INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

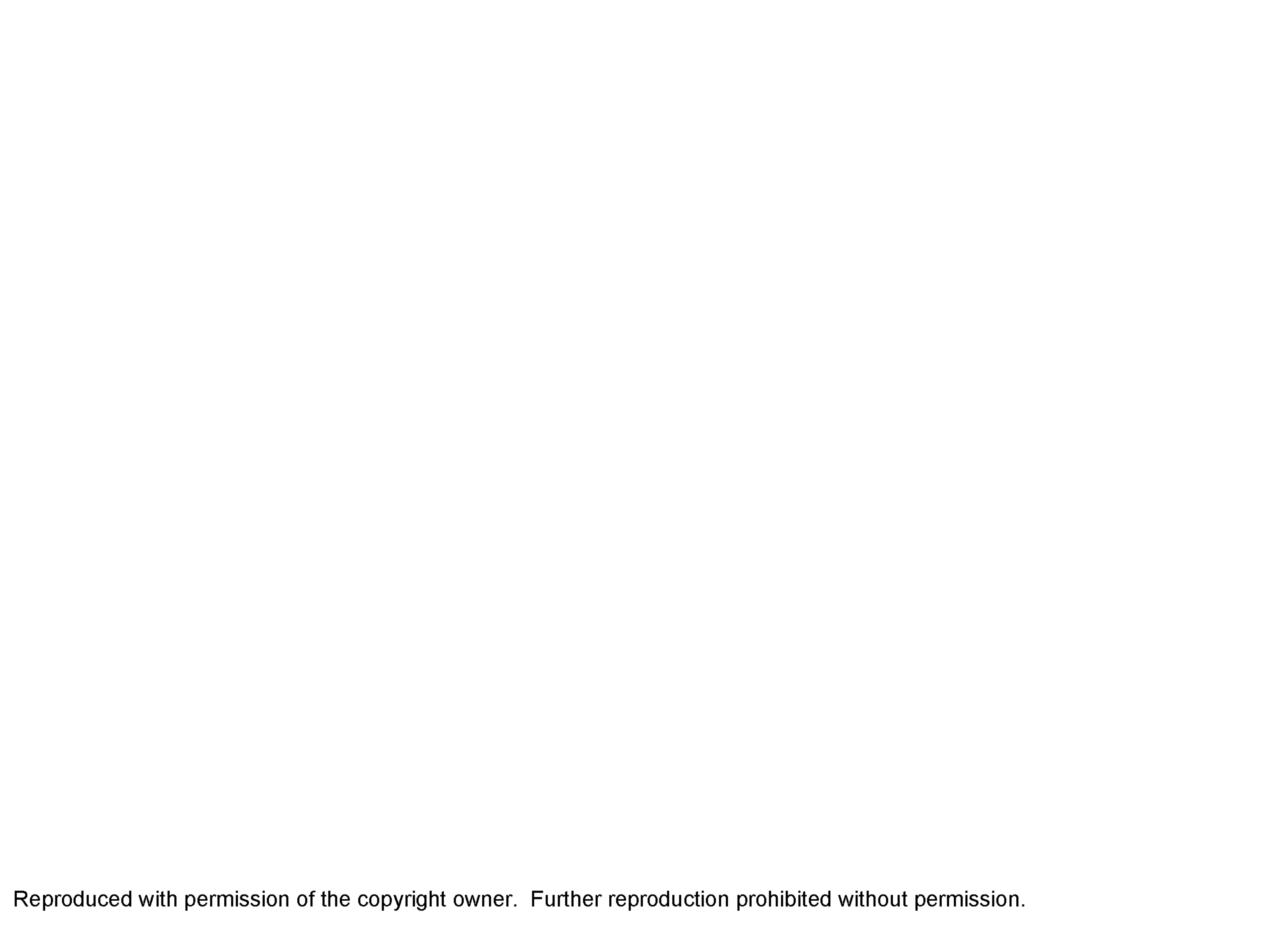
In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

ProQuest Information and Learning
300 North Zeeb Road, Ann Arbor, MI 48106-1346 USA
800-521-0600

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.



Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

UNIVERSITY OF ALBERTA

Enzymatic and Chemical Synthesis of Oligosaccharide Analogs

by

Xiangping Qian

A thesis submitted to the Faculty of Graduate Studies and Research

in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

DEPARTMENT OF CHEMISTRY

Edmonton, Alberta

Spring, 2000

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

National Library of Canada
Bibliothèque nationale du Canada

Acquisitions and Bibliographic Services
Acquisitions et services bibliographiques

395 Wellington Street
Ottawa ON K1A 0N4
Canada

395, rue Wellington
Ottawa ON K1A 0N4
Canada

Your file Votre référence

Our file Notre référence

The author has granted a non-exclusive licence allowing the National Library of Canada to reproduce, loan, distribute or sell copies of this thesis in microform, paper or electronic formats.

L'auteur a accordé une licence non exclusive permettant à la Bibliothèque nationale du Canada de reproduire, prêter, distribuer ou vendre des copies de cette thèse sous la forme de microfiche/film, de reproduction sur papier ou sur format électronique.

The author retains ownership of the copyright in this thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without the author's permission.

L'auteur conserve la propriété du droit d'auteur qui protège cette thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

0-612-60015-7

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

UNIVERISITY OF ALBERTA

LIBRARY RELEASE FORM

NAME OF AUTHOR: **Xiangping Qian**

TITLE OF THESIS: **Enzymatic and Chemical Synthesis of Oligosaccharide Analogs**

DEGREE: **Doctor of Philosophy**

YEAR THIS DEGREE GRANTED: **2000**

Permission is hereby granted to the University of Alberta Library to reproduce single copies of this thesis and to lend or sell such copies for private, scholarly or scientific research only.

The author reserves all other publication and other rights in association with the copyright in the thesis, and except as herein before provided, neither the thesis nor any substantial portion thereof may be printed or otherwise reproduced in any material form whatever without the author's prior written permission.

SIGNED: [signature]

Permanent Address:
c/o Lynne Lechelt
Department of Chemistry
University of Alberta
Edmonton, Alberta
T6G 2G2

DATED: Jan. 25, 2000

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled **Enzymatic and Chemical Synthesis of Oligosaccharide Analogs** submitted by **Xiangping Qian** in partial fulfillment of the requirements for the degree of **Doctor of Philosophy**.

O. Hindsgaul, supervisor

D. R. Bundle

D. L. J. Clive

D. E. Kiely

G. Kotovych

P. Sporns

DATED: 18th January 2000

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Dedicated to the memory of my father

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

ABSTRACT

To explore the possibility of enzymatic glycosylation of analogs in which the C-bonded hydrogen atom is modified (termed carbon-branched sugars), a series of carbon-branched acceptor analogs was chemically synthesized. These analogs had the C-H bond of the ring carbon bearing the OH groups undergoing glycosylation replaced with a C-alkyl bond. Surprisingly, four glycosyltransferases: α1,3/4-fucosyltransferase, α1,3-galactosyltransferase, and the blood group A and B glycosyltransferases were found to be able to recognize the carbon-branched sugar analogs and catalyze their glycosylation. Efficient enzymatic synthesis of glycosides of the complex tertiary sugar alcohols was thus accomplished for the first time.

The glycosides of the tertiary alcohols produced by this chemoenzymatic approach were used as probes for carbohydrate-protein recognition. Analog **83** was found to be a kinetically competent substrate for α-fucosidase which cleaves the fucose residue in the Lewis X structure. Analog **86**, however, had much weaker binding affinity with E-selectin than the natural sialyl Lewis X. Analogs **87** and **89** displayed two and four times stronger binding affinity with the *Griffonia simplicifolia* I B_4 isolectin than their parent trisaccharides. The increased binding affinity may result from a less negative entropic contribution since the analogs may be conformationally more restricted due to the introduction of the bulky methyl group at the glycosidic linkage.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

The second part of the thesis focused on new methods for the stereoselective synthesis of glycosides. An efficient methodology for the synthesis of β-glycosides of 2-amino-2-deoxy sugars was developed employing the *p*-nitrobenzyloxycarbonyl (PNZ) protecting group. The PNZ group was shown to be an orthogonal amine protecting group which can be readily removed either by hydrogenolysis or by sodium dithionite under neutral conditions.

Also described in this thesis is the exploratory work on the development of a method to make hemithio-orthoesters of complex sugar alcohols, which can be further reduced to give β-glycosides with high stereoselectivity. The preliminary results obtained suggest that the synthesis of β-glycosides from the hemithio-orthoesters will not be practical until better thionation chemistry is developed.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

ACKNOWLEDGMENTS

I am most grateful to my supervisor, Professor Ole Hindsgaul, for his excellent guidance, continued enthusiasm and support throughout my studies. I am very grateful to Professor Monica M. Palcic for her advice, encouragement and for generously sharing her knowledge, time and enthusiasm. It has truly been a privilege to work in such a great research environment as they have created. I would also like to thank the Alberta Research Council for a Graduate Scholarship in Carbohydrate Chemistry.

I thank all the members of our research group for their help, friendship and advice. Special thanks go to Drs. Suzanne Hof, Hailong Jiao, Vivek Kamath, Pu-Ping Lu, Carles Malet, Joseph McAuliffe, Ali Mukherjee, Hasan Tahir, Taketo Uchiyama, Jianqiang Wang, Zhi-Guang Wang and Boyan Zhang. In Dr. Palcic's group I am eternally indebted to Dr. Keiko Sujino, Ms. Hong Li, Catherine A. Compston, Iwa Kong and Deanne Cole for all their kind help with the enzymatic experiments. I thank Dr. Warren W. Wakarchuk, National Research Council of Canada, Ottawa, for providing the α1,4-galactosyltransferase. I gratefully acknowledge Dr. Albin Otter for recording the 500 and 600 MHz NMR spectra and sharing his expertise and Dr. Angelina Morales-Izquierdo for her professional cooperation and enthusiasm. As well I sincerely thank Dr. Boyan Zhang for performing the FAC/MS assay reported in this thesis, Dr. Suzanne Hof and Mr. David Rabuka for proof-reading parts of the manuscript and Ms. Lynne Lechelt for her kind help and encouragement throughout my studies.

Finally, my deepest gratitude goes to my wife, my sisters and our parents for their support, understanding and encouragement during my studies.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

TABLE OF CONTENTS

Chapter		Page
PART I.	**Chemoenzymatic Synthesis of Carbon-Branched Oligosaccharides**	
1.	**Introduction**	1
1.1	Biological Roles of Carbohydrates	1
1.2	Naturally Occurring Complex Carbohydrates	2
1.3	Biosynthesis of Oligosaccharides in Mammalian Cells	3
1.4	Glycosyltransferases	5
1.5	Enzymatic Synthesis of Oligosaccharide Analogs	8
1.5.1	β1,4-Galactosyltransferase	10
1.5.2	α1,3-Galactosyltransferase	12
1.5.3	α2,3- and α2,6-Sialyltransferases	14
1.5.4	Human Milk α1,3/4-Fucosyltransferase	17
1.5.7	Human Blood Group A and B Glycosyltransferases	19
1.6	Carbon-Branched Sugars and Glycosides of Tertiary Alcohols	21
1.7	Scope of Project	25
2.	**Chemical Synthesis of Carbon-Branched Sugar Analogs**	29
2.1	Introduction	29
2.2	Chemical Synthesis	31
2.2.1	Preparation of 4-*C*-Branched Glucoside	31
2.2.2	Preparation of 3'-*C*-Branched Lactosides	33
2.2.3	Preparation of 4'-*C*-Branched Lactoside	35

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

2.2.4 Preparation of 3-*C*-Branched LacNAc 37

2.2.5 Preparation of 6',6'-di-*C*-Branched LacNAc 43

2.2.6 Preparation of 3-*C*-Branched H Disaccharides 45

2.3 Experimental 47

2.3.1 General Methods 47

2.3.2 Synthesis of Carbon-Branched Sugar Analogs 48

3. Evaluation of Carbon-Branched Sugar Analogs and Enzymatic Synthesis of Carbon-Branched Oligosaccharides 86

3.1 Introduction 86

3.2 Enzymatic Assays 86

3.2.1 β1,4-Galactosyltransferase 86

3.2.2 α2,3- and α2,6-Sialyltransferases 88

3.2.3 α1,3/4-Fucosyltransferase 89

3.2.4 α1,3-Galactosyltransferase 91

3.2.5 Human Blood Group A and B Glycosyltransferases 92

3.2.6 α1,4-Galactosyltransferase 95

3.3 Preparative Enzymatic Synthesis 97

3.4 Summary 102

3.5 Experimental 105

3.5.1 Enzymatic Assay 105

3.5.1 Preparative Enzymatic Synthesis 108

4. Carbohydrate-Protein Binding Studies with Glycosides of Tertiary Alcohols 120

4.1 Introduction 120

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

4.2 Studies of Lewis X Analog **83** with α-Fucosidase 124

4.3 Studies of Sialyl Lewis X Analog **86** with E-Selectin 125

4.4 Studies of Analogs **88-92** with GSI-B_4 Isolectin 127

PART II. Studies on the Stereoselective Synthesis of Glycosides

5. Introduction 134

5.1 Stereoselective Glycosylation 134

5.1.1 1,2-*trans*-Glycosylation 135

5.1.2 1,2-*cis*-Glycosylation 137

5.1.3 Synthesis of β-Mannosides 138

5.1.4 Synthesis of 2-Deoxy-β-Glycosides 141

5.1.5 Synthesis of α-Sialosides 143

5.2 Synthesis of 2-Amino-2-Deoxy Glycosides 144

6. Studies on the Stereoselective Synthesis of 2-Amino-2-Deoxy Glycosides 149

6.1 The *p*-Nitrobenzyloxycarbonyl (PNZ) Group as an Orthogonal Protecting Group in the Synthesis of β-Glycosides of 2-Amino-2-Deoxy Sugars 149

6.1.1 Installation of the PNZ Group onto 2-Amino-2-Deoxy Sugars 151

6.1.2 Evaluation of the PNZ Protected Imidate Donors 152

6.1.3 Deprotection of the PNZ group 155

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

6.2 Studies on the Synthesis of α-Glycosides of 2-Amino-2-Deoxy Sugars Using *o*-Nitrobenzenesulfonyl and *p*-Nitrobenzenesulfonyl as Amine Protecting Groups 157

6.2.1 Evaluation of an ONS Protected Imidate Donor 159

6.2.2 Evaluation of a PNS Protected Imidate Donor 160

6.3 Experimental 162

7. Studies on the Synthesis of β-Glycosides without Neighboring Group Participation 172

7.1 Introduction 172

7.2 Synthetic Studies 174

7.3 Experimental 180

8. Bibliography 188

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

LIST OF TABLES

Table	Title	Page
Table 2.1	Relevant NOEs obtained from the carbon-branched sugar analogs	33
Table 3.1	Kinetic properties of analogs **5** and **6** with α1,3/4-FucT	90
Table 3.2	Kinetic properties of analogs **2** and **3** with α1,3-GalT	92
Table 3.3	Kinetic properties of analogs **7** and **8a** with GTA and GTB	93
Table 3.4	Relative reaction rates of the analogs with α1,4-GalT	96
Table 3.5	Summary of the enzymatic assay results of the eight C-branched analogs with four retaining and four inverting enzymes	103
Table 4.1	Dissociation constants of the oligosaccharide analogs with GSI-B_4 isolectin	132
Table 6.1	Glycosylation results using PNZ protected imidate donors (1.5 eq.)	153
Table 6.2	Glycosylation results using **96a** as the donor (1.5 eq.) and **99** as the acceptor in different solvents and with different promoters	154

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

LIST OF FIGURES

Figure	Title	Page
Figure 1.1	Some precursor structures for *O*-linked glycoproteins	2
Figure 1.2	Three types of glycan structures of *N*-linked glycoproteins	3
Figure 1.3	Reactions catalyzed by Leloir-type glycosyltransferases	6
Figure 1.4	Nine sugar nucleotide donors used in mammalian systems	7
Figure 1.5	Reaction catalyzed by β1,4-galactosyltransferase	10
Figure 1.6	Non-natural acceptors for β1,4-galactosyltransferase	11
Figure 1.7	Reaction catalyzed by α1,3-galactosyltrasnferase	13
Figure 1.8	Non-natural acceptors for α1,3-galactosyltransferase	14
Figure 1.9	Reaction catalyzed by α2,3-sialyltransferase	15
Figure 1.10	Reaction catalyzed by α2,6-sialyltransferase	16
Figure 1.11	Non-natural acceptors for α2,3-sialyltrasnferase	16
Figure 1.12	Non-natural acceptors for α2,6-sialyltrasnferase	17
Figure 1.13	Reactions catalyzed by human milk α1,3/4-fucosyltransferase	17
Figure 1.14	Non-natural acceptors for human milk α1,3/4-fucosyltransferase	18
Figure 1.15	Reactions catalyzed by blood group A and B glycosyltransferases	20
Figure 1.16	Non-natural acceptors for blood group A glycosyltransferase (left) and B glycosyltransferase (right)	20
Figure 1.17	Some naturally occurring C-branched sugars	22
Figure 1.18	Some naturally occurring glycosides of tertiary alcohols	23
Figure 1.19	Examples on chemical synthesis of glycosides of complex tertiary alcohols	24
Figure 1.20	Schematic representation of the scope of the project	26
Figure 1.21	Carbon-branched analogs synthesized for the enzymatic studies	27
Figure 2.1	Structures of eight *C*-branched acceptor analogs	30

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Figure 2.2	Synthetic approaches to the *C*-branched disaccharides	31
Figure 2.3	Preparation of the 3'-*C*-methyl glucoside **1a**	32
Figure 2.4	Preparation of the 3'-*C*-branched lactosides **2** and **3a**	34
Figure 2.5	Preparation of the 4'-*C*-methyl lactoside **4a**	36
Figure 2.6	First attempt to prepare the 3-*C*-methyl LacNAc **5**	38
Figure 2.7	Literature procedures for nucleophilic addition to the 1,6-anhydro-3-uloses [80]	39
Figure 2.8	Second attempt to prepare the 3-*C*-methyl LacNAc **5**	40
Figure 2.9	Third attempt to prepare the 3-*C*-methyl LacNAc **5**	41
Figure 2.10	Preparation of the 3-*C*-methyl LacNAc **5**	42
Figure 2.11	Attempts to prepare the 6',6'-di-*C*-methyl LacNAc **6**	44
Figure 2.12	Preparation of the 6',6'-di-*C*-methyl LacNAc **6**	45
Figure 2.13	Preparation of the 3-*C*-branched H- disaccharides **7, 8a** and **8b**	46
Figure 3.1	Schematic representation of the radioactive "Sep-Pak assay"	87
Figure 3.2	Hypothetical binding site interaction near OH-4 of Gal	88
Figure 3.3	Proposed transition-state structure for the human milk α1,3/4-fucosyltransferase reaction	91
Figure 3.4	Hypothetical two-step, double-displacement mechanism for the retaining glycosyltransferases	94
Figure 3.5	Enzymatic synthesis of the Lewis X analogs **83** and **84**	97
Figure 3.6	Enzymatic synthesis of the sialyl Lewis X analog **86**	98
Figure 3.7	Enzymatic synthesis of the trisaccharide analogs **87** and **88**	99
Figure 3.8	1D-TROESY spectra obtained from selective excitation of the H-1" resonance (top) and 1D 600 MHz spectra of the trisaccharide analogs **87** and **88** (bottom)	100
Figure 3.9	Enzymatic synthesis of the A and B trisaccharide analogs **89-92**	101
Figure 3.10	Proposed binding models for mammalian glycosyltransferases	104

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Figure 4.1	Schematic representation of carbohydrate-protein binding	121
Figure 4.2	Examples of constrained oligosaccharides used in carbohydrate-protein binding studies	123
Figure 4.3	Schematic representation of spectrophotometric assay for α-fucosidase	124
Figure 4.4	Kinetic properties of Lewis X analog **83** with α-fucosidase	125
Figure 4.5	Top: schematic representation of the binding of sialyl Lewis X to E-selectin. Bottom: the structure of analog **86**	126
Figure 4.6	Schematic representation of the tetrametric structure of the five GS I isolectins consisting of varying proportions of A and B subunits	127
Figure 4.7	Schematic representation of the FAC/MS assay	128
Figure 4.8	Structures of the compounds analyzed by FAC/MS	129
Figure 4.9	Top: Total ion chromatogram (TIC). Middle: Selected ion chromatograms of all ten compounds	130
Figure 5.1	Types of glycosidic linkages	134
Figure 5.2	1,2-*trans*-Glycosylation based on neighboring group participation	135
Figure 5.3	1,2-*trans*-Glycosylation based on glycal epoxides	136
Figure 5.4	1,2-*trans*-Glycosylation in a participating solvent	136
Figure 5.5	1,2-*cis*-Glycosylation based on *in situ* anomerization	137
Figure 5.6	Synthesis of β-mannosides using insoluble catalysts	138
Figure 5.7	Synthesis of β-mannosides by intramolecular aglycone delivery (IAD)	139
Figure 5.8	Synthesis of β-mannosides by intramolecular glycosylation and glycosylation with mannosyl triflate	139
Figure 5.9	Synthesis of β-mannosides via a 1,2-*O*-*cis*-stannylene acetal	140
Figure 5.10	Synthesis of 2-deoxy-β-glycosides using temporary	141

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

participating groups

Figure 5.11 Synthesis of 2-deoxy-β-glycosides using a participating group at C-3 141

Figure 5.12 Synthesis of 2-deoxy-β-glycosides based on migration of an alkylthio aglycone 142

Figure 5.13 Synthesis of 2-deoxy-β-glycosides from a glycal 142

Figure 5.14 Glycosyl donors for the synthesis of α-sialosides 144

Figure 5.15 Synthesis of 2-amino-2-deoxy-β-glycosides using *N*-acetyl and *N*-Phth protecting groups 145

Figure 5.16 Amine protecting groups used in the synthesis of 2-amino-2-deoxy-β-glycosides 146

Figure 5.17 Amine protecting groups used in the synthesis of 2-amino-2-deoxy-α-glycosides 147

Figure 6.1 Glycosylation with benzyloxycarbonyl protected bromide [180] 150

Figure 6.2 The *p*-nitrobenzyloxycarbonyl group as an orthogonal amine protecting group 150

Figure 6.3 Installation of the PNZ group onto D-glucosamine and D-galactosamine 152

Figure 6.4 Deprotection of the PNZ group of **100** using hydrogenolysis 155

Figure 6.5 Deprotection of the PNZ group of **101** using hydrogenolysis 155

Figure 6.6 Deprotection of the PNZ group on **120** using sodium thionite 156

Figure 6.7 Synthesis of 2-amino-2-deoxy-β-glycosides using Sulfonamidoglycosylation 157

Figure 6.8 Deprotection of *o* (or *p*)-nitrobenzenesulfonamide 158

Figure 6.9 Preparation of an ONS protected imidate donor 159

Figure 6.10 Glycosylation with an ONS protected imidate donor 160

Figure 6.11 Preparation of a PNS protected imidate donor 161

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Figure 6.12	Glycosylation with a PNS protected imidate donor	161
Figure 7.1	Synthesis of 2-deoxy-β-glycosides via alkoxy substituted anomeric radicals	173
Figure 7.2	Schematic representation of the objective of the project	174
Figure 7.3	Preparation of the thionolactone **123**	175
Figure 7.4	Attempt to prepare the hemithio-orthoester **126**	176
Figure 7.5	Preparation of the hemithio-orthoester **126**	176
Figure 7.6	Reaction of thionolactone **123** with secondary alkoxides	177
Figure 7.7	Schematic representation of the intramolecular cyclization approach to hemithio-orthoesters	178
Figure 7.8	Preparation of the glucothionates **131** and **132**	179
Figure 7.9	Attempt to prepare the galactothionate **135**	179

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

LIST OF ABBREVIATIONS

α1,3-GalT	α1,3-galatacosyltransferase
α1,3/4-FucT	α1,3/4-fucosyltransferase
α1,4-GalT	α1,4-galatacosyltransferase
α2,3-SialT	α2,3-sialyltransferase
α2,6-SialT	α2,6-sialyltransferase
β1,4-GalT	β1,4-galatacosyltransferase
Ac	acetyl
All	allyl
anal.	analysis
aq.	aqueous
Asn	asparagine
Bn	benzyl
b	broad
BSA	bovine serum albumin
Bu	butyl
Bz	benzoyl
calcd	calculated
CMP	cytidine 5'-monophosphate
d	doublet or day(s)
DMF	*N,N*-dimethylformamide
DMSO	dimethylsulfoxide
DMTST	dimethyl(methylthio)sulfonium triflate
DTBMP	2,6-di-*tert*-butyl-4-methyl-pyridine
E.C.	Enzyme Commission
eq.	equivalent

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Et	ethyl
FAC	frontal affinity chromatography
Fuc	L-fucopyranose
Gal	D-galactopyranose
GalNAc	D-2-acetamido-2-deoxy-galactopyranose
Glc	D-glucopyranose
GlcA	D-glucuronic acid
GlcNAc	D-2-acetamido-2-deoxy-glucopyranose
GDP	guanosine 5'-diphosphate
GTA	blood group A glycosyltransferase
GTB	blood group B glycosyltransferase
h	hour(s)
HEPES	4-(2-hydroxyethyl)-1-piperizineethanesulfonic acid
Hz	hertz
HMBC	heteronuclear multiple bond coherence
HMDS	hexamethyldisilazide
HMQC	heteronuclear multiple quantum coherence
HR-ESMS	high resolution electrospray mass spectrometry
J	coupling constant
m	multiplet
m/z	mass to charge ratio
Man	D-mannopyranose
MCO	8-methoxycarbonyloctyl
Me	methyl
mg	milligram(s)
MHz	megahertz
min	minute(s)

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

mL	milliliter(s)
mol	mole(s)
mmol	millimole(s)
MS	mass spectrometry or molecular sieves
Neu5Ac	*N*-acetylneuraminic acid (sialic acid)
NMR	nuclear magnetic resonance
NOE	nuclear Overhauser effect
ONS	*o*-nitrobenzenesulfonyl
PCC	pyridinium chlorochromate
PDC	pyridinium dichromate
Ph	phenyl
Phth	phthaloyl
PNS	*p*-nitrobenzenesulfonyl
PNZ	*p*-nitrobenzyloxycarbonyl
ppm	parts per million
Pr	propyl
Py	pyridine
q	quartet
quant	quantitative
rt	room temperature
s	singlet
Satd	saturated
Ser	L-serine
t	triplet
TBAF	tetrabutylammonium fluoride
TBS	*tert*-butyldimethylsilyl
Tf	trifluoromethanesulfonyl

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

TFA	trifluoroacetic acid
THF	tetrahydrofuran
Thr	L-threonine
TLC	thin layer chromatography
TMP	2,2,6,6-tetramethylpiperidide
TMS	trimethylsilyl
Troc	2,2,2-trichloroethylformate
TROESY	transverse rotating frame nuclear Overhauser enhancement spectroscopy
Ts	*p*-toluenesulfonyl
UDP	uridine 5'-diphosphate
Xyl	D-xylose

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Part I

Chemoenzymatic Synthesis of Carbon-Branched Oligosaccharides

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Chapter 1

Introduction

1.1. Biological Roles of Carbohydrates

Carbohydrates have long been considered as energy sources, structural components and protective agents. This view, however, is being revised based on the observations that carbohydrates are critically involved in many cellular biological recognition events [1]. Carbohydrates are now well recognized as chemical mediators, receptors and cell-surface markers [2]. They are involved in intercellular communication, recognition and adhesion. Carbohydrates serve as receptors for binding bacteria, toxins, viruses and hormones. These molecules can control vital events in fertilization and early development, regulate many critical immune system recognition events and target aging cells for destruction. Cell-surface carbohydrates also change upon malignant transformation in metastasis and are responsible for significant differences in surface properties between metastatic and non-metastatic cells.

The increased recognition of their biological significance has caused a surge of interest in carbohydrates. As a result, new disciplines described as *Glycobiology*, *Glycochemistry*, *Glycoimmunology*, *Glycoscience* and *Glycotechnology* have appeared in the past decade [3]. Their inherent structural diversity has made carbohydrates superbly effective carriers of information as its component molecules, monosaccharides, can be connected at several positions to form a vast array of branched or linear structures [4]. There is little doubt that many more new roles of carbohydrates will be uncovered for this diverse and complex class of molecules.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

1.2. Naturally Occurring Complex Carbohydrates

The majority of carbohydrates present in cells are covalently attached to proteins or lipids. These carbohydrate-biomolecule adducts are called glycoconjugates which include glycoprotein, glycolipids and proteoglycans. Glycoproteins can be classified as either *O*-linked or *N*-linked glycoproteins [5]. In *O*-linked glycoproteins, saccharides are attached to the hydroxyl group of serine (Ser) or threonine (Thr) (Figure 1.1).

α-D-GalNAc-Ser/Thr

β-D-GlcNAc-β-Ser/Thr

α-L-Fuc-Ser/Thr

β-D-Xyl-Ser

Ser: R = H
Thr: R = CH_3

Figure 1.1. Some precursor structures for *O*-linked glycoproteins.

Carbohydrates in *N*-linked glycoproteins are linked to the amide group of asparagine (Asn) residue, which is part of a consensus sequence of Asn-X-Ser/Thr where X can be any amino acid other than proline. *N*-Linked glycans of glycoproteins can be divided into three types: high mannose type, complex type and hybrid type (Figure 1.2). All three types share a common pentasaccharide core structure, Manα1→6[Manα1→3] Manβ1→4GlcNAcβ1→4GlcNAcβ.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

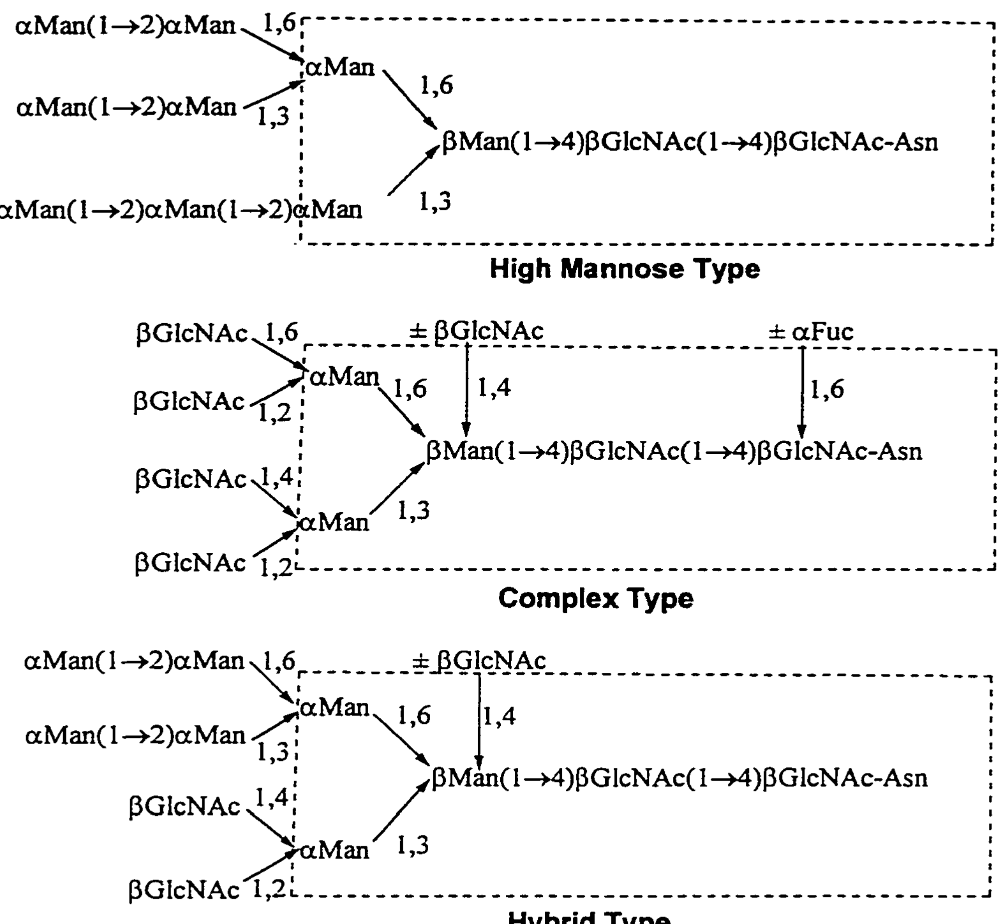


Figure 1.2. Three types of glycan structures of *N*-linked glycoproteins.

The carbohydrates in glycolipids are linked to the hydroxyl group of hydrophobic moieties such as a ceramide or prenyl phosphate [6]. They can be divided into glycoglycerolipids (which are abundant in bacterial and plants), glycosphingolipids (which are the major glycolipids in animals), and glycosyl-phosphatidyl-inositols (GPI) [7]. Proteoglycans, found primarily in connective tissue, contain serine-linked polysaccharides with repeating disaccharide units.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

1.3. Biosynthesis of Oligosaccharides in Mammalian Cells

Whereas proteins and nucleic acids are biosynthesized by well-established template mechanisms, mammalian oligosaccharides, like automobiles, are manufactured on an assembly line in which the monosaccharide building blocks are incorporated sequentially [8]. The elaborate assembly line for the oligosaccharides in glycoproteins and glycolipids consists of the endoplasmic reticulum (ER) and Golgi apparatus where glycosyltransferases and glycosidases are the workers. These two classes of enzymes have complementary functions: glycosyltransferases add carbohydrates to growing oligosaccharide chains while glycosidases cleave them.

The biosynthesis of *N*-linked glycans of glycoproteins is initiated in the ER where the individual sugars, *N*-acetylglucosamine (GlcNAc), mannose (Man) and glucose (Glc) are sequentially added to the lipid carrier, dolichol phosphate, by a series of glycosyltransferases to form $Glc_3Man_9GlcNAc_2$-PP-dolichol. The oligosaccharide is then transferred co-translationally to the Asn residue of proteins. Subsequent trimming of the oligosaccharide chains by glycosidases takes places in the ER to give glycoproteins with a $Man_8GlcNAc_2$ structure, which are then transported to Golgi apparatus. Two mannose residues are then removed and *N*-acetylglucosamine, fucose, galactose and sialic acid residues are sequentially added by glycosyltransferases [8].

The biosynthesis of *O*-linked glycans of glycoproteins starts mainly in the *cis* Golgi apparatus with the transfer of *N*-acetylglucosamine (GalNAc) to serine or threonine. Sugars are transferred from sugar nucleotides to synthesize eight *O*-glycan potential core structures which are further elongated with a variety of glycosyltransferases [9]. Biosynthesis of glycosphingolipids involves the glycosylation of ceramide and

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

sequential additions of monosaccharides from sugar nucleotide donors in the Golgi apparatus [10].

1.4. Glycosyltransferases

Glycosyltransferases are defined as a class of enzymes that catalyze the transfer of a glycosyl residue from a donor to an acceptor. They can be divided into Leloir type and non-Leloir type according to the type of glycosyl donors they use [11]. Leloir glycosyltransferases utilize sugar nucleotides as donors while non-Leloir glycosyltransferases typically use glycosyl phosphates as donors.

Most of the glycosyltransferases responsible for the biosynthesis of glycoproteins and glycolipids are Leloir-type glycosyltransferases. They are typically type II membrane proteins with a short cytoplasmic *N*-terminal domain, a hydrophobic transmembrane domain, a lumenally oriented stem and a large *C*-terminal catalytic domain [12]. Leloir glycosyltransferases have been endowed with a precise specificity for both the sugar nucleotide donor and acceptor. They catalyze the transfer in a regio- and stereospecific manner with either retention (retaining enzymes) or inversion (inverting enzymes) of configuration at the anomeric center of the donor (Figure 1.3). According to the "one linkage-one enzyme" concept [13], there are estimated to be hundreds of glycosyltransferases responsible for elaboration of the diverse and complex oligosaccharide structures found in nature.

Glycosyltransferases are classified according to their nucleotide donor sugars, the type of glycosidic linkage formed (α or β) and the specific hydroxyl group in the acceptor to which the monosaccharide is transferred. For example, β1,4-galactosyltransferase

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

(β1,4-GalT) from bovine milk catalyzes the transfer of a Gal unit with inversion of configuration from UDP-Gal to the 4-OH group of GlcNAcβ-R acceptors generating Galβ1→4GlcNAcβ-R structures. Mammalian glycosyltransferases use only nine main sugar nucleotides as building blocks to construct the complex oligosaccharides (Figure 1.4).

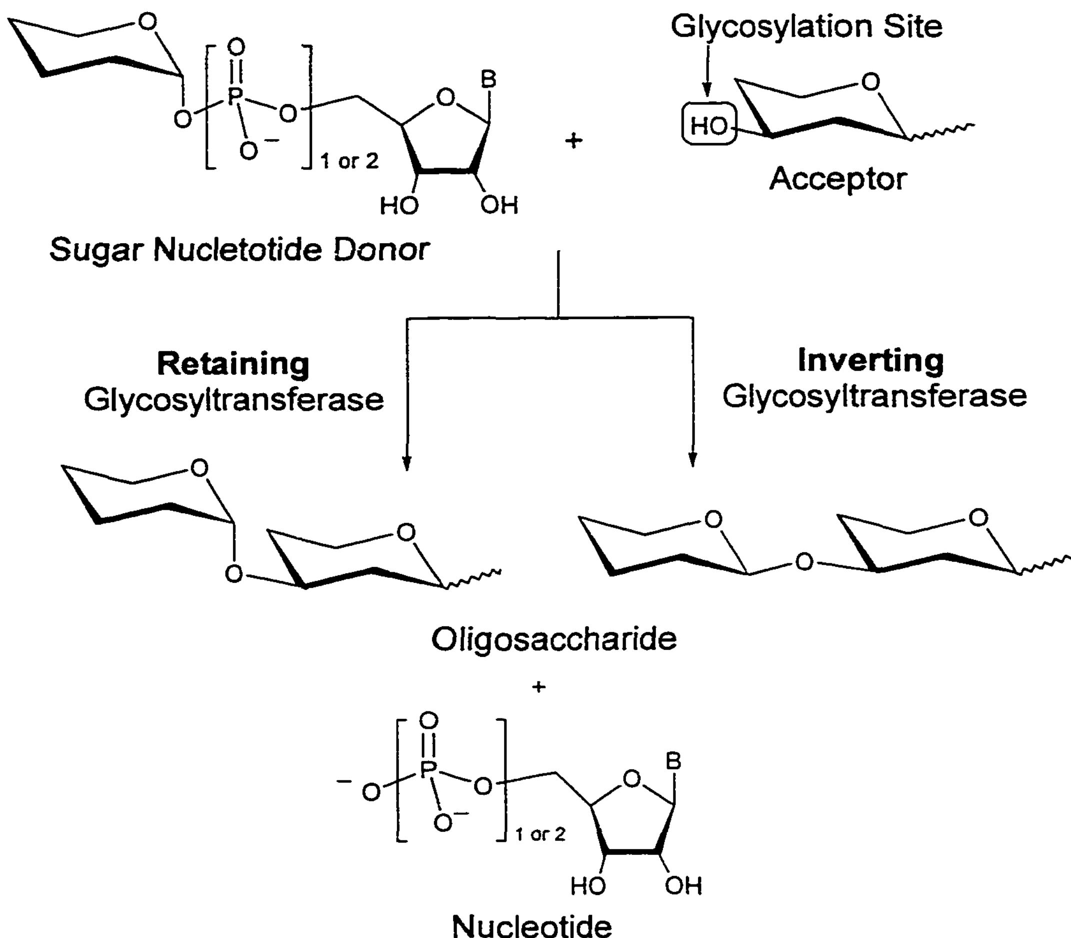


Figure 1.3. Reactions catalyzed by Leloir-type glycosyltransferases (B= Uracil, Guanine, Cytosine).

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

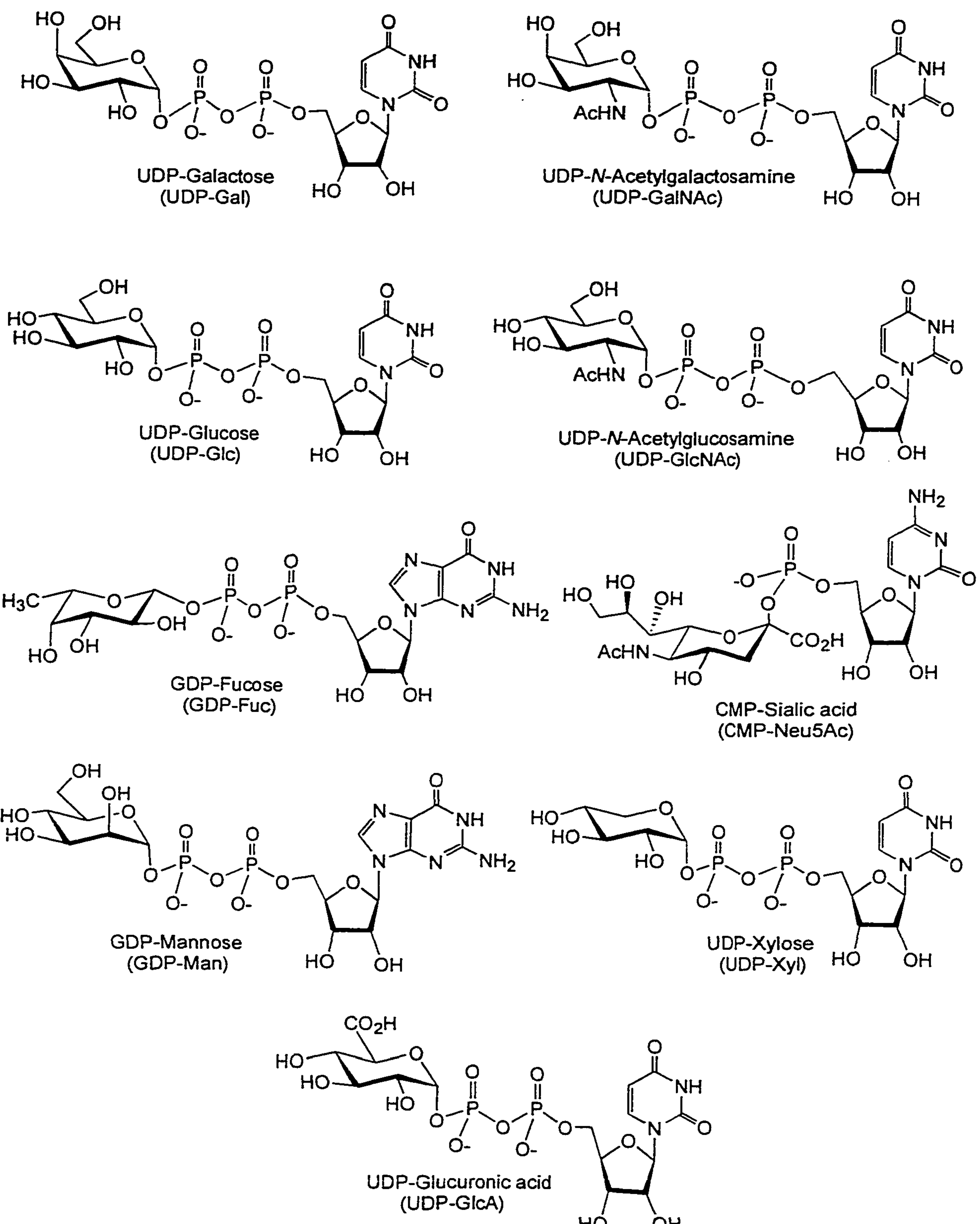


Figure 1.4. Nine sugar nucleotide donors used in mammalian oligosaccharide biosynthesis.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

1.5. Enzymatic Synthesis

The demonstration that oligosaccharides play important roles in diverse biological events has stimulated much interest in the synthesis of oligosaccharides and their analogs. The availability of such molecules can facilitate studies on carbohydrate-protein recognition [14], which further help rational design of novel carbohydrate-based therapeutics [15]. Despite many advances that have been made in the past decades, the chemical synthesis of oligosaccharides still remains a challenge for synthetic carbohydrate chemistry [16, 17]. Reasons for this arise mostly from the inherent chemical difficulties presented by this class of molecules. For one, each monosaccharide carries at least three hydroxyl groups which have to be protected and deprotected during the synthesis. Secondly, the process of glycosylation generates a new stereocenter at the anomeric carbon, and there are no general methods for the introduction of all types of glycosidic linkages in both a stereocontrolled and high-yielding manner. As such, the synthesis of oligosaccharides is very time consuming and requires much expertise. The synthesis of oligosaccharide analogs containing modified sugars is an even more complex task than the preparation of the natural structures. Almost twice as many steps are usually required for the synthesis of oligosaccharide analogs, and the steps are more difficult since most chemical protocols in the literature have been optimized for the natural sugar residues [17].

The enzymatic synthesis of oligosaccharides using glycosyltransferases, in contrast, avoids many of the problems encountered in the chemical synthesis [11, 18]. Multi-step protection/deprotection sequences are no longer required as the glycosyl transfer catalyzed by glycosyltransferases is regiospecific, and the control of configuration of newly formed anomeric center is absolute. With the advances in

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

molecular biology and biotechnology, more than 30 glycosyltransferases from mammalian [8] and bacterial [19] sources have been cloned. Many glycosyltransferases are hence readily available in quantities sufficient for the *in vitro* synthesis of oligosaccharides. The sugar nucleotides required for the synthesis are commercially available and cost limitations for large-scale synthesis are rapidly being overcome with recycling systems [11, 20] or pathway engineering [21]. Enzymatic or combined chemical-enzymatic synthesis has therefore become an increasingly practical alternative to the preparation of oligosaccharides.

Systematic studies probing the specificity of glycosyltransferases using acceptor analogs where the hydroxyl group is replaced with H, OCH_3, NH_2 (or other substituents) have indicated that frequently only a few of the hydroxyl groups on the acceptor, termed "key polar groups" [22], are required for binding to glycosyltransferases. Recognition of the donor substrates by glycosyltransferases, on the other hand, is primarily based on the nucleotide portion. The flexibility in both donor and acceptor specificities has made it feasible to use glycosyltransferases for the synthesis of oligosaccharide analogs starting with non-natural donors or non-natural acceptors [23].

An enzymatic approach to non-natural oligosaccharides greatly simplifies the synthetic scheme since the requirement for the chemistry is then reduced to the synthesis of more readily accessible smaller "primers" (mono- to trisaccharides) which can be elongated in a regio- and stereospecific manner using glycosyltransferases. The term "non-natural", in the present context, is used to indicate that the acceptor or donor is chemically modified and different from the natural ones that are used by glycosyltransferases. A change of aglycone or adding/removing a sugar unit(s) at the reducing end is not considered here as creating a non-natural acceptor. Some enzymatic syntheses of oligosaccharide analogs starting from non-natural acceptors are summarized

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

below with focus on seven glycosyltransferases: β1,4- and α1,3-galactosyltransferases, α2,3- and α2,6-sialyltransferases, human milk α1,3/4-fucosyltransferase and human blood group A and B glycosyltransferases.

1.5.1. β1,4-Galactosyltransferase

β1,4-Galactosyltransferase (β1,4-GalT, E.C. 2.4.1.22/38/90) has been commercially available for many years in unit quantities. It is the most widely studied glycosyltransferase with regard to substrate specificity and use in preparative synthesis. It catalyzes the transfer of Gal from UDP-Gal to OH-4 of terminal β-linked GlcNAc to form *N*-acetyllactosamine (Figure 1.5). In the presence of α-lactalbumin, the enzyme prefers to use glucose as an acceptor to produce lactose.

: key polar group

HO OH O HO OH O—UDP + HO HO NHAc OR O OH

β1,4-GalT

HO OH O HO OH O HO NHAc OR O OH

Figure 1.5. Reaction catalyzed by β1,4-galactosyltransferase.

As shown in Figure 1.6, the acceptor specificity of β1,4-GalT is extremely relaxed since numerous modified GlcNAc analogs are active as acceptors. Basically, β1,4-GalT

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

tolerates modifications everywhere on the sugar ring including the ring oxygen as long as the key polar group OH-4 remains available for glycosylation.

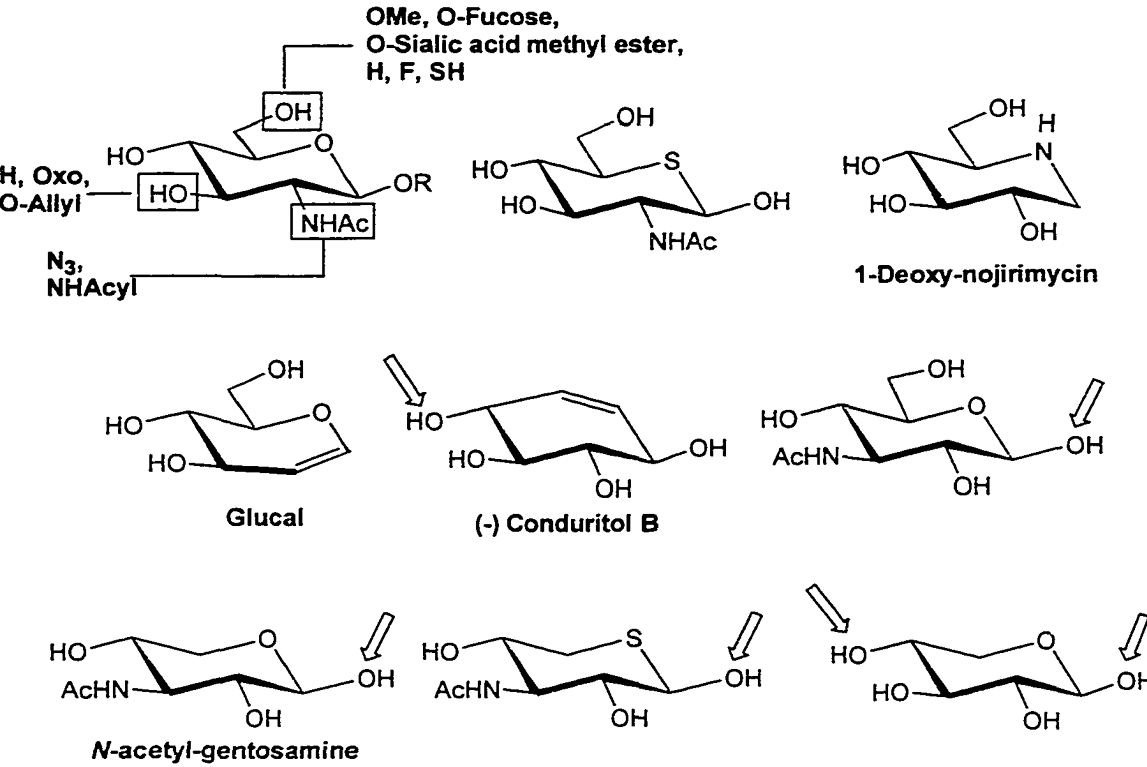


Figure 1.6. Non-natural acceptors for β1,4-galactosyltransferase.

The 2-NHAc group can be replaced with azido [24], *N*-propanoyl [25, 26], *N*-butanoyl [25], allylcarbamate [27] and a large number of amide derivatives [28] including charged groups, highly bulky heterocycles and glycuronamides. However, 2-ethylamino-2-deoxy, 2-*N*-methylacetamido-2-deoxy, and 2-*O*-acetyl-β-D-glucoside were inactive as acceptors and inhibitors for β1,4-GalT [28]. Analogs with the 3-OH group deoxygenated [29, 30], alkylated with a methyl or allyl group, or oxidized to the ketone are active as acceptors although the relative rates of transfer are much lower than *N*-acetylglucosamine [30]. The 6-OH group of GlcNAc can be methylated [26, 30], fucosylated [26],

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

deoxygenated [29] or substituted with F or SH [29]. Addition of α–linked sialic acid to the 6-OH group of GlcNAc is not tolerated [26]. However, when the carboxylic acid of Neu5Ac is derivatized to the methyl ester, the resulting compound proved to be a weak acceptor for β1,4-GalT. The relative rate of transfer is 4% compared to GlcNAc. The residual activity, however, is sufficient activity to carry out preparative synthesis and generate product on the mg-scale [26].

The great tolerance for acceptor modifications exhibited by β1,4-GalT is further exemplified by the fact that it is capable of transferring Gal from UDP-Gal to 5'-thio-Glc and 1-deoxy-nojirimycin which have the ring oxygen modified, and to glucal which has a flattened ring conformation [30]. The enzyme can even resolve racemic (±) conduritol B to give a single galactosylated product of (-) conduritol B [31]. More interestingly, the enzyme transfers galactose to the β-anomeric position of 3-acetamido-3-deoxy-D-glucose acceptors resulting in the formation of an unusual β1→1 (trehalose type) linkage [32]. This "frame-shifted" galactosylation [33] was also observed with *N*-acetyl-gentosamine [34], *N*-acetyl-5'-thio-gentosamine [35] and xylose [36] acceptors.

1.5.2. α1,3-Galactosyltransferase

α1,3-Galactosyltransferase (α1,3-GalT, E.C. 2.4.1.151), a retaining glycosyltransferase, catalyzes the transfer of Gal from UDP-Gal to the 3-OH group of the Gal residue in Galβ1→4GlcNAc-R to form Galα1→3Galβ1→4GlcNAc epitopes [37] (Figure 1.7) that are the major xenoactive antigens responsible for hyperacute rejection in xenotransplantation [38]. Enzyme for preparative synthesis of this sequence has been isolated from porcine and bovine tissues and recombinant porcine α1,3-galactosyltransferase is now commercially available in unit quantities.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Figure 1.7. Reaction catalyzed by α1,3-galactosyltransferase

Besides using Galβ1→4GlcNAc as an acceptor, the enzyme can transfer Gal to Galβ1→3GlcNAc and Galβ1→4Glc [39]. As shown in Figure 1.8, substitutions of 2-NHAc of the GlcNAc residue with azido and succinimido groups are tolerated [40]. The *N*-acetyl group can be replaced with a large number of acyl groups of various sizes, hydrophilicities or lipophilicities [41]. However, replacement of the 2-NHAc with an amino group abolishes activity [40]. Deoxygenation of OH-3 of the GlcNAc residue is tolerated whereas either substitution or derivatization at this position is not tolerated. As shown in Figure 1.8, analogs with modifications (deoxygenation and *O*-alkylation) on OH-6 of GlcNAc, or OH-2 and OH-6 of the terminal Gal residue are substrates [42]. Modification on OH-4 of Gal is not tolerated by the enzyme, suggesting that this group is a key polar group essential for binding to α1,3-GalT [42].

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

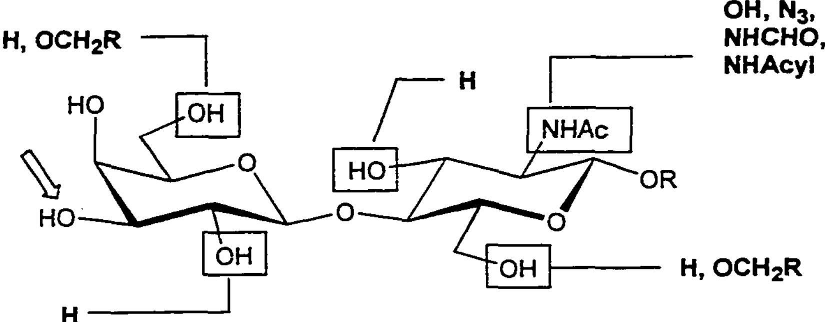


Figure 1.8. Non-natural acceptors for α1,3-galactosyltransferase.

1.5.3. α2,3- and α2,6-Sialyltransferases

Cell-surface sialic acid residues play important roles in diverse biological processes [43]. Sialic acids are usually found at terminal positions and always linked through an α-glycosidic linkage. The stereoselective synthesis of α-sialosides remains a challenge since the glycosides have the thermodynamically unfavorable equatorial orientation and the anomeric carbon is a very hindered quaternary center. Enzymatic sialylation therefore offers an attractive alternative to prepare α-sialosides in an efficient and stereocontrolled manner.

α2,3-Sialyltransferase and α2,6-sialyltransferase from rat liver (α2,3-SialT, E.C. 2.4.99.6; α2,6-SialT, E.C. 2.4.99.1) have been cloned and expressed and are now commercially available in quantities sufficient for use in preparative synthesis. α2,3-SialT from rat liver transfers a sialic acid unit from CMP-Neu5Ac to OH-3 of the terminal Gal residue in Galβ1→3GlcNAc (type I) or Galβ1→4GlcNAc (type II) sequences (Figure 1.9) while rat liver α2,6-SialT transfers the sialic acid to OH-6 of the terminal Gal residue in Galβ1→4GlcNAc (Figure 1.10) [44].

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Figure 1.9. Reactions catalyzed by α2,3-sialyltransferase.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Figure 1.10. Reaction catalyzed by α2,6-sialyltransferase.

Chemical mapping studies have indicated that OH-6 of Gal and the 2-NHAc group are required for binding to rat liver α2,6-SialT while rat liver α2,3-SialT requires an intact 3,4,6-triol system on the Gal residue [45]. Deoxygenation at positions other than those bearing key polar groups is tolerated by α2,3-SialT (Figure 1.11) and α2,6-SialT (Figure 1.12). A wide range of substitutions on the *N*-acetyl group of both type I or type II acceptors were accepted by α2,3-SialT [46]. α2,3-SialT also transfers sialic acid to lactal, lactose and 2-*O*-pivaloyl lactose [24a].

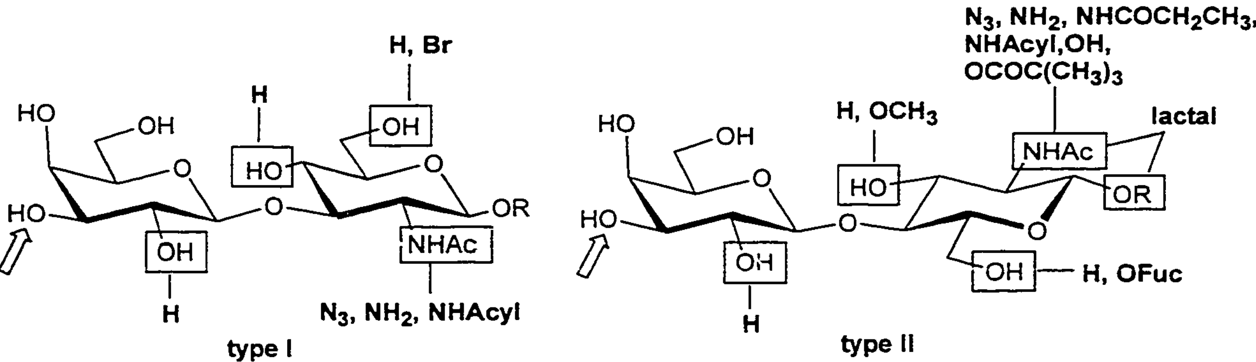


Figure 1.11. Non-natural acceptors for α2,3-sialyltransferase

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

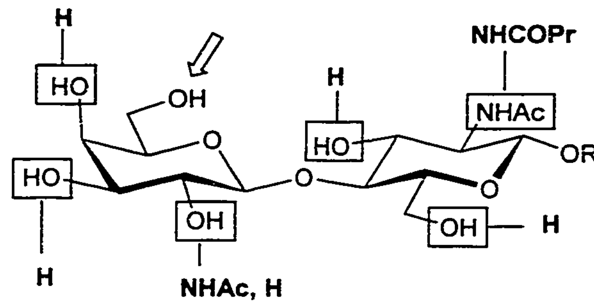


Figure 1.12. Non-natural acceptors for α2,6-sialyltransferase.

1.5.4. Human Milk α1,3/4-Fucosyltransferase

Many antigenic oligosaccharides on the cell surface are fucosylated. These fucose-containing oligosaccharides are considered as oncodevelopmental antigens since they accumulate in numerous human cancers [47]. Along with sialylation, fucosylation by fucosyltransferases is often the last *in vivo* modification of oligosaccharides.

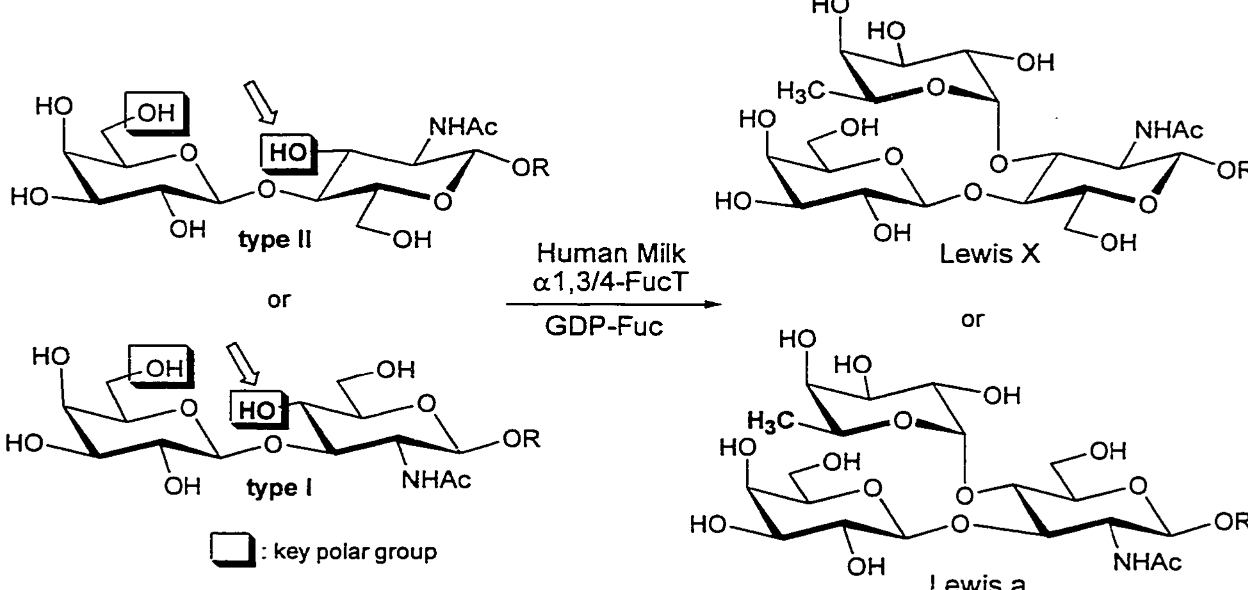


Figure 1.13. Reactions catalyzed by human milk α1,3/4-fucosyltransferase.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Human milk α1,3/4-FucT transfers of a fucose residue from GDP-fucose to Galβ1→3GlcNAc (type I) or Galβ1→4GlcNAc (type II) acceptors to form the blood-group related antigenic dertiminants Lewis a and Lewis X (Figure 1.13). It also use NeuAcα2→3Galβ1→3GlcNAc or NeuAcα2→3Galβ1→4GlcNAc to produce sialyl Lewis a and sialyl Lewis X which are ligands for selectins, a family of adhesion molecules involved in the inflammatory process and in tumor development [48].

Figure 1.14. Non-natural acceptors for human milk α1,3/4-fucosyltransferase.

Chemical mapping studies employing a series of monodeoxygenated and modified acceptor substrates showed that modifications are tolerated at every hydroxyl group in the sugar rings except OH-6 of the Gal and OH-3 or OH-4 of the GlcNAc residue to which the fucose is transferred [49]. As shown in Figure 1.14, the 2-NHAc group of the GlcNAc residue in NeuAcα2→3Galβ1→4GlcNAcβ-OR or NeuAcα2→3 Galβ1→4GlcNAcβ-OR can be replaced with azido, amino or propionamido groups [50]. Thio-linked *N*-acetyllactosamine, where the inter-glycosidic oxygen is replaced by sulfur, is also a good acceptor for the enzyme [51]. Ether- and imino-linked octyl *N*-acetyl-5a'-carba-β-lactosamides were also found to be acceptors for human milk α1,3/4-FucT [52].

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

1.5.5. Blood Group A and B Glycosyltransferases

Human blood group A and B glycosyltransferases (GTA and GTB, E.C. 2.4.1.40 and E.C. 2.4.1.37) are responsible for the biosynthesis of A and B blood-group antigens which are important in cell development, cell differentiation and oncogenesis [53]. Both GTA and GTB are retaining enzymes. GTA catalyze the transfer of GalNAc from UDP-GalNAc to the (O)H antigen (Fucα1→2Galβ-OR) to give the A antigen GalNAcα1→3[Fucα1→2]Galβ-OR. GTB uses the same acceptor but catalyzes the transfer of Gal from UDP-Gal to form the B antigen, Galα1→3[Fucα1→2]Galβ-OR (Figure 1.15).

Chemical mapping studies reveal that the OH-4 of the Gal residue is the key polar group for both GTA and GTB whereas OH-3 of the Gal unit to which the glycosyl residue transfers is not essential for recognition by either enzyme [54, 55]. As shown in Figure 1.16, both enzymes tolerate deoxygenation, substitution and derivatization of the 6-OH group of Gal unit [54]. Deoxygenation of any of the hydroxyl groups on the Fuc residue is tolerated by GTA. Methylation of O-3' and O-4' is tolerated by both GTA and GTB. The arabino derivative, where the CH_3 group of the Fuc residue was replaced by H, was found to be an acceptor for GTA [56].

