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Methods Development for the Glycosylation and Microarraying of L-Rhamnose with Applications Toward the Automated Solution-Phase Synthesis of Homogalacturonan and Rhamnogalacturonan I

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Methods development for the glycosylation and microarraying of L-rhamnose with applications toward the automated solution-phase synthesis of Homogalacturonan and Rhamnogalacturonan I

By

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LIST OF ABBREVIATIONS

AcOH	Acetic acid
Ac_2O	Acetic anhydride
AIBN	2,2'-Azobisisobutyronitrile
BAIB	[bis(acetoxy)iodo] benzene
$BF_3.OEt_2$	Boron trifluoride etherate
BSA	Bovine serum albumin
BSP	1-Benzenesulfinyl piperidine
CAN	Ceric ammonium nitrate
DCM	Dichloromethane
DMF	N, N-Dimethylformamide
DTBMP	2,6-di-*tert*-butyl-4-methylpyrimidine
EtOAc	Ethyl Acetate
FITC-ConA	Fluorescein isothiocyanate concanavalin A
F-tag	Fluorous tag
FSPE	Fluorous Solid-Phase Extraction
Gal	Galactose
GalA	Galacturonic acid
HOBT	Hydroxybenzotriazole
HPLC	High performance liquid chromatography
Man	Mannose
MeCN	Acetonitrile
MeOH	Methanol
NMR	Nuclear magnetic resonance
OMp	–O-*para*-methoxyphenyl
PNB	*para*-nitrobenzoyl
pTSA	*para*-Toluenesulfonic acid
Rha	Rhamnose
RG	Rhamnogalacturonan
TBAB	Tetrabutylammonium bromide

TCA	Trichloroacetimidate
TEA	Triethylamine
TEMPO	2,2,6,6-tetramethyl-1-piperidinyloxyl
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TMSOTf	Trimethylsilyl trifluoromethanesulfonate
TTBP	2, 4, 6-Tri-t*ert*-butylpyrimidine
Tf_2O	Triflic anhydride
TfOH	Triflic acid

CHAPTER 1

Overview of Glycosylation Reactions, Automated Oligosaccharide Synthesis, and Fluorous-Tag Based Microarray Formation

Sahana K. Nagappayya and Nicola L. B. Pohl

Dissertation organization

This dissertation consists of six chapters. Chapter 1 is an overview of the automated solution phase synthesis of oligosaccharides and an introduction to carbohydrate microarrays. Some portions of the microarray preparations have been published in a chapter titled 'Production of fluorous-based microarrays with uncharged carbohydrates' in *Carbohydrate Microarrays: Methods and Protocols*[1]. Chapter 2 describes an approach to synthesizing anomerically-pure α or β–linkages in rhamnopyranosides using a protecting group strategy to direct the glycosylation. Various electron-withdrawing protecting groups are strategically placed at the *O*-3 and *O*-4 of L-rhamnose and the glycosylation of such building blocks with a standard fluorous-tag containing L-rhamnose is performed. Chapter 3 discusses the first automated solution-phase synthesis of fragments of the plant polysaccharide homogalacturonan (HG). The galacturonic acid residues required for the backbone synthesis are derived from galactose; a hexasaccharide of α-linked galacturonic acid is synthesized using the automated solution-phase platform. Chapter 4 describes the development of methods for the synthesis of the plant polysaccharide rhamnogalacturonan – I (RG-I) backbone on the automated platform. α-Linked D-galacturonic acid was glycosylated with a fluorous-tag containing L-rhamnose

[1] Nagappayya, S. K.; Pohl, N. L. B. *Methods in Molecular Biology*, Humana Press **2012**, *88*, 149-155

building block to synthesize the backbone of RG-I. These plant polysaccharide backbones constructed on the automated solution-phase synthesis platform are the first reports of the automated synthesis of both HG and RG-I fragments. Chapter 5 describes the development of a hexyl fluorous tag, which was used for the microarraying of sugars and also describes a comparative study of this hexyl fluorous di-tag with the traditional mono-octylfluorous tag. Parts of this chapter are published in a report in *Chemical Communications* [2]. Chapter 6 is the conclusion of this dissertation and also discusses the future direction of the studies and experiments described thus far.

Glycosylation reactions of carbohydrates

Since the first synthesis of monosaccharides in the late 1800s by Emil Fischer (Fischer, 1902) carbohydrate synthesis methods and their structure/function studies have not developed as quickly as those of proteins and nucleic acids. It is now commonly known that carbohydrates play a crucial role in the development, growth, function, and survival of living cells, and also in cell-cell interactions, especially between a pathogen and its host cells (Varki, 1993). As more information is now being found on the importance of carbohydrates, interest in obtaining pure oligosaccharides is increasing. The task of synthesizing even a fully protected monosaccharide requires several chemical transformations as compared to a nucleic acid or protein building block. There are two important challenges in obtaining pure well-characterized oligosaccharides. First, the hydroxyl functional groups on a monosaccharide display similar reactivities and therefore the synthesis of a building block ready for glycosylation requires a number of functional

[2] Edwards, H. D.; Nagappayya, S. K.; Pohl, N. L. B. *Chem. Comm.* **2012**, *48*, 510-512

group transformations and protection/deprotection strategies. The other challenge in making oligosaccharides is the stereochemistry at the anomeric center. Synthesizing a pure 1,2-*cis* or 1,2-*trans*-linkage is non-trivial and a number of factors have to be taken into account, including protecting groups, solvent conditions, promoters, thermodynamic and kinetic factors and the leaving groups (Davis, 2000).

A typical sugar molecule in its pyranose or furanose form contains multiple hydroxyl groups and to design a monosaccharide building block and chemically synthesize an anomerically-pure carbohydrate linkage, various protecting group transformations have to be performed. A glycoside bond is formed between a glycosyl donor, generally an electrophile, and a glycosyl acceptor, generally a nucleophile that contains a free hydroxyl group (Fig. 1-1). As shown in the figure, the glycosyl donor contains a leaving group or a group that can be converted into a leaving group under the reaction conditions, and upon activation by a promoter, a glycosidic bond is formed between the donor and the acceptor. Even after strategically designing the building blocks to obtain pure α or β linkages at the anomeric position, there is often still some undesired anomeric product obtained. With certain oligosaccharides, some anomeric linkages are extremely challenging to synthesize due to the anomeric effect and the resulting thermodynamic stability of one linkage over the other. A glycosylation reaction is a particularly sensitive reaction. Over time, many researchers have developed a variety of methods for high-yielding and strereochemically-pure glycosylations with various building blocks, protection group schemes, solvents, and promoters (Schmidt, 1986; Zhu et al, 2008). In addition to challenges in the synthesis of oligosaccharides, the purification and characterization of an oligosaccharide with multiple sugars and various anomeric

linkages is also very challenging. The synthetic challenges of carbohydrate chemistry, especially building block synthesis and the rapid assembly of these building blocks, are a major limitation of this field.

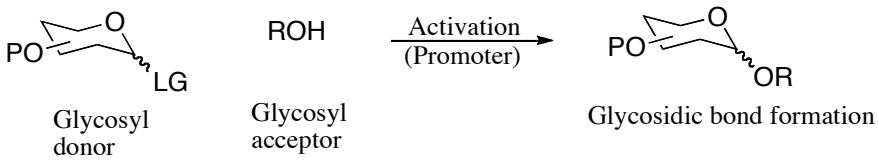


Figure 1-1. Glycosidic bond formation

One of the most popular methods of glycosylation is the use of trichloroacetimidate donors (Schmidt, 1986). This method was first developed by Schmidt and Michel and therefore is also called the Schmidt glycosylation method (Schmidt et al, 1980). The anomeric hydroxyl of a sugar is reacted with trichloroacetonitrile under basic conditions to obtain the trichloroacetimidate donor of the carbohydrate. This donor is reacted with an acceptor in the presence of an appropriate promoter under acidic conditions to obtain the glycosidic bond. The streochemical outcome at the anomeric center is guided by a number of factors like the stereochemistry at the anomeric center of the trichloroacetimidate, neighboring group participation primarily by the protecting group at C-2 position, solvents, thermodynamic and kinetic effects (Schmidt, 1986).

In this dissertation, all the glycosylation reactions discussed have been carried out using a tricholoroacetimidate donor given its broad utility. Chapter 2 discusses the stereochemical outcome of the glycosylation reaction of L-rhamnose to investigate the effect of electron-withdrawing groups farther away from the anomeric center on the α/β

ratio of the glycosylated product. In Chapter 3 and 4, glycosylations with trichloroacetimidate donors are demonstrated on an automated solution-phase synthesis platform.

Automated solution-phase oligosaccharide synthesis

As noted above, the chemical synthesis of oligosaccharides is challenging due to the stereochemical outcome at the anomeric center that can affect the reactivity as well as the bioactivity of the synthesized oligosaccharides (Zhang et al, 2008). Many of these challenges can be tackled by choosing a protecting group that can direct the linkage between two sugars by neighboring group participation; also the choice of the glycosyl donor can sometimes decide the stereochemical outcome of the glycosylation reaction (Schmidt et al, 1980). The synthesis of building blocks is one of the challenges in oligosaccharide synthesis. Once fully-protected glycosyl donor and acceptors are synthesized, the difficulty in the glycosylation reaction and building up the oligosaccharide chain are also many and this process can be lengthy and require up to months to construct an oligosaccharide chain, depending on the number of monomer units.

To ease this problem of the synthesis of polysaccharide chains, there is much interest in the development of synthesis platforms that can automate the assembly of oligosaccharide chains as proposed by Bruce Merrifield in his Nobel address (Merrifield, 1985). Seeberger and co-workers reported the first example of an automated solid-phase oligosaccharide synthesis platform, which was comparable to automated nucleic acid and peptide synthesis (Plante et al, 2001). This platform is a modified peptide synthesizer that

delivers suitable reagents to the reactor vial where the oligosaccharide chain is built up on a solid resin. Once all the sugars have been added in a stepwise manner and the reaction is complete; the final oligosaccharide is cleaved from the resin. Although the method should in theory simplify the assembly of sugar chains, in practice it has a number of disadvantages including the use of large excesses of building blocks (~5 to 20 equivalents) for each coupling cycle. Furthermore, there is no simple way of monitoring the reaction and identifying mistakes during the coupling cycle (Seeberger, 2008).

To overcome some of these issues with oligosaccharide synthesis and to find a more efficient method of carbohydrate synthesis, an automated solution-phase oligosaccharide synthesizer was developed (Pohl, 2008b). The advantages of the solution-phase oligosaccharide synthesis method are that lower numbers of equivalents of donors can be used (1.5 to 3.5 equivalents) and that the reaction can be monitored at each step of the synthesis. A small amount of sample can be withdrawn by the robotic arm and transferred to a vial after each coupling and deprotection reaction and this aliquot can be monitored by thin layer chromatography (TLC) or mass spectrometry (MS), ensuring that the reaction proceeds correctly at each step. This new automated solution-phase oligosaccharide synthesis strategy is based on fluorous-tag affinity purification steps. The lengthening oligosaccharide chain is built upon a fluorous handle and can be easily purified in each step using Fluorous Solid-Phase Extraction (FSPE) (Curran, 2001). The fluorous tag can be attached to the anomeric or other position of a carbohydrate and the chain can be grown from that sugar, as shown in Fig. 1-2. The tag is designed to be orthogonal to the protection and deprotection strategies used for oligosaccharide synthesis and to be stable under the automation cycles. After the desired length of

oligosaccharide is achieved, the fluorous tag can be easily cleaved from the chain or kept for use in fluorous-based microarray formation of the synthesized oligosaccharide as described in the next section.

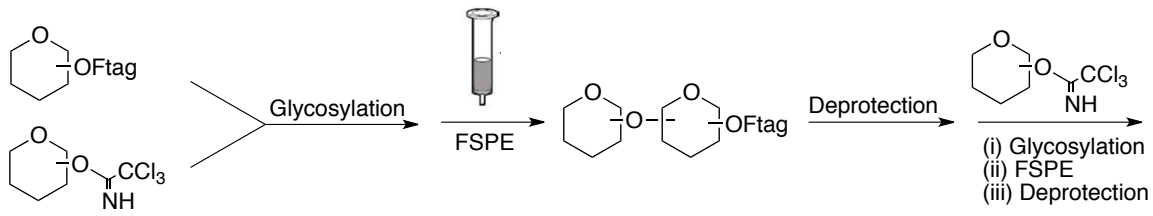


Figure 1-2. Schematic of automated solution phase synthesis using FSPE.

Fluorous-based carbohydrate microarrays

Carbohydrate microarrays are a facile method to qualitatively and quantitatively study carbohydrate binding with various binding partners like proteins and antibodies (Pohl, 2008a). Given the difficulty in obtaining large amounts of pure oligosaccharides from either synthesis or isolation from natural sources, microarray platforms are a particularly good method to study the interaction of these saccharides with other molecules, as only a milligram of sugar can be used to form dozens of microarray spots.

Whereas most microarray platforms require covalent attachment of the analyte to the slide, an alternate non-covalent approach relies on the hydrophobic or "fluorophilic" interactions of a fluorocarbon- tagged compound with a perfluoroalkylsilane-coated glass slide surface. Such fluorous-based microarrays have seen use for probing carbohydrates as well as non-carbohydrate molecules (Nicholson et al, 2007; Vegas et al, 2008). In the case of carbohydrates, a fluorous-tagged carbohydrate obtained through the direct

reaction of the reducing end of an unmodified sugar with a tag (Chen et al, 2007) or through synthesis in which the fluorous tag aids the purification steps throughout (Jaipuri et al, 2008) is spotted onto the fluorous glass slide using a standard robot used for DNA arraying (Fig. 1-3). The fluorous tail of the carbohydrate aligns itself onto the glass in a humidified chamber and then the slide is incubated with a protein of interest, for example the jack bean lectin concanavalin A (ConA) labeled with fluorescein isothiocyanate (FITC) (Jaipuri et al, 2008; Ko et al, 2005). After the slide is washed to remove nonbinding proteins, the binding of FITC- ConA with -mannose is then observed under a standard fluorescence slide scanner (Jaipuri et al, 2008; Ko et al, 2005).

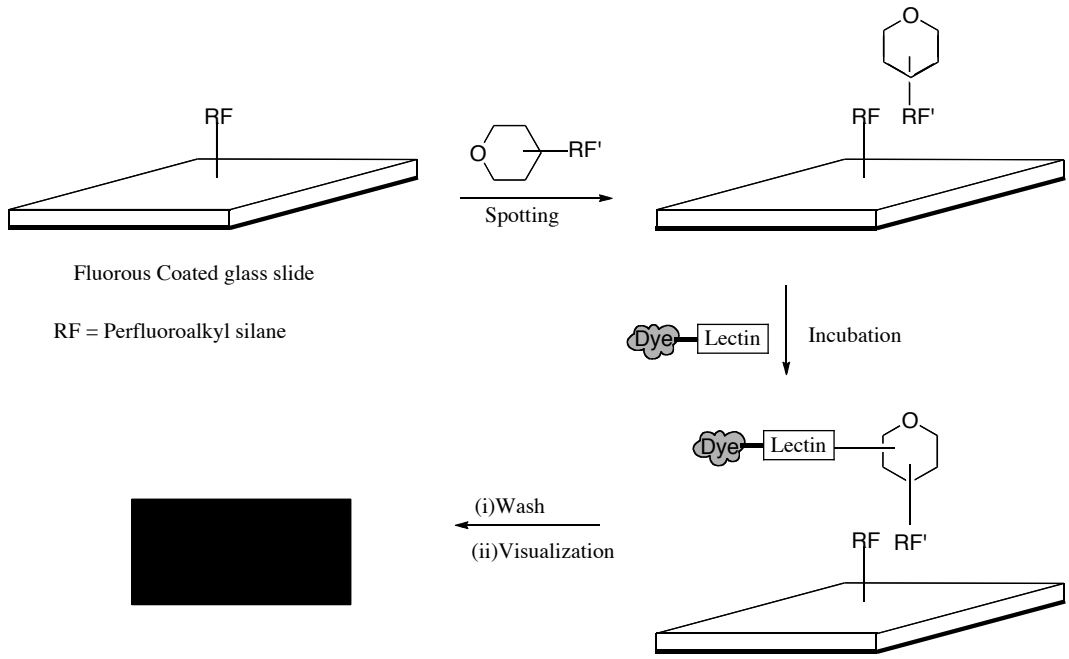


Figure 1-3. Schematic of the work flow for fluorous-based carbohydrate microarray production and screening.

The fluorous-tag containing oligosaccharide obtained after the automated solution-phase synthesis, can be deprotected and the resulting carbohydrate can be used for

microarraying on a fluorous coated glass slide to test the recognition of these sugars with plant lectins or other proteins of interest. The fully deprotected fluorous-tagged oligosaccharide is immobilized on the fluorous-coated glass slide as shown in Fig. 1-4. The spots can then be incubated with the protein of interest, washed and visualized. Useful information about carbohydrate-protein interaction can thus be obtained using fluorous-based microarray. Since minimum amounts of oligosaccharide are required for microarray formation, the multi-milligram-scale synthesis on the automated platform yields sufficient material for a number of arrays. C_8F_{17}- containing oligosaccharides are shown to be efficient for immobilization on the fluorous-coated glass slides for good microarray formation (Jaipuri et al, 2008). More recently, a tag containing two C_6F_{13} moieties has been developed that could be applied to the automated solution-phase synthesis of longer oligosaccharide chains. Microarrays could be performed with these di-C_6F_{13}-tagged sugars to obtain information about carbohydrate-protein binding. The development of this di-C_6F_{13}-tag is discussed more in Chapter 5.

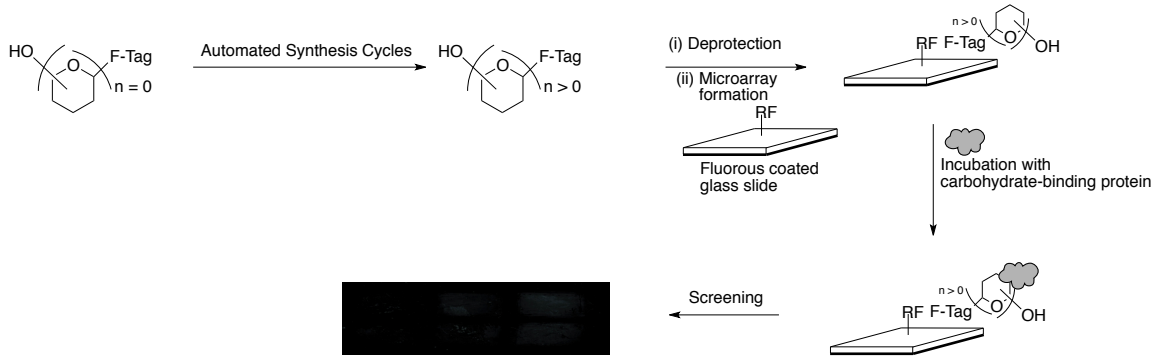


Figure 1-4. Automated synthesis of carbohydrates and direct microarray formation of the fluorous-tagged carbohydrate on the flurous coated glass slide for screening.

CHAPTER 2

Effect of Electron-Withdrawing Groups at O-3 and O-4 Positions of L-Rhamnose Trichloroacetimidate Donors for Rhamnopyranosylation

Sahana K. Nagappayya and Nicola L. B. Pohl*

Portions of this chapter will be submitted to *Journal of American Chemical Society*

Abstract

A study of the effect of the α/β- directing ability of various electron-withdrawing groups on the glycosylation of L-rhamnose trichloroacetimidate donors with a fluorous-taggged rhamnose acceptor is reported. The effect of the electron-withdrawing groups at O-3 and O-4 positions of L-rhamnose is studied to get insight into the mechanism of glycosylation under standard conditions with trichloroacetimidate (TCA) donors for the deoxy sugar L-rhamnose. Both participating and non-participating electron-withdrawing groups are used in this study and a plausible mechanism is proposed.

Introduction

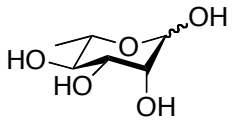
Carbohydrates play important roles in many biological processes in both plants and animals including growth, development and recognition of the cells (Varki, 1993). Development of carbohydrate-based synthetic drug (Petitou et al, 2004) has stimulated more interest in glycoconjugates. Research on carbohydrate-based therapeutics has accelerated in the recent past with carbohydrate inspired drugs like Tamiflu (Weizman, 2003) and heparin, to name a few. The array of glycans found on the cell surface in the form of glycolipids and glycoproteins, modulate cell functions like transcriptional regulation, proliferation and cell migration (Seeberger et al, 2005). Carbohydrates also

play an important role in the development and function of the nervous system (Murrey et al, 2008). Carbohydrate-based vaccines are slowly and steadily gaining importance and the advances in the synthesis of carbohydrate units have helped in such developments (Hakomori, 2001). Glycochemistry and glycoconjugate research are increasingly gaining more and more importance, especially in medicinal chemistry.

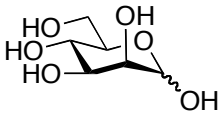
To chemically synthesize a carbohydrate-based therapeutic, one of the most important aspects to take into account is the formation of stereoselective glycosidic linkages. Many attempts have been carried out to synthesize stereospecific glycosidic linkages using various strategies like synthesis of reactive donors or acceptors, use of various promoters and activators and changing glycosylation conditions (Boons, 1996).

Rhamnose is one of the very few naturally available deoxy-sugars. It is also unique as it is one of the very few sugars naturally occurring in L- form. Chemically, L-rhamnose is 6-deoxy-L-mannose (Fig. 2-1). It is found on the lipopolysaccharides of Gram-negative bacteria (Lindberg et al, 1972), and also is a component of many plant sugars including pectin rhamnogalacturonans (Willats et al, 2001) and muricatin A, which has anti-bacterial activity (Khanna et al, 1967). L-rhamnose also occurs in many other natural products like buckthorn (Seebeck et al, 1946). Rhamnose containing oligosaccharides are widely present in immunogenic glycans and lipopolysaccharides and are often the immune-determinant group of the immunogen (Zhang et al, 2001). Recently, high levels of antibodies against L-rhamnose have been discovered in human cells. Due to the ubiquitous presence of L-rhamnose in nature and the discovery of anti-rhamnose

antibodies in humans, L-rhamnose could act as a potential replacement for the α-galactose epitope in cancer therapeutics (Chen et al, 2011).



L-Rhamnose



D-Mannose

Figure 2-1. Structures of L-rhamnose and D-mannose

Although the α-L-rhamnosidic linkage is more common, β-rhamnosidic linkage is also found in nature (Perry et al, 1990). The antigen with β-rhamnosidic linkage was found to be a more efficient inhibitor for the binding of serum to *Salmonella* serogroup lipopolysaccharide than the antigen containing α-rhamnosidic linkage (Chernyak et al, 1993). The 1, 2-*cis* (β) and 1, 2-*trans* (α) linkages in L-rhamnose are shown in Fig 2-2. The importance and abundance of L-rhamnose in the biological systems probe the importance of the study of the chemistry of rhamnose. The stereochemical outcome of glycosylation reactions is very important for the application of these saccharides in any biological systems. Chemical methods to provide exclusive α or β linkages can to be great tools for the synthesis of oligosaccharides important for glycobiological studies as well as for the study of the chemistry of rhamnose.

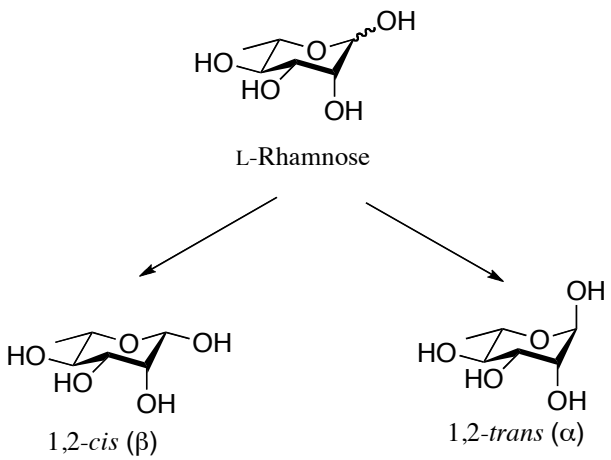


Figure 2-2. 1, 2-*cis* (β) and 1, 2-*trans* (α) linkages in L-rhamnose

The formation of 1, 2- *cis*- glycosidic linkages in L-rhamnose a very challenging problem and is similar to the problem of forming β-D-mannopyranosides (Paulsen, 1982). The traditional method of obtaining a β-linkage is by employing a neighboring participating acyl group at the C-2 position. But neighboring group participation predominantly yields α- product in the case of L-rhamnose and D-mannose (Fig. **2-3**).

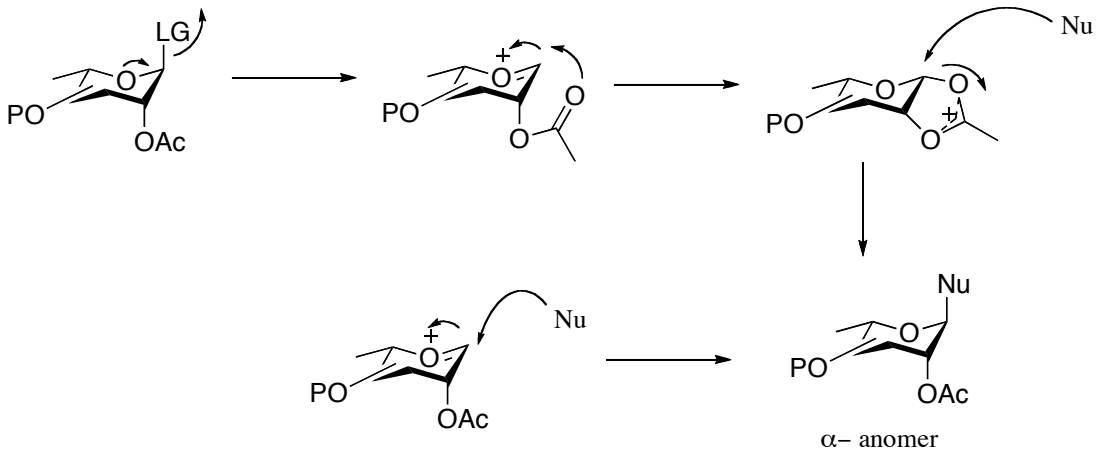


Figure 2-3. Neighboring group participation and formation of α-anomeric linkage in L-rhamnose

Also, the anomeric effect in L-rhamnose and D-mannose guides these sugars to adopt an axial orientation at the anomeric center. The α- anomers are both thermodynamically and kinetically stable in the case of mannose and deoxy-mannose. The 1, 2- *cis* (β) linkage in O-rhamnopyranosylation and O-mannopyranosylation is particularly difficult to synthesize as rhamnosyl and mannosyl donors have a higher selectivity of 1, 2- *trans* (α) linkage due to the steric hindrance by the axial 2-O substituent and also the stereoelectronc effect (Gridley et al, 2000; Ashry et al, 2008).

A closer inspection of the mechanism of glycosylation might have a clue to the synthesis of exclusive 1, 2- *cis* or 1, 2- *trans* glycosidic linkage or a way to increase the ratio of β over α. The glyosidic bond formation reaction as shown in the Fig. 2-4 is a S_N2 type reaction where the glycosyl acceptor (a nucleophile) attacks the glycosyl donor to form the product. During the reaction, an oxocarbenium ion can be generated when the anomeric leaving group forms an intimate ion-pair (Fig. 2-4A). If the acceptor alcohol reacts at this stage, a β-linkage is likely to be formed. When the nucleophile reacts at the solvent separated ion-pair stage (Fig. 2-4B), more α-linkage is likely to be formed due to the anomeric effect. And when the free oxocarbenium ion is formed, the α-glycosidic linkage predominates. So, by shifting the equilibrium shown in fig. 2-4, it is potentially possible to obtain exclusive 1, 2- *cis* or 1, 2- *trans* glycosidic linkage. This can be achieved by utilizing the electronic properties of glycosyl acceptors. In the case of L-rhamnose where the formation of β-linkage is highly challenging, if the equilibrium of the glycosylation reaction is moved more to the left by destabilizing the oxocarbenium ion, a potential excess of β-linkage would be obtained.

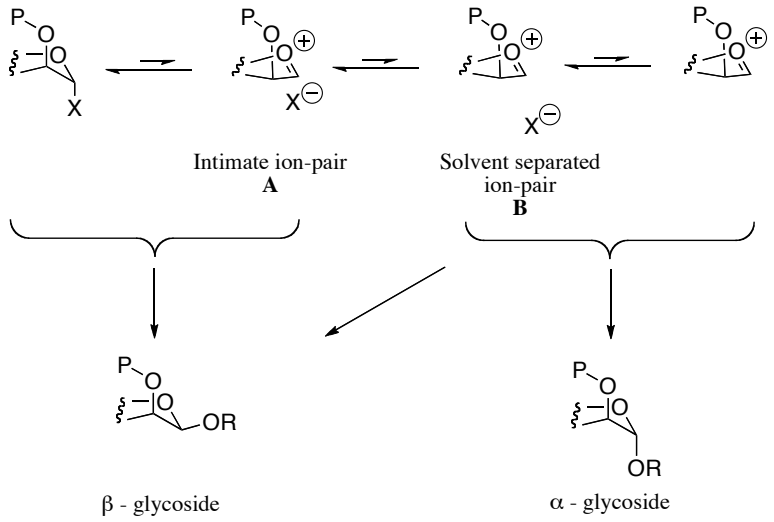


Figure 2-4. Mechanism of glycosylation

One of the recent successful attempts at synthesizing β-D-mannopyranoside was undertaken by Crich and co-workers where they used a 4, 6- O- benzylidene sulfoxide donor of mannose (Crich et al, 1996). In this attempt, the glycosyl donor was activated using triflic anhydride, 2, 6-di-*tert*-butyl-4-methylpyrimidine (DTBMP) at -78 °C (Fig. **2-5**). High β-selectivity of the glycosylation was observed with simple alcohols as well as hindered carbohydrate acceptors. In case of 4, 6-O-benzylidene –protected D-mannose donors, the above equilibrium is shifted greatly toward the covalent glycosyl donor and the intimate ion-pair. This is because the mannopyranoside ring becomes rigid in the presence of the benzylidene and thus, the oxocarbenium ion is difficult to form. In such a case, the β-D-manopyranoside predominates. But since this kind of protecting-group influence is not possible in L-rhamnose due to the lack of 6-hydroxyl function, the formation of the β-linkage in L-rhamnose becomes more challenging.

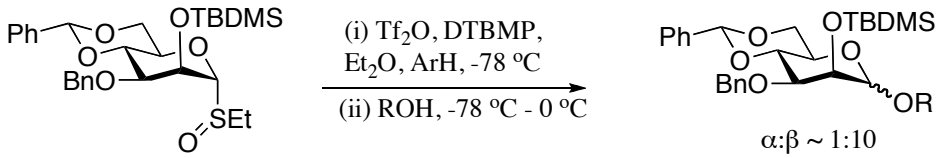


Figure 2-5. β-D-mannopyranoside synthesis using 4, 6-O-benzylidene- protected donor (Crich et al, 1996).

In order to achieve β–L-rhamnopyranosides, the previously attempted methods have utilized 2, 3-O carbonate (Backinowsky et al, 1980) and 2, 3-O alkylidine-protected (Iversen et al, 1980) glycosyl bromides activated by silver-based promoters. 2,3-O-cyclohexylidene derivative of L-rhamnose was converted into its bromide and then reacted under standard Koenigs-Knorr conditions with silver carbonate as the promoter to yield β-L-rhamnopyranoside with simple alcohol acceptors like methanol and also with protected sugar molecules, in moderate to high yield (Fig. 2-6A). 2,3-O cyclic carbonate protecting group has also been used to synthesize rhamnosyl donors to obtain β-glycosidic linkage under standard Koenigs-Knorr condition in the presence of an insoluble silver salt like silver oxide (Fig. 2-6B). If the reaction proceeds in a homogeneous solution with the silver promoter, high α-selectivity was observed (Crich et al, 2003)

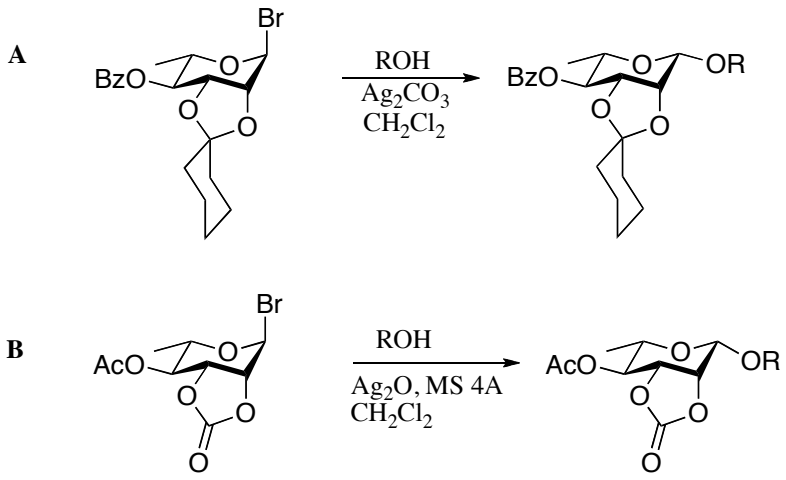


Figure 2-6. Glycosylation with 2, 3-O-benzylidene protected and 2, 3-O-carbonate L-rhamnosyl donor to give β-rhamnosylation (**A**. Backinowsky et al, 1980; **B** Iversen et al, 1980).

A recent attempt at synthesizing β-L-rhamnopyranoside utilized 2-O-sulfonate protecting group of β-L-thiorhamnosides (Crich et al, 2003). The glycosylation method carried out in this work involved the activation of the β-thiorhamnoside with 1-benzenesulfinyl piperidine (BSP) and triflic anhydride in the presence of 2, 4, 6-tri-*tert*-butylpyrimidine (TTBP) (Fig. 2-7). The 2-O-benzene sulfonate groups carried functional groups to make this protecting group electron-rich or electron-deficient. Highest β-selectivity of the glycosyl bond was achieved when the reaction was carried out with 3β-cholestanol as acceptor and *p*-trifluoromethylbenzene sulfonate at the 2-*O* position. Attempts to form the β-rhamnopyranoside linkage with other simple carbohydrate acceptors like glucose 4-OH acceptor, resulted in a 1:1 mixture of α and β linkages. However, this work is a good example of the use of the electronic properties of the protecting group on L-rhamnose donor to direct the stereoelectronic outcome of glycosylation.

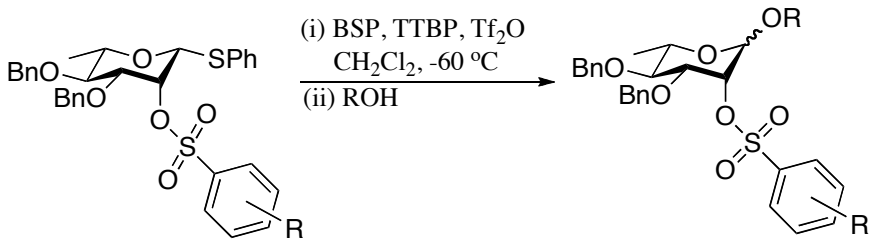


Figure 2-7. β-rhamnosylation with 2-O sulfonate protected rhamnose (Crich et al, 2003).

So far, the majority of attempts to synthesize the β–glycosidic linkage of L-rhamnose, have been using thiorhamnoside donors (Crich et al, 2003) or using the Koenigs-Knorr method (Backinowsky et al, 1980; Iversen et al, 1980). But a widely used method for glycosylation is the Schmidt glycosylation developed by Schmidt and Michel (Schmidt et al, 1980) In this method, trichloroacetonitrile is used to prepare the trichloroacetimidate (TCA) donor at the anomeric center of the sugar under basic conditions, which is reacted with a glycosyl acceptor under mild acidic condition with a promoter, usually TMSOTf (Figure 2-8). This is a very popular method of glycosylation as the trichloroacetimidates are introduced under mild conditions and are formed in excellent yields. They react to form the glycosidic linkage readily to give good yield of the product and in many cases very good α/β selectivity. This method is also orthogonal to a wide range of protecting groups (Schmidt, 1986).

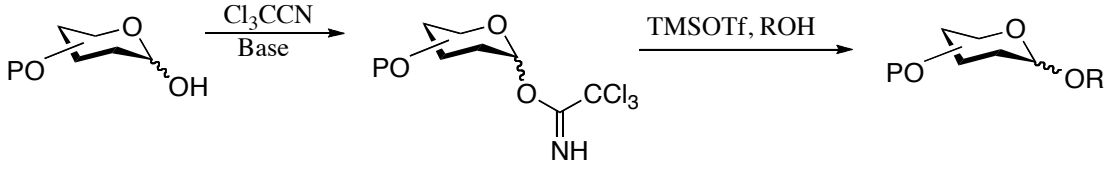


Figure 2-8. Schmidt's trichloroacetonitrile method for glycosylation (Schmidt et al, 1980).

An elegant work using TCA donors of D-mannose with electron-withdrawing groups at the O-3, O-4 and O-6 positions was carried out by Kim and co-workers (Baek et al, 2009). In this study, both participating and non-participating electron withdrawing groups containing D-mannose trichloroacetimidate donors were synthesized and their glycosylation with a number of acceptors was carried out. The electron withdrawing effect and remote participating effect would have opposite effects in the stereochemical outcome of glycosylation reaction. This study is an ingenious example of the use of the electronic property of the protecting groups to guide the stereochemical output of the glycosylation reaction, and in turn, gain knowledge of the mechanism of glycosylation of D-mannose.

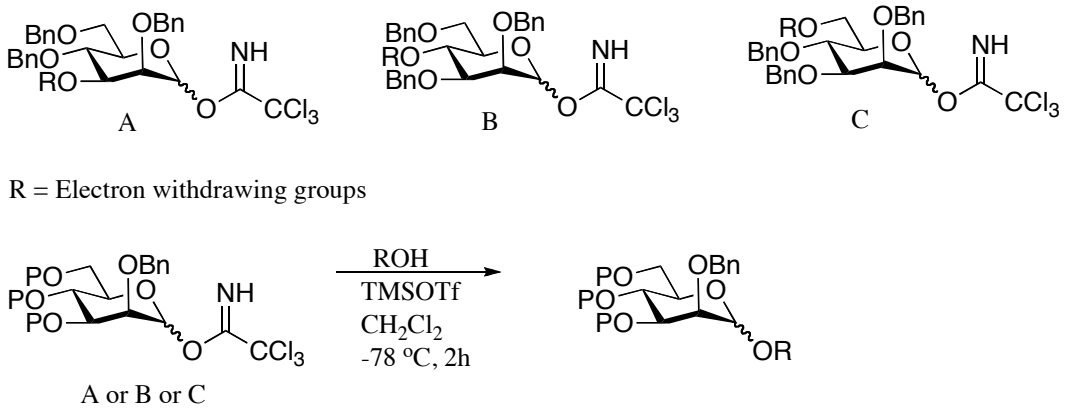


Figure 2-9. Glycosylation with various D-mannose TCA donors containing electron-withdrawing groups (Baek et al, 2005).

The protecting group at the O-2 position of D-mannose was kept constant and those at O-3, O-4 and O-6 were sequentially changed. Both participating and non-participating electron-withdrawing groups were used. Glycosylation with TCA donors were carried out at -78 °C for 2h with various acceptors (summarized in Fig. 2-9). The authors observed

that both participating and non-participating electron withdrawing groups at O-4 position of D-mannose donor predominantly formed the β-glycosidic linkage. Non-participating groups at O-3 yielded high β-selectivity of mannosylation whereas participating groups like acyl groups were α-directing when placed at position 3. At the O-6 position only the acetyl group showed α-directing tendency, all other electron-withdrawing groups yielded β-mannopyranoside.

This methodical and effective study throws some light on the mechanism of the glycosylation of mannose TCA donors. The resulting stereochemical outcome with the various electron-withdrawing group can help in designing a logical building block for stereo-directed glycosylations using the electronic property nature of the protecting groups to D-mannose.

However, such a systematic study has yet not been undertaken in case of L-rhamnose TCA donors. In this chapter, an attempt at such a study on L-rhamnose is described. Firstly, L-rhamnose TCA donors containing both participating and non-participating electron withdrawing groups at the O-3 and O-4 positions are designed and synthesized and then rhamnopyranosylation is carried out to determine the α/β ratio of the product formed. The mechanism of glycosylation with L-rhamnose and also the effect of the electron-withdrawing properties of the various protecting groups are probed in this study.

Results and discussion

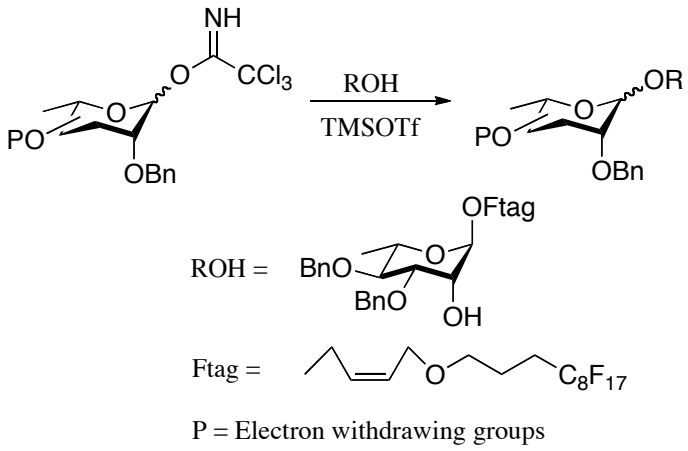
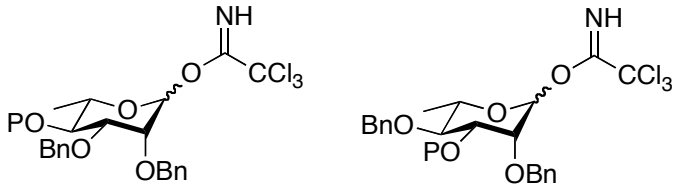


Figure 2-10. Glycosylation strategy for this study

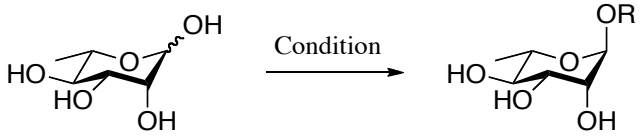
In the work presented in this chapter, trichloroacetimidate donors of L-rhamnose containing different electron withdrawing groups at the O-3 and O-4 positions are synthesized and their glycosylation is carried out with fluorous tag containing L-rhamnose acceptor (Fig. 2-10). The electron withdrawing groups used in this study are participating groups like acetyl, benzoyl and *p*-nitrobenzoyl, and non-participating sulfonylbenzyl at the O-3 and O-4 positions of L-rhamnsoe (Fig. 2-11). The choice of these electron withdrawing groups is inspired by the studies performed on mannose by Kim and co-workers (Baek et al, 2009). The L-rhamnose TCA donors with electron withdrawing groups at position O-4 are labeled type A and those with electron withdrawing groups at position O-3 are labeled type B. The positions not protected by electron withdrawing groups were converted into benzyl ethers.



P = Electron withdrawing group
Ac, Bz, *p*-NO_2Bz, -SO_2Bn

Figure 2-11. Building blocks and the electron-withdrawing protecting groups used for this study.

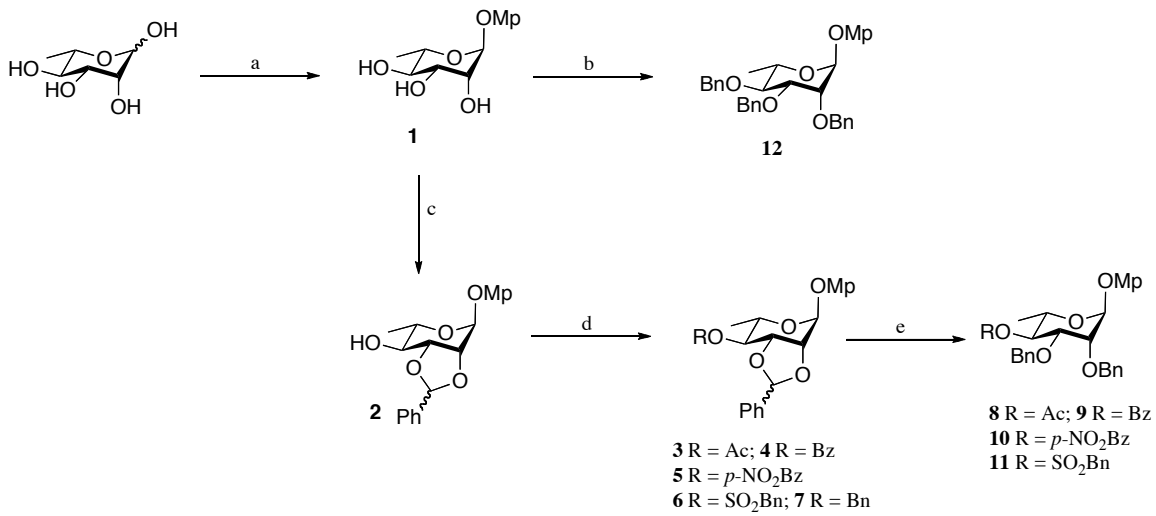
For the synthesis of the L-rhamnose TCA donors, the first reaction sequence was to find a suitable anomeric protecting group which would be robust under the various reaction conditions of protection and deprotection strategies and can itself be protected and deprotected under mild conditions. The anomeric position was protected as the allyl ether, methyl ether and *p*-methoxyphenyl (-OMp) ether (Table 2-1). Although the yields of the reaction were all comparable, the removal condition of the methoxy group was very harsh; and for the removal of the allyl group, two steps were involved which required the use of iridium catalyst and mercury salts. The –OMp group was chosen as the anomeric protecting group of choice because of the ease of the protection and deprotection of this group. One of the other key factors in choosing this group was the ease of recrystallization of the product formed.



R	Condition	Yield (%)	Removal Condition
-Allyl	Allyl Alcohol, CF_3COOH, 65 °C	91	$Ir[COD(PPhMe_2)_2]PF_6$, H_2, THF, 16 h (ii) HgO, $HgCl_2$ Acetone/H_2O
$-CH_3$	Amberhyst® Resin, CH_3OH	86	Conc. H_2SO_4, Heat
-OMp	(i) Ac_2O, Py (ii) *p*-Methoxyphenol, BF_3OEt_2, CH_2Cl_2 (iii) Na, CH_3OH	83 (Over 3 steps)	Ceric ammonium nitrate CH_3CN, H_2O, 45 min

Table 2-1. Choice of anomeric protecting group

To synthesize the type A L-rhamnose building blocks, a 2, 3-O-benzylidene acetal of 1-O-*p*-methoxyphenyl-2, 3, 4-hydroxy-α-L-rhamnopyranoside **1** was first prepared with benzaldehyde dimethyl acetal and *p*TSA as the acid catalyst. Next, the 4-O position was protected with the electron-withdrawing groups like acetate, benzoyl, *p*-nitrobenzoyl and benzylsulfonyl ether under basic conditions followed by the opening of the 2, 3-O-benzylidene acetal with 80% acetic acid as shown in Scheme 2-1. The next step was to form the benzyl ether at the 2 and 3-O positions. The standard basic condition used for the formation of the benzyl ether, using sodium hydride and benzyl bromide was used for the synthesis of compound **19**. With compounds containing base-labile acyl groups, the benzyl ether was formed under acidic condition of triflic acid with freshly prepared trichloroacetimidate of benzyl alcohol in high yields.



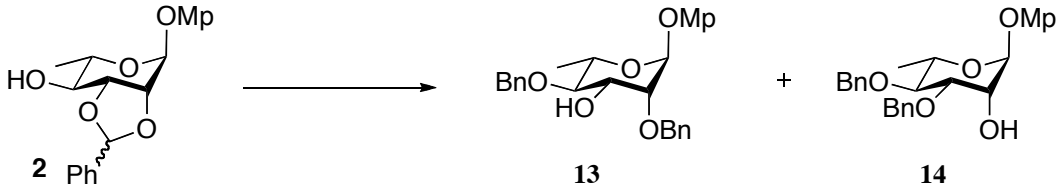
Conditions: a. (i) Ac_2O, py (87%) (ii) *p*-methoxyphenol, $BF_3.OEt_2$, CH_2Cl_2 (82%) (iii) Na, CH_3OH (92%) b. BnBr, NaH, DMF (63%) c. $PhCH(OCH_3)_2$, CH_3CN, *p*TSA (85%) d. For **3**: Ac_2O, py (89%); for **4**: BzCl, Et_3N, CH_2Cl_2 (92%); for **5**: *p*-NO_2BzCl, Et_3N, CH_2Cl_2 (71%); for **6**: $BnSO_2Cl$, 2,6-Lutidine, CH_2Cl_2 (89%); for **7**: BnBr, NaH, DMF (87%) e. (i) 80% CH_3COOH/H_2O (ii) for **8**, **9**, **10**: benzyltrichloroacetimidate, TfOH, CH_2Cl_2 (68%, 59%, 68% respectively); for **11**: BnBr, NaH, DMF (56%)

Scheme2-1. Synthesis of type A building blocks

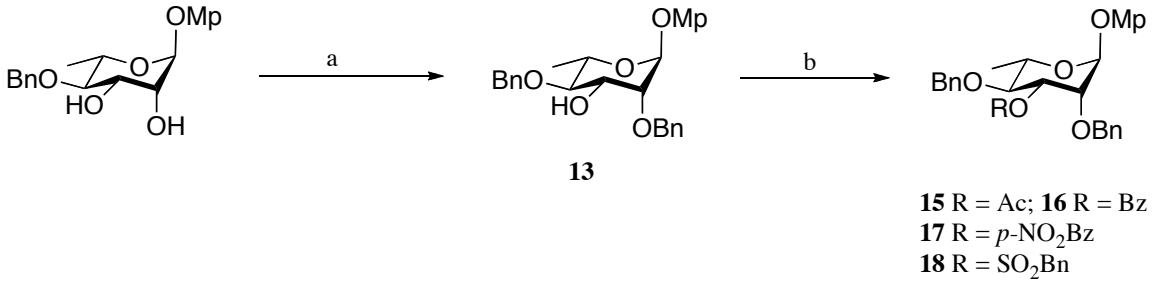
With these building blocks in hand, the next step was to form the trichloroacetimidate donors of these fully protected L-rhamnose. For this the *p*-methoxyphenyl (-OMp) protecting group was deprotected using ceric ammonium nitrate and then converted into the TCA donor under basic conditions with potassium carbonate. The donor containing *p*-nitrobenzoyl at the 4-O position was highly crystalline in nature and did not dissolve in common solvents like dichloromethane and toluene under glycosylation conditions.

The synthesis of type B building blocks was not as straightforward as that of the type A ones. The initial idea in this synthesis was to selectively open the 2, 3-O-benzylidene ester **7** to obtain 2-O benzyl ether and then protect 3-O with the electron-withdrawing

groups (Scheme 2-2). Reduction conditions with $LiAlH_4$-$AlCl_3$, DIBAL-H, Et_3SiH-I_2 and BH_3THF-Bu_2OTf were all used but all the reactions gave a mixture of the desired product **13** along with the undesired product **14** in higher yields. It has been shown that only one of the isomers of the 2, 3-O-benzylidene acetal of L-rhamnose reacts to give the reductive cleavage of the acetal (Liptak, 1976). So, the selective opening of the bezylidine was not carried out, instead another approach to synthesize type B building block was investigated. Finally, compound **13** was synthesized by addition of 1.1 equivalent of benzyl bromide to a solution of the 1-O-*p*-methoxyphenyl-4-O-benzyl-rhamnopyranoside in 5% NaOH solution in CH_2Cl_2 (Scheme 2-3).



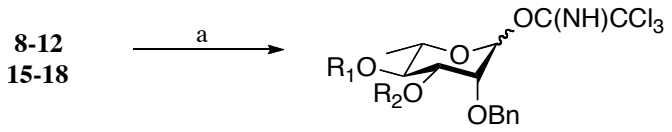
Scheme 2-2. Selective opening of the 2, 3-O-benzylidene acetal



Conditions. a. BnBr (1.1 eq), NaOH (5% aqCH_2Cl_2 (67%) b. For **15** Ac_2O, py (85%); for **16**: BzCl, Et_3N, CH_2Cl_2 (92%); for **17**: *p*-NO_2BzCl, Et_3N, CH_2Cl_2 (76%)for **18**: $BnSO_2Cl$, 2,6-Lutidine, CH_2Cl_2 (86%)

Scheme 2-3. Synthesis of type B building blocks.

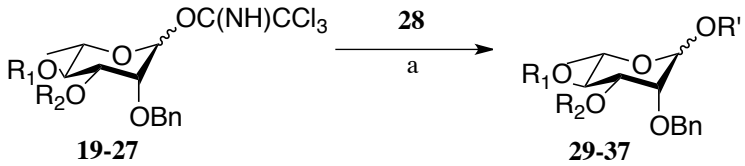
The 2, 4-dibenzyl-3-hydroxy-1-O-*p*-methoxyphenyl-α-L-rhamnopyranoside (**13**) was reacted with the various electron withdrawing groups to synthesize the fully protected type B building block of L-rhamnose (Scheme 2-3). This was converted into the trichloroacetimidate donor with trichloroacetonitrile and potassium carbonate as base. The products and yield of the reaction is shown in Scheme 2-4.



Condition a. (i) CAN, CH_3CN, H_2O (ii) K_2CO_3, $Cl_{3C}CN$, CH_2Cl_2

Compound	**R_1**	**R_2**	**Product**	**Yield (%)**
8	Ac	Bn	**19**	82
9	Bz	Bn	**20**	83
10	*p*-NO_2Bz	Bn	**21**	65
11	SO_2Bn	Bn	**22**	71
12	Bn	Bn	**23**	56
15	Bn	Ac	**24**	77
16	Bn	Bz	**25**	83
17	Bn	*p*-NO_2Bz	**26**	72
18	Bn	SO_2Bn	**27**	73

Scheme 2-4. Synthesis of the L-rhamnose trihloroacetimidate donors.



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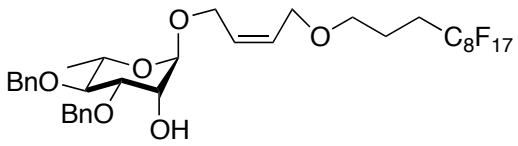


Condition a. TMSOTf (0.15 eq), CH_2Cl_2, 0 °C

Glycosyl Donor	Glycosyl Acceptor	Disaccharide	Yield (%)	α/β ratio
19	**28**	**29**	74	4:1
20	**28**	**30**	61	3:1
22	**28**	**32**	88	8:1
23	**28**	**33**	75	Exclusively α
24	**28**	**34**	68	10:1
25	**28**	**35**	62	9:1
27	**28**	**37**	71	2:1

Scheme 2-5. Glycosylation reaction of the L-rhamnose donors with L-rhamnose acceptor **28**.

Glycosylation of these donors was carried out with a L-rhamnose acceptor containing a fluorous-tag (**28**). The synthesis of this acceptor is discussed in chapter 4 under the synthesis of rhamnogalacturonan-I backbone. The glycosylation of the various L-rhamnose donors with the acceptor along with the α/β ratio of the disaccharides formed is shown in Scheme 2-5.

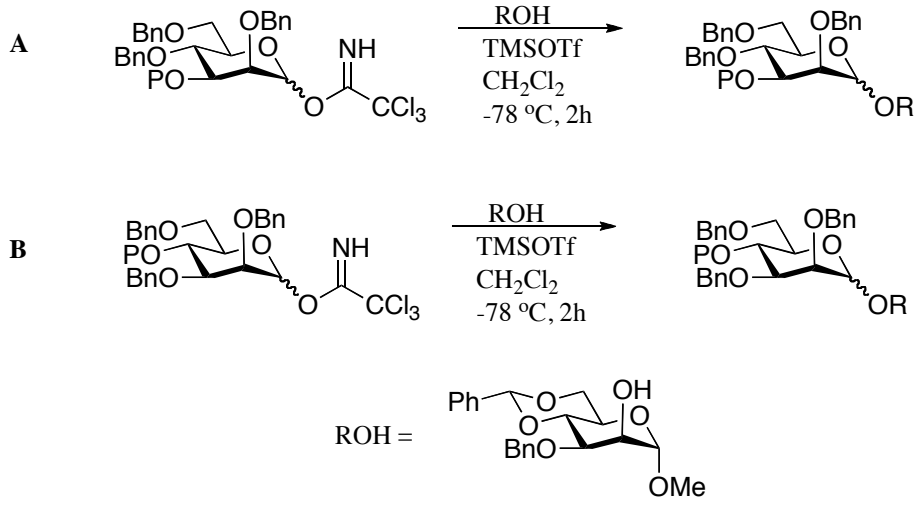


Figure 2-12. Mannopyranosylation with electron withdrawing groups at O-3 (A) and O-4 (B) positions of D-mannose donors (Baek et al, 2009).

The results from these glycosylation reactions of rhamnose are tabulated in Table 2-2 and Table 2-3, along with the results from similar glycosylation reaction of mannose for comparison. In the case of both mannose and rhamnose in the tabulated results, the glycosylation reaction was carried out with a secondary alcohol monosaccharide acceptor as shown in Scheme 2-5 and Fig 2-12, respectively. For D-mannose, the glycosyl acceptor was the secondary alcohol 1-O-methyl-3-O-benzyl-4, 6-O-benzylidene mannopyranoside, and for L-rhamnose in the study presented, the glycosyl acceptor was fluorous-tagged secondary alcohol of rhamnose (**28**).

Electron withdrawing groups (At O-3 position)	**Rhamnopyranosylation α/β**	**Mannopyranosylation α/β**
-SO_2Bn	2:1	1:11.8
-Ac	10:1	40.4:1
-Bz	9:1	19.8:1
-Bn*	Exclusively α	2.7:1

* No electron withdrawing group- a control.

Table 2-2. Results of rhamnopyranosylation with L-rhamnose donor containing electron withdrawing group at O-3, compared with mannopyranosylation (Fig 2-12A).

Electron withdrawing groups (At O-4 position)	**Rhamnopyranosylation α/β**	**Mannopyranosylation α/β**
-SO_2Bn	8:1	1:3.5
-Ac	4:1	1:2.1
-Bz	3:1	1:2.3

Table 2-3. Results of glycosylation reaction of L-rhamnose donor containing electron withdrawing groups at O-4 position compared with mannosylation (Fig 2-12B)

Summarizing some of the key points from these results:

1. The glycosylation with the 2, 3, 4-O-tribenzyl-rhamnose-trichloroacetimidate donor produced exclusive α - product.

2. Non-participating electron withdrawing group (benzylsulfonyl) at O-3 was found to produce more α-glycosidic linkage compared to participating acyl type groups at O-3.

3. Rhamnosylation with donors containing electron withdrawing groups at both 3-O and 4-O are more α-directing. But in comparison to the 3-O position, this α- directing effect at 4-O is less.

With participating electron withdrawing groups (Ac and Bz) at position O-3, of L-rhamnose, more α -directing effect is observed. This is an evidence of remote participation by these groups. Plausible remote participation can take place with the $^{4}C_{1}$ conformer of L-rhamnose as shown in Fig 2-13. Once there is a conformational change from $^{1}C_{4}$ to $^{4}C_{1}$, a six membered acetal type ring can be formed with the acyl type protecting groups. The attack of the incoming nucleophile at this stage occurs from the less hindered face, now is the α -face of the donor. Such remote participation would be absent in case of non-participating group and the destabilization of the oxocarbenium ion will be the deciding factor in the α/β directing effect.

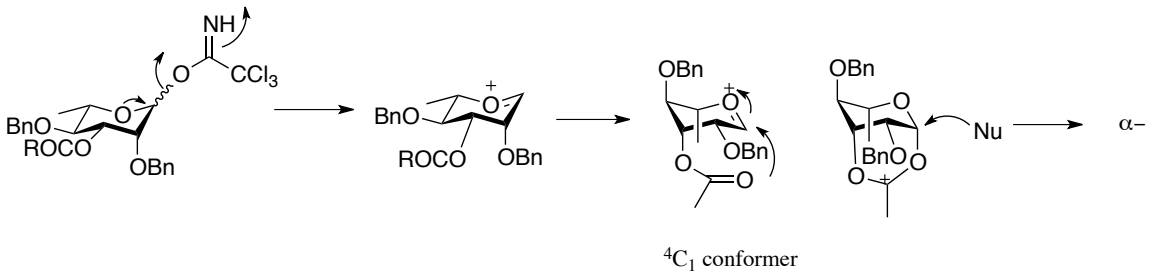


Figure 2-13. Proposed mechanism for the α-directing effect of participating groups at position O-3 of L-rhamnose

At position 4, the remote participation by the acyl type protecting groups is absent as now the conformer change gives a highly unstable structure. The stability of the oxocarbenium ion formed during the reaction would be the main factor in the α/β directing effect of the electron withdrawing groups in this case.

