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Chemical Strategies for Drug Development of Fucose-Truncated Ipomoeassin F Analogues

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Chemical Strategies for Drug Development of Fucose-Truncated Ipomoeassin F Analogues

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science in Chemistry

by

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Bachelor of Science in Chemistry, 2015

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Abstract

Natural product synthesis has many applications in the field of chemical biology, due to the protein binding affinity to the complex three-dimensional structure. However, synthetic and structure-activity relationship studies concerning certain drug targets are scarce. For example, members of the ipomoeassin family have exhibited anti-cancer properties, but cost-effective optimization of these compounds has not yet been extensively studied. Ipomoeassin F, a natural glycoresin isolated from the leaves of the *Ipomoea squamosa* plant in the Suriname rainforest, has shown high cytotoxicity, with IC_{50} values measured at the low nanomolar range. Two studies by Postema and Fuerstner have outlined synthesis pathways for ipomoeassin F using ring closing metathesis (RCM) for macrolactonization. The Shi group improved the synthetic method by introducing the cinnamate and tiglate moieties at different stages and developing novel protecting group strategies to reduce the number of steps. Recently, extensive studies were conducted outlining structure-activity relationship; specifically of the fucose ring, cinnamate and tiglate functional groups, and lipophilic aglycone. Removal of the functional groups on the expensive fucoside moiety did not significantly affect the activity of the compound; therefore monosaccharide and galactoside analogues were designed, synthesized and biologically tested. The overall objective of this research is to explore the monosaccharide and galactoside analogue synthetic pathways in order to better understand the binding affinity and cytotoxic properties of ipomoeassin F.

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CHAPTER 3: Design, Synthesis and Biological Evaluation of Fucose-Truncated Monosaccharide Analogues of Ipomoessin F

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CHAPTER 1: Synthesis and Biological Significance of Ipomoeassin F

1.1: Introduction

Resin glycosides or glycoresins, are secondary metabolites in the extensive *Convolvulaceae* (morning glory) family. These metabolites can be found in either the roots or leaves of the *Convolvulaceae* plants and have been used for centuries as natural remedies for various ailments.¹ Specifically, they have been exploited in traditional medicine for their laxative, antibacterial, antifungal and plant growth inhibitory properties, as well as their strong purgative action.² Research to elucidate the chemical properties of resin glycosides has been undertaken since the 19th century, but the structural complexity of these sugars prevented extensive studies. More recently, modern spectroscopic techniques have provided insight into the structural characteristics and pharmacophores of resin glycosides.³ These amphipathic secondary metabolites are derivatives of monohydroxy- and dihydroxy-fatty acids, and are typically combined with a heteropolysaccharide structure.⁴ Most resin glycosides contain a macrolidic ring resulting from the esterification of a hydroxyl group with the aglycone carboxylic acid.⁵⁻⁷ Their hydrophilic sugar and hydrophobic fatty acid chain render them unique, and their macrolactone architecture, as well as amphiphilic properties, generates a high interest in their synthesis and optimization. Primarily, the tricolorin family, including the naturally occurring compound woodrosin,⁸ has evoked high interest among synthetic chemists due to its antifungal and cytotoxic properties.⁹⁻¹²

In spite of the increasing interest in resin glycoside synthesis, structure-activity relationship studies to determine the mode of action are relatively uncommon.¹³ For example, the ipomoeassin family (resin glycoside derivatives) has lacked significant synthetic studies until recently. In 2005, the glycoresins ipomoeassins A-E were isolated from the leaves of the *Ipomoea squamosa* plant, a species of *Convolvulaceae* found in the Suriname rainforest (*Fig. 1*).¹⁴ In 2007, two years after

ipomoeassins A-E were characterized by the Kingston group, the compound ipomoeassin F was discovered. The entire ipomoeassin family is structurally similar, with only slight differences in the macrolactone aglycone and 4th position of the fucosyl ring; however, ipomoeassin F contains a C16, rather than a C14 hydroxyacid side chain¹⁵ (Fig. 1). Compared to other glycoresins, the ipomoeassins possess an uncommon fucoside anomeric center, as well as an oxygenated aglycone ring structure. Furthermore, the cinnamate and tiglate ester functional moieties replace the typical reduced forms on other glycoresins, including 2-methylbutyrate and 3-hydroxy-2-methylbutyric acid.¹⁶

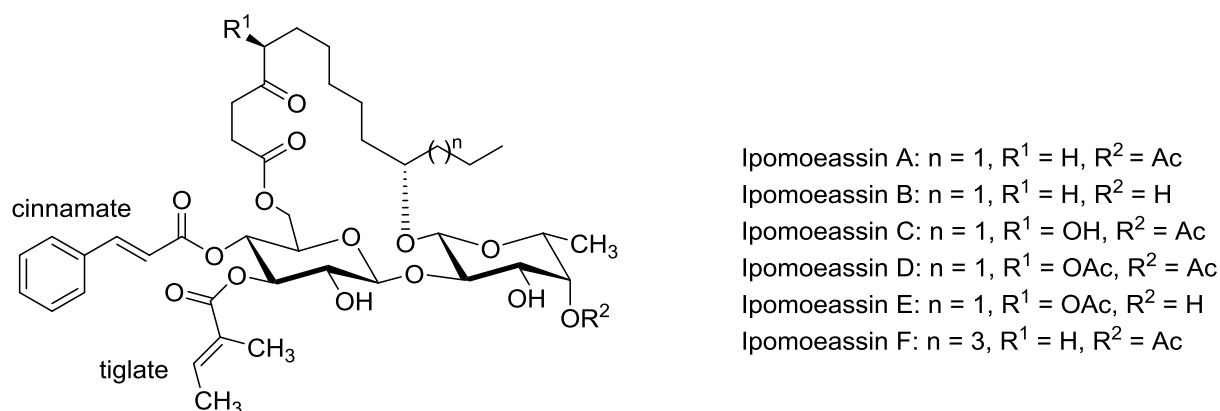


Figure 1: Structural difference among members of the ipomoeassin family

Although resin glycosides are considered potent against certain cancer cell lines, their IC_{50} values are typically in the micromolar range, while certain ipomoeassins have shown IC_{50} values in the nanomolar region.¹⁷ All ipomoeassins exhibit a similar cytotoxicity toward multiple cancer cell lines with structural differences contributing to slight changes in biological properties (Table 1).¹⁸ When ipomoeassin A, the most abundant member of the family, was screened against NCI-60 tumor cell lines, it was shown to be unique among other anticancer agents in the database.¹⁷ Ipomoeassins D and F are the most potent compounds in the family, with relatively low IC_{50} values

against several cancer cell lines, a property thought to be caused by the difference in the 4'OH site in the fucoside moiety and the C-5 within the macrolactone ring.³ Although ipomoeassin F differs from ipomoeassin A only in the two methylene units of the C16 hydroxyacid side chain,¹⁹ it is 14 times more potent against A2780 cells, making ipomoeassin F a prime target for optimization studies.

Table 1: Structures and IC₅₀ Values of Ipomoeassins A-F⁵⁰

ipomoeassin	structure			IC ₅₀ (nM)						
	R ¹	R ²	n	HeLa	L-929	A2780	U937	HT-29	MDA-MB-435	H522-T1
A	H	Ac	1	64	77.8	500	20.2	46.1	42.6	108.9
B	H	H	1	2500		400	134	396	2700	1070
C	OH	Ac	1	1500	> 1000	2900				
D	OAc	Ac	1	32	135	35	7.9	11.8	19.9	23.2
E	OAc	H	1	4300	> 1000	3300	163	393	1633	967
F	H	Ac	3		7.4	36	2.6	4.2	9.4	12.9

1.2: Ipomoeassin F Synthesis Pathways

Currently, three groups have reported total syntheses for ipomoeassin F, all of which employ a ring closing olefin metathesis (RCM) strategy. In 2007, Fuerstner et al. completed the synthesis of ipomoeassins B and E, in an attempt to progress toward more detailed structural editing (*Fig. 2*).²⁰ Previous studies in the Fuerstner lab had shown RCM to be a productive and flexible strategy for the synthesis of several structurally complex resin glycosides.²¹⁻²² After using an *N,N'*-dicyclohexylcarbodiimide (DCC) mediated acylation to attach the tigloyl ester to **1**, the alkene on the macrolactone ring could be reduced with relative selectivity without damaging the tiglic acid functional moiety.²³ An -OPMB ether protecting group for the 4-OH position was selected, due to its orthogonality to the other functional groups, but steric hindrance prevented coordination of the Lewis acid, and, consequently, attachment of the -OPMB ether to the 4th position of glucose on **4**. Further study revealed that a C-silylation strategy to introduce a surrogate

for the cinnamate functional group (*Fig. 2*) prevented the participation of the unsaturated acids in RCM by connecting them to the glucosyl donor at an early stage of the synthesis. The surrogate contained a tri-substituted alkene, rendering it stable during hydrogenation with Wilkinson's catalyst, and the dimethylphenylsilyl group could be deprotected post-RCM reduction.

Subsequent to oxidative cleavage of the ether and Yamaguchi esterification of the cinnamate-substituted intermediate with the aglycone, the olefin was exposed to ruthenium carbene to form macrocycle **5**.²⁴⁻²⁶ Facile deprotection of the C-silyl group was achieved with TASF in MeCN, then subsequent removal of the ether with trifluoroacetic acid to produce ipomoeassin B (**5**). Ipomoeassin E was similarly synthesized by the esterification of the glucose donor with 8-nonenic acid, followed by ring-closing metathesis using the 2nd generation Grubbs catalyst. The use of *t*-BuOOH caused an oxidative rearrangement, preceded by Sharpless-type kinetic resolution for a scalable synthesis route. Although this strategy prevents excessive reduction of the ester groups, it renders structure-activity relationship (SAR) studies illogical, due to the installation of the cinnamate and tiglate functional groups late in the synthesis. Also, deprotection of the silyl and isopropylidene groups over 2 steps with only a 45% yield toward the end of the synthesis is a serious drawback to the practical application of this method.

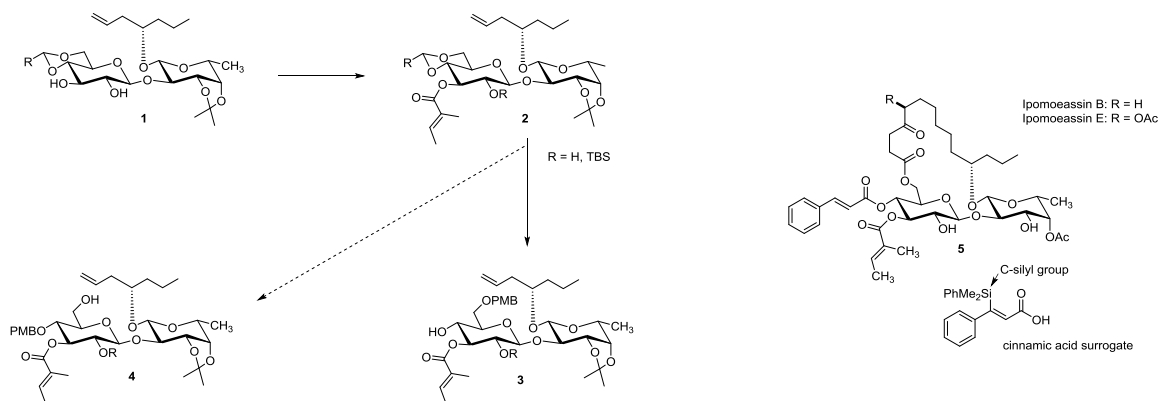
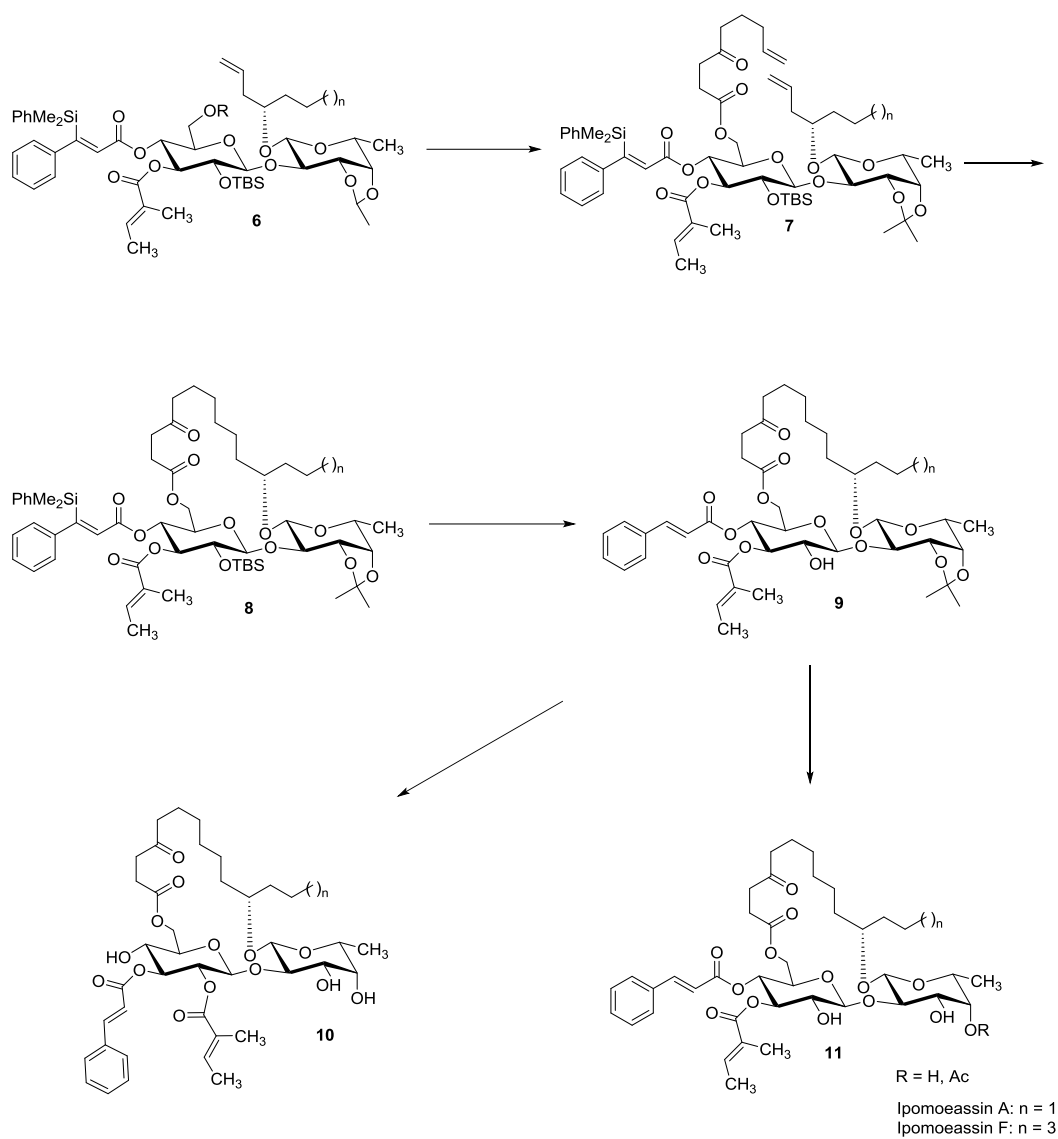


Figure 2: Installation of –OPMB protecting group on the 4th position of glucose,³ and Ipomoeassins B and E with cinnamic acid surrogate²⁰

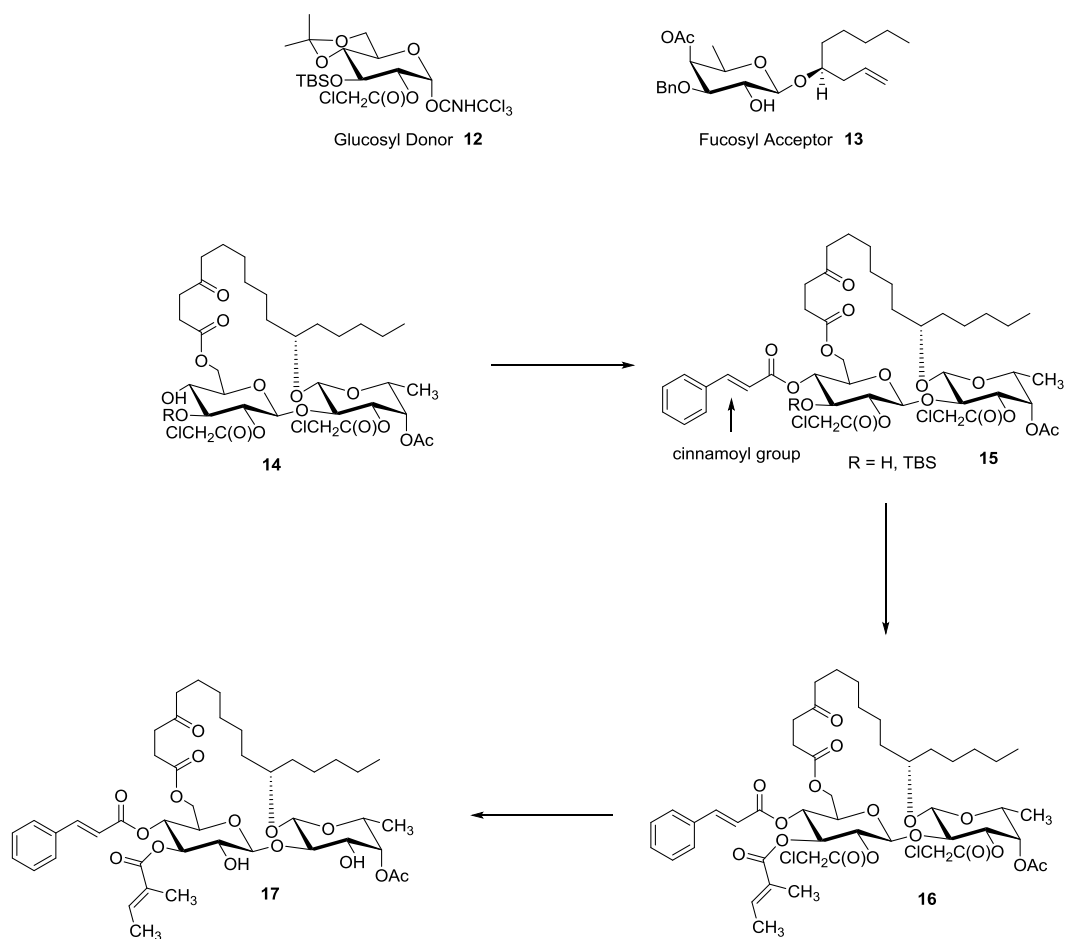
In 2009, Fuerstner applied a similar strategy to the total synthesis of ipomoeassin F (*Sch. I*). Previous studies showed that the cinnamate functional ester is reduced at the same time as the olefin within the macrocycle, while the tri-substituted tiglate ester is reduced at a slower rate.²⁰ Again, they chose to install the tiglate ester at an early stage and create a surrogate for the cinnamate ester by introducing the C-silyl group on **6** through retro-Brook rearrangement.²⁹⁻³² As in the previous study, this method relied on ruthenium-catalyzed RCM to create the macrocyclic structure **8** from diene precursor **7**, and incorporated a tri-substituted alkene for the cinnamic intermediate to achieve chemoselective hydrogenation. Ipomoeassins A-D were synthesized through reductive opening of the single isopropylidene-protected building block **9**. By treating ipomoeassin B with MeC(OEt)₃ and camphorsulfonic acid, an acetate group was installed on the 4th position of fucose through an HOAc-induced rearrangement to give ipomoeassin A (**11**).³³⁻³⁴ Compound **10** was isolated when **9** was subjected to high concentrations of trifluoroacetic acid in an effort to remove the acetal group late in the synthesis. Ipomoeassin F differs from ipomoeassin A only in the two methylene units present on the carbon chain of the aglycone; therefore, a separate fucoside building block with a slightly longer carbon tail was synthesized under the same conditions. Due to the high biological activity of ipomoeassin F, several analogues of this compound were created and tested as probes, but none showed a significant increase in biological activity.



Scheme 1: Synthesis of ipomoeassin F using retro-Brook to install C-silyl³

A slightly improved synthetic methodology was chosen by Postema et al. Similar to Fuerstner, the group exploited RCM instead of macrolactonization as a ring-closing strategy.³⁵⁻³⁷ Schmidt glycosylation³⁸ was used to couple two monosaccharides (glucosyl donor and fucosyl acceptor) using α -chloroacetate and *tert*-butylsilyl protecting groups (*Sch.* 2).³⁹ The Schmidt donor **12** was created by deprotection at the anomeric position with hydrazine acetate, then addition of the trichloroacetimidate group on the glucose fragment.²³ To construct the fucosyl acceptor **13**, the

triol fused with a chiral alcohol⁴⁰ was subjected to equatorial benzylation mediated by stannylidene.⁴¹ The donor and acceptor were then connected in the presence of boron trifluoride etherate to yield the desired β -disaccharide **14** after acetylation and TBS deprotection. The key step in this synthesis was RCM using a Hoveyda-Grubbs catalyst in 1,2-dichloroethane, for which the conditions of 15 mol % catalyst at 45 °C for 3 h at 0.05 M dilution were found to be optimal.⁴² Due to steric hindrance of the bulky TBS group, installation of the cinnamoyl group on **15** via a DCC-mediated coupling reaction in 1,2-dichloroethane proved difficult, but a 62% yield was obtained by evaporating and replacing the solvent.⁴³⁻⁴⁴ After addition of tiglic acid to **15**, removal of the TBS and α -chloroacetate protecting groups on **16** afforded the natural product ipomoeassin F (**17**). Although this synthetic strategy improves upon the methods used by Fuerstner, it also presents several limitations, including low yield during protecting group removal and orthogonality issues. For example, the removal of the chloroacetyl group from the anomeric position of the glucosyl donor **12** and α -chloroacetate protecting groups during the final step **16** \rightarrow **17** only gave yields of 44% and 39% respectively. Also, the steric hindrance from the TBS group on the 3-OH position of the glucosyl donor hindered the addition of cinnamic acid toward the end of the synthesis **14** \rightarrow **15**.



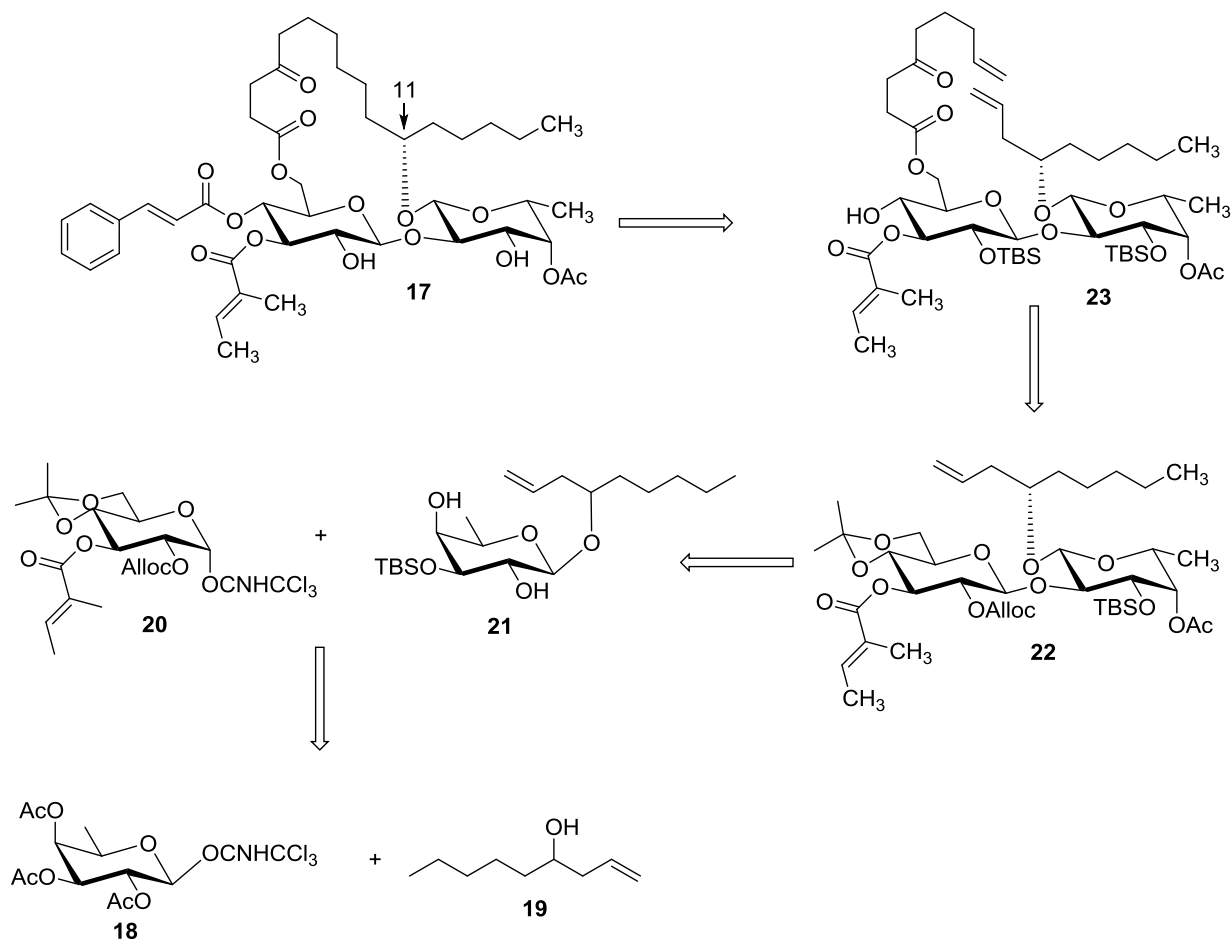
*Scheme 2: Synthesis of ipomoeassin F with Schmidt glycosylation and late ester installation*¹⁹

1.3: Shi Group Synthesis Methodology

To increase overall yield and facilitate further structure-activity relationship studies, the Shi group developed a more scalable and adaptable total synthesis route for ipomoeassin F. Ipomoeassin F was chosen due to its cytotoxicity values in the low nanomolar range and high potency compared to its congeners. In fact, synthetic ipomoeassin F was shown to inhibit growth at least two-fold more than the naturally occurring compound when tested against HT-29, MDA-

MB-435, H522-T1, and U937 cancer cell lines.¹⁹ Further phase contrast microscopy studies showed a rounding up of cells, followed by induced death in H522-T1 lung cancer cells.⁴⁶

Like Postema and Fuerstner, the Shi group employed RCM as the main strategy for macrolactone ring formation, and adopted novel protecting group strategies to prevent reduction of the peripheral ester alkenes (*Sch. 3*). To explore the significant biological properties of phenylpropanoids, or cinnamoyl-containing compounds⁴⁷ and to prevent unwanted reduction during post-RCM hydrogenation, a synthesis route which could successfully install the cinnamate ester at a later stage was required for further SAR studies. Also, it was theorized that, through 1,4-nucleophilic addition, the cinnamate group could modify certain protein structures with its disubstituted, α,β -unsaturated Michael system.⁴⁸ Although protecting group options were limited due to orthogonality and neighboring group participation in Schmidt glycosylation, TBS and allyloxycarbonyl (Alloc) groups were chosen for the glucosyl donor **20** and fucosyl acceptor **21** respectively. Because its mild deprotection conditions could be tolerated by many late-stage functional groups,⁴⁹ Alloc on **22** was selected as a transient protecting group to later be replaced by TBS.⁵⁰ Instead of introducing the tiglate moiety later in the synthesis, it was added directly to the glucosyl donor through a Steglich esterification, reducing the number of protection/deprotection steps. Also, construction of the dissacharide **22** was improved by minimizing the number of protecting groups on both the glucosyl donor **20** and fucoside acceptor **21**, showing phenomenal regio- and stereoselectivity. In addition to the natural product **24**, the 11*R* epimer at C-11 of the macrolactone ring was constructed.



