IOWA STATE UNIVERSITY
Digital Repository

Graduate Theses and Dissertations

Iowa State University Capstones, Theses and Dissertations

2012

Automated solution-phase synthesis of β-mannans

Shu-Lun Tang
Iowa State University

Follow this and additional works at: https://lib.dr.iastate.edu/etd

 Part of the Organic Chemistry Commons

Recommended Citation
Tang, Shu-Lun, "Automated solution-phase synthesis of β-mannans" (2012). *Graduate Theses and Dissertations*. 12481.
https://lib.dr.iastate.edu/etd/12481

This Dissertation is brought to you for free and open access by the Iowa State University Capstones, Theses and Dissertations at Iowa State University Digital Repository. It has been accepted for inclusion in Graduate Theses and Dissertations by an authorized administrator of Iowa State University Digital Repository. For more information, please contact digirep@iastate.edu.

www.manaraa.com

Automated solution-phase synthesis of β-mannans

by

Shu-Lun Tang

A dissertation submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Major: Organic Chemistry

Program of Study Committee:
Nicola L. B. Pohl, Major Professor
Malika Jeffries-EL
Reuben J. Peters
Aaron D. Sadow
Yan Zhao

Iowa State University

Ames, Iowa

2012

Copyright © Shu-Lun Tang, 2012. All rights reserved.

www.manaraa.com

TABLE OF CONTENTS

www.manaraa.com

www.manaraa.com

LIST OF ABBREVIATIONS

Ac	Acetyl
AcOH	Acetic acid
BAIB	(Diacetoxyiodo)benzene
Bn	Benzyl
BSA	Bovine serum albumin
CAN	Ceric ammonium nitrate
CB	Carboxybenzyl
CIP	Contact ion pair
ConA	Concanavalin A
COSY	Correlation spectroscopy
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCE	Dichloroethane
DCM	Dichloromethane
DIBAL-H	Diisobutylaluminum hydride
DMAP	*N*,*N*-Dimethylaminopyridine
DMF	*N*,*N*-Dimethylformamide
DMSO	Dimethylsulfoxide
ESI-MS	Electrospray ionization mass spectrometry
EtOAc	Ethyl acetate
FITC-ConA	Fluorescein isothiocyanate concanavalin A
FITC-PEMV	Fluorescein isothiocyanate pea enation mosaic virus
F-tag	Fluorous tag
FSPE	Fluorous solid phase extraction
ΔH	Enthalpy change
HMQC	Heteronuclear multiple-quantum correlation spectroscopy
HPLC	High-performance liquid chromatography
HSQC	Heteronuclear single-quantum correlation spectroscopy
ITC	Isothermal titration calorimetry
K_d	Dissociation constant
Lev	Levulinate
LiTEBH	Lithium triethylborohydride
MeCN	Acetonitrile
MeI	Methyl iodide
MeOH	Methanol
m/z	Mass/charge ratio
N	Binding stoichiometry
NBS	*N*-Bromosuccinimide
NMR	Nuclear magnetic resonance
PBS	Phosphate buffered saline
*p*ClBn	*p*-Chlorobenzyl
PEMV	Pea enation mosaic virus

www.manaraa.com

Piv	Pivalate
PMB	*p*-Methoxybenzyl
R_f	Retardation factor
ΔS	Entropy change
SPE	Solid phase extraction
SSIP	Solvent-separated ion pair
TBAF	Tetrabutylammonium fluoride
TBS	*tert*-Butyldimethylsilyl
TBSCl	*tert*-Butyldimethylsilyl chloride
TEA	Triethylamine
TEMPO	2,2,6,6-Tetramethyl-1-piperidinyloxy free radical
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TLR	Toll-like receptor
Tom	(Triisopropylsiloxy)methyl
TMSOTf	Trimethylsilyl trifluoromethanesulfonate

www.manaraa.com

CHAPTER 1

Overview of Automated Oligosaccharide Synthesis and the Synthesis of β-Mannosidic Linkages

Shu-Lun Tang and Nicola L. B. Pohl*

1. Strategies for automated synthesis of oligosaccharides

The automated synthesis of oligosaccharides (Seeberger et al. 2008; Hsu et al. 2011) is considered challenging compared to the synthesis of the other two major categories of biomolecule, peptides and nucleotides, for three reasons. First, instead of using limited amounts of building blocks for building up oligopeptides (from 21 canonical amino acids) and oligonucleotides (from 8 major nucleotides), the number of building blocks for chemical oligosaccharide synthesis is tremendously large due to the complexity of the oligosaccharide structures. In order to provide specific stereoselectivity, regioselectivity, and branch points for both linear and branched oligosaccharides, the building blocks require different protecting group strategies for various target glycosidic linkage formations. Second, the amide bond and phosphodiester bond formation reactions have been developed into relatively robust and straightforward reactions; however, glycosylation usually requires anhydrous conditions for higher yield and often low temperatures for better stereoselectivity. These factors set a higher mechanical standard for the robotics platform for carrying out oligosaccharide synthesis. Furthermore, the building blocks for oligosaccharide synthesis usually require multiple synthetic steps (5-14 steps) to synthesize; therefore, the cost of each building block is significantly higher than peptides and nucleotides.

www.manaraa.com

Automated solid-phase synthesis of oligosaccharides

Although the automated synthesis of oligosaccharides has proven more challenging compared to oligopeptides and oligonucleotides, Seeberger and co-workers have developed a similar strategy to the already well-established automated solid-phase synthesis of oligopeptides by using a modified automated peptide synthesizer along the lines proposed by Bruce Merrifield in his Nobel address (Merrifield and Steward 1965; Merrifield 1985). This method was called the automated solid-phase synthesis of oligosaccharides (Plante et al. 2001). After repeating glycosylation and deprotection cycles, the oligosaccharide chain could be built on the polymeric solid support; the subsequent cleavage of the linker releases the oligosaccharide from the solid phase. Although this strategy has the advantage of solid-phase synthesis— namely easy purification by washing of the resin—unfortunately, for each glycosylation, 10 to 20 equivalents of precious building block has to be charged to the synthesizer and at least 9 to 19 equivalents of building block are wasted at each coupling cycle. Moreover, the reactions on the solid phase cannot be easily monitored while the program is running, and, once the saccharide has been cleaved from the resin, if further chain elongation is needed, the reloading of the saccharide on the resin can be problematic.

One-pot oligosaccharide synthesis

The one-pot oligosaccharide synthesis strategy was first introduced by Wong and coworkers in 1999 (Zhang et al. 1999; Hsu et al. 2011). This method relies on building blocks with different anomeric activation group reactivity provided by different protecting groups. The most reactive building block (donor) is activated and reacts with the second reactive building block. Then the anomeric activation group on the second reactive building block is

www.manaraa.com

subsequently activated and reacts with the 3rd reactive building block. In order to apply this strategy to the synthesis of a wide variety of oligosaccharides, a library of building blocks with different anomeric reactivity has been built up and a computer program called Optimer was developed for selecting the building blocks. This one-pot strategy provides a straightforward way to synthesize complex oligosaccharides with no purification steps during the chain elongation. Although the choice of synthetic strategy is computer-assisted, the automation of the synthesis of oligosaccharides using this strategy has not been reported.

Automated solution-phase synthesis of oligosaccharide

In order to synthesize a wider range of oligosaccharides compared to the one-pot strategy and to overcome the drawback of the solid-phase methods, the Pohl group is developing an automated solution-phase synthesis protocol to synthesize oligosaccharides (Pohl 2008). By attaching a C_8F_{17} fluorocarbon chain (F-tag) to the reducing end of saccharides, the reaction can be handled under homogeneous solution-phase conditions similar to ordinary bench-top reactions and the purification can be easily completed by a special robotics platform using fluorous solid phase extraction (FSPE). The F-tag modified saccharide can then be carried on through additional synthetic steps. Since the reaction is in solution-phase, only 1.5 to 3.5 equivalents of precious building block is used for each glycosylation. Compared to the 10 to 20 equivalents of building block required for typical solid-phase protocols, up to 18.5 equivalent of building block could be saved at each coupling cycle by the solution-phase strategy. In addition, just an easy thin layer chromatography (TLC) or mass spectrometry analysis can be used to monitor the reaction. Moreover, any F-tag containing saccharides can be reinjected to the synthesizer for further

www.manaraa.com

reactions, including oligosaccharides already produced from the synthesizer. This advantage provides more flexibility compared to automated or manual solid-phase synthesis strategies. However, the complete scope and utility of this new platform still needs to be demonstrated even with the most synthetically challenging glycosidic linkages such as β-mannosides.

2. The challenges of β-mannoside synthesis

β-Mannoside can be found in a wide variety of natural products including mammalian, insect, plant, fungal, and bacterial saccharides. In order to study these important saccharides, the chemical synthesis of β-mannoside could provide substrates needed for additional biological studies; however, the β-mannosidic linkage is one of the most challenging glycosidic linkages to synthesize (El Ashry et al. 2005). The challenges of the formation of this 1,2-*cis* linkage are attributed to three major factors (Figure 1). After the activation of the mannosyl donor, leaving of the activation group leads to the formation of the oxocarbenium intermediate. However, steric hindrance from the axial C-2 substituent blocks the β face of the pyranose ring and prevents the formation of the β-mannoside. Second, the anomeric effects also favor the thermodynamically more stable α-mannoside over the less stable β-mannoside (El Ashry et al. 2005). Finally, the most reliable way to control the stereochemistry of the anomeric carbon during the glycosylation is to use neighboring-group participation by a group such as an ester. After the formation of the oxocarbenium intermediate, the neighboring ester carbonyl attacks the anomeric position and forms a stabilized five-member ring intermediate, which blocks the α face of the pyranose

www.manaraa.com

ring and does not allow the glycosyl acceptor (nucleophile) to attack from the β face. As result, formation of the α-mannoside is predominant (Figure 1).

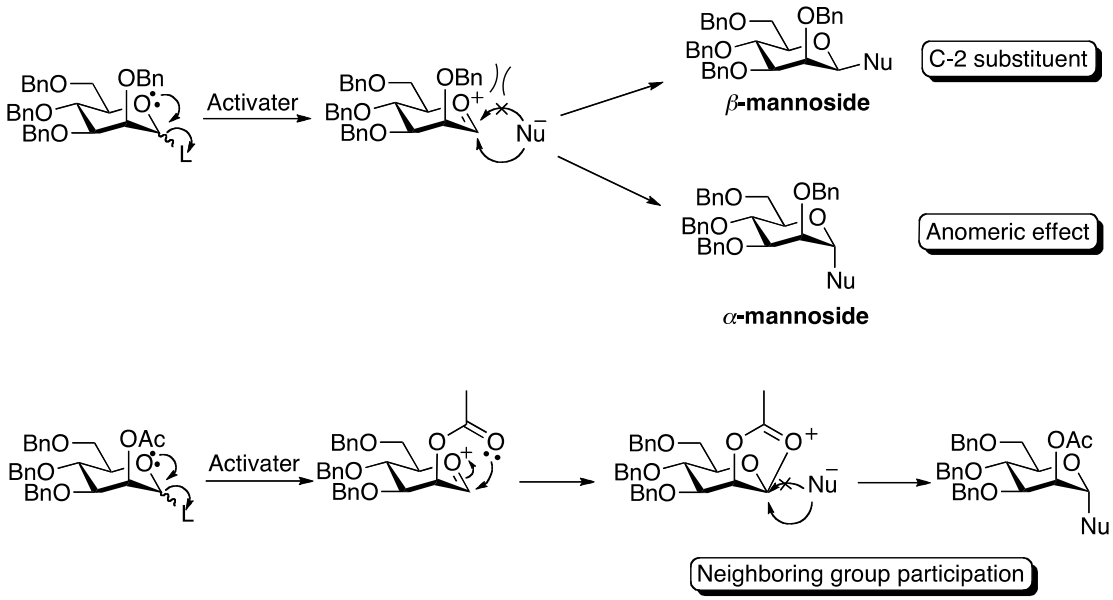


Figure 1. The three-fold challenge of forming β-mannosides.

3. Current synthetic strategies toward β-mannoside synthesis

Crich's 4,6-*O*-benzylidene strategy

In order to synthesize this challenging linkage, various methodologies have been developed. One of the most widely used methods is Crich's 4,6-*O*-benzylidene strategy. The origin of the β-selectivity comes from the crucial 4,6-*O*-benzylidene group. After the mannosyl glycosyl donor is activated by an activator such as triflic anhydride, an equilibrium between the covalent α-anomeric triflate, the contact ion pair (CIP), and the solvent-separated ion pair (SSIP) is formed (Figure 2). The torsional strain of the fused bicyclic

www.manaraa.com

system makes the oxocarbenium intermediate less stable and drives the equilibrium toward the α-anomeric triflate that ultimately results in the formation of a β-mannoside via an S_N2 mechanism. Also, the electron-withdrawing effect of the benzylidene is maximized by the locked C6-O6 bond being antiperiplanar to the C5-O5 bond; therefore the oxocarbenium is destabilized and shifts the equilibrium to the α-triflate that provides the β-mannoside. However, this method needs low activation temperatures in most cases and the selective deprotection of the 4,6-*O*-benzylidene is sometimes tricky. These two factors make automation of this procedure more challenging (Crich 2010; Crich 2011). In addition, the approach does not allow flexibility in the protecting group design for further chain extension. Therefore, protecting group manipulations after glycosylation can add to the length and complexity of syntheses.

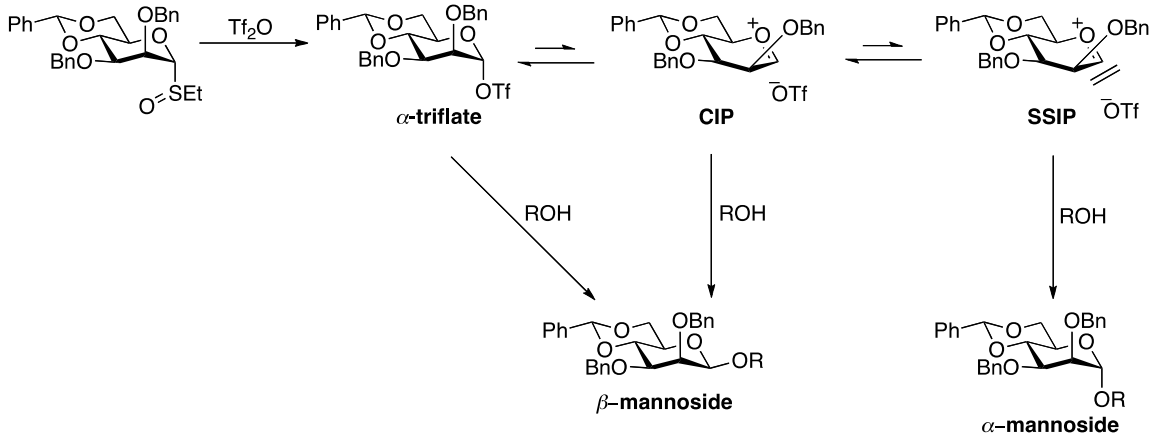


Figure 2. Crich's 4,6-*O*-benzylidene strategy.

Kim's carboxybenzyl strategy

www.manaraa.com

This strategy is based on Crich's 4,6-*O*-benzylidene and provides high *β*-selectivity and has been applied to a wide variety of oligosaccharide synthesis. Except for the sulfoxide/thiol donor, which Crich has reported for the glycosylation, Kim's anomeric carboxybenzyl group (CB) is also used as the leaving group for 4,6-*O*-benzylidene protected donors (Kim et al. 2001; Kim et al. 2008). (Figure 3) The ease of the CB group formation/activation and its high *β*-selectivity made this method the choice for later automated solid-phase synthesis of a *β*-mannoside (Codee et al. 2008). Unfortunately, this method still suffers from problems in facile chain extension just like Crich's original solution.

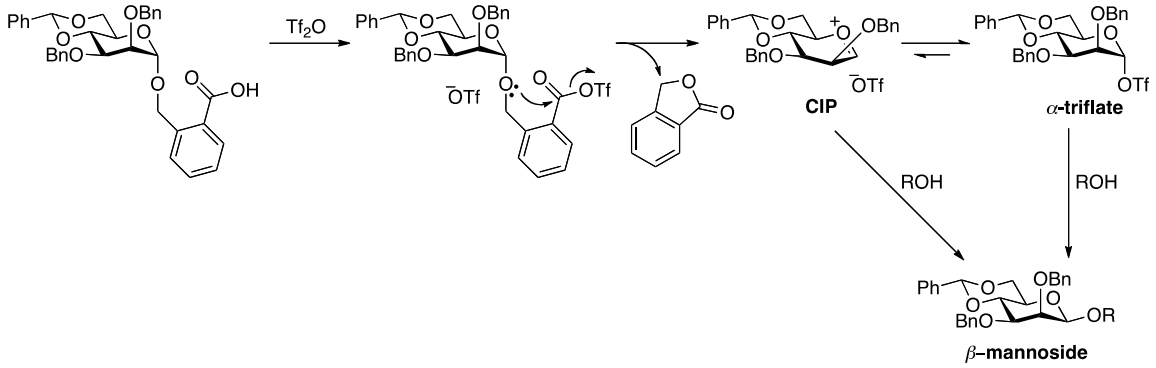


Figure 3. Kim's carboxybenzyl strategy

Oxidation-reduction sequence

Another method for *β*-mannan synthesis relies on an oxidation-reduction sequence and was first reported by Kotchetkov et al (Betaneli et al. 1980). This approach begins from a glucosyl donor with a C-2 participating group (Figure 4). After the glycosylation, the *β*-glucoside is formed followed by a deacylation reaction to furnish a free equatorial hydroxyl

www.manaraa.com

group at the C-2 position. The oxidation gives a C-2 ketone and the following hydride reduction reduces the ketone into an axial hydroxyl to finally yield the desired *β*-mannoside. Bundle and coworkers have utilized this method to construct a variety of fungal *β*-1,2-mannan oligomers for immunology studies related to candidiasis. The advantage of this strategy for application to an automation platform is that there is no need for very low temperature glycosylations (Wu and Bundle 2005). However, conditions for carrying out oxidations and reductions on the automation platform would have to be developed.

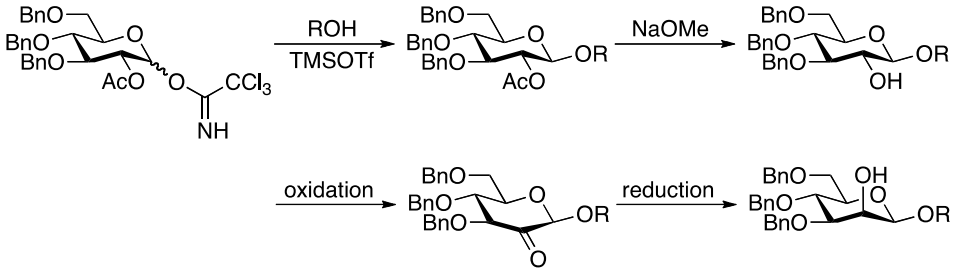


Figure 4. Oxidation-reduction sequence

4. Automated solid-phase synthesis of *β*-mannosides

Seeberger and coworkers have tried to use their modified solid-phase peptide synthesizer for the synthesis of a *β*-mannosidic linkage (Codee et al. 2008). The group utilized Crich's 4,6-*O*-benzylidene strategy with Kim's CB as the anomeric leaving group and also used the less bulky but expensive (triisopropylsiloxy)methyl (Tom) group to protect the 3-OH for better *β*-selectivity. After three cycles of glycosylation-deprotection, a trisaccharide with two *β*-mannosidic linkages was formed. It was worth noting that for each glycosylation step up to 9.0 to 10 equiv. of precious donors have been charged into the

www.manaraa.com

synthesizer, which was both economically and ecologically problematic. In addition, only the simplest chain extension at the 3-position has been attempted. Application of the approach to β-mannosides linked at the 2-, 4-, or 6-positions would require complex protecting group manipulations.

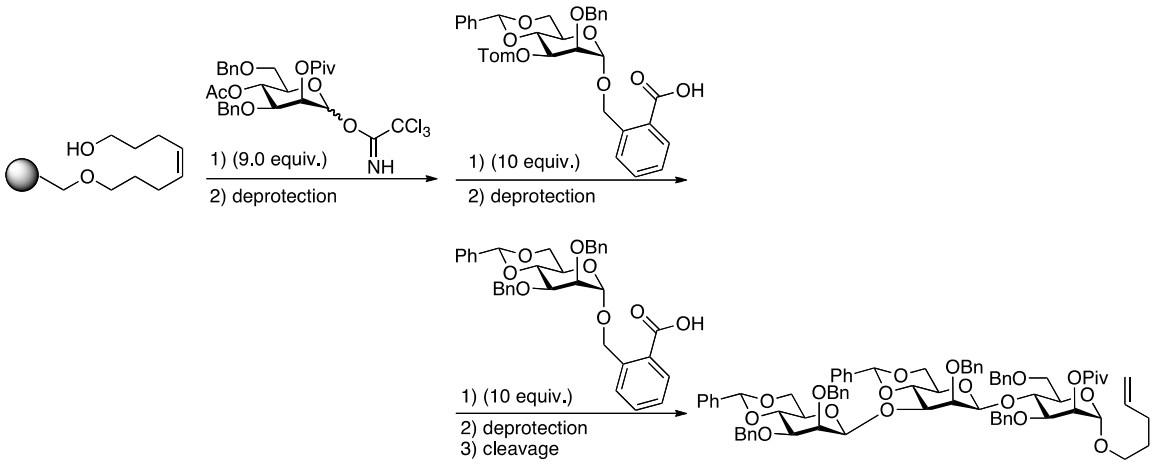


Figure 6. Automated solid-phase synthesis of β-mannan

5. A new approach to the automated synthesis of β-mannans using a solution-phase strategy

In order to provide a strategy which could produce the biologically important β-mannans in a highly efficient and economical way, this dissertation will illustrate the development of the automated solution-phase synthesis of different β-mannans including the fungal β-1,2-mannans by using both oxidation-reduction and β-directing mannuronate strategies, and also reveals a new strategy for β-mannan synthesis by reduction of mannuronate esters to alcohols. The following chapter will show the power of this automated solution-phase method to create β-1,4-mannuronate and β-1,4-mannan oligomers up to

www.manaraa.com

hexasaccharides. The subsequent chapter will demonstrate the application of this new β-mannan synthesis strategy for the synthesis of the insect *N*-glycan terminal trimannosides for fluorous microarray and isothermal titration calorimetry (ITC) studies to investigate the binding affinity of the pea enation mosaic virus (PEMV) in the aphid digestion system for clues to the virus transmission pathway of the virus in these notorious pests. Finally, the automated solution-phase synthesis of β-1,6-mannan and β-1,3-mannan oligomers will present the scope of the automated solution-phase synthesis of β-mannans and its potential for synthesizing other oligosaccharides containing this challenging linkage.

www.manaraa.com

CHAPTER 2

Automated Solution-Phase Synthesis of β-1,2-Mannans

Shu-Lun Tang and Nicola L. B. Pohl*

Abstract

β-1,2-Mannoside is considered one of the most challenging glycosidic linkages to chemically synthesize due to its thermodynamically and kinetically disfavored formation. This linkage can be found in the cell wall polysaccharides of *Candida albicans* and is a target structure for potential vaccines against these yeast infections. To develop the first automated synthetic approach to this important linkage, two approaches were tested in an automated solution-phase synthesis platform: an oxidation-reduction sequence and a β-directing mannuronate strategy. Both strategies effectively produced a β-1,2-mannan disaccharide by automation. However, further chain extension proved problematic with both methods. The β-directing mannuronate strategy was the higher yielding method to synthesize the β-1,2-mannan efficiently and could thereby be the ideal strategy for future automated solution-phase synthesis of 1,2-linked and other β-mannan structures.

Introduction

The β-mannosidic linkage is a 1,2-*cis*-glycosidic linkage considered to be one of the most difficult linkages to synthesize because of the thermodynamically favored formation of α-mannoside and the kinetically disfavored formation of β-mannoside from the steric hindrance from the C-2 substituent (El Ashry et al. 2005). This particular linkage exists in many crucial natural products, including the β-1,2-mannans, which is the immunogenic part

www.manaraa.com

of the cell wall phosphomannan of *Candida albicans*, an organism that mainly infects immunocompromised and long-term antibiotics treatment patients. The antifungal drugs for candidiasis often cause side effects and drug resistance in *C. albicans*. Given these problems, a vaccine against this pathogen would be ideal. To this end, Bundle and co-workers have synthesized bovine serum albumin (BSA) conjugated *β*-1,2-mannans and proven that as small as a disaccharide conjugate could be a potential vaccine for the candidiasis treatment (Nitz et al, 2000; Nitz and Bundle 2001; Wu and Bundle 2005). The group utilized an oxidation-reduction sequence strategy starting from the formation of a 2-*O*-acetyl-*β*-glucoside followed by deacetylation, oxidation, and reduction to convert the C-2 equatorial hydroxyl to axial to become a *β*-mannoside ready for further chain extension. The advantage of this method is the availability of gram scale production of the *β*-1,2-mannans under moderate conditions and a short building block synthesis route (Nitz et al, 2000; Nitz and Bundle 2001; Wu and Bundle 2005). The big disadvantage to this approach, however, is the two additional steps required at each coupling step. This approach would also require an automation strategy that does not just include protocols for glycosylation and deprotection, but would require additional protocols for oxidation and reduction reactions compatible with the automation platform.

For other *β*-mannoside synthesis strategies, in addition to Crich's classic 4,6-*O*-benzylidene methodology (Crich 2010; Crich 2011), van der Marel and coworkers have developed a new strategy to construct the *β*-mannosidic linkage while synthesizing a series of alginate *β*-mannuronic acid oligomers (van den Bos et al. 2006; Codee et al. 2009; Dinkelaar et al. 2009; Walvoort et al. 2009). (Figure 1) The C-5 carboxylate of the mannuronate provides high *β*-selectivity with a wide range of glycosyl acceptors. The origin

www.manaraa.com

of the unique selectivity is believed to be from the electron-withdrawing nature of the carboxylate. When the thiomannoside donor is activated by triflic anhydride, the equilibrium between the α-anomeric triflate, and $^{3}H_{4}$ and $^{4}H_{3}$ conformers of the oxocarbenium intermediate are formed. The electron-withdrawing C-5 carboxylate destabilized the oxocarbenium ion and shifts the equilibrium to the anomeric α-triflate, which leads to the β-mannuronate through a S_N2 mechanism. Because of the through-space stabilization of the cation by the pseudoaxial C-5 carboxylate and the lowest energy conformation of the ring substituents, the $^{3}H_{4}$ conformer is favored over the $^{4}H_{3}$ conformer and gives the β-mannuronate. The spirit of this mannuronate strategy is analogous to Crich's 4,6-*O*-benzylidene strategy, however, without the fused benzylidene ring, the masking strategy of the overall oligosaccharide synthesis has more flexibility without the necessity of the selective benzylidene cleavage. Furthermore, the acid lability of the benzylidene can be a problem during further chain extension (Crich et al. 2004). We reasoned that this strategy to β-mannuronates could possibly provide a new, shorter or more flexible route to β-mannans if a global ester reduction method could be developed successfully. Herein, we report the first automated solution-phase synthesis of the challenging β-mannan linkage by an automated method to the oxidation-reduction sequence and also by a new β-mannan synthesis strategy that relies on a β-directing mannuronate followed by a global hydride reduction of the C-5 esters. Comparison of these two methods showed the latter strategy to be a more efficient method for the automated synthesis of β-mannans.

www.manaraa.com

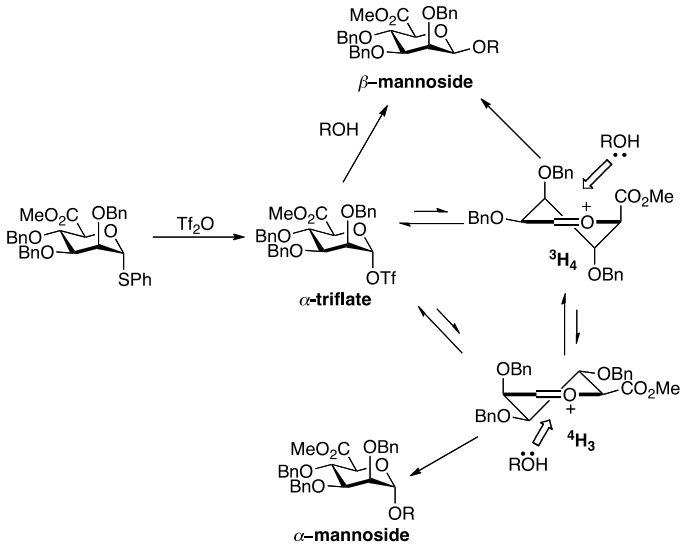


Figure 1. β-Directing effect of a mannuronate building block

Results and discussion

Although the automated solid-phase synthesis of β-mannan up to two β-mannosidic linkages has been reported previously (Codee et al. 2008), a homogeneous automated solution-phase synthesis approach should be a more efficient and economical way to construct oligosaccharides due to the absence of the repeating rinsing cycles and the need for large excesses of glycosyl donors (Codee et al. 2008). The real time TLC or MS monitoring of the reaction and reinjection after bench-top purification also allows more flexibility compared to a traditional automated solid-phase protocol. In order to implement the oxidation-reduction sequence to the automated solution-phase synthesis protocols, the glucosyl donor **4** was synthesized. The *p*-chlorobenzylation of the known orthoester **1** (Tsai et al. 2012) gave the fully protected **2**. The *p*-chlorobenzyl protected sugars are known for their crystalline characteristics that provide convenient purification and storage and, indeed, compound **2** did readily crystalize from dichloromethane (DCM). The orthoester was

www.manaraa.com

hydrolyzed under acidic conditions to yield solid **3**. Subsequent trichloroacetimidate formation gave the desired glycosyl donor **4** for the initial automated solution-phase synthesis run of β-1,2-mannan. (Figure 2) The automation started from the glycosylation of the fluorous allyl tag (F-tag) and the trichloroacetimidate donor **4** (2.0 equiv.) catalyzed by trimethylsilyl trifluoromethanesulfonate (TMSOTf) (0.1 equiv.) at 0 °C under Ar atmosphere. After 30 min a small aliquot of solution was transferred out of the synthesis platform for TLC reaction monitoring. After the reaction was finished, triethylamine was added to quench the reaction and the solvent was removed under reduced pressure generated by the vacuum pump unit. The deacetylation was carried out without further purification by adding sodium methoxide methanol solution to the reactor and the mixture was vortexed for 1 h at 25 °C, and quenched by AcOH/MeOH after the TLC showed the completion of the reaction. The solvent was removed under reduced pressure and the mixture was loaded onto the fluorous solid phase extraction (FSPE) cartridge. The automated synthesis platform finished the elution, collected the product fractions, evaporated the solvent and transferred the product with a free 2-OH to the reactor vial for the oxidation. The Dess-Martin oxidation was chosen for the automated synthesis due to its mild conditions and more complete reaction compared to the previously reported Albright-Goldman oxidation (Nitz and Bundle 2001). The Dess-Martin periodinane (DMP) in 1,2-dichloroethane (DCE) was added to the reactor and vortexed at 50 °C for 45 min. After TLC showed the completion of the reaction, the solvents was removed under reduced pressure and the fluorous-modified product was purified by FSPE. The reduction of the C-2 ketone to an axial alcohol was completed by L-Selectride (Wu and Bundle 2005) followed by another FSPE. At this stage, the F-tag-modified β-mannoside monomer was produced and ready for another glycosylation-

www.manaraa.com

deacetylation-oxidation-reduction cycle. After 2 cycles (8 steps), the desired *β*-1,2-mannan disaccharide **5** was successfully synthesized in 8% yield over 8 steps (73% per reaction step). The further chain extension was tested; however, the formation of trisaccharide **A** from the glycosylation of disaccharide **5** with the donor **4** could not be affected, likely due to the weakened acceptor nucleophilicity from the built-up electron-withdrawing effect from multiple *p*-chlorobenzyl groups when compared to the previously reported similar benzyl groups protected *β*-1,2-di-mannoside acceptor (Wu and Bundle 2005). The deprotection of **5** was accomplished by hydrogenolysis of the *p*-chlorobenzyl ethers catalyzed by 20% $Pd(OH)_2/C$ and the fully deprotected *β*-1,2-mannan **6** was obtained in 87% yield. Although this result represents the first automated solution-phase synthesis of the *β*-mannan, the approach requires too many reactions and transfer steps in between each glycosylation for a high overall yield of the final product. Therefore, a second strategy using a mannuoronate donor with *β*-directing C-5 carboxylate was explored.

www.manaraa.com

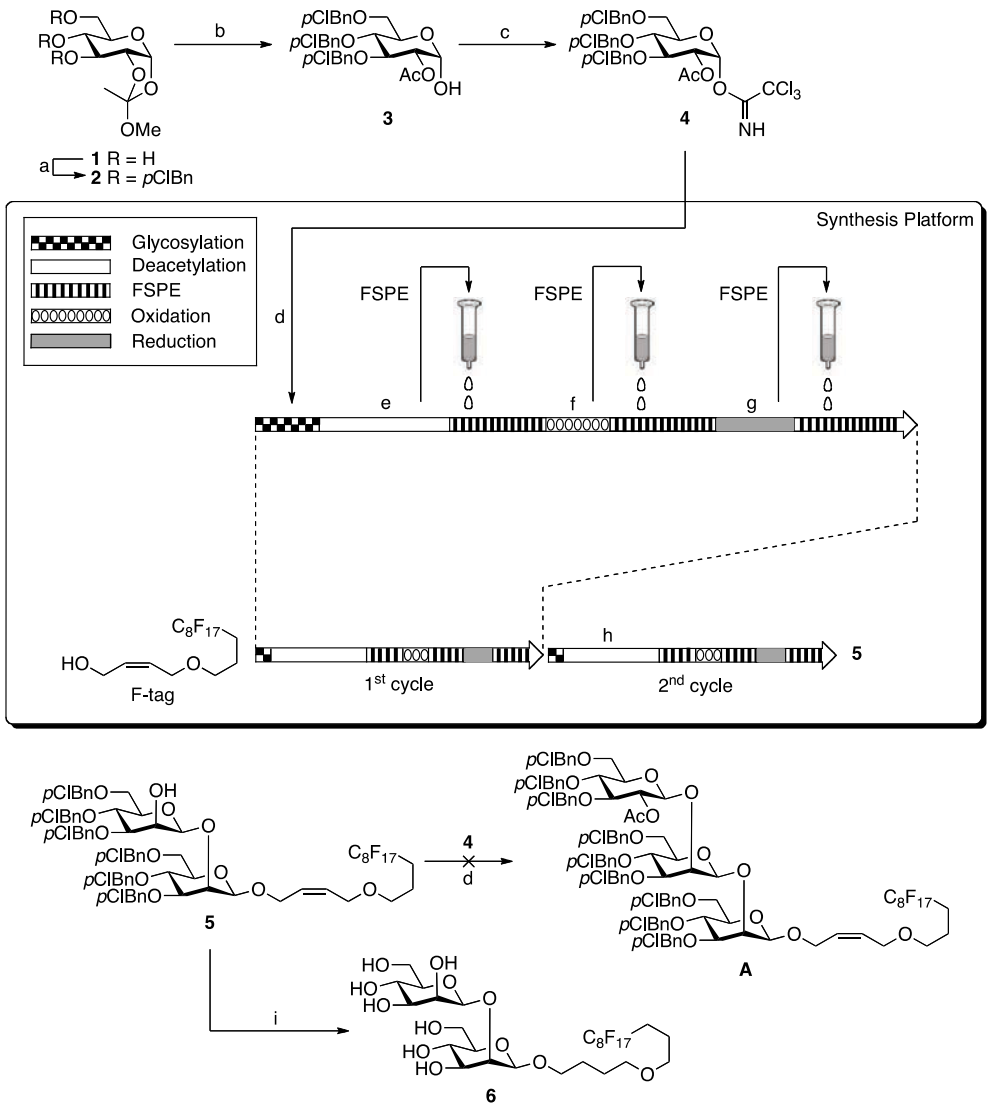


Figure 2. Synthesis of donor **4** and the automated solution–phase synthesis of β-1,2-mannan via oxidation-reduction sequence

Reagents and conditions: (a) *p*ClBnCl, NaH, DMF, 0 °C to rt, 20 h, 86%; (b) 2 N HCl, DCM, rt, 2 h, quant.; (c) Cl_3CCN, DBU, DCM, 0 °C, 3 h, 88%; (d) TMSOTf, DCE, 0 °C 45 min; (e) NaOMe, MeOH, 25 °C, 2 h; (f) DMP, DCE, 50 °C, 45 min; (g) L-Selectride, THF, -20 °C, 45 min; (h) NaOMe, MeOH, 50 °C, 2 h; (i) H_2, $Pd(OH)_2/C$, MeOH, 20 °C, 87%

www.manaraa.com

Compared to the oxidation-reduction sequence strategy, the mannuronate should provide a shorter synthetic route without the inversion of the C-2 position. The *p*-methoxybenzyl (PMB) group was chosen as the masking group for the 2-OH because of its small size, which provides a less hindered *β*-face for the glycosyl acceptor. The synthesis of the mannuronate donor **12** started from the selective opening of the benzylidene acetal of the known compound **7** (Oshitari et al. 1997) with borane tetrahydrofuran complex (BH_3·THF) and dibutylboron triflate (Jiang and Chan 1998) to afford **8** with a free 6-OH. The oxidation of the primary alcohol by 2,2,6,6-tetramethyl-1-piperidinyloxy free radical (TEMPO) and (diacetoxyiodo)benzene (BAIB) (van den Bos et al. 2006) generated the carboxylic acid **9**. The esterification of the carboxylic acid with methyl iodide/K_2CO_3 gave the fully protected methyl mannuronate **10** (van den Bos et al. 2006). *N*-Bromosuccinimide (NBS) was utilized to remove the anomeric thiophenyl group (Liew and Wei 2002) to afford alcohol **11** with a free anomeric hydroxyl and the following trichloroacetimidate formation produced the desired donor **12** for the automated synthesis.

The automated synthesis started from the attachment of the fluorous allyl tag (Mamidyala et al. 2006) and donor **12** (3.0 equiv.) with TMSOTf (0.1 equiv.) at -20 °C. After 30 min, the solvent was removed under reduced pressure and ceric ammonium nitrate (CAN) solution in MeCN/H_2O = 1/9 was added to the mixture and vortexed for 1 h. After the reaction showed completion based on TLC, the mixture was transferred to the SPE station for the FSPE purification. Another glycosylation-deprotection-FSPE cycle was performed and the crude product was transferred out of the synthesis platform and purified manually to afford the desired *β*-1,2-mannuronate dimer **13** in 38% over 4 steps (79% per step). The attempt for further chain extension was also tested using the standard conditions for

www.manaraa.com

glycosylation with the donor **12**. However due to the low reactivity of the acceptor, the reaction did not succeed instead, the only formed product was the hydrolyzed donor. Therefore, a trial for a mixed strategy was initiated that involved attachment of glucosyl donor **C** followed by a deacetylation-oxidation sequence. Interestingly, glycosylation with the glucose building block did take place in 68% yield. Therefore, the inherent reactivity of the growing mannose chain is not as large an issue as matching the correct donor. Unfortunately, the subsequent Dess-Martin oxidation reaction was not successful. Steric hindrance at the glucosyl C-2 position is a likely explanation for the difficulty of this reaction.

To complete the synthesis of the disaccharide, di-mannuronate **13** was reinjected back into the synthesis platform vial and treated with a lithium triethylborohydride (LiTEBH) 1.0 M solution in THF. Fortunately, the esters were reduced quantitatively to afford the desired β-1,2-mannan disaccharide **14**. After hydrogenolysis of the benzyl groups with catalytic 10% Pd/C, the deprotected **6** was obtained in 89% yield. The β-directing mannuronate strategy only required one step to construct one β-mannosidic linkage. Compared to the 4 steps required for the glycosylation-deacetylation-oxidation-reduction sequence, the β-directing mannuronate strategy was more efficient and significantly improved the overall yield of the final β-1,2-mannan. Furthermore, these two strategies provided comparable yields to the automated solid-phase synthesis of β-mannan (Codee et al. 2008) using Crich's 4,6-*O*-benzylidene strategy with 70% to 80% fewer donor equivalents required for each glycosylation. Even in producing a disaccharide, 7.0 equivalents of glycosyl donor were saved with the new mannuronate-based strategy.

www.manaraa.com

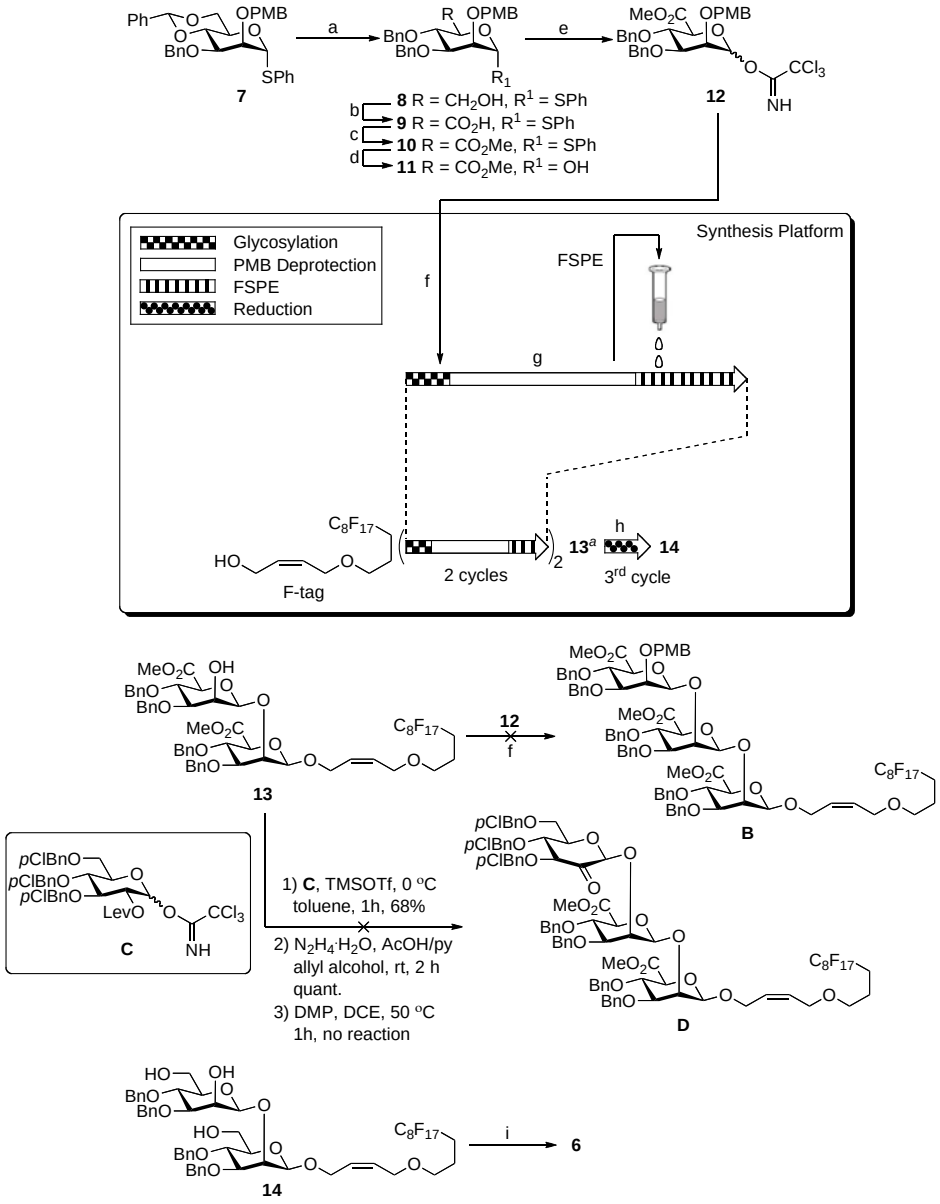


Figure 3. Synthesis of donor **12** and the automated solution–phase synthesis of β-1,2-mannan via mannuronate donor strategy

Reagents and conditions: (a) BH_3·THF, Bu_2BOTf, 0 °C, 3 h, 86%; (b) TEMPO, BAIB, DCM/H_2O, rt, 45 min, 76%; (c) MeI, K_2CO_3, DMF, rt, 6 h, 87%; (d) NBS, $NaHCO_{3(s)}$ acetone/H_2O, rt, 1 h, 87%; (e) Cl_3CCN, DBU, DCM, 0 °C, 3 h, 89%; (f) TMSOTf, DCM, -20

www.manaraa.com

°C, 30 min; (g) CAN, MeCN/H_2O, 25 °C, 4 h; (h) LiTEBH/THF, DCM, 0 °C, 30 min; (i) H_2, Pd/C, MeOH, 20 °C, 89% (*a*: purified on bench-top)

Conclusions

Protocols for the successful synthesis of the challenging *β*-1,2-mannan linkage have now been developed on an automated solution-phase synthesis platform protocol via both an oxidation-reduction sequence and a new *β*-directing mannuronate strategy. The latter proved to be a more efficient way to construct *β*-mannans. Although further work is needed to extend this work to longer polymannan oligosaccharide fragments, the current method now allows the incorporation of the difficult 1,2-linked *β*-mannoside building block into automated solution-phase synthesis strategies.

Experimental section

General materials and methods

Dichloromethane (DCM) and dichloroethane (DCE) for glycosylation were distilled from calcium hydride. Tetrahydrofuran (THF) was collected from PureSolv Micro solvent purification system (Innovative Technology, Inc., Amesbury, MA) before reactions. All other commercial solvents and reagents were reagent grade and used as received without further purification. The reactions were monitored by thin layer chromatography (TLC) with 250 μm Sorbent Technologies silica gel HL TLC plates. The hydrogenation reaction under 1000 psi hydrogen was operated in the Parr model 4766 general purpose vessel high pressure reactor (Parr Instrument Company, Moline, IL). The developed TLC plates were visualized by stain

www.manaraa.com

with *p*-anisaldehyde solution followed by heating on a hot plate. Flash column chromatography was performed with Zeochem ZEOprep 60 silica gel, 40-63 μm particle size. The automated solution phase synthesis was performed in the Chemspeed ASW1000 (Chemspeed, Augst, Switzerland) synthesis platform with hood, 16 reactor vials (13-mL capacity each) and heating/cooling unit (200 °C to -20 °C) machined to hold the SPE cartridges at the Iowa State University Machine Shop. ^{1}H and ^{13}C NMR spectra were obtained at 400 MHz and 100 MHz on a Bruker DRX-400 spectrometer and a Varian MR-400 spectrometer. The C-H coupling constants were measured by the coupled ^{13}C NMR spectra. Chemical shifts (δ) were reported in parts per million (ppm) relative to $CDCl_3$ and CD_3OD as internal references. Mass spectra were obtained on a Finnigan TSQ700 triple quadrupole mass spectrometer (Finnigan MAT, San Jose, CA) fitted with a Finnigan ESI interface.

www.manaraa.com

General procedure for automated synthetic cycles to produce β-1,2-mannosides

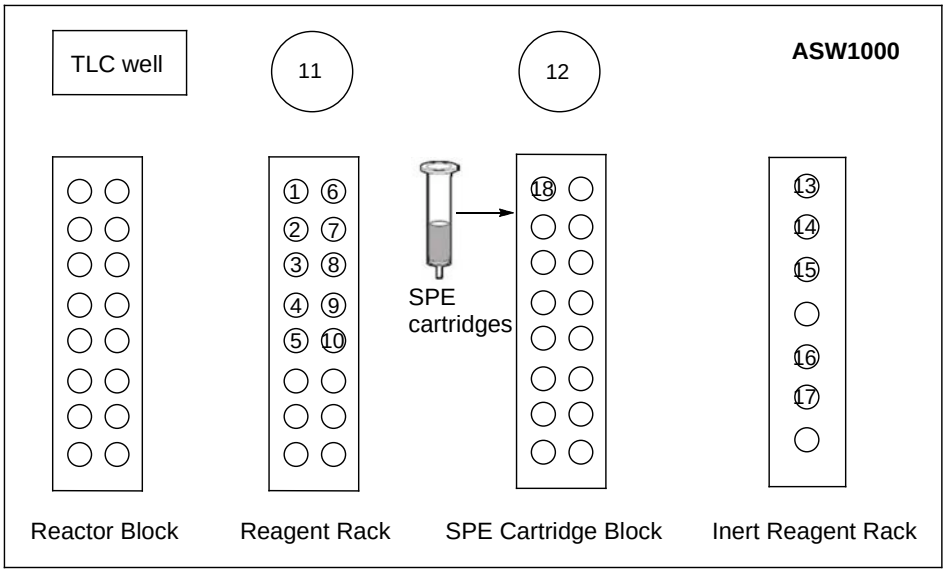


Figure 4. Basic layout of automated solution-phase oligosaccharide synthesizer (ASW1000)

1: F-tagged acceptor (solution in DCM/DCE), 2: TEA, 3: DMF, 4: NaOMe/MeOH, 5: LiTEBH (1.0 M in THF), 6: Dess Martin periodinane/DCE, 7: water, 8: CAN/MeCN, 9: AcOH/MeOH, 10: L-Selectride (1.0 M in THF), 11: MeOH, 12: reservoir (toluene), 13: TMSOTf (0.55 M in DCM/DCE), 14: donor, 15: DCM/DCE, 16: THF, 17: 80 % MeOH, 18: FSPE cartridge.

Part A: Oxidation-reduction sequence

1. Sample Preparation

Donor molecule (148 mg, 0.200 mmol) was dissolved in anhydrous DCE (1.6 mL) in the 13 mL-vial and placed at the inert reagent rack (Donor) under argon atmosphere. A 0.055 M trimethylsilyltrifluoromethanesulfonate (TMSOTf) solution (5.0 mL) in anhydrous DCE

www.manaraa.com

was prepared in an 8 mL-vial and placed as indicated on the inert reagent rack under argon. MeOH (100 mL) was placed in the stock solution bottle at the stock solution station as indicated. Toluene (1.0 L) was placed in the stock solution bottle and placed at the reservoir bottle rack with tubing as reservoir solution for rinsing. Anhydrous DCE (20 mL) was transferred to the 50 mL-vial and placed as indicated on the inert reagent rack under argon. Anhydrous tetrahydrofuran (THF, 10 mL) was transferred to the 50 mL-vial and placed as indicated on the inert reagent rack under argon. 80% methanol/water (100 mL) stock was prepared in the 100 mL-vial and placed as indicated on the inert reagent rack. F-tagged acceptor molecule (50 μmol) was dissolved with anhydrous DCE (0.8 mL) in a Wheaton 8 mL-E-Z extraction vial (conically-bottomed) flushed with argon, capped with septa and placed at the reagent rack as indicated. Triethylamine (TEA, 5.0 mL) was transferred to an 8 mL-vial capped with septa and placed at the reagent rack as indicated. *N*,*N*-dimethylformamide (DMF, 8.0 mL) was transferred to an 8 mL-vial capped with septa and placed at the reagent rack as indicated. A 0.5 M sodium methoxide solution in methanol (5.0 mL) was prepared in an 8 mL-vial capped with septa and placed at the reagent rack as indicated. A 0.2 M acetic acid solution in methanol (5.0 mL) was prepared in an 8 mL-vial capped with septa and placed at the reagent rack as indicated. Dess-Martin periodinane solution (0.21g in 5.0 mL DCE) was prepared in an 8 mL-vial flushed with argon, capped with septa and placed at the reagent rack as indicated. L-Selectride solution in THF (1.0 M, 3.0 mL) was transferred to an 8 mL-vial capped with septa and placed at the reagent rack as indicated. Water (8.0 mL) was transferred to an 8 mL-vial capped with septa and placed at the reagent rack as indicated. A fluorous solid phase extraction (FSPE) cartridge (2.0 g, 10 cc) was preconditioned with 80% methanol/water and placed on the machined FSPE block as

www.manaraa.com

indicated. An empty Wheaton 8 mL-E-Z extraction vial was placed under the FSPE cartridge.

2. Cleaning Cycle

Prior to introduction of reagents, the ASW1000 reactor vials were cleaned, dried and purged with argon by running the cleaning cycle. During the cleaning cycle, each of the 16 reactor vials (13 mL capacity each) was rinsed with toluene (8.0 mL) and methanol (8.0 mL) 3 times each. After the solvents were completely removed, the reactor vials were dried under vacuum and purged with argon for 45 minutes. Reagent solutions were prepared by azeotropic removal of water from each building block with toluene; the resulting building blocks were then dried under high vacuum. After the cleaning cycles were done, the reagents were transferred into the reagent vials respectively, which were then placed on the inert condition reagent rack and general atmosphere reagent rack.

3. Glycosylation

The needle transferred the acceptor molecule (F-tag) solution (0.8 mL) to the reaction vial 1, followed by the transfer of the donor molecule solution (0.8 mL). The mixture was vortexed under ambient temperature at 800 rpm for 20 min. Then the reactor vials were cooled to 0 $^{\circ}$C by the heat transfer oil with 800 rpm vortex rate. The TMSOTf solution (0.1 mL) was transferred into the reactor vial 1 under 200 rpm vortex rate. After each individual transfer, the needle (inside and outside) was rinsed by toluene (2.0 ml) before operating the next task. The reaction mixture was vortexed at 800 rpm for 45 minutes at 0 °C under argon atmosphere. After the reaction time the needle withdrew 20 μL of the solution from the

www.manaraa.com

reaction mixture and placed it into the first well of the microtiterplate for thin layer chromatography monitoring. TEA (0.05 mL) was added to the solution for quenching and the solvent was evaporated under reduced pressure.

4. Deacetylation

To the dried residue after the glycosylation, methanol (0.5 mL) was added to the reactor vial followed by NaOMe solution (0.4 mL). The reaction mixture was vortexed at 800 rpm for 1 hour at room temperature. After the reaction time the needle withdrew 20 μL of the solution from the reaction mixture and placed it into the second well of the microtiterplate for thin layer chromatography monitoring. An acetic acid (0.75 mL) solution added to reactor vial for quenching followed by addition of toluene (1.0 mL) and solvent was evaporated under reduced pressure.

5. Fluorous solid-phase extraction (FSPE)

DMF (0.4 mL) was added to the reactor vials to dissolve the crude mixture and the vials were vortexed at 800 rpm for 3 minutes. The reaction mixture (1.2 mL) was carried to the FSPE cartridge at the FSPE block and dispensed at a speed of 1.0 mL/s via the 10 mL syringe. Then 80% methanol (2.0 mL) was used to rinse the empty reactor vial. The 80% methanol solution was removed from the reactor vial and delivered to the FSPE cartridge. The 80% methanol rinsing and transferring was repeated one more time. Additional 80% methanol solution (4.0 mL, repeated 2 times) was used to rinse the FSPE cartridge. During the 80% methanol rinse, the cartridge was positioned at ‘SPE waste’ for the eluted mixture to be disposed. MeOH (2.0 mL, repeated 3 times) was used to wash the FSPE cartridge for

www.manaraa.com

eluting the desired compound. During the task, the FSPE cartridge was positioned as ‘SPE collect’ to be placed right above the 8 mL vial for collection of the sample. After the task, the position of the SPE rack was changed into ‘SPE direct’ for the needle to withdraw the collected sample from the conically-bottomed vial and deliver it to the clean reactor vial for the next reaction. Toluene (1.0 mL) was added to the solution and solvent was evaporated under reduced pressure. After the evaporation cycle, The MeOH wash step and the evaporation were repeated one more time. Once again toluene (1.0 mL) was added and removed under reduced pressure to remove residual water.

6. Oxidation

Dess-Martin periodinane in DCE (1.5 mL) was added to the sample and vortexed at 800 rpm, 50 °C under argon atmosphere for 45 min. After the reaction time the needle withdrew 20 μL of the solution from the reaction mixture and placed it into the third well of the microtiterplate for thin layer chromatography monitoring. The solvent was removed under reduced pressure.

7. Reduction

L-Selectride 1.0 M in THF (1.0 mL) was added to the sample and vortexed at 800 rpm, -20 °C under argon atmosphere for 45 min. After the reaction time the needle withdrew 20 μL of the solution from the reaction mixture and placed it into the third well of the microtiterplate for thin layer chromatography monitoring. MeOH (0.5 mL) was added to quench the reaction. The solvent was removed under reduced pressure.

www.manaraa.com

Part B: Mannuronate building block

1. Sample Preparation

Donor molecule (0.20 g, 0.30 mmol) was dissolved in anhydrous DCM (1.6 mL) in the 13 mL-vial and placed at the inert reagent rack (Donor) under argon atmosphere. A 0.055 M trimethylsilyltrifluoromethanesulfonate (TMSOTf) solution (5.0 mL) in anhydrous DCM was prepared in an 8 mL-vial and placed as indicated on the inert reagent rack under argon. MeOH (100 mL) was placed in the stock solution bottle at the stock solution station as indicated. Toluene (1.0 L) was placed in the stock solution bottle and placed at the reservoir bottle rack with tubing as reservoir solution for rinsing. Anhydrous DCM (20 mL) was transferred to the 50 mL-vial and placed as indicated on the inert reagent rack under argon. Anhydrous tetrahydrofuran (THF, 10 mL) was transferred to the 50 mL-vial and placed as indicated on the inert reagent rack under argon. 80% methanol/water (100 mL) stock was prepared in the 100 mL-vial and placed as indicated on the inert reagent rack. F-tagged acceptor molecule (50 μmol) was dissolved with anhydrous DCM (0.8 mL) in a Wheaton 8 mL-E-Z extraction vial (conically-bottomed) flushed with argon, capped with septa and placed at the reagent rack as indicated. Triethylamine (TEA, 5.0 mL) was transferred to an 8 mL-vial capped with septa and placed at the reagent rack as indicated. *N*,*N*-dimethylformamide (DMF, 8.0 mL) was transferred to an 8 mL-vial capped with septa and placed at the reagent rack as indicated. A 0.45 M CAN (ceric ammonium nitrate) solution in acetonitrile/water (9/1) (5 mL) was prepared in an 8 mL-vial capped with septa and placed at the reagent rack as indicated. LiTEBH solution in THF (1.0 M, 3.0 mL) was transferred to an 8 mL-vial capped with septa and placed at the reagent rack as indicated. Water (8.0 mL) was transferred to an 8 mL-vial capped with septa and placed at the reagent rack as indicated. A

www.manaraa.com

fluorous solid phase extraction (FSPE) cartridge (2 g, 10 cc) was preconditioned with 80% methanol/water and placed on the machined FSPE block as indicated. An empty Wheaton 8 mL-E-Z extraction vial was placed under the FSPE cartridge.

2. Cleaning cycle (see part A)

3. Glycosylation

The needle transferred the acceptor molecule (F-tag) solution (0.8 mL) to the reaction vial 1, followed by the transfer of the donor molecule solution (0.8 mL). The mixture was vortexed under ambient temperature at 800 rpm for 20 min. Then the reactor vials were cooled to -20 °C during the 60 minutes wait time by the heat transfer oil with 800 rpm vortex rate. The TMSOTf solution (0.1 mL) was transferred into the reactor vial 1 under 200 rpm vortex rate. After each individual transfer, the needle (inside and outside) was rinsed by toluene (2 ml) before operating the next task. The reaction mixture was vortexed at 800 rpm for 30 minutes at 0 °C under argon atmosphere. After the reaction time the needle withdrew 20 μL of the solution from the reaction mixture and placed it into the first well of the microtiterplate for thin layer chromatography monitoring. TEA (0.05 mL) was added to the solution for quenching and the solvent was evaporated under reduced pressure.

4. PMB deprotection

To the dried residue after the glycosylation, The CAN solution (1.0 mL) was added to the reactor vial. The reaction mixture was vortexed at 800 rpm for 1 h at room temperature. After the reaction time the needle withdrew 20 μL of the solution from the reaction mixture

www.manaraa.com

and placed it into the second well of the microtiterplate for thin layer chromatography monitoring.

5. Fluorous solid-phase extraction (FSPE)

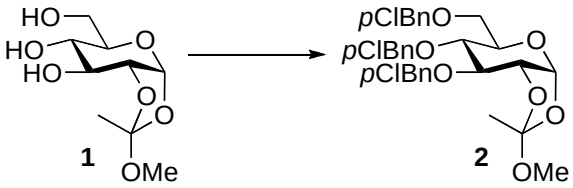
The reaction mixture (1.2 mL) was carried to the FSPE cartridge at the FSPE block and dispensed at a speed of 1 mL/s via the 10 mL syringe. Then 80% methanol (2.0 mL) was used to rinse the empty reactor vial. The 80% methanol solution was removed from the reactor vial and delivered to the FSPE cartridge. The 80% methanol rinsing and transferring was repeated one more time. Additional 80% methanol solution (4.0 mL, repeated 2 times) was used to rinse the FSPE cartridge. During the 80% methanol rinse, the cartridge was positioned at 'SPE waste' for the eluted mixture to be disposed. MeOH (2.0 mL, repeated 3 times) was used to wash the FSPE cartridge for eluting the desired compound. During the task, the FSPE cartridge was positioned as 'SPE collect' to be placed right above the 8 mL vial for collection of the sample. After the task, the position of the SPE rack was changed into 'SPE direct' for the needle to withdraw the collected sample from the conically-bottomed vial and deliver it to the clean reactor vial for the next reaction. Toluene (1.0 mL) was added to the solution and solvent was evaporated under reduced pressure. After the evaporation cycle, The MeOH wash step and the evaporation were repeated one more time. Once again toluene (1.0 mL) was added and removed under reduced pressure to remove residual water.

7. LiTEBH Reduction

www.manaraa.com

Anhydrous THF (1.0 mL) was added to the sample and vortexed at 800 rpm, 0 °C under argon atmosphere for 30 min. The LiTEBH (0.3 mL, 1.0 M in THF) was added to the reaction solution and the mixture was vortexed for 30 min at 800 rpm, 0 °C under argon atmosphere. After the reaction time the needle withdrew 20 μL of the solution from the reaction mixture and placed it into the fourth well of the microtiterplate for thin layer chromatography monitoring. MeOH (0.5 mL) was added to quench the reaction and the mixture was transferred out of the ASW.

Synthetic procedures



3, 4, 6-tri-*O*-*p*-chlorobenzyl-α-D-glucopyranose 1,2-(methyl orthoacetate) (2)

α-D-glucopyranose 1,2-(methyl orthoacetate) (**1**) (1.42 g, 6.00 mmol) was dissolved in anhydrous DMF (20 mL) and 60% sodium hydride in mineral oil (1.20 g, 30.0 Significant figures: check throughout mmol) and *p*-chlorobenzyl chloride (3.86 g, 24.0 mmol) were added slowly at 0 °C under argon atmosphere. The reaction was allowed to warm to ambient temperature and stirred for 20 h. The reaction mixture was quenched with water (20 mL) at 0 °C. The mixture was extracted with EtOAc (3 x 30 mL). The combined organic layers were washed with brine (30 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure and the crude product was purified by flash column chromatography on silica gel using EtOAc/petroleum ether/triethylamine (3/7/0.1) as eluent. The product was obtained as a yellow syrup (3.15 g, 5.16 mmol, 86%).

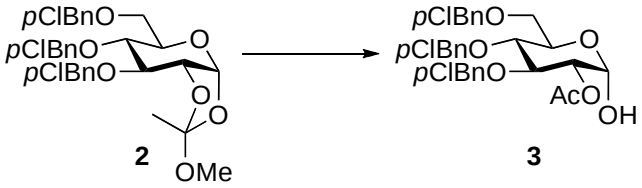
www.manaraa.com

***R*$_f$:** 0.27 (EtOAc/petroleum ether: 3/7)

^{1}H NMR (CDCl$_3$, 400MHz): δ 7.20-7.30 (m, 10H, H$_{arom}$), 7.08 (d, 2H, *J* = 8.4 Hz, H$_{arom}$) 5.75 (d, 2H, *J* = 5.2 Hz, H-1), 4.69 (d, 1H, *J* = 12 Hz), 4.51-4.56 (m, 3H), 4.44 (d, 1H, *J* = 12.4 Hz), 4.37-4.39 (dd, 1H, *J* = 5.2, 4 Hz, H-2), 4.36, (d, 1H, *J* = 11.6 Hz), 3.78-3.80 (dd, 1H, *J* = 4.4, 4.0 Hz, H-3), 3.73-3.77 (m, 1H, *J* = 9.6, 3.2, 2.8 Hz, H-5), 3.62-3.65 (m, 3H), 3.27 (s, 3H), 1.63 (s, 3H).

^{13}C NMR (CDCl$_3$, 400 MHz): δ 136.5, 136.3, 136.1, 133.5, 133.4, 133.2, 129.2, 129.0, 129.0, 128.5, 128.4, 121.3, 97.7, 79.0, 77.6, 77.2, 76.9, 75.9, 74.7, 72.5, 72.1, 70.9, 70.4, 69.0, 50.4, 21.4.

HRMS (ESI): $[M + Na]^+$ calcd for $C_{30}H_{31}Cl_3NaO_7^+$ 631.1028, found 631.1035



2-*O*-acetyl-3,4,6-tri-*O*-*p*-chlorobenzyl-α-D-glucopyranose (3)

3, 4, 6-Tri-*O*-*p*-chlorobenzyl-α-D-glucopyranose 1,2-(methyl orthoacetate) (**2**) (2.00 g, 3.28 mmol) was dissolved in dichloromethane (35 mL); a 2 N HCl solution (30 mL) was then added. The reaction mixture was stirred at ambient temperature for 2 h. The organic portion was separated and washed with a saturated $NaHCO_3$ solution (2 x 30 mL). The organic layer was dried over Na_2SO_4. The solvent was removed under reduced pressure to leave the product as white crystals (1.95 g, 3.28 mmol, 100%).

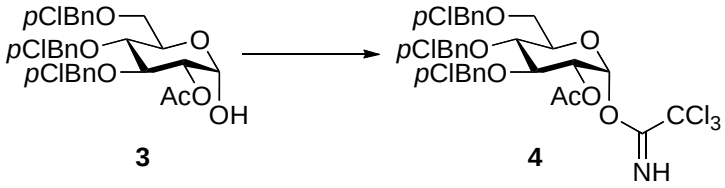
***R*$_f$:** 0.71 (EtOAc/petroleum ether: 2/1)

www.manaraa.com

^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.21-7.29 (m, 10H, H_{arom}), 7.04 (d, 2H, *J* = 8.4 Hz, H_{arom}), 6.20 (d, 1H, *J* = 3.6 Hz, H-1), 4.84 (d, 1H, *J* = 11.6 Hz), 4.79 (d, 1H, *J* = 11.6 Hz), 4.71 (d, 1H, *J* = 11.2 Hz), 4.57 (d, 2H, *J* = 12.4 Hz), 4.47 (d, 1H, *J* = 11.2 Hz), 4.43 (d, 1H, *J* = 12.4 Hz), 3.80-3.82 (m, 1H, H-2), 3.73-3.78 (t, 1H, *J* = 9.2, 8.8 Hz), 3.64-3.71 (m, 2H), 3.59-3.62 (dd, 1H, *J* = 10.8, 2.0 Hz), 2.26 (s, 1H), 2.12 (s, 3H).

^{13}C NMR ($CDCl_3$, 100 MHz): δ 170.0, 137.0, 136.4, 136.2, 133.5, 133.5, 133.4, 129.2, 129.1, 129.0, 128.9, 128.8, 128.6, 92.1, 82.2, 77.5, 77.2, 76.9, 74.5, 74.1, 72.9, 72.7, 71.5, 68.1, 21.1

HRMS (ESI): $[M + Na]^+$ calcd for $C_{29}H_{29}Cl_3NaO_7^+$ 617.0871, found 617.0859



2-*O*-acetyl-3,4,6-tri-*O*-*p*-chlorobenzyl-α-D-glucopyranose trichloroacetimidate (4)

To a solution of 2-*O*-acetyl-3,4,6-tri-*O*-*p*-chlorobenzyl-α-D-glucopyranose (**3**) (0.50 g, 0.84 mmol) in dichloromethane (29 mL) was added trichloroacetonitrile (0.72 g, 5.0 mmol) at 0 °C under argon atmosphere. DBU (0.05 g, 0.33 mmol) was then added and the reaction mixture was stirred at 0 °C under argon atmosphere for 3 h. The solvent was removed under reduced pressure and the crude product was purified by flash column chromatography on silica gel using EtOAc/petroleum ether/triethylamine (3/7/0.1) as eluent. The product was obtained as a yellow syrup (0.55 g, 0.74 mmol, 88%).

***R_f*:** 0.50 (EtOAc/petroleum ether: 3/7)

www.manaraa.com

^{1}H NMR ($CDCl_3$, 400MHz): δ 8.64 (s, 1H, N*H*), 7.23-7.29 (m, 8H, H_{arom}), 7.19 (d, 2H, *J* = 8.0 Hz, H_{arom}), 7.08 (d, 2H, *J* = 8.4 Hz, H_{arom}), 5.76 (d, 1H, *J* = 8.0 Hz, H-1), 5.27 (t, 1H, *J* = 8.0 Hz, H-2), 4.46-4.71 (m, 6H), 3.81 (t, 1H, *J* = 9.2), 3.63-3.73 (m, 4H), 1.96 (s, 3H)

^{13}C NMR ($CDCl_3$, 100 MHz): δ 169.2, 161.3, 136.5, 136.3, 133.8, 133.7, 133.5, 129.3, 129.2, 129.1, 128.7, 128.6, 96.1, 90.7, 82.6, 77.2, 75.9, 74.1, 74.0, 72.7, 71.8, 68.1, 20.9.

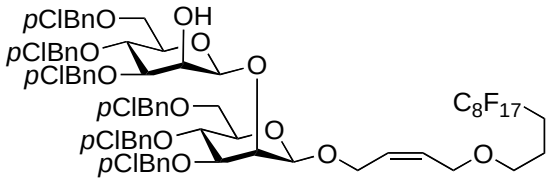
HRMS (ESI): $[M + Na]^+$ calcd for $C_{31}H_{29}Cl_6NNaO_7^+$ 759.9967, found 759.9971

www.manaraa.com

Table 1. Automated synthetic cycles for production of compound **5**

Step	Task	Reagent/ Operation	Operation Time
1	Glycosylation	2.0 equivalent donor (100 μmol) in 0.8 mL DCE, 1.0 equivalent F-tagged acceptor (50 μmol) in 0.8 mL DCE, 0.1 equivalent TMSOTf, 0 °C	45 min
2	TLC sample	20 μL of crude reaction mixture withdrawn	
3	Quenching	0.05 mL TEA	
4	Evaporation	40 °C	45 min
5	Deacetylation	0.4 mL NaOMe/MeOH, 25 °C	1 h
6	TLC sample	20 μL of crude reaction mixture withdrawn	
7	Quenching	0.5 mL AcOH/MeOH, 1 mL toluene	
8	Evaporation	40 °C	45 min
9	FSPE preparation	0.4 mL DMF	
10	Sample loading	0.5 mL crude sample transferred to cartridges	
11	Wash	12 mL 80% methanol wash	
12	Wash	2.0 mL MeOH wash (repeated 3 × 2 times)	
13	Transfer	6.0 mL collected sample to the reactor vial for next cycle	
14	Evaporation	40 °C	45 min
15	Oxidation	1.5 mL DMP in DCE, 50 °C	45 min
16	Evaporation	40 °C	45 min
17	FSPE preparation	0.4 mL DMF	
18	Sample loading	0.5 mL crude sample transferred to cartridges	
19	Wash	12 mL 80% methanol wash	
20	Wash	2.0 mL MeOH wash (repeated 3 × 2 times)	
21	Transfer	6.0 mL collected sample to the reactor vial for next cycle	
22	Evaporation	40 °C	45 min
23	Reduction	1.0 mL THF, 1.0 mL L-Selectride, -20 °C	45 min
24	Quenching	0.5 mL MeOH	
25	Evaporation	40 °C	45 min
25	FSPE preparation	0.4 mL DMF	
26	Sample loading	0.5 mL crude sample transferred to cartridges	
27	Wash	12 mL 80% methanol wash	
28	Wash	2.0 mL MeOH wash (repeated 3 × 2 times)	
29	Transfer	6.0 mL collected sample to the reactor vial for next cycle	
30	Evaporation	40 °C	45 min
31	Glycosylation	2.0 equivalent donor (100 μmol) in 0.8 mL DCE, 0.8 mL DCE, 0.1 equivalent TMSOTf, 0 °C	45 min
32	Repeat the same procedure as step 2-28	Repeat 1 cycle (50 °C for the deacetylation step)	9 h 15 min
33	Transfer	6.0 mL solution transferred out of the synthesis platform	

www.manaraa.com



5

***cis*-4-(1H,1H,2H,2H,3H,3H-perfluoroundecyloxy)-2-butenyl (3,4,6-tri-*O*-*p*-chlorobenzyl-2-*O*-(3,4,6-tri-*O*-*p*-chlorobenzyl-β-D-mannopyranosyl)-β-D-mannopyranoside (5)**

After the 2nd cycle (33rd step) of the automated synthesis, the mixture was transferred out of the synthesis platform and purified by flash column chromatography on silica gel using EtOAc/petroleum ether (1/2) as eluent. The product was obtained as a colorless syrup (6.5 mg, 4.0 μmol, 8% over 8 steps, 73% per step)

***R**f*: 0.31 (EtOAc/petroleum ether: 1/2)

^{1}H NMR ($CDCl_3$, 400MHz): δ (ppm) 7.31-7.00 (m, 24H, H_{arom}), 5.76 (m, 2H, *H*C=C*H*), 4.90 (s, 1H, H-1), 4.87-4.73 (m, 4H, CH*H*Ph), 4.60-4.44 (m, 5H), 4.41 (s, 1H, H-1), 4.40-4.28 (m, 6H), 4.23 (dd, 1H, *J* = 12.8, 8.0 Hz, O-CH*H*C=C), 4.00 (d, *J* = 6.4 Hz, C=CCH_2-O), 3.90 (t, *J* = 9.2 Hz), 3.77-3.65 (m, 4H), 3.60 (dd, J = 10.0, 5.2 Hz), 3.52 (m, 2H), 3.46 (m, 3H), 3.38 (m, 1H), 2.68 (s, 1H, O*H*), 2.22 (m, 2H, C$H_2$$CF_2$), 1.88 (m, 2H, O-$CH_2CH_2$)

^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 135.9, 136.8, 136.7, 136.5, 136.4, 133.7, 133.7, 133.6, 130.4, 129.6, 129.4, 129.4, 129.2, 129.2, 129.1, 128.7, 128.7, 128.7, 128.6, 128.1, 99.6 (C-1, $J_{C1\text{-}H1}$ = 154.7 Hz), 99.3 (C-1, $J_{C1\text{-}H1}$ = 159.0 Hz), 81.6, 80.6, 75.5, 75.0, 74.3, 74.1, 72.8, 72.7, 70.7, 70.1, 69.8, 69.5, 69.2, 69.0, 67.7, 66.6, 64.7, 28.3 (t, $J_{C\text{-}F}$ = 22.1), 21.0

HRMS (ESI): $[M + Na]^+$ calcd for $C_{69}H_{63}Cl_6F_{17}NaO_{12}^+$ 1641.2047, found 1641.2074

HPLC trace of the automated synthesis of **5**

www.manaraa.com

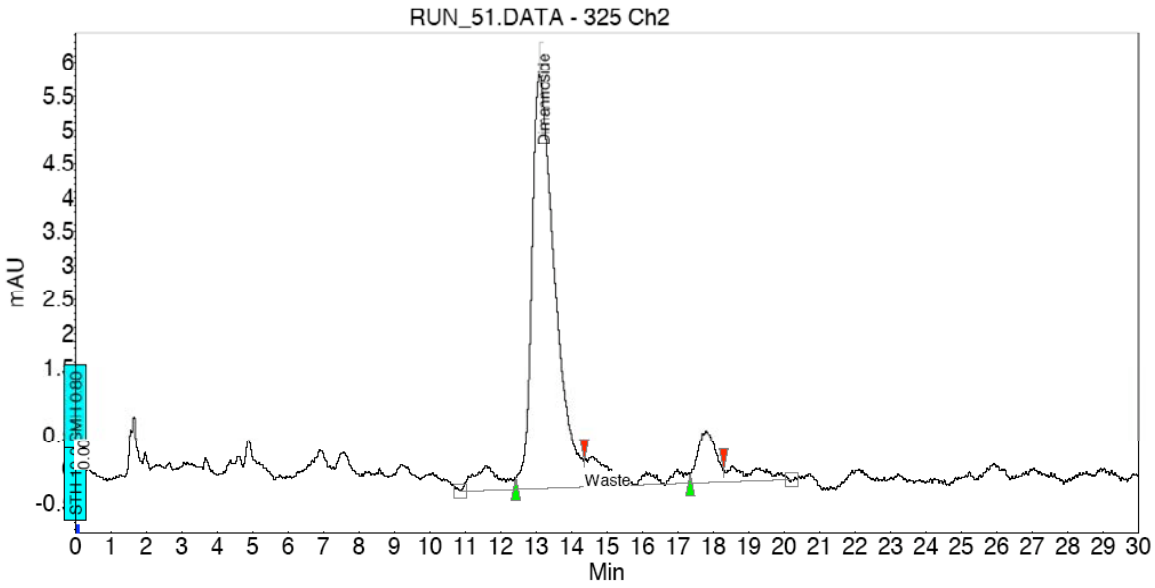
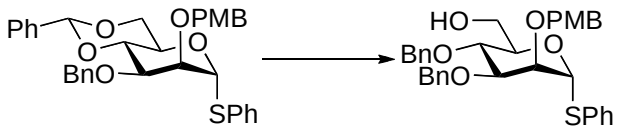


Figure 5. Analytical HPLC chromatogram of the crude product from the synthesis platform after FSPE (1.0 mL/min flowrate, EtOAc/hexanes: 1/4, 15 minutes run, Waters Nova-Pak 4 μm 3.9 × 150 mm silica column)



7 **8**

Phenyl 2-*O*-*p*-methoxybenzyl-3,4-di-*O*-benzyl-thio-α-D-mannopyranoside (8)

To the phenyl 2-*O*-*p*-methoxybenzyl-3-*O*-benzyl-4,6-*O*-benzylidene-thio-α-D-mannopyranoside (**7**) (1.4 mmol) was added borane terahydrofuran complex 1.0 M solution in tetrahydrofuran (14 mL, 14.0 mmol) under argon atmosphere at 0 °C and stirred until the starting material was dissolved. Then the dibutylboryl trifluoromethanesulfonate 1.0 M solution in DCM (1.7 mL, 1.70 mmol) was added dropwise and the reaction mixture was stirred under argon atmosphere at 0 °C for 3 h. Triethylamine (0.3 mL, 2.15 mmol) was

www.manaraa.com

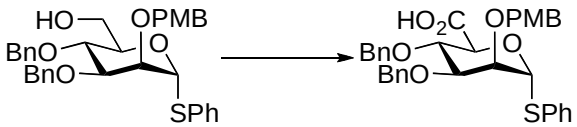
added dropwise to the reaction then methanol was added slowly to quench the reaction under 0 °C. The solvent was removed under reduced pressure and the crude mixture was coevaporated with methanol twice. The crude product was purified by flash column chromatography on silica gel using EtOAc/petroleum ether (1/2) as eluent. The product was obtained as a light yellow syrup (0.69 g, 1.20 mmol, 86%)

***R*$_f$:** 0.14 (EtOAc/petroleum ether: 1/3)

^{1}H NMR (CDCl$_3$, 400 MHz): δ (ppm) 7.40-7.25 (m, 17H, H$_{arom}$), 6.85 (dd, 2H, *J* = 6.8, 2.0 Hz, H$_{arom}$), 5.47 (d, 1H, *J* = 1.6 Hz, H-1), 4.97 (d, 1H, *J* = 10.8 Hz), 4.67-4.57 (m, 5H), 4.13-4.09 (m, 1H, H-5), 4.04 (d, 1H, *J* = 9.2Hz), 3.99 (dd, 1H, *J* = 3.2, 2.0 Hz, H-2), 3.89 (dd, 1H, J = 9.2, 2.8 Hz, H-3), 3.83-3.79 (m, 5H)

^{13}C NMR (CDCl$_3$, 100 MHz): δ (ppm) 159.4, 138.4, 138.2, 134.1, 131.8, 129.8, 129.7, 129.1, 128.4, 128.1, 127.8, 127.7, 125.6, 113.8, 86.1, 80.0, 75.8, 75.3, 74.7, 73.4, 72.1, 72.0, 62.0, 55.2

HRMS (ESI): $[M + Na]^+$ calcd for $C_{34}H_{36}NaO_6S^+$ 595.2125, found 595.2083



8 **9**

Phenyl 2-*O*-*p*-methoxybenzyl-3,4-di-*O*-benzyl-thio-α-D-mannopyranosiduronic acid (9)

To a solution of phenyl 2-*O*-*p*-methoxybenzyl-3,4-di-*O*-benzyl-thio-α-D-mannopyranoside (**8**) (0.50 g, 0.87 mmol) in dichloromethane/water (5.8 mL/2.9 mL) were added TEMPO (0.03 g, 0.19 mmol) and (diacetoxyiodo)benzene (0.70 g, 2.17 mmol) and stirred at ambient temperature. After 45 min, the mixture was diluted with dichloromethane (10 mL) and

www.manaraa.com

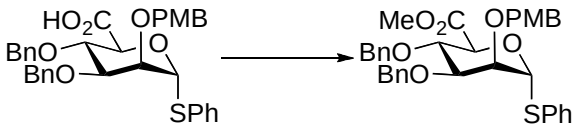
washed with a 10% $Na_2S_2O_3$ solution (10 mL) and water (10 mL). The organic layer was dried over Na_2SO_4. The solvent was removed under reduced pressure and the crude product was purified by flash column chromatography on silica gel using EtOAc/petroleum ether (1/1 → 1/0) as eluent. The product was obtained as a light yellow syrup (0.39 g, 0.66 mmol, 76%).

***R_f*:** 0.11 (EtOAc/petroleum ether: 1/1)

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.50 (s, 1H, H_{arom}), 7.48 (s, 1H, H_{arom}), 7.35-7.21 (m, 15 H, H_{arom}), 6.85 (d, 2H, *J* = 12.0 Hz, H_{arom}), 5.63 (d, 1H, *J* = 4.8 Hz, H-1), 4.74-4.46 (m, 7H), 4.22 (t, 1H, *J* = 7.2 Hz), 3.91 (dd, 1H, *J* = 4.4, 2.8 Hz, H-2), 3.81 (d, 1H, *J* = 3.2 Hz), 3.79 (s, 1H)

^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 174.5, 159.4, 137.8, 137.7, 133.8, 131.4, 129.9, 129.8, 129.1, 128.5, 128.5, 128,0, 128.0, 127.9, 127.9, 127.4, 113.9, 84.1, 75.7, 74.3, 73.8, 72.5, 72.4, 72.0, 55.3, 29.8

HRMS (ESI): $[M + Na]^+$ calcd for $C_{34}H_{34}NaO_7S^+$ 609.1917, found 609.1912



9 **10**

Methyl (phenyl 2-*O*-*p*-methoxylbenzyl-3,4-di-*O*-benzyl-thio-α-D-mannopyranoside) uronate (10)

To a solution of phenyl 2-*O*-*p*-methoxybenzyl-3,4-di-*O*-benzyl-thio-α-D-mannopyranosiduronic acid (**9**) (0.5 g, 0.85 mmol) in anhydrous DMF (4 mL) were added K_2CO_3 (0.12 g, 0.85 mmol) and iodomethane (0.3 g, 2.13 mmol). The reaction mixture was

www.manaraa.com

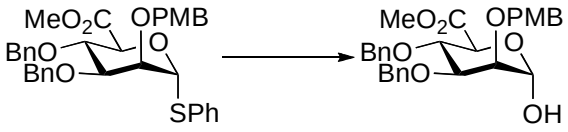
stirred at ambient temperature under argon atmosphere for 6 h. The mixture was diluted with EtOAc (10 mL) and washed with water (10 mL). The aqueous portion was separated and extracted with EtOAc (2 x 10 mL). The combined organic layer was dried over Na_2SO_4. The solvent was removed under reduced pressure and the crude product was purified by flash column chromatography on silica gel using EtOAc/petroleum ether (1/3) as eluent. The product was obtained as a light yellow syrup (0.44 g, 0.74 mmol, 87%).

***R_f*:** 0.80 (EtOAc/petroleum ether: 1/1)

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.54 (d, 2H, *J* = 6.4 Hz, H_{arom}), 7.34-7.21 (m, 15H, H_{arom}), 6.84 (d, 2H, *J* = 8.4 Hz, H_{arom}), 5.65 (d, 1H, *J* = 5.2 Hz, H-1), 4.66-4.59 (m, 4H), 4.55 (s, 2H), 4.48 (d, 1H, *J* = 11.6 Hz), 4.26 (t, 1H, J = 6.4 Hz, H-3), 3.90 (dd, 1H, *J* = 5.6, 3.2 Hz, H-2), 3.79-3.77 (m, 4H), 3.65 (s, 3H)

^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 169.7, 159.4, 138.0, 137.9, 134.0, 131.4, 129.8, 129.8, 129.0, 128.4, 128.4, 127.9, 127.8, 127.8, 127.3, 113.8, 84.1, 76.0, 74.3, 73.7, 73.0, 72.4, 72.0, 55.5, 55.3, 55.2, 55.0, 52.2

HRMS (ESI): $[M + Na]^+$ calcd for $C_{35}H_{36}NaO_7S^+$ 623.2074, found 623.2069



Methyl (2-*O*-*p*-methoxylbenzyl-3,4-di-*O*-benzyl-α-D-mannopyranose) uronate (11)

To a solution of methyl (phenyl 2-*O*-*p*-methoxylbenzyl-3,4-di-*O*-benzyl-thio-α-D-mannopyranoside) uronate (**10**) (0.50 g, 0.83 mmol) in 10% water/acetone (13 mL) were added *N*-bromosuccinimide (1.33 g, 7.47 mmol) and $NaHCO_3$ (1.37 g, 16.3 mmol). The

www.manaraa.com

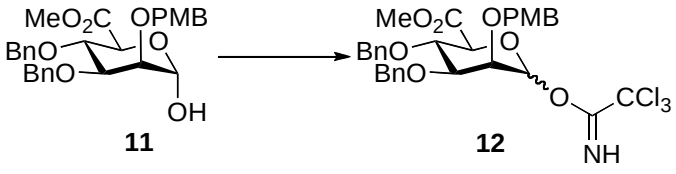
reaction mixture was stirred at ambient temperature for 1 h. The reaction mixture was dilute with EtOAc (30 mL) and washed with a saturated $NaHCO_3$ solution (30 mL). The organic layer was dried over Na_2SO_4. The solvent was removed under reduced pressure and the crude product was purified by flash column chromatography on silica gel using EtOAc/petroleum ether (2/3) as eluent. The product was obtained as a light yellow syrup (0.37 g, 0.72 mmol, 87%).

***R_f*:** 0.52 (EtOAc/petroleum ether: 1/1)

^{1}H NMR ($CDCl_3$, 400MHz): δ 7.25-7.33 (m, 12H, H_{arom}), 6.85 (d, 2H, *J* = 8.4 Hz, H_{arom}), 5.39 (s, 1H, H-1), 4.56-4.73 (m, 6H), 4.46 (d, 1H, *J* = 6.4 Hz, H-5), 4.21 (t, 1H, *J* = 6.8 Hz, H-4), 3.87-3.90 (dd, 1H, *J* = 6.4, 2.8 Hz, H-3), 3.79 (s, 3H), 3.71-3.73 (dd, 1H, *J* = 4.0, 3.2 Hz, H-2), 3.65 (s, 3H), 3.09 (s, 1H).

^{13}C NMR ($CDCl_3$, 100 MHz): δ 170.3, 159.2, 138.2, 138.1, 130.3, 130.0, 129.6, 128.6, 128.4, 128.4, 128.0, 127.9, 127.8, 127.7, 127.6, 114.0, 113.8, 92.8 ($J_{C1\text{-}H1}$ = 167.4 Hz, C-1), 77.8, 75.9, 74.8, 74.0, 72.4, 72.3, 72.1, 55.3, 52.3.

HRMS (ESI): $[M + Na]^+$ calcd for $C_{29}H_{32}NaO_8^+$ 531.1989, found 531.1988



Methyl (2-*O*-*p*-methoxylbenzyl-3,4-di-*O*-benzyl-α/β-D-mannopyranose) uronate trichloroacetimidate (12)

To a solution of methyl (2-*O*-*p*-methoxylbenzyl-3,4-di-*O*-benzyl-α-D-mannopyranose) uronate (0.50 g, 0.98 mmol) in dichloromethane (35 mL) was added trichloroacetonitrile (**11**)

www.manaraa.com

(0.85 g, 5.88 mmol) at 0 °C under argon atmosphere. 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU) (0.03 g, 0.2 mmol) was then added and the reaction mixture was stirred at 0 °C under argon atmosphere for 3 h. The solvent was removed under reduced pressure and the crude product was purified by flash column chromatography on silica gel using EtOAc/petroleum ether/triethylamine (1/1/0.1) as eluent. The product was obtained as a mixture of anomers (α/β = 4/1) (0.57 g, 0.87 mmol, 89%).