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

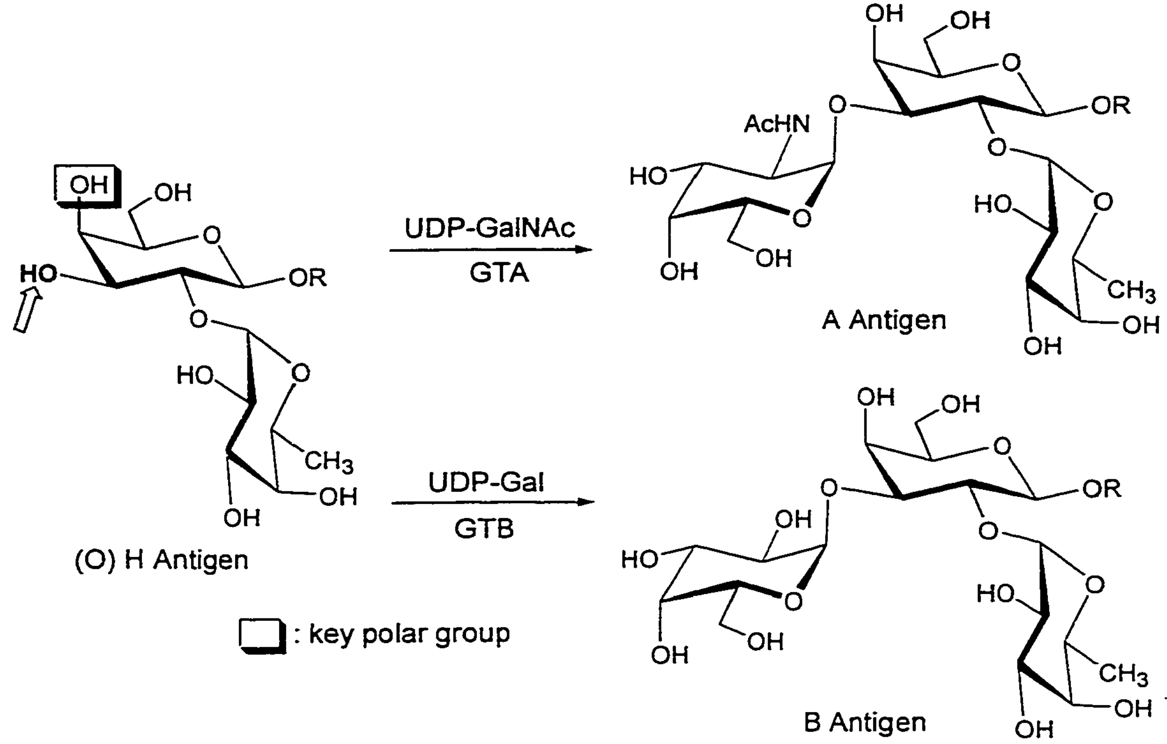


Figure 1.15. Reactions catalyzed by blood group A and B glycosyltransferases.

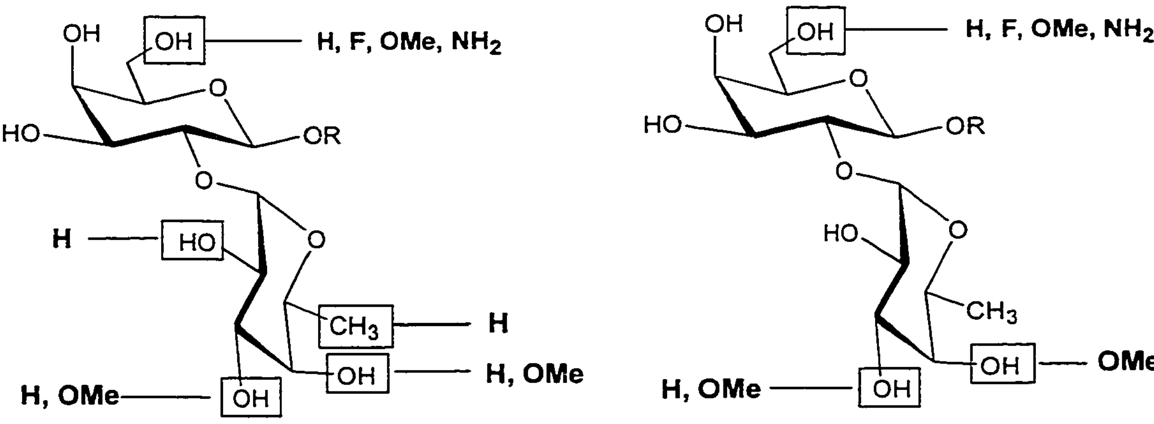


Figure 1.16. Non-natural acceptors for blood group A glycosyltransferase (left) and B glycosyltransferase (right).

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

1.6. Carbon-Branched Sugars and Glycosides of Tertiary Alcohols

Carbon-Branched sugars are a class of rare sugars where the carbon-bonded hydrogen is replaced with substituents such as methyl, hydroxylmethyl or formyl [57]. They are found as components of many natural products in plants and various types of antibiotics produced by microorganisms, mainly strains of *Streptomyces*. For example, apiose and hamamelose, respectively found in parsley and witch hazel early in this century, are now known to occur widely in the plant kingdom. One of the best known antibiotics, streptomycin, used in the treatment of tuberculosis, contains the *C*-formyl branched sugar L-streptose. Erythromycin A has a *C*-methyl branched L-cladinose. The well-known antibiotics vancomycin also includes a *C*-methyl branched vancosamine in the structure (Figure 1.17).

Glycosides of tertiary alcohols are even more rare in nature. There are only a limited number of reported examples such as a mevalonolactone glucoside derivative from the bark of *Prunus burgeriana* [58], ptaquiloside (a bracken carcinogen) [59], saponin D from the leaves of *Hovenia dulcis* (rhamnaceae) [60], and cell-surface glycopeptidolipid-type (GPL) antigen of serovar 19 [61] (Figure 1.18).

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

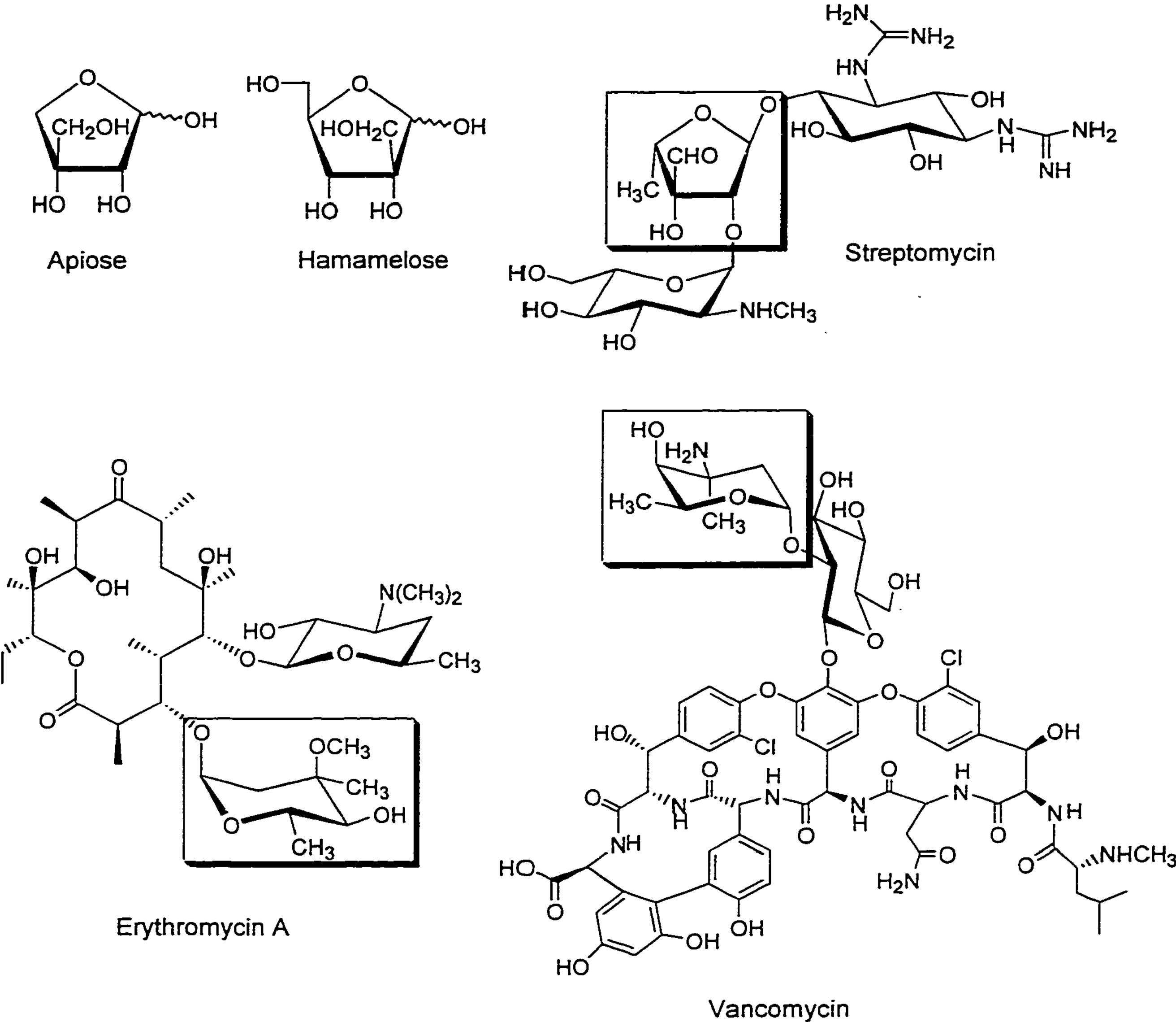


Figure 1.17. Some naturally occurring C-branched sugars.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Mevalonolactone Glucoside

Ptaquiloside

Saponin D

GPL Antigen of Serovar 19

Figure 1.18. Some naturally occurring glycosides of tertiary alcohols.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

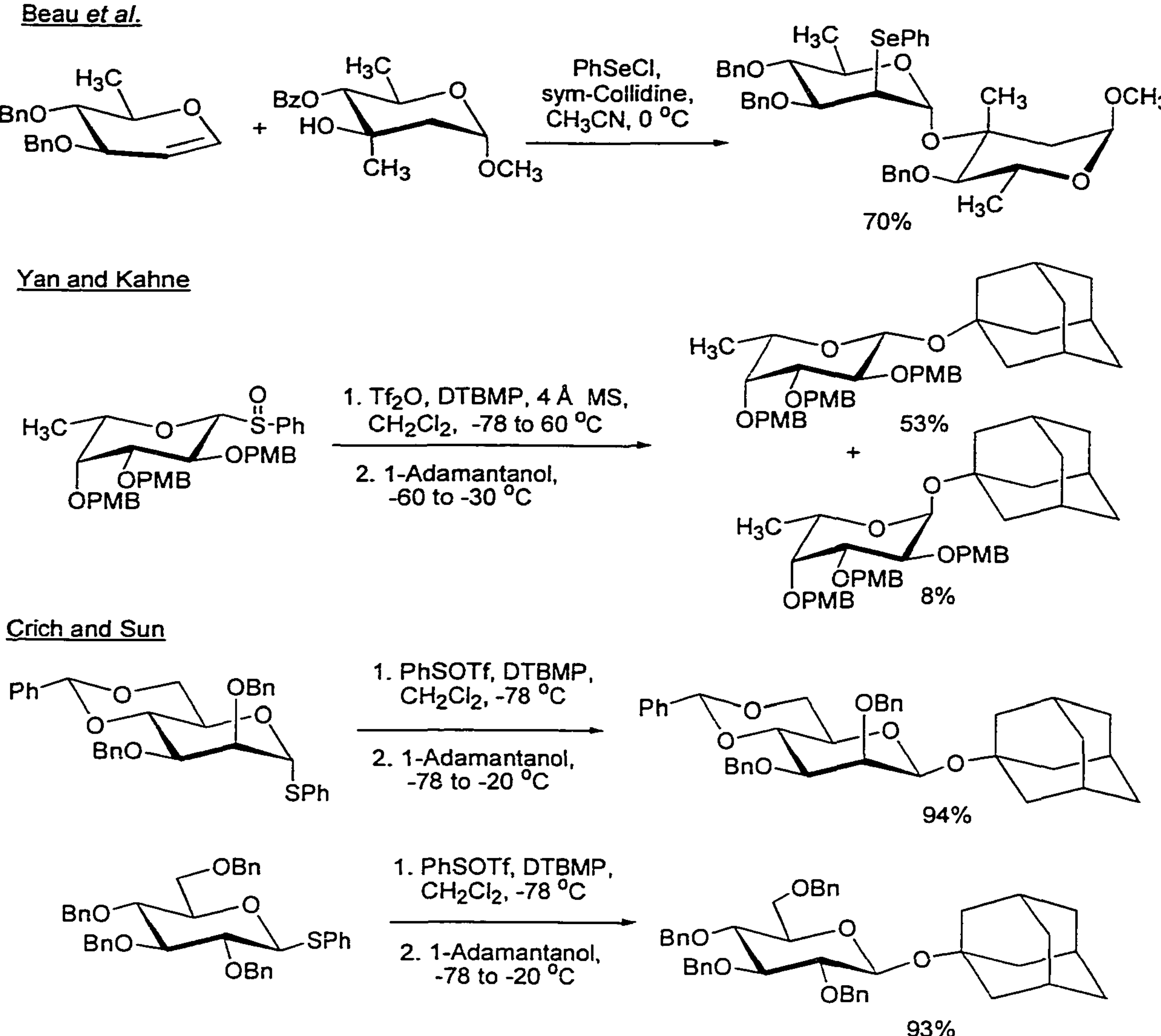


Figure 1.19. Examples on chemical synthesis of glycosides of complex tertiary alcohols.

Chemically, glycosides of tertiary alcohols are extremely difficult to synthesize due to their steric hindrance inherent in the glycosylation. Very few examples of chemical glycosylation of tertiary alcohols have been reported (Figure 1.19). Beau *et al.* used the glycosyloxyseleneation method to condense a glycal with a tertiary alcohol of α-D-evermicoside [62]. Yan and Kahne reported the synthesis of a glycoside of 1-adamantanol using glycosyl sulfoxides as the glycosyl donors [63]. Using thioglycosides as donors, β-

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

mannoside and β-glucosides of hindered tertiary alcohols were obtained in good yields by Crich and Sun [64].

1.7. Scope of Project

Chemical approaches using engineered ligands are still necessary to study carbohydrate-protein interactions [65], since structural information on protein (including enzyme) binding sites is not generally available. Most of the ligand analogs used have been acceptor analogs with hydroxyl groups selectively deoxygenated, derivatized, or substituted with other functional groups [23, 66]. Chemical mapping studies using these analogs help elucidate hydrogen-bonding patterns and delineate the topology of the binding site. However, there are only a few examples known where analogs having a C-bonded hydrogen atom modified (termed C-branched sugars) were used [67]. The scope of chemical mapping on glycosyltransferases and enzymatic synthesis has not yet included such sugar analogs. Probing protein binding domains with C-branched analogs can provide more information on the three-dimensional aspects of carbohydrate-protein interactions, which compliments studies using analogs with modified hydroxyl groups. Functional groups replacing hydrogens could, for example, serve as molecular probes to explore which face of a given substrate is making contact with the binding site and how close the contact is (Figure 1.20).

C-branched sugar analogs, in particular those resulting from branching at the positions bearing a key polar group or a glycosylation site, will be useful for probing the active sites of glycosyltransferases and for studying how steric hindrance affects the binding. The enzyme kinetic properties of these analogs will also shed light on the molecular mechanisms of glycosyltransferase-catalyzed reactions. Since carbon-branched

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Figure 1.20. Schematic representation of the scope of the project.

sugars are found only in plants and microorganisms, it will be interesting to see whether mammalian glycosyltransferases are able to recognize them. Should glycosyltransferases be able to catalyze the glycosyl transfer to theses analogs, this enzymatic approach would provide a new way of access to the C-branched oligosaccharides, especially, glycosides of complex tertiary alcohols which can not be easily made chemically. The resulting C-branched oligosaccharides would potentially be useful in carbohydrate-protein recognition studies. In particular, such C-branched compounds should reveal the steric requirements of the binding sites of proteins near the OH-group where the substitution has occurred. Of potentially greater interest is that the glycosides of tertiary alcohols should be conformationally more restricted due to the increased steric interaction between

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

sugar rings and may thus be useful for evaluating entropic contributions to protein-oligosaccharide binding.

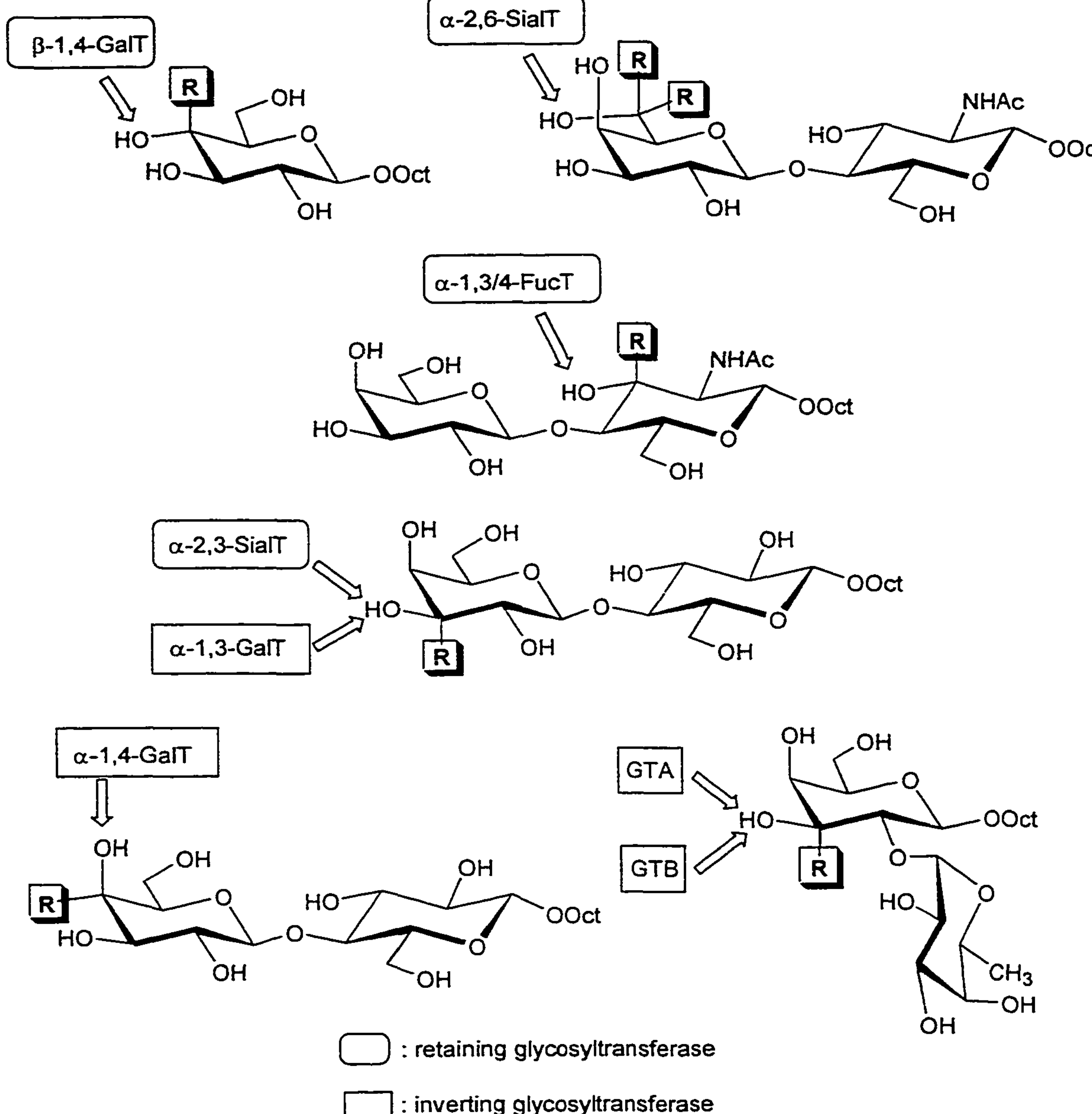


Figure 1.21. Carbon-branched analogs synthesized for the enzymatic studies.

Four retaining glycosyltransferases and four inverting glycosyltransferases were chosen in this study. They are β1,4-GalT, α2,3-SialT, α2,6-SialT, α1,3/4-FucT (retaining enzymes), α1,3-GalT, α1,4-GalT, GTA and GTB (inverting enzymes). The objective was

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

to first chemically synthesize the C-branched acceptor analogs where the H bonded to the carbon bearing an OH group undergoing glycosylation is replaced with a methyl or propyl group (Figure 1.21). The aglycone of the synthetic acceptor analogs was chosen to be octyl, allowing the use of well-established radioactive "Sep-Pak assays" [68] to determine their activities and simplifying the purification of product by absorption onto reverse phase (C18) cartridges.

These novel acceptor analogs would be evaluated as potential acceptors and inhibitors for their respective glycosyltransferases. If several enzymes share the same acceptor substrate, the OH group that is a glycosylation site for one enzyme could also be a glycosylation site or a key polar group for the other enzyme. A single analog would therefore be tested against several different enzymes. For analogs found active as acceptors in the enzymatic assays, enzymatic synthesis would be performed yielding novel carbon-branched oligosaccharides. The analogs produced by this chemoenzymatic approach would be analogs of natural ligands for antibodies, lectin (selectin) and enzymes (glycosyltransferases and glycosidases) and could thus be further used as probes for carbohydrate-protein recognition.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Chapter 2

Chemical Synthesis of Carbon-Branched Sugar Analogs

2.1. Introduction

This chapter describes the chemical synthesis of eight carbon-branched analogs of acceptor substrates for the glycosyltransferases (Figure 2.1). The general strategy involves the selective protection of sugars permitting the oxidation of the required ring carbon to the ketone. The carbon-branching process was achieved by nucleophilic addition of the alkyl group from organometallic reagents onto the ketone. The protection/deprotection procedures followed well-established chemistry [69]. The *O*-benzyl group was used as a protecting group in most of the syntheses, as it is stable to most reaction conditions and, especially, to organometallic reagents. For the synthesis of C-branched disaccharides, two different approaches are used differing in the order of glycosylation and carbon-branching as shown in Figure 2.2. In the "Branching-Glycosylation" approach, the carbon-branching was performed on the monosaccharide residue which was then coupled to another sugar residue by glycosylation to provide the C-branched disaccharide. The "Glycosylation-Branching" approach, involved first the construction of the disaccharide by glycosylation, followed by carbon-branching by nucleophilic addition to a ketone formed on the disaccharide. The stereochemistry at the branching carbon center was determined by NOE studies using one-dimensional TROESY experiments [70].

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Figure 2.1. Structures of eight *C*-branched acceptor analogs.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

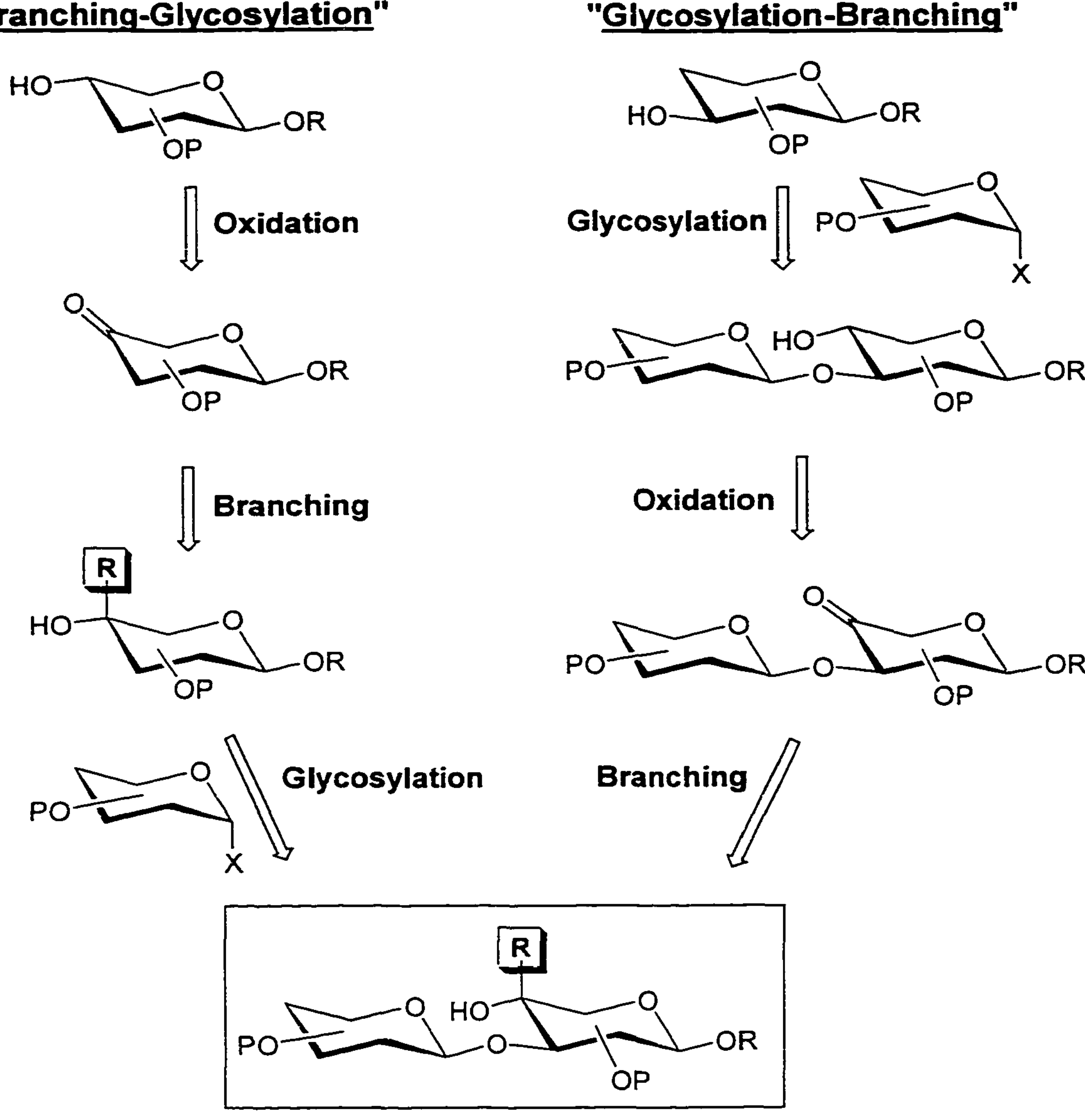


Figure 2.2. Synthetic approaches to the C-branched disaccharides.

2.2. Chemical Synthesis

2.2.1. Preparation of 4-*C*-Branched Glucoside

Compound **9** was prepared according to the literature [54]. Oxidation of **9** with Dess-Martin periodinane [71] gave the ketone compound **10** in 50% yield (Figure 2.3).

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Treatment of **10** with methyllithium gave the C-4 epimers **11a** and **11b** in 1:1 ratio. The low isolated yields were due to the difficult separation of these two diasteromers. Hydrogenolysis using $Pd(OH)_2/C$ (Pearlman's catalyst) [72] under H_2 gave the final 4-*C*-methyl branched glucoside **1a** and **1b** in quantitative yields.

HO
BnO
OBn
O
OOct
OBn
9
Dess-Martin periodinane
(50%)
O
BnO
OBn
O
OOct
OBn
10
MeLi, THF
R_1
R_2
BnO
OBn
O
OOct
OBn
11a $R_1 = CH_3$, $R_2 = OH$, 16%
11b $R_1 = OH$, $R_2 = CH_3$, 17%
H_2, $Pd(OH)_2/C$, MeOH
(quant.)
R_1
R_2
HO
OH
O
OOct
OH
1a $R_1 = CH_3$, $R_2 = OH$
1b $R_1 = OH$, $R_2 = CH_3$

Figure 2.3. Preparation of the 3'-*C*-methyl glucoside **1a**.

As shown in Table 2.1, there are significant NOEs between the 4-*C*-methyl protons and H-2 in **1a** and NOEs between the 4-*C*-methyl protons and H-3 and H-5. The ^{13}C chemical shift of the axial 4-*C*-methyl group in **1a** (14.35 ppm) is shifted upfield compared to that of the equatorial methyl group in **1b** (20.41 ppm), as observed in other 4-*C*-Me branched glucosides [73].

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Table 2.1. Relevant NOEs obtained from the carbon-branched sugar analogs[a]

Molecule	Selectively excited resonance [b]	% NOE [c]
1a	4-*C*-CH_3	1.5 (H-2)
1b	4-*C*-CH_3	1.2 (H-3), 1.3 (H-5)
2	H-1'	2.3 (3'-*C*-CH_3)
3a	H-1'	0.6 and 2.6 (3'-C-C$H_2$$CH_2CH_3$)
3b	H-1'	no NOEs to any propyl resonances
26a	4'-*C*-CH_3	0.9 (H-3'), 0.8 (H-5')
26b	4'-*C*-CH_3	0.5 (H-2')
5	H-1	2.8 (3-*C*-CH_3)
7	H-1	3.3 (3-*C*-CH_3)
8a	H-1	0.5 and 4.0 (3-C-C$H_2$$CH_2CH_3$)
8b	H-2	0.4 and 2.3 (3-C-C$H_2$$CH_2CH_3$)

a) all data obtained at 600 MHz except for **6** (500 MHz), with a mixing time of 200 ms except for **1a**, **1b**, and **7** (400 ms).

b) selective excitation with eburp-1 shaped pulse [74].

c) expressed in % of integral of selectively excited resonance.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

2.2.2. Preparation of 3'-*C*-Branched Lactosides

19 $R_1 = CH_3$, $R_2 = OH$, 90%
20a R_1 = All, R_2 = OH, 43%
20b R_1 = OH, R_2 = All, 48%

2 $R_1 = CH_3$, $R_2 = OH$
3a R_1 = Pr, R_2 = OH
3b R_1 = OH, R_2 = Pr

Figure 2.4. Preparation of the 3'-*C*-branched lactosides **2** and **3a**.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

The readily available disaccharide lactose was used to prepare the *C*-branched lactosides. As described in Figure 2.4, the key step in the synthesis of 3'-*C*-branched lactosides **1** and **2** was the regioselective stannylation [75] of unprotected lactoside **14** resulting in selective 3'-*O*-alkylation. Glycosylation of lactose bromide **13** with octanol followed by Zemplen deacylation (NaOMe/MeOH) gave unprotected disaccharide **14** (37%, two steps). Regioselective allylation at O-3' with dibutyltin oxide and allyl bromide provided **15** in 53% yield [76]. Benzylation of **7** using benzyl bromide and sodium hydride in DMF gave the protected disaccharide **16** (80%). Removal of the allyl group with $PdCl_2$ in MeOH [77] gave **17** with OH-3' free (83%), which was then oxidized with Dess-Martin periodinane to provide the ketone **18** (72%). Nucleophilic addition of methyllithium to the carbonyl group of **18** provided exclusively the product of axial-attack (**19**) in excellent yield (95%), while the reaction of allylmagnesium bromide with **18** gave a mixture of both epimers **20a** (43%) and **20b** (48%) in almost 1:1 ratio. Finally, 3'-*C*-branched lactosides **2** and **3** were obtained by hydrogenolysis where the allyl group in **20** was also converted to the propyl group. NOE studies showed that there are significant NOEs between the 3'-*C*-methyl protons in **2** (3'-*C*-$CH_2CH_2CH_3$ protons in **3a**) and H-1 of the Gal residue while there are no NOEs between the 3'-*C*-propyl protons and H-1' in **3b** (Table 2.1).

2.2.3. Preparation of 4'-*C*-Branched Lactoside

To obtain the lactoside with only OH-4' free, octyl lactoside **14** was first treated with 2,2-dimethoxypropane in the presence of *p*-toluenesulfonic acid (*p*-TSA) to give **21** in 52% yield [78]. Benzylation (84%) followed by acidic hydrolysis of the isopropylidene group gave **23** (quant.). Regioselective benzylation with dibutyltin oxide and benzyl

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

bromide afforded **24** with OH-4' free in 81% yield. Oxidation (72%) followed by nucleophilic addition of MeLi to the resulting 4'-ulose gave both epimers **26a** (26%) and **26b** (24%) in a ratio similar to that obtained in the reaction of **10** with MeLi. The configuration of C-4' was established through NOE studies which showed the strong NOEs between 4'-*C*-CH_3 and H-3' and H-5' in **26a**, and NOEs between 4'-*C*-CH_3 and H-2' in **26b** (Table 2.1). Benzyl groups were removed by hydrogenolysis to give the unprotected 4'-*C*-methyl-branched lactosides **4a** and **4b**.

4a $R_1 = CH_3$, $R_2 = OH$ (88%)
4b $R_1 = OH$, $R_2 = CH_3$ (86%)

26a $R_1 = CH_3$, $R_2 = OH$ (26%)
26b $R_1 = OH$, $R_2 = CH_3$ (24%)

Figure 2.5. Preparation of the 4'-*C*-methyl lactoside **4a**.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

2.2.4. Preparation of 3-*C*-Branched LacNAc

As opposed to the preparation of the C-branched analogs **1-4**, the synthesis of 4'-*C*-branched LacNAc analog proved very problematic. Initial attempts used the "Branching-Glycosylation" approach (Figure 2.2). The first attempt began with the synthesis of the glycosyl acceptor, 3-*C*-methyl branched GlcNAc **33** (Figure 2.6). Starting from *N*-acetylglucosamine, compound **27** was synthesized in four steps according to the well-established procedures. The ketone **30** was obtained in 60% yield by oxidation of **29** with DMSO-Ac_2O. The key step, nucleophilic addition of MeLi to the ketone **30** resulted in very low yield of the desired axial 3-*C*-methyl product **31a** (7%) as the nucleophilic attack seems to favor the equatorial direction. The ratio of axial-attack to equatorial-attack product was 1:9. Use of $Zr(CH_3)_4$, generated *in situ* from ZrCl4 and MeLi, or addition of Yamamoto Lewis acid catalyst "MAD" [methylaluminum bis(2,6-di-*tert*-butyl-4-methylphenoxide)] [79], did not alter the stereoselectivity of the reaction. Regardless, the attempted coupling of **33** with trichloroacetimidate donor **34** or bromide donor **35** failed completely as no desired disaccharide **36** was detected.

In the second approach to **5**, an attempt was made to improve the yield of axial-attack product using 1,6-anhydromannose as the reactant since the nucleophilic addition of MeMgI to the 3-ulose compound **39** provided both axial-attack and equatorial-attack products in a 1:1 ratio as reported by Cerny *et al.* (Figure 2.7) [80]. Inversion of configuration at C-2 by displacement with an azido nucleophile would give the desired *gluco*-configuration and restore the amino function.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Figure 2.6. First attempt to prepare the 3-*C*-methyl LacNAc **5**.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

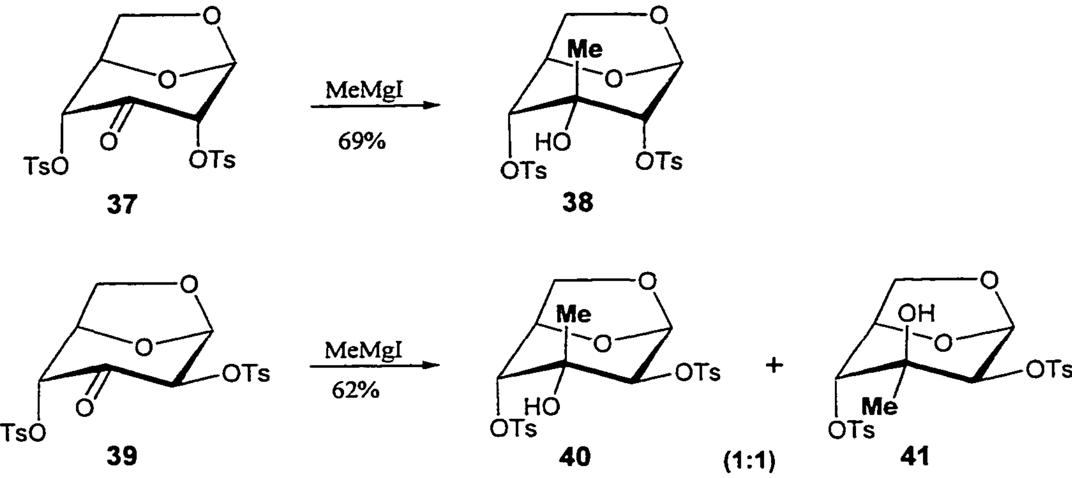


Figure 2.7. Literature procedures for nucleophilic addition to 1,6-anhydro-3-uloses [80].

Compound **43** was prepared according to the procedure of Zottola *et al.* [81]. Benzylation (77%) followed by hydrolysis (74%) of isopropylidene with TFA gave compound **45**. Tosylation of **45** with tosyl chloride in pyridine afforded the mono-tosylated compound **46** (67%) and the di-tosylated compound **47** (16%). Oxidation of **46** with pyridinium dichromate (PDC) gave the ketone **48** (84%). Nucleophilic addition of a series of Grignard reagents (RMgBr, R= Me, Vinyl, Allyl) did give the desired equatorial-attack products **49-51** in over 70% yields. Unfortunately, the attempted displacement of tosylate with azido was unsuccessful. The tosylate was therefore converted to more reactive triflate **53** through ionic radical reduction (sodium naphthalene) [82] followed by triflation (Tf_2O/Py). The displacement of the triflate in **53** by azide unfortunately failed again (Figure 2.8).

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

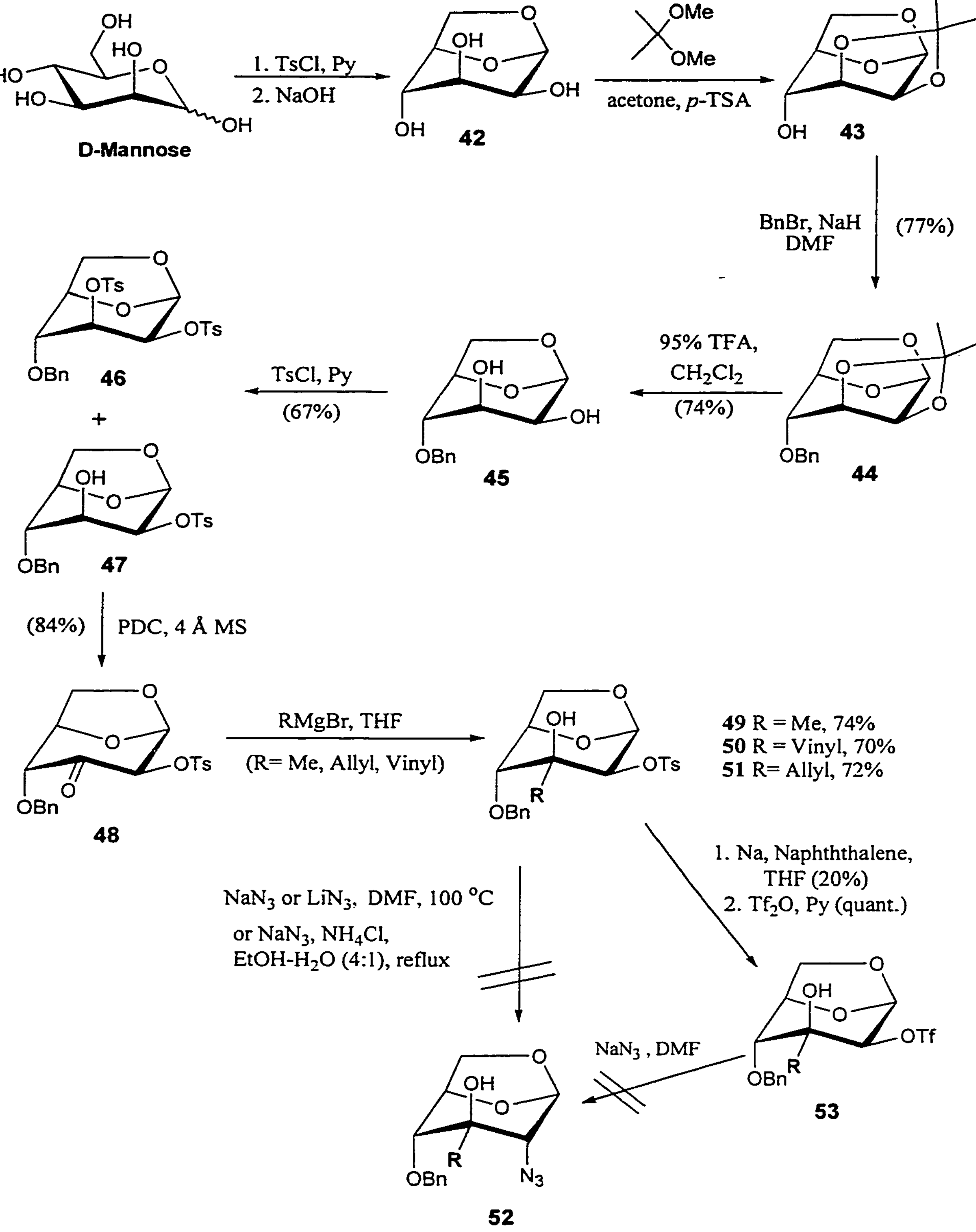


Figure 2.8. Second attempt to prepare the 3-*C*-methyl LacNAc **5**.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

In the third attempt at preparing **5**, the nucleophilic addition to the hex-1-enopyran-3-ulose was explored (Figure 2.9). It was hoped that the nucleophilic addition to the less hindered ketone could improve the stereoselectivity of equatorial attack. The aglycone and *N*-acetyl functional group could be installed on the double bond after nucleophilic addition and glycosylation.

Deacetylation with Amberlite IRN-78 resin (OH^- form) followed by oxidation with PDC in the presence of acetic acid gave glucal **56** [83], which was then protected with the *tert*-butyldimethyl (TBS) group. However, the formed 3-*C*-branched glucals **59** were extremely unstable as decompositions were detected by TLC and NMR. Another route to introduce carbon-branching involved the addition of TMSCN as TMSCN can react with conjugate ketones in a 1,2-addition [84] and the cyano group can be derivatized to many other functionalities [85]. However, the reaction of **57** with TMSCN in the absence or presence of catalyst (ZnI_2) did not proceed, and only starting material was recovered.

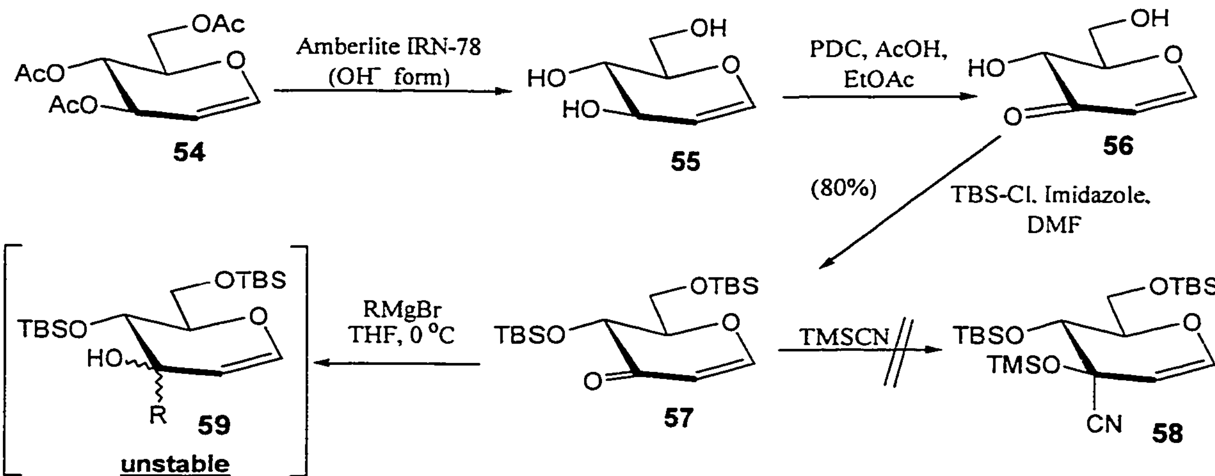


Figure 2.9. Third attempt to prepare the 3-*C*-methyl LacNAc **5**.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

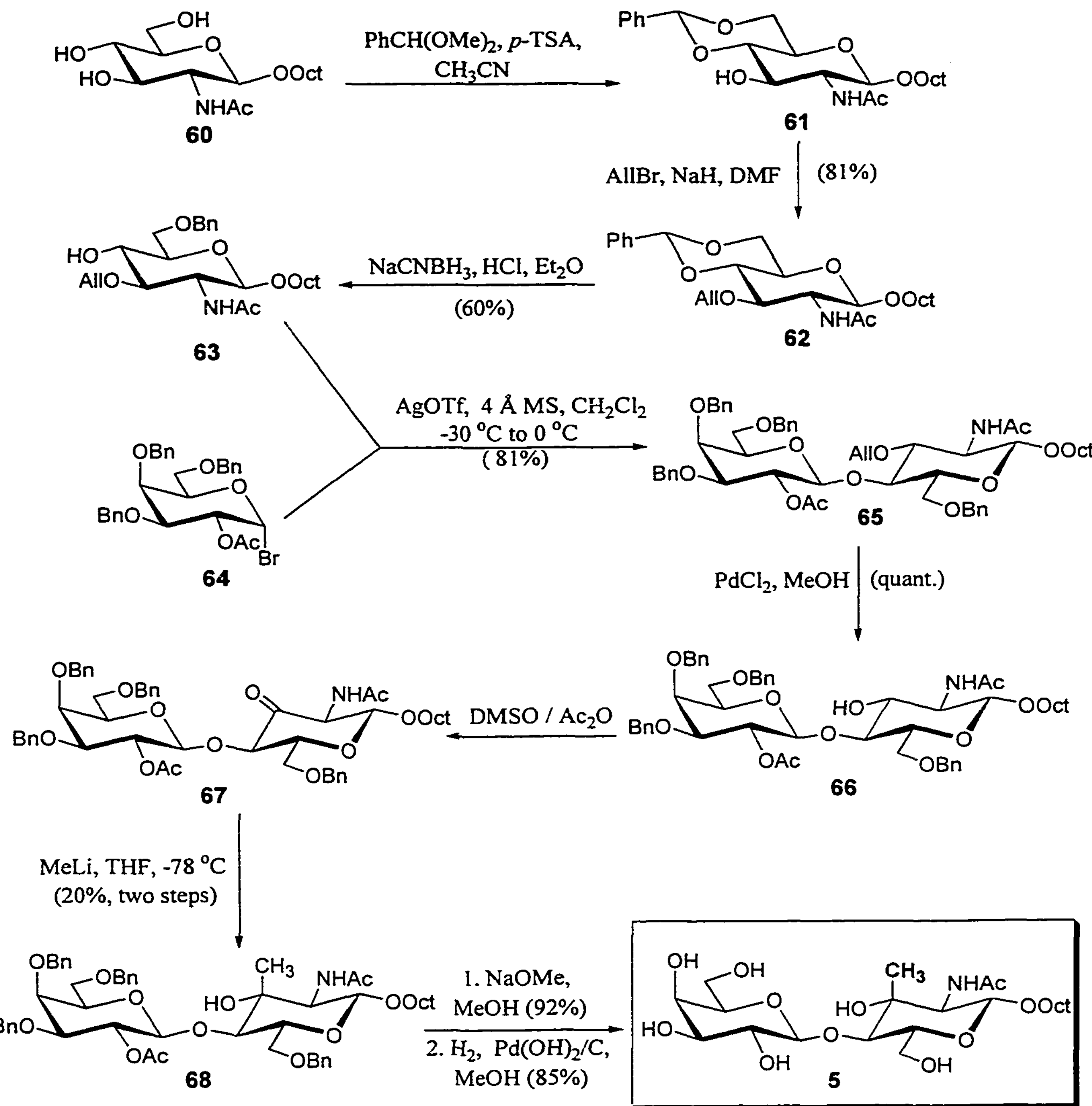


Figure 2.10. Preparation of the 3-*C*-methyl LacNAc **5**.

Finally, the synthesis of the required 3-*C*-methyl LacNAc compound **5** was accomplished via the "Glycosylation-Branching" approach where the branching was performed at the disaccharide level (Figure 2.10). The glycosyl acceptor **63** was prepared from the known compound **60** [86]. Benzylidenation followed by allylation provided **62**.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Regioselective benzylidene opening with $NaCNBH_3$/HCl [87] gave the glycosyl acceptor **63** in 60% yield. Reaction of bromide donor **64** [88] and acceptor **63**, using silver triflate as the promoter, gave the β-linked disaccharide in 81% yield. The allyl group was then removed using $PdCl_2$ to give **66** (quant.), which was then oxidized with DMSO-Ac_2O to give the desired ketone compound **67**. Nucleophilic addition of methyllithium to the ketone gave the C-branched disaccharide **68** with an axial methyl group at C-3 (20%, two steps). The synthesis of the 3-*C*-methyl LacNAc **5** was completed by *O*-deacetylation (92%) followed by hydrogenolysis (85%). The configuration at C-3 was confirmed by the strong NOEs between 3-*C*-methyl protons and H-1 of GlcNAc residue.

2.2.5. Preparation of 6',6'-di-*C*-Branched LacNAc

The synthesis of the 6',6'-di-*C*-methyl LacNAc **6** was achieved using the "Branching-Glycosylation" approach where the 6',6'-di-*C*-methyl groups were installed onto the monosaccharide donor **70** [89] prior to the glycosylation. Initial attempts to couple the bromide donor **70** or the imidate donor **72** with acceptor **63** were unsuccessful (Figure 2.11). This could be caused by the low reactivity of the donors. At this point, it was hoped that the change of some of the acetyl protecting groups on the donor to benzyl groups would enhance the reactivity while still keeping the acetyl group at the C-2 which would act as a neighboring participating group to ensure the formation of β-glycosidic linkage. Acetobromo-donor **70** was thus converted to **76** via the orthoester intermediate [90] (Figure 2.12). Treatment of **70** with methanol and *sym*-collidine in the presence of tetrabutylammonium bromide gave orthoester **70** in 89% yield. *O*-Deacetylation and *O*-benzylation in a one-pot procedure [91] using benzyl bromide and KOH in THF provided **74** with OH-6' unprotected in 71% yield. Treatment of orthoester **74** with acetyl bromide in the presence of tetrabutylammonium bromide gave bromide donor **76** where OH-6'

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

was reacetylated. Compound **76** was then coupled with glycosyl acceptor **62**, using silver triflate as the promoter, to give the expected β-linked disaccharide **77** in 71% yield. *O*-Deallylation (72%) and subsequent *O*-deacetylation (78%) and hydrogenolysis (92%) afforded the required 6',6'-di-*C*-methyl LacNAc **6**.

Figure 2.11. Attempts to prepare the 6',6'-di-*C*-methyl LacNAc **6**.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Figure 2.12. Preparation of the 6',6'-di-C-methyl branched LacNAc 6.

2.2.6. Preparation of 3-C-Branched H Disaccharides

Preparation of 3-C-branched H disaccharides (Fucα1→2Galβ-OR) made use of the known precursor **79** [55] which was available in our laboratory. Oxidation (61%) followed by nucleophilic attack of MeLi onto the carbonyl group gave exclusively **81** in

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

55% yield while reaction of allylmagnesium bromide with **80** afforded both epimers **82a** and **82b** in almost 1:1 ratio. It is quite interesting that the presence of a large tri-*O*-benzyl-fucosyl residue at the 2-position of Gal, compared with the simple *O*-benzyl group in **25**, did not change the stereoselectivity of the nucleophilic addition of either MeLi or AllMgBr to the C-3 ketone. Removal of benzyl groups gave the required 3-*C*-branched H disaccharides **7** and **8**. As shown in Table 2.1, there are strong NOEs between H-1 of Gal and the 3-*C*-methyl protons in **7** (3-*C*-CH_2CH_2CH_3 protons in **8a**), and strong NOEs between H-2 of Gal and 3-*C*-CH_2CH_2CH_3 protons in **8b**.

Dess-Martin Periodinane (61%)

MeLi or AllMgBr

H_2, $Pd(OH)_2$, MeOH (quant.)

7 R_1 = CH_3, R_2 = OH
8a R_1 = Pr, R_2 = OH
8b R_1 = OH, R_2 = Pr

81 R_1 = CH_3, R_2 = OH (55%)
82a R_1 = All, R_2 = OH (33%)
82b R_1 = OH, R_2 = All (29%)

Figure 2.13. Preparation of the 3-*C*-branched H-disaccharides **7, 8a** and **8b**.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

2.3. Experimental

2.3.1. General Methods

Analytical TLC was performed on Silica Gel 60-F254 (E. Merck, Darmstadt) with detection by quenching of fluorescence (aromatic compounds), charring with 5% sulfuric acid in EtOH, ninhydrin, or Morstein reagent. Unless otherwise noted, column chromatography was performed on Silica Gel 60 (E. Merck, 40-63 μm, Darmstadt), and a ratio of ca. 20:1 (silica:mixture, w/w) and up to 100:1 for difficult separations was used. Iatrobeads (beaded silica gel 6RS-8060) were from Iatron Laboratories (Tokyo). Millex-GV (0.22 μm) filter units were from Millipore (Missisauga, ON), C18 Sep-Pak sample preparation cartridges were from Waters Associates (Missisauga, ON). ^{1}H NMR spectra were recorded at 300 MHz (Varian Inova 300), 360 MHz (Bruker AM 360), 500 MHz (Varian Unity 500) or 600 MHz (Varian Inova 600). ^{13}C NMR spectra were recorded at 75 MHz (Bruker AM 300) or 125 MHz (Varian Unity 500). The proton chemical shifts were referenced to solvent residual peaks for solutions in $CDCl_3$ ($CHCl_3$, δ 7.26), CD_2Cl_2 ($CHDCl_2$, δ 5.32), CD_3OD (CHD_2OD, δ 3.30) and DMSO (CHD_2SOCD_3, δ 2.49) or external 1% acetone (δ 2.225). The carbon chemical shifts were referenced to solvent signals for solutions in $CDCl_3$ (δ 77.06), CD_2Cl_2 (δ 53.80), CD_3OD (δ 49.00), DMSO (δ 39.50) or external 1% acetone (δ 31.07) for solutions in D_2O. High resolution electrospray mass spectra were recorded on a Micro-mass ZabSpec Hydroid Sector-TOF.

Hc
Ha
Hb Hd He

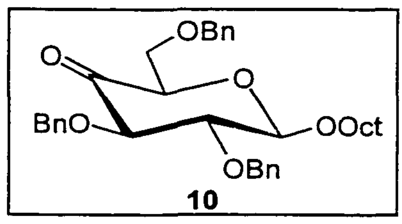
Protons of the allyl group present in the compounds described in the thesis were designated H-a, H-b, H-c, H-d, H-e as defined above. These protons showed the same

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

coupling constants and thus the same multiplicity pattern in all the compounds examined containing the allyl group. Only the chemical shifts varied. The observed couplings were as follows: H-a (dddd, $J_{a,c}$ = 10.5 Hz, $J_{a,b}$ = $J_{a,d}$ = $J_{a,e}$ = 1.5 ± 0.5 Hz); H-b (dddd, $J_{b,c}$ = 17.0 Hz, $J_{a,b}$ = $J_{b,d}$ = $J_{b,e}$ = 1.5 ± 0.5 Hz); H-c (dddd, $J_{b,c}$ = 17.0 Hz, $J_{a,c}$ = 10.5 Hz, $J_{c,d}$ = $J_{c,e}$ = 5.5 Hz); H-d (dddd, $J_{d,e}$ = 13.5 Hz, $J_{c,d}$ = 5.5 Hz, $J_{b,d}$ = $J_{a,d}$ = 1.5 ± 0.5 Hz); H-e (dddd, $J_{d,e}$ = 13.5 Hz, $J_{c,e}$ = 5.5 Hz, $J_{a,e}$ = $J_{b,e}$ = 1.5 ± 0.5 Hz).

2.3.2. Synthesis of Carbon-Branched Sugar Analogs

Octyl 2,3,6-tri-O-benzyl-β-D-xylo-hexopyranosid-4-ulose (**10**).

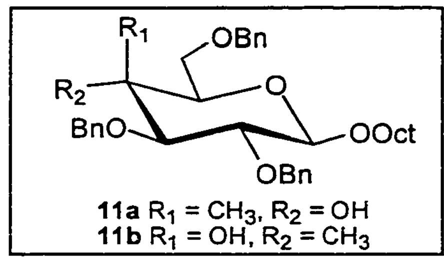


A solution of Dess-Martin periodinane (649 mg, 1.53 mmol) in CH_2Cl_2 (10 mL) was added to a stirred solution of **9** [54] (430 mg, 0.77 mmol) in CH_2Cl_2 (10 mL) and the stirring continued for 3 h. The mixture was poured into a saturated aqueous $NaHCO_3$ containing $Na_2S_2O_3$ and extracted with EtOAc. The organic layer was washed with water and brine, dried (Na_2SO_4), filtered, concentrated and fractionated using column chromatography (6:1 hexanes/EtOAc) to yield **10** (215 mg, 50%) as a white solid: ^{1}H NMR (300 MHz, CD_2Cl_2) δ 7.45-7.22 (m, 15H, ArH), 4.92 (d, 1H, J = 11.5 Hz, PhCH_2), 4.87 (d, 1H, J = 11.5 Hz, PhCH_2), 4.83 (d, 1H, J = 6.7 Hz, H-1), 4.76 (d, 1H, J = 11.5 Hz, PhCH_2), 4.63 (d, 1H, J = 11.5 Hz, PhCH_2), 4.59 (bd, 2H, J= 11.5 Hz, PhCH_2), 4.19 (dd, 1H, J = 6.4, 3.8 Hz, H-5), 4.17 (d, 1H, J = 9.0 Hz, H-3), 4.01-3.92 (m, 2H, H-6a, OCH_2CH$_2$), 3.73 (dd, 1H, J = 10.8, 6.4 Hz, H-6b), 3.71 (dd, 1H, J = 9.0, 6.7 Hz, H-2), 3.61 (dt, 1H, J = 9.6, 6,7 Hz, OCH_2CH$_2$), 1.66 (m, 2H, OCH$_2$CH_2), 1.48-1.26 (m, 10H, CH$_2$ octyl), 0.93 (t, 3H, J = 7.0 Hz, CH$_3$ octyl); ^{13}C NMR (75 MHz, CD_2Cl_2) δ 202.27 (C-4), 138.72,

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

138.64, 138.27 (aromatic quart.), 128.73, 128.67, 128.62, 128.40, 128.14, 128.04 (aromatic CH), 103.32 (C-1), 83.78, 83.70, 77.39 (C-2, C-3, C-5), 74.71, 74.02, 73.92 ($PhCH_2$), 70.44, 68.82 (C-6, OCH_2CH_2), 32.25, 30.09, 29.80, 26.68, 26.54, 23.08 (CH_2 octyl), 14.29 (CH_3 octyl); HR-ESMS calcd for $C_{35}H_{44}O_6Na$ ($M+Na^+$) 583.3036, found 583.3032.

Octyl 2,3,6-tri-O-benzyl-4-C-methyl-β-D-glucopyranoside (**11a**) *and octyl 2,3,6-tri-O-benzyl-4-C-methyl-β-D-galactopyranoside* (**11b**).



Compound **10** (145 mg, 0.26 mmol) was dissolved in THF (8 mL) and cooled to -78 °C under argon. Methyl lithium (1.4 M, 0.39 mL) in diethyl ether was added with stirring. The reaction mixture was stirred for 3 h while it was allowed to warm up to rt. The reaction was poured into saturated NH_4Cl and extracted with EtOAc. The organic layer was washed with water and brine, dried (Na_2SO_4), filtered, concentrated and fractionated using column chromatography (6:1 hexanes/EtOAc) to yield mixtures of **11a** and **11b**, which were then separated by second column chromatography using 19:1 toluene/EtOAc to yield **11a** (24 mg, 16 %) and **11b** (26 mg, 17%), each as a film.

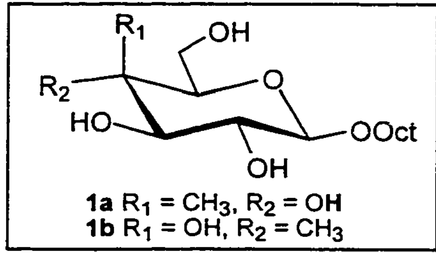
For **11a**: ^1H NMR (600 MHz, CD_2Cl_2) δ 7.40-7.20 (m, 15H, ArH), 4.88 (d, 1H, J = 11.0 Hz, $PhCH_2$), 4.87 (d, 1H, J = 11.5 Hz, $PhCH_2$), 4.79 (d, 1H, J = 11.5 Hz, $PhCH_2$), 4.67 (d, 1H, J = 11.0 Hz, $PhCH_2$), 4.58 (d, 1H, J = 12.0 Hz, $PhCH_2$), 4.54 (d, 1H, J = 12.0 Hz, $PhCH_2$), 4.42 (d, 1H, J = 7.7 Hz, H-1), 3.88 (dt, 1H, J = 9.5, 6.6 Hz, OCH_2CH_2), 3.77 (dd, 1H, J = 10.5, 5.5 Hz, H-6a), 3.64 (dd, 1H, J = 10.0, 6.6 Hz, H-6b), 3.54 (dt, 1H, J = 9.5, 6.6 Hz, OCH_2CH_2), 3.50 (dd, 1H, J = 6.6, 5.5 Hz, H-5), 3.42 (d, 1H, J = 9.7 Hz, H-3), 3.24 (dd, 1H, J = 9.7, 7.7 Hz, H-2), 2.69 (s, 1H, OH), 1.63 (m, 2H, OCH_2CH_2), 1.42-1.22 (m, 10H, CH_2 octyl), 1.20 (s, 3H, 3-*C*-CH_3), 0.88 (t, 3H, J = 7.0

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Hz, CH_3 octyl); ^{13}C NMR (75 MHz, CD_2Cl_2) δ 139.66, 139.38, 138.43 (aromatic quart.), 128.77, 128.65, 128.54, 128.30, 128.14, 127.83, 127.80 (aromatic CH), 104.53 (C-1), 86.73, 81.66, 76.07 (C-2, C-3, C-5), 75.85, 75.01, 74.60, 73.94, 70.47, 69.48 (C-4, C-6, O$CH_2$$CH_2$, Ph$CH_2$ × 5), 32.24, 30.23, 29.81, 29.65, 26.56, 23.06 (CH_2 octyl), 16.50 (3-*C*-CH_3), 14.25 (CH_3 octyl); HR-ESMS calcd for $C_{36}H_{48}O_6Na$ ($M+Na^+$) 599.3349, found 599.3352.

For **11b:** ^1H NMR (600 MHz, CD_2Cl_2) δ 7.40-7.20 (m, 15H, ArH), 4.96 (d, 1H, *J* = 11.0 Hz, PhCH_2), 4.95 (d, 1H, *J* = 11.0 Hz, PhCH_2), 4.69 (d, 1H, *J* = 11.0 Hz, PhCH_2), 4.63 (d, 1H, *J* = 11.0 Hz, PhCH_2), 4.55 (d, 1H, *J* = 12.0 Hz, PhCH_2), 4.38 (d,1 H, *J* = 12.0 Hz, PhCH_2), 4.55 (t, 2H, *J* = 12.0, PhCH_2), 4.38 (d, 1H, *J* = 7.9 Hz, H-1), 3.95 (dt, 1H, *J* = 9.6, 6.7 Hz, OC$H_2$$CH_2$), 3.87 (dd, 1H, *J* = 11.0, 3.0 Hz, H-6a), 3.70 (dd, 1H, *J* = 11.0, 5.7 Hz, H-6b), 3.59 (dd, 1H, *J* = 9.3, 7.9 Hz, H-2), 3.56 (dt, 1H, *J* = 9.6, 6.7 Hz, OC$H_2$$CH_2$), 3.40 (ddd, 1H, *J* = 5.7, 3.0 Hz, 0.7 Hz, H-5), 3.20 (d, 1H, *J* = 9.3 Hz, H-3), 2.58 (s, 1H, OH), 1.66 (m, 2H, OCH_2CH_2), 1.45-1.24 (m, 10H, CH_2 octyl), 1.19 (s, 3H, 3-*C*-CH_3), 0.88 (t, 3H, *J* = 7.0 Hz, CH_3 octyl); ^{13}C NMR (75 MHz, CD_2Cl_2) δ 139.40, 138.88, 138.78 (aromatic quart.), 128.71, 128.65, 128.59, 128.45, 128.42, 128.05, 127.97, 127.84 (aromatic CH), 104.05 (C-1), 84.43, 81.20, 77.84 (C-2, C-3, C-5), 76.51, 75.01, 73.86, 73.81, 70.19, 69.76 (C-4, C-6, O$CH_2$$CH_2$, Ph$CH_2$ × 5), 32.25, 30.28, 29.84, 29.68, 26.64, 23.07 (CH_2 octyl), 22.02 (3-*C*-CH_3), 14.25 (CH_3 octyl); HR-ESMS calcd for $C_{36}H_{48}O_6Na$ ($M+Na^+$) 599.3349, found 599.3347.

