protons which confirmed the presence of one coumaroyl group</p> <p>COSY NMR confirmed the triplet at δ 5.30 ppm to be the proton at C-3. Hence no migration was observed.</p>	<p>^{13}C NMR spectrum of the product showed the absence of distinctive carbon signals corresponding to carbons of TBS, Cbz and PNB groups.</p> <p>The presence of the signal at δ 166.3 ppm, which corresponded to an ester carbon, confirmed that the sinapoyl moiety was not removed during deprotection reactions.</p>	<p>HR-ESI-MS of the product showed a molecular ion m/z value of 651.2281, which corresponded to $[\text{M}+\text{Na}]^+$. This supported the molecular formula of $\text{C}_{29}\text{H}_{40}\text{O}_{15}\text{Na}$: ($m/z$ calcd. for $\text{C}_{29}\text{H}_{40}\text{O}_{15}\text{Na}$: 651.2265).</p>	3-sinap 249

Based on the data from Table 9, the products from these three reactions were confirmed as 3-diOMe **245**, 3-triOMe **246**, 3-caff **247**, 3-feru **247**, 3-coum **248** and 3-sinap **249**. With that, the synthesis of the 3-mono-substituted PSEs series with different phenylpropanoid groups was completed. Next, the synthesis of 3'-mono-substituted PSEs will be discussed in the following subsection.

4.2.3 The synthesis of 3'-mono-substituted PSEs

4.2.3.1 Route 1: Synthesis of 3'-mono-substituted PSEs using di-*O*-PNB **229**

The synthesis of 3'-mono-substituted PSEs can be achieved following the synthetic route as shown in Scheme 63.

Scheme 63: Proposed synthetic route for synthesis of 3'-mono-substituted PSEs

Following the proposed synthetic route, removal of Cbz group on di-*O*-PNB **229** was attempted by treating it with 1.6 equivalents of Et₃SiH in the presence of NEt₃ and Pd(OAc)₂ in CH₂Cl₂ at room temperature for 12 hours (Scheme 64). A single product was obtained in 76% yield.

Reagents and conditions: i. Et₃SiH (1.6 equiv), NEt₃ (0.16 equiv), Pd(OAc)₂ (0.1 equiv), CH₂Cl₂, rt, 12 h

Scheme 64: Removal of Cbz from di-*O*-PNB **229**

The structure of this product was confirmed by ¹H NMR. From its spectrum, the absence of characteristic Cbz peaks at δ 5.30 ppm and 7.30 ppm was observed. This implied the successful removal

of the Cbz group. On the other hand, the distinctive proton peaks of TBS, isopropylidene rings and PNB were still observed from δ 0.00 to 1.00 ppm, δ 1.10 to 1.50 ppm and δ 8.00 to 8.40 ppm respectively. Integration of these peaks confirmed the presence of one TBS group, two isopropylidene rings and two PNB groups. This confirmed that the selective removal of Cbz group was achieved successfully without affecting the other temporary protecting groups. In addition, no migration has occurred since the proton at C-3 and the proton at C-4' were not shifted as compared to that in di-*O*-PNB **229**. Hence, the product was confirmed as compound **250**.

Acylation reaction was then attempted by reacting compound **250** with 1.5 equivalents of diOMe acid **38** in the presence of DCC and DMAP in CH_2Cl_2 at room temperature for 12 hours. A single product was obtained in 86% yield.

Reagents and conditions: i. diOMe acid **38** (1.5 equiv), DCC (1.5 equiv), DMAP (0.1 equiv), CH_2Cl_2 , rt, 12 h

Scheme 65: The acylation of compound **250** with diOMe acid **38**

The ^1H NMR spectrum of the product revealed the distinctive proton peaks corresponding to TBS, PNB and isopropylidene rings, indicating that these groups were not affected during acylation reaction. In addition, integration of the *trans*-alkenyl and aromatic proton peaks from δ 6.41 to 7.82 ppm showed five protons, which confirmed the presence of one dimethoxycinnamoyl group. Furthermore, integration of the distinctive methoxy peak at around δ 3.88 ppm showed six protons, implying the presence of two methoxy groups. This further confirmed the presence of one dimethoxycinnamoyl group.

The COSY spectrum of the product revealed a correlation between the doublet at δ 5.34 ppm and the triplet at δ 5.81 ppm, implying that they are neighbouring protons. Hence, the doublet should correspond to the proton at C-3' while the triplet should correspond to the proton at C-4'. From the spectrum, the proton at C-3' shifted to δ 5.34 ppm as compared to that in di-*O*-PNB **229** (δ 3.50 to 4.20 ppm). This downfield shift can be attributed to the presence of electron withdrawing dimethoxycinnamoyl group, hence implying that the dimethoxycinnamoyl substituent was attached to O-3'. Based on the ^1H and COSY NMR spectra, the product obtained was identified as compound **251**.

Removal of the PNB groups was then attempted by treating compound **251** with 0.1 equivalent of $\text{Mg}(\text{OMe})_2$ in 8:2 MeOH/THF at 4°C (using an ice bath) for an hour (Scheme 66). The reaction was

closely monitored with TLC and quenched immediately with 0.2 equivalent of 1N HCl once the starting material had been consumed. At the end, two products were obtained in 45% and 40% yield.

Reagents and conditions: i. Mg(OMe)₂ (0.5 equiv), 8:2 MeOH/TH, 4°C (ice bath), 1h

Scheme 66: Removal of PNB groups with Mg(OMe)₂

From the ¹H NMR analysis, the first product showed the removal of one PNB while the second product showed the removal of one PNB group and the dimethoxycinnamoyl group. In both products, the PNB group at O-3 was not removed. This implied that the selective removal of both PNB groups cannot be achieved without the removal of dimethoxycinnamoyl group first. As such, this reaction failed since the desired product was not obtained. This failure also prompted us to reconsider the use of di-*O*-PNB **229** as starting material since the selective removal of both PNB groups cannot be achieved without affecting the phenylpropanoid substituent.

4.2.3.2 Route 2: Synthesis of 3'-mono-substituted PSEs using di-*O*-Lev 254

With the failure of the previous synthetic route, another synthetic route was proposed (Scheme 67).

Reagents and conditions: i. Pd(OAc)₂ (0.1 equiv), NEt₃ (0.16 equiv), EtSiH (1.6 equiv), CH₂Cl₂, rt, 12 h; ii. ROOH (1.5 equiv), DCC (1.5 equiv), DMAP (0.1 equiv), rt, CH₂Cl₂; iii. NaBH₄ (4.2 equiv), 1,4-dioxane, rt, 4 h; iv. 1.56 M 3HF·NEt₃ (3.0 equiv), NEt₃ (2.0 equiv), pyridine, rt, 12 h.

Scheme 67: Proposed synthetic route for synthesis of 3'-mono-substituted PSEs

This synthetic route uses di-*O*-Lev **254** as the starting material with the hope that the selective removal of Lev groups can be achieved without the removal of the phenylpropanoid substituent first.

The synthesis of di-*O*-Lev **254** was first attempted by reacting 3'-*O*-Cbz **225** with 2.2 equivalents of LevOH in the presence of DCC and DMAP in CH₂Cl₂ at room temperature for 12 hours (Scheme 68). A white product was obtained in 81% yield.

Scheme 68: Synthesis of di-*O*-Lev 254

In addition to the characteristic proton peaks corresponding to TBS, Cbz and the isopropylidene rings, the ¹H NMR spectrum revealed characteristic peaks of Lev groups from δ 2.10 to 2.80 ppm. Integration

of these peaks showed 14 protons, which confirmed the presence of two Lev moieties. The ^1H NMR spectrum also showed a multiplet at δ 5.16 ppm and a doublet at δ 4.08 ppm. Integration of this multiplet showed two protons, while integration of the doublet showed one proton. From its COSY NMR spectrum, a correlation between the doublet at δ 4.08 ppm and the multiplet δ 5.16 ppm was observed. This implied that they are neighbouring protons, hence one of the protons of the multiplet at δ 5.16 ppm should correspond to the proton at C-4', while the doublet should correspond to the proton at C-3'. From the other correlations observed, the other proton should correspond to the proton at C-3.

Both protons at C-3 and C-4' shifted to δ 5.16 ppm as compared to that in 3'-O-Cbz **225** (from δ 3.50 to 4.20 ppm). This downfield shift can be attributed to the electron withdrawing effects of Lev groups, indicating that the two Lev groups were attached to O-3 and O-4' respectively. Hence, the product was confirmed as di-O-Lev **254**. Comparison of the ^1H NMR spectrum with that of OP-diisopropylidene **224** further confirmed the product as di-O-Lev **254**.

Removal of the Cbz group on di-O-Lev **254** was then attempted by treating it with 1.6 equivalents of Et_3SiH in the presence of $\text{Pd}(\text{OAc})_2$ and NEt_3 in CH_2Cl_2 at room temperature for 12 hours (Scheme 69). A single product was obtained in 75% yield.

Scheme 69: Removal of Cbz group on di-O-Lev 255

The ^1H NMR spectrum of this product showed the absence of the characteristic proton peaks of Cbz group at δ 5.30 and 7.30 ppm respectively. This implied the successful removal of the Cbz group. Furthermore, the presence of the characteristic proton peaks corresponding to TBS, Lev and isopropylidene rings confirmed that the selective removal of the Cbz group was achieved successfully without affecting the other protecting moieties. From the spectrum, it was also observed that the peaks corresponding to the proton at C-3 and the proton at C-3' were not shifted. Hence, this confirmed that no migration has occurred during the deprotection reaction. From these data, the product was confirmed as compound **255**.

To check if the selective removal of Lev groups could be achieved without affecting the phenylpropanoid substituent, we attempted the synthesis of 3'-mono-substituted PSEs with compound **255** using cinnamic acid **30**. Compound **255** was reacted with 1.5 equivalents of cinnamic acid **30** in

the presence of DCC and DMAP in CH₂Cl₂ at room temperature for 12 hours (Scheme 70). A single product was obtained in 87% yield.

Reagents and conditions: i. cinnamic acid (1.5 equiv), DCC (1.5 equiv), DMAP (0.1 equiv), CH₂Cl₂, rt, 12 h

Scheme 70: Synthesis of 3'-mono-substituted PSE 262

The structure of this product was confirmed by ¹H NMR and COSY NMR. In addition to the presence of the distinctive proton peaks of TBS, Lev and isopropylidene rings, additional *trans*-alkenyl and aromatic proton peaks corresponding to that of the cinnamoyl group were observed from δ 6.46 to 7.83 ppm. Integration of these additional proton peaks showed seven protons, which confirmed the presence of one cinnamoyl group. Its ¹H NMR spectrum also revealed the triplet at δ 5.45 ppm and the multiplet at δ 5.11 ppm. Integration of these two peaks showed one proton and two protons respectively. Subsequently, COSY NMR was done to determine the position in which the cinnamoyl moiety was attached to (Figure 39).

Only correlation between protons of C-3' and C-4' was highlighted

Figure 39: COSY NMR spectrum of compound 256

From its COSY spectrum (Figure 39), a correlation between the triplet at δ 5.45 ppm and the multiplet at δ 5.11 ppm. This implied that they are neighbouring protons, hence the triplet should correspond to the proton at C-4', while one of the protons of the multiplet should correspond to the proton at C-3'.

The proton at C-3' shifted to δ 5.11 ppm as compared to that in compound **255** (δ 4.08 ppm). This downfield shift can be attributed to the presence of electron withdrawing cinnamoyl group, hence indicating that the cinnamoyl moiety was attached to O-3'. Since the proton at O-4' couples with the proton at O-3', it shifted to δ 5.45 ppm as compared to that in compound **255** (δ 5.16 ppm). Based on these data, the product was confirmed as compound **256**.

Selective removal of Lev groups was then attempted by treating compound **256** with 4.2 equivalents of NaBH_4 in 1,4-dioxane at room temperature for 4 hours (Scheme 71). A single product was obtained in 79% yield.

Reagents and conditions: i. NaBH₄ (4.2 equiv), 1,4-dioxane, rt, 12 h

Scheme 71: Selective removal of Lev moieties

Structure of the product was confirmed by ¹H NMR. The spectrum revealed the absence of the characteristic Lev proton peaks from δ 2.10 to 2.80 ppm, implying the successful removal of both Lev moieties. In addition, the presence of *trans*-alkenyl and aromatic proton peaks from δ 6.46 to 7.83 ppm confirmed that the cinnamoyl group was not removed during the deprotection reaction. This indicated that the selective removal of Lev groups can be achieved without compromising the cinnamoyl group. In addition, the presence of the characteristic proton peaks corresponding to the TBS group and isopropylidene rings confirmed that the selective removal of the Lev groups did not affect these protecting groups.

Figure 40: COSY spectrum of compound 257

From its COSY spectrum (Figure 40), a correlation was observed between the doublet at δ 5.49 ppm and the triplet at δ 5.13 ppm. This implied that they are neighbouring protons. As such, the doublet should correspond to the proton at C-3' while the triplet should correspond to the proton at C-4'. Due to the removal of electron-withdrawing Lev moieties, both protons at C-4' and C-3 shifted upfield as compared to that in compound **256**. Since the proton at C-3' was not shifted like the other two protons, it can be concluded that the cinnamoyl group was still attached to O-3' and that no migration has

occurred during deprotection reaction. Based on the ^1H and COSY NMR spectra, the product was confirmed as compound **257**. With compound **257**, the last step would be to remove TBS group (Scheme 72).

Reagents and conditions: i. 1.56M $3\text{HF}\cdot\text{NEt}_3$ in pyridine (3.0 equiv), NEt_3 (2.0 equiv), pyridine, rt, 12 h

Scheme 72: Removal of TBS group

However, this reaction was not followed through since another synthetic route which offered greater efficiency was proposed. This newly proposed synthetic will be discussed in the following subsection.

4.2.3.3 Route 3: Synthesis of 3'-mono-substituted PSEs using compound 259

The newly proposed synthetic route for the synthesis of 3'-mono-substituted PSEs is shown in Scheme 73. This new synthetic route is better since it consists of lesser deprotection steps and hence offers greater efficiency in the synthesis of 3'-mono-substituted PSEs.

Reagents and conditions: i. 1,3-Dichloro-1,1,3,3-tetraisopropylidisiloxane (1.1 equiv), pyridine, rt, 6 h; ii. ROOH (1.5 equiv), DCC (1.5 equiv), DMAP (0.1 equiv), CH₂Cl₂, rt, 12 h; iii. 1.56 M 3HF·NEt₃ in pyridine (3.0 equiv), NEt₃ (2.0 equiv), rt, 12 h

Scheme 73: Proposed synthetic route of 3'-mono-substituted PSEs

To achieve the synthesis of TIPDS-diacetonide **259**, 2,1':4,6-di-*O*-isopropylidene sucrose **68** was reacted with 1.1 equivalents of 1,3-dichloro-1,1,3,3-tetraisopropylidisiloxane in pyridine at room temperature for 6 hours (Scheme 74). After completion, pyridine was removed. The crude mixture was then purified with column chromatography. A product was obtained in 75%. The identity of this product was confirmed by ¹H NMR, ¹³C NMR and HR-ESI-MS.

Reagents and conditions: i. 1,3-dichloro-1,1,3,3-tetraisopropylidisiloxane (1.1 equiv), pyridine, rt, 6 h

Scheme 74: Synthesis of TIPDS-diacetonide 259

In addition to the presence of characteristic proton peaks of isopropylidene rings, the ¹H NMR spectrum showed characteristic proton peaks of tetraisopropylidisilyl (TIPDS) from δ 0.81 to 1.11 ppm. Integration of this multiplet revealed 28 protons, indicating the presence of one TIPDS group.

Comparison with the reported literature values of a similar compound confirmed that the TIPDS group was attached at O-4' and O-6' positions.¹⁴⁵

The ¹³C NMR spectrum of the product also revealed additional carbon signals from δ 12.5 to 25.4 ppm as compared to that of 2,1':4,6-di-*O*-isopropylidene sucrose **68**. These additional carbon signals corresponded to the carbons of TIPDS group, hence confirming the presence of the TIPDS group on the product. Furthermore, the HR-ESI-MS of the product showed a molecular ion m/z value of 687.3197, which corresponded to $[M+Na]^+$. This supported the molecular formula of C₃₀H₅₆O₁₂NaSi₂ (m/z calcd. for C₃₀H₅₆O₁₂NaSi₂: 687.3208). From these data acquired, the product was assigned as TIPDS-diacetonide **259**

Acylation reactions were then attempted by treating TIPDS-diacetonide **259** with 1.5 equivalents of different (substituted) cinnamic acid in the presence of DCC and DMAP in CH₂Cl₂ at room temperature for 12 hours (Scheme 75). For each reaction, a single product was obtained with the yields as shown in Scheme 75. The structures of these products were confirmed by ¹H NMR, ¹³C NMR and HR-ESI-MS.

Reagents and conditions: i. ROOH (1.5 equiv), DCC (1.5 equiv), DMAP (0.1 equiv), CH₂Cl₂, rt, 12 h

ROOH = cinnamic acid **30** or OTBS-coum acid **203** or OTBS-feru **234** or OTBS-sinap **235** or OTBS-caff **198** or diOMe acid **38** or triOMe acid **210**

Scheme 75: Acylation reactions of TIPDS-diacetonide **259**

The presence of characteristic proton peaks corresponding to TIPDS group and isopropylidene rings from δ 0.90 to 1.60 ppm were observed across the ¹H NMR spectra of all products. Other observations made from the spectrum of individual products are noted in Table 10.

Table 10: NMR and HR-ESI-MS Analysis of compounds 260 to 266

No	Reaction with	¹ H NMR Spectrum/ COSY Spectrum ^a	¹³ C NMR	HR-ESI-MS	Product
1	Cinnamic acid 30	<p><i>trans</i>-alkenyl and aromatic protons: δ 6.50 to 7.90 ppm Integration of these peaks give seven protons, which confirmed the presence of one cinnamoyl group.</p> <p>COSY NMR: A correlation between the doublet at δ 5.19 ppm and the triplet at δ 4.68 ppm was observed, implying that they are neighbouring protons. Hence, the doublet at δ 5.19 ppm should correspond to the proton at C-3'. Due to the electron withdrawing effect of cinnamoyl group, the proton at C-3' shifted to δ 5.19 ppm as compared to that in TIPDS-diacetonide 259 (δ 3.50 to 4.20 ppm) This implied that the cinnamoyl group was attached to O-3'.</p>	Additional signal at δ 165.1 ppm was observed in the spectrum. This new signal corresponded to the ester carbon, hence confirming the presence of the cinnamoyl group.	The HR-ESI-MS of the product showed a molecular ion <i>m/z</i> value of 817.3598, which corresponded to [M+Na] ⁺ . This supported the molecular formula of C ₃₉ H ₆₂ O ₁₃ NaSi ₂ (<i>m/z</i> calcd. for C ₃₉ H ₆₂ O ₁₃ NaSi ₂ : 817.3627)	260
2	OTBS-coum acid 203	TBS group: δ 0.06 to 1.11 ppm (overlapped with TIPDS). Integration of these peaks showed 43 protons, implying the presence of	Additional signal at δ 171.3 ppm was observed in the spectrum. This new signal corresponded to the ester carbon,	The HR-ESI-MS of the product showed a molecular ion <i>m/z</i> value of 947.4484,	261

		<p>one TBS and one TIPDS moieties.</p> <p>This confirmed the presence of one OTBS-coumaroyl group</p> <p><i>trans</i>-alkenyl and aromatic protons: δ 6.33 to 7.72 ppm</p> <p>Integration of these peaks showed six protons. This confirmed the presence of one OTBS-coumaroyl group.</p> <p>COSY NMR:</p> <p>Correlation between the doublet at δ 5.15 ppm and the triplet at δ 4.64 ppm was observed, implying that they are neighbouring protons. Hence, the doublet at δ 5.15 ppm should correspond to the proton at C-3'.</p> <p>Due to the electron withdrawing effect of OTBS-coumaroyl group, the proton at C-3' shifted to δ 5.15 ppm as compared to that in TIPDS-diacetonide 259 (δ 3.50 - 4.20 ppm). This implied that the OTBS-coumaroyl group was attached to O-3'.</p>	<p>hence confirming the presence of the OTBS-coumaroyl group.</p>	<p>which corresponded to $[M+Na]^+$. This supported the molecular formula of $C_{45}H_{76}O_{14}NaSi_3$ (m/z calcd. for $C_{45}H_{76}O_{14}NaSi_3$: 947.4441)</p>	
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3	OTBS-feru acid 234	<p>TBS group: δ 0.15 to 1.10 ppm (overlapped with TIPDS)</p> <p>Integration of these peaks showed 43 protons, implying the presence of one TBS and one TIPDS moieties.</p> <p>These confirmed the presence of one OTBS-feruloyl group</p> <p>Methoxy peak: δ 3.82 ppm</p> <p>Integration of this peak showed three protons, implying the presence of one methoxy group. This confirmed the presence of one OTBS-feruloyl group.</p> <p>trans-alkenyl and aromatic protons: 6.31 to 7.71 ppm. Integration of these peaks showed five protons, confirming the presence of one OTBS-feruloyl group.</p> <p>COSY NMR:</p> <p>Correlation between the doublet at δ 5.15 ppm and the triplet at δ 4.63 ppm was observed, implying that they are neighbouring protons. Hence, the doublet at δ 5.15</p>	Additional signal at δ 166.7 ppm was observed in the spectrum. This new signal corresponded to the ester carbon, hence confirming the presence of the OTBS-feruloyl group.	<p>The HR-ESI-MS of the product showed a molecular ion m/z value of 955.4745, which corresponded to $[M+Na]^+$. This supported the molecular formula of $C_{46}H_{79}O_{15}NaSi_3$ (m/z calcd. for $C_{46}H_{79}O_{15}NaSi_3$: 955.4727)</p>	262
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		<p>ppm should correspond to the proton at C-3'.</p> <p>Due to the electron withdrawing effect of OTBS-feruloyl group, the proton at C-3' shifted to δ 5.15 ppm as compared to that in TIPDS-diacetonide 259 (δ 3.50 - 4.20 ppm). This implied that the OTBS-feruloyl group was attached to O-3'.</p>			
4	OTBS-sinap acid 235	<p>Methoxy peak: δ 3.83 ppm Integration of this peak showed six protons, implying the presence of two methoxy groups. This confirmed the presence of one OTBS-sinapoyl group.</p> <p>TBS group: δ 0.05 to 1.11 ppm (overlapped with TIPDS). Integration of these peaks showed 43 protons, implying the presence of one TBS and one TIPDS moieties. This confirmed the presence of one OTBS-sinapoyl group.</p> <p><i>trans</i>-alkenyl and aromatic protons: δ 6.31 to 7.71 ppm</p>	Additional signal at δ 166.9 ppm was observed in the spectrum. This new signal corresponded to the ester carbon, hence confirming the presence of the OTBS-sinapoyl group.	<p>The HR-ESI-MS of the product showed a molecular ion m/z value of 1007.4688, which corresponded to $[M+Na]^+$. This supported the molecular formula of $C_{47}H_{80}O_{16}NaSi_3$ (m/z calcd. for $C_{46}H_{79}O_{15}NaSi_3$: 1007.4652)</p>	263

		<p>Integration of these peaks showed four protons, confirming the presence of one OTBS-sinapoyl group.</p> <p>COSY NMR:</p> <p>Correlation between the doublet at δ 5.16 ppm and the triplet at δ 4.63 ppm was observed, implying that they are neighbouring protons. Hence, the doublet at δ 5.16 ppm should correspond to the proton at C-3'.</p> <p>Due to the electron withdrawing effect of OTBS-sinapoyl group, the proton at C-3' shifted to δ 5.16 ppm as compared to that in TIPDS-diacetonide 259 (δ 3.50 - 4.20 ppm). This implied that the OTBS-sinapoyl group was attached to O-3'.</p>			
5	OTBS-caff acid 198	<p>TBS group: δ 0.07 to 0.98 ppm (overlapped with TIPDS)</p> <p>Integration of these peaks showed a total of 58 protons, implying the presence two TBS groups and one TIPDS. These confirmed the presence of one OTBS-caffeoyl group</p>	Additional signal at δ 167.0 ppm was observed in the spectrum. This new signal corresponded to the ester carbon, hence confirming the presence of the OTBS-caffeoyl group.	The HR-ESI-MS of the product showed a molecular ion m/z value of 1077.5242, which corresponded to $[M+Na]^+$. This supported the	264

		<p>Aromatic and <i>trans</i>-alkenyl protons: δ 6.25 to 7.66 ppm Integration of these peaks showed five protons, confirming the presence of one OTBS-caffeoyl group.</p> <p>COSY NMR: Correlation between the doublet at δ 5.13 ppm and the triplet at δ 4.65 ppm was observed, implying that they are neighbouring protons. Hence, the doublet at δ 5.13 ppm should correspond to the proton at C-3'.</p> <p>Due to the electron withdrawing effect of OTBS-caffeoyl group, the proton at C-3' shifted to δ 5.13 ppm as compared to that in TIPDS-diacetonide 259 (δ 3.50 - 4.20 ppm). This implied that the OTBS-caffeoyl group was attached to O-3'.</p>		<p>molecular formula of $C_{51}H_{90}O_{15}NaSi_4$ (m/z calcd. for $C_{51}H_{90}O_{15}NaSi_4$: 1077.5255)</p>	
6	diOMe acid 38	<p>Methoxy: δ 3.84, 3.90 ppm Integration of these peaks showed six protons, implying that the presence of two methoxy groups. This confirmed the presence</p>	Additional signal at δ 166.9 ppm was observed in the spectrum. This new signal corresponded to the ester carbon, hence confirming the	The HR-ESI-MS of the product showed a molecular ion m/z value of 877.3873, which	265

		<p>of the one dimethoxycinnamoyl group.</p> <p>Aromatic and <i>trans</i>-alkenyl protons: δ 6.34 to 7.73 ppm Integration of these peaks showed five protons, confirming the presence of one dimethoxycinnamoyl group</p> <p>COSY NMR: Correlation between the doublet at δ 5.17 ppm and the triplet at δ 4.63 ppm was observed, implying that they are neighbouring protons. Hence doublet at δ 5.17 ppm should correspond to the proton at C-3'.</p> <p>Due to the electron withdrawing effect of dimethoxycinnamoyl group, the proton at C-3' shifted to δ 5.17 ppm as compared to that in TIPDS-diacetonide 259 (δ 3.50 - 4.20 ppm). This implied that the dimethoxycinnamoyl group was attached to O-3'.</p>	presence of the dimethoxycinnamoyl group.	<p>corresponded to $[M+Na]^+$. This supported the molecular formula of $C_{41}H_{66}O_{15}NaSi_2$ (m/z calcd. for $C_{41}H_{66}O_{15}NaSi_2$: 877.3838)</p>	
7	triOMe acid 210	<p>Methoxy: δ 3.87 to 3.90 ppm Integration of this peak showed nine protons,</p>	Additional signal at δ 166.7 ppm was observed in the spectrum. This new	The HR-ESI-MS of the product showed a molecular ion	266

		<p>implying the presence of three methoxy groups. This confirmed the presence of one trimethoxycinnamoyl group.</p> <p>Aromatic and <i>trans</i>-alkenyl protons: δ 6.35 to 7.72 ppm. Integration of these peaks showed four protons, confirming the presence of one trimethoxycinnamoyl group</p> <p>COSY NMR:</p> <p>Correlation between the doublet at δ 5.15 ppm and the triplet at δ 4.64 ppm was observed, implying that they are neighbouring protons. Hence doublet at δ 5.15 ppm should correspond to the proton at C-3'.</p> <p>Due to the electron withdrawing effect of trimethoxycinnamoyl group, the proton at C-3' shifted to δ 5.15 ppm as compared to that in TIPDS-diacetonide 259 (δ 3.50 - 4.20 ppm). This implied that the trimethoxycinnamoyl group was attached to O-3'.</p>	<p>signal corresponded to the ester carbon, hence confirming the presence of the trimethoxycinnamoyl group.</p>	<p>m/z value of 907.3947, which corresponded to $[M+Na]^+$. This supported the molecular formula of $C_{42}H_{68}O_{16}NaSi_2$ (m/z calcd. for $C_{42}H_{68}O_{16}NaSi_2$: 907.2944)</p>	
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^a refer to Appendix for COSY spectra

Based on the spectroscopic data in Table 10, the products formed from acylation reactions were confirmed as compounds **260** to **266**.

With compounds **260** to **266**, the next step would be to remove TIPDS and TBS groups. These two protecting groups can be removed in a single reaction by treating compounds **260** to **266** with 3.0 equivalents of 1.56 3HF·NEt₃ and 2.0 equivalents of NEt₃ in pyridine (Scheme 76). For each reaction, a single product was obtained with the yields as shown in Scheme 76.

Reagents and conditions: i. 1.56 3HF·NEt₃ in pyridine (3.0 equiv), NEt₃ (2.0 equiv), pyridine, rt, 12 h

Scheme 76: Removal of TIPDS and TBS moieties

The identities of these products were confirmed with ¹H NMR, ¹³C NMR and HR-ESI-MS. The ¹H NMR spectra of these products revealed the absence of the characteristic peaks from δ 0.00 to 1.10 ppm, which corresponded to both TBS and TIPDS groups, implying the successful removal of both TIPDS and TBS groups from all the products. In addition, the presence of the characteristic proton peaks of isopropylidene rings from δ 1.20 to 1.50 ppm were observed in the spectra of all products obtained, confirming the selective removal of TIPDS and TBS groups without affecting the isopropylidene rings.

Other than that, the *trans*-alkenyl and aromatic proton peaks were still observed in the spectra for all products, indicating that the phenylpropanoid groups were not affected during the deprotection reactions. Moreover, comparison with the ¹H NMR spectrum of each product with that of their respective starting materials showed the doublet corresponding to the proton at C-3' was not shifted. As such, it can be concluded that no migration has occurred during the deprotection reactions and that the phenylpropanoid group on each product was still attached to O-3'.

The ¹³C NMR spectra of all products obtained also revealed the absence of characteristic carbon signals corresponding to that of TBS and TIPDS groups at δ -5.00 and δ 12.0 to 25.0 ppm respectively. This confirmed the successful removal of both temporary protecting groups as well. Furthermore, the presence of distinctive carbon signals which corresponded to ester carbons at around δ 160.0 to 170.0

ppm were still observed across the spectra of all products obtained. The presence of these ester carbon signals confirmed that the phenylpropanoid moiety on each product was not affected during the deprotection reactions. Finally, the HR-ESI-MS of each product obtained confirmed their molecular formulas (Table 11).

Table 11: HR-ESI-MS of compound 267 to 273

No.	Reaction with	HR-ESI-MS	Product
1	261	The HR-ESI-MS of the product showed a molecular ion m/z value of 553.2266, which corresponded to $[M+H]^+$. This supported the molecular formula of $C_{27}H_{37}O_{12}$ (m/z calcd. for $C_{27}H_{37}O_{12}$: 553.2285)	3'-cinn 267
2	262	The HR-ESI-MS of the product showed a molecular ion m/z value of 569.2247, which corresponded to $[M+H]^+$. This supported the molecular formula of $C_{27}H_{37}O_{13}$ (m/z calcd. for $C_{27}H_{37}O_{13}$: 569.2234)	3'-coum 268
3	263	The HR-ESI-MS of the product showed a molecular ion m/z value of 599.2313 which corresponded to $[M+H]^+$. This supported the molecular formula of $C_{28}H_{39}O_{14}$ (m/z calcd. for $C_{28}H_{39}O_{14}$: 599.2340)	3'-feru 269
4	264	The HR-ESI-MS of the product showed a molecular ion m/z value of 629.2461 which corresponded to $[M+H]^+$. This supported the molecular formula of $C_{29}H_{41}O_{15}$ (m/z calcd. for $C_{29}H_{41}O_{15}$: 629.2445)	3'-sinap 270
5	265	The HR-ESI-MS of the product showed a molecular ion m/z value of 585.2186 which corresponded to $[M+H]^+$. This supported the molecular formula of $C_{27}H_{37}O_{14}$ (m/z calcd. for $C_{27}H_{37}O_{14}$: 585.2183)	3'-caff 271
6	266	The HR-ESI-MS of the product showed a molecular ion m/z value of 613.2489 which corresponded to $[M+H]^+$. This supported the molecular formula of $C_{29}H_{41}O_{14}$ (m/z calcd. for $C_{29}H_{41}O_{14}$: 613.2496)	3'-diOMe 272
7	267	The HR-ESI-MS of the product showed a molecular ion m/z value of 643.2614 which corresponded to $[M+H]^+$. This supported the molecular formula of $C_{30}H_{43}O_{15}$ (m/z calcd. for $C_{30}H_{43}O_{15}$: 643.3602)	3'-triOMe 273

Based on the data above, these products obtained were confirmed as 3'-cinn **267**, 3'-coum **268**, 3'-feru **269**, 3'-sinap **270**, 3'-caff **271**, 3'-diOMe **272** and 3'-triOMe **273**. With that, a complete series of 3'-mono-substituted PSEs were synthesized successfully.

4.2.4 The synthesis of 4'-mono-substituted PSEs

Synthesis of 4'-mono-substituted PSEs can be achieved following the synthetic route as shown in Scheme 77.

Reagents and conditions: i. (substituted) cinnamic acid (1.5 equiv), DCC (1.5 equiv), DMAP (0.1 equiv), CH₂Cl₂, rt, 12 h; ii. Et₃SiH (1.6 equiv), NEt₃ (0.16 equiv), Pd(OAc)₂ (0.1 equiv), CH₂Cl₂, rt, 12 h; iii. 1.56 M 3HF·NEt₃ in pyridine (3.0 equiv), NEt₃ (2.0 equiv), pyridine, rt, 12 h;

Cinn: R₁ = R₂ = R₃ = H
 diOMe: R₁ = R₂ = OMe, R₃ = H
 triOMe: R₁ = R₂ = R₃ = OMe
 OTBS-coum: R₁ = R₃ = H, R₂ = OTBS
 OTBS-feru: R₁ = H, R₂ = OTBS, R₃ = OMe
 OTBS-caff: R₁ = R₂ = OTBS, R₃ = H
 OTBS-sinap: R₁ = R₃ = OMe, R₂ = OTBS

coum: R₁ = R₃ = H, R₂ = OH
 feru: R₁ = H, R₂ = OH, R₃ = OMe
 caff: R₁ = R₂ = OH, R₃ = H
 sinap: R₁ = R₃ = OMe, R₂ = OH

Scheme 77: Proposed synthetic route for synthesis of 4'-mono-substituted PSEs

Acylation reactions were attempted by reacting 3'-O-Cbz **225** with 1.5 equivalents of (substituted) cinnamic acid in the presence of DCC and DMAP in CH₂Cl₂ at room temperature for 12 hours (Scheme 78). For each reaction, a single product was obtained with the yields as shown in Scheme 78.

Reagents and conditions: i. ROOH (1.5 equiv), DCC (1.5 equiv), DMAP (0.1 equiv), CH₂Cl₂, rt, 12 h

ROOH = cinnamic acid **30** or OTBS-coum acid **203** or OTBS-feru **234** or OTBS-sin **235** or OTBS-caff **198** or diOMe acid **38** or triOMe acid **210**

Scheme 78: acylation reactions with 3'-O-Cbz 225

The structures of these products were confirmed by ¹H NMR, ¹³C NMR and HR-ESI-MS. The ¹H NMR spectra of the products obtained showed the presence of the characteristic peaks corresponding to TBS, Cbz and isopropylidene rings, implying that these protecting groups were not affected during the acylation reactions. Other observations made from the ¹H, ¹³C and COSY NMR spectrum of the individual product obtained are shown in Table 12. HR-ESI-MS values of each product were also included in the table

Table 12: NMR and HR-ESI-MS analysis of compounds 274 to 280

No	Reaction with	¹ H NMR Spectrum / COSY Spectrum ^a	¹³ C NMR	HR-ESI-MS	Product
1	Cinnamic acid 30	<p>trans-alkenyl and aromatic protons: δ 6.35 to 7.69 ppm. Integration of these peaks showed seven protons, hence confirming the presence of one cinnamoyl group.</p> <p>COSY NMR: Correlation between the doublet at δ 4.92 ppm and the triplet at δ 5.54 ppm was observed, implying that they are neighbouring protons.</p>	<p>Additional signal at δ 165.7 ppm was observed in the spectrum.</p> <p>This new signal corresponded to the ester carbon, hence confirming the presence of the cinnamoyl group.</p>	<p>The HR-ESI-MS of the product showed a molecular ion <i>m/z</i> value of 901.3481, which corresponded to [M+H]⁺. This supported the molecular formula of C₄₁H₅₇O₁₄Si</p>	274

		<p>Hence, the triplet at δ 5.54 ppm should correspond to the proton at C-4'.</p> <p>Due to the electron withdrawing effect of cinnamoyl group, the proton at C-4' shifted to δ 5.54 ppm as compared to that in 3'-O-Cbz 225 (δ 4.38 ppm). It was implied that the cinnamoyl group was attached to O-4'.</p>		<p>(m/z calcd. for $C_{41}H_{57}O_{14}Si$: 801.3518)</p>	
2	OTBS-coum acid 203	<p>TBS group: δ 0.00 ppm and 0.76 ppm. Integration of these peaks showed six and nine protons respectively, implying the presence of one TBS group. This confirmed the presence of one OTBS-coumaroyl group.</p> <p>trans-alkenyl and aromatic protons: δ 6.35 to 7.69 ppm. Integration of these peaks showed six protons, implying the presence of one OTBS-coumaroyl group.</p> <p>COSY NMR: Correlation between the doublet at δ 4.71 ppm and the triplet at δ 5.32 ppm was observed, implying that they are neighbouring protons. Hence, the triplet at δ 5.32</p>	<p>Additional signal at δ 165.8 ppm was observed in the spectrum. This new signal corresponded to the ester carbon, hence confirming the presence of the OTBS-coumaroyl group.</p>	<p>The HR-ESI-MS of the product showed a molecular ion m/z value of 931.4323, which corresponded to $[M+H]^+$. This supported the molecular formula of $C_{47}H_{71}O_{15}Si_2$ (m/z calcd. for $C_{47}H_{71}O_{15}Si_2$: 931.4332)</p>	275

		<p>ppm should correspond to the proton at C-4'.</p> <p>Due to the electron withdrawing effect of OTBS-coumaroyl group, the proton at C-4' shifted to δ 5.32 ppm as compared to that in 3'-O-Cbz 225 (δ 4.38 ppm). It was implied that the OTBS-coumaroyl group was attached to O-4'.</p>			
3	OTBS-feru acid 234	<p>TBS group: δ 0.00 ppm and 0.82 ppm. Integration of these peaks showed total of six and nine protons respectively, which implied the presence of one TBS group. This confirmed the presence of one OTBS-feruloyl group</p> <p>Methoxy peak: δ 3.71 ppm. Integration of this peak showed three protons, implying the presence of one methoxy group. This confirmed the presence of one OTBS-feruloyl group.</p> <p>trans-alkenyl and aromatic protons: δ 6.08 to 7.46 ppm. Integration of these peaks showed five protons,</p>	<p>Additional signal at δ 165.8 ppm was observed in the spectrum.</p> <p>This new signal corresponded to the ester carbon, hence confirming the presence of the OTBS-feruloyl group.</p>	<p>The HR-ESI-MS of the product showed a molecular ion m/z value of 961.4446, which corresponded to $[M+H]^+$. This supported the molecular formula of $C_{48}H_{73}O_{16}Si_2$ (m/z calcd. for $C_{48}H_{73}O_{16}Si_2$: 961.4437)</p>	276

		<p>confirming the presence of one OTBS-feruloyl group.</p> <p>COSY NMR:</p> <p>Correlation between the doublet at δ 4.75 ppm and the triplet at δ 5.37 ppm was observed, implying that they are neighbouring protons. Hence, the triplet at δ 5.37 ppm should correspond to the proton at C-4'.</p> <p>Due to the electron withdrawing effect of OTBS-feruloyl group, the proton at C-4' shifted to δ 5.37 ppm as compared to that in 3'-O-Cbz 225 (δ 4.38 ppm). It was implied that the OTBS-feruloyl group was attached to O-4'.</p>			
4	OTBS-sinap acid 235	<p>TBS group: δ 0.00 ppm and 0.86 ppm. Integration of these peaks showed total of six and nine protons respectively, indicating the presence of one TBS group. This confirmed the presence of one OTBS-sinapoyl group.</p> <p>Methoxy peak: δ 3.74 ppm. Integration of this peak showed six protons, implying the presence of two</p>	<p>Additional signal at δ 165.9 ppm was observed in the spectrum. This new signal corresponded to the ester carbon, hence confirming the presence of the OTBS-sinapoyl group.</p>	<p>The HR-ESI-MS of the product showed a molecular ion m/z value of 991.4542, which corresponded to $[M+H]^+$. This supported the molecular formula of $C_{49}H_{75}O_{17}Si_2$ (m/z calcd. for</p>	277

		<p>methoxy group. This confirmed the presence of one OTBS-sinapoyl group.</p> <p><i>trans</i>-alkenyl and aromatic protons: δ 6.09 to 7.47 ppm Integration of these peaks showed four protons, which confirmed the presence of one OTBS-sinapoyl group.</p> <p>COSY NMR: Correlation between the doublet at δ 4.79 ppm and the triplet at δ 5.41 ppm was observed, implying that they are neighbouring protons. Hence, the triplet at δ 5.41 ppm should correspond to the proton at C-4'.</p> <p>Due to the electron withdrawing effect of OTBS-sinapoyl group, the proton at C-4' shifted to δ 5.41 ppm as compared to that in 3'-O-Cbz 225 (δ 4.38 ppm). It was implied that the OTBS-sinapoyl group was attached to O-4'.</p>		$C_{49}H_{75}O_{17}Si_2$: 991.4543)	
5	OTBS-caff acid 198	<p>TBS group: δ 0.00 ppm and 0.77 ppm. Integration of these peaks showed total of 12 and 18 protons respectively, implying the</p>	Additional signal at δ 165.8 ppm was observed in the spectrum.	The HR-ESI-MS of the product showed a molecular ion m/z value of	278

		<p>presence of two TBS groups. This confirmed the presence of one OTBS-caffeoyl group.</p> <p>trans-alkenyl and aromatic protons: δ 5.94 to 7.36 ppm. Integration of these peaks showed five protons, implying the presence of one OTBS-caffeoyl group.</p> <p>COSY NMR: Correlation between the doublet at δ 4.72 ppm and the triplet at δ 5.32 ppm was observed, implying that they are neighbouring protons. Hence, the triplet at δ 5.32 ppm should correspond to the proton at C-4'.</p> <p>Due to the electron withdrawing effect of OTBS-caffeoyl group, the proton at C-4' shifted to δ 5.32 ppm as compared to that in 3'-O-Cbz 225 (δ 4.38 ppm). It was implied that the OTBS-caffeoyl group was attached to O-4'.</p>	<p>This new signal corresponded to the ester carbon, hence confirming the presence of the OTBS-caffeoyl group.</p>	<p>1061.5159, which corresponded to $[M+H]^+$. This supported the molecular formula of $C_{53}H_{85}O_{16}Si_3$ (m/z calcd. for $C_{53}H_{85}O_{16}Si_3$: 1061.5145)</p>	
6	diOMe acid 38	<p>Methoxy peak: δ 3.88 ppm. Integration of this peak showed six protons, implying the presence of two</p>	<p>Additional signal at δ 165.7 ppm was observed in the spectrum.</p>	<p>The HR-ESI-MS of the product showed a molecular ion</p>	279

		<p>methoxy groups. This confirmed the presence of one dimethoxycinnamoyl group.</p> <p>trans-alkenyl and aromatic protons: δ 6.21 to 7.37 ppm. Integration of these peaks showed five protons, which confirmed the presence of one dimethoxycinnamoyl group</p> <p>COSY NMR: Correlation between the doublet at δ 4.91 ppm and the triplet at δ 5.51 ppm was observed, implying that they are neighbouring protons. Hence, the triplet at δ 5.51 ppm should correspond to the proton at C-4'.</p> <p>Due to the electron withdrawing effect of dimethoxycinnamoyl group, the proton at C-4' shifted to δ 5.51 ppm as compared to that in 3'-O-Cbz 225 (δ 4.38 ppm). It was implied that the dimethoxycinnamoyl group was attached to O-4'.</p>	<p>This new signal corresponded to the ester carbon, hence confirming the presence of the dimethoxycinnamoyl group.</p>	<p>m/z value of 861.3700, which corresponded to $[M+H]^+$. This supported the molecular formula of $C_{43}H_{61}O_{16}Si$ (m/z calcd. for $C_{43}H_{61}O_{16}Si$: 861.3729)</p>	
7	triOMe acid 210	<p>Methoxy peak: δ 3.85 ppm. Integration of this peak showed nine protons,</p>	<p>Additional signal at δ 165.5 ppm was</p>	<p>The HR-ESI-MS of the product showed</p>	280

		<p>implying the presence of three methoxy groups. This confirmed the presence of one trimethoxycinnamoyl group.</p> <p><i>trans</i>-alkenyl and aromatic protons: δ 6.24 to 7.58 ppm. Integration of these peaks showed four protons, which confirmed the presence of one trimethoxycinnamoyl group</p> <p>COSY NMR: Correlation between the doublet at δ 4.90 ppm and the triplet at δ 5.52 ppm was observed, implying that they are neighbouring protons. Hence, the triplet at δ 5.52 ppm should correspond to the proton at C-4'.</p> <p>Due to the electron withdrawing effect of trimethoxycinnamoyl group, the proton at C-4' shifted to δ 5.52 ppm as compared to that in 3'-<i>O</i>-Cbz 225 (δ 4.38 ppm). It was implied that the trimethoxycinnamoyl group was attached to O-4'.</p>	<p>observed in the spectrum. This new signal corresponded to the ester carbon, hence confirming the presence of the trimethoxycinnamoyl group.</p>	<p>a molecular ion m/z value of 913.3663, which corresponded to $[M+Na]^+$. This supported the molecular formula of $C_{44}H_{62}O_{17}NaSi$ (m/z calcd. for $C_{44}H_{62}O_{17}NaSi$: 913.3654)</p>	
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^a refer to Appendix for COSY spectra

Based on the data shown in Table 12, the products formed from the acylation reactions were confirmed as compound **274** to **280**.