***R_f*:** 0.40 (EtOAc/petroleum ether: 1/3)

^{1}H NMR ($CDCl_3$, 400MHz): δ (ppm) 9.32 (s, 1H, N*H*), 8.62 (s, 1H, N*H*), 7.35-7.22 (m, 12H), 6.87 (d, 2H, *J* = 2.1 Hz), 6.39 (d, 1H, *J* = 2.4 Hz, Ha-1), 5.96 (d, 1H, *J* = 8.0 Hz, Hb-1), 4.83 (d, 1H, *J* = 10.8 Hz), 4.75 (d, 1H, *J* = 12.0 Hz), 4.66 (s, 1H), 4.63-4.48 (m, 6H), 4.39 (d, 1H, *J* = 8.4 Hz, Ha-4), 4.29 (t, 1H, *J* = 8.8, 8.0 Hz, Ha-3), 4.18 (dd, 1H, *J* = 4.4, 2.0 Hz), 3.91-3.84 (m, 2H), 3.81 (s, 3H), 3.71 (s, 3H), 3.62 (s, 3H)

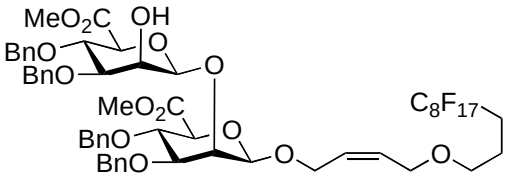
^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 169.4, 169.1, 160.5, 159.5, 159.0, 138.0, 138.0, 137.4, 137.2, 130.1, 129.9, 129.8, 128.7, 128.6, 128.5, 128.3, 128.1, 128.0, 127.9, 113.9, 95.8, 95.2, 90.9, 77.8, 75.9, 75.7, 75.5, 75.0, 74.3, 74.1, 73.2, 73.2, 73.1, 72.9, 72.6, 55.4, 52.7, 52.6

HRMS (ESI): $[M + Na]^+$ calcd for $C_{31}H_{32}Cl_3NNaO_8^+$ 674.1086, found 674.1085

www.manaraa.com

Table 2. Automated synthetic cycles for production of compound **13**

Step	Task	Reagent/ Operation	Operation Time
1	Glycosylation	3.0 equivalent donor (150 μmol) in 0.8 mL DCM, 1.0 equivalent F-tagged acceptor (50 μmol) in 0.8 mL DCM, 0.1 equivalent TMSOTf, -20 °C	30 min
2	TLC sample	20 μL of crude reaction mixture withdrawn	
3	Quenching	0.05 mL TEA	
4	Evaporation	40 °C	45 min
5	Deprotection	1.0 mL CAN/MeCN/H_2O, 25 °C	1 h
6	TLC sample	20 μL of crude reaction mixture withdrawn	
7	Sample loading	1.0 mL crude sample transferred to cartridges	
8	Wash	12 mL 80% methanol wash	45 min
9	Wash	2.0 mL MeOH wash (repeated 3 × 2 times)	
10	Transfer	6.0 mL collected sample to the reactor vial for next cycle	
11	Evaporation	40 °C	45 min
12	Glycosylation	3.0 equivalent donor (150 μmol) in 0.8 mL DCM, 0.8 mL DCM, 0.1 equivalent TMSOTf, 0 °C	30 min
13	Repeat the same procedure as step 2-15	Repeat 1 cycle	4 h 25 min
14			
15	Transfer	6.0 mL solution transferred out of the synthesis platform	



13

***cis*-4-(1H,1H,2H,2H,3H,3H-perfluoroundecyloxy)-2-butenyl (methyl 3,4-di-*O*-benzyl-4-*O*-(methyl 3,4-di-*O*-benzyl-β-D-mannopyranosyl uronate)-β-D-mannopyranoside uronate) (13)**

After the 2nd cycle (17th step) of the automated synthesis, the mixture was transferred out of the synthesis platform and purified by flash column chromatography on silica gel using diethyl ether/benzene (1/4) as eluent. The product was obtained as a colorless syrup (24.5 mg, 19 μmol, 38% over 4 steps, 79% per step)

***R_f*:** 0.19 (diethyl ether/benzene = 1/4 developed for 3 times)

www.manaraa.com

^{1}H NMR ($CDCl_3$, 400MHz): δ (ppm) 7.41-7.22 (m, 20H, H_{arom}), 5.77-5.57 (m, 2H, *H*C=C*H*), 4.95 (s, 1H, C*H*HPh), 4.92 (s, 1H, H-1), 4.88-4.82 (m, 3H, C*H*HPh), 4.69-4.66 (m, 2H, C*H*HPh), 4.61 (d, 1H, *J* = 10.8 Hz, C*H*HPh) 4.48-4.43 (m, 2H, C*H*HPh, H-2), 4.46 (s, 1H, H-1), 4.37-4.33 (m, 2H, H-2, O-CH*H*C=C), 4.30 (t, 1H, *J* = 9.2 Hz, H-4), 4.21 (dd, 1H, *J* = 12.8, 7.6 Hz, O-C-H*H*C=C), 4.11 (t, 1H, *J* = 9.6 Hz, H-4), 4.01 (d, 2H, *J* = 6.4 Hz, C=CCH_2-O), 3.87 (d, *J* = 9.6 Hz, H-5), 3.80 (d, *J* = 9.6 Hz, H-5), 3.73 (s, 3H, CO_2CH_3), 3.61 (s, 3H, CO_2CH_3), 3.58 (m, 1H, H-3), 3.54 (m, 1H, H-3), 3.49 (m, 2H, OCH_2CH_2), 2.76 (s, 1H, O*H*), 2.25 (m, 2H, CH_2CF_2), 1.91 (m, 2H, OCH_2CH_2)

^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 168.8, 168.7, 138.4, 138.2, 138.0, 130.8, 128.7, 128.6, 128.5, 128.5, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.9, 127.7, 100.0 (J_{C1-H1} = 156.3 Hz), 99.8 (J_{C1-H1} = 166.9 Hz), 80.5, 79.6, 76.9, 75.4, 75.3, 75.1, 75.1, 71.0, 70.8, 70.8, 69.1, 67.7, 66.6, 64.9, 52.6, 52.4, 28.3 (t, J_{C-F} = 21.8 Hz), 21.0

HRMS (ESI): $[M + Na]^+$ calcd for $C_{57}H_{57}F_{17}NaO_{14}^+$ 1311.3369, found 1311.3357

HPLC trace of the automated synthesis of **13**

www.manaraa.com

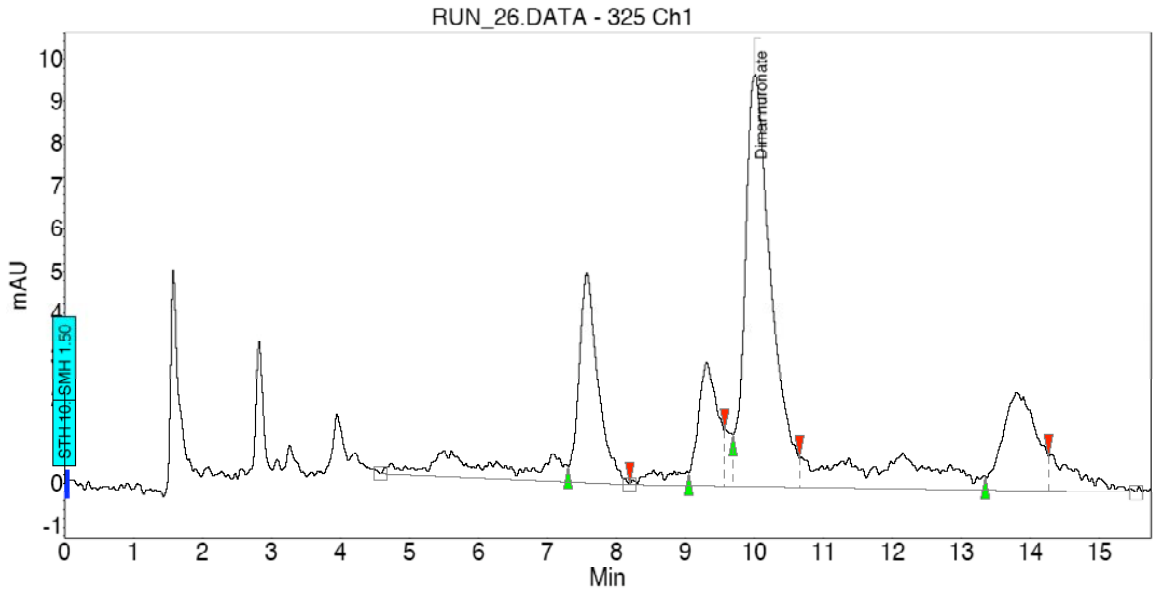
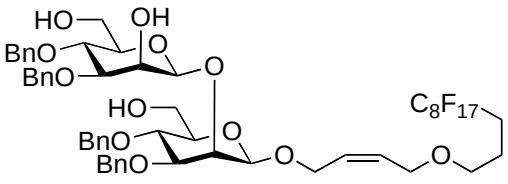


Figure 6. Analytical HPLC chromatogram of the crude product from the synthesis platform after FSPE (1.0 mL/min flowrate, EtOAc/hexanes: 1/4, 15 minutes run, Waters Nova-Pak 4 μm 3.9 × 150 mm silica column)

Table 3. Automated synthetic cycles for production of compound **14**

Step	Task	Reagent/ Operation	Operation Time
18	Transfer **7** to reactor vial	19 μmol in 3.0 mL EtOAc	
	Evaporation	40 °C	45 min
19	Reduction	1.0 mL THF, 0.3 mL LiTEBH, 0 °C	30 min
20	TLC sample	20 μL of crude reaction mixture withdrawn	
21	Quenching	0.5 mL sat. NH_4Cl	
22	Transfer	solution transferred out of the synthesis platform	



14

www.manaraa.com

***cis*-4-(1H,1H,2H,2H,3H,3H-perfluoroundecyloxy)-2-butenyl (3,4-di-*O*-benzyl-2-*O*-(3,4-di-*O*-benzyl-β-D-mannopyranosyl)-β-D-mannopyranoside (14)**

After the 3rd cycle (22nd step) of the automated synthesis, the mixture was transferred out of the synthesis platform, diluted with DCM (10 mL) and washed by sat NH_4Cl solution (10 mL). The organic layer was concentrated and the crude mixture was purified by FSPE. The product was obtained as a colorless syrup (23.4 mg, 19.0 μmol, quant.)

***R**_f*: 0.78 (MeOH/DCM = 1/9)

^{1}H NMR ($CDCl_3$, 400MHz): δ (ppm) 7.41-7.28(m, 20H, H_{arom}), 5.77-5.61 (m, 2H, *H*C=C*H*), 4.92-4.80 (m, 4H, C*H*HPh), 4.87 (s, 1H, H-1), 4.67-4.59 (m, 4H, C*H*HPh), 4.47 (s, 1H, H-1), 4.39 (dd, 1H, *J* = 12.8, 5.6 Hz, O-CH*H*C=C), 4.23-4.19 (m, 3H, O-CH*H*C=C, 2 × H-2), 4.00 (m, 2H, C=CHCH_2O), 3.97 (t, 1H, *J* = 8.4 Hz, H-4), 3.89 (t, 1H, *J* = 9.6 Hz, H-4), 3.85 (m, 2H, 2 × H-6), 3.71 (m, 3H, H-3, 2 × H-6), 3.60 (dd, *J* = 9.2, 2.8 Hz), 3.50-3.44 (m, 3H, H-5, OC$H_2$$CH_2$), 3.31 (m, 1H, H-3), 3.16 (d, 1H, *J* = 2.4 Hz, O*H*), 2.52 (s, 1H, O*H*), 2.23 (m, 1H, C$H_2$$CF_2$), 1.99 (s, 1H, O*H*), 1.89 (m, 2H, OCH_2CH_2)

^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 138.4, 138.3, 137.7, 130.4, 128.7, 128.6, 128.5, 128.5, 128.3, 128.2, 128.2, 128.1, 128.1, 128.0, 127.9, 127.9, 127.9, 127.8, 101.2 (J_{C1-H1} = 161.7 Hz), 99.5 (J_{C1-H1} = 157.2 Hz), 80.8, 80.7, 76.1, 76.0, 75.4, 74.9, 74.4, 74.2, 74.1, 71.7, 71.3, 69.0, 67.9, 66.6, 64.8, 62.3, 62.1, 28.3 (t, J_{C-F} = 22.6 Hz), 20.9

HRMS (ESI): $[M + Na]^+$ calcd for $C_{55}H_{57}F_{17}NaO_{12}^+$ 1255.3471, found 1255.3471

HPLC trace of the automated synthesis of **14**

www.manaraa.com

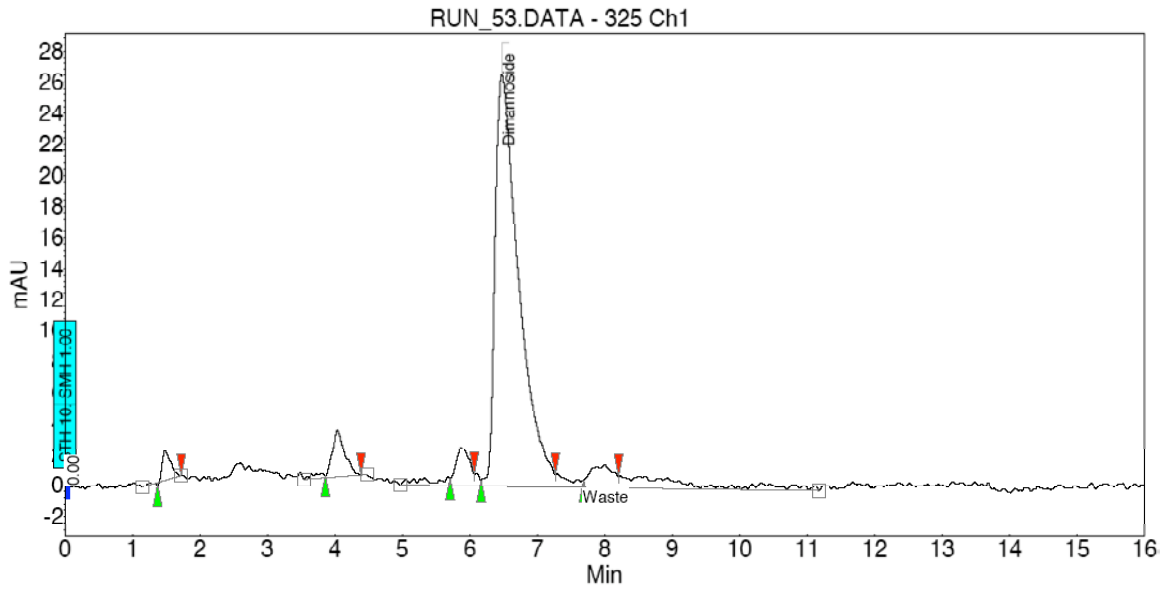
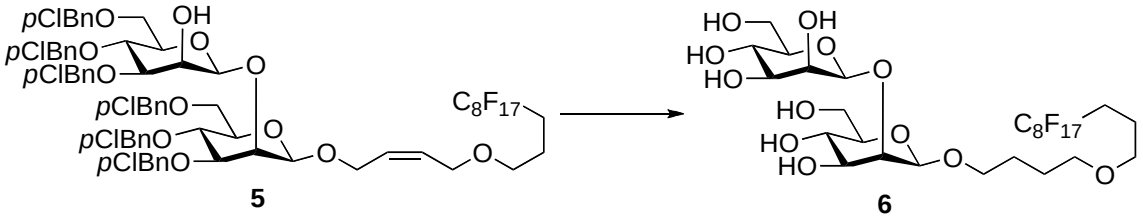


Figure 7. Analytical HPLC chromatogram of the crude product from the synthesis platform after FSPE (1.0 mL/min flowrate, EtOAc/hexanes: 1/4, 15 minutes run, Waters Nova-Pak 4 μm 3.9 × 150 mm silica column)

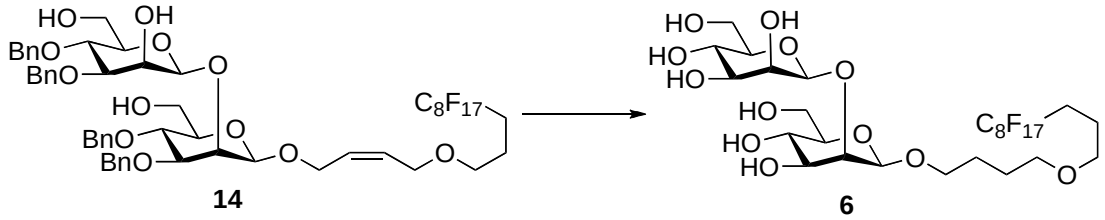


3-(perfluorooctyl)propanyloxybutanyl (2-*O*-(β-D-mannopyranosyl)-β-D-mannopyranoside) (6)

cis-4-(1H,1H,2H,2H,3H,3H-perfluoroundecyloxy)-2-butenyl (3,4,6-tri-*O*-*p*-chlorobenzyl-2-*O*-(3,4,6-tri-*O*-*p*-chlorobenzyl-β-D-mannopyranosyl)-β-D-mannopyranoside (**5**) (32.5 mg, 20.0 μmol), was dissolved in MeOH (2.0 mL) and 20% $Pd(OH)_2/C$ (11 mg) was added and stirred at 20 °C under 500 psi H_2 atmosphere. After 3 h, the mixture was filter through a small pad of silica gel and elute with MeOH. The collected solution was concentrated under

www.manaraa.com

reduced pressure and the product was obtained as a colorless syrup (15.2 mg, 17.4 μmol, 87%).



cis-4-(1H,1H,2H,2H,3H,3H-perfluoroundecyloxy)-2-butenyl (3,4-di-*O*-benzyl-2-*O*-(3,4-di-*O*-benzyl-β-D-mannopyranosyl)-β-D-mannopyranoside (**14**) (30.0 mg, 24.3 μmol) was dissolved in MeOH (2.0 mL) and 10% Pd/C (10 mg) was added and stirred 20 °C under 1000 psi H_2 atmosphere. After 20 h, the mixture was filter through a small pad of silica gel and elute with MeOH. The collected solution was concentrated under reduced pressure and the product was obtained as a colorless syrup (19.0 mg, 21.7 μmol, 89%)

***R**_f*: 0.45 (MeOH/DCM = 1/3)

^{1}H NMR (CD_3OD, 400MHz): δ (ppm) 4.79 (s, 1H, H-1), 4.59 (s, 1H, H-1), 4.13 (d, 1H, *J* = 3.2 Hz, H-2), 3.99 (d, 1H, *J* = 3.2 Hz, H-2), 3.97 (m, 1H), 3.90 (dd, 1H, *J* = 7.6, 2.0 Hz, H-6), 3.88 (dd, 1H, *J* = 8.0, 2.4 Hz, H-6), 3.74 (t, 1H, *J* = 6.0 Hz, H-6), 3.69 (t, 1H, *J* = 6.4 Hz, H-6), 3.58-3.46 (m, 8H), 3.41 (dd, 1H, *J* = 9.2, 3.2 Hz, H-3), 3.23-3.15 (m, 2H, 2 × H-5), 2.33 (m, 2H, CH_2CF_2), 1.90 (m, 2H, O-CH_2CH_2), 1.68 (t, 4H)

^{13}C NMR (CD_3OD, 100 MHz): δ (ppm) 102.3, 102.1, 79.1, 78.7, 78.6, 75.4, 74.6, 72.2, 71.8, 70.3, 69.1, 68.6, 62.9, 62.8, 29.2 (t, $J_{C\text{-}F}$ = 22.4 Hz), 27.7, 27.6, 22.0

HRMS (ESI): $[M + Na]^+$ calcd for $C_{27}H_{35}F_{17}NaO_{12}^+$ 897.1749, found 897.1756

www.manaraa.com

CHAPTER 3

Automated Solution-Phase Synthesis of β-1,4-Mannuronate and β-1,4-Mannan Oligomers

Shu-Lun Tang and Nicola L. B. Pohl*

Abstract

The first automated solution-phase synthesis of β-1,4-mannuronate and β-1,4-mannan oligomers has been accomplished by using the β-directing C-5 carboxylate strategy. By using the fluorous-tag assisting purification after the reaction cycles, the β-1,4-mannuronate was synthesized up to hexamer with relatively small amount of glycosyl donor (3.5 equiv.) for each glycosylation cycle compared to the automated solid-phase protocol due to the homogeneous solution-phase condition. After a global reduction of the esters, the β-1,4-mannan could be produced from the uronates and revealed a new method for the β-mannan synthesis.

Introduction

The β-mannosidic linkage is considered one of the most challenging glycosidic linkages to construct. Both steric hindrance from the 1,2-*cis* configuration and thermodynamic instability from the anomeric effect renders the β-anomer less favorable than the α-anomer (El Ashry et al. 2005). However, this linkage is crucial to a variety of natural oligosaccharides, including *N*-linked glycans (Schmaltz et al. 2011), antigenic bacterial glycans (Crich et al. 2001; Crich et al. 2004a), immunogenic fungal cell wall glycans (Nitz et al. 2000; Nitz and Bundle 2001; Wu and Bundle 2005), and antifreeze xylomannan (Walters

www.manaraa.com

et al. 2009; Crich and Rahaman 2011; Ishiwata et al. 2011). In addition, a variety of natural polysaccharides, such as alginates, contain the related *β*-mannuronic acid. Small oligomers of *β*-1,4-mannuronic acid have been found recently to have immunostimulatory properties by activation of the Toll-like receptors (TLR) 2 and 4 and induction of cytokine production (Flo et al. 2002; Janeway et al. 2002; Iwamoto et al. 2005). Structurally well-defined synthetic alginate fragments can therefore be potential therapeutic agents and useful tools to study the mechanism of TLR-mediated cytokine production. Also, *β*-1,4-mannan in the tagua palm seed, which is also called "ivory nut mannan" forms microfibrils similar to cellulose (Robyt 1998). Herein we report the first strategy for the automated solution-phase synthesis of this class of *β*-linked oligosaccharides and demonstrate the value of mannuronate building blocks as precursors for stereodefined *β*-mannose linkages.

A well-known strategy for the synthesis of *β*-mannosidic linkages has been developed by Crich and co-workers by using a conformationally-constrained 4,6-*O*-benzylidene-protected thiomannoside building block to provide high *β*-selectivity during the glycosylation (Crich 2010; Crich 2011). In fact, this approach was successfully implemented using automated solid-phase synthesis to incorporate up to two *β*-mannosidic linkages (Codee et al. 2008; Seeberger 2008; Hsu et al. 2011). After formation of the *β*-mannosidic bond, a series of protecting group shuffles, including removal of the 4,6-*O*-benzylidene acetal under acidic conditions and formation of the ester protecting group at C-6, must be carried out to provide a free 4-OH as an acceptor for further glycosylation. *β*-1,4-mannan oligomers up to a hexasaccharide were synthesized by this glycosylation-deprotection-esterification sequence (Crich et al. 2004a; Kim et al. 2008). However, a strategy that avoids the acidic deprotection conditions that can also cleave particularly acid-sensitive glycosidic bonds and avoids the

www.manaraa.com

extra protecting step could potentially shorten a synthetic route to *β*-1,4-mannan, make it more general, and render it more amenable to the automated synthesis of structures such as the *β*-mannans and *β*-1,4-mannuronate oligomers that contain more than two such challenging linkages.

To this end, we were inspired by the elegant recent work by van der Marel and co-workers in their construction of *β*-mannosidic linkages with excellent *β*-selectivity in the presence of a C-5 carboxylate. After the thio-mannuronate donor was activated by diphenyl sulfoxide and triflic anhydride, the formed anomeric *α*-triflate, which is stabilized by the C-5 electron-withdrawing carboxylate gave the *β*-anomer via an S_N2-like mechanism (van den Bos et al. 2006; Codee et al. 2009; Dinkelaar et al. 2009; Walvoort et al. 2009; Walvoort et al. 2011). Also, the group addressed that the relatively stable $^3H_4$ half-chair oxacarbenium intermediate in the reaction gave high *β*-selectivity through an S_N1-like manner by the attack of the nucleophile from the *β*-face along a pseudoaxial trajectory. The stability of the $^3H_4$ half-chair oxacarbenium intermediate comes from the through-space stabilization of the cation from the pseudoaxial C-5 carboxylate and the most favorable position of other ring substituents (Walvoort et al. 2009; Walvoort et al. 2011). By using this strategy, *β*-1,4-mannuronate oligomers up to a pentasaccharide were successfully synthesized (Codee et al. 2009). We reasoned that, if conditions could be found for the global reduction of the uronates, the use of mannuronate building blocks would be a unique retrosynthetic strategy to *β*-mannosidic linkages and one also amenable for automation.

Demonstration of a protocol under automated conditions is of particular importance if this difficult linkage is to be readily incorporated in the design of carbohydrate libraries. Previously, in order to adapt to an automated solid-phase synthesis approach, 4,6-*O*-

www.manaraa.com

benzylidene-carboxybenzylmannosyl donors were used for the “non-pre-activation” protocol under relatively higher temperature (-30 °C) compared to the -78 °C to -60 °C needed for good selectivities with mannosyl sulfoxide donors. A less bulky but more expensive (triisopropylsiloxy)methyl (Tom) protecting group was employed at the 3-OH position in order to provide higher *β*-selectivity compared to the bulkier *tert*-butyldimethylsilyl (TBS) group. In addition, a large excess of donors (9 to 10 equivalents per coupling cycle) had to be used (Codee et al. 2008). Even for the recently reported automated solid-phase synthesis of the *β*-mannuronic acid alginates, up to 9 equivalents of donor was charged into the synthesizer. Although 20% of unreacted donor was claimed to be recovered after glycosylation, unfortunately, only 11 % of precious donor could be converted to the desired glycoside, and 71% (6.4 equiv.) of donor was wasted (Walvoort et al. 2012). In contrast, our automated solution-phase synthesis platform for oligosaccharide provides a more efficient way to synthesize oligosaccharides in which bench-top protocols can be readily adapted to automation. We discovered that attachment of C_8F_{17} fluorous-tag (F-tag) to an initial sugar building block provided a strong enough noncovalent interaction for the reliable and standardized automated purification by fluorous solid phase extraction (FSPE) of a variety of growing sugar chains (Mamidyala, et al. 2006). All reactions with the fluorous-modified sugars are homogeneous in solution and can be easily monitored off-line by thin layer chromatography (TLC) of automatically sampled reaction aliquots. Because of the homogeneous reaction environment, significantly lower amounts (1.5 to 3.5 equiv.) of precious glycosyl donors are needed for the glycosylation compared to a heterogeneous solid-phase resin approach.

www.manaraa.com

Results and discussion

To implement an approach to β-mannosidic linkages via mannuronates, a suitably protected building block had to be designed. Of particular importance is the choice of masking group for the 4-OH as it has to be reliably removed for chain extension. The TBS group was chosen as the temporary protecting group on 4-OH for the automated synthesis due to its electron-donating nature, which provides better reactivity to the glycosylation compared to the electron-withdrawing Levulinate group (Zeng et al. 2008). Also, the using of a fluoride reagent to deprotect the TBS group does not affect the C-5 methyl ester compared to Zemplén deacetylation condition that could erode the yield by partial hydrolysis of the methyl ester. The synthesis of the desired mannuronate building block **6** started from the known allyl mannoside **1** (Srivastav et al. 2000) (Figure 1), which was oxidized to the mannuronic acid **2** by the 2,2,6,6-tetramethyl-1-piperidinyloxy free radical (TEMPO)/(diacetoxyiodo)benzene (BAIB) condition then followed by an esterification with methyl iodide/K_2CO_3 to give the methyl mannuronate **3** (van den Bos et al. 2006). The silylation of **3** with the *tert*-butyldimethyl silyl chloride (TBSCl)/imidazole/4-dimethylaminopyridine (DMAP) gave the fully protected mannuronate **4**. The subsequent combination of (1,5-cyclooctadiene)bis(methyldiphenylphosphine)iridium(I) hexafluorophosphate/hydrogen and the following $HgCl_2$/HgO condition completed the deallylation to afford **5** with a free anomeric OH group. The formation of the trichloroacetimidate on the anomeric position furnished the desired building block **6** for trials of the automated solution phase synthesis of both β-1,4-mannuronate and β-1,4-mannan oligomers (Figure 1).

www.manaraa.com

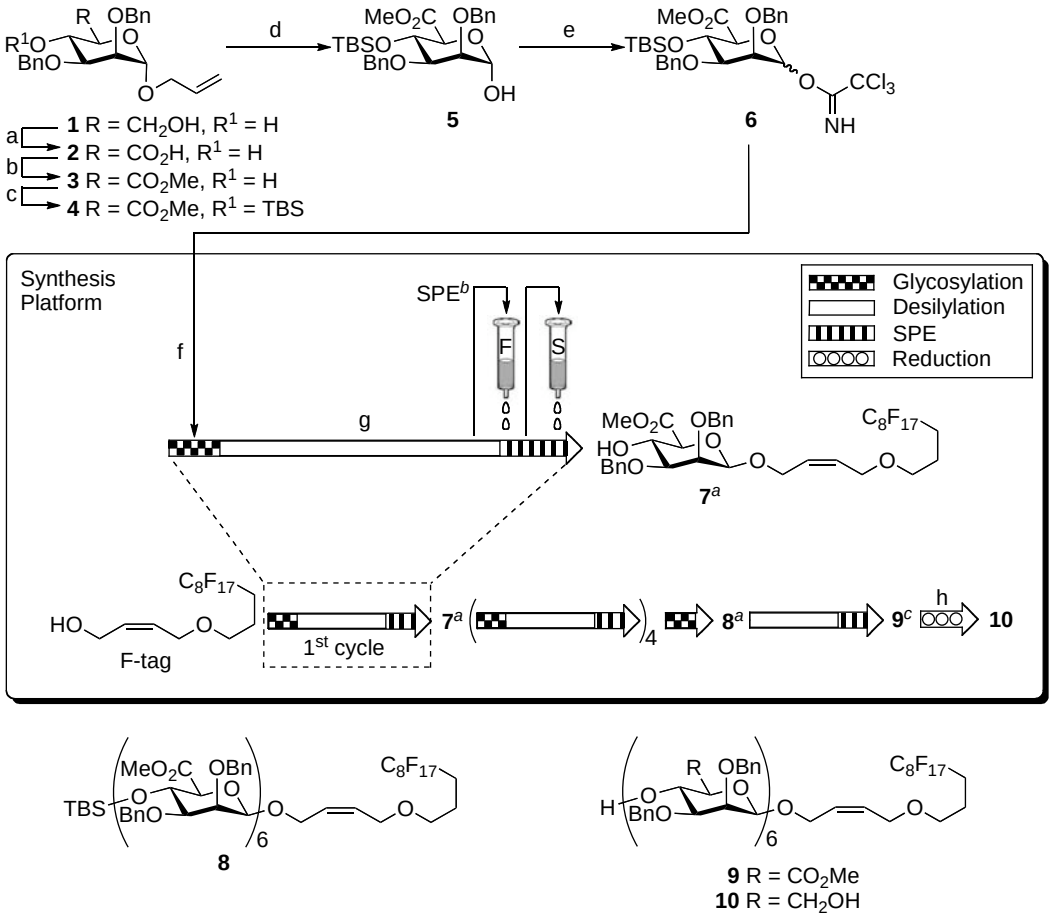


Figure 1. Synthesis of the building block **6** and the automated solution-phase synthesis of β-1,4-mannuronate and β-1,4-mannan hexamers

Reagents and conditions: (a) TEMPO, BAIB, DCM/H_2O, rt, 45 min, 75%; (b) MeI, K_2CO_3, DMF, rt, 6 h; (c) TBSCl, imidazole, DMAP, DMF, 50 °C, 20 h, 69% over 2 steps; (d) H_2, {Ir(COD)[$PCH_3(C_6H_5)_2$]$_2$}PF_6, THF, rt, 20 h, then $HgCl_2$, HgO, acetone/H_2O, rt, 20 h, 93% over 2 steps; (e) Cl_3CCN, DBU, DCM, 0 °C, 40 min, 97%; (f) TMSOTf, DCM, -20 °C, 30 min; (g) TBAF/TEA·3HF (4/1), DMSO/THF, 45 °C, 12 h; (h) DIBAL-H/toluene, DCM, 0 °C, 1 h, 82% over 2 steps. (*a*: purified on bench-top then reinjected to the synthesis platform; *b*: "F" cartridge: FSPE, "S" cartridge: SPE with silica gel; *c*: half of the material was remained to produce **10**)

www.manaraa.com

The synthesis of the β-1,4-mannuronate hexamer **9** and the β-1,4-mannan hexamer **10** in the automated solution-phase synthesis platform (Figure 1) began with the attachment of the allyl F-tag to building block **6**. After the transfer of the solution of the tag and **6** (3.5 equiv.) from the stock solution station, the temperature control unit lowered the temperature to –20 °C prior to addition of the trimethylsilyl trifluoromethanesulfonate (TMSOTf) stock solution (0.1 equiv.). After 30 min, a small aliquot was automatically removed for TLC monitoring. Next, triethylamine (TEA) was added to quench the reaction and the solvent was removed under reduced pressure generated by the vacuum pump unit under elevated temperature. The desilylation was carried on without further purification. Various conditions were tested to cleave the TBS group without degrading the esters and the glycosidic linkage. Also, the reagents should not etch the precision glass reactors. Tetrabutylammonium fluoride (TBAF)/tetrahydrofuran (THF) was found to be too basic for the methyl ester; however, the buffered conditions (TBAF/AcOH) turned out to lack reactivity (Walvoort et al. 2011). The milder HF-pyridine/pyridine condition was eliminated because of the ability of etching glassware. Triethylamine trihydrofluoride (TEA·3HF) did not hurt the glassware but was found too acidic for the glycosidic linkages. Finally, a mixture of TBAF and TEA·3HF (mole ratio = 4/1) provided a mild condition with enough strength for desilylation while also being safe for the glass reactor. After another TLC monitoring showed the completion of the desilylation, the solvent was removed under reduced pressure and the mixture was loaded onto the FSPE cartridge. The automated synthesis platform finished the elution, collected the product fractions, evaporated the solvent, and let the re-dissolved product pass through another silica gel SPE to remove the impurities. The crude product was transferred out of the

www.manaraa.com

synthesis platform and purified to get the monosaccharide **7** (74% over 2 steps). After **7** (50 μmol) was reinjected into the ASW and followed by 4 repeating glycosylaiton-desilylation cycles and one more glycosylation, the mixture was transferred to the bench top and purified to afford the fully-protected hexamannuronate **8** in 7% yield over 9 steps (75% per step). The hexasaccharide **8** was reinjected into the synthesis platform followed by a desilylation cycle to afford **9** with quantitative yield. Half portion of **9** was remained in the platform and added with diisobutylaluminum hydride (DIBAL-H) and stirred for 1 h at 0 °C for the global reduction of the esters into alcohols to afford the *β*-1,4-mannan hexamer **10** in 82% yield over 2 steps (Figure 1)

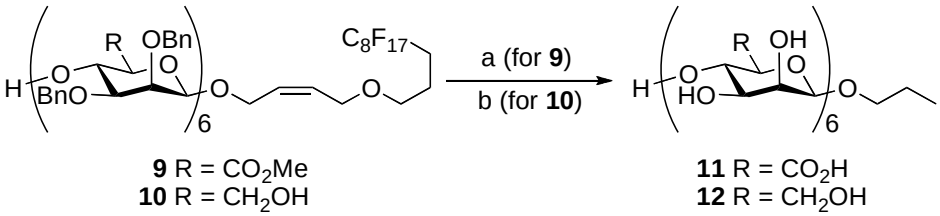


Figure 2. Deprotection of *β*-1,4-mannuronate hexamer **9** and *β*-1,4-mannan hexamer **10** Reagents and conditions: (a) (1) Grubbs catalyst 2nd generation, ethylene, DCM, 20 °C, 24 h; (2) KOH, THF/H_2O, 20 °C, 1 h; (3) H_2, Pd/C, Pd black, MeOH/AcOH, 20 °C, 48 h, 61% over 3 steps; (b) (1) Grubbs catalyst 2nd generation, ethylene, DCM, 20 °C, 24 h; (2) H_2, Pd/C, Pd black, MeOH, 20 °C, 48 h, 73% over 2 steps.

The deprotection of the *β*-mannuronate hexamer **9** started from the cleavage to the F-tag by olefin cross-metathesis, followed by hydrolysis of the esters and hydrogenolysis of the benzyl ethers to afford the fully-depretected *β*-1,4-mannuronic acid hexamer **11** in 61% yield over 3 steps. Also, the F-tag of **10** was cleaved by olefin cross-metathesis and followed by

www.manaraa.com

the removal of the benzyl groups by hydrogenolysis, which provided the fully-deprotected *β*-1,4-mannan hexamer **12** in 73% yield over 2 steps (Figure 2).

Conclusion

In conclusion, we report the strategy for the first automated solution-phase synthesis of the *β*-1,4-mannuronate and *β*-1,4-mannan oligomers. By using significantly limited amount of glycosyl donor per glycosylation cycle, 4-OTBS protecting group with mild desilylation condition, F-tag assisted purification, and real-time reaction monitor, the synthesis oligosaccharides up to hexamers has been successfully achieved. Also, the global reduction of the methyl *β*-mannuuronates revealed a new method to synthesize the *β*-mannans within various natural glycans with critical bioactivities. We are currently exploring the scope of this strategy to apply to the synthesis of other *β*-mannan-containing natural products.

Experimental section

General materials and methods

Dichloromethane (DCM) for glycosylation was distilled from calcium hydride and tetrahydrofuran (THF) was distilled to remove BHT and peroxides right before reactions. All other commercial solvents and reagents were reagent grade and used as received without further purification. The reactions were monitored by thin layer chromatography (TLC) with 250 μm Sorbent Technologies silica gel HL TLC plates. The developed TLC plates were visualized by stain with *p*-anisaldehyde solution followed by heating on a hot plate. Flash

www.manaraa.com

column chromatography was performed with Zeochem ZEOprep 60 silica gel, 40-63 μm particle size. Preparative TLC was performed with Dynamic Adsorbents Prep TLC, Silica Gel, HLO, 20 cm × 20 cm F-254, 1000 micron layer. Fluorous solid-phase extraction was performed with SPE cartridges containing 2.0 g of silica gel bounded with perfluorooctylethylsilyl chains (Fluorous Technologies, Inc., Pittsburgh, PA). The automated solution phase synthesis was performed in the Chemspeed ASW1000 (Chemspeed, Augst, Switzerland) synthesis platform with hood, 16 reactor vials (13-mL capacity each) and heating/cooling unit (200 °C to -20 °C) machined to hold the SPE cartridges at the Iowa State University Machine Shop. The hydrogenolysis reaction under 1000 psi hydrogen was operated in the Parr model 4766 general purpose vessel high pressure reactor (Parr Instrument Company, Moline, IL). ^{1}H and ^{13}C NMR spectra were obtained at 400 MHz and 100 MHz on a Bruker DRX-400 spectrometer; 700 MHz and 175 MHz on a Bruker Avance II 700 spectrometer. The C-H coupling constants were measured by the coupled ^{13}C NMR spectra. Chemical shifts (δ) were reported in parts per million (ppm) relative to $CDCl_3$ and D_2O as internal references. Mass spectra were obtained on a Finnigan TSQ700 triple quadrupole mass spectrometer (Finnigan MAT, San Jose, CA) fitted with a Finnigan ESI interface. HPLC traces were obtained from a Varian ProStar HPLC system using a Waters Nova-Pak 4 μm 3.9 × 150 mm silica column.

General procedure for bench-top fluorous solid-phase extraction (FSPE)

Crude product (less than 300 mg) was dissolved in 1.0 mL dimethyl sulfoxide (DMSO) and loaded onto the 80% MeOH preconditioned 2 g FSPE cartridge. The cartridge was washed with 80% MeOH (4.0 mL × 3 times). Then the product was washed out with

www.manaraa.com

acetone (12 mL). The solvent was removed under reduce pressure to obtain the desired product.

General procedure for automated synthetic cycles to produce β-1,4-mannuronate and β-1,4-mannan oligomers

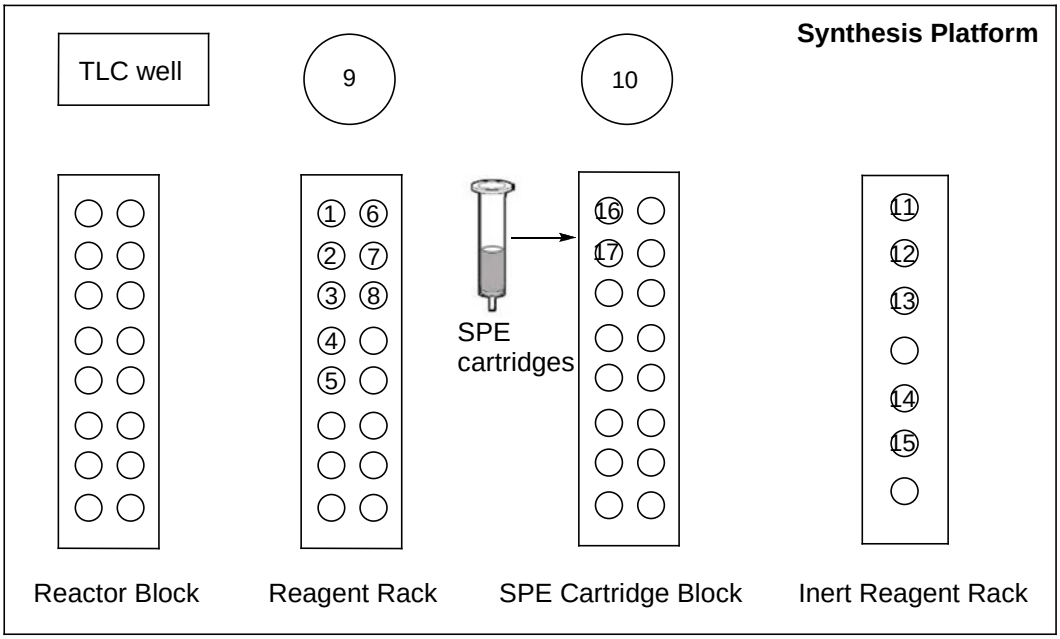


Figure 3. Basic layout of automated solution-phase oligosaccharide synthesis platform

1: F-tagged acceptor, 2: TEA, 3: DMSO, 4: TBAF/TEA·3HF/THF, 5: DIBAL-H (1.0 M in toluene), 6: water, 7-8: MeOH, 9: MeCN, 10: reservoir (toluene), 11: TMSOTf (0.55 M in DCM), 12: donor, 13: DCM, 14: EtOAc/petroleum ether = 2/1, 15: 80 % MeOH, 16: FSPE cartridge, 17: silica gel cartridge.

1. Sample Preparation

www.manaraa.com

Donor molecule (679 mg, 1.05 mmol) was dissolved in anhydrous DCM (5.6 mL) in the 13 mL-vial and placed at the inert reagent rack (Donor) under argon atmosphere. A 0.055 M trimethylsilyltrifluoromethanesulfonate (TMSOTf) solution (5.0 mL) in anhydrous DCM was prepared in an 8 mL-vial and placed as indicated on the inert reagent rack under argon. Acetonitrile (MeCN,100 mL) was placed in the stock solution bottle at the stock solution station as indicated. Toluene (1.0 L) was placed in the stock solution bottle and placed at the reservoir bottle rack with tubing as reservoir solution for rinsing. Anhydrous DCM (20 mL) was transferred to the 50 mL-vial and placed as indicated on the inert reagent rack under argon. 80% methanol/water (100 mL) stock was prepared in the 100 mL-vial and placed as indicated on the inert reagent rack. Ethyl acetate (EtOAc)/petroleum ether (2/1, 100 mL) stock was prepared in the 100 mL-vial and placed as indicated on the inert reagent rack. F-tagged acceptor molecule (50 μmol) was dissolved with anhydrous DCM (0.80 mL) in a Wheaton 8 mL-E-Z extraction vial (conically-bottomed) flushed with argon, capped with septa and placed at the reagent rack as indicated. Triethylamine (TEA, 5.0 mL) was transferred to an 8 mL-vial capped with septa and placed at the reagent rack as indicated. Anhydrous dimethyl sulfoxide (8.0 mL) was transferred to an 8 mL-vial capped with septa and placed at the reagent rack as indicated. Tetrabutylammonium fluoride (TBAF)/triethylamine trihydrofluoride (TEA·3HF)/THF (mole/mole = 4/1) (8.0 mL) was prepared in an 8 mL-vial capped with septa and placed at the reagent rack as indicated. Diisobutylaluminum hydride (DIBAL-H) solution in toluene (1.0 M, 1.0 mL) was transferred to an 8 mL-vial flushed with argon, capped with septa and placed at the reagent rack as indicated. Water (8.0 mL) was transferred to an 8 mL-vial capped with septa and placed at the reagent rack as indicated. A fluorous solid phase extraction (FSPE) cartridge (2.0 g, 10

www.manaraa.com

cc) was preconditioned with 80% methanol/water and placed on the machined FSPE block as indicated. An empty Wheaton 8 mL-E-Z extraction vial was placed under the FSPE cartridge. A silica gel cartridge (2.0 g,) was preconditioned with EtOAc/petroleum ether (2/1) and placed on the machined FSPE block as indicated. An empty Wheaton 8 mL-E-Z extraction vial was placed under the silica gel cartridge.

2. Cleaning Cycle

Prior to introduction of reagents, the reactor vials were cleaned, dried and purged with argon by running the cleaning cycle. During the cleaning cycle, each of the 16 reactor vials (13 mL capacity each) was rinsed with toluene (8.0 mL) and methanol (8.0 mL) 3 times each. After the solvents were completely removed, the reactor vials were dried under vacuum and purged with argon for 45 minutes. Donor solution was prepared by azeotropic removal of water from the glycosyl donor with toluene; the resulting glycosyl donor was then dried under high vacuum. After the cleaning cycles were done, the reagents were transferred into the reagent vials respectively, which were then placed on the inert condition reagent rack and general atmosphere reagent rack.

3. Glycosylation

The needle transferred the acceptor molecule (F-tag) solution (50 μmol, 0.8 mL) to the reaction vial 1, followed by the transfer of the donor molecule solution (0.8 mL). The mixture was vortexed under ambient temperature at 800 rpm for 20 min. Then the reactor vials were cooled to -20 ˚C during the 60 minutes wait time by the heat transfer oil with 800 rpm vortex rate. The TMSOTf solution (5.0 μmol, 0.1 mL) was transferred into the reactor

www.manaraa.com

vial 1 under 200 rpm vortex rate. After each individual transfer, the needle (inside and outside) was rinsed by toluene (2.0 ml) before operating the next task. The reaction mixture was vortexed at 800 rpm for 30 minutes at -20 °C under argon atmosphere. After the reaction time the needle withdrew 20 μL of the solution from the reaction mixture and placed it into the first well of the microtiterplate for thin layer chromatography monitoring. TEA (0.05 mL) was added to the solution for quenching and the solvent was evaporated under reduced pressure.

4. Desilylation

To the residue after the glycosylation (after evaporating solvent), DMSO (0.5 mL) was added to the reactor vial followed by TBAF/TEA·3HF/THF (1.0 mL). The reaction mixture was vortexed at 800 rpm for 12 hours at 45 °C. After the specified reaction time, the needle withdrew 20 μL from the reaction mixture and placed it into the second well of the microtiterplate for thin layer chromatography monitoring. The solvent was evaporated under reduced pressure.

5. Fluorous solid-phase extraction (FSPE)

DMSO (0.5 mL) was added to the reactor vials to dissolve the crude mixture and the vials were vortexed at 800 rpm for 3 minutes. The reaction mixture (1.2 mL) was carried to the FSPE cartridge at the FSPE block and dispensed at a speed of 1.0 mL/s via the 10 mL syringe. Then 80% methanol (2.0 mL) was used to rinse the empty reactor vial. The 80% methanol solution was removed from the reactor vial and delivered to the FSPE cartridge. The 80% methanol rinsing and transferring was repeated one more time. Additional 80%

www.manaraa.com

methanol solution (4.0 mL, repeated 2 times) was used to rinse the FSPE cartridge. During the 80% methanol rinse, the cartridge was positioned at 'SPE waste' for the eluted mixture to be disposed. MeCN (2.0 mL, repeated 3 times) was used to wash the FSPE cartridge for eluting the desired compound. During the task, the FSPE cartridge was positioned as 'SPE collect' to be placed right above the 8 mL vial for collection of the sample. After the task, the position of the SPE rack was changed into 'SPE direct' for the needle to withdraw the collected sample from the conically-bottomed vial and deliver it to the clean reactor vial for the next reaction. Toluene (1.0 mL) was added to the solution and solvent was evaporated under reduced pressure. After the evaporation cycle, The MeCN wash step and the evaporation were repeated one more time. Once again toluene (1.0 mL) was added and removed under reduced pressure to remove residual water.

6. SPE with silica gel cartridge

EtOAc/petroleum ether (2/1, 1.5 mL) was added to the dry residue from the FSPE step and vortexed at 800 rpm for 10 min. The solution (1.7 mL) was carried to the silica gel cartridge at the SPE block and dispensed at a speed of 1.0 mL/s via the 10 mL syringe. During the task, the silica gel cartridge cartridge was positioned as 'SPE collect' to be placed right above the 8 mL vial for collection of the sample. The dissolving with EtOAc/petroleum ether (2/1, 1.5 mL) and transferring to the silica gel cartridge were repeated for 3 times followed by another EtOAc/petroleum ether (2/1, 1.5 mL) transferred to the silica gel SPE cartridge. After the task, the position of the SPE rack was changed into 'SPE direct' for the needle to withdraw the collected sample from the conically-bottomed vial and deliver it to the same reactor vial just rinsed by EtOAc/petroleum ether (2/1). The solvent was removed

www.manaraa.com

under reduced pressure. Another EtOAc/petroleum ether (2/1) wash (2.5 mL, repeated 2 times) was performed and the collected sample was transferred from the conically-bottomed vial and deliver it to the same reactor vial followed by the addition of 1.0 mL toluene. The solvent was removed under reduced pressure.

7. Reduction

DCM (1.0 mL) was added to the sample and vortexed at 800 rpm, 0 °C under argon atmosphere for 10 min. The DIBAL-H (37 μL, 1.0 M in toluene) was added to the reaction solution and the mixture was vortexed for 1 h at 800 rpm, 0 °C under argon atmosphere. After the reaction time the needle withdrew 20 μL of the solution from the reaction mixture and placed it into the seventh well of the microtiterplate for thin layer chromatography monitoring. MeOH (0.1 mL) was added to quench the reaction followed by the addition of 2.0 mL saturated Rochelle salt solution and vortexed for 2 h at 800 rpm. The mixture was transferred out of the synthesis platform.

Synthetic procedures

1 **2**

Allyl-2,3-di-*O*-benzyl-a-D-mannopyranosiduronic acid (2)

To a solution of allyl-2,3-di-*O*-benzyl-a-D-mannopyranoside **1** (0.500 g, 1.25 mmol) in DCM/water (4.0 mL/2.0 mL) were added 2,2,6,6-Tetramethyl-1-piperidinyloxy free radical (TEMPO) (0.04 g, 0.26 mmol) and (diacetoxyiodo)benzene (BAIB) (1.0 g, 3.1 mmol) and

www.manaraa.com

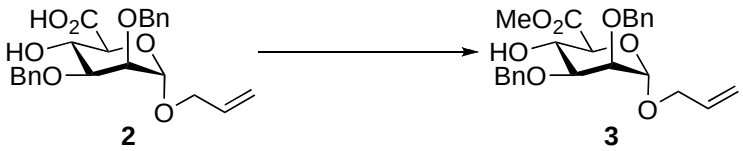
stirred at ambient temperature. After 45 min, the mixture was diluted with dichloromethane (10 mL) and washed with 10% $Na_2S_2O_3$ solution (10 mL) and water (10 mL). The organic layer was dried over Na_2SO_4. The solvent was removed under reduced pressure and the crude product was purified by flash column chromatography on silica gel using EtOAc/petroleum ether/AcOH (1/1/0.02 → 1/0/0.02) as eluent. The product was obtained as a light yellow syrup (0.39 g, 0.94 mmol, 75%) (van den Bos et al. 2006).

***R**_f*: 0.35 (MeOH/dichloromethane: 1/9)

^{1}H NMR ($CDCl_3$, 400MHz): δ (ppm) 7.36-7.30 (m, 10H, H_{arom}), 5.87-5.79 (m, 1H, *H*C=C), 5.26-5.19 (m, 2H, C=C*H*$_2$), 4.98 (d, 1H, *J* = 1.6 Hz, H-1), 4.72-4.62 (m, 4H, 4 × CH*H*Ph), 4.29 (t, 1H, *J* = 9.6 Hz, H-4), 4.21 (dd, 1H, *J* = 13.2, 5.2 Hz, OH*H*CHC=C), 4.16 (d, 1H, *J* = 10.0 Hz, H-5), 4.02 (dd, 1H, *J* = 13.2, 6.4 Hz, OH*H*CHC=C), 3.83 (dd, 1H, *J* = 9.2, 3.2 Hz, H-3), 3.78 (d, 1H, *J* = 2.0 Hz, H-2)

^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 172.8, 138.1, 137.9, 133.3, 128.6, 128.6, 128.4, 128.2, 128.0, 118.0, 97.8 (J_{C1-H1} = 170.1 Hz, C-1), 78.6, 74.4, 73.2, 72.8, 71.2, 68.8, 68.4

HRMS (ESI): $[M + K]^+$ calcd for $C_{23}H_{26}KO_7^+$ 453.1310, found 453.1318



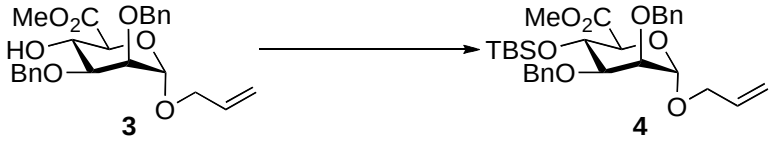
Methyl (allyl-2,3-di-*O*-benzyl-*a*-D-mannopyranoside) uronate (3)

To a solution of allyl-2,3-di-*O*-benzyl-*a*-D-mannopyranosiduronic acid **2** (0.500 g, 1.21 mmol) in anhydrous DMF (6.0 mL) were added K_2CO_3 (0.12 g, 0.87 mmol) and iodomethane (0.64 g, 4.51 mmol). The reaction mixture was stirred at ambient temperature under argon

www.manaraa.com

atmosphere for 6 h. The mixture was diluted with EtOAc (10 mL) and washed with water (10 mL). The aqueous portion was separated and extracted with EtOAc (2 x 10 mL). The combined organic layer was dried over Na_2SO_4. The solvent was removed under reduced pressure and the crude product was obtained as a yellow syrup and ready for the next step without further purification (van den Bos et al. 2006).

***R**_f*: 0.38 (EtOAc/petroleum ether: 1/2)



Methyl (allyl-2,3-di-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-a-D-mannopyranoside) uronate (4)

To a solution of the crude mixture of methyl (allyl-2,3-di-*O*-benzyl-a-D-mannopyranoside) uronate **3** (1.21 mmol) in anhydrous DMF (7.0 mL) was added imidazole (0.330 g, 4.85 mmol), *tert*-butyldimethylsilyl chloride (0.73 g, 4.84 mmol), and 4-(dimethylamino)pyridine (0.03 g, 0.25 mmol). The reaction mixture was stirred at 50 ^{o}C under argon atmosphere for 20 h. The mixture was diluted with EtOAc (10 mL) and washed with sat. $NaHCO_3$ solution (10 mL). The organic layer was dried over Na_2SO_4. The solvent was removed under reduced pressure and the crude product was purified by flash column chromatography on silica gel using EtOAc/petroleum ether (1/6) as eluent. The product was obtained as a light yellow syrup (0.45 g, 0.83 mmol, 69% over 2 steps).

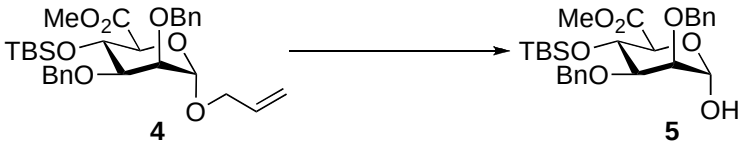
***R**_f*: 0.49 (EtOAc/petroleum ether: 1/6)

www.manaraa.com

^{1}H NMR ($CDCl_3$, 400MHz): δ (ppm) 7.33-7.24 (m, 10H, 10 × H_{arom}), 5.96 (m, 1H, *H*C=C), 5.30 (d, 1H, *J* = 17.2 Hz, C=CH*H*), 5.21 (d, 1H, *J* = 10.4 Hz, C=CH*H*), 5.08 (d, 1H, *J* = 4.0 Hz, H-1), 4.74 (d, 1H, *J* = 12.4 Hz, CH*H*Ph), 4.66 (d, 1H, *J* = 12.0 Hz, CH*H*Ph), 4.61 (d, 1H, *J* = 12.0 Hz, CH*H*Ph), 4.55 (d, 1H, *J* = 12.0 Hz, CH*H*Ph), 4.36 (t, 1H, *J* = 7.2 Hz, H-4), 4.29 (dd, 1H, *J* = 13.2, 5.2 Hz, OH*H*CHC=C), 4.18 (d, 1H, *J* = 6.4 Hz, H-5), 4.08 (dd, 1H, *J* = 12.8, 6.0 Hz, OH*H*CHC=C), 3.73 (t, 1H, *J* = 3.6 Hz, H-2), 3.69 (s, 3H, CO_2CH_3), 3.66 (dd, 1H, *J* = 7.2, 2.8 Hz, H-3), 0.81 (s, 9H, 9 × $H_{t\text{-butyl}}$), 0.01 (s, 3H, 3 × $H_{dimethyl}$), -0.03 (s, 3H, 3 × $H_{dimethyl}$)

^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 169.7, 138.4, 138.1, 133.8, 128.2, 128.2, 128.0, 127.7, 127.5, 127.5, 117.3, 97.9, 79.2, 74.8, 74.2, 72.9, 71.9, 69.4, 68.9, 52.0, 25.7, 17.9, -4.4, -5.3

HRMS (ESI): $[M + Na]^+$ calcd for $C_{30}H_{42}NaO_7Si^+$ 565.2592, found 565.2608



Methyl (2,3-di-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-α-D-mannopyranose) uronate (5)

To a solution of methyl (allyl-2,3-di-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-α-D-mannopyranoside) uronate **4** (0.580 g, 1.06 mmol) in anhydrous THF (14 mL) was added (1,5-cyclooctadiene)bis(methyldiphenylphosphine)iridium(I) hexafluorophosphate (9.0 mg, 10.6 μmol). The solution was stirred at ambient temperature under hydrogen atmosphere until the red solution became light yellow. The hydrogen was replaced by argon atmosphere and the solution was stirred at ambient temperature for 20 h. The solvent was removed under

www.manaraa.com

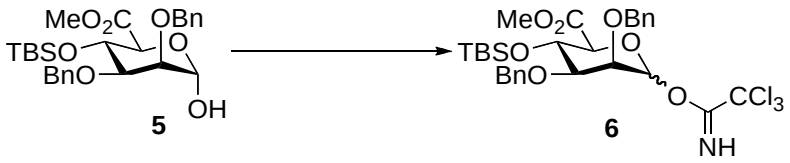
reduced pressure and the mixture was dissolved in acetone/H_2O = 5/1 (13 mL). To the solution of the mixture was added mercury(II) chloride (0.13 g, 0.48 mmol) and mercury(II) oxide (0.13 g, 0.60 mmol). The reaction mixture was stirred at ambient temperature under argon atmosphere for 20 h. The solvent was removed under reduced pressure and the mixture was diluted with chloroform (50 mL) and the solid was filtered off through a celite pad. The solution was washed with 10% KI solution (30 mL) and the organic layer was dried over Na_2SO_4. The solvent was removed under reduced pressure and the crude product was purified by flash column chromatography on silica gel using EtOAc/petroleum ether (1/2) as eluent. The product was obtained as a light yellow syrup (0.50 g, 0.99 mmol, 93% over 2 steps).

***R**_f*: 0.37 (EtOAc/petroleum ether: 3/7)

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.35-7.25 (m, 10H, 10 × H_{arom}), 5.51 (s, 1H, H-1), 4.77 (d, 1H, *J* = 12.0 Hz, CH*H*Ph), 4.68-4.53 (m, 3H, 3 × CH*H*Ph), 4.41 (t, 1H, *J* = 5.2, 4.4 Hz, H-3), 4,32 (d, 1H, *J* = 4.0 Hz, H-4), 3.69 (t, 2H, *J* = 5.6, 4.8 Hz, H-2, H-5), 3.62 (s, 3H, CO_2CH_3), 0.82 (s, 9H, 9 × $H_{t\text{-butyl}}$), 0.03 (s, 3H, 3 × $H_{dimethyl}$), -0.02 (s, 3H, 3 × $H_{dimethyl}$)

^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 170.3, 138.6, 138.2, 128.4, 128.4, 127.9, 127.8, 127.7, 92.9 ($J_{C1\text{-}H1}$ = 168.8 Hz, C-1), 78.4, 75.8, 75.4, 73.0, 72.4, 69.6, 52.2, 25.8, 18.1, -4.6, -5.0

HRMS (ESI): $[M + K]^+$ calcd for $C_{27}H_{38}KO_7Si^+$ 541.2018, found 541.2039



www.manaraa.com

Methyl (2,3-di-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-α/β-D-mannopyranose) uronate trichloroacetimidate (6)

To a solution of methyl (2,3-di-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-α-D-mannopyranose) uronate **5** (0.49 g, 0.98 mmol) in dichloromethane (26 mL) was added trichloroacetonitrile (0.85 g, 5.88 mmol) at 0 °C under argon atmosphere. 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU) (0.03 g, 0.2 mmol) was then added and the reaction mixture was stirred at 0 °C under argon atmosphere for 40 min. The solvent was removed under reduced pressure and the crude product was purified by flash column chromatography on silica gel using EtOAc/petroleum ether/triethylamine (1/3/0.04) as eluent. The product was obtained as a mixture of anomers (α/β = 1.8/1) (0.61 g, 0.95 mmol, 97%).

***R*$_f$**: 0.69 (EtOAc/petroleum ether: 1/3)

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 9.47 (s, 1H, N*H*), 8.66 (s, 1H, N*H*), 7.38-7.26 (m, 10H, 10 × H_{arom}), 6.45 (d, 1H, *J* = 3.6 Hz, H_α-1), 6.03 (d, 1H, *J* = 8.0 Hz, H_β-1), 4.91 (d, 1H, *J* = 12.0 Hz, CH*H*Ph), 4.78-4.58 (m, 4H, 4 × CH*H*Ph), 4.45 (t, 1H, *J* = 7.2 Hz, H_α-4), 4.40 (dd, 1H, *J* = 4.0, 1.6 Hz, H_β-4), 4.36 (s, 1H, H_β-5), 4.31 (d, 1H, *J* = 7.2 Hz, H_α-5), 3.92 (dd, 1H, *J* = 8.0, 2.4 Hz, H_β-2), 3.90 (t, 1H, *J* = 3.2 Hz, H_α-2), 3.72 (m, 12H, 2 × H_3, 2 × CO_2CH_3), 0.87 (s, 9H, 9 × $H_{t\text{-butyl}}$), 0.82 (s, 9H, 9 × $H_{t\text{-butyl}}$), 0.06 (s, 3H, 3 × $H_{dimethyl}$), 0.06 (s, 3H, 3 × $H_{dimethyl}$), 0.03 (s, 3H, 3 × $H_{dimethyl}$), -0.05 (s, 3H, 3 × $H_{dimethyl}$)

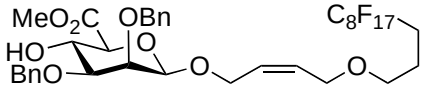
^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 169.4, 169.1, 160.6, 159.1, 138.1, 138.0, 137.8, 137.5, 128.5, 128.5, 128.1, 128.0, 128.0, 128.0, 127.9, 95.9, 95.4, 92.4, 91.1, 78.5, 77.9, 76.6, 73.6, 73.5, 73.4, 73.1, 72.9, 72.6, 69.7, 69.1, 52.6, 52.4, 25.9, 25.8, 18.1, 18.0, -4.1, -5.0, -5.0, -5.1

www.manaraa.com

HRMS (ESI): $[M + Na]^+$ calcd for $C_{29}H_{38}Cl_3NNaO_7Si^+$ 668.1375, found 668.1381

Table 1. Automated synthetic cycles for production of compound **7**

Step	Task	Reagent/ Operation	Operation Time
1	Glycosylation (2 parallel reactions)	3.0 × 2 equivalent donor (150 × 2 μmol) in 0.8 × 2 mL DCM, 1.0 × 2 equivalent F-tagged acceptor (50 × 2 μmol) in 1 mL DCM; 0.11 equivalent TMSOTf, -20 °C	45 min
2	TLC sample	20 μL of crude reaction mixture withdrawn	
3	Quenching	0.05 × 2 mL TEA	
4	Evaporation	40 ˚C	45 min
5	Desilylation ((2 parallel reactions)	1 × 2 mL TBAF/ TEA·3HF/THF, 0.5 × 2 mL DMSO, 45 °C	12 h
6	TLC sample	30 × 2 μL of crude reaction mixture withdrawn	
7	Evaporation	40 °C	45 min
8	FSPE preparation	0.5 × 2 mL DMSO	
9	Sample loading	1.2 × 2 mL crude sample transferred to cartridges	
10	Wash	12 × 2 mL 80% methanol wash	
11	Wash	2 mL MeCN wash (repeated 3 × 2 times)	
12	Transfer	6 × 2 mL collected sample out of the synthesis platform	



7

***cis*-4-(1H,1H,2H,2H,3H,3H-perfluoroundecyloxy)-2-butenyl (methyl 2,3-di-*O*-benzyl-β-D-mannopyranoside) uronate (7)**

The crude product solution was transferred out of the synthesis platform after the FSPE step of the first cycle (12th step of the automated synthesis). The solvent was removed under reduced pressure and the product was purified by flash column chromatography on silica gel using EtOAc/petroleum ether (3/7) as eluent. The product was obtained as a colorless syrup (68 mg, 0.074 mmol, 74% over 2 steps).

***R*$_f$**: 0.24 (EtOAc/petroleum ether: 1/2)

www.manaraa.com

^{1}H NMR ($CDCl_3$, 400MHz): δ (ppm) 7.44-7.42 (d, 2H, *J* = 6.8 Hz, 2 × H_{arom}), 7.32-7.26 (m, 8H, 8 × H_{arom}), 5.77 (m, 2H, *H*C=C*H*), 4.98 (d, 1H, *J* = 12.4, CH*H*Ph), 4.80 (d, 1H, *J* = 12.4, CH*H*Ph), 4.56 (d, 1H, *J* = 12.0, CH*H*Ph), 4.5 (d, 1H, CH*H*Ph), 4.47 (s, 1H, H-1), 4.45 (d, 1H, *J* = 3.2 Hz, OH*H*CHC=C), 4.34 (t, 1H, *J* = 9.4 Hz, H-4), 4.25 (dd, 1H, *J* = 12.9, 5.2 Hz, OH*H*CHC=C), 4.10 (m, 2H, C=CHCH_2O), 3.87 (d, 1H, *J* = 2.2 Hz, H-2), 3.81 (s, 3H, CO_2CH_3), 3.76 (d, 1H, *J* = 9.6 Hz, H-5), 3.50 (t, 2H, *J* = 5.9 Hz, OCH_2CH_2), 3.37 (dd, 1H, *J* = 9.4, 2.6 Hz, H-3), 2.97 (s, 1H, 4-O*H*), 2.26 (m, 2H, CH_2CF_2), 1.91 (m, 2H, OCH_2CH_2)

^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 169.9, 138.6, 138.1, 130.4, 128.6, 128.5, 128.3, 128.3, 128.0, 127.8, 127.7, 101.0 (J_{C1-H1} = 152.3 Hz, C-1), 80.5, 75.4, 74.5, 73.9, 71.9, 69.0, 68.4, 66.7, 65.0, 52.8, 28.4 (t, J_{C-F} = 21.8 Hz), 21.0

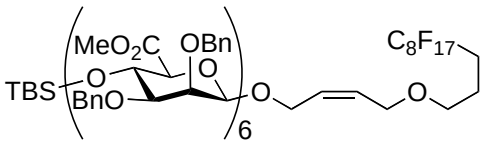
HRMS (ESI): $[M + K]^+$ calcd for $C_{36}H_{35}F_{17}KO_8^+$ 957.1692, found 957.1697

www.manaraa.com

Table 2. Automated synthetic cycles for production of compound **8**

Step	Task	Reagent/ Operation	Operation Time
1	Transfer **7** to reactor vial	50 μmol in 3.0 mL EtOAc	
2	Evaporation	40 °C	45 min
3	Glycosylation	3.5 equivalent donor (150 × 2 μmol) in 1.6 mL DCM 0.11 equivalent TMSOTf, -20 °C	45 min
4	TLC sample	20 μL of crude reaction mixture withdrawn	
5	Quenching	0.05 mL TEA	
6	Evaporation	40 °C	45 min
7	Desilylation	1.0 mL TBAF/ TEA·3HF/THF, 0.5 mL DMSO, 45 °C	12 h
8	TLC sample	20 μL of crude reaction mixture withdrawn	
9	Evaporation	40 °C	45 min
10	FSPE preparation	0.5 mL DMSO	
11	Sample loading	1.2 mL crude sample transferred to cartridges	
12	Wash	12 mL 80% methanol wash	
13	Wash	2.0 mL MeCN wash (repeated 3 times)	
14	Transfer	6.0 mL collected sample to the reactor vial for next cycle	
15	Evaporation	40 °C	45 min
16	Wash	2.0 mL MeCN wash (repeated 3 times)	
17	Evaporation	40 °C	45 min
18	Transfer EtOAc	1.0 mL	
19	Evaporation	40 °C	45 min
20	Silica gel SPE Preparation	1.5 mL EtOAc/petroleum ether (2/1)	
21	Sample loading	1.7 mL sample transfer to the cartridge	
22	Repeating 20 and 21 for 3 times		
23	Transfer	6.0 mL collected sample to the reactor vial for next cycle	
24	Evaporation	40 °C	45 min
25	Transfer	2.5 × 2 mL EtOAc/petroleum ether (2/1) to the cartridge	
26	Transfer	5.0 mL collected sample and 1.0 mL toluene to the reactor vial for next cycle	
27	Evaporation	40 °C	45 min
28	Transfer	1.0 mL toluene	
29	Evaporation	40 °C	45 min
30	Transfer	1.0 mL toluene	
31	Evaporation	40 °C	45 min
32	Repeat the same procedure as step 3-31	Repeat for 4 cycles	81 h
33	Repeat the same procedure as step 3-6	Repeat one cycle	90 min

www.manaraa.com



8

***cis*-4-(1H,1H,2H,2H,3H,3H-perfluoroundecyloxy)-2-butenyl (methyl 2,3-di-*O*-benzyl-4-*O*-(methyl 2,3-di-*O*-benzyl-4-*O*-(methyl 2,3-di-*O*-benzyl-4-*O*-(methyl 2,3-di-*O*-benzyl-4-*O*-(methyl 2,3-di-*O*-benzyl-4-*O*-(methyl 2,3-di-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-β-D-mannopyranosyl uronate)-β-D-mannopyranosyl uronate)-β-D-mannopyranosyl uronate)-β-D-mannopyranosyl uronate)-β-D-mannopyranosyl uronate)-β-D-mannopyranosyl uronate)-β-D-mannopyranoside uronate) (8)**

cis-4-(1H,1H,2H,2H,3H,3H-perfluoroundecyloxy)-2-butenyl (methyl 2,3-di-*O*-benzyl-β-D-mannopyranoside) uronate **7** (46 mg, 50 μmole) was transferred back to the synthesis platform for further synthesis. After the 6th cycle (33rd step of the automated synthesis) was finished the mixture was transferred out of the synthesis platform and purified by FSPE as described by the general procedure for bench-top FSPE. The crude product from the FSPE was purified by preparative TLC using diethyl ether/benzene (1/3). The crude product was collected from the preparative TLC plate and purified by another preparative TLC using diethyl ether/DCM (1/10). The product was collected as a white solid (9.5 mg, 3.3 μmol, 7% over 9 steps, 75 % per step).

***R**$_f$*: 0.23 (EtOAc/petroleum ether: 1/2), 0.59 (diethyl ether/DCM: 1/10), 0.38 (diethyl ether/benzene: 1/3 developed twice)

^{1}H NMR (CDCl$_3$, 700 MHz): δ (ppm) 7.36-7.32 (m, 11H, 11 × H$_{arom}$), 7.28-7.21 (m, 49H, 49 × H$_{arom}$) 5.75 (m, 2H, *H*C=C*H*), 4.83-4.76 (m, 6H, 6 × CH*H*Ph), 4.74-4.66 (m, 11 H, 1 × H-1, 10 × CH*H*Ph), 4.62-4.60 (m, 3 H, 2 × H-1, 1 × CH*H*Ph), 4.58 (s, 1H, H-1), 4.56 (s, 1H, H-

www.manaraa.com

1), 4.53-4.67 (m, 7H, 1 × H-1, 6 × CH*H*Ph), 4.45-4.41 (m, 3 H, 1 × OH*H*CHC=C, 1 × H-4, 1 × CH*H*Ph), 4.33 (m, 5 H, 5 × H-4), 4.20 (dd, 1H, *J* = 12.6, 6.3 Hz, OH*H*CHC=C), 4.07 (m, 2H, C=CHCH_2O), 3.84 (d, 1H, *J* = 8.4 Hz, H-5), 3.80 (d, 1H, *J* = 2.8 Hz, H-2), 3.79 (d, 1H, *J* = 2.8 Hz, H-2), 3.75 (d, 1H, *J* = 5.6 Hz, H-2), 3.74-3.67 (m, 7H, 3 × H-2, 4 × H-5), 3.64-3.52 (m, 8H, 1 × H-3, 1 × H-5, 2 × CO_2CH_3), 3.49-3.37 (m, 18H, 4 × H-3, OCH_2CH_2, 4 × CO_2CH_3), 3.23 (dd, 1H, *J* = 9.8, 2.8 Hz, H-3), 2.20 (m, 2H, CH_2CF_2), 1.88 (m, 2H, OCH_2CH_2), 0.79 (s, 9H, 9 × $H_{t\text{-butyl}}$), -0.03 (s × 2, 6H, 6 × H_{dimethyl})

^{13}C NMR ($CDCl_3$, 175 MHz): δ (ppm) 168.8, 168.8, 139.4, 139.1, 139.1, 138.9, 138.6, 138.1, 130.3, 128.4, 128.3, 128.3, 128.2, 128.0, 127.8, 127.7, 127.6, 127.5, 127.5, 127.5, 127.4, 127.3, 103.0 ($J_{C1\text{-}H1}$ = 155.1 Hz, C-1), 102.7 ($J_{C1\text{-}H1}$ = 157.7 Hz, 3 × C-1), 102.5 ($J_{C1\text{-}H1}$ = 155.8 Hz, C-1), 100.6 ($J_{C1\text{-}H1}$ = 157.0 Hz, C-1), 82.0, 79.9, 79.2, 77.6, 76.4, 76.2, 74.7, 74.7, 74.6, 74.0, 72.8, 72.7, 72.4, 71.4, 69.0, 66.8, 65.1, 52.5, 52.3, 52.3, 52.1, 28.3 (t, $J_{C\text{-}F}$ = 22.2 Hz), 25.9, 21.0, 18.2, -3.8, -5.1

HRMS (ESI): $[M + Na]^+$ calcd for $C_{147}H_{159}F_{17}NaO_{38}Si^+$ 2905.9899, found 2905.9840

HPLC trace of the automated synthesis of **8**

www.manaraa.com

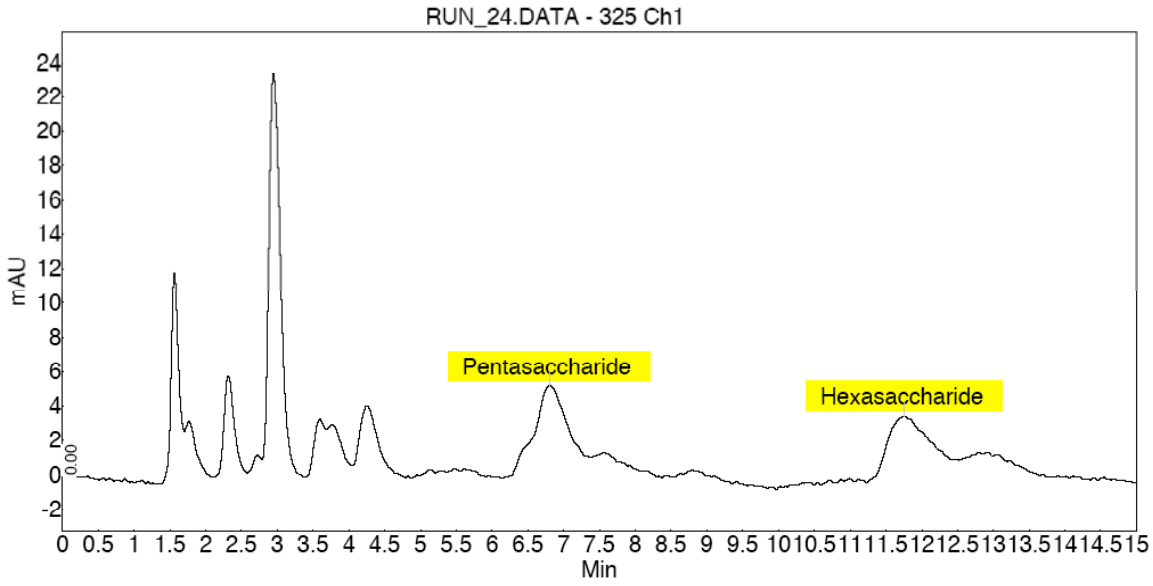
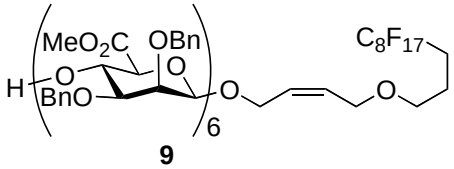


Figure 4. Analytical HPLC chromatogram of the crude product from the synthesis platform after FSPE (1.0 mL/min flowrate, EtOAc/hexanes: 1/4, 15 minutes run, Waters Nova-Pak 4 μm 3.9 × 150 mm silica column)

www.manaraa.com

Table 3. Automated synthetic cycles for production of compound **9** and **10**

Step	Task	Reagent/ Operation	Operation Time
34	Transfer **8** to reactor vial	3.1 μmol in 3.0 mL EtOAc	
35	Evaporation	40 °C	45 min
36	Desilylation	1.0 mL TBAF/ TEA·3HF/THF, 0.5 mL DMSO, 45 °C	12 h
37	TLC sample	20 μL of crude reaction mixture withdrawn	
38	Evaporation	40 °C	45 min
39	FSPE preparation	0.5 mL DMSO	
40	Sample loading	1.2 mL crude sample transferred to cartridges	
41	Wash	12 mL 80% methanol wash	
42	Wash	2.0 mL MeCN wash (repeated 3 times)	
43	Transfer	6.0 mL collected sample to the reactor vial for next cycle	
44	Evaporation	40 °C	45 min
45	Wash	2.0 mL MeCN wash (repeated 3 times)	
46	Evaporation	40 °C	45 min
47	Transfer EtOAc	2.0 mL	
48	Evaporation	40 °C	45 min
49	Silica gel SPE Preparation	1.5 mL EtOAc/petroleum ether (2/1)	
50	Sample loading	1.7 mL sample transfer to the cartridge	
51	Repeating 49 and 50 for 3 times		
52	Transfer	6.0 mL collected sample to the reactor vial for reduction	
53	Evaporation	40 °C	45 min
54	Transfer	2.5 × 2 mL EtOAc/petroleum ether (2/1) to the cartridge	
55	Transfer	5.0 mL collected sample and 1.0 mL toluene to the reactor vial for next cycle	
56	Evaporation	40 °C	45 min
57	Transfer	2.0 mL toluene	
58	Transfer	1.0 mL toluene solution to the reactor vial for Storage of **9**	
59	Evaporation	40 °C	45 min
60	Transfer	1.0 mL toluene to reactor vial for reduction	
61	Evaporation	40 °C	45 min
62	Reduction	1.0 mL DCM, 37 μL DIBAL-H (1.0 M in toluene), 0 °C	4 h
63	TLC sample	20 μL of crude reaction mixture withdrawn	
64	Quenching	0.1 mL MeOH, 0 °C	
65	Transfer	2.0 mL sat. Rochelle salt solution, vortex at 800 rpm	2 h



9

www.manaraa.com

***cis*-4-(1H,1H,2H,2H,3H,3H-perfluoroundecyloxy)-2-butenyl (methyl 2,3-di-*O*-benzyl-4-*O*-(methyl 2,3-di-*O*-benzyl-4-*O*-(methyl 2,3-di-*O*-benzyl-4-*O*-(methyl 2,3-di-*O*-benzyl-4-*O*-(methyl 2,3-di-*O*-benzyl-4-*O*-(methyl 2,3-di-*O*-benzyl-β-D-mannopyranosyl uronate)-β-D-mannopyranosyl uronate)-β-D-mannopyranosyl uronate)-β-D-mannopyranosyl uronate)-β-D-mannopyranosyl uronate)-β-D-mannopyranoside uronate) (9)**

After the 8th cycle (the 65th step of the automated synthesis), the product fraction after FSPE was taken out and the solvent was removed under reduced pressure to afford the compound **9** as a white foam (4.3 mg, 1.6 μmol, quant.).

***R**$_f$*: 0.38 (EtOAc/petroleum ether: 1/1)

^{1}H NMR (CDCl$_3$, 700 MHz): δ (ppm) 7.36-7.20 (m, 60H, 60 × H$_{arom}$), 5.72 (m, 2H, *H*C=C*H*), 4.83-4.69 (m, 15H, 15 × CH*H*Ph), 4.68 (s, 1H, H-1), 4.63-4.59 (m, 5H, 4 × H-1, 1 × CH*H*Ph), 4.56-4.49 (m, 8H, 8 × CH*H*Ph), 4.50 (s, 1H, H-1), 4.45 (dd, 1H, *J* = 12.6, 5.6 Hz, OH*H*CHC=C), 4.34-4.29 (m, 4H, 4 × H-4), 4.19 (dd, 1H, *J* = 13.3, 7.0 Hz, OH*H*CHC=C), 4.17 (t, 1H, *J* = 7.7 Hz, H-4), 4.04 (m, 2H, C=CHC*H*$_2$O), 3.83 (d, 1H, *J* = 8.4 Hz, H-5), 3.76-3.69 (m, 8H, 4 × H-2, 4 × H-5), 3.58-3.57 (m, 7H, 1 × H-3, 2 × CO$_2$C*H*$_3$), 3.48-3.42 (m, 12H, 4 × H-3, OC*H*$_2$CH$_2$, 2 × CO$_2$C*H*$_3$), 3.39 (s, 6H, 2 × CO$_2$C*H*$_3$), 3.28 (dd, 1H, *J* = 9.1, 2.8 Hz, H-3), 2.90 (s, 1H, O*H*), 2.17 (m, 2H, C*H*$_2$CF$_2$), 1.86 (m, 2H, OCH$_2$C*H*$_2$)

^{13}C NMR (CDCl$_3$, 175 MHz): δ (ppm) 170.2, 168.8, 139.1, 138.9, 138.6, 138.2, 130.3, 128.6, 128.4, 128.3, 128.3, 128.3, 128.2, 128.0, 128.0, 127.9, 127.9, 127.7, 127.6, 127.5, 127.4, 127.4, 102.8 (4 × C-1), 102.5 (C-1), 100.6 (C-1), 80.5, 80.0, 80.0, 79.2, 76.3, 76.2, 75.9, 75.3, 74.9, 74.8, 74.7, 74.6, 74.4, 74.0, 72.8, 72.7, 72.6, 72.4, 72.4, 72.0, 69.0, 68.3, 66.8, 65.1, 52.5, 52.5, 52.3, 52.3, 28.3 (t, J_{C-F} = 22.2 Hz), 21.0

www.manaraa.com

HRMS (ESI): $[M + Na]^+$ calcd for $C_{141}H_{145}F_{17}NaO_{38}^+$ 2791.9035, found 2791.9040

HPLC trace of the automated synthesis of **9**

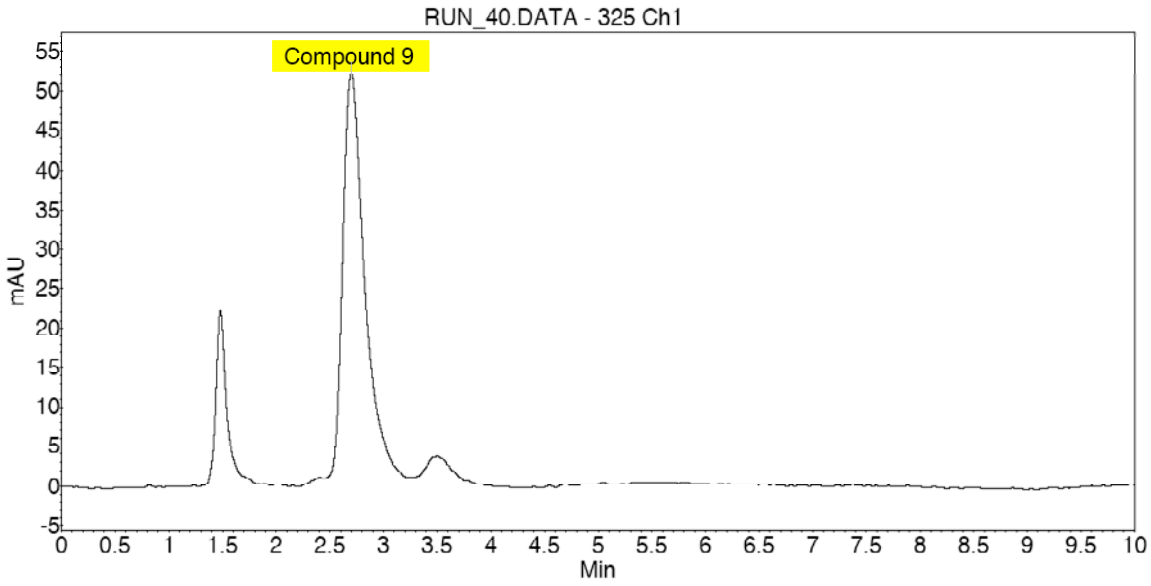
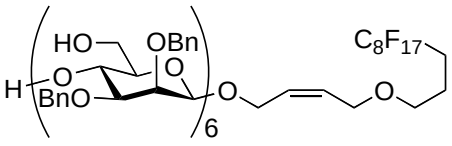


Figure 4. Analytical HPLC chromatogram of the crude product from the synthesis platform after FSPE (1.0 mL/min flowrate, EtOAc/hexanes: 1/1, 10 minutes run, Waters Nova-Pak 4 μm 3.9 × 150 mm silica column).



