SYNTHESIS OF A *C*-LINKED DISACCHARIDE ANALOGUE OF THE THOMSEN FRIEDENREICH (TF)-EPITOPE α-O-CONJUGATED TO L-SERINE AND FORMATION OF A CLUSTER AS POTENTIAL ANTICANCER VACCINE

THÈSE N^{O} 3354 (2005)

PRÉSENTÉE À LA FACULTÉ SCIENCES DE BASE

Institut des sciences et ingénierie chimiques

SECTION DE CHMIE ET GÉNIE CHIMIQUE

ÉCOLE POLYTECHNIQUE FÉDÉRALE DE LAUSANNE

POUR L'OBTENTION DU GRADE DE DOCTEUR ÈS SCIENCES

PAR

Loay AWAD

B.Sc. in Chimistry, Al-Quds University, Palestine
et de nationalité palestinienne

acceptée sur proposition du jury:

Prof. P. Vogel, directeur de thèse
Prof. J.-M. Beau, rapporteur
Prof. S. Castillon, rapporteur
Prof. H. Lashuel, rapporteur

Lausanne, EPFL
2005

ACKNOWLEDGMENT

I would like to express my appreciations and indebtedness to Professor Pierre Vogel, for providing me with the opportunity to study in his laboratories with excellent research facilities. My gratitude should be extended to his invaluable guidance, continuous encouragement throughout my thesis work. His profound insight into the problems of organic chemistry, and the willingness to share his welth of experience on numerous occasions gives me a great learning experience.
I would like to thank my thesis jury: Professor Hilal A. Lashuel, Professor Jean-Marie Beau, and Professor Sergio Castillon for accepting to examine this thesis and for Professor Claude Friedli for being president of the exam commission.
I would like to thank Dr. Maris Turks for having read part of this thesis and making reasonable suggestions, and I would like to thank Dr. Richard Mimna for reading part of this thesis and making language correction, and I would like to thank Mr. Mahmood Al-Khatib for helping me in the typesetting and translation the abstract of this thesis to Arabic.
I would like to use the opportunity to thank all, the former and the present members of Vogel's group that I have met them during my thesis in Lausanne. Especially, I would like to mention Peter, Maris, Laure, Eli, Oana, Dean, Srinivas and freddy.
I wish to thank the postdocs: Jens Riedner, Carmen Murcia, Robert Lysek, and Antonio Moreno who have generously shared their friendship and experience of life and chemistry.
I would like to stress out once again the people with whom together I had an opportunity to write publications: Raynald, Jens, Xiaogen, Oana.
Many thanks to Martial Rey, Francisco Sepulveda and Josef Merkli for technical help concerning NMR, MS and electric equipment, respectively. I would like to thank once again Srinivas Reddy Dubbaka for HRMS technical help.
I wish to thank all secretaries: Christine Kupper, Anita Schori, Ursula Känzig, and Anne Lene Odegaard; the team of Magasin-*BCH* and *Atelier Mécanique* for generous help and problem solving without that the work and life in *BCH* would not be imaginable.

And now in Arabic:

بسم الله الرحمن الرحيم

شكر

في البداية اود ان اشكر ابي وامي واخي عاطف واخواتي فاطمة، عطاف، وعواطف وابنائهم وعائلاتهم على الدعم و التشجيع؛ واود ان اشكر كذلك الاعمام والاخوال والعمة والخالات وابنائهم على التشجيع.
واود ان اشكر ادارة جامعة القدس ممثلة برئيس الجامعة السيد سري نسيبة والدكتور حسن الدويك مساعد الرئيس، والاستاذ عبد الحليم عياد المدير الاداري؛ كما واشكر الاصدقاء محمود الخطيب، مهند قريع، عمر عياد، وعلي جاموس للسؤال عني
كما اتقدم بالشكر الحار لمحمود الخطيب مجددا لمساعدته التقنية في كتابة ملخص البحث بالاحرف العربية ومقترحاته في ترجمتها
كما اتقدم بالشكر الجزيل للاصدقاء: محسن بن حاحا وعبد القادر مزغني من تونس الخضراء، والدكتور عصام خلايلة وزوجتة عطرة من فلسطين، والصديقة العزيزة حنان البيومي على دعمهم الكبير ومساندتي طوال فترة وجودهم في لوزان.
وشكري الجزيل للدكتور جورج شبيطة، والدكتور اشرف العامودي، والسيد اوس السلمان وزوجته كلير للتوجيه والمساندة

This work has been carried out under the supervision of Prof. Pierre Vogel at the "Ecole Polytechnique Fédérale de Lausanne" from October 2000 to June 2005. Parts of the results have been published:

Articles

Awad, L.; Riedner, J.; Vogel, P.; C-Linked Disaccharide Analog of the Thomsen- Friedenreich (T)-Epitope α-O-Conjugated to L-Serine. *Chem. Eur. J.* **2005**,*11*, 3565-3573.

Huang, X.; Craita, C.; **Awad, L.**; Vogel, P. Silyl methallysulfinates-as efficient and powerful agents for the silylation of alcohols, phenols and carboxylic acids. *Chem. Commun.* **2005**, 1297-1299. Accepted as a hot paper.

Demange, R.; **Awad, L.**; Vogel, P. Synthesis of C-linked analogues of β-D-galactopyranosyl-(1→3)-D-galactopyranosides and of β-D-galactopyranosyl-(1→3)-D-galactal. *Tetrahedron: Asymmetry* **2004**, *15*, 22, 3573-3585.

Oral Presentation:

Awad, L.; Vogel, P. Synthesis of a *C*-linked Disaccharide Analog of the Thomsen Friedenreich (T)-Epitope α-O-Conjugated to L-Serine and Formation a Cluster as Potential Anticancer Vaccine. The Fall Meeting of the Swiss Chemical Society, Zürich, Switzerland, 2004. *Chimia*, **2004**, *58*, 462.

Posters:

Awad, L.; Riedner, J.; Vogel, P. Synthesis of a *C*-linked Disaccharide Analog of the Thomsen Friedenreich (T)-Epitope α-O-Conjugated to L-Serine and Formation a Cluster as Potential Anticancer Vaccine. *Chimia*, **2004**, *58*, 468. (The Fall Meeting of the Swiss Chemical Society, Zürich, Switzerland, 2004).

Awad, L.; Riedner, J.; Vogel, P. Synthesis of C-glycosides Analogues of the Thomsen-Friedenreich (T) Antigen and of Non-hydrolyzable Sugar Epitopes for Conjugation with Nanoparticles. Proceedings of Tailored Nanoparticles: The challenge in Diagnostics and Therapeutics, Lausanne, Switzerland, October 16 -17, **2003**.

Awad, L.; Riedner, J.; Vogel, P. Towards the Synthesis of Anticancer Vaccine *Chimia*, **2003**, *57*, 402. (The Fall Meeting of the Swiss Chemical Society, Lausanne, Switzerland, 2003)

Awad, L.; Riedner, J.; Vogel, P. Towards the Synthesis of Non-Hydrolysable Analogues of The T-antigen. Proceedings of Molécules du future: les defis de la synthèse organique, Lyon, France, December 9-11, **2002**.

Award:

Fall Meeting prise of the Swiss Chemical Society, medicinal chemistry section, Zürich, Switzerland, 2004, (for the best poster presentation in medicinal chemistry section).

ABSTRACT

Cell surface glycoproteins and glycolipids are responsible for cellular recognition processes.
Vaccination is the procedure whereby the immune system is induced to create antibodies against a foreign molecule involved in disease or viral infection. In many disease states, the oligosaccharide chains presented on cell surface glycoprotein are altered. In some tumors, the glycan chains of glycoproteins are attenuated to only a few sugar residues. In the case of the TF-antigen, the polysaccharide chains have been shortened to a galactose-β-(1→3)-*N*-acetyl-galactosamine disaccharide structure α-linked to a serine or threonine.

Immunogenicity of this epitope in synthetic vaccines has been demonstrated. However, this disaccharide conjugate is relatively short-lived in the blood stream because of its hydrolysis catalysed by ubiquitous glycosidases *in vivo*.

C-disaccharides are sugar mimetics whose interglycosidic linkage is non-hydrolysable as required for a disaccharide-based vaccine. In the first part of the work, we report the first synthesis of TF-antigen analogues applying the methodology developed by our group for the synthesis of *C*(1→3)-disaccharides. Conjugate addition of diethylaluminium iodide (Et_2AlI) to isolevoglucosenone leads to an aluminium enolate, which reacts with the sugar derived carbaldehyde, 2,6-anhydro-3,4,5,7-tetrakis-*O*-[(*tert*-butyldimethyl)silyl]-D-*glycero*-L-*manno*-heptose, to give an aldol. The convergent and stereoselective synthesis of this adduct allows us to obtain *C*(1→3)-disaccharides. Reduction of the moiety derived from isolevoglucosenone with lithium borohydride, followed by cleavage of the 1,6-anhydro bridge produces *C*-disaccharides with D-*galacto* configuration. Königs-Knorr glycosidation of N-Fmoc-serine *tert*-butylester, followed by reduction of azide moiety to the corresponding acetamido group allows us to obtain TF-antigen analogues linked either by hydroxymethano (-CH(OH)-) or methano ($-CH_2-$) group.

In a second part we report the synthesis of a fully deprotected thio-glycotripeptide based on the TF-antigen -*C*-analogues linked by hydroxymethano - *N*-acetyl-*O*-{α-D-glyco}-D-seryl-*O*-{α-D-glyco}-D-seryl-*rac*-*N*-(3-[(acetylthio)amino]propyl)-*O*-{α-D-glyco}-D-serinamide, which was covalently conjugated to the KLH protein carrier via Michael addition reaction.
Biological trials with the TF (analogues)-KLH are in progress.

We report also the synthesis of fully deprotected TF-antigen -C-analogues linked by hydroxymethano (-CH(OH) and methano ($-CH_2-$) group. their conformational analysis is currently in progress.

Résumé

Les glycoprotéines et les glycolipides du calix des cellules sont responsables de la communication entre cellules et le monde extérieur, et leur sociologie. La vaccination est une opération consistant à stimuler la production d'anticorps qui s'associent à un composé ou un microorganismes étranger comme les bactéries et les virus. Au cours d'une maladie, l'état de la surface cellulaire peut être modifié. Par exemple certaines cellules cancéreuses se trouvent partiellement dénudées au niveau de leurs oligosaccharides de surface. C'est le cas pour les cellules portant l'antigène de Thomsen-Friedenreich où la chaîne polysaccharidique est réduite à un disaccharide, le β-D-Gal*p*1→3-D-GalNAc*p*-α-*O*-serine ou *O*-thréonine. Des vaccins anti-tumoraux artificiels de l'épitope de Thomsen-Friedenreich ont montré une réponse immunitaire intéressante. Toutefois les *O*-disaccharides ne survivent pas longtemps dans le sang à cause de leur hydrolyse catalysée par des glycohydrolases omniprésents *in vivo*.

Les *C*-disaccharides sont des mimes des *O*-disaccharides non hydrolysables. Ils présentent donc un intérêt pour la fabrication de vaccins artificiels anti-cancer. Dans une première partie de ce travail nous avons développé une méthode pour l'obtention de *C*-disaccharides du type *C*(1-3). La méthode utilise la réaction de Oshima-Nozaki qui condense l'isolevoglucosenone et les aldéhydes dérivés de monosaccharides en présence de Et_2AlI, on obtient des aldols qui se laissent convertir en une gamme de *C*-disaccharides de façon hautement stéréosélective, par exemple en système de type β-D-Gal(1→3)-(1,6-anhydrohexose). Les intermédiaires sont utilisés dans la glycosidation selon Königs-Knorr sur une sérine semi-protégée (NFmoc-serine *t*-butyl ester). Après conversion de l'azoture en groupement N-acetamido, on aboutit à des analogues de l'épitope Thomsen-Friedenreich ou le lien interglycosidique est un groupement hydroxyméthano ou un groupement méthano.

Dans une deuxième partie, la synthèse d'un thioglycopeptide déprotégé attaché au disaccharide β-D-Gal 1(CH(OH))→3-D-GalNAc est réalisé. Le cluster - *N*-acetyl-*O*-{α-D-glyco}-D-seryl-*O*-{α-D-glyco}-D-seryl-*rac*-*N*-(3-[(acetylthio)amino]propyl)-*O*-{α-D-glyco}-D-serinamide est ensuite conjugué à la protéine immunogène KLH modifié de façon adéquate pour des additions selon Michael. Les produits ainsi obtenus ont été envoyés pour des tests biologiques.

Les *C*-disaccharides déprotégés mimant les disaccharides Thomsen-Friedenreich ont été préparés pour une étude d'analyse conformationnelle.

الملخص

الجليكوليبيدات و الجليكوبروتينات على سطح الخلية مسؤولة عن عمليات التمييز الخلوية. التلقيح هو إجراء بواسطته يتم حث جهاز المناعة لخلق أجسام مضادة تقاوم الجزيئات الغريبة المتورطة بحدوث الأمراض أو العدوى الفيروسية. في كثير من الحالات المرضية، سلاسل سكرات الأوليجو الموجودة على سطح الجليكوبروتين يتم تغييرها. في بعض الأورام، السلاسل السكرية للجليكوبروتينات يتم تفكيكها لبضعة بقايا سكرية. في حالة المولد المضاد-TF ، سلاسل السكرات العديدة يتم تقصيرها للسكر الثنائي: *جلاكتوز-β -(1←3)-N- اسيتل جلاكتوز أمين* مرتبطا برابطة ألفا بالحمض الأميني *سيرين* أو *ثريونين*.

المناعة الجينية للايبوتوب (الجزيء الغريب) في اللقاحات الصناعية، تم إثبات فعاليته و إظهارها، على الرغم من أن السكر الثنائي المشتق ذو حياة قصيرة نسبيا في مجرى الدم لأنه يتفكك بتحفيز من أنزيم التحليل المائي جلايكوسايديز كلي الوجود في الجسم الحي.
السكريات الثنائية كربونية الارتباط هي سكريات مقلدة، رابطتها الجليكوسيدية (التي تربط جزيئات السكر الأحادية ببعضها البعض) غير قابلة للتحليل المائي كما هو متطلب من اللقاح المعتمد على السكر الثنائي. في الجزء الأول لهذا العمل، قمنا بوصف أول تحضير لمتناظرات المولد المضاد-TF و ذلك بتطبيق المنهج الذي طورته مجموعتنا لتحضير السكريات الثنائية كربونية الارتباط *(1←3)*.

إضافة - ثنائية الازدواج (غير بسيطة)- *بيوديد ثنائي اثيل الألومنيوم (Et_2AlI)* ل *ايزوليفوجلوكوزينون*، تنتج اينولات الألومنيوم، التي تتفاعل مع *كاربلدهايد* مشتق من السكر ، *6,2- أنهيدرو-7,5,4,3- رباعي كيس-O- [(ثالثي- بيوتيل ثنائي مثيل) سيليل]-D- جليسيرو-L- مانوهبتوز*، لإعطاء ال *ألدول*. التحضير الكيميائي المجسم لهذا الناتج يمكننا من الحصول على سكريات ثنائية كربونية الارتباط *(1←3)*. اختزال جزء من المركب المشتق من *ايزوليفوجلوكوزينون* *بالليثيوم بوروهايدرايد* ثم كسر الرابطة (6,1-أنهيدرو) تنتج سكريات ثنائية كربونية الارتباط بتشكل D- جالاكتو. ارتباط "كونيجز- كنور" ب *N- فموك- سيرين ثالثي بيوتيل ايستر*، يتبعه اختزال لجزء *الأزايد* ينتج مجموعة *الأسيتاميدو* المرافقة، هذا يمكننا من الحصول متناظرات المولد المضاد-TF مرتبطة إما بمجموعة *هايدروكسي ميثانو (-CH-(OH)-)* أو بمجموعة *الميثانو ($-CH_2-$)*.

في الجزء الثاني من البحث قمنا بوصف كامل لتحضير *ثيو- جلايكو ثلاثي ببتايد* يعتمد على متناظرات المولد المضاد-TF برابطة كربونية مرتبطة بمجموعة *هايدروكسي ميثانو-N- اسيتل- O-{α-D- جلايكو} -D- سيريل - O-{α-D- جلايكو} -D- سيريل - rac – N- (3-[(اسيتيل ثيو) أمينو] بروبيل)-O-{α-D- جلايكو} -D- سيرين أمايد*، هذا المركب تم ربطه برابطة تساهمية للحامل البروتيني KLH عن طريق تفاعل ميكائيل للإضافة.
الاختبارات الحيوية التي تجري ب متناظرات TF-KLH لم تنتهي بعد.

كذلك فقد وصفنا تحضير كامل لمتناظرات المولد المضاد-TF برابطة كربونية مرتبطة بمجموعة *هايدروكسي ميثانو -(CH-(OH)-)* و بمجموعة *الميثانو ($-CH_2-$)*. يجرى حاليا تحليل للبناء الفراغي لهذه المركبات.

ABBREVIATIONS

Ab	Antibody
Ac	Acetyl
Ag	Antigen
AIBN	2,2'-azobisisobutyronitrile
Anh.	Anhydrous
Aq.	Aqueous
Bn	Benzyl
Boc	*Tert*-butoxycarbonyl
BSA	Bovine serum albumin
CAN	Ceric ammonium nitrate
DAST	Diethylaminosulfurtrifluoride
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCC	Dicyclohexylcarbodiimide
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
DIBAL	Diisobutylaluminium hydride
DIEA	Diisopropylethylamine
DMAP	4-Dimethylaminopyridine
DMDO	3,3-Dimethyldioxirane
DMF	*N*,*N*-dimethylformamide
DMSO	Dimethylsulfoxide
DTBP	Di-*tert*-butylpyridine
EDC	*N*'-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide
ELISA	Enzyme-linked immunosorbant assay
Fmoc	Fluoren-9-ylmethoxycarbonyl
HATU	*N*-[(dimethylamino)-1*H*-1,2,3-triazole[4,5-*b*]-pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate
HMPA	Hexamethylphosphoramide
HOAt	7-Aza-1-hydroxy-1*H*-benzotriazole
Ig	Immunoglobulin
IR	Infrared
IIDQ	2-(2-Methylpropoxy)-1(2H)-quinoline carboxylic acid-(2-

	methylpropyl)ester
KLH	Keyhole limpet hemocyanin
LHMDS	Lithium hexamethyldisilazide
mAb	Monoclonal antibody
MBS	*m*-maleimidobenzoyl-*N*-hydroxysuccinimide
*m*CPBA	*Meta*-chloroperbenzoic acid
MHC	Major histocompatibility complex
Mp	Melting point
MS	Molecular sieves
NMR	Nuclear magnetic resonance
NOE	Nuclear Overhauser effect
Pam_3Cys	Tripalmitoyl-*S*-glycerylcysteinylserine
PMB	*Para*-methoxybenzyl
PPTS	Pyridinium-*p*-toluenesulfonate
*p*TsOH	*Para*-toluenesulfonic acid
SAMA-OPfp	*S*-acetylthioglycolic acid pentafluorophenyl ester
Ser	Serine
TBAF	Tetrabutylammoniumfluoride
TBS	*Tert*-butyldimethylsilyl
TEA	Triethylamine
TES	Triethylsilyl
Tf	Trifluoromethanesulfonyl
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
Thr	Threonine
TIPS	Triisopropylsilyl
TMG	Tetramethylguanidinium
TMS	Trimethylsilyl
UV	Ultraviolet
Z	Benzyloxycarbonyl

1 Carbohydrate based vaccines

1.1 The Carbohydrate

1.1.1 What are the Carbohydrates?

The term carbohydrate was originally derived in the nineteenth century from the French (hydrate de carbon) for the family of compounds possessing the empirical formula $C_n(H_2O)_n$. however, subsequently, the term has been greatly extended to encompass many other materials. In general, carbohydrates are polyhydroxylated aldehydes containing a number of carbon atoms, varying between 3 and 9. some, such as glucose should be very familiar. Others, such as sialic acid, may look rather esoteric, but are in fact crucial throughout biology.

1.1.2 Carbohydrate in nature

One thing is certain, that is, carbohydrates are everywhere, not just in the supermarket in 1 kg bags. Glucose is the most abundant organic molecule on the planet. For example cellulose is simply a polymer of glucose (actually β(1-4) linked). DNA and RNA are of course also made of sugar. Ribose is a sugar containg 5 carbon atoms that is a component of each of bulding block used to make these two nucleic acid, which are fundamental to life it self.
The roles that sugars played in biological systems were thought to be limited to either acting as sources of energy, for example, D-glucose is glycolysis to form ATP, or as good structural bulding blocks, for example, α(1-4) linked D-glucose as a polymer in starch

1.1.3 Biological Properties of Carbohydrates

Carbohydrates have a number of important biological roles which fall into three major functions : important foodstuffs and energy source, structurally important compound , and considered as a key code molecules in biological communication and interaction. Each of

these biological properties is related to the physical and chemical properties of the carbohydrates.

1.1.4 Energy Storage

The first of these roles, energy storage, is the major function of starch in plants and glycogen in animals. These highly branched, glucose-based polysaccharides serve as efficient means in the high density storage of energy for rapid utilisation through enzymatic hydrolysis to the monosaccharide, glucose, when required to maintain the energy balance of cell function.

1.1.5 Structural Roles

Linear polysaccharides that contain highly ordered secondary structures are important in making up structural components in both plant and animal tissues. Cellulose and pectins are the major structural components of plant tissue while the glycosaminoglycan chains of proteoglycan molecules, the major component of the extracellular matrix, serve a similar role in animal tissue.

1.1.6 Cell-Cell Interaction and Cellular Communication

For many decades, carbohydrate molecules were regarded as energy-storage compounds and support structures with no significant biological function, despite their ubiquitous presence on the proteins and membranes of all eukaryotic cells.

The discovery in 1969 that cell-surface oligosaccharides were profoundly altered in cancer cells, and may be related to cancer cell diffusion, marked the beginning of the third revolution in biology, with carbohydrate molecules joining the ranks of proteins and nucleic acids as determinants of biological activity.[1]

During the next 30 years, the biological functions of carbohydrate molecules came under intense scrutiny, resulting in the discovery of their roles in mediating such diverse cell-cell recognition phenomena as viral and bacterial infection,[2] tumor cell metastasis,[3] and leukocyte

[1] Inbar, M.; Sachs, L. *Nature* **1969**, *223*, 710. M. Inbar, L. Sachs, *Proc. Natl. Acad. Sci. USA*, **1969**, *63*, 1418.
[2] Paulson, J. C. "Interaction of animal viruses with cell surface receptors." In *The Receptors. Vol. II.* P. M. Conn, Ed. Academic Press : New York, 1985, 131.
[3] Sell, S. *Human Pathology* **1990**, *21*, 1003 and references cited therein.

adhesion during inflammation[4] (Figure1.1).[5] It is now clear that the mystery of carbohydrates, with their enormous structural complexity, is only just becoming to unravel.

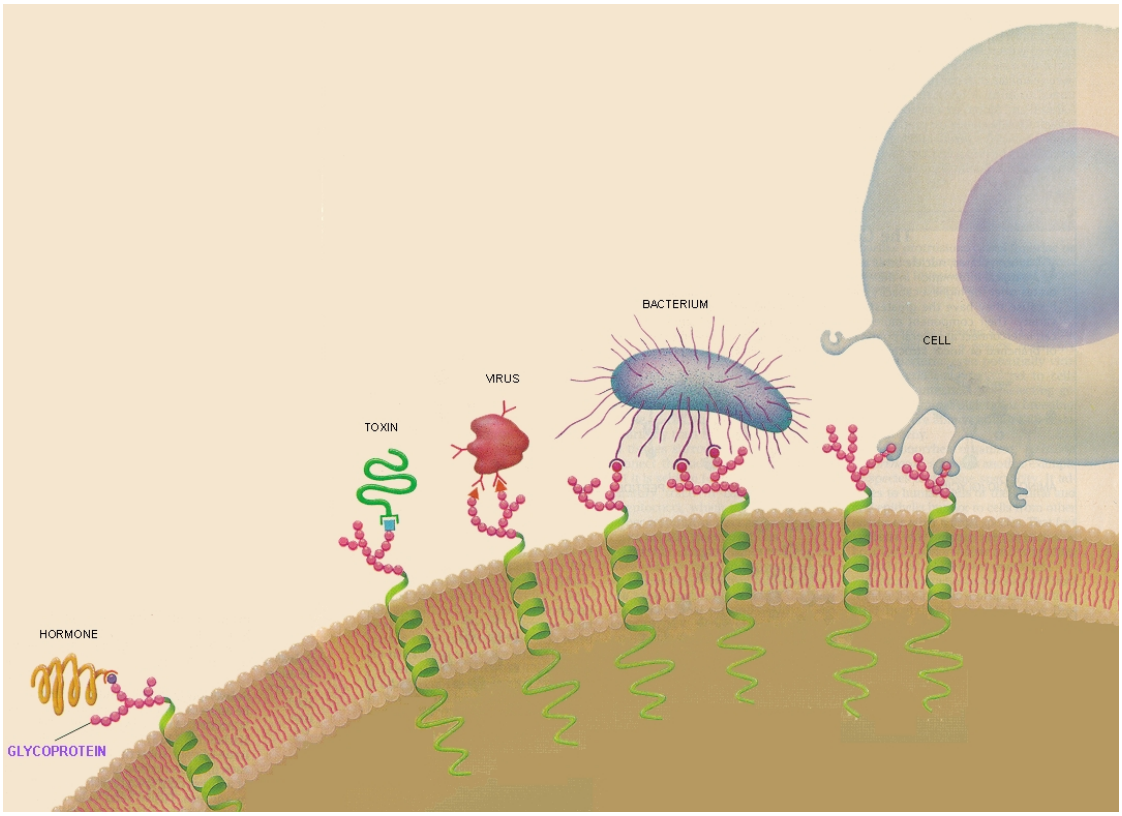


Figure 1.1 Surface Carbohydrates on a cell serve as points of attachment for other cells, infectious bacteria, viruses, toxins, hormones and many other molecules.

[4] Brandley, B. K.; Sweidler, S. J.; Robbins, P. W. *Cell* **1990**, *63*, 861.
[5] Sharon, N.; Lis, H. *Sci. Am.* **1993**, *268*, 74.

1.2 Synthetic Carbohydrate-Based Vaccines

1.2.1 Introduction

The first successful immunization was carried out by Edward Jenner in the late 18th century. He demonstrated that the purposeful inoculation of a young boy with cowpox provided later protection against the related and more deadly pathogen smallpox. He dubbed the process vaccination after the Latin word *vacca* for cow. Jenner's medical breakthrough and subsequent investigations set the stage for the development of modern vaccines to treat numberous diseases such as rabies, diphtheria and polio. Today, scientists continue to research new vaccines that recruit the body's own immune system to treat diseases such as HIV and cancer as well as to treat infections caused by bacteria, viruses, and parasites.

First-generation vaccines still in use today include those of the live-attenuated, killed, and toxoid variety. With the advent of molecular biology and improvements in synthetic and analytical methodologies, the preparation of next-generation vaccines based on purified subunits and the production of attenuated strains containing programmed genetic modifications have recently become possible. Such a strategy of using fully synthetic immunogens with defined compositions affording highly reproducible biological properties marks an important advance in immunology. One important new direction in vaccine research is the use of conjugate vaccines which are vaccines in which a mild antigen (one that is weak enough as to not elicit an immune response) is bound to a stronger antigen (one that will almost definitely elicit an immune response) in order to force the body's immune system to recognize it and develop antibodies. The particular focus of our group involves the covalent attachment of nonhydrolyzable carbohydrate-based antigens to an immunogenic carrier to create a cancer vaccine. Through conjugation with an immunogenic carrier such as a peptide or protein, a normally non-immunogenic carbohydrate antigen can be presented to the body's immune system in an effective way.[6]

There are many factors to consider when designing a carbohydrate-protein conjugate vaccine including the nature of the carrier protein, selection of the carbohydrate antigen, the ratio of protein to carbohydrate, the length and flexibility of the linker binding the two components, as well as the overall homogeneity of the system.

[6] Stacy, J. K. Danishefsky, S. J. *Carbohydrate-Based Druge Discovery* **2003**, *1*, 381.

The various methods for generating the glycoconjugates and for the synthesis of the non-hydrolysable carbohydrate domains will discussed in chapter 4. In this chapter we will focus on vaccines containing completely synthetic carbohydrate antigens.[6]

The carbohydrate-based antigens necessary for cancer vaccines must necessarily be produced synthetically as they are not readily available from natural sources. Their production in quantities sufficient for vaccine research has long been a major hurdle to expansion in the field and it is here that recent advances in glycosylation methods play a pivotal role.[7-9] Recently developed synthetic methodologies afford access to complex oligosaccharides with precisely known structure and stereochemistry and high purity and offer the advantage of being able to introduce new chemical modifications to the carbohydrate domain during the optimization of the vaccine construct. Thus, conjugate vaccines that contain completely synthetic carbohydrate domains are now emerging as an increasingly feasible and powerful way of producing new vaccines targeted to cancer, as well as bacteria and parasites.

1.2.2 Cancer Vaccines

Cells express a multitude of different carbohydrate epitopes on their surfaces. Cancerous cells can often be identified by patterns of aberrant glycosylation and there is typically a direct correlation between the cancer's progression and the level of expression of the antigens associated with it. These can be in high abundance on the surface of cancerous cells, often as high as 10^7 per cell for certain antigens. Tumor antigens are classified into four groups: gangliosides, glycophorins, blood group determinants, and the globo series.[10] Most have been discovered through the use of monoclonal antibodies (mAb), but a few have been isolated by direct extraction from tumors.

Antibodies are the primary mechanism by which the immune system eliminates pathogens from the bloodstream. Thus, the use of vaccines based on synthetic carbohydrate antigens to stimulate the production of antibodies to tumor antigens might be ideally suited for the targeting and elimination of

---

[7] Schmidt, R. R. in *Comprehensive organic synthesis: selectivity, strategy and efciency in modem organic chemistry,* Trost, B. M., Fleming, I., Eds.; Pergamon Press, Inc., **1991**, Vol. 6, pp. 33-64.
[8] Danishefsky, S.J.; Bilodeau, M.T. An*gew. Chem., Int. Ed. Engl*. **1996**, *35*, 1380.
[9] Seeberger, P.H.J. *Carbohydr. Chem.* **2002,** 21, 613.
[10] Hakomori, S.-I. *Adv. Cancer Res.* **1989**, *52*, 257.

circulating tumor cells and mecrometastases[11] There are many factors to be taken into account in considering such a strategy.

Carbohydrates are characterized as T-cell-independent antigens and give rise primarily to IgM antibodies, which are effective for complement activation in the intravascular space. Even with repeated vaccinations, class switching to IgG antibodies (which are the most important complement activators extravascularly) is rarely observed. Complement activation at the cellular surface mediates inflammatory reaction, opsonization for phagocytosis, clearance of antibody complexes from the circulation, and membrane-attack-complex mediated lysis. Conjugating a carbohydrate antigen with an immunogenic carrier protein is critical in surmounting this lack of T cell help. Appropriate conjugation can induce higher IgM antibody counts and partial class switching to IgG antibodies thereby inducing helper T cell activation. The antibodies generated are ideally suited for action in the adjuvant setting where the targets are micrometastatic and circulating tumor cells.

The critical objectives of preclinical cancer vaccine programs can be separated into four stages:

(1) The development of strategies and methodologies for the chemical synthesis of antigen constructs bearing complex oligosaccharide domains.

(2) The selection of an appropriate spacer domain to be introduced between the carbohydrate antigen and the protein domain.

(3) The selection and attachment of the immunogenic carrier protein or other immunostimulant by a covalent bond.

(4) Studies using murine hosts to evaluate the immunogenicity of the construct. Should the results from preclinical studies prove to be positive, petition can then be made for advancing the vaccine to human clinical trials. A detailed review of these approaches developed in the Danishefsky laboratory is available.[12]

1.2.3 Carrier Proteins

As mentioned previously, the use of a suitable carrier is crucial to the development of carbohydrate-based cancer vaccines. While moieties including dendrimers, lipids, and peptidic T-cell epitopes have been explored, most carbohydrate conjugate vaccines rely on the use of carrier proteins. Among the many different carrier proteins that have been investigated in this regard are bovine serum albumin (BSA), keyhole limpet hemocyanin (KLH), and bacillus

[11] Livingston, P.O.; Zhang, S. L.; Lloyd, K.O. *Cancer Immunol. Immunother.* **1997**, *45*, 1.

[12] Danishefsky, S. J.; Allen, J. R. *Angew. Chem., Int. Ed. Engl.* **2000,** *39*, 836.

Calmette-Guérin (BCG). The use of KLH, a very large protein (5 x 10^6 Da) isolated from a marine gastropod,[13] has thus far proven to be superior to others in the majority of cases.[14]

Figure 1.2 shows several gangliosides (GM2, GD2, GM3) that are considered attractive targets for anticancer vaccines since the acidic glycosphingolipids are overexpressed in various cancer types such as melanoma, glioma, seminoma, lung cancer, colon cancer, renal cancer, and prostate cancer.[15-17] GM2 is immunogenic in humans as evidenced by the presence of naturally occurring serum antibodies to GM2 which are cytotoxic to $GM2^+$ cancer cells. Human GM2 mAbs are easily isolated after stimulating their production in melanoma patients by vaccination with GM2-containing vaccines. Those melanoma patients possessing GM2 antibodies appear to have a more favorable prognosis regardless of whether they were induced by vaccination or a natural occurrence. No negative effects have been observed upon vaccination with GM2-based vaccines and early clinical studies of GM2 with different carriers showed that the GM2-KLH conjugate, with QS-21 as an adjuvant,[18] was highly immunogenic, more so than GM2-BCG conjugates with Detox or GM2Lipid A liposomes. The phase III clinical trials with GM2-KLH plus QS-21 currently in progress do not use synthetic GM2. However, a practical and total synthesis of GM2 has been published,[19] and preclinical data from mouse models have shown the synthetic GM2-KLH conjugate to be equally effective. Clinical trials in melanoma patients with the synthetic construct are ongoing.[20]

The first tumor-associated carbohydrate antigens to be discovered were found in epithelial cell mucins[10] which possess extensive serine/threonine α-linked-O-glycosylated domains in clustered form (i.e., glycosylated serine/threonine repeats). The Thomsen-Friedenreich (TF) antigen was first described as a tumor-associated antigen by Springer in 1984.[21] The increased expression of the TF antigen as well as the Tn and sialyl-Tn (STn) antigens is thought to result from changes in the regulation of certain glycosyltransferases.[22] The less studied and more complex glycophorins 2,3-STF, 2,6-STn, and glycophorin represent another interesting group of carbohydrate antigens.

The STn antigen is expressed in more than 80% of cancers of the breast, prostate, and ovaries while its expression in normal tissues is much reduced and restricted to a handful of epithelial tissues at

---

[13] Markl, J.; Lier, B.; Gebauer, W.; AltenHein, B.; Meissner, U.; Harris, J. R. *J. Cancer Res. Clin. Oncol*. **2001,** *127*, R3.
[14] Musselli, C.; Livingston, P.O.; RaguPathi, G. *J. Cancer Res.* Clin. Oncol. **2001,** *127,* R20.
[15] Ragupathi, G. *Cancer Immunol. Immunother.* **1996**, *43*, 152
[16] Livingston, P.O.; Ragupathi, G. *Cancer Immunol. Immunother.* **1997**, *45*, 10.
[17] Zhang, S.; Cordon-Cardo, C.; Zhang, H. S.;Reuter, V E.; Adluri, S.; Hamilton, W. B.; Lloyd, K.O.; Livingston, P. O. *Int. J. Cancer* **1997**, *73*, 42.
[18] Kensil, C. R.; Patel, U.; Lennick, M.; Marciani, D. *J. Immunol.* **1991**, *146*, 431.
[19] Castro-Palomino, J.C.; Ritter, G.; Fortunato, S. R.; Reinhardt, S.; Old, L. J.; Schmidt, R. R. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 1998.
[20] Dullenkopf, W.; Ritter, G.; Fortunato, S. R.; Old, L. J.; Schmidt, R. R. Chem. *Eur. J.* **1999**, *5*, 2432
[21] Springer, G.F. *Science* **1984**, *224*, 1198.
[22] Orntoft, T .F.; Harving, N.; Langkilde, N. C. *Int. J. Cancer* **1990**, *45*, 666.

secretory borders.[23] Overexpression of STn in certain carcinomas is associated with an aggressive phenotype and worsened prognosis.[24] Immunization with STn has been shown to be effective in inducing anti-STn antibodies thereby protecting mice from subsequent tumor risk with syngeneic cancer cell lines expressing STn.[25] The STn antigen has been identified as an attractive target for antibody-mediated cancer immunotherapy by both active and passive immunotherapy studies. This is consistent with a growing body of evidence of the capability of antibodies against defined tumor antigens to protect against circulating tumor cells and micrometastases.[11,16]

Subjects with a variety of epithelial cancers have been immunized with an STn-KLH conjugate with various additional adjuvants resulting in high-titer IgM and IgG anti-bodies against STn. Phase III clinical trials in breast cancer patients with the Theratope® vaccine and Detox as the adjuvant are currently underway.[26] The two domains of the conjugate are linked by ozonization of the STn crotyl monomer to introduce an aldehyde followed by reductive amination with the ε-amino groups of lysine residues on KLH (see Fig. 1.3 for structures).

Monoclonal antibodies against STn have been shown to recognize not only STn monomers, but also STn clusters (STn(c)), indicating the STn antigen is identified in at least two distinct configurations at the cancer cell surface.[27] The STn(c) studied consisted of a linear tripeptide containing serine or threonine residues bearing the carbohydrate antigen attached to the hydroxyl side chain. STn-KLH and STn(c)-KLH conjugates were prepared containing either a two carbon linker or the recently developed heterobifunctional 4-(4-maleimido-methyl)cyclohexane-1-carboxylic acid hydrazide [28] (MMCCH) linker, and their corresponding immunogenicities in mice were evaluated and compared. Conjugation with MMCCH gave a better conjugation efficiency (yield) and higher titers against OSM and STn-positive tumor cells and is currently the method of choice for the preparation of STn(c) vaccines.[29]

---

[23] Zhang, S.; Zhang, H. S.; Cordon-Cardo, C.; Reuter, V. E.; Singhal, A. K.; Lloyd, K.O.; Livingston, P.O. *Int.J. Cancer* **1997,** *73,*50

[24] Werthep, J. L.; Rivera-Macmurray, S.; Bruckner, H.; Tatematsu, M.; Itzkowitz, S. H. *Br. J. Cancer* **1994***, 69,* 613.

[25] Fung, P. Y. S.; Madej, M.; Koganty, R.R.; Longenecker, B. M. *Cancer Res.* **1990***, 50,* 4308.

[26] Holmberg, L.A.; Sandmaier, B. M. *Expert Opin. Biol. Th.* **2001***, 1,* 881.

[27] Zhang, S.L.; Walberg, L. A.; Ogata, S.*;* Itzkowitz, S. H.; Koganty, R. R.; Reddish, M.; Gandhi, S. S.*;* Longenecker, B. M.; Lloyd, K.O.; Livingdton, P.O. *Cancer Res.* **1995,** *55,* 3364.

[28] Ragupathi, G.; Koganty, R. R.; Qiu, D. X.; Lloyd, K.O.; Livingston, P.O. *Glycoconjugate J.* **1998***, 15,* 217.

[29] Ragupathi, G.; Howard, L.; Cappello, S.; Koganty, R.R.; Qiu, D. X.; Longenecker, B.M.; Reddish, M.A.; Lloyd, K.O.; Livingston, P. O. *Cancer Immunol. Immunother.* **1999***, 48,* 1.

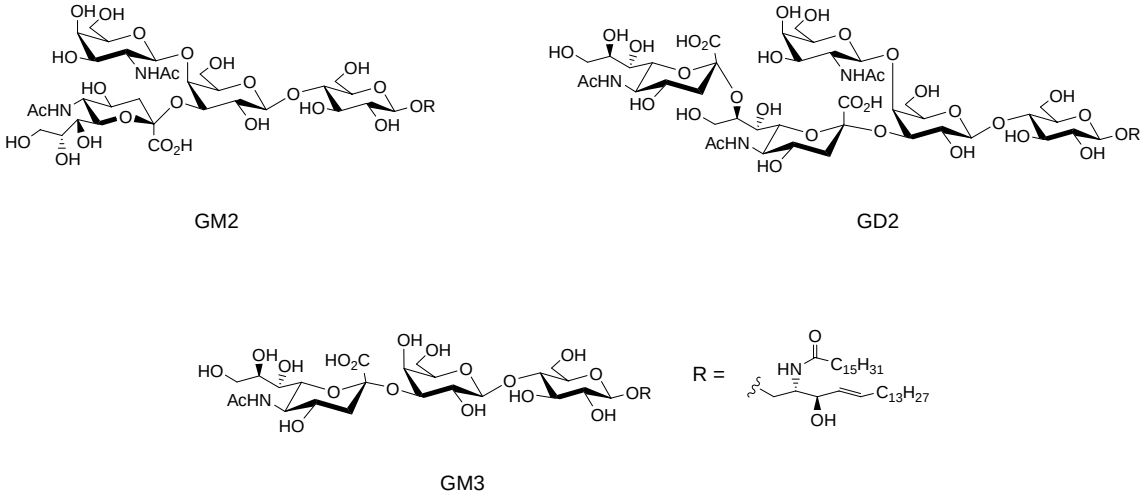


Figure 1.2 Structures of the gangliosides used or considered for use in human cancer vaccines

In an effort to mimick the clustered motifs of the glycophorin carbohydrate antigens recognized by the immune system, Danishefsky et al. developed a general method for the construction of glycoamino acids and their incorporation into glycopeptides. In their "cassette" strategy, essentially a modular rather than a convergent approach, the basic building block, an N-acetylgalactosamine (Ga1NAc) synthon is stereospecifically linked to a serine, threonine, or hydroxynorleucine, with a differentiable acceptor site on the Ga1NAc as shown in Scheme 1.1. This construct serves as a general insert or 'cassette' to be used in subsequent glycosylations with other saccharides.[30] In this way, the synthetically difficult O-linkage step is accomplished early in the synthesis on a simple monosaccharide. Following deprotection of the monosaccharide, a new acceptor site is then exposed for subsequent glycosylations. This method has successfully been used to synthesize various clustered antigen structures built on a peptide backbone.[31-33]

Using the cassette methodology, the synthesis of the clustered epitope of Tn, shown in Fig. 1.4, was realized.[31] A heterobifunctional linker, m-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS), was used to crossconjugate the Tn(c) to KLH or BSA to give the desired vaccine constructs. Results from preclinical ELISA studies showed that the conjugates in combination with QS-21 as the adjuvant were able to generate both IgM and IgG antibodies in mice following three immunizations, with the KLH construct vaccine proving to be the more immunogenic.[31] Tn(c) positive LS-C colon cancer cells and Tn(c) negative LS-B colon cancer cells were used to evaluate the cell surface reactivities of the anti-Tn(c) antibodies. Sera from mice vaccinated with either of the conjugates plus adjuvant showed clear IgM reactivity and significant IgG reactivity by flow

[30] Chen, X.T; Sames, D.; Danishefsky, S. J. J. *Am. Chem. Soc.* **1998***, 120,* 7760.
[31] Kuduk, S. D.; Schwarz, J. B.; Chen, X.T.; Glunz, P.W.; Sames, D.; Ragupathi, G.*;* Livingston, P.O.; Danishefsky, S. J. J. *Am. Chem. Soc.* **1998**, *120,* 12474.
[32] Glunz, P. W; Hintermann, S.; Schwarz, J. B.; Kuduk, S. D.; Chen, X.T.; Williams, L.J.; Sames, D.; Danishefsky, S. J.; Kudryashove, V.; Lloyd, K.O. *J. Am. Chem. Soc.* **1999,** *121,* 10636.

cytometry assays and complement-dependent cytotoxicity assays. A recently completed Phase I clinical trial in patients with prostate cancer using the KLH conjugate yielded positive serological results accompanied by stabilizing or declining prostate specific antigen (PSA) levels.[34]

The cassette approach has also been used to prepare other glycophorin clusters, including TF,[31] STn [33], and 2,6-STF [35] (Fig. 1.5), as well as a Lewis^y (Le^y) cluster. Immunological evaluations of the TF(c)-MBS-KLH and STn(c)-MBS-KLH constructs produced similar results and the evaluation of other glycophorin antigens is currently being pursued.

Le^y is a blood group determinant that has been identified as a critical epitope for eliciting antibodies against cancers of the colon and liver and has also been implicated in breast, prostate, and ovarian tumors. In the first Le^y-containing vaccine, shown in Fig. 1.6, the pentasaccharide Le^y allyl glycoside monomer was linked to the carrier protein by reductive amination while later constructs employed MBS-derivatized KLH for conjugation. Based on preclinical results in mice confirming the increased formation of antibodies that recognized tumor cells expressing the antigen,[36] a Phase I clinical trial in ovarian cancer patients was initiated using the conjugate vaccine in combination with the adjuvant QS-21.[37] The trial was successful at the serological level in that the vaccine induced an antibody response in 75% of the patients.

---

[33] Schwarz, J. B.; Kuduk, S. D.; Chen, X.T; Sames, D.; Glunz, P.W.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1999***, 121,* 2662.
[34] Keding, S; J.; Danishefsky, S. J. *Carbohydrate-Based Drug Discovery* **2003**, *1*, 381.
[35] Sames, D.; Chen, X.T; Danishefsky, S. J. *Nature* **1997***, 389,* 587.
[36] Kudryashov, V.; Kim, H. M.; Ragupathi, G.*;* Danishefsky, S.J.; Livingston, P.O.; Lloyd, K.O. *Cancer Immunol. Immunother*. **1998**, *45,* 281.
[37] Sabbatini, P.*;* Kudryashov, V.*;* Ragupathi, G.*;* Danishefsky, S.*;* Livingston, P.*;* Bornmann, W.; Spassova, M.; Zatorski, A.; Spriggs, D.; Aghajanian, C.; Solgnet, S.; Peyton, M.; O'Flaherty, C.; Curtin, J. ; Lloyd, K. *Clin. Cancer Res*. **2000***,* 6, 450.

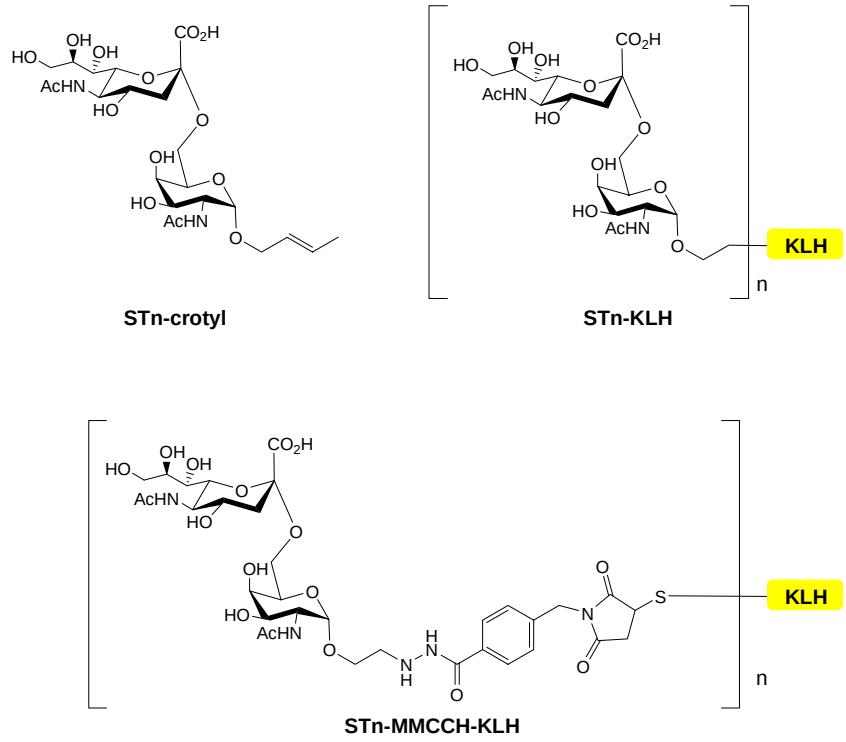


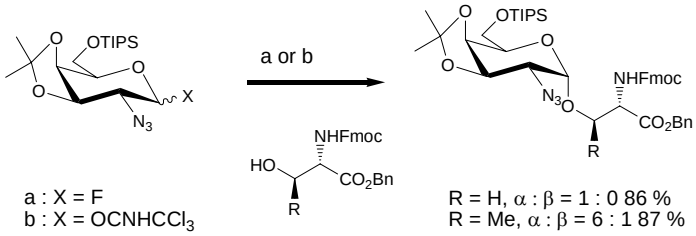
Figure 1.3 Structures of STn monomer and its corresponding conjugate vaccine

Furthermore, the vaccine was well tolerated and no adverse effects related to autoimmunity were reported. In an effort to further promote IgG antibody production in addition to the observed IgM antibodies, a clustered Le^y epitope was explored.[38] Though the Le^y(c)-peptide-MBS-KLH conjugate was indeed capable of eliciting both IgM and IgG responses, the specificity of those responses was limited to the immunizing epitope (ELISA). However, FACS analysis with OVCAR-3 cells did detect moderate activity (-25% positive cells).

The KH-1 antigen contains both the Le^y tetrasaccharide and the Le^x trisaccharide epitopes and its overexpression has been observed in a variety of human adenocarcinomas. The KH-1 nonasaccharide antigen has been synthesized[39] and incorporated into vaccines by conjugation to KLH via the reductive amination procedure or through use of the MMCCH linker as shown in Fig. 1.7.[40] Evaluation of the two constructs in mice showed high titers of both IgM and IgG antibodies for the MMCCH-construct, while the other generated only IgM antibodies.[41] Both constructs elicited antibodies that recognized not only the KH-1 antigen, but also the Le^y antigen,

[38] Kudryashov, V.; Glunz, P.W.; Williams, L.J.; Hintermann, S.; Danishefsky, S. J.; Lloyd, K.O. *Proc. Natl. Acad. Sci. USA*. **2001**, *98,* 3264.
[39] Deshpande, P. P.*;* Danishefsky,*S.* J. *Nature* ***1997,*** *387,* 164
[40] Deshpande, P. P.*;* Kim, H. M.; Zatorski, A.; Park, T K.; Ragupathi, G.*;* Livingston, P.O.; Danishefsky, S. J. *J. Am. Chem. Soc.* ***1998****,120*, 1600.
[41] Ragupathi, G.*;* Deshpande, P. P.*;* Coltart, D. M.; Kim, H. M.; Williams, L. J.; Danishefsky, S.J.; Livingston, P.O. *Int. J. Cancer* **2002**, *99,* 207.

which resembles the four saccharides at the non-reducing end of the KH-1 nonasaccharide. Based on these results, clinical trials are in preparation for the KH-1-KLH vaccine plus adjuvant.



Scheme 1.1 Synthesis of cassette: (a) X = $OCNHCCl_3$, R = H, TMSOTf, THF, -78 ^oC, (b) X = F, R = Me, Cp_2ZrCl_2, AgOTf, CH_2Cl_2

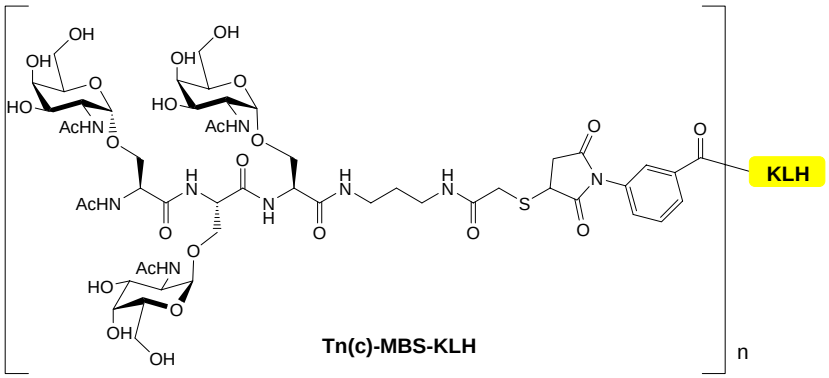
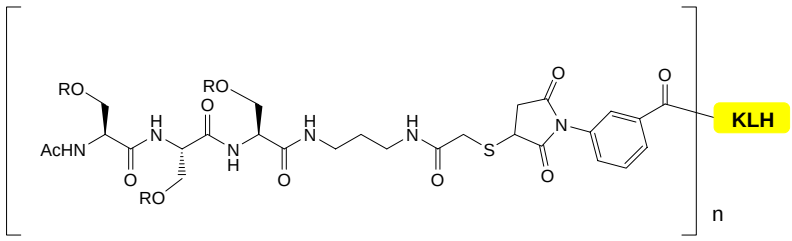
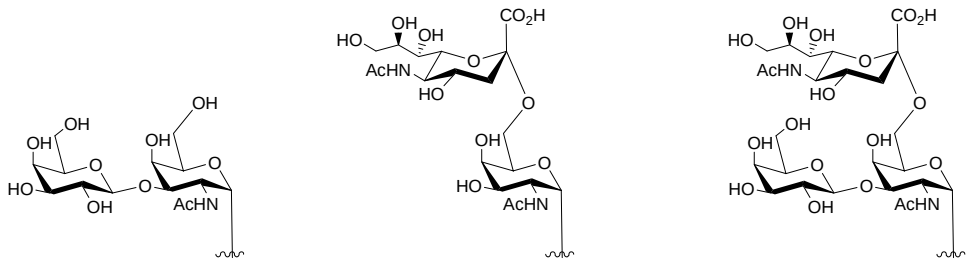


Figure 1.4 Structure of clustered Tn vaccine





TF(c)-MBS-KLH **STn(c)-MBS-KLH** **2,6-STF(c)-MBS-KLH**

Figure 1.5 Structure of other clustered glycophorine vaccine

First isolated in sub-milligram quantities from a human breast cancer cell line, Globo-H is a hexasaccharide that is expressed at the cancer cell surface as a glycolipid. Subsequent

immunocharacterization by means of the monoclonal antibody MBrl and an immunohistological analysis revealed that Globo-H is also expressed in colon, lung, ovary, prostate, and small cell lung cancers. Its structure was later fully elucidated by total chemical synthesis and quantities sufficient for use in vaccine development can now be produced synthetically.[42]

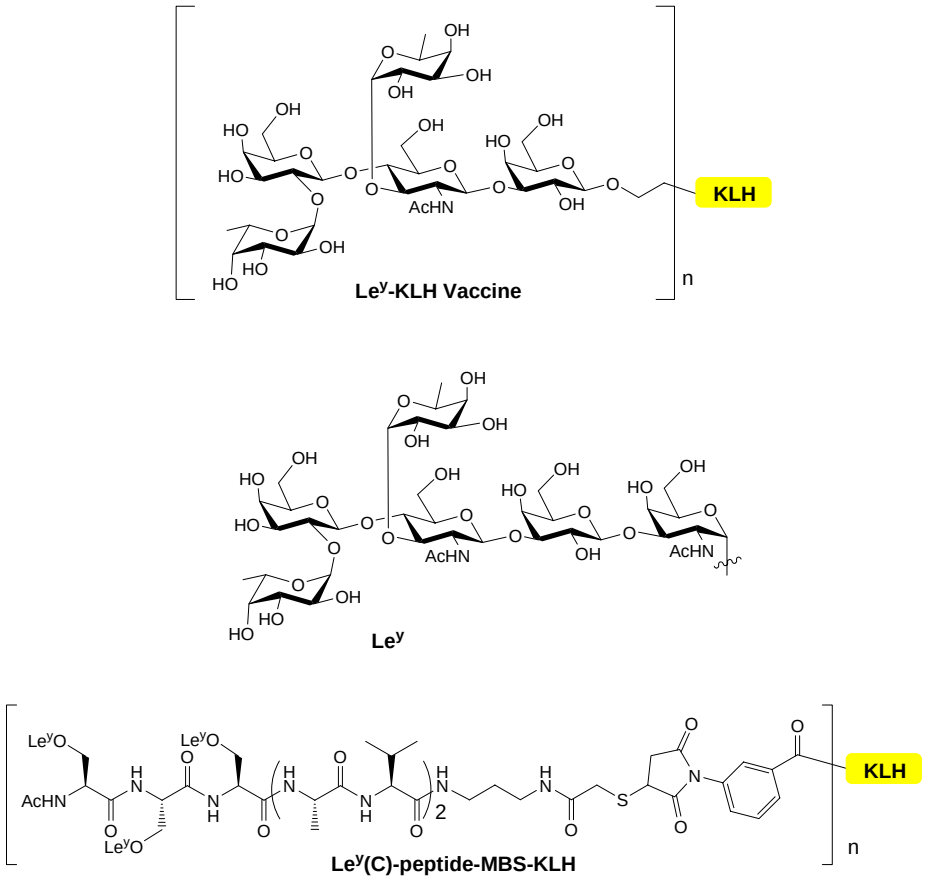


Figure 1.6 Le^y KLH conjugate vaccine

Specifically, both the allyl- and pentenyl-glycosides[43] have been synthesized for attaching the linker to the protein carrier. Again, the KLH conjugate showed greater immunogenicity than the BSA conjugate when administered with QS21 as adjuvant and produced high titer Igm and IgG responses against the Globo-H antigen. The antibodies reacted with MCF-7 cancer cells (Globo-H positive) but not with B78.2 cells (Globo-H negative),[44] and were highly effective at inducing

[42] Park, T. K.; Kim, I.J.; Hu, S.H.; Bilodeau, M.T.; Randolph, J.T.; Kwon, O.; Danishefsky, S.J. *J. Am. Chem. Soc.* **1996**, *118,* 11488.
[43] Allen, J. R.; Allen, J. G.*;* Zhang, X. F.; Williams, L. J.; Zatorski, A.; Ragupathi, G.*;* Livingston, P.O.; Danishefsky, S. J. *Chem. Eur. J.* **2000**, *6,* 1366
[44] Ragupathi, G.*;* Park, T. K.; Zhang, S. L.; Kim, I. J.; Graber, L.; Adluri, S.*;* Lloyd, K.O.; Danishefsky, S. J.; Livingston, P.O. *Angew Chem., Int. Ed. Engl.* **1997**, *36,* 125.

complement-mediated cytotoxicity (48% lysis). Clinical trials with the Globo-H-KLH vaccine are in progress in breast cancer patients.[45]

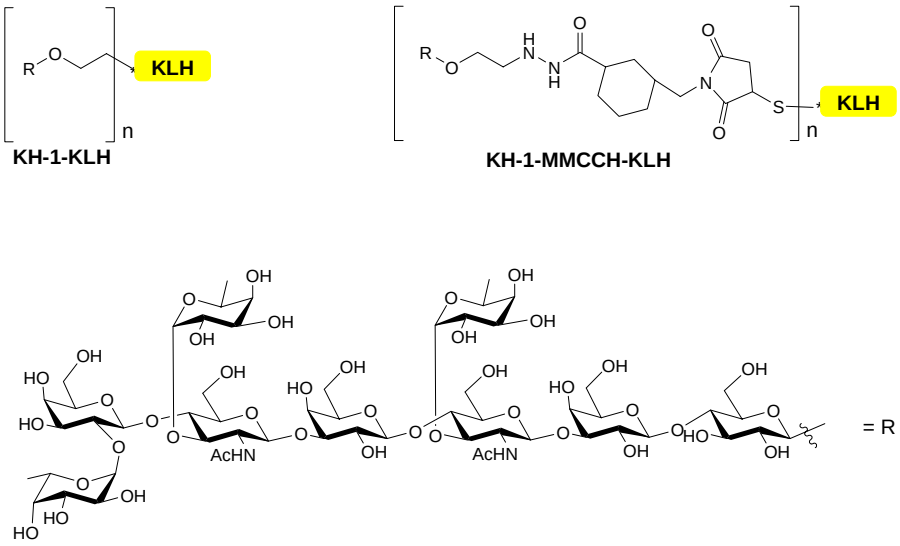


Figure 1.7 KH-1 nonasaccharide vaccine with variable linkage to carrier protien KLH

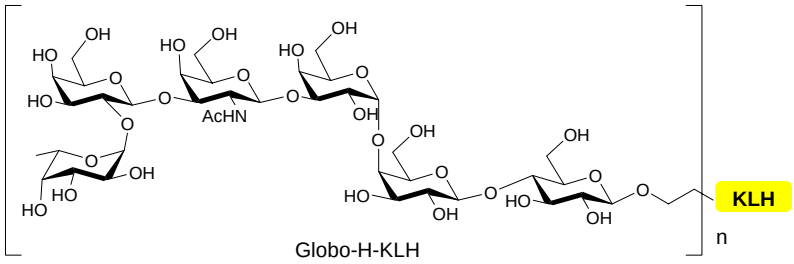


Figure 1.8 Globo-H-KLH vaccine used in clinical trials for treatement of breast, ovarian, and prostate cancer

Completed Phase I trial involving 18 patients with progressive and reoccurring prostate cancer gave promising results,[46,47] Good IgM responses against Globo-H were observed in all immunized patients. The antibodies that were produced recognized Globo-H-expressing cell lines, and in some cases induced complement-mediated lysis. An analysis of the patients' PSA levels was also carried out and showed that the vaccine may have caused a decline in the slope of the plot of log PSA concentration. Furthermore, in those patients initially presented in a non-metastatic state,

[45] Gilewski, T; Ragupathi, G.; Bhuta, S.; Williams, L. J.; Musselli, C.; Zhang, X. F.; Bencsath, K. P.; Panageas, K. S.; Chin, J.; Huis, C. A.; Norton, L.; Houghton, A. N.; Livingston, P.O.; Danishefsky, *S.J. Proc. Natl. Acad. Sci. USA* **2001**, *98,* 3270.
[46] Ragupathi, G.; Slovin, S.F.; Adluri, S.; Sames, D.; Kim, I. J.; Kim, H. M.; Spassova, M.; Bornmann, W .G.; Lloyd, K.O.; Scher, H. L; Livingston, P.O.; Danishefsky, S.J. *Angew Chem., Int. Ed. Engl.* **1999**, *38,* 563

follow up observations conducted six to nine months after vaccine treatment suggested the possibility of favorable changes in PSA slopes having occurred though further studies were needed to confirm these claims. Promising data continues to be obtained from patients who continue to receive booster injections and are disease free though the connection between PSA slopes and early biological efficacy is, at best, anecdotal at this point in time.

Fucosyl GM1 is a complex carbohydrate antigen that has been identified as a highly specific marker associated with small cell lung carcinoma (SCLC) cells. Using methodologies originally developed for the synthesis of the Globo-H antigen, the first total synthesis of this sialyl-containing hexasaccharide was accomplished by the Danishefsky group.[48] By introducing a pentenyl glycoside at the reducing end, more efficient syntheses of potential conjugation precursors could be achieved. The antigen was conjugated with KLH to yield the vaccine shown in Fig. 1.9, and studies in mice are currently ongoing to compare its immunogenicity to earlier non-synthetic fucosyl GM1 vaccines.[49,50]

All of the abovementioned carbohydrate-based cancer vaccines were designed to target one antigen per vaccine. However, it has been well established that several different carbohydrate antigens can be associated with any given cancer type and that these antigens may be expressed at different levels during the various phases of cellular development. Therefore, it is believed strategies that are monovalent in nature may not be sufficient for targeting tumor cells. For these reasons there is an increasing interest in polyvalent strategies that target more than one antigen that could well provide a heightened and more varied immune response. Two different approaches have been taken in this regard. In one, a polyvalent vaccine is produced by the mixing of existing monovalent conjugate vaccines. Alternatively, a multivalent conjugate vaccine is constructed, in which a *single molecule* contains the *full set* of antigens to be targeted.

Immunological evaluation of a polymolecular method involving four KLH conjugate vaccines (GD3-KLH, Le^y-KLH, and two peptidic antigens MUC1-KLH and MUC2-KLH, with adjuvant QS-21) showed promising results in mice. There was no observed decrease in the immunogenicities of the individual components in the polyvalent vaccine as evidenced by high titer IgM and IgG antibody induction regardless of the method of administration (singly in separate mice, separate sites in same mouse, or the same site in mouse).[51] The antibodies showed high specificity and reactivity toward their respective antigens in tumor cells lines expressing the antigens.

---

[47] Slovin, S.F.; Ragupathi, G.; Adluri, S.; Ungers, G.; Terry, K.; Kibornmann, W G.; Fazzari, M.; Dantis, L.; Olkiewicz, K.; Lloyd, Danishefsky, S.J.; Scher, *H. I. Proc. Natl. Acad. Sci. USA* **1999,** *96,* 5710.
[48] Allen, J. R.; Danishefsky, S.J. *J. Am. Chem. Soc.* **1999**, *121,* 10875.
[49] Cappello, S.; Liu, N.X.; Musselli, C.; Brezicka, F.T; Livingston, P.O.; Ragupathi, G. *Cancer* Immunol. *Immunother.***1999**, *48*, 483.
[50] Dickler. M. N.; Ragupathi, G.; Liu, N.X.; Musselli, C.; Martino, D.J.; Miller, V. A.; Kris, M. G.; Brezicka, F. T.; Livingston, P.O.; Grant, S. C. *Clin. Cancer Res.* **1999**, *5*, 2773.

Following these promising results, a series of Phase II clinical trials in breast, ovarian, and prostate cancer patients was initiated in which three to seven individual antigen-KLH conjugates were used (all of which had been previously investigated in Phase I clinical trials) mixed with an immunoadjuvant and administered at a single site. The measured antibody responses will be compared to the antibody response elicited by the same conjugates when administered as monovalent vaccines.

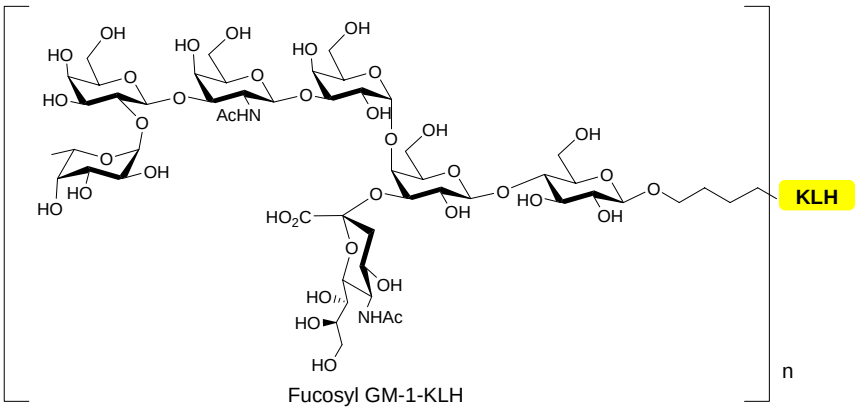


Figure 1.9 Synthetic fucosyl GM-1 conjugate used to investigate development of a SCLC vaccine.

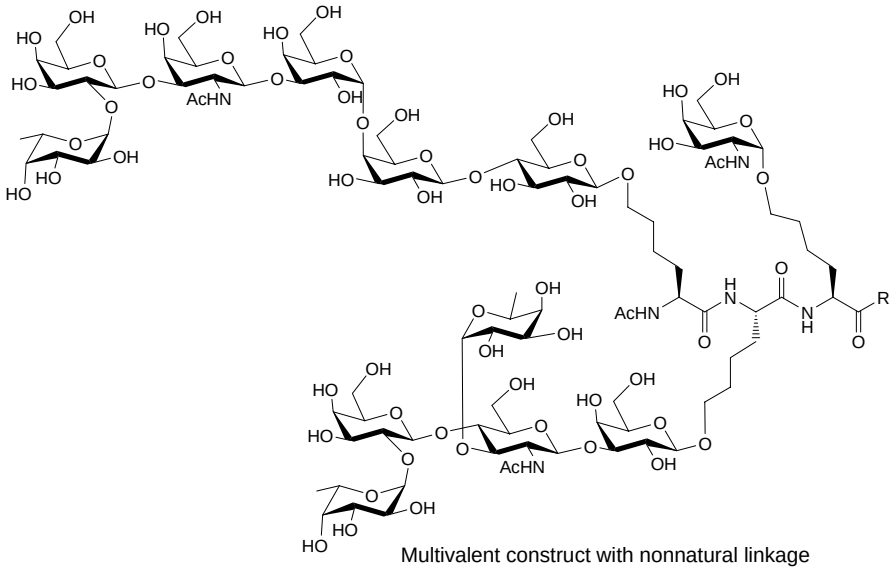
Thanks to various advances in synthetic chemistry, the use of monomolecular multivalent conjugate vaccines for the treatment of cancer is now feasible. Two different glycopeptides have been synthesized, each one containing three different carbohydrate antigens (Fig. 1.10). One construct was based on the natural mucin-type architecture in which the Ley TF, and Tn carbohydrate domains are linked to the peptide backbone via serine hydroxyl groups.[52] In this case the aforementioned serine-Ga1NAc cassette approach was employed. In the other glycopeptide, the Globo-H, Lev, and Tn carbohydrate antigens were linked to the peptide backbone via the non-natural amino acid hydroxynorleucine.[53] Based on preliminary investigations of the two constructs in which the hydroxynorleucine-based construct was found to be considerably more antigenic than the mucin-derived construct, the former was selected for further investigations in murine hosts. The hydroxynorleucine-based conjugate vaccine was observed to generate both IgM and IgG antibodies, and FACS analysis showed that they react selectively with cancer cells expressing those antigens.[54] In addition, GPI-0100,[55] which is structurally similar to QS-21 but considerably less toxic, was tested as an alternative adjuvant and found to be more effective in antibody generation. These findings suggest that a single vaccine construct composed of several

[51] Ragupathi, G.; Cappello, S.; Yi, S. S.; Canter, D.; Spassova, M.; Bornmann, W.G.; Danishefsky, S.J.; Livingston, P.O. *Vaccine* **2002**, *20*, 1030.
[52] Williams, L. J.; Harris, C. R.; Glunz, P. W; Danishefsky, S. J. *Tetrahedron Lett.* **2000**, *41*, 9505.
[53] Allen, J. R.; Harris, C. R.; Danishefsky,S. J. *J. Am. Chem. Soc.* **2001**, *123*, 1890
[54] Ragupathi, G.; Coltart, D. M.; Williams, L.J.; Koide, F.; Kagan, E.; Allen, Ston, P.O.; Danishefsky, S.J. *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 13699.
[55] Marciani, D. J.; Press, J. B.; Reynolds, R. C.; Pathak, A. K.; Pathak, V.; Gundy,L. E.; Farmer, J.T.; Koratich, M. S.; May, R. D. *Vaccine*, **2000**, *18*, 3141.

different carbohydrate-based antigens may be capable of stimulating a multifaceted immune response.

1.2.4 Lipid Carriers

The use of lipid carriers is another approach used in the construction of vaccine and offers a number of potential advantages. Most lipids carriers are B-cell stimulators that are capable of amplifying the proliferation of B-lymphocytes in response to stimulation by antigens. In addition, many lipids are completely synthetic and their combination with synthetic carbohydrate antigens can yield vaccines that are wholly synthetic, thereby making SAR analyses possible. Fig. 1.11 shows several carbohydrate-based vaccines based on lipid carriers.



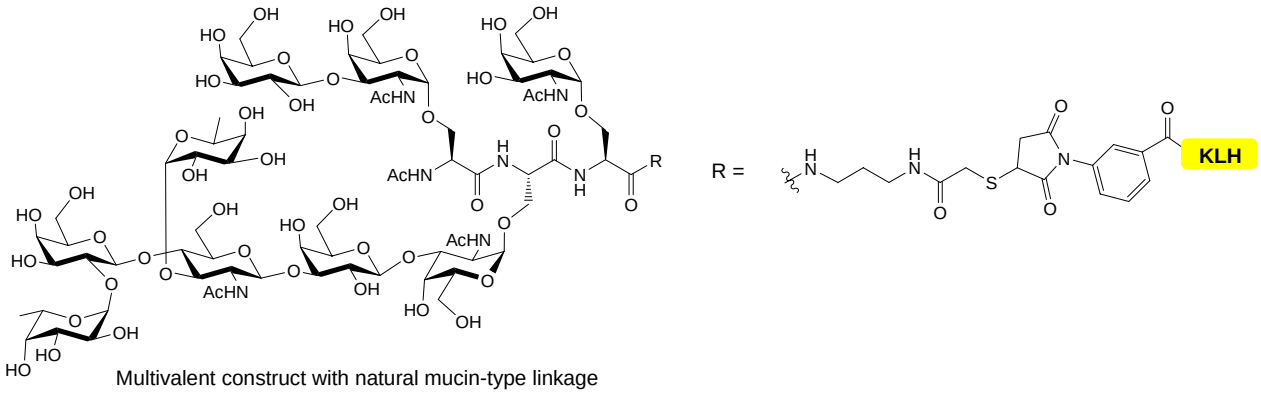


Figure 1.10 structures of two multigenic unimolecular vaccines containing natural and non-natural amino acids in the peptide backbone

In 1994, an entirely synthetic carbohydrate vaccine was developed containing monomeric, dimeric, and trimeric Tn antigens on serine with a 4-aminobutyric acid spacer at the C-terminus.[56] These were conjugated to ovine serum albumin (OSA) and their immunogenicities were evaluated in mice. Conjugates containing dimeric or trimeric Tn antigens elicited a stronger antibody response (IgM and IgG) to a Tn-glycoprotein than the conjugate containing the monomeric Tn antigen. With the aim of generating a completely synthetic vaccine, the dimeric Tn antigen was also conjugated to tripalmitoylglycerylcysteinylserine (Pam_3Cys), a potent B-cell and macrophage activator, derived from the immunologically active N-terminal sequence of the principal lipoprotein of E. coli.[57,58] The resulting Di-Tn-Pam_3Cys lipopeptide conjugate produced not only a high IgM response but also a significant IgG anti-Tn response without the use of a protein carrier or additional adjuvants.[59]

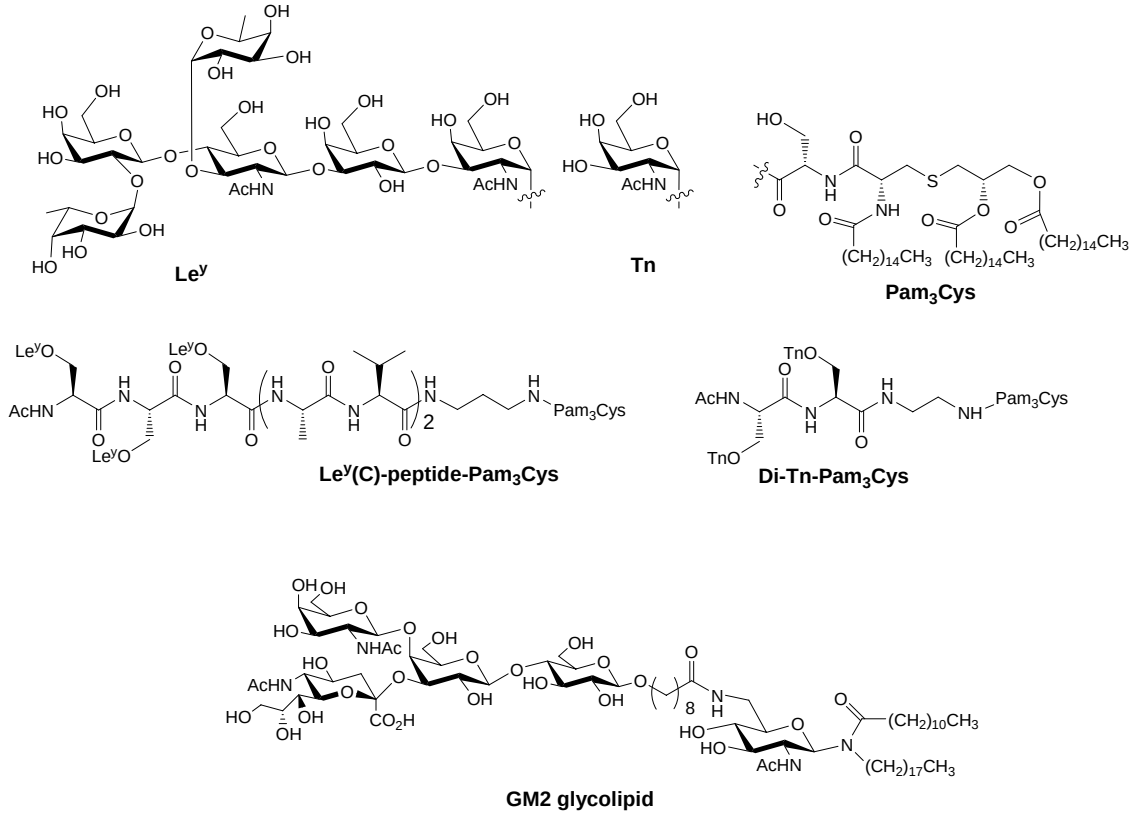


Figure 1.11 Structure of vaccines utilizing lipid carriers.

Pam_3Cys, has also been used in the construction of a Le^y vaccine.[60] The synthetic Le^y(c)-peptide-Pam_3Cys exhibited higher immunogenicity compared to the corresponding Le-Pam_3Cys and Le^y(c)-

[56] Toyokuni, T; Hakomori, S.-I.; Singhal, A. K. *Bioorg. Med. Chem.* **1994**, *2*, 1119.
[57] Bessler, W. G.; Cox, M.; Lex, A.; Suhr, B.; Wiesmuller, K. H.; Jung, G. J. *Im munol.* **1985**, *135*, 1900.
[58] Hoffmann, P.; Wiesmuller, K. H.; Metzger, J.; Jung, G.; Bessler, W .G. *Biol. Chem.* **1989**, *370,* 575.
[59] Toyokuni, T.; Dean, B.; Cal, S. P.; Bolvin, D.; Hakomori, S.; Singhal, A. K. *J. Am. Chem. Soc.* **1994**, *116*, 395.
[60] Glunz, P.W.; Hintermann, S.; Williams, L. J.; Schwarz, J. B.; Kuduk, S. D.; Kudryashov, V.; Lloyd, K.O.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2000**, *122*, 7273.

KLH vaccines when co-administered with adjuvant QS-21, as evidenced by the profile of IgM and IgG antibodies produced, and their recognition of cancer cells bearing Le^y epitopes.[38]

A conjugate molecule containing the ganglioside GM2 antigen linked via a 9-hydroxynonanoate spacer to the B-cell stimulatory glycolipid BAYR1005 has been synthesized.[20] Rabbits vaccinated with this completely synthetic construct in combination with Freund's adjuvant developed IgG antibodies against GM2.

1.2.5 T-Cell Epitopes

In place of an immunogenic protein or lipid carrier, researchers have also investigated the incorporation of known T-cell epitopes into vaccine constructs in an effort to induce a cytotoxic T cell immune response.

The feasibility of this approach was elegantly demonstrated with a synthetic conjugate of a tumor-associated MUC1 glycopeptide antigen and a tetanus toxin epitope connected by a spacer.[61] Tn-, TF-, and sialyl-Tn-antigen (STn) glycopeptides were incorporated into the tandem repeat region of MUC1 at a threonine residue. As only the STn-containing glycopeptide exhibited a proliferating effect on peripheric blood lymphocytes, it was chosen for the further development of a novel conjugate vaccine. The STn-glycododecapeptide tumor-associated antigen was linked with a T-cell epitope of tetanus toxin via a flexible spacer (Fig. 1.12). Proliferation of the vaccine was found to proceed only in the presence of antigen-producing cells and not for purified T cells and this was taken as evidence of an antigen-specific reactivity. FACS analysis determined that the conjugate induced proliferation of up to 100% for $CD3^+$ and 53% for $CD8^+$ T cells. These results lend support to the concept of generating a cytotoxic T-cell response to tumor cells by use of a synthetic conjugate vaccine bearing T-cell epitopes and tumor-associated MUC glycopeptide antigens.

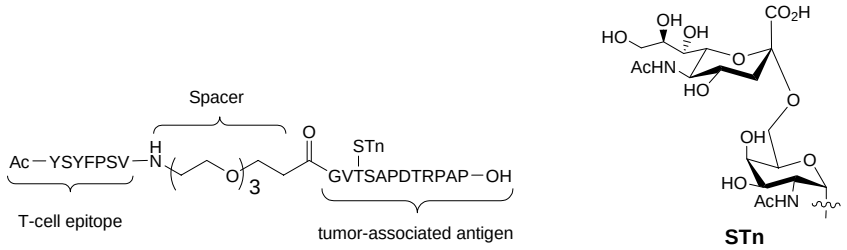


Figure 1.12 vaccine containing a B-cell as well as a T-cell epitope to induce a T-cell-dependent immune response.

[61] Keil, S.; Clus, C.; Dippold, W.; Kunz, H. Angew. *Chem., Int. Ed. Engl.* **2001**, *40*, 366.

The Tn, TF, STn, and 2,3-STF antigens have also been investigated using this approach. One example involved the incorporation of a carbohydrate alkylated homocysteine into a peptide sequence known to bind class I MHC molecules on antigen-presenting cells.[62,63] In another example, four different glycopeptides were synthesized containing variations in both the amino acid sequence and the distance between the T-antigen and the peptide scaffold.[64]

1.2.6 Dendrimers

Dendrimer have also been investigated as scaffolds for conjugation to carbohydrate domains. Like lipids and T cell epitopes, dendrimeric vaccines are completely synthetic and their homogeneity can be strictly controlled. Furthermore, there is less likelihood of carrier-induced immune suppression when dendrimers are used in place of proteogenic carriers.

A dendrimeric glycopeptide bearing multiple O-linked Tn carbohydrate antigens along with a $CD4^+$ T-cell epitope has been described (Fig. 1.13).[65,66] The fully synthetic immunogen possessed a high ratio of saccharidic epitope to carrier ratio and was able to induce anti-Tn IgG antibodies that recognize human tumor cell lines.[67,68] Immunization in tumor-bearing mice significantly increased the survival rate. When used in active specific immunotherapy, the dendrimer bearing the tri-Tn glycotope was found to be far more effective in promoting mouse survival than both the mono-Tn analogue and a linear glycopeptide carrying two copies of the tri-Tn glycotope. A similar approach involving a Starburst dendrimer failed to elicit the desired immune response, perhaps due to the presence of only dimeric-Tn antigens.[56] Well defined synthetic dendrimeric systems represent a versatile and efficient tool explore the importance of both the clustering of the carbohydrate antigens and the way in which they are presented in trying to generate carbohydrate-specific anti-tumor immune responses.

---

[62] George, S. K.; Schwientek, T; Holm, B.; Rets, C.A.; Clusen, H.; Kihlberg, J. *J. Am. Chem. Soc*. **2001**, *123*, 11117.
[63] George, S. K.; Holm, B.; Rets, C.A.; Schwientek, T.; Clausen, H.; Kihlberg, J. *J. Chem. Soc., Perkin Trans. 1* **2001**, 880.
[64] Hilaire, P. M. S.; Cipolla, L.; Franco, A.; Tedebark, U.; Tilly, D.A.; Meldal, M. *J. Chem. Soc., Perkin Trans. 1* **1999**, 3559.
[65] Bay, S.; Lo-Man, R.; Osinaga, E.; Nakada, H.; Leclerc, C.; Cantacuzene, D. *J. Pept.* Res. **1997**, *49*, 620.
[66] Vichier-Guerre, S.; Lo-Man, R.; Bay, S.; Deriaud, E.; Nakada, H.; Leclerc, C.; Cantacuzene, D. *J. Pept. Res.* **2000**, *55*,173
[67] Lo-Man, R.; Bay, S.; Vichier-Guerre, S.; Deriaud, E.; Cantacuzene, D.; Leclerc, *Cancer Res.* **1999**, *59*, 1520.
[68] Lo-Man, R.; Vichier-Guerre, S.; Bay, S.;Eriaud, E.; Cantacuzene, D.; Leclerc, C. *J. Immunol.* **2001**, *166*, 2849.

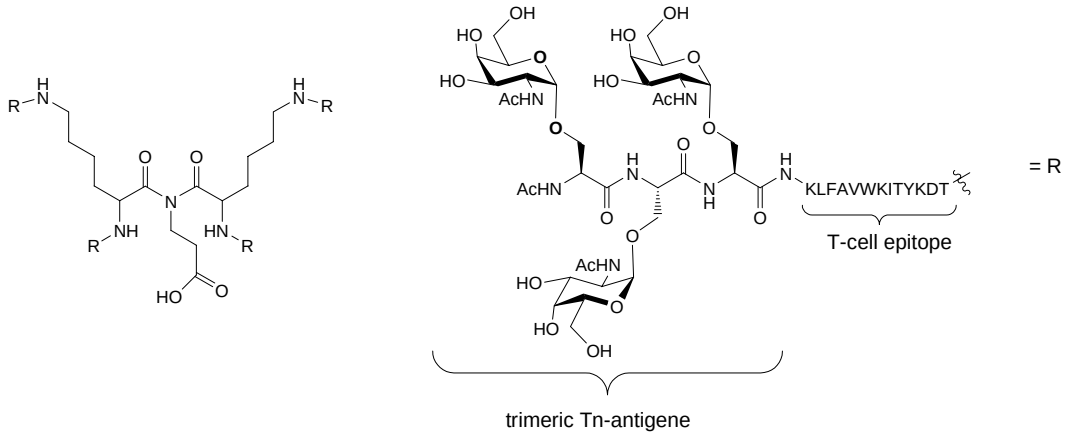
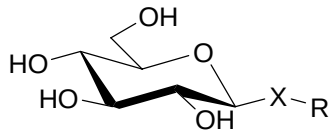


Figure 1.13 Dendrimer containing a clustered Tn motif as well as a T-cell epitope used in vaccine development.

2 *C*-GLYCOSIDES

2.1 The C-Glycosides

It is now well established that cell surface carbohydrates participate in key molecular recognition events with protein receptors. Although such interactions are important elements for cell-to-cell recognition and binding, they may also be harmful in that they provide the initial steps in bacterial, viral infection,[69] as well as cell adhesion in inflammation[70] and metastasis.[71] Strategies for the prevention of such diseases include the preparation of soluble carbohydrate-based pharmaceuticals which may inhibit this recognition event by preferential binding to the protein receptor. An important class of glycomimetics includes the carbon-linked glycosides: *C*-glycosides in which the oxygen atom at the glycosidic linkage has been replaced with a carbon atom (figure 2.1).[72]



X = CH_2, C-glycosid

Figure 2.1 Carbon-linked glycosides

C-Glycosides are resistant to both chemical and enzymatic hydrolysis of the glycosidic bond, and can be readily synthesised from simple sugars. These properties make *C*-glycosides particularly well suited for use as agents and synthetic tools for studying biological events.

[69] Paulson, J. C. "Interaction of animal viruses with cell surface receptors." In *The Receptors. Vol. II.* P. M. Conn, Ed. Academic Press: New York, **1985**, 131.
[70] Brandley, B. K.; Sweidler, S. J.; Robbins, P. W. *Cell* **1990**, *63*, 861.
[71] Sell, S. *Human Pathology* **1990**, *21*, 1003 and references cited therein.
[72] For reviews of the chemistry and biology of *C*-glycosides see: a) Hacksell,U.; Davies, D. *Prog. Med. Chem.* **1985**, *22*, 1. b) Buchanan, J. G. *Prog. Chem. Org. Nat. Prod.* **1983**, *44*, 243. c) Goodchild, J. *Top. Antibiot. Chem.* **1982**, *6*, 99. d) Special issue, *Carbohydr. Res.* **1987**, *171*.

2.2 Physical Properties of C-Glycosides

The potential for *C*-glycosides to mediate biological processes involving carbohydrate-protein recognition depends upon their structural similarity to the native *O*-glycosyl derivatives. The substitution of a methylene group for an oxygen atom results in a change in both the size and electronic properties of the glycosyl linkage (Table 2.1).

Table 2.1: Physical properties of oxygen and carbon linkages.

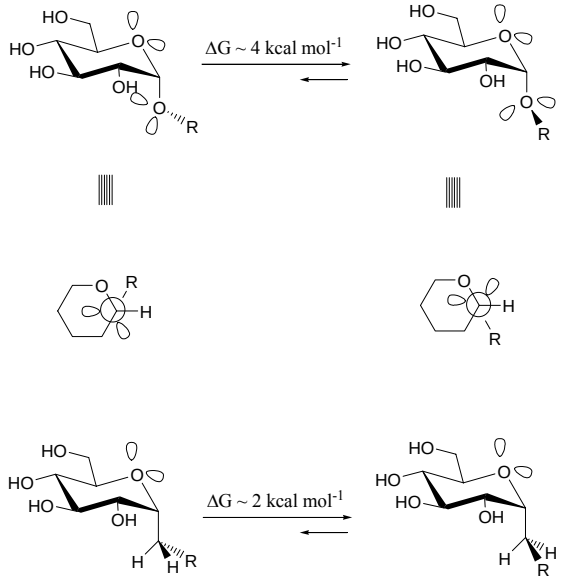
Property	***Oxygen***	***Carbon***
Van der Waals Radius	1.52 Å	2.0 Å
Hydrogen Bonding	Acceptor	None
Electronegativity	3.51	2.35
Dipole Moment	0.8 D (C-O)	0 D (C-C)

Replacement of the oxygen atom with a methylene group destroy the hydrogen bonding ability of the glycosyl side chain, which may reduce the binding affinity of the *C*-glycoside if specific hydrogen bonds to this oxygen atom are involved in recognition. Because oxygen is more electronegative than carbon (3.51 and 2.35 respectively on the Pauling scale), the C-O bond has a strong dipole moment (ca. 0.8 Debye) whereas the C-C bond does not. Therefore, interactions between local dipoles of the receptor and ligand may be affected by substitution of the *C*-glycoside. Finally, the dipole moment and hydrogen bonding ability of the *O*-linkage render it better solvated in an aqueous environment than the *C*-linkage. In this regard, the more hydrophobic *C*-glycoside gains a greater entropic advantage by interacting with a protein receptor and may bind with higher affinity, an effect that is well documented in protein-ligand studies.[73]

Although the differences noted above can affect receptor binding activity, perhaps the most important consideration is the conformational similarity of *C*- and *O*-glycosides in solution. The conformation of *O*-linked glycosides around the glycosidic bond is governed by steric factors and by a stereoelectronic phenomenon termed the "*exo*-anomeric effect". The *exo*-anomeric effect is the antiperiplanar alignment of a lone pair orbital of the glycosidic oxygen and the bond between the ring oxygen and anomeric carbon. As a consequence, the aglycon group (R) tends to be away from the sugar ring substituents as indicated in the *gauche* form to reduce the steric hindrance (Scheme 2.1).

[73] Jencks, W. P. *Catalysis in Chemistry and Enzymology*. Dover Publications, Inc.:New York, 1969.

Though the *exo*-anomeric and steric effects are the main factors that govern the glycosidic torsion angles, the steric effect of *C*-linked saccharides has been found to play a major role in maintaining the conformation of *C*-linked saccharides in the *gauche* form, similar to the conformation in the corresponding *O*-linked sugars.



