Development of transition state analogues targeting chitinases and oligosaccharyl transferase

by

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M.S. Chemistry, Seoul National University, 1999
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Submitted to the Department of Chemistry
in Partial Fulfillment of the Requirements for
the Degree of Doctor of Philosophy

at the

Massachusetts Institute of Technology
September 2006
July 2006

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ABSTRACT

Oligosaccharyl transferase (OT) plays a central role in the biosynthesis of asparagine-linked glycoproteins in eukaryotic systems. The glycosylation step catalyzed by OT involves the co-translational transfer of a tetradecasaccharide from a dolichyl-pyrophosphate carrier to an asparagine side-chain within the Asn-Xaa-Ser/Thr sequence of a nascent polypeptide. Chitinases, which was emerged as a therapeutic target in combating asthma, are β-1,4-N-acetylglucosaminidases that hydrolyze chitin to generate the disaccharide chitobiose

Although the reactions catalyzed by the two enzymes follow different pathways, they are believed to share similar transition states involving an oxocarbenium ion. To understand the mechanism of OT and discover potent and selective inhibitors against different chitinases, our intent was to utilize the common transition state analogue for both enzymes and systematically introduce additional binding determinants. The pseudo-disaccharides containing an imino sugar were designed to target the oxocarbenium ion like transition state.

The pseudo-disaccharides containing imino sugar were synthesized and evaluated at inhibitors for OT and chitinases. Highlights and supporting studies from this work include : (1) the use of the Amadori rearrangement to generate the acyclic substrate; (2) the glycosylation of β-hydroxy ketone; (3) the intramolecular reductive amination between the in-situ generated amine from azido and ketone moieties; (4) the determination of the stereo-chemical outcome by NOE difference experiments.

The pseudo-disaccharides containing imino sugars exhibited IC_{50}s in the low micromolar range versus chitinase, yet significant inhibitory activity against OT was not observed.

Thesis Advisor: Barbara Imperiali
Title: Class of 1922 Professor of Chemistry and Professor of Biology

Acknowledgments

First of all, I would thank my advisor, Professor Barbara Imperiali, for the opportunity to work in the laboratory and for her guidance and support during the past years. I have enjoyed these challenging projects and I believe I have become a better chemist under her guidance. Especially, the pig liver preparation with Barbara was one of the most exciting events in my life. I eagerly await the day I can apply the knowledge gained from that experience. Thanks to my thesis committee members Professor Rick Danheiser and Timothy M. Swager for help and advice through years.

I also would like to thank my former advisor, Professor Eun Lee at Seoul National University. In the end it was his encouragement and support that brought me here.

I cannot express enough thanks to Eranthie Weerapara. She did the all OT assay and helped me to get through my graduate study. As a bench mate, I have occupied her hood and bench space most of time and she has never complained about it. Not a word. I really appreciate that and wish your great success.

Since almost everyone in the lab helped me to write this thesis, it is not fair to say thanks to them in order. But, first, I would thank the LBT (**L**angdon Martin, **B**ianca Sculimbrene and Anne '**T**errific' Reynolds) people for organizing, rewriting and proofreading every chapter. Nelson Oliver helped me a lot with biological aspects. Andreas Aemissegger and Elvedin Lukovic proofread chapter 2 and 4. Beth Vogel helped me to finish introduction. Dora Carrico-Moniz and Wendy Iskendrian kindly corrected the experimental section. (I knew it was boring.) Also thanks to Mettieu Sainlos for computer tips. Last but not least, Galen Loving read and corrected almost every word in my whole thesis, even the acknowledgments "in spite of his busy schedule". I really appreciate it and blame them for remaining typos.

So many of my former and current labmates have been much more than that to me. I am grateful to Mary O'Reilly for introducing me to this lab and for her advice and compassion. She will be my favorite 'O'Reilly' forever. Loving Mister Galen Loving, my best cubby mate. I have really enjoyed time with you and all the conversations and coffee breaks. I am looking forward to transplanting your kidney. As a reward, you are allowed to blame me for your lost items in the mean time. Many thanks to Mark Nitz, for teaching me the carbohydrate chemistry and answering my continuing questions. To Guofeng Zhang for critical thinking and sharing Asian jokes. To Jebrell Glover, for the nice conversation at the lunch time. To Major Nelson, for his wisdom and strength about the research as well as life. To Bianca, a consulting guru, of course for her advise through the years and knowledge of chemistry and baseball. To Elvedin, my first gay friend (that I know of), for his humor in any situation and spirit. Langdon, from protector to extraordinaire. I have been impressed by his unselfishness and congratulate on him on his wedding in advance.

Also I would like to acknowledge everyone during this journey. To all current lab members as well as past members Brenda, Carlos, Christian, Christina, Debbie, Eugenio, Kevin, Maria, Mayssam, Mellisa, Rob, it has been a great pleasure working with you.

Especially, Mark 'tough guy' Chan, Angelyn 'mercury' Larkin and Meredith 'hardly' Hartley, I firmly believe your great success on your project. You are and will remain the true 'glyco' members. You will find your own strength in your journey.

There have been many people outside the lab, who were supportive of me and have not forgotten me during past years. Thanks to Sungho Yoon, Yongwon Jung and Yoona Choi for their friendship and many many coffee breaks.

I would also like to thank my ever supportive parents and my sisters Seung-gu and Seung-lin. My Dad, my biggest fan all the time, I would never been able to finish this journey without his love and help. I am greatly thankful for to my Mom's unconditional love and sacrifice. I thank my sisters for taking care of Mom and Dad and for being proud of their little brother all the time.

Finally, I would like to give special thanks to my wife, Junhee and daughter- my desired product-Emily. Meeting them is the best thing that happened in my life. Their presence has always been a really great consolation and support to me. I apologize that we could not spend more time together over theses last few years. I know it is not enough to write down a few words in acknowledgment. However, don't forget that my heart has been and always will be with them for all time.

Dedication

To Junhee, who showed and taught me the meaning of love.

Table of Contents

List of Figures:

List of Scheme:

List of Tables:

List of Spectra:

List of Abberivations:

Standard three letter codes are used for naturally occurring amino acids, All dolichol-linked disaccharides are in β-1,4 linkages.

Ac	acetyl
AgOTf	silver trifluromethanesulfonate
Alloc	allyloxycarbonyl
Bn	benzyl
Bz	benzoyl
Boc	*tert*-butyloxycarbonyl
CAN	ceric ammonium nitrate
Calcd	calculated
Cbz	benzyloxycarbonyl
DABCO	1,4-diazabicyclo[2,2,2]octane
DBU	1,8-diazabicyclo[5.4.0]-undec-7-ene
DIPEA	*N*,*N*-diisopropylethylamine
DMF	*N*,*N*-dimethylformamide
DMDP	2,5-dideoxy-2,5-imino-D-mannitol
DMSO	dimethylsulfoxide
DPM	disintegrations per minute
Dol-PP	dolichyl pyrophosphate
EC	enzyme category
ER	endoplasmic reticulum
ESI-MS	electrospray ionization mass spectrometry
Fuc-T	fucosyltransferase
GDP	guanosine 5'-diphospho
Glu	glucose
GlcNAc	N-acetylglucosamine
GnT	N-acetylglucosaminyltransferases
h	hour(s)
HEPES	*N*-(2-hydroxyethyl)piperazine-*N*'-(2-ethane-sulfonic acid)
HRMS	high resolution mass spectrometry
LiHMDS	lithium hexamethyldisilazide
min	minute(s)
MeCN	acetonitrile
NaHMDS	sodium hexamethyldisilazide
NIS	*N*-iodosuccinimide
NOE	nuclear Overhauser effect
NMR	nuclear magnetic resonance
N-TFA	N-trifluroacetimide
OT	oligosaccharyl transferase
ppm	parts per million
PMB	*p*-methoxybenzyl
Pth	phthalimide

TBAF	tetra-*n*-butylammonium fluoride
TBAI	tetra-*n*-butylammonium iodide
TBDPSCl	*tert*-butyldiphenylsilyl chloride
TBSOTf	*tert*-butyldimethyl trifluromethanesulfonate
TCA	trichloroacetyl
TFA	trifluroacetic acid
TfOH	trifluoromethanesulfonic acid
THF	tetrahydrofuran
THP	tetrahydropyranyl
TLC	thin layer chromatography
TMSOTf	trimethylsilyl trifluoromethanesulfonate
Troc	trichloroethyloxycarbonyl
p-TsOH	p-toluenesulfonic acid
TUP	theoretic upper phase
UDP-GlcNAc	uridine 5'-diphopho-*N*-acetyl-α glucosamine
Xaa	used to denote any amino acid

Chapter 1- Overview of glycosidase and glycosyltransferases

1-1 Introduction

Carbohydrates are some of the most widely distributed biopolymers in nature, accounting for as much as two-thirds of the carbon found in the biosphere.[1] Traditionally, carbohydrates have been known as a unit for energy storage or as inert polymers that provide structural support in the cellular environment. However, in the past 30 years, with the advent of glycobiology and synthetic carbohydrate chemistry,[2-9] carbohydrates have been found to play important roles in many biological processes such as intercellular communication, cell growth, cell adhesion, immune defense, viral replication, and inflammation.[10-12] Most of the carbohydrates in cells exist as oligosaccharides of complex homo- or hetero-polymers linked to proteins (glycoproteins) or lipid (glycolipids). The assembly of complex glycans is facilitated by carbohydrate processing enzymes including glycosidases (glycosyl hydrolases) and glycosyltransferases.

1-2 Glycosidases

Glycosidases catalyze the hydrolysis of a glycosidic bond by facilitating the attack of a water molecule at the anomeric carbon of a sugar. A glycosidic bond is very stable against hydrolysis, with an estimated half-life of spontaneous hydrolysis of approximately 5 million years.[13] Glycosidases are one of the most efficient and sophisticated catalysts, increasing the reaction rate by a factor of 10^{17}. To achieve such a

rate-acceleration, glycosidases are expected to have very high transition state affinities, on the order of 10^{-22} M.[10] To date more than 6,300 different glycosidase genes have been found and, on the basis of primary sequence homology, these have been classified into more than 100 families.[14] Based on the stereochemistry of the anomeric center in the sugar substrate, glycosidases are termed as α-glycosidases or β-glycosidases. Additionally, the enzymes are classified as retaining or inverting glycosidases according to the stereo chemical outcome of hydrolysis at the anomeric center.

The mechanisms of hydrolysis by both retaining and inverting glycosidases have been well characterized on a structural and biochemical level.[15,16] The majority of glycosidases follow one of these two mechanisms.[17]

For inverting glycosidases, the glycosidic bonds are hydrolyzed *via* single direct displacement, facilitated by two carboxylic acids in the side chains of aspartic acid and/or glutamic acid located in the active site (**Figure 1-1**). One carboxylic acid acts as a general acid activating the leaving group by protonation of the exocyclic oxygen. The other acts as a general base, facilitating the attack of a water molecule on the anomeric carbon by the partial deprotonation.

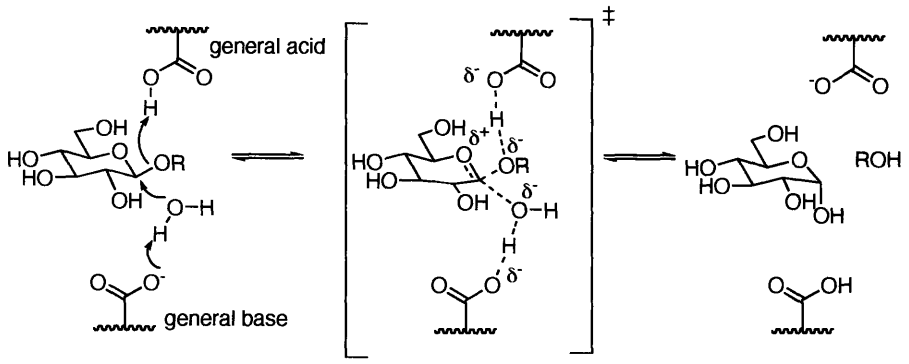


Figure 1-1. The proposed mechanism of the inverting glycosidases in the active site of enzyme.

For retaining glycosidases, the reaction proceeds *via* a double displacement mechanism (**Figure 1-2**). In the first step, a general acid partially protonates the exocyclic oxygen, while the nucleophile carboxylate on the opposite side attacks the anomeric center to displace the leaving group, forming a covalently bonded glycosyl-enzyme intermediate. In the second step, the acidic residue near the active site acts as a general base, facilitating the hydrolysis of the glycosyl-enzyme intermediate, resulting in overall retention. The presence of the glyco-enzyme intermediate has been confirmed by mass spectrometry and protein crystallography.[18,19]

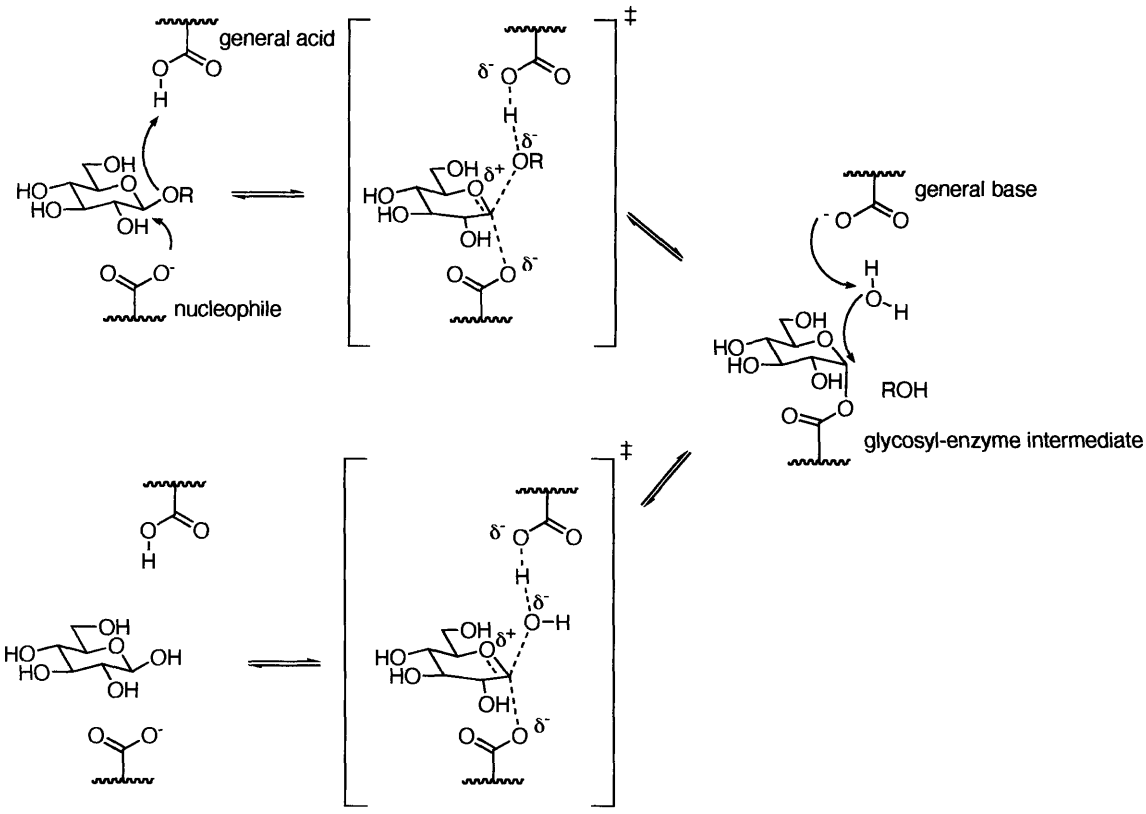


Figure 1-2. The proposed mechanism of the retaining glycosidases in the active site of enzyme.

Reactions catalyzed by both inverting and retaining glycosidases are believed to proceed through an oxocarbenium-ion-like transition state.[20] The degree of concertedness of bond breaking and bond making is not clear. Data from kinetic experiments suggest that the sp^3-hybridized anomeric carbon is converted to an sp^2 carbon, such that cleavage of the glycosidic bond precedes the formation of the new bond.[21,22] In the transition state, the carbohydrate in the active site is believed to adopt a non-chair conformation.[23] Especially for β-glycosidases, the distortion of the carbohydrate substrate from the chair conformation to a boat,[24] skew boat,[25] or half-chair form has been observed in protein crystal structures. The distortion of the carbohydrate directs the departing glycosidic bond

to a pseudo-axial orientation, which is antiperiplanar to the lone pair of the ring oxygen or the nucleophile (**Figure 1-3**).[26]

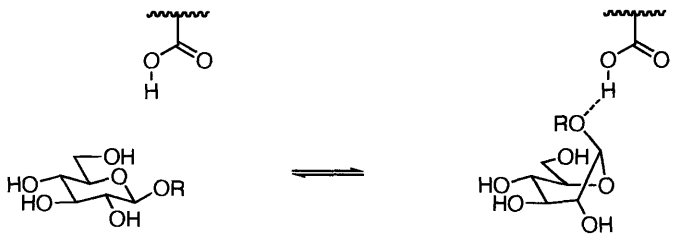


Figure 1-3. The proposed ring distortion in the active site of enzyme.

1-3 Inhibition studies of glycosidases

Since hydrolysis of the glycosidic bond is a ubiquitous biological process, glycosidase inhibitors have many potential applications, including use as agrochemicals and therapeutic agents (**Figure 1-4**).[27,28] α-Glucosidases play an essential role in the control of blood glucose levels in humans, and in the transport of glucose in insects and fungi.[29] Several glucosidase inhibitors are being marketed to treat type II diabetes,[30-32] and others are being developed as powerful insecticides.[33,34] Glycosidases are also involved in the trimming of cell and viral surface complex glycans. Inhibition of these glycosidases can disrupt the biosynthesis of carbohydrates, and hence the cell-cell[35] or the cell-virus recognition process.[36] This principle is the basis for the anti-influenza neuraminidase inhibitors[37] that have recently been marketed as well as the basis for potential HIV inhibitors.[38,39] Genetic disorders,[40,41] hepatitis C,[42] and cancer[43] are other promising targets for glycosidase inhibition.

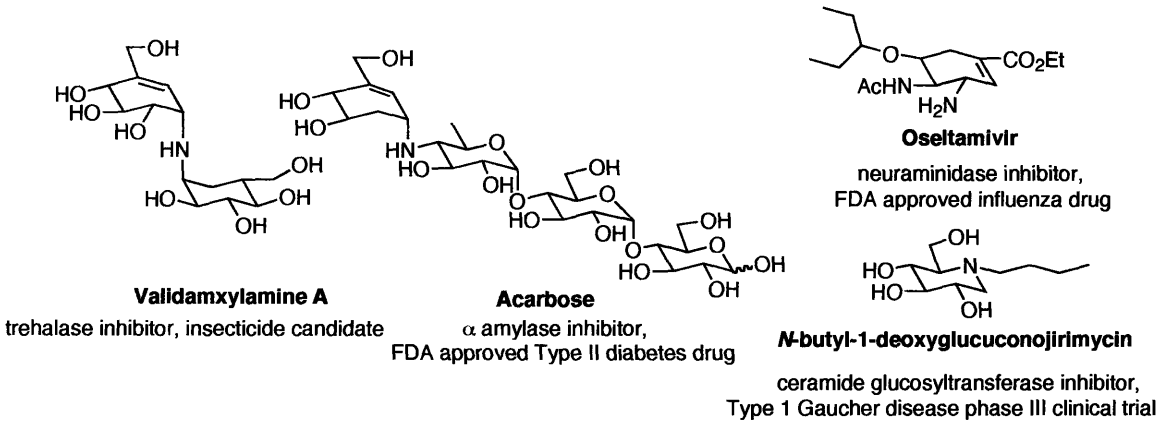


Figure 1-4. The examples of glycosidase inhibitors.

In addition to the practical applications, understanding the mechanism of glycosidase catalyzed reactions has been another motive for the design and synthesis of transition state analogues. A substrate fits into the enzyme's active site, a pocket or groove on its surface. Upon binding, the shape and charge of the active site induce a conformational change of the substrate into its transition-state configuration. Thus, enzymes bind and stabilize the transition state, lowering the activation energy to allow the reaction to proceed at higher rates.[44] In principle, a transition state analogue should bind more tightly than the substrate to the enzyme, resulting in the enzyme inhibition.[45] When considering the design of an enzyme inhibitor, much focus has been placed on mimicking the geometry and charge of the assumed transition state. The relative importance of mimicking the shape and charge of the transition state has been the subject of much debate.[46] It is also noteworthy that shape and charge are interdependent in most cases.[47]

The design of glycosidase inhibitors has focused on mimicking the charge and shape of the oxocarbenium-ion-like transition state (**Figure 1-5**).[48] Natural and synthetic polyhydroxy alkaloids have shown potent and specific inhibitory activity against

glycosidases.[49,50] The ring nitrogen would be protonated under physiological conditions to form a cation. Thus, these compounds are also referred to as imino sugars or aza sugars (**Figure 1-5a, b, c, d**).[51-54] The five-membered ring is assumed to mimic the half-chair structure involved in the transition state, whereas the six-membered ring closely resembles the ground state.[55] Incorporation of amidine (**Figure 1-5e**)[56] and imidazole (**Figure 1-5f**) [57,58] functionality into the sugar ring has been used to mimic the positive charge. To mimic the distorted structure involved in the transition state, a double bond or epoxide group[59] has been introduced into the ring (**Figure 1-5g**). Alternatively, a bicyclic system[60] has been utilized (**Figure 1-5e, h**).

Varying the position of the nitrogen often results in a change in inhibitory activity and selectivity.[53,61] These observations, in conjunction with analysis of a glycosidase crystal structure in the presence of these inhibitors, aided in understanding the mode of inhibition and mechanism for glycosidase catalytic activity.[62-64]

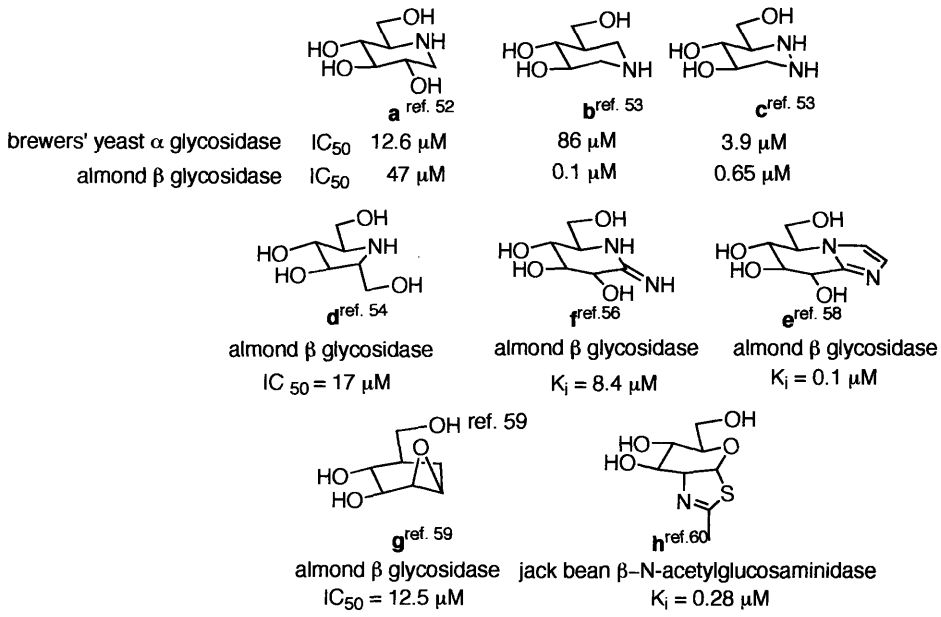


Figure 1-5. The selected examples of the transition state analogues of glycosidases.

1-4 Chitinases

Chitinases (EC 3.2.1.14) are β-1,4-N-acetylglucosaminidases that hydrolyze chitin, a homopolymer of β-1,4-linked N-acetylglucosamine to generate the disaccharide chitobiose (**Figure 1-6**).

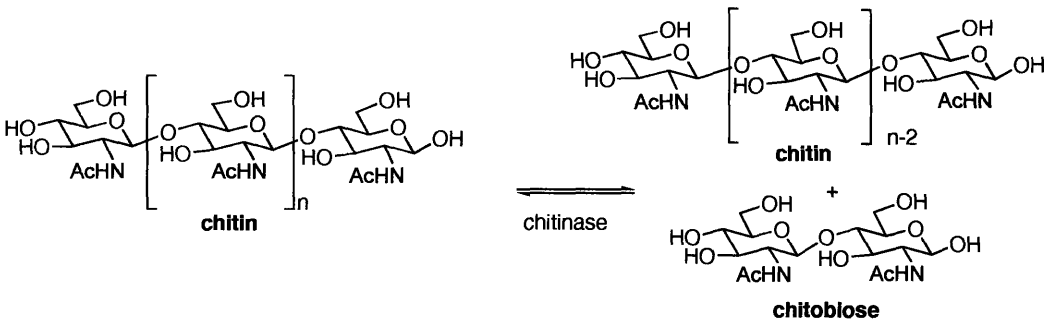


Figure 1-6. The scheme of the reaction catalyzed by chitinase.

Through classification based on amino acid sequence, most chitinases belong to family 18. According to the stereo-chemical outcome of hydrolysis at the anomeric carbon, chitinases are classified as retaining glycosidases. However, chitinases and other glycosidases in family 18 adopt a pathway for enzyme catalysis that differs from the double-displacement mechanism through the glycosyl-enzyme intermediate adopted by other retaining glycosidases.

The mechanism of chitinase-mediated hydrolysis has been extensively studied and is well understood (**Figure 1-7**). All family 18 chitinases contain a conserved DXDXE sequence (Asp-Xaa-Asp-Xaa-Glu) in the active site, which is essential for the enzyme activity.[55] By convention, the core sugar substrates at the binding site are termed as –2, -1, +1, and +2, and chitinases cleave between –1 and +1. Upon chitin binding to chitinase, the -1 sugar adopts a boat conformation to place the leaving group (chitobiose) in the pseudo-axial position, and Asp175 changes its orientation to interact with Glu177.[65-67] Asp175 activates the amide proton of the C2 acetamido group in the –1 sugar. Anchimeric assistance by the neighboring C2 acetamido group produces an oxazoline ion intermediate, liberating chitobiose.[68] Structural and theoretical investigations strongly support the presence of oxazoline ion intermediates.[69,70] The reorientation of Asp175 and Glu177 is believed to play an important role in the stabilization of the high-energy transition state. The hydrolysis of the oxazoline ion intermediate regenerates the N-acetyl group, resulting in overall retention of the anomeric configuration.

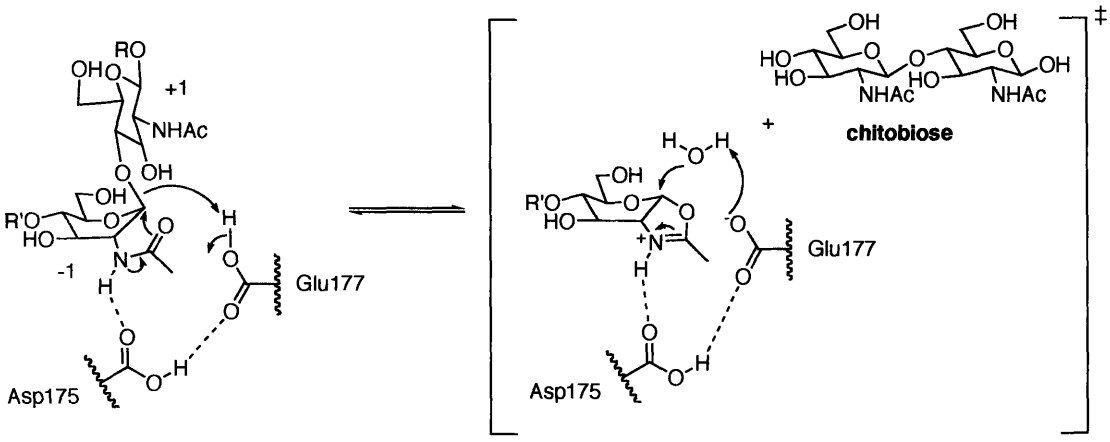


Figure 1-7. The proposed mechanism of family 18 chitinases

1-5 Inhibition studies of chitinases

Family 18 chitinases include enzymes from mammals, insects, plants, nematodes, fungi, and bacteria. Chitinase is implicated in pathogenic fungal cell division,[71] ecdysis of insects[72] and malaria transmission.[73,74] Its involvement in those processes makes chitinases attractive targets for use in the development of fungicides, insecticides and antimalarials.[24] However, chitinases may function in a beneficial role as well. Chitin is one of the main components in the cell walls of fungi, the exoskeletons of insects and other arthropods.[75,76] Since these chitin coats provide protection for pathogens inside the host and chitinases inhibit the growth of chitin containing organisms, a variety of life forms, including plants, insects, and fish, use chitinases as a weapon against chitin-expressing pathogens.[77]

Until a few years ago it was generally assumed that humans lack the ability to produce chitinase. However, a mammalian chitinase has recently been identified.[78] Interestingly, the enzyme substrate, chitin, and chitin syntase have yet to be discovered in mammals. Though the role of chitinase remains to be defined, it was hypothesized that

mammalian chitinase contributes to the immune response on the basis of their functions in other species. The implication of mammalian chitinase in allergic airway responses was reported.[55] The expression of mammalian chitinase by airway epithelia and pulmonary macrophages is dramatically increased with developments of asthma in mice and humans.[79] Moreover, the enzyme activity is critical to disease manifestation in an experimental model of asthma. Inhibition of the chitinase with a natural product, allosamidin, decreases airway inflammation and airway hyperresponsiveness. The detailed mechanisms by which chitinase regulates allergic responses remain unclear. Considering that inhibition of chitinase ameliorates physiological maladies, its regulation is likely to be important. The development of therapeutics that targets mammalian chitinase has been suggested in combating allergic asthma.[80]

To date several natural chitinase inhibitors have been isolated,[81] including the pseudo-trisaccharide allosamidin and its derivatives,[82] the cyclic pentapeptides argifin[83] and argadin,[83] amino acid-derived compounds,[84] and the complex alkaloid styloguanidine.[85]

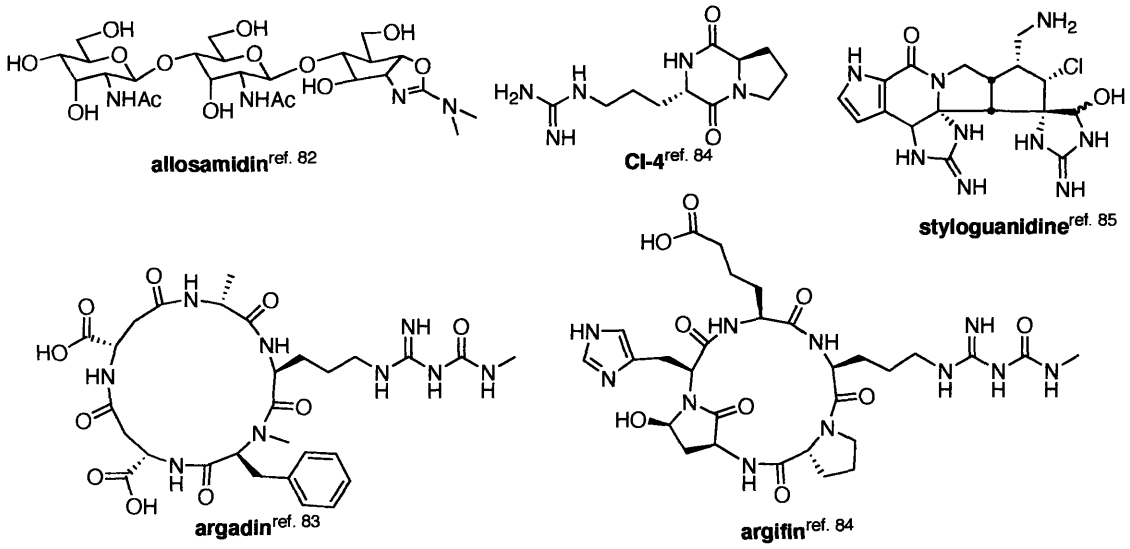
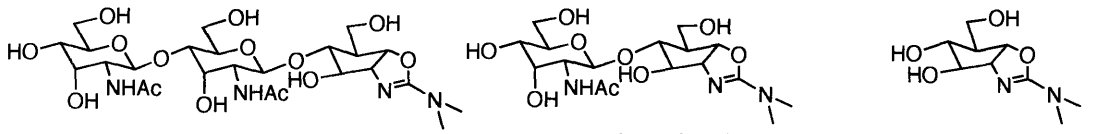


Figure 1-8. Natural product chitinase inhibitors.

Allosamidin, first isolated from mycelial extracts of *Streptomyces* sp. 1713,[82] has been extensively studied due to both its potent inhibitory activity and similarity of the corresponding molecular structure to the proposed transition state.[86-91] As shown in Figure 1-8, allosamidin consists of a di N-acetylallosamine residue and an aminocyclitol aglycone, allosamizoline. Allosamidin strongly inhibits chitinases from human and the silkworm *Bombyx mori*, with IC_{50} values of 40 nM and 48 nM, while it inhibits the yeast chitinases from *Saccharomyces cerevisiae* 500-fold less potently.[81] Interestingly, the pseudo-disaccharide derivative inhibits chitinase from *Bombyx mori* as efficiently as allosamidin. However, allosamizoline showed very little inhibitory activity against chitinases.



allosamidin

IC_{50} = 40 nM *Bombys mori*
IC_{50} = 54 μM *Saccharomyces cerevisiae*

glucoallosamidin A pseudo-disaccharide

IC_{50} = 60 nM *Bombyx mori*
Not active against *Saccharomyces cerevisiaes*

allosamizoline

Very weak inhibition

Figure 1-9. The biological activity of allosamine and its analogues.

1-6 Glycosyltransferases

Glycosyltransferases catalyze the transfer of a sugar moiety from an activated sugar donor onto saccharide or nonsaccharide acceptors such as proteins and lipids. Nucleotide pyrophosphate sugars, nucleotide monophosphate sugars, lipid pyrophosphate sugars, and lipid monophosphate sugars are all utilized as donors. To date 12,000 known and putative glycosyltransferases have been identified, and by amino acid sequence similarities divided into 78 families.[92] Similar to glycosidases, glycosyltransferases are classified as either retaining or inverting, depending on the stereo chemical outcome of reaction at the anomeric center. Glycosyltransferases are believed to follow an analogous mechanistic pathway to that of glycosidases due to the similarity of their catalyzed reactions.[93-95] Unfortunately, a detailed structural and mechanistic understanding of glycosyltransferases is lacking due to difficulties with protein overexpression, purification and crystallization.

The reaction of inverting glycosyltransferases is thought to proceed through single direct displacement, similar to that proposed for the inverting glycosidase reaction. The protein crystal structure of rabbit N-acetylglucosaminyltransferase I (GnT 1) determined to 1.5 Å resolution in the presence of uridine 5'-diphospho-N-acetyl-α-D-glucosamine

(UDP-GlcNAc) and Mn^{2+}, provided insight into the reaction mechanism (**Figure 1-10**).[96] GnT 1 catalyzes the transfer of GlcNAc from UDP-GlcNAc to a mannose residue on a complex N-linked glycoprotein. The general base, Asp291, which is positioned 4.7 Å from C1 of GlcNAc, deprotonates the incoming nucleophile (sugar acceptor), facilitating attack on the anomeric center of UDP-GlcNAc. The Mn^{2+} activates the leaving group (pyrophosphate-nucleotide) by coordination to the pyrophosphate moiety. The structure also revealed the interaction between Mn^{2+} and the metal binding residues. The relative positions of the catalytic base, the metal binding motif and the metal ion show that an in-line attack of the acceptor would lead to inversion of stereochemistry at the C1 position of the donor sugar. Additionally, other structural and kinetic evidence for inverting glycosyltransferases support a direct displacement involving an oxocarbenium ion-like transition state.[97,98]

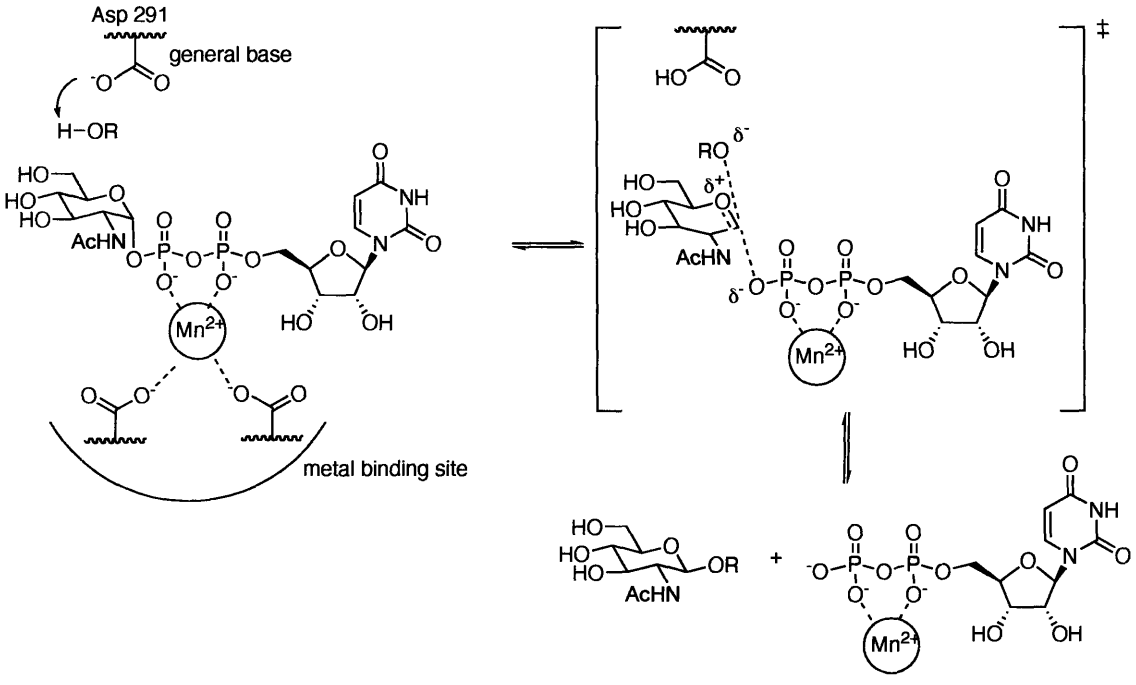


Figure 1-10. The proposed mechanism of GnT I based on the protein crystal structure.

The mechanism of retaining glycosyltransferases remains elusive. A double-displacement mechanism via a covalently bound glycosyl-enzyme intermediate has been postulated based on analogy with retaining glycosidases. However, numerous efforts to trap the glycosyl-enzyme intermediate in the active site have been unsuccessful.[99,100] To date, X-ray crystal structures of retaining glycosyltransferases in six families have been solved.[93] In some of protein crystal structures, no nucleophile candidates, which are required to form glycosyl-enzyme intermediates, were observed in the active site.[101,102] Alternatively, a substitution nucleophilic internal (S_Ni)-like mechanism has been proposed, in which the acceptor attaches on the same side as leaving group.[99] To understand the mechanism of retaining glycosyltransferase, more structural and kinetic experimental evidence is necessary.

1-7 Inhibition studies of glycosyltransferases

In contrast to the many potent inhibitors found for glycosidases, only limited success has been achieved in developing inhibitors of glycosyltransferases.[103] The challenges in the design of glycosyltransferase inhibitors are 1) low binding affinity (K_m) of the enzyme to the reaction substrate and 2) difficulties with rational design due to complex reaction partners, such as sugar donor, sugar acceptor, metal ion and nucleotide; and 3) limited structural information.[104] However, a few natural and synthetic inhibitors have been found or synthesized.[105] They can be divided into one of two categories: nucleotide analogues and transition state analogues.

Construction of inhibitors starting with the nucleotide (or pyrophosphate-nucleotide portion) has become a widely adopted strategy (**Figure 1-11**). The natural product tunicamycin falls into this category of nucleotide analogues.[106,107] Although no clear sequence homology has been found for the nucleotide-binding site, homology in conformation at the binding site has been observed. Since the nucleotide portion is identical to the native substrate, it has a basal binding affinity to the enzyme. Addition of sugar analogues or other functional groups to the nucleotide portion may therefore increase the inhibition and selectivity against other enzymes.[106,108-110]

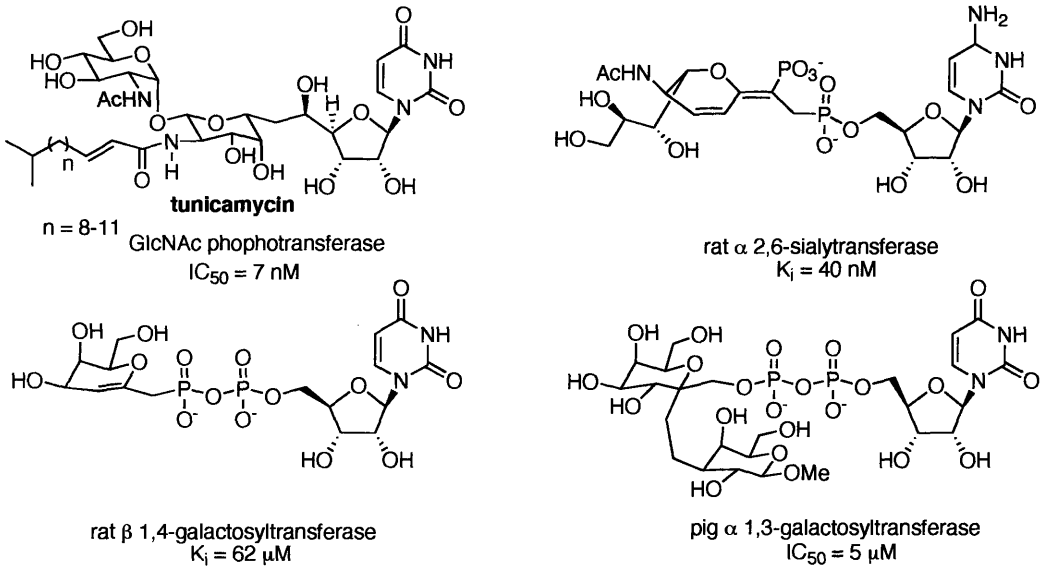
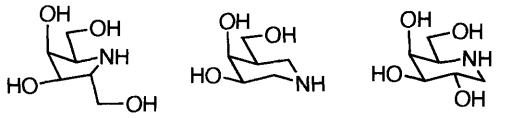


Figure 1-11. Selected inhibitors of glycosyltransferses based on the nucleotide analogue.

As glycosyltransferases are believed to catalyze the reaction *via* similar mechanism, involving an oxocarbenium-ion-like transition state, it is rational to attempt the use of glycosidase inhibitors as glycosyltransferase inhibitors.[111-113] A few synthetic glycosidase inhibitors show micromolar inhibitory activity against glycosyltransferases

(**Figure 1-12**). Similar to glycosidases, the biological activity is very sensitive to changes in the structure of the inhibitor, and addition of functional groups to the core inhibitor has shown to increase the binding affinity.[114]



β-1,4-galactosyltransferase	IC_{50}	22 μM	no inhibition	no inhibition
α-1,3-galatcosyltransferase	IC_{50}	no inhibition	15 μM	no inhibition

Figure 1-12. Selected inhibitors of glycosyltransferses based on the transition state analogue.

Studies of human α-1,3–fucosyltransferase (Fuc-T) inhibition represent both approaches. Fuc-T catalyzes the transfer of L-fucose from guanosine 5'-diphosphate β-L-fucose (GDP-Fuc) to the C3 hydroxyl of GlcNAc in the glycoconjugate acceptor (**Figure 1-13**). The fucosylation is the elaboration step in the biosynthesis of sialyl Lewis x (sLe^x) and sialyl Lewis a (sLe^a), which play essential roles in inflammation and the immune response.[115]

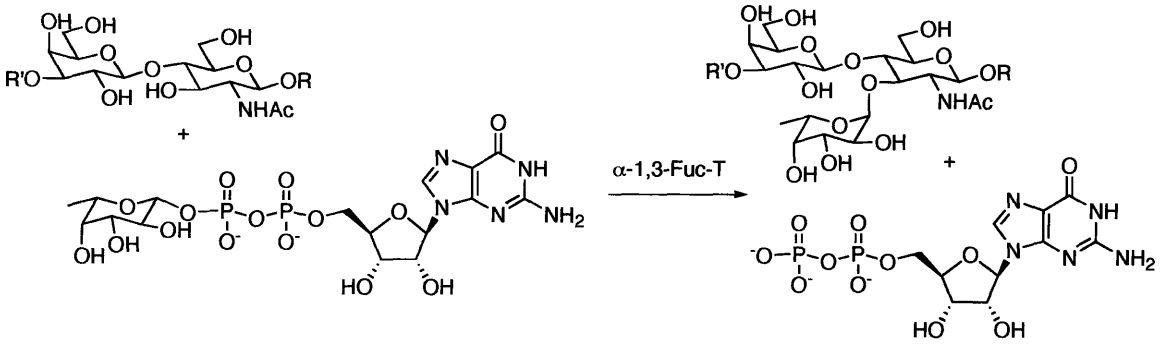


Figure 1-13. The scheme of reaction catalyzed by α-1,3-Fuc-T.

One approach was to generate a GDP analogue library in a combinatorial fashion to increase inhibitory activity (**Figure 1-14**).[116] The synthesis of 85 GDP analogues was achieved by copper-catalyzed cycloaddition between a GDP-alkyne and various azides having different functional groups. The GDP analogue having a hydrophobic biaryl turned out to be the most potent inhibitor (**Figure 1-14c**). Inhibitory activity was increased by 500-fold over GDP. It also showed high selectivity against other fucosyltransferases.

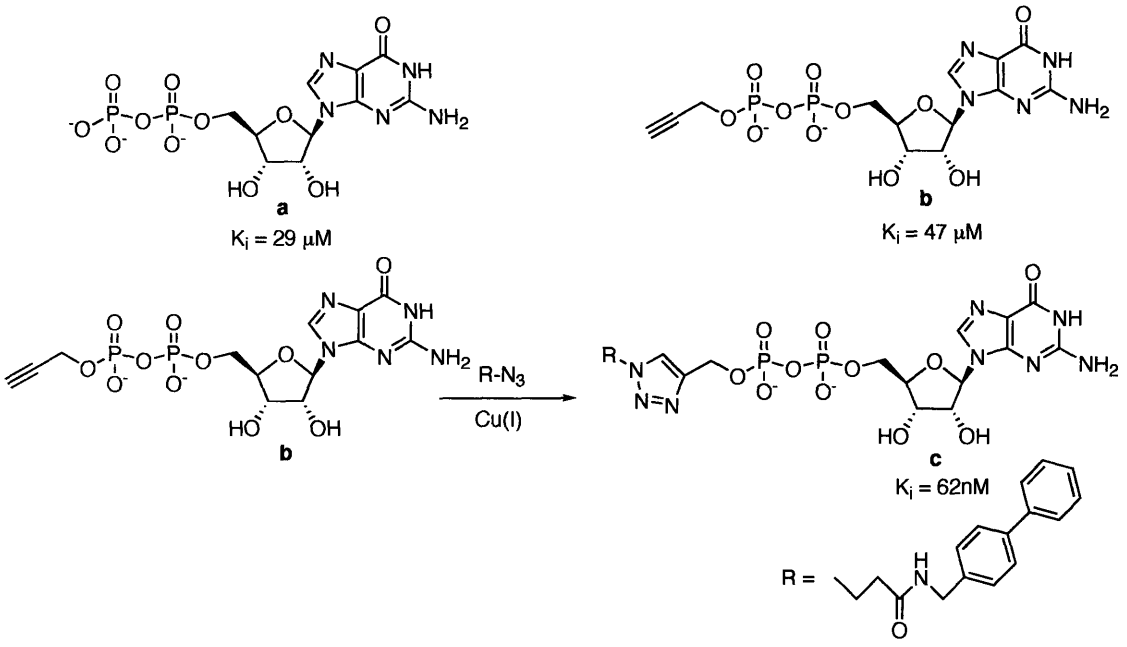


Figure 1-14. the inhibition study of α-1,3-Fuc-T VI with GDP analogues.

The other approach utilized a glycosidase inhibitor as the scaffold (**Figure 1-15**). The compound L-*fuco*-nojirimycin (**Figure 1-15a**) was known as a potent inhibitor against α-L-fucosidase (K_i = 1 nM),[117] but showed very little inhibitory activity against fucosyltransferase. Based on the structure of this transition state analogue, bisubstrate

analogue inhibitors, in which the sugar acceptor and sugar donor were connected by a linker, were synthesized. Addition of the sugar acceptor moiety showed an increase of inhibitory activity. By varying the length and geometry of the linker, the optimal orientation was found (**Figure 1-15d**).[118] Interestingly, the trisaccharide derivative showed poor inhibitory activity, probably due to an unfavorable orientation, despite its close resemblance to the proposed transition state (**Figure 1-15e**).

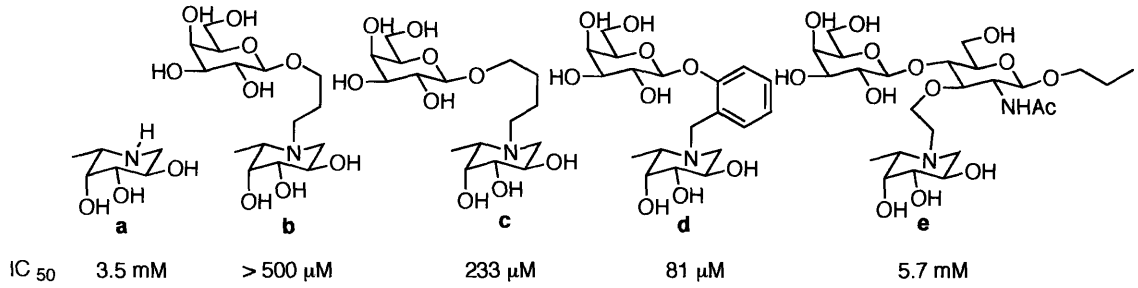


Figure 1-15. The inhibition study of α-1,3-Fuc-T with transition state analogues.

1-9 Oligosaccharyl transferase (OT)

Oligosaccharyl transferase (OT, EC 2.4.1.119) plays a central role in the biosynthesis of asparagine-linked glycoproteins in eukaryotic systems. The glycosylation step catalyzed by OT involves the co-translational transfer of a tetradecasaccharide from a dolichyl-pyrophosphate carrier to an asparagine side-chain within the Asn-Xaa-Ser/Thr sequence of a nascent polypeptide, where Xaa can be any amino acid except proline (**Figure 1-16.**).[119] This is the first step in N-linked glycosylation and the resulting glycoprotein is further processed by various glycosidases and glycosyltransferases.[120]

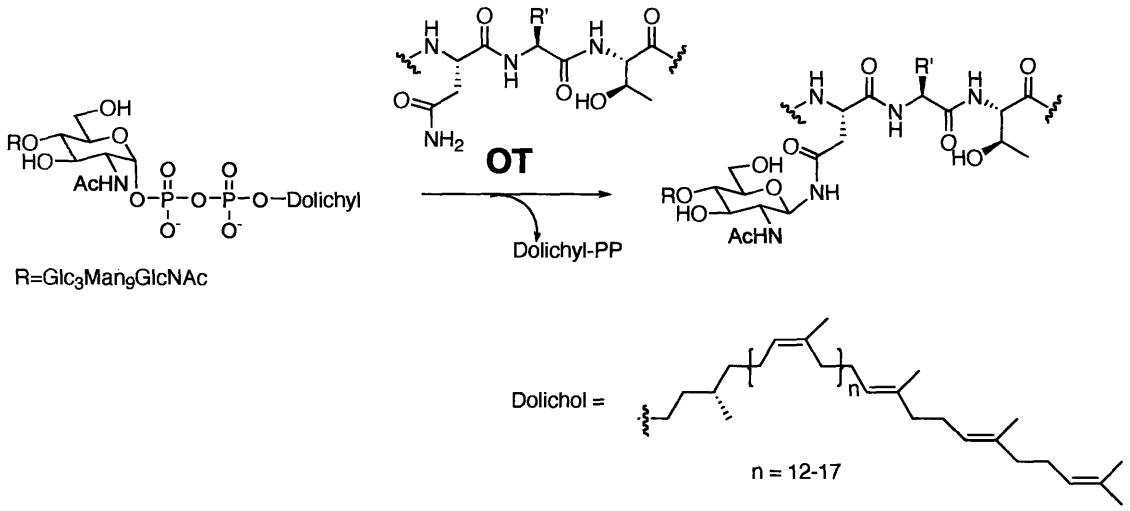


Figure 1-16. N-Linked glycosylation catalyzed by oligosaccharyl transferase.

OT is a membrane-associated multimeric protein localized in the lumen of the endoplasmic reticulum (ER). Eight subunits (Ost1p, Ost2p, Ost3p, Ost4p, Ostp5p, Wbp1p, Swp1p, Stt3) are assembled into a hetero-octameric OT complex in yeast (*Saccharomyces cerevisiae).*[121] Recent evidence indicates that Stt3 is the catalytic subunit of the eukaryotic OT.[122-124] Stt3 family proteins are responsible for the catalytic activity of N-linked glycosylation in eukaryotes, archaebacteria and in selected eubacteria.[125,126] In spite of its essential role in N-linked glycosylation, only limited structural or mechanistic information for Stt3 is available. The highly membrane-associated nature of Stt3, which is predicted to have 11 or 13 transmembrane domains, has hampered the investigation of this subunit.[127,128] Even the topology of the Stt3 protein is hard to predict. Therefore, studies that have focused on substrate specificity *in vivo* and *in vitro* have contributed to our understanding of catalysis.

1-10 Peptide substrate specificity studies of OT

The source of enhanced nucleophilicity of the amide nitrogen of asparagine is a prevailing mechanistic question in N-linked glycosylation, and several mechanisms have been proposed.[129-131] The general agreement is that intramolecular hydrogen bonding activates the carboximide of asparagine.[132]

In addition, the Asx-turn motif is believed to be an important element to activate the peptide substrate (**Figure 1-17**). A peptide having this recognition sequence can adopt two conformations: an Asx-turn or a β-turn. The major difference is the intramolecular hydrogen bonding site of the backbone amide. In the Asx-turn, the backbone amide interacts with the side-chain amide of the asparagine. In contrast, the β-turn is characterized by the hydrogen bonding between backbone amide groups.[133] The hydrogen-bonding network in the Asx-turn facilitates deprotonation of the amide to provide the neutral imidol species. The intermediate could react with the dolichyl-pyrophosphate-linked sugar donor to generate the glycopeptide. Using NMR studies, it was demonstrated that an unglycosylated peptide, based on a short sequence of hemagglutinin, adopts an Asx-turn conformation. Upon glycosylation, the structure changes to induce a type-I-β-turn conformation.[134,135] The fact that proline is not accepted at the Xaa site within Asn-Xaa-Ser/Thr sequence, and that 10-30% of recognition sites are not glycosylated, supports that local conformation plays an important role.[136]

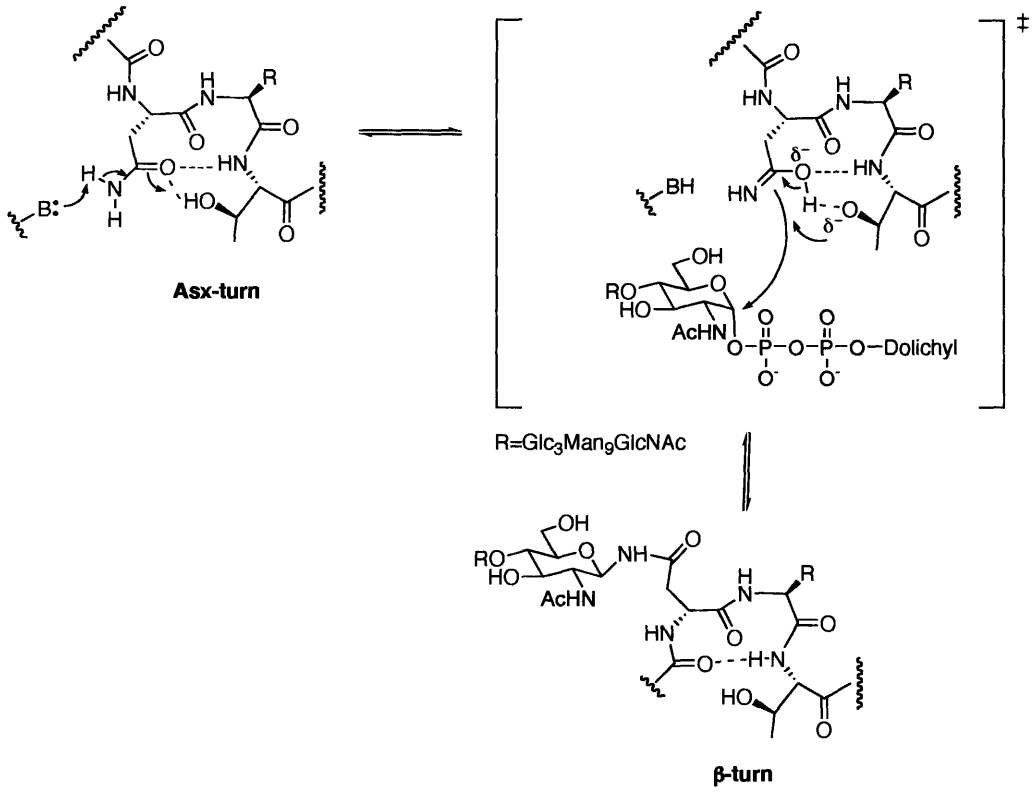


Figure 1-17. The proposed mechanism of OT using Asx turn.

The mechanistic studies of peptide substrates guided the development of inhibitors against OT with low-nanomolar affinity. The establishment of backbone constraint and replacement of the carbonyl of asparagine with methylene unit, gave a tight binding inhibitor with a K_i of 37 nM (**Figure 1-18.**).[137]

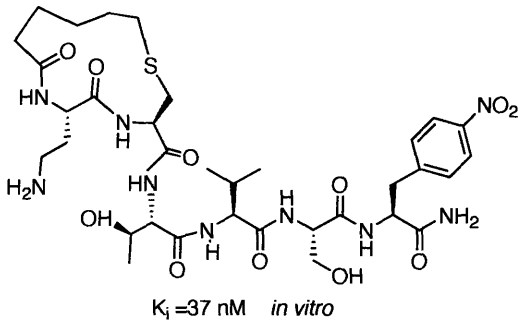


Figure 1-18. The potent OT peptide inhibtior with Asx turn.

1-11 Carbohydrate substrate specificity studies of OT

A tetradecasaccharide linked to dolichyl-pyrophosphate by an α–linkage is the preferred substrate for OT both *in vivo* and *in vitro* (**Figure 1-19a**). Due to the difficulty of obtaining the full substrate, most *in vitro* studies have been carried out using chitobiose linked to a dolichyl-pyrophosphate as the substrate (**Figure 1-19b**). The K_m values for the full substrate (**Figure 1-19a**) and the truncated substrate (**Figure 1-19b**) were determined to be 33 μM and 65 μM respectively.[138] Interestingly, the synthetic substrate Dol-PP-GlcNAc-Glc (**Figure 1-19c**) showed 2.5 fold improvement in binding with a K_m of 26 μM.[139] The replacement of the C2 acetamido group with trifluroacetamide (**Figure 1-19d**) or ethyl ether (**Figure 1-19e**) of the proximal GlcNAc residue leads to total loss of enzyme catalytic activity.[139] These analogues (**Figure 1-19d, e**) were further analyzed as inhibitors of OT, which suggested that OT recognized these synthetic substrates without a C2 acetamide group, but OT could not transfer them to the peptide substrate. In contrast, Dol-PP-GlcNAc (**Figure 1-19f**) was found to be very poor substrate for OT.

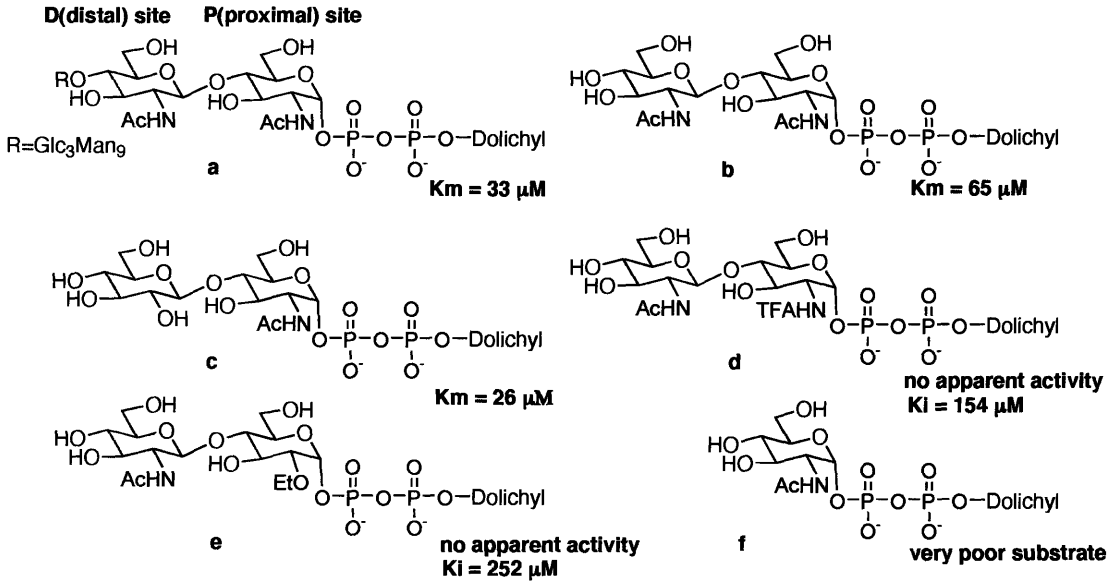


Figure 1-19. The sugar substrate specificity study for OT.

Since OT is classified as an inverting glycosyltransferase, the reaction pathway of OT is believed to proceed *via* single direct displacement involving an oxocarbenium ion-like transition state. Activated asparagine attacks the anomeric center of the GlcNAc in the proximal (P) site, generating a β-linked glycopeptide. Although no metal binding motif has been found in OT, it has been reported that divalent ions such as Mn^{2+} or Mg^{2+} are essential for the catalytic activity.[140] The results of a carbohydrate substrate study suggested that the C2 acetamide group plays an important role in enzyme catalysis, either directly or indirectly.[139] A substrate specificity study of bacterial OT also supports the role of the C2 acetamide group in enzyme activity.[141] Based on these substrate study results, a reaction pathway *via* an oxazoline ion intermediate was proposed.[141] A high-energy oxocarbenium ion might be stabilized by the delocalization of positive charge through the participation of the C2 acetamido group. However, to establish the exact

function of the C2 acetamido related to the mechanism of OT, a detailed structural and kinetic study of OT is required.

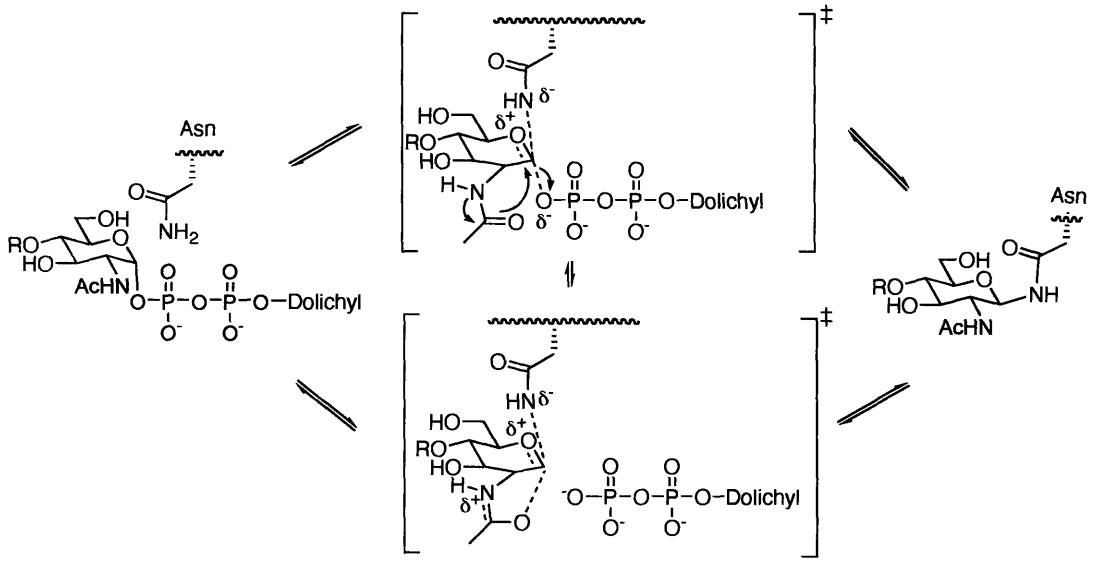
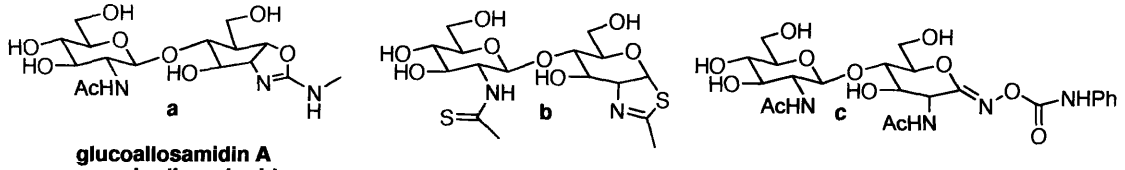


Figure 1-20. The possible transition state of sugar substrate.

Based on the similarity of the proposed transition states of chitinase and OT, the potent chitinase inhibitor glucoallosamdin A pseudo-disaccharide was considered as a potential inhibitor for OT (**Figure 1-21a**). Unfortunately, no inhibitory activity was observed up to 5 mM. Other chitobiose derivatives (**Figure 1-21b, c**) showed no inhibitory activity against OT up to 500 μM concentration.[142]



glucoallosamidin A pseudo-disaccharide

Figure 1-21. The compounds tested in an OT inhibition study.

11 Design of an inhibitor candidate for chitinase and OT

The goal of this study was to design, synthesize, and evaluate potential inhibitors against OT from *Saccharomyces cerevisiae* and chitinase from *Streptomyces grises*. Although the reactions catalyzed by the two enzymes follow different pathways, they are believed to share similar transition states involving an oxocarbenium ion. Our intent was to utilize the common transition state analogue for both enzymes and systematically introduce additional binding determinants.

From the substrate and inhibitor studies and mechanistic considerations of chitinase and OT, common transition state analogues were designed. Polyhydroxypyrrolidine **1**, also known as 2,5-dideoxy-2,5-imino-D-mannitol (DMDP),[54,143] is one of the most prevalent sugar mimics.[49,144] Five-membered imino sugars show potent inhibitory activity against the a broad range of retaining and inverting glycosidases and glycosyltransferases (**Figure 1-22**).[145,146]

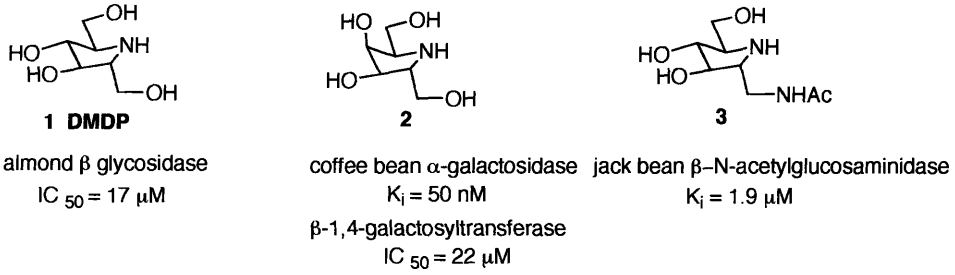


Figure 1-22. Selected examples of five-member imino sugars and their biological activities.

Among imino sugar pyrrolidine analogues, we chose **3** as the target transition state analogue. In addition to its ability to mimic the charge and shape of the oxocarbenium ion, there were several attractive features of iminocyclitol **3**. In chitinase and OT, the C2 N-Ac group of the proximal GlcNAc residue plays an important role in the enzyme catalysis either directly or indirectly. The C1 N-Ac group in the imino sugar could interact with the same key residues in the active site that bind to the C2 N-Ac group in the sugar substrate. The two pseudo-equatorial secondary alcohols have a similar configuration to that of N-acetylglucosamine. Moreover, functionalization of C1 amine or secondary ring amine by acylation or reductive amination have been reported, efficiently generating the corresponding derivatives.[147]

The inhibition study of chitinase and the carbohydrate substrate study of OT suggest that the disaccharide unit is the minimal binding motif for OT and chitinase. To fulfill this requirement, we decided to synthesize a pseudo-disaccharide motif containing the transition state analogue (**Figure 1-23**). To mimic the glycosyl linkage in chitobiose, we planned to add glucose and N-acteylglucosamine to the C4 hydroxyl group of the imino sugar *via* a β–linkage.

Chitinase has emerged as a therapeutic target in combating asthma. To validate the exact role of mammalian chitinase in the allergic response, the effect of exogenous chitinases (from insect or fungi, for example) must be investigated. Although many natural product inhibitors have been discovered, the synthesis of derivatives based on a common motif has proven effective in the discovery of an inhibitor and in improvements to its biological activity.[147,148] To discover potent and selective inhibitors against different

chitinases, the synthesis of pseudo-disaccharide derivatives will be useful. We were particularly interested in exploration of different groups such as aliphatic chains, aromatic and heterocyclic groups on the C1 amine of the imino sugar. These compounds will be valuable tools to elucidate the function of chitinases from various species in allergic response.