10

***cis*-4-(1H,1H,2H,2H,3H,3H-perfluoroundecyloxy)-2-butenyl (2,3-di-*O*-benzyl-4-*O*-(2,3-di-*O*-benzyl-4-*O*-(2,3-di-*O*-benzyl-4-*O*-(2,3-di-*O*-benzyl-4-*O*-(2,3-di-*O*-benzyl-4-*O*-(2,3-di-*O*-benzyl-β-D-mannopyranosyl)-β-D-mannopyranosyl)-β-D-mannopyranosyl)-β-D-mannopyranosyl)-β-D-mannopyranosyl)-β-D-mannopyranoside) (10)**

www.manaraa.com

After the 8th cycle (the 65th step of the automated synthesis), the reaction mixture was taken out and diluted with EtOAc (15 mL), and washed with sat. Rochelle salt solution (20 mL), The combined organic layer was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by preparative TLC using MeOH/DCM (1/19). The product was collected as a colorless syrup (3.3 mg, 1.3 μmol, 82% over 2 steps)

***R**f*: 0.26 (MeOH/DCM: 1/19)

^{1}H NMR ($CDCl_3$, 700 MHz): δ (ppm) 7.44-7.14 (m, 60 H, 60 × H_{arom}), 5.75 (m, 2H, *H*C=C*H*), 4.94-4.89 (m, 3 H, 3 × CH*H*Ph), 4.86-4.72 (m, 15H, 15 × CH*H*Ph), 4.65 (s, 1H, H-1), 4.58 (d, 1H, *J* = 11.9 Hz, CH*H*Ph), 4.54-4.48 (m, 10 H, 5 × H-1, 5 × CH*H*Ph), 4.44 (dd, 1H, *J* = 11.2, 4.2 Hz), 4.36 (d, 1H, *J* = 11.9 Hz), 4.22-4.19 (m, 2H), 4.13 (t, 1H, *J* = 9.1 Hz), 4.09-4.02 (m, 5H), 3.92 (d, 1H, *J* = 2.1 Hz), 3.91 (d, 1H, *J* = 2.8 Hz), 3.89-3.83 (m, 6 H), 3.73-3.60 (m, 7H), 3.51 (t, 2H, *J* = 5.6 Hz, OC*H*$_2$CH$_2$), 3.46-3.42 (m, 7H), 3.41-3.35 (m, 3H), 3.31 (m, 1H), 3.28 (dd, 1H, *J* = 9.8, 2.8 Hz), 3.20-3.16 (m, 5H), 2.33-2.26 (m, 3H, 3 × O*H*), 2.23 (m, 2H, C*H*$_2$CF$_2$), 1.90 (m, 2H, OCH$_2$C*H*$_2$)

^{13}C NMR ($CDCl_3$, 175 MHz): δ (ppm) 139.0, 138.9, 138.8, 138.8, 138.7, 137.9, 129.9, 128.8, 128.6, 128.5, 128.4, 128.4, 128.3, 128.2, 128.1, 127.9, 127.8, 127.6, 127.4, 127.2, 127.0, 101.6 (C-1), 101.4 (C-1), 101.3 (C-1), 101.3 (C-1), 101.1 (C-1), 100.7 (C-1), 82.2, 80.4, 80.4, 80.2, 76.9, 76.6, 76.0, 75.7, 75.6, 75.4, 75.3, 75.1, 74.9, 74.9, 74.7, 74.5, 74.2, 72.9, 72.8, 72.7, 72.4, 71.5, 69.1, 67.6, 66.8, 65.2, 63.0, 62.2, 62.1, 62.0, 32.1, 28.3 (t, $J_{C\text{-}F}$ = 21.9 Hz), 22.9, 21.1, 14.3

HRMS (ESI): $[M + H]^+$ calcd for $C_{135}H_{146}F_{17}O_{32}^+$ 2601.9520, found 2601.9453

HPLC trace of the automated synthesis of **10**

www.manaraa.com

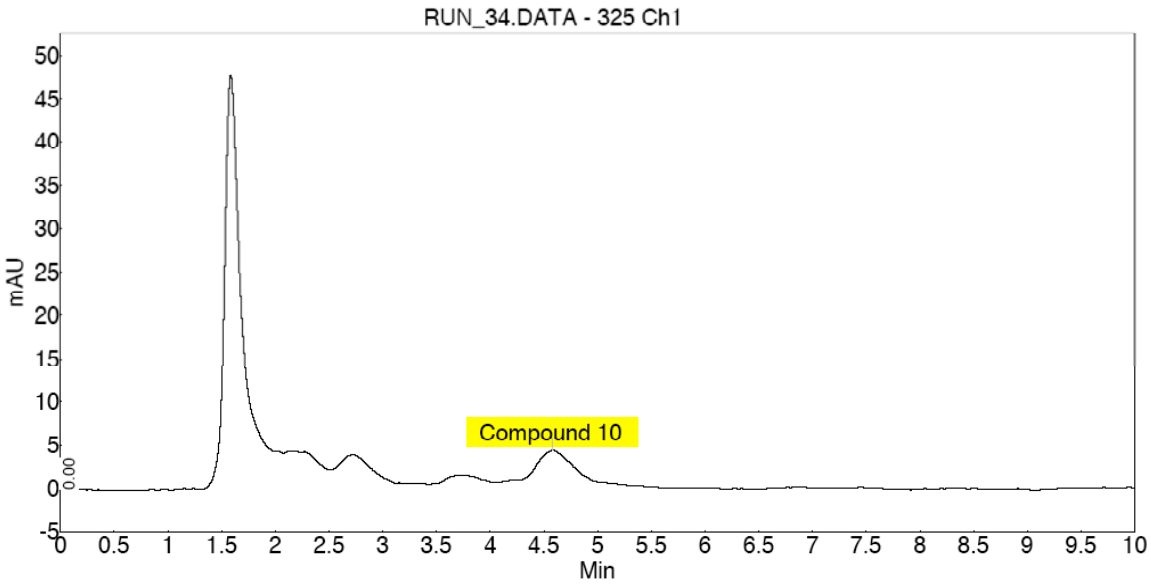
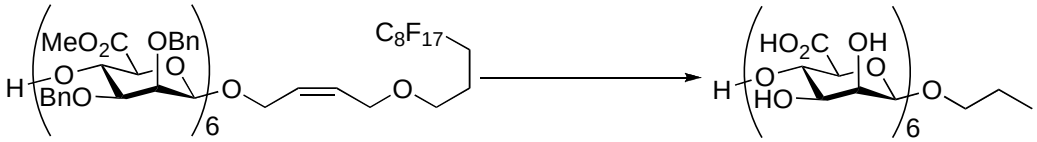


Figure 5. Analytical HPLC chromatogram of the crude product from the synthesis platform after FSPE (1.0 mL/min flowrate, MeOH/DCM: 2.5/97.5, 10 minutes run, Waters Nova-Pak 4 μm 3.9 × 150 mm silica column)



***n*-propyl (4-*O*-(4-*O*-(4-*O*-(4-*O*-(4-*O*-(4-*O*-(β-D-mannopyranosyl uronate)-β-D-mannopyranosyl uronate)-β-D-mannopyranosyl uronate)-β-D-mannopyranosyl uronate)-β-D-mannopyranosyl uronate)-β-D-mannopyranoside) uronic acid (11)**

cis-4-(1H,1H,2H,2H,3H,3H-perfluoroundecyloxy)-2-butenyl (methyl 2,3-di-*O*-benzyl-4-*O*-(methyl 2,3-di-*O*-benzyl-4-*O*-(methyl 2,3-di-*O*-benzyl-4-*O*-(methyl 2,3-di-*O*-benzyl-4-*O*-(methyl 2,3-di-*O*-benzyl-4-*O*-(methyl 2,3-di-*O*-benzyl-β-D-mannopyranosyl uronate)-β-D-mannopyranosyl uronate)-β-D-mannopyranosyl uronate)-β-D-mannopyranosyl uronate)-β-D-

www.manaraa.com

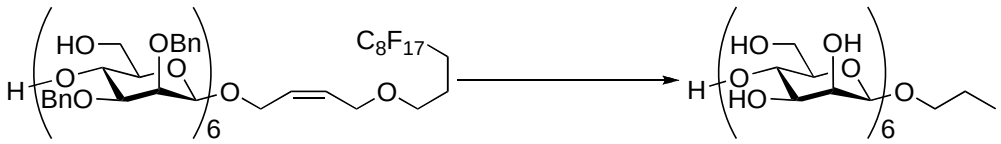
mannopyranosyl uronate)-β-D-mannopyranoside uronate) **9** (18 mg, 6.5 μmol), was dissolved in dry DCM (2.0 mL) and Grubbs catalyst 2nd generation (1.3 mg, 1.5 μmol) was added. The mixture was stirred and bubbled by ethylene gas for 30 min under 20 °C, then an ethylene balloon was attached and the mixture was stirred for 24 h. After the TLC showed the completion of the reaction, the mixture was passed through a short silica column (DCM → EtOAc/petroleum ether = 1/1 → EtOAc/petroleum ether = 3/2). After the product fraction was collected and the solvent was removed under reduced pressure, the crude product was dissolved in freshly distilled THF (1.6 mL), and 0.45 M KOH solution (0.35 mL) and water (0.5 mL) was added under Ar atmosphere. After the mixture was stirred at 20 °C under Ar atmosphere for 1 h, the reaction was neutralized by 0.45 M AcOH in THF (0.4 mL) and diluted with EtOAc (5.0 mL) and washed with water. The aqueous layer was extracted with EtOAc (5.0 mL × 3 times). The organic layer was collected and the solvent was removed under reduce pressure. The crude product was dissolved in MeOH/AcOH (3.0 mL/0.15 mL), and 10% Pd/C (11.0 mg) was added. The mixture was stirred at 20 °C under 1000 psi H_2 atmosphere for 24 h. An additional portion of Pd black (10.0 mg) was added and the reaction was stirred at 20 °C under 1000 psi H_2 atmosphere for another 24 h. The mixture was filtered through a small pad of Celite and the Celite pad was washed with MeOH (10 mL), 80% MeOH (10 mL), and water (10 mL). The collected filtrate was concentrated under reduced pressure and the residue was rinsed with DCM (1.0 mL × 3). The fully-deprotected product **11** was obtained as a white film (4.4 mg, 3.9 μmol, 61% over 3 steps) (van den Bos et al. 2006; Codee et al. 2009).

www.manaraa.com

^{1}H NMR (D_2O, 700 MHz): δ (ppm) 4.79 (s, 2H, 2 × H-1), 4.74 (s, 1H, H-1), 4.71 (s, 2H, 2 × H-1), 4.69 (s, 1H, H-1), 4.07 (d, 5H, *J* = 4.9 Hz), 4.02 (s, 1H), 3.96-3.94 (m, 5H), 3.88-3.77 (m, 13H), 3.70 (d, 1H, *J* = 9.1 Hz), 3.64 (dd, 1H, *J* = 16.8, 7.0 Hz), 1.64 (m, 2H, C*H*$_2$CH$_3$), 0.93 (t, 3H, *J* = 7.7 Hz, C*H*$_3$)

^{13}C NMR (D_2O, 175 MHz): δ (ppm) 174.7, 100.4, 100.3, 100.1, 78.5, 78.2, 78.1, 75.8, 75.5, 72.7, 72.1, 71.7, 71.5, 70.5, 70.1, 70.0, 68.4, 22.3, 9.8

HRMS (ESI): $[M + Na]^+$ calcd for $C_{39}H_{56}NaO_{37}^+$ 1139.2393, found 1139.2374



***n*-propyl (4-*O*-(4-*O*-(4-*O*-(4-*O*-(4-*O*-(β-D-mannopyranosyl)-β-D-mannopyranosyl)-β-D-mannopyranosyl)-β-D-mannopyranosyl)-β-D-mannopyranosyl)-β-D-mannopyranoside) (12)**

cis-4-(1H,1H,2H,2H,3H,3H-perfluoroundecyloxy)-2-butenyl (2,3-di-*O*-benzyl-4-*O*-(2,3-di-*O*-benzyl-4-*O*-(2,3-di-*O*-benzyl-4-*O*-(2,3-di-*O*-benzyl-4-*O*-(2,3-di-*O*-benzyl-4-*O*-(2,3-di-*O*-benzyl-β-D-mannopyranosyl)-β-D-mannopyranosyl)-β-D-mannopyranosyl)-β-D-mannopyranosyl)-β-D-mannopyranosyl)-β-D-mannopyranoside) **10** (9.7 mg, 3.7 μmol), was dissolved in dry DCM (2.0 mL) and Grubbs catalyst 2nd generation (1.3 mg, 1.5 μmol) was added. The mixture was stirred and bubbled by ethylene gas for 30 min under 20 °C, then an ethylene balloon was attached and the mixture was stirred for 24 h. After the TLC showed the completion of the reaction, the mixture was passed through a short silica column (EtOAc → MeOH/DCM = 0.5/9.5). After the product fraction was collected and the solvent was

www.manaraa.com

removed under reduced pressure, the crude product was loaded on a preparative TLC plate and developed by MeOH/DCM = 0.5/9.5. The desired product was collected and dissolved in MeOH (1.5 mL) and 10 % Pd/C (10 mg) was added. The mixture was stirred under 20 °C, 1000 psi H_2 atmosphere. After 24 h, a portion of Pd black (10 mg) was added and the mixture was stirred under 20 °C, 1000 psi H_2 atmosphere for additional 24 h. The mixture was filtered through a small pad of Celite and the Celite pad was washed with MeOH (10 mL), and 80% MeOH (10 mL). The collected filtrate was concentrated under reduced pressure and the residue was rinsed with DCM (1.0 mL × 3). The fully-deprotected product **12** was obtained as a white film (2.8 mg, 2.7 μmol, 73% over 2 steps) (Crich et al. 2004a).

^{1}H NMR (D_2O, 700 MHz): δ (ppm) 4.79-4.78 (m, 4H, 4 × C-1), 4.76 (s, 1H, C-1), 4.72 (s, 1H, C-1), 4.15 (s, 3H), 4.09 (d, 2H, *J* = 9.1 Hz), 3.98-3.92 (m, 8H), 3.86-3.83 (m, 10H), 3.80-3.74 (m, 8H), 3.69-3.63 (m, 3H), 3.59-3.52 (m, 6H), 3.48 (t, 1H, *J* = 9.1 Hz), 1.66 (m, 2H, C*H*$_2$CH$_3$), 0.94 (t, 3H, *J* = 7.0 Hz, C*H*$_3$)

^{13}C NMR (D_2O, 175 MHz): δ (ppm) 100.4, 100.0, 77.0, 76.8, 76.8, 76.6, 75.2, 75.1, 73.0, 71.9, 71.6, 70.7, 70.2, 69.9, 68.7, 66.9, 61.2, 60.7, 23.5, 22.3, 20.2, 9.8

HRMS (ESI): $[M + Na]^+$ calcd for $C_{39}H_{68}NaO_{31}^+$ 1055.3637, found 1055.3620

www.manaraa.com

CHAPTER 4

Automated Solution-Phase Synthesis of Insect Glycans to Probe the Binding Affinity of Pea Enation Mosaic Virus

Shu-Lun Tang and Nicola L. B. Pohl*

Collaboration with Luke Linz and Bryony Bonning, Department of Entomology, Iowa State University

Abstract

Pea enation mosaic virus (PEMV) is an RNA plant virus that infects pea, sweet pea, and related plants. Pea aphids and green pea aphids are the specific vectors for PEMV; however, the transmission pathway within the aphids remains unclear. The endocytosis of the PEMV into the hemocoel happens in the aphid gut, a process that is probably mediated by the heavily glycosylated receptor aminopeptidase N. To investigate the role of the glycans on the receptor in the PEMV transmission pathway, the insect *N*-glycan terminal trimannosides have been synthesized by automated solution-phase synthesis. The insect *N*-glycan terminal trimannosides with fluorous tags (F-tags) attached were then used to form a fluorous microarray to analyze the binding with fluorescein isothiocyanate labeled PEMV (FITC-PEMV). No specific binding was detected between the insect glycan and PEMV on the array. To confirm these microarray results, the fluorous tag was removed from the insect *N*-glycan terminal trimannosides for isothermal titration calorimetry (ITC) studies with unlabeled PEMV. The ITC studies confirmed the microarray results and suggested that this specific interaction in the transmission pathway of PEMV does not rely on the glycan-virus

www.manaraa.com

interaction. Further investigations of the binding of the receptor protein and PEMV are ongoing.

Introduction

Pea enation mosaic virus (PEMV) is a plant virus that infects pea, sweet pea, and other related plants. PEMV is a RNA virus that belongs to the luteovirus family, and the virion is assembled by 180 identical subunit proteins (molecular weight 21.8 KDa for each subunit) (Gray and Gildow 2003; Liu et al. 2006). PEMV has very specific transmission pathway and is transmitted by aphids, which cause serious agricultural losses across the world. However, the transmission pathway of luteovirus within its vectors remains unclear. The transmission of luteovirus is highly specific toward the vectors. For PEMV, only pea aphids and green pea aphids are responsible for the transmission. Also, PEMV is the only luteovirus that can enter the transmission pathway in the pea aphid hemocoel without exiting the aphid in the honeydew excrement. Based on earlier studies, the transmission pathway starts from the endocytosis of the PEMV at the epithelial tissue in the aphid gut (Gray and Gildow 2003; Liu et al. 2006). The most probable receptor protein for triggering the endocytosis of the virus is aminopeptidase N, which is the most abundant protein in the aphid gut and also heavily glycosylated. In order to investigate the specific interaction between the aminopeptidase N and PEMV, studying the binding of the aminopeptidase N glycans and PEMV subunit proteins is necessary since each subunit protein could be a potential glycan binding protein. *N*-linked glycans are found on glycoproteins from bacteria to mammals (Gamblin et al. 2009; Schmaltz et al. 2011). The most common insect *N*-glycan structures share a common terminal trimannoside structure containing a β-mannoside with two α-

www.manaraa.com

mannosides attached at the O-3 and O-6 positions. Glycan microarray and isothermal titration calorimetry have been used to study mammalian virus-mammalian glycan interaction (Neu et al. 2008, Song et al. 2011), however, the plant virus-insect glycan interaction has not been studied by neither of the methods. In order to probe this PEMV-insect glycan binding affinity, the terminal trimannoside was synthesized by automated solution-phase synthesis and the binding affinity was tested by fluorous microarray (Mamidyala et al. 2006; Jaipuri et al. 2008; Ko et al. 2005) and isothermal titration calorimetry (ITC).

Results and discussion

To construct the insect *N*-glycan terminal trimannoside by automated solution-phase synthesis, we designed the mannuronate building block **6** with the C-5 carboxylate as the β-directing group, a 3-*O*-*p*-methoxybenzyl (PMB) group as the temporary capping group for the chain extension at the 3-OH. The methyl ester is acting as a capping protecting group, which can be deprotected by a hydride reduction to release a free 6-OH for the further attachment of the α-mannoside. The synthesis of the building block started from the known 4,6-*O*-benzylidene protected allyl mannoside **1** (Crich et al. 2004b), which could be synthesized from D-mannose in 7 steps. After a selective cleavage of the benzylidene by borane tetrahydrofurane complex (BH_3-THF) and dibutylboron triflate (Bu_2BOTf) (Jiang and Chan 1998), compound **2** with a free hydroxyl group at the 6-position was formed. The combination of 2,2,6,6-tetramethyl-1-piperidinyloxy free radical (TEMPO) and (diacetoxyiodo)benzene (BAIB) oxidized the primary alcohol on **2** and gave the mannuronic acid **3** (van den Bos et al. 2006). After esterification of the carboxylic acid, the anomeric

www.manaraa.com

thiophenyl group of the methyl mannuronate **4** was removed with *N*-bromosuccinimide (NBS) to give **5** with a free anomeric hydroxyl group (Liew and Wei 2002). The subsequent formation of the trichloroacetimidate at the anomeric hydroxyl gave the desired trichloroacetimidate donor **6** for automated solution-phase synthesis (Figure 1).

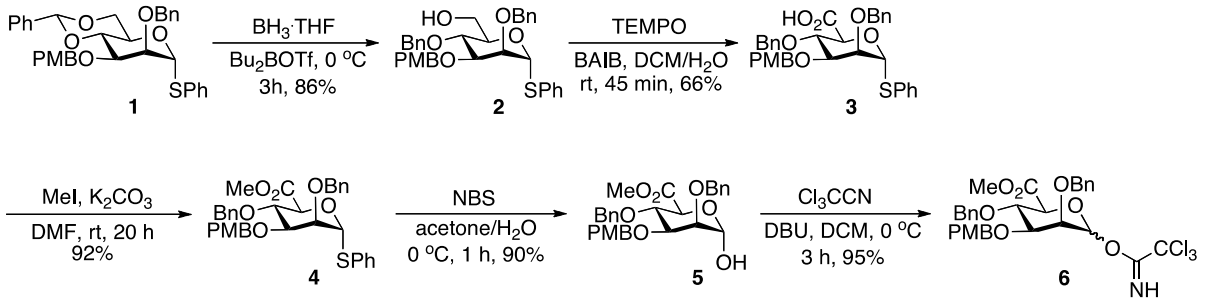


Figure 1. Synthesis of the trichloroacetimidate donor **6**

The automated solution-phase synthesis of the insect glycan started from the glycosylation of the allyl C_8F_{17} fluorous tag (F-tag) (Mamidyala et al. 2006) and the donor **6** (3.0 equiv.) catalyzed by trimethylsilyl trifluoromethanesulfonate (TMSOTf, 0.1 equiv.) at -20 °C. After 45 min, the reaction was quenched by triethylamine (TEA) and the solvent was removed by the evaporation cycle. The PMB group was removed by ceric ammonium nitrate (CAN) and the reaction mixture was purified by fluorous solid phase extraction (FSPE); then the product was transferred out of the synthesis platform for further purification. The β-mannuronate monomer **7** was obtained in 78% yield over 2 steps. The monosaccharide **7** was reinjected into the synthesis platform, and the methyl ester was reduced by lithium triethylborohydride (LiTEBH) at 0 °C. After fluorous solid phase extraction (FSPE), the diol was glycosylated with the known 2-*O*-acetyl-mannosyl-trichloroacetimidate donor **8** (6.0

www.manaraa.com

equiv.) (Song et al. 2010) catalyzed by TMSOTf (0.1 equiv.) to provide the two α-mannosidic linkages via neighboring group participation. The crude trisaccharide **9** was transferred out of the synthesis platform; however, the desired trisaccharide could not be readily isolated from the mixture at this stage. However, after deacetylation, the trimannoside **10** could be isolated and obtained in 50% yield over 2 automation steps and one bench-top deprotection step (Figure 2).

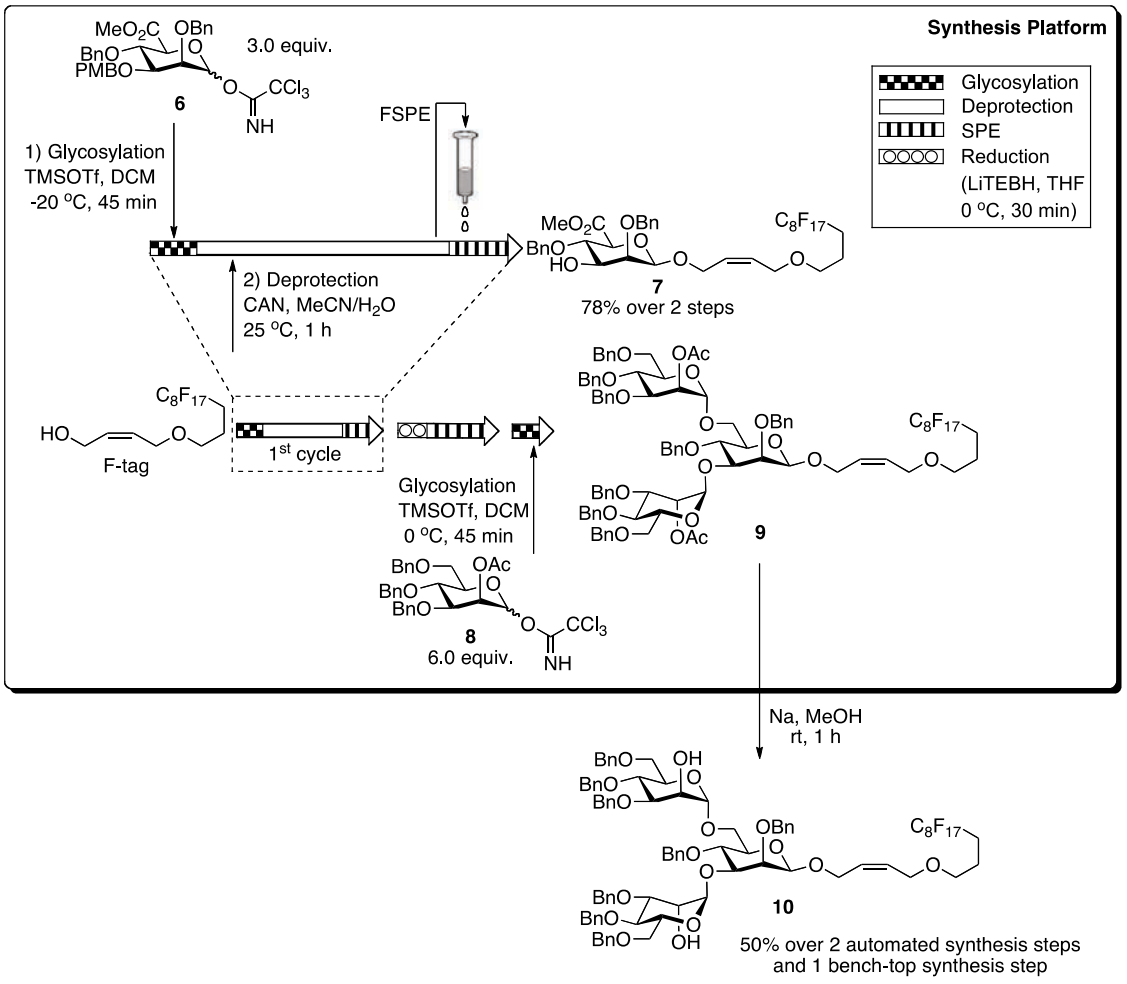


Figure 2. Automated solution-phase synthesis of the insect *N*-glycan terminal trimannoside

www.manaraa.com

The final deprotection of the benzyl groups of **10** was completed by hydrogenolysis catalyzed by 10% Pd/C under 1000 psi hydrogen at 20 °C for 48 h and the fully deprotected trimannoside **11** with fluorous tail was obtained in 51% yield. With the reducing end fluorous tag, the trimannoside **11** was ready for the fluorous microarray experiment with the fluorescein isothiocyanate labeled PEMV (FITC-PEMV). The synthesis of the trimannoside **12** for the ITC experiment started from the cleavage of the F-tag of **10** by olefin cross-metathesis catalyzed by Grubbs 2nd catalyst generation under ethylene atmosphere to provide the allyl trimannoside. The following hydrogenolysis catalyzed by 10% Pd/C, and Pd black under 1000 psi hydrogen atmosphere converted the allyl trimannoside into the fully deprotected *n*-propyl trimannoside **12** in 91% over 2 steps and which was ready for the ITC experiments with unlabeled PEMV (Figure 3).

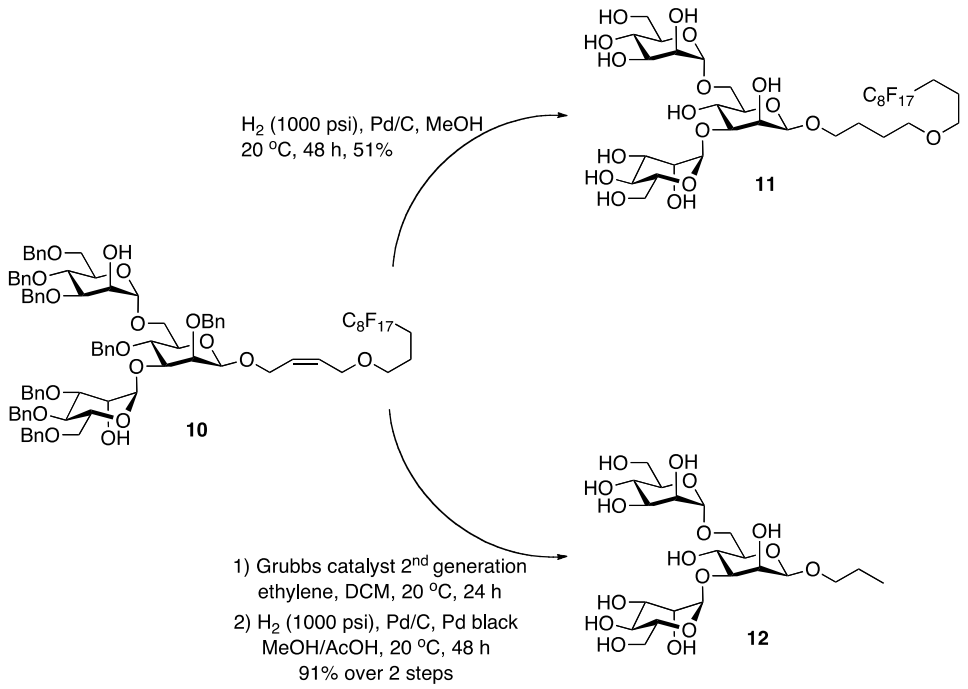


Figure 3. Deprotection of the insect *N*-glycan terminal trimannosides

www.manaraa.com

With the reducing end F-tag, the insect *N*-glycan trimannoside **11** could be immobilized on a C_8F_{17}-coated commercially available glass slide for the proposed microarray experiments with the PEMV. The virus used in the experiment was labeled by FITC for visualizing the possible binding, and the concentration of PEMV subunit protein is used for the experiment since each subunit protein is considered one potential glycan binding protein. The control sugars of the experiment were the known F-tag-modified α-mannoside and β-galactoside; the FITC labeled concanavalin A (FITC-ConA) was the positive control for binding. In the experiment, the F-tagged sugars were spotted on the fluorous glass slide by a microarray spotter and allowed to slowly dry in a humidified chamber. Then, the slide was incubated with solutions of FITC-PEMV or FITC-ConA for 1 h. The following rinsing step removed the unbound protein; then the slide was air dried for the scanning step. From the results of the microarray control experiment of F-tagged α-mannoside (Mamidyala et al. 2006), β-galactoside (Mamidyala et al. 2006), and **11** incubated with FITC-ConA, the α-mannoside and **11** showed strong binding as expected and the β-galactoside showed no binding as expected. However, the FITC-PEMV incubated slide also showed no binding with any of the saccharides even with elevated incubation concentrations (Figure 4).

www.manaraa.com

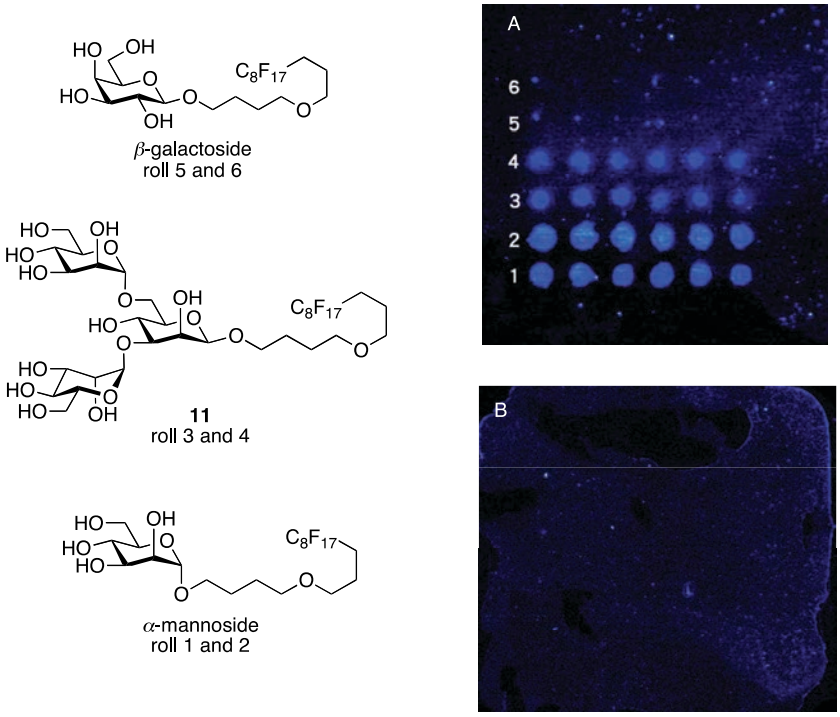


Figure 4. Fluorous microarray of F-tag attached saccharides incubated with A. 0.2 μM of FITC-ConA; B. 1.6 μM of FITC-PEMV subunit protein

To confirm the negative result found in the fluorous microarray experiment, ITC experiments were commenced. The *n*-propyl trimannoside **12** was used for the ITC experiment with the commercially available methyl α-mannoside as the control group. ConA was the positive control for comparison with the PEMV, and also the concentration of PEMV subunit protein is used for the ITC experiments since each subunit protein is considered one potential glycan binding protein. The ITC experiments involved titration of the ligand (saccharide) solution into a cell with the macromolecule solution. By comparing the temperature of the mixing cell and the reference cell, the machine inputs an energy pulse to equalize the temperature in the two cells after each injection of the ligand solution. At the end of each titration experiment, K_d, ΔH, ΔS, and N could be extracted from the resulting

www.manaraa.com

data. In the ITC experiments with the two saccharides and ConA, there was obvious specific binding (K_d = 11.15 μM for methyl α-mannoside, and K_d = 3.27 μM for **12**). However, with PEMV, both methyl α-mannoside and **12** did not show any noticeable binding (see Figure 9-12 in experimental section). This result combined with the fluorous microarray data provides strong evidence that there is no observable specific binding with the insect *N*-glycan terminal trimannoside and the PEMV. Hence, the terminal glycans coated on the aminopeptidase N in the pea aphid gut do not appear to be responsible for the specific binding with the PEMV.

Conclusion

The automated solution-phase synthesis of the insect *N*-glycan trimannoside **11** and **12** was the first automated synthesis of the *N*-glycan related structures by using the β-directing mannuronate strategy. Results from the fluorous microarray and ITC showed no specific binding between the trimannosides and the PEMV and provide evidence that this specific interaction in the transmission pathway of PEMV does not only rely on the terminal glycan-virus interaction. Related studies have been initiated to see whether the aminopeptidase N without the sugar coating has any specific binding interaction with PEMV or whether both the peptide and sugar parts are responsible for this interaction.

Experimental section

General materials and methods

Dichloromethane (DCM) for glycosylation was distilled from calcium hydride. Tetrahydrofuran (THF) was collected from PureSolv Micro solvent purification system

www.manaraa.com

(Innovative Technology, Inc., Amesbury, MA) before reactions. All other commercial solvents and reagents were reagent grade and used as received without further purification. The reactions were monitored by thin layer chromatography (TLC) with 250 μm Sorbent Technologies silica gel HL TLC plates. The hydrogenation reaction under 1000 psi hydrogen was operated in the Parr model 4766 general purpose vessel high pressure reactor (Parr Instrument Company, Moline, IL). The developed TLC plates were visualized by stain with *p*-anisaldehyde solution followed by heating on a hot plate. Flash column chromatography was performed with Zeochem ZEOprep 60 silica gel, 40-63 μm particle size. The automated solution phase synthesis was performed in the Chemspeed ASW1000 (Chemspeed, Augst, Switzerland) synthesis platform with hood, 16 reactor vials (13-mL capacity each) and heating/cooling unit (200 °C to –20 °C) machined to hold the SPE cartridges at the Iowa State University Machine Shop. ^{1}H and ^{13}C NMR spectra were obtained at 400 MHz and 100 MHz on a Bruker DRX-400 spectrometer and a Varian MR-400 spectrometer and also 600 MHz and 150 MHz on a Bruker Avance III 600 spectrometer. The C-H coupling constants were measured by the coupled ^{13}C NMR spectra. Chemical shifts (δ) were reported in parts per million (ppm) relative to $CDCl_3$ and CD_3OD as internal references. Mass spectra were obtained on a Finnigan TSQ700 triple quadrupole mass spectrometer (Finnigan MAT, San Jose, CA) fitted with a Finnigan ESI interface. HPLC traces were obtained from a Varian ProStar HPLC system using a Waters Nova-Pak 4 μm 3.9 × 150 mm silica column.

General procedure for bench-top fluorous solid-phase extraction (FSPE)

Crude product (less than 300 mg) was dissolved in 0.4 mL *N*,*N*-dimethylformamide (DMF) and loaded onto the 80% MeOH preconditioned 2 g FSPE cartridge. The cartridge

www.manaraa.com

was washed with 80% MeOH (4.0 mL × 3 times). Then the product was washed out with acetone (12 mL). The solvent was removed under reduce pressure to obtain the desired product.

General procedure for automated synthetic cycles to produce insect glycan trimannoside

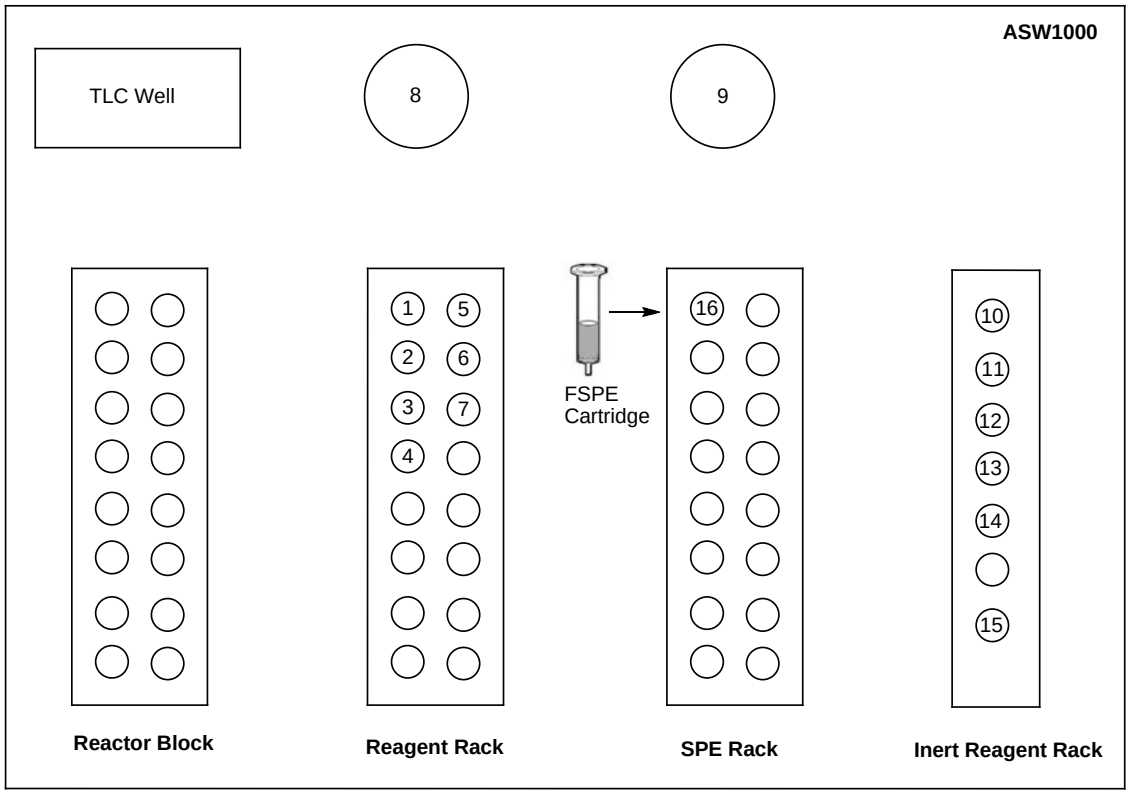


Figure 5. Basic layout of automated solution-phase oligosaccharide synthesizer (ASW1000) 1: F-tagged acceptor (solution in DCM), 2: TEA, 3: DMSO, 4: LiTEBH (1.0 M in THF), 5: water, 6: CAN/MeCN, 7: MeOH, 8: MeCN, 9: reservoir (toluene), 10: TMSOTf (0.55 M in DCM), 11: donor, 12: donor, 13: DCM, 14: THF, 15: 80% MeOH, 16: FSPE cartridge.

www.manaraa.com

1. Sample Preparation

Mannuronate donor molecule (0.20 g, 0.30 mmol) was dissolved in anhydrous DCM (1.6 mL) in the 13 mL-vial and placed at the inert reagent rack (Donor) under argon atmosphere. Mannosyl donor molecule (0.19 g, 0.30 mmol) was dissolved in anhydrous DCM (1.6 mL) in the 13 mL-vial and placed at the inert reagent rack (Donor) under argon atmosphere A 0.055 M trimethylsilyltrifluoromethanesulfonate (TMSOTf) solution (5 mL) in anhydrous DCM was prepared in an 8 mL-vial and placed as indicated on the inert reagent rack under argon. MeOH (100 mL) was placed in the stock solution bottle at the stock solution station as indicated. Toluene (1 L) was placed in the stock solution bottle and placed at the reservoir bottle rack with tubing as reservoir solution for rinsing. Anhydrous DCM (20 mL) was transferred to the 50 mL-vial and placed as indicated on the inert reagent rack under argon. Anhydrous tetrahydrofuran (THF, 10 mL) was transferred to the 50 mL-vial and placed as indicated on the inert reagent rack under argon. 80% methanol/water (100 mL) stock was prepared in the 100 mL-vial and placed as indicated on the inert reagent rack. F-tagged acceptor molecule (50 μmol) was dissolved with anhydrous DCM (0.8 mL) in a Wheaton 8 mL-E-Z extraction vial (conically-bottomed) flushed with argon, capped with septa and placed at the reagent rack as indicated. Triethylamine (TEA, 5 mL) was transferred to an 8 mL-vial capped with septa and placed at the reagent rack as indicated. Dimethylsufoxide (DMSO, 8 mL) was transferred to an 8 mL-vial capped with septa and placed at the reagent rack as indicated. A 0.45 M CAN (ceric ammonium nitrate) solution in acetonitrile/water (9/1) (5 mL) was prepared in an 8 mL-vial capped with septa and placed at the reagent rack as indicated. LiTEBH solution in THF (1.0 M, 3 mL) was transferred to an 8 mL-vial capped with septa and placed at the reagent rack as indicated. Water (8 mL) was

www.manaraa.com

transferred to an 8 mL-vial capped with septa and placed at the reagent rack as indicated. A fluorous solid phase extraction (FSPE) cartridge (2 g, 10 cc) was preconditioned with 80% methanol/water and placed on the machined FSPE block as indicated. An empty Wheaton 8 mL-E-Z extraction vial was placed under the FSPE cartridge.

2. Cleaning Cycle

Prior to introduction of reagents, the ASW1000 reactor vials were cleaned, dried and purged with argon by running the cleaning cycle. During the cleaning cycle, each of the 16 reactor vials (13 mL capacity each) was rinsed with toluene (8 mL) and methanol (8 mL) 3 times each. After the solvents were completely removed, the reactor vials were dried under vacuum and purged with argon for 45 minutes. Reagent solutions were prepared by azeotropic removal of water from each building block with toluene; the resulting building blocks were then dried under high vacuum. After the cleaning cycles were done, the reagents were transferred into the reagent vials respectively, which were then placed on the inert condition reagent rack and general atmosphere reagent rack.

3. Glycosylation

The needle transferred the acceptor molecule (F-tag) solution (0.8 mL) to the reaction vial 1, followed by the transfer of the donor molecule solution (0.8 mL). The mixture was vortexed under ambient temperature at 800 rpm for 20 min. Then the reactor vials were cooled to -20 °C during the 60 minutes wait time by the heat transfer oil with 800 rpm vortex rate. The TMSOTf solution (0.1 mL) was transferred into the reactor vial 1 under 200 rpm vortex rate. After each individual transfer, the needle (inside and outside) was rinsed by

www.manaraa.com

toluene (2 ml) before operating the next task. The reaction mixture was vortexed at 800 rpm for 30 minutes at 0 °C under argon atmosphere. After the reaction time the needle withdrew 20 μL of the solution from the reaction mixture and placed it into the first well of the microtiterplate for thin layer chromatography monitoring. TEA (0.05 mL) was added to the solution for quenching and the solvent was evaporated under reduced pressure.

4. PMB deprotection

To the dried residue after the glycosylation, The CAN solution (1.0 mL) was added to the reactor vial. The reaction mixture was vortexed at 800 rpm for 1 h at room temperature. After the reaction time the needle withdrew 20 μL of the solution from the reaction mixture and placed it into the second well of the microtiterplate for thin layer chromatography monitoring.

5. Fluorous solid-phase extraction (FSPE)

The reaction mixture (1.2 mL) was carried to the FSPE cartridge at the FSPE block and dispensed at a speed of 1 mL/s via the 10 mL syringe. Then 80% methanol (2.0 mL) was used to rinse the empty reactor vial. The 80% methanol solution was removed from the reactor vial and delivered to the FSPE cartridge. The 80% methanol rinsing and transferring was repeated one more time. Additional 80% methanol solution (4 mL, repeated 2 times) was used to rinse the FSPE cartridge. During the 80% methanol rinse, the cartridge was positioned at ‘SPE waste’ for the eluted mixture to be disposed. Acetonitrile (MeCN) (2.0 mL, repeated 3 times) was used to wash the FSPE cartridge for eluting the desired compound. During the task, the FSPE cartridge was positioned as ‘SPE collect’ to be placed

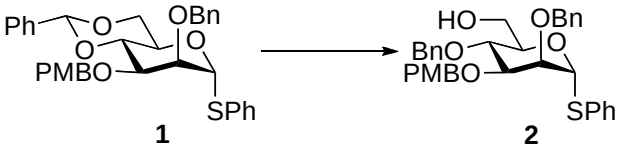
www.manaraa.com

right above the 8 mL vial for collection of the sample. After the task, the position of the SPE rack was changed into 'SPE direct' for the needle to withdraw the collected sample from the conically-bottomed vial and deliver it to the clean reactor vial for the next reaction. Toluene (1 mL) was added to the solution and solvent was evaporated under reduced pressure. After the evaporation cycle, The MeOH wash step and the evaporation were repeated one more time. Once again toluene (1 mL) was added and removed under reduced pressure to remove residual water.

7. LiTEBH Reduction

Anhydrous THF (1.0 mL) was added to the sample and vortexed at 800 rpm, 0 °C under argon atmosphere for 30 min. The LiTEBH (0.3 mL, 1.0 M in THF) was added to the reaction solution and the mixture was vortexed for 45 min at 800 rpm, 0 °C under argon atmosphere. After the reaction time the needle withdrew 20 μL of the solution from the reaction mixture and placed it into the fourth well of the microtiterplate for thin layer chromatography monitoring. MeOH (0.5 mL) was added to quench the reaction and the solvent was removed by evaporation cycle.

Synthetic procedures



Phenyl 2,4-di-*O*-benzyl-3-*O*-*p*-methoxybenzyl-thio-α-D-mannopyranoside (2)

To the phenyl 2-*O*-benzyl-3-*O*-*p*-methoxybenzyl-4,6-*O*-benzylidene-thio-α-D-

www.manaraa.com

mannopyranoside (**1**) (0.80 g, 1.4 mmol) was added borane terahydrofuran complex 1.0 M solution in tetrahydrofuran (14 mL, 14 mmol) under argon atmosphere at 0 °C and stirred until the starting material was dissolved. Then the dibutylboryl trifluoromethanesulfonate 1.0 M solution in DCM (1.7 mL, 1.7 mmol) was added dropwise and the reaction mixture was stirred under argon atmosphere at 0 °C for 3 h. Triethylamine (0.30 mL, 2.2 mmol) was added dropwise to the reaction then methanol was added slowly to quench the reaction under 0 °C. The solvent was removed under reduced pressure and the crude mixture was coevaporated with methanol twice. The crude product was purified by flash column chromatography on silica gel using EtOAc/petroleum ether (1/2) as eluent. The product was obtained as a colorless syrup (0.69 g, 1.2 mmol, 86%)

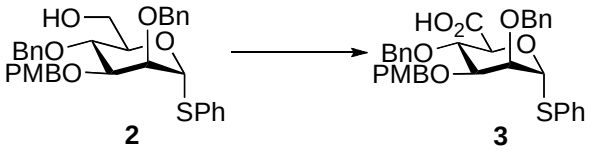
***R**_f*: 0.38 (EtOAc/petroleum ether: 1/2)

^{1}H NMR ($CDCl_3$, 400MHz): δ (ppm) 7.41-7.26 (m, 17H, H_{arom}), 6.89-6.86 (m, 2H, H_{arom}), 5.50 (d, 1H, *J* = 1.6 Hz, H-1), 4.96 (d, 1H, *J* = 10.8 Hz, CH*H*Ph), 4.69-4.64 (m, 2H, CH*H*Ph), 4.58 (s, 2H, CH*H*Ph), 4.13 (m, 1H, H-5), 4.04 (t, 1H, *J* = 9.2 Hz, H-4), 3.98 (dd, 1H, *J* = 2.8, 1.6 Hz, H-2), 3.90 (dd, 1H, *J* = 9.2, 3.2 Hz, H-3), 3.82 (s, 3H, CO_2CH_3), 3.81-3.75 (m, 2H, 2 × H-6)

^{13}C NMR ($CDCl_3$, 150 MHz): δ (ppm) 159.4, 138.5, 138.0, 134.1, 131.9, 130.6, 130.3, 129.6, 129.4, 129.2, 128.5, 128.2, 128.0, 127.9, 127.9, 127.7, 114.0, 86.1, 79.8, 76.6, 75.3, 74.8, 73.4, 72.4, 72.0, 62.2, 55.4

HRMS (ESI): $[M + Na]^+$ calcd for $C_{34}H_{36}NaO_6S^+$ 595.2125, found 595.2119

www.manaraa.com



Phenyl 2,4-di-*O*-benzyl-3-*O*-*p*-methoxybenzyl-thio-α-D-mannopyranosiduronic acid (3)

To a solution of Phenyl 2,4-di-*O*-benzyl-3-*O*-*p*-methoxybenzyl-thio-α-D-mannopyranoside **2** (0.50 g, 0.87 mmol) in dichloromethane/water (5.8 mL/2.9 mL) were added TEMPO (0.03 g, 0.19 mmol) and (diacetoxyiodo)benzene (0.700 g, 2.17 mmol) and stirred at ambient temperature. After 45 min, the mixture was diluted with dichloromethane (10 mL) and washed with a 10% $Na_2S_2O_3$ solution (10 mL) and water (10 mL). The organic layer was dried over Na_2SO_4. The solvent was removed under reduced pressure and the crude product was purified by flash column chromatography on silica gel using EtOAc/petroleum ether (1/2 → 1/0) as eluent. The product was obtained as a light yellow syrup (0.34 g, 0.57 mmol, 66%).

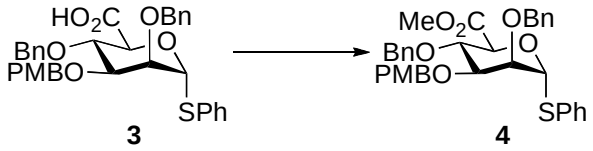
***R**_f*: 0.1 (EtOAc/petroleum ether: 1/2)

^{1}H NMR ($CDCl_3$, 600MHz): δ (ppm) 7.52 (d, 2H, *J* = 6.6 Hz, H_{arom}), 7.33-7.24 (m, 13H, H_{arom}), 7.19 (d, 2H, *J* = 8.4 Hz, H_{arom}), 6.83 (d, 2H, *J* = 9.0 Hz, H_{arom}), 5.67 (d, 1H, *J* = 4.8 Hz, H-1), 4.69-4.65 (m, 3H, CH*H*Ph), 4.64 (d, 1H, *J* = 6.6 Hz, H-5), 4.52 (m, 3H, CH*H*Ph), 4.21 (t, 1H, *J* = 6.6 Hz, H-4), 3.88 (dd, 1H, *J* = 4.8, 2.4 Hz, H-2), 3.82 (m, 1H, H-3), 3.78 (s, 3H, CO_2CH_3)

^{13}C NMR ($CDCl_3$, 150 MHz): δ (ppm) 174.8, 159.3, 137.8, 137.7, 133.8, 131.4, 129.7, 129.6, 129.0, 128.4, 128.4, 128.4, 128.1, 127.9, 127.9, 127.8, 127.3, 113.8, 75.7, 72.6, 72.6, 72.3, 72.0, 55.2

HRMS (ESI): $[M + Na]^+$ calcd for $C_{34}H_{34}NaO_7S^+$ 609.1917, found 609.1904

www.manaraa.com



Methyl (phenyl 3-*O*-*p*-methoxylbenzyl-2,4-di-*O*-benzyl-thio-α-D-mannopyranoside) uronate (4)

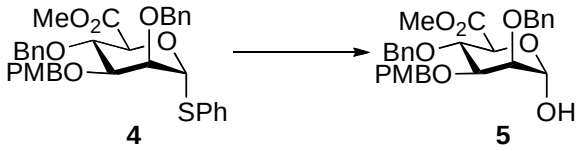
To a solution of phenyl 3-*O*-*p*-methoxybenzyl-2,4-di-*O*-benzyl-thio-α-D -mannopyranosiduronic acid (**3**) (0.50 g, 0.85 mmol) in anhydrous DMF (4.0 mL) were added K_2CO_3 (0.12 g, 0.85 mmol) and iodomethane (0.30 g, 2.1 mmol). The reaction mixture was stirred at ambient temperature under argon atmosphere for 6 h. The mixture was diluted with EtOAc (10 mL) and washed with water (10 mL). The aqueous portion was separated and extracted with EtOAc (2 x 10 mL). The combined organic layer was dried over Na_2SO_4. The solvent was removed under reduced pressure and the crude product was purified by flash column chromatography on silica gel using EtOAc/petroleum ether (1/3) as eluent. The product was obtained as a light yellow syrup (0.47 g, 0.78 mmol, 92%).

^{1}H NMR (CD_3OD, 600MHz): δ (ppm) 7.53 (d, 2H, *J* = 7.2 Hz, H_{arom}), 7.35-7.25 (m, 13H, H_{arom}), 7.25 (d, 2H, *J* = 8.4 Hz, H_{arom}), 6.85 (d, 2H, *J* = 8.4 Hz, H_{arom}), 5.66 (d, 1H, *J* = 4.8 Hz, H-1), 4.66-4.63 (m, 3H, CH*H*Ph), 4.62 (d, 1H, *J* = 6.0 Hz, H-5), 4.52-4.49 (m, 3H, CH*H*Ph), 4.25 (t, 1H, *J* = 6.6 Hz, H-4), 3.87 (d, 1H, *J* = 5.4 Hz, H-2), 3.81 (s, 3H, CO_2CH_3), 3.79 (d, 1H, *J* = 3.0 Hz, H-3), 3.65 (s, 3H, OCH_3)

^{13}C NMR (CD_3OD, 150 MHz): δ (ppm) 169.7, 159.4, 138.1, 137.9, 134.0, 131.5, 130.8, 130.0, 129.6, 129.4, 129.1, 129.0, 128.5, 128.5, 128.4, 128.4, 128.3, 128.3, 128.1, 128.1, 128.1, 128.1, 128.1, 127.9, 127.9, 127.8, 127.4, 127.3, 114.0, 113.8, 76.0, 73.1, 72.4, 72.2, 55.3, 52.3

www.manaraa.com

HRMS (ESI): $[M + Na]^+$ calcd for $C_{35}H_{36}NaO_7S^+$ 623.2074, found 623.2071



Methyl (2,4-di-*O*-benzyl-3-*O*-*p*-methoxylbenzyl-α-D-mannopyranose) uronate (5)

To a solution of methyl (phenyl 2,4-di-*O*-benzyl-3-*O*-*p*-methoxylbenzyl-thio-α-D-mannopyranoside) uronate **4** (0.50 g, 0.83 mmol) in 10% water/acetone (10 mL) were added *N*-bromosuccinimide (0.44 g, 2.5 mmol). The reaction mixture was stirred at ambient temperature for 1 h. The reaction mixture was dilute with EtOAc (30 mL) and washed with a saturated $NaHCO_3$ solution (30 mL). The organic layer was dried over Na_2SO_4. The solvent was removed under reduced pressure and the crude product was purified by flash column chromatography on silica gel using EtOAc/petroleum ether (2/3) as eluent. The product was obtained as a light yellow syrup (0.38 g, 0.75 mmol, 90%).

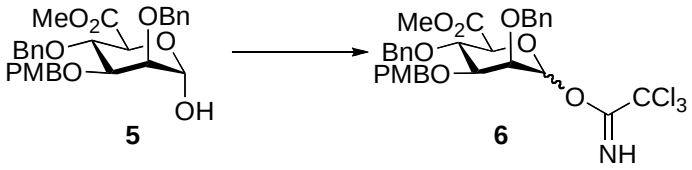
***R**f*: 0.24 (EtOAc/petroleum ether: 1/2)

^{1}H NMR ($CDCl_3$, 400MHz): δ (ppm) 7.36-7.20 (m, 12H, H_{arom}), 6.85 (d, 2H, *J* = 8.4 Hz, H_{arom}), 5.43 (t, 1H, *J* = 4.8 Hz, H-1), 4.75 (d, 1H, *J* = 12.0 Hz), 4.69-4.64 (m, 3H), 4.51 (s. 2H), 4.47 (d, 2H, *J* = 6.0 Hz), 4.22 (t, 1H, *J* = 6.8 Hz), 3.91 (dd, 1H, J = 7.2, 2.8 Hz), 3.80 (s, 3H), 3.71 (m, 1H), 3.65 (s, 3H), 3.18 (d, 1H, *J* = 4.8 Hz), 2.82 (s, 1H)

^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 170.3, 159.3, 138.4, 138.2, 130.3, 129.5, 128.5, 128.5, 128.4, 128.0, 127.9, 127.7, 113.8, 93.0 (J_{C1-H1} = 168.2 Hz, C-1), 77.3, 75.9, 75.5, 74.0, 72.9, 72.4, 72.2, 55.4, 52.4

HRMS (ESI): $[M + Na]^+$ calcd for $C_{29}H_{32}NaO_8^+$ 531.1989, found 531.1985

www.manaraa.com



Methyl (2,4-di-*O*-benzyl-3-*O*-*p*-methoxylbenzyl -α/β-D-mannopyranose) uronate trichloroacetimidate (6)

To a solution of methyl (2,4-di-*O*-benzyl-3-*O*-*p*-methoxylbenzyl-α-D-mannopyranose) uronate (**5**) (0.50 g, 0.98 mmol) in dichloromethane (35 mL) was added trichloroacetonitrile (0.85 g, 5.88 mmol) at 0 °C under argon atmosphere. 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU) (0.03 g, 0.2 mmol) was then added and the reaction mixture was stirred at 0 °C under argon atmosphere for 3 h. The solvent was removed under reduced pressure and the crude product was purified by flash column chromatography on silica gel using EtOAc/petroleum ether/triethylamine (1/2/0.1) as eluent. The product was obtained as a colorless syrup and a mixture of anomers (α/β = 4/1) (0.59 g, 0.93 mmol, 95%).

***R**_f*: 0.56 (EtOAc/petroleum ether: 1/2)

^{1}H NMR ($CDCl_3$, 400MHz): δ (ppm) 9.32 (s, 1H, *H*N), 8.62 (s, 1H, *H*N), 7.41-7.26 (m, 10H, H_{arom}), 7.21 (d, 2H, *J* = 8.8 Hz, H_{arom}), 7.16 (d, 2H, *J* = 8.4 Hz, H_{arom}), 6.83 (d, 2 H, *J* = 8.4 Hz, H_{arom}), 6.40 (d, 1H, *J* = 2.8 Hz, H-1), 5.96 (d, 1H, *J* = 7.6 Hz, H-1), 4.85-4.74 (m, 13H), 4.65-4.45 (m, 18H), 4.39 (d, 1H, *J* = 8.4), 4.28 (t, 1H, *J* = 8.4 Hz), 4.17 (dd, 1H, *J* = 4.4, 2.4 Hz), 3.90-3.87 (m, 2H), 3.83-3.80 (m, 4H), 3.71 (s, 3H), 3.64 (s, 3H)

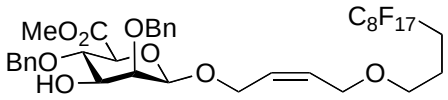
^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 169.4, 169.1, 160.4, 159.5, 159.5, 160.0, 138.0, 137.9, 137.2, 130.0, 129.8, 129.4, 128.7, 128.6, 128.5, 128.3, 128.1, 128.0, 128.0, 113.9, 113.9, 95.8, 95.2, 90.9, 77.3, 75.6, 75.4, 75.2, 75.0, 74.3, 74.1, 73.7, 73.6, 73.0, 72.7, 72.6, 72.3, 55.4, 52.7, 52.6

www.manaraa.com

HRMS (ESI): $[M + Na]^+$ calcd for $C_{31}H_{32}Cl_3NNaO_8^+$ 674.1086, found 674.107

Table 1. Automated synthetic cycles for production of compound **7**

Step	Task	Reagent/ Operation	Operation Time
1	Glycosylation (2 parallel reactions)	3.0 × 2 equivalent donor (150 × 2 μmol) in 0.8 × 2 mL DCM, 1.0 × 2 equivalent F-tagged acceptor (50 × 2 μmol) in 0.8 × 2 mL DCM; 0.11 equivalent TMSOTf, -20 °C	45 min
2	TLC sample	20 × 2 μL of crude reaction mixture withdrawn	
3	Quenching	0.05 × 2 mL TEA	
4	Evaporation	40 °C	45 min
5	PMB deprotection (2 parallel reactions)	1.0 × 2 mL CAN/MeCN/H_2O, 25 °C	1 h
6	TLC sample	20 × 2 μL of crude reaction mixture withdrawn	
7	Sample loading	1.2 × 2 mL crude sample transferred to cartridges	
8	Wash	12 × 2 mL 80% methanol wash	
9	Wash	2 mL MeCN wash (repeated 3 × 2 times)	
10	Transfer	6 × 2 mL collected sample out of the ASW1000	



7

Methyl (*cis*-4-(1H,1H,2H,2H,3H,3H-perfluoroundecyloxy)-2-butenyl -2,4-di-*O*-benzyl-β-D-mannopyranoside) uronate (7)

The crude product solution was transferred out of the ASW1000 after the FSPE step (10th step) of the 1st cycle. The solvent was removed under reduced pressure and the product was purified by flash column chromatography on silica gel using EtOAc/petroleum ether (1/3.5) as eluent. The product was obtained as a colorless syrup (72 mg, 0.078 mmol, 78% over 2 steps).

***R**f*: 0.57 (EtOAc/petroleum ether: 1/2)

www.manaraa.com

^{1}H NMR ($CDCl_3$, 400MHz): δ (ppm) 7.35-7.23 (m, 10H, H_{arom}), 5.74 (m, 2H, *H*C=C*H*), 4.96 (d, 1H, *J* = 12.0 Hz, CH*H*Ph), 4.77 (d, 1H, *J* = 11.2 Hz, CH*H*Ph), 4.63 (s, 1H, H-1), 4.61 (d, *J* = 12.0 Hz, CH*H*Ph), 4.60 (d, *J* = 11.2 Hz, CH*H*Ph), 4.47 (dd, *J* = 12.8, 4.8 Hz, O-CH*H*C=C), 4.24 (dd, *J* = 12.8, 6.4 Hz, O-CH*H*C=C), 4.04 (m, 2H, C=CCH_2-O), 3.99 (t, *J* = 7.6, H-4), 3.90 (d, *J* = 8.0, H-5), 3.81 (d, *J* = 2.4, H-2), 3.78 (m, 1H, H-3), 3.71 (s, 3H, CO_2CH_3), 3.49 (m, 2H, O-CH_2CH_2), 2.69 (d, 1H, *J* = 9.6, 4-O*H*), 2.22 (m, 2H, CH_2CF_2), 1.88 (m, 2H, O-CH_2CH_2)

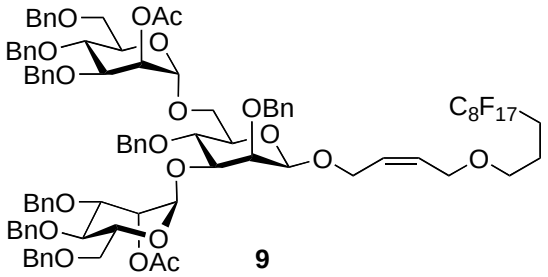
^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 169.4, 138.2, 138.2, 130.6, 128.7, 128.6, 128.4, 128.1, 128.1, 128.0, 100.3 (J_{C1-H1} = 154.8 Hz, C-1), 78.0, 76.0, 74.5, 74.3, 72.3, 69.0, 66.7, 65.1, 52.5, 28.4 (t, J_{C-F} = 21.7 Hz), 21.0

HRMS (ESI): $[M + Na]^+$ calcd for $C_{36}H_{35}F_{17}NaO_8^+$ 941.1953, found 941.1959

www.manaraa.com

Table 2. Automated synthetic cycles for production of compound **9**

Step	Task	Reagent/ Operation	Operation Time
11	Transfer **7** to reactor vial	50 μmol in 3.0 mL EtOAc	
	Evaporation	40 °C	45 min
12	Reduction	1.0 mL THF, 0.3 mL LiTEBH, 0 °C	30 min
13	TLC sample	20 μL of crude reaction mixture withdrawn	
14	Quenching	0.5 mL MeOH	
15	Evaporation	40 °C	45 min
16	FSPE preparation	0.5 mL DMSO	
17	Sample loading	1.2 mL crude sample transferred to cartridges	
18	Wash	12 mL 80% methanol wash	
19	Wash	2.0 mL MeCN wash (repeated 3 times)	
20	Transfer	6.0 mL collected sample to the reactor vial for next cycle	
21	Evaporation	40 °C	45 min
22	Wash	2.0 mL MeCN wash (repeated 3 times)	
23	Evaporation	40 °C	45 min
24	Transfer	1.0 mL toluene	
25	Evaporation	40 °C	45 min
26	Transfer	1.0 mL toluene	
27	Evaporation	40 °C	45 min
28	Glycosylation	6.0 equivalent donor **8** (300 μmol) in 1.6 mL DCM 0.11 equivalent TMSOTf, 20 °C	45 min
29	TLC sample	20 μL of crude reaction mixture withdrawn	
30	Quenching	0.05 mL TEA	
31	Evaporation	40 ˚C	45 min
32	Repeat the same procedure as step 16-23		90 min



9

***cis*-4-(1H,1H,2H,2H,3H,3H-perfluoroundecyloxy)-2-butenyl -2,4-di-*O*-benzyl-3,6-di-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-mannopyranosyl)-β-D-mannopyranoside (9)**

The crude mixture was transferred out of the synthesis platform after the 3rd cycle (32nd step of automated synthesis), diluted with DCM (20mL), and washed with Sat. $NH_4Cl_{(aq)}$. The

www.manaraa.com

organic layer was dried over Na_2SO_4 and the solvent was removed under reduced pressure. Then, the crude product was purified by flash chromatography on silica gel EtOAc/petroleum ether (1/3). The disaccharide by-product could not be separated at this stage and the crude mixture was carried to the next step without further purification.

***R**_f*: 0.47 (EtOAc/petroleum ether: 1/2)

HRMS (ESI): $[M + Na]^+$ calcd for $C_{93}H_{95}F_{17}NaO_{19}^+$ 1861.6088, found 1861.6118

HPLC trace of the automated synthesis of **9**

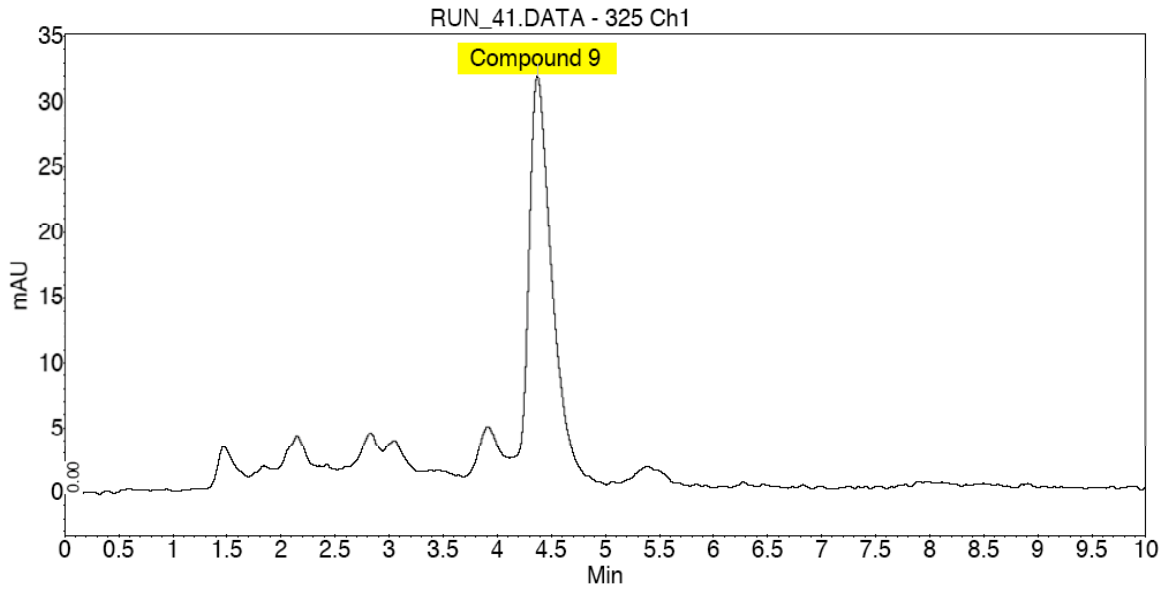
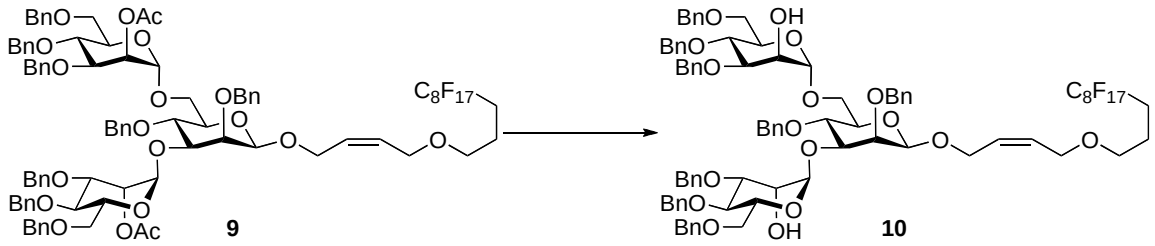


Figure 6. Analytical HPLC chromatogram of the crude product from the synthesis platform after FSPE (1.0 mL/min flowrate, EtOAc/hexanes: 1/2, 10 minutes run, Waters Nova-Pak 4 μm 3.9 × 150 mm silica column).

www.manaraa.com



***cis*-4-(1H,1H,2H,2H,3H,3H-perfluoroundecyloxy)-2-butenyl 2,4-di-*O*-benzyl-3,6-di-*O*-(3,4,6-tri-*O*-benzyl-β-D-mannopyranosyl)-β-D-mannopyranoside (10)**

The crude **9** (58 mg) from the automated synthesis was dissolved in MeOH (2.0 mL) and Na (0.50 mg, 0.22 mmol) was added. The mixture was stirred at ambient temperature for 1 h. The reaction was added by Dowex 50WX8 200 acidic resin until the pH paper showed neutral. The solvent was removed under reduced pressure and the crude product was purified by flash column chromatography on silica gel using EtOAc/petroleum ether (4/5 → 1/0) as eluent. The product was obtained as a colorless syrup (44 mg, 0.025 mmol, 50% over 2 automated synthesis steps and 1 bench-top step).

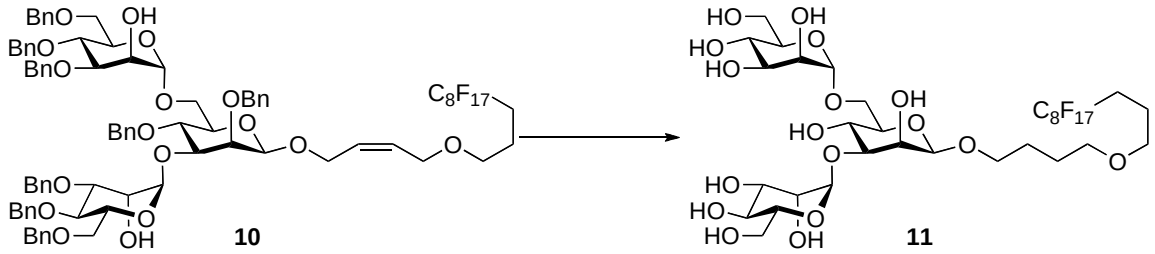
***R*$_f$**: 0.34 (EtOAc/petroleum ether: 1/1)

^{1}H NMR (CDCl$_3$, 600 MHz): δ (ppm) 7.41-7.14 (m, 40 H, H$_{arom}$), 5.67 (m 2H, *H*C=C*H*), 5.23 (d, 1H, *J* = 1.2 Hz, H$_\alpha$-1), 5.08 (d, 1H, *J* = 1.2 Hz, H$_\alpha$-1), 4.99 (d, 1H, *J* = 12.0 Hz, CH*H*Ph), 4.83 (2 × d, 2H, *J* = 10.8 Hz, CH*H*Ph), 4.72 (d, 1H, *J* = 12.6 Hz), 4.66-4.59 (m, 5H, CH*H*Ph), 4.55-4.46 (m, 7H, CH*H*Ph), 4.39 (s, 1H, H$_\beta$-1), 4.35 (dd, 1H, *J* = 12.6, 4.8 Hz, O-CH*H*C=C), 4.11 (d, 1H, *J* = 1.8 Hz, H$_\alpha$-2), 4.09 (dd, 1H, *J* = 13.2, 6.6 Hz, O-CH*H*C=C), 4.02-3.99 (m, 3H, H$_\alpha$-2, C=CC*H*$_2$-O), 3.93 (d, 1H, *J* = 3.0 Hz), 3.88-3.84 (m, 5H), 3.83 (dd, 1H, *J* = 9.6, 3.6 Hz, H$_\alpha$-3), 3.79-3.74 (m, 4H), 3.71 (dd, 1H, *J* = 10.8, 4.2 Hz), 3.65-3.60 (m, 3H), 3.40 (m, 2H, OC*H*$_2$CH$_2$), 3.33 (m, 1H), 2.33 (d, 1H, *J* = 1.8 Hz, 2-OH), 2.30 (d, 1H, *J* = 2.4 Hz, 2-OH), 2.18 (m, 2H, C*H*$_2$CF$_2$), 1.83 (m, 2H, O-CH$_2$C*H*$_2$)

www.manaraa.com

^{13}C NMR ($CDCl_3$, 150 MHz): δ (ppm) 139.2, 138.7, 138.7, 138.5, 138.4, 138.1, 138.0, 130.0, 128.7, 128.6, 128.6, 128.5, 128.4, 128.4, 128.3, 128.1, 128.1, 128.0, 128.0, 128.0, 127.9, 127.9, 127.9, 127.8, 127.8, 127.7, 127.7, 127.5, 101.8 ($J_{C1\text{-}H1}$ = 162.8 Hz, C-1), 100.8 ($J_{C1\text{-}H1}$ = 154.8 Hz, C-1), 100.2 ($J_{C1\text{-}H1}$ = 171.2 Hz, C-1), 81.2, 80.2, 79.8, 78.1, 75.3, 75.2, 75.0, 74.4, 73.6, 73.5, 72.4, 72.1, 71.6, 69.5, 69.1, 68.9, 68.9, 68.1, 66.8, 66.4, 64.9, 28.4 (t, $J_{C\text{-}F}$ = 22.4 Hz), 21.0

HRMS (ESI): $[M + Na]^+$ calcd for $C_{89}H_{91}F_{17}NaO_{17}^+$ 1777.5877, found 1777.5875



3-(perfluorooctyl)propanyloxybutanyl 3,6-di-*O*-(β-D-mannopyranosyl)-β-D-mannopyranoside (11)

cis-4-(1H,1H,2H,2H,3H,3H-perfluoroundecyloxy)-2-butenyl 2,4-di-*O*-benzyl-3,6-di-*O*-(3,4,6-tri-*O*-benzyl-β-D-mannopyranosyl)-β-D-mannopyranoside **10** (44 mg, 0.025 mmol) was dissolved in MeOH (3.0 mL) and 10% Pd/C (10 mg) was added. The mixture was stirred under 1000 psi H_2 atmosphere at 20 °C. After 48 h, the mixture was filtered through a short pad of Celite and the solvent was removed under reduced pressure. The desired product was collected as a white foam (13.2 mg, 0.0130 mmol, 51%).

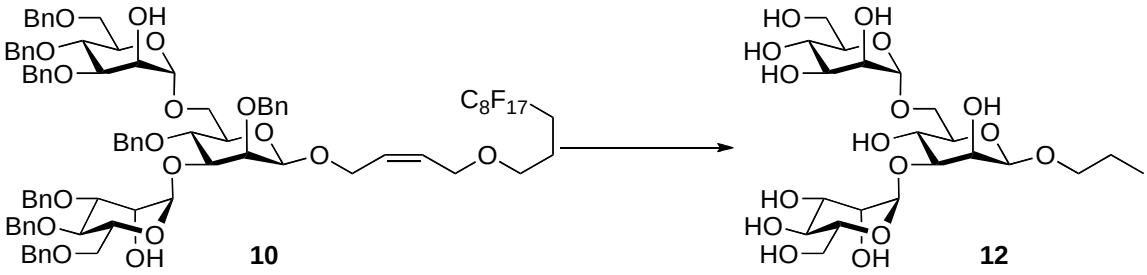
^{1}H NMR ($CDCl_3$, 600MHz): δ (ppm) 4.98 (d, 1H, *J* = 1.2 Hz, H-1), 4.73 (d, 1H, *J* = 1.2 Hz, H-1), 4.40 (s, 1H, H-1), 4.00 (d, 1H, *J* = 3.0 Hz, H-2), 3.88 (dd, 1H, *J* = 3.0, 1.2 Hz, H-2), 3.85-3.81 (m, 2H), 3.78-3.74 (m, 3H), 3.73-3.69 (m, 3H), 3.67 (d, 1H, *J* = 9.6 Hz), 3.64-3.62

www.manaraa.com

(m, 2H), 3.61-3.57 (m, 2H), 3.55-3.52 (m, 2H), 3.49-3.45 (m, 3H), 3.42 (t, 2H, J = 18.0 Hz, -OCH_2CH$_2$CH$_2$CF$_2$), 3.38 (m, 2H, CH$_2$CH_2O-), 3.27 (m, 1H), 2.18 (m, 2H, CH_2CF$_2$), 1.77 (m, 2H, OCH$_2$CH_2CH$_2$CF$_2$), 1.57 (s, 4H, -OCH$_2$CH_2CH_2CH$_2$O-)

^{13}C NMR (CDCl$_3$, 150 MHz): δ (ppm) 104.1, 101.8, 101.6, 83.0, 77.0, 75.1, 74.5, 72.8, 72.6, 72.2, 72.2, 72.1, 71.9, 70.6, 70.2, 69.1, 68.8, 67.7, 67.4, 63.2, 63.1, 29.2 (t, J_{C-F} = 21.8 Hz), 27.6, 27.5, 22.1

HRMS (ESI): [M + Na]$^+$ calcd for $C_{33}H_{45}F_{17}NaO_{17}^+$ 1059.2278, found 1059.2269



***n*-propyl 3,6-di-*O*-(β-D-mannopyranosyl)-β-D-mannopyranoside (12)**

cis-4-(1H,1H,2H,2H,3H,3H-perfluoroundecyloxy)-2-butenyl 2,4-di-*O*-benzyl-3,6-di-*O*-(3,4,6-tri-*O*-benzyl-β-D-mannopyranosyl)-β-D-mannopyranoside **10** (55 mg, 0.031 mmol) was dissolved in dry DCM (4.0 mL) and Grubbs catalyst 2nd generation (5.4 mg, 6.4 μmol) was added. The solution was stirred at 20 °C and ethylene gas was bubbled through for 30 min, then an ethylene balloon was attached, and the mixture was stirred for 24 h. The solvent was evaporated and the mixture was loaded onto a short silica gel column for purification (DCM → EtOAc/petroleum ether = 1/1). The product fraction was collected, the solvent was evaporated, and the crude product was purified by FSPE following the general procedure for bench-top fluorous solid-phase extraction. After the fluorophobic fraction was collected and

www.manaraa.com

the solvent was removed under reduced pressure, the product was dissolved in MeOH (3.0 mL) and 10% Pd/C (25 mg) was added. The mixture was stirred at 20 °C under 1000 psi H_2 atmosphere. After 24 h, another portion of Pd black (20 mg) and AcOH (0.3 mL) was added and the mixture was stirred at 20 °C under 1000 psi H_2 atmosphere for additional 24 h. The mixture was filtered through a small pad of Celite and the Celite pad was washed with MeOH (3 × 10 mL). After the solvent was removed under reduced pressure, the residue was washed with DCM (3 × 1.0 mL) and the fully deprotected product was obtained as a white foam (15.6 mg, 0.0280 mmol, 91% over 2 steps)

^{1}H NMR (CD_3OD, 600MHz): δ (ppm) 4.98 (s, 1H, H-1), 4.74 (d, 1H, *J* = 1.2 Hz, H-1), 4.40 (s, 1H, H-1), 4.00 (d, 1H, *J* = 3.0 Hz, H-2), 3.88 (dd, 1H, *J* = 3.0, 1.8 Hz, H-2), 3.85 (dd, *J* = 10.8, 5.4 Hz), 3.78-3.70 (m, 6H), 3.67-3.61 (m, 7H), 3.49-3.46 (m, 3H), 3.41 (m, 1H), 3.27 (m, 1H), 1.55 (m, 2H, C*H*$_2$CH$_3$), 0.86 (t, 3H, *J* = 7.2 Hz, C*H*$_3$)

^{13}C NMR (CD_3OD, 150 MHz): δ (ppm) 104.1, 101.8, 101.5, 83.0, 76.9, 75.1, 74.5, 72.7, 72.6, 72.5, 72.2, 72.2, 72.1, 69.0, 68.7, 67.6, 67.3, 63.2, 63.0, 24.0, 11.0

HRMS (ESI): $[M + Na]^+$ calcd for $C_{21}H_{38}NaO_{16}^+$ 569.2052, found 569.2040

General materials and methods for fluorous microarray

Microarrays were printed by the MicroarrayerXactII™ (LabNEXT Inc., Glenview, IL 60025) with robotic pin (Xtend MP Microarray Pins 0.35 mm). The F-tag attached sugar solution with concentration 222 μM in MeOH/DMSO/H_2O (v:v:v = 1: 3.5: 1.15) was printed on to the fluorous glass slides (Fluorous Technologies, Inc.; Pittsburgh, PA). The printed slide was dried in a humid chamber for 20 h and ready for incubation with protein solution. The protein solution was prepared in various concentrations in 1X PBS buffer with 1%

www.manaraa.com

bovine serum albumin (BSA, Sigma). For FITC-ConA (Sigma), the solution was with 1.0 mg/mL $CaCl_2$ and 1.0 mg/mL $MnCl_2$. The incubation was carried out in a PC500 CoverWell incubation chamber (Grace Biolabs, Bend, OR) for 1 h, and then washed twice with 1X PBS buffer and once with DI water. Then the slide was dried in the air and scanned at the Iowa State University DNA facility by using a General Scanning ProScanArray 5000 set at 488 nm.

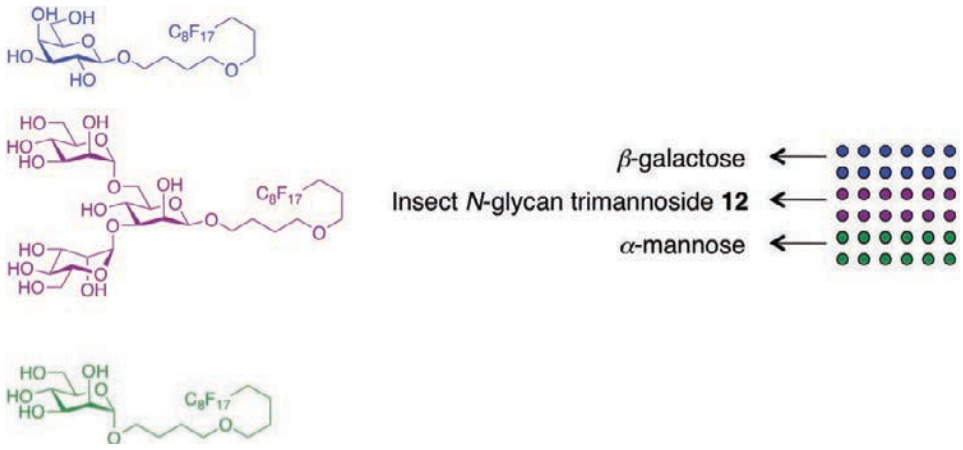


Figure 7. Printing pattern of F-tag attached saccharides on the fluorous glass slide

www.manaraa.com

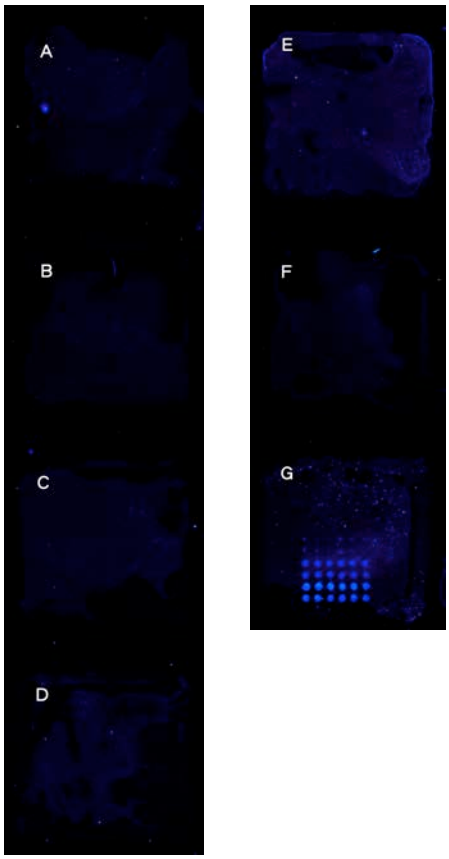


Figure 8. Microarray scanned image with FITC-PEMV subunit protein: A: 0.2 μM, B: 0.27 μM, C: 0.4 μM, D: 0.8 μM, E: 1.6 μM, F: 0.2 μM; with FITC-ConA: G: 0.2 μM

General materials and methods for isothermal titration calorimetry

The ITC experiment was conducted in the VP-ITC MicroCalorimeter (MicroCal, Northampton, MA). The 10 μM protein solution and the 300 μM ligand (sugar) solution were both prepared in pH 7.0 NaOAc buffer. For titration experiments with ConA (Sigma), $CaCl_2$ and $MnCl_2$ (1.0 mg/mL) was added to the buffer for both ConA and ligand solutions. The solutions were degassed prior to the experiment, and the titration was carried out at 26 °C with 310 rpm stirring rate.

www.manaraa.com

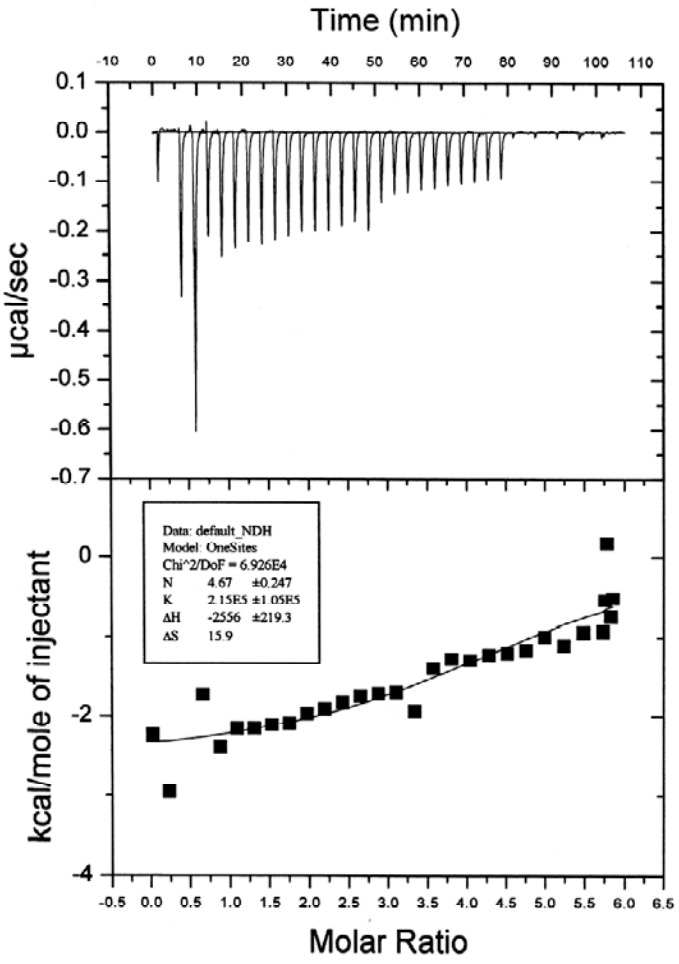


Figure 9. ITC experiment: ConA with methyl α-mannoside

www.manaraa.com

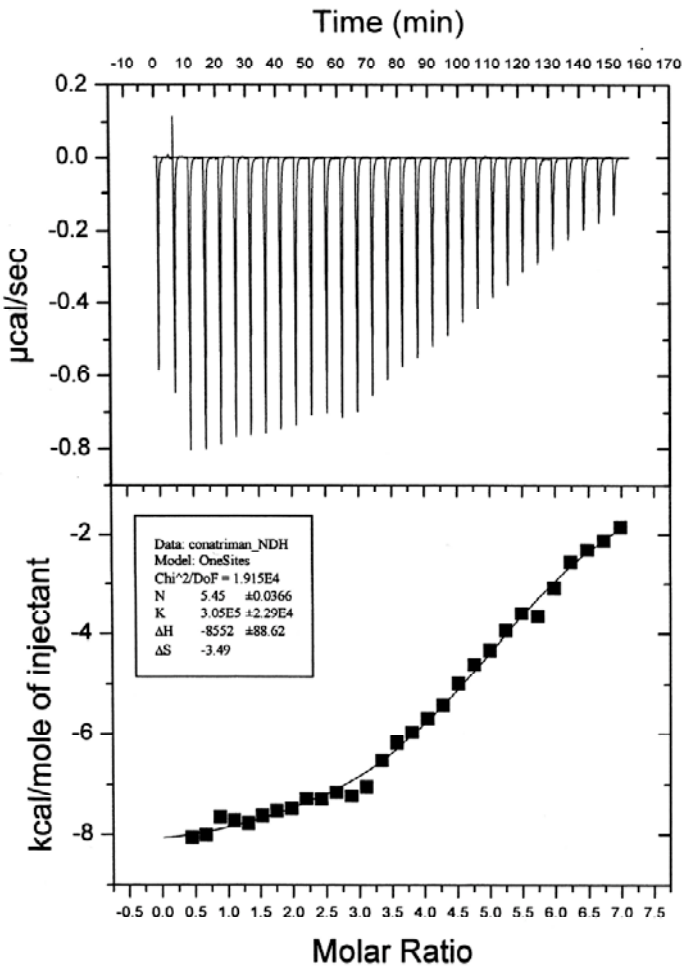


Figure 10. ITC experiment: ConA with **12**

www.manaraa.com

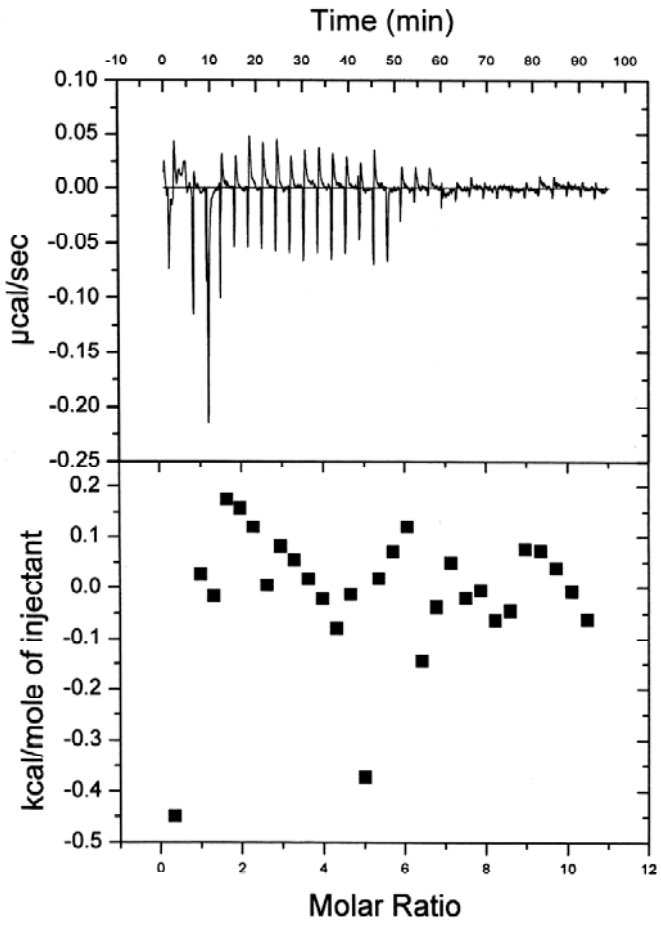


Figure 11. ITC experiment: PEMV with methyl α-mannoside

www.manaraa.com

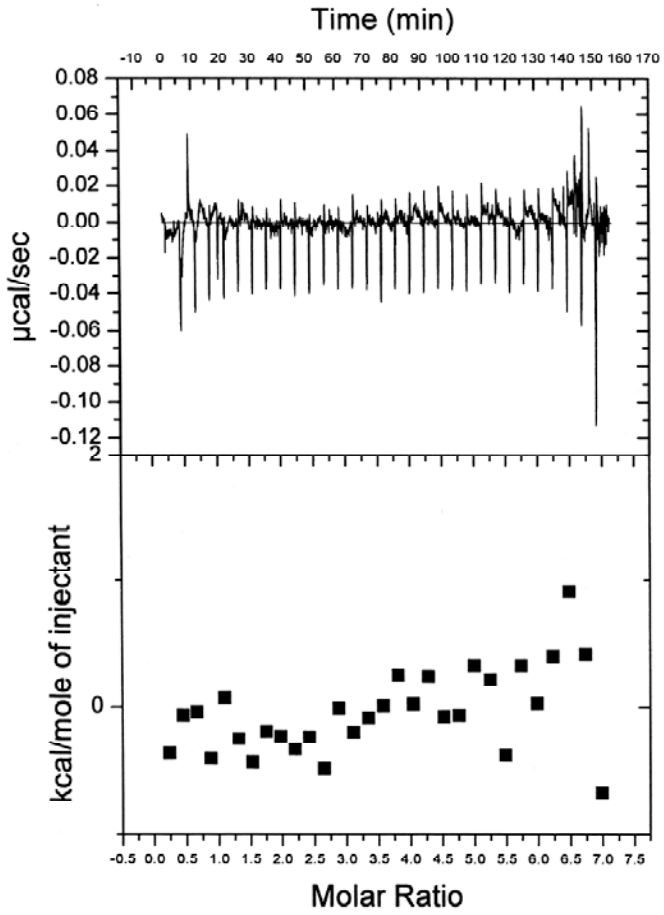


Figure 12. ITC experiment: PEMV with **12**

www.manaraa.com

CHAPTER 5

Automated Solution-Phase Synthesis of *β*-1,6-Mannan and *β*-1,3-Mannan Oligomers

Shu-Lun Tang and Nicola L. B. Pohl*

Abstract

β-Mannan is considered one of the most difficult glycans to chemically synthesize due to its kinetically and thermodynamically disfavored formation. It is also an interesting synthetic target due to the existence in various important natural products. Previously we have reported the automated solution-phase synthesis of different related structures including *β*-1,2-mannan, *β*-1,4-mannan, *β*-1,4-mannuronic acid, and the insect *N*-glycan terminal trimannoside oligomers. Herein we report explorations of the scope of the automated solution-phase synthesis of *β*-mannans by using the *β*-directing mannuronate/global reduction strategy to synthesize *β*-1,6-mannan and *β*-1,3-mannan oligomers. Interestingly, our automated solution-phase synthesis strategy successfully produced both of these *β*-mannan oligomers up to the trisaccharide stage; however, attempts at chain extension to the tetrasaccharides failed, possibly due to the decrease of the acceptor nucleophilicity with the growing chain. A possible future direction to solve this steric hindrance is to use a less bulky protecting group at the 2 or 4 position such as a propargyl group.