Octyl 4-C-methyl-β-D-glucopyranoside (**1a**) *and octyl 4-C-methyl-β-D-galactopyranoside* (**1b**).



The benzyl protected **11a** (16 mg, 28 μmol) was dissolved in MeOH (5 mL) and the solution was stirred under a stream of H_2 in the presence of 20%

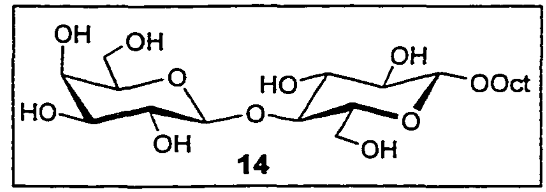
Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

$Pd(OH)_2/C$ (15 mg) for 18 h. The catalyst was filtered removed by filtration through a Millex-GV 0.22 μm filter and the solvent was evaporated. The product was purified by redissolution in water and then passing the solution through a Waters C18 Sep-Pak cartridge. The cartridge was washed with water and eluted with gradient $MeOH/H_2O$ 1:5 to 3:2. The elulant containing the product was concentrated, redissolved in water, filtered through a Millex-GV 0.22 μm filter and lyophilized to yield **1a** (8.1 mg, 95%) as a white solid: ^1H NMR (600 MHz, D_2O) δ 4.43 (d, 1H, J = 7.9 Hz, H-1), 3.97-3.90 (m, 2H, H-6a, OCH_2CH_2), 3.70-3.65 (m, 2H, H-6b, OCH_2CH_2), 3.46 (d, 1H, J = 9.9 Hz, H-3), 3.43 (dd, 1H, J = 8.8, 2.0 Hz, H-5), 3.25 (dd, 1H, J = 9.9, 7.9 Hz, H-2), 1.62 (m, 2H, OCH_2CH_2), 1.38-1.24 (m, 10H, CH_2 octyl), 1.08 (s, 3H, 3-*C*-CH_3), 0.86 (t, 3H, J = 7.0 Hz, CH_3 octyl); ^{13}C NMR (125 MHz, D_2O) δ 103.74 (C-1), 80.11, 78.90, 73.47, 73.04 (C-2, C-3, C-4, C-5), 71.61 (OCH_2CH_2), 60.46 (C-6), 31.87, 29.52, 29.24, 29.14, 25.83, 22.78 (CH_2 octyl), 14.35 (3-*C*-CH_3), 14.18 (CH_3 octyl); HR-ESMS calcd for $C_{15}H_{30}O_6Na$ ($M+Na^+$) 329.1940, found 329.1945.

11b (15 mg, 26 μmol) was deprotected in the same fashion as described above to give **1b** (7.5 mg, 94 %) as a white solid: ^1H NMR (600 MHz, D_2O) δ 4.39 (d, 1H, J = 8.0 Hz, H-1), 3.96-3.91 (m, 2H, H-6, OCH_2CH_2), 3.74 (dd, 1H, J = 12.1, 8.2 Hz, H-6b), 3.67 (dt, 1H, J = 10.0, 7.0 Hz, OCH_2CH_2), 3.50 (dd, 1H, J = 8.2, 2.6 Hz, H-5), 3.44 (dd, 1H, J = 9.7, 8.0 Hz, H-2), 3.33 (d, 1H, J = 9.7 Hz, H-3), 1.63 (m, 2H, OCH_2CH_2), 1.38-1.23 (m, 10H, CH_2 octyl), 1.22 (s, 3H, 3-*C*-CH_3), 0.86 (t, 3H, J = 7.0 Hz, CH_3 octyl); ^{13}C NMR (125 MHz, D_2O) δ 103.28 (C-1), 79.80, 77.12, 73.59, 72.26 (C-2, C-3, C-4, C-5), 71.34 (OCH_2CH_2), 60.76 (C-6), 31.88, 29.55, 29.26, 29.16, 25.86, 22.79 (CH_2 octyl), 20.41 (3-*C*-CH_3), 14.19 (CH_3 octyl); HR-ESMS calcd for $C_{15}H_{30}O_6Na$ ($M+Na^+$) 329.1940, found 329.1945.

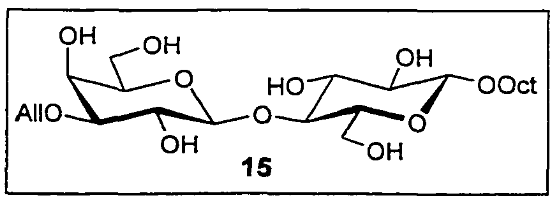
Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Octyl β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (**14**).



Bromide **13** (2 g, 1.76 mmol) and ground 4 Å MS in CH_2Cl_2 (20 mL) were stirred under argon at rt for 20 min and cooled to -50 °C. Octanol (0.45 mL, 2.82 mmol) and silver triflate (725 mg, 2.82 mmol) were added. The reaction mixture was stirred for 4 h while it was allowed to warm to 0 °C. After neutralization with Et_3N, the mixture was filtered through Celite, washed with CH_2Cl_2 and concentrated. The partially purified disaccharide (obtained by column chromatography using 3:1 hexanes/EtOAc) was dissolved in MeOH and a freshly prepared methanolic solution of NaOMe was added. The mixture was stirred at rt for 27 h, neutralized with Amberlite IR-120 (H^+), filtered, concentrated and recrystallized from MeOH to yield **6** (295 mg, 37 % for two steps) as a white solid: ^1H NMR (500 MHz, D_2O) δ 4.48 (d, 1H, J = 8.0 Hz, H-1), 4.45 (d, 1H, J = 8.0 Hz, H-1'), 3.98 (dd, 1H, J = 12.1, 2.1 Hz, H-6a), 3.94-3.88 (m, 2H, H-4', OCH_2CH_2), 3.82-3.56 (m, 9H, H-3, H-4, H-5, H-6b, H-3', H-5', H-6'a, H-6'b, OCH_2CH_2), 3.54 (dd, 1H, J =10.0, 7.8 Hz, H-2'), 3.30 (m, 1H, H-2, virtual order), 1.62 (m, 2H, OCH_2CH_2), 1.39-1.23 (m, 10H, CH_2 octyl), 0.86 (t, 3H, J = 7.0 Hz, CH_3 octyl); ^{13}C NMR (125 MHz, D_2O) δ 103.75, 102.84 (C-1, C-1'), 79.30, 76.16, 75.56, 75.29, 73.67, 73.36, 71.78, 71.58, 69.37 (C-2, C-3, C-4, C-5, C-2', C-3', C-4', C-5', OCH_2CH_2), 61.82, 60.97 (C-6, C-6'), 31.88, 29.53, 29.23, 29.15, 25.84, 22.79 (CH_2 octyl), 14.19 (CH_3 octyl). HR-ESMS calcd for $C_{20}H_{38}O_{11}Na$ (M+Na^+) 477.2311, found 477.2315.

Octyl 3-O-allyl-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (**15**).

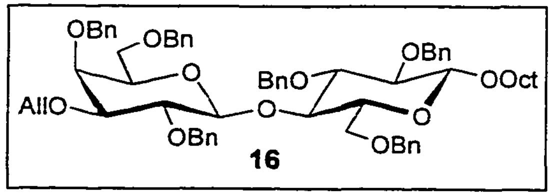


A suspension of **14** (454 mg, 1 mmol) in benzene (25 mL) was refluxed for 24 h in the

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

presence of Bu_2SnO (300 mg, 1.2 mmol) in a flask equipped with a Dean-Stark separator. Allyl bromide (1.6 mL, 18.5 mmol) and tetrabutylammonium iodide (185 mg, 0.5 mmol) were added. The resulting solution was refluxed for 14 h. After evaporation of solvent, the residue was dissolved in hot MeOH. On cooling, a crystalline solid was obtained on filtration and the filtrate was concentrated. Purification on a column of Iatrobeads (10:1 CH_2Cl_2/MeOH) yield **7** (260 mg, 53%) as a white solid: ^{1}H NMR (360 MHz, CD_3OD): δ 5.98 (dddd, 1H, H-c, allyl), 5.32 (dddd, 1H, H-b allyl), 5.15 (dddd, 1H, H-a, allyl), 4.37 (d, 1H, J = 7.8 Hz, H-1), 4.27 (d, 1H, J = 7.8 Hz, H-1'), 4.22 (dddd, 1H, H-d allyl), 4.12 (dddd, 1H, H-e allyl), 3.99 (dd, 1H, J = 3.0, 0.5 Hz, H-4'), 3.92-3.32 (m, 12H, H-3, H-4, H-5, H-6a, H-6b, H-2', H-3', H-5', H-6'a, H-6'b, OCH_2CH_2), 3.23 (dd, 1H, 8.8, 7.8 Hz, H-2), 1.61 (m, 2H, OCH_2CH_2), 1.43-1.23 (m, 10H, CH_2 octyl), 0.89 (t, 3H, J = 7.0 Hz, CH_3 octyl); ^{13}C NMR (125 MHz, CD_3OD) δ 136.47 (CH_2=$CHCH_2O$), 117.41 (CH_2=$CHCH_2O$), 105.08, 104.27 (C-1. C-1'), 82.15, 80.88, 76.93, 76.51, 75.43, 74.79, 71.80, 71.70, 70.98, 67.07 (C-2, C-3, C-4, C-5, C-2', C-3', C-4', C-5', CH_2=$CHCH_2O$, OCH_2CH_2), 62.49, 62.06 (C-6, C-6'), 32.98, 30.78, 30.55, 30.38, 27.10, 23.69 (CH_2 octyl), 14.38 (CH_3 octyl); HR-ESMS calcd for $C_{23}H_{42}O_{11}Na$ ($M+Na^+$) 517.2625, found 517.2625.

Octyl 3-O-allyl-2,4,6-tri-O-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-O-benzyl-β-D-glucopyranoside (**16**).

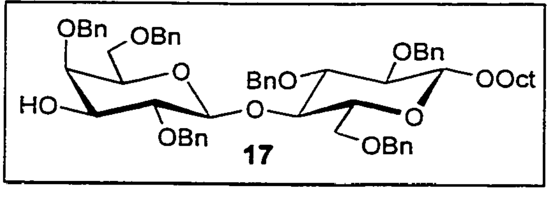


A solution of **15** (240 mg, 0.49 mmol) and benzyl bromide (0.5 mL, 3.92 mmol) in DMF (5 mL) was cooled to 0 °C. Sodium hydride (196 mg, 60% dispersion in mineral oil, 4.9 mmol) was added and the mixture was stirred at 0 °C for 2 h. The reaction was then slowly warmed to rt and stirred overnight. After quenching with MeOH, the mixture was

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

extracted with CH_2Cl_2. The organic layer was washed with water and brine, then dried (Na_2SO_4), filtered and concentrated. The residue was purified by column chromatography (6:1 hexanes/EtOAC) to yield **16** (400 mg, 80%) as a syrup: ^{1}H NMR (360 MHz, $CDCl_3$) δ 7.50-7.10 (m, 30H, ArH), 5.93 (dddd, 1H, H-c allyl), 5.32 (dddd, 1H, H-b, allyl), 5.18 (dddd, 1H, H-a allyl), 5.02 (d, 1H, J = 10.8 Hz, PhCH_2), 4.97 (d, 1H, J = 11.5 Hz, PhCH_2), 4.92 (d, 1H, J = 10.8 Hz, PhCH_2), 4.84-4.71 (m, 4H, PhCH_2), 4.56 (d, 1H, J = 11.5 Hz, PhCH_2), 4.54 (d, 1H, J = 12.0 Hz, PhCH_2), 4.45 (d, 1H, J = 7.7 Hz, H-1), 4.44 (d, 1H, J = 10.8 Hz, PhCH_2), 4.38 (d, 1H, J = 8.3 Hz, H-1'), 4.34 (d, 1H, J = 11.8 Hz, PhCH_2), 4.24 (d, 1H, J = 11.8 Hz, PhCH_2), 4.17 (m, 2H, H-d and H-e allyl), 3.99-3.28 (m, 14H, H-2, H-3, H-4, H-5, H-6a, H-6b, H-2', H-3', H-4', H-5', H-6'a, H-6'b, O$CH_2$$CH_2$), 1.64 (m, 2H, $OCH_2$$CH_2$), 1.46-1.22 (m, 10H, CH_2 octyl), 0.89 (t, 3H, J = 7.0 Hz, CH_3 octyl); ^{13}C NMR (75 MHz, $CDCl_3$) δ 139.30, 139.19, 138.96, 138.83, 138.56, 138.18 (aromatic quart.), 135.06 (CH_2=CHCH_2O), 128.40, 128.26, 128.18, 128.08, 128.04, 127.99, 127.91, 127.89, 127.79, 127.70, 127.57, 127.45, 127.42, 127.35, 127.06 (aromatic CH), 116.43 (CH_2=CHCH_2O), 103.68, 102.84 (C-1, C-1'), 83.08, 82.46, 81.87, 79.63, 76.96, 75.36, 74.99, 74.66, 73.59, 73.45, 73.12, 73.03 (C-2, C-3, C-4, C-5, C-2', C-3', C-4', C-5', PhCH_2 × 6), 71.53, 70.10 (CH_2=CHCH_2O, O$CH_2$$CH_2$), 68.53, 68.19 (C-6, C-6'), 31.88, 29.82, 29.48, 29.31, 26.23, 22.71 (CH_2 octyl), 14.14 (CH_3 octyl); HR-ESMS calcd for $C_{65}H_{78}O_{11}Na$ (M+Na$^+$) 1057.5442, found 1057.5435.

Octyl 2,4,6-tri-O-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-O-benzyl-β-D-glucopyranoside (**17**).

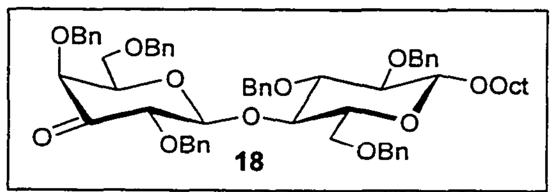


To a solution of **16** (400 mg, 0.39 mmol) in MeOH (15 mL) was added $PdCl_2$ (34 mg, 0.19 mmol). The mixture was stirred at rt for 4 h. After evaporation of MeOH, the residue was

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

purified by column chromatography (4:1 hexanes/EtOAc) to yield **17** (320 mg, 83 %) as a syrup: ^{1}H NMR (360 MHz, $CDCl_3$) δ 7.50-7.10 (m, 30H, ArH), 5.06 (d, 1H, J = 10.8 Hz, $PhCH_2$), 4.96 (d, 1H, J = 10.8 Hz, $PhCH_2$), 4.86 (d, 1H, J = 11.5 Hz, $PhCH_2$), 4.85-4.76 (m, 3H, $PhCH_2$), 4.75 (d, 1H, J = 11.5 Hz, $PhCH_2$), 4.69 (d, 1H, J = 11.2 Hz, $PhCH_2$), 4.63 (d, 1H, J = 11.5 Hz, $PhCH_2$), 4.52-4.39 (m, 4H, H-1, H-1', $PhCH_2$), 4.32 (d, 1H, J = 11.8 Hz, $PhCH_2$), 4.05-3.39 (m, 14H, H-2, H-3, H-4, H-5, H-6a, H-6b, H-2', H-3', H-4', H-5', H-6'a, H-6'b, OCH_2CH_2), 1.69 (m, 2H, OCH_2CH_2), 1.50-1.22 (m, 10H, CH_2 octyl), 0.92 (t, 3H, J = 7.0 Hz, CH_3 octyl); ^{13}C NMR (75 MHz, $CDCl_3$) δ 139.20, 138.77 (2 × C), 138.49, 138.35, 138.08 (aromatic quart.), 128.45, 128.38, 128.29, 128.09, 128.06, 128.00, 127.93, 127.75, 127.72, 127.60, 127.57, 127.49, 127.14 (aromatic CH), 103.69, 102.69 (C-1, C-1'), 82.91, 81.80, 80.65, 76.80, 75.96, 75.34, 75.20, 75.11, 74.96 (2 × C), 74.15, 73.38, 73.25, 73.18 (C-2, C-3, C-4, C-5, C-2', C-3', C-4', C-5', $PhCH_2$ × 6), 70.08 (OCH_2CH_2), 68.40, 68.01 (C-6, C-6'), 31.85, 29.78, 29.45, 29.28, 26.20, 22.68 (CH_2 octyl), 14.12 (CH_3 octyl); HR-ESMS calcd for $C_{62}H_{74}O_{11}Na$ ($M+Na^+$) 1017.5129, found 1017.5137.

Octyl 2,4,6-tri-O-benzyl-β-D-xylo-hex-3-ulopyranosyl-(1→4)-2,3,6-tri-O-benzyl-β-D-glucopyranoside (**18**).

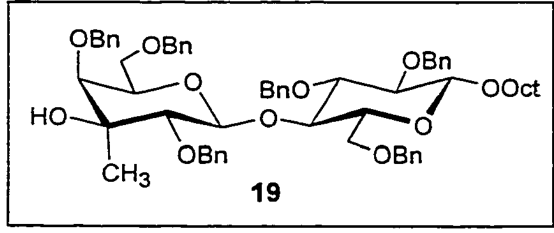


A solution of Dess-Martin periodinane (190 mg, 0.45 mmol) in CH_2Cl_2 (6 mL) was added to a stirred solution of **17** (300 mg, 0.30 mmol) in CH_2Cl_2 (7 mL) and stirring was continued for 1.5 h. The mixture was poured into a saturated aqueous $NaHCO_3$ containing $Na_2S_2O_3$ and extracted with EtOAc. The organic layer was washed with water and brine, dried (Na_2SO_4), filtered, concentrated and fractionated using column chromatography (5:1 hexanes/EtOAc) to yield **18** (215 mg, 72 %) as a solid: ^{1}H NMR (300 MHz, $CDCl_3$)

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

δ 7.40-7.10 (m, 30H, ArH), 4.93 (d, 1H, J = 10.4 Hz, PhCH_2), 4.89 (d, 1H, J = 10.4 Hz, PhCH_2), 4.79 (d, 1H, J = 11.0 Hz, PhCH_2), 4.69 (d, 1H, J = 11.0 Hz, PhCH_2), 4.64 (d, 1H, J = 7.6 Hz, H-2'), 4.63 (d, 1H, J = 11.5 Hz, PhCH_2), 4.48 (d, 1H, J = 12.0 Hz, PhCH_2), 4.46 (d, 1H, J = 12.0 Hz, PhCH_2), 4.44, (d, 1H, J = 11.0 Hz, PhCH_2), 4.40 (d, 1H, J = 12.0 Hz, PhCH_2), 4.37 (d, 1H, J = 8.3 Hz, H-1), 4.34 (d, 1H, J = 12.0 Hz, PhCH_2), 4.31 (d, 1H, J = 11.0 Hz, PhCH_2), 4.30 (d, 1H, J = 7.6 Hz, H-1'), 4.20 (d, 1H, J =12.0 Hz, PhCH_2), 3.96-3.32 (m, 12H, H-2, H-3, H-4, H-5, H-6a, H-6b, H-4', H-5', H-6'a, H-6'b, OCH_2CH$_2$), 1.62 (m, 2H, OCH$_2$CH_2), 1.44-1.20 (m, 10H, CH_2 octyl), 0.86 (t, 3H, J = 7.0 Hz, CH_3 octyl); ^{13}C NMR (75 MHz, $CDCl_3$): δ 204.26 (C-3'), 139.13, 138.65, 138.38, 138.08, 137.39, 136.96 (aromatic quart.), 128.46, 128.41, 128.37, 128.33, 128.18, 128.13, 128.11, 128.08, 127.96, 127.75, 127.62, 127.58, 127.49, 127.21 (aromatic CH), 103.66, 103.57 (C-1, C-1'), 83.27, 83.06, 81.98, 81.02, 77.67, 75.30, 74.98, 74.94, 73.53, 73.33, 73.25, 73.10, 72.53 (C-2, C-3, C-4, C-5, C-2', C-4', C-5', PhCH_2 × 6), 70.14 (OCH_2CH$_2$), 68.55, 66.82 (C-6, C-6'), 31.89, 29.82, 29.47, 29.31, 26.24, 22.71 (CH_2 octyl), 14.14 (CH_3 octyl); HR-ESMS calcd for $C_{62}H_{72}O_{11}Na$ (M+Na$^+$) 1015.4972, found 1015.4972.

Octyl 2,4,6-tri-O-benzyl-3-C-methyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-O-benzyl-β-D-glucopyranoside (**19**).

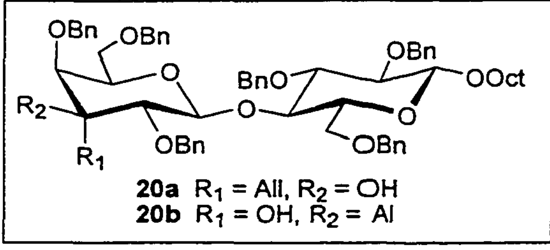


Compound **18** (85 mg, 0.09 mmol) was dissolved in THF (1.5 mL) and cooled under argon to -78 °C. Methyllithium (1.4 M, 0.1 mL) in diethyl ether was added with stirring. The reaction mixture was stirred for 1 h while it was allowed to warm to -40 °C. The reaction was poured into saturated NH_4Cl and extracted with EtOAc. The organic layer was washed with water and brine, dried

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

(Na_2SO_4), filtered, concentrated and fractionated using column chromatography (5:1 hexanes/EtOAc) to yield **19** (78 mg, 90%) as a solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40-7.10 (m, 30H, ArH), 5.10 (d, 1H, J = 11.0 Hz, PhCH_2), 4.89 (d, 1H, J = 11.0 Hz, PhCH_2), 4.77 (d, 1H, J = 11.6 Hz, PhCH_2), 4.76 (d, 1H, J = 11.0 Hz, PhCH_2), 4.71 (d, 1H, J = 11.0 Hz, PhCH_2), 4.67 (d, 1H, J = 11.6 Hz, PhCH_2), 4.64 (d, 1H, J = 11.7 Hz, PhCH_2), 4.60 (d, 1H, J = 11.7 Hz, PhCH_2), 4.53 (d, 1H, J = 8.0 Hz, H-1'), 4.46 (d, 1H, J = 12.3 Hz, PhCH_2), 4.42 (d, 1H, J = 12.3 Hz, PhCH_2), 4.37 (d, 1H, J = 11.8 Hz, PhCH_2), 4.34 (d, 1H, J = 7.7 Hz, H-1), 4.28 (d, 1H, J = 11.8 Hz, PhCH_2), 3.97-3.28 (m, 13H, H-2, H-3, H-4, H-5, H-6a, H-6b, H-2', H-4', H-5', H-6'a, H-6'b, OCH_2CH$_2$), 1.62 (m, 2H, OCH$_2$CH_2), 1.44-1.18 (m, 10H, CH_2 octyl), 1.14 (s, 3H, 3'-*C*-CH_3), 0.86 (t, 3H, J = 7.0 Hz, CH_3 octyl); ^{13}C NMR (75 MHz, $CDCl_3$) δ 139.41, 138.96, 138.79, 138.51, 138.27, 137.94 (aromatic quart.), 128.56, 128.45, 128.30, 128.25, 128.10, 128.04, 127.93, 127.88, 127.81, 127.70, 127.56, 127.44, 127.36, 127.18, 127.13 (aromatic CH), 103.72, 101.93 (C-1, C-1'), 83.15 (2 × C), 82.07, 81.86, 77.27, 75.72, 75.24 (2 × C), 75.13, 74.97, 74.91, 73.50, 72.90, 71.87 (C-2, C-3, C-4, C-5, C-2', C-3', C-4', C-5', PhCH_2 × 6), 70.12 (OCH_2CH$_2$), 68.48, 68.31 (C-6, C-6'), 31.88, 29.81, 29.48, 29.31, 26.22, 22.71 (CH_2 octyl), 19.15 (3'-*C*-CH_3), 14.14 (CH_3 octyl); HR-ESMS calcd for $C_{63}H_{76}O_{11}Na$ (M+Na$^+$) 1031.5285, found 1031.5297.

*Octyl 2,4,6-tri-O-benzyl-3-C-allyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-O-benzyl-β-D-glucopyranoside (**20a**) and Octyl 2,4,6-tri-O-benzyl-3-C-allyl-β-D-gulopyranosyl-(1→4)-2,3,6-tri-O-benzyl-β-D-gluco-pyranoside (**20b**).*



To a solution of **18** (40 mg, 0.04 mmol) in THF (3 mL) at 0 °C was added allyl magnesium bromide (1.0 M, 0.1 mL) in diethyl ether with stirring under argon. The

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

reaction mixture was stirred at 0 °C for 4 h, and quenched with saturated NH_4Cl and extracted with EtOAc. The organic layer was washed with water and brine, dried (Na_2SO_4), filtered, concentrated and fractionated using column chromatography (20:1 toluene/EtOAc) to yield **20a** (16 mg, 39%) and **20b** (21 mg, 51%).

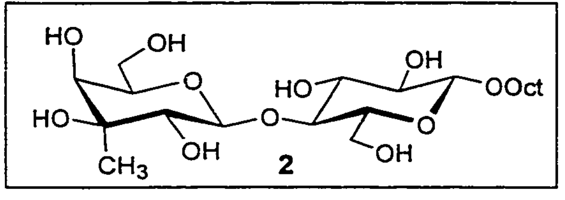
For **20a:** ^{1}H NMR (300 MHz, CD_2Cl_2) δ 7.40-7.20 (m, 30H, ArH), 5.86 (dddd, 1H, H-c allyl), 5.06 (ddd, 1H, H-a allyl), 5.02 (d, 1H, J = 11.0 Hz, PhCH_2), 4.88 (d, 1H, J = 11.0 Hz, PhCH_2), 4.84 (dddd, 1H, J = 17.0, 1.5, 1.5, 1.5 Hz, H-b allyl), 4.78-4.62 (m, 7H, H-1', PhCH_2), 4.53 (d, 1H, J = 12.3 Hz, PhCH_2), 4.45 (d, 1H, J = 12.0 Hz, PhCH_2), 4.43 (d, 1H, J = 12.0 Hz, PhCH_2), 4.36 (d, 1H, J = 8.0 Hz, H-1), 4.33 (d, 1H, J = 12.3 Hz, PhCH_2), 3.94-3.28 (m, 13H, H-2, H-3, H-4, H-5, H-6a, H-6b, H-2', H-4', H-5', H-6'a, H-6'b, OCH_2CH$_2$), 2.76 (dddd, 1H, H-d allyl), 2.44 (d, 1H, J = 1.5 Hz, OH), 1.97 (dddd, 1H, H-e allyl), 1.64 (m, 2H, OCH$_2$CH_2), 1.44-1.22 (m, CH$_2$ octyl), 0.88 (t, 3H, J = 7.0 Hz, CH$_3$ octyl); ^{13}C NMR (75 MHz, CD_2Cl_2) δ 139.90, 139.42, 138.98, 138.75, 138.73 (aromatic CH), 133.75 (CH$_2$=CHCH$_2$), 128.79, 128.66, 128.59, 128.51, 128.30, 128.28, 128.23, 128.13, 128.07, 128.00, 127.92, 127.79, 127.77, 127.71, 127.42, 127.28 (aromatic CH), 118.28 (CH$_2$=CHCH$_2$), 104.06, 101.81 (C-1, C-1'), 83.70, 83.20, 82.07, 78.71, 77.30, 76.39, 75.94, 75.82, 75.34, 75.23, 75.02, 73.56, 73.07, 71.67 (C-2, C-3, C-4, C-5, C-2', C-3', C-4', C-5', PhCH$_2$ × 6), 70.32 (OCH$_2$CH$_2$), 68.83, 68.73 (C-6, C-6'), 36.31 (CH$_2$=CHCH$_2$), 32.24, 30.22, 29.83, 29.67, 26.59, 23.06 (CH$_2$ octyl), 14.25 (CH$_3$ octyl); HR-ESMS calcd for $C_{65}H_{78}O_{11}Na$ (M+Na$^+$) 1057.5442, found 1057.5440.

For **12b:** ^{1}H NMR (300 MHz, CD_2Cl_2) δ 7.40-7.10 (m, 30H), 5.97 (dddd, 1H, H-c allyl), 5.18-5.08 (m, 2H, H-a and H-c allyl), 4.88 (d, 1H, J = 11.0 Hz, PhCH_2), 4.87 (d, 1H, J = 7.9 Hz, H-1'), 4.86 (d, 1H, J = 11.0 Hz, PhCH_2), 4.71 (d, 1H, J =11.0 Hz, PhCH_2), 4.69 (d, 1H, J = 11.0 Hz, PhCH_2), 4.60 (d, 1H, J = 11.0 Hz, PhCH_2), 4.59 (d, 1H, J = 11.0 Hz, PhCH_2), 4.50-4.45 (m, 3H, PhCH_2), 4.37 (d, 1H, J = 12.0 Hz, PhCH_2), 4.36 (d, 1H, J = 7.8 Hz, H-1), 4.13 (m, 12H, H-3, H-4, H-5, H-6a, H-6b, H-2', H-4', H-5', H-6'a, H-6'b, OCH_2CH$_2$), 3.30 (dd, 1H, J = 9.2, 7.8 Hz, H-2), 2.62 (dddd, 1H, H-d

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

allyl), 2.54 (dddd, 1H, H-e allyl), 2.18 (s, 1H, OH), 1.64 (m, 2H, OCH_2CH_2), 1.44-1.20 (m, 10H, CH_2 octyl), 0.89 (t, 3H, J = 7.0 Hz, CH_3 octyl); ^{13}C NMR (75 MHz, CD_2Cl_2) δ 139.85, 139.48, 139.10, 138.81, 138.77, 138.58 (aromatic quart.), 134.19 (CH_2=$CHCH_2$), 128.73, 128.67, 128.60, 128.57, 128.51, 128.50, 128.37, 128.24, 128.22, 128.16, 128.03, 127.93, 127.81, 127.79, 127.75, 127.71, 127.38 (aromatic CH), 118.78 (CH_2=$CHCH_2$), 104.01, 101.52 (C-1, C-1'), 83.14, 81.95, 80.57, 77.61, 76.67, 75.77, 75.42, 75.35, 75.09, 75.05, 73.67, 73.50, 72.27 (C-2, C-3, C-4, C-5, C-2', C-3', C-4', C-5', PhCH_2 × 6), 70.30 (OCH_2CH_2), 68.82, 68.61 (C-6, C-6'), 39.75 (CH_2=$CHCH_2$), 32.25, 30.23, 29.84, 29.67, 26.60, 23.06 (CH_2 octyl), 14.25 (CH_3 octyl); HR-ESMS calcd for $C_{65}H_{78}O_{11}Na$ ($M+Na^+$) 1057.5442, found 1057.5450.

Octyl 3-C-methyl-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (**2**).

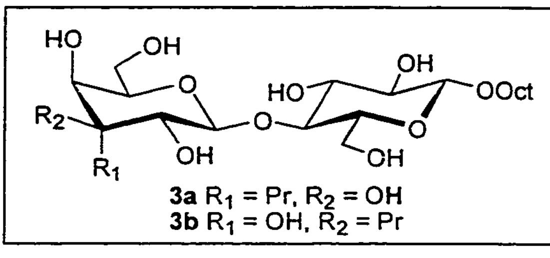


The benzyl protected **11** (21 mg, 21 μmol) was dissolved in MeOH (10 mL) and the solution was stirred under a stream of H_2 in the presence of 20% $Pd(OH)_2/C$ (25 mg) for 10 h. The catalyst was removed by filtration through a Millex-GV 0.22 μm filter and the solvent evaporated. The product was purified by redissolution in water and then passing the solution through a Waters C18 Sep-Pak cartridge. The cartridge was washed with water eluted with gradient MeOH-H_2O 1:4 to 2:1. The elulant containing the compound was concentrated, redissolved in water, filtered through a Millex-GV 0.22 μm filter and lyophilized to yield **2** (9.5 mg, quant) as a white solid: ^1H NMR (500 MHz, D_2O) δ 4.52 (d, 1H, J = 8.1 Hz, H-1'), 4.47 (d, 1H, J = 8.0 Hz, H-1), 3.96 (dd, 1H, J = 12.2, 2.2 Hz, H-6a), 3.94-3.87 (m, 2H, H-5', OCH_2CH_2), 3.81-3.55 (m, 9H, H-3, H-4, H-5, H-6b, H-2', H-4', H-6'a, H-6'b, OCH_2CH_2), 3.30 (m, 1H, H-2, virtual order), 1.62 (m, 2H, OCH_2CH_2), 1.38-1.24 (m, 10H, CH_2 octyl), 1.24 (s, 3H, 3'-C-CH_3), 0.86 (t, 3H, J = 7.0

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Hz, CH_2 octyl); ^{13}C NMR (125 MHz, D_2O) δ 102.82, 102.69 (C-1, C-1'), 79.42, 75.53, 75.39, 75.34, 74.70, 74.09, 74.04, 73.64 (C-2, C-3, C-4, C-5, C-2', C-3', C-4', C-5') 71.58 (OCH_2CH_2), 62.24, 61.03 (C-6, C-6'), 31.88, 29.54, 29.23, 29.15, 25.84, 22.80 (CH_2 octyl), 18.60 (3'-*C*-CH_3), 14.19 (CH_3 octyl); HR-ESMS calcd for $C_{21}H_{41}O_{11}$ ($M+H^+$) 469.2649, found 469.2648.

Octyl 3-C-propyl-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (3a) and octyl 3-C-propyl-β-D-gulopyranosyl-(1→4)-β-D-glucopyranoside (**3b**).



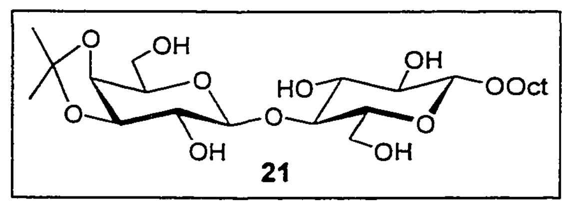
Compound **20a** (7 mg, 6.8 μmol) and **20b** (9 mg, 8.7 μmol) were deprotected in the same fashion as for **2** to give, respectively, **3a** (3.3 mg) and **3b** (4.1 mg) as white solids in quantitative yields.

For **3a:** ^1H NMR (600 MHz, D_2O) δ 4.58 (d, 1H, J = 8.2 Hz, H-1'), 4.47 (d, 1H, J = 7.9 Hz, H-1), 3.96 (dd, 1H, J = 12.3, 2.2 Hz, H-6a), 3.91 (dt, 1H, J = 10.0, 7.0 Hz, OCH_2CH_2), 3.85 (dd, 1H, J = 7.4, 4.8 Hz, H-5'), 3.82 (bs, 1H, H-4'), 3.78 (dd, 1H, J = 12.3, 5.0 Hz, H-6b), 3.75 (dd, 1H, J = 11.8, 7.4 Hz, H-6'a), 3.73 (dd, 1H, J = 11.8, 4.8 Hz, H-6'b), 3.67 (dt, 1H, J = 10.0, 7.0 Hz, OCH_2CH_2), 3.65-3.60 (m, 3H, H-3, H-4, H-2'), 3.57 (m, 1H, H-5), 3.30 (m, 1H, H-2, virtual order), 1.73 (m, 1H, $CH_2CH_2CH_3$), 1.62 (m, 2H, OCH_2CH_2), 1.56 (m, 1H, $CH_2CH_2CH_3$), 1.41 (m, 2H, $CH_2CH_2CH_3$), 1.38-1.22 (m, 10 H, CH_2 octyl), 0.92 (t, 3H, J = 7.0 Hz, $CH_2CH_2CH_3$), 0.86 (t, 3H, J = 7.0 Hz, CH_3 octyl); ^{13}C NMR (125 MHz, D_2O): δ 102.84, 102.45 (C-1, C-1'), 79.38, 76.29, 75.54, 75.41, 74.90, 74.63, 73.63, 71.59, 70.01 (C-2, C-3, C-4, C-5, C-2', C-3', C-4', C-5', OCH_2CH_2), 62.21, 61.03 (C-6, C-6'), 33.01, 31.88, 29.54, 29.23, 29.15, 25.84, 22.79 (CH_2 octyl, $CH_2CH_2CH_3$), 15.89 ($CH_2CH_2CH_3$), 14.77 ($CH_2CH_2CH_3$), 14.19 (CH_3 octyl); HR-ESMS calcd for $C_{23}H_{44}O_{11}Na$ ($M+Na^+$) 519.2781, found 519.2786.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

For **3b:** ^{1}H NMR (600 MHz, D_2O) δ 4.68 (d, 1H, J = 8.0 Hz, H-1'), 4.47 (d, 1H, J = 8.0 Hz, H-1), 4.06 (ddd, 1H, J = 8.0, 4.1, 0.8 Hz, H-5'), 3.97 (dd, 1H, J = 12.2, 4.2 Hz, H-6a), 3.91 (dt, 1H, J = 10.0, 7.0 Hz, OCH_2CH_2), 3.81 (dd, 1H, J = 12.2, 5.1 Hz, H-6b), 3.75 (dd, 1H, J = 12.0, 8.0 Hz, H-6'a), 3.73 (dd, 1H, J = 12.0, 4.1 Hz, H-6'b), 3.67 (dt, 1H, J = 10.0, 7.0 Hz, OCH_2CH_2), 3.65-3.60 (m, 3H, H-3, H-4, H-4'), 3,57 (m, 1H), 3.38 (d, 1H, J = 8.0 Hz, H-2'), 3.30 (m, 1H, H-2, virtual order), 1.76 (m, 1H, $CH_2CH_2CH_3$), 1.66-1.60 (m, 3H, $CH_2CH_2CH_3$, OCH_2CH_2), 1.40-1.22 (m, 12H, $CH_2CH_2CH_3$, CH_2 octyl), 0.92 (t, 3H, J = 7.0 Hz, $CH_2CH_2CH_3$), 0.86 (t, 3H, J = 7.0 Hz, CH_3 octyl); ^{13}C NMR (125 MHz, D_2O): δ 102.86, 102.62 (C-1, C-1'), 79.91, 76.55, 75.52, 75.44, 75.40, 73.65, 72.84, 71.59, 69.59 (C-2, C-3, C-4, C-5, C-2', C-3', C-4', C-5', OCH_2CH_2), 62.23, 61.14 (C-6, C-6'), 36.44, 31.88, 29.54, 29.23, 29.15, 25.84, 22.80 (CH_2 octyl, $CH_2CH_2CH_3$), 15.38 ($CH_2CH_2CH_3$), 14.58 ($CH_2CH_2CH_3$), 14.19 (CH_3 octyl); HR-ESMS calcd for $C_{23}H_{44}O_{11}Na$ (M+Na$^+$) 519.2781, found 519.2786.

Octyl 3,4-O-isopropylidene-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (**21**).

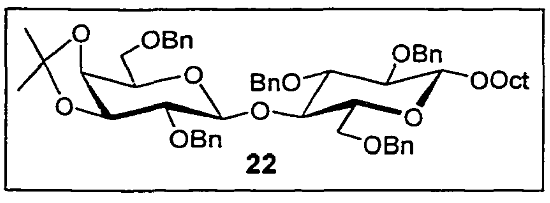


A suspension of **14** (930 mg, 2.05 mmol) in 2,2-dimethoxypropane (3.5 mL, mmol) was refluxed at 80 °C for 15 min in the presence of *p*-toluenesulfonic acid (70 mg, 0.41 mmol). After cooling, the reaction was neutralized with Et_3N, concentrated and co-evaporated with toluene. The resulting residue was then dissolved in 10:1 MeOH/H2O (20 mL) and refluxed for 3 h. The mixture was concentrated and purified on a column of Iatrobeads with 10:1 CH_2Cl_2/MeOH to give **21** (530 mg, 52%) as a solid: ^{1}H NMR (360 MHz, CD_3OD) δ 4.36 (d, 1H, J = 8.2 Hz, H-1'), 4.27 (d, 1H, J = 7.9 Hz, H-1), 4.18 (dd, 1H, J = 5.5, 2.1 Hz, H-3'), 4.05 (dd, 1H, J = 7.5, 5.5, H-2'), 3.97-3.36 (m, 11H, H-3, H-4, H-5, H-6a, H-6b, H-4', H-5, H-6'a, H-6'b, OCH_2CH_2), 3.23 (dd, 1H, J = 8.8, 7.9 Hz, H-2),

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

1.61 (m, 2H, OCH_2CH_2), 1.47 (s, 3H, CCH_3), 1.42-1.22 (m, 13H, CH_2 octyl, CCH_3), 0.89 (t, 3H, J = 7.0 Hz, CH_3 octyl); ^{13}C NMR (75 MHz, CD_3OD) δ 111.06 (CCH_3), 104.15 (2 × C) (C-1, C-1'), 81.05, 80.83, 76.36, 76.30, 75.31, 75.03, 74.80, 74.42 (C-2, C-3, C-4, C-5, C-2', C-3', C-4', C-5'), 70.93 (OCH_2CH_2), 62.38, 61.91 (C-6, C-6'), 32.97, 30.75, 30.54, 30.38, 28.41, 27.08, 26.51, 23.68 (CCH_3 × 2, CH_2 octyl), 14.42 (CH_3 octyl); HR-ESMS calcd for $C_{23}H_{43}O_{11}$ ($M+H^+$) 495.2805, found 495.2809.

Octyl 2,6-di-O-benzyl-3,4-O-isopropylidene-β-D-galactopyranosyl-(1→4)-2,3,6-tri-O-benzyl-β-D-glucopyranoside (**22**).

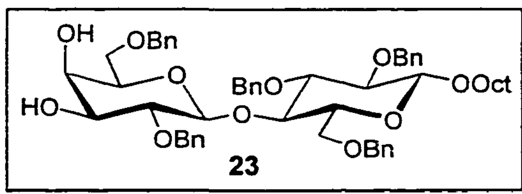


Compound **21** (400 mg, 0.81 mmol) was benzylated as described above for **16** to give compound **22** (640 mg, 84%) as a syrup: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40-7.18 (m, 25H, ArH), 4.91 (d, 1H, J = 11.0 Hz, $PhCH_2$), 4.88 (d, 1H, J = 11.0 Hz, $PhCH_2$), 4.77 (d, 1H, J = 12.0 Hz, $PhCH_2$), 4,73 (d, 1H, J = 11.0 Hz, $PhCH_2$), 4.68 (d, 1H, J = 12.0 Hz, $PhCH_2$), 4.64 (d, 1H, J = 11.0 Hz, $PhCH_2$), 4.55 (d, 1H, J = 12.0 Hz, $PhCH_2$), 4.48 (d, 1H, J = 12.0 Hz, $PhCH_2$), 4.41 (d, 1H, J = 12.0 Hz, $PhCH_2$), 4.39 (d, 1H, J = 8.0 Hz, H-1), 4.36 (d, 1H, J = 7.8 Hz, H-1'), 4.30 (d, 1H, J = 12.0 Hz, $PhCH_2$), 4.09 -3.44 (m, 10H, H-3, H-4, H-6a, H-6b, H-3', H-4', H-5', H-6'a, H-6'b, OCH_2CH_2), 3.42-3.29 (m, 3H, H-2, H-5, H-2'), 1.63 (m, 2H, OCH_2CH_2), 1.40-1.20 (m, 16H, CCH_3 × 2, CH_2 octyl), 0.88 (t, 1H, J = 7.0 Hz, CH_3 octyl); ^{13}C NMR (75 MHz, $CDCl_3$) δ 139.13, 138.77, 138.61, 138.49, 138.41 (aromatic quart.), 128.56, 128.46, 128.35, 128.32, 128.29, 128.21, 128.12, 128.08, 127.99, 127.91, 127.74, 127.65, 127.58, 127.54, 127.46, 127.31 (aromatic CH), 109.90 (CCH_3), 103.74, 101.92 (C-1, C-1'), 83.06, 81.93, 80.72, 79.44, 75.43, 75.16, 74.99, 73.67, 73.59, 73.43, 73.24, 73.27, 72.03 (C-2, C-3, C-4, C-5, C-2', C-3', C-4', C-5', $PhCH_2$ × 5), 70.11 (OCH_2CH_2), 68.99, 68.43 (C-6, C-6'), 31.89, 29.83, 29.49, 29.32,

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

28.01, 26.46, 26.24, 22.72 (CCH_3 × 2, CH_2 octyl), 14.14; HR-ESMS calcd for $C_{58}H_{72}O_{11}Na$ (M+Na$^+$) 967.4972, found 967.4961.

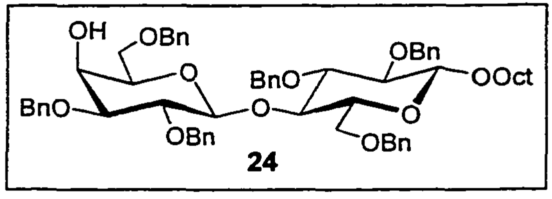
Octyl 2,6-di-O-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-O-benzyl-β-D-glucopyranoside (**23**).



A suspension of **22** (620 mg, 0.66 mmol) in 4:1 AcOH/H_2O was stirred at 65 °C for 19 h. Co-evaporation of the solvent with toluene afforded **22** (570 mg) as a syrup in quantitative yield: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40-7.18 (m, 25H, ArH), 4.97 (d, 1H, J = 11.0 Hz, PhCH_2), 4.90 (d, 1H, J = 11.0 Hz, PhCH_2), 4.81 (d, 1H, J = 11.6 Hz, PhCH_2), 4.78 (d, 1H, J = 11.0 Hz, PhCH_2), 4.70 (d, 1H, J = 11.0 Hz, PhCH_2), 4.66 (d, 1H, J = 11.6 Hz, PhCH_2), 4.59 (d, 1H, J = 12.1 Hz, PhCH_2), 4.46-4.35 (m, 4H, PhCH_2, H-1, H-1'), 4.02-3.31 (m, 14H, H-2, H-3, H-4, H-5, H-6a, H-6b, H-2', H-3', H-4', H-5', H-6'a, H-6'b, OC$H_2$$CH_2$), 2.51-2.20 (b, 2H, OH), 1.64 (m, 2H, OCH_2CH_2), 1.44-1.20 (m, 10H, CH_2 octyl), 0.86 (t, 3H, J = 7.0 Hz, CH_3 octyl); ^{13}C NMR (75 MHz, $CDCl_3$) δ 139.25, 138.73, 138.42, 138.35, 138.06 (aromatic quart.), 128.54, 128.45, 128.31, 128.09, 127.98, 127.95, 127.87, 127.71, 127.64, 127.55, 127.25 (aromatic CH), 103.72, 102.62 (C-1, C-1'), 82.88, 81.86, 80.09, 76.71, 75.25, 75.18, 74.92, 74.91, 73.58, 73.52, 73.25, 72.93, 70.12, 68.85 (C-2, C-3, C-4, C-5, C-2', C-3', C-4', C-5', OCH_2CH_2, PhCH_2 × 5,), 68.73, 68.43 (C-6, C-6'), 31.88, 29.81, 29.47, 29.31, 26.22, 22.70 (CH_2 octyl), 14.13 (CH_3 octyl); HR-ESMS calcd for $C_{55}H_{68}O_{11}Na$ (M+Na$^+$) 927.4659, found 967.4669.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

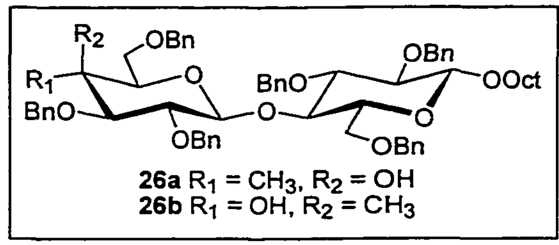
Octyl 2,3,6-tri-O-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-O-benzyl-β-D-glucopyranoside (**24**).



A suspension of **23** (315 mg, 0.35 mmol) in toluene (25 mL) was refluxed for 4 h in the presence of Bu_2SnO (104 mg, 0.42 mmol) in a flask equipped with a Dean-Stark separator. Benzyl bromide (0.29 mL, 2.4 mmol) and tetrabutylammonium iodide (64 mg, 0.17 mmol) were added, and the solution was refluxed for 4.5 h. The mixture was concentrated and the resulting yellow residue was purified by column chromatography (5:1 hexanes/EtOAc) to yield **24** (280 mg, 81%) as a syrup: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40-7.18 (m, 30H, ArH), 4.97 (d, 1H, J = 11.0 Hz, PhCH_2), 4.89 (d, 1H, J = 11.0 Hz, PhCH_2), 4.78-4.66 (m, 6H, PhCH_2), 4.55 (d, 1H, J = 12.0 Hz, PhCH_2), 4.48-4.35 (m, 5H, PhCH_2, H-1, H-1'), 4.01-3.30 (m, 14H, H-2, H-3, H-4, H-5, H-6a, H-6b, H-2', H-3', H-4', H-5', H-6'a, H-6'b, OCH_2CH$_2$), 1.64 (m, 2H, OCH$_2$CH_2), 1.44-1.20 (m, 10H, CH$_2$ octyl), 0.87 (t, 3H, J = 7.0 Hz, CH$_3$ octyl); ^{13}C NMR (75 MHz, $CDCl_3$) δ 139.23, 138.77, 138.69, 138.44, 138.26, 138.00 (aromatic quart.), 128.59, 128.49, 128.40, 128.29, 128.16, 128.08, 127.87, 127.84, 127.80, 127.69, 127.64, 127.55, 127.46, 127.24, 127.01 (aromatic CH), 103.69, 102.58 (C-1, C-1'), 82.96, 81.88, 81.20, 79.46, 76.72, 75.35, 75.28, 75.19, 74.94, 73.55, 73.17, 72.82, 72.06 (C-2, C-3, C-4, C-5, C-2', C-3', C-5', PhCH$_2$ × 6), 70.10 (OCH$_2$CH$_2$), 68.49, 68.41 (C-6, C-6'), 66.20 (C-4'), 31.88, 29.81, 29.48, 29.31, 26.23, 22.70 (CH$_2$ octyl), 14.13 (CH$_3$ octyl); HR-ESMS calcd for $C_{62}H_{74}O_{11}Na$ (M+Na$^+$) 1017.5129, found 1017.5138.

Octyl 2,3,6-tri-O-benzyl-4-C-methyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-O-benzyl-β-D-glucopyranoside (**26a**) and *Octyl 2,3,6-tri-O-benzyl-4-C-methyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-O-benzyl-β-D-gluco-pyranoside* (**26b**).

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.



Compound **24** was oxidized with Dess-Martin Periodinane as described for **10**, and the resulting crude ketone product (75 mg, 0.076 mmol) was treated with MeLi in the same fashion as for **19**. The mixture was purified by column chromatography (5:1 hexanes/EtOAc) to give **26a** (20 mg, 26%) and **26b** (18 mg, 24%).

For **26a:** ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40-7.18 (m, 30H, ArH), 5.01 (d, 1H, J = 11.0 Hz, $PhCH_2$), 4.90 (d, 1H, J = 11.0 Hz, $PhCH_2$), 4.87 (d, 1H, J = 11.0 Hz, $PhCH_2$), 4.80 (d, 1H, J = 11.0 Hz, $PhCH_2$), 4.76 (d, 1H, J = 11.0 Hz, $PhCH_2$), 4.70 (d, 1H, J = 11.0 Hz, $PhCH_2$), 4.68 (d, 1H, J = 11.0 Hz, $PhCH_2$), 4.62 (d, 1H, J = 12.0 Hz, $PhCH_2$), 4.60 (d, 11.0 Hz, $PhCH_2$), 4.42 (d, 1H, J = 7.6 Hz, H-1'), 4.41 (d, 1H, J = 12.0, $PhCH_2$), 4.40 (d, 1H, J = 12.0 Hz, $PhCH_2$), 4.34 (d, 1H, J = 7.7 Hz, H-1), 4.28 (d, 1H, J = 12.0 Hz, $PhCH_2$), 4.00 (dd, 1H, J = 9.3, 9.5 Hz, H-4), 3.90 (dt, 1H, J = 9.4, 6.6 Hz, OCH_2CH_2), 3.81 (dd, 1H, J = 11.0, 4.0 Hz, H-6a), 3.78-3.68 (m, 2H, H-6b, H-6'a), 3.63 (dd, 1H, J = 9.0, 7.7 Hz, H-2), 3.56 (t, 1H, J = 9.0 Hz, H-3), 3.53-3.44 (m, 2H, OCH_2CH_2, H-6'b), 3.42-3.32 (m, 2H, H-2, H-5), 3.09 (dd, 1H, J = 9.8, 3.7 Hz, H-5'), 2.99 (d, 1H, J = 9.2 Hz, H-3'), 1.62 (m, 2H, OCH_2CH_2), 1.42-1.20 (m, 10H, CH_2 octyl), 1.10 (s, 3H, 4'-*C*-CH_3), 0.86 (t, 3H, J = 7.0 Hz, CH_3 octyl); HR-ESMS calcd for $C_{63}H_{76}O_{11}Na$ ($M+Na^+$) 1031.5285, found 1031.5296.

For **26b:** ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40-7.18 (m, 30H, ArH), 4.89 (d, 1H, J = 11.0 Hz, $PhCH_2$), 4.87 (d, 1H, J = 11.5 Hz, $PhCH_2$), 4.86 (d, 1H, J = 11.0 Hz, $PhCH_2$), 4.76 (d, 1H, J = 11.5 Hz, $PhCH_2$), 4.74 (d, 1H, J = 11.2 Hz, $PhCH_2$), 4.72-4.66 (m, 3H, $PhCH_2$), 4.54 (d, 1H, J = 12.0 Hz, $PhCH_2$), 4.46 (d, 1H, J = 7.8 Hz, H-1'), 4.43 (d, 1H, J = 11.2 Hz, $PhCH_2$), 4.40 (d, 1H, J = 11.8 Hz, $PhCH_2$), 4.33 (d, 1H, J = 7.8 Hz, H-1), 4.31 (d, 1H, J = 11.8 Hz, $PhCH_2$), 3.94-3.86 (m, 2H, H-4, OCH_2CH_2), 3.76 (dd, 1H, J =

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

10.8, 4.0 Hz, H-6a), 3.66 (dd, 1H, J = 10.8, 1.6 Hz, H-6b), 3.54-3.28 (m, 8H, H-2, H-3, H-5, H-3', H-5', H-6'a, H-6'b, OCH_2CH$_2$), 3.16 (dd, 1H, J = 9.5, 7.8 Hz, H-2'), 1.62 (m, 2H, OCH$_2$CH_2), 1.40-1.16 (m, 13H, 4'-C-CH$_3$, CH$_2$ octyl), 0.86 (t, 3H, J = 7.0 Hz, CH$_3$ octyl). HR-ESMS calcd for $C_{63}H_{76}O_{11}Na$ (M+Na$^+$) 1031.5285, found 1031.5291.

Octyl 4-C-methyl-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (**4a**) and *Octyl 4-C-methyl-β-D-gulcopyranosyl-(1→4)-β-D-glucopyranoside* (**4b**).

4a R_1 = CH$_3$, R_2 = OH
4b R_1 = OH, R_2 = CH$_3$

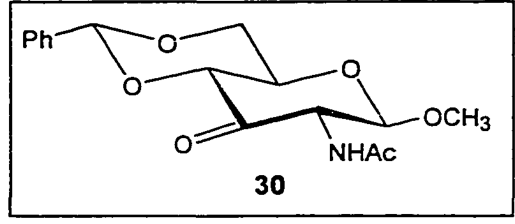
The benzyl protected **26a** (10 mg, 9.9 μmol) and **26b** (5.5 mg, 5.5 μmol) were treated in the same fashion as for **1** to give **4a** (4.1 mg, 88%) and **4b** (2.2 mg, 86%) as white solids respectively.

For **4a:** ^{1}H NMR (300 MHz, D$_2$O) δ 4.49 (d, 1H, J = 8.0 Hz, H-1), 4.47 (d, 1H, J = 7.8 Hz, H-1'), 4.01-3.47 (m, 11H, H-3, H-4, H-5, H-6a, H-6b, H-2', H-5', H-6'a, H-6'b, OCH_2CH$_2$), 3.37 (d, 1H, J = 9.6 Hz, H-3'), 3.31 (m, 1H, H-2, virtual order), 1.63 (m, 2H, OCH$_2$CH_2), 1.42-1.24 (m, 10H, CH$_2$ octyl), 1.23 (s, 3H, 4'-C-CH$_3$), 0.87 (s, 3H, J = 7.0 Hz, CH$_3$ octyl); HR-ESMS calcd for $C_{21}H_{40}O_{11}Li$ (M+Li$^+$) 475.2731, found 475.2736.

For **4b:** ^{1}H NMR (500 MHz, D$_2$O): δ 4.51 (d, 1H, J = 7.8 Hz, H-1), 4.47 (d, 1H, J = 8.0 Hz, H-1'), 3.98 (dd, 1H, J = 12.2, 2.0 Hz, H-6a), 3.96-3.46 (m, 10H, H-3, H-4, H-5, H-6b, H-2', H-5', H-6'a, H-6'b, OCH_2CH$_2$), 3.34 (d, 1H, J = 7.8 Hz, H-3'), 3.30 (m, 1H, H-2, virtual order), 1.64 (m, 2H, OCH$_2$CH_2), 1.42-1.24 (m, 10H, CH$_2$ octyl), 1.11 (s, 3H, 4'-C-CH$_3$), 0.87 (t, 3H, J = 7.0 Hz, CH$_3$ octyl); HR-ESMS calcd for $C_{21}H_{40}O_{11}Li$ (M+Li$^+$) 475.2731, found 475.2730.

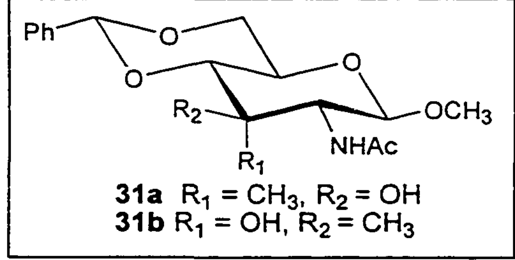
Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Methyl 2-acetamido-4,6-O-benzylidene-2-deoxy-β-D-ribo-hexopyranosid-3-ulose (**30**).



A solution of **29** (2 g, 6.19 mmol) in DMSO (30 mL) and acetic anhydride (15 mL) was stirred at rt for 8h. The mixture was concentrated under high vacuum. Et_2O (100 mL) was then added to the resulting residue to yield the white precipitate. Filtration and washing with Et_2O gave **30** (1.2g, 60%) as a white solid: ^1H NMR (360 MHz, DMSO-d_6) δ 8.20 (d, 1H, J = 8.3 Hz, NH), 7.45-7.35 (m, 5H, ArH), 5.67 (s, 1H, PhC*H*), 4.70-4.64 (m, 2H, H-1, H-4), 4.46 (dd, 1H, J = 8.3, 8.1 Hz, H-2), 4.37 (dd, 1H, J = 10.1, 4.9 Hz, H-6-eq), 3.89 (t, 1H, J = 10.1 Hz, H-6-ax), 3.63 (dt, 1H, J = 10.1, 4.9 Hz, H-5), 3.42 (s, 3H, OCH_3), 1.90 (s, 3H, $COCH_3$); ^{13}C NMR (75 MHz, DMSO-d_6) δ 195.98 (C-3), 169.26 ($COCH_3$), 137.04 (aromatic quart.), 129.06, 128.11, 126.22 (aromatic CH), 103.44 (C-1), 100.37 (Ph*C*H), 80.89, 68.16, 65.64 (C-4, C-5, C-6), 60.69 (C-2), 56.39 (OCH_3), 22.44 ($COCH_3$); HR-ESMS calcd for $C_{16}H_{20}NO_6$ ($M+H^+$) 322.1291, found 322.1282.

Methyl 2-acetamido-4,6-O-benzylidene-2-deoxy-3-C-methyl-β-D-glucopyranoside (**31a**). and *Methyl 2-acetamido-4,6-O-benzylidene-2-deoxy-3-C-methyl-β-D-allopyranoside* (**31b**).



The ketone **30** (710 mg, 2.21 mmol) was treated with MeLi in the same fashion as described as for **11** to give **31a** (52 mg, 6%) and **31b** (469 mg, 63%) as white solids.

For **31a**: ^1H NMR (360 MHz, $CDCl_3$) δ 7.52-7.35 (m, 5H, ArH), 5.89 (d, 1H, J = 10.0 Hz, NH), 5.60 (s, 1H, PhC*H*), 4.50 (d, 1H, J = 8.3 Hz, H-1), 4.39 (dd, 1H, J = 10.1, 4.7 Hz, H-6-eq), 4.03 (dd, 1H, J = 10.0, 8.3 Hz,

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

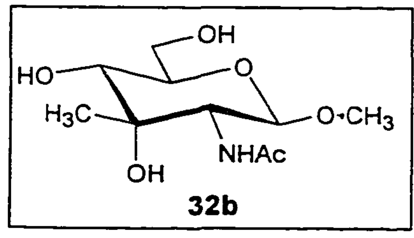
H-2), 3.90 (ddd, 1H, *J* = 10.1, 9.1, 4.7 Hz, H-5), 3.78 (t, 1H, *J* = 10.1, H-6-ax), 3.49 (s, 3H, OCH_3), 3.46 (d, 1H, *J* = 9.1 Hz, H-4), 2.09 (s, 3H, $COCH_3$), 1.30 (s, 3H, 3-*C*-CH_3); HR-ESMS calcd for $C_{17}H_{24}NO_6$ ($M+H^+$) 338.1603, found 338.1593.

For **31b:** ^1H NMR (360 MHz, $CDCl_3$) δ 7.52-7.35 (m, 5H, ArH), 5.61 (d, 1H, *J* = 5.1 Hz, NH), 5.60 (s, 1H, PhC*H*), 4.38 (d, 1H, *J* = 8.5 Hz, H-1), 4.36 (dd, 1H, *J* = 10.1, 5.0 Hz, H-6-eq), 3.79 (t, 1H, *J* = 10.1, H-6-ax), 3.77 (dd, 1H, *J* = 8.5, 5.1 Hz, H-2), 3.70 (d, 1H, *J* = 9.8 Hz, H-4), 3.52 (s, 3H, OCH_3), 3.50 (m, 1H, H-5), 2.11 (s, 3H, $COCH_3$), 1.31 (s, 3H, 3-*C*-CH_3); HR-ESMS calcd for $C_{17}H_{24}NO_6$ ($M+H^+$)

Methyl 2-acetamido-2-deoxy-3-C-methyl-β-D-glucopyranoside (**32a**)

A solution of **31a** (50mg, 0.15 mmol) in 5 mL of HOAc-H_2O (1:1) was stirred at 60 °C for 1.5 h. Co-evaporation with toluene left a residue which was purified on a column of Iatrobeads (6:1 CH2Cl2/MeOH) to give **32a** (30 mg, 81%) as a white solid: ^1H NMR (360 MHz, D_2O) δ 4.51 (d, 1H, *J* = 8.8 Hz, H-1), 3.93 (d, 1H, *J* = 11.1Hz, H-4), 3.84 (d, 1H, *J* = 8.8, H-2), 3.74 (dd, 1H, *J* = 12.2, 4.8 Hz, H-6a), 3.60-3.55 (m, 2H, H-5, H-6b), 3.50 (s, 3H, OCH_3), 2.09 (s, 3H, $COCH_3$), 1.20 (s, 3H, 3-*C*-CH_3); ^{13}C NMR (125 MHz, D_2O) δ 175.54 (*C*OCH_3), 101.52 (C-1), 75.71, 75.70, 73.11 (C-3, C-4, C-5), 62.12 (C-6), 58.85 (C-2), 57.77 (OCH_3), 22.90 (CO*C*H_3), 14.59 (3-*C*-CH_3); HR-ESMS calcd for $C_{10}H_{19}NO_6Na$($M+H^+$) 272.1110, found 272.1110.

Methyl 2-acetamido-2-deoxy-3-C-methyl-β-D-allopyranoside (**32b**).

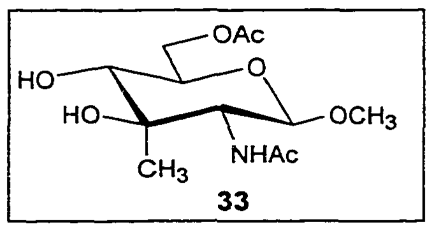


Compound **31b** (102 mg, 0.30 mmol) was deprotected in the same fashion as described above to give **32b** (64 mg, 84%) as a white solid: ^1H NMR (360 MHz, D_2O) δ 4.60

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

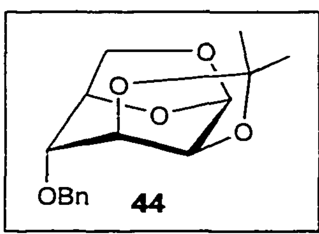
(d, 1H, J = 8.6 Hz, H-1), 3.93 (dd, 1H, J = 15.0, 5.3 Hz, H-6a), 3.78-3.70 (m, 2H, H-5, H-6b), 3.67 (d, 1H, J = 8.6 Hz, H-2), 3.50 (s, 3H, OCH_3), 2.09 (s, 3H, $COCH_3$), 1.20 (s, 3H, 3-C-CH_3); ^{13}C NMR (125 MHz, D_2O) δ 175.33 (COCH$_3$), 101.38 (C-1), 75.33, 74.48, 71.04 (C-3, C-4, C-5), 62.26 (C-6), 57.65 (C-2), 57.19 (OCH_3), 22.75, 22.23 (COCH_3, 3-C-CH_3); HR-ESMS calcd for $C_{10}H_{19}NO_6Na(M+H^+)$ 272.1110, found 272.1111.