In spite of the importance of N-linked glycosylation, the structural and mechanistic study of OT has been limited due to the membrane-associated nature of this multimeric complex. Studies of the inhibition of OT are a more tractable approach to providing insight into the mechanism of transferring an oligosaccharide from the dolichyl lipid to protein. For the design of an OT inhibitor, the addition of a long aliphatic chain to the transition state analogue is expected to increase the binding affinity to the enzyme. The failure of glucoallosamdin A pseudo-disaccharide and chitobiose derivatives as OT inhibitors could be explained based on the possibility that these transition state analogues might not be accessible to the active site of OT. Considering the amphiphilic nature of the dolichyl-pyrophosphate-linked tetradecasaccharide and the membrane-associated nature OT, the active site of glycosylation may be at the interface between the membrane and soluble domains of OT.

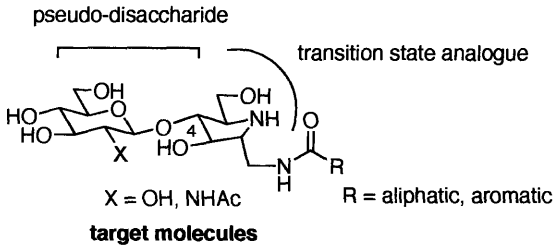


Figure 1-23. The design of inhibitors for chitinase and OT.

A modular approach based on the pseudo-disaccharide core will reveal the critical binding determinants required for inhibition of chitinase and OT. A comparison of biological properties will give insight into the mechanistic differences between OT and chitinase in terms of inhibition.

References

1. Sinnott, M. L. Catalytic Mechanisms of Enzymatic Glycosyl Transfer. *Chem. Rev.*, **1990**, *90*, 1171-1202.
2. Bertozzi, C. R.; Kiessling, L. L. Chemical glycobiology. *Science,* **2001**, *291*, 2357-2364.
3. Alper, J. Glycobiology - Turning sweet on cancer. *Science,* **2003**, *301*, 159-160.
4. Lowe, J. B.; Marth, J. D. A genetic approach to mammalian glycan function. *Annu. Rev. Biochem.*, **2003**, *72*, 643-691.
5. Roseman, S. Reflections on glycobiology. *J. Biol. Chem.*, **2001**, *276*, 41527-41542.
6. Feizi, T.; Mulloy, B. Carbohydrates and glycoconjugates: never a dull moment in glycoscience - Editorial overview. *Curr. Opin. Struct. Biol.*, **2005**, *15*, 479-480.
7. Seeberger, P. H.; Haase, W. -C. Solid-phase oligosaccharide synthesis and combinatorial carbohydrate libraries. *Chem. Rev.*, **2000**, *100*, 4349-4378.
8. Sears, P.; Wong, C. -H. Toward automated synthesis of oligosaccharides and glycoproteins. *Science,* **2001**, *291*, 2344-2350.
9. Nicolaou, K. C.; Mitchell, H. J. Adventures in carbohydrate chemistry: New synthetic technologies, chemical synthesis, molecular design, and chemical biology. *Angew. Chem. Int. Ed. Engl.*, **2001**, *40*, 1576-1624.
10. Helenius, A.; Aebi, M. Intracellular functions of N-linked glycans. *Science,* **2001**, *291*, 2364-2369.
11. Varki, A. Biological Roles of Oligosaccharides - All of the Theories Are Correct. *Glycobiology,* **1993**, *3*, 97-130.
12. Upreti, R. K.; Kumar, M.; Shankar, V. Bacterial glycoproteins: Functions, biosynthesis and applications. *Proteomics,* **2003**, *3*, 363-379.
13. Wolfenden, R.; Lu, X. D.; Young, G. Spontaneous hydrolysis of glycosides. *J. Am. Chem. Soc.*, **1998**, *120*, 6814-6815.
14. Henrissat, B.; Davies, G. Structural and sequence-based classification of glycoside hydrolases. *Curr. Opin. Struct. Biol.*, **1997**, *7*, 637-644.
15. Heightman, T. D.; Vasella, A. T. Recent insights into inhibition, structure, and mechanism of configuration-retaining glycosidases. *Angew. Chem. Int. Ed. Engl,* **1999**, *38*, 750-770.
16. Zechel, D. L.; Withers, S. G. Dissection of nucleophilic and acid-base catalysis in glycosidases. *Curr. Opin. Chem. Biol.*, **2001**, *5*, 643-649.
17. Yip, V. L. Y.; Withers, S. G. Nature's many mechanisms for the degradation of oligosaccharides. *Org. Biomol. Chem.*, **2004**, *2*, 2707-2713.
18. Vocadlo, D. J.; Davies, G. J.; Laine, R.; Withers, S. G. Catalysis by hen egg-white lysozyme proceeds *via* a covalent intermediate. *Nature,* **2001**, *412*, 835-838.
19. Sidhu, G.; Withers, S. G.; Nguyen, N. T.; McIntosh, L. P.; Ziser, L.; Brayer, G. D. Sugar ring distortion in the glycosyl-enzyme intermediate of a family G/11 xylanase. *Biochemistry,* **1999**, *38*, 5346-5354.
20. Zechel, D. L.; Withers, S. G. Glycosidase mechanisms: Anatomy of a finely tuned catalyst. *Acc. Chem. Res.*, **2000**, *33*, 11-18.

21. Vocadlo, D. J.; Wicki, J.; Rupitz, K.; Withers, S. G. Mechanism of *Thermoanaerobacterium saccharolyticum* β-xylosidase: Kinetic studies. *Biochemistry,* **2002**, *41*, 9727-9735.
22. Huang, X. C.; Tanaka, K. S. E.; Bennet, A. J. Glucosidase-catalyzed hydrolysis of β-D-glucopyranosyl pyridinium salts: Kinetic evidence for nucleophilic involvement at the glucosidation transition state. *J. Am. Chem. Soc.,* **1997**, *119*, 11147-11154.
23. Vasella, A.; Davies, G. J.; Bohm, M. Glycosidase mechanisms. *Curr. Opin. Chem. Biol.,* **2002**, *6*, 619-629.
24. Sabini, E.; Wilson, K. S.; Danielsen, S.; Schulein, M.; Davies, G. J. Oligosaccharide binding to family 11 xylanases: both covalent intermediate and mutant product complexes display B-2,B-5 conformations at the active centre. *Acta Crystallogr., Sect. D,* **2001**, *57*, 1344-1347.
25. Ducros, V. M. A.; Zechel, D. L.; Murshudov, G. N.; Gilbert, H. J.; Szabo, L.; Stoll, D.; Withers, S. G.; Davies, G. J. Substrate distortion by a β-mannosidase: Snapshots of the Michaelis and covalent-intermediate complexes suggest a B-2,B-5 conformation for the transition state. *Angew. Chem. Int. Ed. Engl.,* **2002**, *41*, 2824-2827.
26. Sulzenbacher, G.; Driguez, H.; Henrissat, B.; Schulein, M.; Davies, G. J. Structure of the *Fusarium oxysporum* endoglucanase I with a nonhydrolyzable substrate analogue: Substrate distortion gives rise to the preferred axial orientation for the leaving group. *Biochemistry,* **1996**, *35*, 15280-15287.
27. Asano, N. Glycosidase inhibitors: update and perspectives on practical use. *Glycobiology,* **2003**, *13*, 93R-104R.
28. El Ashry, E. S. H.; Rashed, N.; Shobier, A. H. S. Glycosidase inhibitors and their chemotherapeutic value, part 3. *Pharmazie,* **2000**, *55*, 403-415.
29. Elbein, A. D.; Pan, Y. T.; Pastuszak, I.; Carroll, D. New insights on trehalose: a multifunctional molecule. *Glycobiology,* **2003**, *13*, 17R-27R.
30. Joubert, P. H.; Foukaridis, G. N.; Bopape, M. L. Miglitol May Have a Blood-Glucose Lowering Effect Unrelated to Inhibition of α-Glucosidase. *Eur. J. Clin. Pharmacol.,* **1987**, *31*, 723-724.
31. Joubert, P. H.; Venter, H. L.; Foukaridis, G. N. The Effect of Miglitol and Acarbose after an Oral Glucose-Load - a Novel Hypoglycemic Mechanism. *Br. J. Clin. Pharmacol.,* **1990**, *30*, 391-396.
32. Krentz, A. J.; Bailey, C. J. Oral antidiabetic agents - Current role in type 2 diabetes mellitus. *Drugs,* **2005**, *65*, 385-411.
33. Asano, N.; Takeuchi, M.; Kameda, Y.; Matsui, K.; Kono, Y. Trehalase Inhibitors, Validoxylamine-A and Related-Compounds as Insecticides. *J. Antibiot.,* **1990**, *43*, 722-726.
34. Ando, O.; Kifune, M.; Nakajima, M. Effects of Trehazolin, a Potent Trehalase Inhibitor, on *Bombyx-Mori* and Plant-Pathogenic Fungi. *Biosci. Biotechnol. Biochem.,* **1995**, *59*, 711-712.
35. Bucior, I.; Burger, M. M. Carbohydrate-carbohydrate interactions in cell recognition. *Curr. Opin. Struct. Biol.,* **2004**, *14*, 631-637.
36. Kim, C. U.; Lew, W.; Williams, M. A.; Liu, H. T.; Zhang, L. J.; Swaminathan, S.; Bischofberger, N.; Chen, M. S.; Mendel, D. B.; Tai, C. Y.; Laver, W. G.; Stevens, R. C. Influenza neuraminidase inhibitors possessing a novel hydrophobic interaction in the

enzyme active site: Design, synthesis, and structural analysis of carbocyclic sialic acid analogues with potent anti-influenza activity. *J. Am. Chem. Soc.*, **1997**, *119*, 681-690.
37. Moscona, A. Drug therapy - Neuraminidase inhibitors for influenza. *New. Engl. J. Med.*, **2005**, *353*, 1363-1373.
38. Cook, C. S.; Karabatsos, P. J.; Schoenhard, G. L.; Karim, A. Species-Dependent Esterase-Activities for Hydrolysis of an Anti-Hiv Prodrug Glycovir and Bioavailability of Active Sc-48334. *Pharm. Res.*, **1995**, *12*, 1158-1164.
39. Taylor, D. L.; Sunkara, P. S.; Liu, P. S.; Kang, M. S.; Bowlin, T. L.; Tyms, A. S. 6-0-Butanoylcastanospermine (Mdl 28,574) Inhibits Glycoprotein Processing and the Growth of Hiv. *AIDS*, **1991**, *5*, 693-698.
40. Cox, T.; Lachmann, R.; Hollak, C.; Aerts, J.; van Weely, S.; Hrebicek, M.; Platt, F.; Butters, T.; Dwek, R.; Moyses, C.; Gow, I.; Elstein, D.; Zimran, A. Novel oral treatment of Gaucher's disease with *N*-butyldeoxynojirimycin (OGT 918) to decrease substrate biosynthesis. *Lancet*, **2000**, *355*, 1481-1485.
41. Sawkar, A. R.; Cheng, W. C.; Beutler, E.; Wong, C. -H.; Balch, W. E.; Kelly, J. W. Chemical chaperones increase the cellular activity of N370S β-glucosidase: A therapeutic strategy for Gaucher disease. *Proc. Natl. Acad. Sci. U. S. A.*, **2002**, *99*, 15428-15433.
42. Zitzmann, N.; Mehta, A. S.; Carrouèe, S.; Butters, T. D.; Platt, F. M.; McCauley, J.; Blumberg, B. S.; Dwek, R. A.; Block, T. M. Imino sugars inhibit the formation and secretion of bovine viral diarrhea virus, a pestivirus model of hepatitis C virus: Implications for the development of broad spectrum anti-hepatitis virus agents. *Proc. Natl. Acad. Sci. U. S. A.*, **1999**, *96*, 11878-11882.
43. van den Elsen, J. M. H.; Kuntz, D. A.; Rose, D. R. Structure of Golgi α-mannosidase II: a target for inhibition of growth and metastasis of cancer cells. *EMBO J.*, **2001**, *20*, 3008-3017.
44. Kraut, D. A.; Carroll, K. S.; Herschlag, D. Challenges in enzyme mechanism and energetics. *Annu. Rev. Biochem.*, **2003**, *72*, 517-571.
45. Schramm, V. L. Transition state variation in enzymatic reactions. *Curr. Opin. Chem. Biol.*, **2001**, *5*, 556-563.
46. Wolfenden, R. Transition-State Analog Inhibitors and Enzyme Catalysis. *Annu. Rev. Biophys. Bio.*, **1976**, *5*, 271-306.
47. Kraut, D. A.; Sigala, P. A.; Pybus, B.; Liu, C. W.; Ringe, D.; Petsko, G. A.; Herschlag, D. Testing electrostatic complementarity in enzyme catalysis: Hydrogen bonding in the ketosteroid isomerase oxyanion hole. *Plos. Biol.*, **2006**, *4*, 501-519.
48. Lillelund, V. H.; Jensen, H. H.; Liang, X. F.; Bols, M. Recent developments of transition-state analogue glycosidase inhibitors of non-natural product origin. *Chem. Rev.*, **2002**, *102*, 515-553.
49. Asano, N.; Nash, R. J.; Molyneux, R. J.; Fleet, G. W. J. Sugar-mimic glycosidase inhibitors: natural occurrence, biological activity and prospects for therapeutic application. *Tetrahedron: Asymmetry*, **2000**, *11*, 1645-1680.
50. Asano, N. Alkaloidal sugar mimetics: Biological activities and therapeutic applications. *J. Enzym. Inhib.*, **2000**, *15*, 215-234.
51. *Iminosugars as Glycosidase Inhibitors. Nojirimycin and Beyond*; Stütz, A. E., Ed.; Wiley-VCH: **1999**. Weinheim, Germany.

52. Hughes, A. B.; Rudge, A. J. Deoxynojirimycin: synthesis and biological activity. *Nat. Prod. Rep.*, **1994**, *11*, 135-162.
53. Bols, M.; Hazell, R. G.; Thomsen, I. B. 1-azafagomine: A hydroxyhexahydropyridazine that potently inhibits enzymatic glycoside cleavage. *Chem. -Eur. J.*, **1997**, *3*, 940-947.
54. Fleet, G. W. J.; Smith, P. W. Enantiospecific Syntheses of Deoxymannojirimycin, Fagomine and 2R,5R-Dihydroxymethyl-3R,4R-Dihydroxypyrrolidine from D-Glucose. *Tetrahedron Lett.*, **1985**, *26*, 1469-1472.
55. Sears, P.; Wong, C. -H. Carbohydrate mimetics: A new strategy for tackling the problem of carbohydrate-mediated biological recognition. *Angew. Chem. Int. Ed. Engl.*, **1999**, *38*, 2301-2324.
56. Ganem, B.; Papandreou, G. Mimicking the Glucosidase Transition-State - Shape Charge Considerations. *J. Am. Chem. Soc.*, **1991**, *113*, 8984-8985.
57. Tatsuta, K.; Miura, S.; Gunji, H. The total synthesis of a glycosidase inhibitor, nagstatin. *Bull. Chem. Soc. Jpn.*, **1997**, *70*, 427-436.
58. Granier, T.; Panday, N.; Vasella, A. Structure-activity relations for imidazo-pyridine-type inhibitors of β-D-glucosidases. *Helv. Chim. Acta.*, **1997**, *80*, 979-987.
59. Marco-Contelles, J. Cyclohexane Expoxides-Chemistry and Biochemistry of (+)-Cyclophellitol. *Eur. J. Org. Chem.*, **2001**, 1607-1618.
60. Knapp, S.; Vocadlo, D.; Gao, Z. N.; Kirk, B.; Lou, J. P.; Withers, S. G. NAG-thiazoline, an N-acetyl-β-hexosaminidase inhibitor that implicates acetamido participation. *J. Am. Chem. Soc.*, **1996**, *118*, 6804-6805.
61. Dong, W. L.; Jespersen, T.; Bols, M.; Skrydstrup, T.; Sierks, M. R. Evaluation of isofagomine and its derivatives as potent glycosidase inhibitors. *Biochemistry*, **1996**, *35*, 2788-2795.
62. Lohse, A.; Hardlei, T.; Jensen, A.; Plesner, I. W.; Bols, M. Investigation of the slow inhibition of almond β-glucosidase and yeast isomaltase by 1-azasugar inhibitors: evidence for the 'direct binding' model. *Biochem. J.*, **2000**, *349*, 211-215.
63. White, A.; Tull, D.; Johns, K.; Withers, S. G.; Rose, D. R. Crystallographic observation of a covalent catalytic intermediate in a β-glycosidase. *Nat. Struct. Biol*, **1996**, *3*, 149-154.
64. Wiesmann, C.; Hengstenberg, W.; Schulz, G. E. Crystal structures and mechanism of 6-phospho-β-galactosidase from *Lactococcus lactis*. *J. Mol. Biol.*, **1997**, *269*, 851-860.
65. van Aalten, D. M. F.; Komander, D.; Synstad, B.; Gaseidnes, S.; Peter, M. G.; Eijsink, V. G. H. Structural insights into the catalytic mechanism of a family 18 exo-chitinase. *Proc. Natl. Acad. Sci. U. S. A.*, **2001**, *98*, 8979-8984.
66. Tews, I.; vanScheltinga, A. C. T.; Perrakis, A.; Wilson, K. S.; Dijkstra, B. W. Substrate-assisted catalysis unifies two families of chitinolytic enzymes. *J. Am. Chem. Soc.*, **1997**, *119*, 7954-7959.
67. Brameld, K. A.; Goddard, W. A. Substrate distortion to a boat conformation at subsite-1 is critical in the mechanism of family 18 chitinases. *J. Am. Chem. Soc.*, **1998**, *120*, 3571-3580.
68. Brameld, K. A.; Goddard, W. A. The role of enzyme distortion in the single displacement mechanism of family 19 chitinases. *Proc. Natl. Acad. Sci. U. S. A.*, **1998**, *95*, 4276-4281.

69. Papanikolau, Y.; Prag, G.; Tavlas, G.; Vorgias, C. E.; Oppenheim, A. B.; Petratos, K. High resolution structural analyses of mutant chitinase A complexes with substrates provide new insight into the mechanism of catalysis. *Biochemistry,* **2001**, *40*, 11338-11343.
70. Brameld, K. A.; Shrader, W. D.; Imperiali, B.; Goddard, W. A. Substrate assistance in the mechanism of family 18 chitinases: Theoretical studies of potential intermediates and inhibitors. *J. Mol. Biol.,* **1998**, *280*, 913-923.
71. Taib, M.; Pinney, J. W.; Westhead, D. R.; McDowall, K. J.; Adams, D. J. Differential expression and extent of fungal/plant and fungal/bacterial chitinases of *Aspergillus fumigatus. Arch. Microbiol.,* **2005**, *184*, 78-81.
72. Choi, H. K.; Choi, K. H.; Kramer, K. J.; Muthukrishnan, S. Isolation and characterization of a genomic clone for the gene of an insect molting enzyme, chitinase. *Insect Biochem.,* **1997**, *27*, 37-47.
73. Vinetz, J. M. Plasmodium ookinete invasion of the mosquito midgut. *Curr. Top. Microbiol. Immunol.,* **2005**, *295*, 357-382.
74. Vinetz, J. M.; Valenzuela, J. G.; Specht, C. A.; Aravind, L.; Langer, R. C.; Ribeiro, J. M.; Kaslow, D. C. Chitinases of the avian malaria parasite *Plasmodium gallinaceum*, a class of enzymes necessary for parasite invasion of the mosquito midgut. *J. Biol. Chem.,* **2000**, *275*, 10331-10341.
75. Cabib, E.; Silverman, S. J.; Shaw, J. a. Chitinase and Chitin Synthase.1. Counterbalancing Activities in Cell-Separation of *Saccharomyces-Cerevisiae. J. Gen. Microbiol.,* **1992**, *138*, 97-102.
76. Adams, D. J. Fungal cell wall chitinases and glucanases. *Microbiol-Sgm,* **2004**, *150*, 2029-2035.
77. Herrera-Estrella A, C. I. Chitinases in biological contrrol. *EXS,* **1999**, *87*, 171-184.
78. Boot, R. G.; Blommaart, E. F. C.; Swart, E.; Ghauharali-van der Vlugt, K.; Bijl, N.; Moe, C.; Place, A.; Aerts, J. M. F. G. Identification of a novel acidic mammalian chitinase distinct from chitotriosidase. *J. Biol. Chem.,* **2001**, *276*, 6770-6778.
79. Zhu, Z.; Zheng, T.; Homer, R. J.; Kim, Y. K.; Chen, N. Y.; Cohn, L.; Hamid, Q.; Elias, J. A. Acidic mammalian chitinase in asthmatic Th2 inflammation and IL-13 pathway activation. *Science,* **2004**, *304*, 1678-1682.
80. Wills-Karp, M.; Karp, C. L. Chitin checking - Novel insights into asthma. *New. Engl. J. Med.,* **2004**, *351*, 1455-1457.
81. Andersen, O. A.; Dixon, M. J.; Eggleston, I. M.; van Aalten, D. M. F. Natural product family 18 chitinase inhibitors. *Nat. Prod. Rep.,* **2005**, *22*, 563-579.
82. Sakuda, S.; Isogai, A.; Matsumoto, S.; Suzuki, a.; Koseki, K. The Structure of Allosamidin, a Novel Insect Chitinase Inhibitor, Produced by *Streptomyces Sp. Tetrahedron Lett.,* **1986**, *27*, 2475-2478.
83. Shiomi, K.; Arai, N.; Iwai, Y.; Turberg, A.; Kolbl, H.; Omura, S. Structure of argifin, a new chitinase inhibitor produced by *Gliocladium sp. Tetrahedron Lett.,* **2000**, *41*, 2141-2143.
84. Izumida, H.; Imamura, N.; Sano, H. A novel chitinase inhibitor from a marine bacterium, *Pseudomonas sp. J. Antibiot.,* **1996**, *49*, 76-80.

85. Kato, T.; Shizuri, Y.; Izumida, H.; Yokoyama, a.; Endo, M. Styloguanidines, New Chitinase Inhibitors from the Marine Sponge *Stylotella-Aurantium*. *Tetrahedron Lett.*, **1995**, *36*, 2133-2136.
86. Berecibar, A.; Grandjean, C.; Siriwardena, A. Synthesis and biological activity of natural aminocyclopentitol glycosidase inhibitors: Mannostatins, trehazolin, allosamidins, and their analogues. *Chem. Rev.*, **1999**, *99*, 779-844.
87. Griffith, D. A.; Danishefsky, S. J. The total synthesis of allosamidin. Expansions of the methodology of azaglycosylation pursuant to the total synthesis of allosamidin. A surprising enantiotopic sense for a lipase-induced deacetylation. *J. Am. Chem. Soc.*, **1996**, *118*, 9526-9538.
88. Trost, B. M.; Vanvranken, D. L. A General Synthetic Strategy toward Aminocyclopentitol Glycosidase Inhibitors - Application of Palladium Catalysis to the Synthesis of Allosamizoline and Mannostatin-A. *J. Am. Chem. Soc.*, **1993**, *115*, 444-458.
89. Shrader, W. D.; Imperiali, B. Synthesis of the glucoallosamidin pseudo-disaccharide: Use of an efficient Hg(II) mediated cyclization. *Tetrahedron Lett.*, **1996**, *37*, 599-602.
90. Kassab, D. J.; Ganem, B. An enantioselective synthesis of (-)-allosamidin by asymmetric desymmetrization of a highly functionalized meso-epoxide. *J. Org. Chem.*, **1999**, *64*, 1782-1783.
91. Maloisel, J. L.; Vasella, A.; Trost, B. M.; Vanvranken, D. L. Synthesis of Allosamidin. *Helv. Chim. Acta.*, **1992**, *75*, 1515-1526.
92. Coutinho, P. M.; Deleury, E.; Davies, G. J.; Henrissat, B. An evolving hierarchical family classification for glycosyltransferases. *J. Mol. Biol.*, **2003**, *328*, 307-317.
93. Breton, C.; Snajdrova, L.; Jeanneau, C.; Koca, J.; Imberty, A. Structures and mechanisms of glycosyltransferases. *Glycobiology*, **2006**, *16*, 29R-37R.
94. Ünligil, U. M.; Rini, J. M. Glycosyltransferase structure and mechanism. *Curr. Opin. Struct. Biol.*, **2000**, *10*, 510-517.
95. Lairson, L. L.; Withers, S. G. Mechanistic analogies amongst carbohydrate modifying enzymes. *Chem. Commum.*, **2004**, 2243-2248.
96. Ünligil, U. M.; Zhou, S. H.; Yuwaraj, S.; Sarkar, M.; Schachter, H.; Rini, J. M. X-ray crystal structure of rabbit N-acetylglucosaminyltransferase I: catalytic mechanism and a new protein superfamily. *EMBO J.*, **2000**, *19*, 5269-5280.
97. Pedersen, L. C.; Tsuchida, K.; Kitagawa, H.; Sugahara, K.; Darden, T. A.; Negishi, M. Heparan/chondroitin sulfate biosynthesis - Structure and mechanism of human glucuronyltransferase I. *J. Biol. Chem.*, **2000**, *275*, 34580-34585.
98. Chiu, C. P. C.; Watts, A. G.; Lairson, L. L.; Gilbert, M.; Lim, D.; Wakarchuk, W. W.; Withers, S. G.; Strynadka, N. C. J. Structural analysis of the sialyltransferase CstII from *Campylobacter jejuni* in complex with a substrate analog. *Nat. Struct. Mol. Biol.*, **2004**, *11*, 163-170.
99. Persson, K.; Ly, H. D.; Dieckelmann, M.; Wakarchuk, W. W.; Withers, S. G.; Strynadka, N. C. J. Crystal structure of the retaining galactosyltransferase LgtC from *Neisseria meningitidis* in complex with donor and acceptor sugar analogs. *Nat. Struct. Biol.*, **2001**, *8*, 166-175.
100. Lairson, L. L.; Chiu, C. P. C.; Ly, H. D.; He, S. M.; Wakarchuk, W. W.; Strynadka, N. C. J.; Withers, S. G. Intermediate trapping on a mutant retaining α-

galactosyltransferase identifies an unexpected aspartate residue. *J. Biol. Chem.*, **2004**, *279*, 28339-28344.
101. Pedersen, L. C.; Dong, J.; Taniguchi, F.; Kitagawa, H.; Krahn, J. M.; Pedersen, L. G.; Sugahara, K.; Negishi, M. Crystal structure of an α 1,4-N-acetylhexosaminyltransferase (EXTL2), a member of the exostosin gene family involved in heparan sulfate biosynthesis. *J. Biol. Chem.*, **2003**, *278*, 14420-14428.
102. Boix, E.; Zhang, Y. N.; Swaminathan, G. J.; Brew, K.; Acharya, K. R. Structural basis of ordered binding of donor and acceptor substrates to the retaining glycosyltransferase, α-1,3-galactosyltransferase. *J. Biol. Chem.*, **2002**, *277*, 28310-28318.
103. Compain, P.; Martin, O. R. Carbohydrate mimetics-based glycosyltransferase inhibitors. *Bioorg. Med. Chem.*, **2001**, *9*, 3077-3092.
104. Wang, R.; Steensma, D. H.; Takaoka, Y.; Yun, J. W.; Kajimoto, T.; Wong, C.-H. A search for pyrophosphate mimics for the development of substrates and inhibitors of glycosyltransferases. *Bioorg. Med. Chem.*, **1997**, *5*, 661-672.
105. Hu, Y.; Heim, J. S.; Chen, L.; Ginsberg, C.; Gross, B.; Kraybill, B.; Tiyanont, K.; Fang, X.; Wu, T.; Walker, S. Identification of selective inhibitors for the glycosyltransferase *via* high-throughput MurG screening. *Chem. Biol.*, **2004**, *11*, 703-711.
106. Schmidt, R. R.; Frische, K. A New Galactosyl Transferase Inhibitor. *Bioorg. Med. Chem. Lett.*, **1993**, *3*, 1747-1750.
107. Myers, A. G.; Gin, D. Y.; Rogers, D. H. Synthetic Studies of the Tunicamycin Antibiotics - Preparation of (+)-Tunicaminyluracil, (+)-Tunicamycin-V, and 5'-Epi-Tunicamycin-V. *J. Am. Chem. Soc.*, **1994**, *116*, 4697-4718.
108. Hinou, H.; Sun, X. L.; Ito, Y. Systematic syntheses and inhibitory activities of bisubstrate-type inhibitors of sialyltransferases. *J. Org. Chem.*, **2003**, *68*, 5602-5613.
109. Waldscheck, B.; Streiff, M.; Notz, W.; Kinzy, W.; Schmidt, R. R. α-(1-3)-galactosyltransferase inhibition based on a new type of disubstrate analogue. *Angew. Chem. Int. Ed. Engl.*, **2001**, *40*, 4007-4009.
110. Muller, B.; Schaub, C.; Schmidt, R. R. Efficient sialyltransferase inhibitors based on transition-state analogues of the sialyl donor. *Angew. Chem. Int. Ed. Engl.*, **1998**, *37*, 2893-2897.
111. Qiao, L.; Murray, B. W.; Shimazaki, M.; Schultz, J.; Wong, C. -H. Synergistic inhibition of human α-1,3-fucosyltransferase V. *J. Am. Chem. Soc.*, **1996**, *118*, 7653-7662.
112. Kim, Y. J.; Ichikawa, M.; Ichikawa, Y. A rationally designed inhibitor of α-1,3-galactosyltransferase. *J. Am. Chem. Soc.*, **1999**, *121*, 5829-5830.
113. Saotome, C.; Wong, C. -H.; Kanie, O. Combinatorial library of five-membered iminocyclitol and the inhibitory activities against glyco-enzymes. *Chem. Biol.*, **2001**, *8*, 1061-1070.
114. Takayama, S.; Chung, S. J.; Igarashi, Y.; Ichikawa, Y.; Sepp, A.; Lechler, R. I.; Wu, J. Y.; Hayashi, T.; Siuzdak, G.; Wong, C. -H. Selective inhibition of β-1,4- and α-1,3-galactosyltransferases: Donor sugar-nucleotide based approach. *Bioorg. Med. Chem.*, **1999**, *7*, 401-409.
115. Sears, P.; Wong, C. -H. Enzyme action in glycoprotein synthesis. *Cell. Mol. Life. Sci.*, **1998**, *54*, 223-252.

116. Lee, L. V.; Mitchell, M. L.; Huang, S.-J.; Fokin, V. V.; Sharpless, K. B.; Wong, C.-H. A potent and highly selective inhibitor of human α-1,3-fucosyltransferase *via* click chemistry. *J. Am. Chem. Soc.*, **2003**, *125*, 9588-9589.
117. Fleet, G. W. J.; Namgoong, S. K.; Barker, C.; Baines, S.; Jacob, G. S.; Winchester, B. Iminoheptitols as Glycosidase Inhibitors - Synthesis of and Specific α-L-Fucosidase Inhibition by β-L-Homofuconojirimycin and 1-β-C-Substituted Deoxymannojirimycins. *Tetrahedron Lett.*, **1989**, *30*, 4439-4442.
118. Jefferies, I.; Bowen, B. R. Synthesis of inhibitors of α-1,3-fucosyltransferase. *Bioorg. Med. Chem. Lett.*, **1997**, *7*, 1171-1174.
119. Kornfeld, R.; Kornfeld, S. Assembly of Asparagine-Linked Oligosaccharides. *Annu. Rev. Biochem.*, **1985**, *54*, 631-664.
120. Dempski, R. E.; Imperiali, B. Oligosaccharyl transferase: gatekeeper to the secretory pathway. *Curr. Opin. Chem. Biol.*, **2002**, *6*, 844-850.
121. Kelleher, D. J.; Gilmore, R. An evolving view of the eukaryotic oligosaccharyltransferase. *Glycobiology*, **2006**, *16*, 47R-62R.
122. Wacker, M.; Linton, D.; Hitchen, P. G.; Nita-Lazar, M.; Haslam, S. M.; North, S. J.; Panico, M.; Morris, H. R.; Dell, A.; Wren, B. W.; Aebi, M. N-linked glycosylation in *Campylobacter jejuni* and its functional transfer into E-coli. *Science*, **2002**, *298*, 1790-1793.
123. Yan, A. X.; Lennarz, W. J. Unraveling the mechanism of protein N-glycosylation. *J. Biol. Chem.*, **2005**, *280*, 3121-3124.
124. Feldman, M. F.; Wacker, M.; Hernandez, M.; Hitchen, P. G.; Marolda, C. L.; Kowarik, M.; Morris, H. R.; Dell, A.; Valvano, M. A.; Aebi, M. Engineering N-linked protein glycosylation with diverse O antigen lipopolysaccharide structures in *Escherichia coli*. *Proc. Natl. Acad. Sci. U. S. A.*, **2005**, *102*, 3016-3021.
125. Spirig, U.; Glavas, M.; Bodmer, D.; Reiss, G.; Burda, P.; Lippuner, V.; teHeesen, S.; Aebi, M. The STT3 protein is a component of the yeast oligosaccharyltransferase complex. *Mol. Gen. Genet.*, **1997**, *256*, 628-637.
126. Burda, P.; Aebi, M. The dolichol pathway of N-linked glycosylation. *Biochim. Biophys. Acta*, **1999**, *1426*, 239-257.
127. Kim, H.; von Heijne, G.; Nilsson, I. Membrane topology of the STT3 subunit of the oligosaccharyl transferase complex. *J. Biol. Chem.*, **2005**, *280*, 20261-20267.
128. Kelleher, D. J.; Karaoglu, D.; Mandon, E. C.; Gilmore, R. Oligosaccharyltransferase isoforms that contain different catalytic STT3 subunits have distinct enzymatic properties. *Mol. Cell.*, **2003**, *12*, 101-111.
129. Marshall, R. D. Glycoproteins. *Annu. Rev. Biochem.*, **1972**, *41*, 673-702.
130. Bause, E. Structural Requirements of N-Glycosylation of Proteins - Studies with Proline Peptides as Conformational Probes. *Biochem. J.*, **1983**, *209*, 331-336.
131. Imperiali, B.; Shannon, K. L.; Unno, M.; Rickert, K. W. A Mechanistic Proposal for Asparagine-Linked Glycosylation. *J. Am. Chem. Soc.*, **1992**, *114*, 7944-7945.
132. Imperiali, B. Protein glycosylation: The clash of the titans. *Acc. Chem. Res.*, **1997**, *30*, 452-459.
133. Abbadi, A.; Mcharfi, M.; Aubry, A.; Premilat, S.; Boussard, G.; Marraud, M. Involvement of Side Functions in Peptide Structures - the Asx Turn - Occurrence and Conformational Aspects. *J. Am. Chem. Soc.*, **1991**, *113*, 2729-2735.

134. O'Connor, S. E.; Imperiali, B. Conformational switching by asparagine-linked glycosylation. *J. Am. Chem. Soc.*, **1997**, *119*, 2295-2296.
135. Imperiali, B.; O'Connor, S. E. Effect of N-linked glycosylation on glycopeptide and glycoprotein structure. *Curr. Opin. Chem. Biol.*, **1999**, *3*, 643-649.
136. Gavel, Y.; Vonheijne, G. Sequence Differences between Glycosylated and Nonglycosylated Asn-X-Thr Ser Acceptor Sites - Implications for Protein Engineering. *Protein Eng.*, **1990**, *3*, 433-442.
137. Hendrickson, T. L.; Spencer, J. R.; Kato, M.; Imperiali, B. Design and evaluation of potent inhibitors of asparagine-linked protein glycosylation. *J. Am. Chem. Soc.*, **1996**, *118*, 7636-7637.
138. Gibbs, B. S.; Coward, J. K. Dolichylpyrophosphate oligosaccharides: Large-scale isolation and evaluation of oligosaccharyltransferase substrates. *Bioorg. Med. Chem.*, **1999**, *7*, 2121-2121.
139. Tai, V. W. -F.; Imperiali, B. Substrate specificity of the glycosyl donor for oligosaccharyl transferase. *J. Org. Chem.*, **2001**, *66*, 6217-6228.
140. Hendrickson, T. L.; Imperiali, B. Metal ion dependence of oligosaccharyl transferase: implications for catalysis. *Biochemistry*, **1995**, *34*, 9444-9450.
141. Wacker, M.; Feldman, M. F.; Callewaert, N.; Kowarik, M.; Clarke, B. R.; Pohl, N. L.; Hernandez, M.; Vines, E. D.; Valvano, M. A.; Whitfield, C.; Aebi, M. Substrate specificity of bacterial oligosaccharyltransferase suggests a common transfer mechanism for the bacterial and eukaryotic systems. *Proc. Natl. Acad. Sci. U. S. A.*, **2006**, *103*, 7088-,7093.
142. Tai, V. W.-F. Dissertation, Massachusetts Institute of Technology, Cambridge, MA, 2000.
143. Fleet, G. W. J.; Smith, P. W. Methyl 2-Azido-3-*O*-Benzyl-2-Deoxy-α-D-Mannofuranoside as a Divergent Intermediate for the Synthesis of Polyhydroxylated Piperidines and Pyrrolidines - Synthesis of 2,5-Dideoxy-2,5-Imino-D-Mannitol [2R,5R-Dihydroxymethyl-3R,4R-Dihydroxypyrrolidine]. *Tetrahedron*, **1987**, *43*, 971-978.
144. Look, G. C.; Fotsch, C. H.; Wong, C. -H. Enzyme-Catalyzed Organic-Synthesis - Practical Routes to Aza Sugars and Their Analogs for Use as Glycoprocessing Inhibitors. *Acc. Chem. Res.*, **1993**, *26*, 182-190.
145. Saotome, C.; Wong, C. -H.; Kanie, O. Combinatorial library of five-membered iminocyclitol and the inhibitory activities against glyco-enzymes. *Chem. Biol.*, **2001**, *8*, 1061-1070.
146. Takaoka, Y.; Kajimoto, T.; Wong, C.-H. Inhibition of N-Acetylglucosaminyltransfer Enzymes: Chemical-Enzymatic Synthesis of New Five-Membered Acetamido Azasugar. *J. Org. Chem.*, **1993**, *58*, 4809-4812.
147. Liu, J.; Numa, M. M.; Liu, H.; Huang, S. J.; Sears, P.; Shikhman, A. R.; Wong, C. -H. Synthesis and high-throughput screening of N-acetyl-β-hexosaminidase inhibitor libraries targeting osteoarthritis. *J. Org. Chem.*, **2004**, *69*, 6273-6283.
148. Wu, C. -Y.; Chang, C. -F.; Chen, J. -S.; Wong, C. -H.; Lin, C. -H. Rapid diversity-oriented synthesis in microtiter plates for in situ screening: discovery of potent and selective α-fucosidase inhibitors. *Angew. Chem. Int. Ed. Engl.*, **2003**, *42*, 4661-4664.

Chapter 2- The synthetic efforts toward the pseudo-disaccharide containing an imino sugar by the convergent approach

2-1 Introduction

As discussed in Chapter 1-11, our target compounds for both OT and chitinase inhibition studies were pseudo-disaccharide moieties containing the transition state analogue. We were interested in functionalizing the C1 amine of the imino sugar to generate its derivatives. Considering the amphiphilic nature of the dolichyl-pyrophosphate-linked tetradecasaccharide and the membrane-associated nature of OT, we anticipated that a potential OT inhibitor would require a long aliphatic chain to increase the enzyme affinity.

From the outset, we planned to synthesize pseudo-disaccharides containing the imino sugar by a convergent approach (**Figure 2-1**). Thus, the sugar acceptor **2-5** and sugar donor would be synthesized separately. A glucose derivative or a glucosamine derivative donor would be added to the C4 hydroxyl group of the imino sugar building block. Neighboring-group participation of C2' group on the sugar donor would ensure β-linked glycosidic bond formation.[1,2] Imino sugar building block **2-5** would be constructed from unprotected precursor **2-6**, the synthesis of which has been reported.[3-5] Considering its compatibility with glycosylation and facile deprotection, the Cbz (benzyloxycarbonyl) group was chosen as the protecting group for the primary amine and secondary amine. This approach was expected to provide the desired pseudo-disaccharide convergently. Then, inhibitor candidates would be prepared through the protecting group manipulations and functionalization of the resulting primary amine.

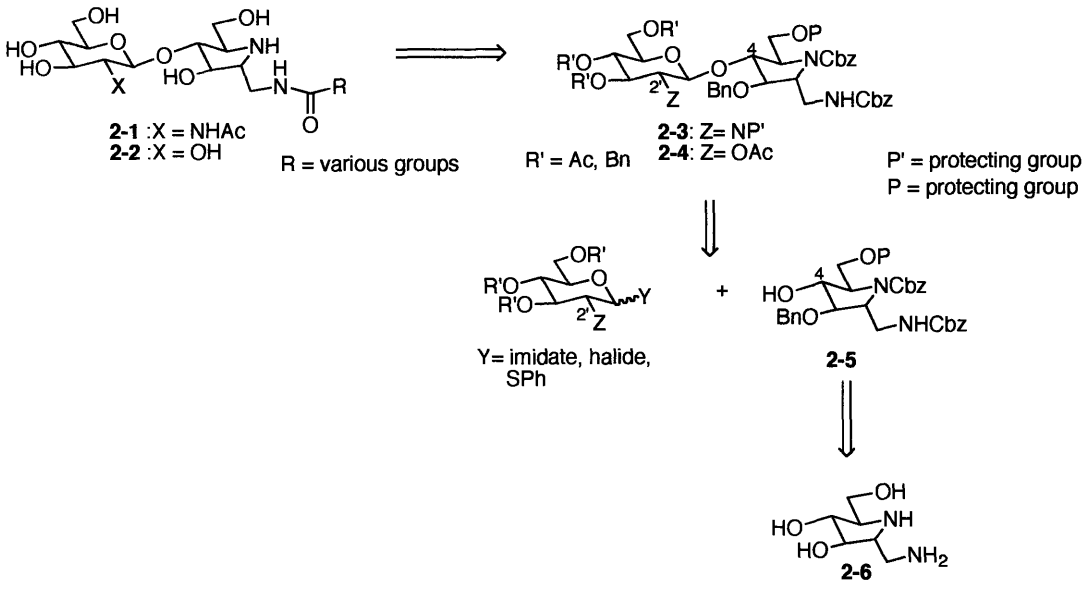
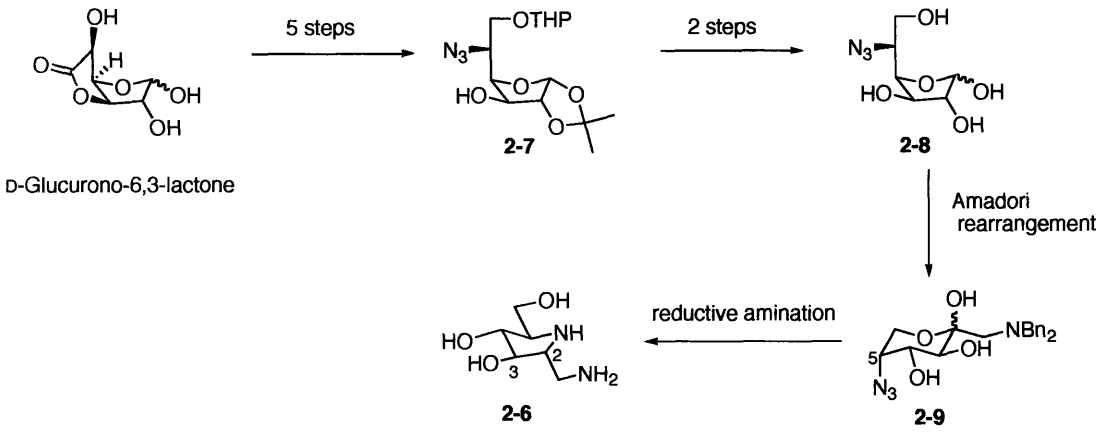


Figure 2-1. Strategy toward the synthesis of pseudo-disaccharides containing an imino sugar.

2-2 Synthesis of the imino sugar and biological evaluations

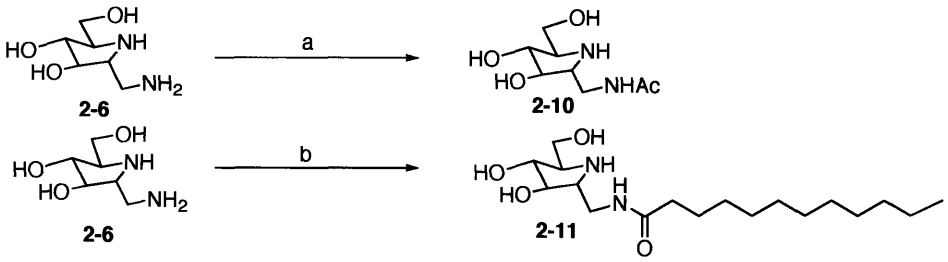
Among the various syntheses of **2-9**, we decided to follow the one developed by Stütz (**Scheme 2-1**).[4,6,7] This route utilizes the Amadori rearrangement[6] and an intramolecular reductive amination as key steps. The synthesis was straightforward and the intermediate was efficiently modified to generate various C1-nitrogen imino sugar derivatives. Beginning with D-glucurono-6,3-lactone, **2-7** was accessible in 5 steps.[8] The sequential removal of protecting groups on **2-7** gave the desired product **2-8**. The conversion of the α-hydroxy lactol to the β-amino ketal was achieved by the Amadori rearrangement[6] of **2-8** with 1.0 equiv. of Bn_2NH in the presence of 1.0 equiv. of acetic acid in methanol. An intramolecular reductive amination between the *in-situ* generated amine from azide group and the ketone followed by concomitant debenzylation gave **2-9**.

The stereo-chemical outcome of the reductive amination was dictated by the approach of hydrogen from the *re* face to avoid torsional strain, generating the *trans*-configuration between C2-C3.[3,4]



Scheme 2-1. The synthesis of imino sugar developed by Stütz.

Following literature procedures,[4] the diamine **2-6** was obtained in 9 steps.[5] Although our target compounds were the pseudo-disaccharide derivatives containing the imino sugar, the facile modification of the primary amine in **2-6** prompted us to investigate the inhibitory activity of the imino sugars against OT and chitinase. The selective acylation of the primary amine in **2-6** with acetic anhydride and dodecanoic anhydride in methanol gave the desired compounds **2-10** and **2-11**, respectively (**Scheme 2-2**).[3,9]



Reagents and conditions: (a) Ac_2O, MeOH, 0 °C, 50%; (b) dodecanoic anhydride, MeOH, 82%.

Scheme 2-2. The synthesis of imino sugar 2-10 and 2-11.

The inhibition assays of **2-10** and **2-11** against chitinase from *Streptomyces griseus* were carried out, following literature procedures (**Figure 2-2**).[10,11] 4-Methylumbelliferyl-β-D-*N*,*N'*-diacetylchitobiose, which is commercially available, was used as a fluorogenic substrate to monitor enzyme activity. Cleavage of 4-methylumbelliferyl-β-chitobiose by chitinase yields the fluorescent molecule 4-methylumbelliferone that emits light at 460 nm when excited at 365 nm. Production of 4-methylumbelliferone was linear with time for the assay incubation time (10 min.). Figure 2-3 shows the fluorescence data at 460 nm for enzyme activity without inhibitor (control experiment) and with **2-10** and **2-11**. The inhibition percentage relative to the control was calculated on the basis of the fluorescence reading at 460 nm after 10 min. No inhibitory activity of **2-10** and **2-11** was observed up to concentration 2 mM.

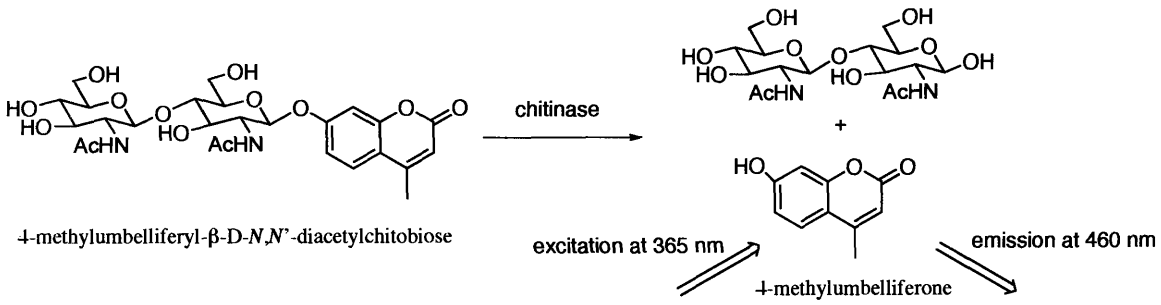


Figure 2-2. The general scheme of the chitinase assay.

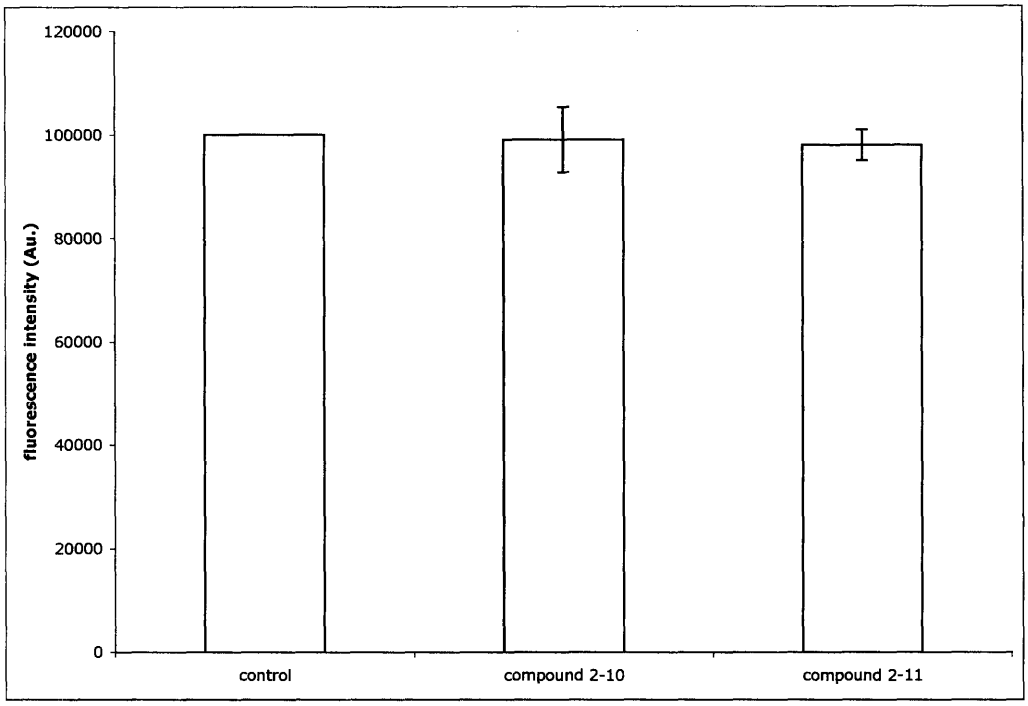


Figure 2-3. The inhibitory activity of 2-10 and 2-11 toward chitinase.

Next, **2-11** was evaluated as an inhibitor for yeast OT from *Saccharomyces cerevisiae* using radiolabeled Dol-PP-GlcNAc-(^{3}H-GlcNAc) as the donor and the consensus sequence tripeptide, Bz-Asn-Leu-Thr-NHMe, as the acceptor (**Figure 2-4**).[12,13] The radiolabeled dolichol-pyrophosphate-linked chitobiose was prepared from the enzyme reaction of the synthetically made Dol-PP-GlcNAc and tritium labeled UDP-(^{3}H)-GlcNAc with Enzyme II from pig liver. The tripeptide was prepared using solution

phase peptide chemistry. An OT activity assay involves monitoring the transfer of radiolabeled chitobiose from the dolichly-pyrophosphate-linked chitobiose to the tripeptide. The difference in partition coefficients of the organic-soluble lipid-linked chitobiose and the aqueous-soluble glycopeptide product allows for separation by a simple aqueous-organic extraction. The radioactivity of the aqueous phase was measured using scintillation counting to determine the amount of radioactivity in the ^{3}H-glycopeptide. The disintegrations per minute (DPM) were plotted as a function of time for the control (without inhibitor) and different inhibitor concentrations (**Figure 2-5**). The percentage inhibition was determined from slope of plot. Compound **2-11** showed no inhibitory activity against OT up to 2 mM.

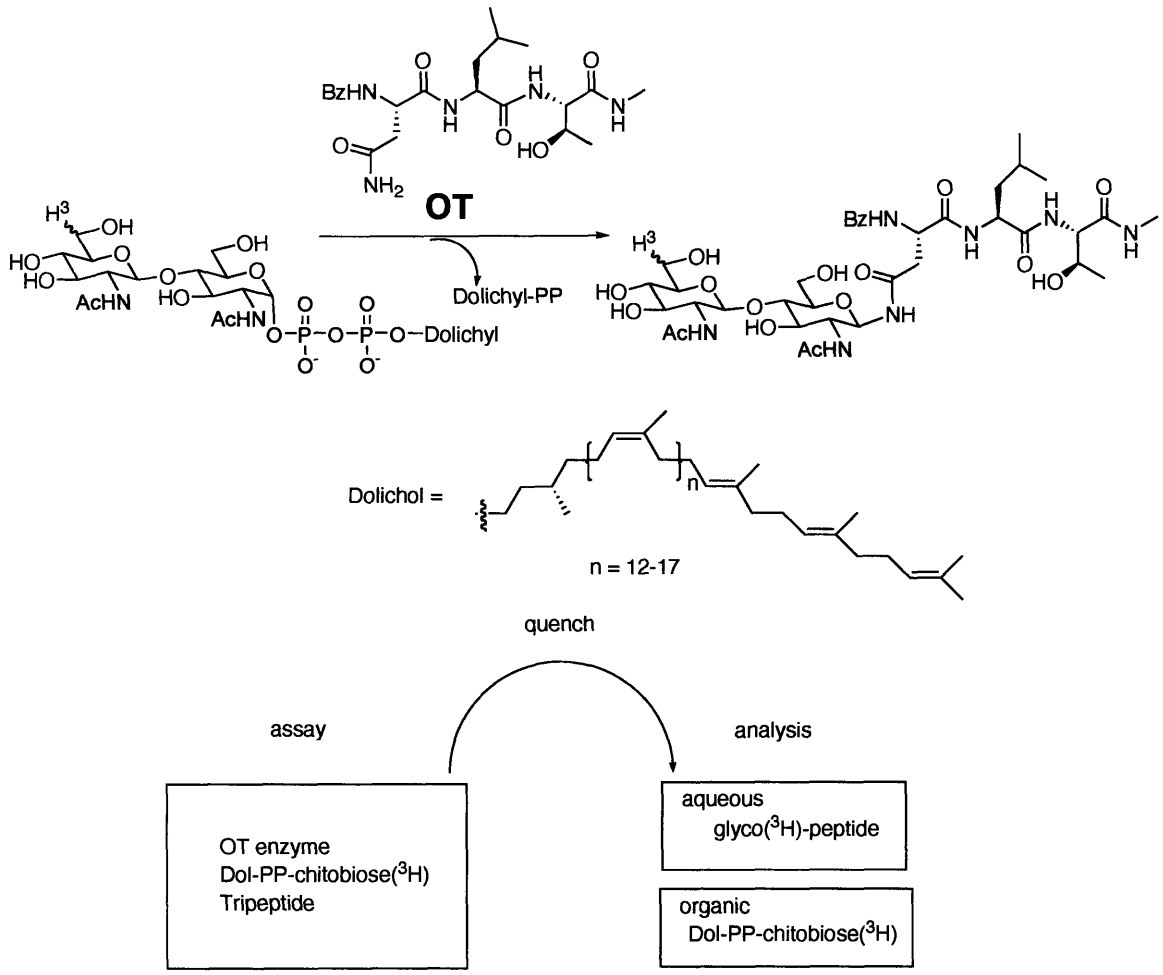


Figure 2-4. The general scheme of OT asssy.

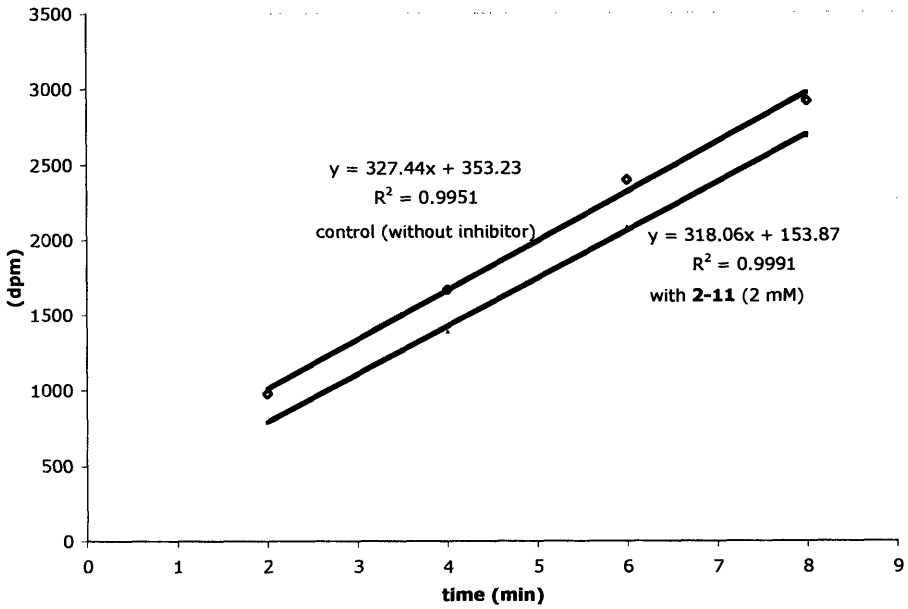


Figure 2-5. The activity assay of 2-11 toward OT

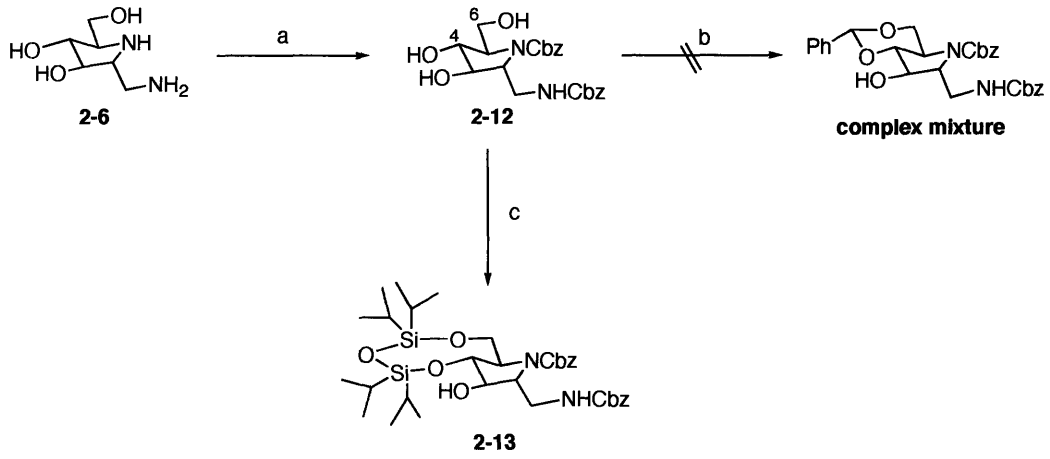
The poor biological activities of **2-10** and **2-11** against chitinase and OT respectively were confirmed our prediction. Considering previous inhibitor studies of chitinases and substrate studies of OT, the disaccharide unit is necessary for the recognition by these enzymes as discussed in Chapter 1-11.[14,15] Therefore, we focused our attention on the synthesis of pseudo-disaccharide containing the imino sugar.

2-3 Synthesis of the imino sugar building block

Compound **2-12** was prepared by treatment of **2-6** with 3.0 equiv. of benzylchloroformate (CbzCl) in the presence of *N*,*N*-diisopropylethylamine (DIPEA) (**Scheme 2-3**). Attempted *trans*-6,4 diol protection of **2-12** employing $PhH(OMe)_2$ in the presence of 0.1 equiv. of *p*-toluenesulfonic acid (*p*-TsOH) gave an inseparable mixture of

reaction products. Modified reaction conditions, such as different solvents (DMF, CH_3CN), excess amounts of the protecting reagents, and increased temperatures did not improve formation of the desired compound. The difficulty of installing this protecting group might be a result of the structural rigidity of the diol, which has a *trans* configuration in the five-membered ring.

Due to the complications with benzylidene acetal formation in **2-12**, we decided to use the disiloxane group,[16] which have been used for protection of a *trans*-3,5 diol in the polyhydroxyfuranoside synthesis.[17] Compound **2-12** was treated with 1.2 equiv. of 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane in pyridine to provide **2-13** in 72% yield.



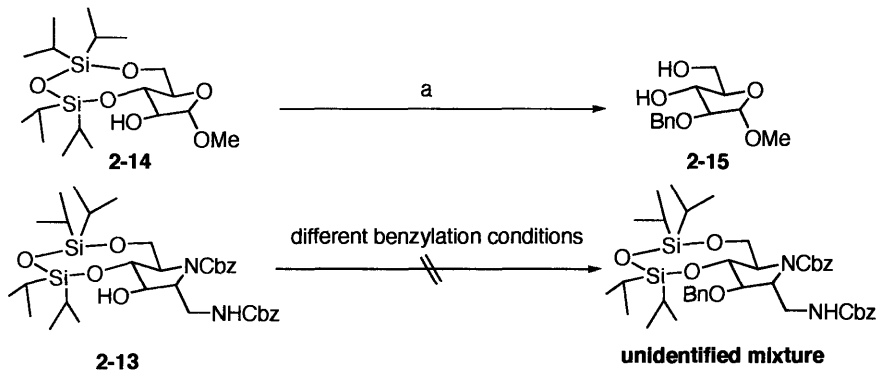
Reagents and conditions: (a) CbzCl, DIPEA, MeOH, 0 °C to RT, 88%; (b) $PhH(OMe)_2$, *p*-TsOH, CH_3CN or DMF; (c) 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane, pyridine, 0 °C to RT, 72%.

Scheme 2-3. The synthesis of 2-13.

In designing the sugar acceptor, the choice of hydroxyl protecting group adjacent to the glycosylation site is often crucial for efficient glycosylation.[18-20] In light of steric and electronic effects, the benzyl group was most suitable for the secondary alcohol at the

C3 position. Attempted benzylation on the secondary alcohol under the various conditions failed to afford the desired product. By modifying a literature procedure,[17,21] benzylation of furanoside **2-14** as a model compound which was synthesized following the literature procedures, was optimized (**Scheme 2-4**). The best conditions were to treat **2-14** with 1.3 equiv. of benzyl bromide (BnBr) and 2.2 equiv. of NaH in DMF. Subsequent deprotection of the disiloxane group using tetrabutyl-*n*-ammonium fluoride (TBAF) in THF provided the desired product **2-15**. Unfortunately, when applying the same reaction conditions to **2-13**, an unidentified complex mixture was obtained. TBAF-mediated removal of the siloxane group did not help to separate the mixture. Changing the amount of benzyl bromide or sodium hydride also led to similar results. Mass spectral analysis indicated that the major constituent in the mixture was the dibenzylated product. The linkage between the silicon and the secondary alcohol has been known to be unstable under basic conditions.[16] One possible explanation is that cleavage of the protecting group took place first resulting in non-selective benzyl ether formation.

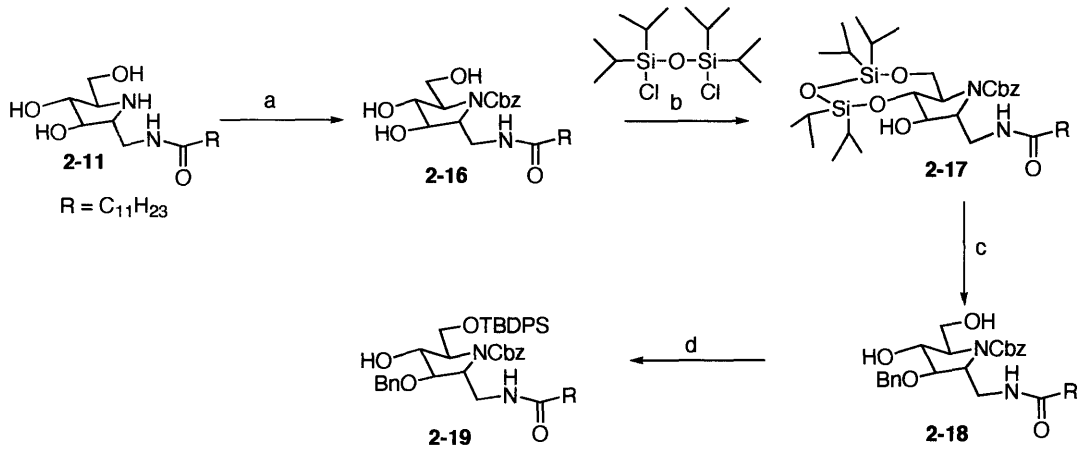
These problems led us to investigate other benzylation conditions. The reaction did not proceed when the solvent was changed to THF. Attempts at using a stronger base such as BuLi, sodium hexamethyldisilazne (NaHMDS), and lithium hexamethyldisilazne (LiHMDS) gave no improvement. Furthermore, no starting material was consumed at low temperatures (-78 °C to 0 °C) and decomposition of the starting material was detected at ambient temperature. Benzylation of **2-13** employing the different base in the solvent mixture of THF and DMF also did not provide any desired product. Finally, benzylation employing silver oxide and benzyl bromide under different solvents (Et_2O or DMF) failed to yield the desired product.



Reagents and conditions: (a) (i) BnBr, NaH, DMF, 0 °C to RT; (ii) TBAF, THF, 2 steps 60 %

Scheme 2-4. Attempted benzylation of 2-13.

Since our primary goal was to synthesize a potential OT inhibitor, the benzylation of **2-17**, prepared by Cbz protection of the secondary amine of **2-11** followed by a subsequent disiloxane protection was attempted. In contrast to **2-13**, the benzylation and subsequent desilylation of **2-17** provided the desired compound **2-18** in 32% yield over 2 steps. It is difficult to rationalize the effect of the C1 amine protection group on benzylation of the C3 alcohol. The TBDPS protection of the primary alcohol in the presence of imidazole furnished **2-19**, which was a substrate of glycosylation. Attempts to improve the yield of benzylation by changing the reaction conditions such as the base (BuLi, NaHMDS, LiHMDS), temperature and solvent (THF, THF/DMF mixture) were not successful. Therefore, it was decided to focus on glycosylation of imino sugar building block **2-19**.



Reagents and conditions: (a) CbzCl, DIPEA, MeOH, 0 °C to RT, 70 %; (b) 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane, pyridine, 0 °C to RT, 75 %; (c) (i) BnBr, NaH, DMF, 0 °C to RT; (ii) TBAF, THF, 2 steps 32 %; (d) TBDPSCl, imidazole, CH_2Cl_2, 85 %.

Scheme 2-5. The synthesis of building block 2-19.

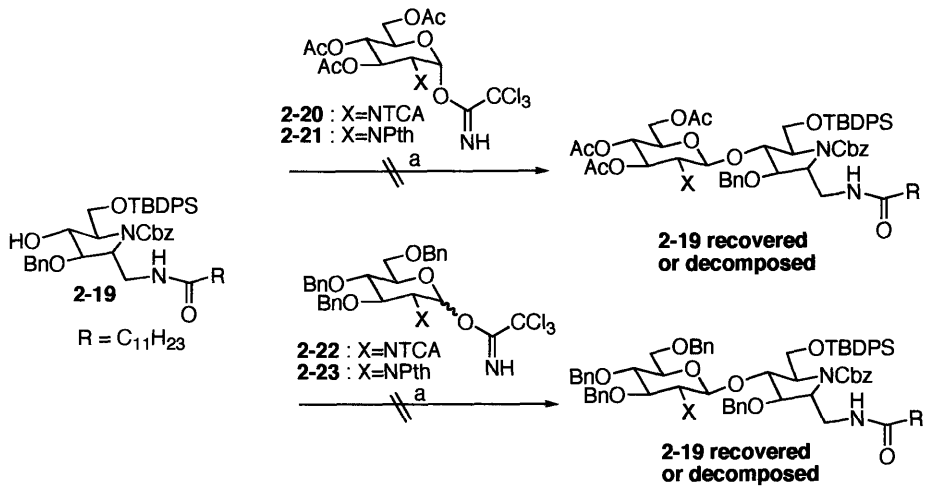
2-4 Glycosylation studies of 2-19

With the sugar acceptor **2-19** in hand, we next turned to the construction of the pseudo-disaccharide linked by a β-glycosidic bond. The selection of a protecting group at the C2' position of the sugar donor was an important consideration to achieve the desired glycosidic linkage.[1,2] For the glycosylation of the glucose derivative, the sugar donor with an acyl protecting group at the C2 alcohol has been used to obtain the β linkage. In glycosylations with the glucosamine derivative as the donor, it is essential to use different protecting groups for the amine at the C2' position rather than using the N-Ac group directly[22] due to its rapid conversion to the corresponding oxazoline.[23] Therefore, a number of protecting groups were investigated, including the N-trichloroethyl carbamate (N-Troc),[24-26] N-trichloroacetimide (N-TCA),[19,27] N-phthalimide (N-Pth),[28,29] and azide.[30-

[32] Neighboring group participation of the N-Troc, N-TCA, and N-Pth groups has been reported to ensure highly selective formation of the glycosidic β-linkage.