Introduction

β-mannan is considered one of the most difficult glycans to chemically synthesize due to its kinetically and thermodynamically disfavored formation (El Ashry et al. 2005). However, because of its existence in many biologically crucial natural products,

www.manaraa.com

methodologies for synthesizing this challenging linkage are an ongoing challenge. Previously we have demonstrated the automated solution-phase synthesis of different β-mannan structures including β-1,2-mannan, β-1,4-mannan, β-1,4-mannuronic acid, and the insect *N*-glycan terminal trimannoside oligomers by incorporating different β-mannoside formation strategies including an oxidation-reduction sequence and a β-directing mannuronate (van den Bos et al. 2006; Codee et al. 2009; Dinkelaar et al. 2009; Walvoort et al. 2009)/global reduction strategy. After comparing the two strategies for the automated solution-phase synthesis, the β-directing mannuronate/final reduction strategy turned out to be the more efficient and flexible approach due to its straightforward β-mannosidic linkage formation and variable protecting group capability compared to the classic Crich 4,6-*O*-benzylidene strategy (Crich and Jayalath 2007). In order to explore the scope of this new automated solution-phase approach to β-mannans utilizing the β-directing mannuronate strategy, and test the robustness of the protocol used for previous β-mannan syntehsis, herein we report the first automated solution-phase synthesis of the unnatural analog oligomers of β-1,6-mannan and β-1,3-mannan.

Results and discussion

In order to construct the β-1,6-mannan oligomer from a mannuronate building block, the fully benzylated mannuronate trichloroacetimidate donor **2** was synthesized from the previously reported lactol **1** (Codee et al. 2009). The automated solution-phase synthesis of the β-1,6-mannan oligomer started with the glycosylation of the trichloroacetimidate donor **2** (3.5 equiv.) and the allyl fluorous tag (F-tag) (Mamidyala et al. 2006) catalyzed by trimethylsilyl trifluoromethanesulfonate (TMSOTf) (0.1 equiv.) at -20 °C. The following

www.manaraa.com

deprotection of the 6-position was furnished by a hydride reduction of the methyl ester by lithium triethylborohydride (LiTEBH) and afforded the monosaccharide **3** followed by a FSPE to afford a (α/β = 1/3) mixture of F-tag-modified anomers, which were transferred out of the automation platform and manually purified—a particular advantage of this solution-phase approach over a traditional solid-phase approach. The pure **3** was reinjected into the automated synthesis platform for the next three repeating cycles of glycosylation and deprotection. The TLC of 2nd and 3rd glycosylation and deprotection showed complete reaction. However, after the 4th cycle the TLC showed that the only product has the same R_f value as the starting material trisaccharide. The mixture was transferred out of the synthesis platform and purified manually, and the isolated product was the trimannoside **4** instead of the expected tetramannoside. The production of this truncated sequence is likely due to the lack of reactivity of the growing glycosyl acceptor because of the possible steric hindrance from the more freely rotated β-1,6-mannan chain. The isolated trisaccharide **4** was deprotected by a 10% Pd/C, Pd black catalyzed hydrogenolysis to afford the fully-deprotected β-1,6-mannan trisaccharide **5** (Figure 1).

www.manaraa.com

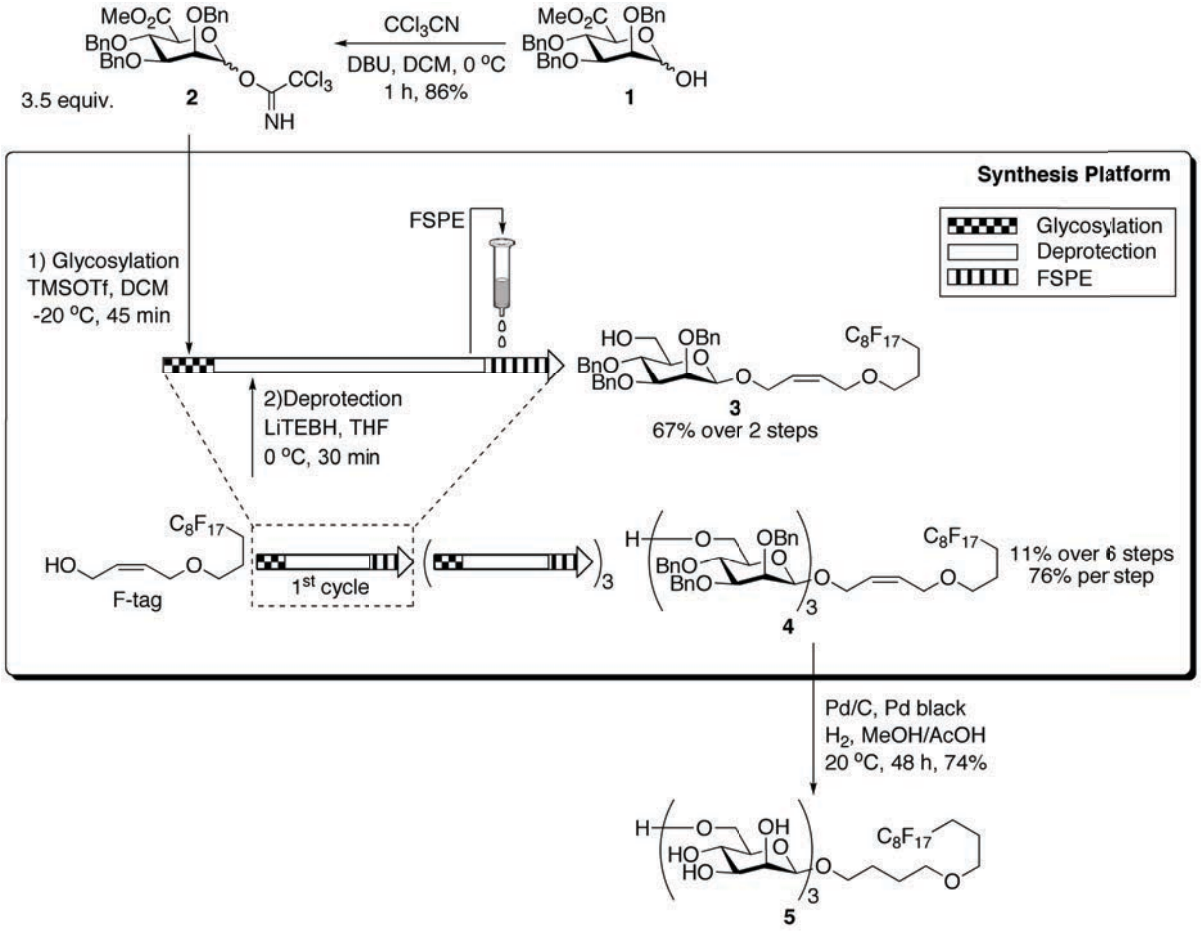


Figure 1. Synthesis of the trichloroacetimidate donor **2** and automated solution-phase synthesis of β-1,6-mannan trisaccharide **5**

The automated solution-phase synthesis of the β-1,3-mannan oligomer began with the glycosylation of the previously reported 3-*O*-*p*-methoxylbenzyl trichloroacetimidate donor **6** (3.5 equiv.) (Chapter 4) and the F-tag catalyzed by TMSOTf (0.1 equiv.) at -20 °C for 45 min. After the glycosylation, the cycle was completed by the removal of the *p*-methoxylbenzyl (PMB) group by ceric ammonium nitrate (CAN) and a fluorous solid phase extraction (FSPE) to give a mixture of anomers ((α/β = 1/3.6) that were separated manually to afford the monosaccharide **7**. The pure **7** was reinjected into the automated synthesis

www.manaraa.com

platform followed by 3 additional cycles of glycosylation/deprotection. The 2nd cycle gave a complete conversion of monosaccharide **7** to the disaccharide from TLC monitor. However, after the 3rd cycle, the TLC showed the product as a mixture of disaccharide and trisaccharide. After the 4th cycle, the mixture was transferred out of the synthesis platform and manually purified. However, instead of having the desired tetrasaccharide, the major product was the trisaccharide **8**. This is probably due to the steric hindrance from the 2,3-cis conformation of the built-up β-1,3-mannan chain, which gives a lower nucleophilicity to the 3-OH. The β-1,3-mannuronate trimer **8** was reinjected into the synthesis platform for the hydride reduction of the methyl esters by LiTEBH, and the β-1,3-mannan trisaccharide **9** was formed. The hydrogenolysis of **9** was catalyzed by Pd/C, Pd black and gave the fully deprotected trisaccharide **10**.

www.manaraa.com

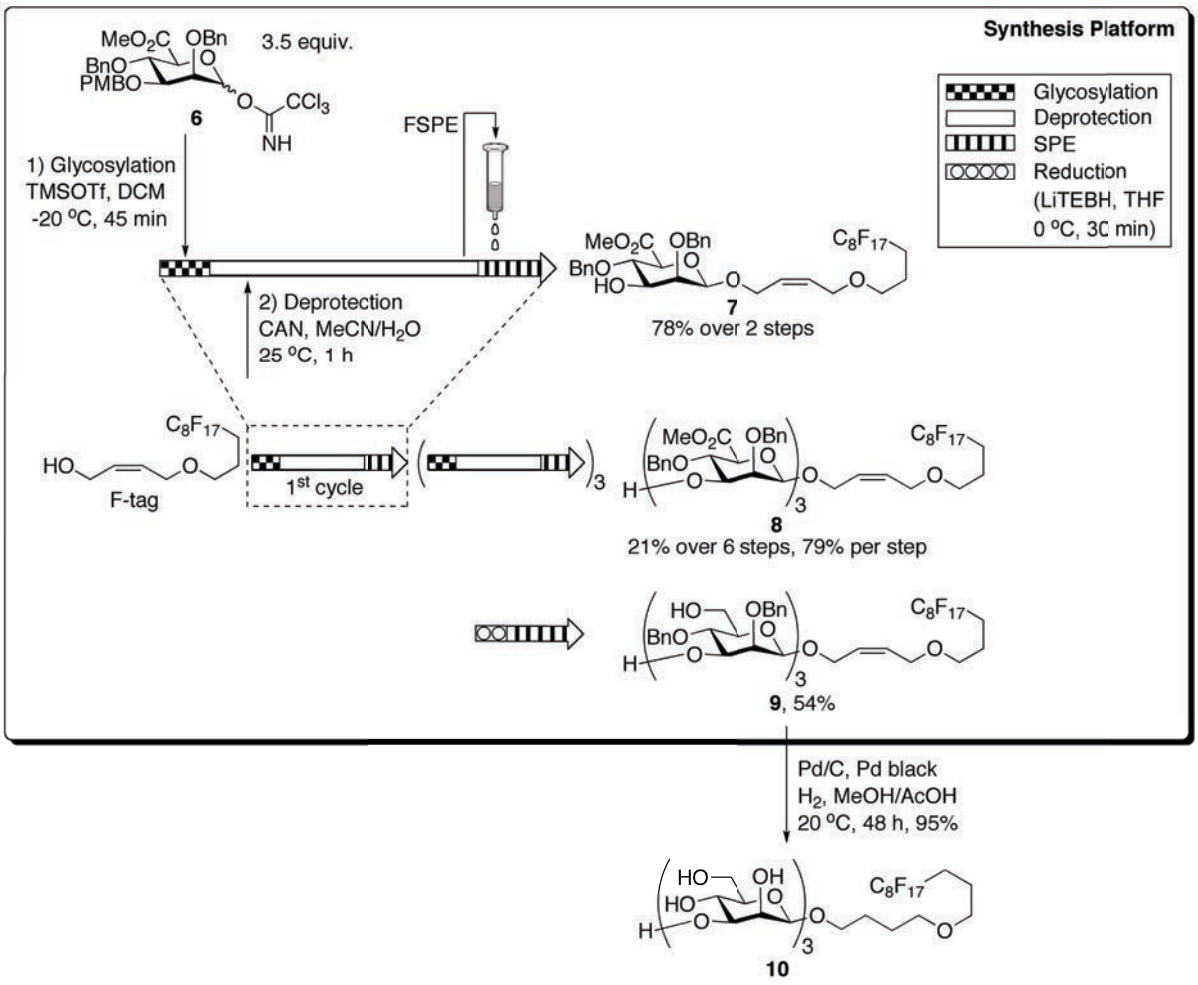


Figure 2. Automated solution-phase synthesis of β-1,3-mannan trisaccharide **10**

Conclusion

The first automated solution-phase synthesis of β-1,6-mannan and β-1,3-mannan oligomers has shown the scope and potential of the automated solution-phase synthesis of different β-mannans, and also the power of the β-directing mannuronate strategy for β-mannan synthesis. In addition, the unfavorable formation of the β-1,6-mannan and β-1,3-mannan tetrasaccharides was from the acceptors larger than the trisaccharide stage, which has insufficient nucleophilicity due to the increasing steric hindrance. Therefore, a donor

www.manaraa.com

which has a less bulky protecting group such as a propargyl group at 4 or 2 position (Crich and Jayalath 2005) could possibly overcome this difficulty and improve the yield of β-mannan structure synthesis.

Experimental section

General materials and methods

Dichloromethane (DCM) for glycosylation reactions was distilled from calcium hydride. Tetrahydrofuran (THF) was collected from PureSolv Micro solvent purification system (Innovative Technology, Inc., Amesbury, MA) before reactions. All other commercial solvents and reagents were reagent grade and used as received without further purification. The reactions were monitored by thin layer chromatography (TLC) with 250 μm Sorbent Technologies silica gel HL TLC plates. The hydrogenation reaction under 1000 psi hydrogen was operated in the Parr model 4766 general purpose vessel high pressure reactor (Parr Instrument Company, Moline, IL). The developed TLC plates were visualized by stain with *p*-anisaldehyde solution followed by heating on a hot plate. Flash column chromatography was performed with Zeochem ZEOprep 60 silica gel, 40-63 μm particle size. Preparative TLC was performed with Dynamic Adsorbents Prep TLC, Silica Gel, HLO, 20 cm × 20 cm F-254, 1000 micron layer. Fluorous solid-phase extraction was performed with SPE cartridges containing 2.0 g of silica gel bonded with perfluorooctylethylsilyl chains (Fluorous Technologies, Inc., Pittsburgh, PA). The automated solution phase synthesis was performed in a modified Chemspeed ASW1000 (Chemspeed, Augst, Switzerland) synthesis platform with hood, 16 reactor vials (13-mL capacity each) and heating/cooling unit (200 °C

www.manaraa.com

to -20 °C) machined to hold the SPE cartridges at the Iowa State University Machine Shop. ^{1}H and ^{13}C NMR spectra were obtained at 600 MHz and 150 MHz on a Bruker Avance III 600 spectrometer, and 700 MHz on a Bruker Avance II 700 spectrometer. The C-H coupling constants were measured by the coupled ^{13}C NMR spectra. Chemical shifts (δ) were reported in parts per million (ppm) relative to $CDCl_3$ and CD_3OD as internal references. Mass spectra were obtained on a Finnigan TSQ700 triple quadrupole mass spectrometer (Finnigan MAT, San Jose, CA) fitted with a Finnigan ESI interface.

www.manaraa.com

General procedure for automated synthetic cycles to produce β-1,6-mannan and β-1,3-mannan oligomers

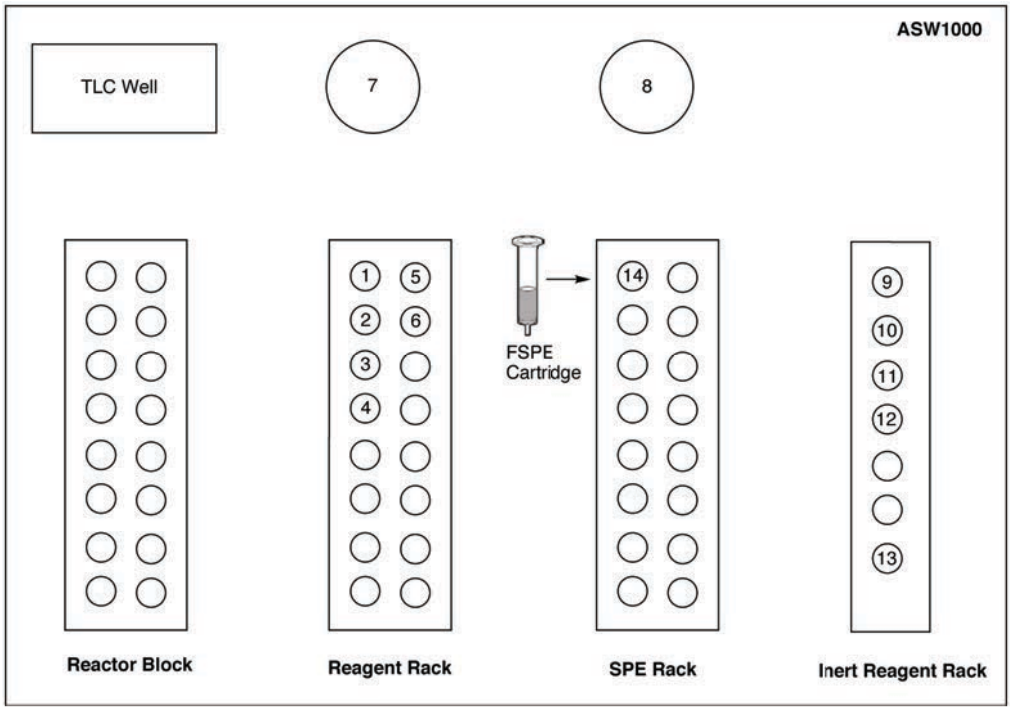


Figure 3. Basic layout of automated solution-phase oligosaccharide synthesizer (ASW1000) 1: F-tagged acceptor (solution in DCM), 2: TEA, 3: DMF, 4: LiTEBH (1.0 M in THF), 5: water, 6: CAN/MeCN, 7: MeOH, 8: reservoir (toluene), 9: TMSOTf (0.55 M in DCM/DCM), 10: donor, 11: DCM, 12: THF, 13: 80 % MeOH, 14: FSPE cartridge.

1. Sample Preparation

Donor molecule (0.20 g, 0.30 mmol) was dissolved in anhydrous DCM (1.6 mL) in the 13 mL-vial and placed at the inert reagent rack location (Donor) under argon atmosphere. A 0.055 M trimethylsilyltrifluoromethanesulfonate (TMSOTf) solution (5.0 mL) in anhydrous DCM was prepared in an 8 mL-vial and placed as indicated on the inert reagent

www.manaraa.com

rack under argon. MeOH (100 mL) was placed in the stock solution bottle at the stock solution station as indicated. Toluene (1.0 L) was placed in the stock solution bottle and placed at the reservoir bottle rack with tubing as reservoir solution for rinsing. Anhydrous DCM (20 mL) was transferred to the 50 mL-vial and placed as indicated on the inert reagent rack under argon. Anhydrous tetrahydrofuran (THF, 10 mL) was transferred to the 50 mL-vial and placed as indicated on the inert reagent rack under argon. 80% methanol/water (100 mL) stock was prepared in the 100 mL-vial and placed as indicated on the inert reagent rack. F-tagged acceptor molecule (50 μmol) was dissolved with anhydrous DCM (0.8 mL) in a Wheaton 8 mL-E-Z extraction vial (conically-bottomed) flushed with argon, capped with septa and placed at the reagent rack as indicated. Triethylamine (TEA, 5.0 mL) was transferred to an 8 mL-vial capped with septa and placed at the reagent rack as indicated. *N*,*N*-dimethylformamide (DMF, 8.0 mL) was transferred to an 8 mL-vial capped with septa and placed at the reagent rack as indicated. A 0.45 M CAN (ceric ammonium nitrate) solution in acetonitrile/water (9/1) (5 mL) was prepared in an 8 mL-vial capped with septa and placed at the reagent rack as indicated. LiTEBH solution in THF (1.0 M, 3.0 mL) was transferred to an 8 mL-vial capped with septa and placed at the reagent rack as indicated. Water (8.0 mL) was transferred to an 8 mL-vial capped with septa and placed at the reagent rack as indicated. A fluorous solid phase extraction (FSPE) cartridge (2 g, 10 cc) was preconditioned with 80% methanol/water and placed on the machined FSPE block as indicated. An empty Wheaton 8 mL-E-Z extraction vial was placed under the FSPE cartridge.

2. Cleaning Cycle

www.manaraa.com

Prior to the introduction of reagents, the ASW1000 reactor vials were cleaned, dried and purged with argon by running the cleaning cycle. During the cleaning cycle, each of the 16 reactor vials (13 mL capacity each) was rinsed with toluene (8.0 mL) and methanol (8.0 mL) 3 times each. After the solvents were completely removed, the reactor vials were dried under vacuum and purged with argon for 45 minutes. Reagent solutions were prepared by azeotropic removal of water from each building block with toluene; the resulting building blocks were then dried under high vacuum. After the cleaning cycles were done, the reagents were transferred into the reagent vials respectively, which were then placed on the inert condition reagent rack and general atmosphere reagent rack.

3. Glycosylation

The needle transferred the acceptor molecule (F-tag) solution (0.8 mL) to the reaction vial 1, followed by the transfer of the donor molecule solution (0.8 mL). The mixture was vortexed under ambient temperature at 800 rpm for 20 min. Then the reactor vials were cooled to 0 °C by the heat transfer oil with 800 rpm vortex rate. The TMSOTf solution (0.1 mL) was transferred into the reactor vial 1 under 200 rpm vortex rate. After each individual transfer, the needle (inside and outside) was rinsed with toluene (2 ml) before operating the next task. The reaction mixture was vortexed at 800 rpm for 45 minutes at 0 °C under argon atmosphere. After the reaction time the needle withdrew 20 μL of the solution from the reaction mixture and placed it into the first well of the microtiterplate for thin layer chromatography monitoring. TEA (0.05 mL) was added to the solution for quenching and the solvent was evaporated under reduced pressure.

www.manaraa.com

4. PMB deprotection

To the dried residue after the glycosylation, the CAN solution (1.0 mL) was added to the reactor vial. The reaction mixture was vortexed at 800 rpm for 1 h at room temperature. After the reaction time the needle withdrew 20 μL of the solution from the reaction mixture and placed it into the second well of the microtiterplate for thin layer chromatography monitoring.

5. Fluorous solid-phase extraction (FSPE)

DMF (0.4 mL) was added to the reactor vials to dissolve the crude mixture and the vials were vortexed at 800 rpm for 3 min. The reaction mixture (1.2 mL) was carried to the FSPE cartridge at the FSPE block and dispensed at a speed of 1.0 mL/s via the 10 mL syringe. Then 80% methanol (2 mL) was used to rinse the empty reactor vial. The 80% methanol solution was removed from the reactor vial and delivered to the FSPE cartridge. The 80% methanol rinsing and transferring was repeated one more time. Additional 80% methanol solution (4.0 mL, repeated 2 times) was used to rinse the FSPE cartridge. During the 80% methanol rinse, the cartridge was positioned at 'SPE waste' for the eluted mixture to be disposed. MeOH (2.0 mL, repeated 3 times) was used to wash the FSPE cartridge for eluting the desired compound. During the task, the FSPE cartridge was positioned as 'SPE collect' to be placed right above the 8 mL vial for collection of the sample. After the task, the position of the SPE rack was changed into 'SPE direct' for the needle to withdraw the collected sample from the conically-bottomed vial and deliver it to the clean reactor vial for the next reaction. Toluene (1.0 mL) was added to the solution and solvent was evaporated under reduced pressure. After the evaporation cycle, the MeOH wash step and the

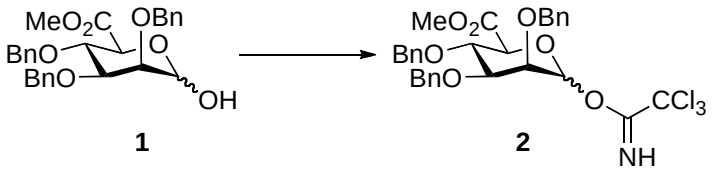
www.manaraa.com

evaporation were repeated one more time. Once again toluene (1.0 mL) was added and removed under reduced pressure to remove residual water.

7. LiTEBH Reduction

Anhydrous THF (1.0 mL) was added to the sample and vortexed at 800 rpm, 0 °C under argon atmosphere for 30 min. The LiTEBH (0.3 mL, 1.0 M in THF) was added to the reaction solution and the mixture was vortexed for 30 min at 800 rpm, 0 °C under argon atmosphere. After the reaction time the needle withdrew 20 μL of the solution from the reaction mixture and placed it into the fourth well of the microtiterplate for thin layer chromatography monitoring. Methanol (0.5 mL) was added to quench the reaction and the solvent was removed under reduced pressure.

Synthetic procedures



Methyl (2,3,4-tri-*O*-benzyl-α/β-D-mannopyranose) uronate trichloroacetimidate (2)

To a solution of methyl (2,3,4-tri-*O*-benzyl-α/β-D-mannopyranose) uronate (**1**) (0.50 g, 1.0 mmol) in dichloromethane (35 mL) was added trichloroacetonitrile (0.85 g, 5.9 mmol) at 0 °C under argon atmosphere. 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU) (0.03 g, 0.2 mmol) was then added and the reaction mixture was stirred at 0 °C under argon atmosphere for 3 h. The solvent was removed under reduced pressure and the crude product was purified by flash column chromatography on silica gel using EtOAc/petroleum ether/triethylamine (1/2/0.1) as

www.manaraa.com

eluent. The product was obtained as a colorless syrup and a mixture of anomers (α/β = 4/1) (0.59 g, 0.93 mmol, 95%).

***R*$_f$**: 0.66 (EtOAc/petroleum ether: 1/2)

^{1}H NMR ($CDCl_3$, 600MHz): δ (ppm) 9.34 (s, 1H, N*H*$_\beta$), 8.62 (s, 1H, N*H*$_\alpha$), 7.4 (d, 2H, *J* = 7.2 Hz, H$_{arom}$), 7.35-7.21 (m, 13H, H$_{arom}$), 6.41 (d, 1H, *J* = 2.4 Hz, H$_\alpha$-1), 5.98 (d, 1H, *J* = 7.8 Hz, H$_\beta$-1), 4.86-4.79 (m, 2H, CH*H*Ph), 4.74 (m, 1H, CH*H*Ph), 4.66-4.56 (m, 3H, CH*H*Ph), 4.54 (d, 1H, *J* = 11.4 Hz, H$_\beta$-4), 4.52 (d, 1H, *J* = 12.0 Hz CH*H*Ph), 4.40 (d, 1H, *J* = 9.0 Hz, H$_\alpha$-3), 4.30 (t, 1H, *J* = 8.4 Hz, H$_\alpha$-3), 4.20 (dd, 1H, J = 4.2, 1.8 Hz, H$_\beta$-3), 3.92-3.88 (m, 3H, H$_\alpha$-5, H$_\beta$-3, H$_\beta$-2), 3.87 (t, 1H, *J* = 3.0 Hz, H$_\alpha$-2), 3.71 (s, 3H, $CO_2CH_{\alpha 3}$), 3.63 (s, 3H, $CO_2CH_{\beta 3}$)

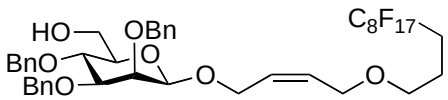
^{13}C NMR ($CDCl_3$, 150 MHz): δ (ppm) 169.3, 169.0, 160.4, 158.9, 138.0, 137.9, 137.9, 137.8, 137.4, 137.1, 128.7, 128.5, 128.5, 128.5, 128.2, 128.2, 128.1, 128.1, 128.0, 128.0, 128.0, 128.0, 128.0, 127.9, 95.7, 95.1, 92.3, 90.9, 77.8, 75.8, 75.6, 75.5, 75.0, 74.3, 74.0, 73.6, 73.5, 73.2, 73.0, 72.6, 72.5, 52.6, 52.5

HRMS (ESI): $[M + H]^+$ calcd for $C_{30}H_{31}Cl_3NO_7^+$ 622.1161, found 622.1145

www.manaraa.com

Table 1. Automated synthetic cycles for production of compound **3**

Step	Task	Reagent/ Operation	Operation Time
1	Glycosylation (2 parallel reactions)	3.5 × 2 equivalent donor (175 × 2 µmol) in 0.8 × 2 mL DCM, 1.0 × 2 equivalent F-tagged acceptor (50 × 2 µmol) in 0.8 × 2 mL DCM; 0.11 equivalent TMSOTf, -20 °C	45 min
2	TLC sample	20 × 2 µL of crude reaction mixture withdrawn	
3	Quenching	0.05 × 2 mL TEA	
4	Evaporation	40 ˚C	45 min
5	Reduction	10 × 2 mL THF, 0.3 × 2 mL LiTEBH, 0 °C	1 h
6	TLC sample	20 × 2 µL of crude reaction mixture withdrawn	
7	Quenching	0.5 × 2 mL MeOH	
8	Evaporation	40 °C	
9	FSPE preparation	0.5 × 2 mL DMF	
10	Sample loading	1.2 × 2 mL crude sample transferred to cartridges	
11	Wash	12 × 2 mL 80% methanol wash	
12	Wash	2 mL MeCN wash (repeated 3 × 2 times)	
13	Transfer	6 × 2 mL collected sample out of the ASW1000	



3

***cis*-4-(1H,1H,2H,2H,3H,3H-perfluoroundecyloxy)-2-butenyl 2,3,4-tri-*O*-benzyl-β-D-mannopyranoside (3)**

The product solution was transferred out of the synthesis platform after the FSPE step of the first cycle (13th step of the automated synthesis). The solvent was removed under reduced pressure and the product was purified by preparative TLC by using diethyl ether/DCM (0.75/9). The product was obtained as a colorless syrup (66 mg, 0.067 mmol, 67% over 2 steps).

***R*$_f$**: 0.35 (diethyl ether/DCM: 0.75/9)

^{1}H NMR (CDCl$_3$, 600MHz): δ (ppm) 7.45 (d, 2H, *J* = 7.2 Hz, H$_{arom}$), 7.34-7.26 (m, 10H, H$_{arom}$), 5.75 (m, 2H, *H*C=C*H*), 4.96 (d, 2H, *J* = 12.0 Hz, CH*H*Ph), 4.85 (d, 1H, *J* = 12.6 Hz, CH*H*Ph), 4.65 (d, 1H, *J* = 10.8 Hz, CH*H*Ph), 4.54 (d, 1H, *J* = 12.0 Hz, CH*H*Ph), 4.49 (d, 1H,

www.manaraa.com

J = 12.0 Hz, CH*H*Ph), 4.45 (s, 1H, H-1), 4.44 (dd, 1H, J = 12.6, 3.6 Hz, OH*H*CHC=C), 4.21 (dd, 1H, *J* = 12.6, 4.2 Hz, OH*H*CHC=C), 4.07 (m, 2H, C=CHC*H*$_2$O), 3.94-3.89 (m, 3H, H-2, H-4, H-6), 3.79 (m, 1H, H-6'), 3.53 (dd, 1H, *J* = 9.6, 3.0 Hz, H-3), 3.48 (t, 2H, *J* = 5.4 Hz, OC*H*$_2$CH$_2$), 3.33 (m, 1H, H-5), 2.22 (m, 2H, C*H*$_2$CF$_2$), 2.09 (t, 1H, *J* = 6.6 Hz, 6-O*H*), 1.89 (m, 2H, OCH$_2$C*H*$_2$)

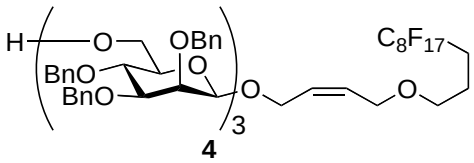
^{13}C NMR (CDCl$_3$, 150 MHz): δ (ppm) 138.8, 138.4, 138.3, 130.0, 128.6, 128.6, 128.5, 128.3, 128.0, 127.8, 127.7, 127.7, 100.8 (J_{C1-H1} = 152.0 Hz, C-1), 82.5, 76.2, 75.5, 75.0, 74.4, 74.3, 71.8, 69.0, 66.7, 65.1, 62.7, 28.3 (t, J_{C-F} = 22.2 Hz), 21.0

HRMS (ESI): $[M + Na]^+$ calcd for $C_{42}H_{41}F_{17}NaO_7^+$ 1003.2473, found 1003.2476

Table 2. Automated synthetic cycles for production of compound **4**

Step	Task	Reagent/ Operation	Operation Time
14	Glycosylation	3.5 equivalent donor (175 μmol) in 0.8 mL DCM, 1.0 equiv. **3** (50 μmol) in 0.8 mL DCM; 0.11 equivalent TMSOTf, -20 °C	45 min
15	TLC sample	20 μL of crude reaction mixture withdrawn	
16	Quenching	0.05 mL TEA	
17	Evaporation	40 °C	45 min
18	Reduction	1.0 mL THF, 0.3 mL LiTEBH, 0 °C	1 h
19	TLC sample	20 μL of crude reaction mixture withdrawn	
20	Quenching	0.5 × 2 mL MeOH	
21	Evaporation	40 °C	
22	FSPE preparation	0.5 × 2 mL DMF	
23	Sample loading	1.2 mL crude sample transferred to cartridges	
24	Wash	12 mL 80% methanol wash	
25	Wash	2.0 mL MeCN wash (repeated 3 × 2 times)	
26	Evaporation	40 °C	45 min
27	Repeat the same procedure as step 14-26	Repeat for 3 cycles	3 h 15 min

www.manaraa.com



4

***cis*-4-(1H,1H,2H,2H,3H,3H-perfluoroundecyloxy)-2-butenyl 2,3,4-tri-*O*-benzyl-6-*O*-(2,3,4-tri-*O*-benzyl-6-*O*-(2,3,4-tri-*O*-benzyl-β-D-mannopyranosyl) -β-D-mannopyranosyl) -β-D-mannopyranoside (4)**

The product solution was transferred out of the synthesis platform after the FSPE step of the first cycle (27th step of the automated synthesis). The solvent was removed under reduced pressure and the product was purified by flash chromatography by using EtOAc/petroleum ether (1/1). Then the crude product was purified by a preparative TLC by using diethyl ether/DCM (1/10 developed for 3 times). The product was collected from the preparative TLC plate and obtained as a colorless syrup (11 mg, 5.7 μmol, 11% over 6 steps).

***R**$_f$*: 0.48 (diethyl ether/DCM: 1.5/8, TLC developed with the solvent twice)

^{1}H NMR ($CDCl_3$, 600MHz): δ (ppm) 7.43 (m, 6H, H_{arom}), 7.31-7.19 (m, 39H, H_{arom}), 5.74 (m, 2H, *H*C=C*H*), 4.92-4.87 (m, 5H, CH*H*Ph), 4.83-4.78 (m, 4H, CH*H*Ph), 4.61 (d, 1H, *J* = 10.8 Hz, CH*H*Ph), 4.55 (d, 1H, J = 11.4 Hz, CH*H*Ph), 4.50-4.46 (m, 2H, CH*H*Ph), 4.43-4.34 (m, 7H, 5 × CH*H*Ph, H-1, OH*H*CHC=C), 4.30 (s, 1H, H-1), 4.26 (s, 1H, H-1), 4.22 (d, 1H, *J* = 10.2 Hz, H-4), 4.10 (m, 2H, H-2, OH*H*CHC=C), 4.00 (m, 2H, C=CHC*H*$_2$O), 3.88-3.83 (m, 3H, H-2, 2 × H-6), 3.81-3.71 (m, 5H, 2 × H-2, 4 × H-6), 3.67 (m, 2H, 2 × H-3), 3.53 (dd, 1H, *J* = 9.0, 2.4 Hz, H-5), 3.49-3.43 (m, 3H, H-3, 2 × H-4), 3.39 (dd, 1H, J = 9.6, 3.0 Hz, H-5), 3.35 (m, 2H, OC*H*$_2$CH$_2$), 3.26 (m, 1H, H-5), 2.34 (t, 1H, *J* = 6.6 Hz, 6-O*H*), 2.13 (m, 2H, C*H*$_2$CF$_2$), 1.79 (m, 2H, OCH$_2$C*H*$_2$)

www.manaraa.com

^{13}C NMR ($CDCl_3$, 150 MHz): δ (ppm) 138.9, 138.8, 138.6, 138.5, 138.5, 138.3, 130.0, 128.8, 128.7, 128.6, 128.6, 128.5, 128.5, 128.3, 128.3, 128.3, 128.0, 127.8, 127.7, 127.7, 127.6, 127.5, 102.6 ($J_{C1\text{-}H1}$ = 151.7 Hz), 102.5 ($J_{C1\text{-}H1}$ = 155.3 Hz), 101.0 ($J_{C1\text{-}H1}$ = 151.5 Hz), 82.5, 82.5, 82.3, 76.1, 75.7, 75.6, 75.4, 75.1, 75.1, 75.0, 74.8, 74.1, 74.1, 74.0, 73.9, 73.7, 73.4, 71.6, 71.5, 71.5, 69.7, 69.6, 68.9, 66.8, 65.1, 62.6, 28.3 (t, $J_{C\text{-}F}$ = 22.5 Hz), 21.0

HRMS (ESI): $[M + Na]^+$ calcd for $C_{96}H_{97}F_{17}NaO_{17}^+$ 1867.6347, found 1867.6322

HPLC trace of the automated synthesis of **4**

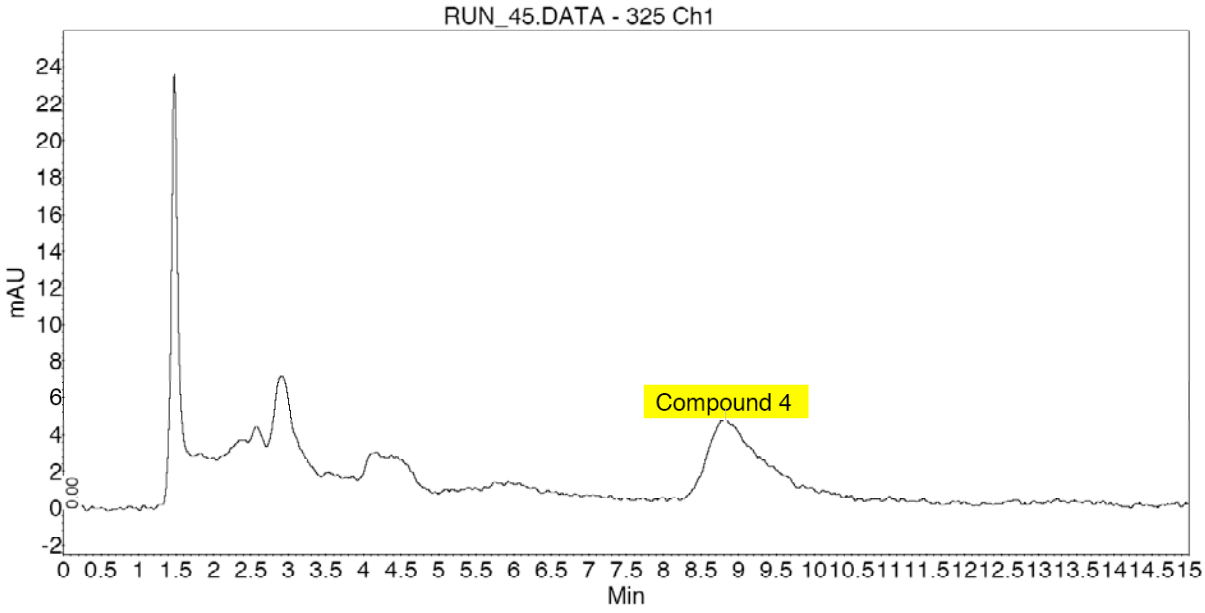
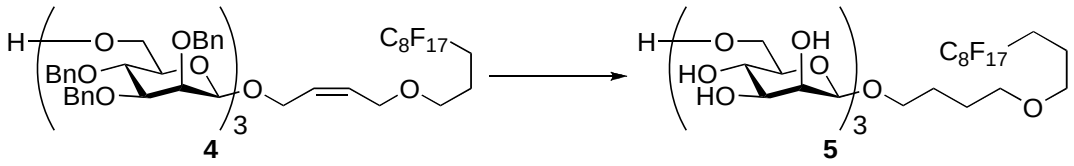


Figure 4. Analytical HPLC chromatogram of the crude product from the synthesis platform after FSPE (1.0 mL/min flowrate, EtOAc/hexanes: 2/3, 15 minutes run, Waters Nova-Pak 4 μm 3.9 × 150 mm silica column)

www.manaraa.com



3-(perfluorooctyl)propanyloxybutanyl 6-*O*-(6-*O*-(β-D-mannopyranosyl)-β-D-mannopyranosyl)-β-D-mannopyranoside (5)

cis-4-(1H,1H,2H,2H,3H,3H-perfluoroundecyloxy)-2-butenyl 2,3,4-tri-*O*-benzyl-6-*O*-(2,3,4-tri-*O*-benzyl-6-*O*-(2,3,4-tri-*O*-benzyl-β-D-mannopyranosyl) -β-D-mannopyranosyl) -β-D-mannopyranoside **4** (11 mg, 5.7 μmol) was dissolved in MeOH/AcOH = 10/1 (2.5 mL) and 10% Pd/C (5.0 mg) and Pd black (5.0 mg) were added. The mixture was stirred under 1000 psi H_2 atmosphere at 20 °C. After 48 h, the mixture was filtered through a short pad of Celite and the solvent was removed under reduced pressure. The desired product was collected as a white foam (4.4 mg, 4.2 μmol, 74%).

^{1}H NMR ($CDCl_3$, 700 MHz): δ (ppm) 4.64 (s, 1H, H-1), 4.62 (s, 1H, H-1), 4.50 (s, 1H, H-1), 4.21 (dd, 1H, *J* = 11.2, 2.1 Hz, H-6), 4.17 (dd, 1H, *J* = 11.2, 1.4 Hz, H-6), 3.93 (d, 1H, *J* = 3.5 Hz, H-2), 3.92 (d, 1H, *J* = 2.8 Hz, H-2), 3.89-3.86 (m, 3H, -OC*H*$_2$CH$_2$CH$_2$CH$_2$O-, H-2, H-6), 3.83 (dd, 1H, *J* = 11.9, 6.3 Hz, H-6), 3.79 (dd, 1H, *J* = 11.2, 5.6 Hz, H-6), 3.74 (dd, 1H, *J* = 11.2, 5.6 Hz, H-6), 3.62-3.55 (m, 4H, 3 × H-4, -OC*H*$_2$CH$_2$CH$_2$CH$_2$O-, 3.53 (t, 2H, *J* = 6.3 Hz, -OC*H*$_2$CH$_2$CH$_2$CF$_2$), 3.45 (m, 2H, CH$_2$C*H*$_2$O-), 3.45 (m, 3H, 3 × H-3), 3.38 (m, 2H, 2 × H-5), 3.23 (m, 1H, H-5), 2.29 (m, 2H, C*H*$_2$CF$_2$), 1.87 (m, 2H, OCH$_2$C*H*$_2$CH$_2$CF$_2$), 1.68 (m, 4H, -OCH$_2$C*H*$_2$C*H*$_2$CH$_2$O-)

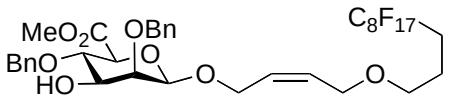
^{13}C NMR ($CDCl_3$, 150 MHz): δ (ppm) 102.6, 102.3, 102.0, 78.4, 77.3, 77.3, 75.3, 75.2, 75.2, 72.5, 72.5, 72.4, 71.9, 70.7, 70.2, 70.0, 69.4, 68.6, 68.5, 68.4, 62.8, 29.1 (t, $J_{C\text{-}F}$ = 22.4 Hz), 27.6, 27.6, 22.0

www.manaraa.com

HRMS (ESI): $[M + Na]^+$ calcd for $C_{33}H_{45}F_{17}NaO_{17}^+$ 1059.2278, found 1059.2291

Table 2. Automated synthetic cycles for production of compound **7**

Step	Task	Reagent/ Operation	Operation Time
1	Glycosylation (2 parallel reactions)	3.0 × 2 equivalent donor (150 × 2 μmol) in 0.8 × 2 mL DCM, 1.0 × 2 equivalent F-tagged acceptor (50 × 2 μmol) in 0.8 × 2 mL DCM; 0.11 equivalent TMSOTf, -20 °C	45 min
2	TLC sample	20 × 2 μL of crude reaction mixture withdrawn	
3	Quenching	0.05 × 2 mL TEA	
4	Evaporation	40 °C	45 min
5	PMB deprotection (2 parallel reactions)	1.0 × 2 mL CAN/MeCN/H_2O, 25 °C	1 h
6	TLC sample	20 × 2 μL of crude reaction mixture withdrawn	
7	Sample loading	1.2 × 2 mL crude sample transferred to cartridges	
8	Wash	12 × 2 mL 80% methanol wash	
9	Wash	2 mL MeCN wash (repeated 3 × 2 times)	
10	Transfer	6 × 2 mL collected sample out of the ASW1000	



7

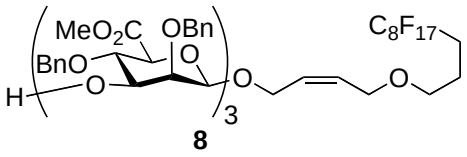
Methyl (*cis*-4-(1H,1H,2H,2H,3H,3H-perfluoroundecyloxy)-2-butenyl 2,4-di-*O*-benzyl-β-D-mannopyranoside) uronate (7)

For the synthetic procedure, and spectral data see compound **7** in chapter 4.

www.manaraa.com

Table 3. Automated synthetic cycles for production of compound **8**

Step	Task	Reagent/ Operation	Operation Time
11	Glycosylation	3.0 equivalent donor (150 μmol) in 0.8 mL DCM, 1.0 equivalent **7** (50 μmol) in 0.8 mL DCM, 0.1 equivalent TMSOTf, -20 °C	30 min
12	TLC sample	20 μL of crude reaction mixture withdrawn	
13	Quenching	0.05 mL TEA	
14	Evaporation	40 °C	45 min
15	Deprotection	1.0 mL CAN/MeCN/H_2O, 25 °C	1 h
16	TLC sample	20 μL of crude reaction mixture withdrawn	
17	Sample loading	1.0 mL crude sample transferred to cartridges	
18	Wash	12 mL 80% methanol wash	45 min
19	Wash	2.0 mL MeOH wash (repeated 3 × 2 times)	
20	Transfer	6.0 mL collected sample to the reactor vial for next cycle	
21	Evaporation	40 °C	45 min
22	Glycosylation	3.0 equivalent donor (150 μmol) in 0.8 mL DCM, 0.8 mL DCM, 0.1 equivalent TMSOTf, 0 °C	30 min
23	Repeat the same procedure as step 2-15	Repeat 2 cycle	8 h 50 min
24			
25	Transfer	6.0 mL solution transferred out of the synthesis platform	



8

***cis*-4-(1H,1H,2H,2H,3H,3H-perfluoroundecyloxy)-2-butenyl (methyl 2,4-di-*O*-benzyl-4-*O*-(methyl 2,4-di-*O*-benzyl-4-*O*-(methyl 2,4-di-*O*-benzyl-β-D-mannopyranosyl uronate)-β-D-mannopyranoside uronate)-β-D-mannopyranoside uronate) (8)**

The product solution was transferred out of the synthesis platform after the FSPE step of the first cycle (25th step of the automated synthesis). The solvent was removed under reduced pressure and the product was purified by flash chromatography by using EtOAc/petroleum ether (1/2). Then the crude product was purified by a preparative TLC by using diethyl ether/benzene (1/5). The product was collected from the preparative TLC plate and obtained as a colorless syrup (14 mg, 11 μmol, 22% over 6 steps).

www.manaraa.com

***R**_f*: 0.35 (diethyl ether/benzene: 1/5)

^{1}H NMR ($CDCl_3$, 600MHz): δ (ppm) 7.43-7.41 (m, 2H, H_{arom}), 7.34-7.18 (m, 28H, H_{arom}), 5.76 (m, 2H, *H*C=C*H*), 4.98-4.96 (m, 3H, CH*H*Ph), 4.93 (d, 1H, *J* = 10.8 Hz, CH*H*Ph), 4.90 (d, 1H, J = 12.0 Hz, CH*H*Ph), 4.86 (d, 1H, *J* = 10.8 Hz, CH*H*Ph), 4.75 (d, 1H, *J* = 12.6 Hz, CH*H*Ph), 4.72 (d, 1H, *J* = 12.0 Hz, CH*H*Ph), 4.61 (s, 1H, H-1), 4.59 (d, 1H, *J* = 4.2 Hz, CH*H*Ph), 4.51-4.47 (m, 3H, OH*H*CHC=C, 2 × CH*H*Ph), 4.44 (d, 1H, *J* = 10.2 Hz, CH*H*Ph), 4.28-4.24 (m, 2H, H-5, OH*H*CHC=C), 4.19 (s, 1H, H-1), 4.14 (s, 1H, H-1), 4.12 (d, 1H, *J* = 9.6 Hz, H-4), 4.07 (d, 2H, *J* = 3.6 Hz, C=CHC*H*$_2$O), 3.99 (dd, 1H, *J* = 8.4, 3.0 Hz, H-3), 3.92-3.87 (m, 3H, H-2, 2 × H-4), 3.74-3.67 (m, 10H, H-5, 9 × OC*H*$_3$), 3.63-3.61 (m, 2H, H-2, H-5), 3.50-3.45 (m, 3H, H-3, OC*H*$_2$CH$_2$), 3.37 (d, 1H, *J* = 3.6 Hz, H-2), 2.34 (d, 1H, *J* = 10.2 Hz, 3-O*H*), 2.21 (m, 2H, C*H*$_2$CF$_2$), 1.90 (m, 2H, OCH$_2$C*H*$_2$)

^{13}C NMR ($CDCl_3$, 150 MHz): δ (ppm) 168.9, 168.8, 168.6, 138.6, 138.4, 138.4, 138.3, 138.1, 130.7, 128.8, 128.8, 128.7, 128.7, 128.6, 128.6, 128.6, 128.6, 128.6, 128.5, 128.5, 128.5, 128.4, 128.4, 128.3, 128.3, 128.2, 128.2, 128.1, 128.1, 128.1, 128.0, 128.0, 127.8, 100.4 (J_{C1-H1} = 154.4 Hz), 98.6 (J_{C1-H1} = 155.3 Hz), 98.2 (J_{C1-H1} = 152.7 Hz), 78.4, 77.8, 77.4, 75.3, 75.3, 75.2, 75.2, 75.2, 75.1, 74.9, 74.9, 74.6, 73.9, 73.6, 73.0, 72.4, 69.0, 66.7, 65.0, 52.6, 52.6, 52.5, 28.3 (t, J_{C-F} = 21.8 Hz), 21.0

HRMS (ESI): $[M + Na]^+$ calcd for $C_{78}H_{79}F_{17}NaO_{20}^+$ 1681.4785, found 1681.4762

HPLC trace of the automated synthesis of **8**

www.manaraa.com

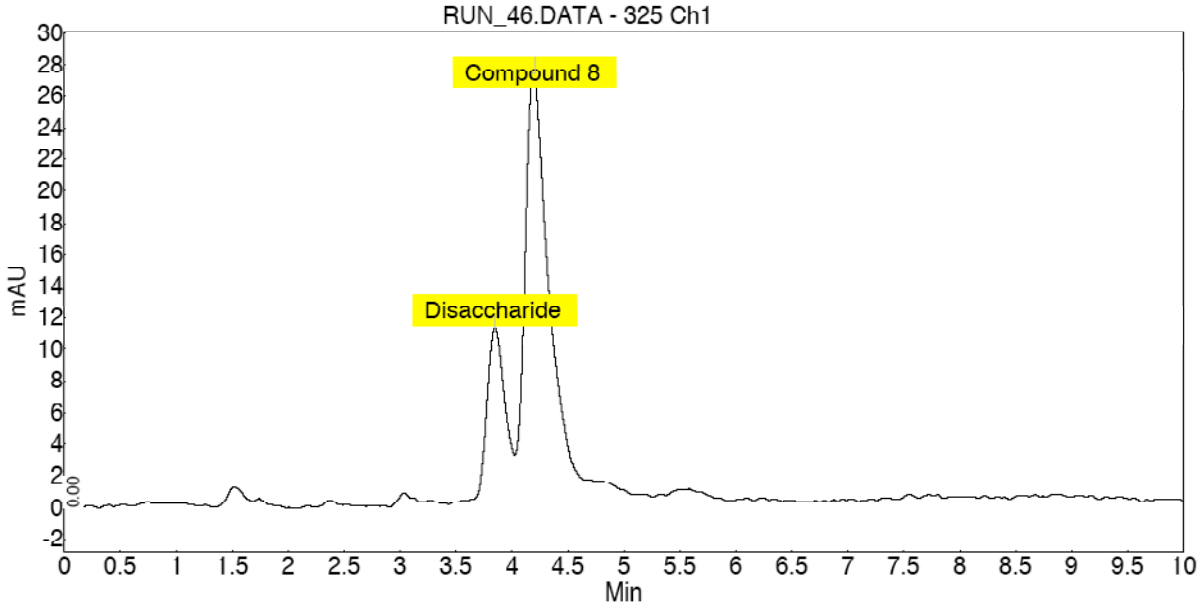
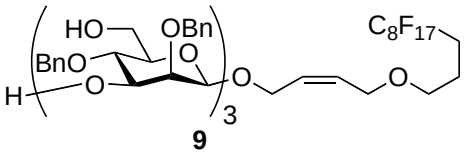


Figure 5. Analytical HPLC chromatogram of the crude product from the synthesis platform after FSPE (1.0 mL/min flowrate, EtOAc/hexanes: 1/2, 10 minutes run, Waters Nova-Pak 4 μm 3.9 × 150 mm silica column)

Table 4. Automated synthetic cycles for production of compound **9**

Step	Task	Reagent/ Operation	Operation Time
26	Reduction	2.0 mL THF, 0.3 mL LiTEBH, **8** (11 μmol), 0 °C	1 h
27	TLC sample	20 μL of crude reaction mixture withdrawn	
28	Quenching	0.5 mL MeOH	
29	Evaporation	40 °C	45 min
30	Transfer	Transfer out of the synthesis platform	



9

www.manaraa.com

cis-4-(1H,1H,2H,2H,3H,3H-perfluoroundecyloxy)-2-butenyl 2,4-di-_O_-benzyl-3-_O_-(2,4-di-_O_-benzyl-3-_O_-(2,4-di-_O_-benzyl-β-D-mannopyranosyl)-β-D-mannopyranosyl)-β-D-mannopyranoside (9)

The product solution was transferred out of the synthesis platform after the FSPE step of the first cycle (30th step of the automated synthesis). The solvent was removed under reduced pressure and the product was purified by flash chromatography by using EtOAc/petroleum ether (3/1 to 1/0). The product was obtained as a colorless syrup (10 mg, 6.3 μmol, 57%).

***R**$_f$*: 0.23 (EtOAc/benzene: 4/1)

^{1}H NMR (CDCl$_3$, 700 MHz): δ (ppm) 7.44 (d, 2H, *J* = 7.7 Hz, H$_{arom}$), 7.35-7.21 (m, 28H, H$_{arom}$), 5.77 (m, 2H, *H*C=C*H*), 5.05-4.99 (m, 4H, CH*H*Ph), 4.95 (d, 1H, *J* = 11.9 Hz, CH*H*Ph), 4.91 (d, 1H, *J* = 11.2 Hz, CH*H*Ph), 4.77 (d, 2H, *J* = 11.2 Hz, CH*H*Ph), 4.63 (d, 1H, *J* = 11.2 Hz, CH*H*Ph), 4.56-4.48 (m, 4H, H-1, 3 × CH*H*Ph), 4.47 (dd, 1H, *J* = 12.6, 3.5 Hz, OH*H*CHC=C), 4.34 (s, 1H, H-1), 4.26 (dd, 1H, *J* = 12.6, 4.9 Hz, OH*H*CHC=C), 4.22 (s, 1H, H-1), 4.07 (m, 2H, C=CHC*H*$_2$O), 3.97-3.89 (m, 5H, H-6), 3.86-3.76 (m, 6H, 3 × H-3), 3.70 (dd, 1H, *J* = 9.8, 2.8 Hz), 3.65-3.60 (m, 3H), 3.59-3.56 (m, 3H, H-6), 3.53 (s, 1H), 3.50 (m, 2H, OC*H*$_2$CH$_2$), 3.38 (m, 1H, H-5), 3.21 (s, 1H, H-5), 3.16 (m, 1H, H-5), 2.31 (d, 1H, *J* = 8.4 Hz, O*H*), 2.21 (m, 2H, C*H*$_2$CF$_2$), 2.13 (s, 1H, O*H*), 1.90 (m, 2H, OCH$_2$C*H*$_2$), 1.82 (s, 1H, O*H*)

^{13}C NMR (CDCl$_3$, 150 MHz): δ (ppm) 138.8, 138.5, 138.5, 138.3, 138.2, 130.2, 128.8, 128.7, 128.7, 128.6, 128.6, 128.5, 128.4, 128.4, 128.3, 128.3, 128.3, 128.2, 128.1, 128.1, 128.0, 127.9, 127.9, 127.9, 100.5, 98.4, 97.8, 79.8, 79.1, 78.3, 76.7, 75.9, 75.6, 75.6, 75.4, 75.2, 75.0, 75.0, 75.0, 74.8, 74.6, 74.5, 74.3, 74.0, 73.8, 73.7, 69.1, 66.7, 65.1, 62.7, 62.4, 62.3, 28.3 (t, J_{C-F} = 22.4 Hz), 21.0

www.manaraa.com

HRMS (ESI): $[M + Na]^+$ calcd for $C_{75}H_{79}F_{17}NaO_{17}^+$ 1597.4938, found 1597.4925

HPLC trace of the automated synthesis of **9**

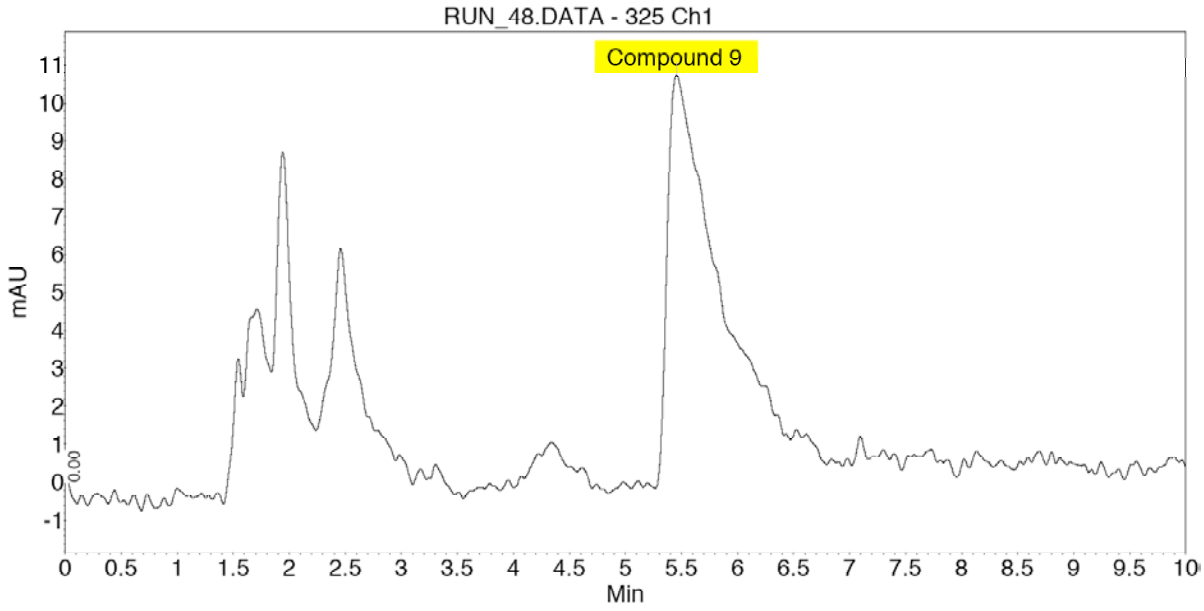
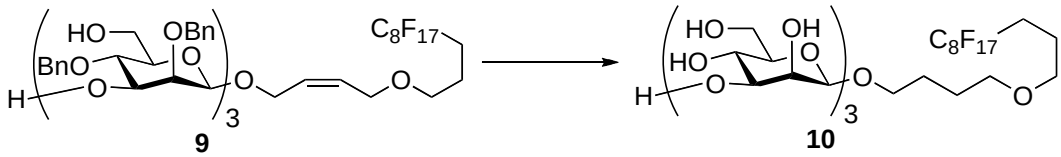


Figure 6. Analytical HPLC chromatogram of the crude product from the synthesis platform after FSPE (1.0 mL/min flowrate, EtOAc/hexanes: 9/1, 10 minutes run, Waters Nova-Pak 4 μm 3.9 × 150 mm silica column)



3-(perfluorooctyl)propanyloxybutanyl 3-*O*-(3-*O*-(β-D-mannopyranosyl)-β-D-mannopyranosyl)-β-D-mannopyranoside (10)

cis-4-(1H,1H,2H,2H,3H,3H-perfluoroundecyloxy)-2-butenyl 2,4-di-*O*-benzyl-3-*O*-(2,4-di-*O*-benzyl-3-*O*-(2,4-di-*O*-benzyl-β-D-mannopyranosyl)-β-D-mannopyranosyl)-β-D-

www.manaraa.com

mannopyranoside **9** (10 mg, 6.3 μmol) was dissolved in MeOH/AcOH = 10/1 (2.5 mL) and 10% Pd/C (5.0 mg) and Pd black (5.0 mg) were added. The mixture was stirred under 1000 psi H_2 atmosphere at 20 °C. After 48 h, the mixture was filtered through a short pad of Celite and the solvent was removed under reduced pressure. The desired product was collected as a white foam (6.2 mg, 6.0 μmol, 95%).

^{1}H NMR ($CDCl_3$, 700 MHz): δ (ppm) 4.74 (s, 1H, H-1), 4.73 (s, 1H, H-1), 4.52 (s, 1H, H-1), 4.15 (d, 1H, *J* = 2.8 Hz, H-2), 4.06 (s, 1H, *J* = 2.8 Hz, H-2), 3.98 (s, 1H, *J* = 2.8 Hz, H-2), 3.96 (dd, 1H, *J* = 6.3, 2.8 Hz, -OC*H*$_2$CH$_2$CH$_2$CH$_2$O-), 3.93-3.89 (m, 3H, 3 × H-6), 3.84-3.81 (m, 2H, 2 × H-3), 3.75-3.72 (m, 2H, 2 × H-6), 3.71-3.66 (m, 3H, 2 × H-4, H-6), 3.59 (dd, 1H, *J* = 5.6, 3.5 Hz, -OC*H*$_2$CH$_2$CH$_2$CH$_2$O-), 3.56 (t, 1H, *J* = 9.8 Hz, H-4), 3.52 (t, 2H, *J* = 6.3 Hz, -OC*H*$_2$CH$_2$CH$_2$CF$_2$), 3.49 (m, 3H, H-3, CH$_2$C*H*$_2$O-), 3.28-3.24 (m, 2H, 2 × H-5), 2.29 (m, 2H, C*H*$_2$CF$_2$), 1.87 (m, 2H, OCH$_2$C*H*$_2$CH$_2$CF$_2$), 1.69 (m, 4H, -OCH$_2$C*H*$_2$C*H*$_2$CH$_2$O-)

^{13}C NMR ($CDCl_3$, 150 MHz): δ (ppm) 101.7, 98.5, 98.1, 81.2, 81.2, 78.7, 78.3, 78.1, 75.3, 72.7, 71.9, 70.6, 70.2, 69.5, 69.3, 68.8, 67.0, 66.9, 63.1, 62.9, 29.1 (t, $J_{C\text{-}F}$ = 21.5 Hz), 27.6, 27.5, 22.0.

HRMS (ESI): $[M + Na]^+$ calcd for $C_{33}H_{45}F_{17}NaO_{17}^+$ 1059.2278, found 1059.2292

www.manaraa.com

CHAPTER 6

Conclusions and Future Directions

This dissertation reports the first automated solution-phase synthesis of the challenging β-mannans including β-1,2-mannan, β-1,4-mannan, the structurally related β-1,4-mannuronates, the insect *N*-glycan terminal trimannoside, β-1,6-mannan, and β-1,3-mannan oligosaccharides by using two different synthetic strategies including an oxidation-reduction sequence and the β-directing mannuronate strategy. The β-directing mannuronate strategy turned out to be a more efficient methodology providing better yields and a shorter synthetic route with high β-selectivities. Also, the solution-phase synthesis is more economical with only 2.0 to 3.5 equivalent of glycosyl donor needed for each glycosylation reaction compared to 9.0 to 10 equivalents for a comparable automated solid-phase protocol. In addition, a simple TLC could monitor the reaction with the homogeneous solution-phase mixture. Plus, the chapters include examples in which the fluorous tag-modified sugars have been transferred out of the synthesis platform, purified on bench top, and reinjected into the synthesis platform for further reactions; such flexibility was not provided by the solid-phase protocol. Besides the successful synthesis of the challenging β-mannans by the automated solution-phase synthesis, the β-mannan-containing insect *N*-glycan trimannoside was also synthesized and used for probing the possible binding of this carbohydrate motif with pea enation mosaic virus (PEMV). Both a fluorous microarray and subsequent isothermal titration calorimetry (ITC) studies did not show any specific interactions between the virus and sugar. Therefore, the results clarified that the specific interaction between PEMV and the receptor protein aminopeptidase N does not rely on the insect *N*-glycan.

www.manaraa.com

With the new protocols developed for automated solution-phase synthesis using the β-directing mannuronate strategy, more β-mannan-containing potentially biologically significant natural products can be synthesized with high efficiency in the future; those molecules can then be used for microarray binding studies, ITC experiments, drug delivery applications, and other biological experiments.

www.manaraa.com

ACKNOWLEDGEMENTS

First, I would like to give my greatest appreciation to my advisor Dr. Nicola Pohl for her inspiration, encouragement, and training me as an independent researcher throughout these years in Iowa State University. And, I would like to thank my POS committees: Dr. Malika Jeffries-EL, Dr. Reuben Peters, Dr. Aaron Sadow, Dr. Yan Zhao, and also my former POS committees Dr. Richard Larock and Dr. Victor Lin for their advices during my PhD work. Also I would like to thank Dr. Jason Chen for his many useful advise of organic synthesis, and Dr. Shu Xu, Dr. Sarah Cady, and Dr. Bruce Fulton for their assistance with NMR spectroscopy, and Dr. Kamel Harrata for the assistance with mass spectroscopy.

I would like to thank my past and present lab mates: Dr. Rahman M. Mizanur, Dr. Beatrice Collet, Dr. Xueshu Li, Dr. Yonghai Chai, Dr. Guosong Chen, Dr. Steve Brokman, Dr. Gulden Camci-Unal, Dr. Eun-Ho Song, Heather Edwards, Sinele Tsabedze, Sahana Nagappayya, Rajarshi Roy Choudhury, Joy Jackson, Xin Liu, Adam Shroyer, Hannah Fuller, Nishad Thambanchandrika, Sayantan Bhaduri, Daniel Kabotso, and Manibarsha Goswami for their help in these years. I would like to especially thank Lin Liu for his support since we joined Iowa Sate University in 2006. Also, I would like to thank Randy Benedict for teaching me knowledge from language, cooking, music, to chemistry. I would also like to thank my friends Isaac Ho, and Dr. Po-Wen Chung for their advise to my life and career.

www.manaraa.com

I would like to give my most special thank to my parents and brother for their love and support. Finally, I would like to thank my greatest supporter: my wife Ya-Lan Yang who has given me so much and made me the luckiest man in the world.

www.manaraa.com

BIBLIOGRAPHY

Betaneli, V. I.; Ovchinnikov, M. V.; Bakinovskii, L. V.; Kochetkov, N. K. Practical synthesis of O-β-D-mannopyranosyl-, O-α-D-mannopyranosyl-, and O-β-D-glucopyranosyl-(1 → 4)-O-α-L-rhamnopyranosyl-(1 → 3)-D-galactoses. *Carbohydr. Res.* **1980**, *84*, 211-24.

Brokman, S. M., Jr. Synthetic standards for mass spectrometry-based carbohydrate sequencing and the automated solution-phase syntheses of beta-glucans. 2009.

Codee, J. D. C.; Kroeck, L.; Castagner, B.; Seeberger, P. H. Automated solid-phase synthesis of protected oligosaccharides containing β-mannosidic linkages. *Chem.—Eur. J.* **2008**, *14*, 3987-3994.

Codee, J. D. C.; van den Bos, L. J.; de Jong, A.-R.; Dinkelaar, J.; Lodder, G.; Overkleeft, H. S.; van der Marel, G. A. The Stereo-Directing Effect of the Glycosyl C5-Carboxylate Ester: Stereoselective Synthesis of β-Mannuronic Acid Alginates. *J. Org. Chem.* **2009**, *74*, 38-47.

Crich, D. Mechanism of a Chemical Glycosylation Reaction. *Acc. Chem. Res.* **2010**, *43*, 1144-1153.

Crich, D.; Banerjee, A.; Yao, Q. Direct Chemical Synthesis of the β-D-Mannans: The β-(1→2) and β-(1→4) Series. *J. Am. Chem. Soc.* **2004**, *126*, 14930-14934.

Crich, D.; Jayalath, P. 2-*O*-Propargyl Ethers: Readily Cleavable, Minimally Intrusive Protecting Groups for β-Mannosyl Donors. *Org. Lett.* **2005**, 7, 2277-2280.

Crich, D.; Li, H.; Yao, Q.; Wink, D. J.; Sommer, R. D.; Rheingold, A. L. Direct Synthesis of β-Mannans. A Hexameric [→)- β-D-Man-(1→4)- β-D-Man-(1]$_3$ Subunit of the

www.manaraa.com

Antigenic Polysaccharides from Leptospira biflexa and the Octameric (1→2)-Linked *β*-D-Mannan of the Candida albicans Phospholipomannan. X-ray Crystal Structure of a Protected Tetramer. *J. Am. Chem. Soc.* **2001**, *123*, 5826-5828.

Crich, D.; Li, W.; Li, H. Direct Chemical Synthesis of the *β*-Mannans: Linear and Block Syntheses of the Alternating *β*-(1→3)- *β*-(1→4)-Mannan Common to Rhodotorula glutinis, Rhodotorula mucilaginosa, and Leptospira biflexa. *J. Am. Chem. Soc.* **2004**, *126*, 15081-15086.

Crich, D.; Rahaman, M. Y. Synthesis and Structural Verification of the Xylomannan Antifreeze Substance from the Freeze-Tolerant Alaskan Beetle Upis ceramboides. *J. Org. Chem.* **2011**, *76*, 8611-8620.

Crich, D.; Sun, S. Are Glycosyl Triflates Intermediates in the Sulfoxide Glycosylation Method? A Chemical and ^{1}H, ^{13}C, and ^{19}F NMR Spectroscopic Investigation. *J. Am. Chem. Soc.* **1997**, *119*, 11217-11223.

Crich, D.; Wu, B.; Jayalath, P. Convergent Synthesis of a *β*-(1→3)-Mannohexaose. *J. Org. Chem.* **2007**, 72, 6806-6815.

Dinkelaar, J.; de Jong, A. R.; van Meer, R.; Somers, M.; Lodder, G.; Overkleeft, H. S.; Codee, J. D. C.; van der Marel, G. A. Stereodirecting Effect of the Pyranosyl C-5 Substituent in Glycosylation Reactions. *J. Org. Chem.* **2009**, *74*, 4982-4991.

Flo, T. H.; Ryan, L.; Latz, E.; Takeuchi, O.; Monks, B. G.; Lien, E.; Halaas, O.; Akira, S.; Skjak-Brak, G.; Golenbock, D. T.; Espevik, T. Involvement of Toll-like Receptor (TLR) 2 and TLR4 in Cell Activation by Mannuronic Acid Polymers. *J. Biol. Chem.* **2002**, *277*, 35489-35495.

Gray, S.; Gildow, F. E. Luteovirus-aphid interactions. *Annual Review of Phytopathology*

www.manaraa.com

2003, *41*, 539-566.

Hsu, C.-H.; Hung, S.-C.; Wu, C.-Y.; Wong, C.-H. Toward Automated Oligosaccharide Synthesis. *Angew. Chem., Int. Ed.* **2011**, *50*, 11872-11923.

Ishiwata, A.; Sakurai, A.; Nishimiya, Y.; Tsuda, S.; Ito, Y. Synthetic Study and Structural Analysis of the Antifreeze Agent Xylomannan from Upis ceramboides. *J. Am. Chem. Soc.* **2011**, *133*, 19524-19535.

Iwamoto, M.; Kurachi, M.; Nakashima, T.; Kim, D.; Yamaguchi, K.; Oda, T.; Iwamoto, Y.; Muramatsu, T. Structure-activity relationship of alginate oligosaccharides in the induction of cytokine production from RAW264.7 cells. *FEBS Lett.* **2005**, *579*, 4423-4429.

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*, 1707-1710.

Janeway, C. A., Jr.; Medzhitov, R. Innate immune recognition. *Annu. Rev. Immunol.* **2002**, *20*, 197-216.

Jiang, L.; Chan, C.-H. Borane/Bu_2BOTf: A Mild Reagent for the Regioselective Reductive Ring Opening of Benzylidene Acetals in Carbohydrate. *Tetrahedron Lett.* **1998**, 39, 355-358.

Kim, K. S.; Baburao Fulse, D.; Baek, J. Y.; Lee, B.-Y.; Jeon, H. B. Stereoselective Direct Glycosylation with Anomeric Hydroxy Sugars by Activation with Phthalic Anhydride and Trifluoromethanesulfonic Anhydride Involving Glycosyl Phthalate Intermediates. *J. Am. Chem. Soc.* **2008**, *130*, 8537-8547.

Kim, K. S.; Kim, J. H.; Lee, Y. J.; Park, J. 2-(Hydroxycarbonyl)benzyl glycosides: a novel

www.manaraa.com

type of glycosyl donors for highly efficient beta-mannopyranosylation and oligosaccharide synthesis by latent-active glycosylation. *J. Am. Chem. Soc.* **2001**, *123*, 8477-81.

Ko, K.-S.; Jaipuri, F. A.; Pohl, N. L. Fluorous-Based Carbohydrate Microarrays. *J. Am. Chem. Soc.* **2005**, *127*, 13162-13163.

Liew, S.-T.; Wei, A. Preparation of Orthogonally Protected Chitosan Oligosaccharides: Observation of an Anomalous Remote Substituent Effect. *Carbohydr. Res.* **2002**, 337, 1319-1324.

Liu, S.; Bonning, B. C.; Allen Miller, W. A simple wax-embedding method for isolation of aphid hemolymph for detection of luteoviruses in the hemocoel. *J. Virol. Methods* **2006**, *132*, 174-180.

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

Merrifield, B. Solid phase synthesis. *Prix Nobel* **1984**, 127-53.

Merrifield, R. B.; Stewart, J. M. Automated peptide synthesis. *Nature* **1965**, *207*, 522-3.

Neu, U.; Woellner, K.; Gauglitz, G.; Stehle, T. Structural basis of GM1 ganglioside recognition by simian virus 40. *Proc. Natl. Acad. Sci. USA.* **2008**, *105*, 5219-5224.

Nitz, M.; Bundle, D. R. Synthesis of Di- to Hexasaccharide 1,2-Linked β-Mannopyranan Oligomers, a Terminal S-Linked Tetrasaccharide Congener and the Corresponding BSA Glycoqconjugates. *J. Org. Chem.* **2001**, *66*, 8411-8423.

Nitz, M.; Purse, B. W.; Bundle, D. R. Synthesis of a β-1,2-Mannopyranosyl Tetrasaccharide Found in the Phosphomannan Antigen of Candida albicans. *Org. Lett.* **2000**, *2*, 2939-

www.manaraa.com

2942.

Oshitari, T.; Shibasaki, M.; Yoshizawa, T.; Tomita, M.; Takao, K.-i.; Kobayashi, S. Synthesis of 2-*O*-(3-*O*-carbamoyl-α-D-mannopyranosyl)-L-gulopyranose: sugar moiety of antitumor antibiotic bleomycin. *Tetrahedron* **1997**, *53*, 10993-11006.

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

Pohl, N. L. Automated solution-phase oligosaccharide synthesis and carbohydrate microarrays: development of fluorous-based tools for glycomics. *ACS Symp. Ser.* **2008**, *990*, 272-287.

Robyt, J. F. *Essentials of Carbohydrate Chemistry*, Springer: New York, 1998.

Schmaltz, R. M.; Hanson, S. R.; Wong, C.-H. Enzymes in the Synthesis of Glycoconjugates. *Chem. Rev.* **2011**, *111*, 4259-4307.

Seeberger, P. H. Automated oligosaccharide synthesis. *Chem. Soc. Rev.* **2008**, *37*, 19-28.

Song, E.-H.; Osanya, A. O.; Petersen, C. A.; Pohl, N. L. B. Synthesis of Multivalent Tuberculosis and Leishmania-Associated Capping Carbohydrates Reveals Structure-Dependent Responses Allowing Immune Evasion. *J. Am. Chem. Soc.* **2010**, *132*, 11428-11430.

Song, X.; Yu, H.; Chen, X.; Lasanajak, Y.; Tappert, M. M.; Air, G. M.; Tiwari, V. K.; Cao, H.; Chokhawala, H. A.; Zheng, H.; Cummings, R. D.; Smith, D. F. A Sialylated Glycan Microarray Reveals Novel Interactions of Modified Sialic Acids with Proteins and Viruses. *J. Biol. Chem.* **2011**, *286*, 31610-22.

Srivastav, S. S.; Khare, N. K.; Khare, A. Stereoselective synthesis of some 1,2-trans-linked oligomers as allyl glycoside using trichloroacetimidate donors. *Indian J. Chem., Sect.*

www.manaraa.com

B: Org. Chem. Incl. Med. Chem. **2000**, *39B*, 346-351.

Tsai, Y.-F.; Shih, C.-H.; Su, Y.-T.; Yao, C.-H.; Lian, J.-F.; Liao, C.-C.; Hsia, C.-W.; Shui, H.-A.; Rani, R. The total synthesis of a ganglioside Hp-s1 analogue possessing neuritogenic activity by chemoselective activation glycosylation. *Org. Biomol. Chem.* **2012**, *10*, 931-934.

van den Bos, L. J.; Dinkelaar, J.; Overkleeft, H. S.; van der Marel, G. A. Stereocontrolled Synthesis of β-D-Mannuronic Acid Esters: Synthesis of an Alginate Trisaccharide. *J. Am. Chem. Soc.* **2006**, *128*, 13066-13067.

Walvoort, M. T. C.; Lodder, G.; Mazurek, J.; Overkleeft, H. S.; Codee, J. D. C.; van der Marel, G. A. Equatorial Anomeric Triflates from Mannuronic Acid Esters. *J. Am. Chem. Soc.* **2009**, *131*, 12080-12081.

Walvoort, M. T. C.; Moggre, G.-J.; Lodder, G.; Overkleeft, H. S.; Codee, J. D. C.; van der Marel, G. A. Stereoselective Synthesis of 2,3-Diamino-2,3-dideoxy-β-D-mannopyranosyl Uronates. *J. Org. Chem.* **2011**, *76*, 7301-7315.

Walvoort, M. T. C.; van den Elst, H.; Plante, O. J.; Kroeck, L.; Seeberger, P. H.; Overkleeft, H. S.; van der Marel, G. A.; Codee, J. D. C. Automated Solid-Phase Synthesis of β-Mannuronic Acid Alginates. *Angew. Chem., Int. Ed.* **2012**, Ahead of Print.

Wu, X.; Bundle, D. R. Synthesis of Glycoconjugate Vaccines for Candida albicans Using Novel Linker Methodology. *J. Org. Chem.* **2005**, *70*, 7381-7388.

Zeng, Y.; Wang, Z.; Whitfield, D.; Huang, X. Installation of Electron-Donating Protective Groups, a Strategy for Glycosylating Unreactive Thioglycosyl Acceptors using the Preactivation-Based Glycosylation Method. *J. Org. Chem.* **2008**, *73*, 7952-7962.