With compounds **274** to **280**, the next step would be to remove the Cbz and TBS groups. This was attempted by treating compounds **274** to **280** first with 1.6 equivalents of Et₃SiH in the presence of Pd(OAc)₂ and NEt₃ in CH₂Cl₂ at room temperature for 12 hours, followed by treatment with 3.0 equivalents of 1.56 M 3HF·NEt₃ and 2.0 equivalents of NEt₃ in pyridine at room temperature for another 12 hours (Scheme 79). At the end, each of the reaction gave a single product with the yield as shown in Scheme 79.

Reagents and conditions: i. Et₃SiH (1.6 equiv), NEt₃ (0.16 equiv), Pd(OAc)₂ (0.1 equiv), CH₂Cl₂, rt, 12 h, then washed twice with 1N HCl; ii. 1.56 M 3HF·NEt₃ in pyridine (3.0 equiv), NEt₃, (2.0 equiv), pyridine, rt, 12 h

Scheme 79: Removal of Cbz and TBS moieties

The ¹H NMR spectra of the products obtained showed the absence of characteristic proton peaks corresponding to the TBS and Cbz groups. This implied the successful removal of both TBS and Cbz groups. The presence of distinctive proton peaks corresponding to isopropylidene rings from δ 1.20 to 1.50 ppm also suggested that the isopropylidene rings were not affected during the deprotection reactions. In addition, distinctive aromatic and *trans*-alkenyl proton peaks were observed for all products, indicating that the phenylpropanoid substituents were not removed during the reactions. Moreover, comparison of the ¹H NMR spectra of the products with that of their respective starting materials showed that the proton at C-4' was not shifted. As such, it can be concluded that no migration has occurred during the reactions and that the phenylpropanoid group on each product was still attached to O-4'.

The ¹³C NMR spectra of all the products also revealed absence of distinctive carbon signals corresponding to both TBS and Cbz groups. This implied the successful removal of both TBS and Cbz groups. Furthermore, their spectra showed the presence of ester carbon signal from δ 160.0 to 170.0 ppm, which indicated the presence of the phenylpropanoid moiety on each product. This implied that

the phenylpropanoid group on each product was not affected during the deprotection reactions. Other than NMR spectra, the HR-ESI-MS of the products confirmed their molecular formulas (Table 13).

Table 13: The HR-ESI-MS of compounds 281 to 288

No.	Reaction with	HR-ESI-MS	Product
1	274	The HR-ESI-MS of the product showed a molecular ion m/z value of 575.2088, which corresponded to $[M+Na]^+$. This supported the molecular formula of $C_{27}H_{36}O_{12}Na$ (m/z calcd. for $C_{27}H_{36}O_{12}Na$: 575.2104)	4'-cinn 281
2	275	The HR-ESI-MS of the product showed a molecular ion m/z value of 569.2239, which corresponded to $[M+H]^+$. This supported the molecular formula of $C_{27}H_{37}O_{13}$ (m/z calcd. for $C_{27}H_{37}O_{13}$: 569.2234)	4'-coum 282
3	276	The HR-ESI-MS of the product showed a molecular ion m/z value of 599.2333 which corresponded to $[M+H]^+$. This supported the molecular formula of $C_{28}H_{39}O_{14}$ (m/z calcd. for $C_{28}H_{39}O_{14}$: 599.2340)	4'-feru 283
4	277	The HR-ESI-MS of the product showed a molecular ion m/z value of 651.2245 which corresponded to $[M+Na]^+$. This supported the molecular formula of $C_{29}H_{40}O_{15}Na$ (m/z calcd. for $C_{29}H_{40}O_{15}Na$: 651.2265)	4'-sinap 284
5	278	The HR-ESI-MS of the product showed a molecular ion m/z value of 585.2163 which corresponded to $[M+H]^+$. This supported the molecular formula of $C_{27}H_{37}O_{14}$ (m/z calcd. for $C_{27}H_{37}O_{14}$: 585.2183)	4'-caff 285
6	279	The HR-ESI-MS of the product showed a molecular ion m/z value of 635.2296 which corresponded to $[M+Na]^+$. This supported the molecular formula of $C_{29}H_{40}O_{14}Na$ (m/z calcd. for $C_{29}H_{40}O_{14}Na$: 635.2316)	4'-diOMe 286
7	280	The HR-ESI-MS of the product showed a molecular ion m/z value of 665.2422 which corresponded to $[M+Na]^+$. This supported the molecular formula of $C_{30}H_{42}O_{15}Na$ (m/z calcd. for $C_{30}H_{42}O_{15}Na$: 665.2421)	4'-triOMe 287

Hence, the products obtained were confirmed as 4'-cinn **281**, 4'-coum **282**, 4'-feru **283**, 4'-sinap **284**, 4'-caff **285**, 4'-diOMe **286** and 4'-triOMe **287**. Hence, a complete series of 4'-mono-substituted PSEs were synthesized successfully.

4.2.5 The synthesis of 6'-mono-substituted PSEs

Synthesis of 6'-cinn **288**, 6'-coum **289** and 6'-feru **290** were achieved by Dr Parthasarathi Panda previously.¹²⁶ To complete the series, 6'-mono-substituted sinapoyl, caffeoyl, dimethoxycinnamoyl and trimethoxycinnamoyl PSE will be synthesized. For this series, orthogonal protecting strategy was not adopted since 6'-OH is a primary OH and direct acylation of 2,1':4,6-di-*O*-isopropylidene sucrose **68** should offer higher selectivity towards this hydroxyl group and give the desired products efficiently.

Synthesis of 6'-mono-substituted PSE was attempted by reacting 1.3 equivalents of oxalyl chloride with 1.2 equivalents of (substituted) cinnamic acid and DMF in CH₂Cl₂ at room temperature for 2 hours. After that, 2,1':4,6-di-*O*-isopropylidene sucrose **68** and pyridine were added to the solution and the reactions were stirred at the ice-bath temperature for 12 hours (Scheme 80). The yields of the products are shown in Scheme 88.

Reagents and conditions: i. oxalyl chloride (1.3 equiv), ROOH (1.2 equiv), pyridine (3.0 equiv), DMF (0.2 equiv), CH₂Cl₂, ice bath (4°C), 14 h; ii. 1.56 3HF·NEt₃ (3.0 equiv), NEt₃ (2.0 equiv), pyridine, rt, 12 h

ROOH = cinnamic acid **30** or OTBS-coum acid **203** or OTBS-feru **234** or OTBS-sinap **235** or OTBS-caff **198** or diOMe acid **38** or triOMe acid **210**

Scheme 80: Synthesis of 6'-mono-substituted PSEs

Mixtures of products were obtained from the reactions. Based on ¹H NMR analysis, these first two products are identified as di-substituted compound and 3'-mono-substituted compounds. Since these are not the desired products, they will not be discussed here. From the spectra of the last more polar products (TLC) obtained from each reaction, the presence of characteristic proton peaks corresponding to isopropylidene rings from δ 1.10 to 1.60 ppm was confirmed. This suggested that the acylation reactions did not affect the isopropylidene rings. Other analysis of the products obtained from each reaction except the one with OTBS-sinap acid **235** are shown in the following table (Table 14).

Table 14: NMR and HR-ESI-MS analysis of compound 288, 291-296

No	Acid	¹ H and ¹³ C NMR Spectra	HR-ESI-MS	Product
1	Cinnamic acid 30	<p><i>trans</i>-alkenyl and aromatic protons: δ 6.10 to 7.66 ppm. Integration of these peaks showed seven protons, hence confirming the presence of one cinnamoyl group.</p> <p>Comparison with the reported values of 6'-cinn 288¹²⁶ confirmed the formation of the product. Basically, to confirm that that substituent is on O-6', no proton peak should be observed from δ 4.50 to 5.80 ppm.</p>	-	6'-cinn 288
2	(OTBS)-coum acid 203	<p>TBS peak: δ 0.00 ppm and 0.75 ppm.</p> <p>Integration of these peaks showed six and nine protons respectively, implying the presence of one TBS group. This confirmed the presence of one OTBS-coumaroyl group.</p> <p><i>trans</i>-alkenyl and aromatic protons: δ 6.08 to 7.45 ppm. Integration of these peaks showed six protons, confirming the presence of one OTBS-coumaroyl group.</p> <p>Comparison with the spectrum of similar compound also confirmed that the OTBS-coumaroyl group was attached on O-6'.</p> <p>¹³C NMR</p> <p>Additional carbon signal at δ 171.2 ppm was observed. This new carbon signal corresponded to the ester carbon, hence</p>	<p>The HR-ESI-MS of the product showed a molecular ion m/z value of 683.3016, which corresponded to $[M+H]^+$. This supported the molecular formula of C₃₃H₅₁O₁₃Si (m/z calcd. for C₃₃H₅₁O₁₃Si: 683.3054)</p>	291

		confirming the presence of OTBS-coumaroyl group.		
3	(OTBS)-feru acid 234	<p>TBS group: δ 0.00 ppm and 0.82 ppm. Integration of these peaks showed six and nine protons respectively, implying the presence of one TBS group. This confirmed the presence of one OTBS-feruloyl group</p> <p>Methoxy peak: δ 3.51 ppm. Integration of this peak showed three protons, which implied the presence of one methoxy group. This confirmed the presence of one OTBS-feruloyl group.</p> <p>trans-alkenyl and aromatic protons: δ 6.13 to 7.47 ppm. Integration of these peaks showed five protons, confirming the presence of one OTBS-feruloyl group.</p> <p>Comparison with the spectrum of similar compound also confirmed that the OTBS-feruloyl group was attached to O-6'.</p> <p>¹³C NMR Additional carbon signal at δ 171.1 ppm was observed. This new carbon signal corresponded to the ester carbon, hence confirming the presence of OTBS-feruloyl group.</p>	The HR-ESI-MS of the product showed a molecular ion m/z value of 713.3122, which corresponded to [M+H] ⁺ . This supported the molecular formula of C ₃₄ H ₅₃ O ₁₄ Si (m/z calcd. for C ₃₄ H ₅₃ O ₁₄ Si: 713.3160)	292
4	(OTBS)-caff acid 198	<p>TBS group: δ 0.00 ppm and 0.78 ppm. Integration of these peaks showed 12 and 18 protons respectively, indicating the presence of two TBS groups. This</p>	The HR-ESI-MS of the product showed a molecular ion m/z value of 813.3830, which corresponded to	294

		<p>confirmed the presence of one OTBS-caffeoyl group.</p> <p><i>trans</i>-alkenyl and aromatic protons: δ 6.02 to 7.40 ppm. Integration of these peaks showed five protons, which confirmed the presence of one OTBS-caffeoyl group.</p> <p>Comparison with the spectrum of similar compound also confirmed that the OTBS-caffeoyl group was attached to O-6'.</p> <p>¹³C NMR</p> <p>Additional carbon signal at δ 167.4 ppm was observed. This new carbon signal corresponded to the ester carbon, hence confirming the presence of OTBS-caffeoyl group.</p>	<p>[M+H]⁺. This supported the molecular formula of C₃₉H₆₅O₁₄Si₂ (<i>m/z</i> calcd. for C₃₉H₆₅O₁₄Si₂: 813.3868)</p>	
6	diOMe acid 38	<p>Methoxy peak: δ 3.80 ppm. Integration of this peak showed six protons, which implied the presence of two methoxy groups. This confirmed the presence of one dimethoxycinnamoyl group.</p> <p><i>trans</i>-alkenyl and aromatic protons: δ 6.21 to 7.55 ppm. Integration of these peaks showed five protons, which confirmed the presence of one dimethoxycinnamoyl group.</p> <p>Comparison with the reported values of similar compounds¹²⁶ like 6'-cinn 288, 6'-coum 289 and 6'-feru 290 confirmed that</p>	<p>The HR-ESI-MS of the product showed a molecular ion <i>m/z</i> value of 635.2324, which corresponded to [M+Na]⁺. This supported the molecular formula of C₂₉H₄₀O₁₄Na (<i>m/z</i> calcd. for C₂₉H₄₀O₁₄Na: 635.2316)</p>	6'-diOMe 295

		<p>the dimethoxycinnamoyl group was attached to O-6'.</p> <p>¹³C NMR</p> <p>Additional carbon signal at δ 167.4 ppm was observed. This new carbon signal corresponded to the ester carbon, hence confirming the presence of dimethoxycinnamoyl group.</p>		
7	triOMe acid 210	<p>Methoxy peak: δ 3.74 ppm. Integration of this peak showed nine protons, which implied the presence of three methoxy groups. Hence, this confirmed the presence of one trimethoxycinnamoyl group.</p> <p>trans-alkenyl and aromatic protons: δ 6.26 to 7.54 ppm. Integration of these peaks showed four protons, which confirmed the presence of one trimethoxycinnamoyl group</p> <p>Comparison with the reported values of similar compounds¹²⁶ like 6'-cinn 288, 6'-coum 289 and 6'-feru 290 confirmed that the trimethoxycinnamoyl group was attached to O-6'.</p> <p>¹³C NMR</p> <p>Additional carbon signal at δ 167.3 ppm was observed. This new carbon signal corresponded to the ester carbon, hence confirming the presence of trimethoxycinnamoyl group.</p>	<p>The HR-ESI-MS of the product showed a molecular ion <i>m/z</i> value of 643.2625, which corresponded to [M+H]⁺. This supported the molecular formula of C₃₀H₄₃O₁₅ (<i>m/z</i> calcd. for C₃₀H₄₃O₁₅: 643.2602)</p>	6'-triOMe 296

Based on the data in Table 14, the products formed were confirmed as compound 6'-cinn **288**, **291**, **292**, **294**, 6'-diOMe **297** and 6'-triOMe **298**.

NMR analysis of the product obtained from reaction with OTBS-sinap acid **235** was not included in Table 14 as the products were not well separated. To avoid loss of desired product from multiple column chromatography, we proceeded straight to TBS removal using the mixture as starting material.

TBS removals were attempted by reacting compounds **291** to **294** with 3.0 equivalents of 1.56 M 3HF·NEt₃ and NEt₃ in pyridine at room temperature (Scheme 80). Each reaction (except the one with compound **293** mixture) gave a single product and the structures of these products were confirmed using ¹H NMR.

The ¹H NMR spectra of all three products (from reactions with compounds **291**, **292** and **294**) showed the absence of the characteristic proton peaks of TBS group from δ 0.00 and 1.00 ppm. The presence of distinctive proton peaks corresponding to the isopropylidene rings from δ 1.10 to 1.60 ppm were also observed. These implied the successful removal of TBS group(s) without affecting the isopropylidene rings. Furthermore, *trans*-alkenyl and aromatic proton peaks from δ 6.20 to 7.60 ppm were still observed in the spectra of all three products. This indicated the presence of phenylpropanoid substituent for all three products and that these substituents were not removed during the deprotection reaction.

Comparison with the reported values of in the literature¹²⁶ confirmed the identities of the first two products (obtained from the reactions with compounds **291** and **292**) as 6'-coum **289** and 6'-feru **290** respectively.

As for the third product (obtained from the reaction with compound **294**), comparison of the ¹H NMR spectrum with that of compound **294** concluded that no migration has occurred during deprotection reaction. Hence, the caffeoyl group was still attached to O-6'. In addition, its ¹³C spectrum revealed the absence of distinctive carbon signals at around δ -5.00 ppm, which corresponded to the silyl carbon of TBS group. This confirmed the successful removal of TBS groups. Furthermore, a carbon signal at δ 173.2 ppm which corresponded to the ester carbon, was observed in the spectrum. This implied that the presence of the caffeoyl substituent and that this substituent was not affected during the deprotection reaction. The HR-ESI-MS of the product also showed a molecular ion *m/z* value of 585.2167, which corresponded to [M+H]⁺. This supported the molecular formula of C₂₇H₃₇O₁₄ (*m/z* calcd. for C₂₇H₃₇O₁₄: 585.2183). Based on these data, the third product was confirmed as 6'-caff **298**.

As for the TBS removal of the product mixture obtained from the reaction with OTBS-sinap acid **235**, two products were obtained at the end. The first product should be the undesired by-product from previous reaction, while the second one should be the desired product. These two products were separated using column chromatography and the structure of the second product was confirmed with ¹H NMR, ¹³C NMR and HR-ESI-MS.

Its ^1H NMR spectrum showed the absence of characteristic TBS peaks at δ 0.00 and 1.00 ppm, implying the successful removal of TBS group. In addition, it revealed the presence of the distinctive proton peaks corresponding to isopropylidene rings from δ 1.10 to 1.60 ppm. This confirmed that the selective removal of the TBS group was achieved without affecting the isopropylidene rings. Furthermore, the spectrum of the product showed the presence of *trans*-alkenyl and aromatic protons, which confirmed that the substituent moiety was not affected during the deprotection reaction. Integration of the aromatic and *trans*-alkenyl protons from δ 6.27 to 7.48 ppm showed four protons, which confirmed the presence of one sinapoyl group. Integration of the methoxy peak at δ 3.78 ppm also showed six protons, implying the presence of two methoxy groups. This confirmed the presence of one sinapoyl group as well. Similarly, comparison with the reported values of similar compounds¹²⁶ confirmed that the group was attached to O-6'.

Other than ^1H NMR, its ^{13}C NMR showed the absence of the distinctive carbon signals at around δ 5.00 ppm, which corresponded to the carbons of TBS group. This confirmed the successful removal of TBS groups as well. In addition, the spectrum showed a signal at δ 167.4 ppm, which corresponded to the ester carbon. The presence of this signal confirmed that the presence of the sinapoyl group. The HR-ESI-MS of the product also showed a molecular ion m/z value of 629.2422, which corresponded to $[\text{M}+\text{H}]^+$. This supported the molecular formula of $\text{C}_{29}\text{H}_{41}\text{O}_{15}$ (m/z calcd. for $\text{C}_{27}\text{H}_{37}\text{O}_{14}$: 629.2445). Hence, the product was confirmed as 6'-sinap **297**.

With this, synthesis of the complete series of 6'-mono-substituted PSEs was achieved successfully. 6'-cinn **288**, 6'-coum **289** and 6'-feru **290** were synthesized again so that they could be screened for their α -glucosidase and α -amylase inhibitory activities as well.

4.3 *In vitro* assays of mono-substituted PSEs against α -Glucosidase and α -Amylase enzymes

After the successful synthesis of mono-substituted PSEs at different positions, these mono-substituted PSEs **243-249**, **267-273**, **281-287**, **288-290** and **295-298** were screened for their α -glucosidase and α -amylase inhibitory activities using the *in vitro* enzyme assays as described (refer to 8.3). The results obtained from the *in vitro* studies will be discussed in the following subsections.

4.3.1 Results and Discussion

4.3.1.1 α -Glucosidase inhibition assay

Mono-substituted PSEs at different positions – O-3, O-3', O-4' and O-6' were screened for their α -glucosidase activities at a concentration of 50 μ g/ml, which was the same concentration at which the tetra-substituted PSEs (except cinnamoyl PSEs) were screened. Values obtained from the screening are presented in Table 15.

Table 15: % α -glucosidase inhibition of mono-substituted PSEs at 50 μ g/ml

α -glucosidase inhibition at 50 μ g/ml							
3-PSEs		3'-PSEs		4'-PSEs		6'-PSE	
3-cinn 243	NA	3'-cinn 267	NA	4'-cinn 281	NA	6'-cinn 288	NA
3-coum 248	NA	3'-coum 268	NA	4'-coum 282	NA	6'-coum 289	NA
3-feru 247	NA	3'-feru 269	NA	4'-feru 283	NA	6'-feru 290	NA
3-sinap 249	NA	3'-sinap 270	NA	4'-sinap 284	NA	6'-sinap 297	NA
3-caff 247	NA	3'-caff 271	NA	4'-caff 285	NA	6'-caff 298	NA
3-diOMe 244	NA	3'-diOMe 272	NA	4'-diOMe 286	NA	6'-diOMe 295	NA
3-triOMe 245	NA	3'-triOMe 273	NA	4'-triOMe 287	NA	6'-triOMe 296	NA

NA = No inhibitory activity

From Table 15, it is evident that none of the mono-substituted PSEs exhibited any α -glucosidase inhibitory activity. This lack of activity for all mono-substituted PSEs contradicted with the previous computational studies for tetra-substituted compounds 4CiI **43** and 4FI **48** which showed the importance of having phenylpropanoid substituent on the O-3 position. One plausible explanation for this could be due to the difference in binding interactions of the mono-substituted PSEs and tetra-substituted PSEs with α -glucosidase. Mono-substituted PSEs which have only a single substituent at one position (either O-3, O-3', O-4' or O-6') may exhibit a greater conformational flexibility as compared to tetra-

substituted PSEs, thus leading to decreased binding interactions with α -glucosidase. Not only that, mono-substituted PSEs may not orient themselves into the binding pocket of α -glucosidase as well as the tetra-substituted PSEs.

The screenings of the PSEs were done at 50 μ g/ml since this was the maximum solubility of tetra-substituted PSEs like 4FI **48**, 4SI **52**, 4FS **58** and 4SS **60**.⁸¹ Since mono-substituted PSEs were more soluble than tetra-substituted PSEs, screenings of these mono-substituted PSEs were repeated again at a concentration of 1000 μ g/ml (Table 16). Likewise, the columns in Table 16 indicated the four different positions at which phenylpropanoid group was attached, while the rows indicated the type of phenylpropanoid group present on the mono-substituted PSE.

Table 16: % α -glucosidase inhibition of mono-substituted PSEs at 1000 μ g/ml

α-glucosidase inhibition at 1000 μg/ml							
3-PSEs		3'-PSEs		4'-PSEs		6'-PSE	
3-cinn 243	NA	3'-cinn 267	10 $\pm$ 5	4'-cinn 281	85 $\pm$ 4	6'-cinn 288	NA
3-coum 248	58 $\pm$ 9	3'-coum 268	NA	4'-coum 282	59 $\pm$ 6	6'-coum 289	NA
3-feru 247	NA	3'-feru 269	28 $\pm$ 11	4'-feru 283	NA	6'-feru 290	NA
3-sinap 249	NA	3'-sinap 270	NA	4'-sinap 284	11 $\pm$ 6	6'-sinap 297	NA
3-caff 247	NA	3'-caff 271	NA	4'-caff 285	NA	6'-caff 298	NA
3-diOMe 244	NA	3'-diOMe 272	NA	4'-diOMe 286	NA	6'-diOMe 295	NA
3-triOMe 245	NA	3'-triOMe 273	NA	4'-triOMe 287	88 $\pm$ 5	6'-triOMe 296	NA

NA = No inhibitory activity

At 1000 μ g/ml, some of the mono-substituted PSEs exhibited α -glucosidase inhibitory activities. These PSEs include 3-coum **248**, 3'-cinn **267**, 3'-feru **269**, 4'-cinn **281**, 4'-coum **282**, 4'-sinap **284** and 4'-triOMe **287**. Their % α -glucosidase inhibition values are shown in Table 16 (highlighted in blue).

Based on Table 16, the following observations can be made:

1. The O-6' position does not contribute to the inhibition of α -glucosidase since none of the 6'-mono-substituted PSEs exhibited any inhibitory activities.
2. The O-3, O-3' and O-4' positions, on the other hand, may contribute to inhibition of α -glucosidase since 3-coum **248**, 3'-cinn **267**, 3'-feru **269**, 4'-cinn **281**, 4'-coum **282**, 4'-sinap **284** and 4'-triOMe **287** exhibited α -glucosidase inhibitory activities (Table 16, highlighted in blue).

3. Out of the three positions, the O-4' position may be the most important since more than half of the 4' mono-substituted PSEs exhibited inhibitory activities. In addition, 4'-cinn **281** and 4'-triOMe **287** exhibited the highest inhibitory activities (at 1000 $\mu\text{g/ml}$) amongst all mono-substituted PSEs at 85 ± 4 and 88 ± 5 respectively.
4. The type of substituent is important as well since not all mono-substituted PSEs at these three positions showed inhibitory activities. It is likely that α -glucosidase inhibitory activities of mono-substituted PSEs are influenced by both factors simultaneously – the position at which substituent is located and the type of substituent.
5. However, no conclusion can be drawn as to which substituent is the most ideal for α -glucosidase inhibition since there is not a single substituent that exhibited α -glucosidase inhibitory activities across all four positions.
6. Interestingly, the presence of the methoxy groups on the aromatic ring of PSE did not decrease the α -glucosidase inhibitory activities of mono-substituted PSEs like that of tetra-substituted PSEs. This is evident from the comparable activities shown by 4'-cinn **281** and 4'-triOMe **287** at 85 ± 4 and 88 ± 5 , respectively.
7. Likewise, the presence of hydroxyl groups on the aromatic rings did not contribute to the α -glucosidase inhibitory activities of mono-substituted PSEs unlike that of tetra-substituted PSEs. This is evident from the comparison of % α -glucosidase values between 4'-cinn **281** and 4'-coum **282**. Despite having a hydroxyl group on its aromatic ring, 4'-coum **282** exhibited a lower inhibitory activity of 59 ± 6 as compared to 4'-cinn **281** at 85 ± 4 .
8. The previous two points suggested that the observations made in the case of tetra-substituted PSEs may not be applicable in the case of mono-substituted PSEs. This is likely due to a difference in binding interactions as mentioned earlier.

4.3.1.2 α -Amylase inhibition assay

Mono-substituted PSEs **243-249**, **267-273**, **281-287**, **288-290** and **295-298** were also screened for their α -amylase activities at a concentration of 50 $\mu\text{g/ml}$. This was the same concentration at which tetra-substituted PSEs (except cinnamoyl PSEs) were screened (Table 17). The columns in Table 17 indicated the four different positions at which the phenylpropanoid group is attached to while the rows indicated the type of the phenylpropanoid group present on the mono-substituted PSE.

Table 17: % α -amylase inhibition of mono-substituted PSEs at 50 μ g/ml

α-amylase inhibition at 50 μg/ml							
3-PSEs		3'-PSEs		4'-PSEs		6'-PSE	
3-cinn 243	NA	3'-cinn 267	NA	4'-cinn 281	NA	6'-cinn 288	NA
3-coum 248	NA	3'-coum 268	NA	4'-coum 282	NA	6'-coum 289	NA
3-feru 247	NA	3'-feru 269	NA	4'-feru 283	NA	6'-feru 290	NA
3-sinap 249	NA	3'-sinap 270	NA	4'-sinap 284	NA	6'-sinap 297	NA
3-caff 247	NA	3'-caff 271	NA	4'-caff 285	NA	6'-caff 298	NA
3-diOMe 244	NA	3'-diOMe 272	NA	4'-diOMe 286	NA	6'-diOMe 295	NA
3-triOMe 245	NA	3'-triOMe 273	NA	4'-triOMe 287	NA	6'-triOMe 296	NA

NA = No inhibitory activity

From Table 17, none of the mono-substituted PSEs exhibited α -amylase inhibitory activity at 50 μ g/ml.

Considering their high solubility, the mono-substituted PSEs **243-249**, **267-273**, **281-287**, **288-290** and **295-298** were also screened for their α -amylase inhibitory activities at a higher concentration of 1000 μ g/ml (Table 18). Similarly, the columns in Table 18 indicated the four different positions at which the phenylpropanoid group was attached to while the rows indicated the type of the phenylpropanoid group present on the mono-substituted PSE.

Table 18: % α -amylase inhibition of mono-substituted PSEs at 1000 μ g/ml

α-amylase inhibition at 1000 μg/ml							
3-PSEs		3'-PSEs		4'-PSEs		6'-PSE	
3-cinn 243	NA	3'-cinn 267	NA	4'-cinn 281	80 $\pm$ 8	6'-cinn 288	NA
3-coum 248	NA	3'-coum 268	NA	4'-coum 282	74 $\pm$ 11	6'-coum 289	NA
3-feru 247	NA	3'-feru 269	NA	4'-feru 283	NA	6'-feru 290	NA
3-sinap 249	NA	3'-sinap 270	NA	4'-sinap 284	NA	6'-sinap 297	NA
3-caff 247	NA	3'-caff 271	53 $\pm$ 8	4'-caff 285	NA	6'-caff 298	NA
3-diOMe 244	NA	3'-diOMe 272	NA	4'-diOMe 286	NA	6'-diOMe 295	NA
3-triOMe 245	NA	3'-triOMe 273	NA	4'-triOMe 287	77 $\pm$ 9	6'-triOMe 296	NA

NA = No inhibitory activity

At 1000 μ g/ml, some mono-substituted PSEs such as 3'-caff **271**, 4'-cinn **281**, 4'-coum **282** and 4'-triOMe **287** exhibited α -amylase inhibitory activities. Their % α -amylase inhibition values are shown in Table 18 and highlighted in blue.

Based on Table 18, the following observations can be made:

1. The O-3 and O-6' positions do not contribute to α -amylase inhibition since none of the 3 and 6' mono-substituted PSEs exhibited any inhibitory activities.
2. The O-3' and O-4' positions, on the other hand, may contribute to the inhibition of α -amylase since as 3'-caff **271**, 4'-cinn **281**, 4'-coum **282** and 4'-triOMe **287** exhibited α -amylase inhibitory activities (Table 18, highlighted in blue).
3. Out of the two positions, the O-4' position may be the most important since more 4' mono-substituted PSEs exhibited inhibitory activities. In addition, 4'-cinn **281**, 4'-coum **282** and 4'-triOMe **287** exhibited the highest inhibitory activities at 80 ± 8 , 74 ± 11 and 77 ± 9 respectively.
4. Likewise, the type of substituent is important as well since not all mono-substituted PSEs at these two positions showed α -amylase inhibitory activities. It is likely that α -amylase inhibitory activities of mono-substituted PSEs is influenced by a combination of the type of substituent and the position at which the substituent is attached.
5. Similarly, no conclusion can be drawn as to which substituent is the most ideal for α -amylase inhibition since none of the substituents showed inhibitory activities across these two positions.
6. The presence of the methoxy groups on the aromatic ring of mono-substituted PSEs did not impede α -amylase inhibition since comparable activities were observed between 4'-cinn **281** and 4'-triOMe **287** at $80\pm 8\%$ and $77\pm 9\%$, respectively.
7. In addition, the presence of a hydroxyl group on the aromatic ring of mono-substituted PSEs did not have effect on α -amylase inhibition. This is evident from the comparable activities observed between 4'-cinn **281** and 4'-coum **282** at $80\pm 8\%$ and $74\pm 11\%$, respectively.
8. Likewise, the previous two points implied that observations made in the case of tetra-substituted PSEs are not applicable to mono-substituted PSEs. This is likely the result of a difference in binding interactions of mono-substituted PSEs and tetra-substituted PSEs with α -amylase.

4.4 Summary

The synthesis of mono-substituted PSEs at O-3, O-3', O-4' and O-6' positions was achieved successfully. Though the synthesis itself did not strictly follow the orthogonal protection strategy devised in all cases (refer to 3.5), the temporary protecting groups were still utilised in the synthetic route (except for synthesis of 6'-mono-substituted PSEs) since selective removal of these protecting groups can be achieved without affecting the isopropylidene rings and phenylpropanoid moieties. Besides, synthesis of mono-substituted PSEs at different positions did not really require full orthogonality of starting material. Strictly following the orthogonal protection strategy in this case will only create unnecessary additional steps in protection and deprotection, leading to decreased efficiency.

Screening of the mono-substituted PSEs at 50 $\mu\text{g/ml}$ concentrations showed no inhibitory activities against both α -glucosidase and α -amylase. However, at a higher concentration of 1000 $\mu\text{g/ml}$, some of the mono-substituted PSEs showed inhibitory activities against α -glucosidase, α -amylase or both. These PSEs are represented in the following graph (Figure 41).

Figure 41: Inhibitory activities of mono-substituted PSEs

Evidently, these mono-substituted PSEs are less potent as compared to tetra-substituted PSEs such as 4CaI **201** and 4CaS **202** (refer to 2.5) and the standard drug Acarbose since they only exhibited activities

at 1000 $\mu\text{g/ml}$. Amongst the mono-substituted PSEs which showed inhibitory activities at 1000 $\mu\text{g/ml}$, 4'-cinn **281** and 4'-triOMe **287** showed the highest inhibitory activities against both α -glucosidase and α -amylase (Figure 41).

In addition, the *in vitro* inhibition studies of the mono-substituted PSEs with different phenylpropanoid groups provided the following information:

1. The O-6' position does not contribute to α -glucosidase and α -amylase inhibitory activity while the O-3 position only contributes to α -glucosidase inhibitory activity.
2. The O-3' and O-4' positions contribute to both α -glucosidase and α -amylase inhibitory activity. Out of all the positions, the O-4' position is the most important for both α -glucosidase and α -amylase inhibitory activity.
3. The position and type of the substituent on mono-substituted PSEs influence the inhibitory activity of both α -glucosidase and α -amylase. However, it cannot be concluded as to which substituent is the best for inhibition of α -glucosidase and α -amylase since no single substituent showed inhibitory activities across all positions.
4. The presence of the methoxy groups on the aromatic ring of PSE did not decrease α -glucosidase and α -amylase inhibitory activities
5. The presence of the hydroxyl group(s) on the aromatic ring of PSE did not contribute to α -glucosidase and α -amylase inhibitory activity of mono-substituted PSEs.

With the SAR of mono-substituted PSEs established, the next step would be to explore the synthesis of PSEs that only inhibit α -glucosidase. This will likely be a mixed PSE since no single phenylpropanoid group showed α -glucosidase inhibitory activities across all positions.

From the information obtained, we know that 4'-cinn **281** and 4'-triOMe **287** showed the highest % α -glucosidase inhibition. However, they also inhibited α -amylase. Hence, cinnamoyl and trimethoxycinnamoyl groups at the O-4' position should be avoided for the synthesis of PSE that only inhibit α -glucosidase. In addition, a coumaroyl ester at O-4' position should be avoided as well since 4'-coum **282** inhibited both α -glucosidase and α -amylase. Furthermore, caffeoyl substituent at the O-3' position should be avoided as well since 3'-caff **272** showed α -amylase inhibitory activity as well.

As for 3-coum **248**, 3'-cinn **267**, 3'-feru **269** and 4'-sinap **284**, they only inhibited α -glucosidase. Amongst these four, 3-coum **248** showed the highest % inhibition of α -glucosidase, followed by 3'-feru

269. Hence for the synthesis of PSE that only inhibit α -glucosidase, a PSE containing a coumaroyl ester at O-3 and a feruloyl ester at O-3' can be considered. These will be discussed in the following chapter.

5 The synthesis of Mixed PSEs

5.1 Introduction

Based on the *in vitro* inhibition studies of mono-substituted PSEs, we envisioned the synthesis of mixed PSEs that would only inhibit α -glucosidase. Since 3-coum **248** and 3'-feru **269** showed the highest % inhibition of α -glucosidase amongst all the mono-substituted PSEs screened, we hypothesize that synthesizing a mixed PSE – such as compound **299** (Figure 42) may give a compound that shows high inhibitory activity of α -glucosidase only.

Figure 42: Proposed mixed PSEs

In addition, we also thought of synthesizing another mixed PSE – compound **300** (Figure 42). This mixed PSE also contains a coumaroyl substituent at O-3 and a feruloyl substituent at O-3'. The only difference is that it has an additional feruloyl ester at O-4'. Though 4'-feru **283** did not show any α -glucosidase inhibitory activity, it did not show any α -amylase inhibitory activity either. It is thus hypothesized that compound **300** was unlikely to exhibit any α -amylase inhibitory activity. We decided to add this feruloyl group at O-4' to check if higher degree of substitution would increase its inhibitory activity against α -glucosidase.

To check the validity of our hypothesis, these two mixed PSEs were synthesized and screened for their α -glucosidase and α -amylase inhibitory activities using the same *in vitro* enzyme assays as described (refer to 8.3).

The naming of these two mixed PSEs will follow the naming style of mono-substituted PSEs – where the position and the substituent on the sucrose core will be part of the naming. As such, the first mixed PSE is called 3-coum-3'-feru PSE, while the second is called 3-coum-3,4'-di-feru. However, since only two mixed PSEs were synthesized in this work, they shall be named Hybrid 1 and Hybrid 2.

5.2 The synthesis of Mixed PSEs

The synthesis of the proposed mixed PSEs, 3-coum-3'-feru PSE (Hybrid 1) **299** and 3-coum-3',4'-di-feru PSE (Hybrid 2) **300**, is discussed in the following two subsections.

5.2.1 The synthesis of 3-coum-3'-feru PSE (Hybrid 1) **299**

Based on previous experiences in the synthesis of mono-substituted PSEs, the following synthetic route was proposed for the synthesis of 3-coum-3'-feru PSE (Hybrid 1) **299**.

Reagents and conditions: i. OTBS-feru acid **234** (1.5 equiv), DCC (1.5 equiv), DMAP (0.1 equiv), CH_2Cl_2 , rt, 12 h; ii. OTBS-coum acid **203** (2.0 equiv), DCC (2.0 equiv), DMAP (0.1 equiv), CH_2Cl_2 , rt, 24 h; iii. 1.56 M $3\text{HF}\cdot\text{NET}_3$, in pyridine (3.0 equiv), NET_3 (2.0 equiv), rt, 12 h

Scheme 81: Proposed synthesis of Hybrid 1 **299**

Since the synthesis of both TIPDS-diacetonide **259** and compound **262** were already discussed previously (refer to 4.2.3.3), the discussion will focus on the synthesis of compound **301**.

The synthesis of compound **302** can be achieved by treating compound **262** with 2.0 equivalents of OTBS-coum acid **203** in the presence of DCC and DMAP in CH_2Cl_2 at room temperature for 24 hours (Scheme 82). A white solid was obtained in 83% yield. The structure of the product was confirmed with ^1H NMR, ^{13}C NMR and HR-ESI-MS.

Reagents and conditions: i. OTBS-coum acid **203** (2 equiv), DCC (2 equiv), DMAP (0.1 equiv), CH_2Cl_2 , rt, 24 h;

Scheme 82: Synthesis of compound **301**

In addition to the characteristic proton peaks of TIPDS and isopropylidene, additional *trans*-alkenyl and aromatic proton peaks from δ 6.22 to 7.52 ppm were observed. This confirmed the presence of an additional phenylpropanoid group, implying that the acylation reaction was successful. Integration of these *trans*-alkenyl and aromatic proton peaks showed 11 protons, which was six more protons as compared to that of compound **262**. This corresponded to the presence of one OTBS-feruloyl and one OTBS-coumaroyl group. Moreover, integration of the characteristic TIPDS and TBS peaks from δ 0.00 to 1.00 ppm showed 58 protons, which was 15 more protons as compared to that of compound **262**. This additional 15 protons corresponded to the presence of one additional TBS group, hence suggesting the presence of one TBS-coumaroyl group.

Furthermore, the spectrum revealed an additional triplet at δ 5.15 ppm that was not present in the spectrum of compound **262**. Its COSY spectrum showed that the triplet at δ 5.15 ppm does not have any correlation with the doublet corresponding to the proton at C-3'. This implied that the triplet should correspond to the proton at C-3 instead of C-4'. The proton at C-3 shifted to δ 5.15 ppm as compared to that in compound **262** (δ 3.50 to 4.20 ppm). This downfield shift can be attributed to the electron withdrawing effect of OTBS-coumaroyl group, hence implying that the OTBS-coumaroyl group was attached to O-3.

Other than that, its ^{13}C NMR spectrum revealed additional carbon signals corresponding to silyl carbon and ester carbon at around δ -5.00 and 165.0 ppm. The presence of these additional signals confirmed that the presence of an additional OTBS-coumaroyl group, indicating that the acylation reaction was a success. The HR-ESI-MS of the product also showed a molecular ion m/z value of 1215.5900, which corresponded to $[\text{M}+\text{H}]^+$. This supported the molecular formula of $\text{C}_{61}\text{H}_{99}\text{O}_{17}\text{Si}_4$ (m/z calcd. for $\text{C}_{61}\text{H}_{99}\text{O}_{17}\text{Si}_4$: 1215.5959). Based on the data obtained, the product was confirmed as compound **301**.

Removal of the TIPDS and TBS groups was attempted by treating compound **301** with 3.0 equivalents of 1.56 M $3\text{HF}\cdot\text{NEt}_3$ and 2.0 equivalents of NEt_3 in pyridine at room temperature for 12 hours (Scheme 83). A white solid was obtained in 85% yield.

Reagents and conditions: i. OTBS-coum acid **203** (1.5 equiv), DCC (1.5 equiv), DMAP (0.1 equiv), CH_2Cl_2 , rt, 12 h;

Scheme 83: Removal of TIPDS and TBS moieties

Its ^1H NMR spectrum revealed the absence of the characteristic proton peaks of TIPDS and TBS groups from δ 0.00 to 1.10 ppm and the presence of the distinctive proton peaks corresponding to the isopropylidene rings from δ 1.10 to 1.60 ppm. These confirmed that the selective removal of TIPDS and TBS groups was achieved successfully without affecting the isopropylidene rings. In addition, integration of the *trans*-alkenyl and aromatic protons from δ 6.19 to 7.79 ppm showed 11 protons, which was the same as that of compound **301**. This implied that both feruloyl and coumaroyl moieties were not affected during the deprotection reaction. Comparison of ^1H NMR spectrum with that of compound **301** also revealed that the triplet at δ 5.45 ppm which corresponded the proton at C-3 and the doublet at δ 4.90 ppm which corresponded to the proton at C-3' were not shifted. This implied that no migration has occurred during the deprotection reaction.

Furthermore, the ^{13}C NMR spectrum of the product showed the absence of characteristic TBS carbon signals from δ -5.00 to 0.00 ppm and characteristic TIPDS carbon signals from δ 17.00 to 25.00 ppm. These confirmed the successful removal of both TIPDS and TBS groups as well. The presence of carbon signals corresponding to the ester carbon at δ 166.3 and 168.5 ppm also confirmed that both feruloyl and coumaroyl groups were not affected during the deprotection reaction.

Other than the NMR spectra, the HR-ESI-MS of the product showed a molecular ion m/z value of 767.2548 corresponding to $[\text{M}+\text{Na}]^+$. This supported the molecular formula of $\text{C}_{37}\text{H}_{44}\text{O}_{16}\text{Na}$ (m/z calcd. for $\text{C}_{37}\text{H}_{44}\text{O}_{16}\text{Na}$: 767.2567). Hence, the product was confirmed as Hybrid 1 **299**.

5.2.2 The synthesis of 3-coum-3',4'-diferu PSE (Hybrid 2)

The synthesis of 3-coum, 3',4'-diferu PSE (Hybrid 2) **300** can be achieved by following the synthetic route as shown in Scheme 84.

Reagents and conditions: i. OTBS-feru acid **234** (2.5 equiv), DCC (2.5 equiv), DMAP (0.1 equiv), CH_2Cl_2 , rt, 12 h; ii. OTBS-coum acid **203** (1.5 equiv), DCC (1.5 equiv), DMAP (0.1 equiv), CH_2Cl_2 , rt, 12 h; iii. 1.56 M $3\text{HF}\cdot\text{NEt}_3$, in pyridine (3.0 equiv), NEt_3 (2.0 equiv), rt, 12 h

Scheme 84: Proposed synthetic route for Hybrid 2

An acylation reaction was attempted by reacting 6'-*O*-TBS **214** with 2.5 equivalents of OTBS-feru acid **234** in the presence of DCC and DMAP in CH₂Cl₂ at room temperature for 12 hours (Scheme 85). A single product was obtained in 81% yield

Reagents and conditions: i. OTBS-feru acid **234** (2.5 equiv), DCC (2.5 equiv), DMAP (0.1 equiv), CH₂Cl₂, rt, 12 h

Scheme 85: Synthesis of compound 307

The structure of this product was confirmed by ¹H NMR, ¹³C NMR and HR-ESI-MS. In addition to the characteristic proton peaks of the isopropylidene rings, the ¹H NMR spectrum of the product revealed distinctive TBS proton peaks at δ -0.09 and 0.82 ppm. Integration of these peaks showed a total of 45 protons which confirmed the presence of three TBS moieties. This confirmed the presence of two OTBS-feruloyl groups since the starting material 6'-*O*-TBS **214** originally had a TBS group. Furthermore, integration of distinctive aromatic and *trans*-alkenyl proton peaks from δ 6.13 to 7.52 ppm showed 10 protons, implying the presence of two OTBS-feruloyl substituents. Integration of the distinctive methoxy peaks also showed six protons, which implied the presence of two methoxy groups. This confirmed the presence of two OTBS-feruloyl groups as well. The positions at which the two OTBS-feruloyl groups are attached to were determined by COSY NMR (Figure 43).