Scheme 2.1: The *exo*-anomeric and steric effects on the conformation of a glycoside, and energetic consequence of changing an *O*- glycoside to a *C*-glycoside.[74]

Kishi and al. demonstraded that "it is possible to predict the conformational behavior around the glycosidic bond of given *C*-saccharides by placing the $C_{1'}$-$C_{2'}$ bond antiperiplanar to the C_{α}-C_n bond and then focusing principally on the steric interaction around the non-glycosidic bond." This postulate was demonstrated utilising a diamond lattice to arrange the glycosidic conformations of various *C*-disaccharides and of the *C*-trisaccharide analogue of blood group determinant (figure 2.2).[75]

However, more rigorous studies performed by Jiménez-Barbero have demonstrated that certain *C*-glycosides are more flexible, possessing other non-*exo*-anomeric conformations.[76]

[74] Lemieux, R. U. *Acc. Chem. Res.* **1996**, *29*, 373

[75] a) Goekjian, P. G.; Wu,T.-C.; Kishi,Y.; *J. Org. Chem.* **1991**, *56*, 6412.b) Goekjian, P. G.; Wu,T.-C.; Kishi,Y.; *J. Org. Chem.* **1991**, *56*, 6422. c) Wang, Y. ; Babirad, S. A.; Kishi,Y. *J. Org. Chem.* **1992**, *57*, 468. d) Wang, Y.; Goekjian, P. G.; Ryckman, D. M.; Miller, W. H.; Babirad, S. A.; Kishi, Y. *J. Org. Chem.* **1992**, *57*, 482.

[76] Espinosa, J. -F; Dietrich, H.; Martin-Lomas, M.; Schmidt, R. R; Jiménez-Barbero, J. *Tetrahedron Lett.* **1996**, *37*, 1467. Espinosa, J. -F.; Cañada, F. J.; Asensio, J. L.; Dietrich, H.; Martin-Lomas, M.; Schmidt, R. R.; Jiménez-Barbero, J. *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 303. Espinosa, J. -F.; Cañada, F. J.; Asensio, J. L.; Martin-Pastor, M.; Dietrich, H.; Martin-Lomas, M.; Schmidt, R. R.; Jiménez-Barbero, J. *J. Am. Chem. Soc.* **1996**, *118*, 10862.

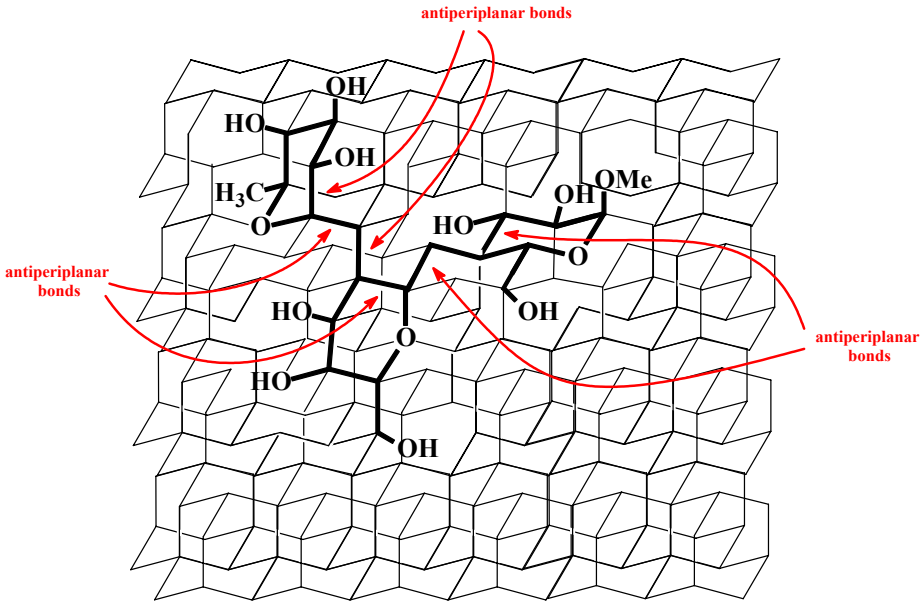


Figure 2.2: *C*-Trisaccharide of blood group determinants in diamond lattice.

2.3 The synthesis of C-glycosides:

2.3.1 Introduction

Carbohydrate analogs in which a carbon atom substitutes for the glycosidic oxygen are called *C*-glycosides. During the last two decades, the synthesis of *C*-glycosides has become an area of intense study among carbohydrate chemists and biochemists because:

1) The discovery of naturally occurring *C*-nucleosides with important pharmacological properties[77] gave impetus to synthetic efforts for preparing active carbohydrate analogs;

2) The requirement of *C*-glycoside chiral building blocks in the synthesis of biologically important macromolecules, such as palytoxin,[78] spongistatin[79,80] and halichondrin B,[81] has stimulated the development of the new synthetic methodologies;

3) *C*-glycosides are potential inhibitors of carbohydrate processing enzymes and are stable analogs of glycans involved in important intra- and inter-cellular processes.[82-85]

C-glycoside synthesis has been reviewed by Postema,[86] Levy,[87] Sinay,[88] Beau,[89] Nicotra,[82] Vogel,[90] and McGarvey.[91] In this chapter we are going to cover some of the latest development published during the last four years and that are not mentioned in the earlier reviews.

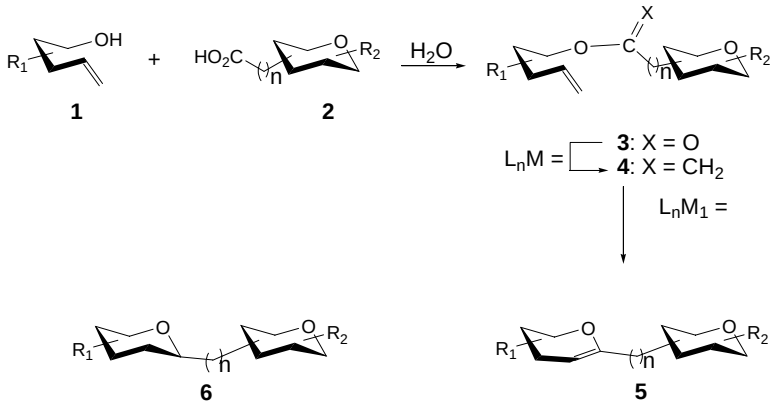
---

[77] Suhadolnik, R. J. *Nucleoside Antibiotics;* Wiley-Interscience: New York, 1970
[78] Lewis, M. D.; Cha, J. K.; Kishi, Y. *J. Am. Chem. Soc.* **1982**, *104*, 4976.
[79] Paterson, L.; Keown, L.E. *Tetrahedron Lett.* **1997**, *38,* 5727.
[80] Smith, III., A.B.; Zhuang, L.; Brook, C.S.; Boldi, A.M.; McBriar, M.D. et. al. *Tetrahedron Lett.* **1997**, *38,* 8667.
[81] Horita, K.; Sakkkurai, Y.; Nagasawa, M.; Hachiya, S.; Yonemitsu, O. *Synlett* **1994**, 43.
[82] Nicotra, F. *Topics Curr. Chem.* **1997**, *187,* 55.
[83] Weatherman, R. V.; Mortell, K. H.; Chervenak, M.; Kiessling, L. L.; Toone, E. J. *Biochemistry* **1996**, *35*, 3619.
[84] Weatherman, R.; Kiessling, L. L. *J. Org. Chem.* 1996, *61,534-538.*
[85] Sutherlin, D. P.; Stark, T. M.; Hughes, R.; Armstrong, R. W. *J. Org. Chem.* **1996**, *61,* 8350.
[86]. a) Postema, M. H. D.; Piper, J. L.; Liu, L.; Shen, J.; Faust, M.; Andreana, P.. *J. Org. Chem.* **2003**, *68*, 4748. b) Liu, Lei; Postema, M. H. D. *J. Am. Chem. Soc.* **2001**, *123*, 8602. c) Postema, M. H. D. *C-Glycoside Synthesis;* CRC press: Boca Raton, **1995**
[87] Levy, D. E.; Tang, C. *The Chemistry of C-glycosides;* Pergamon: Oxford, **1995**
[88] a) Caravano, A.; Mengin-Lecreulx, D.; Brondello, J.-M.; Vincent, S. P.; Sinay, P. *Chem. Eur. J.* **2003**, *9*, 5888. b) Sinay, P. *Pure & Appl. Chem.* 1997, *69,* 459.
[89] a) Abdallah, Z.; Doisneau, G.; Beau, J.-M. *Angew. Chem., In. Ed. Engl.* **2003**, *42*, 5209. b) Beau, J.-M.; Gollagher, T. *Topics Curr. Chem.* **1997**, *187,* 1.
[90] a) Robina, I.; Vogel, P.. *Synthesis*. **2005**, *5*, 675. b) Awad, L.; Riedner, J.; Vogel, P. *Chem. Eur. J.* **2005**, *11*, 3565. c) Steunenberg, P.; Jeanneret, V.; Zhu, Y-H.; Vogel, P. *Tetrahedron: Asymmetry*. **2005**, *16*, 337. d) Demange, R.; Awad, L.; Vogel, P. *Tetrahedron: Asymmetry* **2004**,*15*, 3573. e) Navarro, I.; Vogel, P. *Helv. Chim. Acta*. **2002**, *85*, 152. f) Vogel, P.; Ferritto, R.; Kraehenbuehl, K.; Baudat, A. in *Carbohydrate Mimics, Concepts and Methods*, Ed. Chapleur, Y.; Wiley-VCH, Weinheirn **1998**, Chapter 2, p. 19-48. g) Vogel, P. *Curr. Org. Chem.* **2000**, *4*, 455.
[91] Schmidtmann, F. W.; Bendum, T. E; McGarvey, G. J. *Tetrahedron Lett.* **2005**, *46,* 4681.

2.3.2 Ruthenium-catalyzed cross-metathesis

2.3.2.1 Intramolecular cross-methathesis:

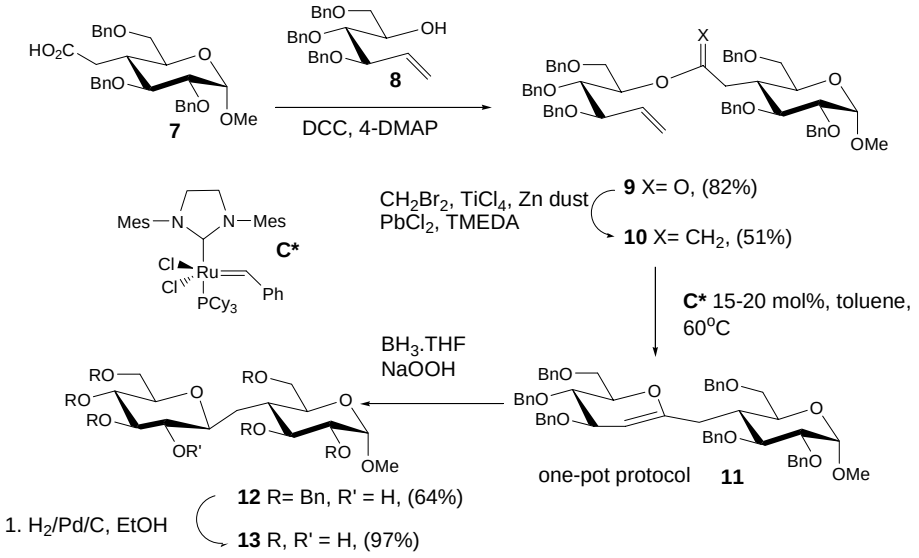
Postema et al. have prepared a small library of differentially-linked *β-C*-disaccharides. They used a radical allylation-RCM strategy.[86a] Acids **2** were prepared by Keck allylation of a suitable carbohydrate-based radical precursor, followed by oxidative cleavage of the formed alkene. Dehydrative coupling of these acids with the known alkenol **1** then gave the corresponding esters **3** in excellent yield. Methylenation of **3** and subsequent RCM and in situ hydroboration/oxidation of the so-formed glycals furnished the corresponding protected *β-C*-disaccharides **6** in good overall yield.



Scheme 2.2: RCM approach to *C*-saccharides

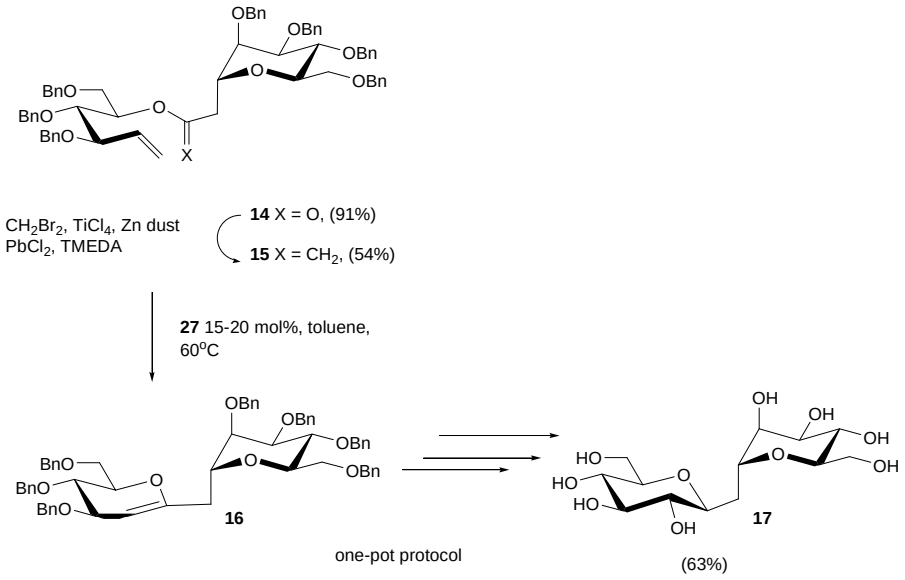
The method developed is a general approach for the synthesis of *C*-glycosides[92] and a variety of β-*C*-disaccharides.[93,94](Scheme 2.3). This approach to *C*-disaccharides synthesis begins with the dehydrative coupling of olefin alcohol **8** with a suitable carbohydrate based acid like **7** that gives the corresponding ester **9**. Methylation,[95] followed by RCM[96] gives glycal **11**. Hydroboration[97] of the alkene moiety affords the *gluco-β-C*-disaccharide **12**. Optimization was realized by the development of the three-step protocol (55% overall yield for the three steps).

[92] Postema, M. H. D.; Calimente D. *J. Org. Chem.* **1999**, *64*, 1770.
[93] Postema, M. H. D.; Calimente D.; Lui, L.; Behrmann, T. L. *J. Org. Chem.* **2000**, *65*, 6061.
[94] Postema, M. H. D.; Calimente D. *Tetrahedon Lett.* **1999**, *40*, 4755.
[95] Takai, K.; Kakiuchi,T.; Kataoka, Y; Utimoto, K. *J. Org. Chem.* **1994**, *59*, 2668.
[96] Trnka, T.; Grubbs, R. H. *Acc. Chem. Res.* **2001**, *34*, 18.
[97] Hanessian, S.; Martin, M.; Desai, R. C., *J. Chem. Soc. Chem. Commun.* **1986**, 926.



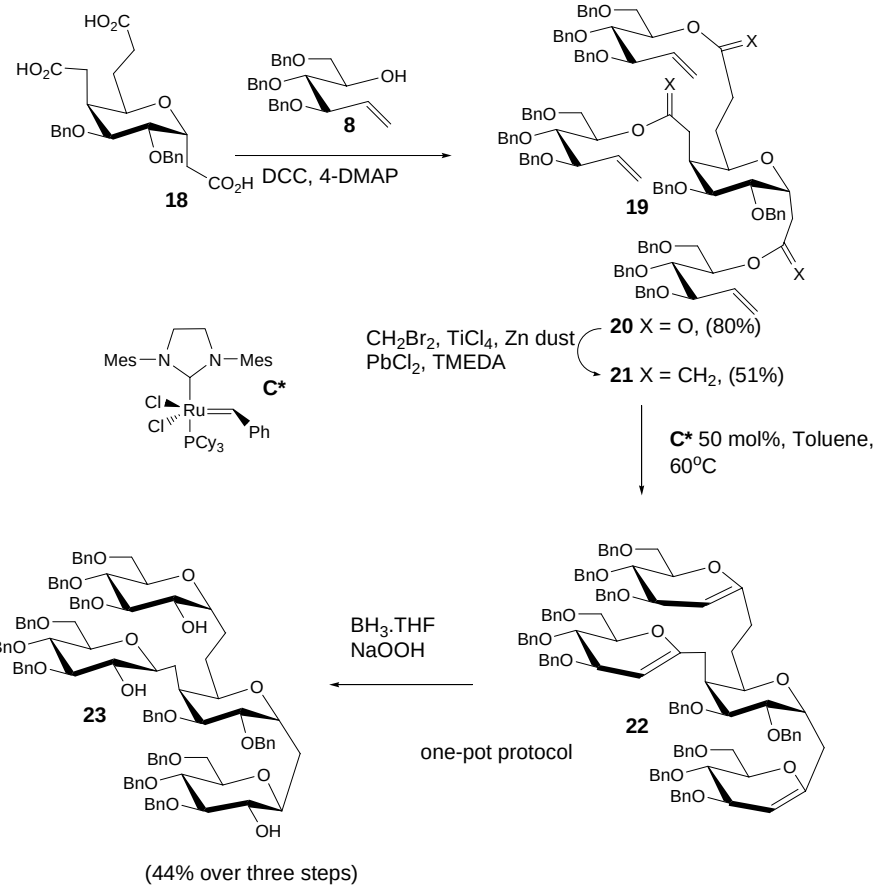
Scheme 2.3: Synthesis of *C*(1'→4) disaccharide using a RCM strategy by Postema and co-workers.

This methodology was also applied to the synthesis of *C*-(1'→1) disaccharides. Ester **14** was methylated and the protocol described above was applied (Scheme 2.3). Finally, deprotection by hydrogenolysis gave the fully deprotected β-*C*-(1'→1)-disaccharide **17** in 64% overall yield (Scheme 2.4). The use of Grubbs catalyst allows the cyclization to be carried out readily with bench top techniques. Together with the one-pot approach this methodology has become quite practical, and, to my opinion, can be seen as an equivalent for the synthesis of *C*-disaccharides to the methodology developed for the synthesis of *O*-disaccharides by Seeberger.



Scheme 2.4: Synthesis of *C*(1'→1) disaccharide using a RCM strategy by Postema et al.

The synthesis of the first branched *C*-tetrasaccharide give a better insight in the impressive work of Postema (Scheme 2.5).[98] Alcohol **8** was coupled with the suitable carbohydrate-based triacid **18**, to provide triester **19** in 80% yield. Methylanation is followed by a *triple* RCM reaction[99] with the second generation Grubbs' catalyst,[100] to furnish the *tris*-*C*-glycal. Hydroboration of the triple alkene bonds afforded the β-*C*-tetrasaccharide **23** in 44% overall yield.



Scheme 2.5: First synthesis of a branched β-*C*-tetrasaccharide using a triple RCM cyclization.

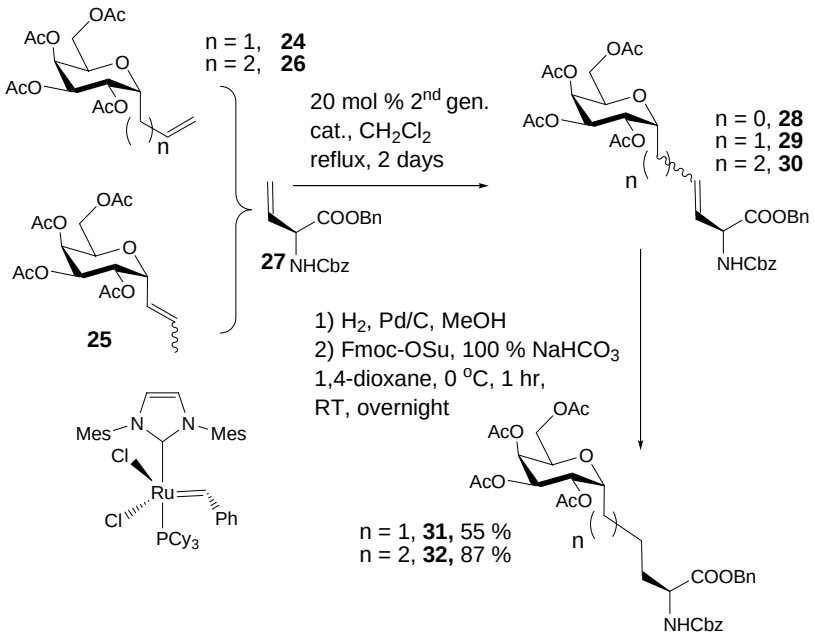
2.3.2.2 Intermolecular cross-methathesis:

Robert N. Ben and co-workers have prepared a series of *C*-linked antifreeze glycoprotein analogues for their evaluation as antifreeze agents as a function of the distance separating the carbohydrate moiety from the polypeptide backbone.[101] The building blocks for these analogues were prepared using either an olefin cross-metathesis or a catalytic asymmetric hydrogenation.

C-Allylated galactose pentaacetate **24** was prepared via a photochemical-mediated allylation in 90% yield.[102a] *C*-Glycoside **26** was obtained by reducing **24** with borane followed by PCC oxidation and

[98] Piper, J. L; Postema, M. H. D. *J. Org. Chem.* **2004**, *69*, 7395.
[99] Grubbs, R. H.; Fu, G. C. *J. Am. Chem. Soc.* **1992**, *114*, 7324.
[100] Scholl, M.; Ding, S.; Lee, C. W.; Grubbs, R. H. *Org. Lett*. **1999**, *1*, 953.
[101] Liu, S.; Ben R. N. *Org. Lett.* **2005**, *7*, 2385
[102] (a) Ponten, F.; Magnusson, G. *J. Org. Chem.* **1996**, *61*, 7463.(b) Perkins, M. V.; Sampson, R. A. *Org. Lett.* **2001**, *3*, 123-126. (c) Abad, A.; Agullo´, C.; Cunat, A. C.; Garcı´a, A. B.; Gimenez-Saiz, C. *Tetrahedron* **2003**, *59*, 9523.

Wittig olefination with methyl triphenyl phosphonium bromide. [102b,c] *C*-(1-Propenyl) glycoside **25**, a key intermediate in the preparation of AFGP analogue, was generated via a palladium-mediated isomerization of **24**. Vinyl glycine derivative **27** was obtained in 34% yield from the orthogonally protected glutamic acid derivative by oxidative decarboxylation.[103] With the requisite building blocks in hand, olefin cross-metathesis of **27** with **24**, **25**, and **26** were conducted using the second-generation Grubbs catalyst (Scheme 2.6). As anticipated, building blocks **29** and **30** were obtained in quantitative yield (Table 2.2).



Scheme 2.6: Preparation of *C*-Linked Building Blocks **31** and **32** blocks **12** and

Unfortunately, cross-metathesis between **27** and **25** furnished building block **28** in only trace quantities. Presumably, this is due to the fact that the carbon-carbon double bond in **25** is too close to the pyranose ring resulting in significant steric interactions during the OCM. A similar effect has been observed by McGarvey *et al*.[104] The carbon-carbon double bonds in enamide esters **29** and **30** were reduced by hydrogenation with palladium over carbon. Under these conditions, the Cbz and benzyl protecting groups were simultaneously removed necessitating reprotection of the amino terminus as an Fmoc carbamate to afford building blocks **31** and **32** in 55% and 85% yield, respectively.

[103] Bacha, J. D.; Kochi, J. K. *Tetrahedron* **1968**, *24*, 2215.
[104] McGarvey, G. J.; Benedum, T. E.; Schmidtmann, F. W. *Org.Lett*. **2002**, *4*, 3591.

Table 2.2: Olefin Cross-Metathesis To Prepare *C*-Linked Building Blocks **29-30**

entery	*C-alkene glycoside*	*product*	*Yield %*
1	**24**	**29**	98
2	**25**	**28**	traces
3	**26**	**30**	100

In a second example McGarvey choses to examine a series of substrates with various olefin substitutions and proximal functionalities[105] (Figure 2.3). Thus *C*-aminoglycoside substrates **A**–**C** were constructed.[106] In addition to the commercially available dihydroalanine **F**, allyl and vinyl glycines, **D** and **E**, were prepared using methods reported by Myers[107] and Hanessian,[108] respectively.

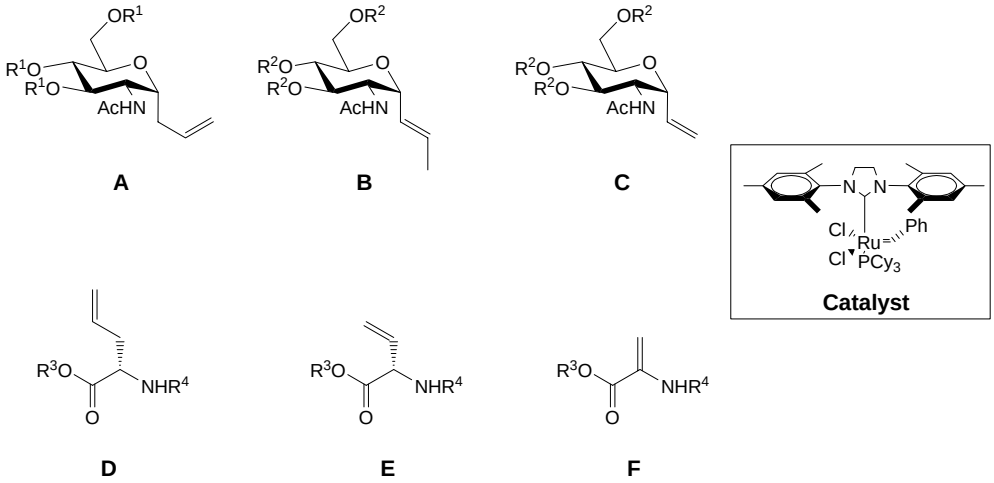


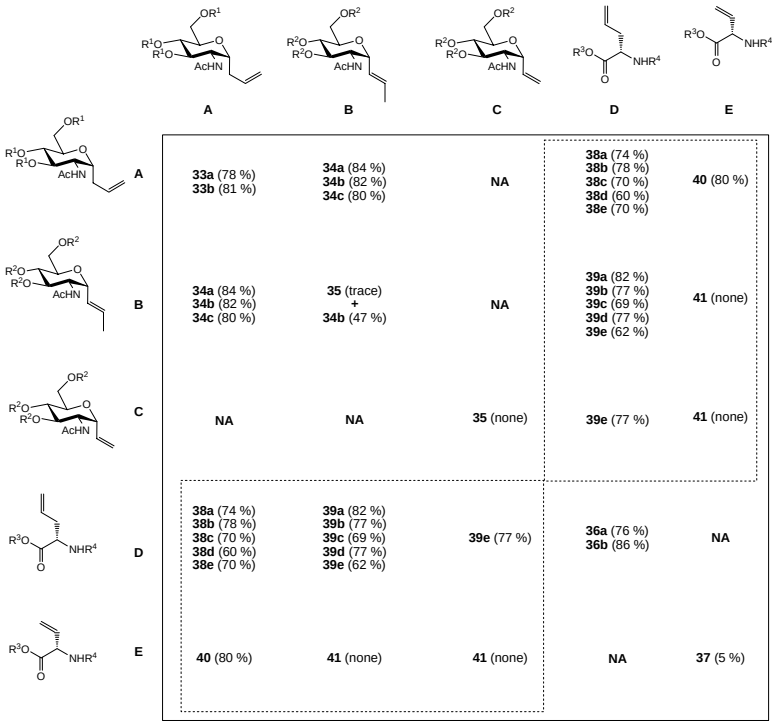
Figure 2.3: Substrates used in the metathesis study and Grubbs second generation catalyst.

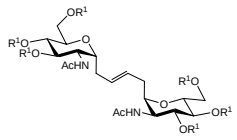
The possible cross- and self-metatheses of these substrates was examined using Grubbs second generation catalyst (Fig. 2.3). The results are summarized in Table 2.3. The cross-metathesis reactions combining *C*-glycosides **A**–**C** with two equivalents of unnatural amino acids **D**–**F** are indicated in the dashed boxes.[109] It was found that allyl glycine **D** is an effective reaction partner for all three *C*-glycosides, giving the various corresponding products **38** and **39** in good yields. It is noteworthy that reactions leading to benzyl protected products **38a,b** and **39a,b** and acetates **38c–e** and **39c–e** required

[105] Maishal, T. K.; Sinha-Mahapatra, D. K.; Paranjape, K.; Sarkar, A. *Tetrahedron Lett.* **2002**, *43,* 2263.
[106] McGarvey, G. J.; Schmidtmann, F. W.; Benedum, T. E.; Kizer, D. E. *Tetrahedron Lett.* **2003**, *44*, 3775.
[107] (a) For the Fmoc protected form: Myers, A. G.; Gleason, J. L.; Yoon, T.; Kung, D. W. *J. Am. Chem. Soc.* **1997**, *119*, 656; For the Boc protected form: (b) Myers, A. G.; Gleason, J. L. *Org. Synth.* **1999**, *76*, 57; See also: Dominique, R.; Liu, B.; Das, S. K.; Roy, R. *Synthesis* **2000**, *6*, 862.
[108] Hanessian, S.; Sahoo, S. P. Tetrahedron Lett. 1984, 25, 1425–1428.
[109] Unless otherwise noted, all cross-metathesis reactions were allowed to proceed until consumption of the starting C-glycoside as noted by TLC analysis. For the case where R^1, R^2 = Bn, 10 mol % of catalyst was used, whereas 20 mol % of catalyst was required for R^1, R^2 = OAc (see Table 2.3).

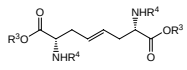
24 and 48 h respectively. This suggests a significant functional group effect on reactivity. In contrast to the allyl amino acids, vinyl glycine **E** was found to be reactive with only allyl *C*-glycoside **A** and afforded **40**. Unfortunately, attempts to access to a product with a linker length corresponding to natural glycoamino acids,**41**, proved unsuccessful as vinylic *C*-glycosides **B** and **C** did not react with **E**.

Table 2.3: Olefin metathesis study

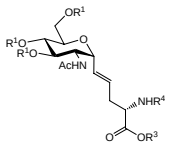




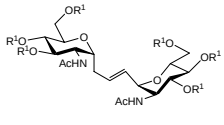
33a: R^1 = Bn
33b: R^1 = Ac



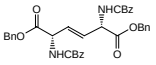
36a: R^3 = Me , R^4 = Boc
36b: R^3 = Me , R^4 = Fmoc



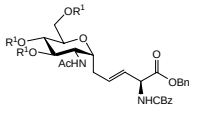
39a: R^1 = Bn, R^3 = Me , R^4 = Fmoc
39b: R^1 = Bn, R^3 = Me , R^4 = Boc
39c: R^1 = Ac, R^3 = Me , R^4 = Fmoc
39d: R^1 = Ac, R^3 = Me , R^4 = Boc
39e: R^1 = Ac, R^3 = Bn , R^4 = CBz



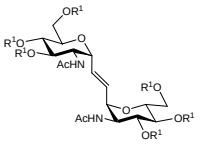
34a: R^1 = R^2 = Bn
34b: R^1 = R^2 =Ac
34c: R^1 = Ac, R^2 = Bn



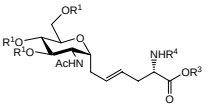
37



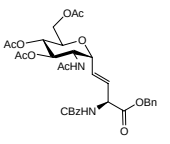
40



35



38a: R^1 = Bn, R^3 = Me , R^4 = Fmoc
38b: R^1 = Bn, R^3 = Me , R^4 = Boc
38c: R^1 = Ac, R^3 = Me , R^4 = Fmoc
38d: R^1 = Ac, R^3 = Me , R^4 = Boc
38e: R^1 = Ac, R^3 = Bn , R^4 = CBz

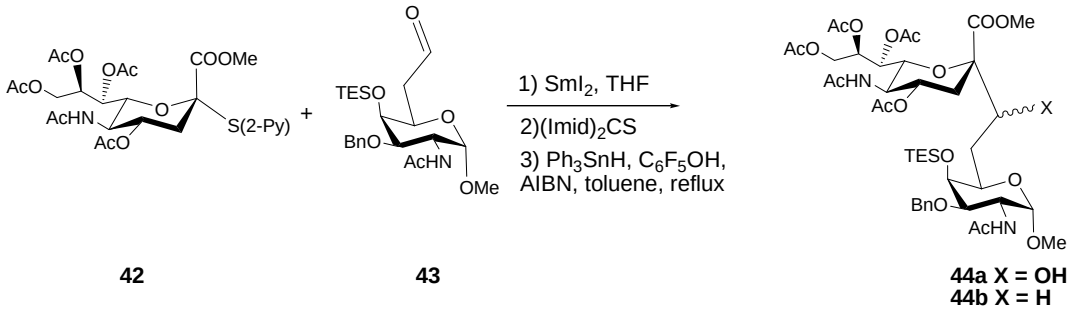


41

2.3.3 Samarium Iodide-Mediated *C*-Glycosylation

Sinaÿ and Beau have shown that glycosylsamarium(III) derivatives, generated from glycosyl chloride or sulfones, undergo Barbier-type condensation with carbonyl compounds to form *C*-glycosides.[110,111] this realizes a very powerful method for the synthesis of *C*-glycosides.

Beau and co-workers have obtains an advanced precursor of STn antigen[89a] using the samarium-Reformatsky coupling procedure[112] with sulfide **42** (1.5equiv) and aldehyde **43**. This afforded the carbon-linked dimeric product **44a** in high yield (93%) as a 1:1 diastereomeric mixture (Scheme 2.7).



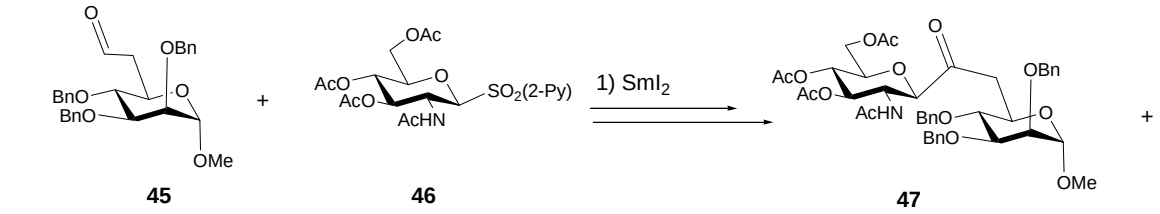
Scheme 2.7: Preparation of the carbon-linked sialyl-N-acetylgalactosaminyl donor **44b**.

Alcohols **44a** were converted into thiocarbonates by treatment with a large excess of N,N' thiocarbonyldiimidazole in refluxing acetonitrile and were then deoxygenated by employing triphenyltin hydride a catalytic amount of AIBN and pentafluorophenol.[113] This yielded the required *C*-disaccharide **44b** as a single compound (65% for the two steps). Desilylation, hydrogenolysis, and acetylation provided the peracetylated C-dimer.

Stereoselective preparation of β-*C*-glycosides has been developed by Beau and co-workers from acetylated glycopyranosyl 2-pyridyl sulfones, involving a samarium-Barbier coupling procedure–oxidation–isomerization sequence (Scheme 2.8).[114]

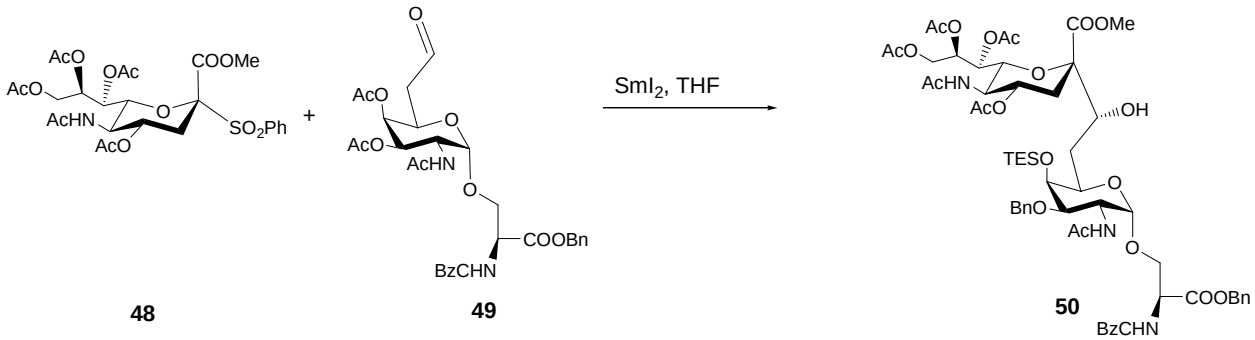
---

[110] de Pouilly, P.; Chenede, A.; Mallet, J.-M.; Sinaÿ, P. *Bull Soc. Chim. Fr.* **1993**, *130,* 256.
[111] Mazeas, D.; Skrydstrup, T.; Beau, J.-M. *Angew. Chem. Int. Ed. Engl.* **1995**, *34,* 909.
[112] For the reductive samariation of glycyl sulfide derivatives, see Ricci, M.; Madariaga, L.; Skrydstrup, T. *Angew. Chem.* **2000**, *112*, 248; *Angew. Chem. Int. Ed.* **2000**, *39*, 242; Ricci, M.; Blakskjaer, P.; Skrydstrup, T. *J. Am. Chem. Soc.* **2000**, *122*, 12413.
[113] Jarreton, O.; Skrydstrup, T.; Beau, J.-M. *Chem. Commun.* **1996**, 1661; Jarreton, O.; Skrydstrup, T.; Beau, J.-M. *Chem. Eur. J.* **1999**,*5*, 430.
[114] Palmier, S.; Vauzeilles, B.; Beau, J.-M. *Org. Blomol. Chem.*,**2003**, *1*, 1097.



Scheme 2.8: Synthesis of β-*C*-glycosides

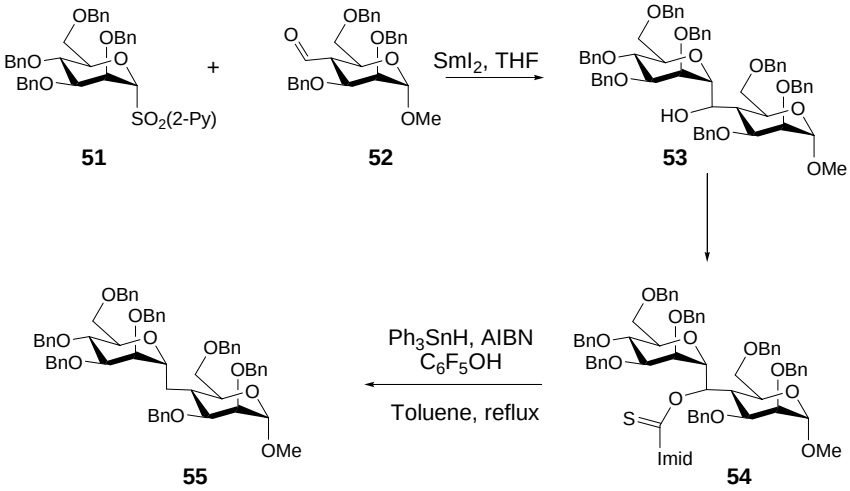
Appling samarium iodide coupling methodology the synthesis of *C*-linked STn antigen was achieved by Linhardt[115] in one enzymatic and 17 chemical steps starting from diisopropylidene galactopyranose and sialic acid (Scheme 2.9).



Scheme 2.9: Synthesis of STn C-glycoside derivatives.

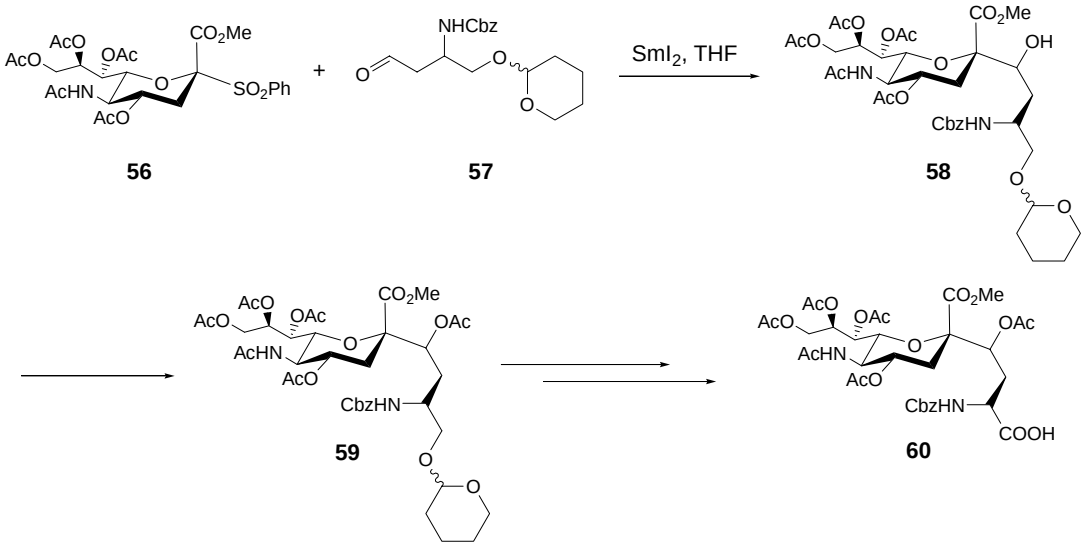
Skrydstrup has used the same methodology to prepare the main building block for a cyclic *C*-oligomer containing repeating units of *C*-dimer. The key step in his synthesis uses a SmI_2-mediated coupling of **51** with **52**, affording the *C*-disaccharide precursor (Scheme 2.10).[116]

[115] Kuberan, B.; Sikkander, S. A.; Tomiyama, H.; Linhardt, R. J. *Angew. Chem. Int. Ed.* **2003**, *42*, 2073.
[116] Mikkelsen, L. M.;Skrydstrup, T. *J. Org. Chem.* **2003**, *68*, 2123.



Scheme 2.10: Synthesis of STn C-glycoside derivatives.

Linhardt prepared a *C*-glycoside linking neuraminic acid to serine by using the SmI_2 methodology. The neuraminic acid derivative **56** was coupled with aldehyde **57** affording the *C*-glycoside **58** in 45% yield. Acetylation of the newly generated hydroxymethano linker afforded the fully protected *C*-glycoside **59**. Removal of THP with pyridinium *p*-toluenesulfonate (PPTS), followed by oxidation of the resulting hydroxyl group, generated L-homoserine *C*-glycoside derivative **60** (Scheme 2.11).[117]

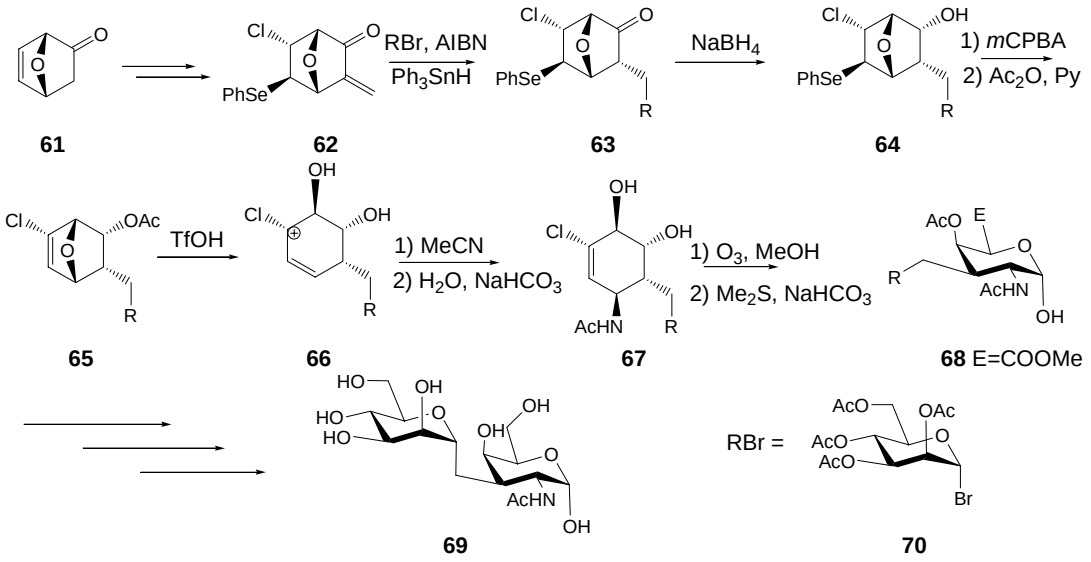


Scheme 2.11: Synthesis of a serine-based neuraminic acid *C*-glycoside

[117] Wang, O.; Linhardt, R. J. *J. Org. Chem.* **2003**, *68*, 2668.

2.3.4 Radical C-glycosidation:

Vogel and co-workers have prepared *C*-disaccharides using enantiomerically pure 7-oxabicyclo[2.2.1]hept-5-en-2-one (**61:** a naked sugars of the first generation).[118] Reaction of **61** are highly diastereoselective. For instance sequence of reactions **61→62→63** have been use to generate *C*-glycosides that are converted into *C*-disaccharides,[119] and analogues.[120] Under modified conditions, the radical *C*-mannosidation of **62** between 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide (**70**) is possible in benzene using Ph_3SnH as hydrogen donor. This gives **63** in 56% yield (Scheme 2.12). This compound can be transformed under standard conditions into the fully deprotected C-linked disaccharide **69**.[121]



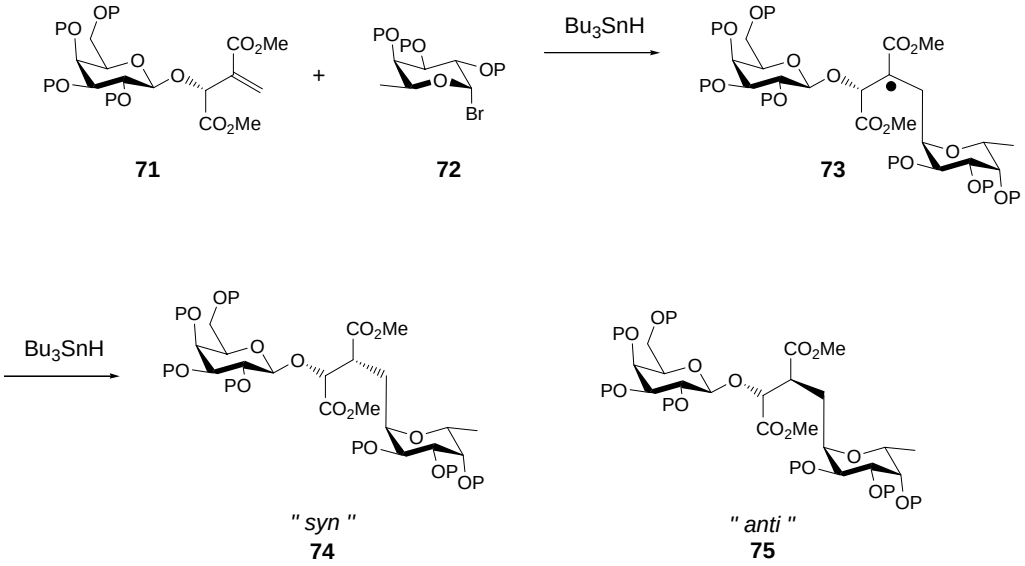
Scheme 2.12: Synthesis of a C-glycoside using the naked sugar methodology.

Guindon and co-workers have shown that the reaction between an anomeric-centered fucosyl-derived radical **72** and a galactosylated hydroxytaconate **71** provides an easy access to C,O-diglycosides as mimics of sLe^X. In this case, two 1,3-distant stereocenters were created with high diastereoselectivity using free radical intermediates in a tandem process (Scheme 2.13).[122]

---

[118] a) Vieira, E.; Vogel, P. *Helv. Chim. Acta* **1983**, *66*, 1865. b) Black, K. A.; Vogel, P. *Ibid.* **1984**, *67*, 1612. c) Reymond, J.-L.; Vogel, P. *Tetrahedron: Asymmetry* **1990**, *1*, 729. d) Vogel, P.; Fattori, D.; Gasparini, F.; Le Drian, C. *Synlett* **1990**, 173. e) Vogel, P. *Bull. Soc. Belg.* **1990**, *99*, 295. f) Forster, A.; Kovac, T.; Mosimann, H.; Renaud, P.; Vogel, P. *Tetrahedron: Asymmetry* **1999**, *10*, 567; see also: g) Saf, R.; Faber, K.; Penn, G.; Griengl, H. *Tetrahedron* **1988**, *44*, 389. h) Ronan, B.; Kagan, H. B. *Tetrahedron: Asymmetry* **1991**, *2*, 75. I) Corey, E. J.; Loh, T.-P. *Tetrahedron Lett.* **1993**, *34*, 3979.
[119] Cossy, J.; Ranaivosata, J.-L.; Bellosta, V.; Ancerewicz, J.; Ferritto, R.; Vogel, P. *J. Org. Chem.* **1995**, *60*, 8351.
[120] Ferritto, R.; Vogel, P. *Synlett* **1996**, 281.
[121] Pasquarello, C.; Picasso, S.; Demange, R.; Malissard, M.; Berger, E. G.; Vogel, P. *J. Org. Chem.* **2000**, *65,* 4251.

[122] Guindon, Y.; Bencheqroun, M.; Bouzide, A. *J. Am. Chem. Soc.* **2005**, *127*, 554.

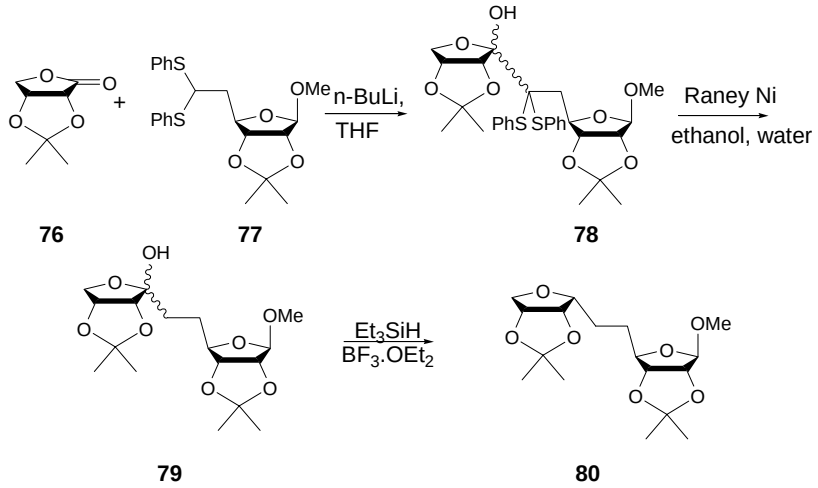


71 72 73 74 75

Scheme 2.13: Synthesis of C,O-diglycosides under radical conditions

2.3.5 *C*-Glycosidation via Lithiated Anions

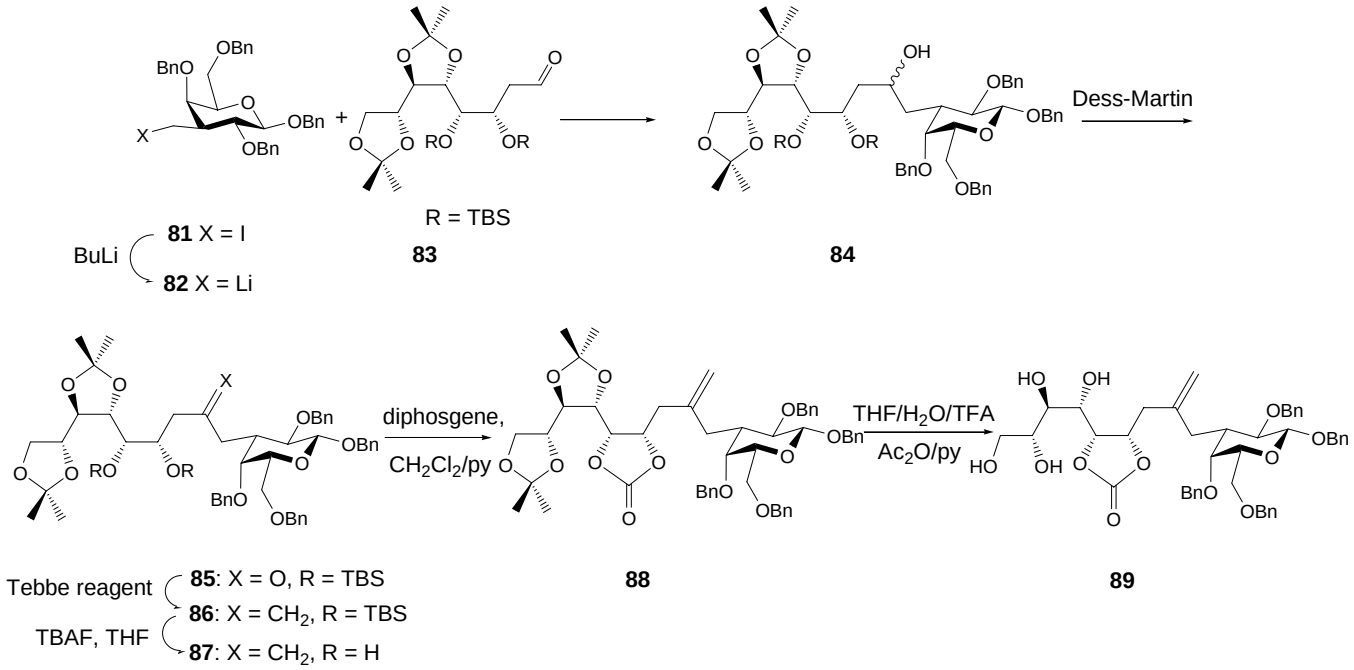
Addition of BuLi and lithiated dithianes **77** generated a polar species that added to 2,3- *O*-isopropylidene-D-erythronolactone **76** affording lactols **78**. The latter are reduced first with Raney nickel and then with Et_3SiH into the corresponding *C*-glycosides **80**. The use of a D-ribose-derived lithiated dithiane nucleophile in this chemistry allows for the convenient construction of a furanose *C*-disaccharide (Scheme 2.14).[123]



Scheme 2.14: Synthesis of furanose C-disaccharide by lithiated dithianes.

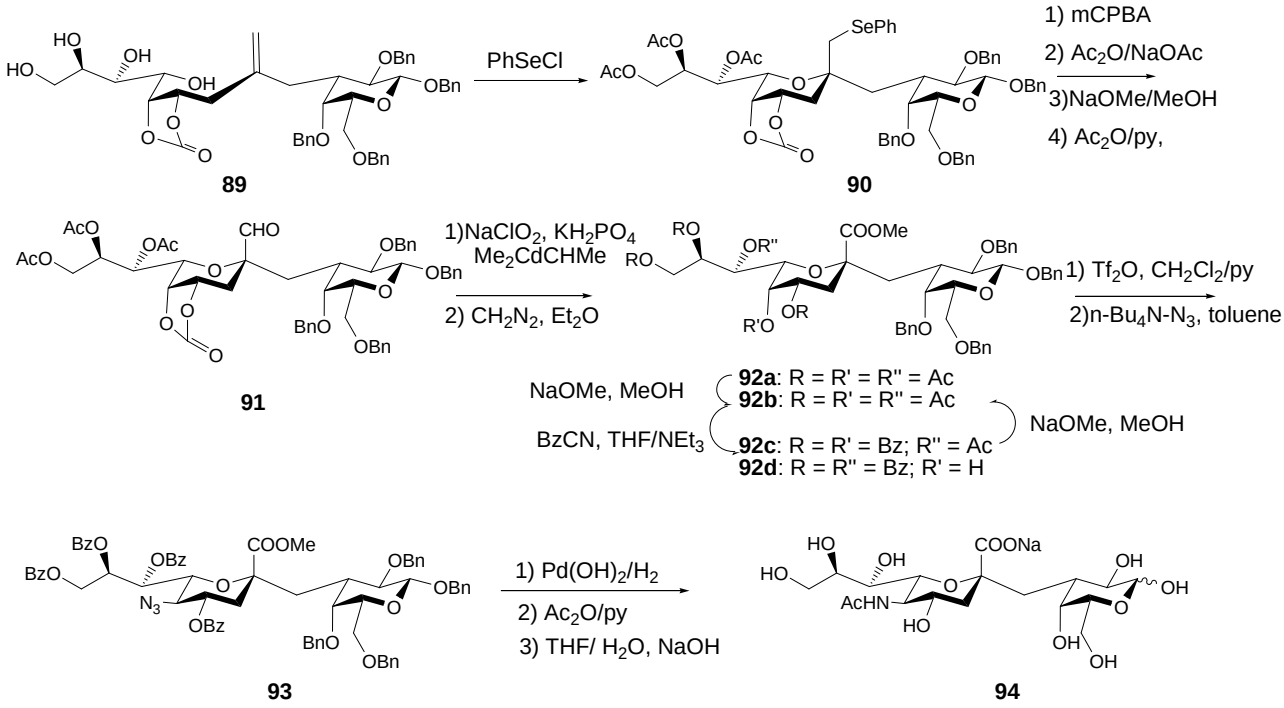
Schmidt and co-workers have used a general strategy toward the synthesis of *C*-ketosides of *N*-acetylneuraminic acid (Neu5Ac).[124] It has been successfully applied to the synthesis of methylene-bridged Neu5Ac-α-(2,3)-Gal *C*-disaccharide **94** (Scheme 2.16). The key strategic element of this novel approach is a stereoselective, 6-*exo-trig* selective, electrophilic cyclization of the appropriate open chain precursor **89** by means of phenylselenyl triflate.[125,126] The open chain precursor was formed by the addition of lithiated iodide **81** accessible from D-galactose to open chain aldehyde **83** obtained from D-glucono-δ-lactone by chain elongation. Subsequent methylation using Tebbe-reagent, subsequent formation of a cyclic carbonate and deprotection of the two isopropylidene ketals afforded tetrol **89** which, upon treatment with phenylselenyl triflate, was stereoselectively cyclized in a 6-*exo-trig* selective manner (Scheme 2.15).

[123] McCartney, J. L.; Meta, C. T.; Cicchillo, R. M.; Bernardina, M. D.; Wagner, T. R.; Norris, P. *J. Org. Chem.* **2003**, *68*, 10152.
[124] Notz, W.; Hartel, C.; Waldscheck, B.; Schmidt, R. R. *J. Org. Chem.,***2001***, 66,* 4250.
[125] a) Nicolaou, K. C.; Petasis, N. A. *Selenium in Natural Products Synthesis*; CIS: Philadelphia, 1984. b) Sharpless, K. B.; Gordon, K. M.; Lauer, R. F.; Patrick, D. W.; Singer, S. P.; Young, M. W. *Chemica Scripta* **1975**, *8A*, 9-13. c) Wirth, T. *Angew. Chem.* **2000**, *112,* 3890; *Angew. Chem., Int. Ed. Engl.* **2000**, *39*, 3740.
[126] Schmidt, R. R.; Vlahov, I. V. *Tetrahedron Asymmetry* **1993**, *4*, 293.



Scheme 2.15: Electrophilic Cyclization

A *selena*-Pummerer rearrangement, oxidation, and esterification readily led to methyl ester **92a** which, after deacetylation, could be regioselectively tetrabenzoylated with benzoyl cyanide. Triflate activation of the axial hydroxyl group in **92d** and nucleophilic displacement by azide ion with inversion of configuration afforded azide **93**, which was reduced with hydrogen and Pearlman's catalyst. Concomitant removal of the benzyl ethers and subsequent saponification of all ester moieties successfully completed the de novo synthesis of the desired methylene bridged Neu5Ac-α-(2,3)-Gal *C*-disaccharide **94** (scheme 2.16).

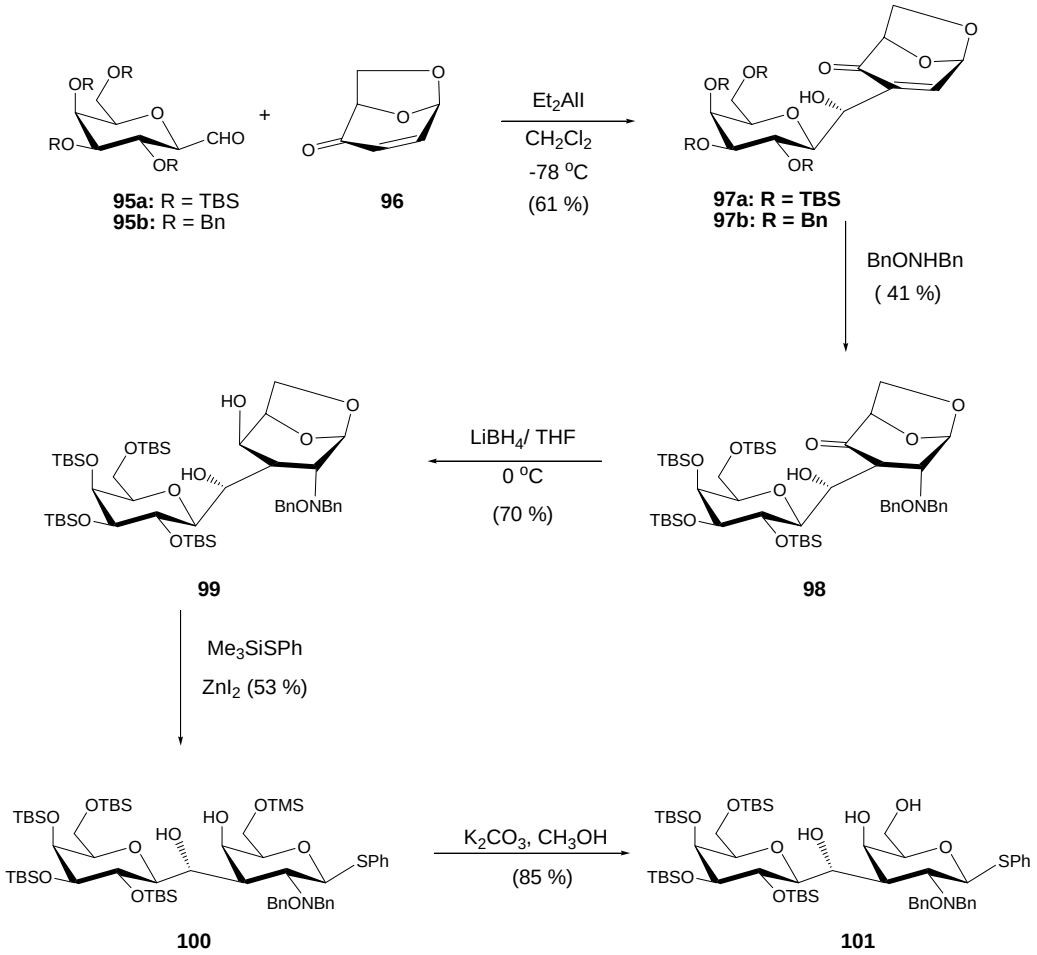


Scheme 2.16: Completion of Neu5Ac-*C*-disaccharide **94**

2.4 Attempts towards the Synthesis of a Non-Hydrolysable Analogue of the (Thomsen-Friedenreich) TF Antigen

2.4.1 The First Attempt of the Synthesis of a *C*-Disaccharide Part of the TF Antigen

The first synthesis of a *C*-disaccharide analogue of the sugar part of the TF antigen was reported in our group by Y.-H. Zhu.[127] Persilylated aldehyde **95** and isolevoglucosenone **96** were reacted with Et_2AlI. Aldol **97a** was obtained as the major product of condensation (61%). Addition of N,O-dibenzyl hydroxylamine gave ketone **98** (41%) which was reduced with $LiBH_4$ to give the galactosamine derivative **99** with good diastereoselectivity in (70%) yield. On treatment with Me_3SiSPh and ZnI_2, **99** was converted into the phenyl thiogalactopyranoside **100**. It was thus treated with K_2CO_3 in MeOH to provide triol **101** which is a partially protected *C*-disaccharide analogue of the glucan portion of the TF antigen (Scheme 2.17).



Scheme 2.17: First approach of the *C*-disaccharide part of T antigen.

[127] Zhu, Y.-H.; Vogel, P. *Synlett* **2001**, 79.

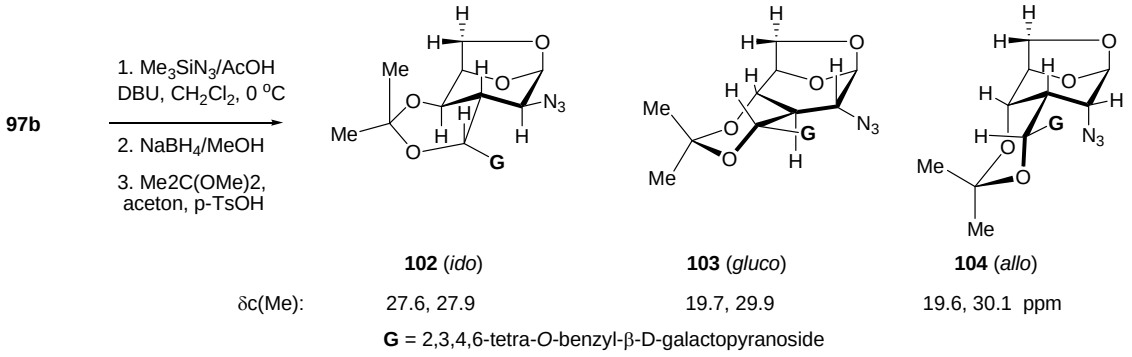
In this approach the elegant 1,4-addition of N,O-dibenzyl hydroxylamine furnished a precursor of GalNAc with good stereoselectivity. Unfortunately the yield of the latter reaction remained low.

The approach remained uncertain concerning the possibility to use **99-101** and derivatives in α-glycosidation of L-serine derivatives.

2.4.2 The Second Attempt of the Synthesis of a *C*-Disaccharide Part of the TF-Antigen

2.4.2.1 1,4-Addition of Azide Anion to a bicyclic enone

Although isolevoglucosenone **96** is known to add NaN_3/AcOH giving the corresponding β-azidoketone,[128] enones **97a** and **97b** failed to react with HN_3 (-40→0 °C). Attempt to add azides to **97b** was reported in our group by R. Demange. He used a 1:1 mixture of Me_3SiN_3 and AcOH containing a catalytical amount of DBU.[129] Under these conditions **97b** gave a mixture of unstable azide after aqueous treatment. The crude reaction mixture obtained at 0 °C was treated with $NaBH_4$/MeOH to give a mixture of azido-diols that were not isolated, but directly converted into the corresponding acetonides under standard conditions (Scheme 2.18). Flash chromatography on silica gel provided the three products **102**, **103** and **104** (52 % yield, 3 steps) with the proportion 1:1.6:1.6. Unfortunately, none of these azides had the desired 1,6-anhydro-D-*galacto* configuration. Their structures were given by their spectral data, in particular by their ^{1}H-NMR (coupling constants between vicinal proton pairs) and ^{13}C-NMR spectra (1',4-*cis vs.* 1',4-*trans* relative configuration).[130]

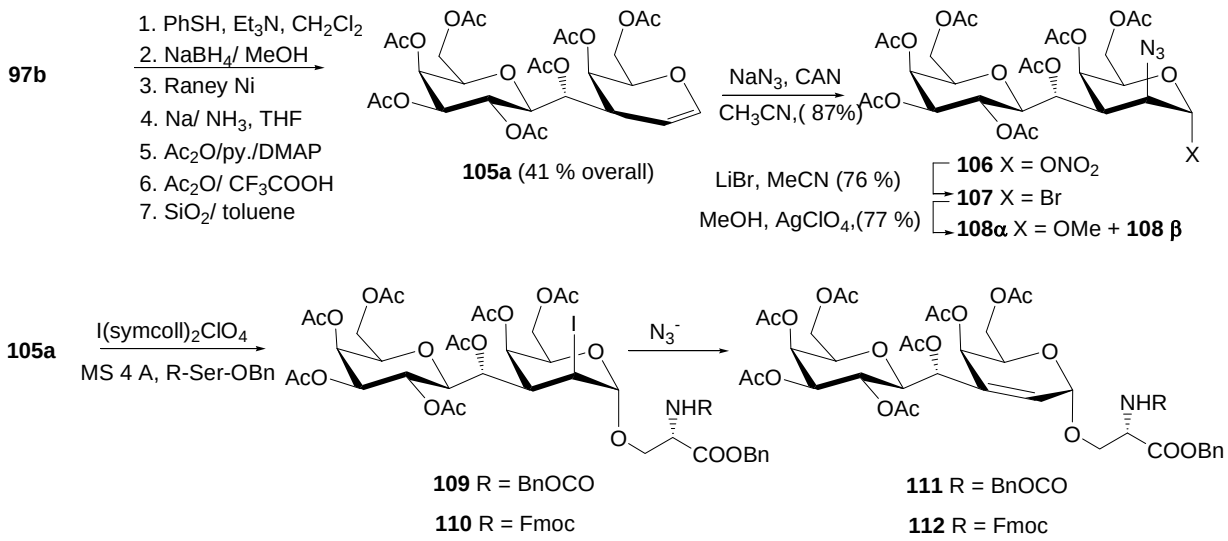


Scheme 2.18 Structure of acetonides products related ^{1}H-NOE effects

[128] Horton, D.; Roski, J. P.; Norris, P. *J. Org. Chem.* **1996**, *61*, 3783.
[129] Guerin, D. J.; Horstmann,T. E.; Miller, S. J. *Org. Lett.* **1999**, *1*, 1107.
[130] Rychnovsky, S. D.; Rogers, B. N.; Richardson, T. I. *Acc. Chem. Res*. **1998**, *31*, 9.

2.4.2.2 Lemieux's Azidonitration of Galactal Derivatives:

Protected D-galactals[131] as well as peracetate of *β*-D-Gal*p*-(1→3)-D-galactal[132] of type **105a** undergo Lemieux's azidonitration ($NaN_3/Ce(NH_4)_2(NO_3)_6$) giving the corresponding α-and β-D-galactopyranosyl nitrates as major products. Our group reported recently the synthesis of the peracetate of β-D-Gal*p*-(1-CH(OH)-3)-D-galactal **105a**.[133] This compound reacted with $Ce(NH_4)_2(NO_3)_6$ and NaN_3 in CH_3CN giving a single *α*-nitrate **106** in 87 % yield. Reaction of **106** with LiBr in CH_3CN gave α-bromide **107** (78%) which underwent Königs-Knorr glycosidation of MeOH furnishing a separable 4.5:1 mixture of *α*-and *β*-galactopyranosides **108*α*** (63%) and **108*β*** (14%). As already reported,[133] iodoglycosidation of semi-protected forms of L-Serine (Cbz-Ser-OBn, Fmoc-Ser-OBn) with **105a** produced 2-deoxy-2-iodo-*talo*-pyranosides **109** and **110**. Unfortunately, S_N2 displacement attempts of the iodides **109** and **110** by all kinds of azides led to decomposition or/and elimination of HI with the formation of enopyranosides **111** and **112**, respectively (Scheme 2.19).

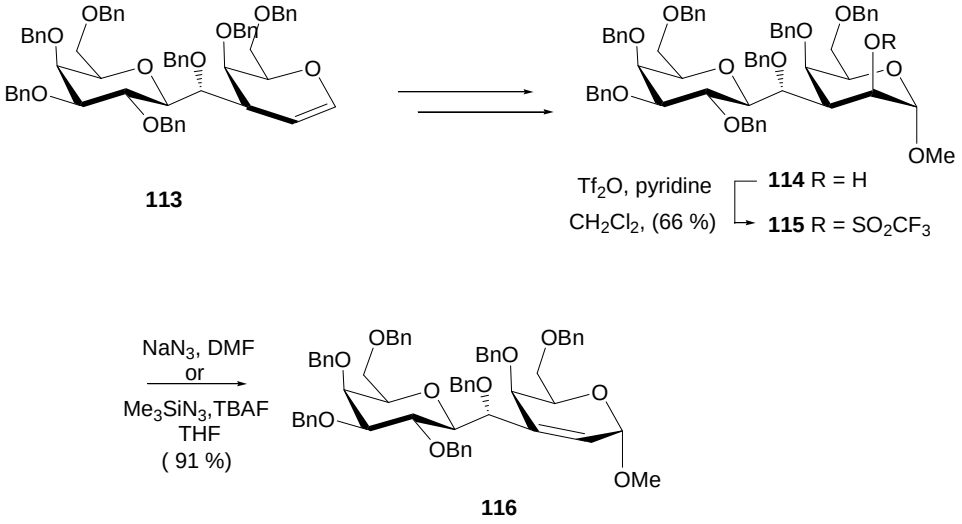


Scheme 2.19: attempts of displacement by azides

Vogel and Demange have also prepared triflate **115** from the talopyranoside **114** derived from **113**. But all attempts to displace the triflate in a S_N2 process failed as the product of elimination **116** were formed readily(Scheme 2.20).[134,135] These failures are in contrast with successes encountered for similar reactions with related monosaccharide derived triflates.

---

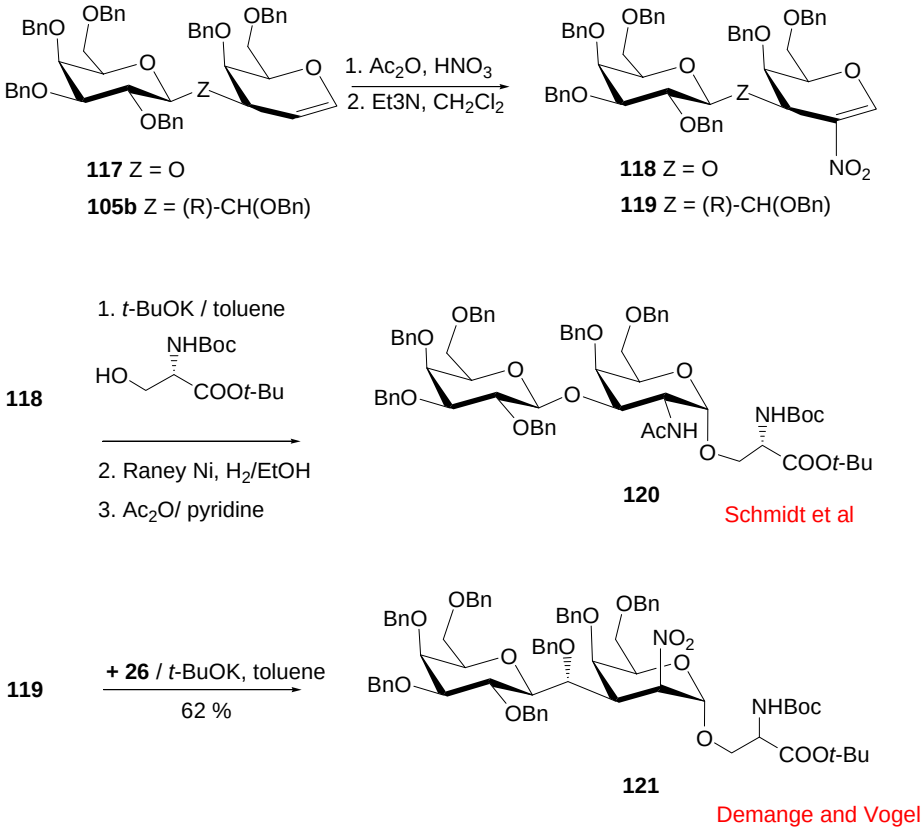
[131] Lemieux, R. U.; Ratcliffe, R. M. *Can. J. Chem*. **1979**, *57*, 1244.
[132] a) Look, G. C.; Ichikawa, Y.; Shen, G. –J.; Cheng, P. –W.; Wong, C. –H. *J. Org. Chem*. **1993**, *58*, 4326. b) Bay, S.; Berthier-Vergnes, O.; Cantacuzene, D. *Carbohydr. Res*. **1997**, *298*, 153.
[133] Demange, R.; Awad, L; Vogel, P. *Tetrahedron: Asymmetry* **2004**, *15*, 3573.
[134] (a) Marra, A.; Gauffeny, F.; Sinaÿ, P. *Tetrahedron* **1991**, *47*, 5149. (b) Tipson, R., S. *Adv. Carbohydr. Res. Chem*. **1953**, *8*, 107.
[135] Karpiesiuk, W.; Banaszek, A; Zamojski, A. *Carbohydr. Res*. **1989**, *186*, 156.



Scheme 2.20: Attempts of S_N2 displacement by trifluoromethanesulphonate derivatives by azides

2.4.2.3 Schmidt's Glycosidation via 2-nitro-D-galactal.

Schmidt and co-workers[136] have prepared perbenzylated T antigen **120** by Michael-type addition of Boc-Ser-O-*t*-Bu to 2-nitro-galactal **118** derived from D-galactal **117** (Scheme4.5). Applying the same reaction sequence to the perbenzylated *β*-Gal*p*-(1-CH(OH)-3)-D-galactal **105b** (derived from enone **97b**[90d]), the nitro-galactal **119** was obtained in (50%) yield which reacted with the semi-protected L-serine (Boc-Ser-O-*t*-Bu). Unfortunately again, only the *α*-talopyranoside **121** was formed. Attempts to epimerize the 2-deoxy-2-nitro-D-talopyranoside **121** into the corresponding 2-deoxy-2-nitro-D-galactopyranoside failed (Et_3N, DBU, DABCO) also (Scheme 2.21).

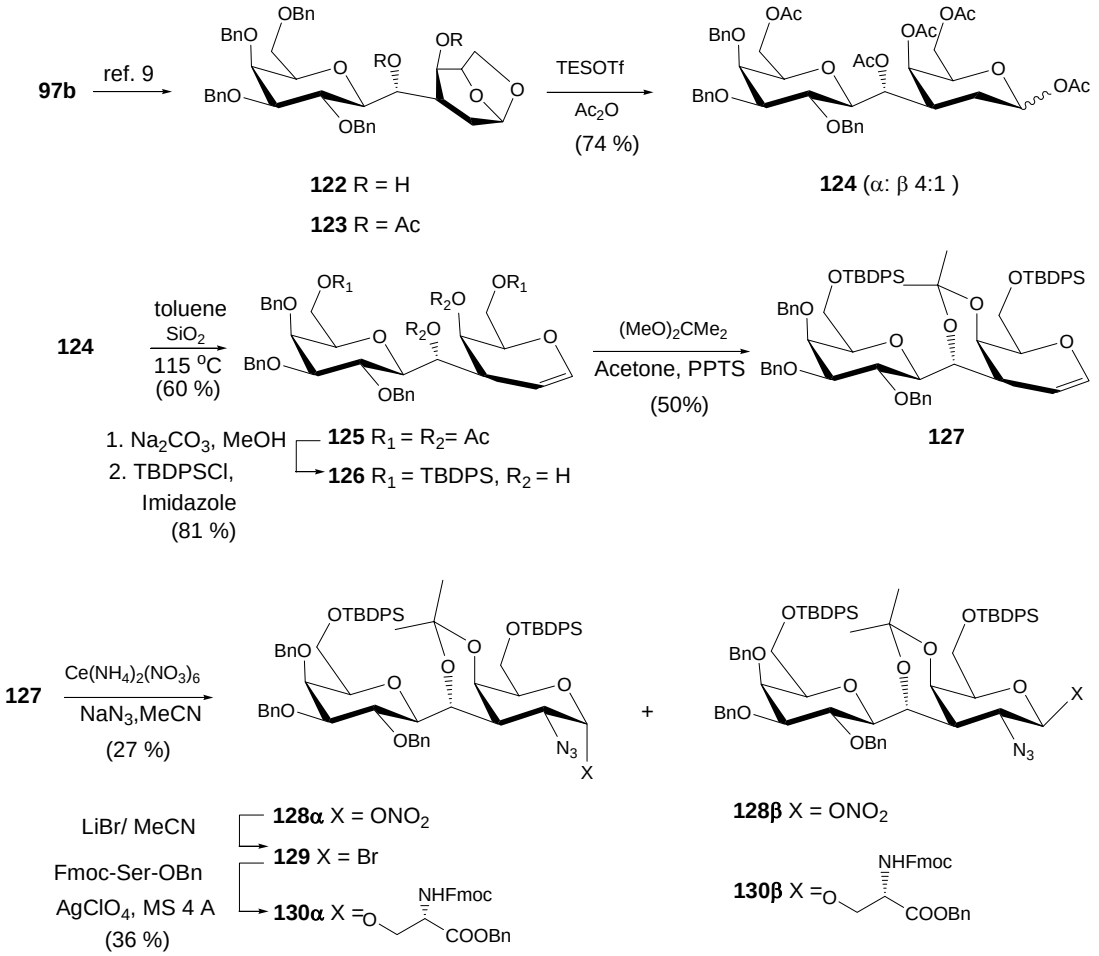


Scheme 2.21: Synthesis of the 2-deoxy-2-nitro-talopyranoside **121**.

[136] Das, J.; Schmidt, R. R. *Eur. J. Org. Chem.* **1988**, 1609. Winterfeld, G. A.; Ito, Y.; Ogawa, T.; Schmidt, R. R. *Eur. J. Org. Chem.* **1999**, 1167. Winterfeld, G. A.; Schmidt, R. R. *Angew. Chem. Int. Ed.* **2001**, *40*, 2654.

2.4.2.4 Azidonitration of a Conformationally Constrained D-galactal Derivative.

Vogel and Demange envisioned that Lemieux's radical azidonitration of a 3-*C*-glycoside of D-galactal, azide may selectively attack from the alpha-side if its 4,6-diol unit could be included in a ring a such as an acetonide. As shown by several authors,[137] the stereoselectivity of this reaction depends strongly on substitution of the galactal and on its conformational mobility. They further envisioned that an acetonide involving the hydroxy groups at C-4 of the galactal moiety and at C-1' of the hydroxymethylene linker might impede radical addition onto the β face of the galactal alkene moiety. Thus, the protected β-*C*-galactopyranosyl-(1→3)-galactal **127** was prepared as shown in Scheme 6. Its azidonitration under Lemieux's conditions gave a 1:3 mixture of 2-azido-2-deoxy-D-*galacto*-pyranosyl nitrates **128α** and **128β** in mediocre yield (27 %). Nitrate **128** was then converted into bromide **129** that was used for the Königs-Knorr glycosidation of Fmoc-Ser-OBn. This led to a 1.5:1 mixture of the desired **α** and undesired **β**-galactosides **130α** and **130β** respectively, with mediocre yield (36 %) also (Scheme 2.22).



Scheme 2.22: Synthesis of a non-hydrolysable analogue of TF (Thomsen-Friedenreich) antigen.

[137] Kinzy, W.; Schmidt, R. R. *Tetrahedron Lett.* **1987**, *28*, 1981. see also a) Tabeur, C.; Machetto, F.; Mallet, J. –M.; Duchaussoy, P.; Petitou, M.; Sinaÿ, P. *Carbohydr. Res*. **1996**, *281*, 253-276. b) Seeberger, P. H.; Roehrig, S.; Schell, P.; Wang, Y.; Christ, W. *J. Carbohydr. Res*. **2000**, *328*, 61. c) Geiger, J.; Barroca, N.; Schmidt, R. R. *Synlett* **2004**, 836.

3 AIM OF THE WORK

3.1 Synthesis of a Non-hydrolysable Mimic of Thomsen-Friedenreich (TF) Antigen:

It can be summarised that oligosaccharides are present at the surface of cellular membranes in the form of glycoconjugates (glycoproteins or glycolipids) and serve as sites of attachment for other cells, bacteria, viruses, toxins and hormones. Mucin-type glycophorin family share a common structural motif possessing an α-glycosidic linkage between Gal-β-(1→3)-GalNAc and the side chain hydroxyl group of serine or threonine (TF antigen, figure 3.1). The T structure in normal cells is further glycosylated to construct complex *O*-linked glycans on mucin-type glycoproteins, whereas in most human carcimonas, this structure is exposed at the cell surface due to the incomplete synthesis of the saccharide chains.

Malignancy is often associated with profound alterations in cell surface bound carbohydrate components of glycoconjugates.[138,139] Such structural changes are due to incomplete glycosylation or novel glycosylation by tumor cells. Among the tumor associated carbohydrate antigens, the Thomsen-Friedenreich antigen (TF antigen) a disaccharide: Gal-β-1→3-GalNAcα→*O* linked to serine or threonine (Figure 3.1) is found in carcimona-associated mucins (Chapter 1).

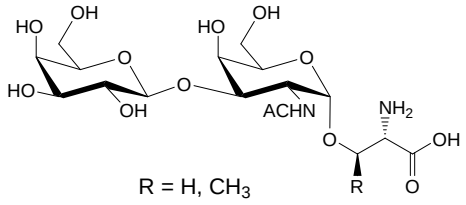


Figure 3.1: Structure of Thomsen-Friedenreich (TF) antigen.

[138] Gray, G. R. *Methods Enzymol.* **1978**, *50*, 155.
[139] Springer, G. F. *Science* **1984**, *224*, 1198. Campbell, B. J.; Finnie, E. F.; Hounsell, E. F.; Rhodes, J. M. *J. Clin. Invest.* **1995**, *95*, 571.

The TF-antigen immunogenicity in conjugate vaccines has been confirmed.[140] The clustered antigen motifs prepared by Danishefsky and coworkers (Chapter 1)[12] have demonstrated the potential for antitumor vaccines based on TF antigen conjugates. [141-143]

Synthetic approaches towards the synthesis of TF antigen and related mucin-type glycosyl amino acids were reported.[144-147] Because of their hydrolysis catalysed by glycosidases *in vivo*, disaccharide conjugates are relatively short-lived in the blood stream. Disaccharide mimetics such as *C*-linked disaccharide analogues (Figure 3.2) offer an improved stability towards hydrolysis as required for a disaccharide-based vaccine.

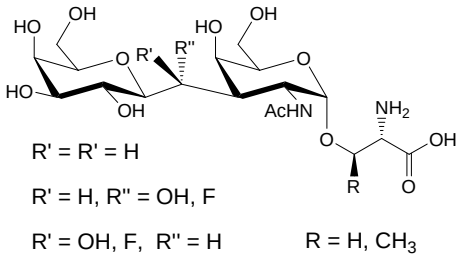


Figure 3.2: *C*-Disaccharide analogues of TF antigen.

[140] Springer, G. F. *Clin. Rev. Oncogenesis* **1995**, *6*, 57. MacLean, G. D.; Reddish, M. A.; Bowen-Yacyshyn, M. B.; Poppema, S.; Longenecker, B. M. *Cancer Invest.* **1994**, *12*, 45. Jennings, H. J.; Ashton, F. E.; Gamian, A.;. Michon, F. in *Towards Better Carbohydrate Vaccines* (Eds.: Roy, R.; Bell, R.; Torrigiani, G.), Wiley, London, 1987, 11.

[141] MacLean, G. D.; Reddish, M. A.; Bowen-Yacyshyn, M. B.; Poppema, S.; Longnecker, B. M. *Cancer Investigat.* **1994**, *12*, 46. Toyokuni, T.; Singhal, A. K.; *Chem. Soc. Rev.* **1995**, *24*, 23. Lo-Man, R.; Bay, S.; Vicher-Guerre, S.; Deriaud, E.; Cantacuzene, D.; Leclerc, C. *Cancer Res.* **1999**, *59*, 1520 and references therein.

[142] Fung, P. Y. S.; Madej, M.; Koganty, R.; Longenecker, B. M. *Cancer Res.* **1990**, *50*, 4308.

[143] MacLean, G. D.; Boyen-Yacyshyn, M. B.; Samuel, J.; Meikle, A.; Stuart, G.; Nation, J.; Poppema, S.; Jerry, M.; Koganty, R.; Wong, T.; Longenecker, B. M. *J. Immunother.* **1992**, *11*, 292.

[144] For examples of previous Tn syntheses, see: Tokoyuni, T.; Hakomori, S.; Singhal, A. K. *Bioorg. Med. Chem.* **1994**, *11*, 1119, and references therein. For examples of Tn glycopeptide clusters, see: Bay, S.; Lo-Man, R.; Osinaga, E.; Nakada, H.; Leclerc, C.; Cantacuzene, D. *J. Pept. Res.* **1997**, *49*, 620, and references therein.

[145] For selected interesting examples of glycoprotein synthesis, see: Bill, R. M.; Flitsch, S. L. *Chem. Biol.* **1996**, *3*, 145. Witte, K.; Sears, P.; Martin, K.; Wong, C.-H. *J. Am. Chem. Soc.* **1997**, *119*, 2114. Tsuda, T.;. Nithimura, S. *Chem. Commun.* **1996**, 2779.

[146] Mono- and diclusters of the T antigen have been reported: Kunz, H.; Birnbach, S.; Wering, P. *Carbohydr. Res.* **1990**, *202*, 207.

[147] Inbar, M.; Sachs, L.; *Nature* **1969**, *223*, 710. Inbar, M.; Sachs, L. *Proc. Natl. Acad. Sci. USA*, **1969**, *63*, 1418.

3.2 Retro-synthetic analysis:

Our group has become interested in preparing non-hydrolysable analogues of TF epitope whose immunogenicity in synthetic vaccines has been demonstrated.[148,149] The synthesis relies on a methodology for the synthesis of *C*(1→3)-disaccharides developed in the group: condensation between a D-galactose derived carbaldehyde and isolevoglucosenone.

From the earlier attempts of synthesis of TF antigen analogues we have seen that the galactal methodology was not convenient for our synthesis: firstly the yield was not suitable for multi-step synthesis especially from product **124** to **130a**; secondly the number of steps is too high; thirdly this methodology led to non compatible protection group (TBDPS, acetonide, OBn) of **130α**; fourthly the poor selectivity in the glycosidation reaction step could not be improved.

All the unsatisfactory results forced us to come back to the Zhu studies during which the 2-amino-2-deoxy-D-galactal moiety was obtained under the protected form of a *N*-benzyl-*O*-(benzyloxy)amino group. We also chose to use the silyl ether protected enone **97a** instead of the benzyl protected derivative **97b**, as the silyl group can be exchanged for acetates more readily than benzyl ethers.