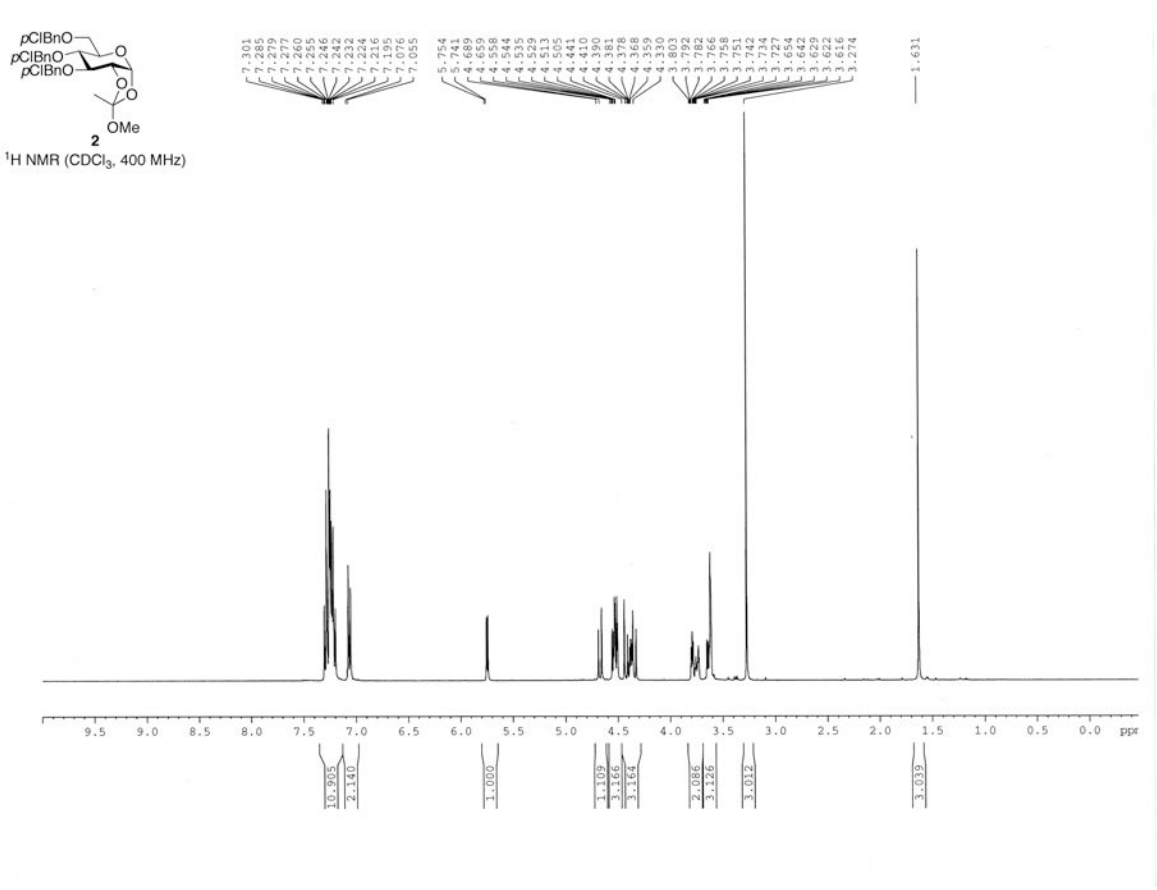
Zhang, Z.; Ollmann, I. R.; Ye, X.-S.; Wischnat, R.; Baasov, T.; Wong, C.-H. Programmable

www.manaraa.com

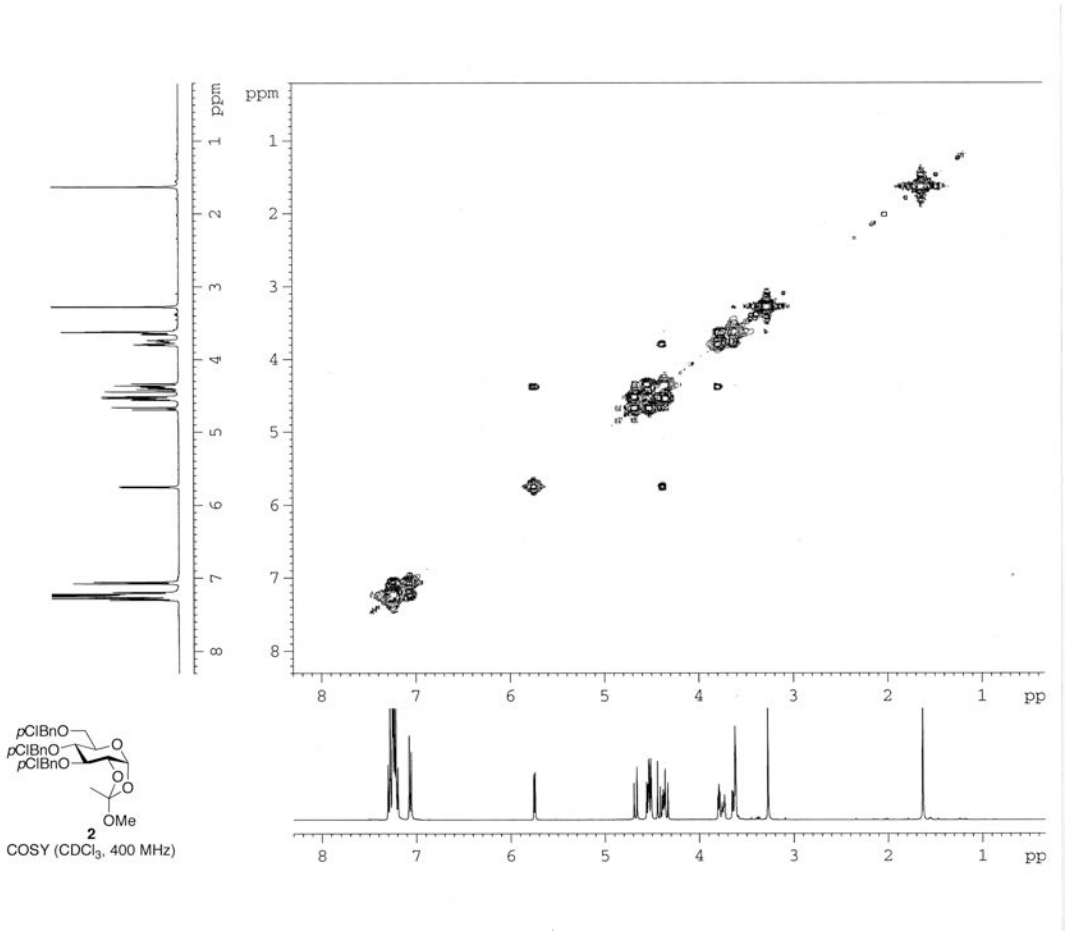
One-Pot Oligosaccharide Synthesis. *J. Am. Chem. Soc.* **1999**, *121*, 734-753.

www.manaraa.com

APPENDIX A. CHAPTER 2 ^{1}H NMR, ^{13}C NMR, COSY, AND HMQC SPECTRA



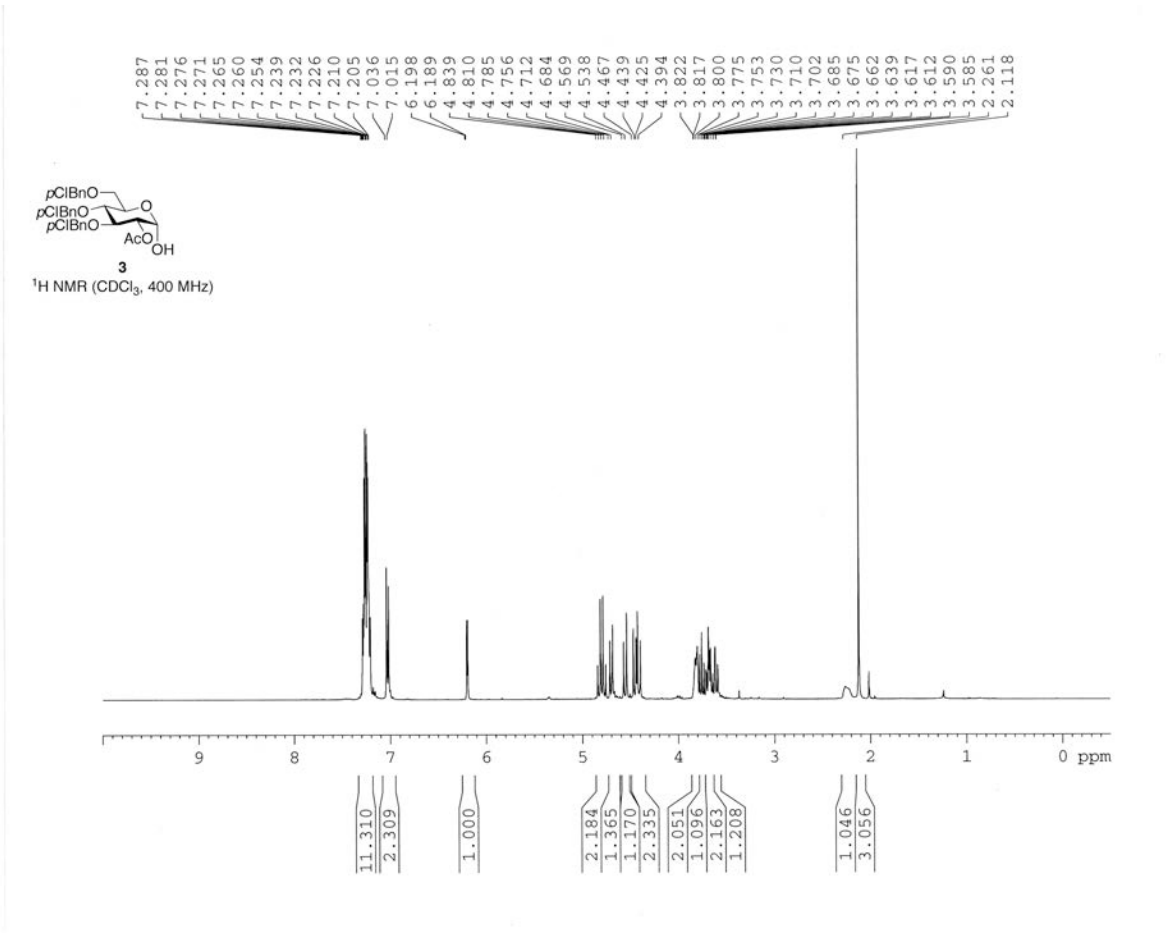
www.manaraa.com



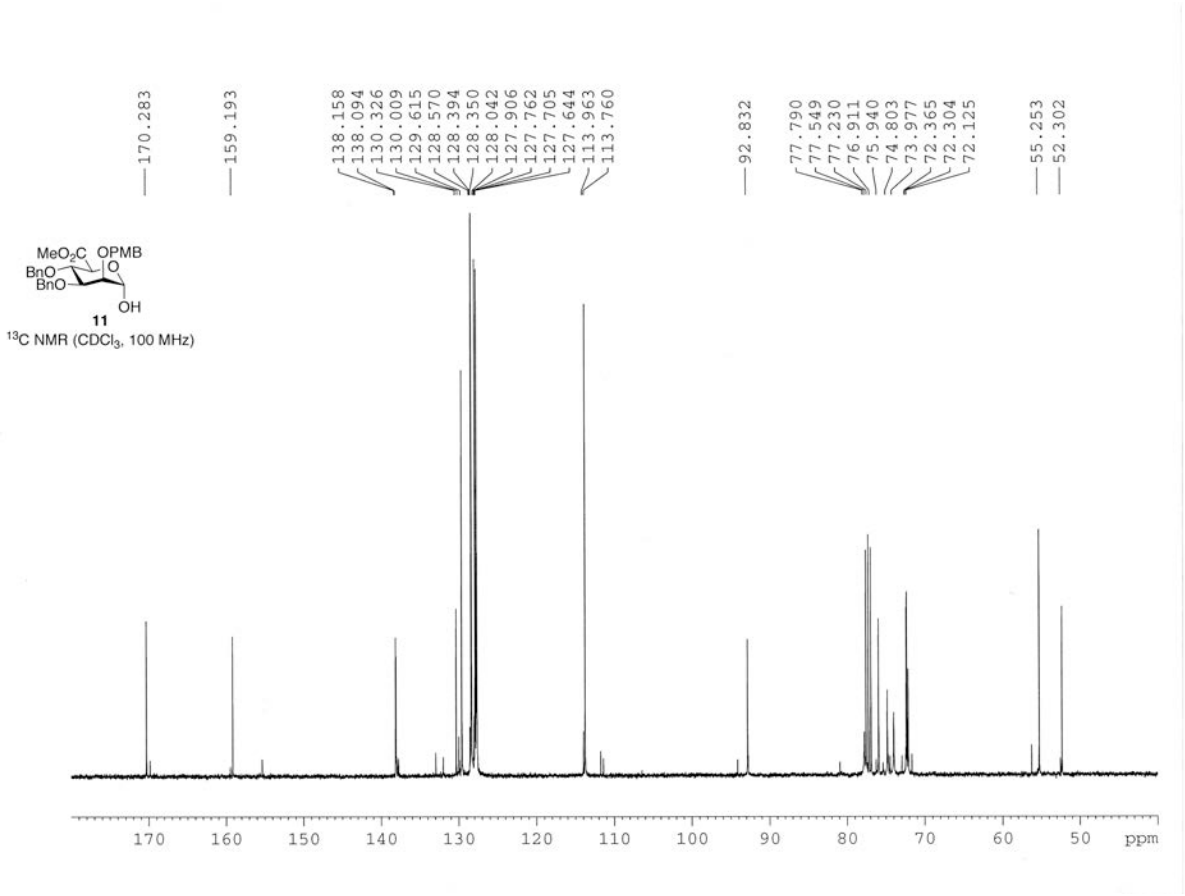
2

COSY ($CDCl_3$, 400 MHz)