The attempted glycosylation of **2-19** with **2-20**[27] in the presence of 0.2 equiv. of BF_3•OEt_2 as the promoter in CH_2Cl_2 did not provide the desired product (**Scheme 2-6**). Compound **2-19** remained unreacted, and most of the imidate **2-20** was converted into the corresponding oxazoline. The attempted coupling reaction of **2-19** and **2-21**[33] also failed to provide the desired product. The decomposition of the sugar donor was observed by thin-layer chromatography (TLC) analysis. Excess amount of the donor did not help to generate the desired product. Use of a different promoter, such as trimethylsilyl trifluoromethanesulfonate (TMSOTf), *tert*-butyldimethylsilyl trifluoromethanesulfonate (TBSOTf), silver trifluromethanesulfonate (AgOTf) and a change of solvent including toluene and acetonitrile had no effect on the outcome of the glycosylation. Addition of excessive amount of the promoter resulted in the decomposition of **2-19**.

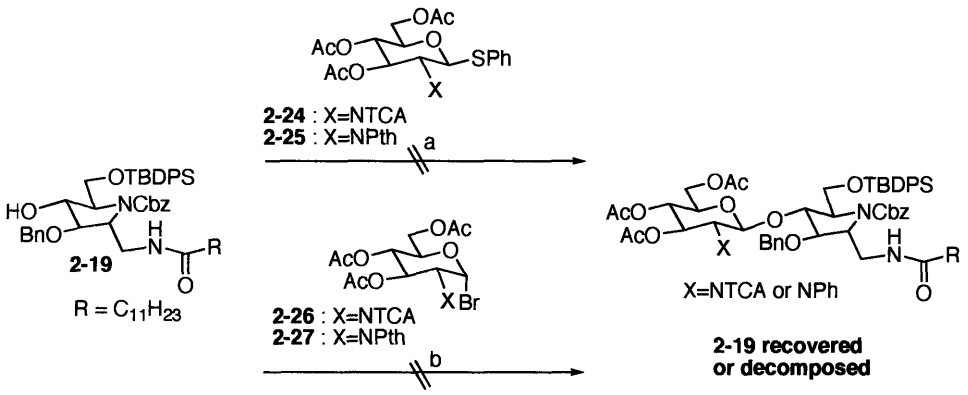
Peracetylated sugar donors have been considered to be weakly reactive reaction partners in the glycosylation. The introduction of benzyl ethers as hydroxyl protection groups is known to increase donor activity.[34,35] The glycosylations of **2-19** with **2-22**[27] or **2-23**[36] was attempted. However, glycosylation of **2-19** with **2-22** employing 0.2 equiv. of BF_3•OEt_2 in CH_2Cl_2 did not afford the desired product and the glycosyl imidate **2-22** was converted to the corresponding oxazoline immediately. Addition of excess imidate did not help to provide the desired product, nor did use of a different promoter (TMSOTf, AgOTf, TBSOTf), solvent (toluene, acetonitrile), or increased reaction time. The desired product was not obtained by glycosylation of **2-19** with different glycosyl imidate donor **2-23**. The imidate **2-23** rapidly decomposed even at – 50 °C.



Reagents and conditions: (a) different promoters (BF_3•OEt_2, TMSOTf, TBSOTf, AgOTf), different solvents (CH_2Cl_2, toluene, CH_3CN), 0 °C for **2-20** and **2-21**, -50 °C for **2-22** and **2-23**.

Scheme 2-6. Attempted glycosylation of 2-19 with glycosyl imidates.

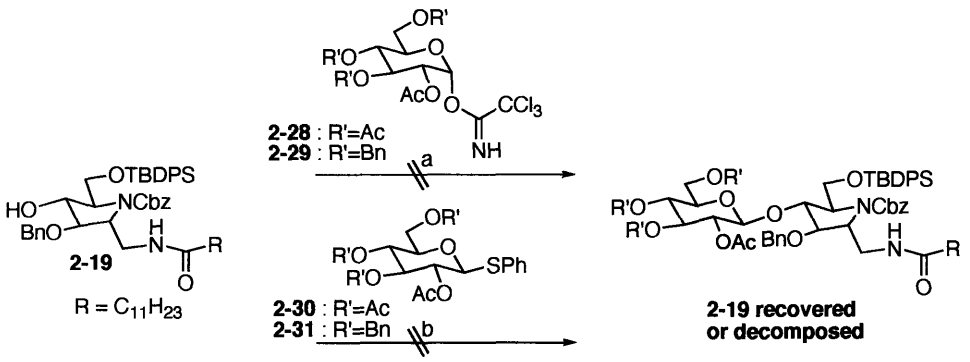
Although glycosyl imidates have been widely used as sugar donors,[37,38] alternative anomeric activating groups are often crucial for the addition of the carbohydrate to a sterically hindered site in a complex sugar synthesis.[18,39-44] Therefore, glycosylations of **2-19** utilizing either thioglycosides or glycosyl halides were attempted (**Scheme 2-7**). Treatment of **2-19** with **2-24**[45] in the presence of *N*-iodosuccinimide (NIS) and trifluoromethanesulfonic acid (TfOH) or silver trifluromethanesulfonate (AgOTf) did not provide the coupled product. Most of the sugar acceptor was recovered and the sugar donor decomposed under reaction conditions. Use of the thioglycoside **2-25**[46] with the NPth group as the glycosyl donor had no effect on the outcome of the glycosylation. The reaction of **2-19** utilizing glycosyl bromide **2-26**[27] or **2-27**[47] using 0.2 equiv. of AgOTf in CH_2Cl_2 did not give the desired product. The unreacted sugar acceptor **2-19** was recovered and most of the sugar donor decomposed.



Reagents and conditions: (a) **2-24** or **2-25**, NIS, TfOH or AgOTf, CH_2Cl_2 or toluene, -50 °C; (b) **2-26** or **2-27,** AgOTf, CH_2Cl_2 or toluene, 0 °C.

Scheme 2-7. Attempted glycosylation of 2-19 with thioglycosides or glycosyl bromides.

Given the failure of glycosylations of **2-19** with glucosamine derivative donors under various reaction conditions, the coupling reaction with a glucose derivative donor was investigated (**Scheme 2-8**). The attempted glycosylations of **2-19** with glycosyl imidates **2-28**[48] and **2-29**[49] or thioglycosides **2-30**[50] and **2-31**[51] using different promoters were explored. However, the desired compound was not obtained under various conditions.

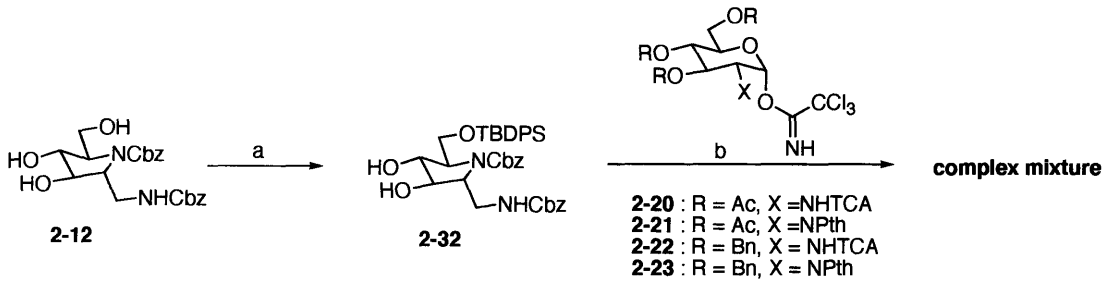


Reagents and conditions : (a) **2-28** or **2-29**, different promoters (BF_3•OEt_2, TMSOTf, TBSOTf), different solvents (CH_2Cl_2, toluene, CH_3CN), 0 °C for **2-28** and - 50 °C for **2-29**; (b) **2-30** or **2-31**, NIS, AfOTf or TfOH, CH_2Cl_2 or toluene, - 50 °C

Scheme 2-8. Attempted glycosylation of 2-19 with glucose derivative donors.

Extensive glycosylation studies of **2-19** employing different glycosyl donors, anomeric activating groups, reaction promoters, and solvents failed to give the desired products. A few glycosylations of the pyrrolidine derivatives were reported.[52,53] The sterically congested glycosyl acceptor **2-19** appears unreactive toward glycosylation. This conclusion prompted us to utilize **2-32** as the sugar acceptor, which was obtained by the treatment of **2-12** with 1.1 equiv. of *tert*-butyldiphenylsilyl chloride (TBDPSCl) in the presence of imidazole in CH_2Cl_2 (**Scheme 2-9**). The sterically less hindered **2-32** would potentially be a more reactive substrate toward the glycosylation. Given its structure, it was not expected to afford the sole desired regioisomer from the glycosylation, but the separation of the regioisomers would enable us to obtain the desired pseudo-disaccharide. Therefore, success of this study depended on the ability to separate glycosylation products. To find out the best separation conditions, the glycosylation of **2-32** and glycosyl imidates **2-20**, **2-21**,**2-22** and **2-23** was carried out employing 0.2 equiv. of BF_3•OEt_2 in CH_2Cl_2. Unfortunately, all of these reactions provided a complex mixture of

products. Mass spectral analyses indicated that mono-glycosylated products were the major components. However, the mixture could not be resolved by flash column chromatography under a variety of different eluents. Further modifications of the crude mixtures, such as removal of the silyl ether, acetate deprotection, and the benzoylation of the remaining secondary alcohol did not facilitate separation.



Reagents and conditions: (a) TBDPSCl, imidazole, CH_2Cl_2, 81 %; (b) different sugar donor, BF_3•OEt_2, CH_2Cl_2, 0 °C.

Scheme 2-9. Attempted selective glycosylation of 2-32.

2-5 Conclusion

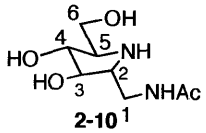
In conclusion, imino sugars **2-10** and **2-11** were synthesized. However, **2-10** and **2-11** showed no inhibitory activity against chitinase and OT. All attempts to construct a pseudo-disaccharide were unsuccessful. Glycosylation studies of **2-19** did not provide any desired products. Attempted selective glycosylations provided inseparable mixtures of products. With these results, we decided to investigate an alternative route for the construction of pseudo-disaccharides containing the imino sugar.

Experimental section

General Synthetic Procedures

Anhydrous dichloromethane (CH_2Cl_2) and toluene were distilled from calcium hydride, and anhydrous tetrahydrofuran, THF, was distilled from sodium/benzophenone. Acetonitrile was pre-dried over anhydrous K_2CO_3 for 24 h, followed by further drying for 24 h over 3Å molecular sieve, followed by distillation. Diethyl ether (Et_2O) was distilled from $LiAlH_4$. DMF was distilled by the fractional distillation under high vaccum. All chemicals were purchased from Sigma/Aldrich and used without further purification unless otherwise noted. Analytical thin-layer chromatography (TLC) was carried out on Merck 60 F_{254} 250-μm silica gel plates. All compounds were visualized on TLC by UV irradiation or an aqueous solution of ceric ammonium molybdate (CAM) staining. Flash column chromatography was carried out using forced flow of the indicated solvent on AdTech Flash Silica Gel, 32-63 μm particle size, 60 Å pore size (Adedge technologies). ^1H NMR spectra were acquired on a Bruker Avance (DPX) 400 MHz spectrometer, Varian INOVA 500 MHz spectrometer and Bruker Avance (DPX) 600 MHz spectrometer. ^{13}C NMR spectra were acquired on a Bruker Avance (DPX) 400 MHz spectrometer and Varian INOVA 500 MHz spectrometer. Chemical shifts (δ) are reported in parts per million (ppm) with chemical shifts referenced to internal standards: $CDCl_3$ (7.27 ppm for ^1H, 77.23 ppm for ^{13}C), CD_3OD (4.87 ppm for ^1H 49.15 ppm for ^{13}C), D_2O (4.80 ppm for ^1H). Coupling constants (*J*) are reported in Hertz (Hz) and multiplicities are abbreviated as singlet (s), doublet (d), triplet (t), multiplet (m), broadened singlet (br) and doublet of doublets (dd). The term "app d" is used to denote a triplet with two similar coupling constants and "app t" is used to denote a doublet of doublets (dd) with similar coupling constants. High resolution Mass Spectra (HRMS) were obtained at the Mass Spectrometry Facility at MIT (Cambridge, MA).

(2*R*,3*R*,4*R*,5*R*)-2-(Acetamide-methyl)-3,4-dihydroxy-5-(hydroxy-methyl)-pyrrolidine (2-10)



2-10 [1]

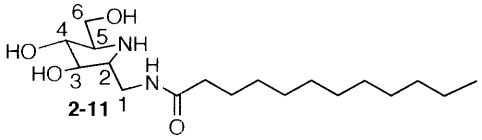
To a solution of **2-6** (120 mg, 0.74 mmol) in MeOH (15 mL), acetic anhydride (0.077 mL, 1.1 equiv.) was added at 0 °C. After 4h, the solvent was removed under reduced pressure, followed by purification *via* flash column chromatography (CH_2Cl_2:MeOH = 5:1 with 5% of NH_4OH to CH_2Cl_2:MeOH = 2:1 with 5% of NH_4OH) to afford **2-10** (76 mg, 50%) as a colorless liquid.

^1H NMR (D_2O, 600 MHz) δ 2.00 (s, 3H, acetyl), 3.05 (m, 1H, H_5), 3.11 (m, 1H, H_2), 3.29 (ABx, 1H, J = 14.0 and 7.1 Hz, H_{1a}), 3.41 (ABx, 1H, J =14.0 and J = 5.1 Hz, H_{1b}), 3.60 (ABx, 1H, J = 11.9 and 6.3 Hz, H_{6a}), 3.70 (ABx, 1H, J = 11.9 and J = 4.3 Hz, H_{6b}), 3.75 (app t, 1H, J = 6.4 Hz, H_3), 3.82 (app t, 1H, J = 6.4 Hz, H_4);

^{13}C NMR (D_2O, 125 MHz) δ 22.4, 42.1, 60.1, 62.2,77.9, 79.6, 175.2;

HRMS (ESI-MS): Calcd for $[C_8H_{16}N_2O_4+H]^+$(M+H): m/z = 205.1183; Found: 205.1189.

(2*R*,3*R*,4*R*,5*R*)-2-(Dodecanoic-acid-amide-methyl)-3,4-dihydroxy-5-(hydroxy-methyl)- pyrrolidine (2-11)



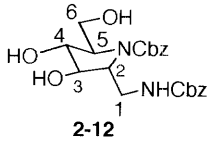
To a solution of **2-6** (250 mg, 1.54 mmol) in MeOH (15 mL), dodecanoic anhydride (693 mg, 1.2 equiv.) was added. After 4h, the solvent was removed under reduced pressure, followed by purification *via* flash column chromatography (CH_2Cl_2:MeOH = 8:1 with 1% of NH_4OH to CH_2Cl_2:MeOH = 5:1 with 1% of NH_4OH) to afford **2-11** (435 mg, 85%) as a colorless liquid.

^{1}H NMR (CD_3OD, 400 MHz) δ 0.92 (t, J = 7.0 Hz, 3H, -CH_2CH_3), 1.31-1.35 (m, 16H, aliphatic chain), 1.62-1.67 (t, 2H, -CH_2CH_3), 2.25 (t, 2H, J = 7.9 Hz, -$COCH_2CH_2$-), 3.05-3.09 (m, 1H, H_5), 3.10-4-14 (m, 1H, H_2), 3.33 (ABx, 1H, J = 13.3 and 7.2 Hz, H_{1a}), 3.41 (ABx, 1H, J =13.9 and 4.8 Hz, H_{1b}), 3.59 (ABx, 1H, J =11.3 and 6.3 Hz, H_{6a}), 3.69 (ABx, 1H, J =11.3 and 4.0 Hz, H_{6b}), 3.72 (app t, 1H, J = 6.3 Hz, H_3), 3.79 (app t, 1H, J = 6.1 Hz, H_4);

^{13}C NMR (CD_3OD, 125 MHz) δ 14.6, 23.9, 27.0, 30.5, 30.6, 30.8, 30.9, 31.0, 33.2, 37.2, 42.4, 62.2, 63.8, 65.3, 78.6, 80.2, 177.6;

HRMS: (ESI-MS): Calcd for $[C_{18}H_{36}N_2O_4+H]^+$(M+H): m/z = 345.2748; Found: 345.2737.

***N*-Benzyloxycarbonyl-[(2*R*,3*R*,4*R*,5*R*)-2-(benzyloxylcarbonylamino-methyl)-3,4-dihydroxy-5-(hydroxy-methyl)]-pyrrolidine (2-12)**

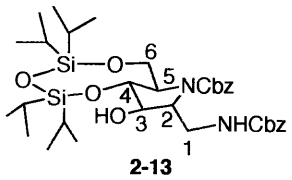


To a solution of **2-6** (520 mg, 3.21 mmol) and DIPEA (2.24 mL, 4.0 equiv.) in methanol (20 mL), CbzCl (1.370 mL, 3.0 equiv.) was added at 0 °C and the reaction mixture was warmed to room temperature for 2 h. The mixture was stirred for 8 h. at room temperature. The solvent was removed under reduced pressure. Flash column chromatography (CH_2Cl_2:MeOH = 15:1 to 10:1) of the crude product gave **2-12** (1.21 g, 88%) as a colorless oil (a mixture of N-rotamers).

^{1}H NMR (CD_3OD, 400 MHz) δ 3.27-3.66 (m, 4H, H_{1a}, H_{1b}, H_2 and H_5), 3.82-4.05 (m, 2H, H_{6a} and H_{6b}), 3.95-4.01(m, 1H), 4.37-4.40 (m, 1H), 4.87-5.11 (m, 4H, -$COOCH_2$Ph *2), 7.26-7.39 (m, 10H, -$COOCH_2Ph$*2);

^{13}C NMR (CD_3OD, 100 MHz) δ 44.1, 46.2, 56.0, 56.9, 59.0, 60.2, 61.3, 64.0, 60.3, 63.0, 65.2, 66.1, 77.5, 78.2, 79.7, 80.0, 128.4, 129.4, 127.7, 128.1, 128.3, 128.4, 127.7, 127.7, 127.9, 133.2, 136.5, 135.2, 138.0, 155.7, 153.0, 170.1, 172.1.

N-Benzyloxycarbonyl-[(2R,3R,4R,5R)-2-(benzyloxylcarbonylamino-methyl)-3-hydroxy-4,6-O-(tetraisopropyldisiloxane-1,3-diyl)]-pyrrolidine (2-13)

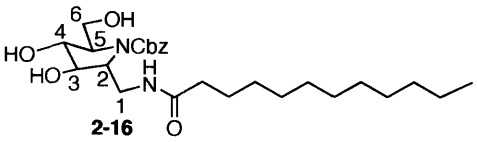


2-13

1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (1.08 mL, 1.2 equiv.) was added to the solution of **2-12** (1.21 g, 2.82 mmol) in pyridine (20 mL) at 0 °C and the reaction mixture was warmed to room temperature for 2 h. The reaction mixture was stirred 24 h. Excess silylating agent was quenched by the addition of 1 mL of methanol. The solvent was removed under reduced pressure and the crude product was purified by flash column chromatography to give **2-13** (1.37 g, 72%) as a colorless oil (a mixture of N-rotamers). (hexane:ethyl acetate = 4:1 to hexane:ethyl acetate = 2:1)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.93–1.08 (m, 28H, 4 isopropyl), 3.27-3.66 (m, 3H, H_{1a}, H_{1b}, H_2 and H_5), 3.82-4.05 (m, 2H, H_{6a} and H_{6b}), 3.95-4.01 (m, 1H, H_3), 4.37-4.40 (m, 1H, H_4), 4.87-5.11 (m, 4H, -COOCH_2Ph*2), 7.26-7.39 (m, 10H, -COOCH$_2$*Ph**2);

N-Benzyloxycarbonyl-[(2R,3R,4R,5R)-2-(dodecanoicacidamide -methyl)-3,4-dihydroxy-5-(hydroxy-methyl)]-pyrrolidine (2-16)



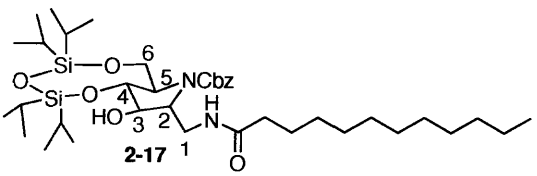
2-16

CbzCl (0.239 mL, 2.0 equiv.) was added to a solution of **2-11** (320 mg, 0.84 mmol) and DIPEA (0.439 mL, 3.0 equiv.) in methanol (20 mL) at 0 °C and the reaction mixture was warmed to room temperature for 2 h. The mixture was stirred for 8 h. at room temperature. The solvent was removed under reduced pressure. Flash column chromatography (CH_2Cl_2:MeOH = 20:1 to 15:1) of the crude product gave **2-16** (281 mg, 70%) as colorless oil (a mixture of N-rotamers).

^{1}H NMR (CD_3OD, 400 MHz) δ 0.96 (t, J = 7.0 Hz, 3H, -CH_2CH_3), 1.32-1.38 (m, 16H, aliphatic chain), 1.62-1.67 (m, 2H, -CH_2CH_3), 2.17-2.25 (m, 2H, -COCH_2CH_2), 3.37-3.48 (m, 1H), 3.76-4.04 (m, 6H), 4.18-4.23 (m, 1H), 5.21-5.23 (m, 2H, -COOCH_2Ph), 7.35-7.50 (m, 5H, -OCH$_2$*Ph*);

^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.6, 23.9, 27.1, 27.2, 30.4, 30.6, 30.7, 30.9, 33.2, 37.4, 37.5, 41.5, 61.1, 67.8, 68.5, 69.5, 79.4, 79.8, 80.1, 129.3, 129.3, 129.7, 129.7, 138.0, 156.7, 156.8, 176.6, 176.7.

N-Benzyloxycarbonyl-[(2R,3R,4R,5R)-2-(dodecanoicacidamide -methyl)- 4,6-O-(tetraisopropyldisiloxane-1,3-diyl)]-pyrrolidine (2-17)



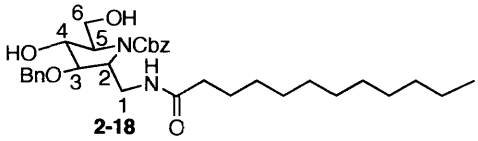
2-17

To the solution of **2-16** (281 mg, 0.59 mmol) in pyridine (10 mL), 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (0.226 mL, 1.2 equiv.) was added at 0 °C and the reaction mixture was warmed to room temperature for 2

h. The reaction mixture was stirred for 24 h. Excess silylating agent was quenched by the addition of 1 mL of methanol. The solvent was removed under reduced pressure and the crude product was purified by flash column chromatography (hexane:ethyl acetate = 3:1 to hexane:ethyl acetate = 1:1) to give **2-17** (319 mg, 75%) as colorless oil (a mixture of N-rotamers).

^{1}H NMR (CD_3OD, 400 MHz) δ 0.85-0.92 (m, 3H, -CH_2CH_3), 0.93-1.15 (m, 28 H, 4 isopropy), 1.22-1.33 (m, 16H, aliphatic chain), 1.51-1.63 (m, 2H, -CH_2CH_3), 2.08-2.20 (m, 2H, -$COCH_2CH_2$-), 3.48-4.05 (m, 6H, H_{1a}, H_{1b}, H_2, H_5, H_{6a}, and H_{6b}), 4.15-4.49 (m, 2H, H_3 and H_4), 5.02-5-15 (m, 2H, -$COOCH_2$Ph), 7.21-7.48 (m, 5H, -$COOCH_2Ph$).

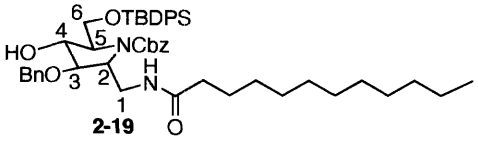
_N_-Benzyloxycarbonyl-[(2_R_,3_R_,4_R_,5_R_)-3-_O_-benzyl-2-(dodecanoicacidamide -methyl)-4-hydroxy-5-(hydroxy-methyl)]-pyrrolidine (2-18)



To a solution of **2-17** (319 mg, 0.56 mmol) in dry DMF (10 mL) were added BnBr (0.086mL, 1.3 equiv.) and then NaH (60% in dispersion in oil, 50 mg, 2.2 equiv.) at 0 °C. After stirring at 0 °C for 2h, CH_3OH (3 mL) was added and the solvent was evaporated. The resulting residue was dissolved into EtOAc (100 mL), and this organic solution was washed with water and brine and then dried over $MgSO_4$. The solvent was evaporated, the residue was dissolved in THF (20 ml) and TBAF (1.0 M solution in THF, 1.12 ml, 2.0 equiv based on **2-17**) was added. After stirring overnight, the solvent was removed under reduced pressure and the crude product was purified by flash column chromatography (hexane:ethyl acetate = 4:1 to 1:2) to yield **2-18** (81 mg, 32%) as a yellow oil (a mixture of N-rotamers).

^{1}H NMR (CD_3OD, 400 MHz) δ 0.93 (t, J = 7.0 Hz, 3H, -CH_2CH_3), 1.23-1.36 (m, 16H, aliphatic chain), 1.42-1.59 (m, 2H, -CH_2CH_3), 2.06-2.14 (m, 2H, -$COCH_2CH_2$-), 3.40-3.52 (m, 1H), 3.58-3.64 (m, 1H), 3,72-4.05 (m, 5H), 4.54-4.59 (m, 1H), 4.54-4.80 (m, 2H, -OCH_2Ph), 5.02-5-18 (m, 2H, -$COOCH_2$Ph), 7.29-7.73 (m, 10H, -OCH_2Ph, -$COOCH_2Ph$).

_N_-Benzyloxycarbonyl-[(2_R_,3_R_,4_R_,5_R_)- 3-_O_-benzyl-2-(dodecanoicacidamide-methyl)--4-hydroxy-5-(_tert_-butyl-diphenyl-silyloxy-methyl)]-pyrrolidine (2-19)

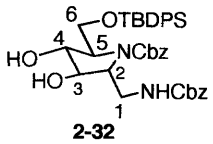


TBDPSCl (0.406 mL, 1.2 equiv.) was added to the solution of **2-18** (74 mg, 0. 130 mmol) and imidazole (117.7 mg, 2.0 equiv.) in CH_2Cl_2 (10 mL) at 0 °C and the reaction mixture was warmed to room temperature for 2 h. The mixture was stirring until no starting material remained. The reaction mixture was poured into satd. $NaHCO_3$ (20 mL). The mixture was extracted with CH_2Cl_2 (3 x 20 mL) and the combined organic layers were washed with satd. brine, dried over $MgSO_4$ and concentrated *in vacuo*. Flash column chromatography (hexane:ethyl acetate = 6:1 to hexane:ethyl acetate = 4:1) of the crude product gave the desired compound **2-19** (82 mg, 78%) as a colorless oil (a mixture of N-rotamers).

^{1}H NMR (CD_3OD, 400 MHz) selected peaks δ 0.96 (t, J = 7.0 Hz, 3H, -CH_2CH_3), 1.07-1.33 (m, 9H, Me_3CPh_2Si-), 1.32-1.38 (m, 16H, aliphatic chain), 1.62-1.64 (m, 2H, -CH_2CH_3), 2.15-2.23 (m, 2H, -$COCH_2CH_2$), 3.45-3.54 (m, 1H), 3.77-4.13 (m, 6H), 4.40-4.52 (m, 1H), 4.53-4.56 (m, 2H, -OCH_2Ph), 5.02-5.10 (m, 2H, -$COOCH_2$Ph), 7.25-7.42 (m, 20H, -OCH_2Ph, -$COOCH_2Ph$, Me_3CPh_2Si-);

^{13}C NMR ($CDCl_3$, 100 MHz) δ 12.3, 13.7, 14.1, 14.7, 18.0, 18.6, 22.3, 22.6, 23.3, 26.6, 29.2, 29.8, 30.0, 32.4, 36.9, 37.6, 39.8, 42.4, 44.3, 44.8, 47.9, 48.3, 51.5, 58.6, 62.4, 63.2, 67.7, 67.9, 68.4, 68.8, 69.1, 69.5, 79.2, 80.3, 92.1, 93.4, 97.0, 97.2, 100.5, 101,9, 118.2, 118.9, 128.8, 129.4, 128.1, 128.8, 135.9, 135.2, 137.8, 154.8, 159.0, 160.3, 163.3, 176.4,177.4.

_N_-Benzyloxycarbonyl-[(2_R_,3_R_,4_R_,5_R_)-2-(benzyloxylcarbonylamino-methyl)-3,4-dihydroxy-5-(*tert*-butyl-diphenyl-silyloxy-methyl)]-pyrrolidine (2-32)



2-32

To the solution of **2-12** (74 mg, 0.130 mmol) and imidazole (118 mg, 2.0 eq.) in CH_2Cl_2 (10 mL), TBDPSCl (0.406 mL, 1.2 eq.) was added to at 0 °C and the reaction mixture was warmed to room temperature for 2 h. The mixture was stirring until no starting material remained. The reaction mixture was poured into satd. $NaHCO_3$ (20 mL). The mixture was extracted with CH_2Cl_2 (3 x 20 mL) and the combined organic layers were washed with satd. brine, dried over $MgSO_4$ and concentrated in *vacuo*. Flash column chromatography (hexane:ethyl acetate = 6:1 to hexane:ethyl acetate = 4:1) of the crude product gave the desired compound **2-32** (82 mg, 78%) as a colorless oil (a mixture of N-rotamers).

^{1}H NMR (CD_3OD, 400 MHz) δ 1.07-1.33 (m, 9H, Me_3CPh_2Si), 3.25-3.66 (m, 4H, H_{1a}, H_{1b}, H_2 and H_5), 3,85-4.07 (m, 2H, H_{6a} and H_{6b}), 4.40-4.62 (m, 2H, H_3 and H_4), 4.87-5.11 (m, 4H, 2"*$COOCH_2$Ph), 7.29-7.75 (m, 20H, 2*$COOCH_2Ph$, Me_3CPh_2Si-).

The chitinase assay

Inhibition studies of chitinase from *Streptomyces griseus* which was purchased from Sigma-Aldrich were carried out using the literature procedures.[10,11] In a final volume of 50 μl, 6 nmol (0.00005 unit) of enzyme was incubated with 6 μM of 4-methylumbelliferyl-β-D-*N*,*N'*-diacetylchitobiose, which is commercially available, in McIlvain buffer (100 mM citric acid, 200 mM sodium phosphate, pH 5.5) containing 0.1 mg/mL BSA, for 10 min at 37 C in the presence of different concentrations of the inhibitor. After 10 min, the reaction was quenched by the addition of 1.5 mL of 0.2 M Na_2CO_3. A 100 μl aliquot was transferred to a cuvette. The fluorescence of the liberated 4-methylumbelliferone was quantified using excitation and emission wavelengths of 360 nm and 460 nm by Fluoromax 3 from Jobin Yvon. Experiments were performed in triplicate.

OT assay

Preparation of solubilized yeast microsomes

Yeast OT was purified to the solubilzed membrane stage according to the literature procedures[54] with the following modification. Sucrose was eliminated from the final solubilzing buffer due to the potential competition of binding sites. The final buffer solution contains 50 mM HEPES, pH 7.5, 10 mM $MgCl_2$, 10% glycerol and protease inhibitor 0.1 mM AEBSF, 0.5 μg/mL pepstain A and 0.5 μg/mL leupetin.

OT Inhibition Assays

The assay buffer consisted of 50 mM HEPES, pH 7.5, 1.2% Triton X-100, 15 mM $MnCl_2$, and 0.5 mg/mL phosphatidylcholine. The radiolabeled sugar substrate (50,000 dpm, 60 Ci/mmol) was aliquoted from a chloroform/methanol stock solution into an eppendorf tube, and the solvent was evaporated under a gentle stream of nitrogen. The peptide substrate Bz-Asn-Leu-Thr-NHMe (5 mM, 10 μL in DMSO, final concentration of 0.25 mM) was mixed well with the sugar substrate in assay buffer (100 μM total volume).

The inhibitor candidate (in 10 μM DMSO) was preincubated with a constant amount of OT in assay buffer (150 μL total volume) for 30 minutes over an ice-water bath. The reaction was initiated by the addition of preincubated inhibitor/OT mixture (100 μL) into the premixed substrates (100 μL). Aliquots (40 μL) of the reaction mixture were quenched into 1.2 mL of chloroform/methanol/4 mM $MgCl_2$ (3:2:1 v/v) after 2,4,6, and 8 min.

The aqueous phase containing N-glycosylated peptide was transferred to 7-mL scintillation vials. A 600-mL aliquot of theoretical upper layer or TUP (2.75% chloroform, 44% methanol, 53.25 % aqueous magnesium chloride (1.55 mM)) was added to the organic layer and the aqueous layer was removed and added to the previously removed aqueous layer. After two extractions, the combined aqueous phases were mixed with Ecolite (5.5 mL) and counted on a Beckman LS-5000TD scintillation counter to give the amount of radioactivity (in units of dpm, disintegrations per minute) for the ^{3}H-glycopeptide.

References

1. Paulsen, H. Advances in Selective Chemical Syntheses of Complex Oligosaccharides. *Angew. Chem. Int. Ed. Engl.*, **1982**, *21*, 155-173.
2. Toshima, K.; Tatsuta, K. Recent Progress in O-Glycosylation Methods and Its Application to Natural-Products Synthesis. *Chem. Rev.*, **1993**, *93*, 1503-1531.
3. Takaoka, Y.; Kajimoto, T.; Wong, C.-H. Inhibition of *N*-Acetylglucosaminyltransfer Enzymes: Chemical-Enzymatic Synthesis of New Five-Membered Acetamido Azasugar. *J. Org. Chem.*, **1993**, *58*, 4809-4812.
4. Wrodnigg, T. M.; Stütz, A. E.; Withers, S. G. Symthesis of 1-Amino-1,2,5-trideoxy-2,5-imino-D-mannitol, a Novel Analogue of the Powerful Glucosidase Inhibitor 2,5-Dideoxy-2,5-imino-D-mannitol, *via* an Amadori Rearrangement of 5-Azido-5-deoxy-D-glucofuranose. *Tetrahedron Lett.*, **1997**, *38*, 5463-5466.
5. Liu, J.; Numa, M. M.; Liu, H.; Huang, S. J.; Sears, P.; Shikhman, A. R.; Wong, C.-H. Synthesis and high-throughput screening of N-acetyl-β-hexosaminidase inhibitor libraries targeting osteoarthritis. *J. Org. Chem.*, **2004**, *69*, 6273-6283.
6. Hodge, J. E. The Amadori rearrangement. *Adv. Carbohydr. Chem. Biochem.*, **1955**, *10*, 169-205.
7. Shu, L.; Wang, P.; Gan, Y.; Shi, Y. Asymmetric epoxidation catalyzed by N-aryl-substituted oxazolidinone-containing ketones: Further evidence for electronic effects. *Org. Lett.*, **2003**, *5*, 293-296.
8. Dax, K.; Gaigg, B.; Grassberger, V.; Kölblinger, B.; Stütz, A. E. Simple Synthesis of 1,5-Dideoxy-1,5-Imino-D-Glucitol(1-Deoxynojirimycin) and 1,6-Dideoxy-1,6-Imino-D-Glucitol from D-Glucofuranurono-6,3-Lactone. *J. Carbohydr. Chem.*, **1990**, *9*, 479-499.
9. Wrodnigg, T. M.; Gaderbauer, W.; Greimel, P.; Hausler, H.; Sprenger, F. K.; Stütz, A. E.; Virgona, C.; Withers, S. G. Biologically active 1-aminodeoxy and 1-*O*-alkyl derivatives of the powerful D-glucosidase inhibitor 2,5-dideoxy-2,5-imino-D-mannitol. *J. Carbohydr. Chem.*, **2000**, *19*, 975-990.
10. Rao, F. V.; Andersen, O. A.; Vora, K. A.; Demartino, J. A.; van Aalten, D. M. Methylxanthine drugs are chitinase inhibitors: investigation of inhibition and binding modes. *Chem. Biol.*, **2005**, *12*, 973-980.
11. Houston, D. R.; Synstad, B.; Eijsink, V. G.; Stark, M. J.; Eggleston, I. M.; van Aalten, D. M. Structure-based exploration of cyclic dipeptide chitinase inhibitors. *J. Med. Chem.*, **2004**, *47*, 5713-5720.
12. Imperiali, B.; Shannon, K. L. Differences between Asn-Xaa-Thr-Containing Peptides - a Comparison of Solution Conformation and Substrate Behavior with Oligosaccharyltransferase. *Biochemistry*, **1991**, *30*, 4374-4380.
13. Imperiali, B.; Zimmerman, J. W. Synthesis of Dolichylpyrophosphate-Linked Oligosaccharides. *Tetrahedron Lett.*, **1990**, *31*, 6485-6488.
14. Berecibar, A.; Grandjean, C.; Siriwardena, A. Synthesis and biological activity of natural aminocyclopentitol glycosidase inhibitors: Mannostatins, trehazolin, allosamidins, and their analogues. *Chem. Rev.*, **1999**, *99*, 779-844.
15. Tai, V. W. -F.; Imperiali, B. Substrate specificity of the glycosyl donor for oligosaccharyl transferase. *J. Org. Chem.*, **2001**, *66*, 6217-6228.

16. Markiewicz, W. T. Tetraisopropyldisiloxane-1,3-Diyl, a Group for Simultaneous Protection of 3'-Hydroxy and 5'-Hydroxy Functions of Nucleosides. *J. Chem. Res., Synop,* **1979**, 24-25.
17. Schaumberg, J. P.; Hokanson, G. C.; French, J. C.; Smal, E.; Baker, D. C. 2'-Chloropentostatin, a New Inhibitor of Adenosine Deaminase. *J. Org. Chem.,* **1985**, *50*, 1651-1656.
18. Nicolaou, K. C.; Mitchell, H. J. Adventures in carbohydrate chemistry: New synthetic technologies, chemical synthesis, molecular design, and chemical biology. *Angew. Chem. Int. Ed. Engl.,* **2001**, *40*, 1576-1624.
19. Love, K. R.; Seeberger, P. H. Solution syntheses of protected type II Lewis blood group oligosaccharides: Study for automated synthesis. *J. Org. Chem.,* **2005**, *70*, 3168-3177.
20. Toshima, K. Novel glycosylation methods and their application to natural product synthesis. *Carbohydr. Res.,* **2006**, *341*, 1282-1297.
21. Yin, H. F.; D'Souza, F. W.; Lowary, T. L. Arabinofuranosides from mycobacteria: Synthesis of a highly branched hexasaccharide and related fragments containing β-axabinofuranosyl residues. *J. Org. Chem.,* **2002**, *67*, 892-903.
22. Williams, J. R.; Gong, H.; Hoff, N.; Olubodun, O. I. Synthesis of the shark repellent pavoninin-4. *J. Org. Chem.,* **2005**, *70*, 10732-10736.
23. Shrader, W. D.; Imperiali, B. Synthesis of the glucoallosamidin pseudo-disaccharide: Use of an efficient Hg(II) mediated cyclization. *Tetrahedron Lett.,* **1996**, *37*, 599-602.
24. Gege, C.; Oscarson, S.; Schmidt, R. R. Synthesis of fluorescence labeled sialyl Lewis^x glycosphingolipids. *Tetrahedron Lett.,* **2001**, *42*, 377-380.
25. Koller, K. M.; Wong, C. -H. Complex carbohydrate synthesis tools for glycobiologists: enzyme-based approach and programmable one-pot strategies. *Glycobiology,* **2000**, *10*, 1157-1169.
26. Nitz, M.; Bundle, D. R. Efficient synthesis of 3,6-dideoxy-β-D-arabino-hexopyranosyl-terminated LacdiNAc glycan chains of the *Trichinella spiralis* parasite. *J. Org. Chem.,* **2000**, *65*, 3064-3073.
27. Blatter, G.; Beau, J. M.; Jacquinet, J. C. The Use of 2-Deoxy-2-Trichloroacetamido-D-Glucopyranose Derivatives in Syntheses of Oligosaccharides. *Carbohydr. Res.,* **1994**, *260*, 189-202.
28. Pratt, M. R.; Bertozzi, C. R. Chemoselective ligation applied to the synthesis of a biantennary N-linked glycoform of CD52. *J. Am. Chem. Soc.,* **2003**, *125*, 6149-6159.
29. Love, K. R.; Andrade, R. B.; Seeberger, P. H. Linear synthesis of a protected H-type II pentasaccharide using glycosyl phosphate building blocks. *J. Org. Chem.,* **2001**, *66*, 8165-8176.
30. Bommer, R.; Kinzy, W.; Schmidt, R. R. Glycosyl Imidates.49. Synthesis of the Octasaccharide Moiety of the Dimeric Le^x Antigen. *Liebigs Ann. Chem.,* **1991**, 425-433.
31. Hung, S. C.; Wang, C. C.; Thopate, S. R. Efficient synthesis of L-altrose and L-mannose. *Tetrahedron Lett.,* **2000**, *41*, 3119-3122.
32. Myers, A. G.; Gin, D. Y.; Rogers, D. H. Synthetic Studies of the Tunicamycin Antibiotics - Preparation of (+)-Tunicaminyluracil, (+)-Tunicamycin-V, and 5'-Epi-Tunicamycin-V. *J. Am. Chem. Soc.,* **1994**, *116*, 4697-4718.

33. Du, Y. G.; Zhang, M. M.; Kong, F. Z. Efficient and practical syntheses of three pentasaccharides core structures corresponding to N-glycans. *Tetrahedron,* **2001**, *57*, 1757-1763.
34. Ye, X.-S.; Wong, C.-H. Anomeric reactivity-based one-pot oligosaccharide synthesis: A rapid route to oligosaccharide libraries. *J. Org. Chem.,* **2000**, *65*, 2410-2431.
35. Mong, T. K.-K.; Huang, C.-Y.; Wong, C.-H. A new reactivity-based one-pot synthesis of N-acetyllactosamine oligomers. *J. Org. Chem.,* **2003**, *68*, 2135-2142.
36. Ohnishi, Y.; Ando, H.; Kawai, T.; Nakahara, Y.; Ito, Y. Synthesis of a novel asparagine-linked heptasaccharide structure via *p*-methoxybenzyl-assisted β-mannosylation. *Carbohydr. Res.,* **2000**, *328*, 263-276.
37. Schmidt, R. R. New Methods for the Synthesis of Glycosides and Oligosaccharides - Are There Alternatives to the Koenigs-Knorr Method. *Angew. Chem. Int. Ed. Eng.,* **1986**, *25*, 212-235.
38. Schmidt, R. R.; Kinzy, W. Anomeric-oxygen activation for glycoside synthesis: the trichloroacetimidate method. *Adv. Carbohydr. Chem. Biochem.,* **1994**, *50*, 21-123.
39. Paulsen, H. Progress in Oligosaccharide Synthesis through a New Orthogonal Glycosylation Strategy. *Angew. Chem. Int. Ed. Eng.,* **1995**, *34*, 1432-1434.
40. Mukaiyama, T. Explorations into new reaction chemistry. *Angew. Chem. Int. Ed. Engl.,* **2004**, *43*, 5590-5614.
41. Blanchard, N.; Roush, W. R. 2-Deoxy-2-iodo-β-glucopyranosyl fluorides: mild and highly stereoselective glycosyl donors for the synthesis of 2-deoxy-β-glycosides from β-hydroxy ketones. *Org. Lett.,* **2003**, *5*, 81-84.
42. Yu, C.-S.; Niikura, K.; Lin, C.-C.; Wong, C.-H. The thioglycoside and glycosyl phosphite of 5-azido sialic acid: Excellent donors for the α-glycosylation of primary hydroxy groups. *Angew. Chem. Int. Ed. Engl.,* **2001**, *40*, 2900-2903.
43. Martichonok, V.; Whitesides, G. M. Stereoselective α-sialylation with sialyl xanthate and phenylsulfenyl triflate as a promotor. *J. Org. Chem.,* **1996**, *61*, 1702-1706.
44. Kroger, L.; Thiem, J. Convenient multigram scale glycosylations of scented alcohols employing phase-transfer reactions. *J. Carbohydr. Chem.,* **2003**, *22*, 9-23.
45. Belot, F.; Jacquinet, J. C. Unexpected stereochemical outcome of activated 4,6-*O*-benzylidene derivatives of the 2-deoxy-2-trichloroacetamido-D-galacto series in glycosylation reactions during the synthesis of a chondroitin 6-sulfate trisaccharide methyl glycoside. *Carbohydr. Res.,* **2000**, *325*, 93-106.
46. Khan, S. H.; Matta, K. L. Synthesis of 4-Nitrophenyl *O*-(2-Acetamido-2-Deoxy-β-D-Glucopyranosyl)-(1-α-2)-*O*-(6-*O*-Methyl-α-D-Mannopyranosyl)-(1-α-6)-β-D-Glucopyranoside and Its 4',6'-Di-*O*-Methyl Analog - Potential Inhibitors of N-Acetylglucosaminyl-Transferase-V (Gnt-V). *Carbohydr. Res.,* **1993**, *243*, 29-42.
47. Mukherjee, D.; Ray, P. K.; Chowdhury, U. S. Synthesis of glycosides *via* indium(III) chloride mediated activation of glycosyl halide in neutral condition. *Tetrahedron,* **2001**, *57*, 7701-7704.
48. Paulsen, H.; Meinjohanns, E. Synthesis of Modified Oligosaccharides of N-Glycoproteins Intended for Substrate-Specificity Studies of N-Acetylglucosaminyltransferases Ii-V. *Tetrahedron Lett.,* **1992**, *33*, 7327-7330.
49. Sakai, K.; Nakahara, Y.; Ogawa, T. Total Synthesis of Nonasaccharide Repeating Unit of Plant-Cell Wall Xyloglucan - an Endogenous Hormone Which Regulates Cell-Growth. *Tetrahedron Lett.,* **1990**, *31*, 3035-3038.

50. Kanie, O.; Ito, Y.; Ogawa, T. Orthogonal Glycosylation strategy in Oligosaccharide Synthesis. *J. Am. Chem. Soc.,* **1994**, *116*, 12073-12074.
51. Yan, L.; Kahne, D. Generalizing glycosylation: Synthesis of the blood group antigens Le^a, Le^b, and Le^X using a standard set of reaction conditions. *J. Am. Chem. Soc.,* **1996**, *118*, 9239-9248.
52. Barrett, A. G. M.; Philipauskas, D. Electrochemical Oxidation of Proline Derivatives: Total Synthesis of Bulgecinine and Bulgecin C. *J. Org. Chem.,* **1991**, *56*, 2787-2800.
53. Griffith, D. A.; Danishefsky, S. J. The Total Synthesis of Allosamidin. Expansion of the Methodology of Azaglycosylation Pursuant to the Total Synthesis of Allosamidin. A Surprising Enantiotopic Sense for a Lipase-Induced Deacetylation. *J. Am. Chem. Soc.,* **1996**, *118*, 9526-9538.
54. Pathak, R.; Hendrickson, T. L.; Imperiali, B. Sulfhydryl Modification of the Yeast Wbp1P Inhibits Oligosaccharyl Transferase-Activity. *Biochemistry,* **1995**, *34*, 4179-4185.

Chapter 3- The synthesis of pseudo-disaccharide containing an imino sugar by the linear approach

3-1 Introduction

In Chapter 2, synthetic efforts toward the pseudo-disaccharide were described. Extensive attempts at glycosylation of the imino sugar building block were unsuccessful in generating the desired product. This failure of glycosylation led us to revise our synthetic route. The new strategy toward the construction of a pseudo-disaccharide containing an imino sugar is shown Figure 3-1. To avoid the previous problems regarding the imino sugar in glycosylation, we decided to conduct the glycosylation step prior to its formation. We chose β-hydroxy ketone **3-5** having an azide group at the C4 position, as the glycosyl acceptor. Toward the synthesis of **3-5**, we planned to use a synthetic procedure modified from the one discussed in Chapter 2. We envisioned utilization of an Amadori rearrangement of lactol **3-6** to generate the acyclic compound **3-5**.[1,2] The glucose and glucosamine derivatives would be added to the C4 hydroxyl group of **3-5**, which was masked as the ring oxygen of the furanoside derivative, to generate **3-3** and **3-4**. The neighboring group participation of the C2' group in the glycosyl donor would ensure high β-selectivity of glycosidic bond formation.[3] After glycosylation, construction of the imino sugar would be achieved by intramolecular reductive amination between in-situ generated amine from azide and ketone moieties.

As an initial study, two pseudo-disaccharides **3-1** and **3-2** having N-Ac groups on the C1 positions of the imino sugars were chosen as target compounds.

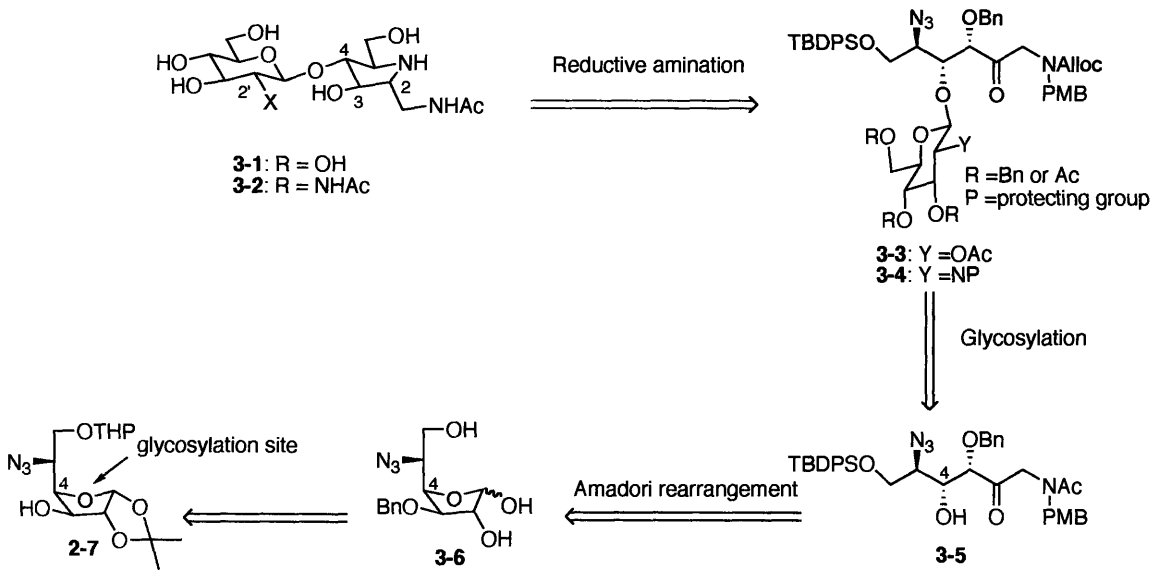
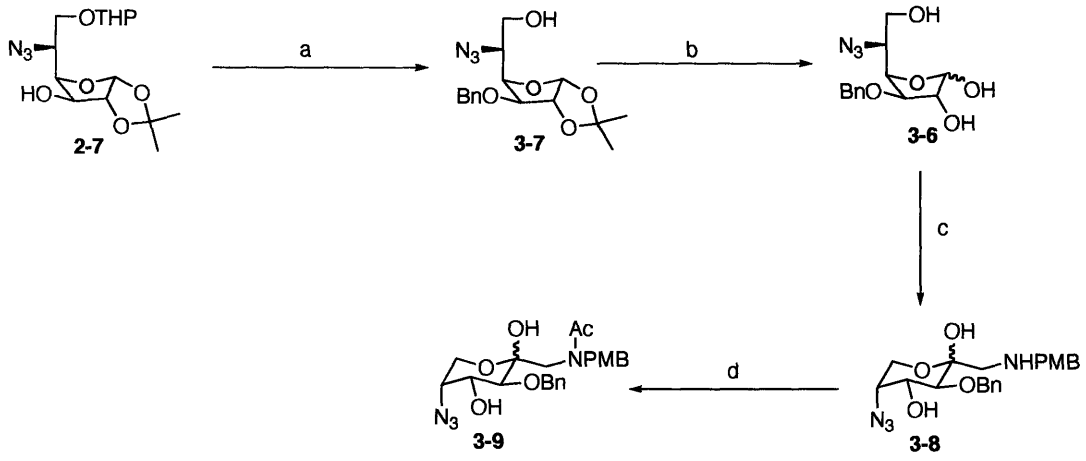


Figure 3-1. The revised route toward the synthesis of the pseudo-disaccharide containing an imino sugar.

3-2 Synthesis of the substrate for glycosylation studies

The synthesis began with **2-7**, which was described in Chapter 2 (**Scheme 3-1**).[4] Benzylation of the secondary alcohol using 1.2 equiv. of benzyl bromide (BnBr) and 1.1 equiv. of NaH in tetrahydrofuran (THF) in the presence of 0.2 equiv. of tetra-*n*-butylammonium iodide (TBAI), followed by the deprotection of the tetrahydropyranyl (THP) group employing 0.2 equiv. of *p*-toluenesulfonic acid (*p*-TsOH) in methanol, provided compound **3-7** in 95% yield over 2 steps. Deprotection of the acetonide utilizing 80% trifluoroacetic acid (TFA) in water gave lactol **3-6** as an α/β mixture. The Amadori rearrangement of **3-6** with 1.0 equiv. of *p*-methoxybenzylamine ($PMBNH_2$) in the presence of 1.0 equiv. of acetic acid in methanol at 40 °C provided the desired ketal **3-8** in 85% yield.[1,2] The subsequent secondary amine protection of **3-8** with 1.2 equiv. of

acetic anhydride in the presence of 2.0 equiv. of *N,N*-diisopropylethylamine (DIPEA) smoothly afforded desired product **3-9** in 76% yield.

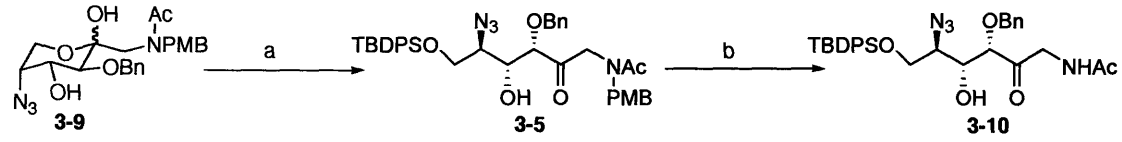


Reagents and conditions: (a) (i) BnBr, NaH, TBAI, THF, 0 °C to RT; (ii) p-TsOH, MeOH, 95 % over 2 steps; (b) TFA:H_2O = 8:2, 0 °C to RT, 82 %; (c) $PMBNH_2$, AcOH, MeOH, 40 °C, 85 %; (d) Ac_2O, DIPEA, MeOH, 76 %.

Scheme 3-1.The synthesis of β-amino ketal 3-9.

From the ^{13}C-NMR spectrum of **3-9**, the predominant structure in solution is the closed form, but this closed form is in equilibrium with the open form. Compound **3-9** in CH_2Cl_2 was treated with 2.0 equiv. of *tert*-butyldiphenylsilyl chloride (TBDPSCl) in the presence of 3.0 equiv. of imidazole to generate acyclic compound **3-5** in 78% yield (**Scheme 3-2**). TBDPSCl reacted selectively with the primary alcohol of the open form to generate the acyclic product **3-5**. Since **3-5** exists as a mixture of N-rotamers, **3-10** was synthesized by the cleavage of the N-PMB using 4.0 equiv. of ceric ammonium nitrate (CAN) in a 10:1 mixture of acetonitrile and water at low temperature,[5] to facilitate

spectroscopic characterization. With glycosylation acceptor **3-5** in hand, we turned our attention to the glycosylation.



Reagents and conditions: (a) TBDPSCl, imidazole, CH_2Cl_2, 78 %; (b) CAN, MeCN:H_2O = 10:1, -20 °C to 0 °C, 63 %.

Scheme 3-2. The synthesis of β-hydroxy ketone 3-10.

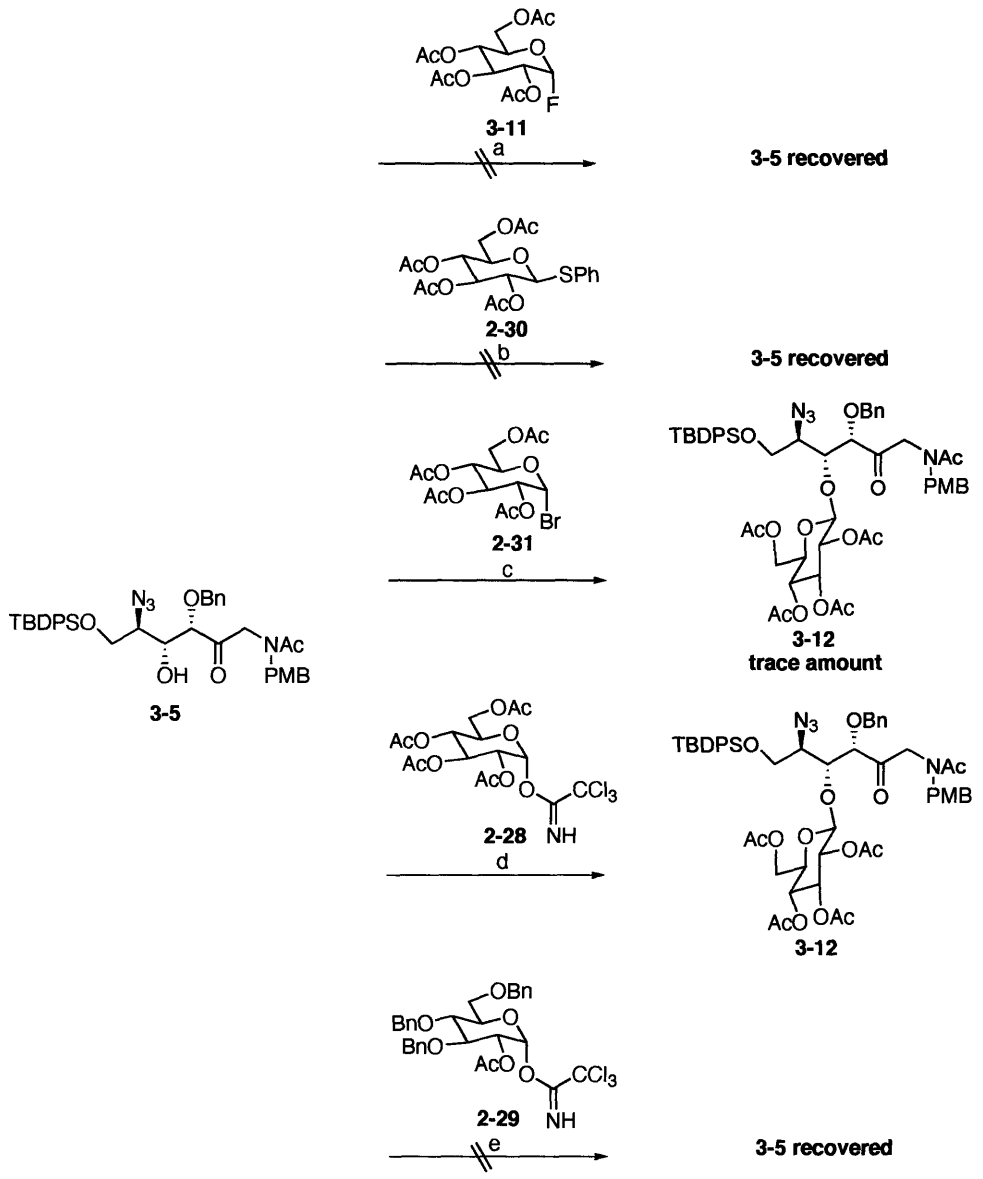
3-3 Glycosylation of the β-hydroxy ketone with a glucose derivative

β–Hydroxy ketones are known to be poor substrates for glycosylation. It has been reported that the intramolecular hydrogen bonding of the β-hydroxy ketone resulted in weak reactivity towards glycosylation.[6] Moreover, the glycosylation of β–hydroxy ketones is sensitive to small changes in reaction conditions.[7-9] Therefore different glycosyl donors and promoters were screened to determine the best coupling partner (**Scheme 3-3**). In the initial screen for glycosylation, 1.0 equiv. of glycosyl donor was used in each reaction.

Glycosidation reaction of **3-5** with glycosyl fluoride **3-11**[10] in the presence of 1.2 equiv. of $AgClO_4$ and 1.2 equiv. of $SnCl_4$ in Et_2O or CH_2Cl_2 did not provide the coupled compound.[6,11] Both acceptor and donor starting materials remained without providing the desired product. The coupling reaction between **3-5** and thioglycoside **2-30**[12] employing 1.2 equiv. of *N*-iodosuccinimide (NIS) and 0.2 equiv. of trifluoromethanesulfonic acid (TfOH) in CH_2Cl_2 led only to decomposition of the thioglycoside. Modification of

reaction conditions employing 1.2 equiv. of NIS and 0.2 equiv. of silver trifluromethanesulfonate (AgOTf) did not facilitate production of the desired product either. The glycosylation of **3-5** with glycosyl bromide **2-31** in the presence of 0.2 equiv. of AgOTf provided only trace amounts of the desired product **3-12** (less than 5% yield). The coupling reaction of **3-5** with glycosyl imidate **2-28**[13] employing 0.2 equiv. of BF_3•OEt_2 as the promoter in CH_2Cl_2 at 0 °C gave the desired product **3-12** in 35% yield with 50% yield of **3-5** recovered. Decomposition of glycosyl imidate **2-28** was also detected by the thin-layer chromatography (TLC) analysis.

Studies have shown that glycosyl donor reactivity can be controlled by the choice of protecting groups.[14-16] The replacement of acetates with benzyl ethers increases reactivity of the glycosyl donor. For this reason, glycosylation between **3-5** and glycosyl imidate **2-29**[17] in the presence of 0.2 equiv. of BF_3•OEt_2 was attempted. Unfortunately, no coupled product was obtained and rapid decomposition of **2-29** was observed by TLC analysis. In the glycosylation of **3–5**, the glycosyl imidate **2-28** was demonstrated to be the best coupling partner in terms of both reactivity and stability. A weak but stable donor seems critical for this reaction to proceed. With these promising results from the initial glycosylation studies, we turned our attention to improving yield.

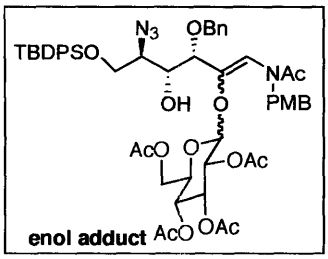


Reagents and conditions: (a) **3-11**, $AgClO_4$, $SnCl_4$,CH_2Cl_2 or Et_2O, -20 °C; (b) **2-30**, NIS, TfOH or AgOTf, CH_2Cl_2, -50 °C; (c) **2-31**, AgOTf,CH_2Cl_2, 0 °C; (d) **2-28**, BF_3•OEt_2, CH_2Cl_2, 35%; (e) **2-29**, BF_3•OEt_2,CH_2Cl_2, -50 °C.

Scheme 3-3. The initial glycosylation studies of β-hydroxy ketone.

To optimize the glycosylation of **3-5** (**Scheme 3-4**), we focused on other variables such as promoter and promoter concentration. Glycosylation utilizing promoters other than BF_3•OEt_2 did not afford the desired product (**Table 3-1, rows 2** and **3**). Employing

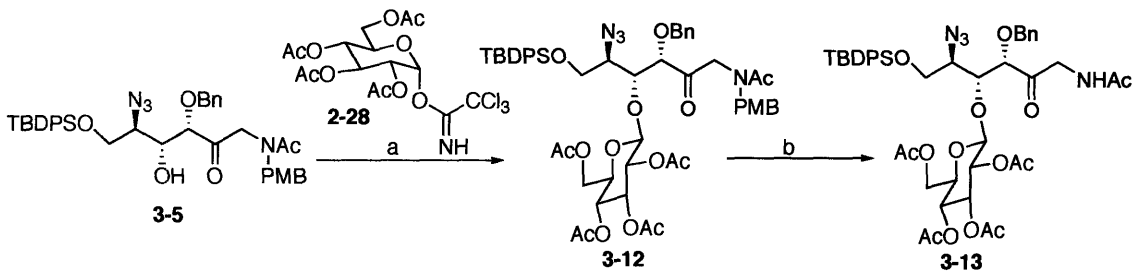
1.0 equiv. of BF_3•OEt_2 improved the yield slightly (**Table 3-1, row 4**), however, using more than one equivalent of BF_3•OEt_2 provided a complex mixture, probably due to the decomposition of both the starting material and product. Since decomposition of imidate **2-28** was observed, the addition of excess glycosyl donor was tested to drive the reaction to complete conversion. The best result was obtained with 3.0 equiv. of glycosyl imidate **2-28**, providing product **3-12** in 65% yield (**Table 3-2, row 6**).



In all of these reactions, more than 10% yield of a side product was isolated. Often, this side product was the major compound. Since mass spectral analysis of this side product indicated an identical mass to the desired product **3-12**, and mild acid hydrolysis employing p-TsOH in CH_2Cl_2 afforded **3-5** and the corresponding lactol of **2-28**, the side product has been speculated to be the enol adduct. To suppress the formation of the enol adduct, different solvents (diethyl ether, acetonitrile and toluene) and solvent mixtures were tested. A longer reaction time was required in toluene (8 h) and 5.0 equiv. of glycosyl imidate was necessary for complete conversion. However, the desired product was obtained in 85% yield without any side product formation (**Table 3-1, row 7**). These reaction conditions were highly reproducible and applicable to large-scale synthesis.

Full assignment of the proton NMR spectrum of **3-12** was difficult due to a mixture of N-rotamers. However, we were able to confirm the anomeric β-linkage from the coupling constant (J = 8.1 Hz) between the anomeric proton and the adjacent proton

at C2'. The oxidative cleavage of the N-PMB group using 4.0 equiv. of CAN gave **3-13** in 83% yield.



Reagents and conditions: (a) **2-28**, BF_3•OEt_2, toluene, 0 °C to RT, 85%; (b) CAN, MeCN:H_2O = 10:1, -20 °C to 0 °C, 83%.

Scheme 3-4. The optimization of glycosylation of β-hydroxyl ketone.

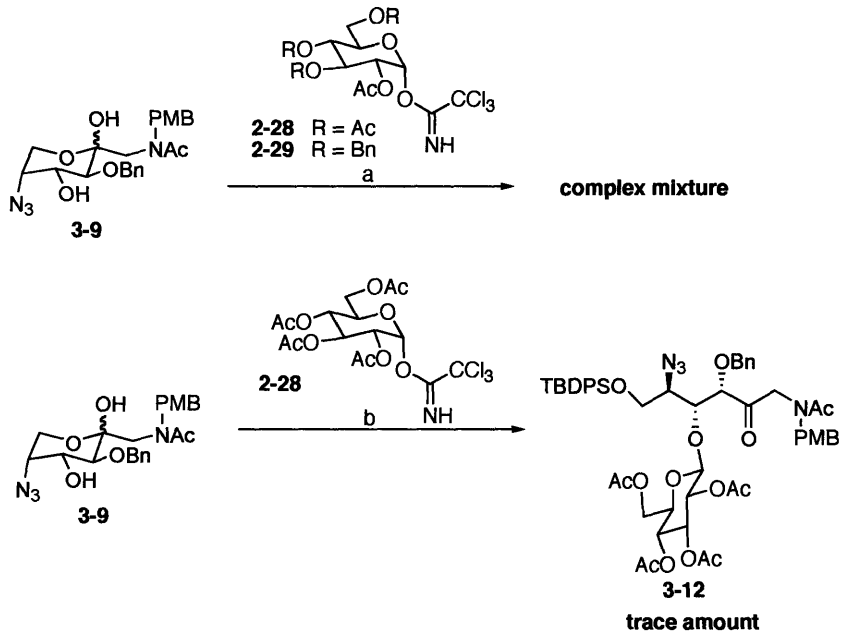
	Equiv. of imidate **2-28**	Promoter	Solvent	Isolated yield of **3-12**
1	1.0 equiv.	0.2 equiv. BF_3•OEt_2	CH_2Cl_2	35%
2	1.0 equiv.	0.2 equiv. TMSOTf	CH_2Cl_2	NR
3	1.0 equiv.	0.2 equiv. TBSOTf	CH_2Cl_2	NR
4	1.0 equiv.	1.0 equiv. BF_3•OEt_2	CH_2Cl_2	45%
5	2.0 equiv.	0.2 equiv. BF_3•OEt_2	CH_2Cl_2	55%
6	3.0 equiv.	0.2 equiv. BF_3•OEt_2	CH_2Cl_2	65%
7	5.0 equiv.	0.2 equiv. BF_3•OEt_2	toluene	85%

Table 3-1. The optimization of glycosylation.

In order to streamline synthesis, we attempted a selective glycosylation on **3-9.** Ketal **3-9** has three possible glycosylation sites: the primary alcohol in the open form, the

secondary alcohol, and the tertiary alcohol. In the previous study, the primary alcohol in the open form selectively reacted with TBDPSCl to generate the acyclic substrate. However, considering its preferential closed form in solution, the sugar might be added selectively to the secondary alcohol of the closed form of **3-9** with appropriate modulation of the activity of the glycosyl donor, as well as other reaction conditions such as temperature and solvent. It was evident that success of this attempted reaction depended on the reactivity of the electrophile toward the secondary alcohol relative to other competing sites. This route would minimize the number of synthetic transformations toward the target compound.

Unfortunately, selective glycosylation of **3-9** under a variety of conditions such as varied amounts (0.5-2.0 equiv.) of sugar donors **2-28** or **2-29**, as well as promoters (BF_3 •OEt_2, TMSOTf), solvents (CH_2Cl_2, toluene) and reaction temperature always provided inseparable mixture of reaction products (**Scheme 3-5**). The isolated compounds were difficult to identify due to their complex proton NMR spectra. The outcome of glycosylation was highly sensitive to variation of the reaction conditions. Exposure of the crude mixture, which was obtained from the coupling reaction between **3-9** and 0.8 equiv. of **2-28** employing 0.2 equiv. of BF_3•OEt_2 in CH_2Cl_2, to TBDPSCl in the presence of imidazole in CH_2Cl_2 provided trace amounts of the desired product **3-12**. These low yields and complex mixtures led us to abandon the selective glycosylation approach. We then shifted focus to the construction of the pyrrolidine ring by intramolecular reductive amination.