Scheme 3: Retrosynthesis of ipomoassin F

The potency of both the natural product ipomoassin F and its epimer were measured through cytotoxicity assays. Five human tumor cell lines, one immortalized normal human mammary epithelial cell line (MCF-10A), and one immortalized normal mouse embryo fibroblast cell line (NIH/3T3) were measured with 4,5-dimethylthiazol-2-yl-2,5-diphenyltetrazolium bromide,⁵¹ using the well-known cancer drug Taxol as a positive control. Although the 11*R*-epimer showed less activity than the natural product, its IC₅₀ value was still in the nanomolar range. The IC₅₀ value against each cancer cell line was comparable to published results, and the cell viability curve demonstrated that it could prevent cell proliferation 20-30% more than Taxol at high concentrations (>100 nM). Furthermore, the selectivity of this epimer was demonstrated by the

~8-fold higher potency of ipomoeassin F against U937 and MCF7, compared to literature values. However, ipomoeassin F showed no selectivity between general cancer cells and normal cells, and its IC₅₀ values at low concentrations were slightly higher than Taxol.

1.4: Structure-Activity Relationship Studies

Recently, the Shi group also conducted extensive structure-activity relationship studies on ipomoeassin F to determine the contribution of each functional group to the overall cytotoxicity (*Fig 3*).⁵² Since ipomoeassin F (**17**) is distinguished from other resin glycosides by the carbonyl at the C-4 position of the macrolactone ring, as well as the tiglate and cinnamate esters on the glucosyl donor, a library was created to determine the connection of these groups to the overall biological activity. Because the ester alkene groups were essential to maintaining low IC₅₀ values, it was crucial that no reduction occurred during hydrogenation of the aglycone after RCM. Consequently, the cinnamate functional group was introduced at a later stage during the synthesis of compounds **25**, **26** and **27**, and the hydrogenation was carefully controlled to avoid reducing the tigloyl alkene. A new route for the glucosyl donor, involving levulinic acid (Lev) esterification of the isopropylidene and *p*-methoxyphenyl protected glucose diol was also created.⁵³ Not only did this route allow the removal of the tiglate functional moiety to examine reactivity, but it also prevented reduction of the ester during the RCM and hydrogenation steps. Because Lev deprotection would damage the ketone on the lipophilic chain, it was replaced with chloroacetyl groups which could be removed under milder conditions of diazabicyclo[2.2.2]octane⁵⁴ after the addition of the aglycone. Subsequent acylation, RCM, hydrogenation, cinnamate esterification, and deprotection afforded analogues **24-27**.

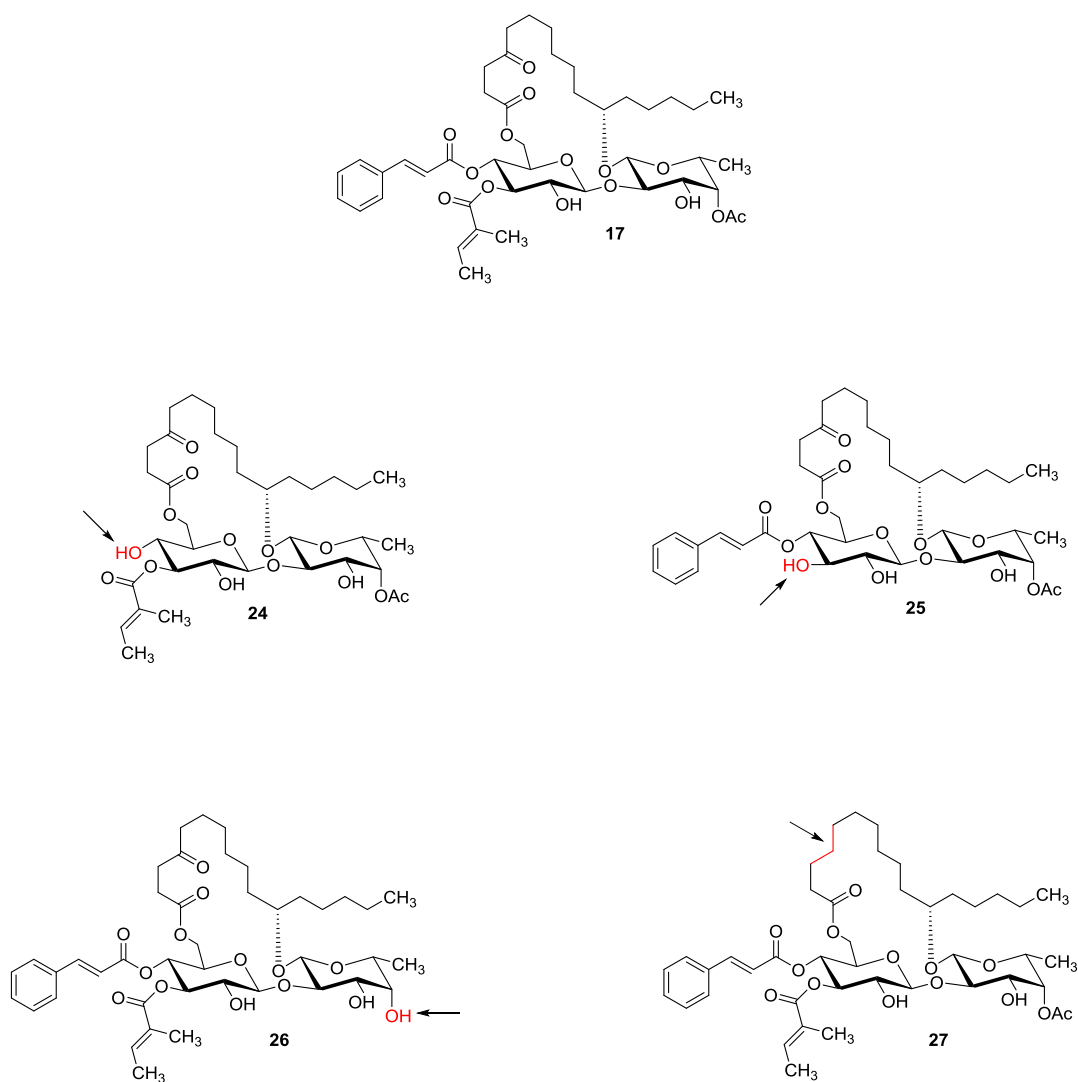


Figure 3: *Ipomoeassin F* analogues for SAR studies⁵³

By synthesizing several analogues of ipomoeassin F, using the optimized procedure developed previously,⁵⁰ it was determined that removal of the two α,β -unsaturated esters, or cinnamate **24** and tiglate **25** functional groups, caused the highest loss of cytotoxicity (Fig. 4). Modification of the carbonyl group on the lipophilic aglycone **27** produced no change in activity when the compound was tested against five different cancer cell lines and compared to ipomoeassin F. Although the acetate-deprived analogue **26** demonstrated a 20-fold decreased potency against MDA MB-231 tumor cells, the decrease was only 2-fold when measured against

MCF7. Moreover, the IC_{50} values of each analogue were compared to both MCF-10A as a non-tumor control, and paclitaxel, which is known to be selective for cancer cells. **27** showed a greater selectivity than the natural product, indicating that structural changes could improve the selectivity as well as cytotoxicity of the compound. Removal of the cinnamate and tiglate functional groups showed both esters to be crucial to the activity of the compound; however, reduction of tiglate caused a slightly lower bioactivity change than reduction of cinnamate. This data indicates that the phenylpropanoid structure, or Michael acceptor system, could contribute to a protein covalent interaction through irreversible pi-pi stacking with the biomacromolecule.⁵⁵

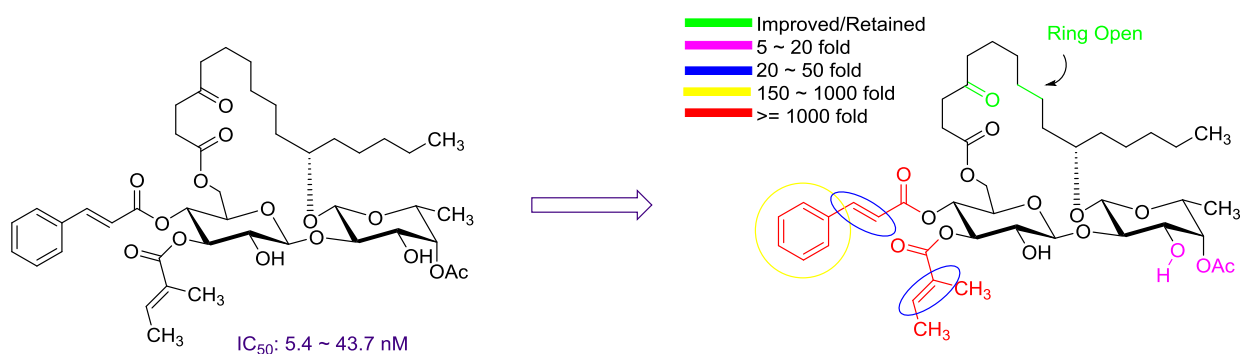


Figure 4: Cytotoxicity loss after removal/modification of functional moieties

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CHAPTER 2: Ipomoessin F Analogue Design, Synthesis and Biological Evaluation

2.1: Introduction

Although fully protected and 3-OH Fucp protected ipomoessin F analogues remained inactive, removal of the acetyl group from the fucoside acceptor caused only a slight cytotoxicity loss, with IC_{50} values still below 150 nM. A 2-23-fold loss was seen when the 4-OH-Fucp was removed and only a 2-14 fold loss for the 3-OH-Fucp (Fig. 5). Because the two free hydroxyl groups represent the only hydrophilic region of the compound, it was theorized that they connect the natural product to the surrounding aqueous environment, without involvement in covalent protein binding. Based on the relative insignificance of the acetyl group, as well as the high cost of fucose (\$427/5g on Sigma Aldrich), removal of the fucoside acceptor ring could potentially improve the synthetic route for ipomoessin F. Not only would the monosaccharide analogues **1** and **2** reduce the overall production expense, but they would also shorten the synthesis, potentially increase cytotoxicity and/or selectivity and provide insight into the structural importance of the fucoside ring moiety.

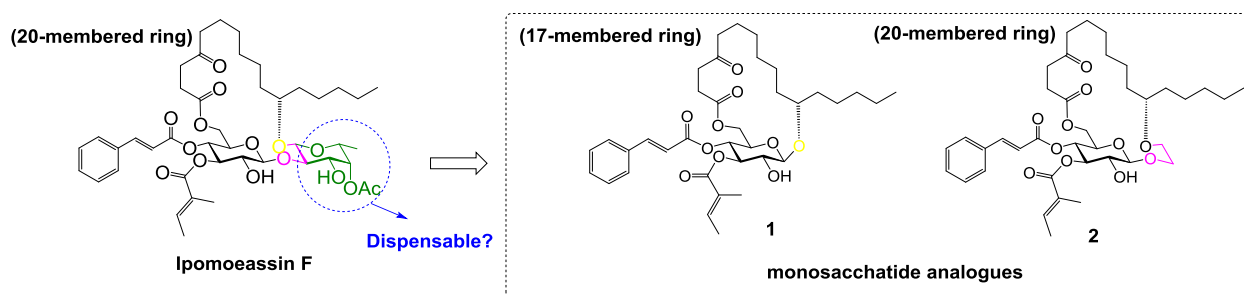
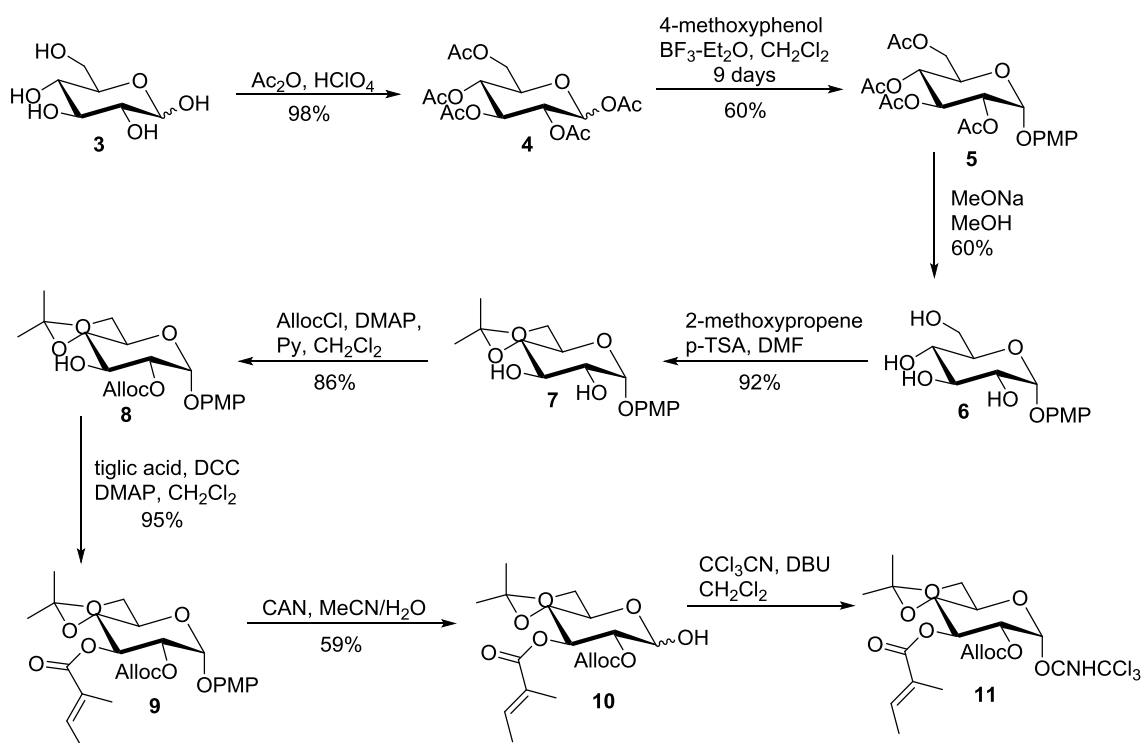


Figure 5: Potential modifications to obtain 17 and 20-membered monosaccharide analogues⁶⁴

2.2: Monosaccharide Analogue Synthesis

Synthesis of the monosaccharide analogue was accomplished using several procedures developed in the previously published “Total Synthesis and Biological Evaluation of Ipomoeassin F and Its Unnatural 11*R* Epimer” and “Revealing the Pharmacophore of Ipomoeassin F through Molecular Editing” papers.^{50,53} As shown in Scheme 4, the glucosyl donor **11** was first synthesized from glucose (**3**), using protecting group strategies to achieve optimal yield and minimize the by-products. Global acetylation to create **4** was accomplished with perchloric acid as a catalyst and acetic anhydride as a solvent to maximize reaction efficiency and minimize pyridine exposure. Following acylation, the para-methoxyphenyl protecting group was introduced to the anomeric position of **4** and allowed to isomerize to the alpha form over a period of 4-5 days in order to direct the allyloxycarbonyl protecting group to the 2-OH position of *D*-glucopyranoside. Due to incomplete isomerization of **5**, a mixture of α/β products was formed, and the yield was relatively low (~60% over two steps). Subsequently, an isopropyl group was introduced to protect the 4th and 6th positions of **6**, using para-toluenesulfonic acid in DMF.⁵⁷ Although 3-OH-Glup is typically more reactive than 2-OH-Glup,⁵⁸ addition of the tigloyl ester moiety was not completely selective. Previous studies⁵⁰ showed that Steglich esterification of (PMP)- β -*D*-glucopyranoside⁵⁹ without a protecting group at the 2-OH-Glup produced a mixture with a ratio of 2.4:1 2-O-tiglate:3-O-tiglate. Since regioselective esterification favored the 2-OH-Glup, allylchloroformate was added to produce an allyloxycarbonyl (Alloc) protecting group on the 2nd position of (PMP)- α -*D*-glucopyranoside (**8**). Because only mild deprotection conditions are necessary for the removal of Alloc, this group was ideal for improving the selectivity and avoiding migration. Early introduction of the tigloyl function group⁶⁰ to **8** prevented extra 3-OH-Glup protection and deprotection, and led to a more facile installation of the cinnamate ester later in the synthesis. Finally, 4-

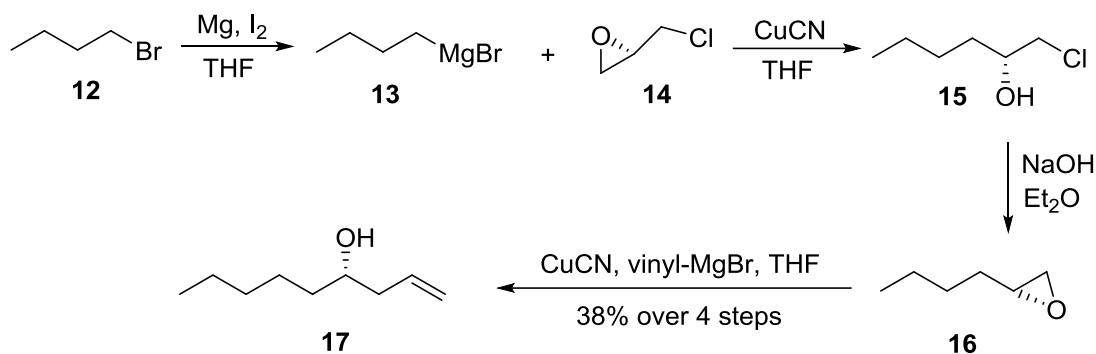
methoxyphenyl (PMP) was cleaved with cerium ammonium nitrate to form **10**, and the trichloroacetonitrile was introduced in the presence of 1,8-diazabicyclo(5.4.0)undec-7-ene (DBU)⁶¹ to the anomeric position to give the trichloroacetimidate donor **11** with a yield of 59% over two steps.



Scheme 4: Synthesis of Alloc-protected glucosyl donor

To replace the fucosyl moiety from the previous synthesis route,⁵⁰ a 2-carbon acceptor was developed as shown in Scheme 5. The acceptor was synthesized in three steps, using the commercially available (*R*)-epichlorohydrin. The butyl magnesium bromide compound **13** was prepared by suspending the alkyl halide **12** in a solution of magnesium, promoting single electron transfer. The resulting alkyl bromide Grignard reagent **13** was added to (*R*)-epichlorohydrin (**14**) through sequential copper-catalyzed epoxide ring opening to form the enantiopure alcohol (*R*)-1-chloro-2-heptanol (**15**).⁶² Following transformation to the alcohol, the epoxide was successfully

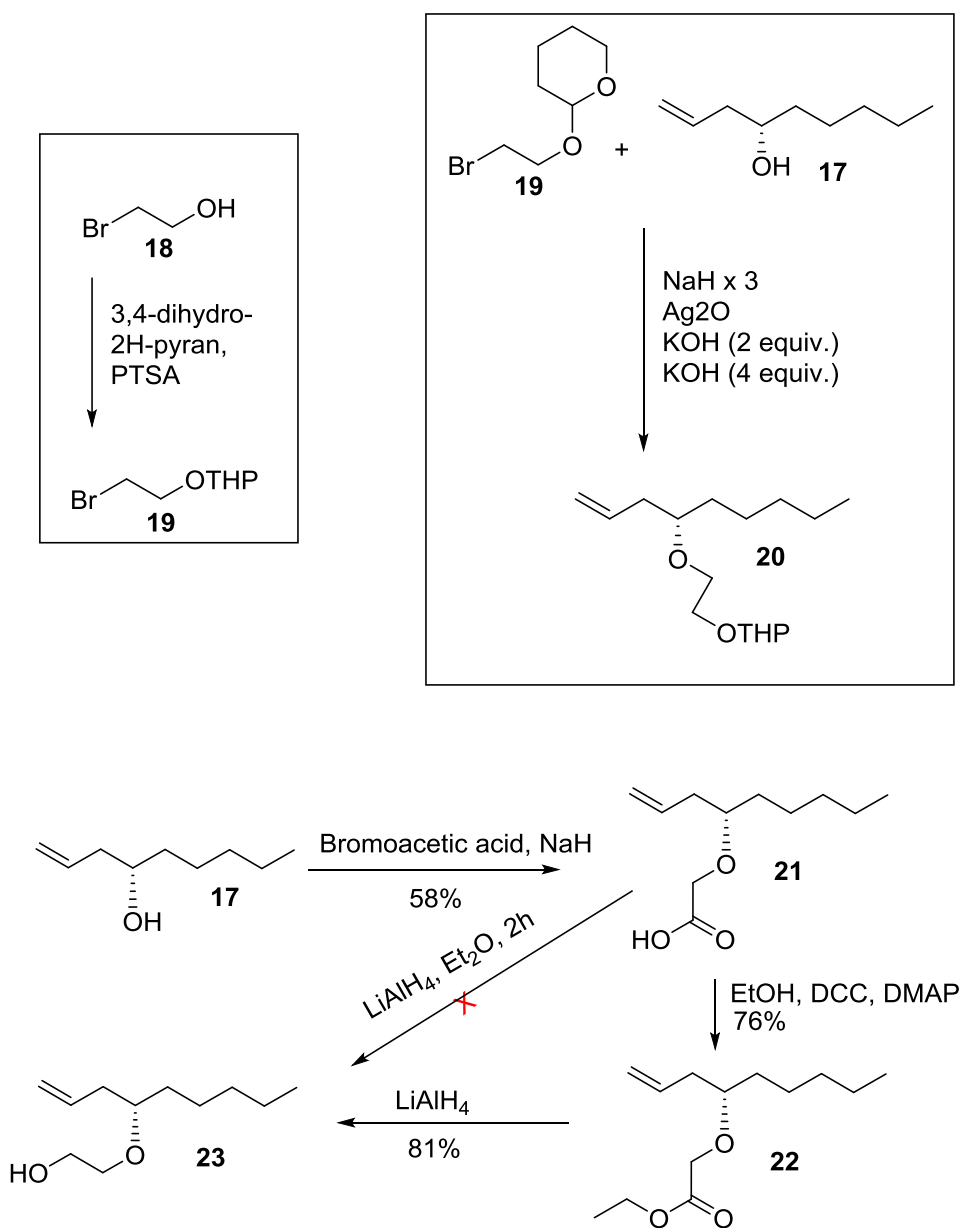
reformed with sodium hydroxide in a 98% yield. Finally, the oxirane species **16** was reopened with vinylmagnesium bromide to give the desired alcohol **17**. When compared to published data, (*R*)-hept-1-en-4-ol showed an enantiomeric excess of 98%.⁶²



*Scheme 5: Synthesis of (*R*)-hept-1-en-4-ol*

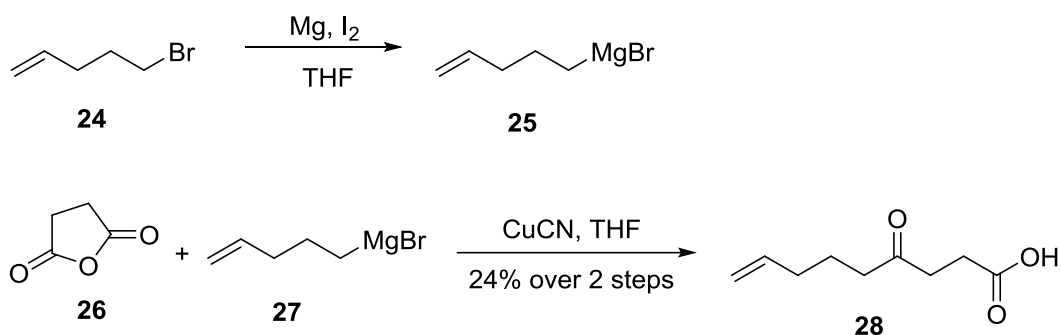
To create the 2-carbon acceptor aglycone shown in Scheme 6, Williamson ether synthesis to connect (*R*)-hept-1-en-4-ol (**17**) with THF-protected 2-bromoethanol (**19**) was attempted. In accordance with literature, a tetrahydrofuran protecting group was attached to 2-bromoethanol (**18**) using para-toluenesulfonic acid as a catalyst to reduce byproduct and promote attack at the halide carbon. Although Gormisky⁶³ reported a Williamson ether synthesis using sodium hydride (NaH) to reduce a secondary alcohol, unfortunately, three attempts using NaH to remove the proton from (*R*)-hept-1-en-4-ol (**17**) and form **20** were unsuccessful. The reaction was attempted several times, by first using two equivalents of NaH, then by varying both the equivalents and time periods of the reaction. Since the harsh conditions caused decomposition of the starting material, an unsuccessful endeavor was made to use the slightly less basic potassium hydroxide (KOH) to remove the secondary hydroxyl group hydrogen. After many futile attempts under several different Williamson ether conditions, including the use of silver oxide (Ag₂O) to connect the alcohol **17** with tetrahydropyranol-protected 2-bromoethanol (**19**), the idea of promoting a

Williamson ether synthesis with a base was abandoned and the indirect route shown in Scheme 6 adopted. Instead of a direct reduction, (*R*)-hept-1-en-4-ol (**17**) was alkylated with bromoacetic acid under basic conditions to give the carboxylic acid **21**. Due to the unsuccessful direct reduction with LiAlH_4 (possibly due to coordination with the ether oxygen), an ethyl ester **22** was created as a precursor to the reduced acceptor product **23**.