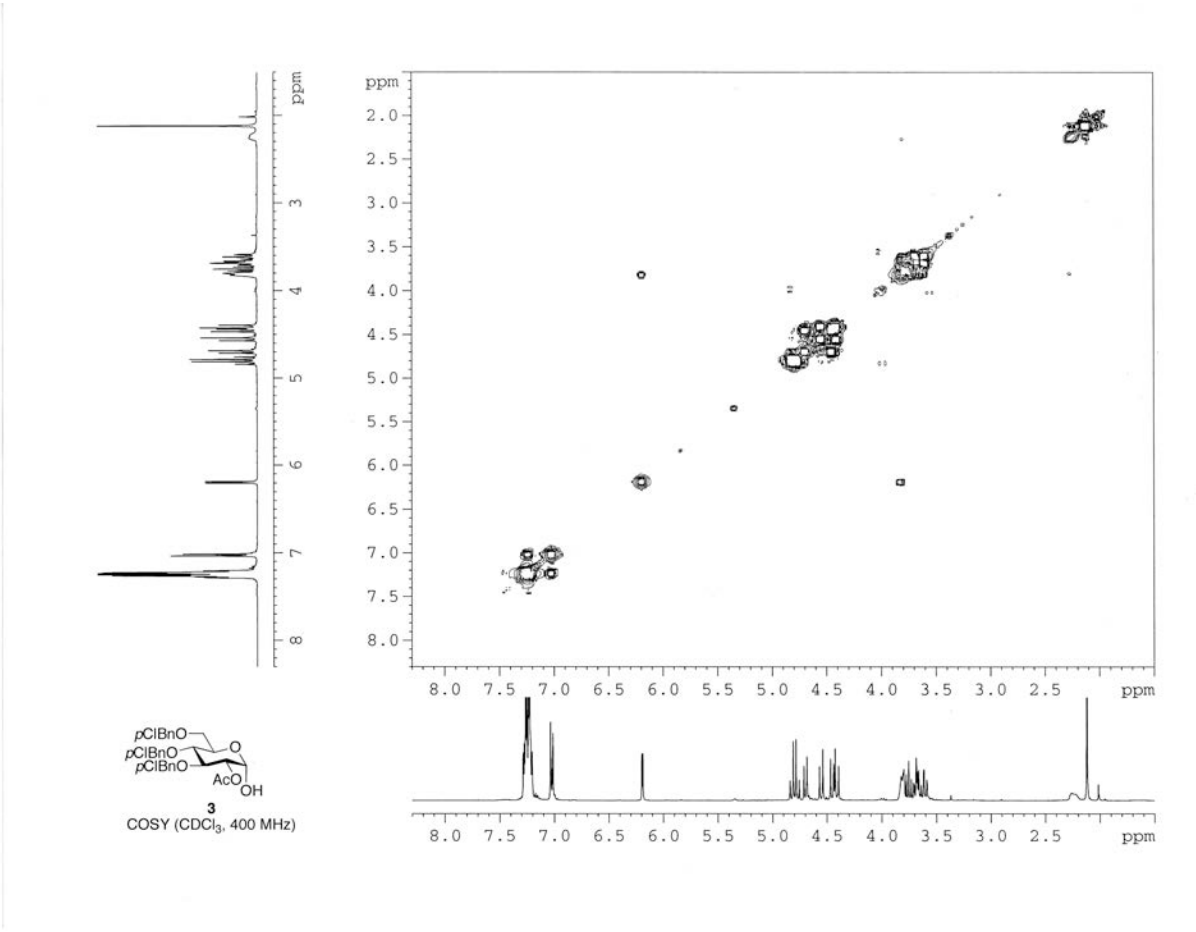
www.manaraa.com



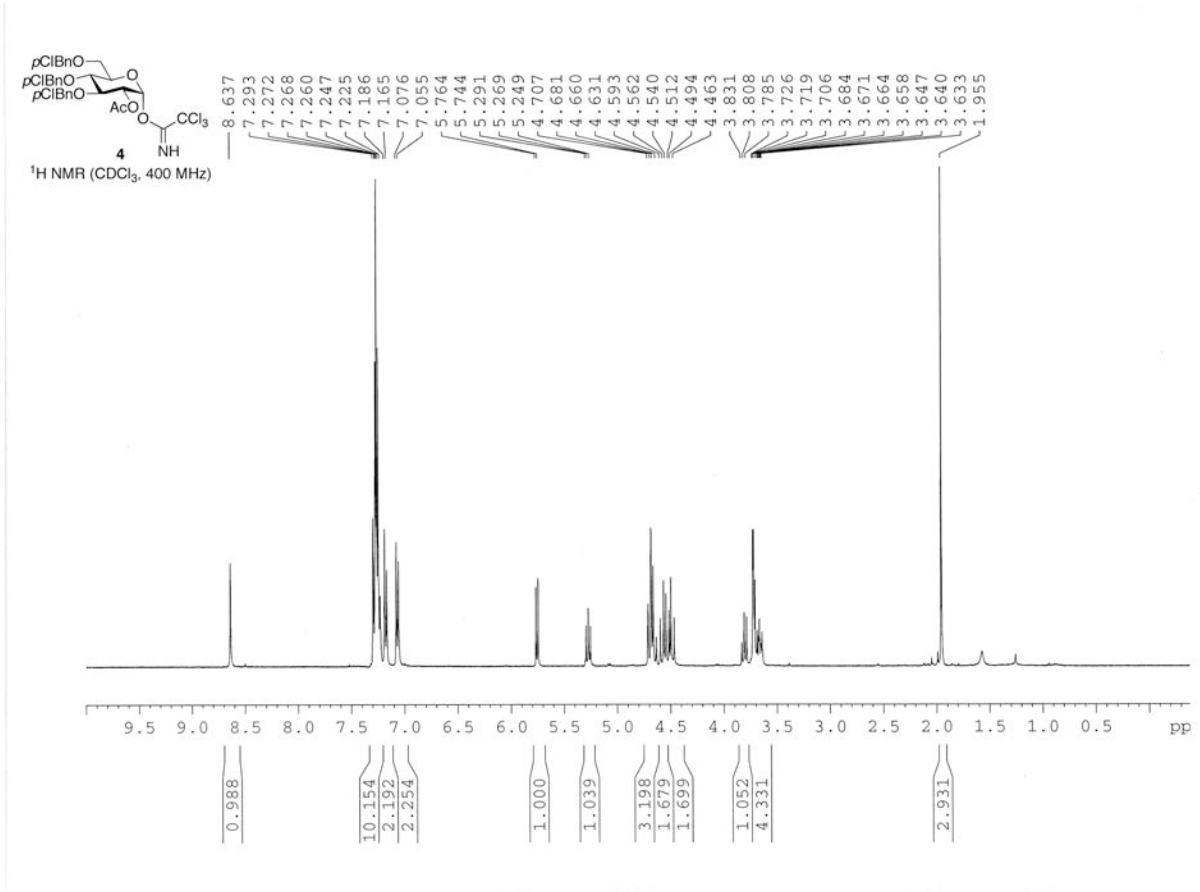
www.manaraa.com



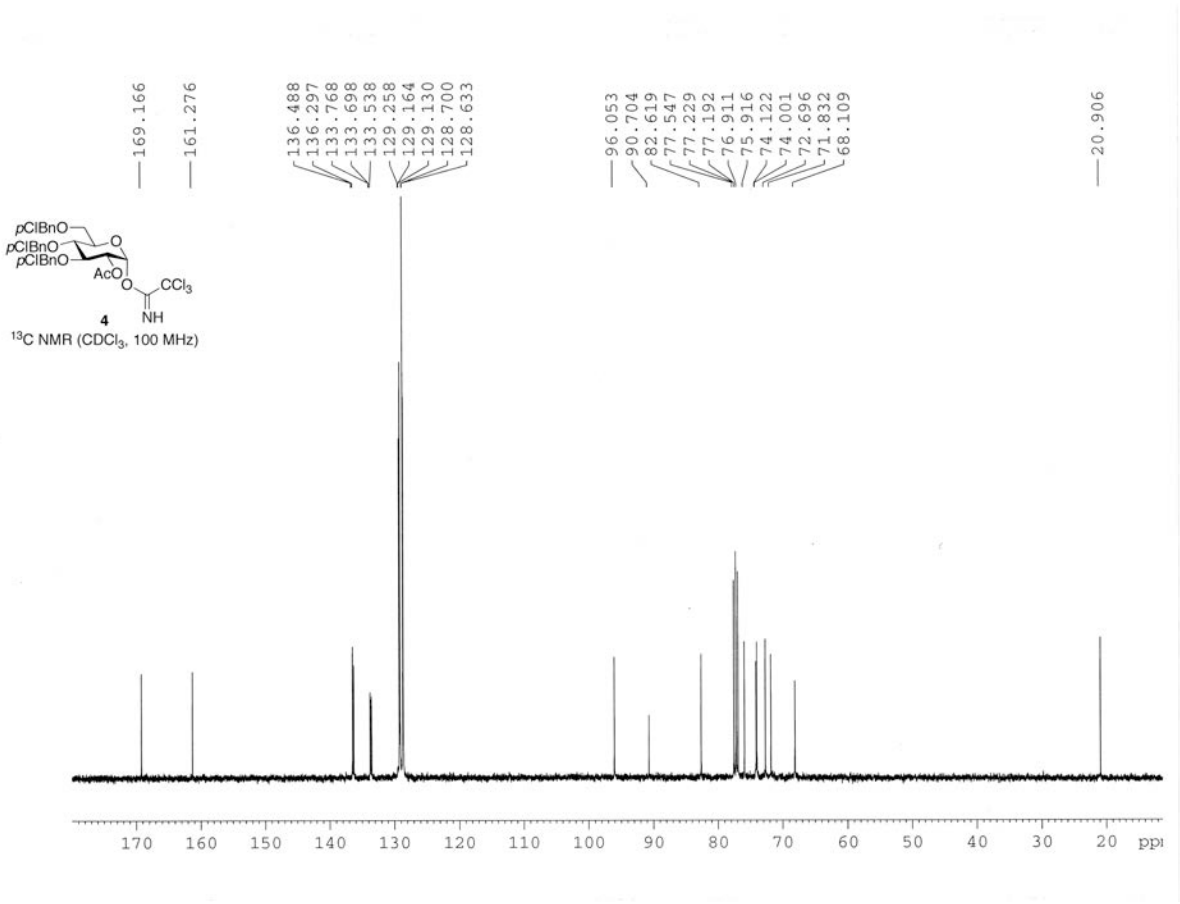
www.manaraa.com



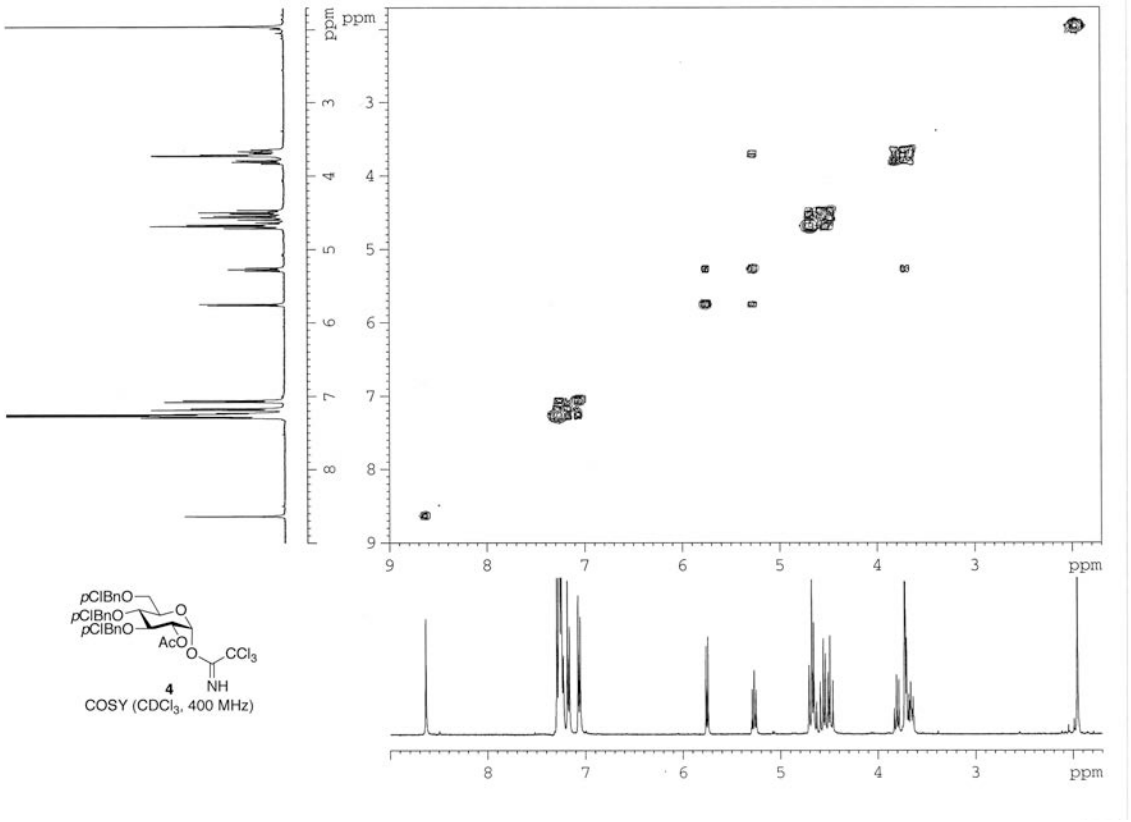
www.manaraa.com



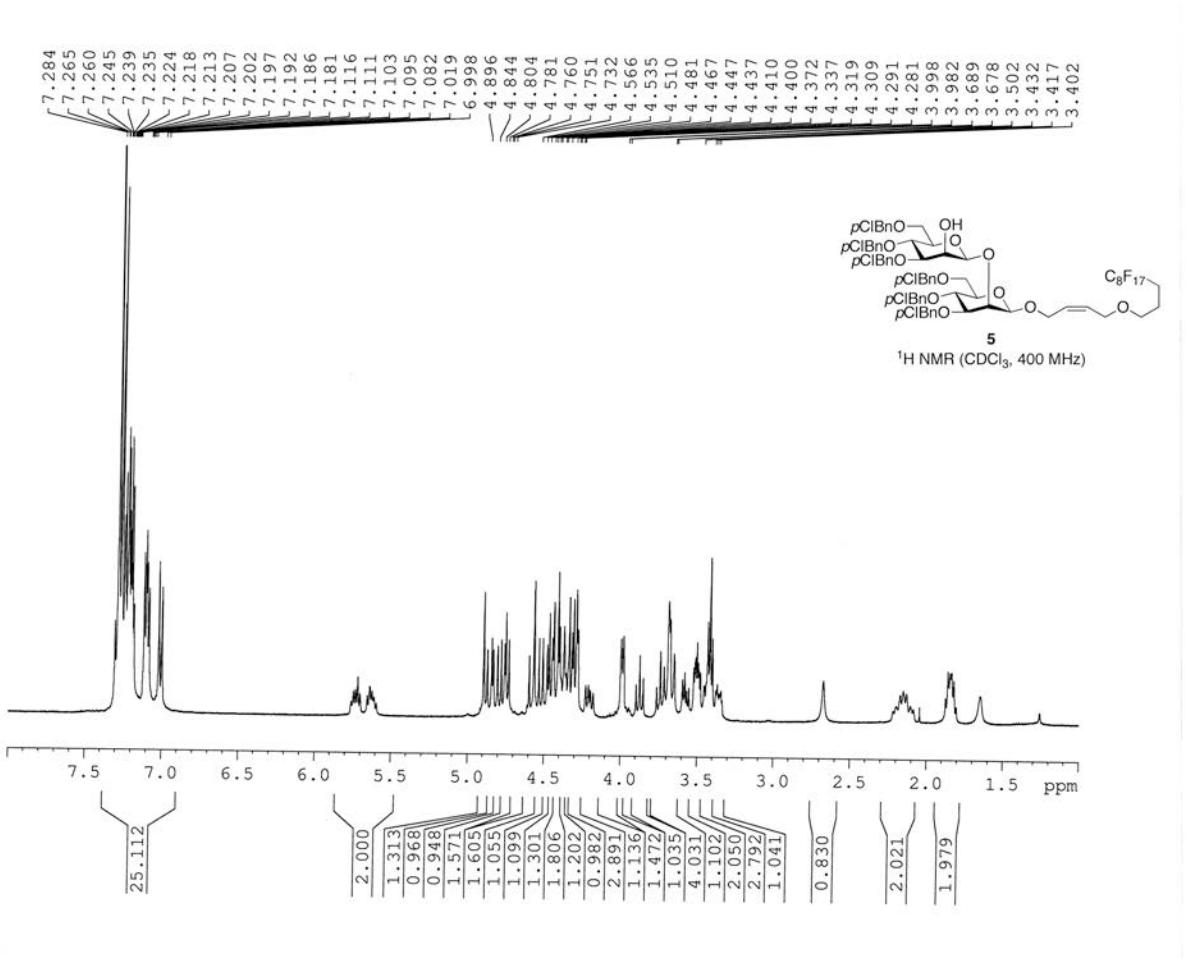
www.manaraa.com



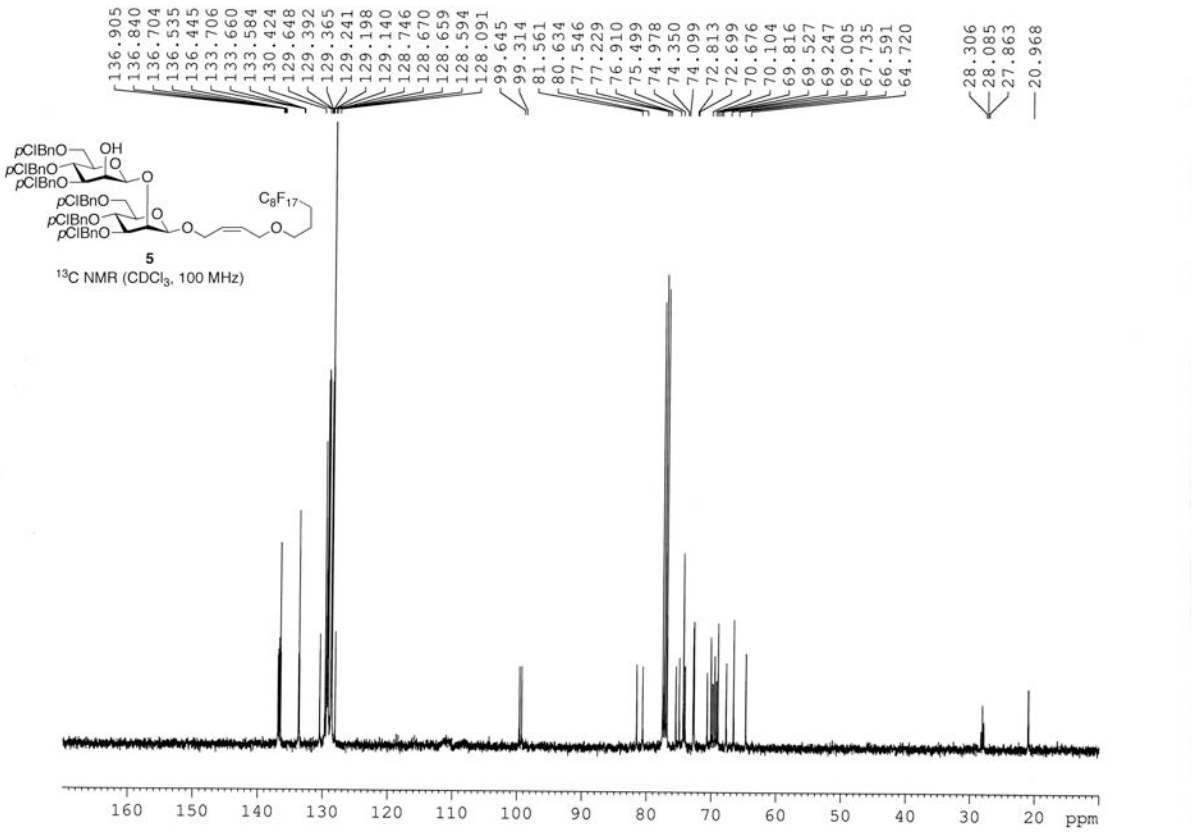
www.manaraa.com



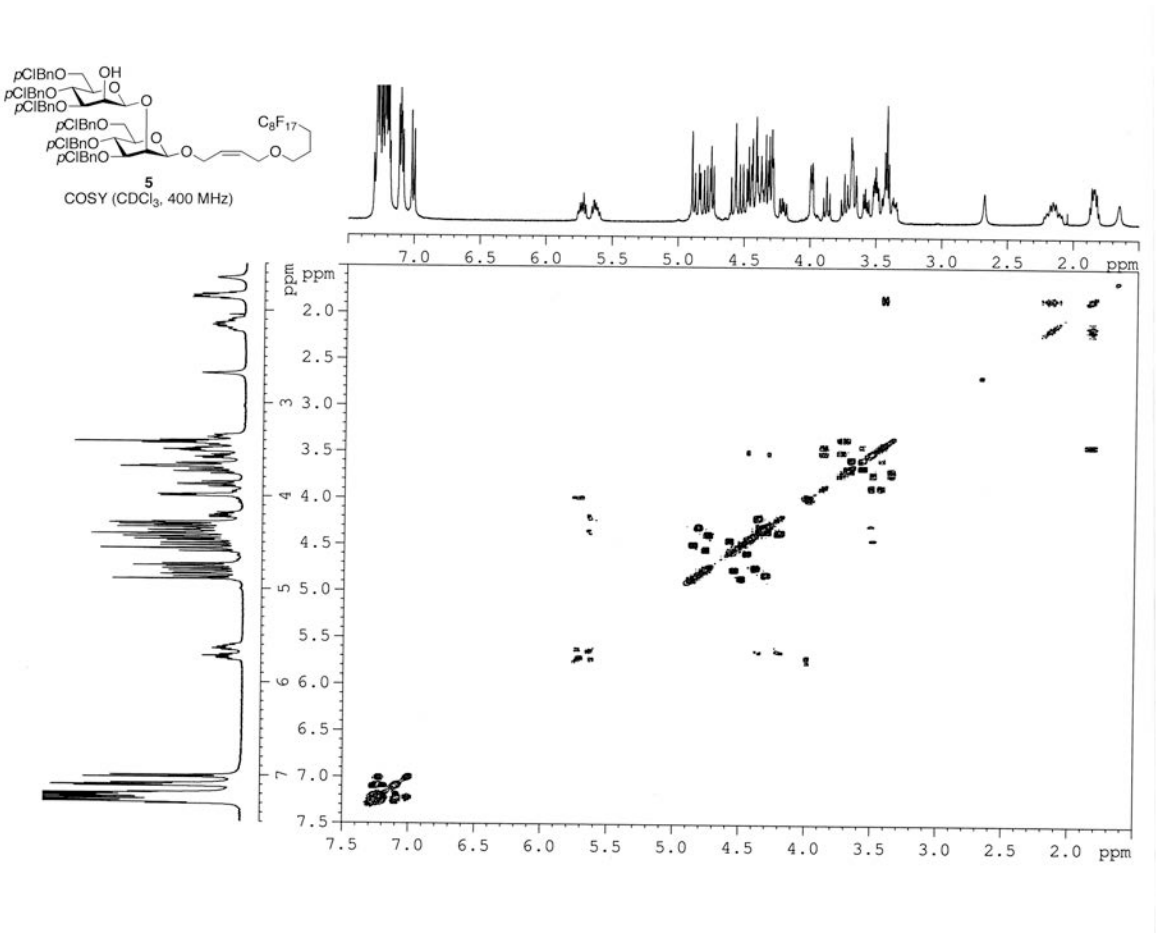
www.manaraa.com



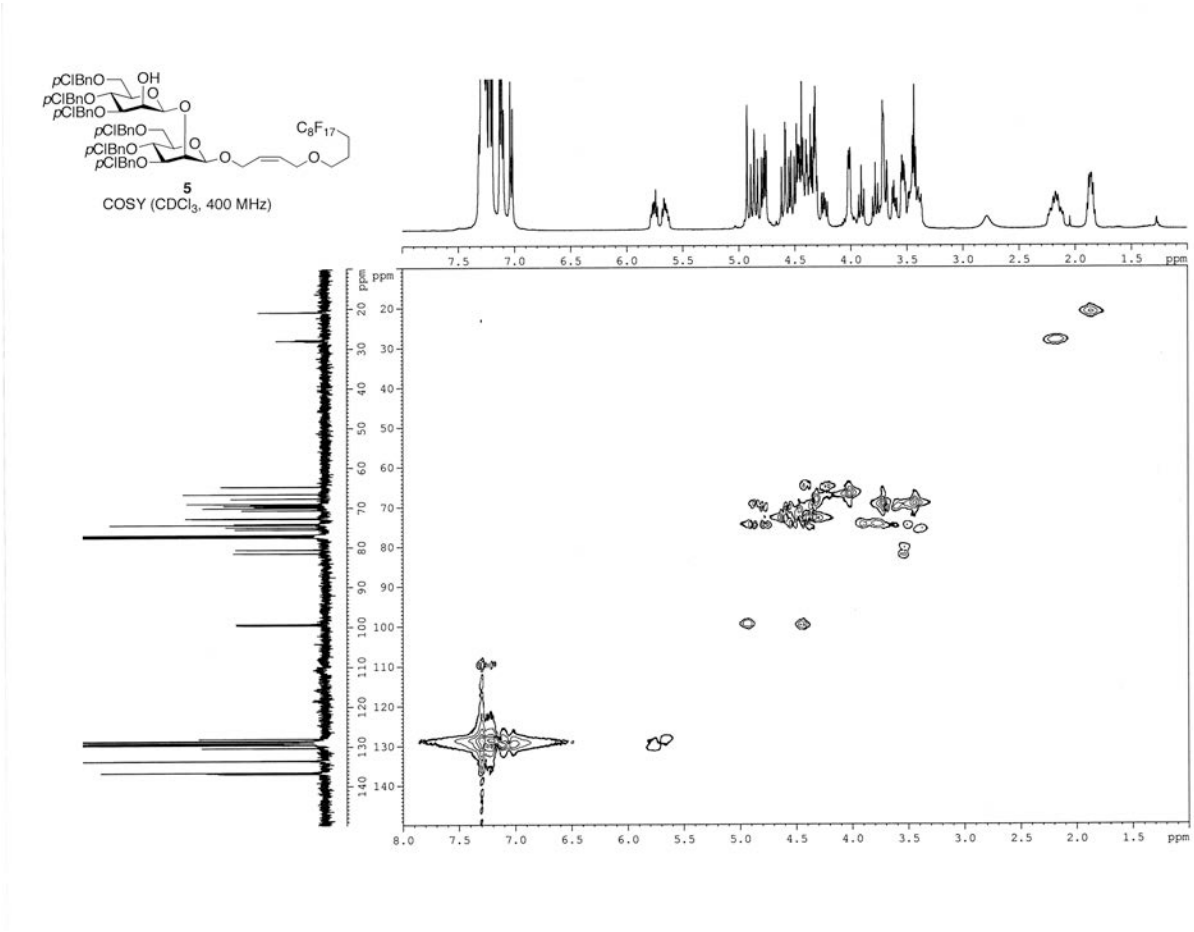
www.manaraa.com



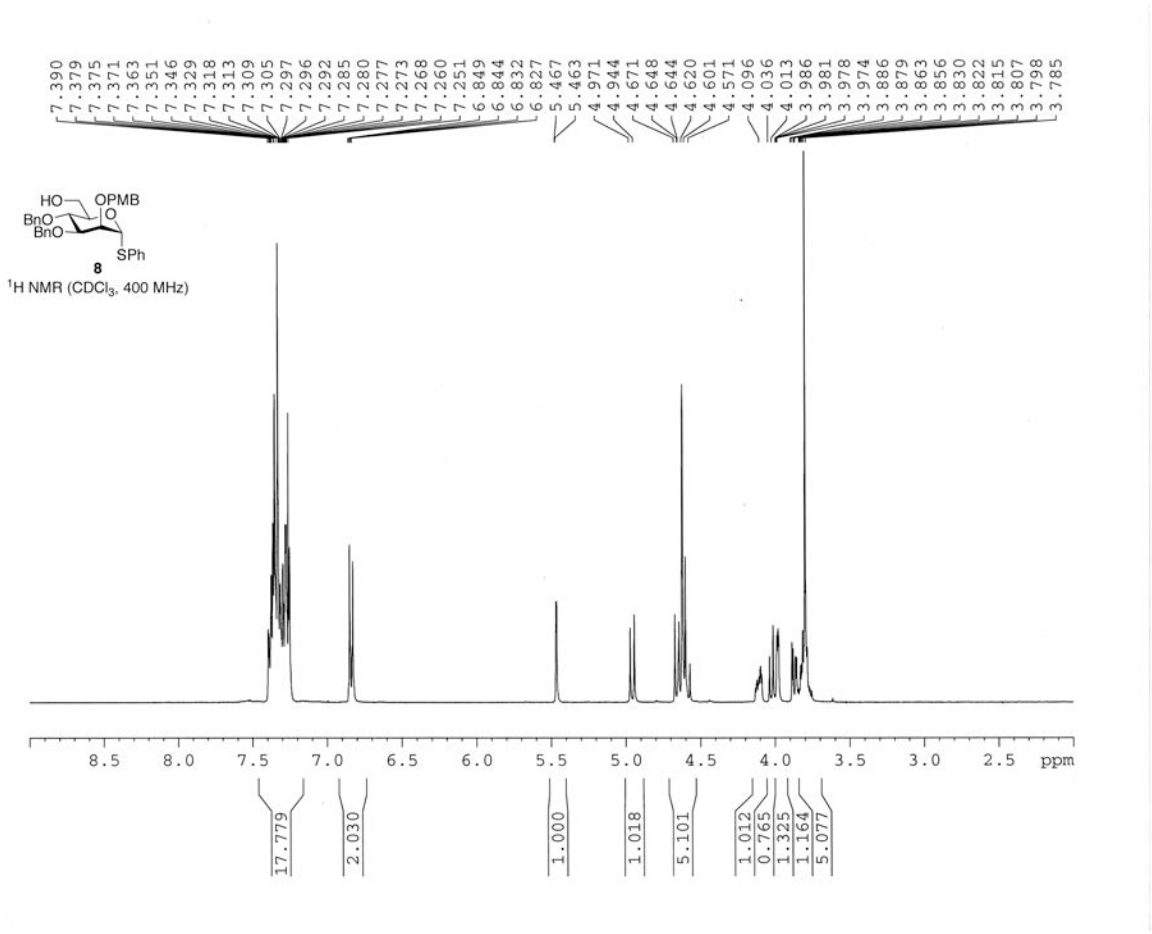
www.manaraa.com



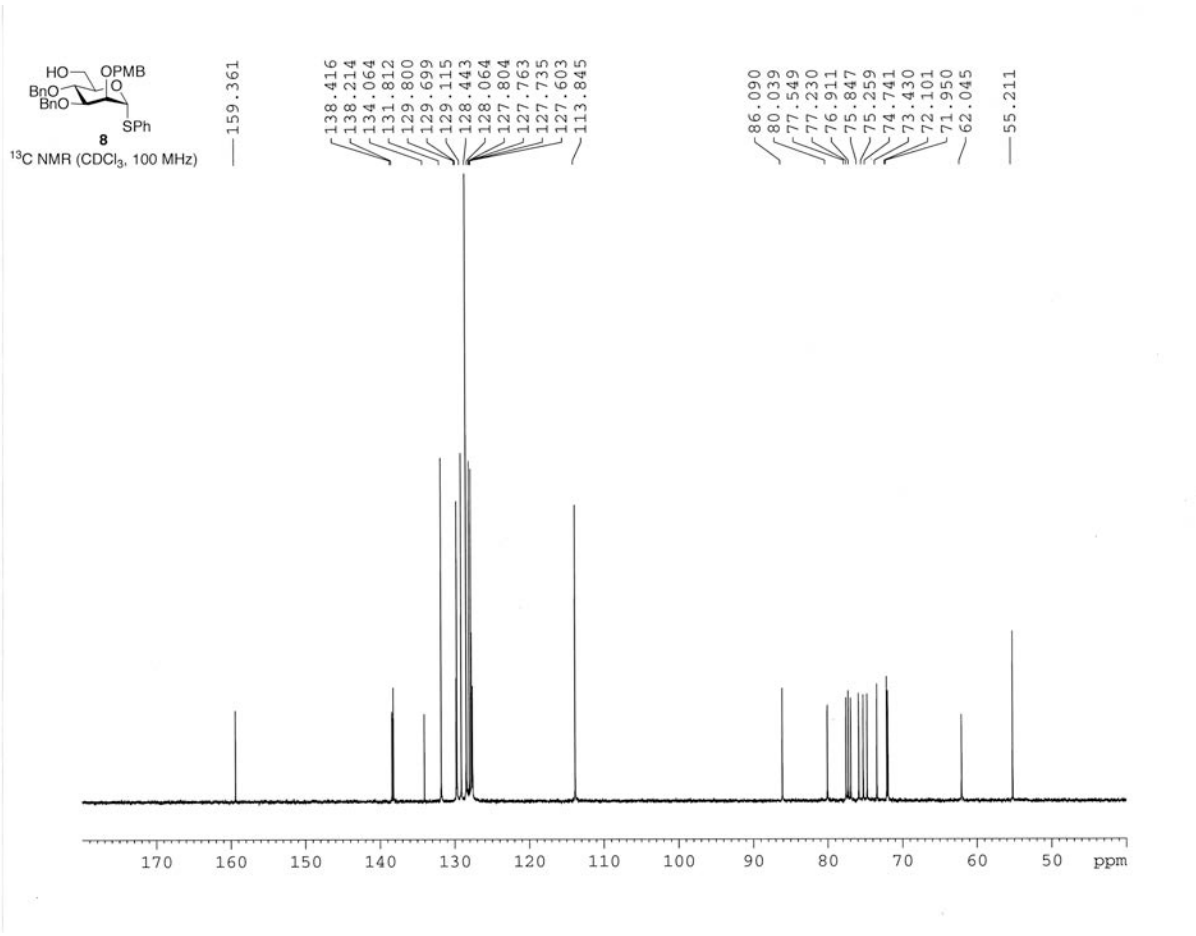
www.manaraa.com



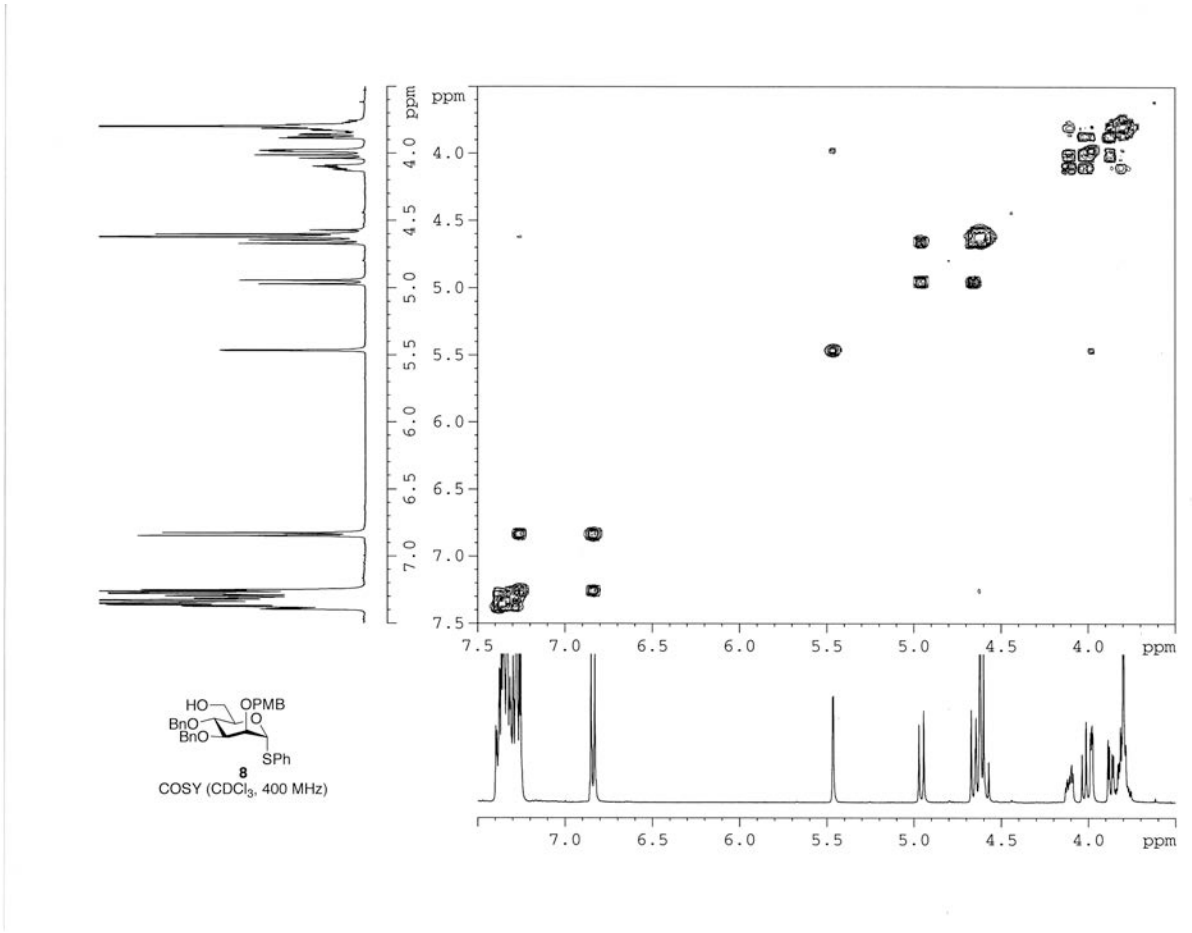
www.manaraa.com



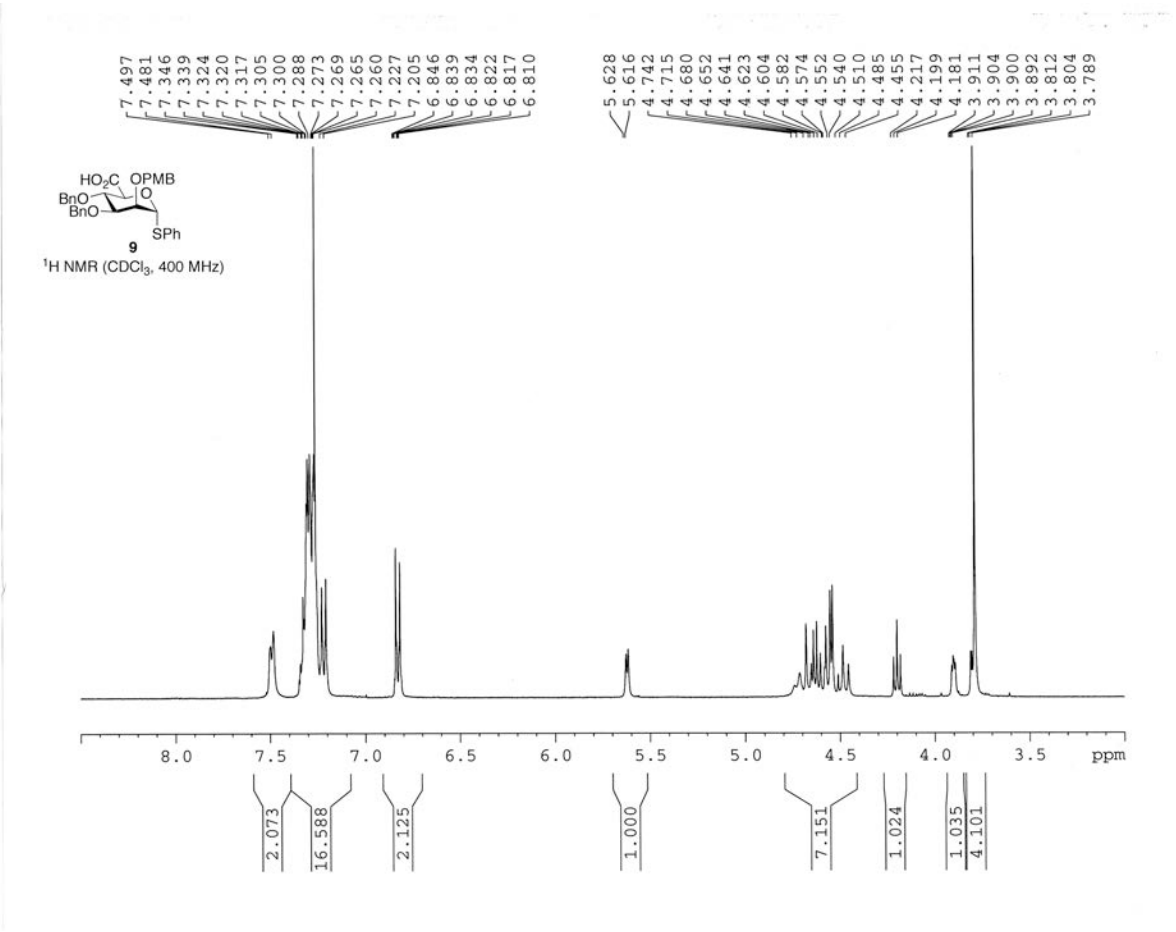
www.manaraa.com



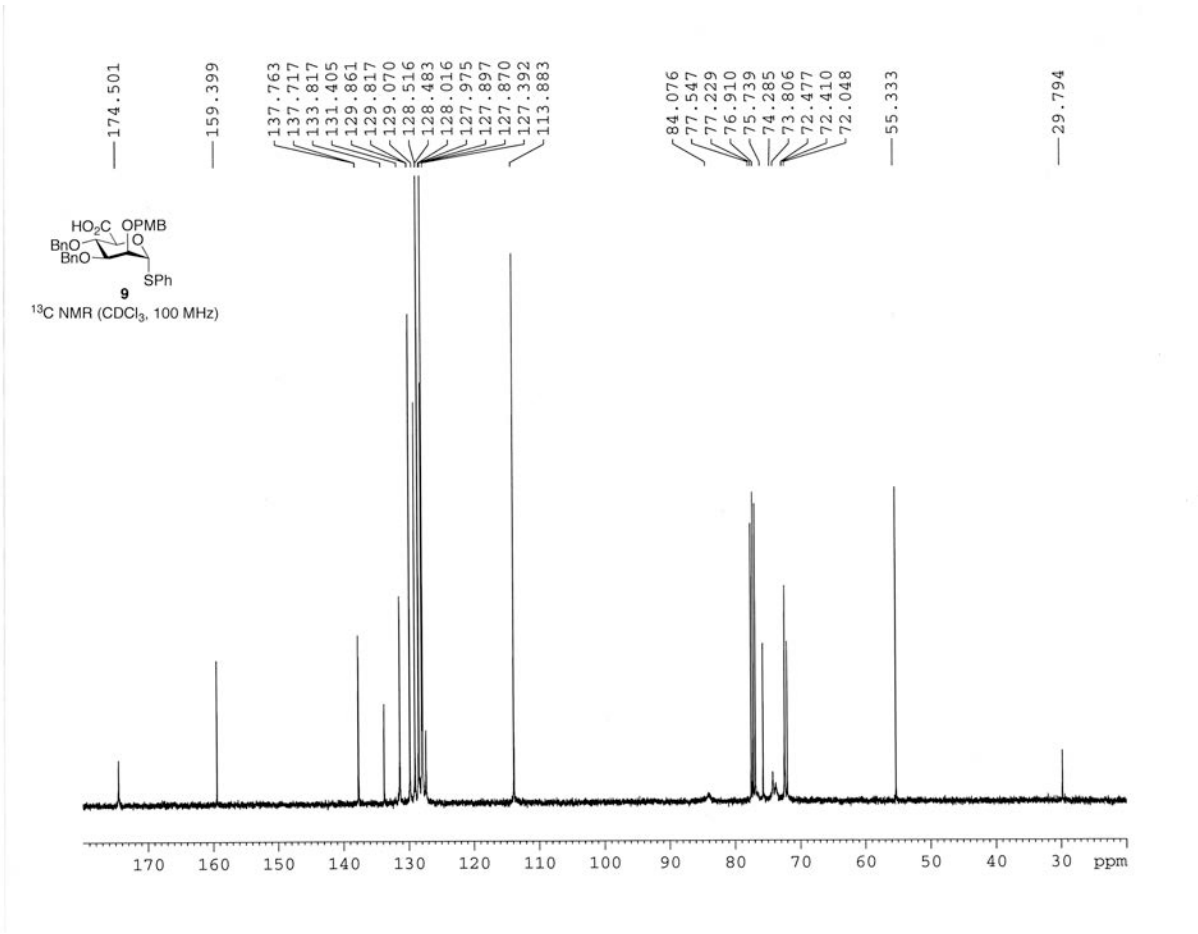
www.manaraa.com



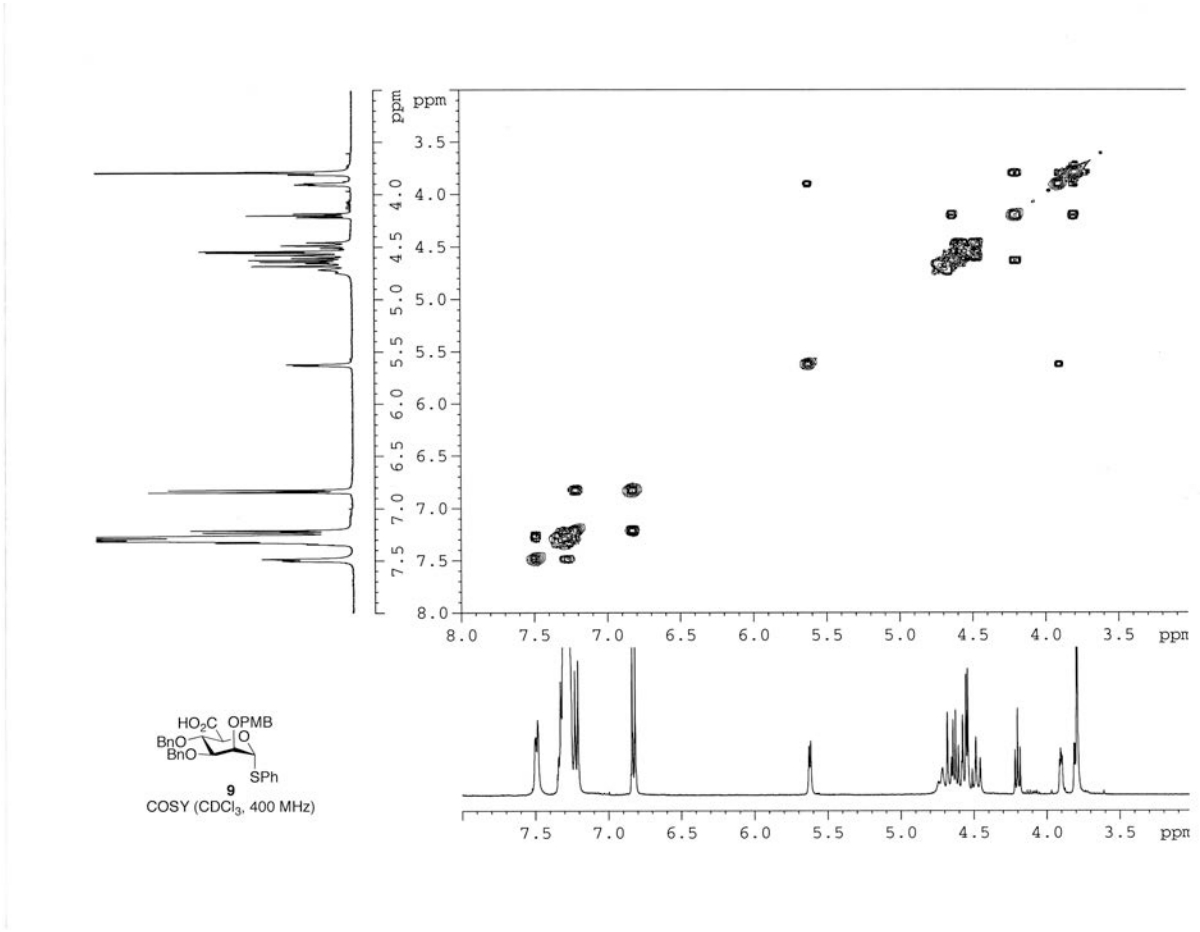
www.manaraa.com



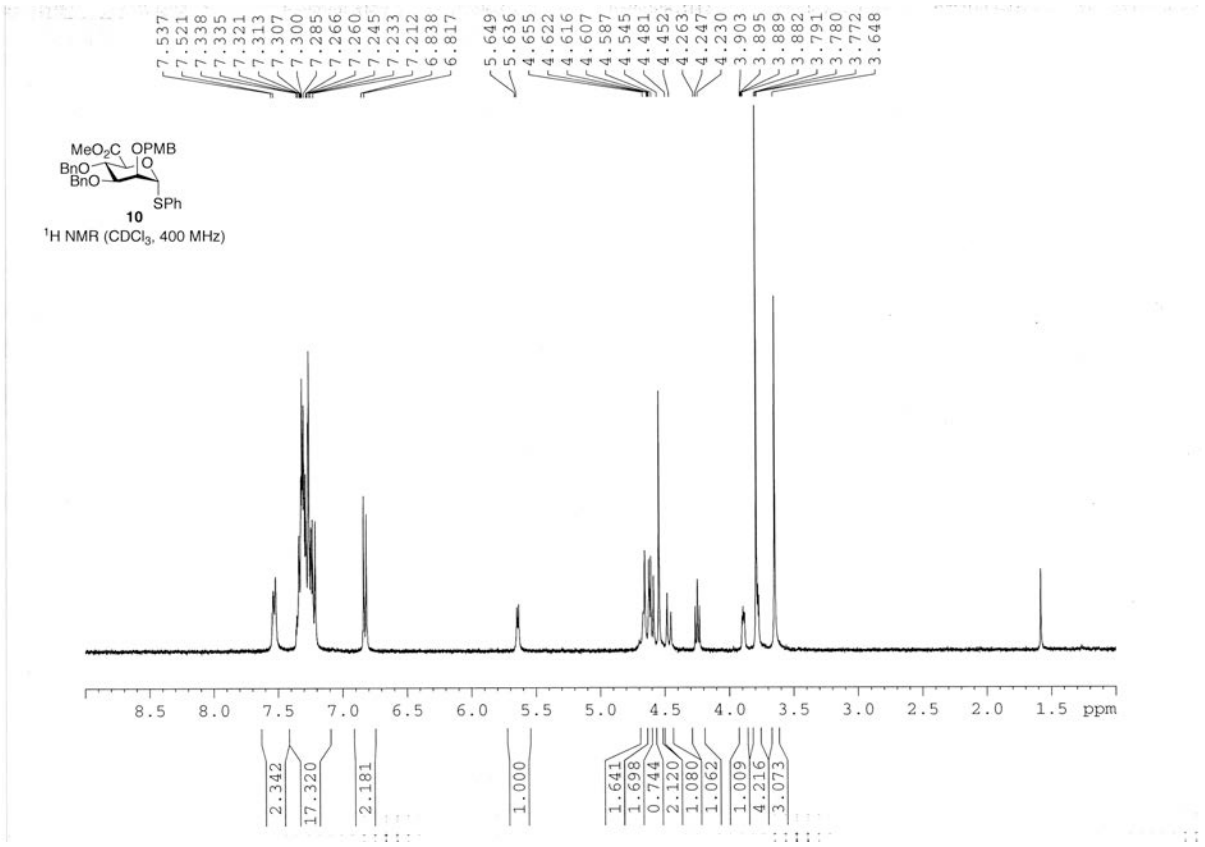
www.manaraa.com



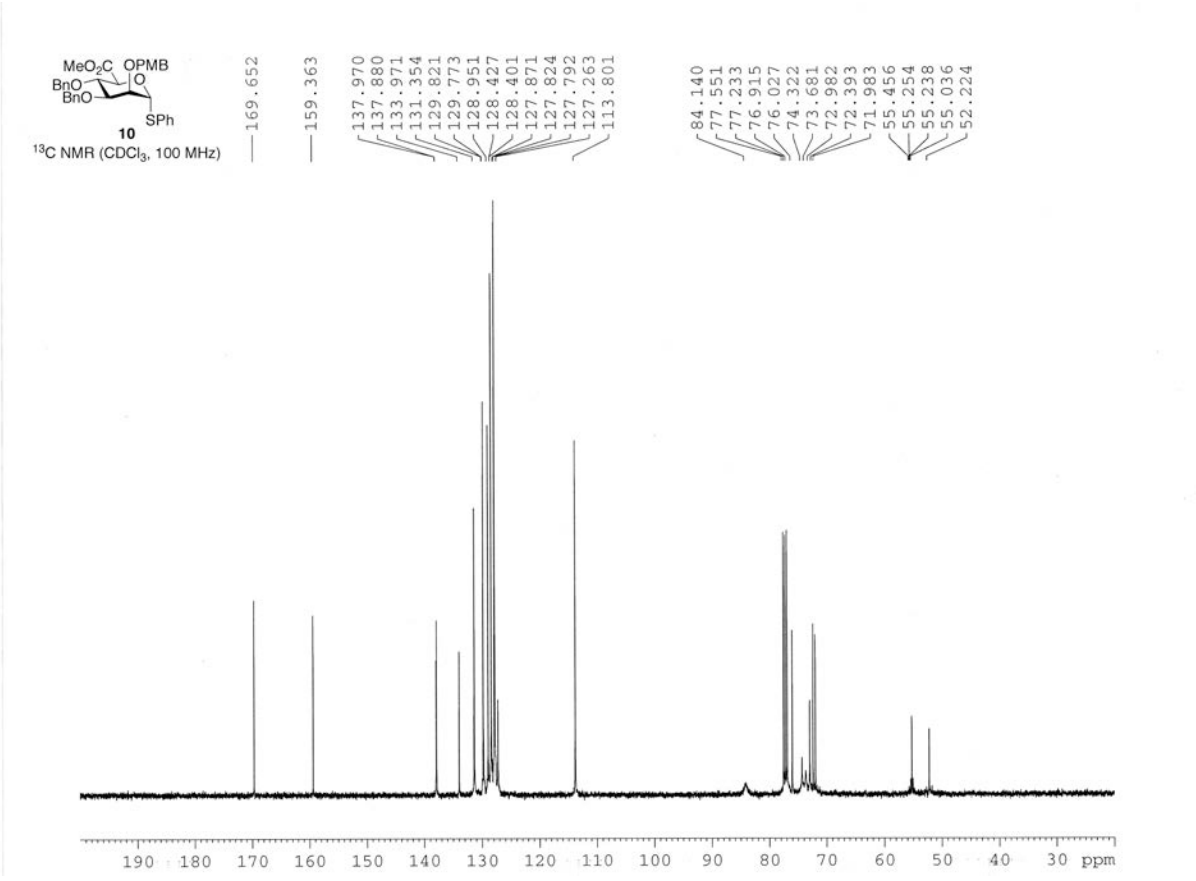
www.manaraa.com



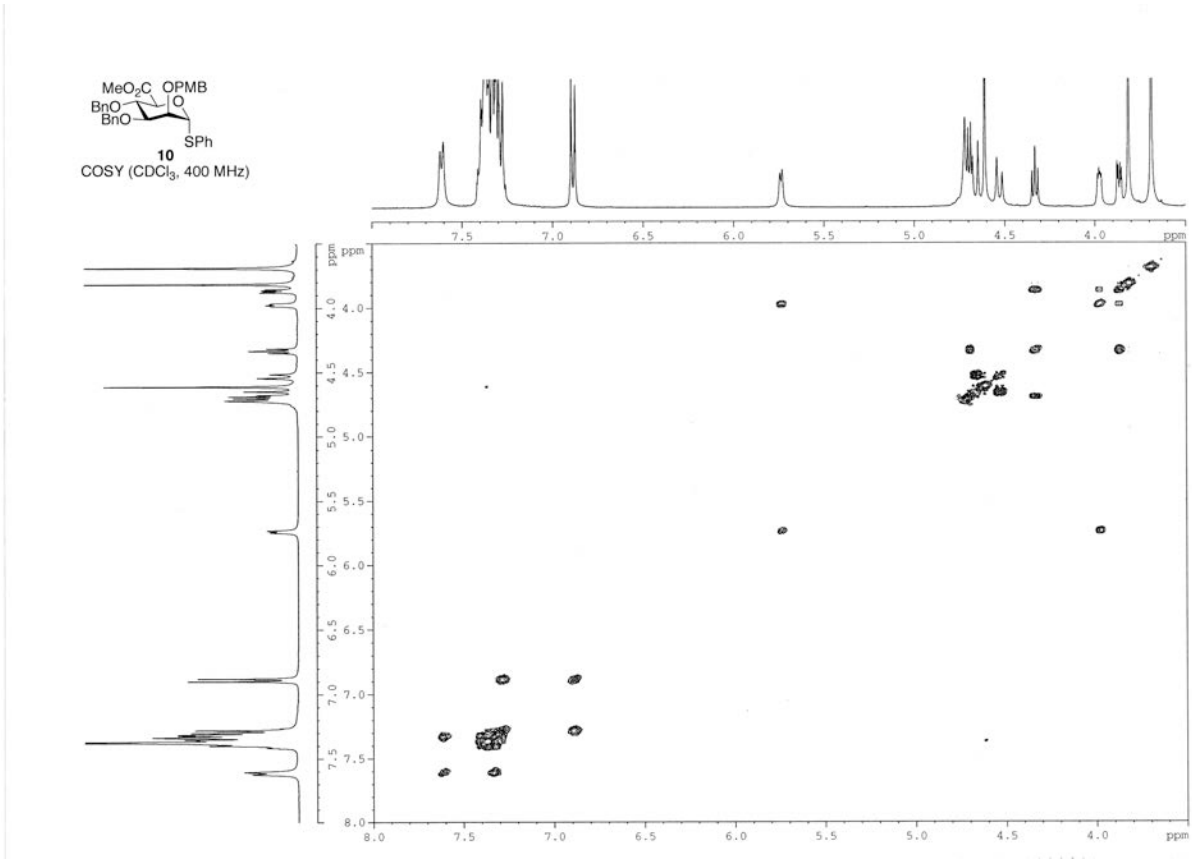
www.manaraa.com



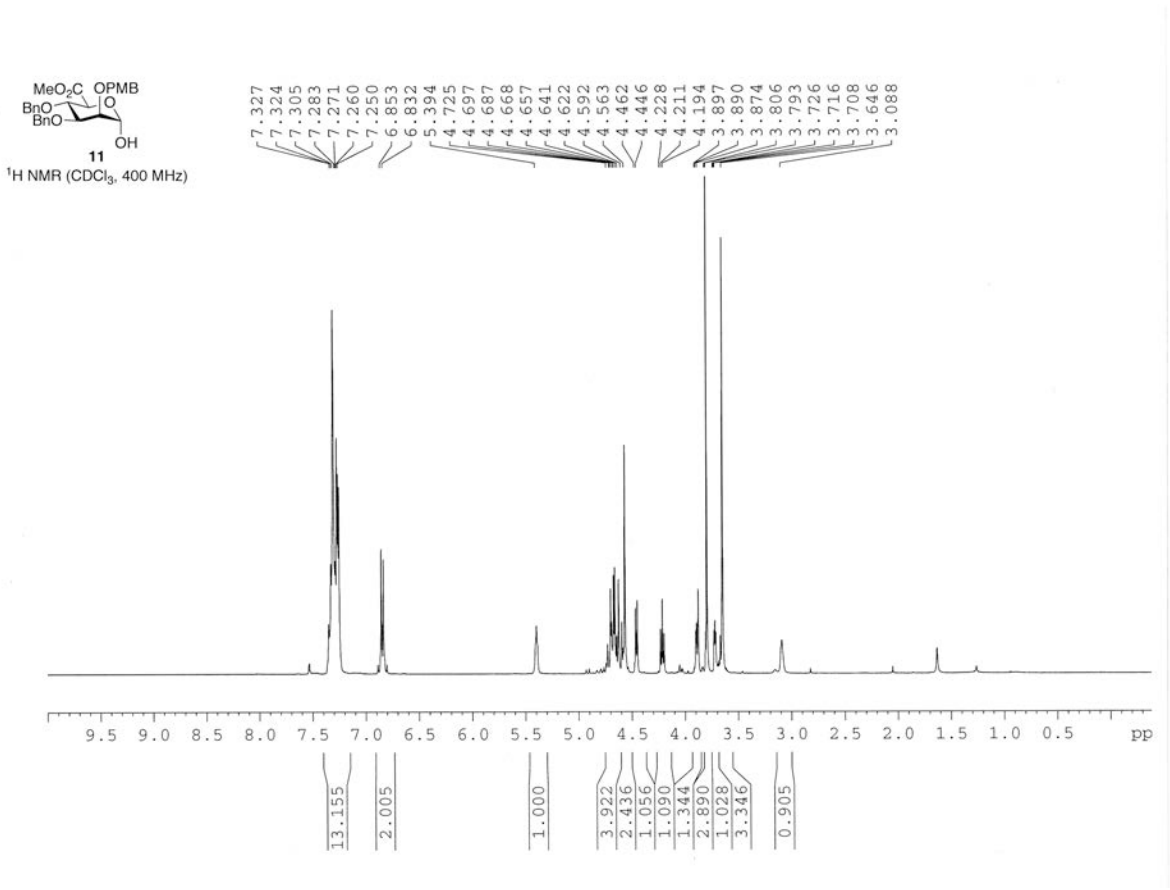
www.manaraa.com



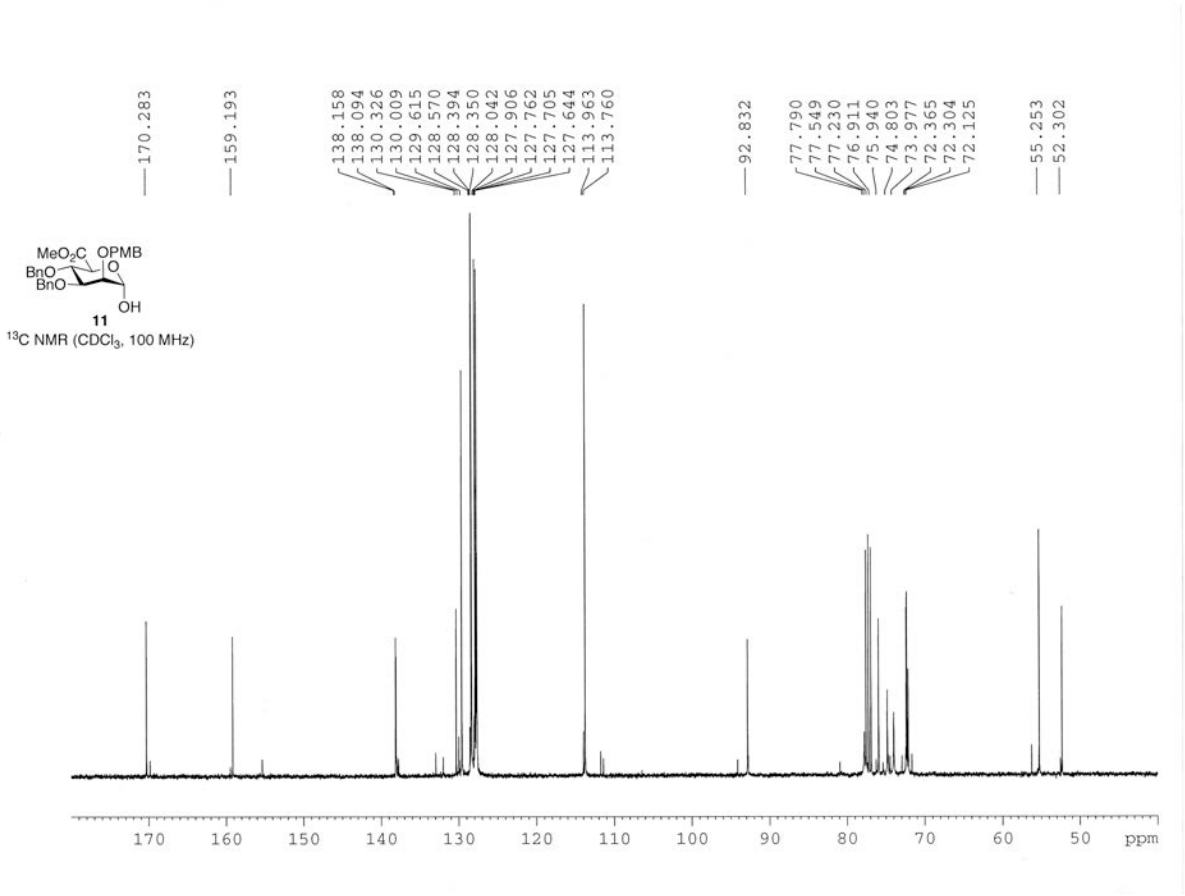
www.manaraa.com



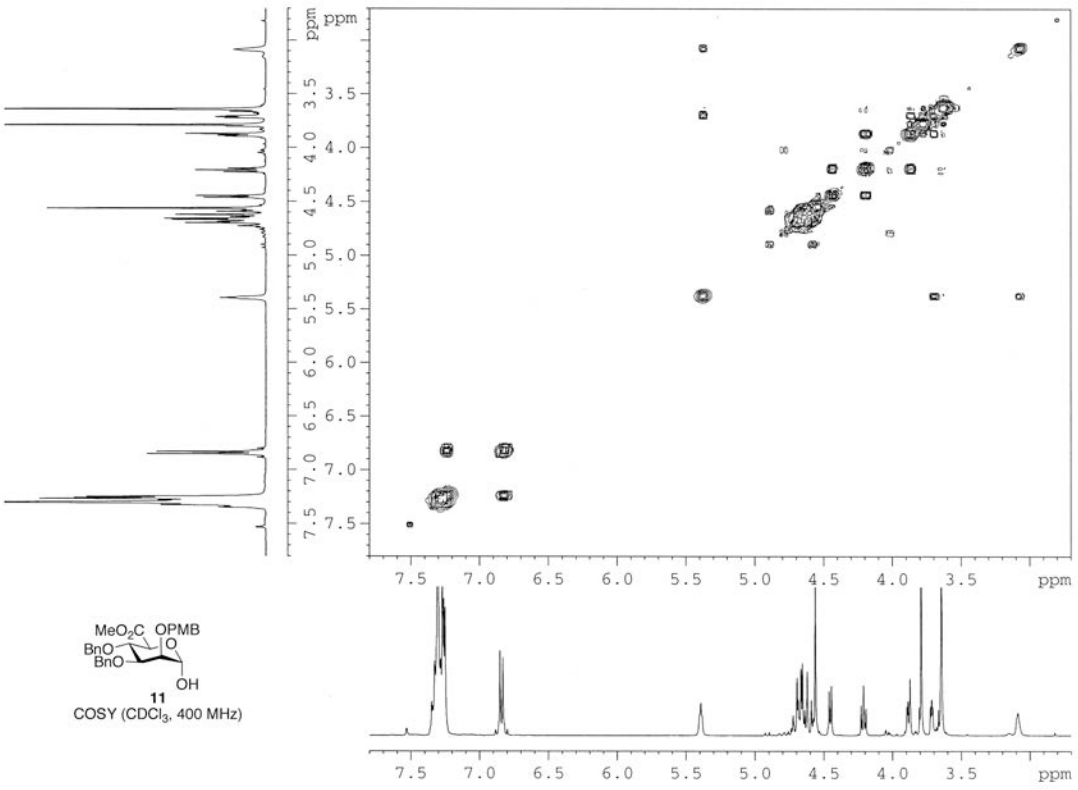
www.manaraa.com



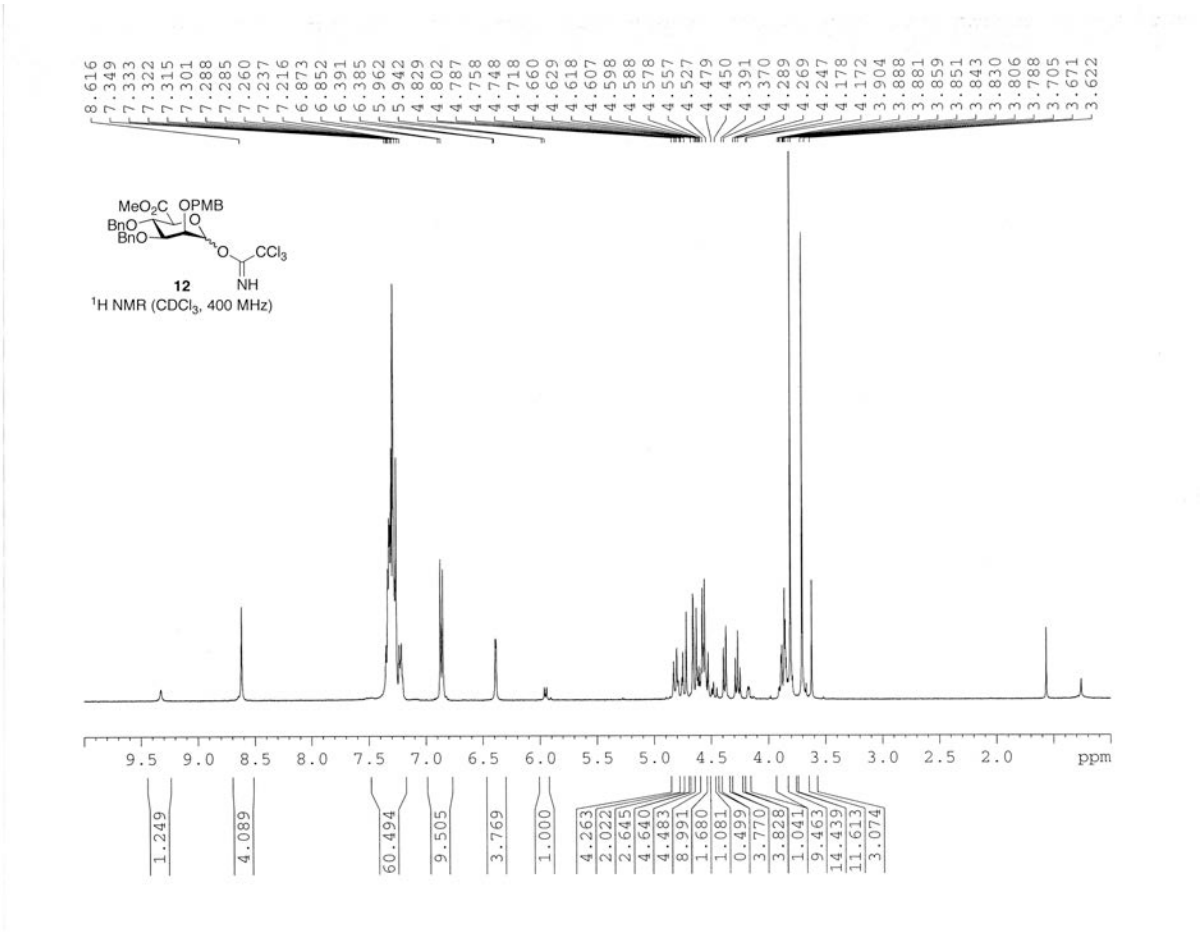
www.manaraa.com



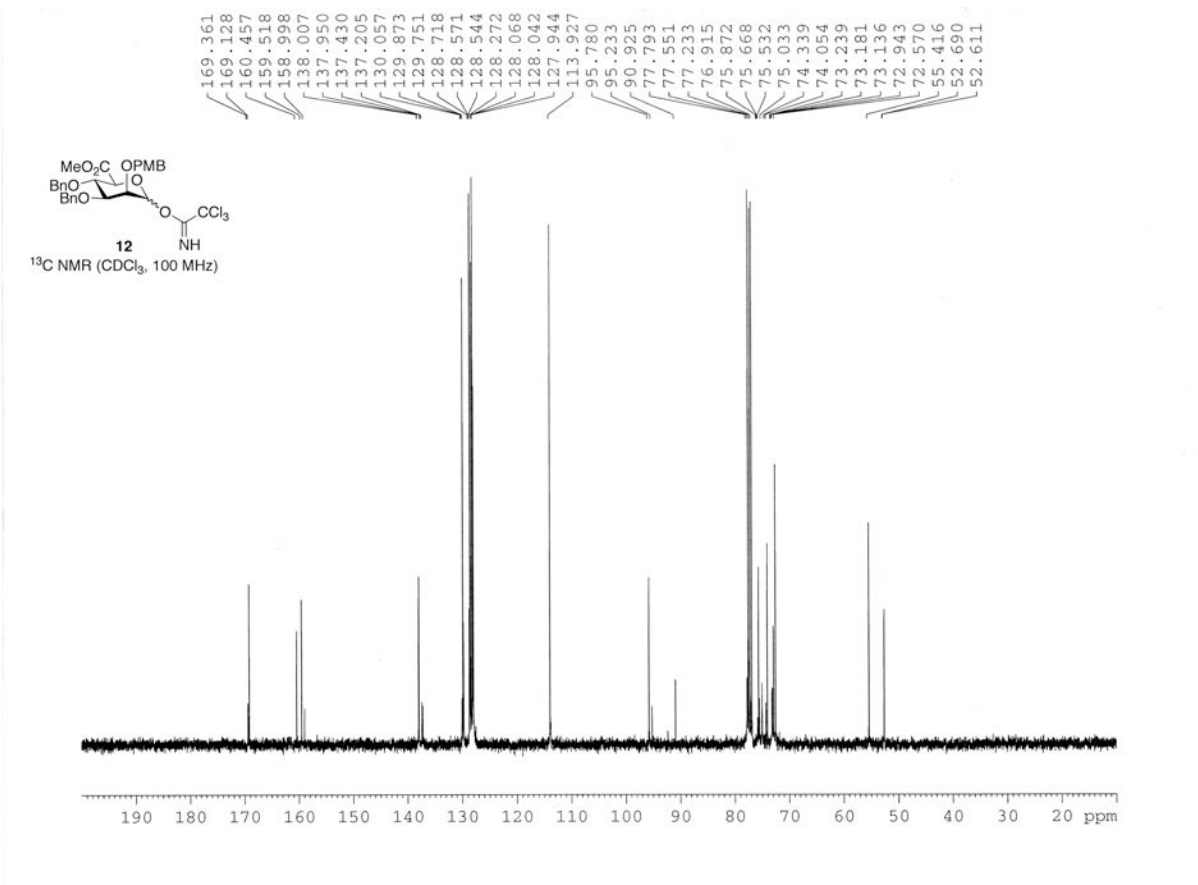
www.manaraa.com



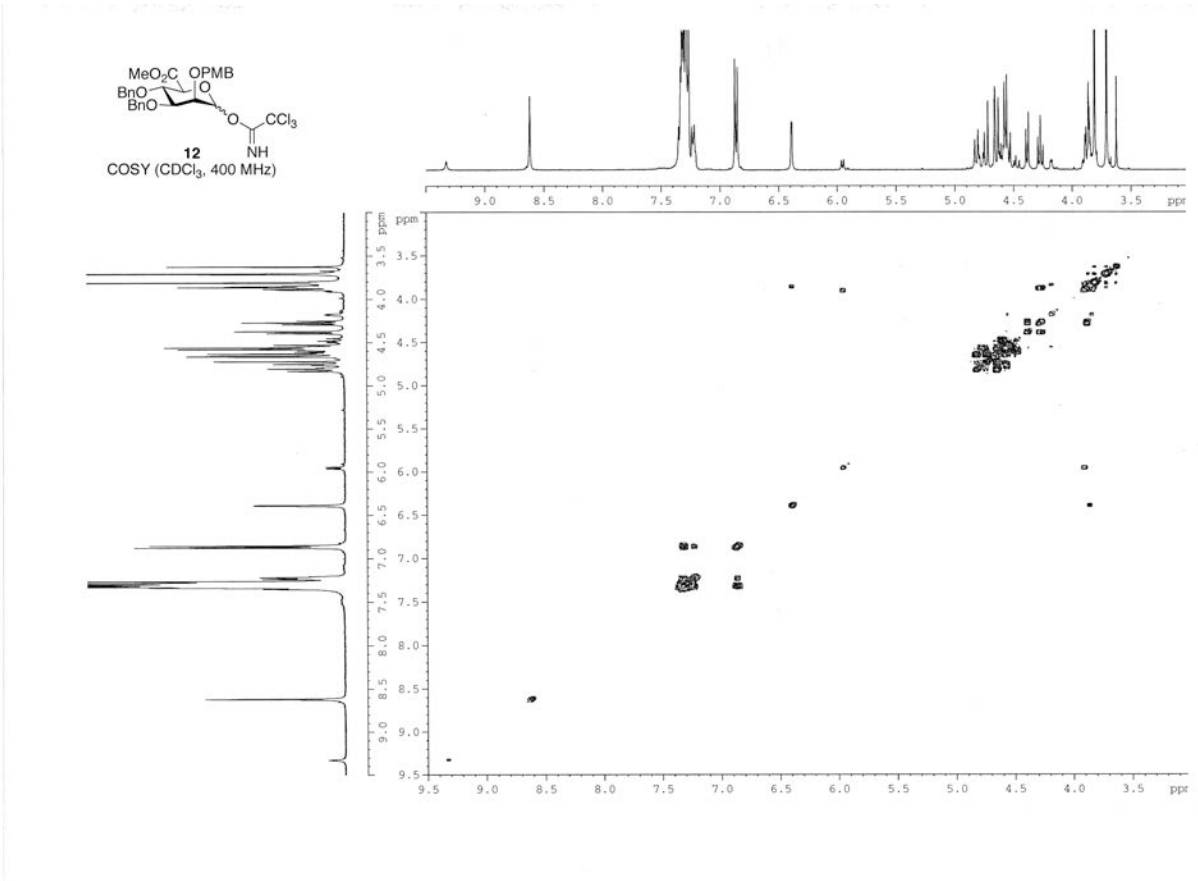
www.manaraa.com



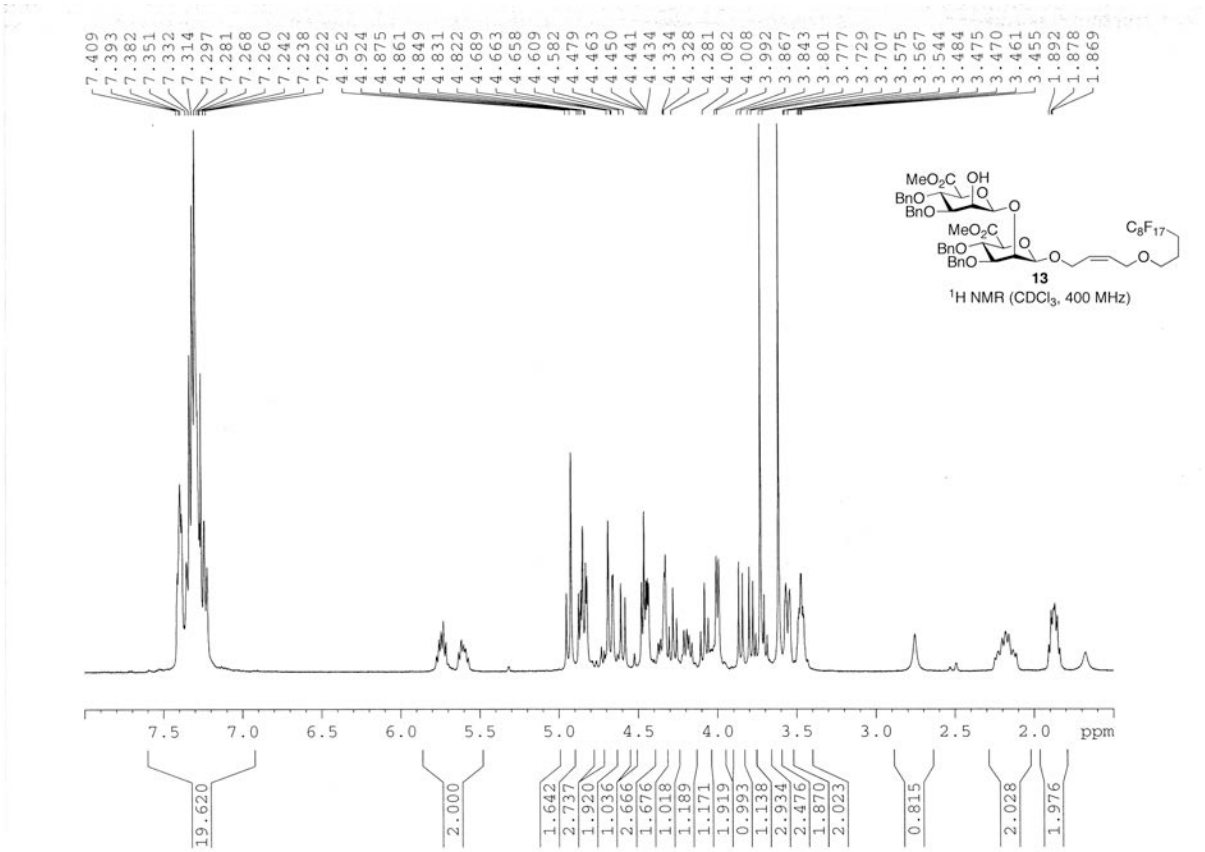
www.manaraa.com



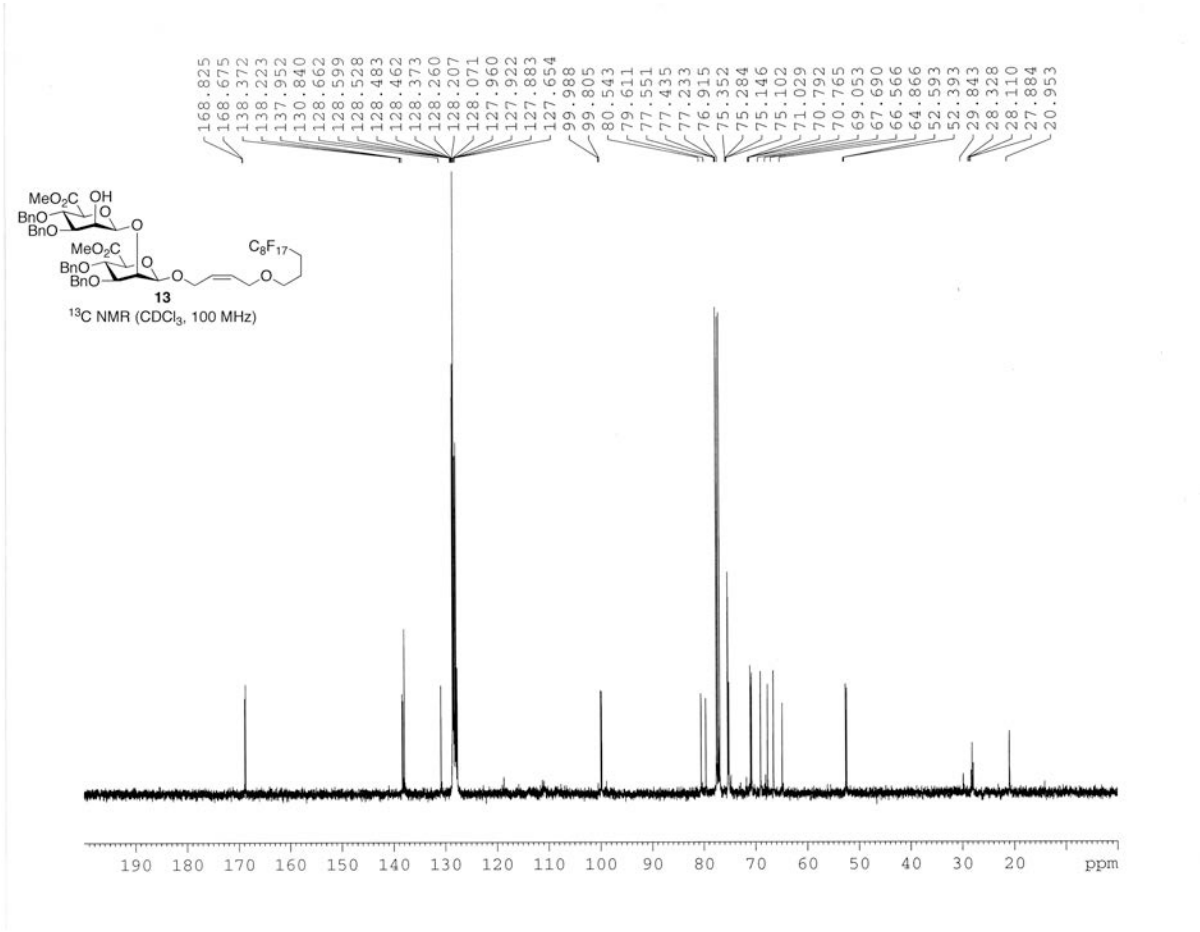
www.manaraa.com



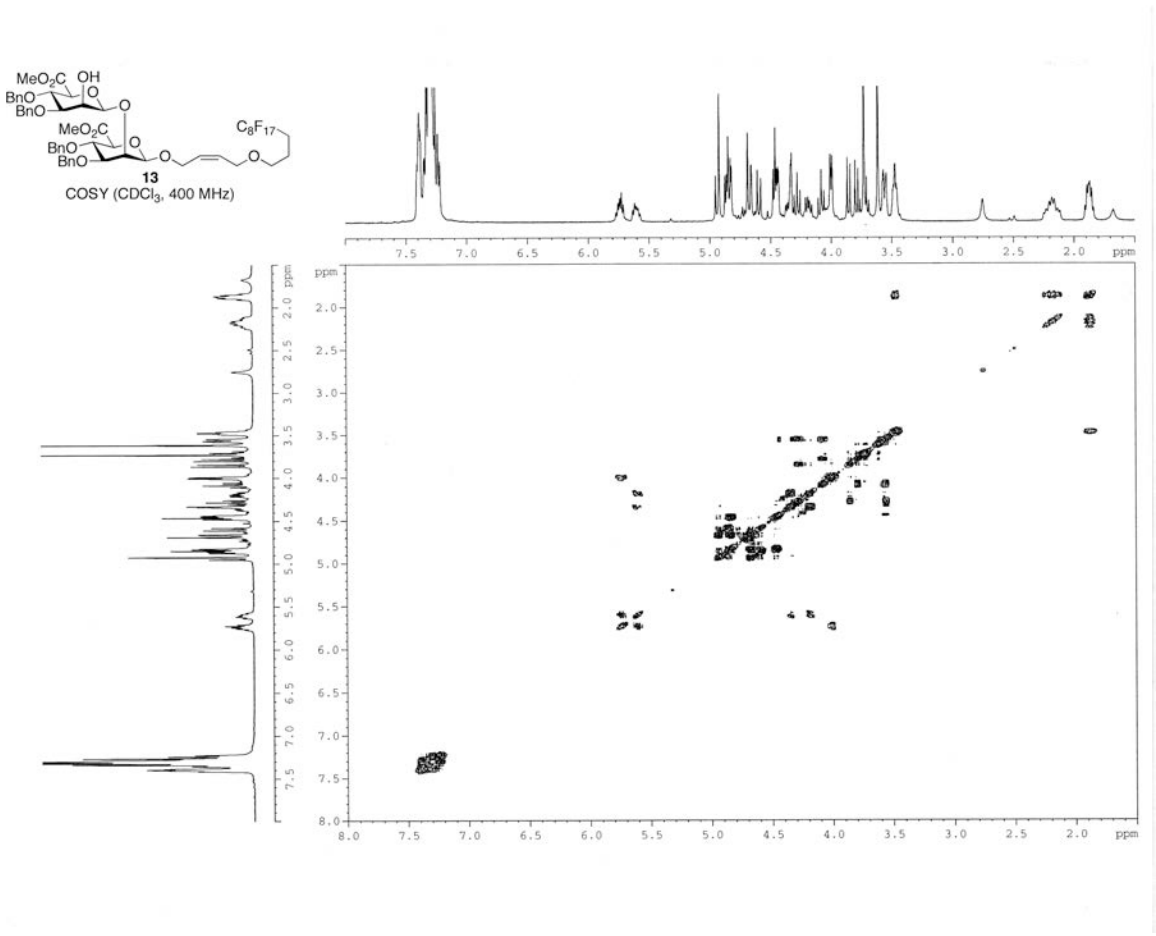
www.manaraa.com



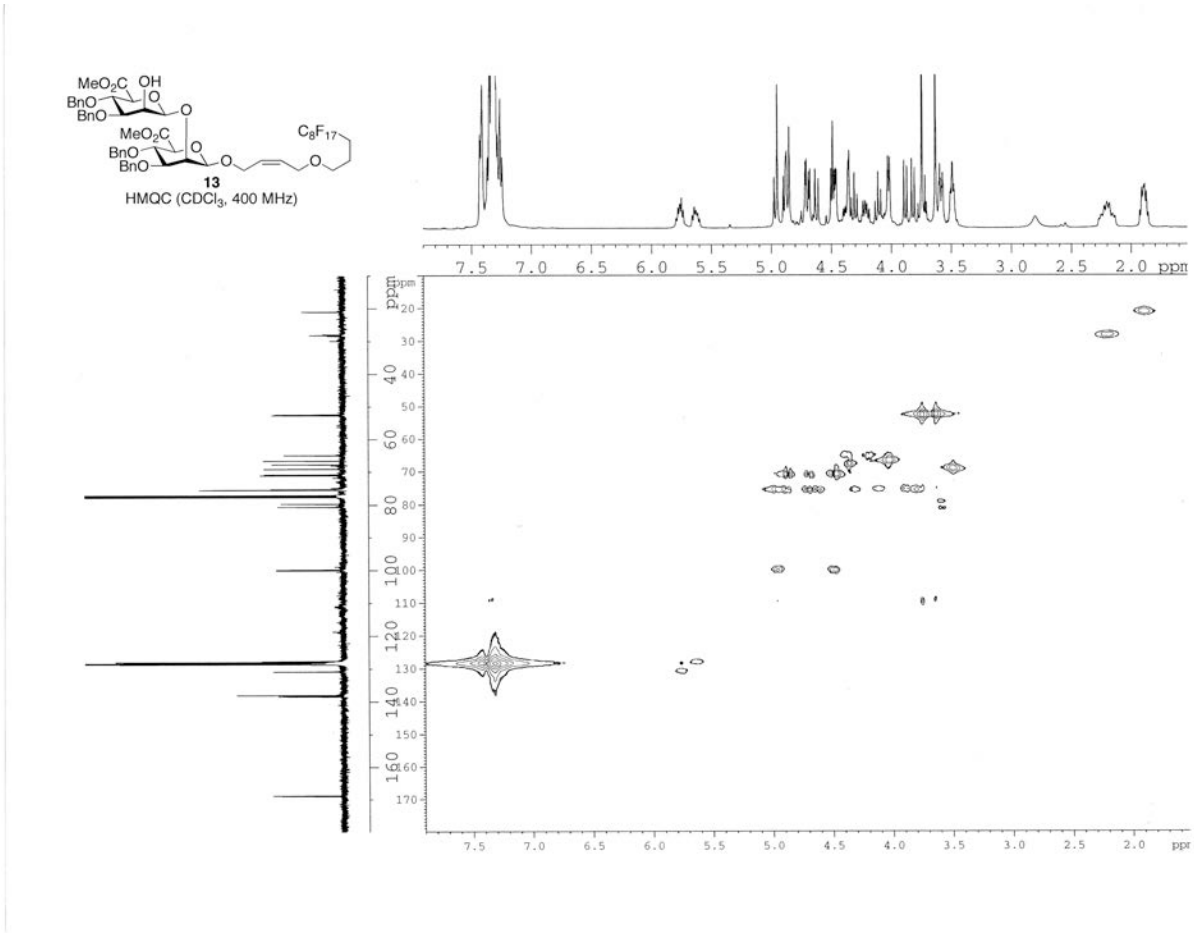
www.manaraa.com



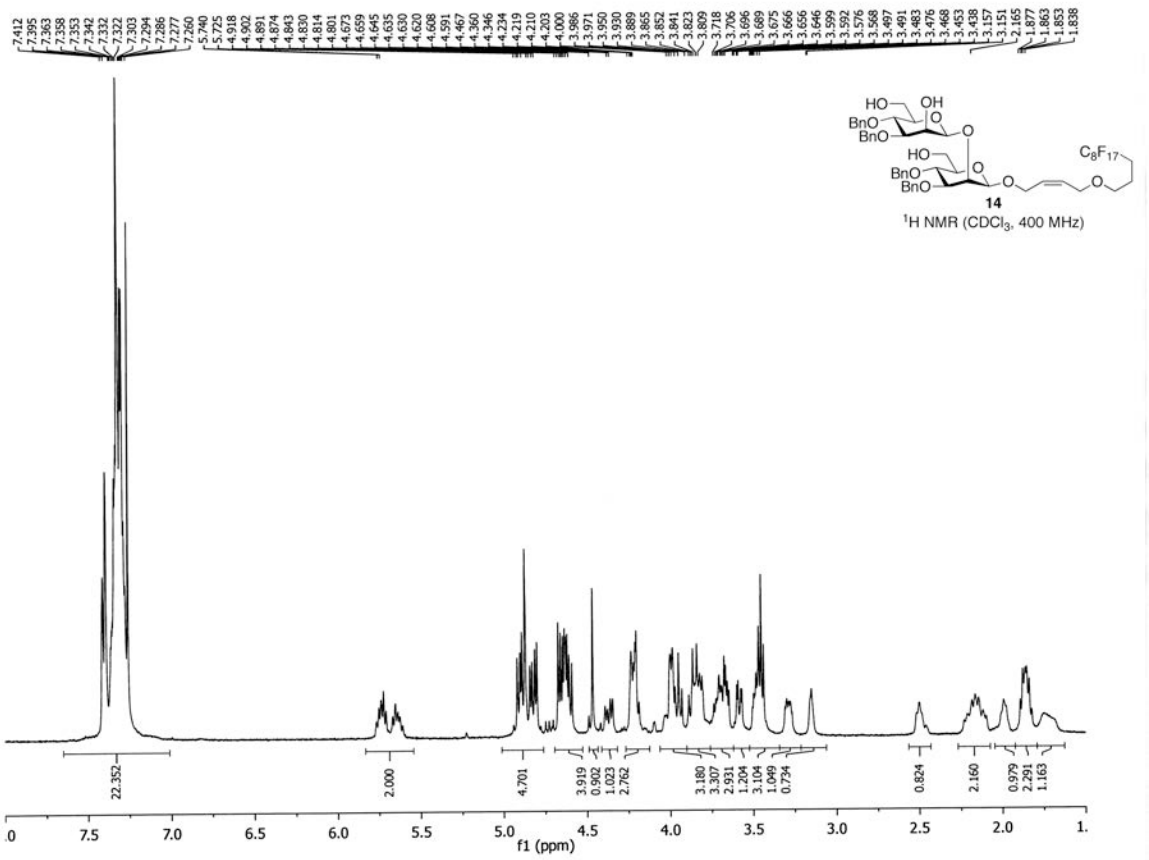
www.manaraa.com



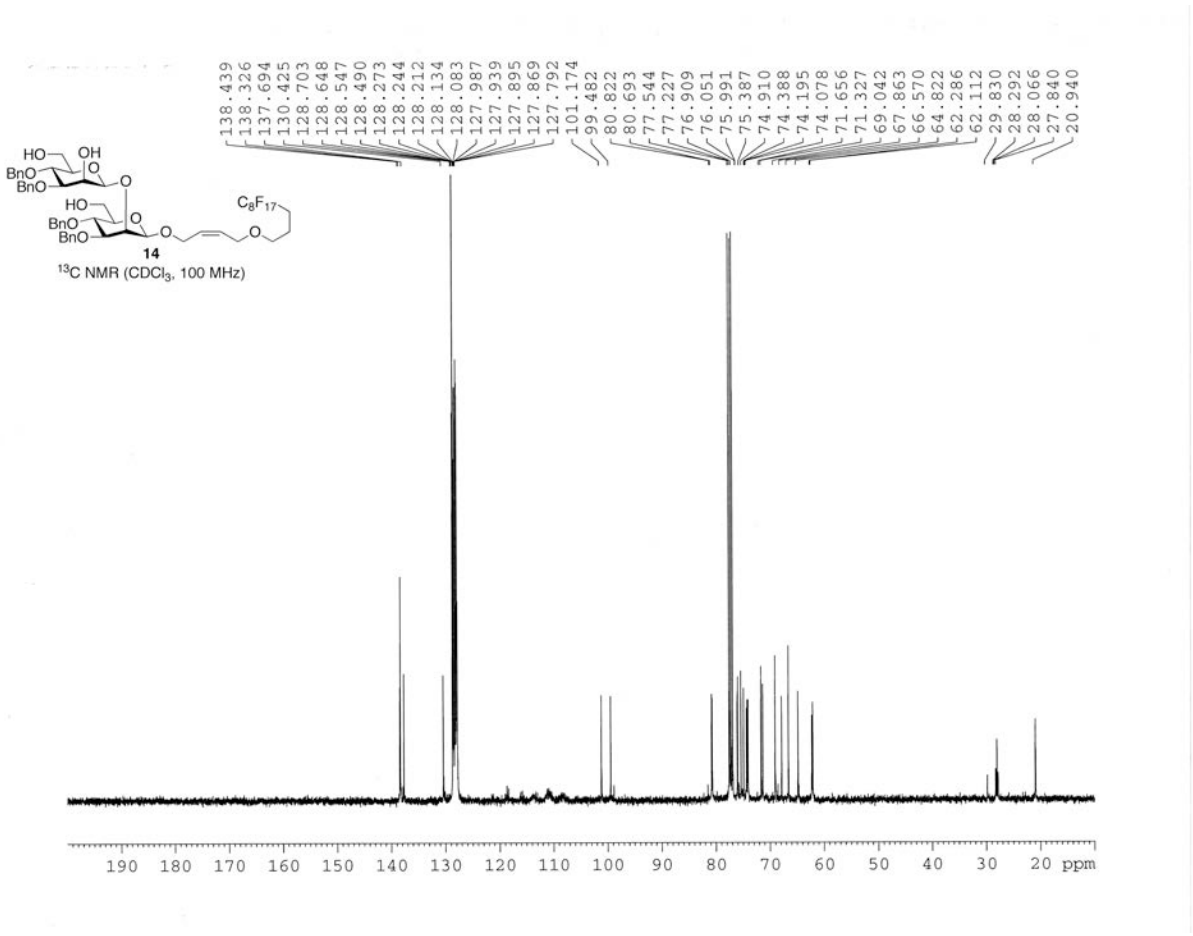
www.manaraa.com



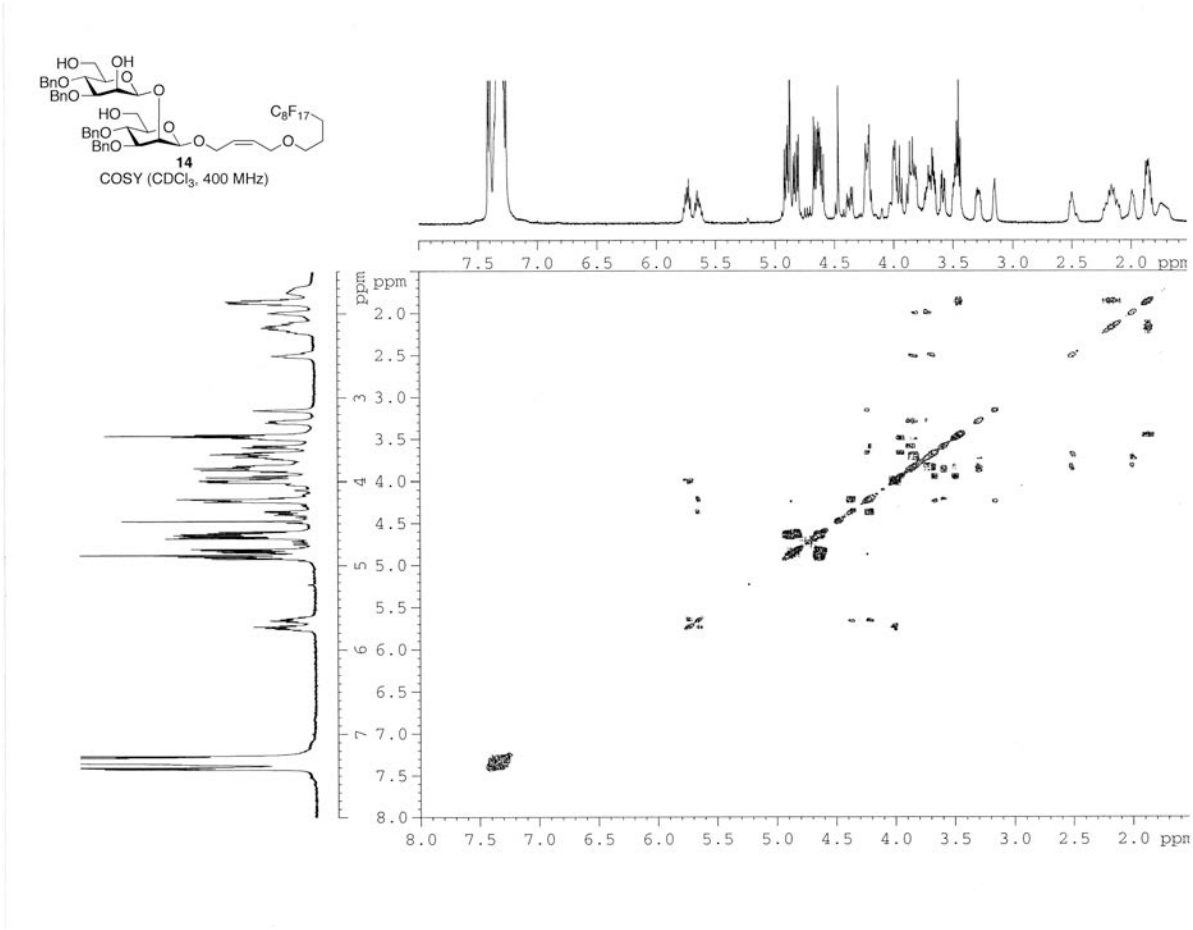
www.manaraa.com



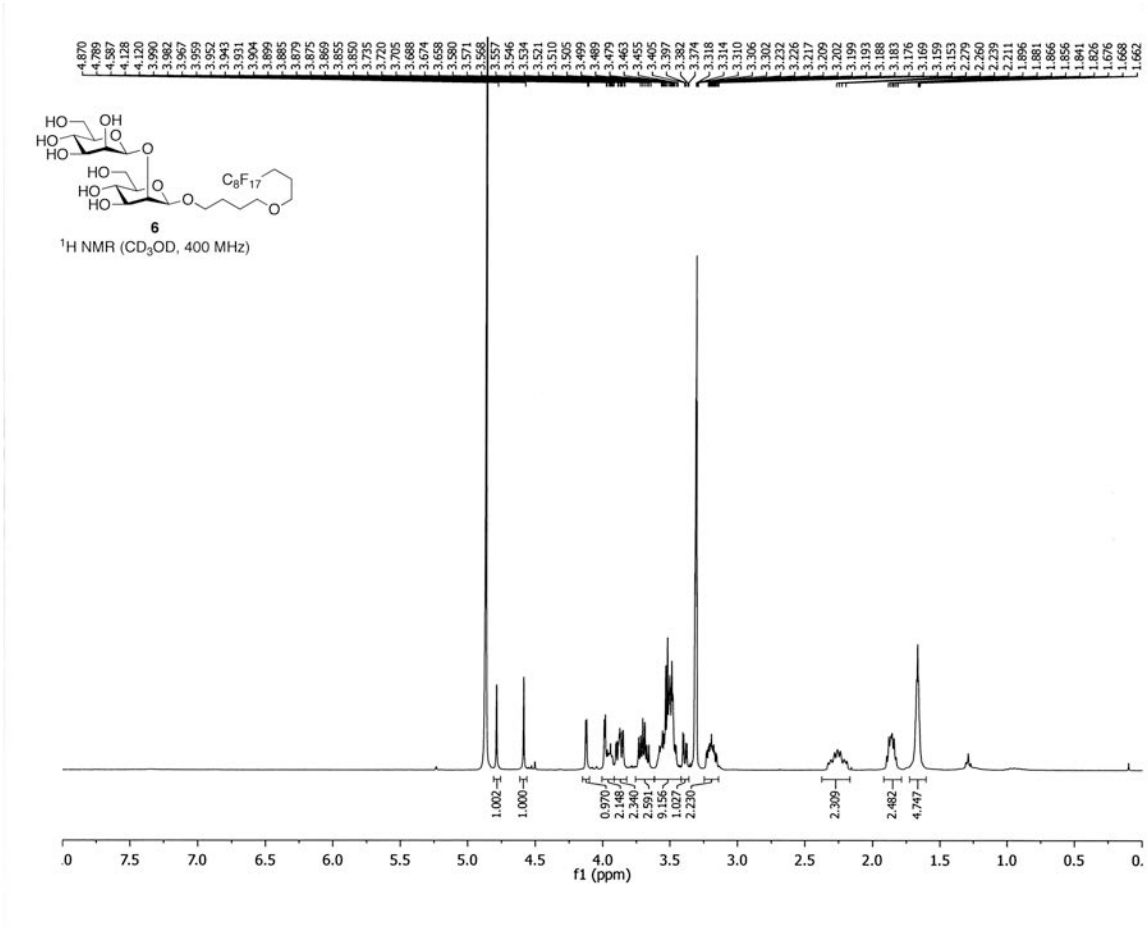
www.manaraa.com



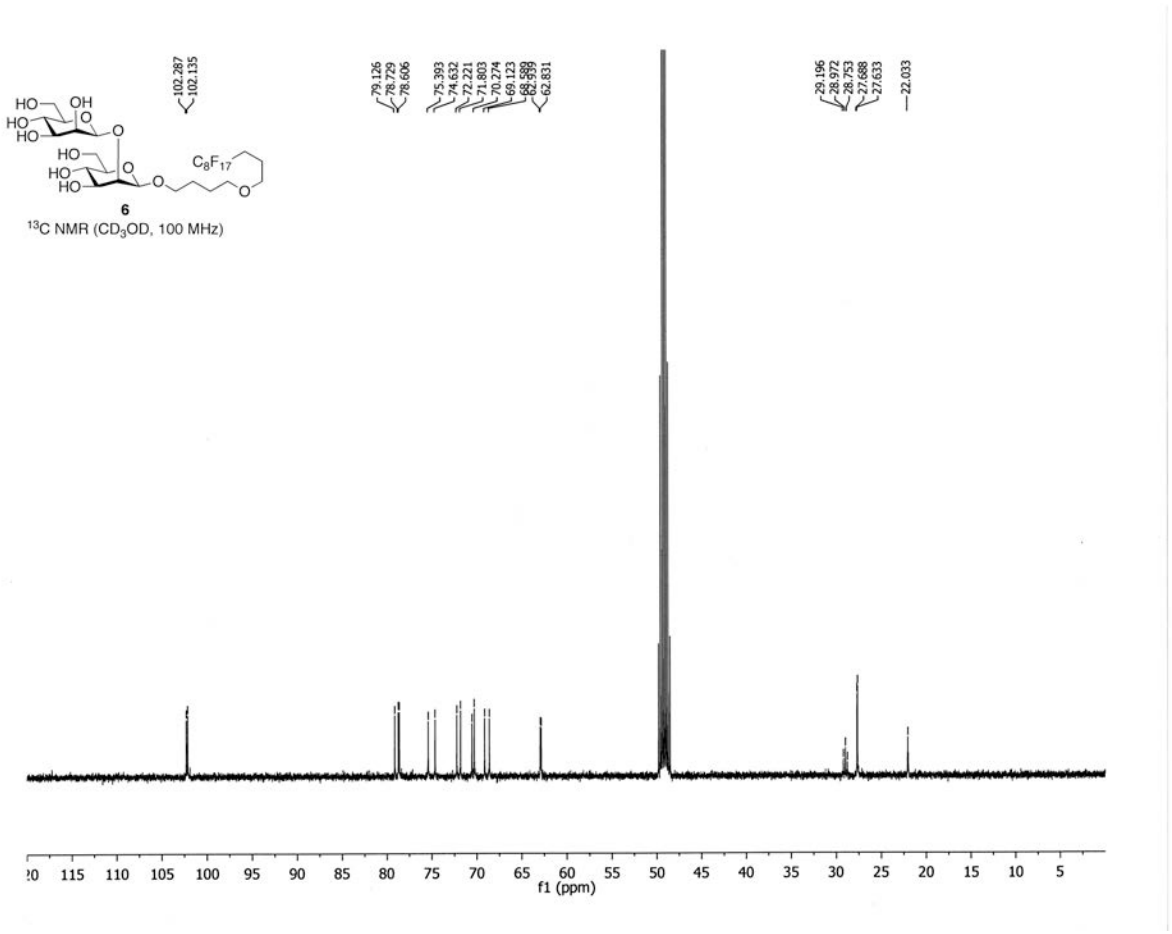
www.manaraa.com



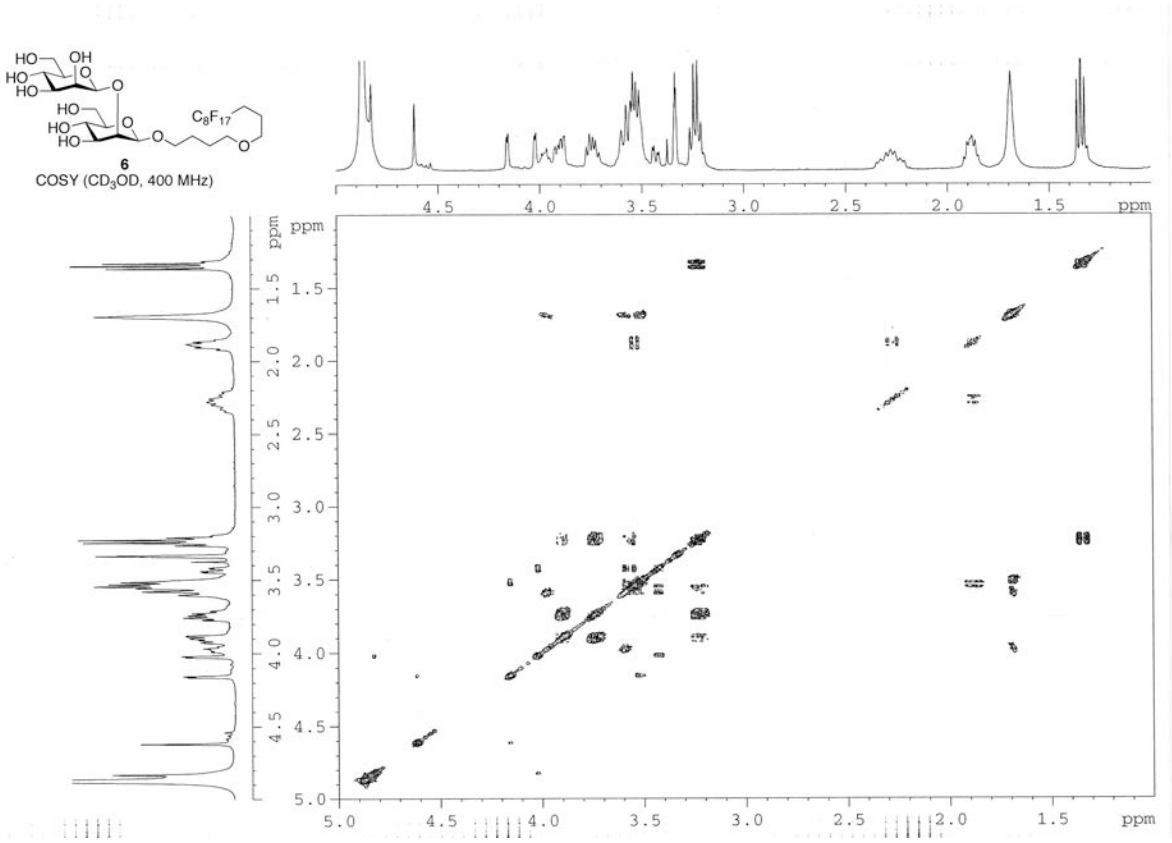
www.manaraa.com



www.manaraa.com

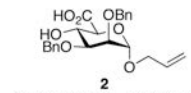


www.manaraa.com



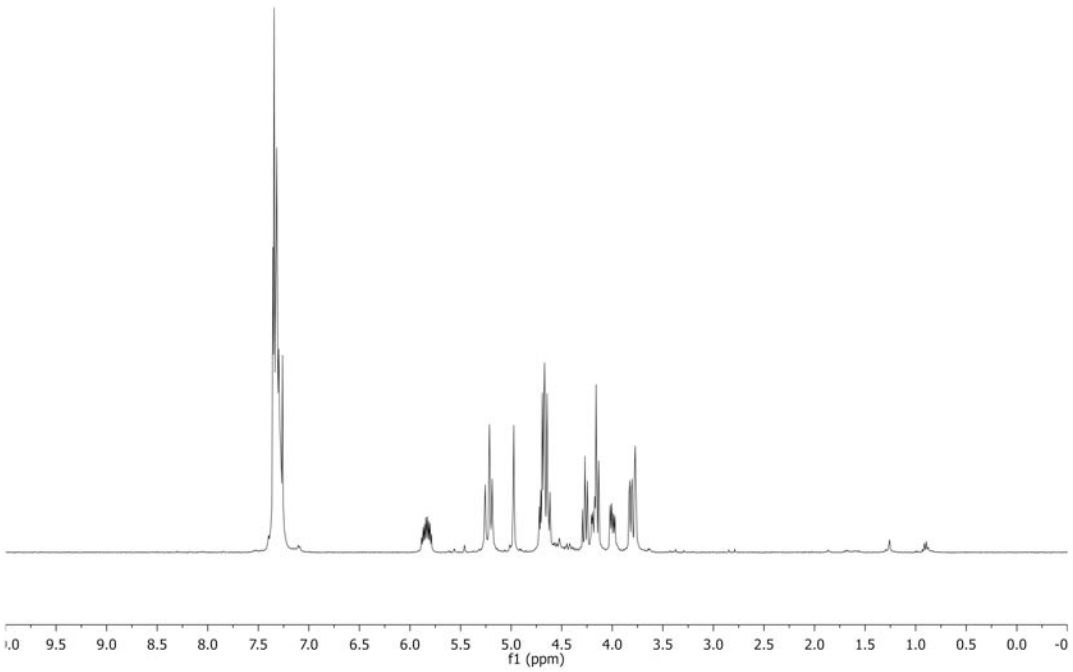
www.manaraa.com

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

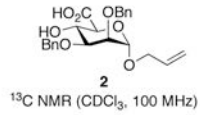


2

^{1}H NMR ($CDCl_3$, 400 MHz)

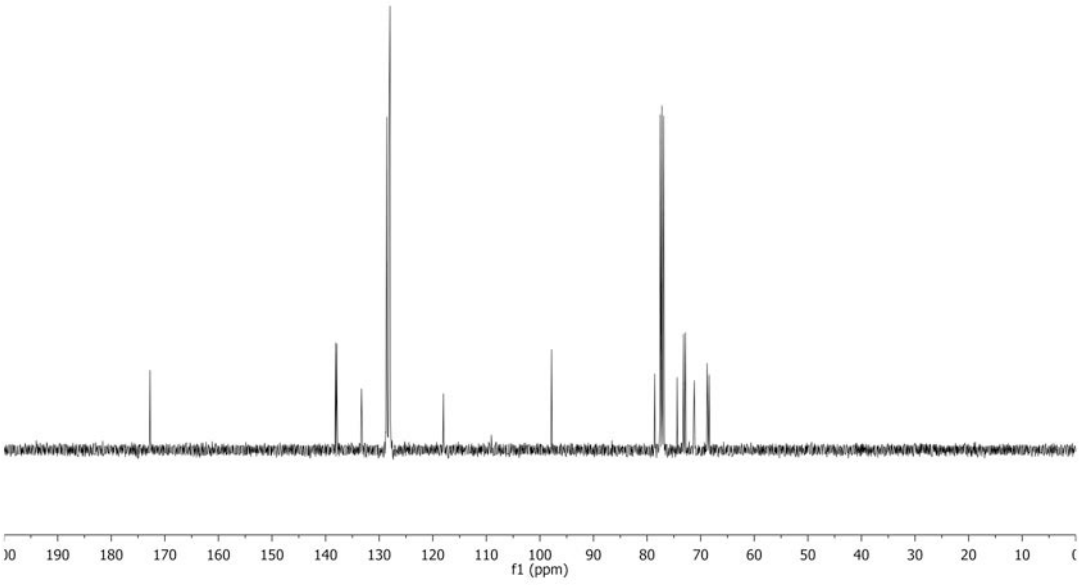


www.manaraa.com

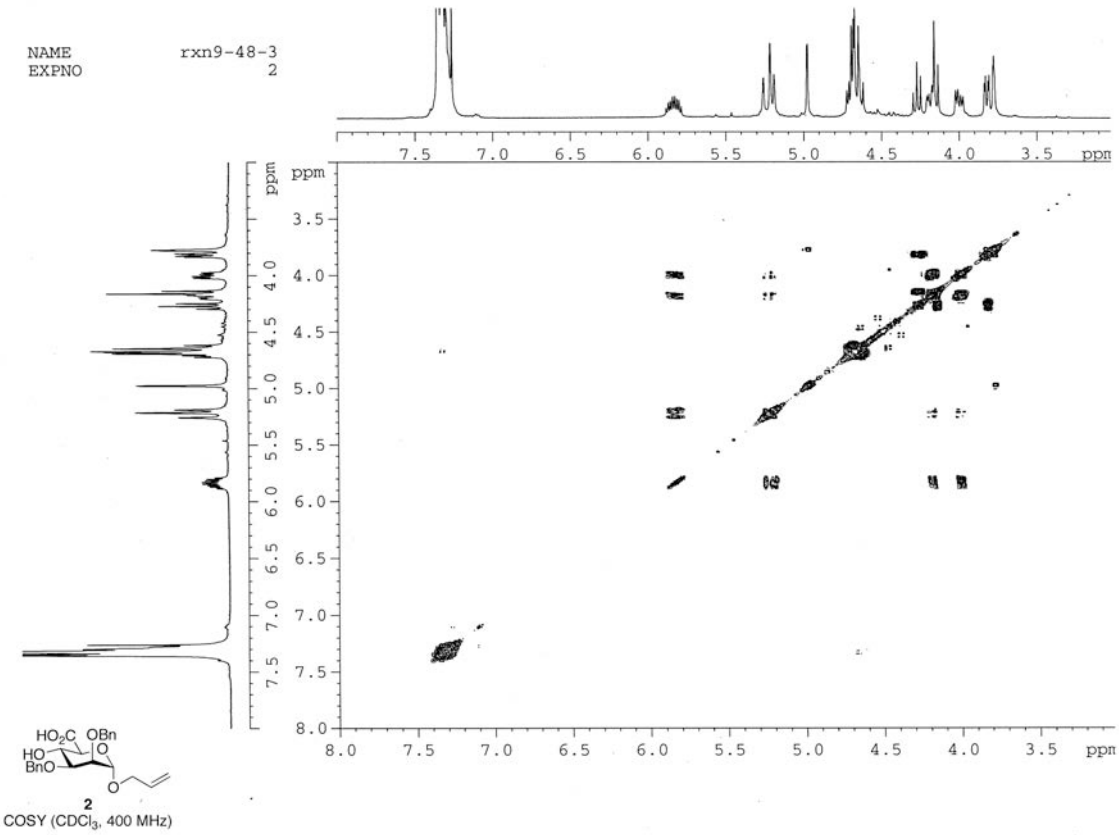


2

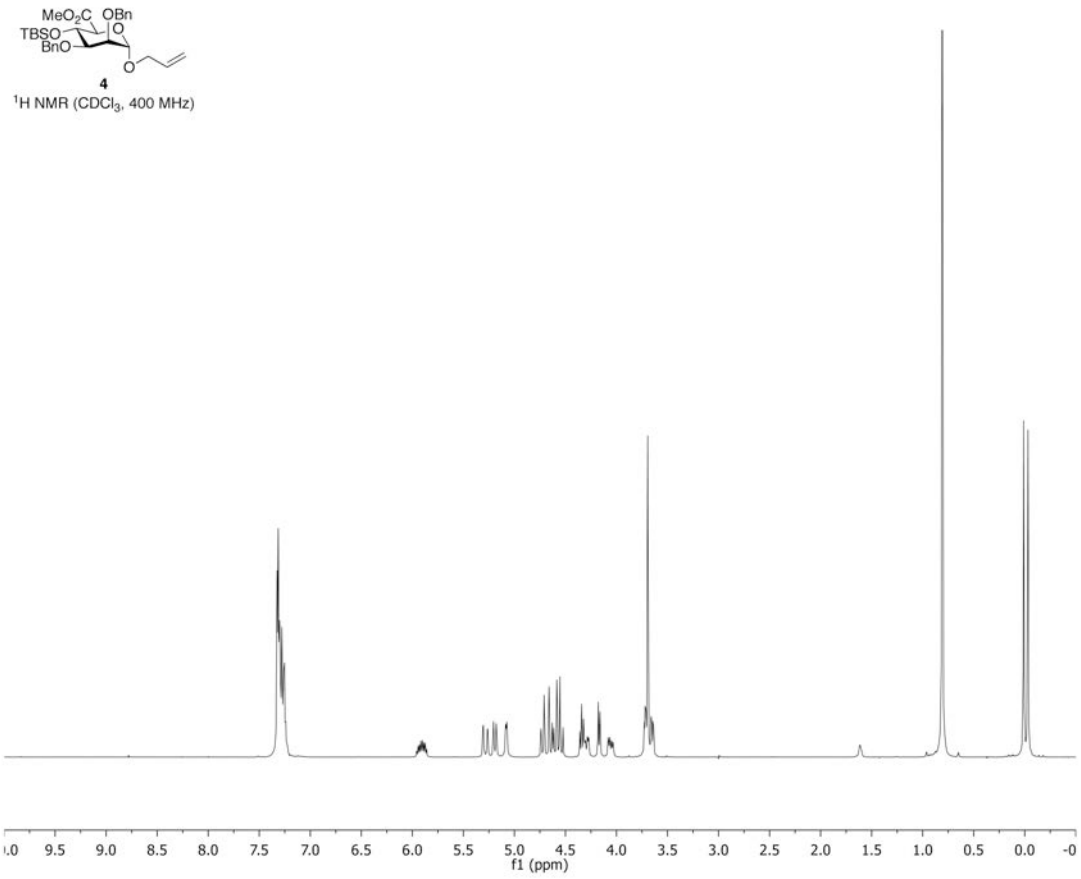
^{13}C NMR ($CDCl_3$, 100 MHz)



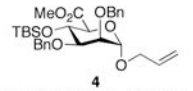
www.manaraa.com



www.manaraa.com

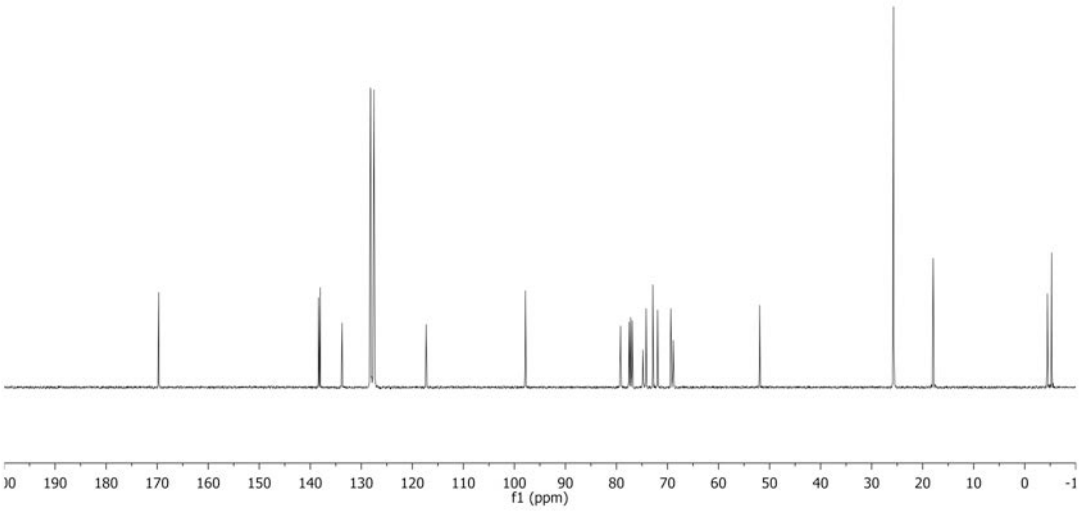


www.manaraa.com

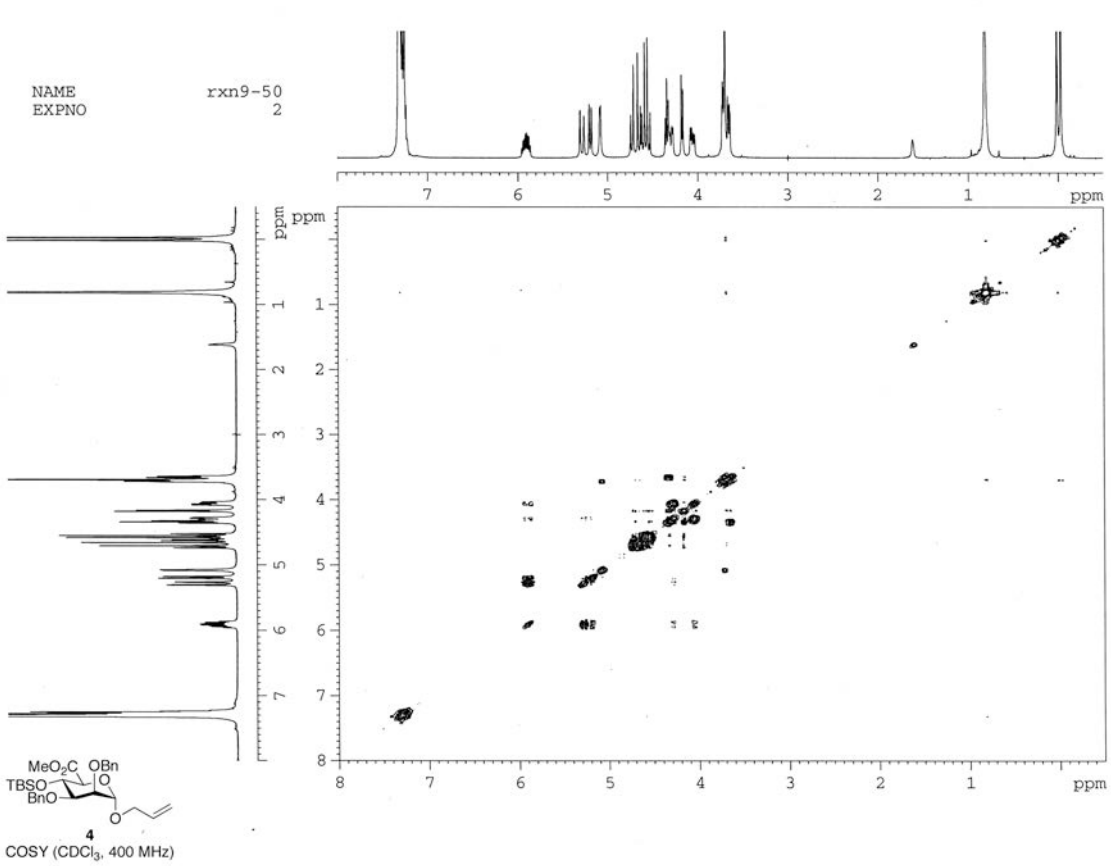


4

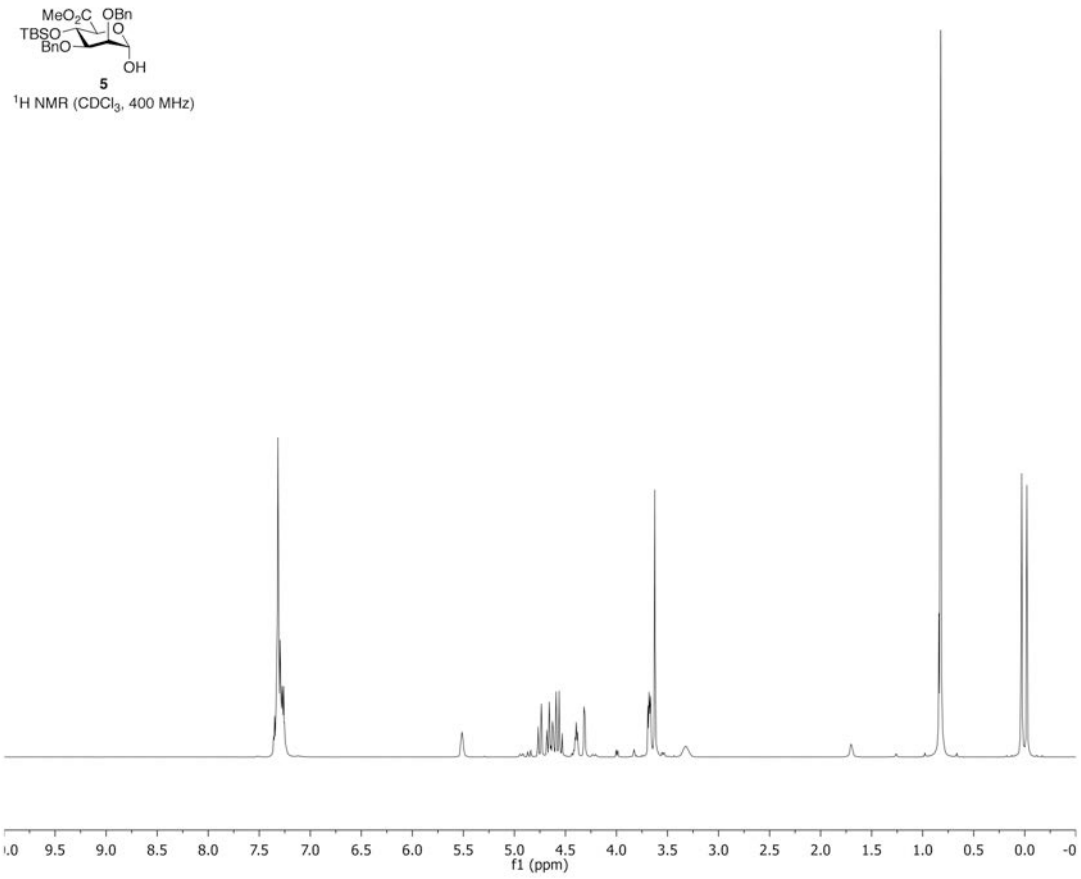
^{13}C NMR ($CDCl_3$, 100 MHz)



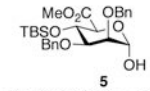
www.manaraa.com



www.manaraa.com

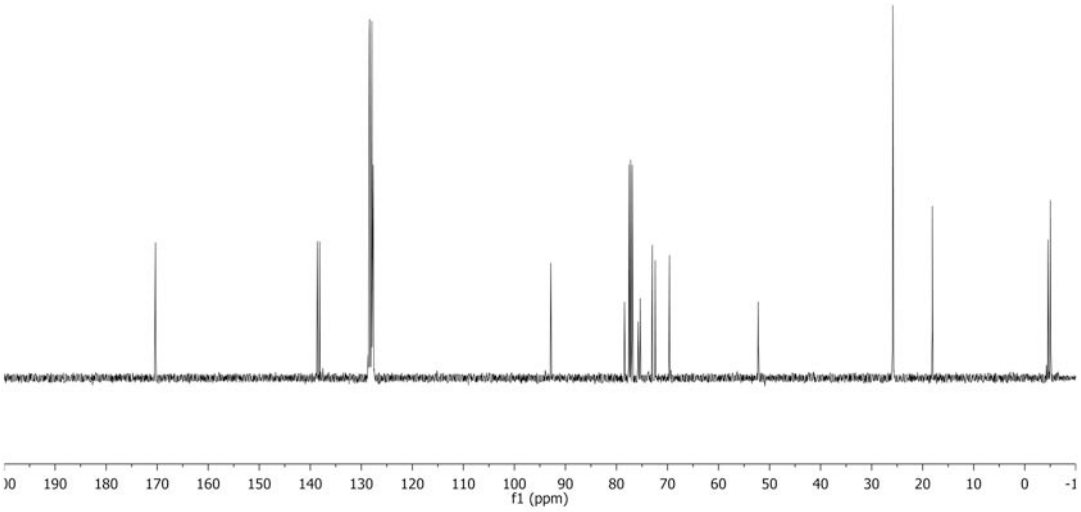


www.manaraa.com

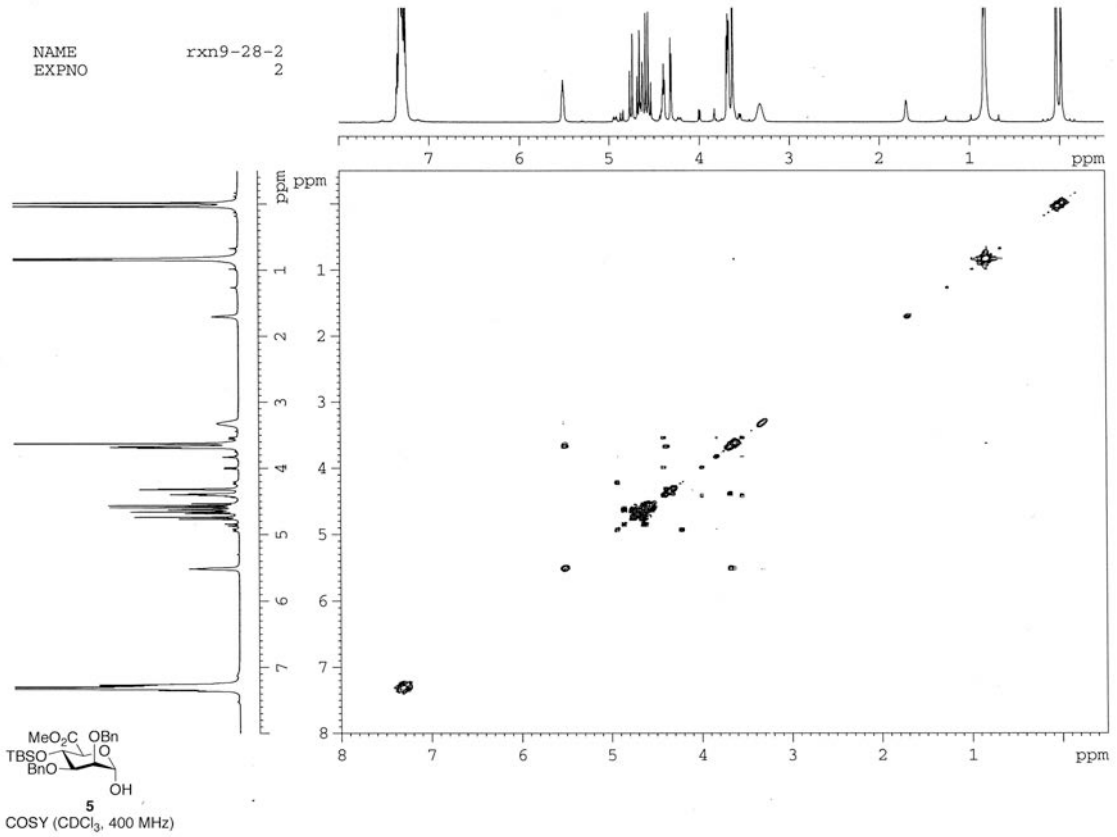


5

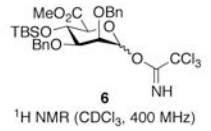
^{13}C NMR ($CDCl_3$, 100 MHz)



www.manaraa.com



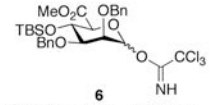
www.manaraa.com



6

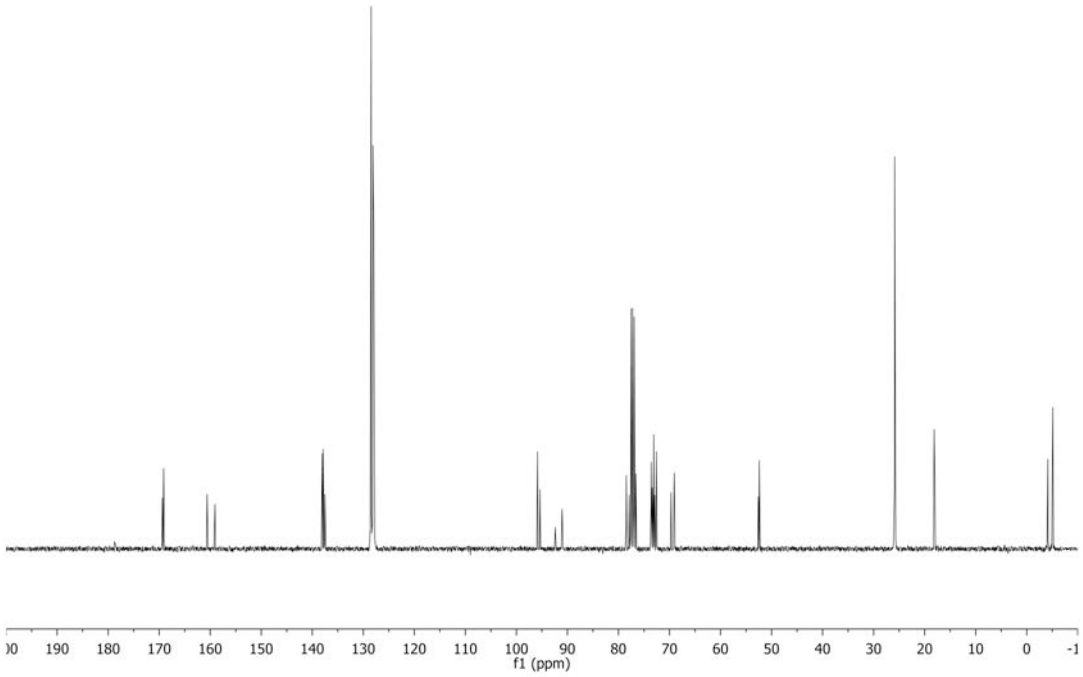
^{1}H NMR ($CDCl_3$, 400 MHz)

www.manaraa.com

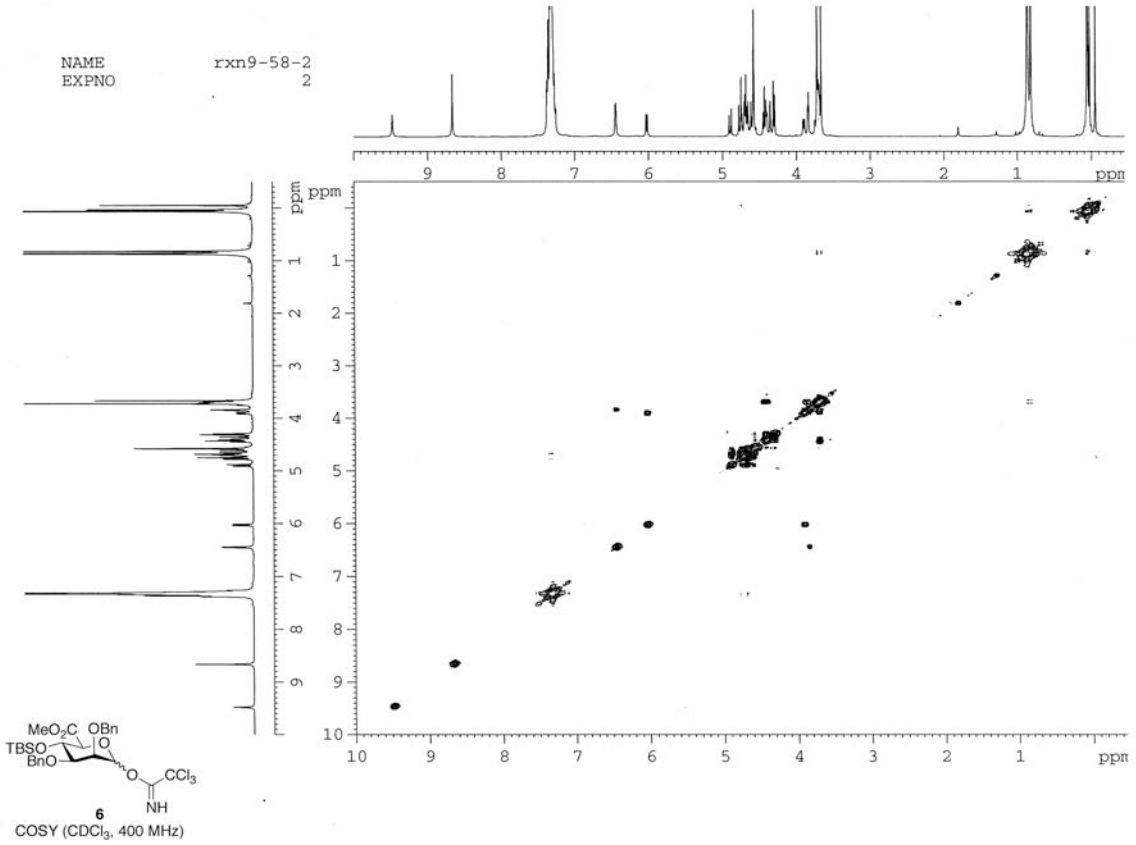


6

^{13}C NMR ($CDCl_3$, 100 MHz)

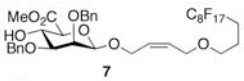


www.manaraa.com



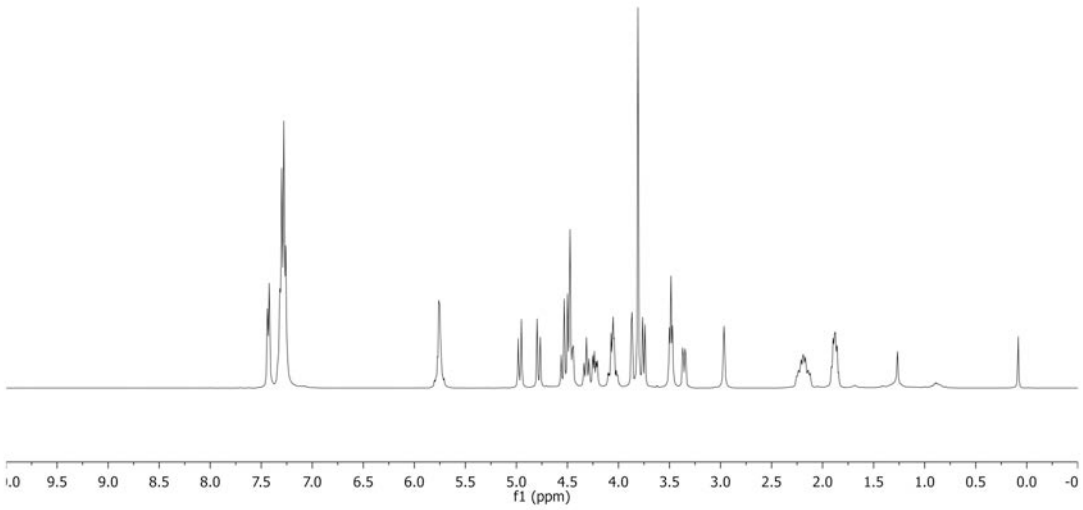
COSY ($CDCl_3$, 400 MHz)

www.manaraa.com

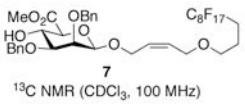


7

^{1}H NMR ($CDCl_3$, 400 MHz)

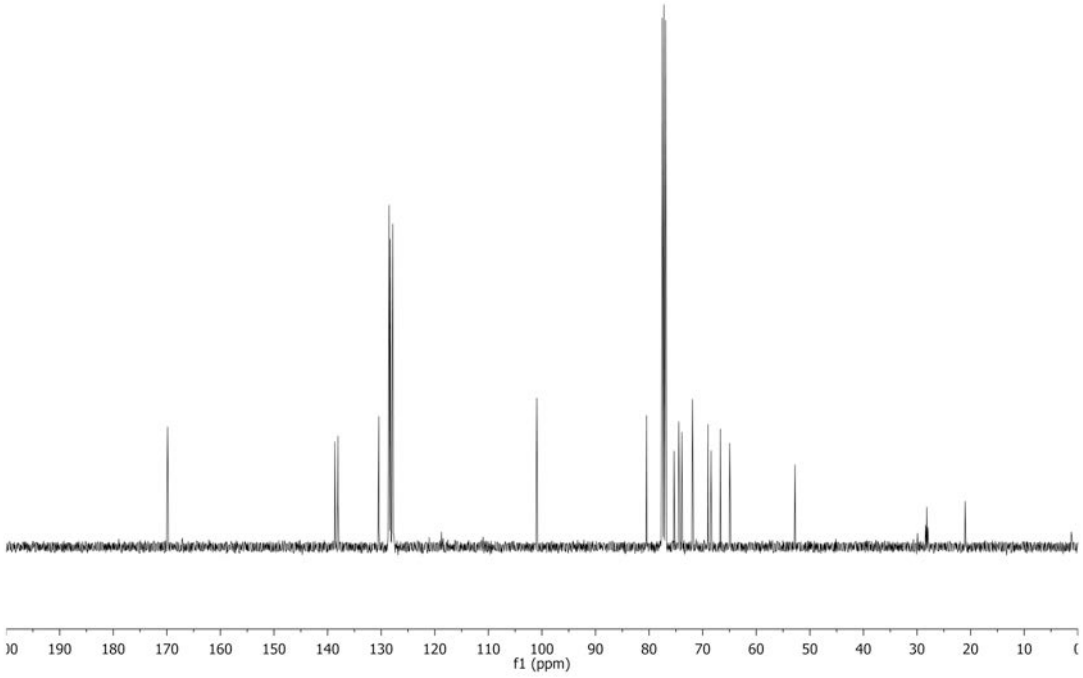


www.manaraa.com

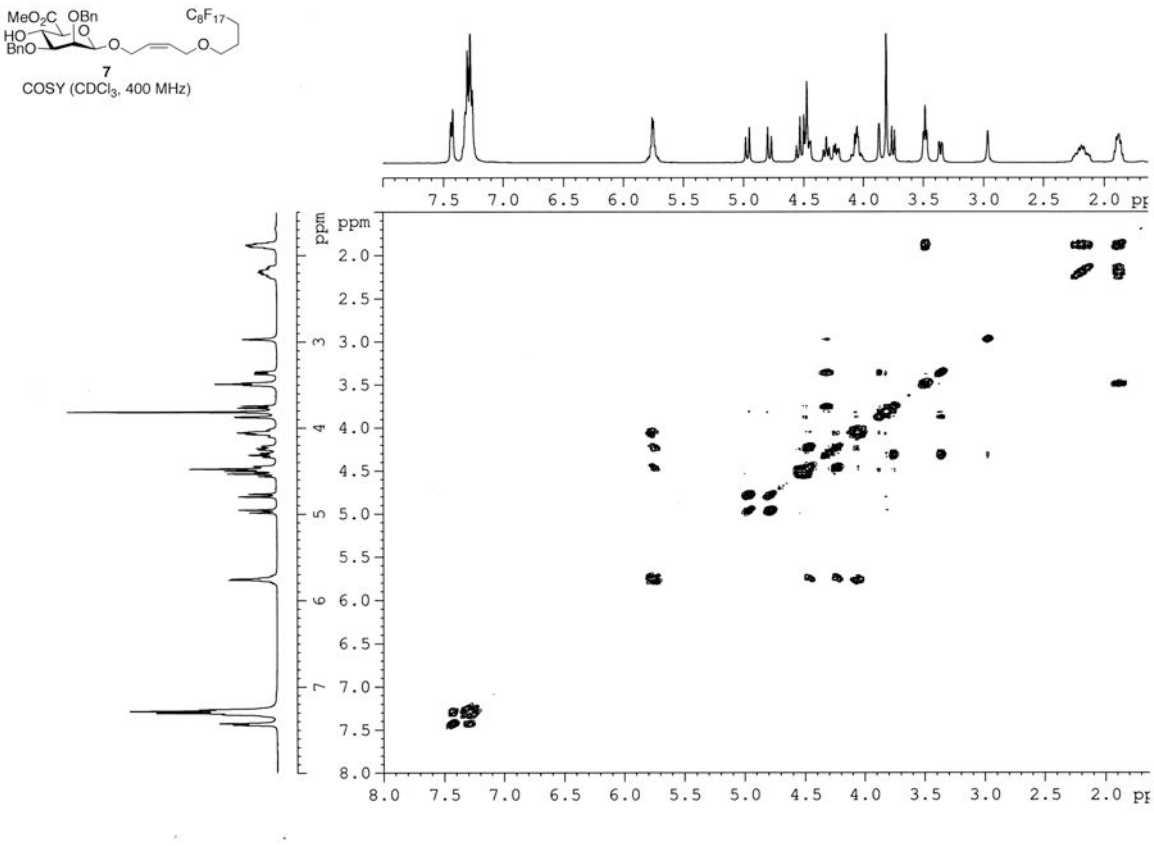


7

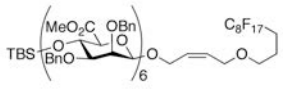
^{13}C NMR ($CDCl_3$, 100 MHz)



www.manaraa.com

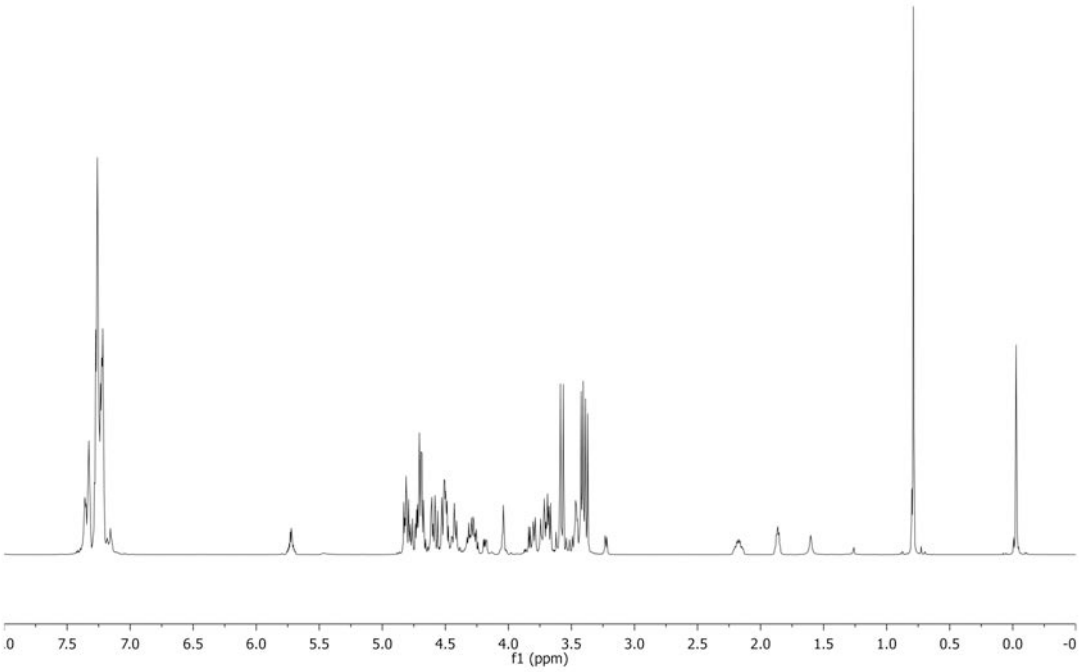


www.manaraa.com

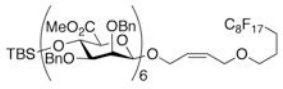


8

^{1}H NMR ($CDCl_3$, 700 MHz)



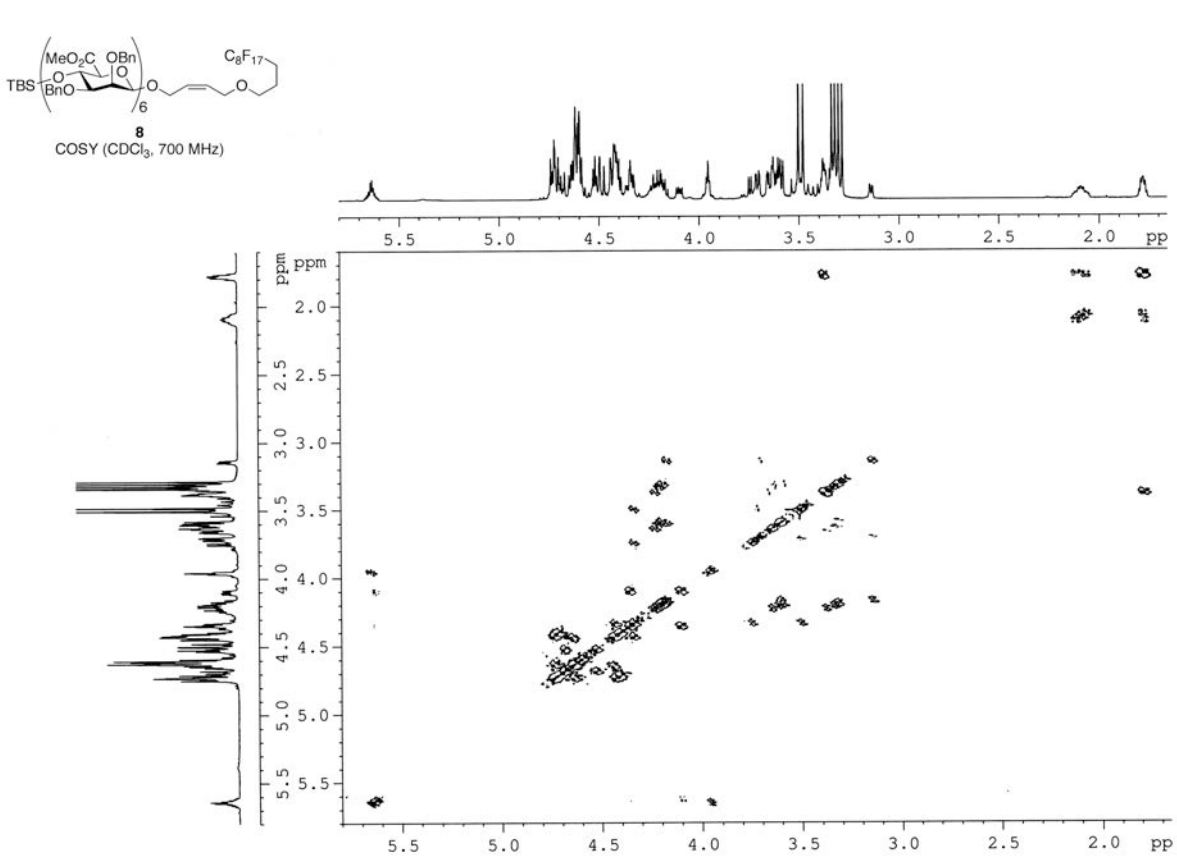
www.manaraa.com



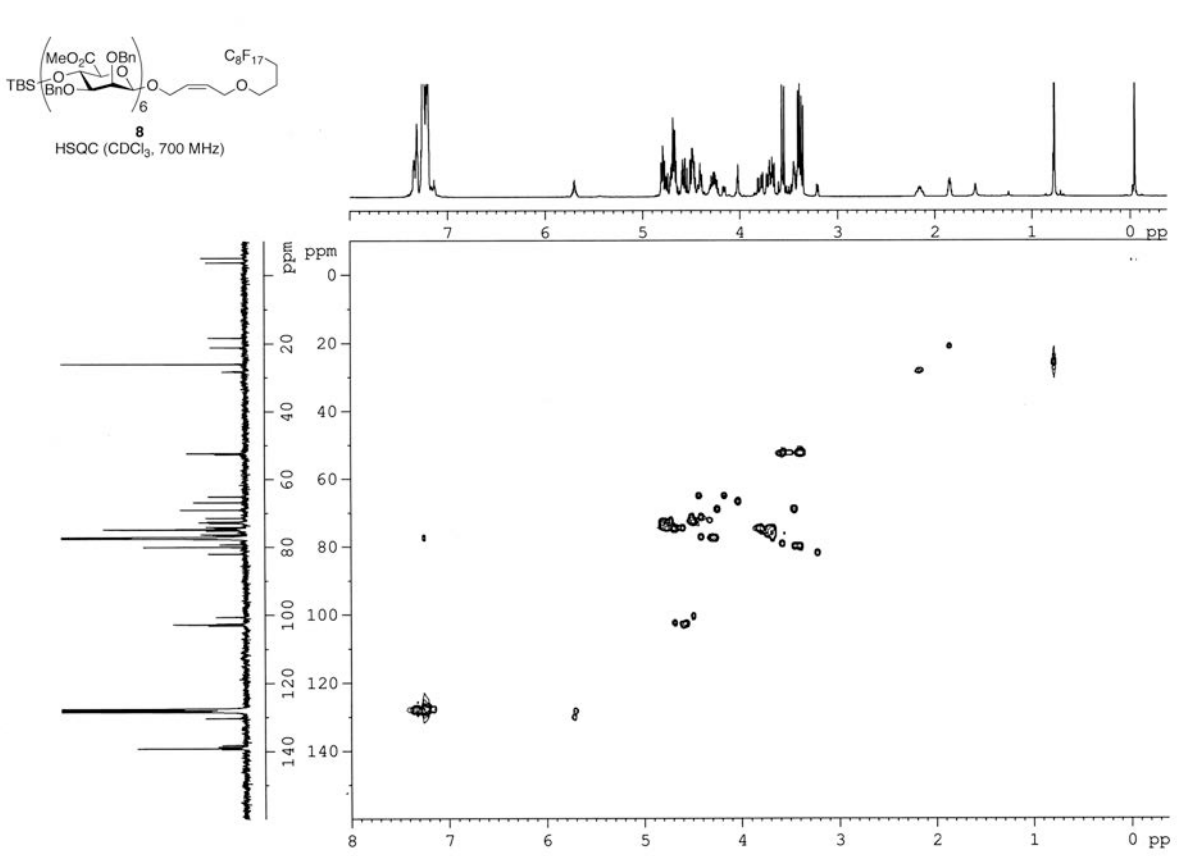
8

^{13}C NMR ($CDCl_3$, 175 MHz)

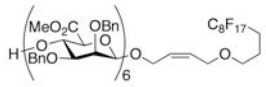
www.manaraa.com



www.manaraa.com

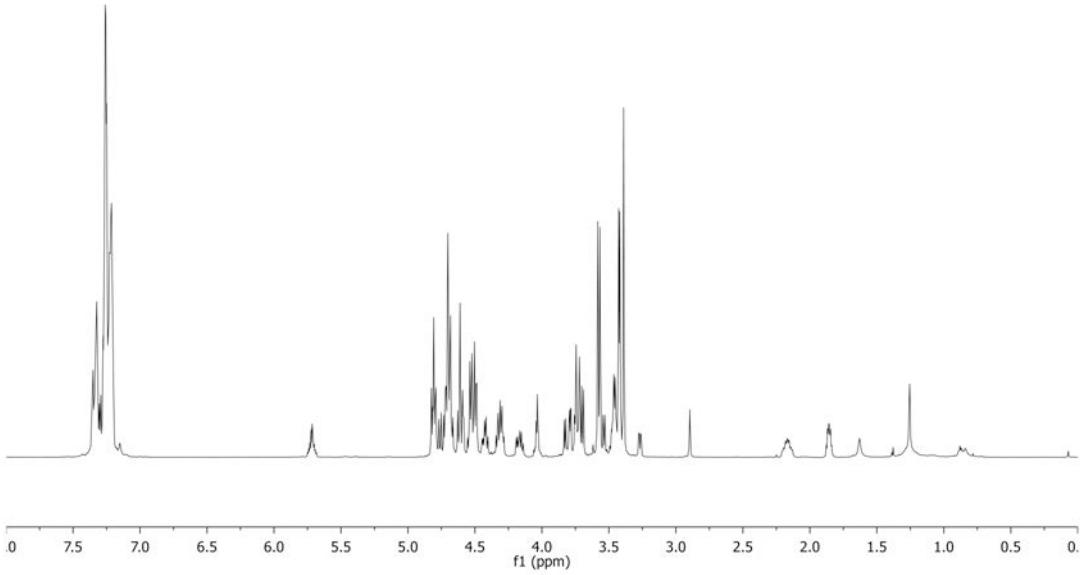


www.manaraa.com

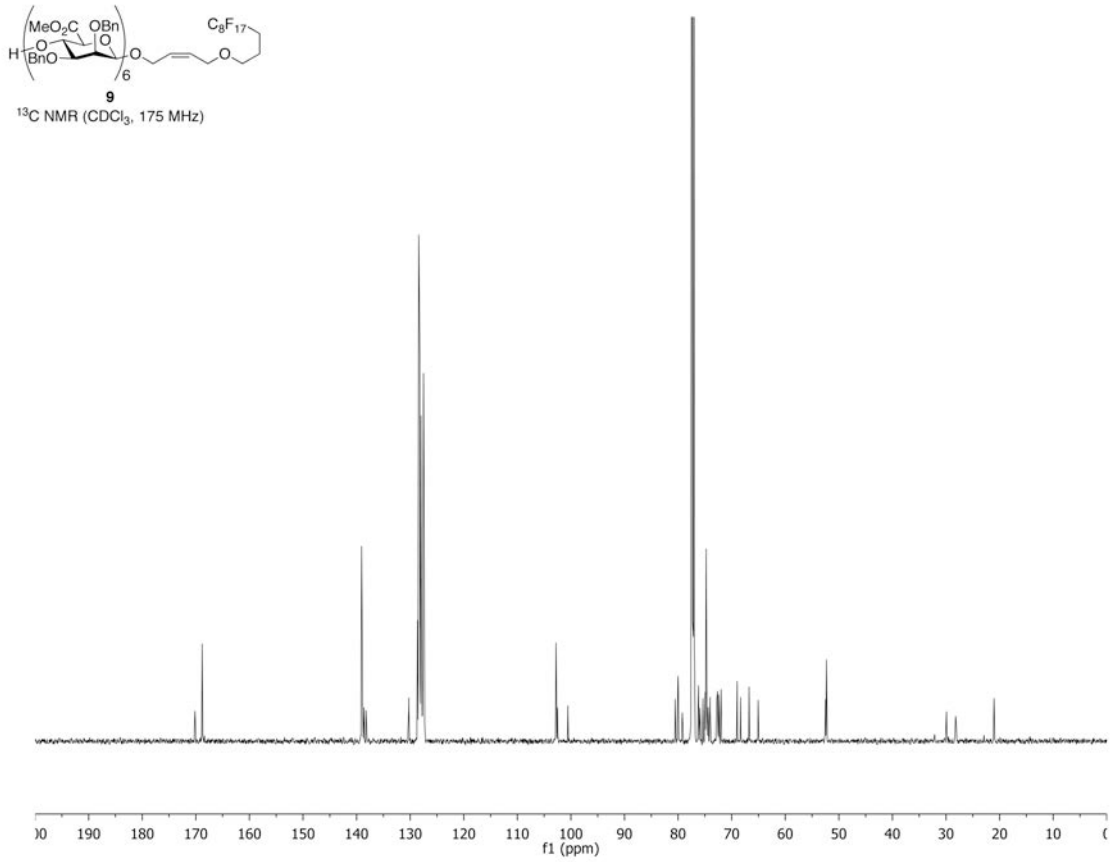


9

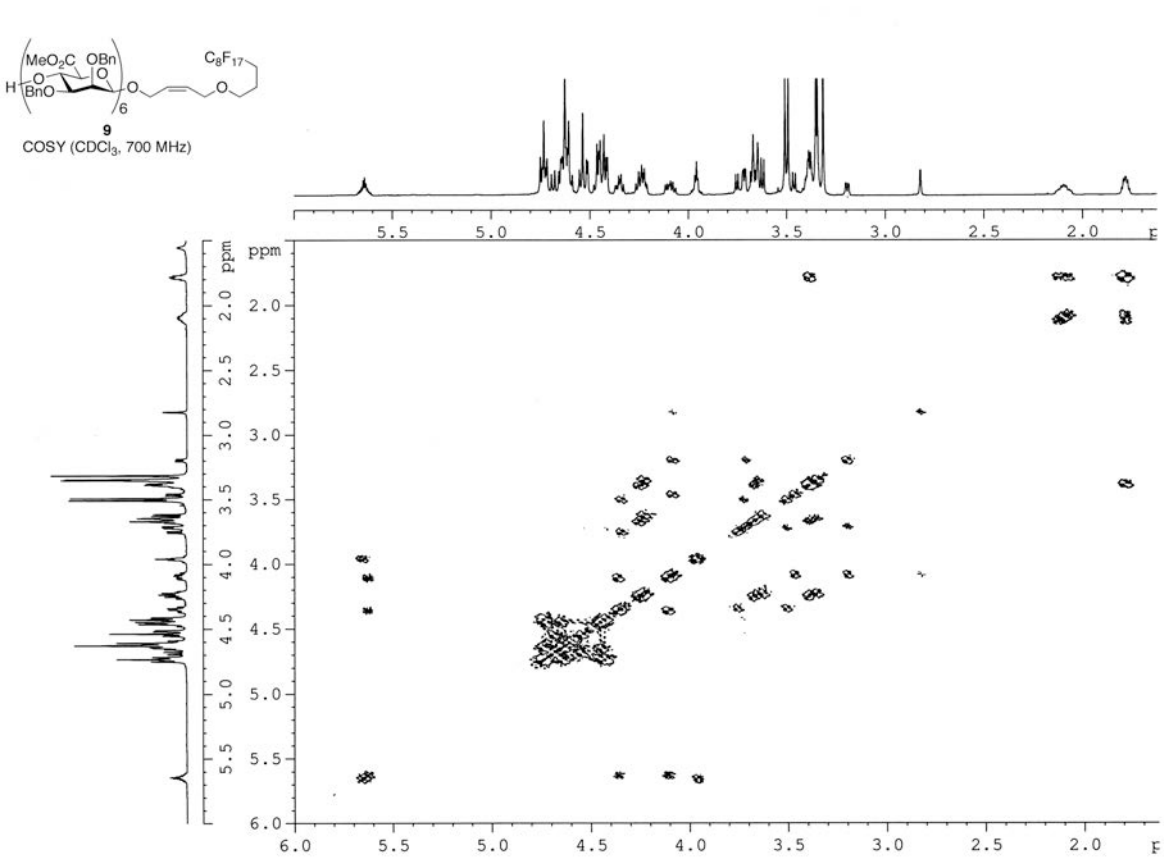
^{1}H NMR ($CDCl_3$, 700 MHz)