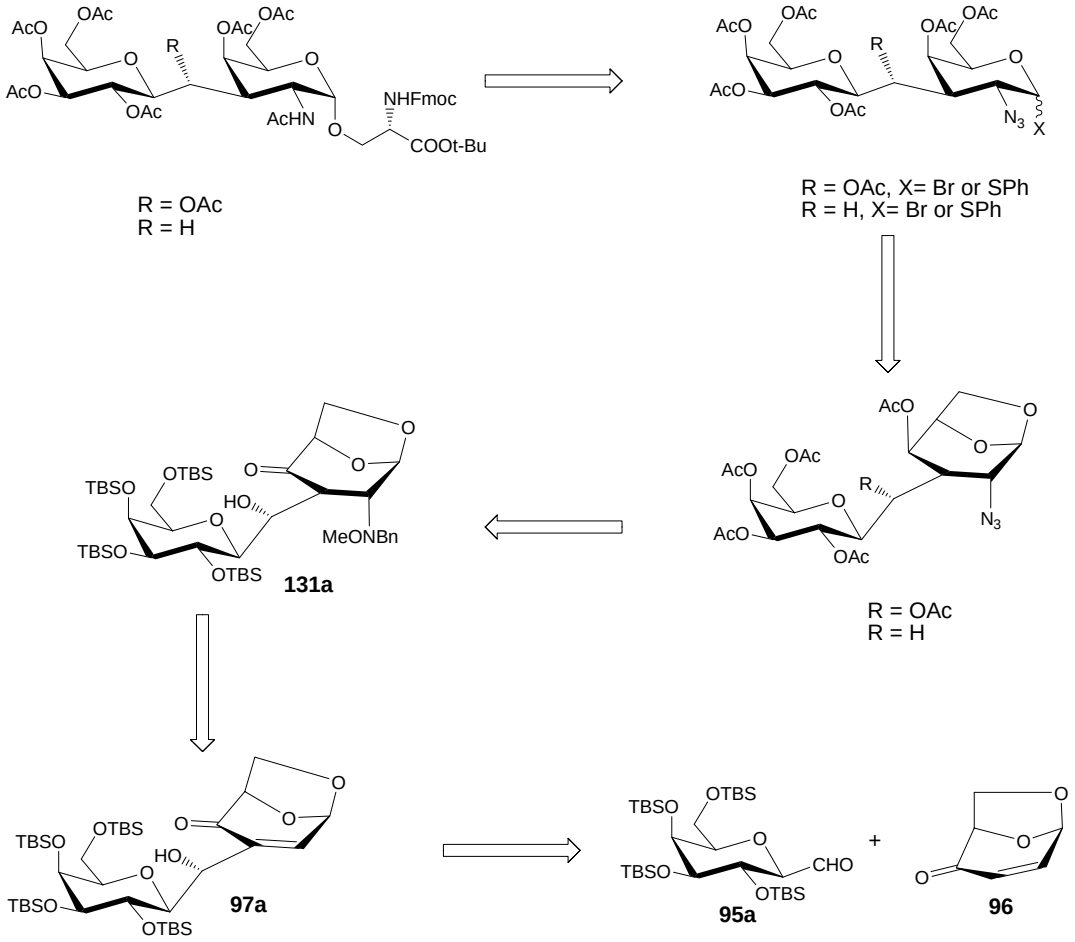
The amine was changed for MeONHBn since this amine, less bulky than the BnONHBn, was expected to improve the yield of the 1,4-addition step. The opening of the 1,6 anhydrosugar unit should be done under adequate conditions to realize α-galactosides with good selectivity. This might require to exchange the MeONBn substitutent for an azide group before the opening of the 1,6 anhydrosugar. A peracetylated intermediate is required since we need to generate clusters of the C-disaccharide. The clustering step required stable protective groups that can be cleaved readely in the last step. At this stage, acetates looked to be the suitable protective groups.

The synthesis start with a *Baylis-Hillmann* type of condensation between the persilylated carbaldehyde **95a** and isolevoglucosenone (**96**) induced with a dialkylaluminium salt. Then we shall use MeONHBn as amine in a 1,4-addition to enone **97a**, hopefully getting better yield than when we using BnONHBn. Subsequent reduction of the adduct into the corresponding alcohol will then be followed by the transformation of the alcohol into a suitable glycosyl donor. Glycosidation of a semi-protected serine will then be carried out.

---

[148] Fung, P. Y. S.; Madej, Koganty, M. R.; Longenecker, B. M. *Cancer Res.* **1990**, *50*, 4308.

[149] MacLean, G. D.; Boyen-Yacyshyn, M. B.; Samuel, J.; Meikle, A.; Stuart,, G.; Nation, J.; Poppema, S.; Jerry, M.; Koganty, R.; Wong, T.; B. M. Longenecker, B. M. *J. Immunother.* **1992**, *11*, 292.



Scheme 3.1: Retro-synthetic analysis toward a TF-antigen analogue

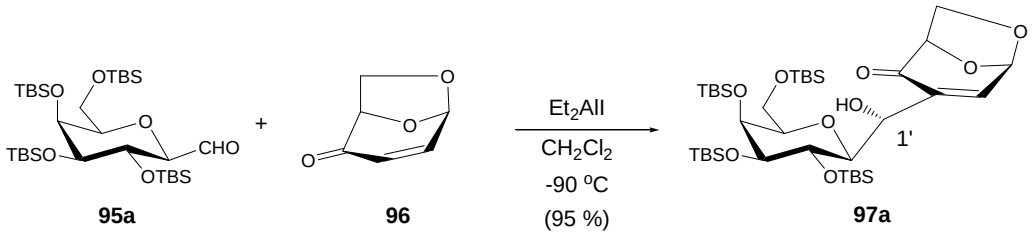
4 RESULTS AND DISCUSSION

4.1 The First Synthesis of C-Disaccharide Analogues of the Thomsen-Friedenreich (TF) Antigen

4.1.1 The Synthesis of a Hydroxymethano-Linked *C*-disaccharide

After all the failures encountered by Zhu and Demange in our group with the route making use of galactal intermediates, we decided to develop the route based on the 1,4-addition of a suitable amine to enone **97a** (See section 2.4.1).

For the synthesis of **97a**, we envisaged to apply the chemistry developed by Zhu which uses isolevoglucosenone (**96**) and aldehyde **95a.** Under previous conditions [127] (0.1 mmol of **95a**, -78 ^{o}C) the yield of the condensation of aldehyde **95a** and isolevoglucosenone **96** was 61%. On scaling up and using high concentration, and on controlling the slow addition of Et_2AlI at -90 ^{o}C, conditions were formed under which **97a** was isolated as a single diasterioisomer in 95 % yield (Scheme 4.2).



Scheme 4.2: *Oshima-Nozaki* coupling reaction.

Table 4.1: Conditions and yield in the coupling reaction

	mmole	**Conc.**	**Temperature**	**yield**
Zhu	0.1 mmole	0.08 M	-78 ^{o}C	61 %
This work	9.4	0.25	-90 ^{o}C	95 %

In the condensation reaction between isolevoglucosenone **(96)** and carbaldehyde **95a** in the presence of Et_2AlI, the *Zimmerman-Traxler* model explains the configuration of C(1') of **97a** (Figure 3).[150]

[150] Zimmerman, H. E.; Traxler, M. D. *J. Am. Chem. Soc.* **1957**, *79*, 1920.

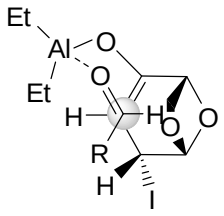


Figure 4.3: Representation of the closed transition state adopted by the aluminium enolate and aldehyde (minimization of steric effects).

For the conjugate addition of amines to enone **97a**, we have chosen hydroxylamine derivatives. Because of the α-effect, their nucleophilicities are much higher than those of similar amines.[151] Initially, a Lewis acid such as Me_2AlCl was used to activate the enone. Unfortunately no reaction occurred when MeONHBn was added to a mixture of **97a** and Me_2AlCl between -78 °C and 0 °C. In fact, we found that a Lewis acid is not necessary for this addition. The best results were obtained using no solvent and no catalyst (Table 4.2).

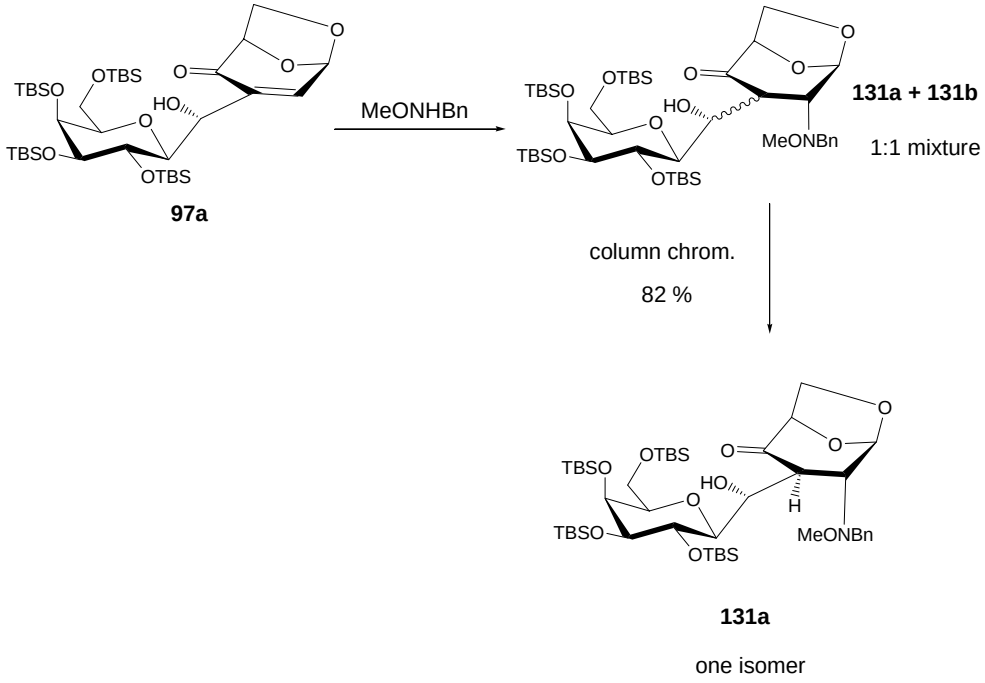
This is a manifestation of the mass law effect for equilibrium **97a**+ MeONHBn $\rightleftharpoons$ **131.**

Table 4.2: Conjugated addition of methoxybenzylamine to enone **97a**

entry	catalyst	solvent	temperature	Yield[152] 131a
1	Me_2AlCl	CH_2Cl_2	-78 → 0 °C	No reaction
2	Me_2AlCl	CH_2Cl_2	25 °C	20 %
3	—	CH_2Cl_2	25 °C	18 %
4	—	No solvent	25 °C	82 %

It was discovered by Dr. Jens Riedner that MeONHBn and enone **97a** give a 1:1 mixture of stereomeric adducts **131a** and **131b** that are isomerized during their slow chromatography on silica gel at room temperature. Thus conditions were found under which adduct **131a** could be obtained pure in 82% yield (Scheme 4.3).

[151] a) Dixon, J. E.; Brucine, T. C. *J. Am. Chem. Soc.* **1971**, *93*, 6592. b) Heo, C. K. M.; Bunting, J. W. *J. Chem. Soc., Perkin Trans* **1994**, *2*, 2279. c) Bunting, J. W.; Mason, J. M.; Heo, C. K. M. *Perkin Trans* **1994**, *2*, 2291.
[152] This value present the isolated yield



Scheme 4.3: Conjugation addition of methoxybenzylamine to enone **97a**.

When pure **131a** was dissolved in THF with MeONHBn, an 8:1 mixture of **131a** and **131b** was obtained upon standing at 25 ^{o}C for 2-3 hours. Fast column chromatography on silica gel of 1:1 mixture of **131a** and **131b** gave the first fraction containing mixture of **131a** and **131b** (major), and a second fraction of pure **131a** (minor). Slow elution led to smaller fraction of **131a** and **131b** and increased amounts of pure **131a** (second fraction). This demonstrated that **131a** and **131b** are in equilibrium in the presence of an amine and that the equilibrium shifts in favor of **131a** when adsorbed on silica gel. Thus a slower elution allows the conversion of absorbed **131b** into absorbed **131a** which is finally recovered pure from the column (Figure 4.4). The structure was confirmed in this stage by the structure of compound **132** obtained after the reduction of the ketone moiety.

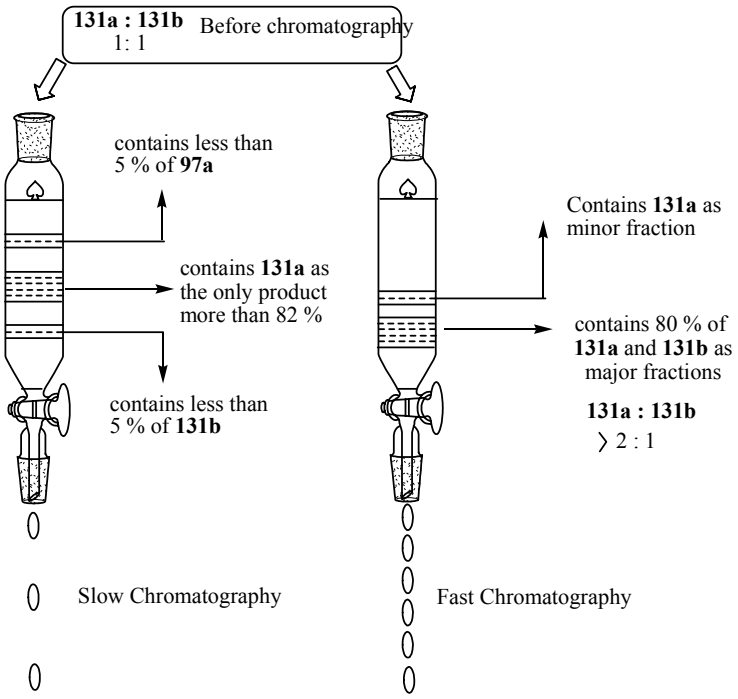
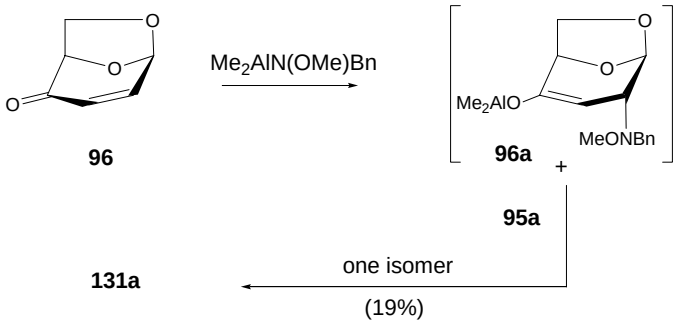


Figure 4.4: Isomerization of **131b** to **131a** during flash chromatograph.

The mechanism of isomerization **131a** $\rightleftharpoons$ **131b** is proposed to involve reversible E_{1cb}-like eliminations **131a** $\rightleftharpoons$ **97a** + MeONHBn and **131b** $\rightleftharpoons$ **97a** + MeONHBn, both catalyzed by MeONHBn.

In the preliminary work of Zhu[153] he had shown that the reaction of isolevoglucosenone **96** with Me_2AlNBn_2 gave an aluminium enolate that could be reacted directly with sugar-derived aldehydes giving, in the same pot, the corresponding 2-amino-2-deoxy-3-C-linked disaccharide derivatives. We thus attempted to obtain **131a** in a similar fashion by reacting isolevoglucosenone **96** with $Me_2AlN(OMe)Bn$, followed by the addition of aldehyde **95a**. Based on related Oshima-Nozaki reactions with **96**, we expected (Zimmerman-Traxler, steric factors)[150] that the preferred double adduct should be **131a** under conditions of kinetic control (Scheme 4.4). After several unfruitful assays we found that the reaction of **96** and $Me_2AlN(OMe)Bn$ in THF at -78 ^oC gives the expected enolate **96a**. After the addition of **95a** to this solution, a slow reaction occurred at -78 ^oC. Subsequent aqueous work-up and purification by chromatography on silica gel gave **131a** in 19 % yield only and the unreacted aldehyde was recovered (Scheme 4.4). Attempts to run the reaction at higher temperature and/or for longer time led to decomposition only.

[153] Zhu, Y. -H.; Demange, R.; Vogel, P. *Tetrahedron: Asymmetry* **2000**, *11*, 263.



Scheme 4.4: One pot double addition

Reduction of ketone **131a** with $LiBH_4$ furnished diol **132** in 90 % yield. Hydride addition to the *exo* face of the bicyclo[3.2.1]octenone system is preferred for steric reasons. When the reduction reaction was run at -78 °C we observed C-3 epimerization. However when run at 0 °C this reduction gave the glucose precursor product. The best way to do this reduction is to add $LiBH_4$ at -78 °C, then let the temperature raise to -30 °C within less than 5 min. This gave product **132** as unique product.

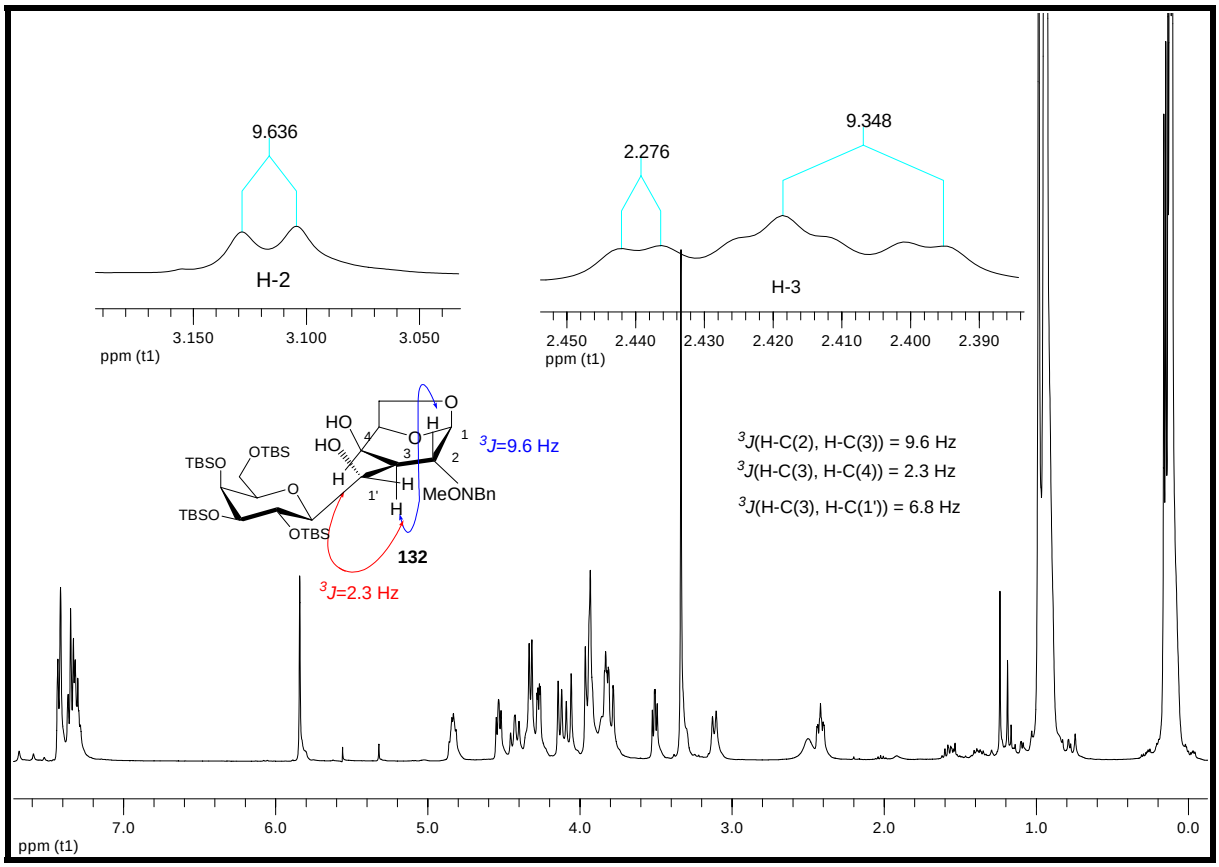
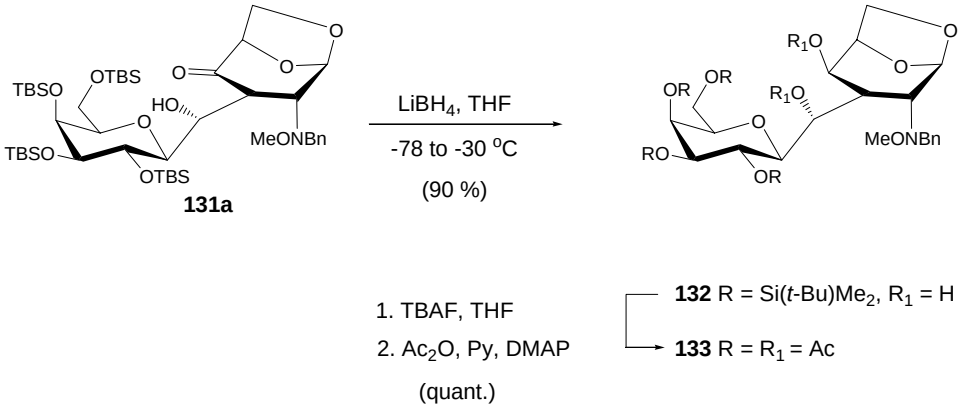


Figure 4.5: the H-NMR (400 MHz) spectrum of diol **132**.

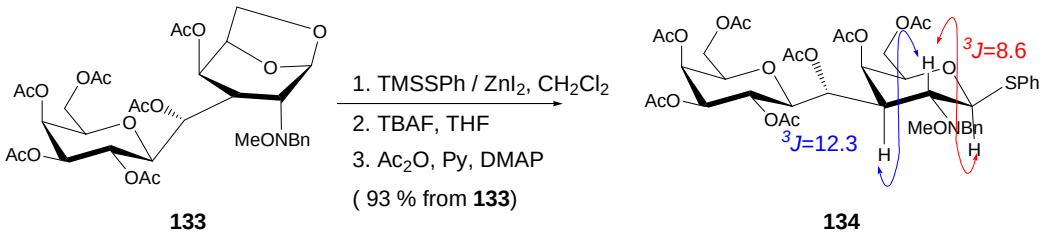
The cross-peaks of the 2-D-NOESY ^{1}HNMR spectrum and the coupling constants of bicyclo[3.2.1]octanol **132** show ^3J(H-2, H-3)= 9.6 Hz, ^3J(H-3, H-1')= 6.8 Hz and ^3J(H-3, H-4) =2.3 Hz which is expected for the galactose configuration.

Treatment of **132** with Bu_4NF (TBAF) in THF (20 °C, 3h) and then with Ac_2O/pyridine and a catalytic amount of 4-dimethylaminopyridine (DMAP) provided the peracetate **133** quantitatively (Scheme 4.5).



Scheme 4.5: Reduction of ketone **131a**

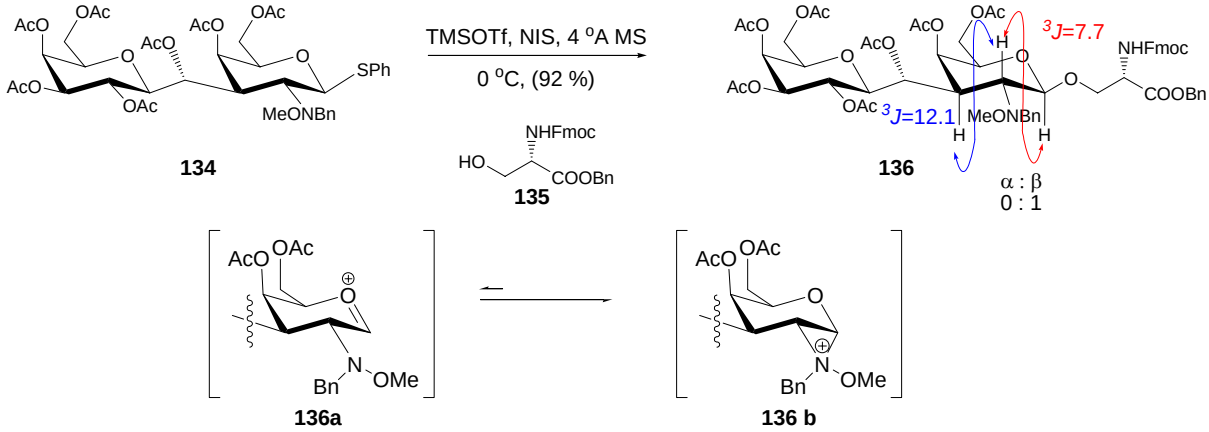
Ring opening of the 1,6-anhydrogalactose moiety of **133** was done using ZnI_2 in CH_2Cl_2.[154] Thus, the crude product so-obtained was not isolated but immediately desilylated (TBAF, THF) and peracetylated under standard conditions to produce thiogalactopyranoside **134** in 93 % yield (Scheme 4.6).



Scheme 4.6: Synthesis of the thio-glycosyl **134**.

[154] Sakairi, N.; Hayashida, M.; Kuzuhara, H. *Tetrahedron Lett.* **1987**, *28*, 2871.

Glycosidation of Fmoc-Ser-OBn **135** with **134** under the conditions of Imamura et al.[155] led exclusively to the β-D-galactoside **136** (92 % yield). The structure of **144** was deduced from its ^{1}H-NMR spectrum which showed ^{3}J(H-1, H-2)= 7.4 Hz, and ^{3}J(H-2, H-3)=12.3 Hz (see Figure 4.6). The high β selectivity during the glycosidation reaction seems to arise from the participation of the amine. Thus, participation of the 2-(N-benzyl-N-methoxy)amino group cannot be avoided (Scheme 4.7).



Scheme 4.7: Imamura glycosidation of **134**

[155] Imamura, A.; Ando, H.; Korogi,S.; Tanabe, G.; Muraoka, O.; Ishida, H.; Kiso, M.*Tetrahedron Lett.* **2003**, *44*, 6725.

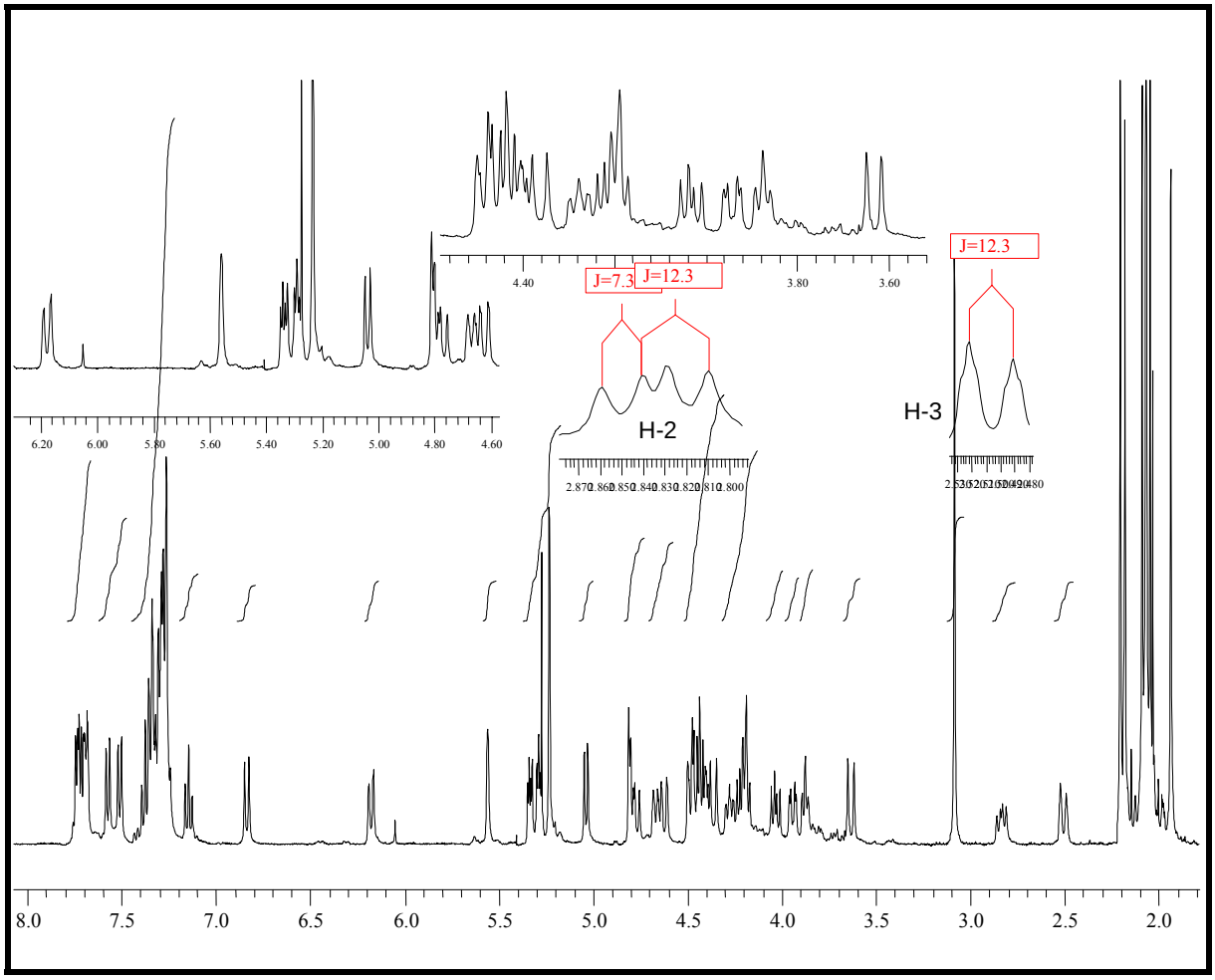
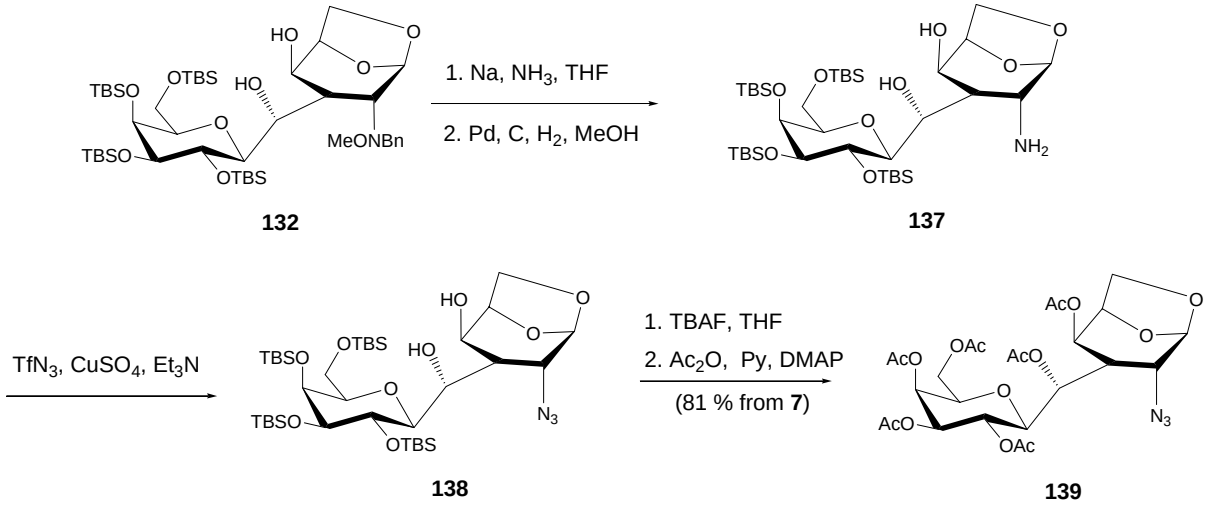


Figure 4.6: the ^{1}H-NMR(400 MHz) spectrum of **136β**.

The bulky and participating 2-BnNOMe subsistituent prohibited the α-glycosidation. Therefore, a non-participating and non-bulky group at C-2 that can be readily converted into a 2-acetamido moiety. Inspired by the work of Wong and co-workers,[156] we converted the *N*-benzyl-*N*-methoxyamino group of **132** into the corresponding primary amine **137**. Under Birch's conditions[157] the methoxy amine group was cleaved and subsequent catalytic (Pd/C, H_2) hydrogenation cleaved the benzylamine group (Scheme 5).[158] The amine **137** so-obtained was not isolated but directly treated with trifluromethanesulfonyl azide, cupric sulfate, and triethylamine in a mixture of H_2O / MeOH / CH_2Cl_2. Desilylation of **138** followed by acetylation provided azide **139** that was isolated in 81 % yield (based on **132**).

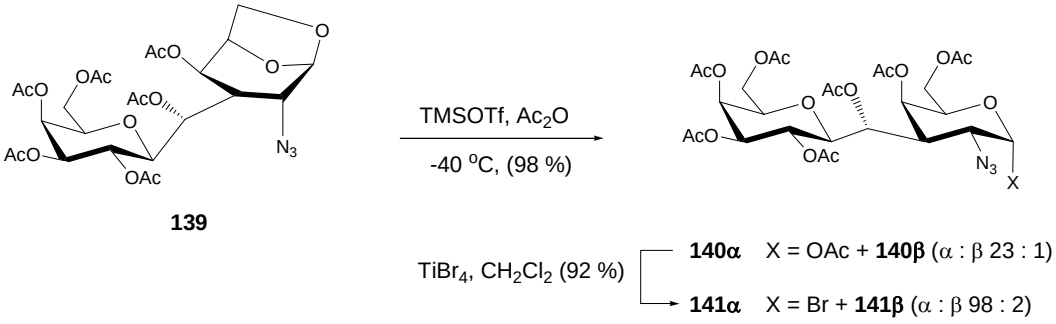
[156] Nyffeler, P. T.; Liang, C.-H.; Koeller, K. M.; Wong, C.-H. *J. Am. Chem. Soc.* **2002**, *124*, 10773.
[157] Jr. Dryden, H. L.; Webber, G. M.; Burtner, R. R.; Cella, J. A. *J. Org. Chem.* **1961**, *26*, 3237.
[158] Direct azidation of enone **97b** failed. See section (4.1.3.1) for more details see R. Demange, Ph. D. Thesis, EPFL, 2002.



132 **137** **138** **139**

Scheme 4.7: Transformation of amine into azide.

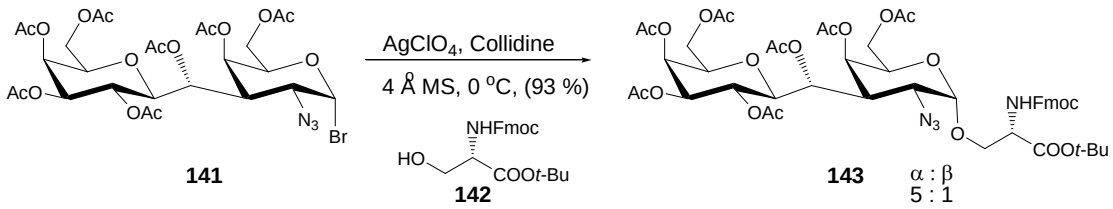
Smooth ring opening of the 1,6-anhydrogalactose moiety of **139** using TMSOTf in Ac_2O gave **140** in 98 % yield and 23:1 α/β selectivity. Conversion of galactosyl acetate **140** into the corresponding bromide **141** (92 %) was done with $TiBr_4$ in CH_2Cl_2 (25 ^oC, 12h) (Scheme 4.8).



139

Scheme 4.8: synthesis of glycosyl bromide.

Königs-Knorr glycosidation ($AgClO_4$ / CH_2Cl_2 , 2,4,6-collidine, 4 Å molecular sieves)[159] of N-Fmoc-serine *tert*-butylester **142** gave a 5:1 mixture of α-**143α** and β-galactoside **143β** in 93 % yield. Flash chromatography does not provided pure **143α**. The preferred formation of the α-galactoside is ascribed to a kinetic anomeric effect (no participating group at C-2) (Scheme 4.9).[160]



141 **142** **143** α : β 5 : 1

Scheme 4.9: Synthesis of a C-disaccharide analogue of TF-antigen precursor.

[159] a)Paulsen, H.; Adermann, K. *Liebigs Ann. Chem.* **1989**, *8*, 751; b) Rothman, D. M.; Vazquez, M. E.; Vogel, E. M.; Imperiali, B. *J. Org. Chem.* **2003**, *68*, 6795.

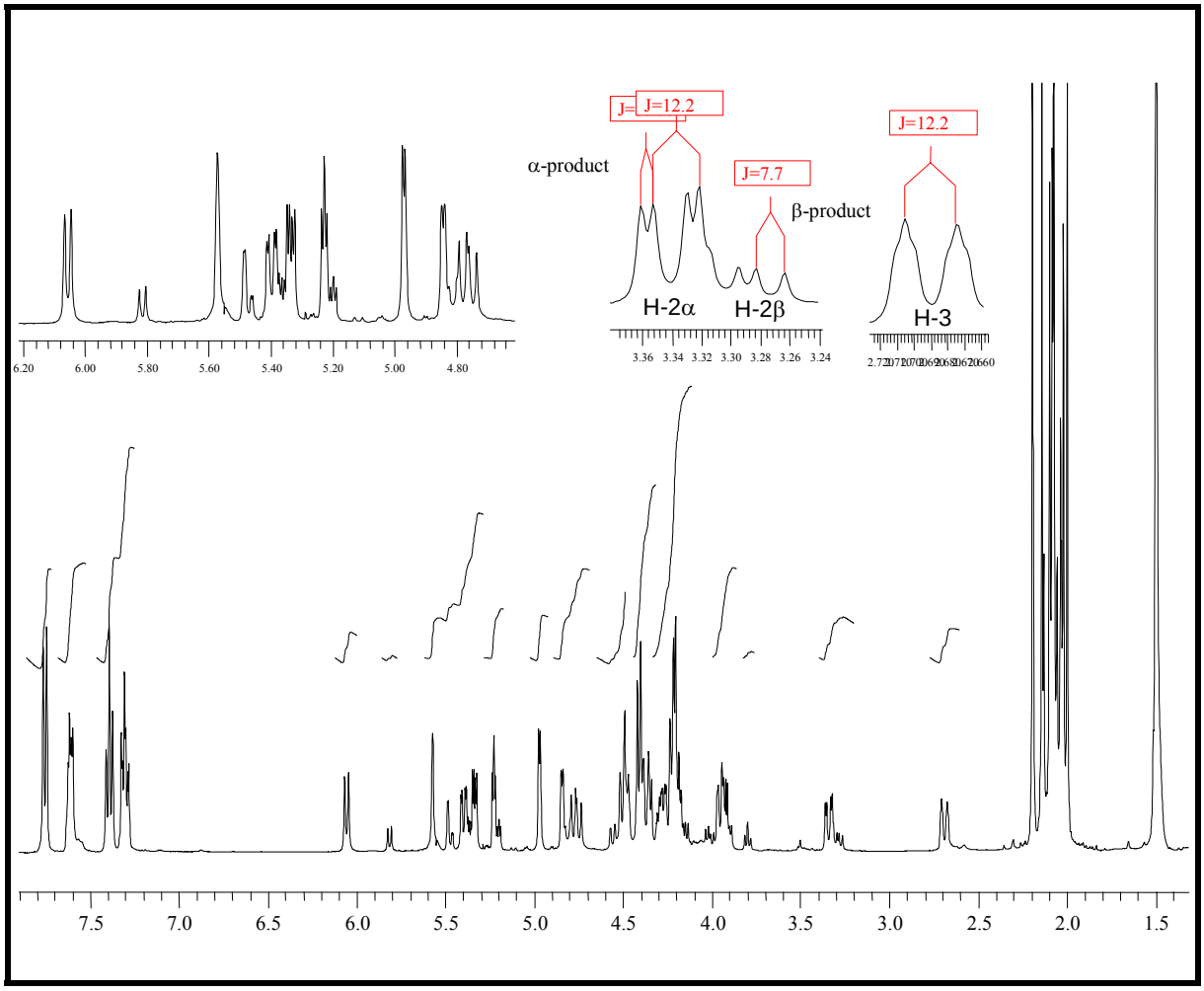
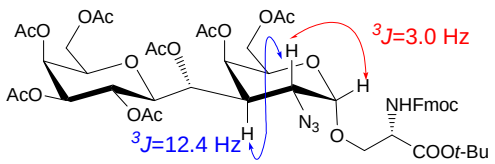


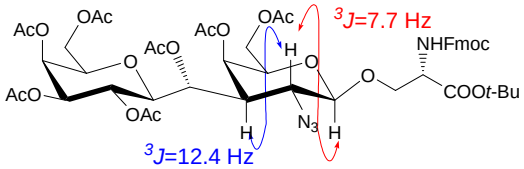
Figure 4.7: The 400 MHz ^{1}H-NMR spectra of **143α,β** mixture.

The structure of **143** was confirmed by its ^{1}H-NMR spectrum. For the major anomer **143α** ^{3}J(H-1, H-2) =3.0 Hz and ^{3}J(H-2, H-3) =12.2 Hz were measured, what is typical for α galactopyranosides. For the minor product ^{3}J(H-1, H-2) =7.7 Hz and ^{3}J(H-2, H-3) =12.2 Hz were found, what is typical for β-galactosides (Figures 4.7, 4.8).



143 α

Typical α galactose



143 β

Typical β galactose

Figure 4.8: ^{1}H-NMR Analysis of α and β glycosides of **143**.

[160] a) Maura, A.; Shun, L. K. S.; Gauffeny, F.; Sinay, P. Synlett **1990**, 445. b) Maura, A.; Gauffeny, F.; Sinay, P. *Tetrahedron* **1991**, *47*, 5149. c) Cheshev, P. E.; Kononov, L. O.; Tsuekkov, Yu. E.; Shashov, A. S.; Nifantiev, N. E. *Russ. J. Bioorg. Chem.* **2002**, *28*, 419.

By *MALDI-HRMS* the molecular weight of **143** was confirmed (Figure 4.9). The purity of the mixture of **143** was confirmed as well by elemental analysis (for more details see the experimental part).

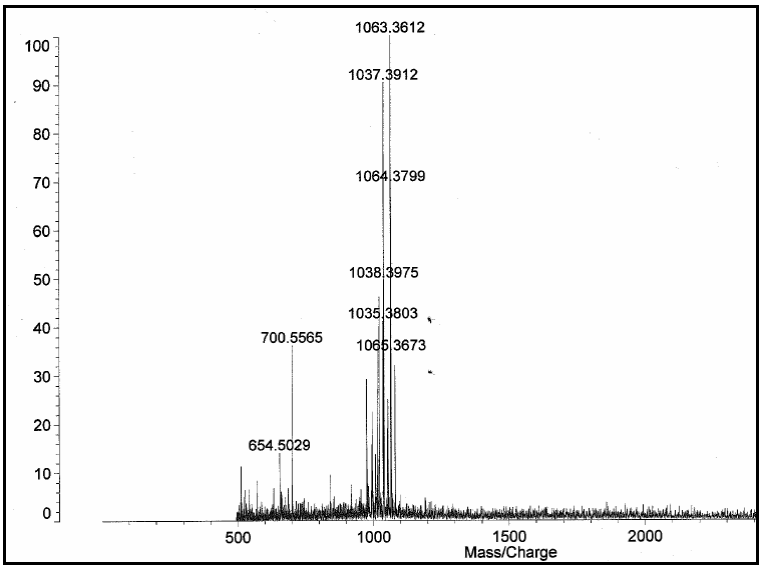
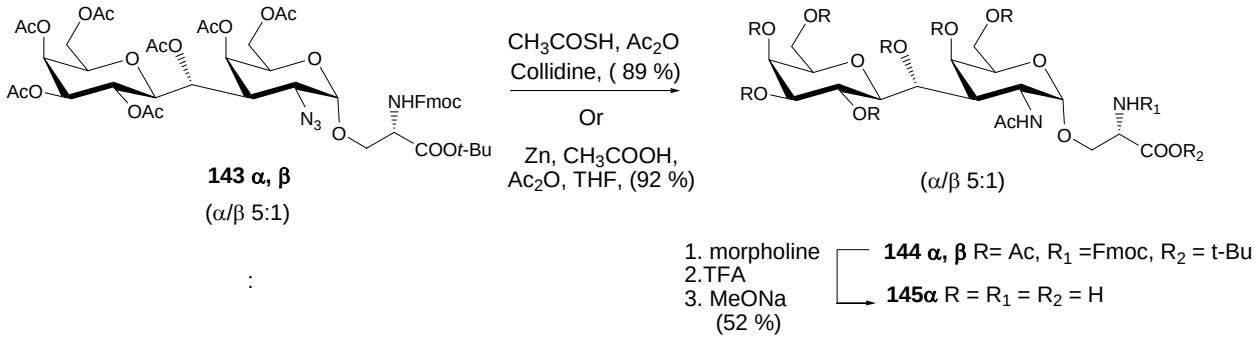


Figure 4.9: *MALDI-HRMS* Analysis of **143**.

Treatment of a 5:1 mixture of **143α** and **143β** with thioacetic acid, collidine and Ac_2O[161] furnished a 5:1 mixture of **144α** and **144β** in 89 % yield. When **143α** and **144β** were treated with zinc AcOH, THF, and Ac_2O the yield climbed up to 92 %. Compound **144α** is a key intermediate of a protected *C*-linked analogue of TF epitope α-O-conjugated to L-serine. It should be suitable for the construction of clusters via peptide synthesis and further conjugation to immunogenic proteins. We have verified that disaccharide **144α** and **144β** can be fully deprotected (Scheme 4.10). When **144α** and **144β** were treated with morpholine in DMF followed by CF_3COOH, and then by MeONa/MeOH, disaccharide **145α** was obtained in 52 % yield after preparative HPLC purification.

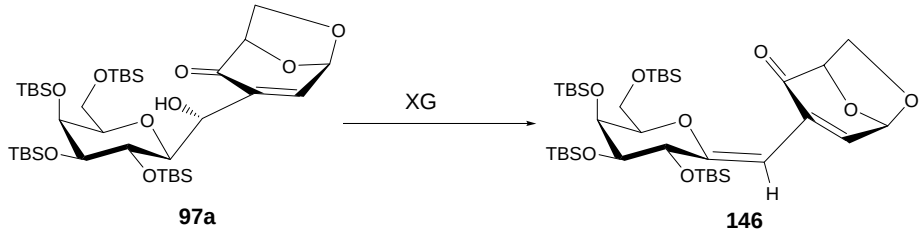


Scheme 4.10: Synthesis of fully protected and deprotected *C*-analogue of TF-antigen.

[161] a) Macindoe, W. M.; Nakahara, Y.; Ogawa, T. *Carbohydr. Res.* **1995**, *271*, 207; b) Kuberan, B.; Sikkander, S. A.; Tomiyama, H.; Linhardi, R. *Angew. Chem. Int. Ed.* **2003**, *42*, 2073.

4.1.2 The Synthesis of a *C*-Disaccharide analogue of the Thomsen-Friedenreich (TF) Antigen Containing a Methano Linker

Attempts to introduce suitable radical leaving group in position C(1') in order to deoxygenate the alcohol under radical conditions all failed. Only degradation products or dienone **146** were obtained (Table 3). The geometric configuration of the double bond present in product **146** has not been established unambiguously.



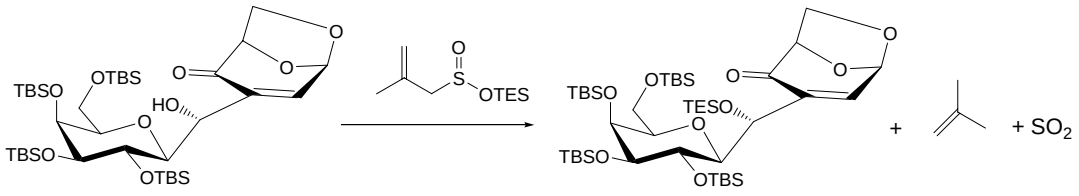
97a **146**

Scheme 4.11: Thioesterification Attempts

Table 4.3: attempt to introduce radical leaving group

entry	solvent	XG	Temperature	yield
1	CH_3CN	$CS(Im)_2$	70 °C	78 % of **146**
2	Pyridine	ClOSCOPh	0 °C	25 % of **146**
3	THF	S_2C, then MeI	0 to 25°C	10 % of **146**
4	Pyridine	TfCl	0 °C	degradation

So far the unique reaction which allowed us to protect the hydroxyl group at C(1') has been Vogel's *triethylsilyl 2-methylprop-2-ene-1-sulfinate*. The latter reagent is a very active silylation agent under neutral and mild conditions (Scheme 4.18).[162] Unfortunately silyl ether **147** was not useful for our synthesis because the selective cleavage of triethylsilylether in later steps could be difficult in the presence of four (t-butyl)dimethylsilylether moieties.



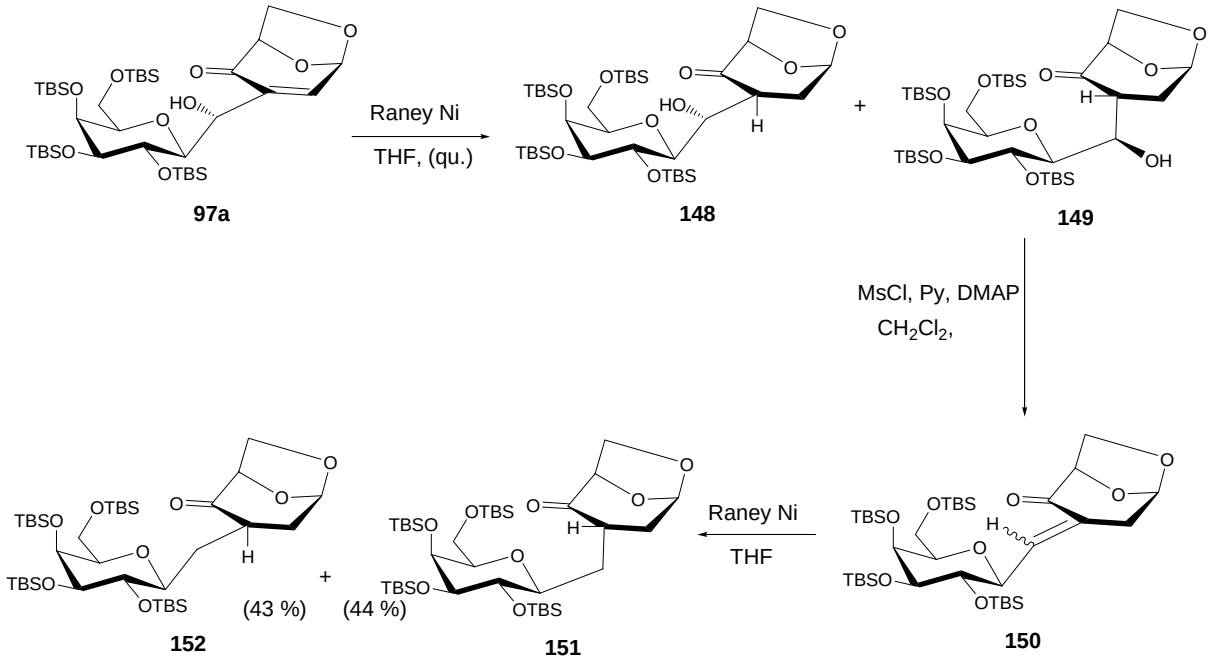
97a **147**

[162] Huang, X.; Craita, C.; Awad , L.; Vogel, P. *Chem. Commun.* **2005**, *10*, 1297.

Scheme 4.12: Silylation of alcohol at C(1')

4.1.2.1 Hydrogenation of enones

In the presence of Raney nickel in THF, the TBS-protected *C*-galactoside **97a** was reduced quantitatively into a mixture of aldols from which **148** and **149** were isolated (flash chromatography) in 57% and 43% yield, respectively. When the crude mixture of **148** and **149** was treated with CH_3SO_2Cl/pyridine and a catalytical amount of DMAP, this led to the formation of a mixture of unstable enones **150**. The latter were not isolated but treated directly with Raney nickel in THF at 25 ^oC. This produced a mixture of ketones from which pure **151** and **152** were isolated in 44% and 43% yield, respectively. Attempts to isomerize **151** into **152** failed applying different isomerization conditions (Et_3N, DBU, KOH, KO*t*-Bu) (Scheme 4.13).

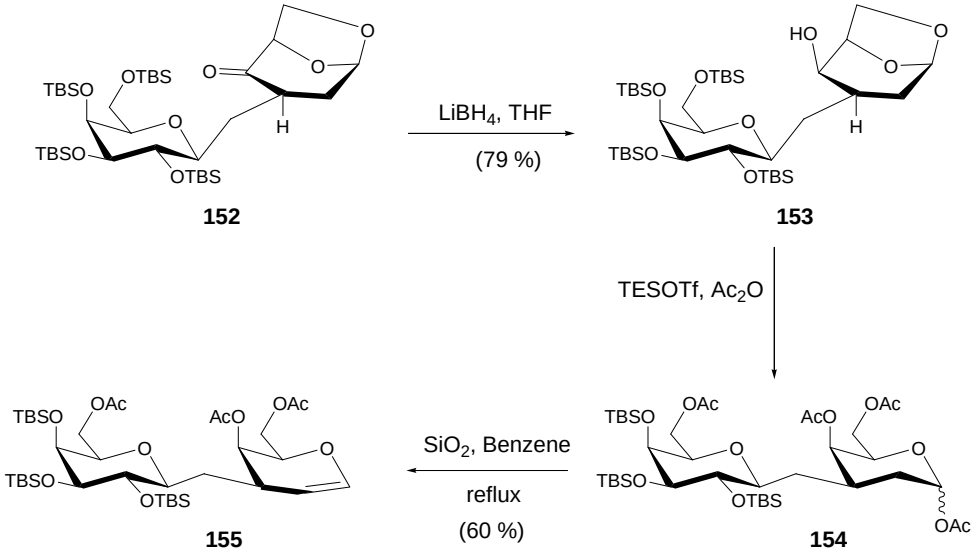


Scheme 4.13: Reduction / elimination / reduction / sequence.

4.1.2.2 Azidonitration of galactal derivatives

Reduction of **152** with $LiBH_4$ in THF gave alcohol **153** in 79% yield, the structure of which (1,6-anhydro-2-deoxy-D-lyxo configuration) was given by its ^{1}H-NMR and the cross-peaks of 2-D-NOESY ^{1}H-NMR spectrum. Treatment of **153** with TESOTf and acetic anhydride lead to the ring opening of 1,6-anhydro moiety and acetylated D-lyxose derivative **154** was obtained without formation of furanosides. The mixture of α- and β-pyranosides **154** thus obtained, was not purified

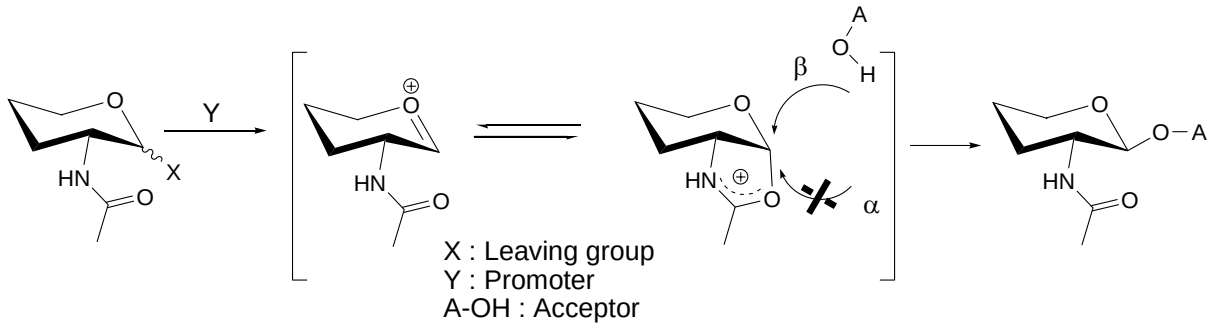
but directly treated with silica gel in benzene heated under reflux. This produced the C-linked analogue of β-D-galactopyranosyl-(1→3)-D-galactal **155** in 60% yield (Scheme 4.14).



Scheme 4.14: Synthesis of galactal **155**

One of the method for the introduction of azido functionality at C-2 is the conversion of glycals into 2-azido-2-deoxyglycosyl nitrates functioning as 2-amino-2-deoxy glycoside precursors. This conversion is commonly effected by the azidonitration reaction developed by Lemieux and Ratcliffe in 1979.[131]

Azides can be considered as masked amino groups and can thus serve as *N* protection in a sense. As non participating groups, they also allow the synthesis of 2-amino-2-deoxy-α-D-glycosides. This is not the case with 2-*N*-acetyl-2-deoxy derivatives and analogues for which β-glycosides are exclusively formed due to the neighbouring group participation at C-2 as shown in scheme 4.15.

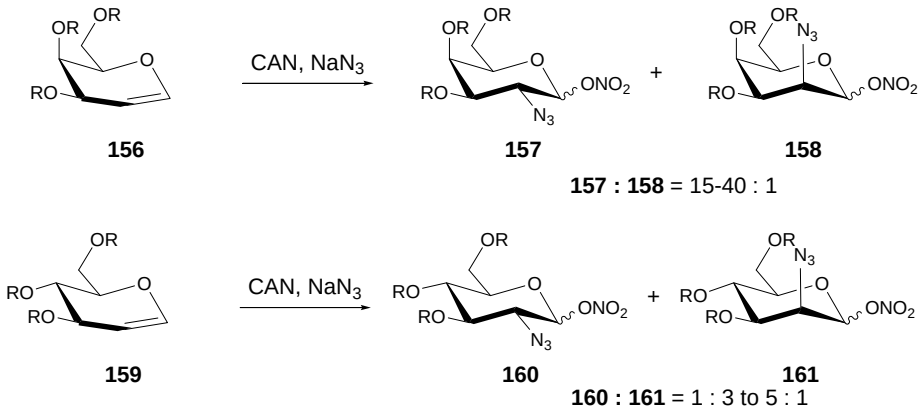


Scheme 4.15: Neighbouring group participation at C-2: complete control over anomeric configuration.

Schmidt has reported that reaction of protected D-galactal **156** with ceric ammonium nitrate (CAN) and sodium azide results in the formation of a mixture of anomers of the desired 2-azido-2-deoxy-D-galactose derivative **157** as well as varying amounts of D-talose side product **158** containing the axial

2-azido moiety (Scheme 4.16). Stereoselectivity of this useful transformation was found to be greatly dependent upon the steric hindrance. In the case of galactal **156**, the axial C-4 substituent guarantees good selectivity for the installation of an equatorial C-2 azido moiety, but selectivities vary considerably depending upon the protecting group (15-40:1 of **157** (D-galacto) to **158** (D-talo)).[131]

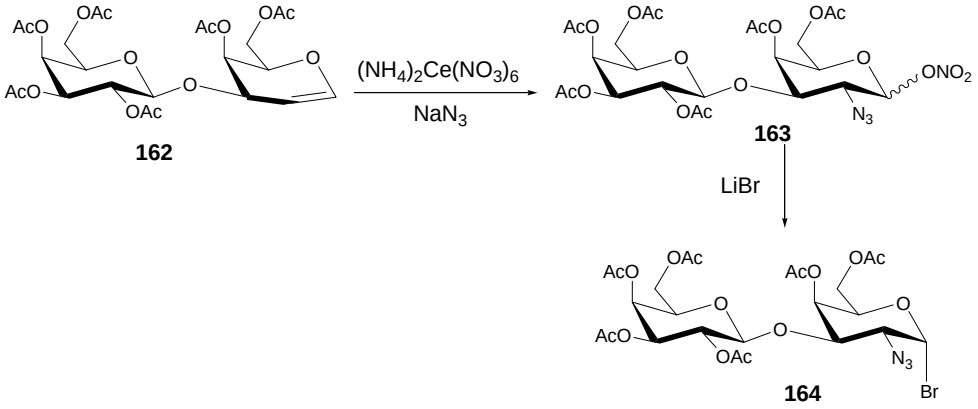
In the case of D-glucal **159**, which carries an equatorial C-4 substituent, unpredictable ratios of products containing an equatorial or an axial C-2 azido group have been obtained. Selectivities ranging from 3:1 in favour of the manno-derivative **161** to a 5:1 ratio in favor of gluco-derivative **160** have been reported.[163]



Scheme 4.16: Selectivity of the azidonitration reaction depending on glycols (Schmidt and co-workers).

The anomeric nitrate group can later be readily converted into a hydroxyl, halide or acetyl functionalty and thus gives access to a variety of glycosyl donors containing a non-participating 2-azido group.

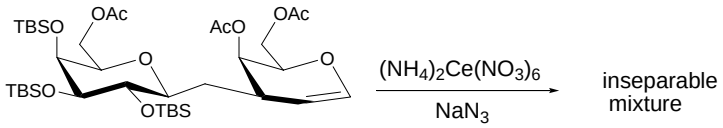
Wong[164] and Cantacuzene[165] reported that azidonitration of peracetylated Gal-β-(1→3)-galactal **162** afforded exclusively the galacto-product **163**. Bromination of nitrate **163** by LiBr provided bromide **164** in 67% overall yield (Scheme 4.17).



Scheme 4.17: Azidonitration of Gal-β-(1→3)-Galactal **162** by Wong and Co-worker.

[163] Schmidt, R. R.; Rembold, H. *Carbohydr. Res.* **1993**, *246*, 137. Kinzy, W.; Löhr, A. *Carbohydr. Res.* **1993**, *245*, 193.
[164] Look, G. C.; Ichikawa, Y.; Shen, G.-J.; Cheng, P.-W.; Wong, C. H. *J. Org. Chem.* **1993**, *58*, 4326.
[165] Bay, S.; Berthier-Vergnes, O.; Cantacuzene, D. *Carbohydr. Res.* **1997**, *298*, 153.

With Gal-β-C(1→3)-galactal **155** in our hands, the reaction of azidonitration can be achieved. Surprisingly, in our case, inseparable complex mixture was obtained (Scheme 4.18); one can explain this because of the steric effect of TBS groups in azidonitration reaction, and concurrent formation of D-talopyranoside, and D-galactopyranoside. The difficulty to reach galactal **155** in sufficient quantity led us to abandon this route.



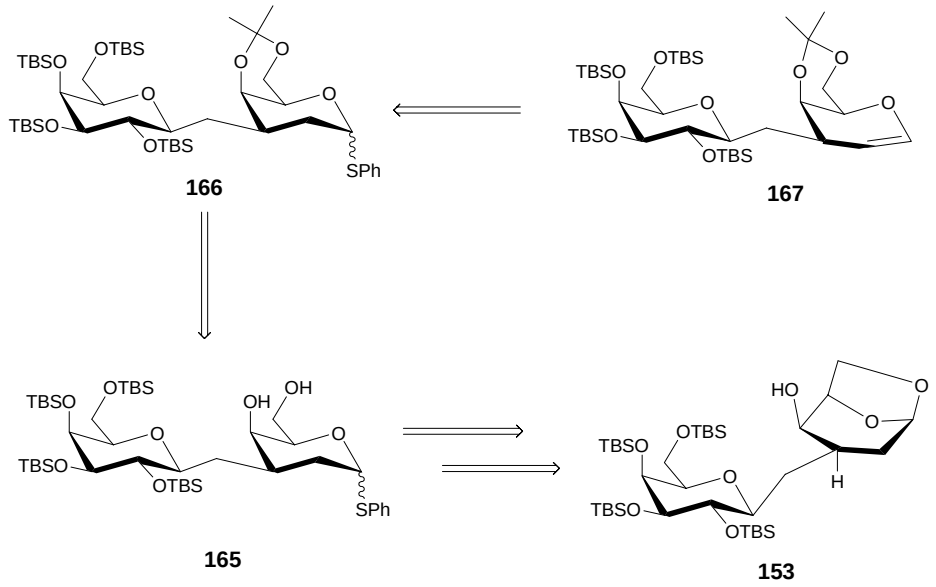
155

Scheme 4.18: Azidonitration of Gal-β-(1→3)-Galactal **155**.

This unexpected result can be attributed to the conformation of the galactal moiety. To over-come this problem, 4,6 diol protection of the galactal part was thought necessary in order to give some rigidity to the galactal moiety and thus favour the equatorial attack of the azide radical.

Thus, we returned to the azidonitration reaction to construct the α-glycosidic bond of 2-azido-2-deoxy-D-galactopyranoside keeping in mind that steric effects and the conformation of galactal are of great influence on the selectivity of this reaction.[137] Indeed, the unexpected mixture obtained in the former experiment of azidonitration of Gal-β-C(1→3)-galactal **155** suggested that the selectivity could be obtained by fixing the structure of **155** by 4,6 diol protection group.

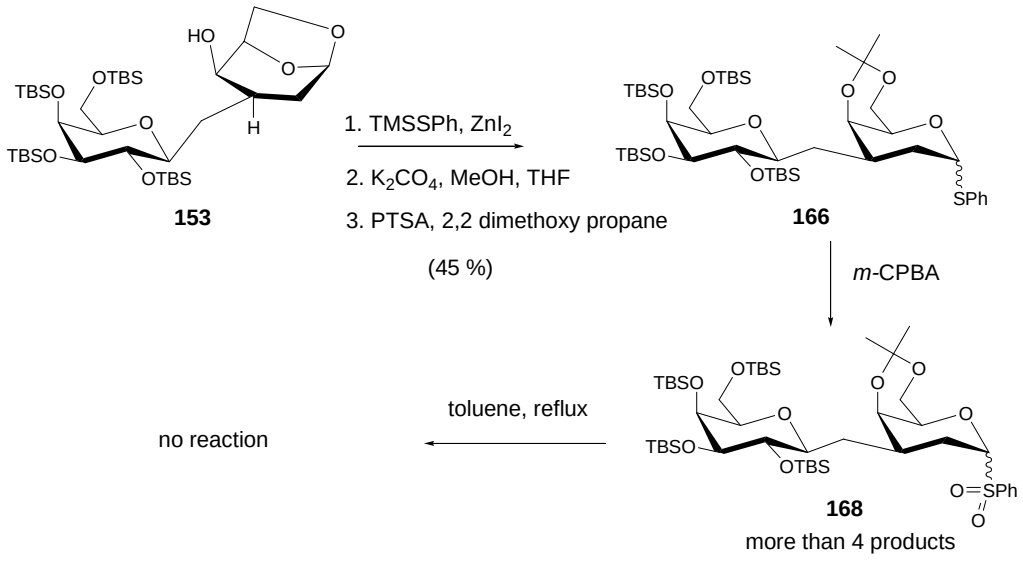
A sterically less-hindered α-face would favour the attack on the galactal onto the bottom face, thus leading to the desired equatorial azide. To this end, the use of conformationally constrained galactal should be adequate. Molecular modelling of structure containing a 4,6-*O*-isopropylidene acetal suggested that the top face was significantly more crowded that the bottom face of the molecule. These remarks prompted us to elaborate the following retrosynthetic scheme (Scheme 4.19).



Scheme 4.19: Retrosynthetic scheme of a galactal **167**

We thus treated **153** with trimethylthiophenylsilane (TMSSPh) in dichloromethane in the presence of ZnI_2. Further desilylation at C(6) and acetonide formation provided a α,β mixture of **166**, in a poor yield.[166]

The thioglycosides **166** were converted into the corresponding sulfone donors **168** by oxidation with *m*-CPBA. Then the mixture of **168** was dissolved in toluene and heated under reflux but unfortunately we did not obtain the desired galactal **167** (scheme 4.20).[167]



Scheme 4.20: Approach toward the synthesis of galactal **167**

[166] Roth, W.; Pigman, W. *Meth. Carbohydr. Chem.* **1963**, *2*, 405.
[167] The mixture of **166** shown in Scheme 4.20 could not be separated by chromatography.

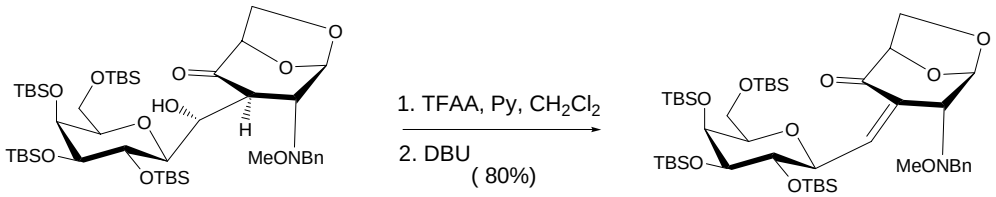
The failure of the later elimination must be put onto the account of conformational constraints that make the syn elimination too difficult.

4.1.2.3 Deoxygenation of –CH(OH)- Linker by an Addition-Elimination-Reduction Sequence.

We have seen in Chapter 4.1.2.2 that attempts to deoxygenate the free alcohol at C(1') was possible by elimination.

Thus, we returned to the product of addition **131a** and tried to eliminate the free alcohol as (Scheme 4.21) keeping in mind the high possibility of the elimination of methoxybenzyl amine at C(2). We tried to find a very small and good leaving group. Trifluroacetate appeared to be the correct choice. Elimination to enone **169** was then effected by trifluoroacylation ($(CF_3CO)_2O$, Et_3N, CH_2Cl_2, 0^oC) followed by addition of DBU. The overall yield for the elimination sequence was 80%.

Attempt to generate mesylate instead of trifluoroacetate led to considerable decomposition. Enone **169** was formed and isolated in 30 % yield only (Scheme 4.21).[168]



131a **169**

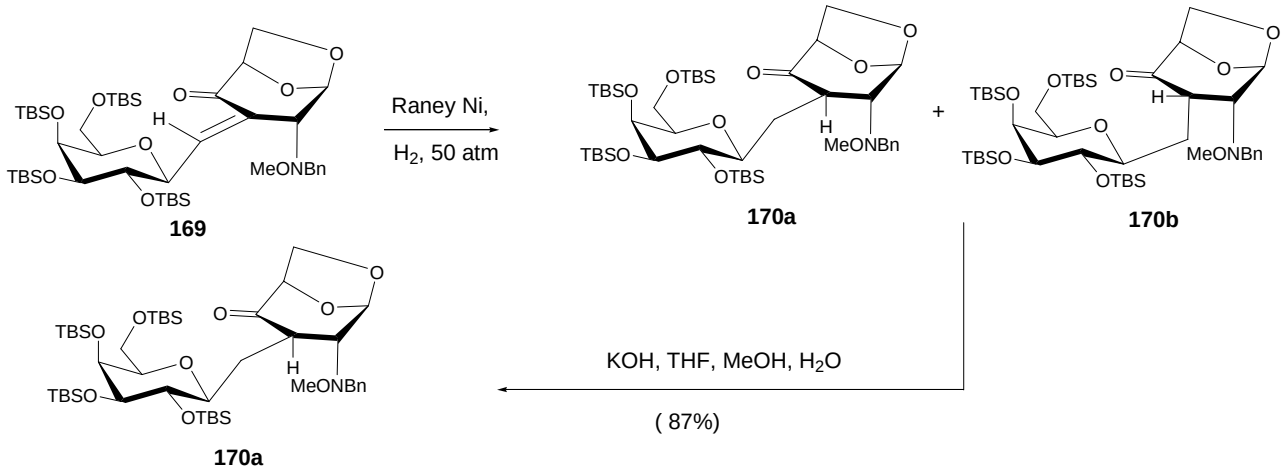
Scheme 4.21: Formation of enone **169**

The geometric configuration of the double bond present in product **169** has not been established unambiguously.[169].

With **169** in our hands, the enone was reduced using Raney Nickel and 50 atm of hydrogen gas in ethanol to give a 3:1 mixture of **170a** and **170b**. Fortunately by treating this mixture with KOH, only epimer **170a** was obtained in 87 % yield (Scheme 4.22). The drawback for this approach is the long reaction time and problems with the scale-up. Smooth conditions for the reduction were found for 3.0 mmol scale. Further scale-up becomes problematic due to considerable amounts of degradation product. The use of CuH as reducing agent led to non-reproducible results and considerable 1,2 reductions.

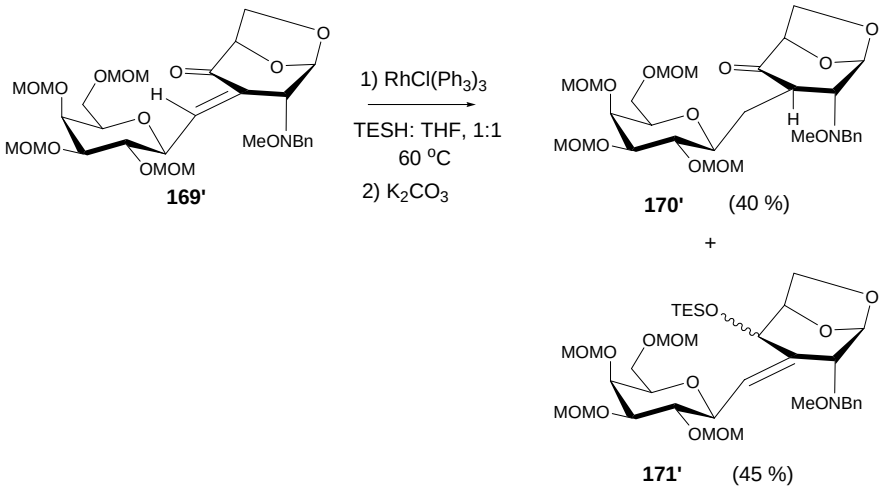
[168] Smith, A. B; Nolen, E. G.; Shirai, G. J. R; Blase, F. R.; Ohta, M.; Chida, N.; Hartz, R. A.; Fitch, D. M.; Clark, W. M.; Sprengeler, P. A. *J.Org. Chem.* **1995**,*60*, 7837.

[169] It is propose to be Z configuration as above in scheme 4.21. Prelimary NMR studies did not show NOE interaction between H-1' and H-2.



Scheme 4.22: Reduction of enone **169**

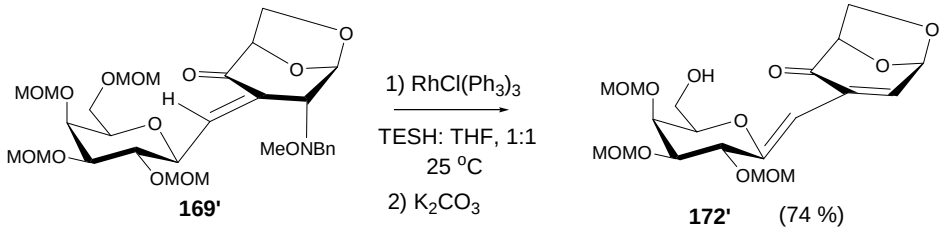
Attempts to exchange the silyl protection groups for methoxymethyl groups (MOM) did not lead to satisfactory results. In order to improve selectivily of the reduction of conjugated carbon-carbon double bond, compound **169'** was treated with triethylsilane and a catalytic amount of tris(triphenylphosphine)rhodium(I) chloride [170] in THF at 65 °C, followed by the addition of K_2CO_3. This provided **170'** in 40 % yield together with the mixture of products **171'** in 45 % yield (Scheme 4.23).



Scheme 4.22: Reduction of enone **169**'

Applying the same reagent at 25 °C gave diene **172'** as unique product (Figure 4.10) in 74 % yield. There results forced us to use the reduction of enone **169** as the route of choice to our target.

[170] Liu, H. J.; Browne, E. N. C. *Can. J. Chem.* **1981**, 59, 601.



Scheme 4.23: Reduction of enone **169**'at room temperature

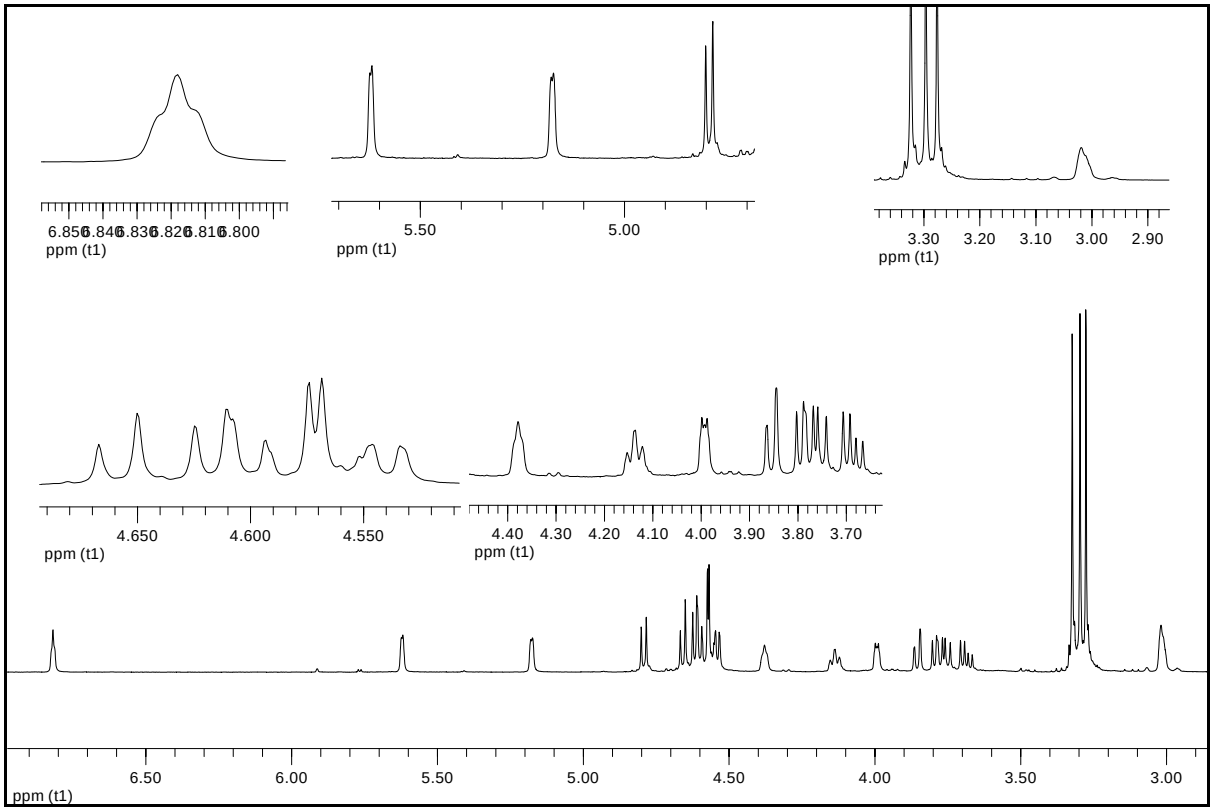
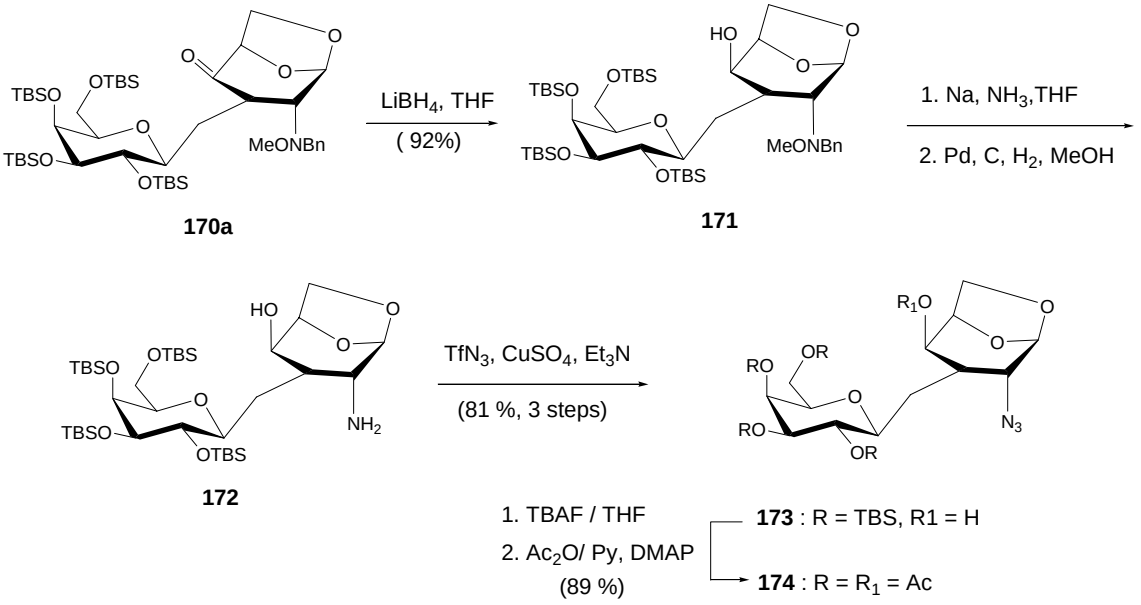


Figure 4.10: The ^{1}H-NMR (400 MHz) spectra for enone **172'**

4.1.2.4 Preparation of a 2-azido-galactosyl donor

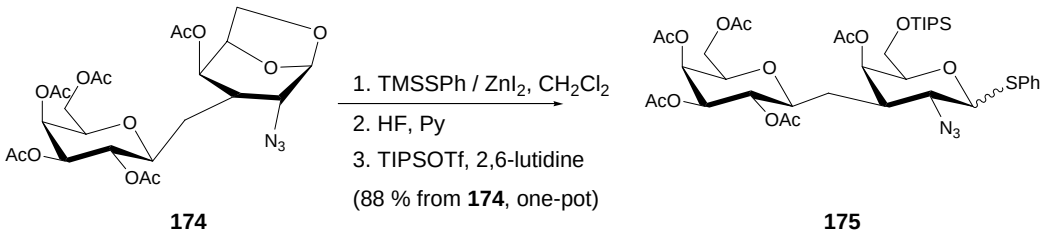
Reduction of ketone **170a** with $LiBH_4$ furnished alcohol **171** in 92 % yield. Hydride addition to the *exo* face of the dioxabicyclo[3.2.1]octonone system is preferred for steric reasons. Then the 2-BnNOMe substituent, which prohibits α-glycosidations, was converted into the corresponding primary amine **172** under Birch's conditions and subsequent catalytic (Pd/C, H_2) hydrogenation (Scheme 4.24). The amine **172** so-obtained was not isolated but directly treated with trifluromethanesulfonyl azide, cupric sulfate, and triethylamine in a mixture of H_2O / MeOH / CH_2Cl_2 to give the azide **173** in 81 % yield. Desilylation of **173** followed by acetylation provided azide **174** in 89 % yield.



Scheme 4.24: Synthesis of azide **174**.

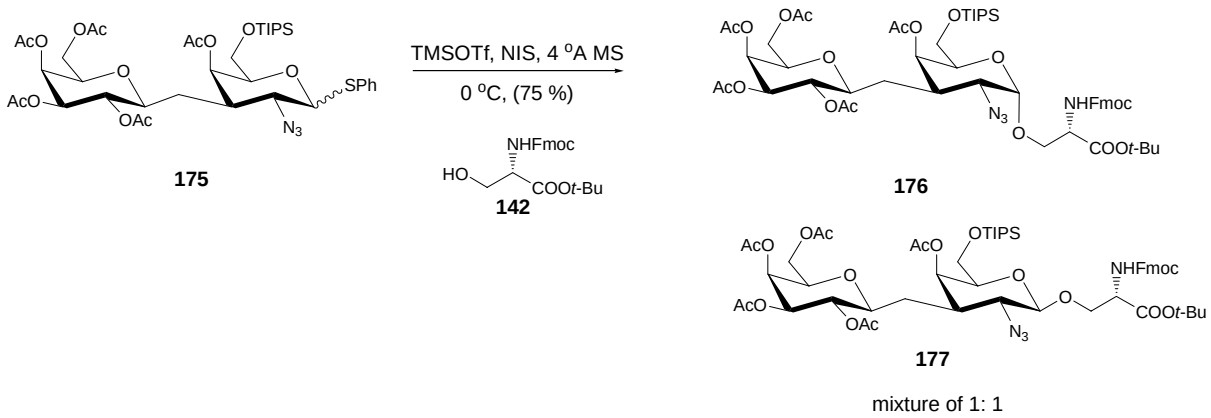
The NMR spectra did not confirm the structure of **174** unambiguously because of signal overlapping nevertheless, its structure was confirmed by the structure of product **179α** and **180α** derived from **174** and described below.

Ring opening of the 1,6-anhydrogalactose moiety of **174** was realized with ZnI_2/TMSSPh in CH_2Cl_2. The crude product so-obtained was not isolated but immediately desilylated (HF, pyridine) and the primary alcohol was reprotected as a triisopropylsilyl ether under standard conditions. This produced thiogalactoside **175** in 88 % yield as an inseparable 1:1 mixture of α and β anomers (Scheme 4.25).



Scheme 4.25: Synthesis of the galactopyranosyl donor**175**

Glycosidation of Fmoc-Ser-O*t*-Bu **142** with **175** under the conditions of Imamura et al. led to a 1:1 mixture of the α- and β-galactopyranosides **176** and **177** these compounds could not be separated (Scheme 4.26).



Scheme 4.26: Imamura glycosidation with **175**.

The structures of compound **176** and **177** were deduced from their ^{1}H-NMR spectra which showed in particular ^{3}J(H-1, H-2)= 3.3 Hz, ^{3}J(H-2,H-3)=12.2 Hz for the α-epimer and ^{3}J(H-1, H-2)= 7.2 Hz, ^{3}J(H-2,H-3)=11.9 Hz for the β-epimer (see Figure 4.11).

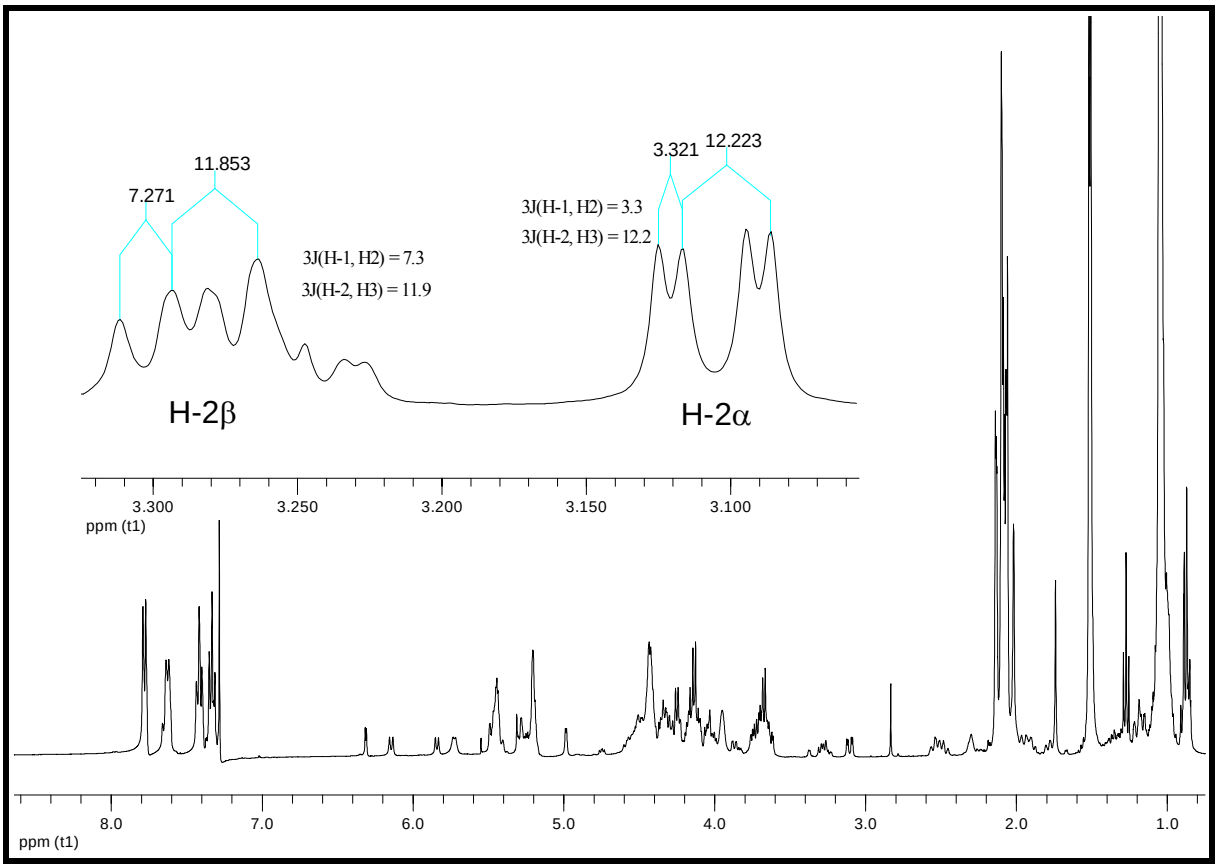
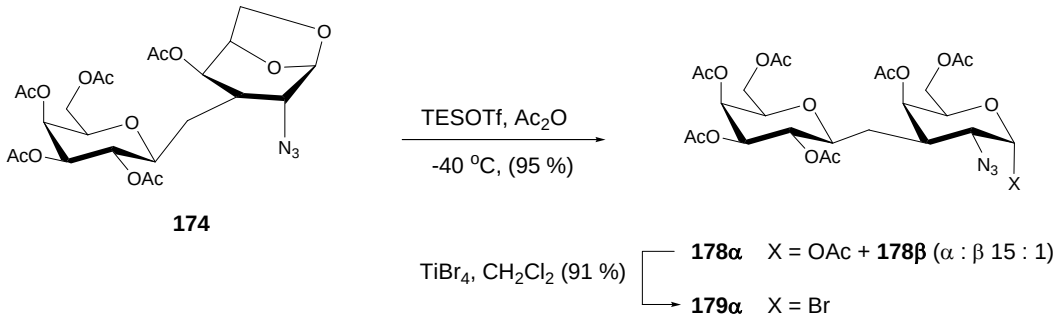


Figure 4.11: The ^{1}H-NMR (400 MHz) spectra of 1:1 mixture of **176** and **177**

Smooth ring opening of the 1,6-anhydrogalactose moiety of **174** using TESOTf in Ac_2O gave a 15:1 mixture of α-(**178α**) and β-galactoside (**178β**) in 95 % yield. Conversion of galactosyl acetate **178** into the corresponding bromide **179** (92 %) was carried out with $TiBr_4$ in CH_2Cl_2 (25 ^oC, 12h). Under these conditions only the α anomer was formed (Scheme 4.27).



Scheme 4.27: Synthesis of galactosyl bromide **179α**

The structure of **179α** was confirmed by ^{1}H-NMR which showed ^3J(H-1, H-2) =3.1 Hz, ^3J(H-2, H-3) =12.3 Hz, ^3J(H-4, H-3) and ^3J(H-4, H-5) less than 2 Hz. This is typical for α-galactopyranosides. Further more the chemical shift of H-4 (5.4 ppm) confirmed the pyranoside structure (figure 4.12, for more details the experimental part) and is not consistent with a furanoside structure.