Methyl 2-acetamido-4-O-acetyl-2-deoxy-3-C-methyl-β-D-glucopyranoside (**33**).



To a solution of **32a** (25 mg, 0.1 mmol) in CH_2Cl_2 (5 mL) was added acetyl chloride (8.5 μL, 0.12 mmol) and *sym*-collidine (20 μL, 0.15 mmol). After stirring at 0 °C for 4 h, the reaction mixture was concentrated and purified on a column of Iatrobeads (5:1 CH_2Cl_2/MeOH) to give **33** (20 mg, 69%) as a white solid: ^1H NMR (360 MHz, $CDCl_3$) δ 5.75 (d, 1H, J = 4.0 Hz, NH), 4.39 (d, 1H, J = 12.0, 2.4 Hz, H-6a), 4.32 (dd, 1H, J = 12.0, 5.1 Hz, H-6b), 4.29 (d, 1H, J = 8.0 Hz, H-1), 3.62 (d, 1H, J = 9.0 Hz, H-4), 3.59 (dd, 1H, J = 8.0, 4.0 Hz, H-2), 3,52 (ddd, 1H, J = 9.0, 5.1, 2.4 Hz, H-5), 3.47 (s, 3H, OCH_3), 2.09, 2.08 (s, 3H, $OCOCH_3$, NHCOCH_3), 1.18 (s, 3H, 3-C-CH_3).

1,6-Anhydro-4-O-benzyl-2,3-isopropylidene-β-D-mannose (**44**).

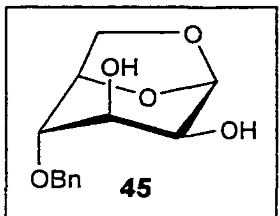


1,6-Anhydro-2,3-isopropylidene-β-D-mannose **43** [81] (5.05 g, 25 mmol) was benzylated in the same fashion as for **16**. The crude product was recrystallized twice in absolute ethanol to give **44** (5.6g, 77%) as a white solid: ^1H NMR (360 MHz, $CDCl_3$) δ 7.40-7.30 (m, 5H), 5.36 (d, 1H, J = 3.0 Hz, H-1), 4.71 (d, 1H, J = 12.0 Hz, PhCH_2), 4.67 (d, 1H, J = 12.0 Hz, PhCH_2), 4.61 (dd, 1H, J = 6.4, 1.4 Hz, H-5), 4.27 (dd,

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

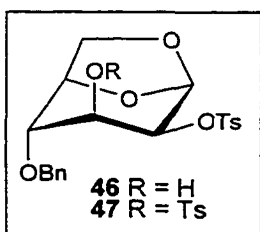
1H, J = 6.3, 0.6 Hz, H-3), 4.11 (dd, 1H, J = 6.3, 3.0 Hz, H-2), 3.91 (dd, 1H, J = 7.0, 1.4 Hz, H-6-endo), 3.73 (dd, 1H, J = 7.0, 6.4 Hz, H-6-exo), 3.68 (d, 1H, J = 0.6 Hz, H-4), 1.55 (s, 3H, CCH_3), 1.35 (s, 3H, CCH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 137.41 (aromatic quart.), 128.59, 128.04, 127.82 (aromatic CH), 109.84 (*C*CH_3) 99.24 (C-1), 76.28, 73.83, 73.46, 72.35 (C-2, C-3, C-4, C-5), 71.65 (Ph*C*H_2), 64.55 (C-6), 26.01 (C*C*H_3), 25.89 (C*C*H_3); HR-ESMS calcd for $C_{16}H_{20}O_5Na$ (M+Na$^+$) 315.1208, found 315.1207.

1,6-Anhydro-4-O-benzyl-β-D-mannose (**45**).



To a solution of **44** (2.8 g, 9.59 mmol) in CH_2Cl_2 (5 mL) was added 6 mL of TFA (95%) at 0 °C. The reaction was stirred for 10 h while it was allowed to warm up to rt. The mixture was concentrated, co-evaporated with toluene and recrystallized in EtOAc to give **45** (1.8 g, 75%) as a white solid: ^{1}H NMR (360 MHz, $CDCl_3$) δ 7.40-7.30 (m, 5H), 5.39 (bs, 1H, H-1), 4.68 (d, 1H, J = 12.2 Hz, PhCH_2), 4.66 (d, 1H, J = 12.2 Hz, PhCH_2), 4.57 (dd, 1H, J = 5.8, 0.6 Hz, H-5), 4.12 (dd, 1H, J = 7.3, 0.6 Hz, H-6-endo), 4.05 (bd, 1H, J = 4.2 Hz, H-2), 3.79 (bd, 1H, J = 4.2 Hz, H-3), 3.72 (dd, 1H, J = 7.3, 5.8, H-6-exo), 3.61 (bs, 1H, H-4); ^{13}C NMR (75 MHz, $CDCl_3$) δ 137.56 (aromatic quart.), 128.58, 128.03, 127.81 (aromatic CH), 101.58 (C-1), 78.40, 74.10, 71.59, 68.80, 66.81 (C-2, C-3, C-4, C-5, PhCH_2), 64.85 (C-6); HR-ESMS calcd for $C_{13}H_{16}O_5Na$ (M+Na$^+$) 275.0895, found 275.0894.

1,6-Anhydro-4-O-benzyl-2-O-p-toluenesulfonyl-β-D-mannose (**46**) and *1,6-Anhydro-4-O-benzyl-2,3-di-O-p-toluenesulfonyl-β-D-mannose* (**47**).



To a solution of **45** (1.58 g, 6.26 mmol) in pyridine (20 mL) was added *p*-toluenesulfonyl chloride at 0 °C. The reaction was stirred

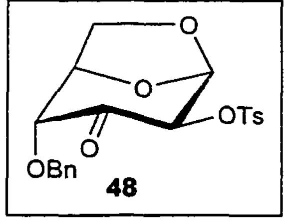
Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

for 3 days while it was allowed to warm up to rt. The mixture was diluted with CH_2Cl_2, washed with H2O, dried over Na2SO4, filtered, concentrated, and fractionated using column chromatography (3: 2 hexanes/EtOAc) to give **46** (1.70 g, 67%) and **47** (0.56 g, 16%) as white solids.

For **46:** ^{1}H NMR (360 MHz, $CDCl_3$) δ 7.83 (m, 2H, ArH), 7.39-7.26 (m, 7H, ArH), 5.27 (bs, 1H, H-1), 4.64 (d, 1H, J = 12.2 Hz, PhCH_2), 4.59 (d, 1H, J = 12.2 Hz, PhCH_2), 4.55-4.51 (m, 2H, H-2, H-5), 4.20 (bd, 1H, J = 7.4 Hz, H-6-endo), 4.13 (dd, 1H, J = 5.0, 1.7 Hz, H-3), 3.68 (dd, 1H, J = 7.4, 5.7 Hz, H-6-exo), 3.62 (bs, 1H, H-4), 2.45 (s, 3H, PhCH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 145.51, 137.28, 132.92 (aromatic quart.), 130.05, 128.51, 127.99, 127.92, 127.72 (aromatic CH), 99.23 (C-1), 78.32, 75.51, 74.36, 71.64, 68.41 (C-2, C-3, C-4, C-5, PhCH_2), 65.11 (C-6), 21.62 (PhCH_3); HR-ESMS calcd for $C_{20}H_{22}O_7NaS$ ($M+Na^+$) 429.0983, found 429.0989.

For **47:** ^{1}H NMR (360 MHz, $CDCl_3$) δ 7.77 (m, 2H, ArH), 7.60 (m, 2H, ArH), 7.39-7.26 (m, 9H, ArH), 5.18 (bs, 1H, H-1), 4.82 (dd, 1H, J = 5.3, 1.5 Hz, H-3), 4.72 (d, 1H, J = 12.2 Hz, PhCH_2), 4.64 (d, 1H, J = 12.2 Hz, PhCH_2), 4.49-4.43 (m, 2H, H-2, H-5), 4.11 (d, 1H, J = 8.0 Hz, H-6-endo), 3.90 (bs, 1H, H-4), 3.68 (dd, 1H, J = 8.0, 5.9 Hz, H-6-exo), 2.44 (s, 3H, PhCH_3), 2.42 (s, 3H, PhCH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 145.37, 145.20, 136.89, 132.66, 132.55 (aromatic quart.), 129.92, 128.55, 128.18, 128.05, 128.01, 127.86 (aromatic CH), 99.64 (C-1), 77.01, 74.49, 74.30, 72.32, 72.01 (C-2, C-3, C-4, C-5, PhCH_2), 65.24 (C-6), 21.62 (PhCH_3), 21.59 (PhCH_3). HR-ESMS calcd for $C_{27}H_{28}O9NaS_2$ ($M+Na^+$) 583.1072, found 583.1076.

1,6-Anhydro-4-O-benzyl-2-O-p-toluenesulfonyl-β-D-arabino-hexopyronos-3-ulose (**48**).

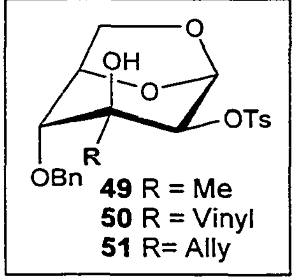


Compound **46** (620 mg, 1.53 mmol), pyridinium dichromate (PDC) (690 mg, 1.84 mmol), and ground 4 Å molecular sieves in CH_2Cl_2 (15 mL) was stirred at rt for 7.5 h. The reaction mixture

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

was filtered through a Celite pad, washed with CH_2Cl_2, concentrated, and fractionated using column chromatography (5:3 hexanes/EtOAc) to give **48** (520 mg, 84%) as a syrup: ^{1}H NMR (360 MHz, $CDCl_3$) δ 7.85 (m, 2H, ArH), 7.39-7.24 (m, 7H, ArH), 5.66 (d, 1H, J = 2.2 Hz, H-2), 5.19 (d, 1H, J = 2.2 Hz, H-1), 4.78 (m, 1H, H-5), 4.57 (d, 1H, J = 12.0 Hz, PhCH_2), 4.38 (d, 1H, J = 12.0 Hz, PhCH_2), 3.82 (dd, 1H, J = 8.5, 5.6 Hz, H-6-exo), 3.75 (d, 1H, J = 2.0 Hz, H-4), 3.68 (dd, 1H, J = 8.5, 1.0 Hz, H-6-endo), 2.45 (s, 3H, PhCH_3).^{13}C NMR (75 MHz, $CDCl_3$) δ 195.61 (C-3), 145.42, 135.99, 133.01 (aromatic quart.), 129.87, 128.68, 128.46, 128.30, 128.19 (aromatic CH), 101.77 (C-1), 82.53, 79.84, 76.60 (C-2, C-4, C-5), 71.91 (PhCH_2), 65.68 (C-6), 21.72 (PhCH_3).

1,6-Anhydro-4-O-benzyl-3-C-methyl-2-O-p-toluenesulfonyl-β-D-mannose (**49**), *1,6-Anhydro-4-O-benzyl-3-C-vinyl-2-O-p-toluenesulfonyl-β-D-mannose* (**50**) and *1,6-Anhydro-4-O-benzyl-3-C-allyl-2-O-p-toluenesulfonyl-β-D-mannose* (**51**).



The ketone compound **48** was treated with methylmagnesium bromide, vinylmagnesium bromide, or allylmagnesium bromide in the same fashion as described for **20** to give **49** (74%), **50** (70%) or **51** (72%).

For **49:** ^{1}H NMR (360 MHz, $CDCl_3$) δ 7.85 (m, 2H, ArH), 7.39-7.24 (m, 7H, ArH), 5.28 (d, 1H, J = 1.8 Hz, H-1), 4.66 (d, 1H, J = 11.8 Hz, PhCH_2), 4.60 (d, 1H, J = 11.8 Hz, PhCH_2), 4.53 (dd, 1H, J = 5.7, 1.7 Hz, H-5), 4.33 (d, 1H, J = 1.8 Hz, H-2), 4.26 (bd, 1H, J = 7.2 Hz, H-6-endo), 3.66 (dd, 1H, J = 7.2, 5.7 Hz, H-6-exo), 3.75 (d, 1H, J = 1.7 Hz, H-4), 2.45 (s, 3H, PhCH_3), 1.15 (3-*C*-CH_3).

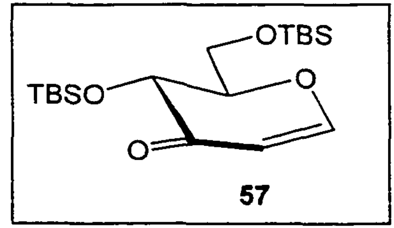
For **50:** ^{1}H NMR (360 MHz, $CDCl_3$) δ 7.75 (m, 2H, ArH), 7.39-7.24 (m, 7H, ArH), 5.80 (dd, 1H, J = 17.0, 10.6 Hz, CH_2=CH), 5.40 (dd, 1H, J = 17.0, 1.4 Hz, CH_2=CH), 5.39 (d, 1H, J = 2.0 Hz, H-1), 5.08 (dd, 1H, J = 10.6, 1.4 Hz, CH_2=CH), 4.61 (d, 1H, J = 12.0 Hz, PhCH_2), 4.52 (d, 1H, J = 12.0 Hz, PhCH_2), 4.46 (m, 1H, H-5), 4.44

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

(d, 1H, J = 2.0 Hz, H-2), 4.30 (bd, 1H, J = 7.2 Hz, H-6-endo), 3.66 (dd, 1H, J = 7.2, 5.8 Hz, H-6-exo), 3.38 (d, 1H, J = 1.8 Hz, H-4), 2.45 (s, 3H, PhCH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 145.43 (aromatic quart.), 138.88 (CH_2=CH), 137.21, 132.95 (aromatic quart.), 129.89, 128.54, 128.34, 128.10 (aromatic CH), 116.47 ($C$$H_2$=CH), 99.17 (C-1), 82.46, 78.10, 75.25 (C-2, C-4, C-5), 74.84 (C-3), 73.23 (Ph$C$$H_2$), 65.60 (C-6), 21.73 (Ph$C$$H_3$); HR-ESMS calcd for $C_{22}H_{24}O_7NaS$ (M+Na^+) 455.1140, found 455.1145.

For **51:** ^{1}H NMR (360 MHz, $CDCl_3$) δ 7.83 (m, 2H, ArH), 7.39-7.26 (m, 7H, ArH), 5.81 (dddd, 1H, H-c allyl), 5.28 (d, 1H, J = 1.8 Hz, H-1), 5.14 (dddd, 1H, H-a allyl), 5.08 (dddd, 1H, H-b allyl), 4.66 (d, 1H, J = 11.3 Hz, PhCH_2), 4.62 (bd, 1H, J = 5.4 Hz, H-5), 4.52 (d, 1H, J = 11.3 Hz, PhCH_2), 4.39 (d, 1H, J = 1.8 Hz), 4.30 (bd, 1H, J = 7.1 Hz, H-6-endo), 3.68 (dd, 1H, J = 7.1, 5.8 Hz, H-6-exo), 3.48 (d, 1H, J = 1.5 Hz, H-4), 2.70 (bs, 1H, OH), 2.45 (s, 3H, PhCH_3), 2.43 (dddd, 1H, H-d allyl), 2.20 (dddd, 1H, H-e allyl); ^{13}C NMR (75 MHz, $CDCl_3$) δ 145.54, 137.31, 133.23 (aromatic quart.), 130.14 (CH_2=CHCH_2), 130.09, 128.48, 127.98, 127.96, 127.73 (aromatic CH), 99.21 (C-1), 79.95, 78.96, 74.84, 73.22 (C-2, C-3, C-4, C-5) 72.05 (PhCH_2), 65.45 (C-6), 40.62 (CH_2=CH$C$$H_2$), 21.67 (Ph$C$$H_3$); HR-ESMS calcd for $C_{23}H_{26}O_7NaS$ (M+Na^+) 469.1297, found 469.1300.

1,5-Anhydro-3-O-tert-butyldimethylsilyl-2-deoxy-D-erythro-hex-1-en-3-ulose (**57**).

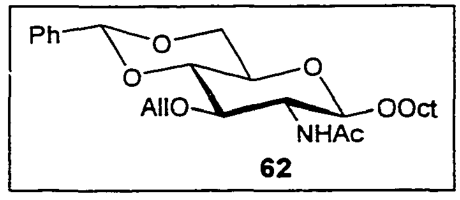


To a solution of **56** (0.70 g, 4.86 mmol) in DMF (15 mL) was added tert-butyldimethylsilyl chloride (TBS-Cl) (1.85g, 12.2 mmol) and imidazole (1.2 g, 17.6 mmol). The reaction was stirred at rt for 14 h. The mixture was concentrated and fractionated using column chromatography (6:1 hexanes/EtOAc) to give **57** (1.2g, 78 %) as a white solid: ^{1}H NMR (360 MHz, $CDCl_3$) δ 7.28 (d, 1H, J = 5.8 Hz, H-1), 5.30 (d, 1H, J = 5.8 Hz, H-2), 4.43 (d, 1H, J = 12.2 Hz, H-4), 4.17 (dt, 1H, J = 12.2,

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

2.7 Hz, H-5), 3.98 (d, 2H, J = 2.7 Hz, H-6a, H-6b), 0.90 (bs, 18H, C(CH_3)$_3$), 0.21 (s, 3H, $SiCH_3$), 0.08 (s, 9H, $SiCH_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 194.06 (C-3), 162.05 (C-1), 104.56 (C-2), 83.73 (C-4), 69.49 (C-5), 61.55 (C-6), 25.92 (C(CH_3)$_3$), 18.53, 18.46 (*C*(CH_3)$_3$), -3.96, -5.14, -5.24, -5.62 ($SiCH_3$); HR-ESMS calcd for $C_{18}H_{36}O_4NaSi_2$ (M+Na$^+$) 395.2050, found 395.2049.

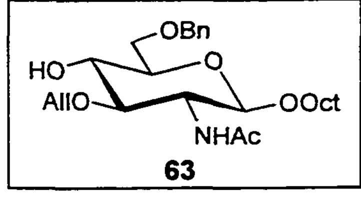
Octyl 2-acetamido-3-O-allyl-4,6-O-benzylidene-2-deoxy-β-D-glucopyranoside (**62**).



To a solution of **61** (3.03 g, 7.19 mmol) in DMF (45 mL) was added allyl bromide (2.4 mL, 28.8 mmol) and sodium hydride (380 mg, 60% dispersion in mineral oil, 9.35 mmol) at 0 °C. The reaction was stirred for 12 h while it was allowed to warm up to rt. After quenching with MeOH, the precipitate was collected by filtration to give **62** (2.70 g, 81%) as a white solid: ^{1}H NMR (360 MHz, $CDCl_3$) δ 7.50-7.30 (m, 5H), 5.88 (dddd, 1H, H-c allyl), 5.71 (d, 1H, J = 7.4 Hz, NH), 5.54 (s, 1H, PhC*H*), 5.24 (dddd, 1H, H-b allyl), 5.14 (dddd, 1H, H-a allyl), 5.08 (d, 1H, J = 8.4 Hz, H-1), 4.39-4.27 (m, 3H, H-3, H-6-eq, H-d allyl), 4.13 (dddd, 1H, H-e allyl), 3.83 (dt, 1H, J = 9.6, 6.4 Hz, OCH_2CH$_2$), 3.77 (t, 1H, J = 10.0 Hz, H-6-ax), 3.61-3.47 (m, 3H, H-4, H-5, OCH_2CH$_2$), 3.11 (m, 1H, H-2), 2.01 (s, 3H, $COCH_3$), 1.52 (m, 2H, OCH$_2$CH_2), 1.36-1.20 (m, 10H, CH_2 octyl), 0.85 (t, 3H, J = 7.0 Hz, CH_3 octyl); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.93 (*C*OCH$_3$), 137.41 (aromatic quart.), 134.93 (CH$_2$=C*H*CH$_2$O), 128.91, 128.24, 128.20, 126.03, 125.99 (aromatic CH), 117.03 (*C*H$_2$=CHCH$_2$O), 101.19 (C-1), 82.66 (C-4), 76.44 (C-3), 73.56 (CH$_2$=CH*C*H$_2$O), 70.29 (PhC*H*$_2$), 68.81 (C-6), 65.93 (C-5), 58.49 (C-2), 31.80, 29.56, 29.30, 29.25, 25.90, 23.13, 22.63 (CH_2 octyl, CO*C*H$_3$), 14.06 (CH_3 octyl); HR-ESMS calcd for $C_{26}H_{39}NO_6Na$ (M+Na$^+$) 484.2675, found 484.2678.

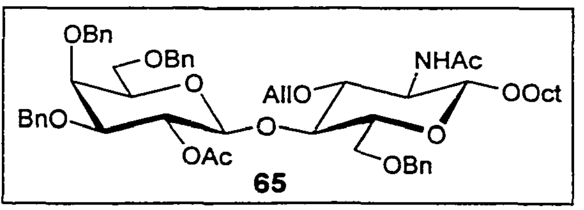
Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Octyl 2-acetamido-3-O-allyl-6-O-benzyl-2-deoxy-β-D-glucopyranoside (**63**).



A mixture of compound **62** (1.50 g, 3.25 mmol), sodium cyanoborohydride (2.25 g, 35.8 mmol), 3 Å molecular sieves (2 g), and a crystal of methyl orange in THF (100 mL) was stirred at 0 °C under argon. Diethyl ether saturated with HCl was added until a persistent pink color was observed. Stirring at 0 °C was continued for 2h and finally at rt overnight. The reaction was diluted with CH_2Cl_2 and poured into aqueous $NaHCO_3$. The organic layer was washed with water and brine, dried (Na_2SO_4), concentrated. The crude product was dissolved in CH_2Cl_2/MeOH (1:1) and deionized with Amberlite MB-1 ion-exchanger resin. The resin was filtered and the solution was concentrated. The resulting residue was purified on a column of Iatrobeads (20:1 CH_2Cl_2/MeOH) to give **63** (0.90 g, 60%) as a white solid: ^{1}H NMR (360 MHz, $CDCl_3$) δ 7.40-7.30 (m, 5H), 5.90 (dddd, 1H, H-c allyl), 5.68 (d, 1H, J = 7.4 Hz, NH), 5.54 (s, 1H, PhC*H*), 5.26 (dddd, 1H, H-a allyl), 5.16 (dddd, 1H, H-b allyl), 4.91 (d, 1H, J = 8. Hz, H-1), 4.60 (d, 1H, J = 12.0 Hz, PhCH_2), 4.57 (d, 1H, J = 12.0 Hz, PhCH_2), 4.26 (dddd, 1H, H-d allyl), 4.18 (dddd, 1H, H-e allyl), 3.97 (dd, 1H, J = 10.3, 8.1 Hz, H-3), 3.82 (dt, 1H, J = 9.6, 6.5 Hz, OCH_2CH_2), 3.75 (d, 2H, J = 4.5 Hz, H-6a, H-6b), 3.62-3.51 (m, 2H, H-4, H-5), 3.46 (dt, 1H, J = 9.6, 6.5 Hz, OCH_2CH_2), 3.15 (m, 1H, H-2), 1.98 (s, 3H, $COCH_3$), 1.52 (m, 2H, $OCH_2C$$H_2$), 1.36-1.20 (m, 10H, CH_2 octyl), 0.85 (t, 3H, J = 7.0 Hz, CH_3 octyl); HR-ESMS calcd for $C_{26}H_{41}NO_6Na$ (M+Na$^+$) 486.2832, found 486.2838.

Octyl 2-O-acetyl-3,4,6-tri-O-benzyl-β-D-galactopyranosyl-(1→4)-2-acetamido-3-O-allyl-6-O-benzyl-2-deoxy-β-D-glucopyranoside (**65**).



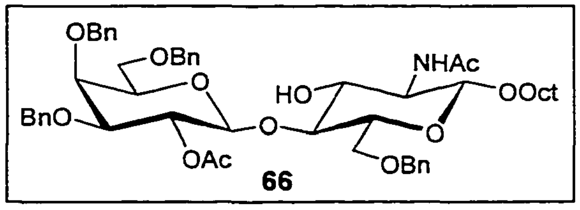
To a stirred solution of **63** (200 mg, 0.43 mmol) and **64** (345 mg, 0.64 mmol) in

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

anhydrous CH_2Cl_2 (10 mL) containing ground 4 Å molecular sieves was added silver triflate (232 mg, 0.90 mmol) at -30 °C with stirring under argon. The reaction mixture was stirred for 4 h while it was allowed to warm up to 0 °C. 10% sodium thiosulfate was added and the mixture was filtered through Celite and washed with CH_2Cl_2. The organic layer was washed with water and brine, dried (Na_2SO_4), concentrated and fractionated using column chromatography (3:2 hexanes/EtOAc) to give **65** (660 mg, 81%) as a white solid: ^1H NMR (360 MHz, $CDCl_3$) δ 7.40-7.20 (m, 20H, ArH), 6.27 (d, 1H, J = 9.0 Hz, NH), 5.82 (dddd, 1H, H-c allyl), 5.29 (dd, 1H, J=10.0, 8.0 Hz, H-2'), 5.20 (dddd, 1H, H-a allyl), 5.07 (dddd, 1H, H-b allyl), 4.93 (d, 1H, J = 11.6 Hz, PhCH_2), 4.67(d, 1H, J = 12.2 Hz, PhCH_2), 4.60 (d, 1H, J = 11.6 Hz, PhCH_2), 4.56 (d, 1H, J = 4.4 Hz, H-1), 4.56 (d, 1H, J = 12.2 Hz, PhCH_2), 4.52 (d, 1H, J = 11.6 Hz, PhCH_2), 4.49 (d, 1H, J = 11.6 Hz, PhCH_2), 4.46 (d, 1H, J = 11.6 Hz, PhCH_2), 4.43 (d, 1H, J = 11.6 Hz, PhCH_2), 4.35 (d, 1H, J = 8.0 Hz, H-1'), 4.13 (dddd, 1H, H-d allyl), 4.07 (dddd, 1H, H-e allyl), 4.01-3.44 (m, 12H, H-2, H-3, H-4, H-5, H-6a, H-6b, H-3', H-4', H-5', H-6'a, H-6'b, OCH_2CH_2), 3.35 (dt, J = 9.4, 7.0 Hz, OCH_2CH_2), 2.01 (S, 3H, $COCH_3$), 1.98 (s, 3H, $COCH_3$), 1.52 (m, 2H, OCH_2CH_2), 1.36-1.20 (m, 10H, CH_2 octyl), 0.88 (t, 3H, J = 7.0 Hz, CH_3 octyl); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.37, 170.14 ($COCH_3$), 138.37, 138.26, 137.91, 137.78 (aromatic quart.), 134.77 (CH_2=$CHCH_2O$), 128.46, 128.44, 128.36, 128.22, 127.89, 127.81, 127.76, 127.65, 127.46 (aromatic CH), 116.43 (CH_2=$CHCH_2O$), 100.14, 99.85 (C-1, C-1'), 79.81, 76.30, 74.60, 74.25, 73.78, 73.65, 73.60, 73.42, 72.60, 72.13, 71.81 (C-3, C-4, C-5, C-2', C-3', C-4', C-5', PhCH_2 × 4), 71.15, 69.96 (CH_2=$CHCH_2O$, PhCH_2), 69.27, 68.19 (C-6, C-6'), 50.23 (C-2), 31.83, 29.55, 29.42, 29.27, 26.07, 23.22, 22.66, 21.10 ($COCH_3$ × 2, CH_2 octyl), 14.10 (CH_3 octyl); HR-ESMS calcd for $C_{55}H_{71}NO_{12}Na$ ($M+Na^+$) 960.4874, found 960.4869.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

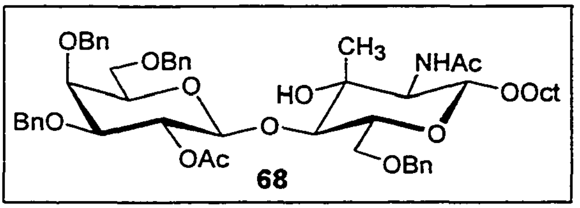
Octyl 2-O-acetyl-3,4,6-tri-O-benzyl-β-D-galactopyranosyl-(1→4)-2-acetamido-6-O-benzyl-2-deoxy-β-D-glucopyranoside (**66**).



Compound **65** (220 mg, 0.23 mmol) was treated with $PdCl_2$ in the same fashion as described for **17**. Purification by column chromatography (4:1 hexanes/EtOAc) gave **66** (202 mg, 96%) as a whit solid: ^{1}H NMR (360 MHz, $CDCl_3$) δ 7.40-7.20 (m, 20H, ArH), 5.50 (d, 1H, J = 7.9 Hz, NH), 5.33 (dd, 1H, J = 10.0, 8.0 Hz, H-2'), 4.90 (d, 1H, J = 11.6 Hz, PhCH_2), 4.70 (d, 1H, J = 8.0 Hz, H-1), 4.66 (d, 1H, J = 12.2 Hz, PhCH_2), 4.65 (d, 1H, J = 12.2 Hz, PhCH_2), 4.53 (d, 1H, J = 11.6 Hz, PhCH_2), 4.49 (d, 1H, J = 12.2 Hz, PhCH_2), 4.48 (d, 1H, J = 12.2 Hz, PhCH_2), 4.41 (d, 1H, J = 11.6 Hz, PhCH_2), 4.38 (d, 1H, J = 11.6 Hz, PhCH_2), 4.35 (d, 1H, J = 8.0 Hz, H-1'), 3.95 (dd, 1H, J = 9.8, 7.5 Hz, H-3), 3.88 (d, 1H, J = 2.2 Hz, H-4'), 3.83 (dt, 1H, J = 9.6, 6.5 Hz, OC$H_2$$CH_2$), 3.70-3.40 (m, 10H, H-2, H-4, H-5, H-6a, H-6b, H-3', H-5', H-6'a, H-6'b, OC$H_2$$CH_2$), 1.94 (bs, 6H, $COCH_3$), 1.54 (m, 2H, OCH_2CH_2), 1.34-1.20 (m, 10H, CH_2 octyl), 0.86 (t, 3H, J = 7.0 Hz, CH_3 octyl).^{13}C NMR (75 MHz, $CDCl_3$) δ 170.20, 169.39 (*C*OCH_3), 138.47, 138.19, 137.78, 137.56 (aromatic quart.), 128.54, 128.39, 128.33, 128.22, 128.02, 127.94, 127.75, 127.70, 127.63, 127.52 (aromatic CH), 101.68, 100.30 (C-1, C-1'), 81.20, 80.23, 74.59, 74.20, 73.80, 73.66, 73.57, 72.34, 72.19, 71.58, 71.27 (C-3, C-4, C-5, C-2', C-3', C-4', C-5', Ph*C*H_2 × 4), 69.65 (O*C*$H_2$$CH_2$), 68.58, 68.31 (C-6, C-6'), 56.7 (C-2), 31.87, 29.59, 29.40, 29.30, 26.01, 23.66, 22.69, 21.00 ($COCH_3$ × 2, CH_2 octyl), 14.13 (CH_3 octyl); HR-ESMS calcd for $C_{52}H_{67}NO_{12}Na$ (M+Na^+) 920.4561, found 920.4593.

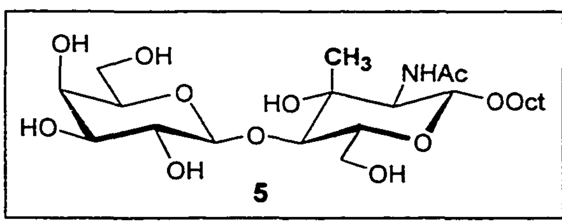
Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Octyl 2-O-acetyl-3,4,6-tri-O-benzyl-β-D-galactosylpyranosyl-(1→4)-2-acetamido-3-C-methyl-6-O-benzyl-2-deoxy-β-D-glucopyranoside (**68**).



A solution of **66** (170 mg, 0.19 mmol) in DMSO (3 mL) and acetic anhydride (1.5 mL) was stirred at rt for 4h. The mixture was concentrated under high vacuum and fractionated using column chromatography (5:2 toluene/acetone). To the resulting crude ketone product (100 mg, 0.11 mmol) in THF (5 mL) was added MeLi (1.4 M, 0.24 mL) in diethyl ether at -78 °C under argon with stirring. The mixture was worked up as described for **11**. Purification by column chromatography (5:2 hexanes/acetone) gave **68** (35 mg, 20% for two steps) as a film: ^{1}H NMR (360 MHz, $CDCl_3$) δ 7.40-7.20 (m, 20H, ArH), 6.05 (d, 1H, J = 9.50 Hz, NH), 5.28 (dd, 1H, J = 10.0, 8.0 Hz, H-2'), 4.88 (d, 1H, J = 11.6 Hz, $PhCH_2$), 4.63 (d, 1H, J = 12.2 Hz, $PhCH_2$), 4.35-4.60 (m, 7H, H-1, $PhCH_2$), 4.25 (d, 1H, J = 8.0 Hz, H-1'), 3.98 (dd, 1H, J = 9.50, 4.90 Hz, H-2), 3.90 (d, 1H, J = 2.1 Hz, H-4'), 3.77 (dt, 1H, J = 9.5, 6.5 Hz, OCH_2CH_2), 3.40-3.72 (m, 9H, H-4, H-5, H-6a, H-6b, H-3', H-5', H-6'a, H-6'b, OCH_2CH_2), 2.04 (s, 3H, $COCH_3$), 1.99 (s, 3H, $COCH_3$), 1.50 (m, 2H, OCH_2CH_2), 1.32-1.15 (m, 10H, CH_2 octyl), 0.85 (t, 3H, J = 7.0 Hz, CH_3 octyl).

Octyl β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-3-C-methyl-β-D-glucopyranoside (**5**).

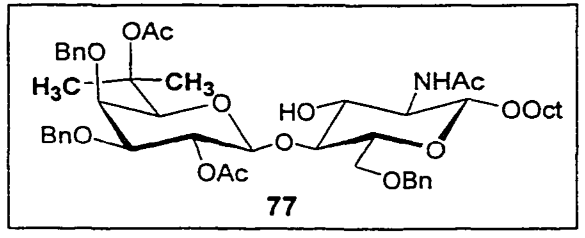


To a solution of **68** (8 mg, 8.77 μmol) in MeOH (3 mL) was added a freshly prepared methanolic solution of NaOMe (0.5 M, 0.3 mL). The mixture was stirred at rt for 27 h,

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

and neutralized with Amberlite IR-120 (H^+), filtered and concentrated. The resulting product was hydrogenolyzed as described for **1a** to yield **5** (3 mg, 67 % for two steps) as a white solid: ^1H NMR (500 MHz, D_2O) δ 4.55 (d, 1H, J = 8.8 Hz, H-1), 4.47 (d, 1H, J = 7.8 Hz, H-1'), 3.96 (dd, 1H, J = 12.1, 2.2 Hz, H-6a), 3.92 (d, 1H, J = 3.2 Hz, H-4'), 3.89 (m, 1H, OCH_2CH_2), 3.87 (d, 1H, J =8.8 Hz, H-2), 3.81 (dd, 1H, J = 12.0, 5.5 Hz, H-6b), 3.78-3.70 (m, 4H, H-4, H-5', H-6'a, H-6'b), 3.66 (dd, 1H, J = 10.0, 3.2 Hz, H-3'), 3.63 (m, 1H, H-5), 3.59-3.54 (m, 2H, H-2', OCH_2CH_2), 2.05 (s, 3H, $COCH_3$), 1.54 (m, 2H, OCH_2CH_2), 1.34-1.22 (m, 13H, 3-C-CH_3, CH_2 octyl), 0.86 (t, 3H, J = 7.0 Hz, CH_3 octyl); HR-ESMS calcd for $C_{23}H_{44}NO_{11}$ ($M+H^+$) 510.2914, found 510.2915.

Octyl 2,6-di-O-acetyl-3,4-di-O-benzyl-6,6'-di-C-methyl-β-D-galacto-pyranosyl-(1→4)-2-acetamido-6-O-benzyl-2-deoxy-β-D-gluco-pyranoside (**77**).

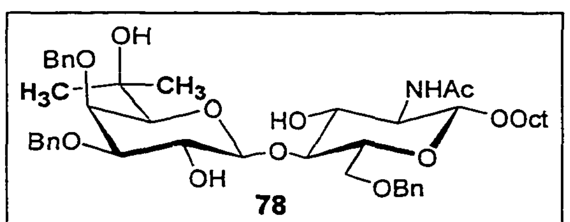


To a stirred solution of **63** (200 mg, 0.43 mmol) and **76** (345 mg, 0.64 mmol) in anhydrous CH_2Cl_2 (10 mL) containing ground 4 Å molecular sieves was added silver triflate (232 mg, 0.90 mmol) at -30 °C with stirring under argon. The reaction mixture was stirred for 4 h while it was allowed to warm up to 0 °C. 10% sodium thiosulfate was added and the mixture was filtered through Celite and washed with CH_2Cl_2. The organic layer was washed with water and brine, dried (Na_2SO_4), concentrated and fractionated using column chromatography (5:2 toluene/acetone) to yield the partially purified disaccharide. Removal of allyl group in the same fashion as for **16**. Purification by column chromatography (1:1 hexanes/EtOAc) gave **77** (192 mg, 51 % for two steps) as a white solid: ^1H NMR (360 MHz, $CDCl_3$) δ 7.40-7.20 (m, 15H, ArH), 5.55 (d, 1H, J = 8.0 Hz, NH), 5.37 (dd, 1H, J = 10.1, 8.0 Hz, H-2'), 4.98 (d, 1H, J = 10.8 Hz, $PhCH_2$), 4.70 (m, 3H, H-1, $PhCH_2$), 4.57 (d, 1H, J = 10.0

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Hz, PhCH_2), 4.51 (d, 1H, J = 12.1 Hz, PhCH_2), 4.50 (d, 1H, J = 11.6 Hz, PhCH_2), 4.35 (d, 1H, J = 8.0 Hz, H-1'), 4.08 (s, 1H, OH), 4.00-3.88 (m, 3H, H-5, H-6a, H-4'), 3.83 (dt, 1H, J = 10.0, 6.6 Hz, OCH_2CH$_2$), 3.73-3.38 (m, 7H, H-2, H-3, H-4, H-6b, H-3', H-5', OCH_2CH$_2$), 1.97 (s, 6H, COCH$_3$), 1.95 (s, 3H, COCH$_3$), 1.55 (m, 2H, OCH$_2$CH_2), 1.48 (s, 3H, 6'-*C*-CH$_3$), 1.44 (s, 3H, s, 3H, 6'-*C*-CH$_3$), 1.35-1.20 (m, 10H, CH$_2$ octyl), 0.87 (t, 3H, J = 7.0 Hz, CH$_3$ octyl); ^{13}C NMR (75 MHz, CDCl$_3$) δ 170.80, 170.25, 169.39 (*C*OCH$_3$), 138.38, 137.67 (2 × C) (aromatic quart.), 128.56, 128.44, 128.35, 128.32, 127.72, 127.67, 127.50 (aromatic CH), 101.99, 100.32 (C-1, C-1'), 82.99 (C-6'), 81.50, 81.14, 74.37, 74.14, 73.86, 73.55, 74.94, 72.90, 71.58, 71.18 (C-3, C-4, C-5, C-2', C-3', C-4', C-5', Ph*C*H$_2$ × 3), 69.63 (O*C*H$_2$CH$_2$), 68.25 (C-6), 56.76 (C-2), 31.84, 29.58, 29.37, 29.29, 25.98, 24.26, 23.68, 22.94, 22.66, 22.42, 20.95 (6'-*C*-CH$_3$ × 2, CO*C*H$_3$ × 3, CH$_2$ octyl), 14.10; HR-ESMS calcd for $C_{49}H_{67}NO_{13}Na$ (M+Na$^+$) 900.4510 found 900.4497.

Octyl 3,4-di-O-benzyl-6,6'-di-C-methyl-β-D-galactopyranosyl-(1→4)-2-acetamido-6-O-benzyl-2-deoxy-β-D-gluco-pyranoside (**78**).

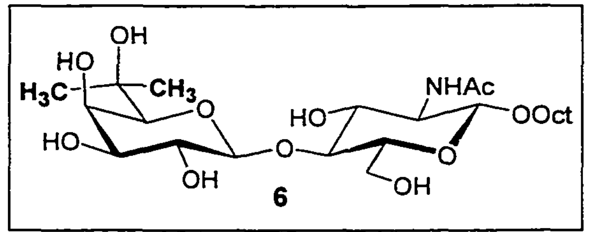


To a solution of **77** (50 mg, 0.057 mmol) in MeOH (10 mL)was added a freshly prepared methanolic solution of NaOMe (0.5 M, 0.4 mL). The mixture was stirred at rt for 48 h, and neutralized with Amberlite IR-120 (H$^+$), filtered, concentrated and fractionated using column chromatography (3:2 hexanes/acetone) to give **78** (35 mg, 78%) as a white solid: ^{1}H NMR (360 MHz, CDCl$_3$) δ 7.40-7.20 (m, 15H, ArH), 5.70 (d, 1H, J = 6.5 Hz, NH), 5.10 (d, 1H, J = 10.7 Hz, PhCH_2), 4.87-4.76 (m, 3H, H-1, PhCH_2), 4.64 (d, 1H, J = 12.2 Hz, PhCH_2), 4.60 (d, 1H, J = 11.1 Hz, PhCH_2), 4.57 (d, 1H, J = 12.5 Hz, PhCH_2), 4.31 (d, 1H, J = 7.7 Hz, H-1'), 4.10-3.24 (m, 12H, H-2, H-3, H-4, H-5, H-6a, H-6b, H-2', H-

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

3', H-4', H-5', OCH_2CH$_2$), 3.18 (bs, 1H, OH), 3.06 (s, 1H, OH), 1.98 (s, 3H, COCH_3), 1.58 (m, 2H, OCH$_2$CH_2), 1.35-1.22 (m, 10H, CH$_2$ octyl), 1.19 (s, 3H, 6'-*C*-CH$_3$), 1.18 (s, 3H, s, 3H, 6'-*C*-CH$_3$), 0.87 (t, 3H, J = 7.0 Hz, CH$_3$ octyl); HR-ESMS calcd for $C_{45}H_{63}NO_{11}Na$ (M+Na$^+$) 816.4299, found 816.4299.

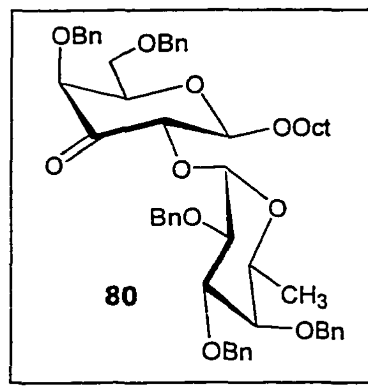
Octyl 6,6'-di-C-methyl-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (**6**).



Compound **78** (28 mg, 0.035 mmol) was treated in the same fashion as described for **1a** to yield **6** (17 mg, 92%) as a white solid: ^{1}H NMR (600 MHz, D$_2$O) δ 4.53 (m, 1H, H-1, virtual order), 4.48 (d, 1H, J = 7.2 Hz, H-1'), 4.13 (d, 1H, J = 3.0 Hz, H-4'), 4.00 (dd, J =12.2, 2.1 Hz, H-6a), 3.90 (dt, 1H, J = 10.0, 7.0 Hz, OCH_2CH$_2$), 3.84 (dd, 1H, J =12.2, 5.0 Hz, H-6b), 3.77-3.70 (m, 3H, H-2, H-3, H-4), 3.65-3.52 (m, 4H, H-5, H-2', H-3', OCH_2CH$_2$), 3.40 (s, 1H, H-5'), 2.04 (s, 3H, COCH$_3$), 1.55 (m, 2H, OCH$_2$CH_2), 1.36-1.22 (m, 16H, 6'-*C*-CH$_3$ × 2, CH$_2$ octyl), 0.87 (t, 3H, J = 7.0 Hz, CH$_3$ octyl); ^{13}C NMR (125 MHz, D$_2$O) δ 175.21 (*C*OCH$_3$), 103.89, 101.90 (C-1, C-1'), 80.10, 79.01, 75.68, 73.87, 73.30, 73.27, 71.80, 71.40, 69.40 (C-3, C-4, C-5, C-2', C-3', C-4', C-5', C-6', OCH$_2$CH$_2$), 60.89 (C-6), 56.03 (C-2), 31.91, 29.38, 29.29, 29.15, 26.49, 25.91, 25.89, 23.06, 22.82 (6'-*C*-CH$_3$ × 2, CO*C*H$_3$, CH$_2$ octyl), 14.20 (CH$_3$ octyl); HR-ESMS calcd for $C_{24}H_{46}NO_{11}$ (M+H$^+$) 424.3071, found 524.3084.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

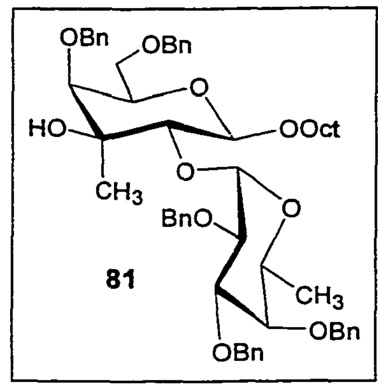
Octyl 2,3,4-tri-O-benzyl-α-L-fucopyranosyl-(1→2)-4,6-di-O-benzyl--β-D-xylo-hexopyranosid-3-ulose (**80**).



Compound **79** [55] (135 mg, 0.15 mmol) was oxidized with Dess-Martin periodinane in the same fashion as for **10**. The mixture was purified by column chromatography (24:1 toluene/EtOAc) to yield **80** (81 mg, 61%) as a syrup: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.50-7.20 (m, 25H, ArH), 5.05 (d, 1H, J = 3.7 Hz, H-1'), 4.99 (d, 1H, J = 11.5 Hz, PhCH_2), 4.98 (d, 1H, J = 11.5 Hz, PhCH_2), 4.92 (d, 1H, J = 11.6 Hz, PhCH_2), 4.73 (d, 1H, J = 11.6 Hz, PhCH_2), 4.70 (d, 1H, J = 7.9 Hz, H-2), 4.69 (d, 1H, J = 11.5 Hz, PhCH_2), 4.64 (d, 1H, J = 11.6 Hz, PhCH_2), 4.60 (d, 1H, 7.9 Hz, H-1), 4.54 (d, 1H, J = 11.8 Hz, PhCH_2), 4.52 (d, 1H, J = 11.8 Hz, PhCH_2), 4.46 (d, 1H, J = 11.8 Hz, PhCH_2), 4.41 (d, 1H, J = 11.8 Hz, PhCH_2), 4.21 (bq, 1H, J = 6.6 Hz, H-5'), 4.10 (dd, 1H, J = 10.2, 3.5 Hz, H-2'), 3.96 (dd, 1H, J = 10.2, 2.9 Hz, H-3'), 3.90 (dt, 1H, J = 9.4, 6.4 Hz, OCH_2CH_2), 3.87 (d, 1H, J = 1.4 Hz, H-4), 3.78-3.72 (m, 2H, H-6a, H-6b), 3.70 (m, 1H, H-5), 3.61 (bd, 1H, J = 2.9 Hz, H-4'), 3.42 (dt, 1H, J = 9.4, 6.4 Hz), 1.52 (m, 2H, OCH_2CH_2), 1.30-1.18 (m, 10H, CH_2 octyl), 1.08 (d, 3H, J = 6.6 Hz, H-6'), 0.86 (t, 3H, J = 7.0 Hz, CH_3 octyl); ^{13}C NMR (125 MHz, $CDCl_3$) δ 203.44 (C-3), 139.23, 138.78, 138.47, 137.88, 136.56 (aromatic quart.), 128.65, 128.57, 128.54, 128.47, 128.39, 128.33, 128.31, 128.30, 128.19, 127.84, 127.73, 127.62, 127.54, 127.45, 127.42 (aromatic CH), 102.58 (C-1), 95.95 (C-1'), 80.48, 79.07, 78.30, 77.63, 76.13, 74.86, 73.78, 73.70, 73.51, 72.58, 72.57 (C-2, C-4, C-5, C-2', C-3', C-4', PhCH_2 × 5), 70.09 (O$CH_2$$CH_2$), 67.83 (C-6), 66.79 (C-5'), 31.89, 29.73, 29.46, 29.36, 26.22, 22.69 (CH_2 octyl), 16.46 (C-6'), 14.13 (CH_3 octyl); HR-ESMS calcd for $C_{55}H_{66}O_{10}Na$ (M+Na$^+$) 909.4554, found 909.4547.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

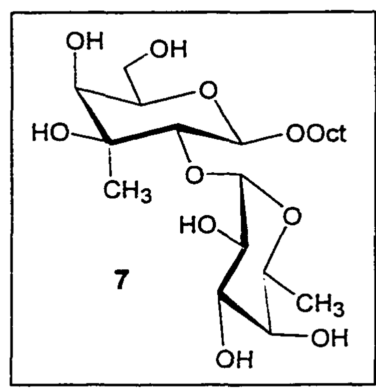
Octyl 2,3,4-tri-O-benzyl-α-L-fucopyranosyl-(1→2)-4,6-di-O-benzyl-3-C-methyl-β-D-galactopyranoside (**81**).



Compound **80** (50 mg, 56 μmol) was treated with MeLi in the same fashion as for **11**. The crude product was purified by column chromatography (25:1 toluene/EtOAc) to yield **81** (28 mg, 55%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.40-7.20 (m, 25H, ArH), 5.33 (d, 1H, J = 3.5 Hz, H-1'), 4.94 (d, 1H, J = 11.6 Hz, $PhCH_2$), 4.82 (d, 1H, J = 11.6 Hz, $PhCH_2$), 4.77 (d, 1H, J = 11.6 Hz, $PhCH_2$), 4.74 (d, 1H, J = 11.6 Hz, $PhCH_2$), 4.72 (d, 1H, J = 11.6 Hz, $PhCH_2$), 4.70 (d, 1H, J = 11.6 Hz, $PhCH_2$), 4.62 (d, 1H, J = 11.6 Hz, $PhCH_2$), 4.61 (d, 1H, J = 11.6 Hz, $PhCH_2$), 4.48 (d, 1H, J = 11.6 Hz, $PhCH_2$), 4.44 (d, 1H, J = 11.6 Hz, $PhCH_2$), 4.26 (d, 1H, J = 7.9 Hz, H-1), 4.16 (bq, 1H, J = 6.5 Hz, H-5'), 4.02 (dd, 1H, J = 10.1, 3.5 Hz, H-2'), 3.96 (dd, 1H, J = 10.1, 2.7 Hz, H-3'), 3.82-3.76 (m, 2H, H-5, OCH_2CH_2), 3.74 (d, 1H, J = 7.9 Hz, H-2), 3.62 (dd, 1H, J = 2.7, 1.0 Hz, H-4'), 3.60-3.57 (m, 2H, H-6a, H-6b), 3.41 (d, 1H, J = 0.9 Hz, H-4), 3.35 (dt, 1H, J = 9.3, 7.0 Hz, OCH_2CH_2) , 1.53 (m, 2H, OCH_2CH_2), 1.28-1.19 (m, 13H, 3-*C*-CH_3, CH_2 octyl), 1.08 (d, 3H, J = 6.5 Hz, H-6'), 0.85 (t, 3H, J = 7.0 Hz, CH_3 octyl); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.97, 138.89, 138.41, 138.20, 138.03 (aromatic quart.), 129.01, 128.67, 128.49, 128.43, 128.39, 128.35, 128.27, 128.23, 128.22, 128.19, 128.14, 127.94, 127.88, 127.84, 127.78, 127.68, 127.49, 127.47 (aromatic CH), 101.52 (C-1), 98.65 (C-1'), 81.84, 79.81, 79.43, 78.03, 77.16, 75.81, 75.73, 74.78, 73.66, 73.58, 72.94, 72.73 (C-2, C-3, C-4, C-5, C-2', C-3', C-4', $PhCH_2$ × 5), 70.12 (OCH_2CH_2), 69.49 (C-6), 66.79 (C-5'), 31.89, 29.75, 29.54, 29.32, 26.14, 22.69 (CH_2 octyl), 20.32 (3-*C*-CH_3), 16.69 (C-6'), 14.13 (CH_3 octyl); HR-ESMS calcd for $C_{56}H_{70}O_{10}Na$ ($M+Na^+$) 925.4867, found 925.4872.

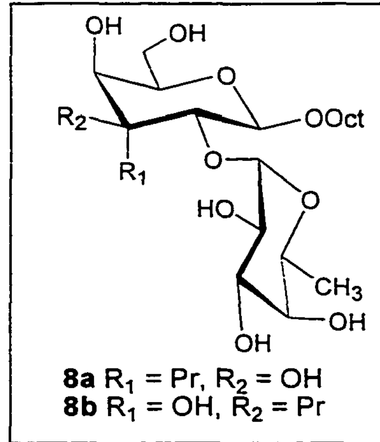
Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Octyl α-L-fucopyranosyl-(1→2)-3-C-methyl-β-D-galactopyranoside (7).



Compound **81** (13 mg, 14 μmol) was deprotected in the same fashion as for **1a** to give **7** (6 mg, 95%) as a white solid: ^{1}H NMR (600 MHz, D_2O) δ 5.35 (d, 1H, J = 4.0 Hz, H-1'), 4.53 (d, 1H, J = 8.1 Hz, H-1), 4.37 (bq, 1H, J = 6.6 Hz, H-5'), 3.91 (dt, 1H, J = 9.6, 6.6 Hz, OCH_2CH_2), 3.89 (dd, 1H, J = 10.5, 3.4 Hz, H-3'), 3.84-3.77 (m, 3H, H-5, H-2', H-4'), 3.76 (dd, 1H, J = 11.5, 7.2 Hz, H-6a), 3.72 (dd, 1H, J = 11.5, 3.8 Hz, H-6b), 3.66 (d, 1H, J = 8.1 Hz, H-2), 3.64 (dt, 1H, J = 9.6, 6.6 Hz, OCH_2CH_2), 3.55 (bs, 1H, H-4), 1.60 (m, 2H, OCH_2CH_2), 1.36-1.24 (m, 13H, 3-C-CH_3, CH_2 octyl), 1.21 (d, 1H, J = 6.6 Hz, H-6'), 0.86 (t, 3H, J = 7.0 Hz, CH_3 octyl); ^{13}C NMR (125 MHz, D_2O) δ 101.63 (C-1), 99.54 (C-1'), 78.31, 76.34, 74.76, 74.50, 72.78, 71.54, 70.27, 69.35, 67.48 (C-2, C-3, C-4, C-5, C-2', C-3', C-4', C-5', OCH_2CH_2), 62.14 (C-6), 31.90, 29.79, 29.35, 29.22, 26.21, 22.80 (CH_2 octyl), 19.30 (3-C-CH_3), 16.18 (C-6'), 14.19 (CH_3 octyl); HR-ESMS calcd for $C_{21}H_{40}O_{10}Na$ ($M+Na^+$) 475.2519, found 475.2526.

Octyl α-L-fucopyranosyl-(1→2)-3-C-propyl-β-D-galactopyranoside (**8a**) and *Octyl α-L-fucopyranosyl-(1→2)-3-C-propyl-β-D-gulopyranoside* (**8b**).



Compound **80** (20 mg, 22 μmol) was treated with allylmagnesium bromide as described for the preparation of **12**. The crude product was purified by column chromatography (8:1 hexanes/EtOAc) to yield **81a** (7 mg, 33 %) and **81b** (6 mg, 29 %). Hydrogenolysis of **81a** (7 mg, 7.5 μmol) and **81b** (4 mg, 4.3 μmol) as described for **1a**

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

gave **8a** (3.4 mg, 94%) and **8b** (1.9 mg, 92 %) as white solids.

For **8a**: ^{1}H NMR (600 MHz, D_2O) δ 5.32 (d, 1H, *J* = 4.0 Hz, H-1'), 4.60 (d, 1H, *J* = 8.2 Hz, H-1), 4.36 (bq, 1H, *J* = 6.6 Hz, H-5'), 3.91 (dt, *J* = 9.9, 6.6 Hz, OCH_2CH_2), 3.89 (dd, 1H, *J* = 10.4, 3.3 Hz, H-3'), 3.81-3.73 (m, 5H, H-4, H-5, H-6a, H-2', H-4'), 3.72 (dd, 1H, *J* = 11.3, 5.0 Hz, H-6b), 3.69 (d, 1H, *J* = 8.2 Hz, H-2), 3.65 (dd, 1H, *J* = 9.9, 6.6 Hz, OCH_2CH_2), 1.92 (m, 1H, $CH_2CH_2CH_3$), 1.67-1.58 (m, 3H, $CH_2CH_2CH_3$, OCH_2CH_2), 1.42 (m, 1H, $CH_2CH_2CH_3$), 1.37-1.24 (m, 11H, $CH_2CH_2CH_3$, CH_2 octyl), 1.22 (d, 3H, *J* = 6.6 Hz, H-6'), 0.93 (t, 3H, *J* = 7.0 Hz, $CH_2CH_2CH_3$), 0.87 (t, 3H, *J* = 7.0 Hz, CH_3 octyl); HR-ESMS calcd for $C_{23}H_{44}O_{10}Na$ ($M+Na^+$) 503.2832, found 503.2838.

For **8b**: ^{1}H NMR (600 MHz, D_2O) δ 5.01 (d, 1H, *J* = 3.8 Hz, H-1'), 4.77 (d, 1H, *J* = 8.0 Hz, H-1), 4.38 (bq, 1H, *J* = 6.6 Hz, H-5'), 4.00 (bdd, *J* = 7.2, 5.1 Hz, H-5), 3.89 (dd, 1H, *J* = 10.5, 3.3 Hz, H-3'), 3.86 (m, 1H, OCH_2CH_2), 3.83-3.79 (m, 2H, H-2', H-4'), 3.76 (dd, 1H, *J* = 11.7, 7.5 Hz, H-6a), 3.73-3.68 (m, 2H, H-6b, OCH_2CH_2), 3.65 (bs, 1H, H-4), 3.42 (d, 1H, *J* = 8.0 Hz, H-2), 1.92 (m, 1H, $CH_2CH_2CH_3$), 1.72-1.60 (m, 3H, $CH_2CH_2CH_3$, OCH_2CH_2), 1.42-1.26 (m, 12H, $CH_2CH_2CH_3$, CH_2 octyl), 1.20 (d, 3H, *J* = 6.6 Hz, H-6'), 0.93 (t, 3H, *J* = 7.0 Hz, $CH_2CH_2CH_3$), 0.86 (t, 3H, *J* = 7.0 Hz, CH_3 octyl); HR-ESMS calcd for $C_{23}H_{44}O_{10}Na$ ($M+Na^+$) 503.2832, found 503.2838.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Chapter 3

Evaluation of Carbon-Branched Sugar Analogs and Enzymatic Synthesis of Carbon-Branched Oligosaccharides

3.1. Introduction

The enzymatic activities of the synthetic carbon-branched sugar analogs **1-8** were evaluated using a well-established radioactive "Sep-Pak assay" schematically represented in Figure 3.1 [68]. This assay takes advantage of hydrophobic properties of acceptors and products to facilitate the removal of unreacted hydrophilic radioactive donor from reaction products. The carbon-branched analogs were first tested as potential acceptors with their respective glycosyltransferases. Analogs that produced radioactive products at or below background counts are considered inactive (non-acceptors). Those analogs showing no activity as acceptors were further evaluated as potential inhibitors. For those analogs that were found active in the radioactive assays, kinetic properties were determined. The structures of the enzymatically produced products were then characterized by mass spectrometry and NMR studies following preparative reactions.

3.2. Enzymatic Assays

3.2.1. β1,4-Galactosyltransferase

β1,4-GalT catalyzes the transfer of galactose from UDP-Gal to the 4-position of GlcNAc or Glc to form Galβ1→4GlcNAc (LacNAc) or Galβ1→4Glc (Lac). As summarized in Chapter 1, the specificity of the enzyme for the acceptor is very flexible as

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

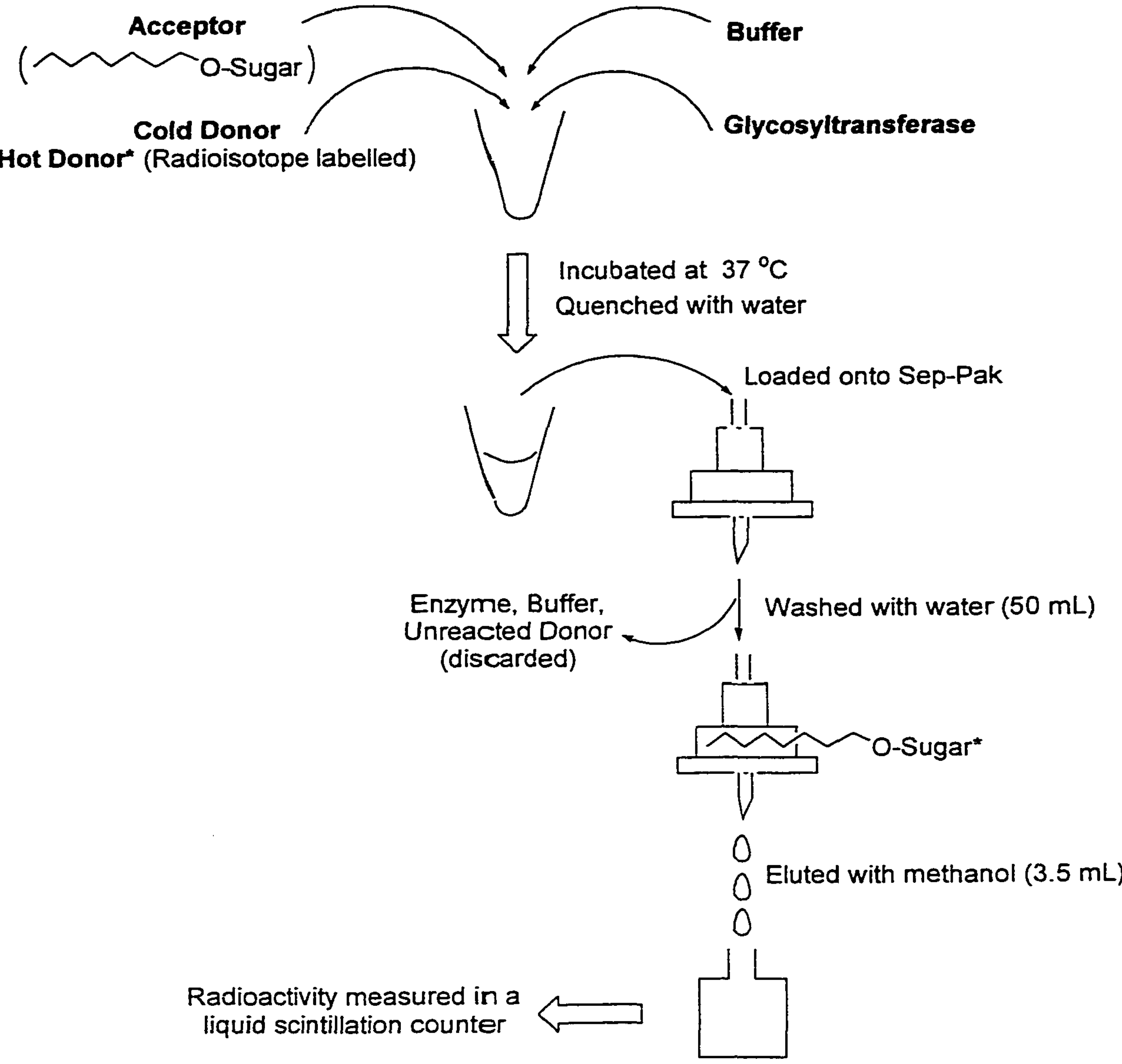


Figure 3.1. Schematic representation of the radioactive "Sep-Pak assay".

a very large number of modified GlcNAc or Glc analogs are active as acceptors. However, deoxygenation or replacement of OH-4 with other functionalities interferes severely with the binding to the enzyme. The 4-OH group is therefore considered to be a key polar group essential for recognition [29].

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

The enzymatic assays showed that the 4-*C*-methyl-branched glucoside **1a** was inactive as an acceptor. This compound was further tested as a potential inhibitor in order to see whether the compound is abie to bind to the enzyme but unable to react because of the steric hindrance. The compound, however, did not show any activity in the inhibition studies. These results suggest that the enzyme binding area is sterically hindered and could only accommodate a C-H bond adjacent to OH-4. The introduction of a C-CH_3 bond caused a steric clash with the active site of the enzyme which abolishes the binding, even though the key polar group was present (Figure 3.2).