Only correlation between protons of C-3' and C-4' was highlighted

Figure 43: COSY spectrum of compound 302

From Figure 43, a correlation was observed between the triplet at δ 5.47 ppm and the doublet at δ 5.08 ppm. This confirmed that they are neighbouring protons. As such, the triplet at δ 5.47 ppm should correspond to the proton at C-4' while the doublet at δ 5.08 ppm should correspond to the proton at C-3'. Due to the electron withdrawing effects of OTBS-feruloyl moieties, both protons shifted to δ 5.47 and δ 5.08 ppm as compared to that in 6'-O-TBS **214** (δ 3.50 to 4.20 ppm). This implied that the two OTBS-feruloyl groups were attached to O-3' and O-4' respectively.

The ^{13}C NMR spectrum of the product also showed additional carbon signals from δ -6.00 to 0.00 ppm, which confirmed the presence of silyl carbons. This implied the presence of TBS groups, which hence confirmed the presence of OTBS-feruloyl groups. Furthermore, new carbon signals at δ 165.8 and 166.1 ppm, which corresponded to ester carbons, were observed. These new carbon signals confirmed the presence of OTBS-feruloyl groups as well. In addition, the HR-ESI-MS of the product showed a molecular ion m/z value of 1117.5414 corresponding to $[\text{M}+\text{H}]^+$, which supported the molecular formula of $\text{C}_{56}\text{H}_{89}\text{O}_{17}\text{Si}_3$ (m/z calcd. for $\text{C}_{56}\text{H}_{89}\text{O}_{17}\text{Si}_3$: 1117.5408). Based on these data, the product was confirmed as compound **302**.

Subsequently, compound **302** was treated with 2.0 equivalents of OTBS-coum acid **203** in the presence of DCC and DMAP in CH_2Cl_2 at room temperature for 24 hours (Scheme 86). A single product was obtained in 81% yield.

Reagents and conditions: i. OTBS-coum acid **203** (2.0 equiv), DCC (2.0 equiv), DMAP (0.1 equiv), CH_2Cl_2 , rt, 24 h

Scheme 86: Synthesis of compound **304**

Other than the characteristic proton peaks of the isopropylidene rings, the ^1H NMR spectrum of the product revealed distinctive TBS proton peaks from δ 0.00 and 0.92 ppm. Integration of these peaks revealed 60 protons, which was 15 more than that of compound **302**. This confirmed the presence of an additional TBS group, which implied the presence of one OTBS-coumaroyl group. In addition, integration of the aromatic and *trans*-alkenyl proton peaks from δ 6.19 to 7.78 ppm showed a total of 16 protons, which was 6 more than that of compound **302**. This confirmed the presence of one OTBS-coumaroyl group and two OTBS-feruloyl groups. Other than the triplet and the doublet observed in the ^1H NMR spectrum of compound **302**, this spectrum showed an additional triplet at δ 5.31 ppm. COSY spectrum of this product showed no correlation between the additional triplet at δ 5.31 ppm and the doublet which corresponded to the proton at C-3'. Hence, this triplet should correspond to the proton at C-3. From the spectrum, it was observed that the proton at C-3 shifted to δ 5.31 ppm as compared to that in compound **302** (δ 3.50 to 4.20 ppm). This can be attributed to the electron withdrawing effect of OTBS-coumaroyl group, thus indicating that the OTBS-coumaroyl group was attached to O-3.

^{13}C NMR spectrum of the product also revealed additional carbon peaks from δ -6.00 to 0.00 ppm and at δ 165.9 ppm, which corresponded to the silyl carbons and the ester carbons respectively. These new

peaks confirmed the presence of OTBS-coumaroyl group. Other than that, the HR-ESI-MS of the product showed a molecular ion m/z value of 1377.6660 corresponding to $[M+H]^+$. This supported the molecular formula of $C_{71}H_{109}O_{19}Si_4$ (m/z calcd. for $C_{71}H_{109}O_{19}Si_4$: 1377.6640). Based on the data, the product was confirmed as compound **303**.

Finally, TBS removal was attempted by treating compound **303** with 3.0 equivalents of 1.56 M $3HF \cdot NEt_3$ and 2.0 equivalents of NEt_3 in pyridine at room temperature for 12 hours (Scheme 87). A single product was obtained in 86% yield.

Reagents and conditions: i. OTBS-coum acid **203** (1.5 equiv), DCC (1.5 equiv), DMAP (0.1 equiv), CH_2Cl_2 , rt, 12 h;

Scheme 87: Removal of TIPDS and TBS moieties

The structure of this product was confirmed using 1H NMR, ^{13}C NMR and HR-ESI-MS. Its 1H NMR spectrum showed the absence of characteristic TBS proton peaks from δ 0.00 to 1.00 ppm and the presence of proton peaks of isopropylidene rings from δ 1.16 to 1.37 ppm. This confirmed the successful removal of TBS groups without affecting isopropylidene rings. Moreover, integration of the aromatic and *trans*-alkenyl proton peaks from δ 6.13 to 7.75 ppm showed 16 protons, which was the same as that of compound **303**. This implied that none of the phenylpropanoid groups was affected during the deprotection reaction. Comparison of the 1H NMR spectrum with compound **303** also showed that the proton peaks corresponding to the protons at C-3, C-3' and C-4' respectively were not shifted. Hence, no migration has occurred and the three phenylpropanoid groups remained attached to O-3, O-3' and O-4' positions respectively.

The ^{13}C NMR spectrum of the product also revealed absence of characteristic TBS peaks from δ -6.00 to 0.00 ppm, confirming the successful removal of TBS groups. In addition, distinctive carbon peaks at δ 165.9, 166.1 and 166.5 ppm corresponding to the ester carbons of phenylpropanoid moieties were observed. These signals confirmed the presence of the coumaroyl and feruloyl groups, implying that these substituents were not affected during the deprotection reaction.

Other than NMR spectra, the HR-ESI-MS of the product showed a molecular ion m/z value of 943.3002, corresponding to $[M+Na]^+$. This supported the molecular formula of $C_{47}H_{52}O_{19}Na$ (m/z calcd. for $C_{47}H_{52}O_{19}Na$: 943.3000). Hence, the product was identified as Hybrid 2 **300**.

5.3 *In vitro* assays of Hybrid 1 299 and Hybrid 2 300 against α -glucosidase and α -amylase enzymes

After the successful synthesis of Hybrid 1 299 and Hybrid 2 300, these mixed PSEs were screened for their α -glucosidase and α -amylase inhibitory activities using the *in vitro* enzyme assays as described previously (refer to 8.3). The results obtained are discussed in the following subsections.

5.3.1 Results and Discussion

5.3.1.1 α -Glucosidase inhibition assay

The mixed PSEs – Hybrid 1 299 and Hybrid 2 300 were screened for their α -glucosidase inhibitory activities at a concentration of 50 μ g/ml. This was the same concentration at which tetra-substituted PSEs were screened previously. The results obtained are tabulated in the following table (Table 19). The value obtained for standard drug Acarbose was also included in the same table (highlighted in blue) for ease of comparison.

Table 19: % α -glucosidase inhibition of mixed PSEs at 50 μ g/ml

Entry	PSE	% α -glucosidase inhibition
1	Hybrid 1 299	87 $\pm$ 6
2	Hybrid 2 300	73 $\pm$ 7
3	Acarbose	19 $\pm$ 5

From Table 19, the following points can be observed:

1. Both mixed PSEs showed α -glucosidase inhibitory activities at 87 $\pm$ 6 and 73 $\pm$ 7 respectively (Table 19, entries 1 & 2) and these activities were much higher as compared to standard drug Acarbose at 19 $\pm$ 5 (Table 19, entry 3). Hence, both PSEs are more potent α -glucosidase inhibitors than standard drug Acarbose.
2. Comparing the inhibition values of both mixed PSEs indicated that an additional feruloyl group at O-4' did not contribute to α -glucosidase inhibitory activity since the % inhibition decreased from 87 $\pm$ 6 in Hybrid 1 299 to 73 $\pm$ 7 in Hybrid 2 300. Hence, higher degree of substitution may not necessarily increase inhibitory activity.

The results from this α -glucosidase inhibition assay proved the hypothesis that these two mixed PSEs do inhibit α -glucosidase. However, to prove that they only inhibit α -glucosidase, these two mixed PSEs needed to be screened for their α -amylase inhibitory activities as well.

5.3.1.2 α -Amylase inhibition assay

Mixed PSEs – Hybrid 1 **299** and Hybrid 2 **300** were also screened for their α -amylase inhibitory activities at the same concentration of 50 $\mu\text{g/ml}$ (Table 20).

Table 20: % α -amylase inhibition of mixed PSEs at 50 $\mu\text{g/ml}$

Entry	PSE	% α -amylase inhibition
1	Hybrid 1 299	NA
2	Hybrid 2 300	77 $\pm$ 5
3	Acarbose	90 $\pm$ 5

NA = no activity

Both mixed PSEs are less potent α -amylase inhibitors as compared to standard drug Acarbose. Hybrid 1 **299** showed no inhibitory activity for α -amylase (Table 20, Entry 1) while, Hybrid 2 **300** exhibited α -amylase 77 $\pm$ 5% inhibitory activity at 50 $\mu\text{g/ml}$ (Table 20, Entry 2).

Based on the results obtained, the hypothesis that Hybrid 1 **299** will only inhibit α -glucosidase was proven true since it did not show any α -amylase inhibitory activity. However, the same cannot be said for Hybrid 2 **300**.

It seemed that the addition of a feruloyl group at O-4' contributed to the inhibition of α -amylase. This contradicted the SAR study of the mono-substituted PSEs which showed that 4'-feru did not exhibit any inhibitory activity for α -amylase (Table 17 & 18). Based on the *in vitro* result obtained for Hybrid 2 **300**, it seemed that SAR study for mono-substituted PSEs cannot be applied entirely across di-substituted and tri-substituted PSEs. Hence to gain a more comprehensive understanding, further SAR study of di-substituted and tri-substituted PSEs will be needed.

5.4 Summary

The % inhibition activities of both Hybrid 1 **299** and Hybrid 2 **300** against both α -glucosidase and α -amylase inhibitory activities are represented in Figure 44.

Figure 44: inhibitory activities of mixed PSEs

From Figure 44, Hybrid 1 **299** showed only α -glucosidase inhibitory activity. With this compound, it is evident that the aim of synthesizing a PSE that only inhibits α -glucosidase was achieved successfully. With a higher inhibitory activity for α -glucosidase as compared to standard drug Acarbose, Hybrid 1 **299** certainly showed the potential for further development as a AGI for treatment of T2D. Prior to that, the efficacy of this drug as compared to Acarbose need to be assessed *in vivo* using diabetic mouse model. In addition, an evaluation of side effects can be done to investigate if the elimination of side effects can be achieved with Hybrid 1 **299**. These are discussed in detail in the next chapter.

Other than that, the surprising results obtained for Hybrid 2 **300** also prompted the need to further the SAR study by screening of di-substituted and tri-substituted PSEs. It is likely that the difference in substitution patterns on PSEs may influence inhibitory activities of either α -glucosidase, α -amylase or both. Hopefully, with SAR studies of di-substituted and tri-substituted PSEs, a comprehensive understanding of a possible correlation of the substitution patterns with inhibitory activities of both α -glucosidase and α -amylase can be obtained.

6 *In vivo* evaluation of lead compounds 4CaS 202 and Hybrid 1 299 using mouse model

6.1 Introduction

From the *in vitro* enzyme inhibition studies of the PSEs, it was observed that several synthetic PSEs showed higher inhibitory activities for α -glucosidase, α -amylase or both as compared to the standard drug Acarbose. Amongst all these PSEs, 4CaS **202** and Hybrid 1 **299** (Figure 45) were selected to be tested *in vivo* to evaluate their activities and side-effects.

Figure 45: Structures of 4CaS 202 and Hybrid 1 304

Hybrid 1 **299** was chosen since it displayed high inhibitory activity for α -glucosidase and no α -amylase activity. This selectivity towards α -glucosidase is desirable in achieving elimination of gastrointestinal side-effects that commonly accompanies AGIs due to inhibition of α -amylase. In addition, 4CaS **202** was also included in the testing since it exhibited high inhibitory activities ($\geq 90\%$) for both α -amylase and α -glucosidase. Therefore, a comparison of these two PSEs will shed light on both activity and side-effects. *In vivo* investigations of these two compounds allow us to determine if inhibition of α -glucosidase alone is effective for treatment of T2D or inhibition of both α -glucosidase and α -amylase is needed. The standard drug Acarbose was also included in the study to check if these PSEs displayed improved efficacies. Prior to conducting *in vivo* experiments, the IC_{50} , solubility and stability of these two lead compounds were tested.

6.2 IC₅₀ of 4CaS **202** and Hybrid 1 **299**

Prior to *in vivo* testing using mouse model, IC₅₀ values of both 4CaS **202** and Hybrid 1 **299** against both enzymes – α -glucosidase and α -amylase were determined (Table 21 and Table 22). The IC₅₀ values for Acarbose were taken from Dr Surabhi Devaraj's screening⁸¹ and are included in the tables (highlighted in blue) for ease of comparison. In addition, all calculations were done in triplicate and values are shown as mean $\pm$ std. deviation.

Table 21: IC₅₀ values of PSEs screened for α -glucosidase

Entry	Compound	IC ₅₀ (μ g/ml)	IC ₅₀ (μ M)
1	4CaS 202	3 $\pm$ 0.3	3 $\pm$ 0.2
2	Hybrid 1 299	29 $\pm$ 2	39 $\pm$ 3
3	Acarbose	212 $\pm$ 5	328 $\pm$ 7

From Table 21, both PSEs showed lower IC₅₀ values than Acarbose. The IC₅₀ of 4CaS **202** at 3 $\pm$ 0 (Table 21, Entry 1) was almost 100 times lower than that of Acarbose at 328 $\pm$ 7 (Table 21, Entry 3). Though the IC₅₀ of Hybrid 1 **299** at 29 $\pm$ 2 (Table 21, Entry 2) was higher than that of 4CaS **202**, it was still 10 times lower than that of Acarbose. These results confirmed that both 4CaS **202** and Hybrid 1 **299** were much more potent α -glucosidase inhibitors than Acarbose.

Table 22: IC₅₀ values of PSEs screened for α -amylase

Entry	Compound	IC ₅₀ (μ g/ml)	IC ₅₀ (μ M)
1	4CaS 202	2 $\pm$ 0.3	2 $\pm$ 0.2
2	Acarbose	-	5 $\pm$ 0.1

From Table 22, the IC₅₀ of 4CaS **202** at 2 $\pm$ 0.2 (Table 22, Entry 1) was comparable to that of Acarbose at 5 $\pm$ 0.1 (Table 22, Entry 2). This implied that 4CaS **202** is just as potent as Acarbose for inhibition of α -amylase. Since Hybrid 1 **299** did not exhibit any α -amylase inhibitory activity, it was not included in this test.

Based on the IC₅₀ values, Hybrid 1 **299** and 4CaS **202** showed great potential to be developed as AGIs for the treatment of T2D. However, further investigation of their *in vivo* efficacies using animal model was required.

6.3 Solubility and stability of 4CaS 202 and Hybrid 1 299 in water

Acarbose, 4CaS 202 and Hybrid 1 299 are intended to be oral drugs and therefore need to be fed to mice orally. Since daily oral gavage is not possible, administering them through drinking water was deemed more feasible. Hence, the solubility and stability of the PSEs in water were tested.

6.3.1 Method

The solubility of the 4CaS 202 and Hybrid 1 299 was assessed by dissolving the respective PSE in water at various known concentrations via sonication at 60°C. A quick visual test for turbidity was done to check the solubility of PSE in water at specific concentrations. Subsequently, solubility was further verified using High Performance Liquid Chromatography (HPLC) with ultraviolet-visible detector using a C18 column (Kromasil 100-3.5). The mobile phase used was 10% 0.1% AcOH in H₂O and 90% 0.1% AcOH in MeCN at a flow rate of 0.4 ml/min with an injection volume of 5 µl. The wavelength for detection was at 320 nm. To determine concentration of PSE in water, a calibration curve was first constructed using different concentrations of the respective PSEs in MeCN (i.e. 10, 25, 50, 75, 125, 250, 500, 750, 1000 and 1500 µg/ml). This calibration curve was a plot of concentration of the PSE in MeCN versus the area under curve for the peak represented by PSE. Using the equation derived from the calibration curve, the area under curve for PSE in water was calculated to check if it tallied with the known concentrations. Finally, these samples were then left for a week and tested again using HPLC to check for any degradation. High resolution mass spectrometry (HRMS-ESI) was also done to confirm that the product had not degraded.

6.3.2 Results and Discussion

6.3.2.1 Solubility and stability of 4CaS 202

The solubility of 4CaS 202 in water was tested by preparing samples where known amounts of 4CaS 202 were added to ultrapure water to achieve the following concentrations: 100, 150, 200, 250, 500, 750 and 1000 µg/ml. Based on turbidity observations, 4CaS 202 was deemed insoluble at 500 µg/ml.

Subsequently, the solubility of 4CaS 202 was further verified with HPLC. Prior to that, a calibration curve of 4CaS 202 in MeCN was plotted as shown in Figure 46.

Figure 46: Calibration curve of 4CaS 202 in MeCN

The exact concentrations present in the prepared samples were then estimated from the calibration curve $Y = 27.159X - 117.74$. Results obtained are represented in Table 23.

Table 23: Calculated concentration of 4CaS in water

Entry	Concentration (µg/ml)	Area under curve		Calculated concentration (µg/ml) ^a
		1 st trial	2 nd trial	
1	100	2520.3	2828.6	100
2	150	3897.3	3928.6	150
3	200	5758.3	5029.5	200
4	250	6654.38	6516.96	250
5	500	7632.59	7955.25	290
6	750	8538.38	9088.58	330

^a calculated with the average value for area under curve for 1st and 2nd trial

It was observed that up to 250 µg/ml, 4CaS **202** is soluble in water as shown above (Table 23, Entry 1 to 4). However, for 500 µg/ml and 750 µg/ml, there were significant differences between observed and calculated concentrations. This indicated that 4CaS **202** was not entirely soluble at these concentrations. Therefore, the maximum solubility for 4CaS **202** in water is 250 µg/ml. After that, these samples were left aside at room temperature for one week.

After one week, these samples were run again using HPLC. From the spectra obtained, a single peak, represented by 4CaS **202**, was observed. Since no additional peaks were seen, this implied that the compound did not degrade. Hence, it can be concluded that 4CaS **202** is stable in water for one week. Mass spectrometry was also done for one sample that was freshly dissolved and one sample that was left for a week. The results obtained are shown in Table 24.

Table 24: Mass Spectrometry Results for 4CaS 202

Entry	Mass of 4CaS 202 [M+H] ⁺	Calculated Mass for fresh sample [M+H] ⁺	Calculated Mass for the week- old sample [M+H] ⁺
1	1013.2328	1013.2325	1013.2330

Evidently, both samples were confirmed as 4CaS **202**. This further confirmed that 4CaS **202** is stable in water for a week.

6.3.2.2 Solubility and stability of Hybrid 1 **299**

Likewise, the solubility of Hybrid 1 **299** in water was tested by preparing samples in which known amounts of Hybrid 1 **299** were added to water to achieve the following concentrations: 125, 150, 200, 250, 500, 750 and 1000 µg/ml. Based on turbidity observations, Hybrid 1 **299** was deemed insoluble from 200 µg/ml onwards.

This was further verified using HPLC. Prior to that, a calibration curve of Hybrid 1 **299** in MeCN was established and as shown in Figure 47.

Figure 47: Calibration curve of Hybrid 1 299 in MeCN

The exact concentrations present in the samples were estimated from the calibration curve $Y = 27.159X - 117.74$ and the results are represented in Table 25.

Table 25: Calculated concentration of Hybrid 1 299 in water

Entry	Concentration (µg/ml)	Area under curve		Calculated concentration (µg/ml) ^a
		1	2	
1	125	8402	8393.3	125
2	150	9979.1	10099.1	150
3	200	10374.5	10709.1	160

^a calculated based on the average area under curve from 1 and 2.

From Table 25 (Entries 1 & 2), Hybrid 1 **299** is soluble in water up to 150 µg/ml, since known and calculated concentrations are the same. However, in the case of 200 µg/ml (Table 25, Entry 3), there was a huge difference between the observed and calculated concentrations. This implied that Hybrid 1 **299** is not entirely soluble in water and that the maximum solubility in water is 150 µg/ml. Likewise, the samples were left aside at room temperature for one week.

After that, these samples were verified again using HPLC. Their spectra revealed a single peak which corresponded to Hybrid 1 **299**. The absence of additional peaks signified that Hybrid 1 **299** is stable in water for a week as no degradation occurred. To add on, mass spectrometry was also done for one

sample that was freshly dissolved and one sample that was left for a week. The results obtained are shown in Table 26.

Table 26: Mass Spectrometry Results for Hybrid 1

Entry	Mass of Hybrid 1 299 [M+Na] ⁺	Calculated Mass for fresh sample [M+Na] ⁺	Calculated Mass for the week- old sample [M+Na] ⁺
1	767.2527	767.2530	767.2532

Evidently, the two samples were confirmed as Hybrid 1 **299**. This further confirmed that Hybrid 1 **299** is stable for one week. No other degradation peaks were observed in the HPLC as well.

6.4 *In vivo* mouse model

6.4.1 Diet induced diabetic mouse model

To investigate the effectiveness of Hybrid 1 **299** and 4CaS **202**, we conducted *in vivo* experiments using mouse models. *In vivo* experimentation is important since it allows one to observe the effect of new therapeutic agent under the complex *in vivo* environment. To date, there are many different types of animal models available for investigation on diabetes and its therapeutic agents.^{118, 146-147}

Amongst all the animal models, mouse models are the most commonly used due to ease of handling and comparatively lower cost. A diabetic mouse model can be derived spontaneously, chemically induced (i.e. streptozotocin), diet induced, surgically induced or via genetic manipulation.¹⁴⁶ For this work, a diet induced diabetic mouse model was chosen. In this model, the mice develop diabetes due to over-nutrition over time.¹⁴⁸ Similar to what was observed for T2D in humans, this model represents a development of diabetes as a combination of both environmental and genetic risk factors. Hence, this model is a good fit for this investigation as PSEs tested are potential AGIs which will be used in T2D treatment.

6.4.2 Method

All procedures involved in this study were approved by IACUC under reference number A0373.

6.4.2.1 Experimental animals

A total of 20 male mice strain black C57BL/6J were used for this study. They were split into four groups – Acarbose, Hybrid 1 **299**, 4CaS **202** and untreated (acting as control). With five mice in one cage respectively, these mice were housed in a light-(12 h on/12 h off) and temperature-controlled room with

food and water provided *ad libitum*. After an initial adjustment period of one week, the mice were fed with the western diet to induce diabetes.

6.4.2.2 Western Diet

In a diet induced mouse model, mice are simply fed with high fat diet for prolonged period to induce diabetes. In this case, we chose to feed them with western diet (catalogue number: D12079B). Western diet offers a good mixture of fats and carbohydrate (mainly sucrose) in a 1:1 ratio. Its high fats and carbohydrate content will help induce diabetes in mice when they are fed over prolonged period. Moreover, the high carbohydrate content is ideal for testing of PSEs since AGIs target the delayed absorption of carbohydrate after a meal.

The weight of the mice was monitored every week. Consistent with the consumption of a high fat diet, an increasing trend in weight was observed across all four cages. After six weeks into the diet, an intraperitoneal glucose tolerance test (IPGTT) was conducted on a weekly basis to check if these mice were diabetic. IPGTT measures the clearance of an intraperitoneally injected glucose load from the body. As such, mice that exhibited slower clearance and marked hyperglycaemia within two hours are considered diabetic and were used for further experiments to evaluate the effectiveness of treatments. The procedure for IPGTT will be discussed in detail in section 6.4.5. At the end of week 9, four mice from each cage were selected and we began with the *in vivo* testing using PSEs and Acarbose respectively.

6.4.2.3 Administration of PSEs via drinking water

Acarbose, Hybrid 1 **299** and 4CaS **202** were administered to the mice of the first three groups via drinking water. Factoring in a dosage of 10mg/kg body weight and the amount of water that may be consumed by one mouse per day¹⁴⁹, a concentration of 15 µg/ml of the respective treatment in drinking water was prepared weekly. The last group, which acts as the control, were not given any treatment. Hence, they were provided with normal drinking water.

For the first two weeks of treatment, the amount of water/treatment consumed was closely monitored. This was done to ensure mice that were being subjected to treatment did not avoid consuming ‘water’. Fortunately, no differences were observed and mice across all four cages were consuming a similar amount daily.

6.4.2.4 Procedure for evaluation of PSEs

In addition to weekly body weight measurement, the fasting blood glucose (FBG) and IPGTT were also measured weekly and biweekly respectively. Prior to the FBG test, all mice were fasted for four hours but given free access to water/treatment. Following this, the FBG was measured.

Likewise, for IPGTT, mice were also fasted for four hours but given free access to water/treatment. Following this, blood glucose was first measured at 'time 0' before 10 μ l/g body weight of 20% glucose solution was intraperitoneally injected into each mouse. Subsequently, blood glucose of each mouse was measured at different time points over two hours at 15, 30, 60, 90 and 120-minute intervals. Instead of treatment, normal drinking water was provided for all cages over this two-hour time frame.

6.4.2.5 Statistical analysis

Statistical analysis was performed using GraphPad Prism and values represented in the graphs are mean $\pm$ standard errors. In addition, the data set was assessed by two-way analysis of variance with a statistical significance set at the 95% confidence level.

6.4.3 Results and Discussion

To induce diabetes, all mice were kept on western diet for a period of nine weeks. Acarbose, Hybrid 1 **299** and 4CaS **202** were then administered in the three cages respectively with one cage left untreated for a total of six weeks. Over these six weeks, body weight, FBG and response to IPGTT was recorded and the results analysed to determine effectiveness of the drug treatments.

6.4.3.1 Body weight

Body weight measurements of mice from all four cages are represented in Figure 48.

Figure 48: Body weight of mice in all four cages over six weeks of treatment

An overall increase in weight was observed for mice across all four cages (Figure 48). This is not surprising since they were still fed on western diet, which is high in fat and carbohydrate. In addition to the overall increase, comparison of weight between each passing week was made. Statistical analysis of mice treated with Acarbose showed significant increase in weight every week as compared to the previous week, except for week 5 to 6. Mice treated with 4CaS **202** also followed a similar trend, displaying significant increase in weight from week 1 to 2, week 3 to 4 and week 5 to 6. Likewise, mice fed with water (untreated) showed significant increase from week 1 to 2, week 2 to 3 and week 5 to 6 as well. Comparison of the results between Acarbose, 4CaS **202** with untreated indicated that both Acarbose and 4CaS **202** are ineffective in controlling weight gain for mice fed on a high fat and high carbohydrate.

However, mice fed with Hybrid 1 **299** differed. Statistical analysis showed that there was no significant increase in weight for each passing week until week 5 to 6. Hence, this implied that Hybrid 1 **299** is

more effective in controlling weight gain of mice fed with a high fat and a high carbohydrate diet in comparison to Acarbose and 4CaS **202**.

6.4.3.2 Fasting blood glucose

Measurements of FBG across six weeks are represented in the following figure:

Figure 49: Fasting Blood Glucose measured over six weeks

Despite variations in FBG over the course of six weeks for all cages, the differences were statistically insignificant. Hence, it can be concluded that there was no significant change to FBG of diabetic mice across all four cages. This implied that all three treatments, including standard drug Acarbose, are ineffective in lowering FBG.

6.4.3.3 IPGTT

The IPGTT test was done biweekly and the results are represented in Figure 50 and 51. Figure 50 groups the results according to their respective treatment group and showed comparison across six weeks, while Figure 51 grouped the results according to the week which IPGTT was conducted and showed comparison across all treatment groups for individual tests.

Legend:

* indicate significant increase (comparison with week 0)

Figure 50: IPGTT (biweekly) for each cage over six weeks

From Figure 50, mice fed with Acarbose showed variations in response to the IPGTT over six weeks. Though variation in week 2 was considered as statistically insignificant, response in week 4 and week 6 showed significant difference at $t = 90$ & 120 mins and $t = 30, 60, 90$ & 120 minutes respectively as compared to week 0. Likewise, mice fed with Hybrid 1 **299** and 4CaS **202** also displayed disparities in response to the IPGTT. However, statistical analysis concluded that such differences for both groups were insignificant except for one time point each. For Hybrid 1 **299**, the IPGTT in week 6 showed a significant difference from that of week 0 at $t = 60$ mins. As for 4CaS **202**, a significant difference was observed in the IPGTT in week 4 at $t = 30$ mins. Despite significant differences at certain time points which implied a worsening response over time, these mice still showed a better response to the IPGTT as compared to untreated mice. From Figure 50, it was observed that the untreated mice showed a poorer response to the IPGTT with each passing week. The IPGTT in week 2 showed a significant difference at $t = 60$ mins, while the IPGTT in week 4 showed significant differences at $t = 60, 90$ & 120 mins. In week 6, the response to the IPGTT was even poorer since significant differences were observed for all time intervals. Hence, comparison between treated groups with the untreated group showed that all three treatments are effective in blood glucose management.

Furthermore, the blood glucose of the untreated mice peaked at 40 mmol/L while the blood glucose of the treated mice only peaked at 30 mmol/L across all three groups. This supports the hypothesis that the three treatments, Acarbose, Hybrid 1 **299** and 4CaS **202**, are effective in managing postprandial blood glucose.

Figure 51: IPGTT for all groups over six weeks

From Figure 51, it was observed that Acarbose, Hybrid 1 **299** and 4CaS **202** help to control the rise in blood glucose following intraperitoneal injection of a 20% glucose solution. From the IPGTT in week 2 (Figure 51), we can see that all four groups showed a rise in blood glucose levels from t=0 min (point of 20% glucose solution administration), peaking at t=30 mins before slowly declining after that. Statistical analysis showed a significant difference for both Hybrid 1 **299** and 4CaS **202** at t = 60 mins in comparison to the untreated group. Likewise, similar trend was observed for the IPGTT in week 4 (Figure 51) as well. For this set of data, a significant difference was observed for both Hybrid 1 **299** and 4CaS **202** at t = 90 mins as compared to untreated group. For the IPGTT in week 6 (Figure 51), a significant difference was observed for 4CaS **202** at t = 30 mins and 60 mins as compared to untreated group. In addition, there was also a significant difference for Acarbose at t = 60 mins. In general, these results denoted the effectiveness of Acarbose, Hybrid 1 **299** and 4CaS **202** in controlling postprandial blood glucose at 30 mins or 60 mins after administration of 20% glucose solution.

Though Hybrid 1 **299** and 4CaS **202** are effective in managing postprandial blood glucose, these novel drug compounds were not found to be more potent than Acarbose since no statistical difference was observed between all three treatments.

6.4.4 Summary

Based on *in vitro* studies, two lead compounds Hybrid 1 **299** and 4CaS **202** were selected to test their abilities to mitigate postprandial blood glucose *in vivo* using a diet induced diabetic mouse model. Results obtained showed that both Hybrid 1 **299** and 4CaS **202** were effective in managing postprandial blood glucose but were not more potent than standard drug Acarbose. Since all three treatments showed comparable abilities in reducing the surge of blood glucose after an intraperitoneal injection of 20% glucose solution, they are seen to be just as effective. However, Hybrid 1 **299** showed better control on weight than 4CaS **202** and Acarbose on a high fat and high carbohydrate diet. This can be an important therapeutic consideration for T2D patients who are overweight or obese. However, further evaluations will be required.

Unlike Acarbose and 4CaS **202** which inhibits both α -glucosidase and α -amylase, Hybrid 1 **299** only inhibits α -glucosidase (based on *in vitro* results). With its selectivity for α -glucosidase, this compound has the potential to eliminate some common side effects like flatulence, diarrhoea and abdominal discomfort which are associated with the inhibition of α -amylase. Hence, future work will involve the evaluation of side effects via analysis of stool samples, colonic pH, short-chain fatty acids and intestinal proliferative indices.¹⁵⁰⁻¹⁵¹ Details are discussed in the next chapter.

7 Future Work

7.1 Evaluation of side effects using *in vivo* mouse model

Evaluation of any side effects via *in vivo* mouse model is important to determine if elimination of side effects can be achieved with Hybrid 1 **299**. These can be done by investigating the effects of respective treatment (Acarbose, Hybrid 1 **299** and 4CaS **202**) on faecal nutrients, colonic pH, short-chain fatty acids composition, intestinal cell proliferation and large-bowel fermentation.¹⁵⁰⁻¹⁵¹

To do this, faecal samples need to be collected. Following procedures by Kooshkghazi and coworkers¹⁵⁰, blood samples can be collected from the portal vein (1 ml) and heart (2 ml) for analysis of short chain fatty acids immediately. In addition, gut (from stomach to rectum) will also be removed and cut into different sections for analysis of weight, length, colonic pH and intestinal cell proliferation. Digesta samples from terminal ileum (large intestine) can also be collected to determine the amount of ingested starch.

The collection of faecal samples has already been completed. This was done by housing the test mice in individual cages and giving them access to water/treatment and food. After two days, faecal samples were collected from individual mice and stored respectively in a -80°C freezer. However, these samples have yet to be processed and analysed, so no results can be presented.

7.2 SAR Study: Substitution pattern

From results of Hybrid 2 **300**, it is evident that substitution patterns have an influence on inhibitory activities against both α -glucosidase and α -amylase. As such, an SAR study should be done on di-substituted and tri-substituted PSEs to determine any correlation between substitution patterns and inhibition of both α -glucosidase and α -amylase. This may potentially give us a more comprehensive understanding on how the different positions contribute to PSE orientation and binding interactions with either α -glucosidase, α -amylase or both.

With four free hydroxyl groups on 2,1':4,6-di-*O*-isopropylidene sucrose **68**, four different substituent patterns can be achieved for synthesis of tri-substituted PSEs. The first will have substituents at O-3', O-4' and O-6' while the second will have substituents O-3, O-3' and O-4'. In addition, the third will have substituents at O-3, O-3' and O-6'. Lastly, the fourth will have substituents at O-3, O-4' and O-6'.

The same applies to di-substituted PSEs. With four free hydroxyl groups available for reaction on 2,1':4,6-di-*O*-isopropylidene sucrose **68**, a total of seven different combinations of di-substituted PSEs can be synthesized. These include substituents on O-3 and O-3', O-3 and O-4', O-3 and O-6', O-3' and

O-4', O-3' and O-6' and O-4' and O-6'. If all these PSEs can be synthesized and screened, an extensive SAR research can be established.

7.3 Improved solubility of PSEs

To date, there are many marketed oral drugs which adopt different drug formulation and delivery technologies to enhance their solubilities.¹⁵² One example will be Calcitriol, a drug taken to regulate calcium. This oral drug employs the formation of an emulsion or microemulsion in the gastrointestinal tract which improves its solubility and bioavailability at the intended site of action. Another example is Paclitaxel, a drug used in chemotherapy. This hydrophobic drug was loaded into a polymeric micelle (that is hydrophilic) to achieve enhanced solubility and higher tolerability. Both drugs, despite being insoluble and hydrophobic, successfully entered the market with the aid of drug formulation and delivery technologies.

To further develop PSEs as lead compounds for the treatment of T2D, there is a need to improve the solubility so that desired concentration of PSEs at target site can be achieved. Similar to the previous examples, solubility of PSEs can be improved using drug formulation and delivery technologies. Several approaches that can be considered are listed in the following points¹⁵³⁻¹⁵⁴:

- Physical modification: to reduce molecule size
Examples include the use of nanosuspension, nanocrystals and polymeric micelles to encapsulate hydrophobic drug
- Chemical modifications
Examples include change of pH, co-crystallization, co-solvent, complexation to enhance solubility or drug release.
- Miscellaneous methods
Examples include use of surfactants, solubilizers, excipients, direct capsule filling and electrospinning.

Each of these methods has its advantages and disadvantages, and the choice depends on several factors – nature of the drug, nature of the formulation selected and the intended dosage form. A thorough literature review and experimental investigation will be required to determine a method that will best fit PSEs.

7.4 Preclinical Toxicity

Preclinical toxicity is also an important area of study for drug development. Prior to clinical trials, a complete toxicity testing on various biological systems must be done. The testing will reveal important

information regarding the species-, organ- and dose specific- toxic effects of the lead compound.¹⁵⁵ Toxicity of a lead compound can be tested *in vitro* using cells or cell lines or *in vivo* using animal models. There are many different toxicity tests available and depending on the drug being tested, some tests are necessary while others may not be as relevant.

In this case, if PSE is a non-systemic drug (currently being tested by another researcher in the same research group using Caco2 transporter assay), liver toxicity or hepatotoxicity will not need to be carried out. However, there is a need to establish the lethal dosage of the lead compound. Examples of other tests to be conducted include acute toxicity test and chronic toxicity test. These two tests are used to determine the effects of a single dose (usually 50% of LD₅₀) on the cells or animals and the effects of the drug on either cells or animals over a prolonged period of time (usually over 90 days) respectively.¹⁵⁵ Since these tests are time consuming and costly, they will only be done when a lead compound showing highest potential for further development as AGI in T2D treatment is isolated.

Hybrid 1 **299**, which showed only α -glucosidase inhibitory activity was synthesized successfully using information derived from SAR studies. Evaluation of its effectiveness was done *in vitro* using enzyme inhibition assays and *in vivo* using diet induced diabetic mouse model. Based on results obtained, it was concluded that Hybrid 1 **299** was just as effective as the standard drug Acarbose in managing postprandial blood glucose. However, this drug showed higher effectiveness in weight management as compared. For future work, evaluation of side effects will be done to check if Hybrid 1 showed elimination of side effect commonly associated with AGIs.

8 Experimental

8.1 General

All commercial materials used in this project were obtained from Sigma-Aldrich, Merck, Alfa Aesar, Acros, Fisher Scientific, TCI, and were used as received unless otherwise indicated. Analytical thin layer chromatography (TLC) were performed using Merck 60 F₂₅₄ pre-coated silica gel plate (0.2 mm thickness) and visualized using either UV radiation (254 nm) or p-anisaldehyde staining solution. Purification of compounds via column chromatography were done using either Merck silica gel 60 (230-400 mesh). ¹H NMR, ¹³C and COSY NMR spectra were recorded at 300 MHz, 75.5 MHz and 300 MHz, using a Bruker 156 Advance DPX 300 machine. ¹H NMR multiplicities were assigned as singlet (s), doublet (d), triplet (t), doublet of doublet (dd) and multiplet (m). All NMR analysis were run in CDCl₃ solvent unless stated otherwise. High resolution mass spectra were recorded using Waters Q-tof Premier mass spectrometer.

8.2 Experimental Data

8.2.1 The synthesis of 2,1':4,6-di-*O*-isopropylidene sucrose **68**

The synthesis of diacetone was done following the same procedure as described by Dr Parthasarathi Panda¹²⁶ but with slight modifications. Drierite (36.0 g) was added to dry DMF (700 ml) and stirred under nitrogen atmosphere. After that, the solvent was filtered under vacuum to remove drierite. Sucrose (65.0g, 190 mmol) was grinded into fine powder before adding into the filtered DMF. The solution was stirred under inert atmosphere, after which, 2-methoxypropene (86.0 ml, 898 mmol) and *p*-TsOH (82.0 mg, 0.476 mmol) were added. The solution was left to stir for a week before adding NEt₃ (7.0 ml, 50.1 mmol) to quench the reaction. DMF was evaporated off and EA (400 ml) was added to the remaining yellow gel and stirred. After 10 minutes, the solution was filtered. the filtrate was collected and dried with anhydrous MgSO₄. Finally, the yellow gel was subjected to flash chromatography using EA as eluent. After purification, white solid 2,1':4,6-di-*O*-isopropylidene sucrose **68** was obtained in 65% yield.

Analytical data for 2,1':4,6-di-*O*-isopropylidene sucrose **68**:

Comparison of ¹H NMR with literature values¹²⁶ confirmed the product to be 2,1':4,6-di-*O*-isopropylidene sucrose **68**.

8.2.2 The synthesis of tetra-substituted PSEs

8.2.2.1 Protection of hydroxyl groups on caffeic acid

The synthesis of 3,4-diacetoxycinnamic acid **196**

The synthesis of 3,4-diacetoxycinnamic (OAc-caff) acid **196** was done following the same procedure as demonstrated by Liu and colleagues³ but with slight modifications as well. Caffeic acid **37** (10.0 g, 55.5 mmol) was dissolved in 4N NaOH (75.0 ml) and acetic anhydride (13.1 ml, 139 mmol) was added dropwise into the mixture. The reaction was left to stir for 12 h. After which, pH value of the mixture was adjusted to 2 using concentrated HCl. The precipitation was collected via vacuum filtration and washed three times with water. The collected precipitation was then recrystallized using hot MeOH and dried under vacuum. A white solid, OAc-caff acid **196** was obtained in 86% yield.

Analytical data for OAc-caff acid **196:** Comparison of ¹H NMR with reported literature values¹²⁰ confirmed the product to be OAc-caff acid **196**.

The synthesis of 3,4-bis(allyloxy)cinnamic acid **197**

The synthesis of 3,4-diallyloxycinnamic (OAllyl-caff) acid **197** was achieved by following procedure described by Liu and colleagues.⁴ Caffeic acid **37** (10.0 g, 55.5 mmol), allyl bromide (12.0 ml, 139 mmol) and K₂CO₃ (38.4 g, 278 mmol) in acetone (100 ml) were stirred under reflux for 12 h. The solids were filtered out and acetone was evaporated. To this residue, MeOH/NaHCO₃ (sat.) (1:1) was added. The mixture was heated under reflux for 2 h. After it was cooled down, the pH value of the mixture was adjusted to 2 with concentrated HCl. The precipitation was collected via vacuum filtration. The precipitation was recrystallized using hot MeOH. After drying under vacuum, yellowish solid, OAllyl-caff acid **197** was obtained in 80% yield.

Analytical data for OAllyl-caff acid **197:** ¹H NMR of **197** corresponded with reported literature values¹²¹ **197** for 3,4-diallyloxycinnamic acid.

The synthesis of 3,4-di-*tert*-butyldimethylsiloxycinnamic acid **198**

Synthesis of 3,4-di-*tert*-butyldimethylsiloxycinnamic (OTBS-caff) acid **198** was done by following the procedure as demonstrated by de Souza and colleagues with slight modifications.⁵ N,N-Diisopropylethylamine (24.2 ml, 139 mmol) and TBSCl (10.9 g, 69.4 mmol) were added to a suspension of caffeic acid **37** (5.00 g, 27.8 mmol) in anhydrous CH₂Cl₂ (30.0 ml) at room temperature. The solution was then left to stir for 12 h. After which, the mixture was diluted with EtOAc (150 ml) and washed twice with 1N HCl (2 x 100 ml). The organic phase collected was then dried with anhydrous MgSO₄

and evaporated to obtain a yellow oil. The oil was then dissolved in 9:1 THF/water (30.0 ml) and K_2CO_3 (422 mg, 2.77 mmol) was added to the solution. After stirring for 4 h, the reaction was again diluted with EtOAc (150 ml) and washed twice with 1N HCl (2 x 100 ml) again. Subsequently, the organic phase was dried with anhydrous $MgSO_4$ and evaporated. Finally, hexane was added to the crude product and shaken. After filtration, OTBS-caff acid **198** was obtained in 91% yield.

Analytical data for OTBS-caff acid 198: Comparison of 1H NMR with reported values in literature¹²² confirmed the formation of OTBS-caff acid **198**.

8.2.3 The synthesis of tetra-substituted caffeoyl PSEs

The synthesis of 3,3',4',6'-tetra-*O*-(3,4-di-*tert*-butyldimethylsiloxycinnamoyl)-2,1':4,6-di-*O*-isopropylidene sucrose **200**

2,1':4,6-di-*O*-isopropylidene sucrose **68** (500 mg, 1.18 mmol), OTBS-caff **198** (3.15 g, 7.70 mmol), DCC (1.59 g, 7.70 mmol) and DMAP (14.4 mg, 0.118 mmol) was dissolved and stirred in CH_2Cl_2 (10.0 ml) at room temperature for two days. Upon completion, the mixture was evaporated and dissolved in cold diethyl ether. After that, filtration was done. The filtrate collected was purified with column chromatography using 8:1 hexane/EtOAc as eluent. Compound **200** (intermediate product) was obtained as yellow solid in 76% yield.