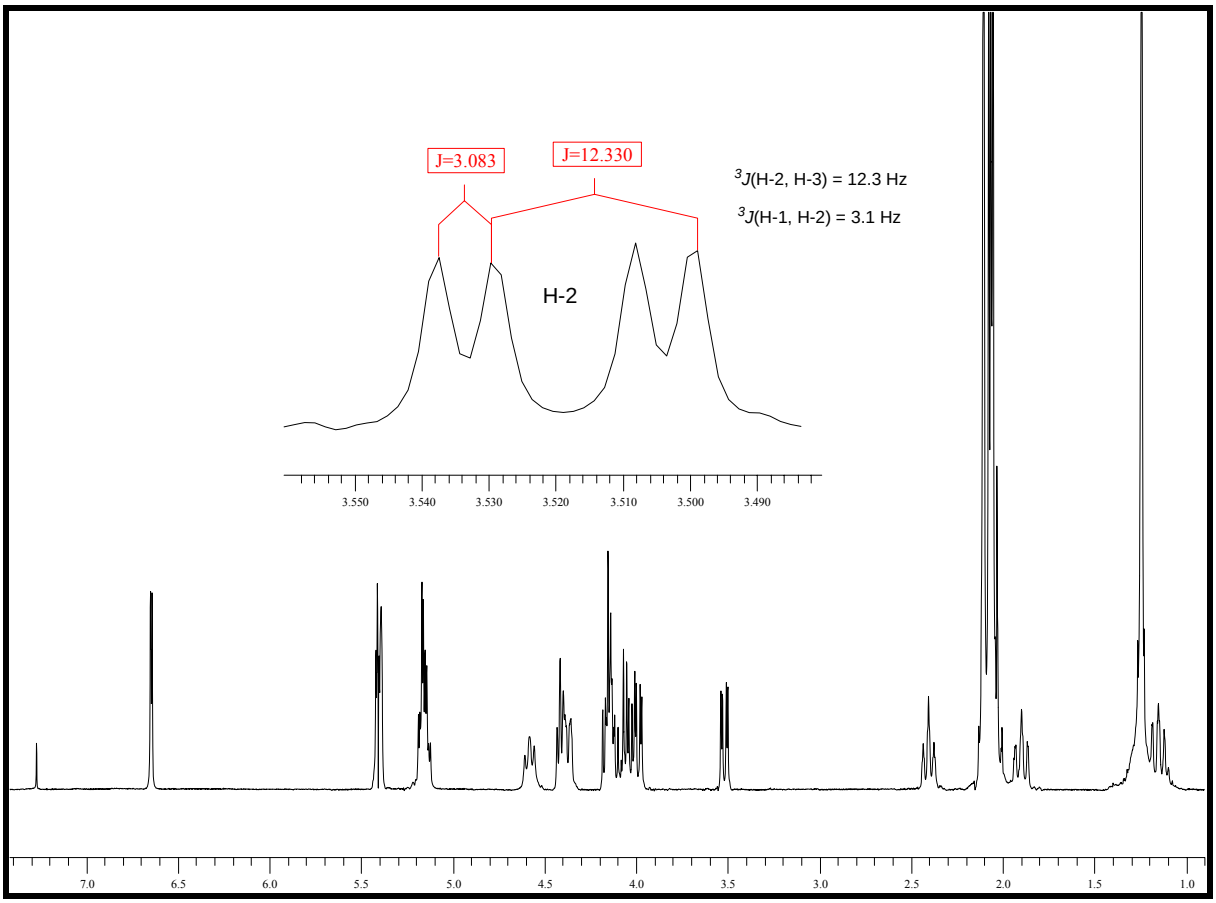
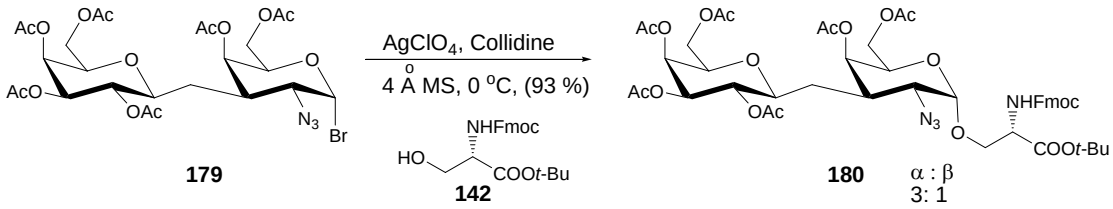


Figure 4.12: The H-NMR spectra of **179α**

Königs-Knorr glycosidation ($AgClO_4$ / CH_2Cl_2 , 2,4,6-collidine, 4 Å molecular sieves) of N-Fmoc-serine *tert*-butylester **142** gave a 3:1 mixture of α-(**180α**) and β-galactoside (**180β** in 73 % yield. Flash chromatography provided pure **180α**. The preferred formation of the α-galactoside is ascribed to a kinetic anomeric effect (no participating group at C-2) (Scheme 4.28).



Scheme 4.28: Synthesis of a precursor of a *C*-disaccharide analogue of the TF-epitope.

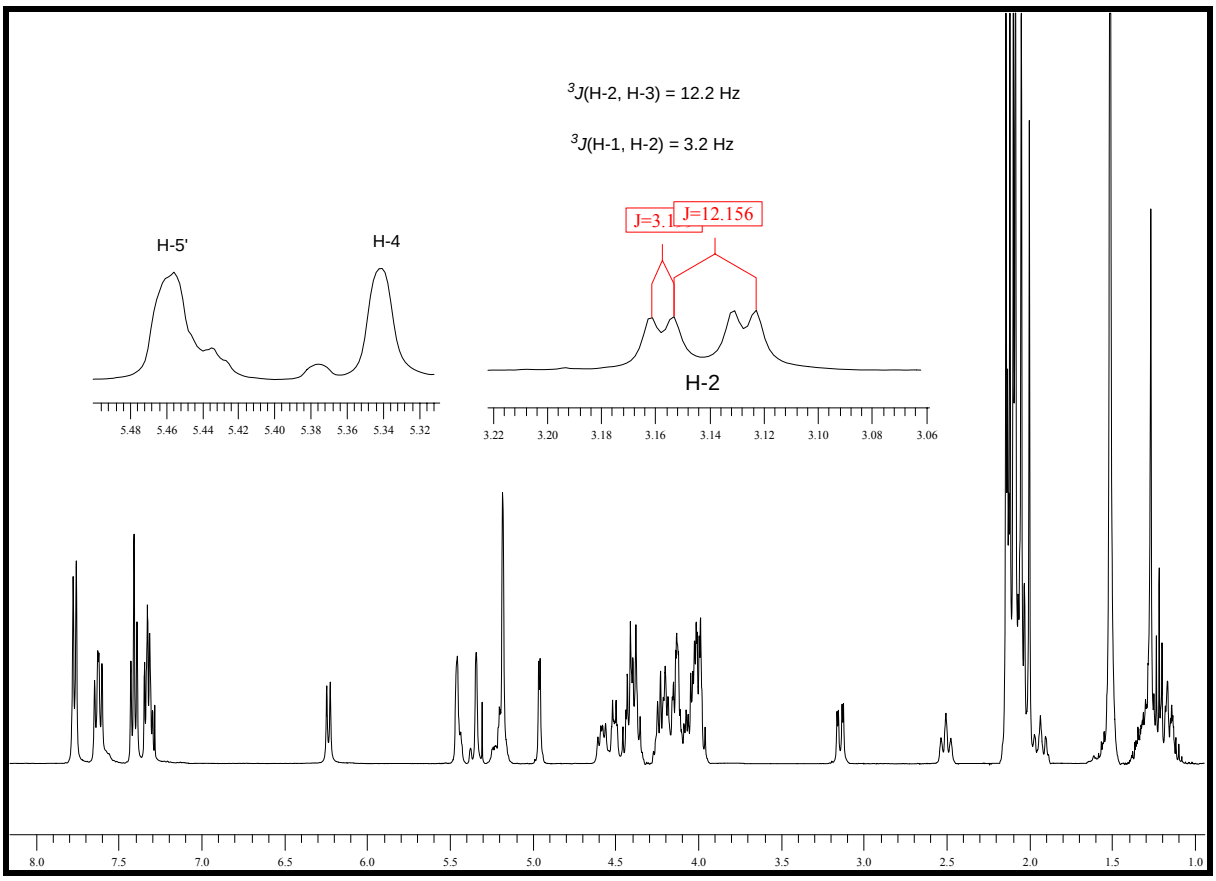
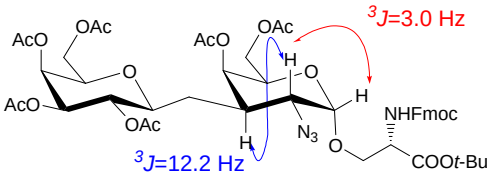


Figure 4.13: the ^{1}H-NMR (400 MHz) spectrum of **180α**.

The structure of **180** was confirmed by its ^{1}H-NMR spectrum (Figure 4.10). The major product shows ^3J(H-1, H-2) =3.2 Hz, ^3J(H-2, H-3) =12.2 Hz, and ^3J(H-4, H-3) and ^3J(H-4, H-5) less than 1 Hz. Additionally 2-D-NOESY ^{1}H-NMR spectrum show cross peak between H-(C3) and H-(C5) which

confirms α-galactopyranoside structure. Further-more the chemical shift of H-4 (5.34 ppm) confirmes the pyranoside structure (Figure 4.13 and 4.14) (for more details see the experimental part).



180 α

Typical α galactose

Figure 4.14: ^{1}H-NMR analysis of **180α**.

By *MALDI-HRMS* the molecular weight of **180α** was confirmed (Figure 4.15). The purity of **180α** was confirmed by elementary analysis (for more details see the experimental part).

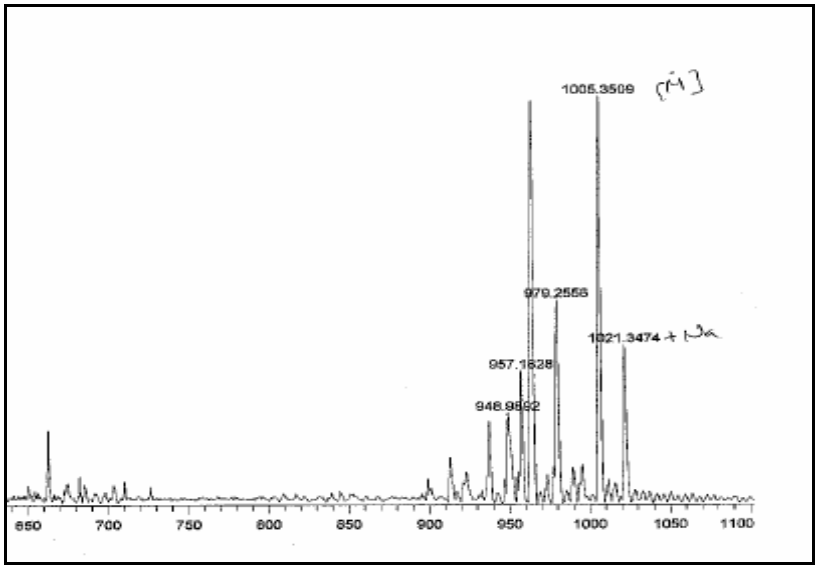
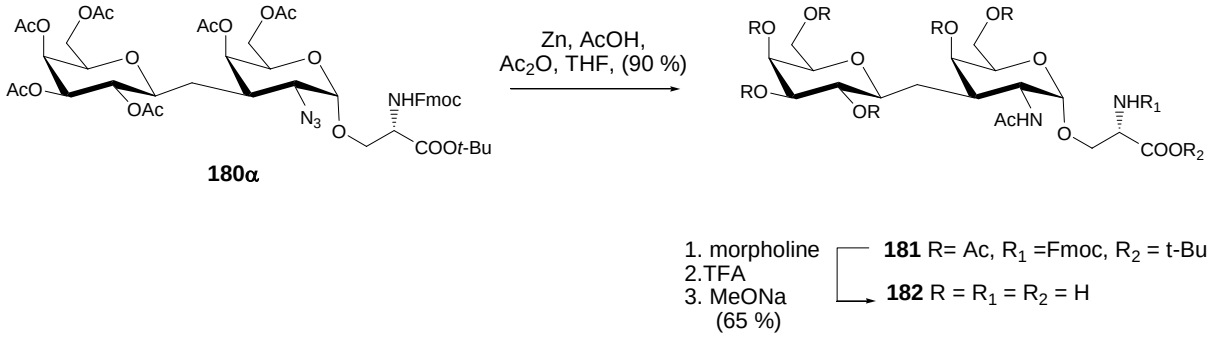


Figure 4.15: *MALDI-HRMS* of **180α**.

Treatment of **180α** with zinc in acetic acid, THF, and Ac_2O furnished **181** in 90 % yield, a protected form of a C-linked analog of the TF epitope α-conjugated to L-serine. This form should be suitable for the construction of clusters via peptide synthesis and further conjugation to immunogenic proteins. We have verified that the key intermediate **181** can be fully deprotected. When the TF-antigen analogue **181** was treated with morpholine in DMF followed by TFA and then with MeONa/MeOH disaccharide **182** was obtained in 65 % yield after HPLC purification (Scheme 4.29).



Scheme 4.29: Synthesis of fully protected and deprotected TF-antigen.

The molecular weight of the fully protected TF-antigen analogue **181** was confirmed by *MALDI-HRMS* (Figure 4.16). The purity of **181** was confirmed by elementary analysis (for more details see the experimental part).

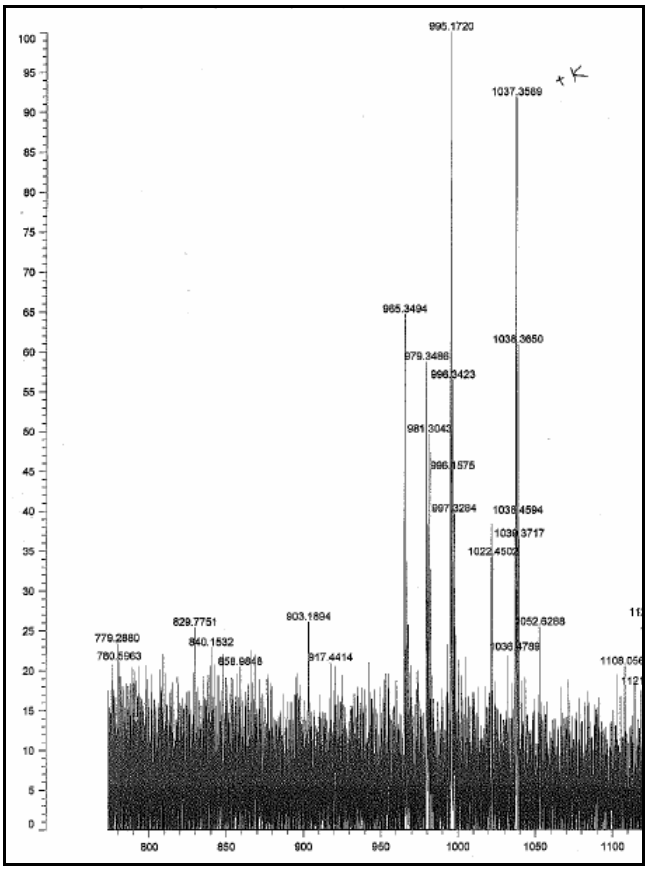


Figure 4.16: *MALDI-HRMS* of **181**.

4.2 Clustering of C-Disaccharide Analogues of the TF-epitope

4.2.1 Introduction

It was determined that multiple repeats or clustering of the carbohydrates was required for a robust and efficient immune response to be generated.[171] The cassette approach to the synthesis of these antigenic glycosylamino acids allowed facile construction of the requisite glycopeptides. Single-antigen vaccines, derived from both clustered (multiple copies of the same antigen displayed on a peptide backbone) and nonclustered (single copy of an antigen).[172]

The strategy envisioned for the construction of vaccines would require the fashioning of a pool of glycosylamino acids in which the carbohydrate entities would be completely protected and the amino terminus blocked with the fluorenylmethyl carbamate (Fmoc) group. Solution-phase Fmoc-based peptide chemistry[173] would be used to assemble the glycopeptide, and to attach a linker domain. Exhaustive deprotection as the last step would provide a final cluster readly for conjugation with KLH.[174]

[171] Gilewski, T., Ragupathi, G., Bhuta, S., Williams, L. J., Musselli, C., Zhang, X. F., Bencsath, K. P., Panageas, K. S., Chin, J., Hudis, C. A. *Proc. Natl. Acad. Sci. USA* **2001**, *98***,** 3270.

[172] Keding S. J; Danishefsky S. J. *Proc. Natl. Acad. Sci. USA* **2004**, *101*,11937.

[173] 1) The coupling steps for the two C-disaccharide antigen amino acids will mediated by HOAt/ HATU coupling reagents 2) cleavage of the Fmoc group using 20 % piperidine in DMF 3) acetylation of the last deprotected N-terminus of the third building block in the cluster is required.

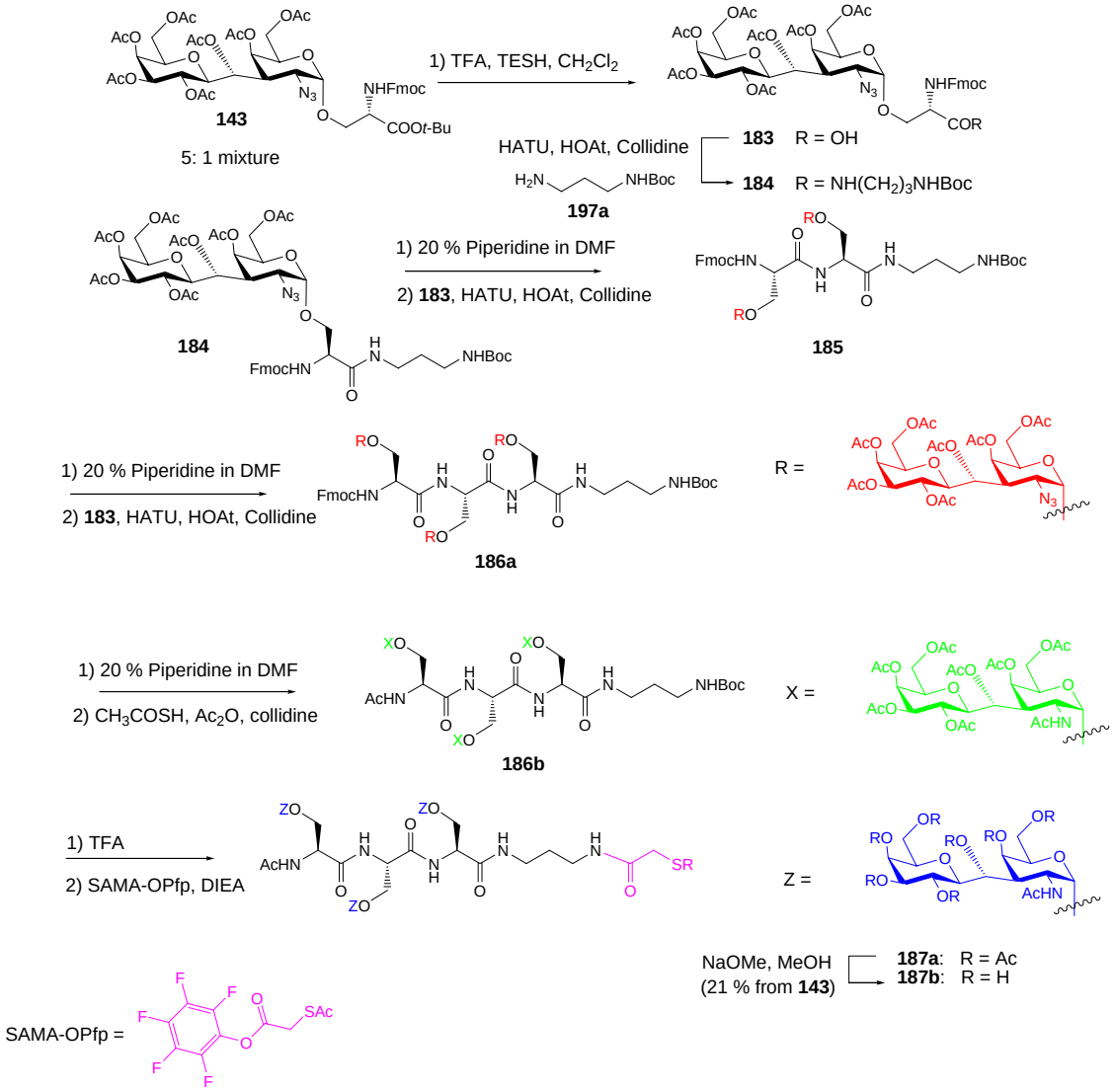
[174] So-Yeop, H.; Young-Ah, K. *Tetrahedron* **2004**, *60*, 2447.

4.2.2 The First Synthesis of a Cluster of a –CH(OH)– *C*-Disaccharide Analogue of the TF Antigen

4.2.2.1 Using a Diamine Linker

The 5:1 mixture of α-and β-galactopyranosides of protected serine **143** was treated with CF_3COOH and Et_3SiH in CH_2Cl_2 at 25 °C to give the free carboxylic acid **183**. Treatment of **183** with (3-amino-propyl)-carbamic acid *tert*-butyl ester **197a** in the presence of HATU, HOAt, and collidine afforded amide **184** in 87 % yield for two steps.[175] Selective removal of Fmoc protection group with 20 % piperidiene in DMF [176] gave the corresponding primary amine that was not isolated but directly treated with with carboxylic acid **183**, HATU, HOAt, and collidine. This produced dipeptide **185** in 81 % for two steps. Repeating the same sequence of reaction (Fmoc cleavage with piperidine, amidification with **183**) led to the tripeptide **186a** in 75 % yield. Removeal of Fmoc protective group of **186a** on treatment with 20 % piperidine in DMF gave the corresponding primary amine which was not isolated but treated directly with excess of $AcSH/Ac_2O$/collidine at 25 °C giving **186b**. The latest operation converted all the three C(2)triazi-azido group in disaccharide moieties into the corresponding C(2)-acetamido system. This compound possesses a terminal NHBoc that was converted into the corresponding amino group on treatment with CF_3COOH. Followed by acylation with SAMA-OPfp in the presence of Hünig's base ($(i\text{-Pr})_2NEt$) gave **187a**. Zemplen's deacetylation (NaOMe/MeOH, 25 °C) liberated fully deprotected C-disaccharide furnishing cluster **187b** in 21 % overall yield based on **143**. Compound **187b** possesses a terminal thiol group ready for conjugation with an adequate modified immunogenic protein such as KLH.

[175] Mono- and diclusters of the natural (O-linked) TF antigen have been reported: see e.g.: Kunz,H.; Birnbach, S.; Wernig, P. *Carbohydr. Res.* **1990**, *202*, 207.
[176] a) Carpino, L. A. *J. Am. Chem. Soc.* **1993**, *115*, 4397. b) Carpino, L. A.; Ionescu, D.; El-Faham, A. *J. Org. Chem.* **1996**, *61*, 2460.



Scheme 4.30: Clustering of the CH(OH)–linked-disaccharide analogue of the TF-epitope

4.2.2.2 Analysis of the Clustering Intermediate Products

As expected, the signals observed in ^{1}H-and ^{13}C-NMR spectra of compounds **185**, **186a**, **186b**, and **187a** were difficulty to attitude to any particular structure fragment due to the molecular complex of these products.

However, the integration of separate groups of signals in ^{1}H-NMR spectra of above mentioned products showed expected ratio.

Thus, this groups of products was characterized exclusively by *MALDI-HRMS* and elementary analysis (Figures 4.17, 4.18).

Additionally, transformation of **186a→186b** was clerly observed by IR spectra of starting material and product. Triazide **186a** was characterized by the presence of strong absorption band at 2115 in its IR spectrum which vanished completely after reduction to **186b**. Instead, relatively strong C=O (st)

characteristic to amides was seen at 1685 cm^{-1} together with classical C=O (st) absorbtion band at 1750 $cm^{-1}$for acetate.

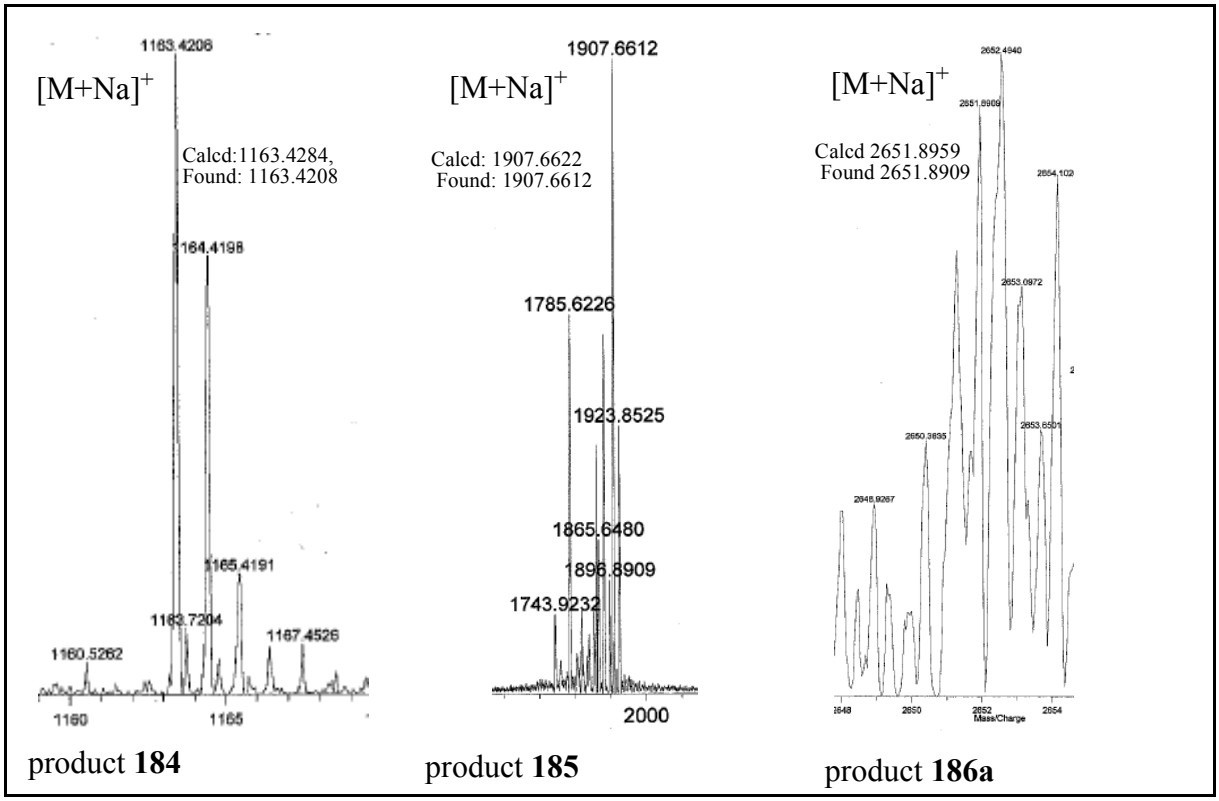


Figure 4.17: *MALDI-HRMS* of **184**, **185**, **186a**.

The molecular weight of tripeptide **187b**, was confirmed by *MALDI-HRMS* (Figure 4.18). Fully deprotected peptide **187b** was purified by HPLC[177]. The fractions were collected according to their *MALDI-HRMS* analysis (for more details see the HPLC chromatogram in the experimental part).

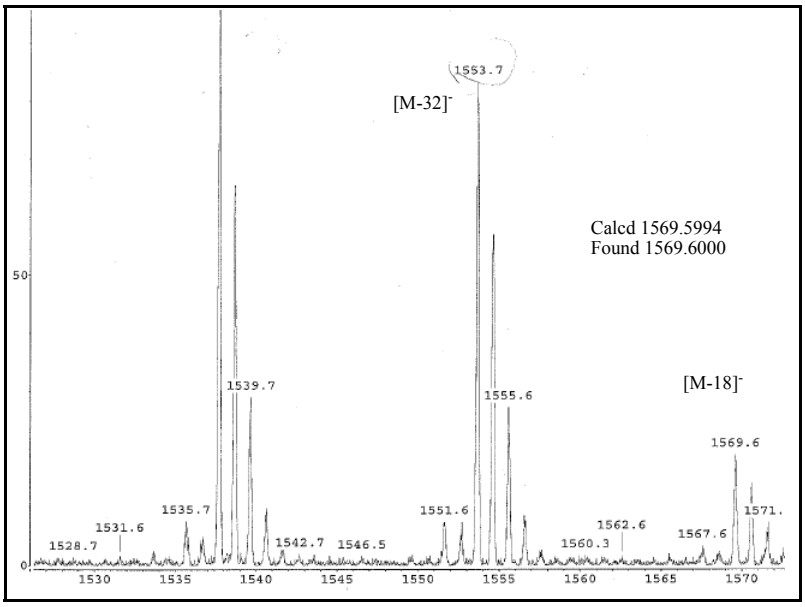
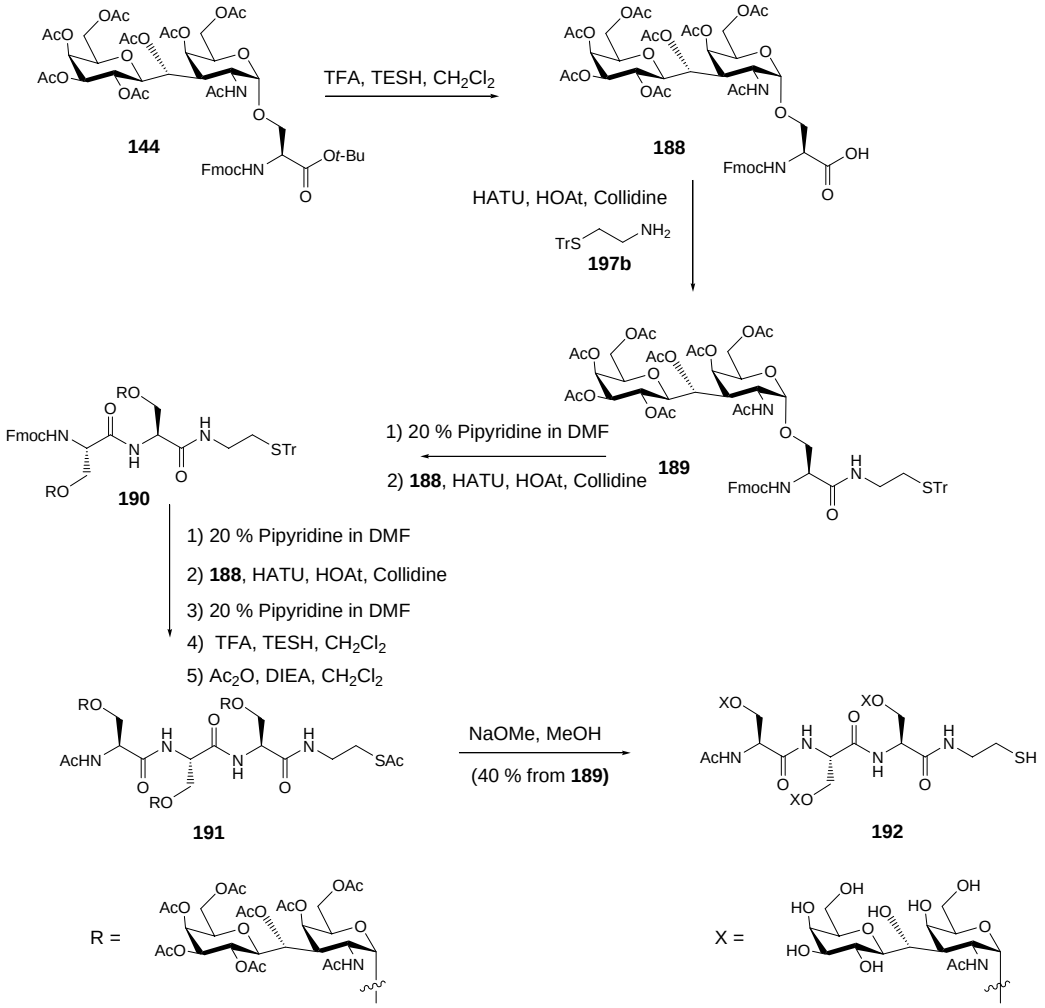


Figure 4.18: *MALDI-HRMS* of **187b**.

4.2.2.3 Using an Aminothio Linker

In a parallel study the fully protected (CH)OH-linked- disaccharide analogue of the TF-epitope **144** was converted into cluster **192** bearing a 2-carboxamidothiol moiety, also ready for conjugation with an adequately modified immunogenic protein. For that, **144** was first treated with CF_3COOH/ Et_3SiH in CH_2Cl_2 at 25 ^oC to liberate the free carboxylic acid **188**. The latter was not isolated but directly submitted to amidification (HATU, HOAt, collidine) with 2-tritylthioethaneamine **(197b)**. providing **189** in 90 % yield. Treatment of **189** with 20% piperidiene in DMF at 25 ^oC liberated the free amine of the L-serine moiety. It was reacted *in situ* with one equivalent of carboxylic acid **188** and HATU, HOAt and collidine at 25 ^oC, giving dipeptide analogue **190** in 85 % yield. Repeating the same reaction sequence, a third unit of **188** was condensed with **190** giving an intermediate product that was treated with 20 % piperidiene in DMF. Subsequent detrytilation with $CF_3COOH/Et_3SiH/CH_2Cl_2$ at 25 ^oC liberated the thiol that was not isolated but acetylated with Ac_2O and Hünig's base in CH_2Cl_2 at 25 ^oC. Zemplen's deacetylation of so-obtained thioacetate **191** with MeONa/MeOH at 25 ^oC provided the fully deprotected cluster **192** in 40 % overall yield (based on **189**).

[177] (100 % water → 0, and 0% CH_3CN → 100 %) used as eluant, reversed-phase column have C-18 stationary phase, and the Rt = 3.05min



Scheme 4.31: Clustering of the CH(OH) disaccharide analogue of the TF-epitope using 2-Amino-ethanethiol linker.

4.2.2.4 Analysis of the Clustering Intermediate Products

As expected like befor, the signals observed in ^{1}H-and ^{13}C-NMR spectra of compounds **190**, and **191** were difficults to attitude to any particular structure fragment due to the molecular complex of these products.

However, the integration of separate groups of signals in ^{1}H-NMR spectra of above mentioned products showed expected ratios.

Thus, this group of products was characterized exclusively by *MALDI-HRMS* and elementary analysis (Figures 4.19, 4.20).

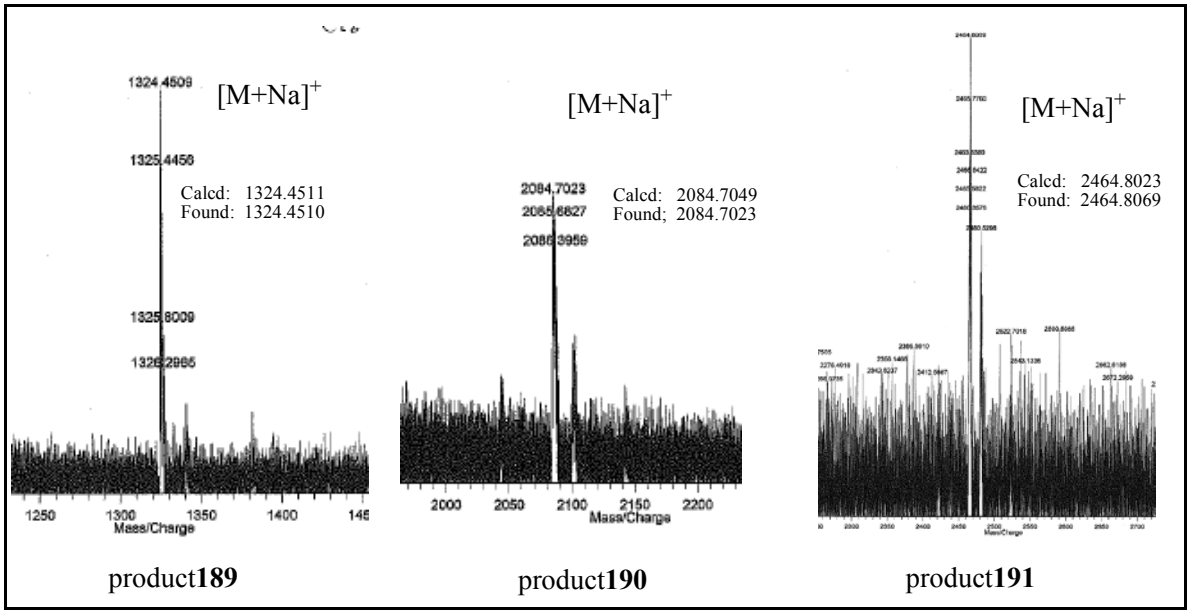


Figure 4.19: *MALDI-HRMS* of **189**, **190**, **191**.

The molecular weight of mono-, di-, and tripeptides **189**, **190** and **191**, was confirmed by *MALDI-HRMS* (Figure 4.18). Fully deprotected peptide **192** was purified by HPLC.[178] The fractions were collected according to their *MALDI-HRMS* analysis, additionally elementary analysis of tripeptide was obtained (for more details see the HPLC chromatogram in the experimental part).

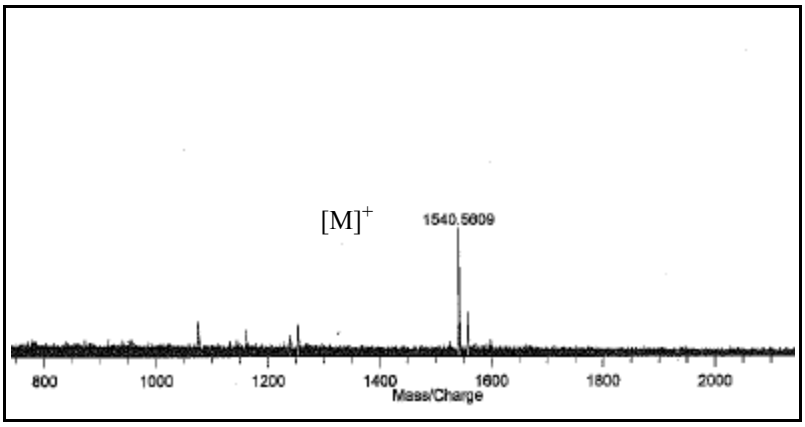


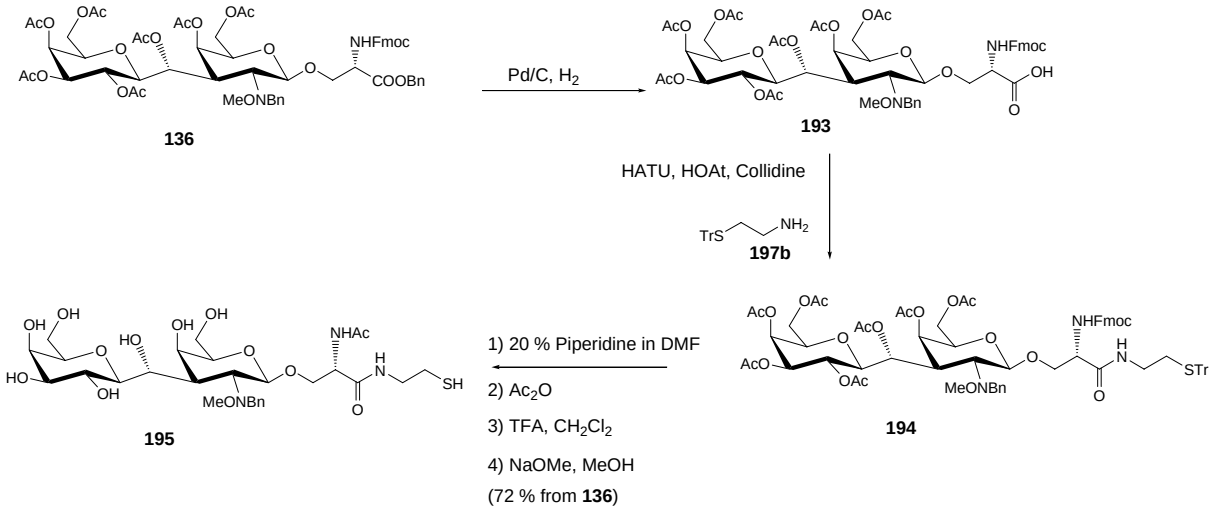
Figure 4.20: *MALDI-HRMS* of **192**.

In a model study, the purpose of which was to find suitable conditions for the conjugation of KLH with a thiol, we used the β-galactoside **136** and converted it into **195**, 2-carboxamidoethanethiol attached to a CH(OH)-disaccharide analogue of TF epitope.

Thus, **136** was hydrogenated (H_2/Pd/C) to liberate the free carboxylic acid **193**. Amidification of **193** with 2-tritylthioethaneamine using HATU/HOAt and collidine at 25 ^{o}C provided **194** in 92 % yield.

[178] (100 % water → 0, and 0% CH_3CN → 100 %) used as eluant, reversed-phase column have C-18 stationary phase, and the Rt = 3.9min

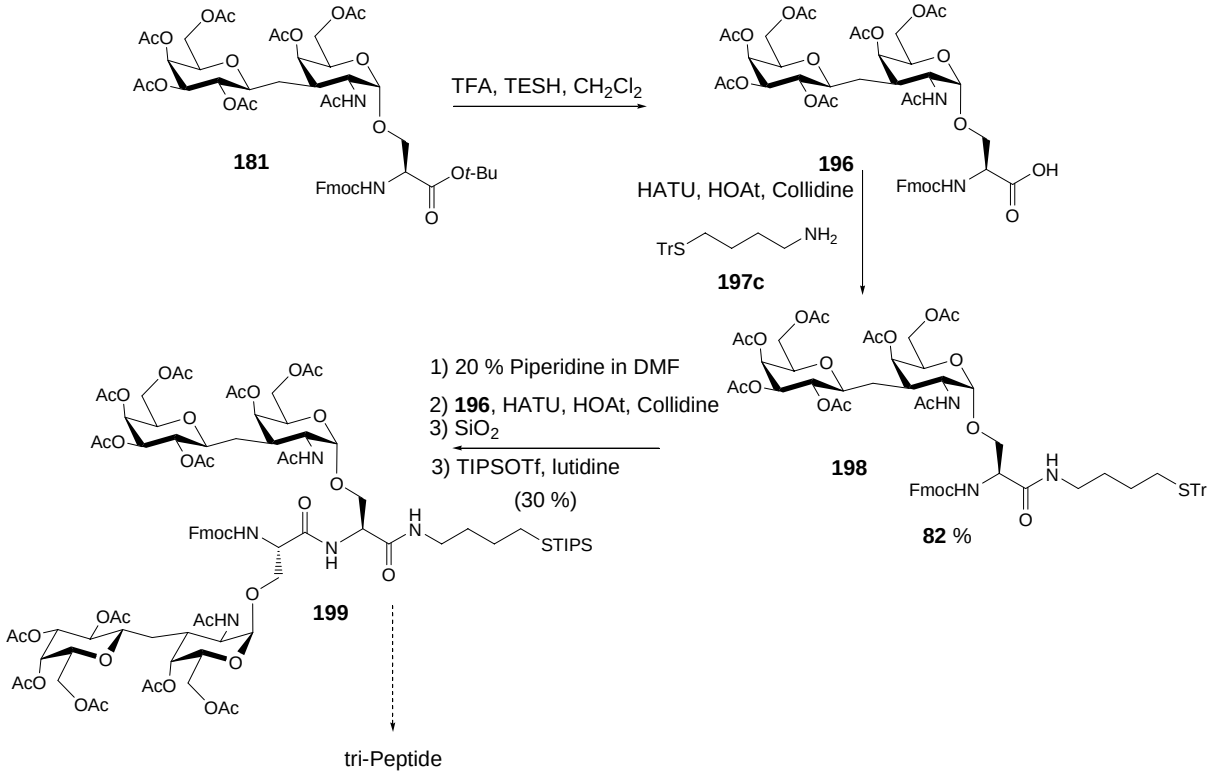
Treatment of **194** with 20 % piperidene in DMF cleaved away the Fmoc protective group. The free amine so-obtained was not isolated but directly acetylated with pure Ac_2O. The intermediate product so-obtained was then detritylated by treatment with $CF_3COOH/Et_3SiH/CH_2Cl_2$ at 25 ^oC. Subsequence deacetylation with MeONa/MeOH gave **195** in 72 % overall yield based on **136**.



Scheme 4.32: the preparation of the thio-linker β-glycoside **195**.

4.2.3 The First Synthesis of a Cluster of a –(CH_2)–Linked-Disaccharide Analogue of the TF Antigen

The fully protected (CH_2)-disaccharide analogue of the TF-epitope **181** was converted into cluster **199** bearing a 4-carboxamidothiol moiety, also ready for third coupling with carboxylic acid **196**. For that, **181** was first treated with CF_3COOH/ Et_3SiH in CH_2Cl_2 at 25 °C to liberate the free carboxylic acid **196**. The latter was not isolated but directly submitted to amidification (HATU, HOAt, collidine) with 2-tritylthiobutylamine **(197c)** producing **198** in 82 % yield. Treatement of **198** with 20% piperidiene in DMF at 25 °C liberated the free amine of the L-serine moiety. It was then reacted *in situ* with one equivalent of carboxylic acid **196**,HATU, HOAt, and collidine at 25 °C, giving dipeptide in which the S-trityl group of the 4-tritylthiobutylamine liker was cleaved during the chromatographical purification on silica gel which then treated by TIPSOTf/lutidine 0 °C, gave dipeptide **199** in 30 % yield.[179]



Scheme 4.33: Clustering of the (CH_2)-linked-disaccharide analogue of the TF-epitope using 4-amino-butanethiol linker.

Di-peptide **199** should undergo in the same manner to get tri-peptide of the (CH_2)-linked-disaccharide analogue of the TF-epitope.

[179] The synthesis is still in progress and the protocol still under further investigation.

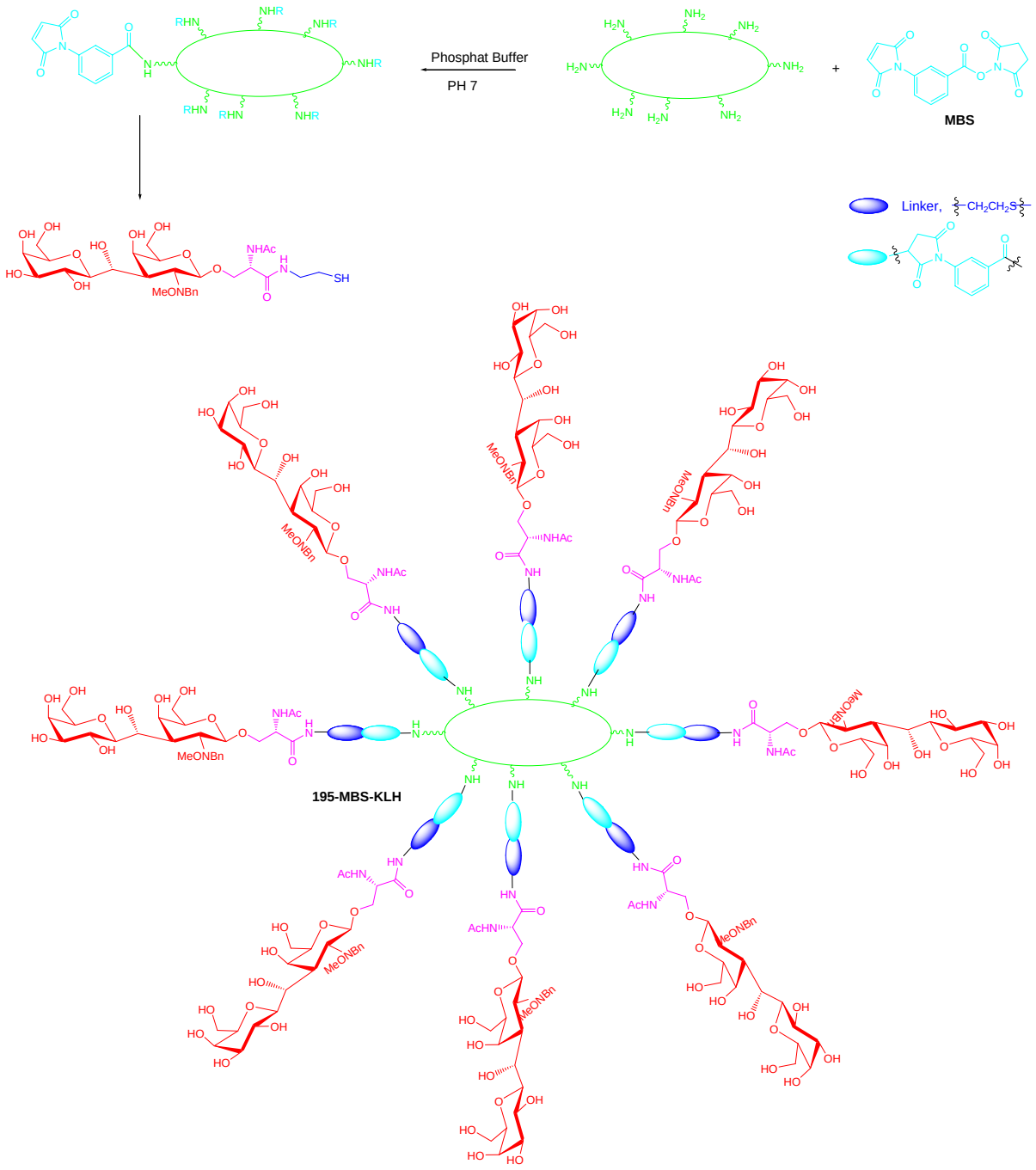
4.3 Preparation of Conjugates of KLH with C-disaccharide Analogues of TF-epitope:

4.3.1 Preparation of Peptide-MBS-KLH

As described in chapter 1.2.1 keyhole limpet hemocyanin[180] has produced up to now the best anti-tumor conjugates with vaccines sugar epitopes. Therefore we chose this immunogenic protein as carrier for our TF-epitope analogues **187b**, **192**, and **195**. The KLH protein was first coupled with MBS (*m*-maleimidobenzoyl-*N*-hydroxysuccinamide ester) by transamidification of lysine terminal NH_2 group. After this transformation the maleimido-modified-KLH is ready to serve as a Michael accepter for following conjugation step.

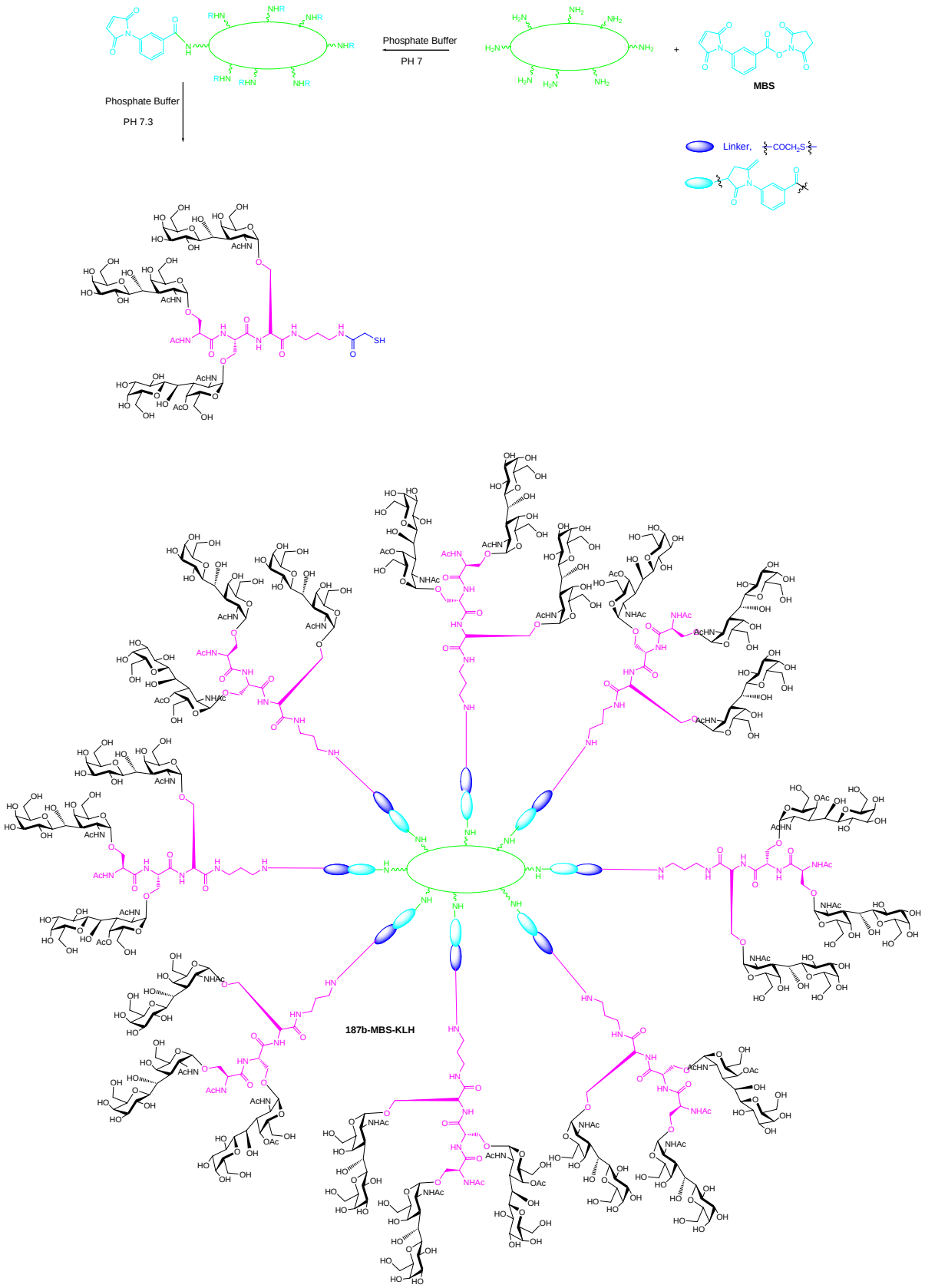
The thiol **195** bearing a CH(OH)-linked disaccharide β-linked to L-serine was reacted with the maleimide moieties of KLH-MBS by 1,4-addition reaction.[181] This generates conjugate **195-MBS-KLH**. By considering the weight increase of KLH used in the experiment the ratio 1**95**: **KLH** was determined to be 360:1 (Scheme 4.34)

[180] (KLH, Molecular weight: 6-8 million)
[181] Zhang, S. L.; Graeber, L. A.; Helling, F.; Ragupathi, G.; Adluri, S.; Lloyd, K. O.; Livingston,P. O. *Cancer Res.* **1996**, *56***,** 3315.



Scheme 4.34: Preparation **195-MBS-KLH**

The same procedure was applied to MBS-KLH using the trimeric cluster **187b**. The ratio of glycopeptide-to-protein for the conjugate (**187b:KLH)** was determined to be 45:1 by considering the weight increase of KLH used in the experiment (Scheme 4.35).



Scheme 4.35: Preparation **187b-MBS-KLH**

4.3.2 Preparation and purification methods of the KLH-Conjugates and Discussion

As the preparation and purification of such kind of KLH-conjugates is not common for the synthetic organic chemists, we would like to focus here on the experimental details of this final key step of our project.

Keyhole limpet hemocyanin KLH[182] (5 mg) was dissolved in phosphate buffer (0.01M, pH 7.0, 0.5 mL). Then *m*-maleimidobenzoyl-*N*-hydroxysuccinimide ester (MBS) (1mg, 0.0032 mmol) was dissolved in DMF(70µL) and added to the KLH solution. The resulting mixture was stirred for 1 h at 20 ^{o}C, and MBS-KLH conjugate was submitted to pre-equilibrated Sephadex G-25 column with phosphate buffer (0.05M, pH 6.0).[183] The mixture was eluted with phosphate buffer (0.05M, pH 6.0), and the first 3.5 ml of elute were collected in 7 Eppendorfs tubes (0.5 ml/Eppendorf). The fractions containing MBS-KLH conjugate were put in dialysis tube,[184] let to equilibrate for 3h and lyophilized.

Synthetic peptide **187b** (5 mg, 0034 mmol) was dissolved in phosphate buffer solution (0.01 M, pH 7.3, 1ml) and added to the resulting MBS-KLH conjugate from the step above. The pH of the resulting solution was adjusted to 7.3,[185] and the reaction mixture was stirred for 3h at 20 ^{o}C. Then it was submitted to pre-equilibrate Sephadex G-25 column with double distilled water, and 3.5 ml of elute was collected in 7 Eppendorf tubes (0.5 ml/Eppendorf). The fractions containing peptide-MBS-KLH conjugates were collected and purified by dialysis tube, let to equilibrate for 3h and lyophilized.

Bradford reagent t [186] was used to determine the presence of peptide-MBS-KLH conjugate in the collected fractions.

Thus in each of 7 wells[187] of microwell plate Bradford reagent (100µL) was diluted with double distilled water (100µL) and after 1min of equilibration, aliquot (10µL) of every chromatography fraction were added respectively in above mentioned 7 wells.

The color change from brown to blue indicated the presence of peptide-MBS-KLH conjugates. The corresponding fractions were collected, submitted to dialysis for 3h at 20 ^{o}C and lyophilized (Scheme 4.21).

[182] *Megathura crenulata*, High Purity, Endotoxin-free, Glycerol, Cat. No **374813** from CALBIOCHEM
[183] Pre-equilibrated mean elute the solvent for 3 times (5ml each time), wait minimum for 15 min befor using the column.
[184] 30,000 cut off dialysis tube
[185] the pH of the solution was adjusted using 0.1M HCl or 0.1M NaOH,
[186] The solution was prepared by dissolving 100 mg Coomassie Brilliant Blue G-250 (100 mg) in 50 ml 95% ethanol followed by addition of 100 ml 85% (w/v) phosphoric acid. The resulting mixture was dilute to 1 liter using double distilled water. When the dye has completely dissolved the final solution was filtered through Whatman #1 paper just before the use (the color of the reagent has to be brown).
[187] The number of wells corresponds to the number of collected fractions.

1. add 100µl Bradford solution, and100µl of double distilled water
2. add 10µl of the collected fractions.
3. the blue color is indicatating the presence of protein exist in corresponding fractions.

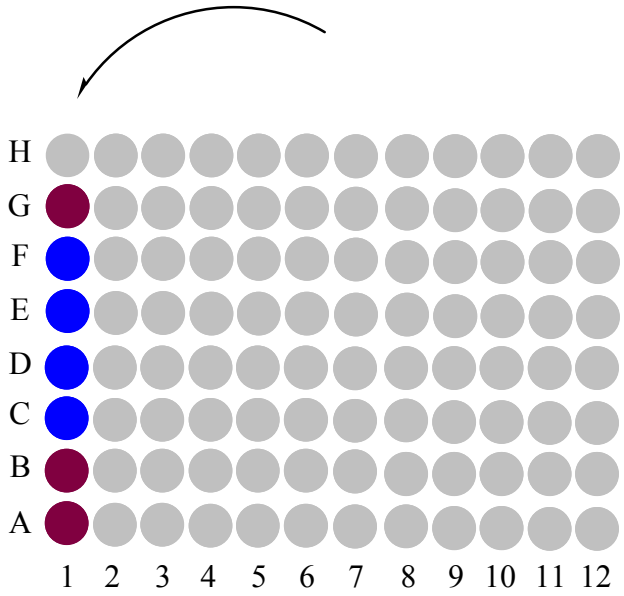


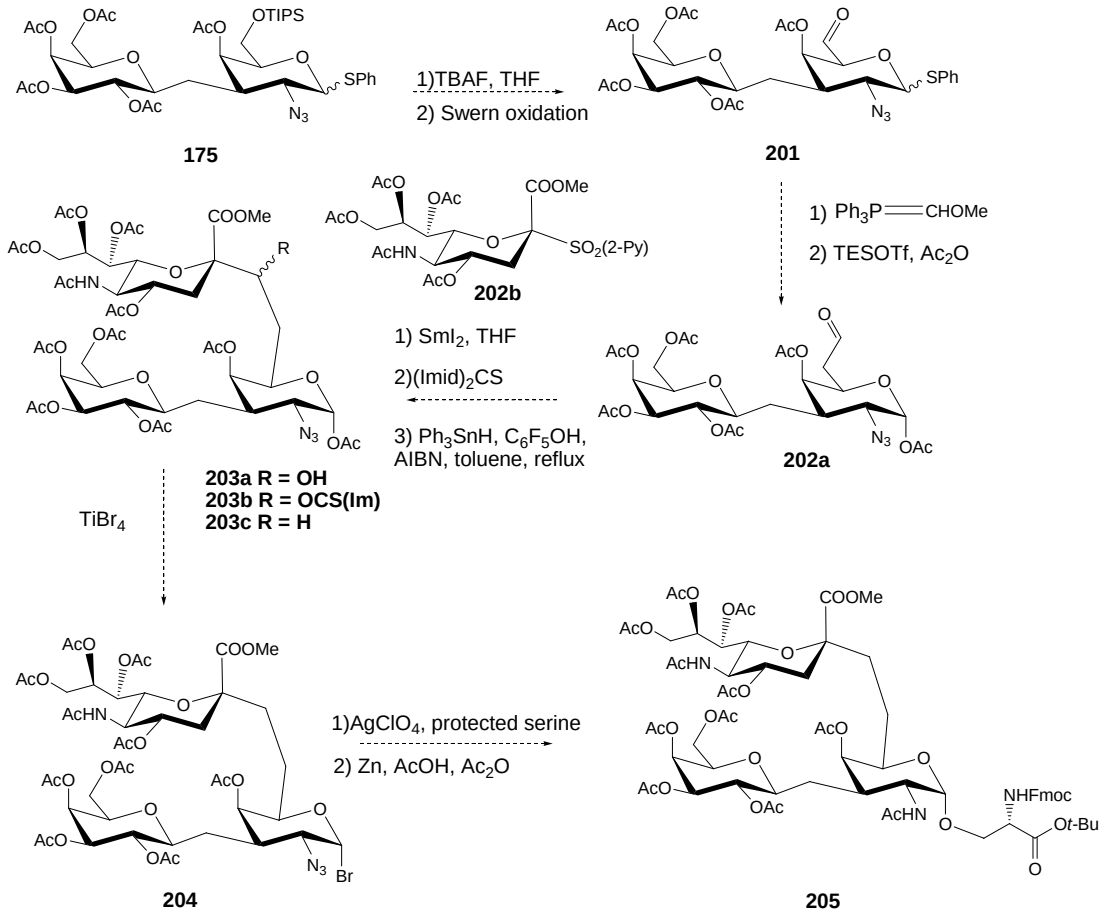
Figure 4.21: microwell plate

.

4.4 Perspectives

4.4.1 Toward a 2,6 ST-antigen analogue

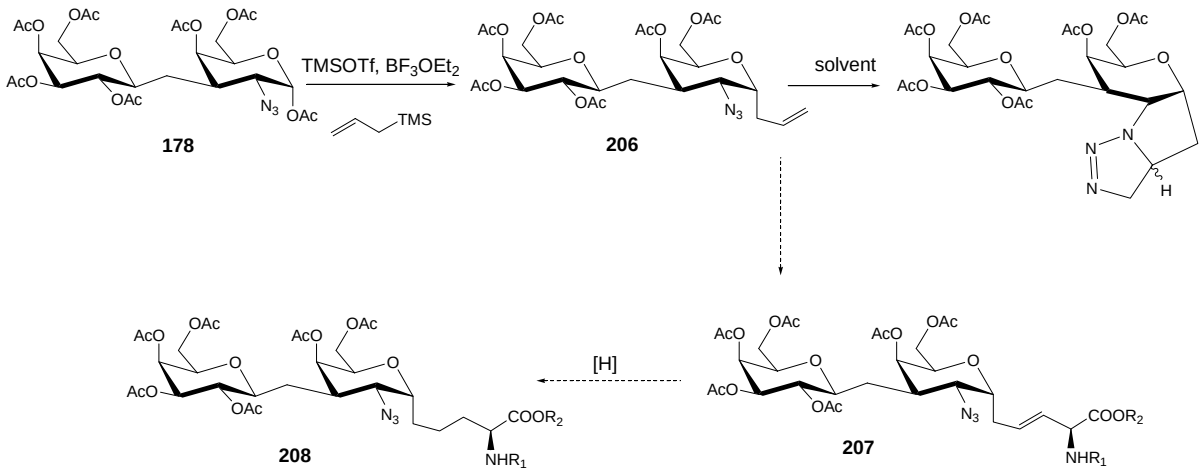
As we have seen in section 1.2.1 several important sugar epitopes containing the 2,6-Sialyl moieties. With the isolation of C-disaccharide **175** we have the possibility, following chemistry developed by Beau and co-workers,[109] to transform it into a *C*,*C*-trisaccharide of type **204** (Scheme 4.36). For that, liberation of the hydroxylmethyl group in **175** by treatement with Bu_4NF and subsequent Swern oxidation should generate aldehyde **201**. one carbon homologation with ylide Ph_3P=CHOMe will provide key aldehyde **202a** which ready for samarium-Reformatsky coupling with a protected sialyl acid **202b**. a mixture of diastereomeric alcohols **203a** should be deoxygenated under standard conditions to generate the protected C,C-trisaccharide **203c**. This compound should be suitable for α-O-glycosidation of semi- protected L-serine. Compound **205** obtained after the reduction of azide moiety should be used to generate clusters and their conjugates with KLH.



Scheme 4.37: Proposal toward the synthesis of 2,6 ST-antigen analogue C

4.4.2 Toward a *Gal-β-C(1→3)-GalNAc-α-C-aminoacid*

Danishefsky and co workers[172] have shown that the vaccines made out of O-disaccharide conjugated with KLH have similar activity independently from the length of linker connecting the disaccharide and the peptide mimetic.[171] We have thus envisioned to use our C-disaccharide **178** in a C-glycosidation with an allylsilane under acidic conditions (Me_3SiOTf, $BF_3{\cdot}OEt_2$). This experiment has been done and generated the expected α-C-glycoside **206**. Unfortunately its purification has been problematic up to now, most probably because of the rapid intermolecular dipolar cycloaddition between the propene moiety and the 2-azido substituent. Once the condition will be found to avoid this intermolecular cycloaddition, well established routs are available to convert alkene **206** into **207** and then further into **208** (Scheme 4.38).



Scheme 4.38: Proposal Toward a Gal-β-C(1→3)-GalNAc-α-C-aminoacid

5 CONCLUSION AND SUMMARY

Our work was initiated on the hypothesis that, the *C*-linked disaccharide would constitute better cancer vaccines as they would be expected to be more stable towards hydrolysis via glycosidase in the blood stream. Given the conformational similarity between *C*- and *O*-disaccharide, and the expected higher biological activity of *C*-linked disaccharide, we sought to design and synthesize potential cancer vaccines based on *C*-linked disaccharide analogue of prostate cancer antigens. Towards achieving this goal, we have been able to accomplish the following:

We have reported an efficient synthesis of *C*-linked disaccharide α-conjugate with L-Serine for the first time. Our flexible synthetic strategy allows us to produce two non-hydrolysable *C*-disaccharide analogues of Thomsen-Friedenreich antigen. The fully protected TF-antigens analogues *N*-[(9*H*-Fluoren-9-yl-methoxy)carbonyl]-{4,6-di-*O*-acetyl-3-*C*-[(1*R*)-2,6-anhydro–1,3,4,5,7-penta-*O*-acetyl-D-*glycero*-L-*manno*-heptitol-1-*C*-yl]-2-[(*N*-acetyl)amino]-2,3-dideoxy-*α*-D-*galacto*pyranosyl}-L-serine *tert*-butyl ester **(144)**, and *N*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-{4,6-*O*-Acetyl-3-*C*-[2,6-anhydro-1-deoxy-3,4,5,7-tetra-*O*-acetyl-D-*glycero*-L-*manno*-heptitol-1-*C*-yl]-2-Acetamido-2,3-dideoxy-*α*-D-*galacto*-pyranosyl}-L-serine *tert*-butyl ester **(181)** have been employed in the preparation of cluster form.

We have employed TF-antigen analogue (**144**) successfully in preparation of a fully deprotected thio-glycotripeptide *N*-acetyl-*O*-{*α*-D-glyco}-D-seryl-*O*-{*α*-D-glyco}-D-seryl-*rac*-*N*-(3-[(acetylthio)amino]propyl)-*O*-{*α*-D-glyco}-D-serinamide (**187b**), the latter was conjugates to the KLH carrier via MBS by Michael addition. The ratio of glycopeptide-to-protein for conjugate **187b-MBS-KLH** was determined to be 45:1. The conjugate molecule derived during the study reported here, will find use as anti-prostate cancer vaccine candidates, molecular probes for epitope mapping of anti-prostate cancer antibodies and for other biological studies, to reveal the minimal structural requirements that convey protection from prostate cancer.

We developed an efficient synthetic strategy of the a key intermediate 4-*O*-acetyl-6-[*O*-(tri-isopropylsyliyl)]-3-*C*-[2,6-anhydro-1-deoxy-3,4,5,7-tetrakis-*O*-acetyl-D-*glycero*-L-*manno*-

heptitol-1-*C*-yl]-2-azido-2,3-dideoxy-α,β-D-*galacto*-hexopyranose thiophenol (**175**) which can be applied to the further synthesis of a *C,C*-trisaccharide analogue of the 2,6 ST-antigen which does not require any complicated synthetic step. The possibility of rapid convergent of intermediate into *C, C*-linked trisaccharide 2,6-ST-antigen analogue should facilitate the construction of a host of glycopeptide conjugates for biological evaluation.

The fully deprotected TF-antigen analogues 3-*C*-[2,6-anhydro-1*R*-D-*glycero*-L-*manno*-heptitol-1-*C*-yl]-2-[(*N*-acetyl)amino]-2,3-dideoxy-α-D-*galacto*-pyranosyl-L-serine **145** and 3-*C*-[2,6-anhydro-1-deoxy-D-*glycero*-L-*manno*-heptitol-1-*C*-yl]-2-[(*N*-acetyl)amino]-2,3-dideoxy-α-D-*galacto*-pyranosyl-L-serine **182** were prepared and will be used in conformational studies to make a comparison between *C*-disaccharide TF-antigen analogue and native TF-antigen, this studied will give us in the future an idea about the possible required change on these analogues in order to reach to maximum biological activity.

We have used successfully for the first time methoxybenzylamine as Michael donor without using any catalyst. Additionally Michael adduct 1,6-anhydro-3-{(1*R*)-2,6-anhydro-3,4,5,7-tetrakis-*O*-[(*tert*-butyl)dimethylsilyl]-D-*glycero*-L-*manno*-heptitol-1-*C*-yl}–2-[(*N*-benzyl-*N*-methoxyl)amino]2,3-dideoxy-β-D-*xylo*-hexopyran-4-ulose **(131a)** has been obtained pure after isomerization during the chromatography on silica gel and, we have employed successfully this amine as azide precursor.

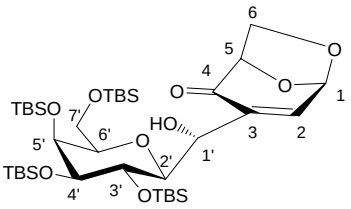
The biological activity of conjugate **187b-MBS-KLH** on prostate cancer is currently under investigation in collaboration with Prof. P. O. Livingston group, USA; Current studies will examine the production of prostate cancer antibodies upon treatment with **187b-MBS-KLH**. We hope that these studies will shed some light on the activities of these compounds and the possibilities of developing effective vaccine based therapeutic strategies using our approach.

EXPERIMENTAL SECTION

General Remarks

Reagents were purchased from Acros, Fluka, Senn, Aldrich or Merk and used without further purification. All solvents for extraction and chromatography were distilled prior to use. Anhydrous THF and Et_2O and toluene were distilled from sodium and benzophenone, CH_2Cl_2 from CaH_2 and methanol magnesium. Reactions were monitored by TLC (Merk Kiesegel 60F254) silica gel plates; detection with UV(254 nm) light or molybdic reagent (21g of $(NH_4)_6Mo_7O_{24}.4H_2O$, 1g of $Ce(SO_4)2$, 31 ml H_2SO_4 and 470 ml of H_2O). Flash chromatography (FC) used 230-400 mesh silica gel (Merk No.9385). Melting points were measured with a Mettler FP52 and were uncorrected. Optical rotations were measured with a JASCO DIP-370 digital polarimeter. UV spectra were recorded on a Kontron Uvikon 810 CW spectrophotometer. IR spectra were recorded on Perkin Elmer Paragon 1000 FT-IR spectrometer. Mass spectra were recorded on a Nermag R 10-10C in chemical ionisation mode. Electron spray mass analyses were recorded on a Finnagan MAT.SSQ 710C spectrometer in positive ionisation mode.^{1}H-NMR spectra were recorded on Bruker DPX-400 FT, Bruker ARX-400 FT, or AMX-600 spectrometers; all ^{1}H signal assignments were confirmed by COSY spectra. ^{13}C-NMR spectra were recorded on Bruker DPX-400 FT (100.61 MHz), Bruker ARX-400 FT (100.61 MHz) machines; all ^{13}C signal assignments were confirmed by HMQC spectra. Chemical shifts in ppm, relative to internal standard such as residual signals of solvents, coupling constants in hertz. High resolution FAB mass spectra were recorded on a FAB-LSIMS (Uniservidad de Sevilla – Spain). Microanalyses were performed by the Ilse Beetz Laboratory, Kronach (Germany).

1,6-Anhydro-3-{(1*R*)-2,6-anhydro-3,4,5,7-tetrakis-*O*-[(*tert*-butyl)dimethylsilyl]-D-*glycero*-L-*manno*-heptitol-1-*C*-yl}-2,3-dideoxy-*β*-D-*glycero*-hex-2-enopyran-4-ulose (97a).



97a

A solution of Et_2AlI 1M in toluene (12 mL, 12.0 mmol) was added dropwise to a mixture of aldehyde **95** (6.0 g, 9.4 mmol) and isolevoglucosenone **96** (2.0 g, 15.9mmol) in CH_2Cl_2 (25 mL) at –78 ^oC. The solution was stirred at this temperature for 2h and diluted with Et_2O (500ml). A 2M aq. soln. HCl (50 mL) was added. The aq. phase was extracted with Et_2O (2 × 200ml). The combined organic phases were washed sequentially with 2M aq. soln. HCl (50 mL), H_2O (50 mL), and brine (50 mL), dried over ($MgSO_4$) and concentrated, FC (9:1, light petroleum ether / EtOAc) afforded 6.89 gm (96 %) of **97a**, colorless oil.

$[\alpha]^{25}_{589}$ = +79, $[\alpha]^{25}_{577}$ = +85, $[\alpha]^{25}_{435}$ = +334, $[\alpha]^{25}_{405}$ = +710 (*c* 0.24, $CHCl_3$).

IR (film) 3400, 2950, 2860, 1695, 1465, 1360, 1260, 1090, 830, 770 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ

7.39 (dd, ^3J (H-C(1), H-C(2)) = 3.5 , ^4J (H-C(2), H-C(1')) = 1.0 , H-C(2)); 5.83 (d, ^3J (H-1, H-C(2)) = 3.5 , H-C(1)); 4.75 (dd, ^3J (H-C(5), H-C(6)) = 6.4 , ^3J (H-C(5), H-C(6)) = 1.3 Hz, H-C(5)); 4.45 (d, ^3J(H-C(3'), H-C(4')) = 8.6, H-C(3')); 4.26 (d, ^2J = 12.4 , H_a-C(7')); 4.23 (ddd, ^3J (H-C(1'), H-C(2')) = 7.0, ^3J (H-C(1'), OH-C(1')) = 6.7, ^4J (H-C(2), H-C(1')) = 1.0, H-C(1')); 4.06 (dd, ^2J=8.3, ^3J (H-C(5), H_{exo}-C(6)) = 6.4, H_{exo}-6); 3.99 (d, ^3J (H-C(3'), H-C(4'))= 8.6, H-C(4')); 3.96 (d, ^3J (H-C(5'), H-C(6')) = 4.1, H-C(5')); 3.91 (t, ^{3}J(H-C(1'), H-C(2')) = ^3J (H-2', H-3') = 7.0, H-C(2')); 3.80 (dd, ^3J (H-C(5'), H-C(6')) = 4.1 , ^3J (H-C(6'), H_b-(7')) = 1.9 Hz, H-C(6')); 3.75 (dd, ^2J=12.7, ^3J (H-C(6'), H_b-C(7')) = 1.9, H_b-(7')); 3.65 (dd, ^2J= 8.3, ^3J (H-C(5), H_{endo}-C(6)) = 1.3, H_{endo}-C(6)); 2.94 (d, ^3J (H-C(1'), OH-C(1') = 6.7, OH-C(1')); 0.94, 0.93, 0.90, 0.90 (4s, 9H 4, ^{t}-Bu × 4), 0.14, 0.12, 0.10, 0.09, 0.08, 0.07, 0.06, 0.05 (8s, 3H × 8, $SiCH_3$ × 8);

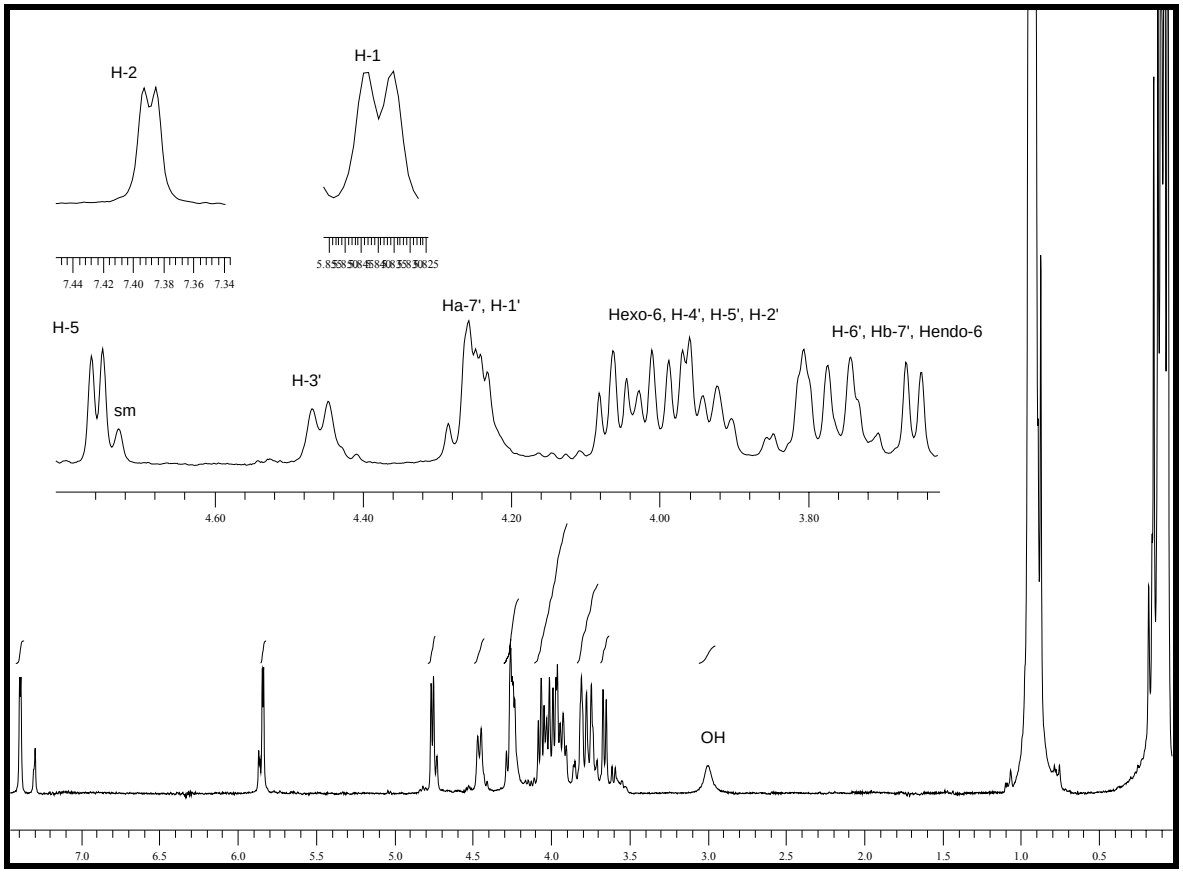
^{13}C NMR (100.6 MHz, $CDCl_3$): δ

194.5 (s, C(4)); 143.6 (d, ^1J(C,H) = 165, C(2)); 137.0 (s, C(3)); 96.9 (d, ^1J(C,H) = 177, C(1)); 79.7 (d, ^1J(C,H) = 141, C(2')); 79.1 (d, ^1J(C,H) = 160, C(5)); 73.1 (d, ^1J(C,H) = 143, C(6')); 70.5 (d, ^1J(C,H) = 144, C(5')); 67.3 (d, ^1J(C,H) = 142, C(3')); 67.1 (d, ^1J(C,H) = 147, C(1')); 66.9 (d, ^1J(C,H) = 138, C(4')); 62.3 (t, ^1J(C,H) = 154, C(6)); 58.2 (t, ^1J(C,H) = 142, C(7')); 25.9, 25.89, 25.85, 25.7(4q, ^1J (C,H) = 125, $(\underline{CH_3})_3$CSi); 18.2; 18.1, 17.9; 17.0 (4s, $(CH_3)_3\underline{C}$Si); -4.4; -4.6; -4.7; -4.9, -5.0; -5.1, -5.3; -5.5; (8q, ^1J (C,H) = 118 , $\underline{CH_3}$Si);

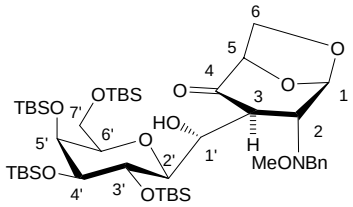
MALDI-HRMS Calcd for (M + Na) $C_{37}H_{74}O_9Si_4Na$ 797.4307; found 797.4310.

Anal. calcd for $C_{37}H_{74}O_9Si_4$ (775.32): C 57.32, H9.62; found: C 57.24, H 9.56.

1H-NMR (400 MHz, $CDCl_3$) spectrum of **97a:**



1,6-Anhydro-3-{(1*R*)-2,6-anhydro-3,4,5,7-tetrakis-*O*-[(*tert*-butyl)dimethylsilyl]-D-*glycero*-L-*manno*-heptitol-1-*C*-yl}–2-[(*N*-benzyl-*N*-methoxyl)amino]2,3-dideoxy-*β*-D-*xylo*-hexopyran-4-ulose 131a.



131a

A solution of **97a** (200 mg, 0.258 mmol) in CH_2Cl_2 (1ml) was stirred at rt. Then BnNHOMe (120 mg, 0.876 mmol) was added. The mixture was allowed to stir at ambient temperature for 12 h. Slow chromatography on silica gel (9:1 , light petroleum ether/ether), afforded 193 mg (82 %) of **131a**, colorless oil.

$[\alpha]^{25}_{589} = -1.7$, $[\alpha]^{25}_{577} = -2$, $[\alpha]^{25}_{435} = -21$, $[\alpha]^{25}_{405} = -37$ (*c* 0.40, $CHCl_3$).

IR (film): 3550, 2955, 2855, 1720,1470, 1265, 1130, 880, 780, 740 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ

7.46-7.43 (m, 2H,.ArH); 7.38-7.26 (m, 3H, ArH); 6.07 (s, H-C(1)); 4.50 (dd, ^3J (H-5, H_{exo}-C(6)) = 5.8, ^3J (H-5, H_{endo}-C(6)) = 0.9 , H-5); 4.27(dd, ^3J (H-C(1'), H-C(2')) = 6.6, ^3J (H-C(1'), H-C(3)) = 2.6, H-C(1')); 4.23 (d, ^2J = 13.0, H_a-C(N$\underline{CH_2}$Ph)); 4.19-4.10(m, 3H, H-C(6'), H_a-C(7'), H-C(5')); 3.95 (d, ^3J(H-C(3'), H-C(4')) = 4.2 , H-C(3')); 3.87 (d, ^2J = 13.0, H_b-C(N$\underline{CH_2}$Ph)); 3.84 (d, ^2J = 7.6, H_{endo}-C(6)); 3.85-3.80 (m, 2H, H-C(4'), H-C(2')); 3.74 (dd, ^2J = 7.6, ^3J (H_{exo}-C(6), H-C(5)) = 5.8 , H_{exo}-C(6)); 3.70 (dd, ^2J = 12.9, ^3J (H_b-C(7'), H-C(6')) = 1.2, H_b-C(7')); 3.45 (d, ^3J (H-C(2), H-C(3)) = 9.83, H-C(2)); 3.25 (m, 4H, H-C(3), 3H-C(O$\underline{CH_3}$)); 0.96, 0.93, 0.92, 0.87 (4s, 36H, 36H-C(SiC($\underline{CH_3}$)$_3$)); 0.14, 0.13, 0.13, 0.12, 0.11, 0.10, 0.10, 090, (8s, 24H, 24H-C(Si$\underline{CH_3}$));

^{13}C NMR (100.6 MHz, $CDCl_3$):δ

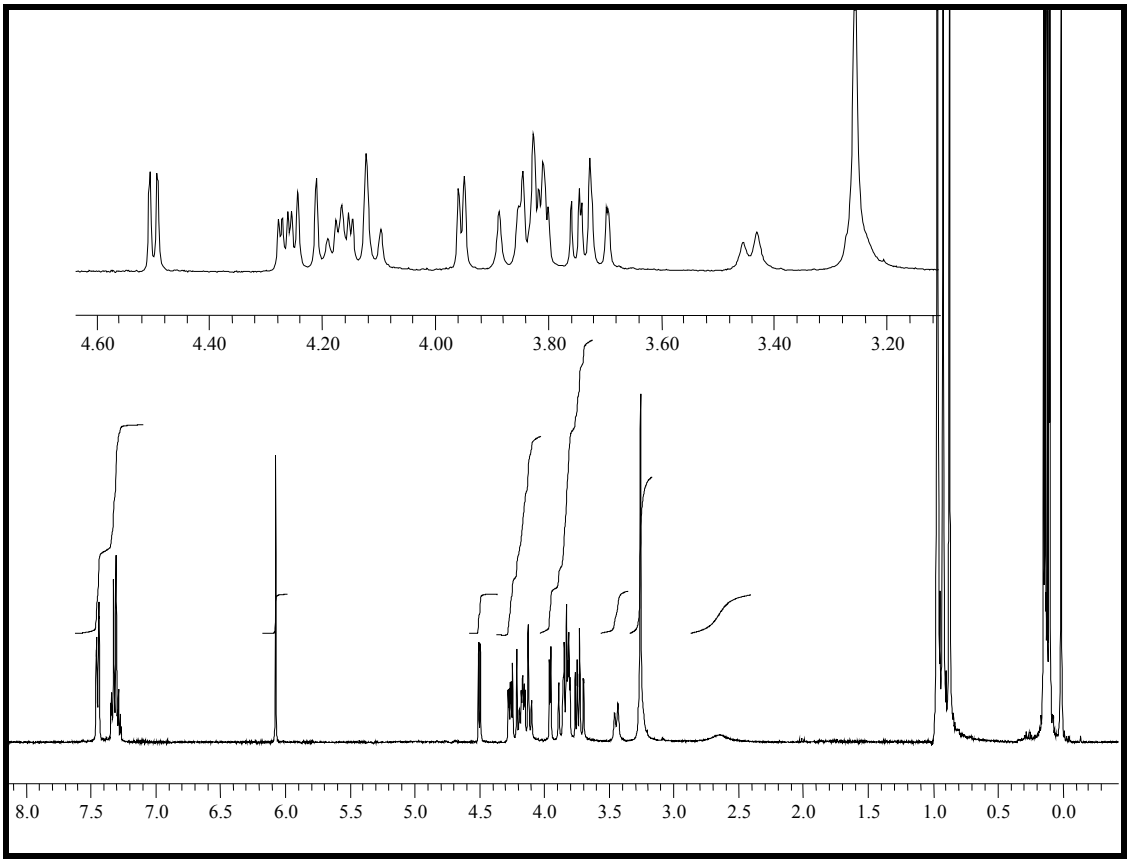
214.0 (s, C(4)); 137.0 (s, C(arom)); 129.8 (d, ^1J(C,H) = 159, C(arom)); 128.1 (d, ^1J(C,H) = 156, C(arom)); 127.3 (d, ^1J(C,H) = 159, C(arom)); 100.3 (d, ^1J (C,H) = 179, C(1)); 79.5 (d, ^1J (C,H) = 146, C(2')); 78.4 (d, ^1J (C,H) = 159, C(5)); 73.3 (d, ^1J (C,H) = 154, C4'); 70.2 (d, ^1J(C,H) = 141, C(3')); 67.4 (d, ^1J(C,H) = 144, C(1')); 70.4 (d, ^1J(C,H) = 146,

C(5')); 67.0 (t, ^1J(C,H) = 154, C(6)); 66.7 (d, ^1J(C,H) = 147, C(6')), 64.9 (d, ^1J(C,H) = 126, C(2)); 62.0 (q, ^1J(C,H) = 125, C(O<u>C</u>H$_3$)); 58.5 (t, ^1J(C,H) = 143, C(7')); 46.3 (d, ^1J(C,H) = 143 , C(3)), 25.9, 25.89, 25.85, 25.7(4q, ^1J (C,H) = 125, (<u>CH$_3$</u>)$_3$CSi); 18.2; 18.1, 17.9; 17.0 (4s, (CH$_3$)$_3$<u>C</u>Si); -4.4; -4.6; -4.7; -4.9, -5.0; -5.1, -5.3; -5.5; (8q, ^1J (C,H) = 118 , <u>CH$_3$</u>Si).

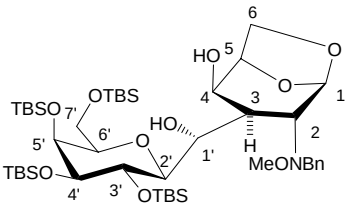
MALDI-HRMS Calcd for (M + Na) $C_{45}H_{85}NO_{10}Si_4Na$ 934.5148; found 934.5134.

Anal. calcd for $C_{45}H_{85}NO_{10}Si_4$ (912.50): C 59.23, H9.39, N 1.53; found: C 59.33, H 9.33, N 1.55.

1H-NMR (400 MHz, $CDCl_3$) spectrum of **131a:**



1,6-Anhydro-3-{(1*R*)-2,6-anhydro-3,4,5,7-tetra-*O*-[(*tert*-butyl)dimethylsilyl]-D-*glycero*-L-*manno*-heptitol-1-*C*-yl}–2-[(*N*-benzyl-*N*-methoxy)amino]-2,3-dideoxy-*β*-D-*galacto*-hexopyranose (132).



132

A THF solution of $LiBH_4$ (7.0 mL, 14.0 mmol) was added dropwise to a solution of **131a** (3.50 mg, 3.84 mmol) in 50 ml of THF at –78 ^oC. The mixture was stirred at - 78 ^oC to 15 ^oC for 4h. An aq. NH_4Cl solution (25 ml) was added. The reaction mixture was warmed to 20 ^oC. An aq. solution of sodium potassium tartrate (25ml) was added. The aq. phase was extracted with CH_2Cl_2 (100 mL/ 3 times). The combined org. phases were dried, concentrated *in vacuo*. FC(4:1 , light petroleum ether/Et_2O) gave 3.22 g (92 %) of **132**, colorless oil.

$[\alpha]^{25}_{589}$ = +26, $[\alpha]^{25}_{577}$ = +28, $[\alpha]^{25}_{435}$ = +49, $[\alpha]^{25}_{405}$ = +57 (*c* 0.28, $CHCl_3$).