Scheme 6: Synthesis of aglycone acceptor

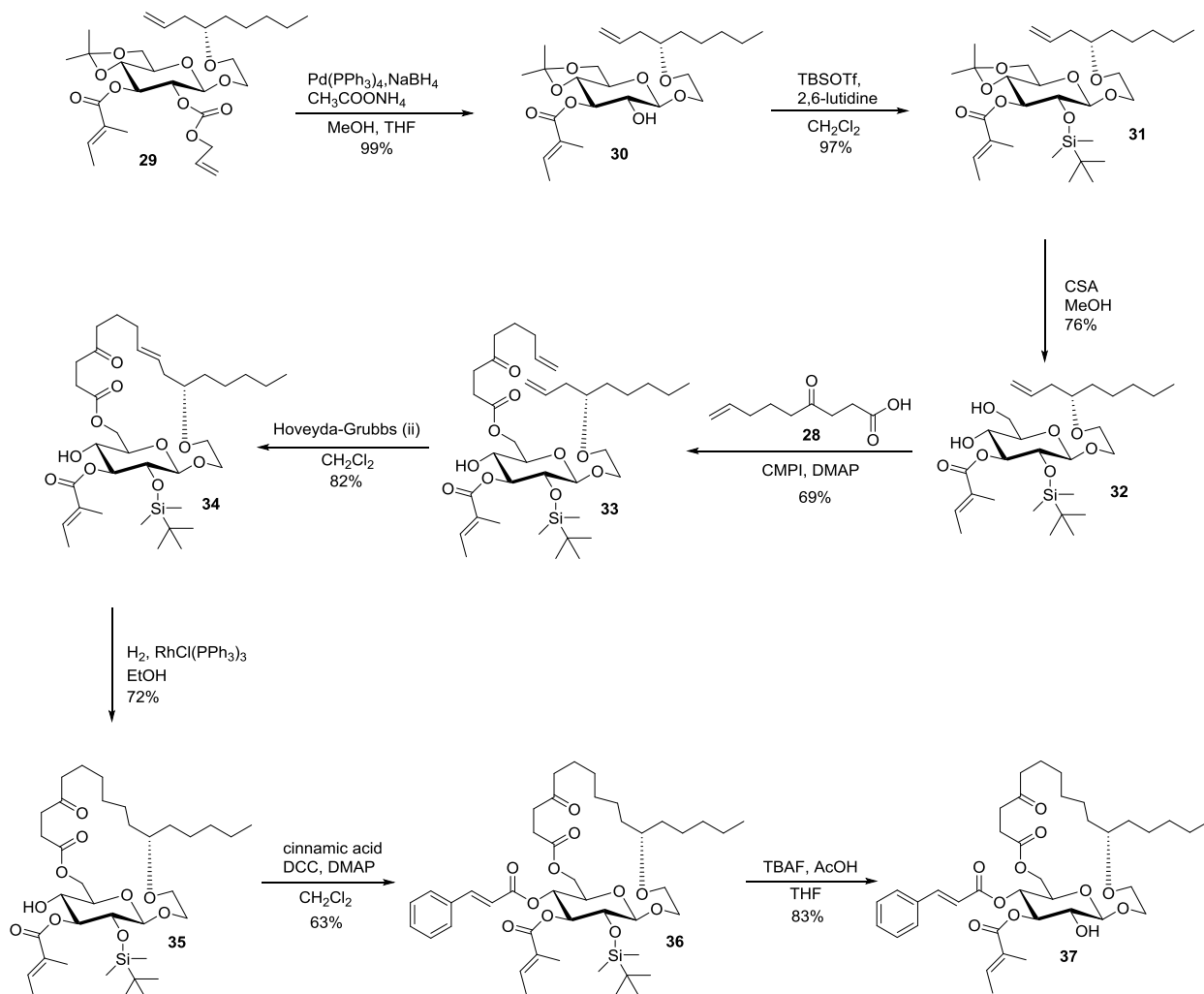
To synthesize the lipophilic aglycone needed to complete the macrocycle of the monosaccharide analogue (*Sch. 7*), an alkyl halide **24** was first suspended in a solution of magnesium and THF. This iodine-catalyzed addition of magnesium produced the Grignard reagent **25**, which was used to selectively open succinic anhydride (**26**). Due to a second copper-catalyzed Grignard reaction with the carbonyl carbon on the aglycone, **28** was the minor product with a yield of 24% over 2 steps.



Scheme 7: Synthesis of lipophilic aglycone chain

After synthesizing the protected donor **11**, 2-carbon acceptor chain **23**, and lipophilic aglycone **28**, Schmidt glycosylation was achieved under the conditions of trimethylsilyl trifluoromethanesulfonate in CH_2Cl_2 , at $-78\text{ }^\circ\text{C}$ to produce **29** (*Sch. 8*). The glycosylation reaction proceeded at a moderate yield of 46%; however, subsequent removal of the Alloc protecting group on **29** afforded a higher yield of 99%. Protection of the 2-OH-Glup on **30** with a tert-butyl dimethylsilyl group also gave an excellent yield and prevented migration of the 4-oxo-9-nonenoic acid chain. Purification of this aglycone was difficult, but after removal of the isopropylidene group with camphorsulfonic acid, 76% of product **32** was obtained. The diene precursor **33** was converted to the 20-membered macrolactone ring structure **34** through RCM using the Hoveyda-Grubbs catalyst under reflux in CH_2Cl_2 . This process was followed by selective

hydrogenation with Wilkinson's catalyst, with limited reduction of the tigloyl alkene on **35**. The cinnamate functional group was then introduced to the glucose ring on **35** by Mukaiyama esterification with a 63% yield. Although an acyl migrated compound was observed for the 17-membered ring **1**, tetra-*n*-butylammonium fluoride was able to successfully remove the TBS protecting group from the 20-membered ring structure **36** after deprotection, producing the final monosaccharide analogue **37**. The structure of this product was confirmed with ¹H, ¹³C, COSY and HMBC NMR, as well as optical rotation measurement.



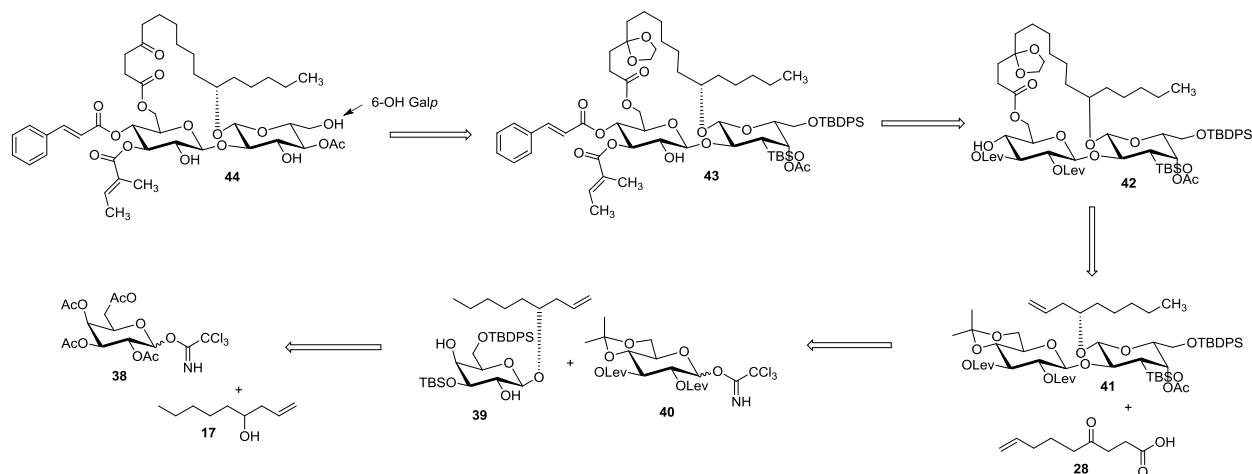
Scheme 8: Synthesis of 20-membered monosaccharide analogue

After completing the synthesis of the 20-membered monosaccharide analogue, the cytotoxicity was tested against several different cancer cell lines. Specifically, the breast cancer cell lines MDA-MB-231 and MCF7 were evaluated, using either the fluorescent alamarBlue or colorimetric MTT assay.⁶⁴ Ipomoeassin F was used as a positive control during this procedure, and the vehicle-treated cells as the negative control. The lack of activity shown by both the 20 and 17-membered ring structures confirmed the assumption that the fucoside moiety is necessary for cytotoxicity retention; in other words, the pyranose ring cannot be removed while retaining the biological activity of the compound. Due to the rigidity and stability of the disaccharide structure, it was theorized that the removal of the right-hand sugar altered the overall conformation to prevent binding to the target protein.

2.3: Galactoside Analogue Synthesis

Although the disaccharide structure is essential to maintaining biological activity through conformational control, the fucose ring could potentially be replaced with a less expensive monosaccharide, such as *D*-galactose. The use of a different carbasugar for the acceptor portion of the disaccharide could not only reduce the expense of the synthesis, but also shorten the number of steps and and/or increase the biological activity. Furthermore, the versatile 6-OH-Galp would allow expansion of the range of functional groups and potential inhibition of cancer cell reproduction. For this reason, the retrosynthesis shown in Scheme 9 was developed to construct an ipomoeassin F analogue with a galactoside acceptor ring. Future studies could exploit glucose or *D*-arabinose as low-cost acceptor sugars; however, galactose was chosen for its similarity to the original structure, with only a slight difference at the 6th position of the acceptor ring on **44**. The removal of the fucoside structure ultimately gave insight into the binding properties of the

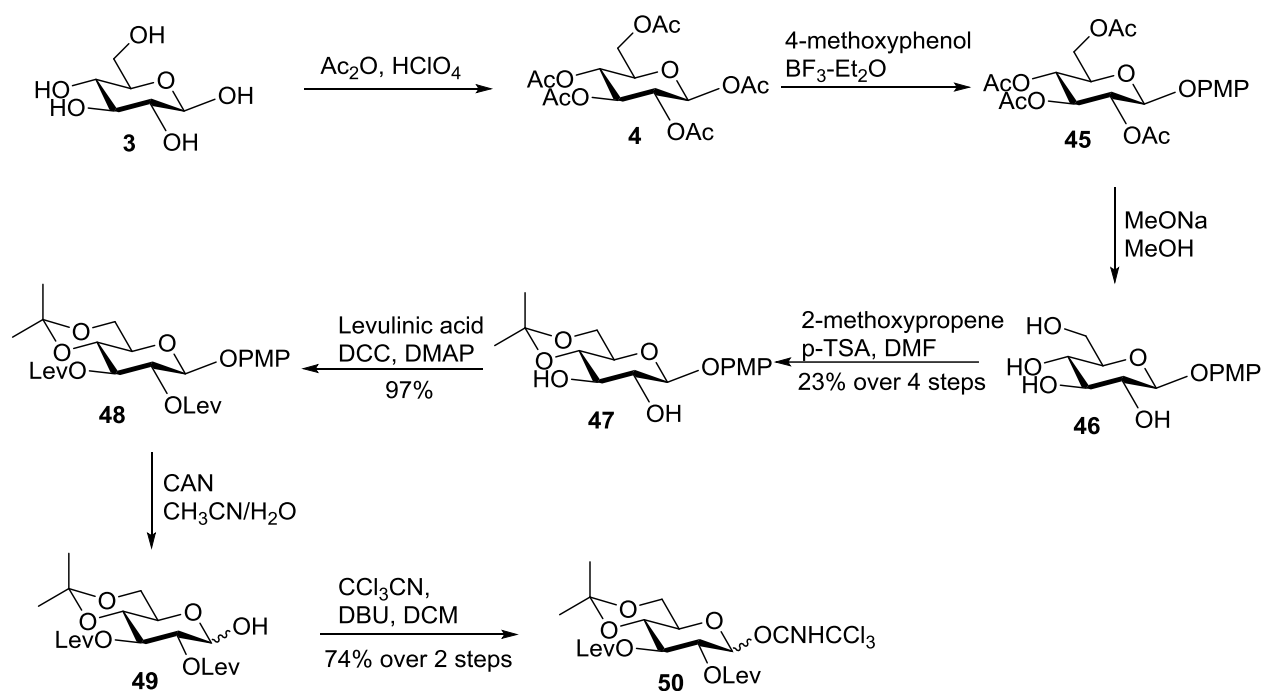
compound and facilitated the development of future synthetic studies and methods involving less expensive carbasugars.



Scheme 9: Retrosynthesis of galactoside analogue

In order to prevent reduction of the cinnamate and tiglate functional moieties after RCM, a new glucosyl donor was designed (*Sch. 10*). Like the procedure outlined in the “Total Synthesis and Biological Evaluation of Ipomoeassin F and Its Unnatural 11*R* Epimer” publication,⁵⁰ which relied on early introduction of the tigloyl ester, global acetylation to produce **4** was achieved in acetic anhydride with perchloric acid acting as a catalyst. Again, the para-methoxyphenyl protecting group was introduced to the anomeric position of glucose; however, isomerization to the alpha form was not required and the major product of this reaction after 12 hours was (PMP)- β -D-glucopyranoside (**45**). Due to minimization of the alpha product and facile separation, the yield of this reaction was significantly higher than the yield in the previous method. Isopropylidene was added to the 5th and 6th positions of glucose to produce **47**, and the levuloyl protecting group was chosen for the 2 and 3-OH positions on **48**. Since an excess of levulinic acid (Lev) was used to protect these positions, the reactivity of the hydroxyl groups was irrelevant. Although the relatively harsh conditions of hydrazine acetate may affect the acetyl ketone and cause a slightly

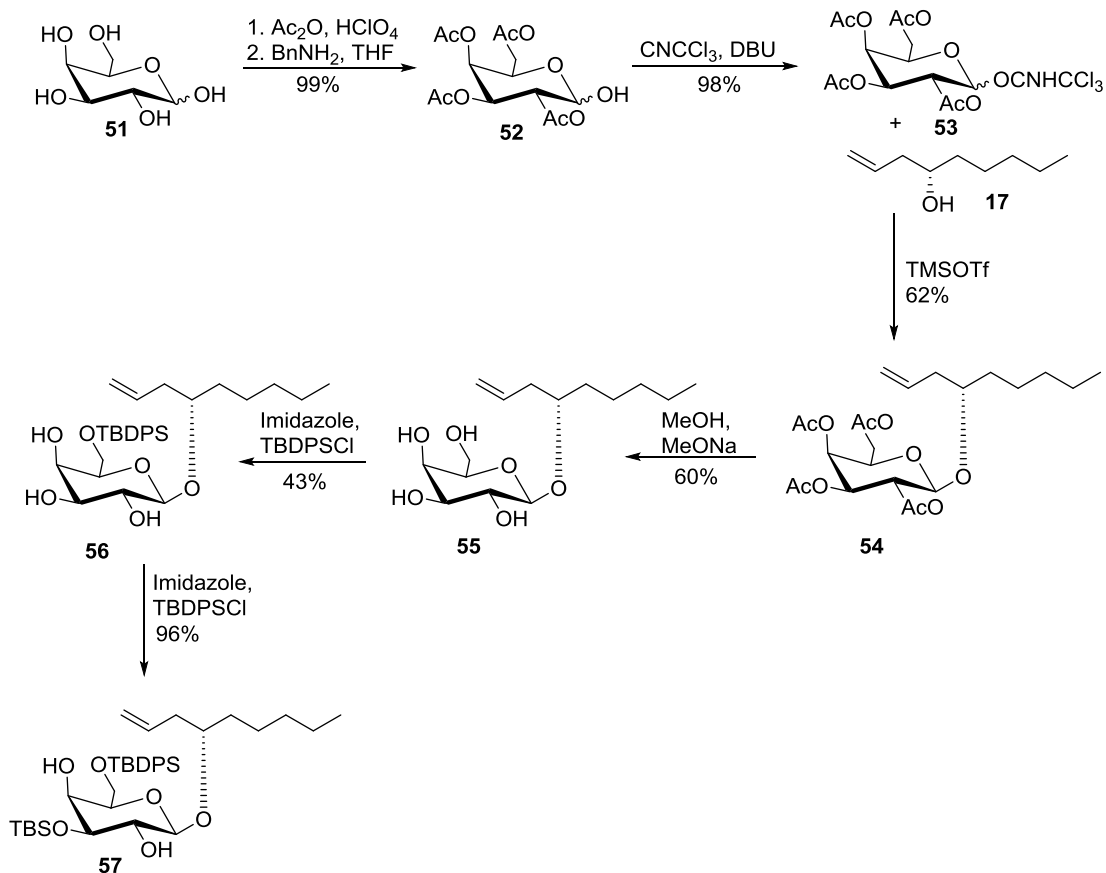
lower yield late in the synthesis, the higher donor yield and lack of tigloyl reduction should compensate for this loss. Lev was attached using DCC and DMAP for esterification of the diol to protect the 2nd and 3rd positions of **47**. Protection was followed by the cleavage of PMP on **48** with cerium ammonium nitrate and introduction of trichloroacetonitrile to **49** to produce the glucosyl donor **50**.



Scheme 10: Synthesis of Lev-protected glucosyl donor

Following synthesis of the Lev and isopropylidene-protected donor, a galactoside acceptor ring **57** was constructed by protecting the 4th and 6th positions prior to glycosylation (Sch. 11). As outlined in Scheme 6, (*R*)-hept-1-en-4-ol (**17**) was synthesized from *R*-epichlorohydrin in excellent yield. After removing the anomeric acetate from globally acetylated galactose to produce **52**, trichloroacetonitrile was connected⁶⁵ to produce the galactosyl donor **53**, which was able to easily undergo TMSOTf-catalyzed glycosylation with the secondary alcohol **17**. Since the 6th position of the globally deprotected galactose is most reactive, the 6-OH-Galp was first protected with *tert*-

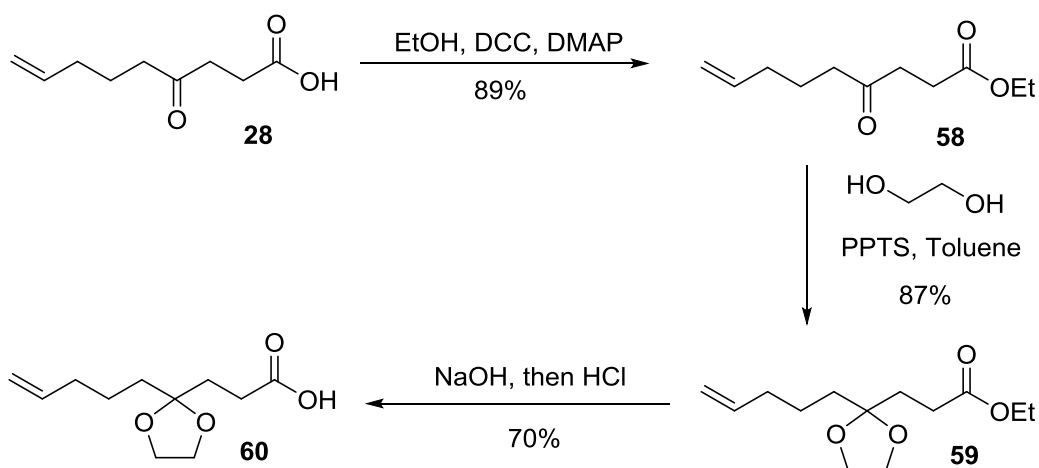
butyldiphenylsilyl (TBDPS) to give **56**. This functional group was chosen due to its stability during the harsh deprotection conditions needed for the removal of the isopropylidene and levuloyl groups. Although *tert*-butylsilyl (TBS) will remain anchored on the 3rd position during addition of a strong base, the more reactive 6-OH-Galp requires a more stable protecting group. As outlined in literature, the 3-OH position can be selectively silylated due to nucleophilicity caused by hydrogen bonding with the proximal hydroxyl group; therefore, the TBS-protected galactose acceptor **57** was obtained in 96% yield.



Scheme 11: Synthesis of galactoside acceptor

Following glycosylation, a ketone-protected aglycone **60** was synthesized over five steps from succinic anhydride, as outlined in literature.⁵³ Ring opening of succinic anhydride (**26**) was accomplished with 4-pentenylmagnesiumbromide to produce the aglycone **28** used for

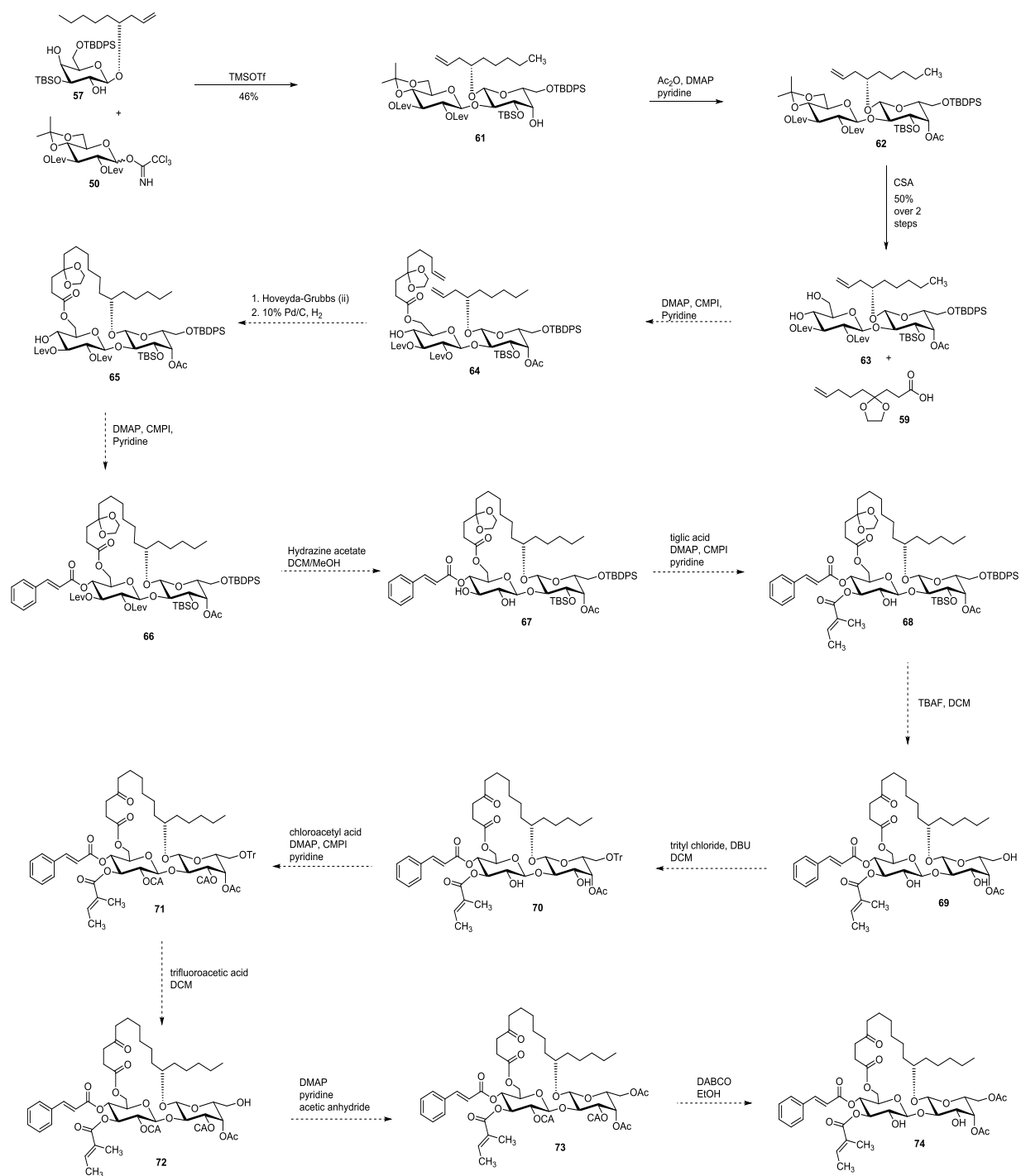
monosaccharide synthesis (*Sch. 12*). However, further modifications were necessary to prevent a reaction of hydrazine acetate with the unprotected ketone. Direct reduction to the ketal **28** using ethylene glycol was not possible, but a more indirect route of esterification with ethanol **28** \rightarrow **58** followed by treatment with ethylene glycol afforded the protected ketone **59**. Deprotection of **59** was achieved by treatment of the ester with sodium hydroxide, followed by hydrochloric acid to give the carboxylic acid **60**.