Analytical data for compound 200: mp 67.5-69.0°C; 1H NMR (300 MHz, $CDCl_3$): TBS peaks: δ -0.01, 0.00 (m, 48H, 8 x $(CH_3)_2Si$), 0.76, 0.78 (m, 72H, 8 x $(CH_3)_3CSi$); isopropylidene rings: δ 0.97, 1.04, 1.06 (m, 6H, $(CH_3)_2C$), 1.21, 1.25 (d, 6H, J = Hz, $(CH_3)_2C$). sucrose unit: δ 3.40, 3.44, 3.53 (m, 2H, H-5, H-1'), 3.70, 3.73, 3.74 (m, 2H, H-6, H-1'), 3.80, 3.82, 3.85 (m, 1H, H-6), 4.04, 4.09 (m, 2H, H-2, H-6'), 4.29, 4.31, 4.33 (m, 2H, H-4, H-6'), 5.12, 5.13, 5.14, 5.17 (m, 1H, H-3), 5.35, 5.36 (m, 1H, H-5'); δ 6.01, 6.03, 6.03, 6.05, 6.08 ppm (m, 4H, H-1, H-3', H-4', H-a¹). *Trans*-

alkenyl and aromatic protons: δ 6.19, 6.24 (d, 1H, J = 15 Hz, H-a²), 6.57, 6.59, 6.60, 6.61, 6.68 (m, 6H, H-a³, H-a⁴, H-g¹, H-g², H-g³, H-g⁴), 6.77, 6.79, 6.80 (m, 4H, H-d¹, H-d², H-d³, H-d⁴), 6.90, 6.91 (d, 1H, J = 3 Hz, H-c¹), 6.91, 6.97, 7.04 (m, 3H, H-c², H-c³, H-c⁴), 7.26, 7.31, 7.35, 7.37, 7.40, 7.42 (m, 3H,

H-b¹, H-b³, H-b⁴), 7.60, 7.65 (d, 1H, J = 15 Hz, H-b²); ¹³C NMR (75.5 MHz, (CDCl₃)): δ -5.83, -5.60, -5.26, -5.22, -4.84, -4.21, -4.19, -0.02, 9.81, 16.6, 16.9, 17.3, 17.3, 17.9, 21.8, 22.7, 24.3, 24.5, 24.6, 24.7, 24.8, 27.7, 27.8, 28.5, 29.2, 37.6, 67.0, 75.5, 75.9, 76.3, 98.3, 100.3, 103.8, 110.5, 112.9, 113.2, 114.3, 116.5, 117.5, 118.5, 119.1, 119.2, 119.3, 119.4, 119.8, 120.0, 120.0, 120.1, 120.9, 121.2, 121.3, 121.5, 121.7, 126.6, 126.9, 127.0, 127.6, 130.2, 135.3, 143.8, 143.9, 145.1, 145.6, 145.7, 145.8, 146.0, 146.0, 146.1, 146.1, 148.1, 148.2, 148.4, 148.6, 164.9, 164.9, 165.0, 165.3, 165.4, 165.4, 165.7, 166.1, 166.1; HRMS (ESI-positive mode): *m/z* calcd. for C₁₀₂H₁₆₆O₂₃Si₈Na 2005.9872; found 2005.9867 [M+Na]⁺

The synthesis of 3,3',4',6'-tetra-*O*-caffeoyl-2,1':4,6-di-*O*-isopropylidene sucrose, 4CaI **201**

Tetra-substituted PSE **200** (200 mg, 0.101 mmol) was dissolved and stirred in pyridine (2.0 ml). After which, NEt₃ (28.1 μL, 0.202 mmol) and 1.56 M 3HF·NEt₃ in pyridine (193 μL, 0.303 mmol) were added. The reaction was left to stir for two days. Upon completion, pyridine was dried off and the crude mixture was subjected to purification via column chromatography using 3:2 EtOAc/Hexane as eluent. 4CaI **201** was obtained as yellow solid in 84% yield.

Analytical data for 4CaI **201:** mp 162-164 °C; ¹H NMR

(300 MHz, (CD₃)₂CO): isopropylidene rings: δ 1.19, 1.21, 1.23 (m, 6H, (CH₃)₂C), 1.46, 1.48 (d, 6H, (CH₃)₂C); sucrose unit: δ 3.63, 3.67, 3.82, 3.99, 4.00, 4.02 (m, 7H, H-5, 2 x H-1', 2 x H-6, H-2, H-6'), 4.41, 4.42, 4.43, 4.44, 4.45, 4.47, 4.48, 4.50 (m, 2H, H-4, H-6'), 4.61, 4.65, 4.70 (m, 1H, H-3), 5.36, 5.38, 5.40 (m, 2H, H-4', H-5'), 5.65 (m, 1H, H-3'), 6.18, 6.19 (d, 1H, J = Hz, H-1); *trans*-alkenyl and aromatic protons: δ 6.25, 6.28, 6.30, 6.31, 6.33, 6.40, 6.45 (m, 4H, H-a¹, H-a², H-a³, H-a⁴), 6.83, 6.86, 6.88, 6.89, 6.89 (m, 4H, H-g¹, H-g², H-g³, H-g⁴), 7.02, 7.03, 7.03, 7.05, 7.05 (m, 4H, H-d¹, H-

4CaI **201**

d², H-d³, H-d⁴) 7.16, 7.18, 7.19, 7.20 (m, 4H, H-c¹, H-c², H-c³, H-c⁴), 7.51, 7.55, 7.57, 7.60, 7.66 (m, 3H, H-b¹, H-b², H-b³), 7.78, 7.83 (d, 1H, H-b⁴); ¹³C NMR (75.5 MHz, (CD₃)₂CO): δ 19.4, 24.3, 25.7, 31.1, 32.0, 36.2, 62.6, 65.2, 65.8, 66.8, 71.5, 72.5, 72.9, 78.2, 80.7, 92.5, 100.2, 102.1, 106.0, 114.2, 114.6, 115.3, 115.4, 115.5, 115.8, 116.3, 122.4, 122.7, 122.9, 123.6, 127.4, 127.6, 127.6, 127.7, 145.7, 146.2, 146.2, 146.3, 147.2, 147.9, 148.8, 149.1, 166.5, 166.7, 166.8, 167.2; HRMS (ESI-positive mode): *m/z* calcd. for C₅₄H₅₅O₂₃ 1071.3134; found 1071.3175 [M+H]⁺

The synthesis of 3,3',4',6'-tetra-*O*-caffeoyl sucrose, 4CaS **202**

4CaI **201** (100 mg, 0.093 mmol) was dissolved in MeCN (3 ml) and stirred at room temperature. After which, Yb(OTf)₃·H₂O (10.0 mg, 0.0187 mmol) was added to the solution and the reaction was left to stir for 12 hours. Upon completion, the reaction was diluted with EtOAc (20.0 ml) and washed with twice with water. Organic layer was collected and dried with anhydrous MgSO₄. After removal of solvent, a yellow gel 4CaS **202** was obtained in 78% yield.

Analytical data for 4CaS **202:** ¹H NMR (300 MHz, (CD₃)₂CO): sucrose unit: δ 3.73, 3.74, 3.76, 3.79, 3.86, 3.88, 3.90 (m, 5 H, H-5, 2 x H-1', 2 x H-6), 4.01, 4.02 (m, 2H, 2 x H-6') 4.21, 4.22, 4.23 (m, 1H, H-4), 4.47, 4.49, 4.57, 4.60, 4.62, 4.65 (m, 3H, H-2, H-3, H-1), 5.43, 5.45, 5.47 (t, 1H, H = 6 Hz, H-5'), 5.62, 5.63 (d, 1H, J = 3 Hz, H-4'), 5.80, 5.82, 5.85, 5.87 (m, 1H, H-3'); *trans*-alkenyl and aromatic protons: δ 6.29, 6.31, 6.34, 6.37, (m, 2H, H-a¹, H-a³), 6.43, 6.50 (d, 1H, J = 21 Hz, H-a⁴), 6.87, 6.90 (m, 3H, H-a², H-g¹, H-g³), 7.06, 7.08, 7.20, 7.31 (m, 6H, H-g², H-g⁴, H-d¹, H-d², H-d³, H-d⁴), 7.58, 7.60, 7.63, 7.66, 7.68 (m, 4H, H-c¹, H-c², H-c³, H-c⁴), 8.23, 8.26 (d, 2H, J = 9 Hz, H-d¹, H-d³), 8.49, 8.52 (d, 2H, J = 12 Hz, H-d², H-d⁴); ¹³C NMR (75.5 MHz, (CD₃)₂CO): δ 55.5, 56.1, 56.2, 60.6, 74.6, 83.6, 83.4, 93.4, 105.7, 105.9, 106.4, 106.5, 111.1, 112.3, 115.3, 115.8, 116.4, 122.7, 122.9, 124.0, 127.7, 128.1, 129.4, 146.3, 146.3, 146.4, 146.5, 146.6, 150.7, 152.8, 166.7, 166.8, 167.1, 171.0; HRMS (ESI-positive mode): *m/z* calcd. for C₄₈H₄₆O₂₃Na 1013.2328; found 1013.2330 [M+Na]⁺

4CaS **202**

8.2.4 Protection of hydroxyl group on coumaric acid

The synthesis of 4-*tert*-butyldimethylsiloxycinnamic acid **203**

N,N-Diisopropylethylamine (15.9 ml, 91.4 mmol) and TBSCl (6.89 g, 45.7 mmol) were added to a suspension of coumaric acid **33** (5.00 g, 30.5 mmol) in anhydrous CH₂Cl₂ (30.0 ml) at room temperature. The solution was then left to stir for 12 h. After which, the mixture was diluted with EtOAc (150 ml) and washed twice with 1N HCl (2 x 100 ml). The organic phase collected was then dried with anhydrous MgSO₄ and evaporated to obtain a yellow oil. The oil was then dissolved in 9:1 THF/water (30.0 ml) and K₂CO₃ (421 mg, 3.05 mmol) was added to the solution. After stirring for 4 h, the reaction was again diluted with EtOAc (150 ml) and washed twice with 1N HCl (2 x 100 ml) again. Subsequently, the

organic phase was dried with anhydrous MgSO_4 and evaporated. Finally, hexane was added to the crude product and shaken. After filtration, 4-*tert*-butyldimethylsiloxycinnamic (OTBS-coum) acid **203** was obtained as yellowish solid in 88% yield.

Analytical data for OTBS-coum acid 203: Comparison of ^1H NMR with reported values in literature¹²⁸ confirmed the formation of OTBS-coum acid **203**.

8.2.5 The synthesis of tetra-substituted coumaroyl PSEs

The synthesis of 3,3',4',6'-tetra-*O*-(4-*tert*-butyldimethylsiloxycinnamoyl)-2,1':4,6-di-*O*-isopropylidene sucrose 204

2,1':4,6-di-*O*-isopropylidene sucrose **68** (500 mg, 1.18 mmol), OTBS-coum **203** (2.14 g, 7.70 mmol), DCC (1.59 g, 7.70 mmol) and DMAP (14.4 mg, 0.118 mmol) was dissolved and stirred in CH_2Cl_2 (10.0 ml) at room temperature for two days. Upon completion, the mixture was evaporated and dissolved in cold diethyl ether. After that, filtration was done. The filtrate collected was purified with column chromatography using 8:1 hexane/EtOAc as eluent. Tetra-substituted PSE **204** (intermediate product) was obtained as white solid in 75% yield.

Analytical data for compound 204: ^1H NMR (300 MHz,

CDCl_3) TBS group: δ -0.01, -0.01, 0.00, 0.01 (m, 24H, 4 x $(\text{CH}_3)_2\text{Si}$), 0.070, 0.76, 0.78, 0.78 (m, 36H, $(\text{CH}_3)_3\text{CSi}$);

isopropylidene rings: δ 1.00, 1.05 (d, 6H, J = Hz, $(\text{CH}_3)_2\text{C}$), 1.22, 1.25 (d, 6H, J = Hz, $(\text{CH}_3)_2\text{C}$); sucrose unit: δ 3.35, 3.46,

3.73, 3.91, 3.93 (m, 7H, H-5, 2 x H-1', 2 x H-6), 4.10, 4.14, 4.13 (m, 2H, H-2, H-6'), 4.40, 4.45, 4.50 (m, 2H, H-4, H-6'),

5.10, 5.15, 5.14, 5.19 (m, 1H, H-3), 5.52, 5.56, 5.57, 5.58 (m, 2H, H-4', H-5'), 5.69, 5.70 (m, 1H, H-3'), 5.96, 5.97 (d, 1H,

J = Hz, H-1) ; *trans*-alkenyl and aromatic protons: δ 6.05, 6.06, 6.07, 6.10, 6.11, 6.12 (m, 3H, H-a¹, H-a², H-a³), 6.23,

6.28 (d, 1H, H-a⁴), 6.58, 6.60, 6.65, 6.65, 6.68, 6.68 (m, 8H, H-d¹, H-d², H-d³, H-d⁴, H-f¹, H-f², H-f³, H-f⁴), 7.16, 7.17, 7.18, 7.19 (m, 4H, H-g¹, H-g², H-g³, H-g⁴), 7.20, 7.21, 7.26, 7.27, 7.29, 7.30, 7.33, 7.35 (m, 4H, H-c¹, H-c², H-c³, H-c⁴) 7.39, 7.40, 7.41, 7.43, 7.45, 7.47 (m, 3H, H-b¹, H-b², H-b³) 7.62, 7.67

(d, 1H, J = 15 Hz, H-b⁴); ^{13}C NMR (75.5 MHz, CDCl_3): δ -4.31, -3.11, -2.00, -0.02, 18.5, 20.1, 23.4, 24.8, 25.3, 28.3, 28.5, 54.6, 61.7, 64.3, 64.9, 65.3, 65.9, 70.6, 71.6, 71.9, 77.2, 77.4, 80.0, 91.6, 99.4, 99.7, 101.2, 105.1, 113.1, 113.4, 114.1, 114.6, 115.8, 115.9, 115.9, 116.0, 122.2, 122.4, 125.6, 125.8, 125.9, 129.4, 129.6, 130.0, 130.2, 130.4, 130.5, 130.7, 144.6, 145.1 146.1, 146.8, 152.6, 159.9, 160.1, **230** | Page

160.2, 160.3, 165.5, 165.7, 166.0, 166.1, 166.5; HRMS (ESI-positive mode): m/z calcd. for $C_{78}H_{111}O_{19}Si_4$: 1463.6752; found 1464.6785 $[M+H]^+$

The synthesis of 3,3',4',6'-tetra-*O*-coumaroyl-2,1':4,6-di-*O*-isopropylidene sucrose, 4CoI 205

Tetra-substituted PSE **204** (200 mg, 0.137 mmol) was dissolved and stirred in pyridine (2.0 ml). After which, NEt_3 (38.1 μL , 0.273 mmol) and 1.56 M $3HF \cdot NEt_3$ in pyridine (263 μL , 0.410 mmol) were added. The reaction was left to stir for two days. Upon completion, pyridine was dried off and the crude mixture was subjected to purification via column chromatography using 3:2 EtOAc/Hexane as eluent. White solid 4CoI **205** was obtained in 82% yield.

Analytical data for 4CoI 205: Comparison of 1H NMR with reported literature values¹²⁶ confirmed the formation of 4CoI **205**;

4CoI 205

The synthesis of 3,3',4',6'-tetra-*O*-coumaroyl sucrose, 4CoS 206

4CoI **205** (100 mg, 0.099 mmol) was dissolved in MeCN (3 ml) and stirred at room temperature. After which, $Yb(OTf)_3 \cdot H_2O$ (10.6 mg, 0.0199 mmol) was added to the solution and the reaction was left to stir for 12 hours. Upon completion, MeCN was removed and the crude product was purified with column chromatography using 2:1 EtOAc/hexane as eluent. A white solid, 4CoS **206**, was obtained in 78% yield.

Analytical data for 4CoS 206: Comparison with reported literature values¹²⁶ confirmed the formation of 4CoS **206**.

4CoS 206

8.2.6 The synthesis of 3,4-dimethoxycinnamic acid 38

Ferulic acid **39** (10.00 g, 51.5 mmol) was dissolved in 4N NaOH (20 ml) and stirred in room temperature. Subsequently, $(CH_3)_2SO_4$ (4.0 ml, 42.2 mmol) was added slowly to the stirring mixture. The yellow

mixture was left to stir for 1 h, before adding another portion of $(\text{CH}_3)_2\text{SO}_4$ (2.0 ml, 21.1 mmol) and 4N NaOH (10 ml). The mixture was left to stir for another 1 hour. After which, a new portion of $(\text{CH}_3)_2\text{SO}_4$ (2.0 ml, 21.1 mmol) and 4N NaOH (10 ml) were added and the mixture was left to stir for 10 hours. Upon completion, the brown mixture was acidified to pH 2 with HCl and the precipitate was collected via vacuum filtration, washed twice with distilled water. After drying under reduced pressure, 3,4-dimethoxycinnamic (diOMe) acid **38** was obtained in 83% yield.

Analytical data for diOMe acid 38: ^1H NMR of diOMe acid **38** corresponded with reported literature values¹²⁹ for 3,4-dimethoxycinnamic acid.

8.2.7 The synthesis of tetra-substituted dimethoxycinnamoyl PSEs

The synthesis of 3,3',4',6'-tetra-*O*-(3,4-dimethoxycinnamoyl)-2,1':4,6-di-*O*-isopropylidene sucrose, **4DI 207**

2,1':4,6-di-*O*-isopropylidene sucrose **68** (500 mg, 1.18 mmol), diOMe acid **38** (1.60 g, 7.70 mmol), DCC (1.59 g, 7.70 mmol) and DMAP (14.4 mg, 0.118 mmol) was dissolved and stirred in CH_2Cl_2 (10.0 ml) at room temperature for two days. Upon completion, the mixture was evaporated and dissolved in cold diethyl ether. After that, filtration was done. The filtrate collected was purified with column chromatography using 4:1 hexane/EtOAc as eluent. White solid 4DI is obtained in 78% yield.

Analytical data for 4DI 207: mp 125.1-126.0°C; ^1H NMR (300 MHz): isopropylidene rings: δ 1.24, 1.29, 1.46, 1.49 (m, 6H, $(\text{CH}_3)_2\text{C}$), (d, 6H, $(\text{CH}_3)_2\text{C}$); methoxy and sucrose unit: δ 3.62, 3.66, 3.73, 3.76, 3.78, 3.79 (m, 4H, H-5, 2 x H-1', H-6), 3.87, 3.90, 3.91, 3.92, 3.96 (m, 24H, 8 x $(\text{CH}_3)\text{O}$), 4.04, 4.05, 4.06 (m, 2H, H-6, H-2), 4.19, 4.24 (d, 1H, $J = 12$ Hz, H-6'), 4.52, 4.55 (m, 3H, H-4, H-3, H-6'), 5.41, 5.42 (m, 2H, H-4', H-5'), 5.65 (s, 1H, H-3'), 6.21, 6.22 (d, 1H, $J = 3$ Hz, H-1); *trans*-alkenyl and aromatic protons: δ 6.28, 6.30, 6.32, 6.33, 6.35, 6.37 (m, 3H, H-a¹, H-a², H-a³), 6.49, 6.54 (d, 1H, $J = 15$ Hz, H-a⁴), 6.82, 6.84, 6.86, 6.88 (m, 4H, H-d¹, H-d², H-d³, H-d⁴), 7.06, 7.07, 7.07, 7.09, 7.10, 7.11 (m, 6H, H-c¹, H-c², H-c³, H-c⁴, H-g¹, H-g³), 7.24, 7.26 (m, 2H, H-g², H-g⁴), 7.63, 7.67, 7.69 (m, 3H, H-b¹, H-b², H-b³), 7.86, 7.91 (d, 1H, $J = 15$ Hz, H-d⁴); ^{13}C NMR (75.5 MHz, CDCl_3): δ 19.9, 24.6, 27.8, 30.9, 50.9, 55.9, 55.9, 55.9,

55.9, 56.0, 62.6, 64.2, 64.7, 70.0, 70.6, 73.7, 76.5, 78.3, 80.0, 92.5, 101.5, 105.0, 105.7, 109.7, 110.3, 111.0, 113.8, 114.0, 114.7, 123.0, 123.2, 123.4, 126.9, 127.0, 127.1, 127.1, 127.2, 146.0, 146.3, 146.8, 148.0, 149.2, 149.3, 151.4, 151.4, 151.6, 166.1, 166.1, 166.4, 167.2, 169.0; HRMS (ESI-positive mode): m/z calcd. for $C_{62}H_{71}O_{23}$ 1183.4386; found 1183.4359 $[M+H]^+$

The synthesis of 3,3',4',6'-tetra-*O*-(3,4-dimethoxycinnamoyl) sucrose, 4DS 208

4DI **201** (100 mg, 0.085 mmol) was dissolved in MeCN (3 ml) and stirred at room temperature. After which, $Yb(OTf)_3 \cdot H_2O$ (9.1 mg, 0.0169 mmol) was added to the solution and the reaction was left to stir for 12 hours. Upon completion, MeCN was removed and the crude product was purified with column chromatography using 2:1 EtOAc/hexane as eluent. A white solid, 4DI **208**, was obtained in 71% yield.

Analytical data for 4DS 208: mp 99.1-100.9°C; 1H

NMR (300 MHz, $CDCl_3$): methoxy and sucrose unit:

δ 3.50, 3.55, 3.60 (t, $J = 15$ Hz, 1H, H-5), 3.70, 3.73 (m, 1H, H-1'), 3.80 (m, 3H, H-1', 2 x H-6), 3.86, 3.87, 3.88, 3.89, 3.90 (m, 24H, 8 x $(CH_3)O$), 4.09, 4.11 (m, 2H, H-2, H-6'), 4.45, 4.47, 4.50, 4.53, 4.60, 4.65, 4.70 (m, 3H, H-6', H-4, H-3), 5.01, 5.05, 5.09 (t, 1H, $J = 12$ Hz, H-1), 5.50, 5.51, 5.54, 5.56 (m, 2H, H-4', H-5'), 5.70 (m, 1H, H-3'); *trans*-alkenyl and aromatic protons: δ 6.27, 6.28, 6.31, 6.32, 6.34, 6.36 (m, 3H, H-a¹, H-a², H-a³), 6.45, 6.51 (d, 1H, $J = 18$ Hz, H-a⁴), 6.79, 6.82, 6.82, 6.84, 6.85 (m, 4H, H-d¹,

H-d², H-d³, H-d⁴), 7.00, 7.01, 7.02, 7.04, 7.06, 7.06, 7.09 (m, 6H, H-c¹, H-c², H-c³, H-c⁴, H-g¹, H-g³), 7.17, 7.19 (m, 2H, H-g², H-g⁴), 7.56, 7.62, 7.63, 7.65, 7.68, 7.70 (m, 3H, H-b¹, H-b², H-b³), 7.80, 7.86 (d, 1H, $J = 18$ Hz, H-b⁴); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 50.9, 55.9, 55.9, 55.9, 55.9, 56.0, 62.6, 64.2, 64.7, 70.0, 70.6, 73.7, 78.8, 80.0, 92.5, 101.5, 105.0, 105.4, 109.7, 110.3, 111.1, 113.8, 114.0, 114.7, 123.2, 123.2, 123.4, 126.9, 127.0, 127.0, 127.2, 127.2, 146.0, 146.3, 146.8, 148.0, 149.2, 149.3, 151.2, 151.3, 151.3, 166.1, 166.1, 166.4, 167.4; HRMS (ESI-positive mode): m/z calcd. for $C_{56}H_{62}O_{23}Na$ 1125.3580; found 1125.3562 $[M+Na]^+$

8.2.8 The synthesis of 3,4,5-trimethoxycinnamic acid **210**

Sinapic acid **209** (10.00 g, 51.5 mmol) was dissolved in 4N NaOH (20 ml) and stirred in room temperature. Subsequently, $(\text{CH}_3)_2\text{SO}_4$ (3.0 ml, 33.5 mmol) was added slowly to the stirring mixture. The yellow mixture was left to stir for 1 h, before adding another portion of $(\text{CH}_3)_2\text{SO}_4$ (1.5 ml, 16.7 mmol) and 4N NaOH (10 ml). The mixture was left to stir for another 1 hour. After which, a new portion of $(\text{CH}_3)_2\text{SO}_4$ (1.5 ml, 16.7 mmol) and 4N NaOH (10 ml) were added and the mixture was left to stir for 10 hours. Upon completion, the brown mixture was acidified to pH 2 with HCl and the precipitate was collected via vacuum filtration, washed twice with distilled water. After drying under reduced pressure, 3,4,5-trimethoxycinnamic (triOMe) acid **210** was obtained in 80% yield.

Analytical data for triOMe acid **210:** Comparison with reported literature values¹³⁰ confirmed product as triOMe acid **210**.

8.2.9 The synthesis of tetra-substituted trimethoxycinnamoyl PSEs

The synthesis of 3,3',4',6'-tetra-*O*-(3,4,5-trimethoxycinnamoyl)-2,1':4,6-di-*O*-isopropylidene sucrose, 4TI **211**

2,1':4,6-di-*O*-isopropylidene sucrose **68** (500 mg, 1.18 mmol), diOMe acid **38** (1.83 g, 7.70 mmol), DCC (1.59 g, 7.70 mmol) and DMAP (14.4 mg, 0.118 mmol) was dissolved and stirred in CH_2Cl_2 (10.0 ml) at room temperature for two days. Upon completion, the mixture was evaporated and dissolved in cold diethyl ether. After that, filtration was done. The filtrate collected was purified with column chromatography using 4:1 hexane/EtOAc as eluent. White solid 4TI **211** was obtained in 74% yield.

Analytical data for 4TI 211: mp 126.9-128.5°C; ^1H NMR (300 MHz, CDCl_3): isopropylidene rings: δ 1.245, 1.25, 1.29, 1.35, 1.39 (m, 12H, 2 x $(\text{CH}_3)_2\text{C}$); methoxy and sucrose unit: δ 3.43, 3.45, 3.47 (t, 1H, $J = 6$ Hz, H-5), 3.63, 3.67, 3.69 (m, 3H, 2 x H-1', H-6), 3.72, 3.76, 3.83, 3.85, 3.87, 3.97, 3.88, 3.89 (m, 36H, 12 x $(\text{CH}_3)\text{O}$) 3.91, 3.93 (m, 1H, H-6), 4.05, 4.07, 4.10 (m, 1H, H-2), 4.50, 4.51, 4.54 (m, 2H, 2 x H-6'), 4.95 (m, 1H, H-4), 5.35 (s, 1H, H-3), 5.45 (m, 2H, H-4', H-5'), 5.60 (s, 1H, H-3'), 6.13 (s, 1H, H-1); *trans*-alkenyl and aromatic protons: δ 6.31, 6.32, 6.34, 6.36, 6.37, 6.38, 6.38, 6.42, 6.43, 6.44, 6.48 (m, 4H, H-a¹, H-a², H-a³, H-

4TI 211

a⁴), 6.70, 6.72, 6.74, 6.75, 6.82, 6.83, 6.85 (m, 8H, H-c¹, H-c², H-c³, H-c⁴, H-g¹, H-g², H-g³, H-g⁴), 7.57, 7.58, 7.60, 7.62, 7.64, 7.65 (m, 3H, H-b¹, H-b², H-b³), 7.69, 7.75 (d, 1H, $J = 18$ Hz, H-b⁴); ^{13}C NMR (75.5 MHz, CDCl_3): δ 19.1, 24.1, 24.1, 25.4, 25.5, 25.6, 28.9, 30.9, 56.1, 56.2, 61.0, 63.9, 64.7, 66.2, 70.2, 72.1, 72.8, 72.9, 73.0, 73.2, 73.8, 74.2, 90.8, 91.4, 92.1, 99.7, 101.7, 101.8, 104.8, 105.3, 105.4, 105.7, 115.5, 115.6, 115.7, 115.8, 116.2, 116.7, 116.9, 129.3, 129.4, 129.4, 129.5, 129.6, 129.6, 129.7, 129.8, 140.3, 145.1, 146.0, 146.4, 146.5, 146.8, 147.0, 153.0, 153.3, 153.4, 165.8, 166.1, 166.4, 167.0, 167.4; HRMS (ESI-positive mode): m/z calcd. for $\text{C}_{66}\text{H}_{79}\text{O}_{27}$ 1303.4809; found 1303.4871 $[\text{M}+\text{H}]^+$

The synthesis of 3,3',4',6'-tetra-*O*-(3,4,5-trimethoxy)cinnamoyl sucrose, 4TS 212

4TI **201** (100 mg, 0.077 mmol) was dissolved in MeCN (3 ml) and stirred at room temperature. After which, $\text{Yb}(\text{OTf})_3 \cdot \text{H}_2\text{O}$ (8.2 mg, 0.0154 mmol) was added to the solution and the reaction was left to stir for 12 hours. Upon completion, MeCN was removed and the crude product was purified with column chromatography using 2:1 EtOAc/hexane as eluent. A white solid, 4TS **212**, was obtained in 78% yield.

Analytical data for 4TS 212: mp 100.2-102.2°C; ^1H NMR (300 MHz, CDCl_3): methoxy and sucrose unit: δ 3.63, 3.67, 3.60 (m, 5H, H-5, 2 x H-1', 2 x H-6), 3.73, 3.74, 3.76, 3.77, 3.78, 3.80 (m, 36H, 12 x $(\text{CH}_3)\text{O}$), 4.32, 4.34 (m, 2H, H-2, H-6'), 4.46 (s, 3H, H-6', H-3, H-4), 5.38, 5.46, 5.48, 5.53, 5.55 (m, 4H, H-1, H-3', H-4', H-5'); *trans*-alkenyl and aromatic protons: δ 6.23, 6.25, 6.28, 6.30, 6.32, 6.38 (m, 4H, H-a¹, H-a², H-a³, H-a⁴), 6.63, 6.65, 6.73 (m, 8H, H-c¹, H-c², H-c³, H-c⁴, H-g¹, H-g², H-g³, H-g⁴), 7.50, 7.55, 7.58, 7.63 (m, 4H, H-b¹, H-b²,

4TS 212

H-b³, H-b⁴); ¹³C NMR (75.5 MHz, CDCl₃): δ 56.1, 56.2, 60.9, 61.0, 64.1, 76.6, 105.6, 105.9, 115.5, 116.1, 129.2, 129.4, 129.4, 140.5, 140.5, 140.7, 146.2, 146.9, 147.7, 153.3, 153.4, 153.5, 165.9, 166.5, 167.0; HRMS (ESI-positive mode): *m/z* calcd. for C₆₀H₇₁O₂₇ 1223.4183; found 1223.4193 [M+H]⁺

8.2.10 Orthogonal Protection of 2,1':4,6-di-*O*-isopropylidene sucrose 68

8.2.10.1 The synthesis of compound 6'-*O*-*tert*-butyldimethylsiloxy-2,1':4,6-di-*O*-isopropylidene sucrose, 6'-*O*-TBS 214

2,1':4,6-di-*O*-isopropylidene sucrose **68** (1.00 g, 2.37 mmol) and DMAP (28.9 mg, 0.237 mmol) were dissolved in CH₂Cl₂ (50.0 ml) and stirred at room temperature. Subsequently, NEt₃ (793 μL, 5.68 mmol) and TBSCl (428 mg, 2.84 mmol) were also added to the stirring solution. The reaction was then left to stir for 12 hours. Upon completion, the reaction was washed twice with 10% NH₄Cl solution and the organic layer collected was then dried with anhydrous MgSO₄. CH₂Cl₂ was removed and the crude mixture was purified with column chromatography using 1:1 EtOAc/hexane as eluent. Finally, white solid 6'-*O*-TBS **214** was obtained in 95% yield.

Analytical Data for 6'-*O*-TBS 214: mp 81.5-83.6°C; ¹H NMR

(300 MHz, CDCl₃): TBS group: 0.01, 0.02 (m, 6H, (CH₃)₂Si), 0.84, 0.85 (m, 9H, (CH₃)₃CSi); isopropylidene rings: δ 1.20 (s, 3H, (CH₃)₂C), 1.27, 1.35, 1.39, 1.41, 1.43 (m, 6H, (CH₃)₂C), 1.48 (s, 3H, (CH₃)₂C); sucrose unit: δ 3.42, 3.46 (d, 1H, J = 12

6'-*O*-TBS 214

Hz, H-5), 3.49, 3.52, 3.54, 3.56, 3.57, 3.59 (m, 2H, 2 x H-1'), 3.61, 3.63, 3.64, 3.66, 3.67, 3.69, 3.70 (m, 3H, H-2, H-6, H-6'), 3.73, 3.75, 3.76, 3.77, 3.78, 3.79, 3.81, 3.82, 3.84, 3.86, 3.88 (m, 6H, H-3, H-4, H-6, H-3', H-6', H-3'), 4.04, 4.06, 4.08 (m, 2H, H-4', H-5'), 5.96, 5.98 (d, 1H, H-1'), ¹³C NMR (75.5 MHz, CDCl₃): δ -5.43, -5.22, 18.4, 19.1, 24.2, 25.3, 26.0, 29.0, 62.4, 63.6, 66.0, 66.0, 69.4, 73.3, 73.9, 78.5, 78.8, 81.5, 90.8, 100.0, 102.0, 103.2; HRMS (ESI-positive mode): *m/z* calcd. for C₂₄H₄₅O₁₁Si 537.2731; found 537.2734 [M+H]⁺

8.2.10.2 The synthesis of compound 3'-*O*-carboxybenzyl-6'-*O*-*tert*-butyldimethylsiloxy-2,1'-di-*O*-isopropylidene sucrose, 3'-*O*-Cbz 225

6'-*O*-TBS **214** (500 g, 0.932 mmol) and DMAP (11.4 mg, 0.0932 mmol) were dissolved in CH₂Cl₂ and stirred at room temperature. After which, CbzCl (199 μL, 1.40 mmol) and TMEDA (420 μL, 2.80 mmol) were added to the stirring solution and the reaction was left to stir for 12 hours. Upon completion, the reaction was washed with twice with water and the organic layer was collected and dried with anhydrous

MgSO₄. CH₂Cl₂ was removed and the crude mixture was subjected to chromatographic purification using a gradient of EtOAc/hexane as eluent (starting from 8:1 Hex/EtOAc, to 6:1 Hexane/EtOAc then to 4:1 Hexane/EtOAc). At the end, white solid product 3'-O-Cbz **225** was obtained in 75% yield.

This reaction also gave 3',4'-di-O-carboxybenzyl-6'-*tert*-butyldimethylsiloxy-2,1': 4,6-di-O-isopropylidene sucrose, di-O-Cbz **226** as white solid in 20% yield.

Analytical data for 3'-O-Cbz 225: mp 73.3-74.9°C; ¹H NMR

(300 MHz, CDCl₃): TBS group: δ 0.02, 0.05 (m, 6H, (CH₃)₂Si), 0.79, 0.83, 0.86, 0.87 (m, 9H, (CH₃)₃CSi); isopropylidene rings: δ 1.21, 1.29, 1.41, 1.43 (m, 12H, 2 x (CH₃)₂C); sucrose unit: δ 3.45, 3.49 (d, 1H, J = 12 Hz, H-5), 3.54, 3.57, 3.60, 3.62, 3.63, 3.64, 3.66 (m, 3H, H-2, 2 x H-1'), 3.68, 3.71, 3.73, 3.75, 3.76, 3.77, 3.80, 3.82, 3.83, 3.85 (m, 5H, H-4, 2 x H-6, 2 x H-6'), 3.94, 3.97, 4.01 (m, 2H, H-3, H-5'), 4.37, 4.38, 4.39 (t, 1H, J = 3 Hz, H-4'), 4.71, 4.73 (d, 1H, J = 6 Hz, H-3'), 5.92, 5.93 (d, 1H, J = 3 Hz, H-1); Cbz group: 5.19 (s, 2H, 2 x H-a), 7.30, 7.32, 7.33, 7.35, 7.37, 7.40 (m, 5H, H-b, H-c, H-d, H-e, H-f); ¹³C NMR (75.5 MHz, CDCl₃): δ -5.46, -5.34, 18.3, 19.1, 24.1, 25.5, 25.7, 25.9, 29.1, 62.3, 63.5, 65.1, 66.2, 70.0, 70.4, 73.0, 73.8, 82.0, 82.3, 90.8, 99.8, 101.6, 103.4, 128.4, 128.7, 128.7, 134.7, 155.2; HRMS (ESI-positive mode): *m/z* calcd. for C₃₂H₅₁O₁₃Si 671.3099; found 671.3102 [M+H]⁺

3'-O-TBS **225**

Analytical data for di-O-Cbz 226: mp 80.0-82.5°C; ¹H NMR

(300 MHz, CDCl₃): TBS group: δ 0.00 (s, 6H, (CH₃)₂Si), 0.83 (s, 9H, (CH₃)₃CSi); isopropylidene rings: δ 1.30, 1.32, 1.43, 1.45 (m, 12H, 2 x (CH₃)₂C); sucrose unit: δ 3.46, 3.50 (d, 1H, J = 12 Hz, H-5), 3.54, 3.58 (d, 1H, J = 12 Hz, H-1'), 3.61, 3.65, 3.66, 3.67, 3.69 (m, 2H, H-2, H-1'), 3.71, 3.75, 3.76, 3.77, 3.78, 3.79, 3.80, 3.82, 3.82, 3.84 (m, 5H, H-4, 2 x H-6, 2 x H-6'), 4.03, 4.07 (d, 1H, J = 12 Hz, H-3), 4.12, 4.13, 4.14, 4.15, 4.16, 4.17, 4.18 (m, 1H, H-5'), 4.90, 4.92 (d, 1H, J = 6 Hz, H-3'), 5.31, 5.33, 5.34 (t, 1H, J = 4.5 Hz, H-4'), 5.96, 5.97 (d, 1H, J = 3 Hz, H-1); Cbz groups: δ 5.17 (s, 2H, 2 x H-a), 5.18, 5.23 (d, 2H, J = 15 Hz, 2 x H-a'), 7.32, 7.33, 7.34, 7.36, 7.37, 7.39 (m, 10H, H-b, H-c, H-d, H-e, H-f, H-b', H-c', H-d', H-e', H-f'); ¹³C NMR (75.5 MHz, CDCl₃): δ -5.55, -5.44, 18.2, 19.1, 62.3, 63.7, 63.8, 66.2, 70.0, 70.1, 70.3, 72.9, 73.9, 80.8, 81.1, 81.5, 91.2, 99.9, 101.6, 104.1, 128.3, 128.4, 128.6, 128.7, 134.8, 134.8, 154.0, 154.5; HRMS (ESI-positive mode): *m/z* calcd. for C₄₀H₅₇O₁₅Si 805.3467; found 805.3452 [M+H]⁺

di-O-TBS **226**

8.2.10.3 The synthesis of compound 4'-O-*para*-nitrobenzoyl-3'-O-carboxybenzyl-6'-O-*tert*-butyldimethylsiloxy-2,1'-di-O-isopropylidene sucrose, 4'-O-PNB **227**

3'-O-Cbz **225** (500 mg, 0.746 mmol) and DMAP (9.1 mg, 0.0746 mmol) were dissolved in CH₂Cl₂ and stirred at 4°C using ice bath. Subsequently, NEt₃ (312 μL, 2.24 mmol) was added to the stirring solution. Then PNBCl (208 mg, 1.12 mmol) was dissolved in CH₂Cl₂ and added to the stirring solution dropwise. The reaction was left to stir for 12 hours. Upon completion, CH₂Cl₂ was removed and the crude product was purified with column chromatography using 4:1 Hexane/EtOAc as eluent. White solid 4'-O-PNB **227** was obtained in 70% yield.

This reaction also gave white solid 3-O-*para*-nitrobenzoyl-3'-O-carboxybenzyl-6'-O-*tert*-butyldimethylsiloxy-2,1'-di-O-isopropylidene sucrose (3-O-PNB) **228** in 10% yield and 3,4'-di-O-*para*-nitrobenzoyl-3'-O-carboxybenzyl-6'-O-*tert*-butyldimethylsiloxy-2,1'-di-O-isopropylidene sucrose (di-O-PNB) **229** in 19% yield.

Analytical data for 4'-O-PNB **227:** mp 87.1-88.9°C; ¹H NMR (300 MHz, CDCl₃): TBS group: δ -0.00 (s, 6H, (CH₃)₂Si), 0.78, 0.83 (m, 9H, (CH₃)₃CSi); isopropylidene rings: δ 1.36, 1.44, 1.49, 1.53 (m, 12H, 2 x (CH₃)₂C); sucrose unit: δ 3.51, 3.56 (d, 1H, J = 15 Hz, H-5), 3.58, 3.61 (d, 1H, J = 9 Hz, H-1'), 3.64, 3.69, 3.71, 3.72, 3.73, 3.74 (m, 3H, H-2, H-1', H-6'), 3.80, 3.82, 3.85, 3.86 (m, 3H, H-4, 2 x H-6, H-6'), 4.09, 4.13 (d, 1H, J = 12 Hz, H-3), 4.22, 4.25, 4.27 (m, 1H, H-5'), 5.02, 5.04 (d, 1H, J = 6 Hz, H-3'), 5.72, 5.73, 5.75 (t, 1H, J = 4.5 Hz, H-4'), 6.01, 6.02 (d, 1H, J = 3 Hz, H-1); Cbz group: δ 5.14, 5.18, 5.20, 5.25 (m, 2H, 2 x H-a), 7.31, 7.33, 7.34, 7.36, 7.38 (m, 5H, H-b, H-c, H-d, H-e, H-f); PNB group: δ 8.13, 8.16, 8.26, 8.29 (dd, 4H, J = 30, 9 Hz, H-a', H-b', H-c', H-d'); ¹³C NMR (75.5 MHz, CDCl₃): δ -5.46, -5.42, 18.2, 19.1, 25.7, 29.1, 70.0, 70.4, 72.9, 73.9, 78.9, 80.8, 81.7, 99.9, 101.7, 104.2, 123.6, 128.2, 128.6, 128.7, 130.9, 134.7, 151.0, 154.6; HRMS (ESI-positive mode): *m/z* calcd. for C₃₉H₅₄NO₁₆Si 820.3212; found 820.3218 [M+H]⁺

4'-O-PNB **227**

Analytical data for 3-O-PNB **228:** mp 85.8-86.9°C; ¹H NMR (300 MHz, CDCl₃): TBS group: δ 0.00 (s, 6H, (CH₃)₂Si), 0.83 (s, 9H, (CH₃)₃CSi); isopropylidene rings: δ 1.10, 1.30m 1.36, 1.40 (m, 3H, 2 x (CH₃)₂C); sucrose unit: δ 3.64, 3.67 (d, 1H, J = 9 Hz, H-5), 3.69, 3.73, 3.76, 3.78 (m, 2H, 2 x H-1'), 3.82, 3.83, 3.85, 3.87, 3.89 (m, 4H, H-2, H-6', 2 x H-6), 3.92, 3.94, 3.96 (m, 2H, H-4, H-6'), 4.04, 4.08 (d, 1H, J = 12 Hz, H-5'), 4.40, 4.41, 4.42 (t, 1H, J = 3 Hz, H-4'), 4.72, 4.74 (d, 1H, J = 6 Hz, H-3'), 5.43, 5.47, 5.50, J = 10.5 Hz, H-

3-O-PNB **228**

3), 6.01, 6.02 (d, 1H, $J = 3$ Hz, H-1); Cbz group: δ 5.27, 5.28, 5.32 (m, 2H, 2 x H-a), 7.30, 7.33, 7.35 (m, 3H, H-c, H-d, H-e) 7.42, 7.45 (m, 2H, H-b, H-f); PNB group: δ 8.12, 8.15, 8.23, 8.26 (dd, 4H, $J = 24, 9$ Hz, H-a', H-b', Hc', H-d'); ^{13}C NMR (75.5 MHz, CDCl_3): δ -5.4, -5.3, 14.1, 18.3, 18.4, 19.0, 19.1, 24.0, 25.4, 25.8, 29.0, 29.7, 29.7, 30.9, 31.9, 62.3, 63.8, 64.6, 64.9, 70.6, 71.5, 71.6, 71.8, 72.6, 82.5, 91.1, 92.0, 99.7, 99.8, 101.3, 103.7, 103.9, 123.5, 123.6, 123.9, 127.4, 128.4, 128.5, 128.6, 128.6, 128.7, 130.7, 130.9, 134.9, 135.7, 135.9, 150.5, 155.2, 163.7, 164.6; HRMS (ESI-positive mode): m/z calcd. for $\text{C}_{39}\text{H}_{54}\text{NO}_{16}\text{Si}$ 820.3212; found 820.3216 $[\text{M}+\text{H}]^+$

Analytical data for di-O-PNB 229: mp 95.0-96.7°C; ^1H NMR

(300 MHz, CDCl_3): TBS group: δ 0.00, 0.03 (d, 6H, $J = 9$ Hz, $(\text{CH}_3)_2\text{Si}$), 0.79, 0.82, 0.84 (m, 9H, $(\text{CH}_3)_3\text{CSi}$); isopropylidene rings: δ 1.17, 1.19, 1.31, 1.39, 1.42, 1.43 (m, 12H, 2 x $(\text{CH}_3)_2\text{C}$); sucrose unit: δ 3.48, 3.52 (d, 1H, $J = 12$ Hz, H-5), 3.71, 3.74 (m, 2H, 2 x H-1'), 3.81, 3.84, 3.87 (m, 4H, H-2, 2 x H-6, H-6'), 3.97, 3.98, 4.00, 4.01 (2H, m, H-4, H-6'), 4.07, 4.09 (d, 1H, $J = 6$ Hz, H-5'), 4.98, 4.99 (d, 1H, $J = 3$ Hz, H-3'), 5.29, 5.35, 5.39 (t, 1H, $J = 12$ Hz, H-4'), 5.70, 5.72, 5.73 (t, 1H, $J = 4.5$ Hz, H-3), 6.09,

di-O-PNB 228

6.10 (d, 1H, $J = 3$ Hz, H-1); Cbz group: δ 5.25, 5.29, 5.35, 3.39 (dd, 2H, $J = 18, 12$ Hz, 2 x H-a), 7.29, 7.31 (m, 3H, H-c, H-d, H-e), 7.42, 7.45 (m, 2H, H-b, H-f); PNB group: δ 8.11, 8.14, 8.18 (m, 4H, H-a', H-b', H-c', H-d'), 8.24, 8.25, 8.28 (m, 4H, H-a'', H-b'', H-c'', H-d''); ^{13}C NMR (75.5 MHz, CDCl_3): δ : δ 8.12, 8.15, 8.23, 8.26 (dd, 4H, $J = 24, 9$ Hz, H-a', H-b', Hc', H-d'); ^{13}C NMR (75.5 MHz, CDCl_3): δ -5.4, -5.3, 14.1, 18.3, 18.4, 19.0, 19.1, 24.0, 25.4, 25.8, 29.0, 29.7, 29.7, 30.9, 31.9, 62.3, 63.8, 64.6, 64.9, 70.6, 71.5, 71.6, 71.8, 72.6, 82.5, 91.1, 92.0, 99.7, 99.8, 101.3, 103.7, 103.9, 123.5, 123.6, 123.7, 123.9, 127.4, 128.4, 128.5, 128.6, 128.6, 128.7, 130.7, 130.9, 131.3, 134.9, 135.7, 135.9, 150.5, 150.6, 154.6 155.2, 163.7, 164.6, 167.1; HRMS (ESI-positive mode): m/z calcd. for $\text{C}_{46}\text{H}_{57}\text{N}_2\text{O}_{19}\text{Si}$ 969.3325; found 969.3352 $[\text{M}+\text{H}]^+$

8.2.10.4 The synthesis of compound 3-*O*-(4-oxopentanoyl)-4'-*O*-*para*-nitrobenzoyl-3'-*O*-carboxybenzyl-6'-*O*-*tert*-butyldimethylsiloxy-2,1'-di-*O*-isopropylidene sucrose, OP-diisopropylidene **224**

4'-*O*-PNB **227** (500 mg, 0.610 mmol) and DMAP (7.5 mg, 0.0610 mmol) were dissolved in CH₂Cl₂ and stirred at room temperature. Subsequently, LevOH (75.0 μ L, 0.732 mmol) and DCC (151 mg, 0.732 mmol) were added to the stirring solution and the reaction was left to stir for 12 hours. Upon completion, CH₂Cl₂ was removed and the crude mixture was dissolved again in cold diethyl ether. After which, the mixture was filtered. After removing diethyl ether, the remaining crude residue was purified by column chromatography using 3:1 Hexane/EtOAc as eluent. White solid OP-diisopropylidene **224** was obtained in 86% yield.