On comparing the results from the glycosylation reactions of mannose and rhamnose with similar secondary alcohols, we find that the preference of L-rhamnose toward α-glycosidic linkage is very high. The results of L-rhamnose and D-mannose with electron withdrawing groups at O-3 are comparable and show the same trend, with both mannose and rhamnose producing excess α-glycosidic linkages. But in case of electron withdrawing groups at O-4, mannose shows a preference for β-glycosidic linkage, unlike L-rhamnose, where the α-linkage still predominates. The absence of the 6-hydroxyl function in L-rhamnose might be a factor in such difference of selectivities.

Although, both these carbohydrates prefer the formation of α-linkage, the trichlororacetimidate driven glycosylation of L-rhamnose with both small and larger acceptors yield the α-linkage in excess.

Conclusion

Various L-rhamnose trichloroacetimidate donors with electron-withdrawing groups at the O-3 and O-4 positions were synthesized. The introduction of the electron-withdrawing groups strategically at various positions in L-rhamnose could be effective in stereo-directed synthesis of glycosidic linkage with L-rhamnose donors and various acceptors. Through this study, a plausible mechanism is proposed for the formation of more α-glycosidc bond with the electron withdrawing group at O-3 with participating acyl type protecting groups compared to these groups at O-4 positions. Unlike β-mannosylations, the problem of formation of β-linkages in rhamnose with tricholoacetimidate assisted glycosylation still has challenges in terms of protecting groups and sterics, that can be an interesting challenge to further probe.

Experimental section

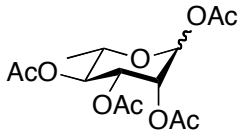
General methods

Reaction solvents were used directly from solvent tower (Swagelok). All other commercial reagents and solvents were used as received without further purification. The reactions were monitored and the *R*f values determined using analytical thin layer chromatography (TLC) with Sorbent Technologies Silica gel HL TLC plates with UV 254 (250 μm). The developed TLC plates were visualized by immersion in a solution of EtOH: conc. H_2SO_4: *p*-anisaldehyde (9:1:1) followed by heating on a hot plate. Silica gel flash chromatography was performed with ZeoPrep 60 Eco 40-63 μm. Fluorous phase chromatography using fluorous solid-phase extraction cartridges containing silica gel bonded with perfluorooctylethylsilyl chains (Fluorous Technologies, Inc.; Pittsburgh,

PA). All other fluorous reagents were also obtained from Fluorous Technologies, Inc. All moisture-sensitive reactions were performed in flame- or oven-dried glassware under a Ar(g) atmosphere. Bath temperatures were used to record the reaction temperature. All reactions were stirred magnetically at ambient temperature unless otherwise indicated. ^{1}H NMR and ^{13}C NMR spectra were obtained with a VarianVXR at 400 MHz and 100 MHz or BrukerDRX at 600 MHz or 100 MHz respectively. ^{1}H NMR spectra were reported in parts per million relative to $CDCl_3$ (7.26 ppm) and ^{13}C NMR spectra were reported in parts per million relative to $CDCl_3$ (77.23 ppm).

Synthetic procedures

1, 2, 3, 4-Tetra-O-acetyl-α/β-L-rhamnopyranoside



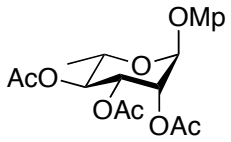
To a solution of pyridine (5.0 mL) and acetic anhydride (5.0 mL) α/β-L-rhamnose (5.0 g, 0.027 mol) was added. The solution was stirred for 2h at ambient temperature and then quenched by pouring the mixture into water. The product was extracted with EtOAc (50 mL). The organic layer was washed with sodium bicarbonate solution (3 X 50 mL), 2N hydrochloric acid solution (1 X 50 mL) and brine (2 X 50 mL), dried over $MgSO_4$ and concentrated under reduced pressure to obtain **1** (8.25g, 87%, colorless oil).

^{1}H NMR (CDCl3, 400 MHz): δ (ppm) 5.94 (s, 1H), 5.24 (dd, J = 10.0 Hz, 1H), 5.18 (dd, J = 3.6 Hz, 1H), 5.05 (t, J = 10.0 Hz, 1H), 3.89 (m, 1H), 2.09 (s, 3H), 2.03 (s, 3H), 1.93 (s, 3H), 1.21 (d, J = 6.0 Hz, 3H)

^{13}C NMR (CDCl3, 100 MHz): δ (ppm) 170.2, 170.0, 169.9, 168.5, 90.7, 70.6, 68.9, 68.7, 68.6, 21.1, 21.0, 20.9, 20.8, 17.6

R_f (Hexane: EtOAc 2:1): 0.42

1-O-*p*-Methoxy-2, 3, 4-tri-O-acetyl-α-L-rhamnopyranoside



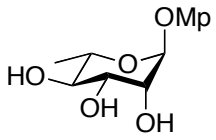
To a solution of peracetylated L-rhamnose (8.25g, 0.025 mol) in dichloromethane, $BF_3.OEt_2$ (3.3 mL, 0.025 mol) was slowly added at 0 ºC followed by the addition of *p*-methoxyphenol (3.75g, 0.031 mol). The reaction mixture was warmed to room temperature and stirred for 4h. The reaction was quenched by adding sodium bicarbonate solution (2 X 25 mL) and extracted with dichloromethane (50 mL). The organic layer was washed with brine (2 X 50 mL), dried with $MgSO_4$ and the solvent removed under reduced pressure. The crude product was purified by flash chromatography (2:1 hexane:ethyl acetate) to obtain 1-O-*p*-methoxy-2, 3, 4-tri-O-acetyl-α-L-rhamnopyranoside.(8.5 g, 81%, white solid).

^{1}H NMR (CDCl3, 400 MHz): δ (ppm) 7.12-7.13 (d, J= 8.0 Hz, 2H), 6.98-6.96 (d, J= 8.0 Hz, 2H 5.94 (s, 1H), 5.86 (s, 1H), 5.23 (dd, 1H), 5.12(t, 1H), 3.69 (m, 1H), 2.12 (s, 3H), 2.09 (s, 3H), 2.03 (s, 3H), 1.32 (d, J = 6.0 Hz, 3H)

^{13}C NMR: CDCl3, 100 MHz): δ (ppm) 157.8, 151.1, 118.8, 118.5, 115.1, 115.2, 97.8, 74.4, 72.0, 71.8, 68.9, 55.8, 17.7

R_f (Hexane: EtOAc 3:2): 0.38

1-O-*p*-Methoxy-α-L-rhamnopyranoside (1)



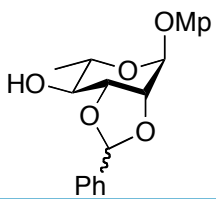
To a solution of 1-O-*p*-methoxy-2, 3, 4-tri-O-acetyl-α-L-rhamnopyranoside (8.5 g, 0.021 mol) in methanol (30 mL) sodium metal was added at ambient temperature and the solution was checked to have a pH of 9 -10 by pH paper. The reaction mixture was stirred for 1h. The reaction was quenched by adding Dowex® till the solution was neutral. The solution was filtered and the solvent removed under reduced pressure. The crude product was recrystallized by ethanol to obtain white needle like crystals of **1** (5.25 g, 92%, white crystal).

^{1}H NMR: ($CDCl_3$, 400 MHz): 7.03-7.01 (d, J= 8.0 Hz, 2H), 6.92-6.90 (d, J= 8.0 Hz, 2H), 5.03 (s, 1H), 4.12 (dd, 1H0, 3.81 (dd, 1H), 3.62 (dd, 1H), 3.33-3.31 (m, 1H), 1.39 (d, 3H)

^{13}C NMR: (CD_3OD, 100 MHz): δ (ppm) 154.1, 151.0, 118.1, 114.2, 99.3, 94.2, 74.2, 73.1, 72.5, 69.4, 68.7, 54.8, 16.7

R_f (CH_2Cl_2: CH_3OH 4:1) 0.32

1-O-*p*-Methoxy-2, 3-O-benzylidine-α-L-rhamnopyranoside (2)



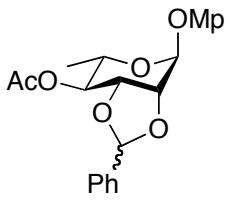
To a solution of **1** (5.25 g, 0.02 mol) in acetonitrile (18 mL), benzaldehydedimethyl acetal (3.0 mL, 0.021 mol) was added, followed by the addition of *p*-toluenesulfonic acid (0.05 g, 0.37 mmol) at ambient temperature. The reaction was allowed to run for 1h and then quenched by the addition of triethylamine (0.15 mL) and the solvent removed under reduced pressure. The crude product was purified by flash chromatography (2:1 hexane:ethyl acetate) to obtain **2** (R/S configuration) as a white solid (5.75 g, 85%, white solid).

^{1}H NMR: (CDCl3, 600 MHz): δ (ppm) 7.18-7.16 (m, 1H), 7.34-7.30 (m, 4H), 6.98-6.94 (d, J = 6 Hz, 2H), 6.78-6.75 (d, J = 6 Hz, 2H), 5.87 (s, 1H), 5.65 (s, 1H), 4.75 (d, 1H), 4.51-4.48 (m, 1H), 4.39-4.37 (m, 1H), 3.80-3.77 (m, 1H), 1.38- 1.36 (d, 3H)

^{13}C NMR: CDCl3, 100 MHz): δ (ppm) 134.8, 130.0, 129.2, 117.2, 114.6, 98.9, 72.4, 72.1, 71.8, 68.2, 56.7, 17.8

R_f (Hexane: EtOAc 3:2) 0.48

1-O-p-Methoxy-2, 3-O-benzylidine-4-O-acetyl-α-L-rhamnopyranoside (3)



To a solution of **2** (0.385g, 1.01 mmol) in pyridine (10.0 mL), acetic anhydride (12.0 mL) was added at ambient temperature. The solution was stirred for 2h and then quenched by pouring the mixture into water. The product was extracted with EtOAc. The organic layer was washed with sodium bicarbonate solution, 2N hydrochloric acid solution and brine,

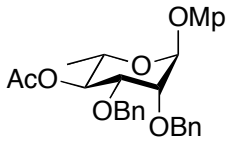
dried over $MgSO_4$ and the solvent removed under reduced pressure. The crude product was purified by flash chromatography (2:1 hexane:ethyl acetate) to obtain **3** (R/S configuration) (0.371g, 89%, colorless oil).

^{1}H NMR: (CDCl3, 400 MHz): δ (ppm) 7.52-7.51 (m, J = 6Hz, 1H), 7.44 (s, 1H), 7.42-7.42 (m, 2H), 7.04-7.03 (d, J= 6.0 Hz, 2H), 6.87-6.88 (d, J= 6.0 Hz, 2H), 6.29 (s, 1H), 5.77 (s, 1H), 5.14-5.12 (dd, 1H), 4.72-4.71 (dd, 1H), 4.57-4.56 (dd, 1H), 4.04-4.02 (m, 1H), 3.81 (s, 3H), 2.15 (s, 3H), 1.29 (d, J=6.0 Hz, 3H)

^{13}C NMR: CDCl3, 100 MHz): δ (ppm) 155.1, 150.1, 138.2, 136.3, 129.7, 128.5, 127.0, 126.3, 117.8, 114.7, 104.8, 96.2, 71.4, 64.8, 60.4, 55.6, 21.0, 17.1, 14.2

R_f (Hexane: EtOAc 2:1): 0.51

1-O-*p*-Methoxy-2, 3-O-di-benzyl-4-O-acetyl-α-L-rhamnopyranoside (8)



80% aqueous acetic acid was added to a solution of **3** (0.48g, 0.95 mmol). The reaction mixture was stirred at 65 ºC for 2h and then the solvent removed under reduced pressure. The crude product was carried on to the next step without purification. Tricholoroacetimidate of benzyl aclcohol (0.24g, 1.04 mmol) was added to the reaction mixture in CH_2Cl_2 maintained at 0 ºC followed by the addition of TfOH (142.5 μL, 9.5 μmol). The reaction was stirred at 0 ºC for 45 min, then quenched by addition of Et_3N (1.32 mL) and concentrated under reduced pressure. The crude mixture was purified by

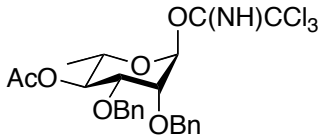
flash chromatography (3:2 Hexane: EtOAc) to obtain **8** (0.72 g, 68% over two steps, yellow oil).

^{1}H NMR: (CDCl3, 400 MHz): δ (ppm) 8.18-8.4 (m, 5H), 7.52-7.49 (m, 5H), 7.25-7.21 (d, 2H), 6.96-6.14 (d, 2H), 5.51 (s, 1H), 5.41-4.39 (dd, 1H), 4.72-4.71 (dd, 1H), 4.57-4.56 (dd, 1H), 4.22-4.19 (m, 1H), 3.91 (s, 3H), 3.86 (s, 2H), 2.10 (s, 3H), 1.29 (d, 3H)

^{13}C NMR: CDCl3, 100 MHz): δ (ppm) 169.2, 159.2, 152.3, 139.1, 138.9, 135.4, 131.2, 131.7, 128.5, 127.0, 124.8, 116.4, 115.8, 95.7, 72.4, 66.8, 65.3, 54.8, 23.0, 18.1, 17.2

R_f (Hexane: EtOAc 2:1): 0.42

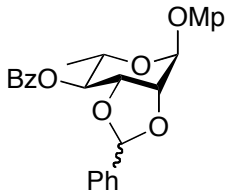
2, 3-O-Di-benzyl-4-O-acetyl-α/β-L-rhamnopyranoside trichloroacetimidate (19)



To a solution of **8** (0.031g, 6.4 mmol) in CH_3CN:H_2O (5:1) ceric ammonium nitrate (0.071g, 0.13 mmol) was added and the solution stirred for 1h. The reaction was quenched by the addition of sodium bicarbonate and extracted with dichloromethane. The organic layer was dried over $MgSO_4$ and the solvent removed under reduced pressure. The crude product was dried under vacuum and carried out to the next step. The crude product obtained was dissolved in dichloromethane under Ar (g) atmosphere. K_2CO_3 (0.044g, 0.32 mmol) was added followed by slow addition of trichloroacetonitrile (0.02mL, 0.20 mmol) and the reaction mixture was stirred for 45 min at 0 ºC. The reaction mixture was quenched by the addition of triethylamine (0.25mL) and filtered

through celite®, washed with dichloromethane and solvent removed under reduced pressure. The crude product was purified by flash chromatography (3:2 hexane:ethyl acetate) to obtain **19** (0.027g, 82% over two steps, oil).

1-O-*p*-Methoxy-2, 3-O-benzylidine-4-O-benzoyl-α-L-rhamnopyranoside (4)



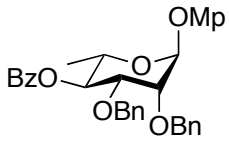
To a solution of **2** (0.436g, 1.2 mmol) in pyridine (12.0 mL), benzoyl chloride (0.15 mL, 1.3 mmol) was added at ambient temperature. The solution was stirred for 2h and then quenched by pouring the mixture into water. The product was extracted with EtOAc. The organic layer was washed with sodium bicarbonate solution, 2N hydrochloric acid solution and brine, dried over $MgSO_4$ and the solvent removed under reduced pressure. The crude product was purified by flash chromatography (2:1 hexane:ethyl acetate) to obtain **4** (R/S configuration) (0.518g, 92%).

^{1}H NMR: (CDCl3, 600 MHz): δ (ppm) 8.23-8.21 (d, 2H), 8.18-8.17 (d, 2H), 7.69-7.64 (m, 1H), 7.63-7.59 (m, 2H), 7.52-7.49 (m, 2H), 7.27-7.25 (d, J = 12 Hz, 2H), 6.81-6.79 (d, J = 12 Hz, 2H), 5.55 (s, 1H), 5.17 (m, 1H), 4.27-4.25 (dd, 1H), 4.21 (m, 1H), 4.19-4.15 (m, 1H), 3.15-3.18 (m, 1H), 1.36-1.34 (d, 3H)

^{13}C NMR: CDCl3, 100 MHz): δ (ppm) 134.9, 134.0, 131.2, 130.1, 129.1, 123.4, 118.3, 115.0, 97.4, 71.8, 71.2, 66.3, 56.4, 46.1, 17.3

R_f (Hexane: EtOAc 2:1) 0.32

1-O-*p*-Methoxy-2, 3-O-di-benzyl-4-O-benzoyl-α-L-rhamnopyranoside (9)



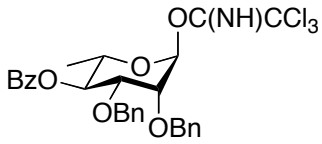
80% aqueous acetic acid was added to a solution of **4** (0.43g, 1.15 mmol). The reaction mixture was stirred at 65 ºC for 2h and then the solvent removed under reduced pressure. The crude product (0.31g) was dissolved in 12.0 mL dichloromethane. Benzyltrichloroacetimidate (0.244g, 1.03 mmol) was added at 0 ºC under Ar(g) atmosphere, followed by the addition of triflic acid (1.29 μL,8.6 μmol). The reaction mixture was stirred for 45 min. The mixture was quenched by adding Et_3N (0.35mL) and concentrated under reduced pressure. The product was purified by silica gel flash chromatography (40% EtOAc in Hexane) to obtain **9** (0.21g, 59% over two steps, yellow oil).

^{1}H NMR: (CDCl3, 600 MHz): δ (ppm) 8.71-8.69 (m, 5H), 7.99-7.94 (m, 5H), 7.65-7.63 (d, 2H), 7.45-7.43 (d, 2H), 7.29-7.25 (m, 5H), 5.65 (s, 1H), 5.42 (m, 1H), 4.89 (s, 2H), 4. 68 (s, 2H), 4.25 (dd, 1H), 3.81 (m, 1H), 1.28 (d, 3H)

^{13}C NMR: CDCl3, 100 MHz): δ (ppm) 164.9, 159.8, 138.4, 138.1, 137.3, 128.6, 128.3, 127.9, 127.4, 127.0, 96.1, 91.2, 76.5, 76.1, 70.3, 69.1, 69.3, 65.6, 17.2

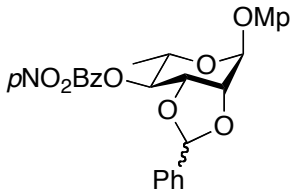
R_f (Hexane: EtOAc 2:1) 0.35

2, 3-O-Di-benzyl-4-O-benzoyl-L-rhamnopyranoside trichloroacetimidate (20)



To a solution of **9** (0.036g, 0.06 mmol) in CH_3CN:H_2O (5:1) ceric ammonium nitrate (0.076g, 0.13 mmol) was added and the solution stirred for 1h. The reaction was quenched by the addition of sodium bicarbonate and extracted with dichloromethane. The organic layer was dried with $MgSO_4$ and the solvent removed under reduced pressure. The crude product (0.02g, 0.05 mmol) was dissolved in dichloromethane under Ar (g) atmosphere. K_2CO_3 (0.03g, 0.26 mmol) was added followed by slow addition of trichloroacetonitrile (0.15 mL, 1.59 mmol) and the reaction mixture was stirred for 45 min at 0 ºC. The reaction mixture was quenched by the addition of triethylamine (0.20 mL) and filtered through celite®, washed with dichloromethane and solvent removed under reduced pressure. The crude product was purified by flash chromatography (3:2 hexane:ethyl acetate) to obtain **20** (0.04 g, 83%, colorless oil).

1-O-*p*-Methoxy-2, 3-O-benzylidine-4-O-*p*-nitrobenzoyl-α-L-rhamnopyranoside (5)



To a solution of **2** (0.45g, 1.26 mmol) in dichloromethane (15.0 mL), *p*-nitrobenzoyl chloride (0.25g, 1.39 mmol) was added followed by the addition of Et_3N (0.21 mL, 1.5 mmol). The solution was stirred for 2h at room temperature and then quenched by

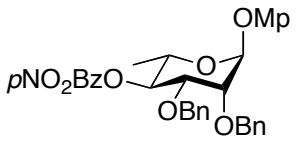
pouring the mixture into water. The product was extracted with EtOAc. The organic layer was washed with sodium bicarbonate solution, 2N hydrochloric acid solution and brine, dried over $MgSO_4$ and the solvent removed under reduced pressure. The crude product was purified by flash chromatography (2:1 hexane:ethyl acetate) to obtain **5** (R/S configuration) (0.442g, 71%, yellow crystal).

^{1}H NMR: (CDCl3, 600 MHz): δ (ppm) 7.91-7.89 (d, 2H), 7.63-7.61 (d, 2H), 7.57-7.54 (m, 5H), 7.15-7.13 (d, J = 12 Hz, 2H), 6.88-6.86 (d, J = 12 Hz, 2H), 6.21 (s, 1H), 5.25 (m, 1H), 4.39-4.34 (dd, 1H), 4.30 (m, 1H), 4.28-4.25 (m, 1H), 3.05-3.02 (m, 1H), 1.38 (d, 3H)

^{13}C NMR: CDCl3, 100 MHz): δ (ppm)) 171.1, 136.8, 136.3, 135.1, 134.2, 133.6, 128.4, 127.5.4, 117.6, 117.0, 96.2, 73.1, 72.2, 68.5, 66.4, 55.3, 18.1

R_f (Hexane: EtOAc 2:1) 0.40

1-O-*p*-Methoxy-2, 3-O-dibenzyl-4-O-*p*-nitrobenzoyl-α-L-rhamnopyranoside (10)



80% aqueous acetic acid was added to a solution of **5** (0.71g, 0.89 mmol). The reaction mixture was stirred at 65 ºC for 2h and then the solvent removed under reduced pressure to obtain crude 1-O-*p*-methoxy-4-O-*p*-nitrobenzoyl-α-L-rhamnopyranoside (0.28g).

To a solution of crude product (0.28 g) in 15.0 mL dichloromethane, benzyltrichloroacetimidate (0.20g, 0.82 mmol) was added at 0 ºC under Ar(g)

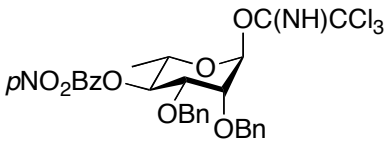
atmosphere, followed by the addition of triflic acid (0.10 mL, 680 μmol). The reaction mixture was stirred for 45 min. The mixture was quenched by adding triethylamine and concentrated under reduced pressure. The product was purified by silica gel flash chromatography (20% EtOAc in Hexane) to obtain **10** (0.63g, 68% over two steps, yellow oil).

^{1}H NMR (CDCl3, 600 MHz): δ (ppm) 8.32-8.30 (d, 2H), 8.29-8.27 (d, 2H), 7.38-7.29 (m, 10H), 7.12-7.10 (d, 2H), 6.88-6.86 (d, 2H), 5.42 (m, 2H), 5.10-5.08 (m, 1H), 4.92 (s, 2H), 4.89 (s, 2H), 4.23-4.20 (m, 1H), 3.81-3.79 (m, 1H), 3.71 (s, 3H), 3.52-3.49 (m, 1H), 1.31 (d, 2H)

^{13}C NMR (CDCl3, 162 MHz): δ (ppm) 165.1, 158.2, 150.9, 138.3, 138.0, 137.6, 137.1, 136.4, 130.9, 130.4, 129.1, 128.4, 113.6, 97.5, 79.2, 79.0, 77.2, 71.6, 65.5, 55.1, 54.2, 16.1

R_f (Hexane: EtOAc 2:1) 0.62

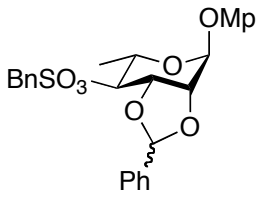
2, 3-O-Dibenzyl-4-O-*p*-nitrobenzoyl-L-rhamnopyranoside trichloroacetimidate (21)



To a solution of **10** (0.036g, 0.075 mmol) in CH_3CN: H_2O (5:1) ceric ammonium nitrate (0.12, 0.18 mmol) was added and the solution stirred for 1h. The reaction was quenched by the addition of sodium bicarbonate and extracted with dichloromethane. The organic

layer was dried over $MgSO_4$ and the solvent removed under reduced pressure to obtain crude product (0.021g), which was dissolved in dichloromethane (10.0 mL) under Ar (g) atm followed by the addition of K_2CO_3 (0.030g, 0.2 mmol). Trichloroacetonitrile (0.013 mL, 0.13 mmol) was added slowly and the reaction mixture was stirred for 45 min at 0 ºC. The reaction mixture was quenched by the addition of triethylamine (0.25 mL) and filtered through celite®, washed with dichloromethane and solvent removed under reduced pressure. The crude product was purified by flash chromatography (3:2 hexane:ethyl acetate) to obtain **21** (0.026g, 65% over two steps, yellow crystals).

1-O-*p*-Methoxy-2, 3-O-benzylidine-4-O-benzylsulfonyl-α-L-rhamnopyranoside (6)



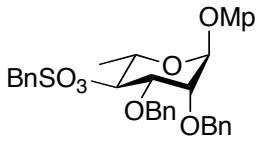
To a solution of **2** (0.365g, 1.05 mmol) in dichoromethane (12.0 mL), phenylmethylsulfonylchloride (0.22g, 1.16 mmol) was added followed by the addition of 2,6 –lutidine (0.24 mL, 2.11 mmol). The solution was stirred for 2h at room temperature and then quenched by pouring the mixture into water. The product was extracted with dichloromethane. The organic layer was washed with sodium bicarbonate solution, 2N hydrochloric acid solution and brine, dried over $MgSO_4$ and the solvent removed under reduced pressure. The crude product was purified by flash chromatography (2:1 hexane:ethyl acetate) to obtain **6** (R/S configuration) (0.48g, 89%, white solid).

^{1}H NMR: (CDCl3, 400 MHz): δ (ppm) 7.56-7.52 (m, 5H), 7.20-7.18 (d, 2H), 7.01-6.99 (d, 2H), 5.99 (s, 1H), 5.71 (s, 1H), 4.89-4.86 (m, 1H), 4.26 (s, 2H), 4.02-3.99 (m, 2H), 3.86-3.83 (m, 1h), 1.39 (d, 3H)

^{13}C NMR: CDCl3, 100 MHz): δ (ppm) 189.1, 131.5, 131.3, 129.4, 128.2, 127.2, 123.1, 117.8, 117.0, 115.3, 114.9, 97.1, 83.4, 71.8, 70.0, 69.9, 66.3, 57.8, 57.1, 56.2, 17.8

R_f (Hexane: EtOAc 2:1) 0.38

1-O-*p*-Methoxy-2, 3-O-dibenzyl-4-O- benzylsulfonyl--α-L-rhamnopyranoside (11)



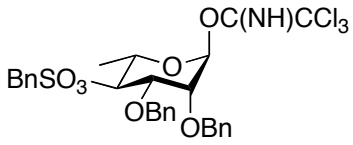
80% aqueous acetic acid was added to a solution of **6** (0.49g, 0.98 mmol). The reaction mixture was stirred at 65 ºC for 2h and then the solvent removed under reduced pressure to obtain crude product (0.23g), which was dissolved in DMF. NaH (0.10g, 3.9 mmol) was added under Ar (g) atmosphere at 0 ºC followed by the addition of BnBr (0.46 mL, 3.9 mmol). The reaction mixture was stirred at 0 ºC for 1h and then warmed to room temperature and stirred for 3h. The reaction was quenched by the addition of water. Organic layer extracted with EtOAc and washed with brine. The organic layer was dried over $MgSO_4$ and the solvent removed under reduced pressure. The crude product was purified by flash chromatography (2:1 hexane:ethyl acetate) to obtain **11** (0.32g, 56% over two steps, colorless oil).

^{1}H NMR: (CDCl3, 600 MHz): δ (ppm) 7.63-7.61 (m, 5H), 7.55-7.51 (m, 5H), 7.29-7.25 (m, 5H), 6.99-6.97 (d, 2H), 6.92-6.90 (d, 2H), 5.10 (s, 1H), 4.91-4.88 (m, 2H), 4.62 (s, 2H), 4.51 (s, 2H), 4.22-4.19 (m, 1H), 3.98 (s, 2H), 3.51-3.47 (m, 1H), 1.29 (d, 3H)

^{13}C NMR: CDCl3, 100 MHz): δ (ppm) 138.1, 134.5, 131.9, 129.4, 129.1, 128.6, 128.7, 127.7, 127.6, 115.2, 95.8, 79.8, 71.2, 70.3, 69.8, 59.1, 56.2, 18.1

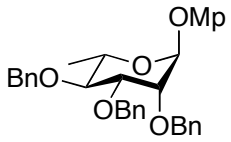
R_f (Hexane: EtOAc 3:2) 0.52

2, 3-O-Di-benzyl-4-O- benzylsulfonyl-α-L-rhamnopyranoside tricholoracetimidate (22)



To a solution of **11** (0.032g, 0.053 mmol) in CH_3CN:H_2O (5:1) ceric ammonium nitrate (0.06g, 0.10 mmol) was added and the solution stirred for 1h. The reaction was quenched by the addition of sodium bicarbonate and extracted with dichloromethane. The organic layer was dried over $MgSO_4$ and the solvent removed under reduced pressure to obtain crude product (0.022g), which was dissolved in dichloromethane (10.0 mL) under Ar (g) atmosphere. K_2CO_3 (0.031g, 0.22 mmol) was added followed by slow addition of trichloroacetonitrile (0.13mL, 1.36mol) and the reaction mixture was stirred for 45 min at 0 °C. The reaction mixture was quenched by the addition of triethylamine (0.15mL) and filtered through celite®, washed with dichloromethane and solvent removed under reduced pressure. The crude product was purified by flash chromatography (3:2 hexane:ethyl acetate) to obtain **22** (0.024g, 71% over two steps, colorless oil).

1-O-*p*-Methoxy-2, 3, 4-tribenzyl-α-L-rhamnopyranoside (12)



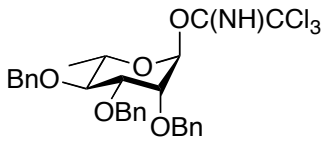
To a solution of **1** (0.82g, 2.1 mmol) in DMF, NaH (0.204g, 8.5 mmol) was added under Ar (g) atmosphere at 0 ºC followed by the addition of BnBr (1.0 mL, 8.5 mmol) The reaction mixture was stirred at 0 ºC for 1h and then warmed to room temperature and stirred for 3h. The reaction was quenched by the addition of water. The organic layer extracted with EtOAc and washed with brine. The organic layer was dried over $MgSO_4$ and the solvent removed under reduced pressure. The crude product was purified by flash chromatography (2:1 hexane:ethyl acetate) to obtain **12** (1.31 g, 63% yield).

^{1}H NMR (CDCl3, 600 MHz): δ (ppm) 7.49-7.46 (m, 5H), 7.38-7.32 (m, 10H), 6.95-6.93 (d, 2H), 6.90-6.88 (d, 2H), 5.22 (s, 1H), 5.14-5.12 (m, 1H), 4.89 (s, 2H), 4.65 (s, 2H), 4.41(s, 2H) 4.31-4.29 (m, 1H), 3.65-3.61 (m, 1H), 3.56 (s, 3H), 3.12-3.10 (m, 1H), 1.22 (d, 2H)

^{13}C NMR (CDCl3, 162 MHz): δ (ppm) 154.2, 129.8, 129.5, 129.2, 128.3, 128.0, 117.6, 115.3, 83.8, 78.2, 77.0, 76.8, 72.6, 63.6, 54.2, 53.8, 18.1

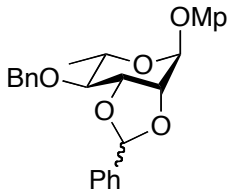
R_f (Hexane: EtOAc 2:1) 0.35

2,3,4-tribenzyl-α-L-rhamnopyranoside trichloroacetimidate (23)



To a solution of **12** (0.054g, 0.10 mmol) in CH_3CN: H_2O (5:1) ceric ammonium nitrate (0.1g, 0.12 mmol) was added and the solution stirred for 1h. The reaction was quenched by the addition of sodium bicarbonate and extracted with dichloromethane. The organic layer was dried over $MgSO_4$ and the solvent removed under reduced pressure to obtain the crude product (0.033g), which was dissolved in dichloromethane under Ar (g) atmosphere. K_2CO_3 (0.052g, 0.37 mmol) was added followed by slow addition of trichloroacetonitrile (0.02mL, 0.2 mmol) and the reaction mixture was stirred for 45 min at 0 ºC. The reaction mixture was quenched by the addition of triethylamine (0.15mL) and filtered through celite®, washed with dichloromethane and solvent removed under reduced pressure. The crude product was purified by flash chromatography (3:2 hexane:ethyl acetate) to obtain **23** (0.035g, 56% over two steps, white solid).

1-O-*p*-Methoxy-2, 3-O-benzylidine-4-O-benzyl-α-L-rhamnopyranoside (7)



To a solution of **2** (2.4g, 6.6 mmol) in DMF, NaH (0.24g, 9.9mmol) was added under Ar (g) atmosphere at 0 ºC followed by the addition of BnBr (1.17mL, 9.9mmol). The reaction mixture was stirred at 0 ºC for 1h and then warmed to room temperature and stirred for 6h. The reaction was quenched by the addition of water. The organic layer was extracted with EtOAc and washed with brine. The organic layer was dried over $MgSO_4$

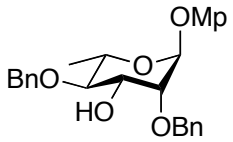
and the solvent removed under reduced pressure. The crude product was purified by flash chromatography (2:1 hexane:ethyl acetate) to obtain **7** (2.56g, 87%, white solid).

^{1}H NMR: (CDCl3, 600 MHz): δ (ppm) 7.61-7.55 (m, 2H), 7.49-7.46 (m, 3H), 7.32-7.27 (m, 3H), 7.17-7.15 (d, 2H), 6.88-6.86 (d, 2H), 6.05 (s, 1H), 5.78 (s, 1H), 5.12-5.10 (dd, 1H), 4.95-4.94 (dd, 1H), 4.65 (m, 1H), 4.01-3.99 (m, 1H), 3.86 (s, 3H), 1.28-1.26 (d, 3H)

^{13}C NMR: (CDCl3, 100 MHz): δ (ppm) 129.9, 129.1, 128.6, 128.4, 127.2, 126.8, 117.6, 115.5, 98.6, 79.9. 78.5, 74.5, 74.2, 55.8, 17.6

R_f (Hexane: EtOAc 2:1) 0.77

1-O-*p*-Methoxy-2, 4-O-di-benzyl-α-L-rhamnopyranoside (13)



80% aqueous acetic acid was added to a solution of **7** (2.56g, 5.71mmol). The reaction mixture was stirred at 65 ºC for 2h and then the solvent removed under reduced pressure to obtain crude product (1.6g), which was dissolved in CH_2Cl_2. NaOH (5% aq) was added at room temperature followed by the addition of BnBr (0.58 mL, 4.8 mmol). The reaction mixture was stirred at 0 ºC for 1h and then warmed to room temperature and stirred for 4h. The reaction was quenched by the addition of water. The organic layer extracted with EtOAc and washed with brine. The organic layer was dried over $MgSO_4$ and the solvent removed under reduced pressure. The crude product was purified by flash

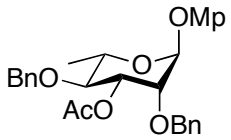
chromatography (2:1 hexane:ethyl acetate) to obtain **13** (1.74g, 67% over two steps, colorless oil).

^{1}H NMR: (CDCl3, 600 MHz): δ (ppm) 7.40-7.36 (m, 10H), 6.98-6.96 (d, 2H), 6.94-6.92 (d, 2H), 5.89 (s, 1H), 4.67 (s, 2H), 4.21(s, 2H), 4.01-3.99 (m, 1H), 3.66-3.63 (m, 1H), 1.36 (d, 2H)

^{13}C NMR: (CDCl3, 162 MHz): δ (ppm) 138.5, 138.3, 128.9, 128.6, 128.4, 127.4, 127.0, 117.9,115.6, 96.6, 94.8, 77.4, 74.3, 73.2, 71.9, 68.5,56.8 20.1

R_f (Hexane: EtOAc 2:1) 0.50

1-O-*p*-Methoxy-2, 4-O-di-benzyl-3-O-acetyl-α-L-rhamnopyranoside (15)



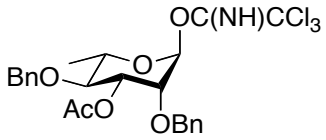
To a solution of **13** (0.43g, 0.61 mmol) in pyridine (10.0 mL), acetic anhydride (12.0 mL) was added at ambient temperature. The solution was stirred for 2h and then quenched by pouring the mixture into water. The product was extracted with EtOAc. The organic layer was washed with sodium bicarbonate solution, 2N hydrochloric acid solution and brine, dried over $MgSO_4$ and the solvent removed under reduced pressure. The crude product was purified by flash chromatography (2:1 hexane:ethyl acetate) to obtain **15** (0.41g, 85% yield).

^{1}H NMR: (CDCl3, 600 MHz): δ (ppm) 7.35-3.26 (m, 10H), 7.12-7.10 (d, 2H), 6.98-6.96 (d, 2H), 4.85 (d, 1H), 4.74 (d, , 1H), 4.68 (d,1H), 4.12-4.07 (m, 2H), 3.93 (dd, J = 12.8 Hz, 6.0 Hz, 1H), 3.75 (m, 1H), 3.36 (t, 1H), 2.56 (d, 1H), 2.0 (s, 3H), 1.32 (d, 3H).

^{13}C NMR: (CDCl3, 162 MHz): δ (ppm) 171.0, 112.5, 1128.0, 107.9, 107.6, 86.6, 81.7, 75.3, 72.9, 70.2, 68.1, 67.5, 21.1, 18.0.

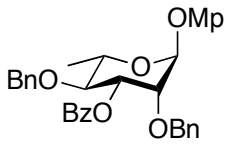
R_f (Hexane: EtOAc 3:2) 0.55

2, 4-O-Di-benzyl-3-O-acetyl-α/β-L-rhamnopyranoside trichloroacetimidate (24)



To a solution of **15** (0.025g, 0.05 mmol) in CH_3CN:H_2O (5:1) ceric ammonium nitrate (0.057g, 0.12 mmol) was added and the solution stirred for 1h. The reaction was quenched by the addition of sodium bicarbonate and extracted with dichloromethane. The organic layer was dried over $MgSO_4$ and the solvent removed under reduced pressure. The crude product (0.017g) was dried under vacuum and carried out to the next step. The crude product obtained was dissolved in dichloromethane under Ar (g) atmosphere, K_2CO_3 (0.031g, 0.22 mmol) was added followed by slow addition of trichloroacetonitrile (0.013mL, 0.13 mmol) and the reaction mixture was stirred for 45 min at 0 ºC. The reaction mixture was quenched by the addition of triethylamine (0.25mL) and filtered through celite®, washed with dichloromethane and solvent removed under reduced pressure. The crude product was purified by flash chromatography (3:2 hexane:ethyl acetate) to obtain **24** (0.021g, 77% over two steps, oil).

1-O-*p*-Methoxy-2, 4-O-dibenzyl-α-L-rhamnopyranoside (16)



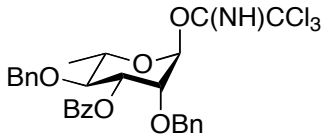
To a solution of **13** (0.436g, 1.2 mmol) in pyridine (12.0 mL), benzoyl chloride (0.15 mL, 1.3 mmol) was added at ambient temperature. The solution was stirred for 2h and then quenched by pouring the mixture into water. The product was extracted with EtOAc. The organic layer was washed with sodium bicarbonate solution, 2N hydrochloric acid solution and brine, dried over $MgSO_4$ and the solvent removed under reduced pressure. The crude product was purified by flash chromatography (2:1 hexane:ethyl acetate) to obtain **16** (0.518g, 92%).

^{1}H NMR (CDCl3, 600 MHz): δ (ppm) 7.67-7.64 (m, 5H), 7.39-7.33 (m, 5H), 6.98-6.96 (d, 2H), 6.92-6.90 (d, 2H), 5.46 (s, 1H), 5.32-5.30 (m, 1H), 4.65 (s, 2H), 4.35 (s, 2H) 4.31-4.29 (m, 1H), 3.44-3.42 (m, 1H), 3.38 (s, 3H), 3.22-3.20 (m, 1H), 1.33 (d, 2H)

^{13}C NMR (CDCl3, 162 MHz): δ (ppm) 201.2, 156.7, 139.2, 139.0, 138.9, 138.1, 137.2, 135.9, 134.4, 128.3, 128.0, 117.6, 96. 95.2,8, 76.2, 76.0, 74.7, 74.2, 68.8, 55.2, 53.8, 19.3.

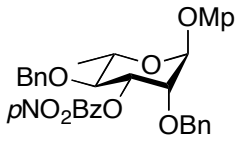
R_f (Hexane: EtOAc 3:2) 0.33

2, 4-O-Di-benzyl-3-O-benzoyl-α-L-rhamnopyranoside trichloroacetimidate (25)



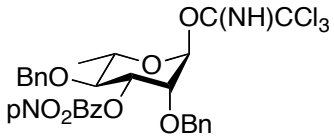
To a solution of **16** (0.036g, 0.64 mmol) in CH_3CN:H_2O (5:1) ceric ammonium nitrate (0.076g, 0.17 mmol) was added and the solution stirred for 1h. The reaction was quenched by the addition of sodium bicarbonate and extracted with dichloromethane. The organic layer was dried with $MgSO_4$ and the solvent removed under reduced pressure. The crude product (0.024g, 0.053 mmol) was dissolved in dichloromethane under Ar (g) atmosphere, K_2CO_3 (0.03g, 0.26 mmol) was added followed by slow addition of trichloroacetonitrile (0.15 mL, 1.59 mmol) and the reaction mixture was stirred for 45 min at 0 ºC. The reaction mixture was quenched by the addition of triethylamine (0.20 mL) and filtered through celite®, washed with dichloromethane and solvent removed under reduced pressure. The crude product was purified by flash chromatography (3:2 hexane:ethyl acetate) to obtain **25** (0.040 g, 83%, colorless oil).

1-O-*p*-Methoxy-2, 4-O-dibenzyl-3-O-*p*-nitrobenzoyl-α-L-rhamnopyranoside (17)



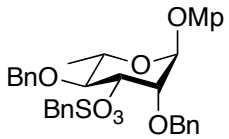
To a solution of **13** (0.051g, 0.13 mmol) in dichloromethane (15.0 mL), p-nitrobenzoyl chloride (0.023g, 0.12 mmol) was added followed by the addition of Et_3N (0.12 mL, 1.35 mmol). The solution was stirred for 2h at room temperature and then quenched by pouring the mixture into water. The product was extracted with EtOAc. The organic layer was washed with sodium bicarbonate solution, 2N hydrochloric acid solution and brine, dried over $MgSO_4$ and the solvent removed under reduced pressure. The crude product was purified by flash chromatography (2:1 hexane:ethyl acetate) to obtain **17** (0.051g, 76%, yellow solid).

2, 4-O-Dibenzyl-3-O-*p*-nitrobenzoyl-α-L-rhamnopyranoside trichloroacetimidate (26)



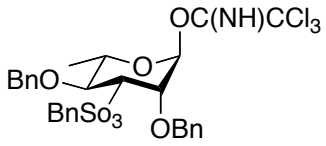
To a solution of **17** (0.051g, 0.086 mmol) in CH_3CN: H_2O (5:1) ceric ammonium nitrate (0.095g, 0.17 mmol) was added and the solution stirred for 1h. The reaction was quenched by the addition of sodium bicarbonate and extracted with dichloromethane. The organic layer was dried over $MgSO_4$ and the solvent removed under reduced pressure to obtain the crude product (0.37g). Dichloromethane (10.0 mL) was added to the crude product under Ar (g) atm followed by the addition of, K_2CO_3 (0.053g, 0.38 mmol) was added followed by slow addition of trichloroacetonitrile (0.023 mL, 0.23 mmol) and the reaction mixture was stirred for 45 min at 0 ºC. The reaction mixture was quenched by the addition of triethylamine (0.25 mL) and filtered through celite®, washed with dichloromethane and solvent removed under reduced pressure. The crude product was purified by flash chromatography (3:2 hexane:ethyl acetate) to obtain **26** (0.035g, 72% over two steps, colorless oil).

1-O-*p*-Methoxy-2, 4-O-dibenzyl-3-O- benzylsulfonyl-α-L-rhamnopyranoside (18)



To a solution of **13** (0.42g, 0.93 mmol) in dichoromethane (12.0 mL), phenylmethylsulfonylchloride (0.19g, 1.02 mmol) was added followed by the addition of 2,6 –lutidine (0.21 mL, 1.86 mmol). The solution was stirred for 2h at room temperature and then quenched by pouring the mixture into water. The product was extracted with dichloromethane. The organic layer was washed with sodium bicarbonate solution, 2N hydrochloric acid solution and brine, dried over $MgSO_4$ and the solvent removed under reduced pressure. The crude product was purified by flash chromatography (2:1 hexane:ethyl acetate) to obtain **18** (0.47g, 86%, white solid).

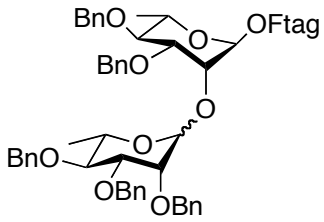
2, 4-O-Di-benzyl-3-O- benzylsulfonyl-α-L-rhamnopyranoside tricholoracetimidate (27)



To a solution of **18** (0.047g, 0.079 mmol) in CH_3CN:H_2O (5:1) ceric ammonium nitrate (0.087g, 0.15 mmol) was added and the solution stirred for 1h. The reaction was quenched by the addition of sodium bicarbonate and extracted with dichloromethane. The organic layer was dried over $MgSO_4$ and the solvent removed under reduced pressure to obtain crude product (0.31g), which was dissolved in dichloromethane (10.0 mL) under Ar (g) atmosphere. K_2CO_3 (0.044g, 0.03 mmol) was added followed by slow addition of trichloroacetonitrile (0.019mL, 0.19 mmol) and the reaction mixture was stirred for 45 min at 0 ºC. The reaction mixture was quenched by the addition of triethylamine (0.15mL) and filtered through celite®, washed with dichloromethane and solvent removed under reduced pressure. The crude product was purified by flash

chromatography (3:2 hexane:ethyl acetate) to obtain **27** (0.037g, 73% over two steps, colorless oil).

***cis*-4-(1H, 1H, 2H, 2H, 3H, 3H-Perfluoroundecyloxy)-2-butenyl 2, 3, 4-tri-O-benzyl-α/β-L-rhamnopyranosyl-(1→2)-3,4-di-O-benzyl-α-L-rhamnopyranoside (33)**



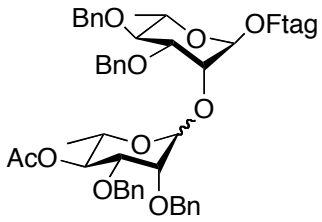
To a solution of **23** (0.035 mg, 0.59 mmol) and cis-4-(1H, 1H, 2H, 2H, 3H, 3H-perfluoroundecyloxy)-2-butenyl-2-O-hydroxyl-3, 4-O-dibenzyl-α-L-rhamnopyranoside (0.020 g, 0.023 mmol) in dichloromethane (4.0 mL), TMSOTf (650 μL, 35 μmol) was added at 0 ºC. The reaction mixture was stirred at 0 ºC for 2 h and then quenched with triethylamine (200 μL) and concentrated under reduced pressure. The crude product was purified by fluorous solid-phase extraction (FSPE) using a fluorous solid-phase extraction cartridge. Non-fluorous compounds were eluted with 80% MeOH/water and the desired product was eluted by 100% MeOH. The solvent was removed under reduced pressure to provide compound **33** (0.023 g, 75%, oil).

^{1}H NMR: (CDCl3, 600 MHz): δ (ppm) 8.64-8.62 (m, 3H), 7.53-7.49 (m, 5H), 7.35-7.31 (m, 5H), 7.22 (m, 2H), 5.68-5.66 (m, 2H), 5.12-5.94 (m, 1H), 4.94-4.88 (m, 1H), 4.84 - 4.70 (m, 1H), 4.67 (s, 2H), 4.63 (s, 2H), 4.16 (s, 2H), 3.94-3.91 (m, 1H), 3.63-3.61

(m,1H), 3.32-3.20 (m, 2H), 2.25-2.21, (m, 2H), 2.08-2.04 (m, 2H), 1.88 (d, 3H), 1.31 (d, 3H)

^{13}C NMR: CDCl3, 100 MHz): δ (ppm) 130.4, 130.2, 128.7, 128.5, 128.3, 127.5, 127.4, 127.2, 121.3, 120.9, 119.9, 119.2, 119.1, 117.4, 116.1, 93.9, 80.5, 80.2, 77.2, 75.4, 74.4, 72.7, 69.9, 68.7, 68.0, 56.5, 46.9, 30.0, 20.2, 18.8, 17.2, 9.0, 8.7

***cis*-4-(1H, 1H, 2H, 2H, 3H, 3H-Perfluoroundecyloxy)-2-butenyl 4-O-acetyl-2, 3-di-O-benzyl- α/β-L-rhamnopyranosyl-(1→2)-3,4-di-O-benzyl-α-L-rhamnopyranoside (29)**



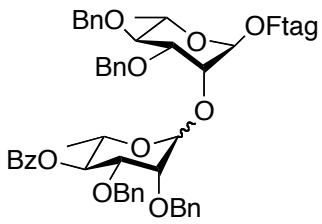
To a solution of **19** (0.027 mg, 0.046 mmol) and cis-4-(1H, 1H, 2H, 2H, 3H, 3H-perfluoroundecyloxy)-2-butenyl-2-O-hydroxyl-3, 4-O-dibenzyl-α-L-rhamnopyranoside (0.018 g, 0.020 mmol) in dichloromethane (4.0 mL), TMSOTf (540 μL, 30 μmol) was added at 0 ºC. The reaction mixture was stirred at 0 ºC for 2 h and then quenched with triethylamine (198 μL) and concentrated under reduced pressure. The crude product was purified by fluorous solid-phase extraction (FSPE) using a fluorous solid-phase extraction cartridge. Non-fluorous compounds were eluted with 80% MeOH/water and the desired product was eluted by 100% MeOH. The solvent was removed under reduced pressure to provide compound **29** (0.018 g, 74%, colorless oil).

^{1}H NMR (CDCl3, 600 MHz): δ (ppm) 7.54-7.46 (m, 5H), 7.43-7.24 (m, 5H), 5.72-5.69 (m, 2H), 5.42 (s, 1H), 4.94-4.93 (d, 1H), 4.81-4.79 (m, 1H), 4.69-4.63 (m, 2H), 4.37 (s, 2H), 4.08 (s, 2H), 3.54-3.47 (m, 2H), 3.35-3.34 (m, 1H), 2.26 (s, 3H), 1.96 (d, 3H), 1.43-1.21 (m,4H), 1.20 (d, 3H)

^{13}C NMR (CDCl3, 162 MHz): δ (ppm) 172.1, 160.0, 153.4, 138.4, 132.6, 132.1, 131.4, 131.1, 129.9, 129.7, 128.7, 127.9, 108.1, 107.2, 96.9, 86.4, 77.0, 76.8, 68.9, 66.2, 65.9, 58.7,47.5, 45.6, 31.2, 29.8, 24.3, 21.4, 20.7, 17.6

HRMS: Calcd for $C_{57}H_{61}O_{11}F_{17}K$: 1242.59, found [M+K+2D]: 1290.44

***cis*-4-(1H, 1H, 2H, 2H, 3H, 3H-Perfluoroundecyloxy)-2-butenyl 4-O-benzoyl-2, 3-di-O-benzyl- α/β-L-rhamnopyranosyl-(1→2)-3,4-di-O-benzyl-α-L-rhamnopyranoside (30)**



To a solution of **20** (0.040 mg, 0.067 mmol) and cis-4-(1H, 1H, 2H, 2H, 3H, 3H-perfluoroundecyloxy)-2-butenyl-2-O-hydroxyl-3, 4-O-dibenzyl-α-L-rhamnopyranoside (0.023g, 0.026 mmol) in dichloromethane (4.0 mL), TMSOTf (700μL, 39 μmol) was added at 0 ºC. The reaction mixture was stirred at 0 ºC for 2 h and then quenched with triethylamine (650 μL) and concentrated under reduced pressure. The crude product was purified by fluorous solid-phase extraction (FSPE) using a fluorous solid-phase extraction cartridge. Non-fluorous compounds were eluted with 80% MeOH/water and the desired

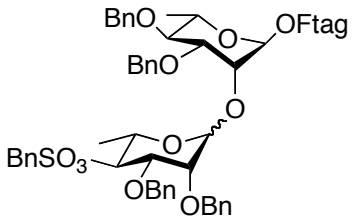
product was eluted by 100% MeOH. The solvent was removed under reduced pressure to provide compound **30** (0.021g, 61%, yellow oil).

^{1}H NMR: (CDCl3, 600 MHz): δ (ppm) 8.27-8.20 (m, 5H), 8.19-8.08 (m, 5H), 7.92 (m, 2H), 7.78 (m, 3H), 7.53- 7.20 (m, 5H), 7.41-7.37 (m,3H), 7.37-7.33 (m, 2H), 5.95 (m, 2H), 5.58 (m, 2H), 4.94 (dd, 1H), 4.80 (dd, 1H), 4.74 (d, 1H), 4.64 (d, 1H), 6.61 (s, 2H), 4.56 (d, 1H), 4.27-4.20 (m,1H), 4.11 (s, 2H), 4.06 (s, 2H), 3.57 (dd, 1H), 3.49 (s, 2H), 2.26-2.22 (m, 2H), 1.91-1.90 (m, 2H), 1.36 (d, 3H), 1.24 (d, 3H)

^{13}C NMR: CDCl3, 100 MHz): δ (ppm) 162.3, 162.3, 162.3, 134.5, 134.5, 134.4, 134.2, 130.6, 130.6, 130.5, 130.5, 128.9, 128.9, 128.8, 128.6, 128.5, 128.3, 128.1, 76.8, 72.9, 71.5, 65.7, 63.5, 60.7, 51.5, 51.2, 29.5, 29.3, 22.7, 13.9, 13.4.

HRMS: Calcd for $C_{62}H_{61}O_{11}F_{17}$: 1304.61, found [M+Na]: 1327.90

***cis*-4-(1H, 1H, 2H, 2H, 3H, 3H-Perfluoroundecyloxy)-2-butenyl 4-O-benzylsulfonyl-2, 3-di-O-benzyl- α/β-L-rhamnopyranosyl-(1→2)-3,4-di-O-benzyl-α-L-rhamnopyranoside (32)**



To a solution of **22** (0.024mg, 0.038 mmol) and cis-4-(1H, 1H, 2H, 2H, 3H, 3H-perfluoroundecyloxy)-2-butenyl-2-O-hydroxyl-3, 4-O-dibenzyl-α-L-rhamnopyranoside (0.013 g, 0.015 mmol) in dichloromethane (4.0 mL), TMSOTf (400 μL, 22 μmol) was

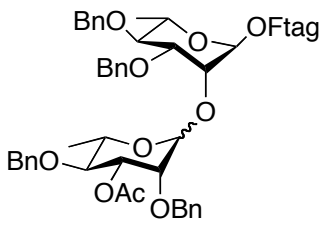
added at 0 ºC. The reaction mixture was stirred at 0 ºC for 2 h and then quenched with triethylamine (220 μL) and concentrated under reduced pressure. The crude product was purified by fluorous solid-phase extraction (FSPE) using a fluorous solid-phase extraction cartridge. Non-fluorous compounds were eluted with 80% MeOH/water and the desired product was eluted by 100% MeOH. The solvent was removed under reduced pressure to provide compound **32** (0.018 mg, 88%, white solid).

^{1}H NMR: (CDCl3, 600 MHz): δ (ppm) 7.56-7.51 (m, 5H), 7.41-7.35 (m, 5H), 7.32-7.27 (m,5H), 7.12-7.06 (m, 5H), 6.98-6.94 (m, 5H), 5.72-5.69 (m. 2H), 5.61 (s, 1H), 4.83-4.81 (m, 1H), 4.79-4.76 (m, 1H), 4.70 (s,2H), 4.62 (s, 2H), 4.62-4.57 (m, 2H), 4.16-4.06 (m, 1H), 3.94-3.89 (m, 1H), 3.67 (m, 1H), 3.19-3.14 (m, 1H), 1.66 (d, 3H), 1.53-1.50 (m, 2H), 1.42-1.39 (m, 2H)

^{13}C NMR: CDCl3, 100 MHz): δ (ppm) 140.1, 138.5, 137.9, 1, 129.4, 128.7, 128.2, 128.1, 127.9, 127.6, 127.5, 127.2, 127.0, 96.1, 82.9, 75.6, 70.4, 69.4, 68.0, 66.2, 56.2, 55.2, 54.5, 53. 2, 51.9, 45.2, 30.0, 29.7, 28.5, 25.2, 25.0, 23.7, 22.9, 13.9, 8.5

HRMS: Calcd for $C_{62}H_{61}O_{12}SF_{17}$: 1322.85, found [M+Na+3D]: 1348.61

***cis*-4-(1H, 1H, 2H, 2H, 3H, 3H-Perfluoroundecyloxy)-2-butenyl 3-O-acetyl-2, 4-di-O-benzyl- α/β-L-rhamnopyranosyl-(1→2)-3,4-di-O-benzyl-α-L-rhamnopyranoside (34)**

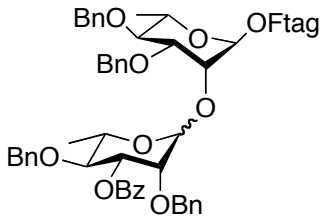


To a solution of **24** (0.021 mg, 0.040 mmol) and cis-4-(1H, 1H, 2H, 2H, 3H, 3H-perfluoroundecyloxy)-2-butenyl-2-O-hydroxyl-3, 4-O-dibenzyl-α-L-rhamnopyranoside (0.014 g, 0.016 mmol) in dichloromethane (4.0 mL), TMSOTf (240 μL, 24 μmol) was added at 0 ºC. The reaction mixture was stirred at 0 ºC for 2 h and then quenched with triethylamine (150 μL) and concentrated under reduced pressure. The crude product was purified by fluorous solid-phase extraction (FSPE) using a fluorous solid-phase extraction cartridge. Non-fluorous compounds were eluted with 80% MeOH/water and the desired product was eluted by 100% MeOH. The solvent was removed under reduced pressure to provide compound **34** (0.013 g, 68%, colorless oil).

^{1}H NMR: (CDCl3, 400 MHz): δ (ppm) 7.37 -7.30 (m, 15H), 7.10-7.04 (m, 5H), 5.79-5.77 (m, 2H), 5.76-5.74 (m, 2H), 5.12 (m, 2H), 4.61 (d, 1H), 4.49 (s, 2H), 4.47 (s, 2H), 4.45-4.43 (m, 3H), 4.03-3.98 (m, 1H), 3.86 (s, 2H), 3.46-3.42 (m, 2H), 2.12 (s, 3H), 1.89 (m, 2H), 1.28 (d, 3H), 1.26 (d, 2H)

^{13}C NMR: CDCl3, 100 MHz): δ (ppm) 170.6, 138.8, 138.5, 120.1, 128.9, 128.8, 128.7, 128.4, 128.0, 99.6, 99.1, 82.0, 77.5, 77.6, 76.4, 75.8, 74.3, 74.2, 68.1, 58.3, 28.5, 25.1, 24.3, 18.9, 18.8

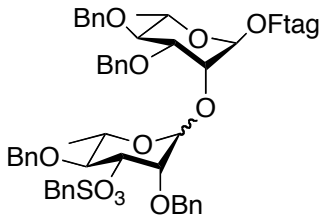
***cis*-4-(1H, 1H, 2H, 2H, 3H, 3H-Perfluoroundecyloxy)-2-butenyl 3-O-benzoyl-2, 4-di-O-benzyl- α/β-L-rhamnopyranosyl-(1→2)-3,4-di-O-benzyl-α-L-rhamnopyranoside (35)**



To a solution of **25** (0.025mg, 0.042 mmol) and cis-4-(1H, 1H, 2H, 2H, 3H, 3H-perfluoroundecyloxy)-2-butenyl-2-O-hydroxyl-3, 4-O-dibenzyl-α-L-rhamnopyranoside (0.015 g, 0.016 mmol) in dichloromethane (4.0 mL), TMSOTf (450 μL, 25 μmol) was added at 0 ºC. The reaction mixture was stirred at 0 ºC for 2 h and then quenched with triethylamine (150 μL) and concentrated under reduced pressure. The crude product was purified by fluorous solid-phase extraction (FSPE) using a fluorous solid-phase extraction cartridge. Non-fluorous compounds were eluted with 80% MeOH/water and the desired product was eluted by 100% MeOH. The solvent was removed under reduced pressure to provide compound **35** (0.013g, 62%, oil).