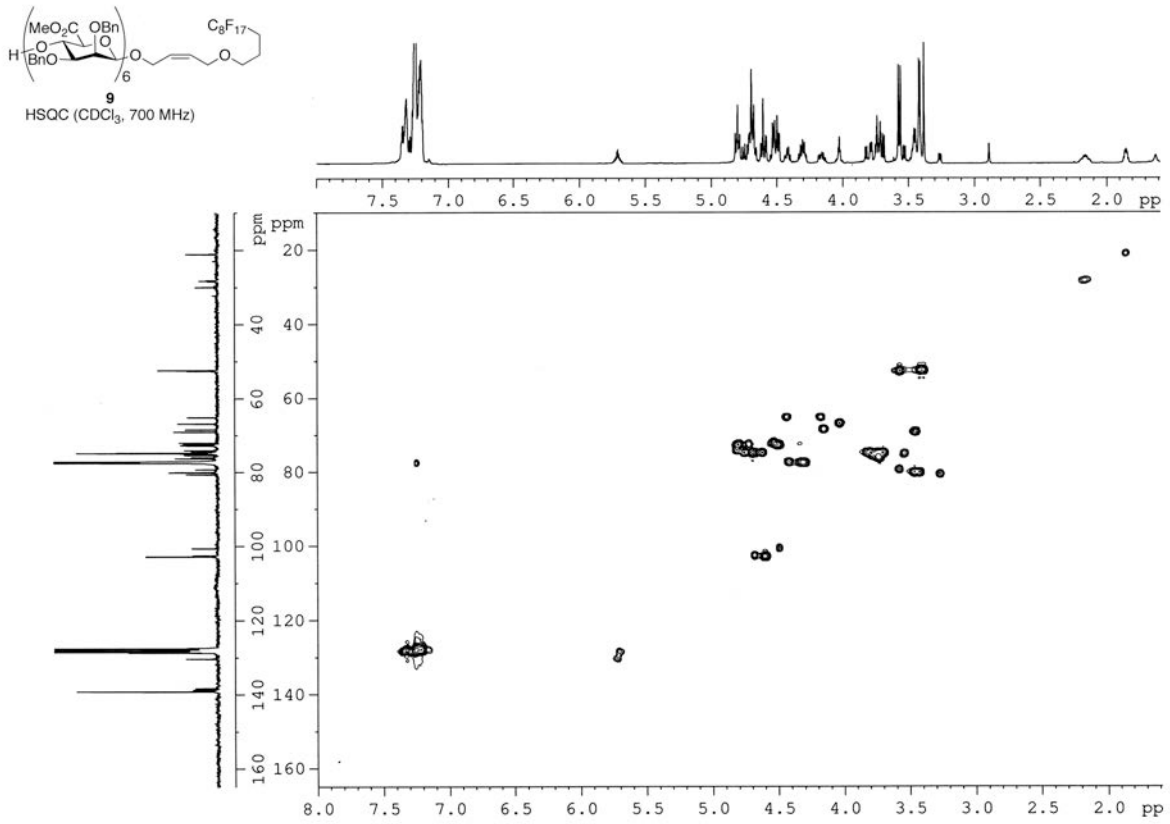
www.manaraa.com



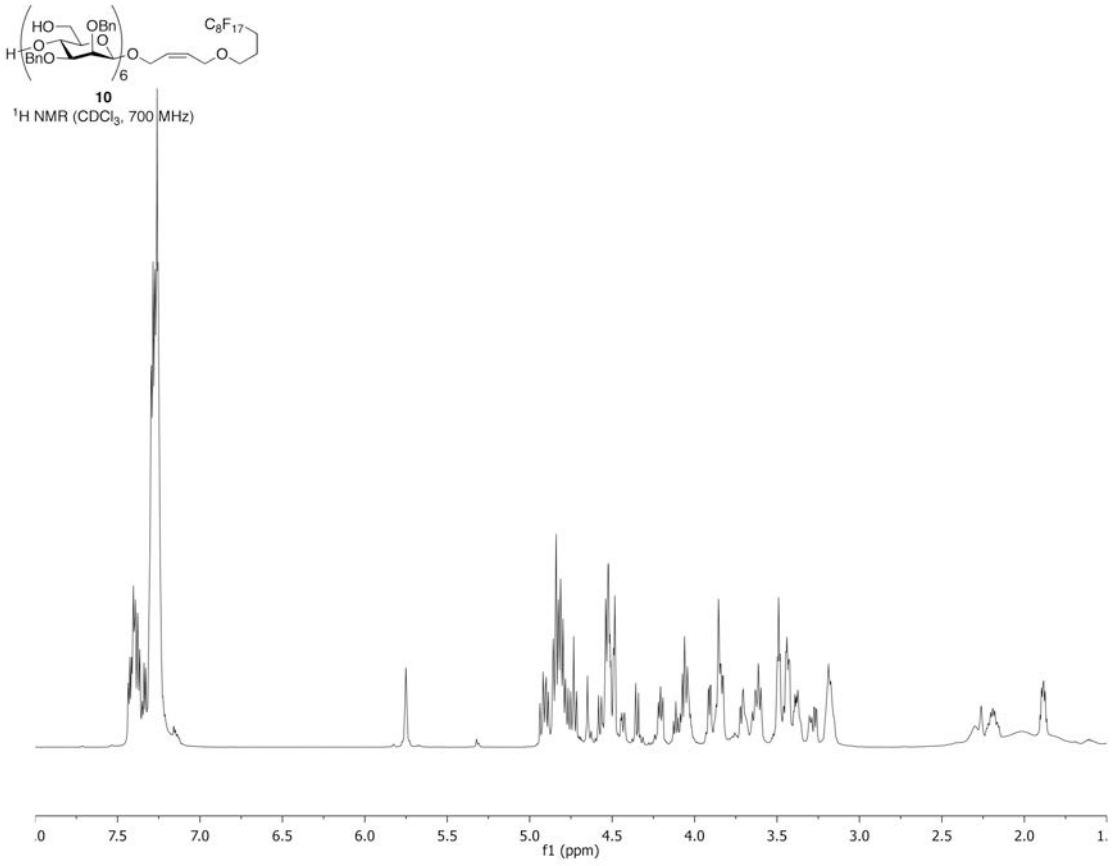
www.manaraa.com



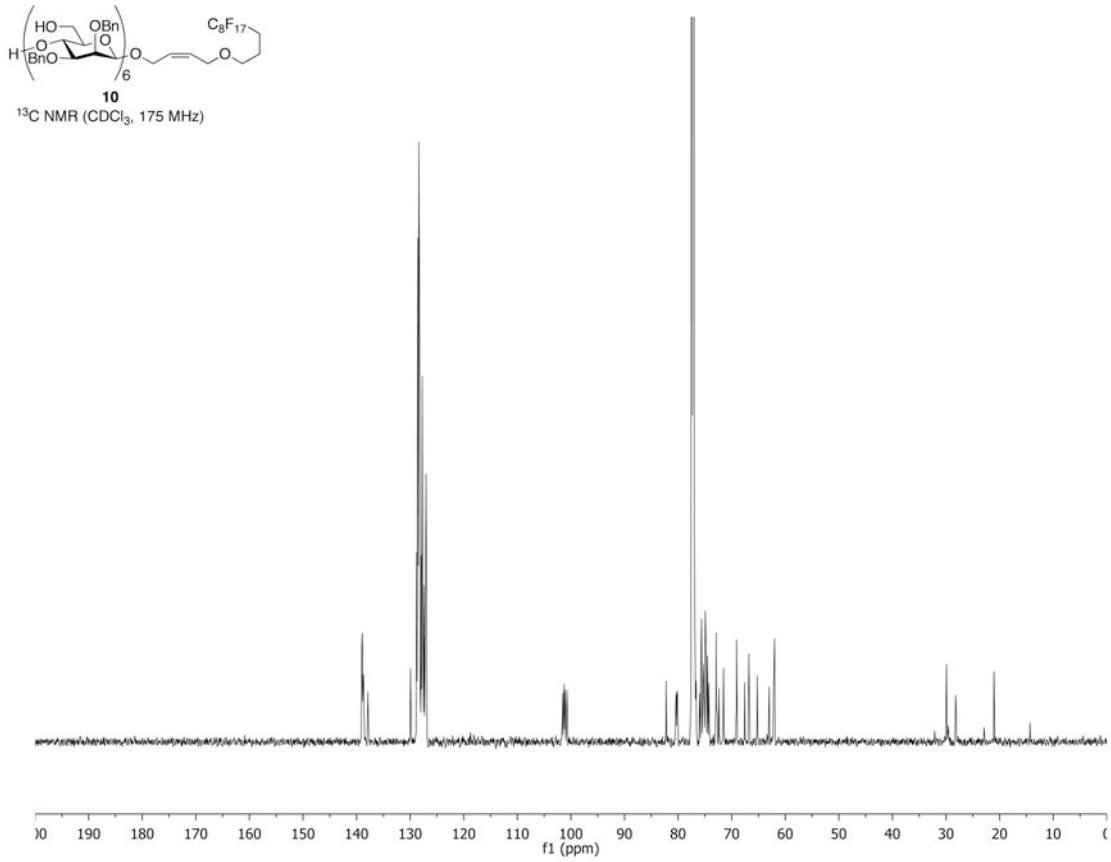
www.manaraa.com



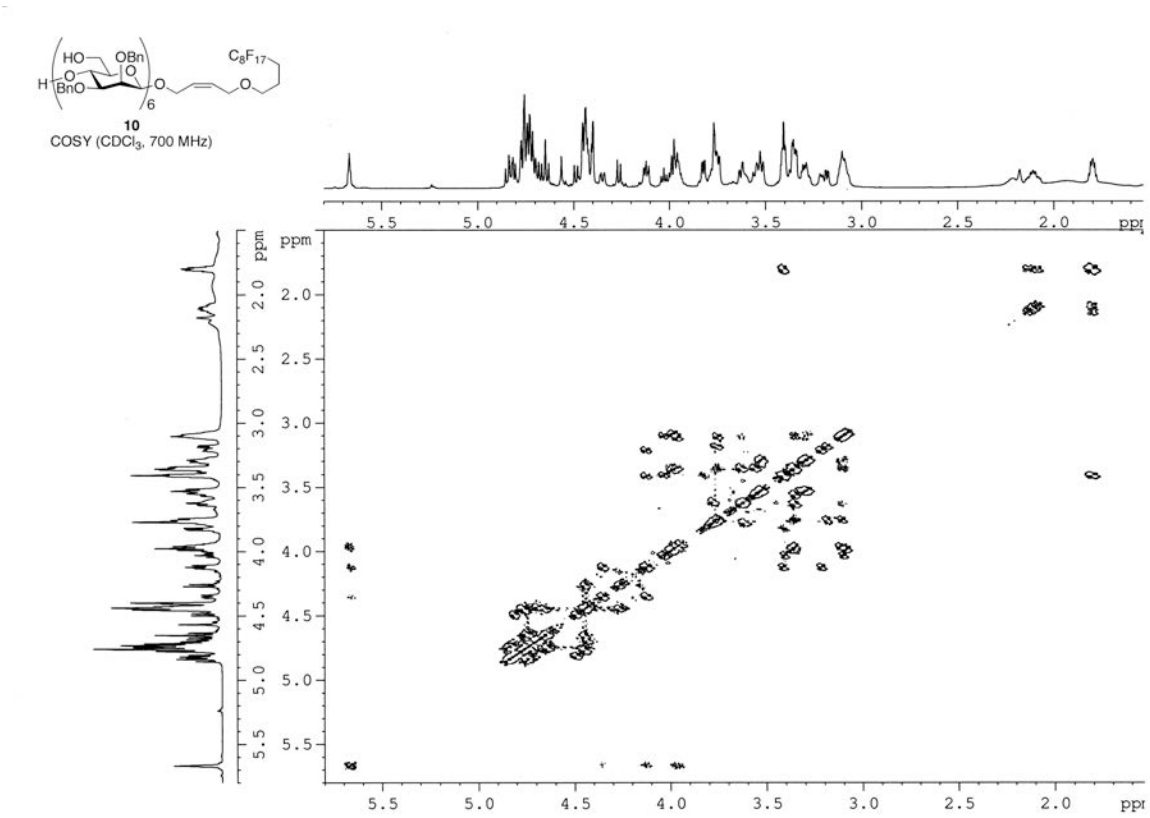
www.manaraa.com



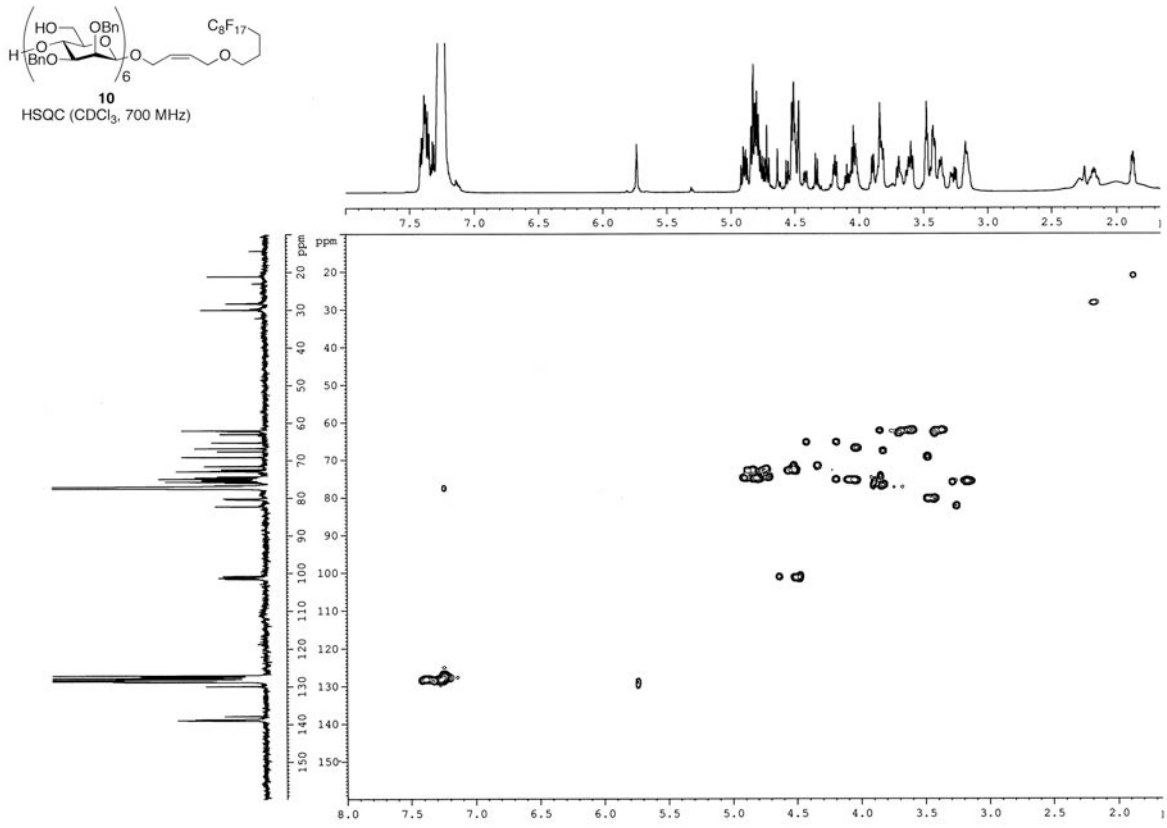
www.manaraa.com



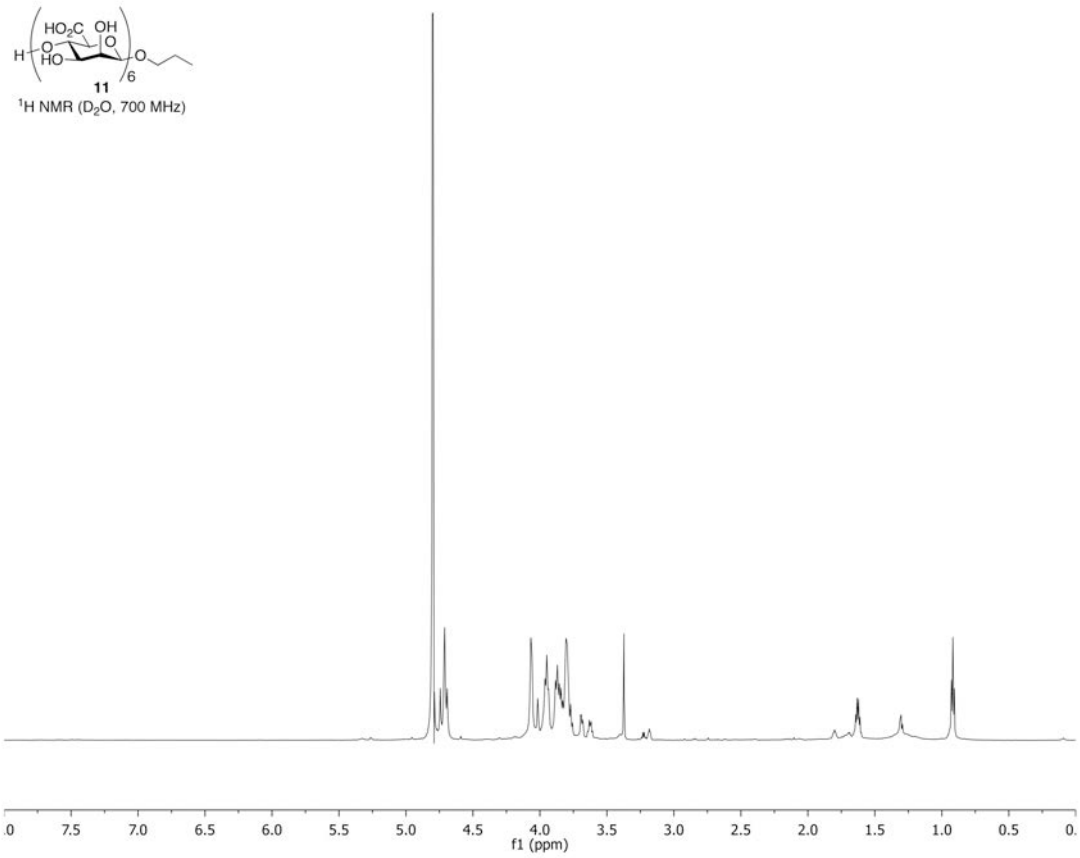
www.manaraa.com



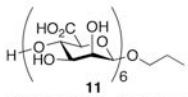
www.manaraa.com



www.manaraa.com

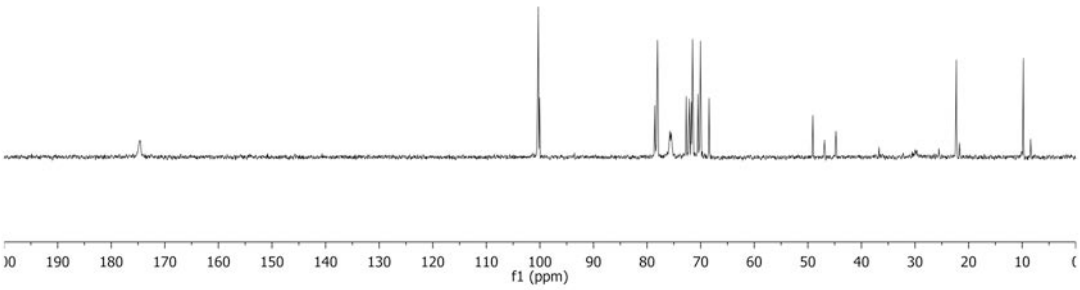


www.manaraa.com

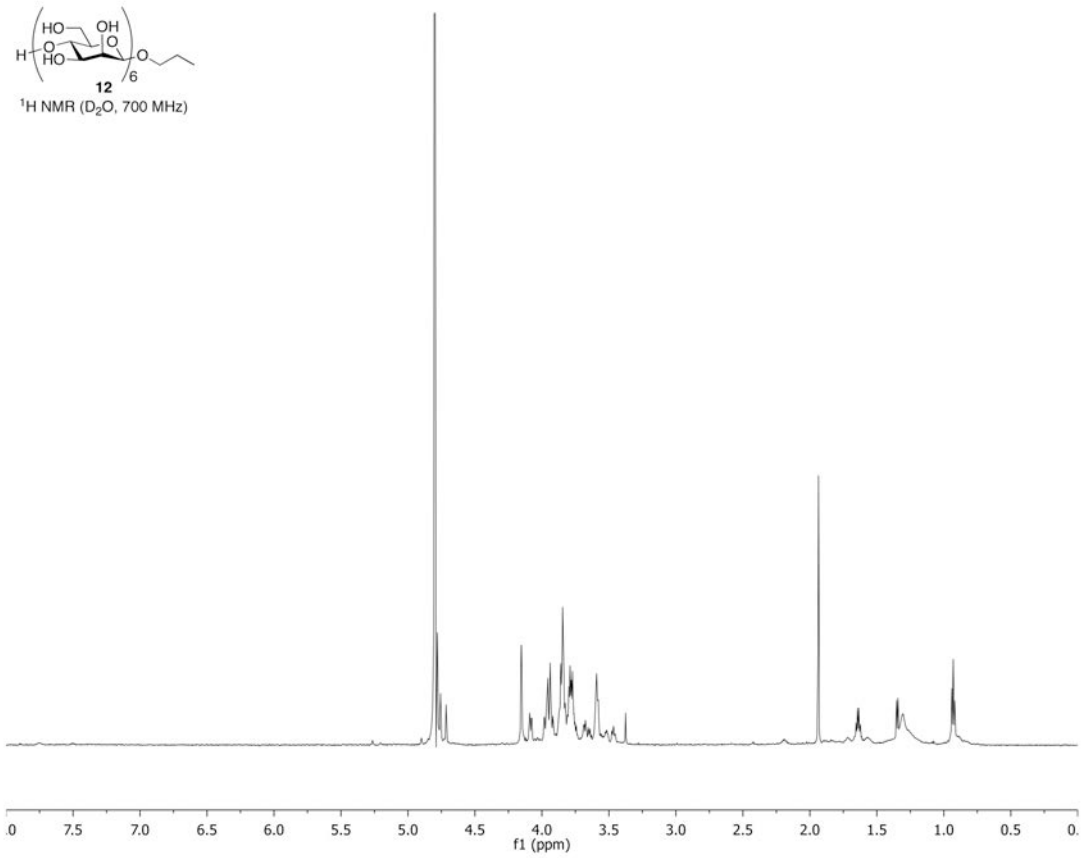


11

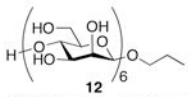
^{13}C NMR (D_2O, 175 MHz)



www.manaraa.com

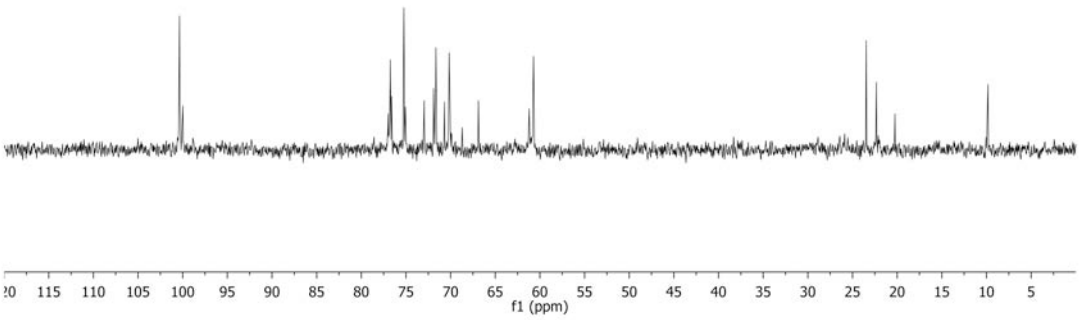


www.manaraa.com



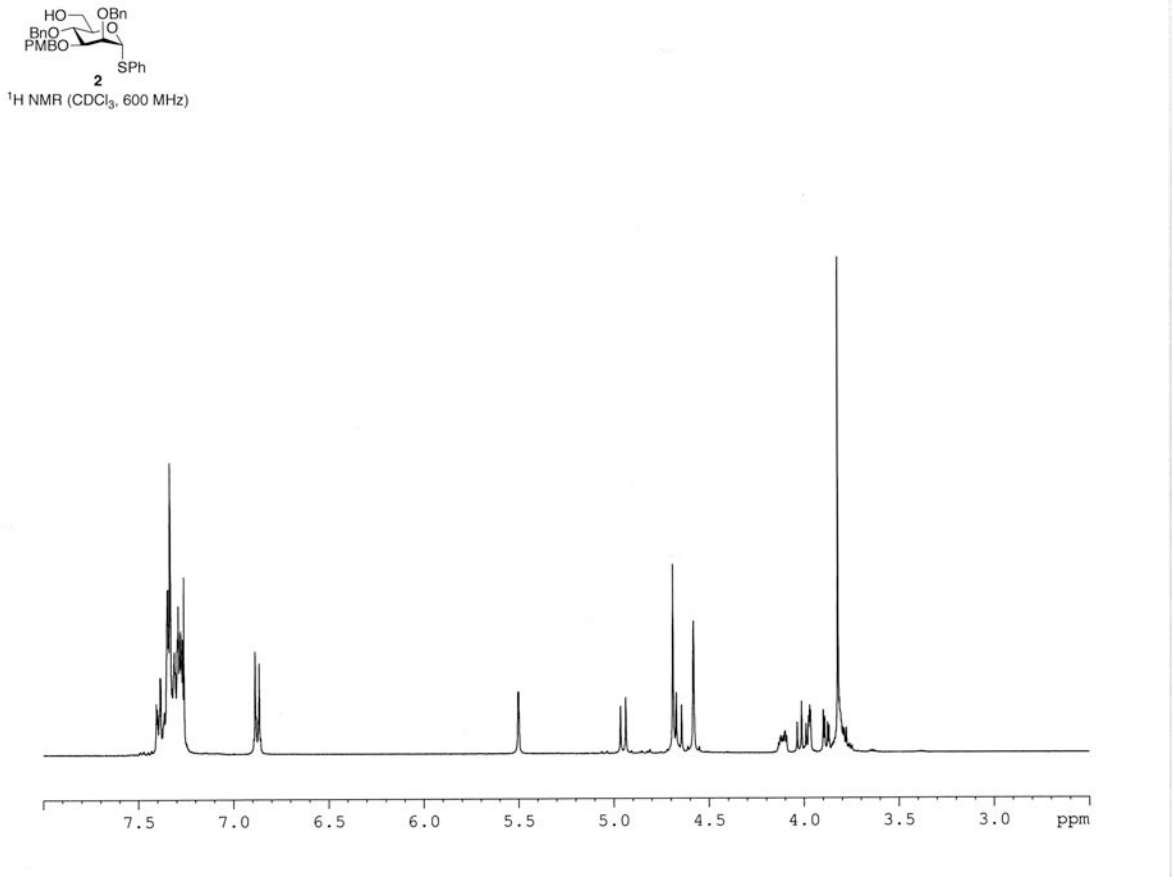
12

^{13}C NMR (D_2O, 175 MHz)

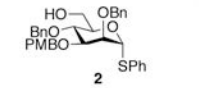


www.manaraa.com

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

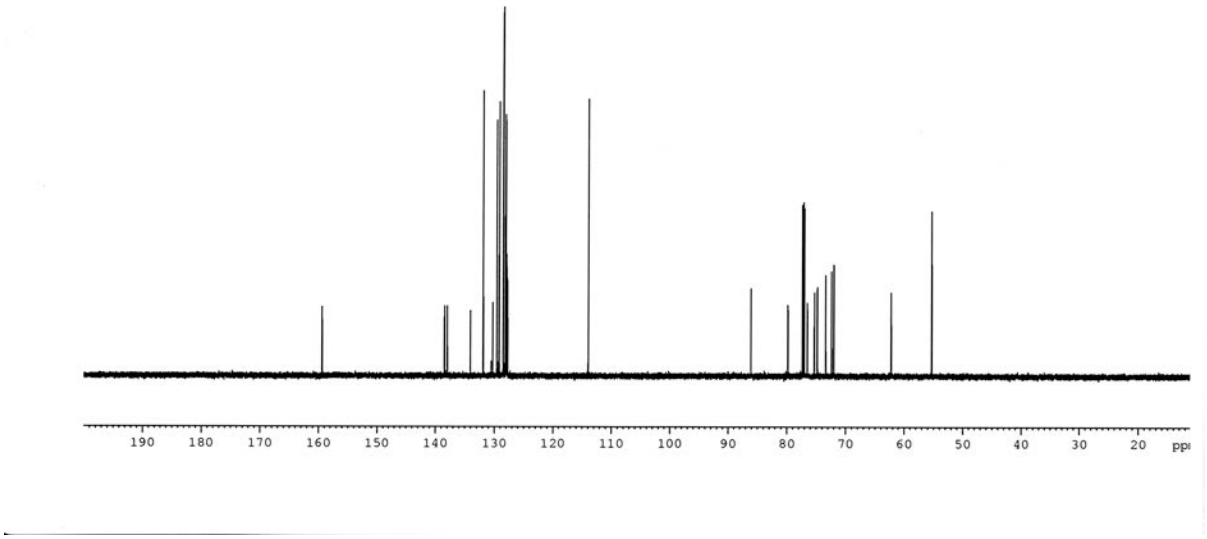


www.manaraa.com

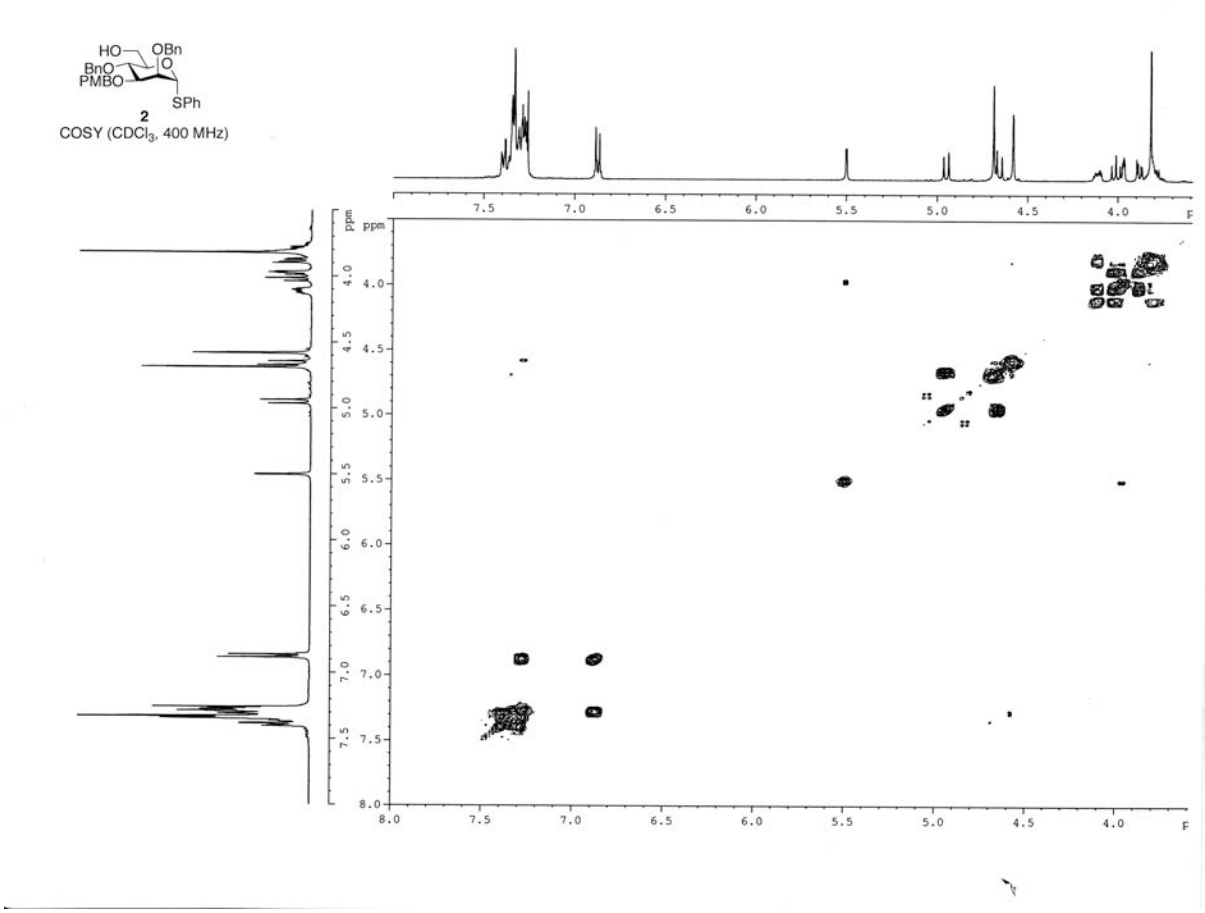


2

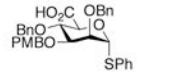
^{13}C NMR ($CDCl_3$, 100 MHz)



www.manaraa.com

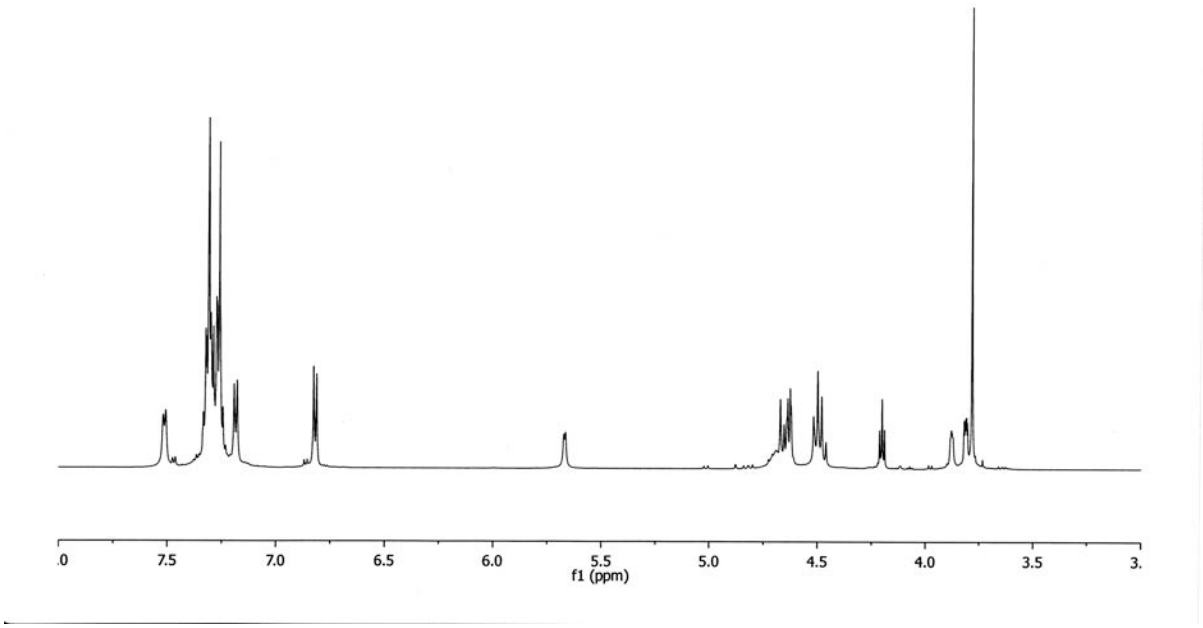


www.manaraa.com

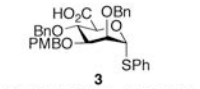


3

^{1}H NMR ($CDCl_3$, 600 MHz)

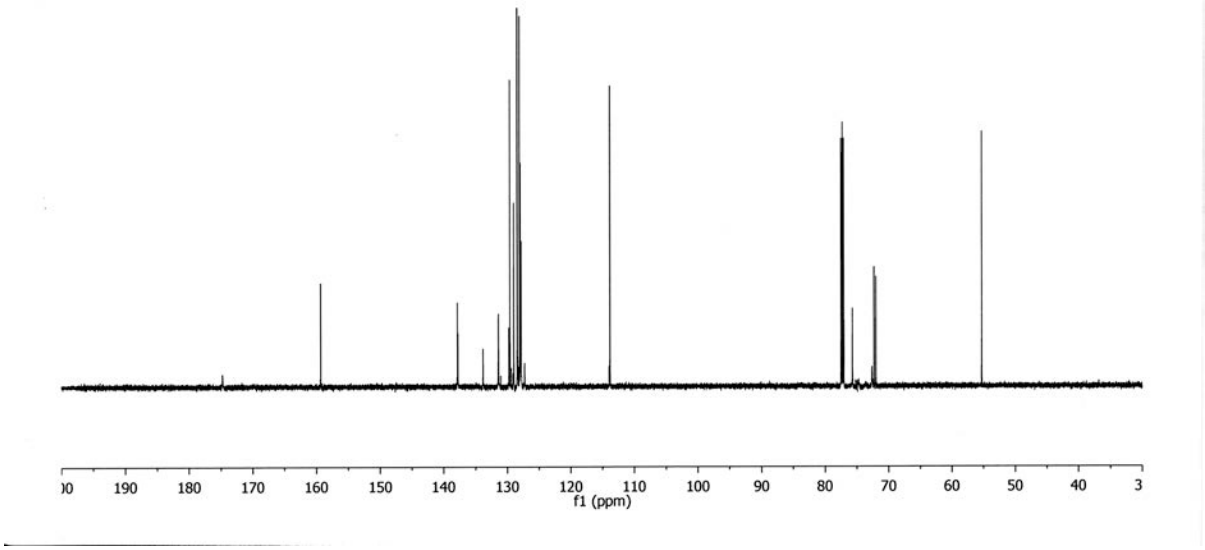


www.manaraa.com

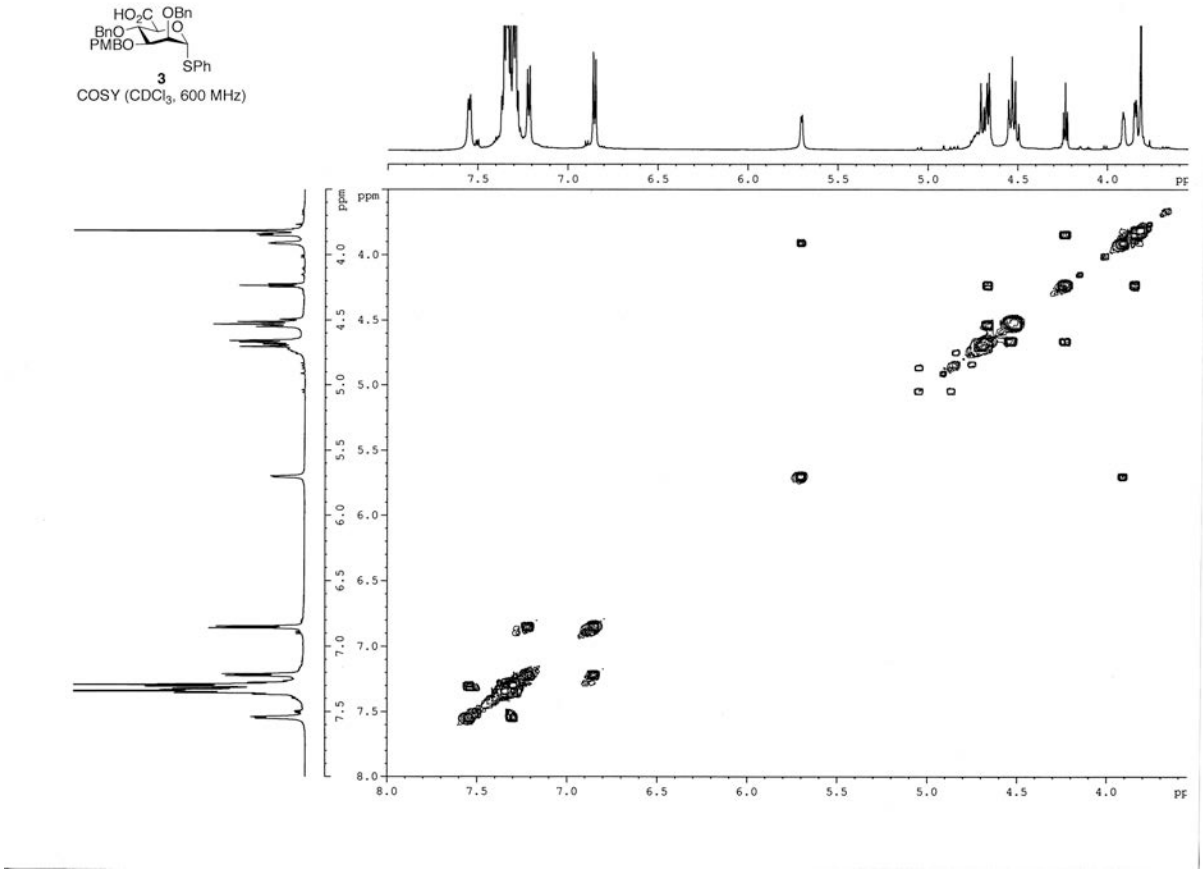


3

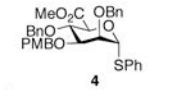
^{13}C NMR ($CDCl_3$, 150 MHz)



www.manaraa.com

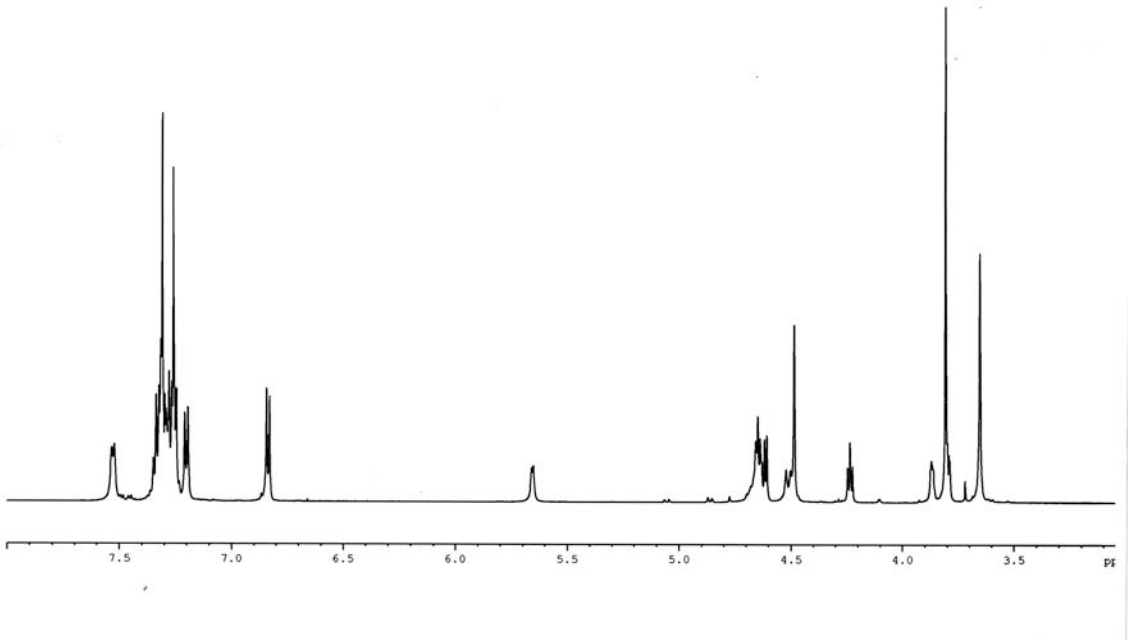


www.manaraa.com

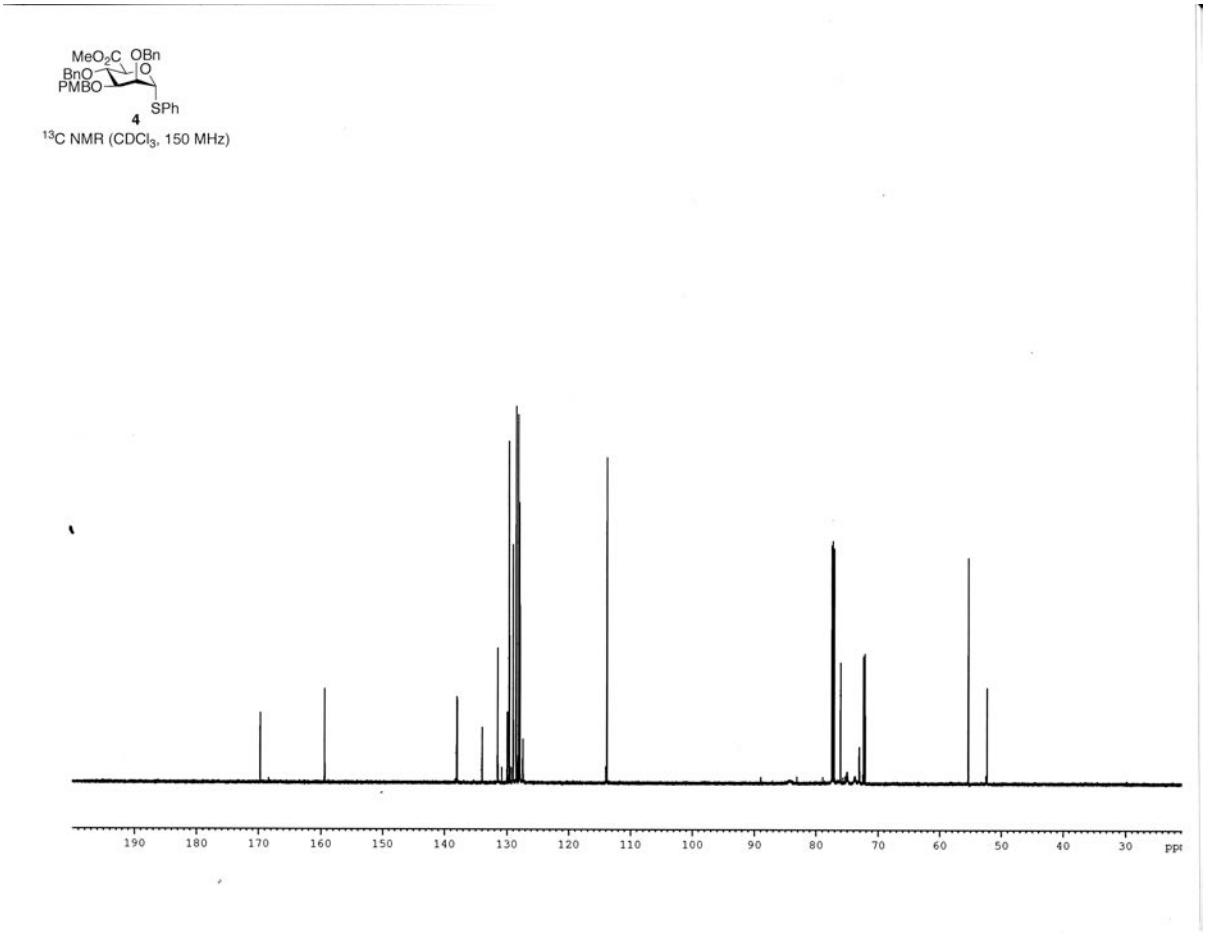


4

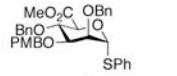
^{1}H NMR ($CDCl_3$, 600 MHz)



www.manaraa.com

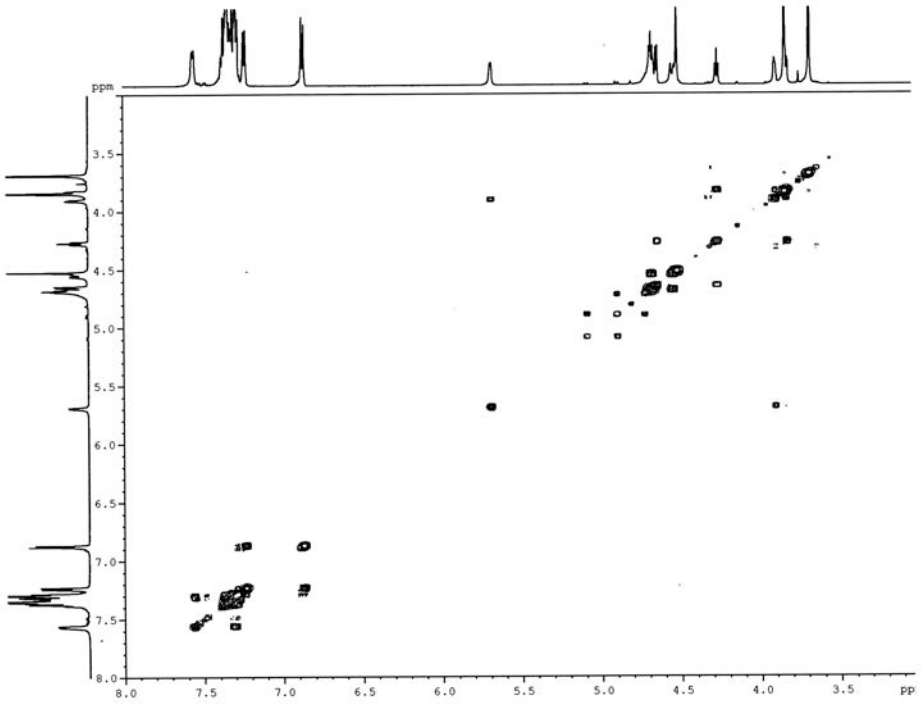


www.manaraa.com

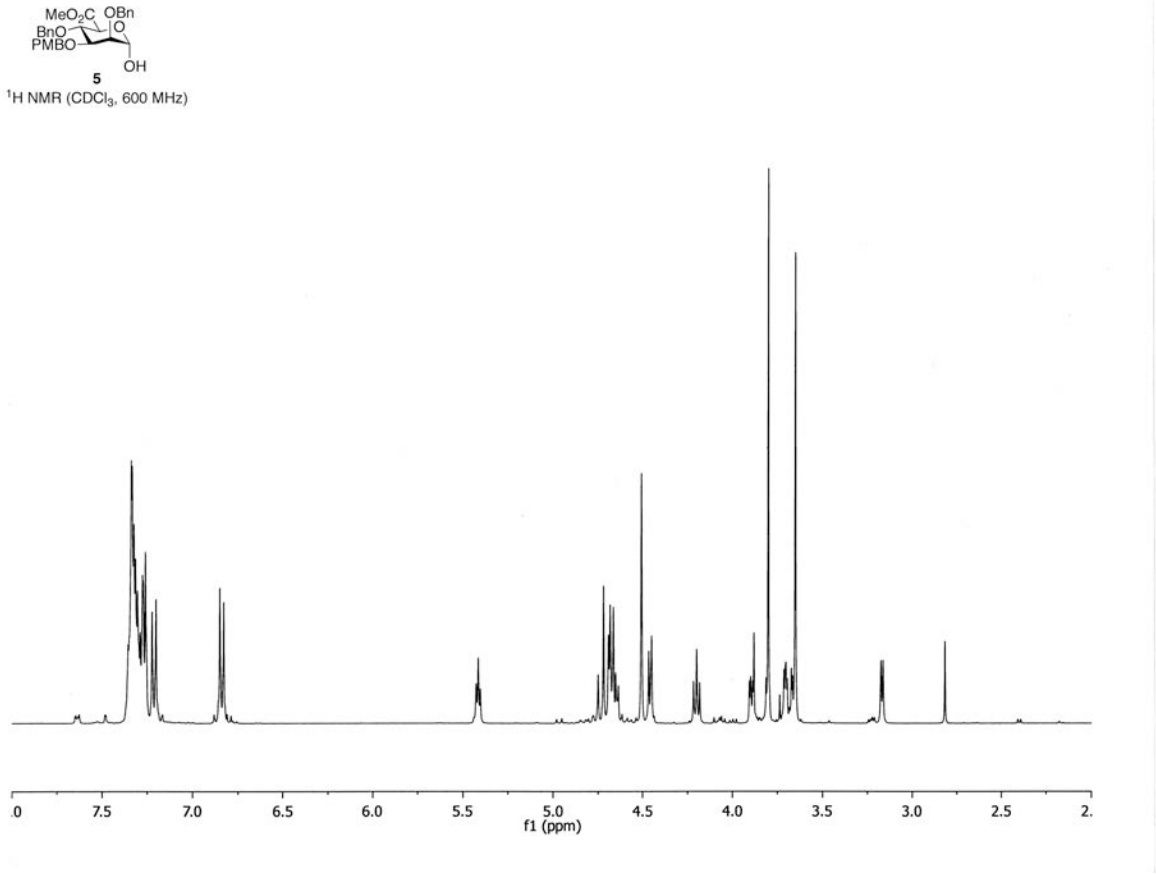


4

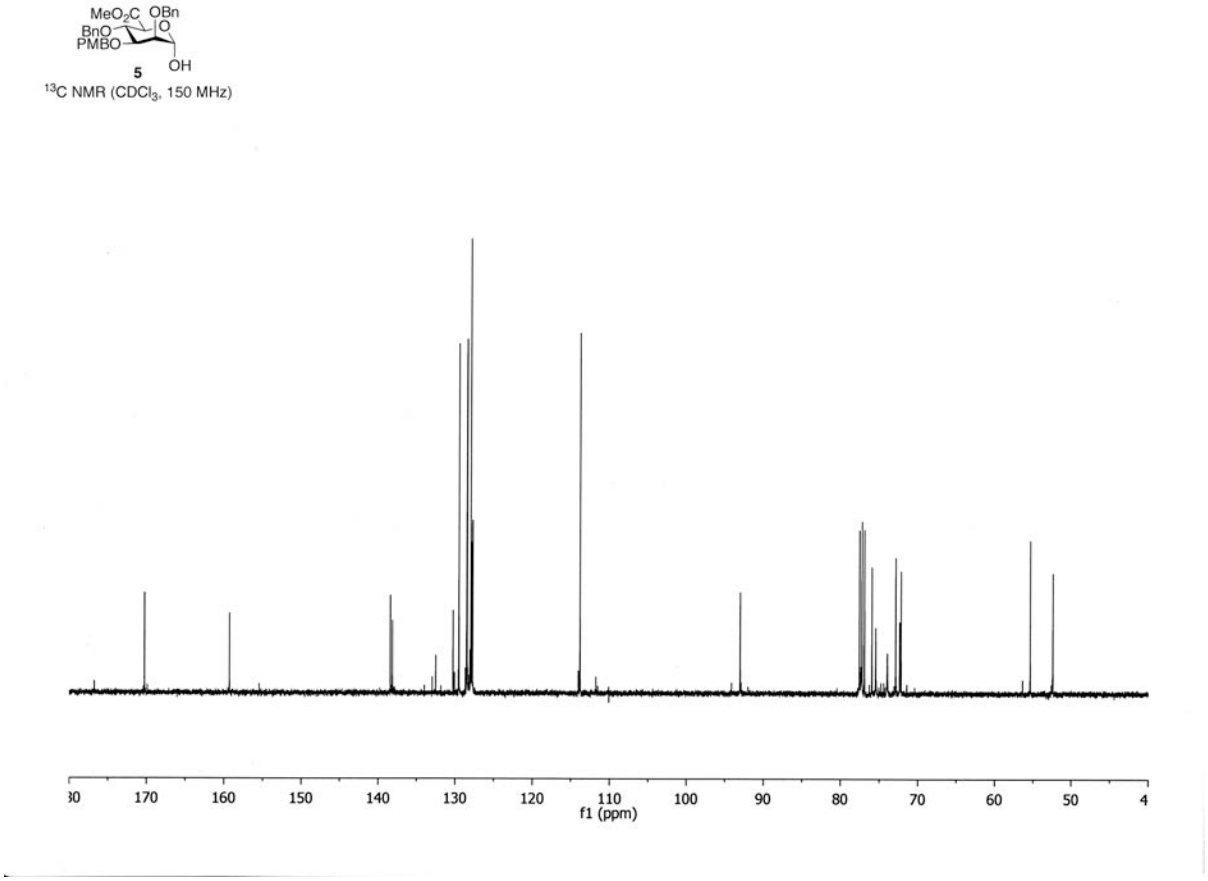
COSY ($CDCl_3$, 600 MHz)



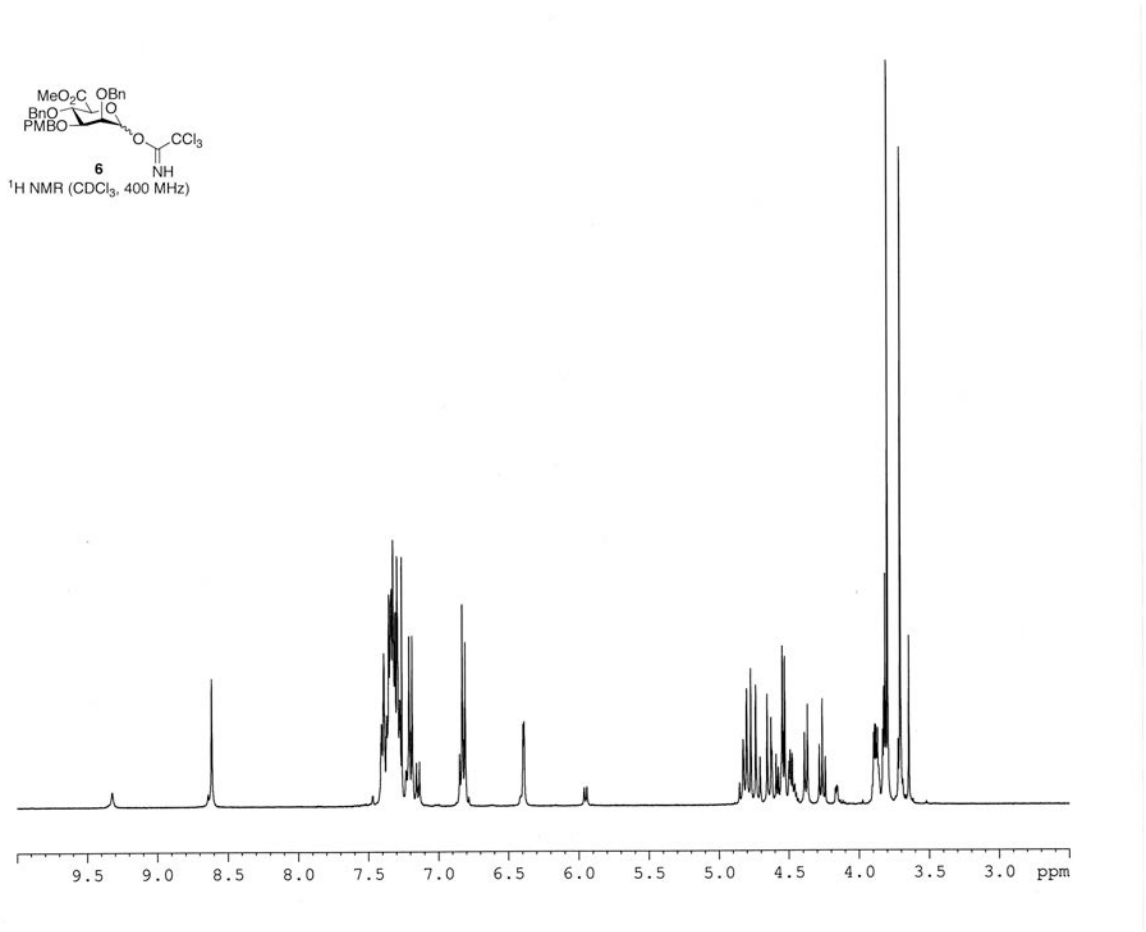
www.manaraa.com



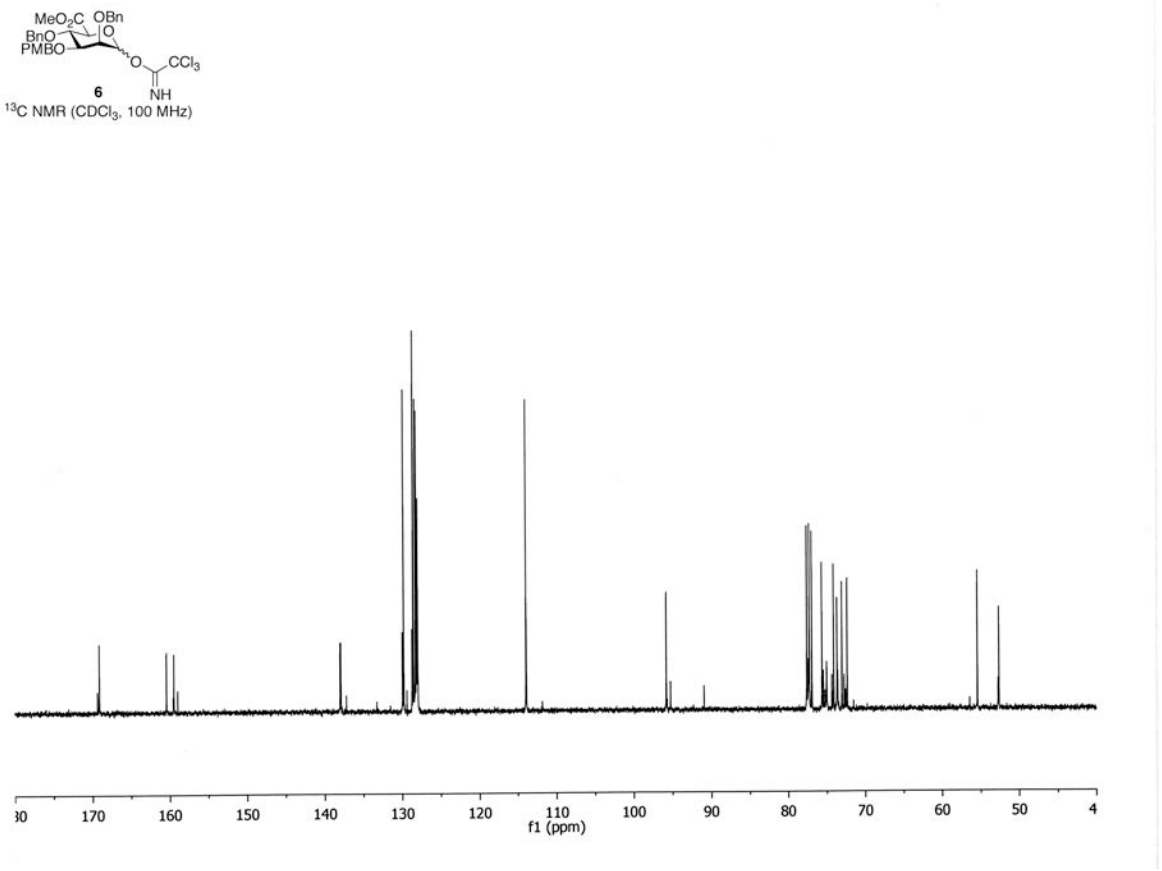
www.manaraa.com



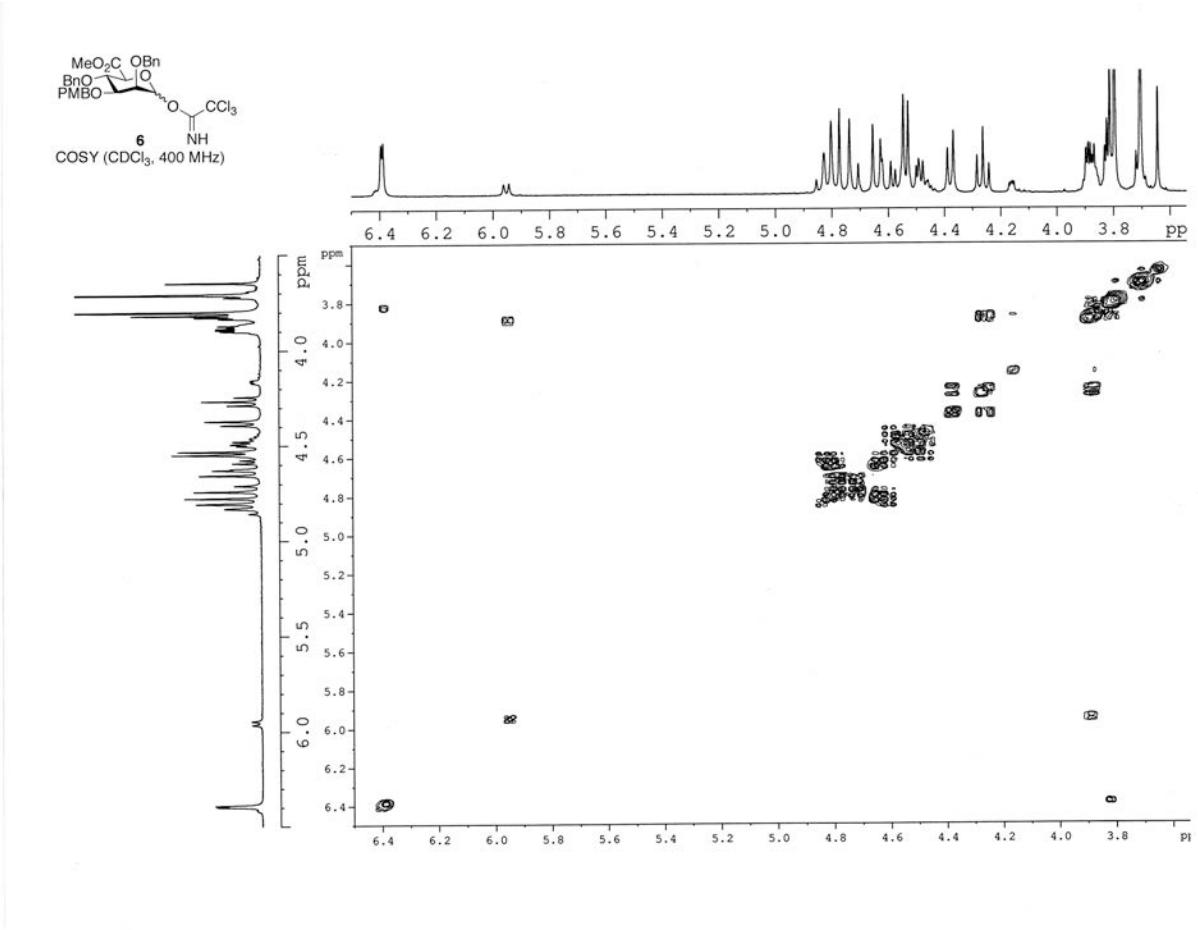
www.manaraa.com



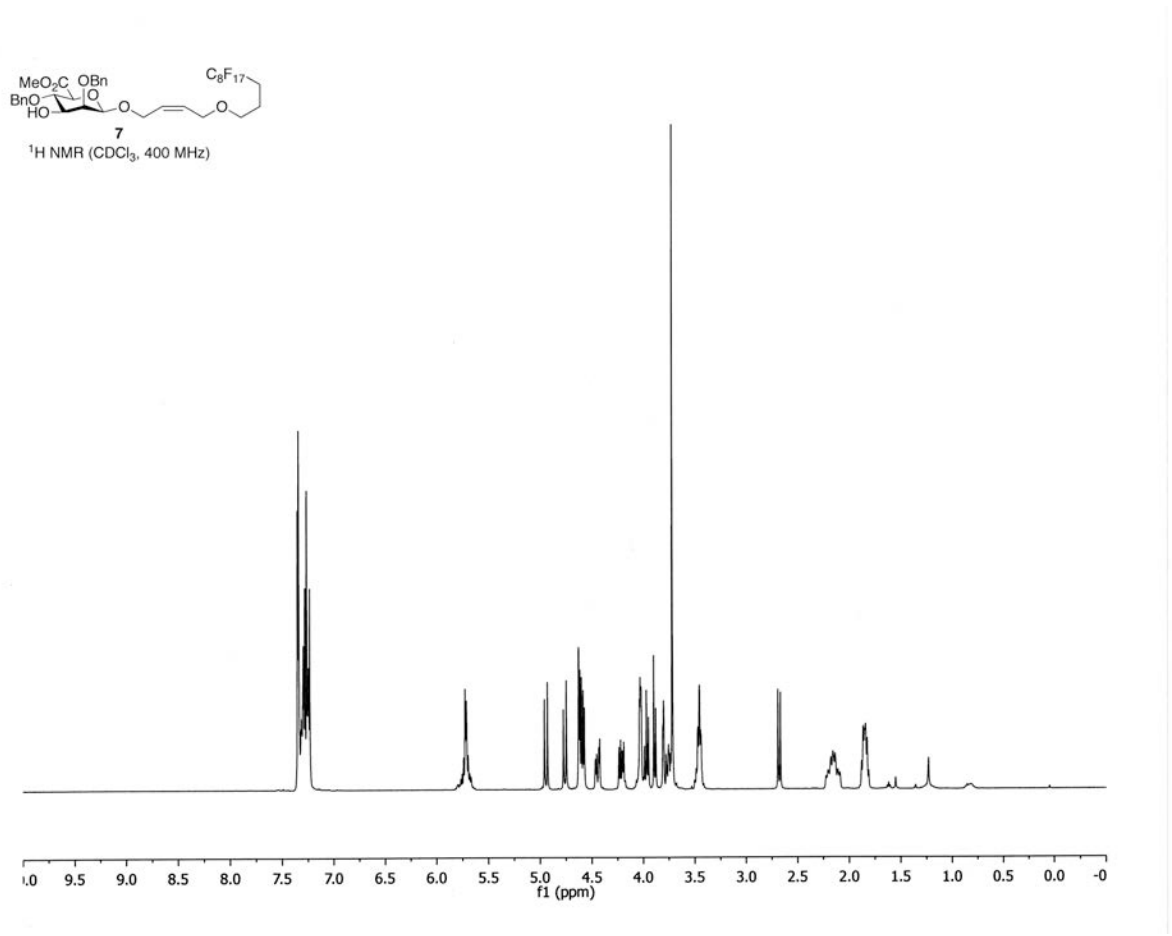
www.manaraa.com



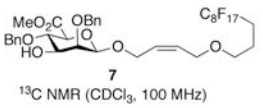
www.manaraa.com



www.manaraa.com

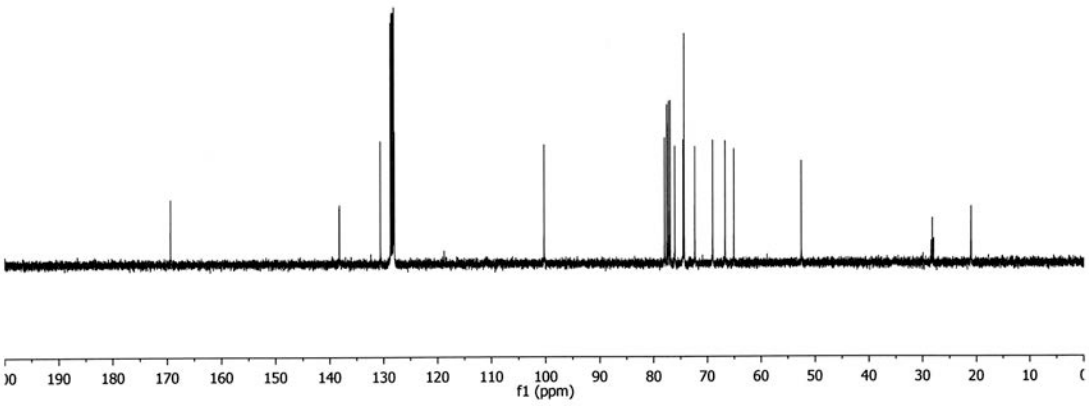


www.manaraa.com

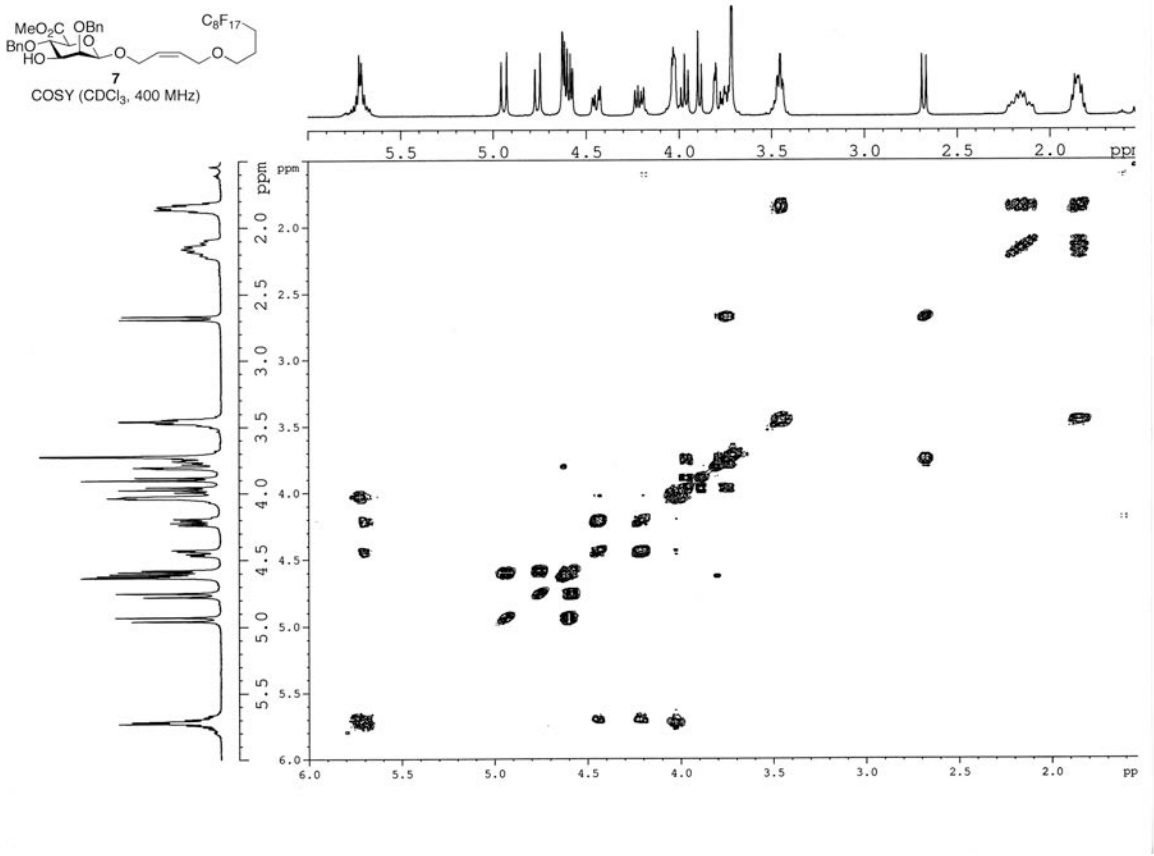


7

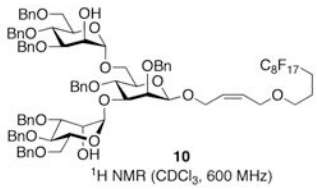
^{13}C NMR ($CDCl_3$, 100 MHz)



www.manaraa.com

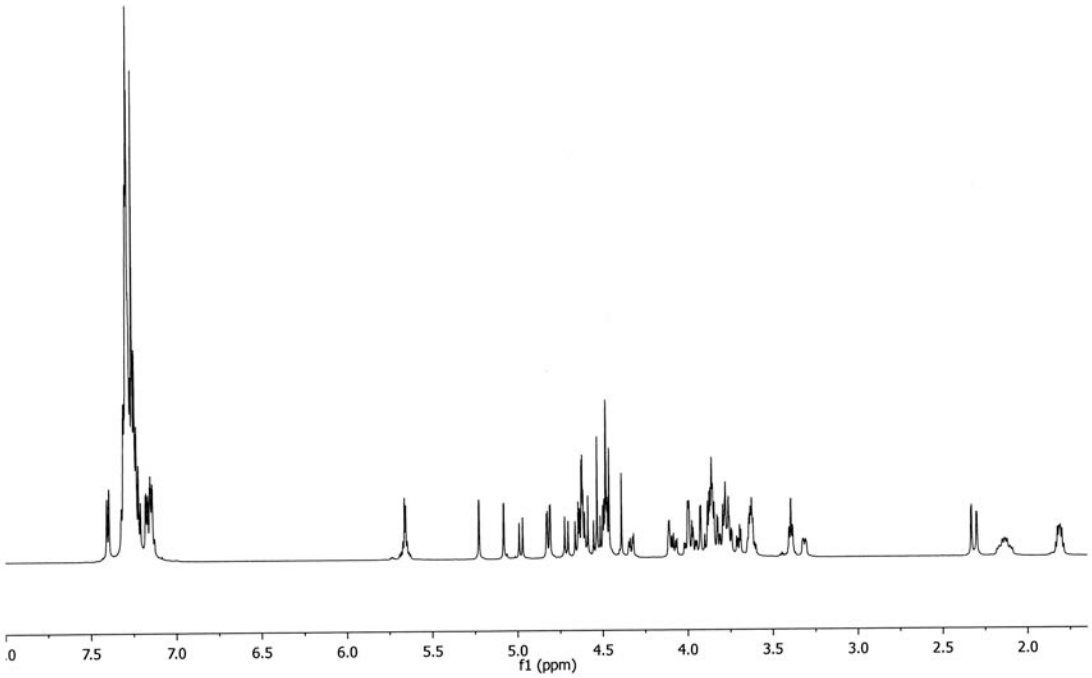


www.manaraa.com

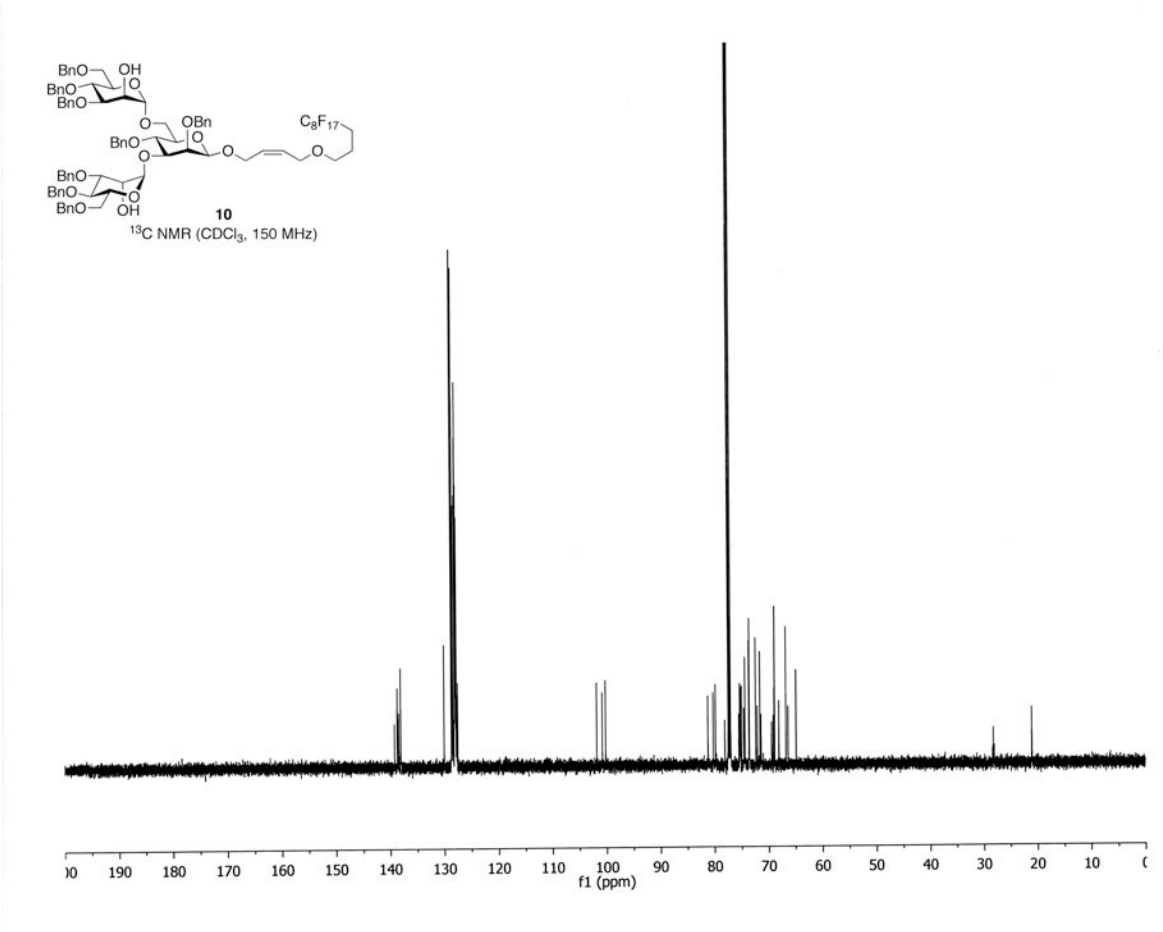


10

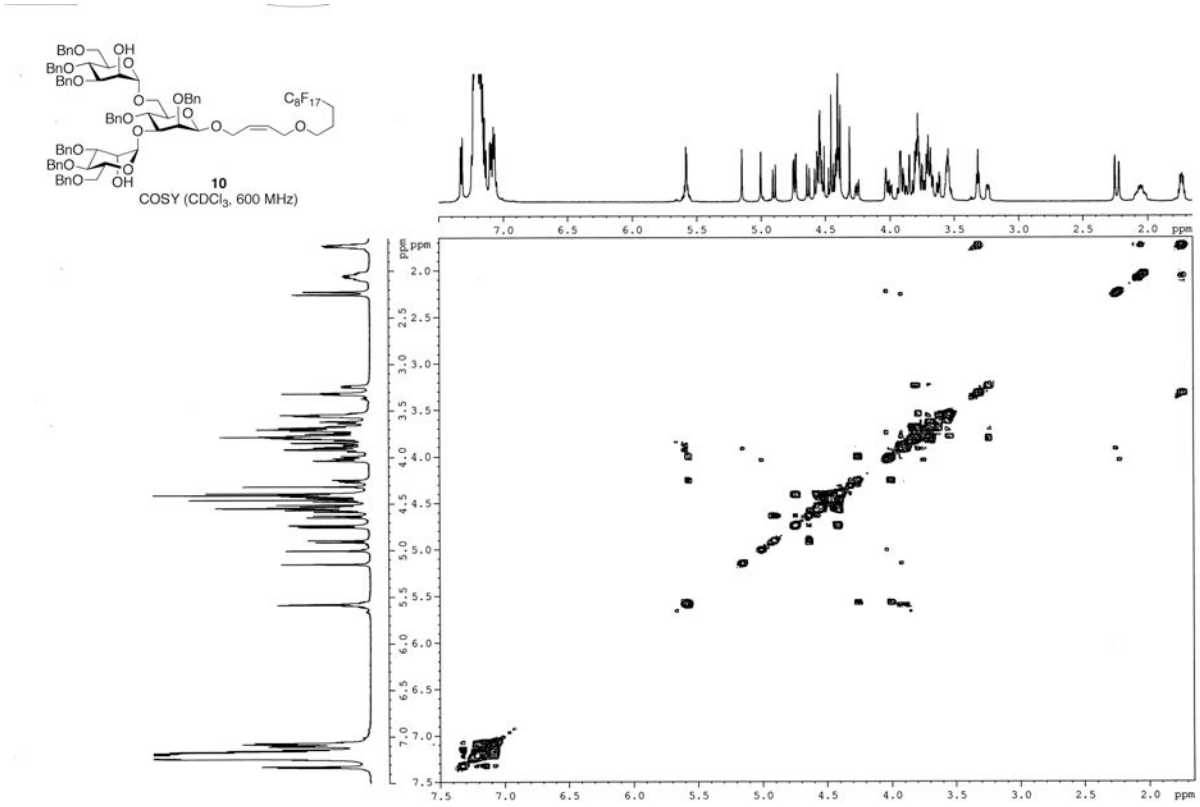
^{1}H NMR ($CDCl_3$, 600 MHz)