Analytical data for OP-diisopropylidene 224: mp 96.1-

98.9°C; ¹H NMR (300 MHz, CDCl₃): TBS group: δ -0.01, 0.00 (m, 6H, (CH₃)₂Si), 0.79, 0.82 (m, 9H, (CH₃)₃CSi); isopropylidene rings: δ 1.23, 1.28, 1.35, 1.46 (m, 12H, 2 x (CH₃)₂C); Lev group: 2.15 (s, 3H, 3 x H-c''), 2.59, 2.61 (m, 2H, 2 x H-b''), 2.73, 2.74 (m, 2H, 2 x H-a''); Cbz and sucrose unit: δ 3.47, 3.51 (d, 1H, J = 12 Hz, H-5), 3.65, 3.67, 3.68 (m, 2H, H-4, H-1'), 3.81, 3.83, 3.96, 3.86 (m, 5H, H-2, 2 x H-6,

OP-diisopropylidene **224**

H-1', H-6'), 4.16, 4.20 (d, 1H, J = 12 Hz, H-6'), 4.22, 4.25, 4.27 (m, 1H, H-5'), 4.98, 5.00 (d, 1H, J = 6 Hz, H-3'), 5.23, 5.29 (m, 3H, H-3, 2 x H-a), 5.70, 5.71, 5.72 (t, 1H, J = 3 Hz, H-4'), 6.03, 6.04 (d, 1H, J = 3 Hz, H-1), 7.30, 7.32 (m, 3H, H-c, H-d, H-e), 7.39, 7.40, 7.42 (m, 2H, H-b, H-f); PNB group: δ 8.12, 8.15, 8.25, 8.27 (dd, 4H, J = 30, 7.5, H-a', H-b', H-c', H-d'); ¹³C NMR (75.5 MHz, CDCl₃): δ -5.45, -5.41, 5.79, 6.59, 18.2, 19.0, 23.8, 25.4, 25.8, 28.0, 29.0, 29.9, 38.1, 62.3, 63.5, 64.0, 66.4, 70.6, 71.0, 71.5, 71.8, 79.1, 81.0, 82.2, 91.7, 99.7, 101.5, 104.6, 123.6, 128.5, 130.9, 134.7, 134.9, 150.7, 154.5, 163.4, 171.6, 206.4; HRMS (ESI-positive mode): *m/z* calcd. for C₄₄H₆₀NO₁₈Si 918.3580; found 918.3589 [M+H]⁺

8.2.10.5 Selective removal of the TBS protecting group

The synthesis of 3-*O*-(4-oxopentanoyl)-4'-*O*-*para*-nitrobenzoyl-3'-*O*-carboxybenzyl-2,1'-di-*O*-isopropylidene sucrose **230**

OP-diisopropylidene **224** (200 mg, 0.218 mmol) was dissolved in pyridine (1.0 ml) and stirred at room temperature. 1.56 M 3HF·NEt₃ (419 μ L, 0.654 mmol) and NEt₃ (60.9 μ L, 0.436 mmol) were added to the stirring solution and the reaction was left to stir for 12 hours. Upon completion, pyridine was

removed, and the crude mixture was subjected to chromatographic purification using 3:2 Hex/EtOAc as eluent. White solid compound **230** was obtained in 86%.

Analytical data for compound 230: mp 85.1-87.9°C; ¹H

NMR (300 MHz, CDCl₃): isopropylidene rings: δ 1.25, 1.31, 1.41, 1.43 (m, 12H, 2 x (CH₃)₂C); Lev group: 2.11 (s, 3H, 3 x H-c''), 2.55, 2.57 (m, 2H, 2 x H-b''), 2.68, 2.70 (m, 2H, 2 x H-a''); Cbz and sucrose unit: δ 3.48, 3.52 (d, 1H, J = 12 Hz, H-5), 3.61, 3.63, 3.66 (m, 2H, H-2, H-1'), 3.80, 3.82, 3.85, 3.86 (m, 5H, H-4, 2 x H-6, H-1', H-6'), 4.15, 4.16 (d, 1H, J = 3 Hz, H-6'), 4.19, 4.20 (m, 1H, H-5'), 5.09, 5.11 (d, 1H, J = 6 Hz, H-3'), 5.15, 5.20, 5.23 (m,

230

3H, H-3, 2 x H-a), 5.71, 5.72, 5.73 (t, 1H, J = 3 Hz, H-4'), 6.17, 6.19 (d, 1H, J = 6 Hz, H-1), 7.24, 7.24, 7.25, 7.26 (m, 3H, H-c, H-d, H-e), 7.34, 7.35, 7.36, 7.37 (m, 2H, H-b, H-f), PNB group: δ 8.06, 8.09, 8.09 (m, 2H, H-a', H-c'), 8.20, 8.21, 8.23 (m, 2H, H-b', H-d'); ¹³C NMR (75.5 MHz, CDCl₃): δ 19.0, 23.9, 24.9, 25.3, 28.0, 29.0, 29.9, 30.9, 34.0, 38.0, 61.8, 61.9, 70.7, 70.8, 71.2, 71.4, 80.4, 83.4, 91.8, 99.8, 101.9, 103.9, 123.7, 128.5, 128.6, 131.0, 134.3, 134.8, 150.8, 154.4, 163.8, 171.6, 206.4; HRMS (ESI-positive mode): *m/z* calcd. for C₃₈H₄₅NO₁₈Na 826.2534; found 826.2546 [M+Na]⁺

8.2.10.6 Selective removal of the Cbz protecting group

The synthesis of 3-*O*-(4-oxopentanoyl)-4'-*O*-para-nitrobenzoyl-6'-*O*-butyldimethylsiloxy-2,1'-di-*O*-isopropylidene sucrose **231**

OP-diisopropylidene **224** (200 mg, 0.218 mmol) and Pd(OAc)₂ (4.1 mg, 0.0182 mmol) were dissolved in CH₂Cl₂ (5.0 ml) and stirred at room temperature. After which, Et₃SiH (46.4 mg, 0.291 mmol) and NEt₃ (4.06 μL, 0.0291 mmol) were added and the reaction was left to stir for 12 hours. Upon completion, CH₂Cl₂ was removed and the crude mixture was subjected to purification via column chromatography. 2:1 Hexane/EtOAc were used as eluent and white solid compound **231** was obtained in 68% yield.

Analytical data for compound 231: mp 84.3-85.9°C; ¹H

NMR (300 MHz, CDCl₃): TBS group: δ 0.00 (s, 6H, (CH₃)₂Si), 0.78 (s, 9H, (CH₃)₃CSi); isopropylidene rings: δ 1.30, 1.40, 1.50 (m, 12H, 2 x (CH₃)₂C); Lev group: 2.20 (s, 3H, 3 x H-c''), 2.59, 2.60, 2.62, 2.62, 2.63, 2.64, 2.65 (m, 2H, 2 x H-b''), 2.73, 2.75, 2.77, 2.80, 2.82 (m, 2H, 2 x H-a''); sucrose unit: δ 3.50, 3.54 (d, 1H, J = 12 Hz, H-5), 3.68, 3.71, 3.74, 3.78, 3.80, 3.81, 3.84, 3.87, 3.88, 3.901,

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3.92, 3.94 (m, 7H, H-2, H-4, 2 x H-6, 2 x H-1', H-6'), 4.09, 4.12 (d, 1H, J = 9 Hz, H-6'), 4.17, 4.20, 4.21, 4.22, 4.22 (m, 2H, H-3', H-5'), 5.23, 5.27, 5.30 (t, 1H, J = H-4'), 5.52, 5.54, 5.56 (t, 1H, J = 6 Hz, H-3), 6.11, 6.12 (d, 1H, H-1'); PNB group: δ 8.22, 8.25, 8.30, 8.33 (dd, 4H, J = 15, 9 Hz, H-a', H-b', H-c', H-d'); ^{13}C NMR (75.5 MHz, CDCl_3): δ -6.98, 0.6.93, -6.91, -6.85, 4.30, 5.11, 16.7, 16.8, 16.8, 17.5, 17.5, 22.4, 23.7, 24.2, 24.3, 24.3, 24.3, 24.5, 26.6, 26.9, 27.5, 28.2, 28.4, 28.4, 29.5, 36.5, 60.8, 62.5 63.6, 69.4, 69.9, 70.2, 78.8, 79.4, 79.9, 89.2, 89.9, 98.3, 98.3, 100.1, 102.6, 103.3, 103.7, 122.0, 122.1, 129.5, 133.4, 133.5, 133.6 149.2, 162.5, 170.6, 206.7; HRMS (ESI-positive mode): m/z calcd. for $\text{C}_{36}\text{H}_{53}\text{NO}_{16}\text{NaSi}$ 806.3031; found 806.3079 $[\text{M}+\text{Na}]^+$

8.2.10.7 Selective removal of PNB

The synthesis of 3-*O*-(4-oxopentanoyl)-3'-*O*-carboxybenzyl-6'-*O*-butyldimethylsiloxy-2,1'-di-*O*-isopropylidene sucrose **232**

OP-diisopropylidene **224** (200 mg, 0.218 mmol) was treated with $\text{Mg}(\text{OMe})_2$ (65.4 μL , 0.0218 mmol) in 8:2 MeOH/THF (5.0 ml) at 4°C (ice bath). The reaction was closely monitored with TLC and quenched immediately with 1N HCl (43.6 μL , 0.0436 mmol) after all starting material was consumed (usually within 1 hour). MeOH/THF were removed and the crude mixture was subjected to purification via column chromatography. 2:1 Hexane/EtOAc was used as eluent and two products – compounds **232** and **233** were obtained as white solid in 70% and 15 % yield respectively.

Since compound **233** is an undesired by-product, its analytical data was not included.

Analytical data for compound 232: mp 85.0-86.9°C;

^1H NMR (300 MHz, CDCl_3): TBS group: δ 0.00 (s, 6H, $(\text{CH}_3)_2\text{Si}$), 0.83 (s, 9H, $(\text{CH}_3)_3\text{CSi}$); isopropylidene rings: δ 1.23, 1.34, 1.41, 1.43 (m, 12H, 2 x $(\text{CH}_3)_2\text{C}$); Lev group: 2.14 (s, 3H, 3 x H-c''), 2.56, 2.58 (m, 2H, 2 x H-b''), 2.69, 2.72 (m, 2H, 2 x H-a'');

Cbz and sucrose unit: δ 3.42, 3.46 (d, 1H, J = 12 Hz,

H-5), 3.60, 3.63, 3.66 (m, 3H, H-2, 2 x H-1'), 3.77, 3.79, 3.81, 3.85 (m, 4H, H-4, 2 x H-6, H-6'), 3.91, 3.93, 3.96, 3.97 (m, 1H, H-6'), 4.02, 4.06 (d, 1H, J = 12 Hz, H-5'), 4.35, 4.36, 4.38 (t, 1H, J = 4.5, H-4') 4.70, 4.73 (d, 1H, J = 9 Hz, H-3'), 5.16, 5.20, 5.23, 5.24 (m, 3H, H-3, 2 x H-a), 5.95, 5.96 (d, 1H, H-1'), 7.29, 7.31, 7.34, 7.36, 7.40, 7.43 (m, 5H, H-b, H-c, H-d, H-e, H-f); ^{13}C NMR (75.5 MHz, CDCl_3): δ -.5.44, -5.34, 18.3, 19.0, 23.9, 25.4, 28.1, 29.0, 29.9, 38.1, 62.3, 63.7, 64.9, 66.2, 70.5, 71.2, 71.5, 82.1, 82.4, 91.1, 99.6, 101.3, 103.6, 128.5, 128.7, 134.9, 155.2, 171.6, 206.4; HRMS (ESI-positive mode): m/z calcd. for $\text{C}_{37}\text{H}_{57}\text{NO}_{15}\text{Si}$ 769.3467; found 769.3460 $[\text{M}+\text{H}]^+$

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8.2.10.8 Selective removal of Lev

The synthesis of compound 4'-O-*para*-nitrobenzoyl-3'-O-carboxybenzyl-6'-O-*tert*-butyldimethylsiloxy-2,1'-di-O-isopropylidene sucrose **227**

OP-diisopropylidene **224** (200 mg, 0.218 mmol) was dissolved in 1,4-dioxane (5.0 ml) and NaBH₄ (18.1 mg, 0.480 mmol) was added to it. The reaction was left stirring for 4 hours and upon completion, 1,4-dioxane was removed. The crude mixture was subjected to purification via column chromatography using 3:2 Hexane/EtOAc as eluent. In the end, compound **227** was obtained as white solid in 72% yield.

Analytical Data for compound 227: Refer to 8.2.10.3

4'-O-PNB 227

8.2.11 Protection of free hydroxyl groups on (substituted) cinnamic acid

8.2.11.1 The synthesis of 4-*tert*-butyldimethylsilyloxy-3-methoxycinnamic acid **234**

N,N-Diisopropylethylamine (13.5 ml, 77.2 mmol) and TBSCl (5.82 g, 38.6 mmol) were added to a suspension of ferulic acid **39** (5.00 g, 25.7 mmol) in anhydrous CH₂Cl₂ (30.0 ml) at room temperature. The solution was then left to stir for 12 h. After which, the mixture was diluted with EtOAc (150 ml) and washed twice with 1N HCl (2 x 100 ml). The organic phase collected was then dried with anhydrous MgSO₄ and evaporated to obtain a yellow oil. The oil was then dissolved in 9:1 THF/water (30.0 ml) and K₂CO₃ (356 mg, 2.57 mmol) was added to the solution. After stirring for 4 h, the reaction was again diluted with EtOAc (150 ml) and washed twice with 1N HCl (2 x 100 ml) again. Subsequently, the organic phase was dried with anhydrous MgSO₄ and evaporated. Finally, hexane was added to the crude product and shaken. After filtration, white solid 4-*tert*-butyldimethylsilyloxy-3-methoxycinnamic (OTBS-feru) acid **234** was obtained in 87% yield.

Analytical data: Comparison of ¹H NMR with reported values in literature¹⁴³ confirmed the formation of OTBS-feru acid **234**.

8.2.11.2 The synthesis of 4- *tert*-butyldimethylsilyloxy-3,5-dimethoxycinnamic acid **235**

N,N-Diisopropylethylamine (11.7 ml, 66.9 mmol) and TBSCl (5.04 g, 33.5 mmol) were added to a suspension of sinapic acid **209** (5.00 g, 22.3 mmol) in anhydrous CH₂Cl₂ (30.0 ml) at room temperature. The solution was then left to stir for 12 h. After which, the mixture was diluted with EtOAc (150 ml) and washed twice with 1N HCl (2 x 100 ml). The organic phase collected was then dried with anhydrous MgSO₄ and evaporated to obtain a yellow oil. The oil was then dissolved in 9:1 THF/water (30.0 ml) and K₂CO₃ (308 mg, 2.23 mmol) was added to the solution. After stirring for 4 h, the reaction was again diluted with EtOAc (150 ml) and washed twice with 1N HCl (2 x 100 ml) again. Subsequently, the organic phase was dried with anhydrous MgSO₄ and evaporated. Finally, hexane was added to the crude product and shaken. After filtration, white solid 4-*tert*-butyldimethylsilyloxy-3,5-dimethoxycinnamic (OTBS-sinap) acid **235** was obtained in 88% yield.

Analytical data: Comparison of ¹H NMR with reported values in literature confirmed the formation of OTBS-sinap acid **235**.¹⁴⁴

8.2.12 The synthesis of 3-mono-substituted PSEs

8.2.12.1 Acylation using 4'-O-PNB **227**

General procedure

4'-O-PNB **227** (500 mg, 0.610 mmol) and DMAP (7.5 mg, 0.0610 mmol) were dissolved in CH₂Cl₂ and stirred at room temperature. After which, respective (substituted) cinnamic acid and DCC (252 mg, 1.22 mmol) were added to the solution and the reaction was left to stir for 24 hours. Upon completion of reaction, CH₂Cl₂ was removed and cold diethyl ether was added to the crude mixture obtained from respective reaction. After filtration, diethyl ether was removed, and the crude product remaining was purified with column chromatography using 8:1 Hexane/EtOAc as eluent. At the end, compounds **236** to **242** were obtained.

The synthesis of 3-*O*-cinnamoyl-4'-*O*-*para*-nitrobenzoyl-3'-*O*-carboxybenzyl-6'-*O*-*tert*-butyldimethylsiloxy-2,1'-di-*O*-isopropylidene sucrose **236**

Following the general procedure, cinnamic acid **30** (181 mg, 1.22 mmol) was added to the solution. After purification, the reaction gave **236** in as white solid in 76% yield.

Analytical data for compound 236: mp 90.3-90.9°C; ¹H NMR (300 MHz, CDCl₃): TBS group: δ 0.00, 0.01 (d, 6H, (CH₃)₂Si), 0.79 (s, 9H, (CH₃)₃CSi); isopropylidene rings: δ 1.23 (s, 3H, (CH₃)₂C) 1.34 (s, 3H, (CH₃)₂C), 1.46, 1.48 (d, 6H, (CH₃)₂C); Cbz and sucrose unit: δ 3.48, 3.52 (d, 1H, J = 12 Hz, H-5), 3.69, 3.73, 3.77, 3.82, 3.84, 3.85, 3.86, 3.88, 3.89, 3.91, 3.93, 3.94, 3.95 (m, 7H, H-2, H-4, 2 x H-6, 2 x H-1', H-6'), 4.21, 4.25, 4.26 (m, 2H, H-5', H-6'), 4.98, 4.99 (d, 1H, J = 3 Hz, H-3'), 5.25, 5.29, 5.35, 5.35, 5.38, 5.40, 5.41 (m, 3H, H-3, 2 x H-a), 5.70, 5.71, 5.73 (t, 1H, J = 3 Hz, H-4'), 6.06, 6.07 (d, 1H, J = 3 Hz, H-1'); Cbz and cinnamoyl protons: δ 6.41, 6.46 (d, 1H, J = 15 Hz, H-a''), 7.29, 7.30, 7.33, 7.35, 7.36, 7.37 (m, 6H, H-b, H-c, H-d, H-e, H-f, H-e''), 7.43, 7.46 (d, 2H, J = 9 Hz, H-d'', H-f''), 7.50, 7.51, 7.52, 7.53 (m, 2H, H-c'', H-g''), 7.65, 7.70 (d, 1H, J = 15 Hz, H-b''); PNB group: δ 8.12, 8.15, 8.24, 8.27 (dd, 4H, J = 27, 9 Hz, H-a', H-b', H-c', H-d'); ¹³C NMR (75.5 MHz, CDCl₃): δ -5.43, -5.39, 18.2, 19.0, 23.8, 25.5, 25.8, 29.0, 63.5, 66.5, 70.8, 71.7, 71.9, 79.2, 81.1, 82.3, 91.8, 99.7, 101.4, 104.7, 118.1, 123.6, 128.0, 128.1, 128.5, 128.6, 128.9, 130.9, 134.5, 135.0, 144.8, 154.5, 163.4, 165.9; HRMS (ESI-positive mode): *m/z* calcd. for C₄₈H₅₉NO₁₇NaSi 972.3450; found 972.3431 [M+Na]⁺

The synthesis of 3-*O*-(3,4-dimethoxycinnamoyl)-4'-*O*-*para*-nitrobenzoyl-3'-*O*-carboxybenzyl-6'-*O*-*tert*-butyldimethylsiloxy-2,1'-di-*O*-isopropylidene sucrose **237**

Following the general procedure, diOMe acid **38** (254 mg, 1.22 mmol) was added to the solution. After purification, the reaction gave compound **237** as white solid in 80% yield.

Analytical data for compound 237: mp 86.7-87.7°C; ¹H NMR (300 MHz, CDCl₃): TBS group: δ 0.00, 0.01 (d, 6H, (CH₃)₂Si), 0.82, 0.85, 0.88 (m, 9H, (CH₃)₃CSi); isopropylidene rings: δ 1.20, 1.21, 1.22, 1.24 (m, 3H, (CH₃)₂C) 1.33, 1.34 (d, 3H, (CH₃)₂C), 1.41, 1.45, 1.46 (m, 6H, (CH₃)₂C); Cbz, methoxy and sucrose unit: δ 3.45, 3.47, 3.50 (t, 1H, J = 7.5 Hz, H-5), 3.70, 3.71, 3.73, 3.77 (m, 3H, H-2, 2 x H-1'), 3.82, 3.84, 3.85, 3.86, 3.89 (m, 11H, 6 x H-h'', H-4, 2 x H-6, 2 x H-6') 4.05, 4.09, 4.13, 4.16, 4.20, 4.25 (m, 1H, H-5'), 5.09, 5.10 (d, 1H,

$J = 3$ Hz, H-3'), 5.29, 5.34, 5.35, 5.38 (m, 3H, H-3, 2 x H-a), 5.68, 5.70, 5.72 (t, 1H, $J = 6$ Hz, H-4'), 6.03, 6.04 (d, 1H, $J = 3$ Hz, H-1'); Cbz and dimethoxycinnamoyl peaks: δ 6.27, 6.32 (d, 1H, $J = 15$ Hz, H-a''), 6.83, 6.85 (d, 1H, $J = 6$ Hz, H-g''), 7.04, 7.05, 7.07 (m, 2H, H-c'', H-f''), 7.20, 7.23, 7.25 (m, 3H, H-c, H-d, H-e), 7.30, 7.33, 7.34 (m, 2H, H-b, H-f), 7.43, 7.45 (d, 1H, $J = 6$ Hz, H-b''); PNB peaks: δ 8.12, 8.15, 8.24, 8.27 (dd, 4H, $J = 27, 9$ Hz, H-a', H-b', H-c', H-d'); ^{13}C NMR (75.5 MHz, CDCl_3): δ -5.43, -5.38, 18.2, 19.0, 23.5, 25.0, 25.6, 25.8, 25.8, 28.0, 56.1, 61.0, 62.2, 64.2, 66.5, 70.7, 70.8, 71.7, 71.9, 76.6, 78.2, 81.2, 82.9, 91.7, 99.7, 99.7, 101.3, 101.4, 104.7, 105.3, 117.3, 121.6, 126.5, 128.5, 128.6, 128.7, 130.0, 130.8, 134.9, 135.0, 140.2, 144.9, 150.8, 153.4, 153.9, 154.5, 165.4, 165.6; HRMS (ESI-positive mode): m/z calcd. for $\text{C}_{50}\text{H}_{63}\text{NO}_{19}\text{NaSi}$ 1032.3661; found 1032.3684 $[\text{M}+\text{Na}]^+$

The synthesis of 3-*O*-(3,4,5-trimethoxycinnamoyl)-4'-*O*-*para*-nitrobenzoyl-3'-*O*-carboxybenzyl-6'-*O*-*tert*-butyldimethylsiloxy-2,1'-di-*O*-isopropylidene sucrose **238**

Following the general procedure, triOMe acid **210** (291 mg, 1.22 mmol) was added to the solution. After purification, the reaction gave compound **238** as white solid in 81% yield.

Analytical data for compound 238: mp 109.7-110.7°C; ^1H NMR

(300 MHz, CDCl_3): TBS group: δ 0.00, 0.01, 0.03 (m, 6H, $(\text{CH}_3)_2\text{Si}$), 0.79, 0.83, 0.85 (m, 9H, $(\text{CH}_3)_3\text{CSi}$); isopropylidene rings: δ 1.21, 1.23, 1.24, 1.25 (m, 6H, $(\text{CH}_3)_2\text{C}$), 1.42, 1.45, 1.46 (m, 6H); Cbz, methoxy and sucrose unit: δ 3.45, 3.50 (d, 1H, $J = 15$ Hz, H-5), 3.73, 3.75, 3.78 (m, 4H, H-2, 2 x H-1', H-6'), 3.82, 3.85, 3.87, 3.90 (m, 12H, 9 x H-h'', H-4, 2 x H-6) 4.25, 4.30 (d, 2H, H-5', H-6'), 4.98, 5.00 (d, 1H, $J = 6$ Hz, H-3'), 5.27, 5.28, 5.30, 5.35, 5.38, 5.40 (m, 3H, H-3, 2 x H-a), 5.68, 5.70, 5.73 (t, 1H, $J = 7.5$ Hz, H-4'), 6.03, 6.05 (d, 1H, $J = 6$ Hz, H-1'); Cbz and

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trimethoxycinnamoyl peaks: δ 6.31, 6.36 (d, 1H, $J = 15$ Hz, H-a''), 6.74 (s, 2H, H-c'', H-g''), 7.31, 7.33, 7.34 (m, 3H, H-c, H-d, H-e), 7.43, 7.43, 7.46 (m, 2H, H-b, H-f), 7.54, 7.56 (d, 1H, H-b''); PNB group: δ 8.12, 8.15, 8.24, 8.27 (dd, 4H, $J = 27, 9$ Hz, H-a', H-b', H-c', H-d'); ^{13}C NMR (75.5 MHz, CDCl_3): δ -5.43, -5.38, 14.2, 18.2, 19.0, 23.8, 25.5, 25.8, 29.0, 56.2, 61.0, 62.4, 63.5, 64.2, 66.5, 70.7, 70.8, 71.7, 71.9, 79.2, 81.1, 82.3, 91.8, 99.7, 101.4, 104.7, 105.3, 117.3, 123.6, 128.5, 128.6, 128.6, 130.0, 130.9, 134.7, 135.0, 140.1, 144.8, 150.8, 153.5, 164.5, 163.4, 165.8; HRMS (ESI-positive mode): m/z calcd. for $\text{C}_{51}\text{H}_{65}\text{NO}_{20}\text{NaSi}$ 1062.3767; found 1062.3792 $[\text{M}+\text{Na}]^+$

The synthesis of 3-*O*-(3,4-di-*tert*-butyldimethylsiloxycinnmoyl)-4'-*O*-*para*-nitrobenzoyl-3'-*O*-carboxybenzyl-6'-*O*-*tert*-butyldimethylsiloxy-2,1'-di-*O*-isopropylidene sucrose **239**

Following the general procedure, OTBS-caff acid **198** (498 mg, 1.22 mmol) was added to the solution. After purification, the reaction gave compound **239** as yellowish solid in 80% yield.

Analytical data for compound 239: mp 100.6-101.3°C; ¹H NMR (300 MHz, CDCl₃): TBS group: δ -0.18, -0.17 (d, 6H, J = 2.1 Hz, (CH₃)₂Si), 0.611 (s, 9H, (CH₃)₃CSi); TBS on OTBS-caffeoyl group: δ 0.00, 0.01 (d, 12H, J = 1.5 Hz, 2 x (CH₃)₂Si), 0.77, 0.78 (d, 18H, J = 2.4 Hz, 2 x (CH₃)₃CSi); isopropylidene rings: δ 1.05, (s, 3H, (CH₃)₂CSi) 1.16 (s, 3H, (CH₃)₂CSi), 1.26, 1.27 (d, 6H, J = 3 Hz, (CH₃)₂Si); Cbz and sucrose unit: δ 3.29, 3.33 (d, 1H, J = 12 Hz, H-5), 3.47, 3.51, 3.52, 3.54, 3.56, 3.59 (m, 2H, H-2, H-1'), 3.63, 3.64, 3.65, 3.70, 3.72, 3.74, 3.75, 3.76, 3.77 (m, H-4, 2 x H-6, H-1', H-6'), 4.02, 4.06 (m, 2H, H-5', H-6'), 4.80, 4.81 (d, 1H, J = 3 Hz, H-3'), 5.06, 5.10, 5.14, 5.16, 5.20 (m, 3H, H-3, 2 x H-a), 5.51, 5.53, 5.55 (t, 1H, J = 6 Hz, H-4'), 5.87, 5.88 (d, 1H, J = 3 Hz, H-1); Cbz and OTBS caffeoyl peaks: δ 6.01, 6.06 (d, 1H, J = 15 Hz, H-a''), 6.60, 6.62 (d, 1H, J = 6 Hz, H-c''), 6.80, 6.84 (m, 2H, H-f'', H-g''), 7.09, 7.12, 7.14, 7.17 (m, 3H, H-c, H-d, H-e), 7.24, 7.27, 7.28 (m, 2H, H-b, H-f), 7.34, 7.39 (d, 1H, J = 15 Hz, H-b''); PNB peaks: δ 7.93, 7.97, 8.06, 8.09 (dd, 4H, J = 27, 10.5, H-a', H-b', H-c', H-d'); ¹³C NMR (75.5 MHz, CDCl₃): δ -5.43, -5.39, -4.10, -4.05, 18.2, 18.5, 18.5, 19.0, 25.5, 25.8, 25.9, 25.9, 29.0, 48.2, 51.7, 63.5, 65.8, 66.7, 70.7, 71.7, 72.0, 81.1, 92.0, 99.7, 101.4, 104.7, 120.7, 123.6, 128.4, 128.6, 130.9, 135.0, 147.3, 154.5, 163.3; HRMS (ESI-positive mode): *m/z* calcd. for C₆₀H₈₇NO₁₉NaSi₃ 1232.5078; found 1232.5082 [M+Na]⁺

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The synthesis of 3-*O*-(4-*tert*-butyldimethylsilyloxy-3-methoxycinnamoyl)-4'-*O*-*para*-nitrobenzoyl-3'-*O*-carboxybenzyl-6'-*O*-*tert*-butyldimethylsilyloxy-2,1'-di-*O*-isopropylidene sucrose **240**

Following the general procedure, OTBS-feru acid **234** (376 mg, 1.22 mmol) was added to the solution. After purification, the reaction gave compound **240** as white solid in 89% yield.

Analytical data for compound 240: mp 85.0-86.2°C; ¹H NMR (300 MHz, CDCl₃): TBS group: δ 0-0.14, -0.13 (d, 6H, J = 3 Hz, (CH₃)₂Si), 0.66 (s, 9H, (CH₃)₃CSi);

TBS on OTBS-feruloyl group: δ 0.00 (s, 6H, (CH₃)₂Si), 0.83 (s, 9H, (CH₃)₃CSi); isopropylidene rings: δ 1.10 (s, 3H, (CH₃)₂C), 1.20 (s, 3H, (CH₃)₂C), 1.31, 1.31 (d, 3H, J = 2.4 Hz, (CH₃)₂C), 1.40 (s, 3H, (CH₃)₂C); Cbz, methoxy and sucrose unit: δ 3.55, 3.59 (d, 1H, J = 12 Hz, H-5), 3.67, 3.70, 3.71, 3.72 (m, 3H, 2 x H-1'), H-6'), 3.75 (s, 3H, 3 x H-h''), 3.76, 3.77, 3.80, 3.81 (m, H-2, 4H, 2 x H-6, H-6') 4.10, 4.11 (m, 2H, H-4, H-5'), 4.85, 4.86 (d, 1H, J = 3 Hz, H-3'), 5.15, 5.17, 5.21, 5.22, 5.24 (m, 3H, H-3, 2 x H-a), 5.57, 5.58, 5.59 (t, 1H, J = 3 Hz, H-4'), 5.92, 5.93 (d, 1H, J = 3

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Hz, H-1); Cbz and OTBS-feruloyl peaks: δ 6.12, 6.18 (d, 1H, J = 15.9, H-a''), 6.67, 6.69 (d, 1H, J = 6 Hz, H-d''), 6.87, 6.88 (m, 2H, H-c'', H-g''), 7.16, 7.19 (m, 3H, H-c, H-d, H-e), 7.29, 7.29, 7.31 (m, 2H, H-b, H-f), 7.44, 7.49 (d, 1H, J = 15 Hz, H-b''); PNB group: δ 7.98, 8.01, 8.10, 8.13 (dd, 4H, J = 27, 9 Hz, H-a', H-b', H-c', H-d'); ¹³C NMR (75.5 MHz, CDCl₃): δ -5.43, -5.38, -4.60, 18.2, 18.5, 19.0, 23.8, 25.5, 25.7, 25.8, 29.0, 55.5, 62.4, 63.5, 64.2, 66.5, 70.6, 70.7, 71.7, 71.9, 79.2, 81.1, 82.3, 91.8, 99.7, 101.4, 104.7, 110.8, 115.8, 121.1, 122.3, 123.6, 128.4, 128.5, 128.6, 128.6, 128.6, 130.9, 134.8, 135.0, 144.9, 147.5, 150.7, 151.2, 154.5, 163.4, 166.1; HRMS (ESI-positive mode): *m/z* calcd. for C₅₅H₇₅NO₁₉NaSi₂ 1132.4370; found 1132.4365 [M+Na]⁺

The synthesis of 3-*O*-(4-*tert*-butyldimethylsilyloxy)-4'-*O*-*para*-nitrobenzoyl-3'-*O*-carboxybenzyl-6'-*O*-*tert*-butyldimethylsilyloxy-2,1'-di-*O*-isopropylidene sucrose **241**

Following the general procedure, OTBS-coum acid **203** (339 mg, 1.22 mmol) was added to the solution. After purification, the reaction gave compound **241** as white solid in 80% yield.

Analytical data for compound 241: mp 84.6-85.6°C; ¹H NMR (300 MHz, CDCl₃): TBS group: δ 0.01, 0.02 (d, 6H, J = 3 Hz, (CH₃)₂Si), 0.80 (s, 9H, (CH₃)₃CSi); TBS on OTBS-coumaroyl group: δ 0.20 (s, 6H, (CH₃)₂Si), 0.96 (s, 9H, (CH₃)₃CSi); isopropylidene rings: δ 1.24 (s, 3H, (CH₃)₂C), 1.35 (s, 3H, (CH₃)₂C), 1.45, 1.46 (d, 6H, (CH₃)₂C); Cbz and sucrose unit: δ 3.70, 3.74 (d, 1H, J = 10.5 Hz, H-5), 3.83, 3.85, 3.86 (m,

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2H, 2 x H-1'), 3.90, 3.94, 3.96, 4.00, 4.01 (m, 5H, H-2, 2 x H-6, 2 x H-6'); 4.25, 4.26 (m, 2H, H-4, H-5'), 4.99, 5.00 (d, 1H, J = 3 Hz, H-3'), 5.30, 5.36, 5.38, 5.40 (m, 3H, H-3, 2 x H-a), 5.71, 5.72, 5.73 (t, 1H, J = 3 Hz, H-4'), 6.06, 6.08 (d, 1H, J = 3.6, H-1); Cbz and OTBS-coumaroyl peaks: δ 6.28, 6.33 (d, 1H, J = 15.9, H-a''), 6.81, 6.84 (d, 2H, J = 8.4 Hz, H-d'', H-f''), 7.31, 7.33 (m, 3H, H-c, H-d, H-e), 7.40, 7.43, 7.44, 7.46 (m, 4H, H-b, H-f, H-c'', H-g''), 7.61, 7.66 (d, 1H, J = 15.9, H-b''); PNB group: δ 8.13, 8.16, 8.25, 8.28 (dd, 4H, J = 27, 9 Hz, H-a', H-b', H-c', H-d'); ¹³C NMR (75.5 MHz, CDCl₃): δ -5.43, -5.39, -4.38, 18.2, 18.3, 19.0, 25.5, 2.6, 25.8, 29.0, 64.2, 70.7, 99.7, 101.4, 104.7, 120.5, 123.6, 127.8, 128.5, 128.6, 128.6, 129.7, 130.9, 134.8, 135.0, 150.7, 157.8; HRMS (ESI-positive mode): *m/z* calcd. for C₅₄H₇₃NO₁₈NaSi₂ 1102.4232; found 1102.4264 [M+Na]⁺

The synthesis of 3-*O*-(4-*tert*-butyldimethylsilyloxy-3,5-dimethoxycinnamoyl)-4'-*O*-*para*-nitrobenzoyl-3'-*O*-carboxybenzyl-6'-*O*-*tert*-butyldimethylsilyloxy-2,1'-di-*O*-isopropylidene sucrose **242**

Following the general procedure, OTBS-sinap acid **235** (413 mg, 1.22 mmol) was added to the solution. After purification, the reaction gave compound **242** as white solid in 80% yield.

Analytical data for compound 242: mp 105.5-106.2°C; ¹H NMR (300 MHz, CDCl₃): TBS group: δ -

0.11, -0.10 (d, 6H, J = 2.1 Hz, (CH₃)₂Si), 0.69 (s, 9H, (CH₃)₃CSi); TBS on OTBS-sinapoyl group: δ 0.00 (s, 6H, (CH₃)₂Si), 0.87 (s, 9H, (CH₃)₃CSi); isopropylidene rings: δ 1.14 (s, 3H, (CH₃)₂C), 1.23 (s, 3H, (CH₃)₂C), 1.34, 1.35 (d, 6H, J = 1.8 Hz, (CH₃)₂C); Cbz, methoxy and sucrose unit: δ 3.38, 3.41 (d, 1H, J = 9 Hz, H-5), 3.59, 3.62, 3.65, 3.66, 3.69 (m, 4H, H-6, H-6', 2 x H-1'), 3.71, 3.71 (m, 6H, 6 x H-h''), 3.73, 3.74, 3.79 (m, H-2, H-6, H-6'), 4.14, 4.15 (m, 2H, H-4, H-5'), 4.88, 4.89 (d, 1H, J = 3 Hz, H-3'), 5.19, 5.24, 5.27 (m, 3H, H-3, 2 x H-a), 5.60, 5.61, 5.62 (t, 1H, J = 3 Hz, H-4'), 5.95, 5.97 (d, 1H, J = 3.6 Hz, H-1); Cbz and OTBS-

sinapoyl peaks: δ 6.16, 6.21 (d, 1H, J = 15.9 Hz, H-a''), 6.55, 6.61 (m, 2H, H-c'', H-g''), 7.13, 7.19, 7.19, 7.22 (m, 3H, H-c, H-d, H-e), 7.32, 7.33, 7.35 (m, 2H, H-b, H-f), 7.45, 7.50 (d, 1H, J = 15 Hz, H-b''); PNB group: δ 8.01, 8.04, 8.13, 8.16 (dd, 4H, J = 27, 9 Hz, H-a', H-b', H-c', H-d'); ¹³C NMR (75.5 MHz, CDCl₃): δ -5.43, -5.39, -4.61, -4.59, -3.57, 18.0, 18.2, 18.8, 19.0, 23.9, 24.7, 25.4, 25.5, 25.7, 25.7, 25.9, 26.3, 29.0, 31.0, 32.8, 55.7, 55.8, 55.9, 62.4, 63.5, 64.1, 70.6, 70.7, 721.7, 71.9, 79.2, 81.1, 82.3, 91.8, 99.7, 101.4, 104.7, 105.2, 105.4, 115.9, 117.4, 123.6, 127.1, 127.4, 128.5, 128.6, 128.6, 130.9, 134.7, 135.0, 136.9, 143.9, 145.3, 150.7, 151.8, 154.5, 163.4, 166.1; HRMS (ESI-positive mode): *m/z* calcd. for C₅₆H₇₇NO₂₀NaSi₂ 1162.4475; found 1162.4419 [M+Na]⁺

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8.2.12.2 Removal of Cbz, PNB, TBS groups

The synthesis of 3-*O*-cinnamoyl-2,1'-di-*O*-isopropylidene sucrose, 3-cinn **243**

Compound **236** (300 mg, 0.316 mmol) was treated with $\text{Mg}(\text{OMe})_2$ (47.4 μL , 0.158 mmol) in 8:2 MeOH/THF at room temperature for 12 hours. Upon completion, 1N HCl (316 μL , 0.316 mmol) was added to the solution to quench the reaction. After removing MeOH/THF, the crude mixture obtained was then treated with 1.56 M $3\text{HF}\cdot\text{NEt}_3$ in pyridine (608 μL , 0.948 mmol) and NEt_3 (88.2 μL , 0.632 mmol) in pyridine (1.0 ml) at room temperature for another 12 hours. After which, pyridine was removed, and the crude product obtained was purified with column chromatography using 2:1 EtOAc/Hexane as eluent. 3-cinn **243** was obtained as a white solid in 70% yield.

Analytical data for 3-cinn 243: mp 70.1-70.9°C; ^1H NMR (300 MHz, CDCl_3): isopropylidene rings: δ 1.34 (s, 3H, $(\text{CH}_3)_2\text{C}$), 1.43 (s, 3H, $(\text{CH}_3)_2\text{C}$), 1.49, 1.50 (d, 6H, $J = 3$ Hz, $(\text{CH}_3)_2\text{C}$); sucrose unit: δ 3.51, 3.52, 3.57 (m, 1H, H-5), 3.68, 3.71, 3.74, 3.76, 3.78, 3.80, 3.83, 3.92, 3.94, 3.96, 3.99, 4.01, 4.02, 4.04 (m, 9H, H-2, 2 x H-6, 2 x H-1', H-3', H-4', 2 x H-6'), 4.24, 4.28 (d, 1H, $J = 12$ Hz, H-5'), 4.58, 4.59, 4.61, 4.64 (m, 1H, H-4), 5.41, 5.44, 5.47 (t, 1H, $J = 9$ Hz, H-3),

3-cinn **243**

6.29, 6.30 (d, 1H, $J = 3$ Hz, H-1); *trans*-alkenyl and aromatic protons: δ 6.44, 6.49 (d, 1H, $J = 15$ Hz, H-a''), 7.40, 7.41, 7.42 (m, 3H, H-d'', H-e'', H-f''), 7.55, 7.56, 7.58 (m, 2H, H-c'', H-g''), 7.68, 7.73 (d, 1H, H-b''); ^{13}C NMR (75.5 MHz, CDCl_3): δ 19.0, 24.13, 25.2, 26.0, 28.9, 61.2, 62.0, 64.1, 66.3, 71.4, 71.5, 73.3, 79.3, 83.0, 91.3, 100.1, 101.8, 103.4, 117.9, 128.2, 128.9, 130.4, 134.4, 145.2, 166.3; HRMS (ESI-positive mode): m/z calcd. for $\text{C}_{27}\text{H}_{37}\text{O}_{12}$ 553.2285; found 553.2278 $[\text{M}+\text{H}]^+$

The synthesis of 3-*O*-(3,4-dimethoxycinnamoyl)-2,1'-di-*O*-isopropylidene sucrose, 3-diOMe **244**

Compound **237** (300 mg, 0.297mmol) was treated with $\text{Mg}(\text{OMe})_2$ (44.6 μL , 0.149 mmol) in 8:2 MeOH/THF at room temperature for 12 hours. Upon completion, 1N HCl (29.7 μL , 0.297 mmol) was added to quench the reaction. Subsequently, MeOH/THF were removed and the mixture was dissolved in pyridine (1.0 ml) and stirred again at room temperature. 1.56 M $3\text{HF}\cdot\text{NEt}_3$ (572 μL , 0.892 mmol) and NEt_3 (83.0 μL , 0.594 mmol) were added to the solution and the reaction was left to stir for 12 hours. Upon completion, pyridine was removed, and the crude mixture was purified using column chromatography with 2:1 EtOAc/Hexane as eluent. 3-diOMe **244** was obtained as white solid in 76% yield.