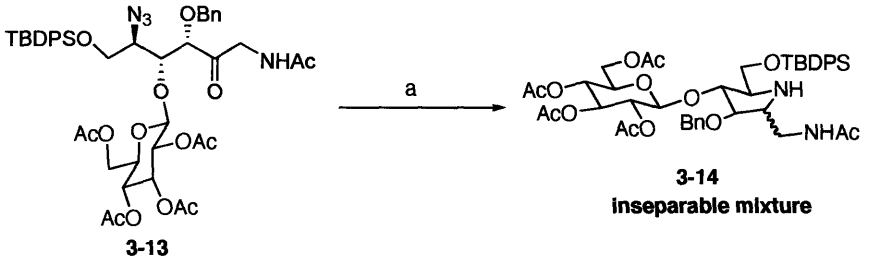
Reagents and conditions: (a) **2-28** or **2-29**, BF_3•OEt_2, or TMSOTf, CH_2Cl_2 or toluene, different reaction temperatures; (b) (i) **2-28**, BF_3•OEt_2, CH_2Cl_2, 0 °C to RT; (ii) TBDPSCl, imidazole, CH_2Cl_2.

Scheme 3-5. Attempted selective glycosylation of 3-9.

3-4 Construction of the imino sugar by intramolecular reductive amination

Initial attempts at the cyclization of **3-13** between *in-situ* generated amine from azide and ketone moieties using palladium hydroxide in the presence of hydrogen at ambient pressure generated various intermediates (**Scheme 3-6**). Mass spectral analysis indicated that most of the material was an uncyclized compound where the azide group had been reduced to the corresponding amine. Addition of AcOH or HCl to the reaction mixture did not facilitate clean conversion to the desired product. Under a high pressure of H_2 (1500 psi), intramolecular reductive amination of **3-13** employing palladium hydroxide in methanol gave the cyclized product **3-14**. However, the isolated product was a mixture of diastereomers, which could not be separated during purification.

Modification of conditions to decrease hydrogen pressure (500 psi or 100 psi) led to no apparent change in results.



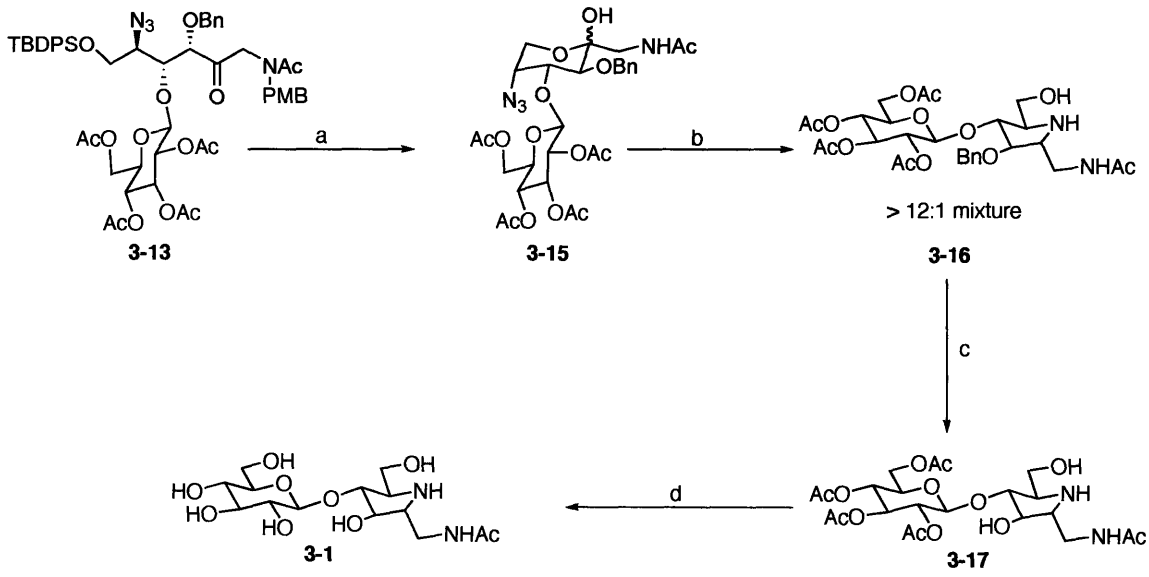
Reagents and conditions: (a) H_2 (50-1500 psi),$Pd(OH)_2$, MeOH, < 34% .

Scheme 3-6. Attempted intramolecular reductive amination of 3-13.

The poor diastereoselectivity in the cyclization of **3-13** prompted us to investigate the intramolecular reductive amination in the absence of the large silyl protecting group (**Scheme 3-7**). The silyl ether was cleaved using 1.0 equiv. of tetrabutyl-*n*-ammonium fluoride (TBAF) in the presence of 1.0 equiv. of AcOH in THF, producing the corresponding ketal **3-15** in 90% yield. The cyclization of **3-15** utilizing palladium hydroxide under H_2 (1500 psi) in methanol gave **3-16** as the predominant product (>12:1 NMR ratio). Under these conditions, only trace amounts of **3-17** in which the benzyl ether was cleaved were isolated. **3-17** was obtained by the treatment of **3-16** with $Pd(OH)_2$ in acetic acid under hydrogen at ambient pressure. The specific details regarding the stereochemistry of structure of **3-17** were confirmed by nuclear Overhauser effect (NOE) difference experiments (**see Chapter 3-6**).

The attempted direct conversion of **3-15** to **3-17** was unsuccessful under various reaction conditions employing higher pressures of H_2 (2000 psi or 2500 psi) or the use of acetic acid as the solvent. Attempted debenzylation of the crude product during the

reductive amination provided only trace amounts of the desired product. The best results were obtained by sequential deprotection using purified compound as a substrate. Deprotection of the acetyl groups under Zampél conditions gave the target molecule **3-1**.



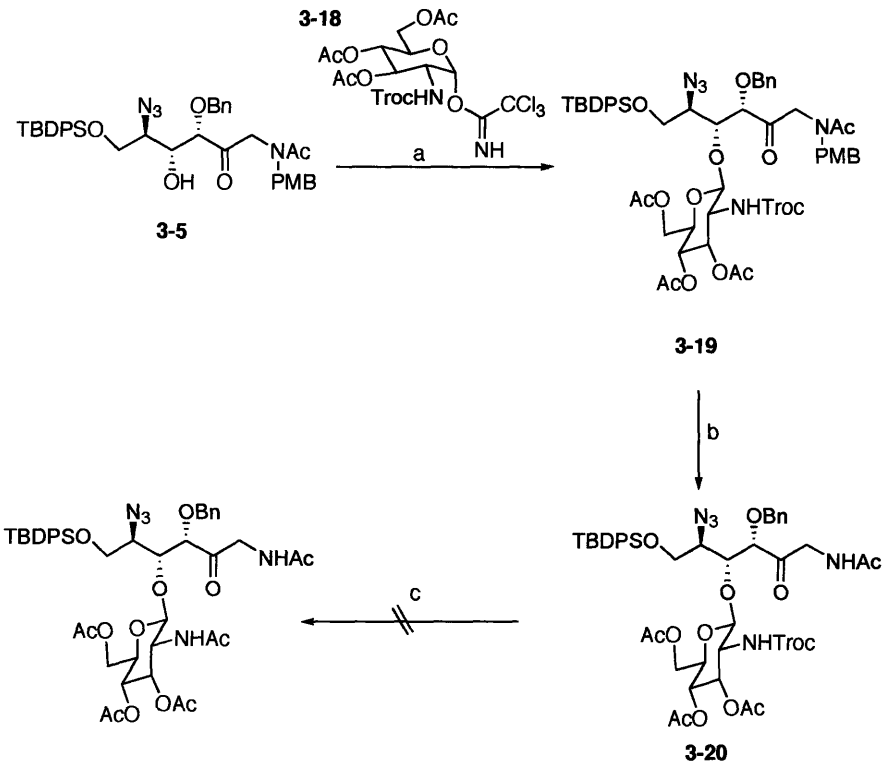
Reagents and conditions: (a) TBAF, AcOH, THF, 0 °C to RT, 90 %; (b) H_2 (1500 psi), $Pd(OH)_2$, MeOH, 70 %; (c) H_2 (balloon), $Pd(OH)_2$, AcOH, 65 %; (d) NaOMe, MeOH, Quant.

Scheme 3-7. Reductive amination and synthesis of pseudo-disaccharide 3-1.

3-5 Synthesis of the imino sugar linked to N-acetylglucosamine

Our focus next turned to the construction of **3-2**, in which the imino sugar was linked to N-acetylglucosamine (GlcNAc). In addition to the stereo-chemical outcome of glycosylation, as discussed in Chapter 2, the choice of amine protecting group was critical for the overall strategy in the complex carbohydrate synthesis. Selection of the proper amine protecting group turned out to be crucial to the completion of this synthesis.

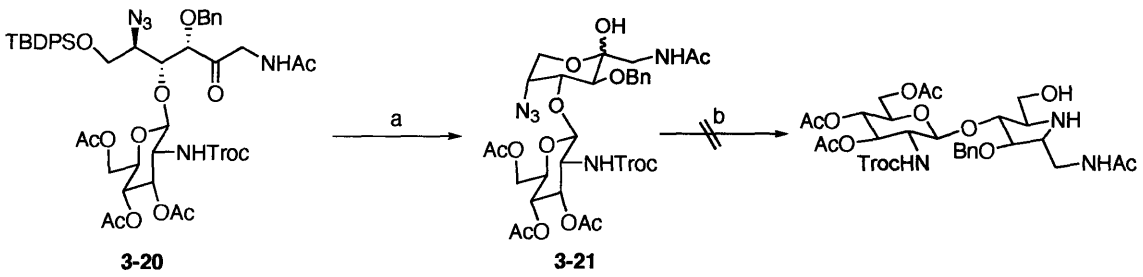
Initially, the coupling between **3-5** and glycosyl imidate **3-18**[18] utilized an N-trichloroethylene carbamate (N-Troc) at the C2' position (**Scheme 3-8**). Glycosylation of **3-5** with 3.0 equiv. of glycosyl imidate **3-18** utilizing 0.2 equiv. of $BF_3 \bullet OEt_2$ in toluene provided the desired product **3-19** in 69% yield. The salient conditions used in the glycosylation of **3-5** with the glucose derivative still proved optimal. Attempted glycosylation of **3-5** with thioglycoside or glycosyl halide provided only a small amount of the desired product or no product at all. Use of a different promoter failed to give the coupled product, as seen for in synthesis of **3-1**. The oxidative removal of the PMB group was achieved by using 4.0 equiv. of CAN at low temperature, affording **3-20** in 65% yield. Initial attempts to convert the N-Troc to an N-Ac before a reductive amination were unsuccessful. Metal-assisted removal of the N-Troc group[19,20] failed to provide the desired compound. These deprotection conditions led to reduction of the azide group and generated a mixture of products.



Reagents and conditions: (a) **3-18**, BF_3•OEt_2, toluene, 0 °C, 69 %; (b) CAN, MeCN: H_2O = 10:1,-20 °C to 0 °C, 65 %; (c) Cd,AcOH, DMF then Ac_2O or Zn, Ac_2O, H_2O.

Scheme 3-8. Attempted conversion of N-Troc to N-Ac group of 3-20.

The incompatibility of the azide group with deprotection of the N-Troc group led us to conduct the reductive amination in the presence of the N-Troc group (**Scheme 3-9**). The removal of the silyl ether using 1.0 equiv. of TBAF in the presence of 1.0 equiv. of acetic acid provided the desired product **3-21**. Unfortunately, reductive amination of **3-21** employing $Pd(OH)_2$ under a high pressure of H_2 (1500 psi) failed to give the desired product. Mass spectral analysis indicated that the carbon-chloride bonds on the N-Troc group were reduced to generate various products. Attempted cyclizations under lower pressures of H_2 (50 psi or 100 psi) were not successful in preventing the reduction of chlorides on the N-Troc group.



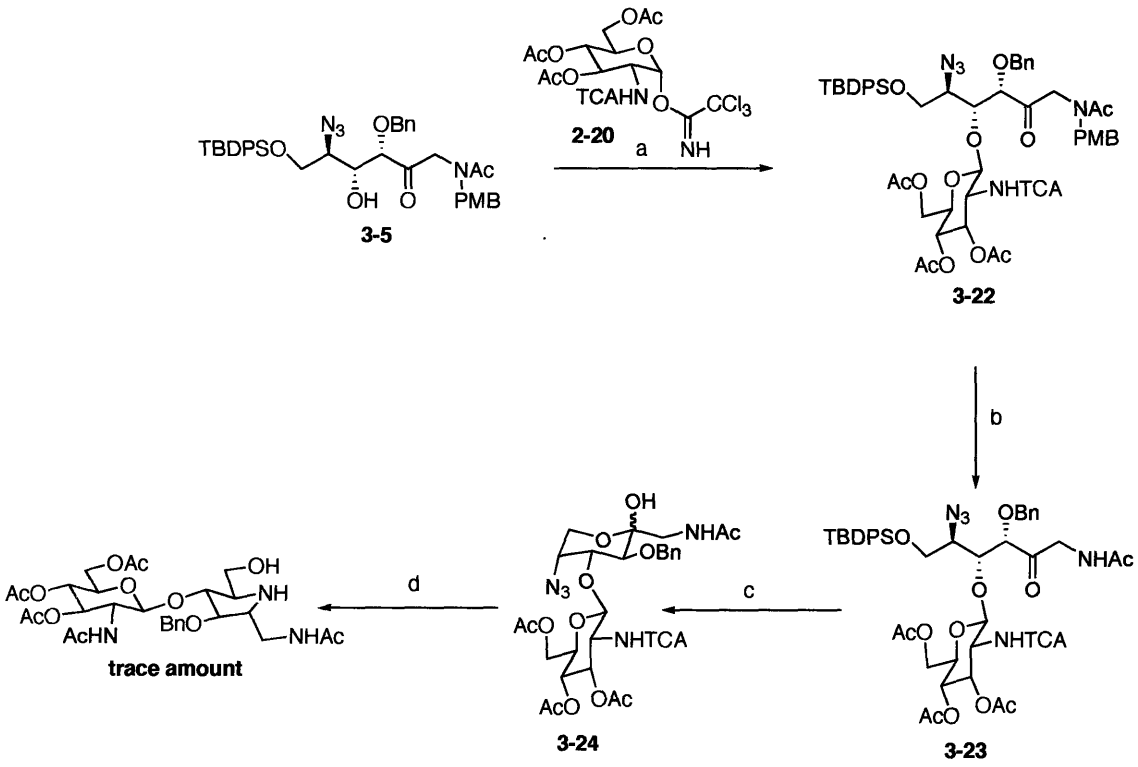
Reagents and conditions: (a) TBAF, AcOH, THF, 0 °C to RT, 83 %; (b) H_2 (50-1500 psi), $Pd(OH)_2$, MeOH.

Scheme 3-9. The attempted intramolecular reductive amination of 3-21.

The problematic chloride reduction during reductive amination prompted us to use the glycosyl imidate having a N-trichloroacetimide (N-TCA) at C2' position. The reduction of chlorides under radical conditions have been used for previously the transformation of N-TCA group to N-Ac group.[21,22] In our synthetic route the radical-mediated transformation could not be applied due to reduction of the azide group. The conversion of N-TCA to N-Ac was accomplished by treatment with H_2 and palladium on carbon in the presence of triethylamine.[23] Our intent was to execute the formation of a pyrrolidine ring and the conversion of the N-TCA group to N-Ac group simultaneously.

Glycosylation of **3-5** with 5.0 equiv. of glycosyl imidate **2-20**[21] in the presence of 0.2 equiv. of $BF_3 \bullet OEt_2$, followed by the deprotection of N-PMB employing 4.0 equiv. of CAN smoothly provided **3-23 (Scheme 3-10)**. The fluoride-mediated removal of the silyl ether of **3-23** in the presence of AcOH provided the desired product **3-24**. Unfortunately, attempted intramolecular reductive amination under high pressures of H_2 (2000-2500 psi) resulted in the formation of only trace amounts of the desired product. A complex

mixture of intermediates at different reduction states of the N-TCA group was generated and the amount of isolated intermediates was very small. The use of increased amounts of $Pd(OH)_2$, $PdCl_2$ or palladium black did not facilitate production of the desired compound as the major product. The addition of triethyamine, DIPEA or ammonium hydroxide similarly did not lead to an improvement in the outcome of the reaction.

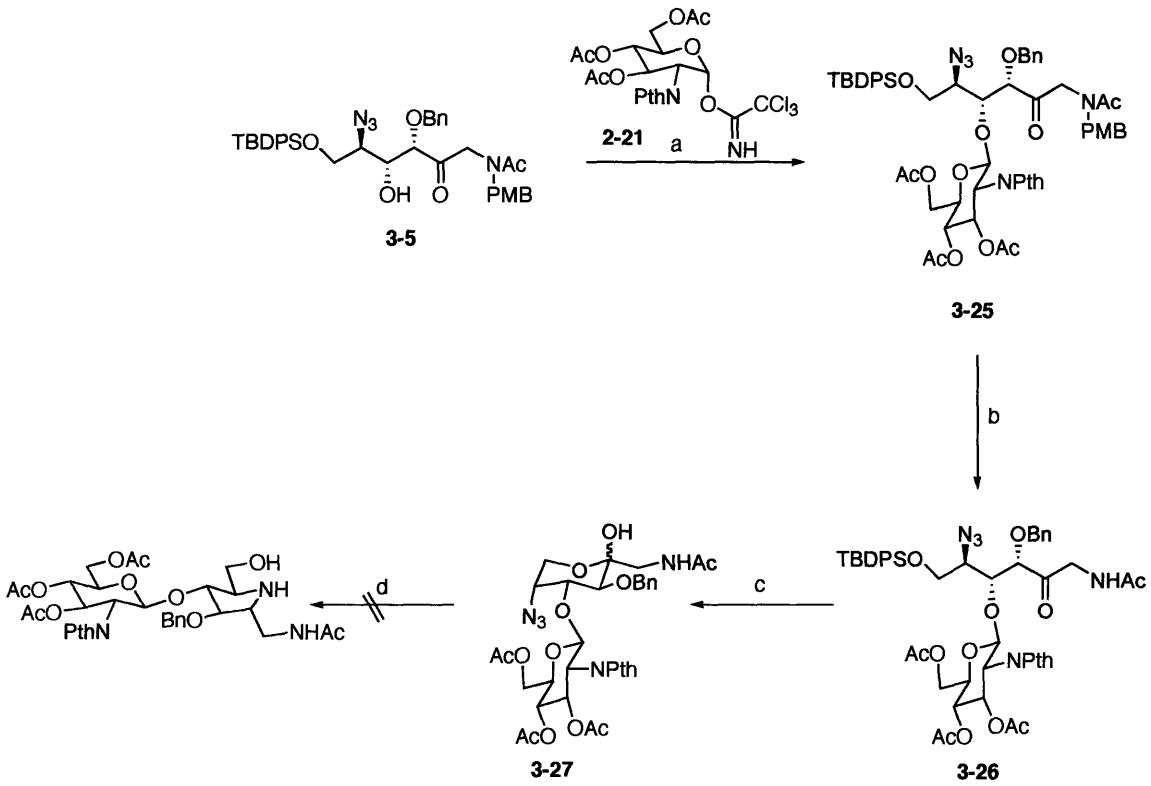


Reagents and conditions: (a) **2-20**, BF_3•OEt_2, toluene, 0 °C to RT, 85%; (b) CAN, MeCN: H_2O = 10:1, -20 °C to 0 °C, 67% ; (c) TBAF, AcOH, THF, 0 °C to RT, 85%; (d) H_2 (1500-2000 psi), $Pd(OH)_2$, MeOH with or without triethylamine.

Scheme 3-10. Attempted intramolecular reductive amination of 3-24.

To avoid the reduction of chlorides during cyclization, a glycosyl imidate having an N-phthalimide (N-Pth) was chosen as the sugar donor. Intramolecular reductive

amination in the presence of the N-Pth group, which was stable under the reducing conditions, was expected to generate the desired pyrrolidine ring without complications. Compound **3-26** was obtained by the glycosylation of **3-5** with 3.0 equiv. of glycosyl imidate **2-21**[24] in the presence of 0.2 equiv. of $BF_3 \bullet OEt_2$, and subsequent deprotection of the PMB group utilizing 4.0 equiv. of CAN (**Scheme 3-11**). Compound **3-27** was prepared by the removal of silyl ether using 1.0 equiv. of TBAF in the presence of 1.0 equiv. of acetic acid. In the attempted intramolecular reductive amination of **3-27**, only unidentified products were isolated. The mass spectrum indicated that the major component of the complex mixture was the uncyclized product. Modification of reaction conditions such as higher pressures of H_2 and increased amounts of $Pd(OH)_2$ did not help to obtain the desired product. One possible explanation is that the intermediate cyclic imine adopts an unfavorable conformation due to the steric hindrance between the bulky phthalimide group and other functional groups, and thereby fails to generate the desired compound.



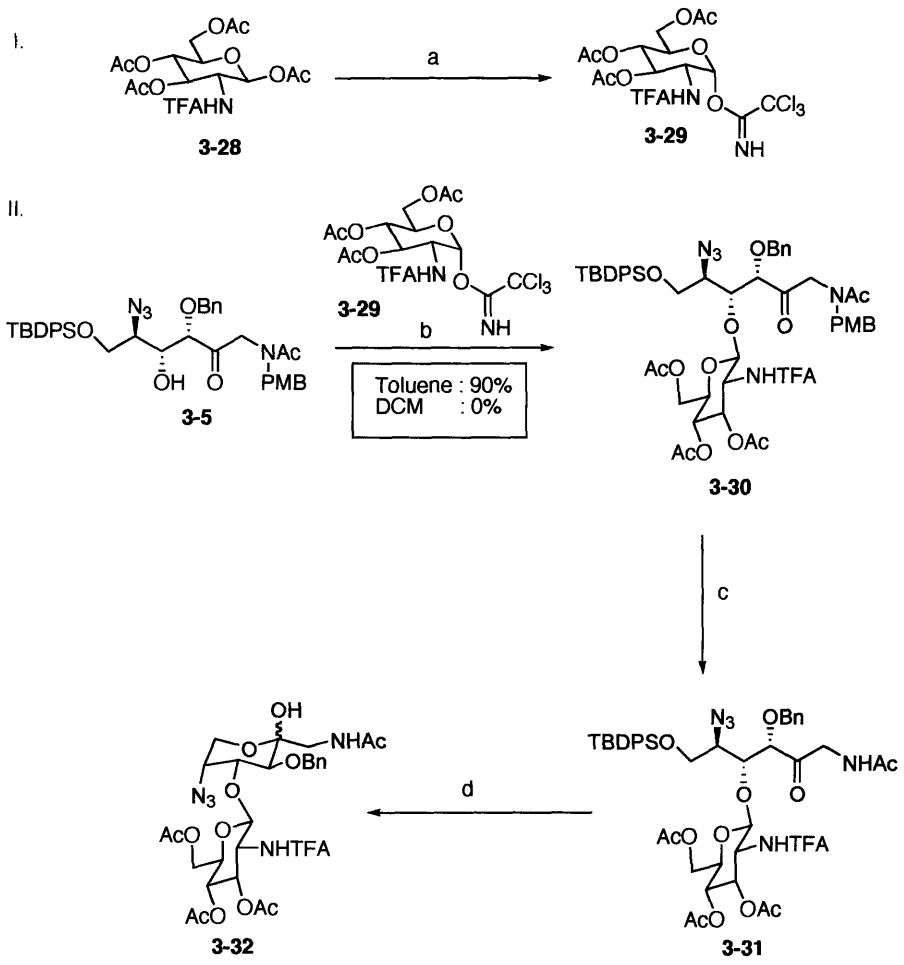
Reagents and conditions: (a) **2-21**, $BF_3{\cdot}OEt_2$, toluene, 0 °C, 73 %; (b) CAN, MeCN: H_2O = 10:1,-20 °C to 0 °C, 62 %; (c) TBAF, AcOH, THF, 0 °C to RT, 87 %; (d) H_2 (50-2000 psi), $Pd(OH)_2$, MeOH.

Scheme 3-11. The attempted reductive aminaton of 3-27.

The attempted conversions of the N-Troc group to an N-Ac group were unsuccessful due to the incompatibility of other functional groups. The attempted cyclizations in the presence of other nitrogen protecting groups failed to generate the desired product due to the chloride reduction of the N-Troc and N-TCA groups, or the large size of the N-Pth group. Considering the other functional groups in the substrate, converting the amine protecting group to N-Ac group before the reductive amination appeared challenging. Therefore, we decided to search for another C2 protecting group suitable for reductive amination.

The glucosamine derivative donor with an N-trifluroacetimide (N-TFA) on the C2 amine was expected to show low reactivity toward glycosylation due to the strongly electron-withdrawing substituent.[25] To the best of our knowledge, there is only one reported example in which an N-TFA group was used as a C2 amine protecting group for a glucosamine derivative donor.[25] However, with regard to its stability under reducing conditions and its relatively small size, the N-TFA group seems to be compatible with a reductive amination. The neighboring group participation of the N-TFA group would ensure high β-selectivity of glycosidic bond formation.

Glycosyl imidate**3-29** was synthesized from known compound **3-28** in 2 steps (**Scheme 3-12-I**). The deprotection of the anomeric acetate of **3-28** with 1.0 equiv. of hydrazine acetate in DMF, followed by treatment with 10 equiv. of CCl_3CN in the presence of 0.1 equiv. of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in CH_2Cl_2 provided the desired **3-29** in 75% yield over 2 steps. As expected, the rate of glycosylation of **3-10** with glycosyl imidate **3-29** in the presence of BF_3•OEt_2 in toluene was much slower than those of previous glycosylations (**Scheme 3-12-II**). For complete conversion, it took 2 days with 8.0 equiv. of glycosyl imidate **3-29**, even when fresh promoter was added every 8 hours. To our delight, however, the desired compound **3-30** was obtained in 90% yield. The synthetic procedure was found to be efficient to a 1-gram scale. It is noteworthy that changing the solvent to CH_2Cl_2 resulted in no desired compound being obtained, as most of the glycosyl imidate was converted to the corresponding oxazoline. Cleavage of the PMB group was achieved by treatment of **3-30** with 4.0 equiv. of CAN to provide **3-31**. The removal of the silyl ether using 1.0 equiv. of TBAF in the presence of 1.0 equiv. of acetic acid provided compound **3-32** in 87% yield.

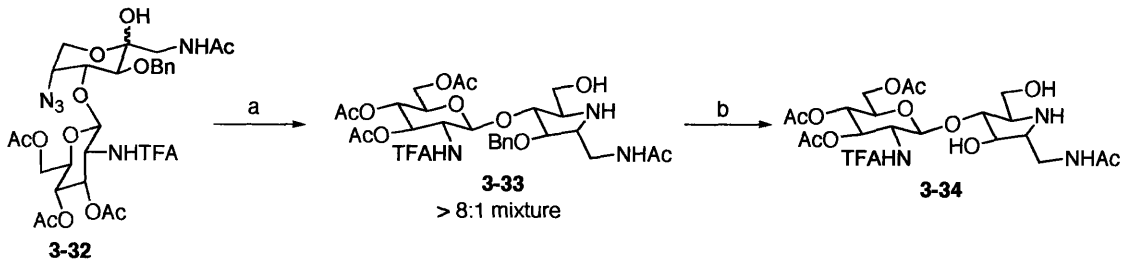


Reagents and conditions: (a) (i) $NH_2NH_2{\cdot}CH_3CO_2H$,DMF, RT; (ii) CCl_3CN, DBU, CH_2Cl_2, 0 °C to RT, 2 steps 75 %; (b) **3-29**, $BF_3{\cdot}OEt_2$, toluene, 0 °C, 90 %; (c) CAN, MeCN: H_2O = 10:1,-20 °C to 0 °C, 62 %; (d) TBAF, AcOH, THF, 0 °C to RT, 87 %.

Scheme 3-12. The synthesis of the ketal 3-32.

Reductive amination of **3-32** gave the desired compound **3-33** as the major product (> 8:1 NMR ratio). However, the yield for the reductive amination of **3-32** was inconsistent (20-80% yield) under the reaction conditions applied (1200 psi of H_2). In contrast, the reductive amination of **3-18**, the analogous glucose derivative, produced reliable results under the same conditions. Under modified conditions employing H_2 at

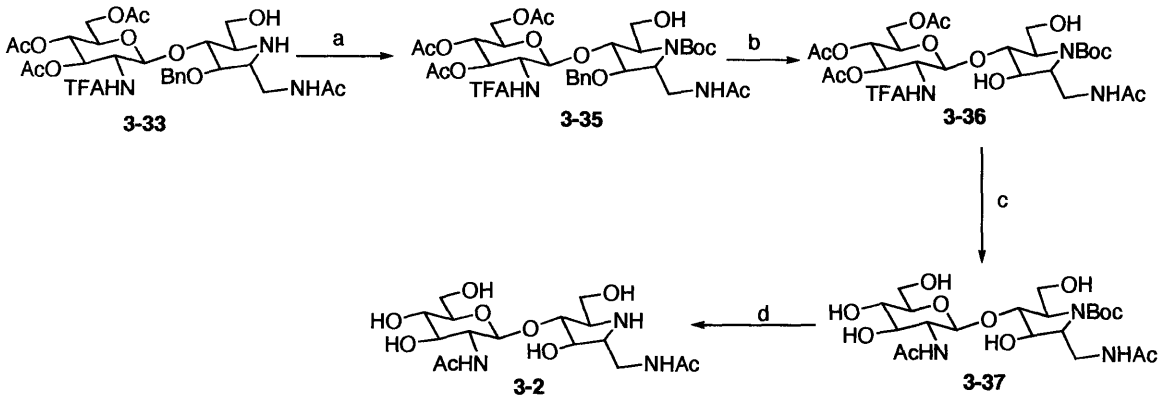
850 psi, the desired product **3-33** was obtained reproducibly (50-60% yield) (**Scheme 3-13**). After deprotection of the benzyl group, specific details of the stereochemistry of **3-34** were confirmed by NOE experiments (**see Chapter 3-6**).



Reagents and conditions: (a) H_2 (850 psi), $Pd(OH)_2$, MeOH, 55%; (b) H_2 (balloon), $Pd(OH)_2$, AcOH, 69 %.

Scheme 3-13. The synthesis of 3-33.

The addition of 1.6 equiv. of di-*tert*-butyl dicarbonate (Boc_2O) to **3-33** in CH_2Cl_2 in the presence of 2.0 equiv. of DIPEA provided desired compound **3-35** (**Scheme 3-14**). The benzyl ether was cleaved under H_2 (50 psi) in the presence of $Pd(OH)_2$ using acetic acid as the solvent to provide the desired compound **3-36**. Removal of the N-trifluroacetimide (N-TFA) group was achieved using ammonia in methanol and subsequent N-acetylation provided the desired product **3-37** in 63% yield over 2 steps.[26] The Boc group was removed using TFA in CH_2Cl_2 to afford the target compound **3-2**.



Reagents and conditions: (a) Boc_2O, DIPEA, CH_2Cl_2, 0 °C to RT, 68%; (b) H_2 (50 psi), $Pd(OH)_2$, AcOH, 80%; (c) (i) NH_3, MeOH; (ii) Ac_2O, DCM:MeOH = 1:1, 2 steps 63%; (d) TFA, CH_2Cl_2, 70%.

Scheme 3-14. The synthesis of the target pseudo-disaccharide 3-2.

3-6 Determination of the stereo-chemical outcome of the reductive amination

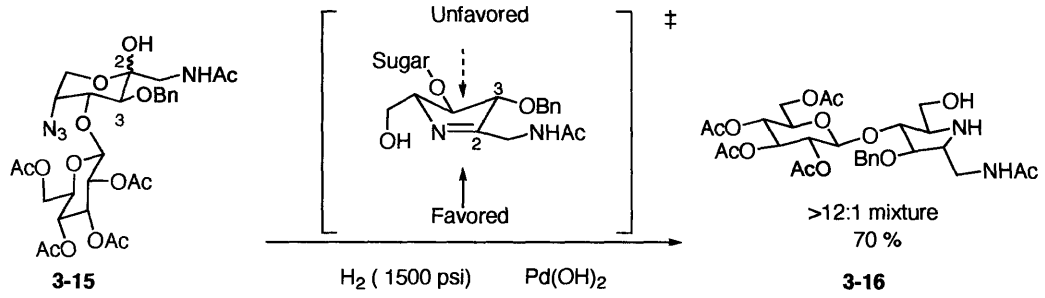


Figure 3-2. The proposed stereo-outcome of reductive amination.

The stereo-chemical outcome of the reductive amination of **3-15** and **3-32** was expected to be the *trans*-configuration between C2-C3 due to the approach of hydrogen from the *re* face to avoid torsional strain (**Figure 3-2**). Other examples of the cyclization between the *in-situ* generated amine from azide and the ketone moieties in similar structures have been confirmed

by NMR spectroscopy utilizing NOE difference experiment.[2,27-30] Unfortunately, an attempted NOE difference experiment of **3-16** in CD_3OD solvent resulted in no noticeable NOE difference between H_2 and H_4 and between H_3 and H_5. Extensive efforts to obtain an NOE signal, such as the use of different solvents (C_6D_6, DMF-d_6, DMSO-d_6), or increased temperature, were unsuccessful. The NOE experiment for **3-33** showed similar results. The efficiency of NOE buildup depends on the distance between the nuclei involved and the tumbling rate of the molecule.[31] The lack of NOE signal for **3-16** and **3-33** might be explained by the unfavorable spatial orientation between the pseudo-equatorial H_2 and pseudo-axial H_4 and the rigid structure of **3-16** due to substituents on the secondary alcohols. This assumption led us to attempt the NOE analysis on **3-17**, which was obtained by the deprotection benzyl ether of **3-16**.

The removal of the benzyl ether would give more flexibility and reduce the size therefore increasing the molecular tumbling rate. In the NOE experiment of **3-17** in CD_3OD solvent, NOE signals between H_3 and H_4 and between H_2 and H_5 were observed. Unfortunately, the peaks for H_3 and H_4 in the proton NMR were not resolved. Thus, the configuration of C2 could not be determined. To find the optimal proton NMR spectrum for the NOE experiment, different NMR solvents and solvent mixtures were tested. Using $CDCl_3$: CD_3OD (4:1), H_3 and H_4 appeared in different regions ($peak_a$ = 3.81 ppm, $peak_b$ = 3.85 ppm) (**Figure 3-3**). Since the resonance for H_2 and H_5 appeared at the same position ($peak_c$ = 3.06 ppm), the exact assignment of $peak_a$ and $peak_b$ as H_3 and H_4 was impossible by COSY. However, the separation between H_3 and H_4 peaks was enough for the NOE experiment to establish the configuration.

In the NOE experiment, we wanted to confirm the configuration of C2 from an NOE difference between H_2 and H_4. On the other hand, an NOE between H_2 and H_4 would confirm

the structure as the possible diastereomer. Since H_2 and H_5 appear in the same region of the spectrum, the selective irradiation of H_2 was difficult. However, the NOE between H_3 and H_5 would be common for both structures. Therefore, two NOE differences between H_2 and H_4 and between H_3 and H_5 would prove the structure of **3-17**, whereas one NOE among H_2, H_3 and H_5 would confirm the other isomer. The control spectrum and three NOE difference spectra of **3-17** are shown in Figure 3-4. The irradiation of $peak_c$ (H_2 and H_5) induced the NOE signal of both $peak_a$ and $peak_b$ (**Figure 3-4b.**). Upon the irradiation of $peak_a$ and $peak_b$ respectively, the NOE signal of $peak_c$ (H_2 and H_5) was observed (**Figure 3-4c, d.**). These data showed that there are NOE differences between H_2 and H_4 and between H_3 and H_5. Therefore, we concluded the **3-17** had the desired *trans*-configuration between C2-C3. The NOE analysis of **3-34** in $CDCl_3$:CD_3OD = 8:1 mixture showed similar results as that of **3-17**. (**See the experimental section for details**)

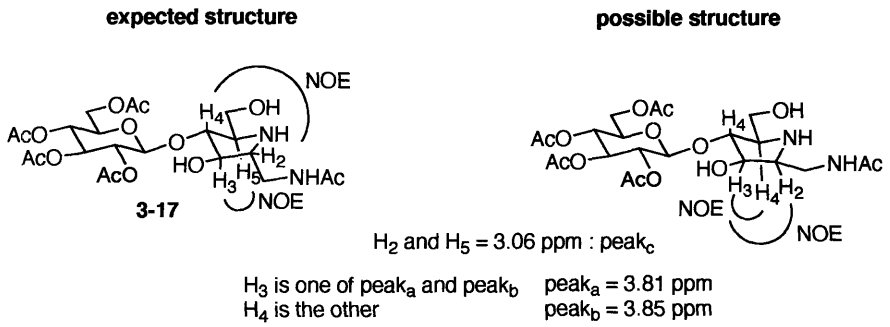


Figure 3-3. The expected structure of 3-17 and possible diastereomers at C2.

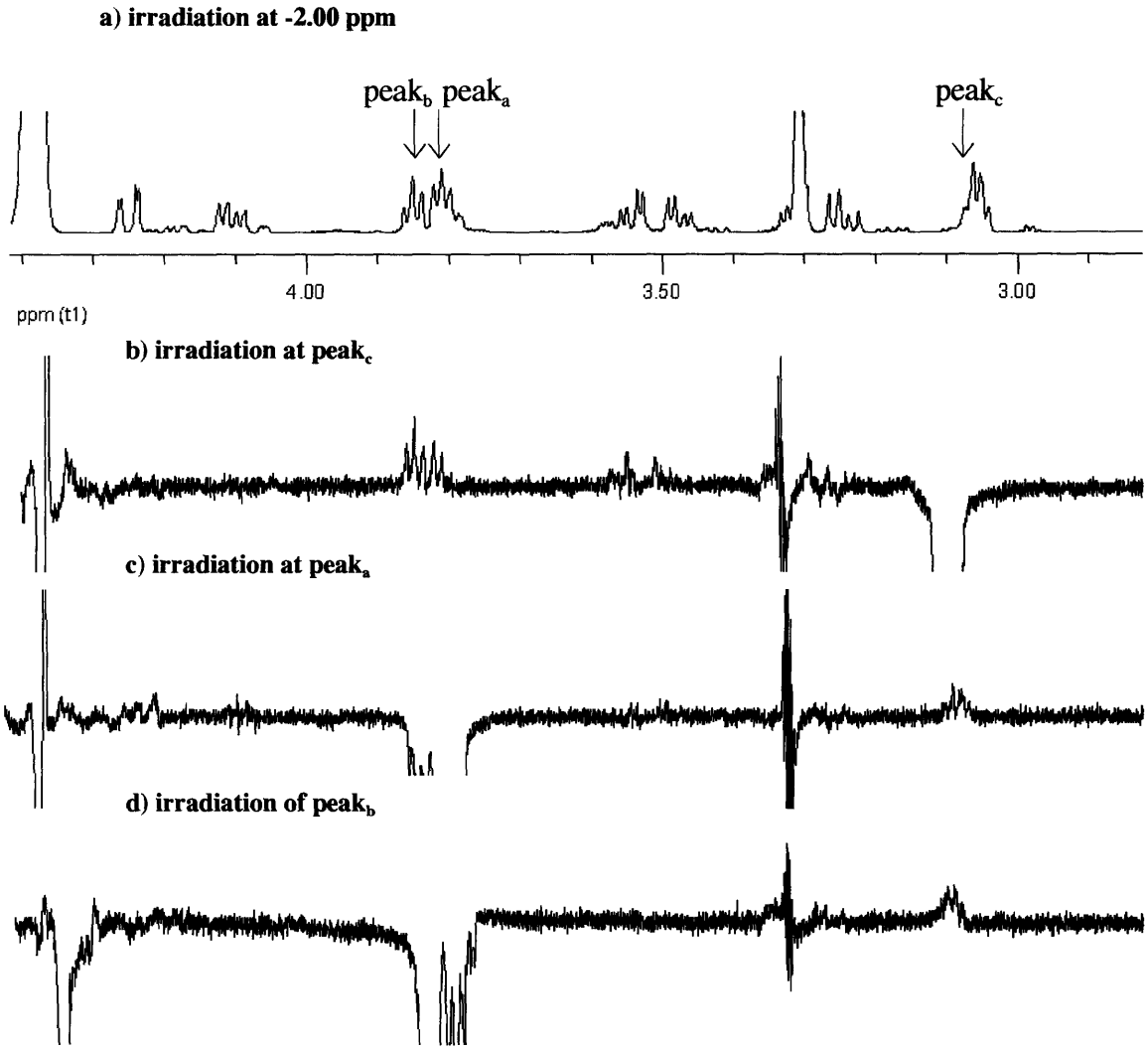


Figure 3-4. The NOE difference spectrum of 3-17.

3-7 The inhibition study of 3-1 and 3-2 against chitinase

Biological evaluation of the inhibitory effects **3-1** and **3-2** against chitinase from *Streptomyces griseus* was conducted. As described in Chapter 2-1, 4-methylumbelliferyl-β-D-N,N'-diacetylchitobiose was used as a fluorogenic substrate to monitor enzyme activity.[32,33] Figure 3-5 shows the fluorescence intensity at 460 nm from control experiment (without the inhibitor) and different concentrations of **3-1**. Depending on the concentration of compound **3-1**, decreased enzyme activity was observed. Experiments were performed in triplicate. The inhibition percentage relative to the control without inhibitor was calculated on the basis of the fluorescence reading at 460 nm. All data points represent the average of three measurements with standard deviations (**Figure 3-6**). **3-1** and **3-2** showed inhibitory activity with IC_{50} values of 3.1 μM and 2.6 μM respectively.

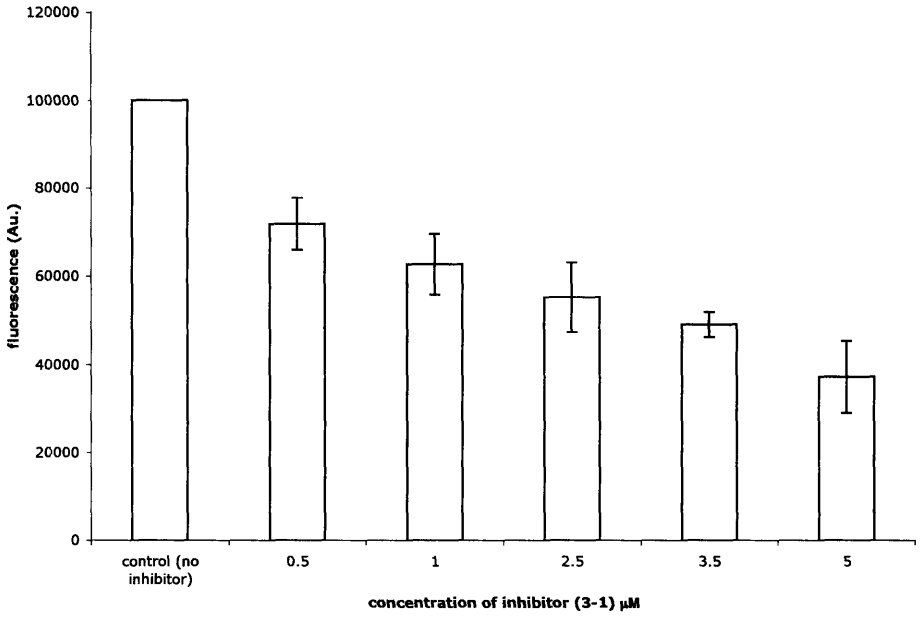


Figure 3-6. Compound 3-1 concentration dependent inhibition toward chitinase.

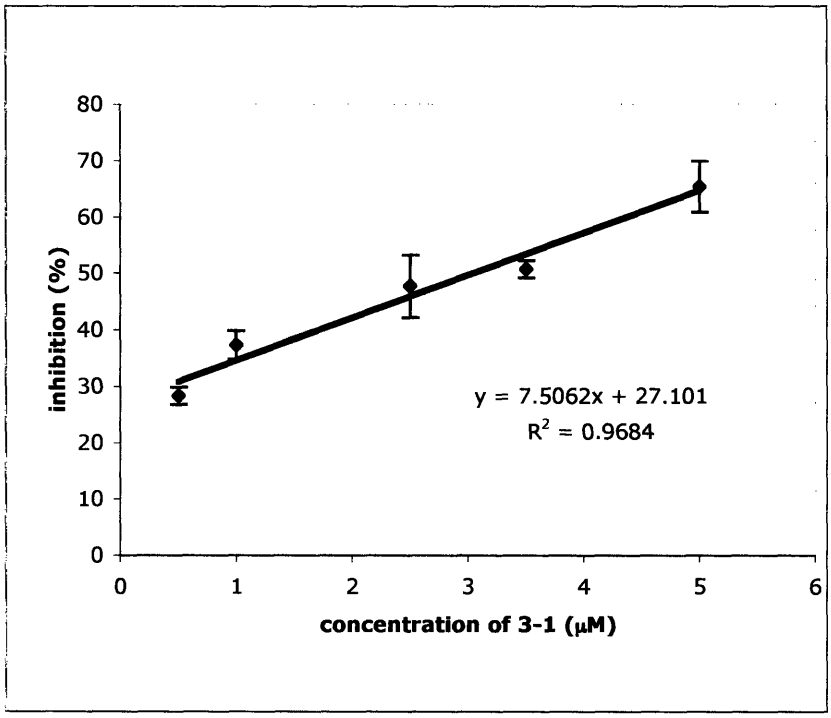


Figure 3-7. Inhibition (%) of 3-1 toward chitinase.

Inhibitory activities of **3-1** and **3-2** could be explained by the fact that the compounds mimic the oxocarbenium-ion-like transition state, specifically the charge and conformation. However, no inhibitory activity of **2-10** was observed up to 1 mM. This trend is also seen when comparing the natural products allosamizoline and allosamidin, which show that the additional carbohydrate moieties were essential for tight binding to the enzyme (**Figure 3-7**).[34] These observations confirmed our prediction that the disaccharide unit is the minimal binding motif for chitinases.

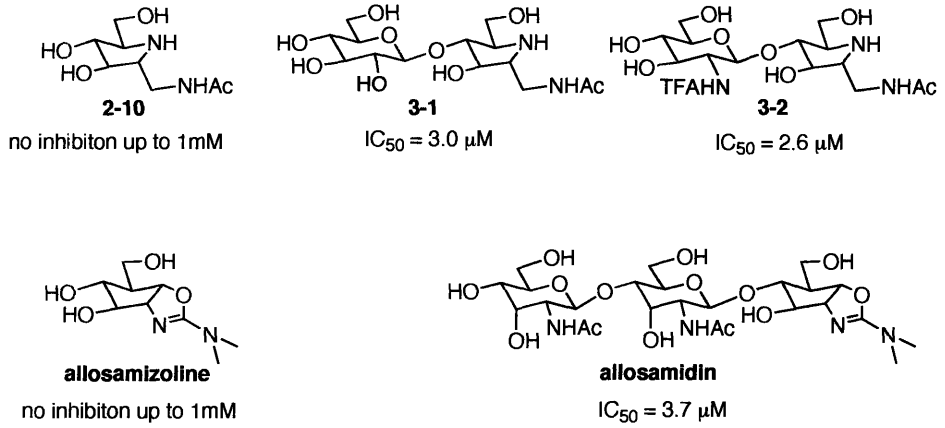


Figure 3-7. The inhibitory actvity of 3-1, 3-2 and other natural products against chitinase from *Streptomyces grises*.

3-8 Conclusion

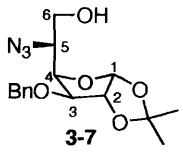
In summary, imino sugar containing pseudo-disaccharides **3-1** and **3-2** were synthesized utilizing an Amadori rearrangement, glycosylation of a β-hydroxyl ketone and intramolecular reductive amination as key steps. These compounds displayed low micromolar inhibitory activities against the glycosidase enzyme chitinase. Applying the synthetic schemes described herein, further derivatives of these pseudo-disaccharide compounds can be synthesized and evaluated for chitinase as well as OT.

Experimental Section

General Synthetic Procedures

Anhydrous dichloromethane and toluene were distilled from calcium hydride, and anhydrous tetrahydrofuran was distilled from sodium/benzophenone. All chemicals were purchased from Sigma/Aldrich and used without further purification unless otherwise noted. Analytical thin-layer chromatography (TLC) was carried out on Merck 60 F_{254} 250-μm silica gel plates. All compounds were visualized on TLC by UV irradiation or an aqueous solution of ceric ammonium molybdate (CAM) staining. Flash column chromatography was carried out using forced flow of the indicated solvent on AdTech Flash Silica Gel, 32-63 μm particle size, 60 Å pore size (Adedge technologies). ^{1}H NMR spectra were acquired on a Bruker Avance (DPX) 400 MHz spectrometer, Varian INOVA 500 MHz spectrometer and Bruker Avance (DPX) 600 MHz spectrometer. ^{13}C NMR spectra were acquired on a Bruker Avance (DPX) 400 MHz spectrometer and Varian INOVA 500 MHz spectrometer. Chemical shifts (δ) are reported in parts per million (ppm) with chemical shifts referenced to internal standards: $CDCl_3$ (7.27 ppm for ^{1}H, 77.23 ppm for ^{13}C), CD_3OD (4.87 ppm for ^{1}H, 49.15 ppm for ^{13}C), D_2O (4.80 ppm for ^{1}H), C_6D_6 (7.16 ppm for ^{1}H, 128.39 ppm for ^{13}C). Coupling constants (*J*) are reported in Hertz (Hz) and multiplicities are abbreviated as singlet (s), doublet (d), triplet (t), multiplet (m), broadened siglet (br) and doublet of doublets (dd). The term "app d" is used to denote a triplet with two similar coupling constants and "app t" is used to denote a doublet of doublets (dd) with similar coupling constants. High resolution Mass Spectra (HRMS) were obtained at the Mass Spectrometry Facility at MIT (Cambridge, MA).

5-Azido-3-O-benzyl-5-deoxy-1,2,-*O*-isopropylidene-α-D-glucofuranose (3-7)



3-7

Sodium hydride (68.2 mg, 1.1 equiv. 60% in dispersion oil) was added to a solution of **2-7** (509 mg, 1.55 mmol) in THF (15 mL) at 0 °C. After the evolution of hydrogen gas ceased, benzyl bromide (0.221 mL, 1.2 equiv.) and TBAI (114 mg, 0.2 equiv.) were added to the reaction mixture at 0 °C, and warmed to room temperature. The reaction mixture was stirred overnight, and poured into water (20 mL). The mixture was extracted with ethyl acetate (3 x 20 mL) and the combined organic layers were washed with satd. $NaHCO_3$ and satd. brine, dried over $MgSO_4$ and concentrated *in vacuo*.

Without further purification, the resulting mixture was dissolved in MeOH: CH_2Cl_2 (10:1, 30 mL) and *p*-TsOH (59 mg, 0.2 equiv.) was added. The reaction was stirred for 4 h at ambient temperature and quenched with triethylamine (0.5 mL). The solvent was removed under reduced pressure, followed by purification *via* flash column chromatography (hexane:ethyl acetate = 8:1 to 2:1) to afford **3-7** (492 mg, 95%) as a colorless liquid.

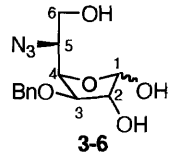
^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.33 (s, 3H, H_3CC-), 1.50 (s, 3H, H_3CC-), 2.52 (brs, alcohol), 3.77 (ABx, 1H, *J* = 5.6 and 11.3 Hz, H_{6a}), 3.89-3.98 (m, 1H, H_5), 3.97 (ABx, 1H, *J* = 3.5 and 11.3 Hz, H_{6b}), 4.08 (app d, 1H, *J* = 3.2 Hz, H_3), 4.13 (dd, 1H, *J* =

3.2 and 9.5 Hz, H_4), 4.62 and 4.70 (ABq, 2H, J = 11.3 Hz, -OCH_2Ph), 4.65 (app d, 1H, J = 3.8 Hz, H_2), 5.92 (d, 1H, J = 3.8 Hz, H_1), 7.30-7.40 (m, 5H, -OCH$_2$*Ph*);

^{13}C NMR ($CDCl_3$, 100 MHz) δ 26.6, 27.2, 60.9, 63.8, 72.4, 79.4, 81.9, 82.1, 105.7, 112.5, 128.4, 128.5, 128.9, 137.4;

HRMS(ESI-MS): Calcd for $[C_{16}H_{21}N_3O_5+Na]^+$(M+Na): m/z = 358.1373; Found: 358.1378.

5-Azido-3-*O*-benzyl-5-deoxy-D-glucofuranose (3-6)



3-6

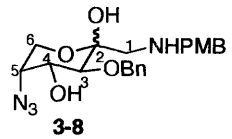
Aqueous trifluoroacetic acid (80%, 10 mL) was added to **3-7** (492 mg, 1.47 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 3 h. The mixture was concentrated *in vacuo* and poured into satd. $NaHCO_3$ (20 mL). The mixture was extracted with ethyl acetate (3 x 30 mL) and the combined organic layers were washed with satd. brine, dried over $MgSO_4$ and concentrated *in vacuo*. Flash column chromatography (hexane:ethyl acetate = 2:1 to ethyl acetate) of the crude oil gave the desired lactol **3-6** (356 mg, 82 %) as a yellow oil (a mixture of anomers).

^{1}H NMR (CD_3OD, 400 MHz) selected peaks δ 4.64-4.82 (m, 2H OCH_2Ph), 5.18 (app s, 0.6H of anomeric mixture, H_1), 5.36 (d, 0.4H of anomeric mixture, J = 3.8 Hz, H_1), 7.26-7.39 (m, 5H, -OCH$_2$*Ph*);

^{13}C NMR (CD_3OD, 100 MHz) δ 63.7, 63.9, 64.3, 64.4, 73.0, 75.1, 78.3, 79.3, 80.8, 84.3, 85.1, 99.0, 105.3, 128.9, 129.0, 129.2, 129.5, 129.6, 129.5, 139.2, 139.4;

HRMS(ESI-MS): Calcd for $[C_{13}H_{17}N_3O_5+Na]^+$(M+Na): m/z = 318.1066; Found: 318.1066.

5-azido-3-*O*-benzyl-1,5-dideoxy-4-hydroxy-1-(*N*-(4-methoxybenzyl)-amino)-D-fructopyranose (3-8)



3-8

p-Methoxybenzylamine (0.160 mL, 1.0 equiv.) was added to a solution of **3-6** (356 mg, 1.21 mmol) and acetic acid (0.139 mL, 1.0 equiv.) in MeOH (20 mL) at room temperature and the reaction mixture was warmed to 40 °C. The mixture was stirred for 2 h at 40 °C. The solvent was removed under reduced pressure. Flash column chromatography (CH_2Cl_2: MeOH = 20:1 to 15:1) of the crude product gave the desired product **3-8**, as the acetic acid salt form (402 mg, 70%) (a mixture of anomers).

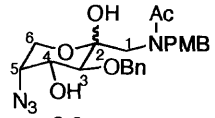
^{1}H NMR ($CDCl_3$, 600 MHz, ca. 3:2 a mixture of anomers) selected peaks δ 2.63 (d, 0.4H of anomeric mixture, J = 12.5 Hz, H_{1a}), 2.77 (d, 0.6H of anomeric mixture, J = 12.1 Hz, H_{1a}), 2.87 (d, 0.6H of anomeric mixture, J = 12.1 Hz, H_{1b}), 3.06 (d, 0.4H of anomeric mixture, J = 12.5 Hz, H_{1b}), 3.50 (d, 0.6H of anomeric mixture, J = 9.5 Hz, H_3), 3.62 (app d, 0.6H of anomeric mixture, J = 12.5 Hz, H_{6a}), 3.80 (s, 3H, -NHCH$_2$PhO*Me*), 3.88 (s, 1.2H of anomeric mixture, -NHCH_2PhOMe), 4.06 (app d, 0.6H of anomeric mixture, J = 12.5 Hz, H_{6b}), 4.34 (dd, 0.6H of anomeric mixture, J = 9.5 Hz, J = 3.8 Hz, H_4), 4.44 and 4.62 (ABq, 0.8H of anomeric mixture, J = 11.6 Hz, -OCH_2Ph), 4.64 and

4.86 (ABq, 1.2H of anomeric mixture, J = 11.6 Hz, -OCH_2Ph), 6.58 (brs, alcohol), 6.85-7.36 (m, 9H, -NHCH$_2$*Ph*OMe and -OCH$_2$*Ph*);

^{13}C NMR (CD_3OD, 100 MHz, ca. 3:2 a mixture of anomers) δ 50.8, 54.6, 61.3, 63.7, 70.7, 75.1, 77.5, 95.9, 114.2, 114.3, 114.4, 127.8, 128.2, 128.2, 128.4, 131.2, 131.5, 138.2, 160.6;

HRMS(ESI-MS): Calcd for $[C_{21}H_{26}N_4O_5+H]^+$(M+H): m/z = 415.1976; Found: 415.1986.

5-Azido-3-*O*-benzyl-1,5-dideoxy-4-hydroxy-1-(*N*-(4-methoxybenzyl)-acetaamide)-D-fructopyranose (3-9)



3-9

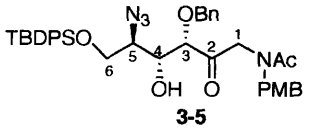
Acetic anhydride (0.160 mL, 2.0 equiv.) was added to a solution of **3-8** (402 mg, 0.847 mmol) and DIPEA (0.237 mL, 2.0 equiv.) in MeOH (20 mL) at 0 °C and the reaction mixture was warmed to room temperature for 2 h. The mixture was stirred for 1 h at room temperature. The solvent was removed under reduced pressure. Flash column chromatography (hexane:ethyl acetate = 2:1 to ethyl acetate) of the crude product gave **3-9** (317 mg, 82%) as a yellow oil (a mixture of anomers and N-rotamers).

^{1}H NMR ($CDCl_3$, 400 MHz) selected peaks δ 2.16 (s, 2.25H of mixtures, acetyl), 2.21 (s, 0.75H of mixtures, acetyl), 2.67 (d, 0.75H of mixtures, J = 14.3 Hz, H_{1a}), 3.35 (d, 0.25H of mixture, J =14.4 Hz, H_{1a}), 3.42 (d, 0.75H of mixtures, J = 9.4 Hz, H_3), 3.55 (d, 0.25H of mixture, J = 14.4 Hz, H_{1b}), 3.69 (app d, 0.75H of mixtures, J = 14.1 Hz, H_{6a}), 3.83 (s, 0.75H of mixtures, -NHCH$_2$PhO*Me*), 3.84 (s, 2.25H of mixture, -NHCH$_2$PhO*Me*), 3.88 (app d, 0. 75H of mixture, J = 3.8 Hz, H_5), 4.12 (d, 0.75H of mixture, J = 14.3 Hz, H_{1b}), 4.20 (app d, 0.75H of mixture, J = 14.1 Hz, H_{6b}), 4.28 (dd, 0.75H of mixtures, J = 9.4 and 3.8 Hz, H_4), 4.37 and 4.63 (ABq, 1.5H of mixtures, J = 11.4 Hz, -NHCH_2PhOMe), 4.70 and 4.82 (ABq, 1.5H of mixtures, J = 11.5 Hz, -OCH_2Ph), 6.87-7.80 (m, 9H, -NHCH$_2$*Ph*OMe and -OCH$_2$*Ph*);

^{13}C NMR (CD_3OD, 100 MHz) δ 21.6, 22.7, 52.4, 54.0, 54.5, 54.6, 54.8, 55.6, 57.2, 61.1, 62.7, 68.6, 70.5, 72.7, 75.1, 76.0, 80.4, 98.6, 99.5, 114.6, 127.6, 127.7, 127.8, 128.1, 128.3, 128.4, 128.4, 128.6, 128.8, 137.0, 137.9, 159.4, 159.5, 175.2, 175.9;

HRMS(ESI-MS): Calcd for $[C_{23}H_{28}N_4O_6+Na](M+Na)]^+$ m/z = 479.1901; Found: 479.1921.

***N*-[(3*R*,4*R*,5*R*)-5-Azido-3-*O*-benzyl-6-*O*-(*tert*-butyldiphenylsilyl)-4-hydroxy-2-oxo-hexyl]-*N*-(4-methoxybenzyl)-acetamide (3-5)**



3-5

TBDPSCl (0.361 mL, 2.0 equiv.) was added to a solution of **3-9** (317 mg, 0. 694 mmol) and imidazole (142 mg, 3.0 equiv.) in CH_2Cl_2 (20 mL) at 0 °C and the reaction mixture was warmed to room temperature for 2 h. The mixture was stirred until no starting material remained (usually for 8 h). The reaction mixture was poured into satd. $NaHCO_3$ (20 mL). The mixture was extracted with CH_2Cl_2 (3 x 20 mL) and the combined organic layers were washed with satd. brine,

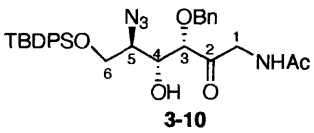
dried over $MgSO_4$ and concentrated *in vacuo*. Flash column chromatography (hexane:ethyl acetate = 4:1 to hexane:ethyl acetate = 2:1) of the crude product gave the desired compound **3-5** (376 mg, 78 %) as a colorless oil (a mixture of N-rotamers).

^{1}H NMR ($CDCl_3$, 400 MHz) selected peaks δ 1.08 (s, 9H, Me_3CPh_2Si), 2.01 (s, 3H, acetyl), 3.49 (d, 1H, J = 18.0 Hz, H_{1a}), 3.63-3.80 (m, 3H, H_5, H_{6a}, and H_{6b}), 3.79 (s, 3H, -$NHCH_2PhOMe$), 4.10 (dd, 1H, J = 8.2 and 2.3 Hz, H_4), 4.12 and 4.33 (ABq, 2H, J = 16.4 Hz, -$NHCH_2PhOMe$), 4.37 and 4.54 (ABq, 2H, J = 18.2 Hz, -OCH_2Ph), 4.51 (d, 1H J = 2.3 Hz, H_3), 4.57 (d, 1H, J = 18.0 Hz, H_{1b}), 6.87 and 7.04 (2*d, 4 H, J = 8.7 Hz, -$NHCH_2PhOMe$), 7.64-7.67 (m, 15H, -OCH_2Ph and Me_3CPh_2Si-);

^{13}C NMR ($CDCl_3$, 100 MHz) δ 19.3, 23.0, 26.9, 48.5, 63.2, 72.0, 74.6, 83.5, 128.0, 128.9, 129.0, 130.1, 132.8, 132.9, 135.8, 136.4, 170.9, 207.7;

HRMS(ESI-MS): Calcd for $[C_{39}H_{46}N_4O_6Si+Na]^+$(M+Na): m/z = 717.3080; Found: 717.3079.

N-[(3R,4R,5R)-5-Azido-3-O-benzyl-6-O-(*tert*-butyldiphenylsilyl)-4-hydroxy-2-oxo-hexyl]acetamide (3-10)



3-10

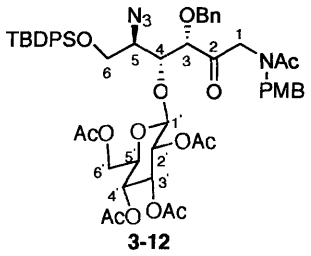
A solution of CAN (185 mg, 3.0 equiv.) in H_2O (1 mL) was added dropwise to a vigorously stirred solution of **3-5** (78 mg, 0.112 mmol) in MeCN (10 mL) at –20 °C and stirred for 3 h at –20 °C. The reaction mixture was treated with additional CAN (61.5 mg, 1.0 equiv.) at –20 °C, warmed within 3 h to 0 °C and stirred for 2 h at 0 °C. The yellow-orange solution was poured into water (20 mL). The mixture was extracted with ethyl acetate (3 x 20 mL) and the combined organic layers were washed with 10 % sodium sulfite solution (30 mL), satd. $NaHCO_3$ (20 mL) and satd. brine (20 mL), dried over $MgSO_4$ and concentrated *in vacuo*. Flash column chromatography (hexane:ethyl acetate = 3:1 to hexane;ethyl acetate = 1:1) of the crude product gave the desired compound **3-10** (41 mg, 63 %) as a colorless oil.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.08 (s, 9H, Me_3CPh_2Si-), 2.01 (s, 3H, acetyl), 3.14 (brs, alcohol), 3.54-3.59 (m, 1H, H_5), 3.74-3.76 (m, 1H, H_4), 3.97 (ABx, 1H, J = 6.6 and 10.9 Hz, H_{6a}), 4.03-4.10 (m, 2H, H_{6a} and H_{1b}), 4.22 (d, 1H, J = 1.3 Hz, H_3), 4.32 (ABx, 1H, J = 4.7 and 19.9 Hz, H_{1b}), 4.57 and 4.75 (ABq, 2H, J =11.3 Hz, -OCH_2Ph), 6.15 (brs, amide), 7.27-7.69 (m, 15H, -OCH_2Ph and Me_3CPh_2Si-);

^{13}C NMR ($CDCl_3$, 100 MHz) δ 19.3, 23.0, 26.9, 48.5, 63.2, 72.0, 74.6, 83.5, 128.0, 128.9, 129.0, 130.1, 132.8, 132.9, 135.8, 136.4, 170.9, 207.7;

HRMS(ESI-MS): Calcd for $[C_{31}H_{38}N_4O_5Si+H]^+$(M+H): m/z = 575.2684; Found: 575.2684.

N-(4-Methoxybenzyl)-[(3R,4R,5R)-5-azido-3-O-benzyl-6-O-(tert-butyldiphenylsilyl)-4-O-(2',3',4',6'-tetra-O-acetyl-β-D-glucopyranosyl)-2-oxo-hexyl]acetamide (3-12)



3-12

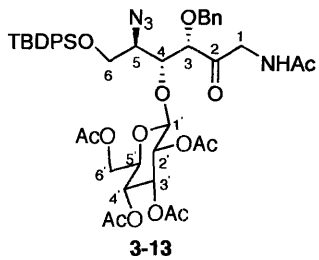
BF_3•OEt_2 (6.1 µl, 0.1 equiv.) was added to a solution of imidate **2-28** (1.19 mg, 5.0 equiv.) and **3-5** (335 mg, 0.482 mmol) in toluene (30 mL) at 0 °C under argon. The mixture was allowed to warm to room temperature. After stirring for 3 h, additional BF_3•OEt_2 (6.1 µl, 0.1 equiv.) was added at 0 °C. Then the mixture was allowed to warm to room temperature again and stirred until no starting material remained (usually for another 5 h at room temperature). The reaction mixture was poured into water (20 mL). The mixture was extracted with CH_2Cl_2 (3 x 40 mL) and the combined organic layers were washed with satd. $NaHCO_3$ and satd. brine, dried over $MgSO_4$ and concentrated *in vacuo*. Flash column chromatography (hexane:ethyl acetate = 3:1 to hexane;ethyl acetate = 2:3) of the crude oil gave the desired compound **3-12** (445 mg, 85 %) as a colorless liquid (a mixture of N-rotamers).

^{1}H NMR (C_6D_6, 400 MHz) selected peaks δ 1.20 (s, 9H, Me_3CPh_2Si-), 1.48 (s, 3H, acetyl), 1.66 (s, 3H, acetyl), 1.85 (s, 3H, acetyl), 1.91 (s, 3H, acetyl), 1.97 (s, 3H, acetyl), 3.04 (s, 3H, -NCH_2PhOMe), 3.96-4.00 (m, 1H, $H_{5'}$), 4.26 (d, 1H, J = 3.0 Hz, H_3), 4.69 (d, 1H, J = 8.1 Hz, $H_{1'}$), 5.01 (app t, 1H, J = 8.9 Hz, $H_{2'}$), 4.83-4.89 (m, 2H, $H_{3'}$ and $H_{4'}$), 6.88-8.57 (m, 19H, -$NHCH_2PhOMe$, -OCH_2Ph and Me_3CPh_2Si-);

^{13}C NMR (C_6D_6, 100 MHz) δ 19.8, 20.4, 20.5, 20.6, 20.9, 21.4, 27.4, 52.8, 53.7, 55.2, 62.4, 63.3, 64.1, 69.2, 72.2, 72.9, 73.8, 74.1, 72.0, 77.0, 84.7, 100.3, 115.2, 128.1, 129.1, 130.6, 130.8, 130.9, 133.2, 133.6, 136.5, 137.9, 160.0, 168.8, 169.6, 170.3, 170.5, 171.1, 206.5;

HRMS(ESI-MS): Calcd for $[C_{53}H_{64}N_4O_{15}Si+H]^+$(M+Na): m/z = 1047.4030; Found: 1047.4007.

N-[(3R,4R,5R)-5-azido-3-O-benzyl-6-O-(tert-butyldiphenylsily)-4-O-(2',3',4',6'-tetra-O-acetyl-β-D-glucopyranosyl)-2-oxo-hexyl]acetamide 3-13



3-13

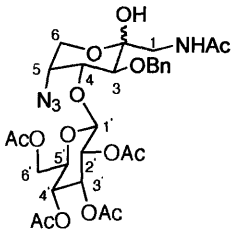
A solution of CAN (1.06 g, 3.0 equiv.) in H_2O (2 mL) was added dropwise to a vigorously stirred solution of **3-12** (660 mg, 0.654 mmol) in MeCN (20 mL) at –20 °C and stirred for 3 h. The reaction mixture was treated with additional CAN (353 mg, 1.0 equiv.), warmed within 3 h to 0 °C and stirred for 2 h at 0 °C. The yellow-orange solution was poured into water. The mixture was extracted with ethyl acetate (3 x 30 mL) and the combined organic layers were washed with 10% sodium sulfite solution, satd. $NaHCO_3$ and satd. brine, dried over $MgSO_4$ and concentrated *in vacuo*. Flash column chromatography (hexane:ethyl acetate = 1:1 to hexane;ethyl acetate = 3:1) of the crude oil gave the desired compound **3-13** as the white liquid (431 mg, 83%).