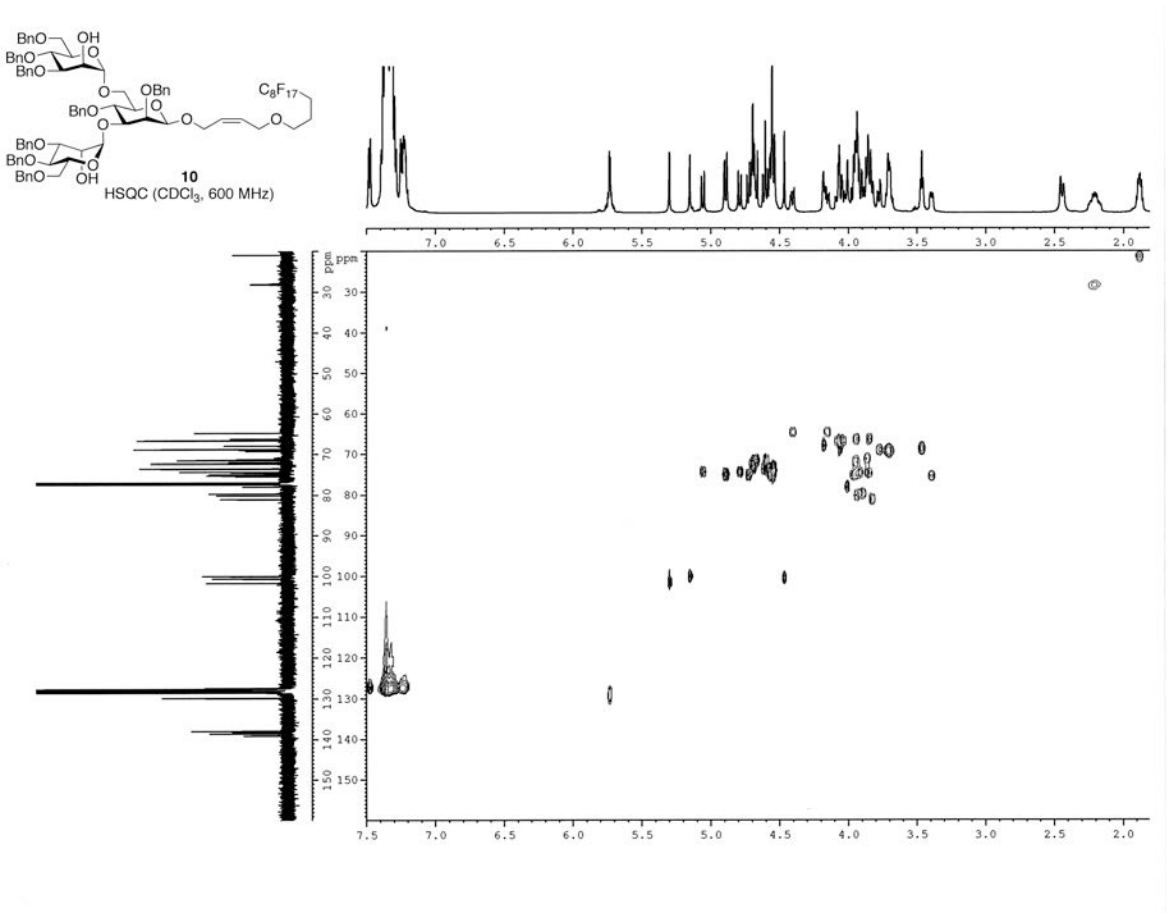
www.manaraa.com



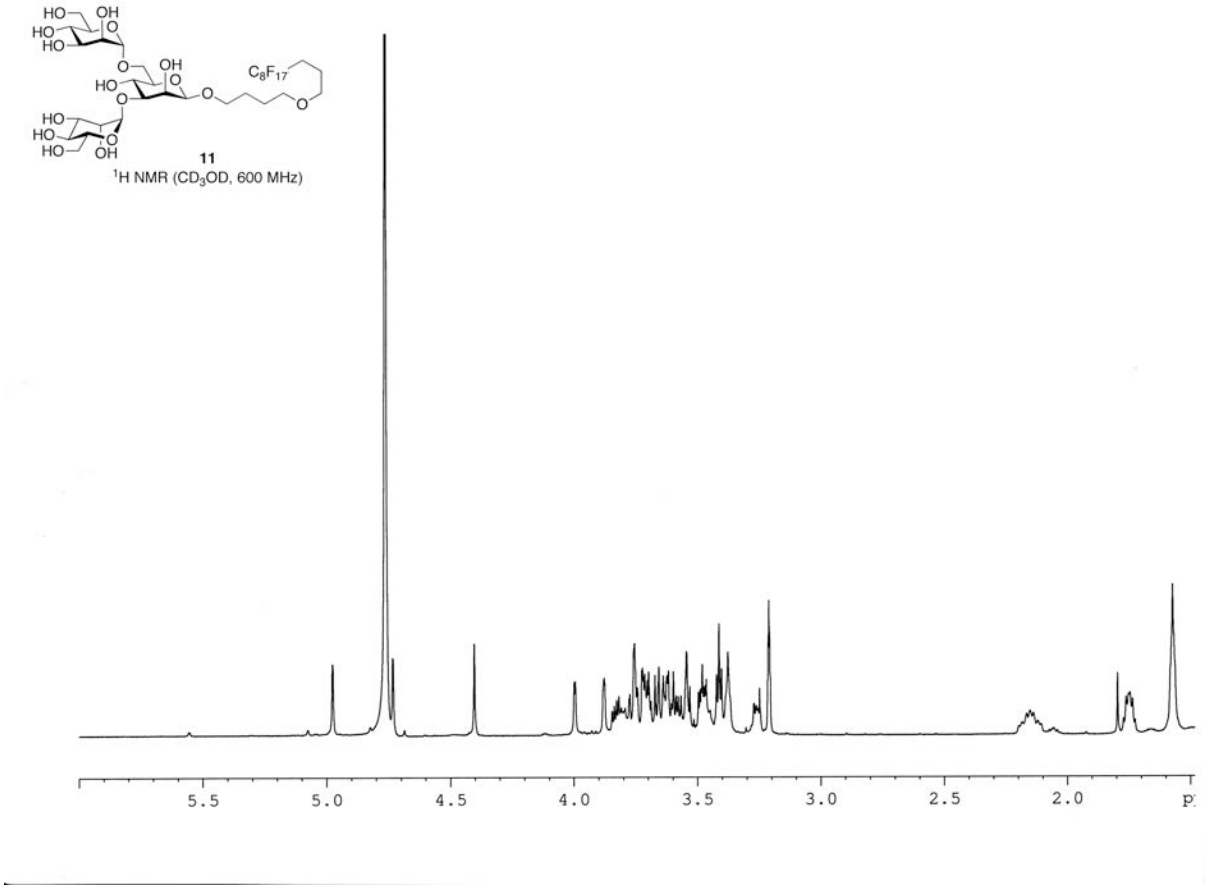
www.manaraa.com



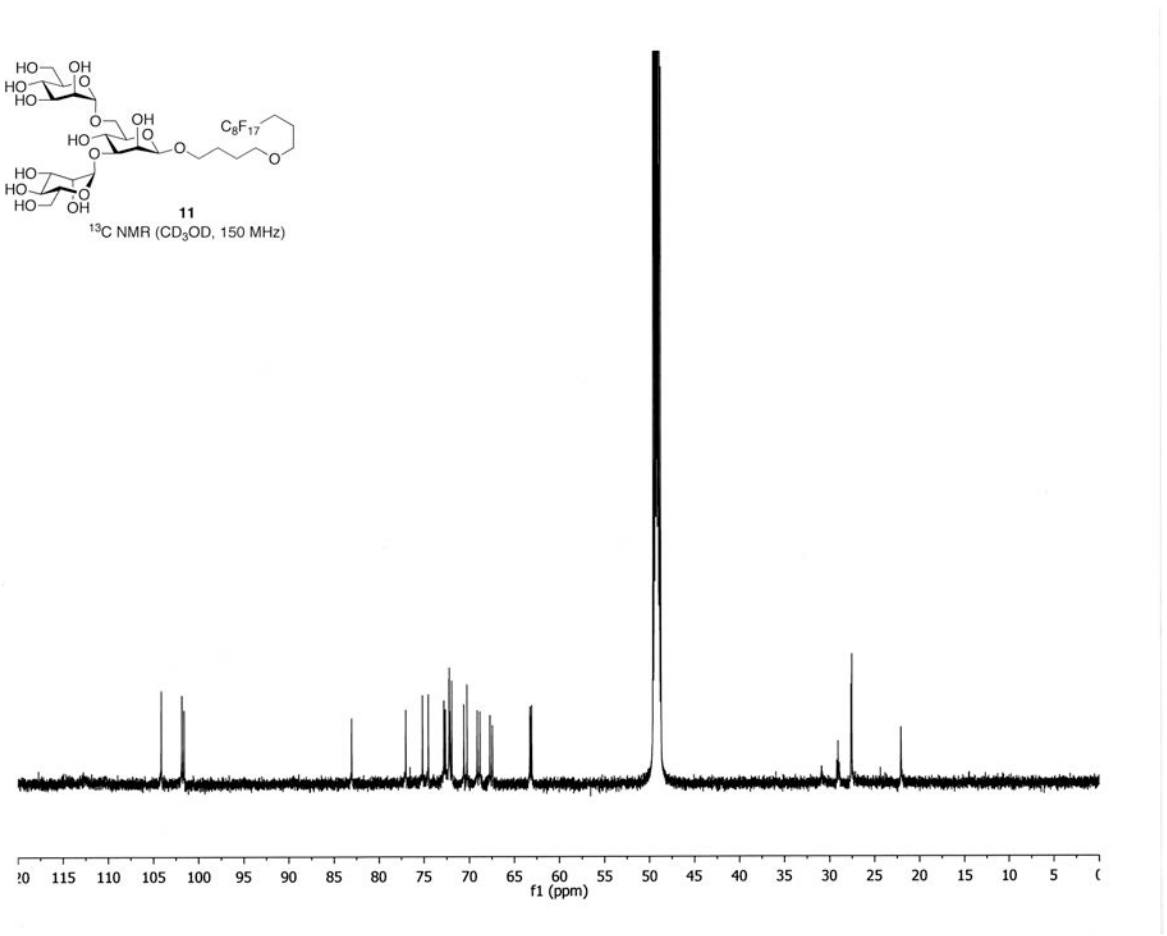
www.manaraa.com



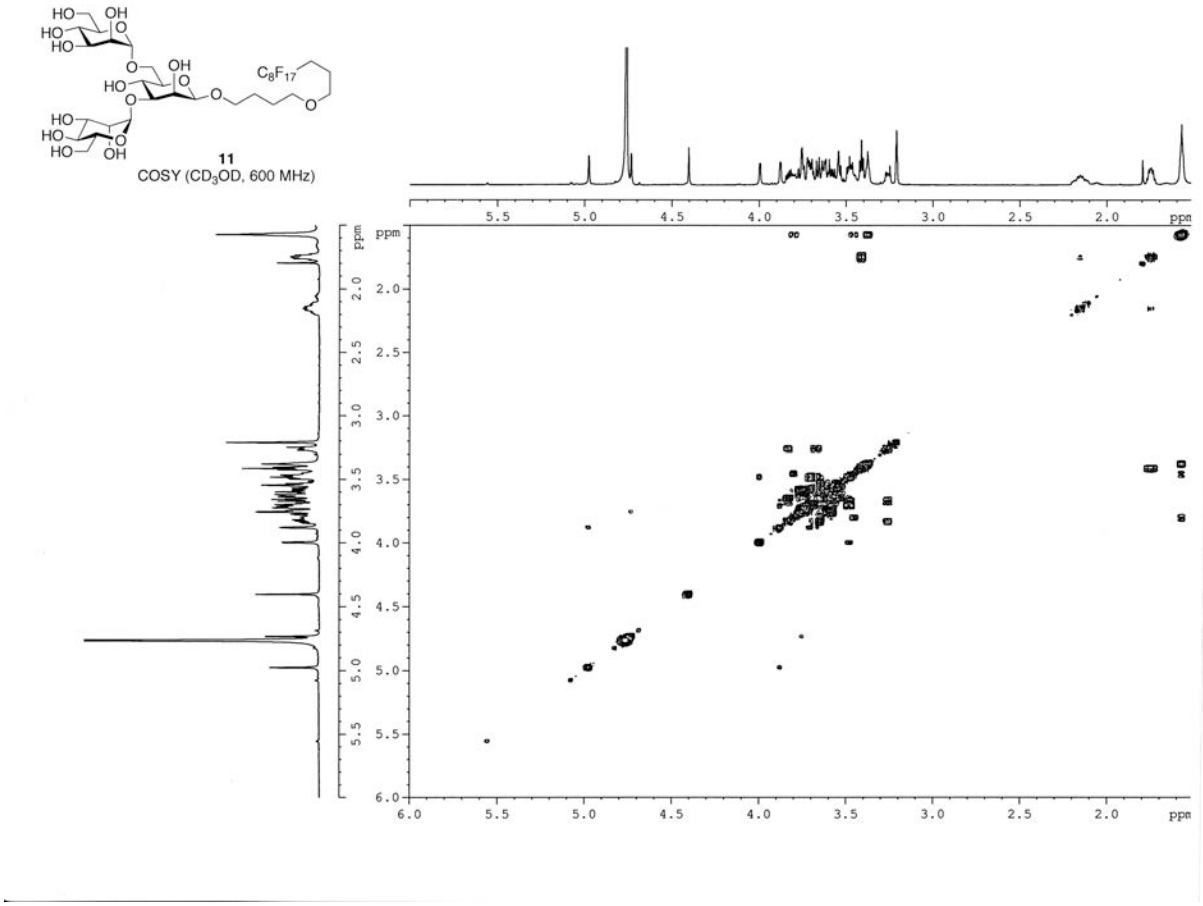
www.manaraa.com



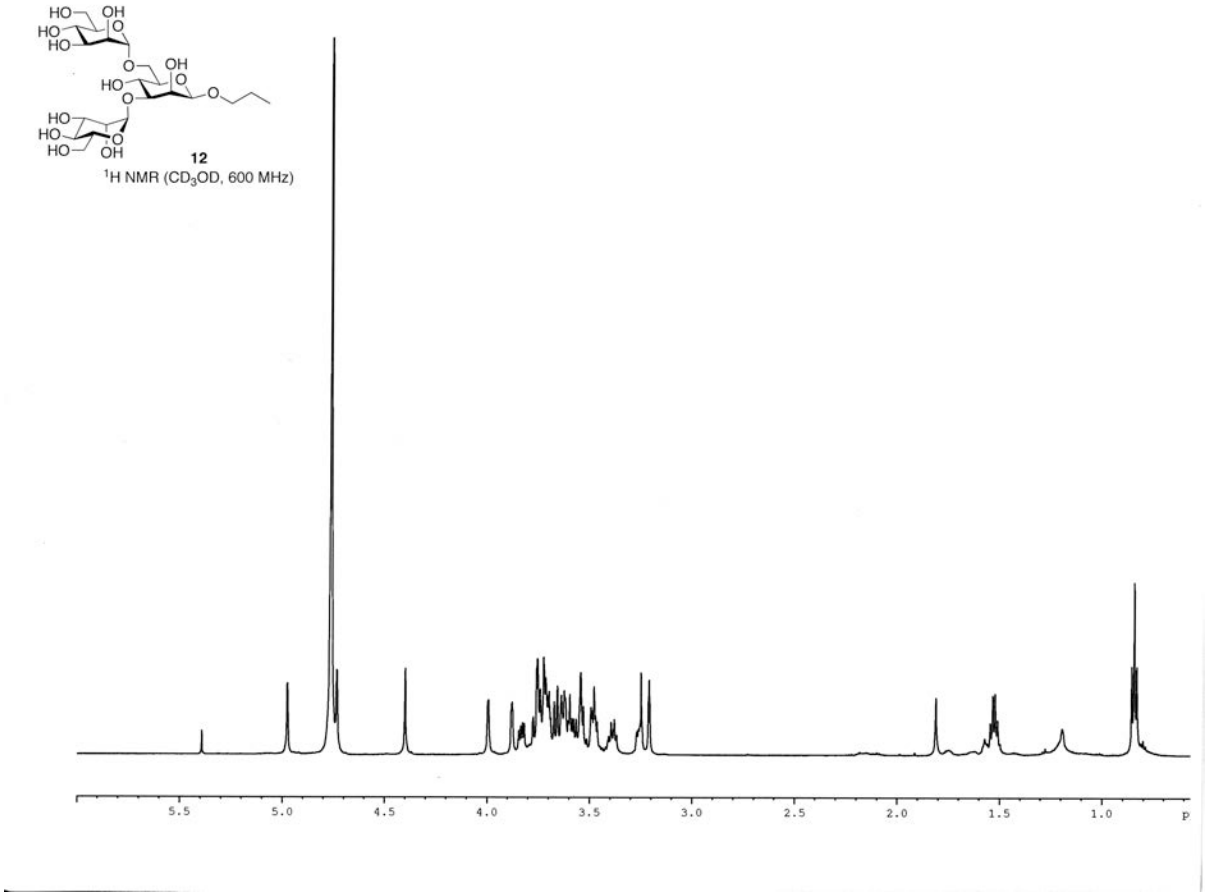
www.manaraa.com



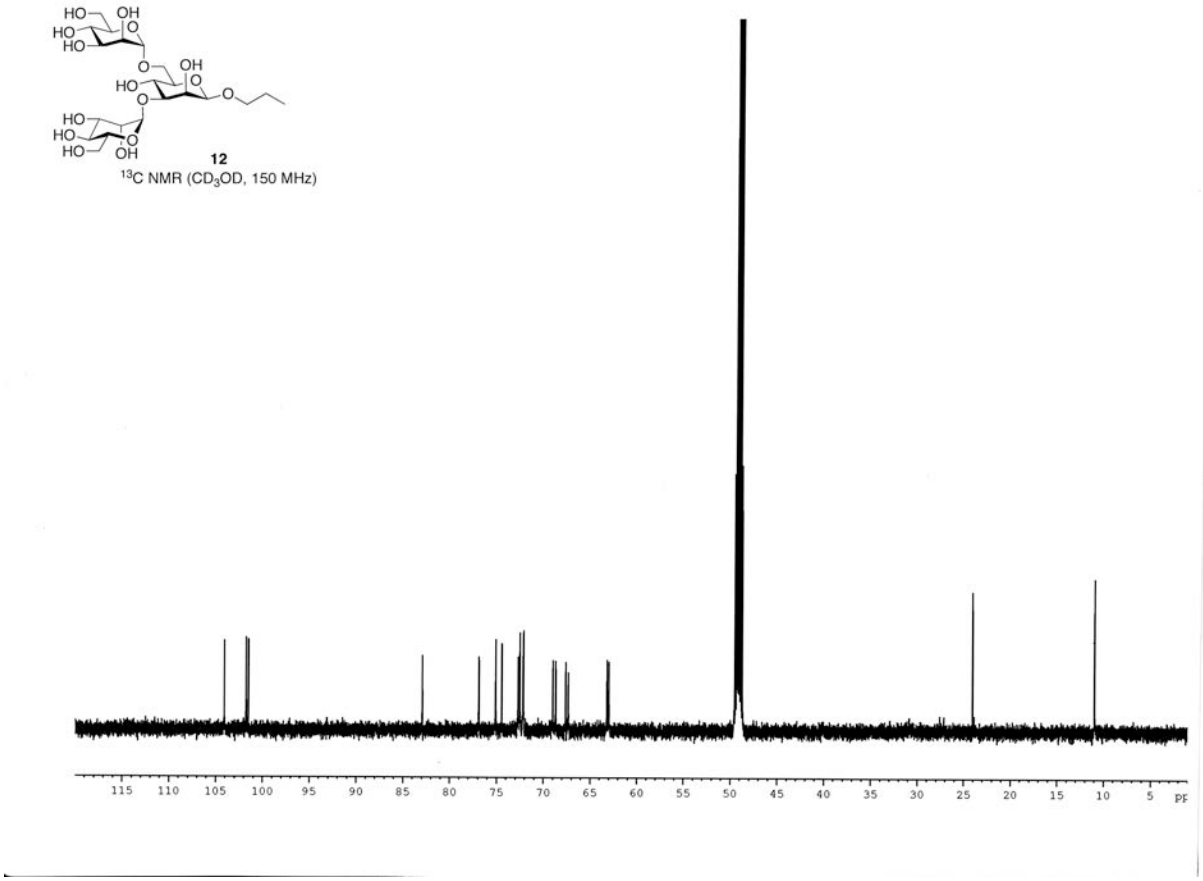
www.manaraa.com



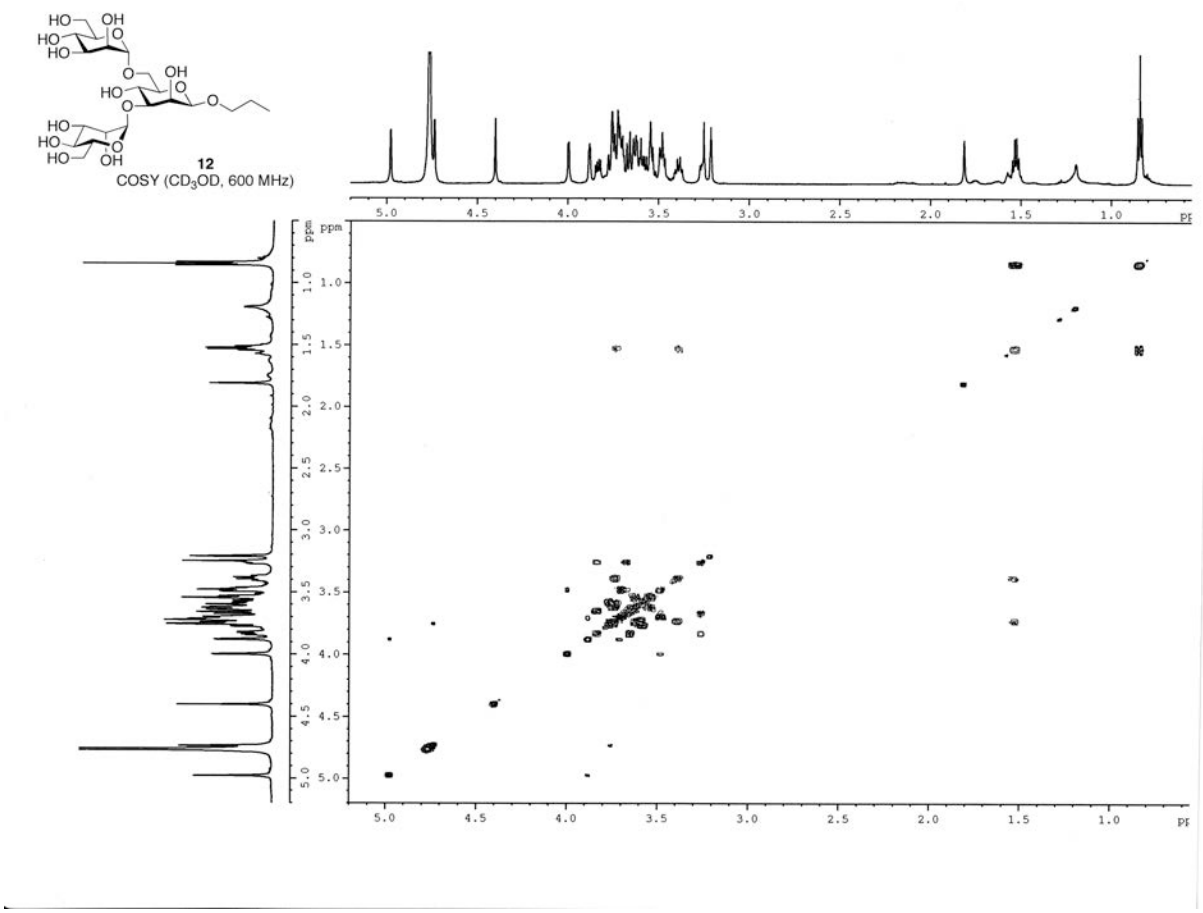
www.manaraa.com



www.manaraa.com

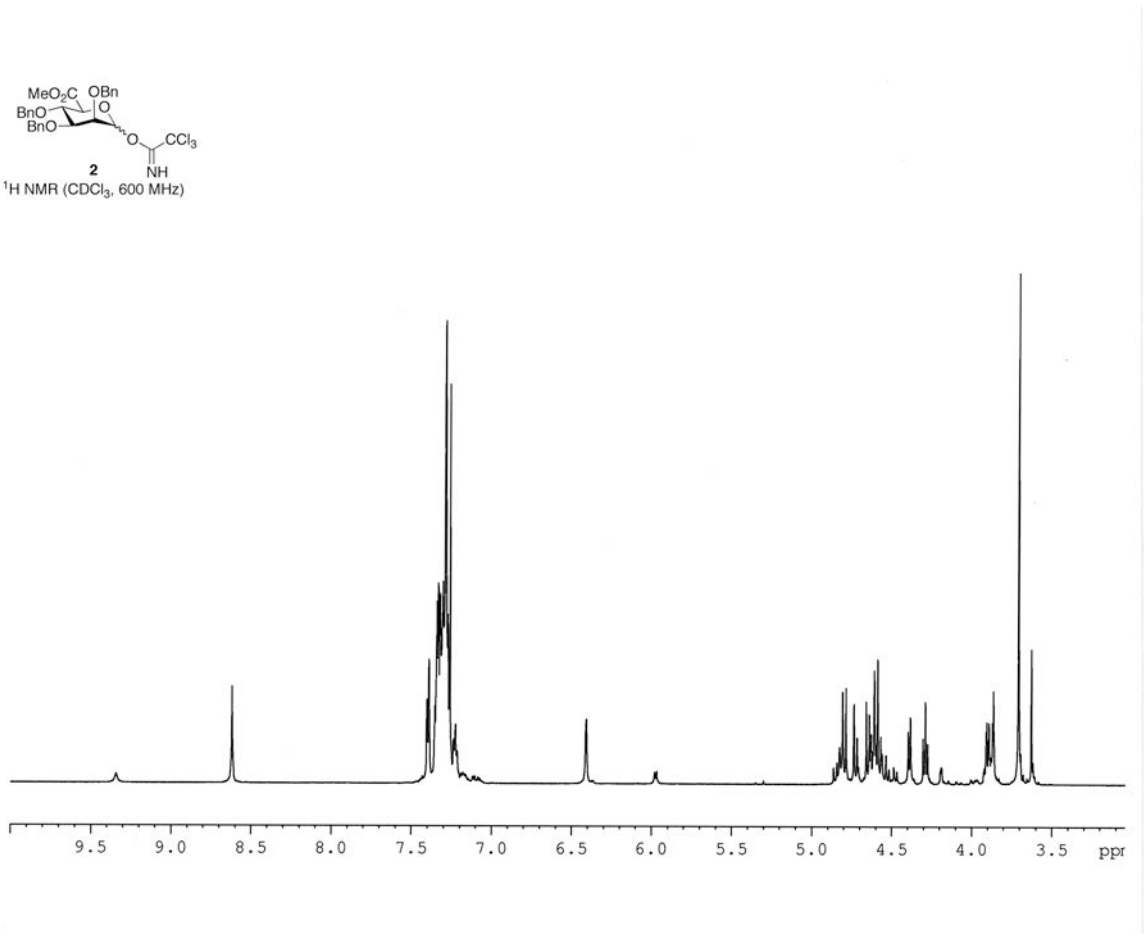


www.manaraa.com

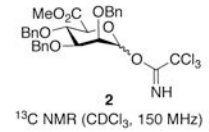


www.manaraa.com

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

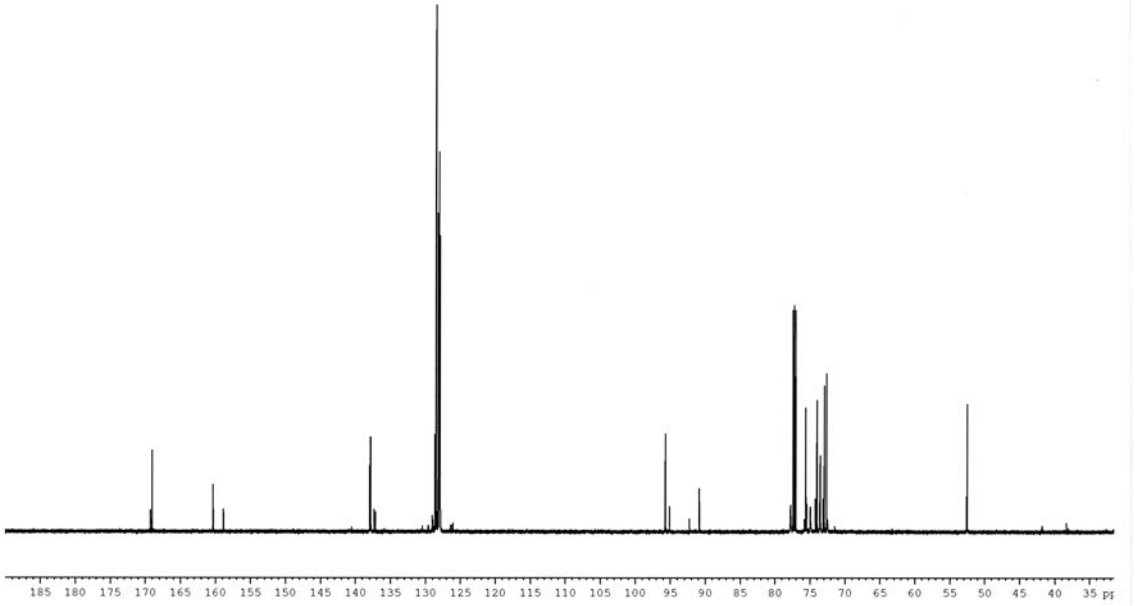


www.manaraa.com

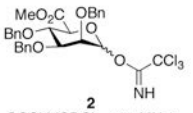


2

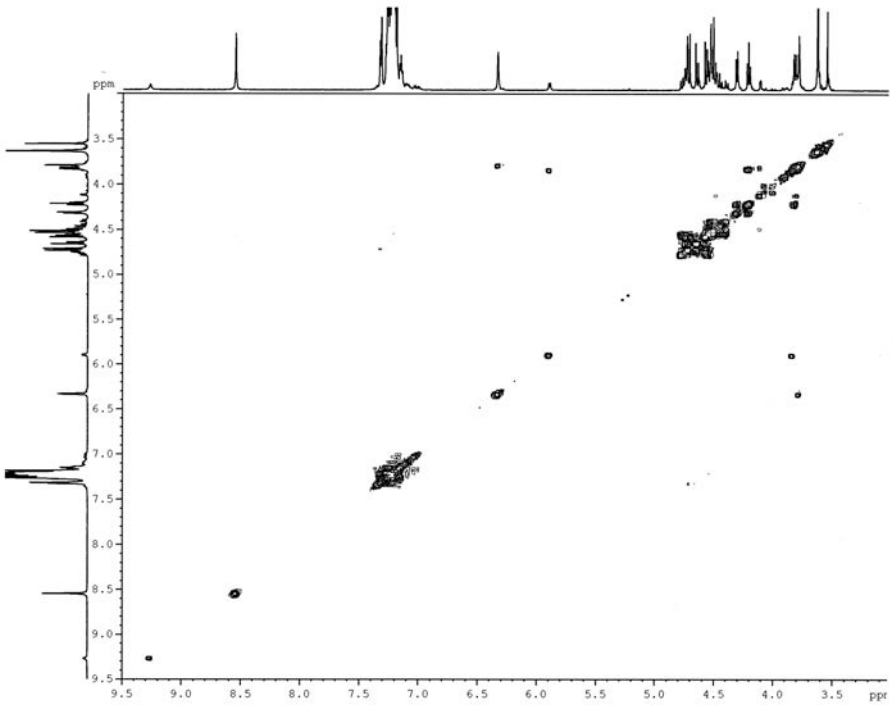
^{13}C NMR ($CDCl_3$, 150 MHz)



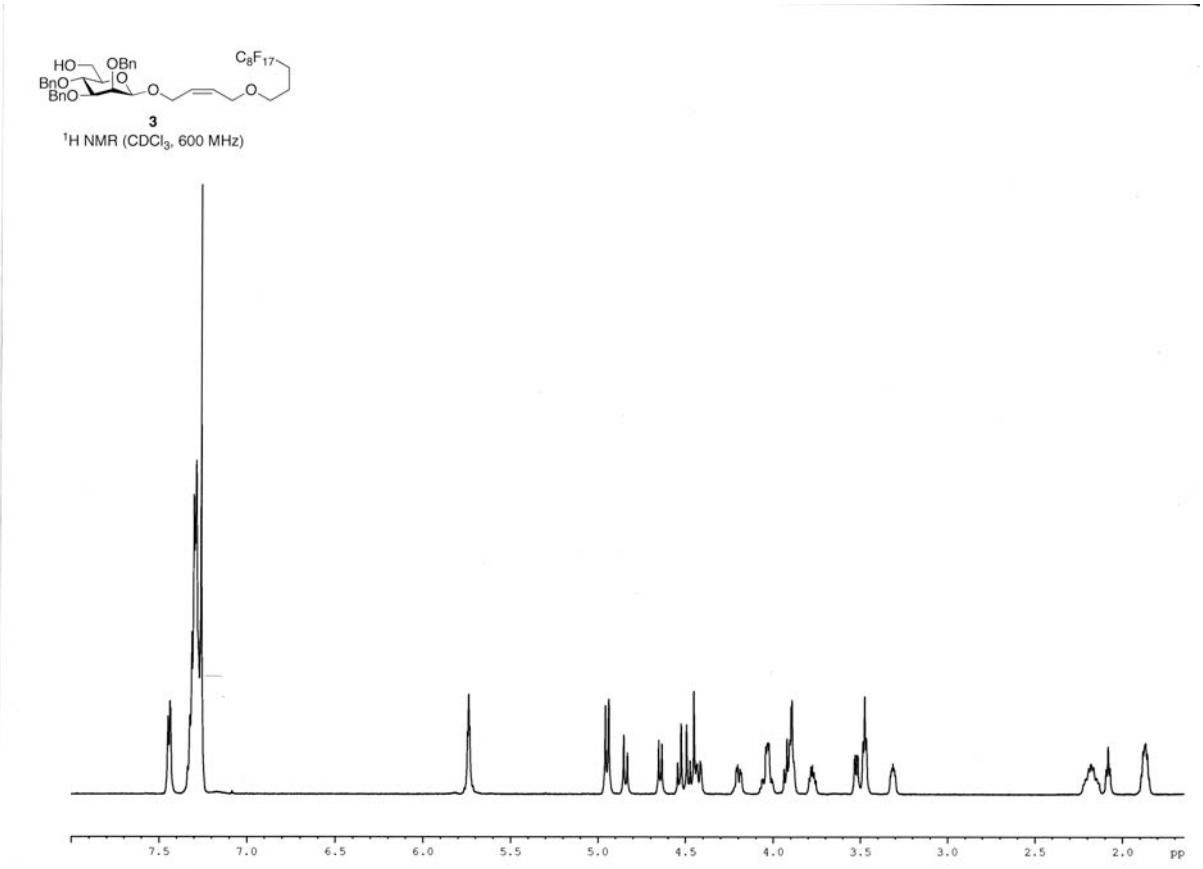
www.manaraa.com



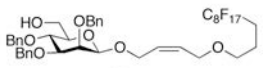
2
COSY ($CDCl_3$, 600 MHz)



www.manaraa.com

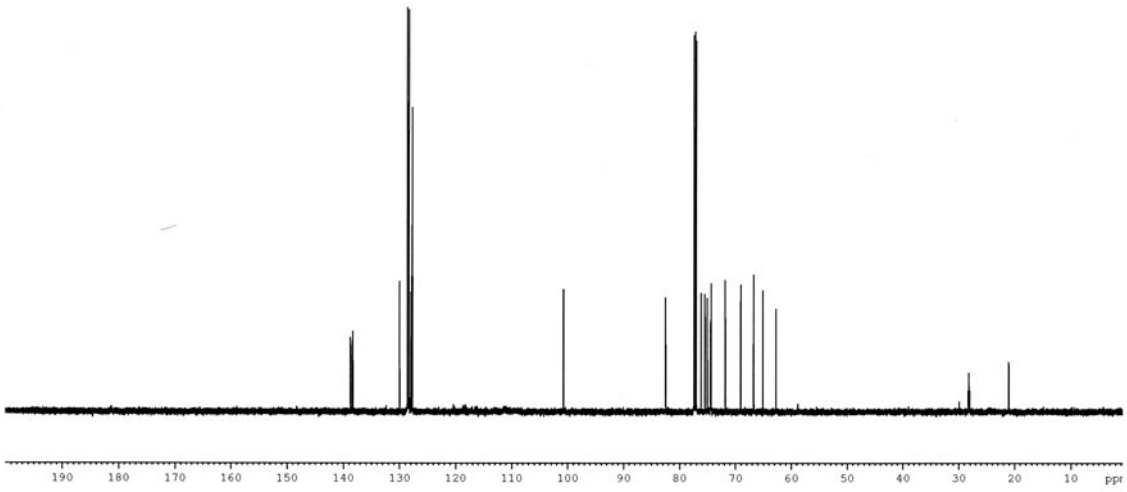


www.manaraa.com

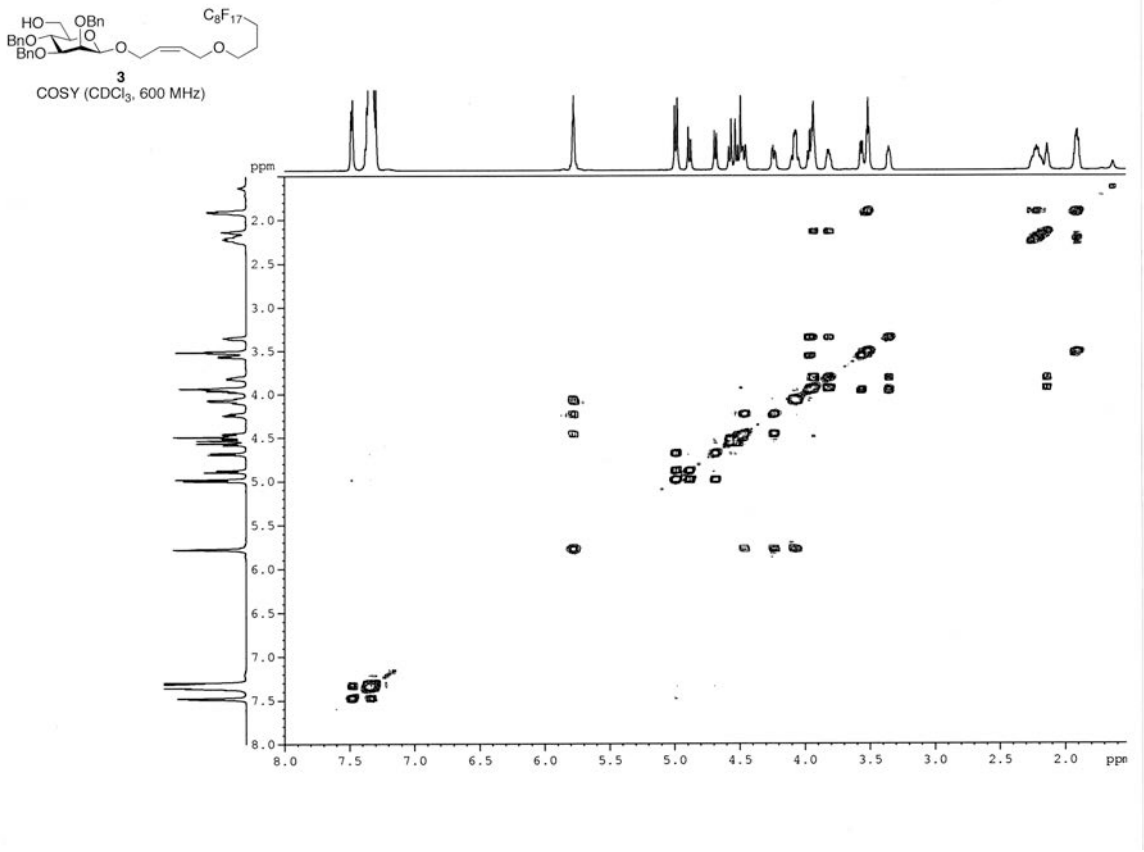


3

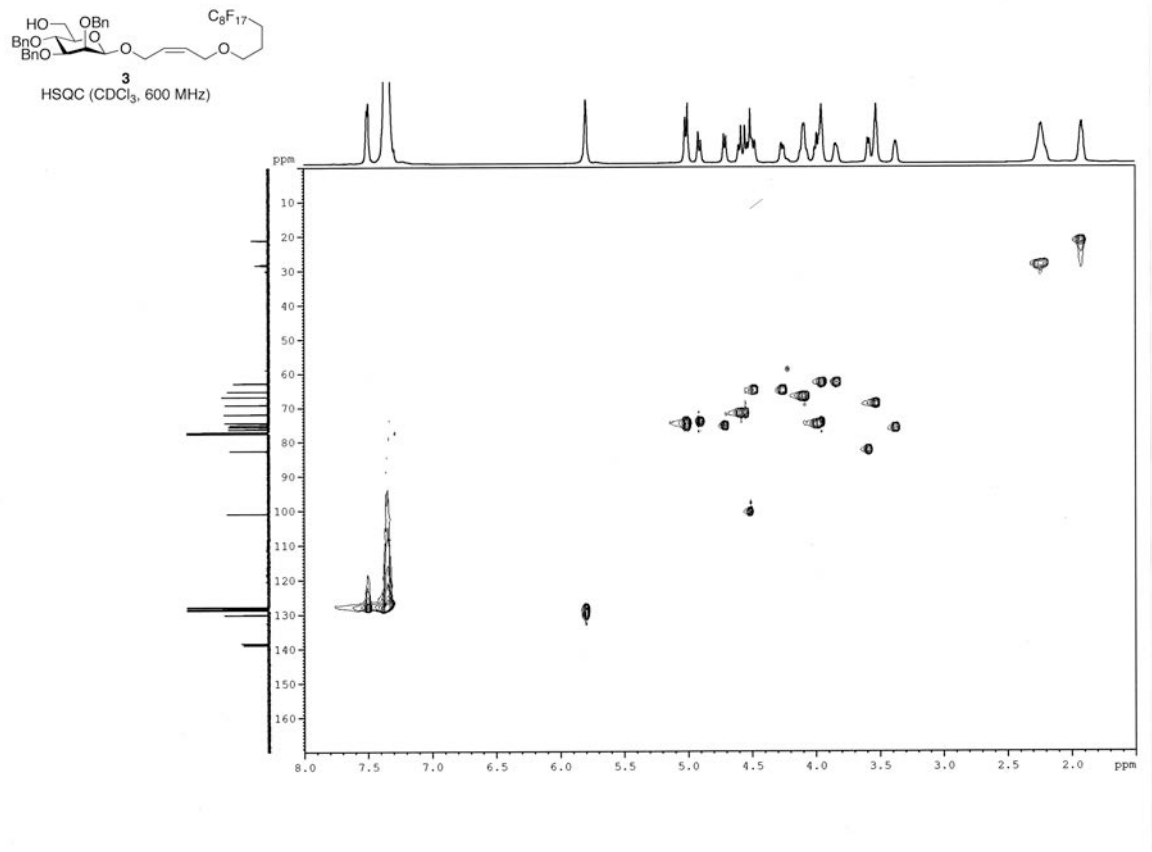
^{13}C NMR ($CDCl_3$, 150 MHz)



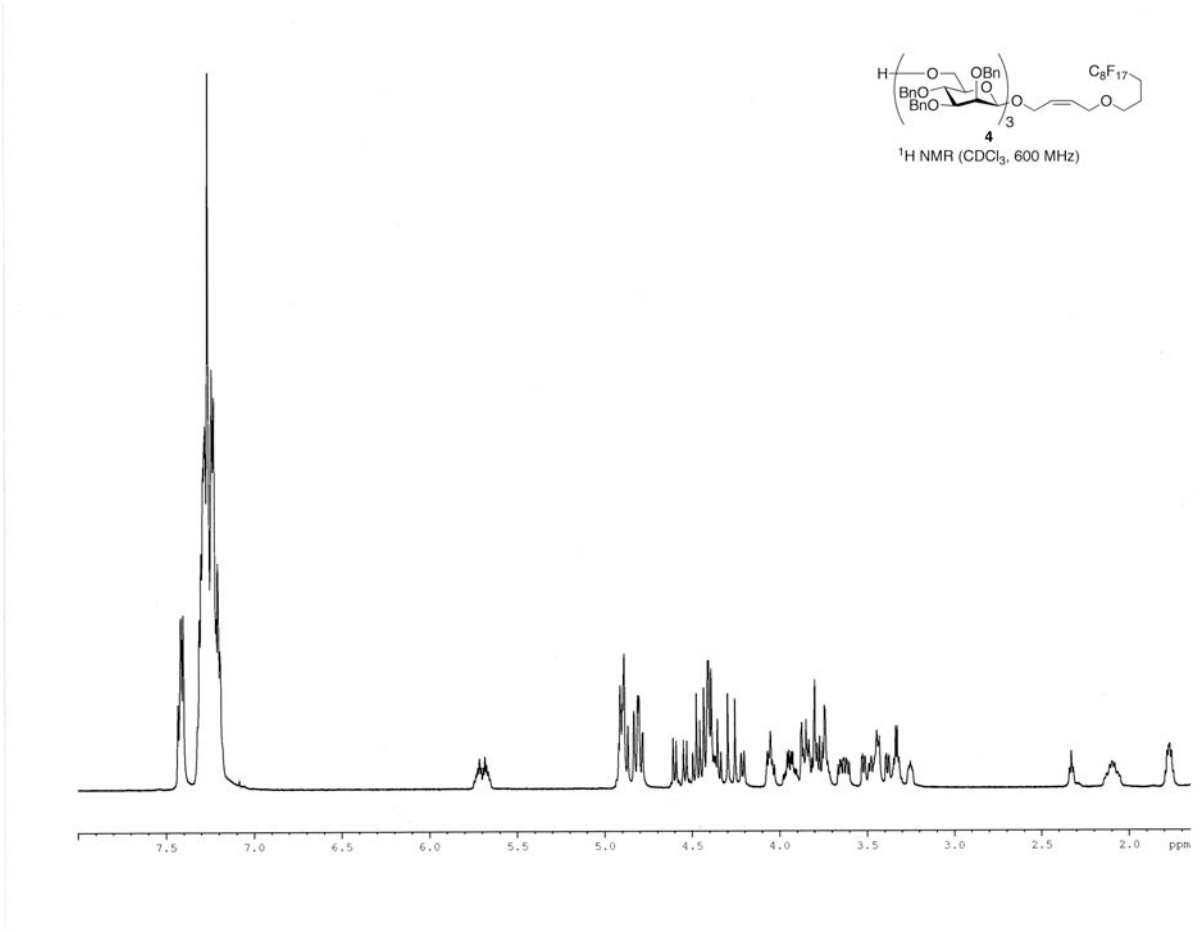
www.manaraa.com



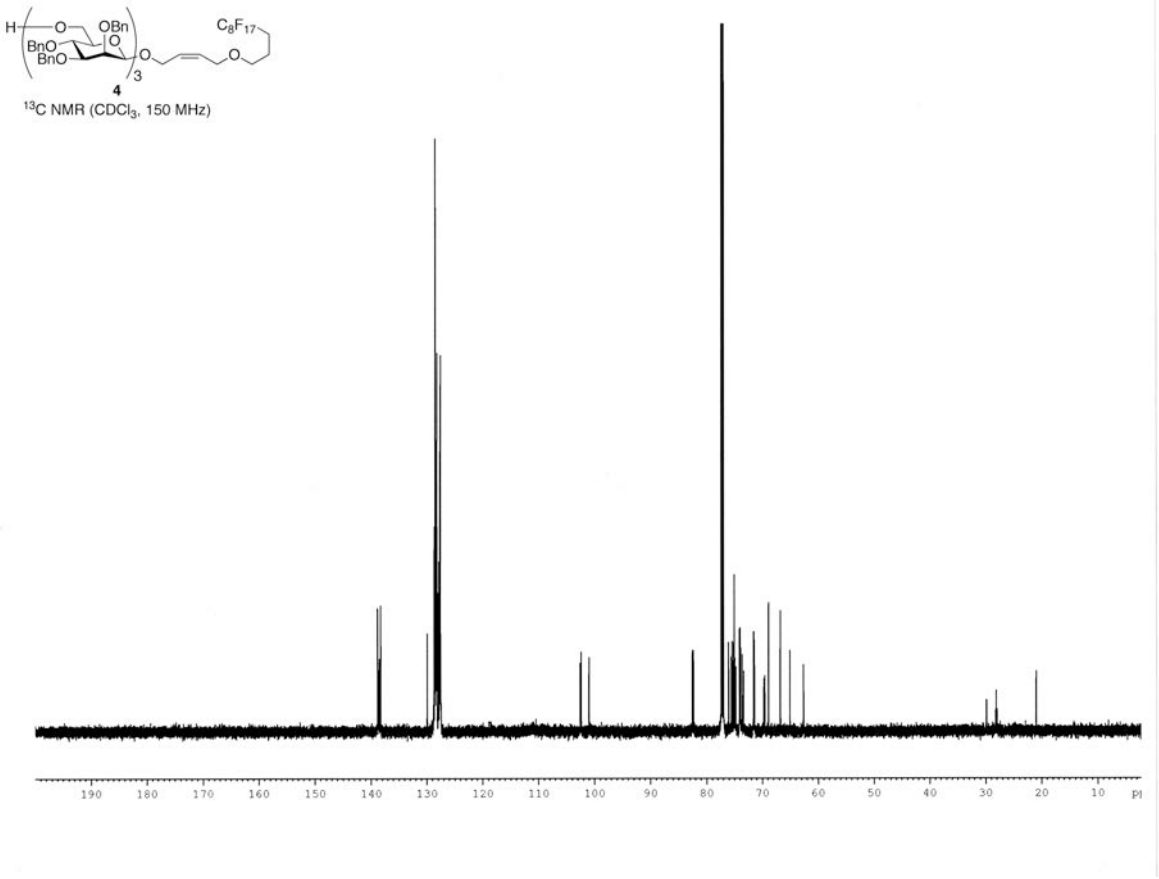
www.manaraa.com



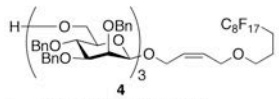
www.manaraa.com



www.manaraa.com

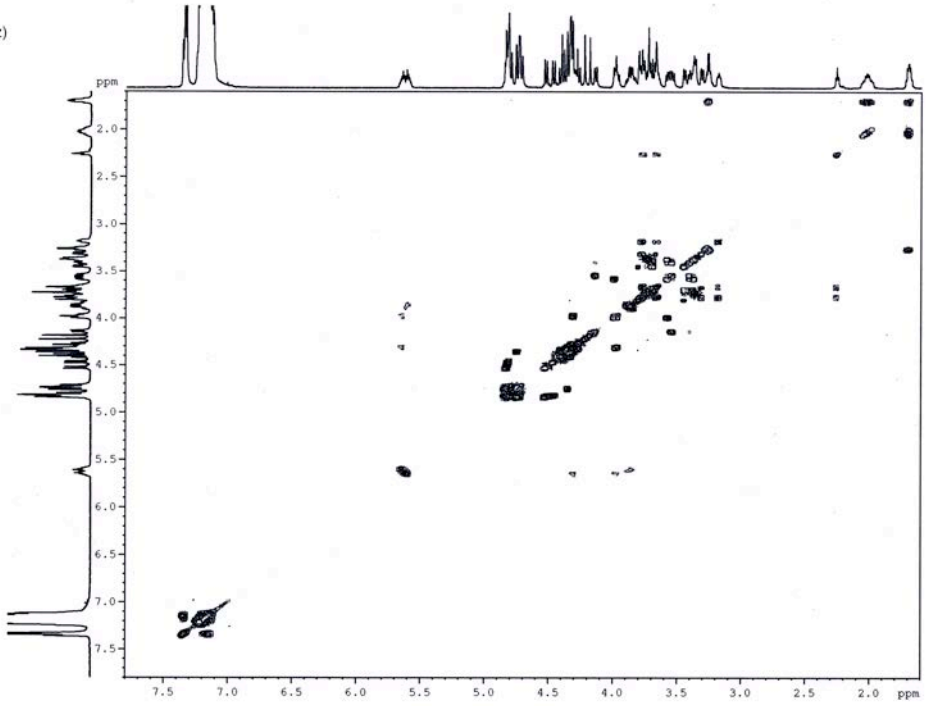


www.manaraa.com

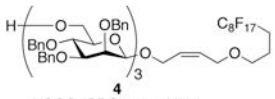


4

COSY ($CDCl_3$, 600 MHz)

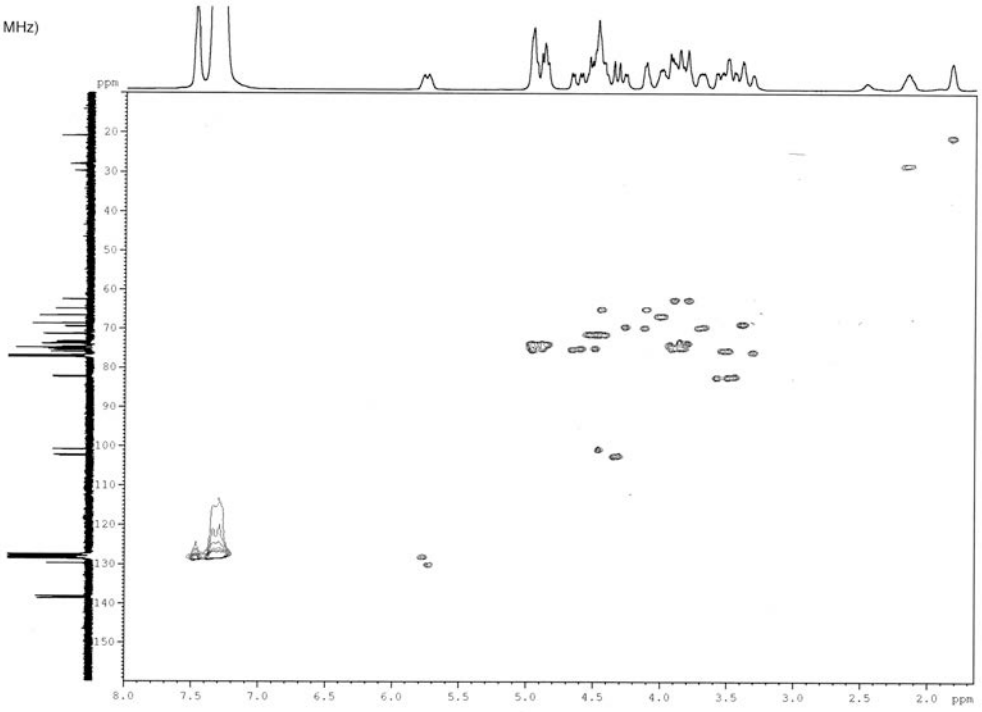


www.manaraa.com

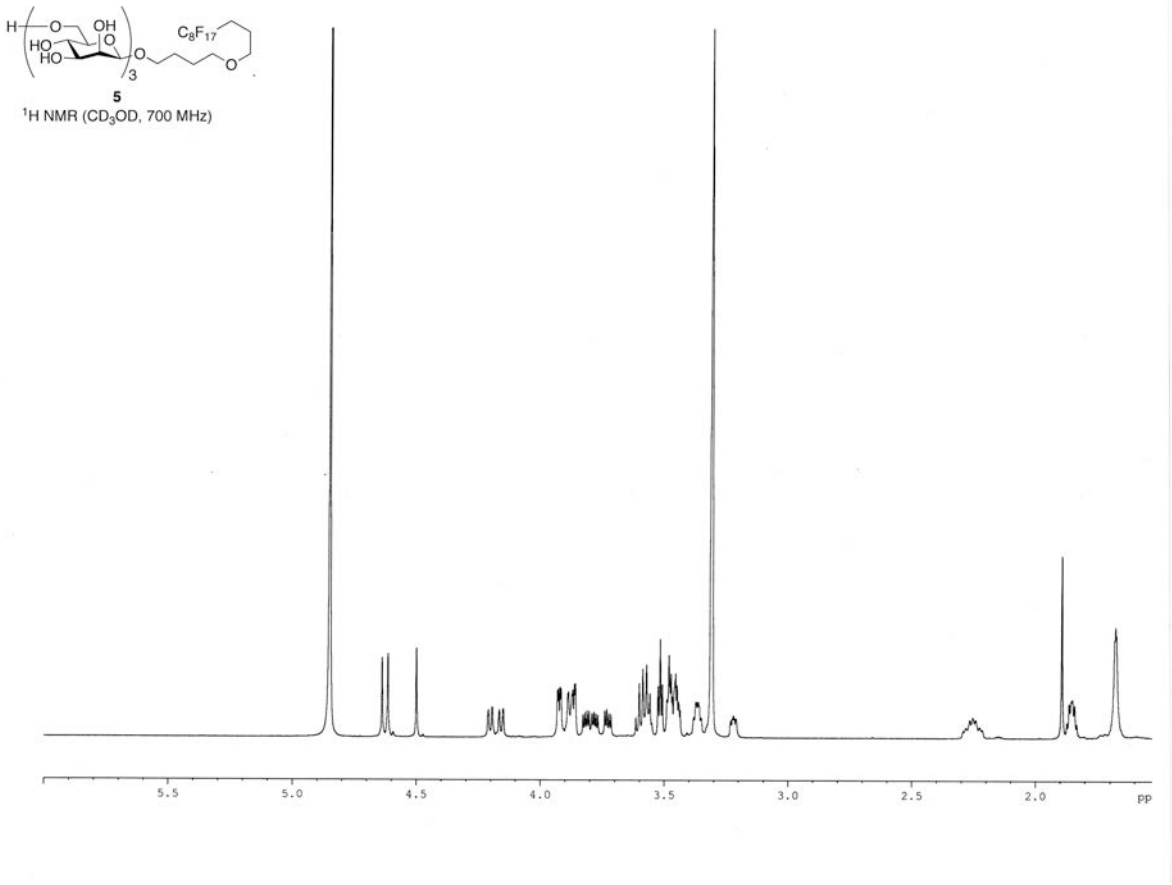


4

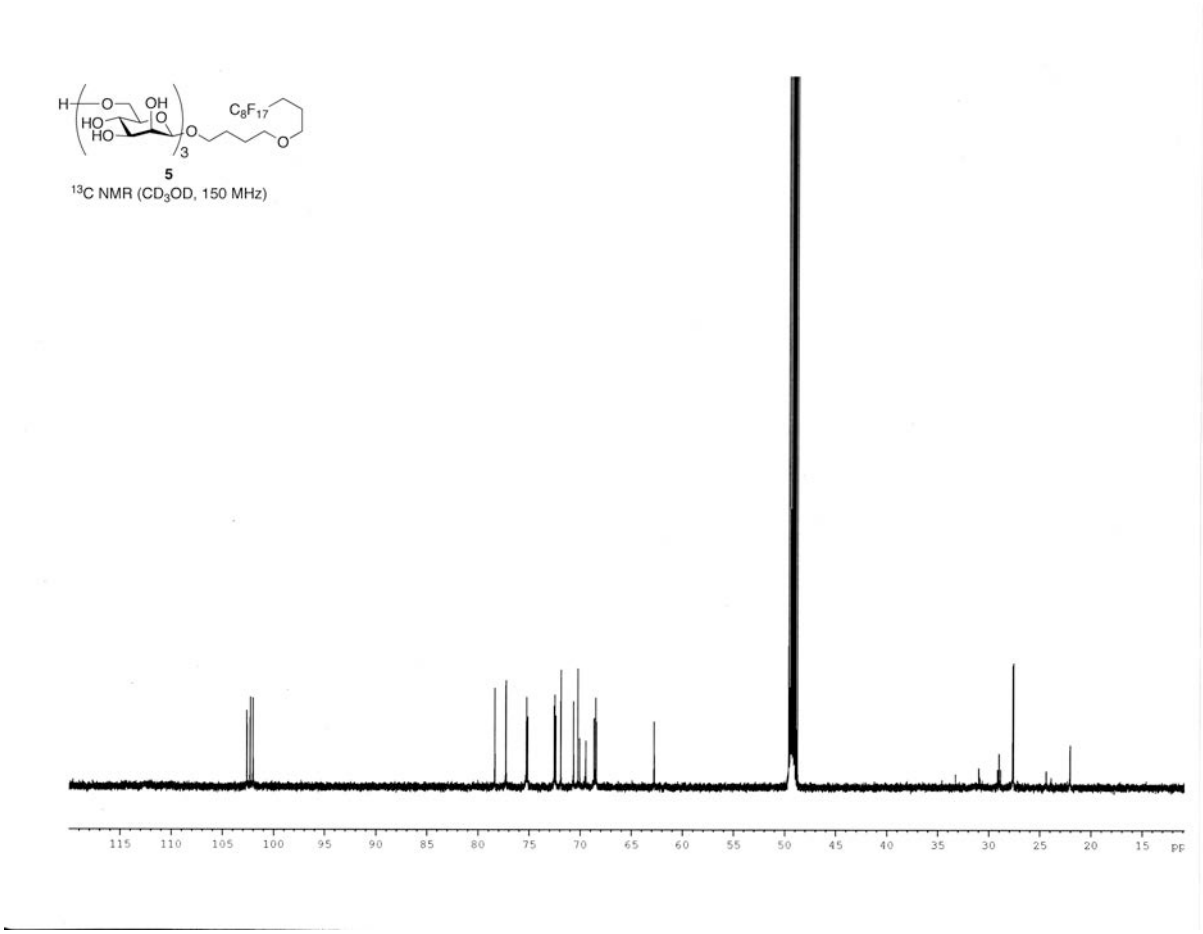
HSQC ($CDCl_3$, 600 MHz)



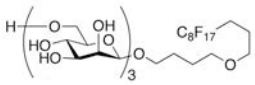
www.manaraa.com



www.manaraa.com

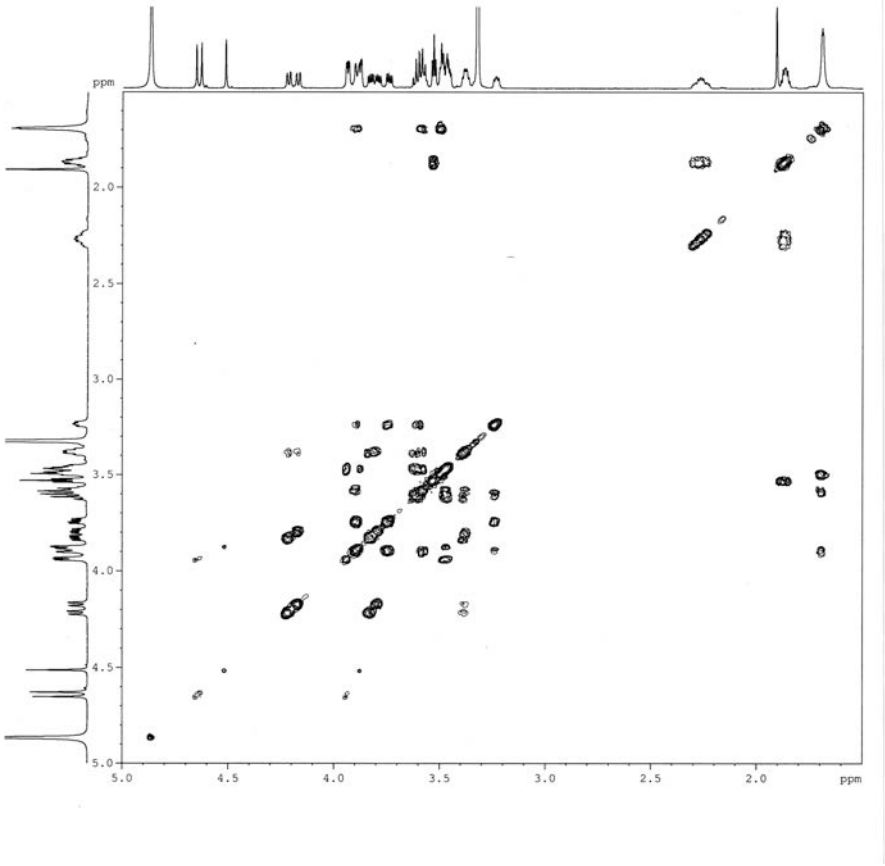


www.manaraa.com

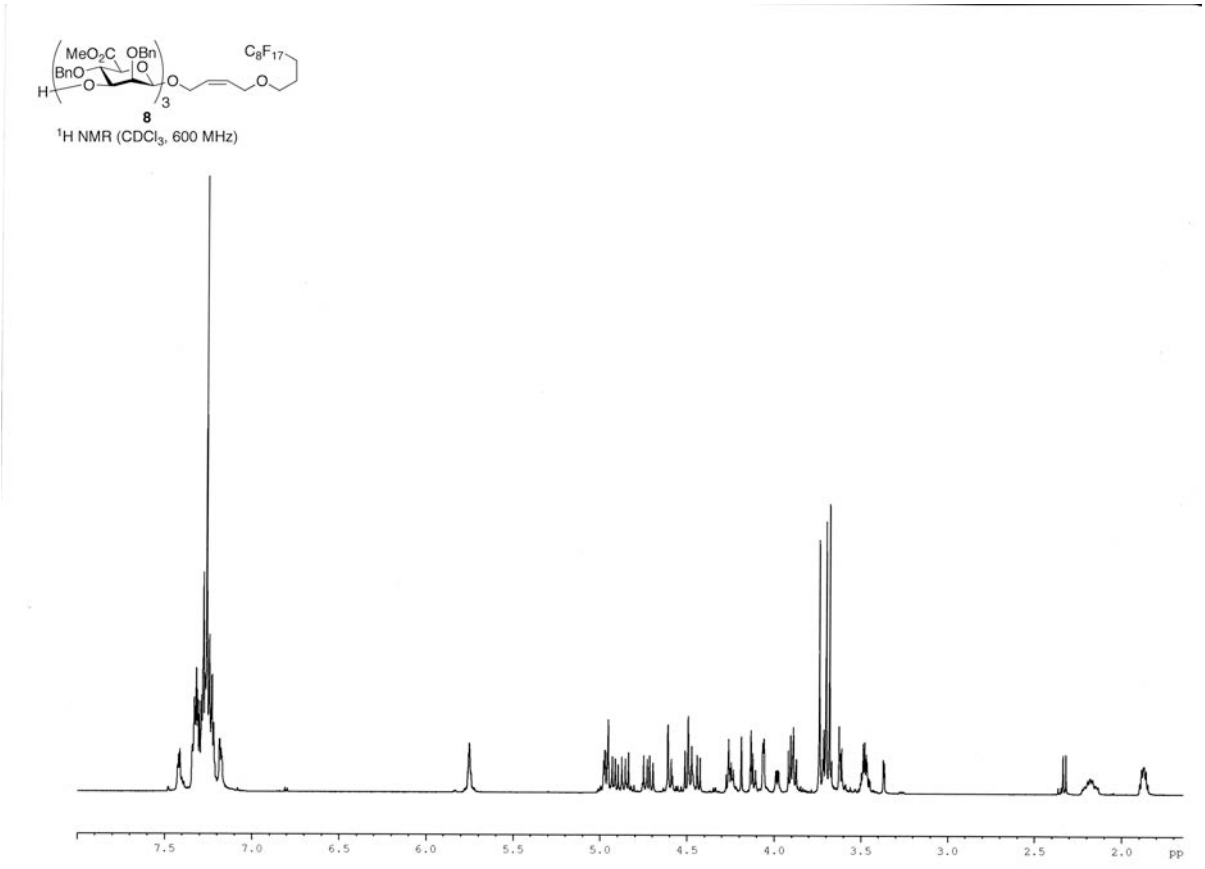


5

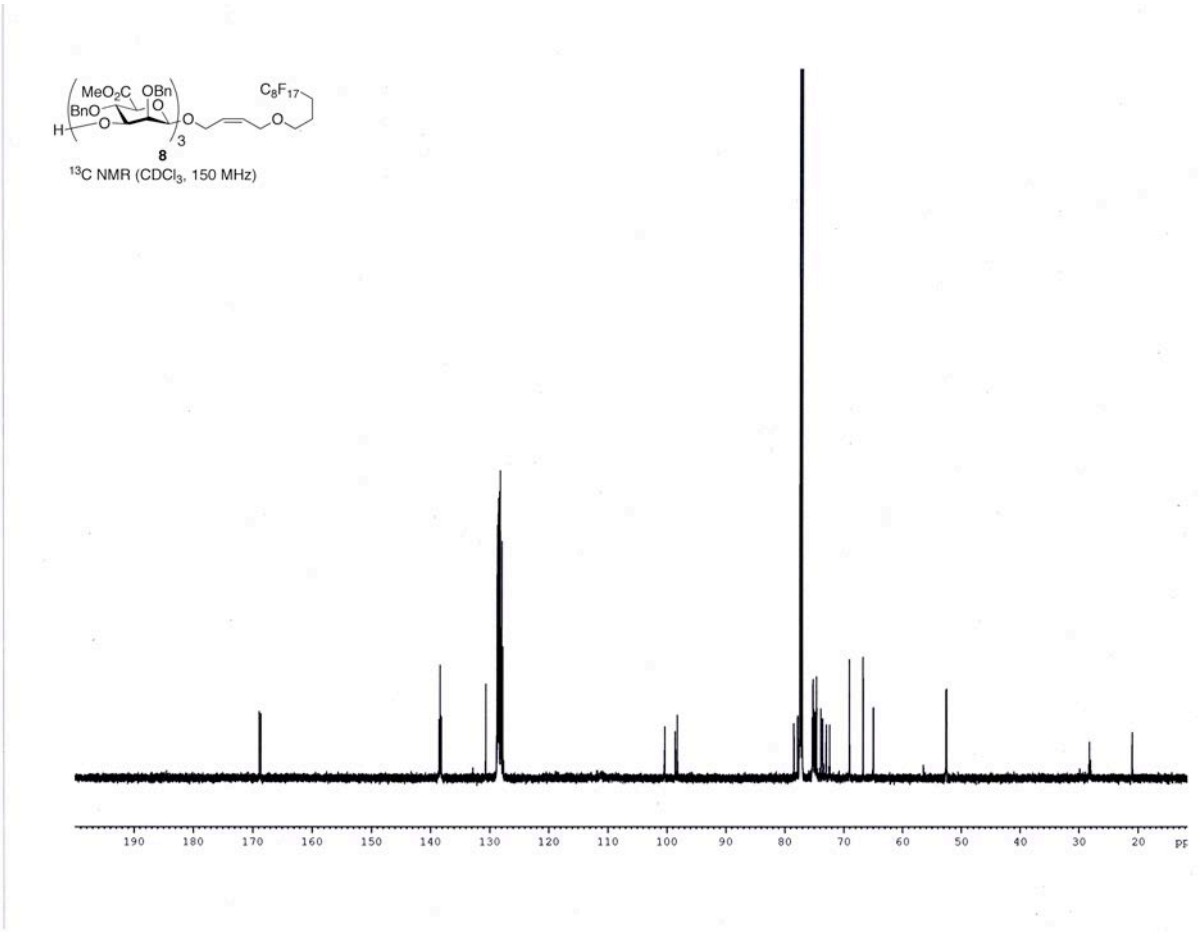
COSY (CD_3OD, 700 MHz)



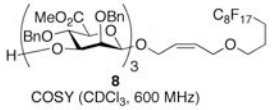
www.manaraa.com



www.manaraa.com

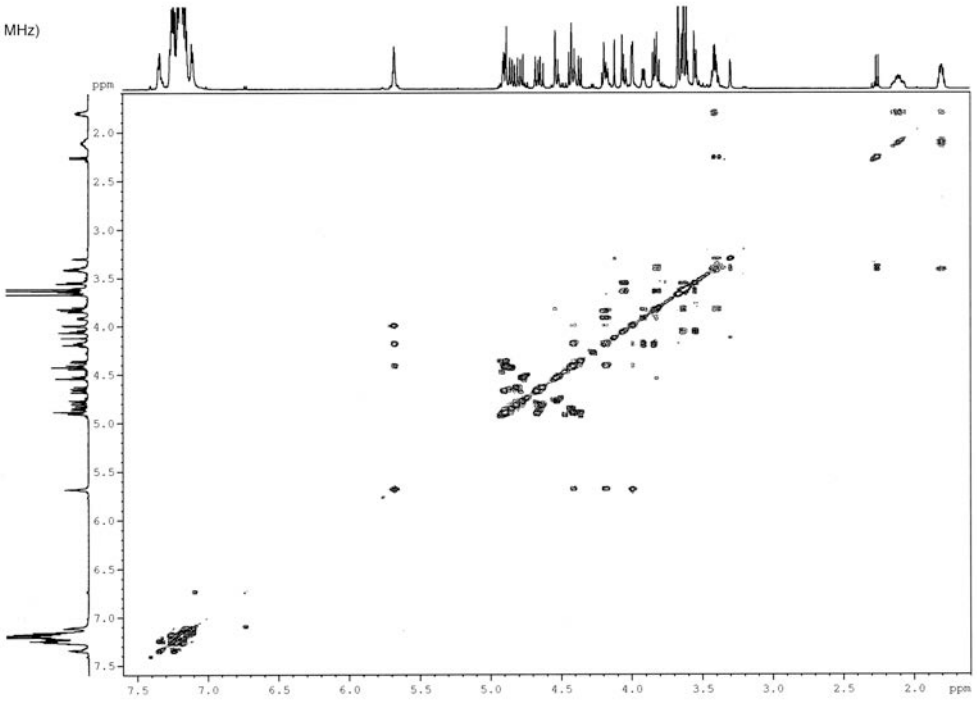


www.manaraa.com

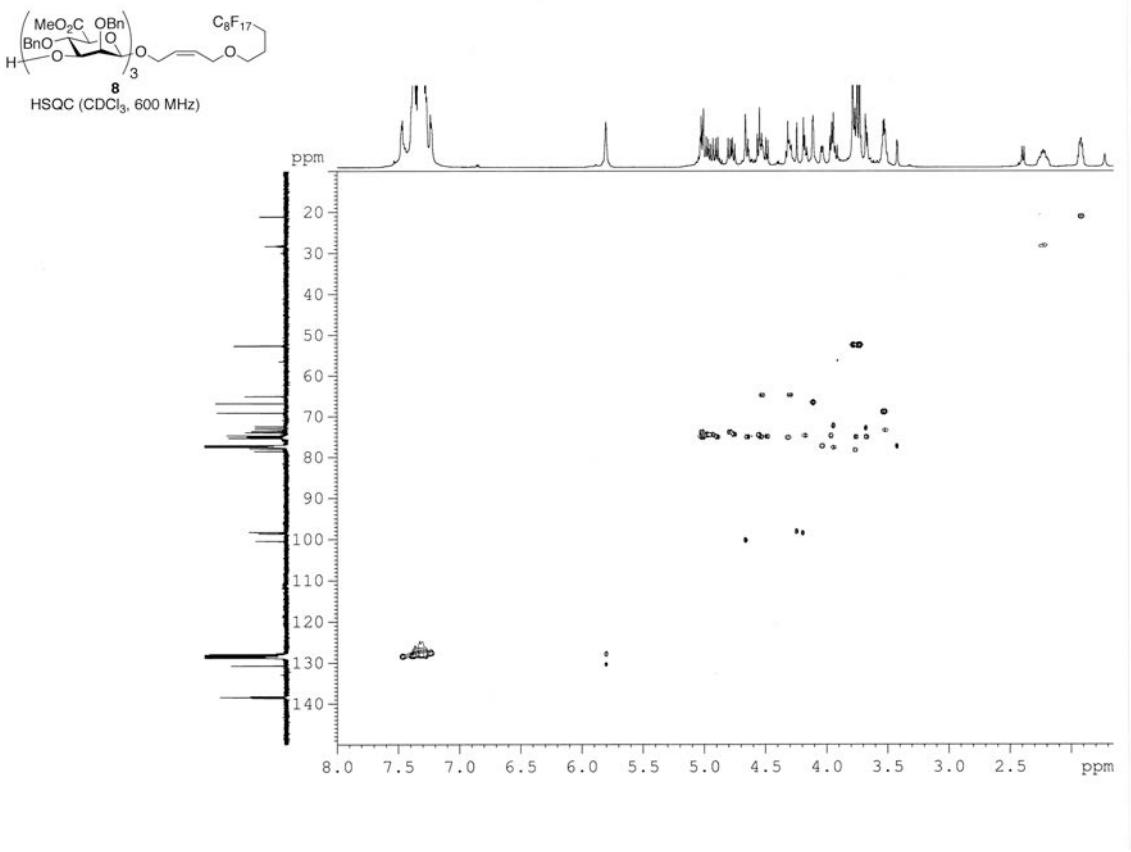


8

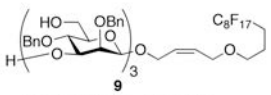
COSY ($CDCl_3$, 600 MHz)



www.manaraa.com

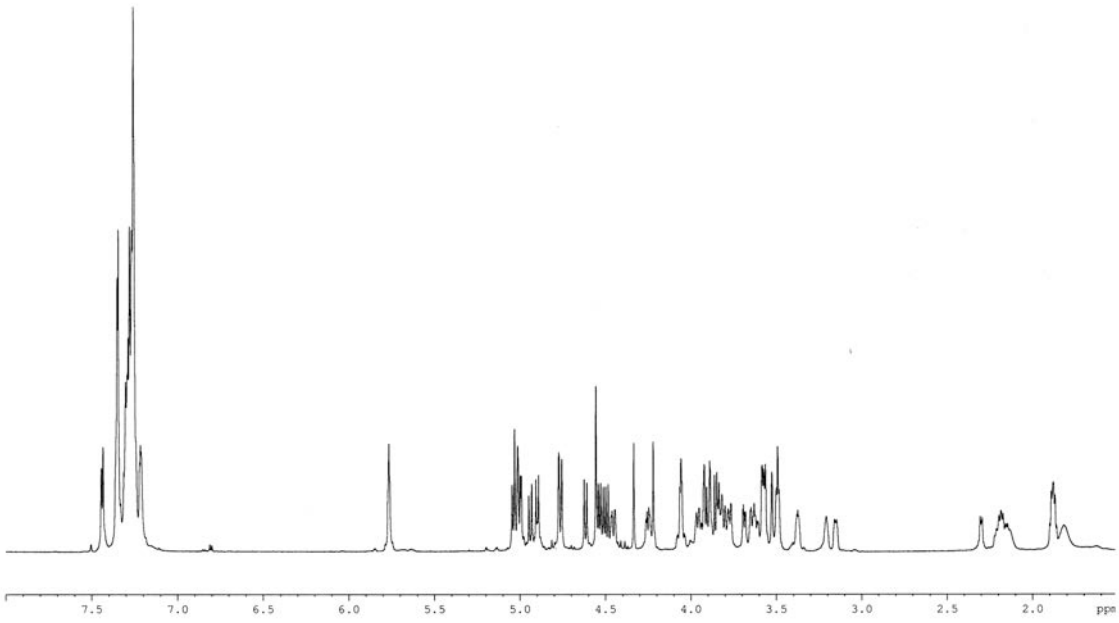


www.manaraa.com

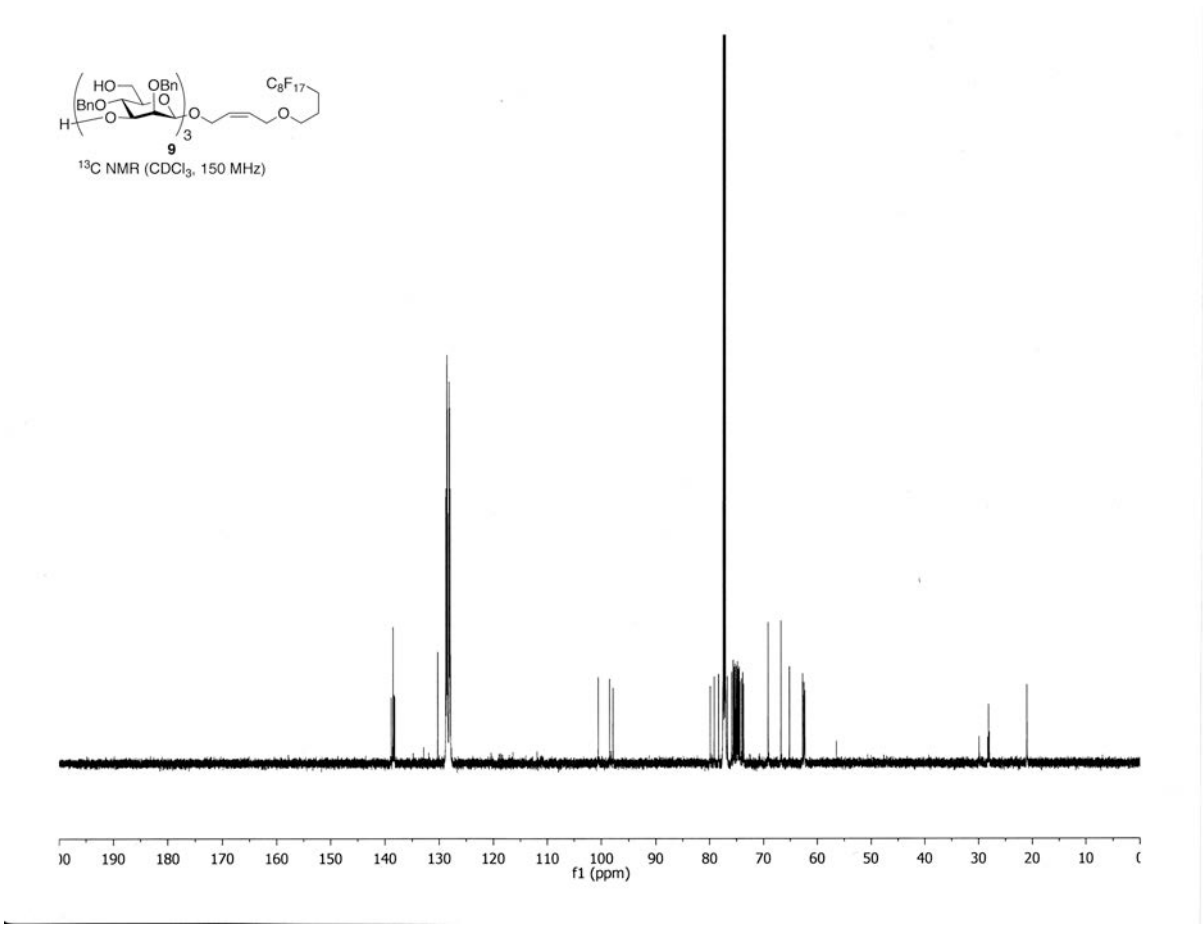


9

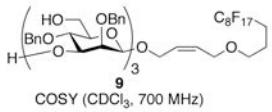
^{1}H NMR ($CDCl_3$, 700 MHz)



www.manaraa.com

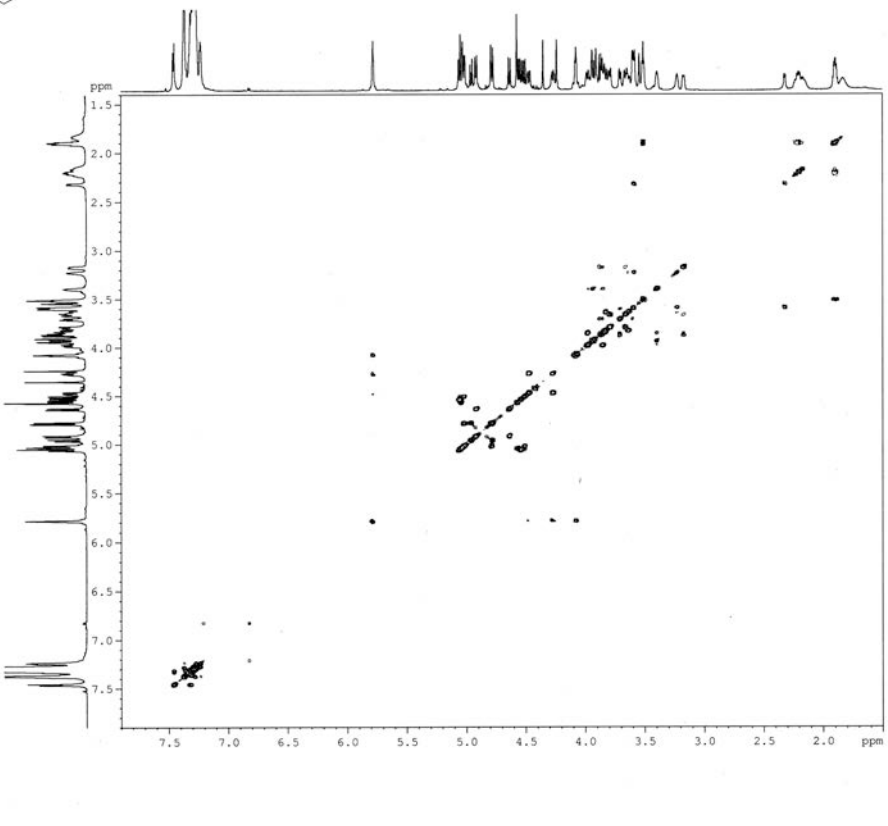


www.manaraa.com

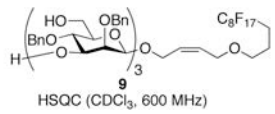


9

COSY ($CDCl_3$, 700 MHz)

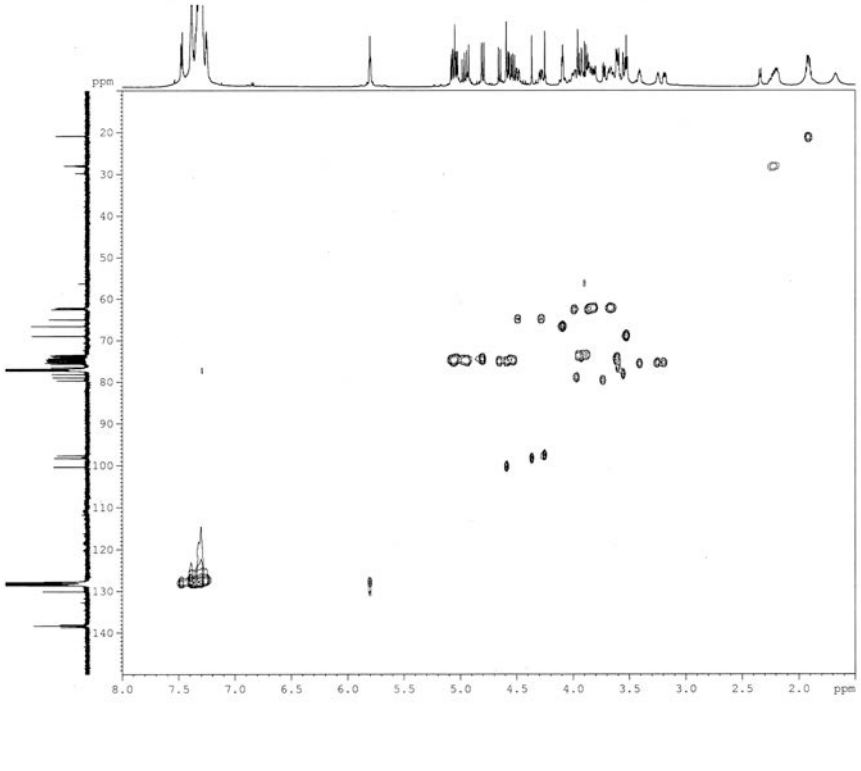


www.manaraa.com

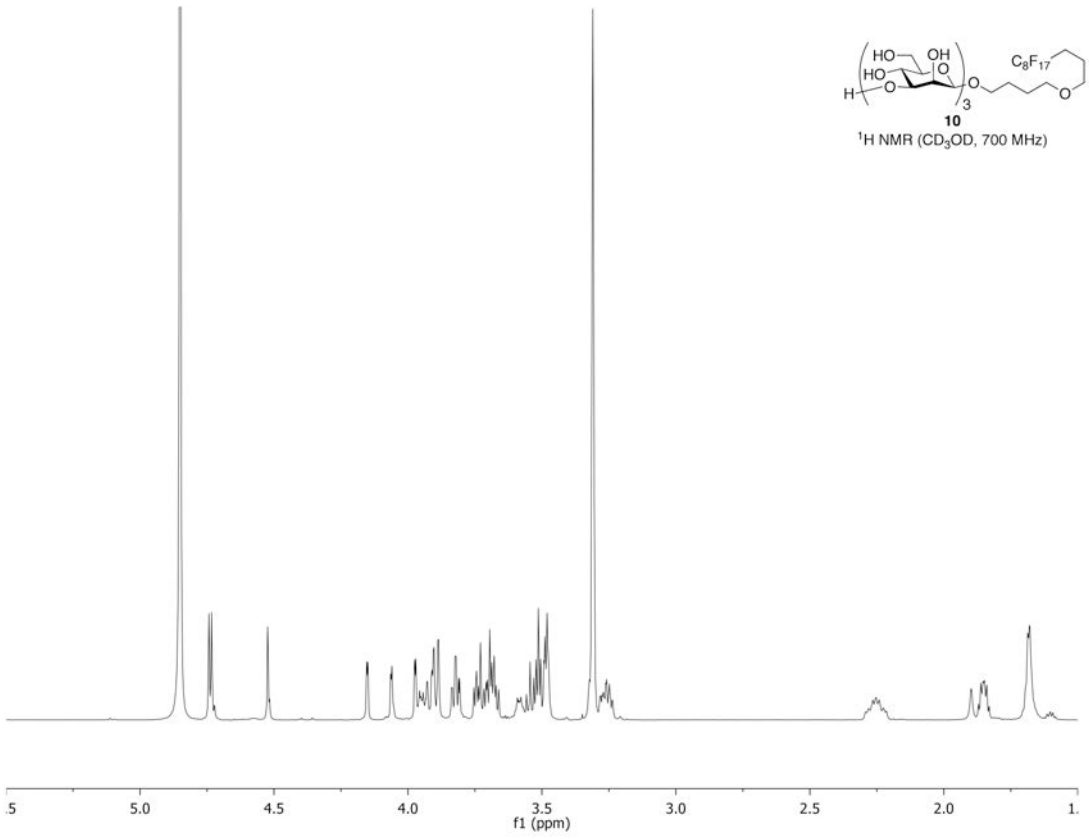


9

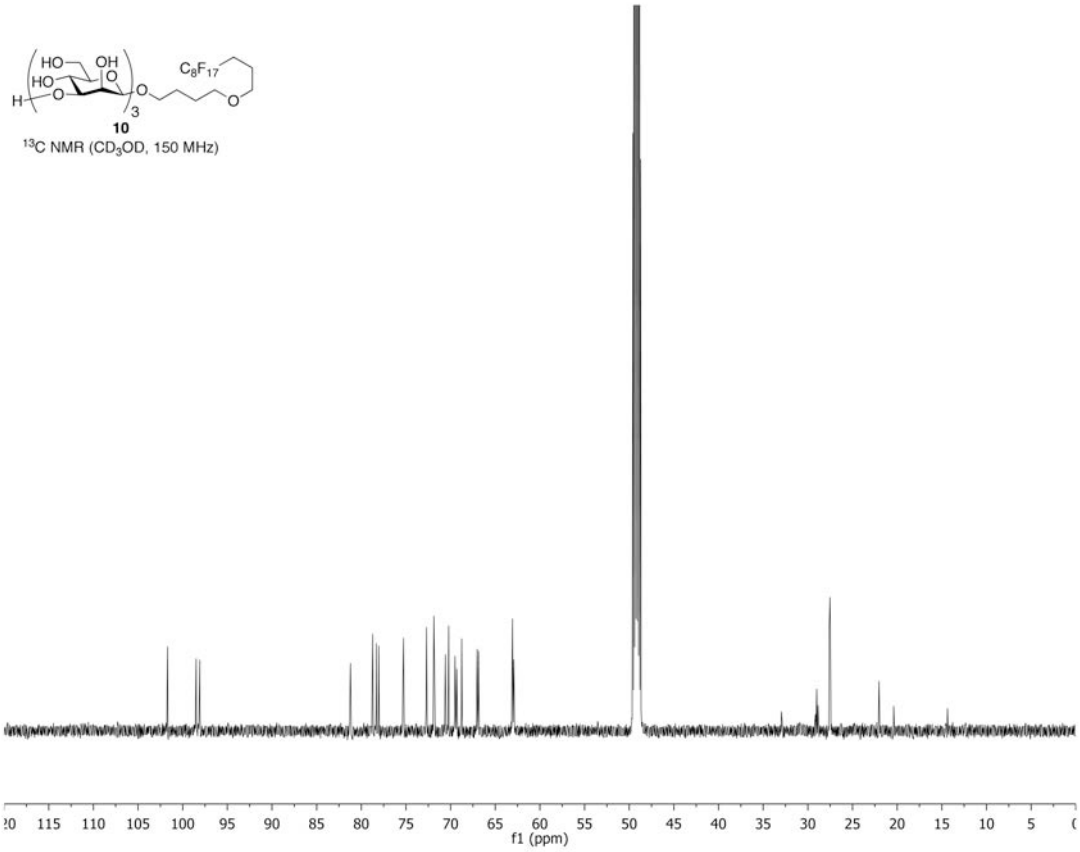
HSQC ($CDCl_3$, 600 MHz)



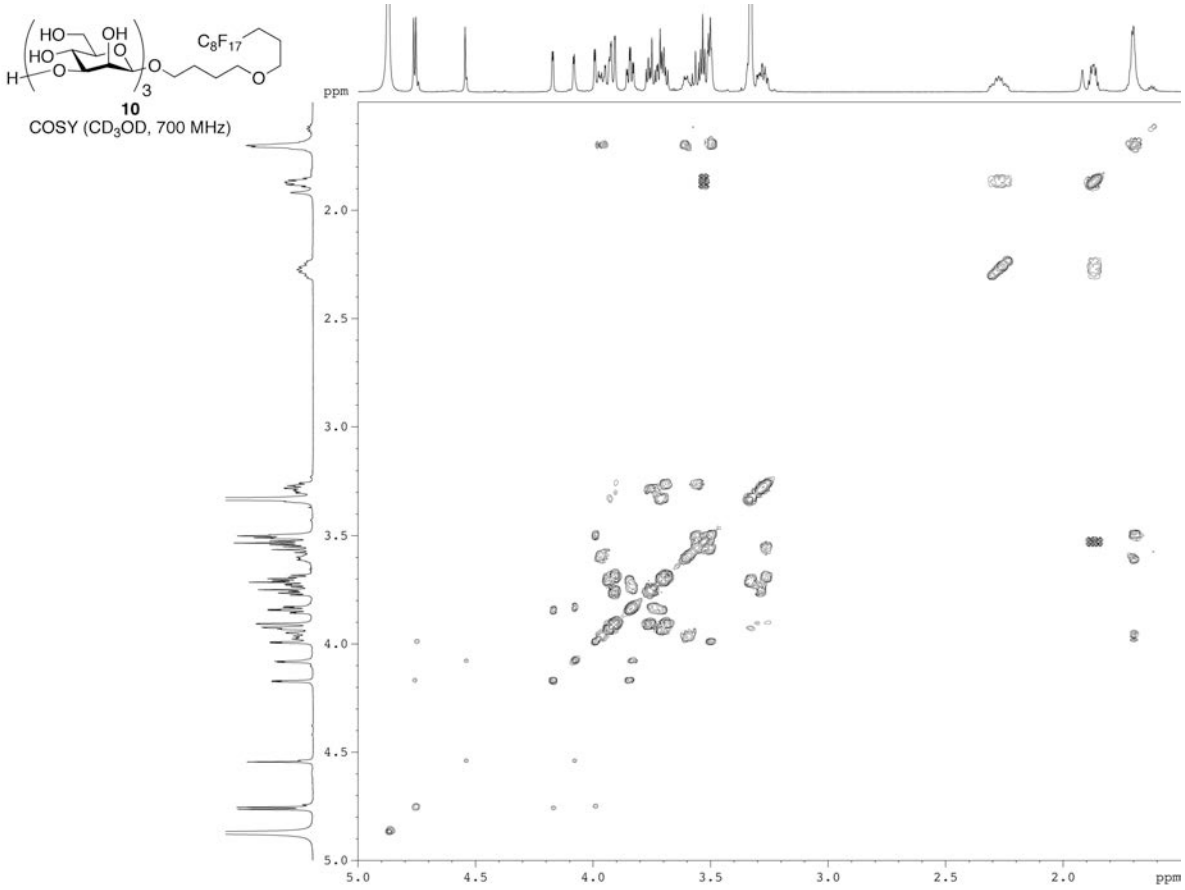
www.manaraa.com



www.manaraa.com



www.manaraa.com



www.manaraa.com