Analytical data for 3-diOMe 244: mp 82.2-83.5°C; ¹H NMR (300 MHz, (CD₃)₂CO): isopropylidene rings: δ 1.18, 1.22, 1.26 (m, 6H, (CH₃)₂C), 1.42, 1.44 (d, 6H, J = 6 Hz, (CH₃)₂C); methoxy and sucrose unit: δ 3.42, 3.46 (d, 1H, J = 12 Hz, H-5), 3.66, 3.69, 3.71, 3.74, 3.77, 3.80, 3.82, 3.84, 3.88 (m, 13H, H-2, H-6, 2 x H-1', H-3', 2 x H-6', 6 x H-h''), 3.94, 3.95, 3.97, 3.98 (m, 2H, H-6, H-4'), 4.07, 4.11 (d, 1H, J = 12 Hz, H-5'), 4.37, 4.39, 4.41 (t, 1H, J = 6 Hz, H-4), 5.29, 5.31, 5.33 (t, 1H, J = 6 Hz, H-3), 6.15, 6.16 (d, 1H, J = 3 Hz, H-1'); *trans*-alkenyl and

3-diOMe 244

aromatic protons: δ 6.38, 6.43 (d, 1H, J = 15 Hz, H-a''), 6.96, 6.99 (d, 1H, J = 9 Hz, H-g''), 7.17, 7.18 (d, 1H, J = 3 Hz, H-f''), 7.31 (s, 1H, H-c''), 7.57, 7.62 (d, 1H, J = 15 Hz, H-b''); ¹³C NMR (75.5 MHz, (CD₃)₂CO): δ 23.6, 24.9, 28.8, 29.8, 33.4, 60.4, 60.5, 66.9, 67.3, 68.6, 71.3, 73.7, 75.7, 76.8, 77.0, 79.0, 84.5, 88.9, 96.3, 104.5, 106.3, 108.7, 115.3, 116.6, 120.9, 128.1, 132.6, 149.8, 154.9, 156.9, 170.9; HRMS (ESI-positive mode): *m/z* calcd. for C₂₉H₄₁O₁₄ 613.2496; found 613.2468 [M+H]⁺

The synthesis of 3-*O*-(3,4,5-trimethoxycinnamoyl)-2,1'-di-*O*-isopropylidene sucrose, 3-triOMe 245

Compound **238** (300 mg, 0.289 mmol) was treated with Mg(OMe)₂ (43.3 μL, 0.144 mmol) in 8:2 MeOH/THF at room temperature for 12 hours. Upon completion, 1N HCl (28.9 μL, 0.289 mmol) was added to quench the reaction. Subsequently, MeOH/THF were removed and the mixture was dissolved in pyridine (1.0 ml) and stirred again at room temperature. 1.56 M 3HF·NEt₃ (555 μL, 0.866 mmol) and NEt₃ (80.6 μL, 0.577 mmol) were added to the solution and the reaction was left to stir for 12 hours. Upon completion, pyridine was removed, and the crude mixture was purified using column chromatography with 2:1 EtOAc/Hexane as eluent. 3-triOMe **245** was obtained as white solid in 79% yield.

Analytical data for 3-triOMe 245: mp 99.0-100.2°C; ¹H NMR (300 MHz, CDCl₃): isopropylidene rings: δ 1.19, 1.22 (d, 6H, J = 9 Hz, (CH₃)₂C), 1.30 (s, 3H, (CH₃)₂C), 1.40 (s, 6H, (CH₃)₂C); methoxy and sucrose unit: δ 3.40, 3.44 (d, 1H, J = 12 Hz, H-5), 3.61, 3.61, 3.66, 3.68, 3.70, 3.72 (m, 3H, H-1', 2 x H-6'), 3.80, 3.81, 3.82, 3.83, 3.84, 3.86, 3.88, 3.89 (m, 12H, 2 x H-6, H-1', 9 x H-h''), 4.02, 4.05, 4.07, 4.09, 4.09, 4.12 (m, 4H, H-4, H-3', H-4', H-5'), 5.28, 5.31, 5.35 (t, 1H, J =

3-triOMe 245

10.5 Hz, H-3), 6.02, 6.03 (d, 1H, J = 3 Hz, H-1); *trans*-alkenyl and aromatic protons: δ 6.26, 6.31 (d, 1H, J = 15 Hz, H-a''), 6.71 (s, 2H, H-c'', H-g''), 7.52, 7.57 (d, 1H, J = 15 Hz, H-b''); ¹³C NMR (75.5 MHz, MeOD): δ 24.5, 26.8, 29.3, 31.0, 61.7, 63.8, 69.4, 71.7, 74.6, 75.8, 77.5, 79.4, 84.2, 93.7, 105.8,

107.2, 118.9, 131.9, 146.5, 155.0, 169.1; HRMS (ESI-positive mode): m/z calcd. for $C_{30}H_{42}O_{15}Na$ 665.2421; found 665.2444 $[M+Na]^+$

The synthesis of 3-*O*-caffeoyl-2,1'-di-*O*-isopropylidene sucrose, 3-caff **246**

Compound **239** (200 mg, 0.301 mmol) was treated with $Mg(OMe)_2$ (24.8 μ L, 0.0827 mmol) in 8:2 MeOH/THF at room temperature for 12 hours. Upon completion, 1N HCl (165 μ L, 0.165 mmol) was then added to quench the reaction. After removal of MeOH/THF, the crude mixture was treated with 1.56 M $3HF \cdot NEt_3$ (318 μ L, 0.496 mmol) and NEt_3 (46.2 μ L, 0.331 mmol) in pyridine (1.0 mL) at room temperature for 12 hours. After which, pyridine was removed, and the crude product obtained was purified using column chromatography with 2:1 EtOAc/Hexane as eluent. White solid 3-caff **246** was obtained in 62% yield.

Analytical data for 3-caff 246: mp 106.0-107.4°C; 1H NMR (300 MHz, $(CD_3)_2CO$): isopropylidene rings: δ 1.11, 1.14 (d, 6H, $J = 9$ Hz, $(CH_3)_2C$), 1.31, 1.33 (d, 6H, $J = 6$ Hz, $(CH_3)_2C$); sucrose unit: δ 3.46, 4.48, 3.50, 3.52, 3.56, 3.57, 3.59, 3.62, 3.63, 3.64, 3.68, 3.69, 3.70, 3.72, 3.73, 3.80 (m, 7H, H-5, 2 x H-6, 2 x H-1', 2 x H-6'), 3.82, 3.83, 3.84, 3.86, 3.87 (m, 2H, H-2, H-3'), 3.97, 4.01 (d, 2H, $J = 12$ Hz, H-4', H-5'), 4.24, 4.26, 4.28 (t, 1H, $J = 6$ Hz, H-4), 5.16, 5.19, 5.22 (t, 1H, $J = 9$ Hz, H-3), 6.04, 6.05 (d, 1H, $J = 3$ Hz, H-1); *trans*-alkenyl and aromatic peaks: δ 6.12, 6.18 (d, 1H, $J = 18$ Hz, H-a''), 6.73, 6.76 (d, 1H, $J = 9$ Hz, H-f''), 6.89, 6.91 (d, 1H, $J = 6$ Hz, H-g''), 7.05 (s, 1H, H-c''), 7.39, 7.44 (d, 1H, $J = 15$ Hz, H-b''); ^{13}C NMR (75.5 MHz, $(CD_3)_2CO$): δ 23.6, 24.6, 35.4, 46.4, 61.7, 62.2, 63.4, 70.5, 71.6, 71.8, 73.9, 70.3, 83.3, 91.4, 99.3, 101.1, 103.5, 114.3, 114.7, 115.5, 121.6, 126.6, 144.9, 145.6, 148.1, 162.2, 165.9; HRMS (ESI-positive mode): m/z calcd. for $C_{27}H_{36}O_{14}Na$ 607.2003; found 607.2007 $[M+Na]^+$

The synthesis of 3-*O*-feruloyl-2,1'-di-*O*-isopropylidene sucrose, 3-feru **247**

Compound **240** (200 mg, 0.180 mmol) was treated with $Mg(OMe)_2$ (27.0 μ L, 0.0901 mmol) in 8:2 MeOH/THF at room temperature for 12 hours. Upon completion, 1N HCl (180 μ L, 0.180 mmol) was then added to quench the reaction. After removal of MeOH/THF, the crude mixture was treated with 1.56 M $3HF \cdot NEt_3$ (347 μ L, 0.541 mmol) and NEt_3 (50.3 μ L, 0.361 mmol) in pyridine (1.0 mL) at room temperature for 12 hours. After which, pyridine was removed, and the crude product obtained was purified using column chromatography with 2:1 EtOAc/Hexane as eluent. In the end, white solid 3-feru **247** was obtained in 80% yield.

Analytical data for 3-feru 247: mp 80.1-82.3°C; ^1H NMR (300 MHz, CDCl_3): isopropylidene rings: δ 1.23, 1.27, 1.30, 1.32 (m, 12H, 2 x $(\text{CH}_3)_2\text{C}$); methoxy and sucrose unit: δ 3.41, 3.44 (d, 1H, $J = 9$ Hz, H-5), 3.58, 3.62, 3.64, 3.67 (m, 2H, 2 x H-6'), 3.70, 3.80, 3.86, 3.89, 3.90 (m, 10H, H-2, H-3', H-4, 2 x H-6, 2 x H-1', 3 x H-h''), 4.12, 4.16 (d, 1H, H-5'), 4.48, 4.51 (m, 1H, H-4), 5.27, 5.31, 5.34 (t, 1H, $J = 9$ Hz, H-3); anomeric, *trans*-alkenyl and aromatic protons: δ 6.17, 6.18, 6.23 (m, 2H, H-1, H-a''), 6.85, 6.88 (d, 1H, $J = 9$ Hz, H-c''), 6.97, 6.99, 7.02 (m, 2H, H-d'', H-g''), 7.51, 7.56 (d, 1H, $J = 15$ Hz, H-b''); ^{13}C NMR (75.5 MHz, CDCl_3): δ 19.0, 24.1, 25.2, 28.9, 46.4, 56.0, 61.9, 63.9, 70.7, 71.5, 71.6, 73.2, 79.2, 83.0, 91.2, 99.9, 101.7, 103.5, 109.6, 114.9, 115.1, 123.1, 126.8, 145.2, 146.9, 148.2, 166.5; HRMS (ESI-positive mode): m/z calcd. for $\text{C}_{28}\text{H}_{38}\text{O}_{14}\text{Na}$ 621.2159; found 621.2175 $[\text{M}+\text{Na}]^+$

The synthesis of 3-*O*-coumaroyl-2,1'-di-*O*-isopropylidene sucrose, 3-coum 248

Compound **241** (200 mg, 0.301 mmol) was treated with $\text{Mg}(\text{OMe})_2$ (24.8 μL , 0.0827 mmol) in 8:2 MeOH/THF at room temperature for 12 hours. Upon completion, 1N HCl (165 μL , 0.165 mmol) was then added to quench the reaction. After removal of MeOH/THF, the crude mixture was treated with 1.56 M $3\text{HF} \cdot \text{NEt}_3$ (318 μL , 0.496 mmol) and NEt_3 (46.2 μL , 0.331 mmol) in pyridine (1.0 mL) at room temperature for 12 hours. After which, pyridine was removed, and the crude product obtained was purified using column chromatography with 2:1 EtOAc/Hexane as eluent. White solid 3-coum **248** was obtained in 81% yield.

Analytical data for 3-coum 248: mp 79.5-80.9°C; ^1H NMR (300 MHz, $(\text{CD}_3)_2\text{CO}$): isopropylidene rings: δ 1.22, 1.26 (d, 6H, $J = 12$ Hz, $(\text{CH}_3)_2\text{C}$), 1.42, 1.44 (d, 6H, $J = 6$ Hz, $(\text{CH}_3)_2\text{C}$); sucrose unit: δ 3.42, 3.46 (d, 1H, $J = 12$ Hz, H-5), 3.59, 3.61, 3.63, 3.66, 3.70, 3.72, 3.74, 3.78, 3.82, 3.83 (m, 6H, H-2, 2 x H-1', H-3', 2 x H-6'), 3.94, 3.94, 3.95, 3.97, 3.98 (m, 2H, H-4', 2 x H-6), 4.07, 4.11 (d, 1H, $J = 12$ Hz, H-5'), 4.35, 4.38, 4.40 (t, 1H, $J = 7.5$ Hz, H-4), 5.27, 5.30, 5.33 (t, 1H, $J = 9$ Hz, H-3), 6.14, 6.16 (d, 1H, $J = 6$ Hz, H-1); *trans*-alkenyl and aromatic protons: δ 6.30, 6.35 (d, 1H, $J = 15$ Hz, H-a''), 6.86, 6.89 (d, 2H, $J = 9$ Hz, H-d'', H-f''), 7.52, 7.54 (d, 2H, $J = 6$ Hz, H-c'', H-g''), 7.56, 7.62 (d, 1H, $J = 18$ Hz, H-b''); ^{13}C NMR (75.5 MHz, $(\text{CD}_3)_2\text{CO}$): δ 18.4, 23.6, 24.6, 25.4, 25.4, 61.7, 62.1, 63.4, 66.1, 70.5, 71.6, 71.8, 73.8, 79.3, 83.4, 91.1, 99.3, 101.1, 103.5, 114.8, 115.8, 116.2, 126.1, 130.1, 144.5, 159.8, 165.8; HRMS (ESI-positive mode): m/z calcd. for $\text{C}_{27}\text{H}_{37}\text{O}_{13}$ 569.2234; found 569.2232 $[\text{M}+\text{H}]^+$

The synthesis of 3-*O*-sinapoyl-2,1'-di-*O*-isopropylidene sucrose, 3-sinap **249**

Compound **242** (200 mg, 0.269 mmol) was dissolved in pyridine (1.0 ml) and stirred at room temperature. 1.56 M 3HF·NEt₃ (518 μL, 0.808 mmol) and NEt₃ (75.2 μL, 0.539 mmol) were added to the solution and the reaction was left to stir for 12 hours. Upon completion, pyridine was removed, and the crude mixture was purified with column chromatography using 2:1 EtOAc/Hexane as eluent. In the end, 3-sinap **249** was obtained as white solid in 80% yield.

Analytical data for 3-sinap 249: mp 85.2-86.4°C; ¹H NMR (300

MHz, CDCl₃): isopropylidene rings: δ 1.23 (s, 3H, (CH₃)₂C), 1.32 (s, 3H, (CH₃)₂C) 1.37, 1.38 (d, 6H, J = 3 Hz, (CH₃)₂C); methoxy and sucrose unit: δ 3.41, 3.45 (d, 1H, J = 12 Hz, H-5), 3.52, 3.55, 3.58, 3.62, 3.64, 3.65, 3.67, 3.70 (m, 2H, 2 x H-6'), 3.74, 3.76, 3.79, 3.82, 3.83, 3.85, 3.86, 3.88, 3.90, 3.93, 3.95, 3.97, 3.98, 4.00, 4.02, 4.03 (m, 13H, H-2, 2 x H-6, 2 x H-1', H-3', H-4', 6 x H-h''), 4.13, 4.18 (d, 1H, J = 15 Hz, H-5'), 4.48, 4.51, 4.53 (t, 1H, J = 7.5 Hz, H-4), 5.31, 5.34,

3-sinap **249**

5.37 (t, 1H, J = 9 Hz, H-3), 6.17, 6.18 (d, 1H, J = 3 Hz, H-1); *trans*-alkenyl and aromatic protons: δ 6.26, 6.31 (d, 1H, J = 15 Hz, H-a''), 6.70 (s, 2H, H-c'', H-g''), 7.50, 7.55 (d, 1H, J = 15 Hz, H-b''); ¹³C NMR (75.5 MHz, CDCl₃): δ 18.3, 23.4, 24.4, 28.2, 55.5, 61.2, 61.5, 63.3, 65.6, 68.1, 68.1, 72.3, 72.8, 81.4, 90.6, 99.1, 101.6, 101.7, 102.5, 102.5, 104.4, 104.4, 113.7, 136.6, 136.6, 145.7, 146.4, 146.4, 166.3; HRMS (ESI-positive mode): *m/z* calcd. for C₂₉H₄₀O₁₅Na 651.2265; found 651.2281 [M+Na]⁺

8.2.13 The synthesis of 3'-mono-substituted PSEs

8.2.13.1 The synthesis of 4',6'-*O*-tetraisopropylidisilyl-2,1':4,6-di-*O*-isopropylidene sucrose, TIPDS-diacetonide **259**

2,1':4,6-di-*O*-isopropylidene sucrose **68** (1.00, 2.37 mmol) was reacted with 1,3-dichloro-1,1,3,3-tetraisopropylidisiloxane (832 μL, 2.61 mmol) in pyridine (5.0 ml) at room temperature for 6 hours. Upon completion, pyridine was removed. Crude mixture was then purified with column chromatography using 3:2 Hexane/EtOAc as eluent. In the end, TIPDS-diacetonide **259** was obtained as white solid in 75% yield.

Analytical data for TIPDS-diacetonide **259:** mp 61.5-62.9°C; ¹H

NMR (300 MHz, CDCl₃): TIPDS 0.89, 0.90, 0.91, 0.92, 0.92, 0.94,

0.96, 0.98, 1.00, 1.03, 1.05, 1.07, 1.09 (m, 28H, 4 x H-a, 8 x

(CH₃)₂CSi); isopropylidene rings: δ 1.24, 1.38, 1.47, 1.48 (m, 12H,

2 x (CH₃)₂C); sucrose unit: δ 3.43, 3.47 (d, 1H, J = 12 Hz, H-5),

3.60, 3.62, 3.63, 3.65, 3.67, 3.71, 3.73, 3.74, 3.77, 3.78, 3.80, 3.81,

3.83, 3.86, 3.88, 3.90, 3.94, 3.95, 3.97 (m, 10H, H-2, H-3, H-4, 2 x H-6, 2 x H-1', H-4', 2 x H-6'), 4.06,

4.10 (d, 1H, J = 12 Hz, H-3'), 4.20, 4.22, 4.23, 4.24 (m, 1H, H-5'), 5.96, 5.97 (d, 1H, H-1); ¹³C NMR

(300 MHz, CDCl₃): δ 12.5, 12.8, 13.1, 13.3, 13.4, 13.5, 17.0, 17.0, 17.1, 17.1, 17.1, 17.3, 17.5, 17.5,

17.5, 17.6, 24.2, 25.4, 29.1, 30.9, 62.2, 64.0, 65.8, 66.2, 69.9, 72.9, 73.7, 80.5, 80.8, 81.9, 91.0, 99.8,

99.9, 101.7, 102.6; HRMS (ESI-positive mode): *m/z* calcd. for C₃₀H₅₆O₁₂NaSi₂ 687.3208; found

687.3197 [M+Na]⁺

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8.2.13.2 Acylation of TIPDS-diacetonide **259**

General procedure

TIPDS-diacetonide **259** (500 mg, 0.753 mmol) and DMAP (9.2 mg, 0.0753 mmol) were dissolved in CH₂Cl₂ at room temperature. (substituted) cinnamic acid and DCC (233 mg, 1.13 mmol) were added and the reaction was left to stir for 12 hours. Upon completion, CH₂Cl₂ was removed. The crude mixture was first dissolved in cold diethyl ether and filtered. After which, diethyl ether was removed. The mixture was then purified with column chromatography using 4:1 Hexane/EtOAc as eluent. Compounds **260** to **266** were obtained.

The synthesis of 3'-*O*-cinnamoyl-4',6'-*O*-tetraisopropylidisilyl-2,1':4,6-di-*O*-isopropylidene sucrose **260**

Following the general procedure, cinnamic acid **30** (167 mg, 1.13 mmol) added to the solution. After purification, the reaction gave white solid compound **260** in 80% yield.

Analytical data for compound **260:** mp 145.4-146.4°C; ¹H

NMR (300 MHz, CDCl₃): TIPDS: δ 0.99, 1.05, 1.09 (m, 28H, 4

x H-a, 8 x (CH₃)₂CSi); isopropylidene rings: δ 1.34, 1.47, 1.47,

1.54 (m, 12H, 2 x (CH₃)₂C); sucrose unit: δ 3.55, 3.57 (d, 1H, J

= 6 Hz, H-5), 3.59, 3.63, 3.66, 3.67, 3.69, 3.70, 3.73, 3.77, 3.79,

3.80, 3.82, 3.83, 3.85 (m, 6H, H-2, 2 x H-6, 2 x H-1', H-6'), 3.93,

3.97, 3.99, 4.03, 4.03 (m, 4H, H-3, H-4, H-5', H-6'), 4.65, 4.68,

4.69 (t, 1H, J = 6 Hz, H-4'), 5.18, 5.20 (d, 1H, J = 6 Hz, H-3'), 5.94, 5.95 (d, 1H, J = 3 Hz, H-1); *trans*-

alkenyl and aromatic protons: δ 6.50, 6.55 (d, 1H, H-a'), 7.40, 7.41, 7.42 (m, 4H, H-c', H-d', H-e', H-

260

f'), 7.58 (s, 1H, H-g'), 7.59, 7.60 (d, 1H, H-b'); ¹³C NMR (300 MHz, CDCl₃): δ 12.6, 13.0, 13.5, 13.6, 17.1, 17.6, 17.8, 19.3, 24.3, 25.7, 29.3, 62.5, 64.0, 66.2, 66.6, 70.2, 73.2, 74.1, 79.2, 81.6, 91.0, 100.0, 101.7, 102.7, 117.2, 128.6, 129.2, 130.9, 134.3, 146.6, 165.1; HRMS (ESI-positive mode): *m/z* calcd. for C₃₉H₆₂O₁₃NaSi₂ 817.3627; found 817.3598 [M+Na]⁺

The synthesis of 3'-*O*-(4-*tert*-butyldimethylsiloxycinnamoyl)-4',6'-*O*-tetraisopropylidisilyl-2,1':4,6-di-*O*-isopropylidene sucrose **261**

Following the general procedure, OTBS-coum acid **203** (314 mg, 1.13 mmol) added to the solution. After purification, the reaction gave white solid compound **261** in 85% yield.

Analytical data for compound 261: mp 97.7-98.7°C;

¹H NMR (300 MHz, CDCl₃): TBS: δ 0.07 (s, 6H, (CH₃)₂Si), 0.88 (s, 9H, (CH₃)₃CSi); TIPDS: δ 0.96, 1.00, 1.02, 1.06, 1.11 (m, 28H, 4 x H-a, 8 x (CH₃)₂CSi); isopropylidene rings: δ 1.31, 1.44, 1.51 (m, 12H, 2 x (CH₃)₂C); sucrose unit: δ 3.52, 3.56, 3.57, 3.60 (m, 2H, H-5, H-1'), 3.63, 3.64, 3.66, 3.70 (m, 2H, H-2, H-1'), 3.75, 3.77, 3.78, 3.80, 3.82 (m, 3H, 2 x H-6, H-6'), 3.91, 3.94, 3.97, 4.01 (m, 4H, H-3, H-4, H-5', H-6'), 4.62,

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4.64, 4.66 (t, 1H, J = 6 Hz, H-4'), 5.14, 5.17 (d, 1H, J = 9 Hz, H-3'), 5.91, 5.92 (d, 1H, J = 3 Hz, H-1); *trans*-alkenyl and aromatic protons: δ 6.33, 6.38 (d, 1H, H-a'), 6.82, 6.85 (d, 2H, H-d', H-f'), 7.45, 7.47 (d, 2H, H-c', H-g'), 7.67, 7.72 (d, 1H, H-b'); ¹³C NMR (300 MHz, CDCl₃): δ -3.91, -3.47, -3.09, 12.0, 12.5, 12.9, 13.4, 13.9, 14.2, 14.3, 17.0, 17.5, 17.6, 17.7, 18.1, 18.3m 19.2, 21.1, 23.1, 24.2, 25.6, 25.7, 25.8, 29.2, 60.5, 62.4, 63.9, 66.1, 66.5, 67.1, 70.0, 73.1, 74.0, 76.8, 78.9, 81.4, 91.0, 99.8, 101.5, 102.6, 114.7, 120.7, 127.6, 130.1, 146.2, 158.3, 166.7, 171.3; HRMS (ESI-positive mode): *m/z* calcd. for C₄₅H₇₆O₁₄NaSi₃ 947.4441; found 947.4484 [M+Na]⁺

The synthesis of 3'-O-(4-*tert*-butyldimethylsiloxy-3-methoxycinnamoyl)-4',6'-O-tetraisopropylidisilyl-2,1':4,6-di-O-isopropylidene sucrose **262**

Following the general procedure, OTBS-feru acid **234** (348 mg, 1.13 mmol) added to the solution. After purification, the reaction gave white solid compound **262** in 81% yield.

Analytical data for compound 262: mp 97.4-

98.4°C; ¹H NMR (300 MHz, CDCl₃): TBS and TIPDS: δ 0.15, 0.16 (m, 6H, (CH₃)₂Si), 0.89, 0.93, 0.93, 0.95, 0.98, 1.00, 1.01, 1.03, 1.07, 1.11, 1.13 (m, 37H, (CH₃)₃CSi, 4 x H-a, 8 x (CH₃)₂CSi); isopropylidene rings: δ 1.33, 1.44, 1.53, 1.59 (m, 12H, 2 x (CH₃)₂C); methoxy and sucrose unit: δ 3.54, 3.58, 3.59, 3.68, 3.78, 3.81, 3.82, 3.84 (m, 7H, H-2, H-5, 2 x H-1', 2 x H-6, H-6'), 3.86 (s, 3H, 3 x H-h')

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3.95, 3.96, 3.98, 4.01, 4.02 (m, 4H, H-3, H-4, H-5', H-6'), 4.64, 4.65, 4.66 (t, 1H, J = 3 Hz, H-4'), 5.16, 5.19 (d, 1H, J = 9 Hz, H-3'), 5.92, 5.93 (d, 1H, J = 3 Hz, H-1'); *trans*-alkenyl and aromatic protons: δ 6.32, 6.38 (d, 1H, H-a'), 6.83, 6.86 (d, 1H, H-d'), 7.05, 7.07 (m, 2H, H-c', H-g'), 7.66, 7.72 (d, 1H, H-b'); ¹³C NMR (300 MHz, CDCl₃): δ -4.58, 12.4, 13.3, 13.4, 16.9, 17.0, 17.0, 17.0, 17.4, 17.5, 17.6, 17.6, 19.1, 24.1, 25.5, 25.7, 29.2, 55.6, 78.8, 99.8, 102.6, 110.7, 121.1, 123.1, 128.0, 148.0, 151.3, 166.7; HRMS (ESI-positive mode): *m/z* calcd. for C₄₆H₇₉O₁₅NaSi₃ 955.4727; found 955.4745 [M+Na]⁺

The synthesis of 3'-O-(4-*tert*-butyldimethylsiloxy-3,5-dimethoxycinnamoyl)-4',6'-O-tetraisopropylidisilyl-2,1':4,6-di-O-isopropylidene sucrose **263**

Following the general procedure, OTBS-sinap acid **235** (382 mg, 1.13 mmol) added to the solution. After purification, the reaction gave white solid compound **263** in 81% yield.

Analytical data for compound 263: mp 100.5-101.5°C; ¹H

NMR (300 MHz, CDCl₃): TBS and TIPDS: δ 0.06 (s, 6H, (CH₃)₂Si), 0.91, 0.94, 0.97, 0.98, 1.00, 1.02, 1.06, 1.10, 1.12 (m, 37H, (CH₃)₃CSi, 4 x H-a, 8 x (CH₃)₂CSi); isopropylidene rings: δ 1.31, 1.42, 1.51, 1.56 (m, 12H, 2 x (CH₃)₂C); methoxy and sucrose unit: δ 3.53, 3.57, 3.58, 3.61, 3.63, 3.64, 3.66, 3.67, 3.70, 3.74, 3.76, 3.78, 3.79 (m, 8H, H-2, H-4, H-5, 2 x H-6, 2 x H-1', H-6'), 3.81, 3.83 (d, 6H, 6 x H-h'), 3.90, 3.94, 3.96 4.00 (m, 3H, H-3, H-5', H-

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6'), 4.62, 4.63, 4.65 (t, 1H, J = 4.5, H-4'), 5.15, 5.17 (d, 1H, J = 6 Hz, H-3'), 5.91, 5.92 (d, 1H, J = 3

The synthesis of 3'-*O*-(3,4-di-*tert*-butyldimethylsiloxy)-4',6'-*O*-tetraisopropyldisilyl-2,1':4,6-di-*O*-isopropylidene sucrose 264

Analytical data for compound 264: mp 102.0-102.8°C;

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The synthesis of 3'-O-(3,4-dimethoxycinnamoyl)-4',6'-O-tetraisopropyldisilyl-2,1':4,6-di-O-isopropylidene sucrose **265**

Following the general procedure, diOMe acid **38** (235 mg, 1.13 mmol) added to the solution. After purification, the reaction gave white solid compound **265** in 88% yield.

Analytical data for compound 265: mp 95.0-96.4°C;

¹H NMR (300 MHz, CDCl₃): TIPDS: δ 0.89, 0.91, 0.93, 0.95, 0.97, 0.98, 1.00, 1.01, 1.03, 1.08, 1.11, 1.13 (m, 28H, 4 x H-a, 8 x (CH₃)₂CSi); isopropylidene rings: δ 1.33, 1.45, 1.53 (m, 12H, 2 x (CH₃)₃C); methoxy and sucrose unit: δ 3.54, 3.58, 3.59, 3.62, 3.65, 3.66, 3.68, 3.69 3.72, 3.76, 3.78, 3.79, 3.81, 3.83, 3.84 (m, 8H, H-5, H-6', 6 x H-h'), 3.90 (s, 3H, 2 x H-1', H-6'), 3.91 (s, 3H, H-4, 2 x H-6), 3.95, 3.97, 3.98, 4.01, 4.02, 4.09 (m, 2H,

265

H-3, H-5'), 4.62, 4.65, 4.67 (t, 1H, J = 7.5 Hz, H-4'), 5.17, 5.19 (d, 1H, J = 6 Hz, H-3'), 5.93, 5.94 (d, 1H, J = 3 Hz, H-1); *trans*-alkenyl and aromatic protons: δ 6.34, 6.39 (d, 1H, J = 15 Hz, H-a'), 6.85, 6.88 (d, 1H, J = 9 Hz, H-g'), 7.10, 7.11 7.13 (m, 2H, H-c', H-f'), 7.68, 7.73 (d, 1H, J = 15 Hz, H-b'); ¹³C NMR (300 MHz, CDCl₃): δ 12.7, 13.0, 13.5, 13.6, 17.1, 17.2, 17.6, 17.7, 17.8, 17.8, 19.3, 24.3, 25.7, 29.4, 31.1, 56.2, 62.5, 64.0, 66.6, 70.2, 73.2, 74.1, 76.8, 79.1, 81.6, 91.04, 100.0, 101.7, 102.8 109.7, 111.2, 114.7, 123.6, 127.4, 146.6, 149.5, 151.7, 166.9; HRMS (ESI-positive mode): *m/z* calcd. for C₄₁H₆₆O₁₅NaSi₂ 877.3838; found 877.3873 [M+Na]⁺

The synthesis of 3'-O-(3,4,5-trimethoxycinnamoyl)-4',6'-O-tetraisopropylidisilyl-2,1':4,6-di-O-isopropylidene sucrose **266**

Following the general procedure, triOMe acid **210** (269 mg, 1.13 mmol) added to the solution. After purification, the reaction gave compound **266** as white solid in 83% yield.

Analytical data for compound 266: mp 77.0-78.2°C; ^1H

NMR (300 MHz, CDCl_3): TIPDS: δ 0.97, 1.00, 1.02, 1.07,

1.10 (m, 28H, 4 x H-a, 8 x $(\text{CH}_3)_2\text{CSi}$); isopropylidene rings:

δ 1.32, 1.43, 1.45, 1.52 (m, 12H, 2 x $(\text{CH}_3)_3\text{C}$); methoxy and

sucrose unit: δ 3.53, 3.55 (d, 1H, $J = 6$ Hz, H-5), 3.58, 3.62,

3.64, 3.65, 3.67, 3.68, 3.71, 3.75, 3.78, 3.79, 3.84 (m, 5H, H-

2, H-4, 2 x H-1', H-6'), 3.86, 3.87, 3.88 (m, 9H, 9 x H-h'),

3.90, 3.95, 3.97, 4.01 (m, 5H, H-3, 2 x H-6, H-5', H-6') 4.61,

4.64, 4.66 (t, 1H, $J = 7.5$ Hz, H-4'), 5.15, 5.18 (d, 1H, $J = 9$

Hz, H-3'), 5.92, 5.93 (d, 1H, $J = 3$ Hz, H-1); *trans*-alkenyl and aromatic protons: δ 6.39, 6.35, 6.37,

6.38, 6.42 (m, 1H, H-a'), 6.74, 7.75, 6.78 (m, 2H, H-c', H-g'), 7.64, 7.69, 7.72 (m, 1H, H-b'); ^{13}C NMR

(300 MHz, CDCl_3): δ 12.6, 13.0, 13.5, 13.6, 17.1, 17.2, 17.2, 17.3, 17.6, 17.7, 17.8, 17.8, 19.3, 24.3,

25.7, 29.3, 56.4, 56.4, 56.5, 61.2, 62.5, 64.0, 66.3, 66.6, 70.2, 73.1, 74.1, 76.8, 79.2, 81.6, 91.1, 100.0,

101.7, 102.7, 105.7, 105.8, 106.0, 116.2, 129.8, 140.7, 146.7, 147.0, 153.7, 153.7, 153.7, 166.7; HRMS

(ESI-positive mode): m/z calcd. for $\text{C}_{42}\text{H}_{68}\text{O}_{16}\text{NaSi}_2$ 907.2944; found 907.3947 $[\text{M}+\text{Na}]^+$

8.2.13.3 Removal of TIPDS and TBS groups (if any)

General procedure

Compounds **260** to **266** were dissolved in pyridine (1.0 mL) at room temperature. Subsequently, 1.56 M $3\text{HF}\cdot\text{NEt}_3$ in pyridine and NEt_3 were added to the solution and the reactions were left to stir for 12 hours. Upon completion, pyridine was removed, and the crude mixtures were subjected to chromatographic purification using 2:1 Hexane/EtOAc as eluent. In the end, 3'-cinn **267**, 3'-coum **268**, 3'-feru **269**, 3'-sinap **270**, 3'-caff **271**, 3'-diOMe **272** and 3'-triOMe **273** were obtained.

The synthesis of 3'-O-cinnamoyl-2,1'-di-O-isopropylidene sucrose, 3'-cinn **267**

Following the general procedure, compound **260** (200 mg, 0.252 mmol) with 1.56 M $3\text{HF}\cdot\text{NEt}_3$ (484 μL , 0.755 mmol) and NEt_3 (70.3 μL , 0.504 mmol) gave 3'-cinn **272** as white solid in 86% yield.

Analytical data for 3'-cinn 267: mp 97.5-98.9°C; ¹H NMR

(300 MHz, CDCl₃): isopropylidene rings: δ 1.31, 1.37, 1.44, 1.45 (m, 12H, 2 x (CH₃)₃C); sucrose unit: δ 3.49, 3.52, 3.54, 3.55, 3.57, 3.58, 3.59, 3.61, 3.63, 3.67, 3.70, 3.71 (m, 5H, H-2, H-5, 2 x H-1', H-6'), 3.76, 3.78, 3.80, 3.81, 3.83, 3.86 (m, 4H, H-4, 2 x H-6, H-6'), 3.97, 4.01, 4.04, 4.06 (m, 2H, H-3, H-5'), 4.76, 4.78, 4.81 (t, 1H, J = 7.5 Hz, H-4'), 4.97, 4.99 (d, 1H, J =

3'-cinn 267

6 Hz, H-3'), 6.13, 6.14 (d, 1H, J = 3 Hz, H-1); *trans*-alkenyl and aromatic protons: δ 6.45, 6.51 (d, 1H, J = 18 Hz, H-a'), 7.33, 7.35, 7.36 (m, 3H, H-d', H-e', H-f'), 7.52, 7.53, 7.54, 7.55 (m, 2H, H-c', H-g'), 7.71, 7.77 (d, 1H, J = 18 Hz, H-b'); ¹³C NMR (75.5 MHz, CDCl₃): δ 18.1, 20.0, 23.1, 24.4, 28.0, 29.9, 59.4, 60.2, 60.9, 63.0, 65.5, 69.1, 70.6, 71.7, 72.5, 79.2, 83.2, 90.0, 99.0, 101.0, 102.5, 115.7, 127.5, 128.0, 129.9, 132.9, 146.0, 166.4; HRMS (ESI-positive mode): *m/z* calcd. for C₂₇H₃₇O₁₂ 553.2285; found 553.2266 [M+H]⁺

The synthesis of 3'-*O*-coumaroyl-2,1'-di-*O*-isopropylidene sucrose, 3'-coum 268

Following the general procedure, compound **261** (200 mg, 0.216 mmol) with 1.56 M 3HF·NEt₃ (416 μL, 0.649 mmol) and NEt₃ (60.4 μL, 0.433 mmol) gave white solid 3'-coum **268** in 85% yield.

Analytical data for 3'-coum 268: ¹H NMR (300 MHz, (CD₃)₂CO): isopropylidene rings: δ 1.12, (s, 3H, (CH₃)₂C), 1.20 (s, 3H, (CH₃)₂C), 1.31, 1.35 (d, 6H, J = 12 Hz, (CH₃)₂C); sucrose unit: δ 3.37, 3.41, 3.43, 3.47, 3.49, 3.54, 3.56, 3.57, 3.58, 3.60, 3.61, 3.62, 3.63, 3.65, 3.67 (m, 9H, H-2, H-4, H-5, 2 x H-6, 2 x H-1', 2 x H-6'), 3.86, 3.87, 3.88, 3.90 (m, 2H, H-3, H-5'), 4.52, 4.54, 4.57 (t, 1H, J = 7.5 Hz, H-4'), 4.97, 4.99 (d, 1H, J = 6 Hz, H-3'), 5.97, 5.98 (d, 1H, J = 3 Hz, H-1); *trans*-alkenyl and aromatic protons: δ 6.27, 6.32 (d, 1H, J = 15 Hz, H-a'), 6.76, 6.79 (d, 2H, J = 9 Hz, H-d', H-f'), 7.47, 7.50 (d, 2H, J = 9 Hz, H-c', H-g'), 7.58, 7.64 (d, 1H, J = 18 Hz, H-b'); ¹³C NMR (75.5 MHz, (CD₃)₂CO): δ 13.2, 17.1, 18.5, 23.5, 24.6, 61.8, 62.0, 63.9, 66.3, 69.7, 72.0, 73.3, 74.0, 78.8, 83.8, 91.0, 99.2, 101.2, 103.5, 125.9, 130.4, 145.9, 160.0, 166.3; HRMS (ESI-positive mode): *m/z* calcd. for C₂₇H₃₇O₁₃ 569.2234; found 569.2247 [M+H]⁺

3'-coum 268

The synthesis of 3'-*O*-feruloyl-2,1'-di-*O*-isopropylidene sucrose, 3'-feru **269**

Following the general procedure, compound **262** (200 mg, 0.210 mmol) with 1.56 M 3HF·NEt₃ (403 μL, 0.629 mmol) and NEt₃ (58.5 μL, 0.419 mmol) gave 3'-feru **269** as white solid in 86% yield.

Analytical data for 3'-feru 269: mp 87.0-88.1 °C; ¹H NMR

(300 MHz, CDCl₃): isopropylidene rings: δ 1.33 (s, 3H, (CH₃)₂C), 1.38 (s, 3H, (CH₃)₂C), 1.45, 1.47 (d, 6H, (CH₃)₂C); methoxy and sucrose unit: δ 3.50, 3.54, 3.57, 3.60 (m, 4H, H-2, H-5, 2 x H-1'), 3.64, 3.67, 3.68, 3.70, 3.71, 3.73 (m, 1H, H-6'), 3.76, 3.78, 3.80, 3.81, 3.82, 3.64, 3.86, 3.88 (m, 6H, H-4, 2 x H-6, H-6'), 3.92 (s, 3H, 3 x H-h'), 3.99, 4.03 (d, 1H,

3'-feru 269

J = 12 Hz, H-3), 4.06, 4.08 (d, 1H, *J* = 6 Hz, H-5'), 4.77, 4.78, 4.79 (t, 1H, H-4'), 4.92, 4.95 (d, 1H, H-3'), 6.14, 6.16 (d, 1H, H-1'); *trans*-alkenyl and aromatic protons: δ 6.29, 6.34 (d, 1H, *J* = 15 Hz, H-a'), 6.86, 6.89 (d, 1H, *J* = 9 Hz, H-d'), 7.05, 7.05, 7.06, 7.07, 7.09, 7.10 (m, 2H, H-c', H-g'), 7.65, 7.70 (d, 1H, *J* = 15 Hz, H-b'); ¹³C NMR (75.5 MHz, (CD₃)₂CO): δ 21.9, 25.6, 55.0, 56.4, 61.2, 67.6, 70.5, 70.9, 71.8, 72.3, 73.6, 73.9, 74.6, 75.0, 75.1, 75.6, 75.9, 76.4, 76.6, 78.6, 78.6, 79.0, 79.2, 79.8, 82.3, 84.2, 86.4, 104.8, 105.2, 105.4, 106.0, 107.1, 109.7, 111.4, 111.7, 112.5, 114.9, 115.0, 115.1, 116.1, 124.3, 127.3, 146.8, 148.9, 150.4, 166.3, 167.4; HRMS (ESI-positive mode): *m/z* calcd. for C₂₈H₃₉O₁₄ 599.2340; found 599.2313 [M+H]⁺

The synthesis of 3'-*O*-sinapoyl-2,1'-di-*O*-isopropylidene sucrose, 3'-sinap **270**

Following the general procedure, compound **263** (200 mg, 0.203 mmol) with 1.56 M 3HF·NEt₃ (391 μL, 0.609 mmol) and NEt₃ (56.7 μL, 0.406 mmol) gave white solid 3'-sinap **270** in 88% yield.

Analytical data for 3'-sinap 270: mp 91.0-92.2 °C; ¹H

NMR (300 MHz, CDCl₃): isopropylidene rings: δ 1.33, 1.35 (d, 6H, (CH₃)₂C), 1.46 (s, 6H, (CH₃)₂C); methoxy and sucrose unit: δ 3.50, 3.54, 3.57, 3.60, 3.63, 3.67, 3.68, 3.69 (m, 4H, H-2, H-5, 2 x H-1'), 3.71, 3.72, 3.76, 3.78, 3.79, 3.81, 3.82, 3.83, 3.84, 3.86, 3.88 (m, 5H, 2 x H-4, H-6, 2 x H-6'), 3.90 (s, 6H, 6 x H-h'), 3.99, 4.03, 4.05, 4.08 (m, 2H, H-3, H-5'), 4.75, 4.78, 4.80 (t, 1H, *J* = 7.5 Hz, H-4'), 4.93,

3'-sinap 270

4.96 (d, 1H, *J* = 9 Hz, H-3'), 6.14, 6.15 (d, 1H, *J* = 3 Hz, H-1); *trans*-alkenyl and aromatic protons: δ 6.31, 6.36 (d, 1H, *J* = 15 Hz, H-a'), 6.78 (s, 2H, H-c', H-g'), 7.63, 7.68 (d, 1H, *J* = 15 Hz, H-b'); ¹³C NMR (75.5 MHz, CDCl₃): δ 19.1, 25.4, 25.5, 25.6, 25.7, 25.9, 29.1, 33.9, 56.5, 21.3, 64.0, 66.4, 70.1,

71.6, 72.7, 80.3, 84.4, 91.0, 100.0, 102.0, 103.5, 105.6, 114.0, 125.4, 137.7, 147.3, 157.8, 162.7; HRMS (ESI-positive mode): m/z calcd. for $C_{29}H_{41}O_{15}$ 629.2445; found 629.2461 $[M+H]^+$

The synthesis of 3'-*O*-caffeoyl-2,1'-di-*O*-isopropylidene sucrose, 3'-caff **271**

Following the general procedure, compound **264** (200 mg, 0.190 mmol) with 1.56 M $3HF \cdot NEt_3$ (365 μL , 0.569 mmol) and NEt_3 (52.9 μL , 0.379 mmol) gave white solid 3'-caff **271** in 89% yield.

Analytical data for 3'-caff **271:** mp 100.2-101.0°C; 1H NMR

(300 MHz, $(CD_3)_2CO$): isopropylidene rings: δ 1.11, 1.12, 1.17, 1.25, 1.36, 1.39 (m, 12H, 2 x $(CH_3)_2C$); sucrose unit: δ 3.41, 3.45, 3.48, 3.51, 3.57, 3.58, 3.61, 3.62, 3.63, 3.64, 3.66, 3.67, 3.69, 3.71 (m, 8H, H-2, H-5, 2 x H-6, 2 x H-1', 2 x H-6'), 3.91, 3.93, 3.95, 3.96, 3.98 (m, 3H, H-3, H-4, H-5'), 4.55, 4.58, 4.60 (t, 1H, $J = 7.5$ Hz, H-4'), 5.01, 5.03 (d, 1H, $J = 6$ Hz, H-3'),

3'-caff **271**

6.02, 6.03 (d, 1H, $J = 3$ Hz, H-1); *trans*-alkenyl and aromatic protons: δ 6.24, 6.27, 6.29 (m, 1H, H-a'), 6.77, 6.79, 6.80 (m, 1H, H-c'), 7.00, 7.02, 7.03 (m, 1H, H-f'), 7.13 (s, 1H, H-g'), 7.56, 7.61 (d, 1H, $J = 15$ Hz, H-b'); ^{13}C NMR (75.5 MHz, $(CD_3)_2CO$): δ 18.5, 23.5, 24.6, 61.8, 62.0, 63.9, 66.3, 73.3, 74.0, 78.8, 83.8, 91.0, 99.2, 101.2, 103.4, 113.8, 114.3, 115.5, 122.3, 126.4, 146.2, 181.8; HRMS (ESI-positive mode): m/z calcd. for $C_{27}H_{37}O_{14}$ 585.2183; found 585.2186 $[M+H]^+$

The synthesis of 3'-*O*-(3,4-dimethoxycinnamoyl)-2,1'-di-*O*-isopropylidene sucrose, 3'-diOMe **272**

Following the general procedure, compound **265** (200 mg, 0.234 mmol) with 1.56 M $3HF \cdot NEt_3$ (451 μL , 0.702 mmol) and NEt_3 (65.3 μL , 0.468 mmol) gave 3'-diOMe **272** as white solid in 83% yield.