^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.07 (s, 9H, Me_3CPh_2Si-), 1.75 (s, 3H, acetyl), 1.99 (s, 3H, acetyl), 2.01 (s, 3H, acetyl), 2.09 (s, 3H, acetyl), 2.22 (s, 3H, acetyl), 3.44-3.48 (m, 1H, H_5), 3.60-3.63 (m, 1H, $H_{5'}$), 3.80 (ABx, 1H, J = 12.2 and 1.7 Hz, H_{6a}), 3.94-3.96 (m, 2H, H_{1a} and H_{1b}), 4.05 (dd, 1H, J = 1.6 and 9.3 Hz, H_4), 4.16 (d, 1H, J = 1.6 Hz, H_3), 4.18 (ABx, 1H, J = 3.4 and 20.8 Hz, $H_{6'a}$), 4.41 (ABx, 1H, J = 12.2 and 7.2 Hz, H_{6b}), 4.43 and 4.75 (ABq, 2H, J = 11.6 Hz, -OCH_2Ph), 4.60 (ABx, 1H, J = 6.2 and 20.8 Hz, $H_{6'b}$), 4.61 (d, 1H, J = 8.1 Hz, $H_{1'}$), 4.83-4.89 (m, 2H, $H_{2'}$ and $H_{4'}$), 4.98 (app t, 1H, J = 9.3 Hz, $H_{3'}$), 6.40 (brs, 1H amide), 7.27-7.69 (m, 15H, -OCH_2Ph and Me_3CPh_2Si-);

^{13}C NMR ($CDCl_3$, 100 MHz) δ 19.7, 20.8, 21.0, 21.0, 21.4, 23.3, 49.3, 61.8, 62.4, 62.7, 68.7, 71.8, 72.4, 73.3, 74.6, 77.7, 83.3, 100.3, 128.5, 128.6, 128.9, 129.0, 129.2, 130.6, 130.7, 132.4, 133.1, 135.9, 136.2, 136.7, 169.1, 169.9, 170.6, 170.9, 171.8, 207.9;

HRMS(ESI-MS): Calcd for $[C_{45}H_{56}N_4O_{14}Si+H]^+$(M+H): m/z = 905.3635; Found: 905.3629.

1-Acetaamide-4-*O*-(2',3',4',6'-tetra-*O*-acetyl-β-D-glucopyranosyl)-5-azido-3-*O*-benzyl-1,5-dideoxy--D-fructopyranose (3-15)



3-15

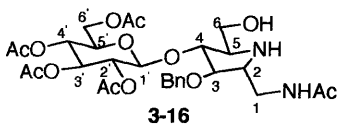
TBAF (1.0 M solution in THF, 0.477 mL, 1.0 equiv.) was added to a solution of **3-14** (431 mg, 0.477 mmol) and acetic acid (0.028 mL, 1.0 equiv.) in THF (10 mL) at 0 °C. The mixture was allowed to warm to room temperature and stirred overnight. The solvent was removed under reduced pressure, followed by the purification *via* flash column chromatography (hexane:ethyl acetate = 4:1 to ethyl acetate following ethyl acetate:methanol = 10:1) to afford **3-15** (286 mg, 90%) as a white oil (a mixtue of anomers).

^{1}H NMR (CD_3OD, 400 MHz) δ 1.93 (s, 3H, acetyl), 1.97 (s, 3H, acetyl), 1.98 (s, 3H, acetyl), 2.00 (s, 3H, acetyl), 2.05 (s, 3H, acetyl), 3.09 (d, 1 H, J = 13.7 Hz, H_{1a}), 3.58 (d, 1H, J = 9.7 Hz, H_3), 3.70-3.78 (m, 1H, H_{6a}), 3.75 (d, 1H, J = 13.7 Hz, H_{1b}), 3.86-3.90 (m, 1H, $H_{5'}$), 4.01 (m, 3H, H_{6b}, $H_{6'a}$ and $H_{6'b}$), 4.32 (app dd, 1H, J = 4.2 and 9.7 Hz, H_5), 4.41 (dd, 1H, J = 9.7 and 3.7 Hz, H_4), 4.60 and 4.94 (ABq, 1H, J = 10.3 Hz, -OCH_2Ph), 4.83-4.89 (m, 2H, $H_{1'}$ and $H_{2'}$), 5.28 (app t, 1H, J = 9.3 Hz, $H_{3'}$), 7.27-7.69 (m, 5H, -OCH_2Ph);

^{13}C NMR (CD_3OD, 100 MHz) δ 19.6, 19.7, 19.8, 19.9, 21.6, 44.8, 59.7, 60.9, 62.1, 68.7, 72.0, 72.1, 73.4, 74.9, 75.3, 77.7, 97.9, 98.1, 127.7, 128.2, 129.0, 139.0, 170.3, 170.4, 170.7, 171.4, 172.6;

HRMS(ESI-MS): Calcd for $[C_{29}H_{38}N_4O_{14}+Na]^+$(M+Na): m/z = 689.2277; Found: 689.2285.

***N*-[((2*R*,3*R*,4*R*,5*R*)-3-*O*-benzyl-4-*O*-(2',3',4',6'-tetra-*O*-acetyl-β-D-glucopyranosyl)-5-(hydroxymethyl)pyrrolidin-2-yl)methyl]acetamide (3-16)**



3-16

To a solution of **3-15** (70 mg, 0.105 mmol) in MeOH (10 mL) was added $Pd(OH)_2$ (35mg). The reaction mixture was stirred under 850 psi hydrogen pressure for 12 h and

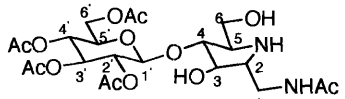
filtered through celite. After the solvent was removed *in vacuo*, the residue was purified by flash column chromatography ($CHCl_3$:MeOH = 10:1 (1 % of ammonium hydroxide) to 8:1 (1 % of ammonium hydroxide)) to afford **3-16** (51 mg, 70%) as a colorless oil.

^{1}H NMR (CD_3OD, 600 MHz) δ 1.93 (s, 3H, acetyl), 1.96 (s, 3H, acetyl), 1.97 (s, 3H, acetyl), 2.00 (s, 3H, acetyl), 2.04 (s, 3H, acetyl), 3.08-3.15 (m, 1H, H_5), 3.28-3.30 (m, 3H, H_2, H_{1a} and H_{1b}), 3.57-3.59 (m, 2H, H_{6a} and H_{6b}), 3.88-3.89 (m, 1H, H_3), 4.11 (ABx, 1H, J = 2.2 and 12.3 Hz, $H_{6'a}$), 4.22 (ABx, 1H, J = 4.4 and 12.3 Hz, $H_{6'b}$), 4.23 (m, 1H, H_4), 4.57 and 4.73 (ABq, 2H, J = 11.7 Hz, -OCH_2Ph), 4.88 (d, 1H, J = 8.0 Hz, $H_{1'}$), 4.90 (dd, 1H, J = 8.0 and 9.5 Hz, $H_{2'}$), 5.02 (app t, 1 H, J = 9.5 Hz, $H_{4'}$), 5.25 (app t, 1H, J = 9.5 Hz, $H_{3'}$), 7.25-7.36 (m, 5H, -OCH$_2$*Ph*);

^{13}C NMR (CD_3OD, 100 MHz) δ 20.7, 20.8, 22.8, 42.8, 62.9, 63.0, 63.1, 65.8, 69.8, 72.8, 73.0, 73.1, 74.4, 87.3, 87.8, 101.7, 128.9, 129.1, 129.5, 139.8, 171.3, 171.4, 171.7, 172.4, 173.9;

HRMS(ESI-MS): Calcd for $[C_{29}H_{40}N_2O_{13}+H]^+$(M+H): m/z = 625.2603; Found: 625.2580.

N-{[(2*R*,3*R*,4*R*,5*R*)-4-*O*-(2',3',4',6'-tetra-*O*-acetyl-β-D-glucopyranosyl)-3-hydroxy-)-5-(hydroxymethyl)pyrrolidin-2-yl]methy]}acetamide (3-17)



3-17

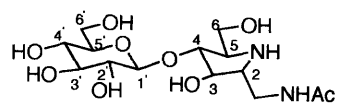
To a solution of **3-16** (30 mg, 0.048 mmol) in acetic acid (5 mL) was added $Pd(OH)_2$ (20 mg). The reaction mixture was stirred under hydrogen at atmosphere pressure for 12 h and filtered through celite. After the solvent was removed *in vacuo*, the residue was purified by column chromatography ($CHCl_3$:MeOH = 7:1 (1 % of ammonium hydroxide) to 5:1 (1 % of ammonium hydroxide)) to give **3-17** (17 mg, 65%) as a colorless oil.

^{1}H NMR (CD_3OD, 400 MHz) δ 1.92 (s, 3H, acetyl), 1.93 (s, 3H, acetyl), 1.98 (s, 3H, acetyl), 2.00 (s, 3H, acetyl), 2.03 (s, 3H, acetyl), 3.08-3.10 (m, 2H, H_2 and H_5), 3.55-3.57 (m, 2H, H_{6a} and H_{6b}), 3.89-3.91 (m, 3H, $H_{5'}$, $H_{6'a}$ and $H_{6'b}$), 4.20-4.21 (m, 2H, H_4 and H_3), 4.75 (d, 1H, J = 8.1 Hz, $H_{1'}$), 4.98 (app t, 1H, J = 9.6 Hz, $H_{3'}$), 5.23 (app t, 1H, J = 9.6 Hz, $H_{4'}$);

^{13}C NMR (CD_3OD, 100 MHz) δ 20.7 20.8, 22.7, 42.9, 63.3, 63.4, 63.7, 64.2, 70.0, 73.0, 73.1, 74.3, 80.1, 89.3, 101.7, 171.3, 171.4, 171.7, 172.5, 174.0;

HRMS(ESI-MS): Calcd for $[C_{22}H_{33}N_2O_{13}+H]^+$(M+H): m/z = 535.2134; Found: 535.2126.

N-{[(2*R*,3*R*,4*R*,5*R*)-3-hydoxy-4-*O*-(β-D-glucopyranosyl))-5-(hydroxymethyl)pyrrolidin-2-yl]methyl}acetamide (3-1)



3-1

To a stirred solution of **3-17** (8.0 mg, 0.0150 mmol) in MeOH at room temperature was added the catalytic amount of NaOMe. After 8h, cation exchange resin was added until the solution was neutralized. The resin was

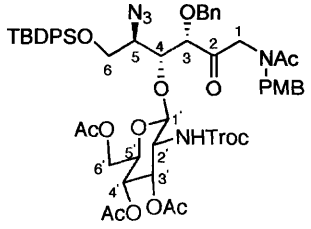
filtered and washed with MeOH. Concentration of the solvent gave the desired product **1** (5.5 mg, quant) as a white liquid.

^{1}H NMR (D_2O, 500 MHz) δ 1.98 (s, 3H, acetyl), 3.11-3.12 (m, 1H, H_5), 3.19-3.20 (m, 1H, H_2), 3.26-3.33 (m, 2H, H_{1a} and $H_{2'}$), 3.34-3.40 (m, 2H, H_{1b} and $H_{3'}$), 3.44-3.49 (m, 2H, $H_{4'}$ and $H_{5'}$), 3.64-3.69 (m, 3H, H_{6a}, H_{6b} and $H_{6'a}$), 3.90-3.95 (m, 3H, H_3, H_4 and $H_{6'b}$), 4.48 (d, 1H, J = 8.0 Hz, $H_{1'}$);

^{13}C HMR (D_2O, 75 MHz) δ22.10, 22.17, 40.99, 61.04, 61.15, 61.31, 61.91, 70.14, 73.33, 75.83, 76.21, 78.30, 86.78, 102.54, 175.12;

HRMS(ESI-MS): Calcd for $[C_{14}H_{26}N_2O_9+H]^+$(M+H): m/z = 367.1711; Found: 367.1718.

_N_-(4-Methoxybenzyl)-[(3_R_,4_R_,5R)-5-azido-3-_O_-benzyl-6-_O_-(_tert_-butyldiphenylsilyl)-4-_O_-(3',4',6'-tri-_O_-acetyl-2'-deoxy-2'-trichloroethyloxycarbonylamine-β-D-glucopyranosyl)-2-oxo-hexyl]acetamide (3-19)

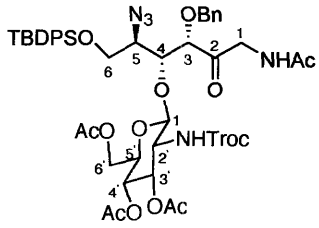


3-19

BF_3•OEt_2 (11.7 μl, 0.2 equiv.) was added to a solution of imidate **3-18** (863 mg, 3.0 equiv.) and **3-5** (320 mg, 0.46 mmol) in toluene (20 mL) at 0 °C under argon. The mixture was allowed to warm to room temperature and stirred for 8 h. The reaction mixture was poured to water (40 mL). The mixture was extracted with CH_2Cl_2 (3 x 30 mL) and the combined organic layers were washed with satd. $NaHCO_3$ and satd. brine, dried over $MgSO_4$ and concentrated *in vacuo*. Flash column chromatography (hexane:ethyl acetate = 3:1 to hexane;ethyl acetate = 1:1) of the crude product gave the desired compound **3-19** (351 mg, 69 %) as a colorless oil (a mixture of N-rotamers).

^{1}H NMR (C_6D_6, 400 MHz) selected peaks δ 1.20 (s, 9H, Me_3CPh_2Si-), 1.66 (s, 3H, acetyl), 1.67 (s, 3H, acetyl), 1.69 (s, 3H, acetyl), 2.05 (s, 3H, acetyl), 3.35 (s, 3H, -NCH_2PhOMe), 3.50-3.65 (m, 2H, H_5 and $H_{5'}$), 5.10 (app t, 1H, J = 9.3 Hz, $H_{4'}$), 5.21 (app t, 1H, J = 9.3 Hz, $H_{3'}$), 6.82-7.87 (m, 19H, -$NHCH_2Ph$OMe, -OCH_2Ph and Me_3CPh_2Si-);

_N_-{(3_R_,4_R_,5R)-5-azido-3-_O_-benzyl-6-_O_-(_tert_-butyldiphenylsilyl)-2'-deoxy-4-_O_-[3',4',6'-tri-_O_-acetyl-2'-trichloroethyloxycarbonylamino-β-D-glucopyranosyl]-2-oxo-hexyl}acetamide (3-20)



3-20

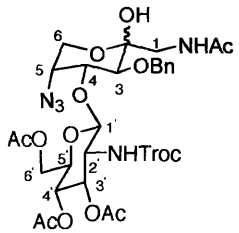
A solution of CAN (665 mg, 3.0 equiv.) in H_2O (3 mL) was added dropwise to a vigorously stirred solution of **3-19** (351 mg, 0.30 mmol) in MeCN (30 mL) at – 20 °C and stirred for 3 h. The reaction mixture was treated with additional CAN (222 mg, 1.0 equiv.), warmed within 3 h to 0 °C and stirred for 2 h at 0 °C. The yellow-orange solution was poured into water. The mixture was extracted with ethyl acetate (3 x 40 mL) and

the combined organic layers were washed with 10 % sodium sulfite solution, satd. $NaHCO_3$ and satd. brine, dried over $MgSO_4$ and concentrated *in vacuo*. Flash column chromatography (hexane:ethyl acetate = 2:1 to hexane;ethyl acetate = 1:2) of the crude product gave the desired compound **3-20** as a white liquid (204 mg, 65 %).

^{1}H NMR (CD_3OD, 400 MHz) δ 1.09 (s, 9H, Me_3CPh_2Si-), 1.96 (s, 3H, acetyl), 1.99 (s, 3H, acetyl), 2.02 (s, 3H, acetyl), 2.08 (s, 3H, acetyl), 3.33-3.42 (m, 1H, H_5), 3.52-3.63 (m, 1H, $H_{5'}$), 3.83-4.12 (m, 4H, H_{6a}, H_{6b}, H_4, $H_{2'}$), 4.23-4.35 (m, 2H, $H_{6'a}$ and $H_{6'b}$) 4.37 (d, 1H, J = 2.0 Hz, H_3), 4.40-4.43 (m, 2H, H_{1a} and H_{1b}), 4.37 and 4.52 (ABq, 2H, J = 10.5 Hz, -OCH_2CCl_3), 4.39 and 4.64 (ABq, 2H, J = 11.0 Hz, -OCH_2Ph), 4.87 (d, 1H, J = 8.3 Hz, $H_{1'}$), 4.77 (app t, 1H, J = 9.2 Hz, $H_{4'}$), 5.27 (app t, 1H, J = 9.1 Hz, $H_{3'}$), 7.69-7.76 (m, 15H, -OCH_2Ph and Me_3CPh_2Si-);

^{13}C NMR ($CDCl_3$, 100 MHz) δ 19.4, 20.8, 21.1, 21.2, 23.1, 27.2, 48.9, 55.1, 62.4, 63.1, 69.5, 72.0, 72.4, 74.6, 76.8, 78.9, 82.5, 89.0, 99.6, 128.1, 128.4, 128.5, 129.1, 130.5, 131.6, 132.8, 134.9, 136.5, 136.8, 136.9, 156.5, 169.5, 170.2, 171.4, 171.9, 206.9;

1-Acetamino-5-azido-3-*O*-benzyl-1,5-dideoxy-4-*O*-[3',4',6'-tri-*O*-acetyl-2'-deoxy-2'-trichloroethyloxycarbonylamino-β-D-glucopyranosyl]-D-fructopyranose (3-21)



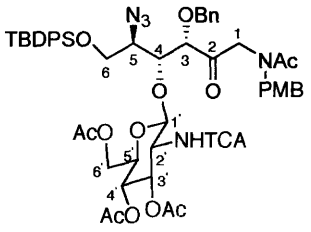
3-21

TBAF (1.0 M solution in THF, 0.130 mL, 1.0 eq.) was added to a solution of **3-20** (135 mg, 0.130 mmol) and acetic acid (8 μl, 1.0 eq.) in THF (10 mL) at 0 °C. The mixture was allowed to warm to room temperature and stirred overnight. The solvent was removed under reduced pressure, followed by the purification *via* flash column chromatography (hexane:ethyl acetate = 2:1 to ethyl acetate, ethyl acetate:MeOH = 10:1) to afford **3-21** (86 mg, 83 %) as a white oil.

^{1}H NMR (CD_3OD, 400 MHz) δ 2.00 (s, 3H, acetyl), 2.01 (s, 3H, acetyl), 2.02 (s, 3H, acetyl), 2.04 (s, 3H, acetyl), 3.05 (d, 1H, J = 12.8 Hz, H_{1a}), 3.72 (d, 1H, J = 9.1 Hz, H_3), 3.83 (dd, 1H, J = 8.3 and 10.4 Hz, $H_{2'}$), 3.68-3.83 (m, 5H, H_{1b}, H_3, H_5, $H_{6'a}$, $H_{6'b}$), 4.31 (app dd, 1H, J = 10.8 and 4.3 Hz, H_5), 4.44 (dd, 1H, J = 9.0 and 4.3 Hz, H_4), 4.60-4.90 (m, 4H, -OCH_2Ph and -OCH_2CCl_3), 4.91 (d, 1H, J = 8.3 Hz, $H_{1'}$), 5.08 (app t, 1H, J = 9.4 Hz, $H_{4'}$), 5.27 (app t, 1H, J = 9.3 Hz, $H_{3'}$), 7.29-7.54 (m, 5H, -OCH_2Ph);

^{13}C NMR (CD_3OD, 100 MHz) δ 20.5, 20.7, 20.9, 22.8, 45.9, 56.4, 60.3, 61.7, 70.1, 73.2, 73.4, 76.0, 76.5, 78.9, 98.7, 99.0, 128.8, 129.3, 129.7, 129.8, 130.1, 140.1, 171.4, 171.8, 172.5, 173.8;

***N*-(4-Methoxybenzyl)-{(3*R*,4*R*,5R)-5-azido-3-*O*-benzyl-6-*O*-(*tert*-butyldiphenylsilyl)-4-*O*-[3',4',6'-tri-*O*-acetyl-2'-deoxy-2'-trichloroacetamide-β-D-glucopyranosyl]-2-oxo-hexyl}acetamide (3-22)**

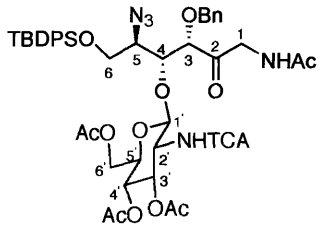


3-22

BF_3•OEt_2 (42.1 µl, 0.3 equiv.) was added to a solution of imidate **2-20** (3.3 g, 4.0 equiv.) and **3-5** (720 mg, 1.11 mmol) in toluene (20 mL) at 0 °C under argon. The mixture was allowed to warm to room temperature and stirred for 24 h. The reaction mixture was poured into water (40 mL). The mixture was extracted with CH_2Cl_2 (3 x 45 mL) and the combined organic layers were washed with satd. $NaHCO_3$ and satd. brine, dried over $MgSO_4$ and concentrated *in vacuo*. Flash column chromatography (hexane:ethyl acetate = 3:1 to hexane;ethyl acetate = 1:1) of the crude product gave the desired compound **3-22** (1.06 g, 85 %) as a colorless oil (a mixture of N-rotamers).

^{1}H NMR (C_6D_6, 400 MHz) selected peaks δ 1.18 (s, 9H, Me_3CPh_2Si-), 1.63 (s, 3H, acetyl), 1.73 (s, 3H, acetyl), 1.74 (s, 3H, acetyl), 1.90 (s, 3H, acetyl), 3.34 (s, 3H, -NCH_2PhOMe), 3.45-3.50 (m, 1H, H_5), 3.80 (d, 1H, J = 13.2 Hz, H_{1a}), 4.32 and 4.42 (ABq, 2H, J = 11.3 Hz, -OCH_2Ph), 4.61 (dd, 1H, J =1.3 and 8.4 Hz, H_4), 4.86 (d, 1H, J = 13.2 Hz, H_{1b}), 4.96 (d, 1H, J = 8.3 Hz, $H_{1'}$), 5.26 (app t, 1H, J = 9.3 Hz, $H_{4'}$), 5.52 (app t, 1H, J = 9.3 Hz, $H_{3'}$), 6.80 and 6.91 (d, 4H, J = 8.6 Hz, -$NHCH_2PhOMe$), 7.08-7.87 (m, 15H, -OCH_2Ph and Me_3CPh_2Si-);

***N*-{(3*R*,4*R*,5R)-5-azido-3-*O*-benzyl-6-*O*-(*tert*-butyldiphenylsilyl)-4-*O*-[3',4',6'-tri-*O*-acetyl-2'-deoxy-2'-trichloroacetamide-β-D-glucopyranosyl]-2-oxo-hexyl}acetamide (3-23)**



3-23

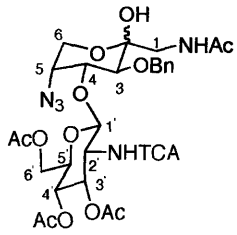
A solution of CAN (1.55 g, 3.0 equiv.) in H_2O (5 mL) was added dropwise to a vigorously stirred solution of **3-22** (1.06g, 0.94 mmol) in MeCN (50 mL) at – 20 °C and stirred for 3 h. The reaction mixture was treated with additional CAN (517 mg, 1.0 equiv.), warmed within 3 h to 0 °C and stirred for 2 h at 0 °C. The yellow-orange solution was poured into water. The mixture was extracted with ethyl acetate (3 x 50 mL) and the combined organic layers were washed with 10 % sodium sulfite solution, satd. $NaHCO_3$ and satd. brine, dried over $MgSO_4$ and concentrated *in vacuo*. Flash column chromatography (hexane:ethyl acetate = 1:1 to hexane;ethyl acetate = 1:2) of the crude oil gave the desired compound **3-23** as the white liquid (634 mg, 67 %).

^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.07 (s, 9H, Me_3CPh_2Si-), 1.97 (s, 3H, acetyl), 2.01 (s, 3H, acetyl), 2.05 (s, 3H, acetyl), 2.13 (s, 3H, acetyl), 3.45-3.56 (m, 1H, H_5), 3.57-3.58 (m, 1H, $H_{5'}$), 3.73-3.81 (m, 3H, H_3, H_{6a} and $H_{2'}$), 3.99 (dd, 1H, J = 11.3 and 2.2 Hz, H_4), 4.07 (d, 1H, J = 2.2 Hz, H_3), 4.10-4.39 (m, 4H, $H_{6'a}$, $H_{6'b}$, H_{1a} and H_{1b}), 4.35 and 4.63 (ABq, 2H, J = 11.0 Hz, -OCH_2Ph), 4.51 (d, 1H, J = 8.3 Hz, $H_{1'}$), 4.82 (app t, 1H, J = 9.3 Hz, $H_{4'}$), 5.02 (app t, 1H, J = 9.3 Hz, $H_{3'}$), 6.32 (brs, 1H, amide), 6.34 (brs, 1H, amide), 7.21-7.66 (m, 15H, -OCH_2Ph and Me_3CPh_2Si-);

^{13}C NMR ($CDCl_3$, 125MHz) δ 19.3, 20.7, 21.1, 23.1, 27.1, 48.8, 56.1, 62.2, 63.1, 62.2, 63.1, 68.7, 71.5, 72.1, 74.2, 76.8, 83.3, 99.6, 128.2, 128.4, 128.4, 128.8, 130.5, 130 .8, 132.5, 135.9, 136.0, 136.5, 161.8, 169.6, 170.6, 170.7, 171.4, 207.2;

HRMS(ESI-MS): Calcd for $[C_{45}H_{54}Cl_3N_5O_{13}Si+Na]^+$(M+Na): *m/z* = 1030.2445; Found: 1030.2427.

1-Acetamino-5-azido-3-*O*-benzyl-1,5-dideoxy-4-*O*-[3',4',6'-tri-*O*-acetyl-2'-deoxy-2'-(trichloroacetamide)-β-D-glucopyranosyl)]-D-fructopyranose (3-24)



3-24

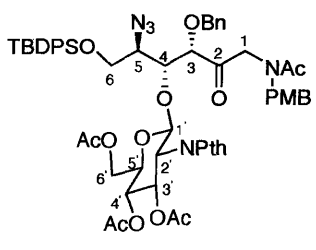
TBAF (1.0 M solution in THF, 0.630 mL, 1.0 equiv.) was added to a solution of **3-23** (634 mg, 0.630 mmol) and acetic acid (0.036 mL, 1.0 equiv.) in THF (10 mL) at 0 °C. The mixture was allowed to warm to room temperature and stirred overnight. The solvent was removed under reduced pressure, followed by the purification *via* column chromatography (hexane: ethyl acetate = 1:1 to ethyl acetate, ethyl acetate:methanol = 10:1) to afford **3-24** (412 mg, 85 %) as a white oil.

^{1}H NMR (CD_3OD, 400 MHz) δ 1.84 (s, 3H, acetyl), 1.90 (s, 3H, acetyl), 1.91 (s, 3H, acetyl), 1.92 (s, 3H, acetyl), 1.99 (s, 3H, acetyl), 3.06 (d, 1H, *J* = 12.8 Hz, H_{1a}), 3.54 (d, 1H, *J* = 9.1 Hz, H_3), 3.67 (d, 1H, *J* = 12.8 Hz, H_{1b}), 3.79-3.82 (m, 2H, H_{6a} and $H_{5'}$), 3.88-3.89 (m, 1H, H_{6b}), 3.94 (dd, 1H, *J* = 8.1 and 10.5 Hz, $H_{2'}$), 4.04-4.07 (m, 2H, $H_{6'a}$ and $H_{6'b}$), 4.32 (app dd, 1H, *J* = 12.4 and 4.1 Hz, H_5), 4.38 (dd, 1H, *J* = 9.1 and 4.1 Hz, H_4), 4.61 and 4.96 (ABq, 2H, *J* = 10.3 Hz, -O*CH*$_2$Ph), 4.97 (d, 1H, *J* = 8.4 Hz, $H_{1'}$), 5.04 (app t, 1H, *J* = 9.3 Hz, $H_{4'}$), 5.44 (dd, 1H, *J* = 10.5 and 9.3 Hz, $H_{3'}$), 7.24-7.48 (m, 5H, -OCH$_2$*Ph*);

^{13}CNMR (CD_3OD, 125 MHz) δ 20.8, 20.9, 20.9, 22.8, 45.9, 57.7, 60.1, 61.6, 70.3 , 73.2, 73.3, 76.1, 76.4, 78.8, 99.0, 99.1, 128.8, 129.3, 129.7, 130.1, 140. 1, 164. 4, 171.4 , 171.8, 172. 5, 173.7;

HRMS: [Calcd for $C_{29}H_{35}Cl_3N_5O_{13}+H]^+$(M+H): *m/z* = 790.12672; Found: 720.1233.

***N*-(4-Methoxybenzyl)-{(3*R*,4*R*,5R)-5-azido-3-*O*-benzyl-6-*O*-(*tert*-butyldiphenylsilyl)-4-*O*-[3',4',6'-tri-*O*-acetyl-2'-deoxy-2'-phthalimide-β-D-glucopyranosyl]-2-oxo-hexyl}acetamide (3-25)**



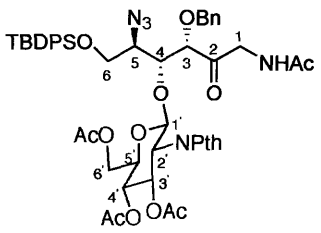
3-25

BF_3•OEt_2 (8.9 μl, 0.2 equiv.) was added to a solution of imidate **2-21** (609 mg, 3.0 equiv.) and **3-5** (214 mg, 0.35 mmol) in toluene (20 mL) at 0 °C under argon. The mixture was allowed to warm to room temperature and stirred for 8h. The reaction mixture was poured into water (40 mL). The mixture was extracted with CH_2Cl_2 (3 x 45 mL) and the combined organic layers were washed with satd. $NaHCO_3$ and satd. brine, dried over $MgSO_4$ and concentrated *in vacuo*. Flash column

chromatography (hexane:ethyl acetate = 4:1 to hexane;ethyl acetate = 2:1) of the crude oil gave the desired compound **3-25** (282 mg, 73%) as a colorless oil (a mixture of N-rotamers).

^{1}H NMR (C_6D_6, 400 MHz) selected peaks δ 1.23 (s, 9H, Me_3CPh_2Si), 1.58 (s, 3H, acetyl), 1.70 (s, 3H, acetyl), 1.93 (s, 3H, acetyl), 1.95 (s, 3H, acetyl), 3.50 (s, 3H, -NH_2CH_2PhOMe), 5.32 (app t, 1H, J = 9.3 Hz, $H_{4'}$), 5.72 (d, 1H, J = 8.2 Hz, $H_{1'}$), 5.63 (app t, 1H, J = 9.8 Hz, $H_{3'}$), 6.89 and 6.97 (d, 4H, J =8.7 Hz, -$NHCH_2PhOMe$), 7.71-8.15 (m, 15H, -OCH_2Ph, Me_3CPh_2Si-);

_N_-{(3_R_,4_R_,5R)-5-azido-3-_O_-benzyl-6-_O_-(_tert_-butyldiphenylsilyl)-4-_O_-[3',4',6'-tri-_O_-acetyl-2'-deoxy-2'-phthalimide-β-D-glucopyranosyl]-2-oxo-hexyl}acetamide (3-26)

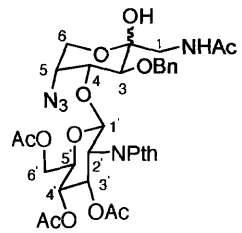


3-26

A solution of CAN (0.428 mg, 3.0 equiv.) in H_2O (2 mL) was added dropwise to a vigorously stirred solution of **3-25** (290 mg, 0.26 mmol) compound in MeCN (20 mL) at – 20 ^{0}C and stirred for 3 h. The reaction mixture was treated with additional CAN (142 mg, 1.0 equiv.), warmed within 3 h to 0 °C and stirred 2 h at 0 °C. The yellow-orange solution was poured into water. The mixture was extracted with ethyl acetate (3 x 30 mL) and the combined organic layers were washed with 10 % sodium sulfite solution, satd. $NaHCO_3$ and satd. brine, dried over $MgSO_4$ and concentrated *in vacuo*. Flash column chromatography (hexane:ethyl acetate = 2:1 to hexane;ethyl acetate = 1:1) of the crude oil gave the desired compound **3-26** as a white liquid (160 mg, 62 %).

^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.95 (s, 9H, Me_3CPh_2Si-), 1.84 (s, 3H, acetyl), 1.96 (s, 3H, acetyl), 2.04 (s, 3H, acetyl), 2.14 (s, 3H, acetyl), 3.55-3.57 (m, 2H, H_5 and $H_{5'}$), 3.73-3.81 (m, 2H, H_3, H_{6a} and $H_{2'}$), 3.99 (dd, 1H, J = 11.3 and 1.9 Hz, H_4), 4.07 (d, 1H, J = 2.2 Hz, H_3), 4.10-4.39 (m, 4H, $H_{6'a}$, $H_{6'b}$, H_{1a} and H_{1b}), 4.18 and 4.50 (ABq, 2H, J = 12.0 Hz, -OCH_2Ph), 4.99 (app t, 1H, J = 9.2 Hz, $H_{4'}$), 5.34 (d, 1H, J = 8.0 Hz, $H_{1'}$), 5.78 (app t, 1H, J = 9.2 Hz, $H_{3'}$), 6.25 (brs, 1H, amide), 7.09-7.76 (m, 19H, -OCH_2Ph, -NPth and Me_3CPh_2Si-);

1-Acetamino-5-azido-3-_O_-benzyl-1,5-dideoxy-4-O-[3',4',6'-tri-O-acetyl-2'-deoxy-2'-phthalimide-β-D-glucopyranosyl]-D-fructopyranose (3-27)



3-27

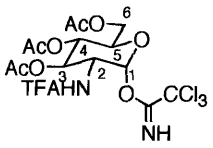
TBAF (1.0 M solution in THF, 0.160 mL, 1.0 equiv.) was added to a solution of **3-26** (160 mg, 0.161 mmol) and acetic acid (9.2 μl, 1.0 equiv.) in THF (10 mL) at 0 °C. The mixture was allowed to warm to room temperature and stirred overnight. The solvent was removed under reduced pressure, followed by the purification *via* column chromatography (hexane: ethyl acetate = 1:1 to ethyl acetate, following ethyl acetate:MeOH = 10:1) to afford **3-27** (105 mg, 87%) as a colorless oil.

^{1}H NMR (CD_3OD, 400 MHz) δ 1.84 (s, 3H, acetyl), 1.87 (s, 3H, acetyl), 2.03 (s, 3H, acetyl), 2.05 (s, 3H, acetyl), 3.06 (d, 1H, J = 11.7 Hz, H_{1a}), 3.44 (d, 1H, J = 10.0 Hz, H_3), 3.57-65 (m, 2H, H_{1b} and $H_{6'a}$), 3.70-3.72 (m, 1H, H_{6a}), 3.94 (dd, 1H, J = 8.1 and 10.5 Hz, $H_{2'}$), 4.00-4.03 (m, 2H, $H_{5'}$ and H_{6b}), 4.04-4.07 (m, 2H, $H_{6'a}$ and $H_{6'b}$), 4.32 (app dd, 1H, J = 12.4 and 4.1 Hz, H_5), 4.38 (dd, 1H, J = 9.6 and 4.1 Hz, H_4), 4.59 and 4.94 (ABq, 2H, J = 10.3 Hz, -O*CH*$_2$Ph), 5.17 (app t, 1H, J = 9.3 Hz, $H_{4'}$), 5.68 (d, 1H, J = 8.1 Hz, $H_{1'}$), 5.85 (app t, 1H, J = 9.3 Hz, $H_{3'}$), 7.24-7.51 (m, 5H, -OCH$_2$*Ph*), 7.81-7.92 (m, 5H, -N*Pth*);

^{13}C NMR (CD_3OD, 100MHz) δ 20.47, 20.75, 20.92, 22.69, 45.82, 56.29, 60.26, 61.18, 63.31, 70.42, 72.15, 73.33, 75.79, 76.42, 78.15, 96.41, 98.88, 124.64, 128. 88, 129.31, 130. 29, 135.90, 140.03, 171.41, 171.81, 172.57, 173.69;

HRMS: [Calcd for $C_{29}H_{35}Cl_3N_5O_{13}$+H]$^+$(M+H): *m/z* = 790.1267; Found: 790.1233.

3,4,6-tri-*O*-Acetyl-2-deoxy-2-trifluoroacetamide-β-D-glucopyranosyl trichloroacetimidate (2-29)

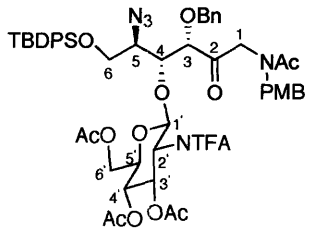


A solution of **2-28** (3.2 g, 7.22 mmol) in DMF (25 mL) was treated with hydrazine acetate (650 mg, 1.0 equiv.). After 8 h, the reaction was diluted with 200 mL of ethyl acetate and washed with satd. $NaHCO_3$, brine and water. The organic layer was dried over $MgSO_4$ and filtered, and the filtrate was concentrated to give 3,4,6-tri-*O*-acetyl-2-deoxy-2-triflouroacetamide-β-D-glucopyranoside as a yellow foam, which was used without further purification. 3,4,6-tri-*O*-Acetyl-2-deoxy-2-triflouroacetamide-β-D-glucopyranoside was azeotroped with toluene (3*10 mL) and then dried under vacuum for 1 h. The residue was dissolved in CH_2Cl_2 and trichloroacetonitrile (7.24 mL, 10 equiv.) was added, followed by DBU (0.10 mL, 0.1 equiv.). After 1h, the solution was concentrated *in vacuo* and the crude residue was purified by flash column chromatography (hexane:ethyl acetate = 4:1 to 2:1) to give **2-29** (3.0 g, 75%) as a white liquid.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.07 (s, 3H, acetyl), 2.08 (s, 3H, acetyl), 2.09 (s, 3H, acetyl), 4.11-4.15 (m, 2H, H_2 and H_{6a}), 4.29 (ABx, 1H, J = 3.8 and 12.5 Hz, H_{6b}), 5.29 (app t, 1H, J = 9.3 Hz, H_4), 5.39 (app t, J = 1H, 9.3 Hz, H_3), 6.46 (d, 1H, J = 3.6 Hz, H_1), 6,74 (br, 1H, amide), 8.88 (s, 1H, imidate);

^{13}CNMR ($CDCl_3$, 100MHz) δ 20.7, 20.8, 20.9, 52.7, 61.4, 66.9, 70.5, 90.0, 93.8, 111.3, 112.8, 117.0, 119.8, 156.9, 157.3, 158.0, 160.2, 169.4, 170.8, 171.9.

N-(4-Methoxybenzyl)-{(3R,4R,5R)-5-azido-3-O-benzyl-6-O-(tert-butyldiphenylsilyl)-4-O-(3',4',6'-tri-O-acetyl-2'-deoxy-2'-triflouroacetamide-β-D-glucopyranosyl)-2-oxo-hexyl]acetamide (3-30)



3-30

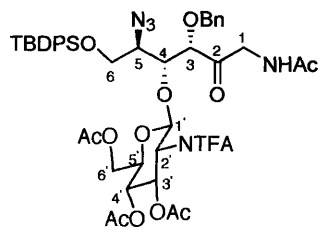
BF_3•OEt_2 (6.6 µl, 0.1 equiv.) was added to a solution of imidate **3-29** (2.25 g, 8.0 equiv.) and **3-5** (335 mg, 0.52 mmol) in toluene (30 mL) at 0 °C under argon. The mixture was allowed to warm to room temperature and stirred. Every 8 h, another BF_3•OEt_2 (6.6 µl, 0.1 eq.) was added at 0 °C. Then the mixture was allowed to warm to room temperature again and stirred until no starting material remained (usual total reaction time 48 h). The reaction mixture was poured into water (20 mL). The mixture was extracted with CH_2Cl_2 (3 x 40 mL) and the combined organic layers were washed with satd. $NaHCO_3$ and satd. brine, dried over $MgSO_4$ and concentrated *in vacuo*. Flash column chromatography (hexane:ethyl acetate = 3:1 to hexane;ethyl acetate = 2:3) of the crude product gave the desired compound **3-30** (504 mg, 90 %) as the colorless liquid (a mixture of N-rotamers).

^{1}H NMR (C_6D_6, 400 MHz) selected peaks δ 1.31 (s, 9H, Me_3CPh_2Si-), 1.69 (s, 3H, acetyl), 1.74 (s, 3H, acetyl), 1.85 (s, 3H, acetyl), 1.95 (s, 3H, acetyl), 3.42 (s, 3H, -NH_2CH_2PhOMe), 3.73-3.81 (m, 2H, H_5 and $H_{5'}$), 3.99 (dd, 1H, J = 11.3 and 1.9 Hz, H_4), 4.07 (d, 1H, J = 2.2 Hz, H_3), 4.10-4.39 (m, 4H, $H_{6'a}$, $H_{6'b}$, H_{1a} and H_{1b}), 4.18 and 4.50 (ABq, 2H, J = 12.0 Hz, -OCH_2Ph), 5.10 (d, 1H, J = 8.3 Hz, $H_{1'}$), 5.34 (app t, 1H, J = 9.8 Hz, $H_{4'}$), 5.63 (app t, 1H, J = 9.8 Hz, $H_{3'}$), 6.89 and 6.97 (2*d, 4H, J =8.7 Hz, -$NHCH_2PhOMe$), 7.71-8.15 (m, 15H, -OCH_2Ph, Me_3CPh_2Si-);

^{13}C NMR (C_6D_6, 100MHz) δ 19.8, 20.4, 20.5, 20.7, 21.1, 27.3, 53.2, 53.6, 55.3, 62.0, 63.1, 64.4. 69.1, 64.4, 69.4, 69.4, 72.9, 73.2, 76.0, 77.1, 84.7, 99.3, 128.7, 128.2, 128.5, 128.7, 129.7, 128.8, 129.0, 129.0, 129.2, 130.7, 133.4, 133.7, 136.7, 137.5, 160.3, 169.5, 170.4, 170.6, 172.5, 204.4;

HRMS: [Calcd for $C_{53}H_{62}F_3N_5O_{14}Si$+Na]$^+$(M+Na): m/z = 1100.3918; Found: 1100.3907.

N-[(3R,4R,5R)-5-azido-3-O-benzyl-6-O-(tert-butyldiphenylsilyl)-4-O-(3',4',6'-tri-O-acetyl-2'-deoxy-2'-triflouroacetamide-β-D-glucopyranosyl)-2-oxo-hexyl]acetamide (3-31)



3-31

A solution of CAN (1.33 g, 3.0 equiv.) in H_2O (4 mL) was added dropwise to a vigorously stirred solution of **3-30** (890 mg, 0.809 mmol) in MeCN (40 mL) at – 20 °C and stirred for 3 h. The reaction mixture was treated with additional CAN (445 mg, 1.0 equiv.), warmed within 3h to 0 °C and stirred for 2 h at 0 °C. The yellow-orange solution was poured into water. The mixture was extracted with ethyl acetate (3 x 30 mL) and the combined organic layers were washed with 10 % sodium sulfite solution, satd. $NaHCO_3$ and satd. brine, dried over $MgSO_4$ and concentrated *in vacuo*. Flash column chromatography (hexane:ethyl acetate

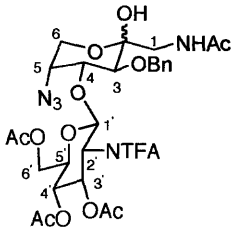
= 3:1 to hexane;ethyl acetate = 1:1) of the crude product gave the desired compound **3-31** as the white liquid (491 mg, 62 %).

^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.11 (s, 9H, Me_3CPh_2Si-), 2.00 (s, 3H, acetyl), 2.04 (s, 3H, acetyl), 2.07 (s, 3H, acetyl), 2.13 (s, 3H, acetyl), 3.44-3.48 (m, 1H, H_5), 3.60-3.63 (m, 1H, $H_{5'}$), 3.69 (ABx, 1H, J = 11.4 and 4.3 Hz, H_{6a}), 3.82 (dd, 1H, J = 12.2 and 2.0 Hz, H_4), 3.86 (dd, 1H, J = 8.4 and 9.3 Hz, $H_{2'}$), 4.06 (ABx, 1H, J = 11.4 and 2.4 Hz, H_{6b}), 4.16 (d, 1H, J = 2.0 Hz, H_3), 4.18-4.24 (m, 2H, $H_{6'a}$ and $H_{6'b}$), 4.29-4.30 (m, 2H, H_{1a} and H_{1b}), 4.42 and 4.68 (ABq, 2H, J = 11.4 Hz, -OCH_2Ph), 4.47 (d, 1H, J = 8.4 Hz, $H_{1'}$), 4.81 (app t, 1H, J = 9.3 Hz, $H_{4'}$), 4.98 (app t, 1H, J = 9.3 Hz, $H_{3'}$), 6.06 (brs, 1H, amide), 6.31 (brs, 1H, amide), 7.24-7.49 (m, 15H, -OCH_2*Ph* and Me_3CPh_2Si-);

^{13}C NMR ($CDCl_3$, 100 MHz) δ 19.5, 20.8, 21.0, 21.3, 23.2, 27.2, 49.0, 54.9, 62.4, 62.8, 68.8, 71.8, 71.9, 72.3, 74.7, 77.0, 83.4, 99.6, 128.4, 128.7, 128.8, 129.0, 130.7, 131.1, 132.6, 133.1, 136.1, 136.2, 136.7, 169.8, 170.9, 171.1, 171.6, 207.2;

HRMS(ESI-MS): Calcd for $[C_{45}H_{54}F_3N_5O_{13}Si+Na]^+$(M+Na): m/z = 980.3332; Found: 980.3331.

1-Acetamino-5-azido-3-*O*-benzyl-1,5-dideoxy-4-O-(3',4',6'-tri-O-acetyl-2'-deoxy-2'-triflouroacetamide-β-D-glucopyranosyl))-D-fructopyranose (3-32)



3-32

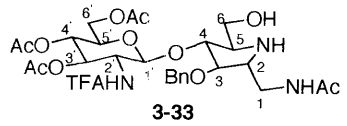
TBAF (1.0 M solution in THF, 0.501 mL, 1.0 equiv.) was added to a solution of **3-31** (491 mg, 0.501 mmol) and acetic acid (0.029 mL, 1.0 equiv.) in THF (10 mL) at 0 °C. The mixture was allowed to warm to room temperature and stirred overnight. The solvent was removed under reduced pressure, followed by the purification *via* flash column chromatography (hexane:ethyl acetate = 4:1 to ethyl acetate, following ethyl acetate:MeOH = 10:1) to afford **3-32** (314 mg, 87%) as a white oil.

^{1}H NMR (CD_3OD, 400 MHz) 1.91 (s, 3H, acetyl), 1.96 (s, 3H, acetyl), 1.98 (s, 3H, acetyl), 2.22 (s, 3H, acetyl), 3.09 (d, 1H, J = 13.7 Hz, H_{1a}), 3.59 (d, 1H, J = 9.6 Hz, H_3), 3.74 (d, 1H, J = 13.7 Hz, H_{1b}), 3.77-3.82 (m, 2H, H_{6a} and $H_{5'}$), 3.88-3.89 (m, 1H, H_{6b}), 3.94 (dd, 1H, J = 8.1 and 10.5 Hz, $H_{2'}$), 4.03-4.07 (m, 2H, $H_{6'a}$ and $H_{6'b}$), 4.32 (app dd, 1H, J = 12.4 and 4.1 Hz, H_5), 4.38 (dd, 1H, J = 9.6 and 4.1 Hz, H_4), 4.61 and 4.96 (ABq, 2H, J = 10.3 Hz, -OCH_2Ph), 4.97 (d, 1H, J = 8.4 Hz, $H_{1'}$), 5.04 (app t, 1H, J = 9.3 Hz, $H_{4'}$), 5.44 (dd, 1H, J = 10.5 and 9.3 Hz, $H_{3'}$), 7.24-7.48 (m, 5H, -OCH_2*Ph*);

^{13}C NMR (CD_3OD, 100 MHz) δ 20.5, 20.7, 20.9, 22.8, 45.9, 56.4, 60.3, 61.7, 70.1, 73.2, 73.4, 76.0, 76.5, 78.9, 98.7, 99.0, 113.2, 116.1, 118.9, 121.8, 128.8, 129.3, 129.7, 129.7, 130.1, 140.1, 159.3, 159.7, 171.4, 171.8, 172.5, 173.8;

HRMS: [Calcd for $C_{29}H_{35}F_3N_5O_{13}+H]^+$(M+H): m/z = 720.2334; Found: 720.2334.

***N*-{[(2*R*,3*R*,4*R*,5*R*)-3-*O*-benzyl-4-*O*-(3',4',6'-tri-O-acetyl-2'-deoxy-2'-trifluroacetamido-β-D-glucopyranosyl)-5-(hydroxymethyl)pyrrolidin-2-yl]methyl}acetamide (3-33)**



3-33

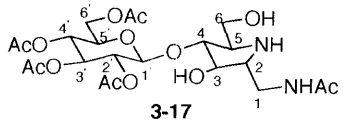
To a solution of **3-32** (70 mg, 0.0973 mmol) in MeOH (10 mL) was added $Pd(OH)_2$(35 mg). The reaction mixture was stirred under hydrogen pressure (850 psi) for 12 h and filtered through celite. After the solvent was removed *in vacuo*, the residue was purified by flash column chromatography ($CHCl_3$:MeOH = 8:1 (1 % of ammonium hydroxide) to 7:1 (1 % of ammonium hydroxide)) to afford **3-33** (36 mg, 55 %) as a colorless oil.

^{1}H NMR (CD_3OD, 400 MHz) δ 1.93 (s, 3H, acetyl), 1.95 (s, 3H, acetyl), 1.96 (s, 3H, acetyl), 1.99 (s, 3H, acetyl), 3.25-3.26 (m, 1H, H_5), 3.56-3.57 (m, 2H, H_6), 3.73-3.77 (m, 1H, $H_{5'}$), 3.89-3.93 (m, 2H, H_3 and $H_{2'}$), 4.13 (ABx, 1H, J = 8.5 and 10.5 Hz, $H_{2'}$), 4.01-4.03 (m, 1H, H_4), 4.13 (ABx, 1H, J = 2.2 and 12.4 Hz, $H_{6'a}$), 4.21-4.24 (m, 2H, H_3 and $H_{6'b}$), 4.58 and 4.73 (ABq, 2H, J = 11.6 Hz, -O*CH*$_2$Ph), 5.03 (app t, 1 H, J = 9.4 Hz, $H_{4'}$), 5.30 (dd, 1H, J = 9.4 and 10.5 Hz, $H_{3'}$), 7.25-7.35 (m, 5H, -OCH$_2$*Ph*);

^{13}C NMR (CD_3OD, 100 MHz) δ 20.52, 20.70, 20.80, 22.78, 42.64, 48.51, 56.18, 62.94, 63.14, 65.46, 69.97, 72.82, 73.26, 73.46, 87.39, 87.90, 101.24, 128.86, 129.03, 129.55, 139.85, 171.36, 171.76, 172.43, 173.8;

HRMS(ESI-MS): Calcd for $[C_{29}H_{37}F_3N_3O_{12}+H]^+$(M+H): *m/z* = 678.2480; Found: 678.2482.

N-{[(2R,3R,4R,5R)-4-O-(2',3',4',6'-tetra-O-acetyl-β-D-glucopyranosyl)-3-hydroxy-)-5-(hydroxymethyl)pyrrolidin-2-yl]methy]}acetamide (3-33)



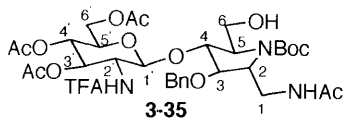
3-17

To a solution of **3-16** (30 mg, 0.048 mmol) in acetic acid (5 mL) was added $Pd(OH)_2$ (20 mg). The reaction mixture was stirred under hydrogen at atmosphere pressure for 12 h and filtered through celite. After the solvent was removed *in vacuo*, the residue was purified by column chromatography ($CHCl_3$:MeOH = 7:1 (1 % of ammonium hydroxide) to 5:1 (1 % of ammonium hydroxide)) to give **3-34** (17 mg, 65%) as a colorless oil.

^{1}H NMR (CD_3OD, 400 MHz) δ 1.90 (s, 3H, acetyl), 1.93 (s, 3H, acetyl), 1.98 (s, 3H, acetyl), 2.05 (s, 3H, acetyl) 3.07-3.24 (m, 2H, H_2 and H_5), 3.45-3.60 (m, 2H, H_{6a} and H_{6b}), 3.84-3.90 (m, 4H, $H_{2'}$, $H_{5'}$, $H_{6'a}$ and $H_{6'b}$), 4.20-4.22 (m, 2H, H_3 and H_4), 4.80 (d, 1H, J = 8.2 Hz, $H_{1'}$), 4.98 (app t, 1H, J = 9.6 Hz, $H_{3'}$), 5.27 (app t, 1H, J = 9.6 Hz, $H_{4'}$);

HRMS(ESI-MS): Calcd for $[C_{22}H_{32}F_3N_3O_{12}+H]^+$(M+H): *m/z* = 588.2011; Found: 588.2021.

N-*tert*-Butyloxycarbonyl-[(2R,3R,4R,5R)-2-(acetamide-methyl)-3-O-benzyl-4-O-(3',4',6'-tri-O-acetyl-2'-deoxy-2'-trifluroacetamido-β-D-glucopyranosyl)-5-(hydroxymethyl)]-pyrrolidine (3-35)



3-35

To a solution of **3-33** (35 mg, 0.0517 mmol) in CH_2Cl_2 in the presence of DIPEA (18 μl, 2.0 eq.) was added Boc_2O (15.8 mg, 1.4 eq.) at 0 °C. The reaction mixture was stirred at room temperature overnight. After

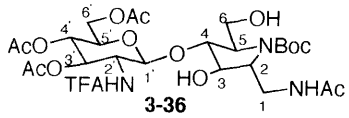
the solvent was removed *in vacuo*, the residue was purified by flash column chromatography (hexane:ethyl acetate = 1:1 to 1:4) to give the desired product **3-35** (27 mg, 68 %) as a white oil (a mixture of N-rotamers).

^{1}H NMR (CD_3OD, 400 MHz) selected peaks δ 1.44-147 (s, 9H, Me_3CCO-), 1.96-2.03 (m, 12H, acetyl), 2.86-2.90 (m, 0.6H of N-rotamers), 3.16-3.24 (m, 0.4H of N-rotamers), 3.50-3.96 (m, 6H), 4.01-4.03 (m, 1H), 4.08-4.16 (m, 1H) 4.25-4.30 (m, 1H, H), 4.45-4.55 (m, 3H, H_4 and -OCH_2Ph), 4.59 (d, J = 8.2 Hz, 0.6H of N-rotamers, $H_{1'}$), 5.01 (app t, 1H, J = 9.6 Hz, $H_{4'}$), 5.28 (app t, 0.6H of N-rotamers, J = 9.6 Hz, $H_{3'}$), 5.37 (app t, 0.4H of N-rotamers, J = 9.6 Hz, $H_{3'}$), 7.24-7.34 (m, 5H, -OCH$_2$*Ph*);

^{13}C HMR (CD_3OD, 100 MHz) δ 20.5, 20.7, 20.9, 22.7, 22.8, 28.7, 28.8, 40.4, 40.6, 55.7, 56.2, 60.3, 61.7, 63.1, 63.2, 64.3, 64.8, 67.0, 67.1, 70.0, 70.1, 72.2, 72.3, 72,9, 73.2, 73.4, 81.8, 82.1, 82.2, 83.0, 84.4, 84.8, 100.0, 128.9, 129.0, 129.1, 129.7, 139.2, 139.3, 155.8, 155.9, 171.4, 171.7, 171.3, 172.4, 173.4, 173.6;

HRMS(ESI-MS): Calcd for $[C_{34}H_{46}F_3N_3O_{14}+Na]^+$(M+Na): m/z = 800.2824; Found: 800.2802.

N-tert-Butyloxycarbonyl-[(2*R*,3*R*,4*R*,5*R*)-2-(acetamide-methyl)-4-*O*-((3',4',6'-tri-*O*-acetyl-2'-deoxy-2'trifluroacetamido-β-D-glucopyranosyl)-3-hydroxy-5-(hydroxymethyl)]-pyrrolidine (3-36)



3-36

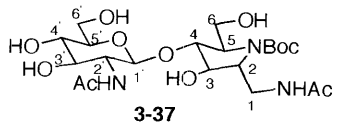
To a solution of **3-35** (30 mg, 0.039mmol) in AcOH (10 ml) was added $Pd(OH)_2$ (30 mg). The reaction mixture was stirred under 50 psi of hydrogen pressure for 24 h and filtered through celite. After solvent removed *in vacuo*, the residue was purified by flash column chromatography (hexane:ethyl acetate = 1:4 to ethyl acetate) to give the desired product **3-36** (21 mg, 80 %) as colorless oil (a mixture of N-rotamer).

^{1}H NMR (CD_3OD, 400 MHz) selected peaks δ 1.41-147 (s, 9H, Me_3CCO-), 1.92-2.05 (m, 12H, acetyl), 2.86-2.90 (m, 0.6H of N-rotamer), 3.16-3.24 (m, 0.4H of N-rotamer), 3.68-3.96 (m, 6H), 4.13-4.32 (m, 4H), 4.77 (d, 0.6H of N-rotamer, J = 8.5 Hz, $H_{1'}$), 5.00 (app t, 1H, J = 9.6 Hz, $H_{4'}$), 5.27 (app t, 0.6H of N-rotamer, J = 9.6 Hz, $H_{3'}$), 5.37 (app t, 0.4H of N-rotamer, J = 9.6 Hz, $H_{3'}$);

^{13}C NMR (CD_3OD, 100 MHz) δ 20.5, 20.7, 20.8, 22.7, 22.8, 28.7, 28.8, 40.5, 40.6, 55.8, 56.3, 60.7, 61.4, 63.1, 66.9, 67.4, 67.9, 70.0, 70.1, 72.9, 75.3, 77.1, 77.4, 81.7, 82.0, 85.7, 86.6, 99.8, 99.9, 155.9, 156.1, 171.4, 171.7, 172.5, 173.4, 173.5;

HRMS(ESI-MS): Calcd for $[C_{27}H_{40}F_3N_3O_{14}+Na]^+$(M+Na): m/z = 710.2355; Found: 710.2358.

N-tert-Butyloxycarbonyl-[(2*R*,3*R*,4*R*,5*R*)-2-(acetamide-methyl)-4-O-(2'-acetamido-2'-deoxy-β-D-glucopyranosyl)- 3-hydroxy- 5-(hydroxymethyl)]-pyrrolidine (3-37)



3-37

NH_3 gas was bubbled to a solution of **3-36** (15 mg, 0.044 mmol) in MeOH overnight. After the solvent was

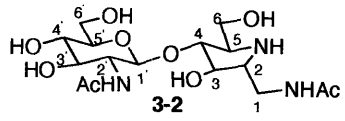
removed *in vacuo*, the residue was dissolved in MeOH:CH_2Cl_2 (1:1, 5 mL) mixture. Acetic anhydride (20 μL) was added to the reaction mixture at 0 °C. After solvent was removed *in vacuo*, the residue was purified by flash column chromatography (CH_2Cl_2:MeOH = 6:1 to 3:1) to give the desired product **3-37** (8.0 mg, 72 %) as a colorless oil (a mixture of N-rotamers).

^{1}H NMR (CD_3OD, 400 MHz) selected peaks δ 1.41-148 (s, 9H, Me_3CCO), 1.91-1.95 (m, 6H, acetyl), 2.97-3.00 (m, 0.6H of N-rotamers), 4.14-4.25 (m, 2H), 4.49 (d, 0.6H of N-rotamers, J = 8.4 Hz, $H_{1'}$), 4.52 (d, 0.4H of N-rotamers, J = 8.2 Hz, $H_{1'}$);

^{13}C NMR (CD_3OD, 100 MHz) δ 22.7, 22.8, 23.1, 28.9, 40.3, 40.5, 57.5, 57.8, 61.0, 61.7, 62.9, 67.3, 67.4, 67.5, 67.7, 69.3, 72.3, 72.4, 75.6, 75.8, 76.5, 76.8, 78.4, 81.6, 81.9, 86.9, 87.8, 102. 2, 102.3, 155.9, 156.2, 173.6, 173.7, 173.9;

HRMS(ESI-MS): Calcd for $[C_{21}H_{37}N_3O_{11}+Na]^+$(M+Na): m/z = 530.2320; Found: 530.2333.

_N_-[{(2_R_,3_R_,4_R_,5_R_)- 4-_O_-(2'-Acetamido-2'-deoxy-β-D-glucopyranosyl)-3-hydoxy-5-(hydroxymethyl)pyrrolidin-2-yl}methyl]acetamide (3-2)



3-2

To a solution of compound **3-37** (4.0 mg, 0.016mmol) in CH_2Cl_2 (3 mL) was added TFA (1 mL) at 0 °C. The reaction mixture was warmed to room temperature. The reaction mixture was stirred at room temperature for 4 h. After solvent was removed *in vacuo*, the residue was filter through Dowex 1X8 200-400 chloride form resin and purified by short flash column chromatography to provide **3-2** (3.2 mg, quant.).

^{1}H NMR (D_2O, 600 MHz) δ 2.01 (s, 3H, acetyl), 2.04 (s, 3H, acetyl), 3.17-3.19 (m, 2H, H_5 and H_2), 3.35-3.37 (m, 2H, H_{1a} and H_{1b}), 3.43 (dd, 1H, J = 10.0 and 8.9 Hz, $H_{3'}$), 3.48-3.50 (m, 1H, H_{6a}), 3.55 (dd, 1H, J = 8.6 and 10.0 Hz, $H_{2'}$), 3.61-3.66 (m, 2H, H_{6b} and $H_{5'}$), 3.72-3.74 (m, 2H, $H_{4'}$ and $H_{6'a}$), 3.93-4.02 (m, 3H, H_3, H_4 and $H_{6'b}$), 4.56 (d, 1H, J = 8.6 Hz, $H_{1'}$);

^{13}C NMR (CD_3OD, 125 MHz) δ 21.9, 22.2, 42.3, 56.8, 62.3, 62.3, 62.6, 62.9, 63.7, 71.9, 75.2, 77.2, 79.5, 88.7, 102.2, 173.1, 173.1;

HRMS(ESI-MS): Calcd for $[C_{16}H_{29}N_3O_9+H]^+$(M+H): m/z = 408.1977; Found: 408.1993.

Determination of the structural configuration of 3-34

Similar to NOE study of **3-17**, we wanted to confirm the configuration of C2 from NOE difference between H_2 and H_4. On the other hand, NOE between H_2 and H_4 would confirm the structure as the possible diastereomer. Since H_2 and H_5 appear in the same region, the selective irradiation of H_2 was difficult. However, NOE between H_3 and H_5 would be common for both structures. Therefore, two NOE differences between H_2 and H_4 and between H_3 and H_5 would prove the structure of **3-33**, whereas one NOE among H_2, H_3 and H_5 would confirm the other isomer. The control spectrum and three NOE difference spectra of **3-34** are shown in Figure 3-10. The irradiation of $peak_C$ (H_2 and H_5) induced the NOE signal of both $peak_A$ and $peak_B$ (**Figure 3-10b.**). Upon the irradiation $peak_A$ and $peak_B$ respectively, the NOE signal of $peak_C$ (H_2 and H_5) was observed (**Figure 3-10c, d.**). These data showed that there are NOE differences between H_2 and H_4 and between H_2 and H_5. Therefore, we concluded the **3-34** had the desired trans-configuration between C2-C3.

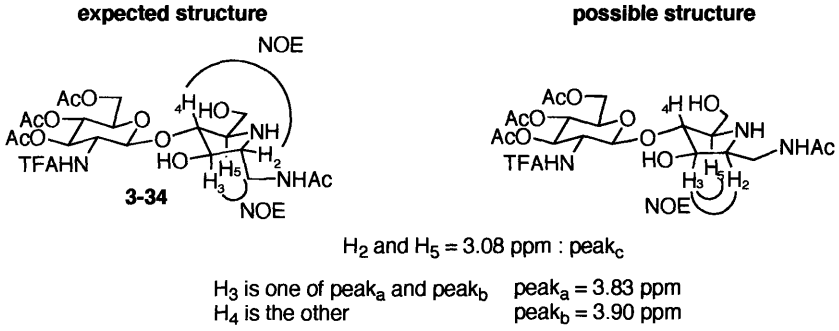


Figure 3-9. The expected structure of 3-34 and possible diastereomers at C2.

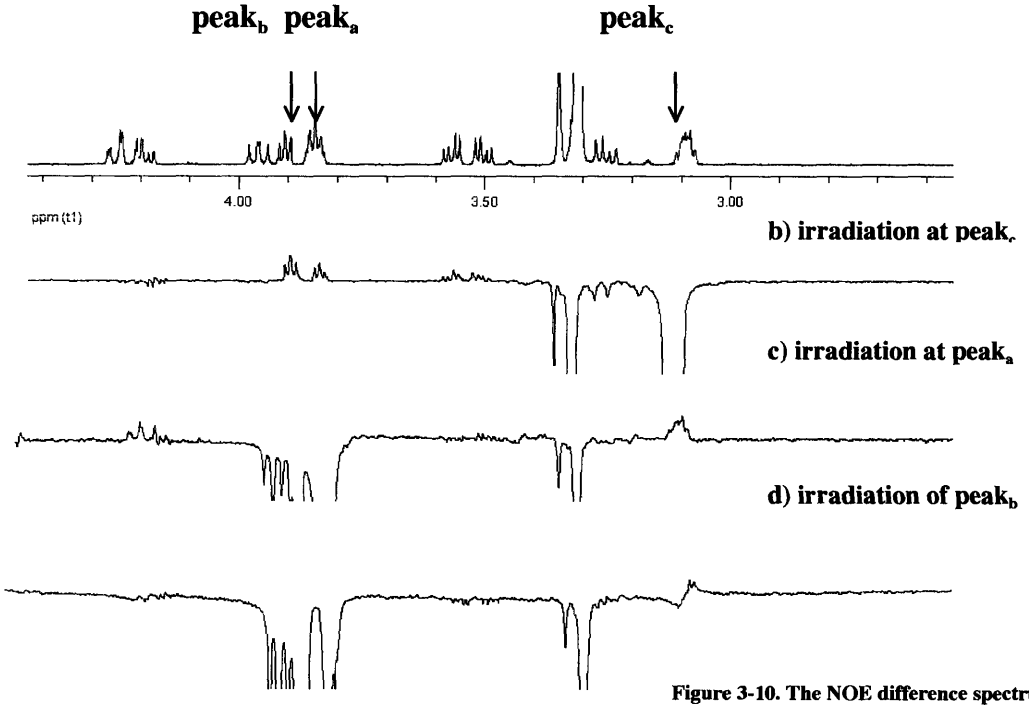


Figure 3-10. The NOE difference spectrum of 3-34

The assay of 3-1 and 3-2 against chitinase.

Biological evaluations of **3-1** and **3-2** against chitinase from *Streptomyces griseus* were carried out, as described in the chapter 2. **3-1** and **3-2** showed inhibitory activity with IC_{50} values of 3.0 μM and 2.6 μM

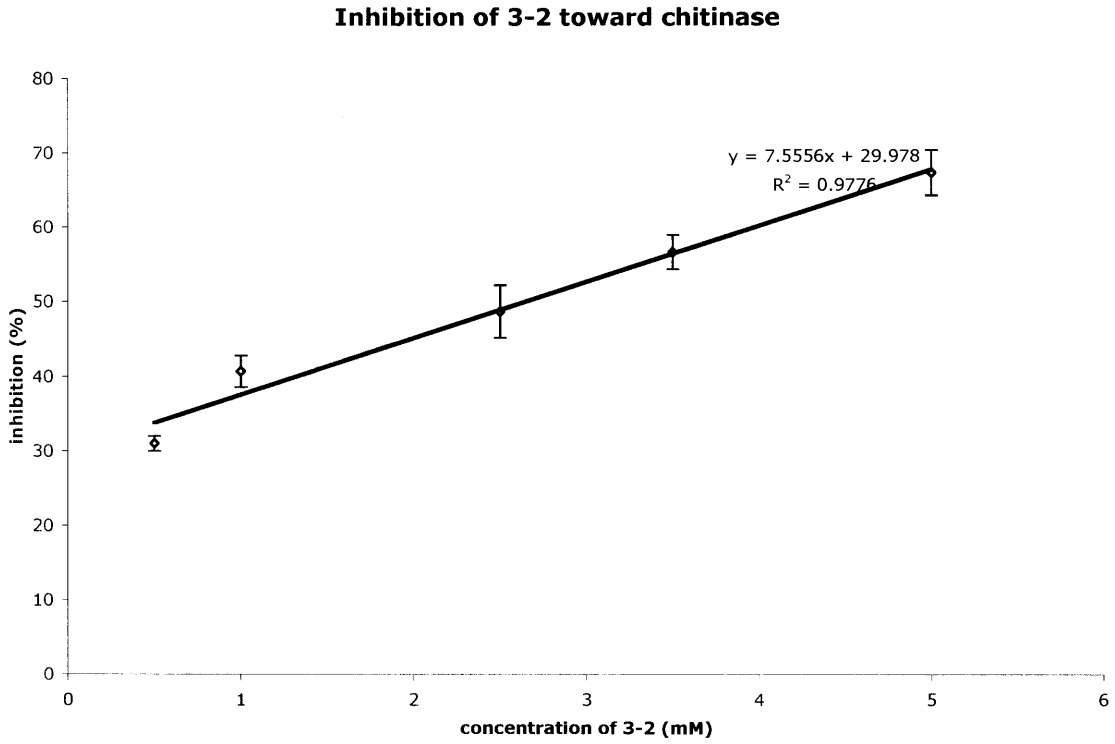


Figure 3-11. Inhibition (%) of 3-2 toward chitinase.