Scheme 12: Synthesis of ketal-protected aglycone

In order to determine the role of hydrophobicity and potentially increase overall cytotoxicity of the compound, a small library of galactoside analogues can be synthesized following the pattern outlined in Scheme 13. After glycosylation of **50** and **57** and addition of an acetyl group to the 4-OH Fucp of **61**, the isopropylidene protecting group can be removed from the 4th and 6th positions of **62** without damage to other functional groups. **59** can be added to the 6th position of the donor, followed by RCM and hydrogenation **64** \rightarrow **65**, as previously described (*Sch. 8*). Due to their installation late in the synthesis, the cinnamoyl and tigloyl moieties can be attached with no olefin reduction. Prior to tiglate addition, the levuloyl functional groups can be deprotected from **66** using hydrazine acetate, with no risk of damaging the ketone on the lipophilic

fatty acid tether. Subsequently, the TBDPS and TBS protecting groups, then the acetal can be deprotected in one pot to give the triol **69**. Previous studies involving a hydroxyl-substituted aglycone chain and protected 6-OH have shown that TBDPS and TBS cannot be selectively deprotected with sufficient yield. However, due to the higher reactivity of the sixth position of galactose, a trityl protecting group can be specifically introduced to 6-Galp of **69**, leaving 3-Galp and 2-Glup of **70** open to protection with α -chloroacetate (CA). The trityl group can then be removed with trifluoroacetic acid, leaving the free hydroxyl group on **72** open to modification. Several analogues, including acetyl and phenyl-substituted functional groups at the sixth position of the fucosyl group on **72** will be created, followed by deprotection of CA from **73** to produce a mini-library, including analogue **74**. These modifications will allow determination of the hydrophobicity and protein-interaction properties of the right-hand carbasugar and further elucidation of the overall conformation and binding mechanisms of ipomoeassin F.



Scheme 13: Synthesis of ipomoeassin F galactose analogue mini-library

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CHAPTER 3: Design, Synthesis and Biological Evaluation of Fucose-Truncated Monosaccharide Analogues of Ipomoessin F

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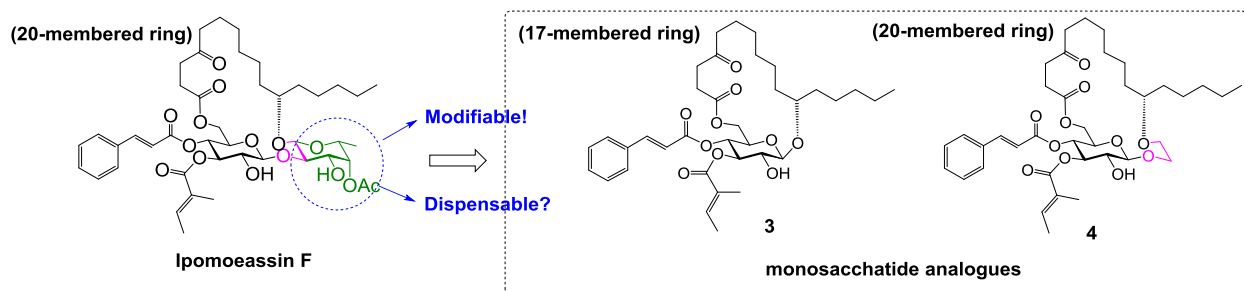
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Notes: The authors report no conflicts of interest.

Table of Content Graphic



Abstract

Ipomoeassin F is a plant-derived macrocyclic glycolipid with single-digit nanomolar IC_{50} values against cancer cell growth. In previous structure–activity relationship studies, we have demonstrated that certain modifications around the fucoside moiety did not cause significant cytotoxicity loss. To further elucidate the effect of the fucoside moiety on the biological activity, we describe here the design and synthesis of several fucose-truncated monosaccharide analogues of ipomoeassin F. Subsequent biological evaluation strongly suggests that the 6-membered ring of the fucoside moiety is essential to the overall conformation of the molecule, thereby influencing bioactivity.

Keywords

Resin glycosides, Ipomoeassin F, Monosaccharide analogues, Macrolide, Cytotoxicity

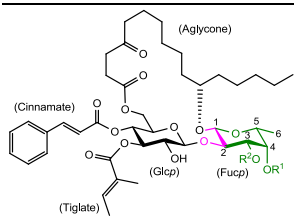
Introduction

Resin glycosides are amphipathic secondary metabolites unique to the morning glory family, *Convolvulaceae*. Their broad bioactivities, such as anticancer, antibacterial, biosurfactant, ionophoretic, purgative and plant growth controlling activities, have attracted more and more attention from phytochemists and pharmaceutical chemists. The common macrolide structure of resin glycosides contains a hydrophobic C-11 hydroxylated fatty acid aglycone and a hydrophilic oligosaccharide. The latter usually comprises two to six sugar units, part or entire portion of which forms the ring structure with the fatty acid.¹ Although monosaccharide cyclic glycolipids have been isolated from other families of plants, no resin glycosides containing a single carbohydrate moiety have been reported to date.

Ipomoeassins, a family of resin glycosides containing an embedded disaccharide, were discovered by Kingston's group in 2005 and 2007.² Whereas most resin glycosides showed micromolar cytotoxicity, several members of the ipomoeassin family exhibited low to single-digit nanomolar IC₅₀ values. Moreover, the naturally most abundant member of the family, ipomoeassin A, was screened against the NCI-60 tumor cell lines and its cytotoxicity profile is well distinguished from those of other anticancer agents in the database.⁵ Therefore, the ipomoeassins quickly inspired synthetic chemists to tackle their total syntheses. In particular ipomoeassin F (Table 1) has been an attractive synthetic target due to its highest potency.^{3,4}

Table 2: Structures and IC₅₀ Values of Ipomoeassin F and Its Analogues.

Compounds	structure		IC ₅₀ (nM)				
	R ¹	R ²	MDA-MB-231	MCF7	HeLa	U937	Jurkat
Ipomoeassin F	Ac	H	6.5	43.7	16.4	5.4	6.1
1	H	H	131	86.7	133	72.5	139
2	Ac	Ac	16.5	216	138	79.8	82.6



Recently, we developed a new efficient gram-scale synthesis of ipomoeassin F and conducted its most systematic structure–activity relationship (SAR) studies to date.^{6,7} During the studies, we found that the two α,β -unsaturated esters in the glucoside moiety, that is, cinnamate and tiglate (Table 1), are the most critical to the cytotoxicity of ipomoeassin F. On the other hand, modifications of the fucoside moiety,⁷ i.e. removal of the acetyl group from 4-OH-Fucp (analogue **1**, Table 1) or introduction of an acetyl group to 3-OH-Fucp (analogue **2**, Table 1), did not cause a dramatic cytotoxicity loss for the five tested cancer cell lines (2–23 fold loss for **1** and 2–14 fold loss for **2**, respectively). The IC₅₀ values of both **1** and **2** are largely still below 150 nM; therefore, the contribution of the whole fucoside moiety to the cytotoxicity is of great interest. In addition, given the high price of D-fucose (\$427/5g, Sigma-Aldrich), relatively potent monosaccharide analogues (IC₅₀ < 0.5 μ M) of ipomoeassin F without the fucoside moiety would significantly decrease the production cost and also shorten the synthetic route, thereby benefiting future ipomoeassin research in drug discovery and chemical biology.

Results and Discussion

Molecular Design. In earlier studies, we proved that some peripheral modifications of the fucoside moiety could be well withstood (analogues **1** and **2**, Table 1). To further elucidate the function of the fucoside moiety, analogue **3** (*Fig. 6*) was first designed, in which the entire fucoside moiety is removed. This analogue also changes the ring size from 20-membered ring in ipomoeassin F to 17-membered ring. Because ring size may have a great impact biological activity, we also designed analogue **4** (*Fig. 6*) in which the fucoside moiety is partially truncated; therefore, **4** maintains the same ring size as ipomoeassin F. Using both analogues **3** and **4**, we hope to address the question of whether the fucoside ring is dispensable or not.

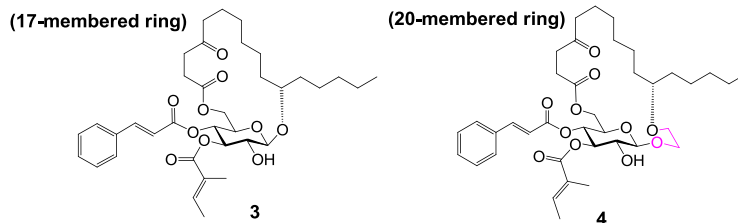
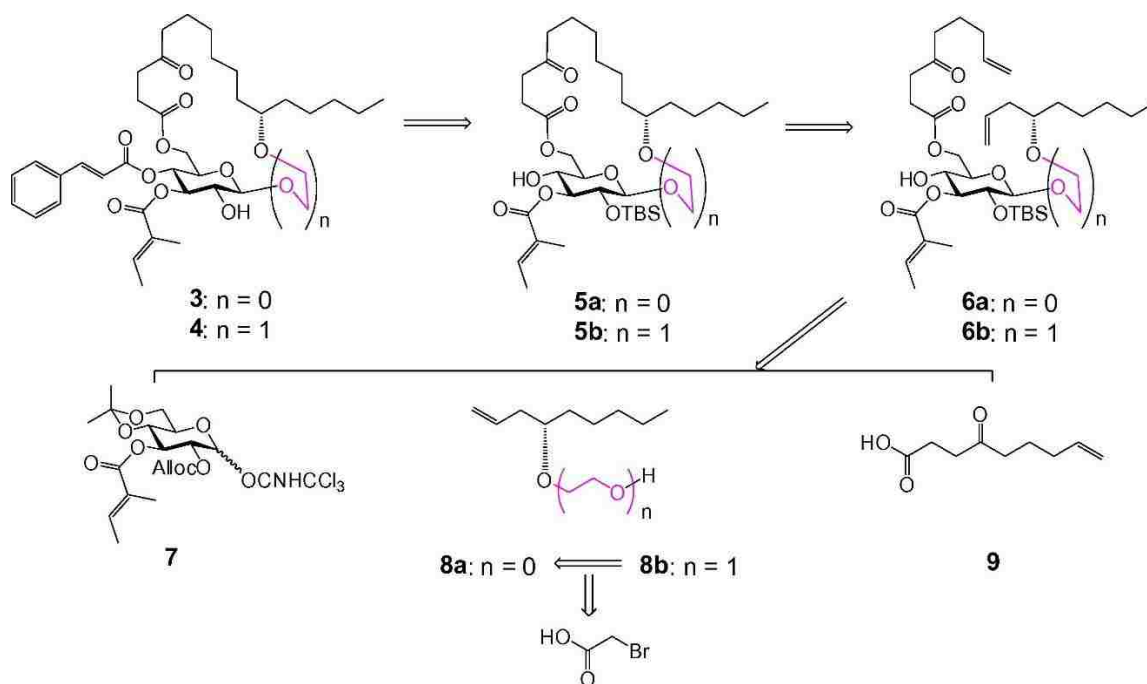


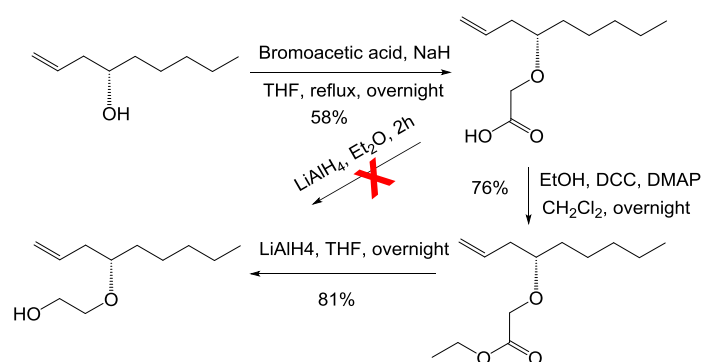
Figure 6: Design of the monosaccharide analogues **3** and **4**.

Synthetic Strategy. The two monosaccharide analogues **3** and **4** can be straightforwardly synthesized by using the same strategy we developed for the total synthesis of ipomoeassin F (Sch. 14).⁶ From the diene intermediate **6a/b**, ring-closing metathesis (RCM) and hydrogenation were still adopted to construct the saturated ring structure in **5a/b**, to which cinnamate could be introduced, followed by removal of the TBS group, to give the desired monosaccharide analogues **3** and **4**. To control the β -linkage in **6a/b**, the glucosyl donor **7**⁶ with Alloc as the neighboring participation group was chosen to couple with the alcohol acceptor **8a/b**. After that, replacement of the Alloc group with TBS, followed by removal of isopropylidene and then chemoselective esterification with 4-oxo-8-nonenic acid **9**,⁸ would lead to the key diene intermediate **6a/b** as the RCM precursor. Synthesis of the acceptor **8b** could be achieved by alkylation of alcohol **8a**³ with bromoacetic acid, followed by the esterification and reduction procedure.



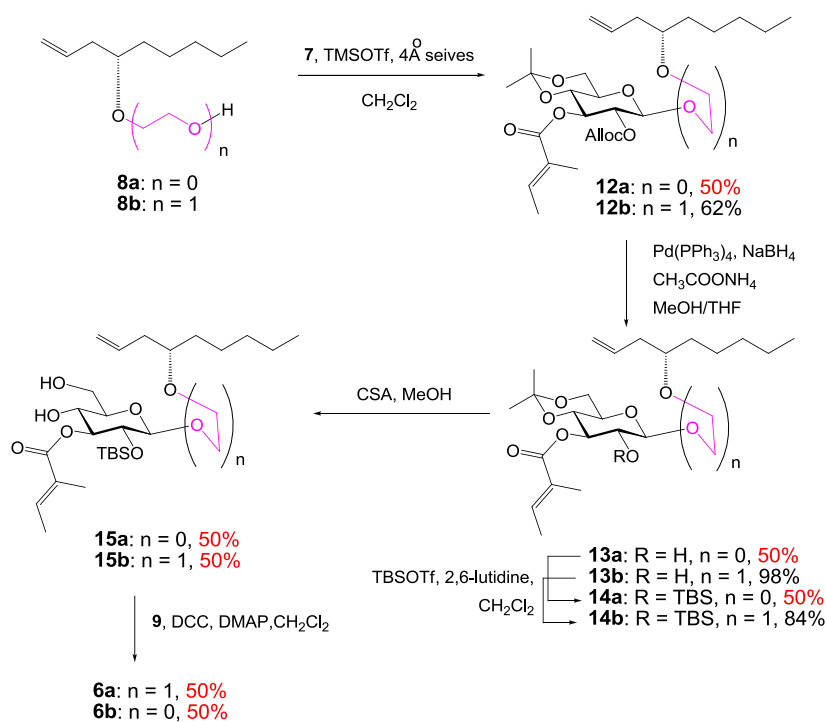
Scheme 14: Retrosynthesis of monosaccharide analogues 3 and 4

Synthesis of the Acceptor 8b. The synthesis of **8b** is outlined in Scheme 15. Alkylation of the other acceptor **8a**³ with bromoacetic acid in the presence of NaH afforded acid **10** in moderate yield. Direct reduction of acid **10** using LiAlH₄ was not successful. Then we converted acid **10** to ethyl ester **11** first, followed by reduction with LiAlH₄ to give **8b** in 62% overall yield over two steps.



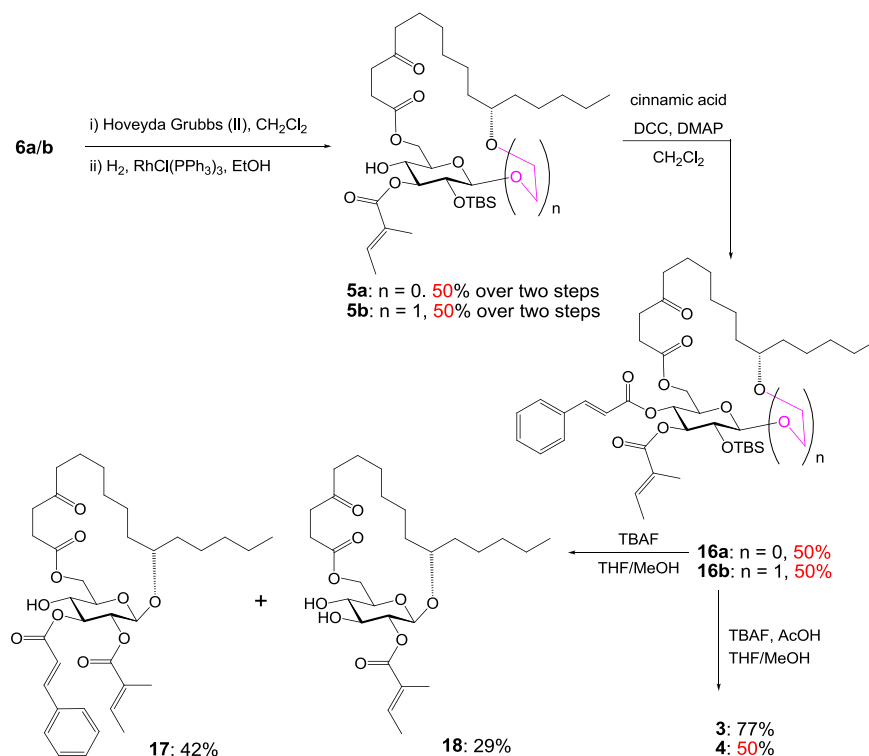
Scheme 15: Synthesis of the acceptor 8b

Synthesis of the RCM Precursor 6a/b. The Glucosyl donor **7** was synthesized by following the route we developed previously. With both glucosyl donor and two acceptors in hand, the RCM precursor **6a/b** was synthesized as shown in Scheme 16. Schmidt glycosylation of **7** with acceptor **8a/b** promoted by TMSOTf in cold CH₂Cl₂ gave β-linked glycoside **12a** in 62% yield or **12b** in 50% yield. Alloc group was then selectively removed in the presence of CH₃COONH₄, Pd[P(C₆H₅)₃]₄, and NaBH₄ in excellent yield to give alcohol **13a/b** within 4 min.⁹ **13a/b** was then treated with *tert*-butyldimethylsilyl triflate (TBSOTf) in the presence of 2,6-lutidine to give silyl ether **14a** in 84% yield or **14b** in 50% yield. Removal of isopropylidene using camphorsulfonic acid (CSA) in MeOH afforded diol **15a/b** without any problem. The subsequent chemoselective Steglich esterification of diol **15a/b** with 4-oxo-8-nonenic acid **9** to give diene **6a/b** as the key RCM precursor.



Scheme 16: Synthesis of diene **6a/b**

Syntheses of Monosaccharide Analogues 3 and 4. RCM of **6a/b** using Hoveyda-Grubbs catalyst (II)¹⁰ (10 mol%) under refluxing in CH₂Cl₂, followed by hydrogenation of the resulting alkene isomers over Wilkinson's catalyst, constructed the desired ring structure (50% for **5a** and 50% for **5b**) over two steps (*Sch. 17*). Cinnamate ester was then introduced to **5a/b** through Mukaiyama esterification to afford the fully protected compound **16a/b** in high yield. In the last step, removal of TBS using TBAF in cold THF/MeOH was first tried on substrate **16a**. Unfortunately, instead of forming the desired monosaccharide analogue **3**, the acyl-migrated compound **17** was obtained as the major product (42% yield) along with **18** as the minor product (29% yield). The structures of compounds **17** and **18** were confirmed by ¹H, ¹³C, COSY and HMBC NMRs (*Fig. 7*). Treatment of **15a/b** with TBAF and acetic acid in THF/MeOH finally delivered the desired monosaccharide analogues in good yield (50% for **3** and 77% for **4**).



Scheme 17: Synthesis of monosaccharide analogues 3 and 4

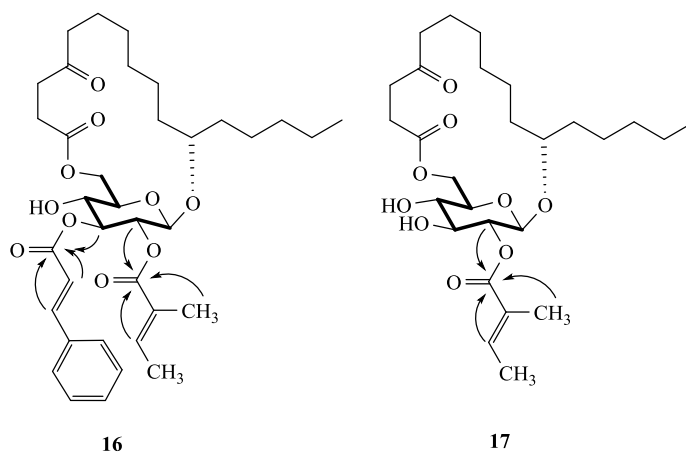


Figure 7: Key COSY (**bold**) and HMBC (arrows) correlations in **17** and **18**.

Cytotoxicity of Ipomoeassin F and analogues. Using the fluorescent alamarBlue or colorimetric MTT assay,¹¹ the cytotoxicity of monosaccharide analogues **3** and **4**, together with the two unexpected analogues **17** and **18**, was evaluated against two breast cancer cell lines (MDA-MB-231 and MCF7) using ipomoeassin F as the positive control and vehicle-treated cells as the negative control. Compared to ipomoeassin F, all the four analogues showed no appreciable inhibition of cell growth at 10 μ M, demonstrating that the fucoside moiety is indispensable for the cytotoxicity of the ipomoeassins.

Conclusions

In summary, we report here the synthesis and biological evaluation of four monosaccharide analogues of ipomoeassin F, **3**, **4**, **17** and **18**, consisting of a truncated carbohydrate core. The fucoside moiety was completely or partially removed from the disaccharide portion of the original natural product. Evaluation of these compounds against two breast cancer cell lines unambiguously revealed the vital role of the pyranose ring of the fucoside moiety in the biological activity of ipomoeassin F, very likely through conformational control. Further studies of using

other economical carbohydrate building blocks to replace expensive D-fucose are planned to consolidate and enrich the SAR data, while improving the production efficiency. In this regard, D-galactose is the most attractive to us (*Fig. 8*). The small 6-OH-Galp may provide an anchor point for introduction of new functional moieties to better biological activities and/or facilitate the discovery of molecular targets.

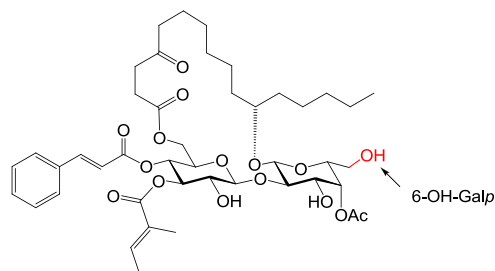


Figure 8: D-galactose-containing analogue of ipomoeassin F.

Acknowledgments

This work was primarily supported by the startup funds from the University of Arkansas and also in part by Grant Number P30GM103450 and R15GM116032 from the National Institute of General Medical Sciences of the National Institutes of Health (NIH) and by the seed money from the Arkansas Biosciences Institute (ABI). We wish to thank Dr. Jianjun Zhang at CAU (China Agricultural University) for providing per-acetylated D-fucopyranose (NKT R&D Program of China, 2015BAK45B01). MH is currently the recipient of a Distinguished Doctoral Fellowship from the University of Arkansas.