^{1}H NMR: (CDCl3, 400 MHz): δ (ppm) 7.397.35 (m, 10H), 7.35-7.28 (m, 10H), 5.86-5.84 (m, 2H), 5.63-5.61 (m, 2H), 4.99 (d, 1H), 4.97 (s, 2H), 4.86-4.84 (m, 2H), 4.82 (s, 2H), 4.64 (s, 2H), 4.46-4.44 (m, 2H), 4.18-4.16 (m, 2H), 4.12 (s, 2H), 3.98 (s, 2H), 3.95 (m, 2H), 3.89-3.87 (m, 2H), 2.12-2.10 (m, 2H), 1.86 (m, 2H), 1.24 (d, 3H), 1.22 (d, 2H)

***cis*-4-(1H, 1H, 2H, 2H, 3H, 3H-Perfluoroundecyloxy)-2-butenyl 3-O-benzylsulfonyl-2, 4-di-O-benzyl- α/β-L-rhamnopyranosyl-(1→2)-3,4-di-O-benzyl-α-L-rhamnopyranoside (37)**



To a solution of **27** (0.037 g, 0.060 mmol) and cis-4-(1H, 1H, 2H, 2H, 3H, 3H-perfluoroundecyloxy)-2-butenyl-2-O-hydroxyl-3, 4-O-dibenzyl-α-L-rhamnopyranoside (0.021 g, 0.024 mmol) in dichloromethane (4.0 mL), TMSOTf (650 μL, 36 μmol) was added at 0 ºC. The reaction mixture was stirred at 0 ºC for 2 h and then quenched with triethylamine (210 μL) and concentrated under reduced pressure. The crude product was purified by fluorous solid-phase extraction (FSPE) using a fluorous solid-phase extraction cartridge. Non-fluorous compounds were eluted with 80% MeOH/water and the desired product was eluted by 100% MeOH. The solvent was removed under reduced pressure to provide compound **37** (0.016 g, 71%, yellow oil).

^{1}H NMR: (CDCl3, 400 MHz): δ (ppm) 7.48-7.45 (m, 5H), 7.41-7.35 (m, 5H), 7.31-7.27 (m,5H), 7.11-7.06 (m, 5H), 6.98-6.94 (m, 5H), 5.72-5.69 (m. 2H), 5.61 (s, 1H), 4.83-4.81 (m, 1H), 4.79-4.76 (m, 1H), 4.70 (s,2H), 4.62 (s, 2H), 4.62-4.57 (m, 2H), 4.16-4.06 (m, 1H), 3.94-3.89 (m, 1H), 3.67 (m, 1H), 3.19-3.14 (m, 1H), 1.66 (d, 3H), 1.53-1.50 (m, 2H), 1.42-1.39 (m, 2H)

CHAPTER 3

Automated Solution-Phase Synthesis of Plant Polysaccharide Homogalacturonan (HG)

Sahana K. Nagappayya and Nicola L.B. Pohl*

Portions of this chapter will be submitted to *Organic and Biomolecular Chemistry*

Abstract

A divergent synthesis of plant polysaccharide homogalacturonan (HG) backbone is developed. The α-linked galacturonic acid (galA) containing HG backbone is synthesized by glycosylation of galA trichloroacetimidate donors with galA fluorous-tagged acceptors on an automated solution phase platform. This is the first example of an automated solution phase synthesis of HG fragments.

Introduction

Plant cell wall consists of nearly 90% saccharides and 10% proteins, which are in the form of glycoproteins (McNeil et al, 1984). Cellulose, xyloglucan, xylans, xylogalacturonan, homogalacturonan, rhamnogalacturonans I and II are some of the polysaccharide components of plant cell wall. The various saccharides found on these polysaccharide components are D-glucose, D-galactose, D-galacturonic acid, L-rhamnose, D-xylose in varied linkages like β-1,4-linked-D-glucosyl residues in cellulose and xyloglucans, β-1,4-linked xylose in xylans and β-1,2- linked in xyloglucans, α-1,4-linked D-galacturonic acid in homogalacturonan and rhamnogalacturon, α-L-rhamnosyl residues, on the backbone along with many other sugar residues like D-galactose, D-

arabinose, L-fucose and unusual sugar like kdo (2-deoxy-D-manno-oct-2-ulosonic acid) on the side-chains of these polysaccharide backbones, shown in Fig 3-1.(Talmadge, et al, 1973; Johansen et al, 2006). The heterogeneity of all the polysaccharides on the plant cell wall makes it one of the most complex natural structures.

Majority of plant cell wall contain pectin polysaccharides (Ridley et al, 2001). Pectin is a class of polysaccharide that consists of 1,4- linked galacturonic acid residue on the backbone. The most abundant classes of pectin are homogalacturonan (HG) and rhamnogalacturonan (RG). Homogalacturonan (HG) is an important pectic polysaccharide containing linear chain consisting of repeating units of α-1, 4- linked galacturonic acid (Fig 3-2). Free acid and methyl esterified D-galacturonic acid residues are found on HG (O'Neil, et al, 1990, Caffall et al, 2009). This methyl esterification of the galacturonic acid seems to have a role in the various functions of the HG.

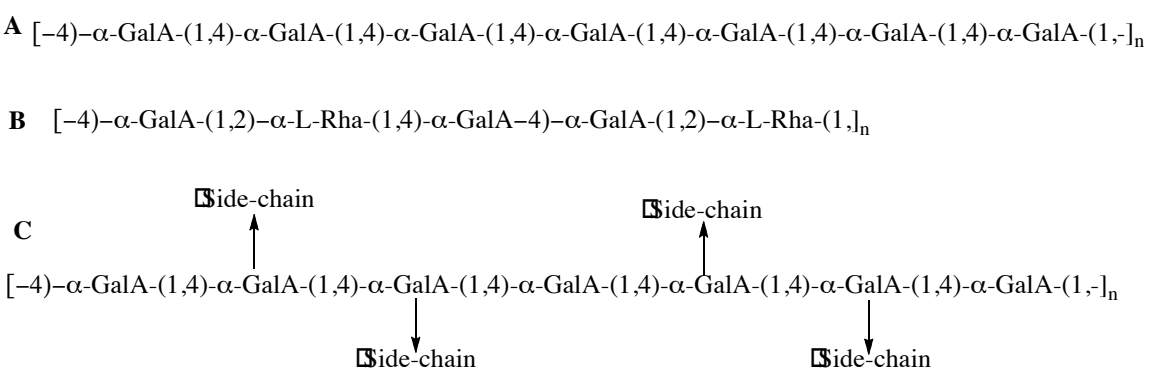


Figure 3-1. Various pectic polysaccharides of the plant cell wall. A. Homogalacturonan (HG) B. Rhamnogalacturonan-I (RG-I) C. Rhamnogalacturonan-II. The side chains on the RG-II framework contains many sugar residues like α-L-fucose, β-D-glucuronic acid, methyl ester of β-D-glucuronic acid, α-L-arabinose, β-D-galactose etc (Mohanen, 2008)

A [–4)–α-GalA-(1,4)-α-GalA-(1,4)-α-GalA-(1,4)-α-GalA-(1,4)-α-GalA-(1,4)-α-GalA-(1,4)-α-GalA-(1,-]$_n$

B

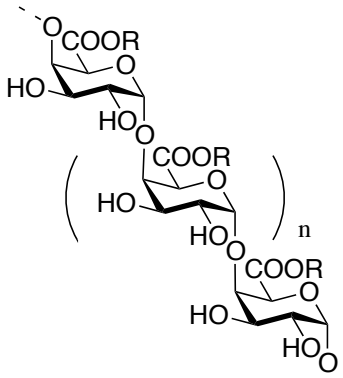


Figure 3-2. Representation of the pectic polysaccharide homogalacturonan. A. Galactuonic acid residues are connected as 1, 4- α - linkages B. HG backbone with 1, 4-α-galacturonic acid linkages shown.

From the studies carried out by the enzymatic digestion of HG backbone, it was found that the highly methyl-esterified portion of the HG could not be cleaved by some enzymes, indicating that the esterification and the pattern of esterification of the galacturonic acid in HG is an important modification of the plant cell-wall (Knox et al, 1990). Also, the highly methyl-esterified portion of the HG was found to be porous and provide permeability to the cell wall whereas the de-esterified portion of the HG was found to bind with Ca^{2+} and form gels, which is responsible for the mechanical strength of the cell wall (Willats et al, 2001). This rigid gel-like structure formation of de-esterified galacturonic acid and the porous nature of the methyl-esterified portions of HG are important contributors to the structural properties of plant cell wall. This esterification and de-esterification of the galacturonic acid in HG take place at various developmental cycle of the plant growth (Knox, 1992).

Along with the structural function of HG, owing to the presence of esters on the galacturonic acid residues, HG is also found to be involved in the defense mechanism of

the plant (McNeil et al, 1984). Not just the long chain polysaccharide of HG, but also smaller fragments of the plant polysaccharide are found to be very important in the defense mechanism of plants. It is well established that HG is involved in the synthesis of phytoalexins, antibiotic compounds produced by plants (McNeil et al, 1984). The production of phyloalexin is considered to be a defense mechanism in plants and the compounds that induce phyloalexin synthesis are termed elicitors (McNeil et al, 1984). It was found that the HG fragments containing α-linked galacturonic acid residues showed elicitor activity (Nothnagel, et al, 1983; Hahn et al, 1981). In this work, the HG fragments were isolated from the plant cell wall by enzymatic digestion. Upon incubation with *endo*-α-1, 4-polygalacturonase, an enzyme that breaks down the α -1, 4- linkage to give free galacturonic acid, the elicitor activity of HG fragment was lost (Nothnagel et al, 1983). This study also implies that the galacturonic acid residues have to be contiguous for the elicitor activity of HG. In another study, the unesterified region of the HG fragment was found to be the elicitor-active portion of the plant polysaccharide (Davis et al, 1986). In this study, the enzyme α-1, 4-D-endopolygalacturonic acid lyase isolated from phytopathogenic bacterium *Erwina carotovora* was allowed to digest fragments of plant cell wall obtained from soybean and after extensive purification of the digested fragments, the authors concluded that the polysaccharide chain containing the α-1, 4-D-galacturonic acid fragment had high elicitor activity.

Another study on fragments of plant polysaccharide extracted from sycamore cell wall has shown that the HG portion of plant cell wall is also involved in hypersensitive cell death (Yamazaki et al, 1983). Hypersensitive cell death is a defense mechanism adopted

by plant cells where, upon invasion of cells by microbes, host cells start to die (Maclean at al, 1974). Strong evidence of the involvement of fragments of HG in inducing this hypersensitive cell death is found by assays of sublethal doses of toxic fragments of HG fragments digested by enzymes (Yamazaki, et al, 1983).

Thus, the role of the HG fragments in the defense mechanism and structural rigidity of plant cell wall is extremely crucial. To study such activity of the HG in plant cell wall, a large polysaccharide of the α-1, 4-linked galacturonic acid HG is not required, a smaller oligosaccharide chain is also potent to study the activity. This makes the chemical synthesis of HG fragment an interesting target for carbohydrate chemists and then subsequently study for their activities. Although the fragments of the HG can be obtained by enzymatic action on the plant polysaccharide and then purification by a number of methods, this enzymatic extraction has many disadvantages. Firstly, not all the enzymes for synthesis of the various epitopes of the plant cell wall are known (Daas et al, 2001). Also, enzymatically cleaved HG fragments are not always homogeneous, random selection of fragments can be obtained and thus, the purification and further study on these fragments become challenging (McNeil et al, 1984). For systematic structure-function relationship study of plant polysaccharides, clear and characterized structures are important. To obtain well-defined, homogeneous fragments of polysaccharides, chemical synthesis from readily available monosaccharides is a viable option. Previous efforts by many researchers to synthesize pure well-characterized fragments of plant polysaccharides have been carried out to obtain varying lengths of oligosaccharide chain from two to six units (Yamamoto et al, 2005; Clausen et al, 2003). In the studies

presented in this chapter, we have synthesized the D-galacturonic acid building blocks and then assembled these building blocks together on an automated solution phase platform developed in the Pohl Labs at Iowa State University.

Automating the process of glycosylation reaction has been of considerable interest to groups across the world in the recent past (Zhang et al, 1999; Plante et al, 2001; Pohl, 2008a). The first programmable synthesis of oligosaccharide was attempted by Wong and co-workers (Zhang et al, 1999). In this work, the researchers utilized a one-pot glycosylation strategy where all the glycosyl donors and acceptors required to build a polysaccharide were introduced into the reaction pot sequentially, eliminating purification at each step. The difference in reactivities of the various acceptors and donors was utilized and glycosyl donors were introduced into the reaction mixture based on their reactivity, with the most reactive donor added first and lesser reactive donors added later. This was a first step toward making oligosaccharide synthesis generally applicable and programmable. Although this is an elegant approach to the synthesis of oligosaccharides, the challenges in synthesizing sequentially less reactive building blocks to be introduced into the reaction pot at specific times, renders this method less applicable.

The first robotically controlled synthesis platform to automate the oligosaccharide synthesis was developed by Seeberger and co-workers (Plante et al, 2001). In this process, a solid-phase peptide synthesizer was converted into a platform for carrying out glycosylation reactions. Once a computer program is set for the synthesizer, a robot

delivers the solution of the glycsoyl acceptors, donors and various reagents required for glycsosylation. During the whole synthesis cycle, the growing oligosaccharide chain is bound to a solid resin and is cleaved from it once the entire cycle of glycosylation reaction is complete. Although an attractive and innovative method for the synthesis of oligosaccharide chains, this method suffers form some fundamental setbacks. Firstly, the number of equivalents of the glycosyl donors for each coupling cycle varies from 10-15, which makes this synthesis extremely expensive. After all the protection-deprotection strategies applied to a monosaccharide to make it a glycosyl donor, the donor becomes expensive and using 10-15 equivalents of the expensive donor for one glycosylation makes this method extremely uneconomical. The other major drawback of this solid-phase automated synthesis is the fact that each glycosylation reaction takes place on a starting material bound to the resin. If there is an error while building the sequence, it cannot be monitored and thus, cannot be rectified during the reaction sequence. Only after the reaction is complete and the final product is cleaved from the resin would the mistake be identified.

Another approach to automate the synthesis of oligosaccharide chain is the use of a solution-phase synthesis platform (Pohl, 2008a). In this method, a robotic platform is used to carry out the entire sequence of glycosylation, deprotection and purification. Solutions of glycosyl donor and acceptors are transferred using a programmable needle to place in appropriate reaction vials to carry out glycosylation reactions. This solution-phase automated synthesis of oligosaccharide requires only about 2-3 equivalents of the glycosyl donor. One of the most ingenious modifications in the solution-phase automated

synthesis is the ability to monitor the reaction sequence after each step. After each glycosylation and deprotection reaction, a small aliquot of the reaction mixture is transferred to the TLC block. A quick TLC of the reaction would give an indication of the progress of the reaction.

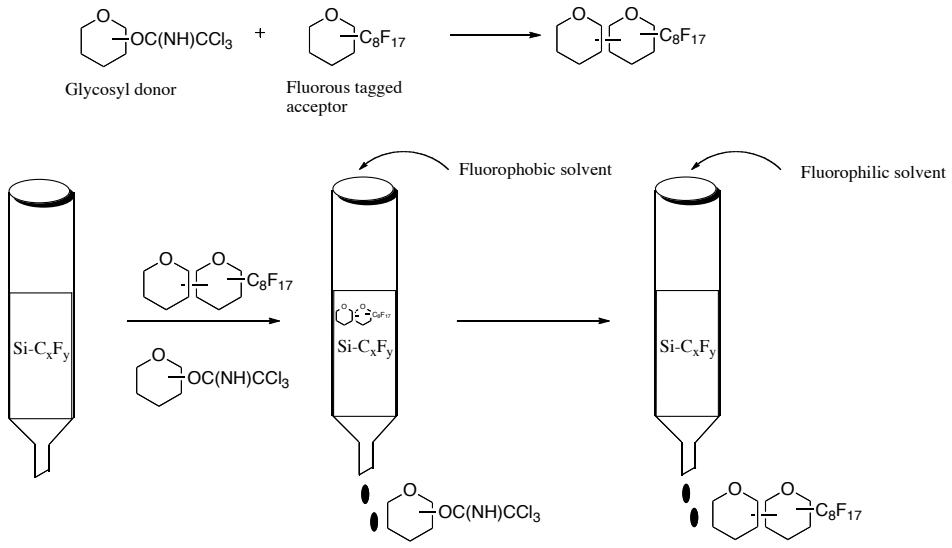


Figure 3-3. Fluorous Solid Phase Extraction (FSPE) for fluorous tagged oligoasaccharide separation

Another advantage of using the automated solution-phase synthesis platform is the use of Fluorous Solid Phase Extraction (FSPE). The FSPE is a separation method where a reverse phase silica gel bonded to fluorocarbon is used to separate out compounds based on their fluorous content (Curran, 1998; Curran, 2001). In the automated solution-phase oligosaccharide synthesis, the growing saccharide chain is attached to a fluorous tag. A fluorous-tagged acceptor is synthesized and the first glycosylation takes place between the trichloroacetimidate donor and the fluorous-tagged acceptor. The purification of the disaccharide built at this stage is carried out by FSPE. A FSPE cartridge is introduced in the automated solution-phase synthesis platform, and after each deprotection cycle, the

reaction mixture is passed through this FSPE cartridge with appropriate fluorophobic and fluorophilic solvents to separate out fluorous-tagged oligosaccharide and the unreacted sugars, as shown in fig. 3-3.

Results and discussions

To synthesize the 1, 4- α linked D- galacturonic acid backbone on the automated solution phase synthesizer, a few factors had to be taken into account to design the right donor and acceptor molecules. The solution phase automated synthesizer is based on Schmidt's tricholoroacetimidate chemistry (Schmidt et al, 1980). This is owing to the milder reaction conditions and reaction times on using the TCA donors compared with sulfur based donors. The activating conditions for using a sulfur based donors, previously used by Dautheau and co-workers to build α-linked galacturonic acid (Magaud et al, 1997), requires the activation condition of NIS and TfOH at -60 ºC. This low temperature is not achieved on the available automated solution phase synthesizer. Although attempts are currently going on to use sulfur based donors on the automated platform, these studies are still in their infancy. On using TCA donors, the glycosylation reactions can be carried out 0 ºC, thus making the automation platform extremely suitable for tandem glycosylations.

The α-selectivity of the glycosylation reaction is extremely important for building the HG backbone. On bechtop, since each step of glycosylation and deprotection can be worked up, the mixture of α/β products can be separated by tedious silica gel chromatography. But in case of glycosylations on the automated platform, the separation of any α/β mixture is avoided as much as possible since the entire reaction sequence is

carried out on the platform and then removed after the final deprotection step. FSPE separation is effective to separate mixtures on the basis of the fluorous content of the compounds; it is not used to separate mixtures of α/β products. This makes the design of the donor and acceptor building blocks extremely crucial.

One of the most important factors in obtaining this selectivity is neighboring group participating groups at position 2-O. It is well known that by employing participating groups at 2-O positions, the β-product predominates due to anchimeric assistance. So, we had to design the D-galA building block with no neighboring group participating group at 2-O position. Thus we used benzyl ether to protect the 2-hydroxyl. Now, the group at position 4-O of the galA building block had to be such that it could be deprotected under mild conditions in the solution phase automated synthesizer and would also give good α-selectivity of the glycosylation reaction.

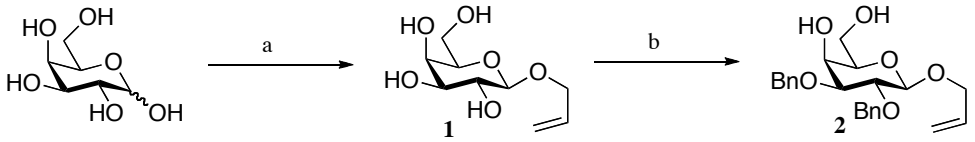
Previously, the 4-O hydroxyls of the D-galA donors have been protected as benzyl ether (Magaud et al, 1997), methoxyphenyl ether (Yamamoto et al, 2005) or allyl ether (Clausen et al, 2003). These protecting groups at 4-O- have provided excellent α-selectivity. But their deprotection conditions are harsh, and require long reaction times. On the automated solution phase synthesizer, shorter reaction times are preferred as the entire assembly of glycosyl donor and acceptors are kept in vials inside the synthesizer platform during the entire process of synthesis for the robotic needle to transfer. Although this assembly is under inert Ar(g) atmosphere, shorter reaction times to minimize any exposure to moisture and air are preferred. On the automated solution phase synthesizer,

the deprotection condition for protecting groups like acetate, levulinic ester, silyl protecting groups etc have been optimized.

With all these factors in mind, we set out to design the D-galacturonic acid building block for our synthesis on the automated platform. Starting with D-galactose, 1-O-allyl-β-D-galactopyranoside was synthesized in 3 steps in high yield. 2, 3-O-benzyl-1-O-allyl-β-D-galactopyranoside was then synthesized by first protecting the 4 and 6- hydroxyls as the benzylidene acetal, and then installing non-participating benzyl ethers at the 2 and 3-O positions followed by opening the benzylidene acetal under standard aqueous acetic acid condition (Scheme 2-1).

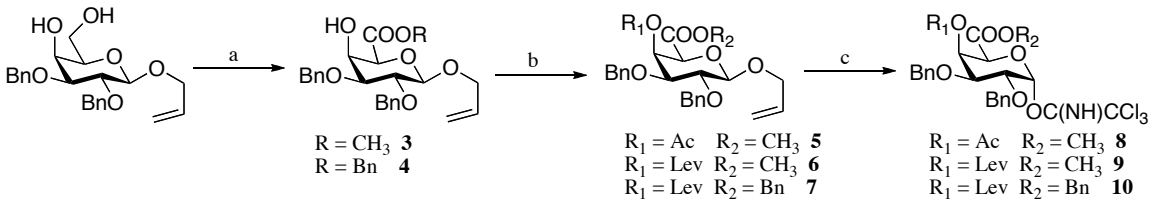
We first synthesized a 4-O acetate building block. To synthesize the galacturonic acid, the 6-O position was oxidized by BAIB/TEMPO at room temperature. After the mild oxidation, the resulting carboxylic acid was methyl esterified under microwave conditions. The allyl anomeric protecting group was removed by first isomerizing the allyl ether to a vinyl ether with Ir(I) catalyst followed by hydrolysis of the vinyl ether with Hg salts, The anomeric hydroxyl was converted to the tricholoroacetimidate donors under basic conditions (Scheme 2-2). Benchtop glycosylation with the 4-O acetate donor **8** with the glycosyl fluorous tag acceptor **12** revealed that there was β-glycosyl linkage also being formed. The α/β selectivity with this building block could be improved. We speculate that this is perhaps due to the long-range participation by the acetate group. In order to avoid synthesizing mixtures of α and β compounds on the synthesizer, we set out to synthesize 4-O levulinic ester protected galA trichloroacetimidate donor **9** and **10**

(Scheme 3-2). The α-selectivity with this building block was excellent. TLC showed that there was no β-glycosylated product formed. Benzyl ester of the 4-O levulinoyl carboxylic acid was also formed by treating the acid with benzyl bromide as shown in Scheme 3-2. We synthesized the benzyl ester of gala to install in the backbone of the HG molecule at specific location. After fully deprotecting all the benzyl groups on the HG fragment obtained from the synthesizer, there would be free acid left at the location of benzyl ester, which could be very useful for any assay with the backbone.



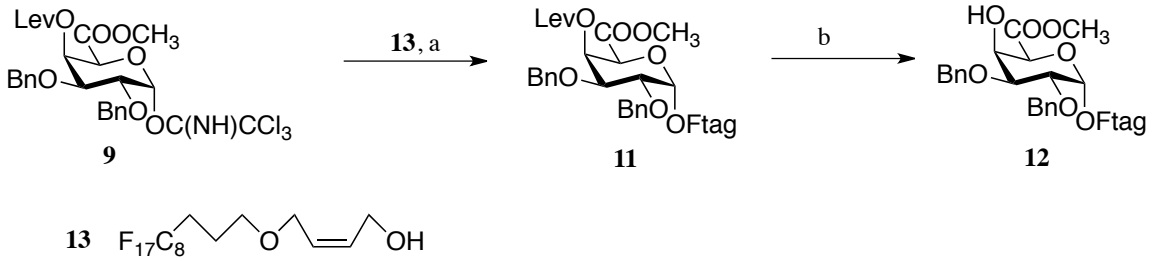
Conditions: a. (i) NaOAc, Ac_2O (86%) (ii) Allyl alcohol, BF_3OEt_2, CH_2Cl_2 (82%) (iii) Na, CH_3OH (85%); b. (i) $PhCH(OCH_3)_2$, *p*TSA, CH_3CN (89%) (ii) BnBr, NaH, DMF (81%) (iii) 80% CH_3COOH/H_2O (76%)

Scheme 3-1. Synthesis of 3, 4-dibenzyl-1-O-allyl-β-D-galactopyranoside (**2**)



Conditions: a. (i) BAIB, TEMPO, CH_2Cl_2, H_2O (ii) For **3** CH_3OH, cat. H_2SO_4, μwave (82% over two steps) For **4** BnBr, TBAI, Et_3N, THF (81% over two steps) b. For **5** Ac_2O, py (64%) For **6** & **7** LevOH, DCC, DMAP (**6** 85%; **7** 92%) c. (i) $Ir[COD(PPhMe_2)_2]PF_6$,H_2, THF (ii) HgO, $HgCl_2$ (ii) Acetone/H_2O **8** (64%), **9** (78%), **10** (85%)

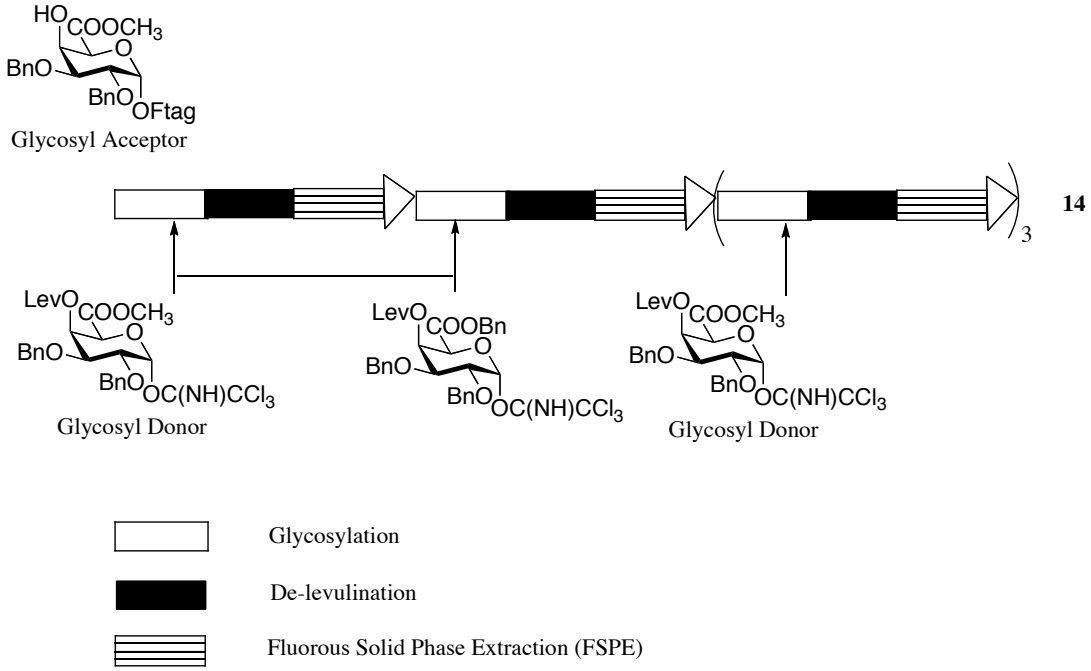
Scheme 3-2. Pre-glycosylation oxidation of D-galactose



Condition: a. **13**, TMSOTf (0.10 equiv), CH_2Cl_2 (77%) b. Hydrazine acetate, CH_2Cl_2 (94%)

Scheme 3-3. Synthesis of the fluorous-tag acceptor of D-galacturonic acid.

Fluorous alcohol **13** was reacted with the trichloroacetimidate donor D-galacturonic acid **8** containing the methyl ester to give the fluorous-tagged acceptor **11** for the reaction on the solution-phase automated platform (Scheme **3-3**). With these building blocks, **9**, **10** and **12** in hand, we set out to synthesize the HG backbone on the automated solution-phase synthesizer. The scheme of the reaction on the automated platform is shown in Scheme 3-4.



Scheme 3-4: A schematic of the automated solution-phase workbench to build the HG hexasaccharide **14** (Note: The protocol for each step is explained in more detail in the experimental section)

We introduced benzyl ester protected galA (**10**) as the second glycosyl donor, and all other positions of the glycosyl donor in the sequence were the methy ester of the galA (**9**). This produced the hexasaccharide **14**, with one benzyl ester and the five methyl ester of the D-galacturonic acid. The benzyl ethers on the backbone were deprotected with H_2/Pd, which also deprotected the benzyl ester of the galacturonic acid, giving the hexamer, **15** with one free acid residue at the backbone of the HG fragment. The hexasaccharide still contains the fluorous-tag at the reducing end of the oligosaccharide chain, so the chain is available for any further arrays based on the fluorous-tag.

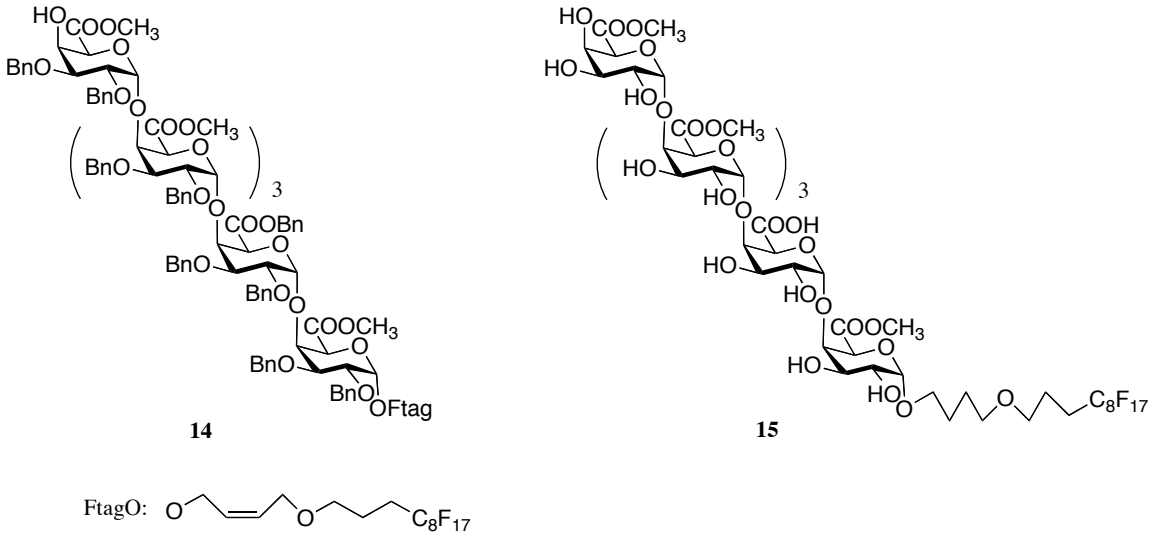


Figure 3-3. The hexasaccharide of homogalacturonan obtained from the automated solution-phase synthesizer (**14**).

Conclusion

In conclusion, homogalacturonan fragment containing six α-D-galacturonic acid residues was successfully synthesized starting with D-galactose in 4% overall yield. This is the first example of an automated synthesis of HG. With the right building blocks in hand, their step-wise assembly on the automated solution phase platform is shown to be high yielding. This is an example of the application of the solution-phase automated synthesis to form linear oligosaccharide chain efficiently. The deprotected hexasacharide synthesized contains one free carboxylic acid, which can be further used in enzymatic assays to study the impact of the methyl esterification of the HG backbone. Also, due to the presence of the fluorous-tag at the reducing end of the oligosaccharide chain, fluorous based arrays are also possible using these HG fragments. Thus, chemical synthesis of polysaccharides can be an important tool in further studies on the importance and applications of these polysaccharides, and automation provides a simpler way by which these oligosaccharides can be assembled.

Experimental section

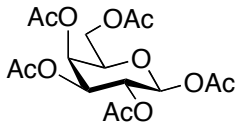
General methods

Reaction solvents were used directly from solvent tower (Swagelok). All other commercial reagents and solvents were used as received without further purification. The reactions were monitored and the *R*f values determined using analytical thin layer chromatography (TLC) with Sorbent Technologies Silica gel HL TLC plates with UV 254 (250 μm). The developed TLC plates were visualized by immersion in a solution of EtOH: conc. H_2SO_4: *p*-anisaldehyde (9:1:1) followed by heating on a hot plate. Silica gel flash chromatography was performed with ZeoPrep 60 Eco 40-63 μm. Fluorous phase

chromatography using fluorous solid-phase extraction cartridges containing silica gel bonded with perfluorooctylethylsilyl chains (Fluorous Technologies, Inc.; Pittsburgh, PA). All other fluorous reagents were also obtained from Fluorous Technologies, Inc. All moisture-sensitive reactions were performed in flame- or oven-dried glassware under a Ar(g) atmosphere. Bath temperatures were used to record the reaction temperature in all cases run without microwave irradiation. Microwave reaction was carried out in CEM Microwave. All reactions were stirred magnetically at ambient temperature unless otherwise indicated. ^{1}H NMR and ^{13}C NMR spectra were obtained with a VarianVXR at 400 MHz and 100 MHz or BrukerDRX at 600 MHz. ^{1}H NMR spectra were reported in parts per million relative to $CDCl_3$ (7.26 ppm) and ^{13}C NMR spectra were reported in parts per million relative to $CDCl_3$ (77.23 ppm).

Synthetic procedure

1, 2, 3, 4, 5, 6- Tetra-O-acetyl-β-D-galactopyranoside



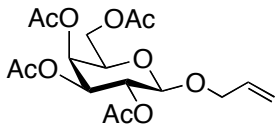
To a solution of sodium acetate (2.27 g; 0.027 mol) in 20 mL acetic anhydride at 80 ºC, β-D-galactopyranoside (1.0 g; 5.5 mmol) was added in portions. After the addition, the reaction mixture was cooled and diluted with EtOAc, washed with sodium bicarbonate solution and brine, dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The crude product was recrystallized with ethanol to obtain peracetylated β-galactopyranoside (1.86g, 86%, white crystals).

^{1}H NMR (400 MHz, CDCl3): δ (ppm) 5.55-5.53 (d, J = 8 Hz, 1H), 5.42 (dd, 1H), 5.39-5.37 (m, 1H), 5.28-5.25 (m, 1H), 4.32-4.29 (m, 2H), 4.27-4.24 (m, 1H), 2.24 (s, 3H), 2.20 (s, 3H), 2.18 (s, 3H), 2.10 (s, 3H)

^{13}C NMR (100 MHz, CDCl3): δ (ppm) 170.2, 170.0, 169.8, 169.7, 169.5, 92.1, 70.2, 70.0, 68.1, 68.0, 61.0, 20.1, 19.8, 19.7

R_f (Hexane: EtOAc 2:1) 0.40

1-O-allyl-2, 3, 4, 6-O-tetra-acetyl-β–D-galactopyranoside



To a solution of peracetylated-β–galactopyranoside (1.86 g, 4.76 mmol) in 20 mL anhydrous dichloromethane at 0 ºC, (5.97 mL, 0.047 mol) $BF_3.OEt_2$ was added dropwise under Ar(g) atmosphere. The reaction mixture was stirred at 0 ºC for 30 minutes followed by the addition of allyl alcohol (1.6 mL, 0.023 mol). This mixture was allowed to warm to room temperature and stirred. After 4h, the mixture was extracted with EtOAc, washed with sodium bicarbonate solution, and brine. Dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. Silica gel flash chromatography with hexane: EtOAC (1:4) was performed to obtain 1-O-allyl-2, 3, 4, 6-O-tetra-acetyl-β–D-galactopyranoside (1.53g, 82 %, oily syrup).

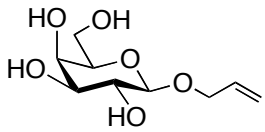
^{1}H NMR (400 MHz, CDCl3): δ (ppm) 5.90-5.83 (m, 1H), 5.39-5.38 9dd, 1H), 5.25-5.24 (dd, 1H), 5.22-5.19 (m 1H), 5.25-5.24 (d, 1H), 4.53-4.51 (d, J=8Hz, 1H), 4.37-4.35 (m,

1H), 4.32-4.31 (m, 1H), 4.21-4.20 (m, 1H), 2.09 (s, 3H), 2.08 (s, 3H), 2.06 (s, 3H), 2.05(s, 3H)

^{13}C NMR (100 MHz, CDCl3): δ (ppm) 206.8, 170.9, 170.7, 169.2, 91.7, 74.1, 70.6, 69.3, 68.0. 62.8, 30.8, 20.6, 20.8

R_f (Hexane: EtOAc 2:1) 0.35

1-O-Allyl-β-D-galactopyranoside (1)



1

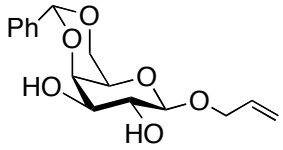
1-O-allyl-2, 3, 4, 6-O-tetra-acetyl-β–D-galactopyranoside (1.5g, 3.96 mmol) was dissolved in 18 mL methanol and 0.24 g of sodium metal was added. The solution was stirred for 1h and quenched by adding Dowex®, and filtered over a pad of Celite®, washed with methanol and concentrated. Flash chromatography with 2% methanol in dichloromethane was carried out to obtain **1** (0.74g, 85%, white solid)

^{1}H NMR (400 MHz, CD3OD): δ (ppm) 6.02-5.99 (m, 1H), 5.30-5.28 (d, 1H), 5.13-5.11 (d, 1H), 4.15-1.13 (dd, 1H), 4.12-4.11 (dd, 1H), 4.03-4.01 (dd, 1H), 3.53 (s, 1H), 3.49 (m, 2H), 3.45-3.44 (m, 1H)

^{13}C NMR (100 MHz, CDCl3): δ (ppm) 125.2, 135.1, 116.7, 98.5, 94.1, 71.2, 69.5, 69.6, 68.3, 68.0, 65.4, 30.1

R_f (CH_2Cl_2: CH_3OH 4:1) 0.61

1-O-Allyl-4, 6- O-benzylidine-β-D-galactoipyranoside



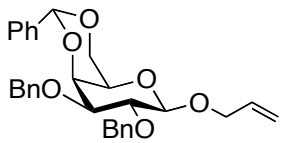
15 mL anhydrous acetonitrile was added to (0.74g, 3.36 mmol) **1** followed by addition of (0.6 mL, 4.1 mmol) benzaldehyde dimethyl acetal. *p*-toluene sulfonic acid (0.025g, 0.13mmol) was added and the mixture was stirred for 1h and then quenched by adding 0.02 mL of triethylamine and concentrated. Flash chromatography with 2% methanol in dichloromethane was carried out to obtain β-1-O-allyl-4, 6- benzylidine-galactopyranoside (0.93 g, 89%, white solid).

^{1}H NMR (400 MHz, CDCl3): δ (ppm) 7.53-7.51 (m, 2H), 7.37-7.32 (m, 3H), 5.97-5.91 (m, 1H), 5.48 (s, 1H), 5.29-5.27 (d, 1H), 5. 22-5.20 (d, 1H), 4.86-4.83 (m, 2H), 4.22-4.19 (m, 1H), 4.15-4.11 (m, 1H), 4.09-4.05 (m, 2H)

^{13}C NMR (100 MHz, CDCl3): δ (ppm) 138.4, 138.0, 133.8, 128.7, 128.6, 125.2, 120.1, 117.6, 95.6, 89.9, 81.8, 76.2, 72.1, 69.2, 68.1, 62.3, 23.1, 21.2, 20.6

R$_f$ (CH_2Cl_2: CH_3OH 4:1) 0.68

1-O-Allyl-2, 3- dibenzyl-4, 6- O-benzylidine-β-D-galactopyranoside



β-1-O-allyl-4, 6- benzylidine-galactopyranoside (0.93g, 3.0 mmol) was dissolved in 15mL anhydrous N, N- dimethylformamide (DMF) followed by the addition of sodium

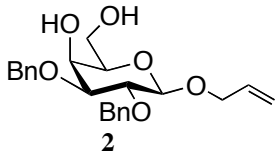
hydride (0.25 g, 0.01 mol) at 0 ºC under Ar(g) atm and stirred for 30 min. Benzyl bromide (1.24 mL, 0.01 mol) was added to the reaction mixture and stirred for 8h at room temperature, and then quenched by the addition of water. Reaction mixture was extracted with EtOAc. The organic layer was washed with sodium chloride solution, dried over anhydrous $MgSO_4$, filtered and concentrated. Excess benzyl bromide was removed by flash chromatography with 2:1 hexane and EtOAc(20%) to obtain β-1-O-allyl-2, 3-benzyl-4, 6-benzylidine galactopyranoside (1.16 g, 81%, white solid).

^{1}H NMR (400 MHz, CDCl3): δ (ppm) 7.56-7.52 (m, 5H), 5.99 – 5.93 (m, 1H), 5.50 (s, 1H), 5.36 (d, 2H), 5.32 (d, 2H), 4.96 (d, 1H), 4.80 (s, 2H), 4.78 (s, 2H), 4.45 (dd, 1H), 4.32 (dd, 1H), 4.00 (m, 1H), 3.55 (m, 1H)

^{13}C NMR (100 MHz, CDCl3): δ (ppm) 138.9, 138.2, 138.1, 133.8, 128.8, 128.7, 127.2, 126.2, 117.2, 95.2, 83.9, 81.3, 80.5, 76.7, 72.1, 72.0, 69.2, 67.8

R_f (Hexane:EtOAc 2:1) 0.40

1-O-Allyl-2, 3- dibenzyl -β-D-galactopyranoside (2)



2

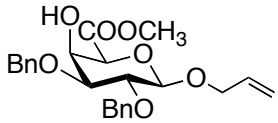
β-1-O-allyl-2, 3-benzyl-4, 6-benzylidine galactopyranoside (1.16 g, 2.4 mmol) was dissolved in 20mL 80 % CH_3COOH/ H_2O and stirred at 65 ºC for 3h. The solution was concentrated under reduced pressure and purified by a quick column with 25% EtOAc in hexane to give **2** (0.73g, 76%, colorless oil).

^{1}H NMR (400 MHz, CDCl3): δ (ppm) 7.43-7.42 (m, 5H), 7.34-7.30 (m, 5H), 6.05-5.95 (m, 1H), 5.26-5.36 (d, 1H), 5.18-5.14 (d, 1H), 4.78 (s, 2H), 4.74 (s, 2H), 4.70 (d, 1H), 4.60 (d, 1H), 4.44-4.40 (dd, 1H), 4.05 (d, 1H), 3.77-3.73 (m, 2H), 3.29-3.26 (m, 1H)

^{13}C NMR (100 MHz, CDCl3): δ (ppm) 138.4, 138.0, 133.8, 128.2, 127.8, 127.5, 117.4, 95.6, 80.5, 81.4, 72.1, 72.4, 73.0, 69.2, 68.7, 62.6

R_f (Hexane:EtOAc 3:2) 0.28

Methyl 1-O-allyl-2, 3- dibenzyl-β-D-galactopyranosyluronate (3)



3

15mL anhydrous dichloromethane was added to (0.73 g, 1.8 mmol) of β-1-O-allyl-2, 3-benzyl-galactopyranoside, followed by the addition of [bis(acetoxy)iodo] benzene (BAIB) (1.46g, 4.5 mmol) and of 2,2,6,6-tetramethyl-1-piperidinyloxyl (TEMPO) (0.05g 0.36 mmol). The reaction mixture was stirred for 3h and quenched by slow addition of aqueous sodium thiosulfate solution. The product was extracted using dichloromethane. The organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to obtain crude β-1-O-allyl-2, 3-benzyl-galacturonic acid (0.76g). This was carried out to the next step without further purification.

The β-1-O-allyl-2, 3-benzyl-galacturonic acid was dissolved in methanol (10 mL) followed by the addition of catalytic amount of sulfuric acid (0.5μL) This reaction was

allowed to stir at 60 ºC in the microwave for 1h. The reaction mixture was allowed to come to room temperature and concentrated under reduced pressure.

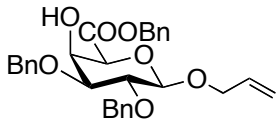
To the residue obtained aqueous sodium bicarbonate solution was added and the organic product was extracted with dichloromethane. The organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash chromatography with Hexane: EtOAc (1:4) give **3** (0.64g, 82%, colorless oil).

^{1}H NMR (400 MHz, CDCl3): δ (ppm) 7.29-7.25 (m, 10H), 5.85-5.81 (m, 1H), 5.44 (dd, 1H), 5.41 (s, 1H), 5.22 (d, 1H), 4.65 (d, 2H), 4.53 (s, 2H), 4.12 (dd, 1H), 4.34 (m, 1H), 3.89 (s, 3H), 3.76 (dd, 1H)

^{13}C NMR (100 MHz, CDCl3): δ (ppm) 171.2, 18.4, 138.1, 138.9, 128.3, 127.8, 127.4, 127.1, 115.2, 95.1, 90.2, 79.8, 78.2, 72.1, 71.2, 65.6, 20.2

R_f (Hexane:EtOAc 2:1) 0.25

Benzyl 1-O-allyl-2, 3- dibenzyl-β-D-galactopyranosyluronate (4)



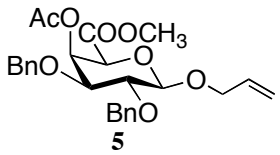
To a solution of 1-O-allyl-2, 3-dibenzyl- β-D-galactopyranoside (0.28g, 0.67 mmol) in THF (10 mL), Et_3N (0.18 mL, 0.34 mmol) was added at room temperature. The solution was stirred under Ar (g) atmosphere and TBAI (0.12g, 0.33 mmol) was added followed by dropwise addition of BnBr (0.22 mL, 1.6 mmol). The solution was allowed to stir for 12 h. The reaction mixture was concentrated and purified by silica gel flash chromatography with Hexane: EtOAc (3:2) to obtain pure **4** (0.29g, 81%, colorless oil).

^{1}H NMR (400 MHz, CDCl3): δ (ppm) 7.38-7.35 (m, 10H), 7.35-7.31 (m, 5H), 6.02-5.89 (m, 1H), 5.37 (dd, 1H), 5.31 (s, 2H), 5.22 (d, 2H), 4.95 (d, 2H), 4.71(s, 2H), 4.44 (dd, 1H), 4.34 (m, 1H), 3.76 (dd, 1H), 3.58 (m, 1H)

^{13}C NMR (400 MHz, CDCl3): δ (ppm) 204.2, 161.2, 160.3. 155.1, 139.3, 139.0, 135.8, 126.2, 126.5, 125.5, 122.3, 121.4, 118.6, 117.2, 96.3, 83.5, 72.1, 72.4, 71.2, 65.3, 65.1, 55.4

R_f (Hexane:EtOAc 2:1) 0.20

Methyl 1-O-allyl-2, 3- dibenzyl- 4-acetyl-β-D-galactopyranosyluronate (5)



5

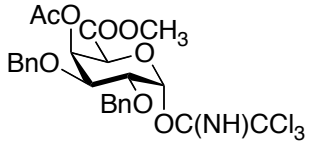
To a solution of methyl 1-O-allyl-2, 3- dibenzyl-β-D-galactopyranosyluronate (0.64g, 1.49 mmol) in dichloromethane (12mL), 4-dimethylaminopyridine (DMAP) (0.01g, 0.03mmol) was added followed by the addition of acetic anhydride (1.6 mL) and for 2h and then diluted with dichloromethane and washed with aqueous sodium bicarbonate solution. The organic layer was dried with anhydrous $MgSO_4$ solution, filtered and concentrated under reduced pressure. The product was purified by a short flash silica gel column with Hexane: EtOAc (1:4) to obtain **5** (0.69g, 88%, colorless syrup).

^{1}H NMR (400 MHz, CDCl3): δ (ppm) 7.39-7.34 (m, 5H), 7.31-7.28 (m, 5H), 6.01 (d, 1H), 5.76-5.72 (m, 1H), 5.21-5.19 (d, 1H), 5.14-5.12 (d, 1H), 4.89 (s, 2H), 4.66 (s, 2H), 4.31 (m, 2H), 3.65-3.62 (m, 1H), 2.01 (s, 1H)

^{13}C NMR (100 MHz, CDCl3): δ (ppm) 171.5, 168.2, 138.1, 137.6, 137.1, 132.3, 132.0, 127.6, 127.4, 116.9, 95.3, 90.2, 80.1, 77.9, 72.0, 68.6, 68.1, 65.3, 21.9, 20.9

R_f (Hexane:EtOAc 3:2) 0.45

Methyl 1-O-trichloroacetimidate-2, 3- dibenzyl- 4-acetyl-β-D-galactopyranosyluronate (8)



Iridium (I) catalyst (0.011g; 0.013 mmol) was added to a solution of methyl 1-O-allyl-2, 3- dibenzyl- 4-acetyl-β-D-galactopyranosyluronate (0.69 g, 1.46 mmol) in anhydrous THF. This solution was hydrogenated for 15 seconds (till the color of the solution changed from red to pale yellow) using a hydrogen gas balloon. After the hydrogenation, the reaction was allowed to run at room temperature under Ar(g) atm for 8h. The solvent was removed under reduced pressure and the residue was dissolved in 10 mL acetone and 2mL H_2O, followed by the addition HgO (0.16g, 0.65 mmol) and $HgCl_2$ (0.35, 1.13 mmol). This mixture was allowed to stir at room temperature for 5h and then filtered through a pad of Celite®. The residue was dissolved in dichloromethane, washed with aqueous KI solution, saturated sodium thiosulfate solution and sodium chloride solution. The organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to obtain methyl-2, 3- dibenzyl- 4-acetyl-β-D-galactopyranosyluronate (0.34g, 0.79 mmol). This was dissolved in anhydrous dichloromethane (10 mL) and of K_2CO_3 (0.41g, 3.0 mmol) was added followed by the addition of trichloroacetonitrile (0.51 mL, 5.1 mmol) under Ar(g) atm. The reaction was allowed to stir at room temperature for 1h

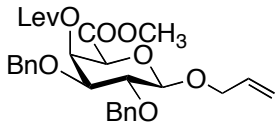
and filtered through a pad of Celite®. The filtrate obtained was concentrated under reduced pressure and purified by a quick flash column with Hexane: EtOAc (3:2) containing 0.1% Et_3N to give **8** (0.33g, 64%, colorless oil).

^{1}H NMR (400 MHz, CDCl3): δ (ppm)) 8.61 (s, 1H), 7.45-7.42 (m, 5H), 7.39-7.35 (m, 5H), 6.13 (d, 1H), 5.14-5.12 (d, 1H), 4.68 (s, 2H), 4.45 (s, 2H), 4.22-4.20 (m, 1H), 3.32--3.29 (m, 1H), 2.11 (s, 1H)

^{13}C NMR (100 MHz, CDCl3): δ (ppm) 200.1, 170.2, 165.1, 138.9, 138.6, 137.6, 130.1, 129.8, 126.5, 125.1, 117.5, 91.3, 79.8, 76.9, 69.4, 68.1, 45.3, 22.1, 19.8

R_f (Hexane:EtOAc 2:1) 0.78

Methyl 1-O-allyl-2, 3- dibenzyl- 4-levulinyl-β-D-galactopyranosyluronate (6)



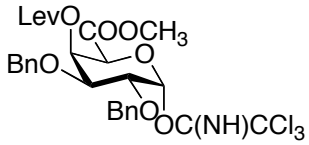
To a solution of methyl 1-O-allyl-2, 3- dibenzyl-β-D-galactopyranosyluronate (0.37g, 0.86 mmol) in dichloromethane, DMAP (0.005 g, 40 μmol) was added followed by the addition of levulinic acid (0.10g, 0.86 mmol) and DIC (0.13 mL, 0.86 mmol) and stirred at room temperature for 6 h. The reaction mixture was filtered through a pad of Celite® and washed with dichloromethane and then concentrated. The crude product was purified by a short silica gel flash chromatography to obtain **6** (0.38g, 85%, yellow oil).

^{1}H NMR (400 MHz, CDCl3): δ (ppm) 7.34-7.28 (m, 10H), 5.96-5.94 (m, 1H), 5.90 (dd, 1H), 5.35 (d, 1H), 5.29 (d, 1H), 4.89 (d, 1H), 4.75 (s, 2H), 4.55 (s, 2H), 4.46 (dd, 1H), 2.80-2.76 (m, 2H), 2.66-2.63 (m, 2H), 2.15 (s, 3H)

^{13}C NMR (100 MHz, CDCl3): δ (ppm) 206.3, 172.4, 170.1, 138.5, 137.2, 127.8, 127.7, 127.5, 127.2, 117.4, 95.0, 85.3, 84.2, 78.6, 72.0, 69.8, 62.8, 29.5, 27.3, 21.2

R_f (Hexane:EtOAc 3:2) 0.25

Methyl 1-O-tricholoroacetimidate-2, 3- dibenzyl- 4-levulinyl-β-D-galactopyranosyluronate (9)



Iridium (I) catalyst (0.032g, 0.039 mmol) was added to a solution of methyl 1-O-allyl-2, 3- dibenzyl- 4-levulinyl-β-D-galactopyranosyluronate (1.1 g, 2.26 mmol) in anhydrous THF. This solution was hydrogenated for 15 seconds (till the color of the solution changed from red to pale yellow) using a hydrogen gas balloon. After the hydrogenation, the reaction was allowed to run at room temperature under Ar(g) atm for 8h. The solvent was removed under reduced pressure and the residue was dissolved in 10 mL acetone and 2mL H_2O, followed by the addition HgO (0.16, 1.12 mmol) and $HgCl_2$ (0.56g, 2.26 mmol). This mixture was allowed to stir at room temperature for 5h and then filtered through a pad of Celite®. The residue was dissolved in dichloromethane, washed with aqueous KI solution, saturated sodium thiosulfate solution and sodium chloride solution. The organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to obtain crude product of methyl 2, 3- dibenzyl- 4-levulinyl-β-D-

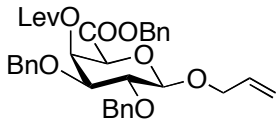
galactopyranosyluronate (0.83g, 1.71 mmol) which was dissolved in anhydrous dichloromethane (10 mL) and K_2CO_3 (1.34g, 8.8 mmol) was added followed by the addition of trichloroacetonitrile (0.51 mL, 5.1mmol) under Ar(g) atm. The reaction was allowed to stir at room temperature for 1h and filtered through a pad of Celite®. The filtrate obtained was concentrated under reduced pressure and purified by a quick flash column with Hexane: EtOAc (3:2) containing 0.1% Et_3N to give **9** (0.91g, 78%, yellow oil).

^{1}H NMR (400 MHz, CDCl3): δ (ppm) 8.64 (s, 1H), 7.36-7.27 (m, 10H), 6.66 (s, 1H), 5.90 (s, 1H), 4.80 (dd, 1H), 4.74 (s, 2H), 4.71 (s, 2H), 4.64 -4.61 (m, 1H), 4.13-4.08 (m, 2H), 3.97-3.90 (m, 2H), 3.79 (s, 1H), 2.73-2.72 (m, 2H), 2.62-2.64 (m, 2H), 2.14 (s, 3H),

^{13}C NMR (100 MHz, CDCl3): δ (ppm) 206.5, 171.3, 168.4, 160.3, 138.1, 137.9, 137.2, 128.8, 128.2, 127.4, 91.3, 90.8, 77.5, 76.2, 72.0, 38.4, 28.7, 32.1, 21.0

R$_f$ (Hexane:EtOAc 3:2) 0.62

Benzyl 1-O-allyl-2, 3- dibenzyl- 4-levulinyl-β-D-galactopyranosyluronate (7)



To a solution of benzyl 1-O-allyl-2, 3- dibenzyl-β-D-galactopyranosyluronate (0.29g, 0.57 mmol) in dichloromethane, DMAP (0.005 g, 40 μmol) was added followed by the addition of levulinic acid (0.06g, 0.57 mmol) and DIC (0.09 mL, 0.57 mmol) and stirred at room temperature for 6 h. The reaction mixture was filtered through a pad of Celite®

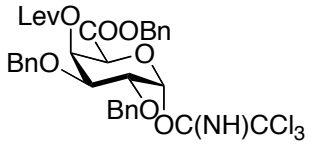
and washed with dichloromethane and then concentrated. The crude product was purified by a short silica gel flash chromatography to obtain **7** (0.32g, 95%, yellow oil).

^{1}H NMR (600 MHz, CDCl3): δ (ppm) 7.36-7.31 (m, 5H), 7.24-7.20 (m, 5H), 6.21 (s, 1H), 5.98-5.93 (m, 1H), 5.65-5.63 (d, 1H), 5.60-5.58 (d, 1H), 5.13-5.10 (m, 1H), 4.95 (dd, 1H), 4.68 (s, 2H), 4.55 (s, 2H), 3.85-3.82 (m, 1H), 2.54-2.51 (t, 2H), 2.33-2.30 (t, 2H)

^{13}C NMR (100 MHz, CDCl3): δ (ppm) 200.9, 172.8, 170.1, 138.9, 138.5, 134.5, 133.2, 129.6, 128.6, 127.1, 116.9, 90.3, 77.3, 75.4, 70.9, 68.6, 66.5, 41.6, 39.5, 29.0, 25.7

R_f (Hexane:EtOAc 3:2) 0.50

Benzyl 1-O-tricholoroacetimidate-2, 3- dibenzyl- 4-levulinyl-β-D-galactopyranosyluronate (10)



Iridium (I) catalyst (0.09g, 0.001 mmol) was added to a solution of benzyl 1-O-allyl-2, 3-dibenzyl- 4-levulinyl-β-D-galactopyranosyluronate (0.32g, 0.54 mmol) in anhydrous THF. This solution was hydrogenated for 15 seconds (till the color of the solution changed from red to pale yellow) using a hydrogen gas balloon. After the hydrogenation, the reaction was allowed to run at room temperature under Ar(g) atm for 8h. The solvent was removed under reduced pressure and the residue was dissolved in 10 mL acetone and 2mL H_2O, followed by the addition HgO (0.05g, 0.27 mmol) and $HgCl_2$ (0.14g, 0.54 mmol). This mixture was allowed to stir at room temperature for 5h and then filtered

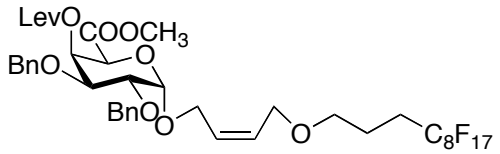
through a pad of Celite®. The residue was dissolved in dichloromethane, washed with aqueous KI solution, saturated sodium thiosulfate solution and sodium chloride solution. The organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to obtain crude product of benzyl 2, 3- dibenzyl- 4-levulinyl-β-D-galactopyranosyluronate (0.31g, 0.55 mmol) which was dissolved in anhydrous dichloromethane (10 mL) and K_2CO_3 (0.42g, 2.8mmol) was added followed by the addition of trichloroacetonitrile (0.16 mL, 1.6 mmol) under Ar(g) atm. The reaction was allowed to stir at room temperature for 1h and filtered through a pad of Celite®. The filtrate obtained was concentrated under reduced pressure and purified by a quick flash column with Hexane: EtOAc (3:2) containing 0.1% Et_3N to give **10** (0.32g, 85%, yellow oil).