IR (film): 3475, 2955, 2855, 1715, 1635, 1470, 1255, 1100, 835, 780, 740 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$): δ

7.45-7.39 (m, 2H,.ArH); 7.38-7.27 (m, 3H, ArH); 5.84 (s, H-C(1)); 4.84 (m, H-C(4)), 4.54 (dd,^3J(H-5, H-C(4)) = 7.0, ^3J (H-C(5), H_{exo}-C(6)) = 5.4, H-C(5)); 4.43 (dd, ^2J = 11.5, ^3J (H_a-C(7'), H-C(6')) = 9.9, H_a-C(7')); 4.37-4.30 (m, 2H, H_{endo}-C(6), H-C(1')); 4.27 (dd, ^3J(H-C(5'), H-C(6')) = 6.4, ^3J (H-C(5'), HC-(4')) = 2.6, H-C(5')); 4.13 (d, ^3J(H-C(2'), H-(1')) = 9.3, H-C(2')); 3.64 (d, ^2J = 13.1, H_a-C(N$\underline{CH_2}$Ph)); 3.98-3.89 (m, 3H, H-C(3'), H-C(6'), H_b-C(N$\underline{CH_2}$Ph)); 3.72 (dd, ^3J(H-C(4'),H-C(3')) = 3.5, ^3J (H-C(4'), H-C(5')) = 2.6, H-C(4')); 3.80 (dd, ^2J = 11.5, ^3J (H_b-C(7'), H-C(6')) = 1.9, H_b-C(7')); 3.51 (dd, ^2J = 7.4, ^3J(H-5, H_{exo}-C(6)) = 5.4, H_{exo}-C(6)), 3.3 (s, 3H, H-C(O$\underline{CH_3}$)); 4.24 (d, ^3J(H-C(2), H-C(3)) = 9.6, H-C(2)); 2.64 (ddd, ^3J(H-3, H-2) = 9.6, ^3J(H-3, H-4) = 6.7, ^3J (H-C(3), H-C(1')) = 2.6, H-C(3)); 0.98, 0.95, 0.94, 0.93 (4s, 36H, 36H-C(SiC($\underline{CH_3}$)$_3$)); 0.16, 0.15, 0.14, 0.13, 0.120, 0.12, 0.11,0.10 (8s, 24H, 24H-C(Si$\underline{CH_3}$)).

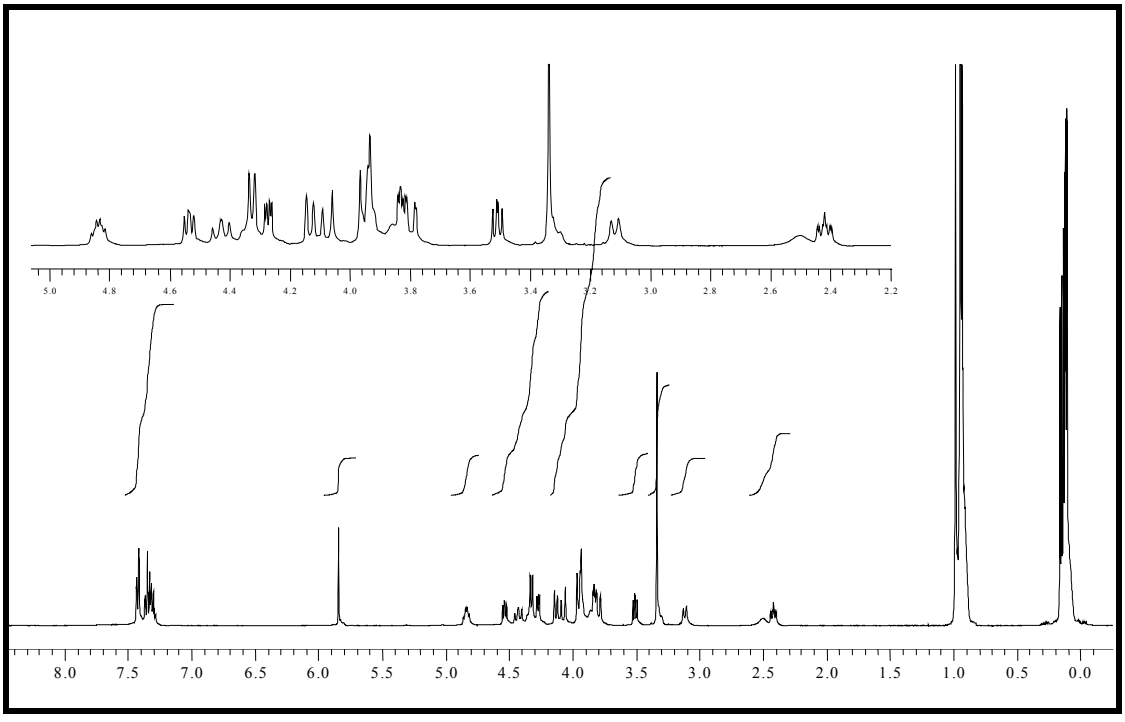
^{13}C NMR (100.6 MHz, $CDCl_3$):δ

137.6 (s, C(arom)); 129.6 (d, ^1J(C,H) = 159, C(arom)); 128.3 (d, ^1J(C,H) = 156, C(arom)); 127.3 (d, ^1J(C,H) = 156, C(arom)); 99.6 (d, ^1J (C,H) = 179, C(1)); 79.1 (d, ^1J(C,H) = 147, C(6')); 73.6 (d, ^1J (C,H) = 159, C(5)); 73.4 (d, ^1J (C,H) = 154, C4'); 70.7 (d, ^1J(C,H) = 141, C(3')); 69.0 (d, ^1J(C,H) = 144, C(1')); 67.4 (d, ^1J (C,H) = 146, C(2')); 67.3 (d, ^1J(C,H) = 146, C(5')); 66.3 (t, ^1J(C,H) = 144, C(4)); 64.2 (d, ^1J(C,H) = 143 , C(2)); 61.6 (t, ^1J(C,H) = 154, C(6)); 61.6 (q, ^1J(C,H) = 122, C(O<u>C</u>H$_3$)); 58.5 (t, ^1J(C,H) = 143, C(7')); 58.5 (t, ^1J (C,H) = 145, C(N<u>C</u>H$_2$Ph); 34.9 (d, ^1J(C,H) = 126, C(3)); 26.0, 25.9, 25.8, 25.7(4q, ^1J (C,H) = 125, (<u>C</u>H$_3$)$_3$CSi); 18.3; 18.0, 17.9; 17.8 (4s, (CH$_3$)$_3$<u>C</u>Si); -4.4; -4.6; -4.8; -4.9, -5.0; -5.1, -5.3; -5.5; (8q, ^1J (C,H) = 118 , <u>C</u>H$_3$Si).

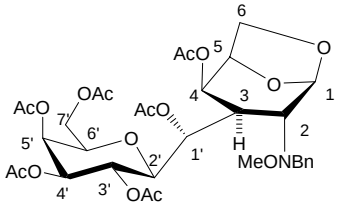
MALDI-HRMS Calcd for (M + Na) $C_{45}H_{87}NO_{10}Si_4Na$ 936.5304; found 936.5304.

Anal. calcd for $C_{45}H_{87}NO_{10}Si_4$ (914.52): C 59.10, H9.59, N 1.53; found: C 59.15, H 9.50, N 1.62.

1H-NMR (400 MHz, $CDCl_3$) spectrum of **132:**



4-O-Acetyl-1,6-anhydro-3-{(1*R*)-1,3,4,5,7-penta-*O*-acetyl-2,6-anhydro-D-*glycero*-L-manno-heptitol-1-*C*-yl}-2-[(*N*-benzyl-*N*-methoxy)amino]-2,3-dideoxy-*β*-D-*galacto*-hexopyranose (133).



133

Compound **132** (1.12g, 1.22 mmol) was dissolved in THF (3 mL), a solution of TBAF in THF (8 ml g, 9.76 mmol) was added, and the solution was stirred for 3 h at 20 ^{o}C. The mixture was evaporated to dryness, then the residue was dissolved in pyridine (5 ml). A catalytic amount of DMAP was added, then acetic anhydride (3ml), and the solution was stirred for 2 days at 20 ^{o}C. MeOH (5 ml) was added, the mixture was evaporated to dryness, then dissolved in EtOAc (25 mL). The solution was quenched with aqueous HCl (10 ml), washed with a sat. aq. solution of $NaHCO_3$ (5 mL), water and brine and dried ($MgSO_4$). Evaporation of the filtrate and FC gave 870 mg (100 %) of **133**, colorless oil.

$[\alpha]^{25}_{589} = +73$, $[\alpha]^{25}_{577} = +77$, $[\alpha]^{25}_{435} = +147$, $[\alpha]^{25}_{405} = +176$ (*c* 0.18, $CHCl_3$).

IR (film): 3060, 2985, 2895, 1750, 1665, 1430, 1370, 735, 700 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ

7.47-7.42 (m, 2H, H-C(arom)); 7.33-7.22 (m, 6H, H-C(arom)); 5.87-5.77 (m, 3H, H-C(1'), H-C(1), H-C(4)); 5.33 (dd, ^3J(H-C(6'), H-C(5')) = 6.2, ^3J(H-C(6'), H$_a$-C(7')) = 3.1, H-C(6')); 5.25 (dd, ^3J(H-C(3'), H-C(4')) = 4.0,^3J(H-C(3'), H-C(2')) = 3.7, H-C(3')); 4.84 (dd, ^3J(H-C(4'), H-C(3')) = 4.0, ^3J(H-C(4'), H-C(5')) = 1.2, H-C(4')); 4.72 (dd, ^3J(H-C(5), H-C(4)) = 7.7,^3J(H-C(5), H$_{exo}$-C(6)) = 4.6,H-C(5)); 4.68 (d, ^2J = 12.9, H$_a$-C(7')); 4.29-4.20 (m, 3H, H$_b$-C(7'), H-C(2'), H-C(5')); 4.02 (d, ^2J = 12.9, H$_a$-C(NCH$_2$Ph); 3.95 (d, ^2J = 7.4, H$_{endo}$-C(6)); 3.79 (d, ^2J = 12.9, H$_b$-C(NCH$_2$Ph); 3.42 (dd, ^2J=7.4, ^3J(H$_{exo}$-C(6),H-C(5)) = 4.6, H$_{exo}$-C(6)); 3.20 (s, 3H, H-C(O<u>CH$_3$</u>); 2.72 (m, H-C(2); 5.31 (ddd, ^3J(H-C(3), H-

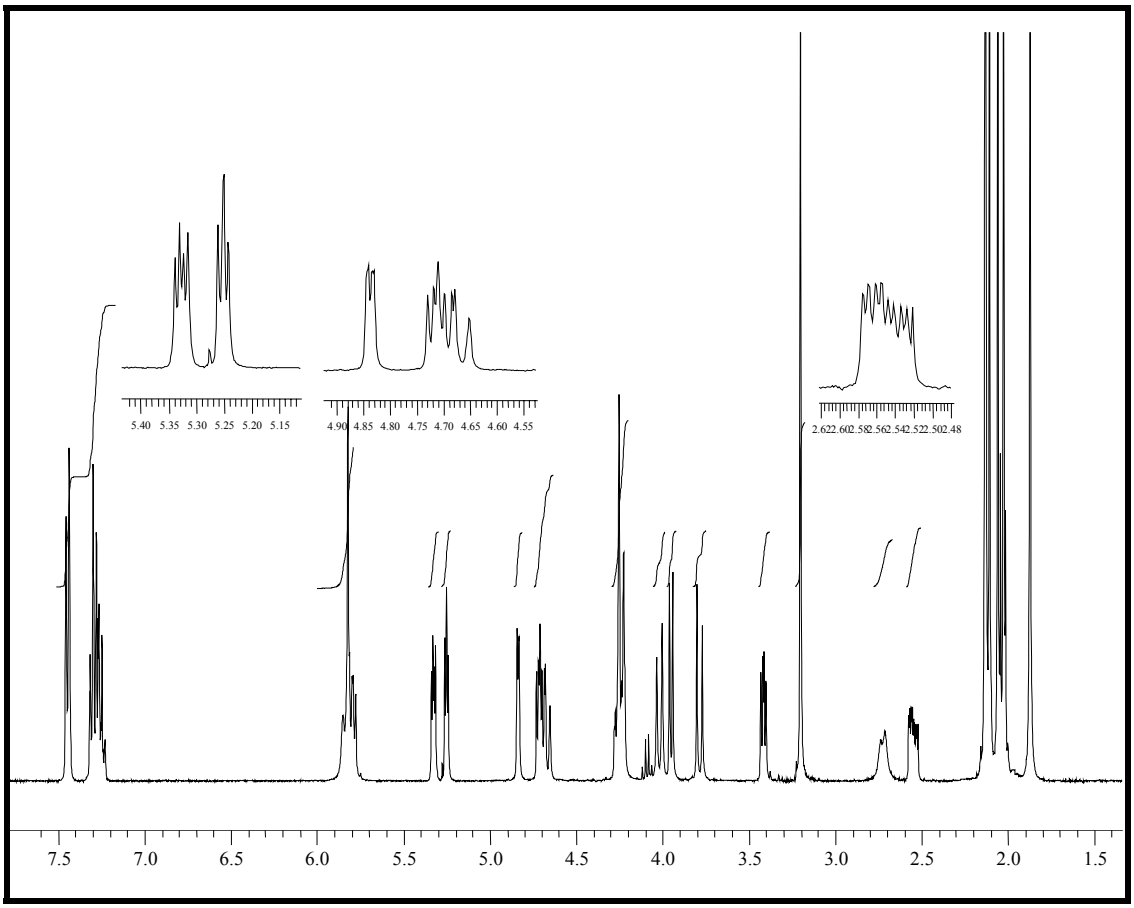
C(2)) = 10.8, ^{3}J(H-C(3), H-C(4)) = 5.5, ^{3}J(H-C(3), H-C(1')) = 2.6, H-C(3)); 2.13-1.87 (6s, 18H, H-C($\underline{CH_3}$COO)).

^{13}C NMR (100.6 MHz, $CDCl_3$):

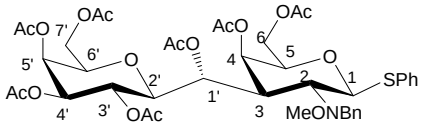
δ 170.7, 170.1, 169.8, 169.4, 169.2, 168.8 (6s, C($CH_3\underline{C}$OO)); 137.3 (s, C(arom)); 129.9 (d, ^{1}J(C,H) = 159, C(arom)); 128.0 (d, ^{1}J(C,H) = 156, C(arom)); 127.2 (d, ^{1}J(C,H) = 156, C(arom)); 99.3 (d, ^{1}J(C,H) = 179, C(1)); 71.9 (d, ^{1}J(C,H) = 159, C(5)); 71.7 (d, ^{1}J(C,H) = 146, C(2')); 67.3 (d, ^{1}J(C,H) = 144, C(1')); 67.1 (d, ^{1}J(C,H) = 154, C(4')); 66.6 (d, ^{1}J(C,H) = 141, C(3')); 66.3 (d, ^{1}J(C,H) = 144, C(4)); 65.5 (d, ^{1}J(C,H) = 147, C(6')); 65.4 (d, ^{1}J(C,H) = 146, C(5')); 64.5 (d, ^{1}J(C,H) = 143, C(2)); 62.6 (t, ^{1}J(C,H) = 154, C(6)); 62.3 (q, ^{1}J(C,H) = 122, O$\underline{C}H_3$); 59.2 (t, ^{1}J(C,H) = 143, C(7')); 59.2 (t, ^{1}J (C,H) = 145, C(N$\underline{C}H_2$Ph), 33.0 (d, ^{1}J(C,H) = 126, C(3)); 21.0-20.5 (6q, ^{1}J(C,H) = 130, H-C($\underline{CH_3}$COO)).

MALDI-HRMS Calcd for (M + Na) $C_{33}H_{43}NO_{16}Na$ 732.2479; found 732.2469.

1H-NMR (400 MHz, $CDCl_3$) spectrum of **133:**



4,6-Di-*O*-acetyl-3-*C*-[(1*R*)-2,6-anhydro–1,3,4,5,7-penta-*O*-acetyl-D-*glycero*-L-*manno*-heptitol-1-*C*-yl]-2-[(*N*-benzyl-*N*-methoxy)amino]-2,3-dideoxy-*β*-D-*galacto*-hexopyranosyl phenylsulfide (134).



134

A mixture of **133** (0.316 g, 0.450 mmol), (phenylthio)trimethylsilane (0.254 mL, 1.345 mmol), and ZnI_2 , (0.42 g, 1.333 mmol) in dry CH_2Cl_2, (7 mL) was stirred at 20 ^oC for 2 h. The mixture was diluted with CH_2Cl_2 (50 mL) and washed successively with sat. aq $NaHCO_3$, (30 mL), water (20 mL, 3 times), and brine (20 mL), dried ($MgSO_4$), and evaporated. The residue was dissolved in THF (10 mL), TBAF (0.450ml, 0.450 mmol) was added, the mixture was stirred at 20 ^oC for 1h. The solvent was evaporated, and the residue was dissolved in pyridine (10mL), then DMAP (40 mg) and Ac_2O (4mL) were added. The mixture was stirred at 20 ^oC for 18h. The solvent was evaporated, FC gave 370 mg (96%) of **134**, colorless oil.

$[\alpha]^{25}_{589}$ = +24, $[\alpha]^{25}_{577}$ = +27, $[\alpha]^{25}_{435}$ = +47, $[\alpha]^{25}_{405}$ = +53 (*c* 0.21, $CHCl_3$).

IR (film): 2965, 1735, 1435, 1370, 1220, 1040, 1025, 940, 890 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ

7.65-7.55 (m, 4H, H-C(arom)); 7.35-7.25 (m, 6H, H-C(arom)); 6.10 (dd, ^3J(H-C(1'), H-C(3)) = 9.9, ^3J(H-C(1'), H-C(2')) = 1.5, H-C(1')); 5.61 (s, H-C(4)); 6.34 (d, ^3J(H-C(1), H-C(2)) = 8.6, H-C(1)); 5.31 (dd, ^3J(H-C(5'), H-C(6')) = 6.5, ^3J(H-C(5'), H-C(4')) = 3.1, H-C(5')); 5.173 (t, ^3J(H-C(4'), H-C(3')) = 3.4,H-C(4')); 4.86 (d, ^3J(H-C(3'), H-C(4')) = 3.4, H-C(3')); 4.75 (dd, ^2J = 12.9, ^3J(H_a-C(7'), H-C(6')) = 9.9, H_a-C(7')); 4.59 (d, ^2J = 12.6, H_a-C(NCH_2Ph); 4.50 (d, ^3J(H-C(2'), H-C(1')) = 10.2, H-C(2')); 4.38 (dd, ^2J = 12.9, ^3J(H_a-C(7'), H-C(6')) = 1.8, H_b-C(7')); 4.30-4.18 (m, 2H, H_{endo}-C(6), H-C(6')); 4.08 (dd, ^2J=11.4, ^3J(H_{exo}-C(6),H-C(5)) = 7.4, H_{exo}-C(6)); 3.90 (t, ^3J(H-C(5), H_{exo}-C(6)) = 6.8,H-

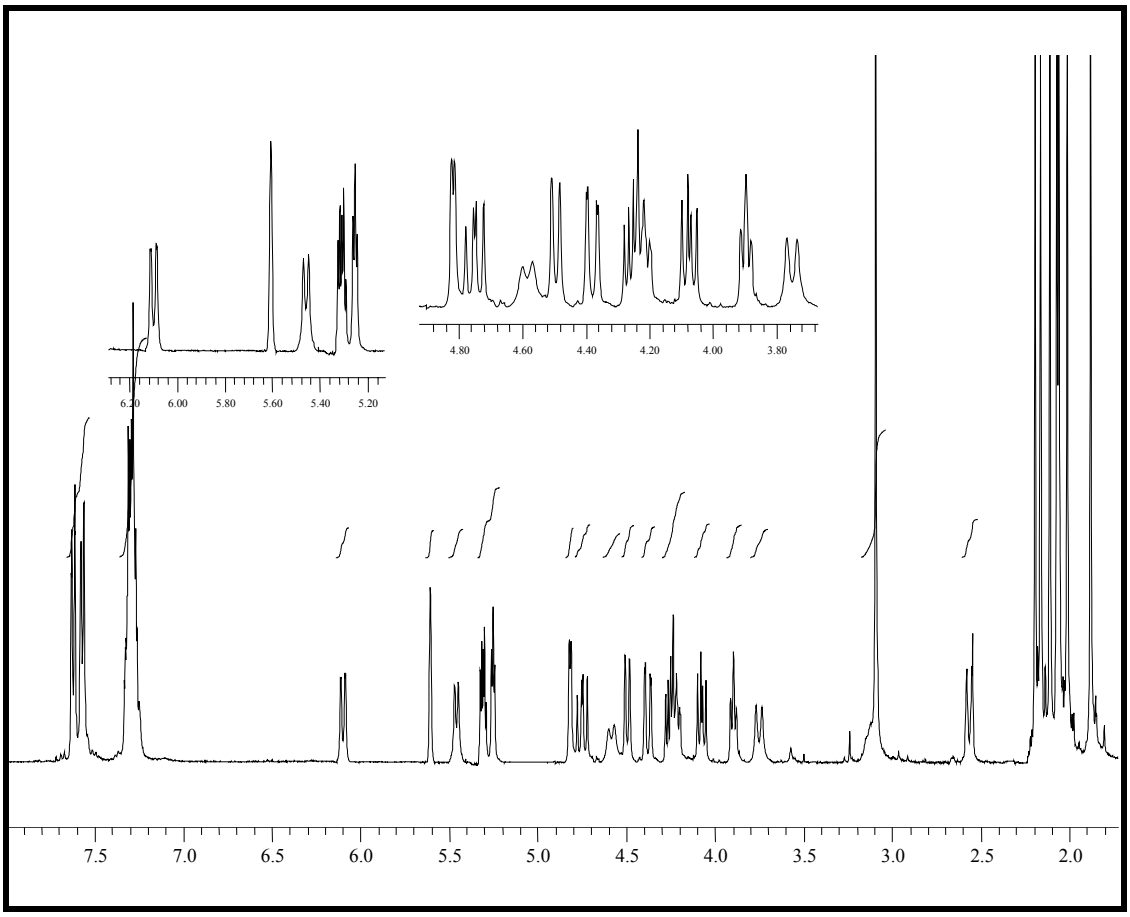
C(5)); 3.75 (d, ^{2}J = 12.6, H_a-C(NCH_2Ph); 3.11-3.08 (m, 4H, H-C($O\underline{C}H_3$), H-C(2)); 2.57 (d, ^{3}J(H-C(3), H-C(2)) = 12.3, H-C(3)); 2.27-1.83 (7s, 21H, CH_3COO).

^{13}C NMR (100.6 MHz, $CDCl_3$): δ *Data for the β anomer:*

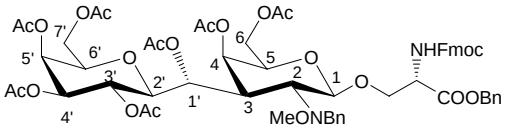
170.6, 170.5, 170.3, 169.8, 169.6, 169.1, 169.0 (7s, -COO); 138.7 (s, C(arom)); 134.0 (s, C(arom)); 131.9 (d, ^{1}J(C,H) = 162, C(arom)); 130.0 (d, ^{1}J(C,H) = 158, C(arom)); 128.7 (d, ^{1}J(C,H) = 159, C(arom)); 128.0 (d, ^{1}J(C,H) = 159, C(arom)); 127.4 (d, ^{1}J(C,H) = 159, C(arom)); 127.3 (d, ^{1}J(C,H) = 159, C(arom)); 83.4 (d, ^{1}J(C,H) = 159, C(1)); 76.2 (d, ^{1}J(C,H) = 139, C(5)); 72.9 (d, ^{1}J(C,H) = 150, C(6')); 67.6 (d, ^{1}J(C,H) = 155, C(1')); 67.1 (d, ^{1}J(C,H) = 155, C(3')); 66.7 (d, ^{1}J(C,H) = 157, C(4')); 65.6 (d, ^{1}J(C,H) = 149, C(5')); 65.4 (d, ^{1}J(C,H) = 150, C(4)); 64.9 (d, ^{1}J(C,H) = 144, C(2')); 63.2 (q, ^{1}J(C,H) = 130, $O\underline{C}H_3$); 62.5 (t, ^{1}J(C,H) = 149, C(6)); 60.8 (d, ^{1}J(C,H) = 139, C(2)); 59.5 (t, ^{1}J(C,H) = 148, C(7')); 41.4 (d, ^{1}J(C,H) = 127, C(3)); 21.3-20.7 (7q, ^{1}J(C,H) = 130, $\underline{C}H_3COO$);

MALDI-HRMS Calcd for (M + Na) $C_{41}H_{51}NO_{17}SNa$ 884.2775; found 884.2776.

1H-NMR (400 MHz, $CDCl_3$) spectrum of **134:**



***N*-[(9*H*-Fluoren-9-ylmethoxy)carbonyl]-{4,6-di-*O*-acetyl-3-*C*-[(1*R*)-2,6-anhydro-1,3,4,5,7-penta-*O*-acetyl-D-*glycero*-L-*manno*-heptitol-1-*C*-yl]-2-[(*N*-benzyl-*N*-methoxy)amino]-2,3-dideoxy-*β*-D-*galacto*pyranosyl}-L-serine benzyl ester (136).**



136

To a solution of **134** (167 mg, 0194 mmol) and *N*-Fmoc-serine-OBn (170 mg, 0407 mmol) in dry CH_2Cl_2 (5 mL) was added pre-activated 4 Å molecular sieves (200 mg) under an Ar atmosphere, then the mixture was stirred for 1 h. After addition of NIS (44 mg, 0194 mmol) and TMSOTf (70 μL, 0.388 mmol) at 0°C, the reaction mixture was continuously stirred at 0°C until **134** was completely consumed on TLC analysis. The mixture was filtered through a pad of Celite, washed with CH_2Cl_2 (50 mL), and the combined filtrate and washings were washed with sat. $NaHCO_3$ aq.(10 mL), sat. $Na_2S_2O_3$ aq. (10 ml) and brine, dried over Na_2SO_4 and concentrated *in vacuo*. FC (hexanes:EtOAc, 4:1 → 3:1) gave 205 mg (92 %) of **136** (β only), white foam.

$[\alpha]^{25}_{589} = +31$, $[\alpha]^{25}_{577} = +32$, $[\alpha]^{25}_{435} = +64$, $[\alpha]^{25}_{405} = +78$ (*c* 0.211, $CHCl_3$).

IR (film): 3330, 2965, 1735, 1515, 1435, 1365, 1215, 1040, 1025, 910 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ

7.81-7.10 (m, 18H, H-C(arom)); 6.84 (d, ^3J(H-N, H-C*(Ser)) = 9.2, H-N); 6.12 (dd, ^3J(H-C(1'), H-C(3)) = 9.9, ^3J(H-C(1'), H-C(2')) = 1.2, H-C(1')); 5.56 (s, H-C(4)); 5.34 (dd, ^3J(H-C(5'), H-C(6')) = 6.5, ^3J(H-C(5'), H-C(4')) = 3.1, H-C(5')); 5.29 (t, ^3J(H-C(4'), H-C(3')) = 3.1,H-C(4')); 5.23 (t, 2H, ^2J = 12.9, H-C(CH_2Ph); 6.34 (d, ^3J(H-C(1), H-C(2)) = 7.7, H-C(1)); 4.81 (m, 1H, H-C(3')); 4.78 (dd, ^2J = 12.5, ^3J(H_a-C(7'), H-C(6')) = 9.9, H_a-C(7')); 4.67 (dt, ^3J(H-C*(Ser),H-N) = 9.2, ^3J(H-C*(Ser),H_2-C(Ser) = 3.1, H-C*(Ser)); 4.63 (d, ^2J = 11.7, H_2-C(Ser)); 4.53-4.39 (m, 4H, H_2- C(Fmoc), H_b-C(7'), H-C(6')); 4.36 (d, ^2J = 12.9, H_a-C(N<u>C</u>H_2Ph); 4.30-4.15 (m, 3H, H-C(2'), H_{endo}-C(6), H-C(Fmoc)); 4.03 (dd, ^2J=11.4, ^3J(H_{exo}-C(6),H-C(5)) = 7.1, H_{exo}-C(6)); 4.63 (dd, , ^2J = 11.7, ^3J(H_2-C(Ser),

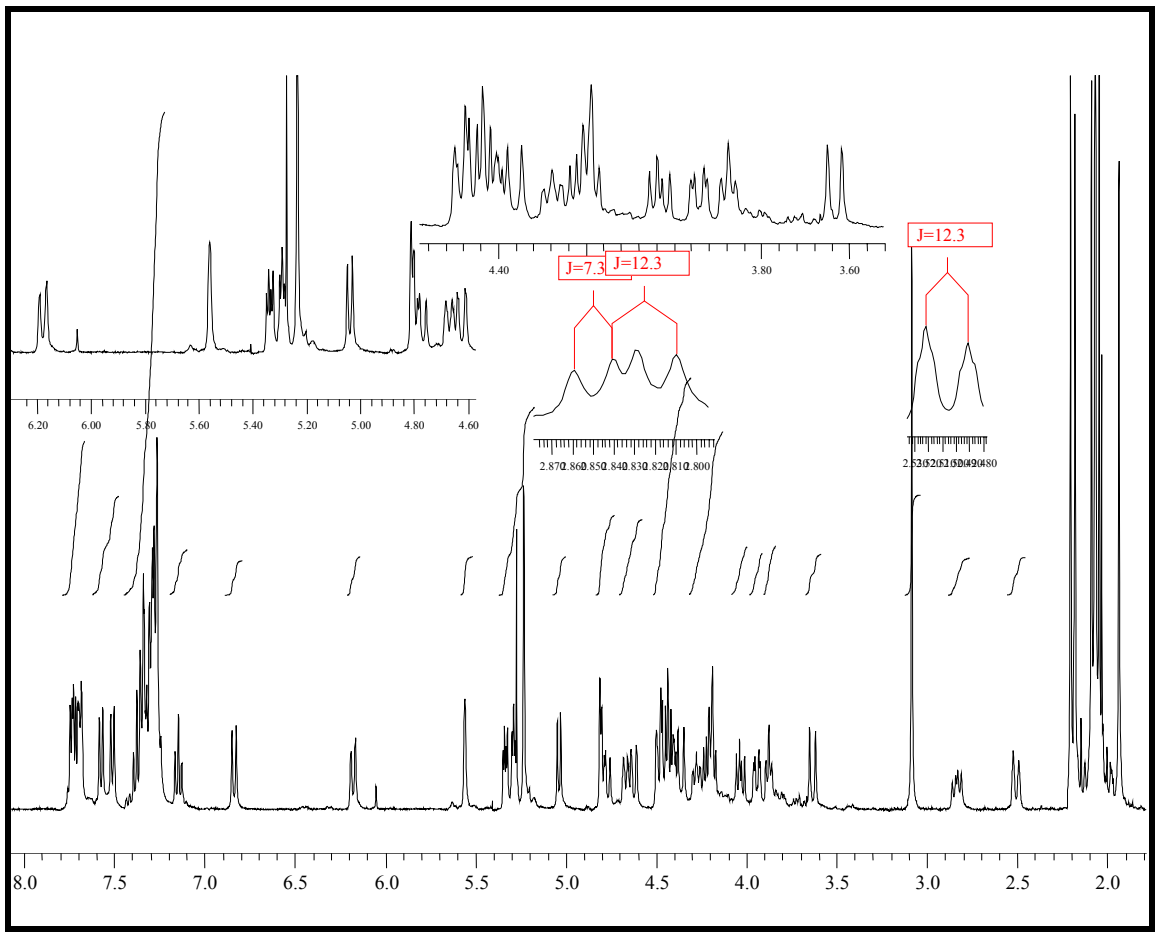
H-C*(Ser)= 3.1 H_2-C(Ser)); 3.88 (t, ^3J(H-C(5), H_{exo}-C(6)) = 6.47,H-C(5)); 3.6 (d, ^2J = 12.6, H_a-C(NCH_2Ph); 3.08 (s, 3H, H-C(OCH_3)); 2.84 (dd, ^3J(H-C(2), H-C(3)) = 12.3, ^3J(H-C(2), H-C(1)) = 7.7, H-C(2)); 2.57 (br dd, ^3J(H-C(3), H-C(2)) = 12.3, H-C(3)); 2.25-1.89 (7s, 21H, CH_3COO).

^{13}C NMR (100.6 MHz, $CDCl_3$): δ

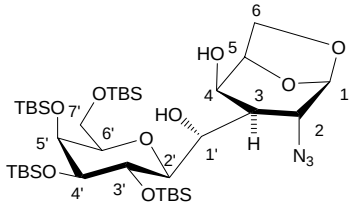
170.5, 170.4, 170.2, 170.1, 169.8, 168.9, 168.7 (7s, -COO); 156.3 (s, C(Carbamat); 143.7 (s, C(arom)); 143.6 (s, C(arom)); 141.2 (s, C(arom)); 141.0 (s, C(arom)); 138.1 (s, C(arom)); 135.1 (s, C(arom)); 130.4 (d, ^1J(C,H) = 162, C(arom)); 128.4 (d, ^1J(C,H) = 158, C(arom)); 128.2 (d, ^1J(C,H) = 158, C(arom)); 128.1 (d, ^1J(C,H) = 158, C(arom)); 128.0 (d, ^1J(C,H) = 159, C(arom)); 128.0 (d, ^1J(C,H) = 159, C(arom)); 127.9 (d, ^1J(C,H) = 159, C(arom)); 127.6 (d, ^1J(C,H) = 159, C(arom)); 127.5 (d, ^1J(C,H) = 159, C(arom)); 127.1 (d, ^1J(C,H) = 159, C(arom)); 126.9 (d, ^1J(C,H) = 159, C(arom)); 124.9 (d, ^1J(C,H) = 159, C(arom)); 119.8 (d, ^1J(C,H) = 159, C(arom)); 103.9(d, ^1J(C,H) = 159, C(1)); 74.3 (d, ^1J(C,H) = 139, C(5)); 72.9 (d, ^1J(C,H) = 144, C(2')); 70.4 (t, ^1J(C,H) = 153, $\underline{C}H_2$(Ser)); 67.4 (t, ^1J(C,H) = 149, $\underline{C}H_2$(Bn)); 67.3 (t, ^1J(C,H) = 150, $\underline{C}H_2$(Fmoc)); 67.3 (d, ^1J(C,H) = 139, C(1')); 67.0 (d, ^1J(C,H) = 155, C(3')); 66.5 (d, ^1J(C,H) = 157, C(4')); 65.6 (d, ^1J(C,H) = 150, C(4)); 65.4 (d, ^1J(C,H) = 149, C(5')); 64.9 (d, ^1J(C,H) = 150, C(6')); 63.4 (q, ^1J(C,H) = 130, O$\underline{C}H_3$); 62.5 (t, ^1J(C,H) = 149, C(N$\underline{C}H_2$Ph)); 61.7 (t, ^1J(C,H) = 148, C(7')); 61.0 (d, ^1J(C,H) = 139, C(2)); 59.5 (t, ^1J(C,H) = 149, C(6)); 54.5 (d, ^1J(C,H) = 138, C*(Ser)); 47.1 (d, ^1J(C,H) = 130, C(Fmoc)); 41.1 (d, ^1J(C,H) = 127, C(3)); 21.1-20.5 (7q, ^1J(C,H) = 130, $\underline{C}H_3COO$).

MALDI-HRMS Calcd for (M + Na) $C_{60}H_{68}N_2O_{22}Na$ 1191.416; found 1191.416.

1H-NMR (400 MHz, $CDCl_3$) spectrum of **136:**



1,6-Anhydro-3-*C*-{(1*R*)-2,6-anhydro-3,4,5,7-tetrakis-*O*-[(*tert*-butyl)dimethylsilyl]-D-*glycero*-L-*manno*-heptitol-1-*C*-yl}-2-azido-2,3-dideoxy-*β*-D-*galacto*-hexopyranose (138)



138

To a stirred mixture of metallic sodium (184 mg, 8.0 mmol, 24 equiv) in liquid NH_3 (10 mL) at -78 °C was added a solution of **132** (300 mg, 0.329 mmol) in dry THF (5 mL). After 30 min at -78 °C, NH_4Cl (540 mg, 10 mmol, 30 equiv) was added and ammonia was allowed to evaporate at 20 °C. After the addition of H_2O (20 ml), the aqueous phase was extracted with CH_2Cl_2 (20 ml, 3 times). The combined org. phases were dried, concentrated in *vacuo*. The residue was dissolved in MeOH (10 ml), then 10% Pd on charcoal (35 mg) was added. The degassed mixture was stirred under H_2 at 20°C for 12 h. The catalyst was filtered off, the solvent evapored and the residue was dissolved in 1 ml of CH_2Cl_2, 1 mg of $CuSO_4$ in 2 ml of H_2O was added, then triethylamine (131 µl, 0.987 mmol) was added, followed by MeOH (7 ml). The dichloromethane solution of trifluoromethanosulfonyl azide freshly prepared (1 ml, 0.6 M, 0.6 mmol) was added at once. The reaction was stirred until TLC showed reaction to be complete. Then the mixture was extracted by CH_2Cl_2 (15 ml, 3 times). These combined org. phases were dried, concentrated *in vacuo*. FC (7:3, light petroleum ether/ether) afforded 220 mg (81 %, 3 steps) of **138**, colorless oil.

$[\alpha]^{25}_{589} = +28$, $[\alpha]^{25}_{577} = +30$, $[\alpha]^{25}_{435} = +57$, $[\alpha]^{25}_{405} = +68$ (*c* 0.296, $CHCl_3$).

IR (film): 3475, 2955, 2855, 2110, 1635, 1470, 1255, 1100, 835, 780, 740 cm^{-1}.

^{1}H NMR (400 MHz, C_6D_6): δ 5.43 (s, H-C(1)); 4.81 (t, ^3J(H-C(4), H-C(3)) = 7.4, H-C(4)), 4.72 (dd, ^2J = 12.3, ^3J (H_a-C(7'), H-C(6')) = 10.5, H_a-C(7')); 4.47 (dd, ^3J(H-C(5'), H-C(6')) = 6.2, ^3J (H-C(5'), HC-(4')) = 2.5, H-C(5')); 4.38-4.28 (m, 3H, H-C(5), H-C(1'), H-C(2')); 3.25 (d, ^2J = 7.4, H_{endo}-C(6)), 4.20 (d, ^3J(H-C(3'), H-(4')) = 4.3, H-C(3')); 4.13

(dt, ^{3}J (H_a-C(7'), H-C(6')) = 10.5, ^{3}J (H-C(6'), H-C(5')) = 6.2, ^{3}J (H-C(6'), (H_b-C(7')) = 2.5, H-C(6')); 3.99 (dd, ^{3}J(H-C(4'), H-C(3')) = 4.3, ^{3}J (H-C(4'), HC-(5')) = 2.5, H-C(4')), 3.96 (dd, ^{2}J = 12.3, ^{3}J (H_b-C(7'), H-C(6')) = 2.5, H_b-C(7')); 3.63 (d, ^{3}J(H-C(2), H-C(3)) = 6.2, H-C(2)); 3.63-3.50 (bs,2H, 2H-C(OH)); 3.27 (dd, ^{2}J = 6.8, ^{3}J(H-5, H_{exo}-C(6)) = 4.9, H_{exo}-C(6)), 2.64 (ddd, ^{3}J(H-3, H-2) = 9.9, ^{3}J(H-3, H-4) = 7.4, ^{3}J (H-C(3), H-C(1')) = 3.1, H-C(3)); 1.05, 1.01, 0.99, 0.96 (4s, 36H, 36H-C(SiC($\underline{CH_3}$)$_3$)); 0.28, 0.21, 0.19, 0.17, 0.16, 0.15, 0.11, 0.06 (8s, 24H, 24H-C(Si$\underline{CH_3}$)).

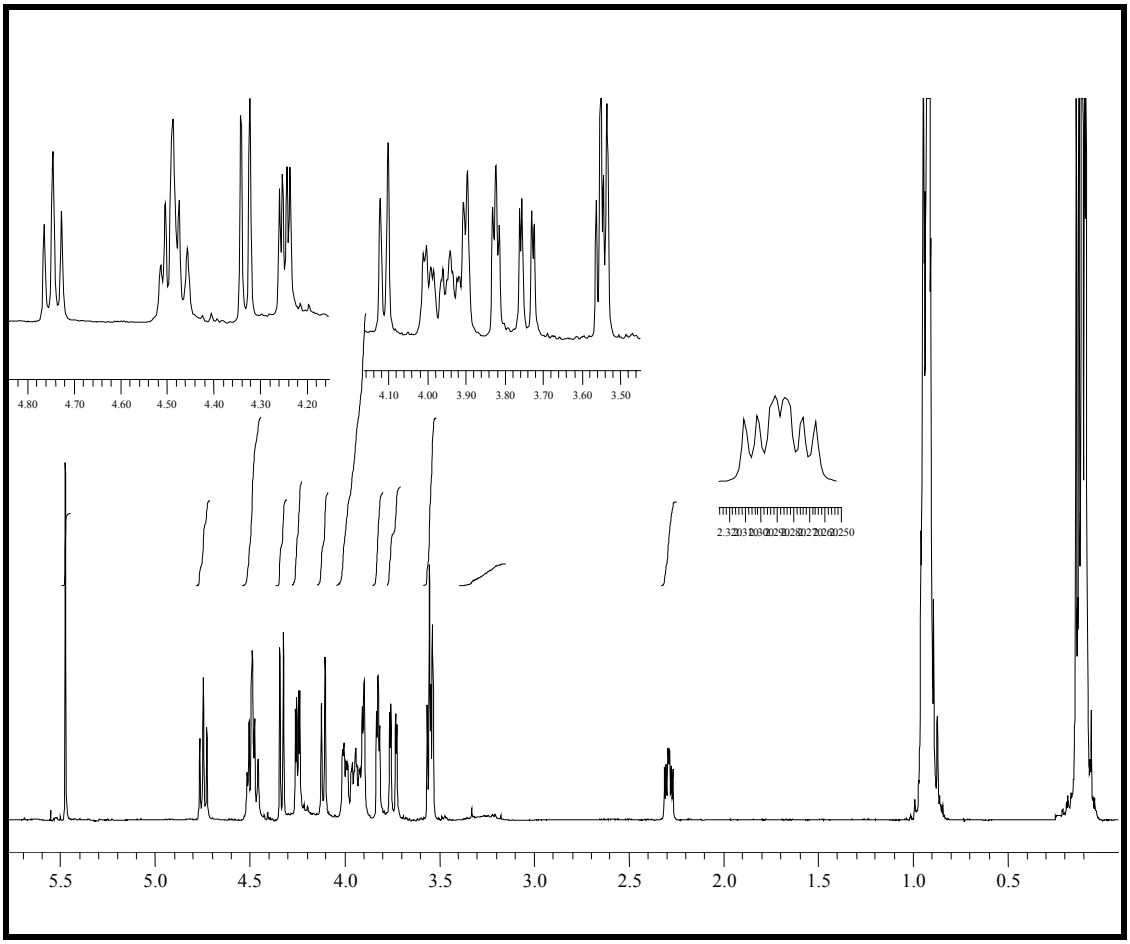
^{13}C NMR (100.6 MHz, C_6D_6):δ

102.5 (d, ^{1}J (C,H) = 179, C(1)); 78.9 (d, ^{1}J(C,H) = 147, C(6')); 74.2 (d, ^{1}J (C,H) = 159, C(5)); 74.1 (d, ^{1}J (C,H) = 154, C4'); 71.5 (d, ^{1}J(C,H) = 141, C(3')); 70.6 (d, ^{1}J(C,H) = 144, C(1')); 68.8 (d, ^{1}J (C,H) = 146, C(2')); 67.9 (d, ^{1}J(C,H) = 146, C(5')); 66.8 (t, ^{1}J(C,H) = 144, C(4)); 63.5 (d, ^{1}J(C,H) = 143 , C(2)); 62.7 (t, ^{1}J(C,H) = 154, C(6)); 59.1 (t, ^{1}J(C,H) = 143, C(7')); 40.4 (d, ^{1}J(C,H) = 126, C(3)); 26.3, 26.1, 26.0, 25.9 (4q, ^{1}J (C,H) = 125, ($\underline{CH_3}$)$_3$CSi); 18.5; 18.2, 18.1; 18.0 (4s, $(CH_3)_3\underline{C}$Si); -4.3; -4.4; -4.4; -4.6, -4.8, -4.9, -5.0; -5.1 (8q, ^{1}J (C,H) = 118 , $\underline{CH_3}$Si).

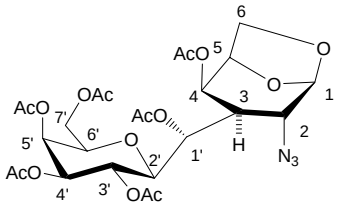
MALDI-HRMS Calcd for (M + Na) $C_{37}H_{77}N_3O_9Si_4Na$ 842.4634; found 842.4631.

Anal. calcd for $C_{37}H_{77}N_3O_9Si_4$ (920.36): C 54.17, H 9.46, N 5.12; found: C 54.19, H 9.38, N 5.12.

1H-NMR (400 MHz, $CDCl_3$) spectrum of **138:**



4-*O*-Acetyl-1,6-anhydro-3-*C*-[(1*R*)-2,6-anhydro-1,3,4,5,7-penta-*O*-acetyl-D-*glycero*-L-*manno*-heptitol-1-*C*-yl]-2-azido-2,3-dideoxy-*β*-D-*galacto*-hexopyranose (139).



139

The *tert*-butyldimethylsilyl-protected **138** (600 mg, 0.733 mmol) was dissolved in THF (4 mL), a solution of TBAF in THF (4 mL, 4.0 mmol) was added, and the solution was stirred for 3 h at room temperature. The mixture was evaporated to dryness, then dissolved in pyridine (5 mL). A catalytic amount of DMAP (10 mg, 0.08 mmol) was added, then acetic anhydride (3 mL, 31.80 mmol) was added, and the solution was stirred for 2 days at 20 ^{o}C. Then MeOH (5 mL) was added, the mixture was evaporated to dryness, then dissolved in ethyl acetate (25 mL), the solution was quenched with aqueous HCl (10 ml), washed successively with saturated aqueous solution of $NaHCO_3$ (5 mL), water and brine and dried ($MgSO_4$). Evaporation of the filtrate, FC (1:1, light petroleum ether/EtOAc) gave 355 mg (89 %) of **139**, white solid,

m.p. 46-47°C.

$[\alpha]^{25}_{589}$ = +51, $[\alpha]^{25}_{577}$ = +56, $[\alpha]^{25}_{435}$ = +107, $[\alpha]^{25}_{405}$ = +131 (*c* 0.124, $CHCl_3$).

IR (film): 3450, 2970, 2150, 1755, 1735, 1440, 1370, 1245, 1140, 1065, 1020, 910 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ
5.74 ((dd, ^3J(H-C(4), H-C(5)) = 8.0, ^3J(H-C(4), H-C(3)) = 5.9, H-C(4)); 5.52 (s , H-C(1)); 5.43-5.36 (m, 2H, H-C(5'), H-C(1')); 5.22 (t, ^3J(H-C(4'), H-C(5')) = 4.0,H-C(4')); 5.03 (dd, ^3J(H-C(3'), H-C(4')) = 4.3, ^3J(H-C(3'), H-C(2')) = 1.8, H-C(3')); 4.82-4.74 (m, 2H, H_a-C(7'), H-C(5)); 4.39-4.27 (m, 2H, H-C(6'), H-C(2')); 4.19 (dd, ^2J = 12.6, ^3J(H_b-C(7'), H-C(6')) = 2.5, H_b-C(7')); 3.97 (d, ^2J=7.4, H_{endo}-C(6)); 3.43 (dd, ^2J=7.4, ^3J(H_{exo}-C(6),H-C(5))= 4.3, H_{exo}-C(6)); 3.25 (d, ^3J(H-C(2), H-C(3)) = 10.2, H-C(2)); 2.18-2 (7s, 22H,, H-C(3), CH_3COO).

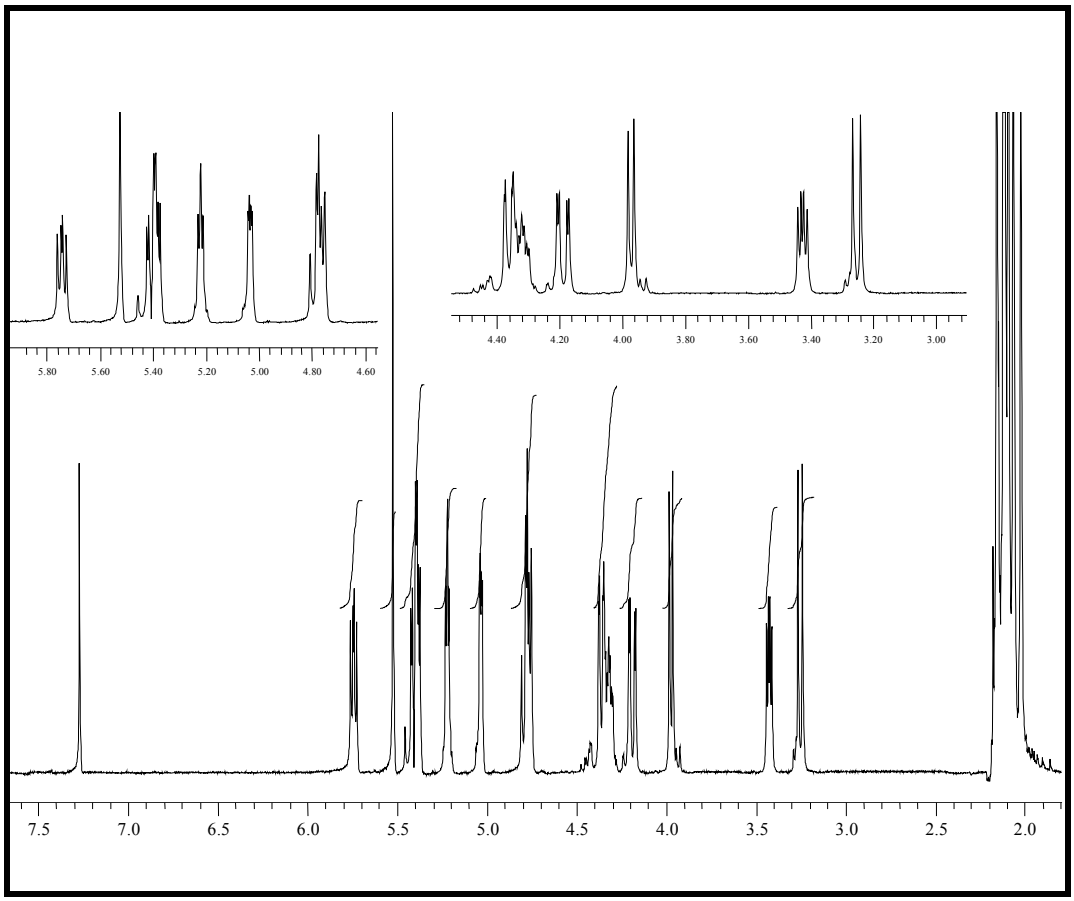
^{13}C NMR (100.6 MHz, $CDCl_3$): δ

167.0, 169.9, 169.8, 169.7, 169.7, 169.4, 168.9 (7s, -COO); 102.9 (d, ^1J(C,H) = 175, C(1)); 72.3 (d, ^1J(C,H) = 149, C(6')); 72.1 (d, ^1J(C,H) = 146, C(5)); 67.5 (d, ^1J(C,H) = 149, C(5')); 67.2 (d, ^1J(C,H) = 148, C(4')); 66.9 (d, ^1J(C,H) = 149, C(3')); 65.6 (d, ^1J(C,H) = 156, C(1')); 65.5 (d, ^1J(C,H) = 144, C(2')); 65.0 (d, ^1J(C,H) = 151, C(4)); 63.2 (t, ^1J(C,H) = 149, C(6)); 62.1 (d, ^1J(C,H) = 129, C(2)); 59.3 (t, ^1J(C,H) = 147, C(7')); 36.4 (d, ^1J(C,H) = 127, C(3)); 21.0- 20.6 (7q, ^1J(C,H) = 130, $\underline{C}H_3COO$).

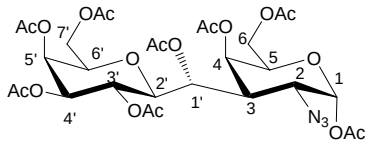
MALDI-HRMS Calcd for (M + Na) $C_{25}H_{33}N_3O_{15}Na$ 638.1809; found 638.1801.

Anal. calcd for $C_{25}H_{33}N_3O_{15}$ (615.54): C 48.78, H 5.40, N 6.83; found: C 48.82, H 5.46, N 6.83.

1H-NMR (400 MHz, $CDCl_3$) spectrum of **139**



4,6-Di-*O*-acetyl-3-*C*-[(1*R*)-2,6-anhydro-1,3,4,5,7-penta-*O*-acetyl-D-*glycero*-L-*manno*-heptitol-1-*C*-yl]-2-azido-2,3-dideoxy-α-D-*galacto*-hexopyranosyl acetate (140α).



140α

139 (0.35 g, 0.57 mmol) was dissolved in Ac_2O (10 mL). After cooling to -40 ^oC, $Me_3SiOSO_2CF_3$ (0.123 mL, 0.683 mmol) was added (syringe) and the mixture stirred for 1 h. CH_2Cl_2 (30 ml) was added and the reaction quenched with a saturated aqueous solution of $NaHCO_3$ (10 mL), the organic phase was collected and extracted with H_2O, and then with brine and dried ($MgSO_4$). Solvent evaporation and FC (hexanes:EtOAc, 2:1 → 1:3) gave 400 mg (98 %) of a 23:1 mixture of **140α** and **140β** (400 mg, 98 %), white foam.

$[\alpha]^{25}_{589}$ = +89, $[\alpha]^{25}_{577}$ = +94, $[\alpha]^{25}_{435}$ = +182, $[\alpha]^{25}_{405}$ = +217 (*c* 0.3775, $CHCl_3$).

IR (film): 2130, 1750, 1435, 1375, 1235, 1035, 910, 600 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ

Data for the α anomer: 6.34 (d, ^3J(H-C(1), H-C(2)) = 3.4, H-C(1)); 5.58 (s, H-C(4)); 5.38 (dd, ^3J(H-C(1'), H-C(3)) = 10.2, ^3J(H-C(1'), H-C(2')) = 2.5, H-C(1')); 5.31 (dd, ^3J(H-C(5'), H-C(6')) = 6.5, ^3J(H-C(5'), H-C(4')) = 3.1, H-C(5')); 5.17 (t, ^3J(H-C(4'), H-C(5')) = 3.1,H-C(4')); 4.86 (dd, ^3J(H-C(3'), H-C(4')) = 4.5, ^3J(H-C(3'), H-C(2')) = 1.5, H-C(3')); 4.77 (dd, ^2J = 12.9, ^3J(H_a-C(7'), H-C(6')) = 10.2, H_a-C(7')); 4.50 (dd, ^3J(H-C(2'), H-C(1')) = 10.2, ^3J(H-C(2'), H-C(3')) = 1.5, H-C(2')); 4.33-4.25 (m, 2H, H_b-C(7'), H-C(6')); 4.14 (t, ^3J(H-C(5), H_{exo}-C(6)) = 6.5,H-C(5)); 4.08 (d, ^2J=11.1, H_{endo}-C(6)); 3.91 (dd, ^2J=11.1, ^3J(H_{exo}-C(6),H-C(5)) = 6.5, H_{exo}-C(6)); 3.61 (dd, ^3J(H-C(2), H-C(3)) = 12.3, ^3J(H-C(2), H-C(1)) = 3.4, H-C(2)); 2.45 (dt, ^3J(H-C(3), H-C(2)) = 12.3, ^3J(H-C(3), H-C(1')) = 2.5, H-C(3)); 2.08-1.99 (8s, 24H, CH_3COO).

^{13}C NMR (100.6 MHz, $CDCl_3$): δ

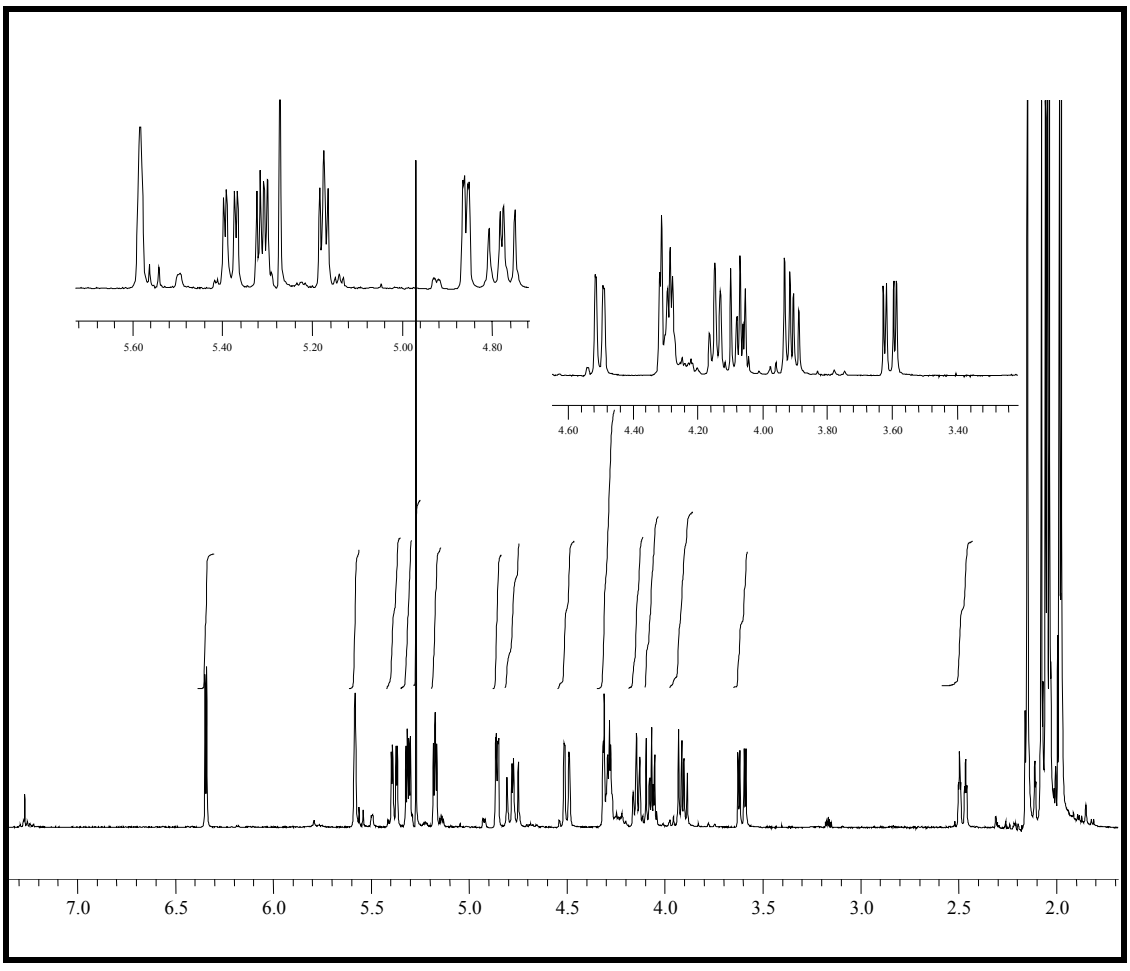
Data for the α anomer: 170.3, 170.2, 169.8, 169.7, 169.3, 168.8, 168.7, 168.6 (8s, -COO); 90.1 (d, ^1J(C,H) = 175, C(1)); 72.7 (d, ^1J(C,H) = 149, C(6')); 69.9 (d, ^1J(C,H) = 146,

C(5)); 66.6 (d, ^{1}J(C,H) = 156, C(1')); 66.6 (d, ^{1}J(C,H) = 148, C(4')); 66.5 (d, ^{1}J(C,H) = 149, C(3')); 65.3 (d, ^{1}J(C,H) = 149, C(5')); 64.8 (d, ^{1}J(C,H) = 151, C(4)); 64.6 (d, ^{1}J(C,H) = 144, C(2')); 61.3 (t, ^{1}J(C,H) = 149, C(6)); 59.3 (t, ^{1}J(C,H) = 147, C(7')); 54.8 (d, ^{1}J(C,H) = 129, C(2)); 37.1 (d, ^{1}J(C,H) = 127, C(3)); 20.8-20.4 (8q, ^{1}J(C,H) = 130, $\underline{C}H_3COO$).

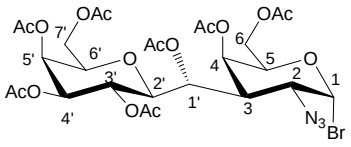
MALDI-HRMS Calcd for (M + Na) $C_{25}H_{33}N_3O_{15}Na$ 740.2126; found 740.2128.

Anal. calcd for $C_{29}H_{39}N_3O_{18}$ (717.63): C 48.54, H 5.48, N 6.86; found: C 48.59, H 5.50, N 5.78.

1H-NMR (400 MHz, $CDCl_3$) spectrum of **140α**



4,6-Di-*O*-acetyl-3-*C*-[(1*R*)-2,6-anhydro–1,3,4,5,7-penta-*O*-acetyl-D-*glycero*-L-*manno*-heptitol-1-*C*-yl]-2-azido-2,3-dideoxy-α-D-*galacto*-hexopyranosyl bromide (141α).



141α

A solution of **140** (0.50 g, 0.697 mmol) and anhydrous $TiBr_4$ (0.99 mg, 1.35 mmol) in anhydrous CH_2Cl_2 (14 mL) was stirred at 20 °C for 12 h. after addition of CH_2Cl_2 (150 mL), the solution was washed with ice-cold H_2O (50 mL, twice) and dried ($MgSO_4$). Solvent evaporation *in vacuo* and quick FC (hexane: EtOAc, 1:1) gave 472 mg (92 %) of **141α**, contaminated by less than 2% of the β-anomer yellow foam, that was dried and stored at -20 °C until its use.

$[\alpha]^{25}_{589}$ = +116, $[\alpha]^{25}_{577}$ = +122, $[\alpha]^{25}_{435}$ = +238, $[\alpha]^{25}_{405}$ = +287 (*c* 0.243, $CHCl_3$).

IR (film): 3475, 2965, 2115, 1750, 1370, 1235, 1050 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ

Data for the α anomer: 6.69 (d, ^3J(H-C(1), H-C(2)) = 3.7, H-C(1)); 5.69 (s, H-C(4)); 5.413 (dd, ^3J(H-C(1'), H-C(3)) = 9.6, ^3J(H-C(1'), H-C(2')) = 1.8, H-C(1')); 5.36 (dd, ^3J(H-C(5'), H-C(6')) = 6.5, ^3J(H-C(5'), H-C(4')) = 3.1, H-C(5')); 5.22 (t, ^3J(H-C(4'), H-C(5')) = 3.7,H-C(4')); 4.97-4.89 (m, 2H,H_{exo}-C(6) , H-C(3')); 4.53 (dd, ^3J(H-C(2'), H-C(1')) = 9.9, ^3J(H-C(2'), H-C(3')) = 1.5, H-C(2')); 4.40 (t, ^3J(H-C(5), H_{exo}-C(6)) = 6.5,H-C(5)); 4.24-4.16 (m, 2H, H_{endo}-C(6) , H_a-C(7')); 4.02 (dd, ^2J = 11.7, ^3J(H_a-C(7'), H-C(6')) = 7.4, H_b-C(7')); 3.70 (dd, ^3J(H-C(2), H-C(3)) = 12.0, ^3J(H-C(2), H-C(1)) = 3.7, H-C(2)); 2.59 (dt, ^3J(H-C(3), H-C(2)) = 12.0, ^3J(H-C(3), H-C(1')) = 1.8, H-C(3)); 2.19-2.00 (8s, 24H, CH_3COO).

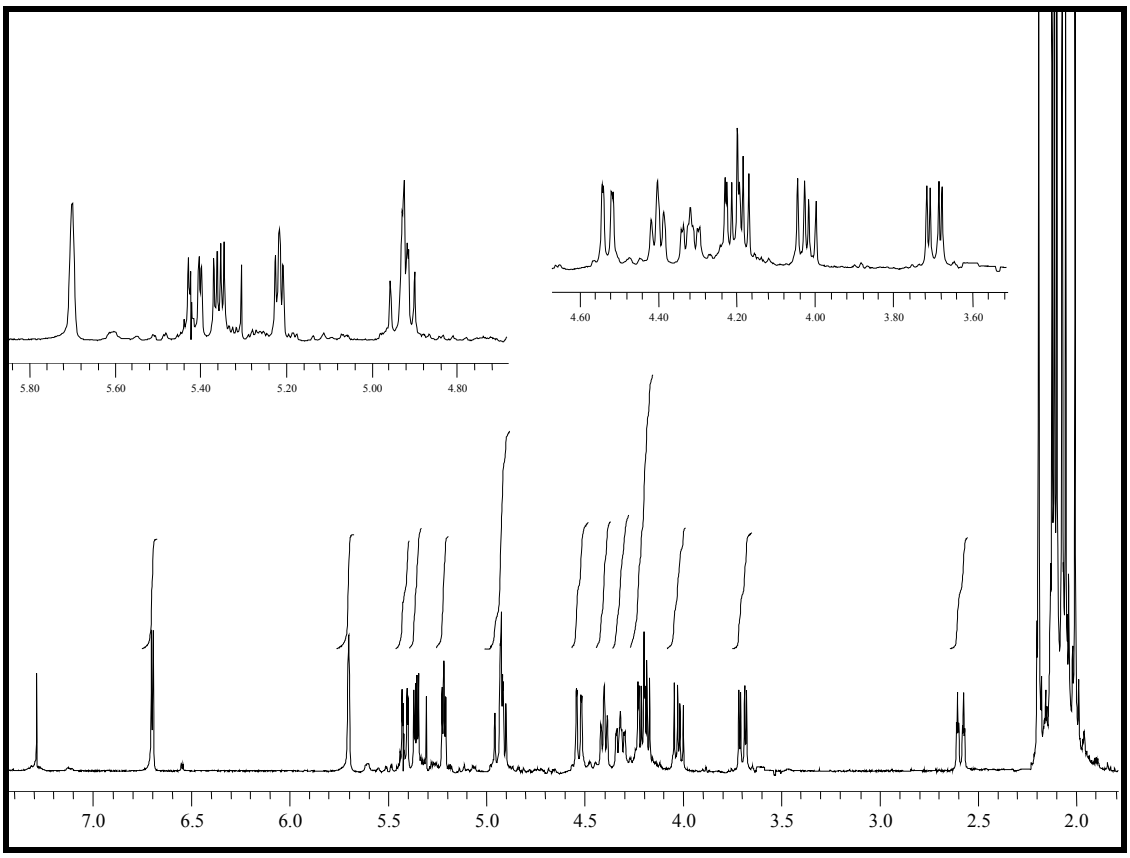
^{13}C NMR (100.6 MHz, $CDCl_3$): δ

Data for the α anomer: 170.7, 170.3, 169.8, 169.6, 169.3, 168.9, 168.8 (7s, -COO); 92.8 (d, ^1J(C,H) = 184, C(1)); 72.9 (d, ^1J(C,H) = 153, C(6')); 72.6 (d, ^1J(C,H) = 146, C(5));

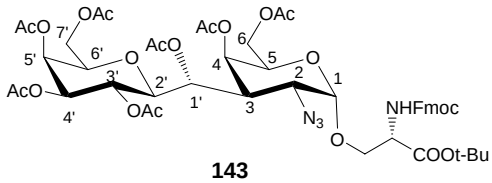
66.8 (d, ^{1}J(C,H) = 149, C(5')); 66.7 (d, ^{1}J(C,H) = 148, C(4')); 66.6 (d, ^{1}J(C,H) = 149, C(3')); 65.4 (d, ^{1}J(C,H) = 139, C(1')); 64.7 (d, ^{1}J(C,H) = 151, C(4)); 64.6 (d, ^{1}J(C,H) = 143, C(2')); 61.1 (t, ^{1}J(C,H) = 151, C(7')); 59.2 (t, ^{1}J(C,H) = 155, C(6)); 57.6 (d, ^{1}J(C,H) = 142, C(2)); 38.0 (d, ^{1}J(C,H) = 129, C(3)); 20.9-20.5 (7q, ^{1}J(C,H) = 130, $\underline{C}H_3COO$).

Anal. calcd for $C_{27}H_{36}BrN_3O_{16}$ (738.49): C 43.91, H 4.91, Br 10.82, N 5.69; found: C 43.81, H 5.05, Br 10.73, N 5.65.

1H-NMR (400 MHz, $CDCl_3$) spectrum of **141α**



5:1 Mixture of *N*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-{4,6-di-*O*-acetyl-3-*C*-[(1*R*)-2,6-anhydro-1,3,4,5,7-penta-*O*-acetyl-D-*glycero*-L-*manno*-heptitol-1-*C*-yl]-2-azido-2,3-dideoxy-α- and-β-D-*galacto*pyranosyl}-L-serine *tert*-butyl ester (143α , 143β).



143

A mixture of *N*-Fmoc-Serine-O^{t}Bu **142** (408 mg, 1.03 mmol), anhydrous CH_2Cl_2 (10 mL), $AgClO_4$ (370 mg, 1.73 mmol), 2,4,6-collidine (240 μL, 1.73 mmol) and 4 Å molecular sieves (dried under vaccum at 200 ^oC, 1 g) was stirred at 20 ^oC for 10 min. A solution of bromide **141**α,β (834 mg, 1.129 mmol) in anhydrous CH_2Cl_2 (10 mL) was added slowly (syringe) to the stirred mixture over 30 min. After stirring at 20 ^oC for 5h, CH_2Cl_2 (100ml) was added and the mixture filtered through a pad of Celite (washing with CH_2Cl_2). Solvent evaporation, FC (hexanes:EtOAc, 4:1 → 3:1) gave 1.1 g (93 %) of **143**α/**143**β 5:1, white foam.

$[\alpha]^{25}_{589}$ = +91, $[\alpha]^{25}_{577}$ = +96, $[\alpha]^{25}_{435}$ = +187, $[\alpha]^{25}_{405}$ = +224 (*c* 0.375, $CHCl_3$).

IR (film): 3660, 2990, 2110, 1735, 1525, 1370, 1220, 1065, 890 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ

Data for the α anomer: 7.80-7.26 (4 m, 8H, ArH); 6.04 (d, ^3J(H-N, H-C*) = 8.0, H-N); 5.56 (s, H-C(4)); 5.38 (dd, ^3J(H-C(1'), H-C(3)) = 9.6, ^3J(H-C(1'), H-C(2')) = 2.6, H-C(1')); 5.32 (dd, ^3J(H-C(5'), H-C(6')) = 6.7, ^3J(H-C(5'), H-C(4')) = 3.2, H-C(5')); 5.22 (t, ^3J(H-C(4'), H-C(3')) = 3.5, H-C(4')); 4.96 (d, ^3J(H-C(1), H-C(2)) = 2.9, H-C(1)); 4.83 (d, ^3J(H-C(4'), H-C(3')) = 3.5, H-C(3')); 4.75 (dd, ^2J = 12.8, ^3J(H-C(6_{exo}), H-C(5)) = 9.9, H-C(6_{exo})); 4.54-4.44 (m, 2H, H-C(2') , H-C*(Ser)); 4.43-4.31 (m, 3H, H_{endo}-C(6) , H_2-C(Fmoc)); 4.30-4.11 (m, 5H, H_a-C(7') , H-C(6'), H-C(5), H-C(Fmoc), H_2-C(Ser)); 4.30-4.11 (m, 2H, H_b-C(7') , H_2-C(Ser)); 3.33 (dd, ^3J(H-C(2), H-C(3)) = 12.2, ^3J(H-C(2), H-C(1)) = 2.9, H-C(2)); 2.59 (d, ^3J(H-C(3), H-C(2)) = 12.2, H-C(3)); 2.25-1.94 (7s, 21H, CH_3COO); 1.48 (s, 9H, $(CH_3)_3C$).

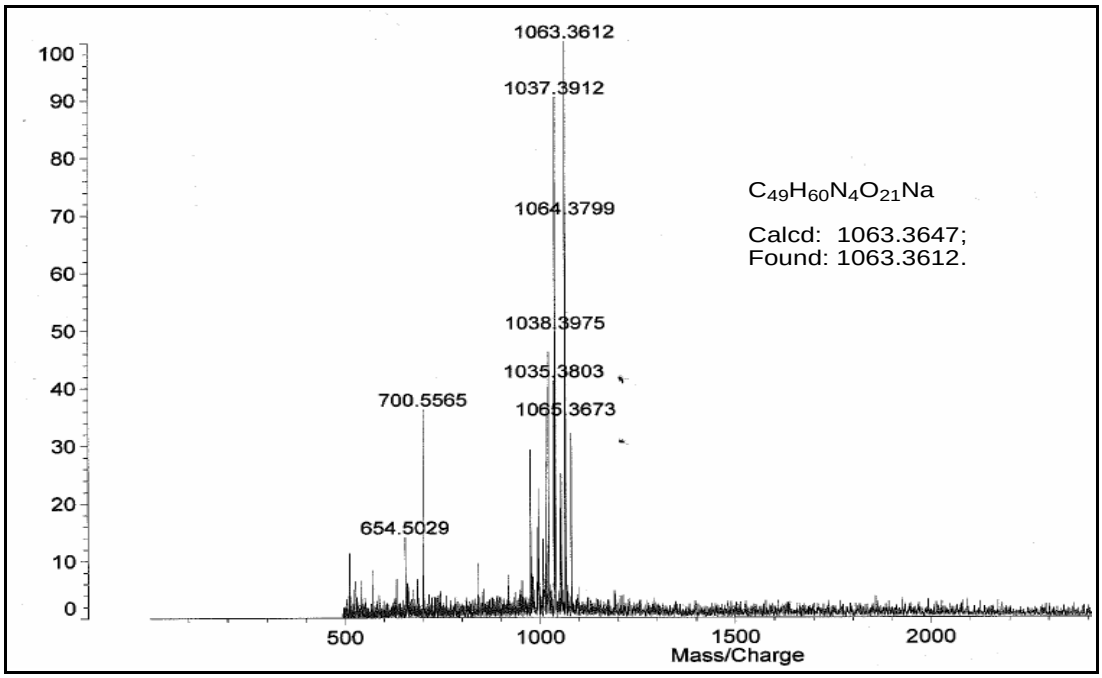
^{13}C NMR (100.6 MHz, $CDCl_3$): δ

Data for the α anomer: 170.7, 170.4, 170.3, 169.9, 169.8, 168.9, 168.7, 168.4 (8s, -COO); 155.8 (s, C(Carbamat); 143.6 (s, C(Fmoc)); 141.2 (s, C(Fmoc)); 127.6 (d, ^1J(C,H) = 159, C(Fmoc)); 126.9 (d, ^1J(C,H) = 159, C(Fmoc)); 125.1 (d, ^1J(C,H) = 158, C(Fmoc)); 119.8 (d, ^1J(C,H) = 158, C(Fmoc)); 99.3 (d, ^1J(C,H) = 184, C(1)); 82.8 (s, $\underline{C}(CH_3)_3$); 72.9 (d, ^1J(C,H) = 153, C(6')); 70.7 (t, ^1J(C,H) = 153, $\underline{C}H_2$(Ser)); 68.8 (d, ^1J(C,H) = 146, C(5)); 66.8 (t, ^1J(C,H) = 150, $\underline{C}H_2$(Fmoc)); 66.6 (d, ^1J(C,H) = 148, C(4')); 66.6 (d, ^1J(C,H) = 139, C(1')); 66.4 (d, ^1J(C,H) = 149, C(3')); 65.8 (d, ^1J(C,H) = 151, C(4)); 65.4 (d, ^1J(C,H) = 149, C(5')); 64.6 (d, ^1J(C,H) = 143, C(2')); 62.1 (t, ^1J(C,H) = 151, C(7')); 59.4 (t, ^1J(C,H) = 155, C(6)); 55.0 (d, ^1J(C,H) = 138, C∗(Ser)); 54.9 (d, ^1J(C,H) = 142, C(2)); 47.0 (d, ^1J(C,H) = 130, C(Fmoc)); 37.0 (d, ^1J(C,H) = 129, C(3)); 27.7 (q, 3C, ^1J(C,H) = 128, ($\underline{C}$H3)CO); 20.9-20.5 (7q, ^1J(C,H) = 130, $\underline{C}H_3COO$).

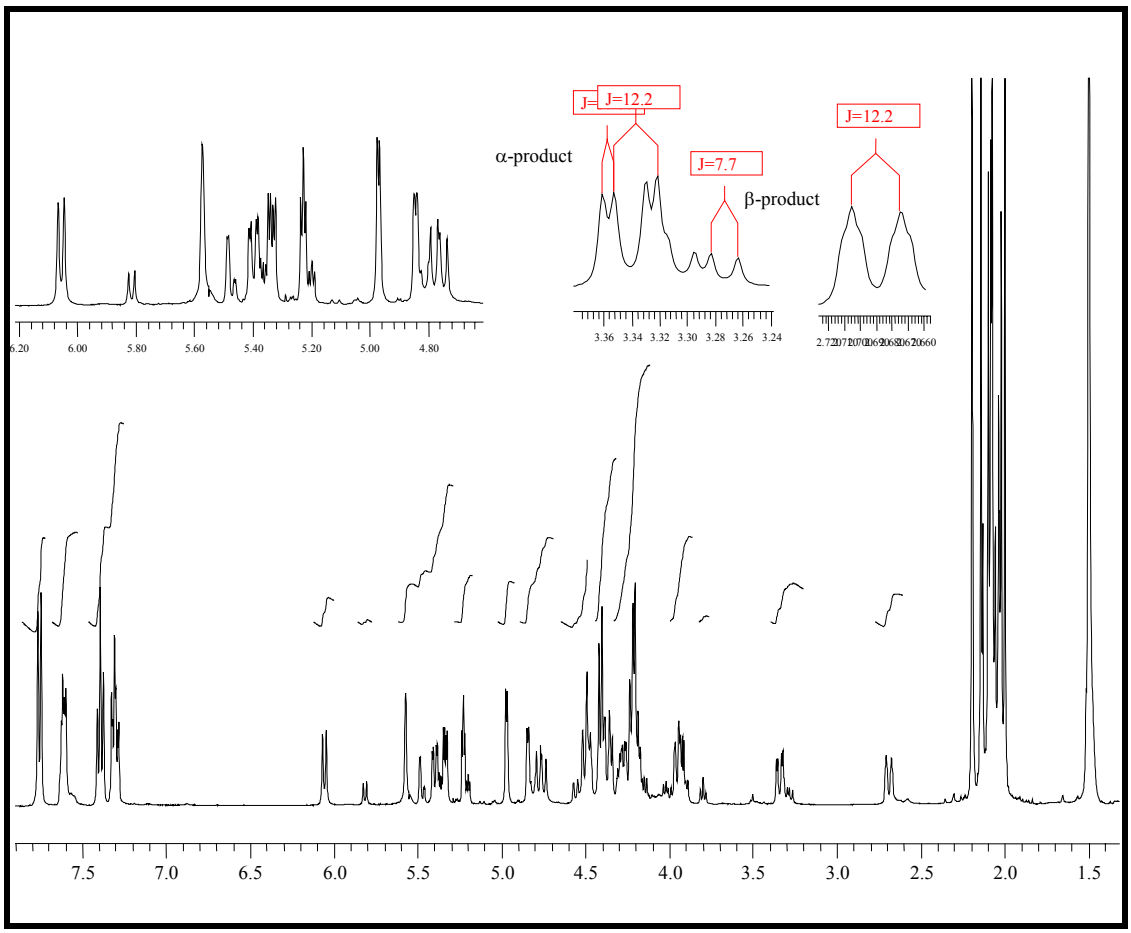
MALDI-HRMS Calcd for (M + Na) $C_{49}H_{60}N_4O_{21}Na$ 1063.3647; found 1063.3612.

Anal. calcd for $C_{49}H_{60}N_4O_{21}$ (1041.02): C 56.53, H 5.81, N 5.38; found: C 56.56, H 5.79, N 5.36.

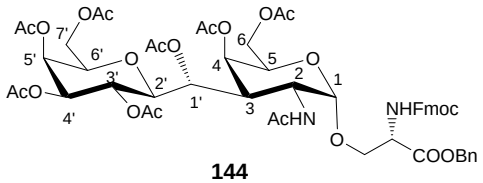
MALDI-HRMS spectrum of **143α,β:**



1H-NMR (400 MHz, $CDCl_3$) spectrum of **143α,β**



***N*-[(9*H*-Fluoren-9-ylmethoxy)carbonyl]-{4,6-di-*O*-acetyl-3-*C*-[(1*R*)-2,6-anhydro–1,3,4,5,7-penta-*O*-acetyl-D-*glycero*-L-*manno*-heptitol-1-*C*-yl]-2-[(*N*-acetyl)amino]-2,3-dideoxy-α-D-*galacto*pyranosyl}-L-serine *tert*-butyl ester (144).**



144

A 5:1 mixture of **143α** and **143β** (120 mg, 0.115 mmol) was dissolved in 4:4:1 mixture of collidine/CH_3COSH/Ac_2O (6 mL). After stirring at 20 ^oC for 18 h the mixture was co-evaporated with toluene under vacuum (10^{-3}Torr). FC (hexanes:EtOAc, 4:1 → 1:2) gave A 5:1 mixture of **144α** and **144β** (107 mg, 89 %) as a white foam.

$[\alpha]^{25}_{589}$ = +65, $[\alpha]^{25}_{577}$ = +69, $[\alpha]^{25}_{435}$ = +129, $[\alpha]^{25}_{405}$ = +152 (*c* 0.21, $CHCl_3$).

IR (film): 3660, 3325, 2965, 1740, 1735, 1520, 1370, 1220, 1055, 800 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ

Data for the α anomer: 7.74-7.23 (4 m, 8H, ArH); 5.89 (d, ^3J(H-N, H-C*) = 8.3, H-N); 5.52 (s, H-C(4)); 5.49 (d, ^3J(H-(NAc), H-C(2)) = 8.7, H-N); 5.33-5.27 (m, 2H, H-C(1'), H-C(5')); 5.23 (t, ^3J(H-C(4'), H-C(5')) = 3.4,H-C(4')); 4.75-4.65 (m, 3H, H-C(1), H_{exo}-C(6), H-C(5')); 4.60 (td, ^3J(H-C(2), H-C(3)) = 12.9, ^3J(H-C(2), H-(NAc)) = 10.0, ^3J(H-C(2), H-C(1)) = 3.4, H-C(2)); 4.52-4.35 (m, 5H, H_2-C(Fmoc), H-C(Ser), H-C(2'), H-C(3'), H_{endo}-C(6),); 4.26-4.17 (m, 3H, H-C(Fmoc), H-C(6'), H_a-C(7')); 4.14-4.06 (m, 1H, H-C(5)); 3.97-3.87 (m, 3H, H_2-C(Ser), H_b-C(7')); 2.53 (d, ^3J(H-C(2), H-C(3)) = 12.9, H-C(2)); 2.22-1.87 (8s, 24H, CH_3COO); 1.47 (s, 9H, $(CH_3)_3C$).

^{13}C NMR (100.6 MHz, $CDCl_3$): δ

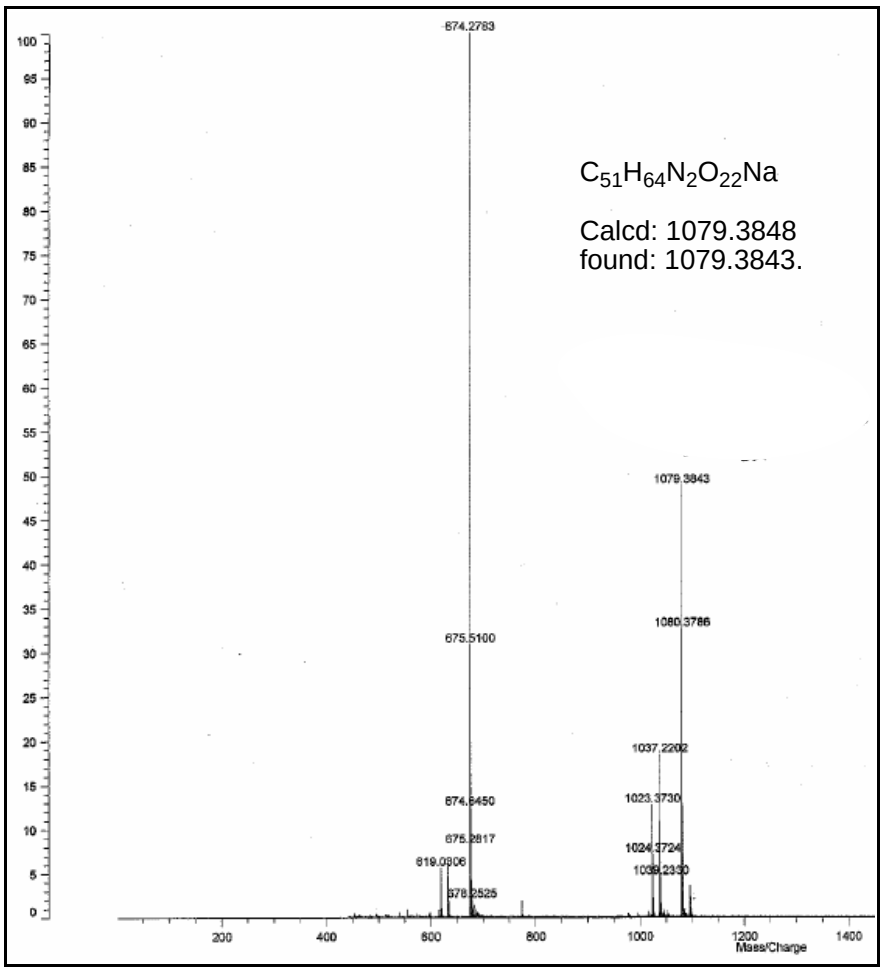
Data for the α anomer: 170.7, 170.3, 170.0, 169.7, 169.5, 169.4, 169.1, 169.0, 168.9 (9s, -COO); 155.9 (s, C(Carbamate); 143.6 (s, C(Fmoc)); 141.3 (s, C(Fmoc)); 127.7 (d, ^1J(C,H) = 159, C(Fmoc)); 127.0 (d, ^1J(C,H) = 159, C(Fmoc)); 124.9 (d, ^1J(C,H) = 158, C(Fmoc)); 120.0 (d, ^1J(C,H) = 158, C(Fmoc)); 98.8 (d, ^1J(C,H) = 169, C(1)); 82.8 (s, $\underline{C}(CH_3)_3$); 72.9 (d, ^1J(C,H) = 149, C(6')); 69.8 (t, ^1J(C,H) = 153, $\underline{C}H_2$(Ser));68.9 (d, ^1J(C,H) = 140, C(5));

67.0 (t, ^{1}J(C,H) = 150, $\underline{C}H_2$(Fmoc)); 67.0 (d, ^{1}J(C,H) = 149, C(3')); 66.4 (d, ^{1}J(C,H) = 149, C(4')); 65.8 (d, ^{1}J(C,H) = 160, C(4)); 65.5 (d, ^{1}J(C,H) = 159, C(5')); 65.2 (d, ^{1}J(C,H) = 148, C(1')); 64.8 (d, ^{1}J(C,H) = 139, C(2')); 62.4 (t, ^{1}J(C,H) = 163, C(7')); 59.7 (t, ^{1}J(C,H) = 151, C(6)); 55.0 (d, ^{1}J(C,H) = 138, C*(Ser)); 47.1 (d, ^{1}J(C,H) = 130, C(Fmoc)); 43.5 (d, ^{1}J(C,H) = 130, C(Ser)); 38.0 (d, ^{1}J(C,H) = 128, C(3)); 28.6 (q, 3C, ^{1}J(C,H) = 128, ($\underline{C}$H3)CO); 23.4 (q, ^{1}J(C,H) = 129, $\underline{C}H_3$CON); 21.1-20.6 (7q, ^{1}J(C,H) = 130, $\underline{C}H_3$COO).

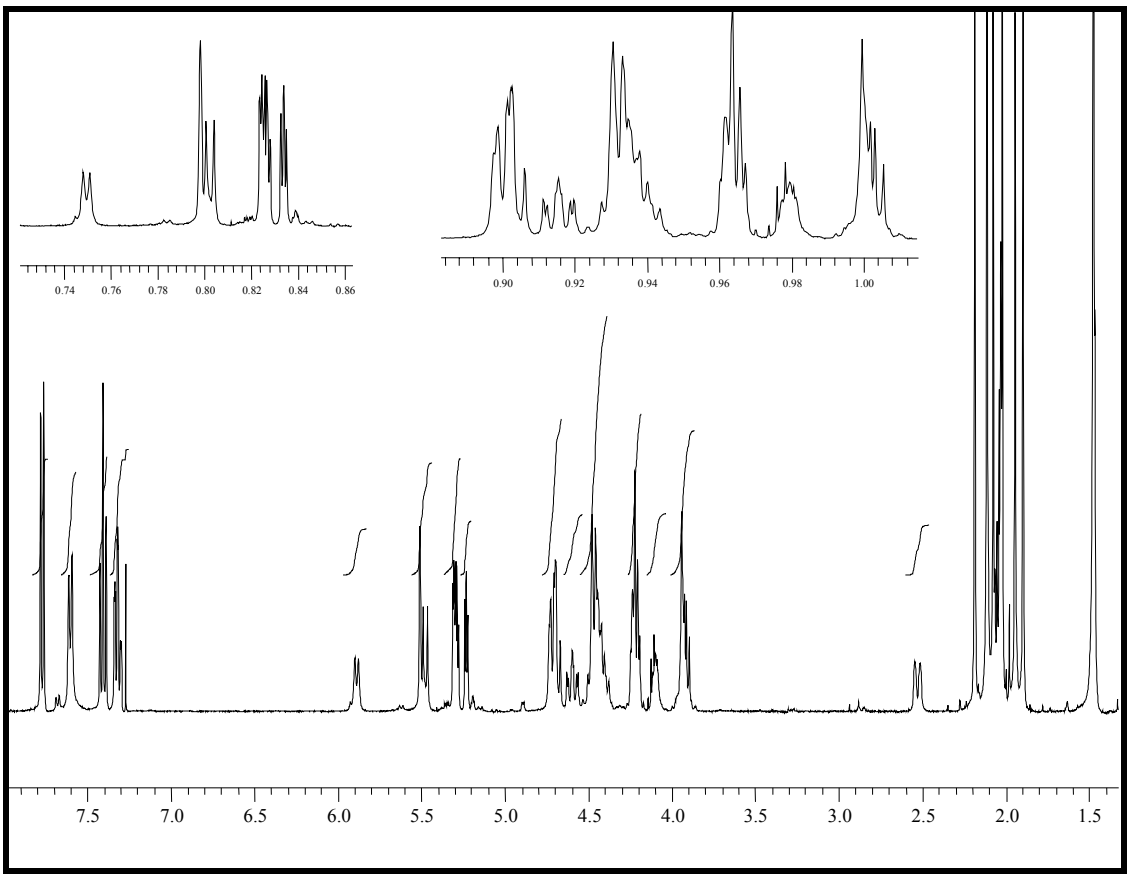
MALDI-HRMS: Calcd for (M + Na) $C_{51}H_{64}N_2O_{22}Na$ 1079.3848; found 1079.3843.