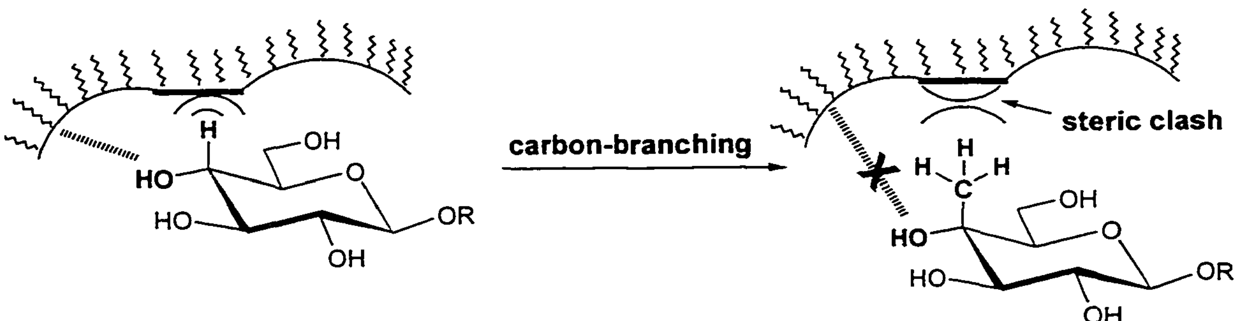


Figure 3.2. Hypothetical binding site interaction near OH-4 of Gal.

3.2.2. α2,3- and α2,6-Sialyltransferases

α2,3-SialT and α2,6-SialT from rat liver use CMP-sialic acid (CMP-Neu5Ac) as the donor and transfer sialic acid to either OH-3 or OH-6 of terminal Gal residue of Galβ1→3/4GlcNAc (α2,3-SialT) or Galβ1→4GlcNAc (α2,6-SialT). Chemical mapping studies by Wlasichuk *et al.* [45] indicate that only OH-6 on the Gal unit and the amide group on the GlcNAc unit are the key polar groups for α2,6-SialT. α2,3-SialT requires an intact 3,4,6-triol system on the Gal residue for the activity.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Carbon-branched compounds **2** and **3a**, analogs of lactoside **13** which is known to be a good substrate of α2,3-SialT [45], turned out to be inactive with α2,3-SialT as either acceptor or inhibitor. LacNAc analog **5** with C-branching at the 6'-position also was inactive toward both α2,3-SialT and α2,6-SialT. These results indicated that both enzymes, similar to β1,4-GalT, could not tolerate changes at the carbon atoms bearing key polar groups. It is likely that the hydrogen atoms bound to the carbon bearing the key polar groups are in close contact to the enzyme binding site.

3.2.3. α1,3/4-Fucosyltransferase

α1,3/4-FucT from human milk catalyzes the transfer of fucose from GDP-Fuc to OH-3 of the GlcNAc residue in the Galβ1→4GlcNAc sequence or OH-4 of the GlcNAc residue in the Galβ1→3GlcNAc sequence. The key polar group mapping studies by Gosselin and Palcic [49] showed that for the Galβ1→4GlcNAc substrate, OH-6 of Gal and OH-4 of the GlcNAc residue to which the enzyme transfers are key polar groups.

Surprisingly, in contrast to the results obtained with β1,4-GalT, α2,3-SialT and α2,6-SialT, both 3-*C*-methyl-branched analog **5** and the 6',6'-di-*C*-methyl-branched analog **6** were found to be acceptors for α1,3/4-FucT in the radioactive assay. As shown in Table 3.1, analogs **5** and **6** had K_m values 15 and 20 times higher, respectively, than the known acceptor Galβ1→4GlcNAcβ-O-MCO (LacNAcβ-O-MCO, MCO = methoxycarbonyloctyl). The maximal rate of transfer (V_{max}) for the parent acceptor was arbitrarily set to 100%. The V_{max} was decreased by 80% for **5** and increased by 70% for **6**. It was very interesting that α1,3/4-FucT, unlike the inverting glycosyltransferases studied above, could tolerate the introduction of *C*-methyl group(s) at both binding sites.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Remarkably, the enzyme could still transfer to the hindered tertiary alcohol produced by the introduction of a *C*-methyl group at the site of transfer, implying that there must be substantial flexibility in the active site even at the transition state of the reaction.

Table 3.1. Kinetic properties of analogs **5** and **6**with α1,3/4-FucT.

Compound	K_m (mM)	V_{max}
Galβ1→4GlcNAcβ-O-MCO	0.4 ± 0.04	100%
5	8.0 ± 1.0	172%
6	6.3 ± 0.7	20%

The molecular mechanism of the reaction catalyzed by human α1,3-fucosyltransferase V, one of the multigene family of human α1,3/4-fucosyltransferases, was recently investigated by the Wong group [92]. α-Secondary isotope effect and inhibition studies suggested that the glycosidic cleavage of GDP-Fuc occurs prior to the nucleophilic attack to form an sp^2-hybridized oxocarbenium intermediate. Based on the rationale that a direct S_N2 type displacement would be extremely difficult for the much hindered tertiary alcohol acceptor, human milk α1,3/4-FucT catalyzed glycosylation would thus also have a significant S_N1 character and sp^2-hybridization would occur prior to the nucleophilic attack from OH-3 of the acceptor.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

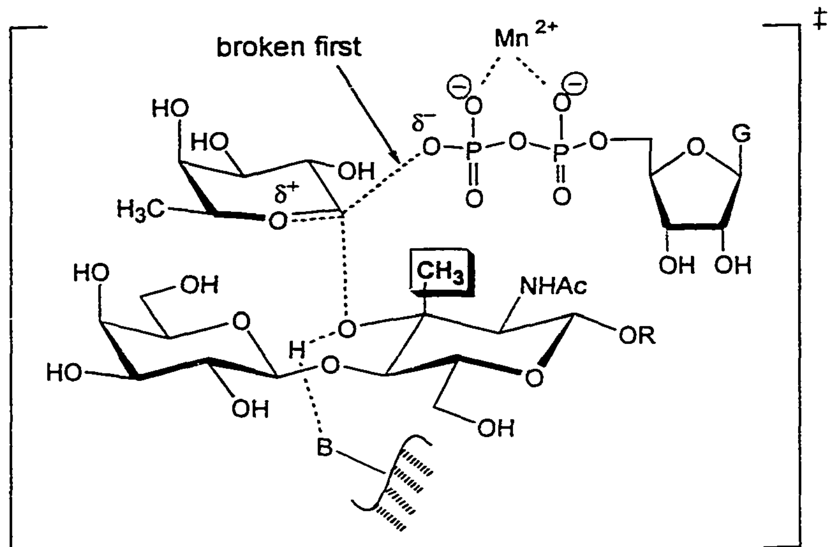


Figure 3.3. Proposed transition-state structure for the human milk α1,3/4-fucosyltransferase reaction.

3.2.4. α1,3-Galactosyltransferase

α1,3-GalT from calf thymus catalyzes the transfer of Gal from UDP-Gal to OH-3 of the Gal residue in Galβ1→4GlcNAc or Galβ1→4Glc [37]. Unlike the glycosyltransferases studied above, α1,3-GalT is a retaining glycosyltransferase. Studies using acceptor analogs indicate that OH-3 of Gal residue (OH-3') is not essential for recognition by α1,3-GalT even though it is the glycosylation site, while OH-4 of the Gal residue (OH-4') is a key polar group.

The carbon-branched analog **4a**, with branching at C-4' bearing the key polar group showed no activity towards α1,3-GalT. This result is similar to that obtained with the β1,4-GalT, α2,3-SialT, α2,6-SialT enzymes that could not tolerate the replacement of C-H bond with C-CH_3 bond at positions bearing key polar groups. Interestingly, the

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

substitution of two H atoms with two methyl group at C-6' also produced the inactive compound **5** whereas deoxygenation or *O*-alkylation at C-6' was shown to be tolerated by the enzyme [42].

To our surprise, the 3'-*C*-methyl-branched analog **2** was active in the assay. The K_m for **2** was 23 mM, about 15 times higher than that of the parent *n*-octyl lactoside **14** which has a K_m of 1.5 mM. The V_{max} is decreased to 38% of that of **14** (Table 3.2). However, replacement of the carbon-bonded hydrogen with more bulky group (a propyl group) produced the inactive compound **3a** when tested as an acceptor at a concentration of 2 mM in a 6-h incubation. Compound **3a** was also inactive as an inhibitor at a concentration as high as 5 mM.

Table 3.2. Kinetic properties of analogs **2** and **3** with α1,3-GalT.

Compound	K_m (mM)	V_{max}
Galβ1→4Glcβ-O-Oct (**14**)	1.5 ± 0.1	100%
2	23 ± 4.9	38%
3a	Inactive	

3.2.5. Human Blood Group A and B Glycosyltransferases

The human blood group A and B glycosyltransferases (GTA and GTB) catalyze the formation of α1→3 galactosidic linkage with retention of configuration, similar to the α1,3-GalT. GTA catalyzes the transfer of GalNAc from UDP-GalNAc to the blood group (O)H-precursor structure Fucα1→2Galβ-OR to give the blood group A trisaccharide GalNAcα1→3[Fucα1→2]Galβ-OR. GTB uses the same acceptor substrate but catalyzes

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

the transfer of Gal from UDP-Gal to produce the blood group B trisaccharide Galα1→3[Fucα1→2]Galβ-OR. Systematic studies have revealed that Gal OH-3 is not essential for recognition by either of these enzymes as was in the case with α1,3-GalT [54, 55]. Furthermore, GTA and GTB are highly homologous glycosyltransferases and differ by only four amino acid residues [53a]. GTB exhibits about 40% sequence identity with α-1,3-GalT [93]. It would thus be reasonable to expect that both GTA and GTB could also tolerate carbon-branching at the 3-position of the Gal residue. In fact, both 3-*C*-methyl and 3-*C*-propyl branched analogs, **7** and **8a**, were found to be acceptors for these two enzymes. As shown in Table 3.3, replacement of the hydrogen atom at the site of the enzymatic reaction with a methyl group did not importantly influence the K_m and V_{max} values. However, substitution with a larger propyl group dramatically altered the kinetic properties. The K_m values were almost 30 and 165 times higher and V_{max} decreased to 8% and 26%, respectively, of the values observed for the parent compound.

Table 3.3. Kinetic properties of analogs **7** and **8a** with GTA and GTB.

Compound	**GTA**		**GTB**	
	K_m (μM)	V_{max}	K_m (μM)	V_{max}
Fucα1→2Galβ-O-Oct	76 ± 13	100%	17 ± 2	100%
7	209 ± 15	60%	39 ± 3	64%
8a	2200 ± 200	8%	2800 ± 300	26%

It is quite remarkable that all the three retaining glycosyltransferases, α1,3-GalT, GTA and GTB, tolerate the introduction of large substituents at the carbon bearing the hydroxyl group that becomes glycosylated. However, as revealed by the kinetic data, the steric increase does make it more difficult for the enzymes to bind and transfer. The larger the alkyl group, the higher the K_m and the lower the V_{max} values. Among the three

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

enzymes, the introduction of an alkyl substituent has more impact on K_m and V_{max} for α1,3-GalT than GTA and GTB since the V_{max}/K_m value for **2** was only 2.5% of that for Galβ1→4Glcβ-O-Oct (**14**) with α1,3-GalT, while the V_{max}/K_m value for **7** was only decreased to 22-28% of that for Fucα1→2Galβ-O-Oct [54] with GTA and GTB. This is also in agreement with the observation that no detectable transfers to the 3'-*C*-propyl branched analog **3a** was observed with α1,3-GalT while **8a** still displayed activity with GTA and GTB. The three enzymes seem to have similar molecular specificity with respect to the β-Gal residue: OH-4 is required for binding while OH-3 is not essential and *C*-methyl-branching is tolerated at C-3 of the β-Gal residue. The similar molecular specificity and the homology of these enzymes indicate that they may have similar three-dimensional structure at the active sites.

Figure 3.4. Hypothetical two-step, double-displacement mechanism for the retaining glycosyltransferases.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

A two-step double-displacement mechanism involving the formation and breakdown of glycosyl-enzyme intermediate is most likely involved for the retaining glycosyltransferases [54] as well as for retaining glycosidases [94]. Since a direct S_N2 substitution is unlikely to occur in the second step for the much hindered tertiary alcohols investigated here, we propose that the breakdown of the glycosyl-enzyme intermediate begins prior to the nucleophilic attack by the acceptor OH group. The process would thus have significant S_N1 character (Figure 3.4) as suggested for β1,4-GalT [95], α1,3-FucT V [92] and α-glucosyltransferase [96].

3.2.6. α1,4-Galactosyltransferase

The interesting and surprising results that α1,3/4-FucT, α1,3-GalT, GTA and GTB could catalyze the glycosylation of a complex tertiary sugar alcohol led us to investigate another retaining glycosyltransferase, α1,4-Galactosyltransferase (α1,4-GalT). Unlike the glycosyltransferases studied above that are mammalian enzymes, the α1,4-GalT investigated here was from the bacterial pathogen *Neisseria menigitidis.* The enzyme was recently cloned and overexpressed in *E. coli* [19b]. The molecular specificity of the enzyme has not been explored in detail.

Preliminary screening showed that the enzyme required the Galβ1→4Glc sequence for recognition (Table 3.4). *n*-Octyl galactoside, Galβ1→3GlcNAcβ–O-MCO, Galβ1→3GlcNAcα-O-MCO, Glcβ1→4Glcβ-O-PNP (PNP = *p*-nitrophenyl) were not substrates. Substitution of OH-2 of the Glc residue by NHAc drastically reduced the activity as the relative rate of transfer (V_{rel}) of Galβ1→4GlcNAcβ-O-MCO (LacNAcβ-O-MCO) is only about 1% of that of Galβ1→4Glc-O-MCO (Lacβ-O-MCO). The 6',6'-di-

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

C-branched LacNAc analog **6** showed no activity towards the enzyme probably due to the intrinsic low activity of the parent LacNAc itself.

Table 3.4. Relative transfer rates of the analogs with α1,4-GalT.

Compound	Relative rate[a]	Compound	Relative rate[b]
Galβ1→4Glcβ-O-MCO	100	Galβ1→4Glcβ-O-MCO	100
Galβ1→4Glcβ-O-Oct	107	Galβ1→4GlcNAcβ-O-MCO	1.4
Galβ1→3GlcNAcβ-O-MCO	3.6	**2**	14
Galβ1→3Galα-O-MCO	0.1	**3a**	0.2
Galβ1→4Glcβ-O-PNP	1.1	**4a**	2.3
Galβ-O-Oct	1.5	**6**	0

a) at a concentration of 3.5 mM.

b) at a concentration of 1.9 mM.

It was hoped that the 4'-*C*-branched lactoside analog **4a** would be active as an acceptor since α1,4-GalT is also a retaining glycosyltransferase and catalyzes the formation of an α(axial)-galactosidic linkage. To our surprise, **4a** displayed poor, if any, activity. Compound **4a** was further confirmed to be inactive since trisaccharide product was undetectable in the preparative synthesis. Interestingly, analog **2** with C-branching at C-3 of the Gal residue was found to be a substrate with V_{rel} of 14%. However, the 3'-*C*-propyl branched analog **5** was found to be inactive as an acceptor. The 6',6'-di-*C*-branched LacNAc analog **6** showed no activity as an acceptor probably due to the intrinsic low activity of the parent LacNAc compound.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

3.3. Preparative Enzymatic Synthesis

To be certain that the analogs which were found to be acceptors in the radioactive "Sep-Pak assay" were indeed glycosylated in the expected manner, preparative syntheses of **2**, and **5**-**7**, **8a** with their corresponding enzymes were performed. Mass spectrometry and extensive NMR studies were employed to confirm the structures of the enzymatic products.

GDP-Fuc
α1,3/4-FucT
(75%)

GDP-Fuc
α1,3/4-FucT
(41%)

Figure 3.5. Enzymatic synthesis of the Lewis X analogs **83** and **84**.

Compounds **5** and **6** were fucosylated using α1,3/4-FucT to give the Lewis X analogs **83** and **84** in 75% and 41% yields (Figure 3.5). NMR studies showed that H-5 of the newly introduced α-Fuc residue was strongly downfield-shifted to 4.85 ppm in **83** and to 4.68 ppm in **84**, which is diagnostic of 3-*O*-fucosyl-*N*-acetyllactosamine sequences [24b, 97]. A novel carbon-branched analog of sialyl Lewis X, **86**, was also enzymatically

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

synthesized from **6** with α-2,3-SialT and α1,3/4-FucT in an overall yield of 41% (Figure 3.6).

Figure 3.6. Enzymatic synthesis of the sialyl Lewis X analog **86**.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Figure 3.7. Enzymatic synthesis of the trisaccharide analogs **87** and **88**.

Compound **87** was produced in 90% yield from the reaction of **2** with UDP-Gal catalyzed by α1,3-GalT (Figure 3.7). As expected, protons of the 3-*C*-methyl group were shifted downfield from 1.24 ppm to 1.37 ppm. The ^{13}C chemical shift of the quaternary branching carbon (C-3') in **87** also shifted downfield from 74.7 ppm to 80.9 ppm with no significant chemical shift changes in other carbon resonances [30]. Trisaccharide **88** was also prepared in 95% yield using α1,4-GalT (Figure 3.7). As can be seen in Figure 3.8, trisaccharide **87** shows the expected H-1"/3'-*C*-methyl NOE as well as stronger H-1"/H-4' interactions, which is consistent with earlier observations in Galα1→3Galβ1→4Glc [98] and the A and B trisaccharides [99], whereas the 1→4 linked trisaccharide **88** shows only

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

the H-1"/H-4' interaction. These NOE results, together with the αGal-H1: βGal-C3 correlation in HMBC [100] experiments of **87** demonstrate the 1→3 linkage in **88**.

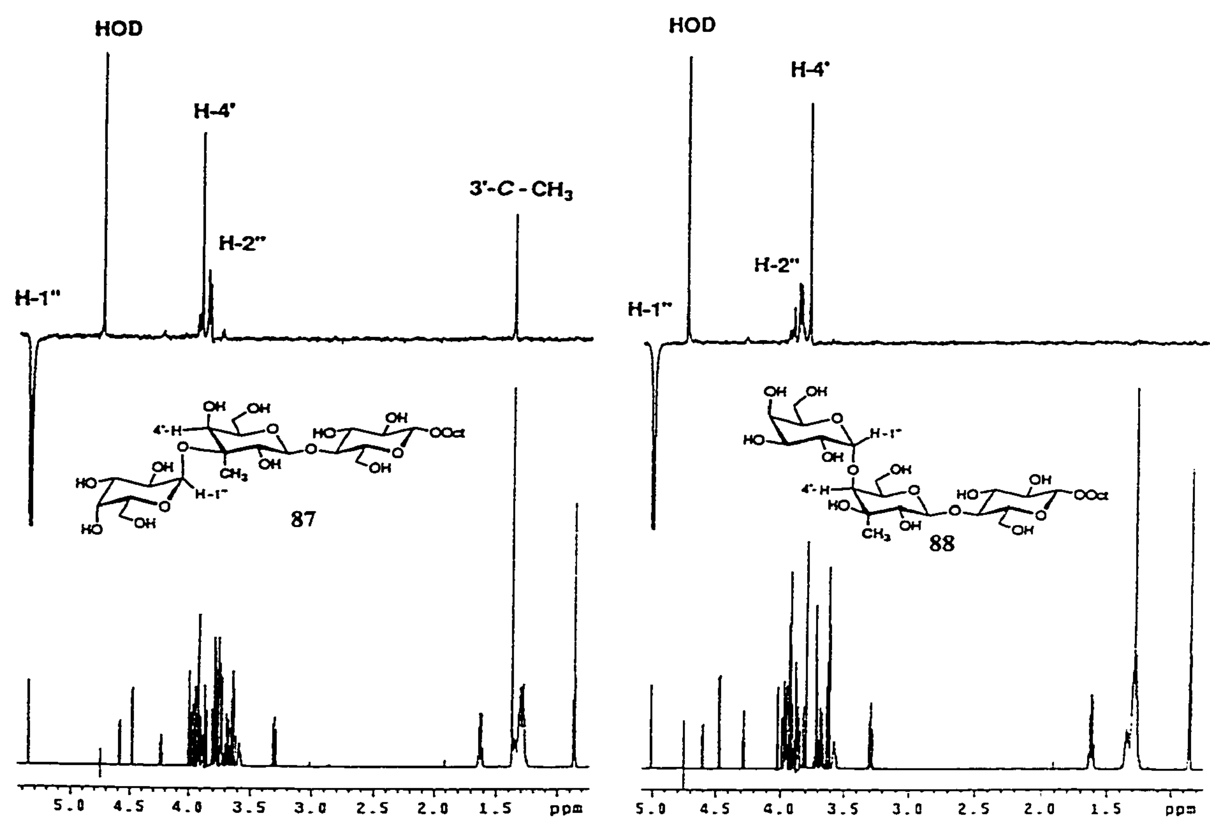


Figure 3.8. 1D-TROESY spectra obtained from selective excitation of the H-1" resonance (top) and 1D 600 MHz spectra of the trisaccharide analogs **87** and **88** (bottom).

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

As shown in Figure 3.9, the A and B trisaccharide analogs **89-92** were obtained in near quantitative yields using GTA and GTB with UDP-GalNAc or UDP-Gal as donors. Complete conversion of the poor substrate **8a** was achieved by increasing the amount of enzyme, donor and the reaction time. Detailed NMR studies revealed the NOE pattern characteristic for the A and B trisaccharides [99]: a stronger NOE from H-1 (α-Gal or α-GalNAc) to H-4 (β-Gal) than from H-1 (α-Gal or α-GalNAc) to protons of the alkyl substituents at C-3. Significant downfield shifts for the protons (~0.15 ppm) of 3-*C*-methyl or 3-*C*-$CH_2CH_2CH_3$ (with the first CH_2 italicized as $C\mathit{H}_2$) and for the carbon resonance C-3 (~7 ppm) were also observed. In addition, conformationally independent evidence for the correct linkages in trisaccharides **89-92** was obtained by HMBC experiments [100] through $^{13}C/^{1}H$ correlations across the glycosidic linkage.

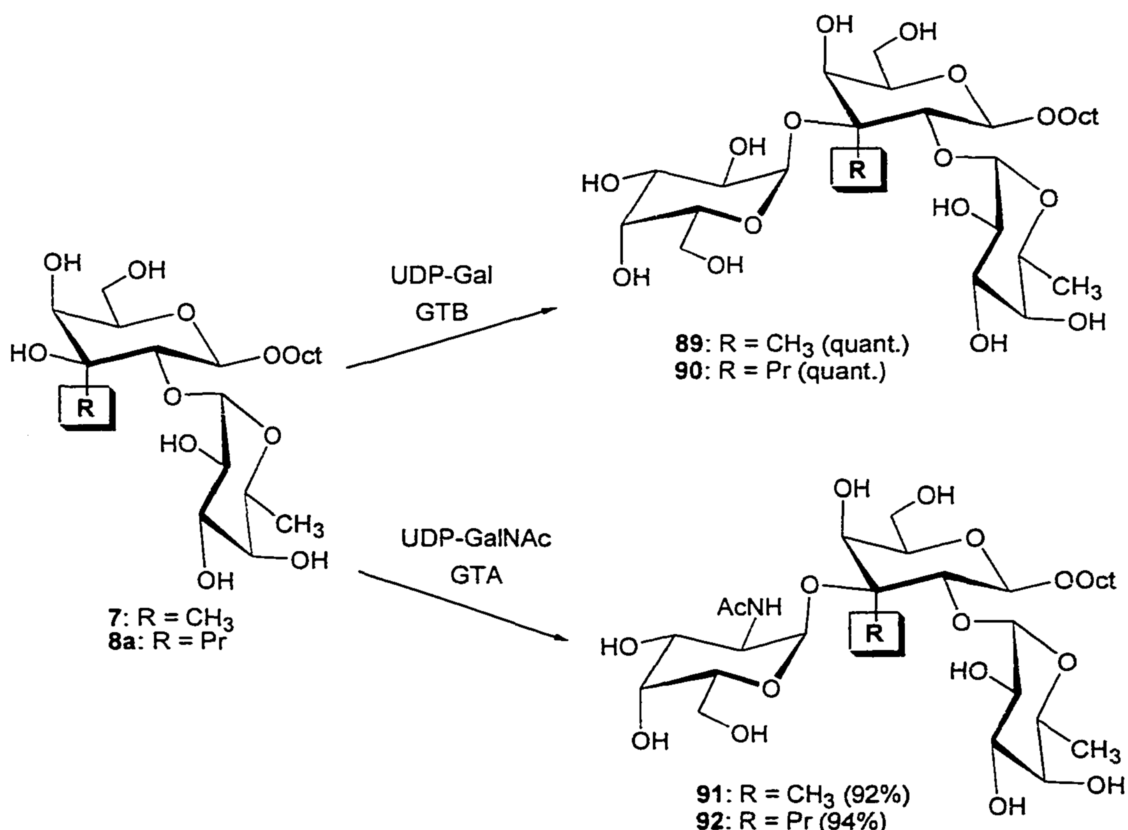


Figure 3.9. Enzymatic synthesis of the A and B trisaccharide analogs **89-92**.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Table 3.5. Summary of the enzymatic assay results of the eight C-branched analogs with four retaining and four inverting enzymes.

Analog	β-1,4-GalT	α-2,3-SialT	α-1,3-GalT	α-1,4-GalT *	α-1,3/4-FucT	α-2,6-SialT
1a	[(X)]					
2		[(X)]	[√]	√		
3a			[X]	X		
4a		(X)	X	[X]		
5					[(√)]	
6		(X)	(X)	X	(√)	[(X)]
		GTA		GTB		
7		[√]		[√]		
8a		[√]		[√]		

carbon-branching at glycosylation site: [ ] ; at position bearing key polar group: ( )
√ : acceptor, X : inactive *. inhibition test not done

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

3.4. Summary

Substitution of hydrogen atom(s) on the carbon atom bearing a hydroxyl group which is either the key polar group or glycosylation site, hinders the binding with the enzyme. This is likely a steric effect resulting from the introduction of a bulky alkyl group. In some cases, this completely inhibits binding with the enzymes. In the cases where enzymes tolerate the C-branching, the C-branched analogs had higher K_m values and lower V_{max} than their parent disaccharides, with the exception that the V_{max} of **5** with α1,3/4-FucT was increased.

Among the seven mammalian enzymes investigated here, the four enzymes: α1,3/4-FucT, α1,3-GalT, GTA and GTB, all catalyze the formation of an α(axial)-glycosidic linkage with either retention or inversion of configuration. They all tolerated C-branching at the glycosylation site. This is in contrast to the three inverting glycosyltransferases: β1,4-GalT, α2,3-SialT and α2,6-SialT, which catalyze the formation of equatorial-linkages and do not tolerate C-branching at the glycosylation site or even at the positions bearing a key polar group (Table 3.5). The different steric requirements at the active sites of the mammalian glycosyltransferases revealed by these studies suggest that glycosyltransferases that make an axial-linkage and glycosyltransferases that make an equatorial-linkage may have very different three-dimensional structures for their binding sites (Figure 3.10).

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

(a) Inverting glycosyltransferases forming equatorial linkage

Acceptor
HO
HO
OR
O-sugar nucleotide
H
β1,4-GalT
α2,3-SiaIT
α2,6-SiaIT
equatorial glycosidic linkage

(b) Inverting glycosyltransferases forming axial linkage

Acceptor
HO
OR
H_3C
O-GDP
OH
HO
OH
axial glycosidic linkage
α1,3/4-FucIT

(c) Retaining glycosyltransferases forming axial linkage

HO
Acceptor
HO
OR
O-UDP
X
HO
Nu
OH
OH
X = OH, NHAc
axial glycosidic linkage
α1,3-GalT
GTA, GTB

Figure 3.10. Proposed binding models for mammalian glycosyltransferases.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

While the reaction-mechanisms of the glycosylglycosyltransferases may be complex, our results demonstrated that these enzymes can be powerful synthetic tools for the preparation of carbon-branched oligosaccharides. The synthesis of glycosides of complex tertiary alcohols using a chemoenzymatic approach was thus accomplished for the first time.

3.5. Experimental

3.5.1. Enzymatic Assay

EcoLite (+) scintillation cocktail was from ICN Radiochemicals (Aurora, OH). UDP-[6-^{3}H]Gal, CMP-[9-^{3}H]Neu5Ac, UDP-[6-^{3}H]GalNAc, GDP-[1-^{3}H]Fuc were from American Radiolabelled Chemicals (St. Louis, MO). Alkaline phosphatase (calf intestine) was from Boehringer Mannheim. Bovine serum albumin was from Sigma.

Enzymatic assays were performed according to a well-established radioactive "Sep-Pak assay" method [68]. The reactions for enzymatic assays were incubated at 37 °C in 450 μL microfuge tubes. After quenching with water, the reaction mixture was transferred to a C18 Sep-Pak cartridge pre-equilibrated with methanol and water. The cartridge was washed with water (50 mL) to remove unreacted donor. The radiolabelled products were then eluted with methanol (3.5 mL) and quantitated in EcoLite (+) scintillation cocktail (10 mL) using a liquid scintillation counter. Kinetic parameters, K_m and V_{max} were derived from the best fit of the Michaelis-Menten equation using nonlinear regression analysis with the SigmaPlot 4.1 program. The V_{max} was arbitrarily set to 100% for the parent (native) acceptors.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

β1,4-GalT Assay

The β1,4-GalT was isolated from bovine milk. The reactions were incubated at 37 °C for 30 min or 3h in 20 μL total volume with 75 mM sodium cacodylate buffer, 7.5 mM $MnCl_2$, pH 7.4, containing 1 mM UDP-Gal, UDP-[6-^{3}H]Gal (about 90n000 dpm), 120 μU β1,4-GalT, and 5 mM Glcβ-O-Oct or 5 mM analog **1a**. When tested as an inhibitor, analog **1** was used at concentration of 5 mM with 0.38 mM of GlcNAcβ-O-MCO, 0.2 mM UDP-Gal.

α2,3-SialT Assay

The α2,3-SialT was expressed using a recombinant baculovirus expression system [101]. The reactions were incubated at 37 °C for 10 min, 35 min and 7h in 20 μL total volume with 50 mM sodium cacodylate buffer, 10 mM $MnCl_2$, 1 mg/mL BSA, pH 7.0, containing 0.2 mM CMP-Neu5Ac, CMP-[9-^{3}H]Neu5Ac (about 90,000 dpm), 2 mU α2,3-SialT, and 2.7 mM **14**, **1**, **2**, **4a**, or **6**. For the inhibition test, analogs **3a**, **4a**, or **6** was used at concentration of 5 mM with 1.5 mM of LacNAcβ-O-MCO.

α2,6-SialT Assay

The α2,6-SialT was isolated from rat liver [45]. The reactions were incubated at 37 °C for 30 min and 1h in 20 μL total volume with 40 mM sodium cacodylate buffer, 7.5 mM $MnCl_2$, 0.1% Triton CF-54, 0.25 mg/mL BSA, pH 7.0, containing 0.2 mM CMP-Neu5Ac, CMP-[9-^{3}H]Neu5Ac (about 90,000 dpm), 6 μU α2,3-SialT, and 5 mM **6**. For the inhibition test, analog **6** was used at concentration of 5 mM with 1.8 mM of LacNAcβ-O-MCO.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

α1,3/4-FucT Assay

The α1,3/4-FucT was isolated from human milk [49]. The reactions were incubated at 37 °C for 10 min in 20 μL total volume with 20 mM HEPES buffer, 20 mM $MnCl_2$, 0.2% BSA, pH 7.0, containing 0.05 mM GDP-Fuc, GDP-[1-^{3}H]Fuc (about 00,000 dpm), 60 μU α1,3/4-FucT. K_m was determined for analog **5** at concentrations of 0.1 mM, 0.2 mM, 0.4 mM, 0.8 mM, 1.7 mM, 3.5 mM. For analog **6** the K_m was determined at concentrations of 1 mM, 2 mM, 4 mM, 8 mM, 16 mM, 32 mM.

α1,3-GalT Assay

The α1,3-GalT was isolated from calf thymus [42]. The reactions were incubated at 37 °C for 30 min and 6h in 20 μL total volume with 100 mM sodium cacodylate buffer, 50 mM MnCl2, pH 6.0, containing 0.5 mM UDP-Gal, UDP-[6-^{3}H]Gal (about 80,000 dpm), 300 μU α1,3-GalT, and 2 mM Lacβ-O-Oct (**14**), **2**, **3a**, **4a**, or **6**. Km was determined for **14** at concentrations of 0.25 mM, 0.5 mM, 1.0 mM, 2.0 mM, 4.0 mM. For analog **2** the K_m was determined at concentrations of 0.5 mM, 1.0 mM, 2.0 mM, 4.0 mM, 8.0 mM. For the inhibition test, analogs **3a**, **4a**, or **6** was used at the concentration of 5 mM with 0.2 mM of LacNAcβ-O-MCO, 0.5 mM UDP-Gal.

GTA and GTB assay

GTA and GTB were expressed and cloned in *E.Coli* [53c]. For GTA assay, the reactions were incubated at 37 °C 30 min in 20 μL total volume with 35 mM sodium cacodylate buffer, 20 mM MnCl2, 1 mg/mL BSA, pH 7.0, containing 70 μM UDP-GalNAc, UDP-[6-^{3}H]GalNAc (about 75,000 dpm), 9 μU GTA. K_m was determined for

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Fucα1→2Galβ-O-Oct at concentrations of 2.5 μM, 5.0 μM, 10.0 μM, 20.0 μM, 40.0 μM, 80.0 μM. For analog **7** the K_m was determined at concentrations of 6.25 μM, 12.5 μM, 25 μM, 50 μM, 100 μM, 200 μM. For analog **8a** the K_m was determined at concentrations of 0.125 mM, 0.25 mM, 0.5 mM, 1.0 mM, 2.0 mM, 4.0 mM.

For GTB assay, the reactions were incubated at 37 °C for 20 min in 20 μL total volume with 35 mM sodium cacodylate buffer, 20 mM MnCl2, 1 mg/mL BSA, pH 7.0, containing 0.2 mM UDP-Gal, UDP-[6-^{3}H]Gal (about 75,000 dpm), 20 μU GTB. K_m was determined for Fucα1→2Galβ-O-Oct at concentrations of 31.25 μM, 62.5 μM, 125 μM, 250 μM, 500 μM, 1000 μM. For analog **7** the K_m was determined at concentrations of 50 μM, 100 μM, 200 μM, 400 μM, 1000 μM, 2000 μM. For analog **8a** the K_m was determined at concentrations of 0.1 mM, 0.2 mM, 0.4 mM, 1.0 mM, 2.0 mM, 5.0 mM.

α1,4-GalT Assay

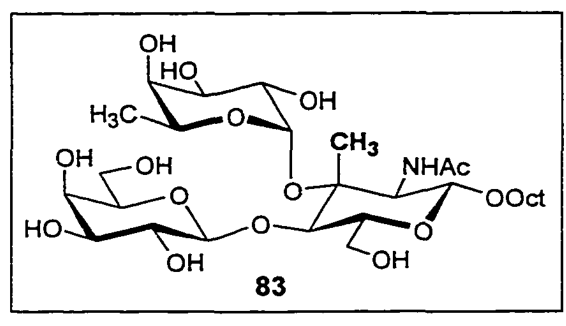
The α1,4-GalT from *Neisseria meningitidis* [19b] was a generous gift from Dr. Warren W. Wakarchuk at National Research Council of Canada, Ottawa. The reactions were incubated at 37 °C for 30 min or 6 h in 20 μL total volume with 50 mM HEPES buffer, 0.1 mg/mL, pH 7.5, containing 1.25 mM UDP-Gal, UDP-[6-^{3}H]Gal (about 70,000 dpm), 47 μU α1,4-GalT, and 1.9 mM Lacβ-O-MCO or analogs.

3.5.2. Preparative Enzymatic Synthesis

General methods used were as described in Chapter 2.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

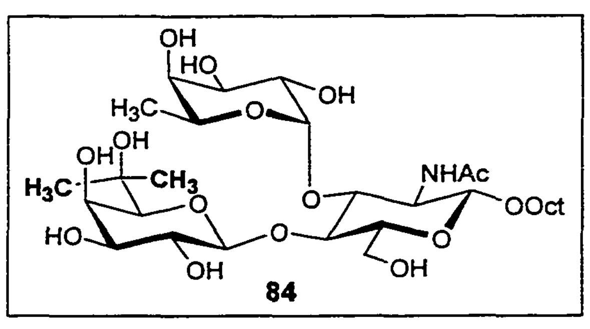
Octyl β-D-galactopyranosyl-(1→4)-[α-L-fucopyranosyl-(1→3)]-2-acetamido-2-deoxy-3-C-methyl-β-D-glucopyranoside (**83**).



The reaction contained **5** (1.0 mg, 2.0 μmol), GDP-Fuc (1.7 mg, 2.9 μmol), 30 mU of human milk α-1,3/4-FucT (in 300 μL of 25 mM sodium cacodylate buffer, pH 6.5, containing 5 mM $MnCl_2$ and 25% glycerol), 30 μL of concentrated buffer (200 mM HEPES, 200 mM $MnCl_2$, 2% BSA, pH 7.0) and 3 μL of alkaline phosphatase (1 U/μL). The reaction was incubated at 37 °C with rotation for 2 days, then at room temperature for 4 days. Additional GDP-Fuc was added daily (total 1.6 mg). The reaction mixture was filtered through a Millex-GV 0.22 μm filter and loaded onto two sequential C18 Sep-Pak cartridges. The cartridges were washed with water (50 mL) and 30% aqueous methanol (20 mL) and then eluted with 50% aqueous MeOH (30 mL). The eluant was concentrated and redissolved in water, filtered through a Millex-GV 0.22 μm filter, and lyophilized to yield **83** (1.0 mg, 75%) as a white solid: ^{1}H NMR (600 MHz, D_2O) δ 5.29 (d, 1H, J = 4.0 Hz, H-1 αFuc), 4.68 (bq, 1 H, J = 6.8 Hz, H-5 αFuc), 4.49 (d, 1H J = 8.4 Hz, H-1 βGlcNAc), 4.48 (d, 1H, J = 7.7 Hz, H-1 βGal), 4.04 (d, 1H, J = 9.9 Hz, H-4 βGlcNAc), 3.99 (d, 1H, J = 8.4 Hz, H-2 βGlcNAc), 3.93 (dd, 1H, J = 12.0, 2.4 Hz), 3.90-3.83 (m, 3H), 3.80 (dd, 1H, J = 12.2, 4.5 Hz), 3.76 (d, 1H, J = 3.8 Hz), 3.74-3.66 (m, 3H), 3.63 (dd, 1H, J = 9.9, 3.5 Hz), 3.59-3.55 (m, 2H), 3.53-3.46 (m, 2H), 2.03 (s, 3H, $COCH_3$), 1.50 (m, 2H, OCH_2CH_2), 1.46 (s, 3H, (3-C-CH_3), 1.30-1.20 (m, 10H, CH_2 octyl), 1.14 (d, 3H, J = 6.8 Hz, H-6 αFuc), 0.84 (t, 3H, J = 7.0 Hz, CH_3 octyl); HR-ESMS calcd for $C_{29}H_{54}NO_{15}$ (M+H$^+$) 656.3493, found 656.3498.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

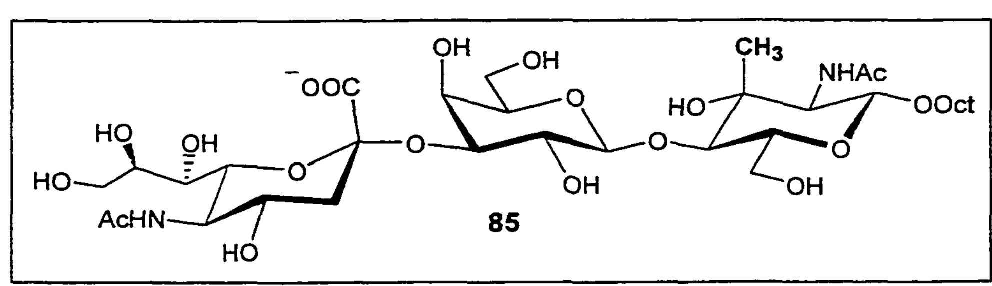
Octyl β-D-6,6'-di-C-methyl-galactopyranosyl-(1→4)-[α-L-fucopyranosyl-(1→3)]-2-acetamido-2-deoxy-β-D-glucopyranoside (**84**).



The reaction mixture contained **6** (1.6 mg, 3.1 μmol), GDP-Fuc (2.0 mg , 3.4 μmol), 20 mU of human milk α-1,3.4-FucT (in 200 μL of 25 mM sodium cacodylate buffer, pH 6.5, containing 5 mM $MnCl_2$ and 25% glycerol), 20 μL of concentrated buffer (200 mM HEPES, 200 mM $MnCl_2$, 2% BSA, pH 7.0) and 1 μL of calf intestine alkaline phosphatase (1 U/μL). The reaction was incubated at 37 °C with rotation for 23 days. Additional GDP-Fuc (2.4 mg), α-1,3.4-FucT (10 mU), and alkaline phosphatase (3 μL) were added during the incubation period. The reaction mixture was filtered through a Millex-GV 0.22 μm filter and loaded onto two sequential C18 Sep-Pak cartridges. The cartridges were washed with water (25 mL), 10% aqueous methanol (25 mL) and then eluted with MeOH (30 mL). The eluant was concentrated and the resulting residue was purified on a column of Iatrobeads with 5:1 CH_2Cl_2/MeOH. The product was then redissolved in water, filtered through a Millex-GV 0.22 μm filter, and lyophilized to yield **84** (0.8 mg, 41%) as a white solid: ^{1}H NMR (600 MHz, D_2O) δ 5.09 (d, 1H, J = 4.2 Hz, H-1 αFuc), 4.85 (bq, 1H, J = 6.6 Hz, H-5 αFuc), 4.54 (d, 1H, J = 8.2 Hz, H-1 βGlcNAc), 4.47 (d, 1H, J = 7.3 Hz, h-1 βGal), 4.22 (d, 1H, J = 2.4 Hz), 4.30 (dd, 1H, J = 10.4, 3.3 Hz), 4.01 (dd, 1H, J = 10.4, 2.2 Hz), 3.96 (t, 1H, J = 9.2 Hz), 3.92-3.82 (m, 4H), 3.71 (bd, 1H, J = 3.5 Hz), 3.68 (dd, 1H, J = 10.6, 4.2 Hz), 3.61-3.55 (m, 4H), 3.28 (s, 1H, H-5 βGal), 2.04 (s, 3H, $COCH_3$), 1.54 (m, 2H, OCH_2CH_2), 1.36-1.26 (m, 16H, 6'-C-CH_3 × 2, CH_2 octyl), 1.18 (d, 3H, J = 6.6 Hz, H-6 αFuc), 0.87 (t, 3H, J = 7.0 Hz, CH_3 octyl); HR-ESMS calcd for $C_{30}H_{55}NO_{15}Na$ (M+Na^+) 692.3469, found 692.3463.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

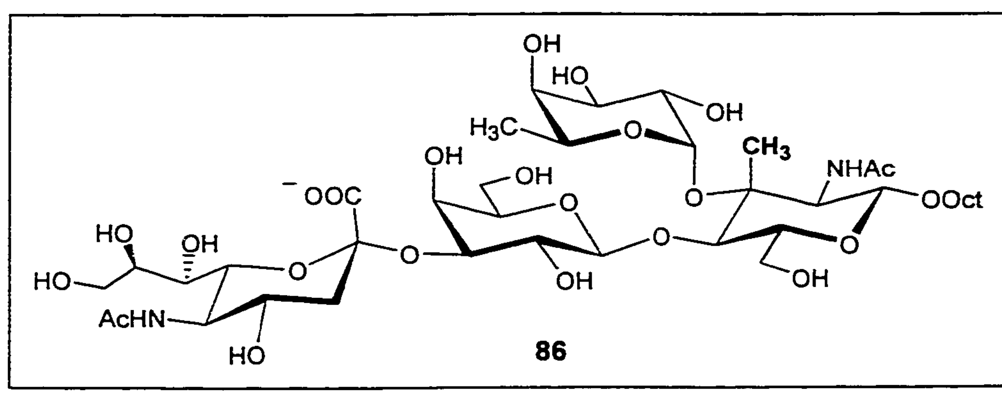
Octyl 5-N-acetyl-neuraminyl-α-(2→3)-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-3-C-methyl-β-D-glucopyranoside (**85**).



The reaction mixture contained **5** (0.8 mg, 1.6 μmol), CMP-Neu5Ac (1.4 mg , 2.1 μmol), 80 mU of cloned α-2,3-SialT (in 360 μL of 50 mM sodium cacodylate buffer, pH 6.5, containing 0.75 mM NaCl, 50% glycerol and 1 mg/ML BSA), 20 μL of concentrated buffer (200 mM HEPES, 200 mM $MnCl_2$, 2% BSA, pH 7.0), 9.6 μL of 1 M $MnCl_2$, 7.7 μL of 100 mg/ml BSA, 7.0 μL of 50 mM sodium cacodylate buffer (pH 7.5) and 2 μL of alkaline phosphatase (1 U/μL). The reaction was incubated at 37 °C with rotation for 25 days. Additional 0.66 mg CMP-Neu5Ac was added. The reaction mixture was loaded onto a C18 Sep-Pak cartridge. The cartridge was washed with water and then eluted with MeOH. The eluant was concentrated and redissolved in 100 μL of water, and loaded onto a syringe containing Biorad AG 50W resin (Na^+ form, 100-200 mesh), and mixed with rotation for 1h. The syringe was then connected to a Sep-Pak, and the resin was washed with water (60 mL). The Sep-Pak was then eluted with 30% aqueous MeOH (50 mL), and the eluant was concentrated and redissolved in water, filtered through a Millex-GV 0.22 μm filter, and lyophilized to yield **85** (0.8 mg, 58%) as a white solid: ^{1}H NMR (600 MHz, D_2O) δ 4.57-4.54 (m, 2H, H-1 βGal, H-1 βGlcNAc), 4.12 (dd, 1H, J = 9.9, 3.1 Hz), 4.00-3.95 (m, 2H), 3.92-3.82 (m, 6H), 3.78 (d, 1H, J = 10.2 Hz), 3.75-3.55 (m, 10H), 2.76 (dd, 1H, J = 12.4, 4.6 Hz, H-3-eq αNeu5Ac), 2.05 (s, 3H, $COCH_3$), 2.04 (s, 3H, $COCH_3$), 1.81 (t, 1H, J = 12.4 Hz, H-3-ax αNeu5Ac), 1.55 (m, 2H, OCH_2CH_2), 1.35-1.24 (m, 13H, 3-C-CH_3, CH_2 octyl), 0.87 (t, 3H, J = 7.0 Hz, CH_3 octyl); HR-ESMS calcd for $C_{34}H_{60}N_2O_{19}Na(M+Na^+)$ 823.3688, found 823.3690.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Octyl 5-N-acetyl-neuraminyl-α-(2→3)-D-galactopyranosyl-(1→4)-[α-L-fucopyranosyl-(1→3)]–2-acetamido-2-deoxy-3-C-methyl-β-D-glucopyranoside (**86**).



The reaction mixture contained **85** (0.8 mg, 1.0 μmol), GDP-Fuc (0.7 mg, 1.2 μmol), 20 mU of human milk α-1,3.4-FucT (in 200 μL of 25 mM sodium cacodylate buffer, pH 6.5, containing 5 mM $MnCl_2$ and 25% glycerol, pH 6.5), 20 μL concentrated buffer (200 mM HEPES, 200 mM $MnCl_2$, and 2% BSA, pH 7.0) and 1 μL alkaline phosphatase (1 U/μL). The reaction was incubated at 37 °C with rotation for 1 day, an then at rt for 5 days. Additional GDP-Fuc (1.1 mg) was added during the incubation period. The reaction mixture was filtered through a Millex-GV 0.22 μm filter and loaded onto a C18 Sep-Pak C18 Sep-Pak cartridge. The cartridge was washed with water and eluted with MeOH. The eluant was concentrated and the resulting residue was purified on a column of Iatrobeads with 65:35:5 CH_2Cl_2/MeOH/H_2O. The product was then redissolved in water, filtered through a Millex-GV 0.22 μm filter, lyophilized to yield **86** (0.7 mg, 70%) as a white solid: ^{1}H NMR (600 MHz, D_2O) δ 5.30 (d, 1H, J = 3.9 Hz, H-1 αFuc), 4.70 (bq, 1H, J = 6.7 Hz, H-5 αFuc), 4.57 (d, 1H, J = 7.8 Hz, H-1 βGal), 4.52 (d, 1H, J = 8.4 Hz, H-1 βGlcNAc), 4.09 (dd, 1H, J = 10.0, 3.3 Hz), 4.07 (d, 1H, J = 10.4 Hz), 4.02 (d, 1H, J = 8.6 Hz), 3.97 (dd, 1H, J = 12.1, 2.2 Hz), 3.95-3.83 (m, 7H), 3.77 (d, 1H, J = 3.4 Hz), 3.73-3.63 (m, 6H), 3.62-3.51 (m, 5H), 2.77 (dd, 1H, J = 12.4, 4.6 Hz, H-3-eq αNeu5Ac), 2.05 (s, 3H, $COCH_3$), 2.04 (s, 3H, $COCH_3$), 1.80 (t, 1H, J = 12.4 Hz, H-3-ax αNeu5Ac), 1.53

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

(m, 2H, OCH_2CH_2), 1.48 (s, 3H, 3-*C*-CH_3), 1.34-1.23 (m, 10H, CH_2 octyl), 1.16 (d, 3H, *J* = 6.6 Hz, H-6 αFuc), 0.86 (t, 3H, *J* = 7.0 Hz, CH_3 octyl); HR-ESMS calcd for $C_{40}H_{70}N_2O_{23}Na(M+H^+)$ 969.4267, found 969.4269.

Octyl α-D-galactopyranosyl-(1→3)-β-D-3-C-methyl-galacto-pyranosyl-(1→4)-β-D-glucopyranoside (**87**).

A solution of 1.5 mL of calf thymus α-1,3-GalT containing 30 mM sodium cacodylate, 20 mM $MnCl_2$, 0.1% Triton X-100, pH 6.5, was concentrated to a total volume of 0.5 mL with the activity of 120 mU, using Slide-A-Lyzer. Disaccharide **2** (3.4 mg, 7.3 μmol), UDP-Gal (8.9 mg, 14.6 μmol), and alkaline phosphatase (1 U/μL, 10 μL) were added to the concentrated solution, and the mixture was incubated with rotation at rt for 5 days. The reaction mixture was filtered through glasswool and loaded onto two sequential C18 Sep-Pak cartridges. The cartridges were washed with water (100 mL), 10% aqueous methanol (40 mL) and then eluted with 50% aqueous MeOH (40 mL). The eluant was concentrated and redissolved in water, filtered through a Millex-GV 0.22 μm filter, and lyophilized to yield **87** (4.1 mg, 90%) as a white slid: ^{1}H NMR (500 MHz, D_2O) δ 5.35 (d, 1H, *J* = 4.0 Hz, H-1 αGal), 4.58 (d, 1H, *J* = 8.2 Hz, H-1 βGal), 4.48 (d, 1H, *J* = 8.0 Hz, H-1 βGlc), 4.23 (m, 1H), 4.00-3.96 (m, 2H), 3.94 (dd, 1H, *J* = 10.2, 3.3 Hz), 3.92-3.87 (m, 3H), 3.86 (dd, 1H, *J* = 10.2, 4.0 Hz), 3.81-3.70 (m, 6H), 3.68 (dt, 1H, *J* = 10.0, 6.8 Hz), 3.64-3.59 (m, 2H), 3.58 (m, 1H), 3.30 (m, 1H), 1.63 (m, 2H, OCH_2CH_2), 1.38-1.24 (m, 13H, 3'-*C*-CH_3, CH_2 octyl), 0.86 (t, 3H, *J* = 7.0 Hz, CH_3 octyl); ^{13}C NMR (125 MHz, D_2O) δ 102.84, 102.35 (C-1 βGal, C-1 βGlc), 93.08 (C-1 αGal), 80.87, 79.69, 75.52, 75.41, 74.93, 73.62, 73.34, 71.93, 71.59, 71.38,

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

70.16, 70.02, 69.04, 62.22, 61.91, 61.07, 31.87, 29.53, 29.22, 29.14, 25.83, 22.79, 15.97, 14.18; HR-ESMS calcd for $C_{27}H_{50}O_{16}Na$ ($M+Na^+$) 653.2998, found 653.2998.

Octyl α-D-galactopyranosyl-(1→4)-β-D-3-C-methyl-galacto-pyranosyl-(1→4)-β-D-glucopyranoside (**88**).

The reaction mixture contained disaccharide **2** (1.8 mg, 3.8 μmol), UDP-Gal (5.0 mg, 8.2 μmol), 20 mU of α-1,4-GalT (in 200 μL containing 52 mM HEPES, 10 mM ammonium acetate), and alkaline phosphatase (1 U/μL, 10 μL). The reaction was incubated with rotation at rt for 4 days. The reaction mixture was filtered through glasswool and loaded onto a C18 Sep-Pak cartridge which was washed with water (50 mL), 10% aqueous methanol (20 mL) and then eluted with 60% aqueous MeOH (30 mL). The eluant was concentrated and redissolved in water, filtered through a Millex-GV 0.22 μm filter, and lyophilized to yield **88** (2.3 mg, 95%) as a while solid: ^{1}H NMR (500 MHz, D_2O) δ 5.01 (d, 1H, J = 4.0 Hz, H-1 αGal), 4.60 (d, 1H, J = 8.0 Hz, H-1 βGal), 4.47 (d, 1H, J = 8.0 Hz, H-1 βGlc), 4.28 (bt, 1H, J = 6.5 Hz), 4.02 (bd, 1H, J = 3.3 Hz), 3.99-3.79 (m, 9H), 3.73-3.60 (m, 6H), 3.57 (m, 1H), 3.29 (m, 1H), 1.62 (m, 2H, OCH_2CH_2), 1.39-1.24 (m, 13H, 3'-C-CH_3, CH_2 octyl), 0.86 (t, 3H, J = 7.0 Hz, CH_3 octyl); ^{13}C NMR (125 MHz, D_2O) δ 103.07, 102.79 (C-1 βGal, C-1 βGlc), 101.80 (C-1 αGal), 84.02, 80.00, 75.66, 75.52, 75.44, 74.67, 74.25, 76.66, 72.42, 69.83, 69.81, 61.64, 61.52, 61.03, 31.88, 29.54, 29.23, 29.15, 25.84, 22.79, 19.27, 14.19; HR-ESMS calcd for $C_{27}H_{50}O_{16}Na$ ($M+Na^+$) 653.3002, found 653.2997.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Octyl α-D-galactopyranosyl-(1→3)-[α-L-fucopyranosyl-(1→2)]-3-C-methyl-β-D-galactopyranoside (**89**).

The reaction mixture contained disaccharide **7** (2.4 mg, 5.3 μmol), UDP-Gal (5.0 mg, 8.2 μmol), 750 mU of blood group B glycosyltransferase (in 150 μL containing 35 mM sodium cacodylate, 20 mM $MnCl_2$, and 1 mg/mL BSA), 40 μL of concentrated buffer (350 mM sodium cacodylate, 200 mM $MnCl_2$, and 10 mg/mL BSA, pH 7.0) and 4 μL of alkaline phosphatase (1 U/μL). The reaction was incubated with rotation at rt for 2 days. The reaction mixture was filtered through glasswool and loaded onto a C18 Sep-Pak cartridge which was washed with water (50 mL), 10% aqueous methanol (20 mL) and then eluted with 80% aqueous MeOH (25 mL). The eluant was concentrated and redissolved in water, filtered through a Millex-GV 0.22 μm filter, lyophilized to yield **89** (3.2 mg, quant.) as a white solid: ^{1}H NMR (500 MHz, D_2O) δ 5.38 (d, 1H, J = 4.0 Hz, H-1 αGal), 5.32 (d, 1H, J = 4.0 Hz, H-1 αFuc), 4.60 (d, 1H, J = 8.0 Hz, H-1 βGal), 4.46 (bq, 1H, J = 6.6 Hz, H-5 αFuc), 4.14 (m, 1H, H-5 αGal), 4.03 (d, 1H, J = 3.1 Hz, H-4 αGal), 3.98 (s, 1H, H-4 βGal), 3.93 (dt, 1H, J = 9.8, 6.6 Hz, OCH_2CH_2), 3.90-3.69 (m, 11H), 3.65 (dt, 1H, J = 9.8, 6.6 Hz, OCH_2CH_2), 1.60 (m, 2H, OCH_2CH_2), 1.48 (s, 3H, 3-C-CH_3), 1.37-1.24 (m, 10H, CH_2 octyl), 1.22 (d, 3H, J = 6.6 Hz, H-6 αFuc), 0.87 (t, 3H, J = 7.0 Hz, CH_3 octyl); ^{13}C NMR (125 MHz, D_2O) δ 101.09 (C-1 βGal), 99.26 (C-1 αFuc), 93.26 (C-1 αGal), 83.84, 76.36, 74.33, 72.80, 72.57, 71.47, 70.58, 70.33, 70.25, 69.94, 69.08, 68.80, 67.51, 62.20, 61.93, 31.92, 29.75, 29.37, 29.23, 26.26, 22.80, 17.24, 16.17, 14.20; HR-ESMS calcd for $C_{27}H_{50}O_{15}Na$ (M+Na$^+$) 637.3047, found 637.3057.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Octyl α-D-galactopyranosyl-(1→3)-[α-L-fucopyranosyl-(1→2)]-3-C-propyl-β-D-galactopyranoside (**90**).

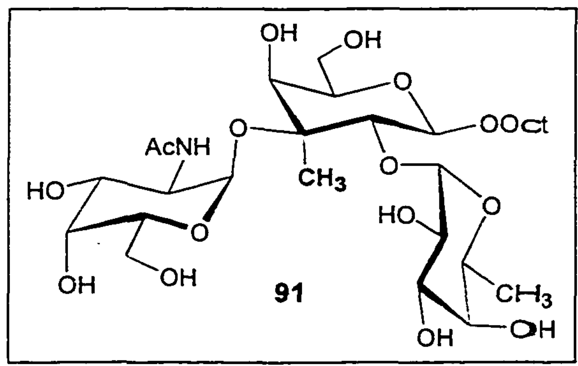
OH OH O OOct O Pr OH HO O HO O O OH OH 90 CH3 OH OH

The reaction mixture contained disaccharide **8a** (1.2 mg, 2.5 μmol), UDP-Gal (3.1 mg, 5.1 μmol), 0.5 U of blood group B glycosyltransferase (in 100 μL containing 35 mM sodium cacodylate, 20 mM $MnCl_2$, and 1 mg/mL BSA), 35 μL of concentrated buffer (350 mM sodium cacodylate, 200 mM $MnCl_2$, and 10 mg/mL BSA, pH 7.0), 5 μL of alkaline phosphatase (1 U/μL) and 200 μL of water. The reaction was incubated with rotation at 37 °C for 22 days. Additional UDP-Gal (6.2 mg), blood group B glycosyltransferase (1.5 U), and alkaline phosphatase (10 μL) were added during the incubation period. The reaction mixture was filtered through glasswool and loaded onto two sequential C18 Sep-Pak cartridge which was washed with water (85 mL), 10% aqueous methanol (45 mL) and then eluted with 60% aqueous MeOH (35 mL). The eluant was concentrated and redissolved in water, filtered through a Millex-GV 0.22 μm filter, and lyophilized to yield **90** (1.6 mg, quant.) as a white solid: ^1H NMR (500 MHz, D_2O) δ 5.43 (d, 1H, J = 4.2 Hz, H-1 αGal), 5.29 (d, 1H, J = 4.0 Hz, H-1 αFuc), 4.68 (d, 1H, J =7.9 Hz, H-1 βGal), 4.44 (bq, 1H, J = 6.6 Hz, H-5 αFuc), 4.19-4.16 (m, 2H, H-5 αGal, H-4 βGal), 4.06 (d, 1H, J = 3.3 Hz, H-4 αGal), 3.92 (dt, 1H, J = 9.9, 6.6 Hz, OCH_2CH_2), 3.91-3.86 (m, 3H), 3.84-3.69 (m, 8H), 3.66 (dt, 1H, J = 9.9, 6.6 Hz, OCH_2CH_2), 2.02 (m, 1H, $CH_2CH_2CH_3$), 1.77 (m, 1H, $CH_2CH_2CH_3$), 1.66-1.56 (m, 3H, $CH_2CH_2CH_3$, OCH_2CH_2), 1.40-1.24 (m, 11H, $CH_2CH_2CH_3$, CH_3 octyl), 1.22 (d, 3H, J = 6.6 Hz, H-6 αFuc), 0.90 (t, 3H, J = 7.0 Hz, $CH_2CH_2CH_3$), 0.87 (t, 3H, J = 7.0 Hz, CH_3 octyl); ^{13}C NMR (125 MHz, D_2O) δ 101.33 (C-1 βGal), 99.47 (C-1 αFuc), 93.53 (C-1 αGal), 84.85, 77.48, 74.10, 72.81, 72.35, 71.51, 70.37, 70.28, 69.80, 69.24, 69.11, 67.58,

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

62.09, 61.40, 34.87, 31.91, 29.75, 29.37, 29.23, 26.25, 22.81, 16.72, 16.16, 15.15, 14.19; HR-ESMS calcd for $C_{29}H_{54}O_{15}Na$ ($M+Na^+$) 665.3360, found 665.3355.

Octyl α-D-2-acetamido-2-deoxy-galactopyranosyl-(1→3)-[α-L-fucopyranosyl-(1→2)]-3-C-methyl-β-D-galactopyranoside **(91)**.

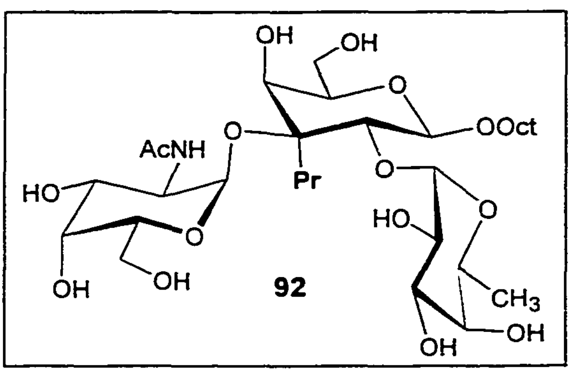


The reaction mixture contained disaccharide **7** (1.2 mg, 2.7 μmol), UDP-GalNAc (5.4 mg, 8.3 μmol), 1.45 mU of blood group A glycosyltransferase (in 490 μL containing 35 mM sodium cacodylate, 20 mM $MnCl_2$, and 1 mg/mL BSA), 80 μL of concentrated buffer (350 mM sodium cacodylate, 200 mM $MnCl_2$, and 10 mg/mL BSA, pH 7.0) and 10 μL of alkaline phosphatase (1 U/μL). The reaction was incubated with rotation at 37 °C for 4 days. The reaction mixture was filtered through glasswool and loaded onto a C18 Sep-Pak cartridge which was washed with water (70 mL), 10% aqueous methanol (45 mL) and then eluted with 60% aqueous MeOH (35 mL). The eluant was concentrated and redissolved in water, filtered through a Millex-GV 0.22 μm filter, and lyophilized to yield **91** (1.6 mg, 92%) as a white solid: ^1H NMR (500 MHz, D_2O) δ 5.36 (d, 1H, $J = 3.8$ Hz, H-1 αFuc), 5.32 (d, 1H, $J = 3.7$ Hz, H-1 αGal), 4.60 (d, 1H, $J = 8.2$ Hz, H-1 βGal), 4.50 (bq, 1H, $J = 6.6$ Hz, H-5 αFuc), 4.22-4.17 (m, 2H, H-2 and H-5 αGal), 4.06 (d, 1H, $J = 2.4$ Hz, H-4 αGal), 4.00 (s, 1H, H-4 βGal), 3.94 (dt, 1H, $J = 9.8, 6.6$ Hz, OCH_2CH_2), 3.90-3.84 (m, 2H, H-3 αFuc, H-2 βGal), 3.84-3.70 (m, 8H), 3.66 (dt, 1H, $J = 9.8, 6.6$ Hz, OCH_2CH_2), 2.02 (s, 3H, $COCH_3$), 1.61 (m, 2H, OCH_2CH_2), 1.38-1.26 (m, 10H, CH_2 octyl), 1.24 (d, 3H, $J = 6.6$ Hz, H-6 αFuc), 0.87 (t, 3H, $J = 7.0$ Hz, CH_3 octyl); ^{13}C NMR (125 MHz, D_2O) δ 175.46, 101.11 (C-1 βGal), 99.09 (C-1 αFuc), 92.65 (C-1 αGal), 83.88, 76.22, 75.138, 72.80, 72.01, 71.64, 70.30, 69.79, 69.20, 69.02, 69.00, 67.64,

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

62.24, 61.71, 50.71, 31.91, 29.78, 29.36, 29.23, 26.28, 22.91, 22.80, 18.85, 16.15, 14.19; HR-ESMS calcd for $C_{29}H_{53}NO_{15}Na$ ($M+Na^+$) 678.3313, found 678.3310.