References

1. Hodge, J. E. The Amadori rearrangement. *Adv Carbohydr Chem,* **1955**, *10*, 169-205.
2. Wrodnigg, T. M.; Stütz, A. E.; Withers, S. G. Symthesis of 1-Amino-1,2,5-trideoxy-2,5-imino-D-mannitol, a Novel Analogue of the Powerful Glucosidase Inhibitor 2,5-Dideoxy-2,5-imino-D-mannitol, *via* an Amadori Rearrangement of 5-Azido-5-deoxy-D-glucofuranose. *Tetrahedron Lett.,* **1997**, *38*, 5463-5466.
3. Toshima, K.; Tatsuta, K. Recent Progress in *O*-Glycosylation Methods and Its Application to Natural-Products Synthesis. *Chem. Rev.,* **1993**, *93*, 1503-1531.
4. Dax, K.; Gaigg, B.; Grassberger, V.; Kölblinger, B.; Stütz, A. E. Simple Synthesis of 1,5-Dideoxy-1,5-Imino-D-Glucitol(1-Deoxynojirimycin) and 1,6-Dideoxy-1,6-Imino-D-Glucitol from D-Glucofuranurono-6,3-Lactone. *J. Carbohydr. Chem.,* **1990**, *9*, 479-499.
5. Störz, T.; Bernet, B.; Vasella, A. β-Lactams from D-Erythrose-Derived Imimes: A Convenient Synthesis of 2,3-Diamino-2,3-dideoxy-D-mannonic-Acid Derivatives. *Helv. Chim. Acta.,* **1999**, *82*, 2380-2412.
6. Blanchard, N.; Roush, W. R. 2-Deoxy-2-iodo-β-glucopyranosyl fluorides: mild and highly stereoselective glycosyl donors for the synthesis of 2-deoxy-β-glycosides from β-hydroxy ketones. *Org. Lett.,* **2003**, *5*, 81-84.
7. Toshima, K.; Misawa, M.; Ohta, K.; Tatsuta, K.; Kinoshita, M. Enantiospecific Synthesis of C20-C28 Segment of Concanamycin-A - Application of Diethylisopropylsilyl Protecting Group. *Tetrahedron Lett.,* **1989**, *30*, 6417-6420.
8. Paterson, I.; Mcleod, M. D. Studies in Macrolide Synthesis - a Sequential Aldol Glycosylation Approach to the Synthesis of Concanamycin-A. *Tetrahedron Lett.,* **1995**, *36*, 9065-9068.
9. Evans, D. A.; Kaldor, S. W.; Jones, T. K.; Clardy, J.; Stout, T. J. Total Synthesis of the Macrolide Antibiotic Cytovaricin. *J. Am. Chem. Soc.,* **1990**, *112*, 7001-7031.
10. Griffith, M. H. E.; Hindsgaul, O. Observation of a Stable Orthoacyl Fluoride in the Reaction of a Reducing Sugar with Diethylaminosulfur Trifluoride. *Carbohydr. Res.,* **1991**, *211*, 163-166.
11. Mukaiyama, T.; Murai, Y.; Shoda, S. An Efficient Method for Glucosylation of Hydroxy Compounds Using Glucopyranosyl Fluoride. *Chem. Lett.,* **1981**, 431-432.
12. Kanie, O.; Ito, Y.; Ogawa, T. Orthogonal Glycosylation strategy in Oligosaccharide Synthesis. *J. Am. Chem. Soc.,* **1994**, *116*, 12073-12074.
13. Paulsen, H.; Meinjohanns, E. Synthesis of Modified Oligosaccharides of N-Glycoproteins Intended for Substrate-Specificity Studies of N-Acetylglucosaminyltransferases II-V. *Tetrahedron Lett.,* **1992**, *33*, 7327-7330.
14. Koeller, K. M.; Wong, C. -H. Complex carbohydrate synthesis tools for glycobiologists: enzyme-based approach and programmable one-pot strategies. *Glycobiology,* **2000**, *10*, 1157-1169.
15. Ye, X. S.; Wong, C. -H. Anomeric reactivity-based one-pot oligosaccharide synthesis: A rapid route to oligosaccharide libraries. *J. Org. Chem.,* **2000**, *65*, 2410-2431.
16. Mong, T. K. -K.; Huang, C. Y.; Wong, C. -H. A new reactivity-based one-pot synthesis of N-acetyllactosamine oligomers. *J. Org. Chem.,* **2003**, *68*, 2135-2142.

17. Sakai, K.; Nakahara, Y.; Ogawa, T. Total Synthesis of Nonasaccharide Repeating Unit of Plant-Cell Wall Xyloglucan - an Endogenous Hormone Which Regulates Cell-Growth. *Tetrahedron Lett.*, **1990**, *31*, 3035-3038.
18. Paulsen, H.; Helpap, B. Components of Oligosaccharides.96. Synthesis of Partial Structures of N-Glycoproteins of the Complex Type. *Carbohydr. Res.*, **1991**, *216*, 289-313.
19. Mong, T. K. -K.; Lee, H. -K.; Duron, S. G.; Wong, C. -H. Reactivity-based one-pot total synthesis of fucose GM(1) oligosaccharide: A sialylated antigenic epitope of small-cell lung cancer. *Proc. Natl. Acad. Sci. USA*, **2003**, *100*, 797-802.
20. Nitz, M.; Bundle, D. R. Efficient synthesis of 3,6-dideoxy-β-D-arabino-hexopyranosyl-terminated LacdiNac glycan chains of the *Trichinella spiralis* parasite. *J. Org. Chem.*, **2000**, *65*, 3064-3073.
21. Blatter, G.; Beau, J. M.; Jacquinet, J. C. The Use of 2-Deoxy-2-Trichloroacetamido-D-Glucopyranose Derivatives in Syntheses of Oligosaccharides. *Carbohydr. Res.*, **1994**, *260*, 189-202.
22. Love, K. R.; Andrade, R. B.; Seeberger, P. H. Linear synthesis of a protected H-type II pentasaccharide using glycosyl phosphate building blocks. *J. Org. Chem.*, **2001**, *66*, 8165-8176.
23. Bélot, F.; Wright, K.; Costachel, C.; Phalipon, A.; Mulard, L. A. Blockwise approach to fragments of the O-specific polysaccharide of *Shigella flexneri* serotype 2a: Convergent synthesis of a decasaccharide representative of a dimer of the branched repeating unit. *J. Org. Chem.*, **2004**, *69*, 1060-1074.
24. Du, Y. G.; Zhang, M. M.; Kong, F. Z. Efficient and practical syntheses of three pentasaccharides core structures corresponding to N-glycans. *Tetrahedron*, **2001**, *57*, 1757-1763.
25. Myszka, H.; Bednarczyk, D.; Najder, M.; Kaca, W. Synthesis and induction of apoptosis in B cell chronic leukemia by diosgenyl 2-amino-2-deoxy-β-D-glucopyranoside hydrochloride and its derivatives. *Carbohydr. Res.*, **2003**, *338*, 133-141.
26. Imazawa, M.; Eckstein, F. Facile Synthesis of 2'-Amino-2'-Deoxyribofuranosyl Purines. *J. Org. Chem.*, **1979**, *44*, 2039-2041.
27. Takaoka, Y.; Kajimoto, T.; Wong, C. -H. Inhibition of N-Acetylglucosaminyltransfer Enzymes: Chemical-Enzymatic Synthesis of New Five-Membered Acetamido Azasugar. *J. Org. Chem.*, **1993**, *58*, 4809-4812.
28. Izquierdo, I.; Plaza, M. T.; Franco, F. Polyhydroxylated pyrrolidines. Part 2: Highly stereoselective synthesis of partially protected DMDP derivatives from D-fructose. *Tetrahedron: Asymmetry*, **2002**, *13*, 1503-1508.
29. Liu, J.; Numa, M. M.; Liu, H.; Huang, S. J.; Sears, P.; Shikhman, A. R.; Wong, C. -H. Synthesis and high-throughput screening of N-acetyl-β-hexosaminidase inhibitor libraries targeting osteoarthritis. *J. Org. Chem.*, **2004**, *69*, 6273-6283.
30. Garcia-Moreno, M. I.; Aguilar, M.; Mellet, C. O.; Fernandez, J. M. G. Intramolecular benzyl protection delivery: A practical synthesis of DMDP and DGDP from D-fructose. *Org. Lett.*, **2006**, *8*, 297-299.
31. Neuhaus, D.; Williamson, M. P. The Nuclear Overhauser Effect in Structual and Conformational Analysis.; Wiley-VCH: **2000**. New-York, U. S, A.

32. Houston, D. R.; Synstad, B.; Eijsink, V. G.; Stark, M. J.; Eggleston, I. M.; van Aalten, D. M. Structure-based exploration of cyclic dipeptide chitinase inhibitors. *J. Med. Chem.*, **2004**, *47*, 5713-5720.
33. Rao, F. V.; Andersen, O. A.; Vora, K. A.; Demartino, J. A.; van Aalten, D. M. Methylxanthine drugs are chitinase inhibitors: investigation of inhibition and binding modes. *Chem. Biol.*, **2005**, *12*, 973-980.
34. Andersen, O. A.; Dixon, M. J.; Eggleston, I. M.; van Aalten, D. M. F. Natural product family 18 chitinase inhibitors. *Nat. Prod. Rep.*, **2005**, *22*, 563-579.

Chapter 4- Toward the library of a potential OT inhibitor: the synthesis of pseudo-disaccharide derivatives

4-1 Introduction

With the successful synthesis of pseudo-disaccharides containing an imino sugar, we turned our efforts to the synthesis derivatives. We were particularly interested in the exploration of different groups on the C1 amine of the imino sugar. Preparation of various pseudo-disaccharide derivatives would be valuable for the discovery of chitinase inhibitors with better biological properties than **3-1** and **3-2**. We also decided to synthesize a pseudo-disaccharide derivative containing a long alkyl chain as a potential OT inhibitor. As discussed in Chapter 1-11, the addition of a long aliphatic chain to the transition state analogue is expected to increase the binding affinity to the membrane-associated enzyme.

We planned to expand the synthetic strategy developed in Chapter 3 for the synthesis of pseudo-disaccharide derivatives (**Figure 4-1**). Since chitinase and OT recognize the chitobiose motif as the main structural determinant in the substrate, we decided to synthesize the imino sugars linked to N-acetylglucosamine. Glycosylation of **4-5** with the glycosyl imidate having N-trifluroacetimide (N-TFA) on C'2 position would provide **4-4**. For the generation of its derivatives, the C1 amine of the imino sugar must be masked until the last step. Given the previous problems regarding the protecting group on C2' protecting group of glucosamine derivatives discussed in Chapter 3, a careful protecting group strategy was required. In light of the functional-group tolerance during

reductive amination, orthogonality to other protecting groups, and facile removal, the *tert*-butyl carbonyl (Boc) group appeared to be the best choice as the protecting group for the C1 primary amine. Therefore, it was imperative to have substrate **4-3** bearing the Boc group on the amine before reductive amination.

Selective acylation of the primary amine in the presence of other functional groups such as secondary amines and alcohols has been investigated by other groups and by our laboratory as outlined in Chapter 2-2.[1-5] This approach enabled us to obtain the pseudo-disaccharide derivatives with various groups such as a long aliphatic chain, aromatic groups, and heterocyclic groups.

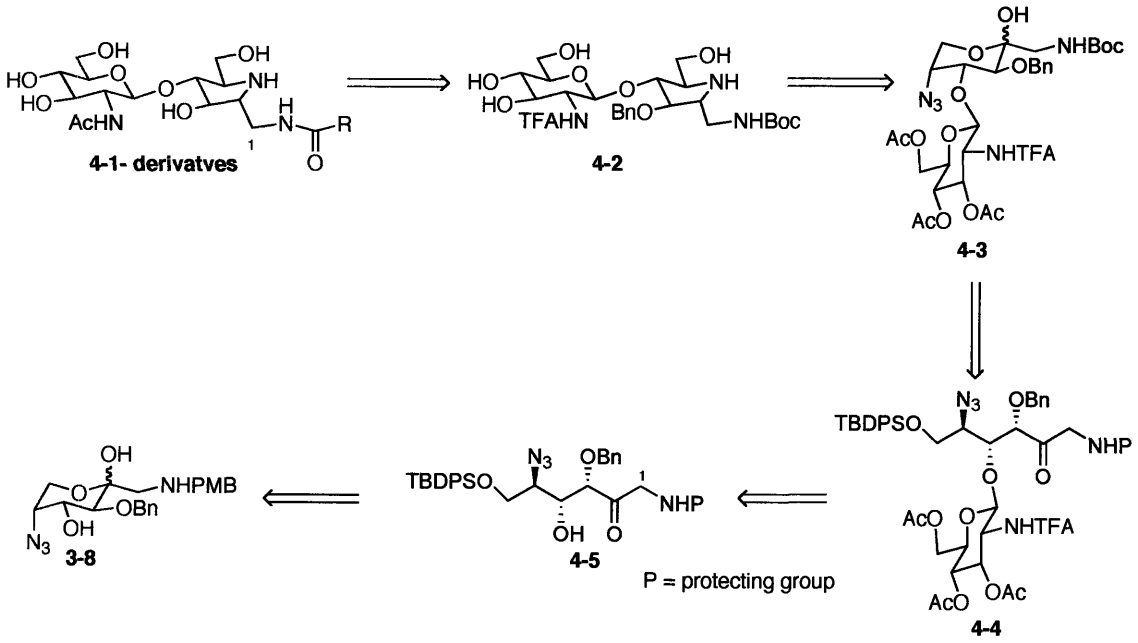
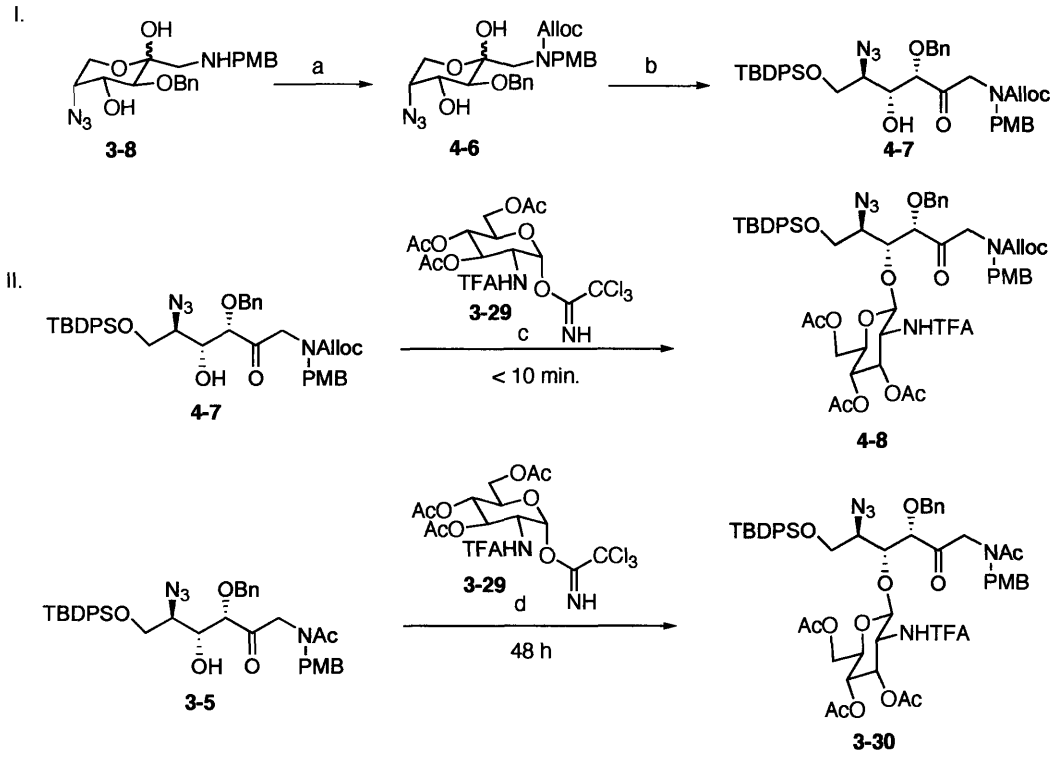


Figure 4-1. The synthetic strategy toward derivatives of the pseudo-disaccharide.

4-2 Synthesis of the pseudo-disaccharide precursor

Considering the glycosylation conditions of similar substrate **3-5** with 8.0 equiv. of glycosyl imidate **3-29** employing 0.6 equiv. of BF_3•OEt_2 for 48 h, it appeared to be quite difficult to execute the glycosylation of the substrate bearing the acid-labile Boc group. Therefore, our efforts focused on the glycosylation of β-hydroxy ketone with the allyloxycarbonyl (Alloc) group on the C1 amine. After glycosylation of **4-7**, the transprotection of the Alloc group to the Boc group would provide the desired intermediate. The protection of the secondary amine of **3-8**, the synthesis of which was described in the Chapter 3, used 1.5 equiv. of AllocCl in the presence of 2.0 equiv. of *N*,*N*-diisopropylethylamine (DIPEA) in methanol to provide **4-6** (**Scheme 4-1-I**). The treatment of **4-6** with 2.0 equiv. of *tert*-butyldiphenylsilyl chloride (TBDPSCl) in the presence of 3.0 equiv. of imidazole in CH_2Cl_2 provided the substrate **4-7** for glycosylation in 80% yield.

Under the reaction conditions reported for **3-5** employing 8.0 equiv. of the glycosyl imidate **3-29** and 0.6 equiv. of BF_3•OEt_2 in the toluene, glycosylation of **4-7** proceeded in less than 10 minutes (**Scheme 4-1-II**). In contrast, the glycosylation of **3-5** was fully completed only after 48 h. The difference in reactivity between two compounds toward glycosylation cannot be explained and further studies need to be done to sufficiently explain this experimental observation.

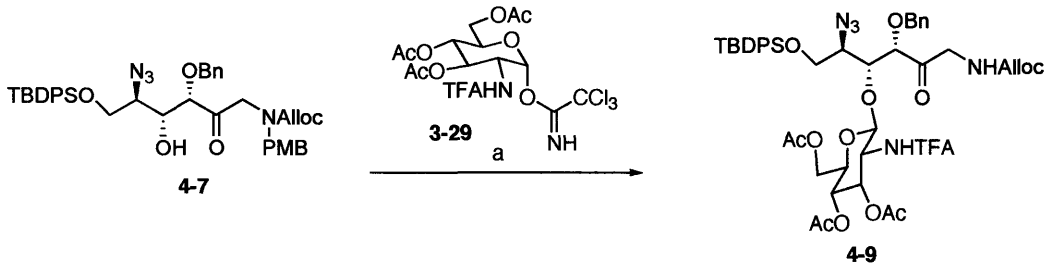


Reagents and conditions: (a) AllocCl, DIPEA, MeOH, 75 %; (b) TBDPSCl, imidazole, CH_2Cl_2, 80 %; (c) **3-29**, BF_3•OEt_2, toluene, 0 °C ; (d) **3-29**, BF_3•OEt_2, toluene, 0 °C to RT, 92 %.

Scheme 4-1. Comparison of the glycosylation results between 3-5 and 4-7.

The increased reactivity of **4-7** allowed for the optimal reaction conditions: 2.0 equiv. of the glycosyl imidate **3-29** and 0.05 equiv. of BF_3•OEt_2 as the promoter in toluene for 8 h (**Scheme 4-2**). The subsequent oxidative cleavage of the *p*-methoxybenzyl

(PMB) group employing 4.0 equiv. of ceric ammonium nitrate (CAN), led to the desired compound **4-9** in 85% yield over 2 steps.



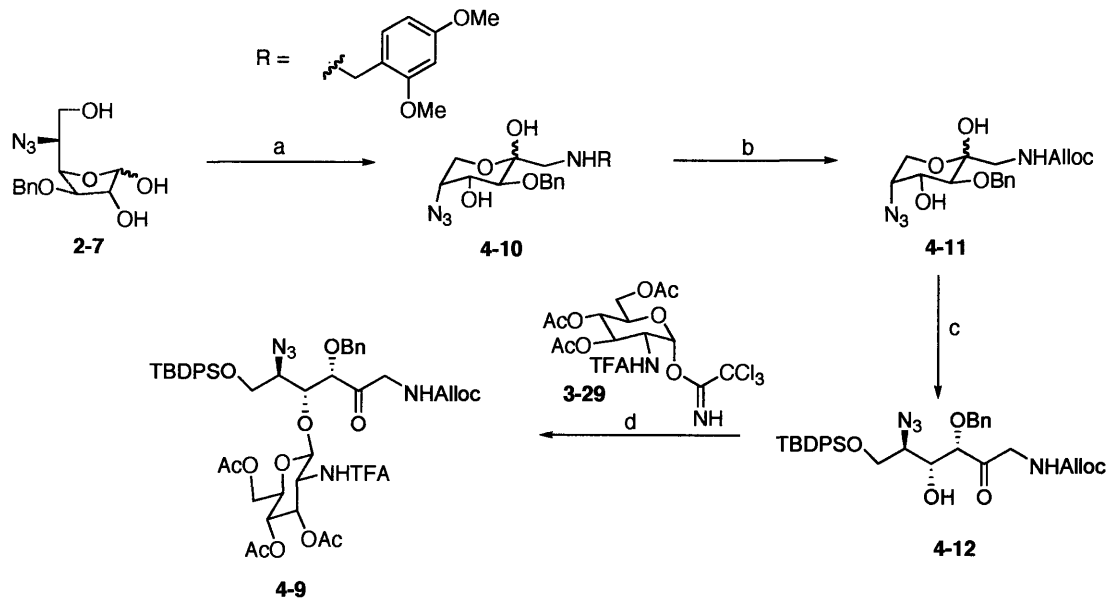
Reagents and conditions: (a) (i) **3-29**, $BF_3 \cdot OEt_2$, toluene, 0 °C to RT; (ii) CAN, MeCN:H_2O = 10:1, - 20 °C to RT, two steps 85 %.

Scheme 4-2. Optimization of the glycosylation of 4-7.

Although **4-9** was obtained successfully, large-scale purification proved to be problematic. R_f values of **4-8** and **4-9** in TLC analysis with various solvents were identical. Therefore, the progress of the reaction was difficult to monitor. Moreover, long exposure of **4-8** to the reaction conditions to drive the complete conversion diminished the yield dramatically (to less than 20%).

One way to circumvent this problem was to cleave the N-substituent prior to glycosylation. To this effect, 2,4-dimethoxybenzylamine was used during the Amadori rearrangement of **2-7** in the presence of 1.0 equiv. of acetic acid (**Scheme 4-3**).[2,6] After the protection of the secondary amine on **4-10** utilizing 1.5 equiv. of AllocCl and 2.0 equiv. of DIPEA, the 2,4-dimethoxybenzyl group was cleaved using trifluoroacetic acid (TFA) to provide the **4-11** in 63% yield over 2 steps.[7] The β-hydroxy ketone **4-12** was obtained by treatment of **4-11** with 2.0 equiv. of TBDPSCl and 3.0 equiv. of imidazole in

CH_2Cl_2. The glycosylation of **4-12** with 2.0 equiv. of glycosyl imidate **3-29** employing 0.05 equiv. of $BF_3{\bullet}OEt_2$ in toluene provided **4-9** in 90% yield.



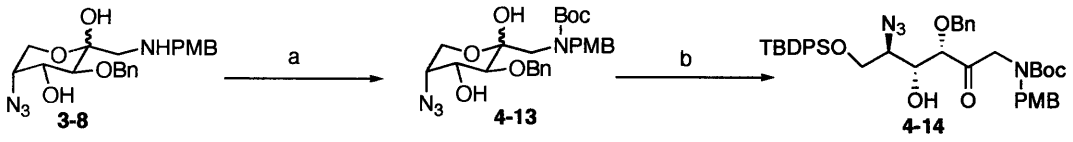
Reagents and conditions: (a) 2,4-Dimethoxybenzylamine, AcOH, MeOH, 40 °C, 85%; (b) AllocCl, DIPEA, MeOH, 0 °C to RT; (ii) TFA, 0 °C to RT, 2 steps 63%; (c) TBDPSCl, imidazole, CH_2Cl_2, 72%; (d) **3-29**, $BF_3{\bullet}OEt_2$, toluene, 0 °C to RT, 90%.

Scheme 4-3. A practical synthesis of 4-9.

The efficient glycosylation of **4-12** encouraged us to attempt the coupling reaction between substrates with the Boc group on the C1 amine and the glycosyl imidate **3-29** minimizing the number of synthetic transformations en route to the target compound Since most glycosylation conditions rely on Lewis acids promoters, glycosylation of the substrate bearing a Boc group was challenging. Glycosylation in the presence of a Boc group could be potentially achieved by utilization of a mild Lewis acid.

The substrate for the glycosylation was synthesized in a manner similar to the route in described for **4-7** (**Scheme 4-4**). The Boc group was introduced by the treatment of **3-8** with 1.2 equiv. of di-*tert*-butyl dicarbonate (Boc_2O) and 1.5 equiv. of DIPEA in

CH_2Cl_2 in 72% yield. The primary alcohol protection of **4-13** employing 2.0 equiv. of TBDPSCl and 3.0 equiv. of imidazole in CH_2Cl_2 gave the desired product **4-14**.



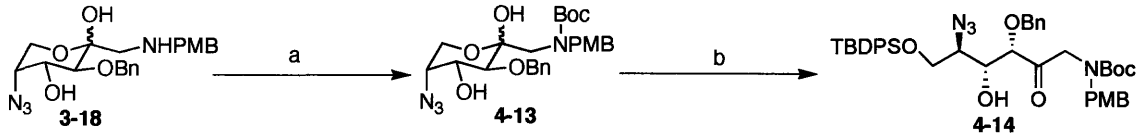
Reagents and conditions: (a) Boc_2O, DIPEA, CH_2Cl_2, 72 %; (b) TBDPSCl, imidazole, CH_2Cl_2, 75 %.

Scheme 4-4. The synthesis of β-hydroxy ketone 4-14 with Boc group.

However, the glycosylation of **4-14** with 3.0 equiv. of the glycosyl imidate **3-29** in the presence of 0.05 equiv. $BF_3 \bullet OEt_2$ in toluene did not yield the desired product, instead decomposed products of **4-14** were isolated (**Scheme 4-5-I**). Attempted coupling reactions employing silver trifluromethanesulfonate (AgOTf) or solvent (CH_2Cl_2, Et_2O) failed with the same result. The use of excess sugar donor did not facilitate the formation of glycosidic bond either. Most of **4-14** remained unreacted and the glycosyl imidate was converted into the corresponding oxazoline.

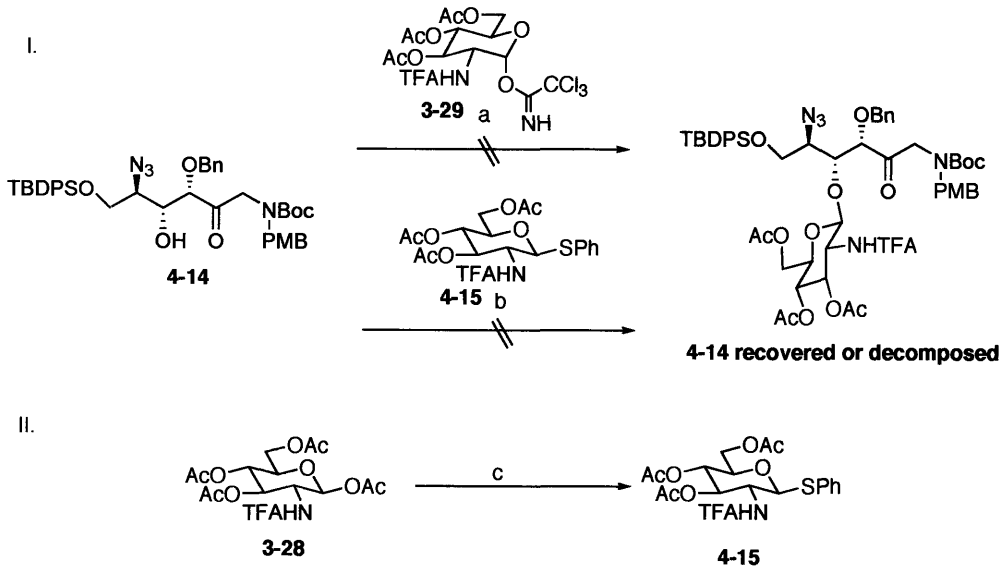
In order to avoid strong Lewis-acids, the coupling reaction of **4-14** under milder conditions utilizing a thioglycoside was also attempted. Thioglycoside **4-15** was prepared by the treatment **3-28**[8] with 1.0 equiv. of PhSH and 1.0 equiv. of $BF_3 \bullet OEt_2$ in CH_2Cl_2 (**Scheme 4-5-II**). Unfortunately, the glycosylation of **4-14** with 3.0 equiv. of thioglycoside **4-15** using 3.0 equiv. of *N*-iodosuccinimide (NIS) and 0.1 equiv. $AgClO_4$ (or AgOTf) in toluene did not provide the desired product. Most of **4-14** remained unreacted and the thioglycoside decomposed. Using CH_2Cl_2 as the solvent, decomposition of the both starting materials was detected by TLC analysis without

generation of the coupled product. Under mild acidic conditions, the electron deficient glycosyl donors having N-trifluroacetimide (N-TFA) on the C2' amine were not reactive in the glycosylation. When more acidic conditions were applied, the N-Boc group was cleaved during the reaction. This conclusion prompted us to focus on the conversion of the N-Alloc to the N-Boc group only after the key glycosylation step.



Reagents and conditions: (a) Boc_2O, DIPEA, CH_2Cl_2, 72 %; (b) TBDPSCl, imidazole, CH_2Cl_2, 75 %.

Scheme 4-4. The synthesis of β-hydroxy ketone4-14 with N- Boc group.

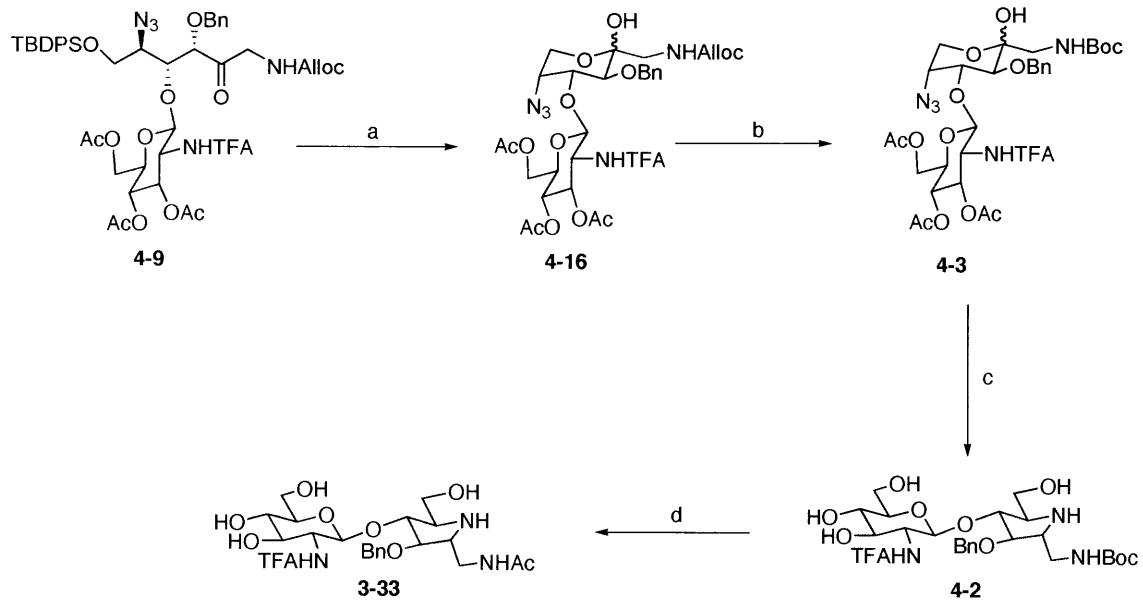


Reagents and conditions: (a) **3-29**, $BF_3{\cdot}OEt_2$ or AgOTf, CH_2Cl_2 or toluene, 0 °C to RT; (b) **4-15**, AgOTf or $AgClO_4$, NIS, CH_2Cl_2 or toluene, -50 °C to RT; (c) PhSH, $BF_3{\cdot}OEt_2$, CH_2Cl_2, 98 %.

Scheme 4-5. Attempted glycosylation of 4-14.

The Alloc protecting group is known to be readily cleaved by catalytic amounts of palladium (0) with a nucleophile ("allyl acceptor" or "allyl scavenger") such as hydride, amine, acid, and thiol.[9] However, using 0.05 equiv. of $Pd(PPh_3)_4$ and $PhSiH_3$ or Bu_3SnH in CH_2Cl_2, only decomposition of substrate **4-9** was observed. The reaction did not proceed with other nucleophiles such as acetic acid,[10] formic acid,[11] diethyl amine,[12] morpholine,[13] 1,4-diazabicyclo[2.2.2]octane (DABCO),[14] and dimedone.[15] The attempted transprotection to the N-Boc group employing catalytic $Pd(PPh_3)_4$ and Bu_3SnH[16] or DABCO[14] in the presence of 1.2 equiv. Boc_2O in CH_2Cl_2 generated a mixture of products. After removal of the silyl ether utilizing 1.0 equiv. of tetra-*n*-butylammonium fluoride (TBAF) and 1.0 equiv. of acetic acid, palladium catalyzed transprotection of ketal **4-16** employing 1.1 equiv. of Bu_3SnH in the presence of 1.2 equiv. Boc_2O in CH_2Cl_2 provided the desired product **4-3** in 92 % yield (**Scheme 4-6**).[16]

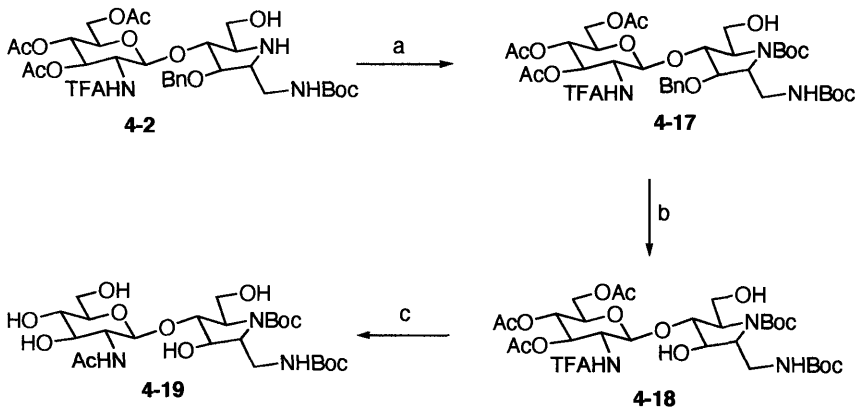
Compound **4-3** was then subjected to reductive amination using palladium hydroxide under 850 psi of H_2. The reaction went smoothly, giving rise to the desired **4-2** as the major product (>15:1, NMR ratio). To confirm configuration of **4-2**, the N-Boc group was removed by the treatment of **4-2** with trifluoroacetic acid in CH_2Cl_2, followed by the acetylation of the primary amine with acetic anhydride in methanol. The obtained compound showed identical spectroscopic properties (^{1}H-NMR, ^{13}C-NMR) as **3-33**.



Reagents and conditions: (a) TBAF, AcOH, THF, 0 °C to RT, 85 %; (b) $Pd(PPh_3)_4$, Bu_3SnH, Boc_2O, CH_2Cl_2, 92 %; (c) H_2 (850 psi), $Pd(OH)_2$, MeOH, 55 %; (d) (i) TFA, CH_2Cl_2, 0 °C to RT; (ii) Ac_2O, MeOH, 0 °C, 50 %

Scheme 4-6. The synthesis of 4-2.

Following the reaction sequence described in Scheme 3-12, protection of the secondary amine was achieved employing Boc_2O and DIPEA in CH_2Cl_2, followed by the removal of the benzyl ether using palladium hydroxide under 50 psi of H_2, generating **4-18** (**Scheme 4-7**). The deprotection of the acetates and conversion of the N-trifluroacetimide (N-TFA) to an N-Ac group afforded **4-19** with 63% yield.



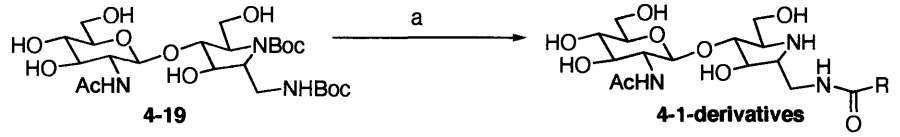
Reagents and conditions: (a) Boc_2O, DIPEA, CH_2Cl_2, 0 °C to RT, 63 %; (b) H_2 (50 psi), $Pd(OH)_2$, AcOH, 80 %; (c) (i) NH_3, MeOH; (ii) Ac_2O, DCM:MeOH = 1:1, 2 steps 63 %.

Scheme 4-7. The synthesis of 4-19.

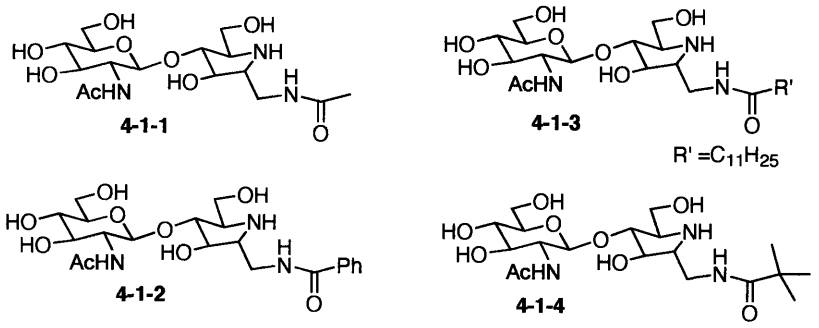
4-3 Synthesis of pseudo-disaccharide derivatives and biological evaluations

We planned to generate pseudo-disaccharide derivatives with various groups on the C1 nitrogen position based on amide formation (**Scheme 4-8**). After deprotection of both Boc groups in **4-19** using TFA in CH_2Cl_2, the primary amine was reacted with 1.0 equiv. of different anhydrides (acetic anhydride, benzoic anhydride, dodecanoic anhydride and Boc anhydride) at 0 °C in methanol to provide the initial library. Existence of products was confirmed by mass spectral analysis. Based on 100 % conversion of starting material in 2 steps, the final inhibitor concentration was 10 μM. As is common practice with library design, the biological activity of the crude mixture was tested.[1,17] To validate reaction results, the chitinase assays as described in Chapter 2[18] were carried out with the crude reaction product of **4-1-1** and the product purified by flash column chromatography. Crude and purified **4-1-1** showed inhibitory activity with IC_{50} values of

7.7 μM and 8.3 μM respectively, which demonstrated that the crude concentration estimation was valid. The inhibitory activities were a slightly worse than **3-2** (IC_{50} = 2.6 μM) which was synthesized in Chapter 3 by the different route. However, these results showed that this approach was useful to identify chitinase inhibitor. At 8.0 μM of inhibitor concentration, **4-1-2**, **4-1-3** and **4-1-4** showed 34 %, 12 % and 19 % inhibitory activities toward chitinase from *Streptomyces grises*.



Reagents and conditions: (a) (i) TFA, CH_2Cl_2, 0 °C to RT; (ii) acid anhydride, MeOH, 0 °C.



Scheme 4-8. The synthesis of pseudo-disaccharide derivatives.

The pseudo-disaccharide derivative with a long alkyl chained was synthesized as the potential OT inhibitor. Compound **4-1-3** was obtained by treatment of **4-19** with TFA, followed by acylation with dodecanoic anhydride. Unfortunately, no inhibitory activity of **4-1-3** was observed up to 200 μM.

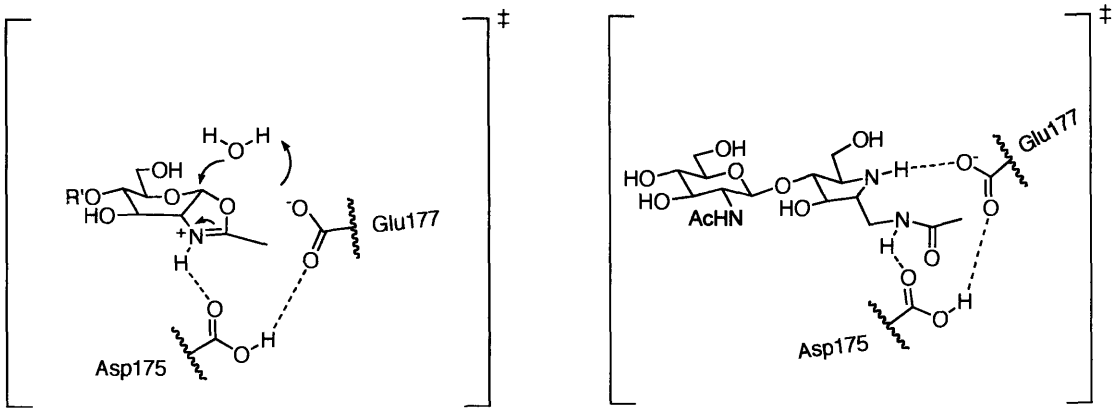
4-4 Conclusion

In summary, the pseudo-disaccharide derivatives including an imino sugar were synthesized based on the expansion of synthetic route developed in Chapter 3, utilizing an Amadori rearrangement, glycosylation of the β-hydroxy ketone and intramolecular reductive amination as key steps. We demonstrated that related derivatives could be generated by the modification of C1 amine by acylation. Their inhibitory activities were evaluated against chitinase and OT. Compound **4-1-2**, **4-1-3** and **4-1-4** showed worse biological activity than **3-2**. Compound **4-1-4** with a long aliphatic chain showed no inhibitory activity up to 200 μM against OT.

4-5 Discussion and future directions

The two pseudo-disaccharides **3-1** and **3-2** exhibited low micromolar inhibitory activity towards the chitinase from *Streptomyces grises*. These results showed the polyhydroxy pyrrolidine moiety effectively mimics the transition state and the carbohydrate linked C4 hydroxyl group of the pyrrolidine increases the binding energy to the active site in the enzyme. In the inhibition study of other enzymes in family 18, the presence of amide bond on the C1 primary amine also played a key role in increasing binding affinity.[1] Therefore, the inhibitory activity of pseudo-disaccharide derivatives against chitinases can be explained by its proper interactions with the key residues Asp 175 and Glu 177, which are essential for the enzyme activity (**Figure 4-2a**). The crystal structure of allosamidin bound to the chitinase supports this mode of inhibition for **3-2**.[19] The reorientation of Asp175 and Glu177 is believed to play an important role in the stabilization of the high-energy transition state of enzyme substrate. The low inhibitory activities of **4-1-2** and **4-1-4** can be explained by a potential interference with the

hydrogen bonding network by bulky groups on the primary amine (**Figure 4-2b**). For the discovery of a potent inhibitor toward chitinase, the addition of functional groups to the transition state analogue linked by the flexible chain will be explored.



a) the proposed transition state of substrate in the active site of chitinase

b) the proposed inhibition mode of 3-2 against chitinase

Figure 4-2. The proposed inhibiton mechanism of pseudo-disaccharide 3-2 against chitinase.

It has been reported that mammalian chitinase is related to allergic responses[20] as discussed in Chapter 1, however, the exact mechanism of action remains unclear. *In vivo* experiments have demonstrated decreased tissue inflammation upon addition of the chitinase inhibitor allosamidin. These observations suggest that chitinase promotes on allergic response in humans. However, several chitinases from insects and fungi also are allergens, and allosamidin has been shown to inhibit chitinases from mammals, insects and fungi. Therefore, the question of whether exogenous chitinases promote the allergic reaction must still be addressed.[21] A synthesis of pseudo-disaccharide derivatives would be a useful strategy for discovering selective inhibitors against chitinases from different

species. The effect of selective inhibitors *in vivo* would elucidate roles that specific chitinases are involved for regulating the allergic responses

The pseudo-disaccharide derivatives showed low micromolar IC_{50} with chitinase yet failed to inhibit OT. These observations can be explained by the mechanistic and structural differences between glycosidases and glycosyltransferases.

A major difference in the mechanisms of glycosyltransferases and glycosidases is that a metal ion plays the role of Lewis acid catalyst in glycosyltransferases, whereas an acidic residue acts as the general acid in glycosidases.[22] The presence of functional groups in glycosidase inhibitors that can interact with the general acid have been shown to be critical for the effective inhibition of glycosidases.[23-25] Therefore, it may be essential to have functional groups that can bind to the active site metal ion, for a strong interaction with OT.

Recently, it has been reported that the essential metal ion induces a conformational change in glycosyltransferases.[26] Upon binding of a sugar substrate to the metal complex in the glycosyltransferase, the induced conformational change switches the catalytic site from open (inactive) to a closed (active) state. A glycosyltransferase with multiple binding sites for ligand moieties changes its structure dramatically to position the anomeric carbon of the sugar donor for nucleophilic attack. The sugar donor-binding site undergoes a marked conformational change to form the closed state. One possible explanation for the lack of inhibition is that the pseudo-disaccharide derivative failed to interact with the open state of OT to induce the conformational change.

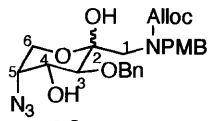
As a future study, the screening of functional groups within the inhibitor candidates, which can effectively induce an enzyme into the active state conformation, will be considered. Malonic and tartaric moieties have been incorporated into the design of potential inhibitors to mimic a pyrophosphate group.[27] Although, these attempts were not successful in increasing binding affinity, we now believed that the investigation of functional groups that can mimic the charge and shape of pyrophosphate is necessary for the effective inhibition of OT and other glycosyltransferases. The synthesis and evaluation of these compounds will provide insight into the mechanism of OT and will serve to guide the design of inhibitors against other glycosyltransferases.

Experimental Section

General Synthetic Procedures

Anhydrous dichloromethane and toluene were distilled from calcium hydride, and anhydrous tetrahydrofuran (THF), was distilled from sodium/benzophenone. All chemicals were purchased from Sigma/Aldrich and used without further purification unless otherwise noted. Analytical thin-layer chromatography (TLC) was carried out on Merck 60 F_{254} 250-μm silica gel plates. All compounds were visualized on TLC by UV irradiation or an aqueous solution of ceric ammonium molybdate (CAM) staining. Flash column chromatography was carried out using forced flow of the indicated solvent on AdTech Flash Silica Gel, 32-63 μm particle size, 60 Å pore size (Adedge technologies). ^{1}H NMR spectra were acquired on a Bruker Avance (DPX) 400 MHz spectrometer, Varian INOVA 500 MHz spectrometer and Bruker Avance (DPX) 600 MHz spectrometer. ^{13} C NMR spectra were acquired on a Bruker Avance (DPX) 400 MHz spectrometer and Varian INOVA 500 MHz spectrometer. Chemical shifts (δ) are reported in parts per million (ppm) with chemical shifts referenced to internal standards: $CDCl_3$ (7.27 ppm for ^{1}H, 77.23 ppm for ^{13}C), CD_3OD (4.87 ppm for ^{1}H, 49.15 ppm for ^{13}C), D_2O (4.80 ppm for ^{1}H), C_6D_6 (7.16 ppm for ^{1}H, 128.39 ppm for ^{13}C). Coupling constants (*J*) are reported in Hertz (Hz) and multiplicities are abbreviated as singlet (s), doublet (d), triplet (t), multiplet (m), broadened singlet (br) and doublet of doublets (dd). The term "app d" is used to denote a triplet with two similar coupling constants and "app t" is used to denote a doublet of doublets (dd) with similar coupling constants. High resolution Mass Spectra (HRMS) were obtained at the Mass Spectrometry Facility at MIT (Cambridge, MA).

5-Azido-3-*O*-benzyl-1,5-dideoxy-1-[*N*-(4-methoxybenzyl)-allyloxycarbonylamino]-D-fructopyranose (4-8)

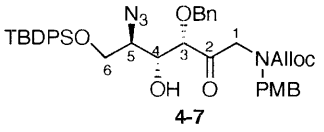


4-8

AllocCl (0.160 mL, 1.5 equiv.) was added to a solution of **3-8** (520 mg, 1.25 mmol) and DIPEA (0.435 mL, 2.0 equiv.) in methanol (20 mL) at 0 °C and the reaction mixture was warmed to room temperature for 2 h. The mixture was stirred for 1 h at room temperature. The solvent was removed under reduced pressure. Flash column chromatography (hexane: ethyl acetate = 2:1 to ethyl acetate) of the crude mixture gave **4-8** (467 mg, 75 %) as a colorless oil (a mixture of anomers and N-rotamers).

^{1}H NMR ($CDCl_3$, 400 MHz) selected peaks δ 2.46 (br, 1H, alcohol), 3.35 (d, 1H, J = 9.7 Hz, H_{1a}), 3.75-3.90 (m, 6H, H_3, H_{6a}, H_{6b}, and $NHCH_2PhOMe$), 3.84 (d, 1H, J = 9.7 Hz, H_{1b}), 4.07-4.81 (m, 3H, $NHCH_2PhOMe$, H_5), 4.61-4.81(m, 5H, H_4, OCH_2Ph and OCH_2CHCH_2) 5.24-5.28 (m, 2H, OCH_2CHCH_2), 5.85-5.96 (m, 1H, OCH_2CHCH_2), 6.87-7.80 (m, 9H, $NHCH_2PhOMe$ and OCH_2Ph).

***N*-[(3*R*,4*R*,5*R*)-5-Azido-3-*O*-benzyly-6-*O*-(*tert*-butyldiphenylsily)-4-hydroxy-2-oxo-hexyl]-*N*-(4-methoxybenzyl)-allyloxycarbonylamine (4-7)**



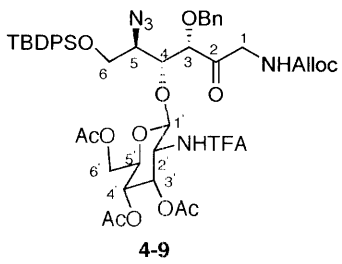
4-7

TBDPSCl (0.487 mL, 2.0 equiv.) was added to the solution of **4-6** (467 mg, 0. 937 mmol) and imidazole (192 mg, 3.0 equiv.) in CH_2Cl_2 (20 mL) at 0 °C and the reaction mixture was warmed to room temperature for 2 h. The mixture was stirred until starting material remained (usually for 8 h.). The reaction mixture was poured into satd. $NaHCO_3$ (20 mL). The mixture was extracted with CH_2Cl_2 (3 x 20 mL) and the combined organic layers were washed with satd. brine, dried over $MgSO_4$ and concentrated *in vacuo*. Flash column chromatography (hexane: ethyl acetate = 4:1 to hexane: ethyl acetate = 2:1) of the crude oil gave the desired compound **4-7** (0.552 mg, 80%) as colorless oil (a mixture of N-rotamers).

^{1}H NMR (C_6D_6, 400 MHz) selected peaks δ 1.17 (s, 9H, Me_3CPh$_2$Si-), 3.27 (s, 3H, -NHCH$_2$PhO*Me*), 4.20 (d, 1H, *J* = 1.7 Hz, H$_3$), 4.25 and 4.36 (ABq, 2H, *J* =12.2 Hz, -O*CH2*Ph), 4.95 (app d, 1H, *J* = 10.4 Hz, -OCH$_2$CH*CH$_2$*), 5.13 (app d, *J* =14.9 Hz 1H) 5.62-5.78 (m, 1H, -OCH$_2$*CH*CH$_2$) 6.72 and 7.00 (2*d, 4H, *J* = 8.2 Hz, -NHCH$_2$*Ph*OMe), 7.15-7.80 (m, 15H, -OCH$_2$*Ph* and Me$_3$C*Ph$_2$*Si-);

^{13}C NMR (C_6D_6, 125 MHz) δ 20.29, 20.33, 27.86, 27.90, 50.30, 52.56, 54.63, 55.68, 55.73, 64.81, 64.87, 65.72, 66.10, 67.39, 67.66, 72.63, 74.74, 75.05, 84.44, 85.68, 115.23, 118.29, 128.37, 129.54, 129.63, 129. 69, 129.78, 130.13, 130.52, 131.03, 131.16, 133.89, 134.14, 134.34, 136.93, 136.98, 137.96, 157.83, 160.60, 207.01, 208.39.

***N*-[(2*R*,3*R*,5*R*)-5-Azido-3-*O*-benzyl-6-*O*-(*tert*-butyldiphenylsilyl)-4-*O*-(3',4',6'-tri-*O*-acetyl-2'-deoxy-2'-trifluroacetamide-β-D-glucopyranosyl)-2-oxo-hexyl]-allyloxycarbonylamine (4-9)**



4-9

BF_3•OEt_2 (1.8 μl, 0.05 equiv.) was added to a solution of imidate **3-29** (0.300 mg, 2.0 equiv.) and **4-7** (205 mg, 0.28 mmol) in toluene (15 mL) at 0 °C under argon. The mixture was allowed to warm to room temperature and stirred at room temperature until no starting material remained (usual total reaction time 8 h). The reaction mixture was poured into water (20 mL). The mixture was extracted with CH_2Cl_2 (3 x 20mL) and the combined organic layers were washed with satd. $NaHCO_3$ and satd. brine, dried over $MgSO_4$ and concentrated *in vacuo*. Flash column chromatography (hexane: ethyl acetate = 4:1 to hexane; ethyl acetate = 2:1) of the crude oil gave the desired compound **4-8** as the colorless liquid.

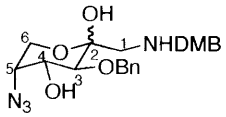
A solution of CAN (452 mg, 3.0 equiv.) in H_2O (1.0 mL) was added dropwise to a vigorously stirred solution of **4-8** in MeCN (10 mL) at – 20 °C and stirred for 3 h at – 20 °C. The reaction mixture was treated with additional CAN (151 mg, 1.0 equiv.), warmed within 3 h to 0 °C and stirred for 2 h at 0 °C. The yellow-orange solution was

poured into water. The mixture was extracted with ethyl acetate (3 x 30 mL) and the combined organic layers were washed with 10 % sodium sulfite solution, satd. $NaHCO_3$ and satd. brine, dried over $MgSO_4$ and concentrated *in vacuo*. Flash column chromatography (hexane:ethyl acetate = 4:1 to hexane;ethyl acetate = 2:1) of the crude oil gave the desired compound **3-31** as the white liquid (238 mg, 2 step 85%).

^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.06 (s, 9H, Me_3CPh_2Si-), 1.94 (s, 3H, acetyl), 1.98 (s, 3H, acetyl), 2.00 (s, 3H, acetyl), 3.15-3.22 (m, 1H, H_2), 3.58-3.61 (m, 1H, $H_{5'}$), 3.69 (ABx, 1H, J = 10.8 and 3.3 Hz, H_{6a}), 3.80-3.85 (m, 2H, H_2, H_4), 4.08-4.20 (m, 4H, H_{1b}, H_3, $H_{6'a}$ and $H_{6'b}$), 4.32-4.40 (m, 3H, $H_{1'}$, -OCH_2CHCH_2) 4.54-4.55 (m, 2H, H_{1a} and H_{1b}), 4.42 and 4.63 (ABq, 2H, J = 11.3 Hz, -OCH_2Ph), 4.83-4.85 (m, 2H, $H_{3'}$ and $H_{4'}$), 4.98 (app t, 1H, J = 9.3 Hz), 5.17 (app d, J = 10.4 Hz, -OCH_2CHCH_2) 5.25 (app d, J = 15.6 Hz, -OCH_2CHCH_2), 5.27 (brs, 1H, amide), 5.75-5.89 (m, 1H, -OCH_2CHCH_2), 7.23-7.70 (m, 15H, -OCH_2Ph and Me_3CPh_2Si-);

^{13}C NMR ($CDCl_3$, 100 MHz) δ 20.73, 21.00, 21.25, 23.20, 27.22, 48.96, 54.92, 61.38, 62.80, 66.21, 68.84, 71.83, 71.93, 72.27, 74.68, 77.01, 83.43, 99.59, 116.54 128.41, 128.66, 128.79, 129.03, 130.68, 131.06, 132.61, 133.06, 136.10, 136.18, 136.68, 152.45, 169.78, 170.88, 171.12, 171.50, 207.24.

5-Azido-3-*O*-benzyl-1,5-dideoxy-1-*N*-(2,4-dimethoxybenzyl-amino)-D-fructopyranose (4-10)



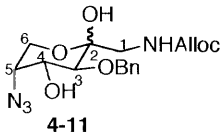
4-10

2,4-Dimethoxybenzylamine (0.504 mL, 1.0 equiv.) was added to a solution of **3-6** (990 mg, 3.35 mmol) and acetic acid (0.192 mL, 1.0 equiv.) in methanol (50 mL) at room temperature and the reaction mixture was warmed to 40 °C. The mixture was stirred for 2 h at 40 °C. The solvent was removed reduced pressure. Flash column chromatography (CH_2Cl_2: MeOH = 20:1 to 15:1) of the crude oil gave the desired prodcut **4-10**, as the acetic salt form (1.26 g, 85%) (a mixture of anomers).

^{1}H NMR ($CDCl_3$, 600 MHz) selected peaks δ 2.65-2.67 (m, 1H, H_{1a}), 2.92 (d, 0.5H of anomeric mixture, J = 12.0 Hz, H_{1b}), 3.06 (d, 0.5H of anomeric mixture, J = 12.3 Hz, H_{1b}), 3.46 (d, 0.5H of anomeric mixture, J = 9.4 Hz, H_3), 3.51-3.52 (m, 0.5H of anomeric mixture, H_5), 3.53-3.54 (m, 0.5H of anomeric mixture, H_5), 3.75 (s, 3H, -$NHCH_2Ph(OMe)_2$), 3.82 (s, 3H, -NH$C H_2$Ph$(OMe)_2$), 3.88-3.95 (m, 2H, -$NHCH_2Ph(OMe)_2$), 4.04 (app d, 0.5H of anomeric mixture, J = 12.4 Hz, H_{6b}), 4.10 (app d, 0.5H of anomeric mixture, J = 12.5 Hz, H_{6b}), 4.35 (dd, 1 of anomeric mixture, J = 9.4 Hz, J = 3.5 Hz, H_4), 4.36 and 4.60 (ABq, 1H of anomeric mixture, J = 11.5 Hz, -OCH_2Ph), 4.66 and 4.83 (ABq, 12H of anomeric mixture, J = 11.5 Hz, -OCH_2Ph), 5.61 (brs, alcohol), 6.44-6.46 (m, 2H, -$NHCH_2Ph(OMe)_2$), 7.04-7.34 (m, 5H, -OCH_2Ph);

HRMS(ESI-MS): Calcd for $[C_{22}H_{28}N_4O_6+H]^+$(M+H): m/z = 445.2082; Found: 446.2155.

1-Allyloxycarbonylamino-5-azido-3-*O*-benzyl-1,5-dideoxy-D-fructopyranose (4-11)



4-11

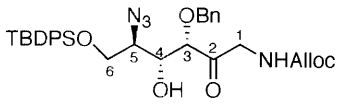
AllocCl (0.451 mL, 1.5 equiv.) was added to a solution of **4-10** (1.26 g, 2.84 mmol) and DIPEA (0.989 mL, 2.0 equiv.) in methanol (40 mL) at 0 °C and the reaction mixture was warmed to room temperature for 2 h. The mixture was stirred for 1 h at room temperature. The solvent was removed under reduced pressure. Without further purification, TFA (30 mL) was added to the crude mixture at 0 °C. The reaction mixture was warmed to room temperature for 2 h. The mixture was stirred for 1 h at room temperature. The solvent was removed under reduced pressure and poured into satd. $NaHCO_3$ (40 mL). The mixture was extracted with ethyl acetate (3 x 50 mL) and the combined organic layers were washed with satd. brine, dried over $MgSO_4$ and concentrated *in vacuo*. Flash column chromatography (hexane:ethyl acetate = 4:1 to ethyl acetate) of the crude oil gave **4-11** (677 mg, 2 steps over 63%) as colorless oil (a mixture of anomers).

^{1}H NMR (CD_3OD, 500 MHz) 3.09 (d, 1H, J = 13.6 Hz, H_{1a}), 3.54-3.61 (m, 2H, H_{1b} and H_3), 3.62 (app d, 1H, J = 12.6 Hz, H_{6a}), 3.81 (d, 1H, J = 1.9 Hz, H_5), 4.07 (app d, 1H, J = 12.6 Hz, H_{6b}), 4.17 (dd, 1H, J = 9.6 Hz and J = 1.9 Hz, H_4), 4.63 and 4.89 (ABq, 2H, J = 10.5 Hz, -O*CH*$_2$Ph), 4.51-4.59 (m, 2H, -O*CH*$_2$CHCH$_2$), 5.15 (app d, 1H, J = 10.5 Hz, -OCH$_2$CH*CH*$_2$), 5.28 (app d, 1H, J = 17.4 Hz, -OCH$_2$CH*CH*$_2$), 5.87-5.92 (m, 1H, -OCH$_2$*CH*CH$_2$), 7.22-7.44 (m, 5H, -OCH$_2$*Ph*);

^{13}C NMR (CD_3OD, 125 MHz) δ 47.64, 62.16, 61.26, 65.47, 66.67, 72.45, 76.66, 78.13, 99.00, 117.61, 128.84, 129.38, 129.84, 134.62, 140.08, 158.93;

HRMS(ESI-MS): Calcd for $[C_{17}H_{22}N_4O_6+Na]^+$(M+Na): m/z = 401.1432; Found: 401.14232.

***N*-[(3*R*,4*R*,5*R*)-5-Azido-3-*O*-benzyl-6-*O*-(*tert*-butyldiphenylsilyl)-4-hydroxy-2-oxo-hexyl]-allylcarbamate (4-12)**



4-12

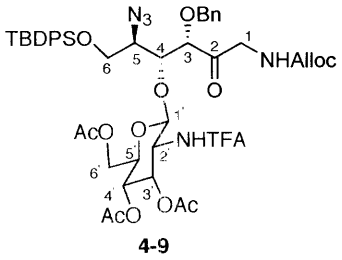
TBDPSCl (0.930 mL, 2.0 equiv.) was added to the solution of **4-11** (677 mg, 1.79 mmol) and imidazole (367 mg, 3.0 equiv.) in CH_2Cl_2 (50 mL) at 0 °C and the reaction mixture was warmed to room temperature for 2 h. The mixture was stirred until no starting material remained (usually for 24 h). The reaction mixture was poured into satd. $NaHCO_3$ (20 mL). The mixture was extracted with CH_2Cl_2 (3 x 20 mL) and the combined organic layers were washed with satd. brine, dried over $MgSO_4$ and concentrated *in vacuo*. Flash column chromatography (hexane:ethyl acetate = 5:1 to hexane:ethyl acetate = 2:1) of the crude oil gave the desired compound **4-12** (795 mg, 72 %) as a colorless oil.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.08 (s, 9H, *Me*$_3$CPh$_2$Si), 2.68 (brs, alcohol), 3.52-3.59 (m, 1H, H_5), 3.76-3.80 (m, 1H, H_4), 3.87 (ABx, 1H, J = 6.5 Hz and J = 10.9 Hz, H_{6a}), 4.03 (ABx, 1H, J = 3.0 Hz, J = 10.9 Hz, H_{6b}), 4.13 (d, 1H, J = 19.9 Hz, H_{1a}), 4.22 (d, 1H, J = 1.3 Hz, H_3), 4.32 (d, 1H, J = 19.9 Hz, H_{1b}), 4.50-4.53 (m, 2H, -O*CH*$_2$CHCH$_2$),

4.51 and 4.70 (ABq, 2H, J =11.3 Hz, -OCH_2Ph), 5.16 (app d, 1H, J = 10.5 Hz, -OCH_2CHCH_2), 5.26 (app d, 1H, J = 17.0 Hz, -OCH_2CHCH_2), 5.49 (brs, amide), 5.80-5.89 (m, 1H, -OCH_2CHCH_2), 7.27-7.69 (m, 15H, -OCH_2Ph and Me_3CPh_2Si-);

^{13}C NMR ($CDCl_3$, 125 MHz) δ 19.32, 26.91, 48.59, 63.17, 64.76, 66.19, 72.03, 74.58, 76.98, 77.23, 77.49, 83.31, 118.09, 128.01, 128.05, 128.06, 128.38, 128.82, 128.86, 128.89, 128.95, 130.10, 130.15, 130.17, 132.65, 132.80, 132.87, 135.78, 136.40, 156.56, 207.73.

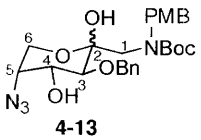
N-[(2R,3R,5R)-5-Azido-3-O-benzyl-6-O-(tert-butyldiphenylsilyl)-4-O-(3',4',6'-tri-O-acetyl-2'-deoxy-2'-trifluroacetamido-β-D-glucopyranosyl)-2-oxo-hexyl]-allylcarbamate (4-9)



4-9

BF_3•OEt_2 (7.0 μl, 0.05 equiv.) was added to a solution of imidate **3-29** (1.20 g, 2.0 equiv.) and **4-12** (680 mg, 1.10 mmol) in toluene (50 mL) at 0 °C under argon. The mixture was allowed to warm to room temperature and stirred until no starting material remained. The reaction mixture was poured into water (20 mL). The mixture was extracted with CH_2Cl_2 (3 x 50mL) and the combined organic layers were washed with satd. $NaHCO_3$ and satd. brine, dried over $MgSO_4$ and concentrated *in vacuo*. Flash column chromatography (hexane:ethyl acetate = 3:1 to hexane;ethyl acetate = 2:3) of the crude oil gave the desired compound **4-9** (990 mg, 90 %) as the colorless liquid, which showed the identical spectroscopic properties(^{1}H NMR and ^{13}C NMR) from the obtained compound from **4-7**.

5-Azido-3-O-benzyl-1,5-dideoxy-1-N-(4-methoxybenzyl-tert-butyloxycarbonylamino)-D-fructopyranose (4-13)

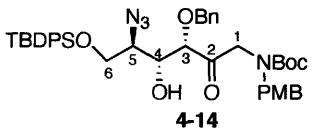


4-13

Boc_2O (200 mg, 1.2 equiv.) was added to a solution of **3-18** (317 mg, 0.77 mmol) and DIPEA (0.267 mL, 2.0 equiv.) in CH_2Cl_2 (20 mL) at 0 °C and the reaction mixture was warmed to room temperature for 2 h. The mixture was stirred for 1 h at room temperature. The solvent was removed under reduced pressure. Flash column chromatography (hexane:ethyl acetate = 2:1 to ethyl acetate) of the crude oil gave **4-13** (285 mg, 75 %) as colorless oil (mixture of anomers and N-rotamers).

^{1}H NMR ($CDCl_3$, 400 MHz) selected peaks δ 1.36-1.40 (m, 9H, Me_3CCO) 2.63 (br, 1H, alcohol), 3.41 (d, 1H, J = 9.3 Hz, H_{1a}), 3.72-3.82 (m, 6H, H_{1b}, H_3, H_{6a}, H_{6b}, and -$NHCH_2PhOMe$), 4.07-4.81 (m, 3H, -$NHCH_2PhOMe$, H_5), 4.57 and 4.85 (ABq, 2H, J = 115. Hz), 4.82-4.80 (m, 1H, H_4), 6.87-7.80 (m, 9H, -$NHCH_2PhOMe$ and -OCH_2Ph).

N-[(3R,4R,5R)-5-Azido-3-O-benzyl-6-O-(tert-butyldiphenylsilyl)-4-hydroxy-2-oxo-hexyl]-N-(4-methoxybenzyl)-tert-butylcarbamate (4-14)



4-14

TBDPSCl (0.192 mL, 2.0 equiv.) was added to the solution of **4-13** (285 mg, 0. 554 mmol) and imidazole (113 mg, 3.0 equiv.) in CH_2Cl_2 (20 mL) at 0 °C and the reaction mixture was warmed to room temperature for 2 h. The mixture was stirred until no starting material remained (usually for 8 h.). The reaction mixture was poured into satd. $NaHCO_3$ (20 mL). The mixture was extracted with CH_2Cl_2 (3 x 20 mL) and the combined organic layers were washed with satd. brine, dried over $MgSO_4$ and concentrated *in vacuo*. Flash column chromatography (hexane: ethyl acetate = 4:1 to hexane:ethyl acetate = 2:1) of the crude oil gave the desired compound **4-7** (315 mg, 80 %) as colorless oil (a mixture of N-rotamers).