References

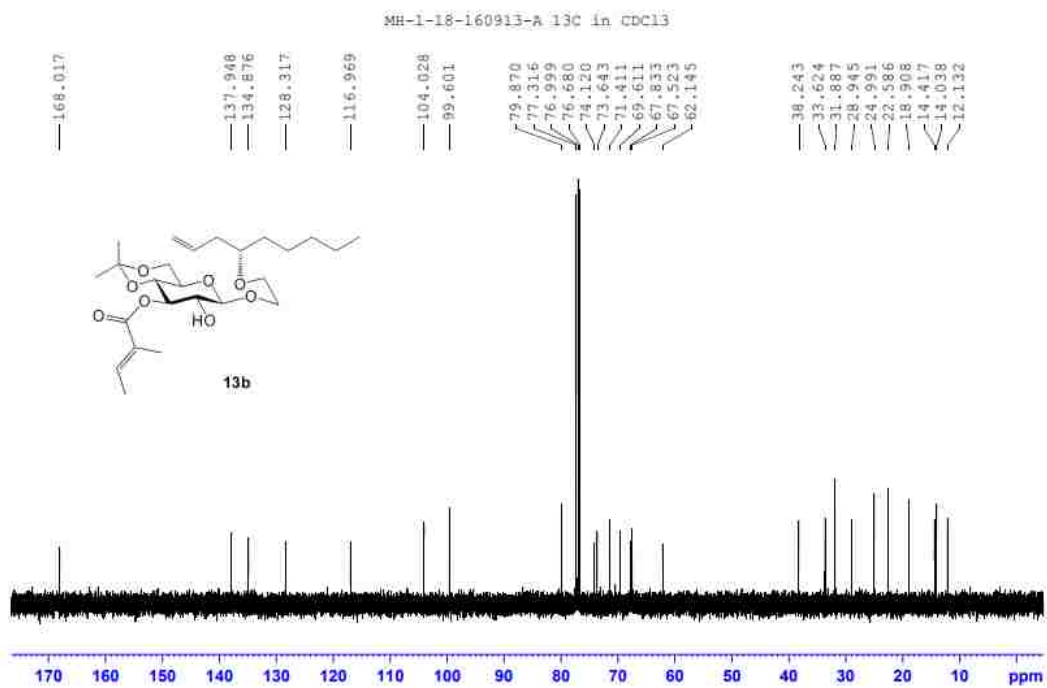
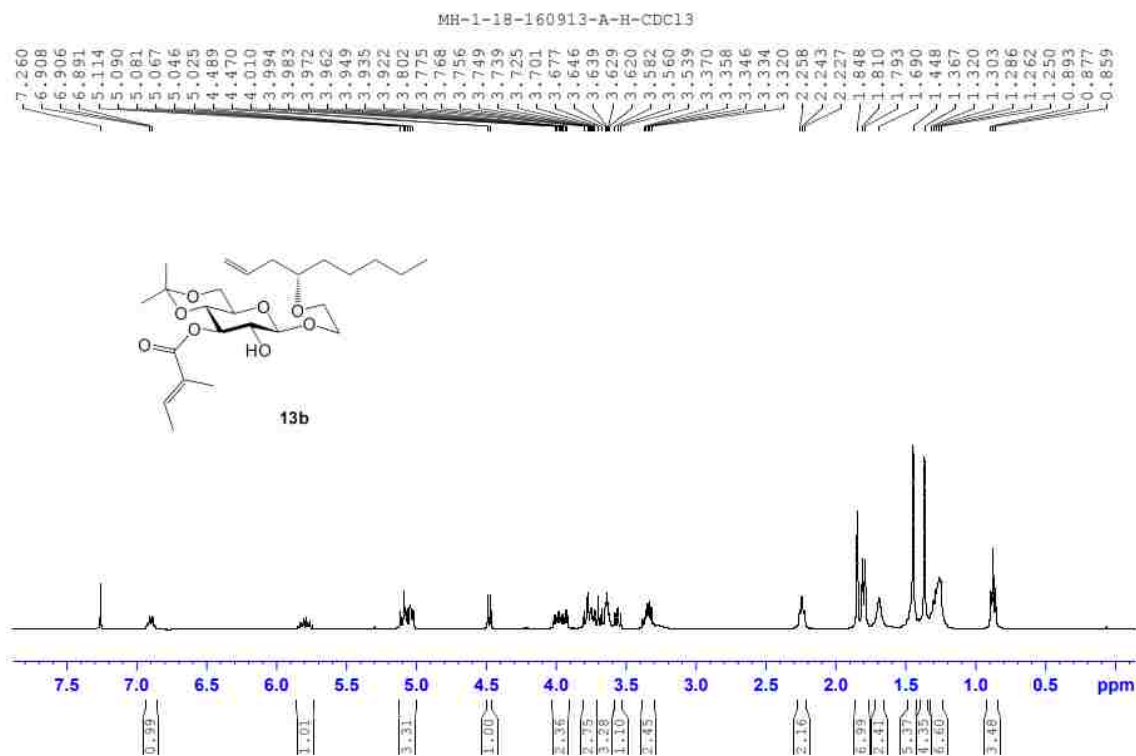
- (1) Pereda-Miranda, R.; Rosas-Ramirez, D.; Castaneda-Gomez, J. *Prog. Chem. Org. Nat. Prod.* **2010**, *92*, 77-153.
- (2) Cao, S.; Norris, A.; Wisse Jan, H.; Miller James, S.; Evans, R.; Kingston David, G. I. *Nat. Prod. Res.* **2007**, *21*, 872-6.
- (3) Nagano, T.; Pospisil, J.; Chollet, G.; Schulthoff, S.; Hickmann, V.; Moulin, E.; Herrmann, J.; Mueller, R.; Fuerstner, A. *Chem. Eur. J.* **2009**, *15*, 9697-9706, S9697/1-S9697/48.
- (4) Postema, M. H. D.; TenDyke, K.; Cutter, J.; Kuznetsov, G.; Xu, Q.. *Org. Lett.* **2009**, *11*, 1417-1420.
- (5) Kingston, D. G. I. *J. Org. Chem.* **2008**, *73*, 3975-3984.
- (6) Zong, G.; Barber, E.; Aljewari, H.; Zhou, J.; Hu, Z.; Du, Y.; Shi, W. Q. *J. Org. Chem.* **2015**, *80*, 9279-9291.
- (7) Zong, G.; Aljewari, H.; Hu, Z.; Shi, W. Q. *Org. Lett.* **2016**, *18*, 1674-1677.
- (8) Lhommet, G.; Freville, S.; Thuy, V.; Petit, H.; Celerier, J. P. *Syn. Comm.* **1996**, *26*, 3897-3901.
- (9) Zong, G. H.; Yan, S. Q.; Liang, X. M.; Zhang, J. J.; Wang, D. Q.; Kong, F. Z. *Chi. Chem. Lett.* **2009**, *20*, 127-130.

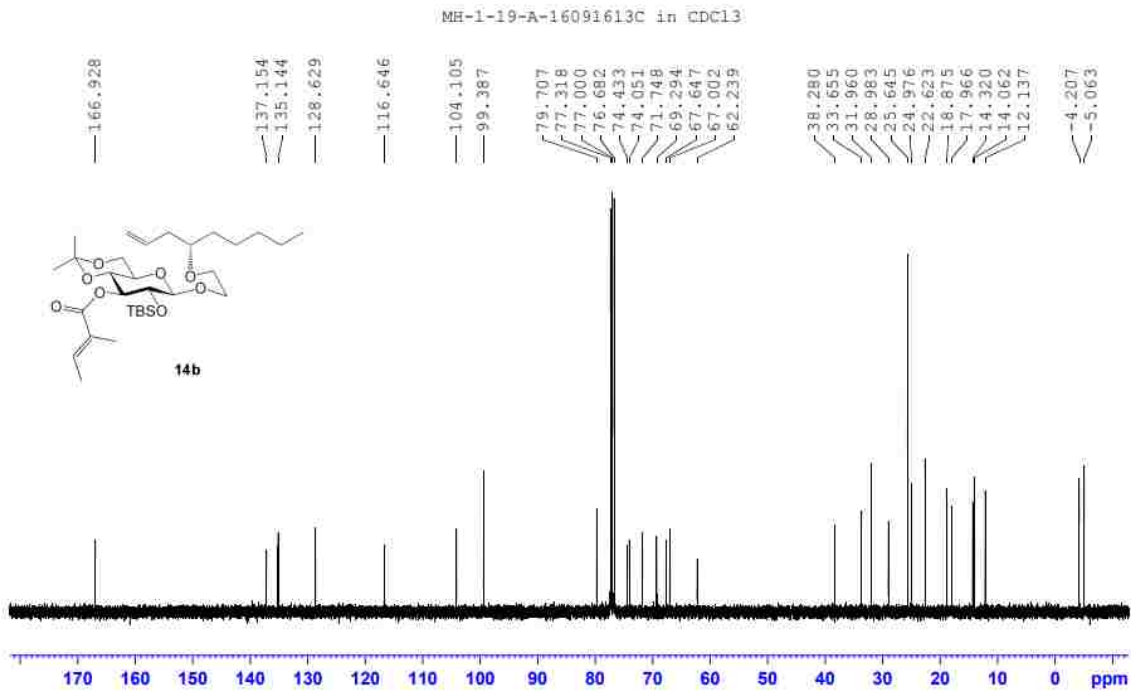
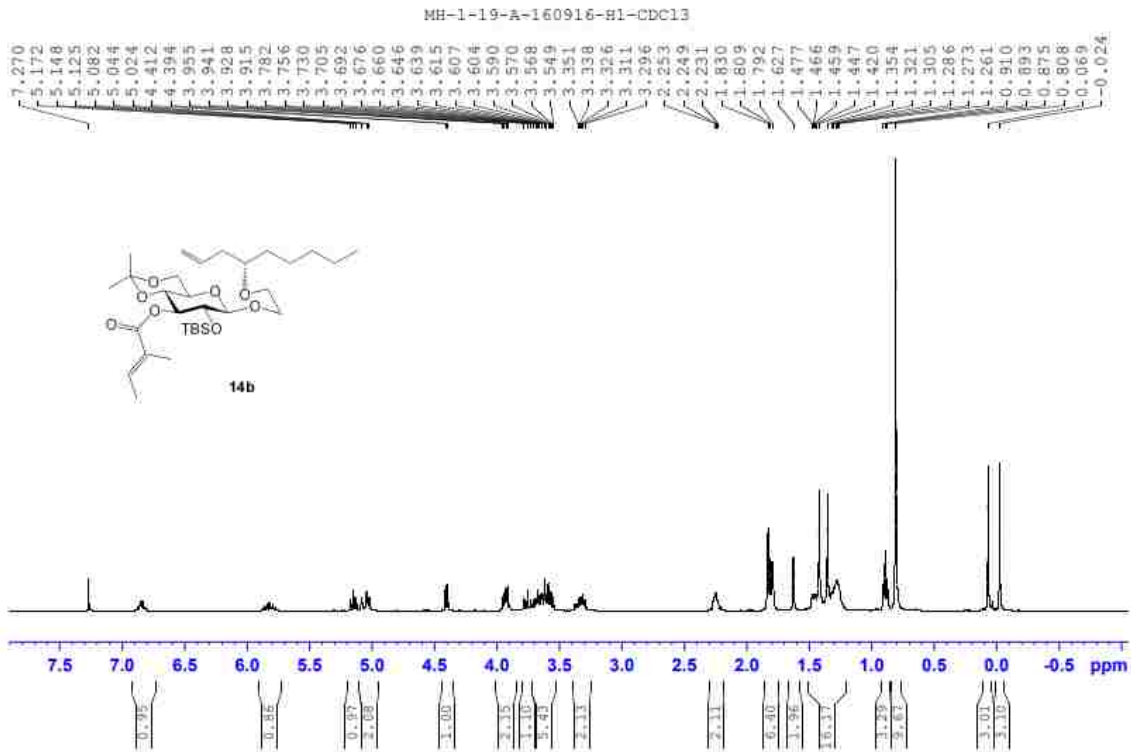
CHAPTER 4: Conclusions

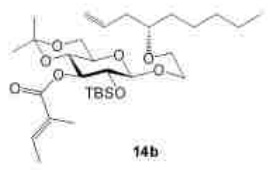
The Postema and Fuerstner groups have reported different methodologies for the synthesis of ipomoeassin F, each with several impediments. The Shi group improved this synthesis route by introducing new protecting group strategies to increase the overall yield and facilitate structure-activity relationship (SAR) studies, which showed the fucoside moiety to be unimportant to the overall cytotoxicity of the compound.

Based on the results of the SAR studies, we successfully completed and published the syntheses of two separate monosaccharide analogues of ipomoeassin F. Key methodologies include the synthesis of the Alloc-protected glucosyl donor (*Sch. 4, p. 20*), carboxylic acid-derived aglycone acceptor (*Sch. 6, p. 22*) and Hoveyda-Grubbs-catalyzed RCM (*Sch. 8, p. 24*). Although SAR studies determined several functional groups on the fucoside acceptor to be unnecessary for cytotoxicity retention, removal of the entire structure resulted in a complete loss of biological activity. Therefore, studies to substitute the fucoside acceptor ring with a less expensive carbasugar such as galactose, glucose or *D*-arabinose are in progress.

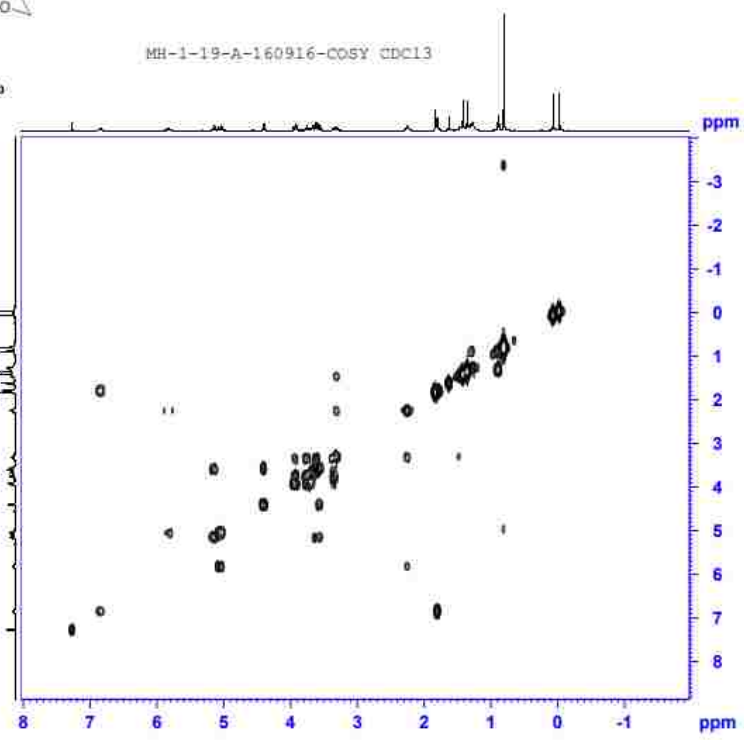
Appendix 1: Experimental—Selected Spectra







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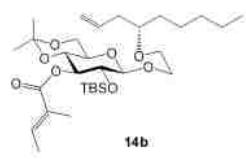
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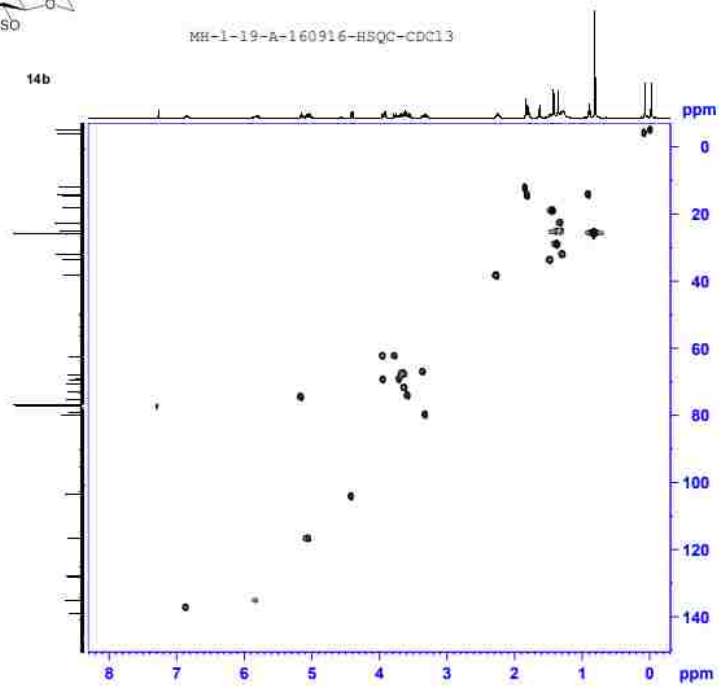
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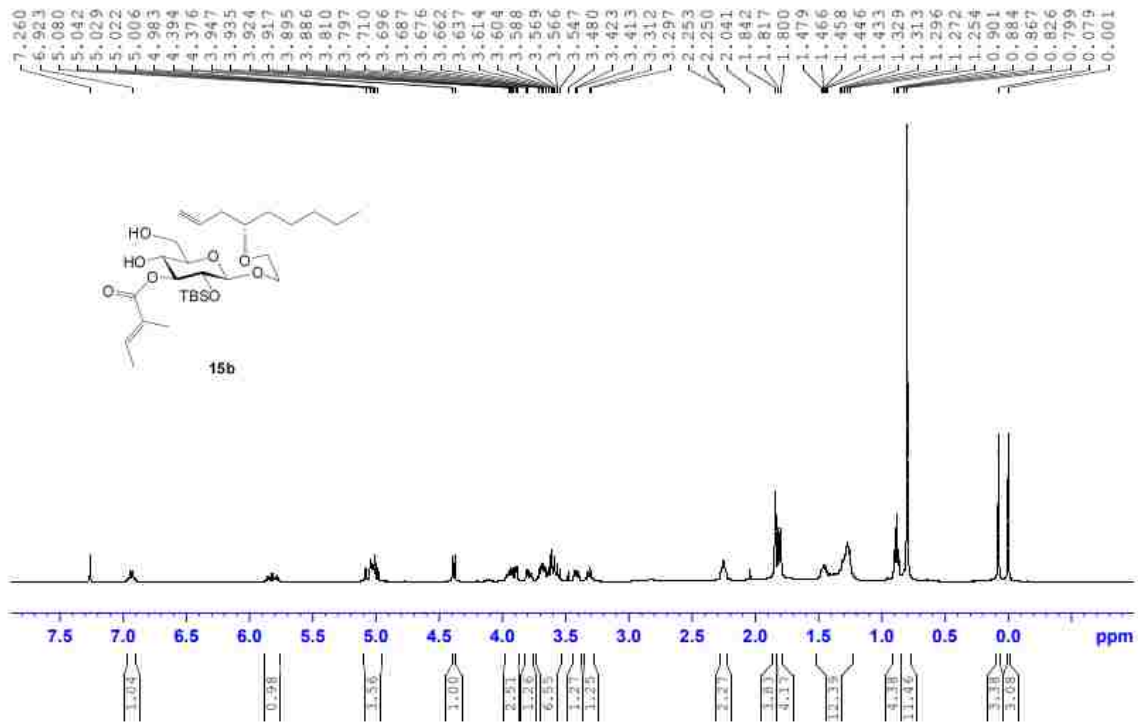
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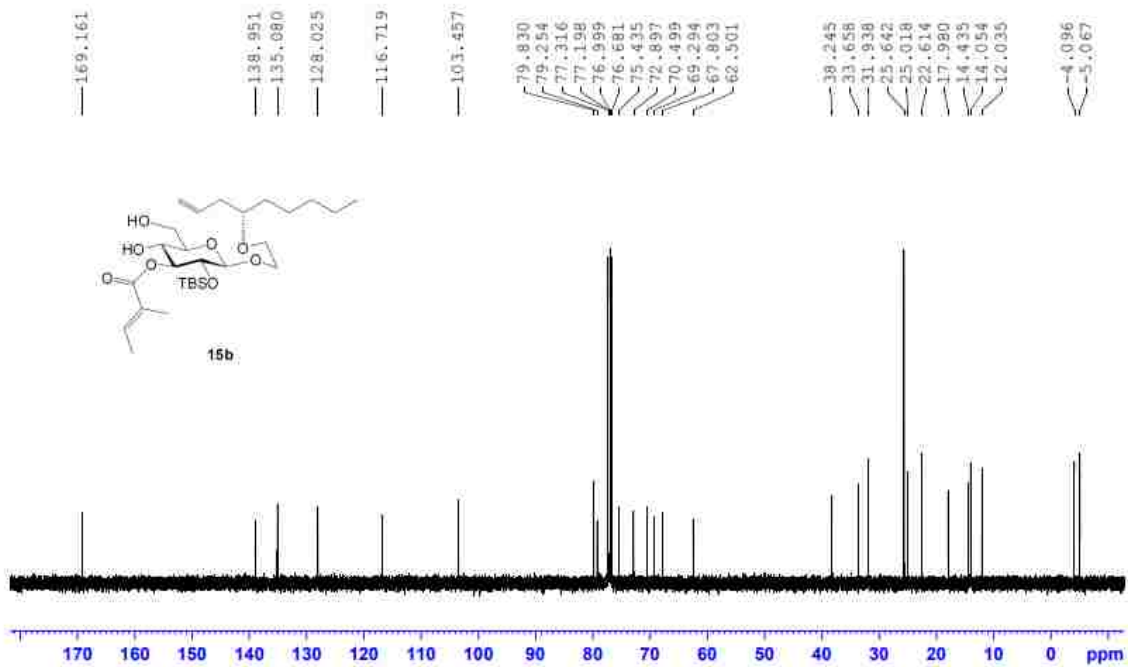
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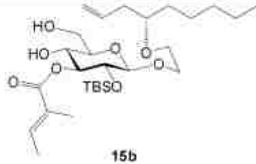
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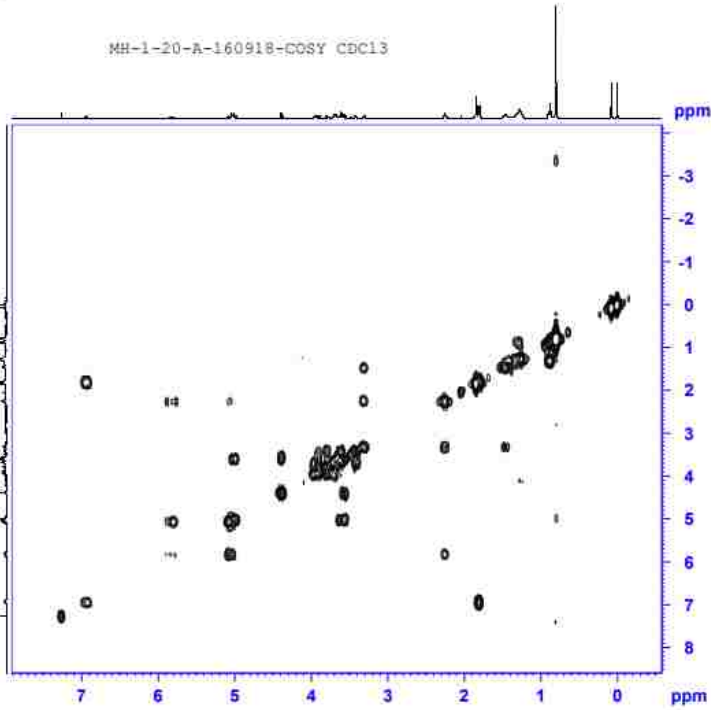


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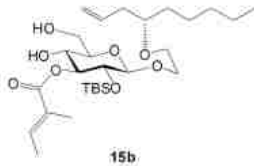
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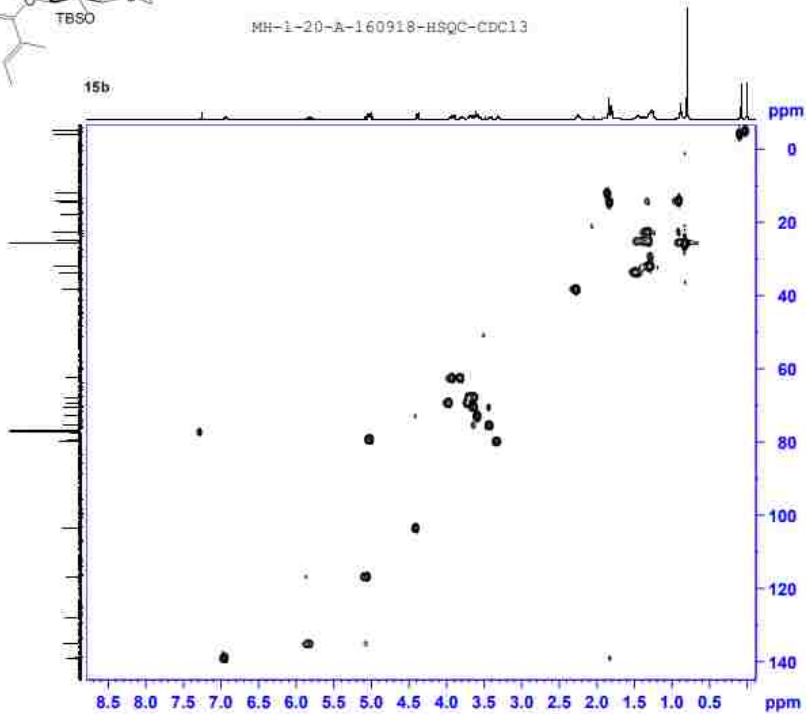
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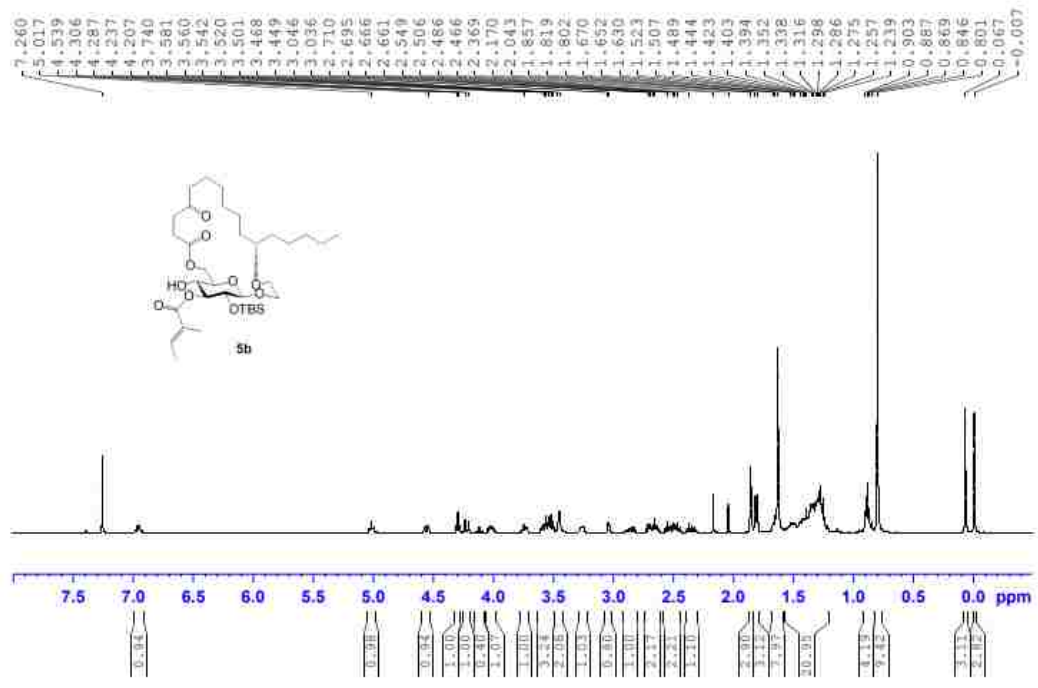