^{1}H NMR (400 MHz, CDCl3): δ (ppm) 8.64 (s, 1H), 7.36 (m, 10H), 6.66 (s, 1H), 5.90 (s, 1H), 4.80 (dd, 1H), 4.74 (s, 1H), 4.71 (s, 2H), 4.64 (dd, 1H), 3.90 (s, 1H), 2.73 (m, 1H), 2.62 (m, 2H), 2.14 (s, 3H)

^{13}C NMR (100 MHz, CDCl3): δ (ppm) 205.3, 172.8, 170.9, 160.1, 138.2, 138.0, 136.4, 136.2, 135.7, 135.1, 134.3, 134.1, 129.7, 129.4, 127.3, 92.1, 90.8, 90.2, 77.3, 77.1, 72.0, 68.2, 40.8, 38.9, 29.3, 28.2

R_f (Hexane:EtOAc 3:2) 0.60

***cis*-4-(1H, 1H, 2H, 2H, 3H, 3H-Perfluoroundecyloxy)-2-butenyl methyl 2, 3-O-dibenzyl-4-O-levulinyl- β-D-galactopyranosyluronate (11)**



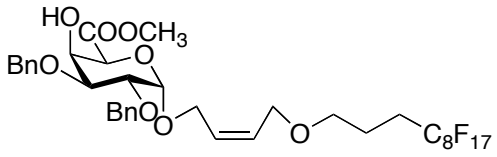
To a solution of the methyl 1-O-tricholoroacetimidate-2, 3- dibenzyl- 4-levulinyl-β-D-galactopyranosyluronate (0.47g, 0.82 mmol) in dichloromethane, a solution of fluorous alcohol (name) (0.18g, 0.32mmol) in 3.0 mL dichloromethane was added at 0 ºC. TMOTf (46 μL, 5.6 μmol) was added and the reaction allowed to run for 1h at 0 ºC. The reaction was quenched by adding Et_3N (0.2mL) and concentrated under reduced pressure. FSPE was performed by dissolving the crude product in DMF (2.0 mL) and eluting out with 80% methanol and methanol to obtain fluorous-tagged methyl 2, 3-O-dibenzyl-4-O-levulinyl- β-D-galactopyranosyluronate (0.25g, 77%, colorless oil).

^{1}H NMR (400 MHz, CDCl3): δ (ppm) 7.45-7.41 (m, 5H), 7.36-7.31 (m, 5H), 6.01 (s, 1H), 5.65 (m, 1H), 5.61 (m, 1H), 5.21 (dd, 1H), 4.65-4.63 (m, 1H), 4.78-4.75 (m, 1H), 4.38 (s, 2H), 4.32-4.31 (m,1H), 4.29 (s, 2H), 4.22-4.19 (m, 2H), 3.68-3.66 (dd, 1H), 2.72-2.71 (t, 2H), 2.22-2.20 (t, 2H), 2.01 (s, 3H), 1.99 (s, 3H)

^{13}C NMR (100 MHz, CDCl3): δ (ppm) 205.1, 172.3, 169.3, 138.9, 138.2, 137.3, 137.0, 128.1, 127.8, 127.5, 119.2, 117.6, 113.0, 112.8, 111.6, 98.7, 96.0, 91.5, 72.0, 68.8, 68.3, 65.4, 61.0, 38.2, 28.1, 20.5, 19.6

R_f (Hexane:EtOAc 3:2) 0.66

***cis*-4-(1H, 1H, 2H, 2H, 3H, 3H-Perfluoroundecyloxy)-2-butenyl methyl 2, 3-O-dibenzyl-β-D-galactopyranosyluronate (12)**



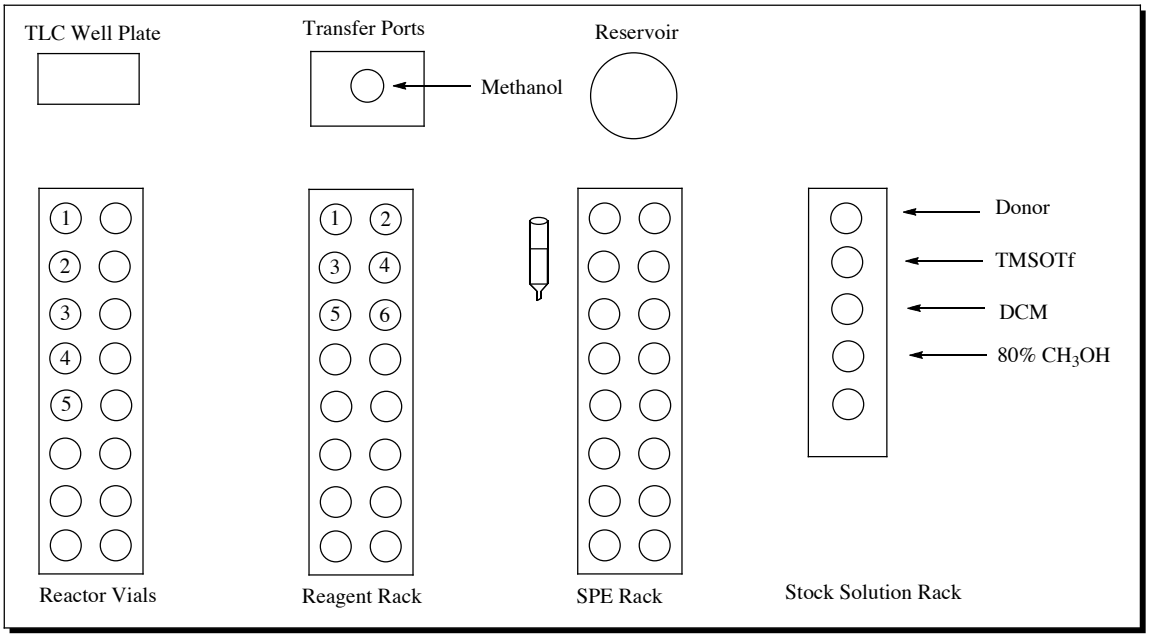
Hydrazine acetate (0.02g, 0.30 mmol) was added to a solution of **11** (0.25 g, 0.24 mmol) in CH_2Cl_2 (8mL)/methanol (8mL) mixture . The reaction mixture was stirred for 3 h at room temperature. The reaction solvent was evaporated and crude product was purified by flash column chromatography on silica gel using 20% EtOAc/hexane to obtain the product **12** (0.21g, 94%, colourless oil).

^{1}H NMR (400 MHz, CDCl3): δ (ppm) 7.39-7.33 (m, 5H), 7.28-7.25 (m, 5H), 6.10 (s, 1H), 5.59 (m, 1H), 5.51 (m, 1H), 5.19-5.22, 1H), 4.68 (s, 2H), 4.65-4.63 (m, 1H), 4.78-4.75 (m, 1H), 4.36 (s, 2H), 4.34-4.31 (m, 2H), 3.77-3.75 (dd, 1H), 2.21 (s, 3H)

^{13}C NMR (100 MHz, CDCl3): δ (ppm) 171.2, 138.9, 138.4, 137.3, 128.7, 127.8, 118.4, 117.3, 117.0, 112.3, 90.5, 80.3, 29.6, 71.4, 67.2, 64.1, 33.2, 20.1

R_f (Hexane:EtOAc 3:2) 0.21

General methods for automated solution phase synthesizer cycles



Scheme 3-5 Schematic of the solution phase automated synthesizer

Number on the reagent rack	**Chemical**
1.	DMF
2.	Triethylamine
3.	Donor (**10**)
4.	F-Tag Acceptor (**12**)
5.	Hydrazine Hydrate/AcOH/Pyridine
6.	Acetone

Table 3-1. The arrangement of chemicals on the reagent rack

Sample preparation

A solution of the donor **9** (0.32 g, 0.53 mmol) was prepared in anhydrous dichloromethane in a 13 mL vial and placed at position 1 of the stock solution rack. 0.3 M TMSOTf solution (1.05 mL) in dichloromethane was prepared in an 8 mL vial and placed at position 2 of the stock solution rack. 500 mL of anhydrous toluene was placed in the stock solution bottle and placed at the reservoir rack with tubing for rinsing the reactor vials. Methanol (100 mL) and 80% methanol (100 mL), triethylamine (8 mL)

were prepared and placed at the indicated positions (Scheme **3-5**), arrangements of the chemicals on the reagent rack of the automated synthesizer is shown in table **3-1**. Fluorous-tagged acceptor **4** (0.086g, 0.14mmol) was dissolved in anhydrous dichloromethane in an 8 mL Wheaton E-Z extraction vial and placed at position 3 of the reagent rack. A solution of hydrazine acetate: AcOH: Pyridine (1:1:1, by volume) was prepared and placed on the reagent rack in an 8 mL vial vial capped with pre-punctured septa. DMF was placed at position 1 of the reagent rack in an 8 mL vial capped with pre-punctured septa. Fluorous Solid Phase Extraction (FSPE) cartridge (2.0g, 10 mL) was activated by washing with 80% methanol in water and placed at SPE block. Underneath the FSPE cartridge, an empty Wheaton 8 mL E-Z extraction vial was placed.

Cleaning cycle

Before the start of the synthesis cycle on the automated solution phase synthesizer, all the reactor vials were washed by running a cleaning cycle, programed for the synthesizer. For the cleaning cycle, firstly, the synthesizer assembly was purged with Ar (g). Then the reactor vials were rinsed with 8 mL toluene (from the reservoir) and 8 mL methanol. The solvents were placed into the reactor vials by the robotic needle, and the reactor vials agitated for 10 minutes before removing the solvents. This was repeated three times. At the end of the third cycle, the reactor vials were dried under vacuum and then Ar (g) was purged for 30 min.

Glycosylation

For the glycosylation cylcle, the reactor vials were cooled to 0 ºC. The solution of the acceptor (0.5 mL) was transferred from its position on the reagent rack to the reactor vial

by the robotic needle. The needle was rinsed with toluene and then the solution of the donor **9** (0.5 mL) was transferred from the stock solution rack into the reactor vial. After rinsing the needle again with toluene, TMSOTf solution (50 μL) was also transferred into the reactor vial. The reactor vial assembly was vortexed for 45 min maintaining the temperature at 0 ºC under Ar (g) atm. At the end of 30 min, 30 μL of the reaction mixture was transferred to the TLC rack. The reaction was quenched by adding triethylamine solution (0.5 mL). This solution was concentrated under reduced pressure at elevated temperature (40 ºC).

Delevulination

The dried residue of the evaporation cycle was diluted with dichloromethane (0.5 mL). A solution of hydrazine acetate/ pyridine and acetic acid (1:1:1) (0.5 mL) was added to the reactor vial and vortexed for 2 h. At the end of the deprotection, 30 μL of solution was transferred o the TLC well plate. Acetone (0.75 mL) was added to the reactor vial to quench the reaction followed by the addition of toluene (1.0 mL). This solution was concentrated under reduced pressure at elevated temperature (70 ºC).

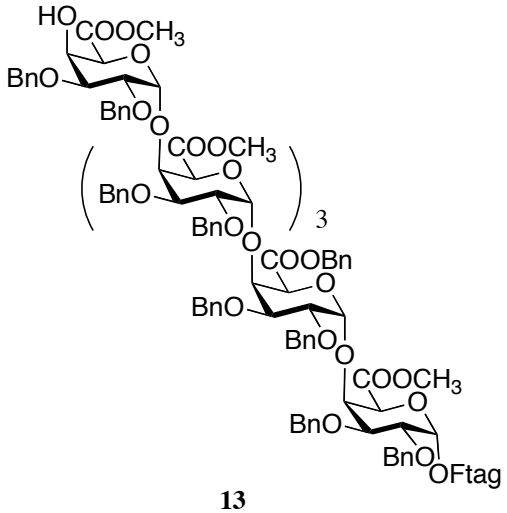
FSPE

For carrying out FSPE, DMF (0.4 mL) was transferred from the reagent rack into the reactor vial and the reaction mixture was agitated for 5 min to dissolve the crude product. 0.7 mL of the reaction mixture was transferred to the FSPE cartridge at the SPE rack. 80% methanol (4.0 mL) was added to the reactor vial. This solution from the reactor vial

was transferred to the FSPE cartridge in three batches of 1.5 mL. 80% methanol solution (1 mL) was used to rinse the FSPE cartridge and the solution was disposed into the 'SPE waste'. Methanol (1.5 mL) was added to the FSPE cartridge and the eluted solution was collected in the 8 mL vial placed underneath the cartridge as 'SPE collect'. This washing with methanol was repeated three times. The collected solution was now transferred via the needle into the next reaction vial for glycosylation. Toluene (1 mL) was added to the reactor vial containing the pure fluorous-tagged glycosylated product and the solution concentrated under reduced pressure at elevated temperature (70 ºC).

HG backbone – from the synthesizer

***cis*-4-(1H, 1H, 2H, 2H, 3H, 3H – Perfluoroundecyloxy) –2-butenyl methyl-2, 3-O-dibenzyl-α-D-galactopyranosyluronate-(1→ 4)-methyl-2, 3-O- dibenzyl-α-D-galactopyranosyluronate-(1→ 4)-methyl-2, 3-O- dibenzyl-α-D-galactopyranosyluronate- (1 →4)- methyl-2, 3-O- dibenzyl-α-D-galactopyranosyluronate-(1→ 4)-benzyl-2, 3-O- dibenzyl-α-D-galactopyranosyluronate-(1→ 4)-methyl-2, 3-O- dibenzyl-α-D-galactopyranosyluronate (13)**



13

^{1}H NMR (400 MHz, CDCl3): δ (ppm) 7.36-7.32 (m, 30H), 7.25-7.15 (m, 15H), 7.12-7.10 (m, 20H), 5.79-5.76 (m, 2H), 5.73 -5.71 (m, 2H), 5.23 (d, 1H), 5.01 (d, 1H), 4.90-4.88 (m, 6H), 4.80-4.76 (m, 6H), 4.68-4.58 (m, 8H), 4.03-3.91 (m, 6H), 3.90 (s, 3H), 3.62 (s, 3H), 3.51 (s, 3H), 2,24-2.15 (m, 2H), 1.85-1.81 (m, 2H)

CHAPTER 4

Synthesis of Plant Polysaccharide Rhamnogalacturonan-I on an Automated Solution-Phase Platform

Sahana K. Nagappayya, Yonghai Chai and Nicola L.B. Pohl

Abstract

An automated solution phase synthesis of plant polysaccharide rhamnogalacturonan-I (RG-I) is reported using flourous-tagged α-L-rhamnopyranoside as the glycosyl acceptor and trichoroacetimidate of α-D-galacturonic acid, synthesized from α-D-galactose as the glycosyl donor. A hexasaccharide of the RG-I backbone is synthesized on this automated solution phase platform and is a first report of an automated synthesis of the RG-I backbone.

Introduction

Rhamnogalacturonan –I (RG-I) is a complex pectic polysaccharide that consists of a backbone of α-D-galacturonic acid and L-rhamnose [-2,)- α-L-Rha-(1, 4)- α-D-GalA-(1-] carrying side-chains of neutral carbohydrates (McNeil et al, 1980; Lau et al, 1985). The roles of such galacturonic acid containing polysaccharide, known as pectins, vary from plant growth and development (McNeil et al, 1984) to disease resistance (Ryan et al, 1981) and they are now also used as gelling agents and stabilizers in food industry (Willats et al, 2006). Recent studies have suggested that changing the side chains on RG-I backbone may have significant roles in various stages of plant growth and development (Ooman, et al, 2002). The side chain of RG- I contains saccharides like single β-D-galactose, and also polysaccharides like α-L-arabinan (Ridley, et al, 2001). The backbone

itself is known to contain both α-D-galacturonic acid and the methyl esterified α-D-galacturonic acid residues (McNeil et al, 1984). But there is little information about the mechanism or specific molecular interaction of the various saccharides present on the RG-I backbone or its side-chains (Mohnen, 2008).

Recently, Davis and co-workers demonstrated the involvement of RG-I backbone in forming Ca^{2+} bridges (Scanlan el al, 2010). In this elegant study, the researchers first chemically synthesized pentasaccharide backbone of RG-I and then set on to perform a solution-phase conformation study in the presence and absence of Ca^{2+} to validate the hypothesis that the methyl esters on the backbone of the pectin polysaccharide is indeed involved in Ca^{2+} binding bridges (Caffall et al, 2009). This is one of the first examples where the chemical synthesis of the plant polysaccharide helped in revealing a molecular basis of the dynamic process that takes place in solution. This study emphasizes the importance of chemical synthesis of pectin polysaccharide over study of inhomogeneous mixture of polysaccharides obtained after enzymatic digestion of pectins (Nakamura et al, 2002).

Fragments of RG have also shown to exhibit proteinase inhibitor inducing factor (PIIF) activity (Ryan et al, 1981). The phenomenon by which plants synthesize large amounts of proteins that inhibit insect and microbial proteinases upon mechanical injury is called the proteinase inhibitor-inducing factor (PIIF) (Green et al, 1972). Fragments of pectic polysaccharides, containing only few oligosaccharide residues, have shown to have PIIF activity, shown in injured cells from tomato leaves (Ryan et al, 1981). In this study, the authors extracted the PIIF from tomato leaves and subjected a batch of young tomato

plants with this solution of the extracted pure PIIF and another batch with a solution of RG-I fragments. Upon incubation and analysis, it was observed that both the batches induced proteinase inhibitors in the leaves of the tomato plant to the same extent. The involvement of RG-I as a PIIF was further confirmed by various analysis by this group.

All the enzymes necessary for the biosynthesis of RG-I are not known yet and many enzymes that break the plant cell wall polysaccharides or synthesize them are still being studied (Nakamura et al, 2002; Sterling et al, 2006) and there is a lack of well-defined structures of RG-I due to the difficulty in isolating pure RG-I from its sources (Ridley et al, 2001). Chemical synthesis, therefore, provides a promising method to better understand the various roles of RG-I in plants and to also extend its utility.

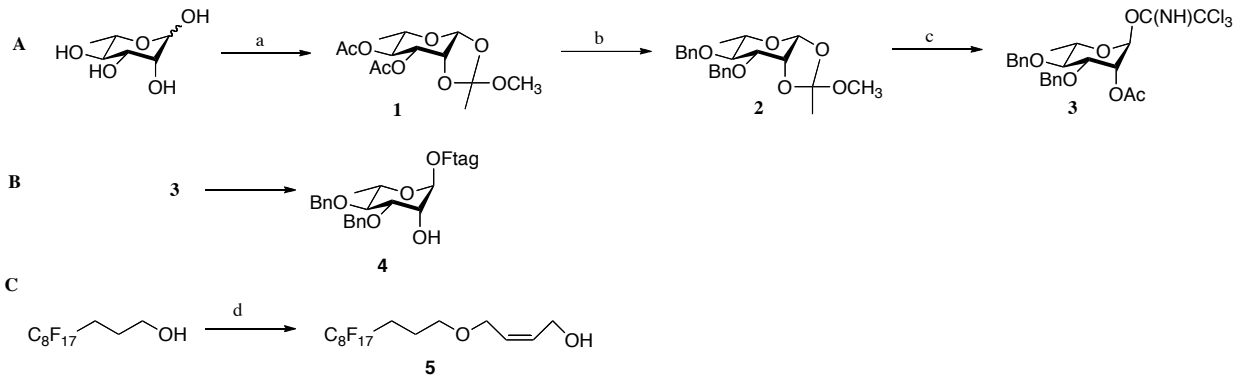
To make the glycosylation reactions consistent by minimizing human errors, an automated solution phase synthesis platform is used in this study. In the automated solution-phase synthesis, the reaction is carried out on a programmable platform using a robotic system where the duration of each reaction and the amounts of reagents added are all controlled by the program thereby minimizing experimental errors. This automated solution phase platform is conveniently based on the Fluorous Solution Phase Extraction (FSPE) method for the purification of the growing oligosaccharide chain (Pohl, 2008a). For the solution-phase automated synthesis, a fluorous tag containing C_8F_{17}- handle is attached to the reducing end of the oligosaccharide chain. During FSPE, the impure mixture is passed through the fluorous silica cartridge, where using a combination of appropriate fluorophilic and fluorophobic solvents, fluorous-tagged compound can be easily separated from un-tagged compounds (Curran, 2001). This flourous-tag based

automated solution phase synthesis is becoming a very useful tool in oligosaccharide synthesis.

Results and discussion

In the synthesis of the rhamnogalacturonan-I backbone fragment, α-D-galacturonic acid tricholoroacetimidate was chosen as the donor and L-rhamnopyranoside with a C_8F_{17}-containing fluorous tag at the anomeric center was chosen as the glycosyl acceptor. The whole assembly of α-D-GalA-1, 2- α-L-Rha-1, 4- repeating unit was synthesized on the solution-phase automated synthesizer. Both post-glycosylation oxidation and pre-glycosylation oxidation strategies have been applied for the synthesis of backbones containing galA (Yamanoto et al, 2005; Clausen et al 2003). In the previous attempts to synthesize RG-I backbone, the researchers started with α-D-galacturonic acid as the starting material and carried out functional group transformations to synthesize the galA donor (Nemati et al, 2008). In our strategy, we used the pre-glycosylation oxidation of D-galactose to synthesize α-D-galacturonic acid tricholoroacetimidate donor **7**. The synthesis of this building block is discussed in detail in Chapter 3. The rhamnopyranosyl acceptor **3** was synthesized starting with L-rhamnose, by first making a 1, 2-orthoester **1** and then benzylating the 3- and 4- O positions to give **2**. Cleavage of this orthoester **2** under acidic conditions give the 2-O acetate and free anomeric hydroxyl, which is coupled with fluorous tag **4** under standard glycosylating conditions (Scheme 4-1A). The fluorous tag **4** was developed by Pohl and co-worrkers (Mamidyala et al, 2008) and is a robust fluorous handle for the solution-phase automated synthesis. The synthesis of this

fluorous tag and is shown in Scheme 4-1B. The assembly of these building blocks on the solution-phase automated synthesizer is shown in Fig 4-1.



Condition: **A**. a. (i) Ac_2O, Py (89%) (ii) HBr/ CH_3COOH, CH_2Cl_2 (81%) (iii) $CH_3CH(OCH_3)_2$, TBAB, CH_2Cl_2, Et_3N (85%) b. (i) Na, CH_3OH (90%) (ii) BnBr, NaH, DMF (83%) c. (i) 2N HCl, CH_2Cl_2 (ii) Cl_3CCN, K_2CO_3, CH_2Cl_2 (76% over two steps) **B.** (i) TMSOTf, CH_2Cl_2 (87%) (iv) Na, CH_3OH (90%) **C.** d. (i) MsCl, ET_3N, TBAB, CH_2Cl_2 (98%) (ii) *cis*-1, 4 butenediol, KOH, CH_2Cl_2 (67%)

Scheme 4-1. Synthesis of fluorous-tagged L-rhamnopyranoside building block

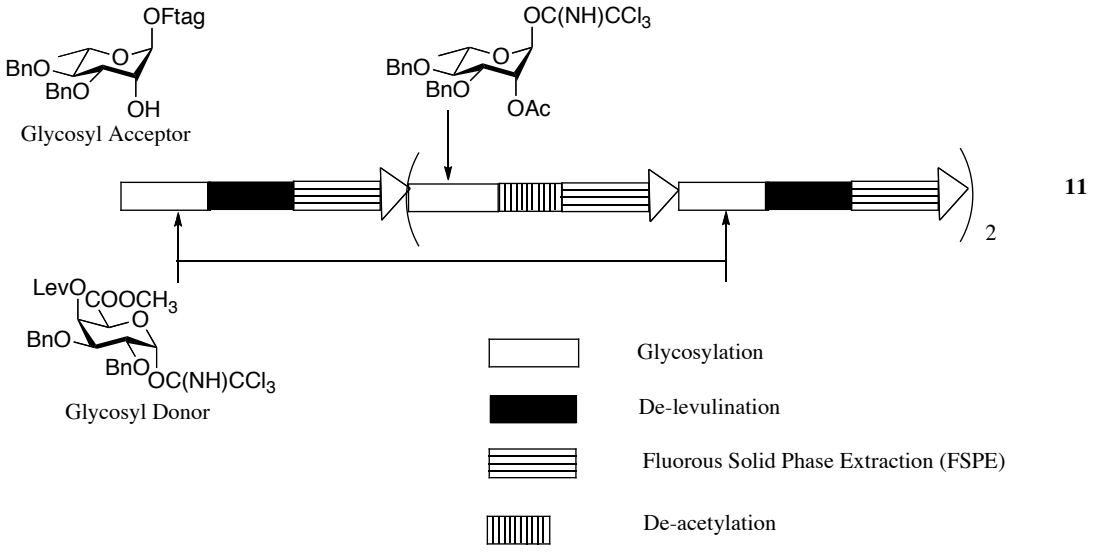
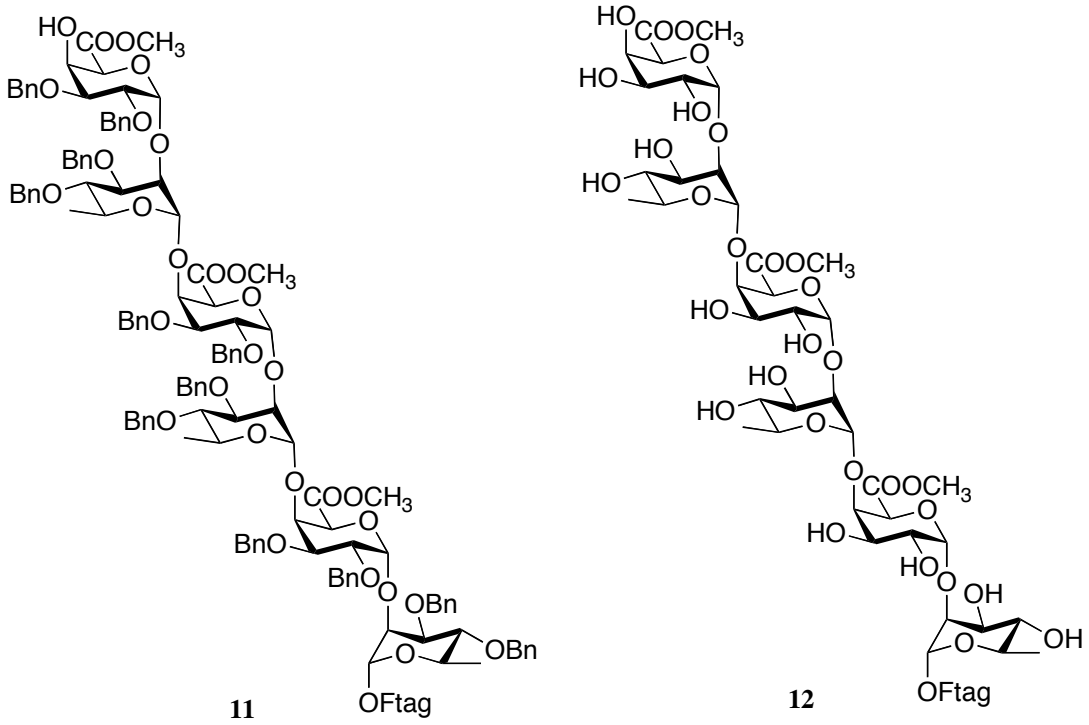


Figure 4-1. Schematic of the automated solution phase synthesis of the RG-I fragment



Scheme 4-3. The hexasaccharide of RG-I **11**- fully protected from the automated solution-phase synthesizer; **12**- after deprotection of the benzyl groups.

First the glycosylation between the fluorous-tagged L-rhamnose and fully protected α-D-galacturonic acid tricholoroacetimidate was carried out by the robot, followed by the deprotection of the levulinate protecting group on the 4-O of the galacturonic acid building block. At this stage, FSPE was performed by dissolving the reaction mixture in DMF and running through the FSPE cartridge with 80% methanol/ water (fluorophobic), followed by 100% methanol (fluorophilic). The resulting disaccharide was glycosylated with L-rhamnopyranosyl tricholoacetimidate donor, and the cycle was continued till a hexasacchride was obtained. Each glycosylation and deprotection cycle was monitored by TLC. The complete successful synthesis of the hexasaccharide of the RG-I backbone was accomplished in 24 hours on the automated solution phase synthesizer. The details and specific requiremnets of the automation assembly are described in the experimental

section. This hexasaccharide was deprotected to remove all the benzyl ethers to obtain a fully deprotected, methyl ester and fluorous tag containing hexasaccharide of RG-I.

Conclusion

A successful synthesis of a hexasaccharide of RG-I was carried out on an automated solution-phase synthesis platform. Post-glycosylation oxidation strategy was used to synthesize D-galacturonic acid, which was glycosylated with L-rhamnosyl glycosyl acceptor on the automated solution phase synthesizer. This synthesis is a stepping-stone in the direction of carrying out RG-I backbone and side chain synthesis. Further studies on this fluorous-tagged RG-I backbone to gain knowledge of the enzymes involved in the synthesis and cleavage of the plant polysaccharide will be carried out along with designing and synthesizing some side-chain oligosaccharides of RG-I.

Experimental Section

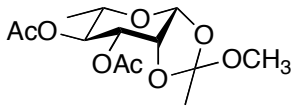
General methods

Reaction solvents were used directly from solvent tower (Swagelok). All other commercial reagents and solvents were used as received without further purification. The reactions were monitored and the *R*f values determined using analytical thin layer chromatography (TLC) with Sorbent Technologies Silica gel HL TLC plates with UV 254 (250 μm). The developed TLC plates were visualized by immersion in a solution of EtOH:conc. H_2SO_4: *p*-anisaldehyde (9:1:1) followed by heating on a hot plate. Silica gel flash chromatography was performed with ZeoPrep 60 Eco 40-63 μm. Fluorous phase chromatography using fluorous solid-phase extraction cartridges containing silica gel bonded with perfluorooctylethylsilyl chains (Fluorous Technologies, Inc.; Pittsburgh,

PA). All other fluorous reagents were also obtained from Fluorous Technologies, Inc. All moisture-sensitive reactions were performed in flame- or oven-dried glassware under a Ar(g) atmosphere. Bath temperatures were used to record the reaction temperature in all cases run without microwave irradiation. All reactions were stirred magnetically at ambient temperature unless otherwise indicated. ^{1}H NMR and ^{13}C NMR spectra were obtained with a VarianVXR at 400 MHz and 100 MHz or BrukerDRX at 600 MHz. ^{1}H NMR spectra were reported in parts per million relative to $CDCl_3$ (7.26 ppm) and ^{13}C NMR spectra were reported in parts per million relative to $CDCl_3$ (77.23 ppm).

Synthetic procedures

3, 4-O-Di-acetyl-1, 2-O-methoxyethylidene-β-L-rhamnopyranose. (1)



Peracetylated L-rhamnose (5.1 g, 8.4 mmol) in dichloromethane (20 mL) at 0 ºC, solution of 33% HBr/CH_3COOH (14.0 mL, 0.23 mol) was added slowly using a dropping funnel. The reaction mixture was allowed to warm up to room temperature and stirred for 3h. The reaction was quenched by pouring in cold water. The organic layer was extracted with dichloromethane, washed with sodium bicarbonate solution, and brine. The dichloromethane layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to obtain 1-bromo-2, 3, 4- tri-O-acetyl-α-L-rhamnopyranoside (4.4g, 81%, yellow oil).

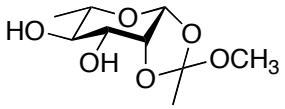
To the crude product obtained, dichloromethane (20.0 mL), TBAB (3.8g, 0.012 mol) and N,N-dimethylformamide dimethyl acetal (1.6 mL, 0.01 mol) at 45 ºC and stirred. After 8h, the reaction mixture was filtered and diluted with dichloromethane. The organic layer

was washed with brine and dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash column chromatography (2:1 hexane and ethyl acetate with 1% triethylamine) to obtain **1** (3.2g. 85%, colorless oil).

^{1}H NMR (CD_3OD, 400 MHz): δ (ppm) 5.67 (d, J = 2.0 Hz), 5.35 (dd, J = 10.0 Hz, 4.0 Hz), 5.12 (t, J = 9.6 Hz), 4.78 (dd, J = 4.4 Hz, 2.4 Hz, 1H), 3.82 (m, 1H), 3.39 (s, 3H), 2.23 (s, 3H), 2.21 (s, 3H), 1.80 (s, 3H), 1.34 (d, J = 6.4 Hz, 3H)

^{13}C NMR (CD_3OD, 100 MHz): δ (ppm) 170.4, 170.4, 124.1, 97.4, 77.4, 70.8, 70.6, 68.4, 50.0, 25.7, 20.9, 20.8, 17.0

1, 2-O-Methoxyethylidene-β-L-rhamnopyranose

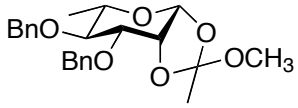


The solution of **1** (3.2g, 0.01 mol) in methanol was stirred with sodium metal to make the solution basic (pH 9-10) for 2h. The solvent was removed under reduced pressure to obtain the crude product, which was dried under high vacuum for 6h and carried out for the next reaction without further purification. (2.1g, 90%, white solid)

^{1}H NMR (CD_3OD, 400 MHz): δ (ppm) 5.48 (d, J = 2.4 Hz), 4.52 (dd, J = 4.0 Hz, 2.0 Hz), 3.72 (dd, J = 8.8 Hz, 4.4 Hz), 3.34-3.29 (m), 1.67 (s, 3H), 1.34 (d, J = 6.0 Hz)

^{13}C NMR: (CD_3OD, 100 MHz): δ (ppm) 125.0, 98.9, 81.6, 73.5, 73.1, 72.0, 49.8, 25.8, 18.1

3, 4-Di-O-benzyl-1, 2-O-methoxyethylidene-β-L-rhamnopyranose. (2)

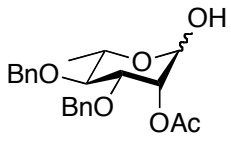


To a solution of 1, 2-O-methoxyethylidene-β-L-rhamnopyranose (2.1g, 9.5 mmol) in 18mL DMF, NaH (1.0g, 0.03 mol) was added under Ar (g) atmosphere at 0 ºC and stirred for 30 min, followed by the addition of BnBr (4.0 mL, 0.03 mol) and stirred for 8 h. The reaction mixture was quenched by addition of water. The product was extracted with ethyl acetate and washed with brine. The organic layer was dried over $MgSO_4$ and solvent removed under reduced pressure. The crude product was purified by a flash silica gel column (2:1 hexane and ethyl acetate with 1% triethylamine) to obtain **2** as white solid. (3.1g, 83% yield, white solid)

^{1}H NMR (CD_3OD, 400 MHz): δ (ppm) 7.53-7.27 (m, 10H), 5.37 (d, J = 2.0 Hz), 4.90 (d, J = 10.4 Hz), 4.79 (d, J = 11.6 Hz), 4.70 (d, J = 10.0 Hz), 4.68 (d, J = 9.2 Hz), 4.57 (dd, J = 4.0 Hz, 2.4 Hz), 3.85 (dd, J = 8.8 Hz, 4.0 Hz), 3.38 (m, 1H), 3.27 (s, 3H), 1.64 (s, 3H), 1.29 (d, J = 6.0 Hz).

^{13}C NMR (CD_3OD, 100 MHz): δ (ppm) 140.0, 139.7, 129.6, 129.5, 129.3, 129.3, 129.0, 128.9, 125.2, 99.1, 81.0, 80.1, 78.9, 76.3, 73.0, 71.3, 50.0, 25.7, 18.4

2-O-Acetyl-3, 4-di-O-benzyl-α/β-L-rhamnopyranose

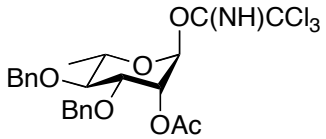


To a solution of 32 in dicholoromethane, 2N hydrochloric acid solution was added in a separatory flask and shaken vigorously. This procedure was repeated twice with 20 mL 2N hydrochloric acid solution. The organic layer was extracted with 20 mL dichloromethane and dried with anhydrous $MgSO_4$ and solvent removed under reduced pressure. Flash silica gel chromatography was carried out with (3:3 hexane and ethyl acetate with 1% triethylamine) to obtain crude product.

^{1}H NMR (CD_3OD, 400 MHz): δ (ppm) 7.34-7.22 (m, 10 H), 5.43 (dd, J = 3.2 Hz, 1.6 Hz, 1H), 4.86 (d, J = 11.2 Hz, 1H), 4.68 (d, J = 11.2 Hz, 1H), 4.63 (d, J = 10.8 Hz, 1H), 4.60 (d, J = 2.0 Hz, 1H), 4.50 (d, J = 11.2 Hz, 1H), 3.87 (dd, J = 9.2 Hz, 3.2 Hz, 1H), 3.66 (m, 1H), 3.42 (t, J = 9.2 Hz, 1H), 3.34 (s, 3H), 2.09 (s, 3H), 1.20 (d, J = 6.0 Hz, 3H).

^{13}C NMR (CD_3OD, 100 MHz): δ (ppm) 172.0, 140.0, 139.5, 129.5, 129.5, 129.4, 129.2, 129.0, 128.9, 100.1, 81.3, 79.4, 72.9, 70.3, 68.9, 55.4, 21.0, 18.4

2-O-Acetyl-3, 4-di-O-benzyl-α/β-L-rhamnopyranoside trichloroacetimidate (34)



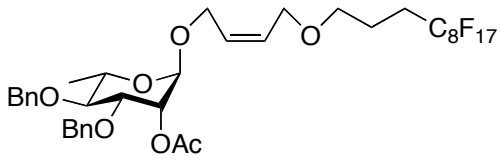
2-O-Acetyl-3, 4-di-O-benzyl-α/β-L-rhamnopyranose was dissolved in 15 mL dichloromethane followed by the addition of K_2CO_3 (3.2g, 0.02 mmol) and trichloroacetonitrile (3.8mL, 0.03 mol) under Ar (g) atmosphere. The reaction was allowed to stir for 1h at room temperature and filtered through a pad of Celite®. The

filtrate obtained was concentrated under reduced pressure and purified by a quick flash column with 20% EtOAc in Hexane containing 0.1% Et_3N to **3**. (3.1g, 76%, colorless oil)

^{1}H NMR: ($CDCl_3$, 400 MHz): δ (ppm) 8.68 (s, 1H), 7.39-7.20 (m, 10H), 6.21 (d, J = 2.0 Hz, 1H), 5.58 (dd, J = 3.2 Hz, 2.4 Hz, 1H), 4.98 (d, J = 10.8 Hz, 1H), 4.78 (d, J = 11.2 Hz, 1H), 4.68 (d, J = 10.8 Hz, 1H), 4.62 (d, J = 11.2 Hz, 1H), 4.04 (dd, J = 9.6 Hz, 3.6 Hz, 1H), 3.98 (m, 1H), 3.58 (t, J = 9.6 Hz, 1H), 2.22 (s, 3H), 1.40 (d, J = 6.4 Hz, 3H)

^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 170.2, 160.3, 138.3, 137.7, 129.2, 128.6, 128.6, 128.5, 128.4, 128.3, 95.4, 91.0, 79.5, 77.3, 75.8, 72.2, 70.9, 67.7, 21.6, 21.2, 18.2

***cis*-4-(1H, 1H, 2H, 2H,3H, 3H-Perfluoroundecyloxy)-2-butenyl-2-O-acetyl-3,4-O-dibenzyl- α-L-rhamnopyranoside**

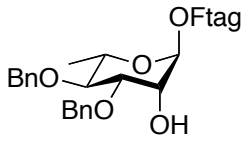


To a solution of **3** (1.5g, 0.02mol) in 18 mL dichloromethane, TMSOTf (21μL, 2.0 μmol) was added under Ar (g) atmosphere, followed by a solution of freshly prepared fluorous tag in toluene. The reaction mixture was stirred for 45 min under Ar (g) atmosphere and quenched by adding triethylamine and filtered through a pad of celite® and solvent removed under reduced pressure. A quick FSPE was carried out by dissolving the product in DMF and loading on the FSPE column and eluting out with 100% CH_3OH (10mL X 2) and 80% aqueous CH_3OH (10mL X 2). The product obtained was concentrated under reduced pressure to obtain **3** (0.94g, 87% yield)

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.31-7.24 (m, 10H), 5.80-5.15 (m, 2H), 5.37 (dd, J = 3.2 Hz, 1.6 Hz, 1H), 4.93 (d, J = 8.1 Hz, 1H), 4.76 (s, 1H), 4.71 (d, J = 8.4 Hz, 1H), 4.62 (d, J = 7.8 Hz, 1H), 4.5 (d, J = 8.4 Hz, 1H), 4.19-4.15 (dd, J = 9.3 Hz, 4.5 Hz, 1H), 4.10-4.06 (dd, J = 9.6 Hz, 5.1 Hz, 1H), 4.04 (d, J = 3.9 Hz, 2H), 3.95-3.92 (dd, J = 6.9 Hz, 2.4 Hz, 1H), 3,79-3,72 (m, 1H), 3.5 (t, J = 5.1 Hz, 2H), 3.44 (t, J = 9 Hz, 1H), 2.30-2.14 (m, 5H), 1.95-1.80 (m, 2H), 1.45 (d, J = 6.0 Hz, 3H)

^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 170.6, 139.1, 138.2, 131.0, 129.1, 128.9, 128.8, 128.2, 98.2, 80.2, 78.34 , 75.5, 72.01, 69.3, 69.0 , 68.4 ,66.8, 62.98, 21.2, 20.8, 18.0

***cis*-4-(1H, 1H, 2H, 2H, 3H, 3H-Perfluoroundecycloxy)-2-butenyl-2-O-hydroxyl-3,4-O-dibenzyl-α-L-rhamnopyranoside**

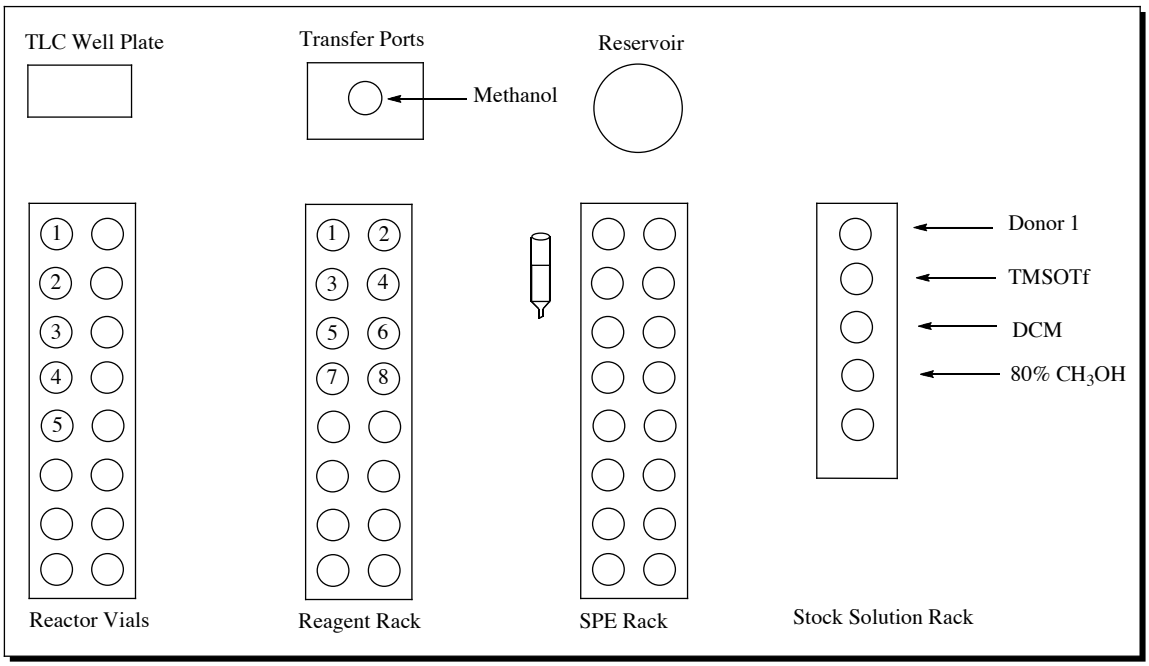


To a solution of *cis*-4-(1H, 1H, 2H, 2H,3H, 3H-Perfluoroundecyloxy)-2-butenyl-2-O-acetyl-3,4-O-dibenzyl- α-L-rhamnopyranoside (0.94g, 1.0 mmol) in methanol (12.0 mL) sodium metal was added at ambient temperature. The reaction mixture was stirred for 1h. The reaction was quenched by adding Dowex® till the solution is neutral, checked by pH paper. The solution was filtered and the solvent removed under reduced pressure. The crude product was purified by flash chromatography (4:1 dichloromethane:methanol) to obtain **4** (0.82g, 90% yield, colorless oil)

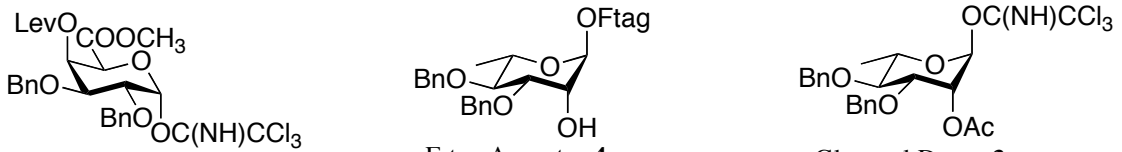
^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.31-7.24 (m, 10H), 5.73-5.66 (m, 2H), 4.92 (d, J = 11.2 Hz, 1H), 4.85 (s, 1H), 4.71 (s, 2H), 4.67-4.65 (d, J = 10.8 Hz, 1H), 4.22-4.17 (dd, J = 12.4 Hz, 5.2 Hz, 1H), 4.12-4.05 (m, 4H), 3.88-3.85 (dd, J = 9.2 Hz, 3.2 Hz, 1H), 3.79-3.72 (m, 1H), 3.51-3.46 (m, 3H), 3.46 (s, 1H), 2.57-2.16 (m, 2H), 1.95- 1.80 (m, 2H), 1.45 (d, J = 6 Hz, 3H)

^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 138.5, 138.2, 130.1, 128.9, 128.7, 128.5, 128.3, 128.3, 128.0, 127.9, 98.2, 80.2, 80.2, 75.5, 72.3, 68.9, 68.7, 67.5, 66.4, 62.6, 28.0, 20.8, 18.0

General methods for automated solution phase synthesizer cycles



Scheme 4-2. Schematic of the automated solution phase synthesizer



Glycosyl Donor **1** F-tag Acceptor **4** Glycosyl Donor **3**

Figure 4-2. Donor and acceptor for the automated solution phase synthesizer.

Number on the reagent rack	**Chemical**
1.	DMF
2.	$NaOCH_3$
3.	F-Tag Acceptor (**4**)
4.	Triethylamine
5.	Donor (**3**)
6.	AcOH
7.	Acetone
8.	Hydrazine Hydrate/AcOH/Pyridine (1:1:1)

Table 4-1. The arrangement of all the chemicals on the automated solution phase synthesizer for the synthesis of RG-I backbone

Sample preparation

A solution of the donor **1** (0.215 g, 0.34 mmol)was prepared in anhydrous dichloromethane in a 13 mL vial and placed at position 1 of the stock solution rack. 0.3 M TMSOTf solution (1.05 mL) in dichloromethane was prepared in an 8 mL vial and placed at position 2 of the stock solution rack. 500 mL of anhydrous toluene was placed in the stock solution bottle and placed at the reservoir rack with tubing for rinsing the reactor vials. Methanol (100 mL) and 80% methanol (100 mL), triethylamine (8 mL) were prepared and placed at the indicated positions (Scheme 4-2) of the automated synthesizer. Acceptor **4** (0.012 g, 0.21 mmol) was dissolved in anhydrous dichloromethane in an 8 mL Wheaton E-Z extraction vial and placed at position 3 of the reagent rack. Solution of donor **3** (0.18 mg, 0.35 mmol) was prepared by dissolving in

dichloromethane in an 8 mL Wheaton E-Z extraction vial and placed at the designated position on the reagent rack.

Sodium methoxide (5.0 mL) solution (Na in methanol) was placed in an 8 mL vial capped with pre-punctured septa and placed at position 3 of the reagent rack. Acetic acid solution (0.45mL) in methanol (5.0 mL) was also placed in the 8 mL vial capped with pre-punctured septa and placed at the assigned position of the reagent rack. A solution of hydrazine acetate: AcOH: Pyridine (1:1:1, by volume) was prepared and placed on the reagent rack in an 8 mL vial vial capped with pre-punctured septa. DMF was placed at position 1 of the reagent rack in an 8 mL vial capped with pre-punctured septa.

Fluorous Solid Phase Extraction (FSPE) cartridge (2.0g, 10 mL) was activated by washing with 80% methanol in water and placed at SPE block. Underneath the FSPE cartridge, an empty Wheaton 8 mL E-Z extraction vial was placed. The positions of the reagents on the reagent rack are shown in table 4-1.

Cleaning cycle

Before the start of the synthesis cycle on the automated solution phase synthesizer, all the reactor vials were washed by running a cleaning cycle, programed for the synthesizer. For the cleaning cycle, firstly, the synthesizer assembly was purged with Ar (g). Then the reactor vials were rinsed with 8 mL toluene (from the reservoir) and 8 mL methanol. The solvents were placed into the reactor vials by the robotic needle, and the reactor vials agitated for 10 minutes before removing the solvents. This was repeated three times. At the end of the third cycle, the reactor vials were dried under vacuum and then Ar (g) was purged for 30 min.

Glycosylation

For the glycosylation cylcle, the reactor vials were cooled to 0 ºC. The solution of the acceptor (0.5 mL) was transferred from its position on the reagent rack to the reactor vial by the robotic needle. The needle was rinsed with toluene and then the solution of the donor **1** or **3** (0.5 mL) was transferred from the stock solution rack into the reactor vial. After rinsing the needle again with toluene, TMSOTf solution (50 µL) was also transferred into the reactor vial. The reactor vial assembly was vortexed for 45 min maintaining the temperature at 0 ºC under Ar (g) atm. At the end of 30 min, 30 µL of the reaction mixture was transferred to the TLC rack. The reaction was quenched by adding triethylamine solution (0.5 mL). This solution was concentrated under reduced pressure at elevated temperature (40 ºC). The glycosylation reaction was performed in reactor vials 1, 2, 3, 4 and 5 (Scheme 4-2)

Delevulination

The dried residue of the evaporation cycle was diluted with dichloromethane (0.5 mL). A solution of hydrazine acetate/ pyridine and acetic acid (1:1:1) (0.5 mL) was added to the reactor vial and vortexed for 2 h. At the end of the deprotection, 30 µL of solution was transferred o the TLC well plate. Acetone (0.75 mL) was added to the reactor vial to quench the reaction followed by the addition of toluene (1.0 mL). This solution was concentrated under reduced pressure at elevated temperature (70 ºC). Delevulination was performed in reactor vials 1, 3 and 5.

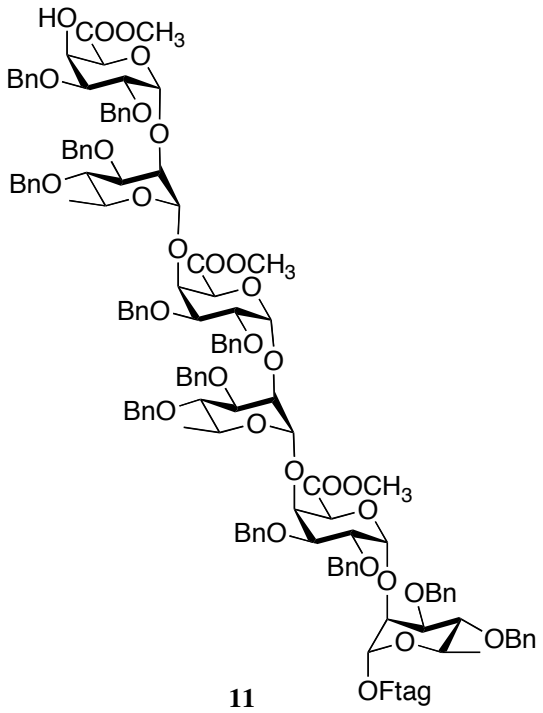
Deacetylation

Methanol (0.5 mL) was added to the dried residue of the glycosylation reaction, followed by the addition of the sodium methoxide solution (0.4 mL). The reaction mixture was vortexed for 2h at room temperature. At the end of the reaction time, 30 μL of the reaction mixture was withdrawn and transferred to the TLC block. The deacetylation reaction was quenchd by adding acetic acid solution (0.75 mL). Toluene (1.5 mL) from the reservoir was added to the reactor vial and the solution concentrated under reducd pressure at elevated temperature (70 ºC). Deacetylation was performed in reactor vials 2 and 4.

FSPE

For carrying out FSPE, DMF (0.4 mL) was transferred from the reagent rack into the reactor vial and the reaction mixture was agitated for 5 min to dissolve the crude product. 0.7 mL of the reaction mixture was transferred to the FSPE cartridge at the SPE rack. 80% methanol (4.0 mL) was added to the reactor vial. This solution from the reactor vial was transferred to the FSPE cartridge in three batches of 1.5 mL. 80% methanol solution (1 mL) was used to rinse the FSPE cartridge and the solution was disposed into the 'SPE waste'. Methanol (1.5 mL) was added to the FSPE cartridge and the eluted solution was collected in the 8 mL vial placed underneath the cartridge as 'SPE collect'. This washing with methanol was repeated three times. The collected solution was now transferred via the needle into the next reaction vial for glycosylation. Toluene (1 mL) was added to the reactor vial containing the pure fluorous-tagged glycosylated product and the solution concentrated under reduced pressure at elevated temperature (70 ºC).

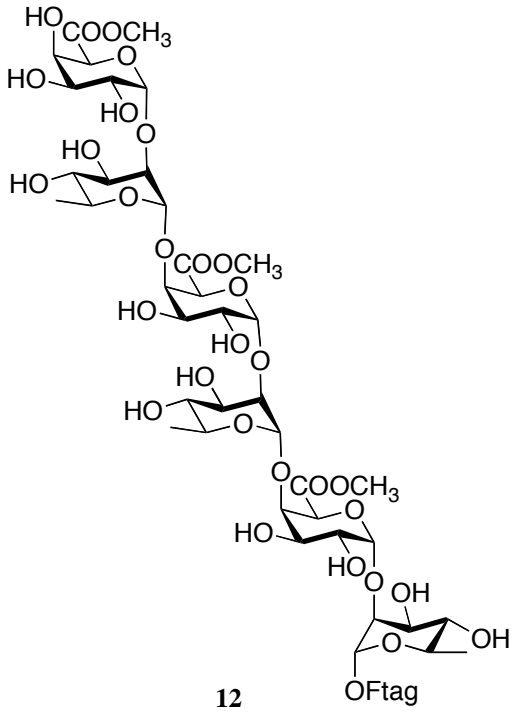
***cis*-4-(1H, 1H, 2H, 2H, 3H, 3H – Perfluoroundecyloxy) –2-butenyl methyl-2, 3-O-dibenzyl-α-D-galactopyranosyluronate-(1→ 2)-3, 4–O-dibenzyl–α-L-rhamnopyranosyl- (1 →4)-methyl-2, 3-O- dibenzyl-α-D-galactopyranosyluronate-(1→ 2)-3, 4–O-dibenzyl–α-L-rhamnopyranosyl- (1 →4)- methyl-2, 3-O- dibenzyl-α-D-galactopyranosyluronate-(1→ 2)-3, 4–O-dibenzyl–α-L-rhamnopyranoside (11)**



11

^{1}H NMR (400 MHz, CDCl3): δ (ppm) 7.39-7.35 (m, 15H), 7.30-7.24 (m, 18H), 7.23-7.21 (m, 15H), 5.70-5.67 (m, 2H), 5.65-5.61 (m, 2H), 5.50-5.48 (m, 2H), 4.99-4.98 (d, 2H), 4.90-4.88 (m, 2H), 4.82-4.78 (m, 4H), 4.78 (s, 4H), 4.63 -4.59 (m, 4H), 4.23-4.19 (m, 3H), 4.03-4.01 (m, 2H), 3.48 (s, 6H), 2.35 -2.32 (m, 2H), 2.13-2.01 (m,4H),1.28 (d, 3H), 1.25 (d, 3H), 1.13 (d, 3H)

4-(1H, 1H, 2H, 2H, 3H, 3H – Perfluoroundecyloxy)-2-butyl methyl-α-D-galactopyranosyluronate-(1→ 2)-α-L-thamnopyranosyl-(1→4)-methyl-α-D-galactopyranosyluronate-(1→ 2)-α-L-thamnopyranosyl-(1→4)-methyl-α-D-galactopyranosyluronate-(1→ 2)-α-L-thamnopyranoside (12)



12

^{1}H NMR (400 MHz, CD3OD): δ (ppm) 5.21-5.19 (m, 8H), 4.39-4.36 (d, 2H), 4.11 (s, 1H), 4.02-4.01 (m, 4H), 3.99 (s, 2H), 3.88 (m, 7H), 3.79-3.76 (m, 4H), 3.66-3.59 (m, 12H), 3.54-3.51 (m, 9H), 3.49-3.44 (m, 6H), 2.34-2.30 (m, 4H), 1.90-1.88 (m, 2H), 1.31 (d, 3H), 1.30 (d, 3H), 1.28 (d, 3H)

CHAPTER 5

Probing the limitations of the fluorous content for tag-mediated microarray formation

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Abstract

The synthesis of a di-perfluorohexyl tag is reported for use in a fluorous-based carbohydrate microarray. A comparative microarray study with this di-perfluorohexyl tag and a mono-perfluorooctyl and mono-perfluorohexyl tag found the increased fluorous content conducive to better spot morphology and easier washing protocols without precluding reuse of the fluorous slide.

Introduction

Perfluorinated compounds (PFCs) have been extensively used in the past decade in commercial, industrial, and research studies, but now PFCs are becoming a major environmental concern (Cheng et al, 2008). The most environmentally persistent PFCs are perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA). Recent studies have found that the growing amounts of PFOS and PFOA found in water correlate with the bioaccumulation of PFCs in human and animals globally (Lopez-Espinosa et al, 2011, Penner et al, 2007). However, unlike the longer PFCs, C_6 and shorter perfluorous chains are not environmentally persistent. (Loi et al, 2011) Growing safety and health issues

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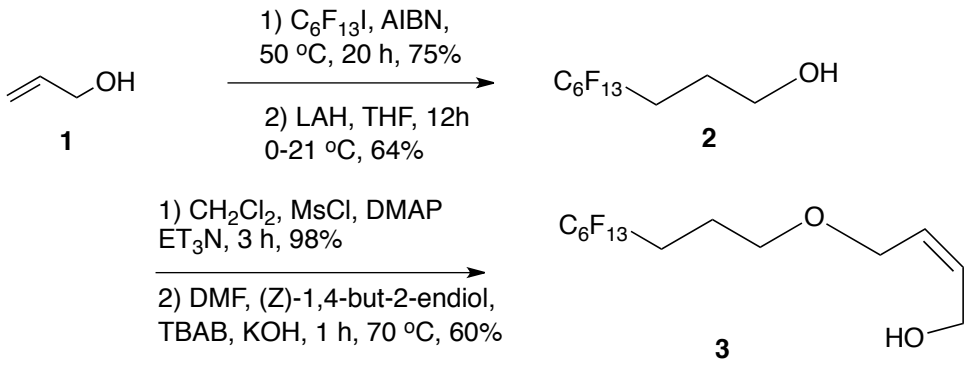
with these 8-carbon-length fluorocarbons threaten their continued bulk manufacturing and therefore also the inexpensive use of this fluorous chain length in a variety of applications in which environmental escape of the PFCs is not a concern. However, such octylfluorous tags have already proven useful not only for efficient separations using fluorous solid phase extraction (FSPE but also as a convenient fluorous handle for microarray formation (Curran, 1998; Curran, 2008).

Fluorous microarrays rely on non-covalent fluorous–fluorous interactions between the fluorous tail linked to the molecule used for screening and the fluorous coated glass slide. (Pohl, 2008b; Zhang et al, 2008) In addition to their use for carbohydrates, such octylfluorous- based microarrays have also been successful for screening other small molecule–protein interactions (Vegas et al, 2007; Liang et al, 2008) More recently, biotin was adhered to a fluorous-coated glass slide and it was demonstrated that C_8F_{17}-tagged molecules were better for fluorous biotin–avidin microarrays than the C_6F_1-tagged molecules in terms of spot intensity, size and spot morphology (Nicholson et al, 2007). Given that a shorter fluorous tag was likely not an option, we set out to discover a fluorous tag that could form strong enough non-covalent interactions for robust fluorous microarray studies without reliance on the C_8F_{17} motif.