Anal. calcd for $C_{51}H_{64}N_2O_{22}$ (1057.05): C 57.95, H 6.10, N 2.65; found: C 57.88, H 6.11, N 2.59.

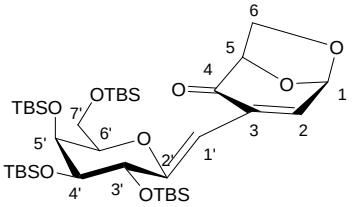
MALDI-HRMS spectrum of **144α,β:**



1H-NMR (400 MHz, $CDCl_3$) spectrum of **144α,β**



(*E*) or (*Z*)-1,6-Anhydro-3-{2,6-anhydro-3,4,5,7-tetrakis-O-[tert-butyl(dimethyl)silyl]-D-*galacto*-hept-1-en-1-ol-1-*C*-yl}-2,3-dideoxy-β-D-*glycero*-hex-2-eno-pyran-4-(ulose((E) or (Z)-146)



146

Solid *N,N'* –thiocarbonyldiimidazole (583 mg, 3.28 mmol) was added to a solution **97a** (500 mg, 0.66 mmol) in THF (3ml). The reaction mixture was gently heated under reflux and nitrogen atm. until TLC indicated complete consumption of the starting material. The solution was cooled to rt. and concentrated. Flash chromatography (9:1, light petroleum ether / EtOAc), afforded a colorless oil **(*E*)** or **(*Z*) 146** (455 mg, 78 %).

R_f =0.50, (9:1, light petroleum ether / Ether).

UV (MeCN): 317 (12625), 244 (4896).

^{1}H NMR (400 MHz, $CDCl_3$): δ

7.93 (d, ^3J (H-(2), H-C(1)) = 3.7, H-(2)); 5.83 (d, ^3J (H-(1), H-C(2)) = 3.7, H-(1)); 5.78 (s, H-C(1')); 4.79 (dd, ^3J (H-(5), H_{exo}-C(6)) = 6.2, ^3J (H-(5), H_{endo}-C(6)) = 1.3 , H-5); 4.46 (d, ^3J (H-(5'), H-C(6')) = 3.2, ^3J (H-(5'), H-C(4')) = 2.1, H-(5')); 4.19 (dt, ^3J (H-(6'), H-C(7')) = 5.3 , ^3J (H-(6'), H-C(5')) = 3.2, H-(6')); 4.03 (d, ^2J = 8.0, ^3J (H_{exo}-(6), H-C(5)) = 6.2 , H_{exo}-C(6)); 4.02 (d, ^3J (H-(3'), H-C(4')) = 4.3, H-(3')); 3.81 (m, 2H, H_a-C(7'), H_b-C(7')); 3.73 (d, ^3J (H-(4'), H-C(3')) = 4.3,, ^3J (H-(4'), H-C(5')) = 2.1, H-C(4')); 3.59 (d, ^2J = 8.9, ^3J (H_{exo}-(6), H-C(5)) = 1.3 , H_{endo}-C(6)); 0.94, 0.93, 0.90, 0.90 (4s, 36H, 36H-C(SiC($\underline{CH_3}$)$_3$)); 0.14, 0.12, 0.10, 0.09, 0.08, 0.07, 0.06, 0.05, (8s, 24H, 24H-C(Si$\underline{CH_3}$)).

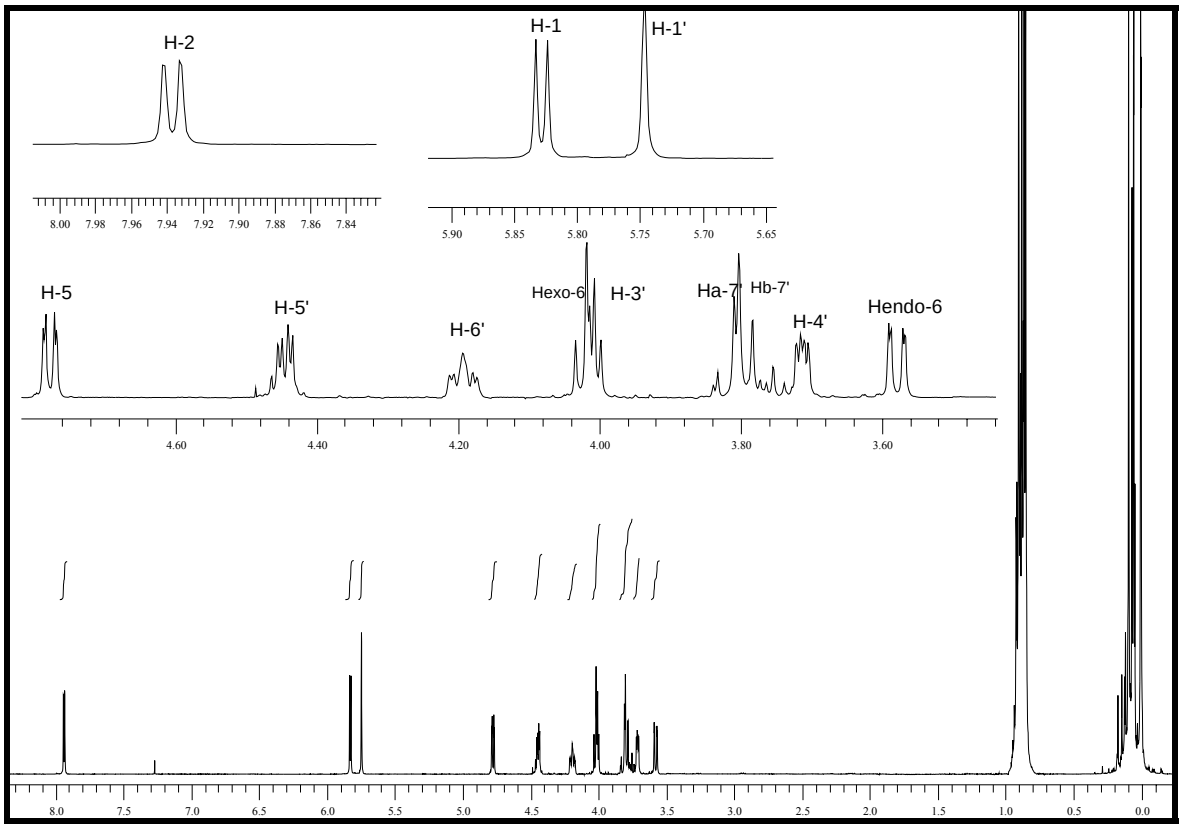
^{13}C NMR (100.6 MHz, $CDCl_3$): δ

194 (s, C(4)); 156.3 (s, C(2')); 141.2 (d, ^1J(C,H) = 170, C(2)); 132.0 (s, C(3)), 102.2 (d, ^1J (C,H) = 159, C(1')); 97.9 (d, ^1J (C,H) = 176, C(1')); 83.4 (d, ^1J(C,H) = 144, C(5')); 79.3 (d, ^1J (C,H) = 164, C(5)); 74.3 (d, ^1J (C,H) = 146, C4'); 74.3 (d, ^1J(C,H) = 147, C(3')); 66.9 (d, ^1J(C,H) = 140, C(6')); 62.4 (t, ^1J(C,H) = 156, C(6)); 62.0 (t, ^1J(C,H) = 143,

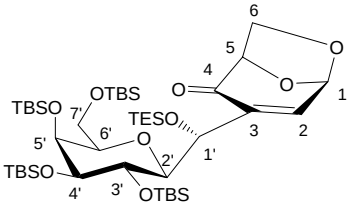
C(7')); 26.0, 26.0, 25.9, 25.85 (4q, ^1J (C,H) = 128, ($\underline{CH_3}$)$_3$CSi); 18.4; 18.3, 18.1; 18.0 (4s, (CH$_3$)$_3$$\underline{C}$Si); -4.4; -4.6; -4.8; -4.9, -5.0; -5.0, -5.3; -5.5; (8q, ^1J (C,H) = 118 , $\underline{CH_3}$Si).

HR-FAB-MS: for $C_{37}H_{72}O_8Si_4$+Na **calcd** 779.4202 **found** 779.4200

^{1}H-NMR (400 MHz, $CDCl_3$) spectrum of **146**:



1,6-Anhydro-3-{(1*R*) -*O*-[(*tert*-butyl)dimediethylsilyl]-2,6-anhydro-3,4,5,7-tetrakis-*O*-[(*tert*-butyl)dimethylsilyl]-1-*O*-triethylsilyl-D-*glycero*-L-*manno*-heptitol-1-*C*-yl}-2,3-dideoxy-*β*-D-*glycero*-hex-2-enopyran-4-ulose (147).



147

IR (film) 2950, 2855, 1695, 1465, 1360, 1265, 1090, 835, 775 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ

6.69 (d, ^{3}J (H-C(1), H-C(2)) = 3.4 , H-C(2)); 5.81 (d, ^{3}J (H-1, H-C(2)) = 3.4 , H-C(1)); 4.82 (br s, H-C(1')), 4.71 (dd, ^{3}J (H-C(5), H-C(6)) = 6.5 , ^{3}J (H-C(5), H-C(6)) = 1.3 Hz, H-C(5)); 4.20 (dd, ^{3}J(H-C(3'), H-C(4')) = 6.8 , ^{3}J (H-C(2'), H-C(3')) = 2.5 , H-C(3')); 4.07 (dd, ^{2}J = 12.0 , ^{3}J (H_a-C(7'), H-C(6')) = 8.32 , H_a-C(7')); 3.99 (dd, ^{2}J=8.0, ^{3}J (H-C(5), H_{exo}-C(6)) = 6.5, H_{exo}-(6)); 3.95(d, ^{3}J (H-C(5'), H-C(6')) = 4.1 , H-C(5')); 3.95-3.77 (m, 4H, H-C(4'), H_{endo}-C(6), H-C(6'), H-C(2')); 3.75 (d, ^{2}J = 12.0 , H_b-C(7')); 1.00- 0.84 (m, 35 H , ^{t}-Bu × 4, $SiCH_2\underline{CH_3}$× 3); 0.53 (q, 6H, $Si\underline{CH_2}$ CH_3); 0.14, 0.12, 0.10, 0.09, 0.08, 0.07, 0.06, 0.05 (8s, 3H × 8, $SiCH_3$ × 8).

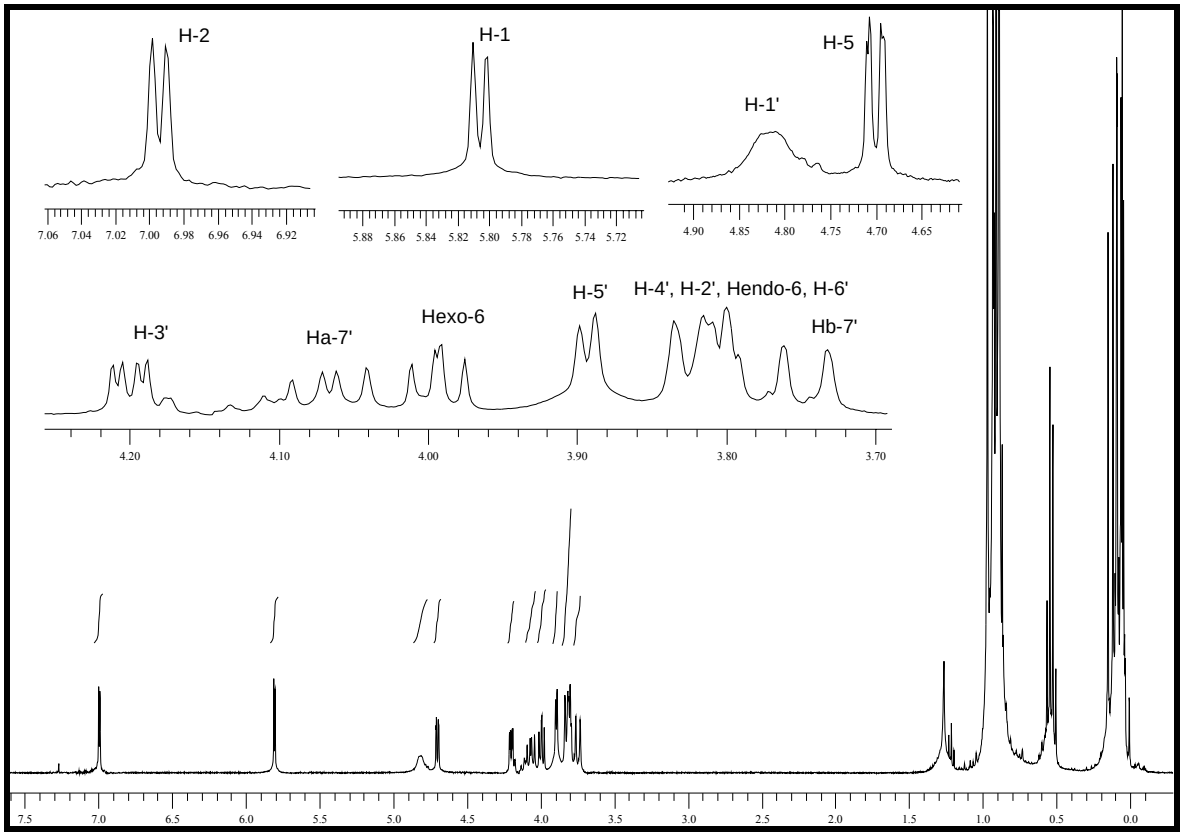
^{13}C NMR (100.6 MHz, $CDCl_3$): δ

193.4 (s, C(4)); 141.9 (d, ^{1}J(C,H) = 165, C(2)); 139.1 (s, C(3)); 96.7 (d, ^{1}J(C,H) = 177, C(1)); 80.5 (d, ^{1}J(C,H) = 145, C(2')); 79.2 (d, ^{1}J(C,H) = 163, C(5)); 73.1 (d, ^{1}J(C,H) = 146, C(6')); 70.4 (d, ^{1}J(C,H) = 149, C(5')); 70.1 (d, ^{1}J(C,H) = 141, C(4')); 67.0 (d, ^{1}J(C,H) = 145, C(3')); 65.4 (d, ^{1}J(C,H) = 146, C(1')); 62.5 (t, ^{1}J(C,H) = 157, C(6)); 58.2 (t, ^{1}J(C,H) = 144, C(7')); 26.1, 25.9, 25.8, 25.7(4q, ^{1}J (C,H) = 123, $(\underline{CH_3})_3CSi$); 18.3; 18.0, 17.9; 17.8 (4s, $(CH_3)_3\underline{C}Si$); 6.9 (q, ^{1}J (C,H) = 125, $\underline{CH_3}CH_2Si$); 5.7 (t, ^{1}J (C,H) = 118, $CH_3\underline{CH_2}Si$); -3.6, -4.3; -4.6; -4.7; -4.8, -5.0; -5.4, -5.5 (8q, ^{1}J (C,H) = 118 , $\underline{CH_3}Si$).

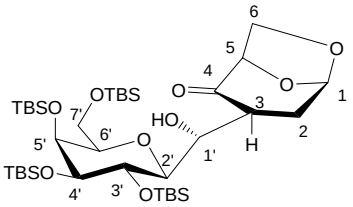
MALDI-HRMS Calcd for (M + Na) $C_{43}H_{88}O_9Si_5Na$ 911.517; found 911.518.

Anal. calcd for $C_{43}H_{88}O_9Si_5$ (889.58): C 58.06, H9.67; found: C 58.03, H 10.05.

^{1}H-NMR (400 MHz, $CDCl_3$) spectrum of **147**:



1,6-Anhydro-3-{(1*S*)-2,6-anhydro-3,4,5,7-tetra-*O*-[(*tert*-butyl)dimethylsilyl]-D-*glycero*-L-*manno*-heptitol-1-*C*-yl}-2,3-dideoxy-*β*-D-*glycero*-hexopyranos-4-ulose (148).



148

103 mg of an aqueous suspension of Raney Nickel was added to a stirred solution of compound **97a** (103 mg, 0.129 mmol) in tetrahydrofuran (1.11 mL) and the mixture was further stirred at 20 °C until complete conversion of starting material (TLC control). The mixture was diluted with Et_2O (200 mL), filtrated through a pad of silica gel. FC (9.5:0.5, light petroleum ether/EtOAc), afforded 59 mg (57%) of **148** and 45 mg (43%) of **149**, both as colorless oil.

Data of **148**:

$\mathbf{R}_f$ = 0.16, (9.5:0.5, light petroleum ether/EtOAc).

$[\alpha]^{25}_{589}$ = +4, $[\alpha]^{25}_{546}$ = +3, $[\alpha]^{25}_{435}$ = -1, $[\alpha]^{25}_{405}$ = -10 (*c* 0.42, $CHCl_3$).

IR (film) 3405, 2955, 2860, 1715, 1460, 1360, 1265, 1095, 835, 775 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ

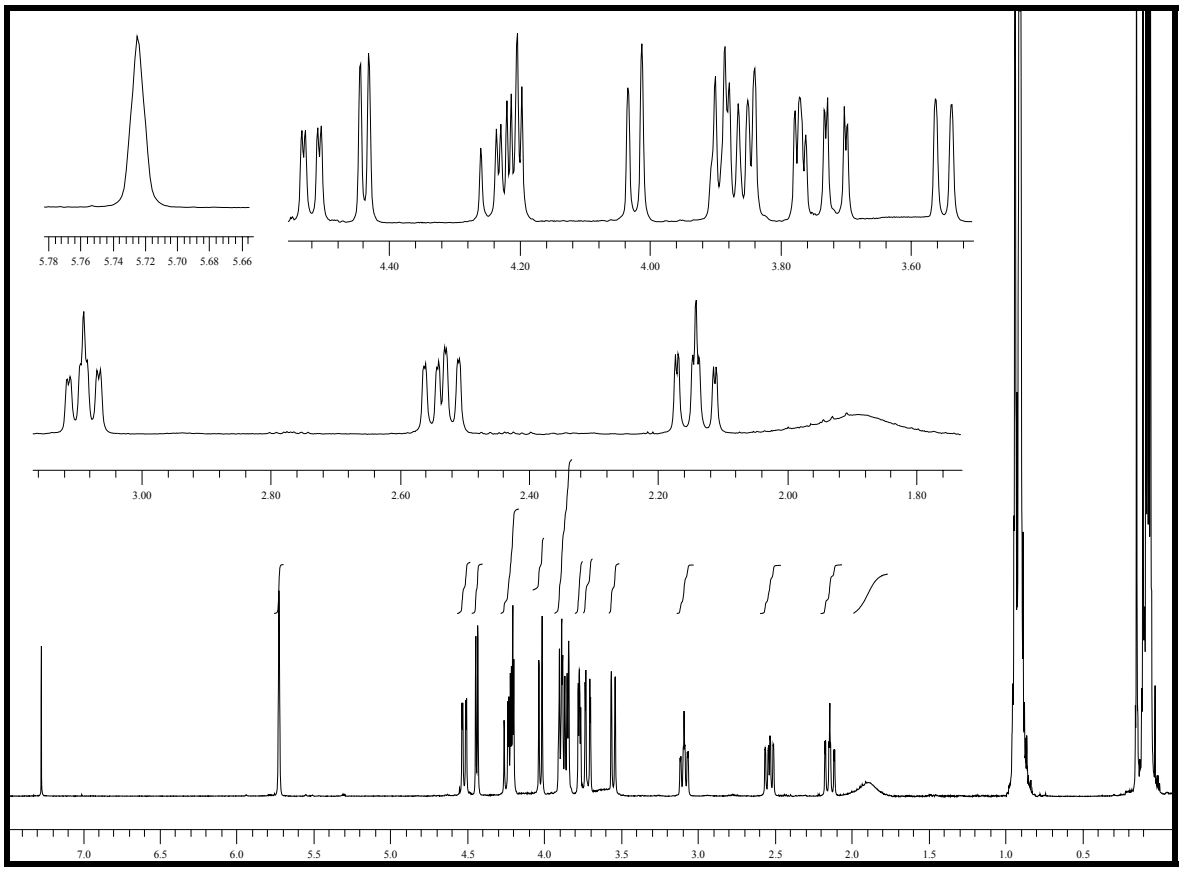
5.73 (br s, H-C(1)); 4.50 (dd, ^3J (H-(6'), H-C(7')) = 9.7 , ^3J (H-(6'), H-C(5')) = 5.8, H-(6')); 4.42 (dd, ^3J (H-(5), H_{exo}-C(6)) = 5.4, ^3J (H-(5), H_{endo}-C(6)) = 0.9 , H-5); 4.22 (dd, ^2J = 12.2, ^3J (H_a-C(7'), H-C(6')) = 9.5, H_a-C(7')); 4.20 (t, ^3J (H-(3'), H-C(4')) = ^3J (H-(3'), H-C(2')) = 2.7, H-(3')); 4.01 (d, ^2J = 8.9, H_{endo}-C(6)); 3.85 (m, 3H, H_{exo}-(6), H-(4'), H-(1')); 3.75 (dd, ^3J (H-(2'), H-C(1')) = 3.2 , ^3J (H-(2'), H-C(3')) = 2.7, H-(2')); 3.70 (dd, ^2J = 12.2, ^3J (H_b-C(7'), H-C(6')) = 1.6, H_b-C(7')); 3.55 (d, ^3J (H-(5'), H-C(6')) = 9.7, H-(5')); 3.07 (m, H-C(3)): 2.51 (m, H_{endo}-C(2)); 2.13 (m, H_{exo}-C(2)); 1.91 (bs, H-(OH));0.97, 0.97, 0.91, 0.88 (4s, 36H, 36H-C(SiC($\underline{CH_3}$)$_3$)); 0.15, 0.14, 0.14, 0.12, 0.08, 0.07, 0.01, 0.01, (8s, 24H, 24H-C(Si$\underline{CH_3}$)).

^{13}C NMR (100.6 MHz, $CDCl_3$):δ

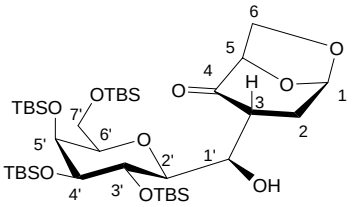
206.0 (s, C(4)); 102.0 (d, ^1J (C,H) = 173, C(1)); 79.5 (d, ^1J (C,H) = 141, C(2')); 79.5 (d, ^1J (C,H) = 163, C(5)); 73.2 (d, ^1J(C,H) = 147, C(1')); 70.2 (d, ^1J (C,H) = 146, C4'); 67.3 (d, ^1J(C,H) = 141, C(3')); 66.8 (t, ^1J(C,H) = 154, C(6)); 65.2 (d, ^1J(C,H) = 146, C(6')); 64.7 (d, ^1J(C,H) = 140, C(5')); 58.5 (t, ^1J(C,H) = 145, C(7')); 44.5 (t, ^1J(C,H) = 140 , C(3)), 32.5 (t, ^1J(C,H) = 125 , C(3)); 26.0, 26.0, 25.9, 25.85 (4q, ^1J (C,H) = 128, ($\underline{CH_3}$)$_3$CSi); 18.4; 18.3, 18.1; 18.0 (4s, (CH_3)$_3$$\underline{C}$Si); -4.4; -4.6; -4.8; -4.9, -5.0; -5.1, -5.3; -5.5; (8q, ^1J (C,H) = 118 , $\underline{CH_3}$Si).

HR-FAB-MS: for $C_{37}H_{76}O_8Si_4$+Na **calcd** 799.4442; **found** 799.4464.

^{1}H-NMR (400 MHz, $CDCl_3$) spectrum of **148**:



1,6-anhydro-3-{(1*R*)-2,6-anhydro-3,4,5,7-tetra-*O*-[(*tert*-butyl)dimethylsilyl]-D-*glycero*-L-*manno*-heptitol-1-*C*-yl}-2,3-dideoxy-*β*-D-*erythro*-hexopyran-4-ulose (149).



149

Data of **149**:

R_f = 0.16 (9.5:0.5, light petroleum ether/EtOAc).

$[\alpha]^{25}_{589}$ = +10, $[\alpha]^{25}_{546}$ = +9, $[\alpha]^{25}_{435}$ = +14, $[\alpha]^{25}_{405}$ = +16 (*c* 0.44, $CHCl_3$).

IR (film) 3405, 2955, 2860, 1715, 1460, 1360, 1265, 1095, 835, 775 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ

5.76 (d, ^3J (H-C(1), H_{exo}-C(2)) = 5.6,H-C(1)); 4.48 (d, ^3J (H-(5), H_{exo}-C(6)) = 4.8, H-5); 4.21 (dd, ^3J (H-C(5'), H-C(6')) = 6.7, ^3J (H-C(5'), H-C(4')) = 2.7, H-C(5')); 4.16 (dd, ^2J = 11.8, ^3J (H_a-C(7'), H-C(6')) = 9.4, H_a-C(7')); 4.09 (d, ^3J (H-(2'), H-C(1')) = 9.7, H-(2')); 3.94-3.86 (m, 2H, H-C(3'), H-C(4')); 3.80-3.75 (m, 2H, H_{endo}-C(6), H-C(6')); 3.73-3.66 (m, 2H, H_{exo}-C(6), H_b-C(7')); 3.53 (dt, ^3J (H-(1'), H-(OH)) = 10.5,^3J (H-(1'), H-C(2')) = 9.7, ^3J (H-(1'), H-C(3)) = 2.7, H-(1')); 3.26 (dt, ^3J (H-(3), H_{exo}-C(2)) = ^3J (H-(3), H_{endo}-C(2)) = 10.2, ^3J (H-(3), H-C(1')) = 2.7, H-(3)); 2.63 (d, ^3J (H-(OH), H-C(1')) = 10.5, H-(OH)); 2.57 (ddd, ^2J = 14, ^3J (H_{exo}-C(2), H-C(3)) = 10.2, ^3J (H_{exo}-C(2), H-C(1)) = 5.6, H_{exo}-C(2)); 1.80 (dd, ^2J = 14, ^3J (H_{endo}-C(2), H-C(3)) = 10.2, H_{endo}-C(2)); 0.97, 0.97, 0.91, 0.88 (4s, 36H, 36H-C(SiC($\underline{CH_3}$)$_3$)); 0.15, 0.14, 0.14, 0.12, 0.08, 0.07, 0.01, 0.01, (8s, 24H, 24H-C(Si$\underline{CH_3}$)).

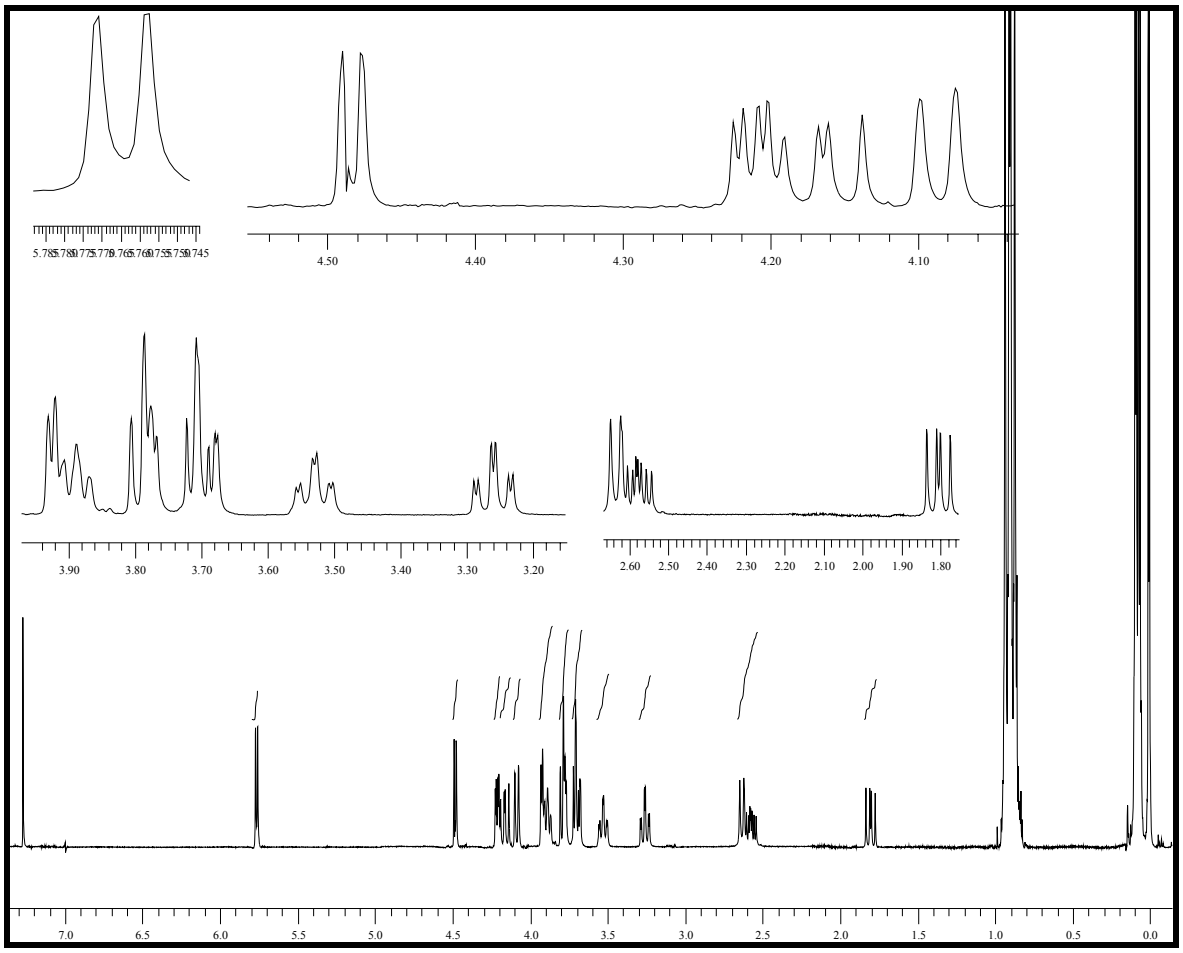
^{13}C NMR (100.6 MHz, $CDCl_3$): δ

216.0 (s, C(4)); 100.5 (d, ^1J (C,H) = 173, C(1)); 79.4 (d, ^1J (C,H) = 141, C(4')); 78.5 (d, ^1J (C,H) = 163, C(5)); 72.9 (d, ^1J(C,H) = 146, C(6')); 70.1 (d, ^1J(C,H) = 141, C(3')); 68.6 (d, ^1J(C,H) = 147, C(1')); 67.3 (d, ^1J (C,H) = 144, C(2')); 66.8 (d, ^1J(C,H) = 140, C(5')); 66.5 (t, ^1J(C,H) = 154, C(6)); 57.6 (t, ^1J(C,H) = 145, C(7')); 42.4 (d, ^1J(C,H) = 125 , C(3));

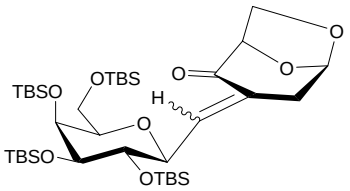
31.7 (t, ^{1}J(C,H) = 154, C(2)); 25.8, 25.8, 25.7, 25.6 (4q, ^{1}J (C,H) = 128, ($\underline{CH_3}$)$_3$CSi); 18.1; 18.0, 17.9; 17.8 (4s, (CH$_3$)$_3$$\underline{C}$Si); -4.5; -4.7; -4.9; -5.0, -5.1; -5.1, -5.4; -5.5; (8q, ^{1}J (C,H) = 118 , $\underline{CH_3}$Si).

HR-FAB-MS: for $C_{37}H_{76}O_8Si_4$+Na **calcd** 799.4442 **found** 799.4451.

^{1}H-NMR (400 MHz, $CDCl_3$) spectrum of **149**:



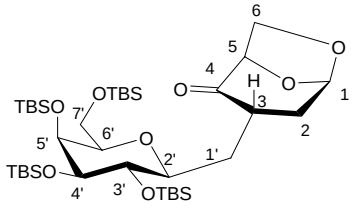
1,6-Anhydro-3-*C*-{2,6-anhydro-3,4,5,7-1-deoxy-tetrakis-*O*-[(*tert*-butyl)dimethylsilyl]-D-*glycero*-L-*manno*-heptitol-1-*C*-ylidene}-2,3-dideoxy-*β*-D-*glycero*-hexopyran-4-ulose (150)



150

Methanesulfonyl chloride (98%, 0.370 mL, 4.76 mmol) was added to a stirred solution of **148** and **149** (0.74 g, 0.959 mmol) in dry pyridine (15 mL) containing DMAP (50 mg) under Ar atmosphere. The mixture was stirred for 14 h and was then quenched by the addition of 20% aq soln of $CuSO_4 \cdot 5H_2O$ solution (30 mL). The solution was extracted with CH_2Cl_2 (3 x 50 mL), and the combined organic phases were washed with 2 M aq HCl (3 x 30 mL), dried ($MgSO_4$), and then concentrated *in vacuo* to yield **150** as unstable colorless oil which was used without further purification in the next step.

1,6-anhydro-3-{2,6-anhydro-1-deoxy-3,4,5,7-tetrakis-*O*-[(*tert*-butyl)dimethylsilyl]-D-*glycero*-L-*manno*-heptitol-1-*C*-yl}-2,3-dideoxy-β-D-*erythro*-hexopyran-4-ulose (151).



151

Raney Nickel (1 g) in aqueous suspension was added to a stirred solution of **150** (0.9 g, 1.19 mmol) in THF (5 mL). The mixture was stirred at 20 °C until complete conversion of **150** (TLC control). The mixture was diluted with Et_2O (200 mL), filtrated through a pad of Celite. FC (9:0.5:0.5 light petroleum ether/Et_2O/CH_2Cl_2): 390 mg (43%) of **152**, and 400 mg (44%) of **151**.

Data of **151**: colorless oil.

IR (film) 2955, 2895, 2855, 1730, 1470, 1255, 1095, 835, 775 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ

5.69 (br s, H-C(1)); 4.53 (dd, ^3J (H-(5), H_{exo}-C(6)) = 5.1, ^3J (H-(5), H_{endo}-C(6)) = 0.5 , H-C(5)); 4.22-4.19 (m, 2H, H_a-(7'), H-(3')); 4.00 (d, ^2J = 8.3, ^3J (H_{endo}-(6), H-C(5)) = 0.5, H_{endo}-C(6)); 3.90 (dd, ^2J = 8.3, ^3J (H_{exo}-(6), H-C(5)) = 5.1, H_{exo}-C(6)); 3.88 (t, ^3J (H-(6'), H_a-C(7')) = ^3J (H-(6'), H-C(5')) = 3.9, H-(6')); 3.80 (d, ^3J (H-(2'), H-C(1')) = 10.5 , ^3J (H-(2')); 3.74 (t, ^3J (H-(4'), H_a-C(3')) = ^3J (H-(4'), H-C(5')) = 3.2, H-(4')); 3.70 (dd, ^2J = 11.8, ^3J (H_a-C(7'), H-C(6')) = 1.61, H_a-C(7')); 3.40 (br s, H-(5')); 3.00 (ddd, ^3J (H-(3), H_{endo}-C(2)) = 10.5, ^3J (H-(3), H_{exo}-C(2)) = 8.1, ^3J (H-(3), H-C(1')) = 3.8, H-(3)); 2.57 (dd, ^2J = 12.6, ^3J (H_{exo}-(2), H-C(3)) = 8.1, H_{exo}-C(2)); 2.37 (ddd, ^2J = 14.8, ^3J (H_a-C(1'), H-C(2')) = 10.7, ^3J (H_a-C(1'), H-C(3)) = 3.8, H_a-C(1')); 1.79 (dd, ^2J = 12.6, ^3J (H_{endo}-(2), H-C(3)) = 10.5, H_{endo}-C(2)); 1.22 (m, H_b-C(1')); 0.97, 0.97, 0.91, 0.88 (4s, 36H, 36H-C(SiC($\underline{CH_3}$)$_3$)); 0.15, 0.14, 0.14, 0.12, 0.08, 0.07, 0.01, 0.01, (8s, 24H, 24H-C(Si$\underline{CH_3}$));

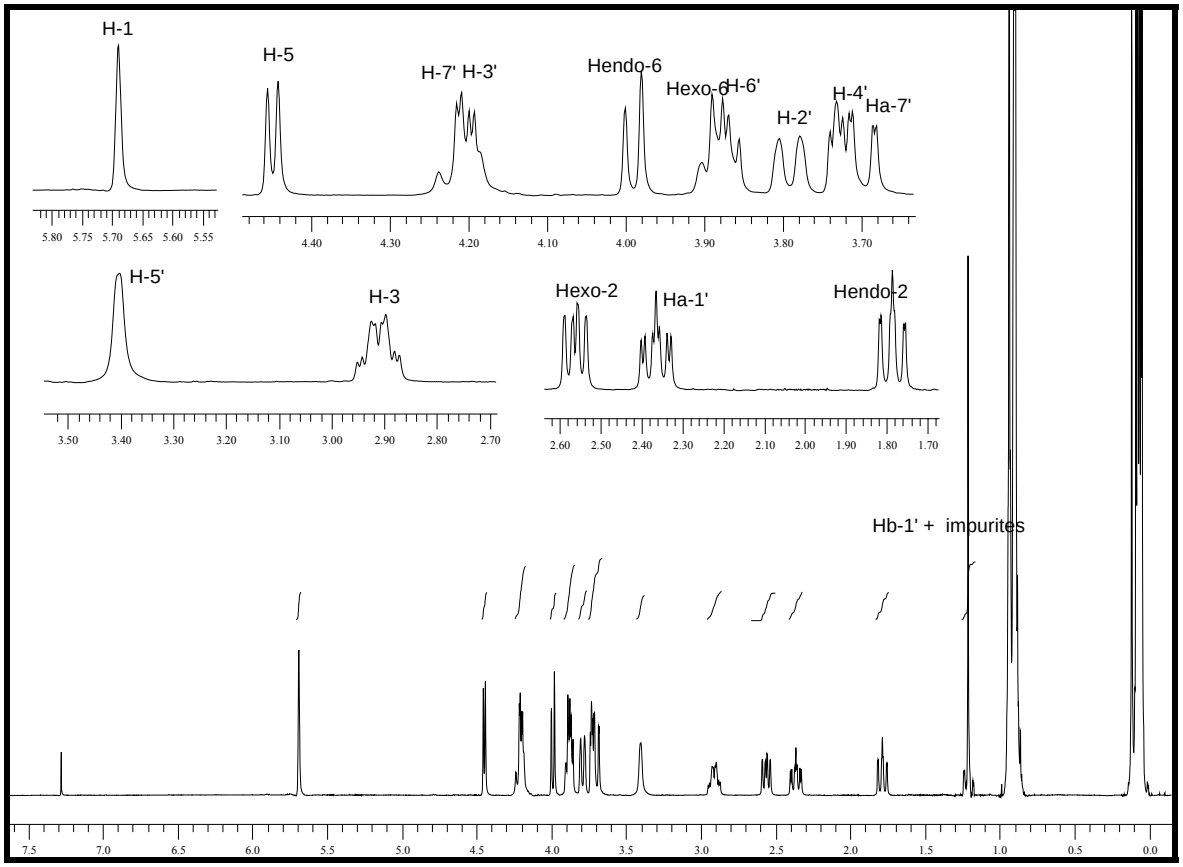
^{13}C NMR (100.6 MHz, $CDCl_3$): δ

204.0 (s, C(4)); 100.8 (d, ^1J (C,H) = 173, C(1)); 79.3 (d, ^1J (C,H) = 141, C(6')); 79.2 (d, ^1J (C,H) = 163, C(5)); 74.5 (d, ^1J(C,H) = 141, C(5')); 73.6 (d, ^1J(C,H) = 146, C(4')); 67.3 (d,

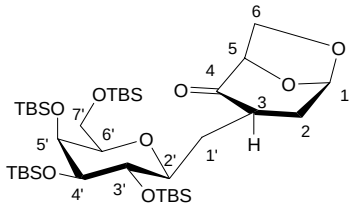
^1J(C,H) = 147, C(3')); 67.0 (t, ^1J(C,H) = 116, C(6)); 62.2 (d, ^1J(C,H) = 146, C(2')); 58.2 (d, ^1J(C,H) = 144, C(7')); 38.6 (d, ^1J(C,H) = 128, C(2)); 38.3 (t, ^1J(C,H) = 125, C(3)); 29.9 (t, ^1J(C,H) = 128, C(1')); 25.9, 25.9, 25.7, 25.6 (4q, ^1J (C,H) = 128, ($\underline{CH_3}$)$_3$CSi); 18.4; 18.3, 18.1; 18.0 (4s, (CH_3)$_3\underline{C}$Si); -4.4; -4.6; -4.8; -4.9, -5.0; -5.1, -5.3; -5.5; (8q, ^1J (C,H) = 118 , $\underline{CH_3}$Si).;

HR-FAB-MS: for $C_{37}H_{76}O_8Si_4$+Na **calcd** 783.4515; **found** 783.4511..

^{1}H-NMR (400 MHz, $CDCl_3$) spectrum of **151**:



1,6-Anhydro-3-{2,6-anhydro-1-deoxy-3,4,5,7-tetrakis-*O*-[(*tert*-butyl)dimethylsilyl]-D-*glycero*-L-*manno*-heptitol-1-*C*-yl}-2,3-dideoxy-β-D-*threo*-hexopyran-4-ulose 152



152

Data of **152**: colorless oil.

$[\alpha]^{25}_{589}$ = -10, $[\alpha]^{25}_{546}$ = -13, $[\alpha]^{25}_{435}$ = -44, $[\alpha]^{25}_{405}$ = -73 (*c* 0.21, $CHCl_3$).

IR (film): 2955, 2895, 2855, 1730, 1470, 1255, 1095, 835, 775 cm^{-1}.

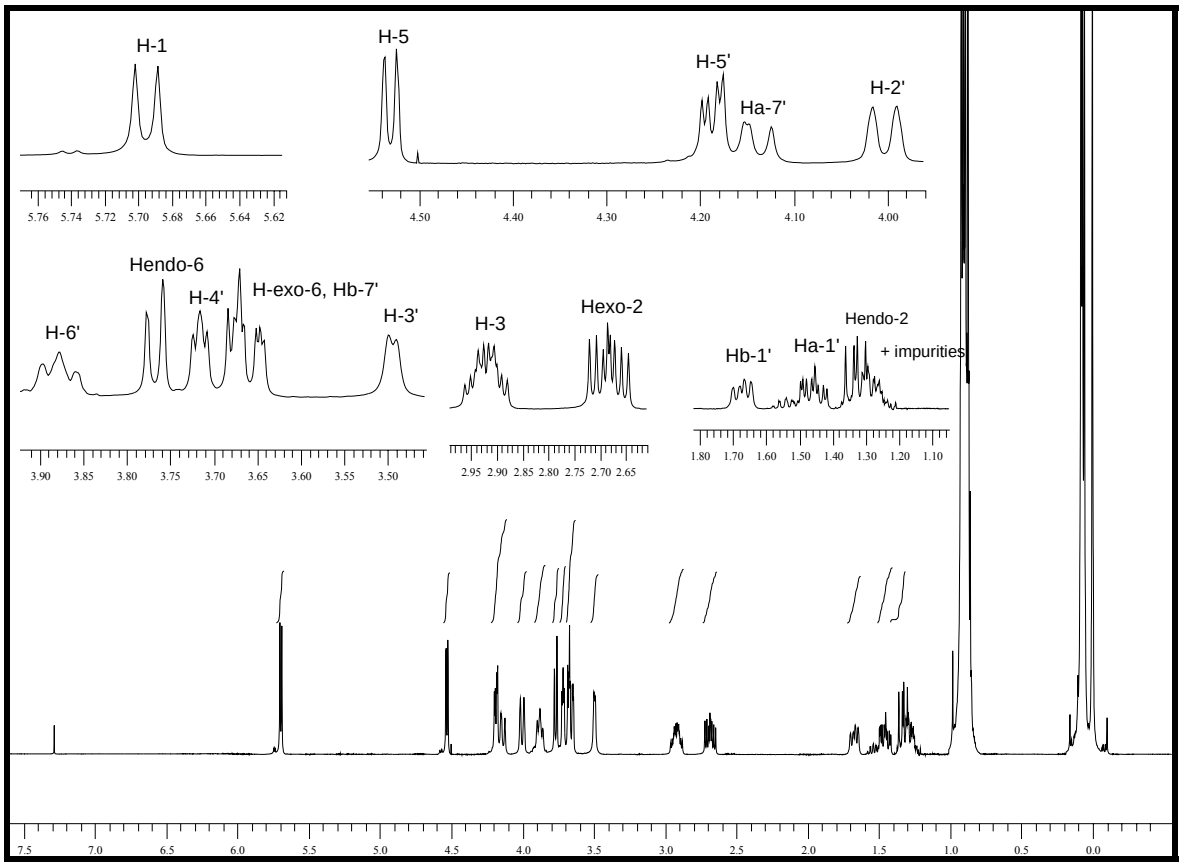
^{1}H NMR (400 MHz, $CDCl_3$): δ
5.70 (d, ^3J (H-(1), H_{exo}-C(2)) = 5.4 , H-C(1)); 4.53 (dd, ^3J (H-(5), H_{exo}-C(6)) = 5.4, ^3J (H-(5), H_{endo}-C(6)) = 0.5 , H-C(5)); 4.19 (dd, ^3J (H-(5'), H-C(4')) = 6.5 , ^3J (H-(5'), H-C(6')) = 2.7, H-(5')); 4.15 (dd, ^2J = 11.8, ^3J (H_a-C(7'), H-C(6')) = 9.7, H_a-C(7')); 4.00 (d, ^3J (H-(2'), H-C(1')) = 10.5 , ^3J (H-(2')); 3.88 (t, ^3J (H-(6'), H_a-C(7')) = ^3J (H-(6'), H-C(5')) = 2.7, H-(6')); 3.77 (d, ^2J = 7.3, ^3J (H-(6), H-C(5)) = 0.5, H_{endo}-C(6)); 3.72 (t, ^3J (H-(4'), H_a-C(3')) = ^3J (H-(4'), H-C(5')) = 3.2, H-(4')); 3.70-3.63 (m, 2H, H_{exo}-(6), H-(7')); 3.49 (d, ^3J (H-(3'), H-C(4')) = 3.5, H-(3')); 2.90 (m, 1H, H-(3)); 2.68 (m, 1H, H_{exo}-(2)); 1.67 (dd, ^2J = 12.9, ^3J (H_b-C(1'), H-C(2')) = 8.0, H_b-C(1')); 1.46 (m, 1H, H_a-C(1')); 1.30 (m, 1H, H_{endo}-C(2)); 0.97, 0.97, 0.91, 0.88 (4s, 36H, 36H-C(SiC($\underline{CH_3}$)$_3$)); 0.15, 0.14, 0.14, 0.12, 0.08, 0.07, 0.01, 0.01, (8s, 24H, 24H-C(Si$\underline{CH_3}$));

^{13}C NMR (100.6 MHz, $CDCl_3$): δ

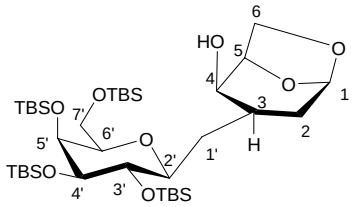
213.0 (s, C(4)); 100.1 (d, ^1J (C,H) = 173, C(1)); 79.3 (d, ^1J (C,H) = 141, C(6')); 79.0 (d, ^1J (C,H) = 163, C(5)); 74.4 (d, ^1J(C,H) = 147, C(3')); 73.6 (d, ^1J(C,H) = 146, C(4')); 67.0 (d, ^1J(C,H) = 141, C(5')); 67.0 (t, ^1J(C,H) = 154, C(6)); 65.0 (d, ^1J(C,H) = 146, C(2')); 58.0 (t, ^1J(C,H) = 145, C(7')); 39.0 (d, ^1J(C,H) = 125, C(3)); 36.0 (t, ^1J(C,H) = 128, C(2)); 30.0 (t, ^1J(C,H) = 128, C(1')); 25.9, 25.9, 25.7, 25.6 (4q, ^1J (C,H) = 128, ($\underline{CH_3}$)$_3$CSi); 18.2; 18.1, 18.0; 17.9 (4s, (CH_3)$_3\underline{C}$Si); -4.4; -4.6; -4.8; -4.9, -5.0; -5.1, -5.3; -5.5; (8q, ^1J (C,H) = 118 , $\underline{CH_3}$Si).

HR-FAB-MS: for $C_{37}H_{76}O_8Si_4$+Na **calcd** 783.4515; **found** 783.4542.

^{1}H-NMR (400 MHz, $CDCl_3$) spectrum of **152**:



1,6-Anhydro-3-*C*-{2,6-anhydro-1-deoxy-3,4,5,7-tetrakis-*O*-[(*tert*-butyl)dimethylsilyl]-D-*glycero*-L-*manno*-heptitol-1-*C*-yl}-2,3-dideoxy-*β*-D-*lyxo*-hexopyranose (153).



153

A THF solution of $LiBH_4$ (0.56 mL, 1.13 mmol) was added dropwise to a solution of **152** (0.140 mg, 0.188 mmol) in THF (5 mL) at -78 °C. The mixture was stirred for 4 h, from -78 °C to 15 °C. Satd aq soln of NH_4Cl (25 mL) was added. The reaction mixture was warmed to 20 °C. A satd soln of sodium potassium tartarate (25 mL) was added. The aqueous phase was extracted with CH_2Cl_2 (3 x 100 mL). The combined organic phases were dried, concentrated *in vacuo*. FC (light petroleum ether/Et_2O 9:1) gave 110 mg of **153** (79%), colorless oil.

$[\alpha]^{25}_{589} = +4$, $[\alpha]^{25}_{546} = +7$, $[\alpha]^{25}_{435} = +9$, $[\alpha]^{25}_{405} = +11$ (*c* 0.08, $CHCl_3$).

IR (film): 3425, 2955, 2855, 1620, 1470, 1360, 1130, 865, 835, 775, 740 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ

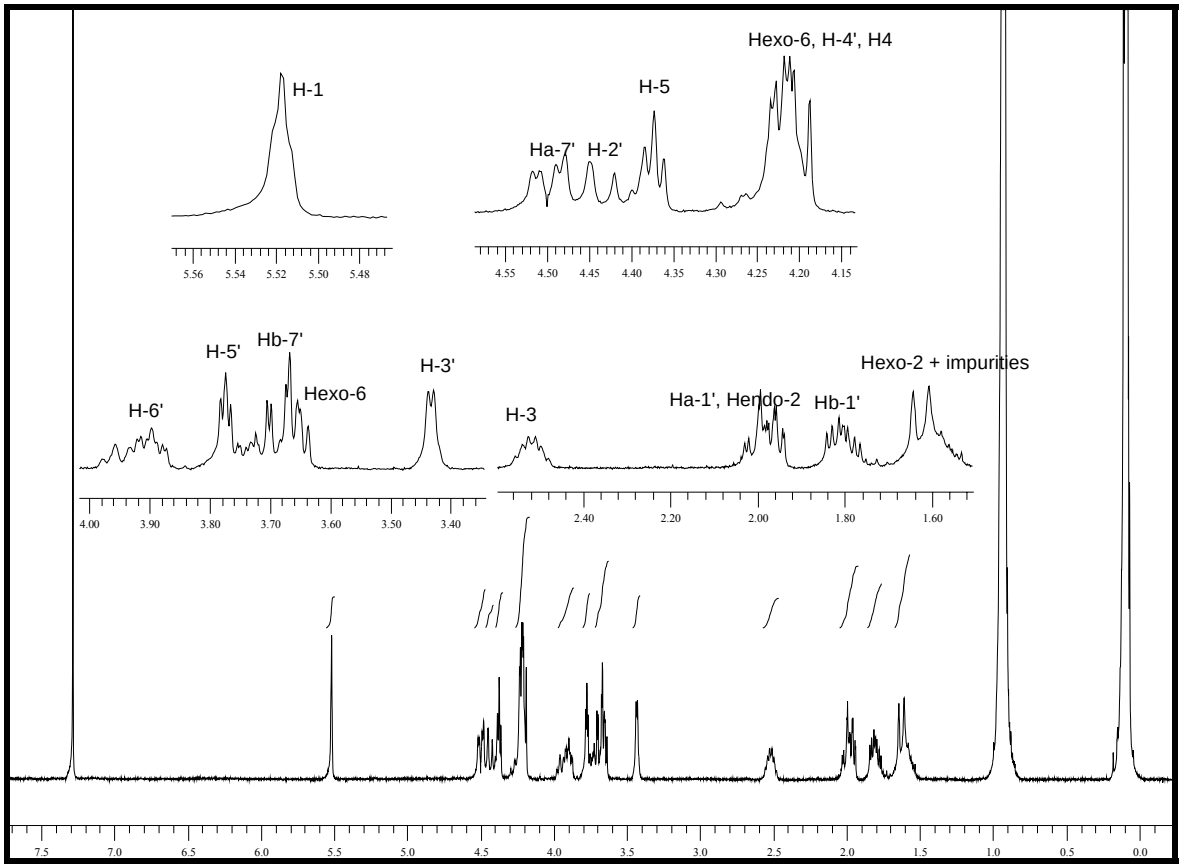
5.57 (br. s, H-C(1)); 4.49 (m, dd, ^2J = 11.3, ^3J (H_a-(7'), H-C(6')) = 4.5, H_a-C(7')); 4.44(d, ^3J (H-(2'), Ha-C(1')) = 11.8, H-C(2')); 4.36(t, ^3J (H-(5), H_{exo}-C(6)) = 4.6, H-C(5)); 4.24-4.17 (m, 3H, H_{exo}-C(6), H-C(4'), H-C(4)); 3.90 (ddt, ^3J (H-(6'), H_a-C(7')) = ^3J (H-(6'), H-C(5')) = 4.5, ^3J (H-(6'), H_b-C(7')) = 2.5 , H-C(6')); 3.88 (t, ^3J (H-(5'), H-C(6')) = 4.5, H-C(5')); 3.73 (dd, ^2J = 11.3, ^3J (H_b-(7'), H-C(6')) = 2.5, H_b-C(7')); 3.70 (dd, ^2J = 7.3, ^3J (H_{exo}-(6), H-C(5)) = 5.1, H_{exo}-C(6)); 3.46 (d, ^3J (H-(3'), H-C(4')) = 3.7, H-C(3')); 2.56-2.42 (m, 1H, H-C(3)); 2.05-1.90 (m, 2H, H_a-C(1'), H_{endo}-C(2)); 1.85-1.75 (m, 1H, H_b-C(1')); 1.68-1.60 (m, 1H, H_{exo}-C(2)); 0.97, 0.97, 0.91, 0.88 (4s, 36H, 36H-C(SiC($\underline{CH_3}$)$_3$)); 0.15, 0.14, 0.14, 0.12, 0.08, 0.07, 0.01, 0.01, (8s, 24H, 24H-C(Si$\underline{CH_3}$));

^{13}C NMR (100.6 MHz, $CDCl_3$): δ

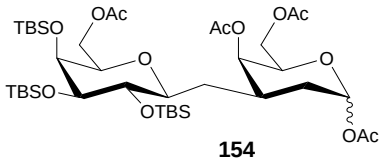
100.1 (d, ^1J (C,H) = 173, C(1)); 78.1 (d, ^1J (C,H) = 141, C(6')); 75.6 (d, ^1J (C,H) = 163, C(5)); 74.4 (d, ^1J(C,H) = 147, C(3')); 63.8 (d, ^1J(C,H) = 141, C(5')); 69.2 (d, ^1J(C,H) = 146, C(4')); 67.5 (d, ^1J(C,H) = 141, C(4)); 64.8 (t, ^1J(C,H) = 144, C(6)); 64.5 (d, ^1J(C,H) = 146, C(2')); 58.6 (d, ^1J(C,H) = 136, C(7')); 36.5 (t, ^1J(C,H) = 128, C(2)); 34.6 (t, ^1J(C,H) = 128, C(1')); 30.8 (d, ^1J(C,H) = 125, C(3)); 25.9, 25.9, 25.7, 25.6 (4q, ^1J (C,H) = 128, ($\underline{CH_3}$)$_3$CSi); 18.4; 18.3, 18.1; 18.0 (4s, (CH_3)$_3\underline{C}$Si); -4.4; -4.6; -4.8; -4.9, -5.0; -5.0, -5.3; -5.5; (8q, ^1J (C,H) = 118 , $\underline{CH_3}$Si).;

HR-FAB-MS: for $C_{37}H_{78}O_8Si_4$+Na **calcd** 785.4671 **found** 785.4662..

^{1}H-NMR (400 MHz, $CDCl_3$) spectrum of **153**:



4,6-Di-*O*-acetyl-3-*C*-{7-*O*-acetyl-2,6-anhydro-1-deoxy-3,4,5-tri-*O*-[(*tert*-butyl)dimethylsilyl]-D-*glycero*-L-*manno*-heptitol-1-*C*-yl}-2,3-dideoxy-*α,β*-D-*lyxo*-hexopyranosyl acetate (154α,β).



154

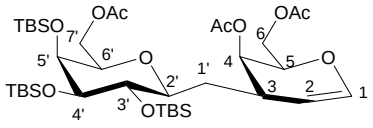
A catalytic amount of triethylsilyl triflate (1.3 μL) was added dropwise via a syringe to a solution of compound **153** (0.12 g, 0.157 mmol) in acetic anhydride (2 mL) cooled at 0 °C. The mixture was stirred at 0°C for 30 min, and then a satd aq soln of $NaHCO_3$ was added. The mixture was extracted with CH_2Cl_2 (3 x 10 mL). The organic layers were dried ($MgSO_4$), concentrated *in vacuo*, coevaporated with toluene *in vacuo*. The oily product so-obtained was used directly in the next step.

$[\alpha]^{25}_{589}$ = +50, $[\alpha]^{25}_{546}$ = +51, $[\alpha]^{25}_{435}$ = +99, $[\alpha]^{25}_{405}$ = +118 (*c* 0.21, $CHCl_3$).

IR (film): 15735, 1435, 1370, 1220, 1030, 910, 600 cm^{-1}.

HR-FAB-MS: for $C_{39}H_{74}O_{13}Si_3$+Na **calcd** 857.4309; **found** 857.4334.

4,6-Di-*O*-acetyl-3-*C*-{7-*O*-acetyl-2,6-anhydro-1-deoxy-3,4,5-tri-*O*-[(*tert*-butyl)dimethylsilyl]-D-*glycero*-L-*manno*-heptitol-1-*C*-yl}-3-deoxy-D-galactal (155).



155

Silica gel (870 mg) was added to a solution of **154*α,β*** (0.286 g, 0.315 mmol) in toluene (10 mL) and the mixture was heated under reflux for 3 h. Filtration, washing with CH_2Cl_2 (100 mL), concentration *in vacuo*, FC (9:0.5:0.5, light petroleum ether/Et_2O/CH_2Cl_2): 160 mg (60%), yellowish oil.

$[\alpha]^{25}_{589}$ = -7, $[\alpha]^{25}_{546}$ = -10, $[\alpha]^{25}_{435}$ = -27, $[\alpha]^{25}_{405}$ = -40 (*c* 0.09, $CHCl_3$).

IR (film): 1955, 1755, 1650, 1370, 1235, 1090, 1035, 905, 600 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ

δ 6.38 (dd, ^3J (H-(1), H-C(2)) = 6.2, ^4J (H-(1), H-C(3)) = 2.7, H-C(1)); 5.49 (d, ^3J (H-(4), H-C(3)) = 3.4, H-C(4)); 5.16 (dd, ^2J = 12.6, ^3J (H_a-(7'), H-C(6')) = 9.9, H_a-C(7')); 4.72 (dt, ^3J (H-(2), H-C(1)) = 6.2, ^3J (H-(2), H-C(3)) = 3.9, H-C(2)); 4.28-4.17 (m, 7H, H_{exo}-C(6), H_{endo}-C(6), H_b-C(7'), H-C(5), H-C(6'), H-C(5'), H-C(2')); 3.77 (t, ^3J (H-(4'), H-C(3')) = 4.6, H-C(4')); 3.40 (d, ^3J (H-(3'), H-C(4')) = 4.6, H-C(3')); 2.70 (br s, H-C(3)); 2.10-2.06 (3s, 9H, CH_3COO); 1.62-1.50 (m, 1H, H_a-C(1'), 1.29-1.20 (m, 1H, H_b-C(1'), 0.95, 0.94, 0.93 (3s, 27H, 27H-C(SiC($\underline{CH_3}$)$_3$)); 0.12, 0.12, 0.11, 0.10, 0.09, 0.07, (6s, 18H, 18H-C(Si$\underline{CH_3}$)).

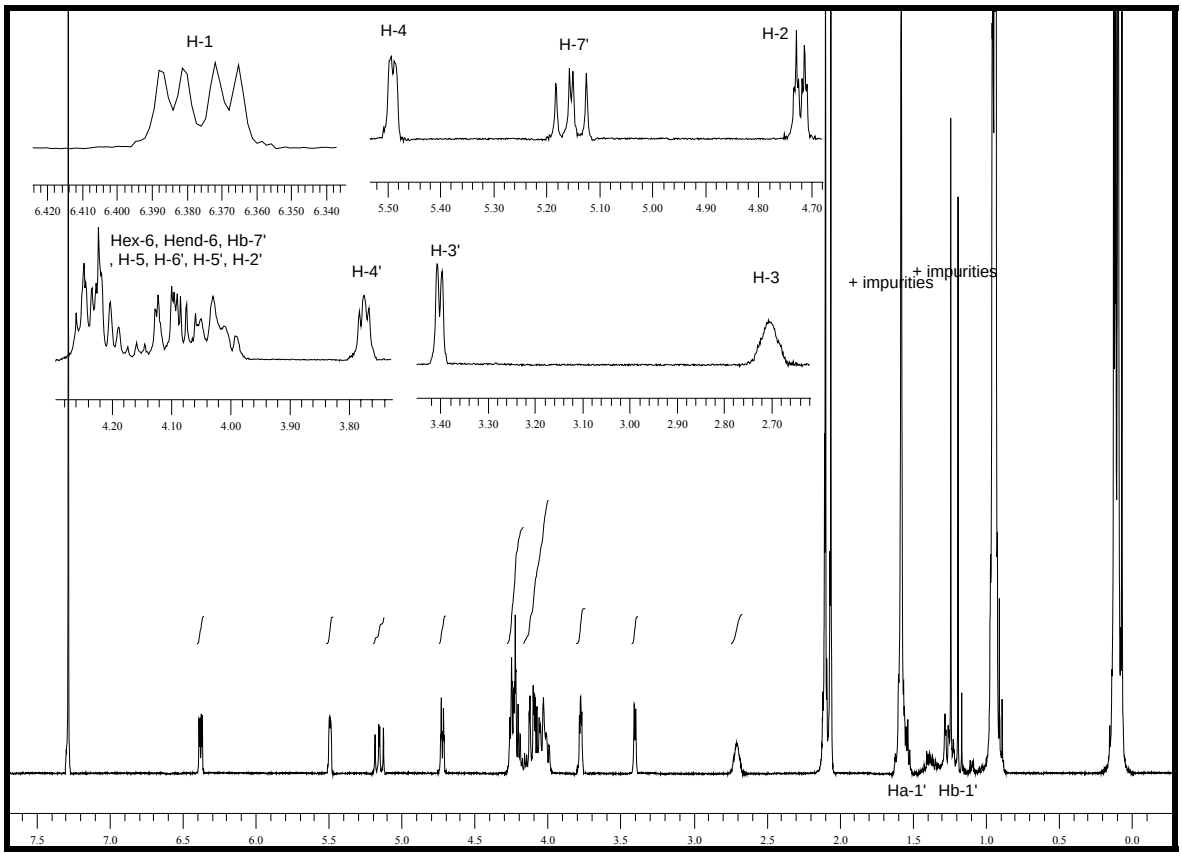
^{13}C NMR (100.6 MHz, $CDCl_3$): δ

δ 171.0, 170.7, 170.3 (3s, -COO); 142.2 (d, ^1J (C,H) = 148, C(1)); 104.0 (d, ^1J (C,H) = 146, C(2)); 75.8 (d, ^1J (C,H) = 142, C(5')); 74.4 (d, ^1J (C,H) = 146, C(4')); 74.1 (d, ^1J (C,H) = 147, C(2')); 73.5 (d, ^1J (C,H) = 147, C(3')); 67.1 (d, ^1J (C,H) = 163, C(5)); 65.6 (d, ^1J (C,H) = 141, C(6')); 65.0 (d, ^1J(C,H) = 142, C(4)); 63.4 (t, ^1J(C,H) = 145, C(7')); 60.2 (t, ^1J(C,H) = 106, C(6)); 32.9 (d, ^1J(C,H) = 125, C(3)); 32.3 (t, ^1J(C,H) = 128, C(1')); 26.1- 25.8 (3q, ^1J(C,H) = 130, $\underline{C}H_3COO$); 25.9, 25.9, 25.7 (3q, ^1J (C,H) = 128,

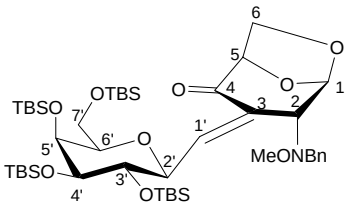
($\underline{CH_3}$)$_{\underline{3}}$CSi); 21.1; 20.8, 20.7 (3s, (CH_3)$_3\underline{C}$Si); -4.4; -4.5; -4.7; -4.7, -5.0; -5.1 (6q, ^1J (C,H) = 118 , $\underline{CH_3}$Si).

HR-FAB-MS: for $C_{37}H_{70}O_{11}Si_3$+Na **calcd** 797.4123; **found** 797.4151.

^{1}H-NMR (400 MHz, $CDCl_3$) spectrum of **155**:



(*E*) or (*Z*) 1,6-Anhydro-3-*C*-{2,6- anhydro-1-deoxy-3,4,5,7-tetra-*O*-[(*tert*-butyl) dimethylsilyl]-D-*glycero*-L- *manno*- heptitol-1-C-ylidene} –2-[(benzyl)(methoxy) amino] 2,3-dideoxy-β-D-*erythro*-hexopyranos-4-ulose (169).



169

Trifluroacetic anhydride (TFAA) (5 mL, 35.95 mmol) was added dropwise at 0°C solution of alcohols **131a** (11.9 g, 13.04 mmol) and pyridine (5 mL, 61.31 mmol).in CH_2Cl_2 (40 ml). Stirring was continued for 1 h, then DBU (15mL, 98.70 mmol) was added, after stirring for 20 min. The mixture was diluted by Et_2O (500 mL) and the solution was quenched with saturated aqueous $NaHCO_3$ (100 mL), washed with HCl (1M) (100 mL), then with saturated aqueous $NaHCO_3$ (100 mL) again, dried ($MgSO_4$), Filtration, evaporation, FC (5% of Et_2O in pentane) gave (**E** or **Z** enone **169**) (9.00 g, 10.04 mmol, 80 %). (Note: NMR information's not enough to confirm Z or E enone)

$[\alpha]^{25}_{589}$ = +11.6, $[\alpha]^{25}_{577}$ = -23.2, $[\alpha]^{25}_{435}$ = -137, $[\alpha]^{25}_{405}$ = -435 (*c* 0.155, $CHCl_3$).

UV (MeCN): 272 (1084), 236 (2137).

IR (cm^{-1}): 2930, 2855, 1705, 1620, 1470, 1255, 1095, 875, 835, 775, 740.

^{1}H NMR (400 MHz, $CDCl_3$): δ

7.34-7.17 (m, 5H, ArH); 6.88 (dd, ^3J(H_a-(1'), H-(2') = 7.1, ^3J(H_a-(1'), H-(3') = 1.9, H-C(1')); 6.04 (s, H-C(1)); 5.33 (d, ^3J (H-(2'), H-(1')) = 7.1, H-C(2')); 4.61 (t, ^3J (H-C(5), H_{exo}-C(6)) = 3.4, H-C(5)); 4.31 (dd, ^3J(H-C(5'), H-C(6')) = 6.5, ^3J (H-C(5'), HC-(4')) = 2.5, H-C(5')); 4.21 (dd, ^2J = 12.3, ^3J (H_a-C(7'), H-C(6')) = 8.9, H_a-C(7')); 4.03 (dd, ^3J (H-(3'), H-(4')) = 4.0, ^4J(H-C(3'), H-(1')) = 1.9, H-C(3')); 3.94 (dd, ^3J (H-(6'), H_a-(7')) = 8.9, ^3J(H-C(6'), H-(5')) = 6.5, H-C(6')); 3.90 (d, ^2J = 13.9, H_{endo}-C(6)); 3.85 (d, 2H, ^2J = 13.9, H-C(N$\underline{CH_2}$Ph)); 3.80 (bs, H-C(2)); 3.76-3.68 (m, 3H, H_{exo}-C(6), H_b-C(7'), H-C(4')); 3.14

(s, 3H, H-(O$\underline{CH_3}$)); 0.97, 0.90, 0.86, 0.75 (4s, 36H, 36H-C(SiC($\underline{CH_3}$)$_3$)); 0.16, 0.09, 0.07, 0.05, 0.02, -0.09, -0.13, -0.13 (8s, 24H, 24H-C(Si$\underline{CH_3}$)).

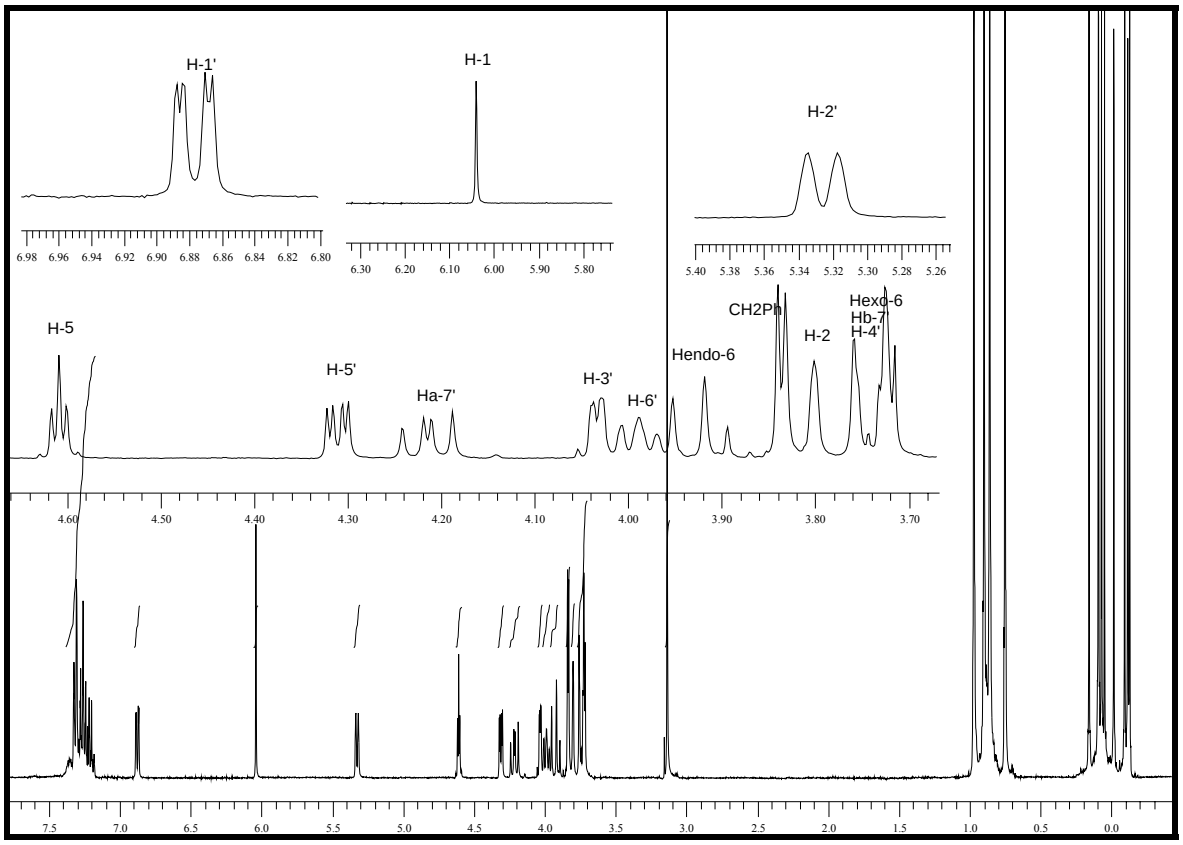
^{13}C NMR (100.6 MHz, $CDCl_3$): δ

195.5 (s, C(4)); 155.3 (d, ^1J(C,H) = 179, C(1')); 138.9 (s, C(3));138.8 (s, C(arom)); 129.8 (d, ^1J(C,H) = 159, C(arom)); 128.0 (d, ^1J(C,H) = 156, C(arom)); 127.3 (d, ^1J(C,H) = 156, C(arom)); 99.9 (d, ^1J (C,H) = 179, C(1)); 79.7 (d, ^1J(C,H) = 147, C(6')); 78.8(d, ^1J (C,H) = 159, C(5)); 73.9 (d, ^1J(C,H) = 154, C(4'));73.6 (d, ^1J (C,H) = 141, C3'); 67.6 (t, ^1J (C,H) = 146, C(N$\underline{C}H_2$Ph); 67.0 (d, ^1J (C,H) = 146, C(2')); 67.0 (d, ^1J(C,H) = 146, C(5')); 64.8 (d, ^1J(C,H) = 143 , C(2)); 60.8 (q, ^1J(C,H) = 140, H(O$\underline{C}H_3$)); 59.7 (t, ^1J(C,H) = 143, C(7')); 56.4 (t, ^1J(C,H) = 154, C(6)); 26.0, 25.9, 25.7, 25.6 (4q, ^1J (C,H) = 125, ($\underline{CH_3}$)$_3$CSi); 18.2; 18.1, 18.0, 17.9(4s, $(CH_3)_3\underline{C}$Si); -4.4; -4.6; -4.7; -4.8, -5.1; -5.3, -5.4; -5.5 (8q, ^1J (C,H) = 118 , $\underline{CH_3}$Si).

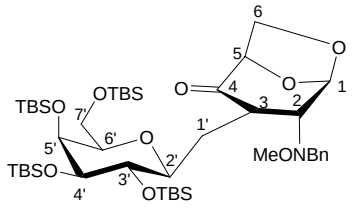
MALDI-HRMS Calcd for (M + Na) $C_{45}H_{83}NO_9Si_4Na$ 916.5043; found 916.5034.

Anal. calcd for $C_{45}H_{83}NO_9Si_4$ (894.48): C 60.42, H9.35, N 1.57; found: C 60.45, H 9.36, N 1.56.

1H-NMR (400 MHz, $CDCl_3$) spectrum of **169:**



1,6-Anhydro-3-{2,6-anhydro-1-deoxy-3,4,5,7-tetra-*O*-[(*tert*-butyl)dimethylsilyl]-D-*glycero*-L-*manno*-heptitol-1-*C*-yl}–2-[(*N*-benzyl-*N*-methoxy)amino]-2,3-dideoxy-*β*-D-*xylo*-hexopyran-4-ulose (170).



170a

Raney Nickel (1g) in aqueous suspension was added to a stirred solution of **169** (2.2 g, 2.46 mmol) in ethanol (5mL). The mixture was stirred at 70 ^{o}C and under 50 atm of H_2 until complete conversion of **169** (TLC control). The mixture was diluted with CH_2Cl_2 (200mL) and filered through a pad of Celite. Evaporation, the residue was disolvend in 20 mL solution of 5:1 THF: MeOH containing 1 % aq.KOH, then the mixture was stirred for 3 h. The mixture was diluted with CH_2Cl_2 (200mL), washed with water (50mL), dried ($MgSO_4$). Filtration, evaporation, FC (9:1 , light petroleum ether/Et_2O) gave 1.9g (87 %) of **170a**, colorless oil.

$[\alpha]^{25}_{589}$ = +9.1, $[\alpha]^{25}_{577}$ = +25.1, $[\alpha]^{25}_{435}$ = +33.7, $[\alpha]^{25}_{405}$ = +16.6 (*c* 0.175, $CHCl_3$).

IR (cm^{-1}): 2895, 2855, 1725, 1255, 875, 835, 775, 740.

^{1}H NMR (400 MHz, $CDCl_3$): δ

7.50-7.24 (m, 5H, ArH); 6.04 (s, H-C(1)); 4.56 (d, ^3J (H-C(5), H_{exo}-C(6)) = 5.3, H-C(5)); 4.31 (d, ^3J = 13.0, H_a-C(N$\underline{CH_2}$Ph)); 4.24 (dd, ^3J(H-C(5'), H-C(6')) = 6.5, ^3J (H-C(5'), HC-(4')) = 2.5, H-C(5')); 4.14 (dd, ^2J = 11.7, ^3J (H_a-C(7'), H-C(6')) = 9.6, H_a-C(7')); 4.01 (d, ^3J (H-(2'), H-(1')) = 10.8, H-C(2')); 3.88-3.78 (m, 3H, H_b-C(N$\underline{CH_2}$Ph), H_{endo}-C(6), H-C(6')); 3.75 (t, ^3J(H-C(4'), H-C(5')) = 2.5, H-C(4')); 3.70 (dd, ^2J = 7.1, ^3J (H_{exo}-C(6), HC-(5)) = 5.3, H_{exo}-C(6)); 3.65 (d, ^2J = 11.7, H_b-C(7')); 3.60 (br d, ^3J (H-(3'), H-(4')) = 3.5, H-C(3')); 3.33 (s, 3H, H-(O$\underline{CH_3}$)); 3.22 (m, 1H, , H-C(3)); 2.62 (br s, 1H, H-C(2)); 2.01 (t,

^{2}J = 12.7, ^{3}J (H_a-C(1'), H-C(2')) = 10.8, H_a-C(1')); 1.47 (m, 1H, H_b-C(1')); 0.97, 0.96, 0.92, 0.87 (4s, 36H, 36H-C(SiC($\underline{CH_3}$)$_3$)); 0.13, 0.11, 0.11, 0.11, 0.10, 0.07, 0.00, -0.01 (8s, 24H, 24H-C(Si$\underline{CH_3}$)).

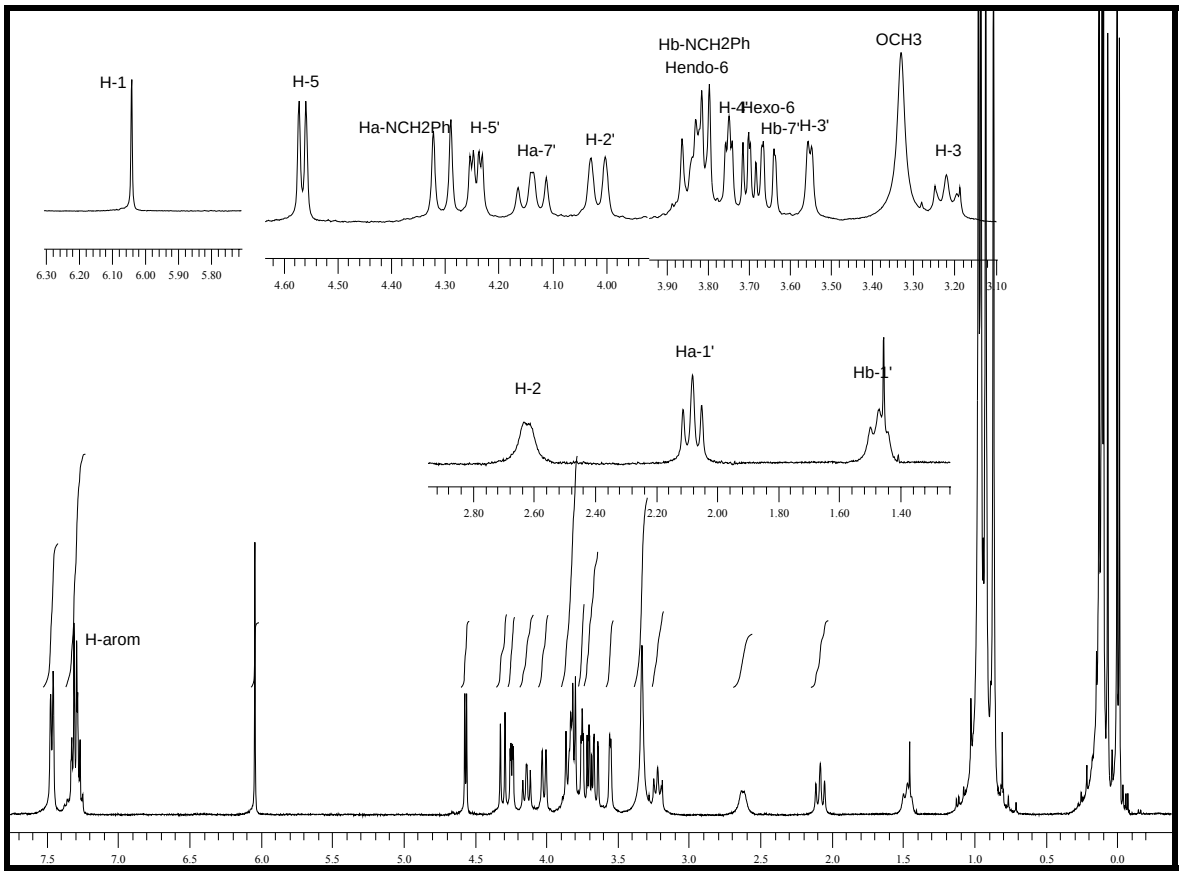
^{13}C NMR (100.6 MHz, $CDCl_3$): δ

213.3 (s, C(4)); 138.8 (s, C(arom)); 129.8 (d, ^{1}J(C,H) = 159, C(arom)); 128.0 (d, ^{1}J(C,H) = 156, C(arom)); 127.0 (d, ^{1}J(C,H) = 156, C(arom)); 99.8 (d, ^{1}J (C,H) = 179, C(1)); 78.8 (d, ^{1}J(C,H) = 147, C(6')); 78.6(d, ^{1}J (C,H) = 159, C(5)); 74.3 (d, ^{1}J (C,H) = 141, C3'); 73.8 (d, ^{1}J(C,H) = 154, C(4')); 67.5 (d, ^{1}J(C,H) = 146, C(5')); 67.4 (d, ^{1}J(C,H) = 143 , C(2)); 67.0 (t, ^{1}J(C,H) = 154, C(6)); 64.4 (d, ^{1}J (C,H) = 146, C(2')); 61.3 (q, ^{1}J(C,H) = 140, H(O$\underline{C}$H$_3$)); 58.5 (t, ^{1}J(C,H) = 143, C(7')); 57.2 (t, ^{1}J (C,H) = 146, C(N$\underline{C}$H$_2$Ph); 42.4 (d, ^{1}J(C,H) = 126, C(3)); 27.8 (d, ^{1}J(C,H) = 126, C(1')); 26.0, 25.9, 25.8, 25.7 (4q, ^{1}J (C,H) = 125, ($\underline{CH_3}$)$_3$CSi); 18.2; 18.1, 18.0, 17.9 (4s, (CH$_3$)$_3\underline{C}$Si); -4.2,-4.4; -4.6; -4.9; -5.0; -5.4, -5.5; -5.8 (8q, ^{1}J (C,H) = 118 , $\underline{CH_3}$Si).

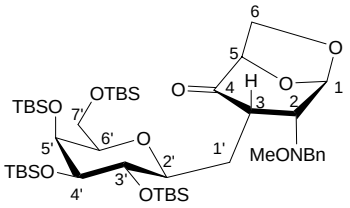
MALDI-HRMS Calcd for (M + Na) $C_{45}H_{85}NO_9Si_4Na$ 918.5199; found 918.5178.

Anal. calcd for $C_{45}H_{85}NO_9Si_4$ (896.49): C 60.29, H9.56, N 1.56; found: C 60.24, H 9.50, N 1.57.

1H-NMR (400 MHz, $CDCl_3$) spectrum of **170a:**



1,6-Anhydro-3-{(2,6-anhydro-1-deoxy-3,4,5,7-tetra-*O*-[(*tert*-butyl)dimethylsilyl]-D-*glycero*-L-*manno*-heptitol-1-*C*-yl}–2-[(*N*-benzyl-*N*-methoxy)amino]-2,3-dideoxy-*β*-D-*erythro*-hexopyran-4-ulose (170b).



170b

Copper cyanide (153 mg, 1.71 mmol) was suspended in THF (7 mL) and chilled to -20 °C. A 1.64 M solution of n-BuLi in hexanes (0.96 mL, 1.53 mmol) was added dropwise. The brown solution was stirred at -20 °C for 30 min, and then the temperature was lowered to -50 °C. A 1 M solution of DIBAL in hexanes (3.4 mL, 3.4 mmol) was added slowly dropwise. The dark brown solution was allowed to stir at -50 °C for 1 h before the enone **169** (150 mg, 1.68 mmol) was added as a solution in 3:1 THF/HMPA (4 mL). The temperature was raised to -20 °C over the next 10 min and then the mixture was allowed to stir at -20 °C for 1 h. The reaction was quenched with a saturated aqueous solution of NH_4Cl at -20 °C and allowed to warm to ambient temperature over 30 min. The mixture was filtered and extracted with ether. The organic layer was evaporated and then partitioned between ether and, sequentially, saturated aqueous solution of NH_4Cl, water, and brine. The organic layer was dried (Na_2SO_4), filtered through silica gel, and evaporated to yield the crude reduction product (120 mg) as a pale-yellow oil. (This procedure is problematic because the reproducibility and the difficultly to avoid 1,2 reduction).

A 0.1 M solution of the Dess-Martin periodinane in CH_2Cl_2 (1 mL, 0.1 mmol) was added to a solution of the crude oil from reduction in CH_2Cl_2. After 30 min, and again after 1 h, additional portions (1 mL each) of periodinane were added. After 2 h, the reaction was complete (TLC). A solution of 1:1 10% aqueous $Na_2S_2O_4$/1 M aqueous NaOH (20 mL) was added and the mixture was allowed to stir for 30 min. The mixture was then partitioned between ether and, sequentially, water and brine. The organic layer was dried

($MgSO_4$), and evaporated, FC (9:1 , light petroleum ether/Et_2O) gave **170b** (100 mg, 1.12 mmol, 65 %), clear oil R_f (20% ether/petroleum ether) = 0.48.

IR (cm^{-1}): 2900, 2865, 1730, 1250, 870, 835, 770, 735.

^{1}H NMR (400 MHz, $CDCl_3$): δ

7.33-7.22 (m, 5H, ArH); 6.05 (s, H-C(1)); 4.50 (d, ^3J (H-C(5), H_{exo}-C(6)) = 5.2, H-C(5)); 4.44 (dd, ^2J = 12.0, ^3J (H_a-C(7'), H-C(6')) = 8.9, H_a-C(7')); 4.29 (d, ^3J (H-C(2), H-C(3)) = 7.7, H-C(2)); 4.34 (dd, ^3J(H-C(5'), H-C(6')) = 6.5, ^3J(H-C(5'), H-C(4')) = 2.7, ^3J (H-C(5')); 4.10 (d, ^3J = 14.5, H_a-C(N$\underline{CH_2}$Ph)); 4.05 (dd, ^2J = 8.3, H_{endo}-C(6)); 3.95 (ddd, ^3J(H-C(6'), H_a-C(7')) = 8.9, ^3J(H-C(6'), H-C(5')) = 6.5, ^3J(H-C(6'), H_b-C(7')) = 2.2, ^3J (H-C(6'); 3.88 (dd, ^2J = 8.3, ^3J (H_{exo}-C(6), HC-(5)) = 5.2, H_{exo}-C(6)); 3.83 (dd, ^3J (H-C(4'), HC-(3')) = 4.1, ^3J (H-C(4'), HC-(5')) = 3.1, H-C(4')); 3.74 (dd, ^2J = 12.0, ^3J (H_b-C(7'), H-C(6')) = 2.2, H_b-C(7')); 3.59 (d, ^3J (H-C(3'), HC-(4')) = 4.1, H-C(3')); 3.54 (d, ^3J = 14.5, H_b-C(N$\underline{CH_2}$Ph); 3.35 (ddd, ^3J(H-C(3), H_a-C(1')) = 11.4, ^3J(H-C(3), H-C(2)) = 7.7, ^3J(H-C(3), H_b-C(1')) = 3.7, ^3J (H-C(3)); 3.23 (s, 3H, H-(O$\underline{CH_3}$)); 2.45 (ddd, ^2J = 14.8, ^3J(H_a-C(1'), H-C(3)) = 11.4, ^3J(H_a-C(1'), H-C(2')) = 3.5, H_a-C(1')); 1.43 (dd, ^2J = 14.8, ^3J(H_b-C(1'), H-C(3)) = 3.7, H_b-C(1')); 0.97, 0.92, 0.92, 0.84 (4s, 36H, 36H-C(SiC($\underline{CH_3}$)$_3$)); 0.16, 0.13, 0.11, 0.10, 0.09, 0.08, 0.07, 0.06 (8s, 24H, 24H, C(Si$\underline{CH_3}$)).