^{1}H NMR (C_6D_6, 400 MHz) selected peaks δ 1.17-1.19 (m, 9H, Me_3CPh_2Si-), 1.35-1.50 (m, 9H, Me_3CCO-), 3.29 (s, 3H, -$NHCH_2PhOMe$), 3.71 (d, 1H, J = 18.5 Hz, H_{1a}), 4.15 (dd, 1H, J = 2.2 and 11.3 Hz, H_4), 4.21 (d, 1H, J = 2.2 Hz, H_3), 4.25 and 4.47 (ABq, J = 10.5 Hz, -OCH_2Ph), 4.33 (d, 1H, J = 18.5 Hz, H_{1b}), 6.73 and 7.09 (2*d, 4 H, J = 8.5 Hz, -$NHCH_2PhOMe$), 7.15-7.80 (m, 15H, -OCH_2Ph and Me_3CPh_2Si-);

^{13}C NMR (C_6D_6, 125 MHz) δ 19.80, 27.33, 28.63, 28.78, 51.13, 52.64, 54.65, 55.14, 55.18, 64.22, 64.32, 65.00, 65.60, 72.04, 72.32, 74.18, 74.51, 80.22, 81.19, 83.53, 85.35, 114.65, 128.68, 128.74, 129.10, 129.18, 129.59, 130.22, 130.44, 130.58, 133.83, 133.87, 136.38, 136.47, 137.82, 155.98, 159.97, 206.14, 208.14.

Phenyl-3',4',6'-tri-*O*-acetyl-2'-deoxy-2'-(triflouroacetamide) -β-D-1-thio-glucopyranoside (4-15)

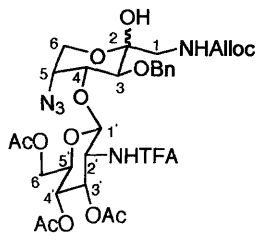
4-15

BF_3•OEt_2 (0.148 ml, 1.0 equiv.) was added to **3-28** (519 mg, 1.17 mmol) and PhSH (0.120 mL, 1.0 equiv) in CH_2Cl_2 (30 mL) at 0 °C under argon. The mixture was allowed to warm to room temperature and stirred for 4 h. The reaction mixture was poured to water (20 mL). The mixture was extracted with CH_2Cl_2 (3 x 40 mL) and the combined organic layers were washed with satd. $NaHCO_3$, dried over $MgSO_4$ and concentrated *in vacuo*. Flash column chromatography (hexane:ethyl acetate = 8:1 to hexane;ethyl acetate = 3:1) of the crude oil gave the desired compound **3-30** (565mg, 98 %) as a white liquid.

^{1}H NMR ($CDCl_3$, 400 MHz) 1.96 (s, 3H, acetyl), 2.07 (s, 3H, acetyl), 2.10 (s, 3H, acetyl), 3.72-3.76 (m, 1H, H_5), 4.07 (dd, 1H, J = 10. 4 and 9.8 Hz, H_2), 4.18-4.26 (m, 2H, H_{6a} and H_{6b}), 4.80 (d, 1H, J = 10. 4 Hz, H_1), 5.05 (app t, 1H, J = 9.8 Hz, H_4), 5.27 (app t, 1H, J = 9.8 Hz, H_3), 6.81 (br, amide), 7.49-7.52 (m, 5H, -SPh);

^{13}C NMR ($CDCl_3$, 100 MHz) δ 20.79, 20.88, 21.15, 22.04, 53.74, 60.83, 68.53, 70.62, 73.67, 80.82, 114.51, 117.38, 129.21, 129.50, 131.48, 133.88, 157.34, 158.71, 159.43, 169.50, 169.67, 171.71.

1-Allyloxycarbonylamino-5-azido-3-*O*-benzyl-1,5-dideoxy-4-*O*-(3',4',6'-tri-*O*-acetyl -2'-deoxy-2'-trifluroacetamido-β-D-glucopyranosyl)-D-fructopyranose (4-16)



4-16

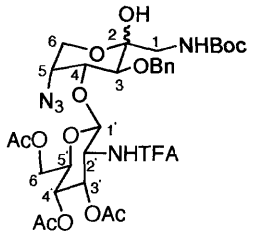
TBAF (1.0 M solution in THF, 0.510 mL, 1.0 equiv.) was added to a solution of **4-9** (515 mg, 0.51 mmol) and acetic acid (0.029 mL, 1.0 equiv.) in THF (10 mL) at 0 °C. The mixture was allowed to warm to room temperature and stirred overnight. The solvent was removed under reduced pressure, followed by purification *via* column chromatography (hexane:ethyl acetate = 4:1 to ethyl acetate) to afford **4-16** (330 mg, 85 %) as an white oil.

^{1}H NMR δ (CD_3OD, 400 MHz) 1.83 (s, 3H, acetyl), 1.87 (s, 3H, acetyl), 1.91 (s, 3H, acetyl), 2.99 (d, 1H, J = 13.7 Hz, H_{1a}), 3.44 (d, 1H, J = 13.7 Hz, H_{1b}), 3.53 (d, 1H, J = 9.6 Hz, H_3), 3.65 (app d, 1H, J = 12.5 Hz, H_{6a}), 3.67-3.69 (m, 1H, $H_{5'}$), 3.83-3.96 (m, 4H, H_4, H_{6b}, $H_{6'a}$, and $H_{6'b}$), 4.20 (app dd, 1H, J = 12.4 and 3.9 Hz, H_5), 4.28 (dd, 1H, J = 9.4 and 3.9 Hz, H_4), 4.36-4.47 (m, 2H, -O*C*H_2CHCH$_2$), 4.51 and 5.23 (ABq, 2H, J = 10.7 Hz, -O*C*H_2Ph), 4.78-4.94 (m, 2H, $H_{1'}$ and $H_{4'}$), 5.04 (app t, 1H, J = 9.4 Hz, $H_{3'}$), 5.16-5.20 (m, 2H, -OCH$_2$CH*C*H_2), 5.76-5.85 (m, 1H, -OCH$_2$*C*HCH$_2$), 7.12-7.37 (m, 5H, -OCH$_2$*Ph*);

^{13}C NMR (CD_3OD, 100 MHz) δ 20.53, 20.73, 20.88, 20.96, 47.35, 56.42, 60.29, 61.48, 63.22, 66.62, 70.07, 73.16, 73.34, 75.80, 76.37, 78.70, 98.68, 99.15, 117.56, 116.34, 117.56, 118.62, 128.83, 129.30, 129.64, 130.13, 134.58, 140.07, 158.91, 159.31, 159.65, 171.35, 171.78, 172.50;

HRMS: [Calcd for $C_{31}H_{38}F_3N_5O_{14}$+Na]$^+$(M+Na): m/z = 784.2260; Found: 784.2279.

5-Azido-3-*O*-benzyl-1-*tert*-butyloxycarbonylamino-1,5-dideoxy-4-*O*-(3',4',6'-tri-*O*-acetyl-2'-deoxy-2'-trifluroacetamido-β-D-glucopyranosyl))-D-fructopyranose (4-3)



4-3

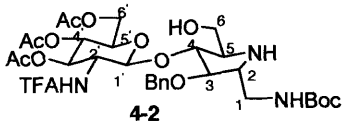
Boc_2O (48 mg, 1.3 equiv.) was added to a solution of **4-16** (130 mg, 0.17 mmol) in CH_2Cl_2. To this mixture a solution of $Pd(PPh_3)_4$ (9.8 mg, 0.05 equiv.) in the same solvent was added, immediately followed by the addition of Bu_3SnH (50 μl, 1.1 equiv.) at 0 °C. The mixture was allowed to warm to room temperature and stirred for 4 h. The solvent was removed under reduced pressure, followed by purification *via* column chromatography (hexane: ethyl acetate = 4:1 to ethyl acetate) to afford **4-3** (122 mg, 92 %) as a colorless oil.

^{1}H NMR (CD_3OD, 500 MHz) 1.46 (s, 9H, *Me*$_3$CCO-), 2.00 (s, 3H, acetyl), 2.02 (s, 3H, acetyl), 2.04 (s, 3H, acetyl), 3.06 (d, 1H, J = 13.8 Hz, H_{1a}), 3.54 (d, 1H, J = 13.8 Hz, H_{1b}), 3.67 (d, 1H, J = 9.6 Hz, H_3), 3.80 (app d, 1H, J =13.1 Hz, H_{6a}), 3.85-3.88 (m, 1H, $H_{5'}$), 3.95-4.09 (m, 4H, H_4, H_{6b}, $H_{6'a}$, and $H_{6'b}$), 4.35 (app dd, 1H, J = 12.3 and 3.9 Hz, H_5), 4.41 (dd, 1H, J = 9.6 and 3.9 Hz, H_4), 4.64 and 4.98 (ABq, 2H, J = 10.3 Hz, O*C*H_2Ph), 5.00 (d, 1H, J = 8.3 Hz, $H_{1'}$), 5.04 (app t, 1H, J = 9.6 Hz, $H_{4'}$), 5.38 (app t, J = 9.6 Hz, $H_{3'}$), 5.98 (br, 1H, amide), 7.29-7.52 (m, 5H, -OCH$_2$*Ph*);

^{13}C NMR (CD_3OD, 100 MHz) δ 20.52, 20.73, 20.87, 20.97, 22.59, 28.87, 29.42, 56.41, 60.22, 61.46, 63.20, 70.05, 73.15, 73.31, 75.84, 76.39, 78.68, 80.47, 98.64, 99.25, 115.05, 116.32, 118.60, 119.88 128.84, 129.30, 129.64, 130.14, 140.05, 158.44, 159.34, 159.64, 171.34, 171.76, 172.48;

HRMS: [Calcd for $C_{32}H_{42}F_3N_5O_{14}$+Na]$^+$(M+Na): *m/z* = 800.257; Found: 800.258.

N-{[(2R,3R,4R,5R)-3-O-benzyl-4-O-(3',4',6'-tri-O-acetyl-2'-deoxy-2'-trifluroacetamido-β-D-glucopyranosyl)-5-(hydroxymethyl)pyrrolidin-2-yl]methyl} *tert*-butylcarmate (4-2)



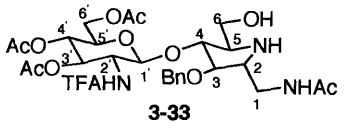
4-2

To a solution of **4-3** (70 mg, 0.090 mmol) in methanol (10 mL) was added $Pd(OH)_2$ (35 mg). The reaction mixture was stirred under hydrogen pressure (850 psi) for 12 h. and filtered through celite. After solvent was removed *in vacuo*, the residue was purified by flash column chromatography (chloroform: methanol = 8:1 (1 % of ammonium hydroxide) to 7:1 (1 % of ammonium hydroxide)) to afford **4-2** (36 mg, 55 %) as a colorless oil.

^{1}H NMR (CD_3OD, 500 MHz) δ 1.43 (s, 9H, Me_3CCO-), 1.94 (s, 3H, acetyl), 1.96 (s, 3H, acetyl), 1.99 (s, 3H, acetyl), 3.15-3.20 (m, 2H, H_2 and H_5), 3.60-3.63 (m, 2H, H_{6a} and H_{6b}), 3.75-3.79 (m, 1H, $H_{5'}$), 3.94 (dd, 1H, *J* = 8.5 and 9.8 Hz, $H_{2'}$), 3.98-3.99 (m, 1H, H_3), 4.10 (ABx, 1H, *J* = 2.1 and 12.3 Hz, $H_{6'a}$), 4.21-4.24 (m, 2H, H_4 and $H_{6'b}$), 4.60 and 4.71 (ABq, 2H, *J* = 11.7 Hz, -OCH_2Ph), 4.89 (d, 1H, *J* = 8.5 Hz, $H_{1'}$), 5.04 (app t, 1 H, *J* = 9.8 Hz, $H_{4'}$), 5.30 (app t, 1H, *J* = 9.8 Hz, $H_{3'}$), 7.25-7.36 (m, 5H, -OCH_2*Ph*) ;

HRMS(ESI-MS): Calcd for [$C_{32}H_{44}F_3N_3O_{13}$+H]$^+$(M+H): *m/z* = 736.2899; Found: 736.2895.

N-{[(2R,3R,4R,5R)-3-O-benzyl-4-O-(3',4',6'-tri-O-acetyl-2'-deoxy-2'-trifluroacetamido-β-D-glucopyranosyl)-5-(hydroxymethyl)pyrrolidin-2-yl]methyl}acetamide (3-33)

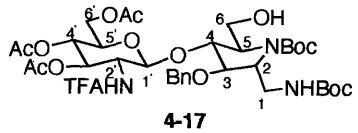


3-33

To a solution of compound **4-2** (40 mg, 0.074mmol) in CH_2Cl_2 (3 mL) was added TFA (1 mL) at 0 °C. The reaction mixture was warmed to room temperature. The reaction mixture was stirred at room temperature for 4 h. After the solvent was removed *in vacuo*, the residue was filter through Dowex 1X8 200-400 chloride form resin and used for the next reaction step without further purification.

To a solution of diamine in MeOH at 0oC was added acetic anhydrde at 0 °C. The reaction mixture was stirred at 0 oC for 1 h. After the solvent removed *in vacuo*, the residue was purified by flash column chromatography ($CHCl_3$:MeOH = 8:1 (1 % of ammonium hydroxide) to 7:1 (1 % of ammonium hydroxide)) to afford **3-33** (20 mg, 50 %) as a colorless oil, which showed the identical spectroscopic properties (^{1}H NMR and ^{13}C NMR) from the obtained compound from **3-32**

***N-tert*-Butyloxycarbonyl-[(2R,3R,4R,5R)- 3-O-benzyl-2-(*N-tert*-butyloxycarbonylamino -methyl)-4-O-(3',4',6'-tri-O-acetyl-2'-deoxy-2'-trifluroacetamido-β-D-glucopyranosyl)-5-(hydroxymethyl)]-pyrrolidine (4-17)**



4-17

To a solution of **4-2** (35 mg, 0.048 mmol) in CH_2Cl_2 in the presence of DIPEA (16.7 μl, 2.0 eq.) was added

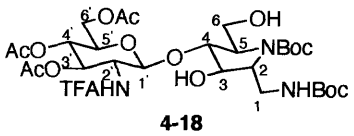
Boc_2O (14.7 mg, 1.4 eq.) at 0 °C. The reaction mixture was stirred at room temperature overnight. After the solvent was removed *in vacuo*, the residue was purified by column chromatography (hexane:ethyl acetate = 3:1 to 1:1) to give the desired product **4-17** (34 mg, 85 %) as a colorless oil (a mixture of N-rotamers).

^{1}H NMR (CD_3OD, 500 MHz) selected peaks δ 1.43-160 (m, 18H, 2*Me_3CCO), 2.01-2.08 (m, 9H, 3*acetyl), 2.87-2.91 (m, 0.6H of N-rotamers), 3.05-3.09 (m, 0.4H of N-rotamers), 3.44-3.93 (m, 7H), 4.02-4.08 (m, 1H), 4,12 and 4.42 (ABq, 2H, J = 11.7 Hz, OCH_2Ph), 4.24-4.27 (m, 1H, $H_{6'b}$) 4.25-4.30 (m, 1H, $H_{6'b}$), 4.52-4.58 (m, 2H, H_3 and H_4), 4.81 (d, J = 8.0 Hz, 0.6H of N-rotamers, $H_{1'}$), 5.01 (m, 1H, $H_{4'}$), 5.28 (app t, 0.6H of N-rotamers, J = 9.6 Hz, $H_{3'}$), 5.37 (app t, 0.4H of N-rotamers, J = 9.6 Hz, $H_{3'}$), 7.34-7.41 (m, 5H, OCH_2Ph);

^{13}C NMR (CD_3OD, 125 MHz) δ 19.64, 19.66, 19.84, 20.04, 20.05, 27.88, 27.95, 28.12, 40.19, 40.69, 55.42, 59.45, 60.07, 62.05, 62.18, 63.83, 64.35, 66.15, 66.29, 68.15, 66.29, 68.97, 69.13, 71.47, 71.51, 71.97, 72.27, 72.44, 79.33, 79.40, 80.85, 81.26, 82.08, 82.61, 83.66, 99.13, 99.42, 113.130, 115.41, 117.69, 119.98, 128.10, 128.14, 128.18, 128.81, 128.84, 138.40, 138.45, 154.90, 155.10, 157.32, 157.45, 158.21, 158.34, 158.52, 158.63, 170.47, 170.83, 170.86, 171.46, 171.50;

HRMS(ESI-MS): Calcd for $[C_{37}H_{52}F_3N_3O_{15}+Na]^+$(M+Na): m/z = 858.3243; Found: 858.3258.

N-tert-Butyloxycarbonyl-[(2_R_,3_R_,4_R_,5_R_)-2-(_N-tert_-butyloxycarbonyl amino-methyl)-4-_O_-(3',4',6'-tri-_O_-acetyl-2'-deoxy-2'trifluroacetamido-β-D-glucopyranosyl)-3-hydroxy-5-(hydroxymethyl)]-pyrrolidine (4-18)



4-18

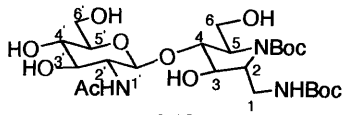
To a solution of **4-17** (45 mg, 0.039 mmol) in AcOH (10 mL) was added $Pd(OH)_2$ (30 mg). The reaction mixture was stirred under 50 psi hydrogen pressure for 24 h and filtered through celite. After the solvent was removed *in vacuo*, the residue was purified by column chromatography (hexane:ethyl acetate =1:1 to ethyl acetate) to give the desired product **4-18** (25 mg, 85 %) (mixture of N-rotamers).

^{1}H NMR (CD_3OD, 600 MHz) δ 1.34-1.42 (m, 18H, 2*Me_3CCO), 1.88-2.05 (m, 9H, 3*acetyl), 2.85-2.89 (m, 0.5H of rotamer), 3.05-3.08 (m, 0.5H of rotamer), 3.32-3.33 (m, 0.5H of rotamer), 3.46-3.47 (m, 0.5H of rotamer), 3.61-3.86 (m, 6H), 4.11-4.22 (m, 4H), 4.83 (d, 0.5H of rotamer, J = 8.3 Hz, $H_{1'}$), 4.94 (app t, 1H, J = 9.6 Hz, $H_{4'}$), 5.23 (app t, 0.5H of rotamer, J = 9.6 Hz, $H_{3'}$), 5.31 (app t, 0.5H of rotamer, J = 9.6 Hz, $H_{3'}$);

^{13}C NMR (CD_3OD, 125 MHz) δ 20.48, 20.68, 20.83, 28.72, 28.79, 28.82, 41.27, 41.52, 55.93, 56.35, 60.70, 71.40, 63.07, 66.93, 67.08, 67.96, 68.38, 69.96, 70.06, 72.84, 73.16, 73.27, 76.94, 77.14, 80.09, 80.19, 81.65, 81.99, 86.26, 86.74, 99.77, 100.08, 113.89, 116.25, 118.54, 120.25,155.86, 155.07, 158.16, 158.03, 171.34, 171.68, 172.42, 172.45;

HRMS(ESI-MS): Calcd for $[C_{30}H_{46}F_3N_3O_{15}+Na]^+$(M+Na): m/z = 768.2773; Found: 768.2760.

***N-tert*-Butyloxycarbonyl-[(2*R*,3*R*,4*R*,5*R*)-2-(*N-tert*-butyloxylcarbonylamino methyl)-4-O-(2'-acetamido-2'-deoxy-β-D-glucopyranosyl)-3-hydroxy-5-(hydroxymethyl)]-pyrrolidine (4-19)**



4-19

NH_3 gas was bubbled to a solution of **4-18** (18 mg, 0.024 mmol) in methanol overnight. After solvent removed *in vacuo*, the residue was dissolved into 1:1 methanol:CH_2Cl_2 (5 mL) mixture. Acetic anhydride (20 μL) was added to the reaction mixture at 0 °C. After the solvent removed *in vacuo*, the residue was purified by column chromatography (MeOH:CH_2Cl_2 = 5:1 to 3:1) to give the desired product **4-19** (8.5 mg, 62 %) as a white oil (a mixture of N-rotamers).

^{1}H NMR (CD_3OD, 600 MHz) selected peaks δ 1.39-1.49 (m, 18H, 2*Me_3CCO), 1.89 (s, 1.5H of N-rotamers, acetyl), 1.96 (s, 1.5H of N-rotamer, acetyl), 2.92-2.96 (m, 0.5H of N-rotamers), 4.14-4.25 (m, 2H), 4.49 (d, 0.6H of N-rotamers, J = 8.4 Hz, $H_{1'}$), 4.52 (d, 0.4H of N-rotamers, J = 8.4 Hz, $H_{1'}$);

^{13}C NMR (CD_3OD, 100 MHz) δ 23.13, 23.26, 28.89, 28.94, 41.16, 41.49, 57.46, 57.76, 61.06, 61.67, 62.81, 67.28, 67.87, 68.19, 72.18, 75.55, 75.76, 76.63, 77.11, 78.28, 79.94, 81.11, 81.65, 81.89, 86.20, 87.16, 101.72, 155.95, 156.29, 158,44, 173.79, 173.90;

HRMS(ESI-MS): Calcd for $[C_{24}H_{43}N_3O_{12}+Na]^+$(M+Na): m/z = 588.2739; Found: 588.2736.

The synthesis of derivatives of pseudo-disaccharide

To a solution of compound **4-19** (8.5 mg, 0.016mmol) in CH_2Cl_2 (3 mL) was added TFA (1 mL) at 0 °C. The reaction mixture was warmed to room temperature. The reaction mixture was stirred at room temperature for 4 h. After the solvent was removed in *vacuo*, the residue was filter through Dowex 1X8 200-400 chloride form resin and used for the next reaction step without further purification. The combined crude product was dissolved in MeOH (2 mL) and divided into 20 different aliquots. The 1.0 equiv. of different acid anhydrides were added to 0 °C to each aliquot. The presence of product was confirmed by mass spectral analysis. The crude product was used as the inhibitor without further purification. The chitinase assay was carried out where the final inhibitor concentration was 8 μM (based on 100 % conversion of 2 steps).

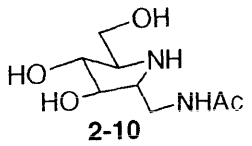
For the synthesis of compound **4-1-3**, to a solution of compound **4-19** (4.2 mg, 0.016mmol) in CH_2Cl_2 (3 mL) was added TFA (1 mL) at 0 °C. The reaction mixture was warmed to room temperature and the reaction mixture was stirred for 4 h. After solvent was removed *in vacuo*, the residue was purified through Dowex 1X8 200-400 chloride form resin and only pure fractions confirmed by mass spectral analysis were combined and used for the next step. The combined product was dissolved in MeOH (2 mL). Dodecanoic anhydride (1.0 equiv. based on 100 % conversion of the first step) was added to the reaction mixture at 0 °C and stirred at room temperature for 4 h. The solvent was removed under reduced pressure. The crude product was purified by flash column

chromatography (CH_3CN:MeOH =10:1 to 1:10). Only pure fractions confirmed by mass spectral analysis were combined (0.5 mg, 0.0009 mmol). The OT assay was carried out as described in Chapter 2, where the final concentration of inhibitor was 200 μM.

References

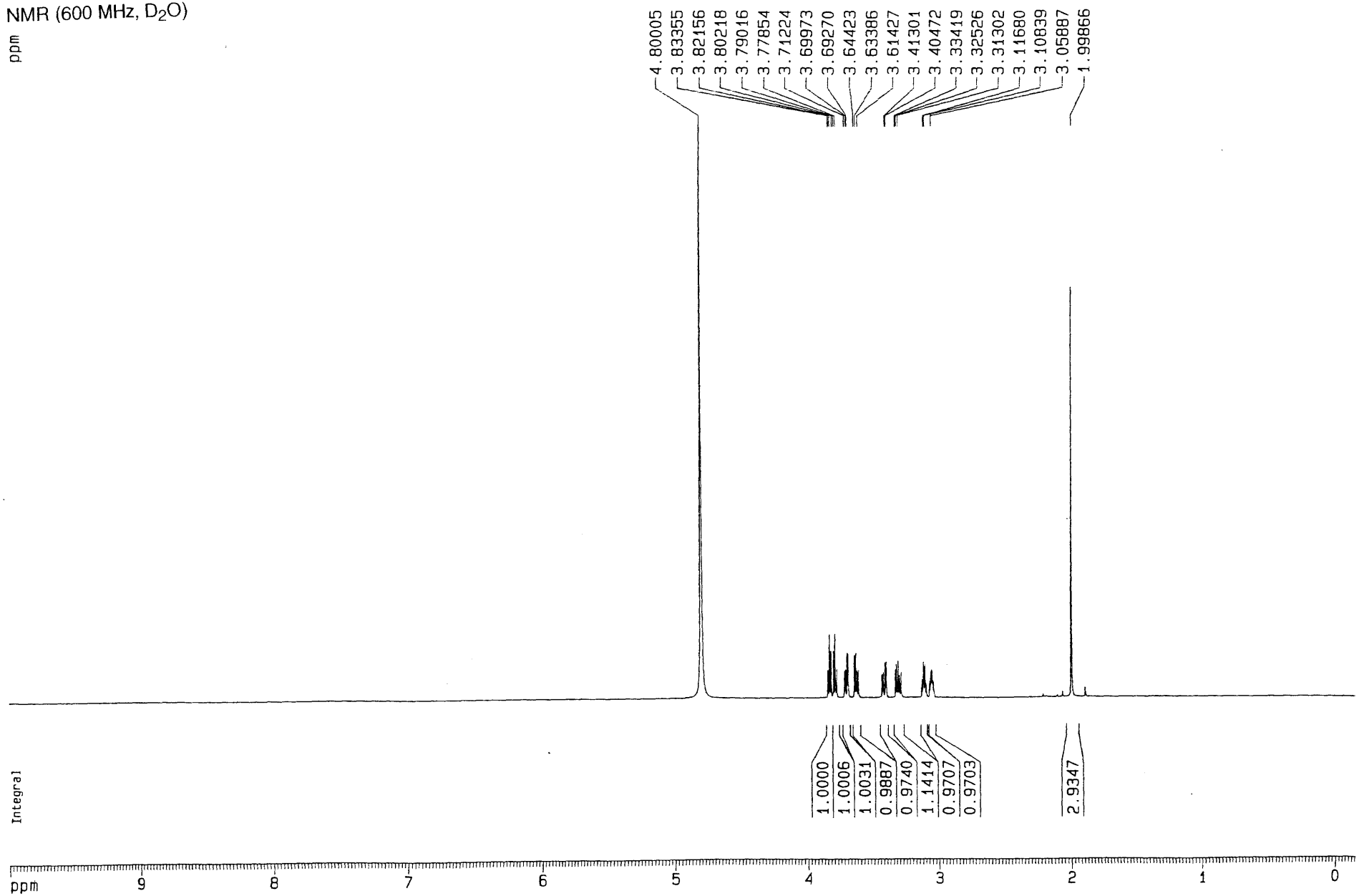
1. Liu, J.; Numa, M. M.; Liu, H.; Huang, S. J.; Sears, P.; Shikhman, A. R.; Wong, C.-H. Synthesis and high-throughput screening of N-acetyl-β-hexosaminidase inhibitor libraries targeting osteoarthritis. *J. Org. Chem.*, **2004**, *69*, 6273-6283.
2. Wrodnigg, T. M.; Stütz, A. E.; Withers, S. G. Symthesis of 1-Amino-1,2,5-trideoxy-2,5-imino-D-mannitol, a Novel Analogue of the Powerful Glucosidase Inhibitor 2,5-Dideoxy-2,5-imino-D-mannitol, *via* an Amadori Rearrangement of 5-Azido-5-deoxy-D-glucofuranose. *Tetrahedron Lett.*, **1997**, *38*, 5463-5466.
3. Lundt, I.; Steiner, A. J.; Stütz, A. E.; Tarling, C. A.; Ully, S.; Withers, S. G.; Wrodnigg, T. M. Fluorescently tagged iminoalditol glycosidase inhibitors as novel biological probes and diagnostics. *Bioorg. Med. Chem. Lett.*, **2006**, *14*, 1737-1742.
4. Hermetter, A.; Scholze, H.; Stütz, A. E.; Withers, S. G.; Wrodnigg, T. M. Powerful probes for glycosidases: Novel, fluorescently tagged glycosidase inhibitors. *Bioorg. Med. Chem. Lett.*, **2001**, *11*, 1339-1342.
5. Liang, P.-H.; Cheng, W.-C.; Lee, Y.-L.; Yu, H.-P.; Wu, Y.-T.; Lin, Y.-L.; Wong, C.-H. Novel five-membered iminocyclitol derivatives as selective and potent glycosidase inhibitors: new structures for antivirals and osteoarthritis. *Chembiochem,* **2006**, *7*, 165-173.
6. Hodge, J. E. The Amadori rearrangement. *Adv. Carbohydr. Chem. Biochem.*, **1955**, *10*, 169-205.
7. Schlessinger, R. H.; Bebernitz, G. R.; Lin, P.; Poss, A. J. Total Synthesis of (-)-Tirandamycin-A. *J. Am. Chem. Soc,* **1985**, *107*, 1777-1778.
8. Myszka, H.; Bednarczyk, D.; Najder, M.; Kaca, W. Synthesis and induction of apoptosis in B cell chronic leukemia by diosgenyl 2-amino-2-deoxy-β-D-glucopyranoside hydrochloride and its derivatives. *Carbohydr. Res.*, **2003**, *338*, 133-141.
9. Guibé, F. Allylic protecting groups and their use in a complex environment - Part II: Allylic protecting groups and their removal through catalytic palladium pi-allyl methodology. *Tetrahedron,* **1998**, *54*, 2967-3042.
10. McCauley, J. A.; Nagasawa, K.; Lander, P. A.; Mischke, S. G.; Semones, M. A.; Kishi, Y. Total synthesis of pinnatoxin A. *J. Am. Chem. Soc.*, **1998**, *120*, 7647-7648.
11. Kanda, Y.; Arai, H.; Ashizawa, T.; Morimoto, M.; Kasai, M. New potent mitomycin derivatives: synthesis and antitumor activity of 7,7-(ethylenedioxy)mitomycins. *J. Med. Chem.*, **1992**, *35*, 2781-2786.
12. Faroux-Corlay, B.; Greiner, J.; Terreux, R.; Cabrol-Bass, D.; Aubertin, A. M.; Vierling, P.; Fantini, J. Amphiphilic anionic analogues of galactosylceramide: synthesis, anti-HIV-1 activity, and gp120 binding. *J. Med. Chem.*, **2001**, *44*, 2188-2203.
13. Yokokawa, F.; Izumi, K.; Omata, J.; Shioiri, T. Total synthesis of amamistatin A, an antiproliferative linear peptide from an actinomycete. *Tetrahedron,* **2000**, *56*, 3027-3034.
14. Zorn, C.; Gnad, F.; Salmen, S.; Herpin, T.; Reiser, O. Deprotection of N-alloc amines by Pd(0)/DABCO - an efficient method for in situ peptide coupling of labile amino acids. *Tetrahedron Lett.*, **2001**, *42*, 7049-7053.
15. Taunton, J.; Collins, J. L.; Schreiber, S. L. Synthesis of natural and modified trapoxins, useful reagents for exploring histone deacetylase function. *J. Am. Chem. Soc,* **1996**, *118*, 10412-10422.

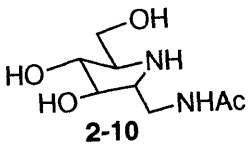
16. Roos, E. C.; Bernabe, P.; Hiemstra, H.; Speckamp, W. N.; Kaptein, B.; Boesten, W. H. J. Palladium-Catalyzed Transprotection of Allyloxycarbonyl-Protected Amines - Efficient One-Pot Formation of Amides and Dipeptides. *J. Org. Chem.*, **1995**, *60*, 1733-1740.
17. Wu, C.-Y.; Chang, C.-F.; Chen, J. S.; Wong, C.-H.; Lin, C.-H. Rapid diversity-oriented synthesis in microtiter plates for in situ screening: discovery of potent and selective α-fucosidase inhibitors. *Angew. Chem. Int. Ed. Engl,* **2003**, *42*, 4661-4664.
18. Rao, F. V.; Andersen, O. A.; Vora, K. A.; Demartino, J. A.; van Aalten, D. M. Methylxanthine drugs are chitinase inhibitors: investigation of inhibition and binding modes. *Chem. Biol.*, **2005**, *12*, 973-980.
19. Vanscheltinga, A. C. T.; Armand, S.; Kalk, K. H.; Isogai, A.; Henrissat, B.; Dijkstra, B. W. Stereochemistry of Chitin Hydrolysis by a Plant Chitinase Lysozyme and X-Ray Structure of a Complex with Allosamidin - Evidence for Substrate Assisted Catalysis. *Biochemistry,* **1995**, *34*, 15619-15623.
20. Zhu, Z.; Zheng, T.; Homer, R. J.; Kim, Y. K.; Chen, N. Y.; Cohn, L.; Hamid, Q.; Elias, J. A. Acidic mammalian chitinase in asthmatic Th2 inflammation and IL-13 pathway activation. *Science,* **2004**, *304*, 1678-1682.
21. Wills-Karp, M.; Karp, C. L. Chitin checking - Novel insights into asthma. *New. Engl. J. Med.*, **2004**, *351*, 1455-1457.
22. Lairson, L. L.; Withers, S. G. Mechanistic analogies amongst carbohydrate modifying enzymes. *Chem. Commun,* **2004**, 2243-2248.
23. Heightman, T. D.; Vasella, A. T. Recent insights into inhibition, structure, and mechanism of configuration-retaining glycosidases. *Angew. Chem. Int. Ed. Engl,* **1999**, *38*, 750-770.
24. White, A.; Tull, D.; Johns, K.; Withers, S. G.; Rose, D. R. Crystallographic observation of a covalent catalytic intermediate in a β-glycosidase. *Nat. Struct. Bio.,* **1996**, *3*, 149-154.
25. Brzozowski, A. M.; Davies, G. J. Structure of the *Aspergillus oryzae* α-amylase complexed with the inhibitor acarbose at 2.0 Å resolution. *Biochemistry,* **1997**, *36*, 10837-10845.
26. Qasba, P. K.; Ramakrishnan, B.; Boeggeman, E. Substrate-induced conformational changes in glycosyltransferases. *Trends Biochem. Sci.,* **2005**, *30*, 53-62.
27. Wang, R.; Steensma, D. H.; Takaoka, Y.; Yun, J. W.; Kajimoto, T.; Wong, C.-H. A search for pyrophosphate mimics for the development of substrates and inhibitors of glycosyltransferases. *Bioorg. Med. Chem. Lett.,* **1997**, *5*, 661-672.



2-10

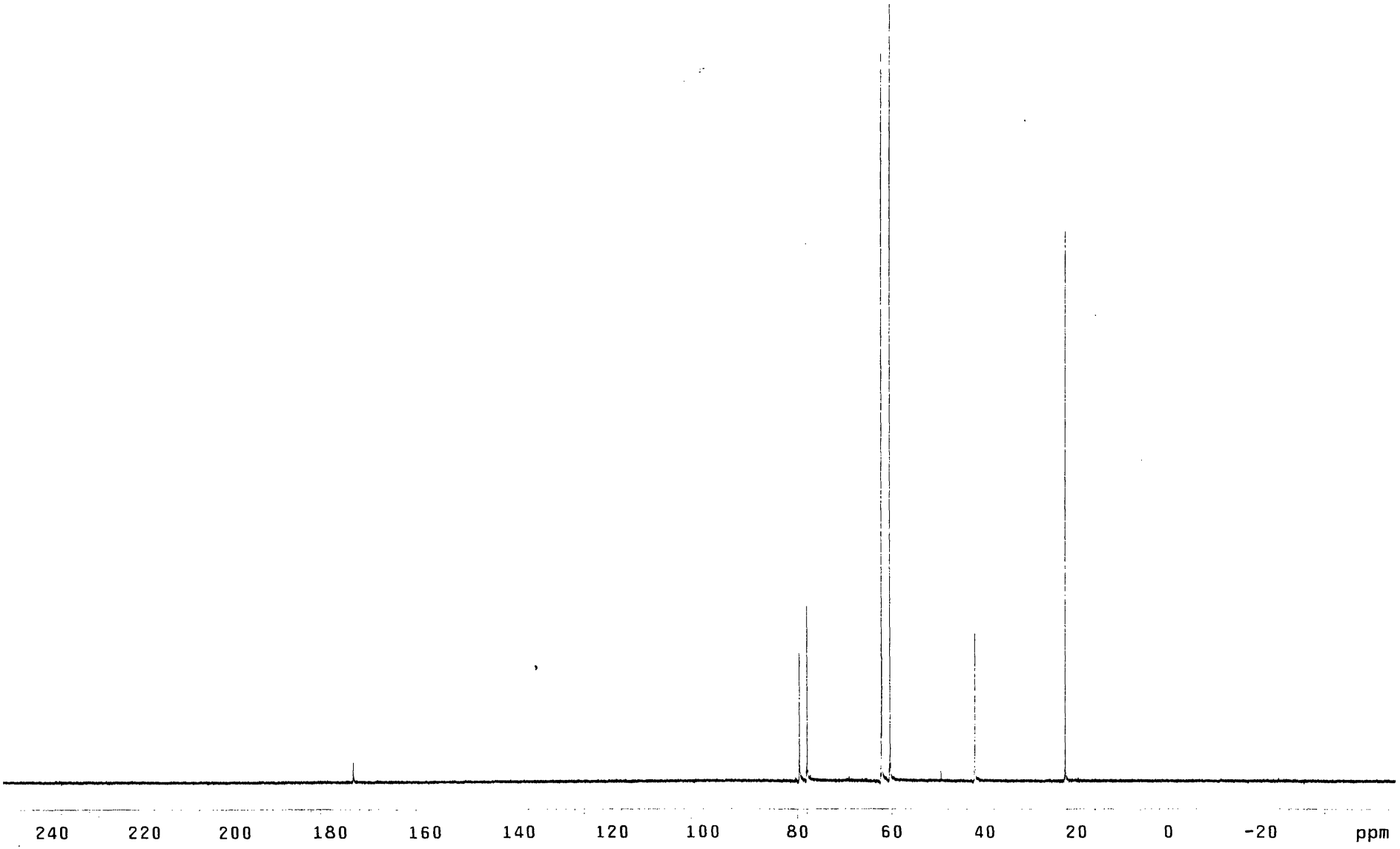
^{1}H NMR (600 MHz, D_2O)

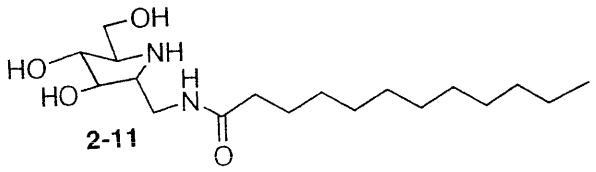




2-10

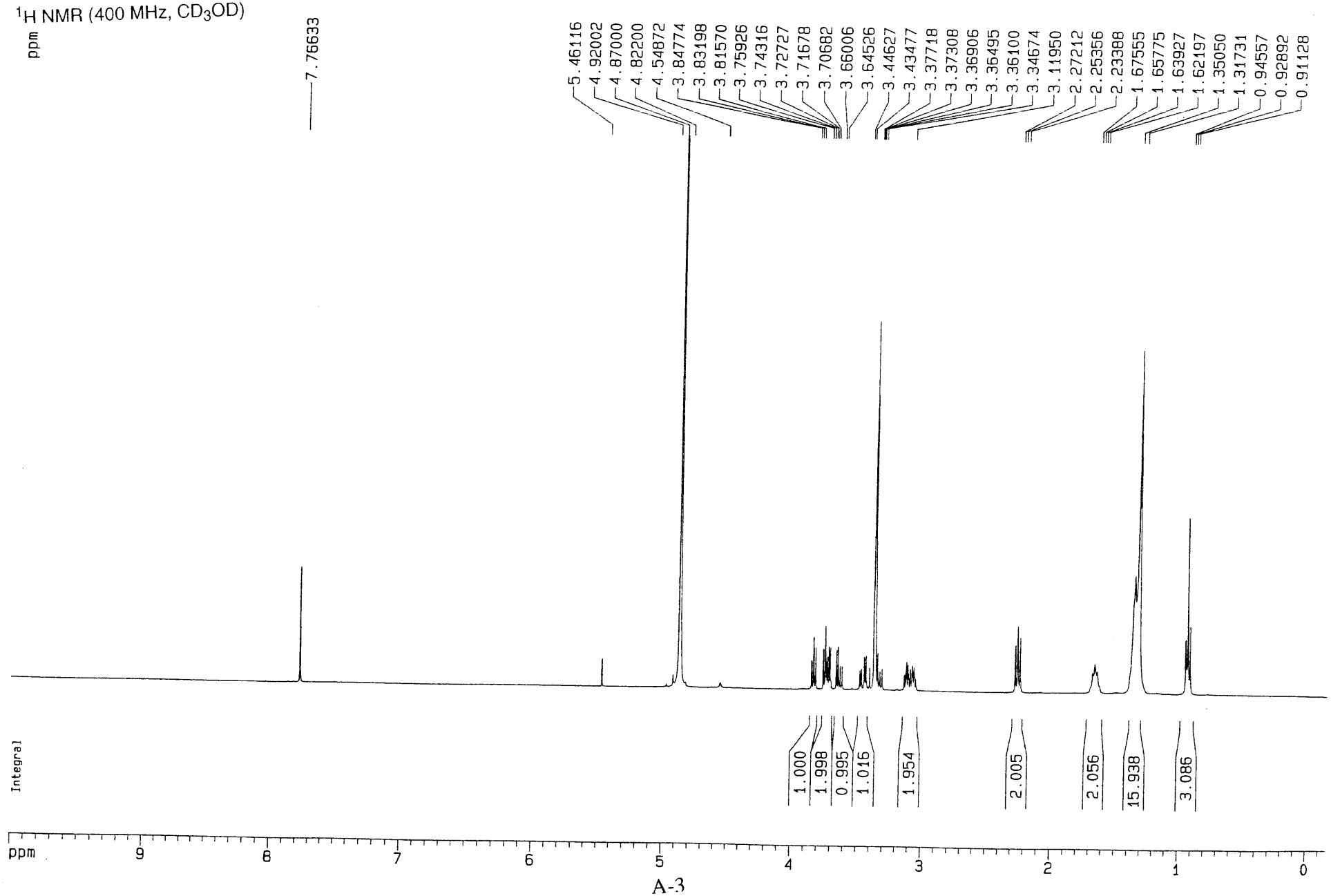
^{13}C NMR (125 MHz, D_2O)

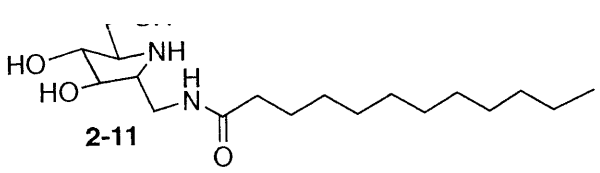




2-11

^{1}H NMR (400 MHz, CD_3OD)





2-11

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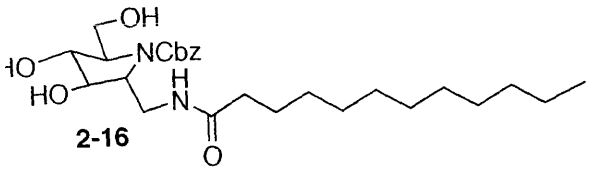

2-16

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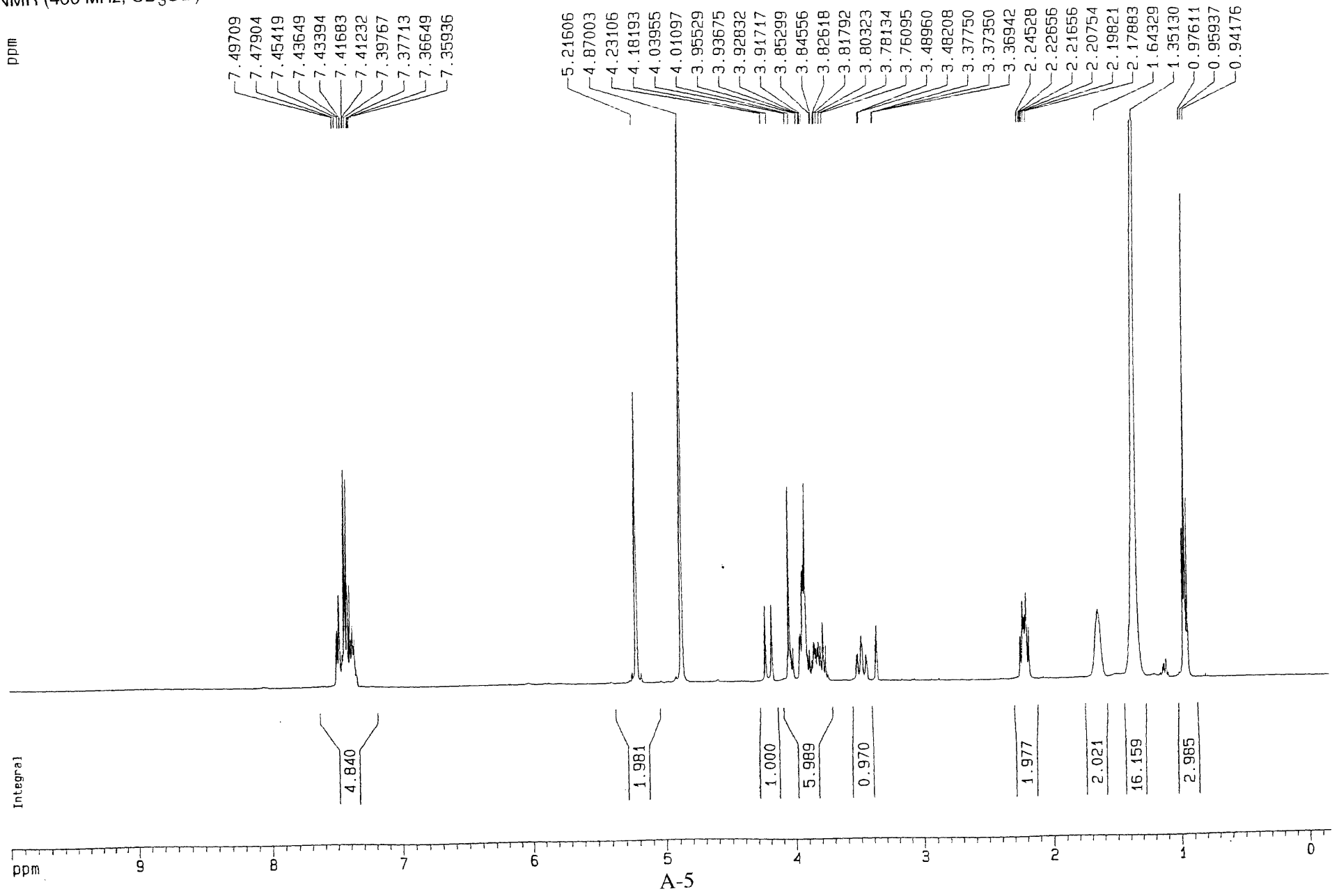

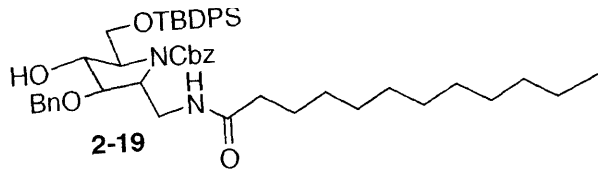

2-19

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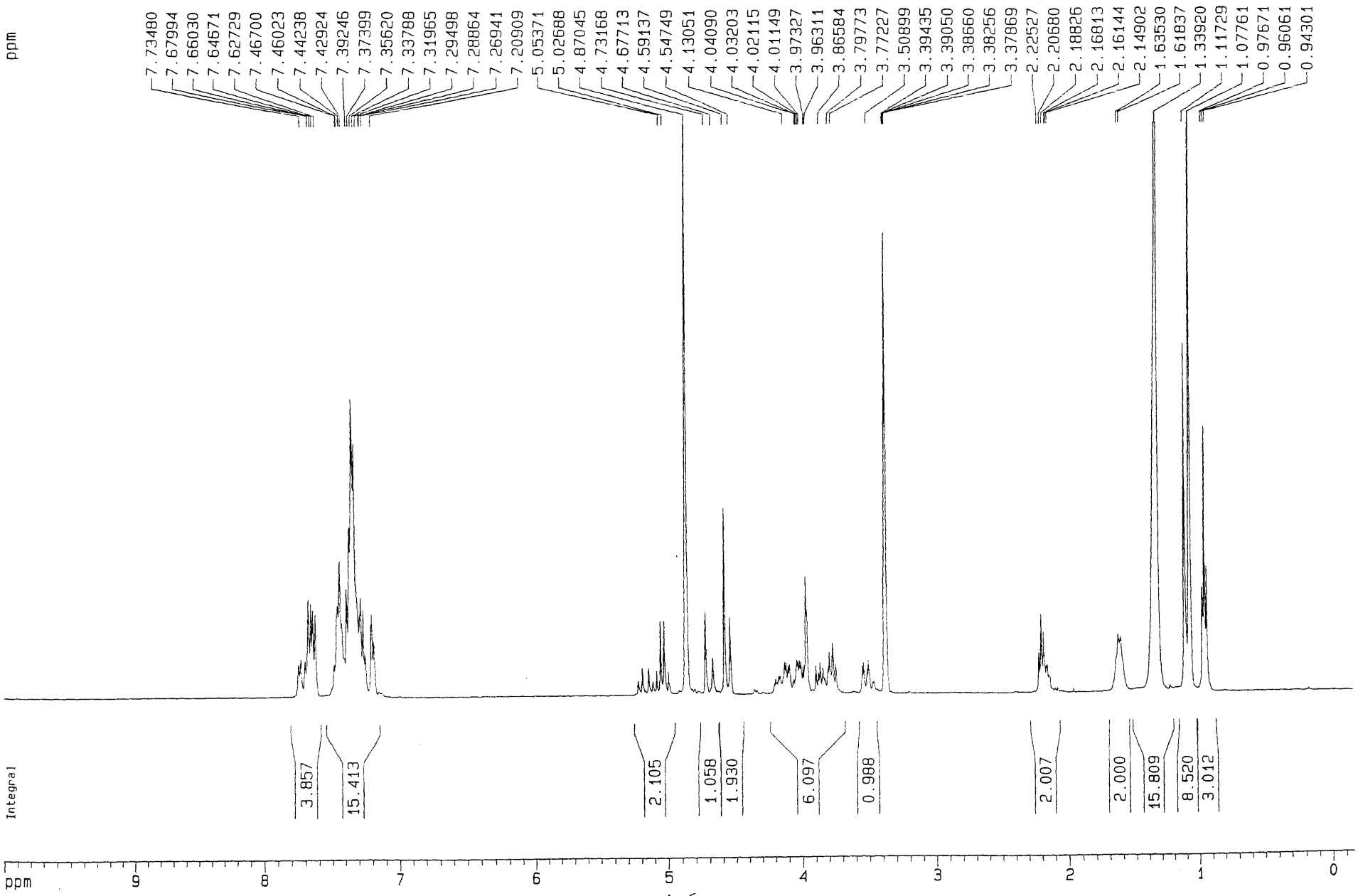
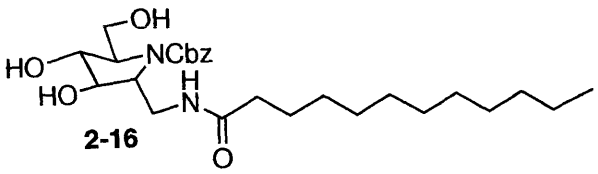
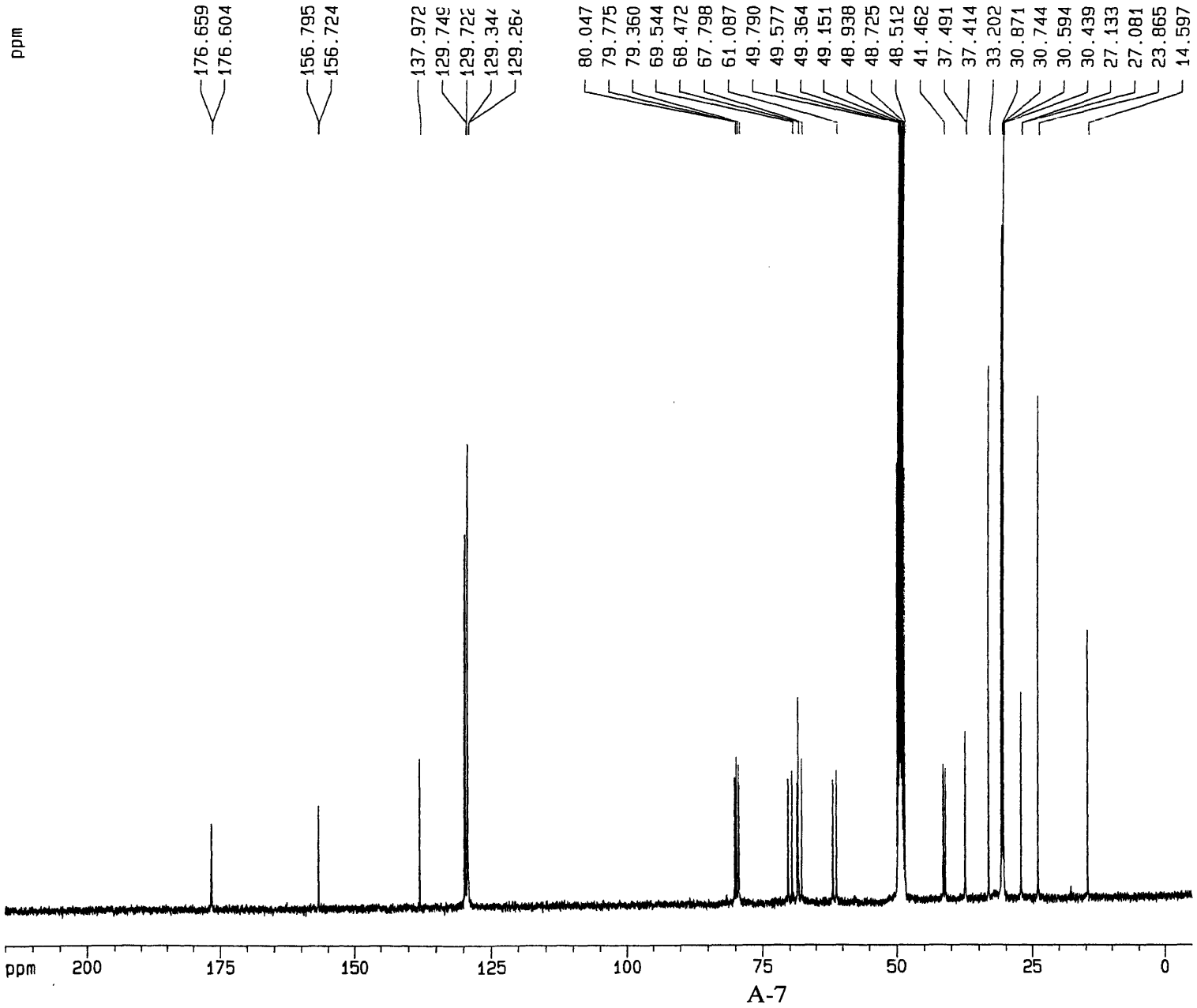




2-16

^{13}C NMR (100 MHz, CD_3OD)



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PL1	3.00 dB
SFO1	100.6237959 MHz

========== CHANNEL f2 ==========

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F2 - Processing parameters

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1D NMR plot parameters

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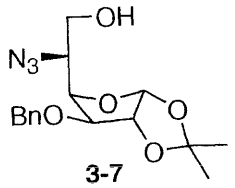
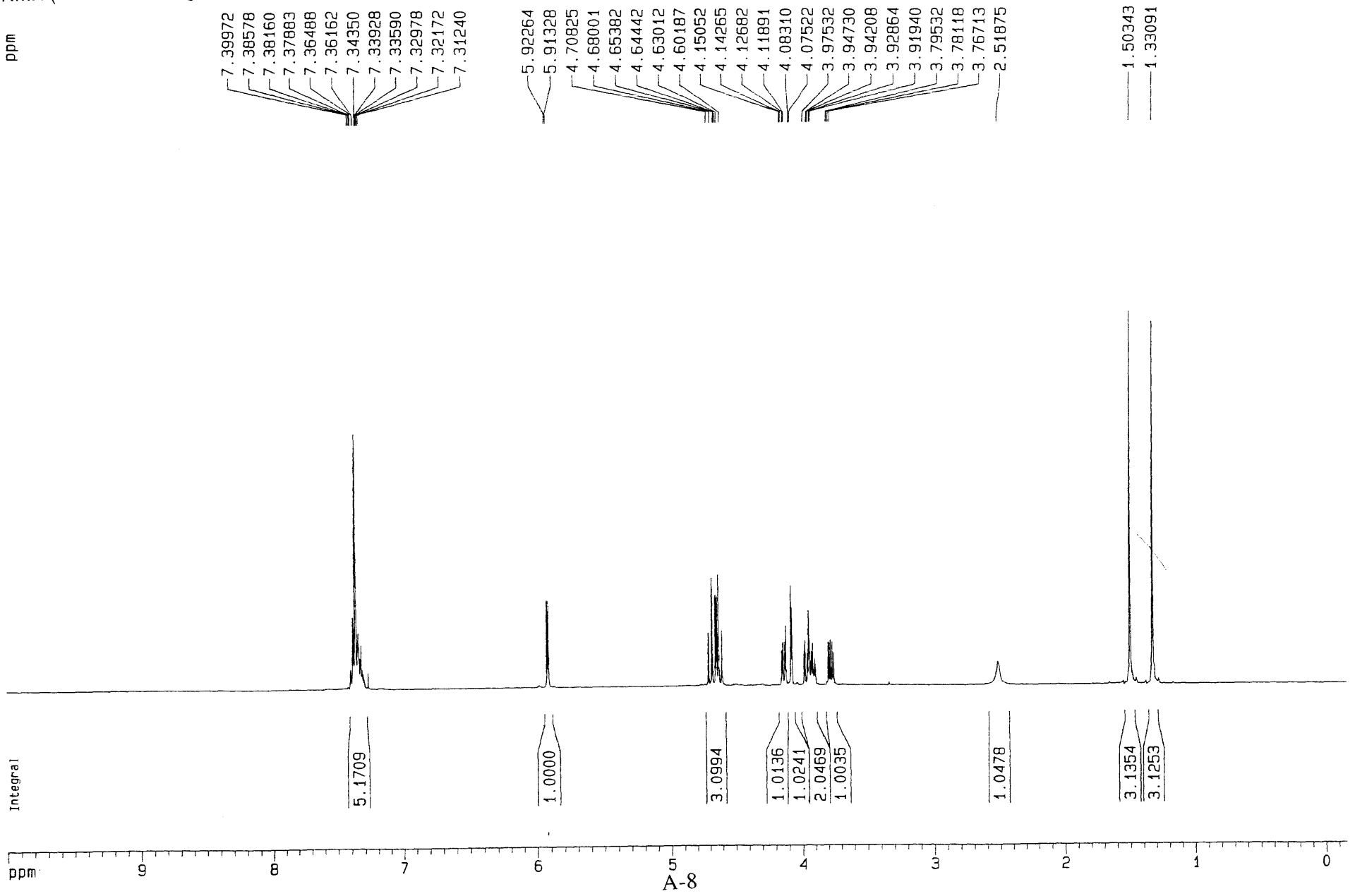
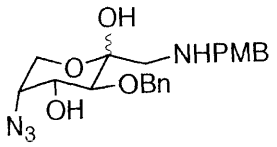


3-7

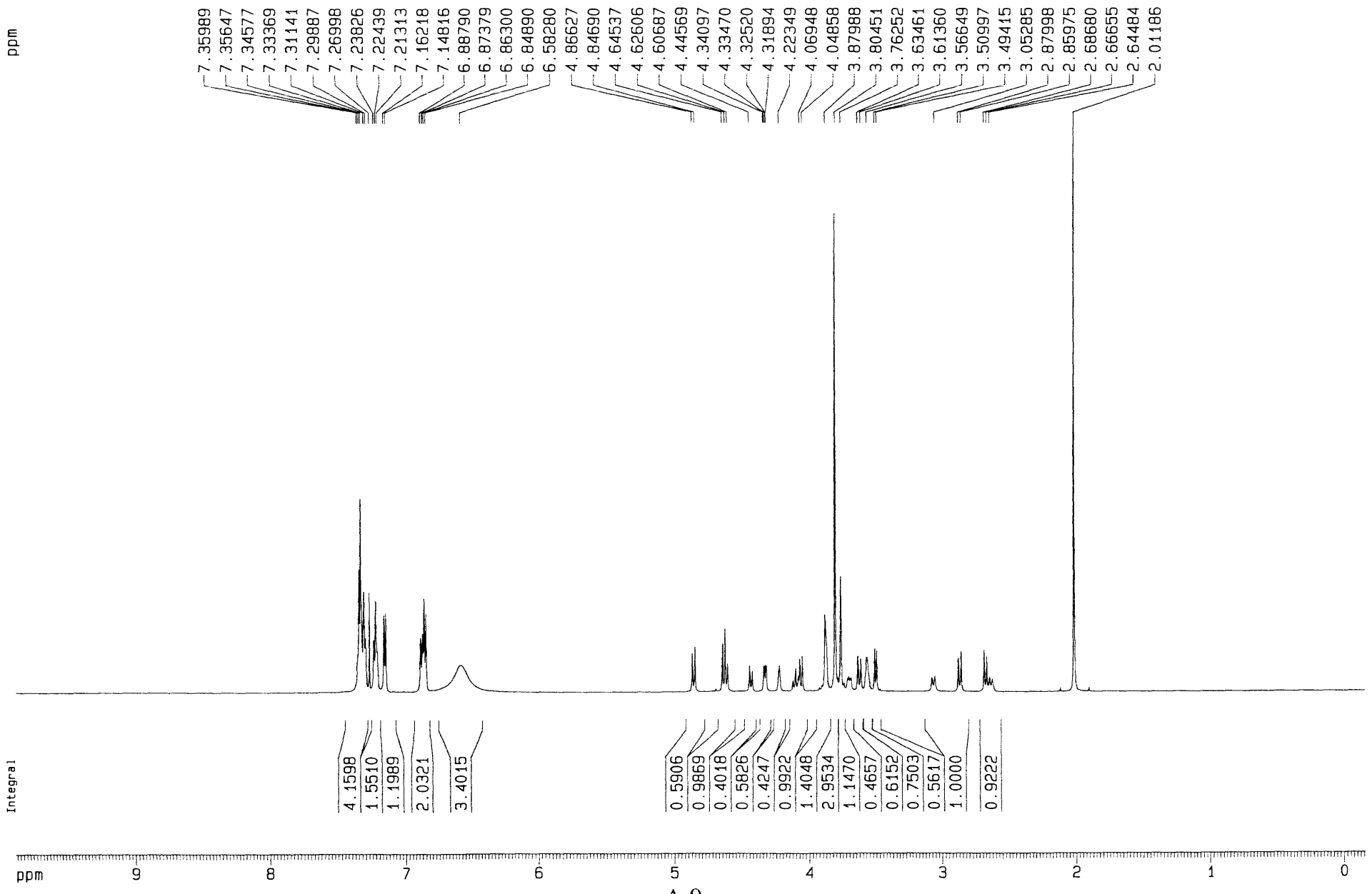
^{1}H NMR (400 MHz, $CDCl_3$)

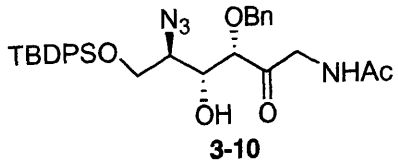




3-8

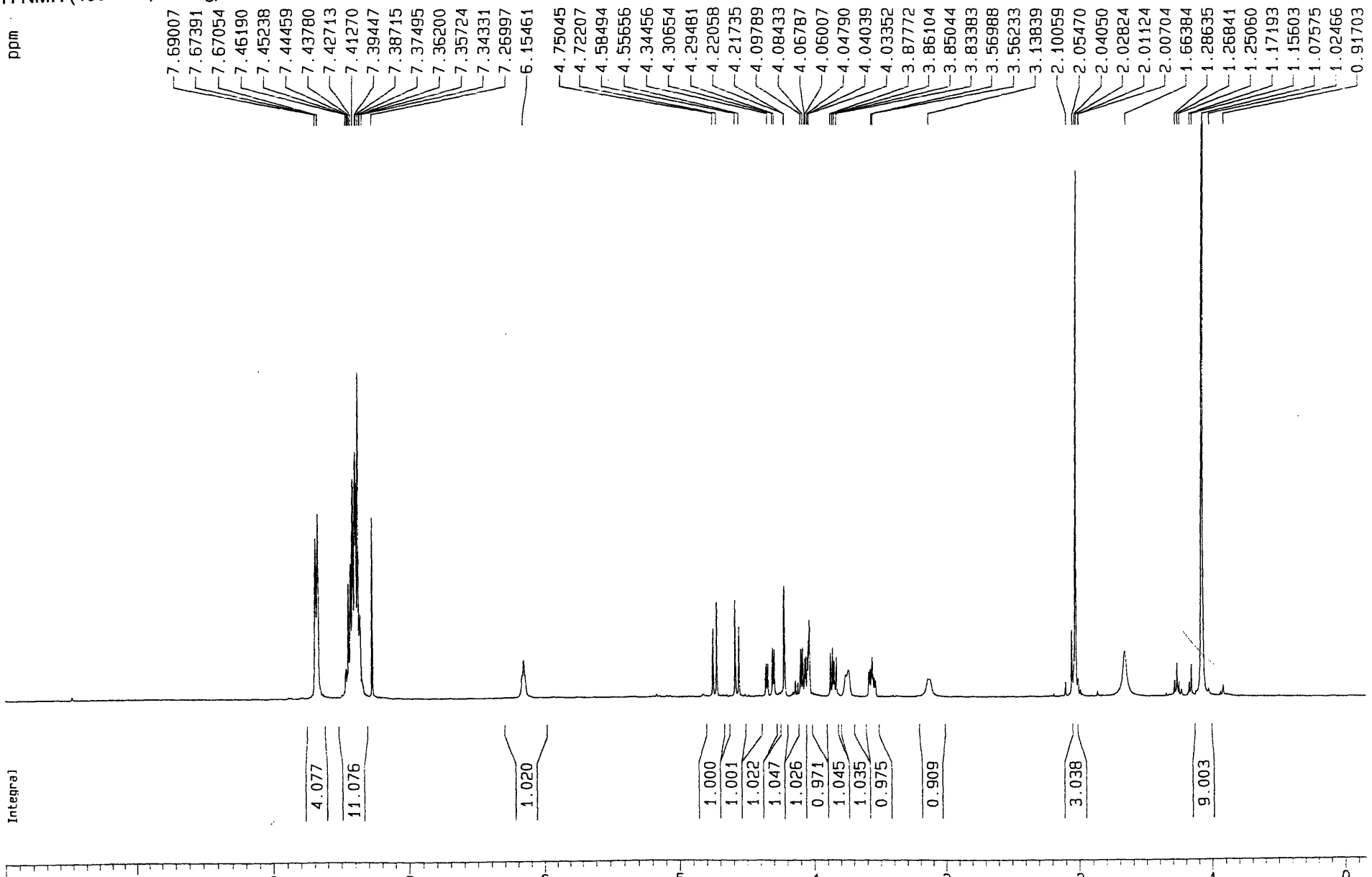
^{1}H NMR (600 MHz, $CDCl_3$)

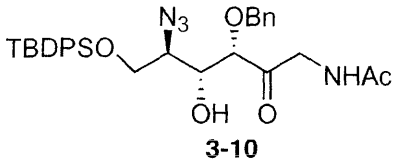




3-10

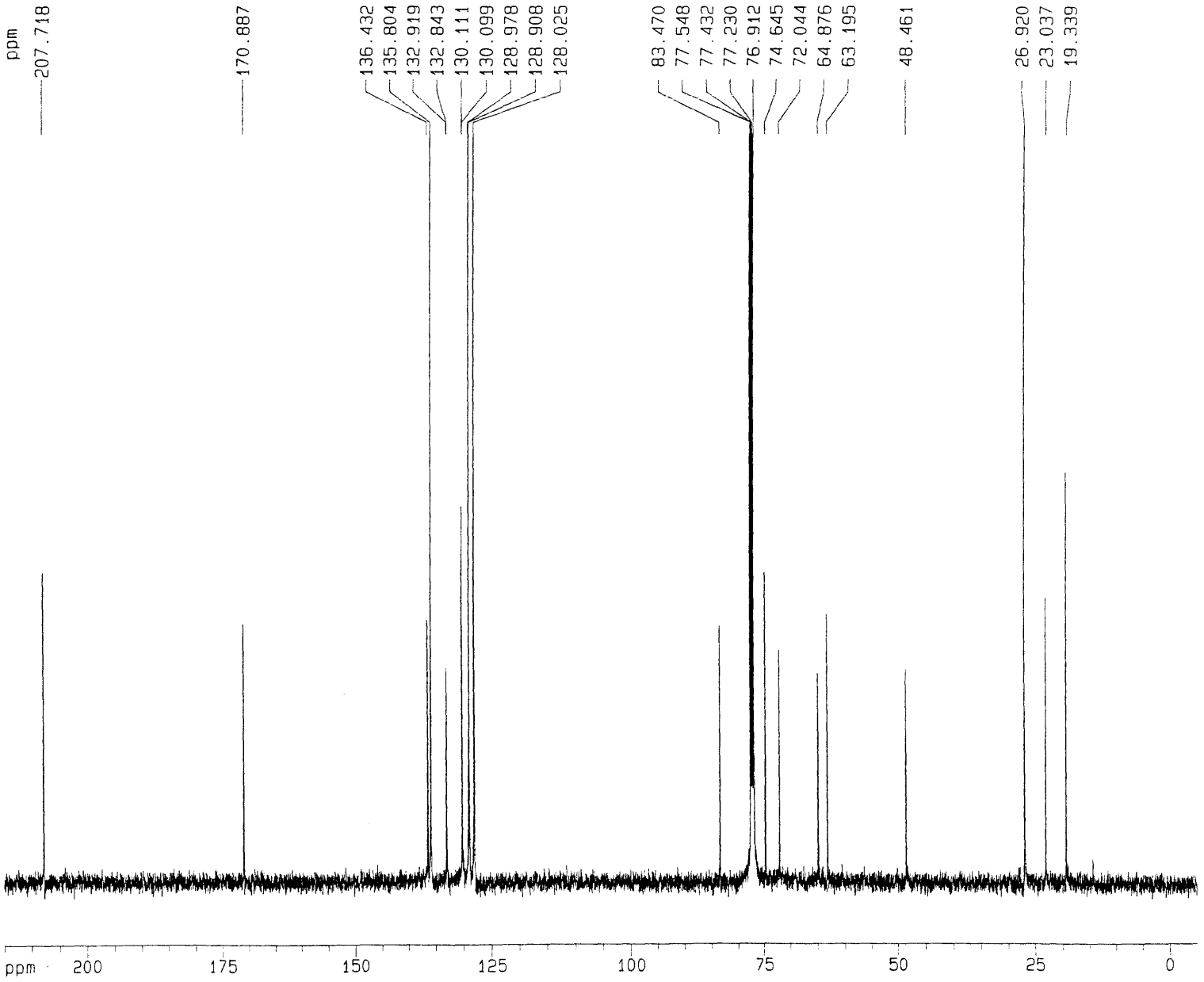
^{1}H NMR (400 MHz, $CDCl_3$)