Results and discussion

First a route to the desired mono-C_6F_{13}-tag was designed based on the synthesis of the known mono-C_8F_{17}-allyl tag for direct comparison (Maidyala, et al, 2006). We started the synthetic route with perfluorohexyl iodide as an inexpensive precursor to obtain our desired product **3** (Scheme 5-1). Radical addition of per- fluorohexyl iodide to allyl

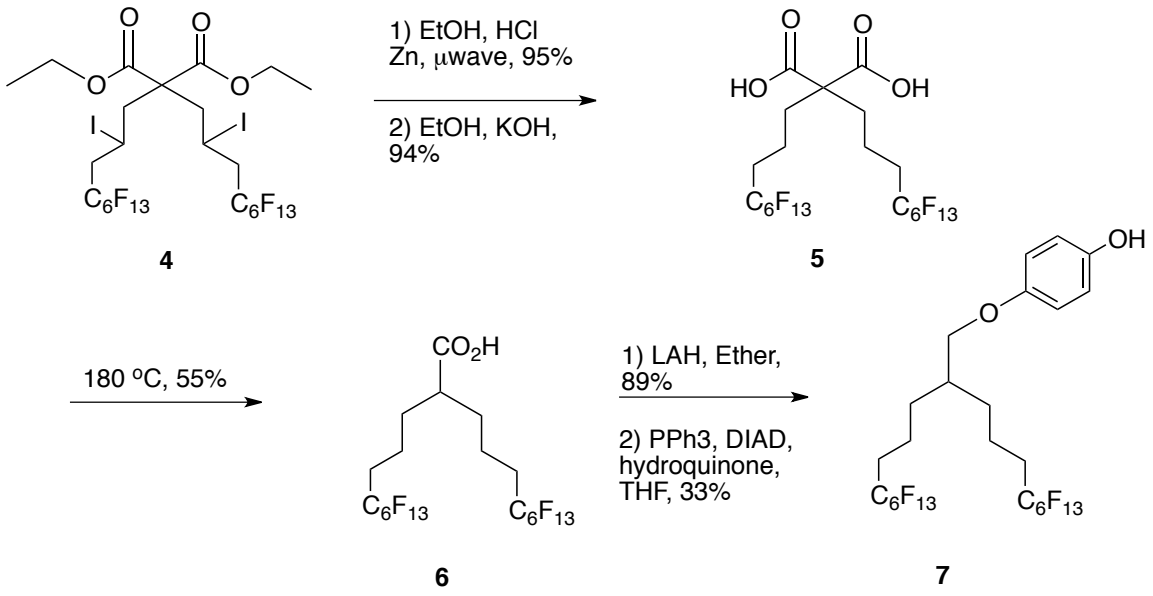
alcohol followed by reduction of the iodide provided perfluorohexyl alcohol **2**. Alcohol **2** was then mesylated for displacement by (Z)-1,4-but-2-endiol to provide the desired allyl tag **3** in 28% overall yield from allyl alcohol.



Scheme 5-1. Synthetic route to the mono-C_6F_{17}-allyl flourous tag.

As one hexylfluorous tag was insufficient for good spot formation on a fluorous slide, we next designed a new tag containing two C_6F_{13}-moieties for direct comparison with the related mono- $C_8F_{17}$and mono- C_6F_{13}-tagged carbohydrates described above. Such a di-C_6F_{13}-tag would still need to allow the attached sugar to orient away from the slide surface in such a way that protein binding could take place. As many sugars come in the form of glycolipids, these structures were seen as inspiration for the design of compound **7** with two fluorous tails attached to a group that served as a UV-active and removable linker (Scheme 5-2). Using ceric ammonium nitrate the fluorous linker could be readily removed from the di- C_6F_{13}-tagged peracetylated glucose (Tojino et al, 2008). The desired di- C_6F_{13}-tag synthesis then started with the known diester 4 made by addition of two allyl groups to diethylmalonate followed by radical addition of perfluorohexyl iodide to the resulting alkenes (Brace, 1999). The presence of the diesters precluded use of lithium aluminium hydride to remove the iodides; therefore, microwave-assisted conditions using

zinc in acidic medium were developed for iodide removal to produce, after base-mediated saponification, diacid **5**. The diacid was immediately subjected to heat-mediated decarboxylation to produce monoacid **6** (Loiseau et al, 2001; Kaplanek et al, 2006). This monoacid was reduced to the alcohol for a Mitsunobu reaction analogous to those previously carried out with other fluorous alcohols (Mizuno et al, 2008) to produce the desired di-fluorous tag **7** in a 14% overall yield from diester **4**. The di-fluorous tag was found to be soluble to at least 1 M concentrations in the common solvents dichloromethane and toluene at room temperature.



Scheme 5-2. Synthetic route to the di-C_6F_{13} fluorous tag.

With the necessary fluorous tags **3** and **7** in hand, the next step was their glycosylation with peracetylated trichloroacetimidate- activated (Schmidt et al, 1980) mannose, glucose, and rhamnose glycosyl donors. After Zemplen deacylation and reduction of the alkene, nine compounds were obtained to perform microarray experiments with

fluorescein isothiocyanate-labeled concanavalin A (FITC-ConA). ConA is known to bind to terminally α-linked D-mannose, whereas β-D-glucose and α-L-rhamnose are not ligands for this plant lectin (Ko et al, 2005) Previously reported FSPE protocols for the octylfluorous tagged monosaccharides were used for the purification of our new fluorous-tagged mono- saccharides (Mizuno et al, 2008). Interestingly, the same solvents could be used for eluting the compounds with a single as for a double fluorous hexyl moiety. The aromatic ring also does not override the fluorous content in the FSPE protocol.

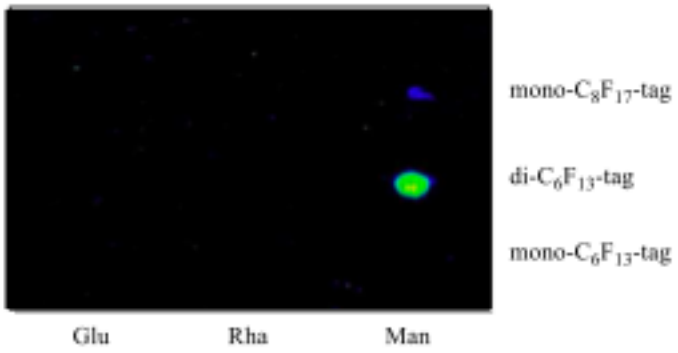


Figure 5-1 Spotting arrangement of carbohydrates on the fluorous coated microarray slide.

In order to compare the performance of the three fluorous-tags on the fluorous-coated glass slide, the carbohydrate microarray study was set up as shown in Figure 5-1. To make a 250 mM concentration of the carbohydrates, the fluorous-tagged monosaccharides were dissolved in methanol/DMSO/water (2 : 6 : 2) (Chen et al, 2008). These monosaccharides were then spotted multiply in groups of nine onto a commercially available fluoroalkylsilane- derivatized glass slide using a standard microarray spotting robot (Chen et al, 2008; Jaipuri et al, 2008). The slides were then incubated with a 200

mM solution of FITC-ConA in phosphate-buffered saline (PBS) for one hour. After incubation, the slide was washed with a 1X PBS containing 1% BSA solution twice and then washed once with distilled water (Liao et al, 2010). Next, the slide was scanned using a General Scanning ProScan Array HT at 488 nm to visualize the carbohydrate–ConA binding (Figure 3). Finally, the data collected from the scan were processed through ImaGenes 8.0 software to obtain the intensities of each fluorous-tagged monosaccharide.

Multiple spotting and scanning experiments revealed several key experimental details. We found that spotting on a new fluorous glass slide resulted in uneven spots; washing the slides with a 1 : 1 dichloromethane : methanol solution before printing solved this problem. The morphology of the spots is also affected by several physical factors like temperature, humidity and drying time. We printed the slides under three different humidity conditions (60%, 65% and 70% humidity) while maintaining the temperature at 22 ºC and observed that the slides printed at 70% humidity showed the best spots with no donut effect. (Note: Details are shown in the experimental section.)

We speculate that the higher water content in air helps the hydrophilic carbohydrates to better orient on the glass slide to create the non-covalent fluorous–fluorous interactions with the fluorous molecules on the slide. Drying of the slide after printing also had an effect on the spot morphology. Less donut effect was observed when the slides were dried for a longer period of time. After testing for various drying times, we concluded that the slides should be kept in the humidity chamber for 18 hours and then outside of it for 2 hours before incubating.

After optimal spotting and drying conditions were found, comparisons among the three different fluorous tags were made. Fluorescence scans after various washing protocols show that the di- C_6F_{13}-tag-containing sugars were robust and could withstand more than two washings with 1X PBS containing 1% BSA. In many cases, the mono- C_6F_{13}-tag containing sugars were being washed away when washed more than once with the PBS solution containing BSA, whereas the intensity of the di- C_6F_{13}-tag containing sugars was the same even after washing multiple times. There was only a slight decrease in the intensity of the – C_8F_{17} tag containing sugars with multiple washings with PBS as shown by the scans. Fig. 1 shows all nine of the fluorous-linked monosaccharides spotted on the same fluorous slide. As shown, only two of these fluorous- linked monosaccharides are seen bound to the ConA-FITC. The relatively weak non-covalent fluorous interaction of the mono- C_6F_{13}-tag with the slide due to less fluorous content precluded its visualization. Note also that the β-D-glucose and α-L-rhamnose compounds did not bind at all, as expected. From the slide we can see also that the di- C_6F_{13}-tag has a larger spot size and is brighter than the mono- C_8F_{17}-tag. Despite the apparent greater adhesion of the sugars attached to the di- C_6F_{13}-tag to the slides, the fluorous slides could still be washed and reused at least five times without significantly increasing background noise.

Conclusion

By software analysis of spot intensities, we can conclude that the spot intensity of the sugars attached to the di- C_6F_{13}-tag is two to four times more intense than that of the mono- C_8F_{17}-tag and its binding ability is superior to both the mono- C_8F_{17}- and - C_6F_{13} tag. The synthesis of the di- C_6F_{13}tag is relatively simple and has high yielding steps, which could be carried out on a larger scale. Clearly, the standard mono-C_8F_{17} tag can be

effectively and efficiently replaced by the di- C_6F_{13}-tag. Given its comparable behaviour on fluorous silica gel, this new tag could also possibly replace the octylfluorous tag in the purification of compounds using FSPE in both manual and automated syntheses.

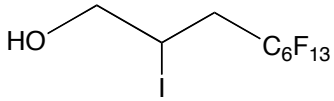
Experimental section

General methods

Reaction solvents were used directly from solvent tower (Swagelok). All other commercial reagents and solvents were used as received without further purification. The reactions were monitored and the *R*f values determined using analytical thin layer chromatography (TLC) with Sorbent Technologies Silica gel HL TLC plates with UV 254 (250 μm). The developed TLC plates were visualized by immersion in a solution of EtOH: conc. H_2SO_4: *p*-anisaldehyde (9:1:1) followed by heating on a hot plate. Silica gel flash chromatography was performed with ZeoPrep 60 Eco 40-63 μm. Fluorous phase chromatography using fluorous solid-phase extraction cartridges containing silica gel bonded with perfluorooctylethylsilyl chains (Fluorous Technologies, Inc.; Pittsburgh, PA). All other fluorous reagents were also obtained from Fluorous Technologies, Inc. All moisture-sensitive reactions were performed in flame- or oven-dried glassware under a Ar(g) atmosphere. Bath temperatures were used to record the reaction temperature in all cases run without microwave irradiation. Microwave reaction was carried out in CEM Microwave. All reactions were stirred magnetically at ambient temperature unless otherwise indicated. ^{1}H NMR and ^{13}C NMR spectra were obtained with a VarianVXR at 400 MHz and 100 MHz or BrukerDRX at 600 MHz. ^{1}H NMR spectra were reported in parts per million relative to $CDCl_3$ (7.26 ppm) and ^{13}C NMR spectra were reported in parts per million relative to $CDCl_3$ (77.23 ppm).

Synthetic procedure

4,4,5,5,6,6,7,7,8,8,9,9,9-tridecafluoro-2-iodononan-1-ol (1)

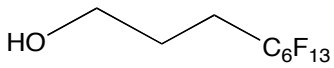


To a mixture of allyl alcohol (1.0 g, 17 mmol) and perfluorohexyl iodide (7.7 g, 17 mmol) was added AIBN (0.30 g, 1.7 mmol) at 20 ºC. The mixture was cooled to -78 ºC to freeze the contents and then degassed, warmed to 20 ºC, and then blanketed with argon. The reaction mixture was heated to 70 ºC and stirred for 20 h. The reaction was then dissolved in hexane and poured through a fritted glass funnel and immediately purified by flash column chromatography with hexane to obtain **1** (82%, 7.1 g, white solid).

^{1}H NMR (CDCl3, 400 MHz): δ (ppm) 3.21-3.19 (m, 2H), 2.98-2.95 (m, 2H), 1.65-1.61 (m, 2H)

^{13}C NMR (CDCl3, 100 MHz): δ (ppm) 116.3, 112.1, 90.2, 56.8, 32.1, 31.9, 30.3, 28.5, 28.1, 28.0, 24.2

4,4,5,5,6,6,7,7,8,8,9,9,9-tridecafluorononan-1-ol (2)



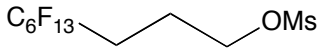
Compound **1** (6.5 g, 13 mmol) was dissolved in tetrahydrofuran (150 mL) and cooled to 0 ºC under argon. Once cooled, lithium aluminum hydride (0.98 g, 26 mmol) was slowly added to the mixture. The reaction vessel was warmed to 22 ºC and stirred for 12 h. The

reaction was quenched very slowly with water (30 mL) by adding it dropwise. The solution was then filtered and washed with $NaHCO_3$ (50 mL) and extracted with ethyl acetate (2 x 80 mL). The organic layer was dried over $MgSO_4$. The solvent was removed under reduced pressure. The crude product was purified by flash column chromatography to obtain **2** (2:1 hexane: ethyl acetate) (83%, 4.1 g, clear oil).

^{1}H NMR (CDCl3, 400 MHz): δ (ppm) 3.13-3.10 (t, 2H), 2.64-2.61 (m, 2H), 1.87-1.82 (m, 2H)

^{13}C NMR (CDCl3, 100 MHz): δ (ppm) 115.6, 110.2, 69.1, 35.5, 26.4, 26.2, 25.4, 22.6, 21.0, 21.0

4,4,5,5,6,6,7,7,8,8,9,9,9-tridecafluorononyl methanesulfonate (4)

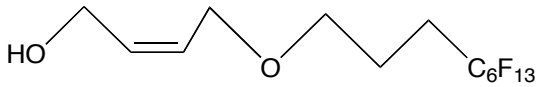


To a solution of 3-(perfluorohexyl)propanol **3** (1.7 g, 4.6 mmol) in dichloromethane (20 mL) was added triethylamine (1.3 mL, 9.1 mmol) and the mixture was cooled to 0 °C. Mesyl chloride (0.7 mL, 9.1 mmol) was added dropwise over 10 min and the reaction mixture was allowed to warm to ambient temperature over 3 h. The reaction mixture was diluted with dichloromethane (20 mL) and the organic layer was washed with water (20 mL) and brine (20 mL), and dried over MgSO4. The solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (2:1 hexane: ethyl acetate) to provide **4** (2.0 g, 98%, pale yellow oil).

^{1}H NMR (CDCl3, 400 MHz): δ (ppm) 4.34-4.30 (t, *J* =12, 2H), 3.05 (s, 3H), 2.32-2.19 (m, 2H), 2.13-2.06 (m, 2H)

^{13}C NMR (CDCl3, 100 MHz): δ (ppm) 117.6, 68.1, 37.7, 27.9, 27.6, 27.4, 23.6, 21.0, 20.9

(Z)-4-((4,4,5,5,6,6,7,7,8,8,9,9,9-tridecafluorononyl)oxy)but-2-en-1-ol

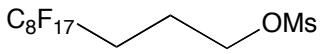


A solution was made of *cis*-1,4-butenediol (3.6 mL, 44 mmol) and 3-(perfluorohexyl)propyl methyl sulfonate (2.0 g, 4.4 mmol) in DMF (20 mL). A second solution was made of tetrabutylammonium bromide (0.16 g, 0.5 mmol) in DMF (20 mL), to which powdered KOH (0.25 g, 4.4 mmol) was added. The first solution was added dropwise over 5 min to the second solution and the reaction mixture was stirred for 12 h at ambient temperature then poured into water (20 mL). The aqueous layer was extracted with ethyl acetate (40 mL) and the organic layer was washed with water (30 mL) and brine (30 mL), and dried over $MgSO_4$. The solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (2:1 hexane: ethyl acetate) to provide **3** (1.2 g, 60%, pale yellow oil).

^{1}H NMR (CDCl3, 400 MHz): δ (ppm) 5.85-5.79 (m, 1H), 5.72-5.66 (m, 1H), 4.07-4.06 (d, *J* = 6 Hz, 2H), 3.53-3.50 (t, *J* = 11.6 Hz, 2H), 2.25-2.12 (m, 2H), 1.92-1.85 (m, 2H), 1.67-1.66 (m, 2H)

^{13}C NMR (CDCl3, 100MHz): δ (ppm) 132.4, 128.5, 123.9, 117.6, 69.1, 66.72, 59.0, 28.2, 23.6, 21.0

4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,11-octadecafluorononyl methanesulfonate

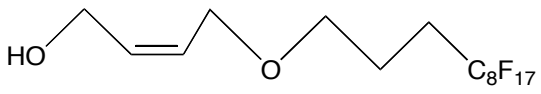


To a solution of 3-(perfluorooctyl) propanol **3** (0.81 g, 1.8 mmol) in dichloromethane (20 mL) was added triethylamine (0.15 mL, 3.6 mmol) and the mixture was cooled to 0 °C. Mesyl chloride (0.28 mL, 3.6 mmol) was added dropwise over 10 min and the reaction mixture was allowed to warm to ambient temperature over 3 h. The reaction mixture was diluted with dichloromethane (20 mL) and the organic layer was washed with water (20 mL) and brine (20 mL), and dried over $MgSO_4$. The solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (2:1 hexane: ethyl acetate) to provide **4** (0.998 g, 97%, pale yellow oil).

^{1}H NMR (CDCl3, 400 MHz): δ (ppm) 4.45-4.40 (t, *J* =12, 2H), 3.01 (s, 3H), 2.34-2.20 (m, 2H), 2.18-2.15 (m, 2H)

^{13}C NMR (CDCl3, 100M Hz): δ (ppm) 67.8, 37.4, 25.4, 26.5, 26.2, 21.4, 21.0, 19.2

(Z)-4-((4,4,5,5,6,6,7,7,8,8,9,9,10,10, 11, 11,11-heptadecafluorononyl)oxy)but-2-en-1-ol

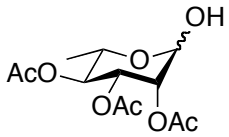


A solution was made of *cis*-1, 4-butenediol (0.998 mL, 0.021 mol) and 3-(perfluorooctyl)propyl methyl sulfonate (1.07 g, 2.42 mmol) in DMF (20 mL). A second solution was made of tetrabutylammonium bromide (0.15 g, 0.48 mmol) in DMF (15

mL), to which powdered KOH (0.27 g, 0.048 mol) was added. The first solution was added dropwise over 5 min to the second solution and the reaction mixture was stirred for 12 h at ambient temperature then poured into water (20 mL). The aqueous layer was extracted with ethyl acetate (40 mL) and the organic layer was washed with water (30 mL) and brine (30 mL), and dried over $MgSO_4$. The solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (2:1 hexane: ethyl acetate) to provide **5** (0.91 g, 67% , pale yellow oil).

^{1}H NMR (CDCl3, 400 MHz): δ (ppm) 6.75-6.72 (m, 1H), 6.58-6.54 (m, 1H), 4.42 (d, 2H), 4.18-4.15 (d, 2H), 3.42-3.40 (t, J = 11Hz, 2H), 2.58-2.55 (m, 2H), 1.54-1.52 (m, 1H)
^{13}C NMR (CDCl3, 100 MHz): δ (ppm) 131.2, 128.7, 128.5, 69.8, 65.4, 58.7, 29.7, 29.5, 29.0, 24.1, 23.8, 22.2

2, 3, 4-Tri-O-acetyl-rhamnopyranoside



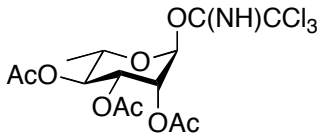
To a solution of the peracetylated L-rhamnose (1.5g, 4.51mmol) in DMF (15.0 mL), freshly prepared hydrazine acetate (0.49g, 5.41 mmol) was added at 50 ºC. The reaction mixture was stirred at 50 ºC for 45 min. The reaction mixture was allowed to come to room temperature and diluted with EtOAc (30 mL); the organic layer was washed with water (30 mL) and brine (30 mL), and dried over $MgSO_4$. The solvent was removed

under reduced pressure to obtain 2, 3, 4-tri-O-acetyl-rhamnopyranoside (1.18g, 90%, white solid).

^{1}H NMR (400MHz, CDCl3): 5.77-5.75 (m. 1H), 5.63 (dd, 1H), 5.32 (dd, 1H), 5.21(dd, 1H), 4.22-4.25 (m, 1H), 2.31 (s, 3H), 2.19 (s, 3H), 2.01 (s, 3H), 1.42 (d, 3H)

^{13}C NMR (100 MHz, CDCl3): δ (ppm) 171.2, 170.9, 168.8, 90.6, 74.2, 73.1, 68.9, 68.3, 20.1

2, 3, 4-Tri-O-acetyl-rhamnopyranoside trichloroacetimidate (14)

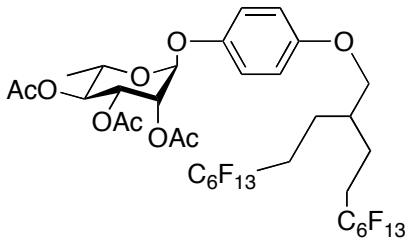


To a solution of 2, 3, 4-tri-O-acetyl-rhamnopyranoside (1.2g, 4.1 mmol) in CH_2Cl_2 (20 mL), trichloroacetonitrile was added (2.2 mL, 0.022 mol) followed by the addition of TMSOTf (74 μL, 0.40 mol) at 0 ºC. The reaction mixture was stirred at O ºC for 1h and then quenched with triethylamine and concentrated under reduced pressure. The crude product was purified by flash column chromatography (4:1 hexane: ethyl acetate) to provide **14** (1.5 g, 85%, pale yellow solid).

^{1}H NMR (CDCl3, 400MHz): δ (ppm) 5.72-5.69 (m, 2H), 5.26 (dd, 1H), 5.10 (dd, 1H), 4.68 (dd, 1H), 4.19-4.17 (m, 1H), 4.13-4.10 (m, 1H), 4.06-4.05 (dd, 1H), 3.89-3.87 (m, 1H), 2.19-2.16 (m, 2H), 2.10 (s, 3H), 2.05 (s, 3H), 2.01 (s, 3H),1.87-1.85 (m, 2H), 1.21 (d, 3H)

^{13}C NMR (100 MHz, CDCl3): δ (ppm) 202.6, 188. 6, 165.2, 166.1, 138.2, 138.2, 115.6, 114.9, 101.8, 101.4, 72.5, 71.8, 68.5, 66.6, 66.1, 46.2, 25.1, 24.8, 20.4, 18.1

4-((6,6,7,7,8,8,9,9,10,10,11,11,11-tridecafluoro-2-(4,4,5,5,6,6,7,7,8,8,9,9,9 tridecafluorononyl)undecyl)oxy)phenyl-2,3,4-tri-O-acetyl-α-L-rhamnopyranoside



To a solution of 2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl trichloroacetimidate **14** (84 mg, 0.19 mmol) and compound **7** [4] (4-((6,6,7,7,8,8,9,9,10,10,11,11,11-tridecafluoro-2-(4,4,5,5,6,6,7,7,8,8,9,9,9- tridecafluorononyl)undecyl)oxy)phenol (81 mg, 0.98 mmol) in toluene (4.0 mL), TMSOTf (0.35 mL, 1.9 μmol) was added at 0 ºC. The reaction mixture was stirred at 0 ºC for 20 min and then quenched with triethylamine (190 μL) and concentrated under reduced pressure. The crude product was purified by fluorous solid-phase extraction (FSPE) using a fluorous solid-phase extraction cartridge. Non-fluorous compounds were eluted with 80% MeOH/water and the desired product was eluted by 100% MeOH. The solvent was removed under reduced pressure to provide compound **19** (56 mg, 51%, oil)

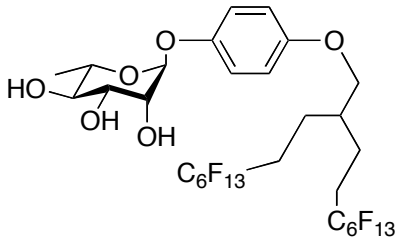
^{1}H NMR (CDCl3, 400MHz): δ (ppm) 7.65-7.63 (dd, *J* = 12Hz, 2H), 7.26-7.23 (dd, *J* =

[4] HDE provided compound **7**

12 Hz, 2H), 5.37-5.35 (m, 1H), 5.26 (dd, 1H), 5.15 (dd, 1H), 5.09-5.07 (m, 1H), 4.12-4.10 (m, 2H), 3.16- 3.14 (d, *J* = 8Hz, 2H), 2.34 (dd, 1H), 2.05 (s, 3H), 2.04 (s, 3H), 2.02 (s, 3H), 1.27-1.24 (m, 4H), 2.07-2.06 (m, 4H), 1.24-1.21 (m, 4H), 1.21-1.20 (d, 3H)

^{13}C NMR (CDCl3, 100 MHz): δ (ppm): 170.4, 157.3, 154.1, 132.9, 132.3, 128.8, 128.6, 116.1, 115.8, 69.1, 68.0, 67.7, 51.0, 35.6, 33.4, 32.1, 29.9, 24.0, 16.6, 14.7, 14.0, 10.2

4-((6,6,7,7,8,8,9,9,10,10,11,11,11-tridecafluoro-2-(4,4,5,5,6,6,7,7,8,8,9,9,9-tridecafluorononyl)undecyl)oxy)phenyl-α-L-rhamnopyranoside



To a solution of compound **19** (42 mg, 37 μmol) in methanol (2.0 mL) K_2CO_3 (30 mg, 22 mmol) was added. The reaction mixture was stirred at ambient temperature for 2 h and then filtered over Celite®. The solvent was removed under reduced pressure to provide compound **19** (28 mg, 71%, oil).

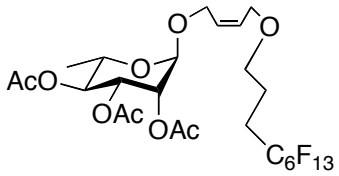
^{1}H NMR (CD_3OD, 400MHz): δ (ppm) 7.67-7.64 (dd, 12Hz, 2H), 7.49-7.46 (dd, 12Hz, 2H), 4.61-4.58 (m, 1H), 4.35-4.28 (m,1H), 4.12-4.10 (m, 1H), 3.75-3.73 (m, 1H), 3.52-3.54 (m, 1H), 3.47 (d, 2H), 2.21-2.10 (m, 4H), 1.72-1.70 (m, 4H), 1.23-1.21 (m, 4H), 1.21- 1.20 (d, 3H)

^{13}C NMR (CD_3OD, 100 MHz): δ (ppm) 127.7, 123.9, 117.6, 114.8, 90.8, 69.9, 69.3,

69.1, 67.7, 65.0, 45.3, 28.4, 23.5, 19.5, 16.23

HRMS calcd. for $C_{32}H_{30}F_{26}O_6Na$: 1027.15, found [M+Na]+2D: 1031.98

***cis*-4-*(1H,* 1H, 2H, 2H,3H, 3H-Perfluorononyloxy)-2-butenyl-2,3,4-O-tri-O-acetyl-α–L-rhamnopyranoside**



To a solution of 2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl trichloroacetimidate (21 mg, 49 μmol) and compound **5** (11 mg, 24 μmol) in toluene (3.0 mL), TMSOTf (9.0 μL, 0.49 μmol) was added at 0 ºC. The reaction mixture was stirred at 0 oC for 20 min and then quenched with triethylamine (0.68 μL) and concentrated under reduced pressure. The crude product was purified by fluorous solid-phase extraction (FSPE) using a fluorous solid-phase extraction cartridge. Non-fluorous compounds were eluted with 80% MeOH/water and the desired product was eluted by 100%MeOH. The solvent was removed under reduced pressure to provide compound **20** (15 mg, 85%).

^{1}HNMR (CDCl3, 400MHz): δ (ppm) 5.74-5.71 (m, 2H), 5.29 (dd, 1H), 5.07 (dd, 1H), 4.75 (dd, 1H), 4.19-4.17 (m, 1H), 4.13-4.10 (m, 1H), 4.06-4.05 (dd, 1H), 3.89-3.87 (m, 1H), 2.19-2.16 (m, 2H), 2.15 (s, 3H), 2.04 (s, 3H), 2.03 (s, 3H), 1.90-1.88 (m, 2H), 1.23-1.21 (d, 3H)

^{13}C NMR (CDCl3, 100 MHz): δ (ppm) 205.6, 184. 6, 167.3, 166.1, 137.1, 136.6, 116.5,

115.9, 103.2, 102.6, 71.5, 70.8, 70.3, 68.5, 66.6, 66.1, 46.2, 26.1, 25.6, 20.4, 17.2

Microarray preparation, screening and notes[5]

Microarrays were printed by the MicroarrayerXactII™ (LabNEXT Inc. 733 Beaver Road, Glenview, IL 60025) robotic pin (Xtend MP Microarray Pins 0.35 mm) deposition of the three different fluorous-tagged sugars in methanol/DMSO/water (v:v:v = 2:6:2) (250 μM solution) from a 384-well plate onto commercially available fluorinated glass slides (Fluorous Technologies, Inc.; Pittsburgh, PA) at 70% humidity and a temperature of 22 ºC. (Note that the slide will work better if it is washed once with a 1:1 solution of dichloromethane:methanol.) The glass slide was allowed to dry for 15 h to 24 h inside a humidity chamber and for another 2 h outside of it. This drying procedure helped in avoiding the donut effect and allowed the molecules to orient themselves on the slides to obtain good spots. The ConA-FITC 200 μM solution was composed of the FITC-labeled ConA (Sigma, 1 mg/1 mL, 200 μL) in a 1X PBS (780 μL) 1 mM $CaCl_2$ (10 μL) and 1 mM $MnCl_2$ (10 μL) were used for the detecting protein-carbohydrate interactions. The arrays were incubated with the protein solution (150 μL) by using a PC500 CoverWell incubation chamber (Grace Biolabs, Bend, OR) for 1 h, and then washed twice with 1X PBS containing 1% BSA followed by washing once with distilled water. They were then dried and scanned at Iowa State University DNA facility. The slides were scanned using a General Scanning ProScanArray 5000 set at 488 nm. The fluorescent intensities were determined using the ImaGene 8.0 software.

[5] The microarray spotting, incubation and screening was carried out with HDE.

During the microarray study we found that the spots had many inconsistencies depending on the environmental conditions. One of the major factors affecting the spot morphology was humidity. So, we did a comparative study by changing humidity and keeping the temperature constant at 22 ºC by using the already known mannose-C_8F_{17} tags. We started with 60% humidity as most microarray studies were previously performed at 60% humidity condition. Then we tested 65% and 70% humidity conditions. From the slides shown below, we can conclude that the slide at 60% humidity has donut effect whereas the slide at 65% humidity has a slightly less donut effect, and the slide at 70% humidity has no donut effect at all. Also, consistently good spots were observed with the slides printed at 70% humidity (Figure 5-2). Based on these results, for all the studies in this paper, we performed the microarray at 70% humidity.

Humidity 60% **Humidity 65%** **Humidity 70%**

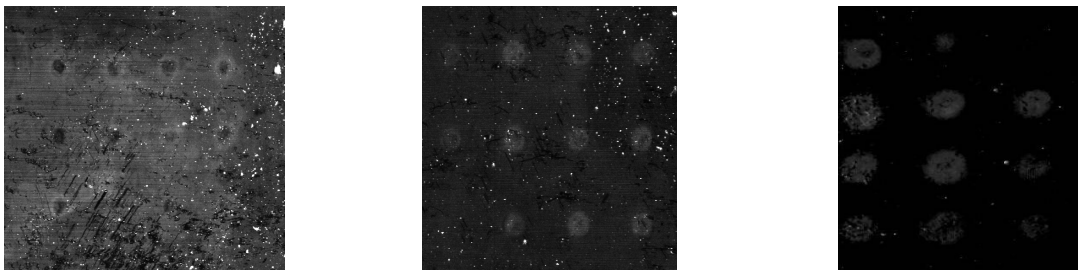


Figure 5-2. Effect of humidity changes on the fluorous coated glass slide.

Also, we found that the use of new slides caused inconsistencies in the spots in terms of binding ability of the carbohydrate to the slide. The new slide always showed prominent donut effect and most of time the spot would be completely washed away. So, we washed

the slides by adopting the method used by Spring and co-workers using 1:1 dichloromethane:methanol (Vegas et al, 2008). We decided to wash the new slide once with the 1:1 solution first before printing. Also, we were able to wash and reuse the slides at least five times.

While printing we observed contamination of the spots from the pins. The washing protocol of the array was not very well set up for carbohydrate microarray studies. So, we had to wash the pins out by hand after each spot with acetone. We used the same pin for each spot to get reliable results.

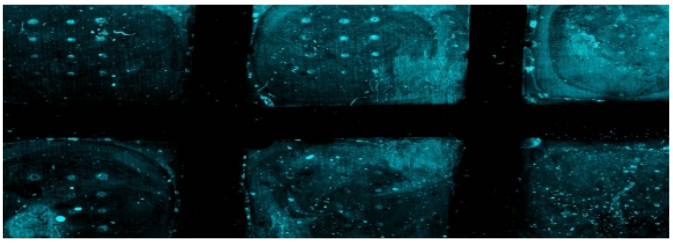
To develop a good washing protocol for the fluorous tagged carbohydrates, we scanned the slide after each washing with 1XPBS containing 1% BSA. We found that the first wash with 1XPBS containing 1% BSA was most effective in clearing the protein from the slide whereas the second wash had little effect. Also, we found that washing with distilled water had no effect on the slide. Each slide was washed twice with 1XPBS containing 1% BSA and once with distilled water to remove any remaining salts that may have deposited on the slide.

The slides below are the results that we obtained from the microarray studies. The β-D-glucose and the α-L-rhamnose slide showed nothing, as expected. The α-D-mannose slide showed that the mono-C_6F_{13}-tag was washed away, while the di-C_6F_{13}-tag and mono-C_8F_{17}-tag were clearly noticeable. The last slide has all nine fluorous-tagged

monosaccharides placed in one well. From this slide there are only two spots in each well indicating what the first three slides data shows.

Mannose Slide:

Mono-C_8F_{17}-Man Di-C_6F_{13}-Man Mono-C_6F_{13}-Man



Rhamnose Slide:

Mono-C_8F_{17}-Rha Di-C_6F_{13}-Rha Mono-C_6F_{13}-Rha

CHAPTER 6

Conclusions and Future Directions

In this dissertation methods development for glycosylation reactions of L-rhamnose using trichloroacetimidate donors, for automated solution-phase synthesis with application toward the synthesis of plant polysaccharides - homogalacturonan and rhamnogalacturonan-I and for a new fluorous tag for microarray studies are reported.

For the development of a method for achieving β-glycosidic linkages of L-rhamnose, the effect of electron-withdrawing protecting groups was analyzed. By employing both participating and non-participating electron-withdrawing groups strategically placed on the glycosyl donor, it was shown that such protecting group manipulations can control the stereochemistry at the anomeric center and the synthesis of difficult anomeric linkages, like β-L-rhamnose, could be achieved. Although the α-glycosylated product predominated in most cases, it was shown that by varying the protecting groups, the amount of β-product could be increased. From the results of glycosylation, a role for remote protecting groups in controlling anomeric selectivities was also proposed. These results are an important step in the study of glycosylation reactions as obtaining the absolute stereochemistry at the anomeric center is a tricky problem and synthesizing β-rhamnopyranosides is particularly challenging. This study with the electron-withdrawing groups paves the way for such protecting group manipulation studies for obtaining better stereoselectivity of glycosylation reactions in other cases, too. The promising results highlighted in chapter 2 of the dissertation can be combined with steric effects of the protecting group and the combined effects of electron withdrawing ability and sterics can

afford methods for better design of building blocks to obtain difficult anomeric linkages.

To automate the glycosylation reactions needed to build carbohydrate chains, the utility of an automated solution phase synthesizer in building the backbone of plant polysaccharides homogalacturonan and rhamnogalacturonan-I was also discussed. Methods for the synthesis of hexasaccharides of HG and RG-I were developed on the automated solution phase synthesis platform. These studies are the first examples of automated syntheses of plant polysaccharides. The well-defined and characterized structures of these plant polysaccharides can be extremely useful in studying the activity of enzymes that synthesize or degrade these polysaccharides in plants. With these convenient new methods for making these backbone chains, and the study of protecting groups in the design and synthesis of building blocks to control anomeric selectivities, the construction of oligosaccharide fragments can be simplified. With such tool sets now available, future studies into the enzymatic synthesis and degradation and possible binding partners of these oligosaccharides can be tackled.

The advantage of the fluorous-based automated solution phase synthesis was discussed in the application of the fluorous-tagged carbohydrates for microarray studies. The fluorous tag containing two C_6F_{13} chains is shown to be a superior tag for fluorous-based microarray formation compared to the previous single C_8F_{17}-containing tag. This di-tag can also be incorporated in the future into the building blocks for the synthesis of longer oligosaccharide chains in the automated solution phase platform to effect their easy separation on the basis of their fluorous tag content. If longer oligoaccharides are targeted for synthesis and the traditional mono-tag is not potent enough for good separation, the di-tag developed can be a very useful handle in such cases. With the flourous di-tag there

are also new possibilities for applications in the selective retention of fluorous content on the fluorous cartridge to separate differentially fluorous-tagged molecules.

The concept of selecting the best method for building block synthesis in terms of the use of protecting groups, combined with automating the process of glycosylation to synthesize longer oligosaccharide chains and the use of fluorous di-tagging strategies discussed in this dissertation all open the possibilities for better understanding of oligosaccharide synthesis and the application of such oligosaccharides to probe the biological roles of these complex rhamnose-containing and other plant oligosaccharides.

BIBLIOGRAPHY

Backinowsky, L. V.; Balan, N. F.; Shashkov, A. S.; Kochetkov, N. K. "Synthesis and ^{13}C-NMR spectra of β-L-rhamnopyranoside." *Carb. Res.* **1980**, *84*, 225-235.

Baek, J. Y.; Lee, B-Y.; Jo, G. M.; Kim, K. S. " β-Directing effect of electron withdrawing groups at O-3, O-4, and O-6 positions and α-directing effect by remote participation of 3-O-acyl and 6-O-acetyl groups of donors in mannopyranosylations." *J. Am. Chem. Soc.* **2009**, *131*, 17705 – 17713.

Bock, K.; Pedersen, C. J. "A study of 13CH coupling constants in hexopyranoses." *J. Chem. Soc. Perkin. Trans. 2.* **1974**, 293-297.

Boons, G-J. "Strategies in oligosaccharide synthesis." *Tetrahedron* **1996**, *52*, 1095-1121.

Brace, N. O. " Cyclization during the dehydrohalogenation of perfluoroalkyl-substituted iodoalkylmalonates. Thermal rearrangement of the derived 2-(perfluoroalkyl)methylcyclopropane-1,1-dicarboxylates." *J. Org. Chem.* **1975**, *40,* 851-858.

Brace, N. O. " Free-radical addition of iodoperfluoroalkanes to terminal alkadienes. Relative reactivity as a function of chain length and reaction conditions." *J. Org. Chem.*

1973, *38*, 3167-3172.

Brace, N. O. "Lactone formation in the free-radical addition of iodoperfluoroalkanes to alkenoic acids and esters." *J. Org. Chem.* **1964**, *29*, 1247-1249.

Brace, N. O. "Synthesis with F-alkyl radicals from F-alkyl iodides: amine and amine salt induced addition to alkenes." *J. Org. Chem.* **1979**, *44*, 212.

Brace, N. O. "Synthesis with perfluoroalkyl iodides. A review: Part III. Addition of R_FI to norbornene esters, acids and anhydrides, alkenoic acids and esters, alkenylsuccinic anhydride or diesters, and to vinyl monomers; lactonization and other reactions of adducts; hydroperfluoroalkylation by R_FI; synthesis and reactions of $I(CF_2)nI$ homologues (n = 1-3); perfluoroalkylation of arenes by R_FI or $[R_FCO_2]_2$; R_FI in the synthesis and reactions of R_FSR and segmented $R_F(CH_2)nSH$; and, useful derivatives therefrom." *J. Fluorine. Chem.* **2001**, *108*, 147-175.

Brace, N. O. "Synthesis with perfluoroalkyl iodides. Part II. Addition to non-conjugated alkadienes; cyclization of 1,6-heptadiene , and of 4-substituted 1,6-heptadienoic compounds: *bis*-allyl ether, ethyl diallylmalonate, *N, N'*-diallylamine and *N*-substituted diallylamines; and additions to homologous *exo*- and *endo*cyclic alkenes, and to bicyclic alkenes." *J. Fluorine. Chem.* **1999**, *96*, 101 -127

Brace, N. O. "Synthesis with perfluoroalkyl radicals from perfluoroalkyl iodides. A rapid

survey of synthetic possibilities with emphasis on practical applications. Part one: alkenes, alkynes and allylic compounds." *J. Fluorine. Chem.* **1999**, *93*, 1-25.

Brace, N. O.; Van Elswyk, J. E. " Evidence for free-radical reductive dehalogenation in reaction of zinc and acid with 1-perfluoroalkyl-2-iodoalkanes and with 1-perfluoroalkyl-2-iodoalkenes." *J. Org. Chem.* **1976**, *41*, 766-771.

Brace, N. O. "Relative reactivities and stereochemistry of addition of iodoperfluoroalkanes to cyclic olefins." *J. Org. Chem.* **1972**, *37*, 2429-2433.

Caffall, K. H.; Mohnen, D. "The structure, function, and biosynthesis of plant cell wall pectic polysaccharides." *Carbohydr. Res.* **2009**, *344*, 1879-1900.

Chen, G.; Pohl, N. L. "Synthesis of fluorous tags for incorporation of reducing sugars in quantitative microarray platform." *Org. Lett.* **2007**, *10*, 785–7885.

Chen, W.; Gu L.; Zhang, W.; Motari, E.; Cai, L.; Styslinger, T. J.; Wang, P.G. " L-rhamnose antigen: a promising alternative to α-gal for cancer immunotherapies." *ACS Chem. Biol.*, **2011**, *6*, 185-191.

Cheng, J.; Vecitis, C. D.; Park, H.; Mader, B. T.; Hoffmann, M. R. " Sonochemical degradation of perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) in landfill groundwater: environmental matric effects." *Environ. Sci. Technol.* **2008**. *42*,

8057-8063.

Chernyak, A.Y.; Weintraub, A; Kochetkov, N. K.; Lindberg, A. A. "The β-configuration of the rhamnosidic linkage in salmonella serogroups C_2 and C_3, lipopolysaccharide is important for the immunochemistry of the O-antigen 8." *Mol. Immunol.* **1993**, *30*, 887-893.

Clausen, M. H.; Jorgensen, M. R.; Thorsen, J.; Madsen, R. "A strategy for chemical synthesis of selectively methyl-esterified oligomers of galacturonic acid." *J. Chem. Soc., Perkin Trans. 1*, **2001**, 543–551.

Clausen, M. H.; Madsen, R. "Synthesis of hexasaccharide fragments of pectin." *Chem. Eur. J.* **2003**, *9*, 3821-3832.

Cramer, F., Pawelzik, K., Baldauf, H. J. "Imidoester. I. Darstellung von trichloroacetimidsäureestern (Imido esters. I. Preparation of trichloroacetamidic acid esters)." *Chemische Berichte*, **1958**, *91*, 1049 -1054.

Crich, D.; "Methodology development and physical organic chemistry:A powerful combination for the advancement of glycochemistry." *J. Org. Chem.* **2011**, *76*, 9193-9200.

Crich, D.; Hutton, T. K.; Banerjee, A.; Jayalath, P.; Picione, J. " Disarming, non-

participating 2-O-protecting groups in manno- and rhamnopyranosylation: scope and limitations of sulfonates, vinylogous esters, phosphates, cyanates, and nitrates." *Tetrahedron: Asymmetry*, **2005**, *16*, 105-119.

Crich, D.; Picione, J. "Direct synthesis of the β-L-rhamnopyranoside." *Org. Lett.* **2003**, *5*, 781-784.

Crich, D.; Sun, S. "Formation of β-mannopyranosides of primary alcohols using the sulfoxide method." *J. Org. Chem.* **1996**, *61*, 4506-4507.

Crich, D.; Vinod, A. U.; Picione, J. " The 3,4-O-carbonate protecting group as a β-directing group in rhamnopyranoylation in both homogeneous and heterogeneous glycosylations as compared to the chameleon-like 2,3-O-carbonates." *J. Org. Chem.* **2003**, *68*, 8453-8458.

Curran, D. "Fluorous reverse phase silica gel. A new tool for preparative separations in synthetic organic and organofluorine chemistry." *Synlett* **2001**, *9*, 1488-1496.

Curran, D. "Separations with fluorous silica gel and related materials." *Handbook of Fluorous Chemistry*, **2005**, 101-127.

Curran, D. P. "Strategy-level separations in organic synthesis: from planning to practice." *Angew. Chem. Int. Ed.*, **1998**, *37*, 1174-1196.

Curran, D. P. "Flourous tags unstick messy chemical biology problems" *Science*, **2008**, *37*, 1645- 1646.

Davis, B. G. " Recent developments in oligosaccharide synthesis." *J. Chem. Soc. Perkin. Trans. 1*, **2000**, 2137-2160.

Davis, K. R.; Darvill, A. G.; Albersheim, P.; Dell A. "Host pathogen interaction: Oligogalacturonides released from sodium polypectate by endopolygalacturonic acid lyase are elicitors of phytoalexins in soybean." *Plant Physiol*. **1986**, *80*, 568-577.

El Ashry E. S. H.; Rashed, N.; Ibrahim, E.S. "Challenges in the stereocontrolled synthesis of β-rhamnoside." *Tetrahedron*, **2008**, *64*, 10631- 10648.

Green, T. R.; Ryan, C. A. " Wound-induced proteinase inhibitor in plant leaves: A possible defense mechanism against insects." *Science* **1972**, *175*, 776-777.

Gridley, J. J.; Osborn, H. M. I. "Recent advances in the construction of β-D-mannose and β-D-mannosamine linkages." *J. Chem. Soc. Perkin Trans 1* **2000**, 1471-1491.

Hahn, M. G.; Darvill, A. G.; Albersheim, P. "Host-pathogen interaction: The endogenous elicitor, a fragment of a plant cell wall polysaccharide that elicits phytoalexin accumulation in soybeans." *Plant Physiol*. **1981**, *68*, 1161-1169.

Hakomori, S. “Tumor-associated carbohydrate antigens defining tumor malignancy: basis for development of anti-cancer vaccines.” *Adv. Exp. Med. Biol.* **2001**, *491*, 369–402.

Iversen, T.; Bundle, D. R. “A new and efficient synthesis of β-L-rhamnopyranosides.” *Carb. Res.* **1980**, *84*, C13-C15.

Jaipuri, F. A.; Collet, B. Y. M.; Pohl, N. L. “Synthesis and quantitative evaluation of glycero-D-manno- heptose binding to Concanavalin A by fluorous-tag assistance.” *Angew Chem Int Ed.* **2008**, *47*, 1708–1710.

Jarvis, M. C.; “Strcucture and properties of pectin gels in plant cell wall.” *Plant Cell Environ.* **1984**, *7*, 153-164.

Johansen, J. N.; Vernhettes, S.; Hofte, H. “The ins and outs of plant cell wall.” *Curr. Opin. Plant Biol.* **2007**, *10*, 616-620.

Kato, K.; Wong, L.-Y.; Jia. L. T.; Kuklenyik, Z.; Calafat, A. M. “ Trends in exposure of polyfluoroalkyl chemicals in the U.S. population: 1999-2008.” *Environ. Sci. Technol.* **2011**, *45,* 8037-8045.

Khanna, S. N.; Gupta, P. C. “Structure of muricatin.” *Phytochemistry*, **1967**, *6*, 735-739.

Knox, J. P. "Cell adhesion, cell separation and plant morphogenesis." *Plant J*. **1992**, *2*, 137-141.

Knox, J. P.; Linstead, P. J.; King, J.; Cooper, C.; Roberts, K. "Pectin esterification is spacially regulated both within cell walls and between developing tissues of root apices." *Planta*, **1990**, *181*, 512-521.

Ko, K-S.; Jaipuri, F. A.; Pohl, N. L. "Fluorous- based carbohydrate microarrays." *J. Am. Chem. Soc*. **2005**, *127*, 12162–13163.

Lau, J. M.; McNeil, M.; Darvill, A. G.; Albersheim, P. " Structure of the backbone of rhamnogalacturonan I, a pectic polysaccharide in the primary cell walls of plants" *Carbohydr. Res*. **1985**, *137*, 111-125.

Lee, H.; Mabury, S. A. " A pilot survey of legacy and current commercial fluorinated chemicals in human sera from United States donors in 2009." *Environ. Sci. Technol*. **2011**, *45*, 8067-8074.

Liang, P.-H.; Wu, C.-Y.; Greenberg, W. A.; Wong, C.-H. "Glycan arrays: biological and medical applications." *Curr. Opin. Chem. Biol*., **2008**, *12*, 86-92.

Liao, H.-Y.; Hsu, C.-H.; Wang, S.-C.; Liang, C.-H.; Yen, H.-Y.; Su, C.-Y.; Chen, C.-H.; Jan, J.-T.; Ren, C.-T.; Chen, C.-H.; Cheng, T.-J, R.; Wu, C.-Y.; Wong, C.-H.

"Differential receptor binding affinities of influenza hemagglutinins on glycan arrays." *J. Am. Chem. Soc.* **2010**, *42*, 14849-14856.

Lindberg, B.; Lonnngren, J.; Thompson, J. L. "Structural studies of the *Klebsiella* type 9 capsular polysaccharide." *Carb. Res.* **1972**, *25*, 49-57.

Liptak, A. "Hydrogenolysis of the dioxolan type exo- and endo- benzylidene derivatives of carbohydrates with the $LiAlH_4$-$AlCl_3$ reagent." *Tett Lett.* **1976**, *39*, 3551-3554.

Loi, E. I. H.; Yeung, W. Y.; Taniyasu, S.; Lam, P. K. S.; Kannan, K.; Yamashita, N. "Tropic magnification of poly- and perfluorinated compounds in a subtropical food web." *Environ. Sci. Technol.* **2011**, *45*, 5506-5513.

Loiseau, J.; Fouquet, E.; Fish, R.H.; Vincent, J.-M.; Verlhac, J.-B. " A new carboxylic acid with branched ponytails for the solubilization of Mn(II) and Co(II) ions in perfluorocarbons." *J. Fluorine. Chem.* **2001**, *108*, 195-197.

Lopez-Espinosa, M.-J.; Fletcher, T.; Armstronfg, B.; Genser, B.; Dhatariya, K.; Mondal, D.; Ducatman, A.; Leonardi, G. " Association of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) with age of puberty among children living near a chemical plant." *Environ. Sci. Technol.* **2011**, *45*, 8160-8166.

MacClean, D. J.; Sargent, J. A.; Tommerup, I. C.; Ingram, D. S. "Hypersensitivity as the primary event in resistance to fungal parasites." *Nature*, **1974**, *249*, 186-187.

Mamidyala, S. K.; Ko, K-S.; Jaipuri, F. A.; Park, G.; Pohl, N. L. "Noncovalent fluorous interactions for the synthesis of carbohydrate microarrays." *J. Flourine. Chem.* **2006**, *127*, 571–579.

McNeal, M.; Darvill, A. G.; Albersheim, P. " Structure of plant cell walls: Identification of seven differently linked glycosyl residues attached to O-4 of the 2, 4-linked L-rhamnosyl residues of rhamnogalacturonan I." *Plant Physiol.* **1982**, *70*, 1586-1591.

McNeil, M.; Darvill, A. G.; Albersheim, P. "Structure of plant cell walls: Rhamnogalacturonan I, a quantitatively major pectin in the cell walls of suspension-cultured sycamore cells." *Plant Physiol.* **1980**, *66*, 1128-1134.

McNeil, M.; Darvill, A. G.; Fry, S. C.; Albersheim, P. "Structure and function of the primary cell walls of plants." *Ann. Rev. Biochem.* **1984**, *53*, 625-663.

Merrifield, B. "Solid phase synthesis." *Biosci. Rep.* **1985**, *5*, 353-376.

Merrifield, B. "Solid phase synthesis." *Science* **1986**, *232*, 341-347.

Mizuno, M.; Kitazawa, S.; Goto, K. " *p*-Alkoxyphenyl-type heavy flourous tag for the

preparation of carbohydrate units." *J. Flourine. Chem* **2008**, *129*, 955-960.

Mohnen, D. "Pectin structure and biosynthesis" *Curr. Opin. Plant Biol.* **2008**, *11*, 266-277.

Murrey, H. E.; Hsieh-Wilson, L. C. "The chemical neurobiology of carbohydrates." *Chem. Rev.* **2008**, *108*, 1708-1731.

Nakamura, A.; Furuta, H.; Maeda, H.; Takao, T.; Nagamatsu, Y. "Structural studies by stepwise enzymatic degradation of the main backbone of soybean soluble polysaccharide consisting of galacturonan and rhamnogalacturonan." *Biosci. Biotechnol. Biochem.* **2002**, *66*, 1310-1313.

Nemati, N.; Karapetyan, G.; Nolting B.; Endress, H-U.; Vogel, C. "Synthesis of rhamnogalacturonan I fragments by a modular design principle." *Carbohydr. Res.* **2008**, *343*, 1730-1742.

Nicholson, R. L.; Ladlow, M. L.; Spring, D. L. "Fluorous tagged small molecule microarrays." *Chem Commun* **2007,** 3906–3908.

Nothnagel, E. A.; McNeil, M.; Albersheim, P.; Dell, A. " Host-pathogen interactions: A galactuuronic acid oligosaccharide from plant cell walls elicits phytoalexins" *Plant Physiol*, **1983**, 71, 916-926.

O'Neil, M.; Albersheim, P.; Darvill, A. "The pectic polysaccharides of primary cell walls." *Methods in Plant Biochemistry*, **1990**, *2*, 415-441.

P. J. H. Daas, B. Boxma, A. M. C. P. Hopman, A. G. J. Voragen, H. A. Schols. "Nonesterified galacturonic acid sequence homology of pectins." *Biopolymers* **2001**, *58*, 1-8.

Paulsen, H. "Advances in selective chemical synthesis of complex oligosaccharides" *Angew Chem. Int. Ed. Engl.* **1982**, *21*, 155- 173.

Penner, R. "PFOA in people." *Environ. Sci. Technol.* **2007**, *41*, 4497-4500.

Perry, M.B.; Richards, J. C. "Identification of the lipopolysaccharide O-chain of *Escgerichia hermannii* (ATCC 33651) as α-D-rhamnan." *Carb. Res.* **1990**, *205*, 371-376.

Petitou, M.; van Boeckel, C. A. A. " A synthetic antithromin III binding pentasaccharide is now a drug! What comes next?" *Angew. Chem. Int. Ed.* **2004**, *43*, 3118-3133.

Pistocchi, A.; Loos, R. " A map of European emissions and concentrations of PFOS and PFOA." *Environ. Sci. Technol.* **2009**, *43*, 9237-9244.

Plante, O. J.; Palmacci, E. R.; Seeberger, P. H. "Automated solid-phase synthesis of oligosaccharides" *Science*, **2001**, *291*, 1523-1527.

a.Pohl, N. L. " Automated solution-phase oligosaccharide synthesis and carbohydrate microarrays: development of fluorous-based tools for glycomics." *Chemical Glycobiology, ACS Symposium Series* **2008**, *990*, 272-2873.

b.Pohl, N. L. "Fluorous tags catching on microarrays." *Angew Chem Int Ed*. **2008** *47*, 3868–3870.

Renner, R. "EPA finds record PFOS, PFOA levels in Alabama grazing fields." *Environ. Sci. Technol*. **2009**, *43*, 1245- 1246.

Ridley, B. L.; O-Neill, M. A.; Mohnen, D. " Pectins: structure, biosynthesis, and oligogalacturonide-related signaling." *Phytochemistry*, **2001**, *57*, 929-967.

Ryan, C. A.; Bishop, P.; Pearce, G.; Darvill, A. G.; McNeil, M.; Albersheim, P. "A sycamore cell wall polysaccharide and a chemically related tomato leaf polysaccharide posses similar proteinase inhibitor-inducing activities." *Plant physiol*. **1981**, *68*, 616-618.

Scanlan, E. M.; Mackeen, M. M.; Wormald, M. R.; Davis, B. G. " Solution-phase conformation of the RG-I fragment of the plant pectin reveals a modification-modulated assembly mechanism." *J. Am. Chem. Soc*. **2010**, *21*, 7238-7239.

Schmidt, R. R. "New methods for the synthesis of glycosides and oligosaccharides. Are there alternatives to Koenigs-Knorr method?" *Ang. Chem. Int. Ed. Engl*. **1986**, *25*, 212-

235.

Schmidt, R. R.; Michel, J. " Facile synthesis of α- and β- glycosyl imidates; preparation of glycosides and disaccharides." *Angew. Chem.* **1980**, *92*, 731-732.

Seebeck, E.; Schindler, O. "Isolation of an emodin bioside from the bark of *Rhamnus fragula*." *Helv. Chim. Acta* **1946**, *29*, 317-324.

Seeberger, P. H.; Wertz, D. B. "Synthesis and medicinal applications of oligosaccharides." *Nature*, **2007**, *446*, 1046-1051.

Stallforth, P.; Lepenies, B.; Adibekian, A; Seeberger, P. H. " Carbohydrates: A frontier in medicinal chemistry." *J. Med. Chem.* **2009**, *52*, 5561-5577.

Talmadge, K. W.; Keegstra, K.; Bauer, W. D.; Albersheim, P. "The structure of plant cell walls." *Plant Physiol.* **1973**, *51*, 158-173.

Varki, A. "Biological roles of oligosaccharides: all of the theories are correct." *Glycobiology*, **1993**, *3*, 97-130.

Varki, A. *Essentials of glycobiology* **1999** Cold Spring Harbor Laboratory Pres (NY).

Vegas, A. J., Bradner, J. E.; Tang, W.; McPherson, O. M.; Greenberg, E. F.; Koehler, A.

N.; Schreiber, S. L. "Fuorous-based small-molecule microarrays for the discovery of histone deacetyase inhibitors." *Angew Chem Int Ed*. **2007**, 46, 7960–7964.

Vincken, J-P.; Schols, H. A.; Oomen, R. J. F. J.; McCann, M. C.; Ulvskov, P.; Voragen, A. G. J.; Visser, R. G. F. " If homogalacturonan were a side-chain of rhamnogalacturonan-I. Implications for cell wall architecture." *Plant Physiology* **2003**, *132*, 1781-1789.

Weizman, H.; Thor, Y. Carbohydrate-Based Drug Discovery Wiley-VCH, Weinheim, **2003**, 661–684.

Willats W.; McCartney, L.; Mackie, W.; Knox, J. "Pectin: cell biology and prospects for functional analysis." *Plant Mol Biol*., **2001**, *47*, 9-27.

Willats, W. G. T.; Orfila, C.; Limberg, G.; Buchholt, H. C.; van Alebeek, G-J. W. M.; Voragen, A. G. J.; Marcus, S. E.; Christensen, T. M. I. E.; Mikkelsen, J. D.; Murray, B. S.; Knox, J. P. "Modulation of the degree and pattern of methyl-esterification of pectic homogalacturonan in plant cell walls." *J. Biol. Chem*. **2001**, *276*, 19404-19413.

Yamamoto, K.; Watanabe, N.; Matsuda, H.; Oohara, K.; Araya, T.; Hashimoto, M.; Miyairi, K.; Okazaki, I.; Saito, M.; Shimizu, T.; Kato, H.; Okumo, T. "Design, synthesis, and enzymatic property of a sulfur-substituted analogue of trigalacturonic acid." *Bioorg. Med. Chem. Lett*. **2005**, *15*, 4932-4935.

Yamazaki, N.; Fry, S. C.; Darvill, A. G.; Albersheim, P. " Host-pathogen interaction: Fragments isolated from suspension-cultured sycamore cell walls inhibit the ability of the cells to incorporate [^{14}C]Leucine into proteins." *Plant Physiol*. **1983**, *72*, 864-869.

Zhag, Z.; Ollman, I. R.; Ye, X-S.; Wischnat, R.; Baasov, T.; Wong, C-H. "Programmable one-pot oligosaccharide synthesis." *J. Am. Chem. Soc*. **1999**, *121*, 734-753.