Octyl α-D-2-acetamido-2-deoxy-galactopyranosyl-(1→3)-[α-L-fucopyranosyl-(1→2)]-3-C-propyl-β-D-galactopyranoside (**92**).



The reaction mixture contained disaccharide **8a** (1.2 mg, 2.5 μmol), UDP-GalNAc (3.3 mg, 5.1 μmol), 1.4 U of blood group A glycosyltransferase (in 200 μL containing 35 mM sodium cacodylate, 20 mM $MnCl_2$, and 1 mg/mL BSA), 10 μL of concentrated buffer (350 mM sodium cacodylate, 200 mM $MnCl_2$, and 10 mg/mL BSA, pH 7.0), 10 μL of alkaline phosphatase (1 U/μL) and 220 μL of water. The reaction was incubated with rotation at 37 °C for 26 days. Additional UDP-GalNAc (9.3 mg), blood group A glycosyltransferase (4.5 U), and alkaline phosphatase (10 μL) were added during the incubation period. The reaction mixture was filtered through glasswool and loaded onto two sequential C18 Sep-Pak cartridge which was washed with water (75 mL), 10% aqueous methanol (40 mL) and then eluted with 60% aqueous MeOH (40 mL). The eluant was concentrated and redissolved in water, filtered through a Millex-GV 0.22 μm filter, and lyophilized to yield **92** (1.6 mg, 94%) as a white solid: ^1H NMR (500 MHz, D_2O) δ 5.36 (d, 1H, J = 4.0 Hz, H-1 αFuc), 5.32 (d, 1H, J = 4.0 Hz, H-1 αGal), 4.67 (d, 1H, J = 7.9 Hz, H-1 βGal), 4.43 (bq, 1H, J = 6.6 Hz, H-5 αFuc), 4.22-4.17 (m, 2H, H-2 and H-5 αGal), 4.10 (s, 1H, H-4 βGal), 4.08 (d, 1H, J = 3.1 Hz, H-4 αGal), 3.93 (dt, 1H, J = 9.7, 6.8 Hz, OCH_2CH_2), 3.91 (d, 1H, J = 7.9 Hz, H-2 βGal), 3.88 (dd, 1H, J = 10.4, 3.3 Hz, H-3 αFuc), 3.85-3.81 (m, 2H, H-3 αGal, H-4 αFuc), 3.79-3.70 (m, 6H), 3.66 (dt, 1H, J = 9.7, 6.8 Hz, OCH_2CH_2), 2.03 (s, 3H, $COCH_3$), 1.89 (m, 1H, $CH_2CH_2CH_3$), 1.79

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

(m, 1H, $CH_2CH_2CH_3$), 1.65-1.54 (m, 3H, $CH_2CH_2CH_3$, OCH_2CH_2), 1.38-1.16 (m, 14 H, H-6 αFuc, $CH_2CH_2CH_3$, CH_2 octyl), 0.91-0.85 (m, 6H, $CH_2CH_2CH_3$, CH_3 octyl). ^{13}C NMR (125 MHz, D_2O) δ 175.40, 101.71 (C-1 βGal), 98.97 (C-1 αFuc), 92.54 (C-1 αGal), 84.76, 77.92, 74.31, 72.81, 72.03, 71.65, 70.46, 69.25, 69.03, 69.00, 67.75, 66.78, 61.95, 61.25, 51.09, 34.71, 31.92, 29.76, 29.37, 29.23, 26.25, 22.96, 22.82, 16.21, 16.19, 14.92, 14.21; HR-ESMS calcd for $C_{31}H_{51}NO_{15}Na$ ($M+Na^+$) 706.3621, found 706.3626.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Chapter 4

Carbohydrate-Protein Binding Studies with Glycosides of Tertiary Alcohols

4.1. Introduction

Natural carbohydrate recognition is mediated by several classes of proteins. The most important classes are: (a) enzymes, both glycosyltransferases [11] and glycosidases [102], which biosynthesize the carbohydrates either assembling or cleaving; (b) antibodies [103], of which over 70% can be normally directed towards oligosaccharide epitopes; and (c) lectins [104], which bind mono- and oligosaccharides with high specificity, but are devoid of catalytic activity. In contrast to antibodies, these are not products of an immune response.

A notable feature of carbohydrate recognition with all these proteins is that binding is often weak, compared to other types of ligands, with dissociation constants rarely below the micromolar range and much more commonly in the millimolar range. The oligosaccharides that mediate biological events can be large, but the actual size of the epitope recognized is usually only three to four sugar residues and, within these, the crucial recognition element can be even smaller.

Carbohydrate-protein recognition is generally established through networks of hydrogen bonds and van der Waals contacts including packing of a hydrophobic sugar face against aromatic amino acid residues (Figure 4.1) [105]. The free energy change (ΔG) during the carbohydrate-protein recognition process comprises both enthalpic (ΔH)

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

and entropic contributions (TΔS). ΔH is usually associated with direct carbohydrate-protein interactions such as hydrogen bonding and van der Waals interactions, and ΔS is associated with solvent reorganization and flexibility of both carbohydrate and protein [106].

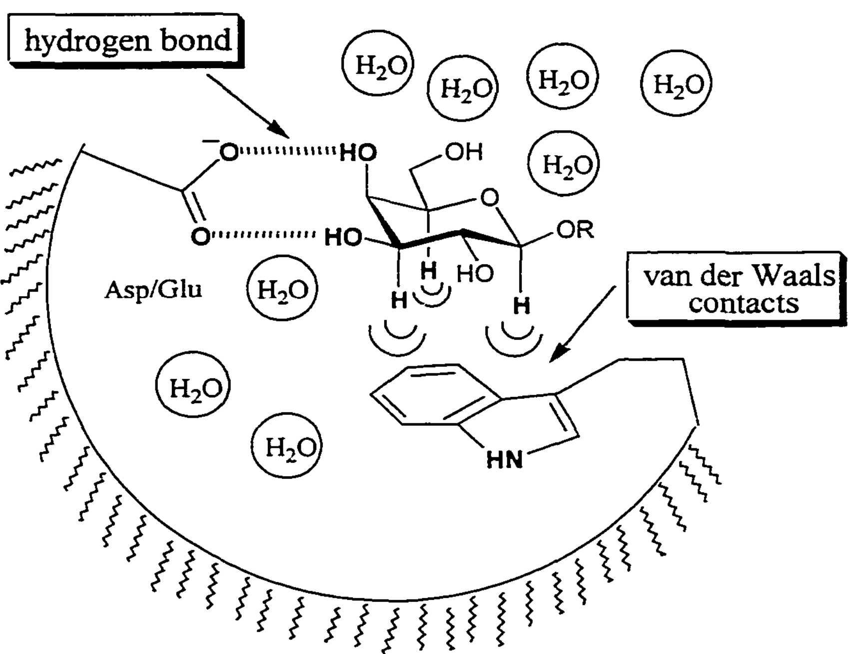


Figure 4.1. Schematic representation of carbohydrate-protein binding.

There is considerable evidence that the majority of oligosaccharides are flexible and that they exist as many conformers [107, 108]. It has been suggested that the loss of flexibility of a carbohydrate in binding to a protein may introduce an unfavorable entropic term, which may partly account for the relatively low binding affinity [108]. The entropic barrier associated with the loss of degrees of freedom when an oligosaccharide binds to a protein has been estimated to be as large as 1-2 kcal mol^{-1} for each glycosidic torsional angle immobilized [108]. Other estimates for freezing single bond rotamers are more conservative at ~0.6 kcal mol^{-1} per torsion angle [109]. It is therefore of great interest to

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

see whether the binding affinity of glycosides of tertiary alcohols will be increased since they are conformationally more restricted due to the steric interactions between sugar rings.

Several attempts have been made to exploit conformational entropy by either locking or biasing conformations toward the bioactive conformation (Figure 4.2). Lindh and Hindsgaul synthesized the fused-ring trisaccharide analogs representing conformationally restricted models for the “gg” and “gt” conformations [67a]. Evaluation of these two analogs with *N*-acetylglucosaminyltransferase V showed that the "gt" analog had a K_m (29 μM) almost identical to the flexible parent acceptor (21 μM) while the "gg" analog had an almost 500-elevated K_m (9.8 mM). Magnusson and co-workers studied bacterial protein recognition with a restricted galabiose derivative where the 6-OH and 2’-OH groups were tethered through a methylene bridge [110]. The restricted analog, however, had a decreased inhibitory efficiency. Kolb reported the binding studies of a macrocyclic mimic of sialyl Lewis X with E-selectin [111]. The compound had about three times lower affinity than the unrestricted parent sialyl Lewis X. Kolb and Ernst further used a computational model to assess the preorganization of a potential inhibitor of E-selectin [112]. The results suggested that only preorganized compounds that populate the bioactive conformations are likely to bind. The Bundle group recently reported thermodynamic studies of a monocolonal antibody with tethered oligosaccharides [113, 114]. Titration calorimetry revealed free energy changes ($\Delta\Delta G^o$) no larger than ± 0.5 kcal mol^{-1} with compensatory changes in enthalpy and entropy [114]. Boons and co-workers designed and synthesized two tethered trisaccharides for studies of lectin binding [115, 116]. The trisaccharide, which contains a methylene acetal bridge that mimics an O-4···O-6" intramolecular bond, was found to have a more favorable entropy of binding, which, however, was offset by a less favorable enthalpy term [116].

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Lindh and Hindsgaul

(gg)

(gt)

Magnusson and co-workers

Kolb

Bundle and co-workers

Boons and co-workers

R = $(CH_2)_n$, Z = O (n= 6, 7,8);
or R = $CH_2C_6H_4CH_2$, Z = S

Figure 4.2. Examples of constrained oligosaccharides used in carbohydrate-protein binding studies.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

This chapter describes protein binding studies with the glycosides of tertiary alcohols produced by the chemoenzymatic approach. The analog of Lewis X, **83**, was tested against α-fucosidase. The sialyl Lewis X analog **86** was tested with E-selectin. The analogs of the α-Gal epitope and blood group A and B trisaccharides were tested with *Griffonia simplicifolia* I (GSI) B_4 isolectin.

4.2. Studies of Lewis X Analog 83 with α-Fucosidase

α-Fucosidase from almond meal (E.C. 3.2.1.111) is a glycosidase that selectively cleaves α1→3 or α1→4 linked fucose residue from glycans [117]. The kinetic parameters for the hydrolysis were determined using a spectrophotometric assay. As shown in Figure 4.3, the fucose released by α-fucosidase could be quantitated by using L-fucose dehydrogenase and $NADP^+$, and monitoring the increase of UV absorbance of NADPH at 340 nm.

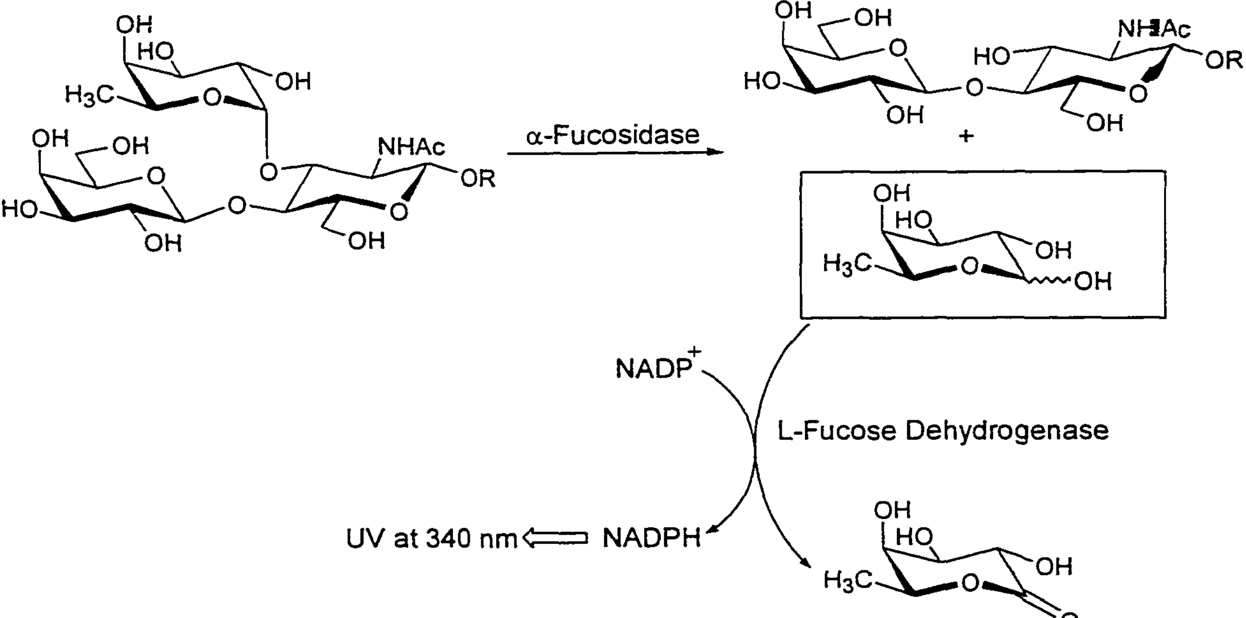


Figure 4.3. Schematic representation of spectrophotometric assay for α-fucosidase.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

As shown in Figure 4.4, the analog **83** had similar kinetic properties as Lewis X. The introduction of the methyl group resulted in slightly improved recognition of the substrate (with a 40% decrease in K_m) and only a modest reduction (30%) in the V_{max}.

Figure 4.4. Kinetic properties of Lewis X analog **83** with α-fucosidase.

4.3. Studies of Sialyl Lewis X Analog 86 with E-Selectin

Interactions of sialyl Lewis X with a family of C-type lectin (Ca^{2+} dependent), the so called selectins (E-, L-, and P-selectin), are responsible for the first step in the multistage process of leukocyte recruitment to sites of injury or inflammation [118]. The results from structure/activity studies reveal that the 2-, 3- and 4-OH groups of the Fuc residue, the 4- and 6-OH groups of the Gal residue and the COO^- group of the sialic acid are essential for binding to E-selectin (Figure 4.5) [119].

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

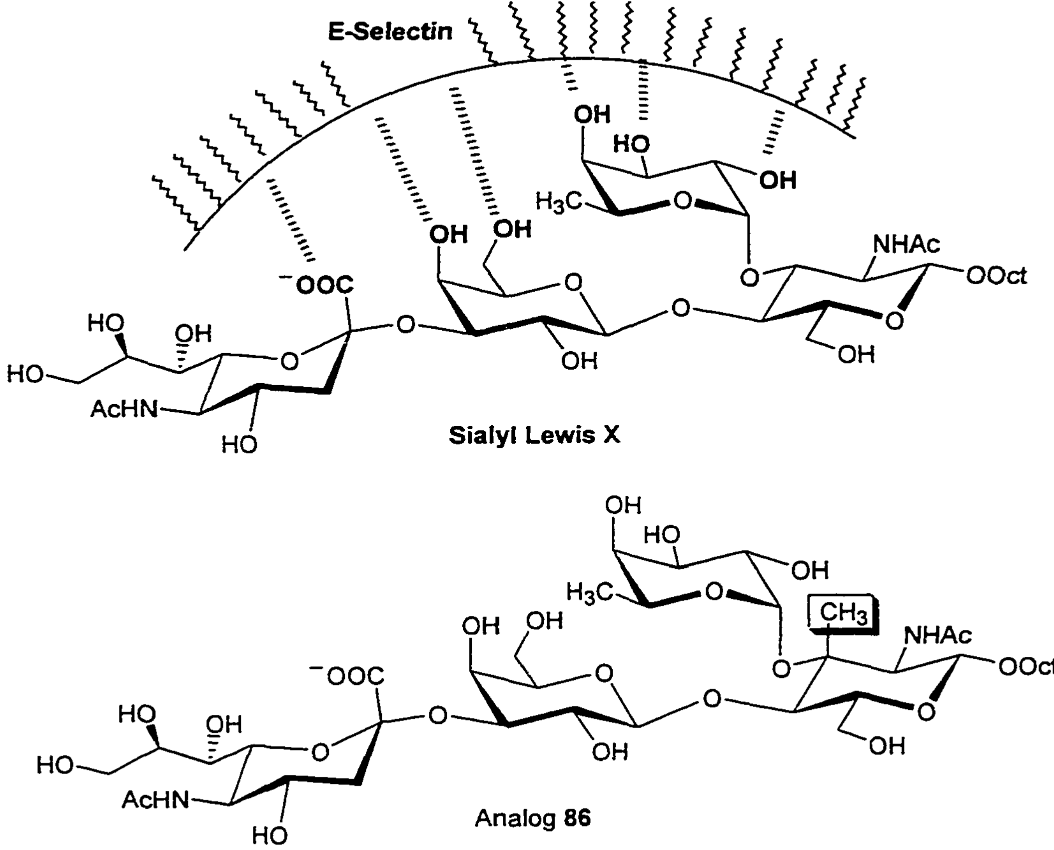


Figure 4.5. Top: schematic representation of the binding of sialyl Lewis X to E-selectin. Bottom: the structure of analog **86**.

The binding assays were performed at Novartis Pharma Ltd. [112]. Analog **86** was found to have very weak binding activity with E-selectin as no inhibition was detected at 10 mM while the IC_{50} for parent sialyl Lewis X was 1.0 mM [120]. The drastic decrease in the bioactivity suggests that **86** may adopt a conformation unsuitable for binding where the fucose orientation could be slightly distorted from the optimum as a result of the introduction of methyl group at the Fucα1$\rightarrow$3GlcNAc linkage. Alternatively, the C-methyl group could itself sterically interfere with the binding.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

4.4. Studies of Analogs 88-92 with GSI-B_4 Isolectin

Griffonia simplicifolia Baill. (formerly known as *Bandeiraea simplicifolia* Benth.) is a leguminous shrub which grows in the rain forests of northwest Africa. The seeds of this plant contain a series of lectins with different carbohydrate binding specificities. These include the *Griffonia simplicifolia* I (GS I) lectins which recognize Gal and GalNAc, GS II lectin which binds to GalNAc, and GS IV which recognizes the Lewis b blood group antigen [121]. GS I lectin consists of a mixture of five isolectins. They are tetrameric structures composed of varying proportions of two different subunits, A and B, and are designated GS I-A_4, -A_3B, A_2B_2, AB_3, B_4 (Figure 4.6). The A subunit recognizes both terminal α-GalNAc and α-Gal residues. The B subunit is very specific for terminal α-Gal residue only [122].

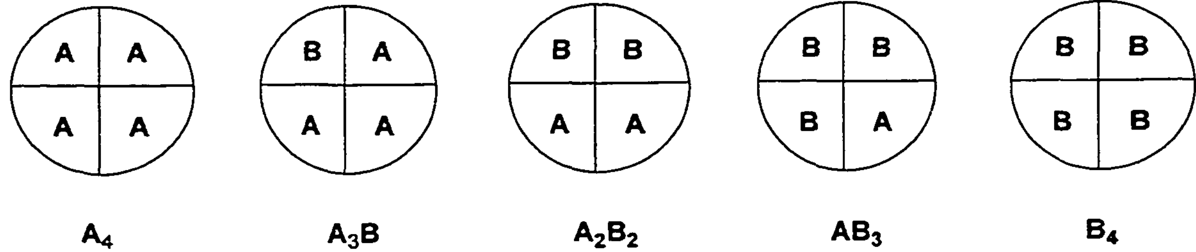


Figure 4.6. Schematic representation of the tetrametric structure of the five GS I isolectins consisting of varying proportions of A and B subunits.

The assays were carried out using frontal affinity chromatography [123] with mass spectrometric detection (FAC/MS), a method developed by Hindsgaul and co-workers [124], and were performed by Dr. Boyan Zhang. In the assay shown schematically in Figure 4.7, controlled porous glass (CPG) beads bearing covalently-coupled streptavidin are packed into a miniature column (10 μL). The GSI-B_4 isolectin is biotinylated and then immobilized by infusing through the column. Compounds with different binding affinities will interact differently with the immobilized protein. All non-binding

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

compounds will elute in the void volume (V_0) at their infusion concentration. Active ligands will bind to the immobilized GSI-B_4 isolectin and eventually exceed the capacity of the column when they will begin eluting at their infusion concentration. The order of elution parallels the order of affinity, with the strongest binding ligands eluting the latest (Figure 4.7). Compounds eluent were detected by mass spectrometry which enables the analysis of a large number of components that have m/z values different from each other.

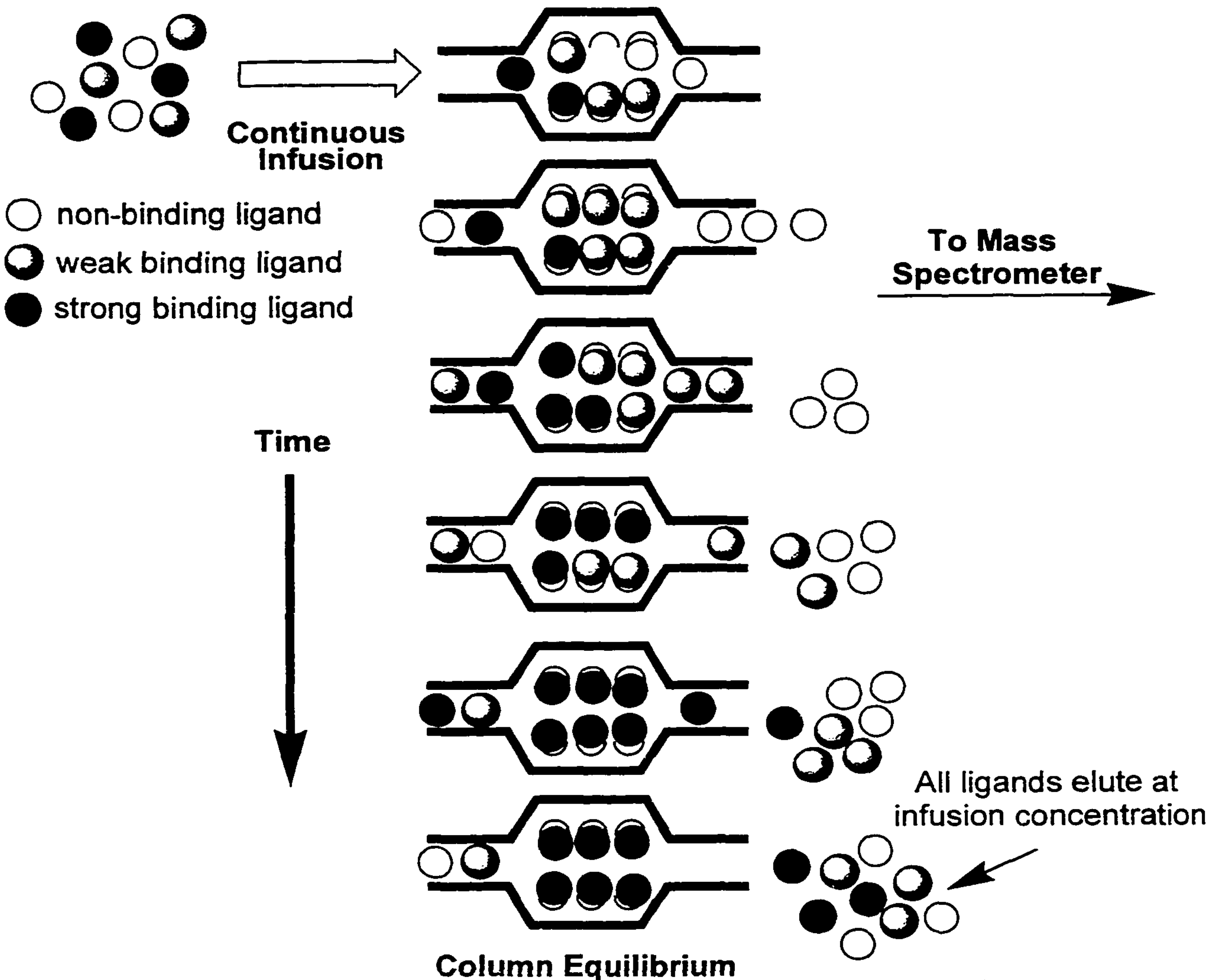


Figure 4.7. Schematic representation of the FAC/MS assay.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

A mixture composed of Glcβ-O-Oct, Lacβ-O-MCO, CD, A and B trisaccharides, **87**, and **89-92** (structures shown in Figure 4.8) was assayed using this FAC/MS method. A total ion chromatogram (TIC) (Figure 4.9, top) was constructed from a 9 min run time. Peaks at specific m/z values could then be identified through the analysis of the TIC to give the selected ion chromatograms (SIC) (Figure 4.9, bottom). As can be seen from the SIC, A trisaccharide, A-Me (**91**) and A-Pr (**92**) with terminal α-GalNAc residue break through the column at almost same time as the Glcβ-O-Oct and Lacβ-O-MCO (void volume markers) while B and CD trisaccharides, CD-Me (**87**) and B-Me (**89**) with terminal α-Gal residue break through later with longer retention times. This is in good agreement with the fact that the GSI-B_4 specifically recognizes the terminal α-Gal residue. Interestingly, the introdution of a methyl group at the Galα1→3Gal linkage of B and CD trisaccharides produced the stronger binding ligands **87** and **89** while analog **90** with a propyl group at the Galα1→3Gal linkage had much weaker binding with the GSI-B_4 isolectin.

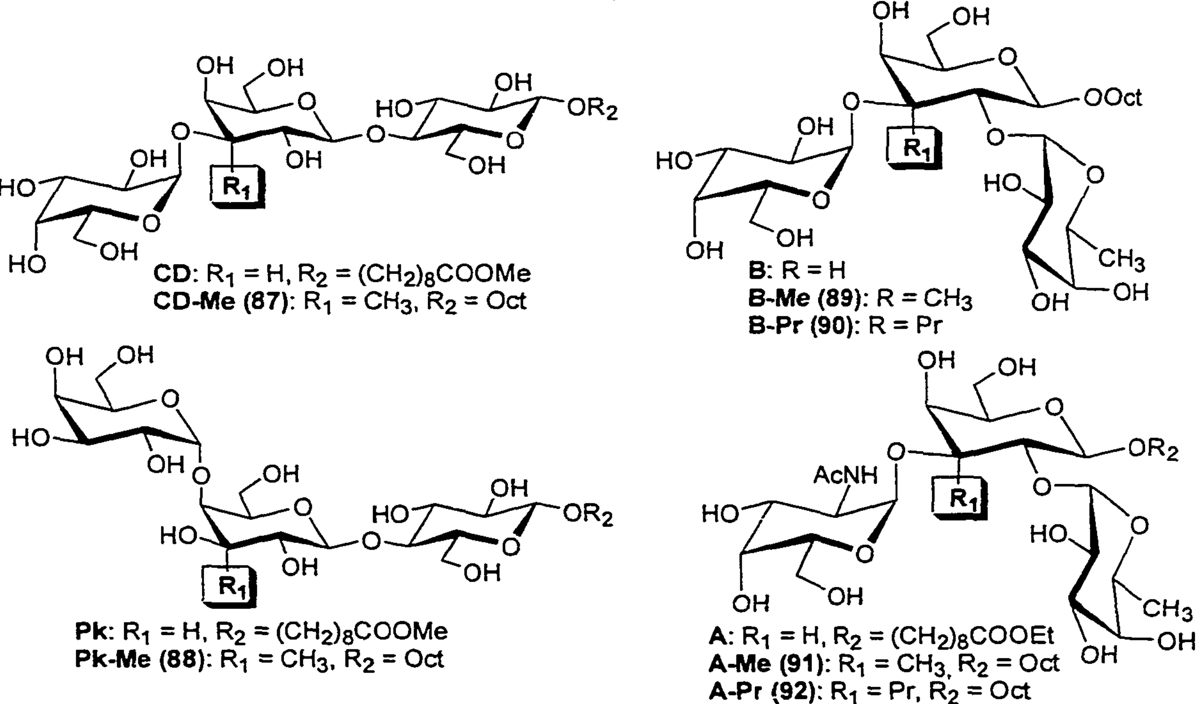


Figure 4.8. Structures of the compounds analyzed by FAC/MS.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

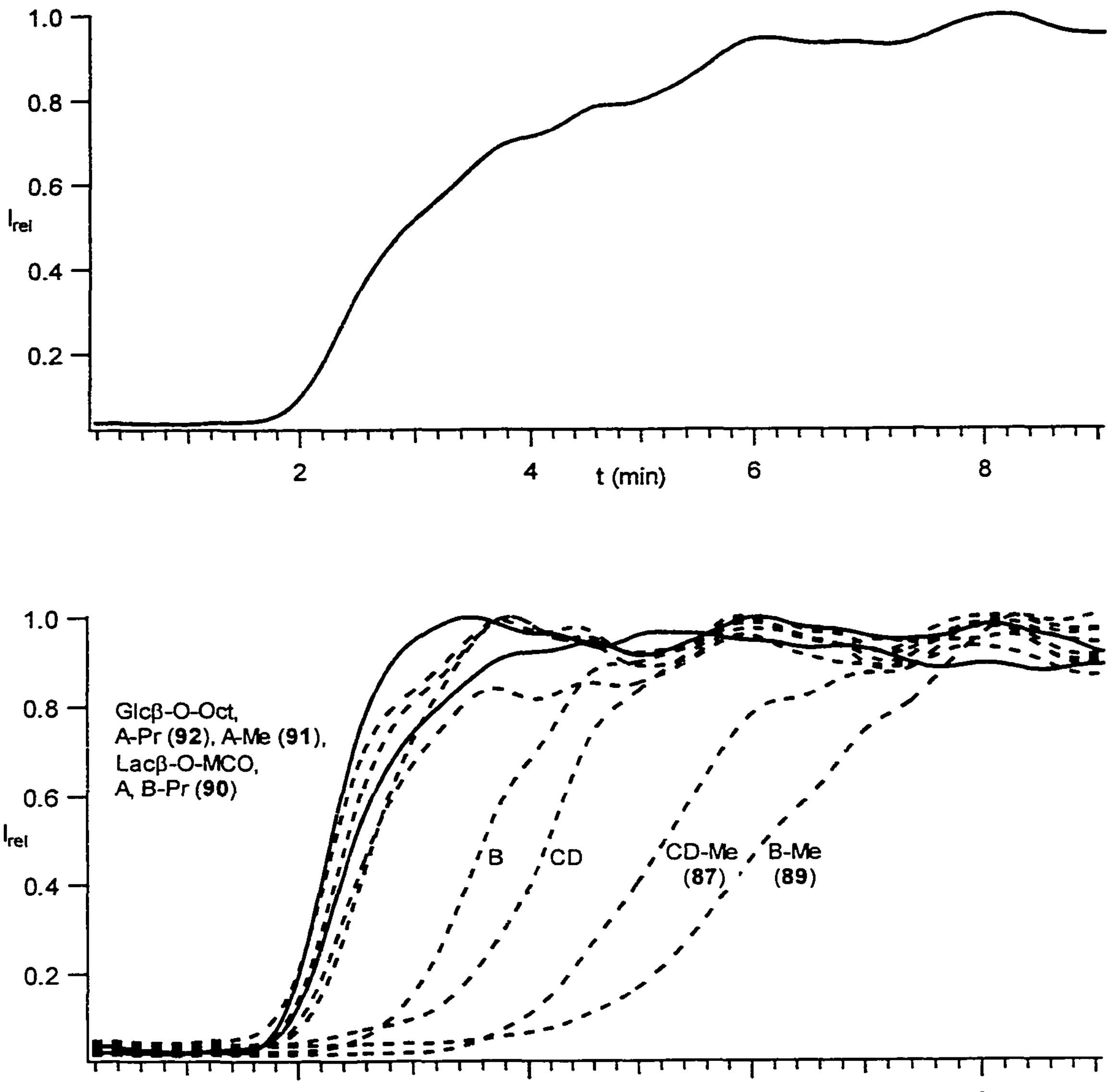


Figure 4.9. Top: Total ion chromatogram (TIC). Middle: Selected ion chromatograms of all ten compounds.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

The dissociation constants (K_d) were further determined individually using equation (1) [123],

$$V_X - V_0 = B_t / \{[X]_0 + (K_d)_X\} \quad (1)$$

where V_X is the elution volume of compound X, V_0 is the elution volume of void marker, and B_t represents the dynamic binding capacity of the column (total amount of the active lectin). The B_t value was determined to be 105 pmol by infusing the CD-Me (**87**) through the column at various concentrations and measuring the V-V_0. As shown in the Table 4.1, the monosaccharide Galα-O-Me had a K_d of 19.3 μM, only about a factor of two different from the value (48.3 μM) determined by equilibrium dialysis where GSI-B_4 isolectin was not immobilized [125]. The disaccharide Galα1→3Galα-O-Me had almost the same K_d value as Galα-O-Me, which was consistent with the results from precipitation studies [122]. While the K_d of its anomer, Galα1→3Galβ-O-Me, was about two times higher. The B trisaccharide containing the Galα1→3Galβ sequence had K_d value similar to Galα1→3Galβ-O-Me, indicating that the Fuc residue on the B trisaccharide had little effect on the binding. It was also interesting that the Pk trisaccharide with Galα1→4Gal sequence was found to be a better ligand for GSI-B_4 than Galα1→3Gal containing compounds.

The K_d values of glycosides of tertiary alcohols **87** and **89**, which showed stronger binding in the assay of the multiple-ligand mixture, were about two and four times lower than those of their parent trisaccharides, with $\Delta(\Delta G^o)$ of - 0.39 and -0.75 kcal mol^{-1} respectively. Since the NMR studies showed that the conformation of **87** and **89** were similar to those of parent CD and B trisaccharides, such an increase in the binding afffinity may be attributed to the less negative entropic contributions as the Galα1→3Gal linkage was much less conformationally flexible. This explanation is also consistent with the fact that Pk-Me (**88**) had almost the same dissociation constant as the Pk trisaccharide since the methyl group at the 3-position of βGal residue could not restrict the flexibility

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Table 4.1. Dissociation constants of the oligosaccharide analogs with GSI-B_4 isolectin.

Carbohydrate	m/z ($M+Na^+$)	K_d (μM)[a]
Galα-O-Me	217.1	19.3 ± 0.8 (48.3)[b]
Galβ-O-Me	217.1	-
Galα1→3Galα-O-Me	379.1	16.5 ± 0.1
Galα1→3Galβ-O-Me	379.1	29.4 ± 6.1
CD	697.3	23.3 ± 4.0
CD-Me (**87**)	653.3	12.0 ± 1.8
B	623.3	29.6 ± 0.2
B-Me (**89**)	637.5	8.3 ± 0.3
B-Pr (**90**)	665.3	-
A	736.5	-
A-Me (**91**)	678.3	-
A-Pr (**92**)	706.4	-
Pk	697.3	9.2 ± 1.1
Pk-Me (**88**)	653.3	8.6 ± 0.3

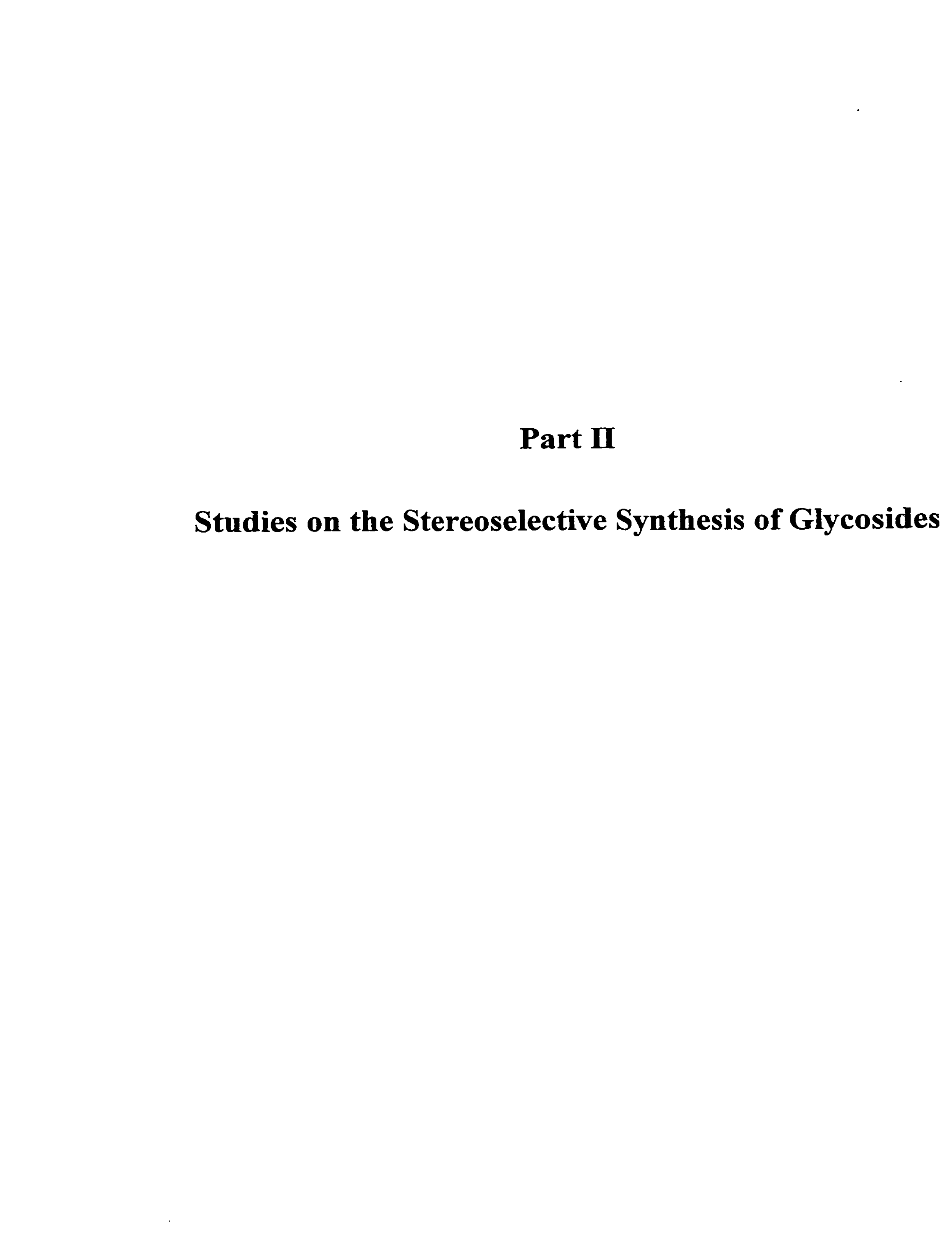
a) values determined from infusion of individual ligand with Lacβ-O-MCO as a void volume marker. b) value determined by equilibrium dialysis [125].

of the Galα1→4Gal linkage. Future measurements on the thermodynamic data using titration calorimetry would be able to reveal whether the increased binding strength is a result of the favorable enthalpic change or entropic change. Interestingly, the introduction of a propyl group to the Galα1→3Gal linkage produced an almost inactive compound. Such a drastic change on the binding activity suggested that there may be a steric clash between the propyl group and the protein, since NMR studies showed that the conformation was not significantly distorted by the propyl group.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

In contrast to the tethering strategy that has been shown to fail to produce stronger binding ligands, our results suggest that the introduction of a small alkyl group at the glycosidic linkage can be a useful strategy to produce a conformationally constrained ligand with stronger binding affinity.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.



Part II

Studies on the Stereoselective Synthesis of Glycosides

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Chapter 5

Introduction

5.1. Stereoselective Glycosylation

Glycosylation is the key reaction in oligosaccharide synthesis [126]. In the process, a glycosyl donor and acceptor are coupled together using an appropriate promoter. Tremendous recent advances in understanding the roles of carbohydrates have highlighted the need to devise efficient and stereoselective syntheses of oligosaccharides and glycoconjugates.

Broadly speaking, there are two general classes of stereochemistry produced by glycosylation reactions: 1,2-*trans* and 1,2-*cis*. In the 1,2-*trans*-glycosylation, a β-glycosidic bond in the *gluco-* series (glucosides and others with O-H-2 equatorial) and an α-glycosidic bond in the *manno-* series are formed. Correspondingly, α-glycosides in the *gluco-* series and β-mannosides are formed in the 1,2-cis-glycosylations (Figure 5.1) [126]. Sialic acids and 2-deoxy sugars lack a neighboring OH group, and special methods are required for stereoselective glycosylation.

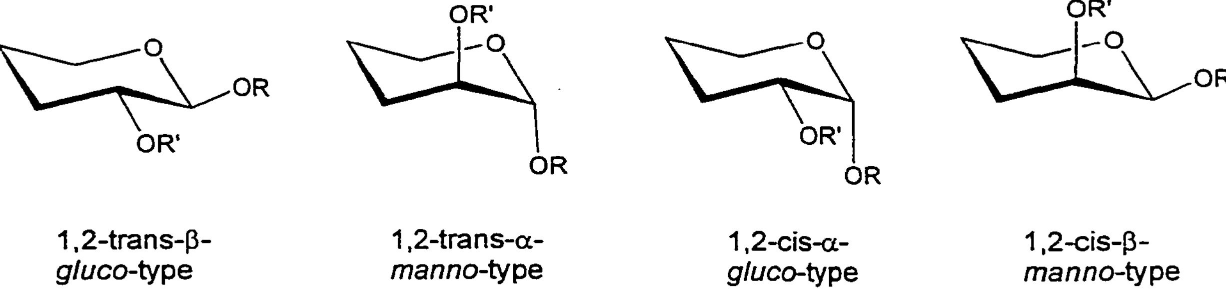


Figure 5.1. Types of glycosidic linkages

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

5.1.1. 1,2-*trans*-Glycosylation

1,2-*trans*-Glycosylation can be generally achieved with neighboring group participation from an ester (acetate, benzoate or pivaloate) at C-2 through the dioxolanium intermediate, which is attacked by the glycosyl acceptor from the less hindered side to give β-glycosides in the *gluco*- series and α-mannosides (Figure 5.2) [126].

Figure 5.2. 1,2-*trans*-Glycosylation based on neighboring group participation.

As shown in Figure 5.3, glycal epoxides resulting from oxidation of glycals are also used to construct 1,2-*trans*-glycosidic linkages [127]. Reaction of epoxide with a glycosyl acceptor in the presence of Lewis acid provides β-glycosides in the *gluco*- series. Gin and co-workers further developed a "one–pot" direct oxidative glycosylation with glycal donors [128].

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Danishefsky and co-workers

OBn BnO BnO O

O—O

OBn BnO BnO O O

ROH, $ZnCl_2$

OBn BnO BnO O OR OH

Gin and co-workers

OBn BnO BnO O

Ph_2SO (3 equiv.)
Tf_2O (1.5 equiv.), DTBMP;
CH_3OH, Et_3N;

ROH, $ZnCl_2$

OBn BnO BnO O OR OH

Figure 5.3. 1,2-*trans*-Glycosylation based on glycal epoxides.

The 1,2-*trans*-glycosidic linkage can also be obtained by a participating solvent such as acetonitrile which changes the selectivity of glycosidic bond formation from α to β, presumably by the mechanism shown in Figure 5.4 [129].

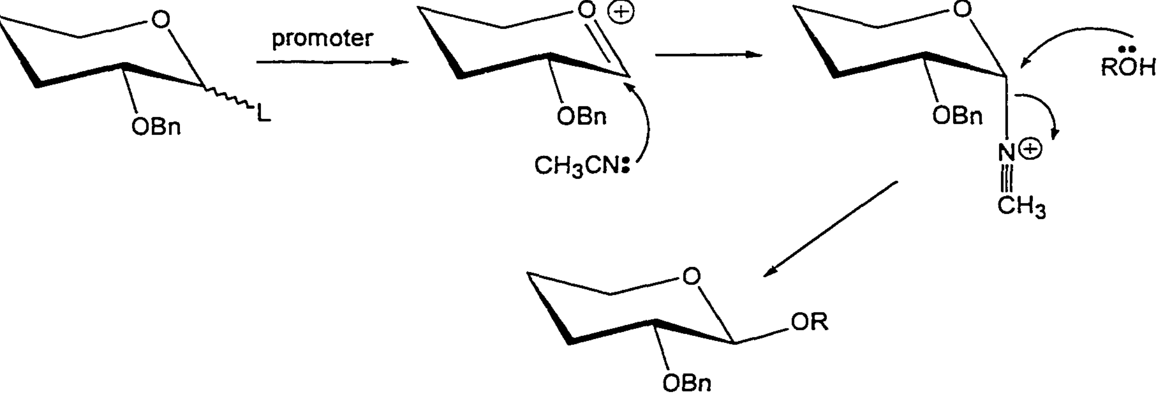


Figure 5.4. 1,2-*trans*-Glycosylation in a participating solvent.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

5.1.2. 1,2-*cis*-Glycosylation

The 1,2-*cis*-glycosylation is more difficult to achieve than the 1,2-*trans*-glycosylation. Instead of using a participating neighboring group, a non-participating group at C-2, usually the persistent *O*-benzyl group, is required for 1,2-*trans*-glycosylation [126]. The S_N2 type of reaction with β-chlorides to make 1,2-*cis*-α-glycoside is not practical because of the instability of β-chlorides caused by the anomeric effect. The *in situ* anomerization of α-halides, introduced by Lemieux [130], was the first reliable method to make 1,2-*cis*-α-glycosides (Figure 5.5). The preferred formation of α-glycosides can be rationalized on the basis of two factors. First, the less thermodynamically stable β-halide is closer to the transition state for glycosylation. Second, reactions at the anomeric center proceed more quickly from the α-face since there is an anti-periplanar arrangement of the incoming nucleophilic atom with the ring oxygen lone pair, which is also called the kinetic anomeric effect [130]. It was later found that a similar reaction without addition of halide anions was possible using catalysts such as mercury salts, silver perchlorate ($AgClO_4$) or silver triflate (AgOTf) [131].

Figure 5.5. 1,2-*cis*-Glycosylation based on *in situ* anomerization.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

5.1.3. Synthesis of β-Mannosides

The 1,2-*cis*-glycosylation is the synthesis of β-mannosides is a special problem. The anomeric effect favors the α-mannosidic linkage both thermodynamically and kinetically, even with a non-participating group at C-2. The β-mannosidic linkage is therefore the most difficult to prepare. One way around this problem involves the use of insoluble silver catalysts (e.g. silver silicate and silver zeolite) that protect the α-side of the donor from attack by glycosyl acceptor (Fig. 5.6) [132].

OBn OBn OBn Br Br Ag ROH δ+ H O R Br Ag O OR OBn

Figure 5.6. Synthesis of β-mannosides using insoluble catalysts.

Recently, a new approach to synthesize β-mannosides, termed intramolecular aglycone delivery (IAD), has been developed by Barresi and Hindsgaul [133]. The general strategy for IAD capitalizes on the principle of intramolecular transfer within a pyranose ring. As shown in Figure 5.7, the method involves the covalent attachment of aglycone to the 2-position of an appropriately derivatized mannose. Carbon acetal [133], silicon tether [134], and methoxybenzylidene acetal [135] were developed as linkers to attach the aglycone onto mannose. Activation of the anomeric leaving group leads to stereocontrolled intramolecular delivery to give only the β-mannosidic linkage.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

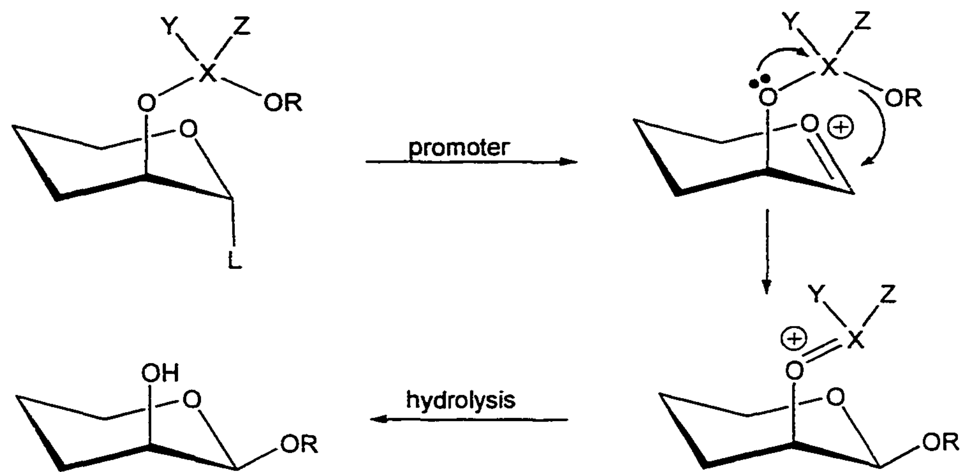


Figure 5.7. Synthesis of β-mannosides by intramolecular aglycone delivery (IAD).

Ziegler and Lemanski described a "true" intramolecular glycosylation protocol where a glycosyl donor and a glycosyl acceptor were covalently linked together by a stable bridge [136]. More recently, Crich and Sun reported the direct stereoselective formation of β-mannosides from mannosyl triflate generated *in situ* at low temperature (Fig. 5.8) [137].

Ziegler and Lemanski

MeOTf, CH_3CN
-30 °C
(55%)
only β

Crich and Sun

1. Tf_2O, DTBMP
CH_2Cl_2, -78 °C
2. ROH, CH_2Cl_2
-78 °C to rt
(>80%)

Figure 5.8. Synthesis of β-mannosides by intramolecular glycosylation and glycosylation with mannosyl triflate.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Hodosi and Kovac described a fundamentally new stereospecific synthesis of β-mannosides via the S_N2 reaction of 1,2-*O*-*cis*-stannylene acetal with triflate of carbohydrate derivatives [138]. Nicolaou and co-workers also reported the stereoselective synthesis of the 1β,1'α-linked dimannoside using 1,2-*O*-*cis*-stannylene acetal (Figure 5.9) [139].

Figure 5.9. Synthesis of β-mannosides via a 1,2-*O*-*cis*-stannylene acetal.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

5.1.4. Synthesis of 2-Deoxy-β-Glycosides

2-Deoxy sugars are frequently found as components in many antibiotics, bacterial polysaccharides and cardiac glycosides [140]. The synthesis of β-glycosides of 2-deoxy sugars is difficult because there is no participating group at C-2 to direct the stereoselectivity. Temporary participating groups such as acetate [141], thiophenyl [142], selenophenyl [143], *N*-formylamino [144], bromo [145, 146] and iodo [146, 147] groups are therefore installed at the C-2 position. These can be removed after glycosylation (Figure 5.10). One interesting example using the *p*-methoxybenzoyl group at C-3 to direct 1,2-*trans*-glycosylation was reported by Wiesner and co-workers (Figure 5.11) [148].

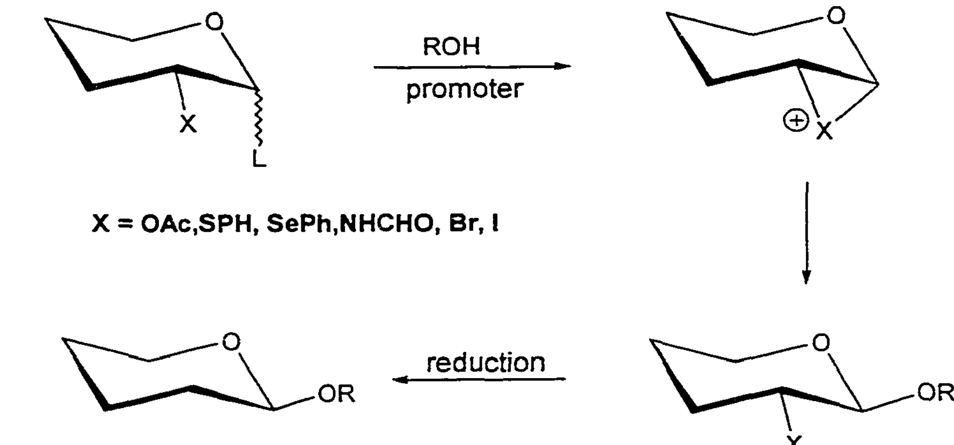


Figure 5.10. Synthesis of 2-deoxy-β-glycosides using temporary participating groups.

Figure 5.11. Synthesis of 2-deoxy-β-glycosides using a participating group at C-3.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

van Boom and co-workers recently developed a method based on migration of alkylthio aglycone [149]. Starting from the mannose thioglycoside, the β-glycoside was formed in high yields. The thioethyl group can be reduced to give 2-deoxy-β-glycosides (Figure 5.12). Glycosylation with a cycloadduct of glucal followed by Raney nickel desulfurization also gave 2-deoxy-β-glycosides with good stereoselectivity (Figure 5.13) [150].

Figure 5.12. Synthesis of 2-deoxy-β-glycosides based on migration of an alkylthio aglycone.

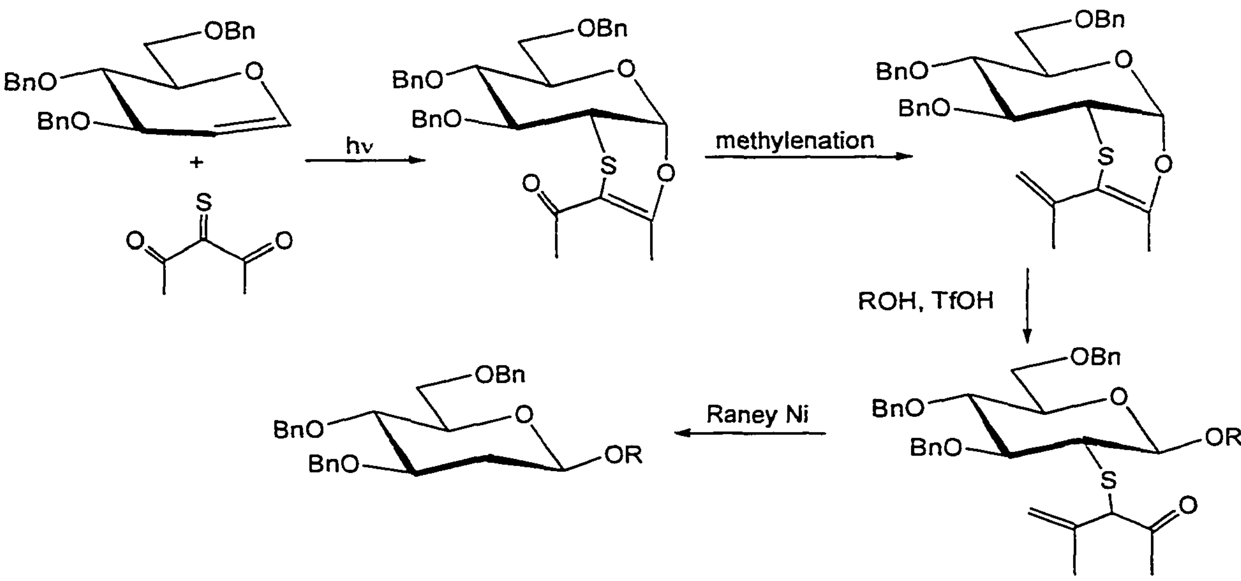


Figure 5.13. Synthesis of 2-deoxy-β-glycosides from a glycal.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

5.1.5. Synthesis of α-Sialoside

Sialooligosaccharides are important constituents of cell-surface glycans. The sialic acids occur exclusively as the α-sialosides [151]. The stereoselective synthesis of α-sialosides remains difficult for several reasons. First, the anomeric center (C-2) is very sterically hindered and formation of a positive (or positive partially) charge at the center is electronically disfavored by the adjacent carboxylate group. Second, the lack of a substituent at C-3 precludes neighboring group participation leading to α-sialosides. Third, the thermodynamically favored product is the β-sialoside due to the anomeric effect. As well, the competitive elimination of the intermediate cation to the glycal is a serious problem.

The classic Koenigs-Knorr method using glycosyl halides as donors give low yield and stereoselectiveity. A stereoselective sialylation using thioglycosides of sialic acid was developed by Hasegawa and co-workers [152]. With the use of dimethyl-(methylthio) sulfonium triflate (DMTST), or NIS/TfOH as promoters, the reactions in acetonitrile gave predominantly α-sialosides (Figure 5.14). Xanthates [153] and phosphites [154] were also found to be useful. α-Sialosides can also be obtained with the participation of 3-thio or 3-seleno substituents [155] (Figure 5.14).

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Figure 5.14. Glycosyl donors for the synthesis of α-sialosides.

5.2. Synthesis of 2-Amino-2-Deoxy Glycosides

Glycosides of 2-amino-2-deoxy sugars are widely distributed in living organisms [156]. The major 2-amino-2-deoxy sugars in nature are *N*-acetylglucosamine (GlcNAc) and *N*-acetylgalactosamine (GalNAc). The synthesis of oligosaccharides of 2-amino-2-deoxy sugars has attracted much attention owing to their biological and medical significance [157]. Due to its nucleophilicity, the amino group has always been protected during glycosylation reactions to avoid *N*-glycosylation. Since the amino group is at the C-2 position, the choice of its protecting group can provide the control of 1,2-*cis*- or 1,2-*trans*-glycosylation. The ideal amine protecting group should be able to direct the stereoselectivity, be stable to commonly employed reactions and reagents and be easy to remove.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

To achieve a 1,2-*trans*-glycosylation with 2-amino-2-deoxy glycosyl donors, a common method is to employ a donor containing a participating group as the amino protective function. Glycosyl donors bearing *N*-acetyl groups, however, have not been generally useful since they tend to form stable oxazoline intermediates which require relatively strong acidic conditions for condensation with glycosyl acceptors [157] (Figure 5.15a). For this reason, other *N*-protecting groups are commonly employed in the synthesis of 2-amino-2-deoxy-β-glycosides. The phthalimido (Phth) group (Fig. 5.15b) has been the most widely used since its introduction by Lemieux in 1976 [158]. However, a major disadvantage of using the *N*-Phth group in oligosaccharide synthesis is that its deprotection requires vigorous conditions: a large excess of hydrazine, butylamine, hydroxylamine, ethylenediamine or $NaBH_4$ is normally used, typically at temperatures as high as 80-100 °C. In some case, even these forcing conditions failed [159].

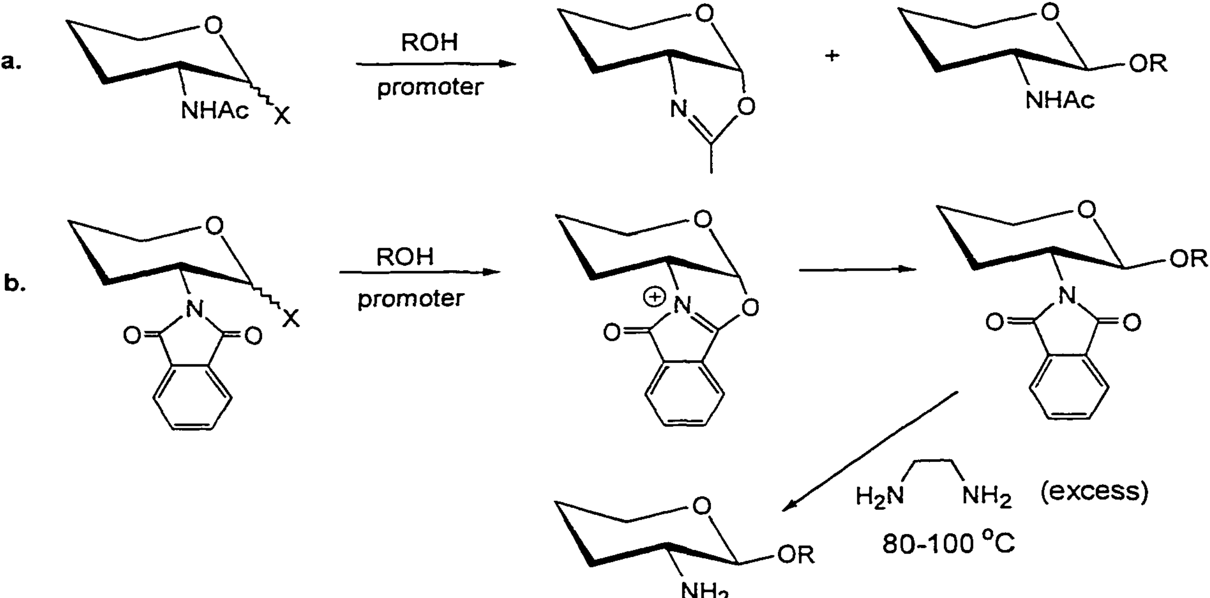


Figure 5.15. Synthesis of 2-amino-2-deoxy-β-glycosides using *N*-acetyl and *N*-Phth protecting groups.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

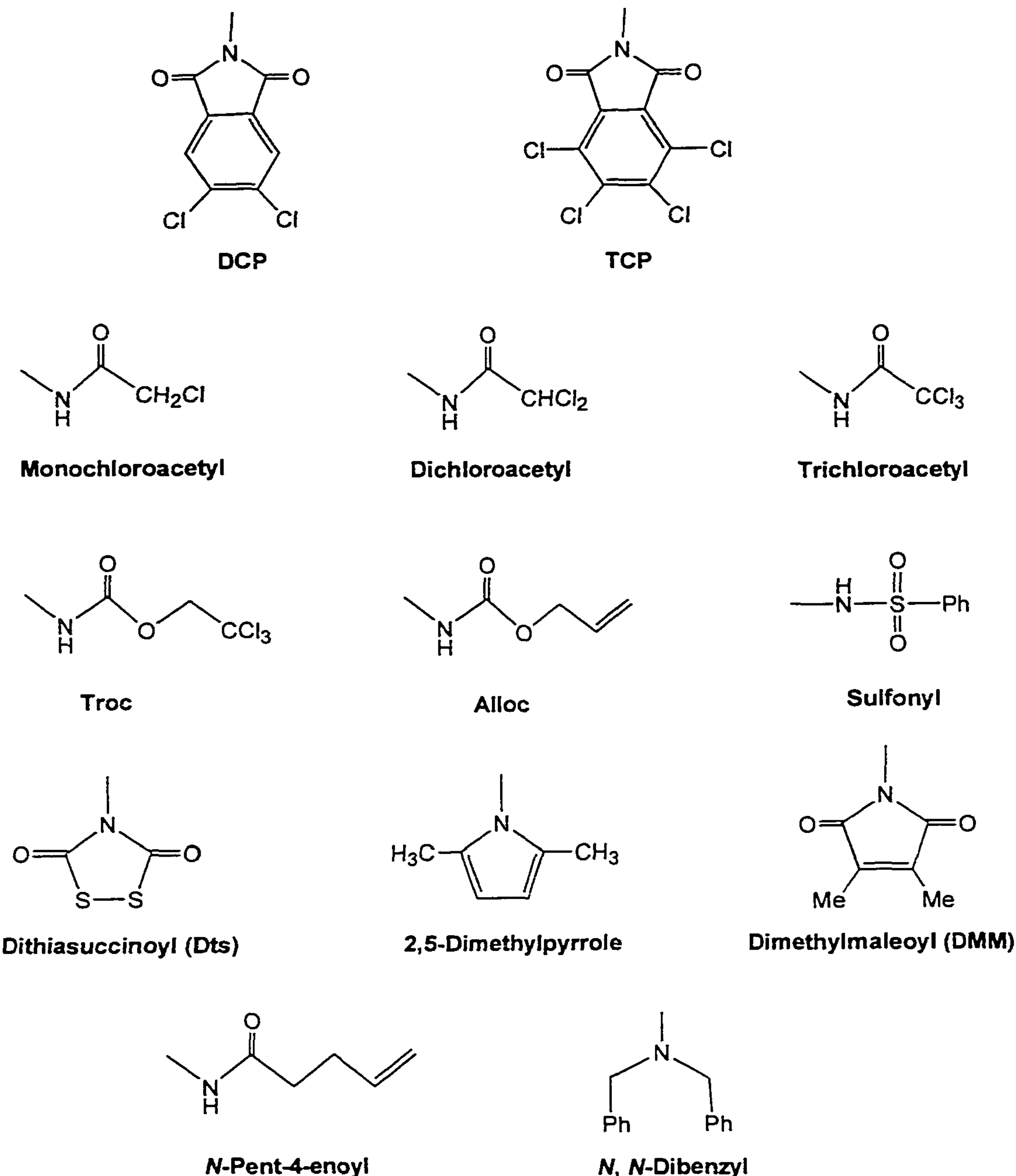


Figure 5.16. Amine protecting groups used in the synthesis of 2-amino-2-deoxy-β-glycosides.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Recently, the more reactive dichlorophthaloyl (DCP) [160] and tetrachlorophthaloyl (TCP) [161] groups were applied as amine protecting groups. Both DCP and TCP groups can be removed under milder conditions than the Phth group by a slight excess of ethylene diamine at a slightly lower temperature. There are a number of other alternative amine protecting groups for the synthesis of 2-amino-2-deoxy-β-glycosides. These include monochloroacetyl [162], dichloroacetyl [163], trichloroacetyl [164], 2,2,2-trichloroethoxycarbonyl (Troc) [165], allyloxycarbonyl (Alloc) [166], phenylsulfonyl [167], pent-4-enoyl [168], dithiasuccinoyl (Dts) [169], 2,5-dimethylpyrrole (DMP) [170], dimethylmaleoyl (DMM) [171], and dibenzyl groups [172], as summarized in Figure 5.16.