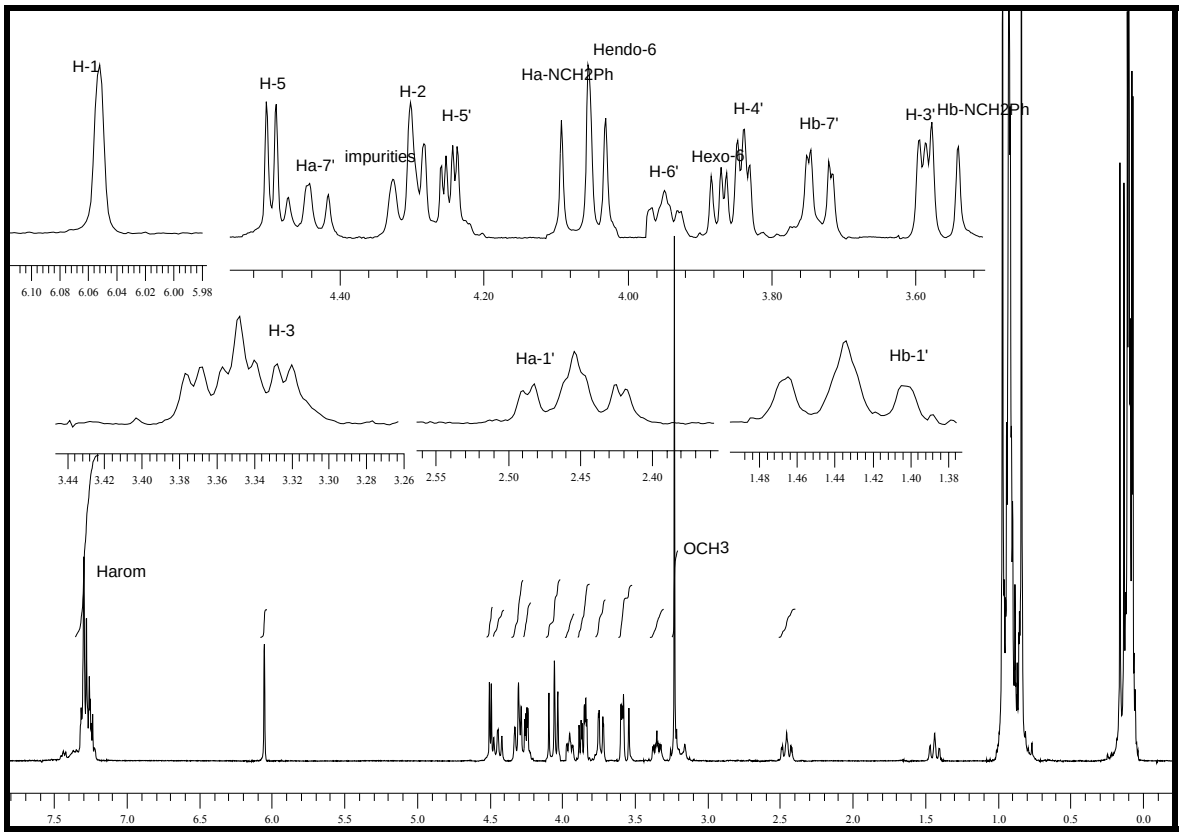
^{13}C NMR (100.6 MHz, $CDCl_3$): δ

204.3 (s, C(4)); 139.1 (s, C(arom)); 129.5 (d, ^1J(C,H) = 159, C(arom)); 128.1 (d, ^1J(C,H) = 156, C(arom)); 127.0 (d, ^1J(C,H) = 159, C(arom)); 100.6 (d, ^1J (C,H) = 179, C(1)); 79.4(d, ^1J (C,H) = 159, C(5)); 79.1 (d, ^1J(C,H) = 147, C(6')); 74.5 (d, ^1J (C,H) = 141, C(3'); 74.0 (d, ^1J(C,H) = 154, C(4')); 67.7 (d, ^1J(C,H) = 146, C(5')); 66.4 (t, ^1J(C,H) = 154, C(6)); 65.5 (d, ^1J(C,H) = 143 , C(2)); 61.8 (d, ^1J (C,H) = 146, C(2')); 61.2 (q, ^1J(C,H) = 140, H(O$\underline{C}$H$_3$)); 58.3 (t, ^1J(C,H) = 143, C(7')); 57.7 (t, ^1J (C,H) = 146, C(N$\underline{C}$H$_2$Ph); 40.2 (d, ^1J(C,H) = 125, C(3)); 24.4 (d, ^1J(C,H) = 128, C(1')); 25.9, 25.8, 25.7, 25.6 (4q, ^1J (C,H) = 125, ($\underline{CH_3}$)$_3$CSi); 18.1; 17.9, 17.4, 17.2(4s, (CH$_3$)$_3$$\underline{C}$Si); -4.4,-4.5; -4.6; -4.8; -4.9; -5.2, -5.3; -5.4 (8q, ^1J (C,H) = 118 , $\underline{CH_3}$Si).

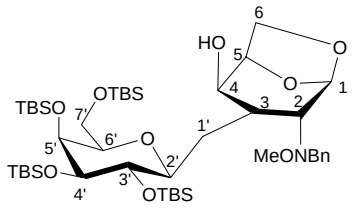
MALDI-HRMS Calcd for (M + Na) $C_{45}H_{85}NO_9Si_4Na$ 918.5199; found 918.5178.

Anal. calcd for $C_{45}H_{85}NO_9Si_4$ (896.49): C 60.29, H9.56, N 1.56; found: C 60.24, H 9.50, N 1.57.

1H-NMR (400 MHz, $CDCl_3$) spectrum of **170b:**



1,6-Anhydro-3-*C*-{2,6-anhydro-1-deoxy-3,4,5,7-tetra-*O*-[(*tert*-butyl)dimethylsilyl]-D-*glycero*-L-*manno*-heptitol-1-*C*-yl}–2-[(*N*-benzyl-*N*-methoxy)amino]-2,3-dideoxy-*β*-D-*galacto*-hexopyranose (171).



171

A THF solution of $LiBH_4$ (7.0 mL, 14.0 mmol) was added dropwise to a solution of **170a** (3.50 mg, 3.84 mmol) in 50 ml of THF at –78 °C. The mixture was warmed to - 40 °C within 5min and stirred for 3h. An aq. NH_4Cl solution (25 ml) was added. The reaction mixture was warmed to 20 °C. An aq. solution of sodium potassium tartarate (25ml) was added. The aq. phase was extracted with CH_2Cl_2 (100 mL/3 times). The combined org. phases were dried, concentrated *in vacuo*. FC(4:1 , light petroleum ether/Et_2O) gave 3.22 g (92 %) of **171**, colorless oil. (If the procedure applied correct, and the reagent was pure ($LiBH_4$), no glucose analogue will obtain).

$[\alpha]^{25}_{589}$ = +33, $[\alpha]^{25}_{577}$ = +35, $[\alpha]^{25}_{435}$ = +60, $[\alpha]^{25}_{405}$ = +66 (*c* 0.37, $CHCl_3$).

IR (film): 3475, 2950, 2855, 1715, 1630, 1475, 1255, 1095, 835, 780, 740 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.45-7.39 (m, 2H,.ArH); 7.38-7.23 (m, 3H, ArH); 5.69 (s, H-C(1)); 4.50 (t, ^3J (H-C(5), H_{exo}-C(6)) = 5.1, H-C(5)); 4.47-4.38 (m, 3H, H-C(2'), H-C(4), H_a-C(7')); 4.24 (dd, ^3J(H-C(5'), H-C(6')) = 6.7, ^3J (H-C(5'), HC-(4')) = 2.8, H-C(5')); 4.22 (d, ^2J = 7.4, H_{endo}-C(6)); 4.05 (d, ^2J = 13.4, H_a-C(N$\underline{CH_2}$Ph)); 3.92 (ddd, ^3J(H-(6'), H_a-(7') = 9.6, ^3J(H-(6'), H-(5') = 6.7, ^3J (H-C(6'), H_b-C(7')) = 2.2, H-C(6')); 3.83-3.76 (m, 2H, H_b-C(N$\underline{CH_2}$Ph), H-C(4')); 3.73 (dd, ^2J = 12.5, ^3J (H_b-C(7'), H-C(6')) = 2.2, H_b-C(7')); 3.56 (dd, ^2J = 7.4, ^3J(H_{exo}-C(6), H-(5)) = 5.4, H_{exo}-C(6)); 3.44 (d, ^3J(H-C(3'),

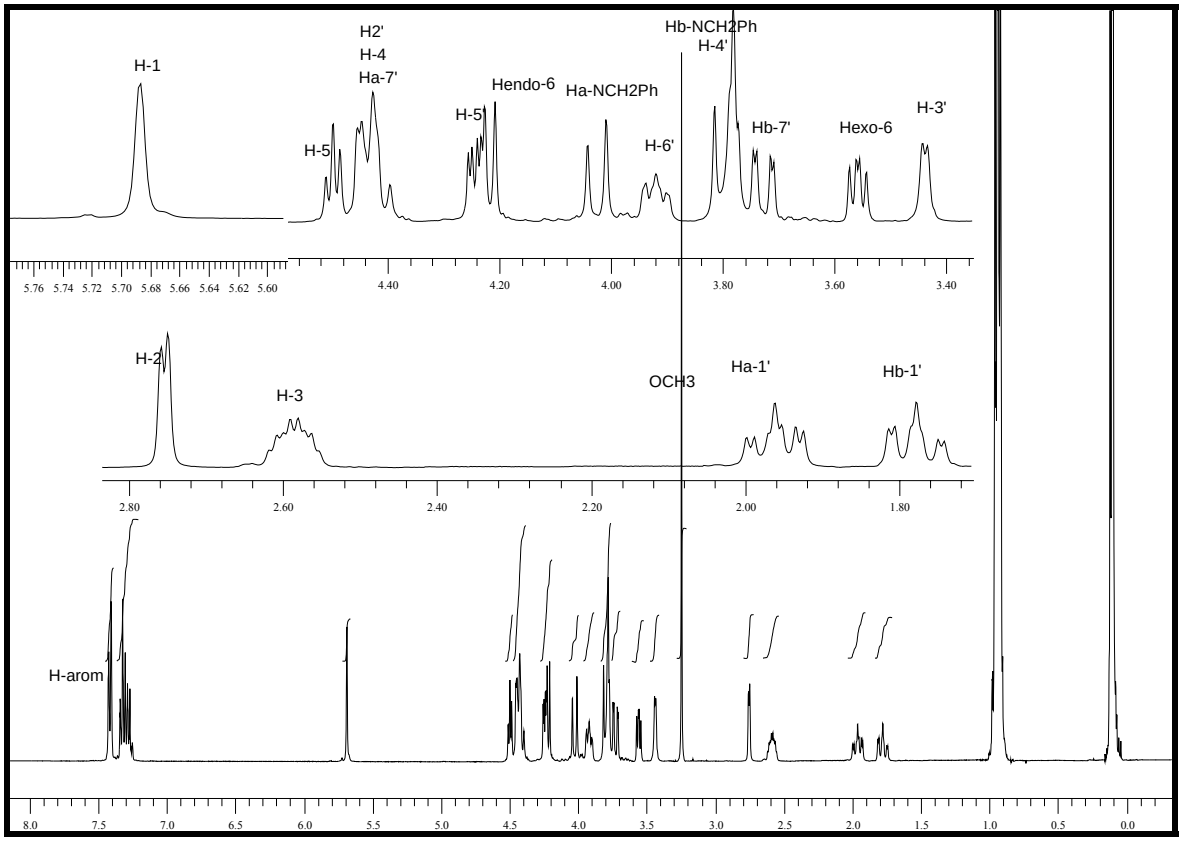
H-(4')) = 3.5, H-C(3')); 3.25 (s, 3H, H-(O$\underline{CH_3}$)); 2.75 (d, ^3J(H-C(2), H-C(3)) = 3.2, H-C(2)); 2.63 (m, 1H, H-C(3)); 1.96 (ddd, ^2J = 14.4, ^3J(H_a-(1'), H-(3) = 10.9, ^3J(H_a-(1'), H-(2') = 3.8, H_a-C(1')); 1.78 (ddd, ^2J = 14.4, ^3J(H_b-(1'), H-(3) = 11.5, ^3J(H_b-(1'), H-(2') = 2.9, H_b-C(1')); 1.00, 0.95, 0.94, 0.90 (4s, 36H, 36H-C(SiC($\underline{CH_3}$)$_3$)); 0.13, 0.12, 0.11, 0.10, 0.08, 0.06, 0.03, 0.01 (8s, 24H, 24H-C(Si$\underline{CH_3}$)).

^{13}C NMR (100.6 MHz, $CDCl_3$): δ_C 138.8 (s, C(arom)); 130.4 (d, ^1J(C,H) = 159, C(arom)); 128.3 (d, ^1J(C,H) = 156, C(arom)); 127.4 (d, ^1J(C,H) = 156, C(arom)); 101.8 (d, ^1J (C,H) = 179, C(1)); 78.7 (d, ^1J(C,H) = 147, C(6')); 75.6(d, ^1J (C,H) = 159, C(5)); 74.8 (d, ^1J(C,H) = 141, C(3')); 74.2 (d, ^1J (C,H) = 154, C4'); 69.8 (d, ^1J(C,H) = 143 , C(2)); 67.8 (d, ^1J(C,H) = 146, C(5')); 67.3 (d, ^1J (C,H) = 146, C(2')); 64.2 (t, ^1J(C,H) = 144, C(4)); 63.3 (t, ^1J(C,H) = 154, C(6)); 61.8 (q, ^1J(C,H) = 122, C(O$\underline{C}H_3$)); 60.2 (t, ^1J (C,H) = 145, C(N$\underline{C}H_2$Ph); 59.1 (t, ^1J(C,H) = 143, C(7')); 33.6 (t, ^1J(C,H) = 130, C(1')); 32.7 (d, ^1J(C,H) = 126, C(3)); 26.4, 26.4, 26.3, 26.2(4q, ^1J (C,H) = 125, ($\underline{CH_3}$)$_3$CSi); 18.7; 18.5, 18.4; 18.4(4s, $(CH_3)_3\underline{C}$Si); -3.9; -4.0; -4.2; -4.4, -4.5; -4.5, -4.8; -4.9; (8q, ^1J (C,H) = 118 , $\underline{CH_3}$Si).

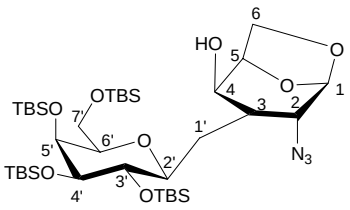
MALDI-HRMS Calcd for (M + Na) $C_{45}H_{87}NO_9Si_4Na$ 920.5356; found 920.5350.

Anal. calcd for $C_{45}H_{85}NO_9Si_4$ (898.52): C 60.15, H9.76, N 1.56; found: C 60.03, H 9.76, N 1.58.

1H-NMR (400 MHz, $CDCl_3$) spectrum of **171:**



1,6-Anhydro-3-*C*-{2,6-anhydro-1-deoxy-3,4,5,7-tetrakis-*O*-[(*tert*-butyl)dimethylsilyl]-D-*glycero*-L-*manno*-heptitol-1-*C*-yl}-2-azido-2,3-dideoxy-*β*-D-*galacto*-hexopyranose (173)



173

To a stirred mixture of metallic sodium (184 mg, 8.0 mmol, 24 equiv) in liquid NH_3 (10 mL) at -78 °C was added a solution of **171** (300 mg, 0.329 mmol) in dry THF (5 mL). After 30 min at -78 °C, NH_4Cl (540 mg, 10 mmol, 30 equiv) was added and ammonia was allowed to evaporate at 20 °C. After the addition of H_2O (20 ml), the aqueous phase was extracted with CH_2Cl_2 (20 ml, 3 times). The combined org. phases were dried, concentrated in *vacuo*. The residue was dissolved in MeOH (10 ml), then 10% Pd on charcoal (35 mg) was added. The degassed mixture was stirred under H_2 atmosphere (1 atm.) at 20°C for 12 h. The catalyst was filtered off, the solvent evapored and the residue was dissolved in 1 ml of CH_2Cl_2, 1 mg of $CuSO_4$ in 2 ml of H_2O was added, then triethylamine (131 µl, 0.987 mmol) was added, followed by MeOH (7 ml). The dichloromethane solution of trifluoromethanosulfonyl azide freshly prepared (1 ml, 0.6 M, 0.6 mmol) was added at once. The reaction was stirred until TLC showed reaction to be complete. Then the mixture was extracted by CH_2Cl_2 (15 ml, 3 times). These combined org. phases were dried, concentrated *in vacuo*. FC (7:3, light petroleum ether/ether): 220 mg (81 %, 3 steps) of **173**, colorless oil.

$[\alpha]^{25}_{589} = +34$, $[\alpha]^{25}_{577} = +22$, $[\alpha]^{25}_{435} = +38$, $[\alpha]^{25}_{405} = +50$ (*c* 0.15, $CHCl_3$).

IR (film): 3470, 2950, 2860, 2120, 1645, 1470, 1250, 1090, 835, 775, 735 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ

5.38 (s, H-C(1)); 4.46-4.34 (m, 3H, H-C(2'), H-C(5), H_a-C(7')); 4.32 (dd, ^{3}J(H-C(4), H-C(3)) = 7.4, ^{3}J(H-C(4), H-C(5)) = 4.9, H-C(4)); 4.20 (dd, ^{3}J(H-C(5'), H-C(6')) = 6.5, ^{3}J (H-C(5'), HC-(4')) = 2.8, H-C(5')); 4.15 (d, ^{2}J = 7.4, H_{endo}-C(6)); 4.13 (m, 1H, H-C(6')); 3.76 (t, ^{3}J(H-C(4'), H-C(3')) = 3.4, H-C(4')); 3.69 (dd, ^{2}J = 12.6, ^{3}J (H_b-C(7'), H-C(6')) = 2.5, H_b-C(7')); 3.63 (dd, ^{2}J = 7.4, ^{3}J(H_{exo}-C(6), H-(5)) = 4.9, H_{exo}-C(6)); 3.41 (d, ^{3}J(H-C(3'), H-C(4')) = 3.4, H-C(3')); 3.25 (br s, H-C(2)); 2.60 (m, 1H, H-C(3)); 1.87 (m, 2H, ^{3}J(H_a-(1'), H_b-C(1')); 0.93, 0.91, 0.90, 0.88 (4s, 36H, 36H-C(SiC($\underline{CH_3}$)$_3$)); 0.11, 0.10, 0.09, 0.08, 0.08, 0.07, 0.07, 0.06 (8s, 24H, 24H-C(Si$\underline{CH_3}$)).

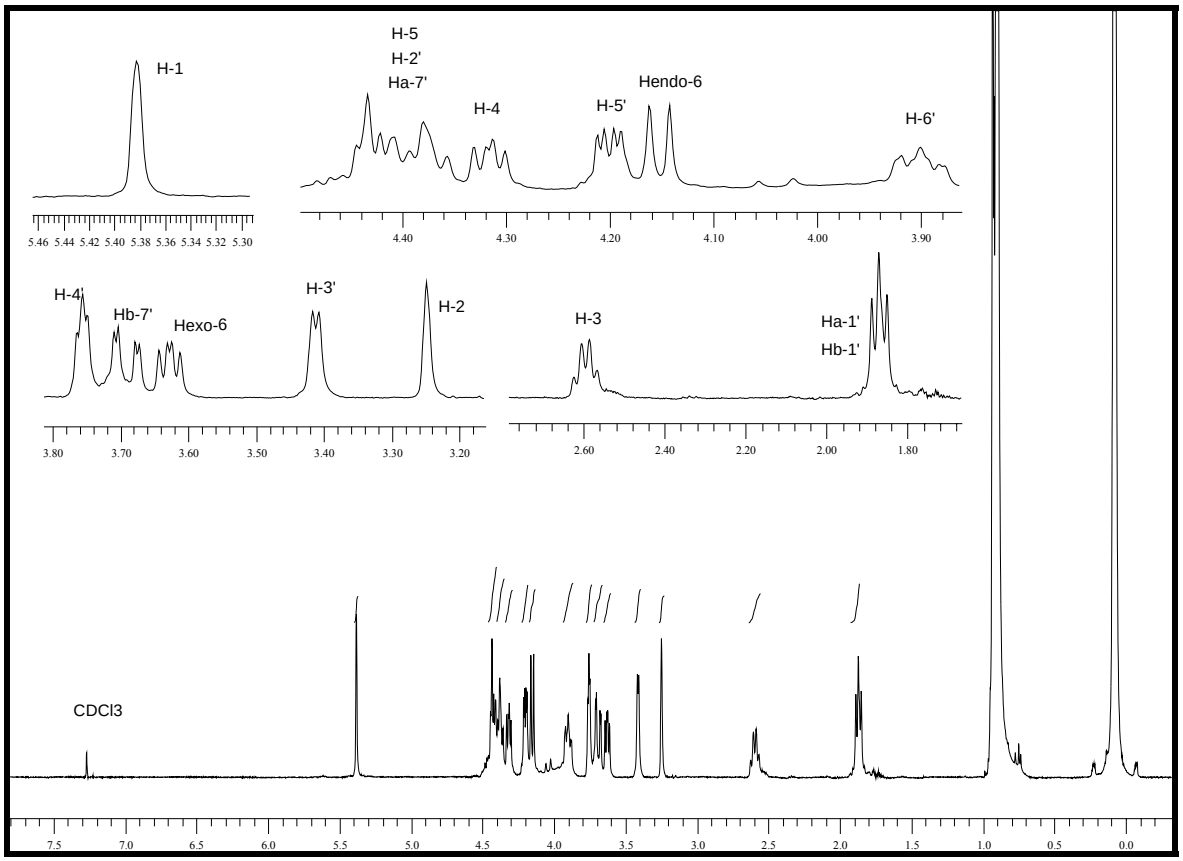
^{13}C NMR (100.6 MHz, $CDCl_3$): δ

101.5 (d, ^{1}J(C,H) = 179, C(1)); 78.3 (d, ^{1}J(C,H) = 147, C(6')); 75.6 (d, ^{1}J(C,H) = 159, C(5)); 74.3 (d, ^{1}J(C,H) = 141, C(3')); 73.8 (d, ^{1}J(C,H) = 154, C4'); 67.3 (d, ^{1}J(C,H) = 146, C(5')); 66.0 (t, ^{1}J(C,H) = 144, C(4)); 64.8 (d, ^{1}J(C,H) = 143 , C(2)); 64.4 (d, ^{1}J(C,H) = 146, C(2')); 63.7 (t, ^{1}J(C,H) = 154, C(6)); 58.6 (t, ^{1}J(C,H) = 143, C(7')); 37.5 (d, ^{1}J(C,H) = 126, C(3)); 32.7 (t, ^{1}J(C,H) = 130, C(1')); 25.9, 25.8, 25.8, 25.6 (4q, ^{1}J(C,H) = 125, ($\underline{CH_3}$)$_3$CSi); 18.2; 18.1, 18.0; 17.9 (4s, $(CH_3)_3\underline{C}$Si); -4.4; -4.6; -4.7; -4.8, -4.9, -5.2, -5.3; -5.4 (8q, ^{1}J (C,H) = 118 , $\underline{CH_3}$Si).

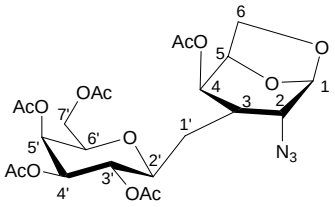
MALDI-HRMS Calcd for (M + Na) $C_{37}H_{77}N_3O_8Si_4Na$ 826.4685; found 826.4650.

Anal. calcd for $C_{37}H_{77}N_3O_8Si_4$ (804.36): C 55.25, H9.65, N 5.22; found: C 55.15, H 9.57, N 5.19.

1H-NMR (400 MHz, $CDCl_3$) spectrum of **173:**



4-*O*-Acetyl-1,6-anhydro-3-*C*-[2,6-anhydro-1-deoxy-3,4,5,7-tetrakis-*O*-acetyl-D-*glycero*-L-*manno*-heptitol-1-*C*-yl]-2-azido-2,3-dideoxy-*β*-D-*galacto*-hexopyranose (174).



174

The *tert*-butyldimethylsilyl-protected **173** (600 mg, 0.733 mmol) was dissolved in THF (4 mL), a solution of TBAF in THF (4 mL, 4.0 mmol) was added, and the solution was stirred for 3 h at room temperature. The mixture was evaporated to dryness, then dissolved in pyridine (5 mL). A catalytic amount of DMAP (10 mg, 0.08 mmol) was added, then acetic anhydride (3 mL, 31.80 mmol) was added, and the solution was stirred for 2 days at 20 ^{o}C. Then MeOH (5 mL) was added, the mixture was evaporated to dryness, then dissolved in ethyl acetate (25 mL). The solution was quenched with 5 % aqueous HCl (10 ml), washed successively with saturated aqueous solution of $NaHCO_3$ (5 mL), water and brine and dried ($MgSO_4$). Evaporation of the filtrate, FC (1:1, light petroleum ether/EtOAc) gave 355 mg (89 %) of **174,** white solid.

m.p. 46-47°C.

$[\alpha]^{25}_{589}$ = +95, $[\alpha]^{25}_{577}$ = +102, $[\alpha]^{25}_{435}$ = +192, $[\alpha]^{25}_{405}$ = +277 (*c* 0.38, $CHCl_3$).

IR (film): 3455, 2965, 2135, 1750, 1735, 1435, 1375, 1235, 1135, 1060, 1025, 910 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ

5.37 (s , H-C(1)); 5.32 (s , H-C(5')); 5.15 ((t, ^3J(H-C(4), H-C(3)) = 6.5, H-C(4)); 5.11-5.08 (m, 2H, H-C(6'), H-C(3')); 4.58 (t, 2H, ^3J (H-C(5), H_{exo}-C(6))= 4.6, H-C(5)); 4.22-4.12 (m, 2H, H_a-C(7'), H-C(2')); 4.10-3.95 (m, 2H, H_b-C(7'), H-C(4')); 3.84 (d, ^2J=8.0, H_{endo}-C(6)); 3.52 (dd, ^2J=8.0, ^3J(H_{exo}-C(6),H-C(5))= 4.6, H_{exo}-C(6)); 3.45 (d, ^3J(H-C(2), H-C(3)) = 4.6, H-C(2)); 2.17 (m, 1H, H-C(3)); 2.04, 2.01, 2.00, 1.99, 1.95 (5s, 15H, CH_3COO);1.90 (m, 1H, H_a-C(1')); 1.64 (m, 1H, H_b-C(1')).

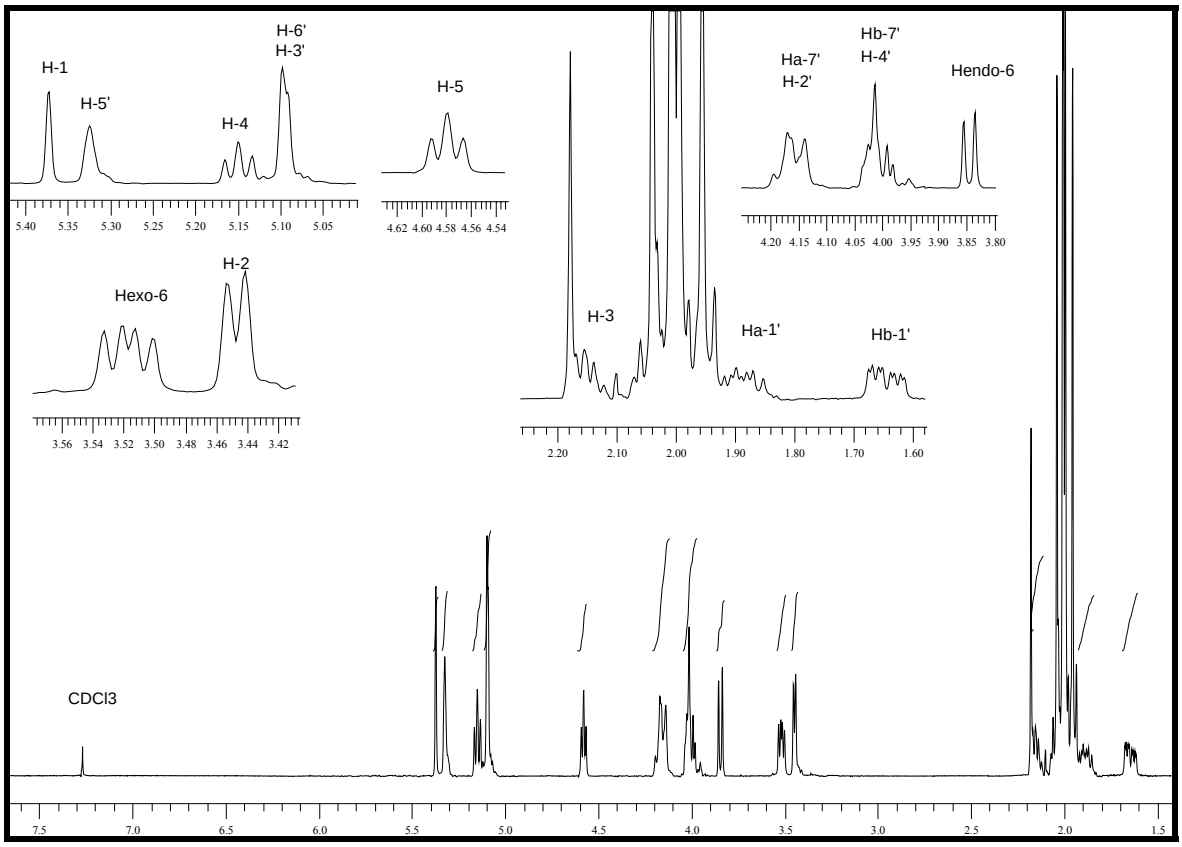
^{13}C NMR (100.6 MHz, $CDCl_3$): δ

δ 170.3, 169.7, 169.6, 169.4, 169.4 (5s, -COO); 101.4 (d, ^1J(C,H) = 176, C(1)); 72.3 (d, ^1J(C,H) = 159, C(5)); 71.3 (d, ^1J(C,H) = 147, C(2')); 68.4 (d, ^1J(C,H) = 145, C(4')); 68.3 (d, ^1J(C,H) = 155, C(6')); 67.4 (d, ^1J(C,H) = 150, C(3')); 67.2 (d, ^1J(C,H) = 153, C(5')); 66.5 (d, ^1J(C,H) = 153, C(4)); 63.4 (t, ^1J(C,H) = 153, C(6)); 63.1 (d, ^1J(C,H) = 149, C(2)); 61.4 (t, ^1J(C,H) = 146, C(7')); 35.3 (d, ^1J(C,H) = 132, C(3)); 23.0 (t, ^1J(C,H) = 127, C(1')); 20.5- 20.2 (5q, ^1J(C,H) = 130, 5 $\underline{C}H_3COO$).

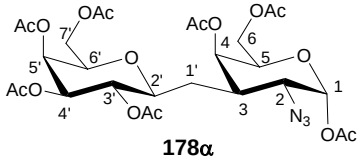
MALDI-HRMS Calcd for (M + Na) $C_{23}H_{31}N_3O_{13}Na$ 580.1755; found 580.1704.

Anal. calcd for $C_{23}H_{31}N_3O_{13}\bullet Ac_2O$ (659.59): C 49.16, H 5.65, N 6.37; found: C 48.99, H 5.45, N 6.28.

1H-NMR (400 MHz, $CDCl_3$) spectrum of **174:**



15:1 Mixture of 1,4,6-*O*-acetyl-3-*C*-[2,6-anhydro-1-deoxy-3,4,5,7-tetrakis-*O*-acetyl-D-*glycero*-L-*manno*-heptitol-1-*C*-yl]-2-azido-2,3-dideoxy-α-and-β-D-*galacto*-hexopyranose (178α,β).



178α

174 (320 mg, 0.575 mmol) was dissolved in Ac_2O (5 mL). After cooling to -40 ^oC, TMSOTf (0.123 mL, 0.683 mmol) was added (syringe) and the mixture stirred for 1 h. CH_2Cl_2 (30 ml) was added and the reaction quenched with a saturated aqueous solution of $NaHCO_3$ (10 mL), the organic phase was collected and extracted with H_2O, and then with brine and dried ($MgSO_4$). Solvent evaporation and FC (hexanes:EtOAc, 2:1 →1:3) gave 400 mg (98 %) of a 15/1 mixture of **178α** and **178β** (360 mg, 95 %), white foam; R_f= 0.53 (PE/EtOAc 1:1);

$[\alpha]^{25}_{589}$ = +131, $[\alpha]^{25}_{577}$ = +141, $[\alpha]^{25}_{435}$ = +276, $[\alpha]^{25}_{405}$ = +320 (*c* 0.075, $CHCl_3$).

IR (film): 2135, 1750, 1445, 1370, 1240, 1030, 910, 600 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$); δ

<u>*Data for the α anomer:*</u> 6.32 (d , ^3J(H-C(1), H-C(2)) = 3.4, H-C(1)); 5.42 (t, ^3J(H-C(5'),H-C(6'))= 2.9, H-C(5')); 5.35 ((br s, H-C(4)); 5.20-5.15 (m, 2H, H-C(3'), H-C(4')); 4.53 (dd, ^2J=11.5, ^3J(H_a-C(7'),H-C(6'))= 7.7, H_a-C(7')); 4.38 (dt, ^3J(H-C(2'),H_a-C(1'))= 8.3, ^3J(H-C(2'),H_a-C(1'))= 2.5, H-C(2')); 4.23 (t, ^3J (H-C(5), H_{exo}-C(6))= 6.2, H-C(5)); 4.15 (m, 1H, H-C(6')); 4.11-3.95 (m, 3H, H_b-C(7'), H_a-C(6), H_b-C(6)); 3.45 (dd, ^3J(H-C(2), H-C(3)) = 12.0, ^3J(H-C(2), H-C(1)) = 3.4, H-C(2)); 2.36 (m, 1H, H-C(3)); 2.17, 2.11, 2.10, 2.07, 2.05, 2.04, 2.02 (7s, 21H, CH_3COO); 1.95 (m, 1H, H_a-C(1')); 1.17 (m, 1H, H_b-C(1')).

^{13}C NMR (100.6 MHz, $CDCl_3$): δ

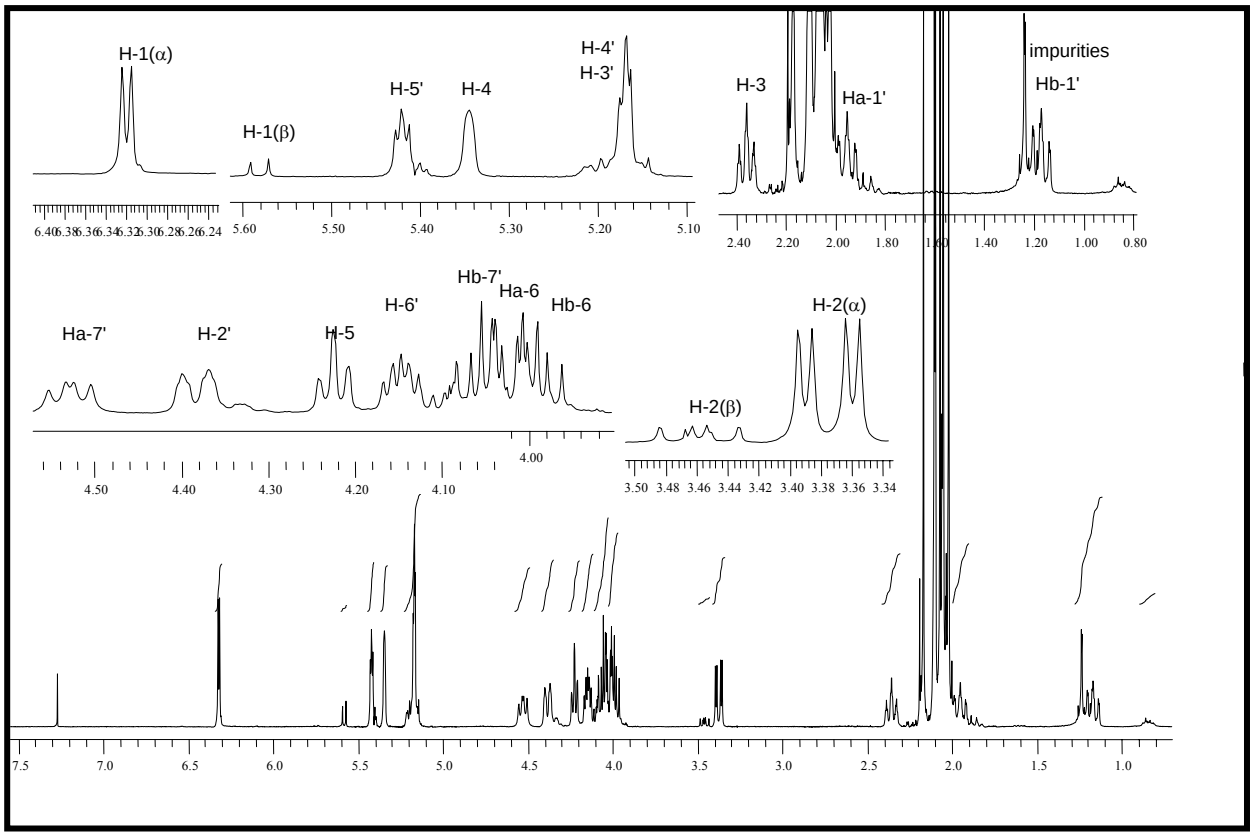
<u>*Data for the α anomer:*</u> 170.4, 170.3, 169.9, 169.8, 169.7, 169.5, 168.8 (7s, -COO); 90.3 (d, ^1J(C,H) = 175, C(1)); 69.7 (d, ^1J(C,H) = 170, C(6')); 69.4 (d, ^1J(C,H) = 162, C(5)); 68.3 (d, ^1J(C,H) = 165, C(4')); 67.7 (d, ^1J(C,H) = 162, C(3')); 66.9 (d, ^1J(C,H) = 151,

C(5')); 66.7 (d, ^1J(C,H) = 164, C(2')); 66.0 (d, ^1J(C,H) = 150, C(4)); 61.8 (t, ^1J(C,H) = 158, C(6)); 60.5 (t, ^1J(C,H) = 153, C(7')); 57.5 (d, ^1J(C,H) = 166, C(2));33.3 (d, ^1J(C,H) = 135, C(3)); 23.0 (t, ^1J(C,H) = 126, C(1')); 21.4- 19.9 (7q, ^1J(C,H) = 120, 7 $\underline{C}H_3COO$).

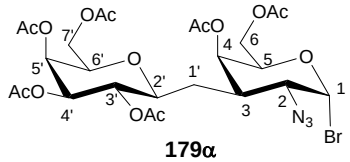
MALDI-HRMS Calcd for (M + Na) $C_{27}H_{37}N_3O_{16}Na$ 682.2072; found 682.2067

Anal. calcd for $C_{27}H_{37}N_3O_{16}$ (659.5932): C 49.16, H5.65, N 6.37; found: C 50.26, H 5.79, N 6.22.

1H-NMR (400 MHz, $CDCl_3$) spectrum of **178α:**



4,6-*O*-Acetyl-3-*C*-[2,6-anhydro-1-deoxy-3,4,5,7-tetrakis-*O*-acetyl-D-*glycero*-L-*manno*-heptitol-1-*C*-yl]-2-azido-2,3-dideoxy-α-D-*galacto*-hexopyranosyl bromide (179α).



179α

A solution of **178α,β** (500 g, 0.759 mmol) and anhydrous $TiBr_4$ (0.99 mg, 1.35 mmol) in anhydrous CH_2Cl_2 (10 mL) was stirred at 20 ^oC for 12 h. After the addition of CH_2Cl_2 (150 mL), the solution was washed with ice-cold H_2O (50 mL, twice) and dried ($MgSO_4$). Solvent evaporation *in vacuo* and quick FC (hexane: EtOAc, 1:1) gave 470 mg (91 %) of **179α**, yellow foam, that was dried and stored at -20 ^oC until its use; R_f= 0.55 (PE/EtOAc 1:1);

$[\alpha]^{25}_{589}$ = +199, $[\alpha]^{25}_{577}$ = +160, $[\alpha]^{25}_{435}$ = +306, $[\alpha]^{25}_{405}$ = +364 (*c* 0.16, $CHCl_3$).

IR (film): 3440, 2970, 2110, 1755, 1375, 1235, 1055 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ

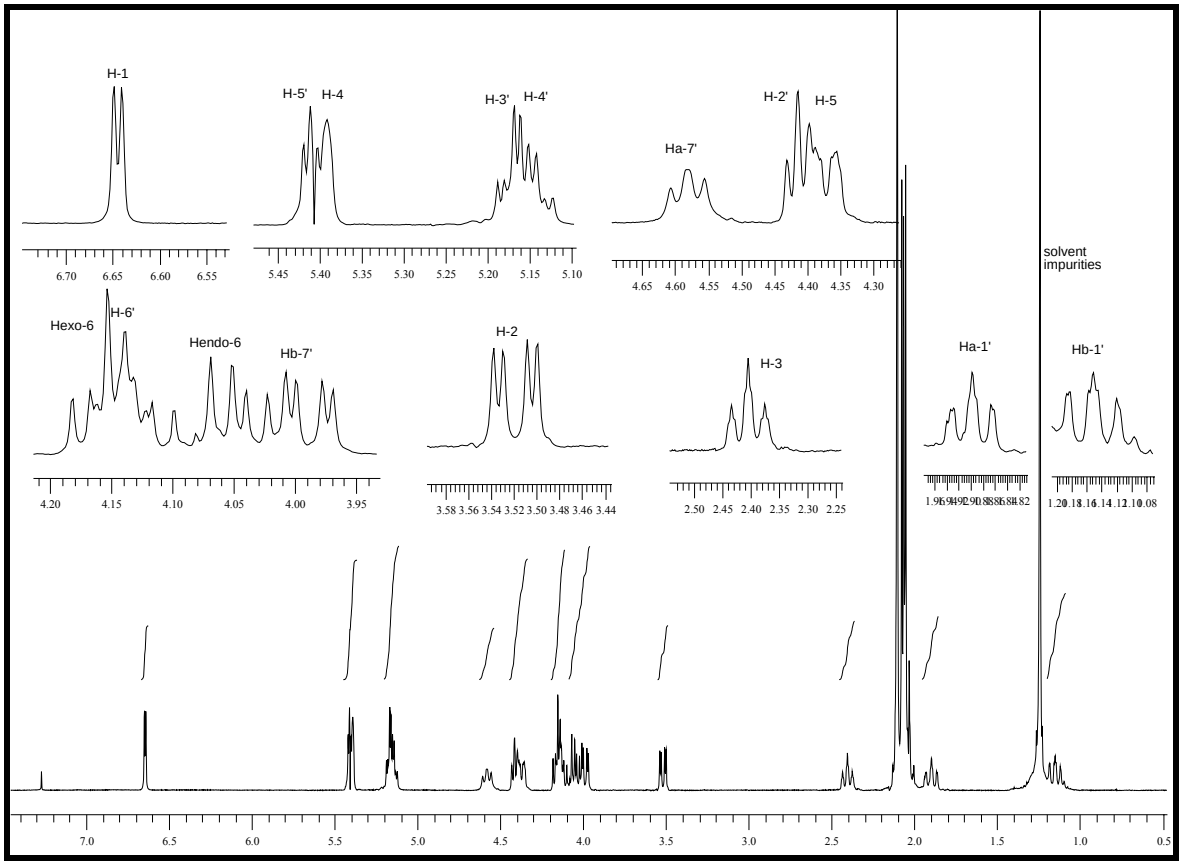
6.64 (d , ^3J(H-C(1), H-C(2)) = 3.1, H-C(1)); 5.41 (t, ^3J(H-C(5'),H-C(6'))= 3.4, H-C(5')); 5.39 ((bs, H-C(4)); 5.20-5.11 (m, 2H, H-C(3'), H-C(4')); 4.53 (dd, ^2J=10.0, ^3J(H_a-C(7'),H-C(6'))= 9.2, H_a-C(7')); 4.45-4.33 (m, 2H, H-C(2'), H-C(5)); 4.20-3.90 (m, 4H, H_{exo}-C(6), H-C(6'), H_{endo}-C(6), H_b-C(7')); 3.52 (dd, ^3J(H-C(2), H-C(3)) = 11.7, ^3J(H-C(2), H-C(1)) = 3.1, H-C(2)); 2.40 (m, 1H, H-C(3)); 2.12-2.04 (6s, 18H, CH_3COO); 1.90 (m, 1H, H_a-C(1')); 1.15 (m, 1H, H_b-C(1'));

^{13}C NMR (100.6 MHz, $CDCl_3$): δ

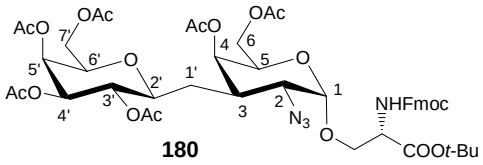
δ 170.8, 170.4, 169.8, 169.8, 169.7, 169.5 (7s, -COO); 92.0 (d, ^1J(C,H) = 184, C(1)); 72.1 (d, ^1J(C,H) = 147, C(5)); 70.0 (d, ^1J(C,H) = 142, C(6')); 68.5 (d, ^1J(C,H) = 163, C(4')); 67.7 (d, ^1J(C,H) = 152, C(3')); 67.0 (d, ^1J(C,H) = 146, C(5')); 66.0 (d, ^1J(C,H) = 146, C(2')); 65.8 (d, ^1J(C,H) = 150, C(4)); 61.4 (t, ^1J(C,H) = 152, C(6)); 60.8 (t, ^1J(C,H) = 149, C(7')); 60.1 (d, ^1J(C,H) = 147, C(2));34.4 (d, ^1J(C,H) = 140, C(3)); 23.3 (t, ^1J(C,H) = 130, C(1')); 21.2- 20.0 (6q, ^1J(C,H) = 123, 6 $\underline{C}H_3COO$).

MALDI-HRMS Calcd for (M + K) $C_{27}H_{38}N_3O_{16}K$ 719.0861; found 719.4910.

1H-NMR (400 MHz, $CDCl_3$) spectrum of **179α:**



3:1 mixturer of *N*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-{4,6-*O*-Acetyl-3-*C*-[2,6-anhydro-1-deoxy-3,4,5,7-tetrakis-*O*-acetyl-D-*glycero*-L-*manno*-heptitol-1-*C*-yl]-2-azido-2,3-dideoxy-α-D-*galacto*-pyranosyl}-L-serine *tert*-butyl ester (180α).



180

A mixture of N-Fmoc-Serine-OtBu (240 mg, 0.606 mmol), anhydrous CH_2Cl_2 (10 mL), $AgClO_4$ (260 mg, 1.21 mmol), 2,4,6-collidine (168 μL, 1.21 mmol), and molecular sieves (4Å, 1 g) was stirred at 25 ^oC for 10 min. A solution of bromide **179** (400 mg, 0.590 mmol) in anhydrous CH_2Cl_2 (10 mL) was added slowly (syringe) to the stirred mixture over 30 min. After the system had been stirred at 25 ^oC for 5 h, CH_2Cl_2 (100 mL) was added and the mixture was filtered through a pad of Celite (washing with CH_2Cl_2). Solvent evaporation and FC (hexanes/EtOAc 1:1) gave a 3:1 mixture of **180α** and **180β** (420 mg, 73%) as a white foam; Rf=0.52 (PE/EtOAc 1:1). (Note gradiant and slow chromatography give pure α).

$[\alpha]^{25}_{589}$ = +81, $[\alpha]^{25}_{577}$ = +91, $[\alpha]^{25}_{435}$ = +197, $[\alpha]^{25}_{405}$ = +236 (*c* 0.12, $CHCl_3$).

IR (film): 3330, 2920, 2850, 2105, 1735, 1515, 1370, 1215, 1040, 730 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ

Data for the α anomer: 7.82-7.29 (4 m, 8H, ArH); 6.23 (d, ^3J(H-N, H-C*Ser) = 8.3, H-N); 5.46 (br s, 1H, H-C(5')); 5.34 (br s, 1H, H-C(4)); 5.26-5.15 (m, 2H, H-C(3'), H-C(4')); 4.97 (d , ^3J(H-C(1), H-C(2)) = 3.4, H-C(1)); 4.59 (dd, ^2J=11.7, ^3J(H_a-C(7'),H-C(6'))= 7.1, H_a-C(7')); 4.51 (dt, ^3J(H-C*(Ser), H-(Nfmoc)) = 8.3, ^3J(H-C*(Ser), H_a-C(Ser)) = ^3J(H-C*(Ser), H_b-C(Ser)) = 3.1, H-C*(Ser)); 4.48-4.34 (m, 3H, H-C(2'), H_a-C(Fmoc), H_b-C(Fmoc)); 4.28-3.90 (m, 8H, H_b(C7'), H(C6'), H_a(C6), H_b(C6), H(C5), H-C*(Fmoc), H_a-C(Ser), H_b-C(Ser); 3.33 (dd, ^3J(H-C(2), H-C(3)) = 12.2, ^3J(H-C(2), H-C(1)) = 3.2, H-C(2)); 2.50 (m, 1H, H-C(3)); 2.12-2.04 (6s, 18H, CH_3COO); 1.93 (m, 1H, H_a-C(1')); 1.52 (s, 9H, $(CH_3)_3C$);1.48 (m, 1H, H_b-C(1')).

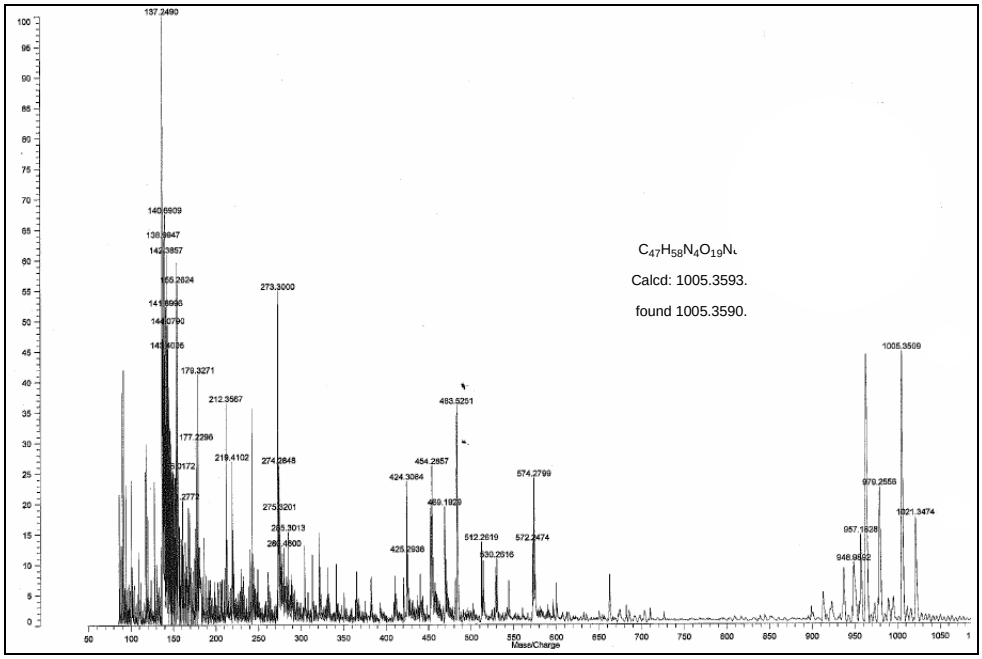
^{13}C NMR (100.6 MHz, $CDCl_3$): δ

. *Data for the α anomer:* 170.9, 170.4, 170.0, 169.9, 169.8, 169.6, (6s, -COO); 156.0 (s, C(Carbamate); 143.6 (s, C(Fmoc)); 141.0 (s, C(Fmoc)); 127.7 (d, ^1J(C,H) = 159, C(Fmoc)); 127.6 (d, ^1J(C,H) = 159, C(Fmoc)); 125.1 (d, ^1J(C,H) = 158, C(Fmoc)); 119.9 (d, ^1J(C,H) = 158, C(Fmoc)); 98.5 (d, ^1J(C,H) = 184, C(1)); 82.7 (s, $\underline{C}(CH_3)_3$); 70.3 (d, ^1J(C,H) = 153, C(6')); 69.5 (t, ^1J(C,H) = 153, $\underline{C}H_2$(Ser)); 68.3 (d, ^1J(C,H) = 148, C(4')); 68.2 (d, ^1J(C,H) = 146, C(5)); 67.2 (t, ^1J(C,H) = 150, $\underline{C}H_2$(Fmoc)); 65.4 (d, ^1J(C,H) = 149, C(5')); 66.9 (d, ^1J(C,H) = 148, C(4)); 66.9 (d, ^1J(C,H) = 149, C(3')); 66.9 (d, ^1J(C,H) = 143, C(2')); 62.4 (t, ^1J(C,H) = 155, C(6)); 60.4 (t, ^1J(C,H) = 151, C(7'); 58.1 (d, ^1J(C,H) = 142, C(2)); 54.7 (d, ^1J(C,H) = 138, C*(Ser)); 47.1 (d, ^1J(C,H) = 130, C*(Fmoc)); 32.9 (d, ^1J(C,H) = 129, C(3)); 27.9 (q, 3C, ^1J(C,H) = 128, (CH3)CO); 24.1 (d, ^1J(C,H) = 139, C(1')); 20.6-20.2 (6q, ^1J(C,H) = 130, $\underline{C}H_3COO$).

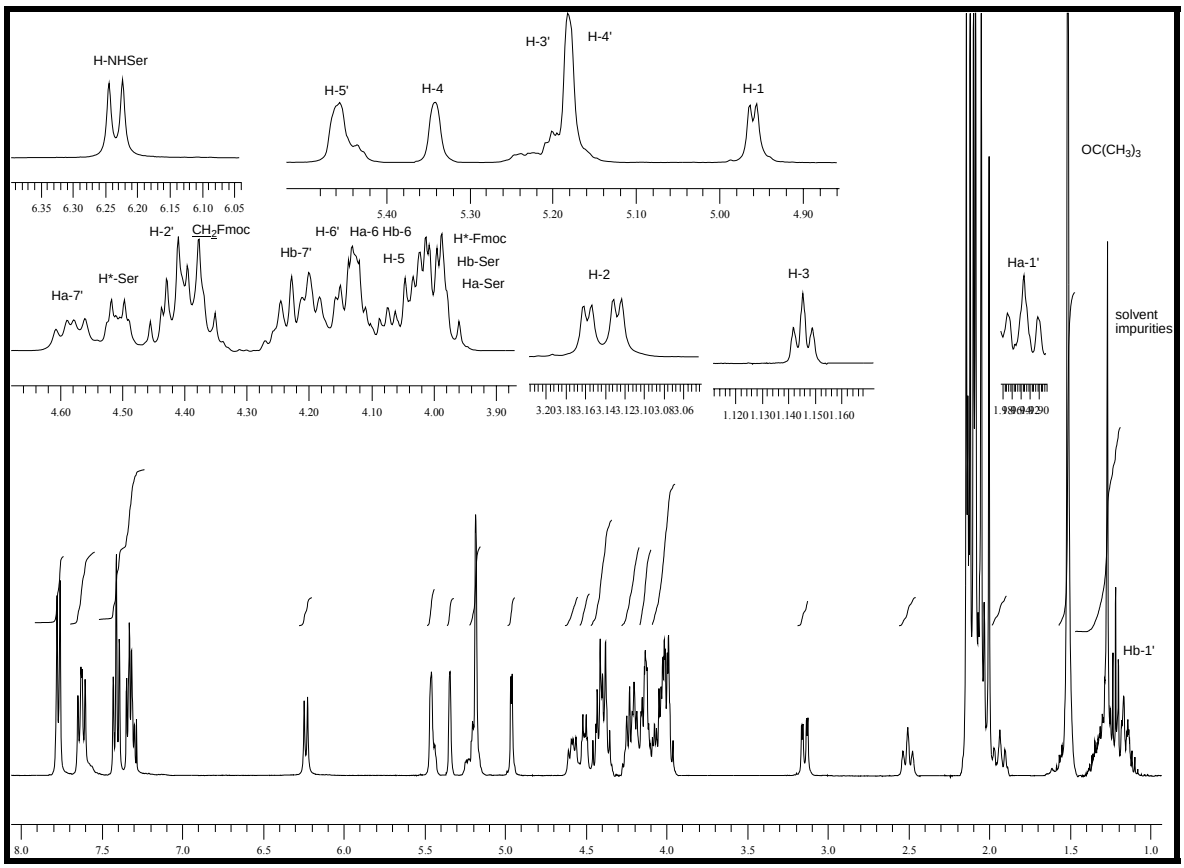
MALDI-HRMS Calcd for (M + Na) $C_{47}H_{58}N_4O_{19}Na$ 1005.3593; found 1005.3590.

Anal. calcd for $C_{47}H_{58}N_2O_{19}\cdot DCCl_3$ (1103.3626): C 52.25, H5.48, N 5.08; found: C 52.36, H 5.52, N 5.56.

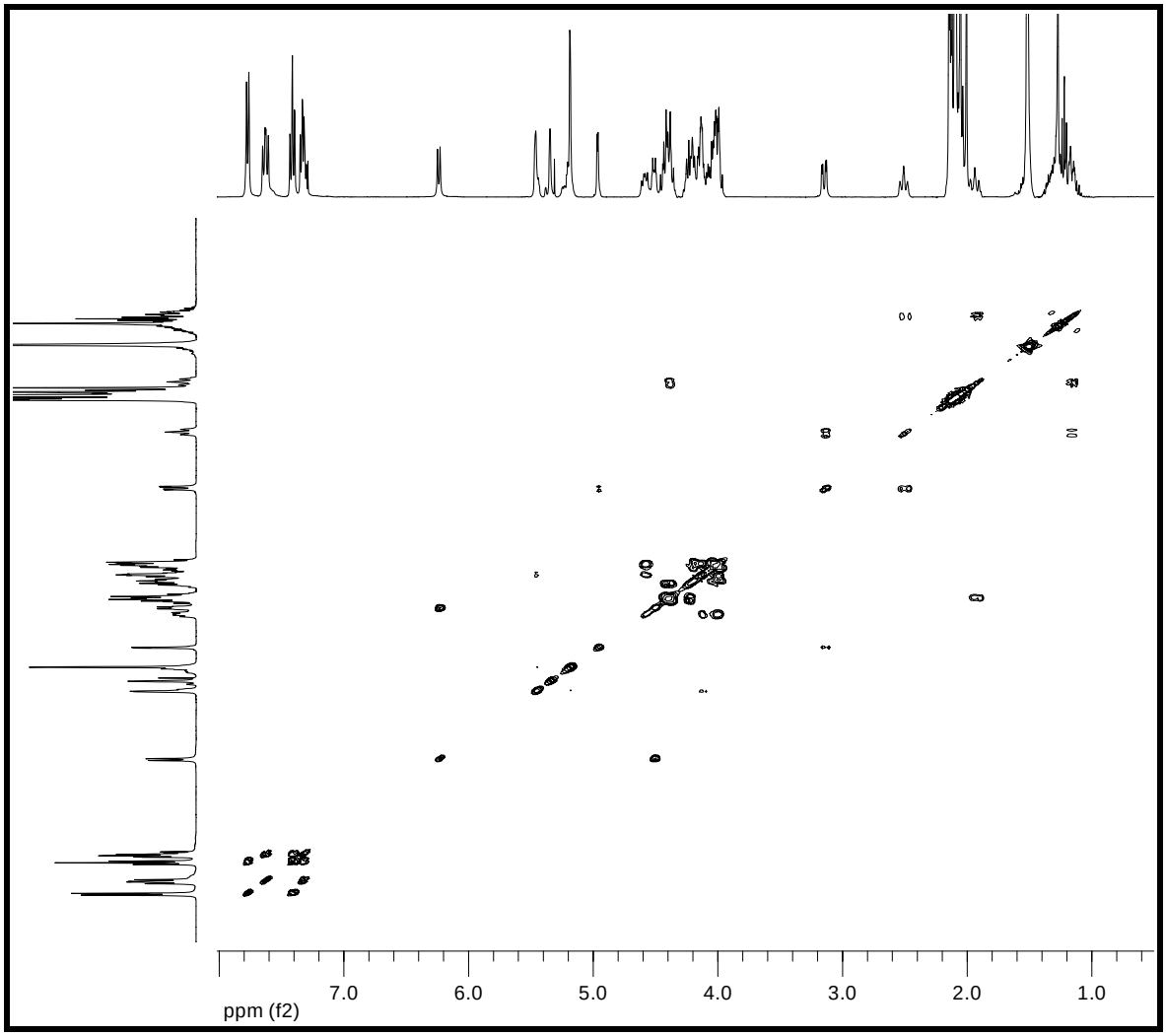
MALDI-HRMS spectrum of **180α:**



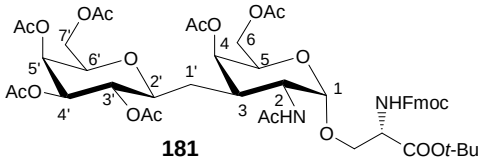
1H-NMR (400 MHz, $CDCl_3$) spectrum of **180α:**



2-D-COSY 1H-NMR (400 MHz, $CDCl_3$) spectrum of ***180α:***



***N*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-{4,6-*O*-Acetyl-3-*C*-[2,6-anhydro-1-deoxy-3,4,5,7-tetra-*O*-acetyl-D-*glycero*-L-*manno*-heptitol-1-*C*-yl]-2-Acetamido-2,3-dideoxy-α-D-*galacto*-pyranosyl}-L-serine *tert*-butyl ester (181).**



181

A solution of 350 mg (0.356 mmol) of **180α** in 15 mL of a 3:2:1 THF/AcOH/Ac_2O was treated with 3.5 g of Zn powder. In 1 h the reaction was filtered through a sintered glass funnel, concentrated, and purified by FC with 95:5 CH_2Cl_2/MeOH to give 320 mg (90%) of **181** (only α) as white foam; Rf=0.52 (CH_2Cl_2/MeOH, 9:1);

$[\alpha]^{25}_{589}$ = -5.5, $[\alpha]^{25}_{577}$ = +29, $[\alpha]^{25}_{435}$ = +91, $[\alpha]^{25}_{405}$ = +127 (*c* 0.055, $CHCl_3$).

IR (film): 3665, 3330, 2970, 2900, 1740, 1730, 1680, 1525, 1450, 1370, 1220, 1045 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ

7.85-7.13 (4 m, 8H, ArH); 6.32 (d, ^3J(H-N, H-C*Ser) = 8.6, H-N); 6.02 (d, ^3J(H-N, H-C(2)) = 9.9, NHAc); 5.41 (t, ^3J(H-C(5'), H-C(6')) = 2.9, H-C(5')); 5.24 (br s, H-C(4)); 5.26-5.15 (m, 1H, H-C(4')); 5.07 (dd, ^3J(H-C(3'),H-C(4'))= 8.8, ^3J(H-C(3'),H-C(2'))= 3.2, H-C(3')); 4.75 (d , ^3J(H-C(1), H-C(2)) = 3.1, H-C(1)); 4.57-4.35(m, 5H, H_a-C(7'), H-C*(Ser), H_a-C(Ser), H_b-C(Ser), H-C(2')); 4.30-3.80(m, 9H, H-C(2), H_a-(C6), H_b(C6), H-C(5), H-C(6'), H-C*(Fmoc), H_a-C(Fmoc), H_b-C(Fmoc), H_b(C7')); 2.22 (m, 1H, H-C(3)); 2.15-1.96 (7s, 21H, CH_3COO); 1.67 (m, 1H, H_a-C(1')); 1.49 (s, 9H, $(CH_3)_3C$);1.14 (m, 1H, H_b-C(1')).

^{13}C NMR (100.6 MHz, $CDCl_3$): δ

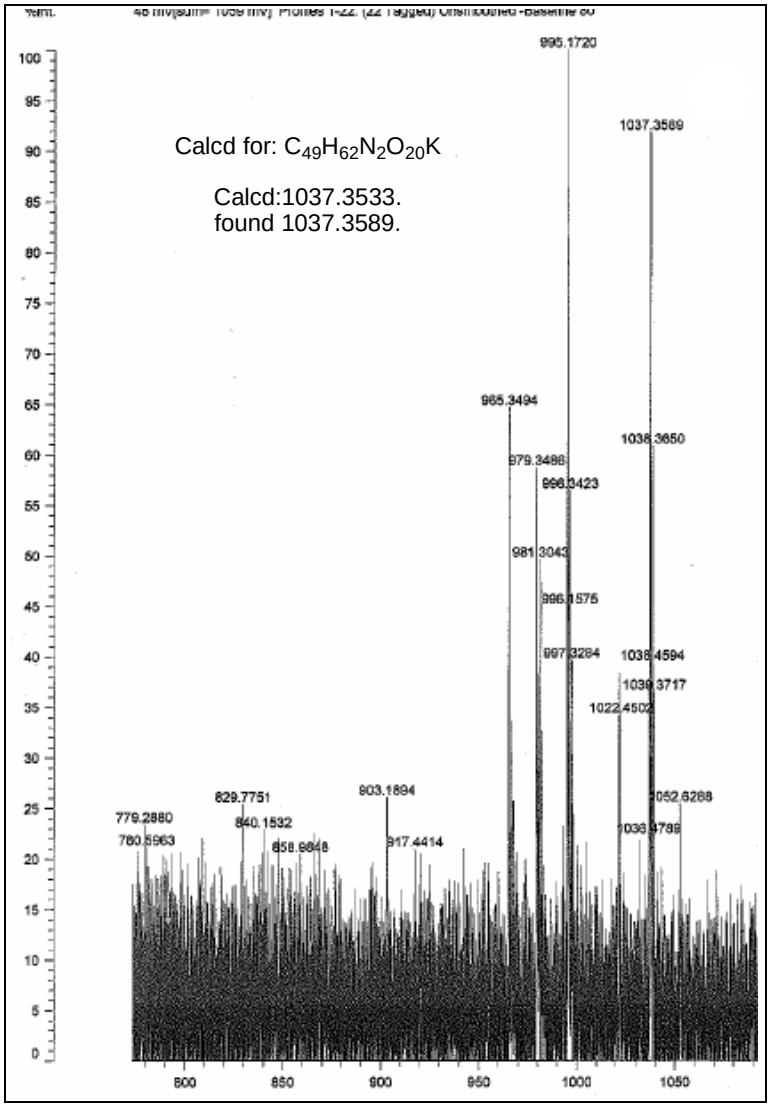
172.4 (s, NHO<u>C</u>CH_3); 170.5, 170.4, 170.1, 169.9, 169.5, 169.3, (6s, -COO); 156.1 (s, C(Carbamat); 143.7 (s, C(Fmoc)); 141.3 (s, C(Fmoc)); 127.8 (d, ^1J(C,H) = 159, C(Fmoc)); 127.0 (d, ^1J(C,H) = 159, C(Fmoc)); 124.8 (d, ^1J(C,H) = 158, C(Fmoc)); 120.1 (d, ^1J(C,H) = 158, C(Fmoc)); 98.01 (d, ^1J(C,H) = 184, C(1)); 82.7 (s, <u>C</u>$(CH_3)_3$); 70.3 (d, ^1J(C,H) =

153, C(6')); 68.6 (t, ^1J(C,H) = 153, $\underline{C}H_2$(Ser)); 68.4 (d, ^1J(C,H) = 148, C(4')); 68.2 (d, ^1J(C,H) = 146, C(5)); 68.1 (d, ^1J(C,H) = 149, C(5')); 68.0 (d, ^1J(C,H) = 148, C(4')); 67.1 (d, ^1J(C,H) = 149, C(3')); 66.9 (t, ^1J(C,H) = 150, $\underline{C}H_2$(Fmoc)); 66.4 (d, ^1J(C,H) = 143, C(2')); 62.7 (t, ^1J(C,H) = 155, C(6)); 61.4 (t, ^1J(C,H) = 151, C(7'); 54.9 (d, ^1J(C,H) = 122, C(2)); 47.2 (d, ^1J(C,H) = 138, C∗(Ser)); 47.1 (d, ^1J(C,H) = 130, C*(Fmoc)); 34.3 (d, ^1J(C,H) = 129, C(3)); 28.1 (q, 3C, ^1J(C,H) = 128, ($\underline{C}H_3$)CO); 22.2 (d, ^1J(C,H) = 139, C(1')); 20.8-20.5 (6q, ^1J(C,H) = 130, $\underline{C}H_3$COO).

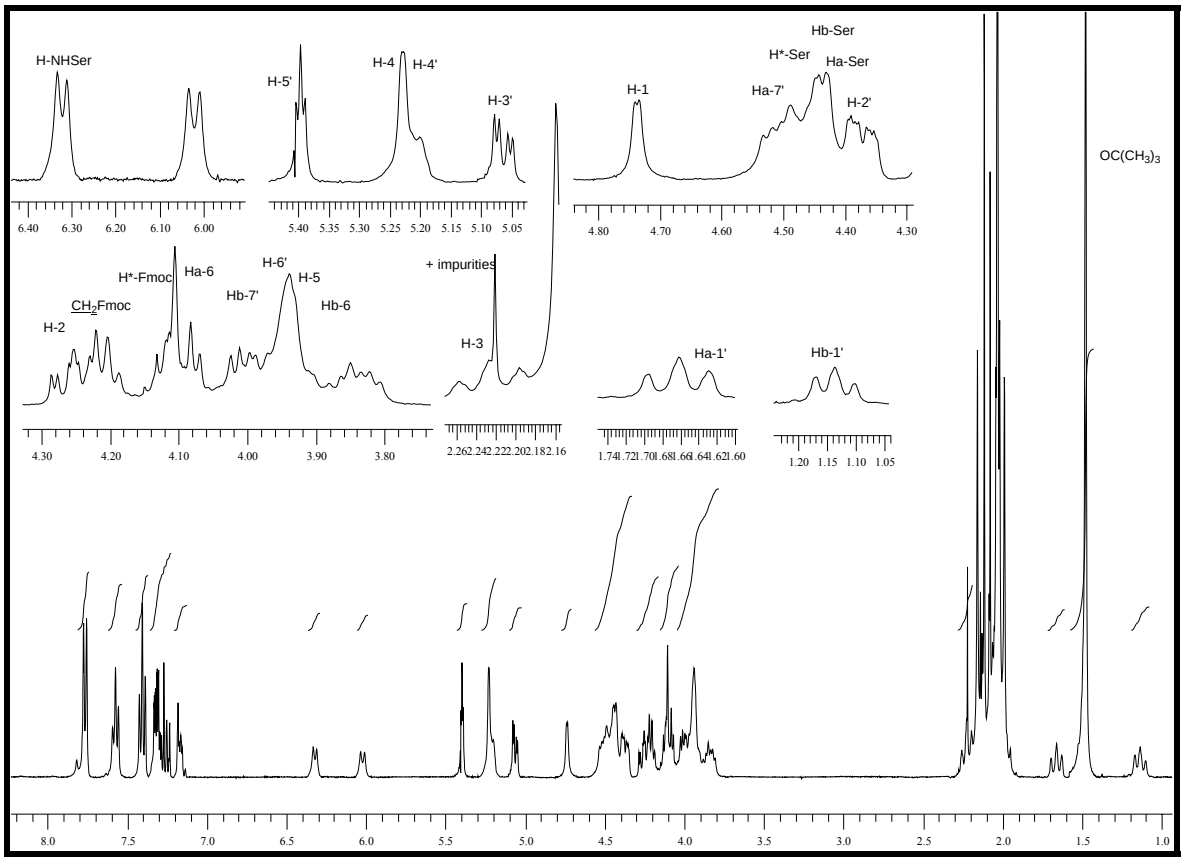
MALDI-HRMS Calcd for (M + K) $C_{49}H_{62}N_2O_{20}K$ 1037.3533; found 1037.3589.

Anal. calcd for $C_{49}H_{62}N_2O_{20}$ (999.02): C 58.91, H6.26, N 2.80; found: C 58.97, H 6.34, N 2.74.

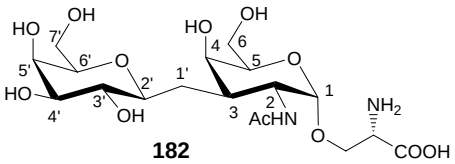
MALDI-HRMS spectrum of **181:**



1H-NMR (400 MHz, $CDCl_3$) spectrum of **181:**



3-*C*-[2,6-anhydro-1-deoxy-D-*glycero*-L-*manno*-heptitol-1-*C*-yl]-2-[(*N*-acetyl)amino]-2,3-dideoxy-α-D-*galacto*-pyranosyl-L-serine182:



182

Product **2** (40 mg, 0.060 mmol) was dissolved in 1:1 mixture of DMF/morpholine (5 mL), after stirring at 20 ^{o}C for 2 h, the mixture was co-evaporated with toluene under vacuum, FC (CH_2Cl_2, MeOH 9:1), then the residue was disolved in 90 % TFA in water and allowed to stir for 1h, then the mixture was co-evaporated with toluene under vacuum, the residue was dissolved in MeOH, 2 drops of NaOMe solution (1M) were added. After 1h the mixture was neutralized by adding Dowex 50WX8-100, the mixture was stirred for 5 min, filtrated,and evaporated HPLC gave **17** (15 mg, 80 %) as a white solid.

m.p. (137°C, discompose).

IR (film): 3285, 2927, 1668, 16645, 1539, 1430, 1186, 1129, 1035, 963, 837 cm^{-1}.

^{1}H NMR (400 MHz, CD_3OD): δ

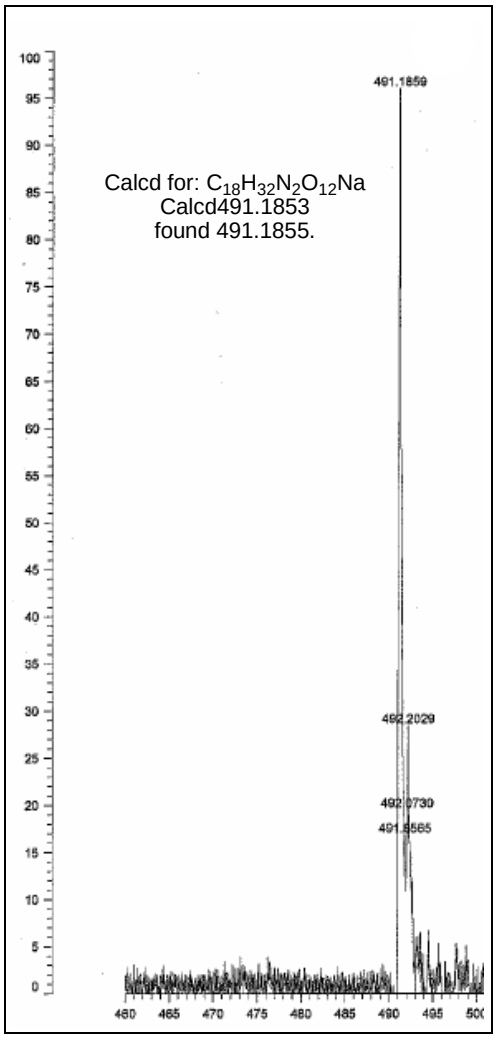
4.75 (d , ^{3}J(H-C(1), H-C(2)) = 3.1, H-C(1)); 4.23 (dd, ^{3}J(H-C(2), H-C(3)) = 12.9, ^{3}J(H-C(2), H-C(1)) = 3.4, H-C(2)); 4.22-4.10(m, 5H, H-C*(Ser), H$_a$-C(Ser), H$_b$-C(Ser), H-C(5'), H-C(4)); 4.23 (dd, ^{2}J = 11.5, ^{3}J(H$_a$-C(7'), H-C(6')) = 4.1, Ha-C(7')); 4.06-3.65(m, 11H, H-C(2'), H-C(3'), H-C(4'), H-C(5'), H-C(6'), H$_b$-C(7'), H-C(5), H-C(4), N<u>H</u>Ac, N<u>H$_2$</u>(Ser)); 3.33(m, 2H, H$_a$-C(6), H$_b$-C(6)); 2.12 (m, 1H, H-C(3)); 2.02(s, 3H, <u>CH$_3$</u>CON);1.70 (m, 1H, H$_a$-C(1'), H$_b$-C(1')).

^{13}C NMR (100.6 MHz, $CDCl_3$): δ

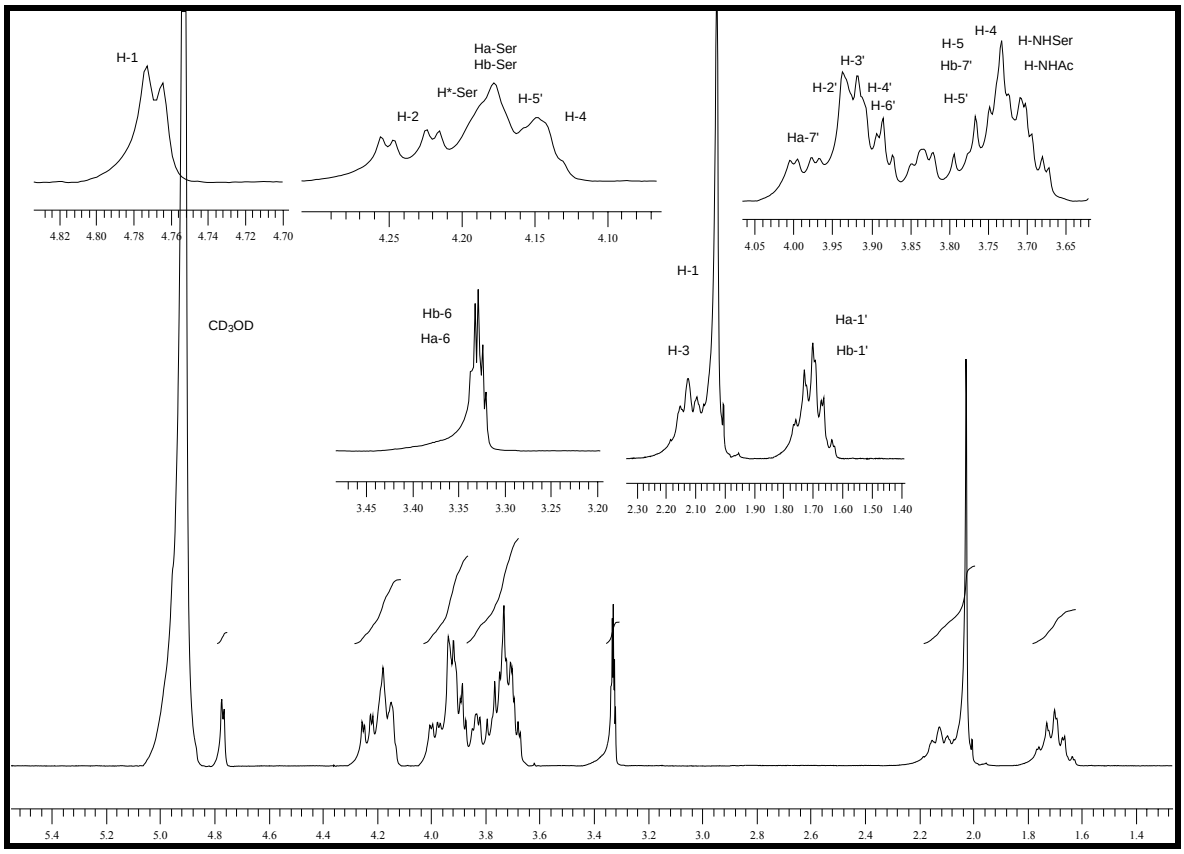
177.0 (s, COOH); 172.4 (s, NHO<u>C</u>CH$_3$); 98.7 (d, ^{1}J(C,H) = 184, C(1)); 73.7 (d, ^{1}J(C,H) = 153, C(6')); 73.0 (d, ^{1}J(C,H) = 148, C(4')); 71.1 (d, ^{1}J(C,H) = 146, C(5)); 69.9 (t, ^{1}J(C,H) = 153, <u>C</u>H$_2$(Ser)); 69.7 (d, ^{1}J(C,H) = 143, C(2')); 69.2 (d, ^{1}J(C,H) = 149, C(3')); 66.0 (d, ^{1}J(C,H) = 149, C(5')); 65.2 (d, ^{1}J(C,H) = 148, C(4)); 62.5 (t, ^{1}J(C,H) = 155, C(6)); 61.7 (t, ^{1}J(C,H) = 151, C(7'); 48.2 (d, ^{1}J(C,H) = 122, C(2)); 47.2 (d, ^{1}J(C,H) = 138, C∗(Ser)); 35.5 (d, ^{1}J(C,H) = 129, C(3)); 22.4 (d, ^{1}J(C,H) = 139, C(1')); 21.9 (q, ^{1}J(C,H) = 130, <u>C</u>H$_3$CON).

MALDI-HRMS Calcd for (M + Na) $C_{18}H_{32}N_2O_{12}Na$ 491.1853; found 491.1855.

MALDI-HRMS spectrum of **182:**



1H-NMR (400 MHz, $CDCl_3$) spectrum of **182:**



General Peptide Coupling Reactions.

To a solution of Fmoc-protected glycosylamino acid (1.05 eq) dissolved in dimethylformamide, at –0°C a mixture of 1-hydroxy-7-aza-1,2,3-benzotriazole (1.5 eq) and *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (1.5 eq), sym-collidine (3 eq) was added, and stirred for 15 min followed by addition of the amine (1.0 eq). The reaction mixture was stirred at 0 °C for 1 h and allowed to slowly warm up to room temperature and stirred for 3 h, during which time the reaction mixture was concentrated *in vacuo* and the residue was purified by flash column chromatography on silica gel using a gradient of methanol in dichloromethane.

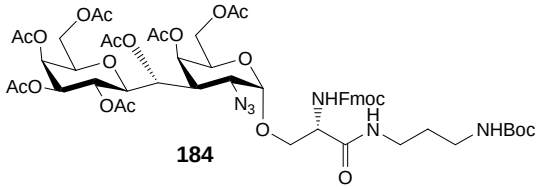
General Fmoc Deprotection:

The Fmoc-protected compound was dissolved in 5:1 dimethylformamide: piperidine and stirred at room temperature for 2 h, time during which the reaction mixture was concentrated *in vacuo* and the residue was purified by flash column chromatography on silica gel using a gradient of methanol in dichloromethane.

General t-Butyl Ester Deprotection:

A solution of t-butyl protected acid was dissolved in 95% aq TFA (10mL) and stirred at room temperature for 1 h. The solution was then co-evaporated with toluene several times to give the corresponding acid which was used without further purification.

***N*-[(9*H*-Fluoren-9-ylmethoxy)carbonyl]-{4,6-di-*O*-acetyl-3-*C*-[(1*R*)-2,6-anhydro–1,3,4,5,7-penta-*O*-acetyl-D-*glycero*-L-*manno*-heptitol-1-*C*-yl]-2-azido-2,3-dideoxy-α-D-*galacto*pyranosyl}-*N*-[3-((*tert*-butoxycarbonyl)amino)propyl]-L-serinamide (184).**



184

To a solution of acid **183** (90 mg, 0.092 mmol) dissolved in DMF (5ml), at 0°C a mixture of HOAt (18 mg, 0.138 mmol) and HATU (53 mg, 0138 mmol), sym-collidine (40 µl, 0.276 mmol) in 1ml DMFwas added, and stirred for 15 min followed by addition of the amine **197a** (17 mg, 0.092). The reaction mixture was stirred at 0 °C for 1 h and allowed to slowly warm up to room temperature and stirred for 1 h, during which time the reaction mixture was concentrated *in vacuo* and FC (light petroleum ether/EtOAc 1:2) gave 92 mg (88 %) of **184**, white foam.

IR (film): 3335,2970, 2110, 1740, 1510, 1450, 1370, 1220, 1025, 935, 880 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ

7.80-7.26 (4 m, 8H, ArH); 6.91 (s, H-N Propyl); 6.04 (d, ^{3}J(H-N, H-C*) = 8.0, H-N); 5.56 (s, H-C(4)); 5.38 (dd, ^{3}J(H-C(1'), H-C(3)) = 9.60, ^{3}J(H-C(1'), H-C(2')) = 2.56, H-C(1')); 5.32 (dd, ^{3}J(H-C-(5'), H-C(6')) = 6.72, ^{3}J(H-C(5'), H-C(4')) = 3.20, H-C(5')); 5.216 (t, ^{3}J(H-C(4'), H-C(3')) = 3.52,H-C(4')); 4.96 (d, ^{3}J(H-C(1), H-C(2)) = 2.88, H-C(1)); 4.83 (d, ^{3}J(H-C(4'), H-C(3')) = 3.52, H-C(3')); 4.75 (dd, ^{2}J = 12.8, ^{3}J(H-C(6_{exo}), H-C(5)) = 9.92, H-C(6_{exo})); 4.54-4.44 (m, 2H, H-C(2'), H-C*(Ser)); 4.43-4.31(m, 3H, H_{endo}-C(6), H_2-C(fmoc)); 4.30-4.11 (m, 5H, H_a-C(7') , H-C(6'), H-C(5), H-C(fmoc), H_2-C(Ser)); 4.30-4.11 (m, 2H, H_b-C(7') , H_2-C(Ser)); 3.43 (dd, ^{3}J(H-C(2), H-C(3)) = 12.16, ^{3}J(H-C(2), H-C(1))= 2.88, H-C(2)); 3.30 (bs, 2H, 2H-C(CONH$\underline{CH_2}$)); 3.15 (br s, 2H, 2H-C(BocNH$\underline{CH_2}$)); 2.59 (d, ^{3}J(H-C(3), H-C(2)) = 12.16, H-C(3)); 2.25-1.94 (7s, 21H, CH_3COO);1.55 (br s, 2H, 2H -C(NHCH$_2\underline{CH_2}$CH$_2$NH);1.42 (s, 9H, $(CH_3)_3C$).

^{13}C NMR (100.6 MHz, $CDCl_3$): δ

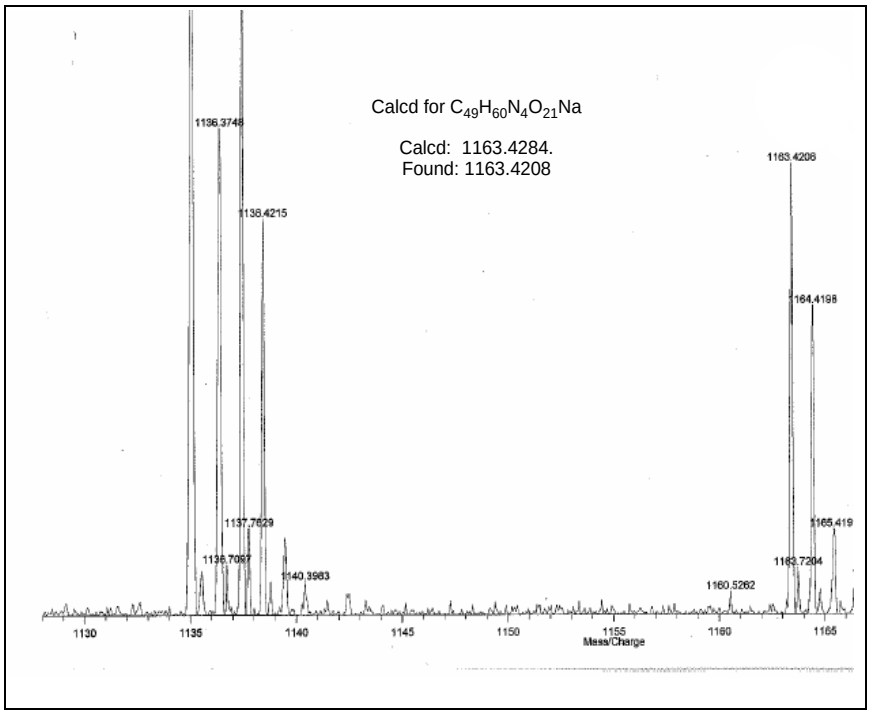
170.7, 170.4, 170.3, 169.9, 169.8, 168.9, 168.7, 168.4 (8s, -CO); 155.8 (s, C(carbamate); 155.0 (s, C(carbamate); 143.6 (s, C(Fmoc)); 141.2 (s, C(Fmoc)); 127.6 (d, ^{1}J(C,H) = 159,

C(Fmoc)); 126.9 (d, ^{1}J(C,H) = 159, C(Fmoc)); 125.1 (d, ^{1}J(C,H) = 158, C(Fmoc)); 119.8 (d, ^{1}J(C,H) = 158, C(Fmoc)); 99.3 (d, ^{1}J(C,H) = 184, C(1)); 79.0 (s, $\underline{C}(CH_3)_3$); 72.9 (d, ^{1}J(C,H) = 153, C(6')); 70.7 (t, ^{1}J(C,H) = 153, $\underline{C}H_2$(Ser)); 68.8 (d, ^{1}J(C,H) = 146, C(5)); 66.8 (t, ^{1}J(C,H) = 150, $\underline{C}H_2$(Fmoc)); 66.6 (d, ^{1}J(C,H) = 148, C(4')); 66.6 (d, ^{1}J(C,H) = 139, C(1')); 66.4 (d, ^{1}J(C,H) = 149, C(3')); 65.8 (d, ^{1}J(C,H) = 151, C(4)); 65.4 (d, ^{1}J(C,H) = 149, C(5')); 64.6 (d, ^{1}J(C,H) = 143, C(2')); 62.1 (t, ^{1}J(C,H) = 151, C(7')); 59.4 (t, ^{1}J(C,H) = 155, C(6)); 55.0 (d, ^{1}J(C,H) = 138, C∗(Ser)); 54.9 (d, ^{1}J(C,H) = 142, C(2)); 47.0 (d, ^{1}J(C,H) = 130, C(Fmoc)); 37.0 (d, ^{1}J(C,H) = 129, C(3)); 36.9 (t, ^{1}J(C,H) = 151, C(BocNH$\underline{C}H_2$));36.1 (t, ^{1}J(C,H) = 151, C(CONH$\underline{C}H_2$)); 29.9 (t, ^{1}J(C,H) = 151, C(NHCH$_2\underline{C}$H$_2$CH$_2$NH)); 28.0 (q, 3C, ^{1}J(C,H) = 128, ($\underline{C}$H3)CO); 20.8-20.4 (7q, ^{1}J(C,H) = 130, $\underline{C}H_3$COO).

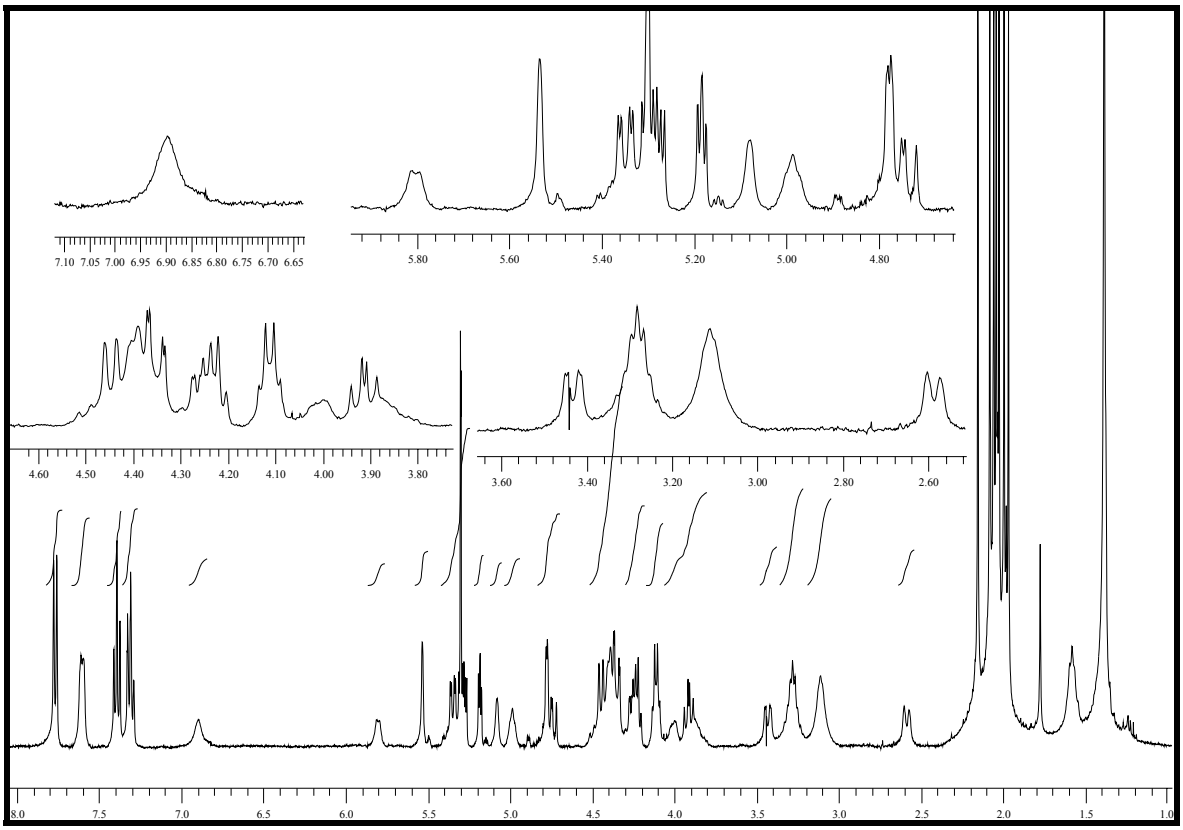
MALDI-HRMS Calcd for (M + Na) $C_{49}H_{60}N_4O_{21}Na$ 1163.4284, Found 1163.4208;

Anal. calcd for $C_{53}H_{68}N_6O_{22}$ (1141.13): C 55.78, H6.01, N 7.36; found: C 55.74, H 5.96, N 7.35.