Zhang, J.; Zhu, Y.; Kong, F.; "Synthesis of an L-rhamnose tetrasaccharide, the common and major structure of the repeating unit if the O-antigenic polysaccharide of a strain of *Klebsiella pneumoniae* and *Pseudomonas holci*" *Carb. Res*. **2001**, *336*, 229-235.

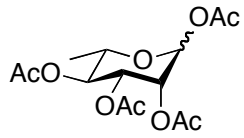
Zhang, W.; Cai, C.; "New chemical and biological applications of flourous technologies." *Chem. Commun*. **2008**, 5686-5694.

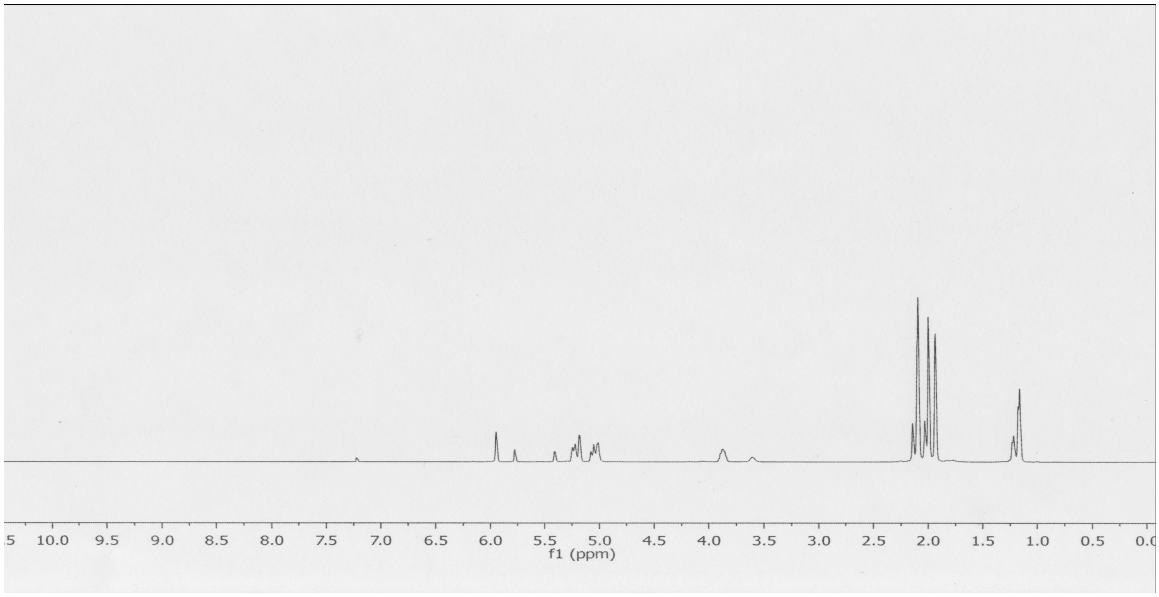
Zhang, Z.; Ollmann, I. R.; Ye, X.-S.; Wischnot, R.; Baasov, T.; Wong, C.-H. "Programmable one-pot oligosaccharide synthesis." *J. Am. Chem. Soc*. **1999**, *121*, 734-753.

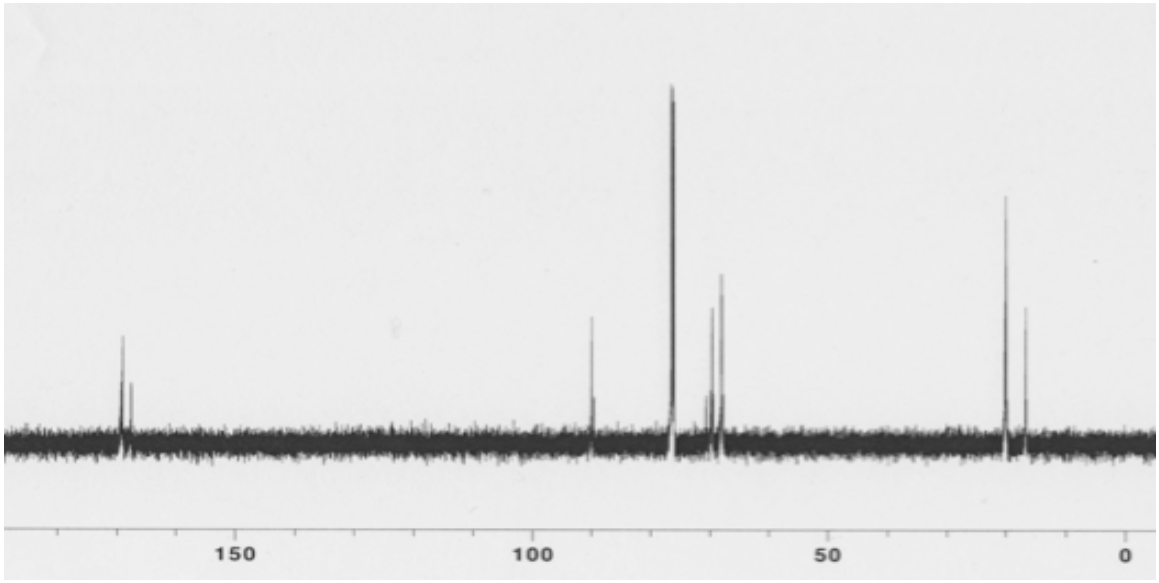
Zhu, X; Schmidt, R. R. " New principles for glycoside-bond formation." *Angew. Chem Int. Ed*. **2009**, *48*, 1900-1934.

APPENDIX A CHAPTER 2 ^{1}H AND ^{13}C NMR SPECTRA

1, 2, 3, 4-Tetra-O-acetyl-α/β-L-rhamnopyranoside







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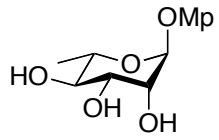

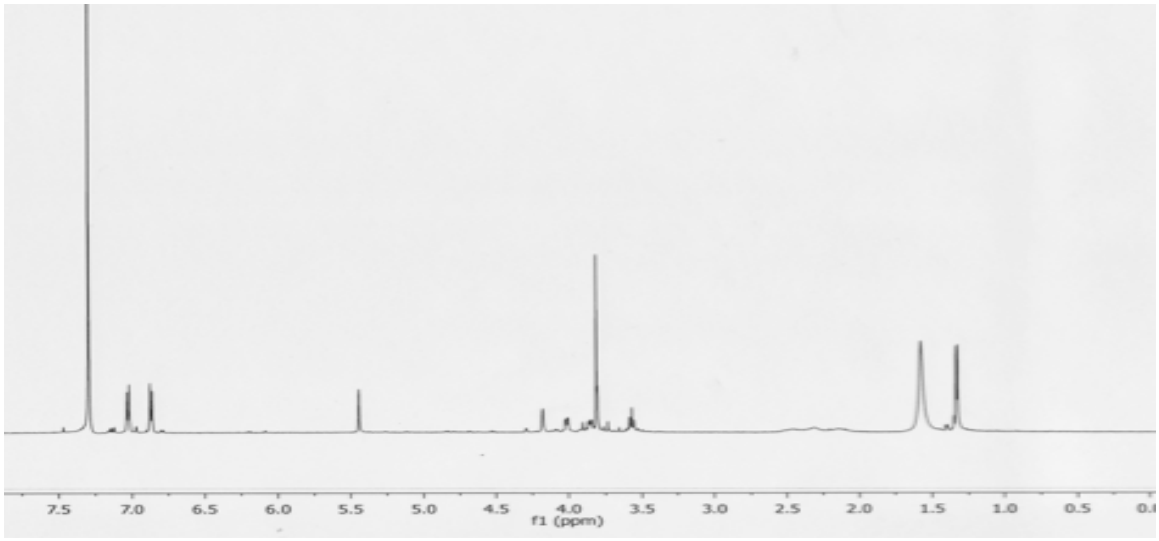

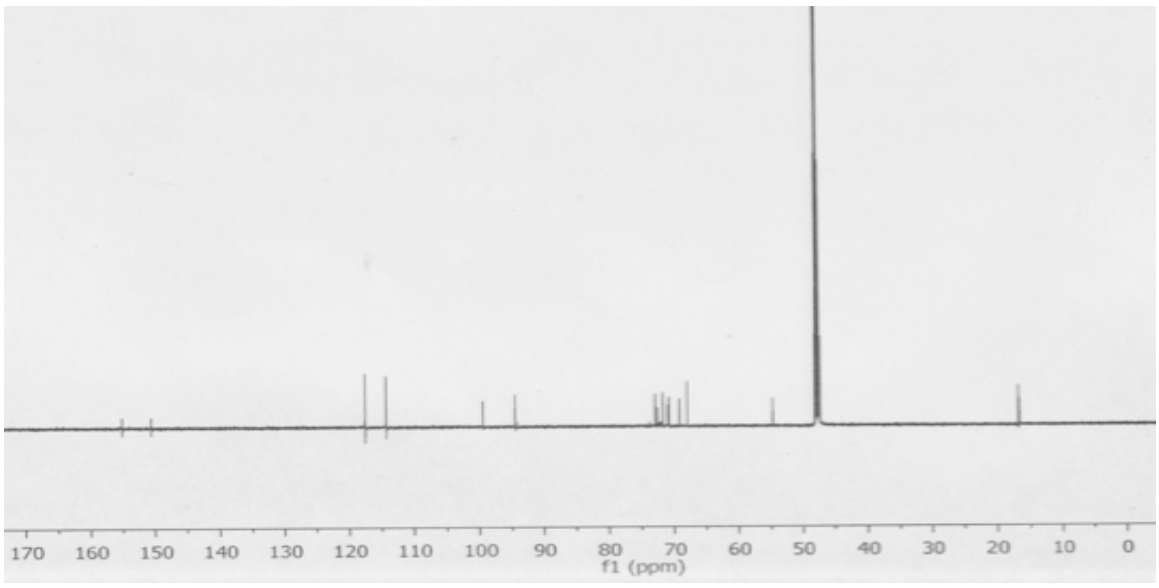

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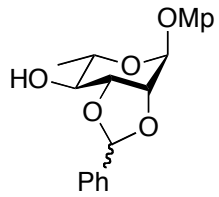

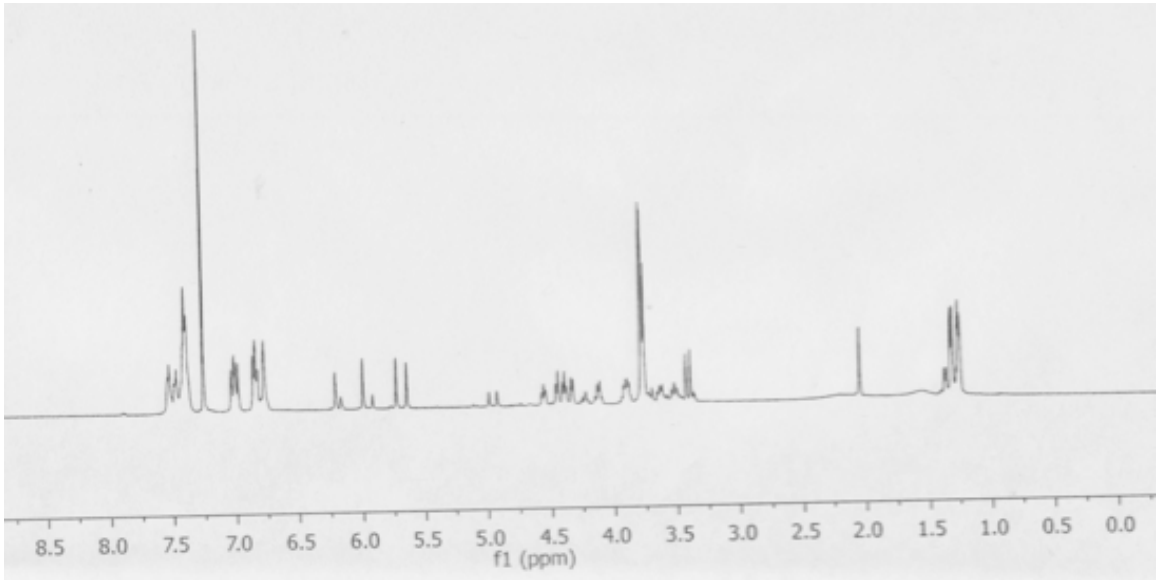

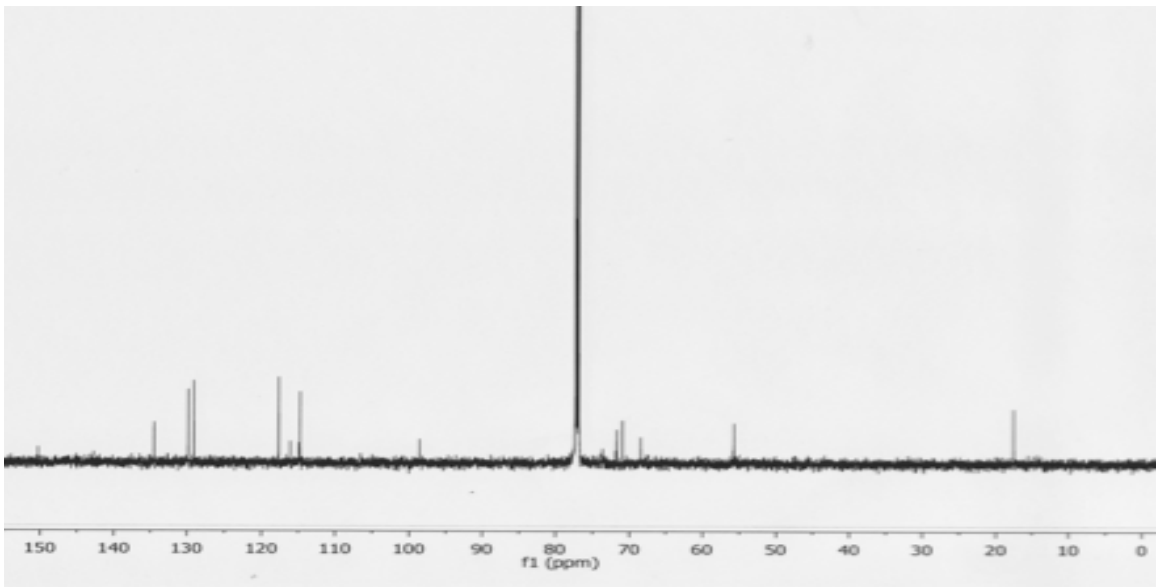

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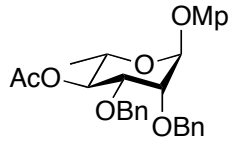

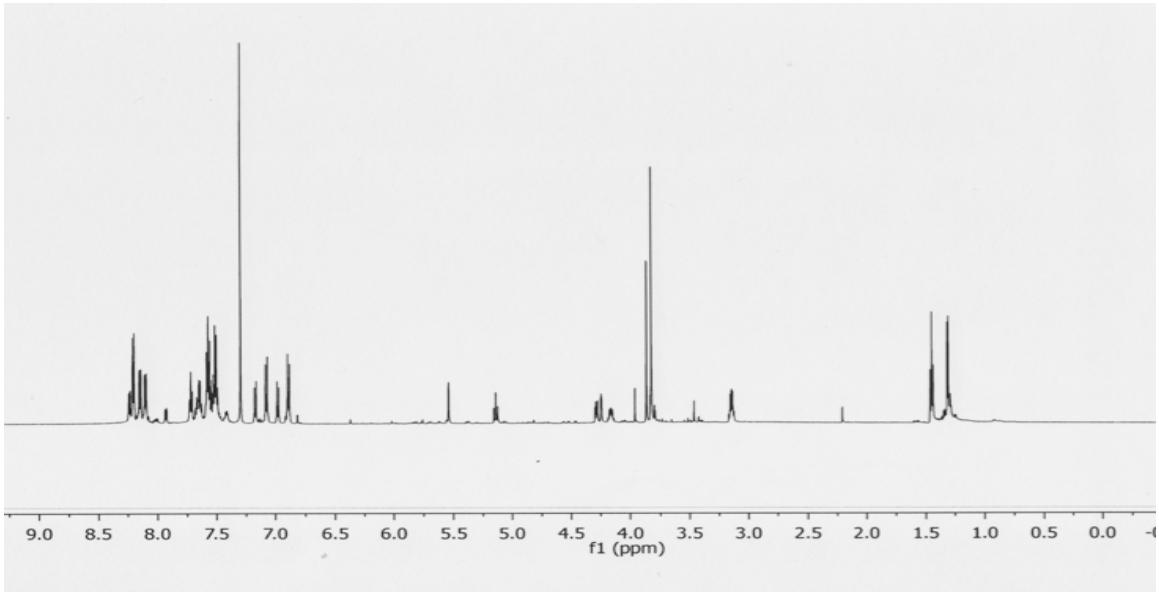

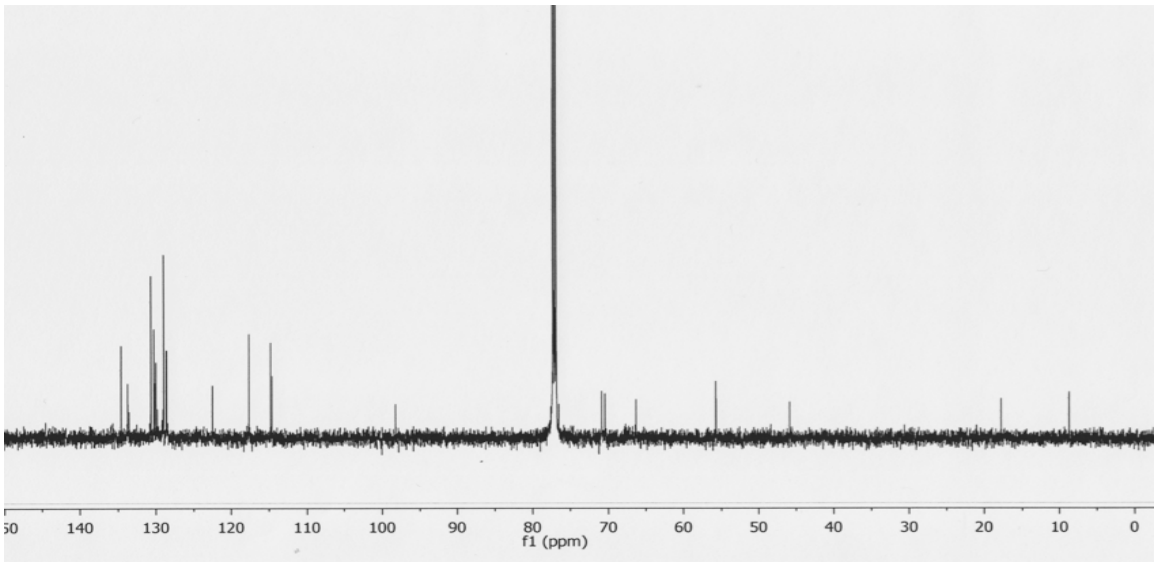

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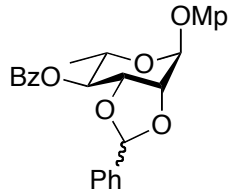

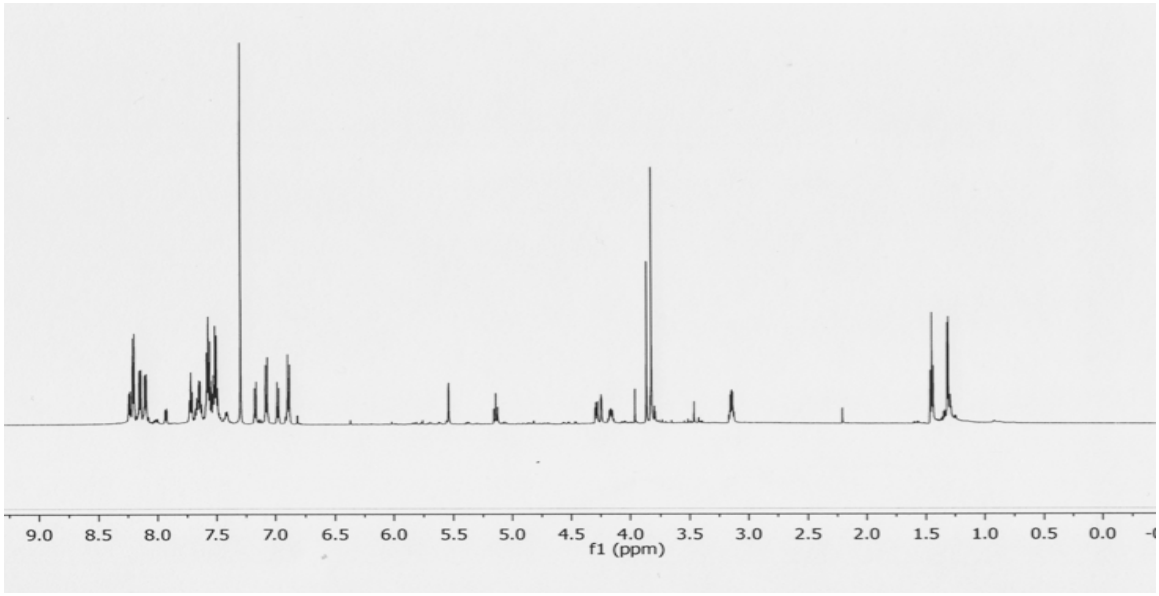

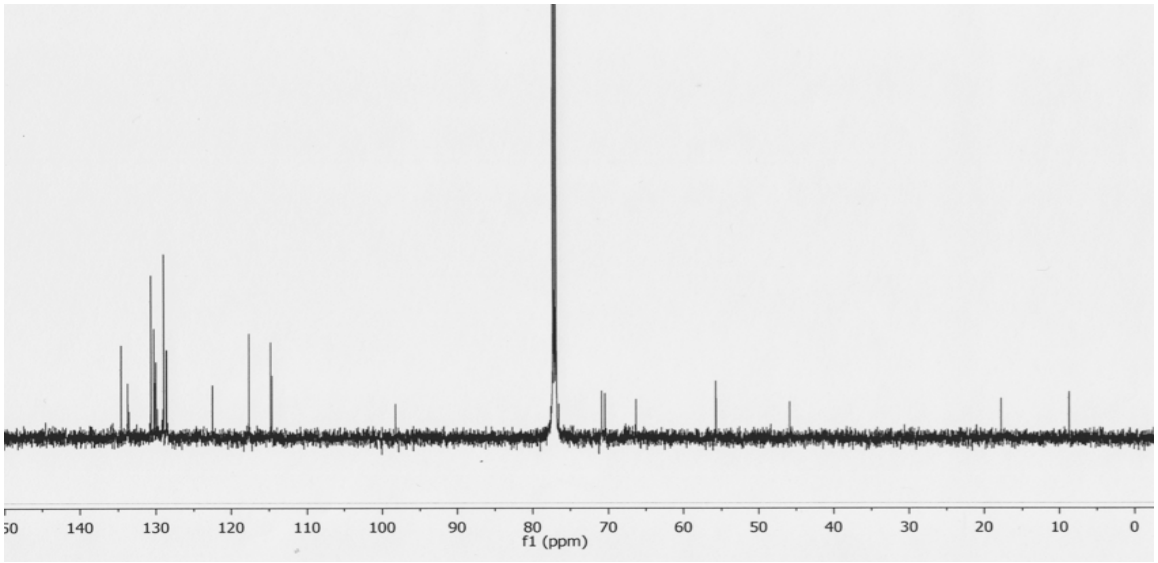

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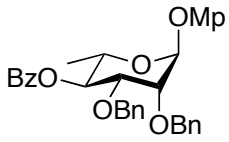

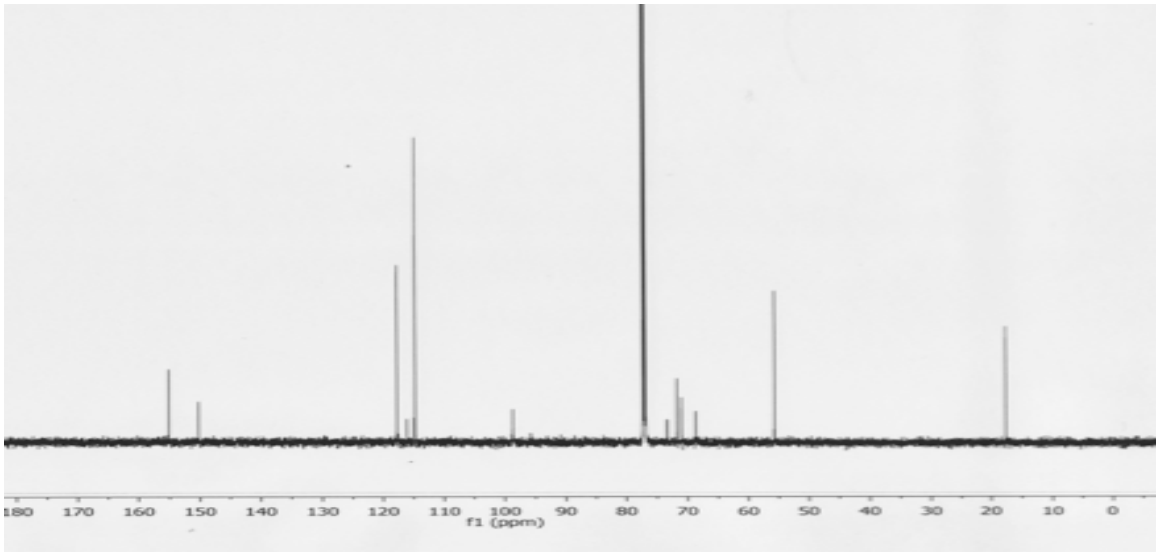

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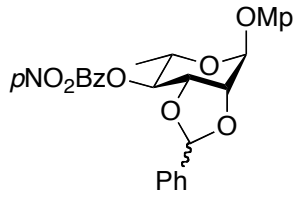

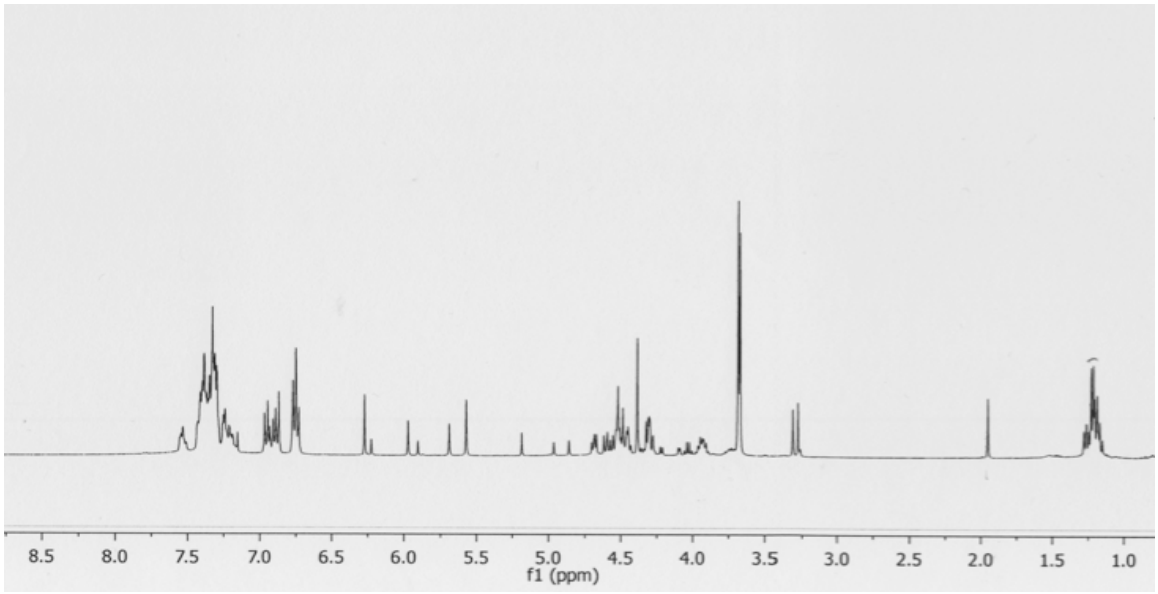

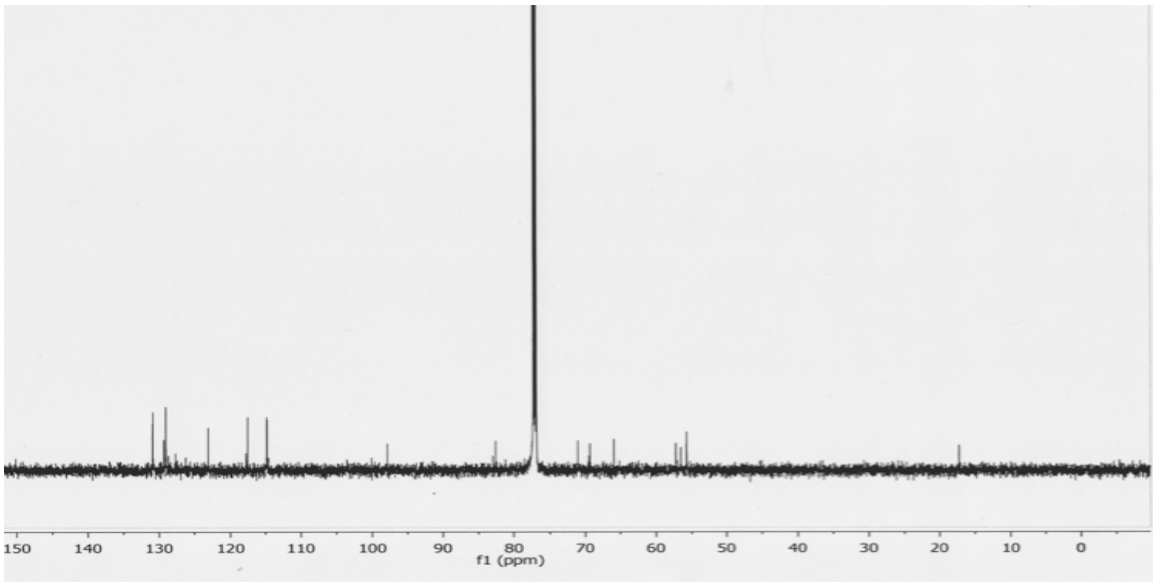

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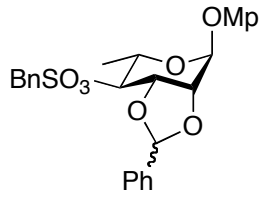
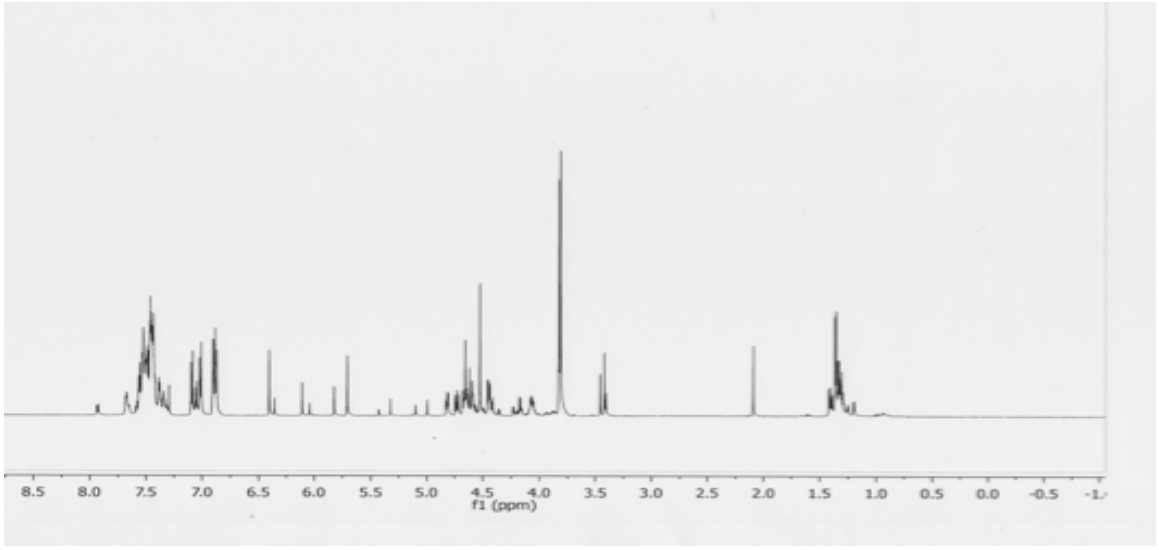
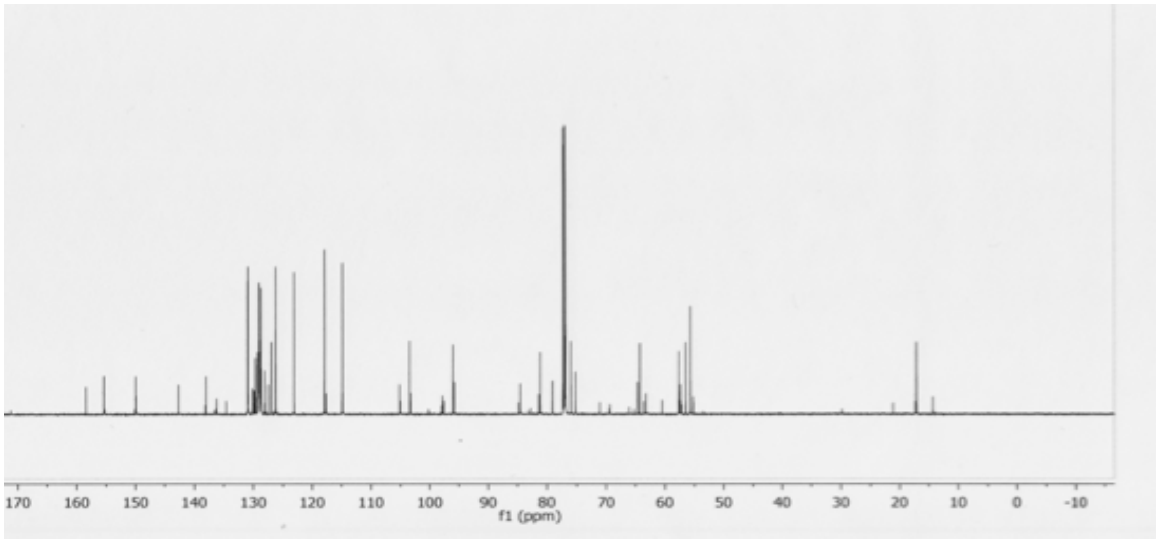
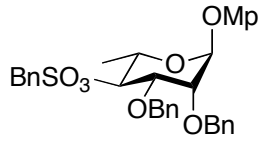
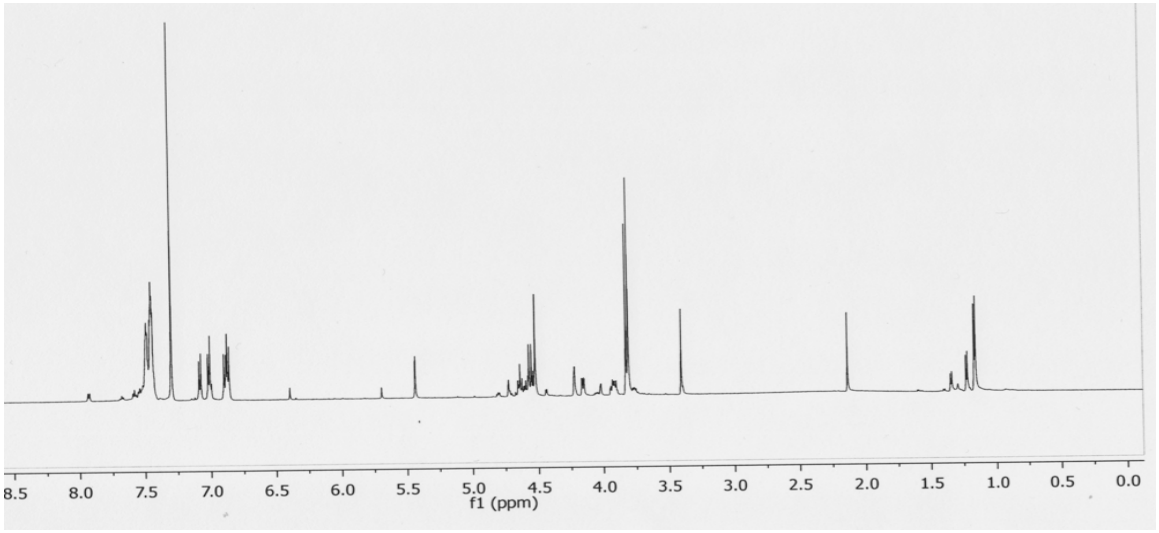


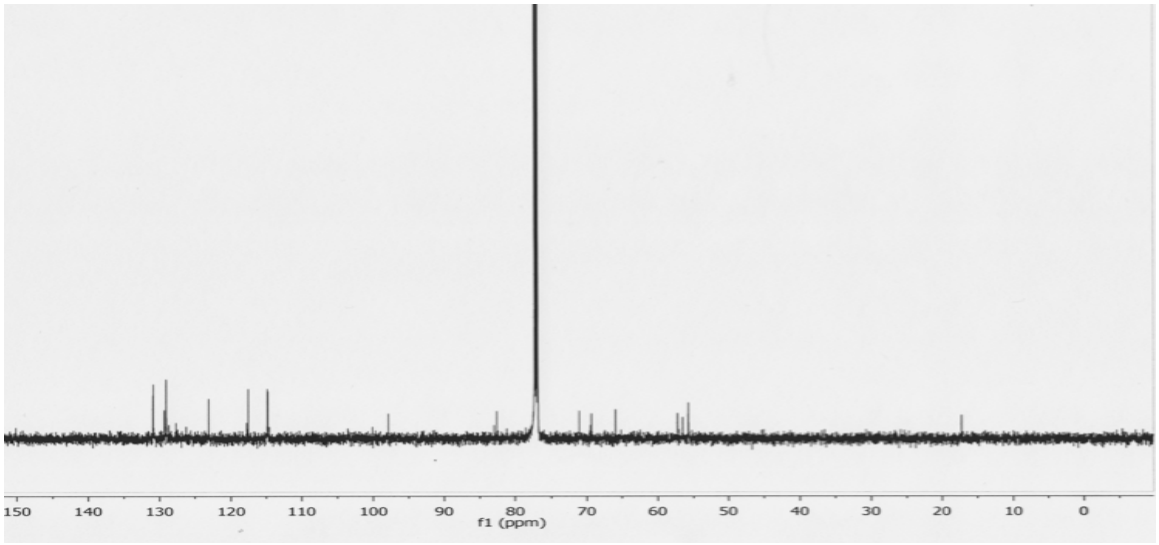




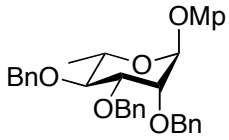
1-O-*p*-Methoxy-2, 3-O-dibenzyl-4-O- benzylsulfonyl--α-L-rhamnopyranoside

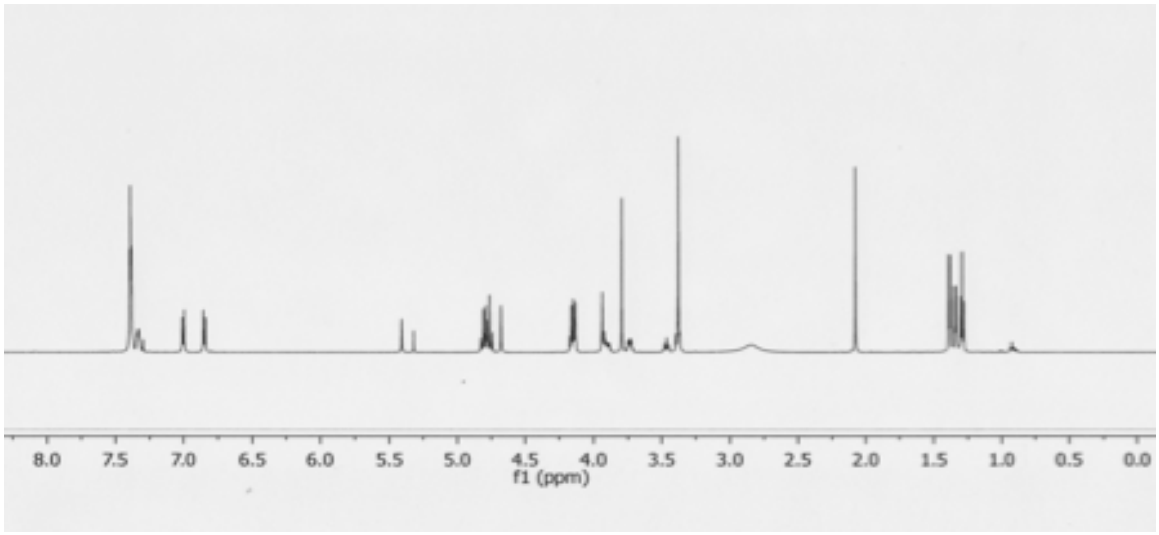


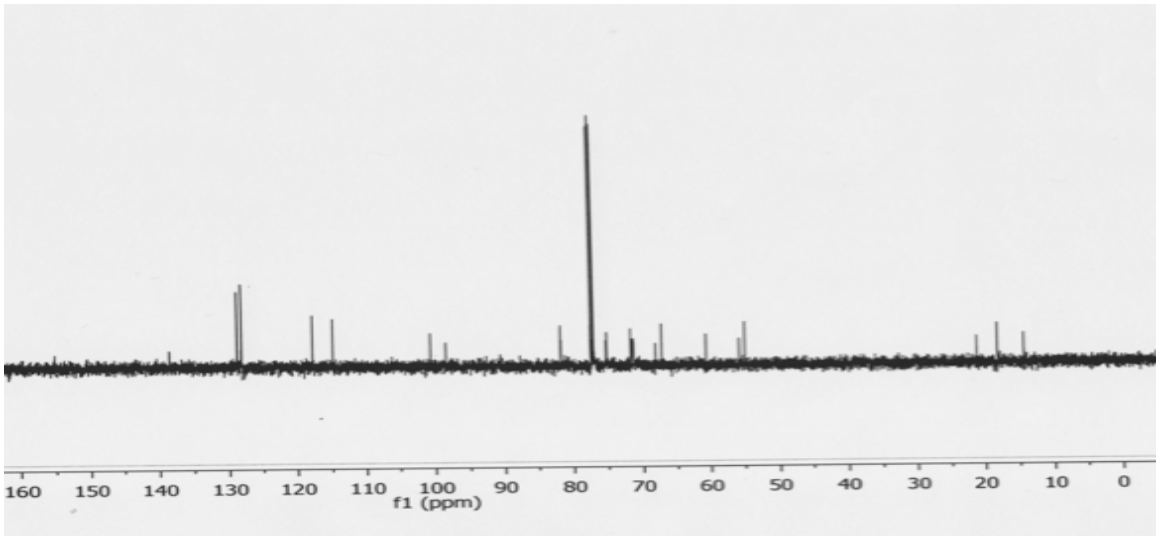




1-O-*p*-Methoxy-2, 3, 4-tribenzyl-α-L-rhamnopyranoside







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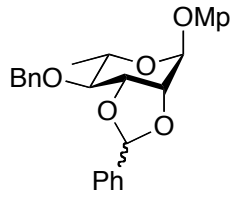

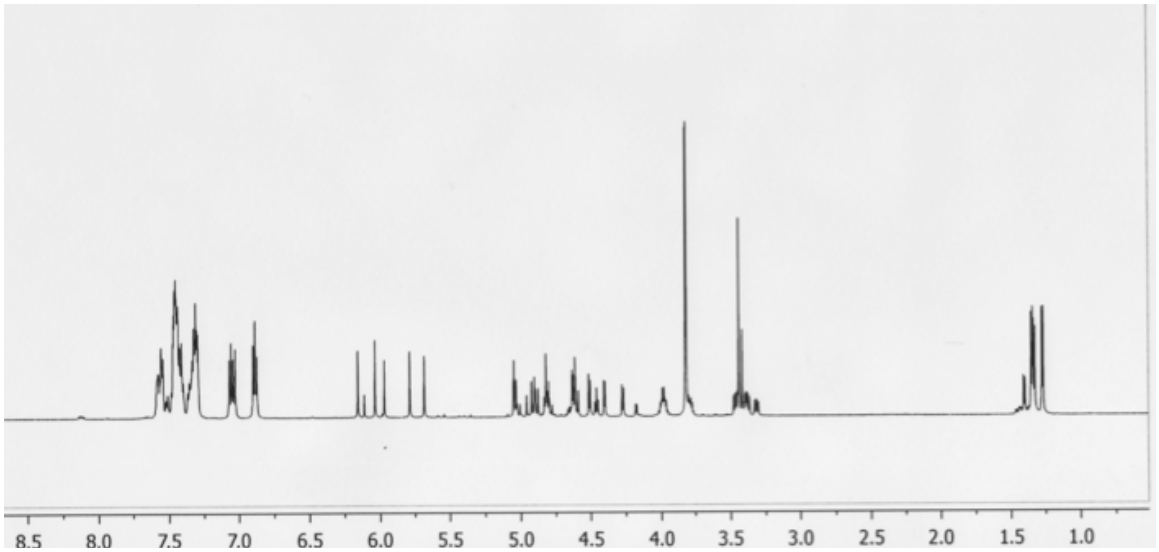

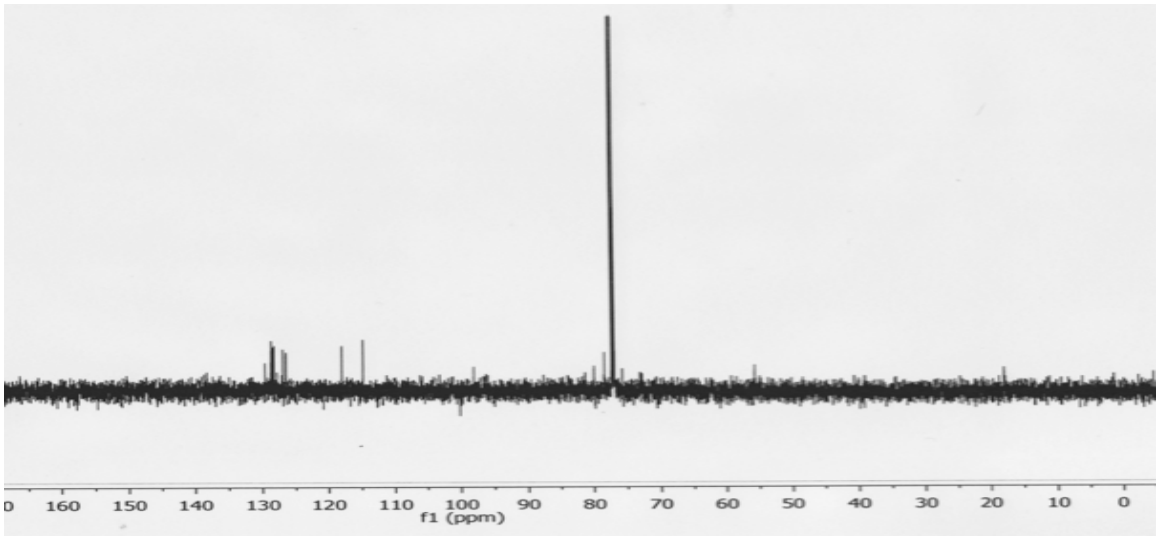

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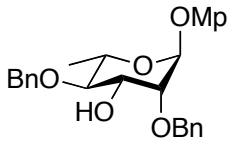
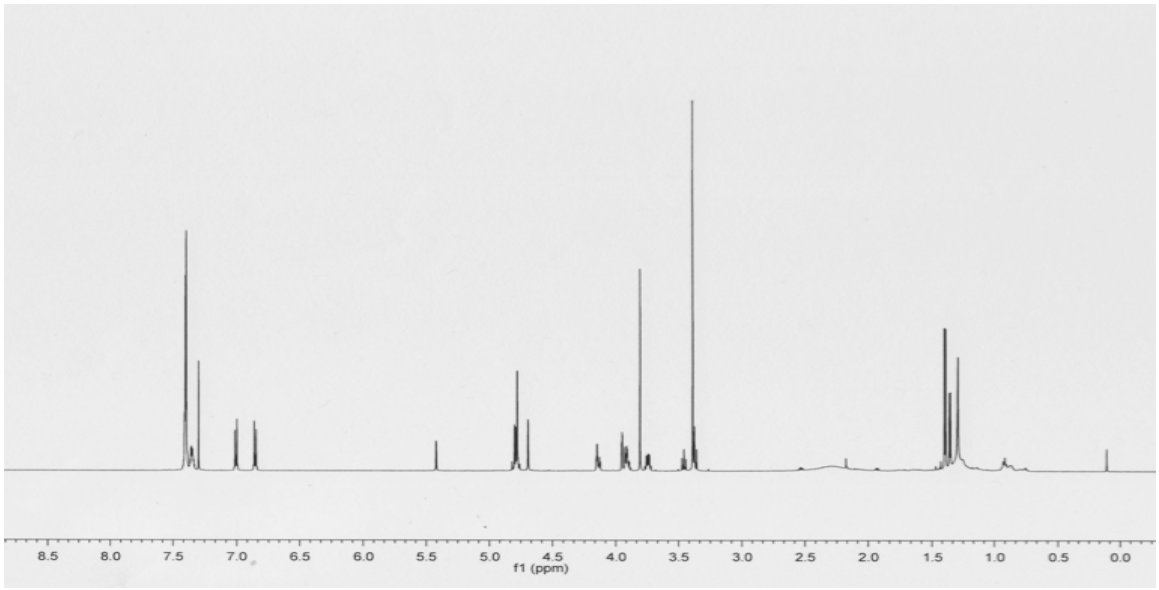
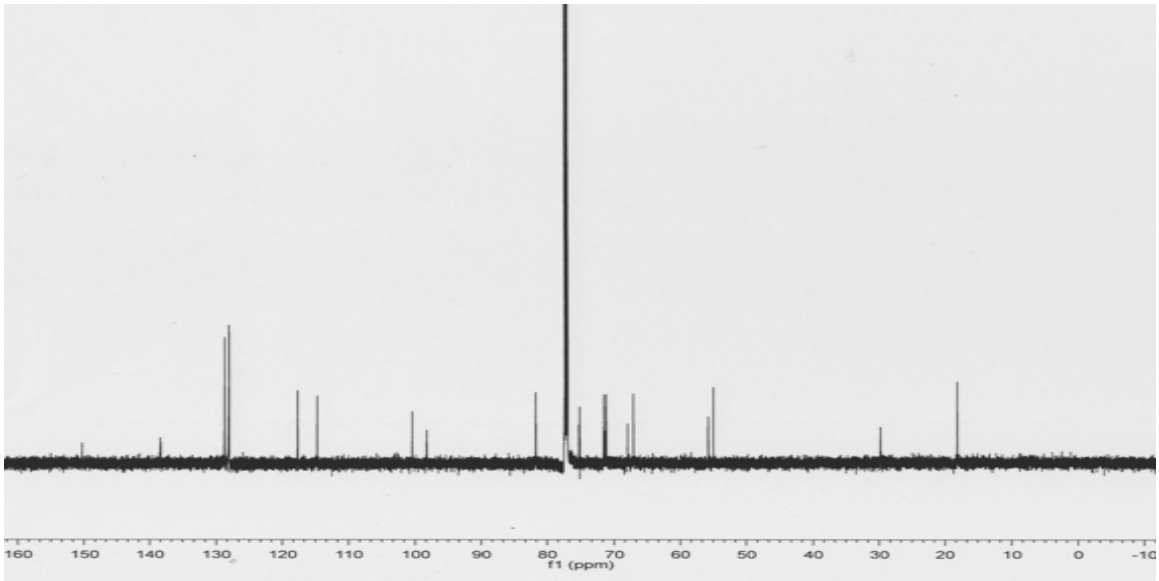
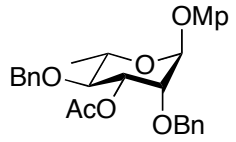
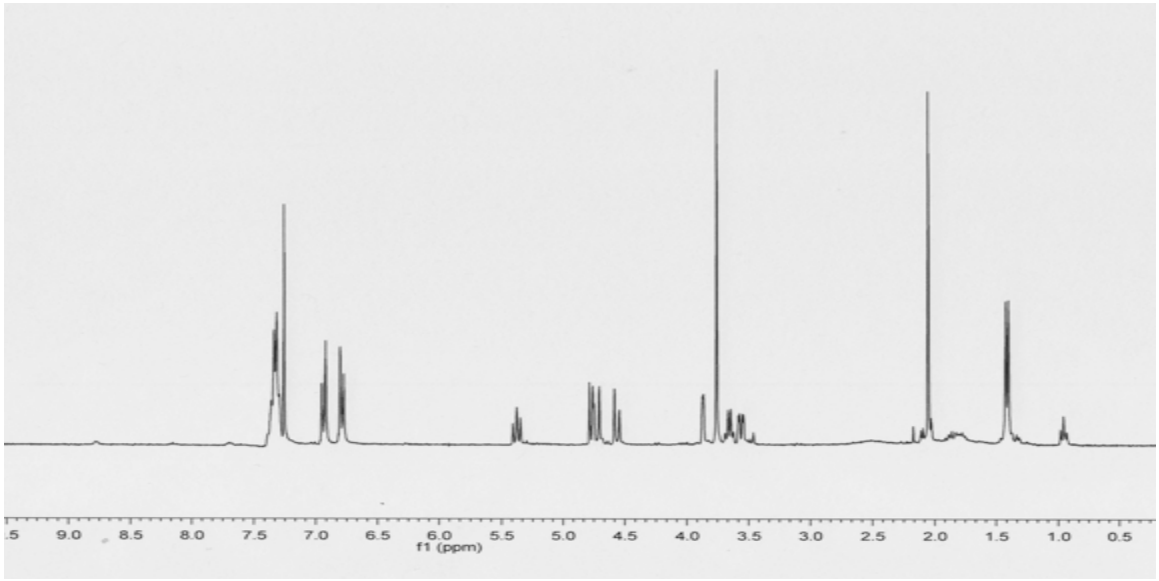


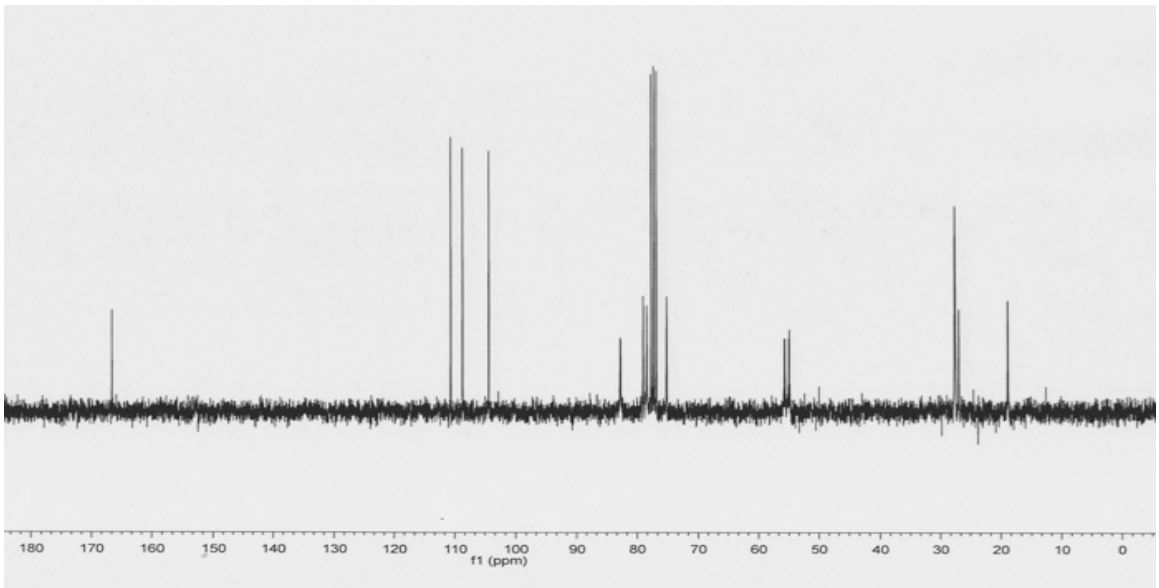




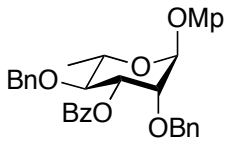
1-O-*p*-Methoxy-2, 4-O-di-benzyl-3-O-acetyl-α-L-rhamnopyranoside

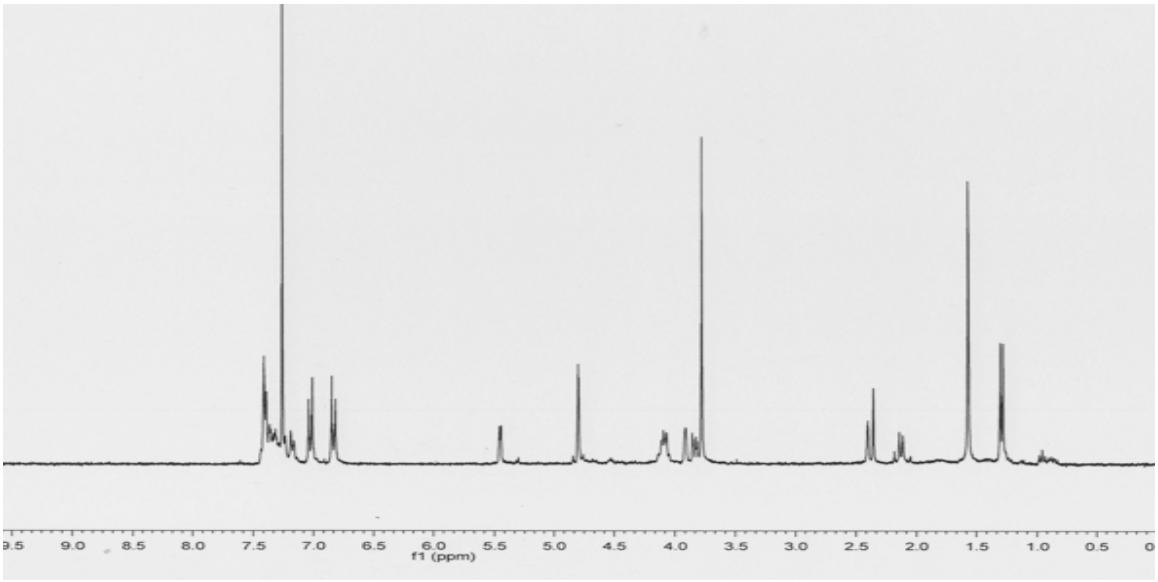




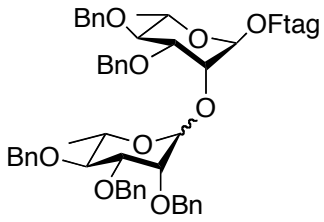


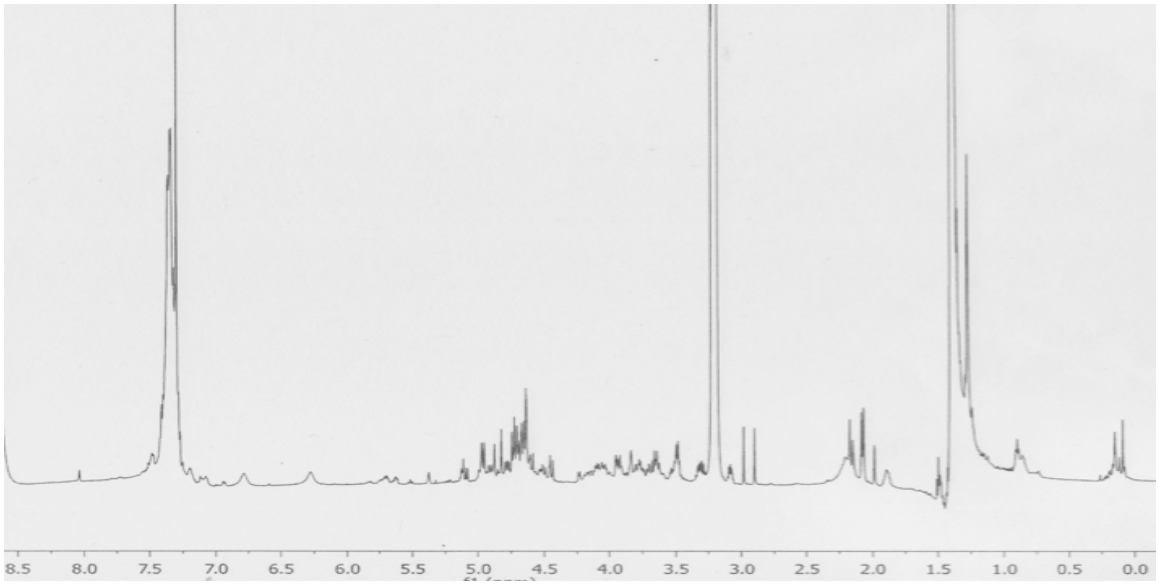
1-O-*p*-Methoxy-2, 4-O-dibenzyl-α-L-rhamnopyranoside