Analytical data for 3'-diOMe **272:** mp 85.9-86.9°C; 1H

NMR (300 MHz, $CDCl_3$): isopropylidene rings: δ 1.33 (s, 3H, $(CH_3)_2C$), 1.38 (s, 3H, $(CH_3)_2C$), 1.45, 1.46 (d, 6H, $J = 3$ Hz, $(CH_3)_2C$); methoxy and sucrose unit: δ 3.50, 3.54, 3.57, 3.60, 3.61, 3.64, 3.67, 3.68, 3.69, 3.71, 3.72 (m, 6H, H-2, H-4, H-5, 2 x H-1', H-6'), 3.76, 3.78, 3.80, 3.81, 3.82, 3.84, 3.85, 3.86, 3.89 (m, 9H, 2 x H-6, H-6', 6 x H-h'), 3.98, 4.03 (d, 1H, $J = 15$ Hz, H-3), 4.05, 4.07 (d, 1H, $J = 6$ Hz, H-5'), 4.76, 4.78,

3'-diOMe **272**

4.81 (t, 1H, $J = 7.5$ Hz, H-4') 4.94, 4.97 (d, 1H, $J = 9$ Hz, H-3'), 6.14, 6.15 (d, 1H, $J = 3$ Hz, H-1); *trans*-alkenyl and aromatic protons: δ 6.31, 6.36 (d, 1H, $J = 15$ Hz, H-a'), 6.81, 6.83 (d, 1H, $J = 6$ Hz, H-g'),

7.06, 7.06, 7.09, 7.10, 7.12, 7.13 (m, 2H, H-c', H-f'), 7.66, 7.71 (d, 1H, J = 15 Hz, H-b'); ¹³C NMR (75.5 MHz, CDCl₃): δ 19.1, 24.3, 25.4, 29.1, 40.0, 41.7, 45.0, 46.2, 56.0, 56.1, 63.9, 66.6, 70.1, 72.7, 73.5, 80.3, 84.3, 100.0, 102.0, 103.5, 111.0, 114.1, 123.6, 126.9, 149.4, 151.7, 167.9; HRMS (ESI-positive mode): *m/z* calcd. for C₂₉H₄₁O₁₄ 613.2496; found 613.2489 [M+H]⁺

The synthesis of 3'-O-(3,4,5-trimethoxycinnamoyl)-2,1'-di-O-isopropylidene sucrose, 3'-triOMe **273**

Following the general procedure, compound **266** (200 mg, 0.226 mmol) with 1.56 M 3HF·NEt₃ (435 μL, 0.678 mmol) and NEt₃ (63.1 μL, 0.452 mmol) gave 3'-triOMe **273** as white solid in 84% yield.

Analytical data for 3'-triOMe 273: mp 72.8-73.9°C; ¹H

NMR (300 MHz, CDCl₃): isopropylidene rings: δ 1.10, 1.11, 1.13, 1.16, 1.18, 1.19, 1.25, 1.34, 1.36, 1.39, 1.40 (m, 12H, 2 x (CH₃)₂C); methoxy and sucrose unit: δ 3.47, 3.51 (d, 1H, J = 12 Hz, H-5), 3.58, 3.60, 3.61, 3.63, 3.65, 3.66 (m, 4H, H-2, 2 x H-1', H-6'), 3.75, 3.77, 3.778, 3.81, 3.82, 3.83, 3.84, 3.86 (m, 13H, H-4, 2 x H-6, H-6', 9 x H-h'), 4.01, 4.05 (m, 2H, H-3, H-5'), 4.73, 4.74, 4.75 (t, 1H, J = 3

3'-triOMe 273

Hz, H-4'), 4.89, 4.91 (d, 1H, J = 6 Hz, H-3'), 6.17, 6.19 (d, 1H, J = 6 Hz, H-1); *trans*-alkenyl and aromatic protons: δ 6.40, 6.45 (d, 1H, J = 15 Hz, H-a'), 6.80 (s, 2H, Hc', Hg'), 7.67, 7.73 (d, 1H, H-b'); ¹³C NMR (75.5 MHz, CDCl₃): δ 18.9, 23.9, 25.1, 28.9, 29.9, 38.0, 46.2, 56.2, 61.0, 64.4, 66.2, 70.7, 71.1, 71.3, 78.2, 82.5, 91.6, 98.6, 99.8, 101.9, 103.7, 105.4, 116.1, 129.6, 140.9, 146.3, 153.5, 166.8, 171.8; HRMS (ESI-positive mode): *m/z* calcd. for C₃₀H₄₃O₁₅ 643.2602; found 643.2614 [M+H]⁺

8.2.14 The synthesis of 4'-mono-substituted PSEs

8.2.14.1 Acylation of 3'-O-Cbz **225**

General procedure

3'-O-Cbz **225** (500, 0.746 mmol) and DMAP (9.2 mg, 0.0746 mmol) were dissolved in CH₂Cl₂ at room temperature. (Substituted) cinnamic acid and DCC (231 mg, 1.12 mmol) were added and the reaction was left to stir for 12 hours. Upon completion, CH₂Cl₂ was removed. The crude mixture was first dissolved in cold diethyl ether and filtered. After which, diethyl ether was removed. The mixture was then purified with column chromatography using 4:1 Hexane/EtOAc as eluent. Compounds **274** to **280** were obtained.

The synthesis of 4'-O-cinnamoyl-3'-O-carboxybenzyl-6'-O-tert-butyldimethylsiloxy-2,1'-di-O-isopropylidene sucrose 274

Following the general procedure, cinnamic acid **30** (166 mg, 1.12 mmol) was added to the solution. After purification, compound **274** was obtained as white solid in 82% yield.

Analytical data for compound 274: mp 88.0-89.5°C; ¹H NMR

(300 MHz, CDCl₃): TBS: δ 0.02 (s, 6H, (CH₃)₂Si), 0.83 (s, 9H, (CH₃)₃CSi); isopropylidene rings: δ 1.33, 1.42, 1.47, 1.51 (m, 12H, 2 x (CH₃)₂C); Cbz and sucrose unit: δ 3.50, 3.54, 3.56, 3.59 (m, 2H, H-5, H-1'), 3.62, 3.67, 3.68, 3.69 (m, 2H, H-2, H-1'), 3.71, 3.72, 3.77, 3.80, 3.80, 3.82, 3.85, 3.86 (m, 5H, H-4, 2 x H-6, 2 x H-6'), 4.08, 4.12 (d, 1H, J = 12 Hz, H-3), 4.18, 4.19, 4.20 (m, 1H, H-5'), 4.92, 4.93 (d, 1H, J = 3 Hz, H-3'), 5.15, 5.19, 5.22, 5.26 (m, 2H, 2 x H-a), 5.53, 5.54, 5.56 (t, 1H, J = 4.5 Hz, H-4'), 6.00, 6.01 (d, 1H, J = 3 Hz, H-1); Cbz and cinnamoyl protons: δ 6.35, 6.41 (d, 1H, J = 18 Hz, H-a'), 7.31, 7.32, 7.32, 7.35, 7.36, 7.37, 7.38, 7.41, 7.41, 7.48, 7.49 (m, 8H, H-c', H-d', H-e', H-f', H-g', H-c, H-d, H-e), 7.50, 7.51 (d, 2H, J = 3 Hz, H-b, H-f), 7.63, 7.69 (d, 1H, J = 18 Hz, H-b'); ¹³C NMR (75.5 MHz, CDCl₃): δ -5.20, -5.18, 18.5, 19.3, 24.2, 25.7, 25.8, 26.0, 29.3, 63.9, 70.3, 705, 73.1, 74.1, 81.2, 82.6, 91.5, 100.0, 101.8, 104.5, 117.3, 128.4, 128.5, 128.7, 128.9, 129.1, 130.8, 134.4, 135.0, 146.2, 145.8, 165.7; HRMS (ESI-positive mode): *m/z* calcd. for C₄₁H₅₇O₁₄Si 801.3518; found 801.3481 [M+H]⁺

The synthesis of 4'-O-(4-tert-butyldimethylsiloxy)cinnamoyl)-3'-O-carboxybenzyl-6'-O-tert-butyldimethylsiloxy-2,1'-di-O-isopropylidene sucrose 275

Following the general procedure, OTBS-coum acid **203** (311 mg, 1.12 mmol) was added to the solution. After purification, compound **275** was obtained as white solid in 87% yield.

Analytical data for compound 275: mp 91.7-92.7°C; ¹H NMR

(300 MHz, CDCl₃): TBS: δ -0.18 (s, 6H, (CH₃)₂Si), 0.63 (s, 9H, (CH₃)₃CSi); TBS on OTBS-coumaroyl group: δ 0.00 (s, 6H, (CH₃)₂Si), 0.76 (s, 9H, (CH₃)₃CSi); isopropylidene rings: δ 1.14, 1.22, 1.28, 1.31 (m, 12H, 2 x (CH₃)₂C); Cbz and sucrose unit: δ 3.30, 3.34, 3.39, 3.42, 3.47, 3.48 (m, 4H, H-2, H-5, 2 x H-1') 3.50, 3.51, 3.52, 3.57, 3.59, 3.60, 3.62, 3.65, 3.66 (m, 5H, H-4, 2 x H-6, 2 x H-6'), 3.88, 3.92 (d, 1H, J = 12 Hz, H-3), 3.96, 3.97, 3.98, 3.99, 4.01 (m, 1H, H-5'), 4.70, 4.72 (d, 1H, J = 6 Hz, H-3'), 4.95, 4.99, 5.03, 5.07 (m, 2H, 2 x H-a), 5.31, 5.32, 5.34 (t, 1H, J = 4.5 Hz, H-4'), 5.80, 5.81 (d, 1H, J = 3 Hz, H-1'); Cbz and dimethoxycinnamoyl protons: δ 6.01, 6.06 (d, 1H, J = 15 Hz, H-a'), 6.61, 6.64 (d, 2H, J = 9 Hz, H-d', H-f'), 7.10, 7.11, 7.11, 7.12, 7.13, 7.15, 7.18, 7.20, 7.23 (m, 7H, H-b, H-c, H-d, H-e, H-f, H-c', H-g'), 7.38, 7.43 (d, 1H, J = 15 Hz, H-b'); ¹³C NMR (75.5 MHz, CDCl₃): δ -5.38, -5.36, -4.37, 18.2, 18.3, 19.2, 24.0, 25.6, 25.6, 25.9, 29.1, 63.7, 63.8, 70.1, 70.3, 72.9, 73.9, 76.6, 81.1, 82.6, 91.3, 99.8, 101.6, 104.3, 114.7, 120.6, 120.6, 127.5, 128.2, 128.5, 128.7, 129.9, 130.0, 134.9, 141.3, 145.7, 154.6, 158.1, 165.8; HRMS (ESI-positive mode): *m/z* calcd. for C₄₇H₇₁O₁₅Si₂ 931.4332; found 931.4323 [M+H]⁺

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The synthesis of 4'-O-(4-tert-butyldimethylsiloxy-3-methoxycinnamoyl)-3'-O-carboxybenzyl-6'-O-tert-butyldimethylsiloxy-2,1'-di-O-isopropylidene sucrose 276

Following the general procedure, OTBS-feru acid **234** (345 mg, 1.12 mmol) was added to the solution. After purification, compound **276** was obtained as white solid in 83% yield.

Analytical data for compound 276: mp 61.8-62.8°C; ¹H NMR

(300 MHz, CDCl₃): TBS: δ -0.13 (s, 6H, (CH₃)₂Si), 0.68 (s, 9H, (CH₃)₃CSi); TBS on OTBS-feruloyl group: δ 0.00 (s, 6H, (CH₃)₂Si), 0.82 (s, 9H, (CH₃)₃CSi); isopropylidene rings: δ 1.19 (s, 3H, (CH₃)₂C), 1.27 (s, 3H, (CH₃)₂C), 1.32, 1.36 (d, 6H, J = 12 Hz, (CH₃)₂C); Cbz, methoxy and sucrose unit: δ 3.41, 3.44 (d, 1H, J = 9 Hz, H-5), 3.47, 3.51, 3.53, 3.54, 3.56, 3.57 (m, 3H, H-2, 2 x H-1') 3.62, 3.65, 3.67, 3.69, 3.71 (m, 7H, H-4, 2 x H-6, H-6', 3 x H-h'), 3.93, 3.97 (d, 1H, J = 12 Hz, H-6'), 4.02, 4.03, 4.04 (m, 2H, H-3, H-5'), 4.75, 4.77 (d, 1H, J = 6 Hz, H-3'), 5.04, 5.07, 5.11 (m, 2H, 2 x H-a), 5.36, 5.37, 5.39 (t, 1H, J = 4.5 Hz, H-4'), 5.85, 5.86 (d, 1H, J = 3 Hz, H-1); Cbz group and OTBS-feruloyl protons: δ

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6.06, 6.11 (d, 1H, $J = 15$ Hz, H-a'), 6.66, 6.69 (d, 1H, $J = 9$ Hz, H-c'), 6.83, 6.86 (m, 2H, H-d', H-g'), 7.15, 7.16, 7.16, 7.17, 7.17, 7.18, 7.20, 7.20, 7.22, 7.23, 7.26, 7.27 (m, 5H), 7.41, 7.46 (d, 1H, $J = 15$ Hz, H-b'); ^{13}C NMR (75.5 MHz, CDCl_3): δ -5.38, -5.36, -4.58, 18.3, 18.5, 19.2, 24.0, 25.6, 25.7, 25.9, 29.1, 55.5, 63.7, 63.9, 66.5, 70.1, 70.3, 72.9, 73.9, 76.6, 81.1, 82.6, 91.3, 99.8, 101.6, 104.3, 110.9, 114.7, 121.1, 122.5, 128.0, 128.2, 128.5, 128.7, 134.9, 146.1, 147.8, 151.2, 154.6, 165.8; HRMS (ESI-positive mode): m/z calcd. for $\text{C}_{48}\text{H}_{73}\text{O}_{16}\text{Si}_2$ 961.4437; found 961.4446 $[\text{M}+\text{H}]^+$

The synthesis of 4'-O-(4-tert-butyldimethylsiloxy-3,5-dimethoxycinnamoyl)-3'-O-carboxybenzyl-6'-O-tert-butyldimethylsiloxy-2,1'-di-O-isopropylidene sucrose **277**

Following the general procedure, OTBS-sinap acid **235** (378 mg, 1.12 mmol) was added to the solution. After purification, compound **277** was obtained as white solid in 84% yield.

Analytical data for compound 277: mp 94.0-94.7°C; ^1H NMR

(300 MHz, CDCl_3): TBS: δ -0.10 (s, 6H, $(\text{CH}_3)_2\text{Si}$), 0.72 (s, 9H, $(\text{CH}_3)_3\text{CSi}$); TBS on OTBS-sinapoyl group: δ 0.00 (s, 6H, $(\text{CH}_3)_2\text{Si}$), 0.86 (s, 9H, $(\text{CH}_3)_3\text{CSi}$); isopropylidene rings: δ 1.22, 1.30, 1.35, 1.39 (m, 12H, 2 x $(\text{CH}_3)_2\text{C}$); Cbz, methoxy and sucrose: δ 3.38, 3.42 (d, 1H, $J = 12$ Hz, H-5), 3.44, 3.47, 3.50 (t, 1H, $J = 9$ Hz, H-1'), 3.55, 3.56, 3.57, 3.59, 3.60 (m, 2H, H-2, H-1'), 3.65, 3.68, 3.70, 3.72, 3.74 (m, 10H, H-4, 2 x H-6, H-6', 6 x H-h'), 3.96, 4.00 (d, 1H, $J = 12$ Hz, H-6'), 4.04, 4.06, 4.08, 4.10 (m, 2H, H-3, H-5'), 4.78, 4.80 (d, 1H, $J = 6$ Hz, H-3'), 5.03, 5.07, 5.11, 5.15, 5.16 (m, 2H, 2 x H-a), 5.40, 5.41, 5.42 (t, 1H, $J = 3$ Hz, H-4'), 5.88, 5.89 (d, 1H, $J = 3$ Hz, H-1); Cbz group and OTBS-sinapoyl protons: δ

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6.09, 6.15 (d, 1H, $J = 18$ Hz, H-a'), 6.58 (s, 2H, H-c', H-g'), 7.18, 7.21, 7.24, 7.26, 7.29 (m, 5H, H-b, H-c, H-d, H-e, H-f), 7.42, 7.47 (d, 1H, $J = 15$ Hz, H-b'); ^{13}C NMR (75.5 MHz, CDCl_3): δ -5.36, -4.59, 18.3, 18.8, 19.1, 24.0, 25.6, 25.7, 25.9, 55.8, 62.5, 63.9, 70.3, 73.0, 83.0, 91.3, 99.8, 101.6, 104.3, 105.5, 126.8, 128.2, 128.5, 128.7, 134.9, 137.2, 146.4, 151.7, 156.6, 165.9; HRMS (ESI-positive mode): m/z calcd. for $\text{C}_{49}\text{H}_{75}\text{O}_{17}\text{Si}_2$ 991.4543; found 991.4542 $[\text{M}+\text{H}]^+$

The synthesis of 4'-O-(3,4-di-*tert*-butyldimethylsiloxylcinnamoyl)-3'-O-carboxybenzyl-6'-O-*tert*-butyldimethylsiloxy-2,1'-di-O-isopropylidene sucrose 278

Following the general procedure, OTBS-caff acid **198** (457 mg, 1.12 mmol) was added to the solution. After purification, compound **278** was obtained as white solid in 86% yield.

Analytical data for compound 278: mp 69.0-70.1 °C; ¹H NMR (300 MHz, CDCl₃): TBS: δ -0.18, -0.17 (s, 6H, (CH₃)₂Si), 0.65 (s, 9H, (CH₃)₃CSi); TBS on OTBS-caffeoyl group: δ 0.00 (s, 12H, 2 x (CH₃)₂Si), 0.77, 0.78, 0.78 (m, 18H, 2 x (CH₃)₃CSi); isopropylidene rings: δ 1.14 (s, 3H, (CH₃)₂C), 1.22 (s, 3H, (CH₃)₂C), 1.28, 1.32 (d, 6H, J = 12 Hz, (CH₃)₂C); Cbz and sucrose unit: δ 3.31, 3.35, 3.38, 3.42 (m, 2H, H-5, H-1'), 3.49, 3.51, 3.53 (m, 2H, H-2, H-1'), 3.58, 3.60, 3.62, 3.65 (m, 5H, H-4, 2 x H-6, 2 x H-6'), 3.88, 3.92 (d, 1H, J = 12 Hz, H-3), 3.99, 4.00 (m, 1H, H-5'), 4.71, 4.73 (d, 1H, J = 6 Hz, H-3'), 4.95, 4.99, 5.03, 5.07 (m, 2H, 2 x H-a), 5.31, 5.33, 5.34 (t, 1H, J = 4.5 Hz, H-4'), 5.80, 5.81 (d, 1H, J = 3 Hz, H-1); Cbz group: δ 7.10, 7.13, 7.16, 7.19, 7.22 (m, 5H); Cbz and OTBS-caffeoyl protons: δ 5.94, 5.99 (d, 1H, J = 15 Hz, H-a'), 6.59, 6.62 (d, 1H, J = 9 Hz, H-f'), 6.78 (s, 2H, H-c', H-g'), 7.31, 7.36 (d, 1H, J = 15 Hz, H-b'); ¹³C NMR (75.5 MHz, CDCl₃): δ -5.39, -5.37, -4.07, -4.05, 18.3, 18.5, 18.5, 19.2, 24.0, 25.6, 25.9, 25.9, 25.9, 29.1, 62.3, 63.7, 63.8, 66.5, 70.1, 70.2, 73.0, 74.0, 76.6, 81.1, 82.6, 91.4, 99.8, 101.6, 104.3, 114.6, 120.5, 121.2, 122.5, 127.8, 128.2, 128.5, 128.7, 134.9, 145.9, 147.2, 149.8, 154.6, 165.8; HRMS (ESI-positive mode): *m/z* calcd. for C₅₃H₈₅O₁₆Si₃ 1061.5145; found 1061.5159 [M+H]⁺

The synthesis of 4'-O-(3,4-dimethoxycinnamoyl)-3'-O-carboxybenzyl-6'-O-*tert*-butyldimethylsiloxy-2,1'-di-O-isopropylidene sucrose 279

Following the general procedure, diOMe acid **38** (233 mg, 1.12 mmol) was added to the solution. After purification, compound **284** was obtained as white solid in 81% yield.

Analytical data for compound 279: mp 91.7-92.7°C; ¹H NMR

(300 MHz, CDCl₃): TBS: δ 0.00 (s, 6H, (CH₃)₂Si), 0.81, 0.83 (m, 9H, (CH₃)₃CSi); isopropylidene rings: δ 1.32, 1.40, 1.45, 1.49 (m, 12H, 2 x (CH₃)₃C); Cbz, methoxy and sucrose unit: δ 3.48, 3.52 (d, 1H, J = 12 Hz, H-5), 3.57, 3.60 (m, 1H, H-1'), 3.65, 3.66, 3.67, 3.69, 3.79 (m, 2H, H-2, H-1'), 3.75, 3.78, 3.80, 3.83, 3.84 (m, 5H, H-4, 2 x H-6, 2 x H-6'), 3.87 (s, 6H, 6 x H-h'), 4.06, 4.10 (d, 1H, J = 12 Hz, H-3), 4.16, 4.18 (m, 1H, H-5'), 4.89, 4.91 (d, 1H, J = 6 Hz, H-3'), 5.13, 5.17, 5.21, 5.25 (m, 2H, 2 x H-a), 5.50, 5.51, 5.53 (t, 1H, J = 4.5 Hz, H-4'), 5.98, 5.99 (d, 1H, J = 3 Hz, H-1); Cbz and

dimethoxycinnamoyl protons: δ 6.21, 6.26 (d, 1H, J = 15 Hz, H-a'), 6.82, 6.85 (d, 1H, J = 9 Hz, H-g'), 6.99 (s, 1H, H-f'), 7.05, 7.08 (d, 1H, H-c'), 7.26, 2.28, 7.31, 7.34, 7.37 (m, 5H, H-b, H-c, H-d, H-e, H-f), 7.55, 7.60 (d, 1H, J = 15 Hz, H-b'); ¹³C NMR (75.5 MHz, CDCl₃): δ -5.39, -5.36, 18.3, 19.2, 24.0, 25.6, 25.9, 29.1, 55.9, 56.0, 63.7, 63.9, 70.1, 70.3, 72.9, 73.9, 82.8, 82.6, 91.3, 99.8, 101.6, 104.3, 109.7, 111.0, 114.7, 122.9, 127.2, 128.2, 128.5, 128.7, 134.9, 145.9, 149.3, 151.4, 154.6, 165.7; HRMS (ESI-positive mode): *m/z* calcd. for C₄₃H₆₁O₁₆Si 861.3729; found 861.3700 [M+H]⁺

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The synthesis of 4'-O-(3,4,5-trimethoxycinnamoyl)-3'-O-carboxybenzyl-6'-O-tert-butylidimethylsiloxy-2,1'-di-O-isopropylidene sucrose 280

Following the general procedure, triOMe acid **210** (266 mg, 1.12 mmol) was added to the solution. After purification, compound **280** was obtained as white solid in 87% yield.

Analytical data for compound 280: mp 92.0-93.1°C; ¹H NMR

(300 MHz, CDCl₃): TBS: δ 0.00 (s, 6H, (CH₃)₂Si), 0.81 (s, 9H, (CH₃)₃CSi); isopropylidene rings: δ 1.29, 1.31, 1.40, 1.41, 1.42, 1.45, 1.49 (m, 12H, 2 x (CH₃)₂C); Cbz, methoxy and sucrose unit: δ 3.45, 3.48, 3.49, 3.52, 3.54, 3.57, 3.60 (m, 4H, H-2, H-5, 2 x H-1'), 3.63, 3.65, 3.66, 3.69, 3.70, 3.75, 3.78, 3.80, 3.83 (m, 4H, H-4, 2 x H-6, H-6'), 3.85 (s, 9H, 9 x H-a), 3.92, 3.94, 3.97, 4.01, 4.06, 4.10 (m, 2H, H-6', H-3), 4.15, 4.16, 4.18 (m, 1H, H-5'), 4.89, 4.91 (d, 1H, J = 6 Hz, H-3'), 5.13, 5.17, 5.19, 5.20, 5.24 (m, 2H, 2 x H-a), 5.51, 5.52, 5.54 (t, 1H, J = 4.5 Hz, H-4'), 5.92, 5.93, 5.98, 5.99 (m, 1H, H-1'); Cbz and trimethoxycinnamoyl protons:

δ 6.24, 6.29 (d, 1H, J = 15 Hz, H-a'), 6.70 (s, 2H, H-c', H-g'), 7.28, 7.31, 7.34, 7.36, 7.37 (m, 5H, H-b, H-c, H-d, H-e, H-f); 7.53, 7.58 (d, 1H, J = 15 Hz, H-b'); ¹³C NMR (75.5 MHz, CDCl₃): δ -5.46, -5.39, -5.36, -5.33, 18.3, 19.1, 19.1, 24.0, 25.5, 25.6, 25.7, 25.9, 25.9, 29.1, 29.7, 30.9, 56.2, 61.0, 62.3, 63.5,

280

63.7, 63.9, 70.0, 70.1, 70.3, 72.9, 73.0, 81.0, 82.0, 82.3, 82.5, 90.8, 91.3, 99.8, 99.8, 101.6, 103.4, 104.3, 105.3, 116.3, 128.2, 128.5, 128.7, 128.7, 129.6, 134.7, 134.8, 140.3, 145.9, 153.5, 154.6, 165.5; HRMS (ESI-positive mode): m/z calcd. for $C_{44}H_{62}O_{17}NaSi$ 913.3654; found 913.3663 $[M+H]^+$

8.2.14.2 Removal of Cbz and TBS groups

The synthesis of 4'-*O*-cinnamoyl-2,1'-di-*O*-isopropylidene sucrose, 4'-cinn **281**

Compound **274** (200 mg, 0.250 mmol) and $Pd(OAc)_2$ (4.7 mg, 0.0208 mmol) were dissolved in CH_2Cl_2 (5.0 ml) and stirred at room temperature. Subsequently, Et_3SiH (53.2 μL , 0.333 mmol) and NEt_3 (4.65 μL , 0.0333 mmol) were added to the solution and the reaction was left to stir for 12 hours. Upon completion, the reaction was diluted with EtOAc (30.0 ml). The reaction mixture was washed twice with H_2O and the organic layer was collected and dried with anhydrous $MgSO_4$. After removal of EtOAc, the crude product obtained were dissolved in pyridine (1.0 ml) and stirred at room temperature. Subsequently, 1.56 M $3HF \cdot NEt_3$ (481 μL , 0.750 mmol) and NEt_3 (69.8 μL , 0.500 mmol) were added to the solution and the reaction was left to stir for 12 hours. Upon completion, pyridine was removed. The crude product was then subjected to purification using 3:2 EtOAc/Hexane as eluent. In the end, 4'-cinn **281** was obtained as white solid in 88% yield.

Analytical data for 4'-cinn **281:** mp 88.1-89.2°C; 1H NMR (300 MHz,

$CDCl_3$): isopropylidene rings: δ 1.38, 1.40, 1.45 (m, 12H, 2 x $(CH_3)_2C$); sucrose unit: δ 3.44, 3.47, 3.48 (m, 2H, H-5, H-1'), 3.52, 3.55, 3.59, 3.62, 3.66, 3.67 (m, 2H, H-2, H-1'), 3.72, 3.73, 3.75, 3.76, 3.78, 3.79, 3.81, 3.83, 3.84, 3.86 3.90 (m, 6H, H-3, H-4, 2 x H-6, 2 x H-6'), 3.93, 3.96, 3.99, 4.02 (m, 1H, H-5'), 4.18, 4.19 (d, 1H, $J = 3$ Hz, H-3'), 5.41, 5.43, 5.46 (t, 1H, $J = 7.5$ Hz, H-4'), 6.18, 6.19 (d, 1H, $J = 3$ Hz, H-1); *trans*-alkenyl and aromatic protons: δ 6.60, 6.70 (d, 1H, $J = 30$ Hz, H-a'), 7.15, 7.17, 7.21 (m, 3H, H-d', H-e', H-f'), 7.23, 7.24, 7.26 (m, 3H, H-

4'-cinn **281**

b', H-c', H-g'); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 17.1, 22.2, 23.2, 27.0, 28.8, 33.7, 59.9, 62.1, 64.2, 67.3, 70.9, 71.4, 80.0, 89.4, 98.0, 100.3, 101.3, 124.4, 126.3, 126.5, 138.0, 170.9; HRMS (ESI-positive mode): m/z calcd. for $C_{27}H_{36}O_{12}Na$ 575.2104; found 575.2088 $[M+Na]^+$

The synthesis of 4'-*O*-coumaroyl-2,1'-di-*O*-isopropylidene sucrose, 4'-coum **282**

Compound **275** (200 mg, 0.215 mmol) and $Pd(OAc)_2$ (4.0 mg, 0.0179 mmol) were dissolved in CH_2Cl_2 (5.0 ml) and stirred at room temperature. Subsequently, Et_3SiH (45.8 μL , 0.287 mmol) and NEt_3 (4.00 μL , 0.0286 mmol) were added to the solution and the reaction was left to stir for 12 hours. Upon

completion, the reaction was diluted with EtOAc (30.0 ml). The reaction mixture was washed twice with H₂O and the organic layer was collected and dried with anhydrous MgSO₄. After removal of EtOAc, the crude product obtained were dissolved in pyridine (1.0 ml) and stirred at room temperature. Subsequently, 1.56 M 3HF·NEt₃ (413 μL, 0.645 mmol) and NEt₃ (60.0 μL, 0.430 mmol) were added to the solution and the reaction was left to stir for 12 hours. Upon completion, pyridine was removed. The crude product was then subjected to purification using 3:2 EtOAc/Hexane as eluent. In the end, 4'-cousm **282** was obtained as white solid in 84% yield.

Analytical data for 4'-cousm 282: mp 75.5-76.9°C; ¹H NMR (300 MHz,

(CD₃)₂CO): isopropylidene rings: δ 1.08, 1.11, 1.13, 1.16, 1.19, 1.31 (m, 12H, 2 x (CH₃)₃C); sucrose unit: δ 3.35, 3.36, 3.39, 3.40, 3.43 (m, 2H, H-5, H-1'), 3.51, 3.55, 3.56, 3.58, 3.59, 3.60 (m, 6H, H-2, H-4, 2 x H-6, H-1', H-6'), 3.68, 3.71, 3.74, 3.75, 3.77 (m, 2H, H-3, H-6'), 3.82, 3.83, 3.84, 3.86, 3.89 (m, 1H, H-5'), 3.97, 4.01 (d, 1H, J = 12 Hz, H-3'), 5.36, 5.39, 5.41 (t, 1H, J = 7.5 Hz, H-4'), 5.98, 5.99 (d, 1H, J = 3 Hz, H-1); *trans*-alkenyl and aromatic protons: δ 6.19, 6.25 (d, 1H, J = 18 Hz, H-a'), 6.74, 6.77 (d, 2H, J = 9 Hz, H-d', H-f'), 7.39, 7.42 (d, 2H, J = 9 Hz, H-c', H-g'), 7.48, 7.53 (d, 1H, J = 15 Hz, H-b'); ¹³C NMR (75.5 MHz, (CD₃)CO): δ 25.8, 62.5, 62.9, 64.1, 71.5, 73.0, 74.4, 74.6, 78.4, 78.9, 83.3, 93.4, 106.4, 114.8, 116.8, 126.7, 131.2, 146.5, 161.0, 167.2; HRMS (ESI-positive mode): *m/z* calcd. for C₂₇H₃₇O₁₃ 569.2234; found 569.2239 [M+Na]⁺

4'-cousm 282

The synthesis of 4'-*O*-feruloyl-2,1'-di-*O*-isopropylidene sucrose, 4'-feru **283**

Compound **276** (200 mg, 0.208 mmol) and Pd(OAc)₂ (3.9 mg, 0.0174 mmol) were dissolved in CH₂Cl₂ (5.0 ml) and stirred at room temperature. Subsequently, Et₃SiH (44.3 μL, 0.278 mmol) and NEt₃ (3.88 μL, 0.0278 mmol) were added to the solution and the reaction was left to stir for 12 hours. Upon completion, the reaction was diluted with EtOAc (30.0 ml). The reaction mixture was washed twice with H₂O and the organic layer was collected and dried with anhydrous MgSO₄. After removal of EtOAc, the crude product obtained were dissolved in pyridine (1.0 ml) and stirred at room temperature. Subsequently, 1.56 M 3HF·NEt₃ (400 μL, 0.625 mmol) and NEt₃ (58.1 μL, 0.416 mmol) were added to the solution and the reaction was left to stir for 12 hours. Upon completion, pyridine was removed. The crude product was then subjected to purification using 3:2 EtOAc/Hexane as eluent. In the end, 4'-feru **283** was obtained as white solid in 82% yield.

Analytical data for 4'-feru 283: mp 77.8-79.0°C; ¹H NMR (300 MHz, (CD₃)₂CO): isopropylidene rings: δ 1.31, 1.34, 1.46 (m, 12H, 2 x (CH₃)₃C); methoxy and sucrose unit: δ 3.49, 3.52 3.53, 3.55, 3.58 (m, 2H, H-5, H-1'), 3.62, 3.64, 3.66, 3.69, 3.71, 3.72, 3.73, 3.73, 3.74, 3.75, 3.77 (m, 5H, H-2, 2 x H-6, H-1', H-6'), 3.82, 3.84, 3.85, 3.86, 3.87, 3.88 (m, 2H, H-4, H-6'), 3.91 (s, 3H, 3 x H-h'), 3.97, 3.98, 3.99, 4.01, 4.02 (m, 2H, H-3, H-5'), 4.12, 4.16 (d, 1H, J = 12 Hz, H-3'), 5.50, 5.52, 5.55 (t, 1H, J = 7.5 Hz, H-4'), 6.12, 6.13 (d, 1H, J = 3 Hz, H-1); aromatic and *trans*-alkenyl protons: δ 6.39, 6.44 (d, 1H, J = 15 Hz, H-a'), 6.85, 6.87 (d, 1H, J = 6 Hz, H-c'), 7.12, 7.13 (d, 1H, J = 3 Hz, H-d') 7.15 (s, 1H, H-g'), 7.33, 7.34 (d, 1H, J = 3 Hz, H-b'); ¹³C NMR (75.5 MHz, (CD₃)CO): δ 18.5, 23.6, 24.6, 25.3, 55.5, 61.8, 63.0, 63.4, 65.8, 69.2, 73.4, 74.1, 76.9, 77.6, 81.9, 91.3, 99.2, 101.4, 103.5, 105.0, 110.6, 114.1, 115.3, 123.3, 126.3, 145.9, 148.0, 149.6, 166.5, 166.7; HRMS (ESI-positive mode): *m/z* calcd. for C₂₈H₃₉O₁₄ 599.2340; found 599.2333 [M+H]⁺

4'-feru 283

The synthesis of 4'-*O*-sinapoyl-2,1'-di-*O*-isopropylidene sucrose, 4'-sinap 284

Compound **277** (200 mg, 0.202 mmol) and Pd(OAc)₂ (3.8 mg, 0.0168 mmol) were dissolved in CH₂Cl₂ (5.0 ml) and stirred at room temperature. Subsequently, Et₃SiH (43.0 μL, 0.269 mmol) and NEt₃ (3.76 μL, 0.0269 mmol) were added to the solution and the reaction was left to stir for 12 hours. Upon completion, the reaction was diluted with EtOAc (30.0 ml). The reaction mixture was washed twice with H₂O and the organic layer was collected and dried with anhydrous MgSO₄. After removal of EtOAc, the crude product obtained were dissolved in pyridine (1.0 ml) and stirred at room temperature. Subsequently, 1.56 M 3HF·NEt₃ (388 μL, 0.606 mmol) and NEt₃ (56.4 μL, 0.404 mmol) were added to the solution and the reaction was left to stir for 12 hours. Upon completion, pyridine was removed. The crude product was then subjected to purification using 3:2 EtOAc/Hexane as eluent. In the end, 4'-sinap **284** was obtained as white solid in 88% yield.

Analytical data for 4'-sinap 284: mp 84.8-86.2°C; ¹H NMR (300 MHz, CDCl₃): isopropylidene rings: δ 1.46, 1.50, 1.52, 1.52 (m, 12H, 2 x (CH₃)₃C); methoxy and sucrose: δ 3.50, 3.54, 3.57, 3.60, 3.63, 3.68, 3.71, 3.79 (m, 5H, H-2, H-5, 2 x H-1', H-6'), 3.81, 3.82 3.83, 3.86, 3.88, 3.89, 3.91 (m, 8H, 2 x H-6, 6 x H-h'), 4.01, 4.03, 4.04, 4.06, 4.07, 4.09, 4.11, 4.14 (m, 4H, H-3, H-4, H-5', H-6'), 4.35, 4.39 (d, 1H, J = 12 Hz, H-3'), 5.57, 5.59, 5.62 (t, 1H, J = 7.5 Hz, H-4'), 5.96 (s, 1H, H-1); *trans*-alkenyl and aromatic protons: δ 6.24, 6.27, 6.28, 6.29 (m, 1H, H-a'), 6.66 (s, 2H, J = H-c', H-g'), 7.48, 7.53 (d, 1H, J = 15 Hz, H-b'); ¹³C NMR

4'-sinap 284

(75.5 MHz, CDCl₃): δ 18.3, 23.4, 24.4, 28.2, 55.5, 61.2, 61.4, 63.3, 65.6, 68.1, 68.1, 72.3, 72.8, 81.4, 90.6, 99.1, 101.6, 101.7, 102.5, 102.5, 104.4, 104.4, 113.7, 124.7, 136.6, 136.6, 145.7, 146.4, 146.4 166.3; HRMS (ESI-positive mode): m/z calcd. for C₂₉H₄₀O₁₅Na 651.2265; found 651.2245 [M+Na]⁺

The synthesis of 4'-*O*-caffeoyl-2,1'-di-*O*-isopropylidene sucrose, 4'-caff **285**

Compound **278** (200 mg, 0.189 mmol) and Pd(OAc)₂ (3.5 mg, 0.0157 mmol) were dissolved in CH₂Cl₂ (5.0 ml) and stirred at room temperature. Subsequently, Et₃SiH (40.2 μ L, 0.251 mmol) and NEt₃ (3.51 μ L, 0.0251 mmol) were added to the solution and the reaction was left to stir for 12 hours. Upon completion, the reaction was diluted with EtOAc (30.0 ml). The reaction mixture was washed twice with H₂O and the organic layer was collected and dried with anhydrous MgSO₄. After removal of EtOAc, the crude product obtained were dissolved in pyridine (1.0 ml) and stirred at room temperature. Subsequently, 1.56 M 3HF·NEt₃ (363 μ L, 0.566 mmol) and NEt₃ (52.6 μ L, 0.377 mmol) were added to the solution and the reaction was left to stir for 12 hours. Upon completion, pyridine was removed. The crude product was then subjected to purification using 3:2 EtOAc/Hexane as eluent. In the end, 4'-caff **285** was obtained as white solid in 84% yield.

Analytical data for 4'-caff **285:** mp 69.5-70.6°C; ¹H NMR (300 MHz, (CD₃)₂CO): isopropylidene rings: δ 1.16, 1.19, 1.32 (m, 12H, 2 x (CH₃)₃C); sucrose: δ 3.35, 3.81, 3.39, 3.40, 3.43, 3.48, 3.49 (m, 3H, H-5, 2 x H-1'), 3.51, 3.55, 3.57, 3.59, 3.60, 3.62, 3.64, 3.69 (m, 4H, H-2, 2 x H-6, H-6'), 3.72, 3.74, 3.75, 3.77 (m, 2H, H-4, H-6'), 3.82, 3.84, 3.85, 3.86, 3.89 (m, 2H, H-3, H-5'), 3.97, 4.02 (d, 1H, J = 15 Hz, H-3'), 5.35, 5.38, 5.40 (t, 1H, J = 7.5 Hz, H-4'), 5.98, 5.99 (d, 1H, J = 15 Hz, H-1); *trans*-alkenyl and aromatic protons: δ 6.13, 6.18 (d, 1H, J = 15 Hz, H-a'), 6.70, 6.73 (d, 1H, J = 9 Hz, H=f'), 6.88, 6.91 (d, 1H, J = 9 Hz, H-g'), 7.02, 7.03 (d, 1H, J = 3 Hz, H-c'), 7.41, 7.47 (d, 1H, J = 18 Hz, H-b');

¹³C NMR (75.5 MHz, (CD₃)₂CO): δ 19.4, 24.5, 25.5, 62.7, 63.8, 64.3, 65.4, 66.8, 70.1, 71.5, 73.7, 74.3, 75.0, 77.7, 78.6, 82.8, 92.2, 93.9, 99.9, 100.0, 102.0, 102.2, 104.3, 104.4, 105.9, 114.9, 115.2, 116.1, 116.4, 120.4, 122.8, 127.4, 146.4, 146.7, 129.1, 167.2, 167.4; HRMS (ESI-positive mode): m/z calcd. for C₂₇H₃₇O₁₄ 585.2183; found 585.2163 [M+H]⁺

4'-caff **285**

The synthesis of 4'-O-(3,4-dimethoxycinnamoyl)-2,1'-di-O-isopropylidene sucrose, 4'-diOMe **286**

Compound **279** (200 mg, 0.232 mmol) and Pd(OAc)₂ (4.3 mg, 0.0193 mmol) were dissolved in CH₂Cl₂ (5.0 ml) and stirred at room temperature. Subsequently, Et₃SiH (49.5 μL, 0.310 mmol) and NEt₃ (4.35 μL, 0.0193 mmol) were added to the solution and the reaction was left to stir for 12 hours. Upon completion, the reaction was diluted with EtOAc (30.0 ml). The reaction mixture was washed twice with H₂O and the organic layer was collected and dried with anhydrous MgSO₄. After removal of EtOAc, the crude product obtained were dissolved in pyridine (1.0 ml) and stirred at room temperature. Subsequently, 1.56 M 3HF·NEt₃ (447 μL, 0.697 mmol) and NEt₃ (64.9 μL, 0.465 mmol) were added to the solution and the reaction was left to stir for 12 hours. Upon completion, pyridine was removed. The crude product was then subjected to purification using 3:2 EtOAc/Hexane as eluent. In the end, 4'-diOMe **291** was obtained as white solid in 85% yield.

Analytical data for 4'-diOMe 291: mp 82.1-83.6°C; ¹H NMR (300 MHz, CDCl₃): isopropylidene rings: δ 1.41, 1.46 (m, 12H, 2 x (CH₃)₃C); sucrose unit: δ 3.45, 3.49 (d, 1H, J = 15 Hz, H-5), 3.53, 3.59, 3.63, 3.66 (m, 3H, H-2, 2 x H-1'), 3.72, 3.73, 3.75, 3.76, 3.78, 3.79 (m, 1H, H-6'), 3.81, 3.83, 3.85, 3.88, 3.90, 3.91 (m, 9H, H-4, 2 x H-6, 6 x H-h'), 3.96, 3.99, 4.02, 4.03, 4.05, 4.06 (m, 3H, H-3, H-5', H-6'), 4.20, 4.24 (d, 1H, J = 12 Hz, H-3'), 5.49, 5.52, 5.54 (t, 1H, J = 7.5 Hz, H-4'), 6.19, 6.20 (d, 1H, J = 3 Hz, H-1); *trans*-alkenyl and aromatic protons: δ 6.22, 6.28 (d, 1H, J = 18 Hz, H-a'), 6.77, 6.80 (d, 1H, J = 9 Hz, H-g'), 6.95 (s, 1H, H-f'), 6.99, 7.00, 7.02, 7.02 (m, 1H, H-c'), 7.55, 7.60 (d, 1H, J = 15 Hz, H-b'); ¹³C NMR (75.5 MHz, CDCl₃): δ 12.0, 17.4, 18.7, 19.1, 24.2, 25.3, 29.0, 41.9, 53.6, 55.9, 56.0, 64.2, 82.4, 91.4, 100.0, 102.2, 103.6, 114.5, 123.0, 127.0, 149.3, 151.5, 167.2; HRMS (ESI-positive mode): *m/z* calcd. for C₂₉H₄₀O₁₄Na 635.2316; found 635.2296 [M+Na]⁺

The synthesis of 4'-O-(3,4,5-trimethoxycinnamoyl)-2,1'-di-O-isopropylidene sucrose, 4'-triOMe **287**

Compound **280** (200 mg, 0.225 mmol) and Pd(OAc)₂ (4.2 mg, 0.0187 mmol) were dissolved in CH₂Cl₂ (5.0 ml) and stirred at room temperature. Subsequently, Et₃SiH (47.8 μL, 0.229 mmol) and NEt₃ (4.18 μL, 0.0229 mmol) were added to the solution and the reaction was left to stir for 12 hours. Upon completion, the reaction was diluted with EtOAc (30.0 ml). The reaction mixture was washed twice with H₂O and the organic layer was collected and dried with anhydrous MgSO₄. After removal of EtOAc, the crude product obtained were dissolved in pyridine (1.0 ml) and stirred at room temperature.