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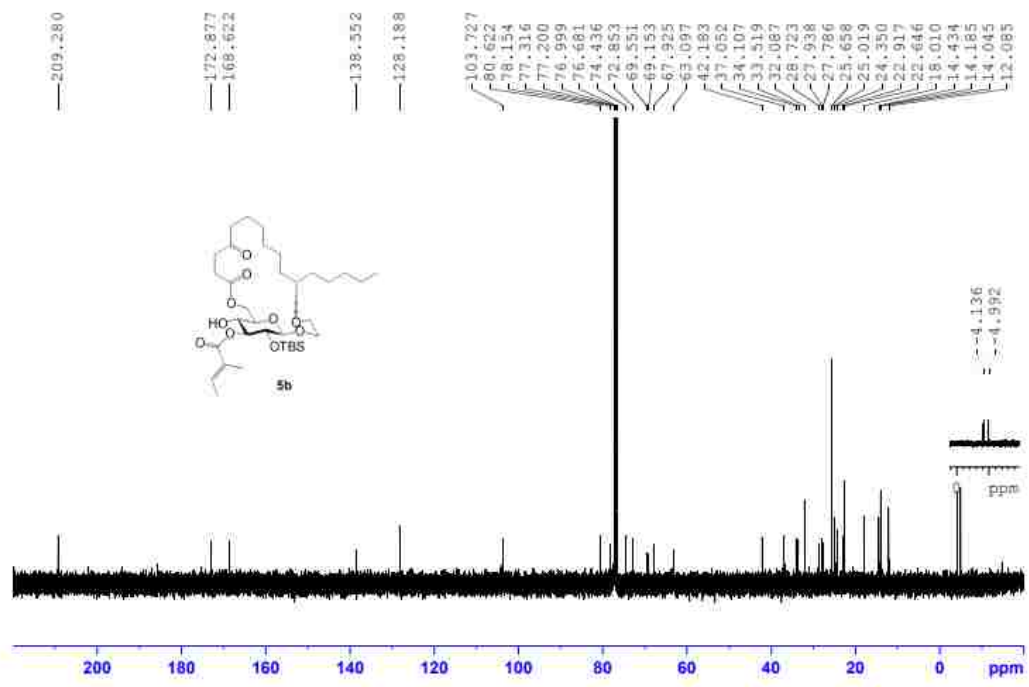
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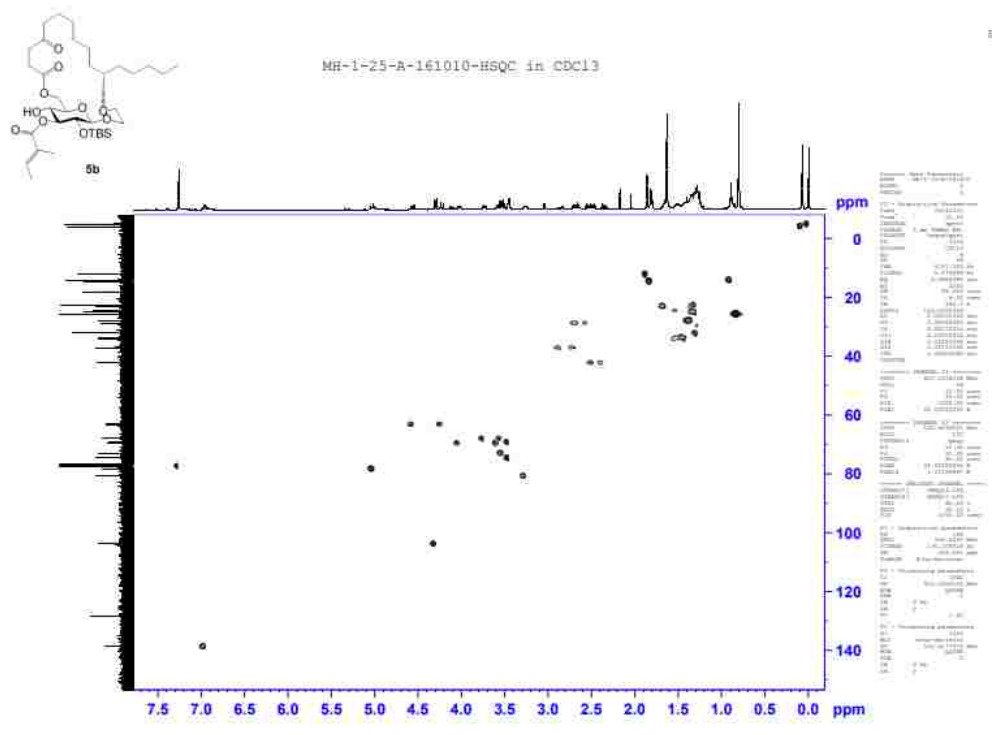
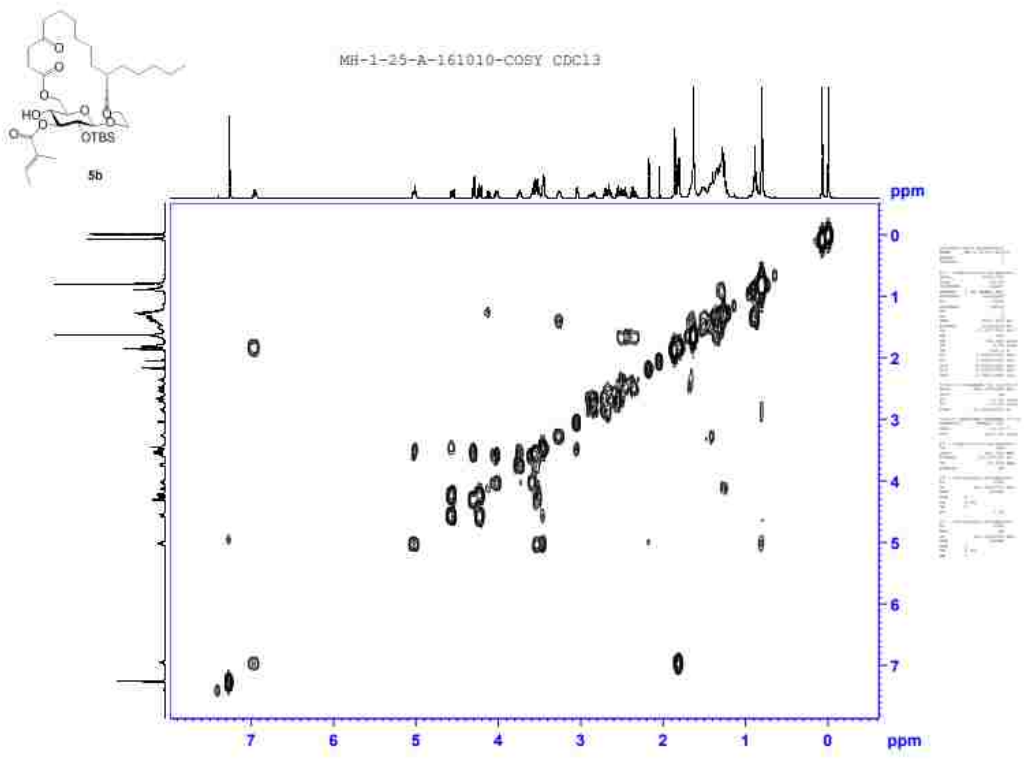
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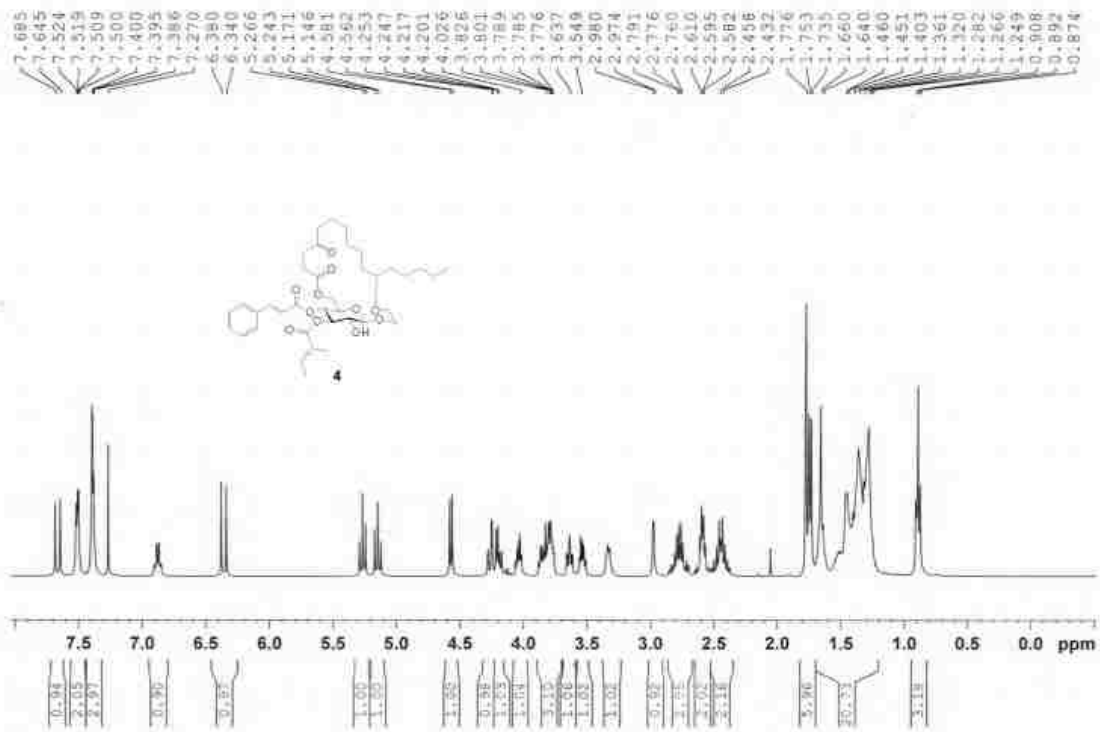
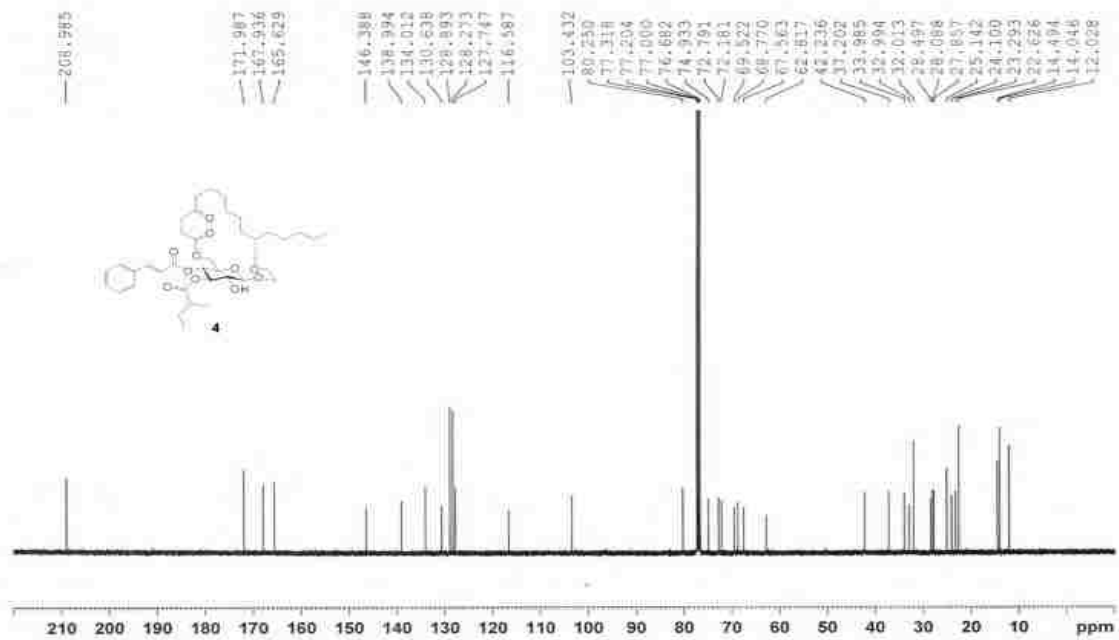

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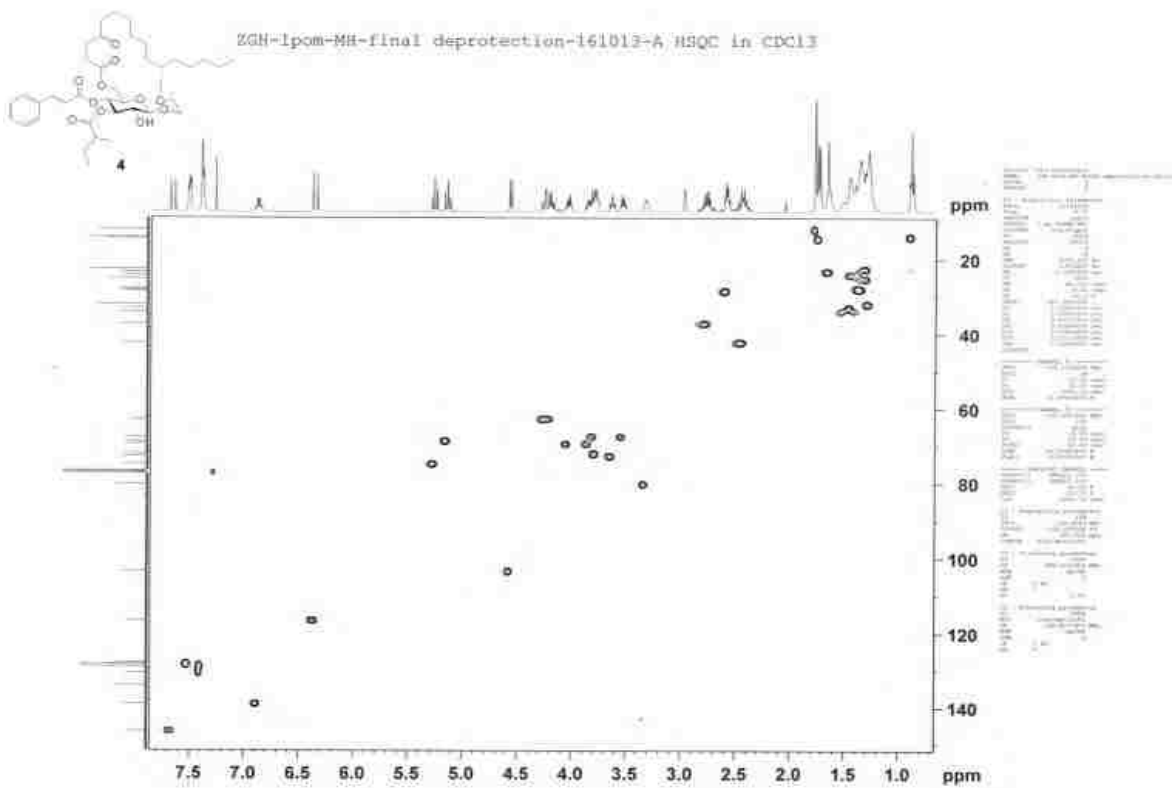
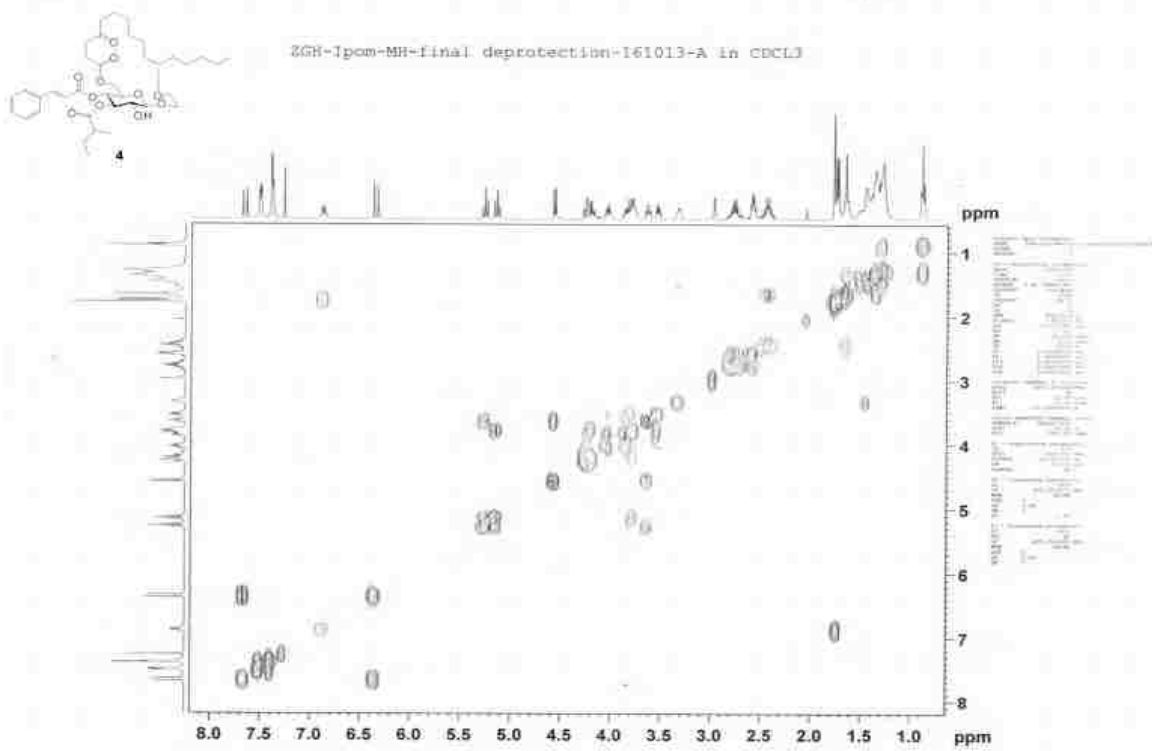


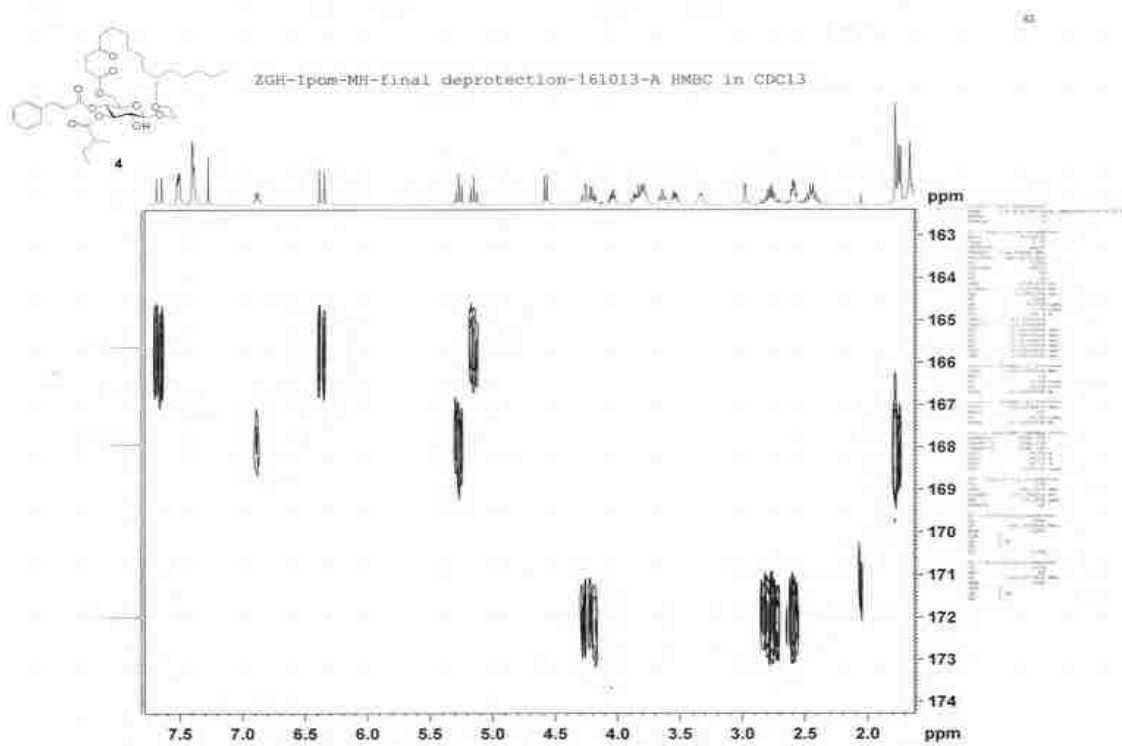
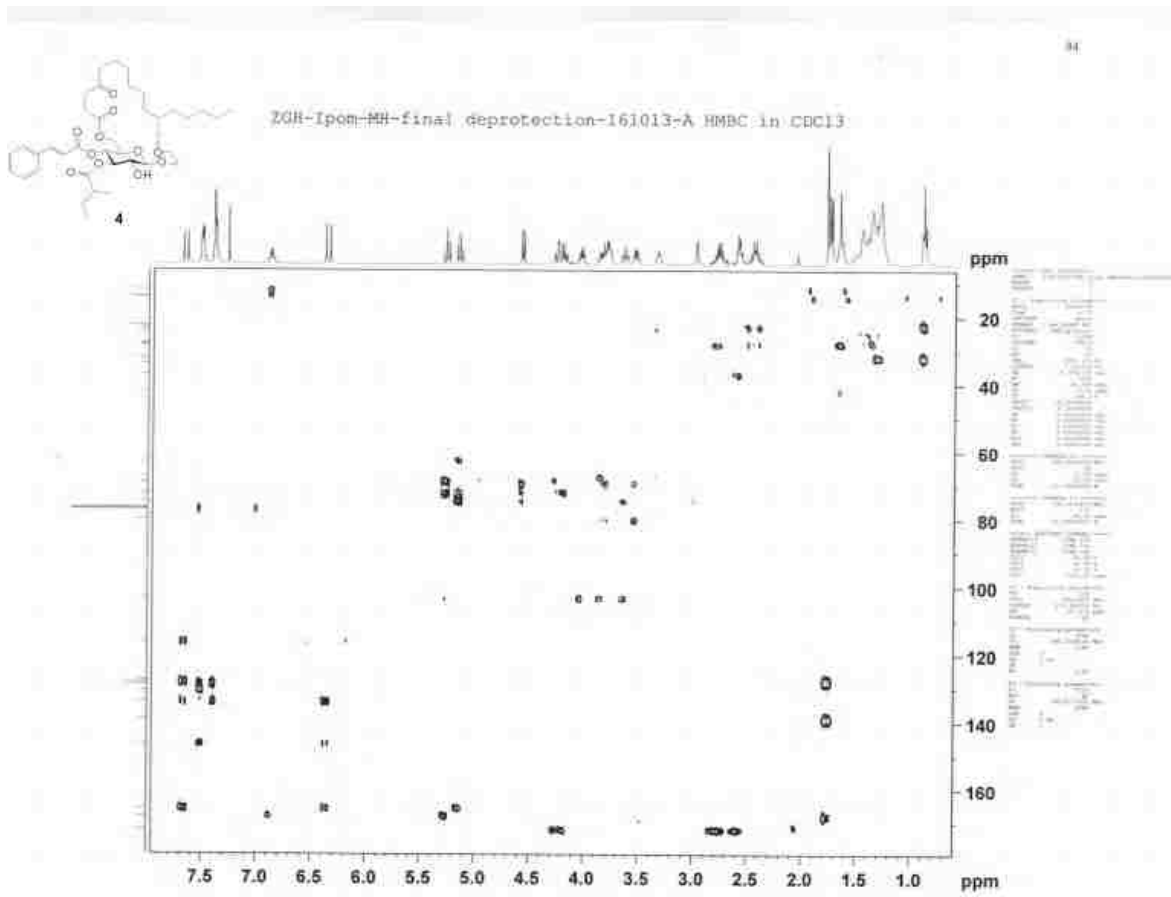
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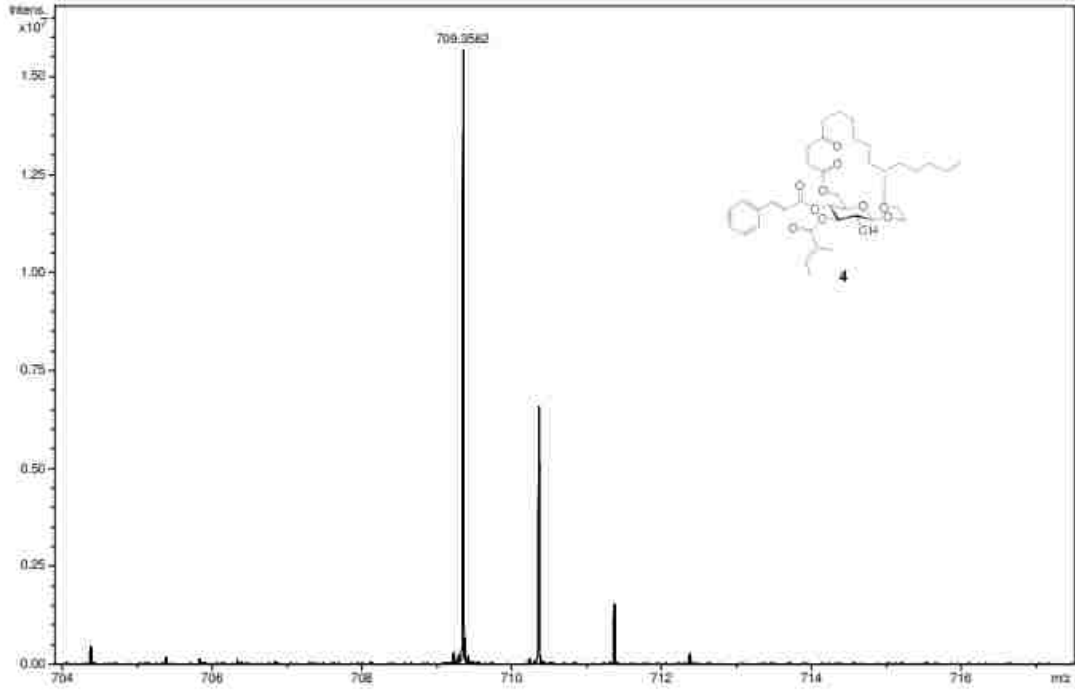


Arkansas Statewide Mass Spectrometry Facility

ESI-FTMS Mass Spectrum

Sample Name: ZF-49
Sample Id#: 917
Instrument: Bruker ApexII-FTMS

2/8/2017



Appendix 2: Experimental—Materials and Methods

General Methods

Reactions were carried out in oven-dried glassware. All reagents were purchased from commercial sources and were used without further purification unless noted. Unless stated otherwise, all reactions were carried out under a nitrogen atmosphere and monitored by thin layer chromatography (TLC) using Silica Gel GF₂₅₄ plates (Agela) with detection by charring with 5% (v/v) H₂SO₄ in EtOH or by visualizing in UV light (254 nm). Column chromatography was performed on silica gel (230–450 mesh, Sorbent). The ratio between silica gel and crude product ranged from 100 to 50:1 (w/w). NMR data were collected on a Bruker 400 MHz NMR spectrometer and a Bruker 400 MHz system. ¹H NMR spectra were obtained in deuteriochloroform (CDCl₃) with chloroform (CHCl₃, $\delta = 7.27$ for ¹H) as an internal reference. ¹³C NMR spectra were proton decoupled and were in CDCl₃ with CHCl₃ ($\delta = 77.0$ for ¹³C) as an internal reference. Chemical shifts are reported in ppm (δ). Data are presented in the form: chemical shift (multiplicity, coupling constants, and integration). ¹H data are reported as though they were first order. The errors between the coupling constants for two coupled protons were less than 0.5 Hz, and the average number was reported. Proton assignments, when made, were done so with the aid of COSY NMR spectra. For some compounds, HSQC and HMBC NMR were also applied to assign the proton signals. Optical rotations were measured on an Autopol III Automatic Polarimeter at 25 ± 1 °C for solutions in a 1.0 dm cell. High resolution mass spectrum (HRMS) and were acquired in the ESI mode. The type of the mass analyzer is FTMS.