Figure 5.17. Amine protecting groups used in the synthesis of 2-amino-2-deoxy-α-glycosides.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

For the synthesis of α-glycosides of 2-amino-2-deoxy sugars, a non-participating amine protecting group is used. The azido group introduced by Lemieux is commonly employed for this purpose [173]. It acts as a masked amino group, and can be transformed to the free amine by various methods. Several other non-participating amine protecting groups have been reported for the synthesis of 1,2-*cis*-glycosides such as 2,4-dinitrophenyl (DNP) [174], *p*-methoxybenzylidene (*p*MB) [175], diphenylphosphoryl (DPP) [176], bis(*p*-nitrobenzyl)-phosphoryl (BNBP) [176], and a nitroso dimer derivative [177] (Figure 5.17). These groups are generally bulky and the stereoselectivity of the glycosylation reactions is accordingly usually poor.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Chapter 6

Studies on the Stereoselective Synthesis of 2-Amino-2-Deoxy Glycosides

6.1. The *p*-Nitrobenzyloxycarbonyl Group as an Orthogonal Amine Protecting Group for the Synthesis of β-Glycosides of 2-Amino-2-Deoxy Sugars

The removal of most of the amine protecting groups developed so far requires conditions that may affect other protecting groups, such as acyl groups, present on the sugar rings. Orthogonal amine protecting groups, which can be readily and chemoselectively removed without affecting other protecting groups or sensitive functionalities, would facilitate the oligosaccharide synthesis.

The carbamate functionality has been used for protection of the amino group in peptide and protein synthesis [178]. A benzyl carbamate of a 2-amino sugar was first used by Zervas and Konstas in 1960 as a glycosyl donor [179]. The reaction of alcohols with 3,4,6-tri-*O*-acetyl-2-benzyloxycarbonylamino-2-deoxy-glucopyranosyl bromide or chloride gave β-glycosides in low or moderate yield due to the problematic formation of an oxazolidinone (Figure 6.1) [180]. The 2,2,2-trichloroethyl carbamate was later found not to generate oxazolidinone side products during glycoside formation [165]. Boullanger *et al.* conducted a systematic study on the glycosylation of *simple* acceptor alcohols with various *N*-alkoxycarbonyl derivatives of glucosamine, including the *p*-nitrobenzyloxycarbonyl (PNZ) group [181]. It was found that β-glycosides were obtained stereoselectively in good yield without the formation of an oxazolidinone when the β-acetate of these carbamate derivatives was used as glycosyl donor in the presence of a Lewis acid. The 2,2,2-trichloroethyl carbamate [165] and allyl carbamate [166] have since been effectively applied in the synthesis of glycoconjugates containing 2-acetamido

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

glycosides. The deprotection of 2,2,2-trichloroethyl carbamate and allyl carbamate can be achieved with zinc dust in acetic acid [165] and with Pd(0) complexes [166], respectively.

Figure 6.1. Glycosylation with benzyloxycarbonyl protected bromide [180].

Fig 6.2. The *p*-nitrobenzyloxycarbonyl group as an orthogonal amine protecting group.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

We chose to investigate the potential of the *p*-nitrobenzyl carbamate of 2-amino sugars because this group might serve as a good participating group to provide 1,2-*trans*-glycosylation while the electron-withdrawing nitro group can prevent the oxazolidinone formation. It should also be selectively cleavable by reduction of the aromatic nitro group to the electron-donating amine substituent followed by 1,6-elimination [182] (Figure 6.2). As there are many methods for reducing an aromatic nitro group, mild and chemoselective deprotection methods should be readily available. Furthermore, unlike the 2,2,2-trichloroacetyl or allyl carbamates, the *p*-nitrobenzyl carbamate can be removed by hydrogenolysis at the end of a synthesis along with the standard *O*-benzyl protecting groups thus obviating the need for an additional deprotection step.

6.1.1. Installation of the PNZ Group onto 2-Amino-2-Deoxy Sugars

The *p*-nitrobenzyloxycarbonyl group can be easily introduced into D-glucosamine and D-galactosamine as shown in Figure 6.3. The free amine was obtained by treatment of glucosamine hydrochloride with one equivalent of NaOMe in MeOH. Reaction of the free amine with *p*-nitrobenzyl chloroformate in the presence of triethylamine give **94a** which was then acetylated with Ac_2O and Py to provide **95a**. Regioselective deacetylation at O-1 with benzylamine followed by treatment of the reducing sugar with CCl_3CN in the presence of K_2CO_3 provided trichloroacetimidate **96a** as a pale yellow crystalline solid (63% overall). The trichloroacetimidate of PNZ-protected galactosamine, **96b**, was prepared in the same fashion in 42% overall yield.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

1. NaOMe, MeOH
2. *p*-Nitrobenzyl chloroformate, Et_3N

93a R_1 = H, R_2 = OH
93b R_1 = OH, R_2 = H

94a R_1 = H, R_2 = OH
94b R_1 = OH, R_2 = H

Ac_2O, Py

95a R_1 = H, R_2 = OAc
95b R_1 = OAc, R_2 = H

1. $BnNH_2$, THF
2. CCl_3CN, K_2CO_3

96a R_1 = H, R_2 = OAc (63%, overall)
96b R_1 = OAc, R_2 = H (43%, overall)

Figure 6.3. Installation of PNZ group onto D-glucosamine and D-galactosamine.

6.1.2. Evaluation of the PNZ Protected Imidate Donors

Imidates **96a** and **96b** were evaluated as glycosyl donors in dichloromethane using $BF_3 \cdot Et_2O$ as the promoter. As shown in Table 6.1, β-glycosides were formed in over 75% yields. The octyl β-glycoside **100** was formed in 82% yield. Glycosylation of **96a** gave the β-(1→6) linked disaccharide **101** in 91% yield. The reaction of **96a** with the less reactive 4-OH groups of **98** and **99** also gave β-(1→4) linked disaccharides **102** and **103** in good yields, 75% and 80%, respectively. Reaction of the imidate donor of PNZ-protected galactosamine, **96b**, with acceptor **99** also provided the β-linked disaccharide **104** in 81% yield. It should be noted that the ^1H NMR spectra of most disaccharides containing the PNZ moiety gave broad peaks which make assignments difficult. This is probably because of the slow rotation of the amide bond. The β-linkage of the

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

disaccharides could be confirmed by ^{13}C-^{1}H HMQC experiments and NMR data of *N*-acetyl disaccharide obtained by *N*-acetylation after the reductive cleavage step.

Table 6.1. Glycosylation results using PNZ protected imidate donors (1.5 eq.). Yields are based on the products purified by column chromatography.

Donor	Acceptor	Product	Yield
96a	Octanol	**100**	82%
96a	**97**	**101**	91%
96a	**98**	**102**	75%
96a	**99**	**103**	80%
96b	**99**	**104**	81%

The imidate donor **96a** was further evaluated in different solvents and with promoters at –30 to 0 °C over a 1.5 h period using **99** as the acceptor. As shown in Table

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

6.2, reactions in CH_2Cl_2 and CH_3CN gave similar glycosylation yields. TMSOTf and $BF_3{\cdot}Et_2O$ were equilvalent.

The results indicate that the combination of PNZ moiety with anomeric trichloroacetimidate activation can afford β-glycosides in high yield with high β-stereoselectivity.

Table 6.2. Glycosylation results using **96a** as the donor (1.5 eq.) and **99** as the acceptor in different solvents and with different promoters. Yields are based on the purified products by column chromatography.

Entry	Promoter	Solvent	Yield
1	$BF_3{\cdot}Et_2O$ (0.5 eq.)	CH_2Cl_2	80%
2	TMSOTf (0.25 eq.)	CH_2Cl_2	76%
3	TMSOTf (0.025 eq.)	CH_2Cl_2	78%
4	$BF_3{\cdot}Et_2O$ (0.5 eq.)	CH_3CN	78%

6.1.3. Deprotection of the PNZ Group

Methods for the deprotection of the PNZ group were examined using the simple octyl glycoside **100** as a model compound. Deacetylation with NaOMe/MeOH gave **105** where the PNZ group remains intact. As expected, deprotection of the PNZ group and acetylation of the resulting amine can be directly achieved in one step by hydrogenolysis using 10% Pd/C under H_2 flow in the presence of Ac_2O to give **106** and *p*-methyl acetanilide in quantitative yield (Figure 6.4). Under same condition, the compound **109** can be obtained from **107** in 83% yield. Free amine **108** could also be isolated (76%) when the hydrogenolysis was performed in the absence of Ac_2O (Figure 6.5).

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

AcO, AcO, OAc, O, OOct, NH, PNZ
100
NaOMe, MeOH
(quant.)
HO, HO, OH, O, OOct, NH, PNZ
105
Pd/C, H_2
MeOH, Ac_2O
(quant.)
HO, HO, OH, O, OOct, NHAc
106
+ H_3C–C_6H_4–NHAc

Figure 6.4. Deprotection of the PNZ group of **100** using hydrogenolysis.

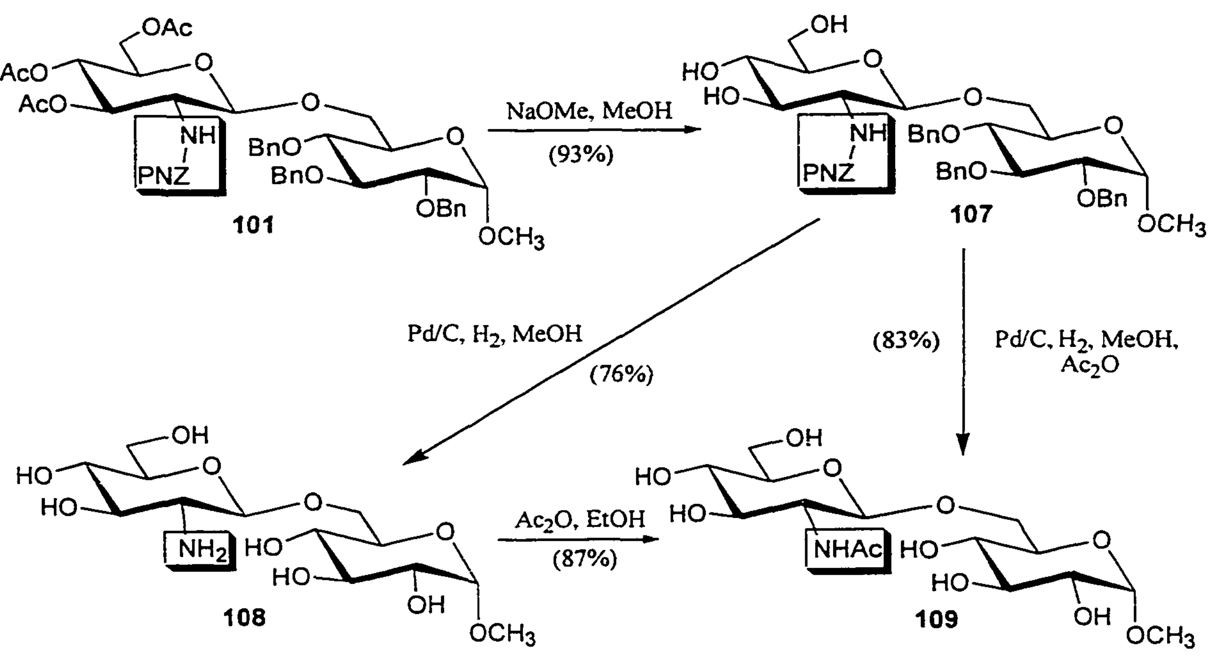


Figure 6.5. Deprotection of the PNZ group of **101** using hydrogenolysis.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Bellamy and Ou have shown that selective reduction of aromatic nitro compounds to amines can be achieved using stannous chloride ($SnCl_2 \cdot H_2O$ or $SnCl_2$) in nonacidic and nonaqueous medium (EtOH or EtOAc) at 70 °C, where other reducible and acid-sensitive groups remain unaffected [183]. The reduction of **100** was examined using this method but the yields were poor.

Figure 6.6. Deprotection of the PNZ group on **120** using sodium thionite.

As reported by Guibe-Jampel and Wakselman [184], *p*-nitrobenzyl esters can be reductively cleaved by sodium dithionite ($Na_2S_2O_4$) under neutral or slightly alkaline conditions. In fact, sodium dithionite was found to be a very effective reducing agent even in the absence of base. The PNZ group of **100** can be removed quickly in quantitative yield. As shown in Figure 6.6, deacetylation of **102** using NaOMe in MeOH followed by cleavage of PNZ and re-acetylation with Ac_2O and Py gave **112.** When disaccharide **102** was treated directly with sodium dithionite in CH_3CN-EtOH-H_2O solution, followed by *N*-acetylation using Ac_2O in MeOH, disaccharide **112** was obtained. This demonstrated the stability of the three *O*-acetate groups to the reduction

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

conditions. The free amine group can also be transformed into the acetamido group in "one pot" by simply adding Ac_2O directly to the reaction mixture. Disaccharide **112** was thus formed in a one-pot reaction in 86% yield (Figure 6.6).

In summary, we have demonstrated that the PNZ group functions as a good participating group for the formation of 2-amino-2-deoxy-β-glycosides. This *N*-protecting group can be conveniently removed either by hydrogenolysis along with *O*-benzyl ethers or selectively by sodium dithionite under neutral conditions where carboxylate esters remain stable. Since *O*-acetyl groups can be removed by treatment with NaOMe/MeOH in the presence of the PNZ group, this group is effectively an orthogonal protecting group and should thus find unique applications in oligosaccharide synthesis.

6.2. Studies on the Synthesis of α-glycosides of 2-Amino-2-Deoxy Sugars Using *o*-Nitrobenzenesulfonyl and *p*-Nitrobenzenesulfonyl as Amine Protecting Groups

Figure 6.7. Synthesis of 2-amino-2-deoxy-β-glycosides using sulfonamidoglycosylation [167].

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Recently, Danishefsky and co-workers developed the sulfonamidoglycosylation method for the preparation of 2-amino-2-deoxy-β-glycosides [167]. The method involves *trans*-diaxial addition of I^+ and sulfonamide to a glycal to form an iodosulfonamide adduct. When an alcohol acceptor and the iodosulfonamide were treated with base (LHMDS or LTMP) and AgOTf, the β-glycoside was obtained presumably through a 1,2-sulfonylaziridine intermediate (Figure 6.7). The 2-sulfonamido group can be reduced to free amine with sodium in liquid ammonia.

More recently Fukuyama used *o*-nitrobenzenesulfonyl (ONS) and *p*-nitrobenzenesulfonyl (PNS) as amine protecting groups in organic synthesis [185, 186]. ONS and PNS groups can be deprotected via Meisenheimer complexes upon treatment with thiolate (Figure 6.8), under conditions which the esters remain unaffected. *o*-Nitrobenzenesulfonamides and *p*-nitrobenzenesulfonamides were also found to be stable under very acidic (HCl 10 eq., MeOH, 60 °C) as well as strong basic (NaOH 10 eq. MeOH, 60 °C) conditions [185].

Figure 6.8. Deprotection of *o* (or *p*)-nitrobenzenesulfonamide.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

The introduction of an electron-withdrawing nitro group should decrease the nucleophilicity of the phenylsulfonamides. Therefore *o*-nitrobenzenesulfonamide and *p*-nitrobenzenesulfonamides might act as non-participating groups for the synthesis of α-glycosides of 2-amino-2-deoxy sugars.

6.2.1. Evaluation of an ONS Protected Imidate Donor

The *o*-nitrobenzysulfonyl group can be easily introduced into D-glucosamine using conditions similar to those used for the introduction of the PNZ group (Figure 6.9). Treatment of glucosamine with NaOMe/MeOH, followed by *p*-nitrobenzenesulfonyl chloride/Et_3N and *O*-acetylation (Ac_2O/Py) gave **2** in 53% yield (three steps). Regioselective deacetylation at O-1 with benzylamine followed by treatment of the reducing sugar with CCl_3CN in the presence of K_2CO_3 provided trichloroacetimidate **114** as a pale yellow crystalline product (50% yield, two steps).

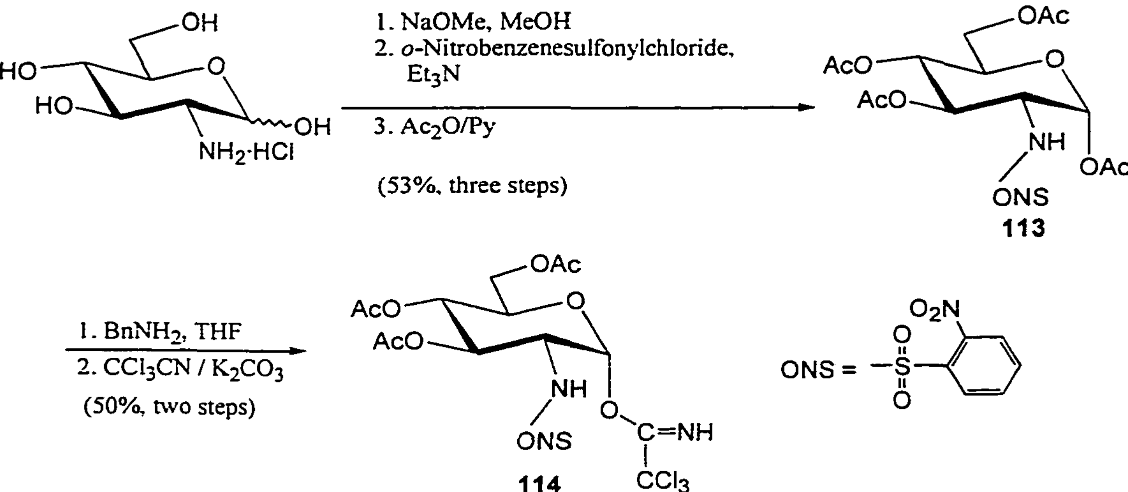


Figure 6.9. Preparation of an ONS protected imidate donor.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Results using **114** as a glycosyl donor, however, were not encouraging. Reaction of the imidate **114** with **97** in dichloromethane using $BF_3 \cdot Et_2O$ as a promoter resulted in a very low yield (30%) of α/β (1:1) mixture, and a large amount of unreacted donor was recovered even after 18h (Figure 6.10).

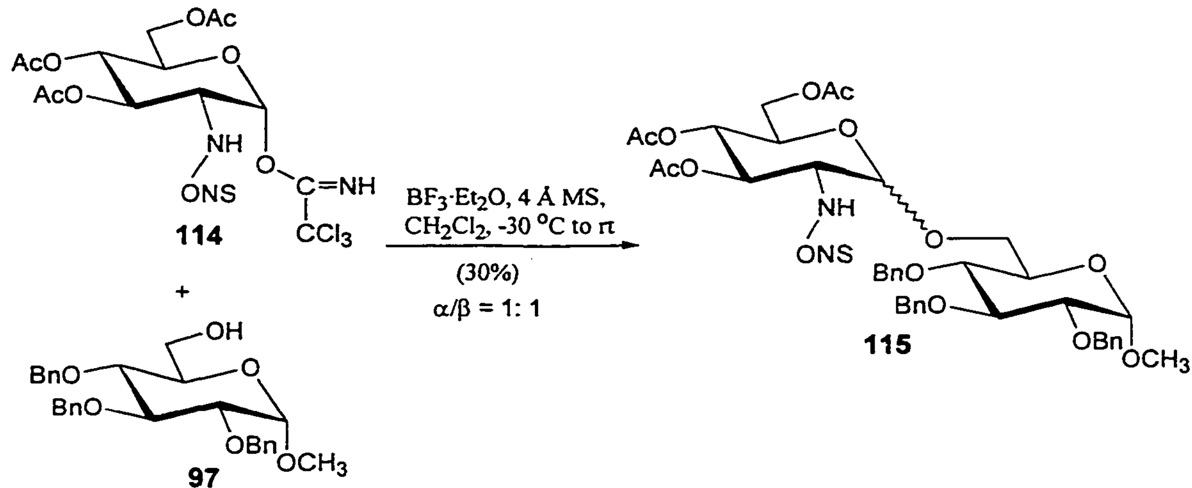


Figure 6.10. Glycosylation with an ONS protected imidate donor.

6.2.2. Evaluation of a PNS Protected Imidate Donor

The PNS group was introduced into D-glucosamine as described above for the ONS group (Figure 6.11). The reaction yields were relatively low. The regioselective deacetylation at anomeric position produced only a 30% yield using $BnNH_2$ and a 40% yield using hydrazine acetate. The imidate **118** was formed in only 25% yield.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

1. NaOMe/MeOH
2. *p*-Nitrobenzenesulfonylchloride, Py
3. Ac_2O/Py
(34%, three steps)

116

$BnNH_2$, THF (30%)
or NH_2NH_2-HOAc, DMF, 60 °C (40%)

117

CCl_3CN, K_2CO_3 (25%)

118

PNS = $-SO_2-C_6H_4-NO_2$

Figure 6.11. Preparation of a PNS protected imidate donor.

Imidate **118** was tested as a glycosyl donor with secondary OH acceptor. It was hoped that the reaction with a less reactive acceptor could increase the α-selectivity [126]. Unfortunately, the glycosylation of imidate donor **118** and acceptor **9** failed completely as no desired disaccharide was isolated (Figure 6.12).

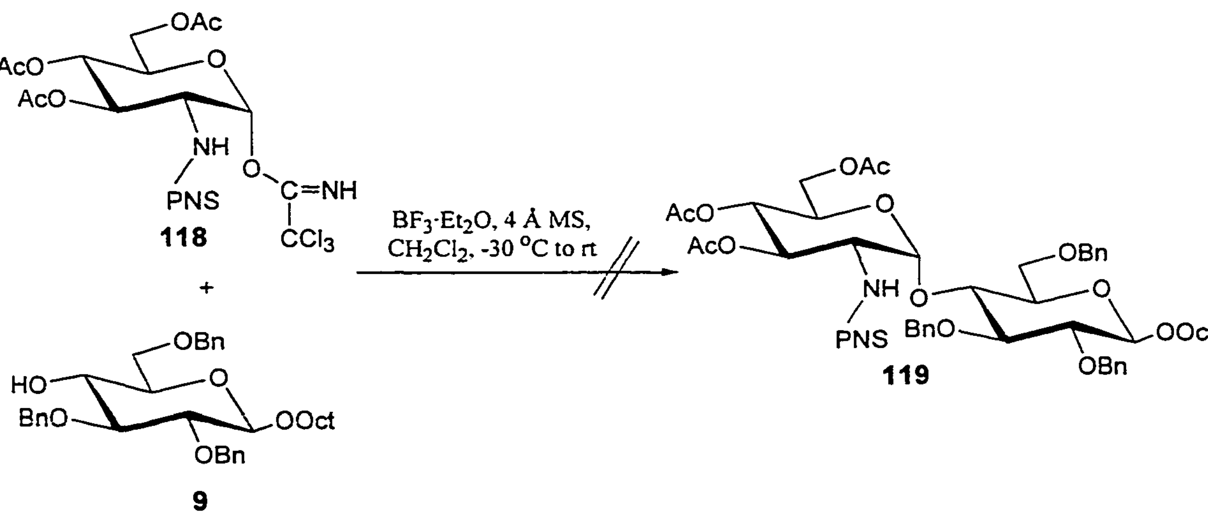


Figure 6.12. Glycosylation with a PNS protected imidate donor.

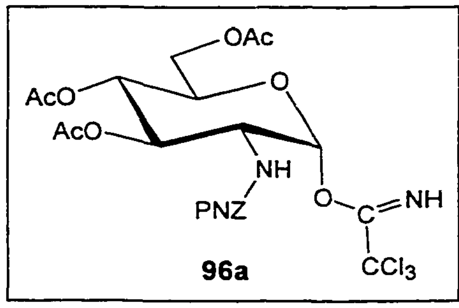
Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

The results indicate that the imidate donors **114** and **118** have extremely low reactivity, probably due to the electron-withdrawing character of the nitro group which makes it difficult to form an oxocarbenium or oxocarbenium-like intermediate.

6.3. Experimental

General methods were the same as described in Chapter 2.

3,4,6-Tri-O-acetyl-2-deoxy-2-(4-nitrobenzyloxycarbonylamino)-β-D-glucopyranosyl trichloroacetimidate (**96a**).

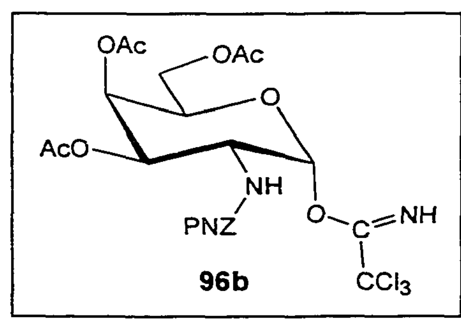


D-Glucosamine hydrochloride (10.8 g, 50 mmol) was added to a freshly prepared solution of sodium methylate, obtained by reaction of methanol (250 mL) and sodium (1.15 g, 50 mmol). The solution was stirred at rt for 10 min. After filtration, *p*-nitrobenzylchloroforamate (10.8 g, 50 mmol) and triethylamine (7 mL, 50 mmol) were added to the filtrate at 0 °C. The reaction was stirred for 3 h while it was allowed to warm up to rt. The mixture was concentrated, dried over vacuum. The resulting residue was dissolved in acetic anhydride (60 mL) and pyridine (120 mL). After stirring overnight, the reaction mixture was concentrated. The residue was dissolved in CH_2Cl_2, washed with dilute HCl, water and brine, dried (Na_2SO_4), filtered, and concentrated. The resulting residue was dissolved in dry THF (200 mL), and benzylamine (5.0 mL) was added. After stirring overnight, the mixture was concentrated. The residue was dissolved in CH_2Cl_2, and K_2CO_3 (20 g) and CCl_3CN (40 mL) were added. The mixture was stirred for 7h. After dilution with CH_2Cl_2, the mixture was filtered. The filtrate was concentrated and

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

submitted to chromatography (1:1 hexanes/EtOAc) to give **96a** (19.8 g, 63% overall) as a pale yellow crystalline: ^{1}H NMR (360 MHz, $CDCl_3$) δ 8.75 (s, 1 H, C=NH), 8.19 (d, 2H, J = 8.6 Hz, ArH), 7.45 (d, 2H, J = 8.6 Hz, ArH), 6.39 (d, 1H, J = 3.6 Hz, H-1), 5.35-5.15 (m, 4H, H-3, H-4, *p*-NO_2PhCH_2), 5.07 (d, 1H, J = 9.3, NHCO), 4.30-4.20 (m, 2H, H-2, H-6a), 4.15-4.06 (m, 2H, H-5, H-6b), 2.03, 2.02, 1.96 (s, 3H, $COCH_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.17, 170.56, 169.24 ($COCH_3$), 160.39, 155.20 (C=NH, NHCO), 147.77, 143.36 (aromatic quart.), 128.19, 123.79 (aromatic CH), 94.75 (C-1), 90.72 (CCl_3), 70.69, 70.30, 67.38 (C-3, C-4, C-5), 65.65, 61.45 (C-6, *p*-NO_2PhCH_2), 53.79 (C-2), 20.68, 20.66, 20.28 ($COCH_3$); HR-ESMS calcd for $C_{22}H_{24}N_3O_{12}NaCl_3$ ($M+Na^+$) 650.0323, found 650.0327.

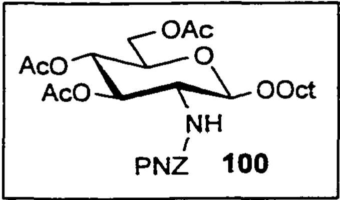
3,4,6-Tri-O-acetyl-2-deoxy-2-(4-nitrobenzyloxycarbonylamino)-β-D-galactopyranosyl trichloroacetimidate (**96b**).



D-Galactosamine hydrochloride (1.08 g) was treated in the same fashion to give **96b** (1.3 g, 42% overall) as a pale yellow crystalline: ^{1}H NMR (360 MHz, $CDCl_3$) δ 8.75 (s, 1 H, C=NH), 8.20 (d, 2H, J = 8.6 Hz, ArH), 7.48 (d, 2H, J = 8.6 Hz, ArH), 6.42 (d, 1H, J = 3.5 Hz, H-1), 5.49 (bd, 1H, J = 2.3 Hz, H-4), 5.24 (dd, 1H, J = 11.4, 3.1 Hz, H-3), 5.28 (bs, 2H, *p*-NO_2PhCH_2), 5.02 (d, 1H, J = 9.6 Hz, NHCO), 4.50 (ddd, 1H, J = 11.4, 9.6, 3.5 Hz, H-2), 4.36 (bt, 1H, J = 6.7 Hz), 4.16 (dd, 1H, J = 11.4, 6.7 Hz, H-6a), 4.06 (dd, 1H, J = 11.4, 6.7 Hz, H-6b), 2.18, 2.03, 1.98 (s, 3H, $COCH_3$).

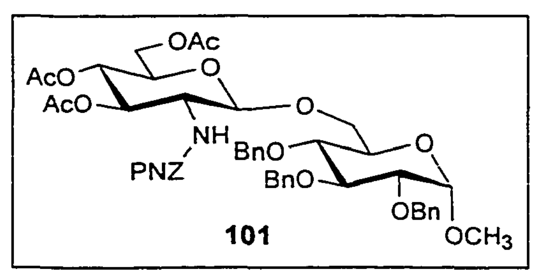
Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Octyl 3,4,6-tri-O-acetyl-2-deoxy-2-(4-nitrobenzyloxycarbonylamino)-β-D-glucopyranoside (**100**).



A mixture of imidate **96a** (1.42 g, 2.3 mmol), octanol (0.24 mL, 1.5 mmol) and powdered 4Å molecular sieves (1.5 g) in dry CH_2Cl_2 (30 mL) was stirred at -30 °C for 10 min under nitrogen. $BF_3 \cdot Et_2O$ (95 µL, 0.75 mmol) in dry CH_2Cl_2 (0.5 ml) was then added, and the reaction mixture was stirred for a further 2 h below 0 °C. After neutralization with Et_3N, the reaction mixture was filtered through Celite, washed with CH_2Cl_2, concentrated, and submitted to column chromatography (3:2 hexanes/EtOAc) to give **100** (736 mg, 82%): ^1H NMR (360 MHz, $CDCl_3$) δ 8.20 (d, 2H, J = 8.6 Hz, ArH), 7.48 (d, 2H, J = 8.6 Hz, ArH), 5.32-5.02 (m, 6H, H-1, H-2, H-3, H-4, p-NO_2PhCH_2), 4.26 (dd, 1H, J = 12.3, 4.8 Hz), 4.11 (dd, 1H, J = 12.3, 2.4 Hz), 3.85 (dd, 1H, J = 9.6, 6.6 Hz, OCH_2CH_2), 3.68 (m, 1H, H-5), 3.45 (dt, 1H, J = 9.6, 6.6 Hz, OCH_2CH_2), 2.08, 2.02, 1.97 (s, 3H, $COCH_3$), 1.53 (m, 2H, OCH_2CH_2), 1.30-1.16 (m, 10H, CH_2 octyl), 0.93 (t, 3H, J = 7.0 Hz, CH_3 octyl).

Methyl 3,4,6-tri-O-acetyl-2-deoxy-2-(4-nitrobenzyloxycarbonylamino)-β-D-glucopyranosyl-(1→6)-2,3,4-tri-O-benzyl-α-D-glucopyranoside (**101**).

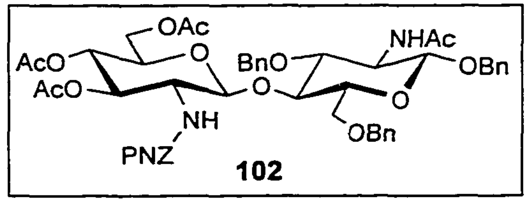


Glycosyl acceptor **97** (116 mg, 0.25 mmol) was coupled with imidate donor **96a** (235 mg, 0.38 mmol) in the same fashion as for **100** to give **101** (211 mg, 91%): ^1H NMR (360 MHz, $CDCl_3$) δ 8.07(d, 2H, J = 7.8 Hz, ArH), 7.42-7.20 (m, 17H, ArH), 5.06-4.72 (m, 8H), 4.70-4.51 (m, 4H), 4.27-3.94 (m, 4H), 3.88-3.45 (m, 6H), 2.05, 2.01, 1.98 (s, 3H, $COCH_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.63, 170.60,

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

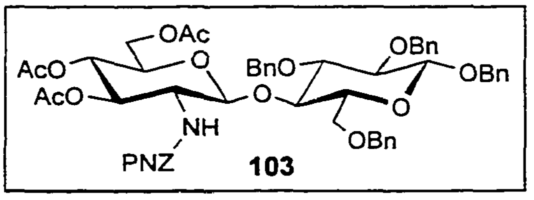
169.38 ($COCH_3$), 154.99 (NHCO), 147.56, 143.30, 138.70, 138.33, 138.06 (aromatic quart.), 128.51, 128.47, 128.41, 128.31, 128.22, 128.11, 128.06, 128.02, 127.98, 127.95, 127.82, 127.71, 123.63 (aromatic CH), 100.74 ($J_{C1'-H1'}$ = 160.4 Hz, C-1'), 98.06 (J_{C1-H1} = 168.5 Hz, C-1), 81.99, 79.80, 77.33, 75.81, 74.73, 73.26, 71.81, 69.50, 68.64 (C-2, C-3, C-4, C-5, C-3', C-4', C-5', $PhCH_2 \times 3$), 68.03, 65.41, 62.08 (C-6, C-6', *p*-NO_2PhCH_2), 56.31, 55.24 (C-2', OCH_3), 20.71, 20.62, 20.60 ($COCH_3$); HR-ESMS calcd for $C_{48}H_{54}N_2O_{17}Na$ ($M+Na^+$) 953.3320, found 953.3348;

Benzyl 3,4,6-tri-O-acetyl-2-dexoy-2-(4-nitrobenzyloxycarbonylamino)-β-D-glucopyranosyl-(1→4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranoside (**102**).



Glycosyl acceptor **98** (125 mg, 0.25 mmol) was coupled with imidate donor **96a** (250 mg, 0.4 mmol) in the same fashion as for **100** to give **102** (182 mg, 75%): ^1H NMR (360 MHz, $CDCl_3$) δ 8.20 (d, 2H, J = 7.8 Hz, ArH), 7.50-7.20 (m, 17H, ArH), 5.32-4.45 (m, 13H), 4.28-3.45 (m, 9H), 2.03, 1.98, 1.96, 1.94 (s, 3H, $COCH_3$); ^{13}C NMR ($CDCl_3$) δ 99.4 ($J_{C1'-H1'}$ = 165.2 Hz, C-1'), 100.6 (J_{C1-H1} =159.4 Hz, C-1).

Benzyl 3,4,6-tri-O-acetyl-2-deoxy-2-(4-nitrobenzyloxycarbonylamino)-β-D-glucopyranosyl-(1→4)-2,3,6-tri-O-benzyl-β-D-glucopyranoside (**103**).

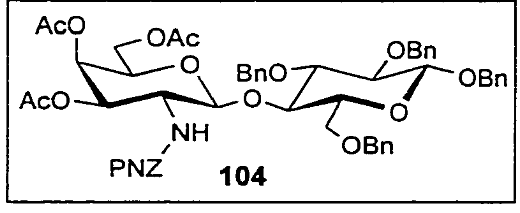


Glycosyl acceptor **99** (108 mg, 0.2 mmol) was coupled with imidate donor **96a** (189 mg, 0.3 mmol) in the same fashion as for **100** to give **103** (161 mg, 80%): ^1H NMR (360 MHz, $CDCl_3$) δ 8.20 (d, 2H, J = 7.8 Hz, ArH), 7.45-7.20 (m, 22H, ArH), 5.17-4.40 (m, 13H),

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

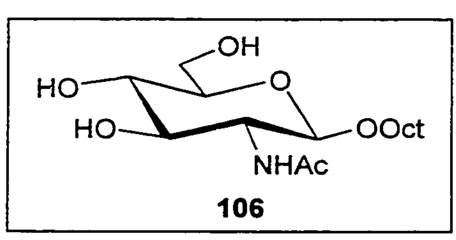
4.12-3.24 (m, 11H), 2.00, 1.94, 1.92 (s, 3H, $COCH_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.59, 170.50, 169.41 ($COCH_3$), 155.18 (NHCO), 147.71, 143.73, 139.22, 138.34, 137.77, 137.41 (aromatic quart.), 128.80, 128.42, 128.23, 128.15, 128.11, 127.90, 127.80, 127.56, 127.25, 127.14, 123.75 (aromatic CH), 102.60 ($J_{C1\text{-}H1}$ = 158.1 Hz, C-1), 100.44 ($J_{C1'\text{-}H1'}$ = 160.0 Hz, C-1'), 82.70, 81.79, 77.30, 74.86 (2 × C), 74.36, 73.69, 72.56, 71.53, 71.15, 68.40 (C-2, C-3, C-4, C-5, C-3', C-4', C-5', PhCH_2 × 4), 68.08, 65.31, 61.77 (C-6, C-6', *p*-NO_2PhCH_2), 56.73 (C-2'), 20.61 (3 × C, COCH_3); HR-ESMS calcd for $C_{54}H_{58}N_2O_{17}Na$ (M+Na$^+$) 1029.3633, found 1029.3634.

Benzyl 3,4,6-tri-O-acetyl-2-deoxy-2-(4-nitrobenzyloxycarbonylamino)-β-D-galactopyranosyl-(1→4)-2,3,6-tri-O-benzyl-β-D-glucopyranoside (**104**).



Glycosyl acceptor **99** (40 mg, 74 μmol) was coupled with imidate donor **96b** (70 mg, 111 μmol) in the same fashion as for **100** to give **104** (64 mg, 86%): ^1H NMR (360 MHz, $CDCl_3$) δ 8.20 (m, 2H, ArH), 7.50-7.20 (m, 22H, ArH), 5.22-4.40 (m, 14H), 4.00-3.45 (m, 10H), 2.08, 1.98, 1.92 (s, 3H, $COCH_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 102.68 ($J_{C1\text{-}H1}$ = 159.5 Hz, C-1), 100.44 ($J_{C1'\text{-}H1'}$ = 162.0 Hz, C-1').

Octyl 2-acetamido-2-deoxy-β-D-glucopyranoside (**106**).

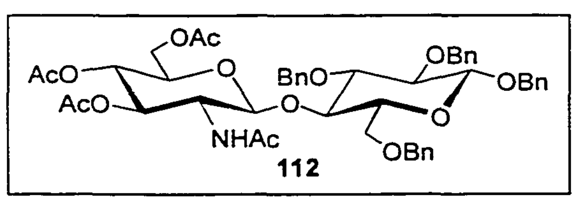


To a solution of compound **100** (480 mg, 0.80 mmol) in MeOH (25 mL) was added a freshly prepared methanolic solution of NaOMe (1 mM, 0.3 mL). After stirring for 6 h, the reaction mixture was concentrated to give **105** (372 mg, quant.). A solution of **105** (42

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

mg, 89 μmol) in MeOH (10 mL) was stirred under a stream of H_2 in the presence of 10% Pd/C (20 mg) and acetic anhydride (0.3 mL) for 2 h. The catalyst was filtered away through a Millex-GV 0.22 μm filter and the solvent evaporated. The product was purified on a column of Iatrobeads (6:1 CH_2Cl_2/MeOH) to yield **106** (29 mg, quant.) as a white solid: ^{1}H NMR (360 MHz, CD_3OD) δ 4.38 (d, 1H, J = 8.4 Hz, H-1), 3.90-3.83 (m, 2H, H-6a, OCH_2CH$_2$), 3.67 (dd, 1H, J = 11.8, 5.6 Hz, H-6b), 3.61 (dd, 1H, J = 10.3, 8.4 Hz, H-2), 3.48-3.40 (m, 2H, H-3, OCH_2CH$_2$), 3.29-3.20 (m, 2H, H-4, H-5), 1.95 (s, 3H, $COCH_3$), 1.54 (m, 2H, OCH$_2$CH_2), 1.40-1.25 (m, 10H, CH_2 octyl), 0.92 (t, 3H, J = 7.0 Hz, CH_3 octyl); ^{13}C NMR (125 MHz, D_2O) δ 175.27 (COCH$_3$), 101.93 (C-1), 76.67, 74.68, 71.34, 70.79 (C-3, C-4, C-5, OCH$_2$CH$_2$), 61.62 (C-6), 56.46 (C-2), 31.91, 29.38, 29.29, 29.15, 25.90, 23.02, 22.82 (CH_2 octyl, COCH$_3$), 14.20 (CH_3 octyl); HR-ESMS calcd for $C_{16}H_{31}NO_6Na$ (M+Na$^+$) 356.2049, found 356.2049.

Benzyl 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl-(1→4)-2,3,6-tri-O-benzyl-β-D-glucopyranoside (**112**).

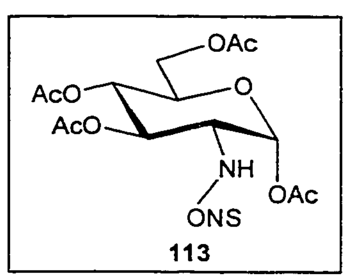


A solution of $Na_2S_2O_4$ (35 mg, 0.2 mmol) in EtOH-H_2O (4:3, 14 mL) was added to a stirred solution of **102** (25 mg, 25 μmol) in CH_3CN (10 mL). After 1h, TLC indicated that the PNZ group was removed. Acetic anhydride (0.2 mL) was added to the solution and stirring continued for another 1h. After dilution with EtOAc, the organic layer was washed with water, dried (Na_2SO_4), filtered, concentrated and submitted to column chromatography (1:5 hexanes/EtOAc) to give **112** (19 mg, 86%): ^{1}H NMR (500 MHz, CD_3Cl) δ 4.4.98-4.88 (m, 4H, H-3', H-4', PhCH_2), 4.83 (d, 1H, J = 11.0 Hz, PhCH_2) 4.81 (d, 1H, J = 12.1 Hz, PhCH_2), 4.79 (d, 1H, J = 9.3 Hz, NH), 4.74 (d, 1H, J = 11.6 Hz, PhCH_2), 4.62 (d, 2H, J = 11.5 Hz, PhCH_2), 4.55 (d, 1H, J = 8.4, H-1), 4.45 (d, 1H, J =

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

12.6 Hz, PhCH_2), 4.44 (d, 1H, J = 7.6 Hz, H-1'), 4.05 (dd, 1H, J = 12.3, 4.3 Hz, H-6'a), 3.90-3.80 (m, 3H, H-4, H-2', H-6'b), 3.64 (m, 2H, H-6a, H-6b), 3.54 (t, 1H, J = 8.9 Hz, H-3), 3.44 (dd, 1H, J = 8.9, 8.4 Hz, H-2), 3.40-3.33 (m, 2H, H-5, H-5'), 1.98, 1.96, 1.90, 1.71 (s, 3H, $COCH_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.76, 170.60, 169.84, 169.27 ($COCH_3$), 139.31, 138.35, 137.86, 137.40 (aromatic quart.), 128.85, 128.75, 128.58, 128.39, 128.18, 128.11, 128.07, 127.88, 127.77, 127.49, 127.16, 127.03 (aromatic CH), 102.55 ($J_{C1'\text{-}H1'}$ =159.2 Hz, C-1), 100.48 ($J_{C1\text{-}H1}$ = 161.8 Hz, C-1), 82.73, 81.82, 77.51, 74.87, 74.82, 74.27, 73.90, 72.98, 71.56, 71.13, 68.38, 68.21, 61.82 (C-2, C-3, C-4, C-5, C-6, C-3', C-4', C-5', C-6', PhCH_2 × 4), 54.68 (C-2'), 23.12, 20.60, 20.59 (COCH_3); HR-ESMS calcd for $C_{48}H_{55}NO_{14}Na$ ($M+Na^+$) 892.3520, found 892.3522.

1,3,4,6-Tetra-O-acetyl-2-deoxy-2-(2-nitrobenzenesulfonylamino)-α-D-glucopyranose (**113**).

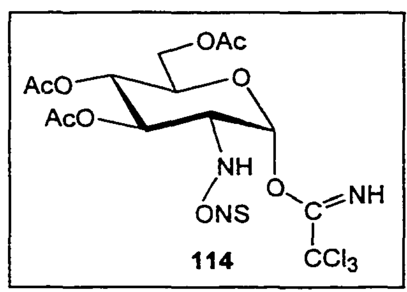


D-Glucosamine hydrochloride (6.48 g, 30 mmol) was added to a freshly prepared solution of sodium methylate, obtained by reaction of methanol (150 mL) and sodium (0.69 g, 30 mmol). The solution was stirred at rt for 10 min. After filtration, 2-nitrobenzenesulfonyl chloride (6.65g, 30 mmol) and triethylamine (4.2 mL, 30 mmol) were added to the filtrate at 0 °C. The reaction was stirred overnight while it was allowed to warm up to rt. The mixture was concentrated, dried over vacuum. The resulting residue was dissolved in acetic anhydride (30 mL) and pyridine (30 mL). After stirring for 24 h, the reaction mixture was concentrated. The resulting residue was dissolved in CH_2Cl_2, washed with dilute HCl, water and brine, dried (Na_2SO_4), filtered, concentrated and submitted to column chromatography (5:4 hexanes/EtOAc) to give **113** (8.4 g, 53%) as a pale yellow crystalline: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.12 (m, 1H, ArH), 7.83 (m, 1H, ArH), 7.78-7.72 (m, 2H, ArH), 5.79

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

(d,1 H, J = 3.8 Hz, H-1), 5.70 (d, 1H, J = 10.2 Hz, NHCO), 5.29 (t, 1H, J = 9.6 Hz, H-3), 5.10 (t, 1H, J = 9.6 Hz, H-4), 4.25 (dd, 1H, J = 13.0, 4.5 Hz, H-6a), 4.09 (dd, 1H, J = 13.0, 7.0 Hz, H-6b), 3.95 (m, 1H, H-5), 3.90 (ddd, 1H, J = 10.2, 9.6, 3.8 Hz, H-2), 2.10, 2.04, 1.99, 1.90 (s, 3H, $COCH_3$); HR-ESMS calcd for $C_{20}H_{24}N_2O_{13}NaS$ ($M+Na^+$) 555.0897, found 555.0893.

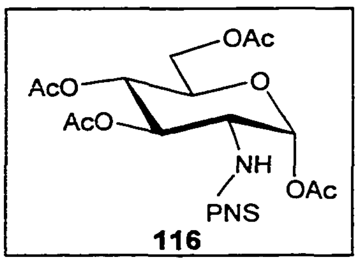
3,4,6-Tri-O-acetyl-2-deoxy-2-(2-nitrobenzenesulfonylamino)-β-D-glucopyranosyl trichloroacetimidate (**114**)



To a solution of **113** (3.04 g, 5.7 mmol) in dry THF (30 mL) was added benzylamine (0.66 mL, 6.3 mmol). After stirring for 2 days, the reaction mixture was concentrated. The resulting residue was dissolved in CH_2Cl_2 (50 mL), and K_2CO_3 (3.06 g) and CCl_3CN (5.55 mL) were added. The mixture was stirred for 14 h. After dilution with CH_2Cl_2, the mixture was filtered through a Celite pad. The filtrate was concentrated and submitted to chromatography (5:4 hexanes/EtOAc) to give **114** (1.8 g, 50%) as pale yellow crystals: ^1H NMR (300 MHz, $CDCl_3$) δ 8.62 (s, 1 H, C=NH), 8.19 (m, 1H, ArH), 7.87 (m, 1H, ArH), 7.80-7.70 (m, 2H, ArH), 6.20 (d, 1H, J = 3.7 Hz, H-1), 5.74 (d, 1H, J = 9.4 Hz, NH), 5.34 (t, 1H, J = 9.6 Hz, H-3), 5.15 (t, 1H, J = 9.6 Hz, H-4), 4.24 (dd, 1H, J = 12.3, 4.0 Hz, H-6a), 4.14-4.01 (m, 3H, H-2, H-5, H-6b), 2.04, 2.01, 1.80 (s, 3H, $COCH_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.49, 170.34, 169.38 ($COCH_3$), 160.22 (C=NH), 147.66, 134.72 (aromatic quart.), 133.94, 133.44, 130.35, 125.63 (aromatic CH), 94.44 (C-1), 90.46 (CCl_3), 70.26, 69.87, 67.66 (C-3, C-4, C-5), 61.32 (C-6), 55.98 (C-2), 20.67, 20.56, 20.36 ($COCH_3$); HR-ESMS calcd for $C_{20}H_{22}N_3O_{12}NaSCl_3$ ($M+Na^+$) 655.9887, found 655.9891.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

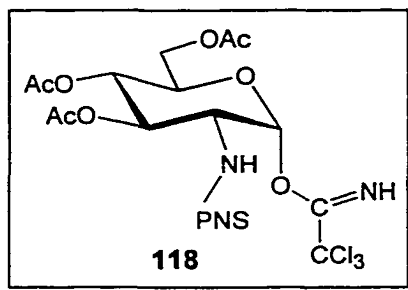
1,3,4,6-Tetra-O-acetyl-2-deoxy-2-(4-nitrobenzenesulfonylamino)-α-D-glucopyranose **(116)**.



D-Glucosamine hydrochloride (10.8 g, 50 mmol) was added to a freshly prepared solution of sodium methylate, obtained by reaction of methanol (200 mL) and sodium (1.15g, 50 mmol). The solution was stirred at rt for 10 min. After filtration, 2-nitrobenzenesulfonyl chloride (11.08g, 50 mmol) and pyridine (4.1 mL, 50 mmol) were added to the filtrate at 0 °C. The reaction was stirred overnight while it was allowed to warm up to rt. The mixture was then concentrated, dried over vacuum. The resulting residue was dissolved in acetic anhydride (30 mL) and pyridine (30 mL). After stirring for 24 h, the mixture was concentrated. The resulting residue was dissolved in CH_2Cl_2, washed with dilute HCl, water and brine, dried (Na_2SO_4), filtered, concentrated and submitted to column chromatography (3:2 hexanes/EtOAc) to give **116** (9.0 g, 34%) as a pale yellow crystalline: ^1H NMR (300 MHz, $CDCl_3$) δ 8.35 (m, 2H, ArH), 8.01 (m, 2H, ArH), 6.01 (d, 1H, J = 3.7 Hz, H-1), 5.42 (d, 1H, J = 9.6 Hz, NHCO), 5.18 (t, 1H, J = 9.6 Hz, H-3), 5.09 (t, 1H, J = 9.6 Hz, H-4), 4.24 (dd, 1H, J = 12.7, 4.2 Hz, H-6a), 3.99 (dd, 1H, J = 12.7, 2.4 Hz, H-6b), 3.94 (m, 1H), 3.74 (ddd, 1H, J = 9.6, 9.6, 3.7 Hz), 2.13, 2.05, 1.99, 1.80 (s, 3H, $COCH_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.19, 170.58, 169.11, 168.28 ($COCH_3$), 150.33, 146.19 (aromatic quart.), 128.27, 124.63 (aromatic CH), 90.57 (C-1), 70.13, 69.63, 67.44 (C-3, C-4, C-5), 61.31 (C-6), 55.30 (C-2), 20.78, 20.67, 20.50, 20.49 ($COCH_3$); HR-ESMS calcd for $C_{20}H_{24}N_2O_{13}NaS$ ($M+Na^+$) 555.0897, found 555.0903.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

3,4,6-Tri-O-acetyl-2-deoxy-2-(4-nitrobenzenesulfonylamino)-β-D-glucopyranosyl tichloroacetimidate (**118**).



To a solution of **116** (3.3 g, 6.2 mmol) in DMF (30 mL) was added hydrazine acetate (0.68g, 7.4 mmol). The reaction was stirred at 50 °C for 8 h. The reaction mixture was diluted with EtOAc, washed with brine, dried (Na_2SO_4), filtered, concentrated and submitted to column chromatography (1:2 hexanes/EtOAc) to give **117** (1.2 g, 40 %). To a suspension of K_2CO_3 (1.1g, 8.0 mmol) in CH_2Cl_2 (20 mL) were added **117** (1.0 g, 2.04 mmol) and CCl_3CN (2.05 mL, 20.4 mol). The mixture was stirred overnight. After dilution with CH_2Cl_2, the mixture was filtered through a Celite pad. The filtrate was concentrated and submitted to chromatography (5:4 hexanes/EtOAc) to give **118** (290 mg, 25%) as a pale yellow crystalline: ^1H NMR (360 MHz, $CDCl_3$) δ 8.69 (s, 1 H, C=NH), 8.32 (m, 2H, ArH), 8.08 (m, 2H, ArH), 6.12 (d, 1H, J = 3.5 Hz, H-1), 5.65 (d, 1H, J = 8.4 Hz, NHCO), 4.94 (dd, 1H, J = 10.1, 9.6 Hz, H-3), 4.22 (dd, 1H, J = 12.6, 4.7 Hz, H-6a), 4.06-3.98 (m, 2H, H-5, H-6b), 3.73 (m, 1H, H-2), 2.09, 2.01, 1.99 (s, 3H, $COCH_3$).

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Chapter 7

Studies on the Synthesis of β-Glycosides without Neighboring Group Participation

7.1. Introduction

Most of the methods for the synthesis of β-glycosides developed so far rely on the assistance of neighboring group. However, these methods are not applicable to some sugars including mannose, rhamnose and 2-deoxy sugar derivatives. Furthermore, the stereoselectivity and yield are often affected by various factors including the leaving group and promoter, reactivity of donor and acceptor, the presence of a participating or non-participating neighboring group and solvent [126].

In 1988, both the Kahne group [187] and the Crich group [188] independently reported the highly stereoselective synthesis of 2-deoxy-β-glycosides via alkoxy-substituted anomeric radicals (Figure 7.1). The β-stereoselectivity was explained as being due to the high stability of the α-anomeric radical which can maximize the overlap with the lone pair of both the ring oxygen and the oxygen atom on the alkoxy substituent [187, 189]. We were attracted to this approach because the preparation of β-glycosides can be dissected into two independent processes: formation of mixed orthoester that includes the new glycosidic bond, and radical reduction which generates β-stereoselectivity. If efficient methods for the preparation of mixed orthoester (Figure 7.2) can be established, the ultimate goal of making β-glycosides in high yield and high stereoselectivity can be achieved. Such an approach should be generally applicable to the synthesis of all types of β-glycosides since it is independent of the presence of a neighboring group.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Figure 7.1. Synthesis of β-glycosides via alkoxy substituted anomeric radicals.

The previous work [187] only reported the preparation and reactions of hemithio-orthoesters of primary alcohols such as methanol. The objective of our studies was to see whether an efficient method to make hemithio-orthoesters of more complex sugar alcohols was plausible.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

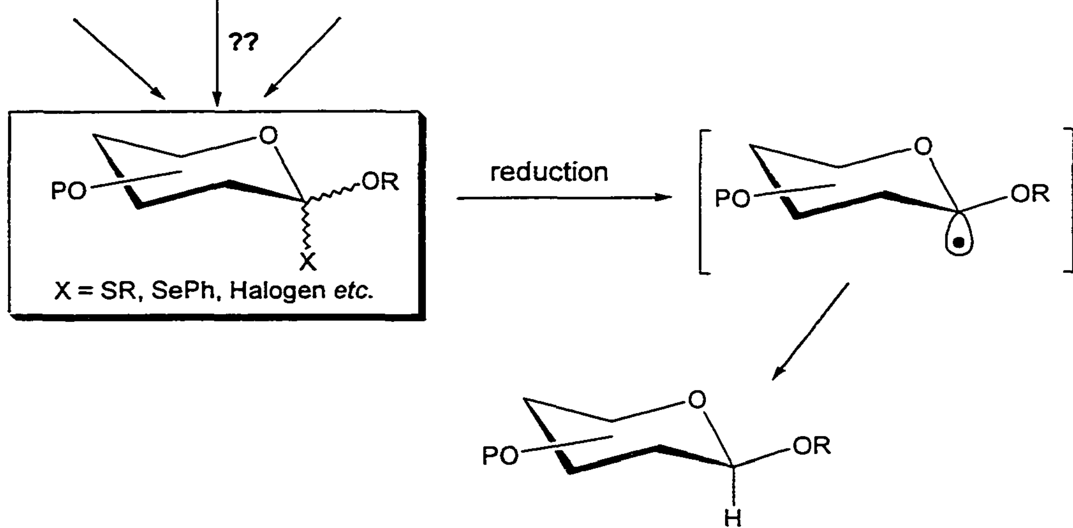


Figure 7.2. Schematic representation of the objective of the project.

7.2. Synthetic Studies

Lactones **120-122** were easily prepared by oxidation of their corresponding hemiacetals with PCC [190]. The thionation of the lactones with Lawesson's reagent [191], however, resulted in the very low yields. The Gal thionolactone **123** was obtained in only 20% yield. The Glc thionolactone **124** could not be purified from the reaction mixture was encountered, and the reaction of mannose derived lactone **122** with Lawesson's reagent failed completely (Figure 7.3).

The synthesis of hemithio-orthoesters from thionolactone **123** was attempted using the method reported by Kahne *et al.* [187] which involved the methylation of thionolactones and trapping of the intermediate oxathienium ions with alcohols as shown in Figure 7.1. Instead of using methyl iodide as the solvent at reflux, we tried

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

dimethyl(methylthio)sulfonium triflate (DMTST) [192, 193] and methyl triflate (MeOTf) [193, 194] as thio-activating agents. However, as shown in Figure 7.4, in both cases the reaction did not give the desired adduct. We therefore tried the direct nucleophilic addition of alkoxide to the thionolactone followed by the trapping of thiolate intermediate with methyl iodide (Figure 7.5).

OBn OBn O BnO OBn O 120 Lawesson's reagent (20%) OBn OBn O BnO OBn S 123

OBn BnO O BnO OBn O 121 Lawesson's reagent OBn BnO O BnO OBn S 124 (not isolated)

OBn OBn BnO O BnO O 122 Lawesson's reagent OBn OBn BnO O BnO S 125

H_3CO S S P P S S OCH_3

(Lawesson's reagent)

Figure 7.3. Preparation of the thionolactone **123**.

The required alkoxide was prepared by the deprotonation of **97** with sodium hydride in THF. Mixing of the alkoxide and **123** followed by addition of methyl iodide provided the desired adduct **126**, the only isomer isolated, in 47% yield (Figure 7.5). The stereochemistry at the newly formed anomeric center was confirmed by NOE studies, where no direct NOEs were observed between H-3 and thiomethyl protons.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

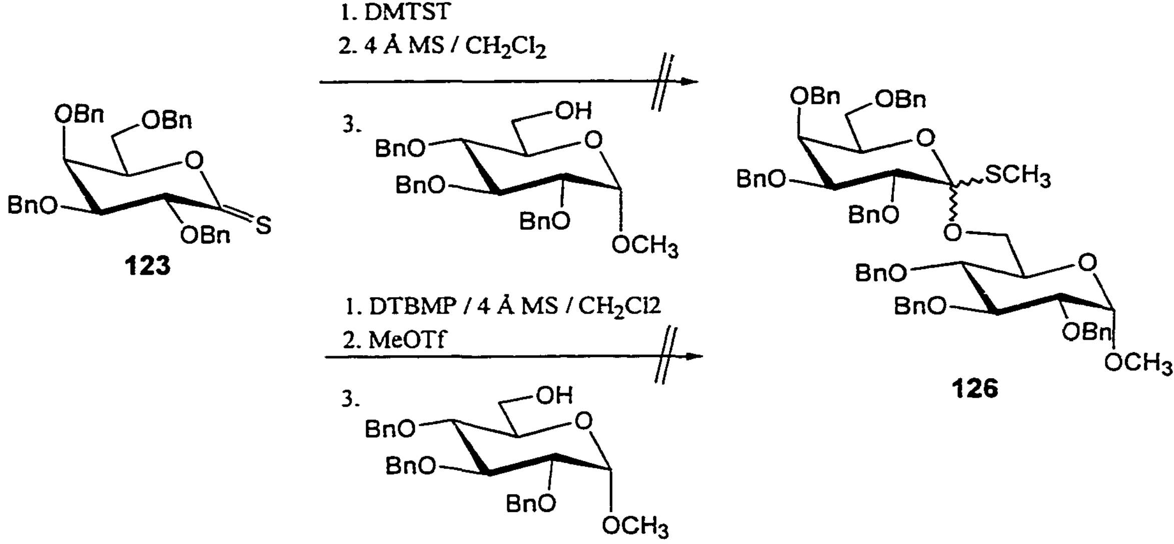


Figure 7.4. Attempt to prepare the hemithio-orthoester **126**.

123

NaH, THF, 5 min

97

CH_3I, 30 min (47%)

126

Figure 7.5. Preparation of the hemithio-orthoester **126**.

The reaction of thionolactone **123** and a hindered C-3 alkoxide, however, gave the product **127** in only 28% yield. No desired adduct was formed in the reaction of **123** with the much more hindered C-4 alkoxide (Figure 7.6). The low yield of the reactions may be

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

due to the elimination and other side reactions caused by the basicity of the alkoxides coupled with steric hindrance to nucleophilic attack.

Figure 7.6. Reaction of thionolactone 123 with secondary alkoxides.

The low yields and difficulties encountered in the preparation of hemithio-orthoesters forced us to consider other approaches. It was thought that the intramolecular cyclization by nucleophilic addition of anionic O-5 to the thionoester could be facile (Figure 7.7). In this approach, the aldose is oxidized to aldonic acid, and the glycosidic C-O bond is established via esterification of the aldonic aid with an alcohol acceptor [195]. Thionation, selective deprotection at O-5, and ring-closure could finally provide the hemithio orthoester. One advantage of this approach is that the esterification-glycosylation could be optimized to give high yields using well-established methods for ester and amide coupling.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Figure 7.7. Schematic representation of the intramolecular cyclization approach to hemithio-orthoesters.

Simple methyl and ethyl gluconates were investigated as model compounds (Figure 7.8). Lactone opening with methanol or ethanol was found to have low yields as the acyclic product and the lactone tend to form an equilibrium in the reaction [196]. During the work-up, the evaporation of methanol or ethanol solvent was carried out at the low temperature to reduce the cyclization of the formed acyclic product. The products were immediately reacted with TBS-Cl and imidazole in DMF to give **129** (26%, two steps) and **130** (20%, two steps). The TBS (*tert*-butyldimethylsilyl) group was chosen to protect the OH-5 since it is compatible with Lawesson's reagent and can be selectively

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

removed without affecting the benzyl groups. The thionation of **129** and **130** with Lawesson's reagent, however, provided the thionoesters, once again, in unacceptably low yields. The attempted removal of the silyl group using TBAF also failed.

Figure 7.8. Preparation of the glucothionates **131** and **132**.

Figure 7.9. Attempt to prepare the galactothionate **135**.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

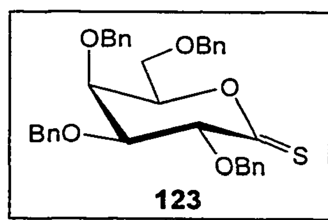
Methyl galactonate **134** was also prepared in the same fashion as for the glucose derivatives (Figure 7.9). Unfortunately, the thionation with Lawesson's reagent failed completely. As studied by Lawesson and co-workers [197], and Nicolaou *et al.* [198], other thionating agents like Belleau's regent [199], Yokoyama's reagent [200] and Davy's reagent [201] do not result in significantly improved yields. At this point, we did not try other thionating agents, and the low yield in the thionation step made it difficult to carry out further studies.

Despite the attractive features of the potential high-yielding esterification-glycosylation and stereocontrolled reduction of hemithio orthoester, the results obtained in this exploratory work suggest that the synthesis of β-glycosides from the hemithio-orthoesters will not be practical until better thionation chemistry is developed.

7.3. Experimental

General methods were the same as described in Chapter 2.

2,3,4,6-Tetra-O-benzyl-D-galactono-1,5-thionolactone (**123**).