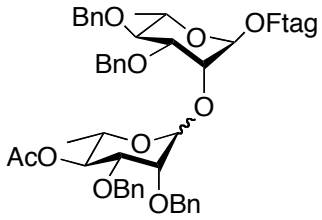


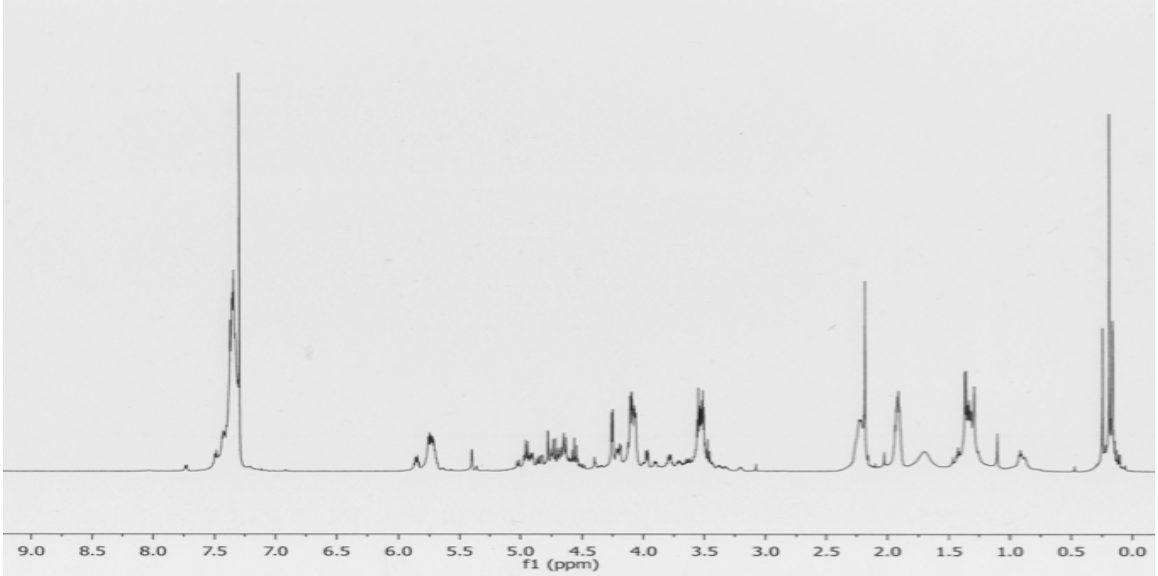
***cis*-4-(1H, 1H, 2H, 2H, 3H, 3H-Perfluoroundecyloxy)-2-butenyl 2, 3, 4-tri-O-benzyl-α/β-L-rhamnopyranosyl-(1→2)-3,4-di-O-benzyl-α-L-rhamnopyranoside**

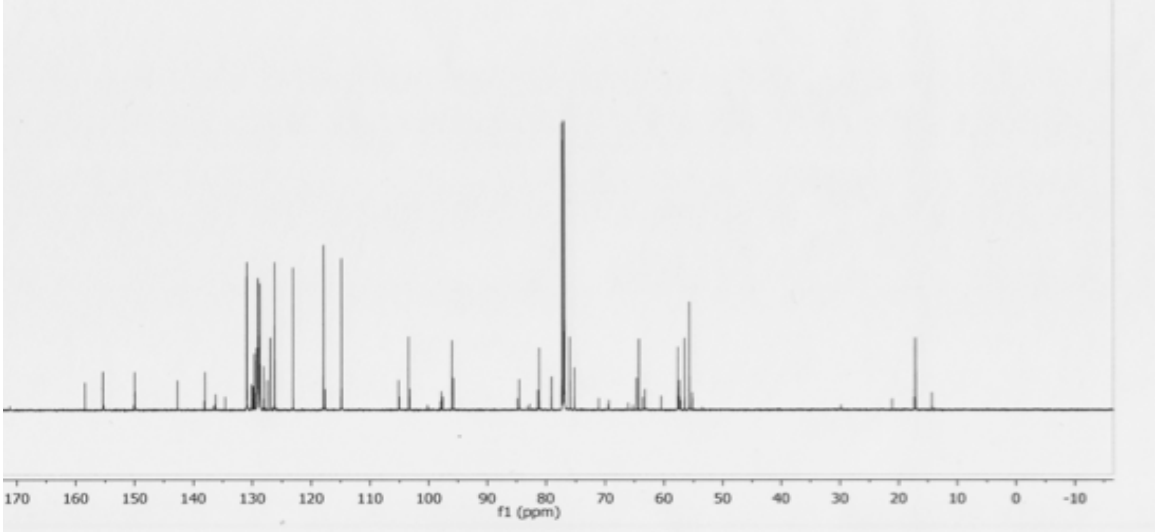




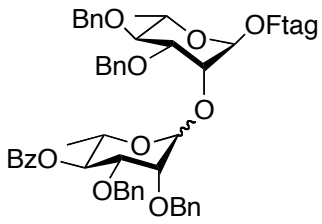
***cis*-4-(1H, 1H, 2H, 2H, 3H, 3H-Perfluoroundecyloxy)-2-butenyl 4-O-acetyl-2, 3-di-O-benzyl- α/β-L-rhamnopyranosyl-(1→2)-3,4-di-O-benzyl-α-L-rhamnopyranoside**

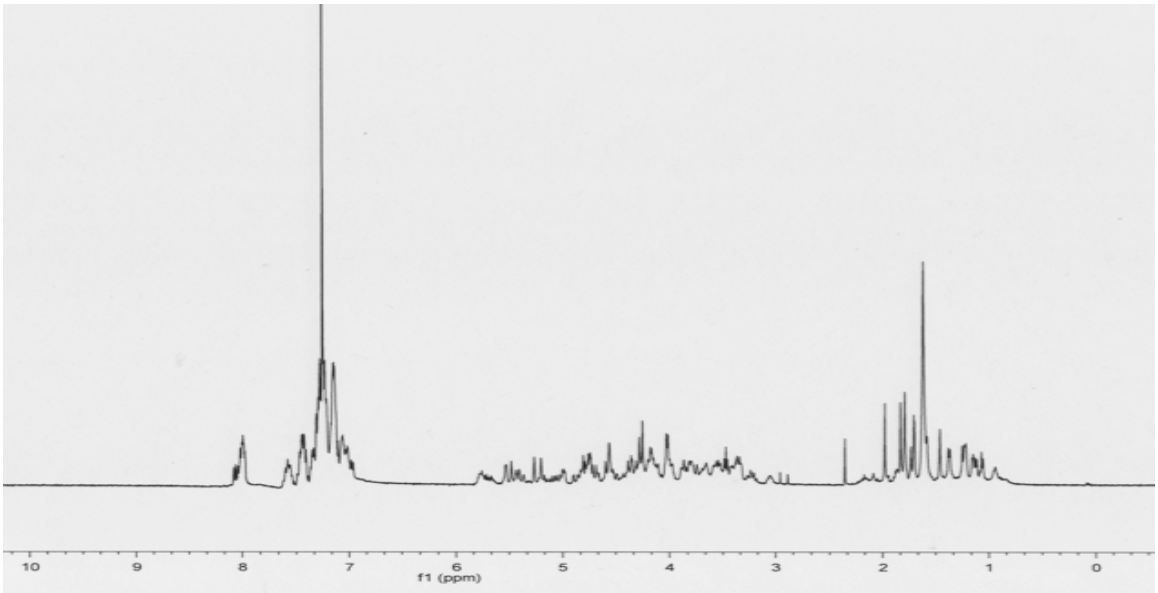




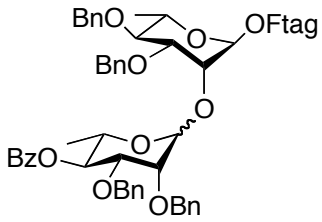


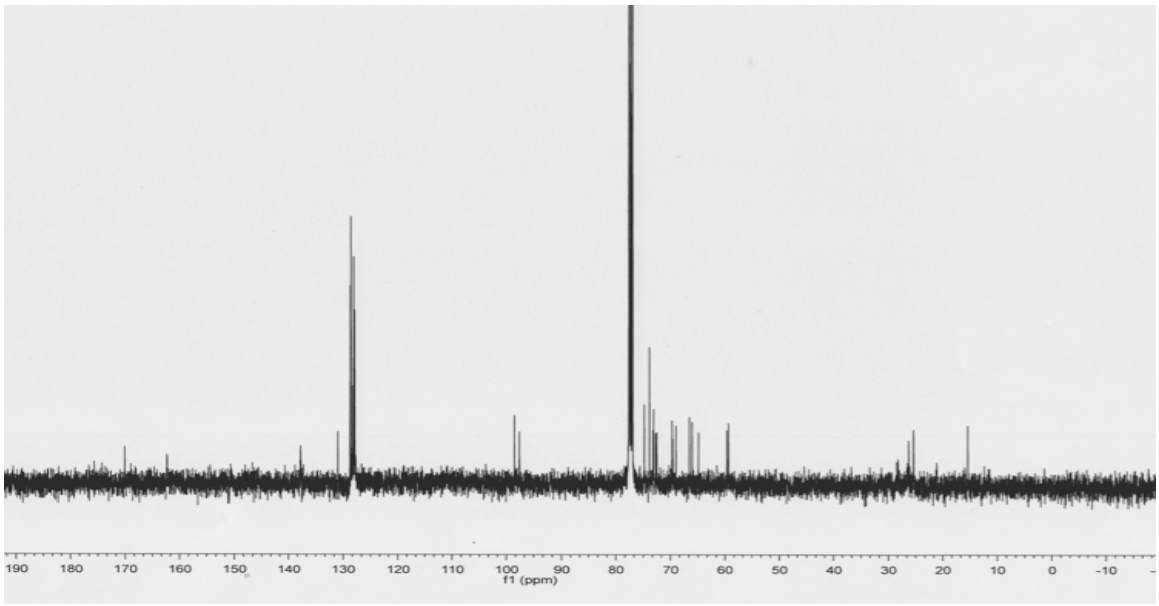
***cis*-4-(1H, 1H, 2H, 2H, 3H, 3H-Perfluoroundecyloxy)-2-butenyl 4-O-benzoyl-2, 3-di-O-benzyl- α/β-L-rhamnopyranosyl-(1→2)-3,4-di-O-benzyl-α-L-rhamnopyranoside**



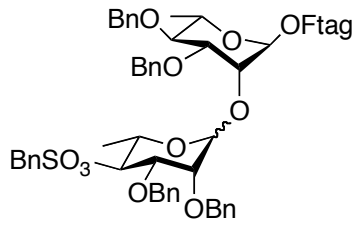


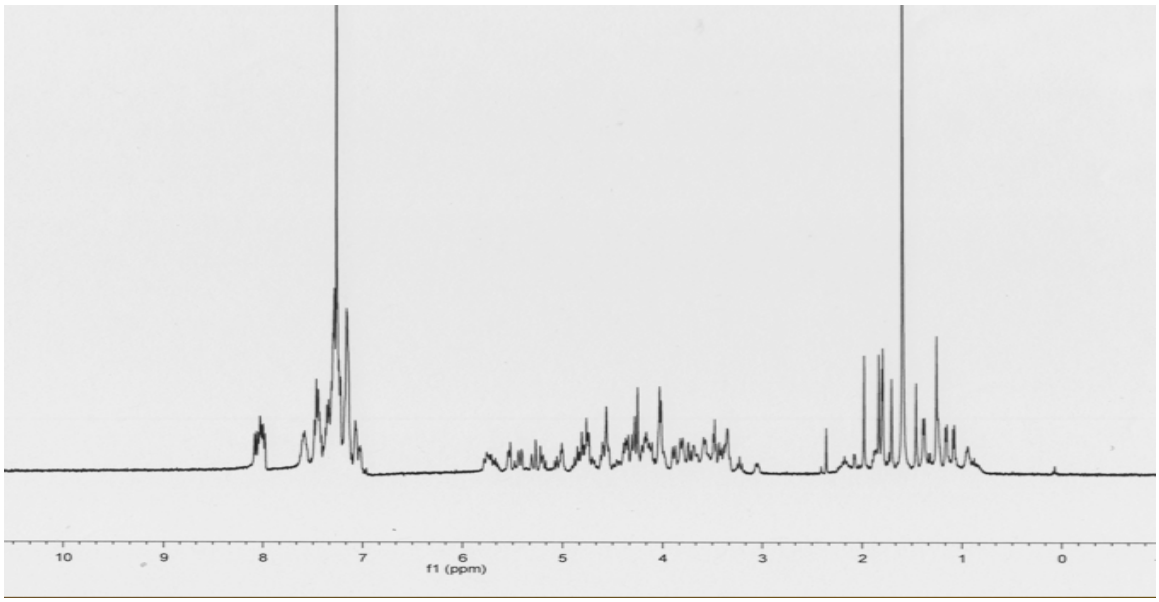
***cis*-4-(1H, 1H, 2H, 2H, 3H, 3H-Perfluoroundecyloxy)-2-butenyl 4-O-benzoyl-2, 3-di-O-benzyl- α/β-L-rhamnopyranosyl-(1→2)-3,4-di-O-benzyl-α-L-rhamnopyranoside**



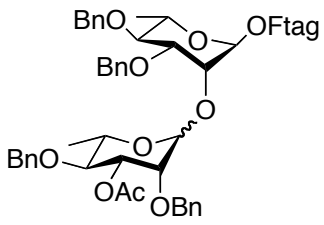


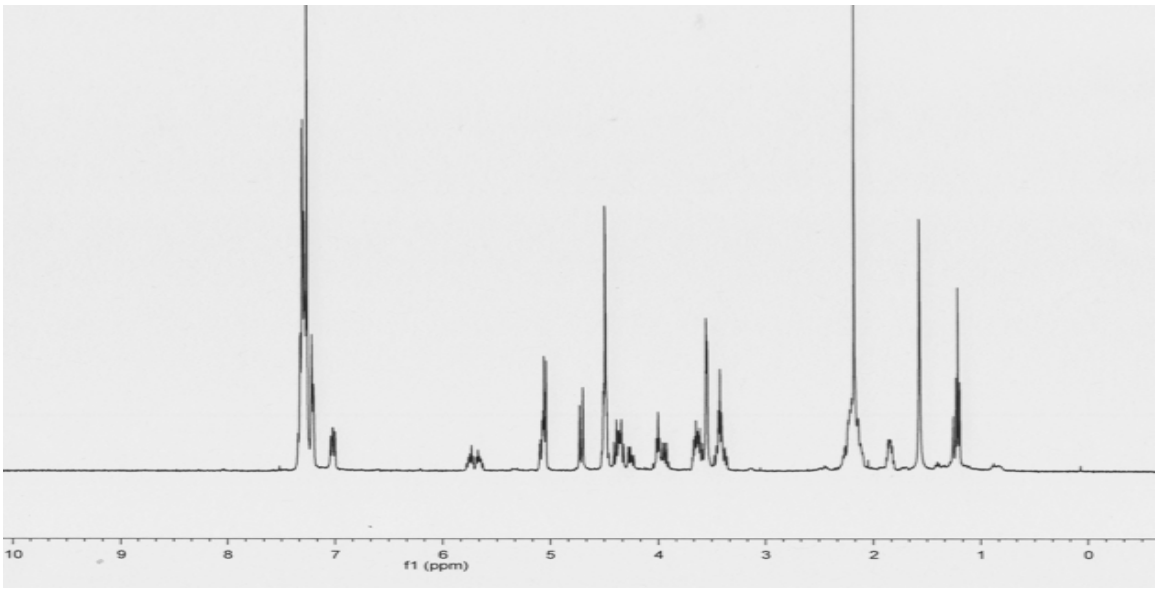
cis-4-(1H, 1H, 2H, 2H, 3H, 3H-Perfluoroundecyloxy)-2-butenyl 4-O-benzylsulfonyl-2, 3-di-O-benzyl- α/β-L-rhamnopyranosyl-(1→2)-3,4-di-O-benzyl-α-L-rhamnopyranoside



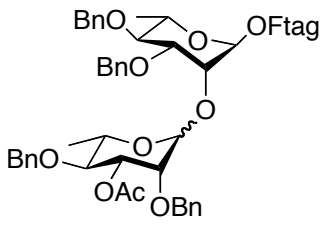


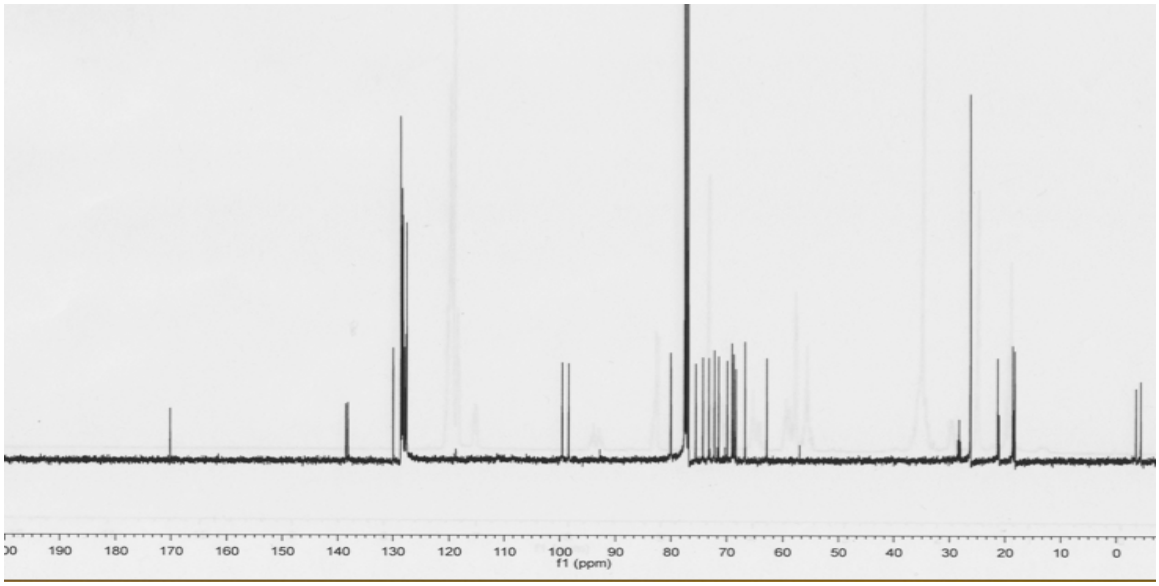
***cis*-4-(1H, 1H, 2H, 2H, 3H, 3H-Perfluoroundecyloxy)-2-butenyl 3-O-acetyl-2, 4-di-O-benzyl- α/β-L-rhamnopyranosyl-(1→2)-3,4-di-O-benzyl-α-L-rhamnopyranoside**



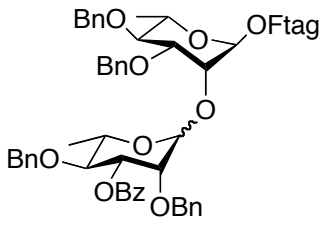


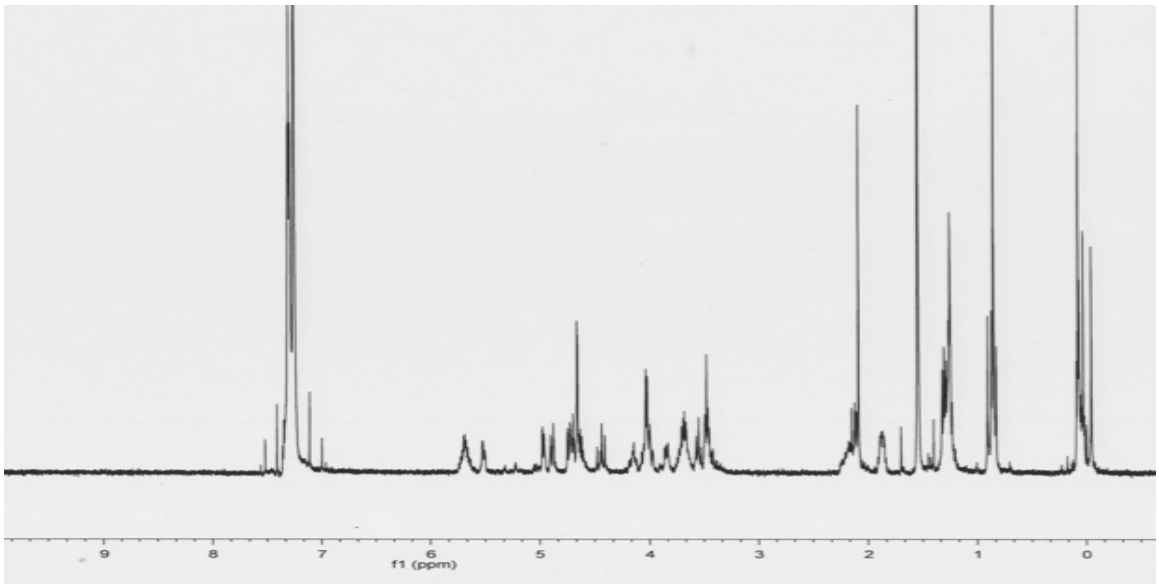
***cis*-4-(1H, 1H, 2H, 2H, 3H, 3H-Perfluoroundecyloxy)-2-butenyl 3-O-acetyl-2, 4-di-O-benzyl- α/β-L-rhamnopyranosyl-(1→2)-3,4-di-O-benzyl-α-L-rhamnopyranoside**



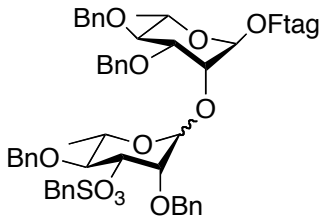


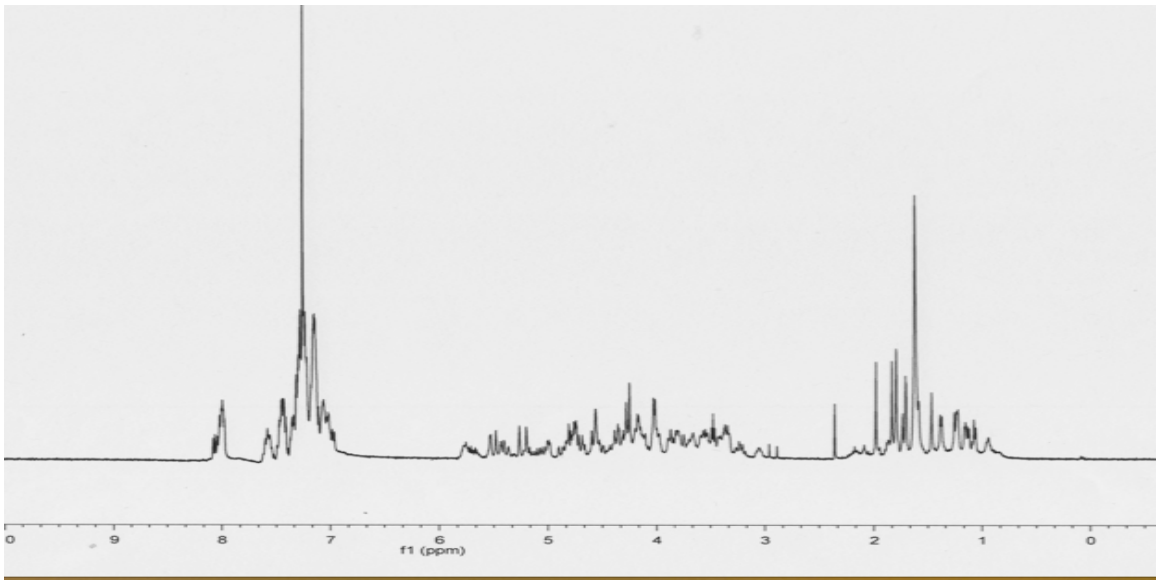
***cis*-4-(1H, 1H, 2H, 2H, 3H, 3H-Perfluoroundecyloxy)-2-butenyl 3-O-benzoyl-2, 4-di-O-benzyl- α/β-L-rhamnopyranosyl-(1→2)-3,4-di-O-benzyl-α-L-rhamnopyranoside**





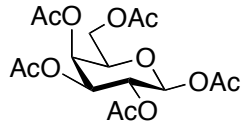
***cis*-4-(1H, 1H, 2H, 2H, 3H, 3H-Perfluoroundecyloxy)-2-butenyl 3-O-benzylsulfonyl-2, 4-di-O-benzyl- α/β-L-rhamnopyranosyl-(1→2)-3,4-di-O-benzyl-α-L-rhamnopyranoside**

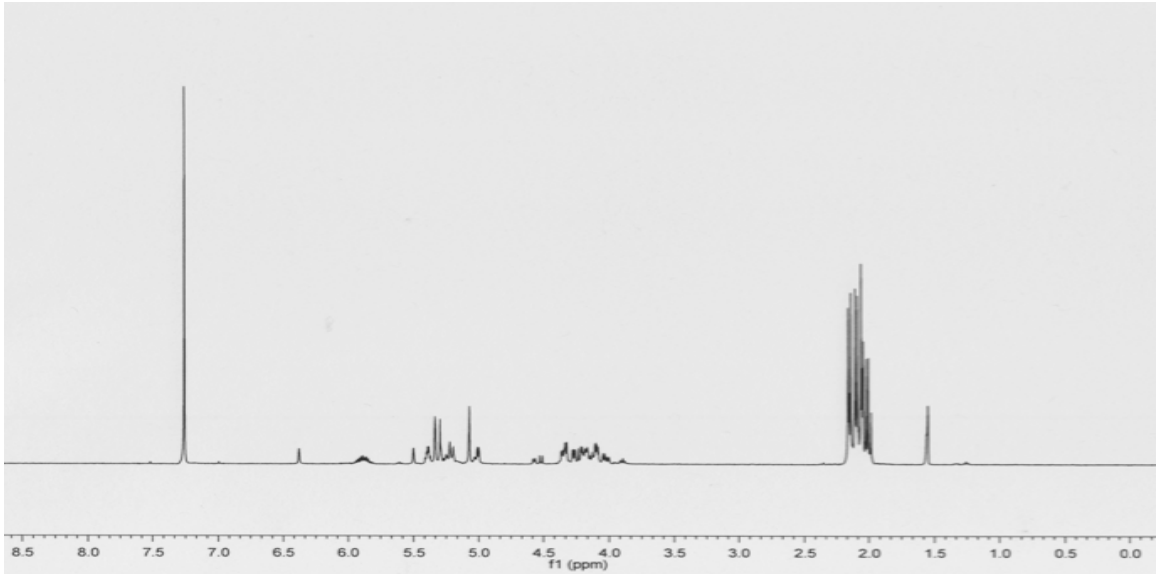


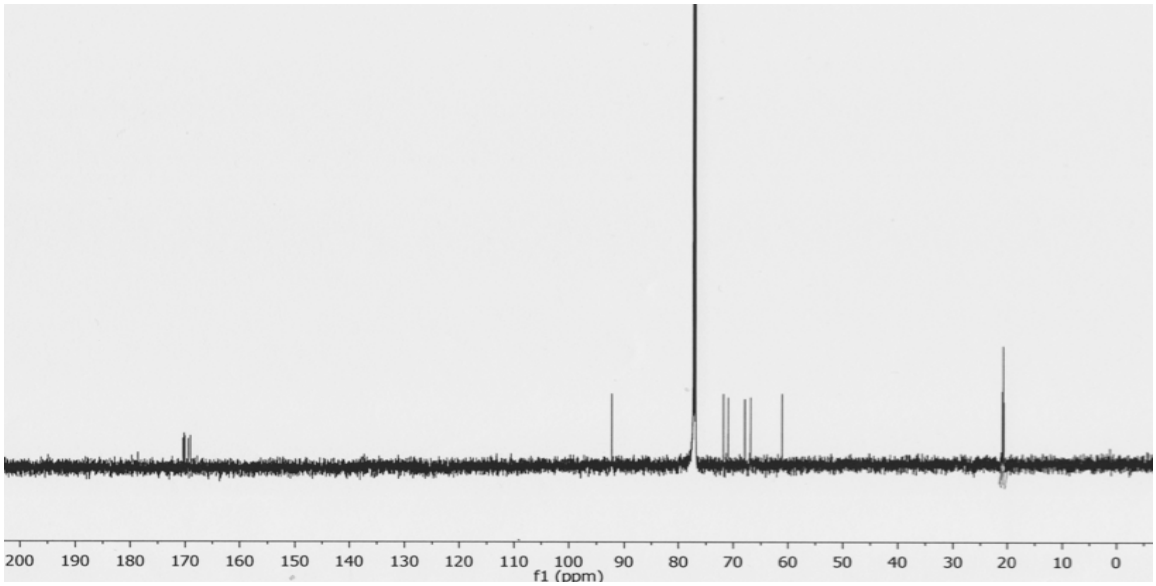


APPENDIX B. CHAPTER 3 ^{1}H AND ^{13}C NMR SPECTRA

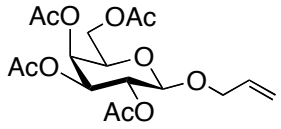
1, 2, 3, 4, 5, 6- Tetra-O-acetyl-β-D-galactopyranoside

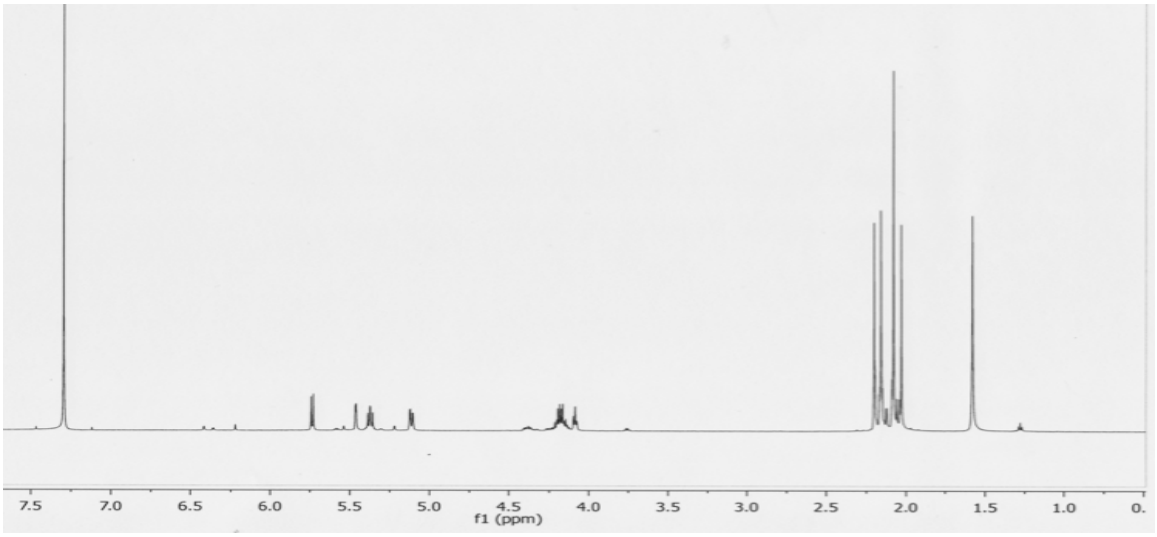




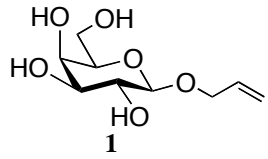


1-O-allyl-2, 3, 4, 6-O-tetra-acetyl-β–D-galactopyranoside

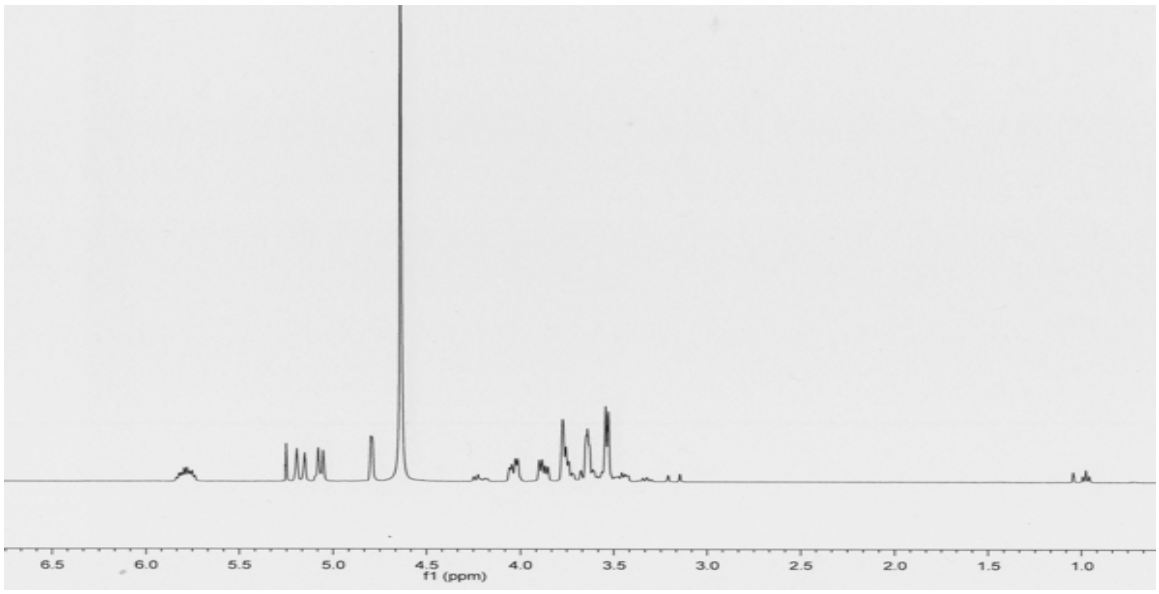




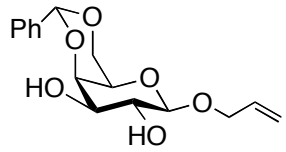
1-O-Allyl-β-D-galactopyranoside (1)

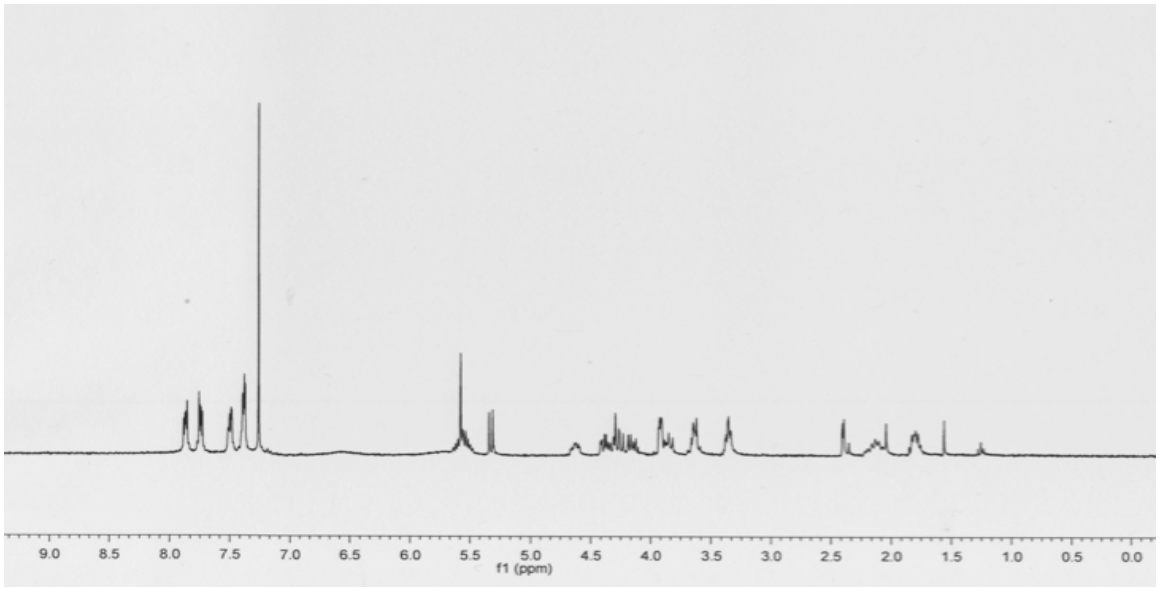


1

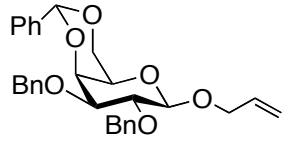


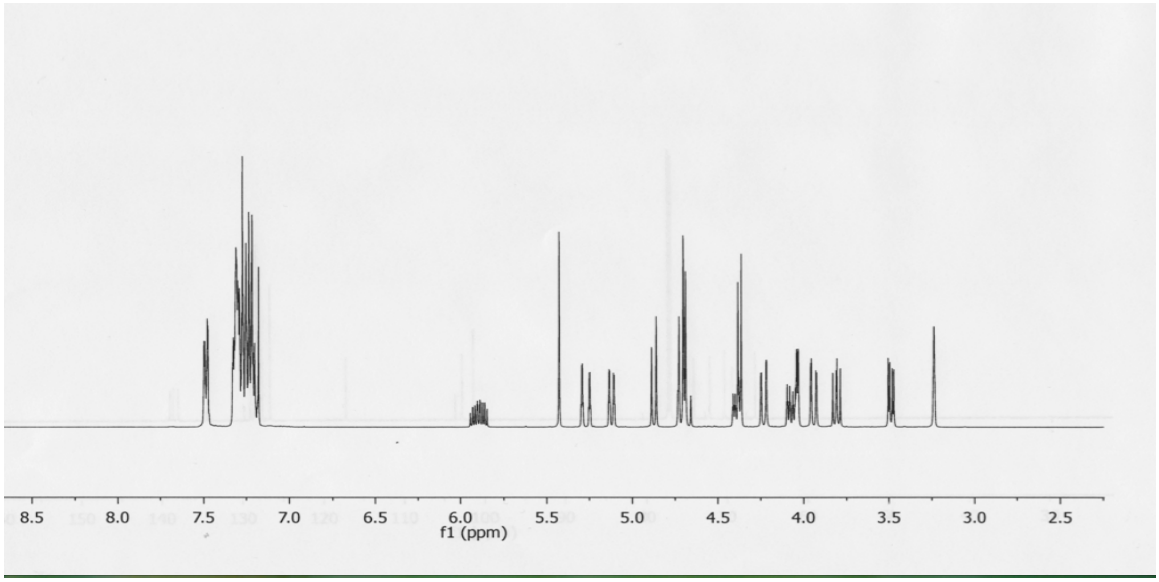
1-O-Allyl-4, 6- O-benzylidine-β-D-galactoipyranoside

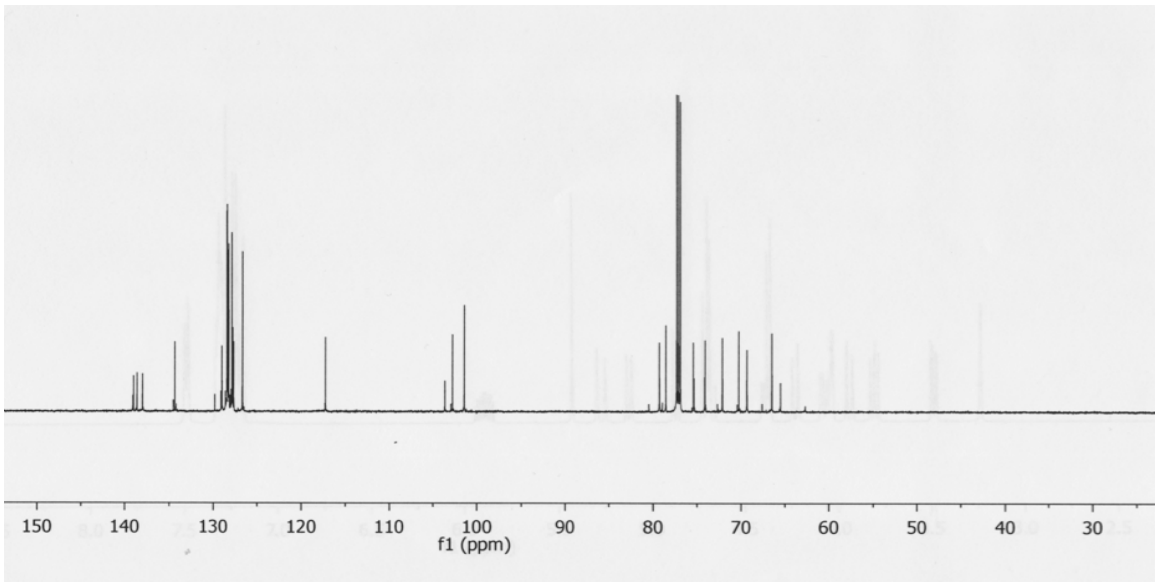




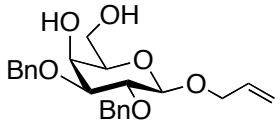
1-O-Allyl-2, 3- dibenzyl-4, 6- O-benzylidine-β-D-galactopyranoside



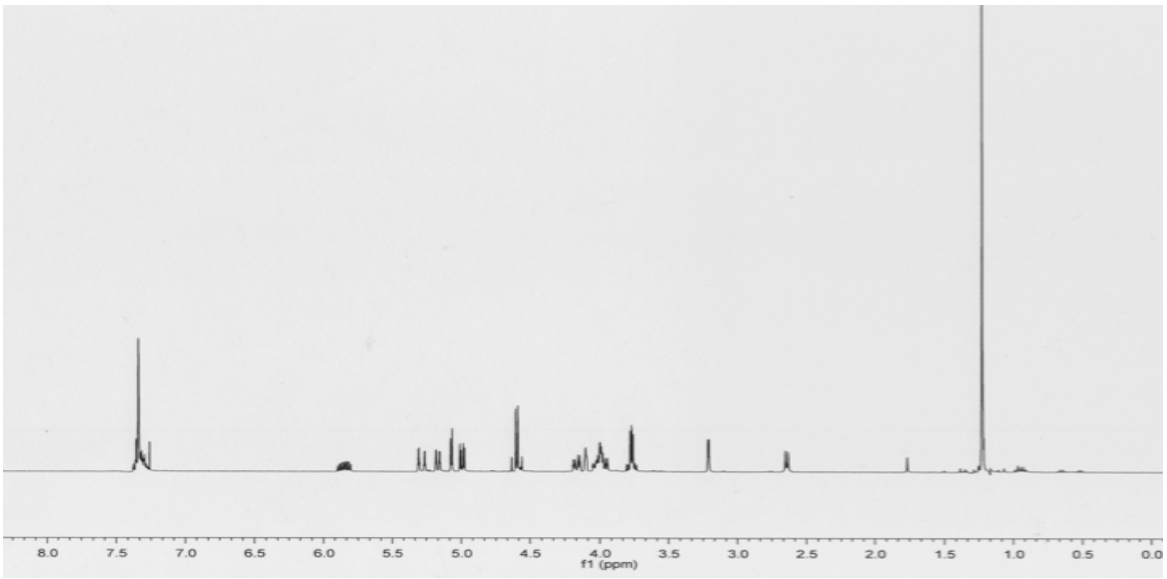




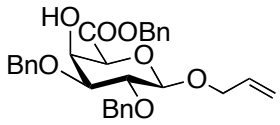
1-O-Allyl-2, 3- dibenzyl -β-D-galactopyranoside (2)

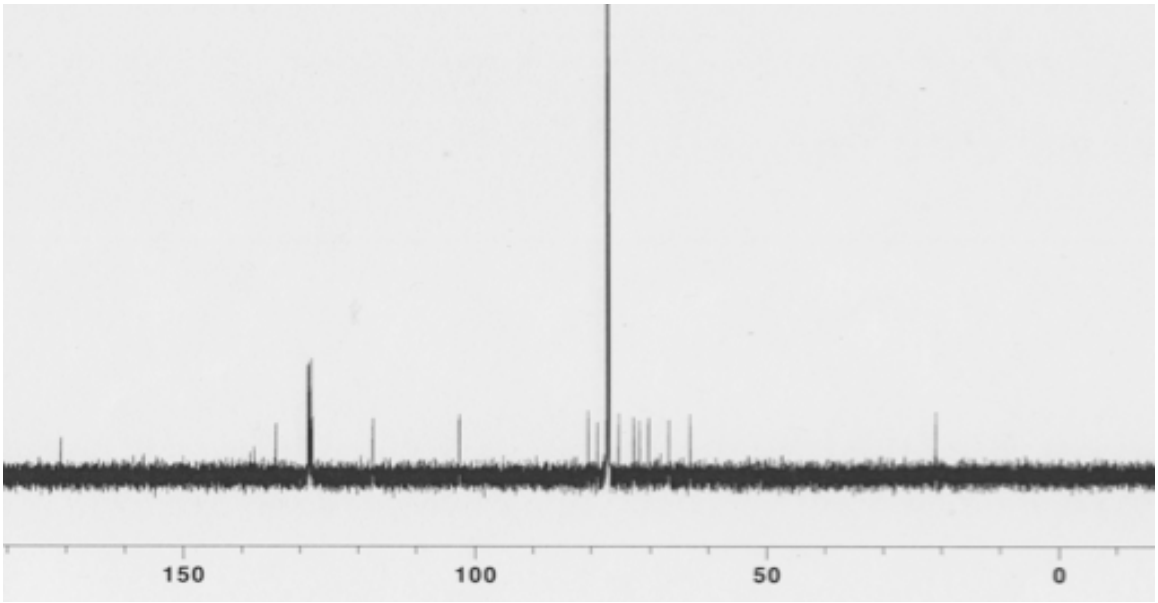


2

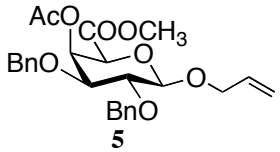


Benzyl 1-O-allyl-2, 3- dibenzyl-β-D-galactopyranosyluronate (4)

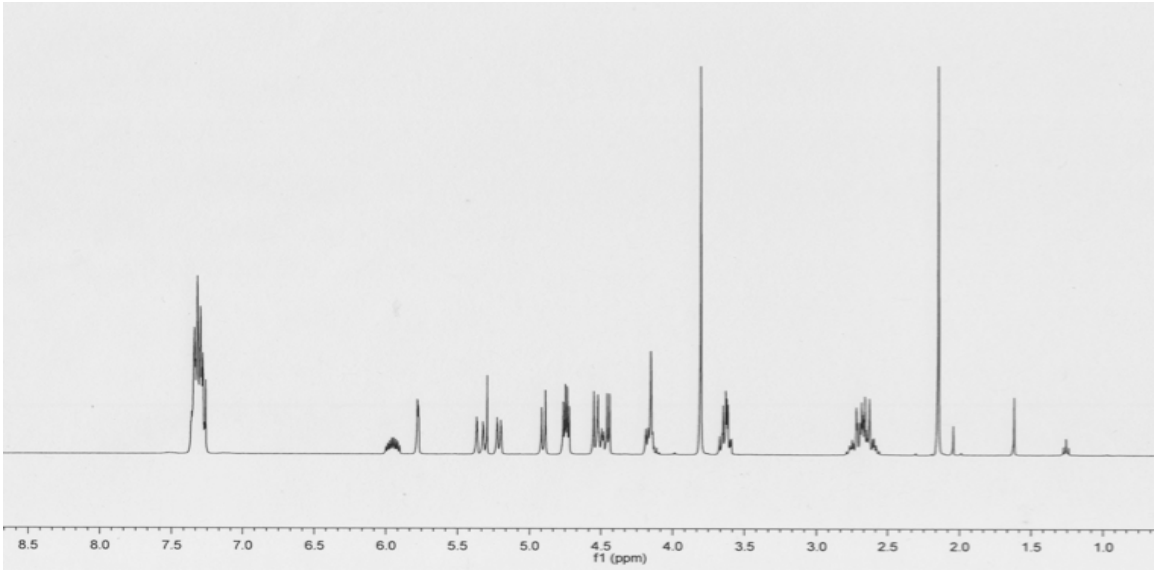




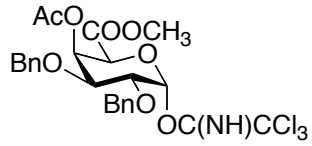
Methyl 1-O-allyl-2, 3- dibenzyl- 4-acetyl-β-D-galactopyranosyluronate (5)

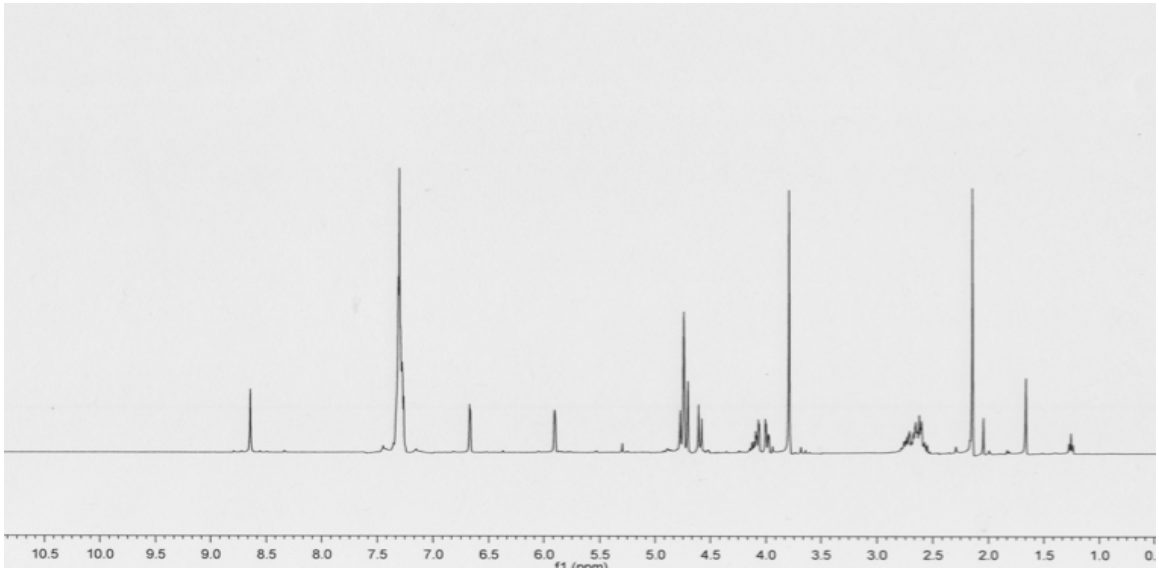


5

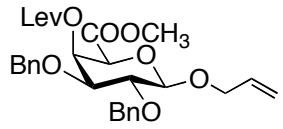


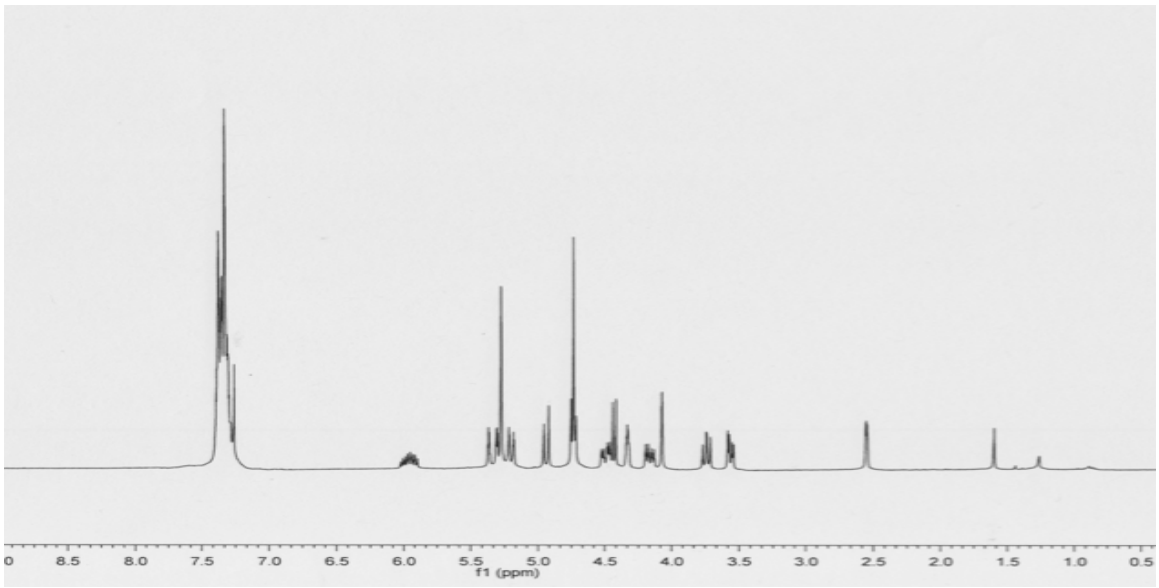
Methyl 1-O-trichloroacetimidate-2, 3- dibenzyl- 4-acetyl-β-D-galactopyranosyluronate (8)



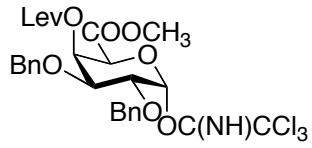


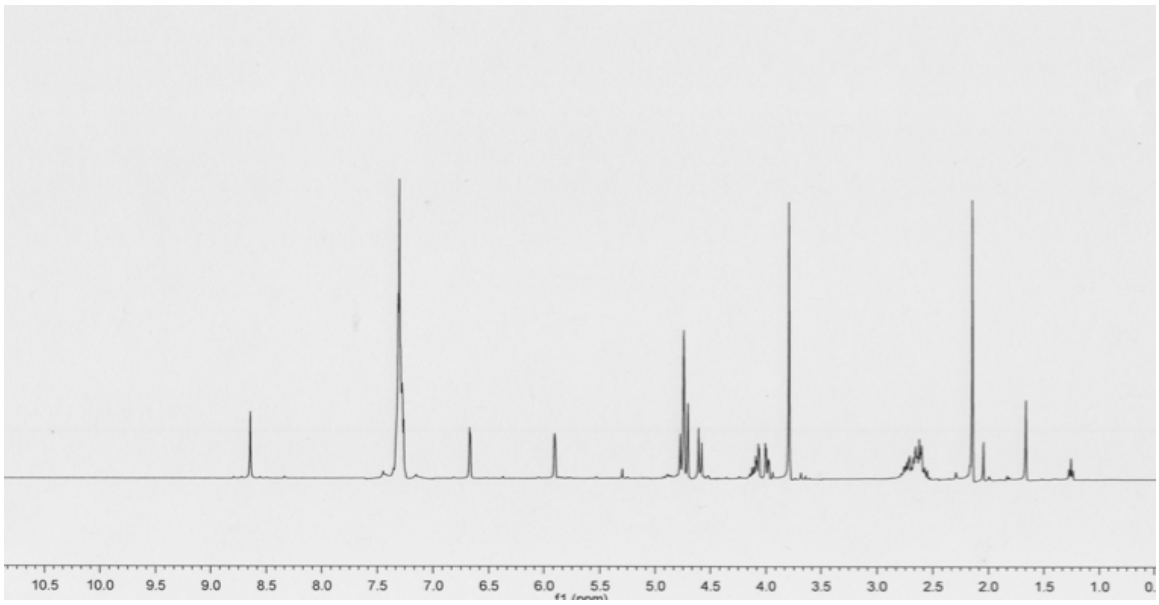
Methyl 1-O-allyl-2, 3- dibenzyl- 4-levulinyl-β-D-galactopyranosyluronate (6)



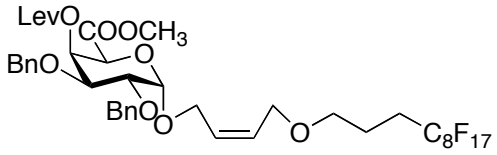


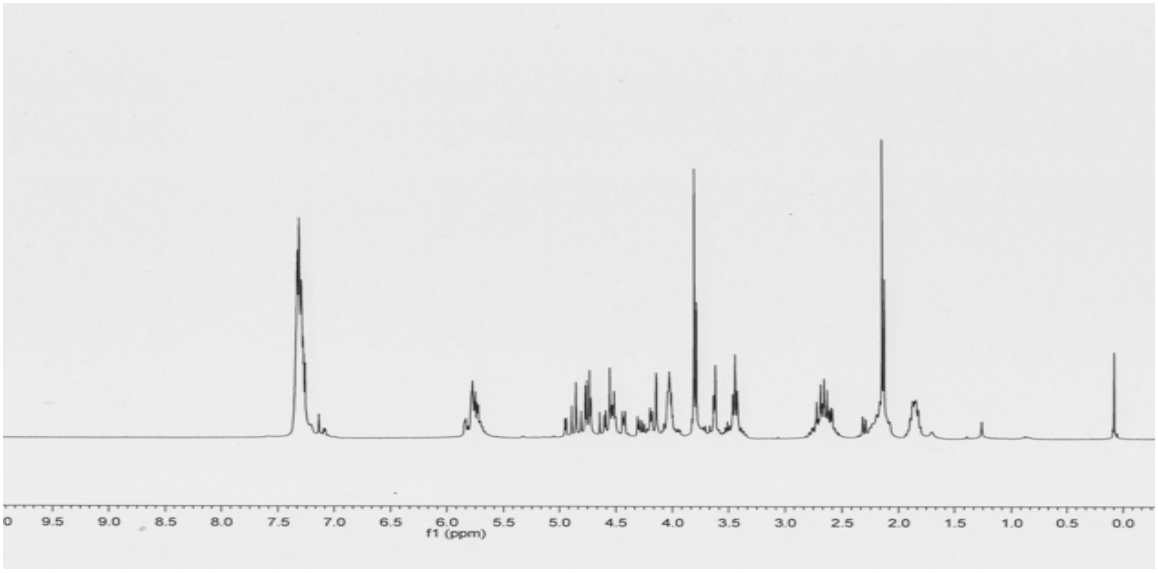
Methyl 1-O-tricholoroacetimidate-2, 3- dibenzyl- 4-levulinyl-β-D-galactopyranosyluronate (9)



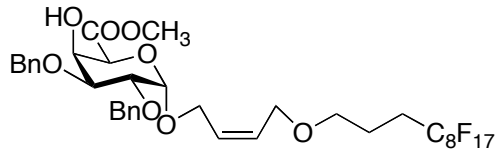


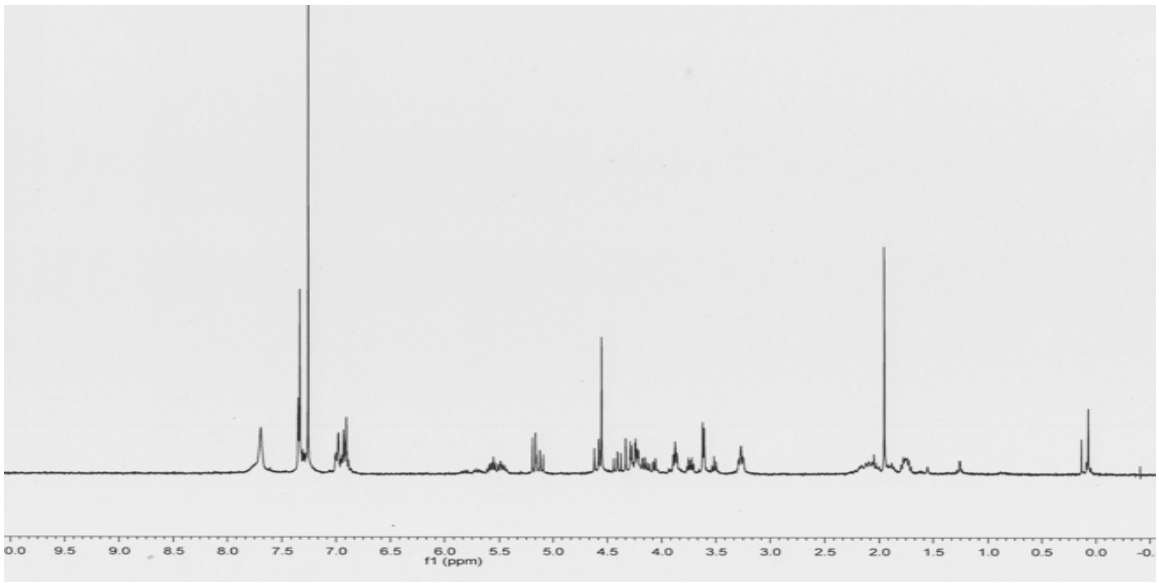
***cis*-4-(1H, 1H, 2H, 2H, 3H, 3H-Perfluoroundecyloxy)-2-butenyl methyl 2, 3-O-dibenzyl-4-O-levulinyl- β-D-galactopyranosyluronate (11)**