Chemistry: Synthetic Procedures and Analytical Data

Compound 10

To an ice-cold, stirred suspension of sodium hydride (111 mg, dry NaH, 95% pure, 4.64 mmol) in THF (20 mL) was added dropwise a solution of **8a** (300 mg, 2.11 mmol) in THF (1 mL). After 30 min, a solution of bromoacetic acid (351 mg, 2.53 mmol) in THF (1 mL) was added dropwise. The resulting mixture was refluxed overnight before cooled to rt. The reaction mixture was diluted with ether (50 mL) and water (50 mL) and stirred for 1 h. The aqueous layer was separated and washed with ether (30 mL \times 2), acidified with concentrated hydrochloric acid and extracted with ethyl acetate (50 mL \times 2). The combined extracts were dried over Na₂SO₄. Evaporation and purification by column chromatography (silica, EtOAc–hexanes, 1:3 containing 0.5% AcOH) to afford compound **10** (258 mg, 58%) as a colorless oil. ¹H NMR (400 MHz, CDCl₃) δ 9.62 (s, 1H), 5.90 – 5.71 (m, 1H), 5.21 – 4.97 (m, 2H), 4.13 (s, 2H), 3.50 – 3.39 (m, 1H), 2.31 (dd, J = 7.2, 6.4 Hz, 2H), 1.64 – 1.45 (m, 2H), 1.44 – 1.19 (m, 6H), 0.89 (t, J = 6.8 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 174.4, 134.2, 117.6, 80.7, 66.0, 38.1, 33.4, 31.8, 24.8, 22.5, 14.0.

Compound 11

DCC (387 mg, 1.88 mmol) was added in one portion to a 0°C CH₂Cl₂ (2 mL) solution of **10** (188 mg, 0.939 mmol), EtOH (274 μ L, 4.69 mmol) and 4-dimethylaminopyridine (11.5 mg, 0.0939 mmol). The reaction was allowed to warm to ambient temperature and stirred overnight. At this point, TLC (silica, 1:9 EtOAc–hexanes) showed the reaction was complete. The reaction mixture was diluted with ether (2 mL) and hexanes (1 mL), stirred for 20 minutes then filtered thru a pad of celite using ether (2 mL) as the eluent and the filtrate concentrated *in vacuo*. The residue was purified by column chromatography (silica, EtOAc–hexanes, 1:15 \rightarrow 1:10) gave **11** (162 mg, 76%) as a colorless oil. ¹H NMR (400 MHz, CDCl₃) δ 5.95 – 5.80 (m, 1H), 5.19 – 5.00 (m, 2H), 4.21

(q, $J = 7.2$ Hz, 2H), 4.16 – 4.01 (m, 2H), 3.50 – 3.30 (m, 1H), 2.43 – 2.17 (m, 2H), 1.63 – 1.16 (m, 11H), 0.88 (t, $J = 6.8$ Hz, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 170.7, 134.6, 117.0, 80.2, 66.6, 60.7, 38.1, 33.5, 31.9, 24.9, 22.6, 14.2, 14.0.

Compound 8b

To an ice-cold, stirred slurry of lithium aluminium hydride (57 mg, 1.5 mmol) in THF (3 mL) was added dropwise a solution of ester **11** (156 mg, 0.683 mmol) in THF (3 mL). The resulting mixture was warmed up to rt and stirred overnight before quenched by the careful addition of 0.5 M aqueous sodium hydroxide (1 mL). The resulting grey suspension was filtered through Celite and the solid washed with THF (3 mL). The combined filtrates were concentrated *in vacuo*. The residue was purified by column chromatography (silica, EtOAc–hexanes, 1:6) gave the alcohol **8b** (103 mg, 81%) as a colorless oil. ^1H NMR (400 MHz, CDCl_3) δ 5.91 – 5.72 (m, 1H, H-2), 5.15 – 5.00 (m, 2H, H-1), 3.80 – 3.64 (m, 2H, H-10), 3.64 – 3.48 (m, 2H, H-11), 3.43 – 3.26 (m, 1H, H-4), 2.27 (dd, $J = 6.8, 6.0$ Hz, 2H, H-3), 2.08 (br, 1H, OH), 1.60 – 1.05 (m, 8H, H-5, H-6, H-7, H-8), 0.89 (t, $J = 6.8$ Hz, 3H, H-9). ^{13}C NMR (100 MHz, CDCl_3) δ 135.0, 117.0, 79.5, 69.9, 62.1, 38.4, 33.8, 31.9, 25.1, 22.6, 14.0.

Compound 12a

A mixture of acceptor **8a** (350 mg, 2.46 mmol), donor **7** (1.31 g, 2.46 mmol), and 4 Å molecular sieves (2 g) in anhydrous, redistilled CH_2Cl_2 (50 mL) was stirred under an N_2 atmosphere for 30 min and then cooled to -60 °C. TMSOTf (45 μL , 0.25 mmol) was added to the mixture. Then the reaction mixture was allowed to gradually warm to -20 °C over 2 h, at the end of which time TLC (silica, 1:6 EtOAc–hexanes) showed it was complete. Then the reaction mixture was quenched with Et_3N (50 μL) and filtrated. The filtrate was evaporated *in vacuo* to give a residue, which was purified by silica gel column chromatography (silica, EtOAc–hexanes, 1:10 \rightarrow 1:8) to give

compound **12a** (778 mg, 62%) as a colorless syrup. $[\alpha]_{\text{D}}^{25} -19.2^{\circ}$ (*c* 1 CHCl₃). ¹H NMR (400 MHz, CDCl₃) δ 6.92 – 6.76 (m, 1H, Me-CH=C(Me)-C=O), 5.95 – 5.80 (m, 1H, CH₂=CH-CH₂-O-), 5.79 – 5.65 (m, 1H, CH₂=CH-CH₂-CH-), 5.35 – 5.16 (m, 3H, H-3-Glup, CH₂=CH-CH₂-O-), 5.09 – 4.95 (m, 2H, CH₂=CH-CH₂-CH-), 4.77 (dd, *J* = 9.2, 8.0 Hz, 1H, H-2-Glup), 4.63 (d, *J* = 7.6 Hz, 1H, H-1-Glup), 4.60 – 4.52 (m, 2H, CH₂=CH-CH₂-O-), 3.93 (dd, *J* = 10.8, 5.6 Hz, 1H, H-6-Glup), 3.83 – 3.72 (m, 2H, H-4-Glup, H-6-Glup), 3.69 – 3.57 (m, 1H, -CH₂-CH-CH₂-), 3.40 – 3.31 (m, 1H, H-5-Glup), 2.23 (t, *J* = 6.4 Hz, 2H), 1.85 – 1.74 (m, 6H, CH₃-CH-C(CH₃)-C=O), 1.55 – 1.41 (m, 5H), 1.39 – 1.18 (m, 9H), 0.88 (t, *J* = 6.8 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 166.9, 154.1, 138.0, 134.1, 131.4, 128.1, 118.5, 117.3, 100.4, 99.6, 80.1, 76.7, 72.0, 71.6, 68.5, 67.2, 62.1, 38.4, 34.4, 31.7, 28.9, 24.6, 22.5, 18.9, 14.4, 14.0, 12.1.

Compound 13a

To a cooled (–10 °C) solution of compound **12a** (383 mg, 0.750 mmol) in 1:1 MeOH–THF (10 mL) was added CH₃COONH₄ (578 mg, 7.50 mmol). With vigorous stirring, NaBH₄ (5.0 mg, 0.13 mmol), Pd[P(C₆H₅)₃]₄ (35 mg, 0.030 mmol), and NaBH₄ (50 mg, 1.3 mmol) was added in 3 portions immediately one after another. Four minutes after the addition of the second portion of NaBH₄, TLC (EtOAc–hexanes, 1:6) indicated that the reaction was complete. The reaction mixture was concentrated under diminished pressure, the residue was dissolved in CH₂Cl₂ (20 mL) and washed with water (10 mL), then the organic layer was dried over Na₂SO₄. Evaporation and purification by column chromatography (silica, EtOAc–hexanes, 1:8) to afford compound **13a** (314 mg, 98%). $[\alpha]_{\text{D}}^{25} -59.6^{\circ}$ (*c* 1 CHCl₃). ¹H NMR (400 MHz, CDCl₃) δ 6.95 – 6.85 (m, 1H, Me-CH=C(Me)-C=O), 5.90 – 5.75 (m, 1H, CH₂=CH-CH₂-), 5.15 – 5.01 (m, 3H, H-3-Glup, CH₂=CH-CH₂-), 4.46 (d, *J* = 7.6 Hz, 1H, H-1-Glup), 3.92 (dd, *J* = 10.8 Hz, 5.2 Hz, 1H, H-6-Glup), 3.78 (t, *J* = 10.8 Hz, 1H, H-4-Glup), 3.74 – 3.65 (m, 2H, H-6-Glup, -CH₂-CH-CH₂-), 3.59 – 3.44 (m, 1H,

H-2-Glup), 3.40 – 3.39 (m, 1H, H-5-Glup), 2.66 (d, $J = 2.0$ Hz, 1H, OH), 2.40 – 2.18 (m, 2H), 1.84 (s, 3H, CH₃-CH-C(CH₃)-C=O), 1.80 (d, $J = 7.2$ Hz, 3H, CH₃-CH-C(CH₃)-C=O), 1.64 – 1.48 (m, 2H), 1.45 (s, 3H, (CH₃)₂C), 1.36 (s, 3H, (CH₃)₂C), 1.33 – 1.19 (m, 6H), 0.88 (t, $J = 6.8$ Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 168.0, 137.9, 134.8, 128.3, 117.7, 103.0, 99.5, 79.8, 74.1, 74.0, 71.3, 67.4, 62.2, 38.6, 34.7, 31.7, 28.9, 24.7, 22.5, 18.9, 14.4, 14.0, 12.1.

Compound 13b

A mixture of acceptor **8b** (100 mg, 0.537 mmol), donor **7** (427 mg, 0.805 mmol), and 4 Å molecular sieves (1 g) in anhydrous, redistilled CH₂Cl₂ (20 mL) was stirred under an N₂ atmosphere for 30 min and then cooled to –60 °C. TMSOTf (9.7 μ L, 0.054 mmol) was added to the mixture. Then the reaction mixture was allowed to gradually warm to –20 °C over 2 h, at the end of which time TLC (silica, 1:6 EtOAc–hexanes) showed it was complete. Then the reaction mixture was quenched with Et₃N (10 μ L) and filtrated. The filtrate was evaporated *in vacuo* to give a residue, which was purified by flash column chromatography to give compound **12b** (138 mg, 46%) as a colorless syrup. To a cooled (–10 °C) solution of compound **12b** (138 mg, 0.25 mmol) in 1:1 MeOH–THF (20 mL) was added CH₃COONH₄ (192 mg, 2.49 mmol). With vigorous stirring, NaBH₄ (1.94 mg, 0.051 mmol), Pd[P(C₆H₅)₃]₄ (11.4 mg, 0.01 mmol), and NaBH₄ (16.9 mg, 0.45 mmol) were added in three portions immediately one after another. Four minutes after the addition of the second portion of NaBH₄, TLC (EtOAc–hexanes, 1:3) indicated that the reaction was complete. The reaction mixture was concentrated under diminished pressure, the residue was dissolved in CH₂Cl₂ (50 mL) and washed with water (25 mL), and then the organic layer was dried over Na₂SO₄. Evaporation and purification by column chromatography (silica, EtOAc–hexanes, 1:8 → 1:7) afforded compound **13b** (116.2 mg, 99%) as a colorless syrup. $[\alpha]_D^{25}$ –24.3° (*c* 1 CHCl₃). ¹H NMR (400 MHz, CDCl₃) δ 6.91 – 6.89 (m, 1H, Me-CH=C(Me)-C=O),

5.83–5.76 (m, 1H, CH₂=CH-CH₂-), 5.15 – 5.00 (m, 3H, H-3-Glup, CH₂=CH-CH₂-), 4.47 (d, *J* = 7.6 Hz, 1H, H-1-Glup), 4.04 – 3.91 (m, 2H, H-6-Glup, -OCH₂CH₂O-), 3.82 – 3.60 (m, 6H, H-4-Glup, H-6-Glup, -CH₂-CH-CH₂-, -OCH₂CH₂O-), 3.59 – 3.53 (m, 1H, H-2-Glup), 3.40 – 3.30 (m, 2H, H-5-Glup, OH), 2.26–2.23 (m, 2H), 1.84 (s, 3H, CH₃-CH-C(CH₃)-C=O), 1.80 (d, *J* = 6.8 Hz, 3H, CH₃-CH-C(CH₃)-C=O), 1.75 – 1.61 (m, 2H), 1.45 (s, 3H, (CH₃)₂C), 1.37 (s, 3H, (CH₃)₂C), 1.35 – 1.20 (m, 6H), 0.88 (t, *J* = 6.8 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 168.0, 137.9, 134.8, 128.3, 117.0, 104.0, 99.6, 79.9, 74.1, 73.6, 71.4, 69.6, 67.8, 67.5, 62.1, 38.2, 33.6, 31.9, 28.9, 25.0, 22.6, 18.9, 14.4, 14.0, 12.1.

Compound 14a

TBSOTf (338 μL, 1.47 mmol) was added to a solution of compound **13a** (314 mg, 0.736 mmol) and 2,6-lutidine (426 μL, 3.68 mmol) in distilled CH₂Cl₂ (10 mL) at 0 °C. The reaction was allowed to warm to ambient temperature and stirred for 2 h. At this point, TLC (silica, 1:9 EtOAc–hexanes) showed the reaction was complete. Evaporation of the solvent followed by purification of the residue by flash chromatography (silica, EtOAc–hexanes, 1:15 → 1:10) gave compound **14a** (334 mg, 84%) as a colorless syrup. $[\alpha]_D^{25} -29.1^\circ$ (*c* 1 CHCl₃). ¹H NMR (400 MHz, CDCl₃) δ 6.89 – 6.76 (m, 1H, Me-CH-C(Me)-C=O), 5.87 – 5.71 (m, 1H, CH₂=CHCH₂-), 5.14 (t, *J* = 9.2 Hz, 1H, H-3-Glup), 5.11 – 4.99 (m, 2H, CH₂=CHCH₂-), 4.50 (d, *J* = 7.4 Hz, 1H, H-1-Glup), 3.91 (dd, *J* = 10.7, 5.3 Hz, 1H, H-6-Glup), 3.78 – 3.68 (m, 2H, H-6-Glup, -CH₂-CH-CH₂-), 3.63 (t, *J* = 9.7 Hz, 1H, H-4-Glup), 3.55 (dd, *J* = 8.7, 7.5 Hz, 1H, H-2-Glup), 3.40 – 3.36 (m, 1H, H-5-Glup), 2.43 – 2.29 (m, 1H), 2.27 – 2.15 (m, 1H), 1.87 – 1.74 (m, 6H, CH₃-CH-C(CH₃)-C=O), 1.59 – 1.14 (m, 14H), 0.88 (t, *J* = 6.8 Hz, 3H), 0.81 (s, 9H), 0.06 (s, 3H, CH₃Si), -0.03 (s, 3H, CH₃Si); ¹³C NMR (100 MHz, CDCl₃) δ 166.9, 137.1, 134.8, 128.7, 117.1, 101.3, 99.3, 77.1, 74.6, 73.9, 72.0, 67.0, 62.4, 38.1, 34.3, 31.8, 29.0, 25.7 (×3), 24.7, 22.6, 18.9, 17.9, 14.3, 14.1, 12.1, -3.8, -4.9.

Compound 14b

TBSOTf (113 μ L, 0.493 mmol) was added to a solution of compound **13b** (116 mg, 0.246 mmol) and 2,6-lutidine (143 μ L, 1.23 mmol) in distilled CH_2Cl_2 (15 mL) at 0 $^\circ\text{C}$. The reaction mixture was allowed to warm to ambient temperature and stirred overnight. At this point, TLC (silica, 1:6 EtOAc–hexanes) showed the reaction was complete. Evaporation of the solvent followed by purification of the residue by flash chromatography (silica, EtOAc–hexanes, 1:15 \rightarrow 1:10) gave compound **14b** (140 mg, 97%) as a colorless syrup. $[\alpha]_{\text{D}}^{25} -26.3^\circ$ (*c* 1 CHCl_3). ^1H NMR (400 MHz, CDCl_3) δ 6.87 – 6.82 (m, 1H, Me-CH-C(Me)-C=O), 5.88 – 5.77 (m, 1H, $\text{CH}_2=\text{CHCH}_2-$), 5.17 (t, *J* = 9.6 Hz, 1H, H-3-Glup), 5.08 – 5.02 (m, 2H, $\text{CH}_2=\text{CHCH}_2-$), 4.40 (d, *J* = 7.2 Hz, 1H, H-1-Glup), 3.96 – 3.90 (m, 2H, H-6-Glup, $-\text{OCH}_2\text{CH}_2\text{O}-$), 3.78 – 3.50 (m, 6H, H-6-Glup, H-2-Glup, H-4-Glup, $-\text{OCH}_2\text{CH}_2\text{O}-$), 3.38 – 3.28 (m, 2H, H-5-Glup, $-\text{CH}_2-\text{CH}-\text{CH}_2-$), 2.30 – 2.19 (m, 2H), 1.88 – 1.75 (m, 6H, $\text{CH}_3-\text{CH}-\text{C}(\text{CH}_3)-\text{C}=\text{O}$), 1.50 – 1.20 (m, 14H), 0.88 (t, *J* = 7.0 Hz, 3H), 0.81 (s, 9H, $\text{C}(\text{CH}_3)_3$), 0.06 (s, 3H, CH_3Si), 0.02 (s, 3H, CH_3Si). ^{13}C NMR (400 MHz, CDCl_3) δ 166.9, 137.2, 135.1, 128.6, 116.6, 104.1, 99.4, 79.7, 74.4, 74.1, 71.7, 69.3, 67.6, 67.0, 62.2, 38.3, 33.7, 32.0, 29.0, 25.6 ($\times 3$), 25.0, 22.6, 18.9, 18.0, 14.3, 14.1, 12.1, -4.2, -5.1.

Compound 15a

CSA (27.4 mg, 0.118 mmol) was added in one portion to a solution of **14a** (319 mg, 0.59 mmol) in MeOH (10 mL) at room temperature. The reaction mixture was stirred for 3 h at which point TLC (silica, 1:4 EtOAc–hexanes) showed it was complete. The reaction was quenched with Et_3N (33 μ L, 0.24 mmol) and concentrated. The residue was purified by column chromatography (silica, EtOAc–hexanes, 1:4 \rightarrow 1:3) gave compound **15a** (263 mg, 89%) as a colorless syrup. $[\alpha]_{\text{D}}^{25} 11.1^\circ$ (*c* 1 CHCl_3). ^1H NMR (400 MHz, CDCl_3) δ 6.98 – 6.87 (m, 1H, Me-CH-C(Me)-C=O), 5.88 – 5.73 (m, 1H, $\text{CH}_2=\text{CHCH}_2-$), 5.14 – 4.99 (m, 3H, H-3-Glup, $\text{CH}_2=\text{CHCH}_2-$), 4.49 (d, *J* = 7.5 Hz, 1H,

H-1-Glup), 3.97 – 3.85 (m, 1H, H-6-Glup), 3.84 – 3.68 (m, 2H, H-6-Glup, -CH₂CHCH₂-), 3.67 – 3.59 (m, 1H, H-4-Glup), 3.55 (dd, *J* = 9.0, 7.5 Hz, 1H, H-2-Glup), 3.45 – 3.36 (m, 1H, H-5-Glup), 2.77 (br, 1H, OH-4-Glup), 2.44 – 2.32 (m, 1H), 2.29 – 2.16 (m, 1H), 2.07 (s, 1H, OH-6-Glup), 1.88 – 1.78 (m, 6H, CH₃-CH-C(CH₃)-C=O), 1.59 – 1.18 (m, 8H), 0.89 (t, *J* = 6.4 Hz, 3H), 0.81 (s, 9H), 0.09 (s, 3H, CH₃Si), 0.00 (s, 3H, CH₃Si); ¹³C NMR (100 MHz, CDCl₃) δ 169.1, 138.8, 134.7, 128.1, 117.2, 100.8, 79.2, 77.4, 75.2, 72.8, 70.8, 62.7, 38.3, 34.3, 31.8, 25.6 (×2), 24.8, 22.6, 17.9, 14.4, 14.1, 12.1, -3.7, -5.0.

Compound 15b

CSA (11.2 mg, 0.0482mmol) was added in one portion to a solution of **14b** (141 mg, 0.241 mmol) in MeOH (10 mL) at room temperature. The reaction mixture was stirred for 3 h at which point TLC (silica, 1:3 EtOAc–hexanes) showed it was complete. The reaction was quenched with Et₃N (15 μL) and concentrated. The residue was purified by column chromatography (silica, EtOAc–hexanes, 1:4 → 1:3) gave compound **15b** (102 mg, 78%) as a white foam. [α]_D²⁵ 8.7° (*c* 1 CHCl₃). ¹H NMR (400 MHz, CDCl₃) δ 6.99 – 6.88 (m, 1H, Me-CH-C(Me)-C=O), 5.89 – 5.76 (m, 1H, CH₂=CHCH₂-), 5.11 – 4.97 (m, 3H, H-3-Glup, CH₂=CHCH₂-), 4.39 (d, *J* = 7.2 Hz, 1H, H-1-Glup), 3.98 – 3.86 (m, 2H, H-6-Glup, -OCH₂CH₂O-), 3.79 (dd, *J* = 12.0, 5.2 Hz, 1H, H-6-Glup), 3.74 – 3.53 (m, 5H, H-2-Glup, H-4-Glup, -OCH₂CH₂O-), 3.46 – 3.37 (m, 1H, H-5-Glup), 3.37 – 3.27 (m, 1H, -CH₂-CH-CH₂-), 2.30 – 2.20 (m, 2H), 1.87 – 1.78 (m, 6H, CH₃-CH-C(CH₃)-C=O), 1.51 – 1.22 (m, 10H), 0.88 (t, *J* = 6.8 Hz, 3H), 0.80 (s, 9H, C(CH₃)₃), 0.08 (s, 3H, CH₃Si), 0.00 (s, 3H, CH₃Si). ¹³C NMR (400 MHz, CDCl₃) δ 169.2, 139.0, 135.1, 128.0, 116.7, 103.5, 79.8, 79.3, 75.4, 72.9, 70.5, 69.3, 67.8, 62.5, 38.2, 33.7, 31.9, 25.6 (×3), 25.0, 22.6, 18.0, 14.4, 14.1, 12.0, -4.1, -5.1.