Subsequently, 1.56 M 3HF·NEt₃ (432 μL, 0.674 mmol) and NEt₃ (62.7 μL, 0.449 mmol) were added to the solution and the reaction was left to stir for 12 hours. Upon completion, pyridine was removed. The crude product was then subjected to purification using 3:2 EtOAc/Hexane as eluent. In the end, 4'-triOMe **287** was obtained as white solid in 85% yield.

Analytical data for 4'-triOMe **287:** mp 87.1-88.3°C; ¹H NMR (300 MHz, CDCl₃): isopropylidene rings: δ 1.38, 1.41, 1.44 (m, 12H, 2 x (CH₃)₃C); methoxy and sucrose: δ 3.42, 3.46 (d, 1H, J = 12 Hz, H-5), 3.49, 3.53, 3.56, 3.57, 3.60 3.64, 3.70 (m, 4H, H-2, 2 x H-1', H-6'), 3.71, 3.73, 3.74, 3.76, 3.79, 3.80, 3.83 (m, 12H, H-4, 2 x H-6, 9 x H-h'), 3.92, 3.93, 3.05, 3.97, 3.98, 3.99, 4.01, 4.04, 4.08 (m, 3H, H-3, H-5', H-6'), 4.25, 4.29 (d, 1H, J = 12 Hz, H-3'), 5.50, 5.53, 5.55 (t, 1H, J = 7.5 Hz, H-4'), 6.19, 6.20 (d, 1H, J = 3 Hz, H-1'); *trans*-alkenyl and aromatic protons: δ 6.22, 6.28 (d, 1H, J = 18 Hz, H-a'), 6.59 (s, 2H, H-c', H-g'), 7.44, 7.49 (d, 1H, J = 15 Hz, H-b'); ¹³C NMR (75.5 MHz, CDCl₃): δ 19.2, 24.2, 25.2, 25.6, 25.9, 29.0, 56.1, 56.2, 61.0, 62.0, 62.3, 64.2, 66.4, 69.0, 73.2, 73.6, 82.2, 91.5, 99.9, 100.0, 102.4, 102.5, 103.4, 105.4, 116.2, 128.2, 129.6, 140.3, 146.3, 153.2, 153.4, 167.0; HRMS (ESI-positive mode): *m/z* calcd. for C₃₀H₄₂O₁₅Na 665.2421; found 665.2422 [M+Na]⁺

4'-triOMe **287**

8.2.15 The synthesis of 6'-mono-substituted PSEs

8.2.15.1 Acylation using 2,1':4,6-di-*O*-isopropylidene sucrose **68**

General procedure

Oxalyl chloride (132 μL, 1.54 mmol) and (substituted) cinnamic acid were dissolved in CH₂Cl₂ (3.0 ml) and stirred at room temperature. DMF (18.3 μL, 0.237 mmol) was added to the solution and the reaction was left to stir for 2 hours. After which, pyridine (286.2 μL, 3.55 mmol) and 2,1':4,6-di-*O*-isopropylidene sucrose **68** (500 mg, 1.18 mmol) dissolved in CH₂Cl₂ (2.0 ml) were added to the stirring solution. The reaction was left to stir for 12 hours. Upon completion, the reaction mixture was evaporated. the crude product obtained was purified using column chromatography using a gradient of solvent to separate (starting from 2:1 Hexane/EtOAc and slowly increase EtOAc). 6'-cinn **288**, **291**, **292**, **293**, **294**, 6'-diOMe **295** and 6'-triOMe **296** were obtained.

The synthesis of 6'-*O*-cinnamoyl-2,1'-di-*O*-isopropylidene sucrose, 6'-cinn 288

Following the general procedure, cinnamic acid **30** (210 mg, 1.42 mmol) will give 6'-cinn **288** as white solid in 43% yield.

Analytical data for 6'-cinn 288: Comparison with reported values in literature¹²⁶ confirmed the formation of 6'-cinn **288**.

6'-diOMe 288

The synthesis of 6'-*O*-(4-*tert*-butyldimethylsiloxycinnamoyl)-2,1'-di-*O*-isopropylidene sucrose 291

Following the general procedure, OTBS-coum acid **203** (395 mg, 1.42 mmol) will give compound **291** as white solid in 37% yield.

Analytical data for compound 291: mp 90.5-

91.5 °C; ¹H NMR (300 MHz, CDCl₃): TBS on OTBS-

coumaroyl group: δ 0.00 (s, 6H, (CH₃)₂Si), 0.75 (s,

9H, (CH₃)₃CSi); isopropylidene rings: δ 1.24, 1.30 (d,

12H, J = 18 Hz, 2 x (CH₃)₂C); sucrose unit: δ 3.29,

3.33 (d, 1H, J = 12 Hz, H-5), 3.36, 3.39, 3.42 (t, 1H,

J = 9 Hz, H-1'), 3.50, 3.53, 3.54, 3.56, 3.57 (m, 2H,

H-2, H-1'), 3.64, 3.66, 3.68, 3.71, 3.74, 3.76, (m, 3H, H-4, 2 x H-6), 3.81, 3.84 (d, 1H, J = 9 Hz, H-3'),

3.92, 3.95, 3.97, 3.99, 4.00 4.01, 4.03, 4.06, 4.09, 4.10, 4.13 (m, 4H, H-3, H-4', H-5', H-6'), 4.27, 4.28,

4.31, 4.32 (m, 1H, H-6'), 6.00 (s, 1H, H-1); *trans*-alkenyl and aromatic protons: δ 6.08, 6.13 (d, 1H, J

= 15 Hz, H-a), 6.59, 6.62 (d, 2H, J = 9 Hz, H-d, H-f), 7.17, 7.20 (d, 2H, J = 9 Hz, H-c, H-g), 7.40, 7.45

(d, 1H, J = 15 Hz, H-b); ¹³C NMR (75.5 MHz, CDCl₃): δ -4.77, -4.39, -4.01, 11.2, 14.2, 18.2, 18.7, 19.1,

19.3, 21.1, 24.2, 25.3, 25.6, 25.9, 29.1, 31.5, 36.6, 53.4, 60.4, 61.8, 62.3, 63.8, 65.9, 66.3, 69.5, 73.3,

73.9, 79.2, 79.8, 91.0, 100.0, 102.1, 103.6, 115.3, 120.5, 127.6, 129.8, 145.2, 157.9, 162.9, 162.8, 171.2;

HRMS (ESI-positive mode): *m/z* calcd. for C₃₃H₅₁O₁₃Si 683.3054; found 683.3016 [M+H]⁺

291

The synthesis of 6'-O-(4-*tert*-butyldimethylsiloxy-3-methoxycinnamoyl)-2,1'-di-*O*-isopropylidene sucrose **292**

Following the general procedure, OTBS-feru acid **234** (437 mg, 1.42 mmol) will give compound **292** in 43 % yield.

Analytical data for compound 292: mp 88.6-89.8

°C; ¹H NMR (300 MHz, CDCl₃): TBS on OTBS-feruloyl group: δ 0.00 (s, 6H, (CH₃)₂Si), 0.83 (s, 9H, (CH₃)₃CSi); isopropylidene rings: δ 1.28, 1.30 1.34 (m, 12H, 2 x (CH₃)₂C); methoxy and sucrose unit: δ 3.32, 3.36, 3.39, 3.42, 3.45 (m, 2H, H-5, H-1'), 3.54, 3.58, 3.60 (m, 3H, H-2, H-6, H-1'), 3.65 (s, 3H, 3 x

H-h), 3.74, 3.77, 3.79 (m, 3H, H-4, H-6, H-3'), 3.92, 3.95, 3.98, 4.03, 4.09, 4.13, 4.17 (m, 4H, H-3, H-4', H-5', H-6'), 4.37, 4.04 (d, 1H, J = 21 Hz, H-6'), 6.04 (s, 1H, H-1); *trans*-alkenyl and aromatic protons: δ 6.14, 6.20 (d, 1H, J = 18 Hz, H-a), 6.64, 6.67 (d, 1H, J = 9 Hz, J=d), 6.82, 6.85 (m, 2H, H-c, H-g), 7.43, 7.48 (d, 1H, J = 15 Hz, H-b); ¹³C NMR (75.5 MHz, CDCl₃): δ -3.60, -3.57, 11.0, 18.5, 19.0, 23.8, 25.2, 25.6, 25.7, 29.0, 29.3, 29.7, 37.8, 38.0, 38.1, 53.5, 55.4, 62.1, 64.2, 64.9, 65.9, 70.9, 71.3, 71.6, 79.5, 91.4, 99.7, 101.5, 104.5, 110.8, 115.4, 121.0, 122.4, 128.3, 145.2, 147.5, 151.2, 166.7, 171.1; HRMS (ESI-positive mode): *m/z* calcd. for C₃₄H₅₃O₁₄Si 713.3160; found 713.3122 [M+H]⁺

The synthesis of 6'-O-(4-*tert*-butyldimethylsiloxy-3,5-dimethoxycinnamoyl)-2,1'-di-*O*-isopropylidene sucrose **293**

Following the general procedure, OTBS-sinap acid **235** (481 mg, 1.42 mmol) will give compound **293**.

To avoid loss of product, the reaction was straightaway treated with 1.56 3HF·NEt₃ in pyridine and NEt₃.

Analytical data for compound 293: Its analytical data shall be made available in the next step.

The synthesis of 6'-O-(3,4-di-*tert*-butyldimethylsiloxycinnamoyl)-2,1'-di-O-isopropylidene sucrose **294**

Following the general procedure, OTBS-caff acid **198** (580 mg, 1.42 mmol) will give compound **294** as white solid in 39% yield.

Analytical data for compound 294: mp 100.2-

101.2°C; ¹H NMR (300 MHz, CDCl₃): TBS on

OTBS-caffeoyl group: δ -0.03, 0.00 (d, 12H, 2 x

(CH₃)₂Si), 0.70, 0.77, 0.80, 0.82 (m, 18H, 2 x

(CH₃)₃CSi); isopropylidene rings: δ 1.18, 1.22, 1.24,

1.30, (m, 12H, 2 x (CH₃)₂C); sucrose unit: δ 3.29,

3.33, (d, 1H, J = 12 Hz, H-5), 3.40, 3.44, 3.45, 3.47,

3.49, 3.50, 3.53, 3.56, 3.57, 3.64, 3.69, 3.72, 3.75

(m, 7H, H-2, H-4, 2 x H-6, 2 x H-1', H-3'), 3.96,

3.97, 4.09, 4.25 (m, 5H, H-3, H-4', H-5', 2 x H-6'), 5.94, 5.95 (d, 1H, J = 3 Hz, H-1); *trans*-alkenyl and

aromatic protons: δ 6.02, 6.07 (d, 1H, J = 15 Hz, H-a), 6.58, 6.61, 6.63 (m, 1H, H-f), 6.78, 6.80, 6.83

(m, 2H, H-c, H-g), 7.34, 7.40 (d, 1H, J = 18 Hz, H-b); ¹³C NMR (75.5 MHz, CDCl₃): δ -4.10, -4.07, -

4.05, 18.5, 18.5, 19.1, 24.2, 25.3, 25.7, 25.9, 25.9, 29.1, 36.1, 62.2, 63.0, 65.8, 68.1, 69.9, 73.1, 73.8,

79.4, 79.9, 91.0, 100.0, 102.0, 103.6, 115.1, 120.4, 121.1, 122.5, 127.9, 145.5, 147.2, 149.6, 167.4;

HRMS (ESI-positive mode): *m/z* calcd. for C₃₉H₆₅O₁₄Si₂ 813.3868; found 813.3830 [M+H]⁺

The synthesis of 6'-O-(3,4-dimethoxycinnamoyl)-2,1'-di-O-isopropylidene sucrose, 6'-diOMe **295**

Following the general procedure, diOMe acid **38** (296 mg, 1.42 mmol) will give white solid 6'-diOMe **295** in 41% yield.

Analytical data for 6'-diOMe 295: mp 88.0-89.2°C;

¹H NMR (300 MHz, CDCl₃): isopropylidene rings: δ

1.34, 1.40, 1.41 (m, 12H, 2 x (CH₃)₂C); methoxy and

sucrose unit: δ 3.38, 3.42 (d, 1H, J = 12 Hz, H-5), 3.50,

3.53, 3.55, 3.57, 3.59, 3.61, 3.63, 3.64, 23.66, 3.67 (m,

5H, H-2, 2 x H-6, 2 x H-1'), 3.73, 3.75, 3.77, 3.77, 3.79

(m, 2H, H-4, H-3'), 3.80 (s, 6H, 6 x H-h), 4.06, 4.07,

4.08, 4.09, 4.12, 4.14, 4.18, 4.21 (m, 5H, H-3, H-4',

H-5', 2 x H-6'), 6.08, 6.08 (d, 1H, J = 2.7, H-1); *trans*-

alkenyl and aromatic protons: δ 6.21, 6.27 (d, 1H, J = 18 Hz, H-a), 6.73, 6.76 (d, 1H, J = 9 Hz, H-g),

6.94, 6.95, 6.96, 6.97 6.99, 7.00 (m, 2H, H-c, H-f), 7.50, 7.56 (d, 1H, J = 18 Hz, H-b); ^{13}C NMR (75.5 MHz, CDCl_3): δ 19.2, 23.7, 24.3, 25.3, 29.1, 29.1, 36.1, 55.9, 56.0, 63.0, 63.8, 68.0, 91.0, 100.1, 102.2, 103.6, 109.6, 111.0, 115.3, 122.8, 127.3, 145.4, 149.2, 151.2, 167.4; HRMS (ESI-positive mode): m/z calcd. for $\text{C}_{29}\text{H}_{40}\text{O}_{14}\text{Na}$ 635.2316; found 635.2324 $[\text{M}+\text{Na}]^+$

The synthesis of 6'-O-(3,4,5-trimethoxycinnamoyl)-2,1'-di-O-isopropylidene sucrose, 6'-triOMe **296**

Following the general procedure, triOMe acid **210** (338 mg, 1.42 mmol) will give white solid compound **296** in 38% yield.

Analytical data for 6'-triOMe **296:** mp 75.8-76.8°C;

^1H NMR (300 MHz, CDCl_3): isopropylidene rings: δ 1.32, 1.35, 1.36, 1.38 (m, 12H, 2 x $(\text{CH}_3)_2\text{C}$); methoxy and sucrose unit: δ 3.38, 3.43 (d, 1H, J = 15 Hz, H-5), 3.50, 3.53 (m, 1H, H-1'), 3.61, 3.64, 3.67, 3.68 (m, 2H, H-2, H-1') 3.73, 3.74, 3.77, 3.78, 3.81, 3.83, 3.97 (m, 14H, H-3, H-4, 2 x H-6, H-3', 9 x H-h), 4.10, 4.12, 4.14, 4.16, 4.17, 4.20 (m, 4H, H-4', H-5', 2 x H-6'), 6.09, 6.10

6'-triOMe **296**

(d, 1H, J = 3 Hz, H-1), *trans*-alkenyl and aromatic protons: δ 6.26, 6.31 (d, 1H, J = 15 Hz, H-a), 6.64 (s, 2H, H-c, H-g), 7.48, 7.54 (d, 1H, J = 18 Hz, H-b); ^{13}C NMR (75.5 MHz, CDCl_3): δ 19.2, 24.2, 25.2, 29.0, 56.2, 61.0, 62.3, 63.7, 65.9, 66.5, 69.3, 73.4, 73.9, 78.9, 79.7, 91.0, 100.2, 102.3, 103.7, 105.3, 116.9, 129.8, 140.2, 145.5, 153.4, 153.4, 167.3; HRMS (ESI-positive mode): m/z calcd. for $\text{C}_{30}\text{H}_{43}\text{O}_{15}$ 643.2602, found 643.2625 $[\text{M}+\text{H}]^+$

8.2.16 The removal of TBS groups

The synthesis of 6'-*O*-coumaroyl-2,1'-di-*O*-isopropylidene sucrose, 6'-coum **289**

Compound **291** (200 mg, 0.293 mmol) was dissolved in pyridine (1.0 ml) and stirred at room temperature. 1.56 M 3HF·NEt₃ (563 μL, 0.879 mmol) and NEt₃ (81.8 μL, 0.586 mmol) were added to the solution and the reaction was left to stir for 12 hours. Upon completion, pyridine was removed. The crude product was then subjected to purification with column chromatography using 2:1 EtOAc/Hexane as eluent. In the end, 6'-coum **289** was obtained as white solid in 81% yield.

Analytical data for 6'-coum 289: Comparison with reported values in literature¹²⁶ confirmed the formation of 6'-coum **289**.

The synthesis of 6'-*O*-feruloyl-2,1'-di-*O*-isopropylidene sucrose, 6'-feru **290**

Compound **292** (200 mg, 0.281 mmol) was dissolved in pyridine (1.0 ml) and stirred at room temperature. 1.56 M 3HF·NEt₃ (540 μL, 0.842 mmol) and NEt₃ (78.4 μL, 0.562 mmol) were added to the solution and the reaction was left to stir for 12 hours. Upon completion, pyridine was removed. The crude product was then subjected to purification with column chromatography using 2:1 EtOAc/Hexane as eluent. In the end, 6'-feru **290** was obtained as white solid in 83% yield.

Analytical data for 6'-feru 290: Comparison with reported values in literature¹²⁶ confirmed the formation of 6'-feru **290**.

The synthesis of 6'-*O*-sinapoyl-2,1'-di-*O*-isopropylidene sucrose, 6'-sinap **297**

Mixture containing compound **293** (200 mg, 0.269 mmol) was dissolved in pyridine (1.0 ml) and stirred at room temperature. 1.56 M 3HF·NEt₃ (518 μL, 0.808 mmol) and NEt₃ (75.2 μL, 0.539 mmol) were added to the solution and the reaction was left to stir for 12 hours. Upon completion, pyridine was removed. The crude product was then subjected to purification with column chromatography using 2:1 EtOAc/Hexane as eluent. In the end, 6'-sinap **297** was obtained as white solid in 81% yield.

Analytical data for 6'-sinap **297:** mp 84.3-84.9°C; ¹H

NMR (300 MHz, (CD₃)₂CO): isopropylidene rings: δ 1.18,

1.19, 1.32 (m, 12H, 2 x (CH₃)₂C); methoxy and sucrose unit:

δ 3.34, 3.37 (d, 1H, J = 9 Hz, H-5), 3.40, 3.49 (m, 1H, H-

1'), 3.55, 3.56, 3.61 (m, 3H, H-2, H-1', H-4), 3.63, 3.64,

3.66, 3.68, 3.70 (m, 3H, 2 x H-6, H-3'), 3.76, 3.78 (d, 6H, 6

x H-h), 3.8, 3.90, 3.90. 3.91, 3.92, 3.95, 3.99, 4.01, 4.03,

4.06, 4.07, 4.10 (m, 4H, H-3, H-4', H-5', H-6'), 4.33, 4.34,

4.36, 4.38 (m, 1H, H-6'), 5.90, 5.91 (d, 1H, J - H-1), aromatic and *trans*-alkenyl protons: δ 6.27, 6.33

(d, 1H, J = 18 Hz, H-a), 6.91 (s, 2H, H-c, H-g), 7.42, 7.48 (d, 1H, J = 18 Hz, H-b); ¹³C NMR (75.5

MHz, (CD₃)₂CO): δ 19.4, 24.5, 25.5, 36.9, 56.7, 62.9, 64.1, 66.7, 67.2, 70.3, 74.5, 75.2, 78.0, 80.4, 81.1,

91.9, 99.9, 102.0, 104.9, 106.9, 115.9, 126.2, 139.5, 146.3, 149.0, 167.4; HRMS (ESI-positive mode):

m/z calcd. for C₂₉H₄₁O₁₅ 629.2445, found 629.2422 [M+H]⁺

6'-sinap **297**

The synthesis of 6'-*O*-caffeoyl-2,1'-di-*O*-isopropylidene sucrose, 6'-caff **298**

Compound **294** (200 mg, 0.246 mmol) was dissolved in pyridine (1.0 ml) and stirred at room temperature. 1.56 M 3HF·NEt₃ (0.473 μL, 0.738 mmol) and NEt₃ (68.7 μL, 0.492 mmol) were added to the solution and the reaction was left to stir for 12 hours. Upon completion, pyridine was removed. The crude product was then subjected to purification with column chromatography using 2:1 EtOAc/Hexane as eluent. In the end, 6'-caff **298** was obtained as white solid in 80% yield.

Analytical data for 6'-caff **298:** mp 96.0-97.3°C; ¹H NMR

(300 MHz, CDCl₃): isopropylidene rings: δ 1.18, 1.22,

1.24, 1.30 (m, 12H, 2 x (CH₃)₂C); sucrose unit: δ 3.29, 3.33,

(d, 1H, J = 12 Hz, H-5), 3.40, 3.44, 3.45, 3.47, 3.49, 3.50,

3.53, 3.56, 3.57, 3.64, 3.69, 3.72, 3.75 (m, 7H, H-2, H-4, 2

x H-6, 2 x H-1', H-3'), 3.96, 3.97, 4.09, 4.25 (m, 4H, H-3,

H-4', H-5', 2 x H-6'), 5.94, 5.95 (d, 1H, J = 3 Hz, H-1);

aromatic and *trans*-alkenyl protons: δ 6.02, 6.07 (d, 1H, J =

6'-caff **298**

15 Hz, H-a), 6.58, 6.61, 6.63 (m, 1H, H-f), 6.78, 6.80, 6.83 (m, 2H, H-c, H-g), 7.34, 7.40 (d, 1H, J = 18 Hz, H-b); ^{13}C NMR (75.5 MHz, MeOD): δ 23.8, 24.4, 25.6, 29.5, 29.6, 30.0, 32.8, 36.6, 50.0, 61.7, 63.1, 63.5, 64.7, 65.4, 66.9, 67.6, 68.5, 71.0, 71.6, 74.3, 74.9, 75.3, 76.8, 78.0, 80.1, 80.9, 81.2, 92.5, 93.6, 94.2, 100.9, 101.0, 102.9, 105.2, 105.7, 105.8, 115.0, 115.3, 116.6, 123.2, 127.8, 127.8, 146.9, 147.3, 147.4, 149.7, 149.8, 149.8, 169.1, 169.1, 169.2, 173.2; HRMS (ESI-positive mode): m/z calcd. for $\text{C}_{27}\text{H}_{37}\text{O}_{14}$ 585.2183, found 585.2167 $[\text{M}+\text{H}]^+$

8.2.17 The synthesis of 3-*O*-coumaroyl-3'-*O*-feruloyl-2,1'-di-*O*-isopropylidene sucrose, Hybrid 1 299

The synthesis of 3-*O*-(4-*tert*-butyldimethylsiloxycinnamoyl)-3'-*O*-(4-*tert*-butyldimethylsiloxy-3-methoxycinnamoyl)-4',6'-*O*-tetraisopropylidisilyl-2,1':4,6-di-*O*-isopropylidene sucrose 301

Compound **262** (300 mg, 0.314 mmol) and DMAP (3.8 mg, 0.0314 mmol) were dissolved in CH_2Cl_2 (5.0 ml) and stirred at room temperature. After which, DCC (130 mg, 0.628 mmol) and OTBS-coum acid **203** (175 mg, 0.628 mmol) were added and the reaction were left to stir for 24 hours. Upon completion, CH_2Cl_2 was removed and the crude product obtained was dissolved in cold diethyl ether. Subsequently, the reaction mixture was filtered. The filtrate collected was concentrated and purified using column chromatography using 8:1 Hexane/EtOAc as eluent. Compound **301** was obtained as white solid in 83% yield.

Analytical data for compound 301: mp 100.5-

101.5°C; ^1H NMR (300 MHz, CDCl_3): TBS and TIPDS groups: δ -0.02, 0.00 (m, 12H, 2 x $(\text{CH}_3)_2\text{Si}$), 0.71, 0.77, 0.78, 0.80, 0.82, 0.83, 0.84, 0.85, 0.90, 0.93, 0.95, 1.01 (m, 46H, 4 x H-a, 8 x $(\text{CH}_3)_2\text{CSi}$, 2 x $(\text{CH}_3)_3\text{CSi}$); isopropylidene rings: δ 1.18, 1.22, 1.28, 1.37 (m, 12H, 2 x $(\text{CH}_3)_2\text{C}$); methoxy and sucrose unit: δ 3.32, 3.36 (d, 1H, J = 12 Hz, H-5), 3.54, 3.57, 3.62, 3.63 (m, 2H, 2 x H-1'), 3.68, 3.72, 3.75, 3.79, 3.79, 3.83 (m, 10H, H-2, H-4, 2 x H-6, H-5', 2 x H-6', 3 x H-h'), 4.46, 4.47, 4.48 (t, 1H, J = 3 Hz, H-4'), 5.03, 5.05 (d, 1H, J = 6 Hz, H-3'), 5.11, 5.14, 5.17 (t, 1H, J

301

= 9 Hz, H-3), 5.78, 5.80 (d, 1H, J = 6 Hz, H-1); *trans*-alkenyl and aromatic protons: δ 6.03, 6.09 (d, 1H, 2 x H-a'), 6.22, 6.27 (d, 1H, J = 15 Hz, H-a''), 6.61, 6.63, 6.64, 6.67 (m, 3H, H-c', H-d'', H-f''), 6.89, 6.90 (d, 1H, J = 3 Hz, H-d'), 6.96, 6.97 (d, 1H, J = 3 Hz, H-g'), 7.18, 7.21 (d, 2H, J = 9 Hz, H-c'', H-g''), 7.34, 7.39 (d, 1H, J = 15 Hz, H-b'), 7.52, 7.57 (d, 1H, J = 15 Hz, H-b''); ^{13}C NMR (75.5 MHz,

CDCl₃): δ -5.43, -5.38, 12.5, 12.8, 13.3, 13.4, 16.9, 17.0, 17.0, 17.4, 17.5, 17.6, 18.2, 18.5, 23.9, 25.4, 25.6, 25.7, 25.7, 29.0, 64.0, 66.2, 70.8, 71.8, 78.5, 81.3, 91.2, 99.6, 101.1, 102.6, 111.4, 114.8, 115.8, 120.5, 121.00, 122.8, 128.4, 129.6, 144.4, 146.7, 147.7, 151.2, 157.8, 166.2, 166.8; HRMS (ESI-positive mode): m/z calcd. for C₆₁H₉₉O₁₇Si₄ 1215.5959, found 1215.5900 [M+H]⁺

The synthesis of 3-*O*-coumaroyl-3'-*O*-feruloyl-2,1'-di-*O*-isopropylidene sucrose, Hybrid 1 299

Compound **301** (200 mg, 0.165 mmol) was treated with 1.56 M 3HF·NEt₃ (317 μ L, 0.495 mmol) and NEt₃ (46.1 μ L, 0.330 mmol) in pyridine (1.0 ml) at room temperature for 12 hours. Upon completion, pyridine was removed, and the crude product was subjected to chromatographic purification using 3:2 EtOAc/Hexane as eluent. Hybrid 1 **299** was obtained as white solid in 85% yield.

Analytical data for Hybrid 1 299: mp 105.0-106.0°C;

¹H NMR (300 MHz, CDCl₃): isopropylidene rings: δ 1.16, 1.19, 1.21, 1.26 (m, 12H, 2 x (CH₃)₂C); methoxy and sucrose unit: δ 3.47, 3.51 (d, 1H, J = 12 Hz, H-5), 3.60, 3.64, 3.67, 3.69, 3.73, 3.76, 3.79, 3.80 (m, 4H, 2 x H-1', 2 x H-6'), 3.84, 3.87, 3.88, 3.89, 3.91, 3.92 (m, 6H, H-2, 2 x H-6, 3 x H-h'), 4.03, 4.07, 4.10, 4.12 (m, 2H, H-4, H-5), 4.74, 4.75, 4.76 (t, 1H, H-4'), 4.87, 4.89 (d, 1H, H-3'), 5.42, 5.46, 5.49 (t, 1H, H-3); H-1, *trans*-alkenyl and

Hybrid 1 299

aromatic protons: δ 6.19, 6.21, 6.24 (m, 2H, H-1, H-a'), 6.36, 6.41 (d, 1H, J = 15 Hz, H-a''), 6.76, 6.79 (d, 3H, J = 9 Hz, H-d'', H-f'', H-c'), 6.85, 6.88 (d, 1H, J = 9 Hz, H-d'), 7.16, 7.18, 7.19, 7.20 (m, 1H, H-g'), 7.34, 7.37 (d, 2H, J = 9 Hz, H-c'', H-g''), 7.49, 7.54 (d, 1H, J = 15 Hz, H-b'), 7.73, 7.79 (d, 1H, J = 18 Hz, H-b''); ¹³C NMR (75.5 MHz, CDCl₃): δ 18.4, 24.0, 25.4, 29.0, 56.1, 61.6, 64.4, 70.6, 71.4, 71.7, 72.3, 81.5, 84.9, 91.3, 99.8, 101.7, 103.9, 111.0, 113.9, 114.7, 115.3, 115.9, 123.4, 126.8, 127.1, 130.0, 144.6, 144.8, 147.7, 148.4, 157.9, 166.3, 168.5; HRMS (ESI-positive mode): m/z calcd. for C₃₇H₄₄O₁₆Na 767.2567, found 767.2548 [M+Na]⁺

8.2.18 The synthesis of 3-*O*-coumaroyl-3',4'-di-*O*-feruloyl-2,1'-di-*O*-isopropylidene sucrose, Hybrid 2 300

The synthesis of 3',4'-di-*O*-(4-*tert*-butyldimethylsiloxy-3-methoxycinnamoyl)-2,1'-di-*O*-isopropylidene sucrose 302

6'-*O*-TBS **214** (200 mg, 0.373 mmol) and DMAP (4.6 mg, 0.0373 mmol) were dissolved in CH₂Cl₂ (5.0 ml) and stirred at room temperature. After which, DCC (192 mg, 0.932 mmol) and OTBS-feru acid **234** (287 mg, 0.932 mmol) were added and the reaction were left to stir for 24 hours. Upon completion, CH₂Cl₂ was removed and the crude product obtained was dissolved in cold diethyl ether. Subsequently, the reaction mixture was filtered. The filtrate collected was concentrated and purified using column chromatography using 4:1 Hexane/EtOAc as eluent. Compound **302** was obtained in 81% yield.

Analytical data for compound 302: mp 129.1-131.2°C; ¹H NMR (300 MHz, CDCl₃): TBS groups: δ -0.09 (s, 6H, (CH₃)₂Si), 0.71 (s, 9H, (CH₃)₃CSi); TBS on OTBS-feruloyl groups: δ -0.01, 0.00, 0.05 (m, 12H, 2 x (CH₃)₂Si), 0.82 (s, 18H, 2 x (CH₃)₃CSi); isopropylidene rings: δ 1.14, 1.20, 1.33, 1.34, 1.42 (m, 12H, 2 x (CH₃)₂C); methoxy and sucrose unit: δ 3.42, 3.46, 3.55 (m, 3H, H-2, H-5, H-1'), 3.65, 3.66, 3.69 (m, 12H, H-4, 2 x H-6, H-1', 2 x H-6', 3 x H-h, 3 x H-h'), 3.94, 3.98 (d, 1H, J = 12 Hz, H-3), 4.03, 4.05, 4.07, 4.10 (m, 1H, H-5'), 5.08, 5.10 (d, 1H, J = 6 Hz, H-3'), 5.46, 5.47, 5.48 (t, 1H, J = 3 Hz, H-4'), 5.88, 5.89 (d, 1H, J = 3 Hz, H-1); *trans*-alkenyl and aromatic protons: δ 6.07, 6.13 (d, 1H, J = 18 Hz, H-a), 6.20, 6.26 (d, 1H, J = 18 Hz, H-a'), 6.65, 6.66, 6.68, 6.69 (m, 3H, H-c, H-d, H-c'), 6.83, 6.84, 6.85, 6.88, 6.92 (m, 3H, H-d', H-g, H-g'), 7.42, 7.48 (d, 1H, J = 18 Hz, H-b), 7.52, 7.57 (d, 1H, H = 15 Hz, H-b'); ¹³C NMR (75.5 MHz, CDCl₃): δ -5.33, -5.32, -4.62, -4.61, -4.59, 18.3, 18.5, 18.5, 19.1, 24.7, 25.5, 25.6, 25.7, 25.9, 31.0, 34.9, 55.4, 55.5, 99.7, 101.6, 104.4, 121.1, 121.2, 122.4, 128.0, 128.1, 147.8, 147.9, 151.3, 165.8, 166.1; HRMS (ESI-positive mode): *m/z* calcd. for C₅₆H₈₉O₁₇Si₃ 1117.5408, found 1117.5414 [M+H]⁺

The synthesis of 3-*O*-(4-*tert*-butyldimethylsiloxycinnamoyl)-3',4'-di-*O*-(4-*tert*-butyldimethylsiloxy-3-methoxycinnamoyl)-2,1'-di-*O*-isopropylidene sucrose **303**

Compound **302** (150 mg, 0.150 mmol) and DMAP (1.8, 0.0150 mmol) were dissolved in CH₂Cl₂ (5.0 ml) and stirred at room temperature. After which, DCC (61.8 mg, 0.300 mmol) and OTBS-coum acid **203** (83.4 mg, 0.300 mmol) were added and the reaction were left to stir for 24 hours. Upon completion, CH₂Cl₂ was removed and the crude product obtained was dissolved in cold diethyl ether. Subsequently, the reaction mixture was filtered. The filtrate collected was concentrated and purified using column chromatography using 8:1 Hexane/EtOAc as eluent. Compound **303** was obtained in 81% yield.

Analytical data for compound 303: mp 105.0-106.0°C; ¹H NMR (300 MHz, CDCl₃): TBS groups: δ 0.00, 0.09, 0.10, 0.14, 0.15 (m, 24H, 4 x (CH₃)₂Si), 0.81, 0.84, 0.92, 0.92 (m, 36H, (CH₃)₃CSi); isopropylidene rings: δ 1.15, 1.18, 1.38 (m, 12H, 2 x (CH₃)₂C); methoxy and sucrose unit: δ 3.50, 3.52 (d, 1H, J = 6 Hz, H-5), 3.64, 3.67 (m, 2H, 2 x H-1'), 3.76, 3.78 (d, 6H, J = 6 Hz, 3 x H-h, 3 x H-h'), 3.81, 3.82, 3.84, 3.86, 3.87 (m, 5H, H-2, 2 x H-6, 2 x H-6'), 4.07, 4.09, 4.15 (m, 2H, H-4, H-5'), 5.22, 5.24 (d, 1H, J = 6 Hz, H-3'), 5.30, 5.31, 5.32 (t, 1H, J = 3 Hz, H-3), 5.57, 5.58, 5.59 (t, 1H, J = 3 Hz, H-4'), 6.03, 6.05 (d, 1H, J = 6 Hz, H-1), *trans*-alkenyl and aromatic protons: δ 6.19, 6.24 (d, 2H, J = 15 Hz, H-a, H-a''), 6.37, 6.42 (d, 1H, J = 15 Hz, H-a'), 6.76, 6.78, 6.78 (m, 4H, H-c, H-c', H-d'', H-f''), 6.93, 6.94, 6.95, 6.96 (m, 2H, H-d, H-d'), 7.01, 7.03 (m, 2H, H-g, H-g'), 7.12, 7.12 (d, 2H, H-c'', H-g''), 7.49, 7.52 (d, 1H, J = 9 Hz, H-a), 7.55, 7.58 (d, 1H, J = 9 Hz, H-a''), 7.73, 7.78 (d, 1H, J = 15 Hz, H-a'); ¹³C NMR (75.5 MHz, CDCl₃): δ -5.32, -4.61, -4.59, -4.39, -4.35, 18.3, 18.3, 18.5, 18.5, 19.0, 25.5, 25.6, 25.7, 25.7, 25.9, 28.9, 55.4, 70.8, 99.6, 101.3, 104.5, 111.7, 114.6, 120.5, 121.1, 122.4, 127.8, 128.2, 128.5, 129.6, 144.4, 147.5, 151.1, 151.2, 165.9, 166.1, 166.5; HRMS (ESI-positive mode): *m/z* calcd. for C₇₁H₁₀₉O₁₉Si₄ 1377.6640, found 1377.6660 [M+H]⁺

The synthesis of 3-*O*-coumaroyl-3',4'-di-*O*-feruloyl-2,1'-di-*O*-isopropylidene sucrose, Hybrid 2 **300**

Compound **303** (100 mg, 0.0726 mmol) was treated with 1.56 M 3HF·NEt₃ in pyridine (139 μL, 0.218 mmol) and NEt₃ (20.3 μL, 0.145 mmol) in pyridine (1.0 ml) at room temperature. The reaction was then left to stir for 12 hours. Upon completion, pyridine was removed, and the crude product was purified with column chromatography using 3:2 EtOAc/Hexane as eluent. Hybrid 2 **300** was obtained in 86% yield.

Analytical data for Hybrid 2 300: mp 156.0-157.0°C; ^1H NMR (300 MHz, CDCl_3): isopropylidene rings: δ 1.16, 1.17, 1.36, 1.37 (m, 12H, 2 x $(\text{CH}_3)_2\text{C}$); methoxy and sucrose unit: δ 3.49, 3.53 (d, 1H, $J = 12$ Hz, H-5), 3.61, 3.64, 3.67, 3.70 (m, 3H, 2 x H-1', H-6'), 3.83, 3.84, 3.86, 3.76, 3.88, 3.89 (m, 9H, 2 x H-6, H-6', 3 x H-h, 3 x H-h'), 4.07, 4.10, 4.15, 4.16 (m, 3H, H-2, H-4, H-5'), 5.29, 5.34, 5.36 (m, 2H, H-3, H-3'), 5.69, 5.71, 5.73 (t, 1H, $J = 6$ Hz, H-4'); H-1, aromatic and *trans*-alkenyl protons: δ 6.13, 6.16, 6.16, 6.18, 6.19, 6.21 (m, 3H, H-1, H-a, H-a''), 6.33, 6.39 (d, 1H, H-a'), 6.72, 6.75, 6.78, 6.80, 6.82, 6.83 (m, 3H, H-c, H-c', H-d''), 6.93, 6.96, 6.99 (m, 2H, H-g'', H-d), 7.05, 7.08, 7.11, 7.16 (m, 3H, H-d', H-g, H-g'), 7.29, 7.32 (d, 2H, $J = 9$ Hz, H-c'', H-g''), 7.44, 7.49 (d, 1H, $J = 15$ Hz, H-b), 7.51, 7.57 (d, 1H, $J = 18$ Hz, H-b''), 7.69, 7.75 (d, 1H, $J = 18$ Hz, H-b'); ^{13}C NMR (75.5 MHz, CDCl_3): δ 19.0, 24.0, 25.4, 25.6, 28.8, 30.9, 56.0, 56.0, 64.5, 71.4, 71.6, 75.1, 76.6, 91.6, 99.8, 101.7, 104.0, 109.5, 110.3, 114.1, 114.6, 114.8, 115.9, 123.5, 123.8, 126.7, 127.0, 130.0, 146.5, 146.7, 146.8, 148.2, 148.3; HRMS (ESI-positive mode): m/z calcd. for $\text{C}_{47}\text{H}_{52}\text{O}_{19}\text{Na}$ 943.3000, found 943.3002 $[\text{M}+\text{Na}]^+$

Hybrid 2 300

8.3 *In vitro* enzyme assays studies

After the successful synthesis of a library of PSEs, these compounds were screened for their inhibitory activities against both α -glucosidase and α -amylase using *in vitro* enzyme assays. Details of the enzyme assays are discussed in the following subsections.

8.3.1 General

α -Glucosidase from *Saccharomyces cerevisiae* (Sigma Aldrich, G5003) and α -Amylase from porcine pancreas (Sigma Aldrich, A3176) were used in all the *in vitro* enzyme assays. Other commercial materials used in the enzyme assays were also purchased from Sigma-Aldrich and used directly unless otherwise stated.

Prior to the assay, buffer solutions were prepared. 1 M sodium phosphate buffer stock solutions at pH 6.8 and pH 7.0 were prepared with the following calculations (Table 27)

Table 27: Preparation of buffer stock solutions

Stock solution \ Volume (mL)	1 M Na ₂ HPO ₄	1 M NaH ₂ PO ₄
1 M sodium phosphate buffer solution, pH 7.0	57.7	42.3
1 M sodium phosphate buffer solution, pH 6.8	46.3	53.8

These stock solutions were then stored in fridge (up to 2 weeks).

From the 1 M sodium phosphate buffer stock solution pH 7.0, 0.1 M sodium phosphate buffer solution pH 7.0 was prepared with a dilution factor of 10 using deionised water. Similarly, 0.05 M sodium phosphate buffer solution pH 6.8 was prepared from its stock solution with a dilution factor of 20 using deionised water. All buffer solutions were stored in the fridge (up to 4 to 5 days) and warmed to 37°C prior to use.

Next, 3,5-dinitro salicylic acid (DNSA) reagent was prepared by dissolving 1.00 g of 3,5-dinitro salicylic acid into 50.0 mL of deionised water. A clear yellow solution was obtained. After which, 30.0 g of sodium potassium tartrate was added slowly to the solution and a milky yellow solution resulted. 20.0 mL of 2N NaOH was then added to the milky yellow solution and the solution was heated gently at 40°C to ensure all solids were dissolved. Finally, the total volume was made up to 100.0 mL with deionised water. DNSA reagent was then stored in fridge (up to 4-5 days) and warmed to 37°C prior to use.

Finally, 0.5% starch solution was prepared by dissolving 0.500 g of starch in 99.5 mL of 0.05 sodium phosphate buffer solution pH 6.8. The solution was heated at 80°C till all starch were completely dissolved. The solution was then left to cool down to 37°C before use.

8.3.2 α -Glucosidase inhibition assay

The procedure employed for testing of α -glucose inhibitory activity is a modified version of the protocol reported by Kang and coworkers¹⁵⁶, and adopted by Dr Surabhi Devaraj⁸¹. 8 μ L of tested compound (dissolved in DMSO), corresponding to 50 μ g/ml final concentration of tested compound, was added to 115 μ L of 0.1 M sodium phosphate buffer pH 7.0 in a 96 well microtiter plate. After which, 50 μ L of α -glucosidase enzyme solution (0.5 U/ml of yeast α -glucosidase in the 0.1 M buffer solution pH 7.0) was added to the mixture. 'Blank' and 'Positive Control' were included in the test. For both, no tested compound (PSE) was added. Instead, 8 μ L of DMSO was added to equalize the volume. In addition,

50 µL of buffer was added instead of the enzyme solution for 'Blank'. Acarbose was used as the standard in this test procedure.

The microtiter plate was shaken at 37°C for 15 minutes. Next, 25 µL of 2.5 mM of 4-nitrophenyl α-D-glucopyranoside (PNPG) substrate (dissolved in the 0.1 M buffer solution pH 7.0) was added to each well. The entire plate was then shaken again at 37 °C for another 15 minutes before measuring absorbance at 405 nm using a microplate reader. The percentage of inhibition is calculated using the following formula:

$$\left(\frac{(Abs_{positive\ control} - Abs_{compound})}{Abs_{positive\ control}} \right) \times 100 \%$$

All measurements were performed in triplicates and the values are represented as mean ± standard deviation.

8.3.3 α-Amylase inhibition assay

The procedure for testing α-amylase inhibitory activity was a modified version of that demonstrated by Phan and colleagues¹⁵⁷, also adapted by Dr Surabhi Devaraj⁸¹. 50 µL of tested compound (dissolved in DMSO), corresponding to a final concentration of 50 µg/ml of tested compound, was added to each test tube. After which, 100 µL of α-amylase (5U/ml of porcine pancreatic α-amylase in 0.05 sodium phosphate buffer pH 6.8) and 460 µL of 0.05 M sodium phosphate buffer pH 6.8 were added respectively. Like α-glucosidase test, both 'Blank' and 'Positive Control' were included in the test. For both, 50 µL of DMSO was added instead of test compound (PSE). In addition, 100 µL of buffer was added instead of the enzyme solution for 'Blank'. Acarbose was also used as a standard in this test.

The test tubes were then left to shake at 37°C for 10 minutes. Subsequently, 450 µL of 0.5% starch solution was added to each tube and all the tubes were left to shake at 37°C for another 20 minutes. Finally, 500 µL of DNSA reagent was added to each tube and the tubes were placed in a boiling water bath for 15 minutes. The absorbance of each tube was recorded at 540 nm and the percentage inhibition is calculated based on the same formula as shown in α-glucosidase enzyme assay.

$$\left(\frac{(Abs_{positive\ control} - Abs_{compound})}{Abs_{positive\ control}} \right) \times 100 \%$$

All measurements were performed in triplicates and the values are represented as mean ± standard deviation.

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10 Appendix

COSY

Note: only the correlation between protons at C-3' and C-4' is highlighted

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COSY

Note: only the correlation between protons at C-3' and C-4' is highlighted

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Note: only the correlation between protons at C-3' and C-4' is highlighted

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Note: only the correlation between protons at C-3' and C-4' is highlighted

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