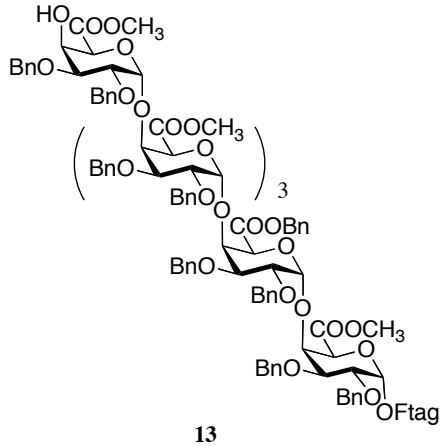


***cis*-4-(1H, 1H, 2H, 2H, 3H, 3H-Perfluoroundecyloxy)-2-butenyl methyl 2, 3-O-dibenzyl-β-D-galactopyranosyluronate (12)**





***cis*-4-(1H, 1H, 2H, 2H, 3H, 3H – Perfluoroundecyloxy) –2-butenyl methyl-2, 3-O-dibenzyl-α-D-galactopyranosyluronate-(1→ 4)-methyl-2, 3-O- dibenzyl-α-D-galactopyranosyluronate-(1→ 4)-methyl-2, 3-O- dibenzyl-α-D-galactopyranosyluronate- (1 →4)- methyl-2, 3-O- dibenzyl-α-D-galactopyranosyluronate-(1→ 4)-benzyl-2, 3-O- dibenzyl-α-D-galactopyranosyluronate-(1→ 4)-methyl-2, 3-O- dibenzyl-α-D-galactopyranosyluronate (13)**



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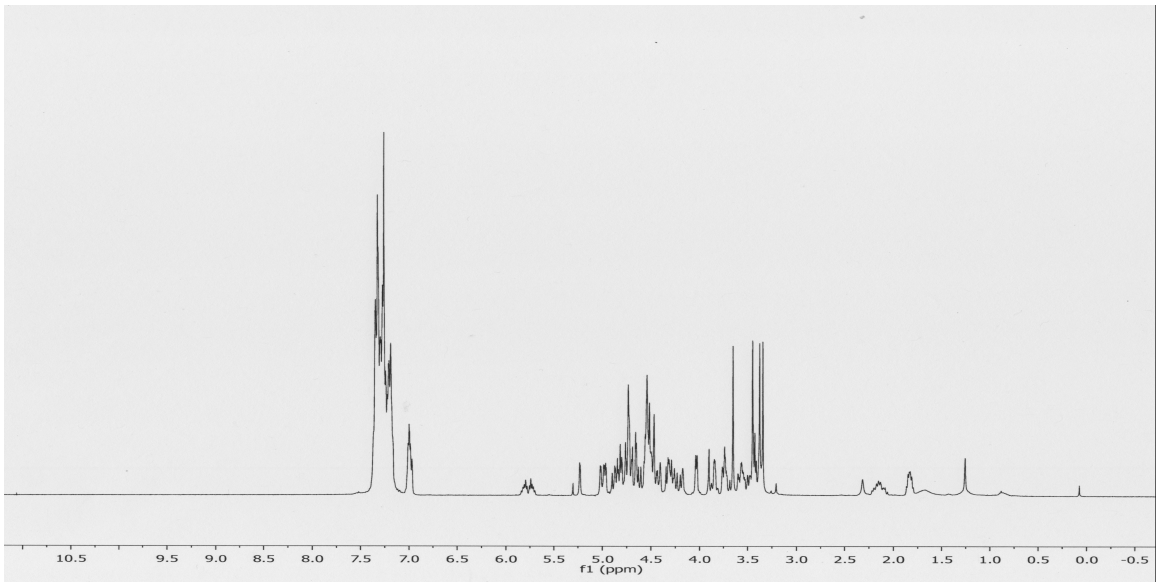
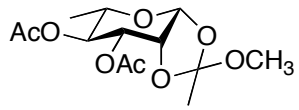
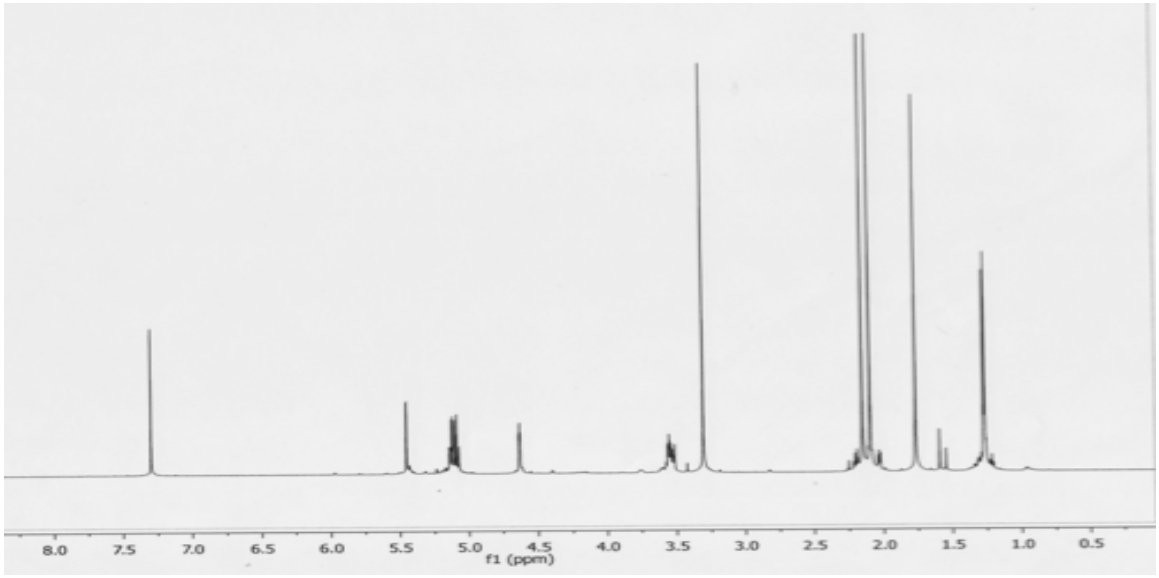
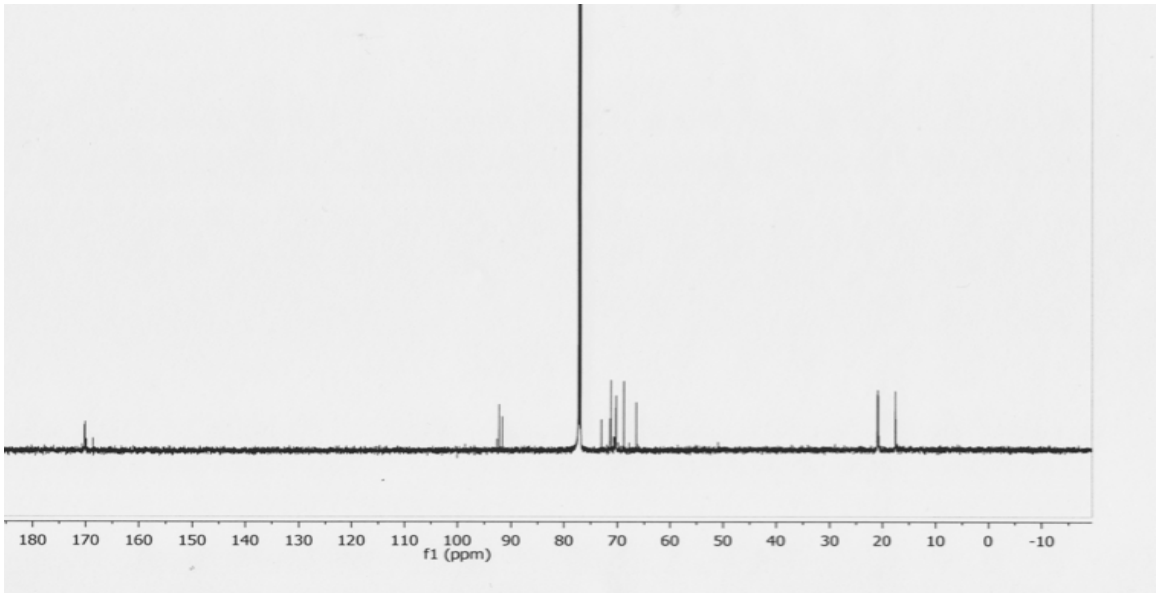


APPENDIX C. CHAPTER 4 ^{1}H AND ^{13}C NMR SPECTRA

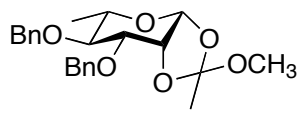
3, 4-O-Di-acetyl-1, 2-O-methoxyethylidene-β-L-rhamnopyranose

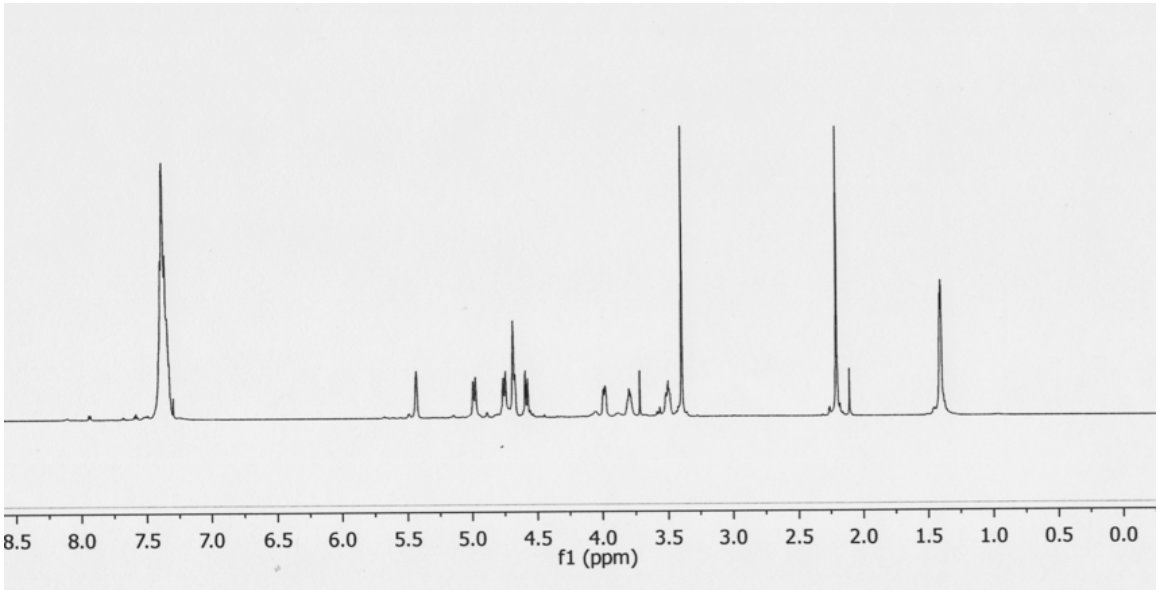


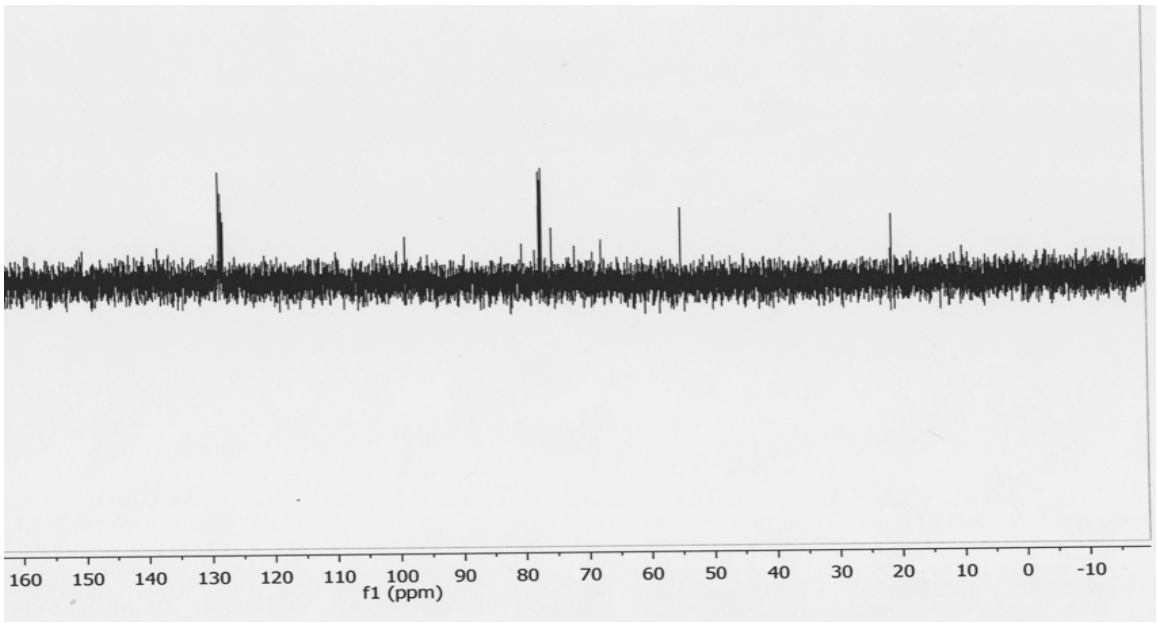




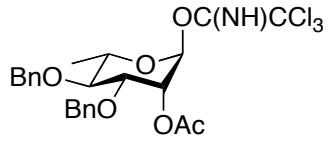
3, 4-Di-O-benzyl-1, 2-O-methoxyethylidene-β-L-rhamnopyranose.

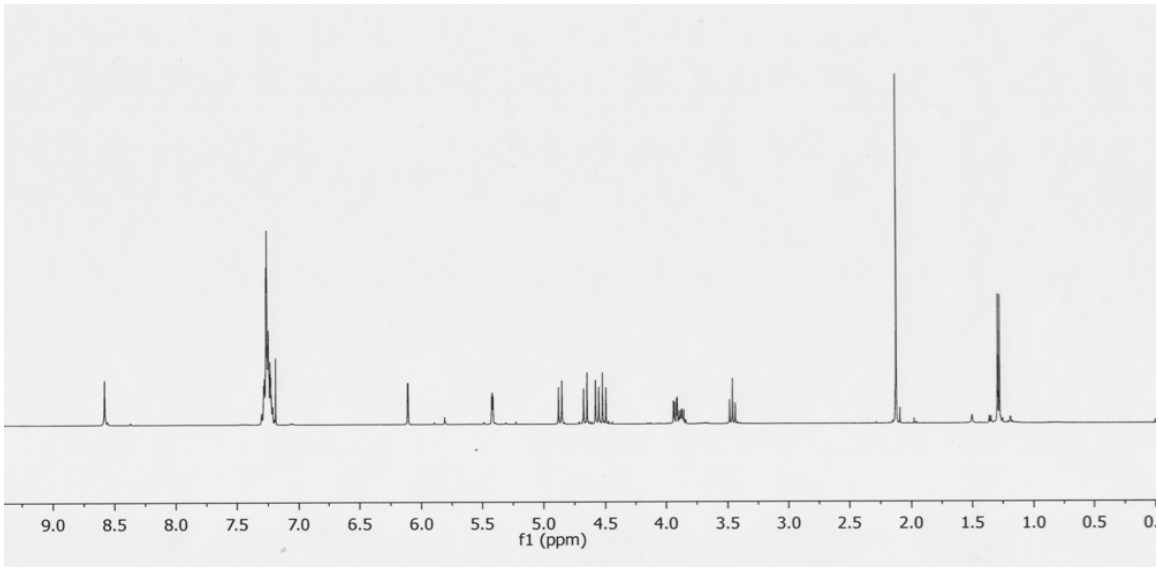


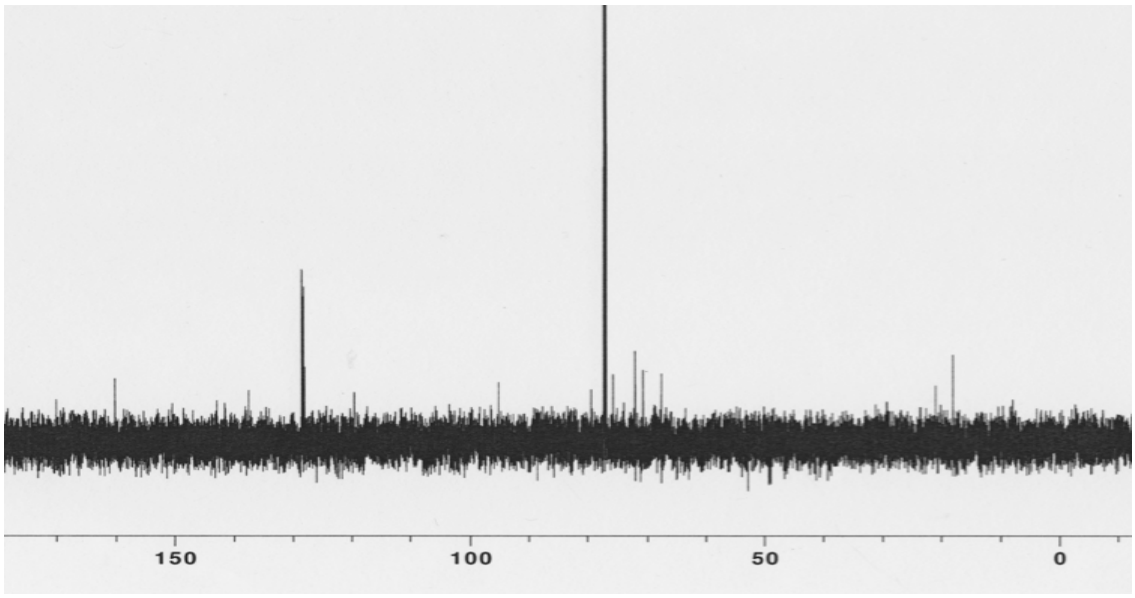




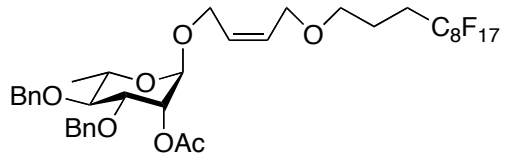
2-O-Acetyl-3, 4-di-O-benzyl-α/β-L-rhamnopyranoside trichloroacetimidate

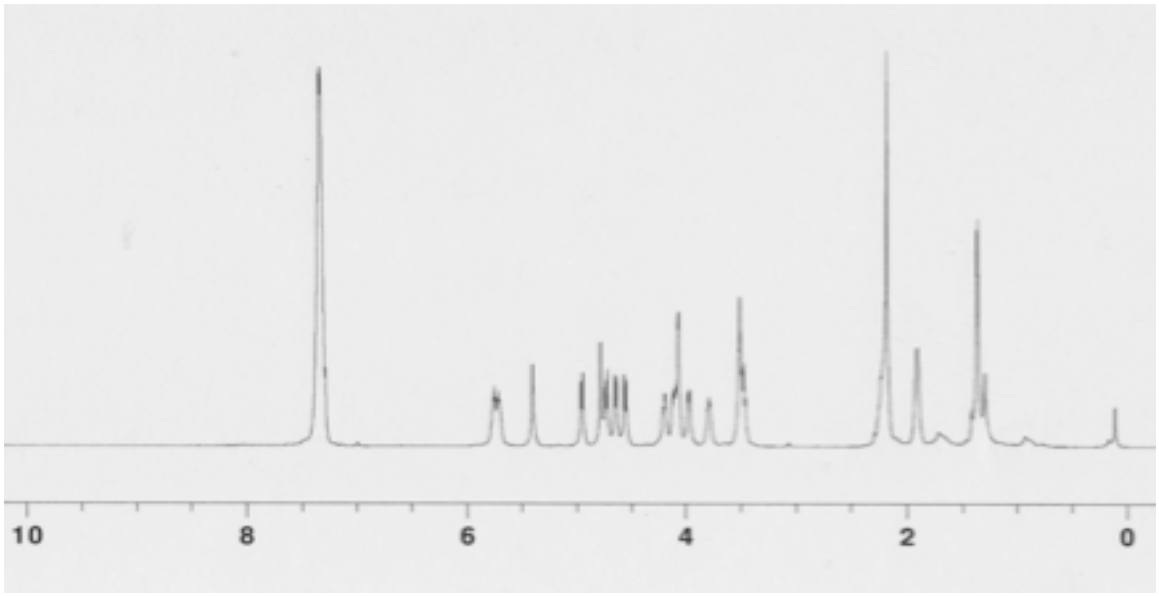


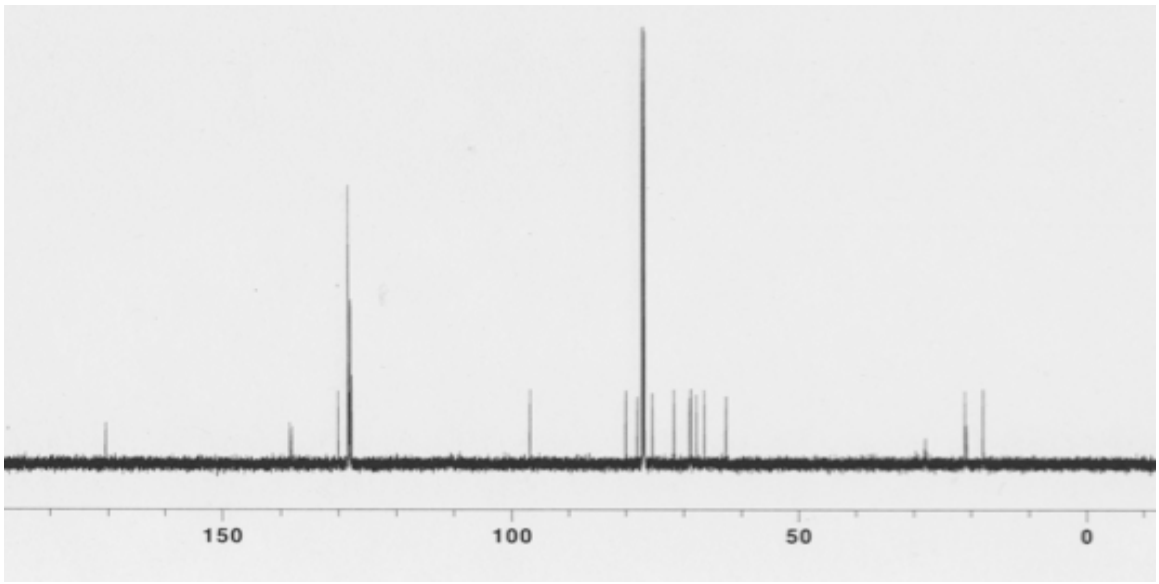




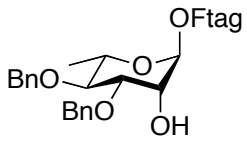
***cis*-4-(1H, 1H, 2H, 2H,3H, 3H-Perfluoroundecyloxy)-2-butenyl-2-O-acetyl-3,4-O-dibenzyl- α-L-rhamnopyranoside**

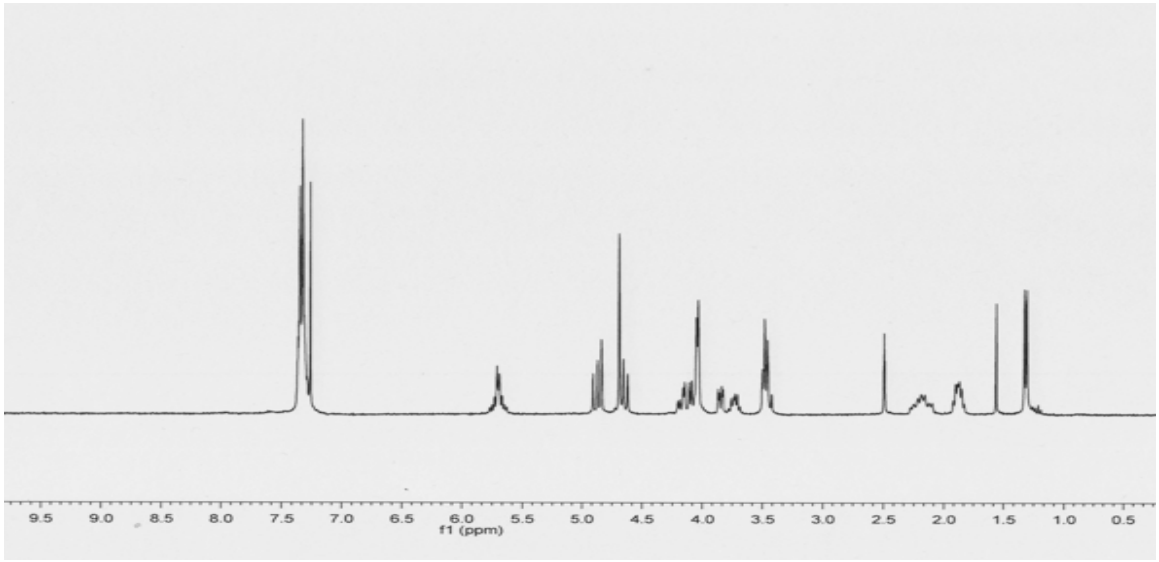


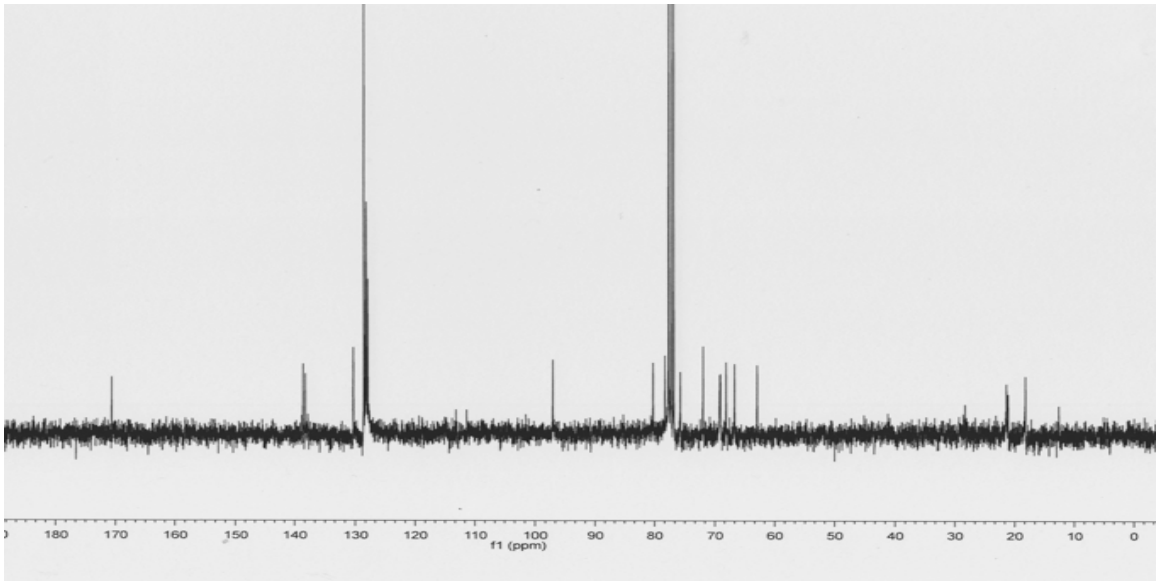




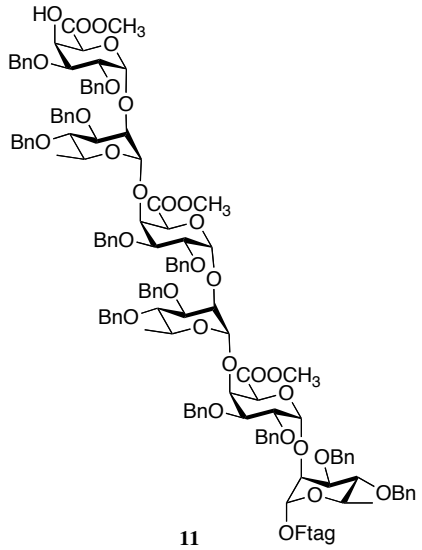
***cis*-4-(1H, 1H, 2H, 2H, 3H, 3H-Perfluoroundecycloxy)-2-butenyl-2-O-hydroxyl-3,4-O-dibenzyl-α-L-rhamnopyranoside**



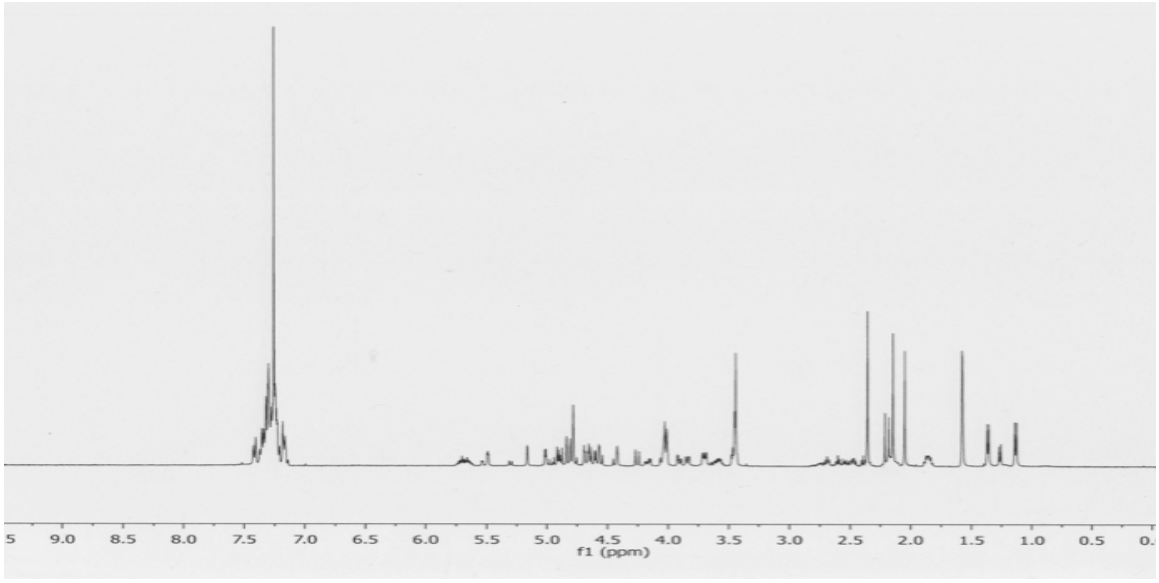




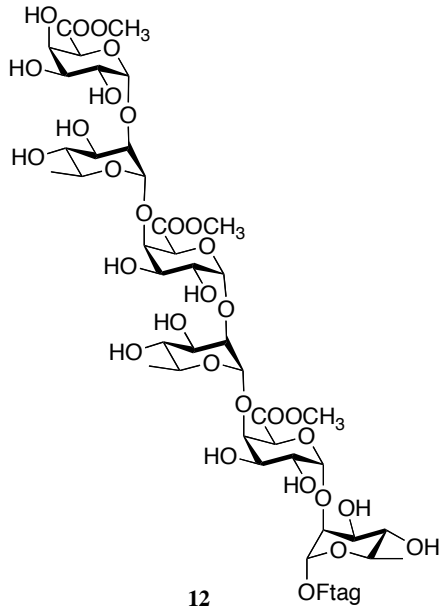
***cis*-4-(1H, 1H, 2H, 2H, 3H, 3H – Perfluoroundecyloxy) –2-butenyl methyl-2, 3-O-dibenzyl-α-D-galactopyranosyluronate-(1→ 2)-3, 4–O-dibenzyl–α-L-rhamnopyranosyl- (1 →4)-methyl-2, 3-O- dibenzyl-α-D-galactopyranosyluronate-(1→ 2)-3, 4–O-dibenzyl–α-L-rhamnopyranosyl- (1 →4)- methyl-2, 3-O- dibenzyl-α-D-galactopyranosyluronate-(1→ 2)-3, 4–O-dibenzyl–α-L-rhamnopyranoside**



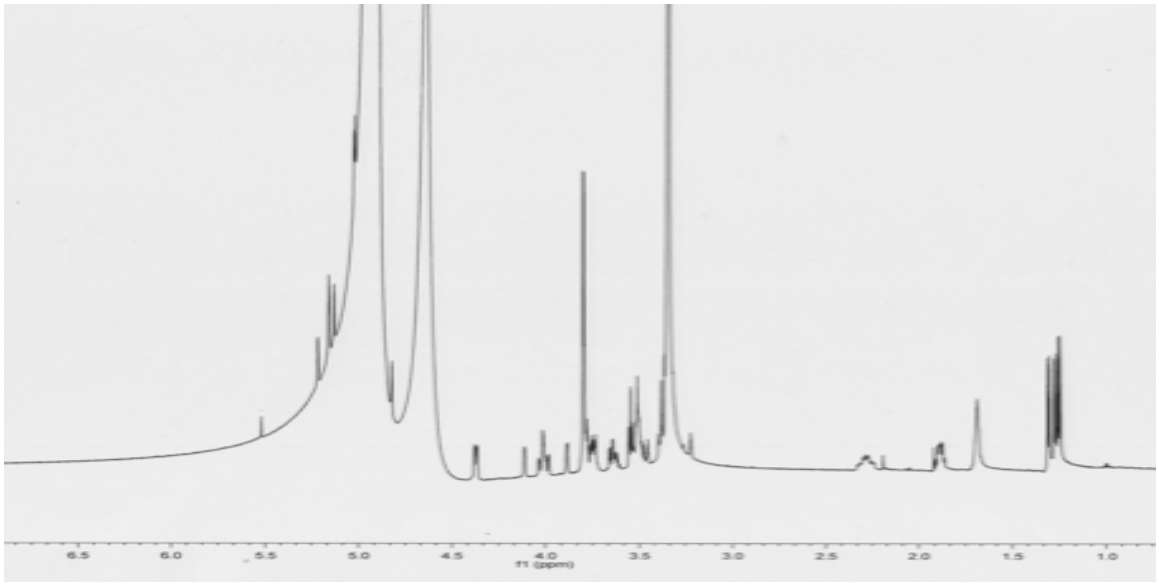
11



4-(1H, 1H, 2H, 2H, 3H, 3H – Perfluoroundecyloxy)-2-butyl methyl-α-D-galactopyranosyluronate-(1→ 2)-α-L-thamnopyranosyl-(1→4)-methyl-α-D-galactopyranosyluronate-(1→ 2)-α-L-thamnopyranosyl-(1→4)-methyl-α-D-galactopyranosyluronate-(1→ 2)-α-L-thamnopyranoside (12).

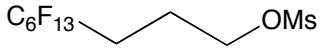


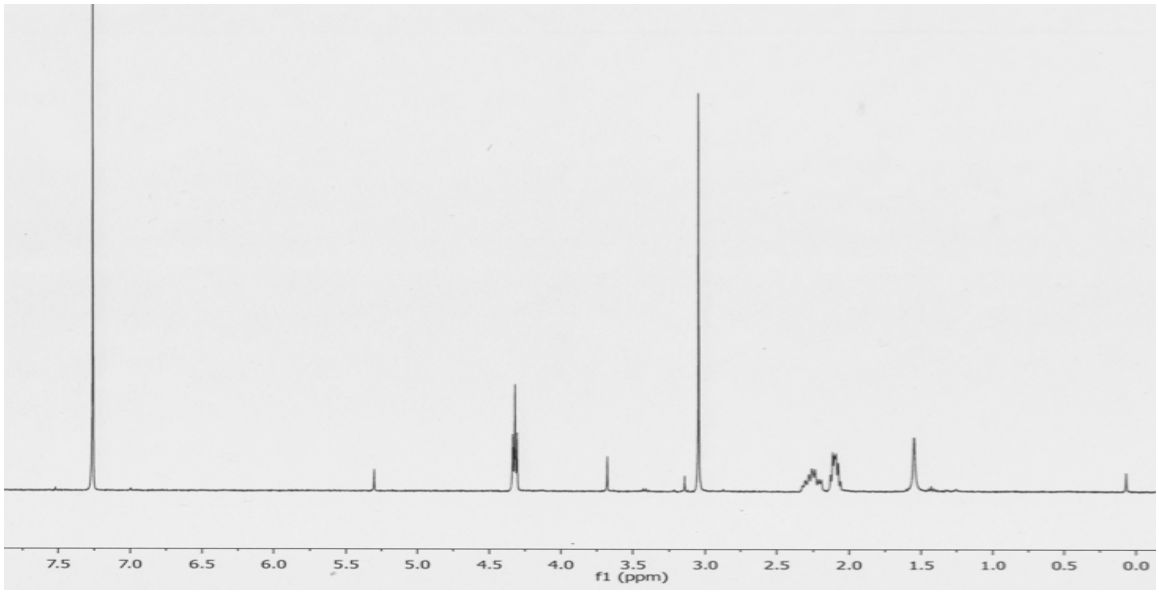
12

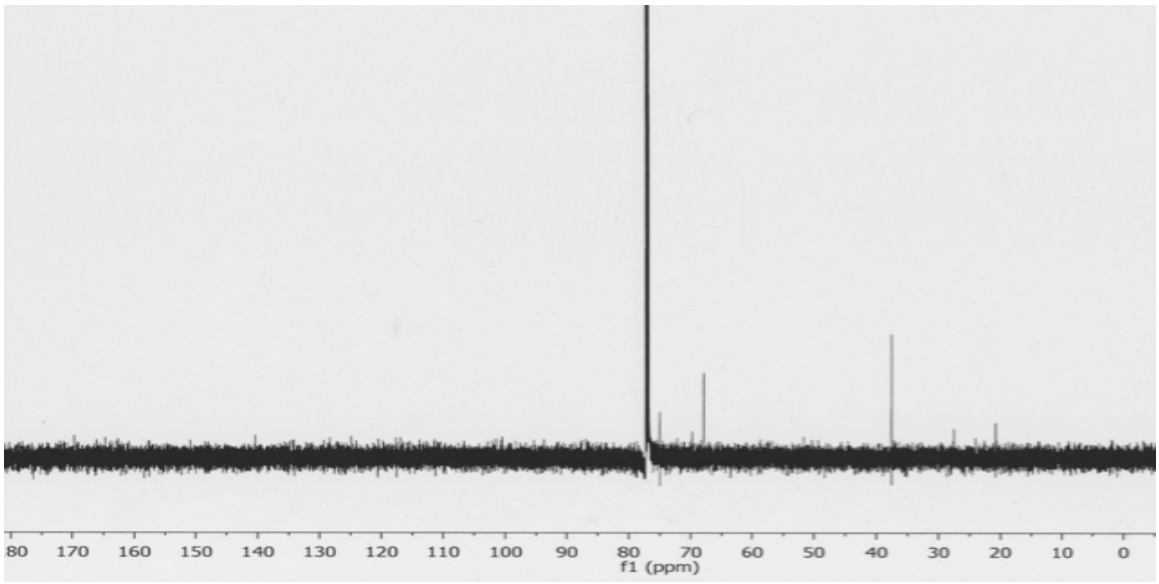


APPENDIX D. CHAPTER 5 ^{1}H AND ^{13}C NMR SPECTRA

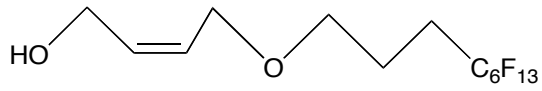
4,4,5,5,6,6,7,7,8,8,9,9,9-tridecafluorononan-1-ol (2)

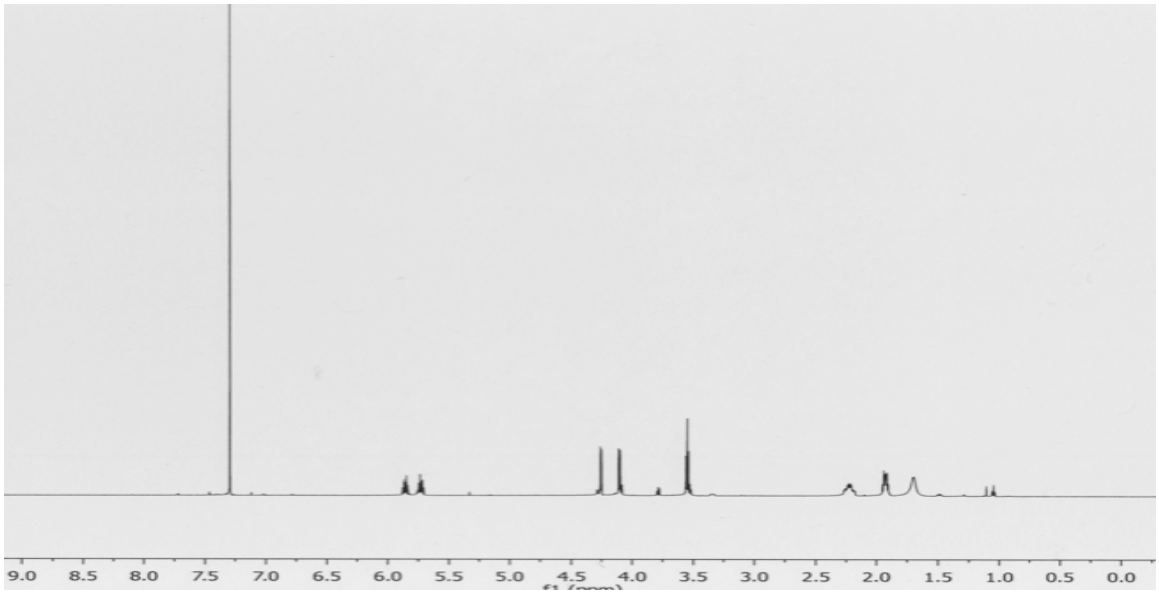


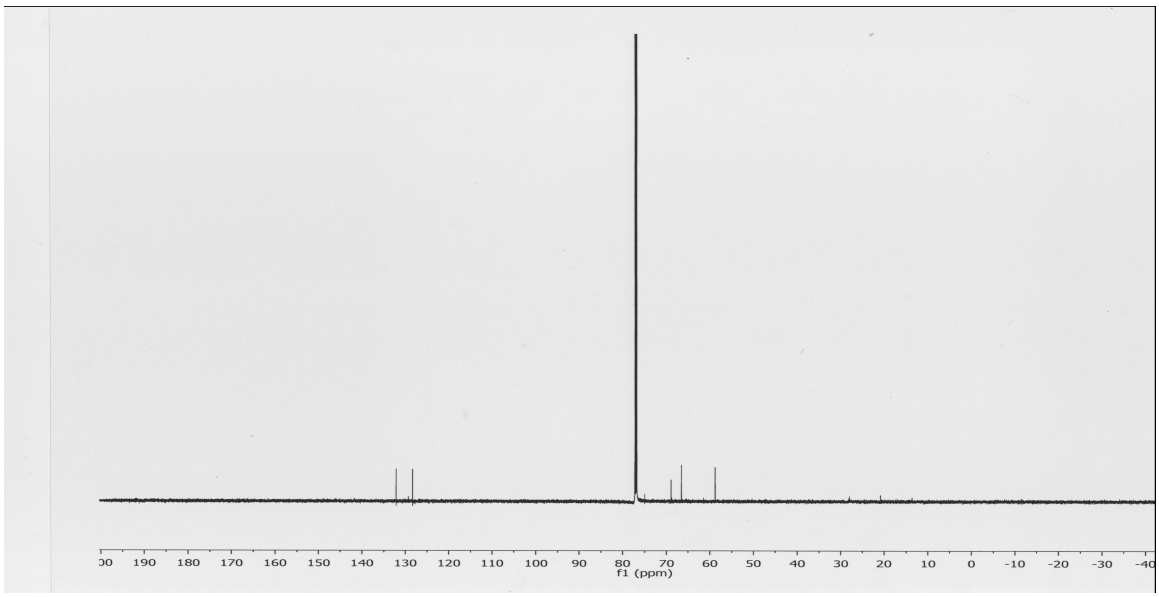




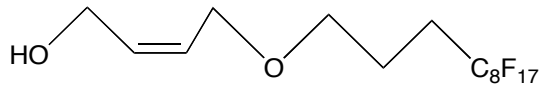
(Z)-4-((4,4,5,5,6,6,7,7,8,8,9,9,9-tridecafluorononyl)oxy)but-2-en-1-ol

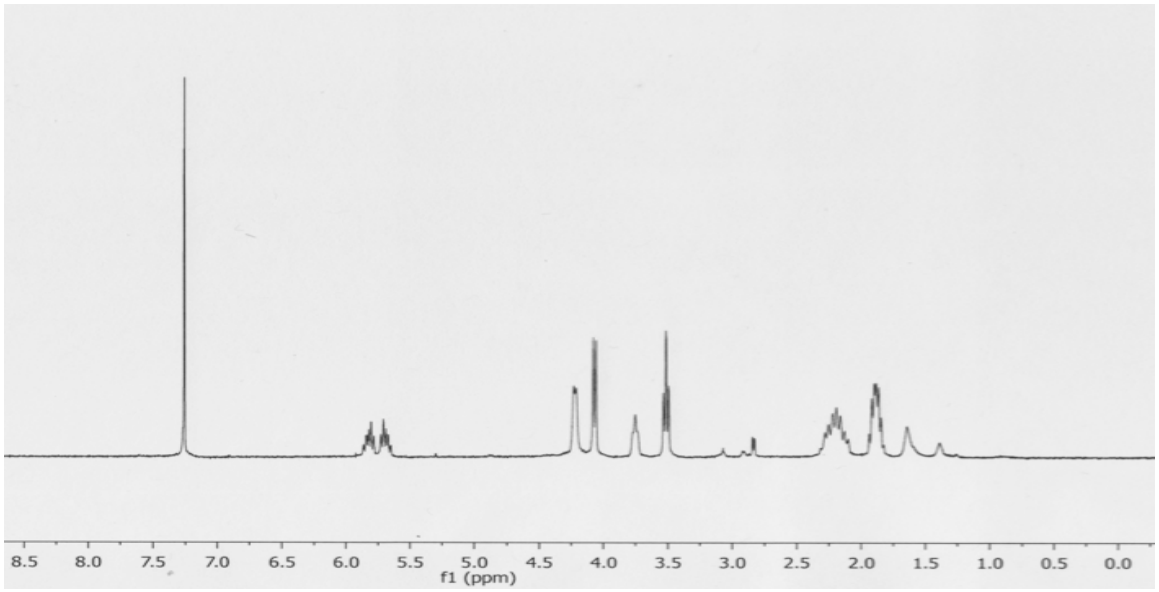


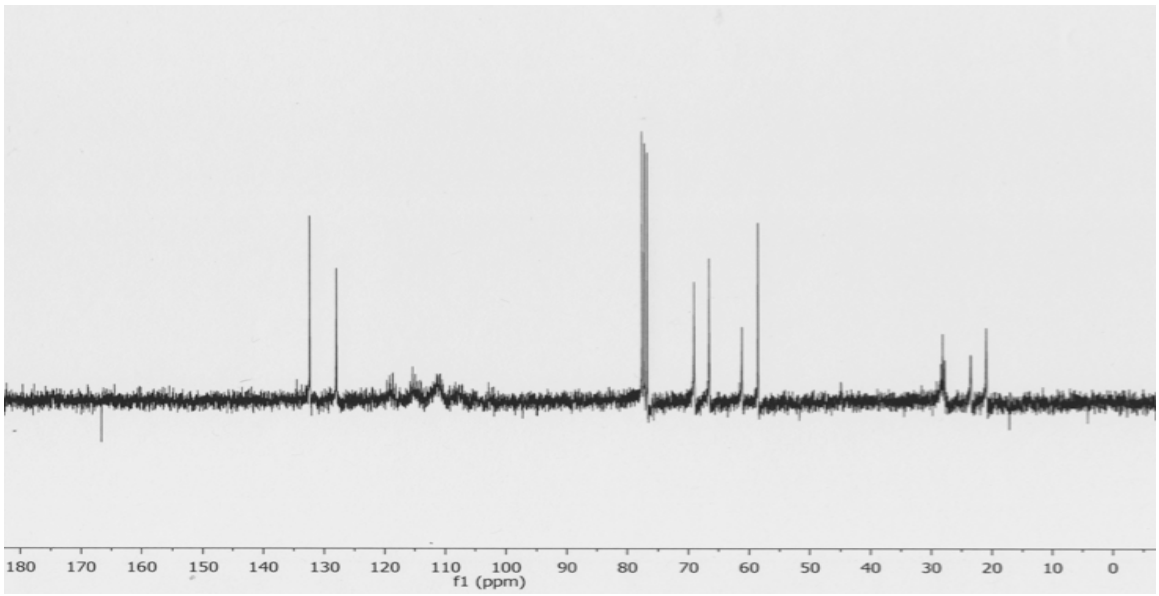




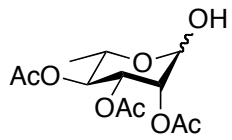
(Z)-4-((4,4,5,5,6,6,7,7,8,8,9,9,10,10, 11, 11,11-heptadecafluorononyl)oxy)but-2-en-1-ol

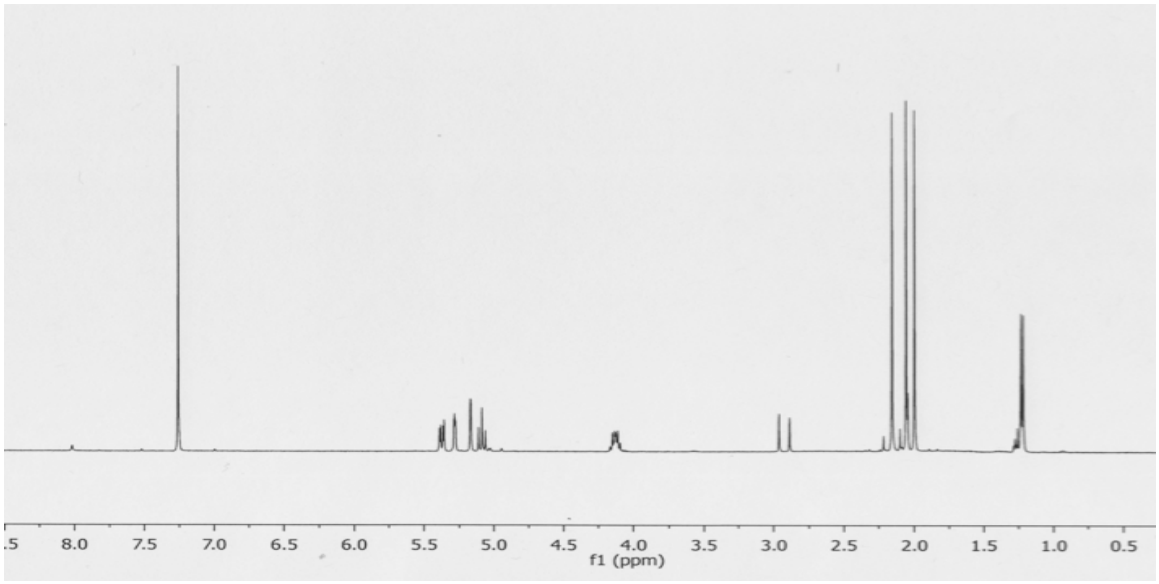


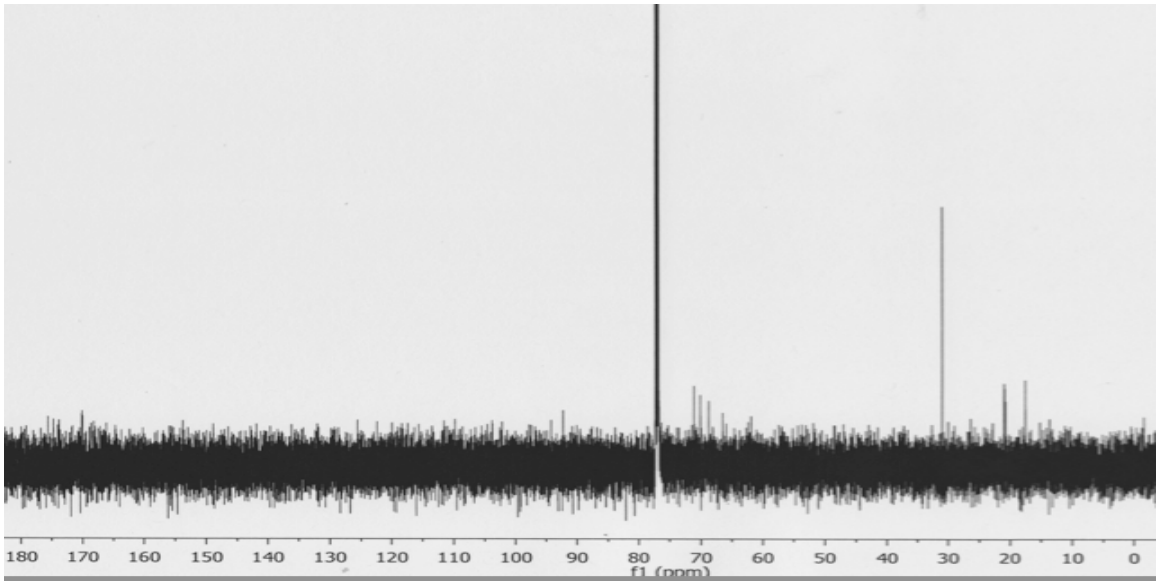




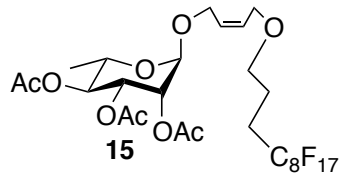
2, 3, 4-Tri-O-acetyl-rhamnopyranoside

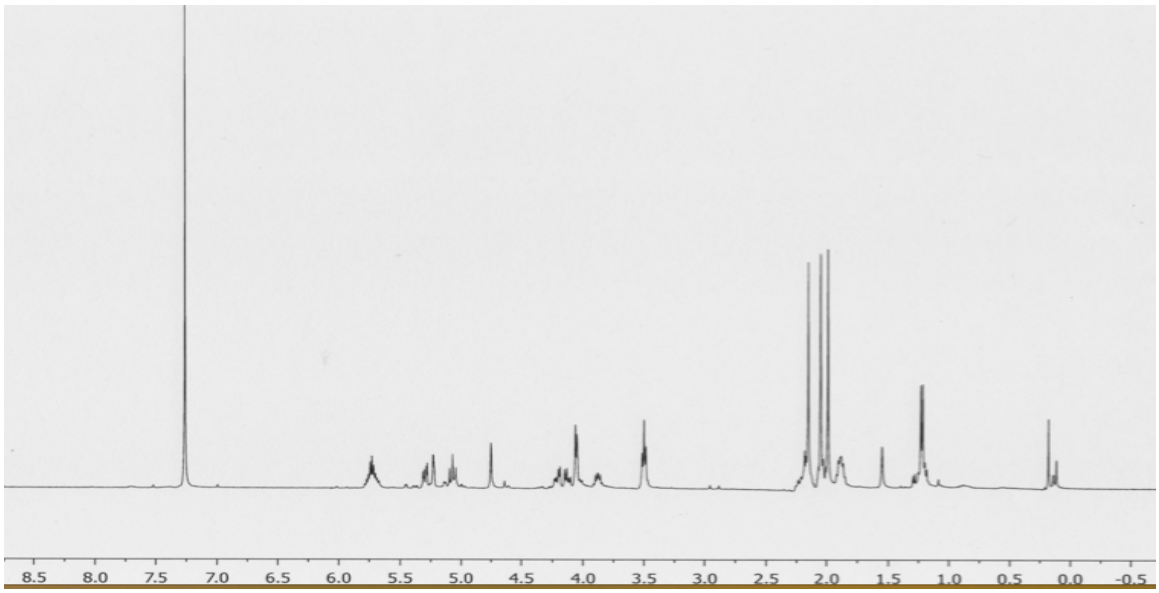






4-((6,6,7,7,8,8,9,9,10,10,11,11,11-tridecafluoro-2-(4,4,5,5,6,6,7,7,8,8,9,9,9 tridecafluorononyl)undecyl)oxy)phenyl-2,3,4-tri-O-acetyl-α-L-rhamnopyranoside





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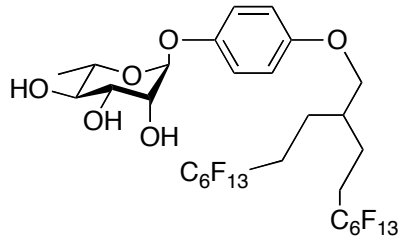

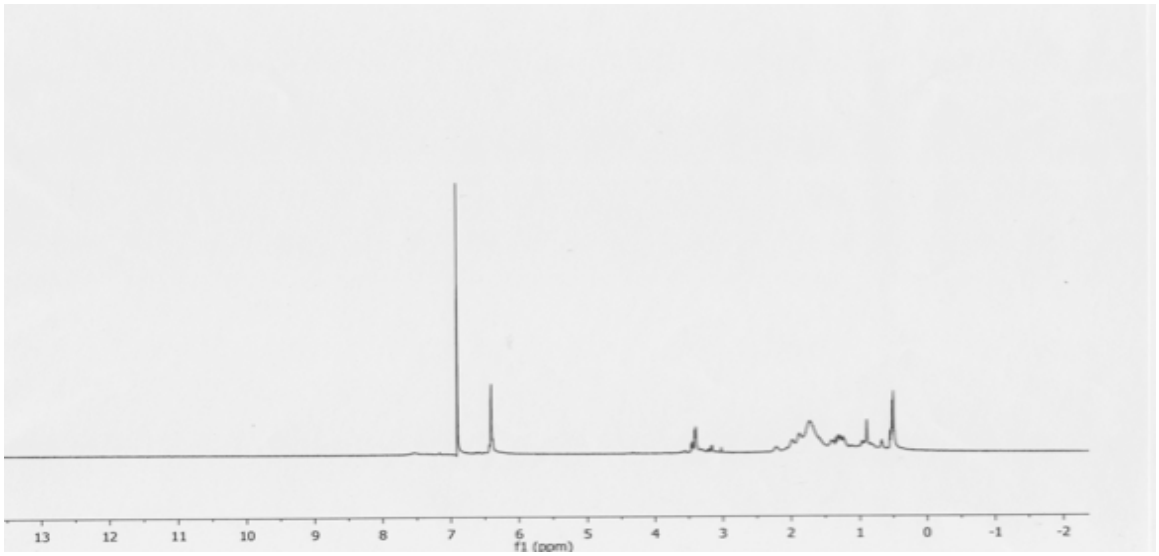

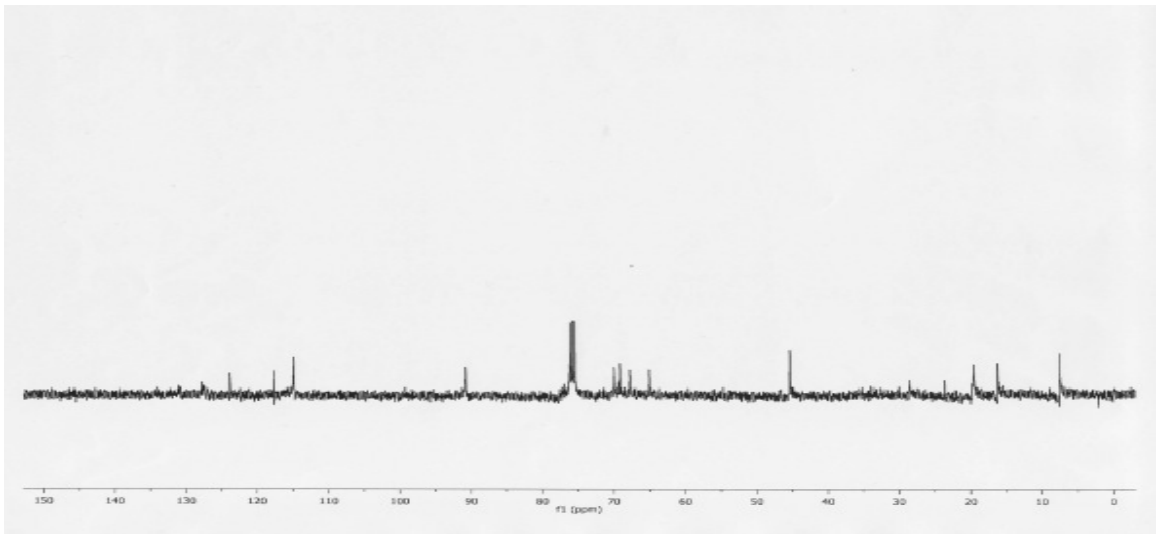

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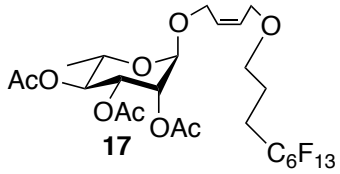

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