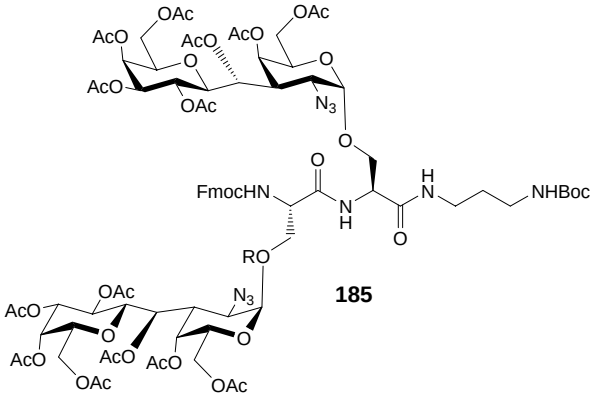
MALDI-HRMS spectrum of **184:**



1H-NMR (400 MHz, $CDCl_3$) spectrum of **184:**



***N*-[(9*H*-Fluoren-9-ylmethoxy)carbonyl]- *O*-{4,6-di-*O*-acetyl-3-*C*-[(1*R*)-2,6-anhydro–1,3,4,5,7-penta-*O*-acetyl-D-*glycero*-L-*manno*-heptitol-1-*C*-yl]-2-azido-2,3-dideoxy-α-D-*galacto*pyranosyl}-D-seryl-*rac*-*N*-[3-({[(1,1-dimethylethyl)oxy]carbonyl} amino)propyl]-*O*-{4,6-di-*O*-acetyl-3-*C*-[(1*R*)-2,6-anhydro–1,3,4,5,7-penta-*O*-acetyl-D-*glycero*-L-*manno*-heptitol-1-*C*-yl]-2-azido-2,3-dideoxy-α-D-*galacto*pyranosyl}-D-serinamide (185).**



The Fmoc-protected compound **184** (70 mg, 0.061 mmol) was dissolved in 5:1 DMF: piperidine (5ml) and stirred at room temperature for 1 h, time during which the reaction mixture was concentrated *in vacuo* and the residue was dissolved in 1 ml DMF. In a separated flask a solution of acid **183** (70 mg, 0.071 mmol) dissolved in DMF (3 ml), at 0°C a mixture of HOAt (14 mg, 0.107 mmol) and HATU (41 mg, 0107 mmol), sym-collidine (31 μl, 0.215 mmol) in 1ml DMFwas added, and stirred for 15 min followed by addition of the solution of amine from the first flask via syringe. The reaction mixture was stirred at 0 °C for 1 h and allowed to slowly warm up to room temperature and stirred for 1 h, during which time the reaction mixture was concentrated *in vacuo* and FC (light petroleum ether/EtOAc 1:2) gave 92 mg (80 % for two steps) of **185**, white foam.

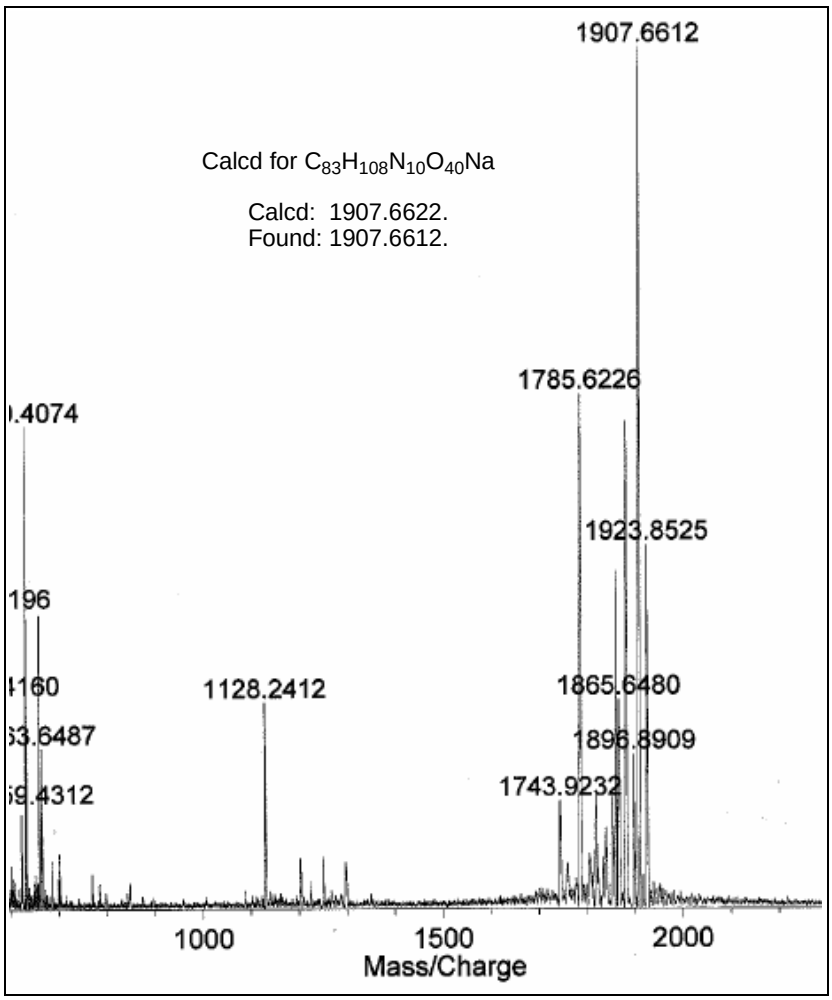
IR (film): 3360,2940, 2110, 1735, 1510, 1450, 1370, 1215, 1040, 940 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.80-7.26 (4 m, 8H, ArH); 6.10-5.80 (m, 2H); 5.60-5.25 (m, 6H); 5.25-4.70 (m, 8H); 4.70-3.80(m, 21H); 3.50-3.00 (m, 6H), 2.70-2.55(m, 2H); 2.30-1.85 (m, 42H); 1.70-1.20 (m, 13H);

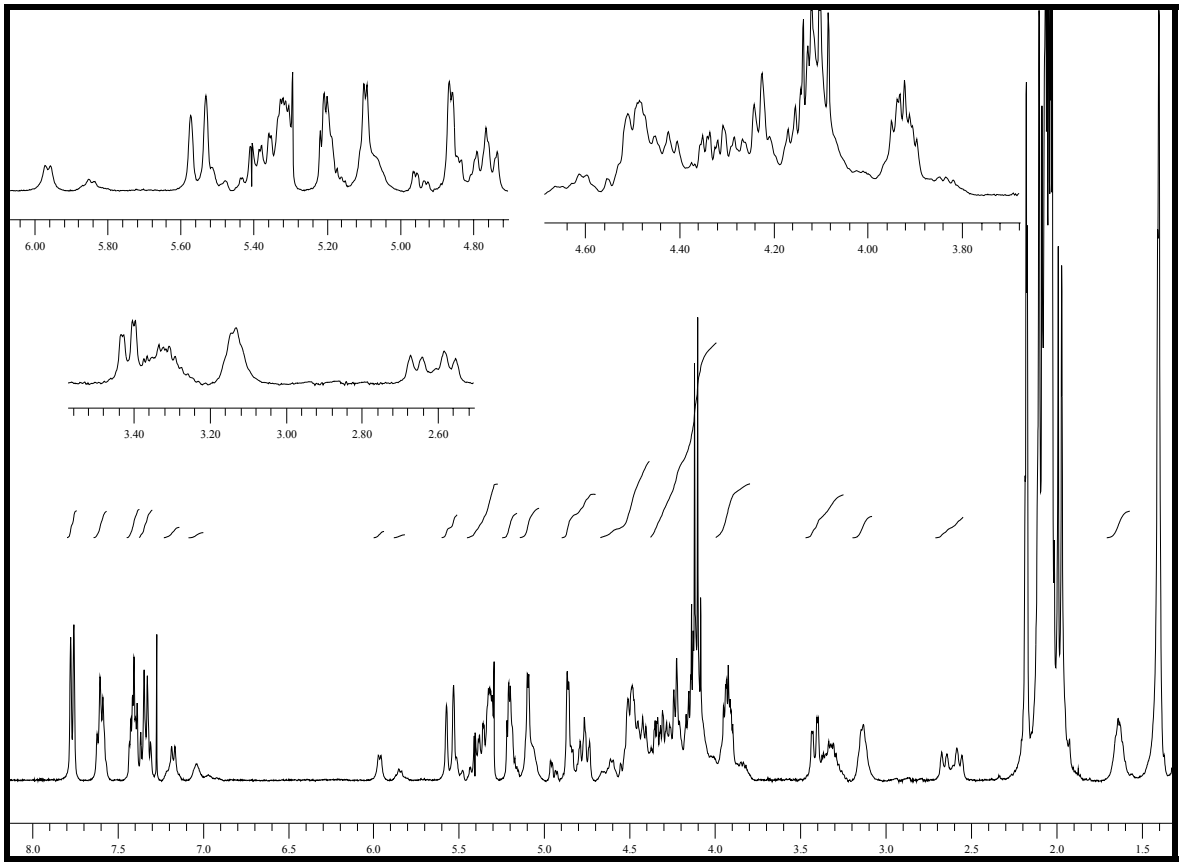
MALDI-HRMS Calcd for (M + Na) $C_{83}H_{108}N_{10}O_{40}Na$ 1907.6622, Found 1907.6612;

Anal. calcd for $C_{83}H_{108}N_{10}O_{40}$ (1884.66): C 52.86, H 5.77, N 7.43; found: C 52.77, H 5.72, N 7.40.

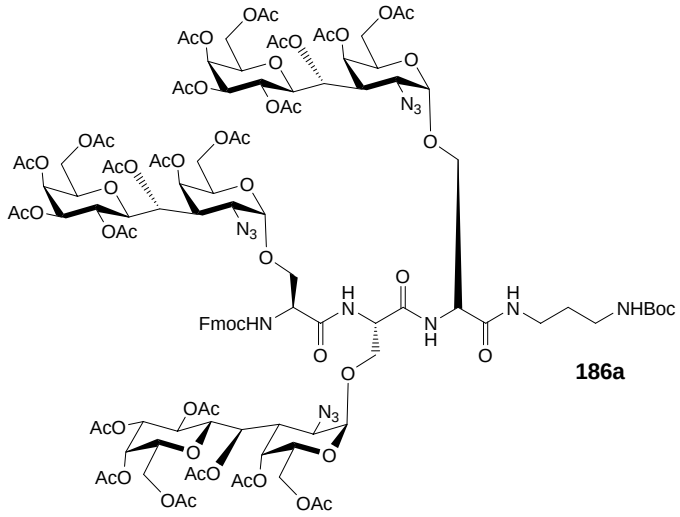
MALDI-HRMS spectrum of **185:**



1H-NMR (400 MHz, $CDCl_3$) spectrum of **185:**



***N*-[(9*H*-Fluoren-9-ylmethoxy)carbonyl]-*O*-{4,6-di-*O*-acetyl-3-*C*-[(1*R*)-2,6-anhydro–1,3,4,5,7-penta-*O*-acetyl-D-*glycero*-L-*manno*-heptitol-1-*C*-yl]-2-azido-2,3-dideoxy-α-D-*galacto*pyranosyl}-D-seryl-*O*-{4,6-di-*O*-acetyl-3-*C*-[(1*R*)-2,6-anhydro–1,3,4,5,7-penta-*O*-acetyl-D-*glycero*-L-*manno*-heptitol-1-*C*-yl]-2-azido-2,3-dideoxy-α-D-*galacto*pyranosyl}-D-seryl-*rac*-*N*-[3-({[(1,1-dimethylethyl)oxy]carbonyl} amino)propyl]-*O*-{4,6-di-*O*-acetyl-3-*C*-[(1*R*)-2,6-anhydro–1,3,4,5,7-penta-*O*-acetyl-D-*glycero*-L-*manno*-heptitol-1-*C*-yl]-2-azido-2,3-dideoxy-α-D-*galacto*pyranosyl}-D-serinamide (186a).**



The Fmoc-protected compound **185** (40 mg, 0.021 mmol) was dissolved in 5:1 DMF: piperidine (3ml) and stirred at room temperature for 1 h, time during which the reaction mixture was concentrated *in vacuo* and the residue was dissolved in 1 ml DMF. In a separated flask a solution of acid **183** (23 mg, 0.024 mmol) dissolved in DMF (1 ml), at 0°C a mixture of HOAt (4.7 mg, 0.036 mmol) and HATU (14 mg, 0.036 mmol), sym-collidine (11 μl, 0.073 mmol) in 1ml DMFwas added, and stirred for 15 min followed by addition of the solution of amine from the first flask via syringe. The reaction mixture was stirred at 0 °C for 1 h and allowed to slowly warm up to room temperature and stirred for 3 h, during which time the reaction mixture was concentrated *in vacuo* and FC (light petroleum ether/EtOAc 1:2) gave 44 mg (72 %, for two steps) of **186a**, white foam.

IR (film): 3355,2935, 2110, 1740, 1510, 1455, 1365, 1215, 1040, 940 cm^{-1}.

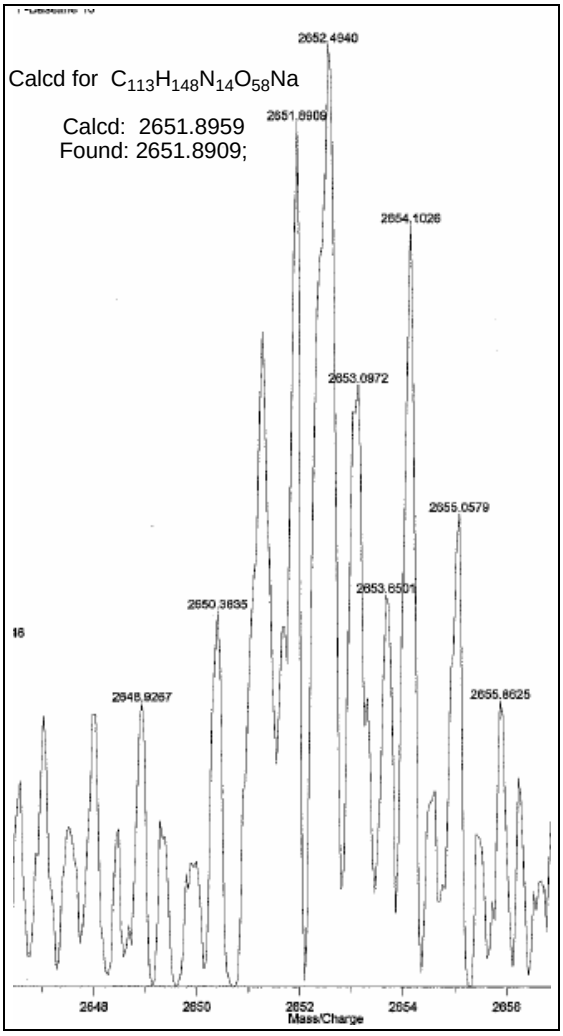
^{1}H NMR (400 MHz, $CDCl_3$): δ

7.80-7.26 (4 m, 8H, ArH); 6.10-5.80 (m, 3H); 5.60-5.25 (m, 9H); 5.25-4.70 (m, 12H); 4.70-3.80(m, 30H); 3.50-3.00 (m, 7H), 2.70-2.55(m, 3H); 2.30-1.85 (m, 63H); 1.70-1.20 (m, 13H);

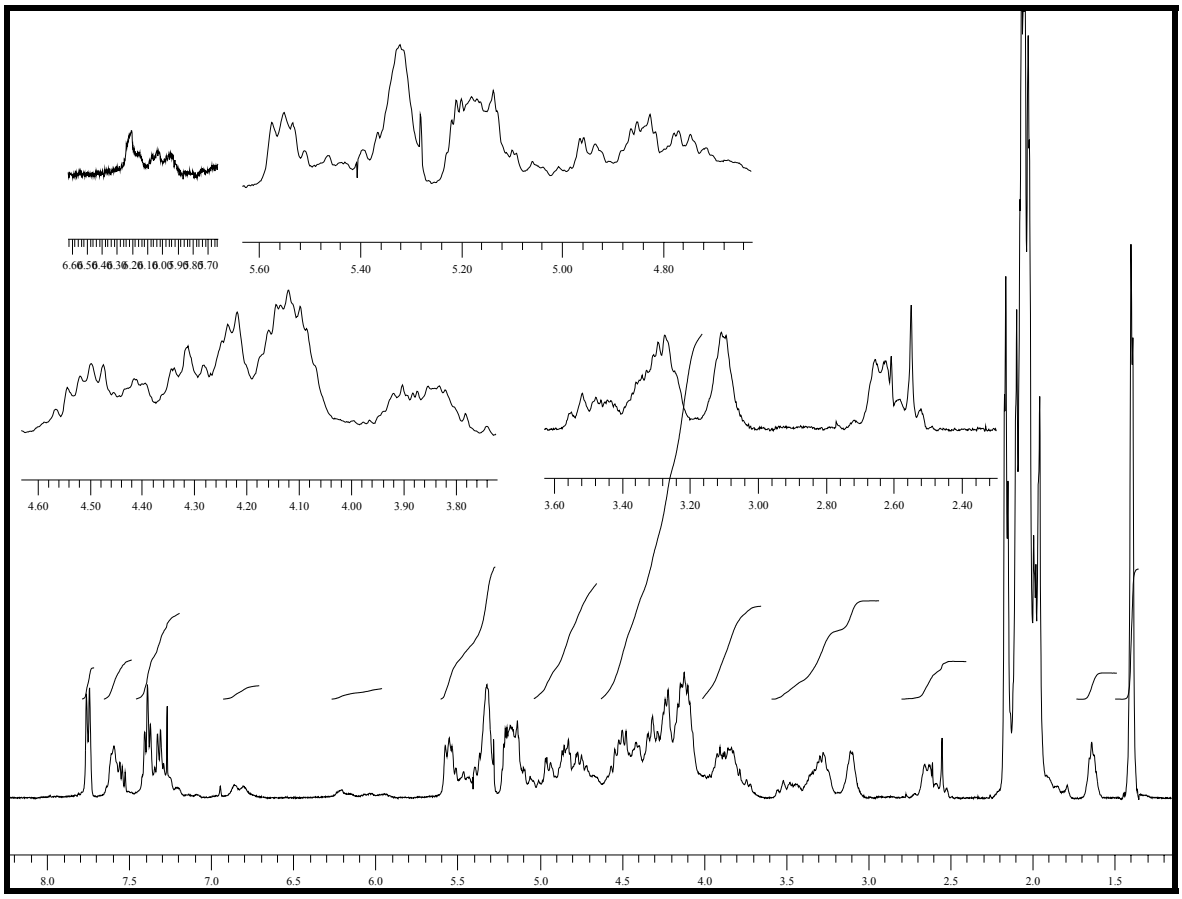
MALDI-HRMS Calcd for (M + Na) $C_{113}H_{148}N_{14}O_{58}Na$ 2651.8959, Found 2651.8909;

Anal. calcd for $C_{113}H_{148}N_{14}O_{58}$ (2628.89): C 51.60, H 5.67, N 7.45; found: C 51.63, H 5.78, N 7.41.

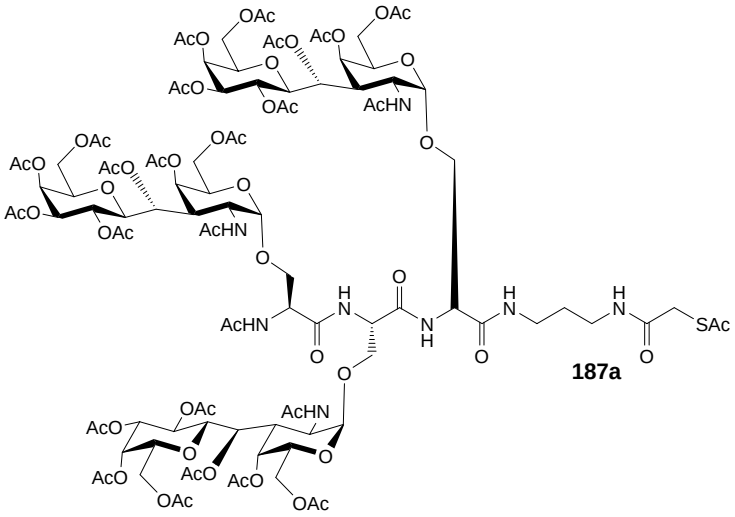
MALDI-HRMS spectrum of **186a:**



1H-NMR (400 MHz, $CDCl_3$) spectrum of **186a:**



***N*-acetyl-*O*-{4,6-di-*O*-acetyl-3-*C*-[(1*R*)-2,6-anhydro–1,3,4,5,7-penta-*O*-acetyl-D-*glycero*-L-*manno*-heptitol-1-*C*-yl]-2-[(*N*-acetyl)amino]-2,3-dideoxy-α-D-*galacto*pyranosyl}-D-seryl-*O*-{4,6-di-*O*-acetyl-3-*C*-[(1*R*)-2,6-anhydro–1,3,4,5,7-penta-*O*-acetyl-D-*glycero*-L-*manno*-heptitol-1-*C*-yl]-2-[(*N*-acetyl)amino]-2,3-dideoxy-α-D-*galacto*pyranosyl}-D-seryl-*rac*-*N*-(3-{[(acetylthio)acetyl]amino}propyl)-*O*-{4,6-di-*O*-acetyl-3-*C*-[(1*R*)-2,6-anhydro–1,3,4,5,7-penta-*O*-acetyl-D-*glycero*-L-*manno*-heptitol-1-*C*-yl]-2-[(*N*-acetyl)amino]-2,3-dideoxy-α-D-*galacto*pyranosyl}-D-serinamide (187a).**



187a

The Fmoc-protected compound **186a** (40 mg, 0.015 mmol) was dissolved in 5:1 DMF: piperidine (3ml) and stirred at room temperature for 1 h, time during which the reaction mixture was concentrated *in vacuo* and the residue was dissolved in 4:4:1 mixture of 2,6-lutidine/CH_3COSH/Ac_2O (2 mL). After stirring at 20 ^oC for 18 h the mixture was co-evaporated with toluene under vacuum (10^{-3}Torr). FC (hexanes:EtOAc, 4:1 →0:1 then 5 % MeOH in CH_2Cl_2), evaporation, the residue dissolved in 95% aq TFA (4 mL) and stirred at room temperature for 1 h. The solution was then co-evaporated with toluene several times to give the corresponding acid which was used without further purification. The formed amine was dissolved in CH_2Cl_2 (2ml), at 0 ^oC DIEA (4 µl, 0.03 mmol) was added then followed by the addition SAMA-OPfp (5mg, 0.017 mmol) then the reaction mixture was stirred at 0 ^oC for 1 h. After the addition of CH_2Cl_2 (10 mL), the solution was washed with ice-cold H_2O (50 mL, twice) and dried ($MgSO_4$). Solvent evaporation *in vacuo* and quick FC (CH_2Cl_2: MeOH, 95:5) gave 30 mg (75 %) of **187a**, yellow foam, that was dried and stored at -20 ^oC until its use.

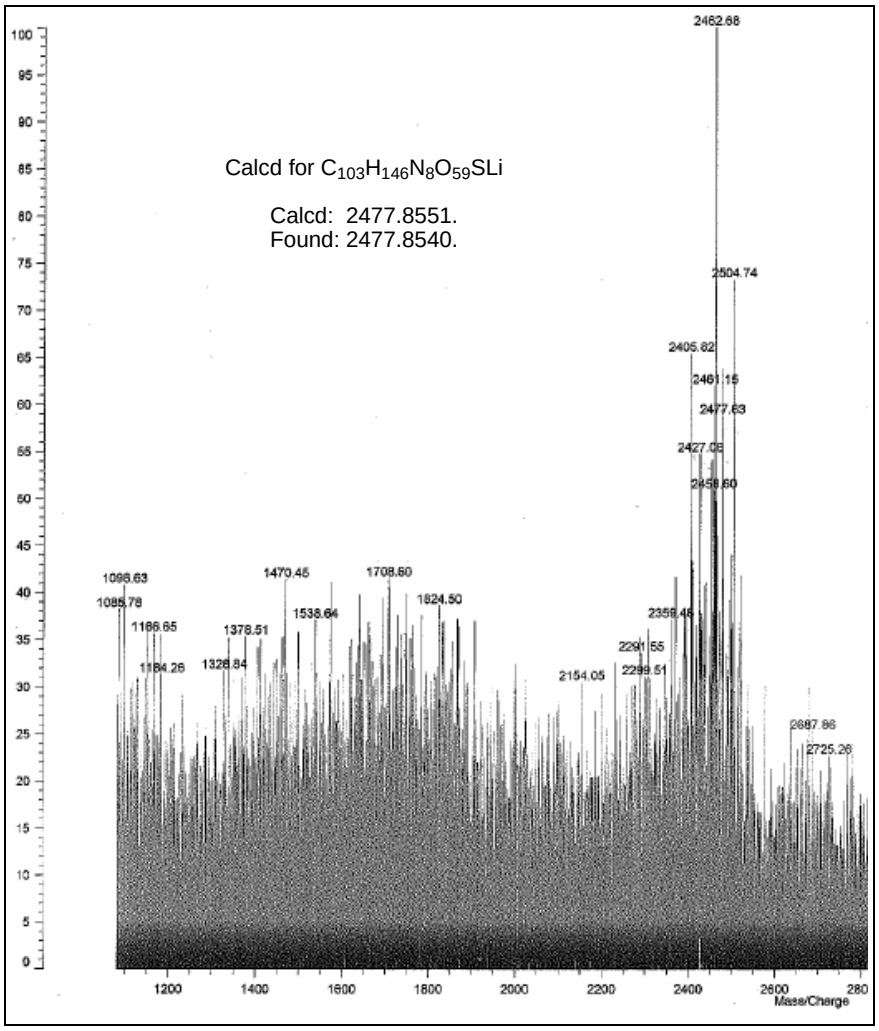
IR (film): 3315, 2960, 2920, 2850, 1735, 1655, 1380, 1245, 1035, 800 cm^{-1}.

^{1}H NMR **(400 MHz, $CDCl_3$); δ**

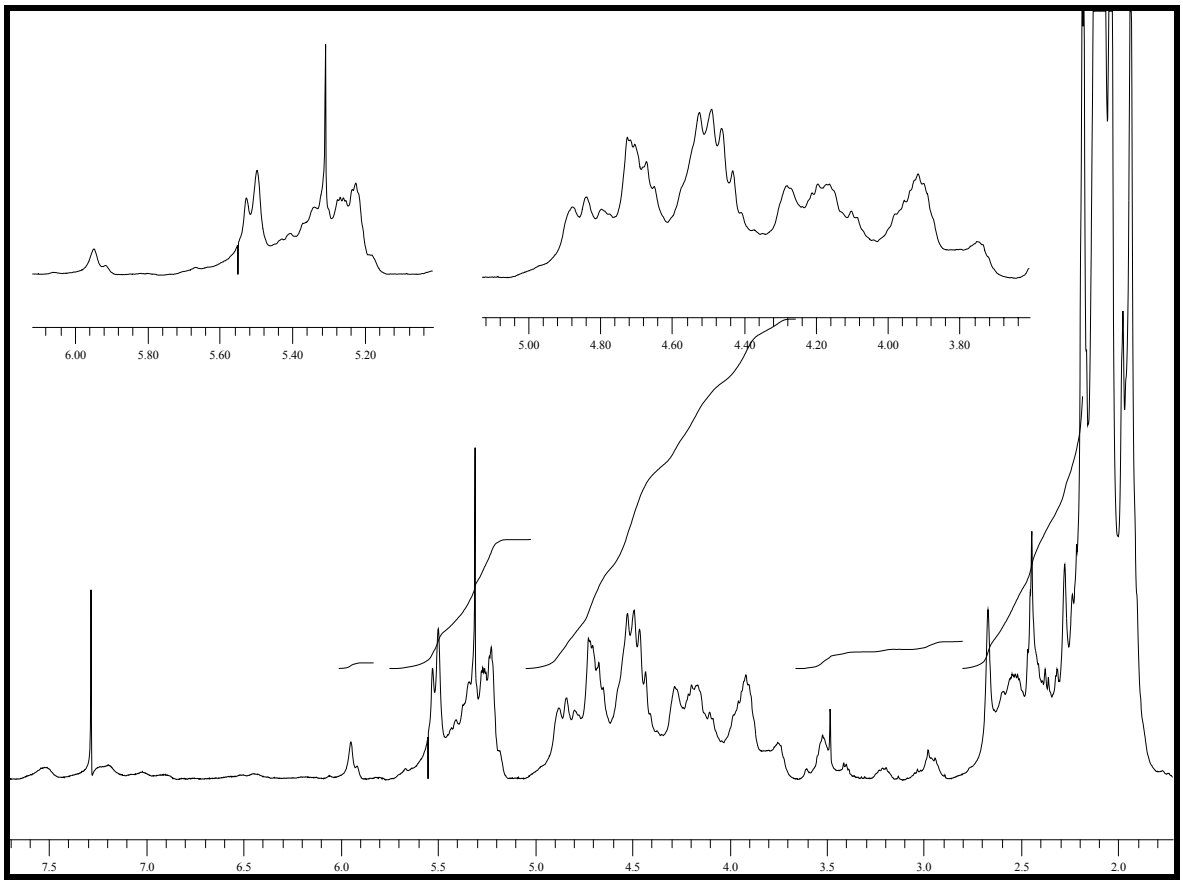
6.02-5.10(m, 14 H); 5.01-2.90(m, 52H); 2.85-1.70(m, 32H).

MALDI-HRMS Calcd for (M + Li-Ac) $C_{103}H_{146}N_8O_{59}SLi$ 2477.8551, Found 2477.8540;

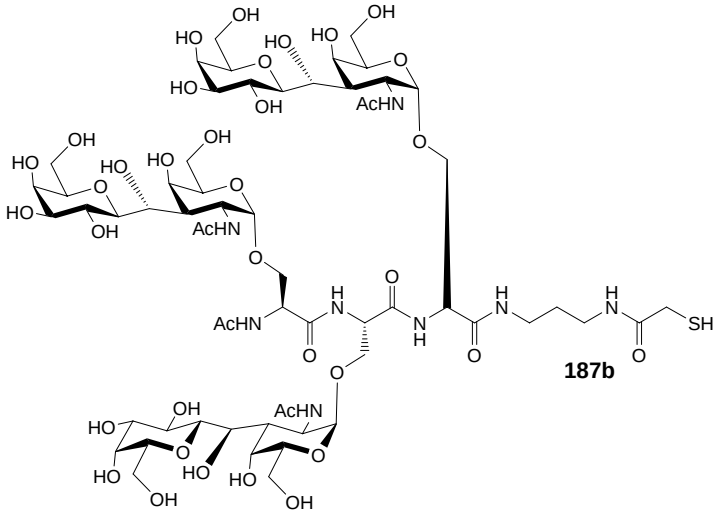
MALDI-HRMS spectrum of **187a:**



1H-NMR (400 MHz, $CDCl_3$) spectrum of **187a:**



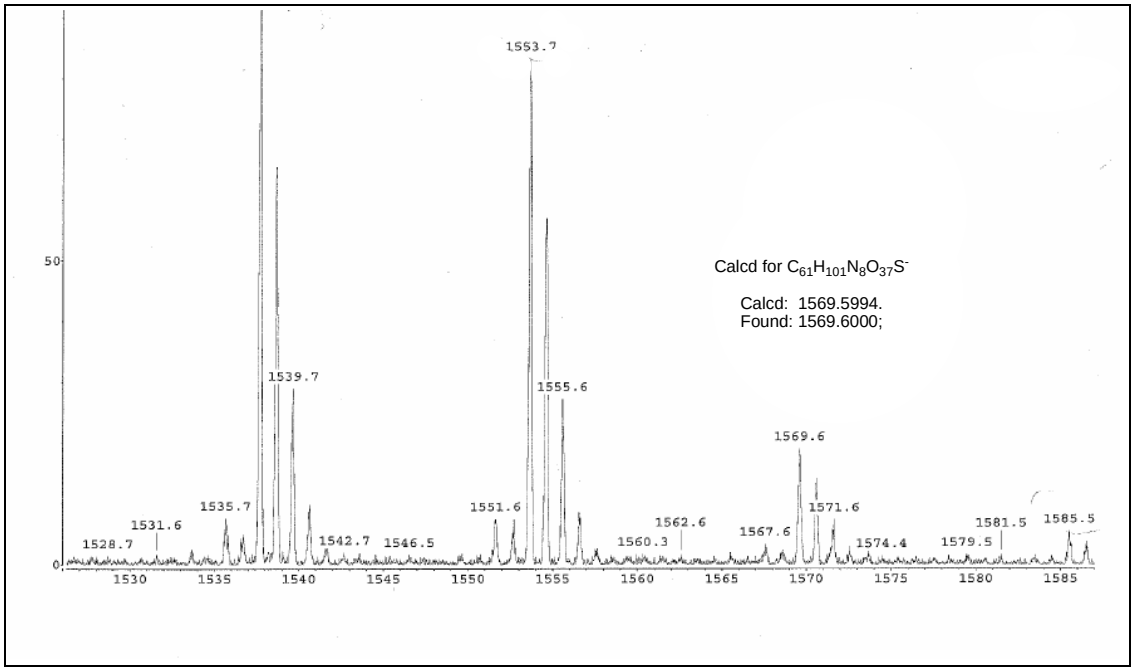
_N_-acetyl-_O_-{3-_C_-[(1_R_)2,6-anhydro-D-_glycero_-L-_manno_-heptitol-1-_C_-yl]-2-[(_N_-acetyl)amino]-2,3-dideoxy-α-D-_galacto_-pyranosyl}-D-seryl-_O_-{3-_C_-[(1_R_)2,6-anhydro-D-_glycero_-L-_manno_-heptitol-1-_C_-yl]-2-[(_N_-acetyl)amino]-2,3-dideoxy-α-D-_galacto_-pyranosyl}-D-seryl-_rac_-_N_-{3-[(mercaptoacetyl)amino]propyl}-_O_-{3-_C_-[(1_R_)2,6-anhydro-D-_glycero_-L-_manno_-heptitol-1-_C_-yl]-2-[(_N_-acetyl)amino]-2,3-dideoxy-α-D-_galacto_-pyranosyl}-D-serinamide (187b).



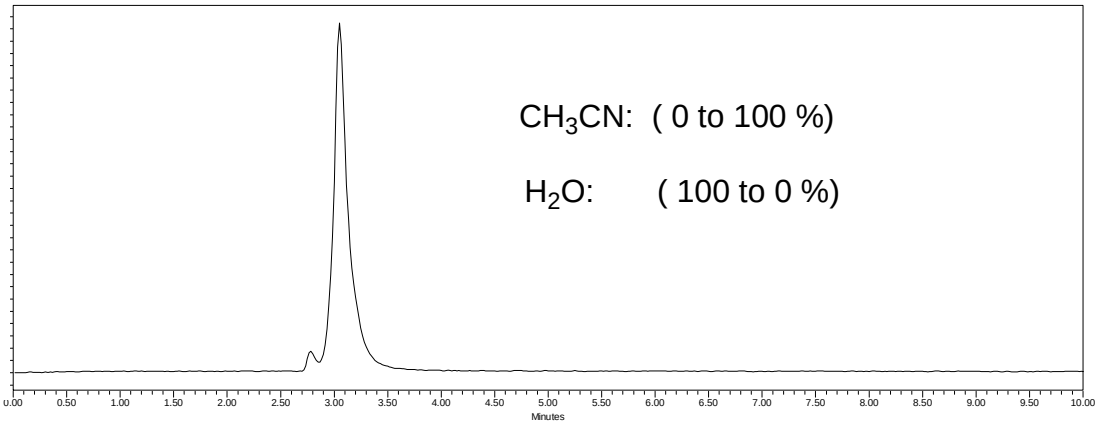
The peracetylated compound **187a** (20 mg, 0.0079 mmol) was dissolved in MeOH, 2 µl of NaOMe solution (1M) were added. After 1h the mixture was neutralized by adding Dowex 50WX8-100, the mixture was stirred for 5 min, filtrated,and evaporated HPLC gave **187b** 6.9 mg (55 %) as a white foam.

MALDI-HRMS Calcd for (M – H_2O) $C_{61}H_{101}N_8O_{37}S^-$ 1569.5994, Found 1569.6000;

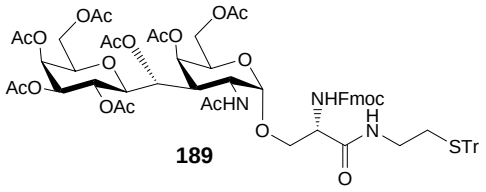
MALDI-HRMS spectrum of **186b:**



HPLC chromatogram of **186b:**



***N*-[(9*H*-Fluoren-9-ylmethoxy)carbonyl]-{4,6-di-*O*-acetyl-3-*C*-[(1*R*)-2,6-anhydro–1,3,4,5,7-penta-*O*-acetyl-D-*glycero*-L-*manno*-heptitol-1-*C*-yl]-2-[(*N*-acetyl)amino]-2,3-dideoxy-α-D-*galacto*pyranosyl}-*N*-[2-(tritylthio)ethyl]-D-serinamide (189).**



189

To a solution of acid **188** (70 mg, 0.07 mmol) dissolved in DMF (3.5ml), at 0°C a mixture of HOAt (13.7 mg, 0.105 mmol) and HATU (40.3 mg, 0.105 mmol), sym-collidine (30.4 µl, 0.210 mmol) in 1ml DMFwas added, and stirred for 15 min followed by addition of the amine **197b** (22 mg, 0.07 mmol). The reaction mixture was stirred at 0 °C for 1 h and allowed to slowly warm up to room temperature and stirred for 1 h, during which time the reaction mixture was concentrated *in vacuo* and FC (5 % MeOH in CH_2Cl_2) gave 80 mg (87 %) of **189**, white foam.

$[\alpha]^{25}_{589}$ = +44, $[\alpha]^{25}_{577}$ = +55, $[\alpha]^{25}_{435}$ = +109, $[\alpha]^{25}_{405}$ = +130 (*c* 0.19, CH_2Cl_2).

IR (film): 3315, 3055, 2960, 1735, 1655, 1505, 1370, 1220, 1030, 845 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ

8.00-7.00 (4 m, 23H, ArH); 6.17 (m, 1H, H-N); 5.72 (d, ^3J(H-N, H-C*) = 10.4, H-N); 5.50 (s, H-C(4)); 5.40-5.30 (m, 3H, H-C(1'), H-C(5'), H-N); 5.26 (t, ^3J(H-C(4'), H-C(5')) = 3.4,H-C(4')); 4.75-4.65 (m, 2H, H-C(1), H_{exo}-C(6)); 4.60 (td, ^3J(H-C(2), H-C(3)) = 12.9, ^3J(H-C(2), H-(NAc)) = 10.0, ^3J(H-C(2), H-C(1)) = 3.4, H-C(2)); 4.52-4.35 (m, 5H, H_2-C(Fmoc), H-C(Ser), H-C(2'), H-C(3'), H_{endo}-C(6),); 4.26-4.17 (m, 3H, H-C(Fmoc), H-C(6'), H_a-C(7')); 4.14-4.06 (m, 1H, H-C(5)); 3.97-3.87 (m, 3H, H_2-C(Ser), H_b-C(7')); 3.13-2.97 (m, 2H, N$\underline{CH_2}$); 2.57-2.36 (m, 3H, H-C(3), S$\underline{CH_2}$); 2.22-1.87 (8s, 24H, CH_3COO);

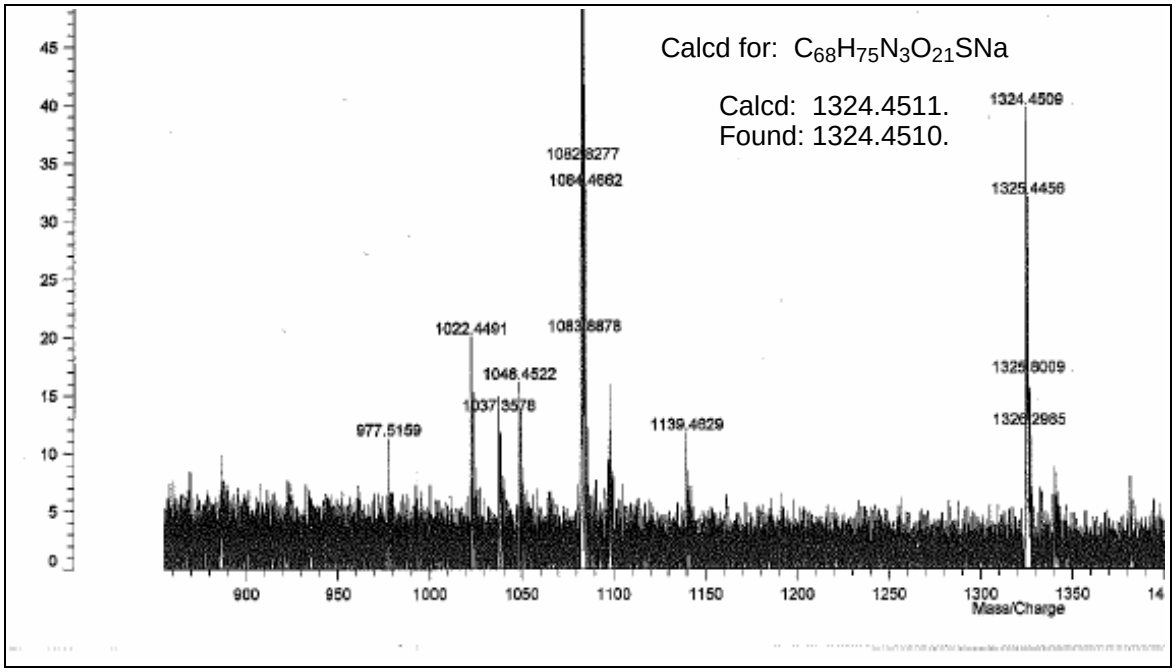
^{13}C NMR (100.6 MHz, $CDCl_3$): δ

170.7, 170.4, 170.3, 170.2, 169.9, 169.5, 169.1, 169.0, 168.9 (9s, -COO); 162.6 (s, C(carbamate); 144.4 (s, C(Tr)); 143.6 (s, C(Fmoc)); 141.3 (s, C(Fmoc)); 129.4 (d, ^1J(C,H)

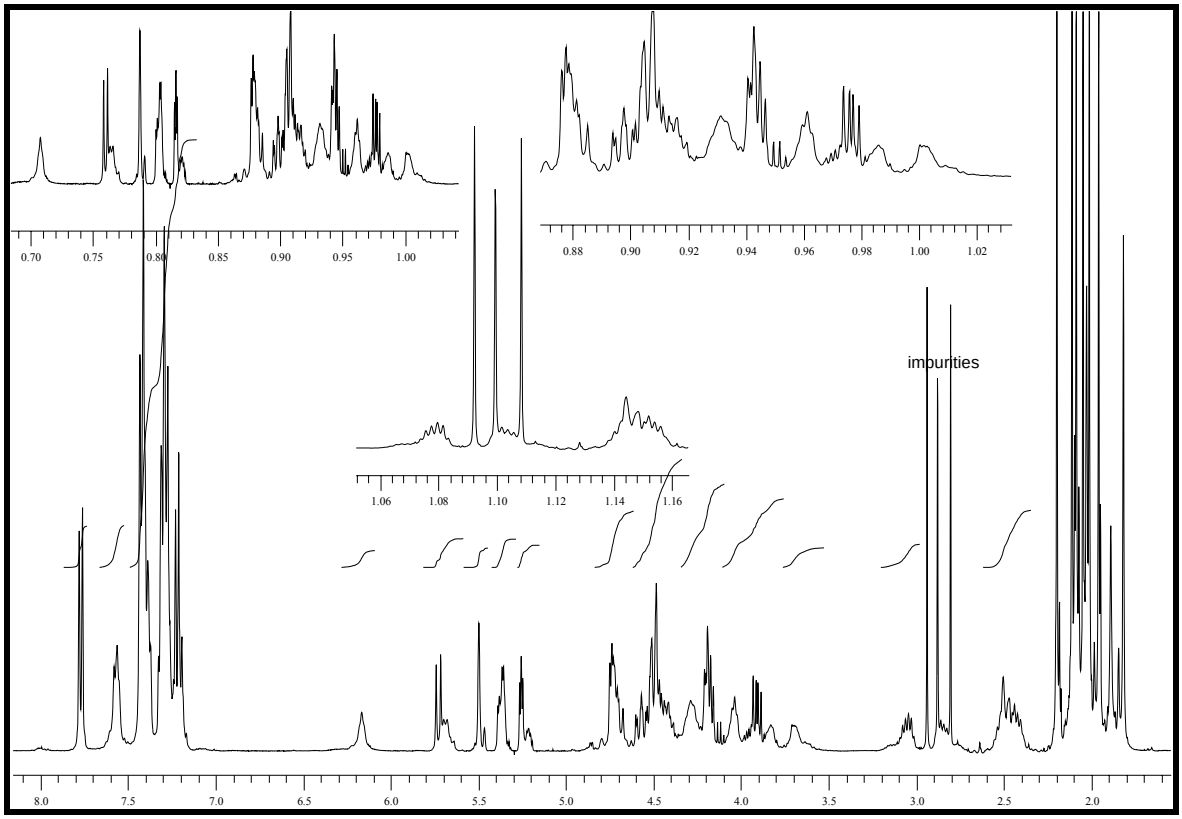
= 155, C(Tr)); 128.1 (d, ^1J(C,H) = 153, C(Tr)); 127.7 (d, ^1J(C,H) = 159, C(Fmoc)); 127.0 (d, ^1J(C,H) = 159, C(Fmoc)); 126.9 (d, ^1J(C,H) = 150, C(Tr)); 124.9 (d, ^1J(C,H) = 158, C(Fmoc)); 120.0 (d, ^1J(C,H) = 158, C(Fmoc)); 98.3 (d, ^1J(C,H) = 169, C(1)); 72.9 (d, ^1J(C,H) = 149, C(6')); 68.8 (d, ^1J(C,H) = 140, C(5)); 68.4 (t, ^1J(C,H) = 153, $\underline{C}H_2$(Ser)); 67.1 (t, ^1J(C,H) = 150, $\underline{C}H_2$(Fmoc)); 67.0 (d, ^1J(C,H) = 149, C(3')); 66.6 (d, ^1J(C,H) = 149, C(4')); 66.4 (d, ^1J(C,H) = 160, C(4)); 65.6 (d, ^1J(C,H) = 159, C(5')); 65.5 (d, ^1J(C,H) = 148, C(1')); 65.2 (d, ^1J(C,H) = 139, C(2')); 64.9(s, $C(C(Ph)_3)$; 62.24 (t, ^1J(C,H) = 163, C(7')); 59.7 (t, ^1J(C,H) = 151, C(6)); 55.0 (d, ^1J(C,H) = 138, C^*(Ser)); 47.1 (d, ^1J(C,H) = 130, C(Fmoc)); 43.5 (d, ^1J(C,H) = 130, C(Ser)); 41.3 (t, ^1J(C,H) = 153, $\underline{C}H_2NH$); 38.0 (d, ^1J(C,H) = 128, C(3)); 36.1 (t, ^1J(C,H) = 153, $\underline{C}H_2STr$); 23.4 (q, ^1J(C,H) = 129, $\underline{C}H_3CON$); 21.1-20.6 (7q, ^1J(C,H) = 130, $\underline{C}H_3COO$).

MALDI-HRMS Calcd for (M + Na) $C_{68}H_{75}N_3O_{21}SNa$ 1324.4511, Found 1324.4510;

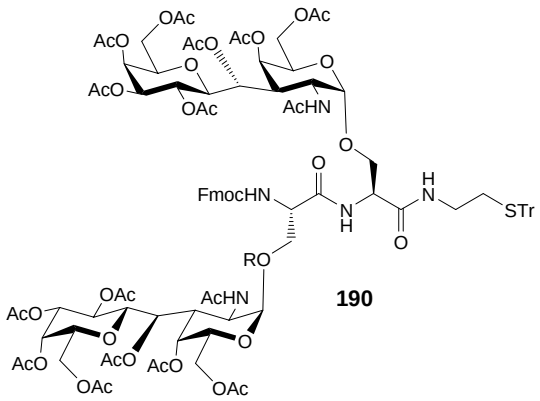
MALDI-HRMS spectrum of **189:**



1H-NMR (400 MHz, $CDCl_3$) spectrum of **189:**



***N*-{[(9*H*-fluoren-9-ylmethyl)oxy]carbonyl}-*O*-{3-*C*-[(1*R*)2,6-anhydro-D-*glycero*-L-*manno*-heptitol-1-*C*-yl]-2-[(*N*-acetyl)amino]-2,3-dideoxy-α-D-*galacto*-pyranosyl}-D-seryl-*rac*-*O*-{3-*C*-[(1*R*)2,6-anhydro-D-*glycero*-L-*manno*-heptitol-1-*C*-yl]-2-[(*N*-acetyl) amino]-2,3-dideoxy-α-D-*galacto*-pyranosyl}-*N*-{2-[(triphenylmethyl)thio]ethyl} -D-serinamide (190).**



190

The Fmoc-protected compound **189** (79 mg, 0.061 mmol) was dissolved in 5:1 DMF: piperidine (5ml) and stirred at room temperature for 1 h, time during which the reaction mixture was concentrated *in vacuo* and the residue was dissolved in 1 ml DMF. In a separated flask a solution of acid **188** (70 mg, 0.07 mmol) dissolved in DMF (3 ml), at 0°C a mixture of HOAt (14 mg, 0.107 mmol) and HATU (41 mg, 0107 mmol), sym-collidine (31 µl, 0.215 mmol) in 1ml DMFwas added, and stirred for 15 min followed by addition of the solution of amine from the first flask via syringe. The reaction mixture was stirred at 0 °C for 1 h and allowed to slowly warm up to room temperature and stirred for 1 h, during which time the reaction mixture was concentrated *in vacuo* and FC (5 % MeOH in CH_2Cl_2) gave 105 mg (83 % for two steps) of **190**, white foam.

$[\alpha]^{25}_{589}$ = +87, $[\alpha]^{25}_{577}$ = +110, $[\alpha]^{25}_{435}$ = +186, $[\alpha]^{25}_{405}$ = +214 (*c* 0.07, $CHCl_3$).

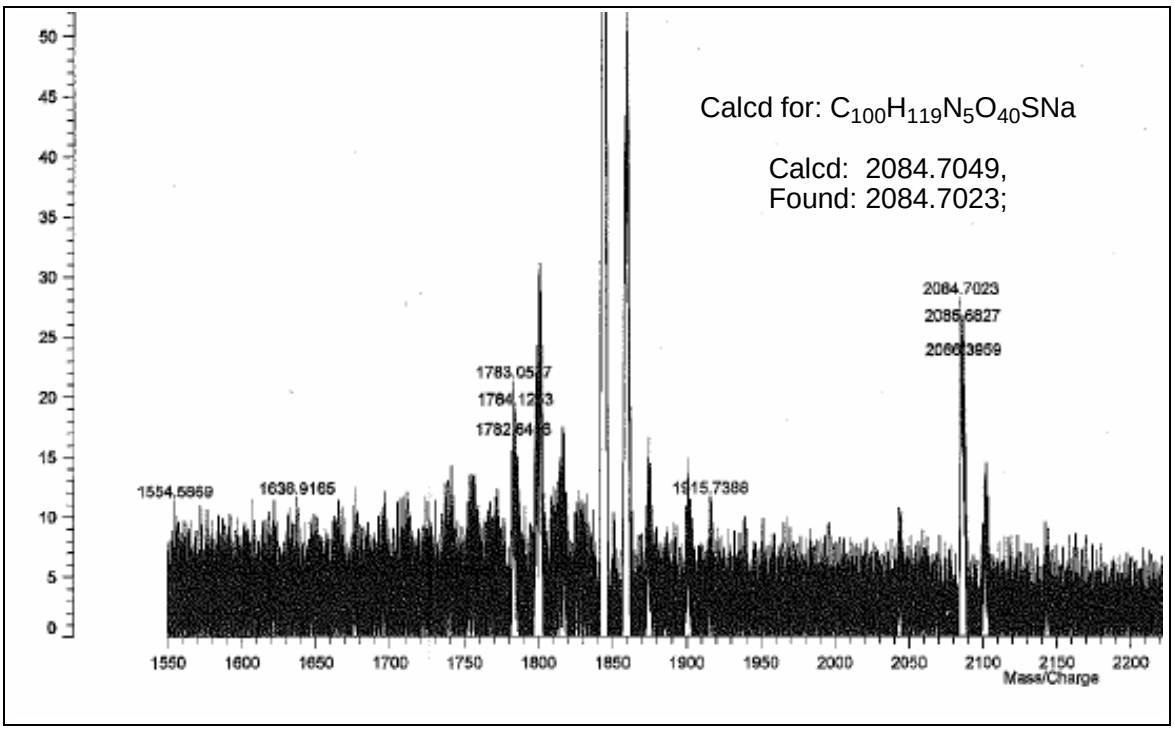
IR (film): 3335, 3060, 2965, 1735, 1655, 1510, 1370, 1215, 1025, 940 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ

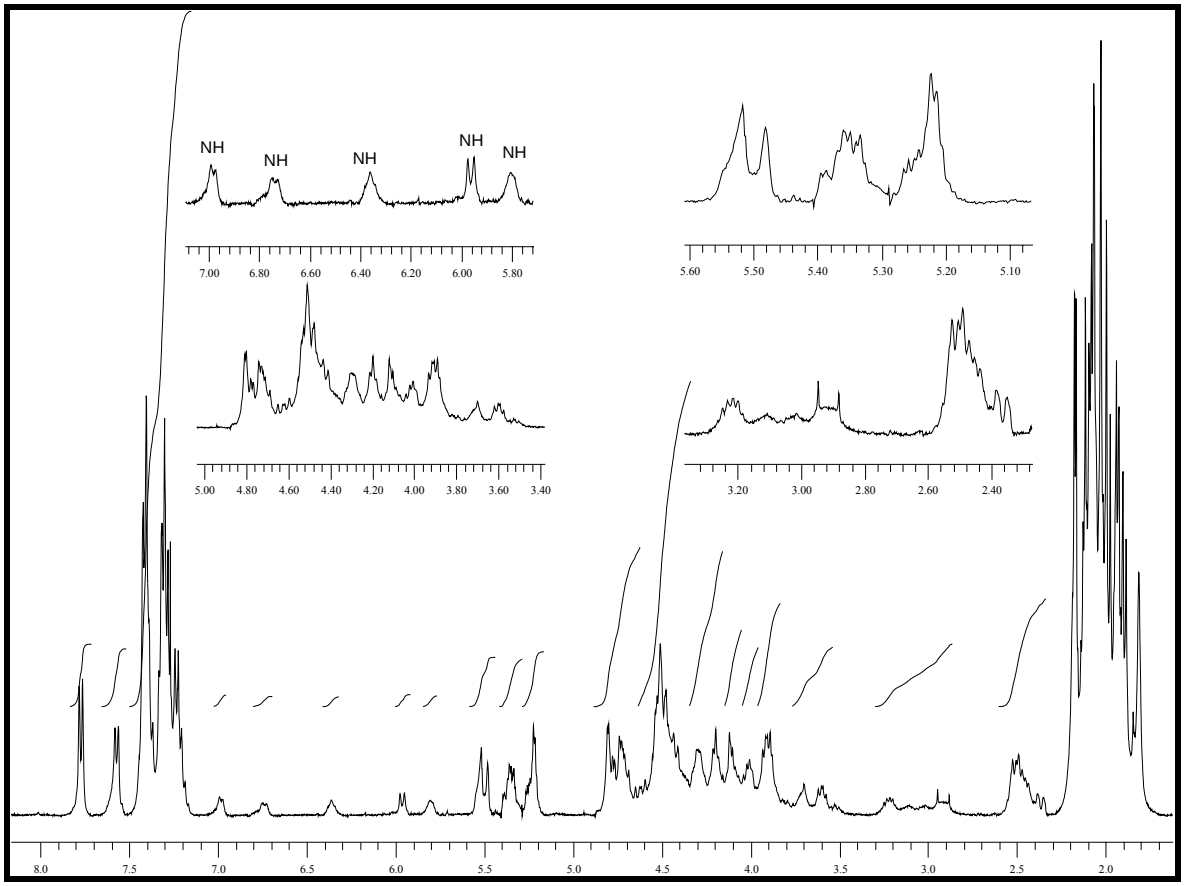
7.90-7.00 (4 m, 23H, ArH); 6.75(m, NH); 6.36(m, NH); 5.97(m, NH); 5.80(m, NH); 5.65-5.10 (m, 4H); 4.89-3.40(m, 27H); 3.31-2.8 (m, 4H), 2.70-2.3(m, 2H); 2.30-1.85 (m, 42H); 1.70-1.20 (m, 48H).

MALDI-HRMS Calcd for (M + Na) $C_{100}H_{119}N_5O_{40}SNa$ 2084.7049, Found 2084.7023;

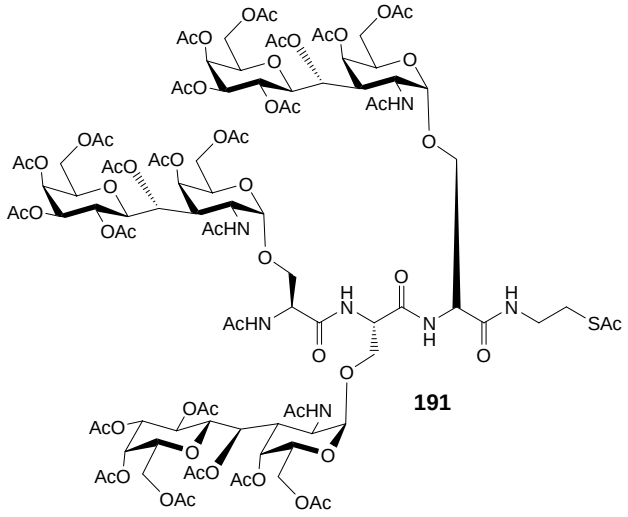
MALDI-HRMS spectrum of **190:**



1H-NMR (400 MHz, $CDCl_3$) spectrum of **190:**



_N_-acetyl-_O_-{3-_C_-[(1_R_)2,6-anhydro-D-_glycero_-L-_manno_-heptitol-1-_C_-yl]-2-[(_N_-acetyl) amino]-2,3-dideoxy-α-D-_galacto_-pyranosyl}-D-seryl-_O_-{3-_C_-[(1_R_)2,6-anhydro-D-_glycero_-L-_manno_-heptitol-1-_C_-yl]-2-[(_N_-acetyl)amino]-2,3-dideoxy-α-D-_galacto_-pyranosyl}-D-seryl-_rac_-_O_-{3-_C_-[(1_R_)2,6-anhydro-D-_glycero_-L-_manno_-heptitol-1-_C_-yl]-2-[(_N_-acetyl) amino]-2,3-dideoxy-α-D-_galacto_-pyranosyl}-_N_-[2-(acetylthio)ethyl]-D-serinamide (191).



191

The Fmoc-protected compound **190** (43 mg, 0.021 mmol) was dissolved in 5:1 DMF: piperidine (3ml) and stirred at room temperature for 1 h, time during which the reaction mixture was concentrated *in vacuo* and the residue was dissolved in 1 ml DMF. In a separated flask a solution of acid **183** (23 mg, 0.023 mmol) dissolved in DMF (1 ml), at 0°C a mixture of HOAt (4.7 mg, 0.036 mmol) and HATU (14 mg, 0.036 mmol), sym-collidine (11 μl, 0.073 mmol) in 1ml DMFwas added, and stirred for 15 min followed by addition of the solution of amine from the first flask via syringe. The reaction mixture was stirred at 0 °C for 1 h and allowed to slowly warm up to room temperature and stirred for 3 h, during which time the reaction mixture was concentrated *in vacuo* and the residue was dissolved in5:1 DMF: piperidine (3ml) and stirred at room temperature for 1 h, time during which the reaction mixture was concentrated *in vacuo* and the residue dissolved in 95% aq TFA (3 mL) and stirred at room temperature for 1 h. The solution was then co-evaporated with toluene several times to give the corresponding acid which was used

without further purification. The residue was dissolved in pyridine (1 ml) and Ac_2O (1m ml) and stirred at room temperature for 2 h. The solution was then co-evaporated with toluene several times then FC (5 % MeOH in CH_2Cl_2) gave 35 mg (68 %) of **186a**, white foam.

$[\alpha]^{25}_{589}$ = +33.3, $[\alpha]^{25}_{577}$ = +66.7, $[\alpha]^{25}_{435}$ = +193.3, $[\alpha]^{25}_{405}$ = +253.3 (*c* 0.015, $CHCl_3$).

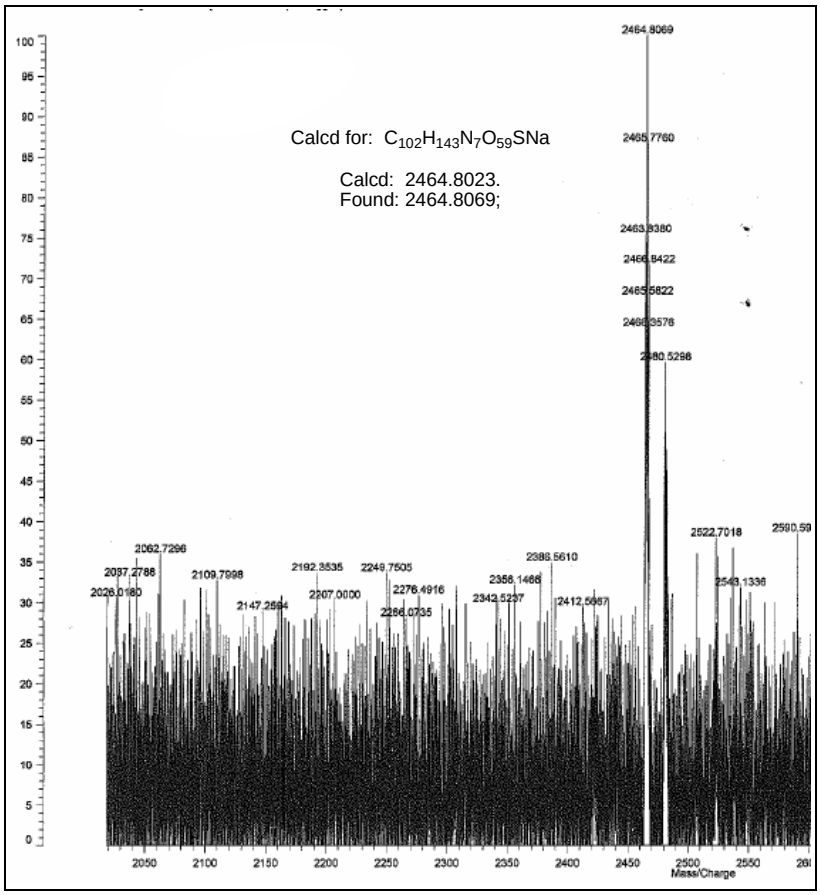
IR (film): 3320, 2960, 2925, 2845, 1740, 1655, 1370, 1220, 1025, 795 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$); δ

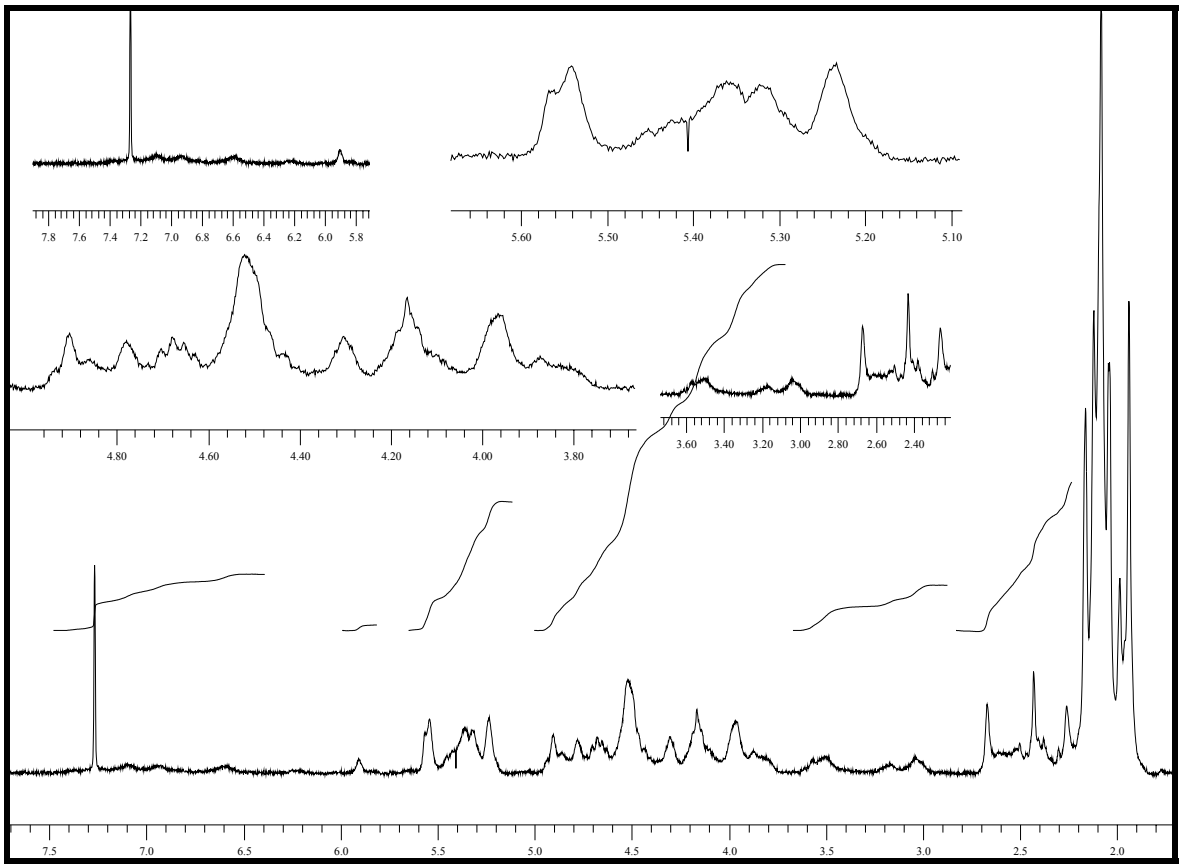
6.02-5.10(m, 14 H); 5.01-2.90(m, 52H); 2.85-1.70(m, 30H).

MALDI-HRMS Calcd for (M + Na) $C_{102}H_{143}N_7O_{59}SNa$ 2464.8023, Found 2464.8069;

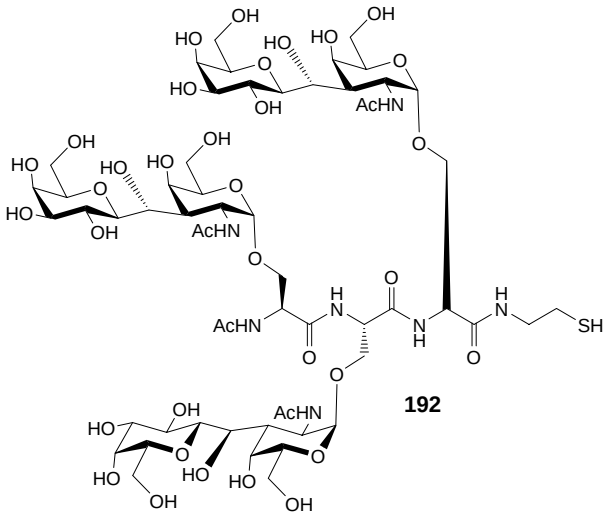
MALDI-HRMS spectrum of **191:**



1H-NMR (400 MHz, $CDCl_3$) spectrum of **191:**



***N*-acetyl-*O*-{3-*C*-[(1*R*)2,6-anhydro-D-*glycero*-L-*manno*-heptitol-1-*C*-yl]-2-[(*N*-acetyl) amino]-2,3-dideoxy-α-D-*galacto*-pyranosyl}-D-seryl-*O*-{3-*C*-[(1*R*)2,6-anhydro-D-*glycero*-L-*manno*-heptitol-1-*C*-yl]-2-[(*N*-acetyl)amino]-2,3-dideoxy-α-D-*galacto*-pyranosyl}-D-seryl-*rac*-*N*-(2-mercaptoethyl)-*O*-{3-*C*-[(1*R*)2,6-anhydro-D-*glycero*-L-*manno*-heptitol-1-*C*-yl]-2-[(*N*-acetyl)amino]-2,3-dideoxy-α-D-*galacto*-pyranosyl}-D-serinamide (192).**



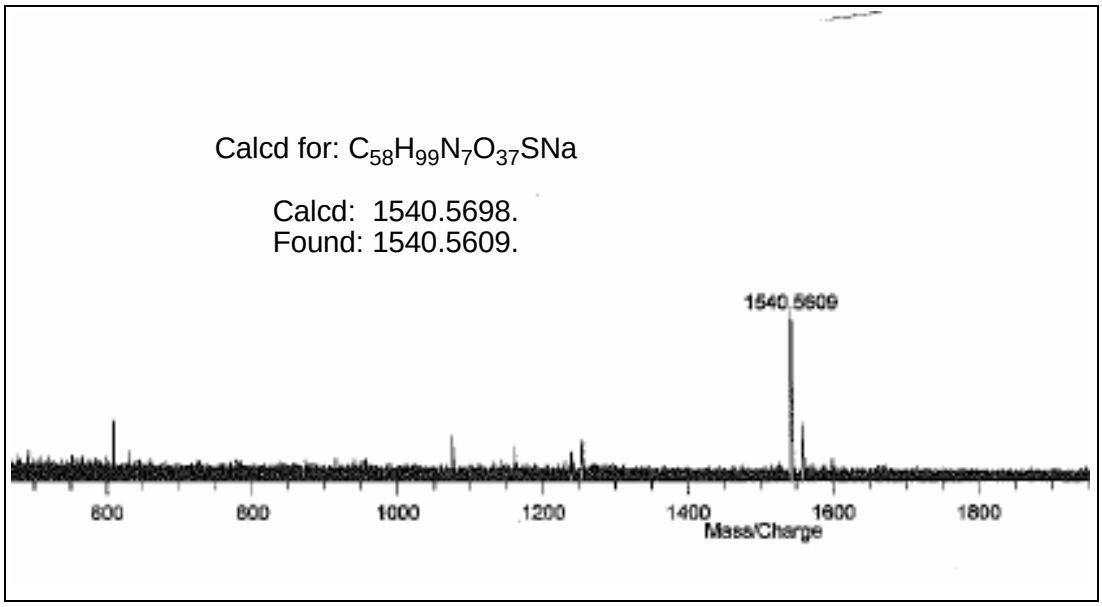
192

The peracetylated compound **191** (20 mg, 0.0082 mmol) was dissolved in MeOH, 2 µl of NaOMe solution (1M) were added. After 1h the mixture was neutralized by adding Dowex 50WX8-100, the mixture was stirred for 5 min, filtrated, and evaporated. HPLC gave **187b** 10.2 mg (82 %) as a white foam.

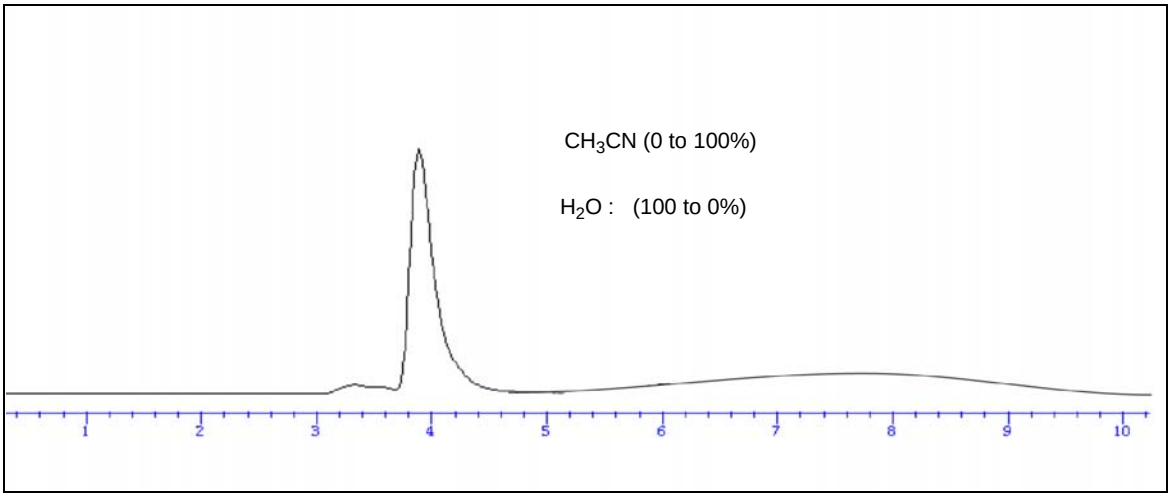
MALDI-HRMS Calcd for (M + Na) $C_{58}H_{99}N_7O_{37}SNa$ 1540.5698, Found 1540.5609;

Anal. calcd for $C_{58}H_{99}N_7O_{37}S$ (1518.50): C 45.88, H6.57, N 6.46; found: C 45.72, H 6.52, N 6.50.

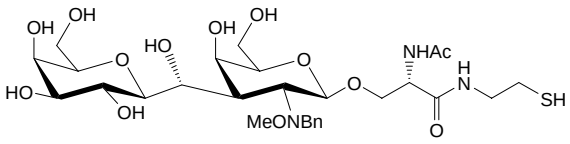
MALDI-HRMS spectrum of **192:**



HPLC chromatogram of **192:**



***N*-acetyl-{3-*C*-[(1*R*)-2,6-anhydro-D-*glycero*-L-*manno*-heptitol-1-*C*-yl]}-2-[(*N*-benzyl-*N*-methoxy)amino]-2,3-dideoxy-*β*-D-*galacto*pyranosyl}-*N*-[2-(thio)ethyl]-D-serinamide (195).**



195

To a solution of acid **193** (75.5 mg, 0.07 mmol) dissolved in DMF (3.5ml), at 0°C a mixture of HOAt (13.7 mg, 0.105 mmol) and HATU (40.3 mg, 0.105 mmol), sym-collidine (30.4 μl, 0.210 mmol) in 1ml DMFwas added, and stirred for 15 min followed by addition of the amine **197b** (22 mg, 0.07 mmol). The reaction mixture was stirred at 0 °C for 1 h and allowed to slowly warm up to room temperature and stirred for 1 h, during which time the reaction mixture was concentrated *in vacuo*. and the residue was dissolved in5:1 DMF: piperidine (3ml) and stirred at room temperature for 1 h, time during which the reaction mixture was concentrated *in vacuo* and the residue dissolved in 95% aq TFA (3 mL) and stirred at room temperature for 1 h. The solution was then co-evaporated with toluene several times to give the corresponding acid which was used without further purification. The residue was dissolved in Ac_2O (1m ml) and stirred at room temperature for 30 min. The solution was then co-evaporated with toluene several times FC (5 % MeOH in CH_2Cl_2) gave peracetylated sugar. The latter was dissolved in MeOH, 2 μl of NaOMe solution (1M) was added. After 1h the mixture was neutralized by adding Dowex 50WX8-100, the mixture was stirred for 5 min, filtrated, and evaporated gave **195** 37 mg (80 %) as a brown foam.

FC (5 % MeOH in CH_2Cl_2) gave 35 mg (68 %) of **186a**, white foam.

$[\alpha]^{25}_{589}$ = -346.7, $[\alpha]^{25}_{577}$ = -560.0, $[\alpha]^{25}_{435}$ = -426.7, $[\alpha]^{25}_{405}$ = -413.3 (*c* 0.0075, MeOH).

^{1}H NMR (400 MHz, CD_3OD): δ

7.44-7.04 (m, 5H, H-C(arom)); 4.88 (d, ^{3}J(H-C(1), H-C(2)) = 8.0, H-C(1)); 4.80 (m, H-C(4)); 4.54 (t, ^{3}J(H-C*(Ser),H_2-C(Ser) = 5.0, H-C*(Ser));4.31-4.22 (m, H-C(1'), H_a-C(CH_2Ph); 4.10 (dd, ^{2}J = 10.6, ^{3}J(H_2-C(Ser), H-C*(Ser)= 5.8, H_2-C(Ser)); 4.06-3.95 (m, 3H, H-C(5), H_a-C(C6), H-C(C3'), H-C(C3')); 3.91-3.72 (m, 3H, H-C(2'), H_b-(CH_2Ph), H-C(C4')); 3.70-3.57 (m,3H, H_b-C(6), H_a-C(7'), H_b-C(7')); 3.50-3.31 (m,3H, H_a-C(N<u>C</u>H_2), H_b-C(N<u>C</u>H_2), H-C(C6')); 3.25.3.11 (m, 4H, H-C(OCH_3), H-C(2)); 2.74-2.63 (m,2H, H_a-C(HS<u>C</u>H_2), H_b-C(HS<u>C</u>H_2)); 2.84 (dt, ^{3}J(H-C(2), H-C(3)) = 12.3, ^{3}J(H-C(2), H-C(1)) = 8.0, H-C(2)); 2.20 (br d, ^{3}J(H-C(3), H-C(2)) = 12.3, ^{3}J(H-C(3)); 1.99 (s, 3H, CH_3COO).

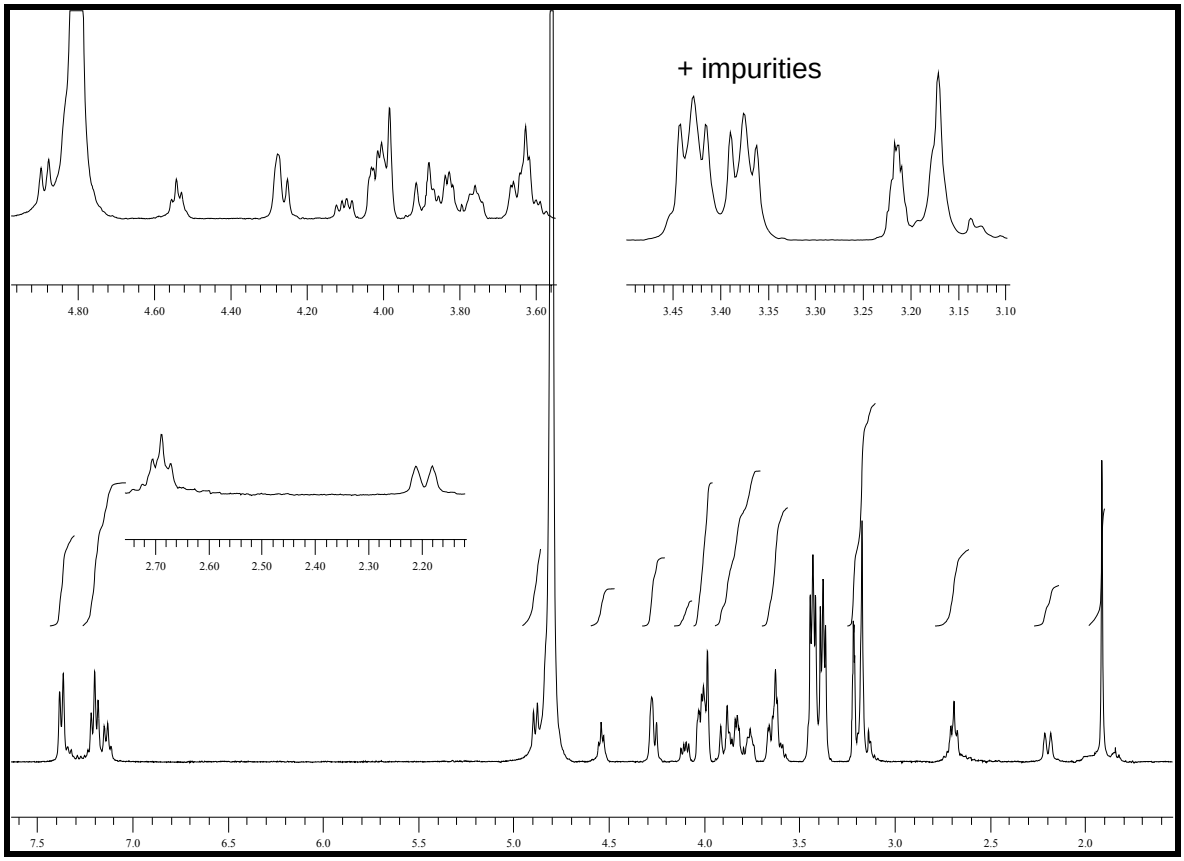
^{13}C NMR (100.6 MHz, $CDCl_3$): δ

173.4(s, -COO); 172.3(s, -COO); 140.5 (s, C(arom)); 130.9 (d, ^{1}J(C,H) = 162, C(arom)); 129.1 (d, ^{1}J(C,H) = 158, C(arom)); 128.1 (d, ^{1}J(C,H) = 158, C(arom)); 104.0(d, ^{1}J(C,H) = 159, C(1)); 79.6 (d, ^{1}J(C,H) = 150, C(6')); 78.3 (d, ^{1}J(C,H) = 157, C(4')); 72.5 (d, ^{1}J(C,H) = 144, C(2')); 70.5 (d, ^{1}J(C,H) = 139, C(5)); 69.8 (d, ^{1}J(C,H) = 149, C(5')); 69.7 (t, ^{1}J(C,H) = 153, <u>C</u>H_2(Ser)); 68.9 (d, ^{1}J(C,H) = 150, C(4)); 68.6 (d, ^{1}J(C,H) = 139, C(1')); 67.6 (d, ^{1}J(C,H) = 155, C(3')); 63.4 (q, ^{1}J(C,H) = 130, O<u>C</u>H_3); 63.0 (t, ^{1}J(C,H) = 149, C(N<u>C</u>H_2Ph)); 63.0 (t, ^{1}J(C,H) = 148, C(7')); 61.5 (d, ^{1}J(C,H) = 139, C(2)); 60.0 (t, ^{1}J(C,H) = 149, C(6)); 55.1 (d, ^{1}J(C,H) = 138, C∗(Ser)); 42.0 (d, ^{1}J(C,H) = 127, C(3)); 39.9 (t, ^{1}J(C,H) = 148, C(N<u>C</u>H_2-)); 39.9 (t, ^{1}J(C,H) = 133, C(HS<u>C</u>H_2-)); 21.1 (q, ^{1}J(C,H) = 130, <u>C</u>H_3COO).

MALDI-HRMS Calcd for (M + Na) $C_{28}H_{45}N_3O_{13}SNa$ 686.2571; found 686.6705.

Anal. calcd for $C_{28}H_{45}N_3O_{13}S$ (663.73): C 50.67, H6.83, N 6.33; found: C 50.69, H 6.82, N 6.22.

1H-NMR (400 MHz, $CDCl_3$) spectrum of **195:**



Protocol: Coupling of Synthetic Peptide to Carrier Protein Using MBS

Coupling of Synthetic Peptide to Carrier Protein Using MBS

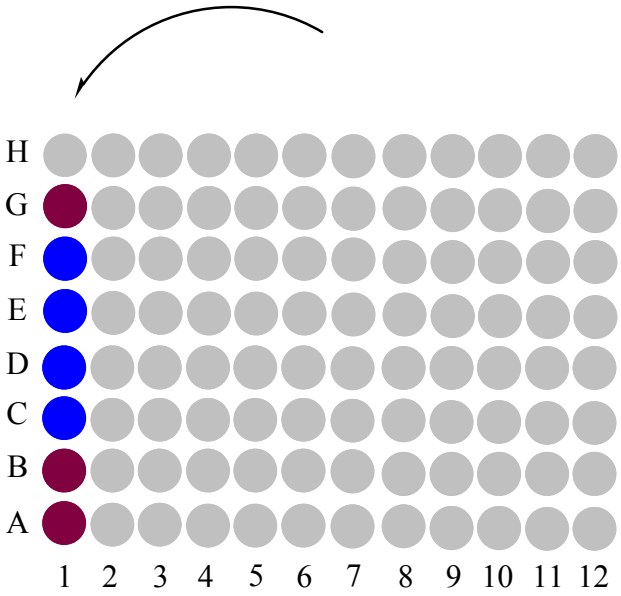
1. Dissolve 5mg keyhole limpet hemocyanin (KLH) in 0.5ml 0.01M Phosphate Buffer (pH 7.0)
2. Dissolve 3mg *m*-maleimidobenzoyl-*N*-hydroxysuccinimide ester (MBS) in 200µl Dimethyl Formamide (DMF), prepared fresh
3. Add 70µl MBS/DMF to KLH solution; gently stir at RT for 30min
4. Pre-equilibrate Sephadex G-25column with 0.05M Phosphate Buffer (pH 6.0); load KLH reaction mixture. Elute the column with 0.05M Phosphate Buffer (pH 6.0)
5. Collect 3.5ml (in 7 fractions) MBS/KLH conjugate solution
6. Dissolve 5mg synthetic peptide in 1ml PBS
7. Add peptide solution to MB/KLH conjugate
8. Adjust pH to 7.3 with 0.1M HCl or 0.1M NaOH
9. Stir 3 hrs at RT
10. Pass through another Sephadex G-25 column to remove the unbound peptide, using water as buffer
11. Collect 3.5ml (in 7 fractions) of cluster-MBS-KLH conjugate solution
12. Lyophilize the conjugate

The collection fraction procedure in point 5 and 11:

1. Mix 100µl of water with 100µl bradford reagent in microwell plate.
2. You will observe a brown color in this mixture.
3. After 1 min add 10 µl from the collected fractions solution to the wells.
4. Collect the fractions that transform the color into dark blue with each other in a small flask, then put this fractions in dialysis tube cutoff 30,000 (look to the following Fiqure).
5. After the dialysis lyophilize the conjugate

Analysis of the collected fraction microwell plate

1. add 100µl Bradford solution, and100µl of double distilled water
2. add 10µl of the collected fractions.
3. the blue color is indicatating the presence of protein exist in corresponding fractions.



CURRICULUM VITAE

AWAD Loay

Av. d'Echallens 68
Lausanne 1004
Tel : +41 021/6939473
Mobile : +41 076/5051405,
Email : loay.awad@epfl.ch

Date of birth:	June 12, 1973
Place of birth:	Jerusalem
Nationality:	Palestinian
Marital status:	Single

Education:

2000-2005	PhD studies, University of Lausanne, Swiss Institute of Technology Lausanne, in the group of Prof. Dr. Pierre Vogel
2001	DEA multinational de Chimie Moléculaire, Ecole Polytechnique, Palaiseau, France
1999-2000	Qualification as high school chemistry teacher.
1995-1999	Bachelor of Chemistry (with distinction), University of Al-Quds, Jerusalem, Palestine
1990-1991	Altwjeehy (State exam for entering the university)

Professional experience:

2001-2004	Teaching assistant, organic chemistry, Swiss Institute of Technology Lausanne
1995-2000	Teaching assistant, Faculty of Chemistry, Al-Quds University, Palestine

Languages:

Arabic	Mother tongue
English	Fluent
French	Good working knowledge
Hebrew	Basic knowledge

Awards and scholarships:
Prize for the best poster presentation at. The Fallmeeting of the Swiss Chemical Society, Zürich, Switzerland, October 7, **2004**.

Peace program scholarships, October, **2000**

.

Publications:

Awad, L.; Riedner, J.; Vogel, P.; C-Linked Disaccharide Analog of the Thomsen-Friedenreich (T)-Epitope α-O-Conjugated to L-Serine. *Chem. Eur. J.* **2005**,*11*, 3565-3573.

Huang, X.; Craita, C.; **Awad, L.**; Vogel, P. Silyl methallysulfinates-as efficient and powerful agents for the silylation of alcohols, phenols and carboxylic acids. *Chem. Commun.* **2005**, 1297-1299. accepted as a hot paper.

Demange, R.; **Awad, L.**; Vogel, P. Synthesis of C-linked analogues of β-D-galactopyranosyl-(1→3)-D-galactopyranosides and of β-D-galactopyranosyl-(1→3)-D-galactal. *Tetrahedron: Asymmetry* **2004**, *15*, 22, 3573-3585.

Oral Presentation:

Awad, L.; Vogel, P. Synthesis of a *C*-linked Disaccharide Analog of the Thomsen Friedenreich (T)-Epitope α-O-Conjugated to L-Serine and Formation a Cluster as Potential Anticancer Vaccine. The Fall Meeting of the Swiss Chemical Society, Zürich, Switzerland, 2004. *Chimia*, **2004**, *58*, 462.

Vogel, P.; **Awad, L.**; Riedner, J.; Demange, R. *C*-Linked disaccharides analogues of the T epitope: Toward an artificial anti-cancer vaccine. Abstracts of Papers, 228th ACS National Meeting, Philadelphia, PA, United States, August 22-26, **2004** (2004),
Presented orally by Prof. P. Vogel

Posters:

Awad, L.; Riedner, J.; Vogel, P. Synthesis of a *C*-linked Disaccharide Analog of the Thomsen Friedenreich (T)-Epitope *α*-O-Conjugated to L-Serine and Formation a Cluster as Potential Anticancer Vaccine. *Chimia*, **2004**, *58*, 468. (The Fall Meeting of the Swiss Chemical Society, Zürich, Switzerland, 2004)

Awad, L.; Riedner, J.; Vogel, P. Synthesis of C-glycosides Analogues of the Thomsen-Friedenreich (T) Antigen and of Non-hydrolyzable Sugar Epitopes for Conjugation with Nanoparticles. Proceedings of Tailored Nanoparticles: The challenge in Diagnostics and Therapeutics, Lausanne, Switzerland, October 16 -17, **2003**.

Awad, L.; Riedner, J.; Vogel, P. Towards the Synthesis of Anticancer Vaccine *Chimia*, **2003**, *57*, 402. (The Fall Meeting of the Swiss Chemical Society, Lausanne, Switzerland, 2003)

Awad, L.; Riedner, J.; Vogel, P. Towards the Synthesis of Non-Hydrolysable Analogues of The T-antigen. Proceedings of Molécules du future: les defis de la synthèse organique, Lyon, France, December 9-11, **2002**.

Invited lecture:

In 08-04-2005 the lecture in title (**Synthesis of a *C*-linked Disaccharide Analog of the Thomsen Friedenreich (T)-Epitope α-O-Conjugated to L-Serine and Formation a Cluster as Potential Anticancer Vaccine)**
Laboratorium für Organische Chemie, ETH, Hönggerberg, HCI F 315, Wolfgang-Pauli-Str. 10, 8093 Zürich.