3-10

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

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EXPNO                       10
PROCNO                       1

F2 - Acquisition Parameters
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Time                     22.06
INSTRUM                  spect
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PULPROG                 zgpg30
TD                       65536
SOLVENT                  CDCl3
NS                       10240
DS                           2
SWH                25125.629 Hz
FIDRES             0.383387 Hz
AQ                 1.3042164 sec
RG                       16384
DW                    19.900 usec
DE                     6.00 usec
TE                     300.0 K
D1                2.00000000 sec
d11               0.03000000 sec
d12               0.00002000 sec

============ CHANNEL f1 ============
NUC1                      13C
P1                     15.25 usec
PL1                     3.00 dB
SFO1             100.6237959 MHz

============ CHANNEL f2 ============
CPDPRG2                waltz16
NUC2                       1H
PCPD2                 107.50 usec
PL2                     0.00 dB
PL12                   24.00 dB
PL13                   24.00 dB
SFO2             400.1316005 MHz

F2 - Processing parameters
SI                         32768
SF               100.6127507 MHz
WDW                           EM
SSB                            0
LB                          1.00 Hz
GB                             0
PC                          1.40

1D NMR plot parameters
CX                         20.00 cm
F1P                       215.000 ppm
F1                      21631.74 Hz
F2P                        -5.000 ppm
F2                       -503.06 Hz
PPMCM                  11.00000 ppm/cm
HZCM                1106.74023 Hz/cm
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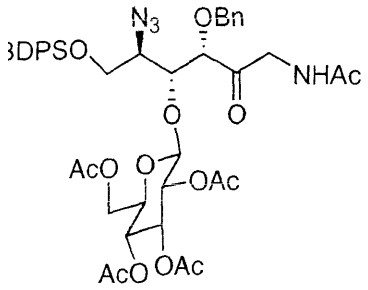
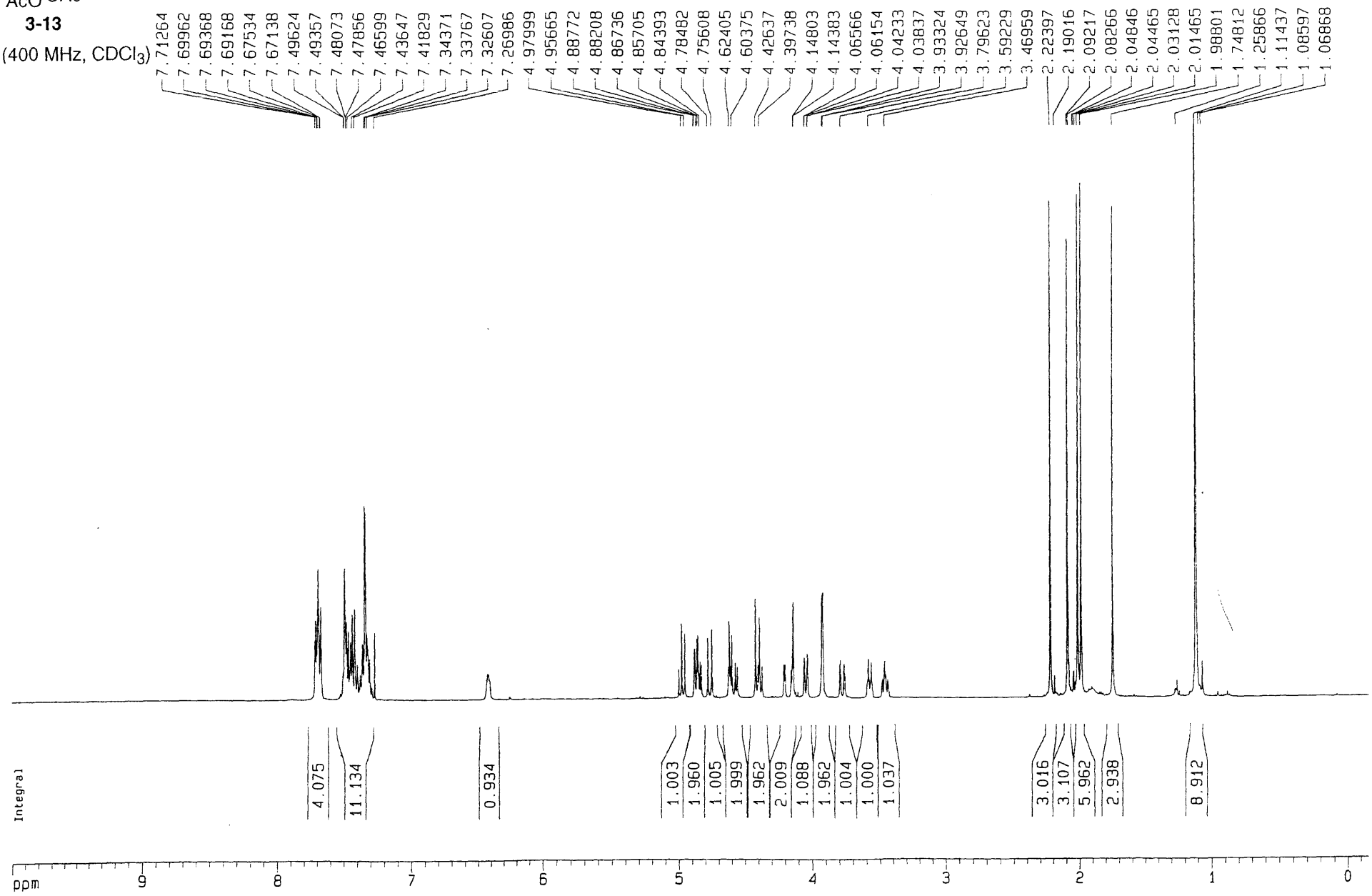
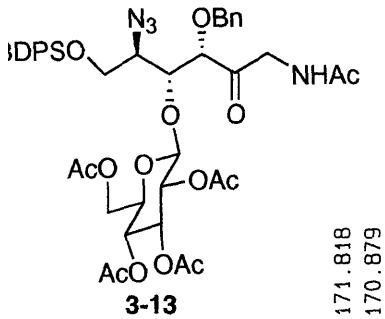


3-13

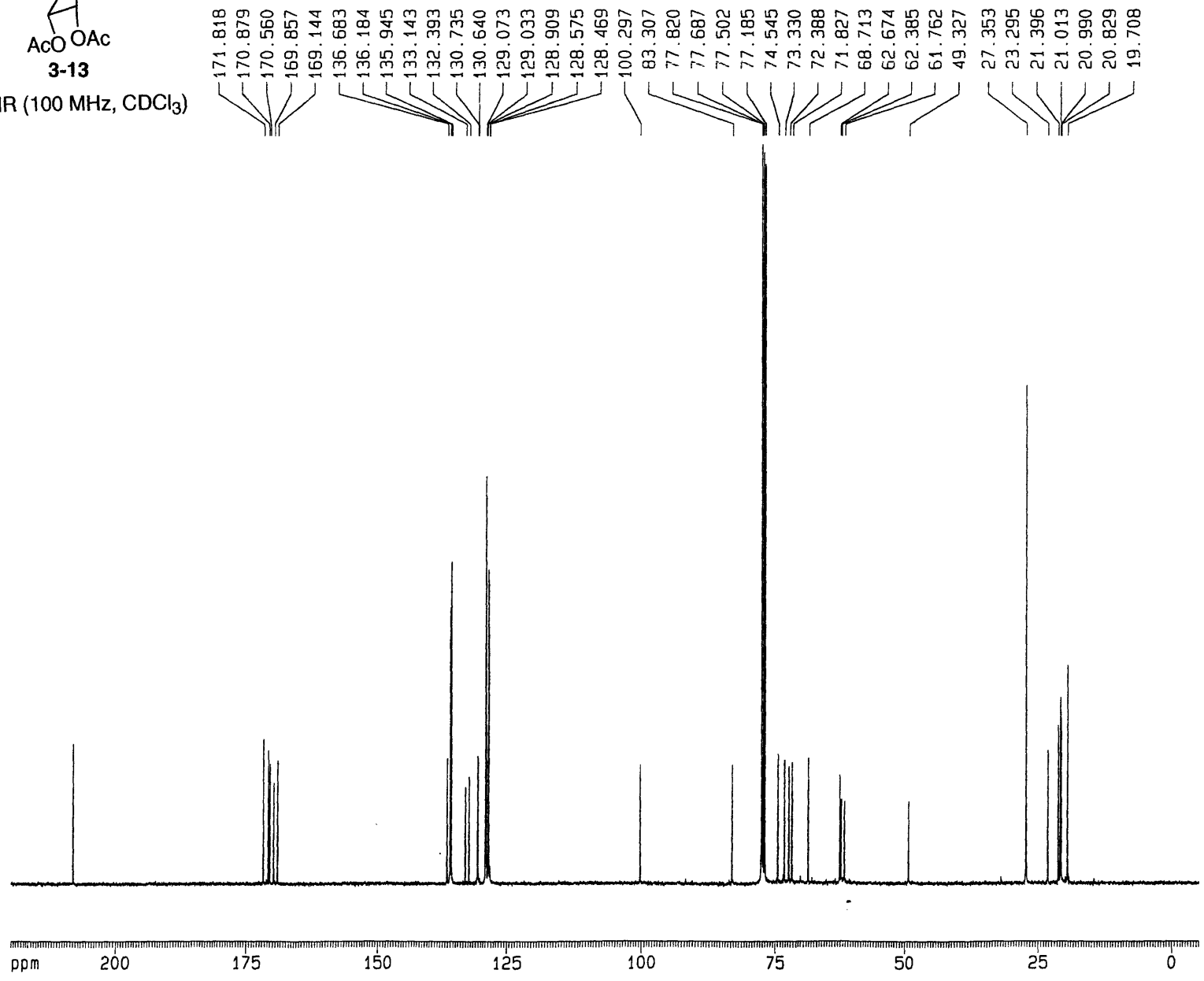
^{1}H NMR (400 MHz, $CDCl_3$)





3-13

C NMR (100 MHz, $CDCl_3$)

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Current Data Parameters
NAME          sj20040829
EXPNO                  2
PROCNO                 1

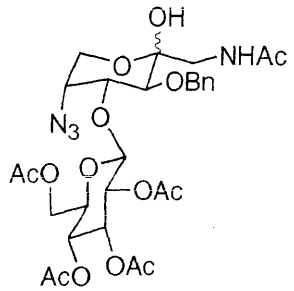
F2 - Acquisition Parameters
Date_           20040829
Time               16.30
INSTRUM             spect
PROBHD     5mm BBO BB-1
PULPROG          zgpg30
TD                65536
SOLVENT           CDCl3
NS                 6180
DS                    2
SWH           25125.629 Hz
FIDRES         0.383387 Hz
AQ             1.3042164 sec
RG                32768
DW               19.900 usec
DE                6.00 usec
TE               300.0 K
D1            2.00000000 sec
d11           0.03000000 sec
d12           0.00002000 sec

========== CHANNEL f1 ==========
NUC1                13C
P1               15.25 usec
PL1               3.00 dB
SFO1        100.6237959 MHz

========== CHANNEL f2 ==========
CPDPRG2         waltz16
NUC2                1H
PCPD2           107.50 usec
PL2               0.00 dB
PL12             24.00 dB
PL13             24.00 dB
SFO2        400.1316005 MHz

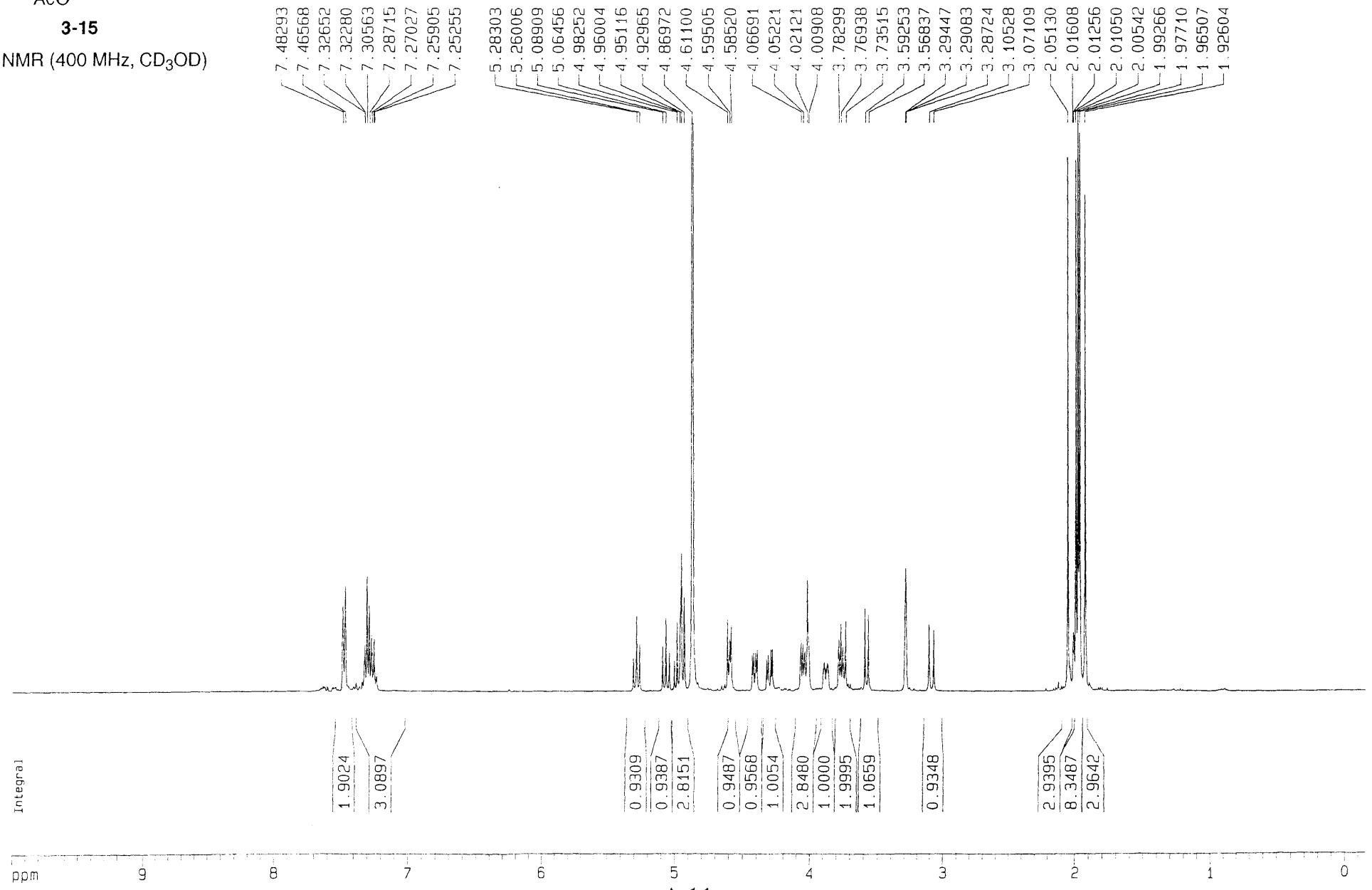
F2 - Processing parameters
SI                 32768
SF          100.6127266 MHz
WDW                   EM
SSB                    0
LB                  1.00 Hz
GB                     0
PC                  1.40

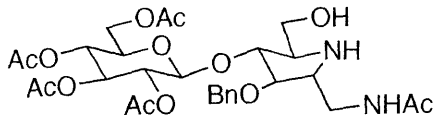
1D NMR plot parameters
CX                 20.00 cm
F1P              220.000 ppm
F1              22134.80 Hz
F2P               -5.000 ppm
F2              -503.07 Hz
PPMCM           11.25000 ppm/cm
HZCM          1131.89319 Hz/cm
```



3-15

^{1}H NMR (400 MHz, CD_3OD)

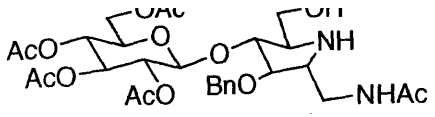




3-16

> 12:1 mixture

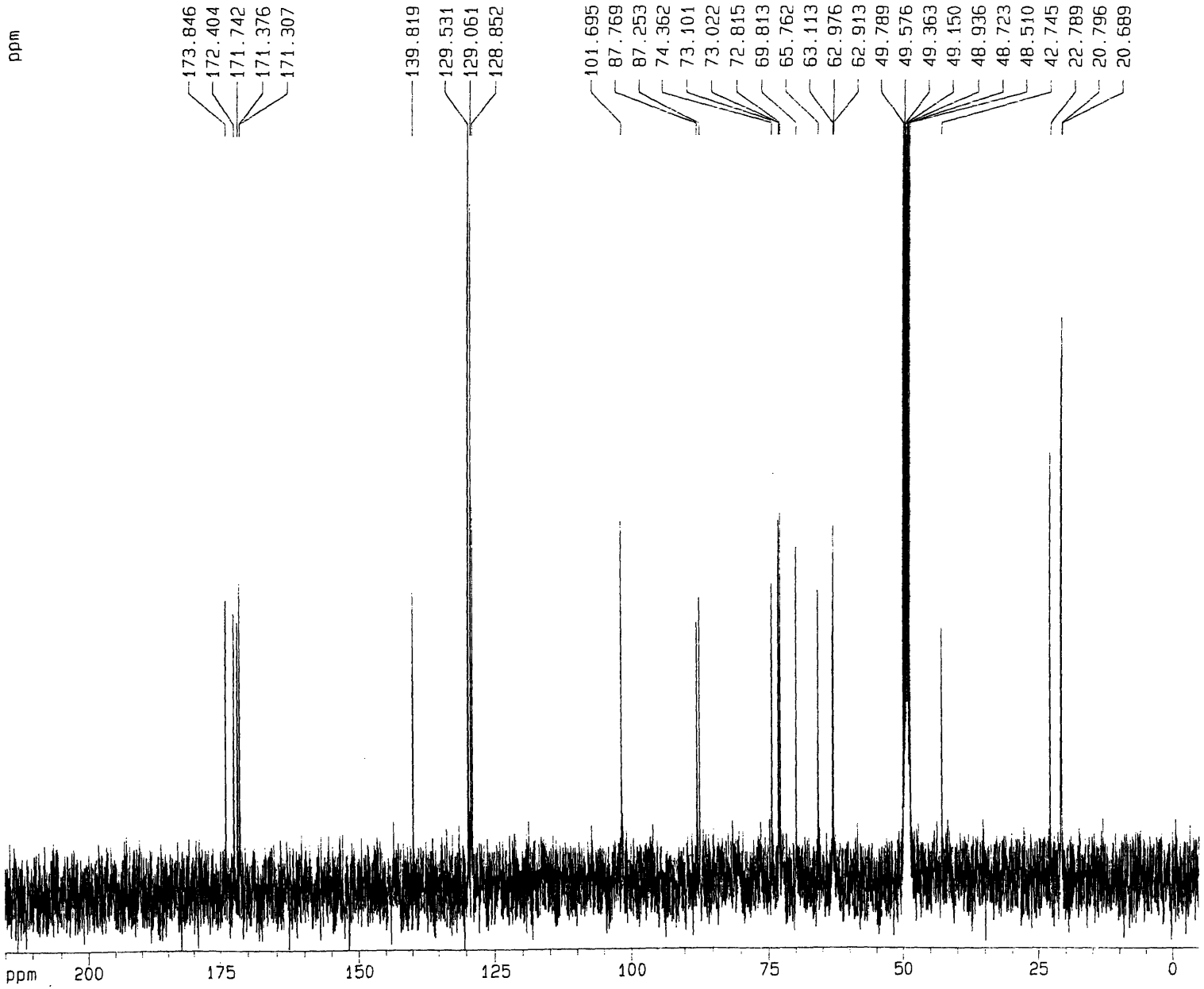
^{1}H NMR (600 MHz, CD_3OD)



3-16

> 12:1 mixture

^{13}C NMR (100 MHz, CD_3OD)




```
Current Data Parameters
NAME                  20050601
EXPNO                       48
PROCNO                       1

F2 - Acquisition Parameters
Date_                 20060122
Time                     19.35
INSTRUM                  spect
PROBHD      5mm BBO BB-1
PULPROG                zgpg30
TD                      65536
SOLVENT                 CDCl3
NS                        822
DS                          2
SWH                 25125.629 Hz
FIDRES                0.383387 Hz
AQ                    1.3042164 sec
RG                       2298 8
DW                       19.900 usec
DE                        6.00 usec
TE                       300.0 K
D1                    2.00000000 sec
d11                   0.03000000 sec
d12                   0.00002000 sec

========== CHANNEL f1 ==========
NUC1                        13C
P1                        15.25 usec
PL1                        3.00 dB
SFO1                100.6237959 MHz

========== CHANNEL f2 ==========
CPDPRG2                 waltz16
NUC2                        1H
PCPD2                    107.50 usec
PL2                        0.00 dB
PL12                      24.00 dB
PL13                      24.00 dB
SFO2                400.1316005 MHz

F2 - Processing parameters
SI                         32768
SF                  100.6126139 MHz
WDW                           EM
SSB                            0
LB                          1.00 Hz
GB                             0
PC                          1 40

1D NMR plot parameters
CX                         20.00 cm
F1P                       215.000 ppm
F1                      21631.71 Hz
F2P                        -5.000 ppm
F2                       -503.06 Hz
PPMCM                   11.00000 ppm/cm
HZCM                 1106.73877 Hz/cm
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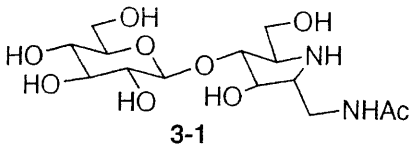
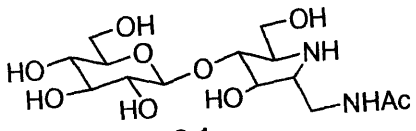


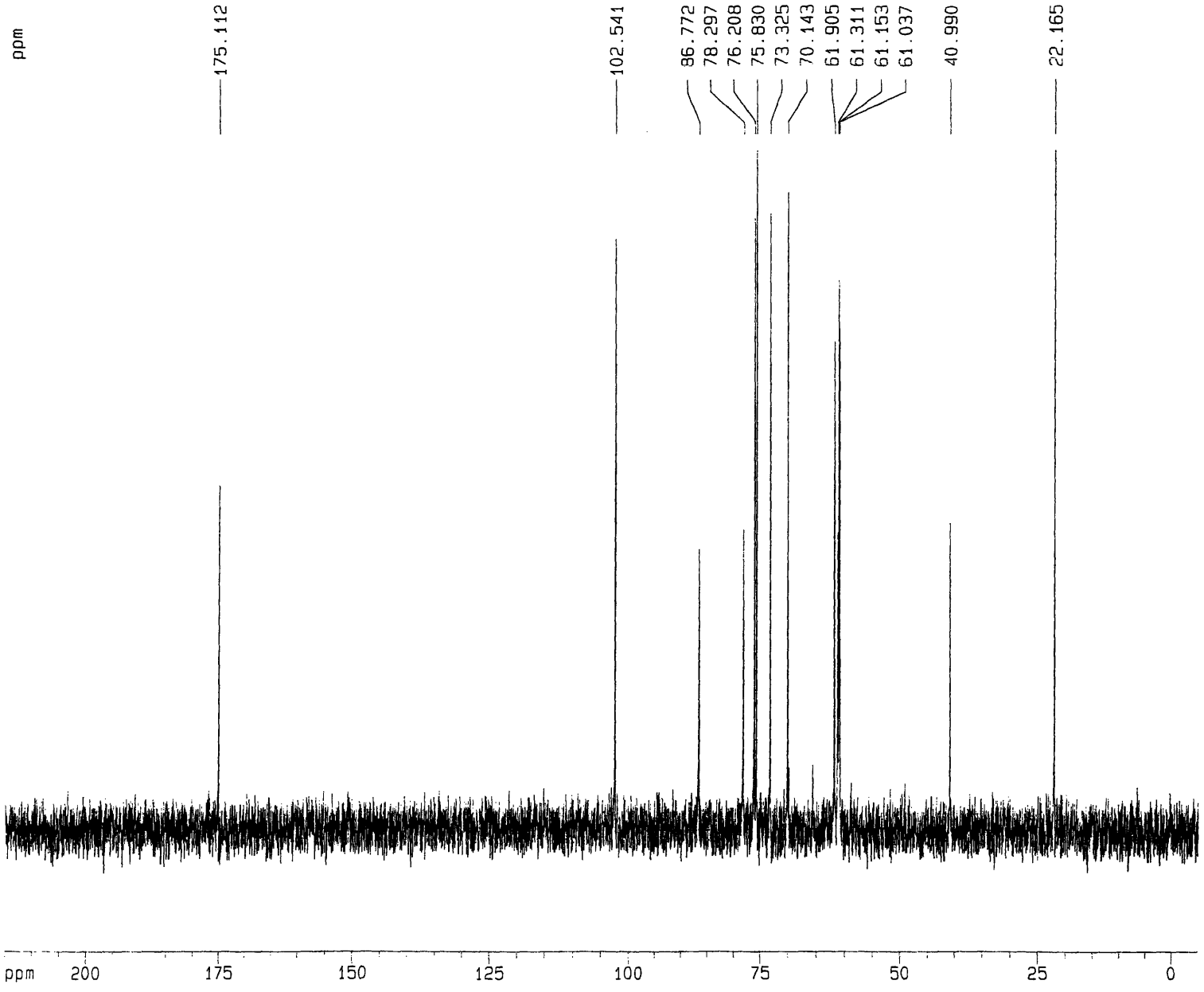
3-1

^{1}H NMR (500 MHz, D_2O)



3-1

^{13}C NMR (100 MHz, D_2O)

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Current Data Parameters
NAME                  20050601
EXPNO                        2
PROCNO                       1

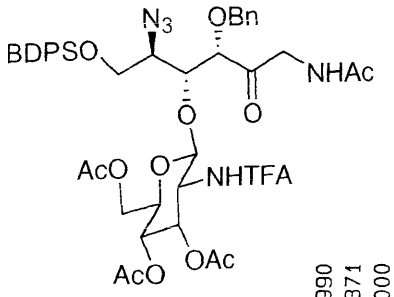
F2 - Acquisition Parameters
Date_                 20050601
Time                     21.51
INSTRUM                  spect
PROBHD     5mm BBO BB-1 BB-1
PULPROG                 zgpg30
TD                       65536
SOLVENT                    D2O
NS                       10770
DS                           2
SWH                  25125.629 Hz
FIDRES                0.383387 Hz
AQ                   1.3042164 sec
RG                      2298.8
DW                      19.900 usec
DE                       6.00 usec
TE                      300.0 K
D1                   2.00000000 sec
d11                  0.03000000 sec
d12                  0.00002000 sec

========== CHANNEL f1 ==========
NUC1                        13C
P1                        15.25 usec
PL1                        3.00 dB
SFO1                100.6237959 MHz

========== CHANNEL f2 ==========
CPDPRG2                 waltz16
NUC2                        1H
PCPD2                    107.50 usec
PL2                        0.00 dB
PL12                      24.00 dB
PL13                      24.00 dB
SFO2                400.1316005 MHz

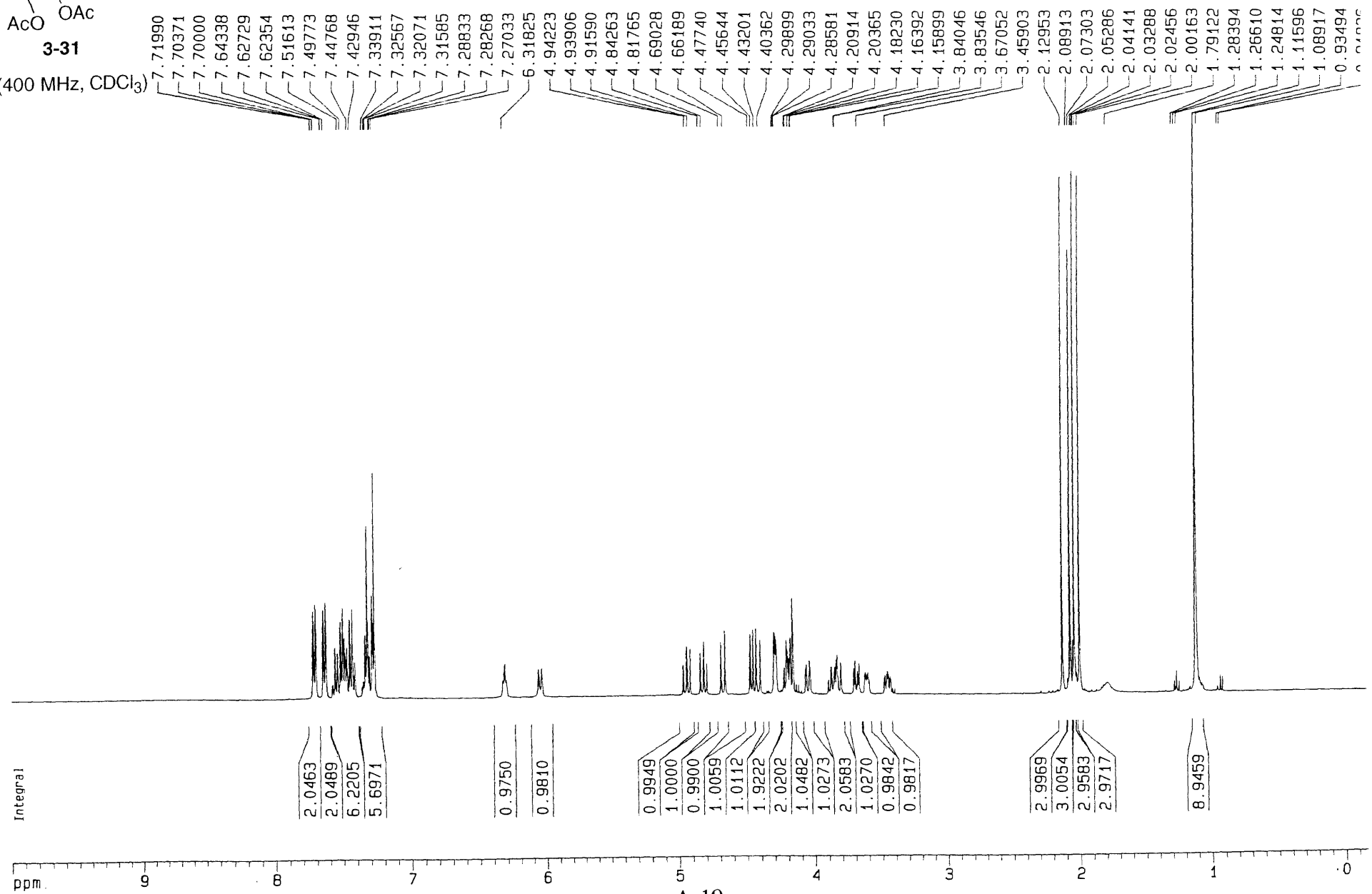
F2 - Processing parameters
SI                         32768
SF                  100.6127290 MHz
WDW                           EM
SSB                            0
LB                          1.00 Hz
GB                             0
PC                          1.40

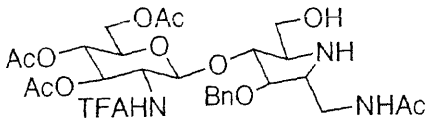
1D NMR plot parameters
CX                         20.00 cm
F1P                       215.000 ppm
F1                      21631.74 Hz
F2P                        -5.000 ppm
F2                       -503.06 Hz
PPMCM                  11.00000 ppm/cm
HZCM                 1106.73999 Hz/cm
```



3-31

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

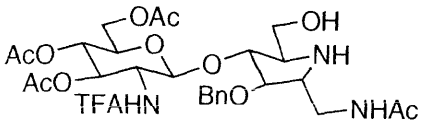




3-33

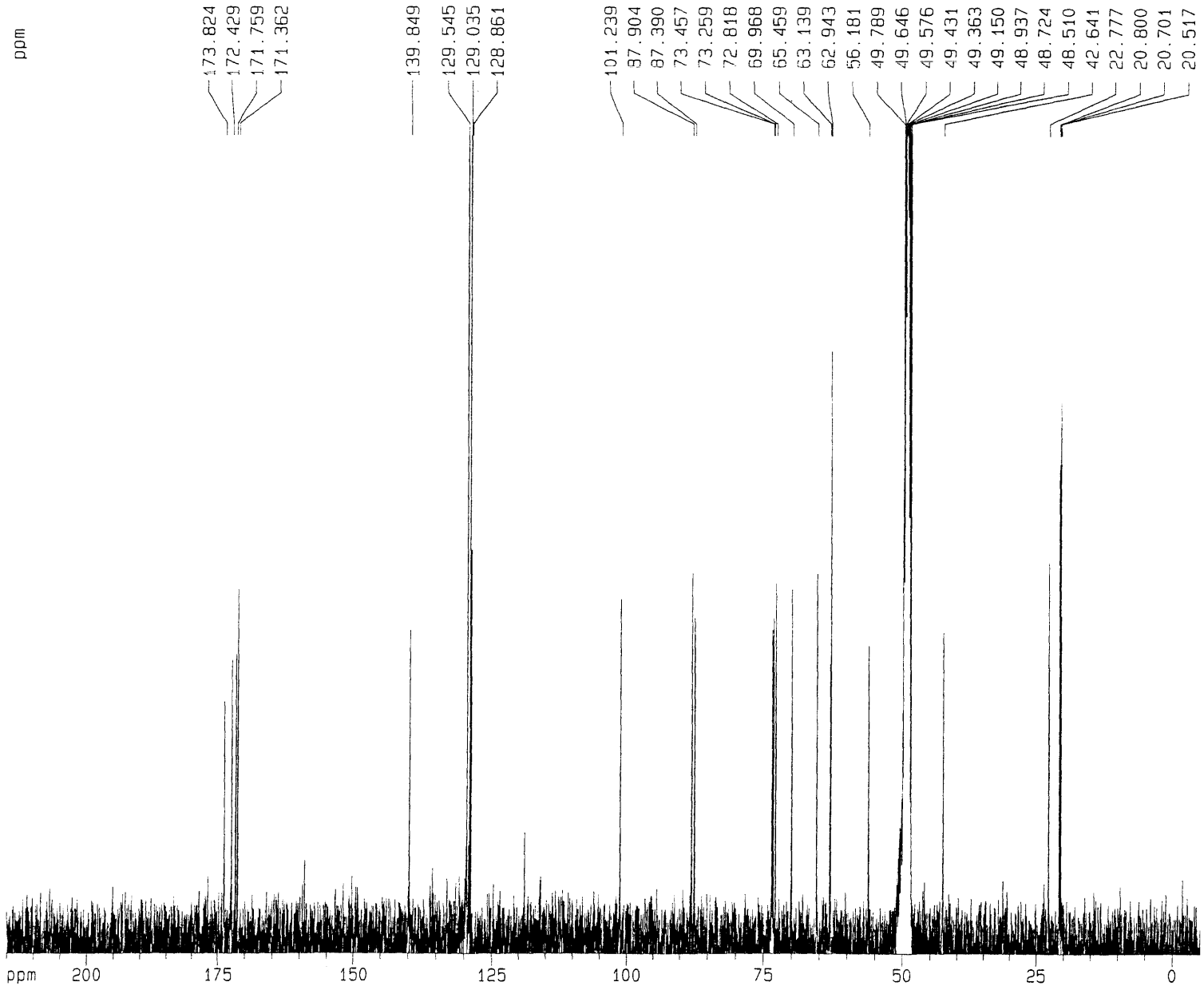
> 8:1 mixture

^{1}H NMR (600 MHz, CD_3OD)



3-33

^{13}C NMR (100 MHz, CD_3OD)

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Current Data Parameters
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EXPNO                       3
PROCNO                      1

F2 - Acquisition Parameters
Date_                20050525
Time                    21.04
INSTRUM                 spect
PROBHD       5mm BBO BB-1
PULPROG                zgpg30
TD                      65536
SOLVENT                  MeOH
NS                       10240
DS                           2
SWH                25125.629 Hz
FIDRES              0.383387 Hz
AQ                  1.3042164 sec
RG                       2048
DW                     19.900 usec
DE                      6.00 usec
TE                     300.0 K
D1                2.00000000 sec
d11               0.03000000 sec
d12               0.00002000 sec

============ CHANNEL f1 ============
NUC1                      13C
P1                      15.25 usec
PL1                      3.00 dB
SFO1              100.6237959 MHz

============ CHANNEL f2 ============
CPDPRG2               waltz16
NUC2                      1H
PCPD2                  107.50 usec
PL2                     0.00 dB
PL12                   24.00 dB
PL13                   24.00 dB
SFO2              400.1316005 MHz

F2 - Processing parameters
SI                       32768
SF                100.6126131 MHz
WDW                         EM
SSB                          0
LB                      1.00 Hz
GB                           0
PC                      1.40

1D NMR plot parameters
CX                     20.00 cm
F1P                  215.000 ppm
F1                 21631.71 Hz
F2P                   -5.000 ppm
F2                  -503.06 Hz
PPMCM                11.00000 ppm/cm
HZCM               1106.73877 Hz/cm
```
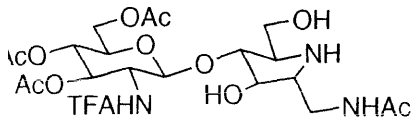
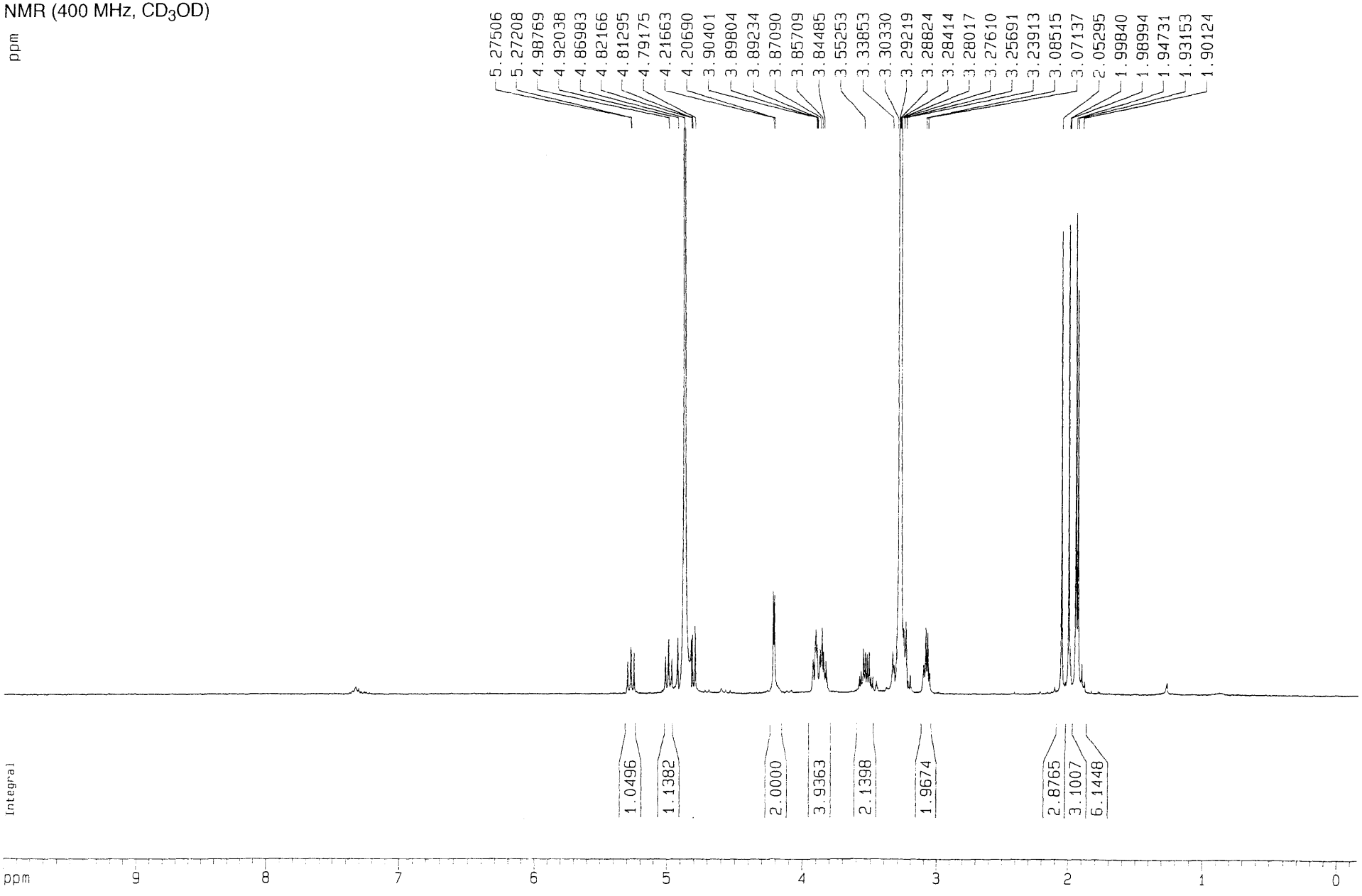
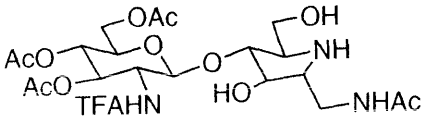



3-34

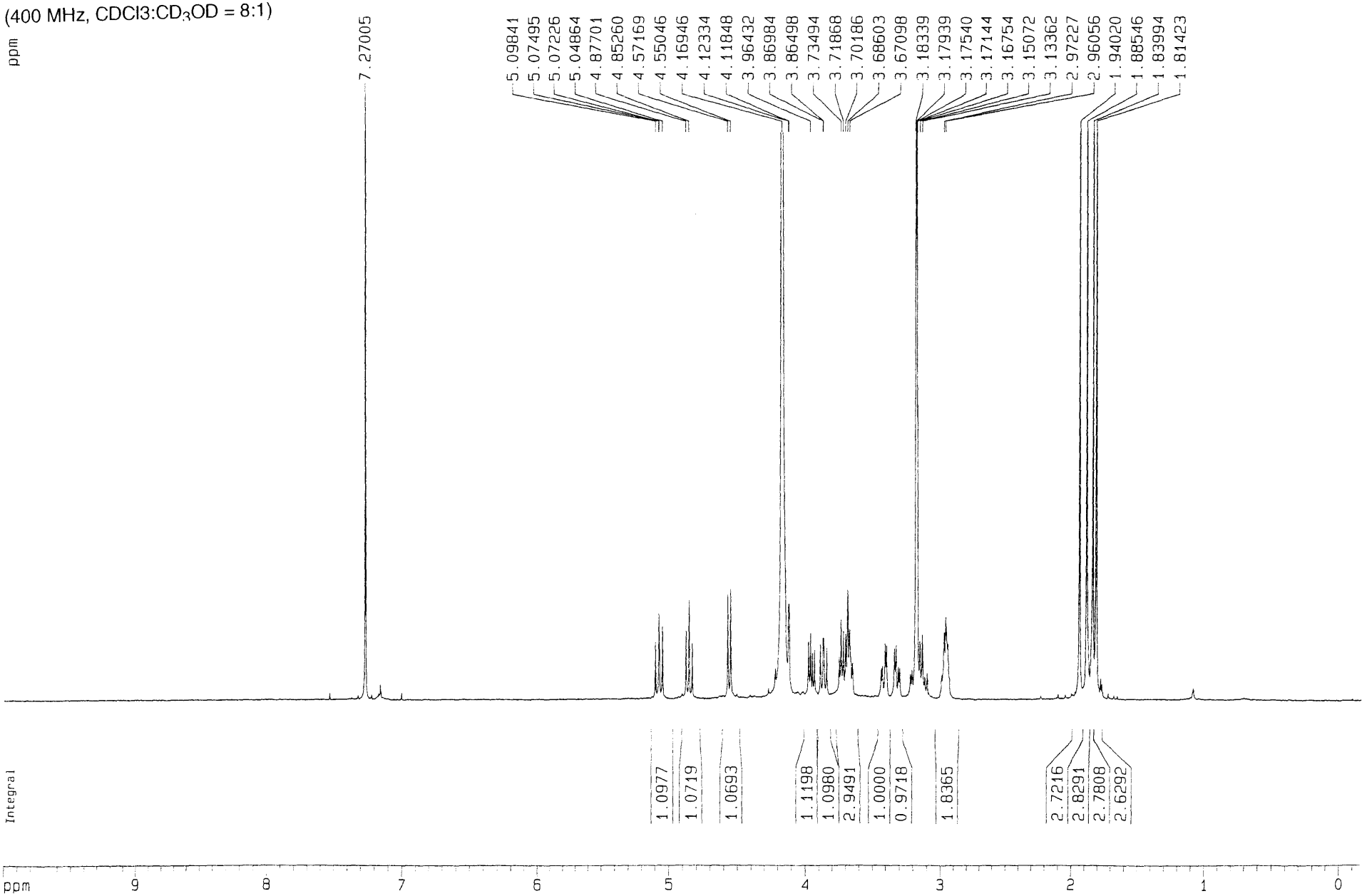
^{1}H NMR (400 MHz, CD_3OD)

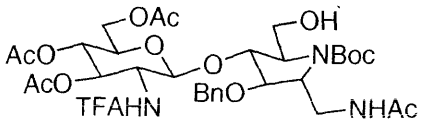




3-34

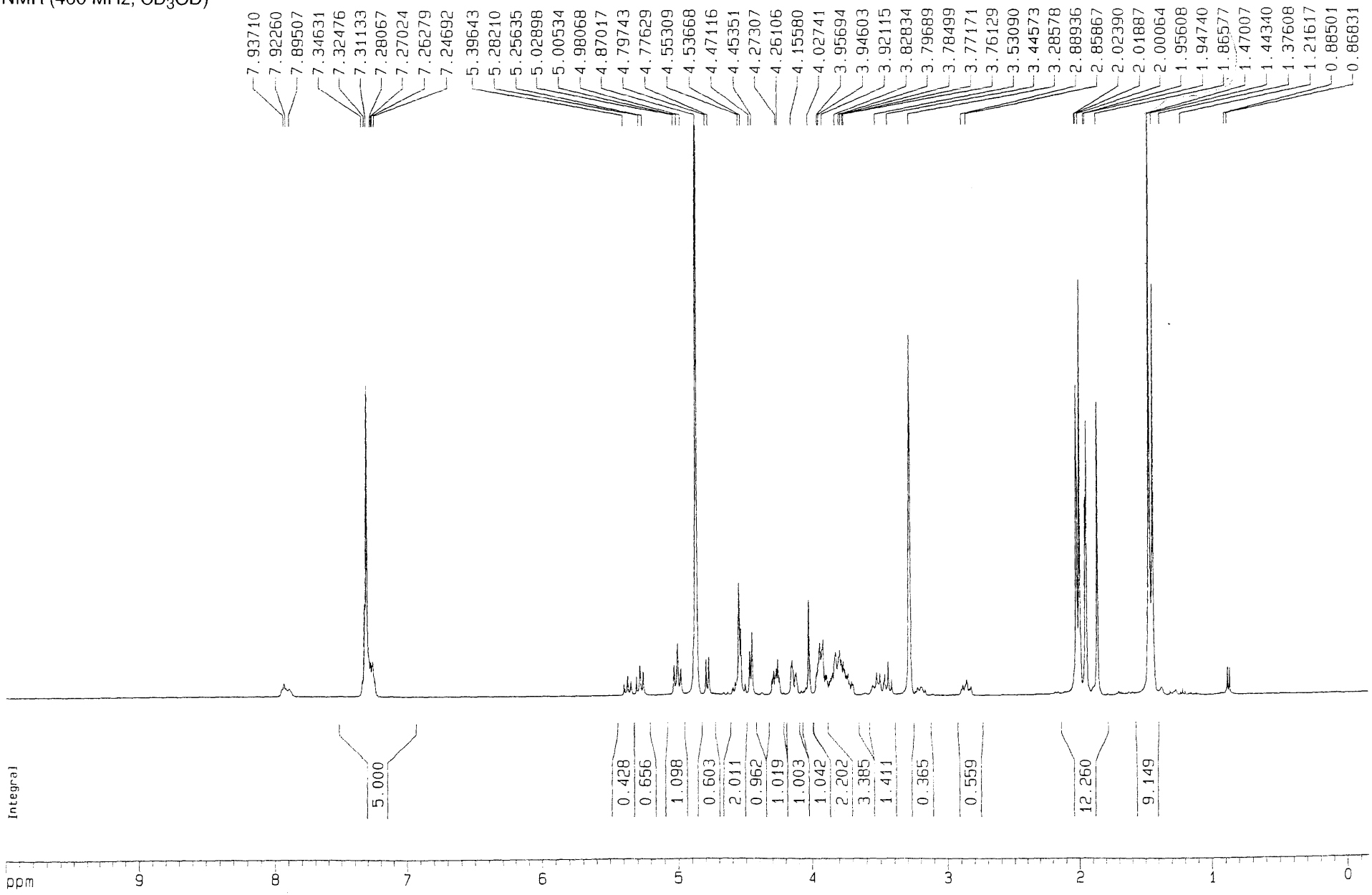
NMR (400 MHz, $CDCl_3$:CD_3OD = 8:1)

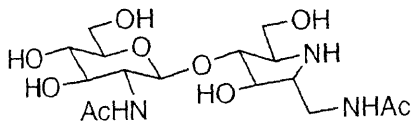




3-35

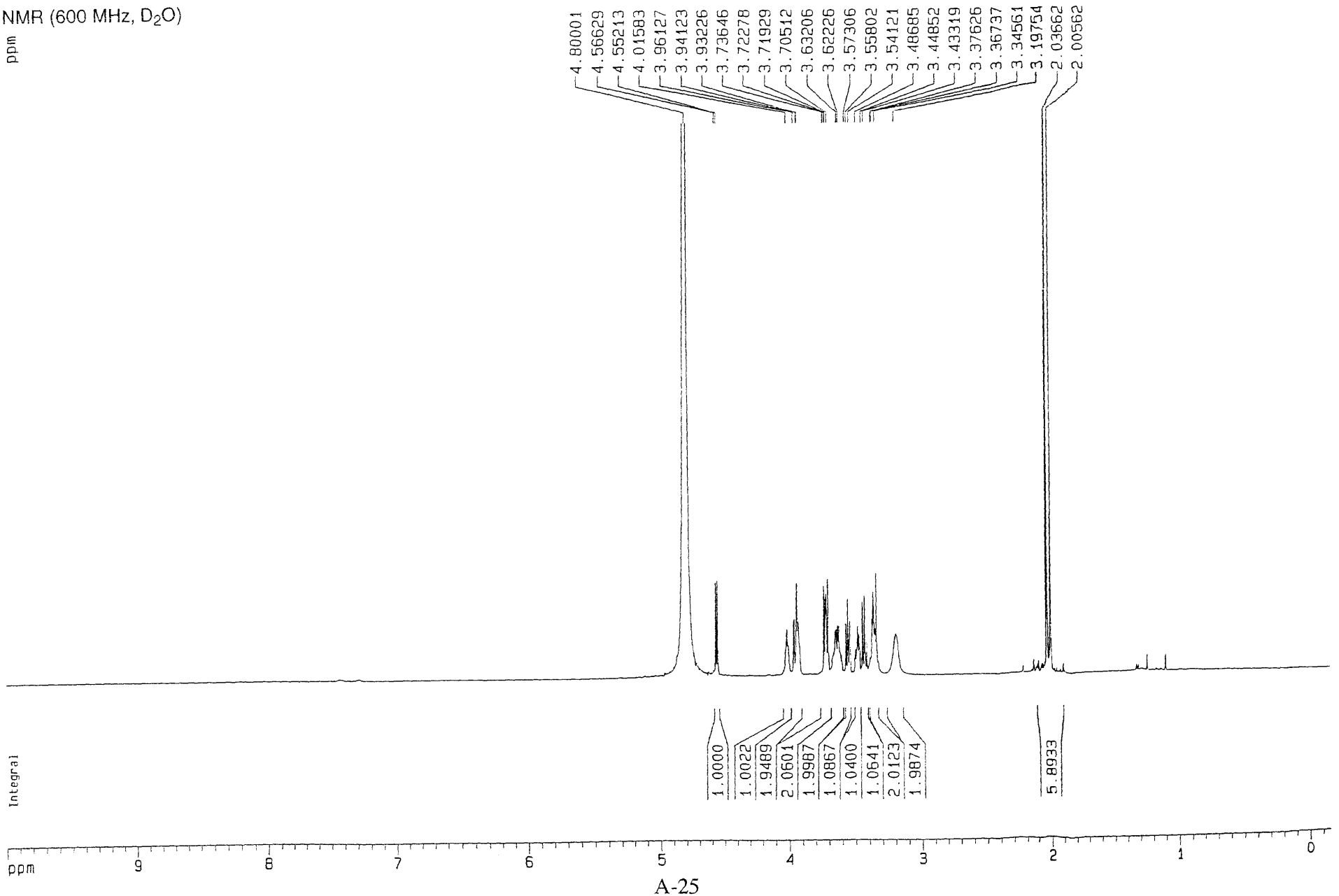
^{1}H NMR (400 MHz, CD_3OD)

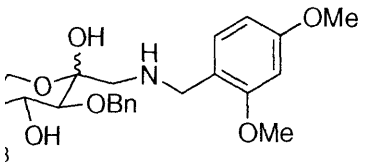




3-2

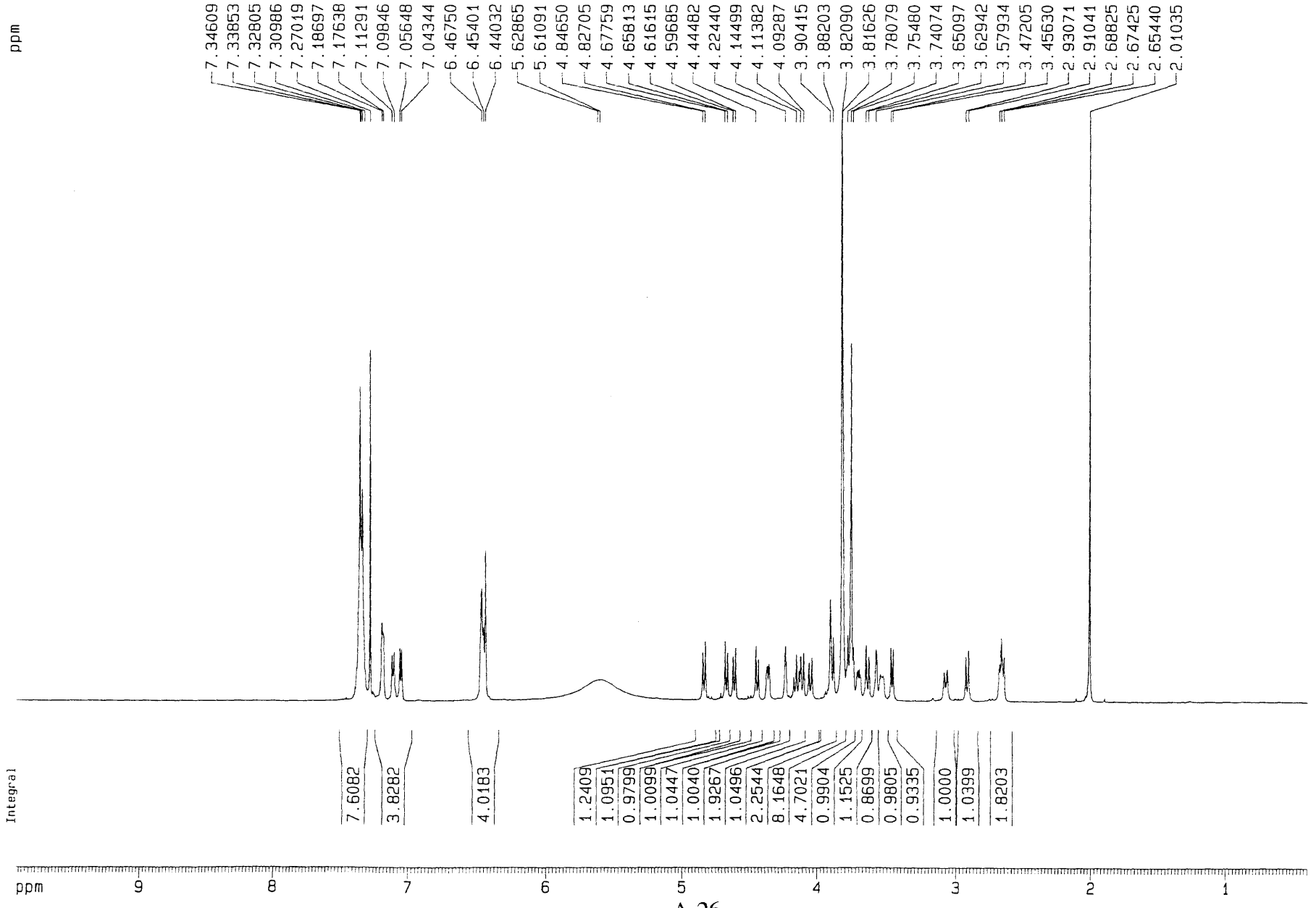
^{1}H NMR (600 MHz, D_2O)

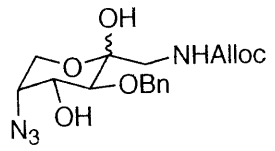




4-10

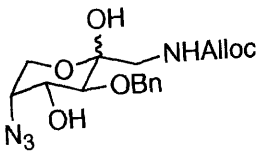
I NMR (600 MHz, $CDCl_3$)





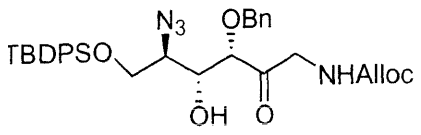
4-11

^{1}H NMR (500 MHz, CD_3OD)



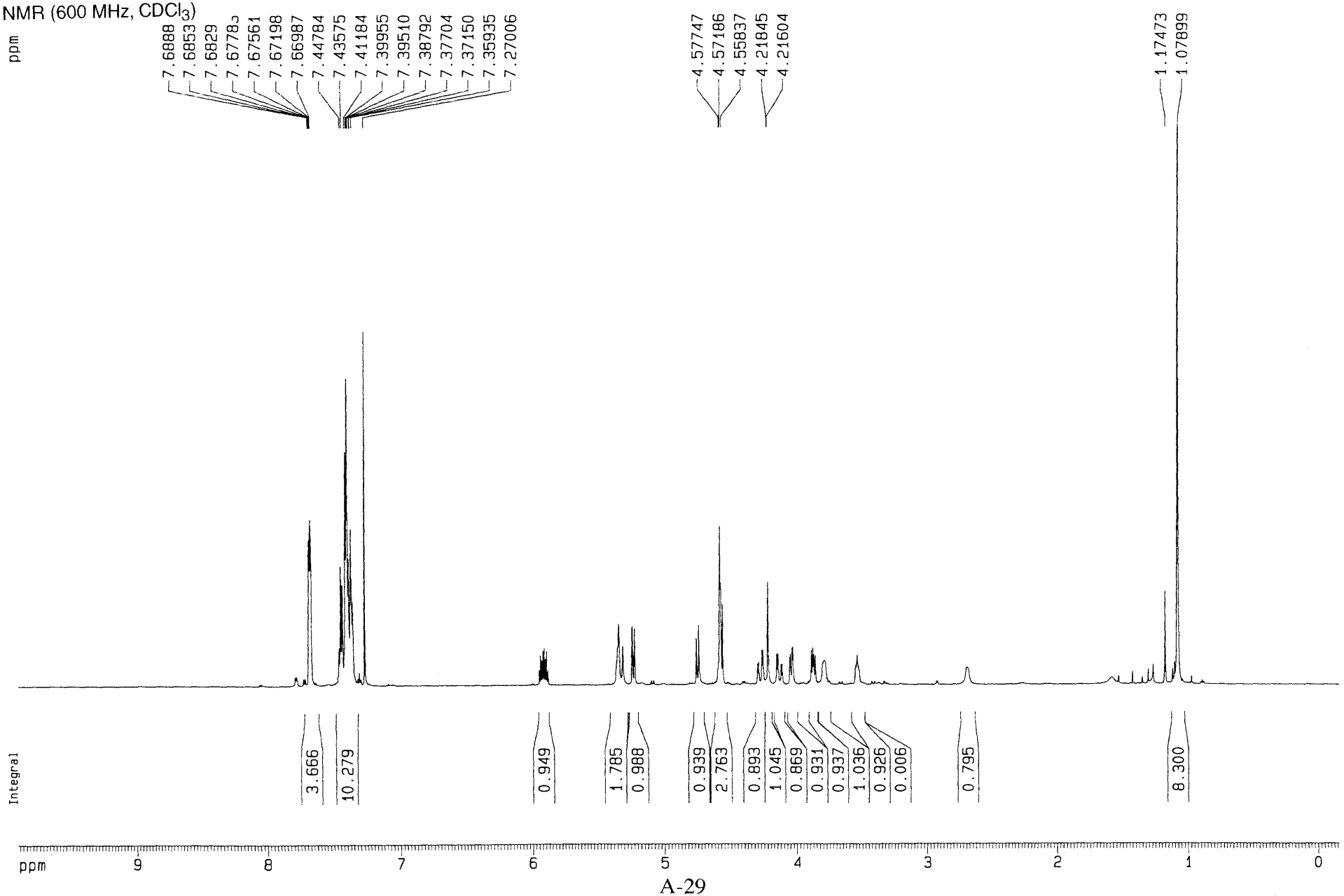
4-11

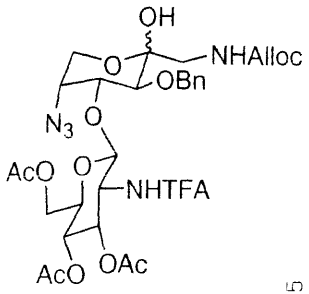
^{13}C NMR (125 MHz, CD_3OD)



4-12

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





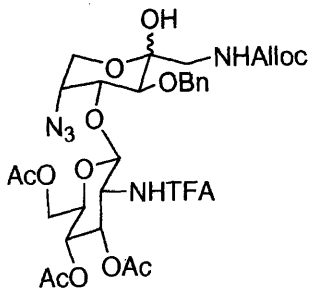
4-16

H NMR (400 MHz, CD_3OD)

7.37545
7.35748
7.31924
7.29849
7.21728
7.20009
7.18145
7.16504
7.14737
7.12912
5.81454
5.79879
5.77215
5.26506
5.24059
5.21505
5.20514
4.94126
4.87001
4.84927
4.82829
4.78112
4.52194
4.49590
4.41997
4.40856
3.96462
3.95922
3.93324
3.90201
3.83431
3.63801
3.63462
3.54339
3.51940
3.46765
3.43317
3.18875
3.18497
3.18188
3.01610
2.98181
1.91361
1.90366
1.88844
1.87799
1.86218
1.83150
1.23485
1.21861
1.13414
1.11616
1.09857
0.78508

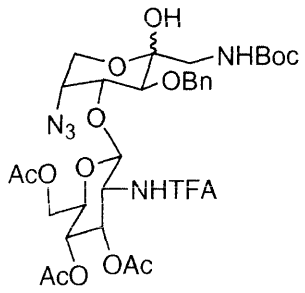
Integral
5.0563
1.0485
2.0810
1.0183
1.1079
1.9042
0.9901
2.1449
1.0364
1.0451
2.0451
2.0279
1.0888
1.0390
1.0714
0.9907
0.9502
9.0000

ppm 9 8 7 6 5 4 3 2 1 0



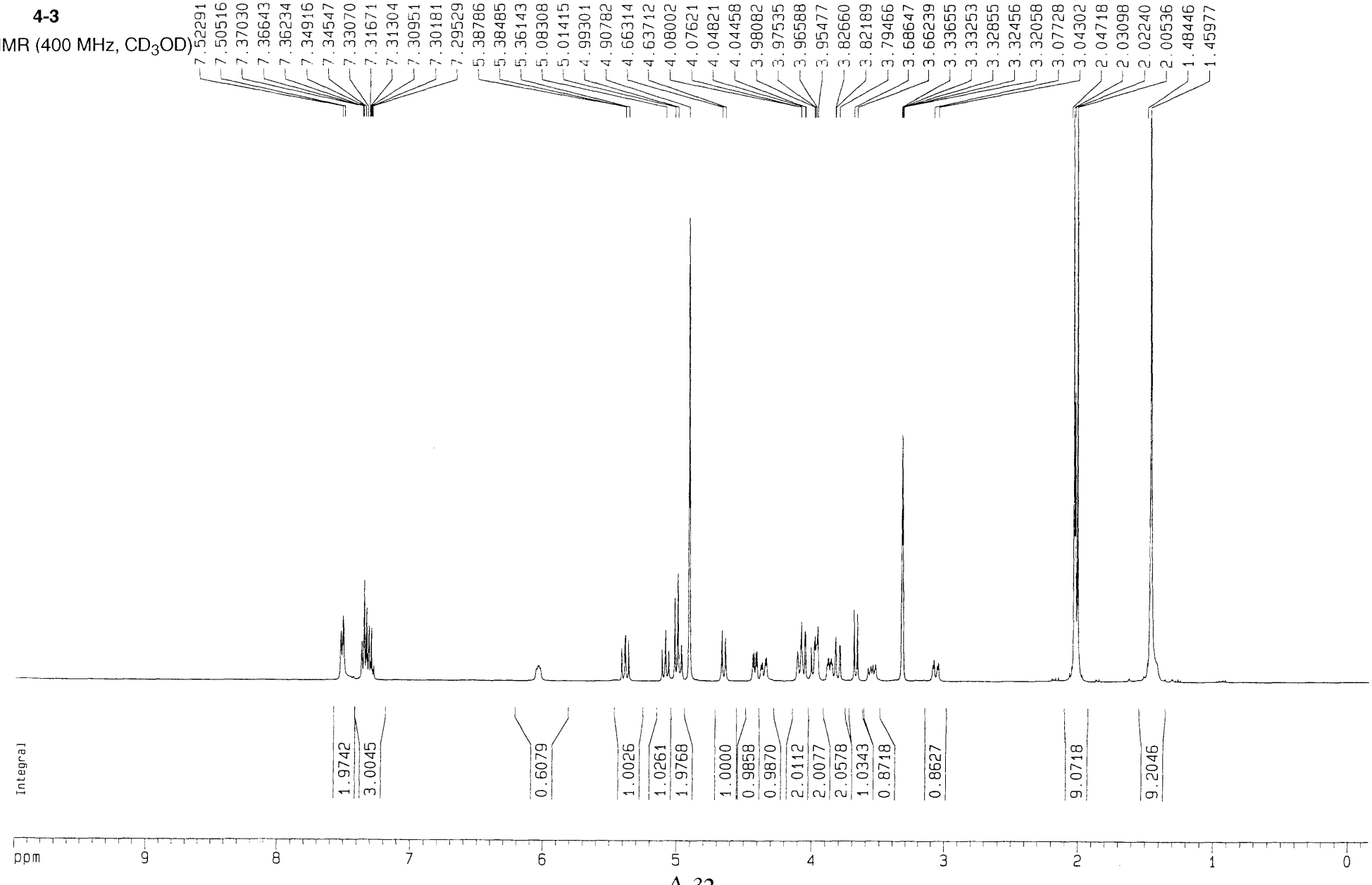
4-16

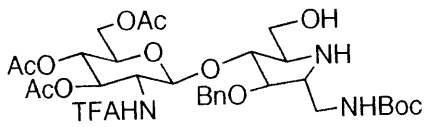
^{13}C NMR (125 MHz, CD_3OD)



4-3

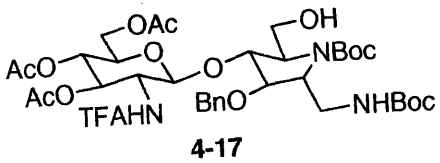
^{1}H NMR (400 MHz, CD_3OD)





4-2

^{1}H NMR (500 MHz, CD_3OD)



4-17

^{13}C NMR (125 MHz, CD_3OD)

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Eun Lee, Seung Jib Choi, Hahn Kim, Hee Oon Han, Young Keun Kim, Sun Joon Min, Sung Hee Son, Sang Min Lim and Won Suk Jang. " Total synthesis of Ambruticin" *Angew. Chem. Int. Ed.* **2002**, 41, 176-178

Eun Lee, Seung Jib Choi. " Radical cyclization of β-alkoxymethacrylates: Expedient synthesis of (+)-Methyl Nonactate" *Org. Lett.* **1999**, 1, 1127-1128