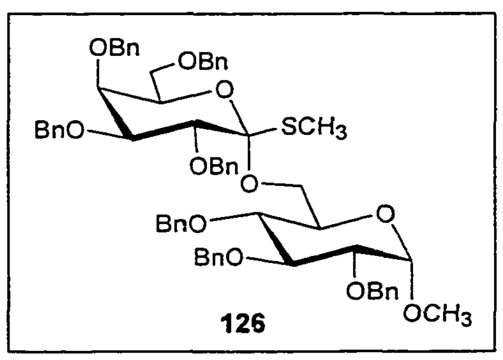


A mixture of 2,3,4,6-tetra-*O*-benzyl-D-galactono-1,5-lactone **120** (1.5 g, 2.8 mmol), Lawesson's reagent (2.2 g, 5.6 mmol) and 3 Å MS (2.5 g) in toluene (40 mL) was refluxed for 4 h. The reaction mixture was filtered through a Celite pad and washed with CH_2Cl_2. The filtrate was concentrated and submitted to column chromatography (15:1 hexanes/EtOAc) to give **123** (250 mg, 24%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42-7.20 (m, 20H, ArH), 5.22 (d, 1H, J = 10.7 Hz, PhCH_2), 4.95

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

(d, 1H, J = 11.5 Hz, PhCH_2), 4.78 (d, 1H, J = 10.7 Hz, PhCH_2), 4.72 (d, 1H, J = 11.9 Hz, PhCH_2), 4.65 (d, 1H, J = 11.4 Hz, PhCH_2), 4.63 (d, 1H, J = 11.9 Hz, PhCH_2), 4.56- 4.45 (m, 4H, PhCH_2, H-2, H-5), 4.22 (t, 1H, J = 2.2 Hz), 3.86-3.78 (m, 3H, H-3, H-6a, H-6b); ^{13}C NMR (125 MHz, $CDCl_3$) δ 217.96 (C=S), 137.83, 137.73, 137.53, 137.40 (aromatic quart.), 128.71, 128.49, 128.47, 128.36, 127.99, 127.96, 127.90, 127.84, 127.58 (aromatic CH), 83.69, 81.62, 79.85, 75.02, 74.59, 73.67, 72.58, 72.51 (PhCH_2 × 4, C-2, C-3, C-4, C-5), 67.33 (C-6); HR-ESMS calcd for $C_{34}H_{34}O_5NaS$ (M+Na$^+$) 577.2025, found 577.2026.

Methyl 2,3,4,6-tetra-O-benzyl-1-thiomethyl-α-D-galactopyranosyl-(1→4)-2,3,4-tri-O-benzyl-α-D-glucopyranoside (**126**).

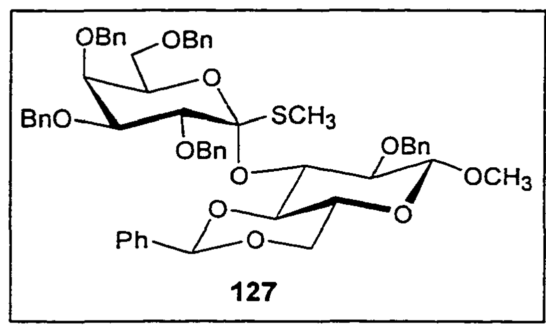


Sodium hydride (12 mg, 60% dispersion in mineral oil, 0.25 mmol) was added to a solution of compound **97** (94 mg, 0.20 mmol) in THF (3 mL). The mixture was stirred at rt for 5 min. A solution of **123** (140 mg, 0.25 mmol) in THF (3 mL) was then added. After 15 min, CH_3I was added to the reaction and stirring continued for another 20 min. The reaction mixture was concentrated and submitted to column chromatography (10:1 hexanes/EtOAc) to give **126** (98 mg, 47%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.40-7.20 (m, 35 H, ArH), 4.98 (d, 1H, J = 11.1 Hz, PhCH_2), 4.97 (d, 1H, J = 11.1 Hz, PhCH_2), 4.96 (d, 1H, J = 10.6 Hz, PhCH_2), 4.88 (d, 1H, J = 11.1 Hz, PhCH_2), 4.86 (d, 1H, J = 11.6 Hz, PhCH_2), 4.79 (d, 1H, J = 3.5 Hz, H-1), 4.78 (d, 1H, J = 12.2 Hz, PhCH_2), 4.77 (d, 1H, J = 10.6 Hz, PhCH_2), 4.73 (d, 1H, J = 12.2 Hz, PhCH_2), 4.70 (t, 2H, J = 8.0 Hz, PhCH_2), 4.62 (d, 1H, J = 11.4 Hz, PhCH_2), 4.54 (d, 1H, J = 11.2 Hz, PhCH_2), 4.50 (d, 1H, J = 12.0 Hz, PhCH_2), 4.48 (d, 1H, J = 12.0 Hz, PhCH_2), 4.22 (d, 1H, J = 10.0 Hz, H-2'), 4.12-4.06 (m, 3H, H-6a, H-4', H-5'),

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

4.00 (dd, 1H, J = 10.0, 2.8 Hz, H-3'), 3.89 (t, 1H, J = 9.1 Hz, H-3), 3.80 (m, 1H, H-5), 3.67 (dd, 1H, J = 10.8, 7.4, H-6b), 3.59 (dd, 1H, J = 6.4, 3.1 Hz, H-6'a), 3.53-3.49 (m, 2H, H-2, H-6'b), 3.34 (dd, 1H, J = 10.2, 8.8 Hz, H-4), 3.28 (s, 3H, OCH_3), 2.09 (s, 3H, SCH_3); ^{13}C NMR (75 MHz, CD_3COCD_3) δ 140.16 (2 × C), 140.00, 139.80, 139.72, 139.61 (2 × C) (aromatic quart.), 129.09, 129.07, 129.04, 128.98, 128.87, 128.56, 128.55, 128.47, 128.37, 128.30, 128.24, 128.16, 128.12, 128.00 (aromatic CH), 109.76 (C-1'), 98.13 (C-1), 83.51, 82.86, 81.33, 80.67, 79.48, 75.99, 75.85, 75.78, 75.38, 75.29, 73.47, 72.94, 72.83 (2 × C), 70.52 ($PhCH_2$ × 7, C-2, C-3, C-4, C-5, C-2', C-3', C-4', C-5'), 69.66, 63.18 (C-6, C-6'), 55.06 (OCH_3), 12.77 (SCH_3); HR-ESMS calcd for $C_{63}H_{68}O_{11}NaS$ ($M+Na^+$) 1055.4380, found 1055.4385.

Methyl 2,3,4,6-tetra-O-benzyl-1-thiomethyl-α-D-galactopyranosyl-(1→4)-2-O-benzyl-4,6-O-benzylidene-β-D-glucopyranoside (**127**).

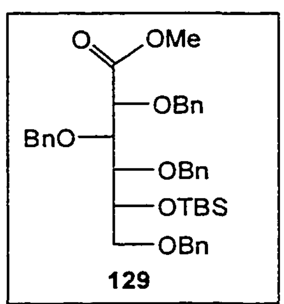


Sodium hydride (8 mg, 60% dispersion in mineral oil, 0.20 mmol) was added to a solution of compound methyl 4,6-*O*-benzylidene-β-D-glucopyranoside (58 mg, 0.16 mmol) in THF (3 mL). The mixture was stirred at rt for 5 min. A solution of **123** (120 mg, 0.22 mmol) in THF (3 mL) was then added. After 15 min, CH_3I was added to the reaction and stirring continued for another 20 min. The reaction mixture was concentrated and submitted to column chromatography (10:1 hexanes/EtOAc) to give **127** (42 mg, 28%): ^1H NMR (300 MHz, CD_2Cl_2) δ 7.60-7.00 (m, 30 H, ArH), 5.57 (s, 1H, Ph*CH*), 4.92 (d, 1H, J = 11.5 Hz, PhCH_2), 4.86 (d, 1H, J = 12.0 Hz, PhCH_2), 4.84 (d, 1H, J = 12.2 Hz, PhCH_2), 4.82 (d, 1H, J = 11.5 Hz, PhCH_2), 4.76 (d, 1H, J = 12.2 Hz, PhCH_2), 4.72 (dd, 1H, J = 8.3, 5.0 Hz, H-3), 4.64-4.50 (m, 4H, PhCH_2, H-1), 4.38-4.20 (PhCH_2, H-6a, H-2', H-5'), 4.00-

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

3.94 (m, 2H, H-4, H-4'), 3.90 (dd, 1H, 10.0, 2.6 Hz, H-3'), 3.76 (t, 1H, J = 5.0 Hz, H-2), 3.73-3.68 (m, 2H, H-5, H-6b), 3.50 (dd, 1H, J = 8.9, 7.6 Hz, H-6'a), 3.37 (s, 3H, OCH_3), 3.35 (dd, 1H, J = 8.9, 3.3 Hz, H-6'b), 2.16 (s, 3H, SCH_3); ^{13}C NMR (75 MHz, CD_3COCD_3) δ 139.53, 139.31, 139.20, 138.66, 138.14 (aromatic quart.), 129.01, 128.63, 128.58, 128.52, 128.30, 128.13, 128.04, 127.93, 127.84, 127.68, 127.63, 127.48, 126.81 (aromatic CH), 111.13 (C-1'), 103.01, 101.83 (C-1, Ph*C*H), 80.67, 80.49, 80.23, 79.98, 75.64, 75.20, 75.05, 74.87, 73.48, 72.84, 72.25, 69.82, 68.63, 65.22 (PhCH_2 × 5, C-2, C-3, C-4, C-5, C-6, C-2', C-3', C-4', C-5', C-6'), 56.33 (OCH_3), 13.55 (SCH_3); HR-ESMS calcd for $C_{56}H_{60}O_{11}NaS$ (M+Na^+) 963.3754, found 963.3743.

Methyl 2,3,4,6-tetra-O-benzyl-5-O-tert-butyldimethylsilyl-D-gluconate (**129**).

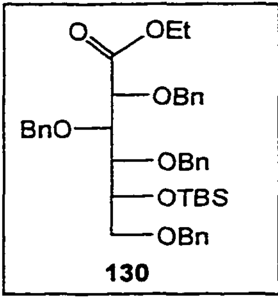


A solution of **121** (3.88 g, 7.21 mmol) in MeOH (10 mL) was stirred at rt for 14 h. The mixture was concentrated at low temperature. The resulting residue was dissolved in DMF (15 mL), and tert-butyldimethylsilyl chloride (2.20 g, 14.4 mmol) and imidazole (1.70 g, 24.9 mmol) were added. After stirring overnight, the reaction mixture was concentrated and submitted to column chromatography (15:1 hexanes/EtOAc) to give **129** (1.31 g, 26%): ^1H NMR (360 MHz, $CDCl_3$) δ 7.40-7.20 (m, 20H, ArH), 4.82 (d, 1H, J = 11.2 Hz, PhCH_2), 4.80 (d, 1H, J = 11.5 Hz, PhCH_2), 4.78 (d, 1H, J = 11.1 Hz, PhCH_2), 4.70 (d, 1H, J = 11.1 Hz, PhCH_2), 4.55 (d, 1H, J = 11.5 Hz, PhCH_2), 4.48-4.42 (m, 3H, PhCH_2), 4.26 (d, 1H, J = 3.5 Hz, H-2), 4.12 (dd,1 H, J = 7.3, 3.5 Hz, H-3), 4.01 (m, 1H, H-5), 3.95 (dd, 1H, J = 7.3, 3.4 Hz, H-4), 3.74 (dd, 1H, J = 9.6, 5.3 Hz, H-6a), 3.54 (s, 3H, OCH_3), 3.46 (dd, 1H, J = 9.6, 5.3 Hz, H-6b), 0.86 (s, 9H, $C(CH_3)_3$), 0.04, 0.01 (s, 3H, $SiCH_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.92 (C=O), 139.15, 138.59, 138.32, 137.35 (aromatic quart.), 128.35, 128.32, 128.31, 128.28, 128.20, 127.99, 127.82, 127.66, 127.46, 127.34 (aromatic CH), 82.05,

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

80.06, 79.17, 75.08, 75.00, 73.40, 73.34, 73.07, 71.84 (PhCH_2 × 4, C-2, C-3, C-4, C-5, C-6), 51.59 (OCH_3), 25.88 (C(CH_3)$_3$), 18.09 (C(CH_3)$_3$), -4.53, -4.72 ($SiCH_3$); HR-ESMS calcd for $C_{41}H_{52}O_7NaSi$ (M+Na^+) 707.3380, found 707.3383.

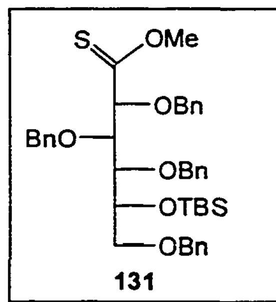
Ethyl 2,3,4,6-tetra-O-benzyl-5-O-tert-butyldimethylsilyl-D-gluconate (**130**).



A solution of **121** (450 mg, 0.84 mmol) in EtOH (40 mL) was refluxed for 2 days. The mixture was concentrated at low temperature. The resulting residue was dissolved in DMF (4 mL), and tert-butyldimethylsilyl chloride (253 mg, 1.68 mmol) and imidazole (114 mg, 1.68 mmol) were added. After stirring overnight, the reaction mixture was concentrate and submitted to column chromatography (10:1 hexanes/EtOAc) to give **130** (120 mg, 20%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40-7.20 (m, 20H, ArH), 4.82 (d, 1H, J = 11.3 Hz, PhCH_2), 4.80 (d, 1H, J = 11.3 Hz, PhCH_2), 4.75 (d, 1H, J = 11.2 Hz, PhCH_2), 4.68 (d, 1H, J = 11.2 Hz, PhCH_2), 4.55 (d, 1H, J = 11.4 Hz, PhCH_2), 4.44 (d, 1H, J = 11.2 Hz, PhCH_2), 4.42 (t, 2H, J = 12.2 Hz, PhCH_2), 4.25 (d, 1H, J = 3.5 Hz, H-2), 4.14-3.90 (m, 5H, H-3, H-4, H-5, C$H_2$$CH_3$), 3.71 (dd, 1H, J = 9.7, 5.1 Hz, H-6a), 3.44 (dd, 1H, J = 9.7, 5.4 Hz, H-6b), 1.13 (t, 3H, CH_2CH_3), 0.84 (s, 9H, C(CH_3)$_3$), 0.024, 0.004 (s, 3H, $SiCH_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.50 (C=O), 139.16, 138.72, 138.38, 137.47 (aromatic quart.), 128.40, 128.30, 128.20, 128.16, 128.04, 127.98, 127.81, 127.64, 127.49, 127.39, 127.34 (aromatic CH), 82.05, 80.24, 79.31, 75.18, 74.95, 73.50, 73.32, 71.92 (PhCH_2 × 4, C-2, C-3, C-4, C-5, C-6), 60.82 (CH_2CH_3), 25.93 (C(CH_3)$_3$, 18.11 (C(CH_3)$_3$), 14.19 (CH_2CH_3), -4.48, -4.68 ($SiCH_3$); HR-ESMS calcd for $C_{42}H_{54}O_7NaSi$ (M+Na^+) 721.3536, found 721.3532.

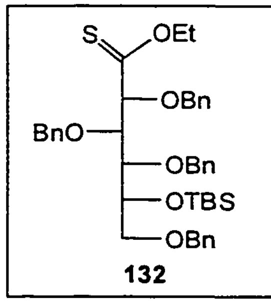
Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Methyl 2,3,4,6-tetra-O-benzyl-5-O-tert-butyldimethylsilyl-D-glucothionate (**131**).



A mixture of **129** (710 mg, 1.04 mmol), Lawesson's reagent (840 mg, 2.08 mmol) and 3 Å MS (900 mg) in toluene (70 mL) was refluxed for 12 h. The reaction mixture was filtered through a Celite pad and washed with CH_2Cl_2. The filtrate was concentrated and submitted to column chromatography (15:1 hexanes/EtOAc) to give **131** (32 mg, 5%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40-7.20 (m, 20H, ArH), 4.73 (d, 1H, J = 11.2 Hz, PhCH_2), 4.71 (d, 1H, J = 11.4 Hz, PhCH_2), 4.70 (d, 1H, J = 11.4 Hz, PhCH_2), 4.63 (d, 1H, J = 11.3 Hz, PhCH_2), 4.60 (d, 1H, J = 11.3 Hz, PhCH_2), 4.56 (d, 1H, J = 4.7 Hz, H-2), 4.46-4.38 (m, 3H, PhCH_2), 4.16 (dd, 1H, J = 6.2, 4.7 Hz, H-3), 3.99 (m, 1H, H-5), 3.94 (s, 3H, OCH_3), 3.80 (dd, 1H, J = 6.2, 3.4 Hz, H-4), 3.74 (dd, 1H, J = 9.8, 4.4 Hz, H-6a), 3.48 (dd, 1H, J = 9.8, 6.0 Hz, H-6b), 0.85 (s, 9H, $C(CH_3)_3$), 0.016, -0.011 (s, 3H, $SiCH_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 75 MHz, $CDCl_3$) δ 219.85 (C=S), 139.11, 138.55, 138.43, 137.43 (aromatic quart.), 128.48, 128.37, 128.28, 128.18, 128.13, 127.90, 127.80, 127.72, 127.45, 127.31 (aromatic CH), 87.73, 82.40, 81.67, 75.59, 74.95, 73.66, 73.29, 72.96, 71.95 (PhCH_2 × 4, C-2, C-3, C-4, C-5, C-6), 58.73 (OCH_3), 25.95 ($C(CH_3)_3$), 18.15 ($C(CH_3)_3$), -4.37, -4.68 ($SiCH_3$); HR-ESMS calcd for $C_{41}H_{52}O_6NaSiS$ (M+Na$^+$) 723.3152, found 723.3149.

Ethyl 2,3,4,6-tetra-O-benzyl-5-O-tert-butyldimethylsilyl-D-glucothionate (**132**).

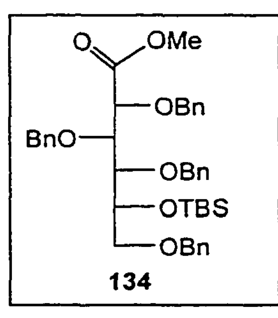


A mixture of **130** (60 mg, 86 μmol), Lawesson's reagent (100 mg, 0.24 mmol) and 3 Å MS (100 mg) in toluene (5 mL) was refluxed for 40 h. The reaction mixture was filtered through a Celite pad and washed with CH_2Cl_2. The filtrate was concentrated and submitted to column chromatography (15:1 hexanes/EtOAc) to

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

give **132** (10 mg, 16%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40-7.20 (m, 20H, ArH), 4.74 (d, 1H, *J* = 11.3 Hz, $PhCH_2$), 4.73 (d, 1H, *J* = 11.3 Hz, $PhCH_2$), 4.70 (d, 1H, *J* = 11.8 Hz, $PhCH_2$), 4.63 (d, 1H, *J* = 11.1 Hz, $PhCH_2$), 4.62 (d, 1H, *J* = 11.3 Hz, $PhCH_2$), 4.56 (d, 1H, *J* = 4.8 Hz, H-2), 4.46-4.38 (m, 3H, $PhCH_2$, CH_2CH_3) , 4.18 (dd, 1H, *J* = 6.2, 4.7 Hz, H-3), 3.99 (m, 1H, H-5), 3.82 (dd, 1H, *J* = 6.1, 3.3 Hz, H-4), 3.75 (dd, 1H, *J* = 9.8, 4.3 Hz, H-6a), 3.49 (dd, 1H, *J* = 9.8, 6.0 Hz, H-6b), 1.14 (t, 3H, CH_2CH_3), 0.82 (s, 9H, $C(CH_3)_3$), 0.02, 0.004 (s, 3H, $SiCH_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 219.27 (C=S), 139.17, 138.73, 138.47, 137.56 (aromatic quart.), 128.86, 128.40, 128.29, 128.18, 128.10, 127.86, 127.79, 127.71, 127.45, 127.40, 127.30 (aromatic CH), 82.88, 82.62, 81.95, 75.69, 74.97, 73.63, 73.27, 72.90, 71.95, 68.49 ($PhCH_2$ × 4, C-2, C-3, C-4, C-5, C-6), 68.22 (CH_2CH_3), 25.98 ($C(CH_3)_3$, 18.16 ($C(CH_3)_3$), 13.57 (CH_2CH_3), -4.35, -4.64 ($SiCH_3$); HR-ESMS calcd for $C_{42}H_{54}O_6NaSiS$ ($M+Na^+$) 737.3308, found 737.3306.

Methyl 2,3,4,6-tetra-O-benzyl-5-O-tert-butyldimethylsilyl-D-galactonate (**134**).



Lactone **120** (1.64 g, 2.88 mmol) was treated in the same fashion as for **129** to give **134** (390 mg, 20%): ^{1}H NMR (360 MHz, $CDCl_3$) δ 7.40-7.20 (m, 20H, ArH), 4.85 (d, 1H, *J* = 11.3 Hz, $PhCH_2$), 4.75 (d, 1H, *J* = 11.1 Hz, $PhCH_2$), 4.65 (d, 1H, *J* = 11.2 Hz, $PhCH_2$), 4.59 (d, 1H, *J* = 12.1 Hz, $PhCH_2$), 4.53 (d, 1H, *J* = 12.1 Hz, $PhCH_2$), 4.51 (d, 1H, *J* = 12.1 Hz, $PhCH_2$), 4.45 (d, 1H, *J* = 12.5 Hz, $PhCH_2$), 4.43 (d, 1H, *J* = 3.4 Hz, H-2), 4.38 (d, 1H, *J* = 11.3 Hz, $PhCH_2$), 4.33 (dd, 1H, *J* = 8.3, 3.4 Hz, H-3), 4.28 (dt,1 H, *J* = 6.1, 2.6 Hz, H-5), 3.95 (dd, 1H, *J* = 8.3, 2.5 Hz, H-4), 3.68 (s, 3H, OCH_3), 3.63 (dd, 1H, *J* = 8.0, 6.1 Hz, H-6a), 3.59 (dd, 1H, *J* = 8.0, 6.1 Hz, H-6b), 0.94 (s, 9H, $C(CH_3)_3$), 0.08, 0.07 (s, 3H, $SiCH_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.08 (C=O), 138.97, 138.53, 138.21, 137.76 (aromatic quart.), 128.31, 128.30, 128.25, 128.21, 128.16, 128.00, 127.69, 127.65, 127.53, 127.43, 127.18, 127.09,

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

127.06, 127.04, 126.98, 126.92 (aromatic CH), 79.16, 78.75, 78.01, 73.51, 73.10, 72.88, 72.85, 71.98, 71.50 (Ph*C*H_2 × 4, C-2, C-3, C-4, C-5, C-6), 51.72 (OCH_3), 26.07 (C(*C*H_3)$_3$), 18.39 (*C*(CH_3)$_3$), -3.82, -4.37 ($SiCH_3$); HR-ESMS calcd for $C_{41}H_{52}O_7NaSi$ ($M+Na^+$) 707.3380, found 707.3388.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Chapter 8

Bibliography

1. (a) Sharon, N.; Lis, H. *Scientific American* **1993**, 82. (b) Varki, A. *Glycobiology* **1993**, *3*, 97.
2. (a) Dwek, R. A. *Chem. Rev.* **1996**, *96*, 683. (b) Ggneux, P.; Varki, A. *Glycobiology* **1999**, *9*, 747.
3. Lee, Y. C. *Trends Glycosci. Glycotechnol.* **1997**, *9*, S1.
4. Laine, R. A. *Glycobiology* **1994**, *4*, 1.
5. Fukuda, M. In *Molecular Glycobiology*; Fukuda, M., Hindsgaul, O. Eds.; Oxford: New York, **1994**; pp 1-52.
6. Kopitz, J. In *Glycosciences*; Gabius, H.-J., Gabius, S. Eds.; Chapman & Hall: Weinheim, **1997**; pp 31-53.
7. Ferguson, M. A. J.; Homans, S. W.; Dwek, R. A.; Rademacher, T. W. *Science* **1988**, *239*, 753.
8. Sears, P.; Wong, C.-H. *Cell. Mol. Life Sci.* **1998**, *54*, 223.
9. Brockhausen, I.; Schachter, H. In *Glycosciences*; Gabius, H.-J., Gabius, S. Eds.; Chapman & Hall: Weinheim, **1997**; pp 79-113.
10. van Echten, G.; Sandhoff, K. *J. Biol. Chem.* **1993**, *268*, 5341.
11. Wong, C.-H.; Halcomb, R. L.; Ichikawa, Y.; Kajimoto, T. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 521.
12. Schachter, H. In *Molecular Glycobiology*; Fukuda, M., Hindsgaul, O. Eds.; Oxford: New York, **1994**; pp 88-162.
13. Hagopian, A.; Eylar, E. H. *Arch. Biochem. Biophys.* **1968**, *128*, 422.
14. Sears, P.; Wong, C.-H. *Angew. Chem. Int. Ed.* **1999**, *38*, 2300.
15. McAuliffe, J.; Hindsgaul, O. *Chem. Ind.* **1997**, 170.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

16. (a) Toshima, K.; Tatsuta, K. *Chem. Rev.* **1993**, *93*, 1603. (b) Barresi, F.; Hindsgaul, O. *J. Carbohydr. Chem.* **1995**, *14,* 1043. (c) Boons, G.-J. *Tetrahedron* **1996**, *52*, 1095. (d) Schmidt, R. R. In *Glycosciences*; Gabius, H.-J., Gabius, S. Eds.; Chapman & Hall: Weinheim, **1997**; pp 31-53.

17. Khan, S. H.; Hindsgaul, O. In *Molecular Glycobiology*; Fukuda, M., Hindsgaul, O. Eds.; Oxford: New York, **1994**; pp 206-229.

18. (a) Wong, C.-H.; Whitesides, G. M. *Enzymes in Synthetic Organic Chemistry*; Elsevier: Oxford, **1994**; pp.252-311. (b) Palcic, M. M. *Methods Enzymol.* **1994**, *230*, 300. (c) Gijsen, H. J. M.; Qiao, L.; Fitz, W.; Wong, C.-H. *Chem. Rev.* **1996**, *96*, 443. (d) Takayama, S.; McGarvey, G. J.; Wong, C.-H. *Chem. Soc. Rev.* **1997**, *26*, 407. (e) Ichikawa, Y. In *Glycopeptides and Related Compounds: Synthesis, Analysis and Application* , Large, D. G., Warren, C. D. Eds.; Dekker: New York, **1997**; pp 79-205.

19. (a) Gilbert, M.; Cunningham, A. M.; Watson, D. C.; Martin, A.; Richards, J. C.; Wakarchuk, W. W. *Eur. J. Biochem.* **1997**, *249*, 187. (b) Wakarchuk, W. W.; Cunningham A.; Watson, D. C.; Young, N. M. *Protein Eng.* **1998**, *11*, 295. (c) Blixt, O.; van Die, I.; Norberg, T.; van den Eijnden, D. H. *Glycobiology* **1999**, *9*, 1061.

20. Wong, C.-H.; Haynie, S. L.; Whitesides, G. M. *J. Org. Chem.* **1982**, *47*, 5416.

21. Koizumi, S.; Endo, T.; Tabata, K.; Ozaki, A. *Nature Biotech.* **1998**, *16*, 847.

22. (a) Lemieux, R. U. In *Proc. VIIIth Int. Symp. Med. Chem.*; Swedish Parmaceutical Press: Stockholm, **1985**; pp 329-351. (b) Lemieux, R. U. *Chem. Soc. Rev.* **1989**, *18*, 347.

23. (a) Crawley, S. C.; Palcic, M. M. In *Modern Methods in Carbohydrate Synthesis;* Khan, S. H., O'Neil, R. A. Eds.; Harwood Academic: Amsterdam, **1995**; pp 492-517. (b) Palcic, M. M.; Hindsgaul, O. *Trends Glycosci. Glycotechnol.* **1996**, *8*, 37

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

and references therein. (c) Elhalabi, J. M.; Rice, K. G. *Curr. Med. Chem.* **1999**, *6*, 93. (d) Öhrlein, R. *Top. Curr. Chem.* **1999**, *200*, 227.

24. (a) Wong, C.-H.; Wang, R.; Ichikawa, Y. *J. Org. Chem.* **1992**, *57*, 4343. (b) Ichikawa, Y.; Lin, Y.-C.; Dumas, D. P.; Shen, G.-J.; Garcia-Junceda, E.; Williams, M. A.; Bayer, R.; Ketcham, K.; Walker, L. E.; Paulson, J. C.; Wong, C.-H. *J. Am. Chem. Soc.* **1992**, *114*, 9283.
25. Berliner, L. J.; Davies, M. E.; Ebner, K. E.; Beyer, T. A.; Bell, J. E. *Mol. Cell. Biochem.* **1984**, *62*, 37.
26. Palcic, M. M.; Srivastava, O. P.; Hindsgaul, O. *Carbohydr. Res.* **1987**, *159*, 315.
27. Öhrlein, R.; Ernst, B.; Berger, E. G. *Carbohydr. Res.* **1992**, *236*, 335.
28. Baisch, G.; Öhrlein, R.; Ernst, B. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 749.
29. Kajihara, Y.; Kodama, H.; Endo, T.; Hashimoto, H. *Carbohydr. Res.* **1998**, *306*, 361.
30. Wong, C.-H.; Ichikawa, Y.; Krach, T.; Gautheron-Le Narvor, C.; Dumas, D. P.; Look, G. C. *J. Am. Chem. Soc.* **1991**, *113*, 8137.
31. Yu, L.; Cabrera, R.; Ramirez, J.; Malinovskii, V. A.; Brew, K.; Wang, P. G. *Tetrahedron Lett.* **1995**, *36*, 2897.
32. Nishida, Y.; Wiemann, T.; Sinnwell, V.; Thiem, J. *J. Am. Chem. Soc.* **1993**, *115*, 2536.
33. Gambert, U.; Thiem, J. *Top. Curr. Chem.* **1997**, *186*, 21.
34. Nishida, Y.; Wiemann, T.; Thiem, J. *Tetrahedron Lett.* **1992**, *33*, 8043
35. Nishida, Y.; Wiemann, T.; Thiem, J. *Tetrahedron Lett.* **1993**, *34*, 2905.
36. Wiemann, T.; Nashida, Y.; Sinnwell, V.; Thiem, J. *J. Org. Chem.* **1994**, *59*, 6744.
37. (a) Blanken, W. M.; Van den Eijnden, D. H. *J. Biol. Chem.* **1985**, *260*, 12927. (b) Joziasse, D. H.; Shaper, N. L.; Salyer, L. S.; Vanden Eijnden, D. H.; vander Spoel, A. C.; Shaper, J. H. *Eur. J. Biochem.* **1990**, *191*, 75. (c) Henion, T. R.; Macher, B. A.; Anaraki, F.; Galili, U. *Glycobiology* **1994**, *4*, 193. (d) Helland, A.-C.;

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Hindsgaul, O.; Palcic, M. M.; Stults, C. L. M.; Macher, B. A. *Carbohydr. Res.* **1995**, *276*, 91. (e) Fang, J.; Li, J.; Chen, X.; Zhang, Y.; Wang, J.; Guo Z,; Zhang, W.; Yu, L.; Brew, K.; Wang, P. G. *J. Am. Chem. Soc.* **1998**, *120*, 6635.

38. (a) Galili, U. *Immunol. Today* **1993**, *14*, 480. (b) Butler, D. *Nature* **1998**, *391*, 320.
39. Blanken, W. M.; Van den Eijnden, D. H. *J. Biol. Chem.* **1985**, *260*, 12927.
40. Stults, C. L. M.; Macher, B. A.; Bhatti, R.; Srivastava, O. P.; Hindsgaul, O. *Glycobiology* **1999**, *9*, 661.
41. Baisch, G.; Öhrlein, R.; Kolbinger, F.; Streiff, M. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1575.
42. Sujino, K.; Malet, C.; Hindsgaul, O.; Palcic, M. M. *Carbohydr. Res.* **1998**, *305*, 483.
43. Reuter, W.; Stache, R.; Stehling, P., Baum, O. In *Glycosciences*; Gabius, H.-J., Gabius, S. Eds.; Chapman & Hall: Weinheim, **1997**; pp 245-259.
44. Harduin-Lepers, A.; Recchi, M.-A.; Delannoy, P.; *Glycobiology* **1995**, *5*, 741.
45. Wlasichuk, K. B.; Kashem, M. A.; Nikrad, P. V.; Bird, P.; Jiang, C.; Venot, A. P. *J. Biol. Chem.* **1993**, *268*, 13971.
46. (a) Baisch, G.; Öhrlein, R.; Streiff, M.; Ernst, B. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 755. (b) Baisch, G.; Öhrlein, R.; Streiff, M. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 157.
47. (a) Hakomori, S.; Nudelma, E.; Levery, S. B.; Kannagi, R. *J. Biol. Chem.* **1984**, *259*, 4672. (b) Hakomori, S. *Adv. Cancer Res.* **1989**, *52*, 257. (c) Feizi, T. *Nature* **1985**, *314*, 53.
48. (a) Philips, M. L.; Neduelman, E.; Gata, F. C. A.; Perez, M.; Singhal, A. K.; Hakomori, S.-I.; Paulsen, J. C. *Science* **1990**, *250*, 1130. (b) Walx, G.; Arrufo, A.; Kolanus, W.; Bevilacqua, M.; Seed, B. *Science* **1990**, *250*, 1132.
49. Gosselin, S.; Palçic, M. M. *Bioorg. Med. Chem.* **1996**, *4*, 2023.
50. Nikrad, P. V.; KAshem, M. A.; Wlasichuk, K. B.; Alton, G.; Venot, A. P. *Carbohydr. Res.* **1993**, *250*, 145.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

51. Ding, Y.; Hindsgaul, O.; Li, H.; Palcic, M. M. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3199.

52. Ogawa, S.; Matsunaga, N.; Li, H.; Palcic, M. M. *Eur. J. Org. Chem.* **1999**, 631.

53. (a) Yamamoto, F.-I.; Clausen, H.; White, T.; Marken, J.; Hakomori, S.-I. *Nature* **1990**, *345*, 229. (b) Yamamoto, F.-I.; Hakomori, S.-I. *J. Boil. Chem.* **1990**, *265*, 19257. (c) Seto, N. O. L.; Palcic, M. M.; Hindsgaul, O.; Bundle, D. R.; Narang, S. N. *Eur. J. Biochem.* **1995**, *234*, 323. (d) Seto, N. O. L.; Palcic, M. M.; Compston, C. A.; Li, H.; Bundle, D. R.; Narang, S. A. *J. Biol. Chem.* **1997**, *272*, 14133.

54. Lowary, T. L.; Hindsgaul, O. *Carbohydr. Res.* **1993**, *249*, 163.

55. Lowary, T. L.; Hindsgaul, O. *Carbohydr. Res.* **1994**, *251*, 33.

56. Mukherjee, A. Ph.D. Dissertation, University of Alberta, **1999**.

57. (a) Watson, R. R.; Orenstein, N. S. *Adv. Carbohydr. Chem. Biochem.* **1975**, *31*, 135. (b) Grisebach, H. *Adv. Carbohydr. Chem. Biochem.* **1978**, *35*, 81. (c) Yoshimura, J. *Adv. Carbohydr. Chem. Biochem.* **1984**, *42*, 69. (d) Lindberg, B. *Adv. Carbohydr. Chem. Biochem.* **1990**, *48*, 279.

58. Shimomura, H.; Sashida, Y.; Mimaki, Y.; Adachi, T.; Yoshinari, K. *Chem. Pharm. Bull.* **1989**, *37*, 829.

59. Yamada, K.; Ojika, M.; Kigoshi, H. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 1818.

60. Kimura, Y.; Kobayashi, Y.; Takeda, T.; Ogihara, Y. *J. Chem. Soc., Perkin Trans.* **1981**, 1923.

61. Chatterjee, D.; Bozic, C.; Aspinall, G. O.; Brennan, P. J. *J. Biol. Chem.* **1988**, *263*, 4092.

62. Beau, J.-M.; Jaurand, G.; Esnault, J.; Sinaÿ, P. *Tetrahedron Lett.* **1987**, *28*, 1105.

63. Yan, L.; Kahne, D. *Synlett* **1995**, 523.

64. Crich, D.; Sun, S. *J. Am. Chem. Soc.* **1998**, *120*, 435.

65. (a) Lemieux, R. U. *Chem. Soc. Rev.* **1989**, *18*, 347. (b) Lee, Y. C.; Lee, R. T. *Acc. Chem. Res.* **1995**, *28*, 321. (c) Lemieux, R. U. *Acc. Chem. Res.* **1996**, *29*, 373. (d)

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Diaz-Maurino, T.; Solis, D. In *Glycosciences*; Gabius, H.-J., Gabius, S. Eds.; Chapman & Hall: Weinheim, **1997**; pp 345-354.

66. (a) Glaudemans, C. P. J. *Chem. Rev.* **1991**, *91*, 25. (b) Sierks, M. R. ; Bock, K.; Refn, S.; Svensson, B. *Biochemistry* **1992**, *31*, 8972. (c) Malet, C.; Hindsgaul, O. *J. Org. Chem.* **1996**, *61*, 4649. (d) Lu, P.-P.; Hindsgaul, O.; Li, H.; Palcic, M. M. *Carbohydr. Res.* **1997**, *303*, 283.

67. (a) Lindh, I.; Hindsgaul, O. *J. Am. Chem. Soc.* **1991**, *113*, 216. (b) Hartmann, M.; Christian, R.; Zbiral, E. *Monatsh. Chem.* **1991**, *122*, 111. (c) Palcic, M. M.; Skrydstrup, T.; Bock, K.; Le, N.; Lemieux, R. U. *Carbohydr. Res.* **1993**, *250*, 87. (d) Sabesan, S.; Neira, S.; Davidson, F.; Duus, J. O.; Bock, K. *J. Am. Chem. Soc.* **1994**, *116*, 1616. (e) Frandsen, T. P.; Stoffer, B. B.; Palcic, M. M.; Hof, S.; Svensson, B. *J. Mol. Biol.* **1996**, *263*, 79. (f) Frandsen, T. P.; Palcic, M. M.; Dupont, C.; Svensson, B. *Carbohydr. Res.* **1998**, *314*, 127.

68. Palcic, M. M.; Heerze, L. D.; Pierce, M.; Hindsgaul, O. *Glycoconjugate J.* **1988**, *5*, 49.

69. Grindley, B. T. In *Modern Methods in Carbohydrate Synthesis;* Khan, S. H., O'Neil, R. A. Eds.; Harwood Academic: Amsterdam, **1995**; pp 225-250.

70. (a) Hwang, T.-L.; Shaka, A. J. *J. Magn. Reson. Ser. B* **1993**, *102*, 155. (b) Bothner-By, A. A.; Stephens, R. L.; Lee, J.; Warren, C. D.; Jeanloz, R. W. *J. Am. Chem. Soc.* **1984**, *106*, 811. (c) Kessler, H.; Mronga, S.; Gemmecker, G. *Magn. Reson. Chem.* **1991**, *29*, 527.

71. (a) Dess, D. B.; Martin, J. C. *J. Org. Chem.* **1983**, *48*, 4156. (b) Dess, D. B.; Martin, J. C. *J. Am. Chem. Soc.* **1991**, *113*, 7277.

72. Pearlman, W. M. *Tetrahedron Lett.* **1967**, 1663. (b) Hanessian, S.; Liak, T. J.; Vanasse, B. *Synthesis* **1981**, 396.

73. Miljkovic, M.; Gligorijevic, M.; Satoh, T.; Miljkovic, D. *J. Org. Chem.* **1974**, *39*, 1379.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

74. Geen, H.; Freeman, R. *J. Magn. Reson.* **1991**, *93*, 93.

75. David, S.; Hanessian, S. *Tetrahedron* **1985**, *41*, 643.

76. Alais, J.; Maranduba, A.; Veyrieres, A. *Tetrahedron Lett.* **1983**, *24*, 2383.

77. Ogawa, T.; Yamamoto, H. *Agric. Biol. Chem.* **1985**, *49*, 475.

78. (a) Youssef, R. H.; Silwanis, B. A.; El-Sokkary, R. I.; Nematalla, A. S.; Nashed, M. A. *Carbohydr. Res.* **1993**, *240*, 287. (b) Barili, P. L.; Catelani, G.; D'Andrea, F. D.; De Rensis, F.; Falcini, P. *Carbohydr. Res.* **1997**, *298*, 75.

79. (a) Maruoka, K.; Itoh, T.; Yamamoto, H. *J. Am. Chem. Soc.* **1985**, *107*, 4573. (b) Maruoka, K.; Itoh, T.; Sakurai, M.; Nonoshita, K.; Yamamoto, H. *J. Am. Chem. Soc.* **1988**, *110*, 2588.

80. Cerny, M.; Kollmann, M.; Pacak, J.; Budesinsky, M. *Coll. Czechoslov. Chem. Commun.* **1974**, *39*, 2507.

81. Zottola, M. A.; Alonso, R.; Vite, G. D.; Fraser-Reid, B. *J. Org. Chem.* **1989**, *54*, 6123.

82. (a) Closson, W. D.; Wriede, P.; Bank, S. *J. Am. Chem. Soc.* **1966**, *88*, 1581. (b) Jarrell, H. C.; Ritchie, R. G. S.; Szarek, W. A.; Jones, J. K. N. *Can. J. Chem.* **1973**, *51*, 1767.

83. Czernecki, S.; Vijayakumaran, K.; Ville, G. *J. Org. Chem.* **1986**, *51*, 5472.

84. Evans, D. A.; Truesdale, L. K.; Carroll, G. L. *J. Chem. Soc., Chem. Commun.* **1973**, 55.

85. Malet, C.; Hindsgaul, O. *J. Org. Chem.* **1996**, *61*, 4649.

86. Malet, C.; Hindsgaul, O. *Carbohydr. Res.* **1997**, *303*, 51.

87. (a) Garegg, P. J.; Hultberg, H.; Wallin, S. *Carbohydr. Res.* **1982**, *108*, 97. (b) Spohr, U.; Lemieux, R. U. *Carbohydr. Res.* **1988**, *174*, 211.

88. Paulsen, H.; Paal, M. *Carbohydr. Res.* **1984**, *135*, 53.

89. Lough, C.; Hindsgaul, O. Lemieux, R. U. *Carbohydr. Res.* **1983**, *120*, 43.

90. Lemieux, R. U.; Morgan, A. R. *Can. J. Chem.* **1965**, *43*, 2199.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

91. Lichtenthaler, F. W.; Schneider-Adams, T. *J. Org. Chem.* **1994**, *59*, 6728.

92. (a) Murray, B. W.; Takayama, S.; Schultz, J.; Wong, C.-H. *Biochemistry* **1996**, *35*, 11183. (b) Murray, B. W.; Wittmann, V.; Burkart, M. D.; Hung, S.-C.; Wong, C.-H. *Biochemistry* **1997**, *36*, 823.

93. Breton, C.; Bettler, E.; Joziasse, D. H.; Geremia, R. A.; Imberty, A. *J. Biochem.* **1998**, *123*, 1000.

94. (a) Sinnott, M. L. *Chem. Rev.* **1990**, *90*, 1171. (b) Kempton, J. B.; Withers, S. G. *Biochemistry* **1992**, *31*, 9961. (c) McCarter, J. D.; Withers, S. G. *J. Am. Chem. Soc.* **1996**, *118*, 241. (d) Heightman, T. D.; Vasella, A. T. *Angew. Chem. Int. Ed.* **1999**, *38*, 750.

95. (a) Kim, C. K.; Singh, A. N.; Raushel, F. M. *Arch. Biochem. Biophys.* **1988**, *267*, 54. (b) Ats, S.-C.; Lehmann, J.; Petry, S. *Carbohydr. Res.* **1992**, *233*, 125.

96. Kakinuma, H.; Yuasa, H.; Hashimoto, H. *Carbohydr. Res.* **1998**, *312*, 103.

97. (a) Hindsgaul, O.; Norberg, T.; Pendu, J. L.; Lemieux, R. U. *Carbohydr. Res.* **1982**, *109*, 109. (b) Yan, L.; Kahne, D. *J. Am. Chem. Soc.* **1996**, *118*, 9239.

98. Li, J.; Ksebati, M. B.; Zhang, W.; Guo, Z.; Wang, J.; Yu, L.; Fang, J.; Wang, P. G. *Carbohydr. Res.* **1999**, *315*, 76.

99. Otter, A.; Lemieux, R. U., Ball, R. G.; Venot, P. A.; Hindsgaul, O.; Bundle, D. R. *Eur. J. Biochem.* **1999**, *259*, 295.

100. Bax, A.; Marion, D. *J. Magn. Reson.* **1988**, *78*, 186.

101. Williams, M. A.; Kitagawa, H.; Datta, A. K.; Paulsen, J. C.; Jamieson, J. C. *Glycoconjugate J.* **1995**, *12*, 755.

102. Legler, G. *Adv. Carobohydr. Chem. Biochem.* **1990**, *48*, 319.

103. (a) Bundle, D. R.; Young, N. M. *Curr. Opin. Struct. Biol.* **1992**, *2*, 666. (b). (c) Bundle, D. R. In *Glycosciences*; Gabius, H.-J., Gabius, S. Eds.; Chapman & Hall: Weinheim, **1997**; pp 311-331.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

104. (a) Liener, I. E., Sharon, N. Goldstein, I. J., Eds.; *The Lectins: Properties, Functions and Applications in Biology in Medicine*; Academic Press: Orlando, **1986**; 600 p. (b) Lis, H.; Sharon, N. *Chem. Rev.* **1998**, *98*, 637.

105. (a) Siebert, H.-S.; von der Lieth, C. L.; Gilleron, M.; Reuter, G.; Wittmann, J.; Vliegenthart, J. F. G.; Gabius, H. -J. In *Glycosciences*; Gabius, H.-J., Gabius, S. Eds.; Chapman & Hall: Weinheim, **1997**; pp 291-310. (b) Davis, A. P.; Wareham, R. S. *Angew. Chem. Int. Ed.* **1999**, *38*, 2978.

106. (a) Toone, E. J. *Curr. Opin. Struct. Biol.* **1994**, *4*, 719. (b)Gupta, D.; Brewer, C. F. In *Glycosciences*; Gabius, H.-J., Gabius, S. Eds.; Chapman & Hall: Weinheim, **1997**; pp 333-343.

107. (a) Montreuil, Adv. Carbohydr. Chem. Biochem. 1980, 37, 157. (b) Homans, S. W.; Foster, S. *Glycobiology* **1992**, *2*, 143.

108. Carver, J. P. *Pure Appl. Chem.* **1993**, *65*, 763.

109. (a) Finkelstein, A. V.; Janin, J. *Protein Eng.* **1989**, *3*, 1. (b) Searles, M. S.; Williams, D. H. *J. Am. Chem. Soc.* **1992**, *114*, 10690.

110. Wilstermann, M.; Balogh, J.; Magnusson, G. *J. Org. Chem.* **1997**, *62*, 3659.

111. Kolb, H. C. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2629.

112. Kolb, H. C.; Ernst, B. *Chem. Eur. J.* **1997**, *3*, 1571.

113. Alibes, R.; Bundle, D. R. *J. Org. Chem.* **1998**, *63*, 6288.

114. Bundle, D. R.; Alibes, R.; Nilar, S.; Otter, A.; Warwas, M.; Zhang, P. *J. Am. Chem. Soc.* **1998**, *120*, 5317.

115. Navarre, N.; van Oijen, A. H.; Boons, G. J. *Tetrahedron Lett.* **1997**, *38*, 2023.

116. Navarre, N.; Amiot, N.; van Oijen, A.; Imberty, A.; Poveda, A.; Jimenez-Barbero, J.; Cooper, A.; Nutley, M. A.; Boons, G. J. *Chem. Eur. J.* **1999**, *5*, 2281.

117. Scudder, P.; Neville, D. C. A.; Butters, T. D.; Fleet, G. W. J.; Dwek, R. A.; Rademacher, T. W.; Jacob, G. S. *J. Biol. Chem.* **1990**, *265*, 16472.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

118. Lowe, J. B. In *Molecular Glycobiology*; Fukuda, M., Hindsgaul, O. Eds.; Oxford: New York, **1994**; pp. 163-205.

119. Simanek, E. E.; McGarvey, G. J.; Jablonowski, J. A.; Wong, C.-H. *Chem. Rev.* **1998**, *98*, 833.

120. Goldstein, I. J.; Winter, H. G. *Subcell. Biochem.* **1999**, *32*, 127.

121. (a) Wood, C.; Kabat, E. A.; Murphy, L. A.; Goldstein, I. J. Arch. Biochem. Biophys. **1979**, *198*, 1. (b) Murphy, L. A.; Goldstein, I. J. *Biochemistry* **1979**, *18*, 4999.

122. Ernst, B. Private Communication.

123. Kasai, K.-I.; Oda, Y. *J. Chromatogr.* **1986**, *376*, 33.

124. (a) Schriemer, D. C.; Bundle, D. R.; Li, L.; Hindsgaul, O. *Angew. Chem. Int. Ed.* **1998**, *37*, 3383. (b) Schriemer, D. C.; Hindsgaul, O.; *Combinatorial Chem. High Throughput Screening* **1998**, *1*, 155.

125. Goldstein, I. J.; Blake, D. A.; Ebisu, S.; Williams, T. J.; Murphy, L. A. *J. Biol. Chem.* **1981**, *256*, 3890.

126. (a) Paulsen, H. *Angew. Chem., Int. Ed. Engl.* **1982**, *21*, 155. (b) Schmidt, R. R. In *Comprehensive Organic Synthesis*; Trost, B. M., Fleming, I. Eds.; Pergamon: New York, **1991**, pp 33-64. (b) Paulsen, H. In *Modern Methods in Carbohydrate Synthesis;* Khan, S. H., O'Neil, R. A. Eds.; Harwood Academic: Amsterdam, **1995**; pp 1-19.

127. Danishefsky, S. J.; Bilodeau, M. T. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1380.

128. Bussolo, V.D.; Kim, Y.-J.; Gin, D. Y. *J. Am. Chem. Soc.* **1998**, *120*, 13515.

129. Sinay, P. *Pure Appl. Chem.* **1991**, *63*, 519.

130. Lemieux, R. U.; Hendriks, K. B.; Stick, R. V.; James, K. *J. Am. Chem. Soc.* **1975**, *97*, 4056.

131. (a) Paulsen, H.; Lockhoff, O. *Chem. Ber.* **1981**, *114*, 3079. (b) Paulsen, H.; Bunsch, H. *Chem. Ber.* **1981**, *14*, 3115.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

132. (a) Paulsen, H.; Lockhoff, O. *Chem. Ber.* **1981**, *114*, 3102. (b) Paulsen, H.; Lebuhn, R.; Lockhoff, O. *Carbohydr. Res.* **1983**, *103*, C7. (c) Garegg, P. J.; Ossowki, P. *Acta. Chem. Scand. B* **1983**, *37*, 249.

133. (a) Barresi, F.; Hindsgaul, O. In *Modern Methods in Carbohydrate Synthesis;* Khan, S. H., O'Neil, R. A. Eds.; Harwood Academic: Amsterdam, 1995; pp 251-276. (b) Barresi, F.; Hindsgaul, O. *J. Am. Chem. Soc.* **1991**, *113*, 9376. (c) Barresi, F.; Hindsgaul, O. *Synlett* **1992**, 759. (d) Barresi, F.; Hindsgaul, O. *Can. J. Chem.* **1994**, *72*, 1447.

134. (a) Stork, G.; Kim, G. *J. Am. Chem. Soc.* **1992**, *114*, 1087. (b) Stork, G.; La Clair, J. J. *J. Am. Chem. Soc.* **1996**, *118*, 247.

135. (a) Ito, Y.; Ogawa, T. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1765. (b) Ito, Y.; Ogawa, T. *J. Org. Chem.* **1995**, *60*, 4680. (c) Ito, Y.; Ogawa, T. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 2510. (d) Ito, Y.; Ogawa, T. *J. Am. Chem. Soc.* **1997**, *119*, 5562.

136. (a) Ziegler, T.; Lemanski, G. *Angew. Chem. Int. Ed.* **1998**, *37*, 3129. (b) Ziegler, T.; Lemanski, G. *Eur. J. Org. Chem.* **1998**, 163.

137. (a) Crich, D.; Sun, S. *J. Org. Chem.* **1996**, *61*, 4506. (b) Crich, D.; Sun, S. *J. Org. Chem.* **1997**, *62*, 1198. (c) Crich, D.; Sun, S. *J. Am. Chem. Soc.* **1997**, *119*, 11217. (d) Crich, D.; Sun, S. *Tetrahedron* **1998**, *54*, 8321. (e) Crich, D.; Sun, S. *J. Am. Chem. Soc.* **1998**, *120*, 435. (f) Crich, D.; Cai, W. *J. Org. Chem.* **1999**, *64*, 4926.

138. Hodosi, G.; Kovac, P. *J. Am. Chem. Soc.* **1997**, *119*, 2335.

139. Nicolaou, K. C.; van Delft, F. L.; Conley, S. R.; Mitchell, H. J.; Jin, Z.; Rodriguez, R. M. *J. Am. Chem. Soc.* **1997**, *119*, 9057.

140. Horton, D.; Pigman, W. *The Carbohydrates*, Academic Press: New York, **1980**, pp 761-778.

141. Collins, P. M.; Munasinghe, V. R. Z. *J. Chem. Soc., Chem. Commun.* **1977**, 927

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

142. Nicolaou, K. C.; Hummel, C. W.; Bockovich, N. J.; Wong, C.-H. *J. Chem. Soc., Chem. Commun.* **1991**, 870.

143. Perez, M.; Beau, J.-M. *Tetrahedron Lett.* **1989**, *30*, 75.

144. Tevecchia, P.; Trumtel, M.; Veyieres, A.; Sinay, P. *Tetrahedron Lett.* **1989**, *30*, 2533.

145. Thiem, J.; Klaffke, W. *Top. Curr. Chem.* **1990**, *154*, 285.

146. Roush, W. A.; Gung, B. W.; Bennet, C. E. *Org. Lett.* **1999**, *1*, 891.

147. Roush, W. A.; Bennet, C. E. *J. Am. Chem. Soc.* **1999**, *121*, 3541.

148. (a) Weisner, K.; Tsai, T. Y. R.; Jin, H. *Helv. Chim. Acta* **1985**, *68*, 300. (b) Weisner, K.; Tsai, T. Y. R.; Kumar, R.; Sivaramakrishnan, H. *Helv. Chim. Acta* **1984**, *67*, 1128.

149. Zuurmond, H. M.; van der Klein, P. A. M.; van der Marel, G. A.; van Boom, J. H. *Tetrahedron Lett.* **1993**, *49*, 6501.

150. Marzabadi, C. H.; Franck, R. W. *Chem. Commun.* **1996**, 2651.

151. Hasegawa, A.; Kiso, M. In *Preparative Carbohydrate Chemistry*; Hanessian, S. Ed.; Marcel Dekker: New York, 1996; pp 357-379.

152. (a) Kanie, O.; Kiso, M.; Hasegawa, A. *J. Carbohydr. Chem.* **1988**, *7*, 501. (b) Hasegawa, A.; Ohki, H.; Nagahama, T.; Ishida, H.; Kiso, M. *Carbohydr. Res.* **1991**, *212*, 277.

153. (a) Marra, A.; Sinay, P. *Carbohydr. Res.* **1990**, *195*, 303. (b) Lonn, H.; Stenvall, K. *Tetrahedron Lett.* **1992**, *33*, 115.

154. (a) Martin, T. J.; Schmidt, R. R. *Tetrahedron Lett.* **1992**, *33*, 6123. (b) Sim, M. M.; Kondo, H.; Wong, C.-H. *J. Am. Chem. Soc.* **1993**, *115*, 2260.

155. (a) Kondo, T.; Abe, H.; Goto, T. *Chem. Lett.* **1988**, 1657. (b) Ito, Y.; Ogawa, T. *Tetrahedron Lett.* **1988**, *29*, 3987. (c) Ito, Y.; Nunomura, S.; Ogawa, T. *J. Org. Chem.* **1992**, *57*, 1821. (d) Martichonok, V.; Whitesides, G. M. *J. Am. Chem. Soc.* **1996**, *118*, 8187.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

156. Horton, D.; Pigman, W. *The Carbohydrates*, Academic Press: New York, **1980**, pp 643-760.

157. Banoub, J.; Boullanger, P.; Lafont, D. *Chem. Rev.* **1992**, *92*, 1167.

158. (a) Lemieux, R. U.; Takeda, T.; Chung, B. Y. *ACS Symp. Ser.* **1976**, *39*, 90 (b) Lemieux, R. U.; Ratcliffe, M. R. *Can. J. Chem.* **1979**, *57*, 1244.

159. Kanie, O.; Crawley, S. C.; Palcic, M. M.; Hindsgaul, O. *Carbohydr. Res.* **1993**, *243*, 139.

160. Lergenmuller, M.; Ito, Y.; Ogawa, T. *Tetrahedron* **1998**, *54*, 1381.

161. (a) Debenham, J. S.; Madsen, R.; Roberts, C.; Fraser-Reid, B. *J. Am. Chem. Soc.* **1995**, *117*, 3302; (*b*) Debenham, J. S.; Fraser-Reid, B. *J. Org. Chem.* **1996**, *61*, 432. (c) Debenham, J. S.; Debenham, S. D.; Fraser-Reid, B. *Bioorg. Med. Chem.* **1996**, *4*, 1909. (d). Castro-Palomino, J. C.; Schmidt, R. R. *Tetrahedron Lett. 1995*, *36*, 5343.

162. (a) Ziegler, T. *Carbohydr. Res.* **1994**, *262*, 195. (b) Kosma, P.; Strobl, M.; Allmaier, G.; Schmid, E.; Brade, H. *Carbohydr. Res.* **1994**, *254*, 105.

163. (a) Shapiro, D.; Acher, A. J.; Rachaman, E. S. *J. Org. Chem.* **1967**, *32*, 3767. (b) Acher, A. J.; Shapiro, D. *J. Org. Chem.* **1969**, *34*, 2652.

164. Blatter, G.; Beau, J.-M.; Jacquinet, J.-C. *Carbohydr. Res.* **1994**, *260*, 189.

165. (a) Kusumoto, S.; Yoshimura, H.; Imoto, M.; Shimamoto, T.; Shiba, T. *Tetrahedron Lett.* **1985**, *26*, 909. (b) Kondo, H.; Aoki, S.; Ichikawa, Y.; Halcomb, R. L.; Ritzen, H.; Wong, C.-H. *J. Org. Chem.* **1994**, *59*, 864. (c) Ellervik, U.; Magnusson, G. *Carbohydr. Res.* **1996**, *280*, 251.

166. (a) Boullanger, P.; Banoub, J.; Descotes, G. *Can. J. Chem.* **1987**, *65*, 1343. (b) Lafont, D.; Boullanger, P.; Banoub, J.; Descotes, G. *Can. J. Chem.* **1990**, *68*, 828.

167. Griffith, D. A.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1990**, *112*, 5811.

168. Madsen, R.; Roberts, C.; Fraser-Reid, B. *J. Org. Chem.* **1995**, *60*, 7920.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

169. (a) Jensen, K. J.; Hansen, P. R.; Venugopal, D.; Barany, G. *J. Am. Chem. Soc.* **1996**, *118*, 3148. (b) Meinjohanns, E.; Meldal, M.; Paulsen, H.; Bock, K. *J. Chem. Soc., Perkin Trans. 1* **1995**, 405.

170. Bowers, S. G.; Coe, D. M.; Boons, G.-J. *J. Org. Chem.* **1998**, *63*, 4570.

171. Aly, M. R. E.; Castro-Palomino, J. C.; Ibrahim, E.-S. I.; El-Ashry, E.-S. H.; Schmidt, R. R. *Eur. J. Org. Chem.* **1998**, 2305.

172. Jiao, H.; Hindsgaul, O. *Angew. Chem. Int. Ed.* **1999**, *38*, 346.

173. Lemieux, R. U.; Ratcliffe, M. R. *Can. J. Chem.* **1979**, *57*, 1244.

174. (a) Horton, D. *J. Org. Chem.* **1964**, *29*, 1776. (b) Reckendorf, W. M. Z.; Wassiliadou-Micheli, N. *Chem. Ber.* **1970**, *103*, 1792.

175. (a) Rude, E.; Meyer-Delius, M. *Carbohydr. Res.* **1968**, *8*, 219. (b) Harayama, A.; Tsuchiya, T.; Umezawa, S. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 3626. (c) Mootoo, D. R.; Fraser-Reid, B.; *Tetrahedron Lett.* **1989**, *30*, 2363.

176. Zervas, L.; Konstas, S. *Chem. Ber.* **1960**, *93*, 435.

177. (a) Lemieux, R. U.; Nagabhushan, T. L. *Methods Carbohydr. Chem.* **1972**, *6*, 487. (b) Lemieux, R. U.; Earl, R. A.; James, K.; Nagabhushan, T. L. *Can. J. Chem.* **1973**, *51*, 19.

178. Greene, T. W.; Wuts, P. G. M. *Protective groups in Organic Synthesis,* John Wiley & Sons: New York, **1991**, pp 339-340.

179. Zervas, L.; Konstas, S. *Chem. Ber.* **1960**, *93*, 435.

180. (a) Heyns, K.; Harrison, R.; Paulsen, H. *Chem. Ber.* **1967**, *100*, 271. (b) Lessard, J.; Driguez, H.; Vermes, J. P. *Tetrahedron Lett.* **1970**, 4887.

181. Boullanger, P.; Jouineau, M.; Bouammali, B.; Lafont, D.; Descotes, G. *Carbohydr. Res.* **1990**, *202*, 151.

182. Wakselmann, M. *Nou. J. Chim.* **1983**, *7*, 439.

183. Bellamy, F. D.; Ou, K. *Tetrahedron Lett.* **1984**, *25*, 839.

184. Guibe-Jampel, E.; Wakselman, M. *Syn. Commun.* **1982**, *12*, 219.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

185. Fukuyama, T.; Jow, C.-K.; Cheung, M. *Tetrahedron Lett.* **1995**, *36*, 6373.

186. Fukuyama, T.; Cheung, M.; Jow, C.-K.; Hidai, Y.; Kan, T. *Tetrahedron Lett.* **1997**, *38*, 5831.

187. Kahne, D.; Yang, D.; Lim, J. J.; Miller, R.; Paguaga, E. *J. Am. Chem. Soc.* **1988**, *110*, 8716.

188. (a) Crich, D.; Ritchie, T. J. *J. Chem. Soc. Chem. Commun.* **1988**, 1461. (b) Crich, D.; Ritchie, T. J. *J. Chem. Soc. Perkin Trans.1* **1990**, 945.

189. (a) Hayday, K.; McKelvey, R. D. J. Org. Chem. **1976**, *41*, 2222. (b) Malatesta, V.; McKelvey, R. D.; Babcock, B. W.; Ingold, K. U. *J. Org. Chem.* **1979**, *44*, 1872.

190. Dondoni, A.; Scherrmann, M.-C. *J. Org. Chem.* **1994**, *59*, 6404.

191. (a) Pedersen, B. S.; Scheibye, S.; Nilsson, N. H.; Lawesson, S.-O. *Bull. Soc. Chim. Belg.* **1978**, *87*, 223. (b) Cava, M. P.; Levinson, M. I. *Tetrahedron* **1985**, *41*, 5061.

192. (a) Fugedi, P.; Garegg, P. J. *Carbohydr. Res.* **1986**, *149*, C9. (b) Fedrik, A.; Fugedi, P.; Garegg, P. J.; Nashed, M. *Tetrahedron Lett.* **1986**, *27*, 3919.

193. Fugedi, P.; Garegg, P. J.; Lonn, H.; Norberg, T. *Glycoconjugate J.* **1987**, *4*, 97.

194. (a) Lonn, H. Carbohydr. Res. 1985, 139, 105. (b) Lonn, H. *J. Carbohydr. Chem.* **1987**, *6*, 301.

195. (a) Barrett, A. G. M.; Bezuidenhoudt, B. C. B.; Howell, A. R.; Lee, A. C.; Russell, M. A. *J. Org. Chem.* **1989**, *54*, 2275. (b) Barrett, A. G. M.; Bezuidenhoudt, B. C. B.; Gasiecki, A. F.; Howell, A. R.; Russell, M. A. *J. Am. Chem. Soc.* **1989**, *111*, 1392. (c) Barrett, A. G. M.; Lee, A. C. *J. Org. Chem.* **1992**, *57*, 2818.

196. Barrett, A. G. M.; Bezuidenhoudt, B. C. B.; Dhanak, D.; Gasiecki, A. F.; Howell, A. R.; Lee, A. C.; Russell, M. A. *J. Org. Chem.* **1989**, *54*, 3321.

197. Yde, B.; Yousif, N. M.; Pederson, U.; Thomsen, I.; Lawesson, S.-O. *Tetrahedron* **1984**, *40*, 2407.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

198. Nicolaou, K. C.; McGarry, D. G.; Somers, P. K.; Kim, B. H.; Ogilvie, W. W.; Yiannikourous, G.; Prasad, C. V. C.; Veale, C. A.; Hark, R. R. *J. Am. Chem. Soc.* **1990**, *112*, 6263.

199. Lajoie, G.; Lepine, F.; Maziak, L.; Belleau, B. *Tetrahedron Lett.* **1983**, *24*, 3815.

200. Yokoyama, M.; Hasegawa, Y.; Hatanaka, H.; Kawazoe, Y.; Imamato, T. *Synthesis* **1984**, 827.

201. Davy, H. *J. Chem. Soc., Chem. Commun.* **1982**, 457.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.