Diene 6a

2-Chloro-1-methylpyridinium iodide (CMPI, 151 mg, 0.590 mmol), N,N-dimethylaminopyridine (DMAP, 30.0 mg, 0.245 mmol) and Et₃N (686 μL, 4.92 mmol) were added to a solution of **15a** (246 mg, 0.492 mmol) and 4-oxo-8-nonenoic acid **9** (92.1 mg, 0.541 mmol) in dry CH₂Cl₂ (20 mL) at 0 °C. The reaction was allowed to warm to ambient temperature and stirred for 1 h. At this point, TLC (silica, 1:4 EtOAc–hexanes) showed the reaction was complete. Evaporation of the solvent followed by purification of the residue by column chromatography (silica, EtOAc–hexanes, 1:5 → 1:3) to give **6a** (227.1 mg, 71%) as a colorless syrup. $[\alpha]_D^{25}$ 9.4° (c 1 CHCl₃). ¹H NMR (400 MHz, CDCl₃) δ 6.97 – 6.86 (m, 1H, MeCH=C(Me)C=O), 5.88 – 5.67 (m, 2H, 2 × CH₂=CHCH₂-), 5.12 – 4.93 (m, 5H, H-3-Glup, 2 × CH₂=CHCH₂-), 4.43 (d, *J* = 7.5 Hz, 1H, H-1-Glup), 4.39 (dd, *J* = 11.9, 4.6 Hz, 1H, H-6-Glup), 4.31 (dd, *J* = 11.9, 2.2 Hz, 1H, H-6'-Glup), 3.78 – 3.67 (m, 1H, -CH₂-CH-CH₂-), 3.64 – 3.43 (m, 3H, H-2-Glup, H-4-Glup, H-5-Glup), 2.90 (d, *J* = 5.2 Hz, 1H, OH), 2.72 (t, *J* = 6.2 Hz, 2H), 2.61 (t, *J* = 6.2 Hz, 2H), 2.45 (t, *J* = 7.4 Hz, 2H), 2.41 – 2.30 (m, 1H), 2.28 – 2.16 (m, 1H), 2.11 – 1.98 (m, 2H), 1.88 – 1.77 (m, 6H, CH₃CH=C(CH₃)C=O), 1.75 – 1.61 (m, 2H), 1.57 – 1.15 (m, 8H), 0.87 (t, *J* = 6.8 Hz, 3H), 0.80 (s, 9H), 0.07 (s, 3H, CH₃Si), -0.01 (s, 3H, CH₃Si); ¹³C NMR (100 MHz, CDCl₃) δ 208.7, 173.0, 168.7, 138.5, 137.8, 134.9, 128.2, 117.0, 115.3, 100.9, 78.6, 77.2, 73.8, 72.8, 70.0, 63.5, 41.8, 38.2, 37.1, 34.3, 33.0, 31.7, 27.8, 25.6 (×3), 24.7, 22.7, 22.6, 17.8, 14.4, 14.0, 12.0, -3.8, -5.0.

Diene **6b**

2-Chloro-1-methylpyridinium iodide (CMPI, 62.2 mg, 0.243 mmol), N,N-dimethylaminopyridine (DMAP, 11.4 mg, 0.094 mmol) and Et₃N (261 μL, 1.87 mmol) were added to a solution of **15b** (102 mg, 0.187 mmol) and 4-oxo-8-nonenoic acid **9** (32.2 mg, 0.206 mmol) in dry CH₂Cl₂ (20 mL) at 0 °C. The reaction was allowed to warm to ambient temperature and stirred for 1 h. At this point, TLC (silica, 1:3 EtOAc–hexanes) showed the reaction was complete.

Evaporation of the solvent followed by purification of the residue by column chromatography (silica, EtOAc–hexanes, 1:5 → 1:3) to give **6b** (90.2 mg, 69%) as a colorless syrup. $[\alpha]_D^{25} -2.5^\circ$ (*c* 1 CHCl₃). ¹H NMR (400 MHz, CDCl₃) δ 6.99 – 6.89 (m, 1H, MeCH=C(Me)C=O), 5.89 – 5.70 (m, 2H, 2 × CH₂=CHCH₂-), 5.13 – 4.94 (m, 5H, H-3-Glup, 2 × CH₂=CHCH₂-), 4.45 (dd, *J* = 12.0, 4.0 Hz, 1H, H-6-Glup), 4.36 (d, *J* = 7.6 Hz, 1H, H-1-Glup), 4.34 – 4.27 (m, 1H, H-6'-Glup), 3.98 – 3.90 (m, 1H, -OCH₂CH₂O-), 3.73 – 3.45 (m, 6H, H-2-Glup, H-4-Glup, H-5-Glup, -OCH₂CH₂O-), 3.36 – 3.28 (m, 1H, -CH₂-CH-CH₂-), 2.95 (br, 1H, OH), 2.76 – 2.69 (m, 2H), 2.66 – 2.57 (m, 2H), 2.46 (t, *J* = 7.2 Hz, 2H), 2.26 (t, *J* = 6.0 Hz, 2H), 2.10 – 2.00 (m, 2H), 1.86 (s, 3H, CH₃-CH-C(CH₃)-C=O), 1.81 (d, *J* = 7.2 Hz, 3H, CH₃-CH-C(CH₃)-C=O), 1.75 – 1.55 (m, 2H), 1.50 – 1.21 (m, 8H), 0.89 (t, *J* = 6.8 Hz, 3H), 0.81 (s, 9H), 0.09 (s, 3H, CH₃Si), 0.01 (s, 3H, CH₃Si); ¹³C NMR (100 MHz, CDCl₃) δ 208.8, 173.2, 168.8, 138.6, 137.9, 135.1, 128.1, 116.7, 115.3, 103.5, 79.7, 78.5, 74.0, 72.9, 69.6, 69.1, 67.7, 63.4, 41.8, 38.2, 37.1, 33.7, 33.0, 32.0, 27.8, 25.7 (×3), 25.0, 22.7, 22.6, 18.0, 14.4, 14.1, 12.1, -4.1, -5.1.

Compound 5a

To a solution of diene **6a** (95.0 g, 0.146 mmol) in CH₂Cl₂ (100 mL) was added Hoveyda-Grubbs catalyst 2nd generation (18.3 mg, 0.029 mmol) in one portion at room temperature. The reaction mixture was refluxed for 4 h. At this point, TLC (silica, 1:2 EtOAc–hexanes) showed the reaction was complete. The reaction was cooled to ambient temperature and then concentrated. Flash chromatography (silica, EtOAc–hexanes, 1:4 → 1:2) gave cycloalkene isomers (86.8 mg) as a colorless syrup. To a solution of the obtained cycloalkene in EtOH (2 mL) was added Wilkinson's catalyst (25.7 mg, 0.028 mmol) in one portion at room temperature. The reaction was then stirred under an atmosphere of hydrogen (1 atm) for 6 h. At this point, TLC (silica, 1:2 EtOAc–hexanes) showed the reaction was complete. The reaction mixture was filtered thru a pad of Celite using

EtOAc (2 mL) as the eluent and the resulting filtrate concentrated. Flash chromatography (silica, EtOAc–hexanes, 1:5 → 1:3) gave **5a** (69.6 mg, 76% over two steps) as a colorless syrup. $[\alpha]_D^{25}$ 5.9° (*c* 1 CHCl₃). ¹H NMR (400 MHz, CDCl₃) δ 6.98 – 6.78 (m, 1H, Me-CH-C(Me)-C=O), 5.01 (t, *J* = 9.2 Hz, 1H, H-3-Glup), 4.63 (dd, *J* = 11.6, 1.2 Hz, 1H, H-6-Glup), 4.42 (d, *J* = 7.6 Hz, 1H, H-1-Glup), 4.04 (dd, *J* = 11.6, 7.2 Hz, 1H, H-6-Glup), 3.84 – 3.71 (m, 1H, -OCH₂CH₂O), 3.60 – 3.49 (m, 2H, H-2-Glup, H-5-Glup), 3.46 – 3.33 (m, 1H, H-4-Glup), 2.80 – 2.69 (m, 3H), 2.64 – 2.56 (m, 2H), 2.55 – 2.36 (m, 2H), 1.85 (s, 3H), 1.81 (d, *J* = 6.8 Hz, 3H), 1.77 – 1.55 (m, 4H), 1.54 – 1.19 (m, 14H), 0.90 (t, *J* = 6.8 Hz, 3H), 0.80 (s, 9H), 0.06 (s, 3H), -0.01 (s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 209.0, 172.2, 169.2, 138.8, 128.1, 99.1, 79.4, 75.2, 74.6, 72.8, 71.1, 65.0, 41.3, 37.3, 34.0, 32.1, 31.7, 28.9, 27.5, 27.0, 25.7 (×3), 25.0, 24.4, 22.8, 22.6, 17.9, 14.4, 14.1, 12.0, -3.8, -5.0.

Compound 5b

To a solution of diene **6b** (82.4 mg, 0.121 mmol) in CH₂Cl₂ (100 mL) was added Hoveyda-Grubbs catalyst 2nd generation (7.6 mg, 0.012 mmol) in one portion at room temperature. The reaction mixture was refluxed for 4 h. At this point, TLC (silica, 1:2 EtOAc–hexanes) showed the reaction was complete. The reaction was cooled to ambient temperature and then concentrated. Flash chromatography (silica, EtOAc–hexanes, 1:4 → 1:2) gave cycloalkene isomers (67.4 mg) as a colorless syrup. To a solution of the obtained cycloalkene in EtOH (2 mL) was added Wilkinson's catalyst (22.5 mg, 0.024 mmol) in one portion at room temperature. The reaction was then stirred under an atmosphere of hydrogen (1 atm) overnight. At this point, TLC (silica, 1:2 EtOAc–hexanes) showed the reaction was complete. The reaction mixture was filtered thru a pad of Celite using EtOAc (2 mL) as the eluent and the resulting filtrate concentrated. Flash chromatography (silica, EtOAc–hexanes, 1:5 → 1:3) gave **5b** (48.8 mg, 62% over two steps) as a

colorless syrup. $[\alpha]_{\text{D}}^{25}$ 7.6° (*c* 1 CHCl_3). $^1\text{H NMR}$ (400 MHz, CDCl_3) δ 6.99 – 6.90 (m, 1H, Me-CH-C(Me)-C=O), 5.01 (t, $J = 9.2$ Hz, 1H, H-3-Glup), 4.63 (dd, $J = 12.2, 3.8$ Hz, 1H, H-6-Glup), 4.29 (d, $J = 7.6$ Hz, 1H, H-1-Glup), 4.26 – 4.18 (m, 1H, H-6-Glup), 4.07 – 3.97 (m, 1H, -OCH₂CH₂O), 3.78 – 3.70 (m, 1H, -OCH₂CH₂O), 3.63 – 3.42 (m, 5H, H-2-Glup, H-4-Glup, H-5-Glup, -OCH₂CH₂O), 3.30 – 3.22 (m, 1H, -CH₂-CH-CH₂-), 3.04 (d, $J = 4.0$ Hz, 1H, OH), 2.91 – 2.80 (m, 1H), 2.73 – 2.60 (m, 2H), 2.58 – 2.42 (m, 2H), 2.40 – 2.30 (m, 1H), 1.86 (s, 3H, CH₃-CH-C(CH₃)-C=O), 1.81 (d, $J = 6.8$ Hz, 3H, CH₃-CH-C(CH₃)-C=O), 1.70 – 1.58 (m, 4H), 1.56 – 1.20 (m, 14H), 0.89 (t, $J = 6.8$ Hz, 3H), 0.80 (s, 9H), 0.07 (s, 3H, CH₃Si), 0.00 (s, 3H, CH₃Si). $^{13}\text{C NMR}$ (100 MHz, CDCl_3) δ 209.3, 172.9, 168.6, 138.6, 128.2, 103.7, 80.6, 78.2, 74.4, 72.9, 69.6, 69.2, 67.9, 63.1, 42.2, 37.1, 34.1, 33.5, 32.1, 28.7, 27.9, 27.8, 25.7 ($\times 3$), 25.0, 24.4, 22.9, 22.6, 18.0, 14.4, 14.2, 12.1, -4.1, -5.0.

Compound 16a

2-Chloro-1-methylpyridinium iodide (CMPI, 42.0 mg, 0.164 mmol), N,N-dimethylaminopyridine (DMAP, 5.0 mg, 0.041 mmol) and Et₃N (115 μL , 0.82 mmol) were added to a solution of **5a** (51.5 mg, 0.082 mmol) and cinnamic acid (24.3 mg, 0.164 mmol) in dry CH_2Cl_2 (2 mL) at 0 °C. The reaction was allowed to warm to ambient temperature and stirred overnight. At this point, TLC (silica, 1:3 EtOAc–hexanes) showed the reaction was complete. Evaporation of the solvent followed by purification of the residue by column chromatography (silica, EtOAc–hexanes, 1:5 \rightarrow 1:3) to give **16a** (51.1 mg, 82%) as a colorless syrup. $[\alpha]_{\text{D}}^{25}$ -78.3° (*c* 0.7 CHCl_3). $^1\text{H NMR}$ (400 MHz, CDCl_3) δ 7.64 (d, $J = 16.0$ Hz, 1H, Ph-CH=C-), 7.55 – 7.47 (m, 2H, $2 \times \text{ArH}$), 7.43 – 7.35 (m, 3H, $3 \times \text{ArH}$), 6.85 – 6.70 (m, 1H, Me-CH-C(Me)-C=O), 6.34 (d, $J = 16.0$ Hz, 1H, Ph-CH=CH-), 5.36 (t, $J = 9.2$ Hz, 1H, H-3-Glup), 4.99 (t, $J = 10.0$ Hz, 1H, H-4-Glup), 4.50 (d, $J = 7.6$ Hz, 1H, H-1-Glup), 4.42 – 3.34 (m, 1H, H-6-Glup), 3.99 (dd, $J = 12.0, 8.4$

Hz, 1H, H-6-Glup), 3.85 – 3.72 (m, 1H, -OCH₂CH₂O-, H-5-Glup), 3.61 (dd, $J = 9.2, 7.6$ Hz, 1H, H-2-Glup), 2.83 – 2.64 (m, 2H), 2.60 – 2.37 (m, 4H), 1.82 – 1.18 (m, 25H), 0.91 (t, $J = 6.8$ Hz, 3H), 0.80 (s, 9H), 0.06 (s, 3H), -0.03 (s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 208.9, 171.8, 167.0, 165.9, 146.3, 138.0, 134.2, 130.5, 128.8 ($\times 2$), 128.3 ($\times 2$), 128.2, 116.8, 99.4, 75.2, 75.1, 73.2, 72.5, 69.8, 64.2, 41.1, 37.4, 34.1, 32.1, 31.6, 28.9, 27.4, 26.9, 25.6 ($\times 3$), 25.0, 24.3, 22.7, 22.6, 17.9, 14.4, 14.1, 12.0, -3.9, -5.1.

Analogue 17 and 18

To a solution of **16a** (15.0 mg, 0.0198 mmol) in THF (2 mL) was added TBAF (1M solution in THF, 119 μ L, 119 μ mol, 6 equiv) at -10 °C. The reaction mixture was stirred at the same temperature for 5 h at which point TLC (silica, 1:1 EtOAc–hexanes) showed it was complete. The reaction mixture was diluted with Et₂O (10 mL), washed with 1M HCl (5 mL), saturated NaHCO₃ (5 mL), brine (5 mL). The aqueous layer was extracted with Et₂O (10 mL). The combined organic layer was dried over Na₂SO₄ and concentrated under reduced pressure. The residue was purified by column chromatography (silica, EtOAc–hexanes, 1:2 \rightarrow 2:1) gave compound **17** (5.3 mg, 42%) as a colorless film and **18** (2.9 mg, 29%) as a colorless film.

17: [α]_D²⁵ 41.3° (*c* 0.2 CHCl₃). ¹H NMR (400 MHz, CDCl₃) δ 7.70 (d, $J = 16.0$ Hz, 1H, Ph-CH=C-), 7.58 – 7.48 (m, 2H, 2 \times ArH), 7.44 – 7.34 (m, 3H, 3 \times ArH), 6.89 – 6.75 (m, 1H, Me-CH-C(Me)-C=O), 6.41 (d, $J = 16.0$ Hz, 1H, Ph-CH=CH-), 5.21 – 5.11 (m, 2H, H-2-Glup, H-3-Glup), 4.75 – 4.66 (m, 1H, H-1-Glup), 4.62 (d, $J = 11.6$ Hz, 1H, H-6-Glup), 4.28 – 4.16 (m, 1H, H-6-Glup), 3.82 – 3.69 (m, 1H, -CH₂-CH-CH₂-), 3.65 – 3.55 (m, 2H, H-4-Glup, H-5-Glup), 3.16 (d, $J = 4.0$ Hz, 1H, OH), 2.90 – 2.36 (m, 6H), 1.81 – 1.69 (m, 6H), 1.64 – 1.52 (m, 4H), 1.47 – 1.16 (m, 14H), 0.88 (t, $J = 6.8$ Hz, 3H). ¹³C NMR (175 MHz, CDCl₃) δ 209.5, 172.6, 167.8, 166.6, 146.6, 138.2, 134.0, 130.7, 128.9 ($\times 2$), 128.3 ($\times 2$), 127.9, 116.7, 97.1, 76.6 ($\times 2$), 74.7, 70.7, 70.0,

64.1, 41.4, 37.3, 33.3, 32.7, 31.7, 29.0, 27.3, 27.2, 24.7, 24.2, 23.0, 22.6, 14.4, 14.1, 12.1. HRMS (ESI) m/z calcd for $C_{36}H_{50}NaO_{10}$ $[M+Na]^+$ 665.3296, found: 665.3299.

18: $[\alpha]_D^{25} -54.5^\circ$ (c 0.2 $CHCl_3$). 1H NMR (400 MHz, $CDCl_3$) δ 6.98 – 6.88 (m, 1H, Me-CH-C(Me)-C=O), 4.47 (dd, $J = 9.2, 8.0$ Hz, 1H, H-2-Glup), 4.65 (d, $J = 8.0$ Hz, 1H, H-1-Glup), 4.59 (dd, $J = 12.0, 1.6$ Hz, 1H, H-6-Glup), 4.18 (dd, $J = 12.0, 6.4$ Hz, 1H, H-6-Glup), 3.80 – 3.71 (m, 1H, -CH₂-CH-CH₂-), 3.64 (td, $J = 8.8, 4.0$ Hz, 1H, H-3-Glup), 3.53 – 3.46 (m, 1H, H-5-Glup), 3.45 – 3.37 (m, 1H, H-4-Glup), 3.20 (d, $J = 3.6$ Hz, 1H, OH), 2.84 (d, $J = 2.8$ Hz, 1H, OH), 2.83 – 2.35 (m, 6H), 1.89 – 1.79 (m, 6H, CH₃-CH-C(CH₃)-C=O), 1.78 – 1.57 (m, 4H), 1.50 – 1.15 (m, 14H), 0.89 (t, $J = 6.4$ Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 209.2, 172.4 ($\times 2$), 168.7, 139.1, 128.1, 97.0, 76.4, 75.9, 74.8, 74.1, 71.6, 64.3, 41.4, 37.3, 33.6, 32.7, 31.7, 28.9, 27.4, 27.2, 24.7, 24.2, 23.0, 22.6, 14.5, 14.1, 12.1. HRMS (ESI) m/z calcd for $C_{27}H_{44}NaO_9$ $[M+Na]^+$ 535.2878, found: 535.2883.

Analogue 3

To a solution of **16a** (30.4 mg, 0.0402 mmol) in THF (2 mL) was added AcOH (230 μ L, 4.02 mmol) and TBAF (1M solution in THF, 2.0 mL, 2.0 mmol) at 0 °C. The reaction was allowed to warm to ambient temperature and stirred overnight. At this point, TLC (silica, EtOAc-hexanes, 1:1) showed the reaction was not complete. Then the reaction mixture was heated to 40 °C and stirred for two days. The reaction mixture was concentrated under reduced pressure. The residue was purified by column chromatography (silica, EtOAc-hexanes, 1:4 \rightarrow 1:3) gave analogue **3** (20.4 mg, 79%) as a white foam. 1H NMR (400 MHz, $CDCl_3$) δ 7.68 (d, $J = 16.0$ Hz, 1H, Ph-CH=C-), 7.55 – 7.48 (m, 2H, 2 \times ArH), 7.44 – 7.34 (m, 3H, 3 \times ArH), 6.91 – 6.81 (m, 1H, Me-CH-C(Me)-C=O), 6.37 (d, $J = 16.0$ Hz, 1H, Ph-CH=CH-), 5.30 (t, $J = 9.2$ Hz, 1H, H-3-Glup), 5.09 (t, $J = 10.0$ Hz, 1H, H-4-Glup), 4.55 (d, $J = 7.6$ Hz, 1H, H-1-Glup), 4.45 – 4.37 (m, 1H, H-6-Glup),

4.02 (dd, $J = 12.0, 8.0$ Hz, 1H, H-6-Glup), 3.90 – 3.77 (m, 2H, H-5-Glup, -CH₂-CH-CH₂-), 3.61 (dd, $J = 9.6, 8.0$ Hz, 1H, H-2-Glup), 2.85 – 2.68 (m, 2H), 2.60 – 2.38 (m, 5H), 1.84 – 1.66 (m, 8H), 1.63 – 1.18 (m, 16H), 0.91 (t, $J = 6.8$ Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 209.0, 171.9, 167.9, 165.9, 146.4, 138.9, 134.1, 130.6, 128.9 ($\times 2$), 128.3 ($\times 2$), 127.8, 116.6, 99.3, 76.0, 74.9, 72.7, 72.6, 69.0, 63.7, 41.0, 37.5, 33.8, 32.5, 31.6, 28.9, 27.2, 26.8, 24.8, 24.0, 22.7, 22.6, 14.5, 14.1, 12.0. HRMS (ESI) m/z calcd for C₃₆H₅₀NaO₁₀ [M+Na]⁺ 665.3296, found: 665.3299.

Analogue 4

2-Chloro-1-methylpyridinium iodide (CMPI, 41.8 mg, 0.062 mmol), N,N-dimethylaminopyridine (DMAP, 3.8 mg, 0.031 mmol) and Et₃N (87 μ L, 0.62 mmol) were added to a solution of **5b** (41.8 mg, 0.062 mmol) and cinnamic acid (18.5 mg, 0.125 mmol) in dry CH₂Cl₂ (2 mL) at 0 °C. The reaction was allowed to warm to ambient temperature and stirred overnight. At this point, TLC (silica, 1:3 EtOAc–hexanes) showed the reaction was complete. Evaporation of the solvent followed by purification of the residue by flash column chromatography to give **16b** (36.8 mg, 74%) as a colorless syrup. To a solution of **16b** (20.0 mg, 0.0250 mmol) in THF (2 mL) was added AcOH (71.4 μ L, 1.25 mmol) and TBAF (1M solution in THF, 0.62 mL, 0.62 mmol) at 0 °C. The reaction was allowed to warm to ambient temperature and stirred overnight. At this point, TLC (silica, EtOAc-hexanes, 1:2) showed the reaction was complete. The reaction mixture was concentrated under reduced pressure. The residue was purified by column chromatography (silica, EtOAc–hexanes, 1:2 \rightarrow 1:1) gave analogue **4** (13.2 mg, 77%) as a colorless film. $[\alpha]_D^{25} - 52.9^\circ$ (c 0.5 CHCl₃). ¹H NMR (400 MHz, CDCl₃) δ 7.67 (d, $J = 16.0$ Hz, 1H, Ph-CH=C-), 7.55 – 7.46 (m, 2H, 2 \times ArH), 7.45 – 7.34 (m, 3H, 3 \times ArH), 6.94 – 6.83 (m, 1H, Me-CH-C(Me)-C=O), 6.36 (d, $J = 16.0$ Hz, 1H, Ph-CH=CH-), 5.27 (t, $J = 9.6$ Hz, 1H, H-3-Glup), 5.15 (t, $J = 9.6$ Hz, 1H, H-4-Glup), 4.57 (d, $J = 7.6$ Hz, 1H, H-1-Glup), 4.26 (dd, $J = 12.0, 2.4$ Hz, 1H, H-6-Glup),

4.19 (dd, $J = 12.0, 6.4$ Hz, 1H, H-6-Glup), 4.10 – 4.00 (m, 1H, -OCH₂CH₂O-), 3.90 – 3.72 (m, 3H, -OCH₂CH₂O-, H-5-Glup), 3.68 – 3.60 (m, 1H, H-2-Glup), 3.58 – 3.49 (m, 1H, -OCH₂CH₂O-), 3.39 – 3.25 (m, 1H, -CH₂-CH-CH₂-), 2.98 (d, $J = 2.4$ Hz, 1H, OH), 2.88 – 2.69 (m, 2H), 2.67 – 2.52 (m, 2H), 2.53 – 2.37 (m, 2H), 1.80 – 1.71 (m, 6H, CH₃-CH-C(CH₃)-C=O), 1.70 – 1.21 (m, 18H), 0.89 (t, $J = 6.8$ Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 209.0, 172.0, 167.9, 165.6, 146.4, 139.0, 134.0, 130.6, 128.9 ($\times 2$), 128.3 ($\times 2$), 127.7, 116.6, 103.4, 80.3, 74.9, 72.8, 72.2, 69.5, 68.8, 67.6, 62.8, 42.2, 37.2, 34.0, 33.0, 32.0, 28.5, 28.1, 27.9, 25.1, 24.1, 23.3, 22.6, 14.5, 14.0, 12.0. HRMS (ESI) m/z calcd for C₃₈H₅₄NaO₁₁ [M+Na]⁺ 709.3558, found: 709.3562.